GENETIC AND PHYSICAL STUDIES OF BACTERIOPHAGE P22 GENOMES CONTAINING TRANSLOCATABLE DRUG RESISTANCE ELEMENTS

by

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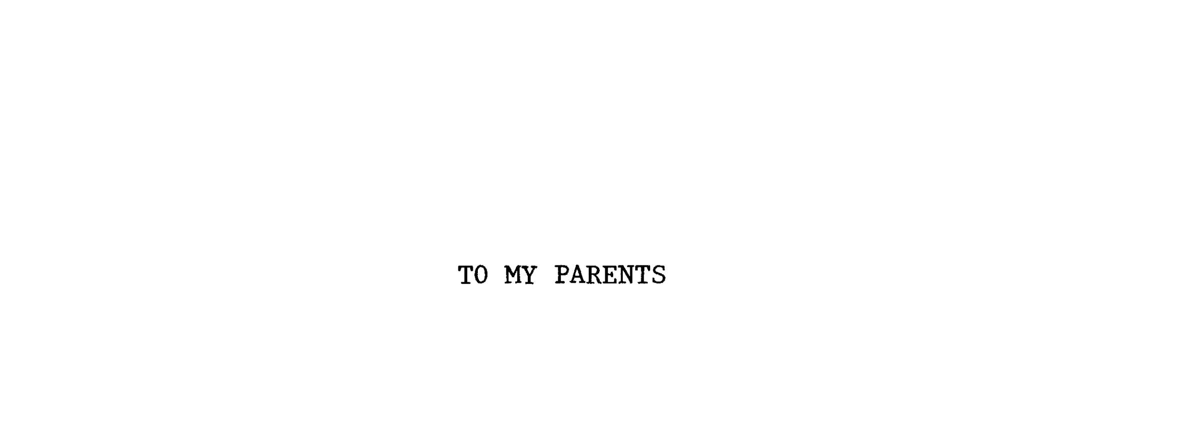

TO MY PARENTS

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by George Matthew Weinstock

Submitted to the Department of Biology on June 20, 1977 in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

ABSTRACT

Translocatable elements are defined segments of DNA with the capacity to integrate into DNA molecules in the absence of any obvious sequence homology. In this thesis the translocatable element Tn1 is studied.

Tn1 contains a gene specifying ampicillin resistance (amp^R). 187 derivatives of the temperate bacteriophage P22, each containing an insertion of Tn1, were identified on the basis of their ability to transduce amp^R at high frequency. 76 of these phages were studied further.

The Tn1 insertions in these phages were not distributed randomly in the genome but tended to cluster in the vicinity of the *ant* gene. However, insertions occurred at many sites within *ant*. Thus, Tn1 integration is regionally, but not locally, specific.

The Tn1 insertions were found to be about 4800 base pairs in length and their terminal 100 bases were identical in sequence but opposite in orientation. Tn1 insertions in structural genes caused irreversible mutations. In one case a deletion was found near the insertion but all other insertions examined appeared to have integrated precisely.

Tn1 insertions also caused polar mutations. The degree of polarity was determined using phages with insertions which were polar on gene *9*, which codes for the phage tail protein. It was found that some insertions reduce gene expression by about 2-fold, while the other reduce expression 20-fold. It was found that the degree of polarity depends on the orientation of the insertion.

Deletions isolated in P22Ap phage genomes were found by genetic mapping to frequently end at the Tn1 insertion. Examination of 5 of these genomes in the electron microscope revealed that 4 contained an intact Tn1 element with an adjacent deletion while the deletion in the fifth, which was isolated by a different procedure, extended into Tn1. The deletions extended from Tn1 to non-random positions in the genome, ending either in the vicinity of gene *12* or *ant*. However, there were many sites

at which the deletions ended in these regions. Thus, deletion formation by Tn1, like integration, is regionally, but not locally, specific. This type of site specificity has not been observed for integration or excision of other translocatable elements.

The fact that Tn1 insertions and deletions exhibit the same form of sequence specificity implies these events occur by related mechanisms. It is proposed that a Tn1 determinant recognizes a rare site in the DNA and then causes the insertion or deletion event to occur at a non-specific, but nearby site.

The Tn1 insertions are also used to study P22. From the phenotypes of polar insertions, evidence is found for the existence of an operon of late phage genes, and it is shown that gene 9 is a member of this operon. This latter finding implies that a) for gene 9 to be expressed transcription may proceed through a 3000 bp region containing genes whose function and regulation is unrelated to the other genes in the late operon, b) there is probably a transcription termination signal between ant and 9, and c) expression of the ant gene may be subject to post-transcriptional control late in infection.

Lastly, by heteroduplex and restriction enzyme analysis, the insertions and deletions in P22 are used to extend the physical map of the P22 genome.

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TABLE OF CONTENTS

TITLE PAGE i

ABSTRACT ii

BIOGRAPHICAL NOTE iv

ACKNOWLEDGEMENTS v

TABLE OF CONTENTS vi

LIST OF FIGURES x

LIST OF PLATES xi

LIST OF TABLES xi

CHAPTER I. INTRODUCTION

I. Translocatable Elements 1

- A. Overview 1
- B. Translocatable Elements Conferring Ampicillin Resistance 2
  - (i) Inroduction 2
  - (ii) Structure 2
  - (iii) Translocation 3
  - (iv) Effects on Gene Expression 4
- C. Deletion Generation by Translocatable Elements 5
- D. Aim and Significance of Thesis 5

II. Bacteriophage P22 6

- A. Overview 6
- B. The Headful Packaging Mechanism 8
- C. Lytic Genes 8
- D. Transduction by P22 10
- E. P22Tc10: A P22 Derivative Containing the Tn10 Translocatable Element 11

F. Lysogeny and the ant Gene 14

G. Gene 9 and the Expression of Late Genes 15

H. Use of P22 to Study Translocatable Elements 16

CHAPTER II. CHARACTERIZATION OF P22 GENOMES CONTAINING Tn1 INSERTIONS 18

I. Materials and Methods 18

A. Bacteria 18

B. Phage 18

C. Media and Solutions 21

D. General Methods 22

E. Isolation of Insertions 24

F. Phenotypes of P22Ap Phages 25

G. Mapping Insertions with P22 virB am am Phages 25

H. Manipulations with P22Ap Phages 27

(i) Growth and Titering 27

(ii) Introduction of Point Mutations by Crossing 27

(iii) Crosses between Different P22Ap Phages 28

I. Complementation of Insertions in Essential Genes 28

J. Preparation of Heads and Tails 29

K. Quantitation of p9 Production by P22Ap Phages 29

L. Deletions 30

M. Physical Analysis of DNA 31

(i) Heteroduplex Analysis 31

(ii) Analysis with Restriction Enzymes 32

II. Results 34

A. Transduction of Amp^R from RP4 34

B. Phenotypes of P22Ap Phages 37

C. Mapping Insertions with P22 virB am am Phages 39

D. Complementation of Insertions in Essential Genes 42

E. P22Ap2: Insertion in the al Region 44

F. Insertions in the ant Gene 46

(i) Mapping 46

(ii) Tail Deficiency of ant⁻ P22Ap Phages 50

G. Polar Effects on Gene 9 53

(i) Tail Production by P22Ap Phages 53

(ii) Complementation Test of Tail Deficiency 58

H. Reversion of Mutations Caused by Tn1 Insertions 58

I. Deletions which Restore Terminal Repetition 60

(i) Plaque Forming Revertants 60

(ii) Low Multiplicity Transductants 62

J. Prophage Deletions Allowing Survival from mnt Induction 66

K. Physical Structure of Genomes Containing Deletions 69

L. Physical Mapping of P22Ap Genomes by Heteroduplex Analysis 75

M. Physical Mapping of P22 Genomes with Restriction Enzymes 78

(i) Digestion with EcoRI 78

(ii) Digestion with BamH1 84

(iii) Double Digestion with EcoRI and BamH1 90

CHAPTER III. DISCUSSION 95

I. Summary of Results 95

II. Characteristics of Tn1 Translocation 96

A. Frequency of Translocation 96

B. Sites of Insertion 96

C. Mechanism of Translocation 98

III. Characteristics of Tn1 Insertions 101

A. Structure of Insertions 101

B. Polarity of Tn1 101

C. Reversion 102

IV. Deletion Generation by Tn1 104

A. Deletions in Transductants Isolated from Low Multiplicity Infections 104

B. Deletions in Survivors of mnt Induction 104

C. Relation of Deletion Formation To Translocation 106

V. Further Discussion Regarding Tn1 108

VI. An Operon of Late Genes in P22 110

VII. The Expression of Gene 9 110

VIII. Physical Map of the P22 Genome 111

IX. Use of P22 to Study Translocatable Elements 113

BIBLIOGRAPHY 115

APPENDIX I. Summary of P22Ap Phages and Their Properties 121

APPENDIX II. Fine Structure Map of Gene 12 125

APPENDIX III. Restriction Fragment Patterns Produced by Digestion of P22 DNA with Various Restriction Enzymes 127

LIST OF FIGURES

FIGURE 1. Life Cycle of P22 DNA 7

FIGURE 2. The P22 Chromosome 9

FIGURE 3. Life Cycle of P22Tc10 DNA 13

FIGURE 4. Mapping of Insertions with P22 virB am am Phages 41

FIGURE 5. Fine Structure Map of the ant Gene 48

FIGURE 6. Models for Transcription of Gene 9 54

FIGURE 7. Assay for Tails Produced by P22Ap Phages 56

FIGURE 8. Prophage Deletions in Low Multiplicity Transductants 64

FIGURE 9. Distribution of Deletion Endpoints in Gene 12 65

FIGURE 10. Prophage Deletions in Survivors of mnt Induction 68

FIGURE 11. Idealized Representations of Heteroduplexes 70

FIGURE 12. Assignment of P22 Genes and Tn1 Insertions to EcoRI Fragments 79

FIGURE 13. Representation of the EcoRI Digestion Patterns of Plate 3. 82

FIGURE 14. Representation of the BamH1 Digestion Patterns of Plate 3. 86

FIGURE 15. Representation of the BamH1 Digestion Patterns of Plate 4. 87

FIGURE 16. Physical Map of P22 from BamH1 Digestion of P22Ap DNAs 91

FIGURE 17. Representation of the EcoRI/BamH1 Double Digestion Patterns of Plate 3. 92

FIGURE 18. Physical Map of P22 94

FIGURE 19. Fine Structure Map of Gene 12 126

LIST OF PLATES

PLATE 1.	Heteroduplexes between P22Tc10 and P22Ap Genomes Containing Deletions	71
PLATE 2.	Heteroduplexes between P22Tc10 and P22Ap Genomes	76
PLATE 3.	Agarose Gel of Digestions of P22, P22Ap2, Ap4, Ap7, Ap9, and Ap63 DNAs with EcoRI, BamH1, and EcoRI+BamH1.	81
PLATE 4.	Agarose Gel of Digestion of P22Ap12, Ap38, and Ap48 DNAs with BamH1	85

LIST OF TABLES

TABLE 1.	Bacterial Strains	19
TABLE 2.	Scheme for Classification of P22Ap Phage Phenotypes	26
TABLE 3.	Ampicillin Resistant Transductants	35
TABLE 4.	Phenotypes of P22Ap Phages	38
TABLE 5.	Complementation of Insertions in Essential Genes	43
TABLE 6.	Mapping the Insertion in P22Ap2	45
TABLE 7.	Crosses between P22Ap Phages	49
TABLE 8.	Tail Deficiency of <u>ant</u>$^-$ P22Ap Phages	51
TABLE 9.	Tail Production by P22Ap Phages	57
TABLE 10.	Complementation Test of P22Ap4's Tail Deficiency	59
TABLE 11.	Plaque Forming Revertants of P22Ap4 <u>sieA</u>-44	61
TABLE 12.	Heteroduplex Analysis of Genomes Containing Deletions	72
TABLE 13.	Heteroduplex Analysis of P22Ap Phage Genomes	77
TABLE 14.	Fragments Produced by BamH1 Digestion of P22Ap DNA	88
TABLE 15.	Action of Restriction Enzymes on P22 DNA	128

CHAPTER I: INTRODUCTION

I. Translocatable Elements

A. Overview

A translocatable element is a segment of DNA which can integrate as a unit into an unrelated DNA sequence. Translocatable elements are natural constituents of phage, plasmid, and bacterial chromosomes and comprise a diverse group of genetic entities. They range in size from the IS sequences, which can be as short at 800 base pairs, to temperate phages such as Mu or λ (more than 40,000 base pairs). Some elements contain antibiotic resistance genes and play a key role in the evolution of R factors. These transposable antibiotic resistance units often have inverted or direct repeats of their terminal sequences. In some cases the repetitious termini are IS sequences.

Besides their capacity for transposition, translocatable elements stimulate the formation of deletions and inversions. Thus, translocatable elements are capable of a number of illegitimate recombination events.

Translocatable elements can also affect gene expression. Integration within a gene is invariably mutagenic and in an operon can be polar. The IS2 element may contain a promoter capable of transcribing external genes. Thus, translocatable elements affect gene expression as well as chromosome structure. The properties of translocatable elements are reviewed by Cohen (1976), Starlinger and Saedler (1976), and Kleckner (1977) and in a monograph (Bukhari et al., 1977).

B. Translocatable Elements Conferring Ampicillin Resistance

(i) Introduction

Plasmid-specified ampicillin resistance is usually due to the production of a β-lactamase, an enzyme which hydrolyzes penicillins and cephalosporins. On the basis of their activities, two classes of β-lactamases have been defined, types O and TEM (Dale and Smith, 1974; Hedges et al., 1974). TEM is far more common and is made by many different R factors from a variety of bacteria. These enzymes form a remarkably homogeneous group and only two types, TEM-1 and TEM-2, are distinguishable by isoelectric focusing (Matthew and Hedges, 1976). This uniformity is reflected in the DNA sequences specifying TEM enzymes. R factors producing a TEM enzyme contain a common DNA segment (Heffron et al., 1975a) which is translocatable and contains the β-lactamase gene (Hedges and Jacob, 1974; Heffron et al., 1975b; Bennett and Richmond, 1976). The translocatability of the β-lactamase gene explains how it can be highly conserved yet widely distributed in nature.

(ii) Structure

The translocatable β-lactamase gene resides in a 4900 base pair (3.2×10^6d) segment of DNA whose terminal 140 base pairs are identical in sequence but opposite in orientation (Heffron et al., 1975b; Rubens et al., 1976). The β-lactamase gene is located near one end of this element (Heffron et al., 1977). Since the TEM enzyme is about 22,000d in molecular weight (Heffron et al., 1975a) its gene need occupy only a small part of the element.

Translocatable sequences coding for the two different TEM enzymes are the same size and contain identical inverted repeats as judged by electron microscopy of heteroduplexes (Rubens et al., 1976). DNA-DNA

hybridization, however, showed that about 15 percent of their sequences are non-homologous and restriction enzyme analysis also revealed minor differences.

In accordance with the nomenclature proposed for translocatable elements (Bukhari et al., 1977) the TEM-2 coding sequence is called Tnl and the TEM-1 coding sequence is called Tn2.

(iii) Translocation

Translocation of amp icillin resistance between plasmids and from plasmids into bacterial chromosomes has been demonstrated in a number of investigations. Heffron et al., (1975b) identified translocations from large R factors into a small plasmid by virtue of the increase in size of the plasmid after insertion of Tnl or Tn2. The inserts all had the characteristic structure, without permutation, of Tnl and Tn2, but could occur in either orientation. Insertions were found at many sites within the plasmid. The distribution of insertions was the same for Tnl or Tn2 but was not random. Because of this it was concluded that Tnl and Tn2 insert at a short, specific sequence. The frequency, structure, and distribution of Tnl and Tn2 insertions were found to be independent of the *recA* gene (Rubens et al., 1976).

Several studies have shown that a recipient of Tnl or Tn2 may act as a donor in a subsequent translocation event (Hedges and Jacob, 1974; Bennett and Richmond, 1976; Heffron et al., 1977). This suggests that translocation determinants are present in the element. To study these determinants, Heffron et al., (1977) enzymatically created deletions in Tn2 which impaired translocation. They found that small deletions removing little more than one of the terminal inverted repeats or lying within the central region of the element reduced translocation at least

a thousand-fold. This shows that both terminal and internal sequences are essential for translocation. In complementation tests, the terminal deletions were cis-dominant while internal deletions were recessive. This suggests the simple model that the terminus contains a site recognized in translocation while internal sequences code for one or more diffusible products necessary for translocation. This is formally analogous to the integration/excision system of bacteriophage λ which requires a site (att) as well as nearby genes (int and xis)(Weisberg et al., 1977). This analogy may be artificial, however: although Tnl inserts at many sites in a small plasmid, the frequency of Tnl integration into other plasmids varies over four orders of magnitude (Bennett and Richmond, 1976). Furthermore, Tnl is reported not to integrate into a plasmid already containing a Tnl sequence except by homologous, recA-dependent recombination (Robinson et al., 1977). These results are not readily explainable by a mechanisms requiring only a short, relatively common sequence in the recipient DNA and genes coding for integration functions in the element. Thus, other factors must influence Tnl translocation.

(iv) Effects on Gene Expression

Rubens et al., (1976) described Tnl and Tn2 insertions in a plasmid conferring resistance to streptomycin and sulfonamide. From the mutant resistance phenotypes caused by these insertions, they concluded that 1) insertion in a gene is mutagenic and 2) insertions are strongly polar in one orientation but weakly polar in the other orientation. The mechanism of this polarity is not known. However, a small deletion within Tnl largely relieves polarity and it has been suggested that a transcription termination signal is deleted. A similar effect of orientation on gene expression has been proposed for IS2 insertions in the gal

operon of E. coli (Saedler et al., 1974). Under certain circumstances, gal genes may be expressed from a promoter within IS2. Promotion of external gene expression from within Tn1 or Tn2 has also been invoked to explain certain complex phenotypes (Rubens et al., 1976; Inselburg, 1977) but no definitive experimental support for this possibility exists.

C. Deletion Generation by Translocatable Elements

Several observations imply that translocatable elements can generate deletions. Inserted elements increase the frequency of deletion of adjacent material (Chan and Botstein, 1972; Reif and Saedler, 1975). These deletions have a characteristic structure. One endpoint is located by genetic criteria at the site of insertion and physical mapping shows the deletions are very near the terminus of the element (Davis and Parkinson, 1971; Hu et al., 1975; Ahmed and Johansen, 1975), implying the ends of translocatable elements are important for deletion generation. The other endpoint is more randomly located outside of the insertion but can have preferred sites (Davis and Parkinson, 1971; Chan, 1974; Reif and Saedler, 1975). In one case, these endpoints are more randomly distributed than the sites of insertion of the element (Kleckner et al., 1977).

The ability of translocatable elements to both insert and form deletions in DNA and the probable importance of their termini in these processes suggests related mechanisms. However, distinctions must exist since the frequency and sequence specificity of these events can be different.

D. Aim and Significance of Thesis

Translocatable elements are biologically significant because of their role in evolution and, possibly, modulation of gene expression.

In addition, they are useful tools for genetic analysis. This is true of Tn1 whose source, the R factor RP4, has a broad host range, allowing Tn1 to be introduced into many interesting bacterial genera. Thus, one object of this thesis, the study of Tn1, is of practical and theoretical significance.

Previous work with Tn1 lacked a well-developed genetic system. In contrast, this thesis describes the solution and characterization of Tn1 insertions in the genome of bacteriophage P22. The processes of integration, excision, deletion generation, and polarity are examined by generally applicable methods.

The other focus of this thesis is the study of P22. Tn1 insertions are used to construct physical and fine structure genetic maps and to deduce the organization of operons. The use of Tn1 to obtain this kind of information, necessary for understanding any genome, provides a precedent for this kind of use of translocatable elements in genetic analysis.

II. Bacteriophage P22

A. Overview

P22 is a temperate, generalized transducing phage of *Salmonella typhimurium*. Its chromosome is a 27 x 10^6 dalton, linear, double-stranded DNA molecule with short terminal repetition and limited circular permutation. Following injection, the chromosome circularizes by a homologous recombination event between its repetitious ends. This circular intermediate is essential in either the lytic or lysogenic cycle (Botstein and Matz, 1970; figure 1).

To lysogenize, the circular DNA molecule integrates at a unique site in the host chromosome, producing a linear, non-permuted prophage. Repression of lytic genes requires two repressors, the product of the *c2*

Figure 1. Life Cycle of P22 DNA

Infection by P22 phage produces circular DNA molecules formed by recombination. The circles either replicate to produce concatemers or integrate in the host chromosome to produce a prophage. When the prophage is induced a circular molecule is produced which replicates to produce concatemers. The concatemers are cut and packaged in phage particles to produce a population of terminally repetitious, circularly permuted molecules.

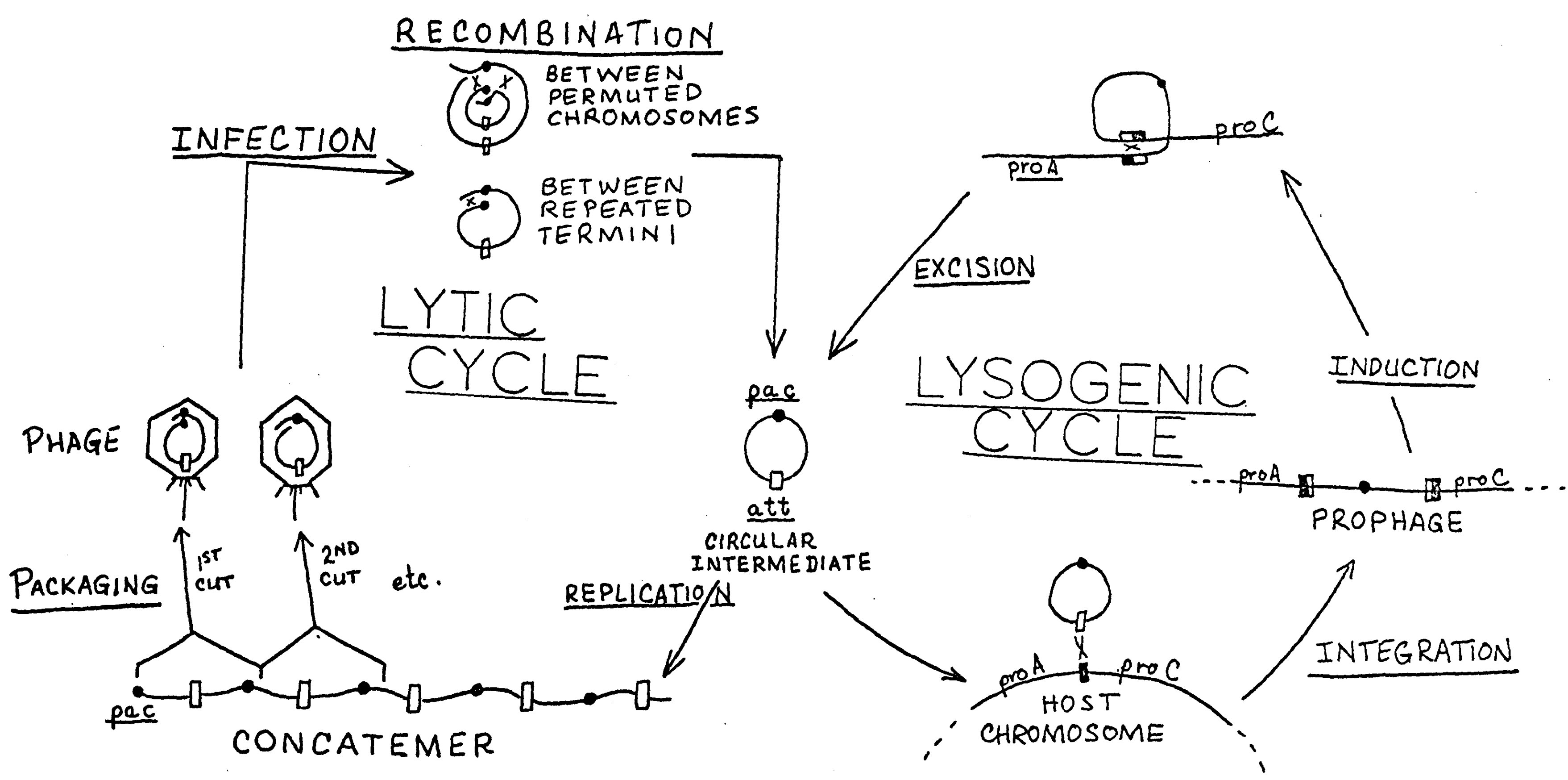


FIGURE 1.
LIFE CYCLE OF P22 DNA

and mnt genes. Upon induction, the prophage excises in a manner which reproduces the essential circular chromosome structure.

During lytic growth the circular DNA replicates to produce molecules which are larger than mature chromosomes (Botstein, 1968); these are called concatemers. Concatemers are thought to contain multiple P22 genomes tandemly repeated in the same orientation. These are converted to mature phage chromosomes by the headful packaging mechanism. Generalized transducing particles are thought to be produced when this mechanism packages host or plasmid DNA instead of phage DNA concatemers.

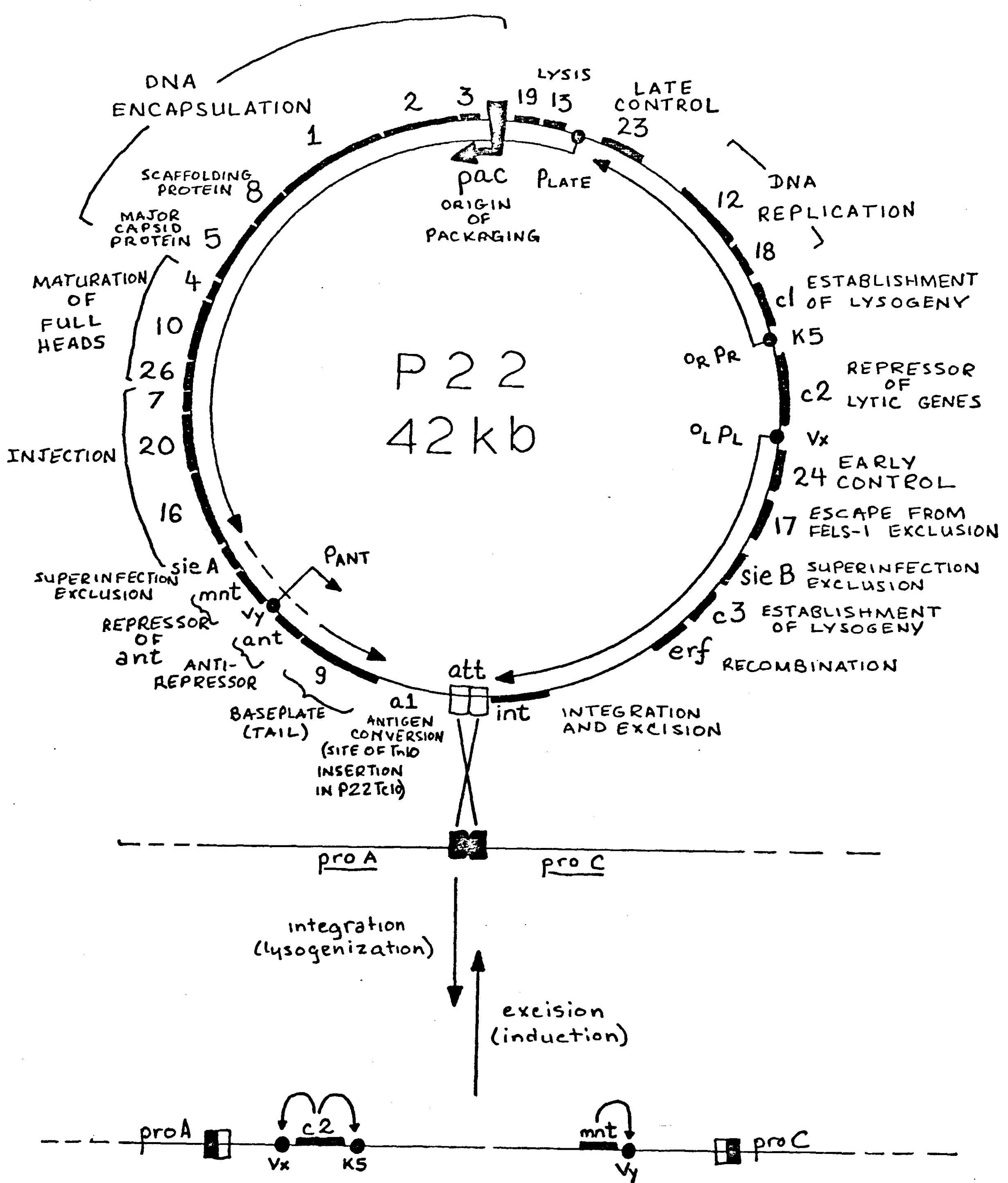
B. The Headful Packaging Mechanism

The terminally repetitious, circularly permuted structure of mature P22 DNA reflects the mechanism of encapsulation (Tye et al., 1974a,b, figure 1). The DNA is cut from long concatemers of P22 genomes. Each head packages a piece of DNA which is about two per cent longer than the P22 genome, thus producing terminally repetitious molecules. The first cut occurs at a unique site, pac, and packaging is sequential so the origin of each headful is displaced two percent from its predecessor. This generates a circularly permuted population of molecules. pac has been mappped genetically in the vicinity of genes 19 and 3 (Chan, 1974; figure 2). Physical mapping of P22 DNA (Jackson, 1977) as well as genetic experiments (Smith, 1968) demonstrate the direction of packaging to be from pac toward gene 2.

