Biogenesis, Structure, and Function of Integral Membrane Proteins in *E. coli*

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Integral membrane proteins, which represent 20-30% of the proteome, consist an intriguing group of molecular nano-machines in living cells. They are unique in almost every aspect: (i) they are biosynthetically dependent on intimate membrane-ribosome relationships; (ii) they zigzag through a complex lipophilic environment of the membrane utilizing a sophisticated membrane-embedded proteinaceous channel; (iii) they fold into active enzymes through mechanisms that are only vaguely understood; (iv) they mediate processes essential to the life of the cell that include vectorial enzymatic reactions and information transfer; and (v) when they require degradation, cells use mechanistically fascinating pathways for recycling. Since many of these proteins are critical for life, various inherited diseases result when mutations occur in membrane proteins. Furthermore, membrane proteins serve as targets for more than 50% of the world's pharmaceuticals at this time.

Currently, our laboratory has several ongoing membrane protein-related research aims. One is to achieve a better understanding of the mechanism underlying secondary multidrug (Mdr) export. A second is to elucidate the biosynthetic pathway of membrane proteins and a third is to unravel the mechanism of integral membrane protein folding and recycling. Mechanistic aspects of Mdr research involve physiologic, genetic, biochemical, and structural approaches, while membrane protein biogenesis studies utilize mainly *in vivo* experiments and the quality control research is studied using both *in vivo* and *in vitro* techniques. To investigate these basic, evolutionarily conserved biological phenomena, our experimental system is the well-characterized Gram-negative bacterium *Escherichia coli*--a "clever" bug that has provided basic insights into innumerable basic biological processes.

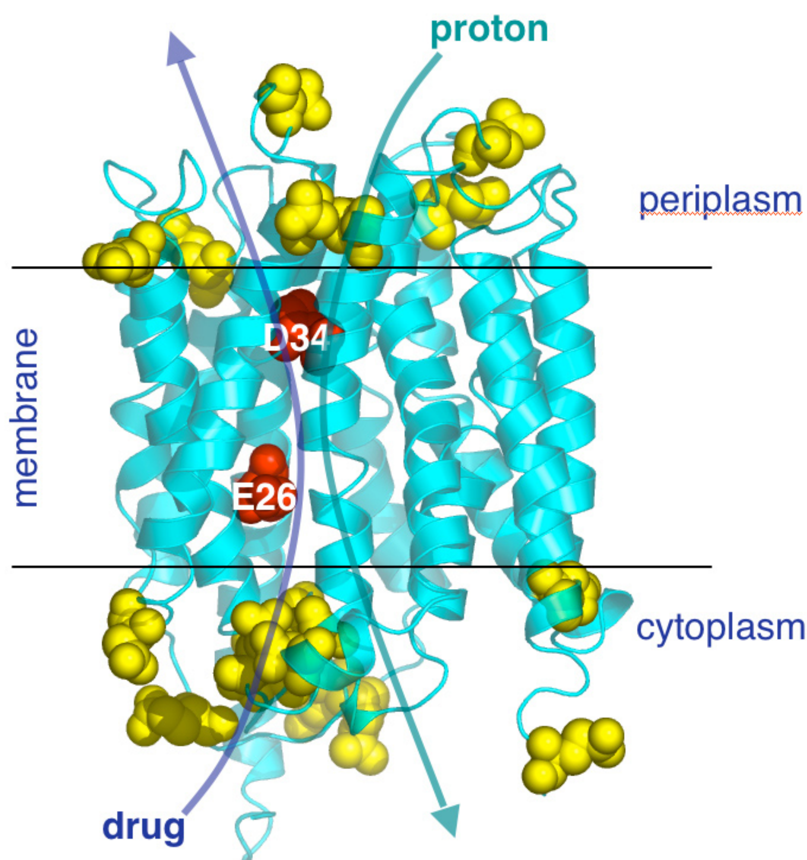

Fig. 1 *Theoretical 3D model of MdfA. All the acidic residues of MdfA are shown in a space filled presentation. Residues D34 and E26 are membrane embedded and can be utilized alternatively for active transport. Arrows illustrate the drug/proton antiport activity.*

Mdr transport: Promiscuity in structure and function

The efflux of multiple drugs by Mdr transporters represents a major obstacle in successfully treating many cancer and infectious diseases. In addition to their clinical importance, Mdr transporters attracted us because they have intriguing mechanistic characteristics that differ substantially from those of substrate-specific transport systems (Lewinson et al, 2006). Several important properties have been revealed during our investigation of the *Escherichia coli* Mdr transporter MdfA (Edgar and Bibi, 1997; Adler and Bibi, 2002; Sigal et al, 2006b). (1) Our studies suggest that MdfA functions as a monomer and has a large, complex, robust and extremely flexible substrate-recognition pocket (Lewinson and Bibi, 2001; Adler and Bibi, 2004; Sigal et al, 2005; Sigal et al, 2007). (2) The importance of electrostatic interactions between Mdr transporters and their cationic substrates was noted in our studies (Edgar and Bibi, 1999; Adler et al, 2004; Adler and Bibi, 2005). (3) We revealed a surprising degree of promiscuity in the transport mechanism of MdfA (Lewinson et al, 2003; Sigal et al, 2006a; Sigal et al, in preparation). (4) We have recently discovered an unanticipated major physiological role for MdfA in alkaline pH homeostasis (Lewinson et al, 2004; Krulwich et al, 2005).

