Biochimica et Biophysica Acta 1843 (2014) 1442–1456

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Review

Chaperone networking facilitates protein targeting to the bacterial cytoplasmic membrane ☆☆

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ARTICLE INFO

Article history:
Received 4 September 2013
Received in revised form 10 November 2013
Accepted 13 November 2013
Available online 21 November 2013

Keywords:
Protein targeting pathway
DnaK/Hsp70
DnaJ/Hsp40
GroEL/Hsp60
Trigger Factor
SecA–SecB

ABSTRACT

Nascent polypeptides emerging from the ribosome are assisted by a pool of molecular chaperones and targeting factors, which enable them to efficiently partition as cytosolic, integral membrane or exported proteins. Extensive genetic and biochemical analyses have significantly expanded our knowledge of chaperone tasking throughout this process. In bacteria, it is known that the folding of newly-synthesized cytosolic proteins is mainly orchestrated by three highly conserved molecular chaperones, namely Trigger Factor (TF), DnaK (HSP70) and GroEL (HSP60). Yet, it has been reported that these major chaperones are strongly involved in protein translocation pathways as well. This review describes such essential molecular chaperone functions, with emphasis on both the biogenesis of inner membrane proteins and the post-translational targeting of presecretory proteins to the Sec and the twin-arginine translocation (Tat) pathways. Critical interplay between TF, DnaK, GroEL and other molecular chaperones and targeting factors, including SecB, SecA, the signal recognition particle (SRP) and the redox enzyme maturation proteins (REMPs) is also discussed. This article is part of a Special Issue entitled: Protein trafficking and secretion in bacteria. Guest Editors: Anastassios Economou and Ross Dalbey.

1. General overview of the protein folding and targeting pathways in bacteria

In bacteria, nascent polypeptide chains emerging from the ribosomal exit tunnel are assisted by molecular chaperones and targeting factors that facilitate their partition as cytosolic, integral membrane, or exported proteins. Although selective binding to such factors is primarily determined by the amino acid composition of the nascent chain, including the presence of secondary structures, signal anchor or signal peptide sequences, it is significantly influenced by the translation machinery itself [1–3]. Indeed, the presence of rare codons in mRNA sequences can modulate the rate of translation and significantly influence both the folding of nascent polypeptides and interactions with downstream chaperones [1,3–5]. Moreover, the ribosomal exit tunnel can promote nascent chain compaction or helical conformation in the lower and upper part of the tunnel, the middle part of the tunnel being constricted by L4 and L22 ribosomal proteins [6]. Tertiary structures of small domain units can also form near the exit port of the tunnel and the ribosome itself can prevent misfolding of nascent chains by modulating their folding rate [7,8]. Key to the early recruitment of molecular chaperones and targeting factors to emerging nascent chains is the ribosomal protein L23, which is located at the ribosomal polypeptide exit and serves as a docking site for several factors, including the molecular chaperones Trigger Factor (TF), the signal recognition particle (SRP), the translocase motor SecA and other proteins involved in enzymatic processing of nascent chains [9,10]. Early binding to such factors strongly impacts the downstream interacting cascades leading to appropriate cellular localization and folding of newly-synthesized proteins.

In the bacterium *Escherichia coli*, newly-made polypeptides of integral cytoplasmic membrane proteins and some presecretory proteins, which contain strong hydrophobic signal anchor or signal peptide sequences, are recognized by SRP as they are emerging from the ribosome and targeted co-translationally to the Sec translocation pore at the cytoplasmic membrane [11]. Yet, certain inner membrane proteins are directly targeted to the insertase YidC at the inner membrane in an SRP-independent manner [12]. In contrast, the majority of presecretory proteins are either translocated folded via the twin-arginine translocation (Tat) pathway or unfolded via the Sec pathway [13,14].

Presecretory proteins of the Sec pathway, which contain mildly hydrophobic signal sequences, need to cross the inner membrane before they acquire their stable tertiary structures. Such proteins are generally targeted post-translationally to the Sec translocon at the inner membrane via a dedicated pathway involving two cytosolic proteins: the

Abbreviations: TF, Trigger Factor; HSP, heat shock protein; SRP, signal recognition particle; DnaKJE, DnaK/DnaJ/GrpE chaperone machine; GroESL, GroEL/GroES chaperonins

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0167-4889
http://dx.doi.org/10.1016/j.bbamcr.2013.11.007

ATPase SecA and the molecular chaperone SecB [15,16]. In this case, the essential SecA protein, which has an increased affinity for proteins containing signal sequences, binds to nascent presecretory proteins, delivers its client to the Sec translocon and drives the ATP-dependent translocation through the Sec translocon [17]. While an early co-translational interaction of SecA with precursors is facilitated by its specific affinity for the ribosomal protein L23 at polypeptide exit [18], the post-translational targeting of precursors by SecA is greatly facilitated by the ATP-independent homotetrameric chaperone SecB, which is conserved in most proteobacteria [19]. SecB is known to bind nonnative substrates either co- or post-translationally, with high affinity and without specificity for signal sequences. SecB can either bind substrates alone and targets them to the Sec translocon via its specific binding site on SecA, or be recruited to early SecA-precursor complexes released from the ribosome: both events leading to post-translational translocation of precursors [16,20].

The Tat protein export pathway acts independently of the Sec pathway to post-translationally translocate proteins and protein complexes that have been previously folded and assembled in the cytoplasm [13,21]. Tat protein substrates possess a classical signal sequence with a consensus sequence (S/T)-R-R-x-F-L-K inserted between the N-region and the hydrophobic core, which mediates specific targeting to the Tat translocon at the inner membrane. Many Tat substrates possess specific chaperones called REMPs (redox enzyme maturation proteins) that interact with the twin-arginine leader peptide, protect Tat substrates from degradation, and assist its folding/assembly and its later engagement with the Tat translocon [22].

The role played by molecular chaperones during the folding of newly-synthetized cytosolic proteins in *E. coli* has been extensively studied during the past 15 years [23] and it has been shown that such a process is mainly orchestrated by the highly conserved molecular chaperones TF, DnaK (HSP70) and GroEL (HSP60) [24–28]. The ribosome-bound TF is the first chaperone to interact co-translationally with nascent polypeptides and it is believed that the majority of the newly synthesized proteins can reach their native state in the cytosol without additional help. Yet, a substantial portion of cytosolic proteins (about 30%) need further co- and/or post-translational assistance by the downstream ATP-dependent molecular chaperones DnaK and GroEL to complete their folding [9]. Interestingly, it has been shown that these three major molecular chaperones play important roles in protein targeting pathways as well. Moreover, significant interplays have been observed between these chaperones and other factors involved in such pathways, including SecB, SecA, SRP and the specific REMPs of Tat substrates. In this review, we wish to examine such specific chaperone tasking of TF, DnaK and GroEL. Following a brief description of their structure, their chaperone cycle, their substrate specificity and their well-established function during the folding of cytosolic proteins (Sections 2 to 5), a detailed analysis of their key contribution to the main protein targeting and translocation pathways will be presented (Sections 6 to 8).

2. The ribosome-bound TF chaperone

The ribosome-bound TF is the first known molecular chaperone to interact with newly synthesized polypeptides in *E. coli* [29]. It is a 48 kDa protein constituted of 3 distinct domains with an elongated topology (Fig. 1; [30]). The N-terminal domain of TF is the ribosome binding domain, which also contributes to its chaperone activity. This domain possesses some structural homology with the molecular chaperone Hsp33 except for the additional loop containing the TF signature motif "GFRxGxxP" involved in the binding of TF to the ribosomal protein L23 close to the polypeptide exit tunnel [31,32]. A long linker region is found between the ribosome binding domain and the active peptidyl-prolyl *cis*/*trans* isomerase domain (PPIase) of TF. The PPIase domain is dispensable for the chaperone function of TF *in vivo* in *E. coli*, but can enhance chaperone activity of TF as a secondary binding site for substrates *in vitro* [33]. The C-terminal domain of TF, which is composed of two protruding helical arms positioned between the N-terminal and the PPIase domains, resembles the N-terminal domain of the periplasmic chaperone SurA. This domain represents the main chaperone domain of TF, providing binding sites for emerging polypeptides [30,33,34].

Although TF is conserved in bacteria and chloroplasts, its PPIase or its N-terminal domain may be absent in some cases [35–37], and certain bacteria such as *Desulfitobacterium hafniense* even possess more than one TF chaperone [37]. TF is an abundant cytosolic protein, with a cellular concentration of ~50 μM, thus exceeding that of ribosome by 2 to 3 fold. It cycles on and off the ribosome in an ATP-independent manner and with a 1:1 stoichiometry. The presence of a nascent chain significantly increases the affinity of TF for the ribosome [35,38–40]. Following release from the ribosome, TF may stay bound to elongating polypeptides, allowing another free TF to bind the ribosome and in some cases to facilitate transfer of its substrate to the downstream chaperones DnaKJE and GroESL [40]. In addition, it has been shown that free TF can dimerize and encapsulate partially folded proteins perhaps facilitating their incorporation into larger protein complexes, thus revealing an additional role for TF in protein complex assembly [41].

TF interaction with the ribosome is crucial for its chaperone activities during co-translational folding of nascent polypeptide chains and it has been shown that *in vitro* TF can interact with nascent polypeptide chains as short as 40 amino acids [33,42,43]. Yet, ribosome profiling analysis of TF-ribosome-nascent chain complexes indicates that *in vivo* TF may preferentially bind to longer nascent polypeptide chains of about 100 amino acids [44]. This relatively late interaction between TF and the nascent chain may facilitate earlier interaction with SRP or with the nascent chain processing enzymes peptide deformylase (PDF) and methionine aminopeptidase (MAP), as shown recently [10,45–47].

It is believed that ribosome-bound TF interacts co-translationally with most of the newly synthesized polypeptides and that 60–70% of the *E. coli* cytosolic proteins interacting with TF could reach their native state without further assistance by the downstream chaperones DnaKJE and GroESL [24,25]. In agreement with such a wide range of interactors, a screening of membrane-bound peptides for TF binding revealed that this chaperone preferentially interacts with short motifs enriched in aromatic residues and basic residues that are frequently found (every 32 residues) in proteins [42,48]. In addition, it was recently shown that TF utilizes several substrate binding surfaces of its three domains to bind continuous and discontinuous hydrophobic sequences in nascent polypeptides as well as hydrophilic regions in folded proteins [41,49]. While the presence of TF arched over the polypeptide exit can facilitate the folding small protein domains [42,50], it has been demonstrated that upon ribosome binding TF can significantly delay the folding of larger proteins in order to minimize misfolding and aggregation [26,51,52], perhaps decreasing the rate of structural rearrangements within the nascent peptide and/or avoiding tertiary structure formation [52]. In addition to folding retardation, TF also exhibits unfolding activity to potentially reroute unproductively folded nascent chains [51]. Remarkably, such chaperone properties of TF might significantly contribute to the post-translational targeting of presecretory proteins that need to reach the Sec translocon in a non-native, translocation competent form (see Section 6 below).

In vivo, mutations in the *E. coli tig* gene encoding TF are tolerated and exert no major effect on *E. coli* growth under standard laboratory conditions [24,25,53]. Nevertheless, *tig* mutants are sensitive to certain antibiotics and detergents and exhibit reduced and increased cell viability at 4 °C and 50 °C respectively [54,55]. Note that the main TF-associated phenotypes in *E. coli* are shown in Table 1. Mutations in *tig* have been characterized in other bacteria as well, and in these cases the absence of TF generally produces modest effects on bacterial growth, as observed for *E. coli*. These bacteria include *Bacillus subtilis* [56,57], *Sinorhizobium meliloti* [58], *Listeria monocytogenes* [59], *Streptococcus mutans* [60,61] and *Streptococcus pyogenes* [62]. In *E. coli*, such a discrepancy between the major contribution of TF during *de*

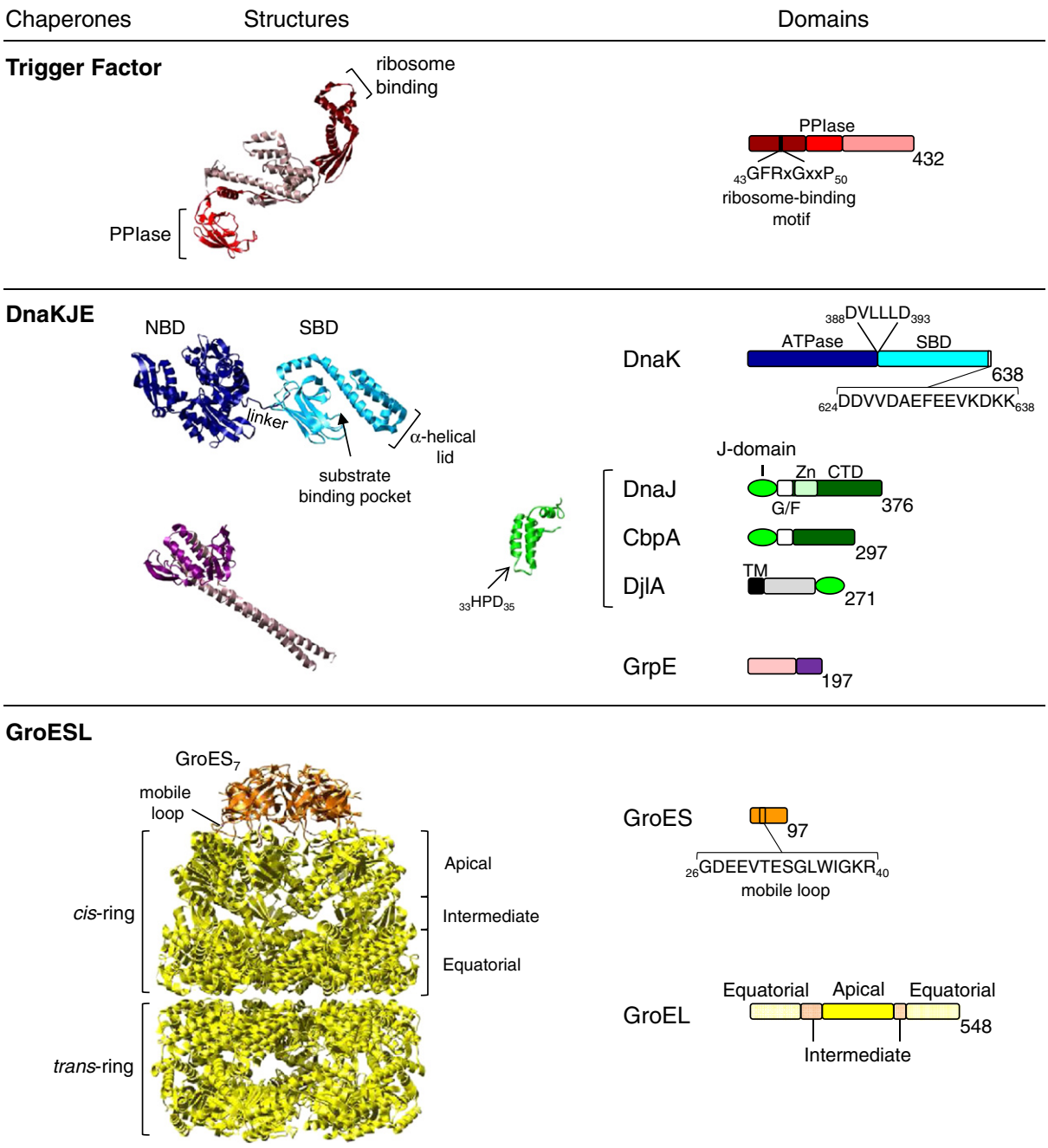

Fig. 1. The TF, DnaKJE and GroESL chaperone machines from *E. coli*. The amino-acid lengths of each protein are indicated. The three-dimensional structures are displayed by chimera. N-terminal domain of TF (dark magenta) contains the ribosome binding motif (sequence presented in the domains diagram part) and a long linker connected to the PPIase domain (red); the C-terminal domain (salmon) inserts between the 2 other domains and is the primary binding-site for nascent polypeptides (Protein Data Bank (PDB) 1W26). The DnaK structure represents the closed state of ADP-bound DnaK (PDB 2KHO); the nucleotide-binding domain (dark blue) is connected by a conserved linker to the substrate-binding domain (light blue) where the α-helical lid is closed over the substrate. Sequences of the linker and the unstructured C-terminal regions are presented. DnaK-cochaperones defined by their J-domains (green) are DnaJ, CbpA and DjlA. Structure of the DnaJ J-domain (PDB 1XBL) is presented with the conserved HPD motif in the loop; other domains are the glycine–phenylalanine-rich domain (G/F, white), the zinc binding domain (Zn, light green), C-terminal domains (CTD, dark green), transmembrane domain (TM, black) and in DjlA, central domain of unknown function (grey). The GrpE NEF is presented in dimeric state (PDB 1DKG) with the long α-helices of the N-terminal domains (light pink) and the compact C-terminal domains (purple). The chaperonin/cochaperonin complex is presented in the ADP-bound state (PBD 1SVT). The heptameric dome of GroES (orange) is connected via the mobile loops (sequence presented in the diagram part) to the apical domains of the ADP-bound-GroEL (yellow) of the *cis*-ring.

novo protein folding and the lack of severe phenotype *in vivo* is largely explained by the fact that TF functions are efficiently substituted by the DnaKJE chaperone machine.

