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Electron Tomography Revealed Compact DNA in the Nucleus-like Compartment, Formed in the Bacterial Cell During Infection by the phiKZ Bacteriophage

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PhiKZ-like phages belong to the Myoviridae family and infect the opportunistic pathogenic bacterium *Pseudomonas aeruginosa*. Notably, phiKZ infection is sustainable against all known bacterial defence systems based on double-stranded DNA damage [1]. Recently, it has been shown that during infection the phiKZ bacteriophage forms a proteinaceous shell in the bacterial host cell [2], [3]. In the second part of the infection, all of the DNA and a part of DNA metabolism proteins are proposed to be packed inside that shell. However, there is no information about the fine structure of this unique nucleus-like shell and the DNA inside it during phage infection.

Here, we used electron tomography to explore the structure and location of the DNA within the nucleus-like shell of *P. aeruginosa* infected by phiKZ bacteriophage.

Samples of non-infected cell and phiKZ-infected cells after 15 and 30 min of infection were chemically fixed and subjected to LR White embedding. Thin sections were cut with a diamond knife (Diatome) on an ultramicrotome Ultracut-UCT (Leica Microsystems), transferred to copper 200 mesh grids, covered with formvar (SPI, USA), and contrasted with lead citrate, according to the Reynolds established procedure (contrasting was in some cases omitted). The grids were studied in a transmission electron microscope JEM-2100 (JEOL, Japan) with an accelerating voltage of 200 kV. Tomograms were obtained with the SerialEm software and processed with IMOD.

Localization and sizes of the pseudo-nuclei differ in the 15- and 30-min infected samples. After 15 min of infection, several elongated or non-connected compartments were visible (fig. 1 A, B). These maturating compartments did not contain noticeable structures inside. We suppose that this is due to the deficiency of phage DNA, as the active phage DNA-replication starts only after 20 min of infection.

After 30 min of infection the pseudo-nuclei were mostly spherical and were located in the middle of the cell, similar to ones observed before [3]. At that time, some new phage capsids on the surface of the inner compartment or near it were revealed (Fig. 1 C, D). The net-like structures inside the pseudo-nucleus compartment were clearly visible (fig. 2). The three-dimensional model of these nets, constructed using the tomographical approach, revealed thin strands interconnected with globular domains. Our results are in consistence with the results of fluorescence microscopy [3], and suggest that observable net-like structures are formed by compacted phage DNA and DNA-binding proteins. Similar organization is known also for bacterial DNA under stress conditions and is called ‘liquid cristalline’ DNA packing [4]. Interactions of DNA with DNA-binding proteins protects the bacterial DNA against adverse environmental conditions.

Here, the unique packaging of the DNA in the pseudo-nucleus in the middle of the live bacterial cell is the main reason for infection sustainability of the phikZ bacteriophage [5].

