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

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## SUPPLEMENTARY DISCUSSION

### The structure of the PCC

#### *The dimeric front-to-front PCC model based on cryo-EM data*

### The back-to-back dimeric PCC: a storage form?

Previously, a back-to-back arrangement of SecYEG/Sec61αβγ heterotrimers was suggested[1], based partly on back-to-back heterotrimer contacts observed in a 2D crystal consisting of two sandwiched lipid bilayers related by a two-fold screw axis ($2_1$) (ref. [2]), and on cross-linking data[3]. These contacts could result from favorable packing interactions in a 2D crystal, or alternatively, could reflect contacts involved in forming 'storage' oligomers in cell membranes of uncomplexed, non-translocating SecYEG/Sec61αβγ heterotrimers. Both EM[4] and FRET[5] studies suggest that

SecYEG/Sec61αβγ heterotrimers undergo rearrangements upon association with a ribosome, possibly to the recently observed front-to-front arrangement[6]. Previously, the ribosome-bound PCC has been suggested to contain a central hole/dimple, and to consist of 3-4 copies of the SecYEG/Sec61αβγ heterotrimer, based on volume calculations on low-resolution cryo-EM reconstructions[7-9]. However, low-resolution (20-30 Å) EM data can lead to the erroneous appearance of structural features, *e.g.*, a fused hole/dimple instead of two resolved, separate pores, and also to erroneous volume calculations, resulting in incorrect estimates of the PCC oligomeric state (see Supplementary Discussion 2.2 in ref. [6]).

## Co-translational translocation

### *A revised model for SRP-SR-mediated docking of the RNC to the PCC: complementary safeguarding*

#### Domain structure of the guanine nucleotide-binding proteins involved in the SRP-SR-mediated docking process

Ffh/SRP54, FtsY and SRα all have three domains, two of which are the N-domain and the GTPase (G-) domain, to which is appended an I-box domain that regulates nucleotide entry into the G-domain[10,11]. The third domain is a methionine-rich (M-) domain in Ffh/SRP54, which recognizes the signal[12], an X-domain (SRX) in SRα, which mediates interaction with the GTPase domain of SRβ [13], and an acidic (A-) domain in FtsY, which is involved in membrane binding[14]. SRβ contains a GTPase domain and a single TMH.

***A new framework for PCC dynamics and translocation of the nascent polypeptide: facilitated discrete states***

**Modeling the nascent polypeptide chain into the cryo-EM map of the functional PCC**

Biochemical experiments demonstrate that the nascent polypeptide can exist in the PCC as a transmembrane hairpin[15]. Cross-linking studies show that while the helical, hydrophobic nascent TMH/peptide signal[16] is positioned close to SecY TMHs 2b and 7 (reference 77), the hydrophilic region of the nascent polypeptide traverses the transmembrane pore inside a SecYEG heterotrimer[17]. Upon placement of the dimeric, front-to-front PCC model into the cryo-EM density of the PCC in the RNC-PCC structure[6], two transmembrane rods of EM density connected by a short loop of density on the exoplasmic side, *i.e.*, a transmembrane hairpin, were observed[18]. The nascent polypeptide was thus modeled into this putative hairpin density, according to the cross-linking data. Regions of the nascent polypeptide were modeled and positioned into the hairpin cryo-EM density as follows: (1) the helical, hydrophobic TMH/signal peptide (residues 35-59) at the front interface of the two heterotrimers, $Sec_1$YEG and $Sec_2$YEG; (2) the polypeptide loop (residues 60-68) into the connecting density on the exoplasmic side; and (3) the extended, hydrophilic segment (residues 69-82) into the bilayer-traversing rod of density, *i.e.*, the transmembrane pore, inside $Sec_2$YEG[18].

**What regulates the opening of linked SecY halves and therefore the PCC state?**

A recent analysis of atomic models fitted into the cryo-EM structure of (1) a PCC-bound, signal peptide-containing ribosome complex, and (2) a ribosome containing a

tetrapeptide[18,19] reveals that the ribosome undergoes specific conformational changes when associated with a nascent polypeptide translocating PCC, and suggests a mechanism by which the conformation of the PCC is modulated. In this model, the folded nascent polypeptide signal within the polypeptide tunnel[20,21] is sensed by ribosomal proteins L4 and L22 (refs. [22-24]), both of which relay this recognition event via conformational changes to rRNA hairpin h24, forming connection C2 with the PCC at the exit site. The movement of h24 with respect to h59 decreases the distance between C1 and C2 (the inter-CFAD distance), thus forcing the two heterotrimers closer together. Since each CFAD is anchored to a relatively rigid C-terminal SecY half, the inter-CFAD distance can be reduced only if both heterotrimers open. The extent of heterotrimer opening, determining whether a consolidated channel or two segregated pores form, thus depends on the inter-CFAD distance: the shorter the distance, the larger the opening angle between linked SecY halves must be. The ability of the PCC to form a single, large consolidated channel explains how the process of orienting a nascent polypeptide TMH inside the PCC could be accommodated in a large enough space that is sheltered from the membrane environment.

