3D structure of the tail complex of *E. coli* bacteriophage DT57C

Rafael Ayala[1], Evgeny Kulikov[2], Alla Golomidova[2], Andrey Moiseenko[3,4], Matthias Wolf[1], Andrey V. Letarov[2,3] and Olga S. Sokolova[3*]

[1.] Molecular Cryo-Electron Microscopy Unit, Okinawa Institute of Science and Technology Graduate University, Onna-son, Okinawa, Japan
[2.] Winogradsky Institute of Microbiology, Research Center of Biotechnology of the Russian Academy of Sciences, Moscow, Russia.
[3.] Department of Biology, Moscow Lomonosov University, Moscow, Russia.
[4.] N.N.Semenov Federal Research Center for Chemical Physics, Russian Academy of Sciences, Moscow, Russia
* Corresponding author: sokolova@mail.bio.msu.ru

Introduction

Under the conditions of intensive livestock farming and fish breeding, the high concentration of animals in limited areas leads to an explosive growth of bacterial infections that ultimately cause significant economic losses. At the same time, the long-term uncontrolled use of antibiotics in agricultural practices has led to the emergence of multidrug-resistant strains of zoopathogenic bacteria that are resistant to a whole group of antibiotics. To solve the problem of preventing bacterial disease outbreaks in agriculture, the development of alternative and/or complementary technologies is necessary. One of the promising technologies in this direction is the biological control of pathogen populations by preparations based on bacteriophages – viruses of pathogenic bacteria. Phages are common in all-natural environments infecting bacteria specific for their habitats. For the purposes of phage therapy, viruses can be selected from environmental objects or obtained by directed modification of phage platforms active against the groups of microorganisms of interest, by methods of genetic engineering and synthetic biology. The second option is the more promising, since it allows the fine-tuning of bacteriophages for specific tasks and target organisms. However, for this purpose, such phage platforms should be comprehensively studied, including their genomics, proteomics, stability, receptor interactions, etc [1]. In this regard, the structural investigation of the T5-like phage DT57C featuring unusual organization of its host-cell recognition devices has both fundamental and applied significance.

Methods

Bacteriophage DT57C was isolated from horse faeces in course of our previous work [2] using *E. coli* C600 as a host. The phage was then maintained using the O-antigen producing host *E. coli* 4s to avoid selection of the mutants depleted of the lateral tail fibers (LTF) that are not required for the infection of the rough *E. coli* strains, such as C600. However, for the final step of phage stock preparation, the virus was grown on *E. coli* C600 because phage particles were shown to aggregate in the presence of *E. coli* 4s debris, hindering the purification. Phage was purified using centrifugation in a CsCl preformed step gradient with subsequence dialysis. The biological activity of the purified phage was confirmed by titration on a lawn of *E. coli* 4s host strain.

For cryo-electron microscopy, 3.5 μL aliquots were applied onto plasma-cleaned Quantifoil grids with hole size of 2 μm (Quantifoil, Germany) and subsequently blotted for 8 s before plunging into liquid ethane-propane using an FEI Vitrobot Mark IV (Thermo Fisher Scientific, TFS). Images were collected on a Titan Krios transmission electron microscope (TFS) operated at 300 kV and using a Falcon 3EC

direct electron detection camera in linear mode at a total electron dose of 70 electrons/Å^2 and a defocus range between -0.7 and -2.5 μm.
We applied classification and reconstruction in cryoSPARC [3] to obtain a reconstruction of the phage DT57C tail complex at 4.7 Å resolution. A total of 34,000 movies were collected and processed. Motion correction was performed with RELION [4]. The phase contrast transfer function was estimated with CTFFIND4 [5].