C. Lytic Genes

The genes of P22 are arranged into operons which are analogous in function to those of bacteriophage λ (Botstein and Herskowitz, 1974; Hilliker and Botstein, 1977; figure 2). P22 has two operons of early genes. One is transcribed from the p_L promoter and contains gene 24, a

FIGURE 2. THE P22 CHROMOSOME



positive regulator of early genes analogous to λ's N gene (Hilliker and Botstein, 1975). The other early operon is transcribed from the p_R promoter and includes genes 18 and 12, required for DNA replication, and gene 23, a positive regulator of late genes analogous to λ's Q gene. The genes 13 to 16 are thought to constitute an operon of late genes transcribed from a promoter, p_{LATE}, located between genes 23 and 13 (Roberts et al., 1976). Genes 13 and 19 are required for cell lysis while the other genes are involved in head morphogenesis (Botstein et al., 1973; Poteete and King, 1977). Genes 3, 2, 1, 8, and 5 are required to cut DNA concatemers to phage size, genes 8 and 5 being the major proteins of the prohead, the structural precursor to DNA-containing heads. Genes 4, 10, and 26 are not required for DNA encapsulation but in their absence the head is unstable and loses its DNA. Mutants in genes 7, 20, or 16 produce stable, DNA-containing particles which are not infections since these functions are necessary for injection of DNA into the cell. Gene 9, which codes for the baseplate (tail) protein, is also expressed at late times but is physically separated from the other late genes (See Ch 1. Section IIG). Gene 23 is required for expression of all late genes including gene 9 (Bostein et al., 1973; Lew and Casjens, 1975).

D. Transduction by P22

There are two ways P22 can introduce host genes into a cell. The genes may become packaged in a particle lacking any phage DNA (generalized transduction) or the genes may become inserted in the phage genome and packaged with phage DNA (specialized transduction). Generalized transducing particles are formed by the action of the headful packaging mechanism on a bacterial or plasmid chromosome. For bacterial genes to be transduced, they must recombine with the chromosome of the recipient.

When plasmid genes are packaged, they will be efficiently transduced only if the plasmid is smaller than a headful in size so a complete genome can be packaged. If the plasmid is larger than a headful, most transductants are abortive and the stable transductants that do appear contained shortened derivatives of the plasmid.

Specialized transducing particles produce transductants by inserting their DNA into the recipient's chromosome via the phage integration system. The transductant consists of a lysogen whose prophage contains an insertion. Thus, a requirement for specialized transduction is that the tranducing genome lysogenize.

Generalized and specialized transductants may be distinguished by the frequency with which they produce transducing particles. A specialized transductant produces particles, all of which contain the transduced gene, and thus, transmits it at a high frequency. In contrast, generalized transductants produce a low frequency of transducing particles.

E. P22Tc10: A P22 Derivative Containing the Tn10 Translocatable Element

Tn10 is a 9300 base pair translocatable element which is found in certain R factors and contains a tetracycline resistance gene ($\underline{tet}^R$). Dubnau and Stocker (1964) were the first to describe P22-mediated transduction of $\underline{tet}^R$. Watanabe et al., (1972) used P22 to transduce this $\underline{tet}^R$ gene and isolated a transductant which produced high frequency transducing (HFT) lysates. The phage in these lysates is a derivative of P22, called Tc10, which contains a Tn10 insertion in the al region (Chan and Botstein, 1976; figure 2).

The Tn10 insertion makes P22Tc10's genome larger than the length of DNA packaged in a phage head. Consequently, P22Tc10 particles contain incomplete, circularly permuted chromosomes which are defective because

they lack terminal redundancy (Tye et al., 1974a; figure 3). When P22Tc10 particles singly infect cells they neither grow nor lysogenize because their DNA is unable to circularize. However, an induced lysogen of P22Tc10 produces a normal burst of particles because prophage excision yields a circular molecule without requiring terminal repetition. Upon multiple infection or infection with helper phage, the permuted chromosomes can recombine with each other to reconstruct a complete circular genome which may either lysogenize to produce a $\underline{tet}^R$ transductant or grow lytically to produce more P22Tc10 particles (Chan et al., 1972). Similarly, if P22Tc10 particles singly infect cells containing a prophage deletion, many of them can recombine with the prophage to reconstruct a complete circular genome and grow normally. This property is used to titer P22Tc10 particles since P22Tc10 plates as a linear function of concentration on prophage deletion strains.

Because of the defectiveness of the particles, it is possible to select deletions in the P22Tc10 genome. When P22Tc10 is plated, the rare, plaque-forming derivatives which appear contain deletions (Chan, 1974). Some of these have lost the Tn10 element while others have deleted non-essential phage DNA and part of Tn10 (Tye et al., 1974a). These deletions shorten the genome so that it can fit completely in a phage head and produce terminally repetitious chromosomes.

A second class of deletions can be isolated when P22Tc10 is used to transduce $\underline{tet}^R$ at low multiplicities of infection. Transduction is rare because most chromosomes fail to circularize. Transductants that do occur have prophages containing a deletion (Chan et al., 1972) which presumably allowed circularization. These deletions have one endpoint at the Tn10 insertion and extend beyond the sieA gene to between genes 20

Figure 3. Life Cycle of Tc10 DNA

Infection by Tc10 particles will not be productive unless the incomplete chromosomes can circularize. This can be achieved by recombination with another genome, present either as a non-immune prophage deletion or as an infecting phage. Unlike wild type, however, circularization cannot occur by self-recombination. The circular, complete genome may lysogenize to produce a tet^R transductant or replicate to produce concatemers. Since the genome of Tc10 is longer than the molecules cut from concatemers, the chromosomes which are packaged form a population of circularly permuted, incomplete genomes.

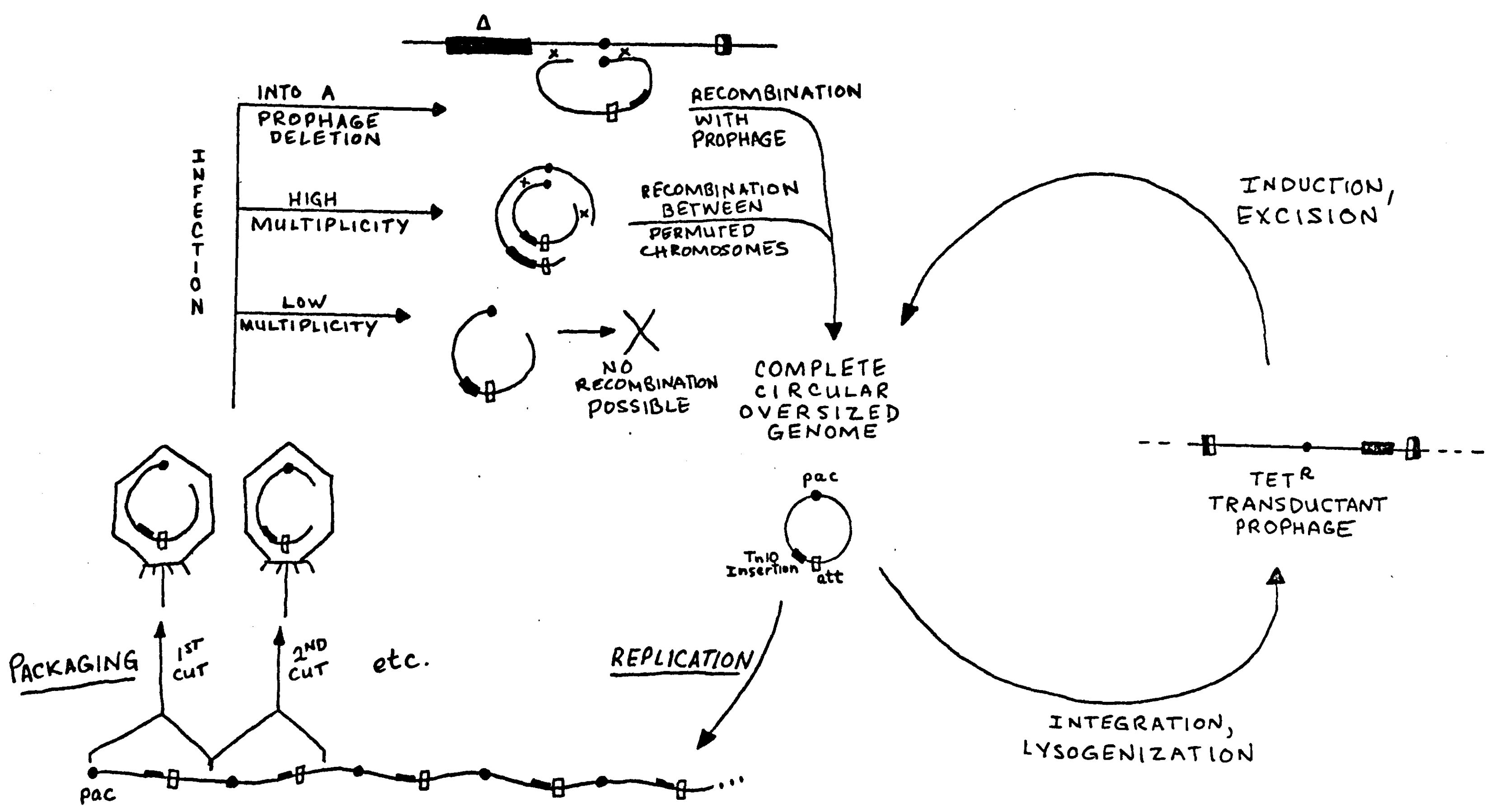


FIGURE 3. LIFE CYCLE OF P22Tc10 DNA

and c2 (Chan and Botstein, 1972). The deletions end at a number of sites in this region but there is a bias to the vicinity of genes 5 and 10 (Chan, 1974).

Thus, deletions can be selected in P22Tc10, in contrast to wild type P22 where a shorter genome offers no selective advantage. Although both of these selections require restoration of terminal repetition, the selection for plaque-formers requires intact essential genes while the selection for low multiplicity transduction demands the tet^R gene and genes for lysogeny to be expressed.

F. Lysogeny and the ant Gene

When P22 infects a cell, it may lysogenize by integrating at a unique site in the bacterial chromosome and turning off its lytic genes. The turn-off of lytic genes requires two repressors, the products of the c2 and mnt genes (Chan and Botstein, 1972; Levine and Smith, 1964; Gough, 1968).

The c2 repressor acts at the operators o_L and o_R to directly repress the expression of lytic genes from the promoters p_L and p_R respectively (Botstein et al., 1975; Levine et al., 1975). These sites of action are defined genetically by the mutations Vx (o_Lp_L) and K5 (o_Rp_R) (Bronson and Levine, 1971). The double mutant Vx K5, called virB3, is able to grow in lysogens (virulent) since the mutations make it insensitive to c2 repression.

P22 has a gene, ant, which codes for an antirepressor, a protein able to inactivate the c2 repressor (Botstein et al., 1975; Levine et al., 1975; Susskind and Botstein, 1975). In order for the c2 gene to effect repression, ant must be turned off. This is accomplished by the mnt gene, which produces a repressor of ant. The mnt repressor prevents expression

from a promoter, p_{ANT}, defined genetically by Vy mutations. Vy mutations allow constitutive expression of ant, independent of mnt. Vy phages are virulent because they can produce antirepressor in a lysogen and remove c2 repression.

The ant gene is dispensable in a non-lysogenic cell as wild type and ant^- phages grow and lysogenize equally well. ant is essential for growth in a lysogen whose prophage contains an intact c2 gene but is deleted for mnt. In this cell ant^+ phages are able to make antirepressor, inactivate c2 repressor, and grow, while ant^- phages are repressed by c2. Such a prophage deletion strain is used to distinguish between wild type and ant^- phages.

G. Gene 9 and the Expression of Late Genes

Gene 9 codes for a protein, p9, of molecular weight 76,000d, which is the major structural component of the phage baseplate or tail (Botstein et al., 1973). 9^- mutants produce normal numbers of phage heads which can be quantitatively converted to infectious phage by incubation *in vitro* with p9 (Israel et al., 1967). The stoichiometry of this reaction is approximately three tails per head, in accord with the observation that phage particles contain more than one molecule of p9 (Botstein et al., 1973). This stoichiometry makes the *in vitro* reaction an extremely sensitive assay for p9 since the number of infectious phage produced is approximately proportional to the cube of the p9 concentration.

Since the tailing reaction is very efficient, it is possible to plate 9^- phages with high efficiency on non-permissive strains by adding p9 to the plate. In this sense, 9 may be regarded as a non-essential gene.

The expression of gene 9 poses a problem not associated with other

late genes. As described earlier (Ch 1., section IIC), genes 13 through 16 are thought to constitute an operon whose promoter, p_{LATE}, lies between genes 23 and 13. Genes 1 and 5 are translated in a direction consistent with this view (Lew, 1974) and expression of this operon requires gene 23 (Botstein et al., 1973; Lew and Casjens, 1975). Similarly, gene 9 is translated in this direction (Lew, 1974) and requires gene 23 for expression (Botstein et al., 1973; Lew and Casjens, 1975). However, gene 9 is separated from the other lates by a number of genes, including sieA, mnt, and ant (figure 2). These genes are regulated differently than 9 since none require 23, sieA and mnt are expressed in lysogens (Susskind et al., 1971; see section 1. IF), and ant is made early in infection (M. Susskind, personal communication). Thus, it is unclear whether 9 belongs to the late operon or a different transcriptional unit.

An observation relevant to this issue is that mnt can affect 9 expression. Under some conditions of prophage induction, the c2 repressor is inactivated while the mnt repressor is intact (Lew, 1974). The particles produced by this procedure are tail deficient (Israel, 1967), implying underproduction of p9. However, if mnt is also inactivated, tail production appears to be normal (Lew, 1974), showing that mnt affects 9 expression. Assuming that the only site at which mnt repressor acts to block transcription is Vy, this result implies that 9 belongs to a transcriptional unit which includes Vy. Thus, 9 could be expressed from p_{LATE} or p_{ANT}, but it is unlikely that a major promoter for 9 exists between ant and 9

H. Use of P22 to Study Translocatable Elements

P22 is a useful organism to use for the study of translocatable drug resistance elements. Insertions into P22 are recognizable as

specialized transducing phages. Since the packaging mechanism can tolerate large insertions, transducing phage DNA can be purified from particles and studied by physical methods. The defectiveness of the chromosomes of these phages allows deletions to be selected, useful for genetic mapping of insertions. Insertions in the non-essential genes, ant and 9, can be used to study the processes of integration and excision. Lastly, the quantitative assay for p9 allows polar effects of insertions to be assessed.

CHAPTER II: CHARACTERIZATION OF P22 GENOMES CONTAINING Tn1 INSERTIONS

I. Materials and Methods

A. Bacteria

The bacterial strains used are listed in table 1. Salmonella typhimurium strains are derivatives of LT2. DB21, DB7000, and DB7136 are the standard Su^- strains and DB7004 is the standard Su^+ strain used in this work. The source of Tn1 was RP4 (Datta et al., 1971), a 34×10^6d (Meyers et al., 1976) self-transmissible R factor conferring resistance to tetracycline, ampicillin, kanamycin, and neomycin. RP4 was transferred from its original host, the E. coli strain DB6292, to DB7011, or a lysogenic derivative of DB7011, to produce DB7189, DB7222, and DB7226.

All lysogens used in superinfection experiments were $sieA^-$ (by point mutation or deletion) to prevent exclusion (Susskind et al., 1971). DB5057 and DB7283, which contain $c2^+mnt^-$ prophage deletions, were used to test for ant (Ch. 1, section II-F). DB147 contains a short ($c2^-mnt^+$) prophage deletion and was used to titer P22Ap phages (see Ch.1, section II-E). DB47 is $recA^-$ and was used to test for erf. DB5000 was the source of P22Tc10 phage. DB7273 is the strain from which the prophage deletions described in Chapter 2, Section II-J were isolated. DB5411, a lac^+ F^- E. coli strain, was used as a recipient in tests for mating transfer of amp^R from Salmonella (Ch.2, section I-D). The other prophage deletions were used for mapping.

B. Phage

The following derivatives of P22 were used: P22 sieA-44: The parent of most P22Ap phages. The sieA-44 mutation eliminates the A supeinfection exclusion system of prophages, allowing superinfecting phage DNA to enter lysogenic cells (Susskind et al., 1971; 1974). The presence of the sieA-44

Table 1. Bacterial Strains

Strain	Genotype	Source/Reference
Salmonella typhimurium:		
DB21	*su*$^-$ prototroph	Botstein and Matz (1970)
DB47	*su*$^-$ *recA* prototroph	Botstein and Matz (1970)
DB53	*su*$^-$ *his C-am*527 *cysA-am*1348	Botstein and Matz (1970)
DB5000	DB21 (P22Tc10)	Watanabe et al. (1972)
DB7000	*su*$^-$ *leuA-am*414	Susskind et al. (1974)
DB7004	*su2* *leuA-am*414	Susskind et al. (1974)
DB7011	*su*$^-$ *leuA-am*414 r^-m^+	D. Botstein
DB7136	*su*$^-$ *leuA-am*414 *hisC-am*527	D. Botstein
DB7189	DB7011 (P22)/RP4	this work
DB7222	DB7011/RP4	this work
DB7226	DB7011 (P22 *sieA*-44)/RP4	this work
DB7273	DB7000 (P22Ap2 *sieA*-44 *mnt-ts*1)	this work
Prophage Deletions:		
DB136	DB53 (P22)Δ see table 6	Chan and Botstein (1972)
DB147	DB53 (P22)Δ [*proA-c*1]	Chan and Botstein (1972)
DB5057	DB21 (P22Tc10 Δ) see table 6	Chan and Botstein (1972)
DB7177	DB7000 (P22Tc221 Δ) see table 6	D. Botstein
DB7241	DB7000 (P22Ap4 Δ) see figures 5 and 8	this work
DB7282	DB7000 (P22Ap2 *sieA*-44 *mnt-ts*1 Δ) see fig. 5 and 10, plate 1	this work
DB7283	DB7000 (P22Ap2 *sieA*-44 *mnt-ts*1 Δ) see fig. 5 and 10, plate 1	this work
DB7293	DB7000 (P22Ap9 Δ) see figures 5 and 8	this work
DB7449	DB7000 (P22Ap18 Δ) see figures 5 and 8	this work

Table 1. Continued

DB7451	DB7000 (P22Ap32 Δ) see figures 5 and 8	this work
DB7452	DB7000 (P22Ap37 Δ) see figures 5 and 8	this work
DB7453	DB7000 (P22Ap48 Δ) see figures 5 and 8	this work
DB7455	DB7000 (P22Ap49 Δ) see figures 5 and 8	this work
DB7457	DB7000 (P22Ap73 Δ) see figures 5 and 8	this work
DB7461	DB7000 (P22Ap2 *sieA*-44 *mnt*-*ts*1 Δ) see fig. 5 and 10, plate 1	this work
DB7464	DB7000 (P22Ap2 *sieA*-44 *mnt*-*ts*1 Δ) see fig. 5 and 10, plate 1	this work
DB7472	DB7000 (P22Ap63 Δ) see figure 5	this work
DB7473	DB7000 (PFR-1) see figure 5	this work
DB7477	DB7000 (PFR-6) see figure 5	this work
DB7480	DB7000 (TDR-3) see figure 5, plate 1	this work
Escherichia coli:		
DB5411	YMEL *suIII* *T7*^{R}	E. Signer
DB6292	J53 *F*$^{-}$ *pro*$^{-}$ *met*$^{-}$ (λ)/RP4	Hedges and Jacob (1974), E. Signer

mutation in P22Ap phages was necessary in order to map lysogens and prophage deletions by superinfection with amber mutants. P22Ap2,4,5,7, and 9 were isolated from wild type P22 and sieA-44 was introduced by crossing (Ch. 2, section I-H).

P22 virB-3: a double mutant (Vx and K5) which is insensitive to c2 repression (Bronson and Levine, 1971). Phages containing virB-3 and two amber mutations (virB am am phages) were constructed by sequential crosses between virB-3 and P22 amber mutants and identified by spot complementation (Ch. 2, section I-D) against the parents. These phages were used to map Tn1 insertions (Ch. 2, section II-C).

P22Ap2 sieA-44 mnt-ts1: Constructed from P22Ap2 sieA-44 and P22 mnt-ts1 (Ch. 2, section I-H). The mnt-ts1 mutation makes the mnt gene thermolabile (Gough, 1968). Lysogens of this phage were used to select prophage deletions as survivors of mnt induction (Ch. 2, section II-J).

P22Tc10: A specialized transducing phage for tetracycline resistance containing a Tn10 insertion (Chan, 1974). DNA from this phage was used as a standard in heteroduplex analysis of P22Ap phages because of the lariat structure of Tn10 in single strands (Tye et al., 1974a).

The amber mutations used in this work are described in Botstein et al., (1972) and Poteete and King (1977), the ant^- mutations in Botstein et al. (1975), and the frameshift mutation in Uomini and Roth (1974).

C. Media and Solutions

Phages were diluted in buffered saline (BS) or dilution fluid (DF) and plated in soft agar on λ plates. Plating cultures were grown in LB broth. These solutions are described in Ebel-Tsipis and Botstein (1971).

M9CAA (Smith and Levine, 1964) is a phosphate buffered mineral medium (M9) containing glucose and a charcoal-clarified amino acid mixture. M9CAA

was used for crosses and complementation tests and for procedures involving UV-irradiation of lysogens. Lysogens can be induced with relatively low doses of UV light in M9CAA since UV-absorbing material has been removed from the medium by adsorption to charcoal.

Green indicator plates (Chan et al., 1972) were used for single colony isolation of bacteria as well as for streak tests for immunity, exclusion, and other prophage markers (See below). Green/amp plates, containing 20 μg/ml of ampicillin trihydrate (generously donated by Bristol Laboratories, Syracuse, New York), were used to select ampicillin resistant cells. Green/amp/EGTA plates contain, in addition, 10 mM [Ethylenebis(oxyethylenenitrilo)]tetraacetic acid (Eastman). EGTA interferes with P22 infection, and this medium was used when amp^R survivors of mnt induction were selected (Ch. 2, section II-J) in order to prevent phage on the plate from killing non-immune cells. Tetracycline was added to green plates to 25 μg/ml and kanamycin to 50 μg/ml to test cells for resistance to these drugs.

M9 plates contain M9 mineral medium, $MgSO_4$ (1mM), a carbon source (0.2% w/v), and 1.5% agar.

Super broth is described in Susskind and Botstein (1975) and allows exponential growth of bacteria to about a ten-fold higher concentration of cells than does M9CAA or LB broth.

D. General Methods

Concentrated phage stocks were prepared by infection (Botstein and Matz, 1970) or, for phages containing an insertion, by UV-induction of lysogens (Chan and Botstein, 1972). Lysates made by induction are tail deficient (Israel, 1967) and were treated with at least 1×10^{10} phage equivalents/ml of p9 at 37° for 1 hour prior to concentration. Phages were purified in either discontinuous CsCl gradients (Botstein, 1967) or equili-

brium CsCl gradients consisting of 9 parts phage stock layered on 11 parts 65% CsCl (in 10 mM Tris buffer, pH = 8) and centrifuged at least 12 hours at 22,000 rpm, 20°C in an SW50.1 rotor.

UV irradiation to destroy immunity of lysogens, either in liquid culture or on plates, was performed at a dose of 200 $ergs/mm^2$.

Phage crosses and complementation tests were performed in M9CAA at 25°C by the method of Botstein et al. (1972).

Spot tests, for mapping insertions with P22 virB am am phages or prophage deletions with P22am phages, and spot complementation tests, used to identify P22 virB am am phages, were performed as described in Botstein et al. (1972).

Streak tests were performed on green indicator plates according to Susskind et al., (1971). When immunity of $sieA^-$ lysogens was tested, the tester phages were P22 virB-3, P22 c2-am08, and P22 c1-7 ant-am16. Immune ($c2^+mnt^+$) lysogens only permit growth of virB-3 phage, $c2^+mnt^-$ non-immune lysogens allow growth of both virB-3 and c2-am08 phage, and $c2^-$ non-immune lysogens allow all three testers to grow. Lysogens of $sieA^+$ phage do not allow any of the testers to grow. Lysogens were purified from the centers of phage spots on plates.

Int and erf function was tested by the methods of Chan (1974).

To plate 9^- phages on hosts which were non-permissive for the 9^- mutation, 10^{11} phage equivalents of p9 were added to the top agar. The 9^- phage plaques observed under these conditions were slightly smaller and clearer than those of wild type P22, but the efficiency of plating was close to 100 per cent.

Mating of RP4 was accomplished by making intersecting lines of donor and recipient cells on a λ plate and incubating this overnight at 37°C.

Cells from the point of intersection were streaked directly on selective medium. To test whether any of the amp^R transductants described in Ch. 2, section II-A could transfer resistance by mating, the recipient used was DB5411, a lac^+ E. coli strain. This recipient was chosen because it cannot be transduced to amp^R by P22 and because its Lac^+ phenotype allows selection against the Lac^- Salmonella donors. Mating was performed as described above and exconjugants were selected on M9 lactose plates containing ampicillin (20 μg/ml).

E. Isolation of Insertions

Transducing lysates were prepared by UV induction of DB7189 or DB7226 or single cycle infection of DB7222. For induced lysates made with helper, P22 sieA-44 was added to a multiplicity of 10 immediately after irradi ation. These lysates were used to infect DB7000 or DB7136 at high multiplicity (>5). About 10^9 infected cells were spread on a green/amp plate and incubated at 30°C. Under these conditions, most cells become lysogens.

The amp^R transductants were screened for production of HFT lysates by the method of Kaye et al. (1974). Unpurified transductants were transferred to a λ plate and a master green/amp plate with a sterile wooden dowel and incubated until patches of cells were visible. Then the λ plate was UV irradiated and incubated at 30°C for 5 hours. Cells were lysed by inverting the plate over a planchet containing $CHCl_3$ for 20 minutes. DB7000 and p9 were spread on a green/amp plate and the lysed cells were immediately replicated onto this lawn. After overnight incubation, an HFT lysate produces a heavy patch of amp^R transductants while generalized transduction usually produces no amp^R cells.

