Hypothesis

A model for co-translational translocation: Ribosome-regulated nascent polypeptide translocation at the protein-conducting channel

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Abstract The protein-conducting channel (PCC) must allow both the translocation of soluble polypeptide regions across, and the lateral partitioning of hydrophobic transmembrane helices (TMHs) into, the membrane. We have analyzed existing structures of ribosomes and ribosome–PCC complexes and observe conformational changes suggesting that the ribosome may sense and orient the nascent polypeptide and also facilitate conformational changes in the PCC, subsequently directing the nascent polypeptide into the appropriate PCC-mediated translocation mode. The PCC is predicted to be able to accommodate one central, consolidated channel or two segregated pores with different lipid accessibilities, which may enable the lipid-mediated partitioning of a TMH from one pore, while the other, aqueous, pore allows translocation of a hydrophilic polypeptide segment. Our hypothesis suggests a plausible mechanism for the transitioning of the PCC between different configurations. © 2006 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.

Keywords: Signal sequence; Transmembrane helix; Membrane protein integration; Translocase; Post-translational translocation; SecA

1. Introduction

Many soluble proteins and most membrane proteins must cross, or integrate into, a membrane to reach their final destination in the cell. Protein translocation/integration at the membrane occurs via a proteinaceous complex, termed the translocase [1], which serves to bypass the energetic barrier posed by the hydrophobic lipid bilayer. At the core of the translocase lies the protein-conducting channel (PCC), which consists of an oligomer of a heterotrimeric integral membrane protein complex, SecYEG in eubacteria and Sec61\textsubscript{E} in eukaryotes [2,3]. The PCC can translocate signal peptide-containing nascent polypeptides while they are still being synthesized on the ribosome, i.e. co-translationally, or translocate fully synthesized preproteins across the membrane post-translationally with the aid of energy-utilizing soluble factors, such as SecA in eubacteria [4] and BiP in eukaryotes [5]. Cryo-electron microscopy (cryo-EM) studies of co-translational ribosome–PCC complexes from various organisms yielded low-resolution reconstructions in which the PCC appeared as a globular ellipsoidal with a central dimple or hole [6–9]. From volume estimates of the globular PCC ellipsoids, the ribosome-bound PCC was posited to consist of between two and four copies of the SecYEG/Sec61\textsubscript{E} heterotrimer. Further details of PCC structure and mechanism of action were provided by the X-ray structure of a monomeric, uncomplexed, inactive, archaean SecYE\textsubscript{B} heterotrimeric complex [10], which revealed that SecY resembles a ‘clam shell’ open laterally to the membrane at the front lateral gate, with the N- and C-terminal halves – each consisting of five transmembrane helices (TMHs) – held together by a plug (TMH2a) domain [10]. Based on this architecture it was proposed that the SecY ‘clam shell’ opens upon displacement of the central plug by a signal peptide, which would then result in both a vectorial pore across and a lateral path into the membrane. It was suggested that the functional PCC consisted of a single heterotrimer, which however was arranged back-to-back with one or more additional heterotrimer(s) when found in complex with the ribosome [10]. The X-ray structure also shows the cytosolic factor-associating domain (CFAD), comprised of the cytoplasmic loops between TMHs 6/7 and 8/9, extending approximately 20 Å above the membrane plane. The CFAD has been shown to interact with ribosomal RNA in the large subunit of the ribosome [11–13]. Although an examination of the X-ray structure of the uncomplexed heterotrimer addressed several structural and mechanistic aspects of the PCC, a detailed image of the functional PCC complexed with the ribosome was necessary for further elucidation of the mechanism of co-translational translocation.

Recently, a cryo-EM reconstruction was obtained of a eubacterial ribosome–nascent polypeptide complex (RNC) bound to both a non-translocating and a translocating PCC. Greatly improving on the globular appearance of the PCC in previous cryo-EM studies [6–9,14] with a lower resolution for the PCC EM density, detailed rod- and lamella-like features, corresponding to groupings of TMHs in the PCC, are discernible in this most recent reconstruction [15]. A fitting technique using normal mode analysis and cross-correlation in conjunction with energy-minimization could be used to demonstrate that a model in which two SecYEG heterotrimers are arranged front-to-front fits the cryo-EM densities better.

