# Genetic Map of Coliphage 186

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by

Stephanie Hocking, B.Sc.(Hons.)

Department of Biochemistry

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## SUMMARY

This thesis describes the construction of a genetic map of phage 186. 186 has enough DNA to code for about 30 proteins and the 61 amber mutants used in this study have been assigned to 22 essential genes by complementation studies. There are in addition three known non-essential genes (*int*, *c*I and *c*II) and thus most of the genes of phage 186 have been identified.

The functions of the essential genes have been studied by *in vitro* reconstitution, electron microscopy and incorporation of [$^{3}$H]thymidine into TCA precipitable counts. All but four of the 21 genes studied were found to be involved in the morphogenesis of the phage particle. Of the other four genes the product of one, gene *A*, is required for phage DNA replication; the product of the second, gene *B*, while not required for DNA replication is required for the synthesis of late proteins; the product of the third gene, *P*, is required for host cell lysis. The function of the fourth gene, gene *O*, is not known.

Attempts to obtain a genetic order for the essential genes of 186 by two-factor crosses were unsuccessful and the genetic map of this phage was constructed using marker rescue experiments. Two methods were used. In the first, hybrid phage consisting of the right-hand section of the 186 genome and the left-hand section of the genome of the closely related phage P2, were used as prophage in marker rescue experiments. These experiments allowed the mapping of the 186 genes *B*, *D*, *E*, *F* and *G* and also allowed the determination of both the P2 and 186 contributions to the various hybrid genomes. In the second method 186 amber mutants were used as prophage in

marker rescue experiments. Using superinfecting amber mutant phage the frequency of marker rescue from these mutant prophage was used to determine the order both of the 186 genes and of their alleles. This second method is more general than the one involving hybrid prophage and allowed the mapping of all the known essential genes of phage 186.

Physical positions on the phage genome for some of the 186 genes have also been determined. Combining the genetic complements of the hybrid phage, determined by marker rescue experiments, with the known physical positions of the P2-186 cross-over points, allowed the assignment of genes to a particular region of the 186 genome. Attempts to produce more P2-186 hybrid phage with cross-over points in different regions were unsuccessful, but further information on physical positions was obtained by the construction of λ-186 *in vitro* recombinants. Using the restriction enzyme R. *Eco*R1, two such recombinants were constructed and marker rescue with these two and with a third (made with the enzyme R. *Hin*dIII by R. B. Saint) allowed the physical positioning of several more genes.

## STATEMENT

Other than in Section 3, where due reference is made in the text, this thesis contains no material which has been accepted for the award of any other degree or diploma in any University. To the best of my knowledge and belief, this thesis contains no material previously published or written by another person except where due reference is made in the text.

STEPHANIE HOCKING

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## NOMENCLATURE

Wherever possible the nomenclature used for phage 186 has been based on that used for phage λ. The 186 *am* mutants used in this study had been given allele numbers upon isolation and, following complementation studies, were given an additional capital letter to designate the gene to which they belonged. Thus the mutant *am*5, when shown by complementation studies to belong to gene *A*, became *Aam*5. (In this thesis the word gene has been used synonymously with the word cistron to refer to the groups of mutations defined by complementation studies.)

Letters were assigned to the various genes only after the completion of the mapping studies and were arranged in alphabetical order, from right to left on the genetic map. The gene letter C was not used in order to avoid confusion with the non-essential genes *c*I and *c*II, defined by clear plaque mutations. The *c*I gene of 186 like the *c*I gene of λ codes for the repressor protein.

The nomenclature used for 186 restriction sites is also based on that used for phage λ (see Murray *et al.*, 1977).

## ABBREVIATIONS

| | | |
|---|---|---|
| *cfu* | - | colony-forming units |
| cpm | - | counts per minute |
| DNA | - | deoxyribonucleic acid |
| EDTA | - | ethylenediamine tetraacetate |
| EM | - | electron microscope |
| *eop* | - | efficiency of plating |
| kb | - | kilobase (i.e. 1,000 base pairs) |
| MC | - | mitomycin C |
| *moi* | - | multiplicity of infection |
| *mrf* | - | marker rescue frequency |
| mRNA | - | messenger ribonucleic acid |
| nm | - | nanometre ($10^{-9}$ metre) |
| NNG | - | *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine |
| $OD_{260}$ | - | optical density, absorbance at 260 nm (1 cm path length) |
| $OD_{600}$ | - | optical density, absorbance at 600 nm (1 cm path length) |
| PEG | - | polyethylene glycol |
| *pfu* | - | plaque-forming units |
| TCA | - | trichloroacetic acid |
| Tris | - | Tris(hydroxymethyl)aminomethane |
| UV | - | Ultraviolet light |

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# SECTION 1

# GENERAL INTRODUCTION

## 1.1 PHAGE 186

Phage 186 is a temperate bacteriophage of *Eschericia coli* and its genome consists of a single piece of non-permuted double stranded DNA of molecular weight 19.7 x $10^6$ daltons (Wang, 1967). It was isolated by Jacob and Wollman (1956) and was characterized by them as belonging to a group of phage known as "non-inducible", in reference to their inability to be induced either by ultraviolet irradiation or as a result of conjugation (zygotic induction).

They found that 186 did not show zygotic induction and, subsequently, that it was unable to recombine with phage λ (Jacob and Wollman, 1961), a property shared by the other "non-inducible" phage but not by the "inducible" phage. However, the UV induction properties of phage 186 were not tested and it has since been found that 186 is, in fact, inducible by UV irradiation (Woods and Egan, 1974). The failure to show zygotic induction is not due to a basic inability of the prophage to excise from the host chromosome, as found for the non-inducible phage P2, but rather to the inability of an exconjugant cell to host a 186 infection (Woods, 1972). The existence of a temperature-inducible mutant of phage 186, isolated by Baldwin *et al.* (1966), again shows that the induction properties of 186 are more like those of λ than like those of the truly non-inducible phage.

Despite the similarities in induction properties 186 clearly does not belong to the same group of phage as the other inducible phage. The other inducible phage are closely related to phage λ in being able to recombine with it and in having similar morphologies and cohesive ends. These cohesive ends can cohere to those of phage λ producing mixed dimers (Baldwin *et al.*, 1966; Yamagishi *et al.*, 1965). By contrast, the cohesive ends of phage 186 are dissimilar to those of phage λ and will not cohere with them to

form mixed dimers (Baldwin *et al.*, 1966; Wang and Schwartz, 1967; Mandel and Berg, 1968). 186 also differs from the other inducible phage in its morphology and its inability to recombine with them.

The two groups of phage defined by Jacob and Wollman (1956) do appear to describe two independent evolutionary groups but the labels "inducible" and "non-inducible" for these groups are misleading since induction properties are not basic to the differences between these phage.

## 1.2 COMPARISON OF PHAGE 186 AND P2

Of the truly non-inducible phage the one which has been most extensively studied is phage P2. There are many similarities between this phage and phage 186 both in the DNA of their genomes and in the morphology of the phage particles.

The P2 phage particle consists of an isometric (and probably icosahedral) head, a rigid tail with a contractile sheath, a base plate and tail fibres (Anderson, 1960; Inman *et al.*, 1971). Additional features of the particle have been described by Lengyel *et al.* (1974) and include a double disc head-connector at the proximal end of the phage tail and a single terminal spike at the distal end. The morphology of phage 186 was described by Baldwin *et al.* (1966). Both the head and the tail structures appear similar to those of phage P2. The tail is rigid and has a contractile sheath and a base plate. Tail fibres are also present although they are generally not well resolved. They can be seen in some of the electron micrographs presented in this thesis. A single terminal spike is also sometimes visible.

The P2 and 186 phage particles are of similar size and some reported dimensions for them are as follows. Phage 186: head diameter 50 nm; tail length 125 nm; tail diameter 12.5 nm (Baldwin

*et al.*, 1966). Phage P2: head dimeter 50-60 nm; tail length 137 nm (or 127 nm of extended sheath, excluding the neck region); tail diameter 16-17 nm (Inman *et al.*, 1971). The size of the P2 phage head varied depending on the method used in the preparation of the sample for electron microscopy. This should also be true for phage 186 and so the figures given for these two phage are not directly comparable. A better comparison would be obtained by including both phage in the same preparation.

The genomes of phage 186 and P2 also show many similarities. Both have single-stranded cohesive ends like those found for λ but while P2 and 186 ends will cohere with each other to form mixed dimers (Mandel and Berg, 1968) neither the 186 nor the P2 ends will cohere with the ends of phage λ (Baldwin *et al.*, 1966; Wang and Schwartz, 1967; Mandel and Berg, 1968). The DNA sequence of the cohesive ends of phage 186 and P2 must therefore be very similar and in fact have been found to differ in only two out of 19 bases (Padmanabhan and Wu, 1972; Murray and Murray, 1973).

The molecular weights of the DNA for each phage are similar: $19.7 \times 10^6$ daltons for 186 (Wang, 1967); $22 \times 10^6$ daltons for P2 (Inman and Bertani, 1969), and considerable homology exists between the DNA of these two phage (Skalka and Hanson, 1972; Younghusband and Inman, 1974). Further evidence for the relatedness of P2 and 186 was provided by the isolation of viable hybrid phage (Bradley *et al.*, 1975), each phage consisting of a section of the P2 genome combined with a section of the 186 genome. Further similarities in the genomes of these two phage have been demonstrated by the genetic mapping described in this thesis.

Although P2 and 186 share many features in common they also show several differences, the main difference being in their induction

properties. 186 is fully inducible by the same agents which cause induction in phage λ, for example UV irradiation, mitomycin C, nalidixic acid (Woods and Egan, 1974) but P2 is non-inducible (G. Bertani, 1968). Two separate phenomena contribute to the non-inducibility of phage P2. Firstly, UV irradiation fails to inactivate the P2 repressor (G. Bertani, 1968) and secondly even if repression had been lifted the prophage would not have been able to excise. This is shown by the results of L. E. Bertani (1968) who isolated P2 mutants with temperature sensitive repressors. Repression could be lifted in these mutants by raising the temperature, but when this was done no phage production resulted due to the failure of the prophage to excise (L. E. Bertani, 1968). By contrast the 186 temperature sensitive repressor mutant isolated by Baldwin *et al.* (1966) is similar to λ mutants of this type in showing both inactivation of repressor and excision of the prophage, at elevated temperatures.

This difference in the induction properties of 186 and of the other phage belonging to the same group is the basis of our interest in phage 186. We wish to compare phage P2 and 186 with a view to determining the processes responsible for this difference.

Bertani (1970) has suggested that the non-excisability of P2 is due to a defect in the production of the *int* protein, which is required for excision (Choe, 1969). She suggested a split operon model in which the defect in *int* production by the prophage is due to a physical separation of the *int* gene from its promoter. The *int* gene and its promoter, while adjacent in the vegetative P2 DNA, would become separated in the prophage state if the recombination event involved in integration of the phage DNA into the host genome occurred at a site located between the *int* gene and its promoter. In this case P2 would be unable to excise even after the lifting of repression

since the prophage would be unable to produce *int* product.

This model requires that the *int* gene and its promoter lie on opposite sides of the phage attachment site, *att*. The *int* gene has been mapped to the right of the *att* site (Lindahl, 1969a) and the promoter must therefore lie to the left, the direction of transcription being left to right in the vegetatively multiplying phage. The direction of transcription of the *int* gene is not known and its determination would provide one method of testing the model. Support for the model would be provided if the direction of transcription was found to be from left to right. However a right to left direction would invalidate the model.

Since 186 is inducible the *int* gene cannot be split from its promoter upon integration. If the split operon model for P2 is correct then the location of the *int* gene or its promoter must be different for the two phage. To compare these two phage a genetic map of 186 is a prerequisite so that any differences in the location or control of genes can be studied.

## 1.3 P2-186 HYBRID PHAGE

The P2-186 hybrid phage isolated by Cliff Bradley in 1972 have been invaluable in the construction of the 186 genetic map. These phage were constructed by recombination between P2 and 186 and selection for 186 immune phage able to plate on an *E. coli* C strain. 186 has a very restricted host range and cannot plate on *E. coli* C. P2 can plate on *E. coli* C and the ability to do this is probably dependent on one of the structural proteins of the P2 phage tail. These hybrids therefore must have the P2 genes responsible for adsorption to *E. coli* C and the 186 immunity region since they were selected on an *E. coli* C strain lysogenic for P2. On the P2 genetic map the

P2 immunity region lies to the right of the region controlling the formation of the phage tail (Lindahl, 1969a). Therefore a hybrid able to plate on *E. coli* C(P2) and resulting from a single cross-over between P2 and 186 should have a section of 186 DNA from the right-hand end of the 186 genome and a section of P2 DNA from the left-hand end of the P2 genome. This has been shown to be the case for hybrids 2 and 5 by heteroduplex analysis (Younghusband *et al.*, 1975). That it is also the case for the other ten hybrids is shown by the results described in Section 6 of this thesis, in which the genetic contributions of P2 and 186 to each of the hybrid phage were determined.

## 1.4 THE P2 GENETIC MAP

The work presented in this thesis describes the construction of a 186 genetic map and the genetic map of P2 will be outlined briefly here for the purpose of comparison.

The basic P2 genetic map was constructed by Lindahl (1969a) by two-factor recombination experiments. Several other essential genes have since been identified and mapped (Lindahl, 1971; Sunshine *et al.*, 1971). The P2 map now consists of a set of 21 essential genes, the products of which are required for lytic infection, and several more non-essential genes whose products are required for the lysogenic cycle only. The P2 map shows a clustering of genes with related functions.

The genes involved in the formation of the phage particle are all located to the left of the phage attachment site, in the left-hand 70% of the phage genome. At the left end of the map there is a group of 6 genes concerned with phage head formation. To the right of this is a gene required for host cell lysis and further right are located the 11 genes involved in the formation of the phage tail. These genes are all "late" genes and are arranged in four transcription

units defined by polarity groups (Lindahl, 1971; Sunshine *et al.*, 1971). Three of these transcription units are transcribed from left to right on the genetic map while the fourth, consisting of the two left-most head genes, is transcribed from right to left (Lindahl, 1971; Sunshine *et al.*, 1971).

Only one other essential gene lies to the left of the phage attachment site. This gene, *ogr*, is located between gene *D*, the right-most known tail gene, and the phage attachment site and is probably concerned with the control of late mRNA production (Sunshine and Sauer, 1975).

The two remaining essential genes *A* and *B* lie to the right of the phage attachment site. The products of both these genes are required for P2 DNA synthesis (Lindqvist, 1971) and gene *A* in addition is required for the expression of all other known essential genes (Lindahl, 1970; Sunshine and Sauer, 1975). By contrast it does not appear to be required for expression of the non-essential P2 genes (Lindahl, 1970). Gene *A* has the unusual feature of only acting in *cis*, that is only on the chromosome which coded for it. A combination of this *cis*-action and the requirement of the *A* gene product for the expression of the other genes leads to the very poor complementation which characterizes gene *A* mutants.

The known non-essential genes of phage P2 are located in three separate regions on the P2 map. In the centre of the genome, between two transcription units containing P2 tail genes, lie the non-essential genes *Z* and *fun*. The product of gene *Z* is required for the formation of stable lysogens (Bertani, 1960, 1976). The presence of prophage P2 converts *E. coli* C to sensitivity to 5-fluoro-deoxyuridine (Bertani, 1964). This conversion does not take place if the prophage carries a *fun* mutation (Bertani and Levy, 1964).

At the extreme right-hand end of the map lies the non-essential gene *old*. The wild-type form of this gene has two phenotypes. Firstly, it causes the killing of *recB* and *recC* cells under conditions where $rec^+$ bacteria survive (Sironi, 1969; Lindahl *et al.*, 1970) and secondly, when present in the prophage state it prevents the growth of phage λ (Lindahl *et al.*, 1970). Mutants in the *old* gene do not cause killing of *recB* and *recC* host cells and when present in the prophage state allow the growth of phage λ (Sironi, 1969; Lindahl *et al.*, 1970).

The remaining known, non-essential genes are clustered in a group mapping to the right of the phage *att* site and to the left of the essential genes *A* and *B*. These genes include the *C* gene coding for the repressor protein (L. E. Bertani, 1968), the *int* gene required both for integration and excision of the prophage (Choe, 1969; Lindahl, 1969b) and the *cox* gene required only for excision (Lindahl and Sunshine, 1972). The repressor binding site, defined by "strong virulent" mutations is also located in this region, in fact between genes *C* and *B* (Lindahl, 1971).

Bertani and Bertani (1974) described the isolation of a tandem duplication and reported the presence of part of the essential gene *B* on the duplicated chromosomal segment. Heteroduplex mapping of the tandem duplication showed that the novel joint was located 80% from the left end of the P2 DNA (Chattoraj and Inman, 1974). Since the novel joint occurs within gene *B* this gene must be located at 80% from the left end of the genome. To the left of gene *B*, that is in the left-hand 80% of the map, there are approximately 24 known genes. This section of the map is therefore well represented. However to the right of gene *B* in the remaining 20% of the genome there are only two known genes, the essential gene *A* and the non-essential gene *old*.

Therefore, either there is a large number of unidentified P2 genes or much of this right-hand DNA does not code for proteins. A deletion, *del1*, deleting approximately 7% of the DNA from the extreme right-hand end of the genome (Chattoraj and Inman, 1972; Chattoraj *et al.*, 1975) is viable and so if this DNA codes for any additional genes they must all be non-essential ones. One known non-essential gene, gene *old*, is known to map within the region deleted by *del1* (Bertani, 1975).

## 1.5 SOME METHODS USED IN THE DETERMINATION OF GENE LOCATION AND GENE FUNCTION

### a. Gene location

The most common methods used in the construction of phage genetic maps are two-factor crosses, three-factor crosses and deletion mapping. Of these the most reliable appears to be deletion mapping. This method involves the recovery of wild-type recombinants from a cross between a point mutant and a deletion mutant. If wild-type recombinants can be recovered then the location of the point mutation is outside the region defined by the deletion. Conversely if no wild-type recombinants can be recovered then the point mutation maps within the region defined by the deletion. This method has the advantage of being independent of the recombination frequency, only the presence or absence of recombinants need be determined.

Deletion mapping is limited by the requirement for a set of overlapping deletions. If several mutants lie within a single deleted region then they cannot be mapped relative to each other unless other deletions can be found covering part but not all of the same region. If such a set of deletion mutants is available then deletion mapping is the method of choice.

For phage λ the existence of the defective λ transducing

phage has provided such a set of overlapping deletions and Campbell (1961) utilized these to construct a genetic map of λ. The map he constructed shows both the power and the limitations of this method. In the left-hand section of the map he was able to obtain not only the order of the 8 genes located in that region but also orders for many of the alleles within those genes. In fact he was able to divide the 8 genes into 25 groups of mutations all of which could be mapped in relation to each other. However, he could not obtain any mapping data on 36 mutants belonging to 5 genes lying within the common core of the deletions.

Three-factor crosses are another reliable method for determining gene order. This method usually involves the selection of recombinants between two genetic markers and then the determination of the relative proportions of the two alternative forms of a third unselected marker, amongst these recombinants. The three markers are then arranged in an order such that a single cross-over would produce a majority of the type of recombinant found to be most numerous. To obtain a unique order for 3 mutations two such crosses are required. However fewer such crosses are needed if some information is already available. If the unselected marker is known to lie at one end of the three mutations the other two can be ordered with a single cross. Parkinson (1968) used this method to map mutations located within the same gene. He used as an unselected marker the *c*I gene, known to map to the right of the mutants he was testing. He scored the number of clear and turbid plaques among his *am*$^+$ recombinants. However, he could not obtain orders when the two *am* mutants mapped very close together. This is due to the high negative interference present in such crosses which tends to cause a random distribution of the unselected marker. A second potential problem in

three-factor crosses is a possible selective advantage to one allele over the other. This would disturb the proportions obtained. Neither of these two phenomena would cause problems in deletion mapping.

Although the use of a non-essential gene as the unselected marker in a three-factor cross is usual and convenient, a variation of the method can be used to map three mutations all with the same selective phenotype. For example P.J. Kretschmer has constructed a map of the staphylococcal phage $\phi$11 solely from three-factor crosses with nonsense mutants (Kretschmer and Egan, 1975). This method involved crossing one mutant with both a double mutant and with the two single mutants from which it was constructed. If the first mutant was located between the markers of the double mutant then two recombination events would be required to form a wild-type recombinant. The recombination frequency obtained should be less than that for a cross between the same mutant and either of the single mutants which together constitute the double mutant. Only a single cross-over would be required if the first mutation was located at one end of the markers in the double mutant and so the frequency of wild-type recombinants would be equivalent to that found in one of the other crosses. By comparisons of the frequencies obtained the single mutation can be mapped either between the double mutant markers or at one end of them.

The third widely used mapping method, two-factor crosses, although the most general of the three methods is also the most unreliable. In this method the frequency of recombination occurring between two markers is considered to be directly proportional to the distance between them. The frequency of recombination is determined by expressing the number of recombinants formed as a percent of the total phage formed. The dependence of the recombination frequency on

this last value introduces a source of error since in many mutants the burst size in "permissive" conditions is still less than that of a wild-type phage. If the burst size varies for different mutants then the measured recombination frequency will vary both as a function of these burst sizes and as a function of the distance between mutations. A reliable genetic map will not, therefore, be obtained.

The *am* mutants isolated by Campbell showed poor burst sizes in permissive conditions and the results of two-factor crosses with these mutants (Campbell, 1959) show that while the recombination frequencies can be used to indicate a possible order for some of the mutations they are not reliable enough to allow the construction of a genetic map. These same mutants, however, were successfully used to construct a λ genetic map both by three-factor crosses and by deletion mapping (Campbell, 1959, 1961). These two methods are not dependent on the burst sizes of the mutants used. Satisfactory results for two-factor crosses with λ *am* mutants have been obtained by Parkinson (1968) and his success was probably due to the use of *am* mutants that produced normal burst sizes under permissive conditions.

For phage P2 the sort of deletion mutants used in the mapping of λ were not available and the map constructed by Lindahl (1969a) relied mainly on two-factor crosses, with some confirmation of order by three-factor crosses. His map was constructed with temperature-sensitive mutants and so the problem of poor suppression encountered for *am* mutants should not occur. However the same type of problem can occur if the burst sizes of the *ts* mutants in permissive conditions vary.

b. <u>Gene function</u>

Bacteriophage such as T4, λ, P2 and 186 have relatively

complex morphologies and most of the essential genes of these phage are concerned with the construction of the phage particle. One of the simplest methods for studying the functions of these genes is *in vitro* reconstitution (also known as *in vitro* complementation). This method was devised by Edgar and Wood (1966) for phage T4 and relies on the fact that several of the steps in the assembly of the phage particle can occur *in vitro*.

For phage λ independent pathways are involved in the assembly of the phage head and phage tail structures and only when complete are these two structures connected. This head-tail joining can occur efficiently *in vitro* and viable phage can therefore be reconstructed *in vitro* by the addition of lysates containing active phage heads to those containing active phage tails. Such lysates can be prepared by the infection of non-permissive cells with mutants in head genes or tail genes. These lysates will contain active phage tails and active phage heads respectively.

Weigle (1966) used this method to divide the late genes of λ into two groups, the defective lysates of one group capable of producing active phage when added to defective lysates of the other group but not when added to any other lysate produced by a mutant in the same group. To determine which were the head donor lysates and which the tail donors the reconstituted phage were tested by complementation with the parental phage. The reconstituted phage will have the genotype of the head donor (or tail mutant) and will therefore complement the parental head mutant but not the parental tail mutant. A direct determination of the genotype of the reconstituted phage can sometimes be made by an observation of its plaque morphology, in cases where the morphologies of the parental phage differ. Other methods can also be used to distinguish between the head donor lysates and the

tail donor lysates. For example the lysates could be viewed in the electron microscope to determine whether heads or tails have accumulated, or the lysates could be tested for sensitivity to UV irradiation. Only tail mutant lysates, containing active head particles, should be sensitive. This last method was the one used to distinguish between the two P2 reconstitution groups defined by Lindahl (1974).

The lysates Weigle used in his experiments were prepared simply by induction of mutant prophage and he was only able to examine one step in phage assembly, namely head-tail joining. However *in vitro* reconstitution can be used to study other steps in phage assembly and depends very much on the conditions used for the preparation of the defective lysates. For example Kühl and Katsura (1975) used highly concentrated lysates prepared by freeze-thawing and were able to demonstrate intra-tail *in vitro* reconstitution for most pairs of lysates produced by 10 of the 11 known λ genes involved in the formation of the phage tail. This intra-tail reconstitution was very efficient, in some cases the efficiency being almost 50% of that obtained for head-tail attachment. Intra-head reconsitution has also been demonstrated (Bode, 1971; Casjens, 1971; Casjens *et al.*, 1972; Hohn and Hohn, 1973, 1974; Kaiser and Masuda, 1973). The results of intra-tail *in vitro* reconstitution have allowed Katsura and Kühl to propose a pathway for the assembly of the tail of phage λ (Katsura and Kühl 1975a,b; Kühl and Katsura, 1975). *In vitro* reconstitution is therefore a very powerful tool in the study of phage morphogenesis.

Electron microscopy on the defective lysates can also produce useful information. While *in vitro* reconstitution may suggest that a tail protein is non-functional, electron microscopy could show the presence of apparently normal tail structures, of aberrant tail

structures or the absence of any tail structure (this last is usually the case for λ tail mutants (Mount *et al.*, 1968; Kemp *et al.*, 1968)). If apparently normal tail particles are present it could be concluded that the mutant does not lie in a gene coding for a major structural component of the tail. If abnormally long tails are found it may suggest that the mutant lies in a gene controlling tail length.

Identification of the protein products of the genes controlling phage morphogenesis is more complicated. One way this can be achieved is to compare, by gel electrophoresis, the proteins made by an *am* mutant infected cell with those made by a wild-type infected cell. If a protein band present in a wild-type infected cell is missing in an *am* mutant infected cell then this protein can be identified as the product of the gene in which the *am* mutant is located. Its molecular weight and relative abundance can also be determined. Genes that code for the structural proteins of the phage can also be identified by comparing the proteins of mutant infected cells with those of the dissociated phage particle. Finally, precursor-product relationships can be identified. An example of this is the finding of Lengyel *et al.* (1973) that the product of the P2 gene *N* is cleaved to form a smaller protein which is the major component of the phage head.

There are, however, many problems associated with this system. For example in phage P2 strong polarity effects operate and this means that a single *am* mutant can eliminate protein formation both from the gene in which it is located and also from the genes distal to it but within the same unit of transcription. This means that a particular phage band could be missing in several mutant lysates and that a single mutant lysate could be lacking more than one protein band. Both these effects have been observed (Lengyel *et al.*, 1973; 1974).

A second problem is the presence of host protein synthesis. This can be reduced by the method of Ptashne (1967) involving UV irradiation of the host prior to infection. However residual synthesis can still be a problem especially in the detection of minor phage bands.

Despite these problems, however, the method has been used successfully for both λ and P2. Murialdo and Siminovitch (1972) have identified the products of 13 of the λ morphogenetic genes. The protein products of several P2 genes have also been identified (Lengyel *et al.*, 1973, 1974; Lengyel and Calendar, 1974).

## 1.6 AIM OF THIS STUDY

The primary aim of the work presented in this thesis was to construct a genetic map of the essential genes of phage 186. A secondary aim was to determine the functions of these genes. A genetic map of 186 was considered a necessary basis for future studies on prophage induction with this phage.

# SECTION 2

# MATERIALS AND METHODS

## 2.1 BACTERIAL STRAINS

The bacterial strains used in this study are described in Tables 2.1 and 2.2. Other lysogenic derivatives used, but not listed, include 594 lysogens of the twelve P2-186 hybrid phage, 594 and C600 lysogens of the 61 186 *am* mutant phage and E251, E261, S26R1e and H12R8A lysogens of several 186 *am* mutant phage.

## 2.2 BACTERIOPHAGE STRAINS

Derivatives of phage 186, P2 and λ have been used in this study and are described below.

### a. Phage 186 strains

186 : Wild-type 186 (Jacob and Wollman, 1956).

186*c*I*ts* : A heat-inducible mutant of 186, previously called 186p (Baldwin *et al.*, 1966).

186*c*I10 : A clear plaque mutant of 186 which fails to complement with 186*c*I*ts* (Huddleston, 1970).

186*c*II8 : A clear plaque mutant of 186 which complements with 186*c*I*ts* and thus defines a second gene (Huddleston, 1970).

186*c*I*am*53*vir*1 : A virulent phage, able to grow on a 186 lysogen. Isolated as a spontaneous clear plaque mutant in a stock of the phage 186*c*I*am*53 (Woods, 1972).

186*Dam*48, 186*Qam*49, 186*Ham*50 : Three amber mutants derived from wild-type 186, obtained as a gift from A. D. Kaiser and previously known as 186*am*4.1, 186*am*6.1 and 186*am*9.1 respectively.

186*Ham*56*c*II : An amber mutant derived from wild-type 186, obtained as a gift from C. P. Georgopoulos and previously known as 186*am*E. It also has a mutation in the *c*II gene.

TABLE 2.1

BACTERIAL STRAINS

| Collection number | Relevant phenotype | Genotype | Origin or reference |
|---|---|---|---|
| *E. coli* K12 | | | |
| 594 | $Su^-$ | $F^-$ *galK galT str594* | Campbell (1965) |
| W3350 | $Su^-$ | $F^-$ *galK galT* | Campbell (1965) |
| C600 | $Su^+$ | $F^-$ *thr leu thi lacY tonA supE* | Appleyard (1954) |
| S26R1e | $Su^+$ | HfrC *rel tonA* $T2^r$ *phoA supD* | Garen *et al.* (1965) |
| H12R8a | $Su^+$ | HfrC *rel tonA* $T2^r$ *phoA supF* | Garen *et al.* (1965) |
| XA7007 | $Su^-$ SuA | $F^-$ (*lac pro*)$\nabla$ *mal thi str rho* | D.E.Morse (no.1013). Isolated by Beckwith (1963) |
| 152 | $Su^-$ *recA* | $F^-$ *galK str recA56* | Gottesman and Yarmolinsky (1968) |
| QR48 | $Su^+$ *recA* | $F^-$ *thi lac supE recA13* | Signer and Weil (1968b) |
| E251 | $Su^-$ | $F^-$ *galK galT str748* | This laboratory, by transduction of the $str^r$ allele from CGSC 4212 into W3350 |

- *continued*

TABLE 2.1 - *continued*

| Collection number | Relevant phenotype | Genotype | Origin or reference |
|---|---|---|---|
| *E. coli* K12 | | | |
| E261 | $Su^-$ $hcr^-$ | HfrP4X *uvrA*6 *thyA*52 *thyR*31 *thi* *str*748 | This laboratory, by transduction of the $str^r$ allele from CGSC 4212 into CGSC 5122 |
| ED8654 | $r_K^-$ $m_K^+$ | $F^-$ *supE supF* $hsdR_K$ *met trpR* | Murray *et al.* (1977) |
| 5K | $r_K^-$ $m_K^+$ | $F^-$ *thr leu thi lacY tonA supE* $hsdR_K$ | Hubacek and Glover (1970) |
| 5KRI | $r_{RI}^+$ $m_{RI}^+$ $r_K^-$ $m_K^+$ | $F^-$ *thr leu thi lacY tonA supE* $hsdR_K$* | N. E. Murray |
| *E. coli* C | | | |
| C-1757 | $Su^+$ | $F^-$ $arg_{am}$ $Tl^r$ *trp str supD* | Sunshine *et al.* (1971) |
| C-1792 | $Su^+$ | $F^-$ $arg_{am}$ $trp_{am}$ *his str supF* | Sunshine *et al.* (1971) |

* In addition this strain carries a plasmid coding for the *Eco*RI restriction and modification enzymes.

## TABLE 2.2

## LYSOGENIC DERIVATIVES OF STRAINS DESCRIBED IN TABLE 2.1

| Collection number | Prophage | Derivative of: |
|---|---|---|
| E573 | $186^+$ | C600 |
| E574 | 186*c*I*ts* | C600 |
| E699 | $186^+$ | 594 |
| E701 | 186*c*I*ts* | 594 |
| E252 | 186*c*I*ts* | E251 |
| E275 | 186*c*I*ts* | E261 |
| E772 | $186^+$ | 152 |
| E511 | $\lambda^+$ | C600 |
| E513 | $\lambda imm^{434}$ | C600 |
| E514 | λ*c*I857 | C600 |
| E663 | λ*c*II*Ram*221 | 594 |
| E811 | $P2^+$ | 594 |

186*am*1 to 186*am*47, 186*am*51, 186*am*52 : Forty seven amber mutants isolated by W. H. Woods in this laboratory following UV mutagenesis of 186*c*I*ts*. (Mutants 3 and 32 are plaque morphology mutants, not amber mutants.)

186*am*57, 186*am*58, 186*am*60 to 186*am*67 : Ten amber mutants isolated in this laboratory following NNG mutagenesis of 186*c*I*ts*.

186*ts*59, 186*ts*68, 186*ts*69 : Three temperature-sensitive mutants isolated in this laboratory following NNG mutagenesis of 186*c*I*ts*.

186*c*I*ts*P*am*16*vir*2 : A virulent phage, able to grow on a 186 lysogen. Isolated as a spontaneous clear plaque mutant in a stock of the phage 186*c*I*ts*P*am*16 (Woods, 1972).

186*c*I*ts*E*ts*7 : A temperature-sensitive "revertant" of the amber mutant 186*c*I*ts*E*am*7.

