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REVIEW

Biogenesis of bacterial inner-membrane proteins

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Abstract All cells must traffic proteins into and across their membranes. In bacteria, several pathways have evolved to enable protein transfer across the inner membrane, the periplasm, and the outer membrane. The major route of protein translocation in and across the cytoplasmic membrane is the general secretion pathway (Sec-pathway). The biogenesis of membrane proteins not only requires protein translocation but also coordinated targeting to the membrane beforehand and folding and assembly into their protein complexes afterwards to function properly in the cell. All these processes are responsible for the biogenesis of membrane proteins that mediate essential functions of the cell such as selective transport, energy conversion, cell division, extracellular signal sensing, and motility. This review will highlight the most recent developments on the structure and function of bacterial membrane proteins, focusing on the journey that integral membrane proteins take to find their final destination in the inner membrane.

Keywords Membrane protein biogenesis · Membrane protein structure · Membrane targeting · Sec complex · Signal recognition particle · Topology · YidC insertase

Introduction

Gram-negative bacteria are surrounded by two distinct membranes: the inner (cytoplasmic) membrane, and outer (periplasmic) membrane. The two membranes, which are separated by a peptidoglycan-containing periplasm, are composed of phospholipids but differ considerably in their structure and composition. The bacterial inner membrane is composed of a symmetrical phospholipid bilayer. The bacterial outer membrane, in contrast, is an asymmetrical bilayer containing mostly phospholipids and lipopolysaccharides in the inner leaflet and in the outer leaflet, respectively. Both membranes contain numerous membrane proteins that are critical for many cellular functions. These proteins play key roles in energy transduction and are involved in the sensing and signal transduction of environmental stimuli as well as the uptake and efflux of substances. The biogenesis of these proteins requires coordinated targeting to the membrane, insertion into or translocation across the membrane, and subsequent assembly into multiprotein complexes. Membrane proteins have to reach their destination either in the inner or the outer membrane and have to be folded correctly in order to function in the cell. To facilitate their targeting, folding, and assembly, a range of molecular chaperones, translocation, and insertion machineries are required. The information of how these membrane proteins reach their final destination is contained within the amino-acid sequence of the protein. In general, proteins with α-helical membrane spanning segments are found in the inner membrane, whereas amphipathic β-barrel proteins (called outer-membrane proteins or Omps) are located in the outer membrane. In *E. coli* approximately 20–30% of all encoded proteins are inner-membrane proteins and approximately 2% are outer-membrane proteins.

Synthesis of membrane proteins

All proteins are synthesized at ribosomes in the cytosol. Since membrane proteins are composed of predominantly hydrophobic amino acids, special attention has to be taken

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after the polypeptide chain leaves the ribosomal tunnel. To prevent aggregation of the hydrophobic parts of the protein in the cytoplasm, chaperone proteins are localized at the exit site of the ribosome, ready to interact with the emerging polypeptide chain. One such chaperone is the trigger factor (TF) that binds to the ribosomal protein L23 [1] (Fig. 1). TF interacts with virtually all emerging nascent chains, especially hydrophobic nascent polypeptides emerging at the ribosomal exit [2, 3]. The chaperone TF is able to prevent premature folding by accommodating newly synthesized polypeptides within its substrate-binding cavity [4]. Subsequently, nascent polypeptides not destined to the inner membrane can interact with the ATP-dependent DnaK and GroEL chaperone systems for further folding of the proteins [5–7]. While *E. coli* can tolerate the deletion of either TF or DnaK, a combined deletion is lethal at temperatures above 30°C because it causes misfolding and aggregation of newly synthesized proteins [8]. In contrast, TF does not generally assist the export of secretory proteins [9]. Presecretory proteins that contain a cleavable signal sequence are assisted by the cytosolic chaperone protein SecB (Fig. 1). SecB binds to unfolded polypeptides after dissociation of TF and delivers them in a translocation-competent conformation to the peripheral membrane component of the protein-secretion apparatus, SecA. It has also been shown that DnaK and GroEL promote the export of several proteins [10, 11]. *E. coli* strains lacking SecB respond by up-regulating DnaK and GroEL and vice versa [12, 13].

Also competing for the same pool of newly synthesized polypeptides is the signal recognition particle (SRP). Likewise, SRP also binds to the ribosomal protein L23 and screens the emerging nascent chain for a hydrophobic signal sequence, such as a transmembrane helix of inner-membrane proteins [14, 15]. If such a sequence emerges, the SRP binds firmly and targets the ribosome-nascent chain (RNC) complex to the Sec complex via an interaction with FtsY, the membrane-associated SRP receptor. In vitro binding and cross-linking studies suggest that TF and SRP are able to bind simultaneously to L23 [16, 17]. It is proposed that TF and SRP sample nascent chains on the ribosome in a nonexclusive fashion, and that binding of FtsY to the ribosome-bound SRP complex excludes TF from the ribosome thereby enabling the docking of the ribosome to the Sec complex [16].

Membrane targeting of proteins

SecB

Most secreted proteins (i.e., proteins that reside in the periplasm or the outer membrane of Gram-negative bacteria) are synthesized as preproteins with a cleavable signal peptide at their N-terminus. These proteins are targeted to the cytoplasmic membrane post-translationally by the molecular chaperone SecB (Fig. 1). The homotetrameric SecB chaperone probably binds to preproteins by recognizing exposed hydrophobic surfaces [18]. While SecB binds to the mature part of a preprotein, the signal sequence is exposed for downstream interactions. The SecB-preprotein complex is then targeted to the Sec complex composed of a peripheral protein, SecA, and the membrane-bound Sec translocase [19]. This interaction is enhanced by binding of the signal sequence to SecA [20]. SecB interacts with the C-terminal domain of SecA (see [21] and references therein). After SecA recognizes the SecB-delivered preproteins, SecB is released from the preprotein [22]. This allows SecB to re-bind to the next newly synthesized nascent secretory protein. The core of the Sec translocase consists of the integral membrane proteins SecY, SecE, and SecG, which constitute a heterotrimeric protein complex. SecA promotes the translocation of the preproteins through the pore by ATP hydrolysis (see [23, 24] for a review). After translocation, the signal peptide is removed proteolytically by the bacterial signal peptidase generating the mature protein. Bacterial signal peptides that target proteins to the secretory pathway are usually characterized by a positively charged amino-terminal (n-) region, a central hydrophobic apolar (h-) region of 7–12 residues and a more polar carboxy-terminal (c-) region that serves as a recognition site for the signal peptidase enzyme [25].

SRP and its receptor

In contrast, most integral membrane proteins do not contain a cleavable signal peptide. The information for integration into the membrane is contained within their hydrophobic transmembrane segments (TMSs). In general, the N-terminal TMS serves as the internal targeting signal or uncleaved signal sequence. The majority of inner-membrane proteins and a few secretory proteins are targeted in a co-translational manner to the SecYEG complex by the SRP and its receptor [26]. The interaction of SRP with the signal peptide is dependent on the hydrophobicity of the h-region [27]. Cross-linking studies have shown that the efficiency of cross-linking to SRP is correlated with the hydrophobicity of the signal sequence [28, 29]. *E. coli* presecretory proteins can be re-routed into the SRP pathway by increasing the hydrophobicity of their signal sequences [30]. In addition to the hydrophobicity of the TMS, basic amino acids are also suggested to promote binding of the signal peptide with SRP through electrostatic interactions [31]. For integral membrane proteins that have a large hydrophilic cytoplasmic domain at their