Several precautions are necessary in this protocol: 1) transduction for amp^R is most reliably accomplished on green/amp plates; LB/amp plates

allow many amp^R cells to survive; 2) it is important that transducing lysates be made at 30°C or lower temperatures; lysates made at 37°C mainly yield transductants which do not produce HFT lysates (table 3); 3) it is important to perform the HFT screening on unpurified transductants since contaminating wild type phage help defective phages to transduce.

F. Phenotypes of P22Ap Phages

Each colony which produced an HFT lysate was purified and picked onto four λ plates with a sterile wooden dowel. The plates were incubated until patches of cells were visible. The plates were then UV irradiated and each patch overlayed with a drop of either DB7000, DB147 + DB53, DB147 + DB53 plus 4 x 10^{10} eq/ml of p9, or DB5057 plus p9. After overnight incubation the spots were scored as ++, +, or - depending on the degree of clearing. The phenotypes observed are shown in table 2 and described in section II-B of this Chapter.

G. Mapping Insertions with P22 virB am am Phages

A set of phages containing virB-3 and two linked amber mutations (virB am am phages) were used to map insertions in P22Ap phages (Ch. 2, section II-C). The amber alleles 12-amN11, 23-amH316, 13-amH715, 19-amN111, 3-amN6, 2-amH200, 1-amN10, 8-amH202, 5-amN114, 10-amN107, 26-amH204, 20-amN20, 16-amN121, and 9-amN9 were used to construct the following double amber mutant virB-3 phages: 12^-23^-, 23^-13^-, 13^-19^-, 19^-3^-, 3^-2^-, 2^-1^-, 1^-8^-, 8^-5^-, 5^-10^-, 26^-20^-, 20^-16^-, and 16^-9^-. To map Tn1 insertions, these phages were spotted on lysogens of $sieA^-$P22Ap phages. Failure of a virB am am phage to grow indicated the Tn1 insertion in the prophage affected the region of the genome spanned by the two amber mutations.

Table 2. Scheme for Classification of P22Ap Phage Phenotypes

Lysogens of P22Ap phages were induced on plates, then overlayed with various indicators as described in Methods, section F.

++ means a large spot of clearing occurred

+ means a small spot of clearing occurred

- means 0 to a few single plaques were seen.

Phenotype	DB7000	DB147	DB147 +p9	DB5057 +p9
wild type	++	++	++	++
mutation in non-essential gene	+	++	++	++
mutation in essential gene	-	-	-	-
ant^-	+	++	++	-
9^-	-	-	++	++
ant^- and 9^-	-	-	++	-

H. Manipulations with P22Ap Phages

(i) Growth and Titering

Stocks of P22Ap phages were prepared by UV-induction of lysogens and titered on a mixture of DB147 and DB53 by the method of Chan et al. (1972). The ratio of titer on DB147 to particle titer, estimated from absorbance at 260nm ($A_{260} = 1 \equiv 5 \times 10^{11}$ particles/ml) for P22Ap2, Ap4, Ap5, Ap7, and Ap9 (purified in discontinuous CsCl gradients) was 1/4, 1/8, 1/7, 1/7, 1/9, and 1/5 respectively. Thus, to approximate the number of particles, observed titers on DB147 were multiplied by 7.

(ii) Introduction of Point Mutations by Crossing

In general, to cross point mutations into P22Ap phages, DB7000 was transduced to amp^R with a low multiplicity (<.01) of P22Ap phage in the presence of a high multiplicity (>10) of helper phage carrying the desired mutation. By this method the *sieA*-44 mutation was crossed into P22Ap2, Ap4, Ap5, Ap7, and Ap9 (Ch. 2, section I-B) and the *mnt*-*ts*1 mutation into P22Ap2 *sieA*-44 (Ch. 2, section II-J). Lysogens of P22Ap *sieA*-44 recombinants were identified by their sensitivity to P22 *virB*-3 in a streak test (Ch. 2, section I-D). Lysogens of the P22Ap2 *sieA*-44 *mnt*-*ts*1 recombinant were identified by their sensitivity to P22 *virB*-3 and their temperature-sensitive phenotype.

To construct lysogens of P22Ap4, Ap9, Ap12, Ap27, Ap31, Ap34, Ap46, and Ap48 containing a mutation in gene *5*, used in Ch. 2, section II-G to measure tail production by P22Ap phages, a modification of this method was used since P22Ap12, Ap27, Ap34, and Ap48 contain insertions in essential genes and will not make infectious particles. Lysogens of these P22Ap *sieA*-44 phages were induced by UV irradiation, then superinfected with P22 5^- *am*N114 and grown at 30°C until lysis. The phage in

these lysates, produced by complementation between the 5^- and P22Ap phages, were used to transduce DB7000 to amp^R. The transductants were streak tested for the $sieA^-$ phenotype, using P22 virB-3, and for the 5^-amN114 allele using P22 virB-3 5^-amN114.

(iii) Crosses between Different P22Ap Phages

Crosses between different ant^- P22Ap phages, performed to determine if the Tn1 insertions were separable by recombination (Ch. 2, section II-F), were done by the standard method used for phages with normal-sized genomes (Botstein et al., 1972). The titer of infectious particles of each parental phage was estimated from its titer on DB147 (see above). The progeny of the cross were plated on DB147 to titer total particles and DB5057 to titer ant^+ recombinants.

I. Complementation of Insertions in Essential Genes

P22Ap phages containing insertions in essential genes do not produce infectious particles and are maintained as prophages. To perform complementation tests with these phages (Ch. 2, section II-D), lysogens of the P22Ap phage were grown in M9CAA to early exponential phase and UV-induced. Immediately after irradiation, P22 amber phages were absorbed to the lysogen for 10 minutes at a multiplicity of 0.1. The cells were diluted 10^{-4} into M9CAA and incubated 2 hours at 37°C. After lysis by $CHCl_3$ and treatment with p9, the lysates were plated on DB7004 to titer total phage and DB7000 to titer wild type recombinants. The P22 amber mutant phage used were c1-7 4-amH1368, c1-7 10-amN107, c1-7 26-amH204, c1-7 7-amH1375, c1-7 20-amN20, c1-7 16-amN121, and c1-7 9-amN110. When the 9^- amber phage was used, the infections were done at 30°C for 3 hours (to minimize tail deficiency caused by mnt) and treatment in vitro with p9 was omitted.

It was observed that with wild type (c1-7) phage, the phage yield

by this method is low, between 1 and 10 (see table 5), with lysogens of phages containing Tn1 in the late genes. This effect is independent of multiplicity of infection (data not shown). However, with lysogens of P22Ap2 sieA-44 (insertion in the a1 region), yields of 100 to 200 were observed (data not shown) implying that the cause of the low yields is neither the conditions of infection nor the Tn1 insertion but is some other aspect of the prophage's genotype.

J. Preparation of Heads and Tails

Tail deficient particles (heads) were prepared from infection of DB7000 with P22 9-amN110 at a multiplicity of at least 5. Heads were purified from lysates in the same manner as phage.

High titer preparations of p9 were made by infection of DB7000 with P22 c2-am08 13-amH101 8-amH202 5-amN114 mnt-1 in super broth for 3 hours at 37°C. Cells were then concentrated 40-fold and lysed with $CHCl_3$. After removing debris by centrifugation (10 minutes; 10,000 rpm; SS-34 Sorvall rotor), any phage present were pelleted (70 minutes; 16,000 rpm; SS-34 Sorvall rotor) and the supernatant used as crude p9. The defects in the quintuply mutant phage cause overproduction of p9 and minimize background phage.

Highly purified heads and tails were generously supplied by Mr. Anthony Poteete.

Head and tail activities were determined according to Israel et al. (1967).

K. Quantitation of p9 Production by P22Ap Phages

A rapid, indirect way to assess tail production is to determine the tail deficiency of particles in a lysate. This was done by titering the lysates before and after tailing. The amount of p9 produced was calculated

from the tailing equation (Israel et al., 1967) log P = 3.3 log T-2.3 log H using P = titer before tailing and H = titer after tailing. This value for T was normalized to the number of heads produced (H) to allow comparis ons between lysates. This method is indirect because it assumes that all tails produced in vivo become bound to heads.

Tails were directly assayed in lysates by using them to convert heads to phage in vitro. Since any heads produced by the tail donor will interfere with the assay, the phage to be tested carried the 5-amN114 mutation which eliminates the major capsid protein. Lysates were made by UV-induction of lysogens at 30°C. Each tailing reaction consisted of a mixture of 0.5 ml of a known amount of heads (usually about 2×10^{8}) and 0.5 ml of a dilution of the lysate, both in M9 buffer, which was incubated at room temperature until tailing was completed as indicated by a constant titer. The tail concentration in each reaction was calculated from the tailing equation using the known value of H and the measured value of P. At least four serial dilutions of each lysate were assayed and a concentration curve (tailing activity vs added lysate) was constructed and used to derive the tailing activity of the lysate. This activity was normalized to the viable count of the original culture.

The tailing equation was empirically derived. During the course of this work, it was observed that purified tails behaved according to this equation but crude lysates followed the empirical curve logP=2.6logT-1.6 logH. The difference between these equations was observed to be small under the conditions used.

L. Deletions

Deletions were selected in P22Ap phage genomes by requiring that terminal repetition be restored. Plaque forming revertants were isolated

by plating P22Ap phages for single plaques on DB7000, either in the presence or absence of p9. Some of these plaques are formed by multiple infection, but these do not produce plaques when they are streaked out. This selection requires essential genes to be functional.

Deletions selected as restoring terminal repetition without requiring function from essential genes were obtained as amp^R transductants from low multiplicity infections as described in Chan and Botstein (1972) and Chan et al. (1972).

Deletions obtained without requiring restoration of terminal repetition were selected as survivors of heat induction of DB7273, a lysogen of P22Ap2 sieA-44 mnt-ts1. From 10^6 to 10^7 cells were spread on green/amp/EGTA plates and incubated at 42° overnight. Colonies which appeared were purified once on green/amp/EGTA plates, then streak-tested for gene 9 against P22 virB-3 9-amN9. Only cells lacking the 9 allele were further characterized.

Mapping of non-immune prophage deletions by spot tests or efficiency of plating was as described in Chan and Botstein (1972). For fine structure mapping of gene 12, the criterion for rescue was at least a three-fold increase in efficiency of plating over that on the non-lysogenic strain, DB7000. Mapping of immune prophage deletions was performed with virulent phages. To map immune deletions in ant, the prophage was induced with UV, the tester phage adsorbed for 10 minutes, and then the infected cells plated with DB7283 to assay selectively for any ant^+ recombinants which might have been formed.

M. Physical Analysis of DNA

(i) Heteroduplex Analysis

Electron microscopy of heteroduplexes was performed by a modification

of the method of Tye et al. (1974a). Purified phages in CsCl were incubated in 0.1M NaOH, 10 mM Tris (pH 8.5 at 1M), 5 mM EDTA for 1 hour at 37°, then neutralized, renatured in 50% formamide, and processed further according to Tye et al. (1974a). Lengths are expressed as (mean)±(standard error) where mean = $\bar{x} = (\Sigma x)/n$ and the standard error is $(\Sigma(x-\bar{x})^2)^{1/2}/(n-1)$ when $\bar{x}$ represents one set of measurements or $(\Sigma(x-\bar{x})^2/(m-1))^{1/2}$ when $\bar{x}$ is taken from m sets of measurements. For heteroduplexes involving Tc10 DNA, the double stranded standard was the Tn10 stem (1390 base pairs) and the single stranded standard was the Tn10 loop (6560 bases)(D. Ross, personal communication). These values for the stem and loop were obtained by comparison with double and single stranded ϕx174 DNA. For the Ap12/Ap30 heteroduplex, used to map Ap30, the double-stranded interval between the Tn1 insertions was calculated with reference to the single-stranded Tn1 loops. The conversion factor between single and double strand lengths was derived from the loop/stem ratio of 60 Tn10 insertions. This ratio was found to be 4.37 ± 0.09 and the conversion used was double strand lengths = 1.08 x single strand lengths. The mol-molecular weight of P22 DNA was taken as 27.45×10^6d (Jackson, 1977) or 42.2 kb.

(ii) <u>Analysis with Restriction Enzymes</u>

DNA was prepared from purified phages by either phenol extraction (Botstein, 1968) or SDS-high salt extraction: sodium dodecyl sulfate (SDS) was added to 0.5 per cent to phages in 10 mM Tris (pH = 8), 5 mM EDTA and the mixture was heated at 65°C for 15 minutes; KCl was then added to 0.25 M and the mixture chilled at 0°C for 15 minutes; the precipitate was removed by centrifugation (10,000 rpm, 20 minutes, in an SS-34 Sorvall rotor) and DNA in the supernatant concentrated by precipi-

tation with at least two volumes of 95 per cent ethanol and resuspension in 10 mM Tris (pH = 8), 5 mM EDTA.

DNA was digested with restriction enzymes according to the manufacturer's (New England Biolabs, Beverly, Mass.) specifications. The fragments were analyzed by agarose gel electrophoresis in E buffer (40 mM Tris, 5 mM sodium acetate, pH = 7.8; 1 mM EDTA). Gels were stained in 0.5 μg/ml ethidium bromide for 15 minutes, then photographed with a Polaroid MP-4 camera under illumination by an ultraviolet light source (mineralight, Ultraviolet Products San Gabrial, Calif.) using a red filter.

To determine the molecular weights of restriction fragments, plots of log (molecular weight) versus distance migrated were constructed using the EcoRI fragments of P22 DNA as markers. The sizes of these fragments, determined by Jackson (1977), are A = 19.9 kb, B = 9.4 kb, C = 7.3 kb, D = 4.1 kb, E = 2.4 kb, F = 1.2 kb, G = 1.1 kb, and H = 0.9 kb.

II. RESULTS

A. Transduction of amp^R from RP4

P22 is able to transduce drug resistances from strains harboring R factors by either generalized or specialized transduction. To isolate insertions of Tn1 into P22 DNA, advantage was taken of the fact that the source of Tn1, the R factor RP4, is larger (52 kb; Meyers et al., 1976) than the P22 headful (43 kb; Tye et al., 1974a). Thus, P22-mediated generalized transduction of amp^R from RP4 is rare and transductants containing a specialized transducing phage can be identified by their ability to produce high frequency transducing (HFT) lysates.

Table 3 shows the results of transduction of amp^R from RP4 by 25 independent P22 lysates prepared by a variety of methods. Transduction was observed by either infection or induction at low frequency. When lysates were made by induction, about half the transductants produced HFT lysates. Lysates made by infection transduced amp^R at higher frequency but a lower proportion of the transductants produced HFT lysates. In general, transductants which produced HFT lysates (i.e. putative insertions of Tn1 into P22) were recovered at about one per 10^{10} phages, regardless of the method of preparation.

Transductants which failed to give HFT lysates were of three types, each containing a wild type prophage. One type had the same resistances as RP4 (amp^R tet^R kan^R) while a second was sensitive to only kanamycin (amp^R tet^R). Both of these types could transfer their resistance together to DB5411 showing the resistances are genetically linked. Since P22 is unable to adsorb to _E. coli_, this transfer cannot be due to transduction but is probably by conjugation. Further evidence for linkage of markers is the observation that amp^R tet^R cells occasionally lose both resistances

Table 3. Ampicillin Resistant Transductants

Lysate	Comment	Frequency of Transduction Amp^R cells/pfu $X10^{10}$	Phenotypes of Transductants: Non-Producers of HFT lysates Amp^R	Amp^R Tet^R	Amp^R Tet^R Kan^R	HFT-lysate Producers
Lysates made by UV induction at 30°C:						
1	DB7189	2.5	0/7	2/7	0/7	5/7
92	DB7189	2.0	2/10	6/10*		2/10
93	DB7226	1.5	3/7	3/7*		1/7
94	DB7226	2.2	5/28	7/28	2/28	14/28
95	DB7226	1.0	5/10	0/10	0/10	5/10
96	DB7226	1.2	1/14	1/14	2/14	10/14
97	DB7226	1.5	5/20	1/20	0/20	14/20
98	DB7226 + helper	1.5	14/70	6/70	18/70	32/70
99	DB7226 + helper	0.9	8/35	3/48	5/48	28/48
100	DB7226 + helper	1.4	12/48	3/48	5/48	28/48
101	DB7226 + helper	1.3	12/44	5/44	5/44	22/44
Lysates made by infection of DB7222 with P22 sieA-44:						
79	37° infection	45	83/99	16/99*		0/99
80	37° infection	40	79/96	17/96*		0/96
81	37° infection	37	70/82	12/82*		0/82
82	37° infection	33	66/81	15/81*		0/81
107	37° infection	>1000	~100%	~13/3000*		0/3000
108	37° infection	>1000	~100%	~7/3000*		0/3000
109	37° infection	>1000	~100%	~14/3000*		0/3000

Table 3. Continued

110	30° infection	>700	~100%	~6/3000*	8/3000
111	30° infection	380	~100%	~3/1500*	0/1500
112	30° infection	440	~100%	~2/2000*	0/2000
87	27° infection	18	36/52	5/52*	11/52
113	20° infection	50	~100%	0/1800*	6/1800
114	20° infection	63	~100%	1/1800*	4/1800
115	20° infection	55	~100%	3/1800*	5/1800

* Kan^R not tested

Transduction of amp^R and identification of HFT producers was as described in Methods (section E). Amp = ampicillin, Tet = tetracycline, Kan = kanamycin.

simultaneously. Because the resistances are linked and transmissible, these transductants probably contain shortened derivations of RP4 and were formed by generalized transduction. Shipley and Olsen (1975) obtained a short derivative of a related plasmid, RP1, using a similar procedure.

The third type of transductants which failed to produce HFT lysates was resistant only to ampicillin and could not transfer the amp^R to E. coli. The frequency of these transductants was higher when lysates were prepared by infection and it is possible that ultraviolet radiation interferes with its formation. These transductants could contain shortened R factors or Tn1 insertions in the bacterial chromosome.

B. Phenotypes of P22Ap Phages

Transductants which produce HFT lysates are presumed to be lysogens of specialized transducing phages (P22Ap phages) consisting of Tn1 inserted into P22 DNA. These lysogens should produce particles which are defective because the insertion inactivates a gene and/or their DNA lacks terminal repetition. This prediction was tested by analyzing the growth properties of particles produced by HFT lysate-yielding amp^R transductants (table 4).

Using different indicators, the Tn1 insertions were classified according to the following rationale (Ch. 2, section I-F):

1) Insertion in a non-essential region of the genome: The particles lack terminal repetition and their growth in a non-lysogen (DB7000) requires multiple infection and biparental recombination during each cycle. Growth is therefore less efficient than wild type. However, in a non-immune prophage deletion (DB147 or DB5057) recombination with the prophage usually permits growth. Thus, these particles grow poorly in a non-lysogen but grow quite well in a prophage deletion strain.

2) Insertion inactivating essential gene: The lysogens do not produce

Table 4. Phenotypes of P22Ap Phages

lysate	Comment	Phenotypes of Prophages						
		NE	E	A	T	AT	WT	Total
1	UV induction, 30°	1	0	0	2	2	0	5
92	UV induction, 30°	2	0	0	0	0	0	2
93	UV induction, 30°	1	0	0	0	0	0	1
94	UV induction, 30°	2	2	2	4	3	1	14
95	UV induction, 30°	3	0	0	1	0	1	5
96	UV induction, 30°	4	2	0	3	1	0	10
97	UV induction, 30°	7	0	2	2	0	3	14
98	induction + helper	19	5	1	4	1	2	32
99	induction + helper	7	0	1	9	3	0	20
100	induction + helper	17	2	1	5	2	1	28
101	induction + helper	8	4	3	6	1	0	22
110	infection, 30°	2	2	1	3	0	0	8
87	infection, 27°	8	1	0	2	0	0	11
113	infection, 20°	2	1	0	2	1	0	6
114	infection, 20°	3	0	0	1	0	0	4
115	infection, 20°	2	1	0	2	0	0	5
Totals:	Induction	39%	8%	8%	24%	12%	10%	51 transductants
	Induction + helper	50%	11%	6%	24%	7%	3%	102 transductants
	Infection	50%	15%	3%	29%	3%	-	34 transductants
	All methods	47%	11%	6%	25%	7%	4%	187 transductants

Phenotypes of purified transductants were determined as in Methods (section F).

NE = insertion in non-essential gene

E = insertion in essential gene

A = ant$^-$ phenotype

T = 9$^-$ phenotype

AT = ant$^-$ and 9$^-$ phenotype

WT = wild type

infectious particles and thus there is no growth on any indicator.

3) Insertions inactivating gene 9: The particles grow on any indicator as long as tails are added in vitro.

4) Insertions inactivating ant: The particles do not grow in DB5057, a prophage deletion strain lacking mnt but retaining c2.

The results of this analysis are shown in table 4. Virtually all (96%) of the amp^R transductants which produced HFT lysates contained a mutant prophage. Those few (4%) transductants with a wild type prophage could be double lysogens containing a wild type and a P22Ap prophage. Few (11%) of the mutations affect essential genes. Most mutations affect ant (13%), 9 (32%), or other non-essential regions (47%). This distribution is essentially the same with lysates made by induction (with or without helper) or infection. Some mutations affect ant and 9 concomitantly. As shown later, these are insertions in ant which are polar on 9.

Thus, the distribution of Tnl insertions in P22, as judged by the phenotypes of P22Ap phages, is non-random since a disproportionate number of insertions affect the ant and 9 genes.

The P22Ap phages described in this thesis were chosen from those amp^R transductants which produced HFT lysates and contained a mutant prophage. All phages with an insertion in ant or essential genes and one or two phages with an insertion in 9 or non-essential regions were picked from each lysate. In all, 76 phages were analysed. Their properties are summarized in Appendix 1.

C. Mapping Insertions with P22 virB am am Phages

Mapping of Tnl insertions in prophages was accomplished by superinfecting with phages carrying two linked mutations. Rescue of both wild type alleles by recombination with the prophage is reduced if the prophage

contains a mutation located between those of the infecting phage.

To this end, pairs of linked amber mutations were crossed into P22 virB-3 to create a set of P22 virB am am phages. Each of these phages contains two amber mutations, in nearby genes, defining an interval of P22's genetic map. The intervals defined by the set of P22 virB am am phages span much of the P22 genetic map. Each of these phages was spotted on a lysogen of a P22Ap phage to determine which interval contained the Tnl insertion. Since the phages were virulent, then growth was limited only by the amber mutations. Failure to grow thus indicated failure to rescue the wild type alleles, due to the presence of an intervening insertion.

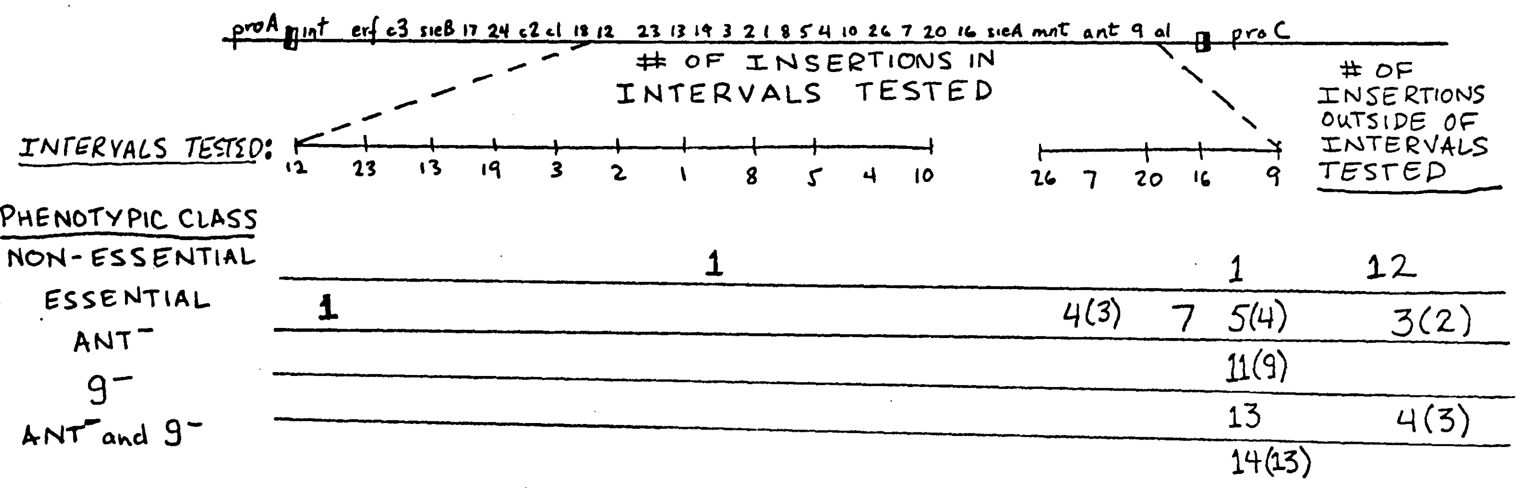
The results of this mapping are shown in figure 4. 76 lysogens were tested and 57 showed altered growth of at least one P22 virB am am phage. In general, agreements were found between the locations of the insertions and the phenotypes of the P22Ap phages. Thus, all ($^{25}/25$) ant^- P22Ap phages and most ($^{13}/17$) 9^- P22Ap phages have mutations between genes 16 and 9. The 9^- P22Ap phages not mutant in this interval could have insertions in gene 9 but to the right of the allele used in the virB 16^-9^- phage. Of the 14 insertions in non-essential regions, 12 fall outside of the intervals tested, one maps between genes 1 and 8, and one between genes 16 and 9. Lastly, of the 20 insertions affecting essential genes, one maps between genes 12 and 23, four between 26 and 20, seven between 20 and 16, five between 16 and 9, and three outside the tested intervals.

Some insertions affecting essential genes gave anomalous results in that they reduced rescue from more than one interval. Thus, the insertion in P22Ap27 only affected the 26-20 interval, while that of P22Ap34 affected 26-20, 20-16, and 16-9. Where anomalous rescue occurred, it always

Figure 4. Mapping Insertions with P22 *virB am am* Phages

P22 *virB am am* phages were spotted on lysogens of P22Ap phages as described in Methods (section G). Since some insertions were from the same lysate, the numbers of independent insertions in a particular interval are shown in parentheses.

FIGURE 4. MAPPING INSERTIONS WITH *virB am am* PHAGES



affected a set of contiguous intervals. It will be shown later that these insertions are polar, indicating that complementation, as well as recombination, is important in the virB am am test. Accordingly, insertions affecting multiple intervals have been placed in the promoter proximal interval affected. It should be noted that polar insertions in the 26-20 interval could, in fact, be in the 10-26 region which was not tested.

Thus, the virB am am mapping results are completely consistent with the phenotypes of the P22Ap phages described in the previous section. Furthermore, the distribution of insertions affecting essential genes is noteworthy. These insertions are mainly in those genes at the right of the prophage map, although they can occur in other regions. These results strengthen the conclusion of the previous section that the distribution of Tn1 insertions in P22 is non-random.

D. Complementation of Insertions in Essential Genes

To verify and extend the mapping of insertions, several insertions in essential genes were complemented against amber mutations. The results are shown in table 5. DB7354 shows reduced growth of 16^- phage, and this is taken to indicate that the Tn1 insertion in P22Ap38 is in gene 16. Similarly, DB7364 shows reduced growth of 20^- phage and DB7343 of 26^- phage, indicating the Tn1 insertion of P22Ap48 is in gene 20 and that of P22Ap27 in gene 26. For DB7328, both 20^- and 16^- phage grow poorly, and this is attributed to a Tn1 insertion in gene 20 of P22Ap12 which is polar on gene 16. By the same reasoning, P22Ap34 is concluded to have a polar insertion in gene 7.

