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Review

Folding mechanisms of periplasmic proteins ☆

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ABSTRACT

More than one fifth of the proteins encoded by the genome of *Escherichia coli* are destined to the bacterial cell envelope. Over the past 20 years, the mechanisms by which envelope proteins reach their three-dimensional structure have been intensively studied, leading to the discovery of an intricate network of periplasmic folding helpers whose members have distinct but complementary roles. For instance, the correct assembly of ß-barrel proteins containing disulfide bonds depends both on chaperones like SurA and Skp for transport across the periplasm and on protein folding catalysts like DsbA and DsbC for disulfide bond formation. In this review, we provide an overview of the current knowledge about the complex network of protein folding helpers present in the periplasm of *E. coli* and highlight the questions that remain unsolved. This article is part of a Special Issue entitled: Protein trafficking and secretion in bacteria. Guest Editors: Anastassios Economou and Ross Dalbey.

1. Introduction

Unraveling the mechanisms by which proteins fold into their correct three-dimensional structure is a fundamental but complex question in basic biology. Although all the information necessary for a protein to attain its native structure is contained in its amino acid sequence, efficient protein folding *in vivo* requires the participation of various factors, including molecular chaperones, folding catalysts and proteases.

In Gram-negative bacteria such as *Escherichia coli*, a relatively well understood quality control machinery is present in the cytoplasm to ensure the proper folding of newly-synthesized polypeptide chains as they emerge from the ribosome. Indeed, successful folding of nascent proteins is essential for bacterial viability. However, although protein synthesis takes place in the cytoplasm, more than 20% of the proteins encoded by the *E. coli* genome are destined to the bacterial cell envelope. In this article, we will review the mechanisms of protein folding in this extracytoplasmic compartment.

The envelope of Gram-negative bacteria is composed of two membranes: the inner membrane (IM), which is in direct contact with the cytoplasm, and the outer membrane (OM), which constitutes the interface between the cell and the external environment [1] (Fig. 1). The IM and the OM have different structures and composition [1]. The IM is a classical phospholipid bilayer and IM proteins often are integral proteins crossing the membrane with one or more hydrophobic α-helices. A few lipoproteins are also anchored to the outer leaflet of the IM via a lipid moiety [2] (Fig. 1). Unlike the IM, the OM is an asymmetric bilayer composed of phospholipids and lipopolysaccharides (LPS) in the inner and outer leaflet, respectively [3]. OM proteins can be of two types: lipoproteins, most of which are anchored by a lipid moiety in the inner leaflet of the OM and face the periplasm [4], and integral membrane proteins, known as OMPs. These latter generally adopt a β-barrel conformation and serve as channels or «porins» that enable free diffusion of ions and hydrophilic molecules across the membrane [5,6] (Fig. 1).

The IM and the OM are separated by the periplasm, a viscous and oxidizing compartment that contains a thin layer of peptidoglycan and represents 10 to 20% of the total cell volume [7]. More than 300 proteins are present in the periplasm [8] where they perform a large variety of physiological functions, such as protein folding, uptake and transport of nutrients and detoxification of harmful substances.

Secreted proteins are synthesized in the cytoplasm as pre-proteins that are translocated across the IM by different secretory machineries, depending on the signal sequence they carry. The majority of secreted proteins carry a signal sequence recognized by the Sec apparatus [9–11], which transports them through the IM in an unfolded conformation. Although some proteins are secreted co-translationally by the Sec machinery, most are targeted post-translationally to the envelope [12,13]. In this latter case, pre-proteins first bind to the chaperone SecB whose role is to maintain them in their fully unfolded state until they reach the translocase [12,13]. In the co-translational targeting mechanism, the signal sequence of the protein is recognized by the signal recognition particle (SRP) while it emerges from the ribosome and the entire SRP–ribosome–nascent protein complex then binds to the Sec translocase [12]. A small subset of approximately 30 proteins

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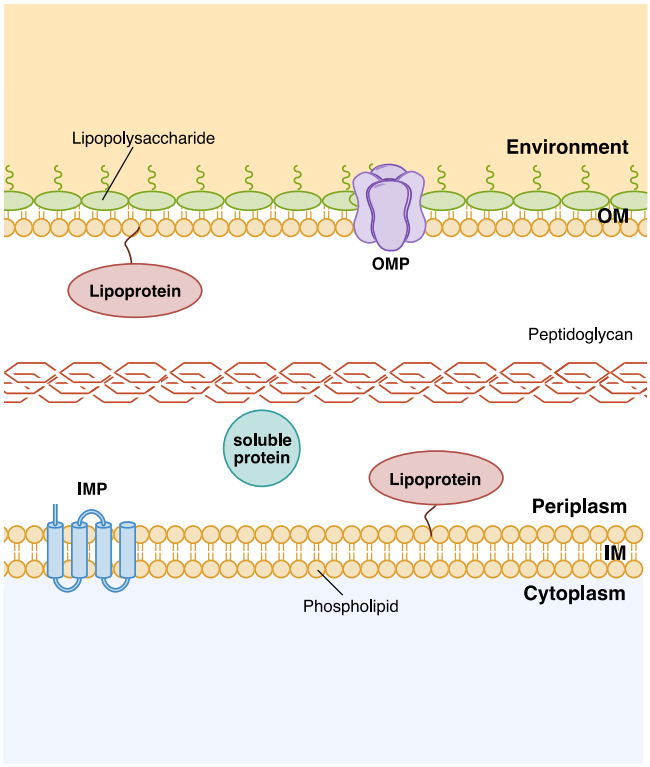

Fig. 1. General structure of the *E. coli* envelope. The envelope of *E. coli* is composed of the inner membrane (IM), the periplasm and the outer membrane (OM). The IM is a symmetric bilayer containing phospholipids and integral membrane proteins (IMP) with α-helical transmembrane domains. The OM is an asymmetric lipid bilayer with phospholipids in the inner leaflet and lipopolysaccharides in the outer leaflet. The OM also contains integral proteins (OMP), known as β-barrels. Both membranes comprise lipoproteins, anchored in the membrane by a lipid moiety and facing the periplasm. The periplasm is a viscous compartment comprised between the two membranes and which contains a thin layer of peptidoglycan.

is transported across the IM by the Twin Arginine transport (Tat) pathway. In this case, the signal peptide presents a characteristic twin-arginine motif [14]. In contrast to the Sec machinery, Tat-translocation substrates fold in the cytoplasm before crossing the membrane and are consequently functional directly after translocation [15]. Noteworthy, Gram-negative bacteria have evolved additional machineries to mediate protein secretion, such as the systems used by pathogenic bacteria to infect host cells. We refer the reader to the chapters of this special issue on protein translocation for more details on these systems.

In this chapter, we will focus on the fate of the polypeptides as they exit the Sec translocon and enter the periplasm, using *E. coli* as a model. The unraveling of the protein quality control mechanisms of the *E. coli* periplasm started in the early 90s with the discovery of catalysts of disulfide bond formation and of peptidyl-prolyl *cis-trans* isomerization [16–19]. The next step was the identification of periplasmic chaperones as a result of the independent work of several groups. Indeed, in 1996, Missiakas *et al.* [20] identified SurA, FkpA and Skp as potential periplasmic chaperones by searching for periplasmic factors decreasing the stress response induced by the accumulation of misfolded proteins in the periplasm [20]. That same year, SurA and Skp were shown to be involved in the folding of OMPs, confirming their identification as periplasmic chaperones [21,22]. Within the next four years, LolA was identified as a general chaperone for most lipoproteins [23] while DegP, which had been known as a protease for a long time, was identified as a protein whose chaperone activity dominates at low temperatures [24]. Other periplasmic chaperones have been identified more recently, including HdeA, a protein that plays an important role in acid survival [25,26], and Spy [27]. We will summarize here these 20 years of multidisciplinary research that led to the discovery of an intricate protein network controlling the folding and integrity of envelope proteins.

2. Periplasmic molecular chaperones

Proper folding of periplasmic proteins requires the assistance of molecular chaperones that are thought to differentiate properly folded proteins from their non-native conformations by recognizing the surface-exposed hydrophobic areas displayed by these latter. A remarkable feature of periplasmic chaperones is that, in contrast to their cytoplasmic counterparts, they assist protein folding without the need of ATP for their activity. So far, periplasmic chaperones have been shown to be involved in two major processes, the maturation of proteins located in the OM (OMPs and lipoproteins) and the protection of periplasmic proteins under stress conditions. In the following section, we will first describe the chaperones involved in OMPs assembly. Then, we will focus on LolA, a chaperone dedicated to lipoprotein transport before describing the stress-induced chaperones.

2.1. Chaperones involved in the biogenesis of OMPs

Newly synthesized OMPs that cross the IM through the Sec machinery need to be escorted by chaperones as they travel through the periplasm to reach the OM. Indeed, since these proteins penetrate the periplasm in an unfolded conformation, they are prone to aggregation in this aqueous compartment. Two parallel folding pathways, which prevent OMPs aggregation during their periplasmic transit, have been described in *E. coli*. The major chaperone pathway involves SurA, while the secondary pathway consists of two proteins, Skp and DegP.

2.1.1. SurA

Originally isolated as a protein essential for survival in stationary phase [28], SurA was later described both as a chaperone that assists the folding of OMPs [20,21,29] and as a peptidyl-prolyl *cis-trans* isomerase (PPIase) [30]. Enzymes with PPIase activity catalyze the *cis-trans* isomerization of peptide bonds involving a proline residue (see Section 3.1).

SurA is composed of four distinct regions: a large N-terminal domain, two PPIase domains of the parvulin family and a short C-terminal helix (Fig. 2). The structure of SurA reveals that the N- and C-terminal domains

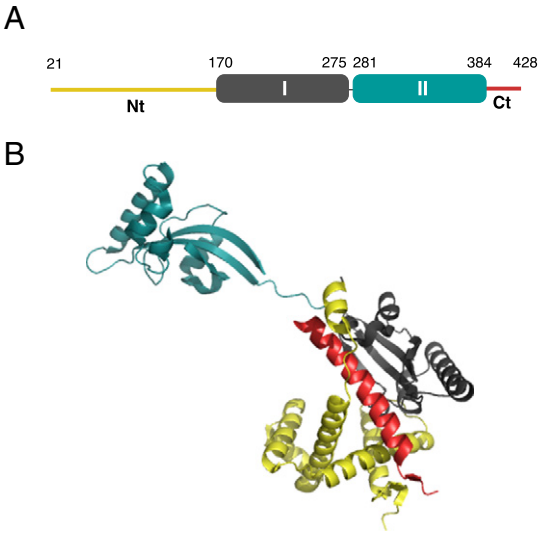

Fig. 2. Structure of *E. coli* SurA. (A) Schematic diagram of the domains of the mature SurA (no signal sequence). The numbers refer to amino acid position. (B) Ribbon representation of SurA (PDB entry code 1M5Y) [31]. The N-terminal domain (yellow) is followed by the two PPIase domains belonging to the parvulin family. The first PPIase domain (grey) has no PPIase activity, whereas the second one (turquoise blue) is active. The C-terminal tail is shown in red. Since polypeptide linkers between domains were poorly ordered, some could not be traced in the structure.

as well as the first PPIase domain are interlaced to form a core structural module with an extended crevice that carries the chaperone activity. The second parvulin domain appears as a satellite module bound to the larger core unit of SurA by two extended polypeptide linkers [31,32] (Fig. 2).