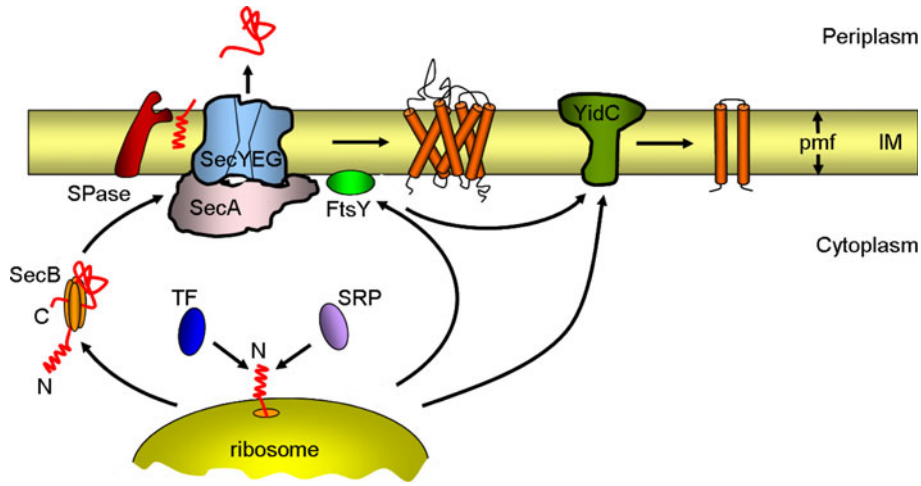

Fig. 1 Schematic overview of bacterial inner-membrane protein biogenesis. Newly synthesized proteins are targeted to the Sec complex either by the signal recognition particle (*SRP*) as soon as they emerge from the ribosome tunnel (co-translational translocation, mainly inner-membrane proteins) or by the tetrameric *SecB* chaperone after translation (post-translational translocation, mainly secretory and outer-membrane proteins). Trigger factor (*TF*) competes with SRP for the binding of the nascent protein. Proteins destined for the inner (*IM*) or outer membrane are transported into or across the inner membrane through the Sec complex. The complex consists of the *SecYEG* protein-conducting channel and the ATPase motor *SecA*. The signal peptidase (*SPase*) cleaves the signal sequence from preproteins at the outer face of the inner membrane. A few membrane proteins insert into the inner membrane via *YidC*. For simplicity, *SecYEG* is shown without its accessory components (SecDFYajC and YidC). *pmf* proton motive force

N-terminus, as in the case of the sensor protein KdpD, the proteins are recognized at an early stage for co-translational targeting. Recent studies have shown that a short amphiphilic sequence at the beginning of the N-terminal cytoplasmic region of KdpD serves as a signal sequence for SRP and is capable of binding to SRP [32]. Most of the analyzed membrane proteins thus far have demonstrated a requirement of SRP for their targeting, including the leader peptidase Lep [33], FtsQ [34], the mannitol permease MtlA [35], SecY [36], and MalF [37].

SRP is conserved in all three kingdoms of life (see [38, 39] for a review). The composition of SRP varies in the different organisms, but the SRP core is a cytosolic ribonucleoprotein particle consisting of the protein SRP54 (Ffh for "fifty-four-homologue" in Bacteria) bound to SRP RNA (helix 8 in Eukarya and Archaea, domain IV in Bacteria) [39, 40]. The bacterial SRP receptor consists of only one protein, FtsY (filamentous temperature sensitive Y), the homologue of the α-subunit of the SRP receptor (SRα) in Eukarya. In Bacteria, there is no known homologue of the eukaryotic β-subunit of the SRP receptor (SRβ), an integral membrane protein that serves to anchor SRα to the membrane. All the bacterial SRP components are essential for cell growth.

SRP binds to the hydrophobic signal sequence of a nascent chain as it emerges from the translating ribosome. The resulting RNC-SRP complex is then targeted to the receptor, FtsY, at the membrane. There an interaction involving two guanosine triphosphatases (GTPases) between SRP and its receptor catalyzes the release of the nascent chain from SRP [41]. The nascent chain, freed from SRP, enters the Sec translocase for insertion into the membrane [38, 42]. After release, SRP can enter the next cycle of protein targeting. The activation of GTP hydrolysis in the targeting complex is essential for protein translocation [43, 44]. In *E. coli*, only a fraction of FtsY is found stably associated with the cytoplasmic membrane [45]. The interaction of FtsY with the cytoplasmic membrane is required for the release of nascent proteins from the SRP-FtsY complex [29, 46]. Although FtsY in *E. coli* lacks a TMS, it binds to membranes by interacting with phospholipids [47–50]. At least two lipid-binding sites have been suggested to exist within FtsY (Fig. 2) [47, 51]. One binding site is located at the N-terminus of FtsY, which probably forms an α-helical conformation (helix 1) [50, 52]. A second binding site is located at the interface between the A- and the N- domain of FtsY, which has been shown to form an amphipathic helix (helix 2) [49]. Although both helices have been shown to interact with anionic phospholipids, it is the cooperative action of the two lipid-binding helices that allows a very stable membrane contact with FtsY [50]. Only helix 2 is essential for FtsY function [49]. Removal of helix 1 reduces the stability of the FtsY membrane contact but only slightly affects the FtsY function [50, 52]. Besides the interaction with membrane lipids, FtsY also interacts directly with the Sec complex most likely via its conserved NG-domains [53, 54].

Both Ffh and the receptor, FtsY, are multi-domain proteins (Fig. 2). Ffh is composed of three domains, an amino-terminal N domain, a central GTPase G domain, and

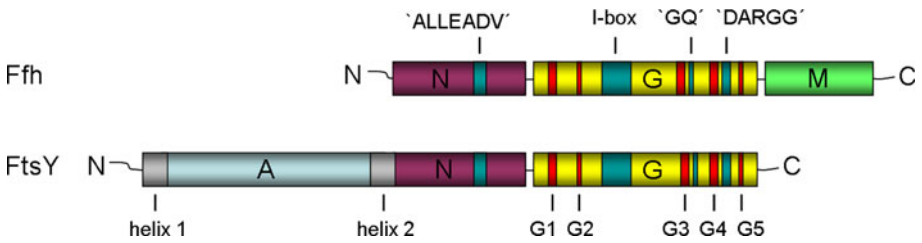

Fig. 2 Schematic representation of the three-domain structure of Ffh and FtsY of *E. coli*. The domains are indicated with different colors: N domain *purple*, G domain *yellow*, M domain *green*, and A domain *light blue*. The five consensus elements responsible for GTP binding and hydrolysis (G1–G5) are highlighted in *red*, whereas the I-box and the inter-domain communication motifs (ALLEADV, DARGG, GQ) are indicated in *blue*. The two lipid-binding sites of FtsY are shown in *grey*

a methionine-rich carboxy-terminal M domain that binds both SRP RNA and signal peptides [39]. FtsY also comprises three domains, an acidic amino-terminal A domain, a central N domain and a carboxy-terminal GTPase G domain. The NG domains of Ffh and FtsY exhibit high sequence similarity to each other, and the crystal structures of the NG domains of Ffh from *Thermus aquaticus* and of FtsY from *E. coli* revealed a high degree of structural similarity [55, 56]. In Ffh and FtsY, the N domain comprises a four-helix bundle and is closely associated with the adjacent G domain. The G domain shows the typical Ras-like GTPase fold with five conserved elements (G1–G5) for nucleotide binding and hydrolysis and an additional α-β-α insertion (I-box or IBD) motif that is unique to SRP GTPases [55]. Inter-domain communication between the N and G domain in Ffh and FtsY involves the ALLEADV motif in the N domain, and the GQ and the DARGG motifs in the G domain (see [40] for a review). The M domain of Ffh is involved in binding the signal sequence of a substrate protein [57] and accommodating it in its hydrophobic groove [58]. The crystal structure of the *E. coli* Ffh M domain bound to the fragment of the SRP RNA showed that a part of the conserved IV domain of the 4.5S RNA lies adjacent to the hydrophobic groove in the M domain and appears to create an extended signal peptide-binding pocket [58]. This suggests that this binding site composed of both protein and RNA would accommodate a signal peptide through a combination of hydrophobic interactions and electrostatic contacts. The presence of a signal sequence in the M domain could trigger the release of the translocating protein into the translocation pore of SecYEG [59].

During the last few years, a number of structures of SRP with its interaction partners, the ribosome [60] and FtsY [61, 62], have been solved using X-ray and cryo-electron microscopy. The crystal structure of the Ffh-FtsY complex from *Thermus aquaticus* revealed that the two GTPases align almost parallel in a symmetrical arrangement that brings their two active sites together to form a shared active site at the interface between them [61, 62]. Although significant progress has been made, the atomic structure of the SRP-RNA complex with a bound signal peptide is required to put the pieces together to show how Ffh and its receptor guide a nascent chain into the translocation pore.

