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# Protein Translocation Is Mediated by Oligomers of the SecY Complex with One SecY Copy Forming the Channel

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## SUMMARY

Many proteins are translocated across the bacterial plasma membrane by the interplay of the cytoplasmic ATPase SecA with a protein-conducting channel, formed from the evolutionarily conserved heterotrimeric SecY complex. Here, we have used purified *E. coli* components to address the mechanism of translocation. Disulfide bridge crosslinking demonstrates that SecA transfers both the signal sequence and the mature region of a secreted substrate into a single SecY molecule. However, protein translocation involves oligomers of the SecY complex, because a SecY molecule defective in translocation can be rescued by linking it covalently with a wild-type SecY copy. SecA interacts through one of its domains with a nontranslocating SecY copy and moves the polypeptide chain through a neighboring SecY copy. Oligomeric channels with only one active pore likely mediate protein translocation in all organisms.

## INTRODUCTION

Many proteins are transported across or are integrated into the endoplasmic reticulum (ER) membrane in eukaryotes or the cytoplasmic membrane in prokaryotes. These proteins are directed to the membrane by cleavable signal sequences or by transmembrane (TM) segments of membrane proteins. Translocation occurs through a hydrophilic channel that is formed from a conserved heterotrimeric membrane protein complex, called the Sec61p complex in eukaryotes and the SecY complex in bacteria and archaea (for review, see Osborne et al., 2005). The complex consists of a multispanning $\alpha$-subunit (Sec61p or SecY) and two smaller $\beta$- and $\gamma$-subunits, called SecG and SecE in bacteria. The channel can associate with different partners that provide the driving force for translocation. In cotranslational translocation, the Sec61p/SecY complex associates with the translating ribosome; in posttranslational translocation in eukaryotes, the Sec61p complex associates with the Sec62/63p complex, and the ER luminal ATPase BiP; in posttranslational translocation in bacteria, the SecY channel associates with the cytoplasmic ATPase SecA. How the Sec61p/SecY complex forms a channel and how it associates with its different partners to translocate polypeptide chains is unclear.

In the simplest model, the channel would be formed from a single copy of the Sec61p/SecY complex. This is supported by the crystal structure of an archaeal SecY complex (Van den Berg et al., 2004). The structure shows a monomer of SecY complex and is likely representative of the closed states of all Sec61p/SecY channels. SecY consists of two linked halves, TMs 1–5 and 6–10, which form a lateral gate at the front and are clamped together at the back by SecE. A cytoplasmic funnel leading into the channel is closed by a short helix, termed the "plug." During initiation of translocation, a signal sequence or TM sequence intercalates into the lateral gate of SecY and causes the plug to move toward the back of SecY (Tam et al., 2005). The open channel would have an hourglass shape, with a pore ring of hydrophobic amino acid residues at its constriction, through which translocating polypeptide chains move, as demonstrated by disulfide bridge crosslinking (Cannon et al., 2005). Hydrophobic segments of membrane proteins would move through the lateral gate into the lipid phase.

The idea that the active translocation pore is formed by a monomer of Sec61p/SecY complex is challenged by observations that the solubilized complex can form oligomers, containing between two and four copies (for discussion, see Eichler and Duong, 2004). Oligomers of the SecY complex have also been observed in intact membranes (Mori et al., 2003; Scheuring et al., 2005). The crystal structure excludes the formation of a hydrophilic channel by the simple association of several SecY copies, because these are entirely hydrophobic in their membrane-facing regions. However, it is conceivable that multiple complexes associate at their front surfaces and open their lateral gates to fuse their pores. This could explain fluorescence-quenching experiments, which indicated a pore diameter of at least 40 Å (Hamman et al., 1997), and it would be consistent with a near front-to-front orientation