Of the amber mutants isolated in this laboratory, four (*am*1, *am*51, *am*52 and *am*62) have an additional clear plaque mutation. *am*40 is a double mutant consisting of two amber mutations. Several of the 186 *am* mutants are poorly suppressed by one or more of the three suppressor hosts used. In particular, mutants *Gam*27 and *Nam*47 fail to grow on the *supE* suppressor host C600, and mutants *Mam*60 and *Pam*16 fail to grow on the *supF* suppressor host H12R8A.

b. Phage P2 strains

P2$vir_1 amF_4$, P2$vir_1 amT_5$, P2$vir_1 amD_6$ : Three amber mutants isolated by Lindahl (1971).

P2$vir_1 amJ_{214}$, P2$vir_1 amE_{30}$, P2$vir_1 amU_{25}$ : Three amber mutants isolated by Sunshine *et al.* (1971).

c. P2-186 hybrid phage

P2-186 hybrid phage Hy1 to Hy11 were isolated by C. Bradley (1972).

The isolation of Hy1 to Hy5 is described in Bradley *et al.* (1975). Hy12 was isolated by Ong, P. L. (1973).

d. Phage λ strains

λ*ind*⁻*c*I857 : A heat-inducible mutant of phage λ (Sussman and Jacob, 1962).

λ*imm*$^{434}$*Nam*7 : An amber mutant derivative of the hybrid phage λ*imm*$^{434}$.

λ641 : An immunity insertion vector for the *Eco*RI restriction system (Murray *et al.*, 1977). Its genotype is: *srI*λ1$^0$ (*srI*λ2-3)∇$^0$ *imm*$^{434}$ *srI*λ4$^0$ *nin*5 *srI*λ5$^0$.

## 2.3 MATERIALS

a. Chemicals

The following chemicals were purchased from the companies indicated:

| | |
|---|---|
| Mitomycin C | - Sigma Chemical Company, St. Louis, USA (Each vial contained 2 mg mitomycin C and 48 mg NaCl.) |
| [6-$^3$H]Thymidine | - The Radiochemical Centre, Amersham, England. (Specific activity 5 Ci/mmol) |
| CsCl | - The Harshaw Chemical Company, Cleveland, Ohio, USA. (Radio Tracer grade) |
| PEG 6000 | - Union Carbide Corporation, New York, USA. |
| T4 polynucleotide ligase | - Miles Laboratories Inc., Elkhart, Ind., USA. |

Bacto-Tryptone, Bacto-Agar, Yeast Extract, Nutrient Broth and Casamino acids were obtained from Difco Laboratories, Detroit, USA. The restriction endonuclease R.*Eco*RI and 186*c*I*ts* DNA were obtained from R. B. Saint. λ641 DNA was obtained from E. J. Finnegan.

Tris buffers were prepared from Trizma base (Sigma

Chemical Company) and the pH was adjusted with HCl.

Scintillation fluid consisted of 3.5 g 2,5-diphenyloxazole (PPO) and 0.35 g 1,4-bis[2-(5-phenyloxazolyl)]benzene (POPOP) dissolved in 1 litre of toluene.

b. Liquid media and buffers

All media and solutions of chemicals were prepared in glass-distilled water and were sterilized by autoclaving for 25 min at a temperature of 120°C and a pressure of 15 $lbs/inch^2$. The compositions of the various media were as follows:

T broth - 2% Bacto-Tryptone, 0.5% NaCl.

L broth - 1% Bacto-Tryptone, 0.5% Yeast Extract, 1% NaCl.

LG broth - L broth supplemented with 0.1% glucose. (The glucose was autoclaved separately as a 20% solution and added to sterile L broth.)

LGC broth - L broth supplemented with 0.1% glucose and 0.0024 M $CaCl_2$. (Both the solutions of glucose (20%) and $CaCl_2$ (0.4 M) were autoclaved separately and added to sterile L broth.)

H-1 - 0.1 M potassium phosphate buffer pH 7.0, 0.015 M $(NH_4)_2SO_4$, 0.001 M $MgSO_4$, 1.8 x $10^{-6}$ M $FeSO_4$. (All components were autoclaved separately and added to sterile $H_2O$.)

H-1+glu - H-1 supplemented with 0.2% glucose.

H-1+glu+$TLB_1$ - H-1 supplemented with glucose (0.2%), threonine (100 μg/ml), leucine (100 μg/ml) and thiamine (10 μg/ml).

TPG-CAA - based on the recipe given by Lindqvist (1971) and prepared as follows: To 900 ml $H_2O$ were added

0.5 g NaCl, 8 g KCl, 1.1 g $NH_4Cl$, 12.1 g Trizma base, 1 g $KH_2PO_4$, 0.8 g sodium pyruvate. The pH was adjusted to 7.4 with HCl, the volume brought to 1 litre and the solution autoclaved. To each 100 ml of this medium were added the following components (which were separately sterilized) : 0.1 ml 0.16 M $Na_2SO_4$, 0.1 ml 1 M $MgCl_2$, 0.1 ml 1 M $CaCl_2$, 0.1 ml 0.1 mg/ml $FeSO_4$, 1 ml 20% glucose, 4 ml 25% Casamino acids (vitamin-free for experiments involving [$^3$H]thymidine incorporation) and 7 ml $H_2O$.

TPM-CAA - equivalent to TPG-CAA except supplemented with 0.2% maltose instead of 0.2% glucose.

TM - 0.01 M Tris pH 7.1, 0.01 M $MgSO_4$.

c. Solid media

i. Soft agar

T soft agar - 1% Bacto-Tryptone, 0.5% NaCl, 0.7% Bacto-Agar.

ii. Stabs

Nutrient stabs - 2.5% Nutrient Broth, 0.3% Yeast Extract, 0.7% Bacto-Agar.

iii. - Plates

Plates were prepared from 30 ml of the relevant mixture, dried (with the lids on) at 37°C overnight and stored at 4°C until required. Unless otherwise indicated T plates were used for all work with phage 186.

T plates - 1% Bacto-Tryptone, 0.5% NaCl, 1.2% Bacto-Agar.

LGC plates - LGC broth with 1.5% Bacto-Agar.

LGcit plates - LG broth with 1.5% Bacto-Agar and 0.1 M tri-sodium

citrate (sterilized separately).

## 2.4 GENERAL METHODS

### a. Storage of bacteria and bacteriophage

Long term storage of bacteria was either in freeze-dried ampoules kept at 4°C or in 40% glycerol kept both in frozen form at -80°C and in liquid form at -15°C. Short term storage of bacteria was in nutrient stabs kept at 4°C. Bacteriophage stocks were stored over chloroform at 4°C.

### b. Growth of indicator bacteria

An overnight bacterial culture was prepared by inoculating broth (usually T broth) with bacteria from a stab and incubating with aeration in a New Brunswick gyratory water bath shaker (usually at 37°C) for approximately 16 hr. This overnight culture was used for inoculating further overnight cultures for approximately 2 weeks before being discarded. The next overnight culture was then prepared from the stab. Indicator bacteria were grown from fresh overnight cultures by diluting 1 in 30 into fresh broth (usually T broth) and incubating with aeration (usually at 37°C) in a New Brunswick gyratory water bath shaker to an $OD_{600}$ of approximately 1, measured on a Zeiss PMQII spectrophotometer. This OD, in a T broth culture, is equivalent to approximately $4 \times 10^8$ *cfu*/ml. The indicator bacteria were chilled and kept on ice until required. The indicator strain used for most 186 phage assays was C600.

### c. Bacterial assays

Bacterial cultures were assayed by diluting in H-1 and spreading 0.1 ml samples on T or LGC plates with a bent glass rod (sterilized by dipping in ethanol and flaming). The plates were

incubated at 37°C (or at 30°C for bacteria lysogenic for heat-inducible phage), and colonies scored.

d. Phage assays

Phage were diluted in TM buffer, 0.1 ml samples were added to 0.2 ml samples of log phase indicator bacteria (4 x $10^8$ *cfu*/ml) and incubated for 20 min at 37°C (for phage adsorption). 3 ml of T soft agar (held at 47°C) was added to each, mixed and then poured over an agar plate. Once the soft agar had solidified the plates were inverted and incubated at 37°C or 30°C overnight. Plaques were scored next day. For assays of 186 phage and λ phage, indicator bacteria were grown in T broth and T plates were used. For assays of phage λ the T soft agar was supplemented with 0.01 M $MgSO_4$. For assays of P2 phage and P2-186 hybrid phage, indicator bacteria were grown in LGC broth and LGC plates were used. When the indicator bacteria were lysogenic for heat-inducible phage the 20 min preincubation was at room temperature rather than at 37°C. For phage 186 adsorption is poor, only approximately 50% adsorb in 20 min.

e. Preparation of lysogens

$Su^+$ lysogens of 186 *am* mutant phage were prepared by taking bacteria from the centre of a turbid plaque and streaking for single colonies. The plates were incubated at 30°C and the resultant colonies were tested either for phage production, by stabbing colonies with sterile toothpicks into lawns of $Su^+$ indicator bacteria and incubating the plates at 37°C, or for immunity to superinfection, by streaking colonies with sterile broad-ended toothpicks against dried streaks of 186*c*I10 and 186*c*I*am*53*vir*1 (from stocks of $10^{10}$ *pfu*/ml) and incubating the plates at 30°C. Lysogenic colonies, showing either

phage activity on $Su^+$ bacteria or resistance to 186*c*I10 and sensitivity to 186*c*I*am*53*vir*1, were purified by an additional two single colony isolation steps with testing for phage production or immunity to superinfection at each step. An LG broth culture was grown from the third single colony isolate and tested for relevant bacterial markers as well as for immunity to superinfection. The culture was stored in stabs and in either freeze-dried ampoules or in 40% glycerol.

$Su^-$ lysogens of 186 *am* mutant phage were prepared by spotting phage from a stock of titre between $10^9$ and $10^{10}$ *pfu*/ml on an $Su^-$ indicator lawn and incubating the plate at 30°C overnight. Next day bacteria were taken from the centre of the spot and streaked for single colonies. Lysogens were purified, tested and stored as described above for $Su^+$ lysogens except that in all cases the presence of the prophage was detected by immunity to superinfection. C600 and 594 lysogens were prepared in this way for all the 186 *am* mutant phage (except *Wam*52 which carries a non-conditional clear plaque mutation).

f. <u>186 phage stocks</u>

Three methods have been used routinely for the preparation of 186 phage stocks and all three usually give phage titres of the order of $10^{10}$ *pfu*/ml.

i. <u>Heat induction</u>

Overnight T broth cultures of $Su^+$ (usually C600) lysogens of 186*c*I*ts am* mutants were diluted 1 in 30 (0.3 ml in 10 ml) into fresh T broth and grown with aeration at 30°C to an $OD_{600}$ = 0.8 (equivalent to 3 x $10^8$ *cfu*/ml). The cultures were transferred to 47°C and shaken by hand for 10 min. They were then incubated with aeration at 37°C until lysis was complete, or for 4 hr if lysis did

not occur. Bacterial debris was removed by centrifugation (10 min, 7800 g, 4°C) and the supernatants stored over chloroform at 4°C.

ii. Liquid infection

186 phage carrying non-conditional clear plaque mutations (for example *vir*2) were usually stocked by this method.

A single plaque of the mutant to be stocked (cut from an agar plate with a pasteur pipette) was added to a flask containing 10 ml of LG broth. 0.6 ml of a log phase ($4 \times 10^8$ *cfu*/ml) T broth culture of C600 (or another $Su^+$ strain) was added to the same flask and the infected culture was incubated with aeration at 37°C until lysis was complete, or (more usually) in the absence of lysis for 4 hr. Bacterial debris was removed by centrifugation (10 min, 7800 g, 4°C) and the supernatant stored over chloroform at 4°C.

iii. Plate stocks

This method was usually used for small plaque mutants. A single plaque of the mutant to be stocked (cut from an agar plate with a pasteur pipette) was added to 1 ml of T broth and left for 15 to 30 min at room temperature to allow the phage to leach out from the agar. 0.1 ml of this solution was plated (on a T plate) with 0.2 ml of a log phase culture ($4 \times 10^8$ *cfu*/ml) of an appropriate $Su^+$ strain, and the plate incubated at 30°C overnight. The plate was chilled at 4°C for 30 min and then flooded with 3 ml of ice-cold TM and left at 4°C for a further 24 hr. The phage solution was removed with a pasteur pipette and filtered through a millipore filter (pore size 0.45 μ) to remove bacteria. The filtrate was stored over chloroform at 4°C.

g. P2 phage stocks

Stocks of P2 wild-type and P2 *am* mutant phage were prepared by

liquid infection as follows.

An overnight T broth culture of C600 was diluted 1 in 10 in LGC broth and grown with aeration at 37°C to a density of approximately $4 \times 10^8$ *cfu*/ml ($OD_{600} = 1$). 0.6 ml was added to 15 ml of LGC broth containing a single plaque of the mutant to be stocked (cut from an agar plate with a pasteur pipette) and incubated with aeration at 37°C. When the cultures started to lyse, as determined by optical density measurements, 0.3 ml of a 0.1 M EDTA solution was added (final concentration 0.002 M) and the incubation continued until lysis was complete. Bacterial debris was removed by centrifugation (10 min, 7800 g, 4°C) and the supernatant stored over chloroform at 4°C.

Although stocks were prepared in an *E. coli* K12 strain the titres were determined on an *E. coli* C suppressor strain, C-1757 or C-1792, since P2 plates better on *E. coli* C than on *E. coli* K12.

h. λ phage stocks

Plate stocks of the λp186 isolates were prepared by the procedure described for 186 mutants in Section 2.4, f, iii, except that the plates were incubated at 30°C for only 7½ hr before flooding with ice-cold TM. These plates were left at 4°C for approximately 16 hr before the phage were harvested. The bacterial strain used was C600 in all cases and stocks of titre between $5 \times 10^{10}$ *pfu*/ml and $8 \times 10^{10}$ *pfu*/ml were obtained.

Stocks of λ*c*I857 were obtained by heat induction as described for 186 mutants (Section 2.4, f, i).

i. High titre phage stocks of 186 *am* mutants

High titre phage stocks of 186 *am* mutants were prepared by polyethylene glycol (PEG) precipitation of phage from low titre

stocks and purification on CsCl gradients. The method used for PEG precipitation was as described by Yamamoto *et al.* (1970) and was found to give almost complete recovery of phage.

500 ml low titre stocks of 186 *am* mutants were prepared by heat induction as described in Section 2.4, f, i, except that LG broth was used and the cells were incubated at 47°C for 20 min instead of 10 min. Lysis of these cultures was usually not observable and after 3 hr NaCl was added (at a final concentration of 0.5 M) and the cultures kept at 4°C for 1 hr. Bacteria and debris were removed by centrifugation (16 min, 8880 g, 4°C) and 50 g of PEG 6000 was dissolved in the supernatant (i.e. at a concentration of 10%). These solutions were decanted into centrifuge pots and left at 4°C for 16 hr (or longer). The precipitate was collected by centrifugation (15 min, 8880 g, 4°C), resuspended in a small volume of TM and purified by equilibrium centrifugation in CsCl ($\rho$ = 1.425 g/$cm^3$, 30000 rpm, 16 hr, 4°C, Beckman Ti50 rotor). The phage band was recovered by piercing the side of the tube just below the band with a syringe needle and withdrawing the band in a volume of 1 to 2 ml. This represents a 250- to 500-fold concentration of the original phage solution.

j. High titre phage stocks of P2-186 hybrid phage

Low titre phage stocks were prepared by induction of 500 ml cultures of 594 lysogens of the P2-186 hybrid phage as described above for 186 *am* mutants, except that LG broth supplemented with 0.08 M $MgSO_4$ was used. No lysis was observed and after 3½ to 4 hr the cultures were removed and processed as described in the previous section. PEG precipitates were resuspended in TM and centrifuged in CsCl gradients of density 1.455 g/$cm^3$. The three hybrid phage Hy9, Hy10 and Hy11, as well as the 186 insertion mutant *hr*22,

produced two bands upon centrifugation (presumably due to breakdown of phage particles, the lower band being composed of detached phage heads as has been shown to be the case for the 186*Lam*21 mutant (see Section 3.3, c)). The increased density was used so that the lower band could be recovered pure, away from any DNA present at the bottom of the tube.

## 2.5 SECTION 3 METHODS

### a. Complementation

#### i. Plate complementation

A drop (∿5 μl) of an amber mutant phage solution of titre between 3 x $10^8$ and $10^9$ *pfu*/ml was streaked with a loop across an indicator lawn of 594 or XA7007 and allowed to dry (∿30 secs). A drop of a second phage solution was then cross-streaked and the plate incubated at 37°C overnight. A square of lysis at the intersection of the streaks indicated complementation. No lysis indicated no complementation. As a control each phage solution was cross-streaked with itself.

For complementation of clear plaque mutants the procedure was essentially the same except that C600 was used as the indicator and less phage were required. A 1 ml solution of a single plaque (∿$10^7$ *pfu*/ml) provided an adequate concentration of phage. Complementation was indicated by a square of turbidity at the intersection of the two clear streaks.

#### ii. Liquid complementation

594 was grown with aeration at 37°C in LG broth to a density of 3 x $10^8$ *cfu*/ml. This culture was infected with a mixture

of two different amber mutants, at an *moi** of approximately 10 for each mutant, incubated without aeration at 37°C for 20 min (to allow adsorption), diluted $10^{-1}$ into LG broth containing 186 antiserum ($K = 8\ min^{-1}$) and incubated at 37°C for a further 5 min. (This antiserum treatment inactivated more than 99.9% of the unadsorbed phage.) The mixtures were then diluted $10^{-3}$ into pre-warmed LG broth and incubated with aeration at 37°C for 90 min. The cultures were chilled and chloroform added. Phage yields were determined on $Su^+$ indicator bacteria (C600 or S26R1e) and on $Su^-$ indicator bacteria (594). The above procedure was repeated for single infections with each phage (at an *moi* of 20) and for infection with the phage 186*cIts* (*moi* = 20). Burst sizes in the complementation tests were expressed as percentages of the burst size of the 186*cIts* control.

b. Preparation of defective lysates

Defective lysates were prepared by heat induction of $Su^-$ lysogens of the various 186 amber mutants. 594 lysogens of the mutants were grown to an $OD_{600} \simeq 0.6$ (3-4 *cfu*/ml) at 30°C in H-1 + glucose. These cultures were transferred to a 47°C water bath, shaken by hand for 15 min and then incubated with aeration at 37°C until lysis was complete (2½ to 3 hr) as determined by optical density measurements on a Zeiss PMQII spectrophotometer. The lysates were centrifuged (10 min, 7800 g, 4°C) to remove bacterial debris.

c. Electron microscopy of defective lysates

The defective lysates prepared above were examined for phage particles in the electron microscope. Samples were mounted on

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* In this thesis the *moi* values given refer to the number of phage added per bacterium. Since 186 adsorbs poorly (only approximately 50% in 20 min) the effective multiplicities of infection are about half those given.

carbon-coated grids, negatively stained with 2% uranyl acetate and viewed in either a Siemens Elmiskop I or a Siemens 102 electron microscope.

d. *In vitro* reconstitution

The procedure used was the one described by Weigle (1966). 0.1 ml of each of two defective lysates (prepared as above) was added to 0.8 ml of H-1 + glucose and incubated (without aeration) at 37°C for 1 hr. Viable phage were measured by assaying the mixture on an $Su^+$ strain. Each lysate was also tested for viable phage by diluting it 0.1 ml into 0.9 ml H-1 + glucose and incubating and assaying as above.

## 2.6 SECTION 4 METHODS

a. Mitomycin C treatment

Mitomycin C (MC) solutions were prepared freshly for each experiment, just prior to use, by dissolving an appropriate amount in 1 ml of TPG-CAA. The MC used was impure, being mixed with an excess of NaCl, and the concentrations required were calculated from the proportion of MC present as indicated on the bottle by the manufacturer. The MC solutions were prepared in the dark and then added to log phase cultures of E261 (see below) giving the final MC concentrations (between 10 and 75 μg/ml) indicated on the facing pages of the figures. The treated cultures were incubated, in the dark and without aeration, for 10 min at 37°C, and then centrifuged (5 min, 7800 g, 4°C). The supernatant was discarded and the pellet resuspended in the same volume of fresh TPG-CAA supplemented with 5 μg/ml thymine.

b. Phage production in mitomycin C treated cells

An overnight culture of E261 was diluted $10^{-2}$ into fresh

TPG-CAA supplemented with 5 μg/ml thymine, and grown at 37°C to a density of 3 x $10^8$ *cfu*/ml. The culture was divided into portions, one or more being treated with MC (see above) and one portion being treated with an equal volume of TPG-CAA. The control culture was incubated, centrifuged and resuspended in fresh medium as described above for the MC treated cultures. All cultures were then infected with 186 at an *moi* = 10, incubated without aeration for 5 min at 37°C, diluted $10^{-1}$ into TPG-CAA containing 186 antiserum (K = 1.5 $min^{-1}$), and incubated for an additional 5 min at 37°C without aeration. The cultures were then diluted $10^{-3}$ and $10^{-5}$ into pre-warmed TPG-CAA plus thymine (5 μg/ml) and incubated with aeration at 37°C. 0.1 ml samples were withdrawn at various times and plated immediately with indicator bacteria (i.e. no adsorption time allowed). These plates were incubated at 37°C overnight.

c. [$^3$H]Thymidine incorporation into TCA precipitable material

Cells to be used in [$^3$H]thymidine incorporation experiments were grown in TPG-CAA medium prepared with vitamin-free casein amino acids. For growth of strain E261 and its derivatives this medium was supplemented with 5 μg/ml thymine and 2 μg/ml thiamine. Other strains were grown in vitamin-free TPG-CAA without additional supplements.

i. Cumulative incorporation

Overnight bacterial cultures were diluted 1 in 50 into TPG-CAA medium (supplemented with thymine and thiamine when required). The cultures were grown with aeration at 37°C to a density of 3 x $10^8$ *cfu*/ml and then divided into two portions. One portion was added to a flask containing 186*cIts* (*moi* = 10) and [$^3$H]thymidine (final concentration 10 μCi/ml). The other portion was added to a

flask containing [$^{3}$H]thymidine alone. Both cultures were incubated with aeration at 37°C. 50 µl samples were withdrawn at 10 min intervals and placed on GF/A filters which were immediately immersed in ice-cold 10% TCA. The filters were bulk-washed 4x in ice-cold 10% TCA and then 2x in ether. Scintillation fluid was added to the dried filters and radioactivity determined in a Packard scintillation spectrometer.

In one experiment cumulative incorporation of [$^{3}$H]thymidine was determined after induction of 186 rather than after infection. In this experiment the cells were grown to log phase at 30°C and then transferred to 39°C. [$^{3}$H]Thymidine was added to one set of cultures at the time of transfer and to the other set 10 min after transfer.

ii. Pulse label incorporation

Overnight bacterial cultures were diluted 1 in 50 into TPG-CAA medium (supplemented when required with thymine and thiamine) and grown with aeration at 30°C to a density of 3 x $10^{8}$ *cfu*/ml. The cultures were then transferred to 39°C and incubation with aeration continued. 0.2 ml samples were withdrawn every 5 min (after transfer to 39°C), added to a 50 µl sample of [$^{3}$H]thymidine in TPG-CAA (final concentration 5 µCi/ml) and incubated without aeration for 2 min at 39°C. The pulse was terminated by taking a 50 µl sample, placing it on a GF/A filter and immediately immersing the filter in ice-cold 10% TCA. The filters were washed and counted as outlined above for cumulative incorporation.

## 2.7 SECTION 5 METHODS

a. 186 two-factor crosses

i. Standard 186 two-factor crosses

An overnight culture of C600 in T broth was diluted 1 in 30 into fresh T broth and grown with aeration at 37°C to a density of 3 x $10^8$ *cfu*/ml. A 0.2 ml sample was added to 0.2 ml of T broth containing a mixture of two different *am* mutants, each at an *moi* of 10. This mixture was incubated without aeration for 20 min at 37°C, then diluted $10^{-2}$ into pre-warmed T broth and incubated at 37°C with aeration for 90 min. The mixtures were chilled and assayed on C600 to measure the total yield of phage and on 594 to measure the yield of wild-type recombinants.

ii. 186 two-factor crosses after pre-irradiation with ultraviolet light

Stocks of mutants used in this type of experiment were prepared by heat induction of C600 lysogens grown in H-1+glu+$TLB_1$.

0.2 ml of a mixture of two mutants (6 x $10^8$ *pfu* of each phage) was placed on a watch glass and irradiated with ultraviolet light. The watch glass was placed 50 cm from a General Electric 15 watt germicidal lamp and was agitated on a rotating table during the irradiation. The dose rate was found to be 15.2 ergs/$mm^2$/sec as determined with a UV dosimeter supplied by Oliphant Bros., Springbank, South Australia.

0.1 ml of the irradiated mixture was added to 0.1 ml of log phase C600 (concentration 3 x $10^8$ *cfu*/ml; prepared as described in Section 2.7, a, i) and incubated without aeration for 20 min at 37°C. This mixture was diluted $10^{-2}$ into pre-warmed T broth and incubated at 37°C with aeration for 110 min. (All work to this stage was done in weak yellow light, to avoid photoreactivation.) The mixtures were chilled and assayed on C600 to measure the total

yield of phage and on 594 to measure the yield of wild-type recombinants. The original mixture of phage was assayed on C600 before and after irradiation to measure the loss in viability.

iii. <u>186 two-factor crosses in the presence of phage λ</u>

An overnight culture of E514 in T broth was diluted 1 in 30 into fresh T broth and grown with aeration at 37°C to a density of $3 \times 10^8$ *cfu*/ml. This culture was heat induced (shaken by hand for 5 min at 47°C) and a 0.2 ml sample added to 0.2 ml of T broth containing a mixture of two different 186 amber mutants, each at an *moi* of 10. This mixture was incubated without aeration for 15 min at 37°C, then diluted $10^{-2}$ into pre-warmed T broth and incubated at 37°C with aeration for 110 min. The mixtures were chilled and assayed on E511 to measure the total yield of 186 phage and on E663 to measure the yield of wild-type 186 recombinants. E511 and E663 are $Su^+$ and $Su^-$ λ lysogens respectively. The presence of a λ prophage in these strains was required to prevent the plating of the induced λ*c*I857 phage.

For the experiments in which λ was introduced by infection (Table 5.6) the method used was essentially that described in Section 2.7, a, i, except that 30 min was allowed for 186 adsorption (before the $10^{-2}$ dilution) and λ*c*I857 was added at an *moi* of 10 during this 30 min incubation. E511 and E663 were used to measure the 186 phage yields.

b. <u>Three-factor crosses</u>

For crosses involving, as the unselected marker, *c*I, *c*II or *vir* mutations, $am^+$ recombinants were obtained by the standard 186 two-factor recombination method described in Section 2.7, a, i, except that in some cases the plates were incubated at 30°C in order to display a difference in morphology between a *c*I*ts* mutant and *c*I, *c*II and

*vir* mutants. The proportions of the alternative forms of the unselected marker among the $am^+$ recombinants were determined by scoring the numbers of clear and turbid plaques present.

For crosses involving the double mutant *Mam60Ets7* the method used was essentially that described in Section 2.7, a, i, except that the 90 min incubation was at 30°C. $am^+$ recombinants were scored by assaying the phage produced on 594 at 30°C while $am^+$ $ts^+$ recombinants were scored on 594 at 38°C or 40°C. The total phage yield was determined on C600 at 30°C.

## 2.8 SECTION 6 AND SECTION 7 METHODS

### a. 186 marker rescue

Overnight T broth cultures of 594 lysogens of the relevant hybrid (or mutant) phage were diluted 1 in 30 into fresh T broth and grown at 30°C with aeration to a density of approximately $4 \times 10^8$ *cfu*/ml ($OD_{600} \doteq 1$ on Zeiss PMQII spectrophotometer). The optical density used varied for some experiments but all the bacterial cultures to be used in a single experiment were grown to optical densities as close to each other as possible. 186 *am vir* double mutants were then assayed on these cultures by the procedure described in Section 2.4, d, except that the 20 min incubation was at room temperature and the plates were incubated overnight at 30°C.

In Section 6 the 186 *am vir* double mutants were assayed on C600, 594 and E699 or E701 in addition to the assays on the 594 lysogens of the P2-186 hybrid phage or the 594 dilysogens of $\lambda imm^{434}$*Nam7* and the λp186 isolates. In Section 7 the 186 *am vir* double mutants were assayed on C600, 594, E701, and 594 lysogens of the various 186 *am* mutant phage. The frequency of recovery of a

particular allele from a prophage was expressed as a percent of the *pfu* obtained for the same mutant either on an $Su^+$ strain (% *mrf*) or on a 594 lysogen of $186^+$ or 186*cIts* (relative % *mrf*).

b. P2 marker rescue

The procedure used was identical with that used for 186 marker rescue except that all bacterial strains were grown in LGC broth and LGC plates were used for the phage assays. Since the P2 mutants used were immunity sensitive no control assays on P2 lysogens could be done. The results were expressed directly as the titre of *pfu* obtained.

## 2.9 SECTION 8 METHODS

### a. Construction of λp186 phage

The methods described in this section are based on those described by Murray *et al.* (1977).

#### i. Restriction

A mixture of λ641 DNA and 186*cIts* DNA was restricted with the restriction endonuclease *Eco*RI. The restriction mixture (30 µl) contained 1.0 µg λ641 DNA, 1.8 µg 186*cIts* DNA, 0.01 M Tris (pH 7.5), 0.1 M NaCl, 0.01 M $MgCl_2$, 0.01 M 2-mercaptoethanol and sufficient *Eco*RI to digest the DNA to completion (as judged by agarose gel electrophoresis; R. B. Saint, personal communication). This mixture was incubated for 60 min at 37°C followed by 5 min at 60°C (to inactivate the restriction enzyme) and stored at 4°C.

#### ii. Ligation

A ligation mixture (30 µl) was prepared on ice and contained 0.5 µg restricted λ641 DNA, 0.9 µg restricted 186*cIts* DNA, 0.066 M Tris (pH 7.5), 0.01 M $MgCl_2$, 0.01 M dithiothreitol, 1 mM EDTA,

0.1 mM ATP and 0.5 units* T4 polynucleotide ligase. Half the volume of the ligation mixture was derived from the restriction mixture and therefore it also contained 0.05 M NaCl. The ligation mixture was incubated for 6 hr at 10°C and stored at 4°C for 24 hr before use.

iii. Transfection

An overnight culture of ED8654 in LG broth was diluted 1 in 50 into fresh LG broth and grown with aeration at 37°C to a density of 2-3 x $10^8$ *cfu*/ml. The cells were chilled on ice for 20 min then sedimented at 4°C and resuspended in 0.5 vol. ice-cold 0.1 M $MgCl_2$. They were immediately resedimented at 4°C and resuspended in one tenth the previous volume of ice-cold 0.1 M $CaCl_2$. The cells were kept on ice for 2 hr before use.

0.1 ml ice-cold DNA in 0.1 M Tris (pH 7.1) was added to 0.2 ml competent cells. This mixture was divided into 3 x 0.1 ml lots and kept at 0°C for 30 min with occasional shaking. The mixtures were then heat shocked at 42°C for 2 min, chilled for 30 min on ice and then warmed to room temperature. They were plated on T plates with the T soft agar layer supplemented with 0.01 M $MgSO_4$. (This procedure should produce about $10^6$ *pfu*/μg λDNA).

iv. Determination of restriction coefficient

Stocks of the λp186 isolates were assayed on log phase cultures (grown in LG broth) of strains 5K and 5KRI. The restriction coefficient was calculated by the titre as measured on strain 5K divided by the titre as measured on strain 5KRI.

v. Preparation of dilysogens

The construction of 594 dilysogens of the λp186 isolates

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* One unit of T4 polynucleotide ligase converts 1 nmole of $^{32}P$-phosphomonoesters into a phosphatase resistant form in 20 min at 37°C.

and the phage $\lambda imm^{434}Nam7$ is described in the text (Section 8.3). After each single colony isolation colonies were tested for phage production on C600 and 594. After 3 or 4 single colony isolations purified cultures were sedimented and the supernatants assayed on C600. The presence of both clear plaques (λp186) and turbid plaques ($\lambda imm^{434}Nam7$) in the supernatants confirmed the presence of both prophage in the lysogenic cells.

b. Attempted preparation of P2-186 hybrid phage

i. "Reverse" hybrids

An overnight culture of C600 in T broth was diluted 1 in 30 into LGC broth and grown with aeration at 37°C to a density of $3 \times 10^8$ *cfu*/ml. A 1 ml sample was co-infected with $P2^+$ and 186*cIts*, each at an *moi* of 10. After incubation with aeration at 37°C for 30 min, 0.1 ml of a potassium phosphate solution was added (final concentration 0.01 M) and the incubation continued for a further 60 min. The above procedure was repeated for single infections by $P2^+$ and 186*cIts*. At the end of 90 min all three lysates were assayed on the following indicators and plates: C600 on LGC plates, C600 on LGcit plates, E573 on LGC plates and E573 on LGcit plates. These measured the titres of total progeny, 186 progeny, P2 progeny and hybrid phage respectively.

ii. "Forward" hybrids

The procedure used was the same as that described above for "reverse" hybrids except that the phage used were amber mutants of P2 and 186, the selective indicators were 594 and E811 and LGC plates were used for all assays. Two crosses were tried, 186*cItsWam*15 x $P2vir_1amE_{30}$ and 186*cItsOam*61 x $P2vir_1amE_{30}$.

c. Heteroduplex analysis

i. Formation of heteroduplex molecules

The method used was basically as described by Davis and Parkinson (1971). A heteroduplex mixture (0.2 ml) was prepared as follows.

