Identification of a TolA-Dependent Bacteriophage

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The Tol system plays a key role in protecting gram-negative cells against environmental toxins. The central protein in this system is TolA, which couples the energy derived at the inner, cytoplasmic membrane to proteins in the outer membrane that play roles in maintaining its barrier function. TolA protein serves as a target that certain toxic bacterial proteins, termed "colicins," and filamentous bacteriophage use to enter the cell. Because of its role in maintaining the integrity of the outer membrane, TolA has the potential of being an antimicrobial target. The Larsen lab is interested in studying the Tol system for two main reasons. First, although colicins and phage target bacteria, their mechanism of cell entry is similar to bacterial toxins and viruses that target animals and humans. Second, molecule specific binding sites on the TolA protein provide potential target sites for engineered molecules that might disable this system and its ability to support the outer defensive barrier of gram negative bacteria, rendering them much more susceptible to antimicrobials and antibiotics.

My project aims to discover a new bacteriophage that uses TolA or another Tol system protein for cell entry. Previous studies had identified TolA as a co-receptor for filamentous enveloped single-stranded DNA bacteriophage that first dock to pilus structures in *E. coli* and *Vibrio cholerae*. The Larsen lab is not equipped to work with filamentous phage, but the lab has an extensive collection of non-enveloped, double-stranded DNA bacteriophage from a range of hosts. The overall goal of this long-term project is to find the specific residues in the TolA protein that allows for cell entry.

From the lab's collection of over 70 different *E. coli*-specific bacteriophage isolated from a variety of environments over the past several years I screened 60 bacteriophages against an *E. coli* mutant that contained a deletion of the *tolA* gene. Successful growth on wild-type *E.coli* but not the *tolA* deletion strain suggests that the phage needs TolA for cell entry. 3 bacteriophages were identified that seem to rely on TolA for cell entry. Efforts were focused on one of these phage, KG31. I isolated and amplified this phage using various plating and liquid culture techniques. During this process it was discovered that KG31 was capable of growing on the Δ*tolA* strain but at such a low rate that their plaques are nearly impossible to see. In liquid cultures, the phage had a much slower growth curve and were unable to fully lyse the cells when in the Δ*tolA* host. Since plaque morphology and rate of infection changed, we can hypothesize that there is another method of cell entry the phage can use at the expense of its virulence. More recently, I have isolated the DNA of KG31 and now have enough to do full-genome sequencing as well as restriction enzyme digests. These will allow us to characterize the phage further. Using the sequence, we can then identify potential residues on the TolA protein that the phage binds to.

Even though KG31 is not completely reliant on TolA for cell entry, the absence of TolA greatly decreases its infection ability. Molecule specific binding sites on the TolA protein provide potential target sites for engineered molecules that might disable this system and its ability to support the outer defensive barrier of gram negative bacteria, rendering them much more susceptible to antimicrobials and antibiotics. With the rise of antibiotic resistance, and the sharp decrease of large pharmaceutical companies researching new antibiotics, studies on phage therapy are becoming increasingly important.