The first evidence of the participation of SurA in OMPs maturation came from the characterization of *surA* mutants. It has indeed been shown by independent studies that *E. coli* cells lacking SurA cannot correctly fold some major porins such as LamB, OmpA, OmpC and OmpF [21,29,33]. Lower amounts of these porins in the OM, reflected by the decreased OM density of *surA* mutants, lead to a highly defective cell envelope [33] and to hypersensitivity to SDS-EDTA, hydrophobic antibiotics and bile salts [21,29].

To gain more insight into the function of SurA, we used a differential proteomic approach to compare the OM proteome of a *surA* mutant to that of a wild type [34]. This method enabled us to identify the subset of OMPs that depend, at least partially, on SurA for proper folding. These OMPs include FadL, FecA, FhuA, LptD, OmpX and the major porins mentioned above, LamB, OmpA and OmpF. However, for most of those proteins, the lower abundance in the *surA* strain could be explained by a decreased synthesis of their gene due to the activation of the σ^E stress response [35]. σ^E is a sigma factor that is activated upon accumulation of misfolded proteins and of intermediates in LPS assembly and transport [36,37]. Interestingly, of all the OMPs listed above, FhuA and LptD were the only proteins for which the lower quantity observed in the *surA* mutant did not correlate with decreased mRNA levels, indicating that those proteins are true SurA substrates. LptD is an essential β-barrel, which associates with the OM lipoprotein LptE to form a complex mediating the insertion of LPS into the outer leaflet of the OM [38]. Low expression of LptD leads to a phenotype reminiscent of that of a *surA* mutant, suggesting that most of the OM perturbations observed in a *surA* strain can be explained by the reduced LptD levels observed in that strain [32,34].

SurA was shown to have a PPIase activity [39], which exclusively resides in the second PPIase domain [40,41]. However, inactivation or deletion of this second PPIase domain has no effect on the *in vivo* function of SurA [29,40]. Furthermore, a SurA variant composed of the N-terminal part directly linked to the C-terminal tail displays chaperone-like activity *in vitro* and almost fully complements the *in vivo* defects of *surA* mutants. These results suggest that the PPIase activity is either not essential for SurA chaperone activity or is complemented by other peptidyl-prolyl *cis-trans* isomerases [40].

SurA preferentially interacts *in vitro* with unfolded porins over other similarly sized proteins [40], further supporting that SurA is involved in the biogenesis of OMPs. In particular, it has been shown that SurA has strong preference for peptides enriched in aromatic residues arranged in specific patterns such as Ar–X–Ar (where Ar is an aromatic residue and X can be any residue) [42], a motif frequently found at the C-terminal part of OMPs. The structure of SurA bound to an artificial peptide mimicking the C-terminus of OMPs was crystallized, which brought new insights into the molecular mechanism of SurA and revealed that the specific peptide binding activity of SurA is located in the first PPIase domain [43]. Since it was shown previously that the first PPIase domain of SurA is dispensable for its *in vivo* function, Xu et al. proposed that this domain is important for the substrate specificity of SurA and that the construct lacking the two PPIase domains could partially complement a *surA* mutant by binding non-specifically to all OMPs [43].

From the work summarized above, it is now clear that SurA acts preferentially as a chaperone involved in the maturation and assembly of OMPs. No evidence indicating that SurA plays a role in the folding of soluble periplasmic proteins has been obtained so far.

2.1.2. Skp

Skp (Seventeen Kilodalton Protein) was originally described as a histone-like protein that binds DNA [44]. The identification of Skp as a molecular chaperone involved in OMPs biogenesis was first suggested by the fact that Skp selectively binds unfolded OmpF covalently linked to sepharose beads [22].

The three-dimensional structure of Skp reveals a trimeric arrangement with "jellyfish" architecture where an inner cavity representing the substrate-binding site is defined by α-helical tentacles diverging from a β-barrel core domain [45] (Fig. 3). Some substrates of Skp, such as the ß-barrel protein OmpA, seem to surpass the capacity of the relatively small substrate-binding cavity. However, NMR experiments on the Skp–OmpA complex have provided an explanation to this apparent discrepancy by showing that only the β-barrel domain of OmpA is found in an unfolded state within the Skp cavity, while the soluble periplasmic part of OmpA remains well-folded outside of the cavity [46].

The overall shape of Skp was surprisingly analogous to that of prefoldin, a eukaryotic cytosolic ATP-independent chaperone from the holdase family (Fig. 3). Holdases do not play an active role in the folding of proteins but rather prevent protein aggregation under stress conditions. The structural similarity between Skp and prefoldin suggests that Skp is also part of the holdase chaperone family [45] (Fig. 3).

The substrate profile of Skp was determined recently using a strep-tag affinity system combined with a proteomic approach. More than 30 envelope proteins were found to interact with Skp [47], suggesting that they are potential Skp substrates. Although the majority of these proteins are β-barrel proteins, a few, like MalE and OppA, are periplasmic proteins. Moreover, the overexpression of Skp was shown to improve the periplasmic expression of recombinant proteins such as scFv antibody fragments as well as phage display [48]. Thus, it appears that Skp can bind a broad range of substrates, including soluble proteins. However, the involvement of Skp in the folding of periplasmic soluble proteins remains to be demonstrated.

Upon deletion of the *skp* gene, a moderate diminution of some OMPs such as LamB, OmpA, OmpC and OmpF was observed in the OM of *E. coli* [22]. However, in a recent differential proteomic study, we showed that the decrease in OMPs levels is only minor and that no OMP appears to preferentially depend on Skp for folding in *E. coli* [49]. Numerous experiments have also been carried out using OmpA as a model substrate. For instance, Skp was shown to increase the folding rate of OmpA *in vitro* and to improve the yield of OmpA insertion into phospholipid membranes [50,51]. Moreover, it was reported that Skp, which interacts with phospholipid monolayers *in vitro* [52], facilitates the secretion of newly synthesized OmpA from spheroplasts [53].

Altogether, these studies confirm the role of Skp as a chaperone involved in OMPs assembly. They also led to the hypothesis that Skp assists the early steps of ß-barrel folding [54,55].

2.1.3. DegP

DegP is a key periplasmic protease. It was discovered as the first member of the serine proteases HtrA family [56], whose representatives have now been identified in many eukaryotic and prokaryotic genomes. DegP is essential for *E. coli* survival at elevated temperatures [57] and is up-regulated both by the σ^E and the Cpx systems in response to heat shock or to other envelope stresses that result in protein misfolding [58,59]. Cpx is a stress response system that controls cell envelope damages via proteases and folding helpers activation [60].

DegP seems to have the uncommon feature to also function as a chaperone. The switch between the chaperone and the protease activity appears to be controlled by the temperature: below 28 °C, DegP mainly functions as a chaperone, whereas the protease activity dominates as the temperature increases [24].

The mature DegP protein is composed of a N-terminal chymotrypsin-like protease domain containing the His-Asp-Ser catalytic site and of two C-terminal PDZ domains (PDZ1 and PDZ2) [61] (Fig. 4). Crystallographic studies have shown that a flexible loop connects the protease domain to PDZ1, another one joining PDZ1 to PDZ2 [62]. PDZ domains are found in a variety of proteins where they are usually involved in protein–protein interactions.

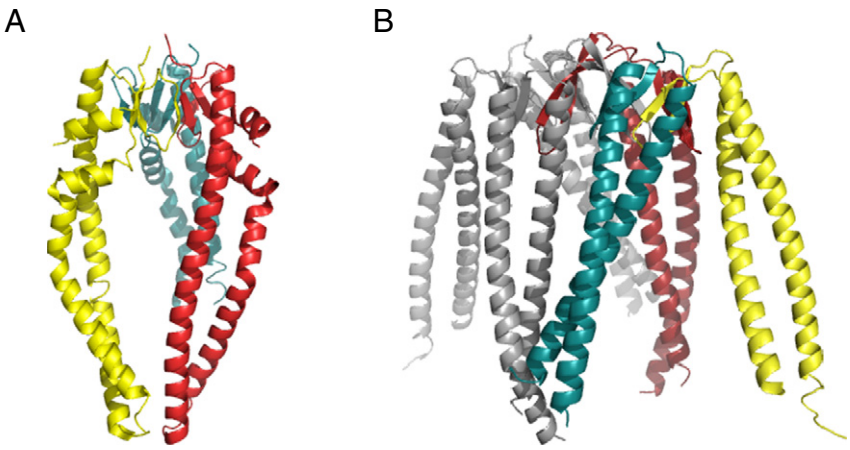

Fig. 3. Structural similarities between the *E. coli* periplasmic chaperone Skp and prefoldin. (A) Crystal structure of the trimeric chaperone Skp from *E. coli* is shown as a ribbon diagram (PDB entry code 1SG2) [141]. The trimerization core is located in the N-terminal part. Each monomer is colored in red, yellow or turquoise blue. (B) Ribbon diagram representing the side view of the prefoldin hexamer with three subunits colored in red, yellow and turquoise blue (PDB entry code 1FXK) [142]. Grey subunits were not defined in the experimental density and were modeled using the PDBePISA software.

In the absence of substrate, DegP forms a homohexamer that consists of two trimeric rings [63] (Fig. 4). This hexamer corresponds to the resting, proteolytically inactive state of DegP, in which the conformation of the active site is structurally disordered. Interestingly, DegP is activated by an allosteric mechanism that involves the binding of the misfolded substrate to the PDZ domains and to the active site, transforming DegP from an inactive oligomer containing two trimers into an active polyhedral cage, typically composed of four or eight trimers [64,65] (Fig. 4). These higher-order 12-meric and 24-meric forms are catalytically active and encapsulate substrates into large central cavities (Fig. 4). It has been recently shown that although the cage assembly and the proteolytic activation are both triggered by substrate binding, these two processes can be uncoupled: DegP cage formation is indeed not required for high temperature survival and proteolysis [66].

As a protease, DegP was shown to degrade a large range of unfolded or misfolded substrates such as PhoA [67], PapA [68], MalS [69] and several OMPs [33,65]. PDZ1, in contrast to PDZ2, is essential for the protease activity of DegP [24,70]. PDZ1 recognizes DegP substrates by binding C-terminal hydrophobic exposed residues before presenting them to the protease domain [71]. Moreover, it was also demonstrated that DegP cleaves its substrates preferentially after Ile, Val, Met, Leu, Thr, Ser and Ala residues by employing a hold-and-cut mechanism [71]: peptide fragments that are released from the proteolytic sites can bind PDZ1 via their newly generated C-termini to undergo another cleavage cycle.

As a chaperone, DegP stimulates folding of the periplasmic α-amylase MalS at low temperatures while at high temperatures, misfolded MalS is degraded by DegP [24]. It was also demonstrated that DegP promotes the *in vitro* refolding both of MalS and of the artificial substrate citrate synthase [69]. Moreover, DegP prevents aggregation of heat-denatured citrate synthase and lysozyme by acting as a holdase chaperone [69]. Furthermore, recent structural data indicate that DegP can accommodate folded monomers of OMPs within its large cavity where they are protected from aggregation and from proteolytic degradation by other proteases during their transport across the periplasm [65].

2.1.4. FkpA

FkpA was originally described in 1995 as a new member of the FKBP family of PPIases (see Section 3.1) due to its C-terminal domain showing 83% sequence identity with the consensus FK506-binding motif [72]. The macrolide FK506 is an immunosuppressant drug that mimics the transition state in the *cis-trans* isomerization reaction and therefore inhibits proteins presenting that motif [73].

FkpA is a V-shaped homodimer composed of two subunits of 245 residues (Fig. 5). Each monomer can be divided into two domains, a N-terminal domain made up of three helices and a C-terminal domain responsible for the PPIase activity. The helices of the N-terminal domain are interwoven with those of the other subunit enabling the dimerization of the protein [73,74] (Fig. 5).

