X-RAY CRYSTALLOGRAPHIC ANALYSIS OF XENOBIOTIC EXPORTER PROTEINS OF *ESCHERICHIA COLI*

The xenobiotic exporter proteins, also known as multidrug efflux transporters, pump a wide variety of noxious compounds out of a cell across the cell membrane. They play an important role in multidrug resistance of pathogenic bacteria and cancer cells [1]. Genomic sequence analysis has revealed the presence of many putative xenobiotic exporter genes in chromosomes from microorganisms to mammalian cells. There are 19 transporter genes in *Escherichia coli*, which actually mediate the efflux of some drugs and toxic compounds. Among them, AcrB is constitutively expressed and is a major contributor for intrinsic drug resistance of *E. coli*. [2]. AcrB cooperates with a membrane fusion protein, AcrA, and an outer membrane channel, TolC. This AcrAB-TolC system exports an unusually wide variety of noxious compounds, *e.g.* dyes, detergents and most lipophilic antibiotics directly out of the cell, bypassing the periplasmic space driven by proton motive force. AcrB is the most important component of this system; it mediates energy coupling and determines substrate specificity (Fig. 1).

First of all we cloned a histidine-tagged AcrB into a multicopy plasmid and overproduced it in *E. coli*. Then, AcrB was solubilized and purified in the presence of a non-ionic detergent, n-dodecyl-β-D-maltopyranoside. Using the vapor diffusion method, we obtained crystals of AcrB (space group *R*32), and we employed the multiple isomorphous replacement method to solve the phase problem. A molecular model was built and refined at a resolution of 3.5 Å. All the diffraction data used for structure determination were collected at beamline **BL44XU** (Institute for Protein Research, Osaka Univ.) [3].

AcrB comprises a trimer of 1,049-residue protomers (Figs. 2(a), 2(b)), and its appearance resembles that of a jellyfish with a three-fold symmetry axis perpendicular to the membrane plane. It comprises an extra-membrane (periplasmic) headpiece approximately 50 Å × 100 Å, and a transmembrane region of dimensions 70 Å × 80 Å. The headpiece is divided into two stacked parts: the upper and lower parts are 30-Å and 40-Å thick, respectively. The side view of the upper part has a trapezoidal appearance that is ~70-Å wide at the bottom and ~40-Å wide at the top. Viewed from above, the upper part is open like a funnel, with an internal diameter of 30 Å. This funnel connected by

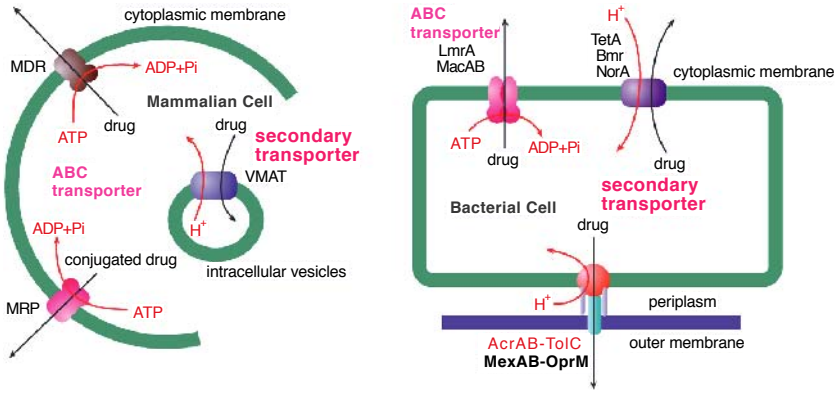

Fig. 1. Xenobiotic exporters world. A mammalian cell and a bacterial cell are illustrated.