Abbreviations: CFAD, cytosolic factor-associating domain; EM, electron microscopy; FSC, Fourier shell correlation; NMF, normal mode-based flexible fitting; NPS, nascent polypeptide signal; PCC, protein-conducting channel; RMSD, root mean squared difference; RNC, ribosome–nascent polypeptide complex; \textit{rRNA}, ribosomal RNA; TMH, transmembrane helix

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than a back-to-back arrangement [15]. A back-to-back arrangement was originally suggested [10] in part based on the organization of uncomplexed, substrateless SecYEG heterotrimers in a 2D crystal, held together by two types of crystal contacts [16]. However, these back-to-back heterotrimer contacts observed in the 2D crystal may be an artifact of crystallization. As an alternative explanation for these contacts, uncomplexed, non-translocating SecYEG heterotrimers may be ‘stored’ in cell membranes as oligomers involving back-to-back contacts, as suggested by cross-linking experiments [17]. Such ‘storage oligomers’ would likely undergo rearrangements upon association with a ribosome, as suggested both by EM [18] and FRET [19] studies, to yield a different (front-to-front) oligomeric structure [15]. The apparent discrepancy between observations of a dimeric [15] and trimeric/tetrameric [8,9,14] PCC in cryo-EM reconstructions of functional ribosome–PCC complexes is resolved when considering that low-resolution EM data can lead to erroneous volume calculations, and subsequently to erroneous estimates of the oligomeric state of the PCC (see Supplementary Discussion 2.2 in Ref. [15]).

According to the front-to-front model of the PCC [15], both heterotrimers in the non-translocating PCC are in their closed conformations, with no transmembrane pores visible. In the translocating PCC, each heterotrimer is observed to open, such that each acquires a pore segregated from the pore in the other heterotrimer. Due to the geometry of the connections that the PCC forms with the ribosome, the two pores are distinguished by their accessibility to lipids: one is accessible, while the other is not [15].

With the model we have obtained, we can attempt to address the following questions:

(i) What conformational changes does the PCC undergo and how are these changes effected?
(ii) What is the mechanism underlying pore/channel formation in the PCC?
(iii) At what stage and by what mechanism are TMH regions of a nascent polypeptide chain oriented with respect to the lipid bilayer?
(iv) How are soluble regions and TMHs of a nascent polypeptide translocated via the PCC? Are TMHs integrated into the lipid bilayer via the lipid-accessible pore in the PCC, while soluble polypeptide regions are transported through the aqueous pore? If so, what directs the polypeptide to the appropriate pore?

Our hypothesis asserts that the PCC conformation may be regulated by nascent polypeptide-induced conformational changes in the ribosome, and that it may be the ribosome – not only the PCC – that plays a pivotal role in ensuring that the nascent polypeptide is properly oriented, and directed to the appropriate pore for translocation across, or integration into, the membrane via the PCC. We base these assertions on three pieces of data not considered in our initial analysis [15], namely: (i) placement of the nascent polypeptide chain and the SecY plug domains into the cryo-EM density of the PCC; (ii) an examination of the behavior of the front-to-front model of the PCC when its major normal mode of motion is extrapolated beyond the states observed experimentally; and (iii) a comparison of existing structures of the PCC-bound ribosome complex and of ribosomes lacking both a PCC and a signal peptide-containing polypeptide.

2. Placement of the nascent polypeptide chain and SecY plug domains into the cryo-EM density of the PCC

Cross-linking experiments have suggested that the helical, hydrophobic nascent polypeptide signal (NPS) [20] is positioned close to SecY TMHs 2b and 7 [21], while the hydrophilic region of a translocating polypeptide has been shown to pass through the pore formed at the interface between linked SecY halves [22]. It has been shown biochemically that the nascent polypeptide can exist as a hairpin upon translocation through the PCC [23]. Biochemical and structural data also suggest specific SecY plug positions in the translocating PCC. When the SecYEG heterotrimer is closed, the plug is positioned at the interface between linked SecY halves, blocking the transmembrane pore, as found in the X-ray structure [10]. During polypeptide translocation the plug has been shown to cross-link to SecE, at the periphery of SecY [24].

Upon our fitting of the front-to-front PCC model, which contained neither the nascent polypeptide chain nor the SecY plug domains, into the cryo-EM density of the translocating PCC [15], a few prominent regions of density were observed to remain unaccounted for (Fig. 1A). These can be classified into two groups: (i) long rods of density traversing the entire bilayer thickness (yellow asterisks), along with a loop of density connecting these rods on the exoplasmic side of the PCC; and (ii) short stretches of density at the exoplasmic side of the PCC (red asterisks).