To a small vial were added 20 µl 0.1 M EDTA pH 8.0, 2-4 µl samples of two phage preparations taken from a CsCl gradient (each containing 0.5 µg of DNA as determined from $OD_{260}$ measurements), $H_2O$ to bring the volume to 80 µl and 10 µl 1 M NaOH. The NaOH treatment results in the extraction and denaturation of the phage DNA. After standing for 10 min at room temperature the solution was neutralized by the addition of 10 µl 2 M Tris (pH 7). Renaturation was effected by the addition of 0.1 ml formamide and standing the mixture at room temperature for 1 hr. Samples of this mixture were used to prepare spreading solutions for electron microscopy. The remainder was dialysed at 4°C against 0.02 M NaCl, 0.005 M EDTA (pH 7.4) for 24 hr and then stored at 4°C.

Preparations of homoduplex DNA were prepared in a similar fashion.

ii. Preparation of DNA specimens for electron microscopy

The procedure used was basically as described by Davis *et al.* (1971).

A spreading (hyperphase) solution (∿100 µl) containing the DNA to be examined was prepared from the following components: 10 µl 1M Tris + 0.1 M EDTA (pH 8.5), 10 µl 5 µg/ml DNA, 50 µl 99% formamide, 1 µl 10 mg/ml cytochrome *c* and 30 µl $H_2O$. 1 µl 6 µg/ml ϕX174 RF DNA was also added, as an internal length standard, to most preparations.

A hypophase solution was prepared in a petri dish and consisted of: 16 ml 99% formamide, 0.8 ml 1 M Tris + 0.1 M EDTA (pH 8.5) and $H_2O$ to fill the dish (∿60 ml).

The hypophase was stirred with a metal ramp which had been cleaned by heating until red hot, in a bunsen burner flame. 50 μl of the spreading solution was then applied to the ramp and allowed to spread across the surface of the hypophase forming a film. The film was allowed to stand for 1 min before being picked up on a parlodian coated grid by momentarily touching the grid to the surface of the film. The grid was stained by dipping for 30 sec in a solution of uranyl acetate prepared approximately 10 min before use by diluting a stock solution ($5 \times 10^{-2}$ M uranyl acetate in 0.05 M HCl, stored in the dark) $10^{-3}$ into 90% ethanol. After staining, the grid was rinsed (10 sec) in isopentane, shadowed with a platinum/palladium alloy (ratio 80 : 20) on a rotating table and examined in a Philips EM 300 electron microscope. For some grids staining with uranyl acetate was omitted.

Electron micrographs were taken on 60 mm glass plates. The negatives were enlarged and length measurements obtained with a Numonics Electronic Graphics Calculator.

## 2.10 CONSTRUCTION OF PHAGE STRAINS

### a. 186 *am vir* double mutants

Two methods have been used to cross the *vir*2 mutation of the phage 186*c*I*ts*P*am*16*vir*2 into the other 186*c*I*ts am* mutants.

#### i. Two-factor crosses

186*c*I*ts*P*am*16*vir*2 and various 186*c*I*ts am* mutants were recombined using the procedure described in Section 2.7, a, i. The

progeny phage were plated on the *supF* suppressor strain H12R8A lysogenic for the mutant *Pam*16, and the plates incubated at 30°C. The parental phage 186*c*I*tsPam*16*vir*2 will not grow on this strain as the *Pam*16 mutation is not suppressed by *supF* suppressors. The recombinant phage 186*c*I*tsPam*16 will also be unable to grow for the same reason. The other parental phage 186*c*I*ts ama* will not grow since the indicator is lysogenic. The only phage able to grow will be the desired 186*c*I*ts ama vir*2 recombinant. *vir*2 derivatives of the mutants *Dam*26, *Fam*20, *Mam*19, *Oam*61 and *Wam*15 were prepared by this method.

As an alternative the mutant *Gam*27*vir*2 can be used in place of *Pam*16*vir*2. In this case the progeny phage should be assayed at 30°C on C600 lysogenic for the mutant *Gam*27 since the mutation *Gam*27 is not suppressed by *supE* suppressors. This was the procedure used to produce *vir*2 derivatives of the mutants *Qam*1, *Ram*51 and *Uam*37.

*Eam*7*vir*2 was prepared from a cross between 186*c*I*tsEam*7 and 186*c*I*tsPam*16*vir*2 as described above except that the selective indicator strain used was H12R8A lysogenic for the mutant *Jam*41. In addition to the *Eam*7*vir*2 recombinants, *Jam*41*vir*2 and $am^+$ *vir*2 phage were also obtained, as a result of marker rescue from the prophage of the indicator strain.

All *vir*2 derivatives constructed were purified by three successive single plaque isolation steps and tested for the presence of the particular *am* mutation by assays on a 594 lysogen of the same *am* mutant and a 594 lysogen of a different *am* mutant. Failure to obtain marker rescue from a particular *am* mutant prophage confirmed the presence of the same allele in the superinfecting phage.

ii. Marker rescue

*vir*2 derivatives of 186 *am* mutant phage can be prepared

by assaying a stock of the phage 186*c*I*tsPam*16*vir*2 on an H12R8A lysogen of the amber mutant, with incubation at 30°C. The superinfecting phage will not grow on this indicator since the *supF* suppressor of strain H12R8A is unable to suppress the *Pam*16 mutation. Any phage which appear on this indicator must therefore be the result of rescue of the *am*16$^+$ allele from the prophage. (Marker rescue is discussed in detail in Sections 6 and 7 and the procedure used is described in Section 2.8.) The phage appearing will also have the *vir*2 mutation since the presence of the prophage in the indicator bacteria will prevent the growth of any non-virulent phage. In recovering the *am*16$^+$ allele from the prophage, coincident recovery of the *am* allele of the prophage may also occur giving rise to the required *am vir* double mutant. Alternatively the *am* mutation of the prophage may not be recovered and the resultant phage will have the genotype 186*c*I*tsvir*2. These two genotypes can be distinguished by testing the phage for ability to grow on an Su$^-$ strain.

This procedure was used to construct a 186*c*I*tsGam*27*vir*2 phage. A similar procedure was used to construct 186*c*I*tsNam*47*vir*2 but due to the lack of an H12R8A lysogen of *Nam*47, an S26R1e lysogen was used instead. The mutant *Pam*16 shows a very reduced *eop* on strain S26R1e and the low level of plaques formed are very tiny. *Nam*47*vir*2 phage were obtained by selecting larger plaques superimposed on a background of these tiny plaques.

A variation of the above procedure involved assays of the phage 186*c*I*tsGam*27*vir*2 (prepared as described above) on C600 lysogens of various 186 *am* mutant phage. This was the procedure used for the construction of *vir*2 derivatives of all 186 *am* mutants other than those discussed above. It was the preferred method as C600 lysogens had

been prepared for most mutants. This procedure is simpler than the two-factor recombination method and since the frequency of *am vir* double mutants was high the presence of the $am^+$ *vir* phage was not a problem. *am vir* double mutants could usually be selected successfully on the basis of plaque size since the plaques produced by these phage were usually smaller than those produced by $am^+$ *vir* phage.

The *vir*2 derivatives obtained were purified and tested as described in Section 2.10, a, i.

b. 186*c*I*ts am* mutants from 186*c*I *am* or 186*c*II *am* mutants

Several of the 186 *am* mutant phage also had a non-conditional mutation in either the *c*I or *c*II gene and it was necessary to cross this mutation out and replace it with a *c*I*ts* $c\text{II}^+$ combination so that lysogens of these phage could be made. The mutants *Qam*1 and *Ram*51 have such additional clear plaque mutations and while the mutant *Uam*37 gave turbid plaques on C600 at 30°C attempts to lysogenise C600 and 594 strains failed. This might be due to an additional mutation in the *int* gene but this has not been examined. These mutants were first converted to *am vir* double mutants as described in Section 2.10, a, i. The double mutants were then recombined with the mutant 186*c*I*tsGam*27 (see Section 2.7, a, i) and the progeny phage plated on C600 at 30°C. Two plaque types were produced; clear plaques produced by the parental *am vir* phage and turbid plaques produced by the desired recombinant, 186*c*I*tsQam*1, 186*c*I*tsRam*51 or 186*c*I*tsUam*37. Turbid plaque isolates were purified by three successive single plaque isolation steps and tested by plating on C600, H12R8A and 594.

The non-conditional clear plaque mutation present in the phage *Oam*62 was removed directly by recombination with 186*c*I*tsPam*16

and plating of progeny phage on strain H12R8A. Two plaque types again resulted; clear plaques produced by the parental *Oam*62 phage and turbid plaques produced by the 186*c*I*ts*O*am*62 recombinants. A turbid plaque isolate was purified and tested as described above. The non-conditional clear plaque mutation has not been crossed out of the mutant *Wam*52 *c*I*ts* *c*II.

The *c*II mutation of the phage 186*Ham*56*c*II was removed by recombining this mutant with 186*c*I*ts*G*am*27 and plating the progeny phage on C600 at 30°C. 11 turbid plaques were obtained although the frequency was very low (1 turbid plaque for every 55,000 clear plaques) due to the presence of the $cI^{+}$ allele in the *Ham*56*c*II phage (see Section 5). These 11 plaques were all produced by 186*c*I*ts*H*am*56 phage and one was selected, purified and tested as described above.

c. *Mam*60*Ets*7

Stocks of *Mam*60 and *Ets*7 were prepared by heat induction of C600 lysogens in the medium H-1+glu+$TLB_1$. (The C600(186*c*I*ts*E*ts*7) culture was returned to 30°C after heat induction.) These mutants were mixed, exposed to 60 seconds UV irradiation and recombined as described in Section 2.7, a, ii. The progeny phage were plated on C600 and the plates incubated at 30°C. The progeny phage were also plated on 594 at 38°C in order to determine the frequency of $am^{+}$ $ts^{+}$ recombinants. This should be the same as the frequency of *am ts* recombinants and was found to be approximately 1 in 1600.

Plaques from the C600 plates incubated at 30°C were tested by toothpicking into lawns of the following bacterial strains and incubating the plates at the temperatures indicated: 594 (30°C), C600 (38°C) and C600 (30°C). The parental mutant *Mam*60 will grow on C600 at either 38°C or 30°C but will not grow on 594 at 30°C. The

parental mutant *Ets*7 will grow on both 594 and C600 at 30°C but will not grow on C600 at 38°C. The double mutant can be distinguished from both parental mutants since it should fail to grow on both 594 at 30°C and C600 at 38°C. It will grow only on C600 at 30°C. 920 plaques were tested; 561 were of the parental type *Mam*60 and 358 were of the parental type *Ets*7. One plaque showed the plating characteristics expected for a double mutant. The phage from this plaque were purified by three successive single plaque isolations and a phage stock prepared. The presence of the two mutations *Mam*60 and *Ets*7 was confirmed by complementation studies. This method could be used to produce other double mutants from any of the combinations of *am* and *ts* mutants.

d. Possible method for the preparation of double amber mutants

The very low frequency of recombination found for phage 186 and the absence of a general screening method for detecting double amber mutants make the production of these mutants by recombination methods impractical. Attempts to utilize the differential plating of the various *am* mutants on the three suppressor strains for this purpose were unsuccessful as very few pairs of mutations met the criteria required, namely, that each mutant should grow on a suppressor strain on which the other mutant cannot grow while both phage should grow on the third suppressor strain. Of the few pairs of mutants meeting these criteria at least one showed a small plaque size on the suppressor strain on which the double mutant was to be isolated and this, together with the low frequency of recombination probably accounts for the failure of attempts to isolate double amber mutants.

A possible method for the production of double amber mutant

phage, which has not as yet been tried, involves transfection with heteroduplex molecules formed from the DNA extracted from two amber mutant phage. If the DNAs from two phage were dissociated into single strands and then reannealed, approximately 50% of the duplex molecules should be heteroduplexes. Upon transfection, repair of the single base mismatches before replication should lead to the production of wild-type phage and double amber mutant phage as well as the single amber mutant phage of the parental types. The proportion of double amber mutants among the transfectants may be as high as 1 in 8 and this frequency is high enough to allow screening for double amber mutants by complementation tests after preparation of phage stocks.

The frequency of double amber mutants produced by this method could be increased by starting initially with heteroduplexes formed from isoalted single strands of the parental phage, separated by the method described by Szybalski *et al.* (1971). This should improve the frequency by at least two-fold.

An easier method for the detection of double amber mutants, obtained by the above procedure, might be possible if the two parental *am* mutant phage each carried the *vir*2 mutation. The transfectant plaques obtained could then be tested for the presence of the *am* mutations by marker rescue from *am* mutant prophage. A double *am* mutant could be distinguished from either single *am* mutant by its failure to produce wild-type recombinants when plated on $Su^-$ lysogens of either parental *am* mutant. By contrast each of the single *am* mutant phage should show marker rescue from one parental *am* mutant prophage, but not from the other.

The frequency of marker rescue would probably be large

enough to allow such a screening method to be used for most pairs of *am* mutants. The frequency of marker rescue from a wild-type prophage is usually between 0.1% and 0.3% (see Table 7.1). A single transfectant plaque should contain approximately $10^7$ *pfu* and should therefore give rise to approximately $10^4$ *pfu* when plated on a wild-type 186 lysogen. The frequency of marker rescue from an *am* mutant prophage would be lower, but the results recorded in Section 7 show that generally a frequency greater than 1% of the marker rescue frequency from a wild-type prophage could be expected. That is, plating the phage from a single transfectant plaque on $Su^-$ lysogens of the parental *am* mutant phage should generally result in more than 100 *pfu* on one of these strains, if the phage tested carried only a single *am* mutation, whereas no plaques would result if the phage tested carried both *am* mutations. If this technique proved satisfactory then any improvement in the frequency of formation of double mutants, obtained by preparing heteroduplexes from separated strands, would be unnecessary.

# SECTION 3

# DEFINITION OF GENES AND DETERMINATION OF FUNCTION

## 3.1 INTRODUCTION

At the beginning of this study on the genetic map of coliphage 186 there was a group of 51 amber (or chain termination) mutants available. 47 of these had been isolated by W. H. Woods in this laboratory, after UV mutagenesis of the heat-inducible mutant 186*c*I*ts*. Three other mutants, *am*48, *am*49 and *am*50, were gifts from A. D. Kaiser and are all derived from wild-type 186. A fourth mutant, *am*56, was a gift from C. P. Georgopoulos and has a mutation in the 186 *c*II gene in addition to the *am*56 mutation. During the course of this work ten more amber mutants and three temperature-sensitive mutants were isolated after NNG mutagenesis of 186*c*I*ts*. All the amber and temperature-sensitive mutations mentioned above are located within essential genes of phage 186.

This section is concerned with the assignment of these mutations to complementation groups and with the determination of the functions of those genes involved in the formation of the phage particle. The work described in this section was initiated while undertaking work for the Honours degree of Bachelor of Science and some of the results obtained during that time have been included here since they are as yet unpublished and the information is required for the discussion of subsequent results. Such results include all liquid complementation results (Table 3.1), many of the plate complementation results, the reconstitution results of Table 3.3 (but not those of Table 3.4) and some of the electron microscopy. Tables 3.2 and 3.5 are summaries of the information about the 186 genes and their functions and have been constructed from a combination of some results presented for the Honours degree of Bachelor of Science and other results obtained while undertaking work for this thesis.

## 3.2 DEFINITION OF THE ESSENTIAL GENES OF PHAGE 186

Two methods have been used to study complementation in phage 186. One method, termed "plate complementation", involves cross-streaking stocks of different phage mutants on a lawn of non-permissive bacteria. If phage activity occurs where the streaks cross then the mutations tested must reside in different genes. If no phage activity is visible then the mutations are deemed to belong to the same gene. The second method, liquid complementation, involves the co-infection of non-permissive bacteria by different mutants in a liquid medium. An increase in the titre of amber mutant phage on co-infection, compared with the titres obtained after infection by each mutant separately, is indicative of complementation and the mutants involved are assigned to different genes. No increase in phage titre suggests that the two mutants belong to the same gene. Plate complementation has the advantage that it is a simple, easy test and many tests can be done in the same time required for a single liquid complementation. However it has the disadvantage that it is not possible to be sure that the phage activity occurring where the streaks cross is due to complementation rather than to recombination. Liquid complementation is more cumbersome but does allow the distinction between complementation and recombination to be made.

### a. Liquid complementation

The results of the initial plate complementation tests suggested the presence of at least 11 genes. A representative mutant from each of these 11 genes was tested by liquid complementation and the results, shown in Table 3.1, confirm that these mutants do belong to different genes.

The increase in phage titre observed after co-infection is due to complementation and not recombination. In all tests not

TABLE 3.1[a]

INTERCISTRONIC LIQUID COMPLEMENTATION TESTS[b]

| | *A* *am*5 | *B* *am*17 | *E* *am*7 | *H* *am*50 | *L* *am*21 | *M* *am*19 | *N* *am*47 | *P* *am*16 | *Q* *am*49 | *T* *am*8 | *W* *am*15 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Aam*5 | 0.03 | 3.8 | 0.52 | 0.56 | 0.29 | 2.1 | 1.3 | 5.8 | 0.69 | 0.42 | 0.34 | *Aam*5 |
| *Bam*17 | | < 0.01 | 63 | 36 | 72 | 48 | 48 | 29 | 34 | 55 | 78 | *Bam*17 |
| *Eam*7 | | | 0.01 | 26 | 32 | 37 | 15 | 38 | 41 | 30 | 16 | *Eam*7 |
| *Ham*50 | | | | < 0.01 | 28 | 30 | 13 | 51 | 51 | 31 | 27 | *Ham*50 |
| *Lam*21 | | | | | < 0.01 | 29 | 16 | 36 | 37 | 43 | 19 | *Lam*21 |
| *Mam*19 | | | | | | < 0.01 | 34 | 37 | 35 | 24 | 32 | *Mam*19 |
| *Nam*47 | | | | | | | < 0.01 | 36 | 16 | 17 | 36 | *Nam*47 |
| *Pam*16 | | | | | | | | 0.05 | 57 | 46 | 32 | *Pam*16 |
| *Qam*49 | | | | | | | | | < 0.01 | 6.5 | 32 | *Qam*49 |
| *Tam*8 | | | | | | | | | | 0.01 | 42 | *Tam*8 |
| *Wam*15 | | | | | | | | | | | < 0.01 | *Wam*15 |

*a* All the results appearing in this table are taken from Hocking (1972).

*b* The figures given are the burst sizes for each co-infection expressed as percentages of the burst size of 186*cIts*. Under the conditions used in the complementation tests the burst size of 186*cIts* was 42. The burst sizes were measured on C600 in all cases except those involving *Nam*47 for which S26R1e was used. For the co-infection *Nam*47+*Pam*16 the burst size was calculated by the addition of the titres obtained on C600 and S26R1e.

involving mutant *Aam*5 the number of wild-type recombinants formed was less than 0.01% of the 186*c*I*ts* burst size. This was also true for the two tests *Aam*5 with *Ram*49 and *Aam*5 with *Ham*50. For other tests involving *Aam*5 the number of recombinants was much higher but in no case did it exceed 0.11% of the burst size of 186*c*I*ts*. Therefore, even the very low burst sizes obtained for tests involving *Aam*5 cannot be accounted for by recombination. However although some complementation appears to be occurring with mutant *Aam*5 it is producing, on average, less than one phage per cell.

b. <u>Plate complementation</u>

In general plate complementation was found to be a satisfactory method for defining the genes of phage 186. Positive complementation results suggested the presence of at least 11 genes and these assignments were confirmed by the liquid complementation results recorded in Table 3.1.

However, apart from clearly positive and clearly negative results a third, intermediate result, was often obtained. In this type of result an increase in phage activity in the region where two streaks crossed would be observed but would be in the form of plaques rather than in the form of a confluent square of lysis.

When mutant *am*5 was tested by plate complementation it gave this intermediate type of result with all mutants belonging to the 10 other genes defined by liquid complementation. This is in agreement with the very poor complementation observed in liquid medium for tests involving this mutant. Amber mutants 11, 12, 13, 24, 30, 33 and 43 also gave intermediate type results when tested with mutants belonging to other genes. When tested amongst themselves these eight mutants failed to show any complementation. They were therefore assigned to the same gene (gene *A*) and poor complementation appears to be a

characteristic of this gene.

Mutant 40 behaved similarly to the mutants of gene *A* in that no squares of confluent lysis were obtained in complementation tests. However rather than producing intermediate types of results, mutant 40 generally gave negative results with other mutants. Subsequent results have shown that this mutant is in fact a double mutant, one amber mutation being in gene *I* and the other in gene *A*. These mutations are referred to as 40a and 40b respectively. When the mutant 40a was separated from 40b by recombination it no longer showed the poor complementation characteristic of gene *A* mutants and complemented all other mutants except mutants 50, 56 and 41.

Intermediate type complementation results were also obtained for various other pairs of mutants in which neither mutation belonged to gene *A*. However in these cases the mutants involved would only show intermediate results with other mutants belonging to one, or at the most two, of the 10 (non-*A*) genes defined in Table 3.1. For example, the fourteen mutations, 14, 23, 26, 48, 7, 35, 46, 20, 9, 25, 27, 29, 10 and 28 showed either negative or intermediate results when tested amongst themselves while showing good complementation with all other mutants. Such mutants were initially assigned to a single gene although it is probable that they belong to more than one gene, the poor complementation being due to polarity effects.

These intermediate results are also obtained in liquid complementation experiments. Mutants *Fam*20 and *Gam*25 when cross-streaked against *Eam*7 showed intermediate results. When co-infected with *Eam*7 in liquid medium they showed burst sizes of 0.40% and 0.33%, of the burst size of 186*c*I*ts*, respectively. This was more than ten-fold higher than background values (0.01% for *Eam*7, 0.01% for *Fam*20 and 0.02% for *Gam*25) but was about 100-fold lower than for mutants giving clearly

positive liquid complementation results. This intermediate complementation represented only about one *pfu* produced for every six or seven infected bacteria. If this sort of result is due to polarity effects then these are of the "strong" type observed in phage P2 (Lindahl, 1971; Sunshine *et al.*, 1971) rather than of the "weak" type observed in phage λ (Parkinson, 1968).

The behaviour of mutants *Lam*21 and *Mam*19 in complementation tests strongly suggested that polarity effects were operating. Complementation on the plate for these two mutants was relatively poor although they clearly belonged to different genes. (Liquid complementation results confirmed this (see Table 3.1).) However there are several other mutants which showed intermediate results in plate complementations with both *Lam*21 and *Mam*19.

To avoid the problem of polarity and to determine the number of genes represented by the known alleles of 186 a different bacterial host was used in complementation tests. This host, XA7007, contains the polarity suppressing mutation known as SuA (Morse and Primakoff, 1970; Beckwith, 1963). When tested by plate complementation on XA7007 the 14 mutations which previously had failed to complement with *Eam*7 could be divided into 4 groups (namely genes *D*, *E*, *F* and *G*), the members of each group complementing the members of the other three groups but not those of their own group. The subdivisions of the other polarity groups resulting from complementation tests on XA7007 are shown in Table 3.2. The genes *A*, *B*, *O*, *P* and *W* defined on strain 594 were not further subdivided when tested on strain XA7007.

Phage 186 appears to have four polarity groups, or four sets of co-transcribed genes. Genes *B* and *W* appear to be quite independent of these four groups. Genes *N*, *O* and *P* although defined in the absence of any suppression of polarity may still belong either

TABLE 3.2[a]

LIST OF THE GENES, THEIR ALLELES AND THE POLARITY GROUPS OF PHAGE 186 DEFINED BY COMPLEMENTATION TESTS

| Gene | Amber alleles | Polarity groups |
|---|---|---|
| *A* | 5, 11, 12, 13, 24, 30, 33, 43 | |
| *B* | 17, 57 | |
| *D* | 14, 23, 26, (48)[b] | |
| *E* | 7, 35, 46 | D, E, F, G |
| *F* | 20 | |
| *G* | 9, 25, 27, 29, (10, 28) | |
| *H* | 50, 56 | |
| *I* | 40a | H, I, J |
| *J* | 41 | |
| *K* | 22, 42, 58[c] | |
| *L* | 2, 21, (44) | K, L, M |
| *M* | 19, 31, 60 | |
| *N* | 47 | |
| *O* | 61, 62 | |
| *P* | 16, 36, 45, 65, 67, (66) | |
| *Q* | 1, 49 | |
| *R* | 6, (51) | |
| *S* | 4, 34, (18) | Q, R, S, T, U, V |
| *T* | 8 | |
| *U* | 37, 63, 64 | |
| *V* | 38 | |
| *W* | 15, 39, 52[d] | |

[a] Many of the complementation results used in the construction of this table are taken from Hocking (1972).

[b] The mutants in brackets appear to be identical with one of the other listed mutants, i.e. 48 with 26, 10 and 28 with 25, 44 with 21, 66 with 67, 51 with 6 and 18 with 4.

[c] Mutant *ts*59 also belongs to gene *K*.

[d] Mutants *ts*68 and *ts*69 also belong to gene *W*.

to the *KLM* operon or to the *QRSTUV* operon, or perhaps to a new operon.

Although the use of strain XA7007 led to the definition of many extra genes in 186 there were several problems associated with its use. Firstly, the appearance of the squares of lysis on this strain was more turbid and indistinct than on strain 594. That is, even mutants in different polarity groups, which produced a very clear square of confluent lysis on 594, showed a poorer (although still positive) response on strain XA7007. Secondly, although there was often a clear-cut improvement in complementation on XA7007 compared with 594 for mutants in the same polarity group, there were also many cases in which only a slight improvement occurred. In some cases no improvement was observed even though the results with other mutants suggested that the two mutants in question belonged to different genes.

These problems seemed particularly prevalent in the polarity group containing genes *Q*, *R*, *S*, *T*, *U* and *V*. The assignment of alleles for this group, shown in Table 3.2, represents the most likely arrangement, although the assignment of the three alleles 37, 63 and 64 to a single gene is doubtful. On strain 594 mutants 37 and 64 showed more phage activity than would be expected for mutants belonging to the same gene. However the use of strain XA7007 led to no improvement in complementation for any pair of mutants from the group 37, 63 and 64.

Quantitative results from liquid complementation experiments in XA7007 might help to resolve these problems. However, quantitative experiments for phage P2 have shown that the level of suppression of polarity by the SuA mutation can be very variable and in some cases no improvement in phage yields was obtained even when the mutations in question were known to belong to different genes (Sunshine *et al.*, 1971).

The gene assignments shown in Table 3.2 are supported by the genetic mapping results described in Sections 6 and 7. Mutations assigned to the same gene by complementation results in the strain XA7007 were also found to be located in adjacent positions on the genetic map.

## 3.3 FUNCTIONAL ASSIGNMENTS

In this section the functions of genes involved in the formation of the phage particle were determined by examination of the defective lysates produced by the various amber mutants.

a. Lysis of a non-permissive host

When Su⁻ lysogens of 186 amber mutants in genes *D*, *E*, *F*, *G*, *H*, *J*, *K*, *L*, *M*, *N*, *Q*, *R*, *S*, *T*, *U*, *V* or *W* were heat-induced, bacterial cell lysis commenced 30 to 60 minutes later, producing lysates containing very few active phage particles (usually less than $10^3$ *pfu*/ml). These lysates have been called "defective lysates" and although they lack infective phage particles they contain many incomplete phage structures (see Sections 3.3b and 3.3c). The single known mutation belonging to gene *I* (*am*40a) has not been tested by this method.

Quite different results were obtained when Su⁻ lysogens of mutants in genes *A*, *B*, *O* and *P* were heat-induced. For mutants in genes *A* and *B* the optical density of the bacterial cultures following heat induction continued to increase, at a rate similar to that of a non-lysogen, for at least 2½ hours. For the mutant *Pam*16 no evidence of cell lysis was obtained although bacterial cell growth appeared to cease at approximately 60 minutes (that is, the OD curve started to plateau at about 60 minutes). For the mutant *Oam*61 cell lysis did occur but was very delayed, starting about 2 hours after induction. (Normally lysis commences between 30 and 60 minutes after induction.)

It appears that mutants in genes *A*, *B*, *O* and *P* have a reduced ability to lyse the bacterial host. Such a failure in cell lysis could be due to a mutation in a gene concerned only with lysis (and therefore not affecting the production of mature phage) or to a mutation in an "early" gene whose product is required for the production both of mature phage and of the proteins required for cell lysis. These two situations differ in that a build-up of mature phage within the host cell should occur in the first case but not in the second. Artificial lysis of the cells should then distinguish between these possibilities, a large increase in phage titre occurring in the former but not in the latter case. This has not been done for 186 and therefore it is not known whether genes *A*, *B*, *O* and *P* are early genes, required for the production of all late proteins, or late genes concerned only with cell lysis.

Apart from the absent or delayed cell lysis, the heat induced $Su^-$ lysogens of mutants in genes *A*, *B*, *O* and *P* also differed from those of mutants in other genes in the numbers of mature phage particles present. At equivalent times after heat induction the lysogenic cultures of mutants in genes *A*, *B*, *O* and *P* contained a large number of active phage ($10^6$ to $10^8$ *pfu*/ml) compared with the numbers obtained in the lysates produced by mutants in the other genes ($<10^3$ *pfu*/ml). However even these relatively large concentrations represent less than one mature phage per bacterial cell. These phage may be due to some spontaneous lysis of cells containing mature phage but alternatively they could be due to "leak", that is, to the misreading of the nonsense mutation allowing the formation of some active protein. Thus the presence of these phage does not distinguish between mutants defective in either "early" or "late" functions.

b. *In vitro* reconstitution

Those mutants capable of lysing a non-permissive host were assumed to be mutant in the phage morphogenetic process. To determine which part of the phage structure is affected by these mutants the defective lysates were tested for *in vitro* reconstitution. In these experiments the defective lysates were added together in pairwise combinations and the increase in phage titre determined.

The results, recorded in Table 3.3, show that functionally there are two main groups of mutations. Mutants belonging to the genes *D*, *E*, *F*, *G*, *K*, *L* and *M* appear to be mutant in the same phage structure since the addition of the defective lysates produced by these mutants, in any pairwise combination, did not lead to any increase in phage titre. Similarly, when tested among themselves, the lysates produced by mutants defective in the genes *R*, *S*, *T* or *W* did not give rise to any increase in phage titre. However, when the lysates produced by the first set of mutants were added to the lysates produced by the second set, a very large (approximately $10^7$ fold) increase in phage titre was observed. This suggests that the products of the two groups of genes, *R*, *S*, *T*, *W* and *D*, *E*, *F*, *G*, *K*, *L*, *M* are concerned with the formation of two different parts of the phage particle.

Complementation studies with the reconstituted phage showed that in all cases the DNA of the reconstituted phage was derived from the lysates produced by mutants in the genes *D*, *E*, *F*, *G*, *K*, *L* and *M* and never from the lysates produced by mutants in the genes *R*, *S*, *T* or *W*. In cases where the plaque morphology of the two parent phage differed, the reconstituted phage always had the morphology of the mutant in the genes *D*, *E*, *F*, *G*, *K*, *L* or *M* and never that of the mutant in the genes *R*, *S*, *T* or *W*. Both these observations suggest that the genes *R*, *S*, *T* and *W* are involved in formation of the phage

TABLE 3.3[a]

*IN VITRO* RECONSTITUTION WITH MUTANTS IN GENES *D*, *E*, *F*, *G*, *K*, *L*, *M*, *N*, *R*, *S*, *T*, *V* AND *W*[b]

| | *W* *am*15 | *V* *am*38 | *T* *am*8 | *S* *am*4 | *R* *am*6 | *N* *am*47 | *M* *am*19 | *L* *am*21 | *K* *am*42 | *G* *am*25[c] | *F* *am*20 | *E* *am*7 | *D* *am*14 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Wam*15 | $<1\times10^{2}$ | $7\times10^{5}$ | $1\times10^{2}$ | $<1\times10^{2}$ | $2\times10^{2}$ | $7\times10^{8}$ | $2\times10^{9}$ | $2\times10^{9}$ | $1\times10^{9}$ | $1\times10^{9}$ | $2\times10^{9}$ | $2\times10^{9}$ | $1\times10^{9}$ | *Wam*15 |
| *Vam*38 | | $<1\times10^{2}$ | $<1\times10^{2}$ | $<1\times10^{2}$ | $5\times10^{5}$ | $2\times10^{9}$ | $2\times10^{9}$ | $1\times10^{9}$ | $2\times10^{9}$ | $9\times10^{8}$ | $1\times10^{9}$ | $2\times10^{9}$ | $1\times10^{9}$ | *Vam*38 |
| *Tam*8 | | | $<1\times10^{2}$ | $<1\times10^{2}$ | $2\times10^{2}$ | $8\times10^{7}$ | $1\times10^{8}$ | $1\times10^{8}$ | $1\times10^{8}$ | $1\times10^{8}$ | $2\times10^{8}$ | $9\times10^{7}$ | $1\times10^{8}$ | *Tam*8 |
| *Sam*4 | | | | $<1\times10^{2}$ | $1\times10^{2}$ | $2\times10^{9}$ | $2\times10^{9}$ | $1\times10^{9}$ | $2\times10^{9}$ | $1\times10^{9}$ | $1\times10^{9}$ | $2\times10^{9}$ | $1\times10^{9}$ | *Sam*4 |
| *Ram*6 | | | | | $2\times10^{2}$ | $2\times10^{9}$ | $2\times10^{9}$ | $1\times10^{9}$ | $2\times10^{9}$ | $1\times10^{9}$ | $1\times10^{9}$ | $2\times10^{9}$ | $1\times10^{9}$ | *Ram*6 |
| *Nam*47 | | | | | | $<1\times10^{2}$ | $6\times10^{4}$ | $5\times10^{5}$ | $6\times10^{5}$ | $4\times10^{5}$ | $9\times10^{5}$ | $6\times10^{4}$ | $7\times10^{5}$ | *Nam*47 |
| *Mam*19 | | | | | | | $2\times10^{2}$ | $5\times10^{2}$ | $6\times10^{2}$ | $2\times10^{2}$ | $2\times10^{2}$ | $5\times10^{2}$ | $1\times10^{2}$ | *Mam*19 |
| *Lam*21 | | | | | | | | $2\times10^{2}$ | $1\times10^{2}$ | $2\times10^{2}$ | $2\times10^{2}$ | $5\times10^{2}$ | $2\times10^{2}$ | *Lam*21 |
| *Kam*42 | | | | | | | | | $<1\times10^{2}$ | $<1\times10^{2}$ | $<1\times10^{2}$ | $3\times10^{2}$ | $<1\times10^{2}$ | *Kam*42 |
| *Gam*25 | | | | | | | | | | $<1\times10^{2}$ | $<1\times10^{2}$ | $2\times10^{2}$ | $<1\times10^{2}$ | *Gam*25 |
| *Fam*20 | | | | | | | | | | | $<1\times10^{2}$ | $2\times10^{2}$ | $<1\times10^{2}$ | *Fam*20 |
| *Eam*7 | | | | | | | | | | | | $2\times10^{2}$ | $2\times10^{2}$ | *Eam*7 |
| *Dam*14 | | | | | | | | | | | | | $<1\times10^{2}$ | *Dam*14 |

[a] All the results appearing in this table are taken from Hocking (1972).