The activity of FkpA as a periplasmic chaperone was first demonstrated using heterologous proteins expressed in *E. coli* and folding-defective mutants of *E. coli* periplasmic proteins. For instance, several studies have reported that the production of soluble and functional single-chain Fv (scFv) antibody fragments in the *E. coli* periplasm was improved by co-expression of FkpA [75,76]. Interestingly, similar refolding rate for scFv fragments devoid of *cis*-proline residues were observed, suggesting that the chaperone function of FkpA is independent of its PPIase activity [75,77]. The independence of the PPIase and chaperone activities was confirmed by other experiments. First, expression of FkpA variants with active-site mutation in the FKBP domain prevents the formation of inclusion bodies from a folding-defective maltose-binding protein (MalE31) [78]. Second, a mutant protein corresponding to the N-terminal part of FkpA where the chaperone activity resides is able to suppress the aggregation of the misfolded mutant MalE31, both *in vitro* and *in vivo* [73]. Third, structural and functional data indicate that the C-terminal domain of FkpA displays similar PPIase activity to that of the wild-type protein and is devoid of chaperone activity [77,78].

Recently, FkpA has been shown to play a role, together with Skp, in the folding of LptD and FhuA, two OMPs that strongly depend on SurA for folding [79]. Accordingly, a *skp fkpA* double mutant exhibits OM defects due to the decrease in LptD levels. Noteworthy, Skp and FkpA are unable to complement the defect in LptD and FhuA assembly observed in the *surA* mutant, suggesting that they perform distinct roles in this process [79].

2.1.5. Model of the periplasmic chaperone network

The molecular chaperones that have been described above seem to function in two parallel, partially redundant pathways (Fig. 6) [33,49]. According to a favorite model, the major pathway involves SurA, which transports the bulk mass of OMPs across the periplasm while Skp and DegP function together in a second pathway, which rescues the OMPs that fall off the SurA pathway. Importantly, *E. coli* needs one of the chaperone pathways for viability as *skp surA* and *degP surA* double-knockout strains have a synthetic lethal phenotype [80]. Although the function of FkpA in the periplasmic chaperone network

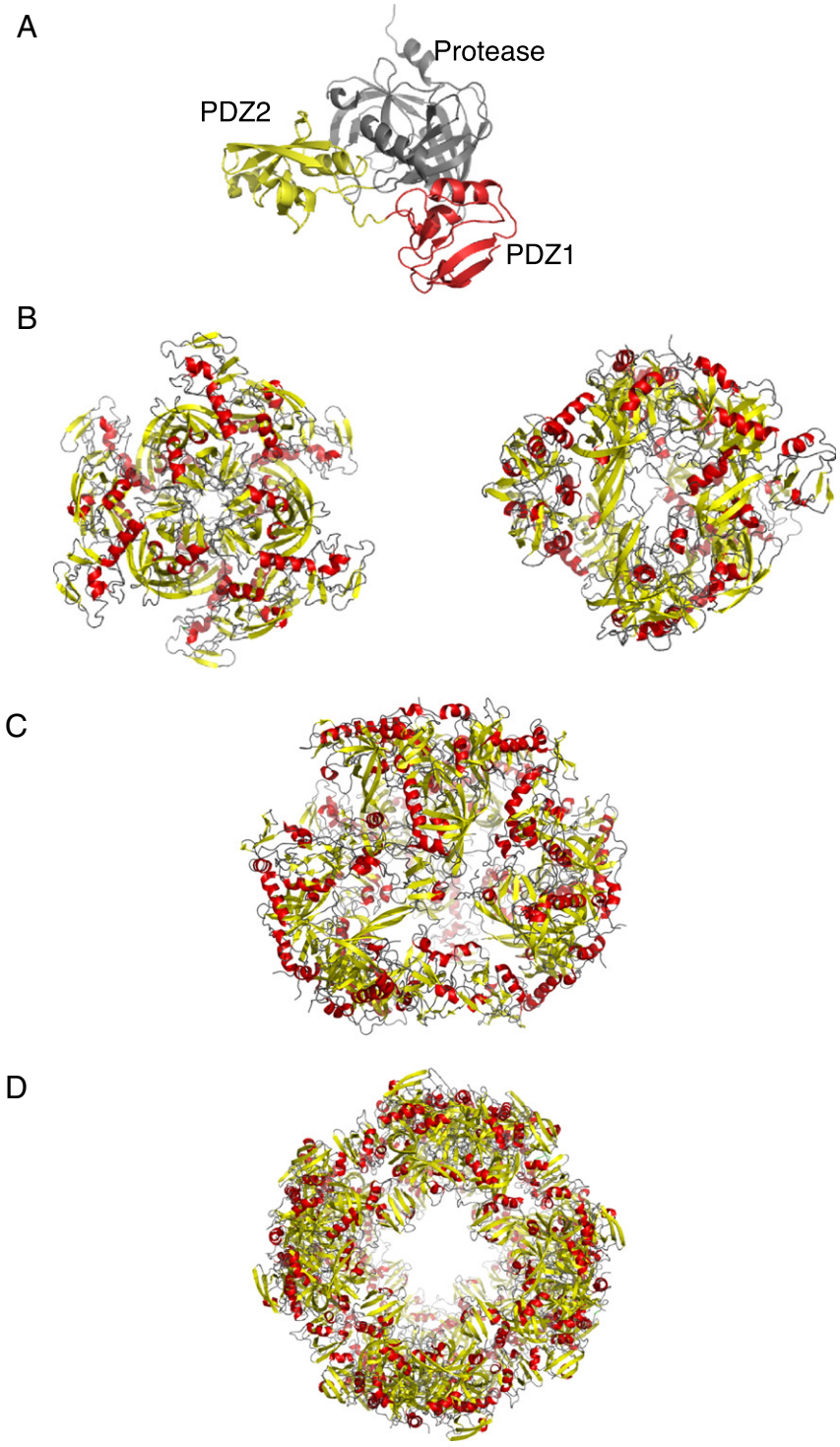

Fig. 4. Architecture of the *E. coli* DegP protease/chaperone. (A) DegP monomer (PDB entry code 1KY9) [63], drawn as a ribbon diagram, consists of a protease domain (grey) and two PDZ domains (PDZ1 in red and PDZ2 in yellow). (B) Top (left) and side (right) views of the resting state hexamer of DegP (PDB entry code 1KY9). (C) Ribbon representation of the active 12-mer form of DegP (PDB entry code 2ZLE) [65]. (D) Ribbon representation of the 24-mer active form of DegP (PDB entry code 2ZLE) [65].

is still unclear, this protein seems to assist Skp in the folding of certain ß-barrel proteins with common structural features [79].

2.2. The assembly machinery of outer membrane proteins into the OM

After crossing the periplasm, proteins that span the OM are assembled locally by the β-barrel assembly machinery (BAM complex) after being released by the periplasmic chaperones (Fig. 6). The BAM machinery facilitates the insertion and assembly of β-barrels into the OM [81,82]. The central element of this machinery is BamA, an essential integral β-barrel protein with the N-terminal extension of five periplasmic POTRA (polypeptide translocation associated) domains. These domains are important for the interaction of BamA with the four BAM lipoproteins facing the periplasm (BamB, BamC, BamD and BamE). However, only BamB and BamD interact directly with BamA, while BamC and BamE form a subcomplex with BamD. Although only BamA and BamD are essential for bacterial survival, the absence of any member of the complex triggers defects in OMP assembly [83].

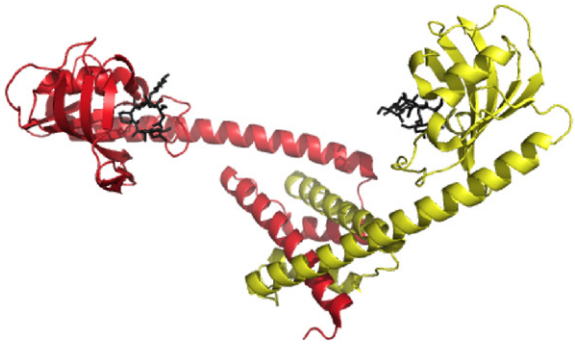

Fig. 5. The crystal structure of *E. coli* FkpA. The three-dimensional structure of FkpA (PDB entry code IQ61) [143] in complex with FK506 is shown as a ribbon diagram. The PPIase FkpA forms a V-shaped dimer with a N-terminal dimerization domain and a C-terminal PPIase domain belonging to the FKBP family. These two domains are linked *via* an α-helix. The bound FK506 (grey) is shown in stick representation and the monomers are shown in yellow and red.

The whole complex has been reconstituted *in vitro*, using the ß-barrel protein OmpT as a model substrate. The *in vitro* reaction was shown to require SurA and all five BAM proteins to proceed efficiently without any energy source [84,85], indicating that the five BAM proteins coupled with the chaperone SurA form the smallest machinery required for OMP insertion [84]. Important to note, the interaction between SurA and the BAM complex was only demonstrated *in vivo* using chemical crosslinkers, indicating a transient association between them [33,86]. The results of additional *in vitro* studies aimed at analyzing the role of the individual components of the BAM complex have been reported recently, using BamA as a substrate. These studies revealed that the lipoproteins BamB and BamD bind to unfolded substrates and facilitate the *in vitro* assembly of BamA in the OM [87].

Although the process of OMPs assembly has been intensively studied, the BAM complex has been discovered quite recently and many questions remain to be addressed regarding its molecular mechanism. We refer the reader to excellent reviews about the BAM machinery for more details [85,88,89] or to the chapter of this special issue describing the BAM complex.

2.3. LolA, a chaperone involved in the transport of lipoproteins

The LolA chaperone is a soluble protein dedicated to assist the transport of OM lipoproteins across the periplasm.

At least 90 lipoproteins are expressed in *E. coli*, most of them being anchored on the periplasmic side of the OM via a N-terminal N-acyl-diacylglycerylcysteine [4,90]. They are synthesized in the cytoplasm as precursors with a signal sequence containing a consensus sequence, Leu-(Ala/Ser)-(Gly/Ala)-Cys, called the lipobox [91]. Lipoproteins are then translocated to the periplasm. Once they reach the periplasm, they are first modified by the addition of diacylglycerol to the cysteine of the lipobox, followed by the cleavage of the signal sequence and the acetylation of the NH_2-terminal group of the cysteine residue [91].