3. The DnaKJE chaperone machine

The multifunctional DnaKJE chaperone machine from *E. coli* is the best characterized member of the HSP70 chaperone family. It is one of the most abundant cytoplasmic chaperones expressed constitutively (with a cellular concentration of ~30 μM) and its expression is induced in response to different stresses [63]. DnaK is a 638 amino acid long protein defined by an N-terminal nucleotide binding domain (NBD) with ATPase activity, a C-terminal substrate binding domain (SBD) subdivided into a β-sandwich subdomain and a α-helical domain forming a lid. Both domains are connected by a linker (aa 381 to 397) which is highly conserved and possesses a characteristic $_{388}$DVLLLD$_{393}$ hydrophobic segment essential for allosteric communication between the NBD and the SBD (Fig. 1; [64–66]). In addition, DnaK possesses a

Table 1
Most relevant phenotypes associated with mutation or overexpression of the TF, DnaKJE and GroESL chaperones of *E. coli*.

Chaperones	Phenotypes[a]
TF	**A**: Sensitive to vancomycin, SDS, dibucaine, and EDTA [44,55]. Suppresses Ts of *secA51* mutant, Cs of *secY40*, *secAR11*, *secB* [125] and *dnaJ* mutants [125] and the cell division defect of *divE42* mutant [198]. Depletion induces cell filamentation [53], reduces viability at 4 °C and increases viability at 50 °C [54]. Ts (above 30 °C) if combined with *dnaK* mutation [31]. SecB overexpression suppresses Ts phenotype of *dnaK tig* [118]. **B**: Toxic [53], increased toxicity in *secB* or *dnaK* mutants [31,125], and reduced toxicity in *ompF* mutant [31]. Severe cell filamentation suppressed by co-overexpression of FtsZ [53]; enhances viability in the cold [54].
DnaKJE	**A**: *dnaK*: Cs below 18 °C, Ts above 35 °C, slow growth, filamentous, flat and translucent colonies. Readily accumulates better growing suppressors. Non-motile, lack flagella, sensitive to nutrient starvation and osmotic stress. Defective for starvation-induced thermotolerance and H_2O_2 resistance. Sensitivity to hydroxyurea, AZT, X-irradiation, novobiocin and fluoroquinolones, defective in plasmid maintenance, resistance to bacteriophages P1, P2 and λ [63]. Reduced persistence and multidrug tolerance [199]. Defective in type VI secretion [162], in export [125], and for growth on several carbon sources such as mannose, maltose and glycerol [200]. Synthetic lethality with *secB* mutation [28]. Induction of the entire heat-shock response [201] and of SecB expression [148]. Synergizes with *tig* and *groESL* mutations ([31]; unpublished data). *dnaJ*: Cs below 14 °C, Ts above 43 °C, filamentous, altered motility, defective in plasmid maintenance,resistance to bacteriophages P1, P2 and λ; suppresses colanic acid induction and UV sensitivity of a *lon* mutant [63]. Sensitive to spectinomycin, copper, low pH, high salt, cobaltazido thymidine [55]. *grpE*: Essential for growth only in the presence of DnaKJ [202]. **B**: DnaK: Toxic and induces mucoidy, filamentation, defect in plasmid maintenance. Defects are partially suppressed by the co-expression of DnaJ [63]. DnaKJ: Suppresses Cs of *secB* mutant. Induces tolerance to mutations [203].
GroESL	**A**: Essential [93]. Point mutations and depletion lead to defects in DNA and RNA synthesis [204], UV sensitivity [205], filamentation [98], increased fluoroquinones susceptibility [206], resistance to bacteriophages T4 and λ [207], induction of the Heat-Shock Response [201], inability to grow on rhamnose [208]. Synergy with *dnaK* mutation (unpublished data). **B**: Suppresses Ts of *secA51* [141,182] and *tig dnaK* mutants [31,117], and Cs of *secB* [182], *secY205*, *secY129*, *secAR11* mutants (unpublished results). Induces tolerance to aminoglycoside [209] and to mutations enabling adaptive evolution [203]. Enhances extreme thermoresistance [210].

[a] Phenotypes associated with mutation (**A**) or overexpression (**B**) of the *E. coli* chaperones described in this work. Ts and Cs stand for temperature- and cold-sensitive phenotype, respectively.

short conserved region at the extreme C-terminal (residues 624–638), which most likely acts as an auxiliary binding site for unfolded substrates [67]. During the DnaK chaperone cycle, the open/closed state of the SBD is controlled by the nucleotide occupancy and status in the ATPase domain of DnaK [64]. The ATP-bound DnaK (lid open) is characterized by a low affinity and fast exchange rate for its substrates whereas the ADP-bound form (lid closed) exhibits a high affinity and low exchange rates. To prevent uncontrolled interaction with random substrates, the DnaK chaperone cycle is tightly regulated by essential co-chaperone partners. The DnaJ (Hsp40) co-chaperone family members stimulate ATP hydrolysis and substrate delivery to DnaK, both events leading to ADP-bound DnaK in complex with its substrate. This family of proteins is characterized by the presence of a small compact domain of approximately 70 amino acid residues, named the J-domain, which is necessary for a functional interaction with DnaK (Fig. 1). These co-chaperones are generally grouped in 3 classes. In addition to their J-domain, class I members share a G/F-rich region, a zinc-binding domain and a C-terminal domain involved in substrate-binding, class II members generally have a similar domain arrangement except that they do not possess a zinc-binding domain. In contrast, class III members only share the J-domain with DnaJ. Whereas classes I and II are considered generic co-chaperones, as judged by their ability to bind a large variety of substrates, class III members often deliver specific substrates or confer specific cellular localization to DnaK chaperones (Section 7). In *E. coli*, DnaK is known to interact with three J-domain co-chaperones (Fig. 1), namely DnaJ (class I), CbpA (class II) and the membrane-bound DjlA (class III). The other co-chaperone that actively contributes to the chaperone cycle of DnaK is the essential nucleotide exchange factor GrpE (Fig. 1), which mediates the dissociation of ADP and the subsequent binding of a new ATP that triggers substrate release from DnaK and resets the chaperone cycle [68–70].

It has been shown that DnaK preferentially binds to short extended hydrophobic polypeptide sequences [71]. Such regions of extended conformation can be accessible to DnaK either during *de novo* protein folding, translocation through biological membranes or following thermal or chemical stresses. Importantly, such peptide segments can also be found within native macromolecular complexes, resulting in DnaK orchestrating oligomeric assembly/disassembly of these complexes. Attesting for such wide range of possible interactors, the recently published *in vivo* interactome of the *E. coli* DnaK chaperone revealed that DnaK interacts with more than six hundred proteins at 37 °C. Among these proteins, ~80% were cytosolic proteins, ~11% inner membrane, ~3% outer membrane and ~3% periplasmic proteins [28]. Analysis of the relative enrichment of substrates on DnaK pointed out that enriched DnaK substrates are generally of low solubility [72], below average cellular abundance and often part of heterooligomeric protein complexes. Remarkably, in addition to its direct involvement as a main chaperone in maintaining protein homeostasis, DnaK masters the entire heat-shock response by acting directly on the stability of the *E. coli* heat-shock factor σ^{32}, which controls the synthesis of all the HSPs, including key chaperones and proteases that often cooperate with DnaK in response to protein aggregation [73].

The central role of DnaK in *E. coli* is additionally revealed by deletion of its gene. Indeed, a *dnaK* mutant exhibits multiple phenotypes, including slow growth at permissive temperature, a cold-sensitive (Cs) phenotype below 20 °C, and a high temperature-sensitive (Ts) phenotype above 35 °C. Note that *dnaK* mutants readily accumulate extragenic suppressors that confer growth advantage [74]. The most relevant DnaK-associated phenotypes are shown in Table 1. Consequences of *dnaK* mutations have been studied in other bacteria as well. While DnaK is most likely essential in *S. mutans* [75] and in *Mycobacterium tuberculosis* [76], *dnaK* mutants generally present severe growth defects similar to the ones observed in *E. coli*, *i.e.*, Ts phenotype and eventually slow growth [77–84]. In addition, it appears that the presence of DnaK often confers selective advantage to pathogens, including *Vibrio cholerae* [79], *Streptococcus intermedius* [80], *Salmonella enterica* serovar Typhimurium [81], *Brucella suis* [82], *L. monocytogenes* [83] and *Staphylococcus aureus* [84].

The consequences of *dnaK* mutations in various bacteria are in complete agreement with the multiple cellular functions attributed to DnaK in *E. coli* [63], thus attesting for its prevailing role in bacterial physiology and virulence.

4. The chaperonin GroESL

The *E. coli* chaperone GroEL and its obligate GroES co-chaperone (together forming the GroESL machine) constitute the best characterized member of the HSP60/HSP10 chaperonin family. GroEL is a large barrel-shaped protein complex composed of two heptameric rings of 57 kDa stacked back-to-back [85]. Each oligomer subunit is divided

into three domains: the equatorial domain responsible for intra- and inter-subunit interactions and for nucleotide-binding, the apical domain involved both in substrate and GroES binding, and the intermediate domain, which relays conformational changes between the other two domains (Fig. 1). Each ring forms a cavity responsible for the binding of non-native protein via interactions with hydrophobic surfaces. GroEL provides both a protected environment and a functional assistance for the folding of polypeptide between 20 and 60 kDa. GroES binding to GroEL depends on a short highly flexible region (residues 16 to 32 of GroES), the mobile loop, that folds into a defined β-hairpin structure after complex formation (Fig. 1) [86]. The flexibility of this short segment is crucial to allow binding of GroES to different GroEL-substrate conformations and the subsequent release of the polypeptide in the central cavity [87].

The GroEL folding chamber can be closed by a 7 GroES molecules lid. This allows confinement of the polypeptide and modification of the cavity properties, corresponding to enlargement and exposure of hydrophilic residues. Productive protein folding requires ATP hydrolysis in the same ring covered by GroES, the *cis*-ring. Finally, the GroES lid dissociates and the protein can either be released into the cytosol or remains bound to the chaperone for a new cycle [88]. The two rings can communicate allosterically, so that events occurring in the *cis*-ring trigger modifications in the *trans*-ring. According to the model the two rings are alternatively binding and hydrolyzing ATP [88]. However under certain conditions (low ADP and high GroES concentrations), both rings could bind nucleotide and GroES producing a symmetric complex named the football complex [88]. How GroEL helps a protein substrate to fold is still unclear and the mechanism may vary with respect to the substrate and/or the physiological conditions. Two main folding models are proposed: the Anfinsen cage model for which the central cavity is acting as a passive cage, so that folding can occur unperturbed by aggregation [89] and the iterative annealing model implying an active role of the cavity through repeated unfolding events (successive binding and release cycles) to reverse kinetically trapped folding intermediates and enhance folding [90,91].

In 2005, Kerner and colleagues identified substrates bound into the central cavity of GroEL under standard laboratory growth conditions [27]. This study showed that approximately 250 different proteins interact with GroEL, representing more than 10% of cytosolic proteins. Further *in vitro* refolding experiments allowed the authors to classify substrates with respect to their GroEL requirement. Starting with the lowest dependence, some can fold independently of GroEL but can utilize the chaperone to improve their folding (Class I), a majority are chaperone-dependent but can use either GroEL or TF and DnaK for folding (Class II). Finally, only 85 were obligate clients of GroEL (Class III). Another study performing proteomic analyses on soluble fractions of GroESL-depleted *E. coli* strains further delimitated obligate GroEL substrates *in vivo* [92]. The authors monitored the presence of Class III proteins in soluble fraction after overproducing them in GroESL-depleted strain, and found that 57 Class III proteins were strictly chaperonin-dependent for their folding *in vivo* (renamed Class IV), as they were not found in the soluble fraction of GroESL-depleted strain. Among them, 6 are essential proteins, providing an explanation for the essentiality of GroESL for cell viability (see below) [93]. Except for size limitation (between 20 and 60 kDa) due to the capacity of the GroEL cavity, few common characteristics were found for GroESL substrates. Yet, an overrepresentation of TIM barrels [27] and more generally aggregation-prone folds [94] was observed among Class IV proteins. Note that two independent *in vivo* studies that did not specifically select for encapsulated GroESL substrates identified a larger repertoire of GroESL interactors [95,96], suggesting that different modes of binding/folding via the GroESL chaperone might exist [97].

The *E. coli* GroESL machine, which is about 10 times less abundant than DnaK, is essential for *E. coli* growth under all conditions tested so far [93]. It was initially discovered as a host factor required for bacteriophage λ morphogenesis in *E. coli* [98] and different *groES* and *groEL* mutations were later used to characterize the involvement of this chaperone machine in *E. coli* physiology. Mutations in *groESL* are generally Ts, induce cell filamentation, and affect both transcription and translation [98,99]. Studies using various strains and inducible promoters controlling GroESL expression resulted in similar results [100,101]. Most of the relevant phenotypes associated with GroESL mutations, depletion or overexpression are shown in Table 1.

Following the initial finding that *groESL* is essential in *E. coli*, similar results were obtained in other bacteria, including *B. subtilis* [102], *S. mutans* [75], *Legionella pneumophila* [103] and *Caulobacter crescentus* [104]. Note that in many bacteria, *groEL* and *groES* have been renamed *cpn60* (chaperonin 60) and *cpn10* (chaperonin 10) respectively. Intriguingly, in approximately 30% of the sequenced bacterial genomes, 2 *groEL*/*cpn60* genes or more are detected along with only one *groES*/*cpn10* gene [105]. The sequence identity between these paralogs is generally high (between 60 and 80%) and their presence might be explained either by overlapping functions or specialization. The first hypothesis implies that each *groEL* gene is individually dispensable but at least one is necessary for growth, as it is the case for *S. meliloti* and *Myxococcus xanthus* [106,107]. Yet, in most cases it seems that the specialization or subfunctionalization of the multiple GroEL is the preferred option, with one essential housekeeping GroEL and a facultative one displaying a more specialized function [108–112]. Remarkably, in numerous Mollicute species, *groESL* is either absent, as shown for *Ureaplasma urealyticum*, a pathogen involved in reproductive tract infections [113,114], or not essential, as demonstrated for *Mycoplasma genitalium* and *Mycoplasma pneumonia* [115]. These bacteria with small genomes have slow growth rates and only 61 proteins homologous to the 250 known *E. coli* GroEL substrates, thus possibly explaining the non-essentiality of the chaperonins [116].

The results gathered from *E. coli* and other bacteria emphasize well the major function of GroESL in maintaining protein homeostasis in the cytoplasmic space. Yet, it is known that efficient protein folding by GroESL *in vivo* relies upon the upstream chaperones TF and DnaKJE, which efficiently deliver substrates to GroEL [28].