Membrane insertion of proteins

The secretory pathway

The general secretory (Sec) pathway is engaged in translocating both secretory proteins across the membrane as well as integrating proteins into the cytoplasmic membrane (Fig. 1). Depending on the substrate, the Sec complex has to switch between two operational modes: a transversal opening for allowing secretory proteins across to the periplasm and a lateral opening for the insertion of TMSs. The SecYEG complex is highly conserved and homologues have been found in Eukaryotes (Sec61p, comprising α, γ and β-subunits) and Archaea (SecYEβ) [63]. The peripheral SecA protein is the motor subunit of the SecYEG complex. It is required for the translocation of secretory proteins and insertion of inner-membrane proteins with large ($\geq$60 amino acids) hydrophilic periplasmic domains [64, 65]. SecA like FtsY also binds to membrane phospholipids [66], and to the Sec complex via a direct interaction with SecY [67]. SecA appears to bind to anionic lipids with its C-terminal domain [68].

The SecA ATPase is a multi-domain protein that contains two nucleotide-binding domains (NBD1 and NBD2), a polypeptide crosslinking domain (PPXD), an α-helical wing domain (HWD), and an α-helical scaffold domain (HSD) [69]. The crystal structure of SecA bound to the SecY complex from *Thermotoga maritima* revealed a "two-helix finger" formed by two helices of SecA's HSD inside the cytoplasmic funnel of the SecY channel [70]. It is suggested that a polypeptide chain moves from the tip of the two-helix finger of SecA into the SecY pore by movements inside the cytoplasmic funnel during the ATP hydrolysis cycle [71] (Fig. 3). SecA binds in its

ATP-bound state to the polypeptide chain, pushes it into the SecY pore, and then releases it in its ADP-bound state [72, 73]. Backsliding of the chain is reduced by its interactions with the SecY pore. Repeated cycles of ATP binding and hydrolysis, as well as polypeptide binding and release, result in the stepwise translocation of the polypeptide across the membrane. Each additional ATP hydrolysis cycle is accompanied by the translocation of 20–30 residues of polypeptide [74]. After completion of translocation, SecA can dissociate from the SecYEG complex. Once polypeptide translocation has been initiated, the proton motive force (pmf) can further drive the reaction in the absence of ATP when SecA is not bound with the translocating polypeptide at SecYEG [75]. Although it is generally accepted that SecA is dimeric in the cytosol, there is controversy concerning the oligomeric state of SecA during active protein translocation. Likewise, the stoichiometry of the SecYEG heterotrimers within the active translocase complex is still a topic of debate. Numerous biochemical [76–78] and structural [79–81] studies demonstrate that the SecYEG complex (and the Sec61p complex) forms higher-order oligomers, predominantly dimers and tetramers. Electron microscopic images of purified Sec complexes from different species revealed ring-like structures containing between two and four copies of the Sec complex [79, 80]. Similar to the bacterial system, multimeric ring-link structures were also observed for the Sec61p complex [82]. Further biochemical analysis by blue native gel electrophoresis, fluorescence resonance energy transfer, and chemical cross-linking revealed that the SecYEG complex exists as a monomer or dimer [76, 83, 84]. A recent cross-linking study of the *E. coli* SecA-SecYEG system proposes that in a SecYEG dimer one copy of SecYEG acts as a docking station for SecA, whereas the other copy is used as the translocation pore [85]. If a single copy of the Sec complex can form a translocation pore for exiting polypeptides, what is the role of oligomerization? The answer is not yet known, but one possibility is that the formation of monomeric, dimeric, and tetrameric states of the SecYEG complex (and the Sec61p complex) might be influenced by its interaction with ligands, like SecA or the ribosome [80–82].

SecY, the pore-forming component of the complex, consists of ten transmembrane (TM) helices (TM1–TM10), whereas SecE has three transmembrane helices and SecG only two transmembrane helices in *E. coli* [86]. SecY and SecE are essential for viability in *E. coli*, whereas SecG is not required for cell viability at 37°C. SecY forms a stable complex with SecE that protects it from degradation by the membrane-bound protease FtsH [87, 88]. The crystal structure of the SecYEβ complex from the Archaeon *Methanococcus jannaschii* in a closed state revealed an hourglass-shaped channel with aqueous funnels and a ring of hydrophobic amino acids at its constriction near the center of the membrane within the SecY subunit [89]. The external funnel is gated by a plug formed by a short helix (helix 2a) (Fig. 3). This plug hinders the passage of small molecules during translocation. It was proposed that when the channel opens for polypeptide translocation, helix 2a swings outward, opening the extracellular channel. The TM2b, TM3, TM7, and TM8 regions of SecY were proposed to comprise the lateral gate, for the exit of hydrophobic segments of newly synthesized proteins. Cross-linking data revealed that the translocating chain mainly contacts residues in the narrowest part of the hourglass-shaped channel [90]. Recently, another crystal structure of the SecYE complex from *Thermus thermophilus* was resolved [91]. This crystal structure in a "pre-open" state identified a SecA-SecYE interface that comprises SecA contacting the SecYE protein.

SecD, SecF, and YajC are additional translocase subunits that can interact with the SecYEG core [92]. Both SecD and SecF consist each of six TMSs in *E. coli* [93]. The single-spanning protein YajC, which has no known function in the Sec complex, has been found associated also with AcrB, which is a component of the multidrug efflux complex AcrB:AcrA:TolC [94]. Both SecD and SecF facilitate membrane insertion and export of several proteins [95, 96].

YidC

Not all inner-membrane proteins use SecYEG for insertion, a small subset of integral membrane proteins are targeted to YidC where they are inserted into the membrane in a Sec-independent manner (Fig. 1). This alternative Sec-independent insertion pathway is used by a number of small inner-membrane proteins, including the phage coat proteins M13 [97] and Pf3 [98], the endogenous inner-membrane proteins F_oc (subunit *c* of the F_1F_o-ATP synthase) [99], and MscL (mechanosensitive channel of large conductance) [100]. A common feature of these membrane proteins is their small size and the presence of short hydrophilic periplasmic domains. YidC can also function together with the Sec complex to promote membrane insertion of larger proteins. YidC has been shown to interact with the Sec complex via the SecDFYajC complex [101, 102]. In the Sec-dependent pathway, YidC most likely acts downstream of the Sec channel in the lateral transfer of TMSs from the Sec pore into the lipid bilayer [103–105]. Photo-cross-linking studies using ribosome-bound nascent chains of the single-spanning membrane protein FtsQ revealed that FtsQ first binds to SecY and at a later stage moves towards YidC [105]. The in vivo depletion of YidC shows only a minor effect on the Sec-dependent proteins requiring YidC such as Lep [97], FtsQ [105], and MtlA [104]. Recent studies on

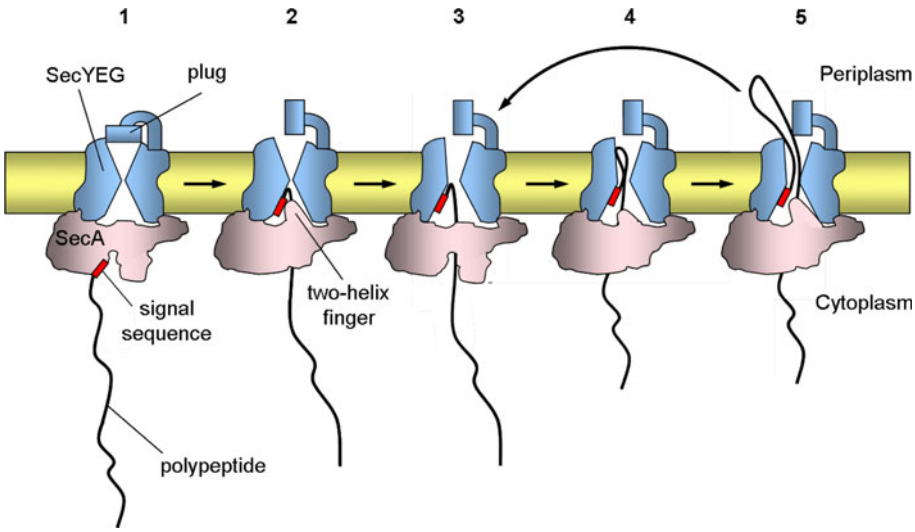

Fig. 3 SecA-mediated protein translocation through the SecY pore. The scheme shows a model for the different steps in translocation. *1* SecA binds to a polypeptide substrate bearing an N-terminal signal sequence. *2* SecA opens toward SecY and transfers the signal sequence and early mature region of the polypeptide into SecY with the help of the two-helix finger. The polypeptide inserts as a loop structure. This step requires ATP hydrolysis. The insertion of the signal sequence of the polypeptide into SecY causes the plug to move away from the pore, which leads to a fully activated channel. *3* For further translocation, SecA releases the polypeptide into the pore and the helix finger is pulled back and reset. *4* SecA binds the next section of the polypeptide and moves toward the translocationally active SecY. *5* SecA then pushes the next section of the polypeptide into the pore by the helix finger. The signal sequence remains stationary, while the mature part of the polypeptide passes through the pore. These steps are coupled to ATP hydrolysis cycles and steps *3–5* are repeated until the polypeptide is fully translocated (not shown). For simplicity, SecA and SecYEG are shown as monomers and the initial stages of the process with SecB are not shown. The *thick black line* represents the polypeptide and the *red rectangle* represents the N-terminal signal sequence

the biogenesis of the endogenous *E. coli* inner-membrane protein CyoA (subunit II of the cytochrome bo_3 ubiquinol oxidase complex) have shown that YidC can also function upstream of the Sec complex. The N-terminal region of CyoA was shown to require YidC, whereas the large C-terminal periplasmic domain was Sec-dependent [106, 107].