The low yields of phage in this experiment are unexplained (see Ch. 2, section I-I) and make the results less clear-cut than standard P22 comple-

Table 5. Complementation of Insertions in Essential Genes

lysogen	Prophage	Insertion Location by vir am am Test	Yield upon infection with: 4^-	10^-	26^-	7^-	20^-	16^-	9^-	cl	Conclusion
DB7354	P22Ap38	16-9	-	-	-	-	1.3*	0.41*	1.9*	4.1*	16^-, non-polar
DB7328	P22Ap12	20-16	-	-	-	2.3	.38	.28	-	7	20^-, polar
DB7364	P22Ap48	20-16	-	-	-	1.5	≃.04	6	-	10	20^-, non-polar
DB7350	P22Ap34	26-20	2.0	0.9	1.6	0.24	0.67	0.64	-	1.3	7^-, polar
DB7343	P22Ap27	26-20	5.0	3.9	0.37	2.1	2.7	3.6	-	7.0	26^-, non-polar
DB7000	-	-	<0.8	<.3	<.12	.02	.1	.06	.15*	1000	-

*Test performed at 30° without tailing

Lysogens of P22Ap phages were induced, then superinfected with amber mutant phage at a multiplicity of 0.1 as described in Methods, section I.

$$\text{Yield} = \frac{\text{output phage titer on DB7004}}{\text{input phage titer on DB7004}}$$

mentation tests (compare with table 10). Nevertheless, the conclusions bolster the virB am am mapping results of the previous section. Thus, both P22Ap27 and P22Ap34 mapped in the 26-20 interval but P22Ap34 also affected the 20-16 and 16-9 intervals whereas P22Ap27 did not. Analogous results were found with P22Ap12 and P22Ap48. In section G it will be shown that the polarity of these insertions also affects gene 9.

E. P22Ap2: Insertion in the al Region

The P22Ap2 genome contains an insertion in a non-essential region mapping outside of the intervals tested with virB am am phages. When P22Ap2 particles are plated on a non-lysogen, two types of plaques appear. Most plaques are small and semi-clear while, at most, two per cent of the plaques are large and turbid. The titer of small plaques does not dilute linearly whereas the big plaque titer does. This indicates that small plaques are formed by multiple infections while big plaques are formed by single infection, probably by revertants which have recovered terminal repetition (Chan, 1974). This interpretation is supported by the observation that phages in a small plaque produce both small and large plaques while phages in a large plaque breed true.

Since plaque size and morphology revert together, they must both be caused by the insertion. This deduction formed the rationale for mapping the insertion. P22Ap2 was plated on a number of prophage deletions at low multiplicity of infection so that growth depended on recombination with the prophage (Ch. 1 section II-E). If the site of insertion was deleted in the prophage, the plaques formed by recombinants would still be semi-clear, while if the site was present in the prophage, some recombinants would reconstruct a wild type genome and form turbid plaques. The results (table 6) show that all genes to the left of ant can be deleted

Table 6. Mapping the Insertion in P22Ap2

Host	Extent of Deletion: proA	att	19	20	16	ant	al	att	proC	Relative Plating Efficiency	semi-clear	turbid
DB7000		—	—	—	—	—	—	—		(7×10^{-4})	47/48	1/48
DB136	—	—	—	—	—					1.0	0/672	672/672
DB7177			—	—	—	—				0.11	0/77	77/77
DB5057				—	—	—	—			0.36	228/243	15/243

P22Ap2 was plated on the various prophage deletion strains and the relative number of semi-clear and turbid plaques measured. The horizontal bar represents deleted material: DB136 deletes from the left on proA (outside the prophage) to gene 16; DB7177 (derived from a Tn10 insertion between genes 19 and 3) deletes from the right of gene 19 to ant; DB5057 (derived from P22Tc10) deletes from gene 20 to the al region.

in the prophage (DB136, DB7177) and rescue of turbid morphology is still efficient. With DB5057, plating efficiency is still high, and rescue still occurs, but at a reduced frequency. This indicates that the site of insertion is present in DB5057 but is near the deletion endpoint. The deletion in DB5057, derived from P22Tc10 (Chan and Botstein, 1972), is believed to end at or near the Tn10 insertion, which is located in the a1 region. Thus, the Tn1 insertion in P22Ap2 most probably lies in the a1 region, to the right of the Tn10 insertion in P22Tc10. This conclusion is supported by the genetic and physical evidence presented in later sections.

F. Insertions in the ant Gene

ant is a pivotal gene in this study. Since it is conditionally essential, mutations are easily detected and analyzed, while because it is one of the genes separating 9 from the other late genes, insertions in ant can be used to study 9 expression.

(i) Mapping

Intergration of a translocatable element within a gene creates a mutation genetically located at the site of insertion. Furthermore, since translocatable elements stimulate deletion generation, insertions within a gene can be used to obtain partial deletions of the gene. Thus, from a set of insertions within a gene it is possible to construct a deletion map of the gene which can be used to order the insertions. Such a self-generating approach was used to map ant^- insertions.

When an ant^+ phage infects a lysogen whose prophage lacks mnt, the infecting phage produces antirepressor which inactivates c2 repressor and allows phage growth. When an ant^- phage infects such a lysogen, growth will occur if the infecting phage can recombine with the prophage to

construct an ant$^+$ gene. If the ant$^+$ allele is deleted in the prophage, however, the infecting ant$^-$ phage will become repressed. With this rationale, ant$^-$ phages were plated on c2$^+$ mnt$^-$ prophage deletions, and the mutations mapped within the ant gene.

From ant$^-$ insertions and one insertion (P22Ap2) to the right of ant, deletions were generated entering ant from either side (Ch. 2, sections II-I and J). The deletions were oriented with the P22 map by scoring for markers outside of ant (16 or mnt to the left; 9 to the right). To order ant$^-$ mutations, their efficiency of plating, which should reflect rescue of the ant$^+$ alleles, was measured on these deletions. This information was sufficient to construct a self-consistent map of ant (figure 5).

The 21 insertions analyzed map at at least 13 different positions. 12 map between ant$^-$ base change (point) mutations and thus must be within the structural gene. The seven insertions mapping to the right of all point mutations are most likely within the gene since none revert to ant$^+$ (see Ch. 2, section II-H). P22Ap29's insertion maps to the left of all mutations and also does not revert to ant$^+$. It could be within the structural gene or possibly the promoter.

Crosses between ant$^-$ P22Ap phages were performed as a second method of demonstrating that these insertions were at different sites. The results (table 7) show that the ant$^-$ phages P22Ap4, Ap32, Ap49, and 63 all produce ant$^+$ recombinants when crossed with each other, and wild type recombinants when crossed with P22Ap7 (insertion in 9). The frequency of ant$^+$ recombinants varies from 0.4% (P22Ap4 x P22Ap32) to several per cent in crosses with P22Ap49. These frequencies are, in general, consistent with the deletion map of figure 5.

One phage, P22Ap14, was found to contain a deletion. P22Ap14 does

Figure 5. Fine Structure Map of the ant Gene

ant^{-} mutations were mapped by spot tests and efficiency of plating as described in Methods, sections D and L. All deletions were derived from P22Ap phages.

Tn1 insertions which are polar on gene 9 are represented by ⚲, non-polar insertions by ⚲, point mutations by a vertical line |, and deleted material by a solid bar ▬. The allele number of each mutation is given above or below its symbol.

Some data on P22Ap14, Ap29, Ap50, and Ap53 and the point mutations 3, 13, 14, 15, and 20 were personal communications from Dr. M. Susskind.

Parent insertions of deletions isolated as low multiplicity transductants (Ch. 2, section I) have been placed at the endpoint of the deletion.

The point mutants 18 and 16 are tentatively ordered on the basis of their recombination frequencies with DB7449.

P22Ap60 and Ap62 are omitted since they appear to be identical to P22Ap63; P22Ap11 is omitted since it appears to be identical to P22Ap14; P22Ap42 is not included.

The map is not intended to display physical distance, only topology.

FIGURE 5. FINE STRUCTURE MAP OF THE ant GENE

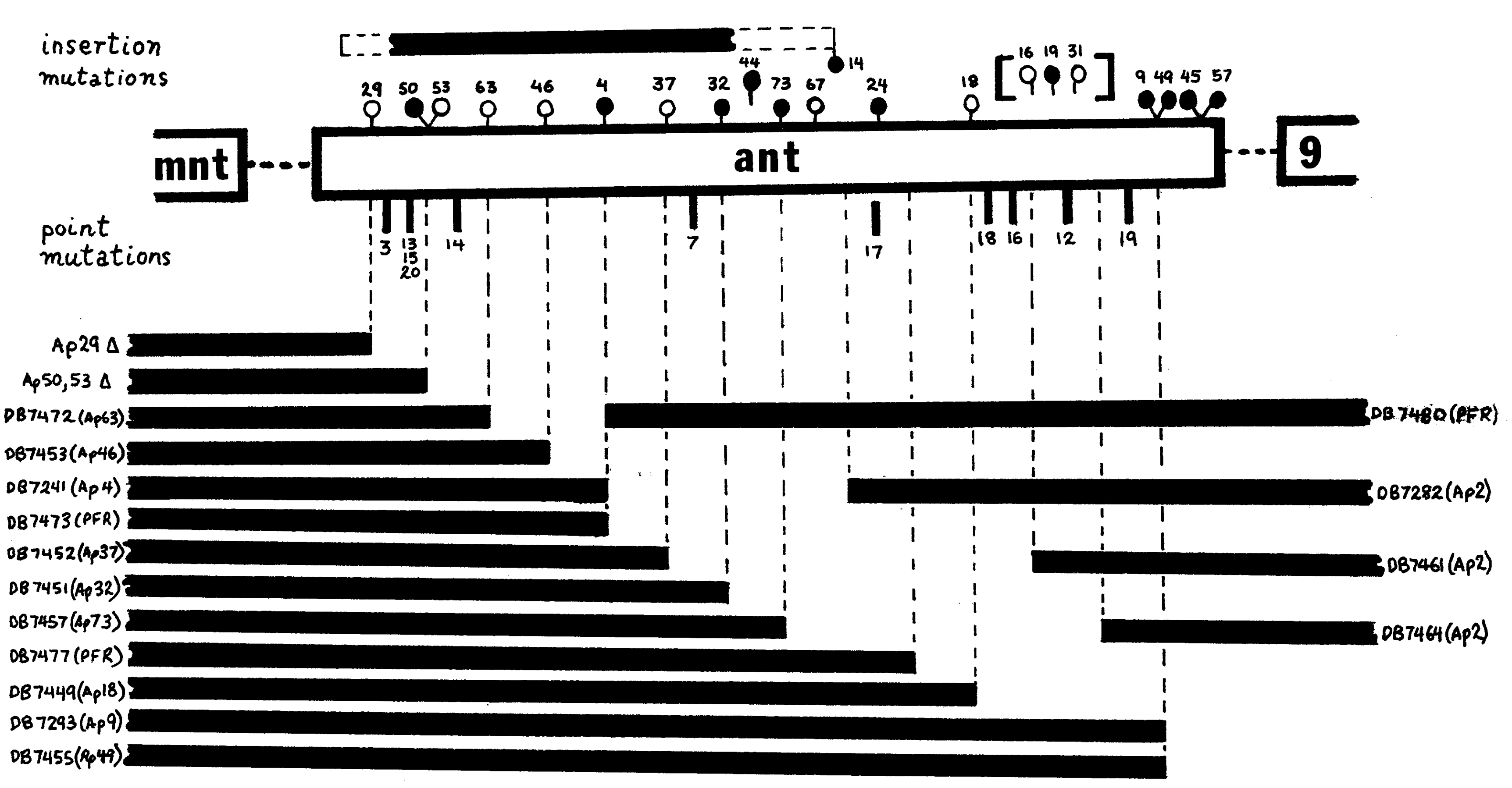
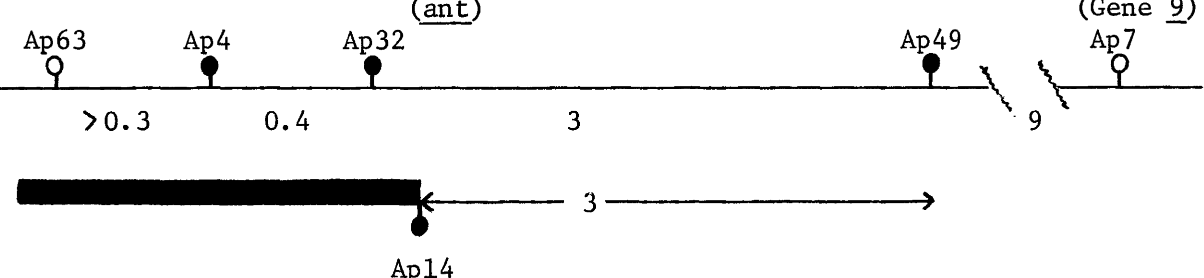


Table 7. Crosses between P22Ap Phages

	% Recombination					
	P22Ap4	P22Ap7	P22Ap14	P22Ap32	P22Ap49	P22Ap63
P22Ap4	$<1x10^{-4}$					
P22Ap7	6	$<1x10^{-4}$				
P22Ap14	$<1x10^{-4}$	8	$<1x10^{-4}$			
P22Ap32	0.4	7	$<1x10^{-4}$	$<1x10^{-4}$		
P22Ap49	4	9	3	3	$<1x10^{-4}$	
P22Ap63	>0.3	6	$<1x10^{-4}$	>0.3	2	$<1x10^{-4}$



P22Ap phages were crossed as described in Methods, section H. Polar insertions are represented by , non-polar insertions by , and the deletion in P22Ap14 by . P22Ap7 has an insertion in gene _9_, the other phage have insertions in _ant_.

$$\% \text{ Recombination} = \frac{\text{titer on DB5057}}{\text{titer on DB147}} \times 100$$

not produce <u>ant</u>$^+$ recombinants (less than 10^{-4} per cent) with P22Ap4, Ap32, or Ap63, although these phages recombine with each other to produce at 0.3 per cent <u>ant</u>$^+$ recombinants. P22Ap14 does recombine normally with P22Ap49 and Ap7. P22Ap14 also fails to produce <u>ant</u>$^+$ recombinants in crosses with phages containing the <u>ant</u>$^-$ point mutations 3, 7, 13, 14, 15, and 20 but recombines with other point mutations (data not shown). The simplest explanation for this behavior is that P22Ap14 contains a deletion. Since lysogens of P22Ap14 are immune, the deletion must not affect <u>mnt</u>.

(ii) <u>Tail Deficiency of ant$^-$ P22Ap Phages</u>

In section II-B, some <u>ant</u>$^-$ P22Ap phages were found to be phenotypically <u>9</u>$^-$. To quantitate this deficiency, lysates of <u>ant</u>$^-$ P22Ap phages were prepared by UV-induction and the tail deficiency of the particles determined by titering before and after incubation <u>in vitro</u> with p9. The results shown in table 8 corroborate the earlier finding. The tail deficient phages make a normal burst of particles (column(b)) very few of which are infectious (column(a)). These phages produce about 3 per cent as much p9 as is needed to tail all the particles they produce (column c). In contrast, the <u>9</u>$^+$ phages produce the same number of heads but make about 17 times as much p9. Since phages produced by UV-induction of a wild type lysogen are slightly tail deficient (Israel, 1967) it is not possible to determine whether the tail deficiency of the <u>9</u>$^+$ phages is in part due to the Tn1 insertion.

This analysis assumes the tail deficiency of particles quantitatively reflects the production of p9 in the cell. The results of a direct measurement of p9 production (see next section) verify this assumption. It will also be shown that the polarity of Tn1 insertions on gene <u>9</u> depends on their orientation.

Table 8. Tail Deficiency of ant^- P22Ap Phages

Phage	PFU/CELL (a) Without Tailing	PFU/CELL (b) With Tailing	(c) Tail Production [Tails(ϕEquiv.)/Head]x100
ant^- 9^- Phenotypic Class:			
Ap4*	$8x10^{-5}$	12.8	3.1
Ap9*	$1x10^{-4}$	16.	2.5
Ap11	$5x10^{-4}$	4.2	7.1
Ap14	$6x10^{-4}$	10.	5.0
Ap19	$7x10^{-6}$	4.0	1.8
Ap24	$3x10^{-5}$	3.8	2.6
Ap32	$1x10^{-5}$	3.3	2.1
Ap42	$4x10^{-5}$	2.6	3.8
Ap44	$4x10^{-5}$	7.3	2.7
Ap45	$2x10^{-5}$	4.2	2.4
Ap49	$1x10^{-5}$	2.1	2.9
Ap50	$2x10^{-5}$	5.7	1.8
Ap57	$4x10^{-5}$	6.5	3.1
Ap73	$1x10^{-5}$	1.9	2.6
ant^- 9^+ Phenotypic Class:			
Ap16	0.4	2.7	56
Ap18	1.0	11.	48
Ap29	0.3	2.2	50
Ap31	0.5	4.0	52

Ap37	0.5	2.8	61
Ap46	0.6	8.0	45
Ap53	0.3	3.0	50
Ap62	0.2	2.1	47
Ap63	1.0	11.	48
Ap67	0.2	2.3	43
Averages:			
$ant^- \ 9^-$	$1x10^{-4}$	4.6	3.1
$ant^- \ 9^+$	0.5	5.0	50

Lysates of Ap phages were prepared and tail deficiency determined as described in Methods, section K. The titers and bursts are not corrected for the plating efficiency of P22 Ap phages on DB147. Thus the actual numbers of particles produced are 7 times the values in the table.

* The tail deficiency of P22Ap4 and Ap9 was measured in separate experiments. The rest of the phages were tested in a single experiment.

Several other points are noteworthy. First, about one half of the insertions analyzed are polar and one half non-polar on 9, and both types of insertions map throughout the ant gene (figure 5). Thus there is no apparent site or orientation specificity among Tn1 insertions in ant. Secondly, P22Ap11 and P22Ap14 appear to produce about four times as much p9 as the other polar insertions. These phages are not independent and are apparently identical since both contain a similar deletion (data not shown). It is not known if this increase in p9 synthesis is due to the deletion.

G. Polar Effects on Gene 9

(i) Tail Production by P22Ap Phages

The observation that some insertions in ant cause tail deficiency is consistent with either p_{ANT} or p_{LATE} being the promoter for gene 9 transcription (Ch. 1, section II-G; figure 6). To distinguish between these possibilities, tail production by P22Ap phages with insertions in essential genes (26, 7, and 20) in the late phage operon was measured. If gene 9 is promoted from p_{ANT}, none of these insertions should affect tail production, but if 9 is promoted from p_{LATE}, a polar insertion in the late operon will cause tail deficiency.

Tail production by P22Ap phages was measured in this experiment by using lysates from induced lysogens to convert P22 heads to phage in vitro (Methods, section J). To prevent tails from becoming bound to defective heads produced by the P22Ap phage, and thus being undetectable, P22Ap prophages carried an amber mutation in gene 5, which codes for the phage's major capsid protein. Dilutions of the lysates were added to a known number of heads and the phage produced were measured. From this, the number of tails present in each dilution was calculated.

Figure 6. Models for Transcription of Gene 9

Illustration of the two models for transcribing gene 9. Insertions in ant affect gene 9 transcription by either model, while insertions in genes 26, 7, or 20 only affect transcription of gene 9 from p_{LATE}.

FIGURE 6. MODELS FOR TRANSCRIPTION OF GENE 9

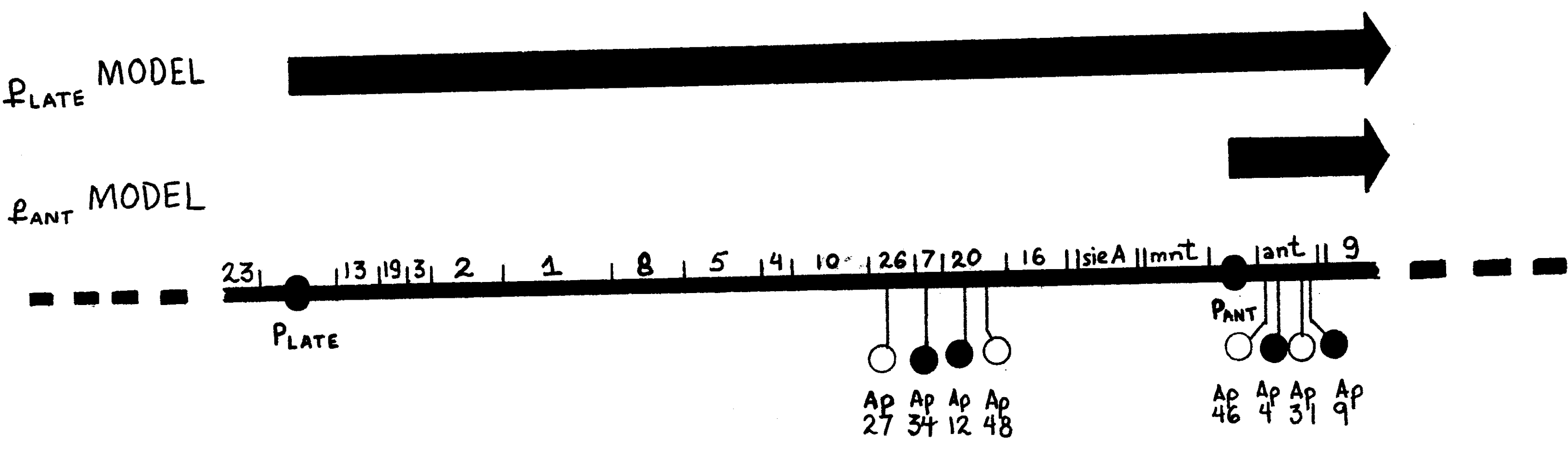


Figure 7 shows the results for $\underline{5}^-$ phages with no insertion or insertions in ant or 20. The calculated amounts of tails are plotted against the corresponding amount of lysate present in each reaction. Since the calculated tail concentration is found to be proportional to the amount of lysate used, these dilution curves can be used to quantitate the tails present in each lysate. Figure 7 shows that all the P22Ap phages produced fewer tails than a phage without an insertion, but P22Ap9 and P22Ap12 were more reduced than P22Ap31 and 48.

These and other results are summarized in table 9. The P22Ap phages fall into two classes, either strongly polar, producing a few per cent of the wild type level of tails (P22Ap4, 9, 12, and 34), or weakly polar, producing about half as many tails as wild type (P22Ap31, 46, 48, and 27). There are strongly polar insertions in ant, 20, and 7 and weakly polar insertions in ant, 20, and 26. Thus, the degree of polarity of these insertions is not due to their location but, as will be shown later, is due to their orientation.

The strongly polar insertions in P22Ap12 (gene 20) and P22Ap34 (gene 7) were previously found to reduce expression of other late genes located promoter-distal to the insertions (table 5). Since Tn1 insertions in the late operon are polar on late genes, including 9, gene 9 expression must use $\underline{p}_{LATE}$ and be a member of the late operon. Furthermore, since strongly polar insertions reduce 9 activity by at least 95%, no more than 5% of 9 expression can occur from other promoters. Thus $\underline{p}_{LATE}$ is the major promoter for gene 9.

Thoughout this thesis, strongly polar insertion s are referred to as "polar" and weakly polar insertions as "non-polar".

Figure 7. Assay for Tails Produced by P22Ap Phages

Assays were performed by mixing dilutions of lysates of P22Ap 5-am phages with phage heads as described in Methods, section K. Insertions in ant ●-●; insertions in 20 0-0; 5-am without an insertion □-□.

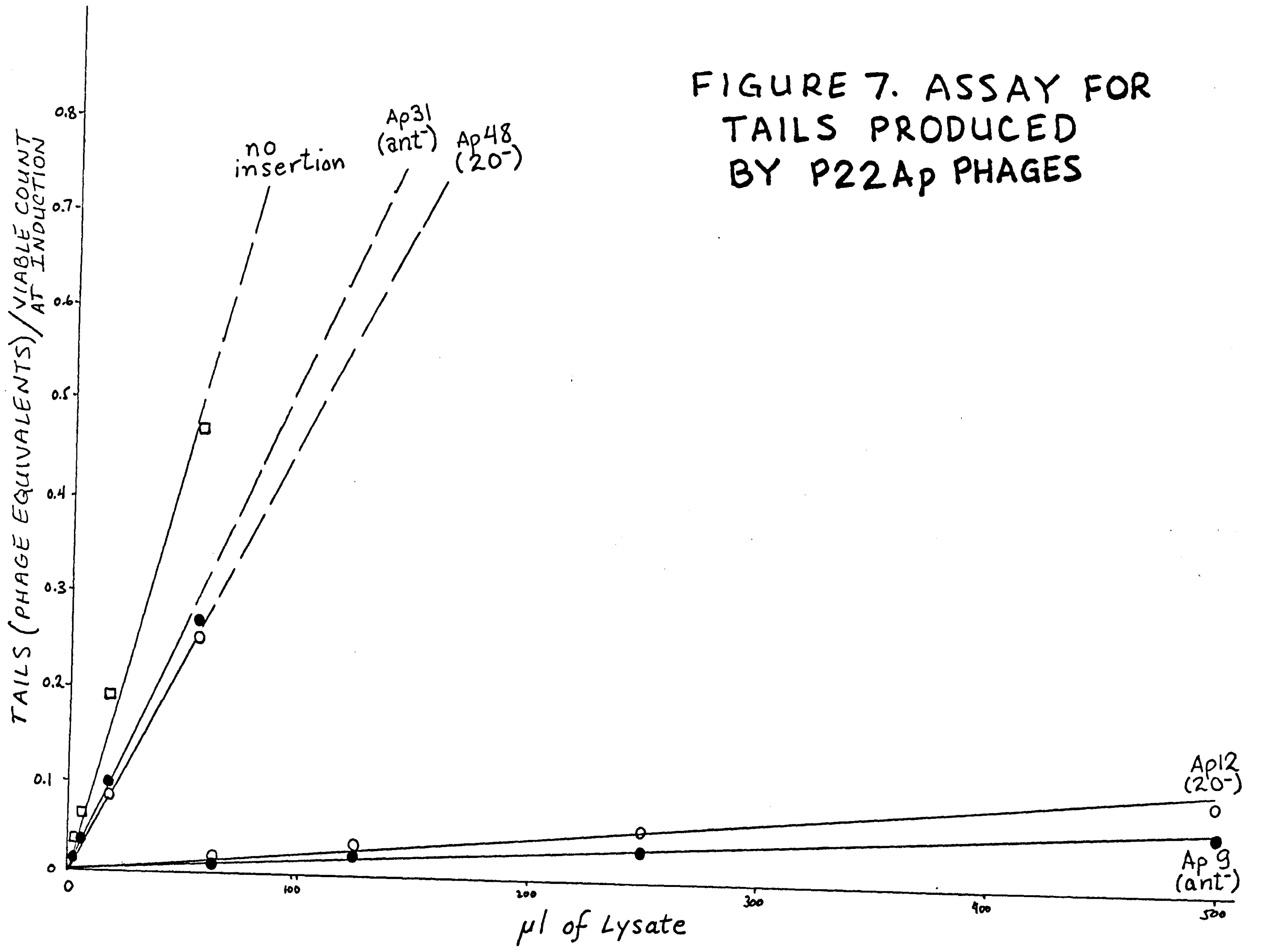


FIGURE 7. ASSAY FOR TAILS PRODUCED BY P22Ap PHAGES

Table 9. Tail Production by P22Ap Phages

Prophage	Site of Tn1 Insertion	Relative Amount of Tails Produced
P22 5-am("wild type")	-	100
P22Ap4 5-am	ant	4.5
P22Ap9 5-am	ant	1.5
P22Ap31 5-am	ant	54
P22Ap46 5-am	ant	56
P22Ap12 5-am	20	2.3
P22Ap48 5-am	20	51
P22Ap34 5-am	7	5.4
P22Ap27 5-am	26	53

Tails were assayed as described in Methods, section K and dilution curves (see figure 7) were constructed. The amount of tails produced was determined from these dilution curves. The absolute amount of tails produced by P22 5-am was about 9 phage equivalents/cell.

