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Co- and post-translational translocation through the protein-conducting channel: analogous mechanisms at work?

Kakoli Mitra, Joachim Frank & Arnold Driessen

Many proteins are translocated across, or integrated into, membranes. Both functions are fulfilled by the 'translocon/translocase', which contains a membrane-embedded protein-conducting channel (PCC) and associated soluble factors that drive translocation and insertion reactions using nucleotide triphosphates as fuel. This perspective focuses on reinterpreting existing experimental data in light of a recently proposed PCC model comprising a front-to-front dimer of SecY or Sec61 heterotrimeric complexes. In this new framework, we propose (i) a revised model for SRP-SR-mediated docking of the ribosome-nascent polypeptide to the PCC; (ii) that the dynamic interplay between protein substrate, soluble factors and PCC controls the opening and closing of a transmembrane channel across, and/or a lateral gate into, the membrane; and (iii) that co- and post-translational translocation, involving the ribosome and SecA, respectively, not only converge at the PCC but also use analogous mechanisms for coordinating protein translocation.

Many hydrophilic, soluble proteins and most membrane proteins have to traverse and/or integrate into the cytoplasmic membrane in prokaryotes or the endoplasmic reticulum (ER) membrane in eukaryotes; from there, they are sorted by various mechanisms to their final destinations^{1,2}. To bypass the energetic barrier of the lipid bilayer, proteins cross cellular membranes via a proteinaceous complex³ that is termed the 'translocon' or 'translocase'⁴. At the core of the translocase lies the PCC, which consists of an oligomer of a heterotrimeric integral membrane protein complex, SecYEG in eubacteria and Sec61 $\alpha\beta\gamma$ in eukaryotes. As the PCC does not use nucleotides to generate energy, it must associate with cellular components that provide the driving force necessary for polypeptide translocation or insertion. The translocase can participate in two types of processes: cotranslational and post-translational translocation (reviewed in ref. 5). Polypeptides that are still in the process of being translated (nascent polypeptides) are cotranslationally translocated by the translocase while the ribosome is bound to the PCC.

The energy for translocation comes from the hydrolysis of GTP on the ribosome during polypeptide elongation. During post-translational translocation, a fully translated but only partially folded polypeptide, a preprotein, is transported through the translocase with the aid of energy-using soluble factors, namely SecA in eubacteria⁶ and BiP in eukaryotes⁷. In addition to the energy-intensive directional translocation of the polypeptide, translocation also requires the ability of the PCC to interact dynamically with its binding partners and the nascent polypeptide. The PCC must be able to mediate both translocation of hydrophilic regions of polypeptides through an aqueous channel running perpendicularly to the membrane plane and integration of hydrophobic transmembrane helices (TMHs) by regulating their lateral partitioning into the plane of the lipid bilayer.

A growing number of biochemical, genetic and biophysical studies attest to the versatility of the PCC, in terms of both the variety of cytosolic and membrane components it binds and the effects the PCC has on these binding partners. Furthermore, structural work, especially the X-ray crystallographic structure of an uncomplexed archaeal SecYEG heterotrimer⁸ and the recent cryo-EM structure of a functional eubacterial PCC bound cotranslationally to a ribosome-nascent polypeptide complex (RNC)⁹, reveals aspects of the highly dynamic nature of the PCC that enable it to mediate both protein translocation across membranes and protein integration into membranes. Here we focus on the central role of the PCC in these processes, with an emphasis on translocation in eubacteria and elaborations on the eukaryotic and archaeal systems where appropriate.