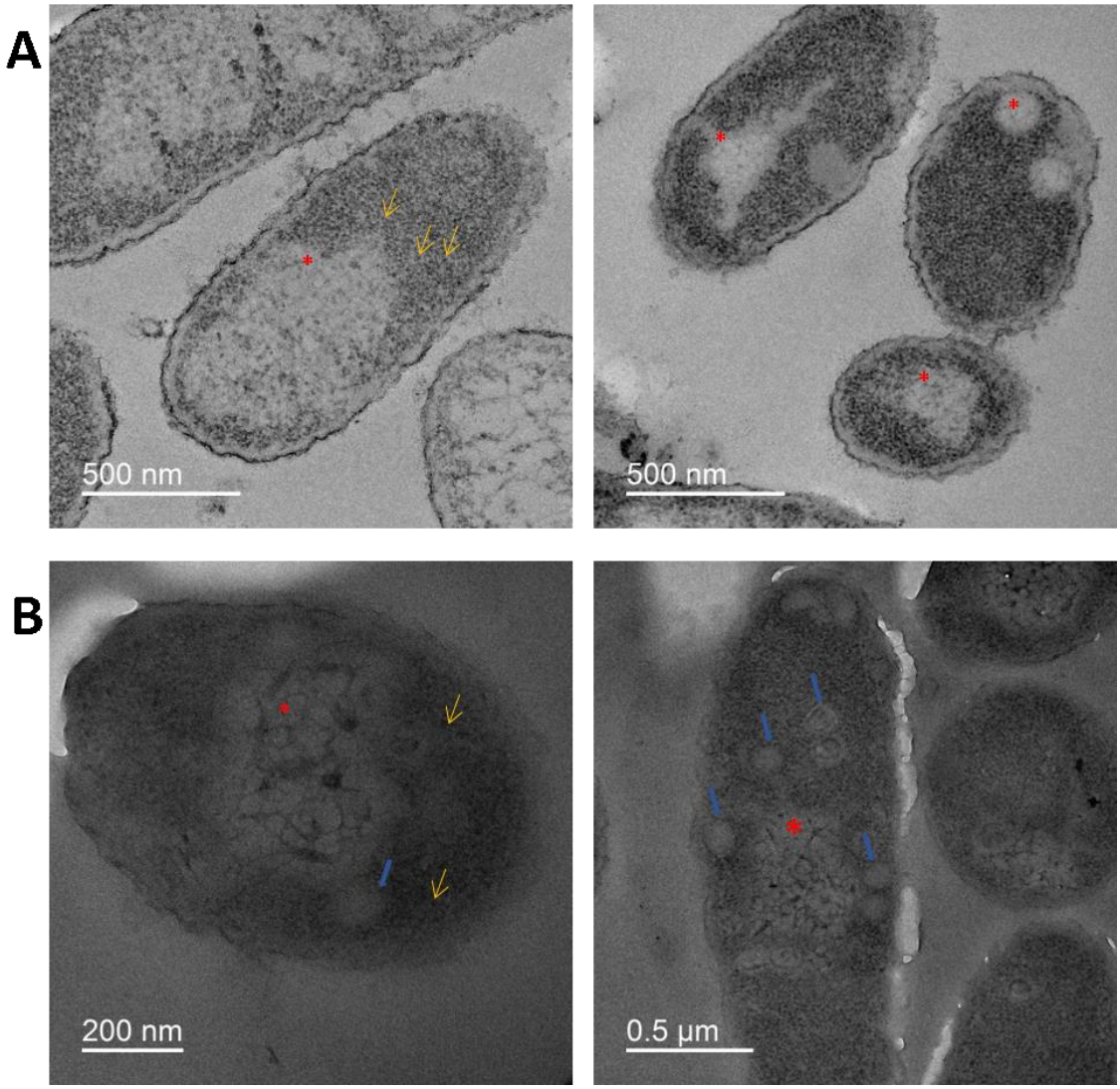

Figure 1. Morphology of *P. aeruginosa* cells after infection by phiKZ bacteriophage (A) 15 min after infection: maturating of the compartment. Variety of localization and size of the compartment of the cells (B) 30 min aftrer infection. The net-like structures inside the pseudo-nucleus compartment are clearly visible. New phage capsids on the surface of the inner compartment or near it were revealed. Solid arrows are pointing to the ribosomes, filled arrows – to phage capsids, stars mark compartments

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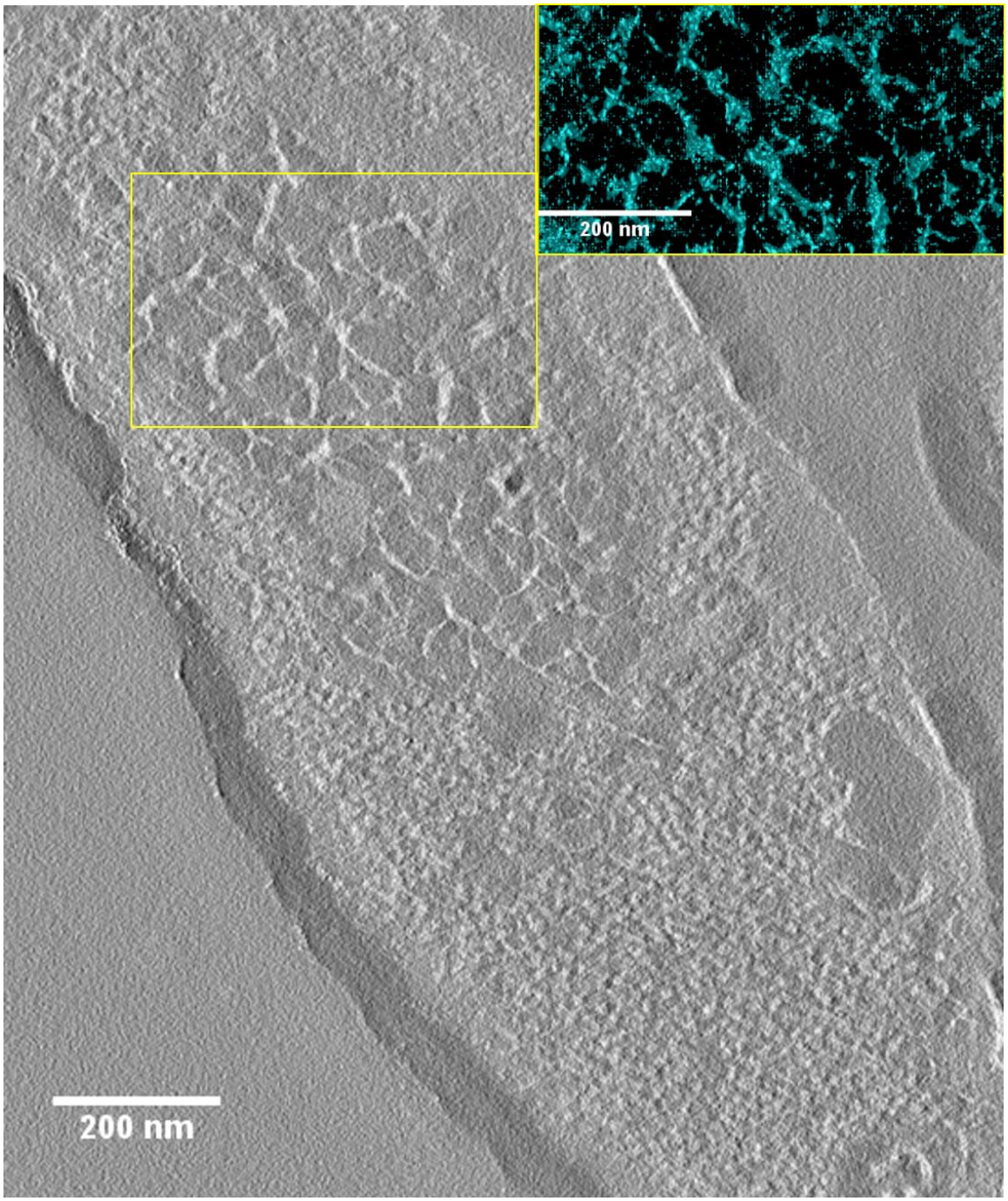

Figure 2. Central slice through the tomogram *P. aeruginosa* cells infected with phiKZ, 30 min of infection. Insert – three-dimensional model of the net, formed by DNA and DNA-binding proteins.

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