Collectively, by using MdfA as a paradigm for secondary Mdr

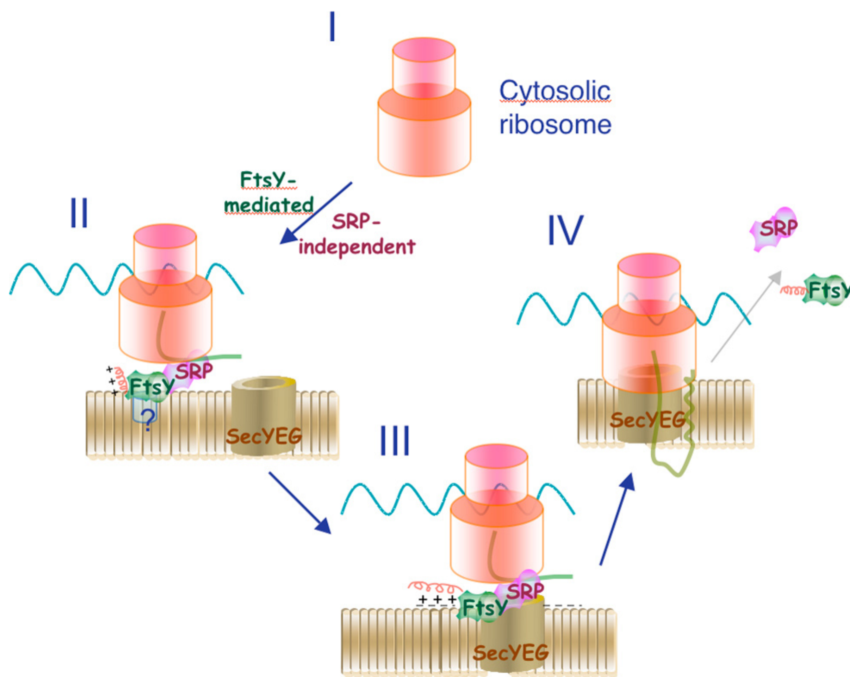

Fig. 2 *A model for targeting ribosomes translating membrane proteins to the translocon in E. coli. SRP= Signal recognition particle; FtsY= SRP-receptor. (I) targeting ribosomes to the membrane. (II) The ribosome docking site after incorporation of an mRNA encoding a membrane protein. (III) Interaction with a free translocon (SecYEG). (IV) Dissociation of SRP & FtsY and assembly of the ribosome translating a membrane protein on the translocon. The positively charged amphipathic helix in FtsY is required for GTPase stimulation and dissociation of SRP from its receptor.*

transporters (Sigal et al, 2006b), our studies demonstrated that in order for these transporters to function in multidrug resistance, they must be exceptionally flexible in structure and function. As a major goal for the future, we hope to resolve the high-resolution 3D structure of MdfA and elucidate the multidrug transport mechanism, with an emphasis on the novel proton recognition and translocation mechanism.

Membrane protein biogenesis: Surprises *in vivo*

All living cells utilize conserved systems responsible for membrane protein biogenesis, including the signal recognition particle (SRP) system, consisting of the SRP protein-RNA complex, which recognizes nascent hydrophobic peptides in the process of translation, and its membrane-bound receptor (the SRP receptor). Since the SRP pathway has been elucidated mainly through a remarkable series of *in vitro* studies, we were attracted by the findings that *E. coli* utilizes a similar pathway and by the possibility of studying the targeting pathway *in vivo*. Our initial results were both surprising and unpredicted (Herskovits et al, 2000). (1) We demonstrated that of the major components of the system only the SRP-receptor is essential for membrane protein expression and membrane targeting of ribosomes (Seluanov and Bibi, 1997; Herskovits and Bibi, 2000). The SRP itself was found to be inessential for expression and targeting, but is required for proper insertion and assembly of membrane proteins (Bochkareva et al, in preparation). Given its central role in the process, we focused our later efforts in elucidating the role of the SRP-receptor *in vivo*. (2) We identified the functional core of the SRP-receptor (Eitan and Bibi, 2004). (3) We demonstrated that, despite the fact that the majority of the SRP receptor molecules are located in the cytoplasm, only the membrane-bound receptor represents the functional form (Zelazny et al, 1997; Herskovits et al, 2001). (4) Importantly, we discovered that the SRP-receptor forms a complex with membrane-bound ribosomes, even in the absence of SRP or the translocon (Herskovits et al, 2002). (5) In collaboration with the group of prof. Irmgard Sinning at Heidelberg University, we identified a lipid-responsive domain in the receptor (Parlitz et al, 2007; Bahari et al, 2007). Collectively, our results support an alternative order of events in the *E. coli* system, compared to the current SRP model. Due to the complexity of *in vivo* experiments, we are evaluating the predictions of our hypothesis, utilizing a broad variety of approaches and methods, including genetic, biochemical, structural, and imaging tools, with the aim of better understanding how ribosome targeting and membrane protein synthesis are regulated *in vivo*.