[b] The figures given are the phage titres (in *pfu*/ml) obtained after 60 minutes incubation of $10^{-1}$ dilutions of two defective lysates.

[c] Mutants *Gam*27 and *Gam*28 were also tested and the results were found to be qualitatively identical with those for *Gam*25.

head while genes *D*, *E*, *F*, *G*, *K*, *L* and *M* are involved in formation of the phage tail.

These results also suggest that on the addition of the two defective lysates a functional head, complete with DNA, is attached to a functional tail. If the packaging of DNA occurred *in vitro* during the final assembly of the phage particle then DNA from either lysate could be included and both genotypes should appear among the reconstituted phage. This is not the case and suggests that one of the lysates contains DNA already packaged in the phage head.

Mutants *am*47 and *am*38 (in genes *N* and *V* respectively) behaved differently in reconstitution tests. The lysate produced by *Nam*47 when added to those produced by mutants in genes *R*, *S*, *T* or *W* gave rise to a large (approximately $10^7$ fold) increase in phage titre. This lysate therefore appears to be as efficient a head donor as the lysates produced by mutants in genes *D* to *M*. However some reconstitution was also observed when the lysate produced by *Nam*47 was added to those produced by mutants in genes *D* to *M*. This reconstitution was not nearly as efficient as usual (approximately $10^3$ fold instead of $10^7$ fold) but was still well above background.

The plaques obtained after reconstitution of *Nam*47 with *Fam*20 showed two different morphologies. Complementation studies showed that one of these was produced by phage whose DNA possessed the *Nam*47 mutation while the other was produced by phage with the *Fam*20 mutation. This is to be expected since both *Nam*47 and *Fam*20 are known to be head donors. It also suggests that the products of genes *N* and *F* take part in the formation of the phage tail prior to its attachment to the phage head. If either the *N* or the *F* product had attached firstly to the head and then to the rest of the tail only one genotype would have been represented among the reconstituted phage.

Reconstitution between *Nam*47 and mutants in the genes *R*, *S*, *T* or *W* produced phage which invariably contained the DNA of mutant *Nam*47. (Results from complementation and plaque morphology.) All the above results suggest that the mutant *Nam*47 is capable of producing fully active, DNA containing, head particles but is defective in the production of a protein involved in tail formation.

By contrast the mutation *Vam*38 showed normal reconstitution with all tail mutants, including *Nam*47, (approximately a $10^7$ fold increase in *pfu*) but a lower reconstitution with some head mutants (approximately a $10^3$ fold increase in *pfu*). Complementation and plaque morphology studies showed that when *Vam*38 was reconstituted with the tail mutants of genes *D* to *N* the genotype of the reconstituted phage was that of the mutants in genes *D* to *N* and not that of *Vam*38.

When *Vam*38 was reconstituted with the head mutants *Wam*15 or *Ram*6 the reconstituted phage all had the genotype of *Vam*38. Therefore even at this low level of reconstitution *in vitro* packaging of DNA is not occurring. Instead it appears that in the absence of the protein coded for by gene *V* formation of a few non-functional but DNA containing heads can occur. Completion of head formation must then occur on the addition of the other lysate. Although *Vam*38 reconstituted with the head mutants *Wam*15 and *Ram*6 it did not reconstitute with other head mutants, *Tam*8 and *Sam*4. This could perhaps be due to polarity effects since *V*, *T*, *S*, and *R* are all in the same polarity group, or to the complexities of the formation of the 186 head. However, a second lysate produced by the mutant *Vam*38 failed to show reconstitution with the lysate produced by mutant *Wam*15 (see Table 3.4) so the "head donor" properties of this mutant are in doubt.

From this work it appears that *Vam*38 is capable of producing active phage tails at a normal efficiency but is defective in a

protein involved in head formation.

Table 3.4 shows the results of additional *in vitro* reconstitution experiments involving mutants in genes *H*, *J*, *O*, *Q* and *U*. The lysates produced by mutants *Ham*56 and *Jam*41 showed reconstitution with the lysate produced by the head mutant *Wam*15 but not with the lysate produced by the tail mutant *Kam*42. Genes *H* and *J* must therefore be involved in 186 tail formation. The plaque morphology of the reconstituted phage in both cases was that of *Jam*41 or *Ham*56, not that of *Wam*15 and this supports the above conclusion. The reverse situation occurred for the mutants *Qam*1, *Uam*37, *Uam*64 and *Uam*63. For these mutants reconstitution occurred with the tail mutant lysate but not with the head mutant lysate. Genes *Q* and *U* must therefore be involved in the formation of the 186 head particle.

The results for the mutant *Oam*61 were quite different. This mutant showed very late lysis and a large number of mature, active phage in the lysate. No reconstitution was obtained when the *Oam*61 lysate was added to the *Wam*15 lysate but a tenfold increase in phage titre was obtained when it was added to the *Kam*42 lysate. Identical results were obtained with a second lysate of *Oam*61 (see footnote Table 3.4). Again a ten-fold increase in phage titre occurred when the *Oam*61 lysate was added to the tail mutant lysate. The plaque morphologies of *Oam*61 and *Jam*41 are easily distinguishable and the reconstituted phage in this test had the plaque morphology of *Jam*41 indicating that the increase in phage titre is due to genuine reconstitution. (The 5 plaques with the morphology of *Oam*61 are to be expected since one in ten of the phage present after reconstitution should have been derived as a complete phage from the *Oam*61 lysate.) It would appear that there is an excess of phage tails in the *Oam*61 lysate, perhaps suggesting a defect in head formation. However, the

TABLE 3.4

*In vitro* RECONSTITUTION WITH MUTANTS IN GENES *H*, *J*, *K*, *O*, *Q*, *U*, *V* AND *W*[a]

| Defective lysate | Head donor lysate *Kam*42 | Tail donor lysate *Wam*15 | Control |
|---|---|---|---|
| *Ham*56 | $< 1 \times 10^2$ | $1.5 \times 10^9$ | $< 1 \times 10^2$ |
| *Jam*41 | $3.5 \times 10^3$ | $1.9 \times 10^9$ | $1.8 \times 10^3$ |
| *Qam*1 | $9.1 \times 10^7$ | $< 1 \times 10^2$ | $< 1 \times 10^2$ |
| *Uam*37 | $7.0 \times 10^7$ | $< 1 \times 10^2$ | $< 1 \times 10^2$ |
| *Uam*64 | $1.6 \times 10^9$ | $< 1 \times 10^2$ | $< 1 \times 10^2$ |
| *Uam*63 | $1.7 \times 10^9$ | $1 \times 10^2$ | $< 1 \times 10^2$ |
| *Oam*61[b] | $7.3 \times 10^6$ | $5.6 \times 10^5$ | $5.7 \times 10^5$ |
| *Kam*42 | $< 1 \times 10^2$ | $1.5 \times 10^9$ | $< 1 \times 10^2$ |
| *Wam*15 | $1.5 \times 10^9$ | $< 1 \times 10^2$ | $< 1 \times 10^2$ |
| *Vam*38 | $1.1 \times 10^9$ | $< 1 \times 10^2$ | $< 1 \times 10^2$ |

*a* The figures given are the phage titres (in *pfu*/ml) obtained after 60 minutes incubation of $10^{-1}$ dilutions of two defective lysates (columns 2 and 3) or of a $10^{-1}$ dilution of the defective lysate alone (column 4).

*b* In a $10^{-1}$ dilution of another lysate produced by *Oam*61 there were $4.8 \times 10^5$ *pfu*/ml. Following incubation with the lysates produced by the head mutant *Wam*15 and the tail mutant *Jam*41 there were $6.5 \times 10^5$ *pfu*/ml and $6.8 \times 10^6$ *pfu*/ml respectively. In the latter case, of the 68 plaques scored, 5 had the morphology of *Oam*61 while 63 had the morphology of *Jam*41.

level of reconstitution obtained was very much lower than the normal level and it is possible that an excess of phage tails is normal for a 186 infection. (For phage λ Kemp *et al.* (1968) have observed that in lysates produced by the wild-type phage the number of free tails is 10 times greater than the number of free heads.) It appears likely that gene *O* is not involved in phage morphogenesis.

c. Electron Microscopy

To confirm the functional assignments obtained from *in vitro* reconstitution experiments, the defective lysates were observed directly in the electron microscope. Table 3.5 records the phage particles seen together with the behaviour of each mutant in reconstitution experiments.

Genes *D*, *E*, *F*, *G*, *K*, and *L* are clearly involved in formation of the phage tail since head particles but no tail particles were visible in the defective lysates produced by mutants in these genes. In addition to the DNA containing heads visible in these lysates there were also "empty" heads present. Plate 3.1b is a typical view of a tail mutant lysate showing both "full" and "empty" heads and both types of head were present in all tail mutant lysates examined. In addition to phage heads the lysate produced by *Gam*27 did show one particle which appeared to be a tail core without a sheath (see Plate 3.2a). Mutants in all the above genes donated heads in reconstitution experiments, as expected for mutants in tail genes. The defective lysate produced by *Mam*19 contained heads but no normal tails. However some tail-like protein (possibly a sheath protein) was observed (see Plate 3.2b). *Mam*19 behaved as a head donor in reconstitution experiments and is clearly a tail mutant.

The lysates produced by mutants in genes *R*, *S* and *W* contained normal tails but the heads observed were obviously defective

TABLE 3.5[a]

186 GENES INVOLVED IN PHAGE HEAD AND TAIL FORMATION

| Gene | Allele | Particles observed in defective lysates | Active phage particles[b] | Function |
|---|---|---|---|---|
| *D* | *am*14 | Heads[c] | Heads | Tail |
| *E* | *am*7 | Heads | Heads | Tail |
| *F* | *am*20 | Heads | Heads | Tail |
| *G* | *am*25[d] | Heads | Heads | Tail |
| *H* | *am*56 | Heads, Tails | Heads | Tail |
| *J* | *am*41 | Heads, Tails | Heads | Tail |
| *K* | *am*42 | Heads | Heads | Tail |
| *L* | *am*21 | Heads | Heads | Tail |
| *M* | *am*19 | Heads, Tail protein[e] | Heads | Tail |
| *N* | *am*47 | Heads, Defective tails[f] | Heads (many)<br>Tails (few) | Tail |
| *Q* | *am*1 | Heads, Tails | Tails | Head |
| *R* | *am*6 | Tails, Defective heads[g] | Tails | Head |
| *S* | *am*4 | Tails, Defective heads[g] | Tails | Head |
| *T* | *am*8 | Tails | Tails | Head |
| *U* | *am*37[h] | Tails | Tails | Head |
| *V* | *am*38 | Defective tails[f]<br>Defective heads[g] | Tails (many)<br>?Heads (few) | ?Head |
| *W* | *am*15 | Tails, Defective heads[g] | Tails | Head |

*a* Many of the results appearing in this table are taken from Hocking (1972).

*b* As determined by *in vitro* reconstitution.

*c* In all lysates this refers to the presence of both full and empty heads.

*d* *G* alleles *am*27 and *am*28 gave identical results.

*e* See Plate 3.2b.

*f* Defective tails show variation in length (see Plate 3.3).

*g* This refers to the presence of empty heads only (see Plate 3.1c).

*h* *U* alleles *am*63 and *am*64 gave identical results.

*Plate 3.1* ELECTRON MICROGRAPHS OF WILD-TYPE 186 AND OF DEFECTIVE LYSATES PRODUCED BY THE MUTANTS *Gam*28 AND *Sam*4

(a) Wild-type 186. Magnification 240,000x

Tail fibres can be seen attached to several of the phage particles in this electron micrograph. A base plate and a single terminal spike can be seen on one of the phage tail particles. This electron micrograph also shows two phage with contracted tail sheaths.

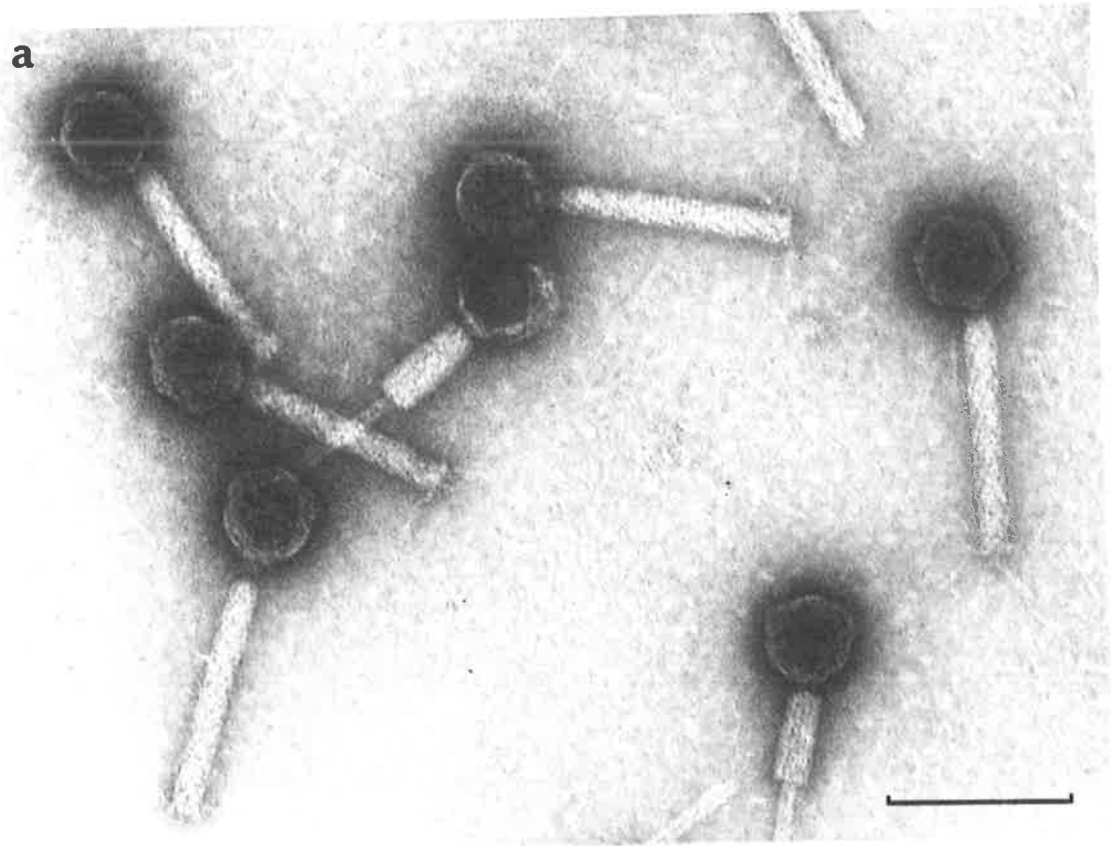
(b) Defective lysate produced by the mutant *Gam*28.
Magnification 180,000x

A typical view of a lysate produced by a tail mutant, showing head particles but no tail particles. Both full heads (bottom left-hand corner) and empty heads (top, and bottom right-hand corner) can be seen.

(c) Defective lysate produced by the mutant *Sam*4.
Magnification 180,000x

A typical view of a lysate produced by a head mutant, showing normal tail particles and defective head particles (inset).

Magnification bars represent 100 nm.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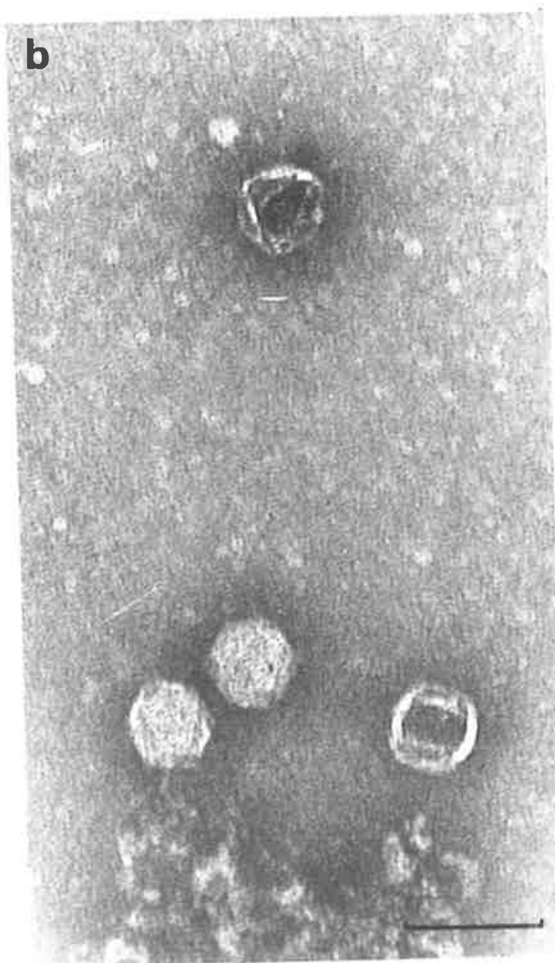
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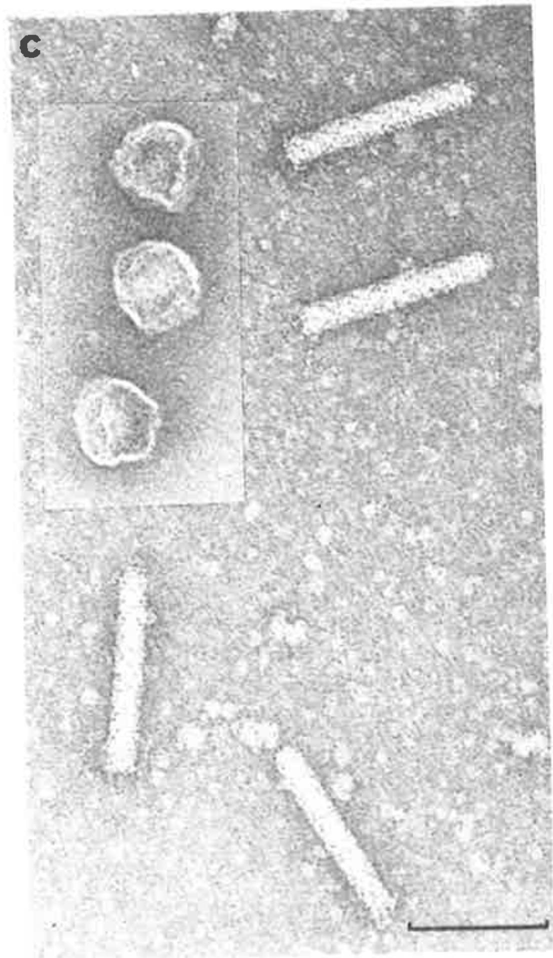

c

*Plate 3.2* ELECTRON MICROGRAPHS OF DEFECTIVE LYSATES PRODUCED BY THE MUTANTS *Gam*27, *Mam*19 AND *Ham*56.

(a) Defective lysate produced by the mutant *Gam*27.
Magnification 120,000x

In addition to the full and empty head particles, which are typical of tail mutant lysates, this micrograph shows another particle which appears to be a tail core without a sheath.

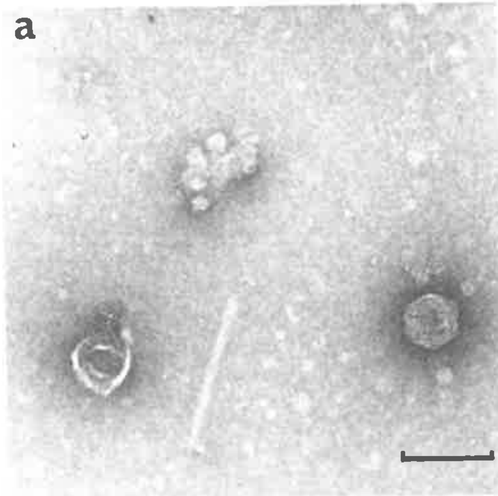
(b) Defective lysate produced by the mutant *Mam*19.
Magnification 120,000x

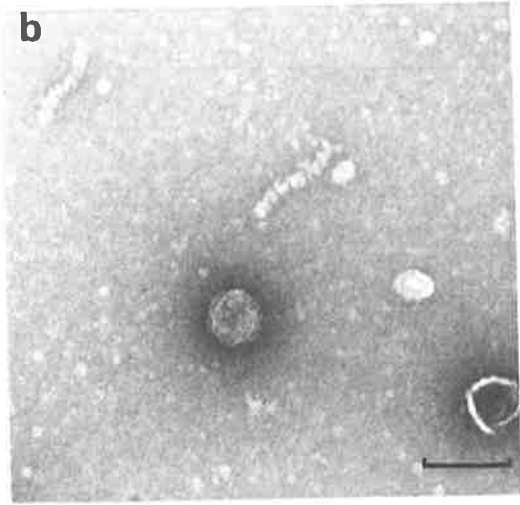
A full and an empty head, typical of a tail mutant lysate, can be seen in this micrograph. In addition, two particles can be seen which might be tail proteins (e.g. perhaps sheath proteins).

(c) Defective lysate produced by the mutant *Ham*56.
Magnification 120,000x

Apparently normal phage tail particles can be seen in this micrograph. This lysate also produced full and empty heads (not shown in this micrograph).

Magnification bars represent 100 nm.




c

(see Plate 3.1c). No full heads were observed in any of these lysates. The results of reconstitution experiments also suggested that the heads in these lysates were not active. It can therefore be concluded that genes *R*, *S* and *W* are involved in the formation of the phage head although the presence of head related structures in the lysates shows that these genes do not code for the major capsid protein.

Only tail particles were visible in the lysates produced by mutants in genes *T* and *U* and these genes must therefore be involved in head formation. Since no head related structures were observed in these lysates it is possible that one of these genes codes for the major capsid protein. The absence of head related structures in the other lysate could be due to polarity effects since genes *T* and *U* belong to the same polarity group. The results of reconstitution also suggested that genes *T* and *U* are involved in head formation.

Both phage heads and phage tails were visible in the lysates produced by mutants *Ham*56 and *Jam*41. *In vitro* reconstitution results showed that these two mutants were able to donate active phage heads but not active phage tails, suggesting that the formation of phage tails by these mutants is defective. However the tails appeared normal in the electron microscope (see Plate 3.2c) and it therefore appears that genes *H* and *J* are not involved in the formation of the major tail structures, the core and the sheath. A minor structure, such as a collar or the tail fibres may instead be involved. The number of tails appearing in the *Ham*56 lysate was considerably greater than the number of heads but since no quantitative studies have been done on any of the lysates it is not known whether this is significant. It may be that an excess of tails is normal for a 186 infection.

The lysate produced by mutant *Qam*1 showed very few phage structures but both phage heads and phage tails were observed. The

heads appeared normal despite the fact that the results of *in vitro* reconstitution showed that the lysate produced by this mutant was unable to donate active heads. A head gene function has been assigned to gene *Q* on the basis of the reconstitution results. The level of reconstitution with the *Qam*1 and *Kam*42 lysates was lower than the normal level of reconstitution and this is consistent with the low numbers of phage particles observed in the *Qam*1 lysate.

The lysate produced by *Nam*47 contained full, apparently active phage heads (together with the usual empty heads) but the tail structures seen varied greatly in length and seemed to be unstable, the core often protruding from the sheath (see Plate 3.3a). Some apparently normal tails were also observed. The results of electron microscopy and *in vitro* reconstitution are therefore in agreement and both suggest that some step in the assembly of the phage tail is defective in this mutant.

The electron microscopy results with *Vam*38 are hard to reconcile with the reconstitution results. Reconstitution suggested that *Vam*38 was an efficient tail donor yet under the electron microscope very few tails of normal length were observed (see Plate 3.3b). Most varied in length, some being exceedingly long (see Plate 3.3c). It is not known whether these long tails are capable of attaching to heads and if so whether the resultant phage are infectious. Defective heads were seen in these lysates and this is in agreement with reconstitution results. The function of gene *V* is therefore rather obscure, both heads and tails appearing to be abnormal.

For the mutant *Lam*21 the results of both *in vitro* reconstitution and EM observation of the defective lysate suggested that the tail particle was defective. Further confirmation of this was provided by EM observation of a stock of *Lam*21 grown in the suppressor host C600. *Lam*21 produces very small plaques on this host and the

*Plate 3.3* ELECTRON MICROGRAPHS OF DEFECTIVE LYSATES PRODUCED BY THE MUTANTS *Nam*47 AND *Vam*38

(a) Defective lysate produced by the mutant *Nam*47.
Magnification 90,000x

This micrograph shows abnormal phage tail structures.

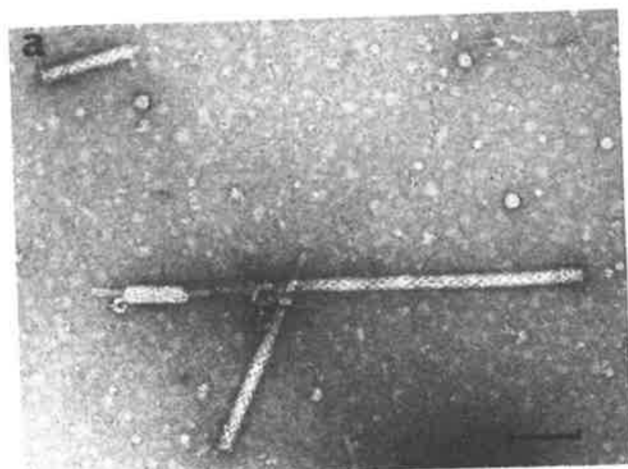
(b) Defective lysate produced by the mutant *Vam*38.
Magnification 90,000x

Abnormal tail structures, varying in length, and defective head particles (at the top of the frame) can be seen in this micrograph.

(c) Defective lysate produced by the mutant *Vam*38.
Magnification 120,000x

This micrograph shows an abnormally long phage tail particle.

Magnification bars represent 100 nm.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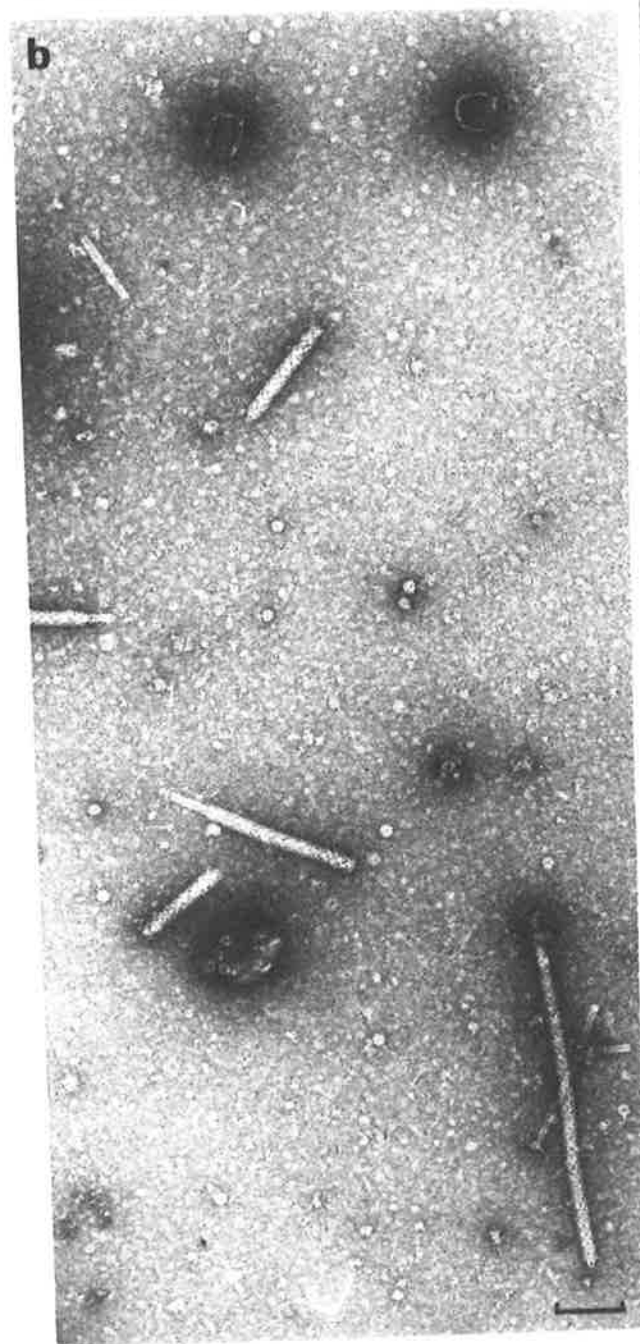
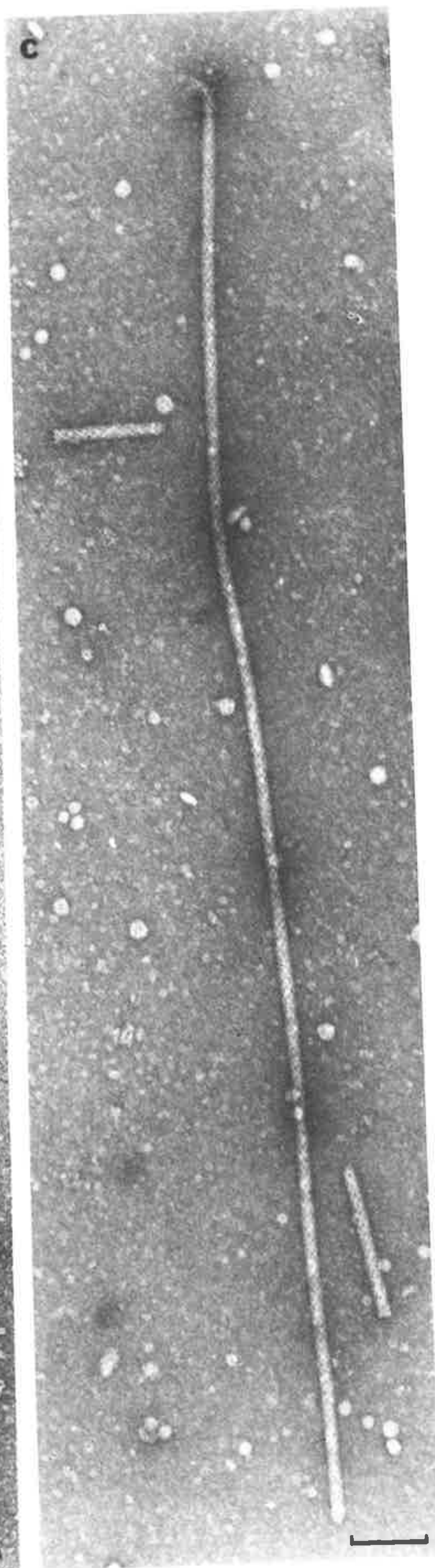

b


c

tails of many of the phage produced are visibly defective (see Plate 3.4a). When a stock of *Lam*21 grown in C600 was centrifuged in a CsCl gradient two bands were produced. The upper (less dense) band consisted of whole 186 phage (Plate 3.4a) while the lower band consisted of 186 phage heads only (Plate 3.4b). This instability of the 186*c*I*tsLam*21 phage can be used to purify 186 phage heads. The tail particles, if still intact, did not band under the conditions used.

## 3.4 DISCUSSION

Mutants in gene *A* of phage 186 are characterized by their very poor complementation with mutants in all other genes. This sort of behaviour has also been reported for mutants belonging to gene *A* of phage P2 (Lindahl, 1970). From the failure of gene *A* mutants to complement mutants in other genes he concluded, firstly, that the product of gene *A* must be *cis*-acting (that is, only able to act on the chromosome which coded for it) and secondly that it must be required for the expression of all other known P2 essential genes. In the absence of either of these properties complementation should have occurred. These two properties should also apply to gene *A* of phage 186 in view of the very poor complementation observed with mutants in this gene. The product of the P2 gene *A* does not appear to be required for the expression of non-essential P2 genes (Lindahl, 1970). For phage 186 $Su^-$ lysogens of amber mutants in gene *A* have been prepared and are stable. It therefore appears that the 186 gene *A* product is not required for the expression of the non-essential genes *c*I, *c*II and *int*, whose products are required for lysogen formation.