If the mature lipoproteins contain an Asp residue adjacent to the acylated cysteine, they will remain in the IM whereas lipoproteins lacking this Asp residue will be transferred to the OM by the Lol system [4]. OM lipoproteins are first extracted from the IM by the action of LolCDE, an ATP-binding cassette (ABC) transporter that releases lipoproteins from the membrane and transfers them to the periplasmic chaperone

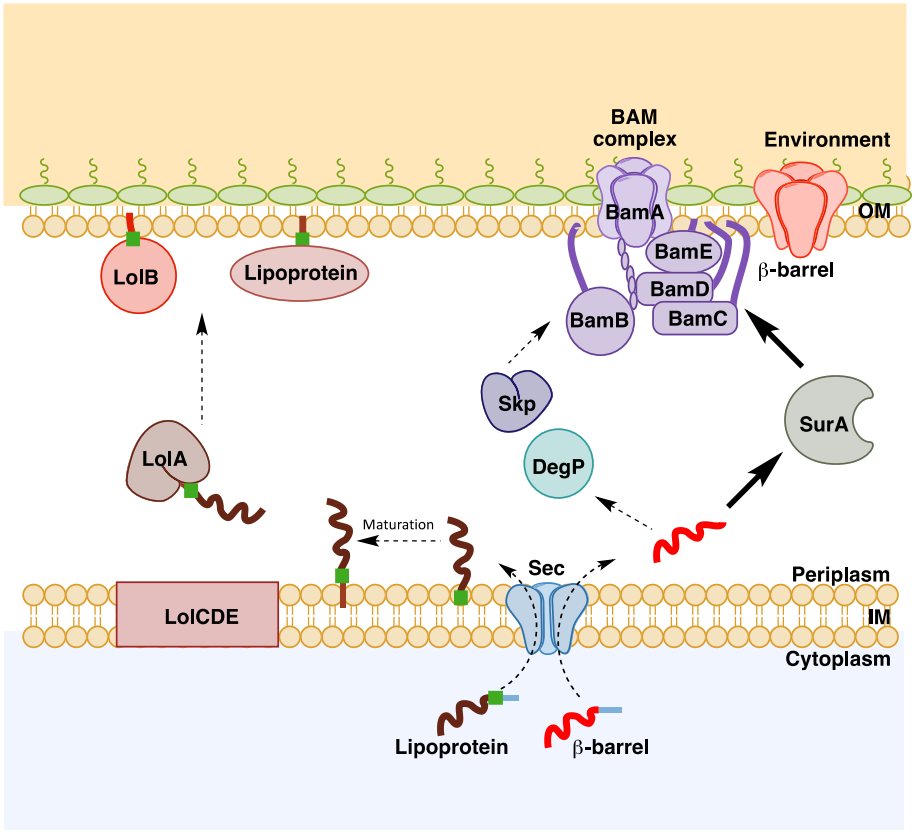

Fig. 6. A model for the periplasmic chaperone network. Unfolded proteins are transported across the inner membrane (IM) by the Sec-machinery. After cleavage of the signal sequence, proteins are assisted by chaperones before they reach their final destination. β-barrel proteins are escorted to the BAM complex in the OM. The BAM complex is composed of the essential β-barrel protein BamA and of four additional lipoproteins BamB, BamC, BamD, BamE. Four chaperones have been shown to interact with β-barrels in the periplasm. SurA functions in the major folding pathway, while Skp and DegP serve in a back-up folding pathway. FkpA assists Skp in the assembly of certain ß-barrel proteins such as LptD (not shown). OM lipoproteins precursors are first matured in the IM before being extracted from the IM with the help of the LolCDE complex, which transfers them to LolA. LolA binds lipoproteins via the acyl chain, covalently attached to the lipobox (green square) and transports them across the periplasm to the OM lipoprotein LolB.

LolA in an ATP-dependent manner [92,93] (Fig. 6). LolA binds to the acyl chain of the lipoproteins, protecting this hydrophobic moiety from the hydrophilic environment of the periplasm. Then, the soluble complexes formed between LolA and the lipoproteins cross the periplasm to reach the OM where LolA transfers its protein substrates to LolB, an OM receptor that inserts lipoproteins into the membrane [93] (Fig. 6). Important to note, the function of LolA seems to be restricted to the transport of the lipoprotein across the periplasm and not to the folding of the soluble domain of the lipoprotein. How this latter gets folded in the periplasm remains unclear.

The structures of LolA and LolB show a high similarity despite a weak sequence homology [94] (Fig. 7). They both comprise a novel fold consisting of eleven antiparallel β-strands and of three α-helices assembled in an incomplete β-barrel covered by a lid. Interestingly, the hydrophobic cavity of LolA was recently found to undergo opening and closing upon the binding and release of lipoprotein substrates, respectively [95–97].

Despite their high similarity, the superimposition of the structures of LolA and LolB revealed significant differences between these two proteins. First, LolA possesses an additional C-terminal loop consisting of a short α-helix and a twelfth β-strand (Fig. 7). This region is important as reflected by the fact that deletion of the twelfth β-strand or of the short α-helix prevents the release of the substrate from the chaperone [98]. Second, the properties of their hydrophobic cavity are different: the cavity of LolB is principally made of leucine and isoleucine residues, while the hydrophobic cavity of LolA mainly contains aromatic residues. Moreover, the LolA cavity is closed by hydrogen bonds between Arg43 (in the β-barrel) and some residues in the lid [94]. It was demonstrated that this arginine residue plays a crucial role in the efficient transfer of lipoproteins from LolA to LolB by reducing the strength of the hydrophobic interaction between LolA and lipoproteins [99,100].

Interestingly, researchers recently identified MAC13243, a molecule belonging to a new chemical class, as a specific inhibitor of LolA [101]. This compound represents thus a novel chemical probe that may lead to the development of new drugs to overcome antibiotic resistance in Gram-negative bacteria.

2.4. Stress-induced chaperones

In addition to the previously described chaperones for which a role in the biogenesis of OMPs and of OM lipoproteins has been demonstrated, three periplasmic chaperones that seem to specifically function under stress conditions were recently discovered in *E. coli*.

2.4.1. HdeA

To survive in very acidic environments, such as the mammalian stomach where the pH varies between 1 and 3, bacteria have evolved many strategies that help them tolerate acidic conditions. The bacterial periplasm, because of its immediate proximity to the external environment and the selective permeability of the OM, is more vulnerable to acid stress than the cytoplasm.

Recently, HdeA, a periplasmic protein of about 9 kDa, was identified as a key factor supporting acid resistance in the *E. coli* periplasm [25,26,102]. The identification of HdeA as a chaperone came from the observation that it promotes the resolubilization and refolding of acid-denatured substrates and suppresses the aggregation of several periplasmic proteins at low pH [103,104]. HdeA probably acts in concert with a second protein, HdeB, transcribed from the same operon [105]. The expression of both proteins is induced in response to low pH [106]. In addition, a *hdeA hdeB* deletion strain of *E. coli* is highly acid sensitive, while complementation of this double mutant by either HdeA or HdeB was reported to partly restore the acid resistance phenotype [26].

The three-dimensional structures of HdeA and HdeB have been solved at neutral pH and reveal in both cases a homodimeric conformation [102,107,108] (Fig. 8). Whereas HdeA and HdeB share only about 17% sequence identity, the structures of their subunits, which consist of a compact single-domain protein with a hydrophobic core of four α-helices, display high similarity (Fig. 8). Nevertheless, they show distinct hydrophobic interfaces that may accommodate different substrates. It was demonstrated that HdeA can interact with a broad range of periplasmic proteins such as chaperones (SurA, DegP, FkpA, PpiD), lipoproteins, proteases, transport proteins and others [109].

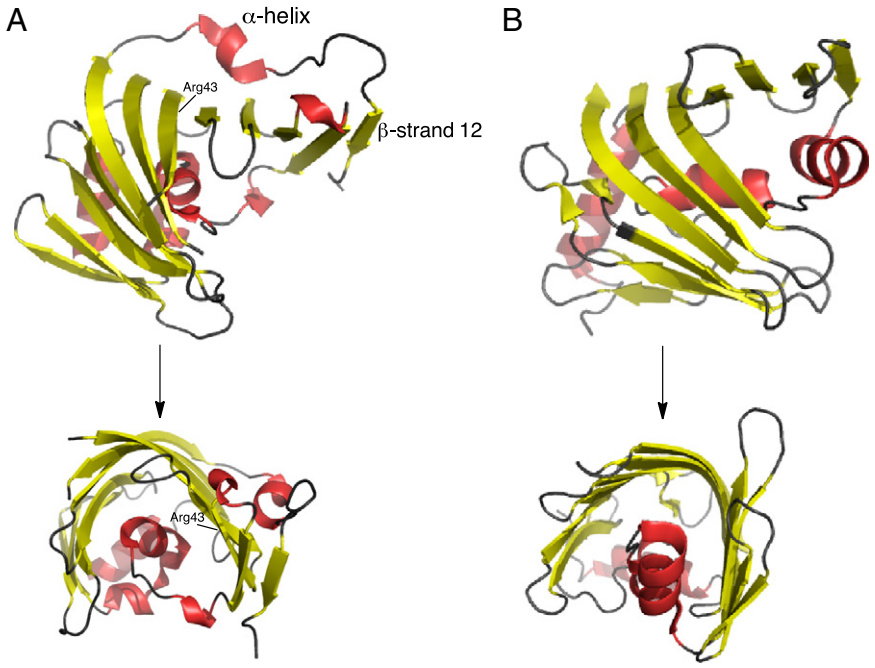

Fig. 7. The three-dimensional structures of *E. coli* LolA and LolB. Despite their low amino acid sequence identity, the structure of (A) LolA (PDB entry code 1IWL) [94] and (B) LolB (PDB entry code 1IWM) [94] are similar. Their hydrophobic cavity consists of an unclosed β-barrel and an α-helical lid. However, LolA contains an additional C-terminal loop composed of a short α-helix and a twelfth β-strand, which are important for the correct delivery of lipoproteins to the OM.

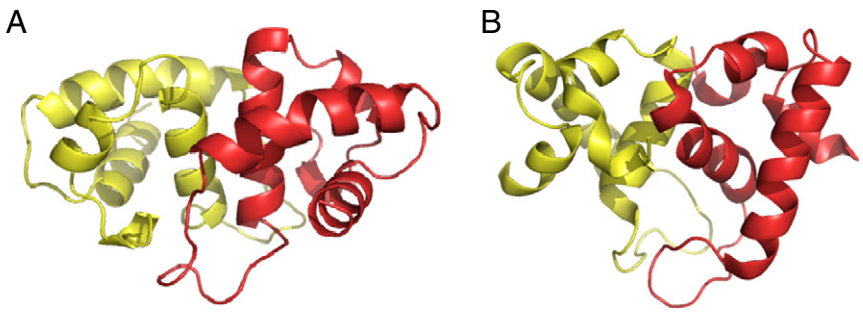

Fig. 8. Comparison between the structures of *E. coli* HdeA and HdeB chaperones. Ribbon diagram of the structures of (A) HdeA dimer (PDB entry code 1BG8) [108] and (B) HdeB dimer (PDB entry code 2XUV) [107]. Each monomer (colored in yellow or red) consists of four α-helices. Despite their low sequence identity, their monomeric structure display high similarity.

In contrast, the substrates of HdeB have not been identified so far and the exact function of this protein remains to be determined.

HdeA has a unique and intriguing activation mechanism (reviewed in [110]). At neutral pH, it exists as a homodimer and, once exposed to acidic conditions, it separates into chaperone-active monomers. The acid-induced dissociation exposes the hydrophobic patches of the dimer interface enabling HdeA to bind unfolded substrates [25,109,111]. The shift back to higher pH induces the slow release of the substrates, enabling the refolding of the proteins. Further investigation demonstrated that the dimer is in an inactive folded conformation whereas the two monomers become partially unfolded and active upon pH decrease [111]. Recent studies have also shown that mutation of two aspartic acid residues responsible for the pH-dependent monomerization of HdeA partially unfolds the protein and turns on the activity at neutral pH [112].

2.4.2. Spy

The small protein Spy (spheroplast protein Y) was recently shown to be a periplasmic chaperone by Bardwell and co-workers [27]. They found that overexpression of Spy increases the folding yields of otherwise unstable proteins and that Spy is involved in defending bacteria from tannin-induced protein aggregation [27]. Moreover, Spy is capable of preventing the aggregation and of promoting the renaturation of a wide range of protein substrates, even at sub-stoichiometric concentrations. Interestingly, in a mutant lacking Skp and FkpA, overexpression of Spy was shown to be necessary and sufficient to restore LptD assembly [79].

The expression of Spy is under the control of the Bae and Cpx periplasmic stress response pathways, which are both activated by protein unfolding and aggregation [113]. Although Spy has 30% identity with CpxP, an inhibitory factor of the Cpx system, both proteins appear to play different functions in protein quality control [27]. Moreover, it has been reported that *spy* deletion leads to a slight induction of *degP* and *rpoH*, both involved in the Cpx system [27].