5. Interplay among TF, DnaKJE, GroESL and other chaperones during *de novo* protein folding

It has been shown that TF, DnaKJE and GroESL work as an interactive network of molecular chaperones assisting the folding of newly-synthesized proteins (Fig. 2). Seminal work has shown that deletion of the *tig* gene greatly enhances the number of newly-synthesized polypeptides interacting with DnaK and enables DnaK to bind to considerably shorter nascent chains [24,25]. Accordingly, the number of DnaK interactors significantly increases (>35%) *in vivo* in the absence of TF, including ribosomal and small basic proteins. Supporting such a functional redundancy, DnaK endogenous level is significantly increased in the absence of TF [28]. Remarkably, newly-synthesized large multi-domain proteins can sequentially interact with both TF and DnaK chaperones and in this case, TF and DnaK actively cooperate to facilitate their folding *in vitro* [26]. Such a group of proteins seems to interact less extensively with DnaK in the absence of TF *in vivo* [28]. Previous genetic studies have shown that the simultaneous deletion of both the *tig* and *dnaK* genes causes synthetic bacterial lethality at temperatures ≥30 °C and provokes a severe accumulation of aggregated proteins [31,117]. Noticeably, more than one thousand proteins, pre-existing and *de novo* synthesized, were shown to aggregate at 30 °C in this double *tig dnaK* mutant, indicating that interplay between the two chaperones is indeed crucial for the overall cellular protein homeostasis.

In contrast with the double *tig dnaK* mutant, Ts mutant alleles of *groEL* (*groEL44* and *groEL140*) did not present any synergistic effect with *tig* mutations (unpublished data). Nevertheless, overexpression of GroESL efficiently suppressed both the Ts phenotype and the protein folding defect observed in the absence of both TF and DnaK [31,117]. Accordingly, in the absence of both chaperones, 150 additional *E. coli*

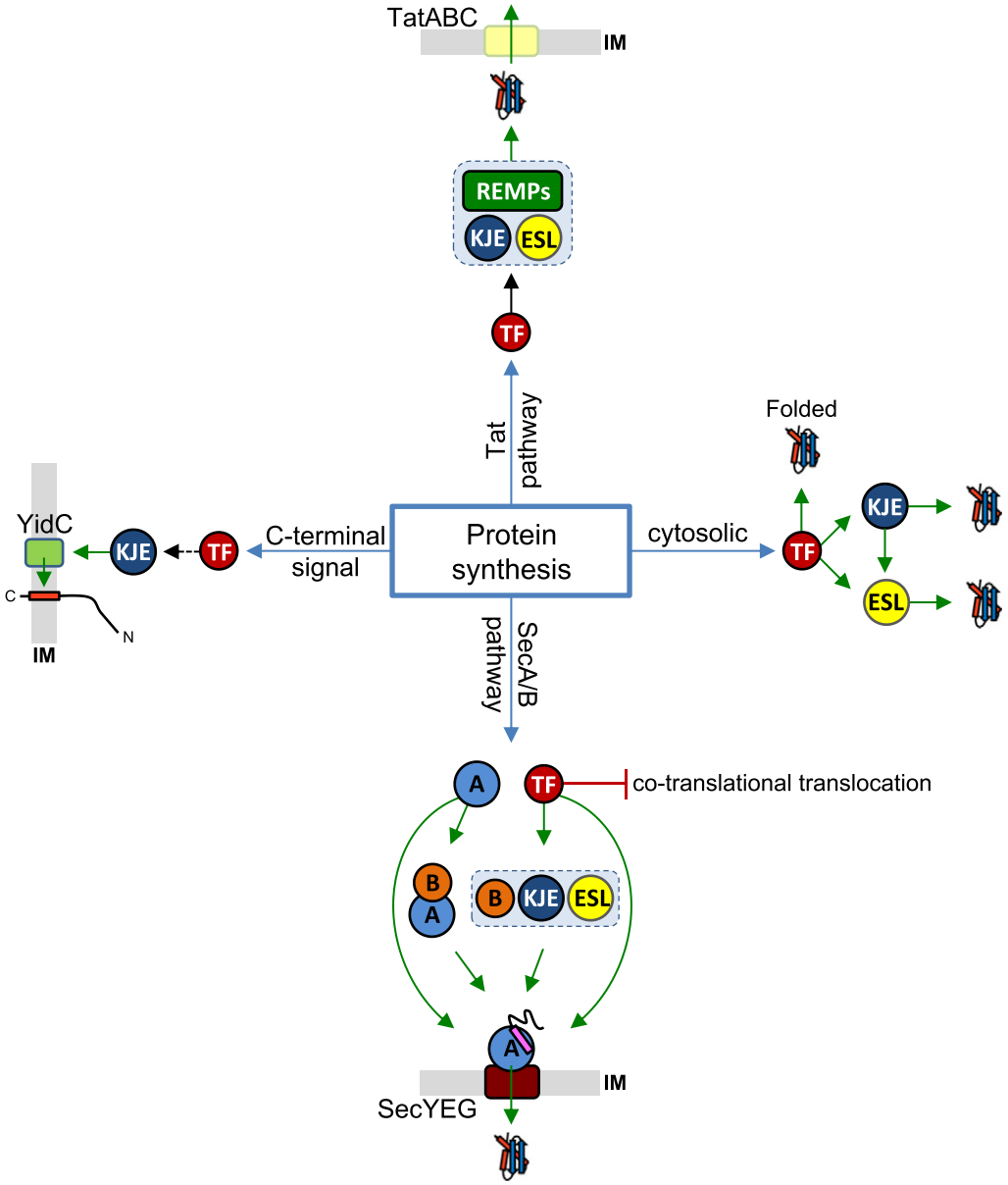

Fig. 2. Chaperoning of *de novo* protein folding and targeting by TF, DnaKJE and GroESL, and their interplay with other chaperones and targeting factors in *E. coli*. *De novo* protein synthesis (center), cytosolic protein folding (right), Sec pathway (bottom), Tat pathway (top), targeting of C-tail anchored proteins (left). Abbreviations for the chaperones and targeting factors described are: Trigger Factor (TF), DnaKJE (KJE), GroESL (ESL), SecA (A), SecB (B), and redox enzyme maturation proteins (REMPs). IM stands for inner membrane. A green arrow indicates a previously established involvement (direct or indirect) of the chaperone or the targeting factor concerned, a filled black arrow indicates that an interaction between TF and Tat substrates was found but no involvement described yet, and a dashed black arrow indicates a possible interaction between TF and C-tail anchored proteins that was not investigated. The Sec and Tat signal peptides are shown in pink. See text for details.

proteins were found to interact with GroEL *in vivo* [27]. In addition, DnaK was shown to interact with more than 40% of the obligate GroEL substrates, indicating that GroEL and DnaK substrates overlap significantly [28]. This is in agreement with a proposed model in which DnaK is often working upstream of GroEL, stabilizing substrates for subsequent productive interaction with GroEL. Furthermore, significantly more GroEL substrates were found to interact with DnaK *in vivo* upon GroEL depletion and about ~70% of GroEL substrates aggregate in the double *tig dnaK* mutant, thus arguing for a central role of DnaK in targeting protein substrates to GroEL [28]. The strong genetic synergy observed between a *dnaK* mutation and a sensitive *groEL44* mutant allele, *i.e.*, a drop of at least 5 °C in the permissive temperature of growth, further attests to such intricate interplay between the two chaperones (unpublished data).

As observed for GroEL, overexpression of the SecB chaperone efficiently suppresses both the Ts phenotype and the severe aggregation of cytoplasmic proteins observed in the absence of both TF and DnaK [118,119]. In this case, suppression by SecB was independent of its productive interaction with the SecA subunit of the Sec translocon, and a direct interaction was observed between SecB and short nascent chains of RpoB, a cytosolic model protein known to interact with both TF and DnaK [118]. These results suggest that in addition to its specific role in the chaperoning of presecretory proteins, SecB has the capacity to perform generic chaperone functions in *E. coli*. Accordingly, it is interesting to note that both GroEL and SecB were recently isolated as highly enriched DnaK interacting proteins [28].

Yet, interplay among TF, DnaKJE, GroESL and other chaperones like SecB is not restricted to newly-synthesized cytosolic proteins. Indeed,

significant fractions of proteins that either interact with these chaperones or accumulate as aggregates in their absence were recently identified as inner membrane and exported proteins [27,28,41]. Furthermore, mutations in chaperone-encoding genes often affect protein export and show strong genetic interactions with mutations in genes involved in protein targeting and translocation pathways. The following parts focus on such emerging functions of TF, DnaKJE and GroESL.

6. TF functions in protein targeting pathways

6.1. TF and the Sec pathway

Seminal works from the Wickner's lab identified TF as *E. coli* factor that forms a stable 1:1 complex with purified precursor of the outer membrane protein OmpA and triggers it into a translocation competent protein [39,120,121]. Further work by other groups showed that TF can interact co-translationally with several nascent presecretory proteins, including PhoA, PhoE [29], OmpA [49], β-lactamase and MBP [51]. Yet, although TF could bind and assist proOmpA in a manner comparable to that of the SecB chaperone, it could neither bind nor retain the outer membrane precursor of PhoE competent for translocation [122]. This is in sharp contrast with the known translocation competent SecB–prePhoE complex, thus suggesting differences in substrate specificity between the two chaperones.

Although TF efficiently stabilized translocation competent proOmpA *in vitro*, the deletion of the *tig* gene exhibited no major defect on proOmpA processing *in vivo*. On the contrary, the absence of TF slightly accelerates export of known SecB substrates, *i.e.*, OmpA, OmpF and OmpC, as well as the SecB-independent substrate preBla (β-lactamase) [53,123]. This suggests that TF can maintain a prolonged interaction with precursors and delay their targeting or transfer to downstream chaperones. Thus, overexpression of TF strongly retards translocation of these proteins [31,53,123–125]. Remarkably, the impact of ribosome-bound TF on protein export was even more pronounced in the absence of the chaperone SecB. Indeed, several studies have shown that *tig* mutation fully suppressed the SecB-dependent defect in proOmpA, proOmpF and proOmpC processing, and the Cs and Ts phenotypes of a *secB* null strain [31,53,123,125]. In addition, toxicity induced by overexpressed TF was significantly intensified in the absence of endogenous SecB [31,125]. Further highlighting such a strong connection between TF and the Sec pathway, mutations in *tig* additionally suppressed both the Ts and Cs phenotypes of other sensitive *sec* alleles, *i.e.*, *secA51*, *secAcsR11* and *secY40*, and induced an increased expression (about 1.5 fold) of endogenous SecA [28,44,125].

Using an *in vivo* ribosome profiling method based on the purification of *E. coli* ribosome-nascent chain complexes (RNC) cross-linked to TF and the subsequent analysis of the whole fraction mRNA footprint fragments protected by the ribosome, Oh and colleagues [44] showed that OMPs mRNA were highly enriched in such complexes. This included OmpA, OmpC/F, LptD and LamB proteins as the most enriched mRNA (top 25) out of the approximately 2000 genes examined [44]. These results indicate that OMPs are strong TF interactors in growing *E. coli* cells. In support of this, the outer membrane usher protein AfaC, OmpA and the periplasmic proteins OpgG and PstS were also isolated as enriched TF substrates *in vivo* by affinity purification using TF as bait [41]. In addition, two independent studies performing SILAC (stable isotope labeling with amino acids in cell cultures) followed by mass spectrometry on both whole cell lysates and purified outer membrane fractions of *E. coli* wild-type and *tig* mutant revealed that a significant fraction of OMPs and periplasmic proteins was significantly decreased in the absence of TF [28,44]. This included the known OMPs OmpA, Tsx, OmpC, TolC, the periplasmic proteins DegP, Spy, FkpA, GlnH, LolA, OppA and PbpG, and several outer membrane subunits of the essential Bam complex involved in the insertion of β-barrel proteins in the outer membrane [28,44,126]. As proposed by the authors, the fact that *E. coli tig* mutants are sensitive to SDS, EDTA and to the antibiotic vancomycin that weaken the outer membrane, is in agreement with the observed decrease in OMPs (Table 1; [55]). In addition, the sensitive profile of a *tig* mutant obtained after a chemical screen of 300 different conditions correlates well with profiles of certain *bam* mutants, suggesting contribution to common pathways [44].

Together these results indicate that presecretory proteins represent a significant fraction of TF interactors and that ribosome-bound TF may play a key role at early stage during their biogenesis. A very likely model is that TF facilitates post-translational targeting of precursors by maintaining them in a form competent for binding to SecA [127] or by transferring them to downstream chaperones like DnaK or SecB (Fig. 2). In such a model, TF would prevent unproductive co-translational translocation, assuring proper functioning of the Sec translocon and indirectly facilitating later folding or interaction with other factors in the periplasmic compartment [128]. In agreement with such a hypothesis, the fraction of membrane-bound ribosomes and SecA was significantly increased in the absence of TF, suggesting increased co-translational translocation [125]. Recently, Oh and colleagues [44] used an elegant method previously developed by Linda Randall's group [129] to estimate the fraction of polypeptides that are translocated co- or post-translationally by visualizing the amount of processed and unprocessed N-terminal regions within a population of incomplete nascent chains *in vivo* both in wild-type and *tig* mutant cells. Using LamB as model, an OMP that is processed almost entirely post-translationally in wild-type cells, they could show that in the absence of TF, the export of this protein significantly switches to a more co-translational mode. This strongly supports a model in which TF favors a post-translational engagement of precursors with the Sec translocon (Fig. 2).

Decision about co- or post-translational targeting takes place at the ribosome, early during polypeptide synthesis. Both TF and SRP can bind simultaneously to the L23 protein at the ribosomal polypeptide exit tunnel and their recruitment to translating ribosome is modulated by the nascent chain itself [6,130,131]. Previous work suggested that TF could provide a discriminatory role early during the targeting process by preventing interaction of SRP with mildly hydrophobic signal peptides, thus orienting presecretory proteins toward a post-translational mode of translocation. On the other hand, the high affinity of SRP for strongly hydrophobic signal sequences of emerging nascent chains clearly outcompetes TF to initiate co-translational targeting of ribosome-nascent chain complexes to the Sec translocon [132–135]. *In vivo*, the relevance for such a possible interplay between TF and SRP is not known and initial work performed in *E. coli* showed that the lack of TF did not reroute proOmpA toward SRP [136], thus suggesting that the co-translational targeting of presecretory proteins observed in the absence of TF is not mediated by SRP.

Another factor capable of competing with TF for binding to emerging polypeptide chains of presecretory proteins is SecA, which also binds to L23 at the ribosomal polypeptide exit, as observed for TF and SRP [18,137]. It has been shown that SecA preferentially binds to nascent chains of presecretory proteins with mildly hydrophobic signal peptides and targets them mainly post-translationally to the Sec translocon (reviewed in [20]). Yet, it is not known whether ribosome-bound TF cooperates with SecA or in contrast, prevents unproductive binding of SecA to nascent precursors that must transit via downstream chaperones like DnaK, SecB or perhaps GroESL, before interacting with membrane-bound SecA (Fig. 2). Alternatively, the presence of TF might also prevent the SecA-dependent co-translational targeting of ribosome-nascent chain complexes to the Sec translocon [125,137]. In agreement with such a hypothesis, SecA overexpression can partially suppress the Cs phenotype of a *secB* mutant observed in the presence of TF, albeit significantly less efficiently than *tig* mutations [125]. On the other hand, excess of SecA *in vivo* might well be sufficient to partially bypass the need for downstream SecB and insure post-translational targeting in spite of the presence of TF. In this case, suppression by SecA overexpression or *tig* mutations would thus occur by different mechanisms. More work is clearly needed to shed light on such possible

interplays between TF and SecA during initial stages of the post-translational targeting cascade.

6.2. TF and the Tat pathway

In vitro cross-linking experiments initially revealed that TF can interact with signal peptides of two different Tat substrates, the trimethylamine *N*-oxide reductase TorA and SufI, a suppressor of *ftsI* mutations belonging to the multicopper oxidase superfamily [138]. However, further *in vivo* experiments showed that deletion of the *tig* gene neither perturbed kinetics of preSufI processing via Tat nor favored accumulation of unprocessed precursors at steady state [138]. In addition, overexpression of TF, which is known to severely delay the export of some Sec-dependent substrates, did not affect translocation of SufI via Tat, both at steady state and by pulse-chase analysis [138]. Although TF does not seem to play a major role in the *E. coli* Tat pathway, the amount of data available is very limited and we cannot exclude a contribution of this chaperone in this process (Fig. 2).

