CHAPTER V

SUMMARY

In many bacteriophages, phage-induced lysis is mediated by a dual lysis system, which consists of a lysin and a holin. Phage lysins degrade the cell wall peptidoglycan to release the progeny phage. Because these lysins lack secretory signal sequences, they cannot reach the cell wall without the function of a holin. Phage holins form lesions in the cytoplasmic membrane of the host, facilitating the release of lysins to the cell wall. Most holin genes are located upstream of the lysin genes. Regardless of their primary sequences, all known holins possess common structural features. In the present study, the upstream region of a lysin gene (*lytA*) of bacteriophage ϕ11 of *S. aureus* was analyzed to determine the mechanism of lysin secretion in phage ϕ11.

The upstream region (6.2 kb) of the *lytA* gene was cloned into the vector pTZ18R . The nucleotide sequence of the cloned region was determined by the dideoxy chain termination method. Computer-assisted nucleotide sequence analysis revealed 13 open reading frames (ORFs). No substantial sequence similarities were detected when the nucleotide sequences and the deduced amino acid sequences of the 13 ORFs were compared to known sequences in the NCBI Entrez database. However, the predicted protein product of ORF1 showed some structural similarities (a pair of potential trans-membrane domains and a highly charged C-terminus) to

phage holins.

ORF1 is located immediately upstream of the *lytA* gene, and it overlaps the *lytA* gene by 17 bp. ORF1 consists of 435 bp and is preceded by a putative Shine-Dalgarno sequence and -10 and -35 promoter sequences. A potential transcription terminator sequence is located downstream of the coding sequence of ORF1. The predicted protein product of ORF1 consists of 145 amino acids and has a molecular mass of 15.7 kDa and a pI of 4.85.

ORF1 of a ϕ11 lysogen was disrupted by inserting an Em^r marker gene into it. Four of the ORF1 mutants were induced for lysis, and cell lysis and phage titers were determined. Upon induction, 2 of the mutants did not lyse. The other 2 mutants showed very little lysis and produced very low phage titers. The ORF1 mutants that did not lyse upon induction were complemented in *trans* with plasmid clones containing the intact ORF1. The ORF1 mutants that are complemented with the intact ORF1 in *trans* were induced for lysis. Upon induction, they lysed, producing high phage titers comparable to the wild-type ϕ11 lysogen. The above observations suggested that the product of ORF1 is involved in phage ϕ11-induced lysis of the host.

Using an *E. coli* S30 extract system, in vitro transcription-translation was carried out to determine whether the ORFs revealed by the nucleotide sequence are being expressed. Discrepancies between the results obtained from the in vitro transcription-translation analysis and the nucleotide sequence analysis were observed. The DNA sequence should be verified in order to determine whether these discrepancies are due to an error in the DNA sequence. No protein product that corresponds to the ORF1 product was observed when in vitro transcription-translation was done. It is possible

that certain factors that are nessesary for ORF1 expression are absent in the *E. coli* S30 extract. It also is possible that ORF1 is expressed at a very low level, so that the product cannot be detected under the conditions employed.

Based on the structural features of the predicted product of ORF1, its location in relation to the *lytA* lysin gene, mutational analysis of ORF1, and complementation studies, I speculate that ORF1 codes for a putative holin, which permeabilizes the cytoplasmic membrane to help the *lytA* lysin to gain access to the host cell wall.