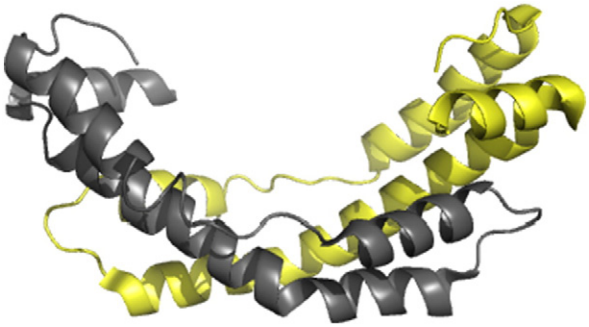

Fig. 9. Crystal structure of the *E. coli* Spy chaperone. Ribbon diagram of the structure of the Spy dimer (PDB entry code 3OEO) [144]. Each monomer (in yellow or grey) consists of four α-helices.

The crystal structure of Spy, which is similar to the one of CpxP [114], reveals interesting features of the protein. Indeed, Spy forms a cradle shaped dimer (each monomer is composed of four α-helices) that is different from any other chaperone whose structure has been solved (Fig. 9). This particular shape combined with its high flexibility may allow Spy to enclose and accommodate bigger proteins, enabling them to fold correctly while protecting them from harsh conditions [27].

3. Periplasmic folding catalysts

All the chaperones described above participate in the protein network taking care of envelope proteins in order to assist their folding and prevent their aggregation. However, the proper folding of secreted proteins also requires the intervention of periplasmic folding catalysts involved in the formation of disulfide bonds between cysteine residues and in the *cis-trans* isomerization of peptidyl-prolyl bonds. These proteins will be described briefly in the following section.

3.1. Peptidyl-prolyl cis-trans isomerases

Whereas most peptide bonds are in the *trans* configuration, proline residues can be found in peptide bonds that adopt the *cis* configuration. Because this *cis-trans* isomerization reaction is a slow natural process that depends on the nature of the amino acid prior to the proline residue, enzymes with PPIase activity catalyze the interconversion of peptidyl-prolyl imide bonds in protein substrates.

Four PPIases belonging to three distinct families have been identified so far in the *E. coli* periplasm: PpiA is from the cyclophilin group, FkpA is related to the FKBP (FK506 binding protein) family and PpiD and SurA both belong to the parvulin group. All these enzymes are able to catalyze the *cis-trans* isomerization of peptidyl-prolyl bonds that are important for the refolding of RNase T1 *in vitro* [115]. Importantly, the quadruple mutant *ppiD ppiA surA fkpA* does not exhibit a severe phenotype indicating that the periplasmic PPIases are not essential for viability of the bacterium [72,116].

Although SurA and FkpA have been first identified as peptidyl-prolyl *cis-trans* isomerases, they seem to preferentially function as periplasmic chaperones as described above (see Sections 2.1.1 and 2.1.4). However, we would like to highlight here the recent finding that the toxicity of imported colicin M depends on the PPIase activity of FkpA [117,118]. Colicins M are toxic proteins interfering with peptidoglycan biosynthesis, which are produced and released by *E. coli* strains carrying a pColBM plasmid [119]. It has been shown that FkpA is needed for the proper folding of colicin M after its entry into the periplasm of the target cells [117,118].

PpiA, also called *E. coli* cyclophilin A or RotA, is a globular protein of 164 amino acids [17]. While its three-dimensional structure looks like the one of the well-characterized human cyclophilin A [120], PpiA exhibits three-fold less PPIase enzymatic activity and 1000-fold less affinity for the inhibitor cyclosporin A compared to the human enzyme [17,18,121]. Moreover, an *E. coli ppiA* mutant has normal levels of

periplasmic or OM proteins and does not exhibit any particular phenotype [116]. The sole indication that PpiA may contribute in protein quality control is the fact that the *ppiA* gene is regulated by the Cpx system [122]. However, the exact *in vivo* function of PpiA is still not known.

PpiD is anchored in the IM via a N-terminal helix followed by three soluble domains facing the periplasm. Whereas the first and the third periplasmic parts are likely to have a chaperone activity, the second domain adopts a parvulin-like fold that strongly resembles the first parvulin domain of SurA [123]. PpiD was initially isolated in 1998 as a multicopy suppressor of a *surA* deletion strain [39]. The *ppiD* mutant was reported to have a phenotype similar to that of a *surA* deletion strain and the simultaneous deletion of *surA* and *ppiD* to be synthetically lethal [39]. However, more recent studies have shown that the *ppiD* mutant and the *surA ppiD* double mutant do not display any growth defect [124]. Moreover, by investigating the functional interactions between PpiD and other folding factors such as Skp, SurA and DegP, it was recently shown that PpiD plays no major role in the biogenesis of OMPs and that the protein cannot compensate for the absence of SurA in the periplasm [125]. Thus, the function of PpiD remains unclear, although the protein has been proposed to be a periplasmic gatekeeper assisting the initial folding events of newly translocated proteins due to its localization in the IM together with the Sec translocon [126].

3.2. Oxidoreductases involved in disulfide bond formation

Together with peptidyl prolyl *cis-trans* isomerization, disulfide bond formation is the second rate-limiting step in protein folding. Therefore, cells contain enzymes that catalyze disulfide bonds in order to ensure fast and correct folding *in vivo*. In *E. coli*, these enzymes are involved in two major pathways: the oxidation pathway (DsbA and DsbB) and the isomerization pathway (DsbC and DsbD). These pathways have been the focus of several recent reviews [127–130]. For that reason, we summarize here the most important features of the *E. coli* Dsb proteins and we refer the reader to the above-referenced reviews for in-depth information.

3.2.1. DsbA and DsbB

E. coli DsbA is the first catalyst of disulfide bond formation identified in the bacterial periplasm [16]. It is a soluble monomeric protein that adopts a thioredoxin fold and has a CXXC catalytic motif. The cysteine residues of this motif are maintained in the oxidized state *in vivo* [131], which enables DsbA to react with proteins entering the periplasm to oxidize them [132] (Fig. 10). This reaction occurs in two successive steps. First, a cysteine residue from the substrate performs a nucleophilic attack on the first cysteine of DsbA, leading to the formation of a mixed-

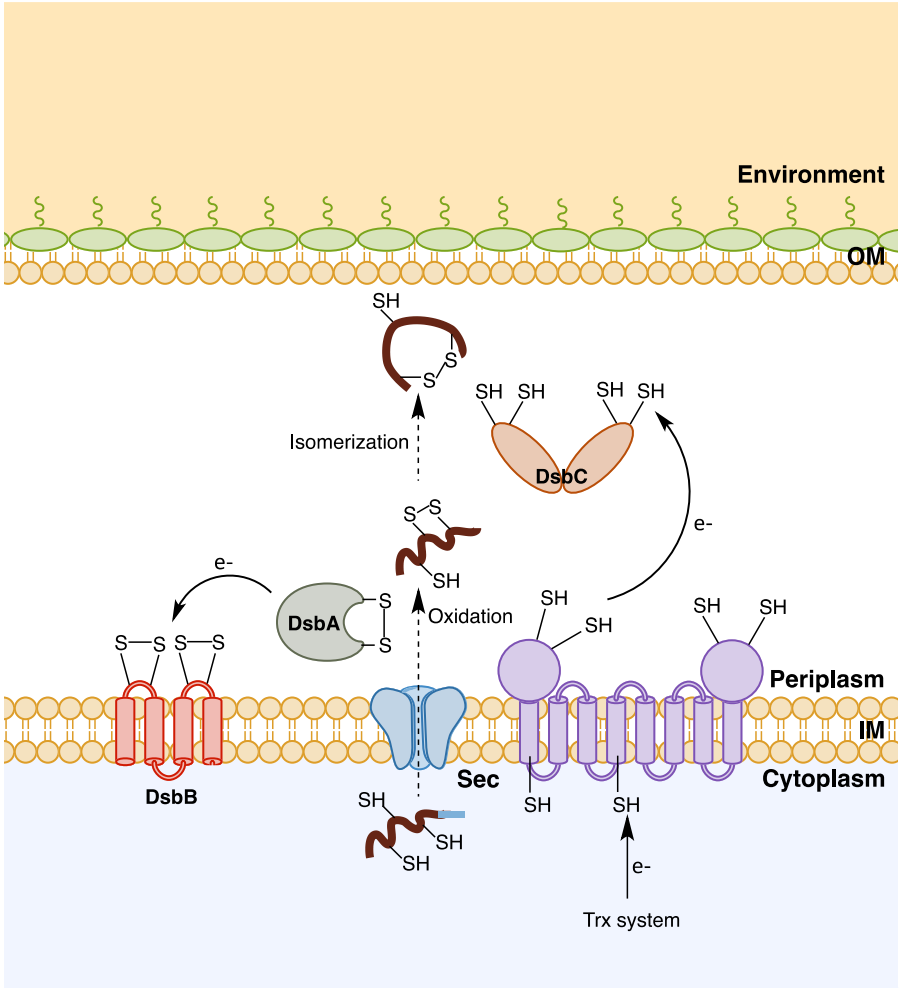

Fig. 10. Disulfide bond formation and isomerization in the periplasm of *E. coli*. After crossing the inner membrane (IM) via the Sec-machinery, disulfide bonds are introduced in newly synthesized proteins by DsbA, which then transfers electrons originating from the substrate to the IM protein DsbB. Then, DsbB transfers electrons to the respiratory chain (not shown). When DsbA introduces non-native disulfide bonds into its substrates, they are corrected by the soluble homodimeric protein DsbC, which is kept reduced by the IM protein DsbD. DsbD receives reducing equivalents from the cytoplasmic thioredoxin system (Trx system). The black arrows show the electron flow.

disulfide complex. Then, this complex is resolved by the attack of a second cysteine from the substrate, resulting in the oxidation of the substrate and the reduction of DsbA. About 30 substrates of DsbA have been identified so far, but the number of proteins predicted to depend on this protein for folding is much higher [133,134].

After donating its disulfide, DsbA is recycled back to its oxidized form by the IM protein DsbB [135]. DsbB has four transmembrane segments and two small hydrophilic loops, both containing a pair of cysteines exposed to the periplasm and maintained in an oxidized state. DsbB uses these cysteine residues to channel the electrons away from DsbA and deliver them to bound quinone molecules, connecting the disulfide bond formation pathway to the electron transport chain [16] (Fig. 10).

3.2.2. DsbC and DsbD

DsbA preferentially introduces disulfide bonds in a vectorial manner into proteins entering the periplasm, i.e. between cysteines that are consecutive in the primary sequence of the protein [136]. Thus, DsbA often incorrectly oxidize proteins whose three-dimensional structure involves the formation of disulfide bonds between cysteines that are non consecutive in the sequence. The function of the disulfide isomerization system present in the periplasm of *E. coli* is to catalyze the rearrangement of these incorrect disulfide bonds [137]. The major player in this pathway is the soluble, V-shaped, homodimeric protein DsbC [138]. Each monomer of DsbC consists of two domains: an N-terminal dimerization domain and a C-terminal thioredoxin-like domain with a CXXC motif found predominantly reduced *in vivo* [131]. DsbC can function as an isomerase or as a reductase, in which case, the reaction results in the reduction of the substrate and the oxidation of DsbC (Fig. 10).

