Could the Next Antibiotic Emerge from Bacteria?

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ABSTRACT

An intrinsic bacterial mechanism could play a fundamental role in the future of antibiotics. Using cryo-EM, the structural resolution of the effector protein complexed with its chaperone and other accessory proteins reveals the mechanism of action of type VI bacterial secretion system. The importance of the chaperone protein, used to prime the toxic effector protein, was previously identified. Future research efforts should encompass the immunity protein that may allow bacteria to evade the lethal effects of this mechanism.

Keywords: T6SS, Tse6, antibiotic resistance, antibiotics, antimicrobials, effector protein

The microscopic world is fascinating, with many variables that grant it much flexibility and a range of outcomes. Like in the macroscopic world, bacteria compete for resources, and in the process, utilize various mechanisms to outcompete and kill other bacteria. One such mechanism is the type VI secretion system (T6SS) as stated by Quentin and others.[1] Molecular insight into the various subcomplexes of this biochemical pathway have been elucidated by single particle electron cryomicroscopy (cryo-EM).[2,3] In this study, the structure of the chaperone paired with the effector protein, Tse6-EagT6, in complex with VgrG1 is resolved to further understand the T6SS mechanism of action in killing bacteria.[1]

T6SS Structure and Mechanism of Action

Bacterial pathogens commonly use protein secretion to facilitate interactions with their environment and other microorganisms.[4] T6SS is a protein secretion apparatus used by many gram-negative bacteria such as *Pseudomonas aeruginosa*. T6SS is encoded up to six times into the bacterial genome and is comprised of 13 core components and several accessory genes that can vary between bacteria, allowing for varying target specificity and firing modalities.[5,6] The apparatus is structurally homologous to a contractile phage tail attached to the prokaryotic membrane. It is composed of an inner tail tube complex made of stacked hexameric rings of the hemolysin coregulated protein (Hcp). This is capped with a spike complex made of a valine-glycine repeat protein G (VgrG) trimer and a Pro-Ala-Ala-Arg (PAAR) repeat-containing protein.[5] This tube-like complex is engulfed by a sheath attached to a baseplate which is adhered to the membrane of the bacterium. The T6SS structure can be seen in Figure 1A. Similar to phages, T6SS functions by contracting and injecting an effector protein through the protrusion of the inner tube which is capped with the spike complex and has the effector attached to it.[1] Effector proteins can kill bacteria as the Tse6 exerts its functions in the cytoplasm through hydrolyzing nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$).[1] There is evidence that the effector proteins are injected into the periplasm of the target bacterium where a small subset moves to the cytoplasm.[7] The effector-producing bacterium and their sister bacteria are not affected by the toxicity of the effector protein due to the co-expression of another immunity protein that renders the effector inert.[1]

The effectors are shuttled to the target-competing bacteria through two methods. The smaller effectors that are less than 40 kDa are bound and stabilized by the lumen of the Hcp hexamers, while the larger multi-domain effectors are bound to the spike, VgrG.[1]

In a 2019 study, Unterweger et al. determined that a

small subset of effector proteins require the assistance of chaperones to be loaded on VgrG.[8] EagT6 is one such example and is required for the loading of Tse6. Although the precise function of this chaperone is not yet elucidated, it is known to be essential for intracellular effector stability. Furthermore, Tse6 also requires interaction with the elongation factor Tu (EF-Tu), to be delivered to its target bacterium.[1]

Biochemical studies suggest that the association of the chaperone's homodimer to the transmembrane domain of Tse6 allows its protection from the cytoplasmic hydrophilic environment that might cause the effector to aggregate and degrade.[1] This chaperone-effector association also allows the PAAR domain on the effector to interact and associate with the VgrG, which is followed by its loading on the T6SS. A study conducted by Quentin et al. (2019), reported on the ability of Tse6 to translocate across a lipid bilayer. This led the research team to hypothesize that the effector is injected into the periplasm, where it passively crosses the inner membrane layer and translocate to the cytoplasm to exert its deleterious effects,[1] as shown in Figure 1. The effects of the association of the effector with EF-Tu is not yet fully understood. It was previously hypothesized that the elongation factor in the antagonist bacterium functions as a lock.[9] Through this mechanism of action, the effector binds to EF-Tu once it is in the cytoplasm, preventing it from being expelled to the extracellular space.

The experimental data also suggests a mechanism of membrane translocation, where the transmembrane hydrophobic domain (TMD) of Tse6 forms a small pore. This allows the unfolded large toxic domain of the Tse6 to cross the membrane where it folds again and kills the antagonist bacteria.