Results

DT57C is a T5-like coliphage that recognizes the outer membrane transporter (OM) protein of *E. coli* BtuB as the final receptor. The phage was identified as a member of the *Siphoviridae* family. Here we demonstrated that the DT57C phage possesses a morphology related to the T5 coliphage [6] with an icosahedral head and a long flexible tail (**Fig. 1a-b**). We used single particle analysis to solve a reconstruction of the tail tip (**Fig. 1c**) with C3 symmetry applied.
Phage DT57C has two large genes encoding for the LTF proteins, *ltfA* and *ltfB*, responsible for the interaction with different O-antigen types of *E. coli* [PMID: 26805872]. Genetic data indicate that the lateral fibrils of phage DT57C are branched and include two proteins carrying different receptor-recognition domains [PMID: 26805872]. Our reconstruction (**Fig. 1c**) suggests that LtfA and LtfB trimers both make contacts with the tail tip complex. Further work is required to identify complete topology of the complex LTF structure and to determine the contacts (if present) between the LtfA and LtfB moieties.

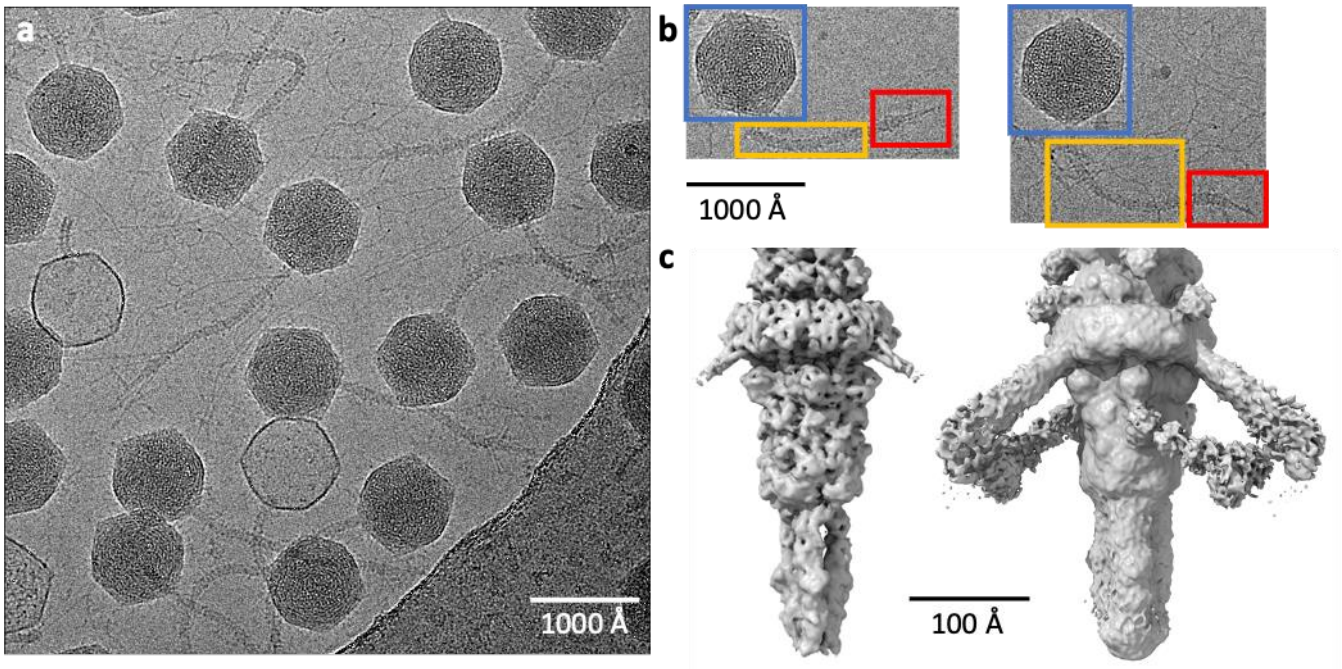

Figure 1. Cryo-EM reconstruction of DT57C phage tail tip complex. (**a**) Representative cryo-EM field-of-view; (**b**) Representative DT57C phage particles, with different regions highlighted: blue – capsid; yellow – tail; red – tail tip; (**c**) Reconstruction of the tail tip (left panel: high threshold; right panel: low threshold).

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[7] The authors acknowledge funding from the RSF (21-44-07002) and the Japanese Ministry of Agriculture and Fisheries (MAFF) (to MW and RAH).