doi:10.1017/S1431927620019455
Microsc. Microanal. 26 (Suppl 2), 2020

Structural Study of the *Legionella pneumophila* Dot/Icm T4SS Using Cryo-electron Microscopy

Clarissa Durie[1], Michael Sheedlo[2], Jeong Min Chung[1], Brenda Byrne[3], Thomas Knight[3], Michele Swanson[3], Dana Lacy[2] and Melanie Ohi[1]

[1]University of Michigan, Ann Arbor, Michigan, United States, [2]Vanderbilt University Medical Center, Nashville, Tennessee, United States, [3]University of Michigan Medical School, Ann Arbor, Michigan, United States

Bacterial pathogens are a threat to global health and have evolved elaborate strategies to infect their hosts. One potent bacterial weapon in the war between host and pathogen is the Type IV secretion system (T4SS). In Gram negative bacteria these complexes can deliver: 1) effector proteins into eukaryotic cells, 2) DNA into other bacteria, or 3) toxins into bacterial neighbors. T4SSs are found in a wide variety of bacterial species including those that cause human disease, such as *Legionella pneumophila, Helicobacter pylori*, *Bordetella pertussis, Brucella, Bartonella,* and *Coxiella.* When inhaled, *L. pneumophila* replicates in alveolar macrophages and can cause a progressive and potentially fatal pneumonia, called Legionnaire's disease (1, 2). Infection begins when macrophages phagocytose the opportunistic pathogen which then co-opts the host cell phagosome to create a replicative niche (3). The Defect in Organelle Transport (Dot) T4SS is required for this process and essential for *L. pneumophila* pathogenesis. This complex secretes hundreds of bacterial proteins into the host cell that work to subvert host cellular machinery, inhibiting phagosome fusion with the lysosome and bacteria degradation.

T4SSs are challenging complexes to work with because they span the inner and outer membranes of Gram-negative bacteria and contain a minimum of 12 proteins. Studies in prototype systems, such as *E. coli*plasmids and *A. tumefaciens* show that T4SSs are generally organized into an outer membrane core complex (OMCC), an inner membrane complex (IMC), and in some species an extracellular pilus(4-6). Recent biochemical and *in vivo* cryo-electron tomography (cryo-ET) studies of *L. pneumophila* and *H. pylori* T4SSs show these complexes have distinctive structural features compared to previously characterized T4SSs (7-12). Differences include: 1) the OMCCs contain at least five proteins instead of three, 2) the OMCCs are larger and more intricately organized than those in *X. citri* and conjugation systems, and 3) the T4SSs have periplasmic sub-complexes not seen in other bacteria. Since *L. pneumophila* and *H. pylori* T4SSs secrete proteins rather than DNA, we predict that these structural differences are essential for function and will provide molecular insight into the mechanism of T4SS substrate specificity.

The *L. pneumophila* T4SS is composed of ~26 components and low resolution (~20-40 Å) cryo-electron tomography studies show that this T4SS shares some architectural characteristics with the *H. pylori* T4SS (8, 9, 11-16). However, the lack of detailed structural information limits our mechanistic understanding of how the *L. pneumophila* T4SS functions and contributes to pathogenesis. I have biochemically purified the *L.pneumophila* T4SS core complex and visualized it by cryo-EM. From this analysis it is clear that while similarities between the Cag and Dot T4SSs exist, the *L. pneumophila* T4SS is structurally distinct from other T4SS complexes.

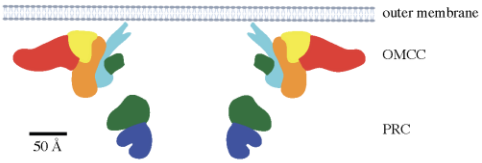

Figure 1. Figure 1. Cartoon schematic showing the organization of the L. pneumophila Dot T4SS outer membrane core complex (OMCC) and periplasmic ring complex (PRC) in relation to the outer membrane. The central axial slice view is shown. Different protein core components are shown in different colors.

Identified Proteins	Spectral Counts		
	Prep 1	Prep 2	Prep 3
DotG	112	114	195
DotF	94	69	101
DotA	38	65	60
DotO	37	38	47
DotH	28	19	28
IcmF	15	18	36
IcmX (IcmY)	19	13	28
DotL	10	26	20
DotC	9	11	16
DotD	11	9	14
DotB	16	11	7
DotM	2	14	3
DotK	2	6	10
IsmW	5	7	5
DotN	2	6	4
DotI	1	3	5
IcmS		4	1

Figure 2. Table 1. T4SS components identified by mass spectrometry. Spectral counts of all Dot/Icm proteins are shown across three biological replicates of complex isolation.

References

1. Molofsky AB, Swanson MS. 2004. *Mol Microbiol* 53: 29-40
2. Swanson MS, Hammer BK. 2000. *Annu Rev Microbiol* 54: 567-613
3. Nora T, Lomma M, Gomez-Valero L, Buchrieser C. 2009. *Future Microbiol* 4: 691-701
4. Christie PJ, Cascales E. 2005. *Mol Membr Biol* 22: 51-61
5. Grohmann E, Christie PJ, Waksman G, Backert S. 2018. *Mol Microbiol* 107: 455-71
6. Waksman G. 2019. *EMBO Rep* 20
7. Frick-Cheng AE, Pyburn TM, Voss BJ, McDonald WH, Ohi MD, Cover TL. 2016. *MBio* 7: e02001-15
8. Chang YW, Shaffer CL, Rettberg LA, Ghosal D, Jensen GJ. 2018. *Cell Rep* 23: 673-81
9. Ghosal D, Chang YW, Jeong KC, Vogel JP, Jensen GJ. 2017. *EMBO Rep* 18: 726-32
10. Chung JM, Sheedlo MJ, Campbell AM, Sawhney N, Frick-Cheng AE, et al. 2019. *Elife* 8
11. Chetrit D, Hu B, Christie PJ, Roy CR, Liu J. 2018. *Nat Microbiol* 3: 678-86
12. Park D, Chetrit D, Hu B, Roy CR, Liu J. 2020. *mBio* 11
13. Segal G, Purcell M, Shuman HA. 1998. *Proc Natl Acad Sci U S A* 95: 1669-74
14. Segal G, Shuman HA. 1999. *Infect Immun* 67: 2117-24
15. Vogel JP, Andrews HL, Wong SK, Isberg RR. 1998. *Science* 279: 873-6
16. Hu B, Khara P, Song L, Lin AS, Frick-Cheng AE, et al. 2019. *mBio* 10

https://doi.org/10.1017/S1431927620019455 Published online by Cambridge University Press