Identification of nascent polypeptides on the ribosome

Nascent polypeptides designated to be translocated across, or integrated into, the membrane are identified cotranslationally by a cleavable signal sequence or a TMH that acts as a signal anchor¹⁰. The signal consists of a hydrophobic core flanked by polar residues with positive charges on the N-terminal end¹¹. As the signal emerges from the polypeptide exit tunnel, it binds the large ribosomal subunit proteins L23 and L29 (ref. 12; the eukaryotic equivalents are L23a and L35). The length and hydrophobicity of the signal's hydrophobic core determines which of a variety of cytosolic factors interacts with it, and that in turn determines which translocation pathway the nascent polypeptide will use: cotranslational or post-translational^{13,14}. In eubacteria, various cytosolic factors crowd around the ribosomal polypeptide exit site to probe the nascent polypeptide upon its egress^{12,13}. The signal-recognition particle (SRP) seems to be the first factor to recognize and transiently bind the signal when it emerges from the polypeptide exit tunnel¹². If the signal has strong hydrophobicity¹³ and helicity¹⁵, it is bound tightly by the SRP, which then

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shunts the RNC to the cotranslational translocation pathway¹⁴. If the signal is not hydrophobic enough for tight SRP binding, predominantly trigger factor associates with the nascent polypeptide until completion of translation, shunting the preprotein to the post-translational translocation pathway involving SecA and the chaperone SecB¹².

The structure of the PCC

The X-ray structure of an uncomplexed SecYEG. From the X-ray structure of archaeal SecYEG uncomplexed with any cytosolic factor or substrate polypeptide, the architectural features of a single inactive heterotrimer are evident⁸. The α subunit, SecY, resembles a clam shell formed by the N-terminal and C-terminal halves (TMH1–TMH5 and TMH6–TMH10, respectively). The two halves are hinged/linked on one side by the loop between TMH5 and TMH6 and clamped together by the γ subunit, SecE, leaving the other side—the lateral gate—unconstrained. SecY interacts with SecG, the β subunit, near the lateral gate (that is, the opening of the ‘clam shell’). The transmembrane funnel-like cavity in the center of the heterotrimer is formed by both SecY halves and, in the nontranslocating state, is blocked by a plug (TMH2a; Fig. 1a). The X-ray structure also shows that the long cytoplasmic loops between TMH6 and TMH7 and between TMH8 and TMH9 (cytosolic factor–associating domain, or CFAD)^{16,17} extend ~20 Å above the membrane plane (Fig. 1b). On the basis of this structure, it has been proposed that the functional PCC is formed by a single copy of the heterotrimer. Translocation is hypothesized to be initiated when the nascent polypeptide or preprotein signal displaces the plug and lodges itself into the lateral gate, prying open the two SecY halves in the plane of the membrane. This would enlarge the diameter of the hydrophilic funnel enough for the translocation of the hydrophilic region of the nascent polypeptide or preprotein, and enable a nascent TMH to partition laterally into the lipid bilayer⁸ (Fig. 1c).

Problems with the monomeric PCC model. Although this model addresses several aspects of translocation, key issues are unresolved. (i) If a single copy of the SecYEG or Sec61 $\alpha\beta\gamma$ heterotrimer forms the functional PCC, why have oligomers been observed biochemically and biophysically^{18,19} as well as in structures^{20–22}? (ii) Upon cleavage²³ and dissociation²⁴ of the signal from the PCC, how do the linked SecY halves remain wedged open for maintenance of a pore wide enough for polypeptide translocation? (iii) How are lipids from the lipid-channel interface prevented from entering the pore, which would drive up the energetic cost of translocating a hydrophilic polypeptide region across the membrane (Fig. 1d)? (iv) How does the pore in one heterotrimer provide a sheltered enclave for maneuvering the inversion of alternating TMHs (along with their flanking hydrophilic regions) in multipass membrane proteins, to yield proper membrane protein topology (Fig. 1e)? (v) How does a monomer of the SecYEG complex maintain a channel large enough to translocate the disulfide-bonded hairpins of preproteins²⁵ without exposure to the lipid bilayer (Fig. 1f)?