## Post-translational translocation

### *ATP-binding and hydrolysis-driven SecA activity*

#### SecA domain structure and biochemistry

In eubacteria, the entrance to the cleft between NBD1 and NBD2 (also called intramolecular regulator of ATPase or IRA2) is covered by a flap[25], which has been implicated in the regulation of nucleotide binding[26]. NBD2 suppresses ATP hydrolysis at

NBD1[26]. The DEAD domain has been shown to bind with high affinity to the PCC[27]. Interestingly, the C-terminal domain synergistically promotes PCC binding of the DEAD domain but cannot bind on its own[28]. The SSD is extended out as a projection from NBD1 and is involved in preprotein binding[29,30]. SSD can be divided into N- and C-terminal subdomains. The C-domain contains four substructures, the scaffold domain (SD), the helical wing domain (HWD), IRA1, and a C-terminal zinc-binding domain (CTD). SD spans almost the entire length of SecA, intimately connecting the DEAD domain with the other domains (Suppl. Fig. 1a). IRA1 has been implicated in globally regulating protein translocase activities[28], while the CTD, which is expendable in SecA, has been shown to bind SecB[31,32].

Nucleotides induce a variety of conformational changes in SecA: (1) Alteration of the NDB1-NDB2 interface, conformation and stability[26,33]; (2) change in SSD conformation[28]; (3) tighter interaction between DEAD motor and C-domain and stabilization of the C-domain[34,35]; fluorescence studies demonstrate that nucleotide-driven conformational changes of the SecA DEAD motor are transferred to the C-domain[28,36,37]; (4) binding and release of IRA1 to SD[28]; (5) HWD disassociation from, and re-association with, the compact core of SecA[37]; and (6) changes in CTD conformation[33,38]. Energy conversion to mechanical work is expected to involve cross-talk between the DEAD motor and specificity domains of SecA. SecA without a tightly bound C-domain is a hyperactivated ATPase that is incompetent in translocation[26]. Binding of the C-domain to the DEAD motor suppresses its ATPase, and this regulation requires

specifically IRA1[28]. Finally, the C-domain also modulates the DEAD motor ligand binding affinities and catalysis in trans[28].

**Does SecA function like a 'piston' during preprotein translocation?**

SecA has an intrinsic ability to interact with, and penetrate into, phospholipid bilayers[39,40]. It forms dumbbell-shaped elongated and ring-like pore structures in the presence of lipid membranes[41]. SecA bound to the PCC is, however, not in contact with the bulk lipid[42], and at this stage the function of lipid-bound SecA is unknown. Bulk lipids likely affect the conformation and activity of the PCC and may indirectly affect SecA activity, as well[43]. The ATP binding and hydrolysis cycle of SecA has been linked with the concept that SecA – or domains thereof – inserts and de-inserts into the lipid membrane through the PCC[44,45]. This hypothesis is based on observations that SecA domains become highly protease-resistant during preprotein translocation, while this protease-resistance can be alleviated by disruption of the membrane by detergents or sonication. SecA is susceptible to some detergents that cause it to denature[39], while protease-resistance of the domains has also been observed in detergents that preserve the interaction between SecA and the PCC[46]. Therefore, it appears that the protease-resistant fragments correspond to catalysis-related conformational states of the SecA domains rather than membrane-protected fragments; *e.g.*, a domain may become more compact during a given stage in translocation and therefore become resistant to proteases. The accessibility of SecA to membrane-impermeable agents or proteases added from the periplasmic face of the membrane[47,48] may be a consequence of these agents gaining access to SecA from the periplasmic membrane face through the PCC.

## SUPPLEMENTARY FIGURE

**Suppl. Figure 1** Architectural features of monomeric and dimeric SecA. **(a,d)** Structure of the SecA protomer from *B. subtilis*[37], colored according to the various subdomains and functional regions of SecA (**d**). **(b,c)** In the SecA dimer from *M. tuberculosis*[25] **(b)**, the distance between the putative PCC-binding domains, NPN1s (N-terminal portion of NBD1), is larger than in the SecA dimer from *B. subtilis* **(c)**. **(c)** The *B. subtilis* SecA dimer is shown docked onto the SecYEG dimer (forming the functional PCC), with the inter-NPN1 distance in SecA matching the inter-CFAD distance in the PCC. **(d)** Schematic of the subdomains and functional regions of SecA. **(e,f)** The SecA dimer from *M. tuberculosis* **(e)** and *B. subtilis* (**f**) shown in a view perpendicular to **b**, and **c**, respectively, with the PCC (not shown) behind the SecA dimer.

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