YidC was first identified in bacteria based on the sequence homology with Oxa1 in mitochondria (see [108] for a review). In addition to bacteria, YidC homologues are also found in mitochondria (Oxa1 and Cox18/Oxa2) [109, 110] and chloroplasts (Alb3 and Alb4) [111, 112], where they all are thought to promote membrane insertion. The YidC homologues Oxa1, Cox18, Albino 3, and Albino 4 have been shown to complement YidC insertase activity when expressed in *E. coli* [113–116]. Unlike the eukaryotic YidC homologues, which span the membrane five times, *E. coli* YidC spans the membrane six times, and has a large periplasmic loop (P1) between its first two TMSs. Multiple sequence alignments reveal that the second, third, and fifth TMSs of YidC contain conserved residues in the YidC/Oxa1/Alb3 family [117]. Site-specific cross-linking revealed that the conserved third TMS of YidC contacts the first TMS of both nascent Sec-dependent and Sec-independent substrates [118]. In addition, the TMS of the coat protein of bacteriophage Pf3 can be cross-linked in vivo to the first and third TMS of YidC shortly after synthesis [119]. The membrane insertion of Pf3 coat protein can also be reconstituted with proteoliposomes containing only YidC. This demonstrates that YidC functions catalytically as a membrane insertase [120].

YidC seems to also play a role as a membrane chaperone for more complex membrane proteins by assisting in the release of transmembrane helices from the Sec complex, in helix packing, and in the proper folding of polytopic membrane proteins [121]. An in vitro study using lactose permease (LacY) suggests that YidC functions in the co-translational folding of this inner-membrane protein [122]. In the absence of YidC, it appears that LacY cannot achieve its final tertiary structure because the newly synthesized protein is not correctly inserted in the bilayer to fold properly. In addition, YidC is also required for the folding and assembly of the inner-membrane protein MalF, which is part of the maltose transport complex ($MalFGK_2$) in *E. coli* [37]. The importance of YidC in membrane protein folding and assembly is also suggested by the observation that YidC depletion leads to the induction of the Cpx stress response pathway, which senses protein misfolding of membrane proteins [123]. In addition, both the phage shock protein family and the cold shock protein family genes, especially *pspA* and *cspA*, are highly up-regulated following YidC depletion [124]. The

depletion of YidC also affects the pmf by hindering the functional assembly of F_1F_o-ATPase and the cytochrome *o* oxidase [125].

The integration of YidC itself into the inner membrane of *E. coli* requires SecYEG and the coordinated activity of both SRP and SecA [126]. Although SecA does not contribute to the SRP-targeting pathway, when long, hydrophilic periplasmic domains are encountered, as in the case of the large periplasmic P1 domain of YidC, SecA is required to catalyze their translocation across the membrane.

Folding and assembly of membrane proteins

Less understood is the folding and assembly of polytopic membrane proteins into their final tertiary conformation and, thus helix orientation and packing arrangements. Many integral membrane proteins function in multi-protein complexes including the Sec complex, the F_1F_o ATP synthase complex, the ABC-transporters and the respiratory chain complexes. After membrane insertion, the subunits of these membrane protein complexes must locate each other and assemble correctly to function properly in the cell. If a polypeptide is unstable and fails to assemble correctly, the proteins in the complex are often degraded (see [127] for a review). Protein misfolding triggers specific responses such as the expression of chaperones, proteases, and other folding catalysts to counteract protein misassembly. Protein misfolding and aggregation lead to a significant reorganization of the membrane and lipid composition in the cell [128]. The folding of the outer membrane protein (OmpA) is strongly dependent on the biophysical properties of the membrane [129]. Mechanosensitive channels such as the widely studied MscL, whose function is to respond to mechanical stress applied to the cell membrane, have altered activities in different bilayers [130, 131].

In *E. coli*, FtsH is a cytoplasmic membrane protein and ATP-dependent zinc metalloprotease involved in the degradation of unstable membrane proteins. FtsH is known to play a central role in membrane protein quality control. It degrades the SecY subunit of the protein-conducting channel [88] and subunit *a* of F_1F_o ATP synthase [132] when they exist in unassembled states in the membrane. Another membrane protein degraded by FtsH is YccA, which spans the membrane seven times [133]. FtsH can initiate processive proteolysis of membrane protein substrates by recognizing their ends when they protrude sufficiently into the cytosol [134, 135]. The ATP-dependent protease starts proteolysis from a specific initiation site on the substrate protein and then continues to degrade the entire molecule of the substrate [127].

Phospholipids and the composition of the lipid bilayer play an important role in the regulation of integral membrane protein folding and function. The natural lipid bilayer environment consists of a hydrophobic interior region and a polar or charged headgroup region with aqueous solvent on either side of the bilayer. Integral membrane protein must interact with all these different solvents. At the same time, the lipids form a tight seal around the membrane proteins so that proton and chemical gradients are maintained across the membrane. The cytoplasmic membrane of *E. coli* contains ~70–75 mol% phosphatidylethanolamine (PE) lipids, with the rest being dominated by anionic phosphatidylglycerol (PG, 20%), and to a lesser extent cardiolipin (CL, 5%). Certain lipids such as PE are required for correct topology of integral membrane proteins in *E. coli* [136–139]. In the absence of PE, defects occur in the function and topological organization of LacY [136] (Fig. 4a). LacY consists of 12 transmembrane helices with both the N- and C-termini exposed to the cytoplasm. LacY inserts into the membrane in the absence of PE in an inverted orientation of the first six transmembrane helices and associated extramembrane domains with respect to the membrane bilayer [136, 139]. This LacY protein is able to carry out facilitated, but not active, transport [140]. A similar requirement for PE was observed with phenylalanine permease (PheP) [137] and γ-aminobutyric acid permease (GabP) [138]. For both proteins, assembly in the absence of PE resulted in the inversion of their N-terminal TMSs and their adjoining cytoplasmic and periplasmic domains.

Changes in lipid composition also affect the lateral pressure profile of the membrane. An increase in PE, which affects the chain lateral pressure within the bilayer, lowers the folding yield of bacteriorhodopsin [141] and the multidrug-proton antiporter EmrE [142]. The increased lateral chain pressure caused by PE lipids has been found to decrease the rate of insertion of a transmembrane helix or protein across a bilayer. Furthermore, anionic lipids were found to be important in the membrane interactions of SecA [66, 143, 144].