The long rod of density unaccounted for at the front interface of the two heterotrimers, Sec1YEG and Sec2YEG, is adjacent to SecY TMHs 2b and 7 of both heterotrimers (see Fig. 1A and Ref. 15), and thus likely corresponds to the NPS, as suggested by cross-linking [21]. We generated an atomic model of the NPS and placed it rigidly into this long rod of density. The other long rod of density unaccounted for is found between the two linked SecY halves of Sec2YEG; i.e., at the transmembrane pore, and thus likely corresponds to the hydrophilic region of the translocating polypeptide chain, again as suggested by cross-linking [22]. Hence, we modeled the remaining hydrophilic region of the nascent polypeptide and placed it into the density inside the cavity of Sec2YEG, with the polypeptide loop between the two bilayer-traversing stretches docked into the connecting region of density at the exoplasmic side of the PCC, resulting in a nascent polypeptide hairpin [23]. The two short stretches of density unaccounted for at the exoplasmic side of the PCC are observed (i) at the interface of the two linked SecY halves in Sec1YEG, as seen in the X-ray structure of the non-translocating, closed heterotrimer [10], and (ii) at the periphery of Sec2Y, close to the region of Sec2E to which cross-linking with the plug has been demonstrated [24] (Fig. 1B). Therefore, we placed the Sec2Y plug in its closed-state position, i.e., into the density at the interface of linked SecY halves in Sec1YEG, while placing the Sec2Y plug in its open-state position, i.e., into the density at the periphery of Sec2Y. The full model, the nascent polypeptide chain and the SecY plug domains observed to be regulated by nascent polypeptide hairpin barriers observed to form the nascent polypeptide ‘hook’ domains of both SecYEG heterotrimers, which separates two segregated pores (Fig. 1B and D).
3. Conformational changes of the PCC predicted by normal mode analysis

Previously, the translocating PCC model was obtained from the non-translocating PCC model by following the trajectory of the major inter-domain – i.e., between linked SecY halves – normal modes calculated for the PCC, which lead to an increase in the angle of opening between linked SecY halves (pink arrows) narrower when transitioning from the non-translocating (C) to the translocating (D) PCC, shown in the same view as in (A,B). 180° rotation shows the change in distance (grey arrows) between the CFADs (forming connections C1/C2 to the ribosome) in the two states. SecYEG is colored in hues of red and Sec2YEG in hues of blue.

4. Comparison of structures of ribosomes with and without an NPS

We compared the atomic model of the 50S subunit fitted into the RNC–PCC cryo-EM reconstruction [15] with the 50S atomic model fitted – with real-space refinement using rigid bodies – into the cryo-EM reconstruction of a conformationally similar PCC-unbound, pretranslocational ribosome containing a dipeptide [25,26] (Fig. 2A), and other atomic models of 50S subunits lacking a nascent polypeptide that were obtained by X-ray crystallography [27–30]. Positions of ribosomal elements near the polypeptide exit site are relatively conserved among all PCC-unbound 50S subunit models that we superimposed [26–30], while there are marked differences between the PCC-unbound, pretranslocational model [25,26] and the PCC-bound model [15]. The positions of ribosomal elements to the left of the exit site, i.e., rRNA hairpin h59 (connection C1) and ribosomal proteins L23 and L29, remain fixed (average RMSD <1 Å), while elements to the right of the exit site, namely rRNA helices h19/20, rRNA hairpin h24 and proteins L24, L22 and L4, display significant movement (average RMSD ~8 Å) upon NPS recognition and PCC binding (Fig. 2A). The protein and rRNA elements on the right are physically interconnected, making concerted movements possible. L4 movement affects h19/20, which interacts both with L24 and h24. Additionally, repositioning the body of L22, which sits directly on h24, also influences the position of this hairpin at connection C2, thus altering the relative geometry and distance between C1 and C2.

We have thus the result that the ribosome adopts different conformations at the polypeptide exit site, depending on whether the ribosome is bound to a translocating PCC and is thus translating a signal peptide-containing nascent polypeptide, or whether the ribosome lacks an NPS. These two conformations favor either a short distance between ribosomal elements forming connections C1 and C2 or a large distance, respectively.

It should be noted that atomic models fitted into moderate to low resolution EM maps have indicated that accuracies of 4- to 5-fold better than the nominal resolution can be expected when the modeling is restrained/constrained to agree with standard stereochemistry determined from more detailed, e.g. X-ray crystallography, studies of molecules [31–34]. Thus, the EM map of the PCC-ribosome complex (Fourier shell correlation (FSC) characteristics ~15 Å at 0.5, ~11 Å at 3σ) can be interpreted at a level of detail beyond the nominal resolution estimated by FSC characteristics, i.e., atomic models can be fitted into our EM map with an accuracy of 2-3 Å, using fitting methods such as NMFF in conjunction with energy-minimization [15], and real-space refinement using rigid bodies [26].
5. Hypothesis: modulation of PCC conformation and pore/channel formation by the ribosome