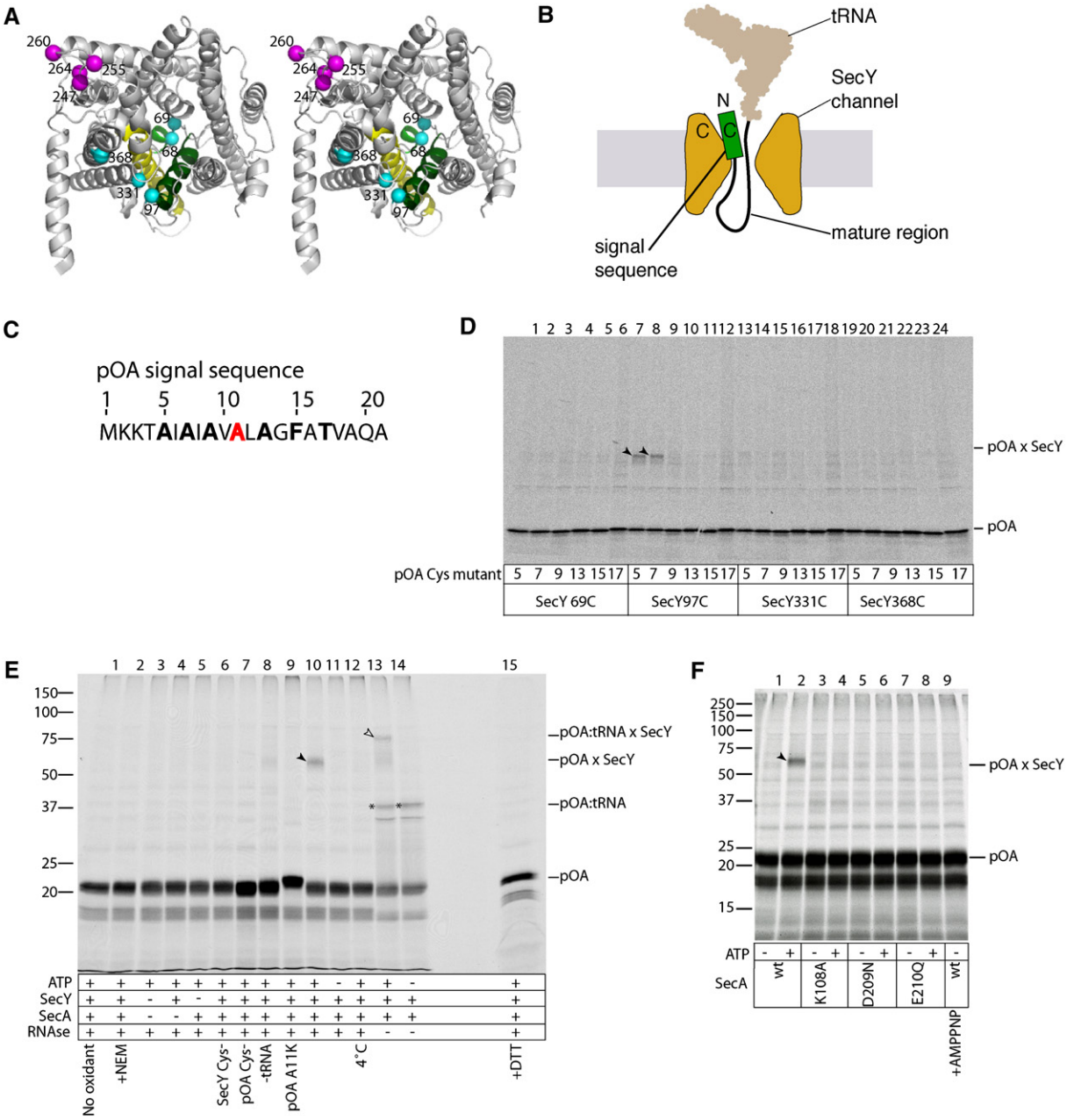


**Figure 1. Crosslinking of SecY to the Signal Sequence of a Translocation Substrate**

(A) Stereo view from the cytoplasm of the *M. jannashii* SecY complex. The plug (TM2a) is colored light green, and helices in the lateral gate (TM2b and TM7) are dark green and yellow, respectively. Cyan-colored spheres mark cysteines introduced into *E. coli* SecY for pOA-SecY crosslinking experiments. Magenta-colored spheres indicate cysteines placed into the 6/7 loop for SecA-Y crosslinking. Residues are numbered as in *E. coli* SecY. *E. coli* residue 255 is in an insertion; the closest position is labeled.

(B) Strategy of crosslinking. A translocation intermediate of proOmpA (pOA) was generated with a bulky tRNA at the C terminus (pOA:tRNA) to prevent its complete translocation through the SecY channel. Single cysteine residues (C) were placed into SecY and pOA to test their proximity by disulfide bond formation.

(C) Signal sequence of pOA. Residues mutated to cysteines are in bold and the inactivating A11K mutation in red.

(D) Proteoliposomes containing purified SecY mutants with cysteines at various positions were mixed with purified SecA, ATP, and $^{35}$S-methionine-labeled pOA:tRNA, containing 120 residues and cysteines at different positions of the signal sequence. After incubation at 37°C, the samples were treated on ice with an oxidant. NEM was added and the samples were digested with RNase prior to nonreducing SDS-PAGE and autoradiography. Filled arrows, crosslinked products.

(E) As in (D), with SecY containing a cysteine at position 97 (SecY-97C) and a pOA:tRNA substrate, containing 206 residues and a cysteine at position 5 (pOA-5C). The samples were incubated under different conditions, as indicated. +NEM, NEM added prior to the oxidant; SecY Cys- and pOA