Secrets of a Secretin

Dirk W. Heinz[1,*]
[1]Helmholtz Centre for Infection Research, Inhoffenstraße 7, D-38124 Braunschweig, Germany
*Correspondence: dirk.heinz@helmholtz-hzi.de
http://dx.doi.org/10.1016/j.str.2013.11.002

Secretins are major constituents of bacterial type III secretion systems (T3SS). In this issue of *Structure*, Kowal and colleagues report on the cryo-EM structure of the native YscC secretin from *Yersinia*, revealing its internal symmetry and mode of length adaptation.

Type III secretion systems (T3SS), also called injectisomes, are important virulence factors in several well-known Gram-negative bacterial pathogens, including *Shigella flexneri*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, and *Yersinia sp*. By acting as sophisticated molecular needles, T3SS actively translocate effector proteins from the bacterial cytoplasm directly into the host cell to support an infection by specifically subverting host cell pathways and processes (Coburn et al., 2007). The T3SS forms a >3 MDa syringe-shaped structure, which is made up of less than 25 distinct proteins forming the bacterial inner and outer membrane-spanning basal body, the extracellular needle, and the translocon, which is inserted into the host cell membrane.

Over the last 10 years, considerable progress has been made on the structural elucidation of T3SS from different bacteria, including *Shigella*, *Salmonella*, pathogenic *E. coli*, and *Yersinia*. The clever combination of electron microscopy (EM), X-ray crystallography, and nuclear magnetic resonance (NMR) spectroscopy led to strikingly detailed models of assembled needle complexes (reviewed by Kosarewicz et al., 2012; Bergeron et al., 2013). The recent in situ structure determination of whole injectisomes from *Shigella flexneri* and *Yersinia enterocolitica* revealed remarkable structural dynamics, with the size of the basal body being able to fluctuate by up to 20% (Kudryashev et al., 2013).

Secretins are well conserved multidomain proteins that form large cylindrical homo-oligomeric assemblies in T3SS as well as a number of other multiprotein secretion systems (Korotkov et al., 2011). In its assembled homooligomeric structure, it constitutes the outer membrane pore of the basal body, where it provides a channel for the injectisome needle. Secretins directly interact with the inner membrane ring components, thus bridging the periplasmic space. Abundant structural information is available on various secretins from different secretion systems with high resolution structures of individual domains and EM reconstructions leading to models of secretin ring complexes with different spatial resolutions (Korotkov et al., 2011).

In this issue of *Structure*, Kowal et al. (2013) contribute new structural insights on the secretin YscC from *Yersinia enterocolitica*, which have important functional implications for injectisome dynamics and assembly. The 12 Å cryo-EM structure of the native YscC complex reconstituted into proteoliposomes not only revealed its overall dodecameric symmetry, but also showed pronounced periplasmic extensions, named petals, linked by flexible regions to the outer membrane secretin core (Figure 1). The authors suggest that the loosely ordered and protease-sensitive petals not only link YscC to inner membrane component YscD, but also contribute to length adaptation of the basal body, e.g., under osmotic stress. In addition, experimental data are presented for the first time, demonstrating how a secretin integrates into the lipid bilayer. The structure nicely supports the previously suggested outside-in assembly model (Diepold et al., 2010) in which the outer membrane protein YscC serves as an assembly platform for the entire injectisome. Compared to the structures of the secretins InvG from *S. typhimurium* (Schraidt and Marlovits, 2011) and MxiD from *S. flexneri* (Hodgkinson et al., 2009), both the size of the petals and the arrangement of the

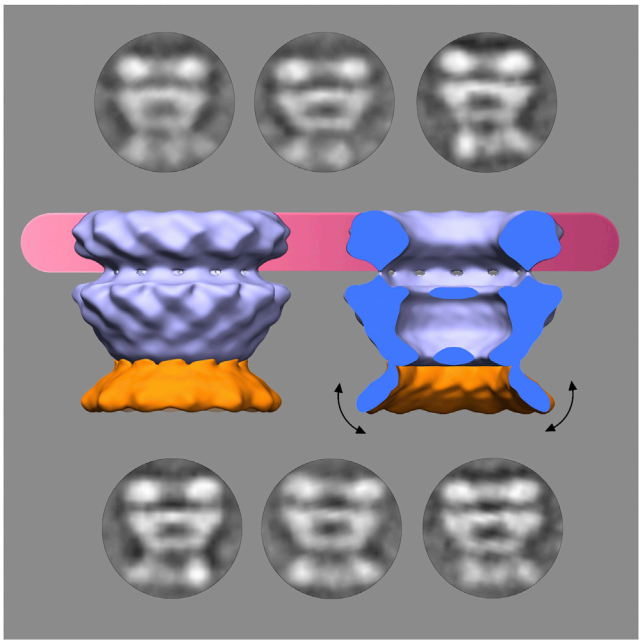

Figure 1. Class Averages of Side Views Demonstrate Substantial Flexibility of the YscC Secretin Peripheral Extensions
These petals (dark yellow) represent the N-terminal domain and are attached to the protease-resistant YscC core (purple/blue) by flexible hinges. The outer membrane ring is embedded in the lipid bilayer, indicated in pink. Figure courtesy of Kowal et al. (2013).

inner gates show remarkable differences, while the outer membrane parts are well conserved.