(ii) Complementation Test of Tail Deficiency

Insertions in ant which affect the expression of gene 9 could be polar on 9 or could affect a diffusible regulator of 9. To distinguish between these possibilities, complementation tests were performed between P22Ap4, containing a polar insertion in ant, and phages containing amber mutations.

The results of this experiment (table 10) show that P22Ap4 does not complement P22 9^-am (burst = 0.4) although normal amounts of 9^- particles are produced (burst = 129). Both of these phages, however, complement P22 16^-am phage (bursts of 8.1 and 15). The fact that P22Ap4 complements P22 16^-am shows that the tail deficiency of P22Ap4 is recessive in trans since it does not cause P22 16^-am to become tail deficient, and thus is not due to the production of a diffusible negative regulator of 9. The failure of P22Ap4 to complement P22 9^-am shows that the tail deficiency of P22Ap4 is dominant in cis since P22 9^-am cannot induce the good copy of gene 9 in P22Ap4 to be expressed. Thus the tail deficiency of P22Ap4 is not due to the failure to produce a diffusible activator of gene 9. Moreover, the Tnl insertion of P22Ap4 does not affect a diffusible regulator of gene 9 and most likely is directly polar on gene 9.

H. Reversion of Mutations Caused by Tnl Insertions

As discussed in section E, the phenotypes caused by the Tnl insertion in P22Ap2 are capable of reverting. However, when 11 independent plaque forming revertants (PFRs) were isolated from P22Ap2, none were wild type since, unlike P22Ap2 they could not stably lysogenize. In fact, none of the 25 ant^- insertions revert to ant^+ and neither of two 9^- insertions (P22Ap5 and Ap7) revert to 9^+. These experiments were capable of detecting one revertant in 10^{10} phages, as judged by reconstruction experiments in

Table 10. Complementation Test of P22Ap4's Tail Deficiency

Infection	Burst Untailed	Tailed in vitro
P22Ap4	⩽0.2	77
9-am	⩽0.2	81
16-am	⩽0.2	1
P22Ap4 + *16-am*	8.1	10
9-am + *16-am*	15	22
P22Ap4 + *9-am*	0.4	129

DB7000 was infected with phages at a multiplicity of 5 and the standard procedure for P22 complementation tests was followed (Methods, section D). The infection by P22Ap4 was titered on DB147 and the titer multiplied by seven; the other infections were titered on DB7004 and hence the bursts only reflect the amber mutant phages.

which a few wild type phages were plated in the presence of large numbers of P22Ap phages. Thus, Tn1 integration within a gene causes irreversible loss of function.

I. Deletions which Restore Terminal Repetition

The DNA in P22Ap phage particles is not terminally repetitious because the Tn1 insertion makes the phage's genome too large to fit completely into a phage head. Consequently, any process which requires circularization of P22Ap phage DNA by self-recombination will select for those genomes which have acquired enough terminal repetition to allow recombination to occur. These terminally repetitious P22Ap phage derivatives contain deletions which shorten the genome size. In this section the results from different selections for terminal repetition are described.

(i) Plaque Forming Revertants

When P22Ap phages are plated at low multiplicity of infection on a non-lysogenic strain, only those phages with terminally repetitious DNA and intact essential genes (PFRs) will make plaques. In section H, PFRs isolated from P22Ap2 were described. These phages have lost the amp^R gene and do not form stable lysogens and thus probably contain deletions.

The nature of PFRs was investigated in more detail with P22Ap4, which contains a polar insertion in ant. Twelve independent PFRs were isolated by plating P22Ap4 on the non-lysogen DB7000. This selects for deletions which restore terminal repetition and relieve polarity on gene 9. The frequency of these PFRs was about one per 10^6 particles. Their properties are summarized in table 11.

All of these PFRs have lost the amp^R gene, but only 7 could stably lysogenize. The defect in lysogenization has not been characterized. Two of the PFRs capable of lysogenization (DB7473 and DB7477 in figure 5)

Table 11.

amp = ampicillin

res = resistant

sens = sensitive

stable lysogeny = tested by streaking from the center of a phage spot and testing the colonies obtained for immunity; immune colonies were restreaked and tested for immunity.

ant = growth on DB7283 was tested

9 = growth on DB7000 ± p9 was tested

mnt = identified by streak tests (Methods, section D)

ND = not determined

Table 11. Plaque Forming Revertants of P22Ap4 sieA-44

	amp	Plaque Size	Stable Lysogeny	ant	9	mnt	#	Strain Number
P22Ap4 parent	Res	Medium	+	–	–	+		
PFRs isolated on DB7000	Sens	Large	+	–	+	–	2	DB7473, DB7477
	Sens	Large	+	–	+	+	5	
	Sens	Large	–	–	+	ND	2	
	Sens	Small	–	–	+	ND	1	
	Sens	Very small	–	–	+	ND	2	
							12	
PFRs isolated on DB7000 + p9	Res	Medium	+	–	–	+	1	DB7480
	Res	Medium	–	–	–	ND	8	
	Sens	Medium	–	–	–	ND	16	
	Sens	Medium	+	–	–	ND	1	
	Sens	Medium	+	–	+	ND	12	
							38	

contain deletions extending from within ant to between 16 and mnt. The endpoint in ant of DB7473 is near the site of the Tnl insertion in the P22Ap4 parent while the DB7477 deletion is farther away. The other five PFRs capable of lysogenization retained the wild type alleles of all ant^- point mutations but none would revert to ant^+. Thus, these contain deletions which remove amp^R, relieve polarity, and restore terminal repetition.

When plaque forming revertants of P22Ap4 are selected on DB7000 in the presence of p9, the requirement for relief of polarity is removed. Out of 38 revertants so selected (table 11), 26 were still tail deficient. Of these tail deficient revertants, 24 formed unstable lysogens, 8 of which were amp^R. One of the tail deficient revertants able to form stable lysogens also retained the amp^R gene, and contained a deletion extending from the Tnl insertion to the al region (DB7480 in figure 5). It is shown later that this deletion starts within the Tnl insertion (section K).

The fact that many of the PFRs have lost the amp^R gene suggests that deletions in PFRs occur in the vicinity of the Tnl insertion but do not leave it intact. The deletions in DB7477 (figure 5) and DB7480 (see section K) are examples of this. Thus some deletions in P22Ap phages do not end at the terminus of the Tnl insertion.

(ii) Low Multiplicity Transductants

Chan et al (1972) have shown that transduction of tet^R by P22Tcl0, whose DNA lacks terminal repetition, requires double infection to construct a circular genome capable of lysogenization (see Ch. 1, section II-E). However at very low multiplicities, when double infection is rare, the transductants obtained do not contain complete genomes but are prophage deletions. These shortened genomes were able to circularize and lysogen-

ize in single infection but occur at a low frequency in the phage stock and are only detectable when transduction by double infection is rare.

Such prophage deletions have been isolated from P22Ap phages after low multiplicity transduction for ampicillin resistance. The transductants were screened for immunity and those lacking either mnt or c2 were analyzed further. The results of mapping the prophage deletions in 43 independent transductants obtained from 15 P22Ap phages are shown in figure 8. In general, the frequency at which these deletions were recovered was about one in 10^5 particles.

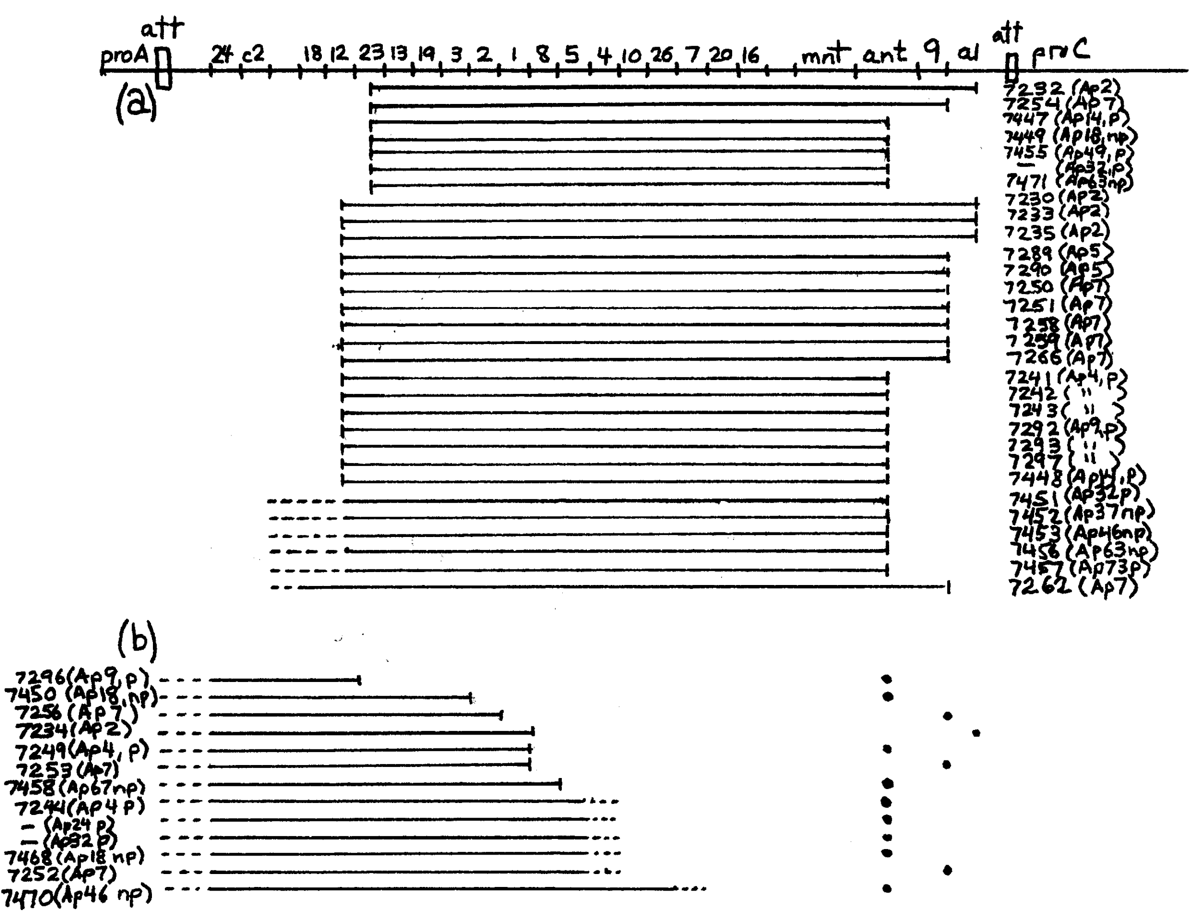
Two types of deletions were found. One class, shown in figure 8a, are tightly linked genetically to the site of the Tnl insertion in the parent phage. Thus, deletions in P22Ap2 have an endpoint in the al region, while deletions from 10 ant^- P22Ap phages all have an endpoint in ant. In figure 5, the fine structure of this endpoint is shown for 9 of the ant^- P22Ap phages. In no case is the deletion separable from the site of insertion. Thus, these deletions have an endpoint at or within the Tnl insertion, suggesting their formation involves Tnl functions.

The other endpoint of insertion-linked deletions is not randomly located, but is limited to the vicinity of gene 12. This is true of deletions generated from insertions at many sites and in either orientation. A number of the endpoints in gene 12 have been mapped with respect to 42 amber mutations and one frameshift mutation within this gene. The results, shown in figure 9, shows that the 14 deletions mapped have at least 8 different endpoints. These are apparently broadly distributed across the gene, although there may be a preferred region. The fact that these deletions end in the same region, around gene 12, is an example of long range specificity, but since there are many different endpoints w ithin gene 12

Figure 8. Prophage Deletions in Low Multiplicity Transductants

Deletions were isolated and mapped as described in Methods, section L. p = polar insertion; np = non-polar insertion. Deleted material is indicated by a horizontal line ———; a broken horizontal line -----, indicates uncertainty as to the endpoint.

FIGURE 8. PROPHAGE DELETIONS IN LOW MULTIPLICITY TRANSDUCTANTS



• = SITE OF Tn1 INSERTION IN PARENT

Figure 9. Distribution of Deletion Endpoints in Gene 12

Fine structure mapping of deletion endpoints in gene 12 was done by measuring efficiencies of plating of 12^- mutants as described in Methods, section L. The horizontal bar at the top of the figure represents gene 12 and the vertical lines indicate the positions of mutations. All mutations were amber except for one frameshift mutation indicated by (fs). Deleted material is indicated by a horizontal bar.

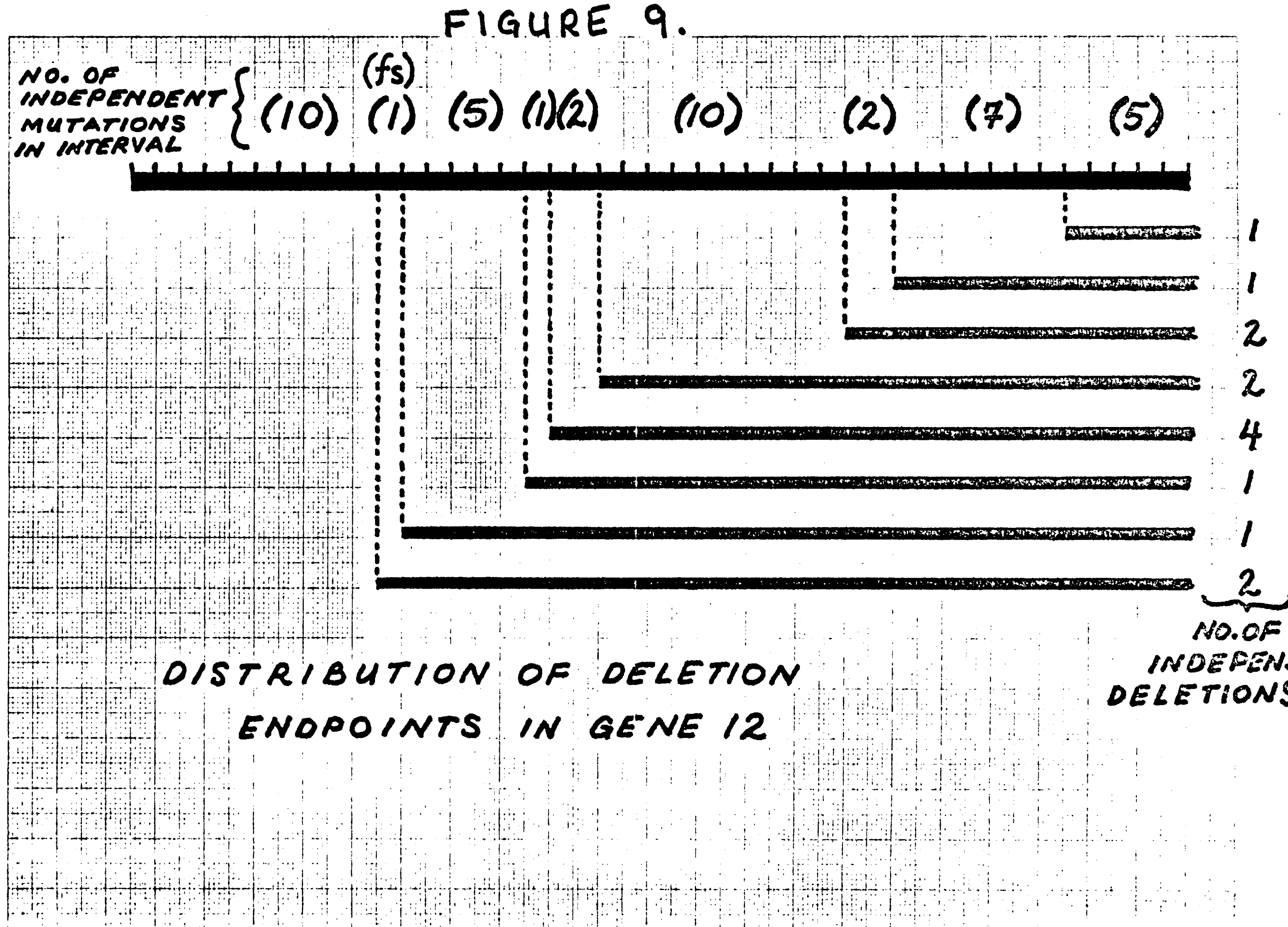


FIGURE 9.

DISTRIBUTION OF DELETION ENDPOINTS IN GENE 12

there is also local non-specificity.

A second class of deletions obtained from the low multiplicity transduction selection is shown in figure 8b. These deletions are not limited to the site of Tn1 insertion of the parent phage. Some of these deletions were mapped with the virB am am phages and found to contain a mutation in the same interval as the parent phage, suggesting the Tn1 insertion had not moved. These deletions all have one endpoint to the left of gene 24 and extend beyond c2 to at least 7 sites in the late operon, with a possible preference for the region between genes 1 and 10. It is possible these deletions are generated by phage functions since they are linked to the region containing the phage attachment site, but if they delete the int gene, they should not be able to intergrate. Thus their origin is unknown.

J. Prophage Deletions Allowing Survival from mnt Induction

Because the non-randomness of the deletion endpoints described in the previous section could have resulted from the selection procedure, a second, unrelated method was used to obtain deletions from P22Ap phages. From DB7273, a lysogen of P22Ap2 sieA-44 mnt-ts1 (containing an insertion in the a1 region), temperature-resistant, amp^R survivors were selected. Lysogens of this phage are temperature-sensitive since at high temperature mnt repressor is inactivated, allowing ant to be expressed which induces the prophage and kills the cell. Thus, temperature resistant survivors are ant^- mutants, mnt^+ revertants or deletions removing the killing functions near c2. The survivors were screened for the presence of gene 9 and only 9^- prophages were characterized. Deletions which are associated with the P22Ap2 insertion must extend from the a1 region to ant and will

thus be $\underline{9}^-$ whereas $\underline{ant}^-$ point mutants, $\underline{mnt}^+$ revertants, or deletions not linked to the insertion will be $\underline{9}^+$.

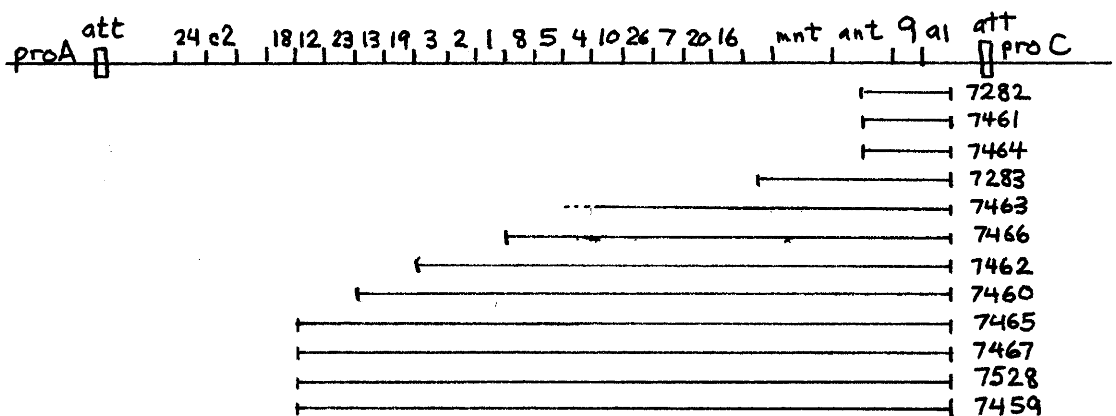
The $\underline{ant}^-$ $\underline{9}^-$ amp^R prophage deletions were recovered at a frequency of about one per 10^7 induced cells. The results of genetic mapping are shown in figure 10. All deletions have an endpoint in the al region, as demanded by the selection procedure. The other endpoint is distributed more randomly than in the previous selection. Nevertheless, there is a bias to the vicinity of gene 12, supporting the notion that the long-range site specificity of deletion formation is not an artifact of the selection procedure. Deletions also appear to frequently end in the mnt-ant region, which was previously shown to be a preferred location for Tnl integration (Ch. 2, section II-C). Most deletions in this region would have been missed in the selection for low multiplicity transductants since they would have been immune ($\underline{c2}^+$ $\underline{mnt}^+$) and possibly not removed enough DNA to restore terminal repetition to the genome. Three of these deletions end in ant (DB7282, DB7461, and DB7464) and their endpoints are mapped at different positions in figure 5. Thus, like the deletions in gene 12, these also may be locally non-specific. The correlation between preferred regions for Tnl integration and deletion formation suggests a common mechanism for these processes.

Those deletions with an endpoint in the central region of the prophage, between genes 12 and ant, were not observed in the low multiplicity selection and appear to be randomly distributed. Since they occur at about one per cent of the frequency of low multiplicity transductants, it is possible they could have been missed previously.

Figure 10. Prophage Deletions in Survivors of mnt Induction

Isolation and mapping of deletions was as described in Methods, section L.

FIGURE 10. PROPHAGE DELETIONS IN SURVIVORS OF *mnt* INDUCTION.



K. Physical Structure of Genomes Containing Deletions

Some of the deletions described previously do not affect phage genes which are essential for particle formation. When lysogens of these genomes are induced, they produce particles which can be purified and their DNA analyzed. In this section, the structure of the genomes of DB7480, a tail-deficient plaque forming revertant of P22Ap4 (Ch. 2, section II-I), and DB7282, DB7283, DB7461, and DB7464, deletions in P22Ap2 obtained by the _mnt_-_ts_ selection (Ch. 2, section II-J) are described.

Heteroduplexes were prepared between these DNAs and P22Tc10 and studied by electron microscopy. Such heteroduplexes should be double stranded except for two non-homologous single strand regions (figure 11a) one consisting of Tn1 DNA in the deletion genome which is not present in P22Tc10, and the other consisting of P22 material present in P22Tc10 but removed by the deletion. Each of the deletions spans the region of P22Tc10 which contains the Tn10 insertion, thus these two single strand regions are distinguishable since the strand corresponding to the deleted P22 material contains Tn10, which forms a characteristic lariat-shaped structure in single strands due to its terminal inverted repeats (Tye et al., 1974a).

Representative heteroduplexes are shown in plate 1 and measurements are summarized in table 12. The heteroduplex between DB7480 and P22Tc10 DNAs (plate 1e, figure 11a) contains a deletion/substitution loop, one strand of which contains the Tn10 lariat. The amp^R insertion in DB7480 is about 2960 bp and, thus, about 1800 bp have been deleted from the original Tn1 insertion. A total of 5560 bp of P22 DNA have been deleted. Thus, the total deletion in DB7480 is 7360 bp or about 17.4 per cent of the P22 genome. Since the Tn1 insertion amounts to 11.3 per cent of the P22 genome (see next section), the genome of DB7480 is about 94 per cent

Figure 11. Idealized Representations of Heteroduplexes

At the top of the figure, the physical structure of the genomes which were studied is shown.

The heteroduplexes shown in a), b), c), d), and e) are circular because the single strands are circularly permuted. Each line represents a single strand; thick lines represent the inverted repeat region of the insertions.

FIGURE 11. IDEALIZED REPRESENTATIONS OF HETERODUPLEXES

GENOME STRUCTURES:

Tn10
P22Tc10
Tn1
P22Ap4
DB7480
Tn1
P22Ap2
DB 7282 7283 7461 7464

HETERODUPLEX STRUCTURES:

a) P22Tc10/DB7480
Deletion Ends within Tn1

Tn1
Tn10

b) P22Tc10/DB7282, 7283, 7461, or 7464
Deletion Ends at Tn1 Terminus

Tn1
open structure
Tn10

e) No Deletion
P22Tc10/P22Ap

c)

closed structure

Tn1
Tn10

d) Deletion End away from Tn1

Tn1
Tn10

Tn1
Tn10

Plate 1. Heteroduplexes between P22Tc10 and P22Ap Genomes Containing Deletions

a) P22Tc10/DB7283 (closed single strand loops)

b) P22Tc10/DB7282 (closed single strand loops)

c) P22Tc10/DB7464 (closed single strand loops)

d) P22Tc10/DB7283 (open single strands)

e) P22Tc10/DB7480 (open single strands)

The interpretation of these structures is shown in figure 11.

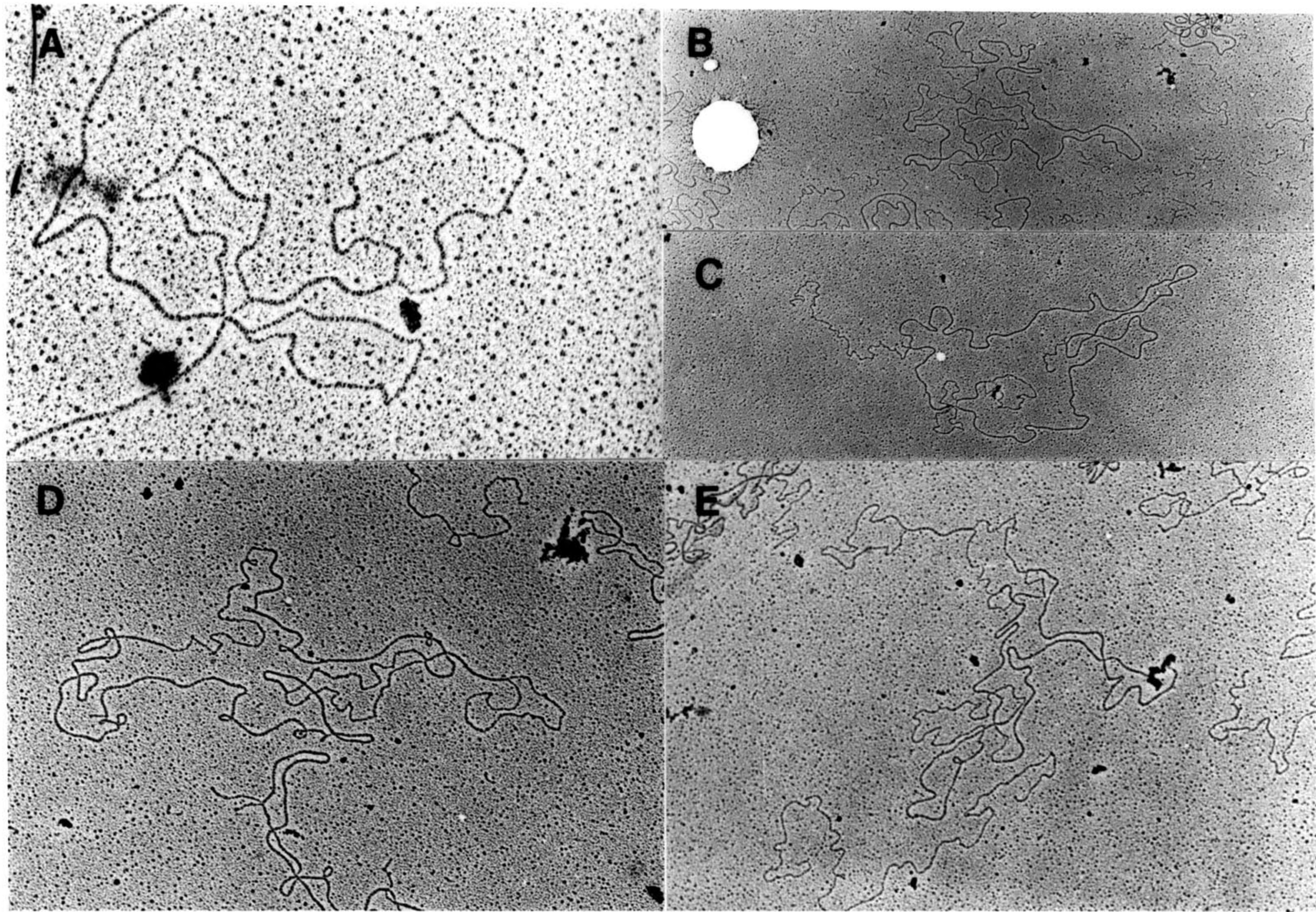

A
B
C
D
E

Table 12. Heteroduplex Analysis of Genomes Containing Deletions

proA c2 Tn10 (L R) proC

Deletion	Isolation Selection	Tn1 Material* Remaining	Tn1 Material* Deleted	P22 Material Deleted Total	P22 Material Deleted L-Tn10	P22 Material Deleted Tn10-R
		(bp)	(bp)	(bp)	(bp)	(bp)
DB7480	PFR	2960±79	1800	5560	3110±26	2450±105
DB7282	mnt-ts	4970±361	∿0	3260	3020±92	240±28
DB7283	mnt-ts	4970±504	∿0	4780	4570±282	200±58
DB7461	mnt-ts	4780±118	∿0	3550	3360±295	190±20
DB7464	mnt-ts	4440±213	∿0	3120	2880±92	240±29

* size of Tn1 = 4760 bp (Table 13)

Summary of results from the heteroduplexes illustrated in figure 11 and plate 1. The deletions were isolated as either plaque forming revertants (PFR) or survivors of mnt induction (mnt-ts). The results are based on 5 heteroduplexes of DB7282, 5 heteroduplexes of DB7283, 5 heteroduplexes of DB7461, 6 heteroduplexes of DB7464, and 3 heteroduplexes of DB7480.

bp = base pairs

of P22, thus accounting for its restored terminal repetition.

