



HAL
open science

Inside the Chamber of Secrets of the Type III Secretion System

E. Cascales

► **To cite this version:**

E. Cascales. Inside the Chamber of Secrets of the Type III Secretion System. Cell, 2017, 168 (6), pp.949 - 951. 10.1016/j.cell.2017.02.028 . hal-01780709

HAL Id: hal-01780709

<https://amu.hal.science/hal-01780709v1>

Submitted on 27 Apr 2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Inside the chamber of secrets of the Type III secretion system

Eric Cascales^{1,*}

¹Laboratoire d'Ingénierie des Systèmes Macromoléculaires (LISM, UMR 7255), Institut de Microbiologie de la Méditerranée (IMM), Aix-Marseille Université (AMU) - Centre National de la Recherche Scientifique (CNRS), 31 chemin Joseph Aiguier, 13402 Marseille Cedex 20, France.

*Correspondence: cascales@imm.cnrs.fr

The bacterial Type III secretion system is a specialized machine that injects effectors into eukaryotic cells to manipulate the host cell physiology. In this issue of *Cell*, Hu *et al.* use electron cryo-tomography to reveal an unprecedented level of details regarding the architecture of this machine and the conformational changes that occur during its assembly.

Bacteria have evolved sophisticated mechanisms to transport proteins across membranes, specifically to deliver protein effectors into the milieu or directly inside target cells. These different mechanisms rely on specialized machineries, known as *secretion systems*, which are usually constituted of 10-20 soluble and membrane proteins. The Type III secretion system (T3SS) is an important virulence device used by plant and animal pathogens to inject effectors upon contact with eukaryotic cells (Figure 1A). This secretion apparatus comprises four structures: the cytoplasmic platform, the export apparatus, the envelope-spanning basal body, and the extracellular needle - the last two collectively known as needle complex (Burkinshaw and Strynadka, 2014; Galan *et al.*, 2014)(Figure 1B). Although the T3SS needle and flagellar filament are structurally distinct, the cytoplasmic platform, export apparatus and most of the basal body of these two organelles share functional similarities (Galan *et al.*, 2014). The needle and basal body are very well characterized, especially due to the ease to purify needle complex particles for *in vitro* structural approaches (Schraidt and Marlovits, 2011; Fujii *et al.*, 2012). The basal body is constituted of several rings - two rings flanking the inner membrane (IR1 and IR2) and two rings close to the outer membrane (OR1 and OR2), the IR and OR rings being separated by the neck (Burkinshaw and Strynadka, 2014; Galan *et*

al., 2014; Diepold and Armitage, 2015)(Figure 1B). A recent high-resolution cryo-electron microscopy structure of the basal body demonstrated that the secretin is the main component of the OR1, OR2 and neck, whereas the periplasmic domains of SctD in complex with SctJ and the cytoplasmic domain of SctD constitute the IR1 and IR2 rings, respectively (Worrall et al., 2017). The cytoplasmic components, that include the SctRSTU export apparatus, the SctV export gate, the cytoplasmic platform and the SctN ATPase, associate with the basal body (Diepold and Wagner, 2014).

During T3SS biogenesis, the basal body is assembled first and recruits the export apparatus and cytoplasmic platform prior to needle polymerization and effector transport (Diepold and Wagner, 2014). The cytoplasmic platform sorts the needle subunits and effectors, renders them competent for secretion and hierarchizes their transport to the export apparatus (Galan et al., 2014). The secretion process is a highly controlled mechanism: early substrates, comprising the inner rod and needle subunits, are selected and exported first, and form the conduit for the secretion of the needle tip proteins, the translocon components and the effectors *per se* (Galan et al., 2014). This hierarchical substrate specificity process is regulated by switches that occur at the level of the export apparatus and cytoplasmic platform (Galan et al., 2014). Although the cytoplasmic complex is critical for effector selection and specificity switching, less data are available for this portion of the T3SS as it has been recalcitrant to purification attempts and it does not stably co-purify with the basal body. Hence, understanding how the cytoplasmic complex and the basal body are connected and how docking of the cytoplasmic complex impacts the conformation of the basal body are of critical importance.

In this issue of *Cell*, Hu et al. (2017) use electron cryo-tomography (ECT), a powerful methodology to image intact, unperturbed complexes directly inside their native cellular environment. They report the structure of the fully assembled *Salmonella* SPI-1 T3SS with a

remarkable 17-Å resolution. Although ECT studies have been recently reported for several T3SSs including those of *Yersinia* and *Shigella* (Kudryashev et al., 2013; Hu et al., 2015; Nans et al., 2015), none of them reached the level of details defined here. First, the current study demonstrates that - with the exception of the IR2 - the basal body and needle are superimposable with isolated needle complex particles. Interestingly, as shown for the *Myxococcus* Type IVa pilus (Chang et al., 2016), they noticed that the insertion of the T3SS in the cell envelope induces a deformation of the outer membrane yielding a ~ 3-nm constriction of the periplasm thickness. More importantly, this study uncovers unprecedented details on the export apparatus and cytoplasmic platform. Previous subtomogram averaging of the *Shigella* T3SS revealed that the cytoplasmic platform forms a cage that comprises six pod-like structures connected to a hub by spokes. The level of definition did not however permit to unambiguously assign each of the densities to known components (Hu et al., 2015). Indeed, correlating densities of ECT reconstructions to atomic structures is always challenging, but the authors of the current study successfully used a combination of deletion mutants and fusion proteins to precisely locate the components of the export apparatus and cytoplasmic platform. While deletion mutants might be useful, they often prevent assembly or cause instability of the apparatus - or at least of part of it. Cells producing functional T3SS subunits fused to large domains are more powerful, as extra-densities seen in the map indicate their position and their orientation. Indeed, *sctV* mutant cells and cells producing GFP-SctV were imaged to properly position the 9-mer export gate and the export apparatus that docks underneath IR2. The cytoplasmic platform is a 23-nm long sixfold symmetry cage-like structure that protrudes from the IR2 and assembles and docks to the basal body independently of the export apparatus (Figure 1B). By fusing SctK, SctL, SctN, SctO and SctQ to the GFP or PAFP fluorescent protein, Hu et al. provide a detailed map of the cytoplasmic platform. The hub is constituted of the SctN ATPase on which is bound the SctO

