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Structural Analysis of Proteobacteria-Phage Interactions

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The interaction between bacteriophages and their hosts plays a pivotal role in bacterial adaptation and represents a tractable model system to determine the evolution of infection and disease and the general principles governing host-pathogen associations [1]. Our goal was to determine how bacterial developmental processes trigger specific events in bacteriophage infection. Using cryo-electron microscopy and cryo-electron tomography (cryo-ET) we investigated the process by which phage ϕCb13 interacts with *Caulobacter crescentus*, a fresh-water, Gram–negative, alpha-proteobacterium, with a dimorphic cell cycle [2]. We utilized 2D images and 3D reconstructions of phage-infected cells, as well as microbiology protocols to understand the mechanism of infection.

We found that ϕCb13 can preferentially interact with the flagellum with its head (Fig. 1) to consequently attach to bacteriophage receptors. It is evident that this interaction takes place through a filament of unknown nature that connects the phage to the flagellum (Fig. 2). Our results indicate that a function of this head filament is to mediate initial (reversible) adsorption to the host.

In addition, infectivity assays were carried out utilizing several motility mutants of *C. crescentus* (Table 1). Our results indicate that flagellar rotation mutants NS26, SC1057 and SC1163 exhibit resistance to infection by ϕCb13 with more than 90 % of bacterial cells remaining viable after 2.5 hours of exposure (Fig. 3). These findings imply that flagellar rotation is important for infection and more specifically suggest that directionality of rotation could play a role in this process. Similarly, the reduction on viable Δ*pil*A cell numbers indicates that pili subunits are important in the infection process but may not be the sites for final (irreversible) attachment to the host.

Future research will include structural studies to characterize temperate *Caulobacter*-specific phages, on which no studies have been carried out. If, as suggested, susceptibility to bacteriophages is an important motor for adaptation in bacteria [3] and considering the importance of both flagellar and pili machineries on phage infection in *Caulobacter*, a comparative evolutionary analysis of the genes involved in flagellum and pili assembly in alpha-proteobacteria would illustrate the importance of bacteriophages in driving evolutionary processes in bacteria.

References

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TABLE 1. Mutant strains investigated on this study.

Strain name	Phenotype
C. crescentus NA1000	Wild type strain
C. crescentus Cb13b1A	S layer deficient strain
C. crescentus NS26 (*mot*A)	Incomplete basal body. Non motile cells
C. crescentus Δ*pil*A	Pili not present
C. crescentus Δ*pfl*I (*p*olar-*f*lagellum-*l*inked gene)	Misplaced flagellum or multiple flagella
C. crescentus SC1057	Counter-clockwise flagellar rotation
C. crescentus SC1163	Counter-clockwise flagellar rotation

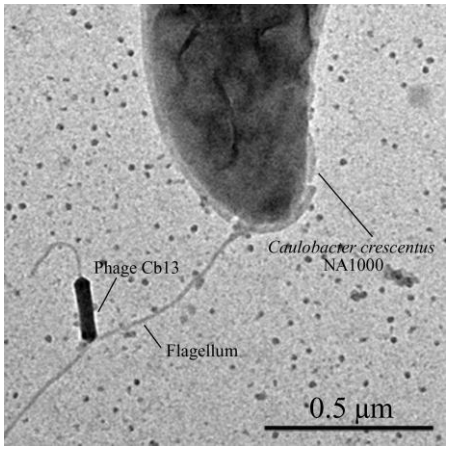

FIG. 1. Image from a negatively stained culture of ϕCb13-infected, wild-type *C. crescentus*. Notice the orientation of the phage in contact with the flagellum. Stain: 1% uranyl acetate for 15 seconds.

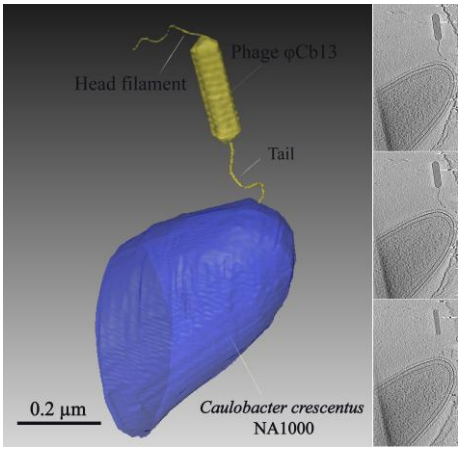

FIG. 2. Segmentation of wild type *C. crescentus* infected with ϕCb13 (Amira 5.2.0 (Visage Imaging, Inc, Andover, MA)). Notice the filament emerging from phage head. Right panel corresponds to serial, 13 nm slices from the original tomogram showing the head fiber. Tomogram reconstruction: IMOD 3.13.6. [4].

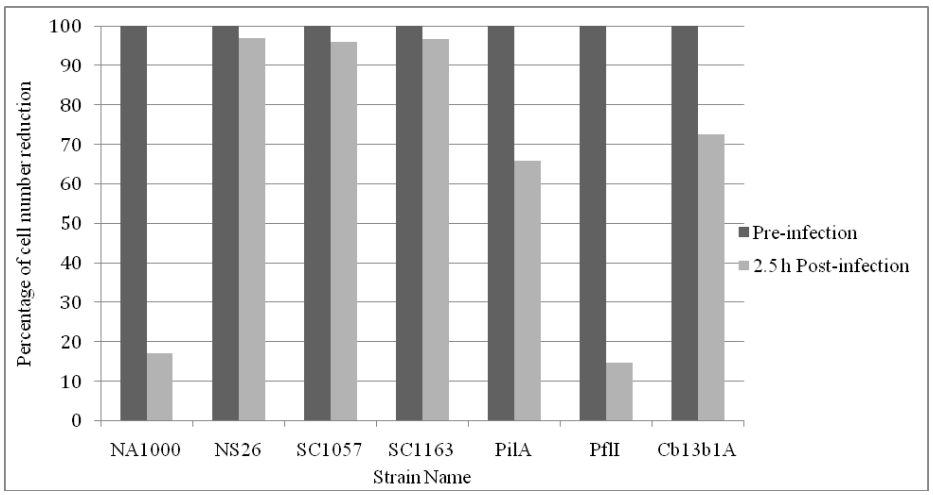

FIG. 3. Reduction in viable *C. crescentus* cell numbers after 2.5 hours of infection with ϕCb13.

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