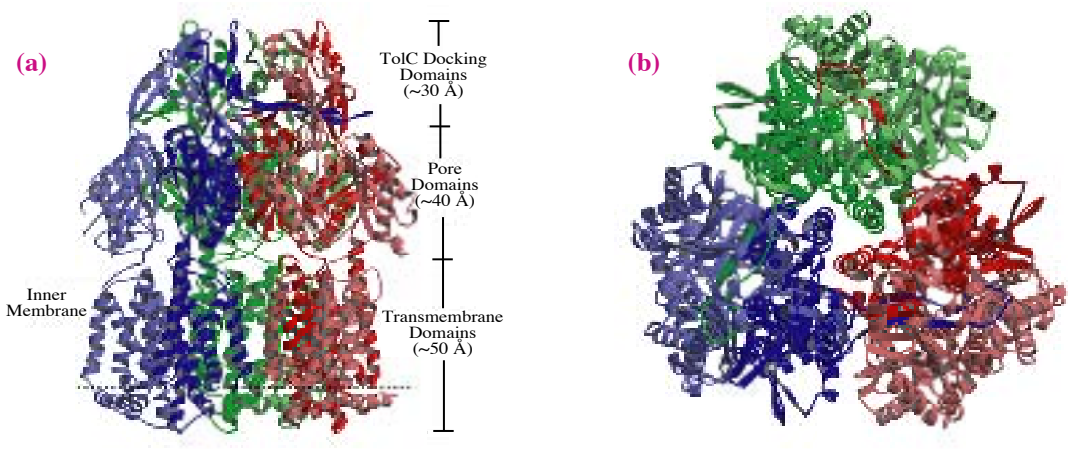

*Fig. 2. Structure of AcrB. **(a)** Side view of a ribbon representation. Three protomers are individually coloured. The N-terminal and C-terminal halves of the protomers are depicted by dark and pale colours, respectively. The extra-membrane (periplasmic) headpieces are at the top. **(b)** Top view of a ribbon representation. The protomers are individually colored as in **(a)**.*

a pore, located between the headpieces of the three protomers to a large central cavity at the interface of the headpiece and transmembrane region of the protomers. Within the membrane, the protomers are arranged like a ring with a 30-Å hole between them, which may be filled with phospholipids.

The protomers appear to be interlocked by three hairpin-like structures *ca.* 35 Å long, each protruding from one protomer into the next protomer. To achieve this mutual insertion, the protomers are tightly packed as a trimer. Between every protomer in the periplasmic domain, there are three vestibules that open wide into the periplasm and lead to the central cavity inside the headpiece, and a substrate located in the periplasmic space or on the membrane plane might gain access to the cavity through any of these vestibules (Fig. 3). The funnel opened at the top of the AcrB trimer seems to be a perfect fit for the proximal end of the TolC protein (Fig. 4(a)). The crystal structure of TolC protrudes 100 Å into the periplasm [4], thus, the sum of the periplasmic length of AcrB and TolC is ~170 Å, which is just enough to cross the periplasmic space, indicating that AcrB and TolC might direct dock with each other (Fig. 4(a)).

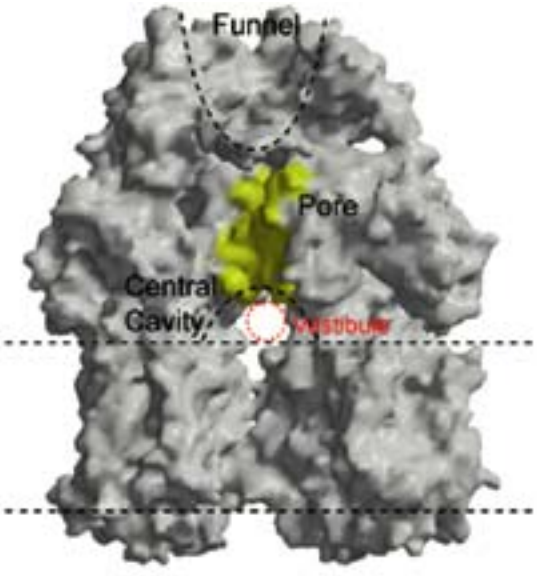

Fig. 3. A cutaway view displaying the solvent-accessible surface of AcrB. The front protomer is removed. The yellow areas of the surface are colored according to residues belonging to the pore helix.

