TRANSCRIPTIONAL STUDIES OF BACTERIOPHAGE 186

A Thesis submitted for
the Degree of
Doctor of Philosophy

at the
University of Adelaide

by
Elizabeth Jean Finnegan, B.Sc.(Hons.)
Department of Biochemistry

May, 1979.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUMMARY</td>
<td>ix</td>
</tr>
<tr>
<td>STATEMENT</td>
<td>xi</td>
</tr>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>xii</td>
</tr>
<tr>
<td>ABBREVIATIONS</td>
<td>xiii</td>
</tr>
<tr>
<td>INDEX OF FIGURES AND TABLES</td>
<td>xiv</td>
</tr>
<tr>
<td>SECTION I - GENERAL INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>1. Phage 186</td>
<td>1</td>
</tr>
<tr>
<td>2. Comparison of P2 and 186</td>
<td>1</td>
</tr>
<tr>
<td>3. Transcription Studies</td>
<td>4</td>
</tr>
<tr>
<td>(a) Deletion studies</td>
<td>4</td>
</tr>
<tr>
<td>(b) Sheared DNA</td>
<td>5</td>
</tr>
<tr>
<td>(c) Isolated phage DNA strands</td>
<td>6</td>
</tr>
<tr>
<td>(d) Restriction fragments</td>
<td>6</td>
</tr>
<tr>
<td>(i) Restriction enzymes</td>
<td>7</td>
</tr>
<tr>
<td>(ii) Cloning of restriction fragments</td>
<td>10</td>
</tr>
<tr>
<td>4. Transcriptional Controls</td>
<td>16</td>
</tr>
<tr>
<td>5. Aim of This Work</td>
<td>20</td>
</tr>
<tr>
<td>SECTION II - GENERAL MATERIALS AND METHODS</td>
<td>21</td>
</tr>
<tr>
<td>A. MATERIALS</td>
<td>21</td>
</tr>
<tr>
<td>1. Bacterial strains</td>
<td>21</td>
</tr>
<tr>
<td>2. Bacteriophage strains</td>
<td>21</td>
</tr>
<tr>
<td>(a) Phage 186 strains</td>
<td>21</td>
</tr>
<tr>
<td>(b) Phage P2 strains</td>
<td>24</td>
</tr>
<tr>
<td>(c) P2.186 hybrid phage</td>
<td>24</td>
</tr>
<tr>
<td>(d) Phage λ strains</td>
<td>24</td>
</tr>
<tr>
<td>3. Plasmid vector</td>
<td>24</td>
</tr>
<tr>
<td>4. Chemicals</td>
<td>25</td>
</tr>
<tr>
<td>5. Liquid media and buffers</td>
<td>25</td>
</tr>
<tr>
<td>6. Solid media</td>
<td>27</td>
</tr>
</tbody>
</table>
B. GENERAL METHODS

1. Storage of bacteria and bacteriophage 29
2. Growth of bacterial cultures 28
3. Preparation of lysogens 29
4. Preparation of T₁, T₅ resistant bacteria 29
5. Titration of phage stocks 30
6. Preparation of phage stocks 30
   (a) Low titre λ stocks 30
      (i) Plate stocks 30
      (ii) Liquid infection 31
   (b) Low titre λ stocks 31
   (c) Low titre λ stocks 32
      (i) Plate stocks 32
      (ii) Liquid infection 32
   (d) High titre λ stocks 33
      (i) Heat induction 33
      (ii) Liquid infection 33
   (e) High titre λ stocks 34
   (f) High titre λ stocks 34
   (g) High titre λ,186 hybrid phage stocks 34

7. Extraction of bacteriophage DNA 35

SECTION III - HYBRIDIZATION OF λDNA TO HV5 AND λDNA

A. INTRODUCTION 36

B. MATERIALS 40

C. METHODS 42

1. Latent period of λ860Ttse following heat induction 42
2. Incorporation of $^3$H-uridine into TCA precipitable material 42
3. Total RNA synthesis following 186It's heat induction 43
4. Preparation of phage DNA filters for hybridization 43
5. Amount of DNA/filter 44
6. Labelling and isolation of phage $^3$H-RNA 44
7. Filter hybridization 45

