STUDY OF BACTERIOPHAGE EXCLUSION PROTEIN SieA

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Bacteriophages are viruses that infect bacteria. They do so by injecting their DNA into the host cell using specialized proteins called ejection proteins. This study examines a bacteriophage named P22 that infects and can lysogenize *Salmonella* strains. SieA is a protein that is expressed from the P22 prophage that blocks DNA injection by P22 and other phages by an unknown mechanism. When SieA is present, P22-like phage DNA is injected into the periplasm of the host cell, suggesting a block of DNA transfer at the inner membrane (Susskind *et al.*, 1974), and the SieA protein has been reported to be in the cell's membrane fraction (Hofer *et al.,* 1995). The long-range goal of this research is to determine the mechanism by which SieA blocks infection by certain bacteriophages.

Our hypothesis is that SieA blocks the infection of bacteriophages by inhibiting the creation of a temporary channel that extends from the infecting virus particle (virion) to the target cell's cytoplasm. This putative channel is thought to be built from the virion's "ejection proteins", specifically the products of genes 7, *16* and *20*. These problems are being approached genetically, largely through the study of phage mutants that overcome SieA mediated superinfection exclusion. The ejection proteins of the P22-like phages vary greatly among different phage (e.g., p16 from P22 and the *Shigella* P22-like phage Sf6 are only 27% identical). Previous unpublished Casjens laboratory results showed that mutants in p20 and p16 overcome SieA exclusion.

We used GalK recombineering to insert the phage P22 SieA gene into bacterial chromosomes. We inserted the P22 sieA gene along with its natural promoter into the *Salmonella* chromosome. *Salmonella* P22-like phages L, LP7, MG178, and MG40 are blocked, but other phage types such as the short tailed SP6 or long tailed Det7, ES18 and 9NA are not. This shows for the first time that no other P22 gene products are required for SieA mediated exclusion. P22 carrying the ejection protein genes from the *E. coli* P22-like phages CUS-3 of HK620 or *Shigella flexneri* P22-like phage Sf6 are all blocked by SieA in spite of their very different ejection protein amino acid sequences. These results suggest that SieA is specific for P22-like short tailed phages, and it can function on a surprisingly wide range of quite different ejection proteins to block infection. We also inserted the SieA gene into chromosomes of the different *E. coli* hosts of phage CUS-3 and HK620. SieA blocked phage CUS-3, but did not block phage HK620. This suggests that SieA can function in *E. coli* since it blocks CUS-3 infection. The failure to block HK620 means that either SieA does not work in that *E. coli* strain or it is not expressed there. (Recall P22 with HK620 ejection protein genes is blocked in *Salmonella*). Future plans include testing the HK620 host for SieA expression using epitope tagged SieA and inserting the SieA gene into the *Shigella* chromosome to test for Sf6 exclusion.

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