The protein that recycles DsbC back to the reduced state is the IM protein DsbD [137]. It is a monomeric protein composed of three distinct structural domains: an N-terminal periplasmic domain (DsbDα), a membrane-embedded domain with eight transmembrane segments (DsbDβ) and a C-terminal periplasmic thioredoxin-like domain (DsbDγ). Each of these domains contains a pair of conserved redox-active cysteines, essential for the function of DsbD [139] that catalyze the transfer of reducing equivalents from the cytoplasmic thioredoxin system to DsbC [137] (Fig. 10).

4. Conclusions

Polypeptides that are destined to the bacterial envelope need to fold in an environment where there is no obvious energy source. Moreover, once folded, they will be particularly exposed to environmental changes and to toxic molecules present in the surrounding medium.

Understanding how envelope proteins fold and maintain their native conformation in this extracytoplasmic compartment is therefore a fascinating problem of high biological relevance. Moreover, a better characterization of these processes should open the way to the design of new antibacterial molecules, which are required to fight multiresistant bacteria.

The work of the past decades led to the identification of multiple chaperones and protein folding catalysts that participate in the assembly of envelope proteins. However, numerous questions remain unsolved and need to be explored. For instance, we only have a partial understanding of how the periplasmic folding helpers cooperate in the assembly pathway of envelope proteins. The complexity of the problem is illustrated by the LPS insertion protein LptD. The folding of LptD has indeed been shown to require the assistance of several proteins including the periplasmic chaperones Skp, FkpA and SurA [79]. However, the exact role of the various players involved is far to be clear [34,79,140]. Moreover, it is also not known whether the periplasmic soluble proteins emerging from the Sec translocon need the assistance of chaperones to reach their proper three-dimensional conformation and what are the chaperones involved. Another unresolved problem is the function of proteins such as PpiA and PpiD, which also needs clarification. Further studies are thus required to allow a complete understanding of the mechanisms of periplasmic protein folding.

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References

[1] N. Ruiz, D. Kahne, T.J. Silhavy, Advances in understanding bacterial outer-membrane biogenesis, Nat. Rev. Microbiol. 4 (2006) 57–66.
[2] H. Tokuda, Biogenesis of outer membranes in Gram-negative bacteria, Biosci. Biotechnol. Biochem. 73 (2009) 465–473.
[3] W.T. Doerrler, Lipid trafficking to the outer membrane of Gram-negative bacteria, Mol. Microbiol. 60 (2006) 542–552.
[4] H. Tokuda, S. Matsuyama, Sorting of lipoproteins to the outer membrane in *E. coli*, Biochim. Biophys. Acta 1693 (2004) 5–13.
[5] W.C. Wimley, The versatile beta-barrel membrane protein, Curr. Opin. Struct. Biol. 13 (2003) 404–411.
[6] H. Nikaido, Molecular basis of bacterial outer membrane permeability revisited, Microbiol. Mol. Biol. Rev. 67 (2003) 593–656.
[7] J.E. Van Wielink, J.A. Duine, How big is the periplasmic space? Trends Biochem. Sci. 15 (1990) 136–137.
[8] J.H. Weiner, L. Li, Proteome of the *Escherichia coli* envelope and technological challenges in membrane proteome analysis, Biochim. Biophys. Acta 1778 (2008) 1698–1713.
[9] W. Wickner, A.J. Driessen, F.U. Hartl, The enzymology of protein translocation across the *Escherichia coli* plasma membrane, Annu. Rev. Plant Physiol. Plant Mol. Biol. 60 (1991) 101–124.
[10] A.R. Osborne, T.A. Rapoport, B.v.d. Berg, Protein translocation by the Sec61/SecY channel, Annu. Rev. Cell Dev. Biol. 21 (2005) 529–550.
[11] A.J.M. Driessen, E.H. Manting, C. van der Does, The structural basis of protein targeting and translocation in bacteria, Nat. Struct. Biol. 8 (2001) 492–498.
[12] J. Luirink, G. von Heijne, E. Houben, J.W. de Gier, Biogenesis of inner membrane proteins in *Escherichia coli*, Annu. Rev. Microbiol. 59 (2005) 329–355.
[13] A.J.M. Driessen, SecB, a molecular chaperone with two faces, Trends Microbiol. 9 (2001) 193–196.
[14] B.C. Berks, T. Palmer, F. Sargent, Protein targeting by the bacterial twin-arginine translocation (Tat) pathway, Curr. Opin. Microbiol. 8 (2005) 174–181.
[15] P. Natale, T. Bruser, A.J. Driessen, Sec- and Tat-mediated protein secretion across the bacterial cytoplasmic membrane-distinct translocases and mechanisms, Biochim. Biophys. Acta 1778 (2008) 1735–1756.
[16] J.C. Bardwell, K. McGovern, J. Beckwith, Identification of a protein required for disulfide bond formation *in vivo*, Cell 67 (1991) 581–589.
[17] J. Liu, C.T. Walsh, Peptidyl-prolyl *cis-trans*-isomerase from *Escherichia coli*: a periplasmic homolog of cyclophilin that is not inhibited by cyclosporin A, Proc. Natl. Acad. Sci. U. S. A. 87 (1990) 4028–4032.
[18] T. Hayano, N. Takahashi, S. Kato, N. Maki, M. Suzuki, Two distinct forms of peptidylprolyl-*cis-trans*-isomerase are expressed separately in periplasmic and cytoplasmic compartments of *Escherichia coli* cells, Biochemistry 30 (1991) 3041–3048.
[19] C. Wülfing, A. Plückthun, Protein folding in the periplasm of *Escherichia coli*, Mol. Microbiol. 12 (1994) 685–692.
[20] D. Missiakas, J.M. Betton, S. Raina, New components of protein folding in extracytoplasmic compartments of *Escherichia coli* SurA, FkpA and Skp/OmpH, Mol. Microbiol. 21 (1996) 871–884.
[21] S.W. Lazar, R. Kolter, SurA assists the folding of *Escherichia coli* outer membrane proteins, J. Bacteriol. 178 (1996) 1770–1773.
[22] R. Chen, U. Henning, A periplasmic protein (Skp) of *Escherichia coli* selectively binds a class of outer membrane proteins, Mol. Microbiol. 19 (1996) 1287–1294.
[23] Terutaka Tajima, Naoko Yokota, Shin-ichi Matsuyama, Hajime Tokuda, Genetic analyses of the *in vivo* function of LolA, a periplasmic chaperone involved in the outer membrane localization of *Escherichia coli* lipoproteins, FEBS Lett. 439 (1998) 51–54.
[24] C. Spiess, A. Beil, M. Ehrmann, A temperature-dependent switch from chaperone to protease in a widely conserved heat shock protein, Cell 97 (1999) 339–347.
[25] W. Hong, W. Jiao, J. Hu, J. Zhang, C. Liu, X. Fu, D. Shen, B. Xia, Z. Chang, Periplasmic protein HdeA exhibits chaperone-like activity exclusively within stomach pH range by transforming into disordered conformation, J. Biol. Chem. 280 (2005) 27029–27034.
[26] R. Kern, A. Malki, J. Abdallah, J. Tagourti, G. Richarme, *Escherichia coli* HdeB is an acid stress chaperone, J. Bacteriol. 189 (2007) 603–610.
[27] S. Quan, P. Koldewey, T. Tapley, N. Kirsch, K.M. Ruane, J. Pfizenmaier, R. Shi, S. Hofmann, L. Foit, G. Ren, U. Jakob, Z. Xu, M. Cygler, J.C. Bardwell, Genetic selection designed to stabilize proteins uncovers a chaperone called Spy, Nat. Struct. Mol. Biol. 18 (2011) 262–269.
[28] A. Tormo, M. Almiron, R. Kolter, surA, an *Escherichia coli* gene essential for survival in stationary phase, J. Bacteriol. 172 (1990) 4339–4347.
[29] P.E. Rouviere, C.A. Gross, SurA, a periplasmic protein with peptidyl-prolyl isomerase activity, participates in the assembly of outer membrane porins, Genes Dev. 10 (1996) 3170–3182.

