

CHARACTERIZATION OF MEMBRANE-ASSOCIATED SECA

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ABSTRACT

Proteins are synthesized in the cytoplasm of bacterial cells and approximately 30 percent of these are exported across the inner membrane and are integrated into the inner membrane or reside in the periplasm or the outer membrane. One system of protein export is the general secretory system which exports precursor proteins through the transmembrane protein complex SecYEG that acts as the selective pore in the inner membrane through which the ATPase SecA translocates the substrate proteins. We have developed an *in vitro* translocation assay where purified SecYEG and SecA are reconstituted simultaneously into proteoliposomes. Quantifying the number of active translocons in both the proteoliposomes and inverted membrane vesicles with two different precursor proteins revealed that the stoichiometry of SecYEG in the active translocase depends upon the precursor being translocated.

We investigated the nature of the interaction between SecA and the membrane using mutant SecA species that lacked the N-terminal ten residues, the C-terminal twenty-one residues, or that lacked the coordinated zinc in the C-terminal twenty-one residues. We observed that SecA species that have the N-terminal ten residues had a higher affinity for the membrane than the SecA deletion mutant SecAdN10 that lacks the ten N-terminal residues. We also identified a role for the N-terminal ten residues of SecA in the translocation process. SecAdN10 is deficient in a step of the translocation process where a 2-kDa segment of the precursor protein is translocated through SecYEG. This deficiency is abolished if a SecA species that contains the N-terminal ten residues is added, and when SecA species that contain the N-terminal ten residues are added to assays with wild-type SecA the rate of the 2-kDa translocation step increases.