The respiratory chain complexes

A prominent family of membrane protein complexes is the members of the respiratory chain that enable cells to oxidize various chemical compounds and collect the energy in a transmembrane potential. In eukaryotic cells, these complexes are exclusively localized in the mitochondrial membrane, which pinpoints to their bacterial origin. Bacteria have evolved a variety of different respiratory chains that vary from sulfate to nitrate, fumarate, and oxygen as their terminal electron acceptors. Each system, expressed

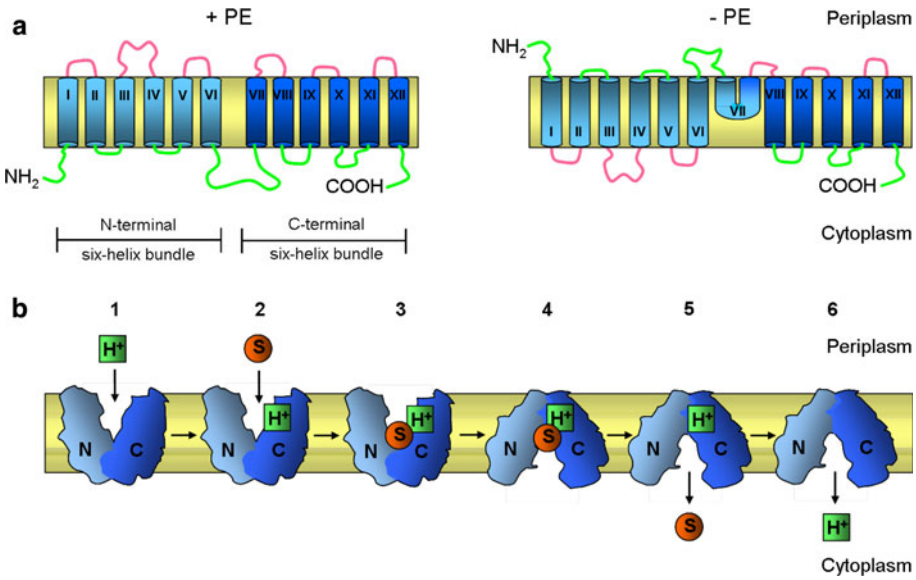

Fig. 4 Membrane topology and function of lactose permease (LacY) of *E. coli*. **a** Topological organization of LacY in *E. coli* membranes with (+*PE*) and without (−*PE*) phosphatidylethanolamine (*PE*). LacY consists of 12 transmembrane helices divided into two six-helix bundles. In the absence of PE (−*PE*), the N-terminal six-helix bundle with their associated extramembrane domains adopts an inverted topology with respect to the membrane bilayer, whereas the C-terminal six-helix bundle, except for TM VII, retains its native topology. The topological organization of TM VII in −PE cells remains unknown. The transmembrane helices are labeled in roman numerals consecutively from the N-(NH_2) to C-(COOH) terminus. The cytoplasmic (*green*) and periplasmic (*pink*) extramembrane domains are indicated. **b** Schematic representation of conformational changes in LacY upon sugar binding. LacY uses a symport mechanism to couple the transport of lactose and H^+ across the membrane. The proposed mechanism of transport alternates between the outward-facing conformation (exposed to the periplasmic side) shown in steps 1–3 and the inward-facing conformation (exposed to the cytoplasm) shown in steps 4–6. Starting from the outward-facing conformation *1* LacY is protonated *2* prior to substrate binding. *3* The binding of substrate to the binding site probably induces a conformation change in the two halves of the protein (N- and C-terminal six-helix bundles) that results in the inward-facing conformation. *4* The substrate is then released into the cytoplasm *5* followed by release of the H^+. *6* After releasing the H^+ inside, LacY returns to the outward-facing conformation *1*. The N- and C-terminal six-helix bundles of LacY are colored *light* and *dark blue*, respectively. Substrate and H^+ are represented by *orange circles* and *green squares*, respectively

in certain bacterial strains, provides the metabolic basis for their existence in distinct ecological niches.

The respiratory chain complexes are localized in the inner membrane. Depending on the availability of oxygen, *E. coli* expresses two different NADH dehydrogenases, NDH-1 and NDH-2, and two different ubiquinol oxidase complexes, cytochrome bo_3 complex (contains heme *b* and heme *o*) and cytochrome *bd* complex (contains heme *b* and heme *d*). The bo_3 ubiquinol oxidase from *E. coli* contains four subunits, CyoABCD (or subunits II, I, III, IV, respectively), and is the predominant oxidase when the oxygen levels are high, whereas under low-oxygen conditions, the *bd* ubiquinol oxidase is more abundant.

When oxygen is limiting, the proton-coupled dehydrogenase NDH-1 is expressed that consists of seven transmembrane and six peripheral subunit proteins designated as NuoA to NuoN, where NuoC and NuoD are fused proteins in *E. coli* [145]. NDH-1 is one of the largest protein complexes in the bacterial membrane with a total mass of ~550 kDa. Electron microscopy has shown that the protein complex has an L-shaped structure, with the hydrophobic arm embedded in the membrane and the hydrophilic peripheral arm protruding into the cytoplasm [146, 147]. Recently, the crystal structure of the hydrophilic peripheral arm of NDH-1 from *Thermus thermophilus* has been solved [148]. The crystal structure revealed that this subcomplex consists of eight subunits and contains all the redox centers of the enzyme, including nine iron-sulfur clusters. The assembly of the NDH-1 complex is impaired in YidC-depleted cells [149], suggesting that YidC is involved in the insertion or assembly process. In contrast, NDH-2 consists of a single protein, NdhA, of 434 amino acids and is not capable of translocating protons across the membrane. It is presumably membrane-anchored at the C-terminus [150]. So far, no tertiary structure is available for NDH-2. Both enzymes oxidize NADH and reduce ubiquinone to ubiquinol.

The bo_3 ubiquinol oxidase is a terminal oxidase in *E. coli* that is closely related to the cytochrome oxidase of mitochondria. The structure of the complex has been solved at 3.5 Å resolution, which allows to follow the path of the electron from the bound ubiquinol to the

oxygen [151]. It involves a heme *b*, a heme o_3 and a copper center (Cu_B) in the CyoB subunit (subunit I) that is a 15-spanning membrane protein. The ubiquinol binding site is localized in CyoA (subunit II) that has two transmembrane helices and an N-terminal lipid moiety. In the bo_3 ubiquinol oxidase, the subunits CyoC (subunit III) and CyoD (subunit IV) span the membrane five and three times, respectively. The membrane insertion of CyoA has been extensively studied and involves YidC to translocate the first periplasmic loop and SecYEG to translocate the C-terminal region [106, 107]. The protein is synthesized with a signal sequence that is cleaved by signal peptidase II after its acylation [152]. Depletion of YidC also revealed a reduction in the level of CyoB in the inner membrane of *E. coli* [125]. Recently, the assembly pathway of bo_3 ubiquinol oxidase of *E. coli* was proposed to occur in a preferred order, in which subunits CyoC and CyoD assemble first, followed by CyoB and CyoA [153].

In *E. coli*, anaerobic respiration occurs with various systems, all involving a NADH dehydrogenase and ubiquinol [154]. Ubiquinol is oxidized by the fumarate reductase, FrdABCD, whereby the C and D subunits are three-spanning integral membrane proteins that have their N-termini located in the cytoplasm and their C-termini in the periplasm [155]. The remaining two subunits, the flavoprotein FrdA and the iron protein FrdB, comprise the cytoplasmic part of the complex. Since in YidC-depleted cells fumarate reduction is inhibited and the assembly of FrdABCD is strongly reduced, the insertion of subunits C and D might require YidC [149]. Likewise, the nitrate reduction is hampered in the absence of YidC. In *E. coli,* the nitrate reductase is composed of three subunits, NarGHI, where NarI spans the membrane five times and harbors two *b*-type cytochromes that transport the electrons to the subunits NarH and NarG at the cytoplasmic face of the membrane [156].

The photosynthesis complexes

Since photosynthesis is an evolutionary old process that converts solar energy into chemical energy, a number of bacteria have relatively simple systems when compared to plants. In particular, the photosynthetic systems in purple bacteria, such as *Rhodopseudomonas viridis* and *Rhodobacter sphaeroides* have been extensively investigated. The photosynthetic reaction center was one of the first membrane protein complexes to be crystallized and structurally characterized [157, 158]. It consists of three protein subunits, the M, L and H proteins. Two of these protein subunits, M and L, span the membrane five times and contain carotenoid and bacteriochlorophylls. Only the L-linked bacteriochlorophylls transfer the electrons to the bound ubiquinone.

Light-harvesting complexes (LHC) are present in photosynthetic membranes to increase the efficiency of light energy harvesting. In *Rhodospirillum rubrum*, the LHC surrounds the reaction center as a ring structure composed of two small proteins, the α and β subunits. Both are single-spanning proteins that assemble into ring structures of 16 heterodimers [159]. Bacteriochlorophylls and carotinoids are bound to the heterodimers that absorb the light and transfer the excitation energy to the reaction center.

The evolution of the photosynthetic system in cyanobacteria to use water as a source of electrons was made possible by combining two reaction centers in tandem, the photosystem II and I. A wealth of structural data demonstrate the complexity of these systems in *Synechococcus* (see [160] for a review). Recent studies have shown that the cpSRP (SRP in chloroplasts) and the YidC homologue (Alb3) are important for the assembly of several light-harvesting chlorophyll-binding proteins into thylakoid membranes [111, 161]. In the cyanobacterium *Synechocystis* sp. PCC6803, a deletion of the YidC homologue causes severe defects in the assembly of the photosystems [162].