[30] J.U. Rahfeld, A. Schierhorn, K. Mann, G. Fischer, A novel peptidyl-prolyl *cis/trans* isomerase from *Escherichia coli*, FEBS Lett. 343 (1994) 65–69.
[31] E. Bitto, D.B. McKay, Crystallographic structure of SurA, a molecular chaperone that facilitates folding of outer membrane porins, Structure 10 (2002) 1489–1498.
[32] S. Abe, T. Okutsu, H. Nakajima, N. Kakuda, I. Ohtsu, R. Aono, n-Hexane sensitivity of *Escherichia coli* due to low expression of imp/ostA encoding an 87 kDa minor protein associated with the outer membrane, Microbiology 149 (2003) 1265–1273.
[33] J.G. Sklar, T. Wu, D. Kahne, T.J. Silhavy, Defining the roles of the periplasmic chaperones SurA, Skp, and DegP in *Escherichia coli*, Genes Dev. 21 (2007) 2473–2484.
[34] D. Vertommen, N. Ruiz, P. Leverrier, T.J. Silhavy, J.F. Collet, Characterization of the role of the *Escherichia coli* periplasmic chaperone SurA using differential proteomics, Proteomics 9 (2009) 2432–2443.
[35] J. Johansen, A.A. Rasmussen, M. Overgaard, P. Valentin-Hansen, Conserved small non-coding RNAs that belong to the sigmaE regulon: role in down-regulation of outer membrane proteins, J. Mol. Biol. 364 (2006) 1–8.
[36] C. Dartigalongue, D. Missiakas, S. Raina, Characterization of the *Escherichia coli* sigma E regulon, J. Biol. Chem. 276 (2001) 20866–20875.
[37] S. Lima, M.S. Guo, R. Chaba, C.A. Gross, R.T. Sauer, Dual molecular signals mediate the bacterial response to outer-membrane stress, Science 340 (2013) 837–841.
[38] E. Freinkman, S.S. Chng, D. Kahne, The complex that inserts lipopolysaccharide into the bacterial outer membrane forms a two-protein plug-and-barrel, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 2486–2491.
[39] C. Dartigalongue, S. Raina, A new heat-shock gene, *ppiD*, encodes a peptidyl-prolyl isomerase required for folding of outer membrane proteins in *Escherichia coli*, EMBO J. 17 (1998) 3968–3980.
[40] S. Behrens, R. Maier, H. de Cock, F.X. Schmid, C.A. Gross, The SurA periplasmic PPIase lacking its parvulin domains functions *in vivo* and has chaperone activity, EMBO J. 20 (2001) 285–294.
[41] S. Behrens-Kneip, The role of SurA factor in outer membrane protein transport and virulence, Int J Med Microbiol 300 (2010) 421–428.
[42] E. Bitto, D.B. McKay, The periplasmic molecular chaperone protein SurA binds a peptide motif that is characteristic of integral outer membrane proteins, J. Biol. Chem. 278 (2003) 49316–49322.
[43] X. Xu, S. Wang, Y.X. Hu, D.B. McKay, The periplasmic bacterial molecular chaperone SurA adapts its structure to bind peptides in different conformations to assert a sequence preference for aromatic residues, J. Mol. Biol. 373 (2007) 367–381.
[44] A. Holck, K. Kleppe, Cloning and sequencing of the gene for the DNA-binding 17 K protein of *Escherichia coli*, Gene 67 (1988) 117–124.
[45] T.A. Walton, M.C. Sousa, Crystal structure of Skp, a prefoldin-like chaperone that protects soluble and membrane proteins from aggregation, Mol. Cell 15 (2004) 367–374.
[46] T.A. Walton, C.M. Sandoval, C.A. Fowler, A. Pardi, M.C. Sousa, The cavity-chaperone Skp protects its substrate from aggregation but allows independent folding of substrate domains, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 1772–1777.
[47] S. Jarchow, C. Luck, A. Gorg, A. Skerra, Identification of potential substrate proteins for the periplasmic *Escherichia coli* chaperone Skp, Proteomics 8 (2008) 4987–4994.
[48] H. Bothmann, A. Pluckthun, Selection for a periplasmic factor improving phage display and functional periplasmic expression, Nat. Biotechnol. 16 (1998) 376–380.
[49] K. Denoncin, J. Schwalm, D. Vertommen, T.J. Silhavy, J.-F. Collet, Dissecting the *Escherichia coli* periplasmic chaperone network using differential proteomics, Proteomics 12 (2012) 1391–1401.
[50] G.J. Patel, S. Behrens-Kneip, O. Holst, J.H. Kleinschmidt, The periplasmic chaperone Skp facilitates targeting, insertion, and folding of OmpA into lipid membranes with a negative membrane surface potential, Biochemistry 48 (2009) 10235–10245.
[51] P.V. Bulieris, S. Behrens, O. Holst, J.H. Kleinschmidt, Folding and insertion of the outer membrane protein OmpA is assisted by the chaperone Skp and by lipopolysaccharide, J. Biol. Chem. 278 (2003) 9092–9099.
[52] H. De Cock, U. Schafer, M. Potgeter, R. Demel, M. Müller, J. Tommassen, Affinity of the periplasmic chaperone Skp of *Escherichia coli* for phospholipids, lipopolysaccharides and non-native outer membrane proteins. Role of Skp in the biogenesis of outer membrane protein, Eur. J. Biochem. 259 (1999) 96–103.
[53] U. Schafer, K. Beck, M. Muller, Skp, a molecular chaperone of gram-negative bacteria, is required for the formation of soluble periplasmic intermediates of outer membrane proteins, J. Biol. Chem. 274 (1999) 24567–24574.
[54] D.M. Walther, D. Rapaport, J. Tommassen, Biogenesis of beta-barrel membrane proteins in bacteria and eukaryotes: evolutionary conservation and divergence, Cell. Mol. Life Sci. 66 (2009) 2789–2804.
[55] J. Tommassen, Assembly of outer-membrane proteins in bacteria and mitochondria, Microbiology 156 (2010) 2587–2596.
[56] B. Lipinska, M. Zylicz, C. Georgopoulos, The HtrA (DegP) protein, essential for *Escherichia coli* survival at high temperatures, is an endopeptidase, J. Bacteriol. 172 (1990) 1791–1797.
[57] B. Lipinska, O. Fayet, L. Baird, C. Georgopoulos, Identification, characterization, and mapping of the *Escherichia coli* htrA gene, whose product is essential for bacterial growth only at elevated temperatures, J. Bacteriol. 171 (1989) 1574–1584.
[58] P.N. Danese, W.B. Snyder, C.L. Cosma, L.J. Davis, T.J. Silhavy, The Cpx two-component signal transduction pathway of *Escherichia coli* regulates transcription of the gene specifying the stress-inducible periplasmic protease, DegP, Genes Dev. 9 (1995) 387–398.
[59] B.M. Alba, C.A. Gross, Regulation of the *Escherichia coli* sigma-dependent envelope stress response, Mol. Microbiol. 52 (2004) 613–619.
[60] N.L. Price, T.L. Raivio, Characterization of the Cpx regulon in *Escherichia coli* strain MC4100, J. Bacteriol. 191 (2009) 1798–1815.
[61] T. Clausen, C. Southan, M. Ehrmann, The HtrA family of proteases: implications for protein composition and cell fate, Mol. Cell 10 (2002) 443–455.
[62] J. Iwanczyk, D. Damjanovic, J. Kooistra, V. Leong, A. Jomaa, R. Ghirlando, J. Ortega, Role of the PDZ domains in *Escherichia coli* DegP protein, J. Bacteriol. 189 (2007) 3176–3186.
[63] T. Krojer, M. Garrido-Franco, R. Huber, M. Ehrmann, T. Clausen, Crystal structure of DegP (HtrA) reveals a new protease-chaperone machine, Nature 416 (2002) 455–459.
[64] J. Jiang, X. Zhang, Y. Chen, Y. Wu, Z.H. Zhou, Z. Chang, S.F. Sui, Activation of DegP chaperone-protease via formation of large cage-like oligomers upon binding to substrate proteins, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 11939–11944.
[65] T. Krojer, J. Sawa, E. Schafer, H.R. Saibil, M. Ehrmann, T. Clausen, Structural basis for the regulated protease and chaperone function of DegP, Nature 453 (2008) 885–890.
[66] S. Kim, R.T. Sauer, Cage assembly of DegP protease is not required for substrate-dependent regulation of proteolytic activity or high-temperature cell survival, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 7263–7268.
[67] H. Kadokura, H. Kawasaki, K. Yoda, M. Yamasaki, K. Kitamoto, Efficient export of alkaline phosphatase overexpressed from a multicopy plasmid requires degP, a gene encoding a periplasmic protease of Escherichia coli, J. Gen. Appl. Microbiol. 47 (2001) 133–141.
[68] C.H. Jones, P. Dexter, A.K. Evans, C. Liu, S.J. Hultgren, D.E. Hruby, *Escherichia coli* DegP protease cleaves between paired hydrophobic residues in a natural substrate: the PapA pilin, J. Bacteriol. 184 (2002) 5762–5771.
[69] J. Skorko-Glonek, E. Laskowska, A. Sobiecka-Szkatula, B. Lipinska, Characterization of the chaperone-like activity of HtrA (DegP) protein from *Escherichia coli* under the conditions of heat shock, Arch. Biochem. Biophys. 464 (2007) 80–89.
[70] A. Jomaa, D. Damjanovic, V. Leong, R. Ghirlando, J. Iwanczyk, J. Ortega, The inner cavity of *Escherichia coli* DegP protein is not essential for molecular chaperone and proteolytic activity, J. Bacteriol. 189 (2007) 706–716.
[71] T. Krojer, K. Pangerl, J. Kurt, J. Sawa, C. Stingl, K. Mechtler, R. Huber, M. Ehrmann, T. Clausen, Interplay of PDZ and protease domain of DegP ensures efficient elimination of misfolded proteins, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 7702–7707.
[72] S.M. Horne, K.D. Young, *Escherichia coli* and other species of the Enterobacteriaceae encode a protein similar to the family of Mip-like FK506-binding proteins, Arch. Microbiol. 163 (1995) 357–365.
[73] F.A. Saul, J.P. Arie, B. Vulliez-le Normand, R. Kahn, J.M. Betton, G.A. Bentley, Structural and functional studies of FkpA from *Escherichia coli*, a *cis/trans* peptidyl-prolyl isomerase with chaperone activity, J. Mol. Biol. 335 (2004) 595–608.
[74] M.K. Rosen, R.F. Standaert, A. Galat, M. Nakatsuka, S.L. Schreiber, Inhibition of FKBP rotamase activity by immunosuppressant FK506: twisted amide surrogate, Science 248 (1990) 863–866.
[75] H. Bothmann, A. Pluckthun, The periplasmic *Escherichia coli* peptidylprolyl cis, trans-isomerase FkpA. I. Increased functional expression of antibody fragments with and without cis-prolines, J. Biol. Chem. 275 (2000) 17100–17105.
[76] Z. Zhang, L.P. Song, M. Fang, F. Wang, D. He, R. Zhao, J. Liu, Z.Y. Zhou, C.C. Yin, Q. Lin, H.L. Huang, Production of soluble and functional engineered antibodies in *Escherichia coli* improved by FkpA, Biotechniques 35 (2003) 1032–1038(1041–1032).
[77] K. Ramm, A. Pluckthun, The periplasmic Escherichia coli peptidylprolyl cis, trans-isomerase FkpA. II. Isomerase-independent chaperone activity in vitro, J. Biol. Chem. 275 (2000) 17106–17113.
[78] J.P. Arie, N. Sassoon, J.M. Betton, Chaperone function of FkpA, a heat shock prolyl isomerase, in the periplasm of *Escherichia coli*, Mol. Microbiol. 39 (2001) 199–210.
[79] J. Schwalm, T.F. Mahoney, G.R. Soltes, T.J. Silhavy, A role for Skp in LptD assembly in *Escherichia coli*, J. Bacteriol. 195 (2013) 3734–3742.
[80] A.E. Rizzitello, J.R. Harper, T.J. Silhavy, Genetic evidence for parallel pathways of chaperone activity in the periplasm of *Escherichia coli*, J. Bacteriol. 183 (2001) 6794–6800.
[81] R. Voulhoux, M.P. Bos, J. Geurtsen, M. Mols, J. Tommassen, Role of a highly conserved bacterial protein in outer membrane protein assembly, Science 299 (2003) 262–265.
[82] I. Gentle, K. Gabriel, P. Beech, R. Waller, T. Lithgow, The Omp85 family of proteins is essential for outer membrane biogenesis in mitochondria and bacteria, J. Cell Biol. 164 (2004) 19–24.
[83] J.C. Malinverni, J. Werner, S. Kim, J.G. Sklar, D. Kahne, R. Misra, T.J. Silhavy, YfiO stabilizes the YaeT complex and is essential for outer membrane protein assembly in *Escherichia coli*, Mol. Microbiol. 61 (2006) 151–164.
[84] C.L. Hagan, S. Kim, D. Kahne, Reconstitution of outer membrane protein assembly from purified components, Science 328 (2010) 890–892.
[85] C.L. Hagan, T. Silhavy, D. Kahne, β-Barrel membrane protein assembly by the Bam complex, Annu. Rev. Biochem. 80 (2011) 189–210.
[86] D. Bennion, E.S. Charlson, E. Coon, R. Misra, Dissection of beta-barrel outer membrane protein assembly pathways through characterizing BamA POTRA 1 mutants of *Escherichia coli*, Mol. Microbiol. 77 (2010) 1153–1171.
[87] C.L. Hagan, D.B. Westwood, D. Kahne, Bam lipoproteins assemble BamA *in vitro*, Biochemistry 52 (2013) 6108–6113.
[88] D.P. Ricci, T.J. Silhavy, The Bam machine: a molecular cooper, Biochim. Biophys. Acta 1818 (2012) 1067–1084.
[89] N.W. Rigel, T. Silhavy, Making a beta-barrel: assembly of outer membrane proteins in Gram-negative bacteria, Curr. Opin. Microbiol. 15 (2012) 189–193.
[90] S. Narita, S. Matsuyama, H. Tokuda, Lipoprotein trafficking in *Escherichia coli*, Arch. Microbiol. 182 (2004) 1–6.
[91] S. Okuda, H. Tokuda, Lipoprotein sorting in bacteria, Annu. Rev. Microbiol. 65 (2011) 239–259.