Cryo-EM data suggest a dimeric front-to-front PCC model. Recently, a cryo-EM structure of a eubacterial, cotranslationally translocating PCC bound to an RNC, containing a signal anchor and hydrophilic polypeptide regions, has been solved to moderate resolution (Fourier shell correlation characteristics of 11 Å at 3 σ and 15 Å at 0.5)⁹. This has enabled the observation of structural details at the level of groupings of TMHs⁹, as opposed to featureless, globular regions^{20,21}. The existence of substantial structural detail in this most recent PCC cryo-EM map allowed the normal mode-based flexible fitting⁹ of the X-ray structure of the SecY complex⁸ and the development of a model of the PCC²⁶ that addresses the key issues discussed above. This fitting favors a model in which the functional PCC consists of two SecYEG or Sec61 $\alpha\beta\gamma$

Figure 1 The structure of the PCC. (a,b) The clam shell architecture of the PCC (a) and the CFAD extending above the membrane plane (b) are revealed in the X-ray structure⁸. (c) Model in which a monomeric PCC is the functional unit for polypeptide translocation. (d–f) Problems with the monomeric PCC model. (g) The dimeric PCC in the context of a functional ribosome–PCC complex. The large frontal opening between the ribosome and PCC and the three connections (C1, C2 and C3) are indicated. Shown is the ‘front’ view with the frontal opening facing the reader. (h) Directional architecture of the translocating dimeric PCC containing two segregated pores. Possible (green) and blocked (yellow) trajectories of the nascent polypeptide from the two pores are indicated by arrows. In a, c–f and h, view is within the plane of the membrane, with the ribosome behind the plane. See text for discussion.

heterotrimers in a front-to-front arrangement—that is, with the lateral gates facing each other (see **Supplementary Discussion** online for an exposition of the back-to-back dimeric PCC model). There are three PCC-ribosome connections around the ribosomal polypeptide exit tunnel. Two quasisymmetrical connections (C1 and C2) are at either side, each comprised of the CFAD of one heterotrimer interacting with a specific ribosomal RNA hairpin (helix h24 in C1 and h59 in C2). A third connection (C3) is made at the back of the complex to ribosomal proteins, as also previously suggested²⁰. A large opening at the front (~20 Å × 40 Å) renders the nascent polypeptide and the CFADs accessible from the cytosol (Fig. 1g). Surprisingly, in this structure of a PCC containing the hydrophobic signal anchor/TMH as well as the hydrophilic nascent polypeptide regions, the authors observed not one central consolidated channel^{20–22,27} but two segregated pores, one in each heterotrimer, termed Sec₁YEG and Sec₂YEG (and containing CFAD₁ and CFAD₂, respectively)⁹. The ‘hook’-shaped N-terminal SecY half in each heterotrimer and the back ‘wall’ formed by connection C3 give each pore a lateral directionality within the plane of the membrane. Sec₁YEG is suited for hydrophobic TMH integration into the membrane^{24,28}, as the hook directs a path to the bulk lipid in the front. Sec₂YEG is suited for translocating hydrophilic nascent polypeptide

regions through an aqueous pore²⁷, as the hook forces a path to the back that the C3 wall blocks to lipid (Fig. 1h). The use in the same PCC of two segregated pores with different solvent environments provides a novel and unexpected paradigm for both lipid-mediated TMH integration and hydrophilic region translocation through an aqueous medium, by physical separation of these two processes.

Cotranslational translocation

Complementary safeguarding: a revised SRP-SR docking model. The role of the SRP is to recognize and bind the hydrophobic signal of a nascent polypeptide as it emerges from the ribosome, forming an SRP-RNC complex. This function is fulfilled mainly by the protein Ffh in eubacteria²⁹, the homolog of mammalian Srp54 (ref. 30). The SRP-RNC complex is then targeted to membranes containing the signal-particle receptor (SR) bound to a translocation-competent PCC. Interaction of the SRP with its receptor thus transfers the RNC to the PCC^{31,32}. The SR in eubacteria consists of membrane-associated FtsY³³, and in eukaryotes it is a heterodimer³⁴ composed of a soluble protein, SR α , and an integral membrane protein, SR β . These components of the SRP-SR targeting pathway are guanine nucleotide-binding proteins (see **Supplementary Discussion** for a description of the domain structure of these proteins) that interact with each other in a concerted way in the activated, GTP-bound form and dissociate upon GTP hydrolysis³². Successful targeting of the RNC to a receptive PCC is ensured by using both the RNC³⁵ and PCC³⁶ as guanine nucleotide-exchange factors (GEFs) for the SRP and SR components.