A possible contribution of TF-like chaperones to the Tat pathway has emerged from work performed in the obligate anaerobic Gram-positive bacterium *D. hafniense*, which has the ability to grow using organohalide respiration [37]. Key to such an anaerobic respiration is the reductive dehalogenase enzyme PceA, which harbors a Tat signal sequence and which is most likely translocated folded via the Tat pathway. The PceT protein, whose gene is present in the same operon as *pceA*, is an atypical TF chaperone without the N-terminal ribosome-binding domain but retaining both the PPIase domain and C-terminal chaperone domain of classical TF chaperones. Note that this bacterium encodes two additional full-length TF that may function as generalized chaperones. Purified PceT exhibits both PPIase and chaperone activities *in vitro* and co-immunoprecipitation experiments performed on *D. hafniense* cell extracts revealed that PceT indeed interacts with PceA precursors harboring the Tat signal peptide but not with the mature form [139]. Accordingly, an interaction between PceT and the Tat signal peptide of PceA was further confirmed using a bacterial two hybrid assays performed in *E. coli* [37]. Remarkably, overexpression of PceT efficiently solubilized the aggregation prone reductive dehalogenase PceA and prevented its degradation in *E. coli*, thus highlighting a new class of dedicated TF-like chaperones involved in the processing of Tat-dependent substrates.

6.3. TF and protein export in other bacteria

Although not well documented, a role for TF in protein secretion has been observed in the Gram-positive human pathogens *S. mutans* and *S. pyogenes*. In *S. mutans*, mutations in *tig*/*ropA* impaired activity of the cell wall-associated fibrillar surface protein P1, which mediates adherence to the tooth and which is secreted in a SecA-dependent manner. In this case, the *tig*/*ropA* mutation did not significantly affect the amount of secreted P1, thus suggesting a role for TF in the maturation of P1 precursors [60]. In *S. pyogenes*, a bacterium responsible for group A streptococcal infections in human, the cysteine protease SpeB (exotoxin B) is one of the most abundant secreted proteins that contribute to pathogenesis. A genetic screen for proteinase deficient mutants of this bacterium using transposon mutagenesis identified the *tig*/*ropA* gene encoding TF as a main chaperone involved in SpeB biosynthesis. Indeed, TF was required for both the secretion of the protease and the productive maturation of the protease via its PPIase activity [62]. In this case, TF was acting on a single prolyl residue in the prodomain of the exotoxin [140]. No defect in the secretion of other proteins, including streptolysin O or DNAses was observed in the absence of TF [62]. This work provided the first evidence for a role of TF in protein secretion involving both its chaperone and PPIase activity.

All together the studies presented in this part highlight a key emerging role for TF in assisting Sec-dependent presecretory proteins, which is in agreement with TF chaperone properties described in Section 2. Yet, it remains to be determined how TF efficiently delivers competent substrates to the Sec machinery and perhaps to downstream chaperones before translocation occurs, and to what extent its PPIase activity contributes to this process. In addition, deciphering early interplays between TF, SRP and SecA at the ribosomal polypeptide exit will undoubtedly help to understand such TF functions in protein targeting. Finally, potential involvement of TF in the Tat export pathway or in the biogenesis/assembly of inner membrane protein complexes remains largely unexplored and merits further attention.

7. Multitasking DnaKJE facilitates post-translational protein targeting

7.1. DnaKJE and the SecA–SecB pathway

The first evidence for a role of DnaKJE in protein export came for the work by Phillips and Silhavy [141], who showed that the export of LamB–LacZ hybrid protein that normally causes jamming of the Sec translocon was facilitated by overexpressed DnaK. In this case, the DnaK-dependent relief of the jammed translocon enabled the processing of other exported proteins, thus suggesting a major function of DnaK in response to stress affecting the Sec translocon. This is in agreement with other studies showing that accumulation of presecretory proteins induces the synthesis of HSPs [142,143] and that overexpression of the heat-shock factor σ^{32} facilitates the processing of MBP and of an export-deficient LamB mutant in a *secB* null strain [144]. Direct evidence for DnaKJE function during protein export under physiological conditions was provided by Wild and colleagues [145], who showed that processing of alkaline phosphatase (AP), a SecB-independent secretory protein, was significantly impaired in the absence of a functional DnaK. As expected, a mutation in *dnaJ* also affected AP export, albeit less severely [145]. Strongly supporting such an involvement of DnaKJE in protein export, several outer membrane proteins and lipoproteins (17 in total, including OmpA, OmpC/F, OmpX, OmpT, lpp, BamA/D, NlpD and Antigen 43) and periplasmic proteins (23 in total, including DppA, OppA, ThiB, ZnuA and DegP) have been recently identified as *bona fide* DnaK substrates *in vivo* [28]. Finally, DnaKJE was also required for the export of β-lactam degrading enzymes [146].

Most of our knowledge concerning the DnaKJE chaperone machine and the Sec-dependent secretion originates from the study of *sec*-sensitive mutant alleles. Indeed, processing of OmpA, OmpC and OmpF relied on a functional DnaK when protein translocation was compromised following SecA depletion. In this case, a direct interaction between DnaK and proOmpA was observed [147]. In addition, it has been shown that overexpression of DnaKJ efficiently suppresses the Cs phenotype of a *secB* null strain and facilitates export of SecB-dependent substrates such as LamB or MBP (unpublished data; [125,145]). While processing of these SecB substrates was not affected by *dnaK* mutations in the presence of SecB [145], the depletion of DnaKJ in the absence of SecB induced both a further decrease in the processing of these proteins and a massive accumulation of protein aggregates in the cytoplasmic space [125,145]. These aggregated proteins included known DnaK cytoplasmic substrates as well as several outer membrane proteins, namely OmpX, OmpC, OmpA, PhoE and YaeT [125]. Further supporting such interplay between DnaK and SecB, mutations in *secB* and *dnaK*(or *dnaJ*) show synthetic lethality (Table 1) and expression of each chaperone is upregulated in the absence of the other [125,148]. Together these results suggest a model in which DnaKJE significantly contributes to the general Sec secretion pathway, both by assisting the post-translational translocation of a subset of specific substrates and by rescuing SecB substrates that accumulate under stress conditions affecting the Sec translocon, most likely maintaining them in an export competent form for a prolonged period until normal growth conditions resume [149]. In agreement with such a model (Fig. 2), the absence of ribosome-bound TF, which induces an artificial co-translational mode

of translocation of presecretory proteins (see Section 6) partially suppresses the synthetic lethality of a double *secB dnaJ* [125,145,149].

Intriguingly, it was recently shown that alteration of the conserved C-terminal end of DnaK, which is involved in weak substrate-binding but not in interdomain allostery, severely affects bacterial growth in the absence of SecB [67]. This suggests that this region could be critical for DnaK's function in protein export. Together with the physical interaction recently found between SecB and DnaK *in vivo*, these data suggest that the two chaperones might work in concert more than previously thought (Section 5).

7.2. DnaKJE and the chaperoning of autotransporters

A role for DnaK in chaperoning the export of autotransporter proteins has been suggested. Autotransporters are cell-surface-exposed and secreted proteins that are widely spread among Gram-negative bacteria and which often contribute to bacterial virulence [150,151]. They generally possess an N-terminal signal sequence that mediates inner membrane targeting and translocation through the Sec translocon, a C-terminal translocator domain that forms a β-barrel structure in the outer membrane, and the central passenger domain that translocates across the outer membrane to the bacterial surface. Most likely, successful secretion and folding of such proteins is tightly coupled to translocation across the two membranes and relies on several generic factors, including molecular chaperones, in both cellular compartments [152]. The bacterium *Aggregatibacter actinomycetemcomitans*, a Gram-negative human pathogen associated with severe periodontitis produces collagen-binding cell surface structures composed of the extracellular matrix protein adhesion A (EmaA), which belongs to the autotransporter family of proteins. Recent work suggests that membrane targeting of EmaA requires DnaK [153]. Indeed, overexpression of a dominant negative *dnaK* mutation significantly affected EmaA secretion in the absence of endogenous SecB chaperone. Interestingly, Janakiraman and colleagues [154] showed that DnaK was necessary both for secretion and polar localization of the autotransporter IcsA, which is required for actin assembly following *Shigella* infection of host cells. Similar role for DnaK was observed with another *Shigella* autotransporter, the serine protease autotransporters SepA, suggesting a more general role for DnaK in chaperoning autotransporters [154].

7.3. DnaKJE and the biogenesis of inner membrane proteins

Although mostly found in the cytosol, preliminary work using immunogold labeling techniques indicated that a significant fraction of DnaK localizes at the *E. coli* inner membrane under physiological conditions [155]. Such intriguing observation was more recently confirmed by two independent proteomic studies of the *E. coli* inner membrane complexosome [156,157]. The presence of DnaK at the membrane suggests that in addition to its role in protein export, DnaK could contribute to the biogenesis of inner membrane proteins or protein complexes by facilitating their targeting to Sec and YidC, or their subsequent assembly. The fact that a significant fraction (about 10%) of the *in vivo* DnaK interactors in *E. coli* are inner membrane localized proteins is in line with such a hypothesis [28]. Yet, a direct involvement of DnaK has been very difficult to tackle and very little data have been provided to date.

Possible links between DnaK and SRP have been investigated but did not reveal a major contribution of DnaK in this pathway [158,159]. Bernstein and Hyndman [159] first observed that depletion of SRP induced a σ^{32}-dependent heat-shock response and that the resulting synthesis of HSPs was essential for viability under low SRP concentration. Accordingly, SRP depletion increases the inner membrane fraction of DnaK and GroEL (Section 8) and was accompanied by an increased accumulation of protein aggregates in the cytoplasm [158]. However, both chaperones do not significantly contribute to bacterial survival at low SRP levels, as mutations in either *dnaK* or *groEL* did not further compromise the viability of SRP deficient strains. In this case, other HSPs, mainly proteases, were responsible for survival in response to low SRP levels [159].

An intriguing connection was recently found between DnaK and the essential protein insertase YidC, which assists the biogenesis of inner membrane proteins alone or in concert with the Sec translocon [160,161]. Indeed, a proteomic analysis performed upon YidC depletion revealed that an increased fraction of DnaK was found at the inner membrane, thus suggesting a redistribution of both chaperones toward the membrane. Note that in contrast with SRP, YidC depletion neither provoked an accumulation of aggregated proteins nor induced the synthesis of HSPs. Although the significance for such a probable redistribution remains to be determined, it suggests that DnaK, and perhaps GroEL (Section 8), could actively participate in pathways involving YidC [160]. Recently, a role for DnaK in the efficient targeting of the TssL C-tail anchored inner membrane protein of the Type VI secretion system of enteroaggregative *E. coli* has been found. The so called C-tail anchored proteins possess a single C-terminal transmembrane domain and are relatively rare in bacteria [161]. Their targeting, most-likely post-translational, and insertion into the inner membrane remains largely unexplored. Aschtgen and co-workers [162] show that mutation in *dnaK*, but not in *secB* or *tig*, significantly impaired insertion of the TssL protein. Interestingly, membrane insertion of TssL was YidC-dependent but SRP-independent, thus providing a first evidence for a pathway involving DnaK and the downstream YidC for post-translational insertion of C-tail anchored proteins in bacteria (Fig. 2; [162]). Further work is needed to pin point a more general contribution of such a DnaK/YidC pathway in the biogenesis of C-tail anchored proteins.

7.4. Membrane recruitment of DnaK by specialized cochaperones

Although most of the DnaK functions described above seem to rely on its DnaJ cochaperone partner, DnaK can be recruited to the inner membrane by a specialized class of membrane anchored DnaJ-like cochaperones [63]. These proteins especially present in Gram-negative bacteria belong to the class III J-domain proteins that only share the short J-domain sequence (about 70 aa) with DnaJ. The DjlA cochaperone (DnaJ-like protein A) from *E. coli* is the best characterized member of this class of proteins (Fig. 1). It is a dimeric class III inner membrane protein that possesses a single N-terminal transmembrane domain, a central domain of unknown function and a cytoplasmic C-terminal J-domain [163–166]. The *djlA* gene is not essential in *E. coli* but it can act synergistically with *dnaJ* in supporting *E. coli* growth since a *djlA dnaJ* double mutant exhibited a pronounced growth defect above 40 °C [167]. As a *bona fide* DnaJ-like cochaperone, it has been demonstrated that DjlA interacts and controls DnaK chaperone function via its functional J-domain but its cellular function remains unknown [166]. A mild overexpression of DjlA enhanced sensitivity to certain drugs such as novobiocin and the anticalmodulin W7, and induced the synthesis of mucoid colanic acid capsule in a strictly DnaK-dependent manner. However, a stronger overexpression of DjlA was rapidly fatal for *E. coli* ([164]; unpublished data) and such toxicity relied on both DjlA's proper membrane localization and a functional interaction with DnaK (unpublished data). Whether too high concentration of relocalized DnaK directly interferes with uncharacterized membrane transport processes or is hijacked from its multiple cytoplasmic functions remains unknown.

A role for DjlA in bacterial virulence has been described in the Gram-negative pathogens *L. pneumophila* and *Legionella dumoffii* [168,169]. The *djlA* gene was first identified as a gene required for intracellular growth of *L. dumoffii* in murine macrophage-like cells or in human epithelial cells. In this case the lack of DjlA resulted in a failure to inhibit phagosome/lysosome fusion as well as an increased susceptibility to stresses [169]. In *L. pneumophila*, DjlA was also needed for intracellular survival and growth in macrophages and it has been proposed that it may play a role in the assembly of the organelle trafficking intracellular multiplication (Dot/Icm) type IV protein translocation apparatus

needed for the secretion of a set of bacterial proteins into the host cytoplasm, perhaps controlling the DnaK-dependent assembly/disassembly of this secretion apparatus. Finally, mutation of the *djlA* gene in the Gram-negative clam pathogen *Vibrio tapetis* inhibited both cytotoxicity against clam hemocytes and the development of the brown ring disease, further demonstrating a major role for DnaK–DjlA chaperone/cochaperone pairs in virulence [170].

The cell wall-less bacterium *M. pneumonia* is a human pathogen that causes pharyngitis and bronchitis. Gliding motility and attachment, which contribute to successful colonization of host cells are predominantly mediated by the terminal organelle, a polar and membrane-bound structure containing proteins gathered at its terminal cap [171]. In this bacterium, the atypical type III J-domain protein TopJ, which localizes to the base of the terminal organelle, is necessary for gliding motility and attachment. Except for the J-domain, TopJ shares no other domain with DjlA or DnaJ [171]. Adjacent to its N-terminal J-domain, TopJ possesses (*i*) a short aromatic and glycine residue domain (EAGR box) and (*ii*) a central large acidic and proline-rich (APR) domain, both present in other terminal organelle proteins, and (*iii*) a less conserved C-terminal domain. While the APR and C-terminal domains of TopJ were shown to contribute to the localization and stability of TopJ, a functional J-domain capable of recruiting DnaK was absolutely essential for gliding motility and adherence [171]. In this case, it has been proposed that the TopJ/DnaK cochaperone/chaperone partners might be involved in the maturation process of the terminal organelle, perhaps acting on the translocation or folding of the membrane-anchored primary adhesin P1 [171].

These studies illustrate well the diversity and the key cellular functions played by specialized DnaJ-like co-chaperones capable of triggering relocalization of the entire DnaK chaperone power to the inner membrane.

7.5. DnaKJE and the Tat pathway

Although DnaKJE generally appears critical for Tat, its precise contribution remains obscure. Several groups have shown that DnaK interacts with precursors of different Tat substrates or with protein chimeras containing Tat signal sequences [28,172–174], and that DnaK binding sites critical for folding and proper targeting of Tat-dependent substrates might also be present outside leader sequences [172,173]. In addition, interactions have been revealed between DnaK and several REMPs, suggesting that DnaK could act in concert with these Tat-dedicated chaperones [28,175]. Co-exported partners of the Tat-dependent substrates were also identified as *bona fide* DnaK interactors *in vivo*, thus further extending the involvement of DnaK in Tat [28]. Finally, both translocase subunits TatA and TatB were recently identified as DnaK interactors, suggesting that DnaK could directly contribute to substrate transfer to the Tat machinery at the inner membrane [28].

