Type III secretion system (T3SS) is a protein injection nano-machine consisting of syringe and needle-like structure spanning bacterial inner and outer membranes. Bacteria insert the tip of T3SS needle to host cell membranes, and deliver effector proteins directly into host cells via T3SS to prime the host cell environment for infection. Thus inhibition of T3SS would be a potent strategy for suppressing bacterial infection. We previously demonstrated that T3SS needle rotates by proton-motive force (PMF) with the same mechanism as two evolutionally related rotary protein motors, flagellum and ATP synthase (FASEB J., 27, 2013, Ohgita et al.). Inhibition of needle rotation resulted in suppression of effector secretion, indicating the requirement of needle rotation for effector export. Simulation analysis of protein export by the T3SS needle suggests the importance of a hydrophobic helical groove formed by the conserved aromatic residue in the needle components. Based on these results, we have proposed a novel model of protein export by the T3SS needle, in which effector proteins are exported by PMF-dependent needle rotation oppositely to the hydrophobic helical groove in the needle. Quantitative examinations of the correlation between the speeds of T3SS rotation and the amount of effector export support this model. In this review, we summarize our current understanding of T3SS, and discuss our novel model of the protein export mechanism of T3SS based on the needle rotation.

Key words type III secretion system; protein export; rotary motor; bacterial infection
3. Similarities of T3SS with Bacterial Flagellum and ATP Synthase

In 1998, Kubori et al. first succeeded in observing the structure of T3SS in *Salmonella typhimurium* by electron microscopy. The obtained structure was highly similar to that in bacterial flagellum except for the exterior region, in which T3SS has thin and short needle (approximately 100 nm in length and 10 nm in width) whereas flagellum has thick and long filament (µm-ordered length and approximately 20 nm in width). High sequence homology of some components indicates the evolutional relationship of these two machineries. Recently, structural similarities of cytosolic components of T3SS with *F*₀*F*₁-ATP synthase have also been indicated: the ATPase unit FliI/SctN, the central stalk FliJ/SctO, and the negative regulator FliH/SctL of T3SS resemble the α/β subunits, the γ subunit, and the b/δ subunits of the ATP synthase, respectively. Flagellum rotates its filament with proton-motive force (PMF) derived from the concentration gradient of protons across the inner membrane. The γ subunit of *F*₀*F*₁-ATP synthase also rotates depending on PMF generated by mitochondrial electron transport system, and the rotation promotes ATP synthesis on α/β subunits. Since T3SS is known to require PMF for effector secretion, we hypothesized that effector proteins are exported by the T3SS needle similarly to PMF-dependent needle rotation.

4. PMF-Dependent Rotation of T3SS Needle

Whether T3SS rotates was debatable. Büttner denied the possibility of T3SS rotation because T3SS does not have a stator unit, whereas flagellum has the corresponding MotAB. To examine the possibility of T3SS rotation, we attempted to observe the motion of T3SS needle. According to the system to observe the motion of biological nanomachines, we constructed an observation system for T3SS needle motion as shown in Fig. 2A. In this system, PcrV, the tip component of T3SS of *Pseudomonas aeruginosa* PAO1 strain, was genetically modified with Strep tag II peptide, and streptavidin-coated fluorescent microbead was specifically attached to the tip via Strep tag II-streptavidin interaction. As a result, the motion of T3SS needle was observable as that of the attached microbead under the condition of effector secretion. When PMF was dissipated by the protonophore carbonyl cyanide m-chlorophenylhydrazone, the rotation was largely inhibited, indicating the requirement of PMF for T3SS rotation. In addition, when the rotation was inhibited by viscous polymers (e.g. polyethylene glycol 8000 or alginate), the effector secretion was also suppressed. These results strongly indicate a correlation between PMF-dependent T3SS rotation and effector transport by T3SS.

5. Model of Effector Export Based on T3SS Needle Rotation

Based on the importance of hydrophobic helical groove inside the needle and the correlation between the PMF-dependent needle rotation and the exported amount of effector proteins by T3SS, we have proposed a novel model of effector protein export by T3SS (Fig. 3A). In this model, the helical groove in the needle functions as a rail for one-directional export of effector proteins set to the rail when T3SS needle rotates oppositely to the helical rail.
To verify this model, we analyzed correlations between the kinetics of effector export and T3SS rotation. The speed of effector export by T3SS was estimated using genetically modified effector proteins with an epitope tag 3FLAG to quantify the tag in supernatant by a competitive enzyme-linked immunosorbent assay (ELISA) with FLAG-tagged bacterial alkaline phosphatase. Almost same kinetics of export of these effector proteins suggest that primary structures are not important for the export speed by T3SS (Fig. 3B). Since these measurements were performed at OD\textsubscript{600} of 0.3 (corresponding to a bacterial density of \(3 \times 10^8\) colony-forming units mL\textsuperscript{−1}),\textsuperscript{13} the average speed of effector export in T3SS was estimated 2184 amino acids min\textsuperscript{−1} under the assumption that the rotation of needle is 345 Å and the number of turns of 3FLAG to quantify the tag in supernatant by a competitive enzyme-linked immunosorbent assay (ELISA) with FLAG-tagged bacterial alkaline phosphatase. Almost same kinetics of export of these effector proteins suggest that primary structures are not important for the export speed by T3SS (Fig. 3B). Since these measurements were performed at OD\textsubscript{600} of 0.3 (corresponding to a bacterial density of \(3 \times 10^8\) colony-forming units mL\textsuperscript{−1}),\textsuperscript{13} the average speed of effector export in T3SS was estimated 2184 amino acids min\textsuperscript{−1} under the assumption that the rotation of needle is 345 Å and the number of turns of needle width versus 1 μm of bead width). This estimated rotation speed of microbead is in good agreement with the actual observation (approximately 3 rpm),\textsuperscript{30} strongly supporting our model of effector export shown in Fig. 3A.

### 6. Conclusion

Using T3SS, pathogenic bacteria export effector proteins for efficient infection into host cells across the membrane barriers. Although recent studies are uncovering the molecular mechanism of T3SS, there are still many unanswered questions. One such question is how effector proteins are exported by T3SS. Recently, we have proposed a novel model of export of effector proteins by T3SS based on our observation of T3SS needle rotation. We demonstrated good correlation between the needle rotation and the exported amount of effector proteins, strongly supporting the effector export mechanism by T3SS needle rotation. Our results provide new insights into the development of novel inhibitors of bacterial infection to target T3SS.

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### Conflict of Interest
The authors declare no conflict of interests.

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