The complementation results for mutants in other genes of phage 186 were complicated by the presence of polarity. Polarity describes the phenomenon in which a nonsense mutation in one gene can

*Plate 3.4* ELECTRON MICROGRAPHS OF TWO BANDS FROM A CsCl GRADIENT OF THE MUTANT PHAGE *Lam*21 PROPAGATED ON C600

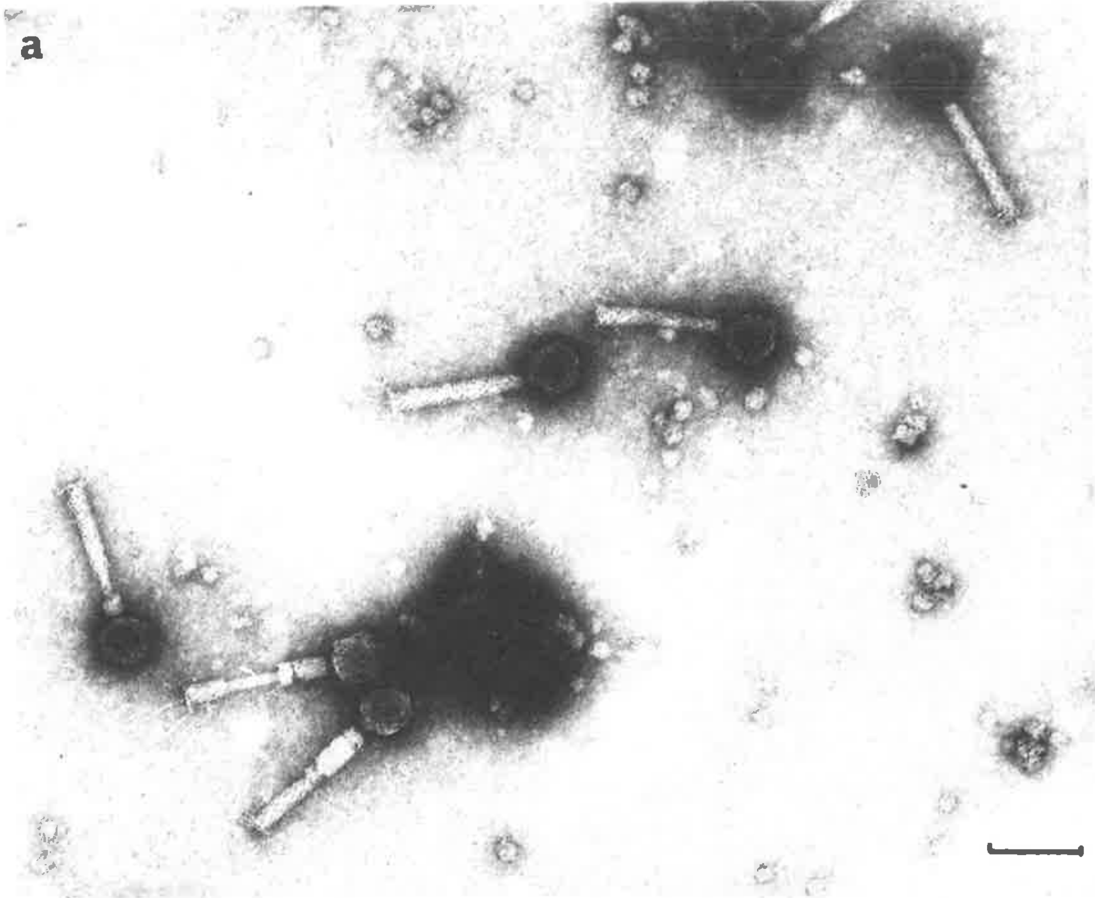
(a) Less dense band. Magnification 120,000x

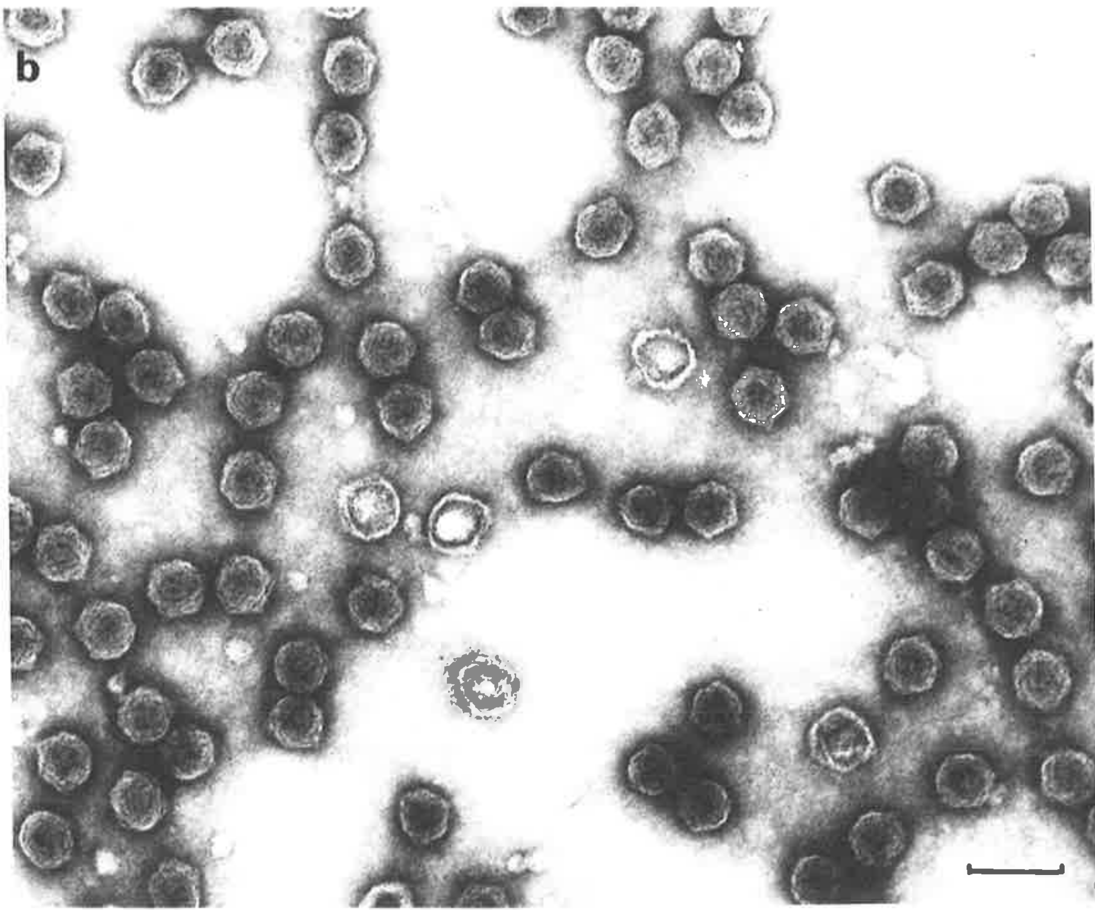
This micrograph shows some apparently normal phage particles together with several particles with obviously defective tails. A base plate and tail fibres are clearly visible on one of the phage particles.

(b) Denser band. Magnification 120,000x

This micrograph shows phage head particles. Most of these heads are full.

Magnification bars represent 100 nm.


a


b

reduce the expression of other genes located distal to it within the same transcription unit. In phage λ polarity effects causing a reduction in inter-gene complementation have been described by Parkinson (1968). These polarity effects were relatively weak in that the phage yields obtained in co-infections with mutants affected by polarity were still 100 to 300 times greater than the yields obtained for mutants in the same gene. By contrast, much stronger effects have been observed for phage P2, phage yields in some cases for mutants in different genes being no greater than those for mutants in the same gene (Lindahl, 1971; Sunshine *et al.*, 1971).

The polarity effects operating in 186 infections are of the "strong" P2-type rather than of the "weak" λ-type. Without the use of a polarity suppressing host the number of genes represented by the known amber mutants would have been greatly underestimated. The use of the polarity suppressing host overcame this problem and allowed the definition of many additional genes. However, as mentioned in the text, the level of suppression by strain XA7007 appears to be very variable. For 186 the increase in phage yield could not be quantitated as all results with strain XA7007 were obtained from plate complementation tests. However Sunshine *et al.* (1971) have reported some liquid complementation results for phage P2, using the polarity suppressing mutation SuA, and the results they obtained were also extremely variable. They reported increases in phage activity of up to 5,000 fold but the increase was usually less than this and in some cases no increase could be obtained even for mutants in different genes. Thus while the SuA mutation is extremely useful it is not completely reliable. Doubtful results could be checked by the isolation of temperature-sensitive "revertants" of the amber mutations. Temperature-sensitive mutants do not show polarity effects and so should fail

to complement only if located in the same gene.

The strong polarity present in P2 complementation tests and the availability of both *am* and *ts* mutations have allowed the determination of the direction of transcription for the various polarity groups. *am* and *ts* mutations in different genes will only fail to complement if both genes are within the same transcription unit and if the *am* mutation is proximal to the *ts* mutation. By observing which combinations of *am* and *ts* mutants fail to complement a direction of transcription can be determined. This could not be done for phage 186 since all mutants used were of the *am* type. Although with the use of the SuA mutation it could be demonstrated that polarity was operating it was not certain which of the two *am* mutants was responsible for the lack of complementation, that is, which was the proximal mutation.

Of the 22 essential genes of phage 186 defined by complementation only 4 affected the ability of the phage to lyse the host cell. For phage λ it has been found that mutants in genes *N*, *O*, *P*, *Q*, *R* and *S* are unable to cause lysis of the host cell in non-permissive conditions (Campbell, 1961; Brooks, 1965; Harris *et al.*, 1967). Of these, only two, *R* and *S*, seem to be directly involved in lysis of the host cell. *R* is the structural gene for the phage lysozyme (Campbell and del Campillo-Campbell, 1963) while the function of gene *S* is to alter the cytoplasmic membrane in a way that enables the phage lysozyme to degrade the cell wall (Reader and Siminovitch, 1971b). Mutants in gene *S* synthesize normal amounts of the phage lysozyme (Harris *et al.*, 1967). Mutants in the other genes *N*, *O*, *P* and *Q* all show defects in the production of lysozyme (Dove, 1966; Protass and Korn, 1966). Gene *N* controls the synthesis of early proteins (including gene *Q* protein) while gene *Q* controls the production

of late mRNA (Joyner *et al.*, 1966). Low levels of lysozyme should therefore be expected for mutants in these two genes. Genes *O* and *P* are required for DNA replication (Joyner *et al.*, 1966) and it has been found for both λ (Dove, 1966; Protass and Korn, 1966) and T4 (Epstein *et al.*, 1963) that DNA synthesis is required for adequate production of late proteins. Thus mutations in genes with a wide variety of functions can lead to a failure in host cell lysis.

The functions of the 186 genes *A*, *B*, *O* and *P* could be analogous to any of the λ functions described above. After heat induction of prophage mutant in genes *A* or *B* the optical density of the bacterial cultures continued to increase during the 2½ hours of the experiment. By contrast, after induction of a prophage in gene *P* the optical density remained relatively constant at times beyond the normal lysis time. This behaviour is characteristic of the λ gene *R* mutants (Campbell, 1961) while continued growth is observed for mutants in genes *N*, *O*, *P*, *Q* and *S* (Brooks, 1965; Harris *et al.*, 1967). This suggests that the 186 gene *P* may be analogous in function to the λ gene *R*, that is it may be the structural gene for the phage lysozyme, while genes *A* and *B* could have functions analogous to those of the λ genes *N*, *O*, *P*, *Q* or *S*. For phage P2, of the mutants which fail to cause host cell lysis, a plateau in optical density has been observed after infection with mutants in gene *K*, while continued cell growth occurs after infection with mutants in the early genes *A* and *B* (Lindahl, 1974). Studies on DNA synthesis with mutants in the 186 genes *A*, *B*, *O* and *P* are presented in the next section and future work on these mutants should include a direct assay for phage lysozyme production. This may help to clarify the function of the 186 gene *O*.

Mutants in the remaining 18 essential genes of phage 186 all show host cell lysis. *In vitro* reconstitution results with

lysates produced by these mutants has allowed their division into two groups, the genes of one group controlling the formation of the phage head while the gens of the other group control the formation of the phage tail. 186 head and tail particles combine *in vitro* at a very high efficiency. From Table 3.3 it can be seen that a combination of undiluted head and tail mutant lysates should contain approximately $10^{10}$ *pfu*/ml of reconstituted phage. This compares with a level of less than $10^8$ *pfu*/ml obtained for phage λ by Weigle (1966) in conditions almost identical with those used for 186. *In vitro* reconstitution has also been reported for phage P2 (Lindahl, 1974) but a comparison with 186 can not be made since the titres of reconstituted phage were not given and the results were complicated by the presence of a large number of active phage in the lysates ($10^7$ to $10^8$ *pfu*/ml).

The results of Table 3.3 also provide evidence of some intra-tail reconstitution (between the product of the tail gene *N* and those of the other tail genes). As described in Section 1 both intra-tail and intra-head reconstitution can be demonstrated for phage λ by varying the methods used in the preparation of the lysates. This might also be possible for phage 186. 186 appears to be a very suitable phage for the study of morphogenesis since it has a relatively complex morphology (but simpler than that of T4); *in vitro* reconstitution is very efficient; the *am* mutants available are not at all leaky; and the phage is inducible, allowing the preparation of defective lysates by induction and thus eliminating complications due to unadsorbed phage.

The results of electron microscopy of the defective lysates provided some additional information about the functions of the 186 genes. Lysates produced by mutants in the head genes *R*, *S* and *W* all contained recognizable, although empty, head structures. Thus

these genes do not code for the major structural protein of the head. The lysates of mutants in genes *T* and *U* did not show head related structures but this needs to be confirmed by quantitative studies on more lysates. If confirmed then it is possible that one of these genes codes for the major head protein. The product of the other gene might be required for the assembly of the major capsid protein. Alternatively the absence of head related structures in the second lysate might be due to polarity effects. Defective lysates produced in a polarity suppressing host could help to distinguish between these possibilities.

Apparently normal, DNA containing head particles were observed in the lysate produced by the mutant *Qam*1, although the results of *in vitro* reconstitution showed that these particles must be defective. DNA containing, but defective, head particles have also been found to be intermediates in the assembly of the head particles of T4 (King, 1968), λ (Casjens *et al.*, 1972) and P2 (Pruss, 1977). If examination of further lysates produced by the mutant *Qam*1 confirm the presence of DNA containing heads then it could be concluded that the product of gene *Q* is required for one of the final steps in the assembly of the 186 head particle. A gene with a mutant phenotype like that of *Vam*38 (namely, defective heads and abnormally long tails) has not been found for either λ or P2.

As found for phage λ (Mount *et al.*, 1968; Kemp *et al.*, 1968; Kühl and Katsura, 1975) and P2 (Lengyel *et al.*, 1974) mutants in most 186 tail genes show no tail related structures. Quantitative studies would be required to determine whether the apparent tail core found in the *Gam*27 lysate and the possible sheath protein found in the *Mam*19 lysate are characteristic of mutants in these genes. Exceptions to the absence of tail structures in tail mutant lysates

were the lysates produced by mutants in genes *N*, *H* and *J*. The tails in the gene *N* lysate were obviously defective, most being too long, but the tails in the gene *H* and gene *J* lysates appeared to be normal. Gene *N* may have a function similar to those of genes *U* of λ (Mount *et al.*, 1968) and *R* of P2 (Lengyel *et al.*, 1974), mutants in both of which show abnormally long tails in defective lysates. Genes *H* and *J* may perhaps be involved in the synthesis of a part of the tail structure which is normally not visible or only occasionally resolved (for example, the tail fibres of 186 which are rarely seen).

No attempt has so far been made to study the production of serum blocking antigens by 186. Genes involved in the production of these antigens can be readily tested for by the methods used for phage λ (Dove, 1966; Buchwald and Siminovitch, 1969) and this determination would lead to an identification of at least one of the genes coding for a protein present in the phage particle.

## SECTION 4

## 186 DNA SYNTHESIS

## 4.1 INTRODUCTION

Of the known essential genes of phage 186 there are only four (genes *A*, *B*, *O* and *P*) which appear not to be involved in the formation of the phage particle. As discussed in Section 3 a defect in any of several different phage functions could account for the absent or delayed lysis observed for cells infected with mutants in these genes. For example these genes could be involved in early gene control (like gene *N* of phage λ), in DNA replication (genes *O* and *P* of λ), in production of late mRNA (gene *Q* of λ) or in host cell lysis (λ genes *R* and *S*). In this section 186 DNA synthesis is studied in order to determine whether the products of 186 genes *A*, *B*, *O* and *P* are required for phage DNA replication.

It has been found for several other phage that when an $hcr^-$ (*h*ost *c*ell *r*eactivation) bacterial cell is treated with mitomycin C (MC) and then infected with the phage, bacterial DNA synthesis is impaired while phage DNA synthesis proceeds. This therefore provides a good system in which phage DNA synthesis can be studied and has been used for many phage including λ (Young and Sinsheimer, 1967), P2 (Lindqvist, 1971) and φX174 (Lindqvist and Sinsheimer, 1967). For λ, P2 and φX174, if an $hcr^+$ cell is treated with MC then neither the bacteria nor the phage can replicate. The reason for this is not understood. For all work with 186 in MC treated cells an $hcr^-$ host was used.

## 4.2 PHAGE PRODUCTION AND DNA SYNTHESIS IN MITOMYCIN C TREATED CELLS

### a. Effect of mitomycin C on 186 phage production

Before proceeding with a study of 186 DNA synthesis in an MC treated cell the effect of MC on 186 phage production was first determined. Contrary to the results obtained for phage λ and P2, it was found that MC had a very marked effect on the ability of the host

cell to support a 186 infection. The burst size in the MC treated cell was 2.5 compared with a burst size in the non-treated cell of 48. The concentration of MC in this experiment was 75 μg/ml, within the range of 50-100 μg/ml used in the study of other phage.

The effect of lowering the concentration of MC on the production of 186 phage was determined. The results (Figure 4.1) show that very little improvement in burst size occurs for MC concentrations within the range used for other phage. The burst sizes obtained were:

| | | |
|---|---|---|
| 3.4 | for | 75 μg/ml MC |
| 4.2 | for | 50 μg/ml MC |
| 12 | for | 25 μg/ml MC |
| 41 | for | 0 μg/ml MC |

For 25 μg/ml MC an improvement in burst size did occur, the burst size increasing to about a quarter of that obtained for non-treated cells, but this concentration is not in the range used by other workers and may fail to adequately inhibit bacterial DNA synthesis. The drop in phage titre observed at later stages of the infectious cycle in non-treated cells is probably due to a loss of viability of the free phage when present at a very low concentration in the medium TPGCAA. Under the conditions of the experiment the concentration of phage in the final solution was only 1,000 to 5,000 *pfu*/ml. The burst size in this and all other experiments was calculated from the maximum titre obtained after induction.

It is known that after MC treatment the bacteria do not resume DNA synthesis (Young and Sinsheimer, 1967). However this may be due to the damaged DNA and it is possible that the cell may recover, with time, the function which 186 requires in order to replicate. To test this possibility MC treated cells were incubated for 30 minutes and 90 minutes before the addition of phage and phage production

*Figure 4.1* EFFECT OF MITOMYCIN C CONCENTRATION ON 186 PHAGE PRODUCTION

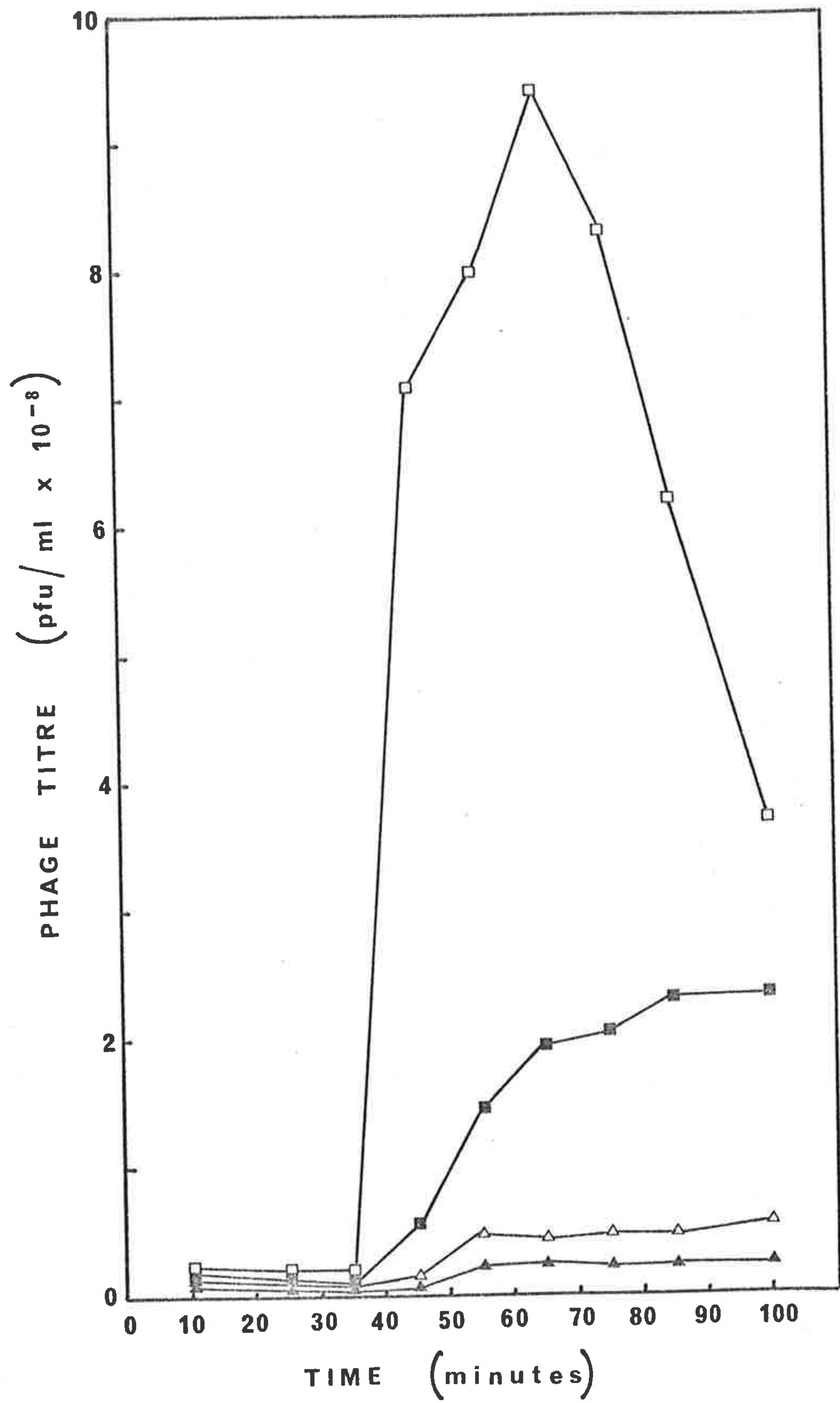
The figure shows the phage titre (measured on C600) at various times after infection of MC treated, and non-treated, E261 by 186*cIts*. The procedure used is described in Sections 2.6, a and 2.6, b. The titres given are calculated per ml of the initial infected cultures.

□ 0 μg/ml MC

■ 25 μg/ml MC

△ 50 μg/ml MC

▲ 75 μg/ml MC



PHAGE TITRE (pfu/ml x $10^{-8}$)
10
8
6
4
2
0
0
10
20
30
40
50
60
70
80
90
100
TIME (minutes)

compared with that for cells infected immediately after MC treatment and for infected, non-treated cells. 50 μg/ml MC was used in this experiment. The burst size obtained for the non-treated cells was 79. For cells infected immediately after the MC treatment the burst size was 3.1. For cells infected 30 minutes and 90 minutes after MC treatment the burst sizes were found to be 3.6 and 3.5 respectively. Therefore the bacterial function which 186 requires in order to replicate is not regenerated within the 90 minutes following the MC treatment.

Phage λ can replicate in an MC treated cell and it is possible that the function required by 186 for replication is coded for by the λ chromosome. To determine whether this was the case, MC treated cells were co-infected with λ and 186 and the production of both phage measured. The results are shown in Figure 4.2 and Table 4.1.

In cells infected with λ only, the production of λ was reduced by about half in the presence of MC. This is consistent with literature values which vary between no reduction and 50% reduction (Young and Sinsheimer, 1967). λ production in MC treated cells

TABLE 4.1

EFFECT OF THE PRESENCE OF λ ON 186 PRODUCTION IN MITOMYCIN C TREATED CELLS

| Phage added | Treatment | Burst size of λ | Burst size of 186 |
|---|---|---|---|
| λ | - | 43 | - |
| λ | MC | 20 | - |
| 186 | MC | - | 4 |
| λ,186 | MC | 21 | 8 |

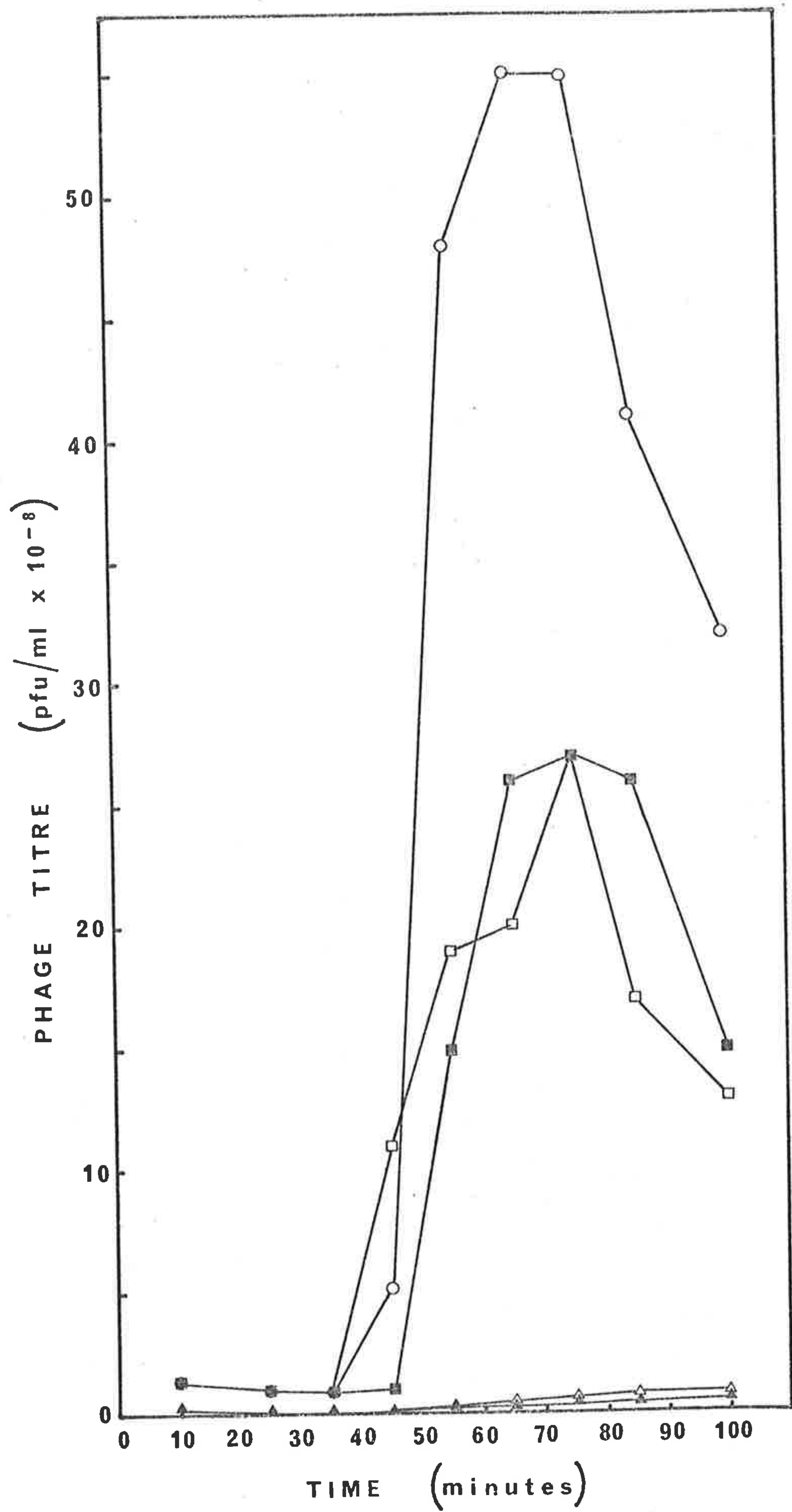
*Figure 4.2* EFFECT OF CO-INFECTION WITH λ ON 186 PHAGE PRODUCTION FOLLOWING MITOMYCIN C TREATMENT

The figure shows the phage titres at various times after infection of MC treated E261 by 186*c*I*ts*, λ*c*I857 and 186*c*I*ts* + λ*c*I857. The procedure used was as described in Sections 2.6, a and 2.6, b except that TPM-CAA was used instead of TPG-CAA.

Cells infected singly with 186 or λ were treated with 186 antiserum or λ antiserum respectively. Cells co-infected with both 186 and λ were treated with a mixture of 186 and λ antisera. Both antisera were used at a K of 1.5 $min^{-1}$.

Phage titres for the single infections were measured on C600. The phage titre in the co-infection was measured on E511 and on E573, C600 lysogens of $\lambda^{+}$ and $186^{+}$ respectively. The titres given are calculated per ml of the initial infected cultures.

- ○ λ production in a single infection : No MC
- ■ λ production in a single infection : 50 μg/ml MC
- □ λ production in a λ,186 coinfection : 50 μg/ml MC
- ▲ 186 production in a single infection : 50 μg/ml MC
- △ 186 production in a λ,186 coinfection : 50 μg/ml MC


PHAGE TITRE (pfu/ml x 10⁻⁸)
0
10
20
30
40
50
0 10 20 30 40 50 60 70 80 90 100
TIME (minutes)

was not affected by the presence of 186. A slight improvement in the burst size of 186 was obtained for the co-infection compared with infection by 186 alone. However even if the increase were significant, the burst size obtained was still too low to be useful in studies of DNA replication.

The same bacteria were used in this experiment for the λ and the 186 infections. Yet 10 minutes after infection the number of 186 infectious centres was only 10% of the number of λ infectious centres. This could be due to a difference in the *moi* but this is unlikely as the titres of both phage stocks were known and enough phage were added (*moi* = 10) for every cell to be infected.

b. Effect of mitomycin C on 186 DNA synthesis

When studying phage Mu DNA replication in MC treated cells, Waggoner *et al.* (1974) used much lower concentrations of MC than usual (namely 10 μg/ml) and obtained a difference in incorporation of [$^3$H]thymidine for Mu-infected and non-infected cells. 25 μg/ml MC did allow some 186 phage production so 10 μg/ml may allow a workable level. Incorporation of [$^3$H]thymidine into TCA precipitable counts was therefore followed for 186 infected and non-infected hcr$^-$ cells pre-treated with either 10 μg/ml or 50 μg/ml MC.

The results (Figure 4.3) show that very little DNA synthesis occurs when the cells are treated with 50 μg/ml of MC. The presence of phage made no difference to the incorporation. With 10 μg/ml MC considerable DNA synthesis occurred for the uninfected culture but the presence of phage caused no stimulation. In fact less DNA synthesis occurred in the presence than in the absence of phage. Therefore it seems that concentrations of MC that inhibit bacterial DNA synthesis severely inhibit 186 DNA synthesis also. Lower concentrations which allow some 186 production fail to adequately inhibit bacterial synthesis.

*Figure 4.3* CUMULATIVE INCORPORATION OF [$^{3}$H]THYMIDINE INTO TCA PRECIPITABLE COUNTS FOLLOWING MITOMYCIN C TREATMENT OF 186 INFECTED AND NON-INFECTED hcr$^{-}$ CELLS

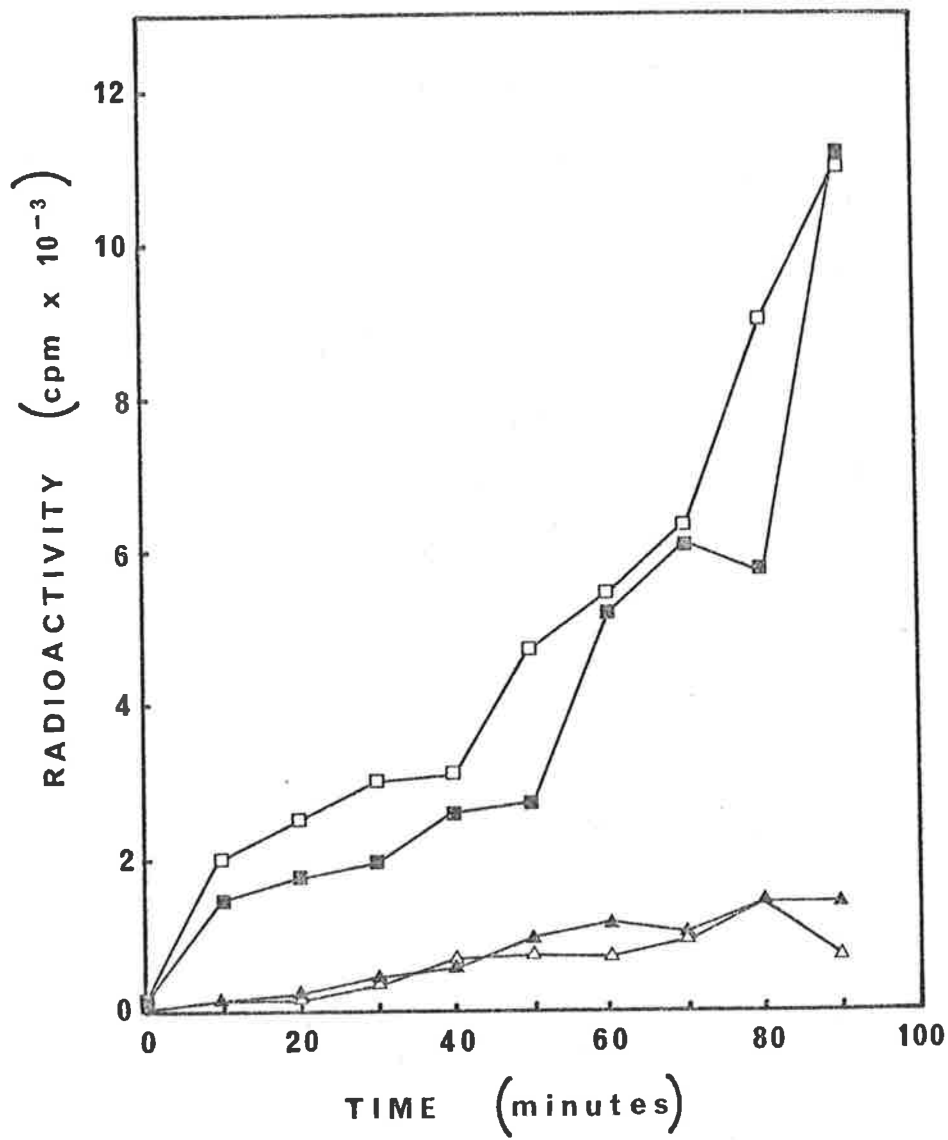
MC treatment and cumulative incorporation of [$^{3}$H]thymidine into TCA precipitable material was as described in Sections 2.6, a and 2.6, c, i, except 1) the concentration of [$^{3}$H]thymidine was 1 μCi/ml and 2) 0.5 ml samples were withdrawn and added to 1 ml of ice-cold 15% TCA (final concentration of TCA = 10%). The TCA precipitates were filtered onto GF/A filters, washed with ice-cold TCA and then with ethanol. Radioactivity was determined as described in Section 2.6, c, i.