Impact on Antibiotic Resistance Research

Ever since the discovery of penicillin in 1928, this antibiotic has been used to treat serious bacterial infections. Following this scientific breakthrough, antibiotics have transformed modern medicine and saved millions of lives.[10] Penicillin was successful in treating infections during World War II but its efficacy was reduced shortly after, upon the emergence of antibiotic resistance. Antibiotic resistance became a growing global health concern, threatening penicillin's utility as

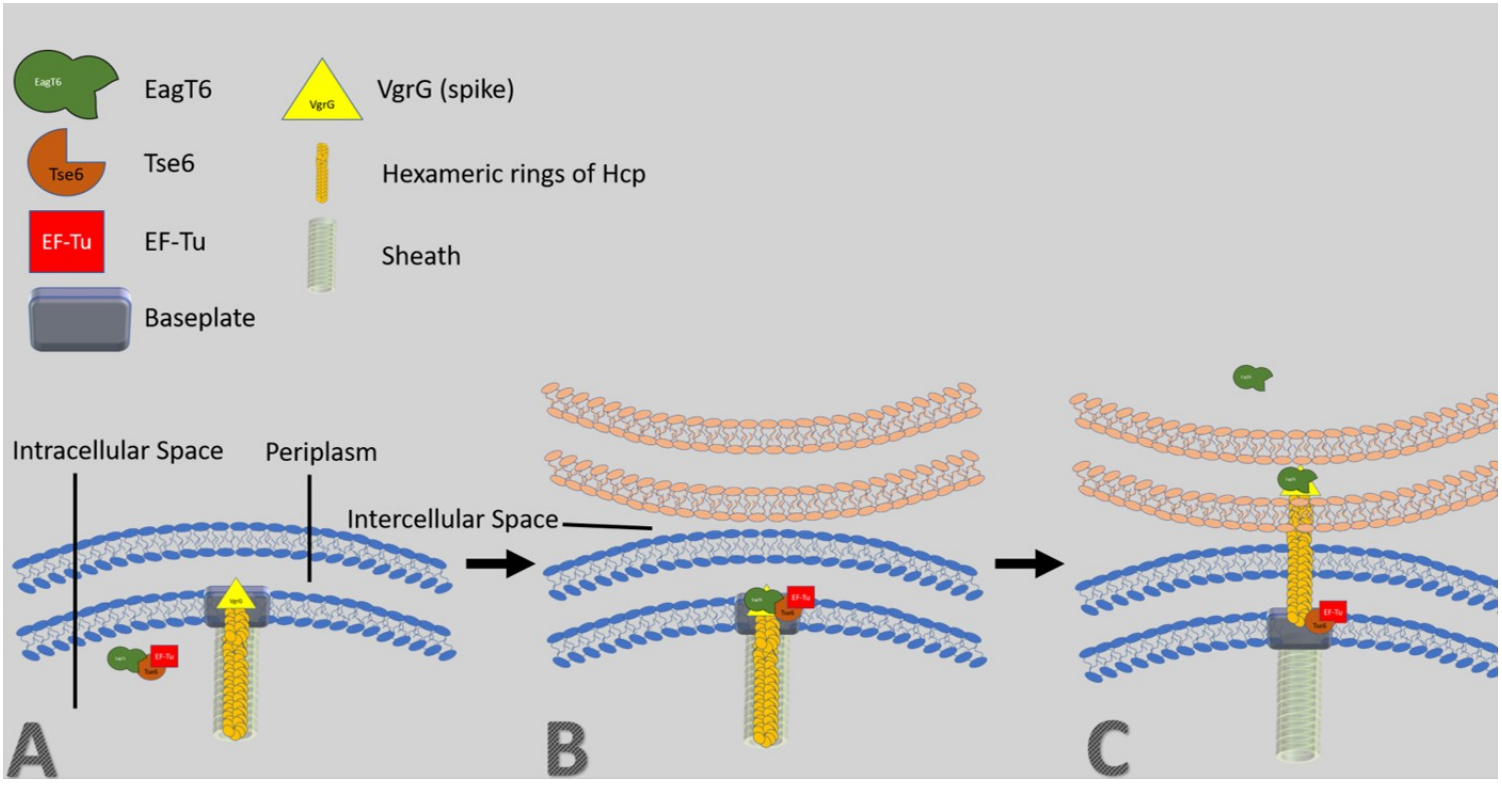

Figure 1. Interaction and loading of the Tse6-EagT6 complex onto the T6SS. The T6SS assembly can be seen in A-C. The mechanism of function of the T6SS is initiated by the association of the effector protein, Tse6, to the homodimer chaperone EagT6 (A). The assembly of the complex prevents the hydrophobic domain of the effector to aggregate with other Tse6 proteins and degrade in the hydrophilic environment of the cytoplasm (B).[1] The complex is then loaded on the T6SS spike formed by the VgrG protein and upon contact with an antagonist bacterium, the T6SS complex contracts and injects the effector into the periplasm of the antagonist bacterium (C).[1] The antagonist then crosses the inner membrane bilayer to the cytoplasm where it can exert its toxic effects, hydrolyzing NAD^+ and $NADP^+$.[1]

an effective treatment for infections.[10] This has created an "arms race" between modern medicine and bacterial infections, with continued development of new antibiotics which are rendered ineffective in a few generations due to bacterial resistance.