Our placement of the nascent polypeptide chain into the cryo-EM density of a translocating PCC bound to the ribosome suggests that the NPS-polypeptide hairpin straddles the lateral gate barrier – formed by the tips of the SecY N-terminal ‘hook’ domains of both SecYEG heterotrimers, which separates two segregated pores. How might the nascent polypeptide adopt a straddling hairpin conformation relative to the PCC? Analysis of the major inter-domain normal mode of the PCC suggests that the PCC can adopt at least three different configurations: (i) a closed-state, in which linked SecY halves are tightly juxtaposed in each heterotrimer, leaving a large lateral gate barrier; (ii) a segregated-pores state, in which linked SecY halves are separated (open), such that pores form in each heterotrimer, but are segregated from each other due to the presence of a small lateral gate barrier; and (iii) a consolidated channel state, in which linked SecY halves are open, such that the lateral gate barrier disappears, leaving one large central consolidated channel in the PCC. An NPS-polypeptide hairpin could conceivably insert into the PCC when the PCC is in its consolidated channel state (see movie in Supplementary Material). Subsequently, upon partial closing of linked SecY halves, the hairpin would then adopt a configuration in which it straddles the lateral gate barrier (see Section 6 below).

How would the PCC transition between these three configurations, i.e., how would the linked SecY halves separate (open)? It has been hypothesized that the short hydrophobic plug domain located at the interface between the two linked SecY halves in a heterotrimer needs to be displaced to enable the two halves to separate for formation of a translocation pore [10]. Normal mode analysis on the heterotrimer structure suggests, however, that displacement of the plug is not sufficient for the opening of linked SecY halves, and that the two halves need to be wedged open at the lateral gate [15]. The NPS has been hypothesized to both displace the plug and wedge open the linked SecY halves [10]. Accessible surface area calculations [35] of a SecY molecule suggest that the free energy (using 15 cal/mol per Å² of buried hydrophobic surface [36]) of opening of linked SecY halves, not considering entropic effects such as lipid displacement, is between 65 and 80 kcal/mol, depending on the extent of opening. Experimental measurements of insertion free energies of a hydrophobic helix from water into a lipid bilayer, which has a dielectric constant that is lower than that of the interior of a membrane protein, range from −5 to −8.8 kcal/mol [37–39]. Thus, NPS insertion alone cannot account for the opening of the linked SecY halves in the PCC. The mode of attachment of the functional, dimeric PCC to the ribosome may enable the ribosome to provide the free energy necessary to facilitate the separation of linked SecY halves, concomitantly with or, more likely, prior to plug displacement by NPS insertion into the PCC. By positional rearrangements of ribosomal elements on one side of the polypeptide exit site the distance between connections C1 and C2 can be altered, directly affecting the inter-CFAD distance on the PCC. Since the lateral gates of the two heterotrimers face each other, a decrease in the inter-CFAD distance can only be accommodated if linked SecY halves separate (open). NPS/TMH-induced conformational changes in the ribosome could thus provide the free energy necessary for sep-
aration of linked SecY halves, and thus the facilitation of pore/channel formation in the PCC.

How might ribosomal elements at the polypeptide exit site be induced to undergo positional changes when a signal peptide-containing nascent polypeptide is being translated and a translocating PCC is bound? Studies have shown that ribosomal elements along the tunnel, namely proteins L4, L22, and L23 [40,41], can interact with a translocating nascent polypeptide [42,43], which in turn induces conformational changes within the ribosome [26,44]. Furthermore, sequence-specific nascent polypeptide folding within the tunnel has been demonstrated [40,45]. Proteins L4 and L22 send projections into the polypeptide exit tunnel of the 50S subunit (Fig. 2B and C) and, together with the projection of L23, comprise the major protein components lining the tunnel formed predominantly by rRNA [27–30]. Thus, a possible explanation for the observed positional changes in the globular bodies of L24, L22 and L4 is that the projections of L4 and L22 may sense distinct folded regions of the nascent polypeptide, e.g. a helical hydrophobic NPS/TMH [20,40], within the tunnel and transduce this information through conformational changes to the polypeptide exit site.