□ E261 treated with 10 μg/ml MC

■ E261 treated with 10 μg/ml MC and infected with 186*cIts*

△ E261 treated with 50 μg/ml MC

▲ E261 treated with 50 μg/ml MC and infected with 186*cIts*



RADIOACTIVITY (cpm x $10^{-3}$)
TIME (minutes)
0
2
4
6
8
10
12
0
20
40
60
80
100

Treatment with MC therefore appears to be of no value in the study of 186 DNA synthesis.

## 4.3 186 DNA SYNTHESIS IN THE ABSENCE OF MITOMYCIN C TREATMENT

### a. 186 DNA synthesis after infection with phage 186*cIts*

Although λ DNA synthesis can be studied using MC it can also be studied in the absence of MC since the incorporation of [$^3$H]thymidine into TCA precipitable material for a bacterium infected with phage λ greatly exceeds the incorporation for a non-infected bacterium (Cohen and Chang, 1970). Furthermore it has been found that during λ infection bacterial DNA synthesis is inhibited to some extent and in fact most of the total incorporation obtained (between 81% and 89% according to the calculations of Waites and Fry (1964)) is due to λ DNA synthesis (Waites and Fry, 1964; Cohen and Chang, 1970).

This system was tried for 186 DNA synthesis. The same strain (E261) was used although the *uvrA* mutation was no longer required. Strain W3350 which is *thy*$^+$ was also used and the cumulative incorporation of [$^3$H]thymidine into TCA precipitable material for infected and non-infected cultures was determined (Figure 4.4).

For the *thy*$^+$ strain incorporation was initially extremely rapid but after the first ten minutes very little more incorporation occurred. This is due to the induction of the enzyme thymidine phosphorylase which breaks thymidine down to thymine (see Section 4.4). The use of the *thy*$^-$ strain led to a lower, but more regular, incorporation over a longer period. However there was no indication of any difference in the rate of incorporation for infected and non-infected cultures.

In the experiment (Section 4.2a) in which cells were infected separately with λ and with 186, the number of infectious

*Figure 4.4* CUMULATIVE INCORPORATION OF [$^3$H]THYMIDINE INTO TCA PRECIPITABLE COUNTS FOR 186-INFECTED AND NON-INFECTED BACTERIA OF STRAINS W3350 AND E261

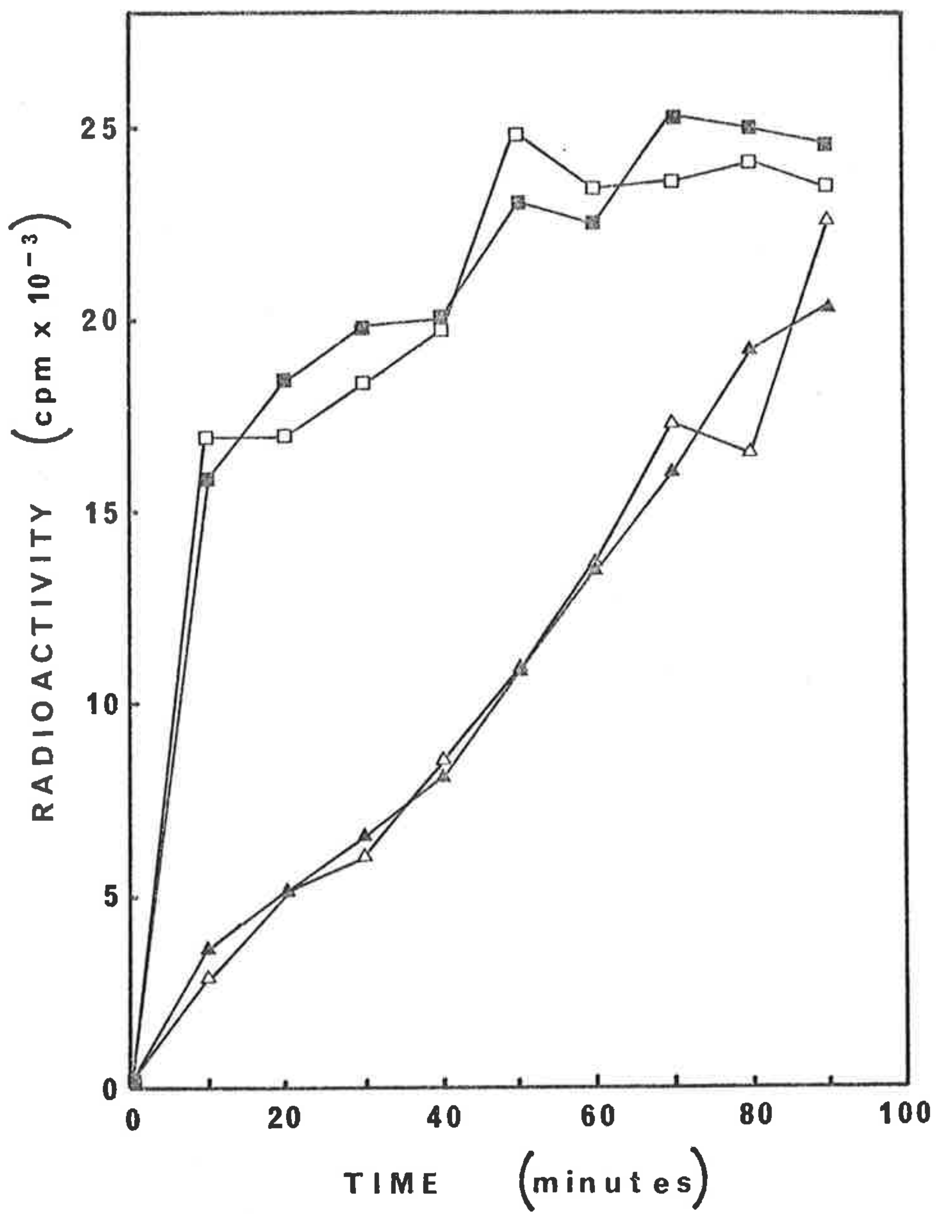
The procedure used is described in Section 2.6, c, i. [$^3$H]Thymidine was added at 0 min (final concentration 10 µCi/ml).

□ W3350

■ W3350 infected with 186*cIts*

△ E261

▲ E261 infected with 186*cIts*



RADIOACTIVITY (cpm x 10$^{-3}$)
25
20
15
10
5
0
0
20
40
60
80
100
TIME (minutes)

centres for λ was ten times the number for 186. This low formation of infectious centres for 186 might have been due to the unusual conditions, namely MC treatment and centrifugation. However a low proportion of infectious centres was obtained even in the absence of these conditions. This could account for the lack of difference in the infected and non-infected cultures of Figure 4.4 since if a large majority of cells fail to produce infectious centres there would be very few cells able to contribute to a difference between infected and non-infected cultures. This low proportion of infectious centres is not obtained in 186 heat-induction experiments. In these experiments 100% of the original cells become infectious centres. Therefore it is much better to study 186 DNA synthesis after induction than after infection, and induction of 186 has been used in all future [$^3$H]thymidine incorporation experiments.

b. <u>186 DNA synthesis after induction of prophage 186*c*I*ts*</u>

[$^3$H]Thymidine incorporation into TCA precipitable material was followed after transfer of lysogenic (E275) and non-lysogenic (E261) cultures from 30°C to 39°C. Under these conditions the latent period of 186 production was 40 minutes and the burst size 44. When the [$^3$H]thymidine was added immediately before transfer from 30°C to 39°C the incorporation curves for the lysogenic and non-lysogenic cultures appeared similar. However if the label was added 10 minutes after the transfer then the lysogenic culture showed somewhat less incorporation than the non-lysogenic culture. It was apparent from these results that cumulative incorporation is not a very good method for studying DNA synthesis, especially if the observed difference between lysogenic and non-lysogenic cells varies depending on the time at which label is added. A much better method is to pulse label the cells so that incorporation at a particular time can be measured.

All future experiments involved pulse-labelling with [$^3$H]thymidine rather than cumulative incorporation.

The results for incorporation of [$^3$H]thymidine after transfer of strains E261 and E275 from 30°C to 39°C, measured by pulse-labelling, are shown in Figure 4.5. The results for the non-lysogenic strain E261 show that initially there is rapid synthesis, but that after 5 minutes the rate of synthesis is depressed and does not recover until about 40 minutes when once again it starts to increase. This depression in synthesis must be due to the heat shock that the cells experienced at 0 minutes when they were transferred from 30°C to 39°C. The cells of strain E261 appear to take 40 minutes to adjust to the new temperature before the rate of synthesis once more increases.

For the lysogenic culture the rate of DNA synthesis rather than being greater than for the non-lysogenic culture as it would be if the replicating phage were λ, was considerably less. At times beyond 5 minutes there appears to be a severe inhibition of bacterial DNA synthesis, greatly exceeding the depression caused by the heat shock. However after reaching a low point at 25 minutes DNA synthesis starts to increase again until 35 minutes when once again the rate decreases. This peak of DNA synthesis occurs at a time which would be consistent with the production of 186 DNA. The latent period of 186 under these conditions is 40 minutes by which time the rate of DNA synthesis in the induced lysogen is once again decreasing. However it is possible that this small peak of synthesis is due to restimulation of bacterial DNA replication.

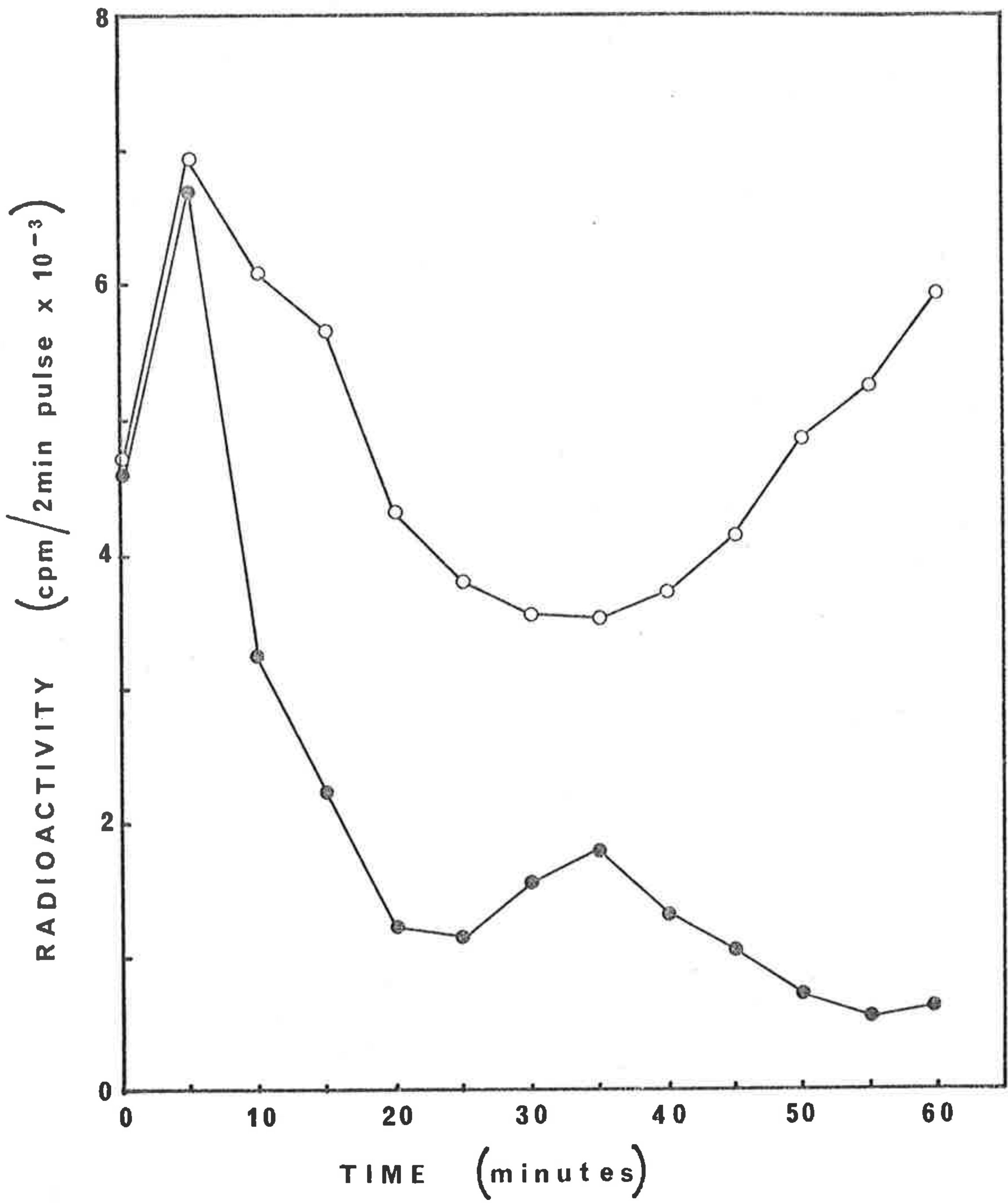
This experiment was repeated using two other sets of strains, strain 594 and its lysogenic derivative E701 and strain E251 and its lysogenic derivative E252. Strain 594 is an $Su^-$ strain containing a $Sm^r$ allele which, under certain conditions, causes a low production of

*Figure 4.5* PULSE LABEL INCORPORATION OF [$^3$H]THYMIDINE INTO TCA PRECIPITABLE COUNTS FOLLOWING INDUCTION OF STRAINS E261 AND E275.

The procedure used is described in Section 2.6, c, ii. Samples were pulsed with [$^3$H]thymidine (final concentration 10 µCi/ml) at the times indicated. The pulses were terminated 2 min later. 0 min is the time at which the cells were transferred from 30°C to 39°C.

○ E261 : a non-lysogen

● E275 : a 186*cIts* lysogen of E261



RADIOACTIVITY (cpm/2min pulse x 10⁻³)
8
6
4
2
0
0
10
20
30
40
50
60
TIME (minutes)

186 phage. The results with this strain (Figure 4.6) show that the depression due to heat shock is probably still present although not nearly as severe as it was for strain E261. The inhibition of bacterial synthesis due to 186 is also probably still present but is not nearly as obvious as in strain E261. Nick Gough (personal communication) has shown that the presence of the $Sm^r$594 allele adversely affects transcription of phage 186. This could account for the reduction in inhibition if this inhibition were due to a protein coded for by 186.

The third set of strains (E251 and E252) appear to be the most promising for studying 186 DNA replication (Figure 4.7). E251 is isogenic with strain 594 the only difference being the $Sm^r$ allele. E251 has the same $Sm^r$ allele as E261 ($Sm^r$748) and this allele does not interfere with 186 production. Once again there is a depression of bacterial DNA synthesis in the non-lysogenic cells but it is not as severe and does not last nearly as long, that is, the cells seem to recover from the heat shock more rapidly. The inhibition of bacterial DNA synthesis by phage 186 is also present in strain E252. Total DNA synthesis does not reach as low a point in this strain as it did in strain E275 but this is probably because the peak of 186 synthesis is very much larger in this strain and may conceal the extent of the inhibition.

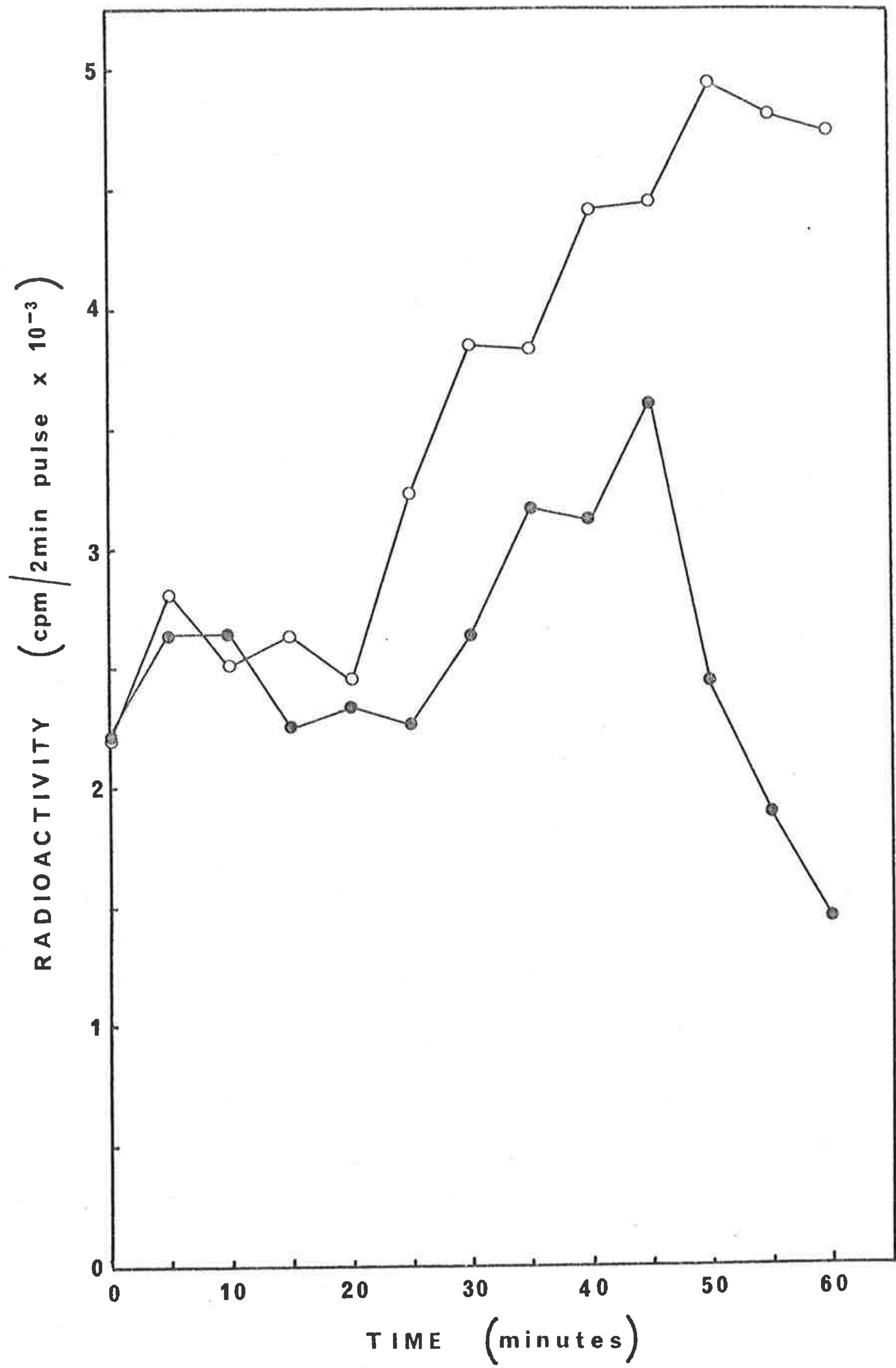
The poor DNA synthesis peak observed in strain E275 appears to be typical of this strain and not due to the conditions of induction used. Different conditions were tried for both E275 and E252, namely, transfer of cells from 30°C to 41°C for 10 minutes to ensure induction, and then growth of cells and pulse-labelling at 30°C. No change in the shape of the curves was obtained for either strain under these conditions. However the rate of incorporation at 30°C was only half that

*Figure 4.6* PULSE LABEL INCORPORATION OF [$^3$H]THYMIDINE INTO TCA PRECIPITABLE COUNTS FOLLOWING INDUCTION OF STRAINS 594 AND E701

The procedure used is described in Section 2.6, c, ii. Samples were pulsed with [$^3$H]thymidine (final concentration 10 μCi/ml) at the times indicated. The pulses were terminated 1 min later. 0 min is the time at which the cells were transferred from 30°C to 39°C.

○ 594 : a non-lysogen

● E701 : a 186*cIts* lysogen of 594



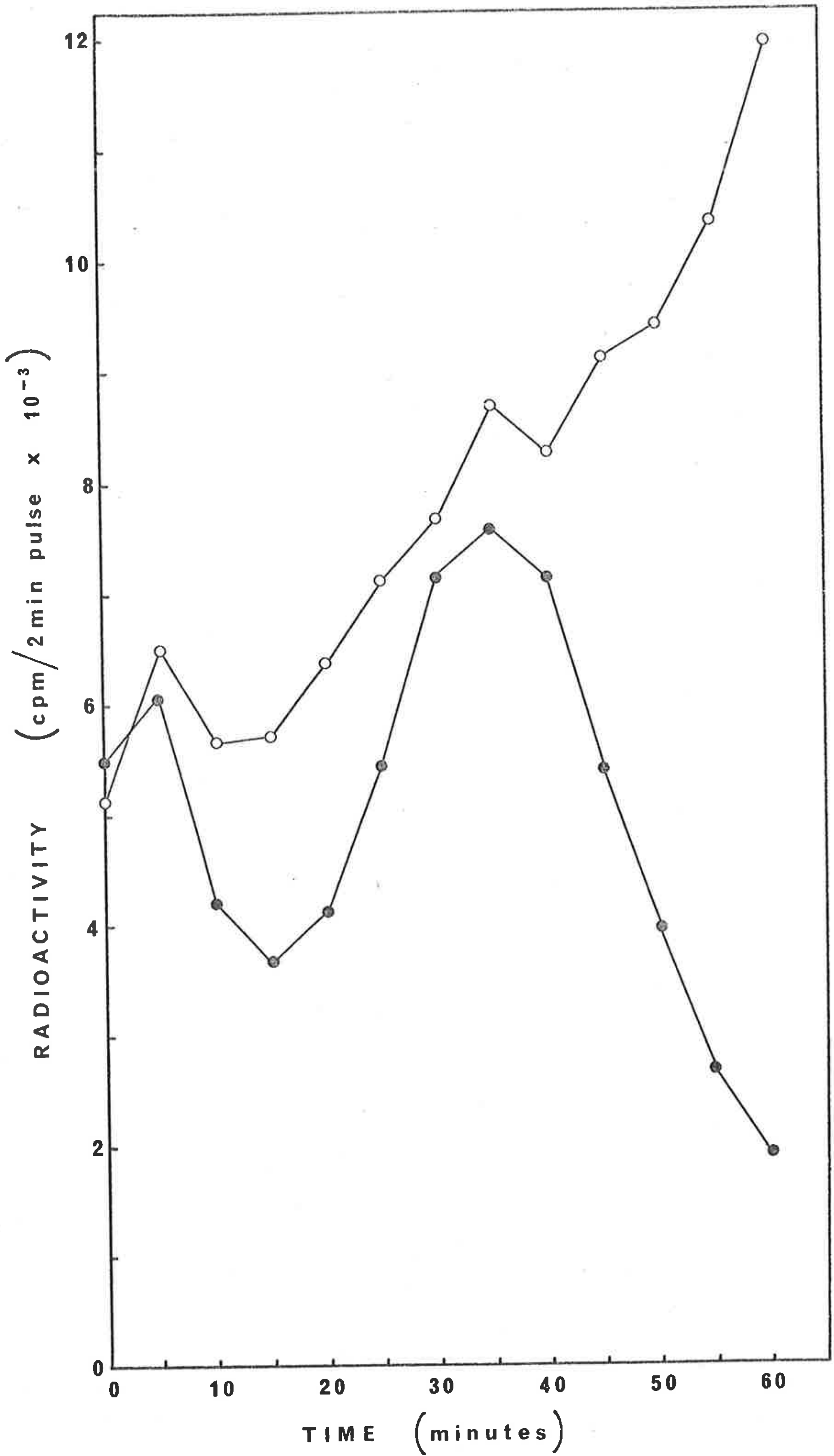
RADIOACTIVITY (cpm/2min pulse x $10^{-3}$)
TIME (minutes)
0
1
2
3
4
5
0
10
20
30
40
50
60

*Figure 4.7* PULSE LABEL INCORPORATION OF [$^3$H]THYMIDINE INTO TCA PRECIPITABLE COUNTS FOLLOWING INDUCTION OF STRAINS E251 AND E252

The procedure used is described in Section 2.6, c, ii. Samples were pulsed with [$^3$H]thymidine (final concentration 5 μCi/ml) at the times indicated. The pulses were terminated 2 min later. 0 min is the time at which the cells were transferred from 30°C to 39°C.

○ E251 : a non-lysogen

● E252 : a 186*cIts* lysogen of E251


RADIOACTIVITY (cpm/2min pulse x 10⁻³)
TIME (minutes)
0
2
4
6
8
10
12
0
10
20
30
40
50
60

at 39°C so the original conditions are more favourable.

All three strains mentioned above could probably be used to study 186 DNA synthesis. However strain E251 and its derivatives seem most suitable due to the very large peak of synthesis observed after induction of the 186 lysogen. Of all future experiments only one (the one described in Figure 4.9) used derivatives of a strain other than E251.

Strain E251 is a *thy*$^{+}$ strain and incorporation will only be linear in the few minutes before the induction of the enzyme thymidine phosphorylase (see Figure 4.4). It is important therefore to make sure that the pulse time does not exceed the time for which incorporation is linear. The maximum rate of DNA synthesis was observed after induction of 186*c*I*tsBam*17 and Figure 4.8 shows a time course of incorporation for this strain and also for the non-lysogen E251. The label was added to these two strains 50 minutes and 60 minutes after induction respectively, times at which near maximal rates of incorporation were occurring. For 3 minutes after this time incorporation of label was linear but beyond 3 minutes the rate was reduced, presumably due to the action of the enzyme thymidine phosphorylase since the total label added was well in excess of that incorporated within the 3 minutes. A 2 minute pulse is therefore satisfactory since it lies well within the limits of linear incorporation.

c. <u>186 DNA synthesis after induction of 186 *am* mutant prophage</u>

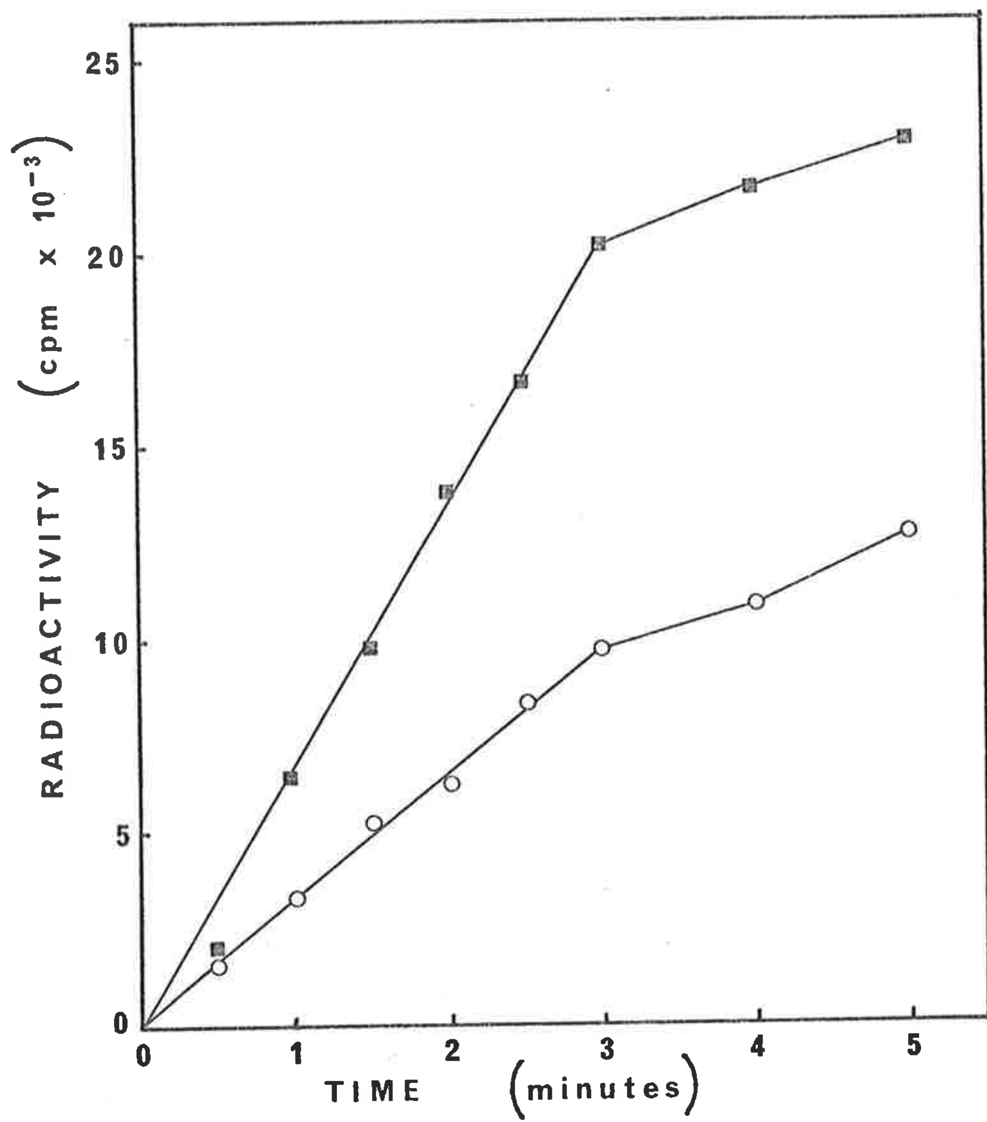
The results for the incorporation of [$^{3}$H]thymidine after induction of E261 lysogens of the mutants 186*c*I*tsAam*5 and 186*c*I*tsBam*17 are shown in Figure 4.9. It can be seen that after induction of 186*c*I*tsAam*5 the peak of synthesis occurring at about 35 minutes is completely absent. By contrast, after induction of 186*c*I*tsBam*17 a

*Figure 4.8* TIME COURSE OF INCORPORATION OF [$^3$H]THYMIDINE INTO TCA PRECIPITABLE COUNTS AFTER INDUCTION OF STRAINS E251 AND E251 LYSOGENIC FOR 186*c*I*tsBam*17

E251 and E251(186*c*I*tsBam*17) cultures were grown to log phase as described in Section 2.6, c, ii. The cultures were transferred to 39°C and incubated with aeration for a further 60 min and 50 min respectively. At those times 0.4 ml samples were withdrawn, added to 100 µl samples of [$^3$H]thymidine in TPG-CAA (final concentration 5 µCi/ml) and incubated without aeration at 39°C. 50 µl samples were withdrawn at the times indicated and placed on GF/A filters which were immediately immersed in ice-cold 10% TCA. The filters were washed and counted as described in Section 2.6, c, i.