The ATP synthase complex

The F_1F_o ATP synthase of *E. coli* is comprised of two subcomplexes, the membrane-integral F_o complex ($a_1b_2c_{10}$)

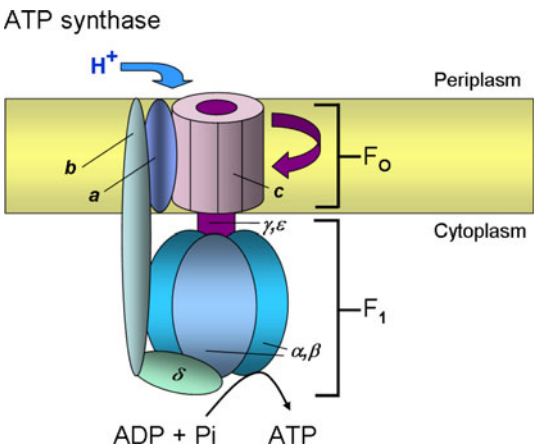

Fig. 5 Model of *E. coli* F_1F_o-ATPase. The ATP synthase consists of two subcomplexes with eight different proteins, the membrane-integral F_o complex ($a_1b_2c_{10}$) and the peripheral F_1 complex ($\alpha_3\beta_3\gamma\delta\varepsilon$). The *c* subunit of F_o is linked to the γ and ε subunits to form the central rotor (*purple*). Subunits *b* and δ form a stator which ensures that subunits *a* in F_o and the $\alpha_3\beta_3$ hexamer of F_1 do not rotate with the central rotor ($\gamma\varepsilon c_{ring}$). The proton pathway lies between the *a* and *c* subunits. Either H^+ translocation through F_o or ATP hydrolysis in F_1 leads to the rotary movement of the central rotor element ($\gamma\varepsilon c_{ring}$)

and the peripheral F_1 complex ($\alpha_3\beta_3\gamma\delta\varepsilon$) (Fig. 5). The ATP synthase plays a key role in biological energy metabolism. The F_1F_o ATP synthase converts the energy stored in a transmembrane electrochemical gradient of protons or Na^+ ions into ATP or generates a transmembrane ion gradient at the expense of ATP hydrolysis under conditions of low driving force. While the peripheral membrane F_1 complex synthesizes or hydrolyzes ATP, the integral membrane F_o complex serves as a proton pump that is sensitive to the antibiotic oligomycin. The crystal structure of the mitochondrial F_1 complex has been solved at high resolution [163]. Recently, two crystal structures of the rotor ring from a Na^+-translocating bacterium, *Ilyobacter tartaricus* [164] and from a proton-translocating cyanobacterium, *Spirulina platensis* [165] have been solved. The complete structure of the ATP synthase complex of *E. coli* has been modeled [166].

Subunit *a* (F_oa) of the F_o complex is a polytopic membrane protein with five TMSs with a N-out/C-in orientation. Subunit *b* (F_ob) is a single-spanning membrane protein with a short N-terminal region located in the periplasm and a longer C-terminus in the cytoplasm. Subunit *c* (F_oc) consists of two transmembrane helices that form a helical hairpin. The short N- (eight residues) and C-termini (three residues) are located in the periplasm. The subunit *c* forms an oligomeric ring in the membrane that rotates in response to the pmf. In the center of the c ring, the $\gamma\varepsilon$ proteins bind and extend the rotation into the peripheral F_1 complex. Two *b* subunits and one *a* subunit form the membrane-embedded stator that binds to the peripheral $\alpha_3\beta_3$ complex via the δ protein.

Recent studies have shown that the membrane insertion of F_oc is strictly dependent on YidC [99, 167, 168], whereas F_oa and F_ob of the F_1F_o ATP synthase require both YidC and SecYEG for membrane insertion [169, 170]. The membrane insertion of F_oa and F_ob is also dependent on SRP [169, 170]. The translocation of the N-terminal tail of F_oa requires the pmf, whereas the translocation of the second periplasmic loop is pmf-independent [169]. Previous studies showed that F_oa is unstable in the absence of the partner molecules, F_ob and F_oc, and is readily degraded by the ATP-dependent membrane protease FtsH [132, 171].

The positive-charged residues in the cytoplasmic loop (M-region) of F_oc appear to be important determinants for YidC binding and subsequent membrane insertion [172]. Removal of these M-region charges affects the binding of F_oc to YidC and membrane insertion. Previously, it had been demonstrated that proteoliposomes containing only YidC support both the stable membrane insertion and oligomerization of F_oc [99].

The permeases

Transmembrane ion gradients are used to transport nutrients and other solutes into the interior of the bacterial cell. In contrast to primary active transporters, which utilize a primary energy source (i.e., ATP hydrolysis, photon absorption or electron flow) to catalyze the transport of substrates, secondary active transporters utilize the free energy stored in the electrochemical ion gradient to drive the transport of substrates. These secondary transporters include the permeases which are operating either as symporters or antiporters, respective of their transport direction and ion movement. The most prominent permease is the lactose permease of *E. coli* that transports β-galactosides with protons. A crystal structure of LacY [173] combined with a wealth of biochemical data (see [174] for a review) has led to an alternating access model for lactose/H^+ symport (Fig. 4b). The N-terminal and C-terminal half of LacY fold into two pseudosymmetrical six-helix bundles. The interface of these N- and C-terminal six-helix bundles forms an internal hydrophilic cavity accessible to one side of the membrane [173]. The crystal structure shows the sugar-binding sites in detail in the center of the hydrophilic cavity. A possible mechanism of transport is suggested by first binding a proton, then binding of lactose followed by a conformational change where the N- and C-terminal six-helix bundles move to close the periplasmic opening and open a cytoplasmic cavity. The substrate and the proton are then released and the conformation may return to the outward-facing cavity. Proton translocation involves residues primarily in the C-terminal six-helix bundle. Intriguingly, the presence of the substrate on the periplasmic side controls the opening of the outward cavity [175]. The basic structure and transport mechanism observed for the lactose permease is likely to be valid for other permeases and members of the major facilitator superfamily (MFS) of membrane transporters [176]. These include the multidrug transporters AcrB and EmrD [177, 178], the melibiose transporter MelB [179], and GlpT [180], a glycerol phosphate antiporter.

LacY is targeted to the membrane co-translationally by the SRP pathway and requires the SecYEG complex for insertion into the inner membrane (see [122] and references therein). The Sec complex anchors the protein in the membrane as verified by its resistance to urea extraction. YidC does not appear to be required for the insertion of LacY into the membrane, but is important in the folding of LacY into its final tertiary conformation in the membrane [122]. In vitro experiments with monoclonal antibodies, directed against conformational epitopes on LacY, revealed that YidC is required for exposing the antigenic epitopes of LacY in the correct conformation.

Other secondary transporters that support the alternating access model for membrane transport are the Na^+-coupled symporters LeuT (Na^+/leucine transporter) [181], BetP (Na^+/betaine transporter) [182], vSGLT (Na^+/galactose transporter) [183], and Mhp1 (benzyl-hydantoin transporter) [184]. Crystal structures of the different Na^+ symporters revealed a similar overall architecture in the structures, which is not reflected in sequence similarity. Although their number of transmembrane helices differ from 12 (LeuT, BetP, Mhp1) to 14 (vSGLT), they all share a core structure of inverted repeats of five transmembrane helices each. The structures of these different Na^+ symporters indicate an alternating mechanism of transport involving at least three states: open to outside, occluded, and open to inside. The different conformations interconvert to bring about membrane transport. To date, nothing is known about the synthesis, insertion, and assembly of these transporters. Most likely, they are operating as monomers in the membrane. However, BetP is organized as trimers and it has been shown that the C-terminal region and helix seven of one monomer make contact to the next monomer [182].

The ABC transporters

ATP-binding cassette (ABC) transporters are a large family of membrane protein complexes that use the binding and hydrolysis of ATP to power the transport of solutes across the membrane [185, 186]. The ATP-driven transporters are three component systems, involving a periplasmic substrate-binding protein, a transmembrane transporter unit, and a cytoplasmic ATP-binding component. These primary transporters are mainly involved in sugar and amino-acid uptake, but some are known to export lipo-oligosaccharides, lipidA, and teichoic acids [187]. In bacteria, the majority of ABC transporters are importers. The best-studied ABC transporter is the maltose importer $MalFGK_2$ (Fig. 6).

