

Biogenesis, Structure and Function of Membrane Transport Proteins

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1. On the travel of ribosomes toward and along the cytoplasmic membrane: An essential pathway for proper biosynthesis of integral membrane proteins.

Cytoplasmic membrane proteins have indispensable functions in cellular physiology, metabolism, structure and communication. In all living cells the biogenesis of these proteins requires

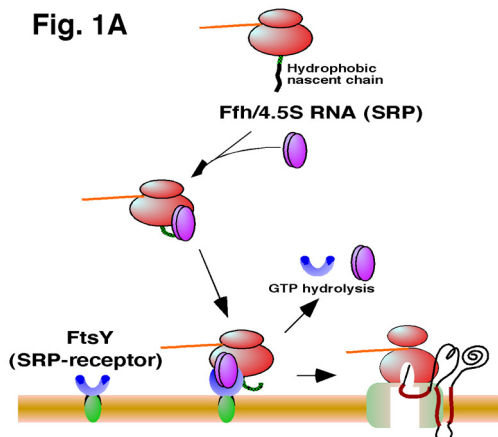
the Signal Recognition Particle (SRP) system (Fig. 1A), which mediates their translation on membrane-bound ribosomes.

We are studying central mechanistic and structural aspects of the SRP system in *E. coli*, in the context of the biosynthetic pathway of membrane proteins. We have demonstrated that the bacterial homologue of the mammalian SRP receptor (FtsY) is essential for targeting ribosomes to the membrane and for expression of membrane proteins. In contrast, in the absence of the SRP itself, ribosome targeting and membrane protein synthesis continue, although their proper assembly is severely impaired.

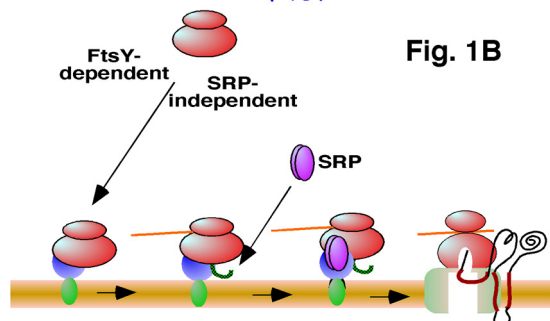
These observations, combined with additional considerations, led us to propose a testable model for ribosome targeting in *E. coli* (Fig. 1B). We propose that ribosomes are targeted to the membrane in an SRP-receptor-dependent, SRP-independent manner. After targeting, the transfer of ribosomes to the membrane insertion machinery is mediated by SRP. This scenario implies that the SRP might function downstream to the SRP receptor during the biosynthesis of membrane proteins.

This model is currently being investigated using genetic screens, biochemical tools and structural analyses. These studies will clarify the order of events during the biosynthetic pathway of membrane proteins and the mechanism by which membranes are equipped with ribosomes.

The signal recognition particle (SRP) pathway



Hypothesis: SRP might act downstream to FtsY



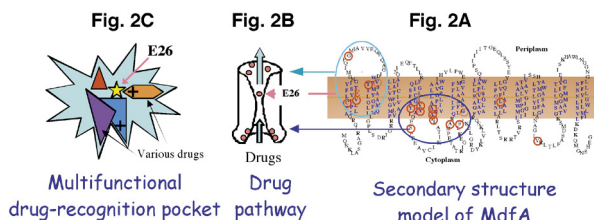
2. Studying the intriguing promiscuity of multidrug (Mdr) transporters, using MdfA from *E. coli* as a model.

Eukaryotic and prokaryotic cells often become multidrug resistant due to elevated levels of expression of Mdr transporters, which expel chemically unrelated toxic compounds from the resistant cells. We study various mechanistic aspects of Mdr transport. (I) How the driving force is

coupled stoichiometrically to the export process. (II) How a single transport protein can handle such an extremely broad spectrum of chemically unrelated species. (III) What is the physiological role of MdfA.

We have identified and cloned the *E. coli* Mdr transporter MdfA. This integral membrane protein (Fig. 2A) that recognizes a large variety of charged and uncharged substrates. MdfA is a drug/proton antiporter, which catalyzes discrete transport reactions (with electrically dissimilar substrates) that differ in their electrogenicity.

An extensive topological analysis has so far identified a single membrane-embedded charged amino acid residue (E26) in MdfA. The negative charge at position 26 is specifically important for transport of cationic drugs. By searching for second-site suppressors of inactive E26 mutations, two substrate-recognition domains were identified in MdfA (Fig. 2A, B). Direct substrate-binding assays with purified MdfA, showed that MdfA can



bind two dissimilar substrates simultaneously, in a cooperative manner. Collectively, our studies of the multidrug recognition properties of MdfA suggest a multifunctional recognition pocket as depicted in Fig. 2C.

Future studies of the MdfA substrate recognition properties and transport mechanism, will implement a combination of genetic, bioinformatics, biochemical, biophysical, and physiological tools. Specific efforts are dedicated to identify conditions under which high-resolution structural studies can be initiated using Cryo-EM and X-ray crystallography.

Selected Publications

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