○ E251

■ E251(186*c*I*tsBam*17)



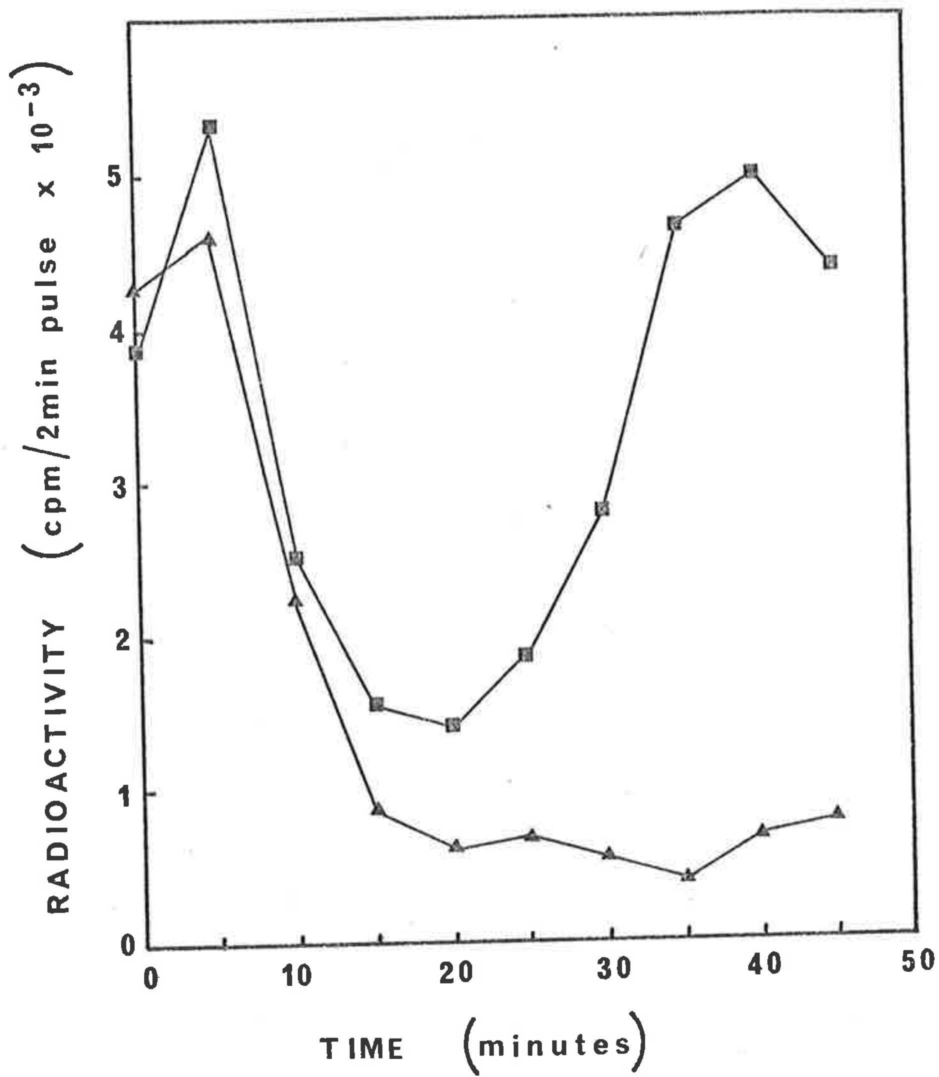
RADIOACTIVITY (cpm x $10^{-3}$)
TIME (minutes)
0
5
10
15
20
25
0
1
2
3
4
5

*Figure 4.9* PULSE LABEL INCORPORATION OF [$^3$H]THYMIDINE INTO TCA PRECIPITABLE COUNTS FOLLOWING INDUCTION OF E261 LYSOGENS OF 186*c*I*tsAam*5 AND 186*c*I*tsBam*17

The procedure used is described in Section 2.6, c, ii. Samples were pulsed with [$^3$H]thymidine (final concentration 10 µCi/ml) at the times indicated. The pulses were terminated 2 min later. 0 min is the time at which the cells were transferred from 30°C to 39°C.

▲ E261(186*c*I*tsAam*5)

■ E261(186*c*I*tsBam*17)


RADIOACTIVITY (cpm/2min pulse x $10^{-3}$)
TIME (minutes)
0
1
2
3
4
5
0
10
20
30
40
50

peak of synthesis is observed and the height of the peak is many times greater than that observed when the prophage is 186*c*I*ts* itself (see Figure 4.5).

Qualitatively identical results were obtained for derivatives of strain E251. (Results shown in Figure 4.10.) Once again the peak of synthesis observed after induction of 186*c*I*ts* is absent after induction of the *Aam*5 mutant prophage. DNA synthesis in the induced *Bam*17 lysogen appears normal initially but instead of reaching a maximum at 35 minutes it continues unabated throughout the 60 minutes of the experiment. The rate of increase is also considerably greater than that for the non-lysogen.

Other alleles of genes *A* and *B* show the same behaviour (Figure 4.11). The results for *Bam*57 are almost identical with those for *Bam*17. Those for mutants *Aam*33 and *am*40 are almost identical with those for *Aam*5. (In complementation and recombination *am*40 behaves like a double mutant, one mutation being in gene *A* to the right of *att* and the other in gene *I* to the left of *att*.)

These results suggest that the product of gene *A* is required for 186 DNA synthesis, while the product of gene *B* is not required. However the decrease in DNA synthesis occurring after 35 minutes is dependent, directly or indirectly, on the product of gene *B*.

The product of gene *B* may itself act to turn off DNA synthesis. However in the absence of the *B* protein late 186 mRNA is not produced (E. J. Finnegan, personal communication) and so it could be any one of the late proteins which is responsible for the turnoff of 186 DNA synthesis. This late protein might be specifically involved in turning off DNA synthesis, or it might be the lysozyme protein (after cell lysis no incorporation could occur) or it might be any one

*Figure 4.10* PULSE LABEL INCORPORATION OF [$^{3}$H]THYMIDINE INTO TCA PRECIPITABLE COUNTS FOLLOWING INDUCTION OF E251 LYSOGENS OF 186*c*I*ts**Aam*5 AND 186*c*I*ts**Bam*17

The procedure used is described in Section 2.6, c, ii. Samples were pulsed with [$^{3}$H]thymidine (final concentration 5 μCi/ml) at the times indicated. The pulses were terminated 2 min later. 0 min is the time at which the cells were transferred from 30°C to 39°C.

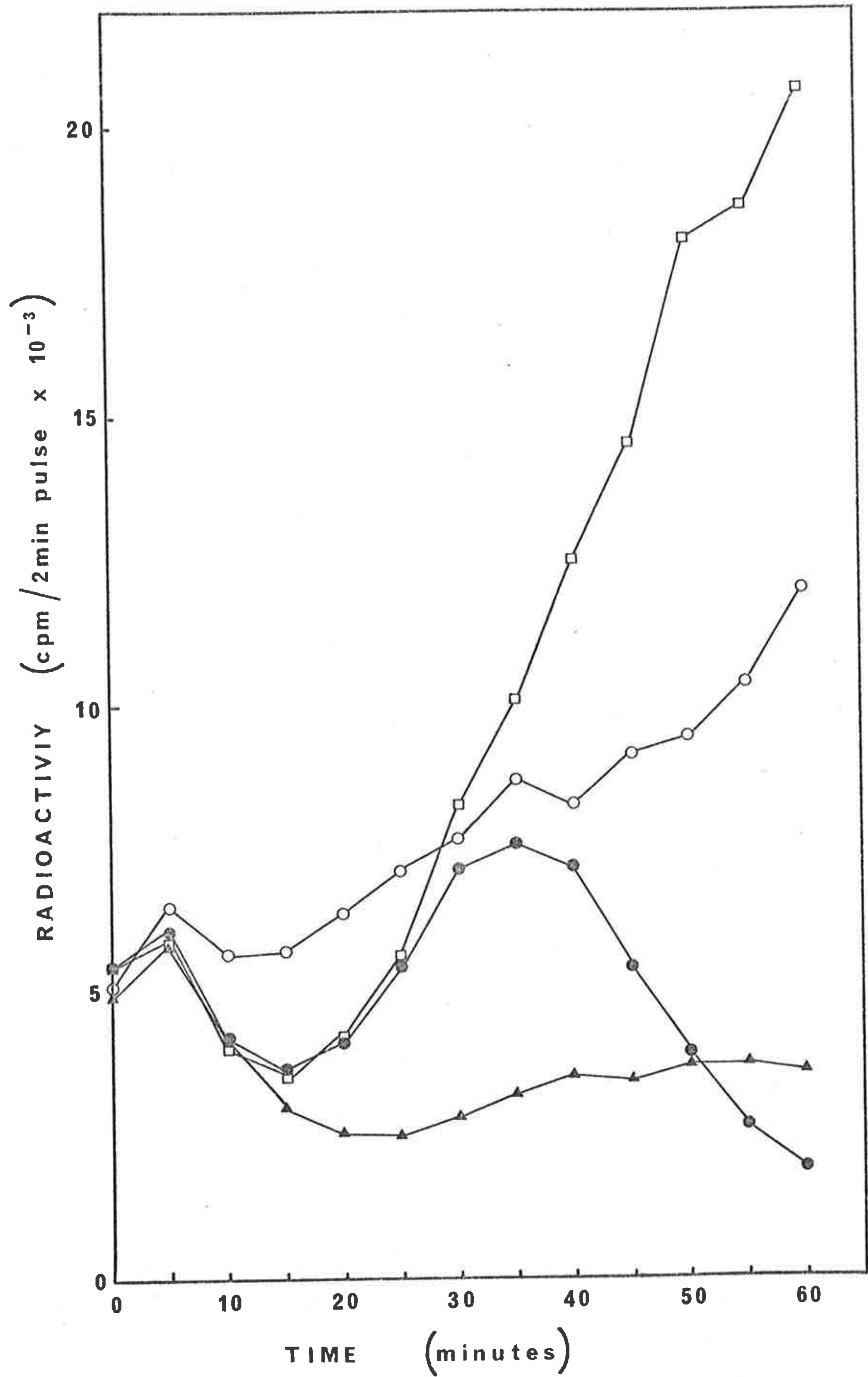
○ E251

● E251(186*c*I*ts*)

▲ E251(186*c*I*ts**Aam*5)

□ E251(186*c*I*ts**Bam*17)

The results for induction of the strain E251(186*c*I*ts*) are the average of 11 experiments. The results for induction of the other strains are the average of 2 experiments.

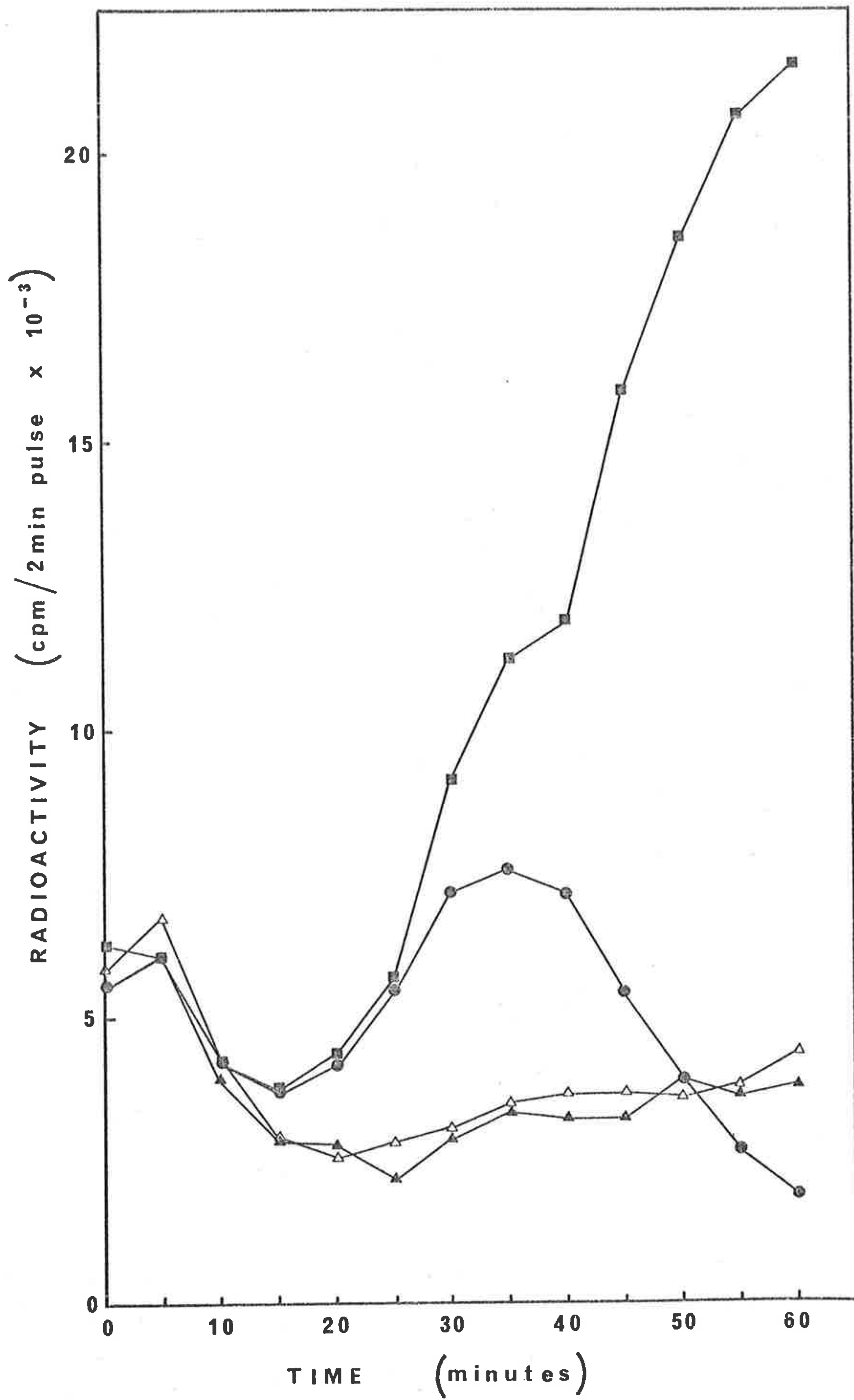


RADIOACTIVIY (cpm/2min pulse x 10⁻³)
20
15
10
5
0
0
10
20
30
40
50
60
TIME (minutes)

*Figure 4.11* PULSE LABEL INCORPORATION OF [$^{3}$H]THYMIDINE INTO TCA PRECIPITABLE COUNTS FOLLOWING INDUCTION OF E251 LYSOGENS OF 186*c*I*tsBam*57, 186*c*I*tsAam*33 AND 186*c*I*tsAam*40b*Iam*40a

The procedure used is described in Section 2.6, c, ii. Samples were pulsed with [$^{3}$H]thymidine (final concentration 5 μCi/ml) at the times indicated. The pulses were terminated 2 min later. 0 min is the time at which the cells were transferred from 30°C to 39°C.

- ● E251(186*c*I*ts*)
- △ E251(186*c*I*tsAam*33)
- ▲ E251(186*c*I*tsAam*40b*Iam*40a)
- ■ E251(186*c*I*tsBam*57)


RADIOACTIVITY (cpm/2min pulse x $10^{-3}$)
20
15
10
5
0
0
10
20
30
40
50
60
TIME (minutes)

of the head proteins, without which a functional head capable of packaging DNA could not be formed and the unpackaged DNA might continue to act as a template for DNA synthesis.

Some of these possibilities can be tested by [$^3$H]thymidine incorporation experiments using amber mutations in various genes. Incorporation after induction of a prophage mutant in the lysozyme gene *P* was identical with that after induction of a non-mutant prophage (Figure 4.12a). Therefore the reduction in DNA synthesis 35 minutes after the transfer of cells from 30°C to 39°C is not due to the lysis of the bacterial cells.

If the reduction in synthesis is due to packaging of the DNA within the phage head, thus removing the template for DNA synthesis, an *am* mutant in a head gene should show uncontrolled DNA synthesis as in the case of a *Bam* mutant. By contrast an *am* mutation in a tail gene which does not interfere with DNA packaging should lead to an incorporation curve identical with that observed for 186*c*I*ts*. The results for incorporation experiments with a head mutant *Wam*15 and a tail mutant *Eam*7 are shown in Figure 4.12b. As expected the curves for 186*c*I*tsEam*7 and 186*c*I*ts* are identical. The curve for 186*c*I*tsWam*15 is not identical with that for 186*c*I*ts*. The time at which DNA synthesis is turned off appears to be retarded by about 5 minutes. This may suggest that the normal turnoff mechanism is not acting, but this difference may not be significant since it is the result of one experiment only.

In practice the result for *Wam*15 could not be expected to be equivalent to that for *Bam*17 since infection by *Wam*15 results in lysis of the cells, and although lysis itself is not responsible for the normal turnoff of DNA synthesis, once the cells have lysed no more incorporation should occur. Therefore a better way to test the hypothesis that packaging is causing the turnoff of DNA synthesis

*Figure 4.12* PULSE LABEL INCORPORATION OF [$^3$H]THYMIDINE INTO TCA PRECIPITABLE COUNTS FOLLOWING INDUCTION OF E251 LYSOGENS OF 186*c*I*ts*, 186*c*I*tsPam*16, 186*c*I*tsOam*61, 186*c*I*tsEam*7 AND 186*c*I*tsWam*15

The procedure used is described in Section 2.6, c, ii. Samples were pulsed with [$^3$H]thymidine (final concentration 5 μCi/ml) at the times indicated. The pulses were terminated 2 min later. 0 min is the time at which the cells were transferred from 30°C to 39°C.

(a) ● E251(186*c*I*ts*)

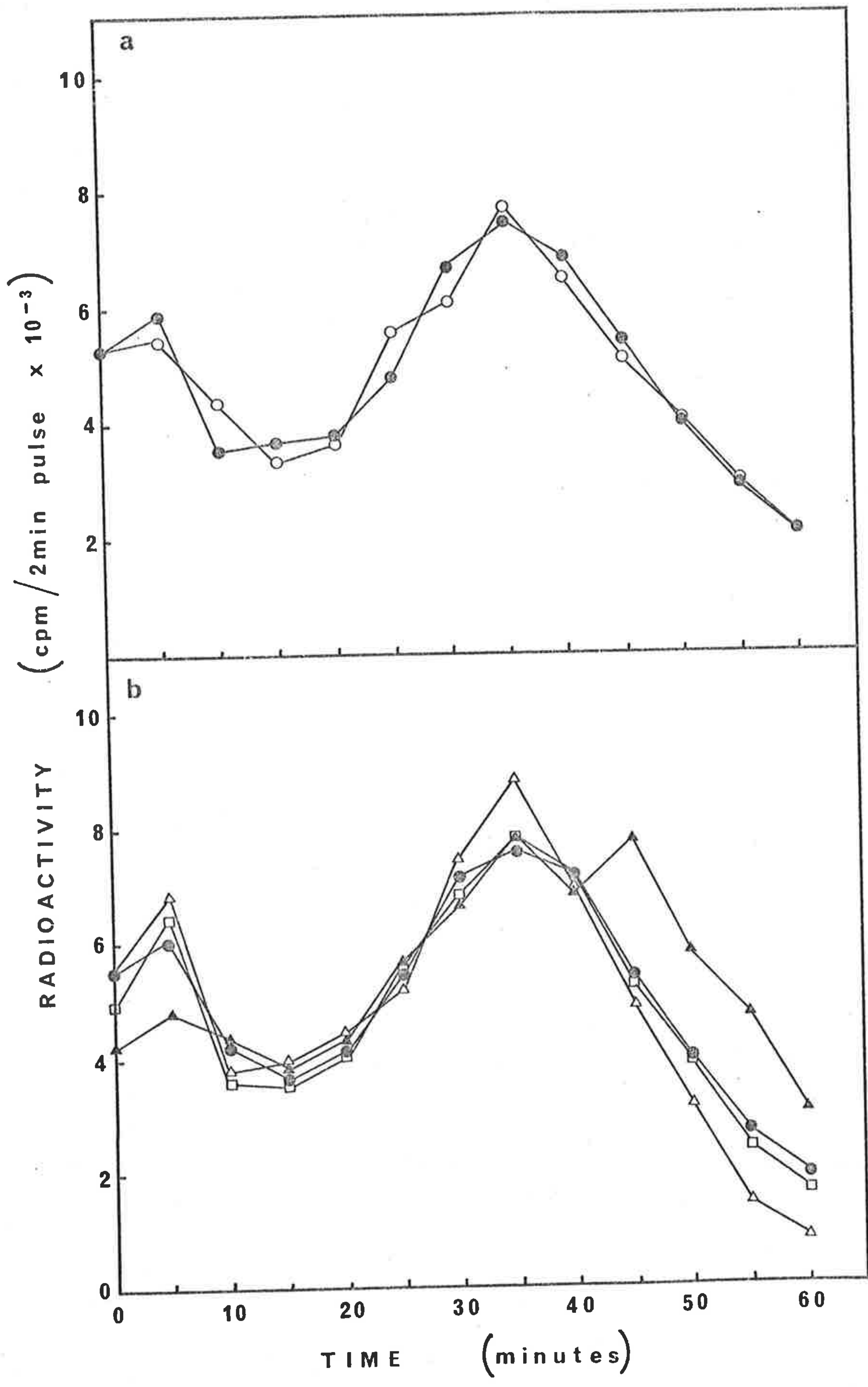
○ E251(186*c*I*tsPam*16)

(b) ● E251(186*c*I*ts*)

△ E251(186*c*I*tsOam*61)

□ E251(186*c*I*tsEam*7)

▲ E251(186*c*I*tsWam*15)


a
b
RADIOACTIVITY (cpm/2min pulse x 10⁻³)
TIME (minutes)
0
2
4
6
8
10
20
30
40
50
60

would be to use a double mutant, one mutation in a head gene and the other in the lysozyme gene, *P*. The incorporation curve produced by this mutant should then be identical with the one produced by *Bam*17 if it is packaging which is causing the turnoff of DNA synthesis. This experiment could not be done due to the lack of such a double mutant and awaits its construction. The construction of double mutants in 186 is unfortunately very difficult but a possible method is outlined in Section 2.

Figure 4.12b also shows the results of incorporation with the mutant 186*c*I*ts**O**am*61. This mutant shows delayed lysis (see Section 3) but the incorporation results (Figure 4.12b) show that it is able to replicate its DNA normally. The gene *O* product therefore appears to act later in the infectious cycle than the products of the early genes *A* and *B* but the exact function of this gene is unknown.

## 4.4 DISCUSSION

The results described in this section have shown that: (1) bacterial DNA synthesis is inhibited after induction of phage 186; (2) 186 DNA synthesis is dependent on the product of gene *A* but not on the product of gene *B*; and (3) the reduction in DNA synthesis commencing at 35 minutes is dependent, directly or indirectly, on the product of gene *B*. (This reduction in synthesis is not due simply to lysis of the host cell.)

Inhibition of bacterial DNA synthesis following induction or infection is common among phage and has been found for both λ (Séchaud, 1960; Waites and Fry, 1964; Young and Sinsheimer, 1967; Cohen and Chang, 1970) and P2 (Lindqvist, 1971). Cohen and Chang (1970) found that mutations in the λ *N* gene showed a reduced or absent ability to inhibit bacterial DNA synthesis. It therefore appears that

a λ protein is involved in this inhibition, either the gene *N* protein, or another protein under the control of gene *N*. By contrast a gene *O* mutation (which prevents λ DNA synthesis) was still able to cause inhibition of host DNA synthesis.

The results for phage 186 were similar in that mutants in gene *A*, the product of which is required for 186 DNA synthesis, still caused inhibition of host DNA synthesis. In fact all 186 mutants tested showed this inhibition and so it appears that if inhibition by phage 186 is due to a diffusable phage product as it is for phage λ (Cohen and Chang, 1970) then the 186 gene responsible for this phenomenon has not yet been identified. Evidence suggesting that a 186 protein is involved in host DNA inhibition was provided by the observation that this inhibition was reduced in strains carrying the $Sm^r$594 allele (Figure 4.6), an allele whose presence has been shown to cause a reduction in 186 transcription (Nick Gough, personal communication).

No 186 gene corresponding to gene *N* of phage λ has yet been found and the "host inhibition" protein may be the product of such a gene or of another gene under the control of this gene. All that can be said at the moment is that the "inhibition protein" is not under the control of 186 gene *A* and that it must be the product of an "early" gene since inhibition commences within 10 minutes after induction.

Inhibition of host DNA synthesis following infection has been reported for phage P2 by Lindqvist (1971). He found that the amount of incorporation of [$^3$H]thymidine in P2 infected cells was no greater (and in fact was somewhat less) than the amount in non-infected cells. (In the absence of host inhibition, incorporation should have been greater in the P2 infected cells.) He also found that, after a lag of approximately 15 minutes, the rate of P2 DNA synthesis (measured

in a mitomycin C treated, infected cell) was as great as the rate of P2 and host synthesis combined (measured in a non-treated, infected cell). He interpreted this as indicating a cessation of host DNA synthesis by about 15 minutes after P2 infection. Lindqvist also showed that P2 DNA synthesis depends on the products of the P2 genes *A* and *B* but since these results were obtained in MC treated cells (that is in the absence of host DNA synthesis) it is not possible to say whether or not host inhibition depends on the products of these genes. By analogy with the results for phage 186 it is likely that host inhibition would occur for mutants in both P2 genes *A* and *B*.

The shape of the DNA synthesis curve obtained for phage 186 (see Figure 4.7) appears quite different from that obtained under similar conditions for phage λ (Adhya *et al.*, 1971). The main difference appears to be in the rate of synthesis obtained. For phage 186 maximal DNA synthesis occurs 35 minutes after the start of induction and the rate of synthesis at this time is slightly lower than that occurring in a non-infected cell. By contrast the rate of DNA synthesis after induction of λ is very much greater than that of a non-infected cell, even at times as early as 10 minutes after the start of induction. These high rates of synthesis were also obtained following infection by phage λ (Cohen and Chang, 1970).

A second difference between the λ and 186 synthesis curves is the time at which synthesis commences. For phage λ DNA synthesis is apparent by 10 minutes after the start of induction (Adhya *et al.*, 1971) or infection (Cohen and Chang, 1970). A similarly early start in phage synthesis was observed after infection by phage P22 (Smith and Levine, 1964). However 186 synthesis appears to commence later, between 15 and 20 minutes after the start of induction. (It is possible that 186 synthesis starts earlier than this but if so then it is concealed by the residual host DNA synthesis.)

Between 20 and 35 minutes 186 synthesis continues at an ever-increasing rate but after 35 minutes the rate of synthesis rapidly declines. Optical density measurements showed that lysis commenced between 35 and 40 minutes and so it appears that DNA synthesis is occurring very close to the time of lysis. This has also been observed for phage λ (Young and Sinsheimer, 1967) and P22 (Smith and Levine, 1964). For both these phage DNA synthesis, following infection with clear plaque mutants, continued (at an increasing rate) reaching a maximum just before cell lysis. The results for phage P2 differed slightly in that DNA synthesis appeared to reach a maximum by about 15 minutes after infection. This maximal rate was then maintained, again until very close to the time of lysis (Lindqvist, 1971). However the P2 results were obtained by cumulative incorporation and are therefore not directly comparable with the results for 186, λ and P22 obtained by pulse-labelling.

The final decrease in 186 DNA synthesis occurring after 35 minutes has been shown not to be due to cell lysis since a mutant in gene *P*, which is unable to lyse the host cell, still showed this reduction in synthesis. This behaviour is similar to that found for gene *R* mutants of phage λ. An identical decrease in DNA synthesis occurred after induction of λ wild-type and λ *R* mutant prophage, the reduction occurring at about the same time as cell lysis in the induced λ wild-type lysogen (Reader and Siminovitch, 1971a; Adhya *et al.*, 1971). By contrast the results for a λ gene *S* mutant were quite different, DNA synthesis continuing beyond the normal times of lysis (Reader and Siminovitch, 1971a; Adhya *et al.*, 1971). It would therefore appear that the product of gene *S* is responsible for the final decrease in λ DNA synthesis. Lindqvist (1971) reported that for phage P2 a lysis defective mutant (belonging to gene *K*) did not show continued DNA

synthesis after the normal time of lysis of a P2*vir*$_1$ infected cell. In this respect the behaviour of mutants in genes *R* of λ, *P* of 186 and *K* of P2 is similar.

The switch-off of 186 DNA synthesis at about 35 minutes appears to be under the control of the 186 gene *B*, either directly by the action of the gene *B* product or indirectly by the action of one or more of the proteins under the control of gene *B*. Such a protein might be the product of a late gene equivalent in function to the λ gene *S*, although no such gene in 186 has so far been identified. (186 gene *P* appears equivalent in function to λ gene *R* and 186 gene *O* whose function is not known shows the normal decrease in DNA synthesis after 35 minutes.) However other explanations are also possible. For example, packaging of the DNA inside the phage head, prior to the release of mature phage, could be responsible for the termination of DNA synthesis since it would remove DNA that might otherwise be used as a template for replication. Control of termination by the product of gene *B* should then be expected since in the absence of the *B* product no late proteins should be made, and this includes the phage head proteins required for the packaging of DNA.

The use of double mutants could help to distinguish between these possibilities. If packaging was the explanation for the termination of DNA synthesis then all head gene-gene *P* double mutants (which would be unable to package DNA) should show the uncontrolled DNA synthesis observed for gene *B* mutants, whereas all tail gene-gene *P* double mutants should show the normal decrease in incorporation after 35 minutes. However if a specific protein was involved then this difference between head and tail genes should not occur. If this specific protein was the product of gene *B* then mutants in this gene would be the only ones to show uncontrolled synthesis. If the specific

protein was the product of an unidentified gene of the λ *S* type then either no other mutants would show uncontrolled synthesis or else mutants belonging to the same polarity group might show the same effect. In this case only mutants belonging to a single polarity group would be affected.

For phage P2 Lindqvist (1971) reported that a polar mutant, *am*7, continued to synthesize DNA beyond the normal lysis time. This mutant also showed delayed lysis of non-permissive bacteria (Lindahl, 1971). The first result might suggest that there is an unidentified λ *S* type gene present in phage P2 also but the second result is more in keeping with Lindahl's suggestion of polarity over gene *K* than of a λ *S* type mutant since lysis was delayed rather than eliminated. However polarity over gene *K* does not explain the overproduction of DNA.

Although the results for λ and P22 clear plaque mutants are basically similar to those for 186, quite different results were obtained for wild-type λ and P22. In these experiments (Young and Sinsheimer, 1967; Smith and Levine, 1964) a large percentage of infected cells became lysogenic. Peaks of phage synthesis were obtained early in the infectious cycle but were followed by an inhibition in net DNA synthesis. Net DNA synthesis later recovered, approaching the level found in uninfected cells, presumably due to the formation of lysogens.

For a λ*c*I*ts* mutant Young and Sinsheimer (1967) obtained a result intermediate between that for λ$c^+$ and λ$c^-$ phage. Although lysis did eventually occur for this mutant it was delayed and the level of DNA synthesis was depressed. Young and Sinsheimer attributed this to a partial activity of the temperature sensitive repressor at 37°C. All 186 mutants tested were derived from a 186*c*I*ts* mutant (rather than from a 186*c*$I^-$ mutant) but there was no apparent delay in lysis and it is probable that there is very little if any activity of the 186*c*I*ts*

repressor protein at 39°C.

The method used to study 186 DNA synthesis (involving pulse labelling with [$^3$H]thymidine) is essentially the same as that used by other workers for phage P22 and λ. As mentioned by Smith and Levine (1964), "The rate of incorporation of label into acid-insoluble material is an accurate measure of the true rate of DNA synthesis only when entry of labelled thymidine into the cell and equilibration with the endogenous pool are not rate-limiting." That this was the case in their experiments was supported by the fact that the rate curves they obtained were in excellent agreement with the physiological events of P22 infection, such as cell growth, phage production and cell lysis. The same assumption has been made by other workers with phage λ and has been made for the 186 results obtained in this section.

The technique of following DNA replication by pulse labelling is obviously superior to following it by cumulative incorporation, especially in a case such as with 186 where increases and decreases in DNA synthesis are interspersed. This method can be used for either induction or infection but induction has been used for all 186 experiments since it was found that only a small proportion of cells became infectious centres after infection with phage 186.

Incorporation of [$^3$H]thymine or [$^3$H]thymidine into DNA occurs via the salvage pathway and not by the usual pathway involved in DNA synthesis (the *de novo* pathway). In the salvage pathway thymine is initially converted into deoxythymidine which is then converted to deoxyTMP, an intermediate in the normal, *de novo*, pathway of DNA synthesis.

If thymine is added to the growth medium of a wild-type cell only a very small amount is incorporated into DNA but, by contrast, if thymidine is added it is rapidly incorporated. However although

thymidine is incorporated rapidly initially, over an extended period very little of the radioactive thymidine appears in the DNA, most of it being broken down to thymine (Rachmeler *et al.*, 1961).

The breakdown of deoxythymidine into thymine and deoxyribose-1-phosphate is catalysed by the enzyme thymidine phosphorylase. This enzyme is induced by deoxythymidine but there is a basal level of activity even in non-induced cells. After the addition of thymidine to the cells there is a three minute lag before the appearance of thymidine phosphorylase. Incorporation of [$^3$H]thymidine into DNA continues until all the thymidine has been broken down into thymine. Incorporation then ceases (Rachmeler *et al.*, 1961).

From these results it is obvious that if a wild-type cell is to be used for DNA synthesis studies then the label added must be in the form of [$^3$H]thymidine and not in the form of [$^3$H]thymine since a wild-type cell will synthesize DNA by the *de novo* pathway rather than utilize added thymine. If thymidine is used it is important to keep the pulse time to within 3 minutes since after this time the enzyme thymidine phosphorylase will be induced converting thymidine to thymine which will not be incorporated. The results of Figure 4.8 show that linearity of incorporation is maintained for approximately 3 minutes and this correlates well with the appearance of the enzyme thymidine phosphorylase (Rachmeler *et al.*, 1961). Cumulative incorporation cannot be used for a wild-type cell since for times greater than 3 minutes the rate of incorporation reflects the activity of the enzyme, not that of DNA replication. In the case of a *thy*$^-$ mutant the presence of thymine or thymidine is essential for growth, since the *de novo* pathway has been blocked by mutation, and incorporation of label into DNA must occur whether it is present as [$^3$H]thymine or [$^3$H]thymidine.

In summary, if a *thy*$^-$ cell is used in incorporation

experiments then DNA synthesis can be followed either by cumulative incorporation or by pulse labelling and the radioactive label can be present as either thymine or thymidine. However if a wild-type cell is used then the method must involve pulse labelling with pulse times less than or equal to 3 minutes and the radioactive label must be in the form of thymidine.

Although all the results of DNA incorporation experiments described in this section are consistent with the DNA synthesis between 20 and 35 minutes being due to the replication of 186, it has not been shown that this synthesis is in fact 186 synthesis. It is possible, although unlikely, that it is bacterial DNA synthesis. If this were the case it would mean that after induction of 186, bacterial synthesis is at first inhibited (5 - 15 minutes) then stimulated (15 - 35 minutes) and then once more inhibited (after 35 minutes). The product of 186 gene *A* would be required for the stimulation of bacterial synthesis between 15 and 35 minutes, and in the absence of the product of gene *B*, the gene *A* product (or another 186 coded protein) would be capable of stimulating bacterial synthesis at a rate far in excess of that observed in uninfected bacteria. Finally, bacterial synthesis would be switched off by the gene *B* protein or by another 186 protein whose production is controlled by the gene *B* protein. This possibility could be eliminated by hybridization experiments, that is by showing that the DNA made 35 minutes after induction of 186 hybridizes to 186 DNA and not to *E. coli* DNA. This has not been done and the conclusions drawn in this section, namely that the product of gene *A* is required for 186 DNA synthesis while the product of gene *B* is not, await final confirmation.