The maltose transporter $MalFGK_2$ of *E. coli* is composed of two integral membrane proteins, MalF and MalG, which form the translocation pathway, and two copies of the ATP-binding cassette subunit MalK [188]. A periplasmic maltose-binding protein (MBP), MalE acts as a maltose receptor in delivering maltose to $MalFGK_2$. The MBP also stimulates the ATPase activity of $MalFGK_2$ [189]. The crystal structure of the intact transporter $MalFGK_2$ with the MBP was determined to high resolution in an outward-facing conformation (open to the periplasm) [190] and recently, another structure of this complex has been resolved in an inward-facing conformation (open to the cytoplasm) where both ATP-binding sites of the MalK dimer are closed with ATP [191]. Comparison of the two structures of the transporter reveals details of the transport mechanism for ABC

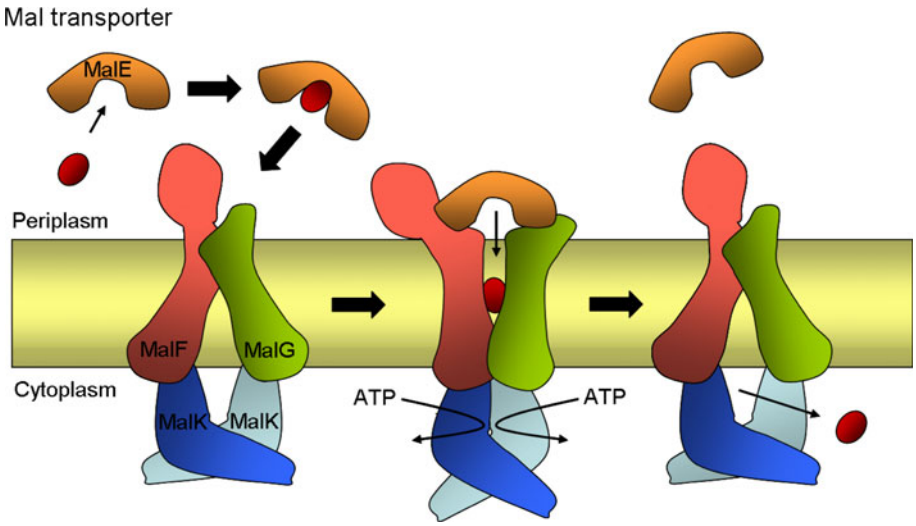

Fig. 6 Schematic drawing of inward- and outward-facing structures of the maltose transporter, $MalFGK_2$, of *E. coli*. The maltose transporter complex is composed of a periplasmic maltose-binding protein (*MalE*), two integral membrane proteins, MalF and MalG, and two copies of the cytoplasmic ATP-binding cassette subunit MalK. In the transport cycle, MalE binds and delivers maltose to the resting state transporter in the closed conformation (*shown on the left*). Interactions with MalE in the presence of maltose stimulate ATPase activity of the transporter by triggering major conformational changes in which the two cytosolic MalK subunits close and the transmembrane helices of MalF and MalG reorient to receive the substrate from MalE (*shown in the middle*). After ATP hydrolysis, the outward-facing conformation is no longer stable and the transporter returns to the inward-facing resting state releasing maltose (*shown on the right*). Maltose is indicated by a *red sphere*

importers, which involves global re-arrangements of the integral membrane proteins MalF and MalG with the closure and opening of the ATP-binding sites of the MalK dimer.

The integral membrane subunit MalF consists of eight TMSs with both N- and C-termini located in the cytoplasm [192]. MalF includes a large periplasmic domain (MalF-P2) between the third and the fourth TMS. In the crystal structure, MalF-P2 is in contact with MalE [190]. For its biogenesis, MalF is targeted to the inner membrane via the SRP pathway and insertion is SecAYE-dependent [37]. YidC is required for the folding stability of MalF before it is incorporated into the $MalFGK_2$ complex and contacting the six-spanning MalG subunit [37].

PTS transporters

In bacteria, the transport of some sugars across the membrane is mediated by the phosphoenolpyruvate dependent phosphotransferase system (PTS) (see [193] for a review). PTS is a bacterial signal-transduction pathway that involves phosphoryl transfer from phosphoenolpyruvate to enzyme I, HPr, enzyme II to the membrane-bound sugar. Enzyme II consists of an integral membrane component, termed enzyme IIC or IICD, and two associated components in the cytoplasm, enzyme IIA and enzyme IIB, that transfer the energy-rich phosphate to the substrate, which is bound to IIC [194]. Enzyme II is sugar-specific and of modular architecture. Depending on the sugar, enzyme II exists as a single protein (mannitol PTS), as two proteins IIA and IICB (glucose PTS), or as three proteins IIAB, IIC, and IID (mannose PTS), where IIC and IID are localized in the membrane. The glucose enzyme IICB spans the membrane eight times, whereas the mannose IIC is a six-spanning and IID a single-spanning inner-membrane protein [195]. To date, structural data exist only for the soluble PTS components showing details of the phosphoryl transfer sites (see [196] and references therein). A 5-Å two-dimensional projection map is available for the membrane-embedded C-domain of the mannitol-specific enzyme II-Mtl [197]. EIIC functions as a dimer that has one binding site for mannitol [198]. The biosynthesis and membrane insertion of EIIC has not been studied in detail. However, circularly permuted variants of the *E. coli* EIICB-Glc have been constructed and analyzed for activity [199]. One of them, CL3, has the TMSs 7 and 8 at the N-terminus and the TMSs 1–6 at the C-terminus. Although the order of the transmembrane regions is changed, the activity of the protein reached 70% of the wild-type, suggesting that the initiation of membrane insertion is not restricted to TM1 at the N-terminus.

Aquaporins and mechanosensitive channels

Bacteria are often exposed to osmolarity changes in their natural habitats. Aquaporins (AQPs) are a large family of membrane channels that are highly specific for water (AQPs) or small uncharged hydrophilic solutes such as glycerol or urea (aquaglyceroporins) [200, 201]. These channel proteins do not allow the permeation of small or charged solutes or even protons, thereby preserving proton gradients. Sequence analysis suggested that the members of the AQP family arose by tandem gene duplication [202] where the N-terminal segment has $\sim$20% conservation with the C-terminal segment [203]. The structures of several AQPs in diverse life forms have been determined to date (see [204] and references therein). The crystal structures of AQPs revealed a common molecular architecture containing four monomeric subunits, each consisting of six transmembrane spanning α-helices, assembled into a homotetrameric structure. The six TMSs from each subunit form an independent aqueous pore, whereby helices 1–3 and helices 4–6 sit in the membrane in opposite orientations. A seventh transmembrane region is created by two unusually long loops, which form half helices in the channel from opposite sides of the membrane, creating a constitutively open, narrow aqueous pathway. Both ends of these half helices contain the highly conserved Asn-Pro-Ala signature motif near its center. Studies have suggested that the two asparagines in the Asn-Pro-Ala motif form hydrogen bonds with water molecules and mediate the exchange of waters between the two halves of the channel by flipping the water molecule into a different orientation (see [205] and references therein). How AQP Z of *E. coli* is inserted into the membrane is presently unknown. The protein has been synthesized in vitro and was inserted into liposomes, suggesting that it might do this without an insertase or translocase [206]. The addition of purified FtsY increased the binding of the protein to the liposomes but not its insertion.

Bacteria also have the ability to sense physical stress within its environment with the help of mechanosensitive ion channels. For example, the mechanosensitive channels, MscL and MscS, play a primary role in protecting cells exposed to osmotic downshock [207]. Determination of the crystal structure of a mechanosensitive channel of large conductance, MscL, from *Mycobacterium tuberculosis* revealed a homopentameric channel in an apparently closed state [208]. Each subunit consists of two transmembrane α-helices, TM1 and TM2, and a cytoplasmic N- and C-terminal domain. The closed pore is lined by the five tightly packed TM1 segments, whereas the five TM2 segments form an external surrounding ring. The pore of the closed channel is approximately 18 Å in diameter at the periplasmic side but narrows to approximately 2 Å at

the cytoplasmic side. The constriction is believed to be the channel gate, which is formed by a group of amino acids (five Val and five Ile) at the cytoplasmic end of TM1. The diameter of the protein is found to increase by 16 Å upon channel activation. In contrast, the crystal structure of MscL from *Staphylococcus aureus* revealed a tetrameric channel in an expanded intermediate state [209]. It is not uncommon in different species that certain multimeric membrane proteins form distinct oligomers. In *E. coli*, MscL requires only SRP and YidC for membrane targeting and insertion [100].

