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Structural Organization and Dynamic Assembly of TOL membrane proteins

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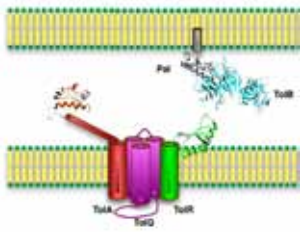
Introduction

The *tolQRAB-pal* operon is conserved in Gram-negative genomes. The Tol system is essential for bacteria as it maintains the outer membrane integrity and is involved in a late step of the the cell division process. Tol is composed of five proteins: TolA, TolQ and TolR form a complex in the inner membrane, Pal is a lipoprotein anchored in the outer membrane, and TolB is a soluble periplasmic protein. It is thought that TolQ and TolR associate to form an ion channel, and that the energy derived from the ion passage through the channel is transformed into mechanical movement of TolA that interacts with TolB/Pal. Very little is known about the nature of the movement and the long distance energy transduction. The atomic structure of the soluble part of different proteins of the complex has been solved, but the structural organisation of the transmembrane helices (TMH) of the TolA-TolQ-TolR was not described. Our goal was to investigate the architecture and the dynamic of the TMH fragments of the TolQ-TolR complex.

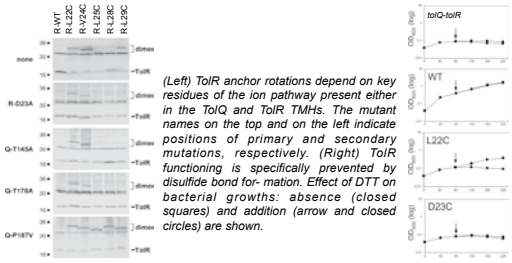


The TolA-TolQ-TolR inner membrane proteins are shown with the TMHs (cylinders) and the structures of the soluble protein domains. The outer membrane associated TolB-Pal protein complex is also shown.

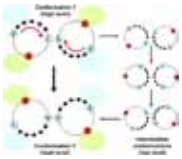
Results

To investigate the structural organisation of the TolQ and TolR TMHs, we used a systematic cysteine mutagenesis approach, coupled to physiological tests.

- We have evidenced that the TMHs of two TolR in the complex are in close proximity, and that these two TMHs rotate. Mutations of critical residues present in the suspected TolQR ion pathway prevent rotation (1) .

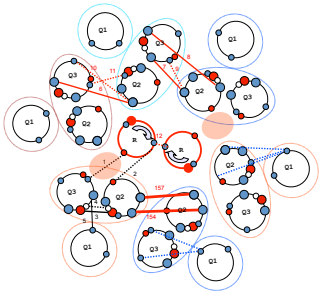


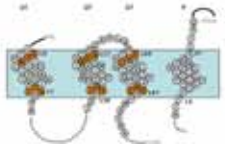
(Left) TolR anchor rotations depend on key residues of the ion pathway present either in the TolQ and TolR TMHs. The mutant names on the top and on the left indicate positions of primary and secondary mutations, respectively. (Right) TolR functioning is specifically prevented by disulfide bond for- mation. Effect of DTT on bacterial growths: absence (closed squares) and addition (arrow and closed circles) are shown.



A model for the TolR anchor rotary motion. The TMHs from two distinct TolR molecules are represented at the extreme positions of the dimerization interface (corresponding to high levels of dimerization) and oscillate between conformations 1 and 2 (red arcs). Leu22, Asp23 and Val24 residues and suspected intermediate conformations (corresponding to low levels of dimerization) are indicated.

- TolQ was shown to exist as a multimer in the complex, that could contain up to 6 copies of TolQ. Mapping of the TolQ TMH interactions deduced from single or tandem cysteine scanning suggest that the TMHs of TolQ are dynamic and can accomodate several conformation (3).





(Top) Lateral view of TolQ and TolR TMHs in which the residues used for the tandem cysteine mutagenesis are indicated. (Left) Schematic model of the TolQ and TolR TMH interactions. TolQ-TolR residues in contact are shown in a model containing six TolQ molecules, two TolR molecules, and two putative ionic channels. TolQ TMH organization (obtained by genetic suppressive data) was kept identical in each TolQ molecule. Balls represent cysteine-substituted positions involved in disulfide bond formation

- First attempts to purify solubilised TolQ and TolQ-TolR complexes have been performed and we have initiated a collaboration with the laboratory of William Cramer (West Lafayette, USA) who has expertise in channel electrophysiology to explore the ability of TolQ and TolQ-TolR to form ion channels (2).

Conclusions and perspectives

Through extensive molecular cloning (more than 150 cysteine substitutions), functional and biochemical analyses, we were able to propose a dynamic model of TMH interaction of the TolQ-TolR complex that reflects the modification of these interactions during the energisation cycle. To continue the structural investigation of this type of complex, we are currently working on the purification of the TolQ-TolR system to obtain two- and/or three-dimensional crystals suitable for structure determination by electron microscopy or Xray crystallography. In this respect we are working on the purification of the TonB system, which is highly homologous of the Tol system, but invovled in iron and nutrient uptake. We are currently purifying the ExbB-ExbD complex of the TonB system, equivalent to the TolQ-TolR complex. We have obtained two-dimensional crystals, as well as three-dimensional crystals in collaboration with the laboratory of Susan Buchanan (NIH Bethesda, USA). Refinement of the crystallisation conditions are underway.

Publications

(1) Zhang X, Goemaere E, Thomé R, Gavioli M, Cascales E, Lloubes R, 2009, Mapping the interactions between escherichia coli tol subunits: rotation of the TolR transmembrane helix. *The Journal of biological chemistry*, 284:4275-4282.

(2) Zakharov S, Célia H, Datsenko K, Santamaria M, Whitelegge J, Lloubes R, Cramer W, 2010, Components of E. Coli Energy-Transducing Complexes, ExbB and TolQ, Display Ion Channels. *Biophysical Journal, 98 (3 Supplement 1),* 540a.

(3) Zhang X, Goemaere E, Seddiki N, Célia H, Gavioli M, Cascales E and Lloubes R, 2011, Mapping the interactions between Escherichia coli TolQ transmembrane segments. *The Journal of biological chemistry,* 286:11756-11764

Publications resulting from material or expertise derived from the ANR grant

Barnéoud-Arnoulet A, Gavioli M, Lloubes R, Cascales E, 2010, Interaction of the colicin K bactericidal toxin with components of its import machinery in the periplasm of Escherichia coli. *Journal of bacteriology*, 192:5934-5942.

Zhang X, Lloubes R, Duché D, 2010, Channel domain of colicin A modifies the dimeric organization of its immunity protein. *The Journal of biological chemistry*, 285: 38053-38061.

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