Cell

Inside the Chamber of Secrets of the Type III Secretion System

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http://dx.doi.org/10.1016/j.cell.2017.02.028

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 cryo-electron 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 are collectively known as the needle complex (Burkinshaw and Strynadka, 2014; Galán 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 (Galán et al., 2014). The T3SS needle and basal body are very well characterized, especially due to the ease of purifying 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; Galán et al., 2014) (Figure 1B). A recent high-resolution cryo-electron microscopy structure of the basal body demonstrated that 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., 2016). The cytoplasmic components, which 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 (Galán 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 (Galán et al., 2014). This hierarchical substrate specificity process is regulated by switches that occur at the level of the export apparatus and cytoplasmic platform (Galán 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 because 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 cryo-electron tomography (cryo-ET), 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 cryo-ET studies have been recently reported for several T3SSs, including those of *Yersinia* and *Shigella* (Kudryashev et al., 2013; Hu et al., 2015), none of them have 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 an ~3-nm constriction of the periplasm thickness (Figure 1B). More importantly, this study uncovers unprecedented details on the export apparatus and cytoplasmic platform. Previous sub-tomogram 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 unambiguous assignment of each of the densities to known components (Hu et al., 2015). Indeed, correlating densities of cryo-ET reconstructions to atomic structures is always challenging, but the authors of the current study successfully use a combination of deletion

CELL 9482_9485