To date, the best characterized model for DnaK involvement in the Tat export pathway is the biogenesis of the Tat-dependent multicopper oxidase CueO [172,173]. Indeed, it has been shown that mutations in *dnaK* inhibit translocation of chromosomally-encoded CueO, without significantly affecting its stability in the cytoplasmic space. This is in agreement with *in vitro* and *in vivo* affinity purification experiments showing that DnaK efficiently binds CueO precursors. The fact that endogenous CueO precursor was not degraded in the absence of DnaK suggests that the chaperone may participate in folding and/or assembly, or directly modulate interaction with the Tat translocon [172]. Intriguingly, overexpressed CueO, and other Tat substrates including SufI and Tat-GFP chimeras, were very efficiently degraded in the absence of DnaK, indicating that the chaperone may also contribute to the stabilization of Tat substrates and prevent degradation by proteases [173]. The fact that the absence of DnaK does not stimulate degradation of chromosomally-encoded low levels of CueO suggests that other chaperones, including GroEL and SlyD that also bind Tat signal sequences efficiently could contribute to stability [172,175]. Overexpression of Tat substrates would thus rapidly overwhelm both the chaperones and the Tat translocon, and subsequently be targeted for degradation. In agreement with such hypothesis, overexpression of DnaKJ improved both, expression and translocation of several overexpressed Tat substrates [173], perhaps increasing the folding yield of Tat substrate competent for export and/or avoiding premature engagement with the Tat translocase.

DnaK was isolated as one of the main proteins that bind the Tat leader sequence of the catalytic subunit DmsA of the dimethyl sulfoxide reductase, despite the presence of its specific REMP DmsD [174]. Later work revealed that DnaK also interacts with both the REMP DmsD and the DmsB subunit B, which is co-exported with DmsA [28,175], thus highlighting intricate interactions between DnaK and several components of the dimethyl sulfoxide reductase. Yet, *dnaK* mutation only partially affected DmsA translocation *in vivo* [172], suggesting that other generic chaperones could partially replace DnaK as proposed for CueO. Nevertheless, all these experiments were performed at permissive temperature for a *dnaK* mutant (30 °C) and it is very likely that DnaK function in the biogenesis of the dimethyl sulfoxide reductase will become essential at higher physiological temperature or under stress conditions.

More Tat-dependent substrates seem to rely on DnaK for their export. Indeed, translocation of SufI was also affected, albeit very weakly, in the absence of DnaK. In this case, a significant fraction of SufI precursors accumulated in an export-incompetent conformation, perhaps due to impaired folding or premature targeting [138]. In addition, while membrane localization of the formate dehydrogenase N subunit G (FdnG) was not affected by the absence of DnaK, the resulting formate dehydrogenase activity was significantly lower, suggesting that translocation or assembly of the enzyme complex is partially affected under these conditions [173]. In contrast, translocation of both the high potential iron–sulfur protein HiPIP and the unknown YcdB protein was not affected by mutations in *dnaK*, although DnaK was shown to interact with their Tat signal sequences [172,176].

The fact that DnaK is capable of interacting (*i*) with the signal sequence and/or the mature region of Tat-dependent precursors, (*ii*) with specific REMP chaperones, (*iii*) with other co-exported protein subunits and (*iv*) with the Tat complex itself, suggests an attractive model in which DnaK acts at multiple steps in the maturation process, including stabilization, folding, assembly, as well as the timely and efficient targeting to the Tat complex at the inner membrane (Fig. 2). Whether DnaK contributes alone or within an expanded cooperation with other chaperones like the chaperonin GroEL, the PPIase and metallochaperone SlyD or the specialized REMPs, remains to be determined [27,172]. Finally, regulatory proteins such as CueR, the activator of copper-responsive regulon genes, including *cueO*, or the TorR DNA-binding response regulator of the *torCAD* operon have been recently identified as DnaK substrates *in vivo* [28]. Therefore, it is likely that the DnaKJE chaperone machine additionally contributes to Tat by indirectly modulating the expression of genes encoding Tat-dependent proteins, thus adding one extra level of control by DnaK.

The discoveries discussed in this section clearly demonstrate that in addition to its pivotal role in assisting *de novo* protein folding and in maintaining protein homeostasis in the cytoplasmic space, DnaK equally contributes to the post-translational targeting of both folded and unfolded translocation competent presecretory proteins to the inner membrane. Such a variety of interactors and interactor properties is in line with DnaK's abilities to interact with both native and unfolded proteins, as well as with oligomeric protein complexes (Section 3).

8. GroESL in protein targeting pathways

8.1. GroESL and the Sec pathway

A direct involvement of the *E. coli* chaperonin GroESL in the processing of Sec-dependent substrates has been observed in some cases. Indeed, overexpression of GroESL or GroEL alone improved the export of the LamB–LacZ fusion protein and decreased jamming of the *E. coli* protein export machinery, as observed for DnaK [141]. In addition, GroEL was able to interact with urea-denatured proOmpA and prePhoE *in vitro*, and to stabilize proOmpA for transit into inner membrane vesicles [177]. Furthermore, mutations in *groESL* (*groES619* or *groEL44*) significantly delayed β-lactamase processing *in vivo* [178]. Yet, in contrast with SecB, the effect of such *groESL* mutations on protein export could not be generalized to other presecretory proteins, as processing of both proOmpA and proOmpF was unaffected under these conditions. More recently, 5 outer-membrane proteins, namely CirA, Lpp, OmpA, OmpC and OmpF, and 11 periplasmic proteins, including BlaT, FrdA, OppA, YncE and the periplasmic chaperone Skp involved in outer membrane protein biogenesis were identified as GroEL interactors *in vivo* [27]. Noticeably, 4 of these interactors are known SecB substrates, *i.e.*, OppA, YncE, OmpA and OmpF, further highlighting possible overlaps between the two chaperones [179,180].

Interplays between GroESL and the Sec machinery were further revealed by genetic studies using translocation-compromised *E. coli* cells. Indeed, overexpression of GroESL efficiently rescued most of the *sec*-sensitive mutations, including the Ts *secA51* and *secY24*, and the Cs *secY39*, *secE15*, *secE501*, *secD1* and *secF62* alleles [181,182]. Accordingly, we have confirmed and extended such suppression by GroESL using the *secY205*, *secY129*, *secAR11* and Δ*secB* Cs alleles (unpublished data). The fact that endogenous SecB levels increase in strains with impaired GroESL is in line with such findings [148]. Importantly, the discovery that GroEL is efficiently targeted to membrane-bound SecA *in vitro* strongly supports such a link between the chaperone and Sec [183]. In addition, the increased fraction of membrane-localized GroEL observed both in *E. coli* following SecE, YidC or SRP depletion, and in *B. subtilis* in response to ethanol stress indicates that re-localization of GroESL is intensified under stress conditions affecting membrane integrity [160,184,185].

Together these data demonstrate that GroESL actively contributes to the Sec-dependent export process, perhaps acting directly on precursors to facilitate their transfer to SecA at the inner membrane, as observed for SecB or DnaK (Fig. 2). The fact that precursors need to be in a non-native form in order to be translocated via Sec suggests that the robust *in vitro* unfoldase activity of GroEL previously observed in the absence of GroES might be critical in this case [97,186,187]. In such a scenario, GroEL might act as a quality control device performing partial unfolding and release of unproductive, folded precursor proteins that have missed a timely interaction with the Sec translocon, thus offering additional opportunities for translocation [97,186,187]. This is in contrast with the active folding of cytosolic proteins that are bound inside the cavity of GroESL as described in Section 4.

8.2. GroESL and the Tat pathway

Despite some experimental evidence, the role of GroESL in Tat remains elusive. A study performed under anaerobic conditions first demonstrated a negative effect of *groESL* mutations (*groEL673* and *groES619*) on the biosynthesis of the Tat-dependent NiFe-hydrogenase 1, which catalyzes the reversible oxidation of hydrogen under anaerobic condition [188]. NiFe-hydrogenase 1 is composed of 2 subunits: HyaA and HyaB, the former being a known Tat substrate and the latter a co-exported partner [189]. Yet, evidence for a direct interaction between GroEL and HyaA or HyaB is lacking, perhaps due to the fact that GroEL interactome was performed aerobically. In contrast with *dnaK* mutations, expression and localization of hybrid proteins containing the Tat signal sequence of TorA fused to either GFP or MBP were variably affected by *groESL* mutations, as only the TorA-GFP hybrid seemed to partially rely on GroEL [173]. Similarly, overexpression of GroESL enhanced translocation via Tat of the heterologous OPH (organophosphorus hydrolase) fused to the TorA signal sequence [190] but did not help CueO or SufI [173].

A direct interaction between GroESL and the Tat substrate named AmiA was found *in vivo*. AmiA is one of the three N-acetylmuramyl-L-alanine amidases present in *E. coli*, which is classified as one of the obligate Class III GroEL client by Kerner and colleagues [27]. To date, there is no physiological relevance for such interaction. The cytoplasmic protein TatD, which is encoded by the *tatD* gene present in the *tatABCDE* operon was also isolated as a *bona fide* GroEL substrate *in vivo* [92]. However, so far there is no Tat function assigned to this protein. In addition, GroEL was shown to interact with several Tat signal sequences including those of AmiA, CueO and HiPIP, both *in vivo* and *in vitro*. Yet, interactions of GroEL with Tat signal sequences seems to occur mainly in the absence of DnaK, thus revealing possible overlaps between the two chaperones during the processing of Tat substrates, with a selective advantage for DnaK [172]. In addition to Tat substrates, it was shown that GroEL is capable of interacting with both NapD and DmsD, which are the REMPs for the Tat-dependent proteins NapA and DmsA, respectively [175,191]. Together these results suggest a role for GroESL in the stabilization and folding of some Tat substrates, perhaps cooperating with REMPs or other chaperones and facilitating insertion of cofactors. Yet, in contrast with the proposed multistep and generic involvement of DnaKJE (Section 7), GroESL function in Tat might be directed towards more specific substrates.

9. Concluding remarks

We have highlighted several lines of evidence that demonstrate how, in addition to their major role in the folding of newly-synthesized cytosolic proteins, the chaperones TF, DnaKJE and GroESL contribute to post-translational protein targeting (Fig. 2). Remarkably, the fact that these chaperones can participate at almost every step in translocation pathways that either export folded or unfolded proteins is in complete agreement with their known abilities to interact with various substrate conformations and to cooperatively assist the folding of certain proteins. The fact that both DnaKJE and GroESL are strongly induced in response to various types of stress, including those affecting protein export or inner membrane integrity, suggests that their contribution might become predominant under these circumstances. Yet, it remains to be determined how these chaperones precisely drive precursor proteins to their correct translocation pathway and perhaps directly help translocation through the cytoplasmic membrane. Likewise, active cooperation with other chaperones and targeting factors such as SecB, SecA or the specialized REMPs still needs to be demonstrated.

Interestingly, adding to their central role in protein export pathways, it has been shown that both DnaK and GroEL are found as abundant extracellular proteins in certain bacteria [192–194]. Studies from various bacteria indicate that these secreted chaperones can recognize and bind ligands, thus contributing to cell adherence, biofilm formation and pathogenesis [111,194], or exhibit activities similar to intercellular signaling molecule, as it is the case for GroEL2 from *M. tuberculosis*, which acts as an alternative macrophage activator [195] and GroEL from *Enterobacter aerogenes* that functions as insect toxins [196,197]. To date, it is not known how these chaperones are exported to the cell surface and to what extent dedicated export systems exist. In addition, nothing is known about the oligomeric status of these secreted chaperones and whether chaperone-bound substrates are involved [193]. More work is clearly warranted to shed light on such fundamental process involving DnaK and GroEL.

Acknowledgements

We thank Joen Luirink for critical reading of part 7.3. This work was supported by a joint Region Midi-Pyrénées/CNRS grant (to NB) and an ANR Grant mycoTAC (to PG).

