

Some Self-Assembly Required



IN SHORT > Acting like microscopic lunar landers, bacteriophages, the viruses that infect bacteria, recognize and bind to specific molecules on the surface of their bacterial hosts. In an infection cycle very similar to that of the viruses that infect humans, once they are bound to the surface of the bacterial cell, bacteriophages break through the bacterial cell wall, inject their genomes, and proceed to take over the internal machinery of the cell. Within a short time, bacterial molecules have been hijacked to manufacture the proteins encoded by the viral genome. Once the viral proteins are made, the viruses go through a complex process of self-assembly, becoming particles that are ready to leave the cell to find new victims to infect. A deeper understanding of these complex infection processes is vital to designing treatments against viral infections in people.

MORE > Structural studies of the tailed bacteriophage, $\phi 29$, have shed light on two steps in this infection cycle. The work, performed at the BioCARS 14-ID, SER-CAT 22-ID, and GM/CA-CAT 23-ID-D beamlines at the APS, has revealed the mechanism by which the tail spike of this bacteriophage is assembled in the host cell and provided clues to how it binds its host. Bacteriophage $\phi 29$ uses a 12-appendage tail spike to recognize glycopolymers on the cell wall of its bacterial host, *Bacillus subtilis*. Each of the 12 appendages consists of three copies of a protein called gp12 that assemble to form a trimeric coil containing the recognition sites for the *B. subtilis* cell wall. This complex structure is assembled inside the bacterium during infection and incorporated into new viral particles that are released from the cell. Elucidation of the structure of

one of the trimeric appendages of the tail spike showed that the $\phi 29$ tail spike is assembled by a process involving a special autochaperone “base” region at the C-terminal end of the protein sequence that aids in formation of the gp12 trimer. Then, once its work is done, it neatly removes itself in an autocatalytic step that involves ATP hydrolysis.

The researchers in this study, from Purdue University and the University of Minnesota, first generated crystals of the gp12 protein before and after the autochaperone region had been removed, and then solved the structure of the protein using single anomalous dispersion phasing to a resolution of 2.0 Å (Fig. 1). The full-length structure reveals a coiled-coil trimeric domain with a globular trimeric base at one end. During assembly within the host cell, this base portion of the

> Fig. 1 Model of bacteriophage $\phi 29$ structures approaching their bacterial host, *Bacillus subtilis*. Shown are fully assembled particles, in white and purple, and multicolored particles showing bacteriophage with tail spike assemblies, showing the autochaperone structure before and after autocatalytic processing.

molecule is crucial for guiding the formation of the trimer by shielding certain water-insoluble amino acids (that will reside within the interior of the structure) from the watery milieu of the bacterial cytosol. If the base portion of the molecule is deleted, for example, the trimeric structure does not coil properly and results in an improperly folded, non-functional protein. Once self-assembly has completed, the chaperone base moves neatly over a portion of the coiled domain onto a putative proteolytic site, where it is cleaved and then released with the hydrolysis of a molecule of ATP. The researchers were able to identify and test possible amino acids in the structure to confirm that both the removal of the base portion by autocatalytic cleavage and the ATP hydrolysis could be accounted for by elements of the viral protein structure rather than external elements. This fascinating self-assembly and processing sequence appears to be conserved among other viruses that contain bacterial tail spikes similar to that of $\phi 29$ and may provide insight into how self-assembly of viruses could be blocked.

The structure also revealed information about the ability of

Landing of a dsDNA Bacteriophage



the $\phi 29$ tail spike to recognize host cell-wall molecules. In experiments where bacterial cell-wall analogs were bound to the gp12 trimeric structure, the researchers were able to show which specific regions of gp12 bind to the host, and that this interaction is dependent on metal ions in the viral protein structure. Their hypothesis, that each of the

12 appendages binds the host cell wall in succession to drive the virus through the bacterial cell wall, will be the subject of future investigations.

— *Sandy Field*

See > Ye Xiang¹, Petr G. Leiman^{1,§}, Long Li¹, Shelley Grimes², Dwight L. Anderson², and Michael G. Rossmann^{1*}, “Crystallographic Insights into the Autocatalytic Assembly Mechanism of a Bacteriophage Tail Spike,” *Mol. Cell* **34**, 375 (May 15, 2009). DOI:10.1016/j.molcel.2009.04.009

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• Accepting general users

> 23-ID-D • GM/CA-CAT • Life science • Macromolecular crystallography, microbeam, microdiffraction, anomalous diffraction (MAD/SAD), subatomic (<0.85 Å) resolution • 3.0-cm undulator • Accepting general users