A synthesis of existing data in the literature leads us to propose a revised 'complementary safeguarding' model for SRP-SR-mediated docking of the RNC to the PCC (Fig. 2). The SRP undergoes a conformational change on the ribosome upon signal binding to the Ffh M domain³⁷ (Fig. 2a,b, steps 1 and 2). The SRP may approach the ribosome from the back of the polypeptide tunnel exit (opposite the frontal opening), as this would enable SRP to displace the PCC in the event that the RNC were docked onto a nontranslocating PCC (Fig. 2b, step 1). The GEF activity of the RNC³⁵ may lead to displacement of the I box in Ffh, allowing GTP entry into the G domain, which would result in tighter binding between the RNC and the SRP³⁸. This SRP-RNC complex would then be primed (Fig. 2a,b, step 2), SRP being GTP bound, to interact with a similarly primed SR-PCC complex (Fig. 2a,b, step 5). In eubacteria, one protein, FtsY, performs two sets of functions: (i) membrane association and PCC binding³⁹ (Fig. 2a, steps 3 and 4) and (ii) GTP binding and hydrolysis, GTPase-domain regulation and interaction with Ffh (Fig. 2a,b, step 5). In eukaryotes, by contrast, these tasks are subdivided between SR β and SR α , respectively

(Fig. 2c). The PCC (probably through Sec61 β) acts as the GEF for FtsY or SR β , stimulating GTP binding and ultimately strengthening the interaction with the PCC³⁶. In eukaryotes, SR β probably binds a nontranslocating PCC before the association of SR α (Fig. 2c), as GTP binding by SR β is required for association with SR α ⁴⁰. SR α then interacts through its SRX domain with the GTPase domain of SR β ⁴¹ (Fig. 2c). This, in turn, may elicit the necessary conformational changes for GTP binding by SR α , as SR β is thought to act as a GEF for SR α .

Thus, the role of the PCC as a GEF for FtsY or SR β ensures that SR components are stimulated to bind GTP only when associated with a translocation-competent PCC. As the affinity of SR components for SRP is highest in the GTP-bound state⁴², successful SRP-RNC targeting to the SR-PCC complex is ensured. It has been suggested that membrane-associated FtsY interacts directly with the cytoplasmic loops between

Figure 2 Proposed 'complementary safeguarding' model for SRP-SR-mediated docking of the RNC to the PCC. (a–d) Interactions of SRP and SR components with the ribosome and/or PCC, shown in 'front' view (a,c,d) and in a different view parallel to the membrane plane, with the ribosome behind the PCC (b). The probable sequence of SR α and SR β interaction with the PCC-RNC complex is shown in c. FtsY or SR α cannot bind a translocating PCC-bound RNC complex (d). See text for discussion.

Figure 3 Nontranslocating and translocating models of the PCC fitted into experimental cryo-EM densities. (a,b) The angle between linked SecY halves (yellow angles) becomes larger and the lateral gate barrier (pink arrows) narrower when transitioning from the nontranslocating (a) to the translocating (b) PCC. The view is within the plane of the membrane, with the ribosome behind the PCC. The N-terminal hook domain (TMH1–TMH5) and C-terminal domain (TMH6–TMH10) of SecY are rendered opaque and semitransparent, respectively. Bottom, 180° rotation shows the change in distance (gray arrows) between the CFADs (forming connections C1 and C2 to the ribosome) in the two states. ‘Front’ indicates the side of the frontal opening between the ribosome and PCC. Sec₁YEG is colored in hues of red and Sec₂YEG in hues of blue. Figure is adapted, by permission of the Federation of the European Biochemical Societies, from ref. 26.

