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Analysis of Phage-Pilus Interactions in *Caulobacter crescentus*

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Many bacterial species express external filamentous structures known as pili that are essential to numerous biological processes . Because of their roles in motility, biofilm formation, and surface colonizaion, pili often serve as important virulence factors for pathogenic bacteria [1]. *Caulobacter crescentus* is a Gram-negative, oligotrophic bacterium that expresses polar type IVb pili in the swarmer stage of its dimorphic life cycle [2]. These pili are known to be involved in surface attachment at the swarmer to sessile transition [3] and are additionally utilized by the prolate siphophage ϕCbK in the initial stages of infection by attaching to the pilus portals [4]. Although there is no known homologue to the type IVa retraction ATPase in the *C. crescentus* genome, it has been hypothesized that pilus retraction aids in both of these functions [2,5]. We investigated the role of pilus retraction in ϕCbK attachment using bacteriophage adsorption assays, cryo-correlative light and electron microscopy (cryo-CLEM), and cryo-electron microscopy and tomography (cryo-EM and cryo-ET).

Bacteriophage adsorption assays were carried out after the addition of carbonyl cyanide m-chlorophenylhydrazone (CCCP), a proton-ionophore that dissipates the proton motive force (PMF). Our results indicate that ϕCbK adsorption is severely compromised at CCCP concentrations above 5 μM, implying that ϕCbK adsorption is dependent upon the PMF, which has been shown to be involved in type IVa pilus retraction [6]. We then utilized cryo-CLEM and cryo-ET to further characterize the interactions between pili and ϕCbK. *C. crescentus* NA1000 *spmX::spmX-mCherry* cells were infected with GFP-ϕCbK in which GFP is fused with the major capsid protein, then applied to copper 200 mesh grids with R 2/1 Quantifoil carbon supports and plunge frozen in liquid ethane. Grids were imaged using a Leica EM Cryo CLEM system and a JEOL JEM-2200FS 200 kV TEM equipped with an in-column Omega energy filter and Direct Electron DE-20 camera. Tilt series were acquired at 2° increments with a range of -62° to +62° using SerialEM [7] and tomographic reconstructions were generated with IMOD [8].

Cryo-CLEM experiments allow us to identify events in which mCherry-labeled cells are being infected with GFP-labeled ϕCbK by cryo-fluorescence microscopy (Fig. 1A and 1B), then correlate and locate these events for cryo-EM data collection (Fig. 1C). Using cryo-ET, we found that phage tail fibers interact with extended pilus filaments at early timepoints of infection (Fig. 2). Combined with adsorption assays, these results suggest that the ϕCbK tail fiber adsorbs to the pilus filament of the host cell and pilus retraction brings the phage to the site of irreversible attachment at the pilus portal. We therefore hypothesize that the type IVb pili of *C. crescentus* are capable of retraction. Future research will investigate the possible mechanisms that control pilus retraction and determine the structure of the machinery associated with pilus secretion and retraction.

References:

[1] N Van Gerven, G Waksman, and H Remaut, Prog Mol Biol Transl Sci **103** (2011), p. 21-72.
[2] JM Skerker and L Shapiro, EMBO J **19** (2000), p. 3223-3234.
[3] D Bodenmiller, E Toh, and YV Brun, J. Bacteriol **186** (2004), p. 1438-1447.
[4] RC Guerrero-Ferrerira, PH Viollier, B Ely, JS Poindexter, M Georgieva, GJ Jensen, and ER Wright, Proc Natl Acad Sci U S A **108** (2011), p. 9963-9968.
[5] A Persat, HA Stone, and Z Gitai, Nat. Commun. **5** (2014), p. 3824.
[6] R Kurre, N Kouzel, K Ramakrishnan, ER Oldewurtel, and B Maier, PLoS One **24** (2013), p. 2556-2563.
[7] DN Mastronarde, J. Struct. Biol. **152** (2005), p. 36-51.
[8] JR Kremer, DN Mastronarde, and JR McIntosh, J. Struct. Biol. **116** (1996), p. 71-76.
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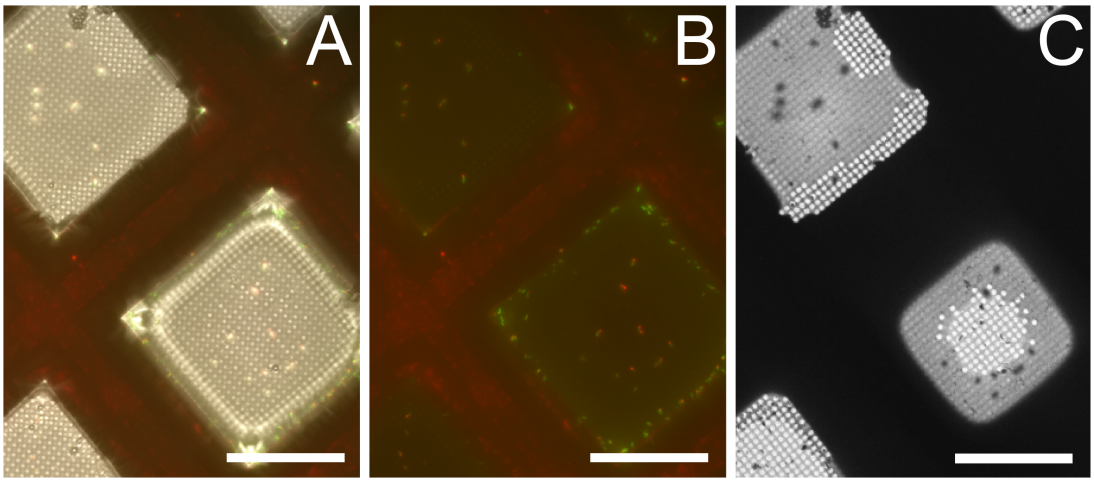

Figure 1. Cryo-CLEM of GFP-ϕCbK infected *C. crescentus* NA1000 *spmX::spmX-mCherry* cells. (A and B) Cryo-fluorescent light microscopy and (C) cryo-EM. Scale bars 50 μm.

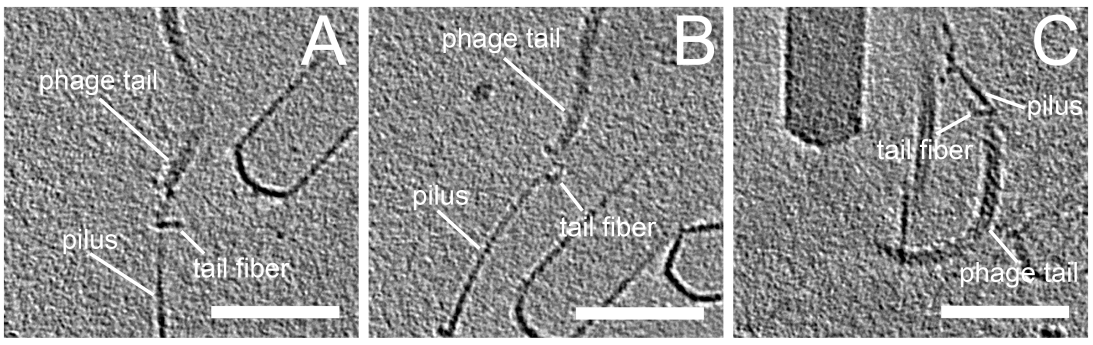

Figure 2. Cryo-ET slices of ϕCbK tail fibers wrapping around extended *C. crescentus* pilus filaments (A-C). Scale bars 200 nm.

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