Kowal et al. (2013) have achieved a breakthrough in deciphering the internal rotational symmetry of the YscC complex despite the moderate spatial resolution. The previously published 13-fold symmetry of YscC (Burghout et al., 2004) was rather inconsistent, considering the prevalent 12-fold symmetry of many other secretin complexes. To determine the rotational symmetry of a cylindrical complex by electron microscopy, image contrast and structural preservation of the sample have to be optimal. Negative staining provides high contrast but does not always warrant sample conservation. Erroneous results may emerge from such samples: in particular, when a reference with presumed symmetry is used for image alignment. Because the contrast in images of frozen, well preserved YscC complexes was too small for symmetry determination, Kowal et al. (2013) analyzed axial projections of negatively stained YscC cylinders using multiple algorithms to substantiate the 12-fold symmetry. Mass measurements of the YscC complex by STEM and cryo-electron tomography of lipid-embedded YscC confirmed the dodecameric nature of the YscC secretin. Subsequent refinements of the structure calculated from projections of vitrified YscC secretins imposing symmetries of order 3, 4, and 6 demonstrated that the complex is, in fact, a dodecamer.

The spatial resolution of the described YscC complex structures currently precludes the precise localization of individual YscC domains. Molecular mechanisms, such as gating for translocation of needle components or effectors as well as secretin assembly, can only be explained by much higher resolution structures. However, the observation of the flexible periplasmic domains has clear functional implications. As the protease-resistant C-terminal core of YscC is well defined, labeling this core with nanogold clusters could provide a strong signal for aligning projections and achieving a much higher resolution. Alternatively, embedding YscC in two-dimensional regular arrays suitable for electron crystallography should ultimately provide atomic resolution.

Infections by Gram-negative bacteria pose a dramatically increasing problem in the clinic due to their resistance to multiple antibiotics and lack of novel antibiotics. The T3SS represents an attractive target for novel antibiotics against Gram-negative bacteria in multiple ways (Raska and Sperandio, 2010). The detailed knowledge on both the conserved structure of the secretin core as well as the assembly process of the basal body could pave the way for new strategies in this direction.

REFERENCES

Bergeron, J.R.C., Worrall, L.J., Sgourakis, N.G., DiMaio, F., Pfuetzner, R.A., Felise, H.B., Vuckovic, M., Yu, A.C., Miller, S.I., Baker, D., and Strynadka, N.C.J. (2013). PLoS Pathog. *9*, e1003307.

Burghout, P., van Boxtel, R., Van Gelder, P., Ringler, P., Müller, S.A., Tommassen, J., and Koster, M. (2004). J. Bacteriol. *186*, 4645–4654.

Coburn, B., Sekirov, I., and Finlay, B.B. (2007). Clin. Microbiol. Rev. *20*, 535–549.

Diepold, A., Amstutz, M., Abel, S., Sorg, I., Jenal, U., and Cornelis, G.R. (2010). EMBO J. *29*, 1928–1940.

Hodgkinson, J.L., Horsley, A., Stabat, D., Simon, M., Johnson, S., da Fonseca, P.C., Morris, E.P., Wall, J.S., Lea, S.M., and Blocker, A.J. (2009). Nat. Struct. Mol. Biol. *16*, 477–485.

Korotkov, K.V., Gonen, T., and Hol, W.G.J. (2011). Trends Biochem. Sci. *36*, 433–443.

Kosarewicz, A., Königsmaier, L., and Marlovits, T.C. (2012). Philos. Trans. R. Soc. Lond. B Biol. Sci. *367*, 1140–1154.

Kowal, J., Chami, M., Ringler, P., Müller, S.A., Kudryashev, M., Castaño-Díez, D., Amstutz, M., Cornelis, G.R., Stahlberg, H., and Engel, A. (2013). Structure *21*, this issue, 2152–2161.

Kudryashev, M., Stenta, M., Schmelz, S., Amstutz, M., Wiesand, U., Castano-Diez, D., Degiacomi, M.T., Munnich, S., Bleck, C.K., Kowal, J., Diepold, A., Heinz, D.W., Dal Peraro, M., Cornelis, G.R., and Stahlberg, H. (2013). eLife *2*, e00792.

Raska, D.A., and Sperandio, V. (2010). Nat. Rev. Drug Discov. *9*, 117–128.

Schraidt, O., and Marlovits, T.C. (2011). Science *331*, 1192–1195.