TMH8 and TMH9 on SecY³⁹ (within the CFAD) either when the PCC is in a nontranslocating, preassembled¹⁸ empty state or by displacing a nontranslating ribosome bound to a PCC³¹. In the latter case, FtsY or SRβ probably gains access to the cytoplasmic CFAD(s) through the frontal opening⁹, an idea supported by studies showing that SRβ associates with Sec61β³⁶ (Fig. 2a,b, step 4). The large frontal opening (~20 Å × 40 Å) may thus constitute an important architectural feature of the PCC–ribosome complex, providing access for cytosolic factors to the CFADs of the PCC. FtsY or SRβ interaction with the CFAD(s) may disrupt the CFAD–ribosome interaction and displace the empty ribosome to the back (Fig. 2a, step 4), resulting in an FtsY/SRβ–PCC complex. It has been suggested that FtsY or SRαβ cannot interact with a translocating PCC³⁶ (Fig. 2d). When Ffh or Srp54 (complexed with the RNC) and FtsY or SRαβ (complexed with the PCC) associate via their N and G domains⁴³ (Fig. 2a,b, step 5), conformational changes occur, and GTP hydrolysis is stimulated, as the proteins act as mutual GTPase-activating proteins (GAP)⁴⁴. The resulting series of conformational changes⁴⁵ includes (i) release of the nascent polypeptide signal from the SRP M domain, (ii) dissociation of the SRP–SR complex from the RNC and the PCC and (iii) diffusion of the SRP and SR components away from the RNC–PCC, probably through the frontal opening. The ribosome⁴⁶ together with the PCC⁴⁷ may act as a GAP for SRβ to effect its dissociation from the complex (Fig. 2a,b, steps 6 and 7).

Facilitated discrete states: Translocation through the PCC. In line with biochemical data, the nascent polypeptide has been modeled into the cryo-EM density of the functional PCC⁹ as a hairpin⁴⁸ (Fig. 3). This hairpin straddles the lateral gate barrier formed by the tips of the N-terminal SecY hook domains of both SecYEG heterotrimers, which separates the two segregated pores²⁶ (Fig. 3b; see **Supplementary Discussion** for description of the modeling). How does the nascent polypeptide end up in this conformation relative to the PCC? The translocating conformation (Fig. 3b) of the PCC can be obtained from the nontranslocating conformation (Fig. 3a) by following the trajectory of the major interdomain (between the linked N- and C-terminal halves of SecY hinged by the loop between TMH5 and TMH6) normal modes calculated for the PCC. This transition leads to an increase in the angle of opening between linked SecY halves, which results in narrowing of the lateral gate barrier and a concomitant reduction in the distance between the two SecY CFADs in the PCC²⁶. Thus, when normal-mode trajectories are followed from the closed state to the ‘segregated pores’ state of the PCC (corresponding to the nontranslocating and translocating cryo-EM densities of the PCC observed experimentally⁹), the lateral gate barrier becomes smaller (Fig. 3a,b). If this trend is extrapolated, the barrier disappears, resulting in a PCC in which both heterotrimers form a single, central, consolidated channel. The consolidated channel in the PCC is large enough to accommodate the insertion of the nascent polypeptide as a hairpin. Subsequent partial closing of linked SecY halves in the PCC would then result in the hairpin straddling the lateral gate barrier (see **Supplementary Discussion** for details on what might regulate the opening of linked SecY halves).