Finally, a function of cardinal importance that the ribosome–PCC complex must perform is to correctly orient the NPS/TMH with respect to the lipid bilayer. The PCC by itself does not appear to fulfill this function, since from its structure [10,15] a plausible mechanism is not apparent. The possibility that this helix-orienting function may lie in part with the ribosome is supported by the observations that (1) the translocation of most membrane proteins, especially those with multiple TMH segments, occurs co-translationally, and (2) the ribosome is the first to encounter an NPS/TMH. Upon its exit from the polypeptide tunnel the NPS has been demonstrated to bind to L23 and L29 [46], presumably to the hydrophobic binding surface (Fig. 3A). It is interesting to note that the surface of the projection of L24, which is extended into the polypeptide tunnel exit, is hydrophilic [28] or negatively charged [27–30] (Fig. 3A). In the RNC–PCC structure the hydrophilic surface of the L24 projection is aligned with Sec2YEG to the right of the polypeptide exit site, whereas the hydrophobic surfaces of L23/L29 are aligned with Sec1YEG on the left (Fig. 3B). Furthermore, the PCC is arranged in such a way that the pore in Sec1YEG is accessible to membrane lipids in the front, whereas the pore of Sec2YEG is blocked to lipids in the back [15] (Fig. 1).

6. A new proposed framework for co-translational translocation at the PCC

Our analysis leads us to propose a synergistic model for co-translational translocation through the PCC (Fig. 4): (1) The sensing of an NPS/TMH within the polypeptide tunnel by the projections of L4 and L22 may be relayed through conformational changes to the respective globular protein bodies sitting at the polypeptide exit site, on the surface of the large ribosomal subunit. Repositioning of these proteins effects a movement of rRNA h19/20 and h24, and protein L24 on the L24-side of the polypeptide exit site, which in turn changes the relative geometry of connections C1 and C2 to the PCC. Thus, NPS/TMH-induced conformational changes in the ribosome may facilitate the separation of linked SecY halves – concomitantly with, or more likely, prior to plug displacement by NPS insertion – and the formation of a central, consolidated channel in the PCC for the purpose of nascent polypeptide loop insertion. The hydrophobic projection of L23 near the tunnel exit could serve as a ‘lever’, attracting a hydrophobic NPS/TMH toward it and guiding it to the L23/L29 hydrophobic surfaces (Fig. 3B, 4). The same ‘lever’ would repel a hydrophilic/charged nascent polypeptide region toward h24 and the hydrophilic projection of L24, which, due to the ribosomal conformational changes described, is brought into alignment with the polypeptide exit for interaction with the exiting nascent polypeptide (Fig. 2A and B). (2) The orientation of an NPS/TMH is governed predominantly by the balance between
hydrophobicity and flanking charges [47,48], with the more positively charged end generally residing in the cytoplasm [49,50]. The segregated ribosomal surface characteristics at the polypeptide exit site may orient an NPS/TMH such that the positively charged flanking region is sequestered by L24, and possibly by negatively charged rRNA h24 or other rRNA elements near the exit site, while L23/L29 – on the opposite side of L24 – sequester the hydrophobic helix. The insertion of such a ribosome-bound NPS/TMH into the PCC may then be driven by lipid-mediated partitioning in the consolidated channel [51]. The orientation of insertion is governed by the balance/interplay of the electrostatic and hydrophobic forces involved in the interaction of the NPS/TMH with the ribosome, the PCC, and both the negatively charged headgroup and hydrophobic acyl chain regions of lipids [47,48,51–53].

(3) Upon insertion, the hydrophobic NPS/TMH favors partitioning into the lipid-accessible region of the consolidated channel, i.e., toward Sec1YEG, while the hydrophilic nascent polypeptide segment – aided by interaction with L24 – may partition into the more aqueous region toward Sec2YEG. Concomitant closing of SecYEG heterotrimers results in a PCC with segregated pores exposed to different solvent environments. (4) The NPS/TMH partitions from Sec1YEG into the lipid bilayer [54], a process which might involve SecG/Sec61β [55] and/or YidC/TRAM [56,57], while translocation of the hydrophilic nascent polypeptide continues through the aqueous Sec2YEG pore.

7. Conclusions

The model presented here suggests that conformational changes in the PCC, most importantly channel formation, may be regulated and facilitated by the ribosome via nascent polypeptide signal-induced ribosomal RNA and protein dynamics at the SecY/Sec61α CFAD attachment sites of the dimeric PCC. It is possible that the bacterial posttranslational motor protein, SecA, may also regulate PCC conformation via polypeptide-induced modulation of the inter-CFAD distance. The proposed ability of the PCC to form one central, consoli-

Fig. 4. Model of polypeptide translocation through the ribosome–PCC complex. The hydrophobic NPS/TMH is shown as a green cylinder with the hydrophilic portion shown as a yellow line/open circle. Grey arrows indicate inter-CFAD distance. The view in the upper panel is as in Fig. 3B, and in the lower panel as in Fig. 1B. See text for discussion.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2006.05.019.

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