Intra-membrane proteolysis

We were attracted by the relatively recent discovery of proteolytic enzymes, which catalyze cleavage of integral membrane proteins inside the lipid environment. This activity is crucial for many biological and pathological processes. One group of such proteases includes Rhomboids, which are evolutionarily widespread intra-membrane serine proteases that cleave unrelated sequences inside membrane proteins with a single trans-membrane helix. To test whether rhomboids may have a general role in cleavage of unfolded membrane proteins, we utilized the *E. coli* rhomboid GlpG (of unknown function) and several *E. coli* integral membrane proteins. Our studies demonstrated that indeed, the protease cleaves unfolded membrane proteins both *in vivo* and in detergent solution, and various aspects of this activity are currently investigated (Erez and Bibi, in preparation). We suggest that, in addition to their specific functions, rhomboids may potentially play a role in membrane quality control, by initiating cleavage of unfolded integral membrane proteins.

The intriguing question of how a water requiring reaction (peptide bond

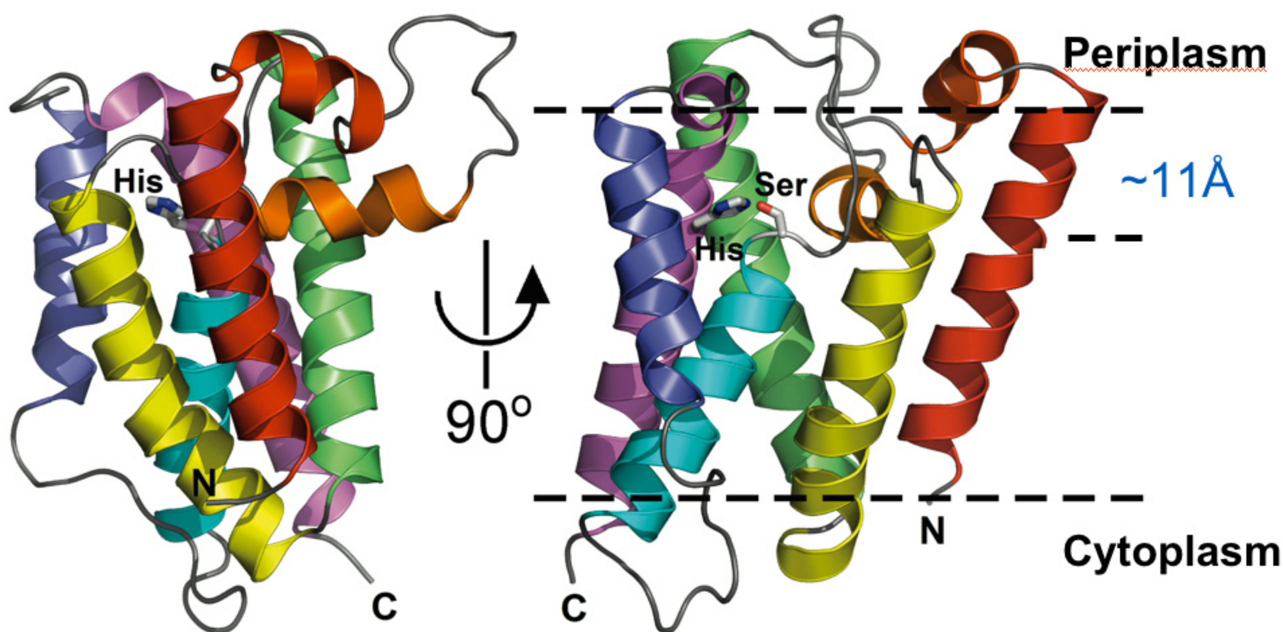

Fig. 3 *Crystal structure of the E. coli rhomboid, GlpG, an intra-membrane serine protease. Shown are side views of the protease with the active site catalytic diad residues Ser201 and His254. The depth of the active site in the membrane is approximately 11Å.*

hydrolysis) occurs inside the lipophilic environement, was studied by x-ray crystallography. Recently, we presented the 2.3 Å resolution crystal structure of GlpG, which answers the questions of how water enter the membrane embedded active site and how the structure of the trans-membrane helical substrate might be disrupted to enable accessibility of the scissile bond to the active site serine-histidine diad. Our results revealed a novel mechanism to enable water-dependent catalysis at the depth of the hydrophobic milieu of the membrane (Ben-Shem et al, 2007).

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