UNDER THE LENS

Freeze! Secretion systems caught in the act

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This month's Under the Lens discusses electron cryotomography (ECT) as a powerful tool for investigating host–pathogen interactions.

To colonize their hosts, pathogenic bacteria have developed highly efficient strategies, including the delivery of virulence factors into host cells via sophisticated secretion systems. As secretion systems are composed of multiple proteins that span at least one bacterial membrane, determining the structure of these dynamic macromolecular complexes has been challenging. Two recent papers show that the application of ECT can give insights into the mechanistic basis of secretion in vivo and understanding bacterial pathogenesis. ECT produces high-resolution 3D reconstructions of molecular complexes by immobilizing biological specimens in non-crystalline ice and imaging samples under cryogenic conditions. By collecting series of 2D images of a sample in a defined set of tilts, ECT allows the reconstruction of 3D volumes of the field of view.

The type III secretion system (T3SS) is a syringe-like nanomachine that translocates effectors from bacteria into the cytoplasm of host cells. The system is composed of a basal body embedded in bacterial membranes, a needle and a translocon that is inserted into the host plasma membrane^{1,2}. Although

engagement of the translocon is a crucial step for an intimate bacterium–host interaction and the delivery of effectors through T3SSs, its insertion into the plasma membrane remains largely uncharacterized. Park et al.³ used ECT to image *Salmonella enterica* subsp. *enterica* serovar Typhimurium minicells interacting with epithelial cells through the T3SS. Interaction of the needle tip complex of the T3SS with the host cell induces curvature in the plasma membrane, without penetration of the needle or a conformational change in the T3SS. Moreover, in situ structural analysis confirmed that the needle tip complex, composed of only SipD, is necessary to sense the host cell, prior to inserting the remainder of the translocon into the plasma membrane. A subtomogram revealed that a hemispherical hole appears in the translocon once it has partially embedded into the plasma membrane, potentially acting as the channel for effectors to translocate into the host cell.

Using a combination of cryo-focused ion beam milling and ECT, Böck and colleagues⁴ revealed, for the first time, the architecture of a type VI secretion system (T6SS) in bacteria interacting with eukaryotic cells. The T6SS is a contractile macromolecular apparatus used by Gram-negative bacteria to deliver toxins into prokaryotic competitors or eukaryotic hosts. The structure and dynamics of the T6SS in whole-cell were first revealed by ECT in *Vibrio cholerae* spp. and consists of an inverted phage tail-like structure anchored in the bacterial membrane via a cytoplasmic baseplate and a periplasmic membrane complex⁵. Using ECT,

Böck et al. identified a novel T6SS, denominated 'T6SS subtype 4', in '*Candidatus Amoebophilus asiaticus*', an intracellular

bacterial symbiont of amoebae. Bacteriophage tail-like structures were observed together with a baseplate and an anchoring complex in the bacterial cytoplasm. In contrast to the canonical T6SS, the tail sheaths were organized in bundles of 2–34 individual structures as hexagonal arrays and lacked a clear transmembrane complex. Cryotomograms of bacteria inside their host revealed that the contact between the bacterial outer membrane and the phagosomal membrane was associated with contraction of at least one of the T6SS-like structures within the array, suggesting that the T6SS helps to disrupt the phagosome, allowing bacteria entry into the amoeba cytoplasm.

In summary, ECT is a powerful tool to study the function and importance of secretion systems of bacteria in their native environment. However, this technique is not only restricted to macromolecular complexes, but can be applied to many other biological systems in which the relevant structural reorganization of the membranes, transient binding partners or specific protein conformations may only occur during host–pathogen interactions.

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Competing interests

The authors declare no competing interests.