References

[1] I. Fedyunin, L. Lehnhardt, N. Bohmer, P. Kaufmann, G. Zhang, Z. Ignatova, tRNA concentration fine tunes protein solubility, FEBS Lett. 586 (2012) 3336–3340.
[2] N. Bruel, M.P. Castanie-Cornet, A.M. Cirinesi, G. Koningstein, C. Georgopoulos, J. Luirink, P. Genevaux, Hsp33 controls elongation factor-Tu stability and allows *Escherichia coli* growth in the absence of the major DnaK and trigger factor chaperones, J. Biol. Chem. 287 (2012) 44435–44446.
[3] A.A. Komar, A pause for thought along the co-translational folding pathway, Trends Biochem. Sci. 34 (2009) 16–24.
[4] P. Cortazzo, C. Cervenansky, M. Marin, C. Reiss, R. Ehrlich, A. Deana, Silent mutations affect in vivo protein folding in *Escherichia coli*, Biochem. Biophys. Res. Commun. 293 (2002) 537–541.
[5] T. Crombie, J.P. Boyle, J.R. Coggins, A.J. Brown, The folding of the bifunctional TRP3 protein in yeast is influenced by a translational pause which lies in a region of structural divergence with *Escherichia coli* indoleglycerol-phosphate synthase, Eur. J. Biochem. 226 (1994) 657–664.
[6] J. Lu, C. Deutsch, Folding zones inside the ribosomal exit tunnel, Nat. Struct. Mol. Biol. 12 (2005) 1123–1129.
[7] A. Kosolapov, C. Deutsch, Tertiary interactions within the ribosomal exit tunnel, Nat. Struct. Mol. Biol. 16 (2009) 405–411.
[8] C.M. Kaiser, D.H. Goldman, J.D. Chodera, I. Tinoco Jr., C. Bustamante, The ribosome modulates nascent protein folding, Science 334 (2011) 1723–1727.
[9] G. Kramer, D. Boehringer, N. Ban, B. Bukau, The ribosome as a platform for co-translational processing, folding and targeting of newly synthesized proteins, Nat. Struct. Mol. Biol. 16 (2009) 589–597.
[10] A. Sandikci, F. Gloge, M. Martinez, M.P. Mayer, R. Wade, B. Bukau, G. Kramer, Dynamic enzyme docking to the ribosome coordinates N-terminal processing with polypeptide folding, Nat. Struct. Mol. Biol. 20 (2013) 843–850.
[11] J. Luirink, I. Sinning, SRP-mediated protein targeting: structure and function revisited, Biochim. Biophys. Acta 1694 (2004) 17–35.
[12] R.E. Dalbey, P. Wang, A. Kuhn, Assembly of bacterial inner membrane proteins, Annu. Rev. Biochem. 80 (2011) 161–187.
[13] J. Frobel, P. Rose, M. Muller, Twin-arginine-dependent translocation of folded proteins, Philos. Trans. R. Soc. Lond. B Biol. Sci. 367 (2012) 1029–1046.
[14] R. Kudva, K. Denks, P. Kuhn, A. Vogt, M. Muller, H.G. Koch, Protein translocation across the inner membrane of Gram-negative bacteria: the Sec and Tat dependent protein transport pathways, Res. Microbiol. 164 (2013) 505–534.
[15] A.J.M. Driessen, N. Nouwen, Protein translocation across the bacterial cytoplasmic membrane, Annu. Rev. Biochem. 77 (2008) 643–667.
[16] L.L. Randall, S.J. Hardy, SecB, one small chaperone in the complex milieu of the cell, Cell. Mol. Life Sci. 59 (2002) 1617–1623.
[17] C. Mao, C.E. Cheadle, S.J. Hardy, A.A. Lilly, Y. Suo, R.R. Sanganna Gari, G.M. King, L.L. Randall, Stoichiometry of SecYEG in the active translocase of *Escherichia coli* varies with precursor species, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 11815–11820.
[18] D. Huber, N. Rajagopalan, S. Preissler, M.A. Rocco, F. Merz, G. Kramer, B. Bukau, SecA interacts with ribosomes in order to facilitate posttranslational translocation in bacteria, Mol. Cell 41 (2011) 343–353.
[19] A. Sala, V. Calderon, P. Bordes, P. Genevaux, TAC from *Mycobacterium tuberculosis*: a paradigm for stress-responsive toxin–antitoxin systems controlled by SecB-like chaperones, Cell Stress Chaperones 18 (2013) 129–135.
[20] K.E. Chatzi, M.F. Sardis, S. Karamanou, A. Economou, Breaking on through to the other side: protein export through the bacterial Sec system, Biochem. J. 449 (2013) 25–37.
[21] T. Palmer, B.C. Berks, The twin-arginine translocation (Tat) protein export pathway, Nat. Rev. Microbiol. 10 (2012) 483–496.
[22] C. Robinson, C.F. Matos, D. Beck, C. Ren, J. Lawrence, N. Vasisht, S. Mendel, Transport and proofreading of proteins by the twin-arginine translocation (Tat) system in bacteria, Biochim. Biophys. Acta 1808 (2011) 876–884.
[23] F.U. Hartl, M. Hayer-Hartl, Converging concepts of protein folding in vitro and in vivo, Nat. Struct. Mol. Biol. 16 (2009) 574–581.
[24] S.A. Teter, W.A. Houry, D. Ang, T. Tradler, D. Rockabrand, G. Fischer, P. Blum, C. Georgopoulos, F.U. Hartl, Polypeptide flux through bacterial Hsp70: DnaK cooperates with trigger factor in chaperoning nascent chains, Cell 97 (1999) 755–765.
[25] E. Deuerling, A. Schulze-Specking, T. Tomoyasu, A. Mogk, B. Bukau, Trigger factor and DnaK cooperate in folding of newly synthesized proteins, Nature 400 (1999) 693–696.
[26] V.R. Agashe, S. Guha, H.C. Chang, P. Genevaux, M. Hayer-Hartl, M. Stemp, C. Georgopoulos, F.U. Hartl, J.M. Barral, Function of trigger factor and DnaK in multidomain protein folding: increase in yield at the expense of folding speed, Cell 117 (2004) 199–209.
[27] M.J. Kerner, D.J. Naylor, Y. Ishihama, T. Maier, H.C. Chang, A.P. Stines, C. Georgopoulos, D. Frishman, M. Hayer-Hartl, M. Mann, F.U. Hartl, Proteome-wide analysis of chaperonin-dependent protein folding in *Escherichia coli*, Cell 122 (2005) 209–220.
[28] G. Calloni, T. Chen, S.M. Schermann, H.C. Chang, P. Genevaux, F. Agostini, G.G. Tartaglia, M. Hayer-Hartl, F.U. Hartl, DnaK functions as a central hub in the *E. coli* chaperone network, Cell Rep. 1 (2012) 251–264.
[29] Q.A. Valent, D.A. Kendall, S. High, R. Kusters, B. Oudega, J. Luirink, Early events in preprotein recognition in *E. coli*: interaction of SRP and trigger factor with nascent polypeptides, EMBO J. 14 (1995) 5494–5505.
[30] L. Ferbitz, T. Maier, H. Patzelt, B. Bukau, E. Deuerling, N. Ban, Trigger factor in complex with the ribosome forms a molecular cradle for nascent proteins, Nature 431 (2004) 590–596.
[31] P. Genevaux, F. Keppel, F. Schwager, P.S. Langendijk-Genevaux, F.U. Hartl, C. Georgopoulos, In vivo analysis of the overlapping functions of DnaK and trigger factor, EMBO Rep. 5 (2004) 195–200.
[32] G. Kramer, T. Rauch, W. Rist, S. Vorderwulbecke, H. Patzelt, A. Schulze-Specking, N. Ban, E. Deuerling, B. Bukau, L23 protein functions as a chaperone docking site on the ribosome, Nature 419 (2002) 171–174.
[33] S.K. Lakshmipathy, S. Tomic, C.M. Kaiser, H.C. Chang, P. Genevaux, C. Georgopoulos, J.M. Barral, A.E. Johnson, F.U. Hartl, S.A. Etchells, Identification of nascent chain interaction sites on trigger factor, J. Biol. Chem. 282 (2007) 12186–12193.
[34] F. Merz, A. Hoffmann, A. Rutkowska, B. Zachmann-Brand, B. Bukau, E. Deuerling, The C-terminal domain of *Escherichia coli* trigger factor represents the central module of its chaperone activity, J. Biol. Chem. 281 (2006) 31963–31971.
[35] A. Hoffmann, B. Bukau, G. Kramer, Structure and function of the molecular chaperone Trigger Factor, Biochim. Biophys. Acta, Mol. Cell. Res. 1803 (2010) 650–661.
[36] O. Kristensen, M. Gajhede, Chaperone binding at the ribosomal exit tunnel, Structure (Camb) 11 (2003) 1547–1556.
[37] J. Maillard, P. Genevaux, C. Holliger, Redundancy and specificity of multiple trigger factor chaperones in Desulfitobacteria, Microbiology 157 (2011) 2410–2421.
[38] A. Raine, M. Lovmar, J. Wikberg, M. Ehrenberg, Trigger factor binding to ribosomes with nascent peptide chains of varying lengths and sequences, J. Biol. Chem. 281 (2013) 28033–28038.
[39] E. Crooke, B. Guthrie, S. Lecker, R. Lill, W. Wickner, ProOmpA is stabilized for membrane translocation by either purified *E. coli* trigger factor or canine signal recognition particle, Cell 54 (1988) 1003–1011.
[40] C.M. Kaiser, H.C. Chang, V.R. Agashe, S.K. Lakshmipathy, S.A. Etchells, M. Hayer-Hartl, F.U. Hartl, J.M. Barral, Real-time observation of trigger factor function on translating ribosomes, Nature 444 (2006) 455–460.
[41] E. Martinez-Hackert, W.A. Hendrickson, Promiscuous substrate recognition in folding and assembly activities of the trigger factor chaperone, Cell 138 (2009) 923–934.
[42] F. Merz, D. Boehringer, C. Schaffitzel, S. Preissler, A. Hoffmann, T. Maier, A. Rutkowska, J. Lozza, N. Ban, B. Bukau, E. Deuerling, Molecular mechanism and structure of Trigger Factor bound to the translating ribosome, EMBO J. 27 (2008) 1622–1632.
[43] E.N. Houben, R. Zarivach, B. Oudega, J. Luirink, Early encounters of a nascent membrane protein: specificity and timing of contacts inside and outside the ribosome, J. Cell Biol. 170 (2005) 27–35.
[44] E. Oh, A.H. Becker, A. Sandikci, D. Huber, R. Chaba, F. Gloge, R.J. Nichols, A. Typas, C.A. Gross, G. Kramer, J.S. Weissman, B. Bukau, Selective ribosome profiling reveals the cotranslational chaperone action of trigger factor in vivo, Cell 147 (2011) 1295–1308.
[45] L.A. Ball, P. Kaesberg, Cleavage of the N-terminal formylmethionine residue from a bacteriophage coat protein in vitro, J. Mol. Biol. 79 (1973) 531–537.
[46] R. Bingel-Erlenmeyer, R. Kohler, G. Kramer, A. Sandikci, S. Antolic, T. Maier, C. Schaffitzel, B. Wiedmann, B. Bukau, N. Ban, A peptide deformylase-ribosome complex reveals mechanism of nascent chain processing, Nature 452 (2008) 108–U113.
[47] R.J. Keenan, D.M. Freymann, R.M. Stroud, P. Walter, The signal recognition particle, Annu. Rev. Biochem. 70 (2001) 755–775.
[48] H. Patzelt, S. Rudiger, D. Brehmer, G. Kramer, S. Vorderwulbecke, E. Schaffitzel, A. Waitz, T. Hesterkamp, L. Dong, J. Schneider-Mergener, B. Bukau, E. Deuerling, Binding specificity of *Escherichia coli* trigger factor, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 14244–14249.
[49] S.K. Lakshmipathy, R. Gupta, S. Pinkert, S.A. Etchells, F.U. Hartl, Versatility of trigger factor interactions with ribosome-nascent chain complexes, J. Biol. Chem. 285 (2010) 27911–27923.
[50] A. Hoffmann, F. Merz, A. Rutkowska, B. Zachmann-Brand, E. Deuerling, B. Bukau, Trigger factor forms a protective shield for nascent polypeptides at the ribosome, J. Biol. Chem. 281 (2006) 6539–6545.
[51] A. Hoffmann, A.H. Becker, B. Zachmann-Brand, E. Deuerling, B. Bukau, G. Kramer, Concerted action of the ribosome and the associated chaperone trigger factor confines nascent polypeptide folding, Mol. Cell 48 (2012) 63–74.
[52] E.P. O'Brien, J. Christodoulou, M. Vendruscolo, C.M. Dobson, Trigger factor slows co-translational folding through kinetic trapping while sterically protecting the nascent chain from aberrant cytosolic interactions, J. Am. Chem. Soc. 134 (2012) 10920–10932.
[53] B. Guthrie, W. Wickner, Trigger factor depletion or overproduction causes defective cell division but does not block protein export, J. Bacteriol. 172 (1990) 5555–5562.
[54] O. Kandror, A.L. Goldberg, Trigger factor is induced upon cold shock and enhances viability of *Escherichia coli* at low temperatures, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 4978–4981.
[55] R.J. Nichols, S. Sen, Y.J. Choo, P. Beltrao, M. Zietek, R. Chaba, S. Lee, K.M. Kazmierczak, K.J. Lee, A. Wong, M. Shales, S. Lovett, M.E. Winkler, N.J. Krogan, A. Typas, C.A. Gross, Phenotypic landscape of a bacterial cell, Cell 144 (2011) 143–156.
[56] D.Y. Reyes, H. Yoshikawa, DnaK chaperone machine and trigger factor are only partially required for normal growth of *Bacillus subtilis*, Biosci. Biotechnol. Biochem. 66 (2002) 1583–1586.
[57] S.F. Gothel, C. Scholz, F.X. Schmid, M.A. Marahiel, Cyclophilin and trigger factor from *Bacillus subtilis* catalyze in vitro protein folding and are necessary for viability under starvation conditions, Biochemistry 37 (1998) 13392–13399.

