DEFINING THE
EARLY LYTIC REGION
OF COLIPHAGE 186
AND THE CONTROL OF
MIDDLE GENE TRANSCRIPTION

A Thesis submitted for
for the degree of
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by

HELENA ELIZABETH RICHARDSON (B.Sc.Hons.)

Adelaide Centre for Gene Technology,
Department of Biochemistry,
University of Adelaide,
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DEFINING THE EARLY LYtic REGION OF COLIPHAGE 186 AND THE CONTROL OF MIDDLE GENE TRANSCRIPTION.

This thesis describes work carried out to provide an understanding of the expression of the early lytic and middle genes of the temperate coliphage 186. The specific aims of this study were to identify the 186 early lytic genes, and to investigate the mechanism of control of middle gene transcription.

The DNA sequence of the early lytic region was completed. Computer-assisted analysis of the DNA sequence led to the prediction that the early lytic transcript encoded four genes: CP75, CP76, CP77 and CP78. This transcript was predicted to terminate after the CP78 gene at a potential rho-independent terminator structure, t31. The gene, CP79, following the terminator t31, was predicted to be the first gene in the middle region. These predicted genes were cloned into a plasmid expression vector and their protein products were identified by SDS-polyacrylamide gel electrophoresis.

The functions of the CP75 and CP76 genes have been determined by other members of the laboratory and are involved in the lysis-lysogeny decision. Thus, the assignment of functions to CP77 and CP78 was required. Two functions have been previously described that are likely to be encoded by CP77 and CP78: Dhr, which results in an inhibition of E. coli DNA replication, and Tom, which was postulated to be an essential function required for 186 middle gene transcription. The investigation of the Dhr function revealed that it was encoded by CP78. CP78 is a non-essential gene but appears to be important in 186 lytic development. It was expected that the CP77 gene would encode the Tom function, however this study also revealed that CP77 is a non-essential gene, the expression of which results in an inhibition of E. coli cell division. CP77 was named the fil gene.
Thus, it appeared that the predicted Tom function was not encoded in the early lytic region.

Previous studies carried out in this laboratory, led to the prediction that middle gene transcription occurs either by antitermination of the early lytic transcript or by promoter activation of a new transcript. As a first step towards understanding the control of middle gene transcription, Northern analysis was used to identify, size and determine the approximate 5'‐ends and 3'‐ends of the in vivo transcripts from the 186 early lytic and middle regions. The transcription pattern of the early lytic and middle regions was consistent with a mechanism for middle gene transcription involving antitermination and RNaseIII processing.

Studies were carried out to determine whether an antitermination mechanism for middle gene transcription was likely. This study did not provide evidence for the existence of a control mechanism for 186 middle gene transcription, and it is likely that middle gene transcription occurs simply by transcription passing through the relatively weak early terminators. However, these studies revealed that translation was important for transcription of the 186 early lytic and middle regions and it was postulated that an attenuation‐type mechanism may be involved in the control of middle gene transcription. The work presented in this thesis provides the basis for further studies concerning 186 middle gene expression.