The structure of a mechanosensitive channel of small conductance, MscS, revealed a homoheptameric channel [210]. Each homoheptameric subunit consists of three transmembrane helices (TM1–TM2–TM3) with the N-terminus in the periplasm and the C-terminus in the cytoplasm. In this channel, the TM3 helices line the channel pore, whereas the TM1 and TM2 helices are in a direct contact with the surrounding lipids.

The sensor proteins

Various signals, particularly the presence of nutrients such as amino acids and sugars, are detected at the periplasmic surface of bacteria by integral sensor proteins. In *E. coli*, five different membrane-bound chemotactic sensor proteins are known: Tsr, Tar, Tap, Trg, and Aer that sense serine, aspartate and maltose, dipeptides, ribose and galactose, and oxygen, respectively. A number of other bacteria have more transmembrane chemoreceptors, also known as methyl-accepting chemotaxis proteins, e.g., *Vibrio cholerae* has 45 different chemotactic sensor proteins [211]. The chemotactic sensor proteins form arrays at the cell poles (see [212] for a review). Each subunit of the transmembrane chemoreceptor dimer spans the inner membrane twice creating a membrane-spanning 4-α-helix bundle. The homodimers assemble into trimers of dimers and associate with CheW and CheA in the cytoplasm. CheW is a linker protein and CheA is a histidine kinase. The ligand binds to the periplasmic domain of the receptor [213, 214]. Depending on the external stimuli (attractant or repellent), the receptor signaling decreases or increases the autophosphorylation activity of CheA. In the case of autophosphorylation, the phosphoryl group is transferred to a response regulator, CheY, which in turn controls the rotational direction of the flagellar motor. It has been reported that the serine chemoreceptor Tsr of *E. coli*, which consists of a large periplasmic domain of about 150 amino acids, requires SecA for membrane insertion [215]. Three-dimensional images of Tar-GFP have shown that the aspartate chemoreceptor Tar colocalizes transiently with the Sec machinery that forms a helical structure in the cell [216].

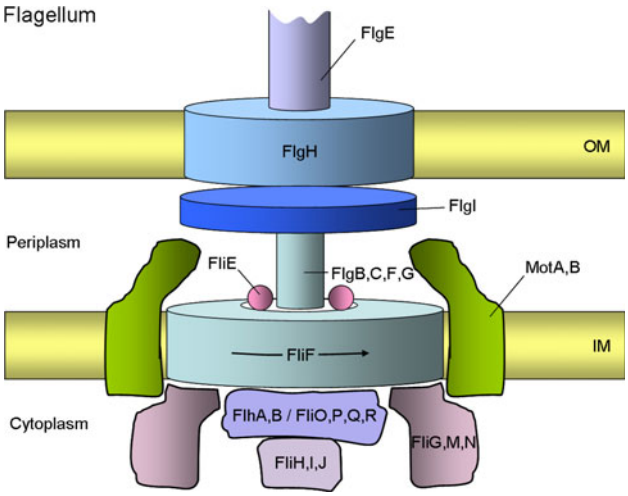

Fig. 7 A schematic representation of a bacterial flagellum basal body. The flagellum, which works as a rotary motor, consists of a motor component called the basal body, which spans from the cytoplasm to the outer membrane (*OM*), a flexible hook, and a filament. The basal body is composed of a rotor, a rod, a bushing, and a switch regulator. The core of the rotor is made up of FliF. The rod components are composed of four proteins, FlgB, FlgC, FlgF, and FlgG, and a rod adapter protein, FliE. The outer ring structures assembled around the rod consists of FlgI and FlgH and are thought to act as a molecular bushing of the rotary axial structure of the flagellum. The direction of rotation is controlled by the switch proteins, FliG, FliM and FliN. The stator part of the motor is composed of MotA and MotB, which span the inner membrane (*IM*). MotB is anchored to the peptidoglycan layer with its C-terminal periplasmic domain. Together both proteins form the proton pathway that powers the flagellum motor. The flagellar secretion apparatus (*T3SS*) consists of six integral membrane proteins FlhA, FlhB, FliO, FliP, FliQ, and FliR, and three soluble components, FliH, FliI, and FliJ, at the cytoplasmic face of the flagellar basal body. These proteins facilitate the export of flagellar substrates and are dependent on ATP hydrolysis by the ATPase FliI protein. The basal body of the flagellum is linked to a flexible hook consisting of FlgE and to a helical filament (*FliC*). The flagellum can rotate in either a clockwise or a counter-clockwise (*arrow*) direction

The KdpD protein of *E. coli* is a membrane component of the KdpD/E two-component signal transduction system involved in maintaining the intracellular osmolarity. The integral sensor protein KdpD is unusual, because it consists of a large cytoplasmic N-terminal domain, four closely spaced transmembrane regions, and an extended cytoplasmic C-terminal domain. The membrane insertion of KdpD occurs independent of the Sec complex and YidC, but requires the pmf [217] and SRP [32].

The flagellar motor complex

Motile bacteria express one or many flagella that rotate clockwise or counter-clockwise depending on their regulation by chemotactic signaling proteins. The flagellar motor component, which represents the basal body, is

located in the inner membrane and structured as an oligomeric ring structure (Fig. 7). The central component of the rotor is the two-spanning inner-membrane protein FliF that assembles into a 26mer to form the ring structure with a diameter of about 24 nm [218]. To this ring, three soluble proteins, FliGMN, bind at the cytoplasmic side. In the center of the ring, the flagellar export complex is situated composed of six membrane proteins, FlhAB and FliOPQR and a soluble part composed of FliHIJ in the cytoplasm. On the periplasmic side, FlgBCFG, and a rod adapter protein (FliE), bind to the rotor forming the rod that connects to the flagellar filament. The stator surrounding the ring structure is composed of only two inner-membrane proteins, the four-spanning MotA and the single-spanning MotB protein that is bound to the peptidoglycan at the C-terminus. 4MotA/2MotB form hetero-hexameric complexes and provide the proton pathway. In *E. coli*, an aspartic acid residue (Asp32) in the transmembrane region of MotB is functionally critical and might be protonated [219]. About a dozen of these complexes couple the proton movement with torque generation of one ring. To do this, specific electrostatic contacts between MotA and FliG occur [219]. However, the detailed mechanism of how the ring turns relative to the stator is presently unknown.

The assembly of the flagellar motor complex has been extensively studied for the later steps that occur from the inside to the outside. First, the FliF ring is assembled in the inner membrane to which the flagellar export complex, the rod, hook, and filament is added on [220]. The assembly of the flagellum involves YidC and the Sec complex and no flagella are observed after depletion of YidC or SecE [124, 221]. The L and P ring is composed of FlgH and FlgI, respectively. These proteins are exported by the Sec complex whereas the other components of the late assembly steps are translocated by the flagellar export complex [222]. The flagellar export complex is related to the type III secretion system and the FliI component harbors the ATP-binding site that might drive the export of the individual flagellar components [223]. ATP hydrolysis is controlled by FliH and it interacts with the FliI hexamer. FlhA and FlhB interact with each other and could build the transmembrane export pore since they are located in the membrane and interact with a substrate [224]. FlhA and FliR are eight-spanning membrane proteins; FlhB is a five-spanning and FliP a four-spanning membrane protein with a cleavable signal sequence.

Conclusions

Investigating the biogenesis of membrane proteins has clearly shown that there is not a uniform pathway for these proteins to insert into the membrane bilayer. Different targeting and integrating factors such as SRP, SecB, Sec(A)YEG, and YidC can be envisioned as modules. Various combinations of these modules result in the different biogenesis pathways of membrane proteins. In the last two decades, major progress has been made in understanding the membrane transport and function of membrane proteins due to recent high-resolution structures, revealing a more detailed insight into overall domain organization and the function of possible conformational changes. In particular, the new structures of ABC transporters and permeases show how these fascinating proteins may function. This research is now providing a first glimpse into how membrane proteins move to transport ions, water, and sugar across the bilayer. Although our knowledge has improved significantly, many questions still remain unanswered about membrane proteins, for example how they tightly fold and assemble into multi-subunit complexes, but these are challenges for the future.

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