D. RESULTS 46
1. Latent period of 186It's following heat induction 46
2. Incorporation of $^3$H-uridine into TCA precipitable material 47
3. Does 186 affect host RNA synthesis? 47
4. Conditions of DNA excess 55
5. Time course of hybridization 55
6. Non-specific hybridization 58
   (i) Cross hybridization between P2 DNA and 186 mRNA 59
   (ii) Cross hybridization between host RNA and 186, My5 or P2 DNA 58
7. Transcription of 186It's, 186It'sAcm5 and 186It'sAcm17 58

E. DISCUSSION 62
1. 186 and host RNA synthesis 62
2. Non-specific hybridization 68
3. Transcription of 186\(\text{Its}\) 71
4. Transcription of 186\(\text{Its}\)\(\text{Aam5}\) 73
5. Transcription of 186\(\text{Its}\)\(\text{Bam17}\) 75
6. Summary 77

SECTION IV - HYBRIDIZATION OF 186 mRNA TO 186 DNA
DIGESTED WITH RESTRICTION ENDONUCLEASES 79

A. INTRODUCTION 79
B. MATERIALS 80
C. METHODS 81
1. Restriction endonuclease digestion 81
2. Agarose gel electrophoresis 81
3. Transfer of DNA from a gel to nitrocellulose 82
4. Hybridization to transferred DNA 83
5. Autoradiography 84

D. RESULTS 84
1. Hybridization of 186\(\text{Its}\) mRNA to 186 DNA digested with \(\text{EcoRI}\) and \(\text{KpnI}\) 84
2. Hybridization to 186 DNA digested with \(\text{EcoRI}, \text{KpnI}\) and \(\text{BglII}\) 89
   (a) Hybridization of 186\(\text{Its}\) late mRNA 91
   (b) Hybridization of 186\(\text{Its}\)\(\text{Bam17}\) mRNA 91
   (c) Hybridization of 186\(\text{Its}\)\(\text{Aam5}\) mRNA 93

E. DISCUSSION 95
1. Hybridization of 186\(\text{Its}\) RNA to restricted 186 DNA 95
2. Hybridization of 18S rRNA to restricted DNA
3. Hybridization of 18S rRNA to restricted DNA

SECTION VII - THE PHYSICAL MAP OF 18S

A. Introduction
B. Materials
C. Methods

1. Cloning of 18S restriction fragments into pBR322
   (a) Restriction endonuclease digestion
   (b) Ligation
   (c) Transformation
   (d) Transfection

2. Purification of DNA from low melting agarose

3. Agarose gel electrophoresis

4. Marker rescue
   (a) Spot testing
   (b) Assay testing

5. Plasmid DNA preparation

6. Hybridization to recombinant clones represented by single plaques
   (a) Filter preparation
   (b) Hybridization and autoradiography

7. Preparation of PstI restriction endonuclease
D. RESULTS

1. Physical map for the late functions of 186
2. Physical map of the early functions of 186
3. Cloning of pJF18 into 

E. DISCUSSION

1. Physical map of 186
2. Recloning of pJF18
3. Expression of 186 functions from cloned fragments
4. Restriction fragments for transcription mapping

SECTION VI - HYBRIDIZATION OF 186 mRNA TO CLONED RESTRICTION FRAGMENTS OF 186 DNA

A. INTRODUCTION

B MATERIALS

C. METHODS

1. Filter hybridization with cloned DNA
2. Incorporation of $^3$H-leucine into TCA precipitable material
3. Chloramphenicol inhibition of protein synthesis
4. Isolation of chloramphenicol mRNA
   (a) Heat induction
   (b) Infection
5. Burst size of 1650ts following infection of dnaCts host
6. Preparation of mRNA in the absence of DNA synthesis 146