Since DB7480 was derived from P22Ap4, the interval from Tn10 to one end of the single strand region in which it is found represents the distance from Tn10 to the site of Tn1 insertion in P22Ap4, while the interval from Tn10 to the other end of this single strand region represents the distance the deletion extends beyond Tn10 toward the attachment site. In the next section, the Tn1-Tn10 interval in P22Ap4 is found to be 3390 bp from heteroduplexes between P22Ap4 and P22Tc10. Thus, I take the 3110 bp interval in the DB7480/P22Tc10 heteroduplex (table 12) to be the Tn1-Tn10 interval and the 2450 bp distance to be the extent of the deletion beyond Tn10 toward the attachment site. This latter distance corresponds to 5.8 per cent of P22's genome. Chan and Botstein (1976) found the Tn10 insertion in P22Tc10 to be 6.0 per cent from the attachment site. Thus, the deletion in DB7480 ends very near the attachment site. Since DB7480 is able to lysogenize, no functions essential to P22 integration are located in this deleted region.

In summary, the deletion in DB7480, which was selected as a plaque forming revertant, extends from within the Tn1 insertion to a point near the attachment site. This structure is similar to plaque forming revertants of P22Tc10 (Tye et al., 1974a) whose deletions also remove part of the Tn10 insertion.

In contrast to DB7480, the deletions of DB7282, DB7283, DB7461, and DB7464, isolated as survivors of induction of <u>mnt-ts</u>, appear to end very near the terminus of the Tn1 insertion. Several observations support this view. First, the Tn1 segment remaining in these strains is similar in size to the insertion in the P22Ap2 parent (table 12), indicating that the deletion removes little, if any, Tn1 DNA. Second, the ends of the

single strands in deletion/substitution loops were usually adjoining, resulting in the appearance of two closed single strand loops (figure 11c), suggesting that the terminal inverted repeats of Tn1 are still present in these strains. In plate 1a,b, and c are shown examples of such structures from heteroduplexes between P22Tc10 DNA and DB7283, DB7282, and DB7464 respectively. It is apparent that the ends of the single strand regions are adjacent, unlike the P22Tc10/DB7480 heteroduplex of panel e, in which the ends are separated and the two single strands are open. Structures with closed single strand regions were seen in 4/5 heteroduplexes of P22Tc10 DNA with DB7282, 3/5 with DB7283, 3/5 with DB7461, and 6/6 with DB7464, but were not observed (0/3) in heteroduplexes with DB7480. An example of a P22Tc10/DB7283 heteroduplex with open single strand regions (figure 11b) is shown in plate 1d. Since the stem formed by the inverted repeats of Tn1 is small (estimated to be 140 bp by Heffron et al., 1975b) it is not unexpected that the stem is relatively unstable; thus the structure of plate 1d could be generated by denaturation of the stem.

The closed structures could also occur if the deletion in P22Ap genomes ended outside of the Tn1 insertion (figure 11d) leaving a short stretch of P22 DNA between the deletion and the Tn10 insertion. Measurements of the distance from the ends of these deletions to the Tn10 insertion show one end of each deletion to be about 200 bp from Tn10 (table 12) which is close to the Tn1-Tn10 distance observed for P22Ap2 in the next section, indicating again that the deletion ends very near the Tn1 insertion. Furthermore, in all heteroduplexes examined, the Tn1 insertion does not appear to be displaced from the deletion. Thus, the deletions in DB7282, DB7283, DB7461, and DB7464 all appear to end at or very near the Tn1 insertion of P22Ap2.

The deletions DB7282, DB7461, and DB7464 delete similar amounts of P22

material (table 12) which is consistent with the fact that they all end in the ant gene (figure 5). DB7282 is longer than DB7461 by genetic mapping, and the fact that the opposite result is obtained from physical measurements is most probably a reflection of the poor resolution of the heteroduplex technique relative to genetic mapping. DB7283 was found by genetic mapping to extend beyond ant and mnt, and the physical size of this deletion is seen to be larger than the deletions ending in ant. The deletion in DB7283 removes about 11.3 per cent of the P22 genome and thus should compensate for the 11.3 per cent Tn1 insertion. This has been verified since particles from DB7283 plate with high efficiency on DB7000 in the presence of p9 (data not shown), indicating that DNA in DB7283 particles has terminal repetition.

L. Physical Mapping of P22Ap Genomes by Heteroduplex Analysis

Heteroduplexes between DNAs of P22Tc10 and either P22Ap2 (insertion in the a1 region), P22Ap4 (ant), P22Ap7 (9), P22Ap12 (20), or P22Ap38 (16) were prepared and analyzed by electron microscopy. Representative heteroduplexes of P22Tc10 and P22Ap7 or P22Ap38 are shown in plate 2 and diagrammed in figure 11e. The measurements of heteroduplexes (table 13) were derived using the Tn10 stem as a double strand standard and the Tn10 loop as a single strand standard.

In the molecules shown in plate 2, the single stranded Tn1 loop is connected to P22 duplex DNA by a short stem which results from intramolecular annealing of Tn1's terminal inverted repeats. This stem was visible in only half of the heteroduplexes examined. The size of the stem given in table 12, 101 ± 58 bp, does not include those molecules where the stem was not visible and is thus a maximum estimate. By examining single stranded molecules in which the inverted repeats had annealed, Heffron et

Plate 2. Heteroduplexes between P22Tc10 and P22Ap Genomes

A = P22Tc10/P22Ap38 (insertion in 16)

B = P22Tc10/P22Ap7 (insertion in 9)

The interpretation of these heteroduplexes is shown in figure 11.

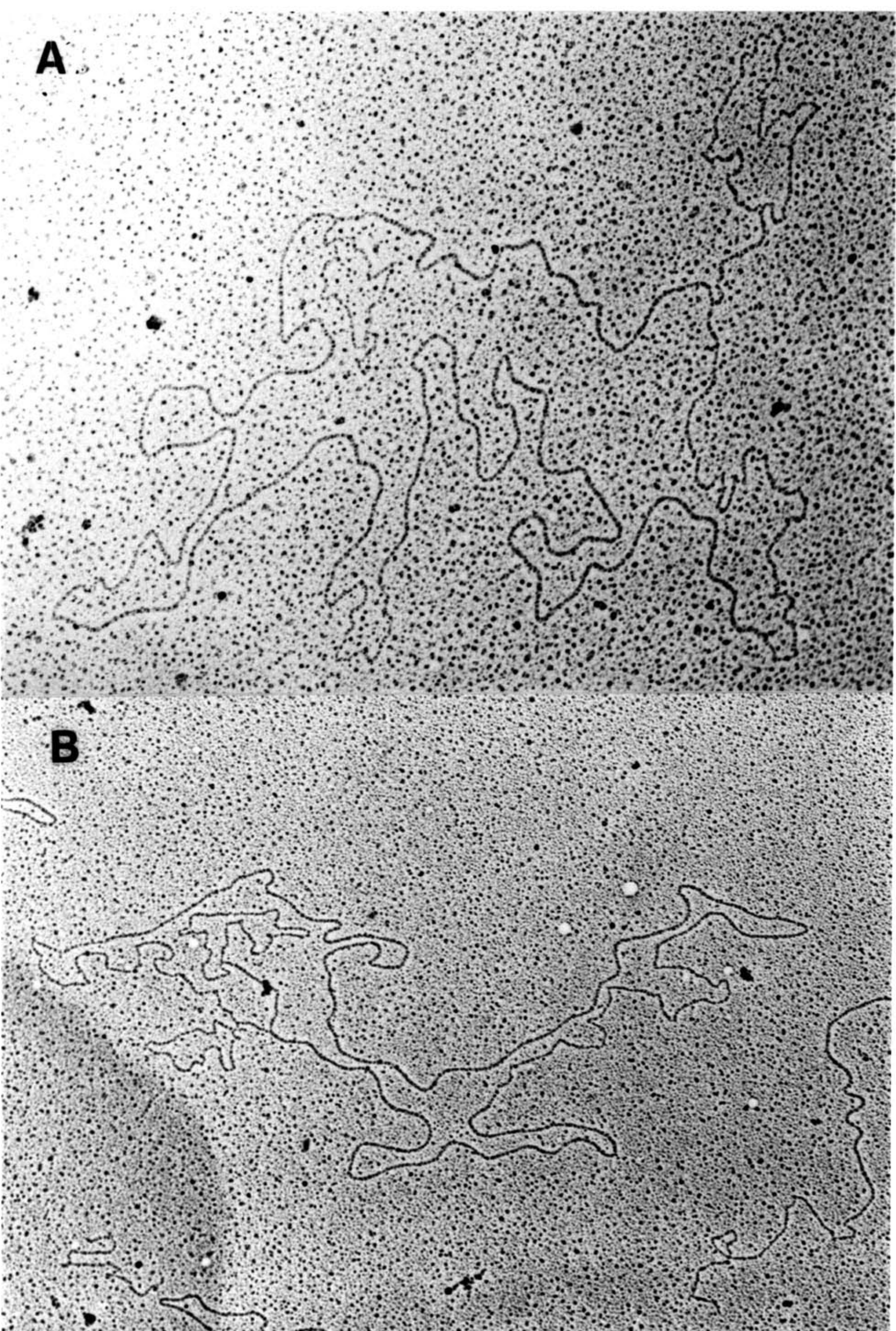

A
B

Table 13. Heteroduplex Analysis of P22Ap Phage Genomes

Phage	Site of Tn1 Insertion	Size of Tn1 Insertion(bp)*	Size of Tn1 stem(bp)**	Short Tn1-Tn10 bp	Distance % P22
P22Ap2	al region	4720±212	93±13	160±29	0.4
P22Ap7	9	4610±138	42±13	510±38	1.2
P22Ap4	ant	4770±140	160±68	3390±110	8.0
P22Ap38	16	4680±172	160±82	5880±155	13.9
P22Ap12	20	5030±228	48	7480±167	30.6
means values		4760±160	100±58		

* Size of Tn1 Insertion = (Loop)+ 2x(Stem)

** Does not include molecules where no stem was visible

The number of molecules measured was 5 for P22Ap2, 12 for P22Ap4, 4 for P22Ap7, 6 for P22Ap12, and 6 for P22Ap38. The standard error for each P22Ap phage was calculated as $S.E. = (\Sigma(x-\bar{x})^2)^{1/2}/(n-1)$ where x = a particular measurement

$\bar{x}$ = the mean value = $(\Sigma x)/n$

n = number of measurements.

The standard deviation of the mean values was calculated as $S.D. = (\Sigma(x-\bar{x})^2/(m-1))^{1/2}$ where x = the individual mean value

$\bar{x}$ = the mean value of the means = $(\Sigma X)/m$

m = the number of means.

al. (1975b) estimated the maximum size of the repeat to be 140 ± 39 bp. Because this stem is not reproducibly seen, it would appear that a DNA segment of this size is near the limit of resolution of the heteroduplex method.

The size of the Tn1 insertion in each P22Ap phage was calculated as the sum of the length of the single stranded loop plus twice the length of the double strand stem. The values for the five phages listed in table 13 do not differ greatly from each other and give a mean value of 4760 ± 160 bp (3.09×10^6d or 11.3% of the P22 genome). This value agrees the value of 4800 ± 450 bp reported by Rubens et al. (1976).

The P22Tc10/Ap heteroduplexes studied did not show deletion loops. Thus, if Tn1 integration caused deletion of P22 material (i.e. is not precise), the deletion must be less than 100 bp long.

The distances from the Tn10 insertion in P22Tc10 to the Tn1 insertions in 9, ant, 16, 20, and the a1 region are given in table 13. The Tn1 insertion in P22Ap2 is found to lie very near Tn10 (160 bp away) as predicted by genetic mapping (Ch. 2, section II-E). It is noteworthy that recombination between two large non-homologies (Tn10 = 9.3 kb; Tn1 = 4.8 kb) separated by only 160 bp is readily detectable. The locations of the other P22Ap Tn1 insertions are discussed in more detail in later sections.

M. Physical Mapping of P22 Genomes with Restriction Enzymes

(i) Digestions with EcoRI:

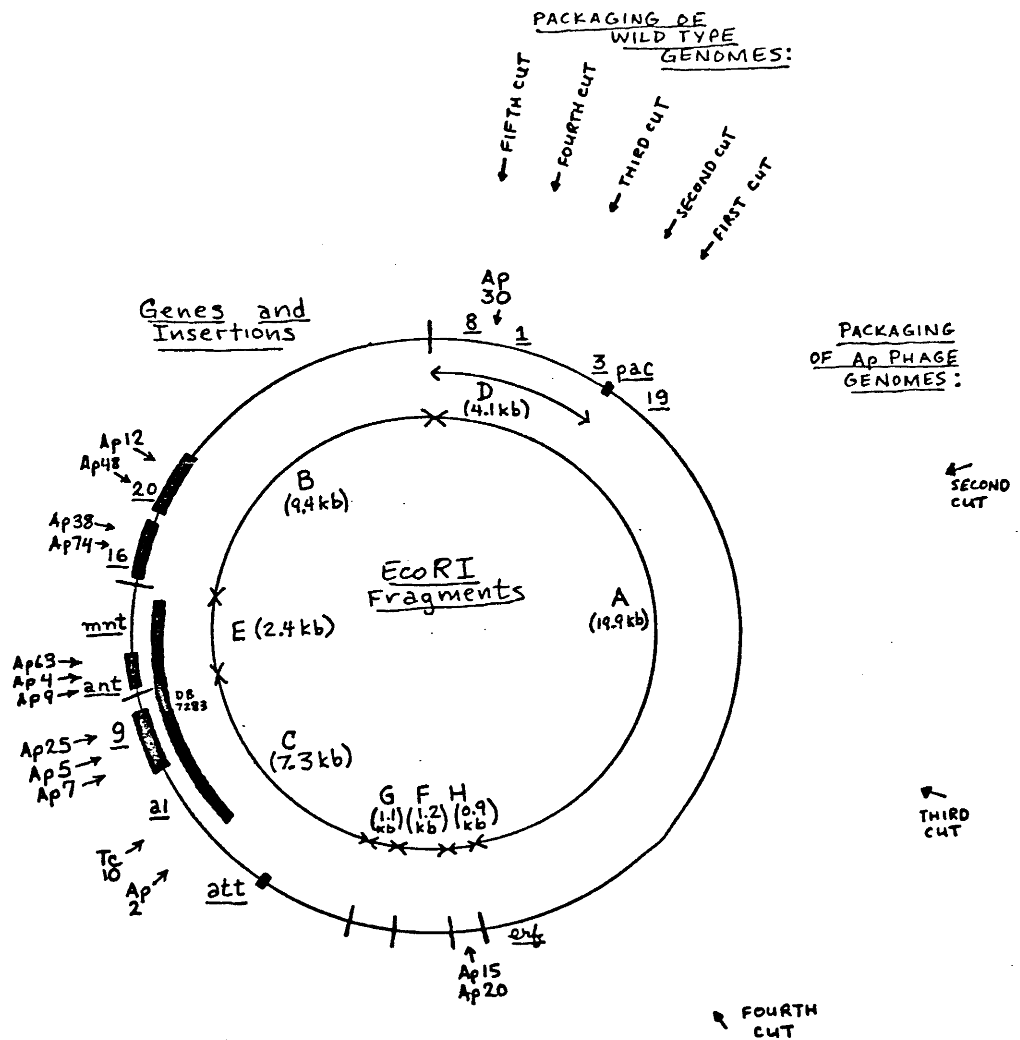
Jackson (1977) has shown that EcoRI cleaves linear, mature P22 DNA at 7 sites to produce 8 fragments and has ordered these fragments with respect to each other as shown in figure 12. The D fragment results from EcoRI cleavage of a molecule which was packaged into a phage head from pac, the origin of packaging (see Ch. 1, section B) and thus has pac at

Figure 12. Assignment of P22 Genes and Tn1 Insertions to EcoRI Fragments

The locations of P22 genes and Tn1 insertions in P22Ap phage relative to the eight EcoRI fragments of P22 and the theoretical locations of packaging cuts in wild type P22 and P22Ap phage DNA are shown.

kb = kilobases

FIGURE 12. Assignment of P22 Genes and Tn1 Insertions to EcoRI Fragments



one terminus. However, since P22 chromosomes are circularly permuted, not all molecules have pac at their terminus, and consequently the D fragment is present in lesser amounts than other fragments. Since packaging is sequential, and the DNA is believed to enter the head starting from pac and proceeding counter-clockwise around the map shown in figure 12, the subsequent cuts in wild type genomes occur within the D region since each headful is about two per cent larger than a complete genome (see figure 12). Eventually, a complete A fragment can be produced, but since the previous fragments are only slightly smaller than A, and molecular weights of this size are poorly resolved on agarose gels, these fragments produce a diffuse band at the position of A. A representative gel of an EcoRI digest of P22 is shown in plate 3. In this gel the dispersion of the A fragment is less apparent because of the poor resolution of high molecular weight DNAs, but the non-stoichiometric D fragment is clear.

When mature DNA from an oversized genome, such as a P22Ap phage, is digested with EcoRI, a different pattern is found (plate 3; figure 13). The non-stoichiometric D fragment is still produced since the first cut is fixed at pac, but because the genome is larger than a headful, the subsequent cuts occur within the A fragment as shown in figure 12. This generates a series of fragments which are present in non-stoichiometric amounts and which are sufficiently different in size to be resolved in the gel. Some of these fragments are plainly seen in the gel of plate 3.

Given this expectation for the EcoRI digestion pattern of P22Ap phage DNA, it was possible to determine which fragment contained the Tn1 insertion in each genome. Since there is no EcoRI cleavage site in Tn1 (Heffron et al., 1975b), the fragment containing the insertion will be absent from the digestion pattern and a new fragment will appear whose molecular length

Plate 3. Agarose Gel of Digestions of P22, P22Ap2, Ap4, Ap7, Ap9, and Ap63 DNAs with EcoRI, BamH1, and EcoRI+BamH1

A = BamH1 digestion of P22Ap9 DNA

B = EcoRI/BamH1 digestion of P22Ap9 DNA

C = EcoRI/BamH1 digestion of P22Ap9 DNA

D = BamH1 digestion of P22Ap7 DNA

E = EcoRI/BamH1 digestion of P22Ap7 DNA

F = EcoRI digestion of P22Ap7 DNA

G = BamH1 digestion of P22Ap63 DNA

H = EcoRI/BamH1 digestion of P22Ap63 DNA

I = EcoRI digestion of P22Ap63 DNA

J = BamH1 digestion of P22 DNA

K = EcoRI/BamH1 digestion of P22 DNA

L = EcoRI digestion of P22 DNA

M = BamH1 digestion of P22Ap4 DNA

N = EcoRI/BamH1 digestion of P22Ap4 DNA

O = EcoRI digestion of P22Ap4 DNA

P = BamH1 digestion of P22Ap2 DNA

Q = EcoRI/BamH1 digestion of P22Ap2 DNA

R = EcoRI digestion of P22Ap2 DNA

1% agarose gel

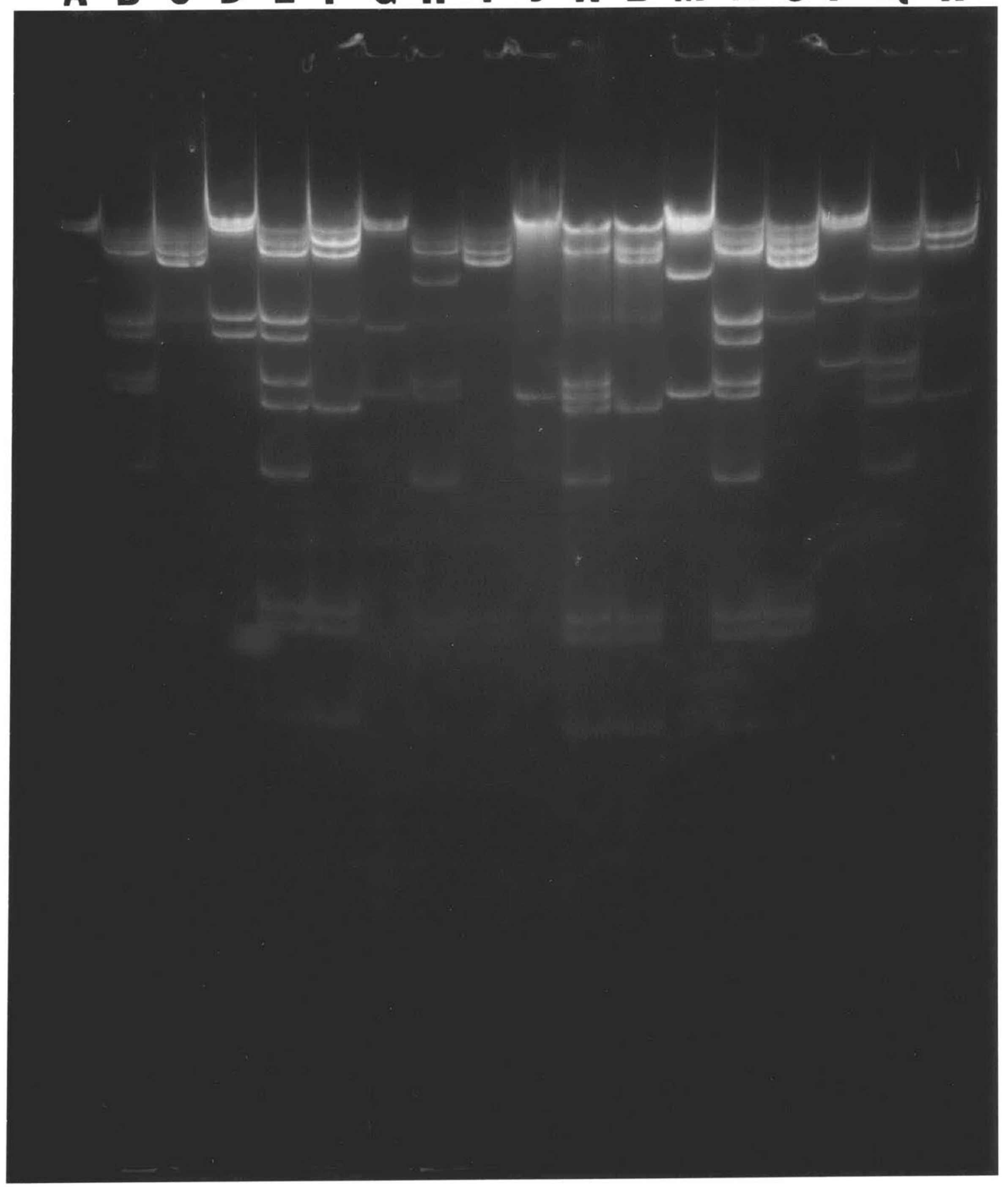

Ap9
Ap7
Ap63
P22
Ap4
Ap2
A B C D E F G H I J K L M N O P Q R

Figure 13. Representation of the EcoRI Digestion Pattern of Plate 3

C = P22Ap9 DNA

F = P22Ap7 DNA

I = P22Ap63 DNA

L = P22 DNA

O = P22Ap4 DNA

R = P22Ap2 DNA

FIGURE 13. EcoRI Digests of Plate 3

equals the sum of Tn1 and the missing fragment. The patterns from several P22Ap phages are shown in plate 3 and diagrammed in figure 13. The EcoRI pattern of P22Ap2 or Ap7 DNA lacks the C fragment and contains a new fragment about 12.4 kb in size. Since the C fragment is 7.3 kb, the Tn1 insertion must be 5.1 kb. The patterns from P22Ap4, Ap9, or Ap63 lack the E fragment but no new fragment appears. This is because the composite Tn1 + E fragment of these genomes is not resolved from the C fragment. Using the 5.1 kb value for Tn1 deduced above, the new fragment would be 7.5 kb (since the E fragment is 2.4 kb in size) which would not be resolved from the 7.3 kb C fragment. It can be seen in plate 3 that the C fragment of these patterns is more intense than the nearby B fragment. Thus the insertion in P22Ap2 and Ap7 is in C while that of P22Ap4, Ap9, and Ap63 is in E.

The location of the insertions in a number of Ap phages is summarized in figure 12. Since the genetic location of these insertions is known, the EcoRI map can be oriented to the genetic map of P22. The C fragment contains the insertions in P22Ap5, 7, and 25 which are $\underline{9}^-$ and that of P22Ap2, in the al region. Jackson (1977) showed this fragment contained the Tn10 insertion in P22Tc10 as well as the attachment site. The E fragment contains the insertions in P22Ap4, Ap9, and Ap63 which are $\underline{ant}^-$. In addition, the EcoRI pattern of DB7283 DNA (containing a deletion which is $\underline{mnt}^-$) lacks E and C, but not B. Thus, the mnt and ant genes are in E. The $\underline{16}^-$ phages P22Ap38 and Ap74 and the $\underline{20}^-$ phages P22Ap12 and Ap48 have insertions in the B fragment while P22Ap30, whose insertion maps in a non-essential region between genes 1 and 8, removes the D fragment. Lastly, two phages with insertions in non-essential regions, P22Ap15 and Ap20, lack the H fragment, which is in the vicinity of the erf gene (Jackson,

1977).

The molecular length of the Tn1 element derived from these and subsequent digestions was found to be 5.1 ± 0.1 kb, about 7 per cent larger than the value found from heteroduplex analysis. Consequently, in the subsequent restriction enzyme analyses the 5.1 kb value is used in calculations. This lack of agreement between the two methods of analysis may reflect a discrepancy between the standards used.

(ii) <u>Digestion with BamH1</u>

When P22 DNA is digested with BamH1, two bands are seen in agarose gels (plate 3). One band (the A fragment) is large (>20 kb) and diffuse while the other (the B fragment) is 2.6 kb in size. This is interpreted to mean that BamH1 cuts P22 DNA at two sites, 2.6 kb apart, generating a small fragment and two large, poorly resolved, fragments whose sizes are variable due to the permuted ends of the chromosome. Rubens et al. (1976) showed that BamH1 cuts Tn1 at a single site located asymmetrically within the element. Thus, P22Ap phage genomes contain three BamH1 sites and digestions will produce three fragments whose sizes reflect the orientation and position of the Tn1 insertion relative to the BamH1 sites in P22 DNA.