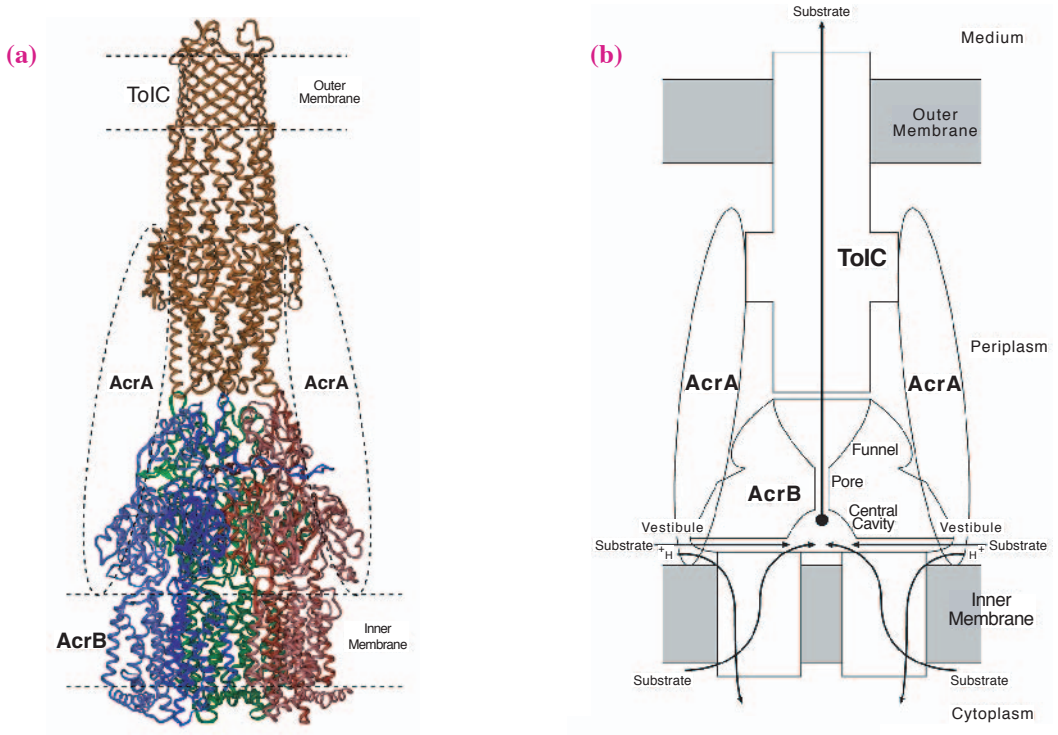

*Fig. 4. **(a)** Proposed model of the AcrB-AcrA-TolC complex. **(b)** The schematic mechanism of xenobioic export mediated by AcrAB-TolC system. The TolC structure dock with the AcrB.*

Xenobiotic substrates from the cytoplasm or inner leaflet of the membrane are transported through every protomer across the membrane and collect in the central cavity. Substrates from the periplasmic surface of the inner membrane or the outer leaflet of the membrane also gain access in the cavity through the vestibules. It appears, then, that substrates in the cavity are actively exported through the pore into the funnel and then on into the TolC tunnel.

The energy for active transport is captured in the transmembrane region and transmitted to the extra-membrane pore by a remote conformational coupling (Fig. 4(b)). This beautiful AcrB structure provides a great deal of important insight into the function of multidrug resistance mediated by xenobiotic exporters. This structure is not only the first structure of a multidrug exporter, but also the first atomic-level structure of a membrane transporter that couples with proton translocation across the membrane. Therefore, we believe that this work is a real milestone for understanding active membrane transport mechanisms based on molecular structure.

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