The Bacteria-Infecting Virus P22 Tail Needle

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Salmonella bacteriophage P22 is a well-studied model system that is used in the study of the molecular basis for tail-attached bacteriophage infection. The P22 virion has a protein "needle" at the base of its tail that is composed of the phage gene 26 protein (gp26) and is thought to make first contact with the bacterial outer membrane during the initiation of an infectious cycle. Our goal is to genetically characterize the gp26 protein by locating its functional domains involved in virion attachment, membrane contact and internalization. The recently determined gp26 crystal structure (obtained in collaboration with Dr. Gino Gengel, SUNY Upstate Medical University) shows that it is a long thin trimeric rod, but which end is attached to the virion is currently unknown.

To begin our analyses, a set of oligohistidine-tagged N- and C-terminal truncations have been constructed using the expression vector plasmid pET-15b. The C-terminus was shortened by 9, 16, and 30 amino acids, and the N-terminus was shortened by 10, 25, and 30 amino acids. These truncations will first be analyzed in vitro to determine whether they retain full biological activity. The truncated proteins will be expressed in Salmonella, and these cells infected with P22 that has a defective gp26 gene. If the plasmid-expressed gp26 is fully functional, biologically active phage virions will be formed as measured by plaque formation. This approach will determine the minimum portion of the protein that is fully capable of binding and forming active infectious phage. The ability of these different mutant proteins to attach to virions in vivo will also be determined to find out which end of the gp26 trimer attaches to virions.

The ability of mutant gp26's to trimereize will be studied in vitro as follows. The oligohistidine tagged proteins will be purified by virtue of their histidine tag on a nickel column, and their monomer or trimer status measured by their mobility during polyacrylamide electrophoresis (trimers migrate much more slowly than monomers).

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