Today, any research that explores the mechanisms of targeting and killing bacteria is valuable, considering the continuing need for novel and unique antibiotics. Thus, the aim of the Quentin et al. paper is to study this lethal bacterial mechanism and its machinery, which may lead to novel therapeutics that are capable of conquering bacterial infections. One such novel therapeutic that may emerge could be in the form of a genetically engineered agent that exhibits the T6SS apparatus with lethal effectors to circumvent current antibiotic resistance to some antimicrobials.

Prospective Research and Considerations

The researchers of the study attempted -yet failed- to resolve the structure of the toxic domain of the Tse6-EF-Tu-Tsi (immunity protein that neutralizes the effector) complex, concluding that it is too flexible to be determined using cryo-EM. Future research should aim to identify the structure of the complex to further understand or confirm the researchers' hypothesis about the elongation factor Tu acting as a lock, in order to prevent the expulsion of the effector. This would be an essential characteristic of effective therapeutic. Additional studies must be conducted on the mechanism of action of the immunity protein, as it seems to be the most likely mechanism of antibiotic resistance that may arise if the effector is to be used as an antibiotic. Another potential avenue of this field of research could be to study whether this type VI secretion system is lethal against gram-positive bacteria in addition to its functionality against gram-negative bacteria.

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REFERENCES

(1) Quentin D, Ahmad S, Shanthamoorthy P, Mougous JD, Whitney JC, Raunser S. Mechanism of loading and translocation of type VI secretion system effector Tse6. Nat Microbiol [Internet]. [cited 2019 Feb 17]; Available from: https://doi.org/10.1038/s41564-018-0238-z

(2) Kudryashev M, Yu-Ruei Wang R, Egelman EH, Basler Correspondence M, Brackmann M, Scherer S, et al. Structure of the Type VI Secretion System Contractile Sheath Article Structure of the Type VI Secretion System Contractile Sheath. Cell [Internet]. 2015 [cited 2019 Feb 17];160:952–62. Available from: http://dx.doi.org/10.1016/j.cell.2015.01.037http://dx.doi.org/10.1016/j.cell.2015.01.037

(3) Ge P, Scholl D, Leiman PG, Yu X, Miller JF, Zhou H, et al. Atomic structures of a bactericidal contractile nanotube in its pre-and postcontraction states HHS Public Access Author manuscript. Nat Struct Mol Biol [Internet]. 2015 [cited 2019 Feb 17];22(5):377–82. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4445970/pdf/nihms-692005.pdf

(4) Mougous JD, Cuff ME, Raunser S, Shen A, Zhou M, Gifford CA, Goodman AL, Joachimiak G, Ordoñez CL, Lory S, Walz T. A virulence locus of Pseudomonas aeruginosa encodes a protein secretion apparatus. Science. 2006 Jun 9;312(5779):1526-30.

(5) Chang Y-W, Rettberg LA, Ortega DR, Jensen GJ. In vivo structures of an intact type VI secretion system revealed by electron cryotomography. EMBO Rep [Internet]. 2017 [cited 2019 Feb 17];18:1090–9. Available from: http://embor.embopress.org/content/embor/18/7/1090.full.pdf

(6) Ho BT, Dong TG, Mekalanos JJ. A view to a kill: the bacterial type VI secretion system. Cell host & microbe. 2014 Jan 15;15(1):9-21.

(7) Russell AB, Hood RD, Bui NK, LeRoux M, Vollmer W, Mougous JD. Type VI secretion delivers bacteriolytic effectors to target cells. Nature. 2011 Jul;475 (7356):343.

(8) Unterweger D, Kostiuk B, Pukatzki S. Adaptor Proteins of Type VI Secretion System Effectors. 2017 [cited 2019 Feb 17]; Available from: http://dx.doi.org/10.1016/j.tim.2016.11.012

(9) Whitney JC, Quentin D, Sawai S, LeRoux M, Harding BN, Ledvina HE, Tran BQ, Robinson H, Goo YA, Goodlett DR, Raunser S. An interbacterial NAD (P)+ glycohydrolase toxin requires elongation factor Tu for delivery to target cells. Cell. 2015 Oct 22;163(3):607-19.

(10) Lee Ventola C. The Antibiotic Resistance Crisis Part 1: Causes and Threats [Internet]. Vol. 40. 2015 [cited 2019 Feb 16]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4378521/pdf/ptj4004277.pdf