

The ABC transporter HlyB and its C39 peptidase-like domain – an appendix without function?

Christian Schwarz¹, Justin Lercher², Thorsten Jumpertz¹, Matthias Stoldt²,
Dieter Willbold², Sander H.J. Smits¹ and Lutz Schmitt¹

¹Institute of Biochemistry, Heinrich-Heine-University, Düsseldorf, Germany;

²Institute of Structural Biology, Research Center Jülich, Jülich, Germany

A paradigm of Type I secretion is the haemolysin A (HlyA) secretion system of *E. coli*. The ATP-binding cassette (ABC)-transporter haemolysin B (HlyB), a central element of this Type I secretion machinery, acts in concert with the membrane fusion protein haemolysin D (HlyD) and the outer membrane protein TolC to translocate the 107 kDa toxin HlyA in one step from the cytoplasm to the exterior.

Like all other ABC transporters, functional HlyB is supposed to contain two copies of a nucleotide binding domain (NBD) and a transmembrane domain (TMD). Since HlyB is a so-called “half-size” transporter, the functional and transport-competent unit is a dimer of HlyB. In striking contrast to other ABC transporters, a C39 peptidase-like domain of 123 amino acids is additionally fused to the N-terminus of HlyB, which is conserved in many Type I secretion systems.

Generally, C39 peptidase domains hydrolyze their substrates after a GG motif, via an active site composed of a highly conserved catalytic dyad build up by a cysteine and histidine residue. The C39 domain of HlyB however lacks the catalytic cysteine and is therefore named a “C39-like” domain. The function of this domain is, besides being necessary for translocation, not known.

In a C39 knockout strain the secretion of HlyA is completely abolished, pointing towards a crucial role of C39 in the secretion process. To understand the role of this domain in substrate secretion via the HlyA machinery, we have analyzed the “C39-like” domain of HlyB in its isolated state by functional and structural approaches. Here, we will describe our recent results which will shed a new light on C39 and will finally reveal its mystery.