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Biogenesis, structure and function of membrane transport proteins

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On the travel of ribosomes toward and along the *E. coli* 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 eukaryotic cells the biogenesis of membrane proteins requires the Signal Recognition Particle (SRP) system (Fig. 1A), and they are synthesized on membrane-bound ribosomes. In *E. coli*, many membrane proteins are also inserted into the membrane co-translationally by membrane-bound ribosomes. Therefore, membrane-bound ribosomes represent an evolutionary conserved phenomenon, and have a central role in every living cell.

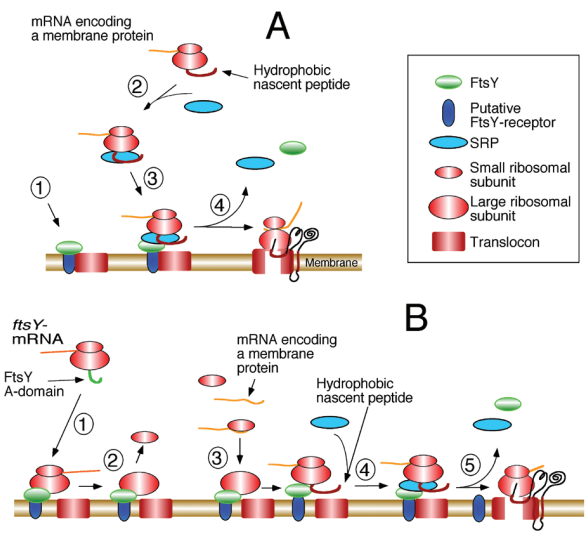

Fig. 1 *Models for targeting ribosomes to the E. coli membranes and the SRP-dependent synthesis of membrane proteins.*

We are studying 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. Interestingly, our recent studies suggest that for proper function, FtsY has to be targeted to the membrane co-translationally.

The two main observations: (1) FtsY is essential for targeting ribosomes to the membrane, and (2) FtsY is targeted to the membrane co-translationally, combined with additional considerations, led us to propose a hypothetical model for ribosome-targeting (Fig. 1B). This model is based on the idea that if FtsY is targeted to the membrane co-translationally, then both FtsY and the ribosomes translating it are delivered together to the membrane. In the original model (Fig. 1A), after its co-translational targeting, FtsY interacts with a new, SRP-targeted ribosome. Alternatively (Fig. 1B), after termination of FtsY translation the large ribosomal subunit may remain membrane-bound, ready to translate a new mRNA. Provided that the new mRNA encodes a membrane protein, transfer of this ribosome to the translocon requires recognition of the hydrophobic nascent peptide by SRP. This scenario implies that the SRP can also function downstream to the SRP receptor during the biosynthesis of membrane proteins. This model is preliminary and additional studies are currently underway in order to investigate and establish the order of events during the biosynthetic pathway of membrane proteins and the mechanism by which membranes are equipped with ribosomes

Studying the fascinating multidrug resistance (Mdr) phenomenon using a model Mdr transporter, MdfA from *E. coli*.

Eukaryotic and prokaryotic cells often become multidrug resistant due to elevated levels of expression of Mdr transporters, which remove chemically unrelated toxic compounds from the cell. However, the mechanism of substrate recognition and transport by Mdrs remained unresolved. A few major aspects are investigated in our laboratory. (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.

Recently, we have identified an *E. coli* Mdr transporter gene termed *mdfA*. MdfA is an integral membrane protein (Fig. 2A), which represents Mdr transporters that recognize a large variety of charged and uncharged substrates. Drug export is driven by

the proton electrochemical potential, and MdfA is a drug/proton antiporter.

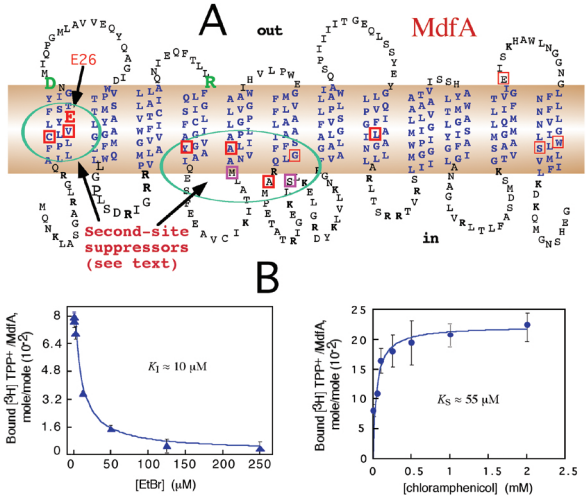

Fig. 2 *A) Secondary structure model of the Mdr transporter MdfA. B) Binding of TPP to MdfA: Effect of a cationic substrate (EtBr, left panel) and a neutral substrate (chloramphenicol, right panel).*

An extensive topological analysis has so far identified only one membrane-embedded charged amino acid residue (E26) in MdfA. We have shown that the negative charge at position 26 is essential only for transport of cationic drugs. A genetic approach was devised to identify additional residues that might participate in the drug recognition pocket of MdfA, by searching for second-site suppressors of inactive E26 mutations. The location of second-site suppressors, has so far revealed two substrate recognition-related regions in MdfA (Fig. 2A).

In order to characterize further the multidrug recognition phenomenon, we use direct substrate-binding assays with purified MdfA. We showed that the specific binding of the cationic substrate tetraphenylphosphonium (TPP) is inhibited competitively by other positively charged substrates. Surprisingly however, the neutral substrate chloramphenicol markedly stimulated the affinity of TPP binding to MdfA (Fig. 2B). These studies demonstrated that MdfA binds chloramphenicol and TPP simultaneously, and that the cooperativity in binding indicates that the two binding sites must communicate with each other.

In the future, we plan to address additional aspects of the structure of MdfA, its substrate recognition properties and transport mechanism, by a combination of genetic and biochemical tools. Specific efforts will be dedicated to identify conditions under which high-resolution structural studies can be initiated.

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