Plates 3 and 4 (represented diagramatically in figures 14 and 15) show the results of digesting P22Ap phage DNAs with BamH1. The sizes of the fragments present in these digests are listed in table 14. The 2.6 kb B fragments found in wild type DNA is absent from digests of P22Ap2 and Ap7 DNAs and two new larger bands appear in each of these digests. This is taken to mean that the Tn1 insertions in these phages lie within the B fragment region of the genome. This conclusion is supported by the fact that the sum of the sizes of the new fragments is 7.8 kb in each case,

Plate 4. Agarose Gel of Digestions of P22Ap12, Ap38, and Ap48 DNAs with BamH1

A = EcoRI digest of P22 DNA

B = BamH1 digest of P22Ap12 DNA (polar insertion in 20)

C = BamH1 digest of P22Ap48 DNA (non-polar insertion in 20)

D = BamH1 digest of P22Ap38 DNA (non-polar insertion in 16)

1.4% agarose gel

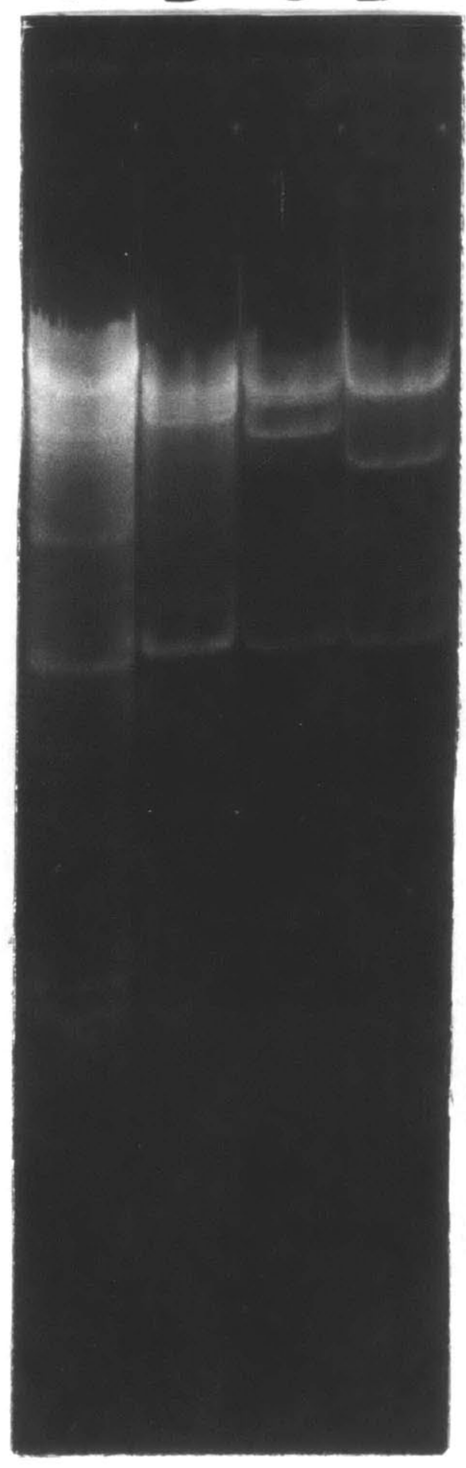

A B C D

Figure 14. Representation of the BamH1 Digestion Pattern of Plate 3

A = P22Ap9 DNA

D = P22Ap7 DNA

G = P22Ap63 DNA

J - P22 DNA

M = P22Ap4 DNA

P = P22Ap2 DNA

FIGURE 14. BamH1 Digestions of Plate 3

Ap9 A

Ap7 D

Ap63 G

W T J

Ap4 M

Ap2 P

Bam A

BamB

Figure 15. Representation of the BamH1 Digestion Pattern of Plate 4

A = EcoRI digest of P22 DNA

B = BamH1 digest of P22Ap12 DNA

C = BamH1 digest of P22Ap48 DNA

D = BamH1 digest of P22Ap38 DNA

FIGURE 15. REPRESENTATION OF PLATE 4

Bam H I

RI/WT A

Ap12 B

Ap48 C

Ap38 D

RI-A

RI-B

RI-C

RI-D

RI-E

RI-F

RI-G

Bam B

Table 14. Fragments Produced by BamH1 Digestion of P22Ap DNA

Source of DNA	Site of Tn1 Insertion	Polar(P) or Non-polar(NP)	Size of Fragments (kb)
wild type	-	-	>13, 2.6
P22Ap2	a1 region	-	>13, 4.8, 3.0
P22Ap4	ant	P	>13, 6.2 2.6
P22Ap7	9	-	>13, 4.1, 3.7
P22Ap9	ant	P	>13, 5.5 2.6
P22Ap12	20	P	>13, 12.3, 2.6
P22Ap38	16	NP	>13, 6.2, 2.6
P22Ap48	20	NP	>13, 8.9, 2.6
P22Ap63	ant	NP	>13, 3.8, 2.6

Summary of the results shown in plates 3 and 4.

which is close to the value of 7.7 kb expected for the sum of the B fragment and Tnl. Since the insertion in P22Ap2 is in the al region and that of P22Ap is in 9, the Bam-B fragment must span this region of the genome.

All the other P22Ap phage DNAs have an intact B fragment (table 14) and thus their Tnl insertions probably lie outside of this region. These digests contain, in addition to the A and B fragments, a fragment whose size is different for each phage. It can be seen that the size of this fragment is considerably different in P22Ap4 and Ap63 DNAs, although the Tnl insertions in these genomes are near each other in ant (figure 5). Thus, the difference in size of this fragment between these phages reflects the different orientations, rather than positions, of these insertions. Rubens et al. (1976) found that polar and non-polar Tnl insertions are in opposite orientation and, in accord with this, in section F it was shown that P22Ap4 contains an insertion which is polar on 9, while P22Ap63's insertion is non-polar. Assuming the insertion to be at the same position in these two phages, I calculate that the BamHl site in Tnl is 1.3 kb from the terminus and when Tnl is in the polar orientation, this 1.3 kb segment is on the promoter proximal side of the element. This location of the BamHl site within Tnl is close to that (1.4 kb from the terminus) reported by Rubens et al. (1976).

A second example of the relationship between polarity and orientation is seen in plate 4 (represented in figure 15) with P22Ap12, containing a polar insertion in 20, and P22Ap48, containing a non-polar insertion in 20 (sections D and G). The variable third fragments of these digestions differ in size by 3.4 kb (table 14) which is much larger than the size of gene 20 (1.4 kb) as calculated from the apparent molecular weight of the gene 20 protein (50,000d)(Botstein et al., 1973). Thus, the difference

in size of the new BamH1 band must again reflect the different orientation of Tn1 in these genomes. When the position of the BamH1 site in Tn1 derived above is taken into account, these insertions are found to be 0.9 kb apart, which is within the extent of gene 20.

Because the orientation of Tn1 insertions on the promoter proximal side of gene 9 can be deduced from their polar properties (Ch. 2, section G), and their genetic location from complementation tests (section D), it was possible to construct a physical map of this region of the genome (figure 16) from the BamH1 digestion data. To locate the insertions in P22Ap2 and Ap7, which are on the promoter distal side of gene 9 and whose orientations cannot be determined from polar effects, the BamH1 data was combined with the results from heteroduplex analysis and fitted to the four possible models for the orientations of these insertions. The only model which agreed was when the insertions were in the same orientation as in P22Ap63 (non-polar).

The relative positions of insertions, as derived from heteroduplex analysis, are included in figure 16 for comparison. In general there is good agreement between the BamH1 and heteroduplex methods. The worst case occurs with P22Ap12, whose insertion is found to be 7.5 kb from Tn10 by heteroduplex analysis but 9.3 kb from Tn10 by BamH1 digestion, a discrepancy of 20 to 25 per cent. Since the Tn1 insertion in P22Ap12 is farther than the other insertions from both the Bam-B fragment and Tn10, it is expected to be subject to the greatest error.

(iii) Double Digestions with EcoRI and BamH1

To combine the results of heteroduplex, EcoRI, and BamH1 analyses, double digestions with EcoRI and BamH1 were performed on several P22Ap phage DNAs (plate 3, figure 17). The double digest of P22 DNA lacks the

Figure 16. Physical Map of P22 from BamH1 Digestions of P22Ap Phage DNAs

Summary of the BamH1 digestions shown in plates 3 and 4 and the heteroduplex data of table 13.

FIGURE 16. Physical Map of P22 from BamHI Digestions of P22Ap Phage DNAs

distances in kilobases

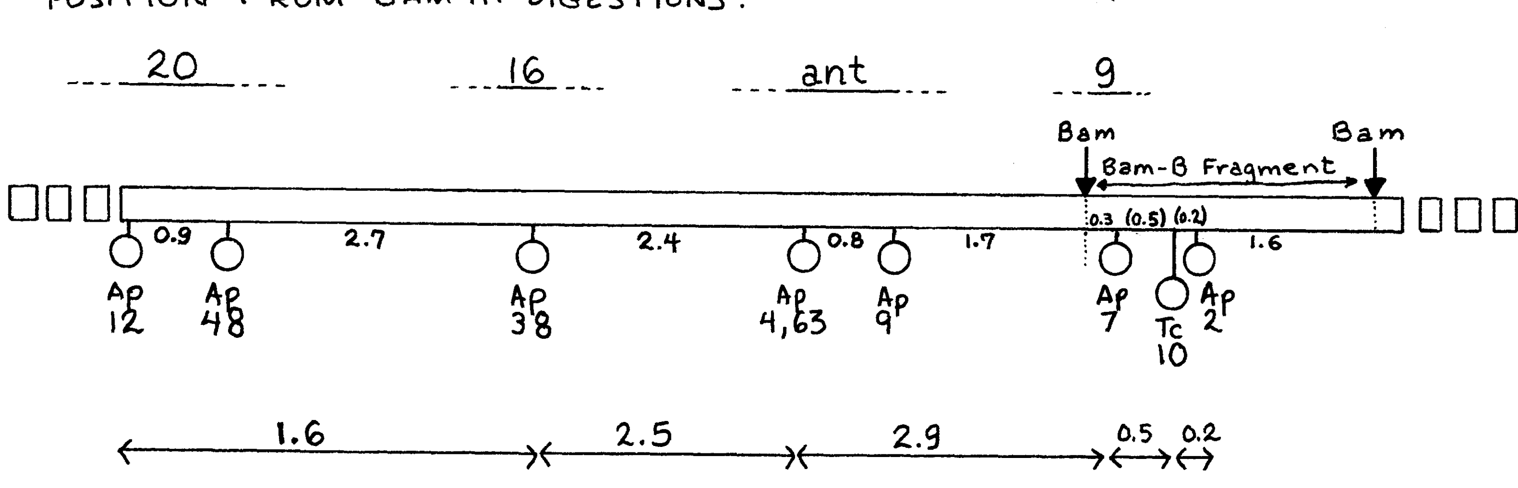


Figure 17. Representation of the EcoRI/BamH1 Double Digestion Patterns of Plate 3.

B = P22Ap9 DNA

E = P22Ap7 DNA

H = P22Ap63 DNA

K = P22 DNA

N = P22Ap4 DNA

Q = P22Ap2 DNA

FIGURE 17. EcoRI + BamHI Double Digestions of Plate 3

Ap9 B | Ap7 E | Ap63 H | WT K | Ap4 N | Ap2 Q

RI-B

RI-D

new

BamB

RI-E

new

RI-F

RI-G

RI-H

--- NON-STOICHIOMETRIC BAND

EcoRI-C fragment and contains two new fragments, one slightly larger (2.8 kb) than the Bam-B fragment and the other (1.8 kb) smaller than the EcoRI-E fragment. This shows that the two BamH1 sites in P22 DNA lie within the EcoRI-C fragment since the sum of the sizes of the new fragments and Bam-B (7.2 kb) is close to the size of the EcoRI-C fragment (7.3 kb). Since the two new fragments are also found in double digests of P22Ap and P22Ap7 DNAs, which lack the Bam-B fragment, these new fragments flank the Bam-B fragment within the EcoRI-C fragment.

When the sizes of the new P22Ap4 fragments (4.0 and 3.5 kb) and P22Ap63 fragments (5.6 and 1.8 kb) found in these double digests were used to compute the location of the BamH1 site in Tn1, as was done in the previous section, the Bam site is found to be 1.5 kb from the Tn1 terminus, and this segment to lie promoter proximal in the polar orientation, confirming the results of the previous section. Using this value for the location of the BamH1 cut in Tn1, and combing the data of previous sections, the physical map of P22 shown in figure 18 was constructed. This is discussed at length in the discussion section.

Figure 18. Physical Map of P22

The data from heteroduplex and restriction enzyme analyses have been combined with the data of Jackson (1977) to construct this map. The size of the Tn1 element is also shown for reference.

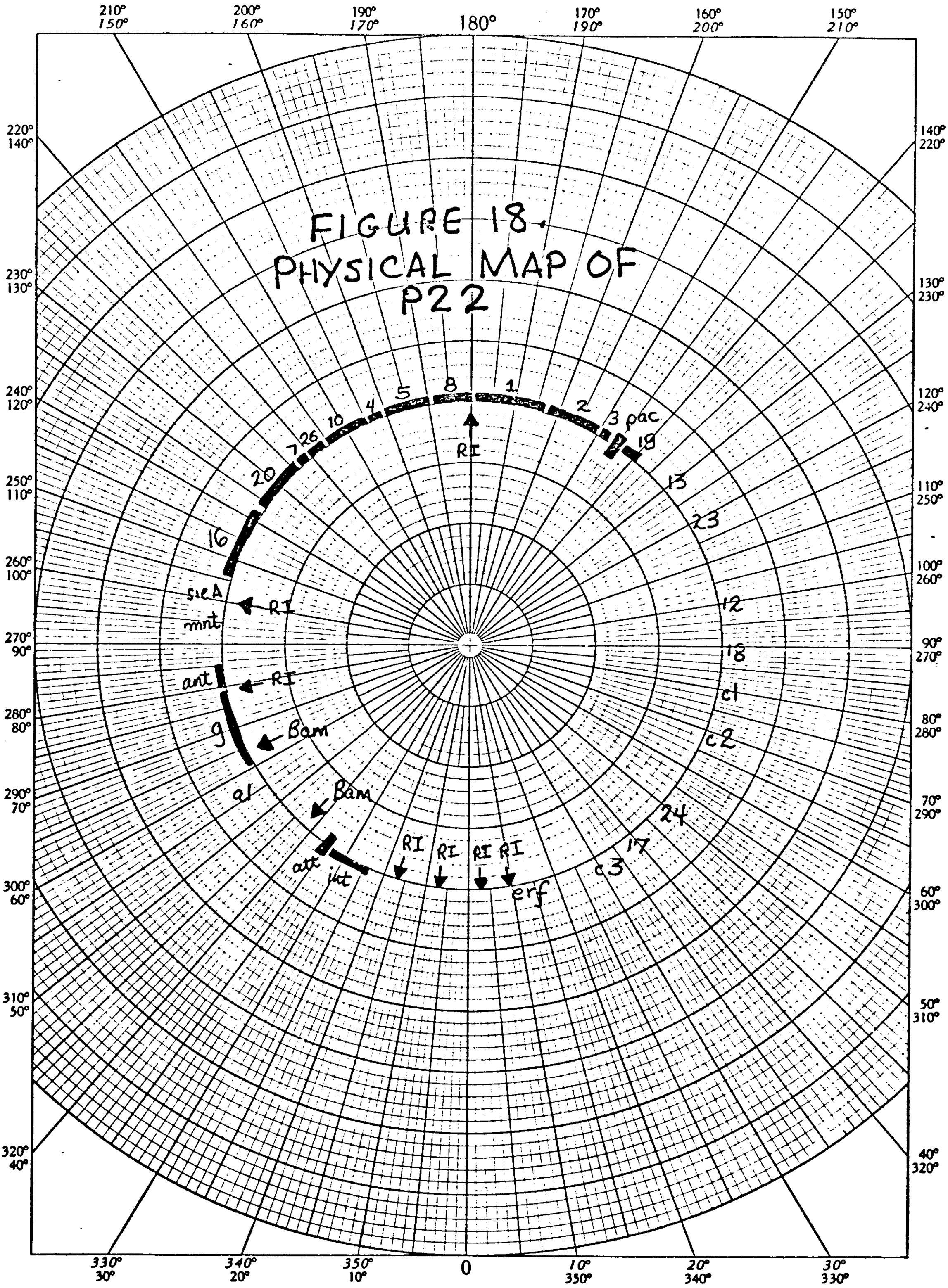


FIGURE 18.
PHYSICAL MAP OF
P22
8
1
5
4
10
2
3
pac
7
26
19
RI
20
13
16
23
sieA
RI
mnt
12
18
ant
RI
c1
g
Bam
c2
a1
Bam
24
17
RI
RI
RI
RI
att
int
erf
c3
180°
0

CHAPTER 3. DISCUSSION

I. Summary of Results

In chapter 2, the isolation and characterization of 76 insertions of the translocatable element Tnl into bacteriophage P22 was described. The major results relating to Tnl are:

(1) Insertions of Tnl into P22 are rare (10^{-10}/phage) and are non-randomly distributed in the genome, being found mainly in the vicinity of the *ant* gene.

(2) Insertions of Tnl within the *ant* gene are found at many (at least 13) sites and cause irreversible loss of *ant* function.

(3) Deletions generated by Tnl have non-randomly distributed endpoints. Prophage deletions isolated as amp^R transductants after low multiplicity infections by P22Ap phages extend from a point at or near the Tnl insertion to the vicinity of gene *12*. These deletions can end at many (at least eight) sites within gene *12*. Prophage deletions in P22Ap2, isolated as survivors of *mnt* induction, extend from the terminus of the Tnl insertion to various positions in the genome, but tend to occur in the neighborhoods of either gene *12* or *ant*.

(4) Tnl insertions can be strongly or weakly polar, depending on their orientation.

The major results relating to P22 are:

(1) Evidence supporting the existence of an operon of late genes has been obtained using polar Tnl insertions.

(2) Insertions of Tnl in either the phage's late operon or the *ant* gene can be polar on gene *9*, implying gene *9* is a member of the operon of late genes.

(3) A physical map of the P22 genome has been constructed using P22Ap

phages.

II. Characteristics of Tnl Translocation

A. Frequency of Translocation

Translocations of Tnl from the plasmid RP4 into P22 DNA were recovered at a frequency of about 10^{-10}/phage (table 3). This was not affected by temperature or irradiation of the cells with ultraviolet light. This contrasts sharply with the frequency of Tnl translocation betweeen plasmids, which varies from 10^{-6} to 10^{-2}/plasmid (Bennett and Richmond, 1976; Rubens et al., 1976). This difference in frequency is not understood but might reflect a requirement for supercoil ed recipient DNA. Mizuuchi and Nash (1976) found that bacteriophage λ will integrate only into supercoiled chromosomes. If this were also true for Tnl integration, P22 DNA would be a poor recipient since there is little supercoiled P22 DNA present during lytic growth (Botstein, 1968; Weaver and Levine, 1977).

B. Sites of Insertion

The locations of Tnl insertions in the P22 genome were inferred from the phenotypes of P22Ap phages (table 4), mapping with *virB am am* phages (figure 4) and prophage deletions (table 6, figure 5), and complementation tests with P22 amber mutants (table 5). Out of a total sample of 187 insertions, from 16 different lysates, 13 per cent of the insertions were found to map in the *ant* gene, which spans about 1.7 per cent of the genome (based on the apparent molecular weight of the antirepressor protein; Susskind, personal communication), while about 40 per cent of the insertions lie between gene *16* and the al region, an interval I estimate to be about 13 per cent of the P22 chromosome from the physical map. In contrast, only about 15 per cent of the insertions map in the essential region between gene *12* and *16*, which encompasses about 50 per cent of the chromo-

some. Thus, the Tn1 insertions are not randomly distributed throughout the P22 genome, but are concentrated in the region around genes 9 and ant.

A priori, there is reason to expect that the procedure used to isolate P22Ap phages will select against phages with insertions in some regions of the genome. Thus, phages with insertions which inactivate the c2 or mnt genes will not be able to lysogenize and form amp^R transductants, while insertions in the vicinity of genes 18 and 12; by analogy with bacteriophage λ , might cause a cis-dominant defect by interfering with the transcription required for initiation of replication (Dove et al., 1971). Thus, phages with insertions in these regions might not be recovered by the procedure employed.

On the other hand, insertions in the other essential genes should cause recessive mutations which can be complemented by wild type genomes. Nevertheless, these phages might also be underrepresented since complementation is usually not completely efficient. Thus, if Tn1 integrates at random sites in P22 DNA, it is reasonable to expect that insertions in essential genes will be less frequent than insertions in non-essential regions. However, in this case the distribution of insertions should be random among essential genes, and this was not found. Rather, insertions occurred mainly in those essential genes nearest the ant-9 region (figure 4). Thus, I conclude that the non-random distribution of the Tn1 insertions in P22's genome is not an artifact of the procedure used to obtain these insertions. It is possible, however, that insertions in the vicinity of either genes 18 and 12, c2, or mnt would not have been recovered.

In contrast to the regional specificity described above, insertions within the ant gene (estimated to be 700-800 bp in size) were found at at least 13 different sites (figure 5). It is not known how these inser-

tions are distributed throughout the gene, but the fact that they are interspersed among the ant$^-$ point mutations suggests they are not tightly clustered. These insertions occur in either orientation with equal frequency, which may reflect the symmetry of the terminal inverted repeats, as these sequences are important for translocation (Heffron et al., 1977). Thus, on a more local level, Tn1 integration into P22 DNA is non-specific with respect to both location and orientation.

These results are similar to those reported by Rubens et al. (1976) for Tn1 translocation between plasmids. However, the type of distribution of insertion sites seen for Tn1 is fundamentally different from that seen for the temperate phages λ and P22, or another translocatable drug resistance element, Tn10. Kleckner et al. (1977) isolated a number of Tn10 translocations from P22Tc10 into the histidine operon (10 kb) of *Salmonella typhimurium* and found that about half of the insertions were at the same site and the remainder were distributed among several other discrete sites. Similarly, bacteriophage λ and P22 integrate almost exclusively at a single site in the bacterial chromosome, although λ can integrate at lower frequency into other sites when its hot spot is absent (Shimada et al., 1972). Thus, whereas λ, P22, or Tn10 integration occurs frequently at a hot spot (local specificity), Tn1 integration occurs at many sites (local non-specificity) in a hot region (regional specificity).

C. Mechanisms of Translocation

There are 2 general types of explanations for the non-random distribution of insertions in P22: either this distribution reflects an inherent characteristic of Tn1 translocation or else the distribution results from some constraint imposed by P22's physiology. Perhaps it is not possible to recover insertions in most essential genes because the structure of

the P22 chromosome, as a "folded chromosome", membrane complex, transcription complex, replication complex, or possibly a complex with packaging proteins, may make only certain regions of the DNA accessible for translocation. This particular model would require further assumptions since all regions of P22 DNA are available for genetic recombination, an exchange reaction analogous to translocation. However, this certainly does not rule out the idea that selection could be imposed by P22's physiology.

On the other hand, the distribution of insertions may reflect some inherent aspect of the Tnl translocation mechanism. In this case it is necessary to resolve the paradox that insertion sites are randomly distributed within a small region (for instance, the _ant_ gene) but much more non-random over the larger region of P22's chromosome. A plausible mechanism resolving these observations is provided by the restriction enzyme of _E. coli_ K (Murray et al., 1973). This enzyme recognizes a specific, relatively rare, DNA sequence which is the only site at which it can bind to the DNA. The sequences at which it cleaves DNA, however, are non-specific and occur frequently near the binding site but less frequently farther away, as if once bound, the K restriction enzyme travels along the DNA for a (normally distributed?) distance before it cleaves. This generates a high frequency of cuts in the region near the binding site and a low frequency in regions farther away. DNA cleavage must also occur during Tnl integration and it is possible the cleavage determinant operates in an analogous manner to the _E. coli_ K restriction nuclease. P22 DNA would then have a site, in the vicinity of the _ant_ gene, at which the translocation determinants can recognize the DNA, but there would be no sites in the late genes. These determinants would travel along the DNA before integrating Tnl, thus accounting for the high

concentration of insertions in _ant_, the low concentration in the nearby essential genes _16_ and _20_, and the extreme scarcity in essential genes even more removed from _ant_. Heffron et al. (1975b) remarked on the fact that Tn1 insertions seemed to occur more frequently in an AT-rich region of a small plasmid. The partial denaturation map of P22 (Tye et al., 1974b) is consistent with the notion that the _ant_ and a1 regions of P22 DNA are the AT-rich regions of the molecule. Perhaps the sequence recognized by Tn1 is more apt to occur in these regions.

The experiments presented in this thesis do not allow a conclusive choice to be made between these explanations. However, I prefer the second explanation for several reasons. The restriction enzyme model has precedent, and offers insights into several observations about Tn1. Heffron et al. (1975b) also observed the non-random insertion specificity of Tn1 into a small plasmid and suggested that Tn1 inserts at a short, specific sequence which occurred frequently in the DNA. Bennett and Richmond (1976), however, observed large variations in the frequency of translocation of Tn1 into various large plasmids, including one where translocation was not detectable. This variation is not consistent with integration at a widespread sequence, since one would expect uniformly higher frequencies of translocation into larger plasmids. However, these observations may both be explained by a model invoking rare binding sites and frequent, nearby, integration sites. Thus, the small plasmid of Heffron et al. would contain one or more clustered sites, analogous to P22, while the plasmids of Bennett and Richmond would contain varying numbers of binding sites, perhaps some in regions essential for the maintenance of the plasmid, and thus the different frequencies of translocation would reflect the number and location of the binding sites in different plasmids.

A final reason for preferring the restriction enzyme model is that deletion generation by Tnl exhibits a similar site specificity. This is discussed more fully below, but the tendency for deletions to end in or near gene 12, yet at many sites within gene 12, is analogous to the insertion specificity. As discussed in the introduction, insertion and deletion generation by translocatable elements probably occur by related mechanisms. Thus the sequence specificity exhibited by deletion endpoints probably reflects a general characteristic of illegitimate recombination events catalyzed by Tnl.

III. Characteristics of Tnl Insertions

A. Structure of Insertions

Tnl insertions in P22 DNA were found to be about 4.8 kb in size and are flanked by short (about 100 bp) inverted repetitions (table 13). Although one phage, P22Ap14 with an insertion in ant, was found to contain a deletion of P22 DNA (figure 5), no further evidence for imprecise insertion was found among the six insertions analyzed by electron microscopy.

B. Polarity of Tnl

Tnl insertions in the P22 genome were found to be polar on late genes by complementation tests (table 5). This phenotype was quantitated by directly measuring the production of p9, the product of gene 9 (table 9). It was found that Tnl insertions reduced p9 production by either about two-fold or twenty-fold, depending on their orientation (Ch. 2, section M; Rubens et al., 1976).

The degree of polarity shown by Tnl in the weakly polar orientation is in the range shown by nonsense mutations (Newton et al., 1965). However, the degree of polarity of nonsense mutations is dependent on their location within an operon and this is not true of Tnl in the weakly polar orienta-

tion.

Polar mutations caused by insertions of IS sequences are reported to reduce gene expression at least 100-fold (Saedler et al., 1972). Thus, in the strongly polar orientation, although Tnl is more polar than nonsense mutations, it may be less so than IS sequences. It should be noted that insertions of IS sequences were selected on the basis of strong polarity.

Both nonsense - and IS-mediated polarity is suppressed by mutants of the transcription termination factor rho (Das et al., 1976). Thus these types of polarity involve transcription termination by rho factor. P22's gene 23 appears to code for a protein whose function is to act at a termination signal to allow transcription to proceed into the late operon (Roberts et al., 1976). Thus, Tnl causes polarity in the presence of the antiterminator produced by gene 23. If, by analogy with nonsense mutations and IS sequences, the mechanism of Tnl-mediated polarity involves transcription termination, either gene 23 product has no effect on this termination or, if gene 23 product does affect it, the polarity would be much greater in the absence of gene 23 product. Thus, because of the possibility of antitermination by gene 23 product, it is not possible to interpret clearly the degrees of polarity observed with Tnl. However, the difference between orientations remains a striking feature as observed in the present case as well as in the plasmid experiments of Rubens et al. (1976) where, presumably, no 23-like product is involved.

C. Reversion

Insertions of Tnl in the *ant* gene were found to cause non-reverting mutations of *ant* (Ch. 2, section II-H). This provides a convenient criterion for determining whether a mutation caused by Tnl is due to integration within a structural gene or polarity since phenotypes caused solely

by the polarity of Tn1 revert readily (Ch. 2, section II-I).