D. RESULTS

1. Hybridization to cloned restriction fragments of 186 DNA 147
2. Transcription of the prophage 148
3. Transcription of 186 site following heat induction 151
4. Transcription of 186 site following induction 154
5. Transcription of 186 site following induction 158
6. Inhibition of bacterial protein synthesis by chloramphenicol 158
7. Transcription in the presence of chloramphenicol 162
8. Burst size of 186 site in the absence of DNA synthesis 165
9. DNA synthesis and 186 transcription 167

Z. DISCUSSION

1. Transcription of the prophage 172
2. Transcription following prophage induction 173
3. The role of the A gene in transcription 174
4. B protein and late transcription 177
5. Protein synthesis and transcription 178
   (a) Transcription from the fragments known to require A function 180
(b) Transcription from pJF17.2 and pJF15 160
(c) Transcription of the immunity region 183
(d) Transcription of the cohesive ends 164
(e) Summary of chloramphenicol RNA 184

6. DNA synthesis and transcription around omt 185

7. Transcription of the cohesive ends 188

8. Control of B gene transcription 191

SECTION VII - GENERAL DISCUSSION 193

1. Controls of 186 transcription 193
   (a) Transcription of the prophage 193
   (b) Heat induction 193
   (c) Lytic development 196
   (d) Genes under repressor control 200

2. A comparison between 186 and P2 transcription 201

3. Comparison of control elements in 186 and 1 transcription 210

4. Int gene and the split operon model 215

5. The future 217

APPENDIX I - EXPRESSION OF NON-ESSENTIAL FUNCTIONS FROM CLONED 186 DNA 221

(a) Expression of c1 221
(b) Expression of int 225
(c) Expression of dio 229

REFERENCES 236
SUMMARY

This thesis describes an investigation of the control of transcription of bacteriophage 186 during lytic development following heat induction of a 186-six prophage.

Preliminary characterization of transcripts as control or structural was based on filter hybridization to DNA from two phage, Rys and 186. A hybrid between the right 34.6% of 186 and the left 70% of the closely related phage P2 encodes the tail genes D and E (the exchange occurs in gene E) and all of the known control functions namely genes A and B, the origin of replication, cI, cII, ini, cII and dho the gene proposed to initiate the inhibition of host DNA synthesis. Early transcripts originated exclusively from this part of the 186 chromosome. During the late phase structural genes were also transcribed. Early transcription of ham phage was reduced and phage defective in either A or B function did not have normal late gene expression.

Attempts to analyse Ham and Bam transcripts by hybridization to Southern transfers of restriction endonuclease generated fragments of 186 DNA were unsuccessful, and the need to isolate pure fragments of known genetic function arose. Restriction fragments of 186 were cloned into the plasmid vector pBR322 and the genes encoded by each fragment were identified by marker rescue experiments. In this way the recombination and restriction maps were correlated to provide a physical map of the 186 chromosome.

Transcription of genes A, B, cI and ini, cII and dho,
the cohesive ends (which had been cloned into ) and the
two late genes, W and P, cloned on separate fragments, was
studied by filter hybridization to recombinant DNA. Trans-
scripts of wild type phage and phage with a mutation in gene
A or gene B were analysed. Genes A and B were both
required, directly or indirectly, for the normal transition
to the late phase; a protein was also needed for the trans-
scription in the early region 87.0 to 93.8% but was early
transcription was identical to wild type. The dependence
of phage directed transcription on protein synthesis and
DNA replication was investigated by the addition of chlor-
amphenicol to inhibit protein synthesis or by the use of a
dnaC+ host at a non-permissive temperature to inhibit
phage DNA synthesis.

Transcripts made by the prophage were examined to
test the hypothesis that B gene expression is constitutive
in the prophage. No B gene transcripts were detected but
RNA from the fragment coding for αI and also in the 87.0 to
93.8% region was found.

The DNA in the region 87.0 to 93.8% has two distinct
functions; during lytic development transcription in this
fragment is associated with DNA synthesis and is dependent
on the A gene, while in the prophage state this fragment
is active suggesting a role in maintaining stable lysogeny.