On the basis of the analysis of multiple cryo-EM and X-ray structures of ribosomes, a new ‘facilitated discrete states’ framework for cotranslational translocation through the PCC has been described, which incorporates both biochemical and structural observations^{26,49} (Fig. 4). In stage 1, the ribosome senses the nascent polypeptide signal within the polypeptide tunnel through the extensions of ribosomal proteins L4 and L22. Interaction with the signal induces conformational changes in L4 and L22, which are propagated to the polypeptide exit site, in particular to rRNA hairpin h24, which forms connection C2 with the PCC. The repositioning of h24 decreases the distance between C1 and C2 (the inter-CFAD distance), which facilitates the transition of the PCC from the closed state to the ‘consolidated channel’ state, concomitantly with—or, more likely, preceding—the insertion of the signal into the PCC. In stage 2, the hydrophobic signal and downstream hydrophilic region of the nascent polypeptide are inserted as a hairpin into the central, consolidated channel of the PCC. In stage 3, immediately upon hairpin insertion, the linked SecY halves in the PCC partially close, with the hydrophobic signal partitioning into the lipid-accessible pore in Sec₁YEG and the hydrophilic polypeptide region partitioning into the lipid-inaccessible, aqueous pore in Sec₂YEG. In stage 4, the signal leaves the pore in Sec₁YEG to position itself at the front interface between the two heterotrimers. If the signal constitutes a signal anchor or TMH, partitioning into the lipid bilayer may occur in a process involving SecG or Sec61β⁵⁰ and other membrane proteins, such as YidC or TRAM^{28,51}, while translocation of the hydrophilic nascent polypeptide continues through the aqueous Sec₂YEG pore.

Post-translational translocation

ATP-binding and hydrolysis-driven SecA activity. Post-translational translocation in bacteria involves the association of the motor protein SecA with the PCC to facilitate the translocation of a signal-containing preprotein across the membrane. Biochemical and structural studies^{52,53} show that the SecA protomer is composed primarily of three interconnected structural units: (i) the DEAD motor domain, from which protrudes (ii) the substrate-specificity domain (SSD), and (iii) the

Figure 4 'Facilitated discrete states' model of polypeptide translocation through the ribosome–PCC complex. The hydrophobic nascent polypeptide signal/TMH is shown as a green cylinder, with the hydrophilic portion shown as a yellow line or open circle. Gray arrows indicate inter-CFAD distance. Dashed black arrows indicate possible trajectories of motion. The view in the upper panel is as in **Figure 1g** and that in the lower panel is as in **Figure 1h**. The PCC schematics for the nontranslocating (1) and translocating (4) states are derived from atomic models fitted into experimental cryo-EM densities, whereas states 2 (consolidated channel) and 3 (segregated pores) are predicted from normal mode analysis of the PCC structure. See text for discussion. Figure is adapted, by permission of the Federation of the European Biochemical Societies, from ref. 26.

C-domain (**Supplementary Fig. 1** online). The DEAD domain consists of two nucleotide-binding domains, NBD1 and NBD2, which form an intersubdomain cleft that binds ATP. Upon binding to the PCC, SecA is primed for nucleotide exchange⁵⁴, and it is activated for ATP hydrolysis upon binding of a preprotein⁵⁵ (see **Supplementary Discussion** for a summary of SecA domain structure and biochemistry). A complete ATP-binding and hydrolytic cycle results in the translocation of about 40 residues of a partially folded preprotein through the PCC into the periplasmic space^{56,57}. Consecutive cycles of ATP binding and hydrolysis are thought to 'drive' the incremental translocation of the entire preprotein, whereas in the presence of a proton motive force, rapid SecA-independent translocation occurs once the translocation reaction has been initiated by SecA and ATP⁵⁶.

In eubacteria, the preprotein is maintained in a partially unfolded state by being wrapped around a tetramer of SecB⁵⁸, which interacts via hydrophobic pockets on the preprotein⁵⁹. The SecB–preprotein complex then binds with high affinity to the C-terminal zinc-binding domain (CTD) of SecA^{60,61}, which is docked on the PCC⁶². The N-terminal signal of the preprotein—and a region of the mature preprotein—is transferred to and recognized by the SSD site on SecA⁶³. ATP binding by SecA releases SecB from the CTD of SecA⁶⁰.