[92] T. Yakushi, K. Masuda, S. Narita, S. Matsuyama, H. Tokuda, A new ABC transporter mediating the detachment of lipid-modified proteins from membranes, Nat. Cell Biol. 2 (2000) 212–218.
[93] S. Okuda, H. Tokuda, Model of mouth-to-mouth transfer of bacterial lipoproteins through inner membrane LolC, periplasmic LolA, and outer membrane LolB, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 5877–5882.
[94] K. Takeda, H. Miyatake, N. Yokota, S. Matsuyama, H. Tokuda, K. Miki, Crystal structures of bacterial lipoprotein localization factors, LolA and LolB, EMBO J. 22 (2003) 3199–3209.
[95] S. Watanabe, Y. Oguchi, N. Yokota, H. Tokuda, Large-scale preparation of the homogeneous LolA lipoprotein complex and efficient *in vitro* transfer of lipoproteins to the outer membrane in a LolB-dependent manner, Protein Sci. 16 (2007) 2741–2749.
[96] S. Watanabe, Y. Oguchi, K. Takeda, K. Miki, H. Tokuda, Introduction of a lethal redox switch that controls the opening and closing of the hydrophobic cavity in LolA, J. Biol. Chem. 283 (2008) 25421–25427.
[97] S. Nakada, M. Sakakura, H. Takahashi, S. Okuda, H. Tokuda, I. Shimada, Structural investigation of the interaction between LolA and LolB using NMR, J. Biol. Chem. 284 (2009) 24634–24643.
[98] S. Okuda, S. Watanabe, H. Tokuda, A short helix in the C-terminal region of LolA is important for the specific membrane localization of lipoproteins, FEBS Lett. 582 (2008) 2247–2251.
[99] A. Miyamoto, S. Matsuyama, H. Tokuda, Mutant of LolA, a lipoprotein-specific molecular chaperone of *Escherichia coli*, defective in the transfer of lipoproteins to LolB, Biochem. Biophys. Res. Commun. 287 (2001) 1125–1128.
[100] N. Taniguchi, S. Matsuyama, H. Tokuda, Mechanisms underlying energy-independent transfer of lipoproteins from LolA to LolB, which have similar unclosed beta-barrel structures, J. Biol. Chem. 280 (2005) 34481–34488.
[101] R. Pathania, S. Zlitni, C. Barker, R. Das, D.A. Gerritsma, J. Lebert, E. Awuah, G. Melacini, F.A. Capretta, E.D. Brown, Chemical genomics in *Escherichia coli* identifies an inhibitor of bacterial lipoprotein targeting, Nat. Chem. Biol. 5 (2009) 849–856.
[102] K.S. Gajiwala, S.K. Burley, HDEA, a periplasmic protein that supports acid resistance in pathogenic enteric bacteria, J. Mol. Biol. 295 (2000) 605–612.
[103] T.L. Tapley, T.M. Franzmann, S. Chakraborty, U. Jakob, J.C. Bardwell, Protein refolding by pH-triggered chaperone binding and release, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 1071–1076.
[104] A. Malki, H.T. Le, S. Milles, R. Kern, T. Caldas, J. Abdallah, G. Richarme, Solubilization of protein aggregates by the acid stress chaperones HdeA and HdeB, J. Biol. Chem. 283 (2008) 13679–13687.
[105] J.W. Foster, *Escherichia coli* acid resistance: tales of an amateur acidophile, Nat. Rev. Microbiol. 2 (2004) 898–907.
[106] D.L. Tucker, N. Tucker, T. Conway, Gene expression profiling of the pH response in *Escherichia coli*, J. Bacteriol. 184 (2002) 6551–6558.
[107] W. Wang, T. Rasmussen, A.J. Harding, N.A. Booth, I.R. Booth, J.H. Naismith, Salt bridges regulate both dimer formation and monomeric flexibility in HdeB and may have a role in periplasmic chaperone function, J. Mol. Biol. 415 (2012) 538–546.
[108] F. Yang, K.R. Gustafson, M.R. Boyd, A. Wlodawer, Crystal structure of *Escherichia coli* HdeA, Nat. Struct. Biol. 5 (1998) 763–764.
[109] M. Zhang, S. Lin, X. Song, J. Liu, Y. Fu, X. Ge, X. Fu, Z. Chang, P.R. Chen, A genetically incorporated crosslinker reveals chaperone cooperation in acid resistance, Nat. Chem. Biol. 7 (2011) 671–677.
[110] W. Hong, Y.E. Wu, X. Fu, Z. Chang, Chaperone-dependent mechanisms for acid resistance in enteric bacteria, Trends Microbiol. 20 (2012) 328–335.
[111] T.L. Tapley, J.L. Korner, M.T. Barge, J. Hupfeld, J.A. Schauerte, A. Gafni, U. Jakob, J.C. Bardwell, Structural plasticity of an acid-activated chaperone allows promiscuous substrate binding, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 5557–5562.
[112] L. Foit, J.S. George, B.W. Zhang, C.L. Brooks, J.C. Bardwell, Chaperone activation by unfolding, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 1254–1262.
[113] S. Bury-Mone, Y. Nomane, N. Reymond, R. Barbet, E. Jacquet, S. Imbeaud, A. Jacq, P. Bouloc, Global analysis of extracytoplasmic stress signaling in *Escherichia coli*, PLoS Genet. 5 (2009).
[114] G.L. Thede, D.C. Arthur, R.A. Edwards, D.R. Buelow, J.L. Wong, T.L. Raivio, J.N. Glover, Structure of the periplasmic stress response protein CpxP, J. Bacteriol. 193 (2011).
[115] C. Scholz, G. Scherer, L.M. Mayr, T. Schindler, G. Fischer, F.X. Schmid, Prolyl isomerases do not catalyze isomerization of non-prolyl peptide bonds, Biol. Chem. 379 (1998) 361–365.
[116] M. Kleerebezem, M. Heutink, J. Tommassen, Characterization of an *Escherichia coli rotA* mutant, affected in periplasmic peptidyl-prolyl cis/trans isomerase, Mol. Microbiol. 18 (1995) 313–320.
[117] S. Helbig, S.I. Patzer, C. Schiene-Fischer, K. Zeth, V. Braun, Activation of colicin M by the FkpA prolyl cis-trans isomerase/chaperone, J. Biol. Chem. 286 (2011) 6280–6290.
[118] J. Hullmann, S.I. Patzer, C. Romer, K. Hantke, V. Braun, Periplasmic chaperone FkpA is essential for imported colicin M toxicity, Mol. Microbiol. 69 (2008) 926–937.
[119] E. Cascales, S.K. Buchanan, D. Duche, C. Kleanthous, R. Lloubes, K. Postle, M. Riley, S. Slatin, D. Cavard, Colicin biology, Microbiol. Mol. Biol. Rev. 71 (2007) 158–229.
[120] R.T. Clubb, S.B. Ferguson, C.T. Walsh, G. Wagner, Three-dimensional solution structure of *Escherichia coli* periplasmic cyclophilin, Biochemistry 33 (1994) 2761–2772.
[121] J. Liu, C.M. Chen, C.T. Walsh, Human and *Escherichia coli* cyclophilins: sensitivity to inhibition by the immunosuppressant cyclosporin A correlates with a specific tryptophan residue, Biochemistry 30 (1991) 2306–2310.
[122] J. Pogliano, A.S. Lynch, D. Belin, E.C. Lin, J. Beckwith, Regulation of *Escherichia coli* cell envelope proteins involved in protein folding and degradation by the Cpx two-component system, Genes Dev. 11 (1997) 1169–1182.
[123] U. Weininger, R.P. Jakob, M. Kovermann, J. Balbach, F.X. Schmid, The prolyl isomerase domain of PpiD from *Escherichia coli* shows a parvulin fold but is devoid of catalytic activity, Protein Sci. 19 (2010) 6–18.
[124] S.S. Justice, D.A. Hunstad, J.R. Harper, A.R. Duguay, J.S. Pinkner, J. Bann, C. Frieden, T.J. Silhavy, S.J. Hultgren, Periplasmic peptidyl prolyl *cis-trans* isomerases are not essential for viability, but SurA is required for pilus biogenesis in *Escherichia coli*, J. Bacteriol. 187 (2005) 7680–7686.
[125] Y. Matern, B. Barion, S. Behrens-Kneip, PpiD is a player in the network of periplasmic chaperones in *Escherichia coli*, BMC Microbiol. 10 (2010) 251.
[126] R. Antonoaea, M. Furst, K. Nishiyama, M. Muller, The periplasmic chaperone PpiD interacts with secretory proteins exiting from the SecYEG translocon, Biochemistry 47 (2008) 5649–5656.
[127] S.-H. Cho, J.-F. Collet, Many roles of the bacterial envelope reducing pathways, Antioxid. Redox Signal. 18 (2013) 1690–1698.
[128] K. Denoncin, J.-F. Collet, Disulfide bond formation in the bacterial periplasm: major achievements and challenges ahead, Antioxid. Redox Signal. 19 (2013) 63–71.
[129] K. Ito, K. Inaba, The disulfide bond formation (Dsb) system, Curr. Opin. Struct. Biol. 18 (2008) 450–458.
[130] H. Kadokura, J. Beckwith, Mechanisms of oxidative protein folding in the bacterial cell envelope, Antioxid. Redox Signal. 13 (2010) 1231–1246.
[131] J.C. Joly, J.R. Swartz, *In vitro* and *in vivo* redox states of the *Escherichia coli* periplasmic oxidoreductases DsbA and DsbC, Biochemistry 36 (1997) 10067–10072.
[132] J. Messens, J.F. Collet, Pathways of disulfide bond formation in *Escherichia coli*, Int. J. Biochem. Cell Biol. 38 (2006) 1050–1062.
[133] R.J. Dutton, D. Boyd, M. Berkmen, J. Beckwith, Bacterial species exhibit diversity in their mechanisms and capacity for protein disulfide bond formation, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 11933–11938.
[134] D. Vertommen, M. Depuydt, J. Pan, P. Leverrier, L. Knoops, J.P. Szikora, J. Messens, J.C. Bardwell, J.F. Collet, The disulphide isomerase DsbC cooperates with the oxidase DsbA in a DsbD-independent manner, Mol. Microbiol. 67 (2008) 336–349.
[135] J.C. Bardwell, J.O. Lee, G. Jander, N. Martin, D. Belin, J. Beckwith, A pathway for disulfide bond formation *in vivo*, Proc. Natl. Acad. Sci. U. S. A. 90 (1993) 1038–1042.
[136] H. Kadokura, J. Beckwith, Detecting folding intermediates of a protein as it passes through the bacterial translocation channel, Cell 138 (2009) 1164–1173.
[137] A. Rietsch, P. Bessette, G. Georgiou, J. Beckwith, Reduction of the periplasmic disulfide bond isomerase, DsbC, occurs by passage of electrons from cytoplasmic thioredoxin, J. Bacteriol. 179 (1997) 6602–6608.
[138] A.A. McCarthy, P.W. Haebel, A. Torronen, V. Rybin, E.N. Baker, P. Metcalf, Crystal structure of the protein disulfide bond isomerase, DsbC, from *Escherichia coli*, Nat. Struct. Biol. 7 (2000) 196–199.
[139] F. Katzen, J. Beckwith, Transmembrane electron transfer by the membrane protein DsbD occurs via a disulfide bond cascade, Cell 103 (2000) 769–779.
[140] T. Wu, J. Malinverni, N. Ruiz, S. Kim, T.J. Silhavy, D. Kahne, Identification of a multicomponent complex required for outer membrane biogenesis in *Escherichia coli*, Cell 121 (2005) 235–245.
[141] I.P. Korndorfer, M.K. Dommel, A. Skerra, Structure of the periplasmic chaperone Skp suggests functional similarity with cytosolic chaperones despite differing architecture, Nat. Struct. Mol. Biol. 11 (2004) 1015–1020.
[142] R. Siegert, M.R. Leroux, C. Scheufler, F.U. Hartl, I. Moarefi, Structure of the molecular chaperone prefoldin: unique interaction of multiple coiled coil tentacles with unfolded proteins, Cell 103 (2000) 621–632.
[143] F.A. Saul, M. Mourez, B. Vulliez-Le Normand, N. Sassoon, G.A. Bentley, J.M. Betton, Crystal structure of a defective folding protein, Protein Sci. 12 (2003) 577–585.
[144] E. Kwon, D.Y. Kim, C.A. Gross, J.D. Gross, K.K. Kim, The crystal structure *Escherichia coli* Spy, Protein Sci. 19 (2010) 2252–2259.