stalk, and is attached to the six SctQ pods by six SctL spokes. Finally, SctK docks the cytoplasmic platform to the basal body IR2 by interacting with both SctQ and the SctD cytoplasmic domain. Comparison of wild-type and platformless T3SS also revealed that docking of the cytoplasmic platform causes significant rearrangements of IR2.

Overall, the structure of the *Salmonella* T3SS cytoplasmic platform is similar to the one observed in *Shigella* but does not share homologies with the flagellar C-ring. However, it is supposed that other T3SS from different species such as *Yersinia* and *Pseudomonas* exhibit significant variability compared to the *Salmonella* and *Shigella* T3SSs. It is likely that future ECT imaging of these T3SSs will reveal the structural diversity that exists within this family of secretion nanomachines. Similarly, comparing T3SS and flagellar structures from diverse bacteria will likely provide further understanding on how T3SS emerged from flagellum and on how these two structures co-evolved. The overall cage-like structure of the cytoplasmic platform and the proximity of the SctN ATPase with the SctV export gate led the authors to propose that it could constitute a chamber in which substrates are selected and prepared for secretion. Imaging the T3SS with substrates trapped in this chamber using point mutations in the ATPase or export gate is an exciting prospect to this work. Similarly to the hidden chambers in the Giza great pyramid or in the Tutankhamun tomb, the T3SS cytoplasmic chamber preserves its secrets. But for how long?

ACKNOWLEDGMENTS

Work in E.C. laboratory is supported by the Centre National de la Recherche Scientifique, the Aix-Marseille Université and grants from the Agence Nationale de la Recherche (ANR-14-CE14-0006-02, ANR-15-CE11-0019-01). I apologize for the lack of citation of primary reports owing to space limitations.

REFERENCES

- Burkinshaw, B.J., and Strynadka, N.C. (2014). *Biochim Biophys Acta* 1843, 1649–1663.
- Chang, Y.W., Rettberg, L.A., Treuner-Lange, A., Iwasa, J., Søgaard-Andersen, L., and Jensen, G.J. (2016). *Science*. 351, aad2001.
- Diepold, A., and Wagner, S. (2014). *FEMS Microbiol Rev.* 38, 802–822.
- Fujii, T., Cheung, M., Blanco, A., Kato, T., Blocker, A.J., and Namba, K. (2012). *Proc Natl Acad Sci U S A*. 109, 4461–4466.
- Galán, J.E., Lara-Tejero, M., Marlovits, T.C., and Wagner, S. (2014). *Annu Rev Microbiol* 68, 415–438.
- Hu, B., Lara-Tejero, M., Kong, Q., Galán, J.E., and Liu, J. (2017). *Cell this issue*.
- Hu, B., Morado, D.R., Margolin, W., Rohde, J.R., Arizmendi, O., Picking, W.L., Picking, W.D., and Liu, J. (2015). *Proc Natl Acad Sci U S A* 112, 1047–1052.
- Kudryashev, M., Stenta, M., Schmelz, S., Amstutz, M., Wiesand, U., Castaño-Díez, D., Degiacomi, M.T., Münnich, S., Bleck, C.K., Kowal, J., et al. (2013). *Elife* 2, e00792.
- Schraidt, O., and Marlovits, T.C. (2011). *Science* 331, 1192–1195.
- Worrall, L.J., Hong, C., Vuckovic, M., Deng, W., Bergeron, J.R., Majewski, D.D., Huang, R.K., Spreter, T., Finlay, B.B., Yu, Z., and Strynadka, N.C. (2017). *Nature*. doi: 10.1038/nature20576.

Figure caption.

Figure 1. Architecture of the *Salmonella* Type III secretion injectisome.

(A) Schematic representation of a *Salmonella* cell with assembled SPI-1 Type III secretion systems. A magnification of the secretion apparatus is shown in panel B. (B) Architecture of the *Salmonella* Type III secretion systems (T3SS) injectisome revealed by electron cryotomography. The localizations of the different subunits (see color code on left) are indicated (OM, outer membrane; PG, peptidoglycan; IM, inner membrane; OR, outer ring; IR, inner ring; CP, cytoplasmic platform). For each protein, the unified T3SS Sct nomenclature is indicated as well as the name of the orthologs in *Yersinia* (Ysc), *Shigella* (Spa/Mxi),

Salmonella (Inv/Prg/Org) and in the flagellum (Fli, Flh).