Support for SecA functioning as a dimer. Biochemical studies demonstrate that the N-terminal DEAD motor domain of SecA interacts with SecYEG⁶⁴, and genetic studies map the SecA-interacting region to the SecY CFAD⁶⁵. In the presence of AMP-PNP, a nonhydrolyzable analog of ATP, a dimer of SecYEG has been shown to bind a dimer

of SecA⁶⁶. Many studies have also demonstrated that it is the dimeric form of SecA that is functional in preprotein translocation through the PCC^{67,68}. SecA has been shown to become compact upon binding of SecYEG, changing from a more loose conformation in solution⁶⁹. The X-ray structure of the physiological antiparallel SecA dimer⁵² from *Bacillus subtilis* shows that the N-terminal portion of NBD1 (NPN1) in one protomer is separated from the same substructure in the other protomer by approximately 40 Å (**Supplementary Fig. 1**). This corresponds to the distance between SecY CFADs in the translocating PCC state²⁶. In line with observations on the structure of the functional PCC⁹, we propose that a SecA dimer⁶⁷ associates with a front-to-front–arranged dimeric SecYEG through two symmetric interactions of the CFAD with NPN1 (**Supplementary Fig. 1**).

In solution and bound to the lipid bilayer, the oligomeric state of SecA has been shown to be altered by the ligands that it interacts with during protein translocation. It has been suggested that the interaction of SecA with the preprotein signal may drive the formation of new intermolecular contacts distinct from those stabilizing the physiological dimer⁷⁰. Indeed, the X-ray structure of SecA from *Mycobacterium tuberculosis*⁵³ reveals a different possible dimerization interface for two SecA protomers, resulting in a flatter conformation, in which the two NPN1s are much farther apart (**Supplementary Fig. 1**). Comparison of the *M. tuberculosis* and *B. subtilis* SecA dimers reveals the presence of a channel in the central interface between the two protomers: in the former structure, the channel is open (that is, it is large enough to fit unfolded regions of a preprotein; **Supplementary Fig. 1**), whereas in the latter structure, the channel is closed (**Supplementary Fig. 1**).

Figure 5 Proposed 'molecular peristalsis' model for the mechanism of SecA-mediated preprotein translocation through the PCC. (a,b) Components of the bacterial, post-translational translocation machinery, shown in the 'front' view (a) and in a view parallel to the membrane plane, with the PCC behind the SecA dimer (b). SecA2 and SecYEG2 denote SecA dimer and SecYEG dimer, respectively. The open and closed conformations of SecA illustrated are those observed for the *M. tuberculosis*⁵³ and *B. subtilis*⁵² X-ray structures, respectively. Although the physiological SecA structures at the various stages may differ in detail from the X-ray structures, they may be similar qualitatively—that is, a flat, open-channel, large-cavity conformation for the open SecA state and a compact, closed-channel, small-cavity conformation for the closed SecA state. See text for discussion.

Molecular peristalsis: a new SecA translocation model. These structural observations, combined with existing biochemical data, lead to the suggestion of the following novel and testable model for SecA-mediated protein translocation through the PCC (Fig. 5). In stage 1, the SecA dimer binds to the functional PCC, a front-to-front-associated dimer of SecYEG, through two symmetric NPN1-CFAD interactions. The PCC-bound SecA dimer is in a flat, 'open channel' conformation, with a large inter-NPN1 distance (~55 Å), thus maintaining a large inter-CFAD distance (~50 Å) in the PCC, which remains closed. The preprotein, alone or in association with SecB, binds via the signal to the SSDs at the top of the SecA-PCC complex. The preprotein also promotes SecB binding to the CTD of SecA^{60,61}. Upon binding, SecB passes the preprotein onto SecA through a cascade of events that do not depend on nucleotide triphosphates⁶⁰. In stage 2, the partially unfolded preprotein passes through the open central channel of the SecA dimer into the cavity formed at the SecA-PCC interface until the cavity is filled (approximately 40 amino acid residues). In stage 3, binding of ATP to SecA results in (i) release of SecB and (ii) a conformational change in SecA that shortens the inter-NPN1 distance and results in the closing of the central SecA channel. This, in turn, causes a shortening of the inter-CFAD distance in the PCC, thus facilitating the formation of a consolidated PCC channel. Concomitantly, the cavity size at the SecA-PCC interface is greatly reduced, forcing the

