Dynamically Regulated Assembly of the Bacterial Xenobiotic Efflux Complex

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The resistance-nodulation-division (RND)-type xenobiotic efflux system, consisting of the inner membrane transporter (IMT), the membrane fusion protein (MFP), and the outer membrane channel (OMC), plays a major role in the multidrug resistance of gram-negative bacteria by utilizing proton-motive force to export a wide variety of chemicals, such as antibiotics and detergents, out of the cell. Among five RND-type xenobiotic efflux systems of *Escherichia coli*, the only constitutively expressed one consists of AcrB (IMT), AcrA (MFP), and TolC (OMC). The latter two components are shared with another IMT AcrD, whose expression is induced by environmental stimuli. We aimed at understanding how an RND-type ternary complex, which spans two membranes and the cell wall, form *in vivo*.

Total internal reflection fluorescence (TIRF) microscopy revealed that most fluorescent foci formed by AcrB fused to green fluorescent protein (GFP) were stationary in the presence of TolC but showed lateral displacements when *tolC* was deleted. The fraction of stationary AcrB-GFP foci decreased with increasing levels of AcrD. We propose that the AcrB-containing complex becomes unstable upon the induction of AcrD, which presumably replaces AcrB, a process we call "transporter exchange" (Fig. 1). This instability is suppressed by AcrB-specific substrates, suggesting that the ternary complex is stabilized when it is in action (Fig. 1).

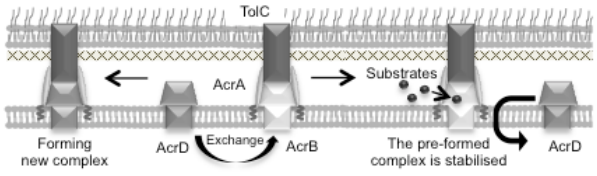

Fig. 1. Transporter exchange model.

We next visualized two IMTs (MdtB and MdtC) of the MdtABC-TolC complex that are induced upon the exposure to indole. When expressed alone in the presence of MdtA (MFP) and TolC, MdtB-GFP formed a stationary trimer, whereas MdtC-RFP rarely formed a trimer and was mobile. When co-expressed, a heterotrimer consisting of two MdtB-GFP molecules and one MdtC-RFP molecule was formed predominantly and at least some of them were stationary. These results suggest that MdtB and MdtC favor to form the 2:1 heterotrimer, which has been proposed, by the study on tandem-linked trimers, to have the highest drug efflux activity among all possible trimers.

Altogether, these results suggest that the assembly of the RND-type efflux system is dynamically regulated, shedding new light on the adaptive antibiotic resistance of bacteria.