[58] M. Miller-Williams, P.C. Loewen, I.J. Oresnik, Isolation of salt-sensitive mutants of *Sinorhizobium meliloti* strain Rm1021, Microbiology 152 (2006) 2049–2059.
[59] A. Bigot, E. Botton, I. Dubail, A. Charbit, A homolog of *Bacillus subtilis* trigger factor in *Listeria monocytogenes* is involved in stress tolerance and bacterial virulence, Appl. Environ. Microbiol. 72 (2006) 6623–6631.
[60] P.J. Crowley, T.B. Seifert, R. Isoda, M. van Tilburg, M.W. Oli, R.A. Robinette, W.P. McArthur, A.S. Bleiweis, L.J. Brady, Requirements for surface expression and function of adhesin P1 from *Streptococcus mutans*, Infect. Immun. 76 (2008) 2456–2468.
[61] Z.T. Wen, P. Suntharaligham, D.G. Cvitkovitch, R.A. Burne, Trigger factor in *Streptococcus mutans* is involved in stress tolerance, competence development, and biofilm formation, Infect. Immun. 73 (2005) 219–225.
[62] W.R. Lyon, C.M. Gibson, M.G. Caparon, A role for trigger factor and an rgg-like regulator in the transcription, secretion and processing of the cysteine proteinase of *Streptococcus pyogenes*, EMBO J. 17 (1998) 6263–6275.
[63] P. Genevaux, C. Georgopoulos, W.L. Kelley, The Hsp70 chaperone machines of *Escherichia coli*: a paradigm for the repartition of chaperone functions, Mol. Microbiol. 66 (2007) 840–857.
[64] M.P. Mayer, B. Bukau, Hsp70 chaperones: cellular functions and molecular mechanism, Cell. Mol. Life Sci. 62 (2005) 670–684.
[65] J.F. Swain, G. Dinler, R. Sivendran, D.L. Montgomery, M. Stotz, L.M. Gierasch, Hsp70 chaperone ligands control domain association via an allosteric mechanism mediated by the interdomain linker, Mol. Cell 26 (2007) 27–39.
[66] M. Vogel, M.P. Mayer, B. Bukau, Allosteric regulation of Hsp70 chaperones involves a conserved interdomain linker, J. Biol. Chem. 281 (2006) 38705–38711.
[67] R.G. Smock, M.E. Blackburn, L.M. Gierasch, Conserved, disordered C terminus of DnaK enhances cellular survival upon stress and DnaK *in vitro* chaperone activity, J. Biol. Chem. 286 (2011) 31821–31829.
[68] K. Liberek, J. Marszalek, D. Ang, C. Georgopoulos, M. Zylicz, *Escherichia coli* DnaJ and GrpE heat shock proteins jointly stimulate ATPase activity of DnaK, Proc. Natl. Acad. Sci. U. S. A. 88 (1991) 2874–2878.
[69] C.J. Harrison, M. Hayer-Hartl, M. Di Liberto, F. Hartl, J. Kuriyan, Crystal structure of the nucleotide exchange factor GrpE bound to the ATPase domain of the molecular chaperone DnaK, Science 276 (1997) 431–435.
[70] D. Brehmer, S. Rudiger, C.S. Gassler, D. Klostermeier, L. Packschies, J. Reinstein, M.P. Mayer, B. Bukau, Tuning of chaperone activity of Hsp70 proteins by modulation of nucleotide exchange, Nat. Struct. Biol. 8 (2001) 427–432.
[71] S. Rudiger, L. Germeroth, J. Schneider-Mergener, B. Bukau, Substrate specificity of the DnaK chaperone determined by screening cellulose-bound peptide libraries, EMBO J. 16 (1997) 1501–1507.
[72] G.G. Tartaglia, C.M. Dobson, F.U. Hartl, M. Vendruscolo, Physicochemical determinants of chaperone requirements, J. Mol. Biol. 400 (2010) 579–588.
[73] A. Mogk, D. Huber, B. Bukau, Integrating protein homeostasis strategies in prokaryotes, Cold Spring Harb. Perspect. Biol. 3 (2011).
[74] B. Bukau, G.C. Walker, Delta dnaK52 mutants of *Escherichia coli* have defects in chromosome segregation and plasmid maintenance at normal growth temperatures, J. Bacteriol. 171 (1989) 6030–6038.
[75] J.A. Lemos, Y. Luzardo, R.A. Burne, Physiologic effects of forced down-regulation of dnaK and groEL expression in *Streptococcus mutans*, J. Bacteriol. 189 (2007) 1582–1588.
[76] J.E. Griffin, J.D. Gawronski, M.A. Dejesus, T.R. Ioerger, B.J. Akerley, C.M. Sassetti, High-resolution phenotypic profiling defines genes essential for mycobacterial growth and cholesterol catabolism, PLoS Pathog. 7 (2011) e1002251.
[77] A. Schulz, B. Tzschaschel, W. Schumann, Isolation and analysis of mutants of the dnaK operon of *Bacillus subtilis*, Mol. Microbiol. 15 (1995) 421–429.
[78] K. Selby, M. Lindstrom, P. Somervuo, J.T. Heap, N.P. Minton, H. Korkeala, Important role of class I heat shock genes hrcA and dnaK in the heat shock response and the response to pH and NaCl stress of group I *Clostridium botulinum* strain ATCC 3502, Appl. Environ. Microbiol. 77 (2011) 2823–2830.
[79] S. Chakrabarti, N. Sengupta, R. Chowdhury, Role of DnaK in in vitro and in vivo expression of virulence factors of *Vibrio cholerae*, Infect. Immun. 67 (1999) 1025–1033.
[80] T. Tomoyasu, A. Tabata, H. Imaki, K. Tsuruno, A. Miyazaki, K. Sonomoto, R.A. Whiley, H. Nagamune, Role of *Streptococcus intermedius* DnaK chaperone system in stress tolerance and pathogenicity, Cell Stress Chaperones 17 (2012) 41–55.
[81] A. Takaya, T. Tomoyasu, H. Matsui, T. Yamamoto, The DnaK/DnaJ chaperone machinery of *Salmonella enterica* serovar Typhimurium is essential for invasion of epithelial cells and survival within macrophages, leading to systemic infection, Infect. Immun. 72 (2004) 1364–1373.
[82] S. Kohler, E. Ekaza, J.Y. Paquet, K. Walravens, J. Teyssier, J. Godfroid, J.P. Liautard, Induction of dnaK through its native heat shock promoter is necessary for intramacrophagic replication of *Brucella suis*, Infect. Immun. 70 (2002) 1631–1634.
[83] T. Hanawa, M. Fukuda, H. Kawakami, H. Hirano, S. Kamiya, T. Yamamoto, The *Listeria monocytogenes* DnaK chaperone is required for stress tolerance and efficient phagocytosis with macrophages, Cell Stress Chaperones 4 (1999) 118–128.
[84] V.K. Singh, S. Utaida, L.S. Jackson, R.K. Jayaswal, B.J. Wilkinson, N.R. Chamberlain, Role for dnaK locus in tolerance of multiple stresses in *Staphylococcus aureus*, Microbiology 153 (2007) 3162–3173.
[85] H.R. Saibil, W.A. Fenton, D.K. Clare, A.L. Horwich, Structure and allostery of the chaperonin GroEL, J. Mol. Biol. 425 (2013) 1476–1487.
[86] F. Shewmaker, K. Maskos, C. Simmerling, S.J. Landry, The disordered mobile loop of GroES folds into a defined beta-hairpin upon binding GroEL, J. Biol. Chem. 276 (2001) 31257–31264.
[87] T. Nojima, T. Ikegami, H. Taguchi, M. Yoshida, Flexibility of GroES mobile loop is required for efficient chaperonin function, J. Mol. Biol. 422 (2012) 291–299.
[88] A.I. Jewett, J.E. Shea, Reconciling theories of chaperonin accelerated folding with experimental evidence, Cell. Mol. Life Sci. 67 (2010) 255–276.
[89] A.L. Horwich, W.A. Fenton, Chaperonin-mediated protein folding: using a central cavity to kinetically assist polypeptide chain folding, Q. Rev. Biophys. 42 (2009) 83–116.
[90] Y.C. Tang, H.C. Chang, A. Roeben, D. Wischnewski, N. Wischnewski, M.J. Kerner, F.U. Hartl, M. Hayer-Hartl, Structural features of the GroEL–GroES nano-cage required for rapid folding of encapsulated protein, Cell 125 (2006) 903–914.
[91] Z. Lin, D. Madan, H.S. Rye, GroEL stimulates protein folding through forced unfolding, Nat. Struct. Mol. Biol. 15 (2008) 303–311.
[92] K. Fujiwara, Y. Ishihama, K. Nakahigashi, T. Soga, H. Taguchi, A systematic survey of in vivo obligate chaperonin-dependent substrates, EMBO J. 29 (2010) 1552–1564.
[93] O. Fayet, T. Ziegelhoffer, C. Georgopoulos, The groES and groEL heat shock gene products of *Escherichia coli* are essential for bacterial growth at all temperatures, J. Bacteriol. 171 (1989) 1379–1385.
[94] T. Niwa, B.W. Ying, K. Saito, W. Jin, S. Takada, T. Ueda, H. Taguchi, Bimodal protein solubility distribution revealed by an aggregation analysis of the entire ensemble of *Escherichia coli* proteins, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 4201–4206.
[95] E. Chapman, G.W. Farr, R. Usaite, K. Furtak, W.A. Fenton, T.K. Chaudhuri, E.R. Hondorp, R.G. Matthews, S.G. Wolf, J.R. Yates, M. Pypaert, A.L. Horwich, Global aggregation of newly translated proteins in an *Escherichia coli* strain deficient of the chaperonin GroEL, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 15800–15805.
[96] M. Arifuzzaman, M. Maeda, A. Itoh, K. Nishikata, C. Takita, R. Saito, T. Ara, K. Nakahigashi, H.C. Huang, A. Hirai, K. Tsuzuki, S. Nakamura, M. Altaf-Ul-Amin, T. Oshima, T. Baba, N. Yamamoto, T. Kawamura, T. Ioka-Nakamichi, M. Kitagawa, M. Tomita, S. Kanaya, C. Wada, H. Mori, Large-scale identification of protein–protein interaction of *Escherichia coli* K-12, Genome Res. 16 (2006) 686–691.
[97] S. Priya, S.K. Sharma, V. Sood, R.U. Mattoo, A. Finka, A. Azem, P. De Los Rios, P. Goloubinoff, GroEL and CCT are catalytic unfoldases mediating out-of-cage polypeptide refolding without ATP, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 7199–7204.
[98] C.P. Georgopoulos, H. Eisen, Bacterial mutants which block phage assembly, J. Supramol. Struct. 2 (1974) 349–359.
[99] A.L. Horwich, K.B. Low, W.A. Fenton, I.N. Hirshfield, K. Furtak, Folding in vivo of bacterial cytoplasmic proteins: role of GroEL, Cell 74 (1993) 909–917.
[100] M. Kanemori, H. Mori, T. Yura, Effects of reduced levels of GroE chaperones on protein metabolism: enhanced synthesis of heat shock proteins during steady-state growth of *Escherichia coli*, J. Bacteriol. 176 (1994) 4235–4242.
[101] N. McLennan, M. Masters, GroE is vital for cell-wall synthesis, Nature 392 (1998) 139.
[102] M. Li, S.L. Wong, Cloning and characterization of the groESL operon from *Bacillus subtilis*, J. Bacteriol. 174 (1992) 3981–3992.
[103] G.K. Nasrallah, E. Gagnon, D.J. Orton, R.A. Garduno, The htpAB operon of *Legionella pneumophila* cannot be deleted in the presence of the groE chaperonin operon of *Escherichia coli*, Can. J. Microbiol. 57 (2011) 943–952.
[104] M.F. Susin, R.L. Baldini, F. Gueiros-Filho, S.L. Gomes, GroES/GroEL and DnaK/DnaJ have distinct roles in stress responses and during cell cycle progression in *Caulobacter crescentus*, J. Bacteriol. 188 (2006) 8044–8053.
[105] P.A. Lund, Multiple chaperonins in bacteria—why so many? FEMS Microbiol. Rev. 33 (2009) 785–800.
[106] A.N. Bittner, A. Foltz, V. Oke, Only one of five groEL genes is required for viability and successful symbiosis in *Sinorhizobium meliloti*, J. Bacteriol. 189 (2007) 1884–1889.
[107] J. Li, Y. Wang, C.Y. Zhang, W.Y. Zhang, D.M. Jiang, Z.H. Wu, H. Liu, Y.Z. Li, *Myxococcus xanthus* viability depends on groEL supplied by either of two genes, but the paralogs have different functions during heat shock, predation, and development, J. Bacteriol. 192 (2010) 1875–1881.
[108] F. Rodriguez-Quinones, M. Maguire, E.J. Wallington, P.S. Gould, V. Yerko, J.A. Downie, P.A. Lund, Two of the three groEL homologues in *Rhizobium leguminosarum* are dispensable for normal growth, Arch. Microbiol. 183 (2005) 253–265.
[109] W.T. Lee, K.C. Terlesky, F.R. Tabita, Cloning and characterization of two groESL operons of *Rhodobacter sphaeroides*: transcriptional regulation of the heat-induced groESL operon, J. Bacteriol. 179 (1997) 487–495.
[110] M. Sato, K. Nimura-Matsune, S. Watanabe, T. Chibazakura, H. Yoshikawa, Expression analysis of multiple dnaK genes in the cyanobacterium *Synechococcus elongatus* PCC 7942, J. Bacteriol. 189 (2007) 3751–3758.
[111] A. Ojha, M. Anand, A. Bhatt, L. Kremer, W.R. Jacobs Jr., G.F. Hatfull, GroEL1: a dedicated chaperone involved in mycolic acid biosynthesis during biofilm formation in mycobacteria, Cell 123 (2005) 861–873.
[112] Y. Hu, B. Henderson, P.A. Lund, P. Tormay, M.T. Ahmed, S.S. Gurcha, G.S. Besra, A.R. Coates, A *Mycobacterium tuberculosis* mutant lacking the groEL homologue cpn60.1 is viable but fails to induce an inflammatory response in animal models of infection, Infect. Immun. 76 (2008) 1535–1546.
[113] J.I. Glass, E.J. Lefkowitz, J.S. Glass, C.R. Heiner, E.Y. Chen, G.H. Cassell, The complete sequence of the mucosal pathogen *Ureaplasma urealyticum*, Nature 407 (2000) 757–762.
[114] A. Barre, A. de Daruvar, A. Blanchard, MolliGen, a database dedicated to the comparative genomics of Mollicutes, Nucleic Acids Res. 32 (2004) D307–310.
[115] C.A. Hutchison, S.N. Peterson, S.R. Gill, R.T. Cline, O. White, C.M. Fraser, H.O. Smith, J.C. Venter, Global transposon mutagenesis and a minimal *Mycoplasma* genome, Science 286 (1999) 2165–2169.
[116] G.W. Clark, E.R. Tillier, Loss and gain of GroEL in the Mollicutes, Biochem. Cell Biol. 88 (2010) 185–194.
[117] S. Vorderwulbecke, G. Kramer, F. Merz, T.A. Kurz, T. Rauch, B. Zachmann-Brand, B. Bukau, E. Deuerling, Low temperature or GroEL/ES overproduction permits growth of *Escherichia coli* cells lacking trigger factor and DnaK, FEBS Lett. 559 (2004) 181–187.

[118] R.S. Ullers, J. Luirink, N. Harms, F. Schwager, C. Georgopoulos, P. Genevaux, SecB is a bona fide generalized chaperone in *Escherichia coli*, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 7583–7588.

[119] P. Bordes, A.M. Cirinesi, R. Ummels, A. Sala, S. Sakr, W. Bitter, P. Genevaux, SecB-like chaperone controls a toxin–antitoxin stress-responsive system in *Mycobacterium tuberculosis*, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 8438–8443.

[120] E. Crooke, W. Wickner, Trigger factor: a soluble protein that folds pro-OmpA into a membrane-assembly-competent form, Proc. Natl. Acad. Sci. U. S. A. 84 (1987) 5216–5220.

[121] E. Crooke, L. Brundage, M. Rice, W. Wickner, ProOmpA spontaneously folds in a membrane assembly competent state which trigger factor stabilizes, EMBO J. 7 (1988) 1831–1835.

[122] S. Lecker, R. Lill, T. Ziegelhoffer, C. Georgopoulos, P.J. Bassford Jr., C.A. Kumamoto, W. Wickner, Three pure chaperone proteins of *Escherichia coli*-SecB, trigger factor and GroEL-form soluble complexes with precursor proteins in vitro, EMBO J. 8 (1989) 2703–2709.

[123] H.C. Lee, H.D. Bernstein, Trigger factor retards protein export in *E. coli*, J. Biol. Chem. 29 (2002) 29.

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

[125] R.S. Ullers, D. Ang, F. Schwager, C. Georgopoulos, P. Genevaux, Trigger Factor can antagonize both SecB and DnaK/DnaJ chaperone functions in *Escherichia coli*, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 3101–3106.

[126] D.P. Ricci, T.J. Silhavy, The Bam machine: a molecular cooper, Biochim. Biophys. Acta 1818 (2012) 1067–1084.

[127] G. Gouridis, S. Karamanou, I. Gelis, C.G. Kalodimos, A. Economou, Signal peptides are allosteric activators of the protein translocase, Nature 462 (2009) 363–367.

[128] H. Kadokura, J. Beckwith, Detecting folding intermediates of a protein as it passes through the bacterial translocation channel, Cell 138 (2009) 1164–1173.

[129] L.G. Josefsson, L.L. Randall, Different exported proteins in *E. coli* show differences in the temporal mode of processing in vivo, Cell 25 (1981) 151–157.

[130] T. Bornemann, J. Jockel, M.V. Rodnina, W. Wintermeyer, Signal sequence-independent membrane targeting of ribosomes containing short nascent peptides within the exit tunnel, Nat. Struct. Mol. Biol. 15 (2008) 494–499.

[131] K.F. Lin, C.S. Sun, Y.C. Huang, S.I. Chan, J. Koubek, T.H. Wu, J.J. Huang, Cotranslational protein folding within the ribosome tunnel influences trigger-factor recruitment, Biophys. J. 102 (2012) 2818–2827.

[132] G. Eisner, H.G. Koch, K. Beck, J. Brunner, M. Muller, Ligand crowding at a nascent signal sequence, J. Cell Biol. 163 (2003) 35–44.

[133] K. Beck, L.F. Wu, J. Brunner, M. Muller, Discrimination between SRP- and SecA/SecB-dependent substrates involves selective recognition of nascent chains by SRP and trigger factor, EMBO J. 19 (2000) 134–143.

[134] R.S. Ullers, E.N. Houben, A. Raine, C.M. ten Hagen-Jongman, M. Ehrenberg, J. Brunner, B. Oudega, N. Harms, J. Luirink, Interplay of signal recognition particle and trigger factor at L23 near the nascent chain exit site on the *Escherichia coli* ribosome, J. Cell Biol. 161 (2003) 679–684.

[135] R.S. Ullers, E.N. Houben, J. Brunner, B. Oudega, N. Harms, J. Luirink, Sequence-specific interactions of nascent *Escherichia coli* polypeptides with trigger factor and signal recognition particle, J. Biol. Chem. 281 (2006) 13999–14005.

[136] H.C. Lee, H.D. Bernstein, The targeting pathway of *Escherichia coli* presecretory and integral membrane proteins is specified by the hydrophobicity of the targeting signal, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 3471–3476.

[137] A.L. Karamyshev, A.E. Johnson, Selective SecA association with signal sequences in ribosome-bound nascent chains: a potential role for SecA in ribosome targeting to the bacterial membrane, J. Biol. Chem. 280 (2005) 37930–37940.

[138] W.S. Jong, C.M. Hagen-Jongman, P. Genevaux, J. Brunner, B. Oudega, J. Luirink, Trigger factor interacts with the signal peptide of nascent Tat substrates but does not play a critical role in Tat-mediated export, Eur. J. Biochem. 271 (2004) 4779–4787.

[139] Y. Morita, T. Futagami, M. Goto, K. Furukawa, Functional characterization of the trigger factor protein PceT of tetrachloroethene-dechlorinating *Desulfitobacterium hafniense* Y51, Appl. Microbiol. Biotechnol. 83 (2009) 775–781.

[140] W.R. Lyon, M.G. Caparon, Trigger factor-mediated prolyl isomerization influences maturation of the *Streptococcus pyogenes* cysteine protease, J. Bacteriol. 185 (2003) 3661–3667.

[141] G.J. Phillips, T.J. Silhavy, Heat-shock proteins DnaK and GroEL facilitate export of LacZ hybrid proteins in *E. coli*, Nature 344 (1990) 882–884.

[142] J. Wild, W.A. Walter, C.A. Gross, E. Altman, Accumulation of secretory protein precursors in *Escherichia coli* induces the heat shock response, J. Bacteriol. 175 (1993) 3992–3997.

[143] K. Ito, Y. Akiyama, T. Yura, K. Shiba, Diverse effects of the MalE–LacZ hybrid protein on *Escherichia coli* cell physiology, J. Bacteriol. 167 (1986) 201–204.

[144] E. Altman, C.A. Kumamoto, S.D. Emr, Heat-shock proteins can substitute for SecB function during protein export in *Escherichia coli*, EMBO J. 10 (1991) 239–245.

[145] J. Wild, E. Altman, T. Yura, C.A. Gross, DnaK and DnaJ heat shock proteins participate in protein export in *Escherichia coli*, Genes Dev. 6 (1992) 1165–1172.

[146] J. Moran-Barrio, A.S. Limansky, A.M. Viale, Secretion of GOB metallo-beta-lactamase in *Escherichia coli* depends strictly on the cooperation between the cytoplasmic DnaK chaperone system and the Sec machinery: completion of folding and Zn(II) ion acquisition occur in the bacterial periplasm, Antimicrob. Agents Chemother. 53 (2009) 2908–2917.

[147] H.Y. Qi, J.B. Hyndman, H.D. Bernstein, DnaK promotes the selective export of outer membrane protein precursors in SecA-deficient *Escherichia coli*, J. Biol. Chem. 277 (2002) 51077–51083.

