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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.

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 Sec61αβγ 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/Sec61αβγ heterotrimer. Further details of PCC structure and mechanism of action were provided by the X-ray structure of a monomeric, uncomplexed, inactive, archaeal SecYEβ 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

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Abbreviations: CFAD, cytosolic factor-associating domain; EM, electron microscopy; FSC, Fourier shell correlation; NMFF, normal mode-based flexible fitting; NPS, nascent polypeptide signal; PCC, protein-conducting channel; RMSD, root mean squared difference; RNC, ribosome–nascent polypeptide complex; 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, Sec_1YEG and Sec_2YEG, 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 Sec_2YEG; 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 Sec_2YEG, 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 Sec_1YEG, as seen in the X-ray structure of the non-translocating, closed heterotrimer [10], and (ii) at the periphery of Sec_2Y, close to the region of Sec_2E to which cross-linking with the plug has been demonstrated [24] (Fig. 1B). Therefore, we placed the Sec_1Y plug in its closed-state position, i.e., into the density at the interface of linked SecY halves in Sec_1YEG, while placing the Sec_2Y plug in its open-state position, i.e., into the density at the periphery of Sec_2YEG. We then performed normal mode-based flexible fitting (NMFF) on the complete front-to-front PCC model, which gave a correlation coefficient of 0.79. In the full model, the nascent polypeptide chain is observed to exist as a hairpin straddling the lateral gate barrier – formed by the tips of the SecY N-terminal 'hook' domains of both SecYEG heterotrimers, which separates two segregated pores (Fig. 1B and D).