Failure to revert to wild type, i.e. reconstruct an intact gene, could be due to either imprecise integration or excision. Although Tn1 insertions examined with the electron microscope appeared to have integrated without causing deletion of any P22 material (Ch. 2, section II-L), P22Ap14 (which was not examined by electron microscopy) was found by genetic criteria to contain a deletion (Ch. 2, section II-F). If all Tn1 insertions caused small deletions upon integration, they would not revert to wild type. However, except for P22Ap14, there is no evidence that this occurs in P22Ap phages.

On the other hand, integration could be precise but excision of Tn1 could be imprecise, leaving a small insertion, deletion, or both. Among plaque forming revertants of P22Ap4 (Ch. 2, II-I, K), $\underline{ant}^-$ phages were found which, by genetic criteria, had lost the amp^R phenotype but no $\underline{ant}$ markers (putative small insertions), had a deletion removing Tn1 and $\underline{ant}$ material on either side of the insertion (small deletions), or, by physical mapping, contained a deletion starting within Tn1 and extending into P22, thereby leaving a part of the insertion while also deleting some P22 material. Thus, it seems that all possible types of imprecise excisions can occur. Kleckner et al. (1977) have shown that no more than 1 in 10,000 excisions of Tn10 from within the histidine operon of *Salmonella typhimurium* are precise, and thus, if precise excision of Tn1 does occur, it may simply be too rare to detect.

Insertion mutations caused by bacteriophage Mu also do not revert to wild type (Bukhari, 1975). However, certain derivatives of Mu, containing mutations, do revert to wild type. Thus, failure to revert does not necessarily imply an inability to integrate or excise precisely.

IV. Deletion Generation by Tn1

A. Deletions in Transductants Isolated from Low Multiplicity Infec-Infections

Two types of deletions were found in transductants isolated from low multiplicity infections (figure 8). The minority class extended from a point near the phage attachment site to a variety of positions in the genome. These deletions did not appear to be linked to a Tn1 insertion and it is not known if their formation required Tn1 or was due solely to phage and bacterial functions.

The major (75-80 per cent) class of deletions all ended at a point that was, by genetic criteria, at or very near to a Tn1 insertion. For this reason the formation of these deletions is believed to involve Tn1 functions. These deletions always extended to the region of the genome around gene 12; thus both endpoints are non-randomly located. However, although one endpoint is limited to the region of gene 12, it can be at many sites within 12 (figure 9).

Deletions isolated by the same technique in P22Tc10 (containing a Tn10 insertion) also had one endpoint fixed at the insertion, while the other endpoint was much more randomly distributed throughout the genome (Chan and Botstein, 1972). This shows that the deletion specificity observed with P22Ap phages is due to the Tn1 insertion and is not a procedural artifact.

The observation that Tn1 seems to show regional (but not local) specificity both in translocation and deletion formation fortifies the notion that these two processes are related mechanistically.

B. Deletions in Survivors of mnt Induction

Deletions isolated in P22Ap2 (insertion in the a1 region) as survivors

of mnt induction were required to be ant$^-$ and 9$^-$ (Ch. 2, section II-J). Thus, the fact that these deletions all have an endpoint near the Tn1 insertion could be an artifact of this procedure and need not imply that the deletions were generated by Tn1 functions. However, when four of these genomes were analyzed with the electron microscope (Ch. 2, section II-K), in each case the deletion was found to end at the terminus of an intact Tn1 element. Since there is about 700 bp between gene 9 and the Tn1 insertion in P22Ap2 (table 12), and the resolution of the heteroduplex methods is at least 100 bp, if the deletions had ended at random sites between 9 and Tn1 they would have been clearly resolved. Thus, at least four of these deletions end at the Tn1 terminus, strongly implying that Tn1 functions were involved in their formation. Furthermore, these four deletions extend to the ant region and consititute a significant fraction (4/12) of the deletions isolated. Thus it appears that both Tn1 translocation and deletion generation have a tendency to occur in this region. The deletions, like the insertions, occur at locally different sites (figure 5), showing that this specificity cannot be accounted for by a single hot spot.

In addition to the deletions which end in the ant region, a significant fraction (5/12) end near gene 12. These deletions also have an endpoint at or near the Tn1 insertion but, as discussed above, this was demanded by the selection. Nevertheless, I conclude that these deletions are formed by Tn1 functions since the tendency for deletions to end preferentially around gene 12 was shown above to be a characteristic of Tn1 generated deletions.

Thus, by this selection it is shown that Tn1 generated deletions frequently contain an intact insertion and extend from the terminus of

Tn1 to either the region around ant or 12. This selection is less demanding than that of the previous section since it does not require deletions to be long enough to restore terminal repetition. Because of this length requirement, deletions ending in the ant region would have been missed by the procedure of the previous section.

Three deletions were also found by the mnt-ts selection which ended at different positions in the late genes. It is not possible to tell if formation of these deletions required Tn1 functions since genetic linkage to Tn1 was demanded by the selection. Deletions isolated by this procedure occur at about one per cent of the frequency of the deletions isolated as low multiplicity transductants. This decreased frequency of deletions could be related to the observation that UV irradiation of P22Tc10 stimulates the formation of deletions (Chan, 1974). In any case, it is possible that these three deletions are not generated by Tn1 but are the background level of spontaneous deletions which are seen because the frequency of Tn1 generated deletions is so low.

C. Relation of Deletion Formation to Translocation

The fact that deletions end at the terminus of an intact Tn1 element suggests that these termini are important in deletion formation. Heffron et al. (1976) presented evidence that the terminus contains a site recognized in translocation. By analogy with bacteriophage λ integration, this site might determine the integration site specificity of Tn1. Thus, the fact that Tn1 deletions show a tendency to end in the ant region, which is also a preferred region for insertion, indicates that the same site is recognized in deletion formation and translocation. A further similarity between these processes is that, although in each case this is a hot region, it does not contain a hot spot for either insertion or deletion end points.

Thus both insertions and deletions could be generated by a mechanisms like that of the _E. coli_ K restriction enzyme.

The Tnl generated deletions also show a striking tendency to end in the region of gene _12_ and, since this is a hot region without a hot spot, the same type of mechanism may be operating. However, only one out of 76 insertions mapped in this region and thus translocation does not appear to reflect this specificity. One explanation for this is that insertions in this region are rarely recovered because they cause a defect in replication which cannot be complemented (see Ch. 3, section II-B). Thus, if this is a hot region for translocation, the procedure employed for isolating insertions could miss it.

On the other hand, insertions may not occur in this region. This would imply that the mechanisms of translocation and deletion generation have different site specificities although they are otherwise related, (i.e. regional specificity, local non-specificity, and an essential role for the Tnl termini).

One speculative way to account for this assumes the site specificities for deletion and insertion formation are truly different. Then, deletions ending in _ant_ would be generated by translocation of Tnl from its location in the parent into a new site, followed by recombination between an insertion in another phage, located at the parental site, and the newly located Tnl, to generate the deletion. Deletions ending in _12_ would be generated by a mechanism independent of translocation with a different site specificity. Alternatively, the specificity of translocation may derive from a sequence formed when the termini are adjacent while that of deletion generation derives from a sequence at the terminus when termini are apart, i.e. "half-site specificity". In this case the deletion mechanism might be expected

to recognize the translocation sites as well as some additional half-sites, not recognized in translocation.

In conclusion, deletions generated by Tn1 are formed by a mechanism similar to translocation but possibly differing somewhat in site specificity. Kleckner et al. (1977) have shown that deletion generation by Tn10 appears to have a different specificity than Tn10 translocation but in this case, insertion specificity (which shows hot spots) is less random than deletion specificity, where strong hot spots have not been observed (Chan, 1974).

V. Further Discussion Regarding Tn1

As discussed previously, the non-random distribution of Tn1 insertions and deletion endpoints (regionally specific but locally non-specific) contrasts with the non-random distributions seen with Tn10 or bacteriophage λ (locally specific). Furthermore, other translocatable elements, such as bacteriophage Mu or Tn5 (kanamycin resistance), integrate with little or no site specificity (regionally and locally non-specific)(Bukhari and Zipser, 1972; Berg, 1977). Thus, the mode of site specificity of Tn1 is unique among the translocatable elements studied to date.

The fact that this same type of non-random distribution occurs for both deletions and insertions of Tn1 implies that a similar mechanism operates in these two processes. This contrasts with Tn10, whose insertions, but not deletions, occur at a restricted number of sites. In addition, I have shown by heteroduplex analysis and genetic mapping that deletions are often located adjacent to an intact Tn1 insertion. This strongly suggest that the terminus of Tn1 plays a role in deletion generation. Heffron et al. (1977) presented evidence which implied that the Tn1 terminus contains a site which is recognized during translocation,

and it is probable that this site has a similar function during deletion formation.

To reconcile the regional specificity with the local non-specificity of Tn1 integration and deletion generation, I have suggested a model based on the properties of the E. coli K restriction enzyme. This model asserts that Tn1 determinants recognize a few specific sites in DNA and cause insertions or deletions to occur at non-specific sites nearby. However, during translocation or deletion formation, cleavage of DNA must also occur at the Tn1 element. Since the element is found to be intact following integration or deletion, and since deletions lie adjacent to the element, these cuts must occur at specific sites at the Tn1 termini. Thus, both site specific and non-specific cleavages occur during translocation or deletion formation by Tn1.

Another property of Tn1 insertions which may be unique is their failure to produce wild type revertants. Among most other translocatable elements, at least 98 per cent of the insertions can produce wild type revertants (Kleckner, 1977). An exception is bacteriophage Mu, which normally causes an irrversible mutation upon integration. However, there is a class of mutants of Mu which can integrate and excise precisely (Bukhari, 1975) and thus resemble other translocatable elements.

Lastly, one P22Ap phage was found to contain a deletion. It is possible the formation of this deletion was related to the integration of Tn1. As many as 25 per cent of the insertions of Mu have associated deletions (Kleckner, 1977) and, thus, this may be a more general property of Tn1.

VI. An Operon of Late Genes in P22

The late genes of bacteriophage λ constitute an operon whose transcription requires the product of the Q gene (Herskowitz and Signer, 1970a; Skalka et al., 1967). It is believed that the Q gene product is an antiterminator which allows a short leader transcript to be extended into the late operon (Roberts et al., 1976). Similarly, the late genes of phage P22 are coordinately controlled by gene 23 (Lew and Casjens, 1975). Hilliker (1974) showed that genes 23 and Q are functionally, if not structurally, equivalent since they can complement and recombine with each other. Moreover, Roberts et al. (1976) presented evidence for a leader RNA and suggested that gene 23 allows this transcript to be extended into the P22 late genes. Thus, by analogy with λ , these results strongly suggest that the P22 late genes are organized in an operon.

Using polar insertions, I have found direct evidence for the existence of this operon of late genes. Complementation tests (table 5) show that a polar insertion in gene 7 reduces expression of genes 20 and 16 while a polar insertion in gene 20 affects 16. In addition, insertions in genes 26, 7 or 20 reduce gene 9 expression (table 9). These results show that genes 26, 7, 20, 16, and 9 are coordinately transcribed and provide further evidence for a single operon of late genes.

VII. The Expression of Gene 9

The fact that polar insertions in the late operon can reduce expression of gene 9 by at least 95 per cent (table 9) shows that p_{LATE} is the major, if not only, promoter for gene 9 transcription. This result has interesting implications for the expression of the ant and 9 genes.

Since antirepressor is produced early in infection (M. Susskind, personal communication) it might be expected that transcription from p_{ANT}

could extend into gene 9. However, since p9 is not seen until late in infection (Botstein et al., 1973) and polar insertions in late genes prevent essentially all expression of gene 9, transcription of gene 9 from p_{ANT} must not occur to a significant extent. One explanation for this is that there is a transcription termination site between genes ant and 9. Since this site must be overcome in order for transcription of gene 9 from p_{LATE} to occur, it might be sensitive to antitermination by gene 23 product. In this case, the structure of the late operon of P22 can be thought of as being analogous to the rightward early operon of bacteriophage λ (Herskowitz and Signer, 1970b). In each case there are two termination signals within the operon, and the promoter-distal signal occurs at a region of DNA which is unrelated to the function of the operon (the P-Q region in λ; the sieA-mnt-ant region in P22).

In order for gene 9 to express from p_{LATE}, RNA polymerase must traverse the sieA-mnt-ant region and thus it might be expected that ant is expressed from p_{LATE} at late times during infection. However, this is not true because ant is turned off late in infection (M. Susskind, personal communication). Nevertheless, since insertions in ant are polar on gene 9, and polarity is thought to involve transcription termination (see Ch. 3, section II-B), it is probable that ant is transcribed, though not expressed. This suggests that the turn-off of ant at late times occurs through a post-transcriptional control mechanism.

VIII. Physical Map of the P22 Genome

The physical map shown in figure 18 was constructed from the data in this thesis, the EcoRI map of Jackson (1977), and the apparent molecular weights of P22 proteins as judged by acrylamide gel electrophoresis (Botstein et al., 1973; Poteete and King, 1977).

The only genes whose positions are known with any certainty are those between gene 7 and att. The insertion in P22Ap30, which mapped between genes 1 and 8, was found to be 8.1 kb from the insertion in P22Ap12 by heteroduplex analysis (data not shown) but this is probably too large a distance to be reliably measured (see Ch. 2, section II-M).

Several other points are worthy of note:

(1) The late operon: The interval from pac (between genes 19 and 3) to the end of gene 9 is about 43 per cent of the P22 chromosome. Since the late operon begins between genes 23 and 13, the size of the late operon (and its transcript) is probably about 50 per cent of the chromosome (21 kb).

(2) The sieA-mnt-ant region: This region is about 7 per cent of the chromosome (3 kb). The deletion in DB7283 ends about 800 bp from the end of the EcoRI-E fragment and, since this strain is mnt^-, mnt must lie in this fragment.

(3) The ant gene: This gene is at the end of the EcoRI-E fragment. The insertion in P22Ap9, which maps at the end of the ant gene, is located in the E-fragment but is very close to the end and its distance from this EcoRI site cannot be determined precisely. It is possible that this EcoRI site lies within the ant gene.

(4) Gene 9: The distance from P22Ap9's insertion in ant to the insertion of P22Ap7, at the C-terminal side of gene 9, is about 5 per cent of the chromosome. This is approximately the size of gene 9, hence there is not a large space between ant and 9. In addition, since the P22Ap7 insertion is within the Bam-B fragment, but very near the end, it is likely that BamH1 cuts within gene 9.

(5) The phage attachment site (att): Based on the fact that the Tn10

insertion in P22Tc10 is 6 per cent from att (Chan and Botstein, 1976), I calculate that att is within the EcoRI-C fragment, in accord within Jackson (1977), and outside of the Bam-B fragment.

IX. Use of P22 to Study Translocatable Elements

The experiments of this thesis show that P22 is a useful vehicle for studying translocatable drug resistance elements. The isolation of large numbers of insertions is reliably achieved with the HFT test and the size of the element is not a barrier since insertions of large elements can be readily accommodated by the headful packaging mechanisms.

Once integrated, the insertion can be treated like a point mutation and crosses and complementation tests performed. Mapping of large numbers of insertions can be reliably accomplished with virB am am phages and the phenotype test. Further fine structure mapping is possible with the large number of mutants and prophage deletions of P22. The fine structure map of ant is an example of this procedure.

Polar effects of insertions can be determined by complementation tests and quantitation of the production of p9. The p9 assay is extremely sensitive, being reliable at least to the femptomolar range. The possibility of antitermination effects by genes 23 or 24 could obscure polarity but, if this can be proved, it would give an insight into the mechanism of polarity.

Insertions in ant and 9 are useful for studying reversion and imprecise excision. Several selections exist for the isolation of deletions in P22 transducing phages and mapping of deletions can be performed by the methods mentioned above. Furthermore, since the regions adjacent to ant or 9 are not essential for particle formation, genomes containing deletions within this interval can be purified in large quantities for

physical analysis. These physical studies can be performed by either heteroduplex or restriction enzyme analysis.

Because of the importance of translocatable elements in the evolution of bacterial genomes, particularly antibiotic resistance plasmids, and as models for the regulation of gene expression, the development of the bacteriophage P22 system as a vehicle for studying translocatable elements adds a significant tool to the store of molecular genetics.

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Appendix I: Summary of P22Ap Phages and their Properties

Phage	Lysogen	Lysate	Phenotype	Location			Orientation
				virB am am	E. O. P. or Complementation	Conclusion	
Ap2	7197	1	non-essential	outside	al region	al region	
Ap4	7198	1	ant^- 9^-	16-9	ant	ant	P
Ap5	7199	1	9^-	outside	9	9	
Ap7	7200	1	9^-	outside	9	9	
Ap9	7201	1	ant^- 9^-	16-9	ant	ant	P
Ap10	7326	94	9^-	16-9	9	9	
Ap11	7327	94	ant^- 9^-	16-9	ant	ant, also has deletion	P
Ap12	7328	94	essential	20-16	20	20 or between 7 and 20	P
Ap13	7329	94	9^-	16-9		9	
Ap14	7330	94	ant^- 9^-	16-9		ant also has deletion	
Ap15	7331	94	non-essential	outside			
Ap16	7332	94	ant^-	16-9	ant	ant	NP
Ap17	7338	94	essential	16-9			
Ap18	7334	94	ant^-	16-9	ant	ant	NP
Ap19	7335	94	ant^- 9^-	16-9	ant	ant	P
Ap20	7336	95	non-essential	outside			
Ap21	7337	95	9^-	16-9		9	
Ap22	7338	96	9^-	16-9		9	
Ap23	7339	96	non-essential	outside			

Ap24	7340	96	ant^{-} 9^{-}	16-9	ant	ant	P
Ap25	7341	96	9^{-}	16-9	9	9	
Ap26	7342	96	essential	20-16			P
Ap27	7343	96	essential	10-20	26	26	NP
Ap28	7344	97	9^{-}	16-9		9	
Ap29	7345	97	ant^{-}	16-9	ant	ant	NP
Ap30	7346	97	non-essential	1-8		between 1 and 8	NP
Ap31	7347	97	ant^{-}	16-9	ant	ant	NP
Ap32	7348	98	ant^{-} 9^{-}	16-9	ant	ant	P
Ap33	7349	98	non-essential	outside			
Ap34	7350	98	essential	10-20	7	between 7 or 26 + 7	P
Ap35	7351	98	9^{-}	16-9	9	9	
Ap36	7352	98	essential	10-20			P
Ap37	7353	98	ant^{-}	16-9	ant	ant	NP
Ap38	7354	98	essential	16-9	16	16	NP
Ap39	7355	98	essential	16-9			
Ap40	7356	98	essential	20-16			P
Ap41	7357	99	9^{-}	16-9		9	
Ap42	7358	99	ant^{-} 9^{-}	16-9	ant	ant	P
Ap43	7359	99	non-essential	outside			
Ap44	7360	99	ant^{-} 9^{-}	16-9	ant	ant	P
Ap45	7361	99	ant^{-} 9^{-}	16-9	ant	ant	P
Ap46	7362	99	ant^{-}	16-9	ant	ant	NP

Ap47	7363	100	9^-	outside		9	
Ap48	7364	100	essential	20-16	20	20	NP
Ap49	7365	100	ant^- 9^-	16-9	ant	ant	P
Ap50	7366	100	ant^- 9^-	16-9	ant	ant	P
Ap51	7367	100	non-essential	outside			
Ap52	7368	100	essential	20-16			P
Ap53	7369	100	ant^-	16-9	ant	ant	NP
Ap54	7370	101	essential	outside			
Ap55	7371	101	essential	outside			
Ap56	7372	101	essential	10-20			P
Ap57	7373	101	ant^- 9^-	16-9	ant	ant	P
Ap58	7374	101	essential	20-16			P
Ap59	7375	101	non-essential	outside			
Ap60	7376	101	ant^-	16-9	ant	ant	NP
Ap61	7377	101	9^-	16-9		9	
Ap62	7378	101	ant^-	16-9	ant	ant	NP
Ap63	7379	101	ant^-	16-9	ant	ant	NP
Ap64	7383	87	essential	16-9			
Ap65	7384	87	9^-	16-9		9	
Ap66	7385	87	non-essential	outside			
Ap67	7411	110	ant^-	16-9	ant	ant	NP
Ap68	7412	110	9^-	16-9		9	
Ap69	7413	110	non-essential	16-9			NP
Ap70	7414	110	essential	20-16			NP

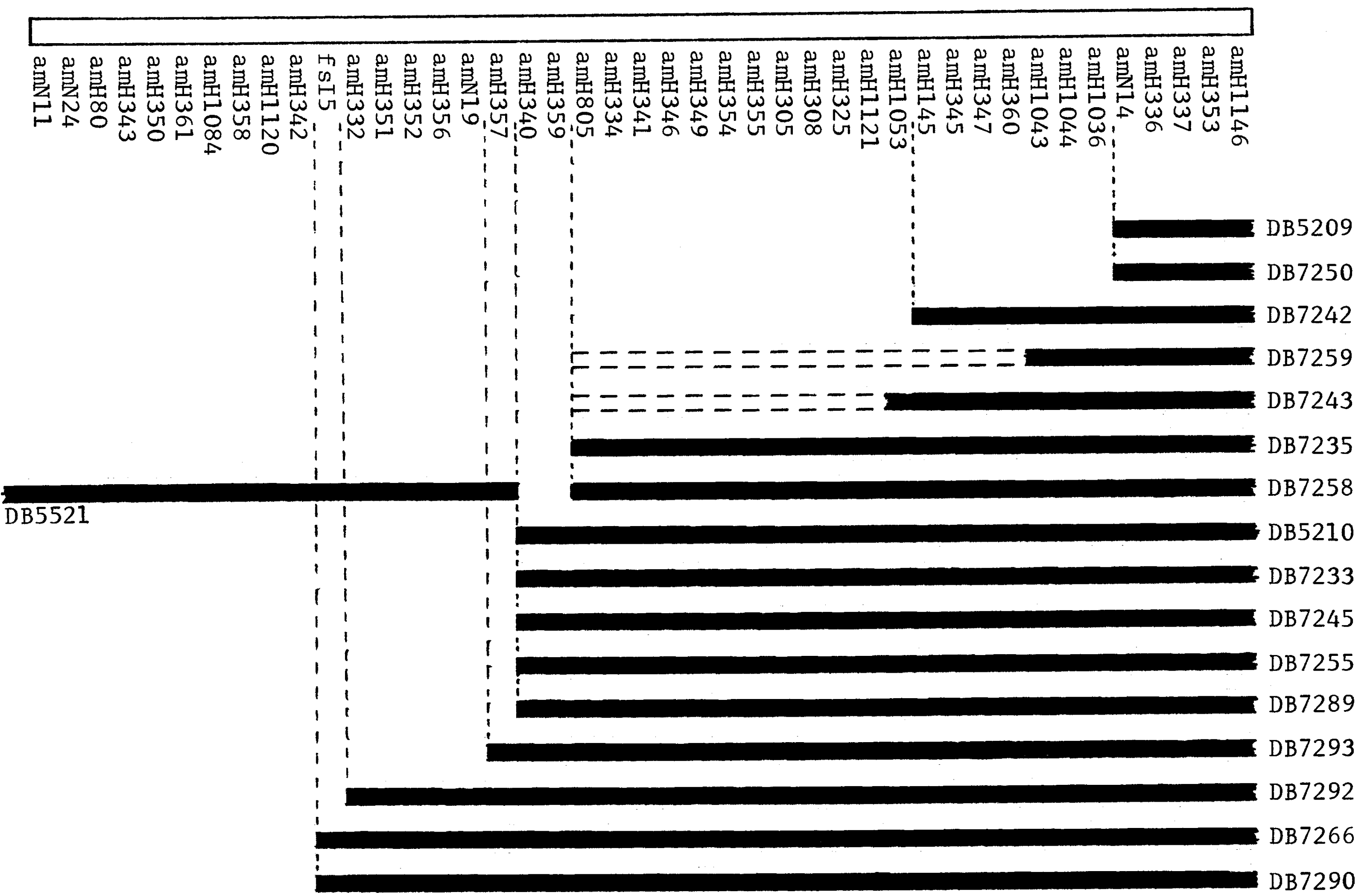
Ap71	7415	110	essential	outside			
Ap72	7416	113	9^-	16-9		9	
Ap73	7417	113	ant^- 9^-	16-9	ant	ant	P
Ap74	7418	113	essential	16-9			NP
Ap75	7419	113	non-essential	outside			
Ap76	7420	114	9^-	outside		9	
Ap77	7421	114	non-essential	outside			
Ap78	7422	115	non-essential	outside			
Ap79	7423	115	9^-	16-9		9	
Ap80	7424	115	essential	12-23			

Appendix II: Fine Structure Map of Gene 12

The fine structure map of gene _12_ is presented in figure 19. This map is based on the efficiencies of plating on prophage deletions of 42 amber mutant and one frameshift mutant phage (see Methods, section L). The amber mutations are all independently derived but do not necessarily represent different mutations. The prophage deletions DB5209 and DB5210 (Chan and Botstein, 1972) were isolated as low multiplicity tet^R transductants from P22Tc10. The deletion is DB5521 (Chan, 1974) was derived, by a different procedure, from a lysogen of P22Tc10 and extends into the bacterial chromosome to the left of the prophage. The other deletions are described in chapter 2, section II-I.

The deletions of DB7259 and DB7243 gave ambiguous results for rescue of those alleles in the region indicated by the dotted line; thus the precise endpoints of these deletions cannot be determined. These ambiguous results could occur if the mutations are very near to the deletion endpoint or if some further chromosome aberration (a small inversion for example) were present in this region.

Figure 19. Fine Structure Map of Gene 12



Appendix III: Results of Digestions of P22 DNA with Various Restriction Enzymes

The results of restriction enzyme digestions of P22 DNAs are summarized in table 15. The enzymes Bgl II, Kpn I, Xba I, and Xho I do not appear to cleave P22 DNA since only a large (> 20kb) band appears in gels of these digests.

Digestion by Sal I produces one large (> 20kb) band and one smaller (7.5kb) band which appears to be non-stoichiometric. In double digests of Sal I and EcoRI, both the 7.5kb Sal I band and the EcoRI-B band, (see figure 12) are missing. Thus it appears that Sal I cleaves P22 DNA at one site, 7.5kb (counterclockwise in figure 12) from the _pac_ site.

BamH1 digestion is described in chapter 2, section II-M.

Sma I produces a large (> 20kb) and a small (2.1kb) fragment which has not been mapped.

Bgl I produces two or three large (10-15kb) pieces and a small (0.8kb) piece. In double digests by Bgl I and EcoRI, the RI-A,E,F, and H bands (see figure 12) as well as the small Bgl I band are missing. Thus, Bgl I cleaves within each of these EcoRI fragments and EcoRI cleaves within the small Bgl I fragment. In addition, Bgl I cuts at three sites within Tn1 to produce a 1.5kb and 1.0kb fragment of Tn1 DNA.

The EcoRI digest is described in chapter 2, section II-M.

The Pst I digest contains one fragment of 15-20kb and 7 fragments ranging in size from about 4kb to less than 0.9kb.

The Hpa I digest contains fragments from 10kb to about 1kb in size. The largest ($\simeq$ 10kb) fragment spans the region of P22 containing the BamH1 cleavage sites.

Table 15. Action of Restriction Enzymes on P22 DNA

Restriction Enzyme	Number of Bands Seen on Gel	Number of Cuts in P22	Number of Cuts in Tn1
Bgl II	1	0	ND
Kpn I	1	0	0
Xba I	1	0	ND
Xho I	1	0	ND
Sal I	2	1	0
BamH1	2-3	2	1
Sma I	2	1-2	0
Bgl I	3-4	4	3
EcoRI	8	7	0
Pst I	8	7	ND
Hpa I	12	11	0

ND = not determined