amino acid residues of the preprotein present in the cavity to translocate through the consolidated PCC channel. In stage 4, subsequent ATP hydrolysis elicits a conformational change in SecA, such that it acquires a flat conformation, which partially closes the PCC channel. Concomitantly, the SecA channel reopens for renewed passing through of subsequent preprotein regions, in line with the observation that ATP hydrolysis releases the bound preprotein from SecA⁵⁶. At this stage, SecA is probably in an ADP-bound state. A new cycle is initiated upon ADP-ATP exchange, and the process is repeated until the entire preprotein has translocated through the PCC.

During the post-translational process, the SecA dimer may undergo a series of ligand- and nucleotide-induced conformational changes resulting in a variety of open and closed states. Thus, the four translocation stages described in our 'molecular peristalsis' model may each encompass structural and functional intermediates. The major open and closed conformations of SecA (Fig. 5) posited in this model may correspond qualitatively to the X-ray structures of SecA from *M. tuberculosis*⁵³ and *B. subtilis*⁵², respectively. SecA in its major open state may be characterized by an open channel, large cavity and flat conformation⁵³, whereas SecA in its major closed state may have a closed channel, small cavity and compact conformation⁵². Changes in the SecA dimerization interface are proposed to effect conformational changes in SecA that regulate PCC conformation. In our 'molecular peristalsis'

model, SecA does not physically push the preprotein through the PCC, but instead facilitates directional transport of the preprotein through the PCC (see **Supplementary Discussion** for an exposition of the 'piston' model of SecA). Interestingly, the step size of this translocation event is dictated by the size of the cavity. Furthermore, in a manner analogous to that of the ribosome, SecA is suggested to modulate PCC conformation by regulating the inter-CFAD distance, emphasizing the unifying nature of our framework regarding co- and post-translational translocation.

Summary

Polypeptides are both co- and post-translationally translocated across, or integrated into, membranes via the PCC, composed of the heterotrimeric SecYEG or Sec61 $\alpha\beta\gamma$ complex. Recent cryo-EM work, in conjunction with previous X-ray crystallography studies, suggests that the PCC is a dimer of front-to-front-arranged SecYEG or Sec61 $\alpha\beta\gamma$. The large frontal opening at the ribosome-PCC junction serves the important function of providing access to the SecY or Sec61 α CFADs. CFADs are the probable interaction sites for soluble factors, such as the SR components, which we propose mediate docking of the RNC to the PCC via a 'complementary safeguarding' mechanism. In the 'facilitated discrete states' framework, the PCC can maintain two segregated pores with different lipid accessibilities and also form a consolidated channel. Such a channel is necessitated, for instance, by nascent polypeptide hairpin insertion during cotranslational translocation or translocation of partially folded polypeptide regions during post-translational translocation. The attachment of the PCC to the ribosome occurs pseudo-symmetrically via the CFAD in each heterotrimer, and suggests a mechanism for the formation and regulation of the consolidated PCC channel through nascent polypeptide-induced conformational changes in the ribosome. An analogous mechanism of PCC channel regulation is proposed for the SecA dimer, which is suggested to bind the CFADs in the PCC symmetrically. A new 'molecular peristalsis' model for SecA-mediated preprotein translocation through the PCC is proposed, in which SecA does not physically push the preprotein but instead directs its translocation. We present here an integrated framework for protein translocation, suggesting that analogous mechanisms are at work at the PCC in both the co- and post-translational pathways.

Note: Supplementary information is available on the Nature Structural & Molecular Biology website.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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