Tripartite assembly of RND multidrug efflux pumps

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1. INTRODUCTION

Tripartite multidrug efflux systems of Gram-negative bacteria export a large variety of antimicrobial compounds at the expense of ATP or the proton motive force, thereby conferring resistance to a wide variety of antibiotics. *Pseudomonas aeruginosa* MexAB-OprM and *Escherichia coli* AcrAB-TolC, are prototypic proton motive force-driven efflux systems from Resistance Nodulation and cell Division (RND) family. These efflux systems, composed of an inner membrane transporter, an outer membrane channel and a periplasmic adaptor protein, are assumed to form ducts inside the periplasm, facilitating drug exit across the outer membrane. We previously studied OprM and MexA reconstituted in lipid membrane, reporting the possibility to control their orientation using solid support [1-3] and determined the architecture of OprM/MexA complex reconstituted into lipid membranes, using cryo-electron tomography[4]. To date there are only few studies reporting the assembly of the tripartite complex.

2. RESULTATS

2.1 Conditions expérimentales

The rationale for the reconstitution of tripartite complexes was based on the insertion of the integral membrane proteins (*i.e.* OprM/TolC or MexB/AcrB) into nanodiscs (NDs). Upon detergent removal, the membrane proteins (MexB, AcrB, OprM, TolC) were inserted into a 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) containing NDs. Subsequently, the separately ND-reconstituted efflux components were mixed with native lipidated AcrA or MexA.

2.2 Resultat 1

We present here the reconstitution of native MexAB-OprM and AcrAB-TolC in a ND system. Single particle analysis by electron microscopy revealed the lipid ND-embedded inner and outer membrane protein components linked together via the periplasmic adaptor protein, this forming a tripartite setup. This intrinsic *in vitro* self-assembly of the native components was emphasized by the formation of a stable interspecies AcrA-MexB-TolC complex, providing evidence for a common mechanism of tripartite assembly with cognate and non-cognate components. The projection structures of all three RND complexes presented here emphasize the role of the periplasmic adaptor protein as part of the exit duct with no physical interaction between the inner and outer membrane components.

3. CONCLUSION

This approach can be extended to a vast number of RND pump systems and open the field for further structural analysis at atomic level. In addition, the development of an inhibitor class targeting the tripartite assembly process can be approached more systematically.

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