[148] J.P. Muller, Influence of impaired chaperone or secretion function on SecB production in *Escherichia coli*, J. Bacteriol. 178 (1996) 6097–6104.

[149] S. Sakr, A.M. Cirinesi, R.S. Ullers, F. Schwager, C. Georgopoulos, P. Genevaux, Lon protease quality control of presecretory proteins in *Escherichia coli* and its dependence on the SecB and DnaJ (Hsp40) chaperones, J. Biol. Chem. 285 (2010) 23504–23512.

[150] N. Dautin, H.D. Bernstein, Protein secretion in gram-negative bacteria via the autotransporter pathway, Annu. Rev. Microbiol. 61 (2007) 89–112.

[151] J. Grijpstra, J. Arenas, L. Rutten, J. Tommassen, Autotransporter secretion: varying on a theme, Res. Microbiol. 164 (2013) 562–582.

[152] W.S. Jong, C.M. ten Hagen-Jongman, E. Ruijter, R.V. Orru, P. Genevaux, J. Luirink, YidC is involved in the biogenesis of the secreted autotransporter hemoglobin protease, J. Biol. Chem. 285 (2010) 39682–39690.

[153] X. Jiang, T. Ruiz, K.P. Mintz, Characterization of the secretion pathway of the collagen adhesin EmaA of *Aggregatibacter actinomycetemcomitans*, Mol. Oral Microbiol. 27 (2012) 382–396.

[154] A. Janakiraman, K.R. Fixen, A.N. Gray, H. Niki, M.B. Goldberg, A genome-scale proteomic screen identifies a role for DnaK in chaperoning of polar autotransporters in *Shigella*, J. Bacteriol. 191 (2009) 6300–6311.

[155] B. Bukau, P. Reilly, J. McCarty, G.C. Walker, Immunogold localization of the DnaK heat shock protein in *Escherichia coli* cells, J. Gen. Microbiol. 139 (1993) 95–99.

[156] M. Klepsch, S. Schlegel, D. Wickstrom, G. Friso, K.J. van Wijk, J.O. Persson, J.W. de Gier, S. Wagner, Immobilization of the first dimension in 2D blue native/SDS-PAGE allows the relative quantification of membrane proteomes, Methods 46 (2008) 48–53.

[157] F. Stenberg, P. Chovanec, S.L. Maslen, C.V. Robinson, L.L. Ilag, G. von Heijne, D.O. Daley, Protein complexes of the *Escherichia coli* cell envelope, J. Biol. Chem. 280 (2005) 34409–34419.

[158] D. Wickstrom, S. Wagner, L. Baars, A.J. Ytterberg, M. Klepsch, K.J. van Wijk, J. Luirink, J.W. de Gier, Consequences of depletion of the signal recognition particle in *Escherichia coli*, J. Biol. Chem. 286 (2011) 4598–4609.

[159] H.D. Bernstein, J.B. Hyndman, Physiological basis for conservation of the signal recognition particle targeting pathway in *Escherichia coli*, J. Bacteriol. 183 (2001) 2187–2197.

[160] D. Wickstrom, S. Wagner, P. Simonsson, O. Pop, L. Baars, A.J. Ytterberg, K.J. van Wijk, J. Luirink, J.W. de Gier, Characterization of the consequences of YidC depletion on the inner membrane proteome of *E. coli* using 2D blue native/SDS-PAGE, J. Mol. Biol. 409 (2011) 124–135.

[161] N. Borgese, E. Fasana, Targeting pathways of C-tail-anchored proteins, Biochim. Biophys. Acta 1808 (2011) 937–946.

[162] M.S. Aschtgen, A. Zoued, R. Lloubes, L. Journet, E. Cascales, The C-tail anchored TssL subunit, an essential protein of the enteroaggregative *Escherichia coli* Sci-1 Type VI secretion system, is inserted by YidC, Microbiologyopen 1 (2012) 71–82.

[163] D.J. Clarke, A. Jacq, I.B. Holland, A novel DnaJ-like protein in *Escherichia coli* inserts into the cytoplasmic membrane with a type III topology, Mol. Microbiol. 20 (1996) 1273–1286.

[164] W.L. Kelley, C. Georgopoulos, Positive control of the two-component RcsC/B signal transduction network by DjlA: a member of the DnaJ family of molecular chaperones in *Escherichia coli*, Mol. Microbiol. 25 (1997) 913–931.

[165] C.M. Toutain, D.J. Clarke, J.A. Leeds, J. Kuhn, J. Beckwith, I.B. Holland, A. Jacq, The transmembrane domain of the DnaJ-like protein DjlA is a dimerisation domain, Mol. Genet. Genomics 268 (2003) 761–770.

[166] P. Genevaux, A. Wawrzynow, M. Zylicz, C. Georgopoulos, W.L. Kelley, DjlA is a third DnaK co-chaperone of *Escherichia coli*, and DjlA-mediated induction of colanic acid capsule requires DjlA–DnaK interaction, J. Biol. Chem. 276 (2001) 7906–7912.

[167] P. Genevaux, F. Schwager, C. Georgopoulos, W.L. Kelley, The djlA gene acts synergistically with dnaJ in promoting *Escherichia coli* growth, J. Bacteriol. 183 (2001) 5747–5750.

[168] C.D. Vincent, B.A. Buscher, J.R. Friedman, L.A. Williams, P. Bardill, J.P. Vogel, Identification of non-dot/icm suppressors of the *Legionella pneumophila* DeltadotL lethality phenotype, J. Bacteriol. 188 (2006) 8231–8243.

[169] H. Ohnishi, Y. Mizunoe, A. Takade, Y. Tanaka, H. Miyamoto, M. Harada, S. Yoshida, *Legionella dumoffii* DjlA, a member of the DnaJ family, is required for intracellular growth, Infect. Immun. 72 (2004) 3592–3603.

[170] F. Lakhal, S. Bury-Mone, Y. Nomane, N. Le Goic, C. Paillard, A. Jacq, DjlA, a membrane-anchored DnaJ-like protein, is required for cytotoxicity of clam pathogen *Vibrio tapetis* to hemocytes, Appl. Environ. Microbiol. 74 (2008) 5750–5758.

[171] J.M. Cloward, D.C. Krause, *Mycoplasma pneumoniae* J-domain protein required for terminal organelle function, Mol. Microbiol. 71 (2009) 1296–1307.

[172] W. Graubner, A. Schierhorn, T. Bruser, DnaK plays a pivotal role in Tat targeting of CueO and functions beside SlyD as a general Tat signal binding chaperone, J. Biol. Chem. 282 (2007) 7116–7124.

[173] R. Perez-Rodriguez, A.C. Fisher, J.D. Perlmutter, M.G. Hicks, A. Chanal, C.L. Santini, L.F. Wu, T. Palmer, M.P. DeLisa, An essential role for the DnaK molecular chaperone in stabilizing over-expressed substrate proteins of the bacterial twin-arginine translocation pathway, J. Mol. Biol. 367 (2007) 715–730.

[174] I.J. Oresnik, C.L. Ladner, R.J. Turner, Identification of a twin-arginine leader-binding protein, Mol. Microbiol. 40 (2001) 323–331.

[175] H. Li, L. Chang, J.M. Howell, R.J. Turner, DmsD, a Tat system specific chaperone, interacts with other general chaperones and proteins involved in the molybdenum cofactor biosynthesis, Biochim. Biophys. Acta 1804 (2010) 1301–1309.

[176] T. Bruser, T. Yano, D.C. Brune, F. Daldal, Membrane targeting of a folded and cofactor-containing protein, Eur. J. Biochem. 270 (2003) 1211–1221.

[177] S. Lecker, R. Lill, T. Ziegelhoffer, C. Georgopoulos, P.J. Bassford, C.A. Kumamoto, W. Wickner, 3 pure chaperone proteins of *Escherichia coli*—SecB, trigger factor and Groel—form soluble complexes with precursor proteins invitro, EMBO J. 8 (1989) 2703–2709.

[178] N. Kusukawa, T. Yura, C. Ueguchi, Y. Akiyama, K. Ito, Effects of mutations in heat-shock genes groES and groEL on protein export in *Escherichia coli*, EMBO J. 8 (1989) 3517–3521.
[179] T. Watanabe, S. Hayashi, H.C. Wu, Synthesis and export of the outer membrane lipoprotein in *Escherichia coli* mutants defective in generalized protein export, J. Bacteriol. 170 (1988) 4001–4007.
[180] L. Baars, A.J. Ytterberg, D. Drew, S. Wagner, C. Thilo, K.J. Van Wijk, J.W. de Gier, Defining the role of the *Escherichia coli* chaperone SecB using comparative proteomics, J. Biol. Chem. 281 (2006) 10024–10034.
[181] T.K. Van Dyk, A.A. Gatenby, R.A. LaRossa, Demonstration by genetic suppression of interaction of GroE products with many proteins, Nature 342 (1989) 451–453.
[182] P.N. Danese, C.K. Murphy, T.J. Silhavy, Multicopy suppression of cold-sensitive sec mutations in *Escherichia coli*, J. Bacteriol. 177 (1995) 4969–4973.
[183] E.S. Bochkareva, M.E. Solovieva, A.S. Girshovich, Targeting of GroEL to SecA on the cytoplasmic membrane of *Escherichia coli*, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 478–483.
[184] L. Baars, S. Wagner, D. Wickstrom, M. Klepsch, A.J. Ytterberg, K.J. van Wijk, J.W. de Gier, Effects of SecE depletion on the inner and outer membrane proteomes of *Escherichia coli*, J. Bacteriol. 190 (2008) 3505–3525.
[185] G. Seydlova, P. Halada, R. Fiser, O. Toman, A. Ulrych, J. Svobodova, DnaK and GroEL chaperones are recruited to the *Bacillus subtilis* membrane after short-term ethanol stress, J. Appl. Microbiol. 112 (2012) 765–774.
[186] S. Sharma, K. Chakraborty, B.K. Muller, N. Astola, Y.C. Tang, D.C. Lamb, M. Hayer-Hartl, F.U. Hartl, Monitoring protein conformation along the pathway of chaperonin-assisted folding, Cell 133 (2008) 142–153.
[187] A.A. Laminet, T. Ziegelhoffer, C. Georgopoulos, A. Pluckthun, The *Escherichia coli* heat shock proteins GroEL and GroES modulate the folding of the beta-lactamase precursor, EMBO J. 9 (1990) 2315–2319.
[188] A. Rodrigue, N. Batia, M. Muller, O. Fayet, R. Bohm, M.A. Mandrand-Berthelot, L.F. Wu, Involvement of the GroE chaperonins in the nickel-dependent anaerobic biosynthesis of NiFe-hydrogenases of *Escherichia coli*, J. Bacteriol. 178 (1996) 4453–4460.
[189] K. Hatzixanthis, T. Palmer, F. Sargent, A subset of bacterial inner membrane proteins integrated by the twin-arginine translocase, Mol. Microbiol. 49 (2003) 1377–1390.
[190] D.G. Kang, C.S. Kim, J.H. Seo, I.G. Kim, S.S. Choi, J.H. Ha, S.W. Nam, G. Lim, H.J. Cha, Coexpression of molecular chaperone enhances activity and export of organophosphorus hydrolase in *Escherichia coli*, Biotechnol. Prog. 28 (2012) 925–930.
[191] G. Butland, J.M. Peregrin-Alvarez, J. Li, W. Yang, X. Yang, V. Canadien, A. Starostine, D. Richards, B. Beattie, N. Krogan, M. Davey, J. Parkinson, J. Greenblatt, A. Emili, Interaction network containing conserved and essential protein complexes in *Escherichia coli*, Nature 433 (2005) 531–537.
[192] A. Knaust, M.V. Weber, S. Hammerschmidt, S. Bergmann, M. Frosch, O. Kurzai, Cytosolic proteins contribute to surface plasminogen recruitment of *Neisseria meningitidis*, J. Bacteriol. 189 (2007) 3246–3255.
[193] C.K. Yang, H.E. Ewis, X. Zhang, C.D. Lu, H.J. Hu, Y. Pan, A.T. Abdelal, P.C. Tai, Nonclassical protein secretion by *Bacillus subtilis* in the stationary phase is not due to cell lysis, J. Bacteriol. 193 (2011) 5607–5615.
[194] B. Henderson, M.A. Fares, P.A. Lund, Chaperonin 60: a paradoxical, evolutionarily conserved protein family with multiple moonlighting functions, Biol. Rev. Camb. Philos. Soc. 88 (2013) 955–987.
[195] T.B. Hickey, H.J. Ziltener, D.P. Speert, R.W. Stokes, *Mycobacterium tuberculosis* employs Cpn60.2 as an adhesin that binds CD43 on the macrophage surface, Cell. Microbiol. 12 (2010) 1634–1647.
[196] N. Yoshida, K. Oeda, E. Watanabe, T. Mikami, Y. Fukita, K. Nishimura, K. Komai, K. Matsuda, Protein function. Chaperonin turned insect toxin, Nature 411 (2001) 44.
[197] M.C. Joshi, A. Sharma, S. Kant, A. Birah, G.P. Gupta, S.R. Khan, R. Bhatnagar, N. Banerjee, An insecticidal GroEL protein with chitin binding activity from *Xenorhabdus nematophila*, J. Biol. Chem. 283 (2008) 28287–28296.
[198] H. Nakano, Y. Yamada, H. Ishikura, H. Inokuchi, A mutation in the gene for trigger factor suppresses the defect in cell division in the divE42 mutant of *Escherichia coli* K12, Mol. Gen. Genet. 260 (1998) 75–80.
[199] S. Hansen, K. Lewis, M. Vulic, Role of global regulators and nucleotide metabolism in antibiotic tolerance in *Escherichia coli*, Antimicrob. Agents Chemother. 52 (2008) 2718–2726.
[200] J. Spence, A. Cegielska, C. Georgopoulos, Role of *Escherichia coli* heat shock proteins DnaK and HtpG (C62.5) in response to nutritional deprivation, J. Bacteriol. 172 (1990) 7157–7166.
[201] E. Guisbert, C. Herman, C.Z. Lu, C.A. Gross, A chaperone network controls the heat shock response in *E. coli*, Genes Dev. 18 (2004) 2812–2821.
[202] D. Ang, C. Georgopoulos, 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 (1989) 2748–2755.
[203] M.A. Fares, M.X. Ruiz-Gonzalez, A. Moya, S.F. Elena, E. Barrio, Endosymbiotic bacteria: groEL buffers against deleterious mutations, Nature 417 (2002) 398.
[204] M. Wada, H. Itikawa, Participation of *Escherichia coli* K-12 groE gene products in the synthesis of cellular DNA and RNA, J. Bacteriol. 157 (1984) 694–696.
[205] C.E. Donnelly, G.C. Walker, groE mutants of *Escherichia coli* are defective in umuDC-dependent UV mutagenesis, J. Bacteriol. 171 (1989) 6117–6125.
[206] Y. Yamaguchi, T. Tomoyasu, A. Takaya, M. Morioka, T. Yamamoto, Effects of disruption of heat shock genes on susceptibility of *Escherichia coli* to fluoroquinolones, BMC Microbiol. 3 (2003) 16.
[207] J. Zeilstra-Ryalls, O. Fayet, L. Baird, C. Georgopoulos, Sequence analysis and phenotypic characterization of groEL mutations that block lambda and T4 bacteriophage growth, J. Bacteriol. 175 (1993) 1134–1143.
[208] B.P. Burnett, A.L. Horwich, K.B. Low, A carboxy-terminal deletion impairs the assembly of GroEL and confers a pleiotropic phenotype in *Escherichia coli* K-12, J. Bacteriol. 176 (1994) 6980–6985.
[209] L. Goltermann, L. Good, T. Bentin, Chaperonins fight aminoglycoside-induced protein misfolding and promote short-term tolerance in *Escherichia coli*, J. Biol. Chem. 288 (2013) 10483–10489.
[210] B. Rudolph, K.M. Gebendorfer, J. Buchner, J. Winter, Evolution of *Escherichia coli* for growth at high temperatures, J. Biol. Chem. 285 (2010) 19029–19034.