Oral presentation

Structure of the nonameric type III secretion export gate with bound substrate-chaperone complex

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Many bacterial pathogens employ a type III secretion system (T3SS) to inject cytotoxic effector proteins into the cytoplasm of human host cells. A T3SS is a large multi-protein complex resembling a macromolecular syringe. T3SS assembly and function relies on the correctly timed selection of proteins for secretion. Secreted proteins usually require binding to a cognate chaperone that remains in the bacterial cytoplasm after secretion of the substrate. Chaperones are involved in targeting secretion substrates to the injectisome. The export gate SctV, the major protein of the inner membrane export apparatus, is involved in selection and export of secreted proteins. Up to now, there was no structural information on how substrate-chaperone complexes interact with the injectisome of virulence-associated T3SS. We addressed this question using X-ray crystallography.

Structures of binary complexes consisting of the secreted protein SctX and its chaperone SctY revealed that SctX has an unusual extended hydrophobic C-terminal helix that might be involved in targeting to the export gate [1]. Crystals of the isolated cytosolic domain of the export gate protein ($SctV_C$) and crystals of ternary complexes consisting of substrate, chaperone and $SctV_C$ from various species mostly diffracted poorly but revealed rings with eight-, nine-, or ten-fold symmetry [2]. The ternary complex of the substrate:chaperone (SctX:SctY) pair bound to $SctV_C$ from pathogenic *Yersinia enterocolitica* facilitated structure determination [3]. $SctV_C$ forms a central nonameric ring to which nine SctX:SctY complexes bind on the outside with 9:9:9 stoichiometry (Fig. 1). The structure reveals interactions between the export gate protein and both the secretion substrate SctX and the chaperone SctY. The secretion substrate SctX interacts with $SctV_C$ via both its N- and its C-terminus. This nicely fits previously published functional data showing that alterations of both the N- and the C-terminus of SctX impede T3SS function [4].

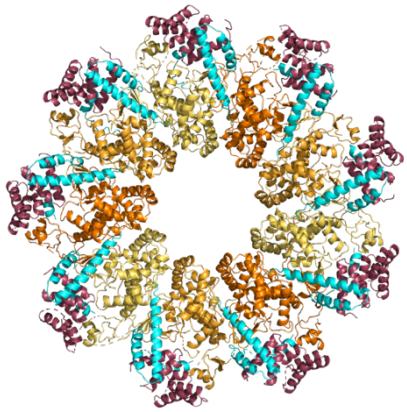

Figure 1. View on the membrane-distal side of the nonameric $SctV_C$:SctX:SctY complex.
The SctV cytosolic domain is shown in yellow, orange and dark orange to distinguish the protomers. The high affinity binary complex of SctX (secreted protein; cyan) and SctY (chaperone; red) binds to the outside of the nonameric $SctV_C$ ring. The C-terminal helix of SctX protrudes into the ring between two adjacent $SctV_C$ protomers. This interaction is essential for a functional T3SS.

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[2] Gilzer, D., Baum, E., Lieske, N., Kowal, J.L. Niemann H.H. (2022). Acta Crystallogr. F Struct. Biol. Commun., ***78****, 386.*

[3] Gilzer, D., Schreiner, M., Niemann, H.H. (2022). Nat. Commun. ***13****, 2858.*

[4] Day J.B., Plano G.V. (2000). J. Bacteriol. ***182****, 1834.*