



A unique bacterial secretion machinery with multiple secretion centers

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Edited by Jorge Galán, Yale University, New Haven, CT; received November 3, 2021; accepted March 22, 2022

The *Porphyromonas gingivalis* type IX secretion system (T9SS) promotes periodontal disease by secreting gingipains and other virulence factors. By in situ cryoelectron tomography, we report that the *P. gingivalis* T9SS consists of 18 PorM dimers arranged as a large, caged ring in the periplasm. Near the outer membrane, PorM dimers interact with a PorKN ring complex of ~52 nm in diameter. PorMKN translocation complexes of a given T9SS adopt distinct conformations energized by the proton motive force, suggestive of different activation states. At the inner membrane, PorM associates with a cytoplasmic complex that exhibits 12-fold symmetry and requires both PorM and PorL for assembly. Activated motors deliver substrates across the outer membrane via one of eight Sov translocons arranged in a ring. The T9SSs are unique among known secretion systems in bacteria and eukaryotes in their assembly as supramolecular machines composed of apparently independently functioning translocation motors and export pores.

cryoelectron tomography | type IX secretion | protein transport | proton motive force | porphyromonas

Bacteria deploy at least 11 different translocation systems to export macromolecules across their cell envelopes (1–3). Of these, the recently discovered type IX secretion systems (T9SSs) carried by many species of the *Fibrobacteres–Chlorobi–Bacteroidetes* (FCB) superphylum play various critical roles in pathogenesis and colonization (4–7). Two bacterial models have emerged for detailed mechanistic and structural analyses of the T9SS. The Por T9SS in *Porphyromonas gingivalis*, a key contributor to human periodontitis and an emerging systemic pathogen, transports various heme-binding proteins and toxins aiding in colonization and invasion of gingival and other tissues (8, 9). The Gld T9SS in *Flavobacterium johnsoniae* secretes adhesins SprB and RemA and dozens of other effector proteins, but also functions as a rotary motor to direct the movement of a surface-bound adhesin along helical tracks, resulting in a form of gliding motility (10, 11). Studies have shown that these T9SSs are large, cell envelope–spanning nanomachines composed of at least 14 essential subunits (Fig. 1A) (6, 7). They translocate substrates, exported to the periplasm by the general secretory (Sec) pathway, across the outer membrane (OM). At the cell surface, they release their substrates to the milieu or deliver them to an “attachment complex,” which covalently links the substrates to anionic lipopolysaccharides (A-LPSs) for surface display (5, 12).

Three stable subassemblies have been identified through biochemical or single-particle microscopy approaches: 1) An inner membrane (IM) molecular motor composed of the PorL and PorM subunits (13, 14) with structural and functional homologies with the MotAB/PomAB flagellar and ExbBD/TolQR PMF-dependent motors (15–18); 2) a large ~50-nm ring-shaped complex composed of periplasmic PorN and OM-associated lipoprotein PorK (19, 20); and 3) an OM-spanning translocon, termed Sov or SprA, configured as a 36-strand β -barrel with a central pore of ~70 Å (21). Despite this progress, it is not yet known how these substructures interact or coordinate their activities, in part because there is no overarching view of the T9SS in the native context of the bacterial cell envelope. Here, we visualized the intact T9SS of *P. gingivalis* by in situ cryoelectron tomography (cryo-ET). Remarkably, the T9SS presents not as a single functional entity, as shown for all known bacterial and eukaryotic protein transport systems, but rather as a composite of apparently independent translocation motors and Sov translocons arranged symmetrically as distinct rings in the cytoplasm, periplasm, and cell surface.

Results and Discussion

The T9SS: A Large, Caged-Ring Structure. *P. gingivalis* cells are small (~0.5 μ m in diameter) and well suited for cryo-ET imaging (Fig. 1B and *SI Appendix, Fig. S1A*).

Significance

The newly described type IX secretion systems (T9SSs) translocate virulence factors and can mediate specialized gliding motility among bacterial pathogens of the *Fibrobacteres–Chlorobi–Bacteroidetes* superphylum. We visualized the spatial organization of the T9SS in its native context in the *Porphyromonas gingivalis* cell by cryoelectron tomography. The T9SS exhibits distinct symmetries across the bacterial cell envelope: a cytoplasmic complex requiring PorL and PorM for assembly exhibits 12-fold symmetry; a periplasmic complex composed of PorM exhibits 18-fold symmetry and attaches to a PorKN ring near the outer membrane; and eight Sov translocons are arranged with 8-fold symmetry at the cell surface. The T9SS is the largest of the known bacterial secretion systems and evidently arranges as multiple, independently functioning translocation motors.

Author contributions: R.J.L. and B.H. designed research; L.S., J.D.P., C.W., T.D., Z.N., J.P., E.C., and B.H. performed research; L.S., J.D.P., P.J.C., E.C., R.J.L., and B.H. analyzed data; and P.J.C., E.C., R.J.L., and B.H. wrote the paper.

The authors declare no competing interest.

This article is a PNAS Direct Submission.

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This article contains supporting information online at <http://www.pnas.org/lookup/suppl/doi:10.1073/pnas.2119907119/-DCSupplemental>.

Published April 26, 2022.