# SECTION 5

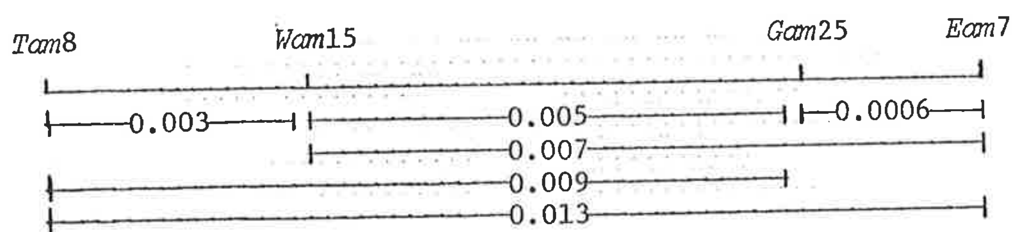
# TWO- AND THREE-FACTOR CROSSES

## 5.1 INTRODUCTION

This section describes the attempts made to construct a 186 genetic map from the results of two-factor crosses. The frequency of recombination obtained in standard two-factor crosses was found to be extremely low (see Section 5.2) and Sections 5.3 and 5.4 describe attempts to increase this frequency, either by the addition of phage λ or by the ultra-violet irradiation of the parental phage before infection. Finally the results of several three-factor crosses are described in Sections 5.5, 5.6 and 5.7.

## 5.2 STANDARD 186 TWO-FACTOR CROSSES

In two-factor recombination experiments involving pairs of mutations from 186 genes *B* to *W*, the frequency of recombination was invariably less than 0.05% and usually around 0.01% or less. Some examples of the frequencies obtained can be seen in Tables 5.1 and 5.2. The results of Table 5.1 suggest the following order for the mutations tested.




The results with the mutant *Qam*1 are anomalous as it appears to be a long way from *Eam*7 but quite close to *Gam*25. There is no position for this mutant which would be consistent with the results of Table 5.1.

Table 5.2 shows the results of two-factor crosses with another set of mutants. The order suggested by this set of data is as follows:

TABLE 5.1

TWO-FACTOR CROSSES WITH ALLELES OF GENES *E*, *G*, *Q*, *T* AND *W*

| Cross | | | Number of experiments | Average recombination frequency (%)[a] |
|---|---|---|---|---|
| *Eam*7 | x | *Tam*8 | 4 | 0.013 |
| *Eam*7 | x | *Wam*15 | 1 | 0.007 |
| *Eam*7 | x | *Gam*25 | 1 | 0.0006 |
| *Tam*8 | x | *Wam*15 | 2 | 0.003 |
| *Tam*8 | x | *Gam*25 | 1 | 0.009 |
| *Wam*15 | x | *Gam*25 | 1 | 0.005 |
| *Qam*1 | x | *Eam*7 | 2 | 0.010 |
| *Qam*1 | x | *Tam*8 | 2 | 0.003 |
| *Qam*1 | x | *Wam*15 | 2 | 0.004 |
| *Qam*1 | x | *Gam*25 | 1 | 0.002 |

[a] The recombination frequency is calculated as:

$$2 \times \frac{pfu \text{ on } Su^-}{pfu \text{ on } Su^+} \times 100$$

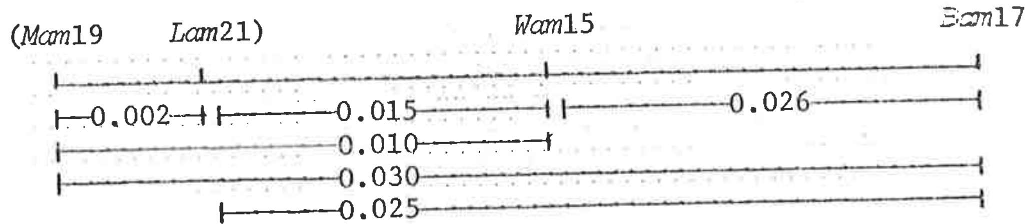
The factor of 2 allows for the presumed double *am* reciprocal recombinants which would not plate on the $Su^-$ host. The recombination frequencies shown in Tables 5.2 to 5.13 have all been calculated using this formula.

TABLE 5.2

TWO-FACTOR CROSSES WITH ALLELES OF GENES *B*, *L*, *M* AND *W*

| Cross | Number of experiments | Recombination frequency (%) |
|---|---|---|
| *Wam*15 x *Bam*17 | 1 | 0.026 |
| *Wam*15 x *Mam*19 | 1 | 0.010 |
| *Wam*15 x *Lam*21 | 1 | 0.015 |
| *Bam*17 x *Mam*19 | 1 | 0.030 |
| *Bam*17 x *Lam*21 | 1 | 0.025 |
| *Mam*19 x *Lam*21 | 1 | 0.002 |

Reversion frequencies for the mutants were determined following single infections. For the mutants *Wam*15, *Mam*19 and *Lam*21 the reversion frequencies were less than 0.0001%. The reversion frequency for the mutant *Bam*17 was 0.0008%.




These results are not consistent but suggest that *Mam*19 and *Lam*21 map fairly close together and that *Wam*15 probably lies between them and *Bam*17.

In the two sets of results described above problems were encountered due to the very low frequency of recombination. In order to measure this low frequency so many phage had to be plated on the $Su^-$ indicator plates that an opportunity arose for recombination to occur on the plates themselves. This type of recombination gave the indicator lawns a speckled appearance.

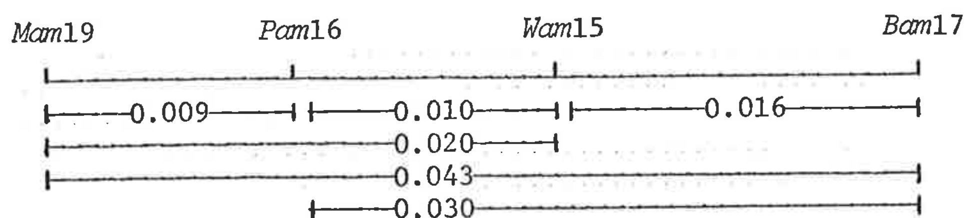
For the results recorded in Table 5.2 the crosses *Wam*15 x *Bam*17, *Wam*15 x *Mam*19, *Bam*17 x *Mam*19 and *Bam*17 x *Lam*21 all showed a very large number of "specks", about ten times as many as the number of "true" recombinants. For example, for the cross *Wam*15 x *Mam*19, 100 recombinants were scored on a plate and there were an additional 1,000 specks. Only large plaques were scored as recombinants in the calculations of the recombination frequencies.

For phage P2, which also has a very low recombination frequency, Lindahl found that to avoid recombination on the indicator plate it was necessary to keep the product of the number of recombinant plaques on a plate, and the total number of phage added to the plate to $\leqslant 3 \times 10^7$ (Lindahl, 1969a). This probably also applies to phage 186 and for the four crosses mentioned above this product was in excess of $10^8$. However for the cross 15 x 21, where this product was again in excess of $10^8$, specks did not create a problem. In general the number of specks increased with the recombination percent

but this was not always so, for example, fewer specks were observed for the cross 15 x 21 (recombination % = 0.015) than for the cross 15 x 19 (recombination % = 0.010). For the cross 19 x 21 the product of recombinants and total phage added was less than 3 x $10^7$ and no problem with specks was encountered.

In an attempt to reduce the occurrence of recombination on the plate, two alterations were made in the procedure used for two-factor crosses. Firstly, an $Su^-$ *recA* bacterial strain was used to measure the recombinant phage. The use of this strain should reduce any *rec* dependent recombination occurring on the plate, without affecting the recombination to be measured, that is the recombination occurring during the 90' incubation of the phage with strain C600 in the liquid medium. Secondly, a much more dilute phage mixture was added to each plate, ten plates being used to score the total number of recombinant plaques. These were the conditions used for all the crosses recorded in Table 5.3.

In Table 5.3 if the crosses involving the four mutants *Mam*19, *Pam*16, *Wam*15 and *Bam*17 are considered, a consistent map can be drawn suggesting the order 19-16-15-17.




The results obtained with mutant *Eam*7 are consistent with the above order (7 x 15 gave a lower frequency than 7 x 19), and it appears to map fairly close to *Bam*17. However from these results it is not possible to say which side of 17 it lies. Mutant 21 appears to map close to 19 but its frequencies with 15 and 17 were lower than they should be if this were the case. Mutant 8 showed a low frequency

TABLE 5.3

TWO-FACTOR CROSSES WITH ALLELES OF GENES *B*, *E*, *L*, *M*, *P*, *T* AND *W*

| Cross | X[a] | Individual recombination frequencies (%) | Average recomb. freq.(%) |
|---|---|---|---|
| *Wam*15 x *Pam*16 | 8 | 0.0043, 0.0080, 0.0053, 0.0068, 0.018, 0.012, 0.012, 0.015 | 0.010 |
| *Wam*15 x *Bam*17 | 5 | 0.016, 0.013, 0.010, 0.023, 0.016 | 0.016 |
| *Wam*15 x *Lam*21 | 5 | 0.027, 0.0042, 0.0077[b], 0.010, 0.0058[b] | 0.011 |
| *Pam*16 x *Bam*17 | 3 | 0.017, 0.041, 0.033 | 0.030 |
| *Pam*16 x *Lam*21 | 7 | 0.011, 0.013, 0.018, 0.014, 0.0044, 0.0038, 0.0081 | 0.010 |
| *Bam*17 x *Lam*21 | 3 | 0.038, 0.013, 0.024 | 0.025 |
| *Eam*7 x *Wam*15 | 1 | 0.029 | 0.029 |
| *Eam*7 x *Bam*17 | 1 | 0.0057 | 0.0057 |
| *Eam*7 x *Mam*19 | 1 | 0.041 | 0.041 |
| *Eam*7 x *Lam*21 | 1 | 0.037 | 0.037 |
| *Mam*19 x *Wam*15 | 1 | 0.020 | 0.020 |
| *Mam*19 x *Pam*16 | 2 | 0.0079, 0.0092 | 0.0086 |
| *Mam*19 x *Bam*17 | 1 | 0.043 | 0.043 |
| *Mam*19 x *Lam*21 | 1 | 0.0052 | 0.0052 |
| *Wam*15 x *Tam*8 | 1 | 0.0020[b] | 0.0020 |

[a] X represents the number of experiments used in the calculation of the average recombination frequency shown.

[b] In these crosses problems in scoring recombinants arose due to the presence of specks, formed by recombination on the plate.

Reversion frequencies for the mutants were determined following single infections. For the mutants *Wam*15, *Pam*16 and *Lam*21 the reversion frequencies were less than 0.0001%. For the mutants *Eam*7, *Mam*19, *Bam*17 and *Tam*8 the reversion frequencies were <0.0007%, <0.0005%, 0.0002% and 0.0004% respectively. The numbers of experiments used to calculate the reversion frequencies for these mutants were 7, 6, 5, 1, 1, 4 and 1 respectively.

with mutant 15 and so should map nearby. However due to the many inconsistencies in this set of results it was not possible to obtain a unique order for the mutations.

For 18 of the 41 crosses recorded in Table 5.3, the product of the number of recombinants and the total number of phage added to the plate was less than or equal to $3 \times 10^7$. For the remaining 23 crosses this product was greater than $3 \times 10^7$ but less than $1 \times 10^9$. In the crosses in which this product was less than $3 \times 10^7$ only a few specks were observed for each cross, and it seems that keeping this product low has almost eliminated recombination on the plate. Even for the crosses with products higher than $3 \times 10^7$ very few specks were observed and in only three crosses were there enough specks to cause problems.

Although the reduction in recombination on the plate observed in these crosses is probably mainly attributable to the reduced *moi* on the Su⁻ indicator plate, the presence of the *recA* mutation in the indicator bacteria may also have helped to reduce recombination on the plate. When a mixture of the phage *Bam*17 and *Mam*19 was assayed on a *rec*$^+$ strain (594) and on a *recA* strain (152) the number of specks on strain 594 was about ten-fold greater than the number on 152, suggesting that recombination on the plate is reduced in the absence of the *recA* product. However, in two other tests involving mixtures of the phage *Tam*8 and *Wam*15, and *Wam*15 and *Mam*19, very little difference was observed in the number of specks appearing on the *rec*$^+$ and *recA* indicators. In these last two tests $10^6$ *pfu* of each phage were added to plates containing about $6 \times 10^7$ *cfu* of either 594 or 152. For the mixture 15 + 19 approximately 1,000 specks appeared on both the 594 and 152 plates. For the mixture 8 + 15 fewer specks appeared and this is probably the result of the smaller recombination frequency

obtained for this pair of mutants.

A greater reduction in the appearance of specks due to the use of the *recA* strain was anticipated since the results of a two-factor cross using *rec*$^+$ and *recA* strains suggested that the majority of recombination in 186 two-factor crosses depends on the host *rec* system. For the cross *Eam*7 x *Mam*19 a recombination frequency of 0.029% was obtained in the *rec*$^+$ strain C600, while for the same cross a frequency of 0.0014% was obtained in the *recA* strain QR48. That is, recombination is about 20 fold lower in a *recA* strain. However, the two strains used are not isogenic and further crosses in an isogenic pair of strains would be required to determine the effect on 186 recombination of the host *rec* system.

In conclusion, it appears that to avoid recombination on the plate it is important to keep the product of the number of recombinants per plate and the total number of phage added per plate to a minimum (preferably about, or less than, the 3 x $10^7$ level recommended for P2 by Lindahl (1969a)). However, even under conditions in which recombination on the plate was not a problem (as in Table 5.3) a consistent order for the mutations could not be obtained. Furthermore, the order obtained for the four mutants, 19, 16, 15 and 17 (which was internally consistent) seemed unlikely since it would mean that in phage 186 genes whose functions are related do not all map together. The mutants *Mam*19 and *Eam*7 are defective in genes involved in the synthesis of the phage tail while the mutant *Wam*15, mapping between them, is defective in the formation of the phage head (see Section 3).

Although low recombination frequencies (of the order of 0.01%) were obtained in all the two-factor crosses described so far, frequencies ten to fifty times greater than this were obtained in crosses in which one of the parental mutants was a gene *A* mutant.

Table 5.4 shows several examples of the frequencies obtained in crosses involving gene *A* mutants. Other crosses between the mutant *Aam*5 and the mutants *Bam*17, *Fam*20, *Gam*25, *Lam*21, *Mam*19 and *Tam*8, also showed similarly high recombination frequencies. It would appear that there is a very large distance between gene *A* and all the other genes or that recombination in the region between gene *A* and the other genes is unusually efficient. In either case the result is the appearance of a long region in the 186 genetic map which is devoid of mutations. A much smaller region at one end of the long blank region contains all but one of the known 186 essential genes, the remaining gene (gene *A*) mapping at the other end of the long blank region.

However these elevated recombination frequencies for gene *A* mutants were only obtained if both parental phage had, apart from an amber mutation in an essential gene, a mutation in the repressor (or *c*I) gene. This can be seen from the results of experiments recorded in Table 5.5 in which mutants in gene *A* (derived from 186*c*I*ts*) were crossed with mutants in other genes derived from wild-type 186. These results suggest that the presence of the $cI^+$ allele reduces recombination in the region between gene *A* and the other genes. The alternative explanation, that genes *D*, *Q* and *H* in fact lie on the same side of the long blank region as gene *A*, is unlikely since the last two crosses of Table 5.5 show that *Qam*49 gives low recombination frequencies with *Eam*7 and *Tam*8, both of which give recombination frequencies of between 0.1% and 0.2% with *Aam*5 (see Table 5.4 for cross *Aam*5 x *Eam*7). *Dam*48 and *Ham*50 also showed low recombination frequencies with several mutants all of which give large frequencies with *Aam*5 (data not shown). Finally, other genes in the polarity groups to which genes *D* and *Q* belong are separated from gene *A* by the long blank region.

## TABLE 5.4

### TWO-FACTOR CROSSES WITH ALLELES OF GENE *A*

| Cross | Number of experiments | Recombination frequency (%) |
|---|---|---|
| *Aam*5 x *Eam*7 | 1 | 0.13 |
| *Aam*5 x *Wam*15 | 1 | 0.18 |
| *Aam*11 x *Pam*16 | 1 | 0.34 |
| *Aam*12 x *Pam*16 | 1 | 0.45 |
| *Aam*13 x *Pam*16 | 1 | 0.45 |

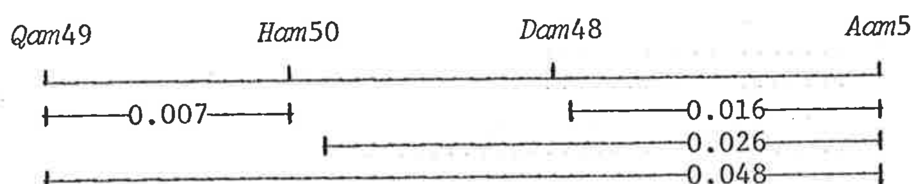
## TABLE 5.5

### TWO-FACTOR CROSSES WITH MUTANTS DERIVED FROM $186^+$

| Cross | Number of experiments | Recombination frequency (%) |
|---|---|---|
| *Aam*5*c*I*ts* x *Dam*48*c*$I^+$ | 1 | 0.016 |
| *Aam*5*c*I*ts* x *Qam*49*c*$I^+$ | 1 | 0.048 |
| *Aam*5*c*I*ts* x *Ham*50*c*$I^+$ | 1 | 0.026 |
| *Qam*49*c*$I^+$ x *Ham*50*c*$I^+$ | 1 | 0.007 |
| *Qam*49*c*$I^+$ x *Eam*7*c*I*ts* | 1 | 0.007 |
| *Qam*49*c*$I^+$ x *Tam*8*c*I*ts* | 1 | 0.0006 |

The fact that recombination frequencies between gene *A* and the other genes can be reduced by the presence of the *c*I$^+$ gene suggests that the long blank region is not, in fact, a physical entity but rather an artifact resulting from the unusually high recombination frequency occurring in this region. The recombination frequency 0.007% for the cross *Qam*49 x *Ham*50 is in the normal range of frequencies obtained, as is 0.007% for *Qam*49 x *Eam*7 and 0.0006% for *Qam*49 x *Tam*8, suggesting that the *c*I$^+$ allele may not affect recombination in other regions.

In crosses where both parental phage have been derived from 186*c*I*ts*, recombination frequencies obtained for crosses involving alleles of gene *A* are so much higher than for crosses not involving gene *A* alleles that gene *A* cannot be mapped relative to the other genes. However, gene *A* can be mapped relative to mutations *Dam*48, *Ham*50 and *Qam*49 by virtue of the reduced frequency of recombination. From the results of Table 5.5 the following order is suggested:




The frequencies are not additive and it may be that frequencies with gene *A* alleles are still a little inflated even in the presence of one *c*I$^+$ gene. *Tam*8 gave a very low recombination frequency with *Qam*49 and so maps close to it.

In summary, the results of the two-factor crosses described in this section suggest that the arrangement of genes on the 186 chromosome is: tail genes - lysozyme gene - head genes - tail genes - early genes, very different from the arrangement found for the closely

related phage P2, which is head genes - lysozyme gene - tail genes - early genes.

## 5.3 186 TWO-FACTOR CROSSES IN THE PRESENCE OF PHAGE λ

It has been reported by Mandel and Kornreich (1972) that the presence of the λ *red* gene increases the frequency of recombination in phage 186. An increase in recombination frequency would be advantageous in that it would help to reduce the problem of recombination on the plate. Two-factor crosses in the presence of λ would also provide another system in which to check the order of mutations obtained in standard two-factor crosses.

The presence of replicating λ in a 186 two-factor cross only slightly reduced the number of 186 progeny phage produced, and increased the number of 186 recombinant phage formed. Table 5.6 compares the recombination frequencies obtained for a 186 two-factor cross into which λ has been introduced either by induction or by infection. The conditions of induction used were sufficient to produce a good stock of phage λ*c*1857 ($\geqslant 10^{10}$ *pfu*/ml). For the various conditions recorded in Table 5.6 it can be seen that the highest recombination frequency was obtained when λ was introduced by induction, and this was the method used in all future experiments.

Table 5.7 records the results of two-factor crosses in the presence of phage λ. Each cross was repeated several times to obtain an average recombination frequency. At least 100 and generally 200 or more recombinants were scored for each cross. Under the conditions used the frequency of 186 recombination was only two- to three-fold greater than in the standard two-factor crosses of Section 5.2. (For

TABLE 5.6

RECOMBINATION FREQUENCIES FOR THE CROSS *Bam*17 x *Mam*19 FOLLOWING INDUCTION OF OR CO-INFECTION WITH PHAGE λ

| Host | Addition of λ | Time when added (mins)[a] | Number of experiments | Average recombination frequency (%) |
|---|---|---|---|---|
| E514 | Induction | -5 | 5 | 0.091 |
| C600 | Infection | 0 | 2 | 0.044 |
| C600 | Infection | 10 | 2 | 0.048 |
| C600 | Infection | 20 | 2 | 0.027 |

[a] Time relative to addition of phage 186 at zero minutes.

TABLE 5.7

186 TWO-FACTOR CROSSES IN THE PRESENCE OF PHAGE λ

| Cross | Individual recombination frequencies (%) | | | | | Average recomb. freq.(%) |
|---|---|---|---|---|---|---|
| *Eam*7 x *Tam*8 | 0.044 | 0.034 | 0.045 | 0.019 | 0.042 | 0.037 |
| *Eam*7 x *Wam*15 | 0.017 | 0.038 | 0.083 | 0.034 | 0.049 | 0.044 |
| *Eam*7 x *Bam*17 | 0.018 | 0.011 | 0.011 | 0.0072 | 0.012 | 0.012 |
| *Eam*7 x *Mam*19 | 0.075 | 0.048 | 0.073 | 0.054 | 0.051 | 0.060 |
| *Tam*8 x *Wam*15 | 0.0041 | 0.0048 | 0.013 | 0.0050 | 0.015 | 0.0084 |
| *Tam*8 x *Bam*17 | 0.050 | 0.045 | 0.057 | 0.050 | 0.038 | 0.048 |
| *Tam*8 x *Mam*19 | 0.033 | 0.0019 | 0.021 | 0.014 | 0.023 | 0.019 |
| *Wam*15 x *Bam*17 | 0.016 | 0.0080 | 0.056 | 0.058 | 0.112 | 0.050 |
| *Wam*15 x *Mam*19 | 0.019 | 0.022 | 0.040 | 0.037 | 0.058 | 0.035 |
| *Bam*17 x *Mam*19 | 0.117 | 0.070 | 0.089 | 0.105 | 0.075 | 0.091 |
| *Pam*16 x *Eam*7 | 0.071 | 0.037 | 0.045 | 0.088 | 0.065 | 0.061 |
| *Pam*16 x *Tam*8 | 0.010 | 0.0021 | 0.0051 | 0.0078 | 0.0069 | 0.0064 |
| *Pam*16 x *Wam*15 | 0.036 | 0.010 | 0.023 | 0.022 | 0.044 | 0.027 |
| *Pam*16 x *Bam*17 | 0.104 | 0.072 | 0.080 | 0.092 | 0.112 | 0.092 |
| *Pam*16 x *Mam*19 | 0.044 | 0.029 | 0.019 | 0.029 | 0.040 | 0.032 |
| *Oam*61 x *Eam*7 | 0.141 | 0.147 | 0.195 | 0.142 | 0.162 | 0.157 |
| *Oam*61 x *Tam*8 | 0.026 | 0.029 | 0.053 | 0.043 | 0.028 | 0.036 |
| *Oam*61 x *Wam*15 | 0.062 | 0.053 | 0.081 | 0.064 | 0.108 | 0.074 |
| *Oam*61 x *Pam*16 | 0.027 | 0.032 | 0.033 | 0.042 | 0.031 | 0.033 |
| *Oam*61 x *Bam*17 | 0.088 | 0.095 | 0.157 | 0.155 | 0.183 | 0.136 |
| *Oam*61 x *Mam*19 | 0.032 | 0.048 | 0.036 | 0.051 | 0.035 | 0.040 |
| *Jam*41 x *Eam*7 | 0.015 | 0.033 | 0.033 | 0.027 | 0.032 | 0.028 |
| *Jam*41 x *Tam*8 | 0.0048 | 0.0063 | 0.013 | 0.027 | 0.017 | 0.014 |
| *Jam*41 x *Wam*15 | 0.049 | 0.027 | 0.034 | 0.056 | 0.050 | 0.043 |
| *Jam*41 x *Pam*16 | 0.024 | 0.029 | 0.056 | 0.065 | 0.042 | 0.043 |
| *Jam*41 x *Bam*17 | 0.018 | 0.015 | 0.035 | 0.037 | 0.033 | 0.028 |
| *Jam*41 x *Mam*19 | 0.013 | 0.012 | 0.024 | 0.036 | 0.014 | 0.020 |
| *Jam*41 x *Lam*21 | 0.023 | 0.0072 | 0.036 | 0.048 | 0.035 | 0.030 |
| *Jam*41 x *Oam*61 | 0.041 | 0.043 | 0.068 | 0.078 | 0.084 | 0.063 |

*continued* -

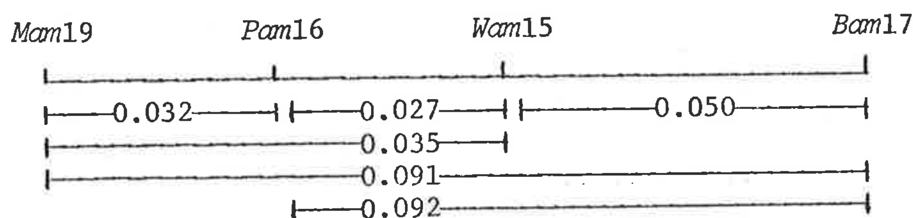
TABLE 5.7 *(continued)*

| Cross | Individual recombination frequencies (%) | | | Average recomb. freq.(%) |
|---|---|---|---|---|
| *Lam*21 x *Eam*7 | 0.115 | | 0.058 | 0.087 |
| *Lam*21 x *Tam*8 | 0.017 | 0.0065 | 0.0034 | 0.0090 |
| *Lam*21 x *Wam*15 | 0.040 | 0.022 | 0.011 | 0.024 |
| *Lam*21 x *Pam*16 | 0.068 | | 0.030 | 0.049 |
| *Lam*21 x *Bam*17 | 0.097 | | 0.034 | 0.066 |
| *Lam*21 x *Mam*19 | 0.0061 | 0.0020 | 0.0025 | 0.0035 |
| *Lam*21 x *Oam*61 | 0.057 | | 0.037 | 0.047 |

Reversion frequencies for all mutants were determined following single infections. For the mutants *Eam*7, *Tam*8, *Wam*15, *Pam*16, *Mam*19 and *Oam*61 the reversion frequencies were less than 0.0001%. The reversion frequencies for the mutants *Bam*17, *Lam*21 and *Jam*41 were 0.0008%, 0.0002% and 0.002% respectively. The reversion frequencies given are an average of values obtained in two experiments.

a comparison see Table 5.8.) This was only a moderate increase and meant that recombination on the plate remained a problem. However by counting only larger plaques and ignoring all specks it was hoped that a reproducible frequency could be obtained. Most results were reasonably reproducible though in some cases large variations did occur. For example in the cross *Wam*15 x *Bam*17 results varied from 0.008% to 0.112%. By taking an average of five results a better estimate should be obtained. However, to draw a map of the positions of 186 mutations consistent with all the results of Table 5.7 is quite impossible.

In the absence of λ the order 19-16-15-17 was consistent with the results obtained (see Table 5.3). In the presence of λ this order is also the one which fits the results best.




The recombination frequencies suggest that *Eam*7 maps closer to *Bam*17 than to *Wam*15 and closer to *Wam*15 than to *Pam*16 or *Mam*19. As for the results obtained in the absence of λ, *Eam*7 appears to map in the vicinity of *Bam*17. When mutant 7 was crossed with mutants 15, 16 or 19 lower recombination frequencies were obtained than when mutant 17 was crossed with mutants 15, 16 or 19. It therefore appears that *Eam*7 lies to the left of *Bam*17.

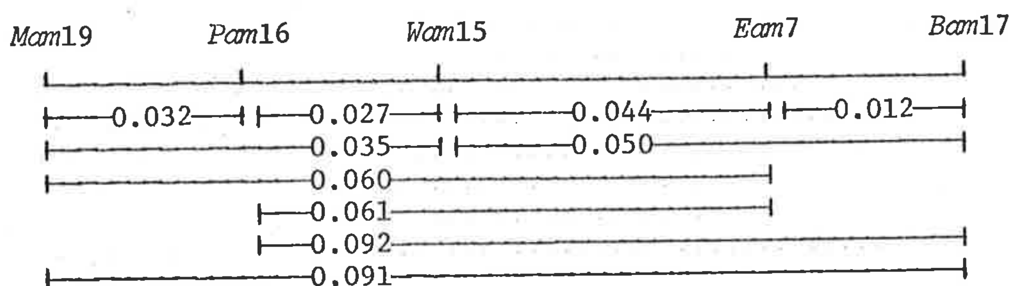


TABLE 5.8

COMPARISON OF 186 RECOMBINATION FREQUENCIES IN THE PRESENCE AND ABSENCE OF PHAGE λ

| Cross | Without λ [a] | | With λ [b] | | Ratio[c] |
|---|---|---|---|---|---|
| | X[d] | Average recomb. freq.(%) | X[d] | Average recomb. freq.(%) | |
| *Wam*15 x *Pam*16 | 8 | 0.010 | 5 | 0.027 | 2.7 |
| *Wam*15 x *Bam*17 | 5 | 0.016 | 5 | 0.050 | 3.1 |
| *Wam*15 x *Lam*21 | 5 | 0.011 | 3 | 0.024 | 2.2 |
| *Pam*16 x *Bam*17 | 3 | 0.030 | 5 | 0.061 | 2.0 |
| *Pam*16 x *Lam*21 | 7 | 0.010 | 2 | 0.049 | 4.9 |
| *Bam*17 x *Lam*21 | 3 | 0.025 | 2 | 0.066 | 2.6 |
| *Eam*7 x *Wam*15 | 1 | 0.029 | 5 | 0.044 | 1.5 |
| *Eam*7 x *Bam*17 | 1 | 0.0057 | 5 | 0.012 | 2.1 |
| *Eam*7 x *Mam*19 | 1 | 0.041 | 5 | 0.060 | 1.5 |
| *Eam*7 x *Lam*21 | 1 | 0.037 | 2 | 0.087 | 2.4 |
| *Wam*15 x *Mam*19 | 1 | 0.020 | 5 | 0.035 | 1.8 |
| *Pam*16 x *Mam*19 | 2 | 0.0086 | 5 | 0.032 | 3.7 |
| *Bam*17 x *Mam*19 | 1 | 0.043 | 5 | 0.091 | 2.1 |
| *Lam*21 x *Mam*19 | 1 | 0.0052 | 3 | 0.0035 | 0.7 |
| *Tam*8 x *Wam*15 | 1 | 0.0020 | 5 | 0.0084 | 4.2 |

*a* Results from Table 5.3

*b* Results from Table 5.7

*c* Ratio of frequencies obtained in the presence of phage λ to those obtained in its absence. The average ratio is 2.5.

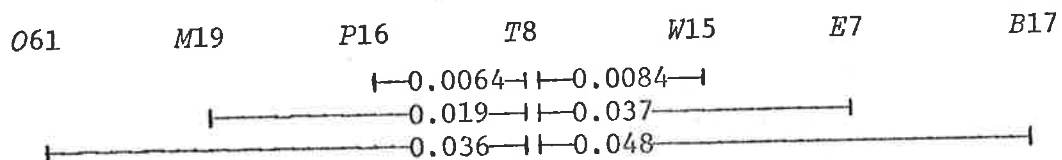
*d* X represents the number of experiments used in the calculation of the average recombination frequency shown.

Recombination frequencies suggest that *Oam*61 lies closer to *Pam*16 and *Mam*19 than to *Wam*15, and closer to *Wam*15 than to either *Eam*7 or *Bam*17. 61 must therefore map to the left of 15 and because of its large values with 15, 7 and 17, probably to the left of 16 and 19 as well. That is, the most likely order is:

*O*61 - *M*19 - *P*16 - *W*15 - *E*7 - *B*17

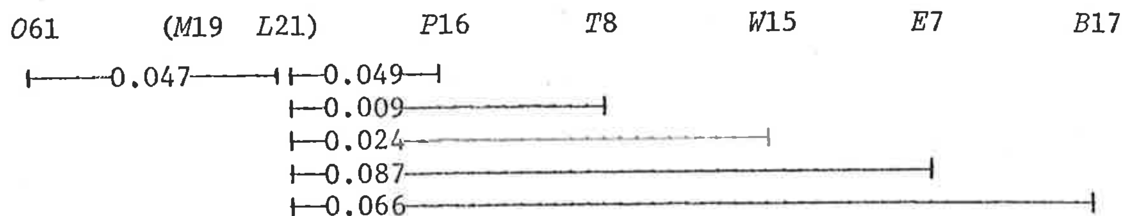
However 61 appears closer to 16 than to 19 and the ordering of 16 and 19 is unsatisfactory in a couple of other crosses, so these two mutants may be misplaced.

*Tam*8 appears to map closest to mutants 15 and 16. Assuming the order *O*61-*M*19-*P*16-*W*15-*E*7-*B*17, the results for *Tam*8 are consistent with a position between 15 and 16.




However inconsistencies appear when all combinations are considered. For example if this order is correct then the frequency for 8 x 7 (0.037%) should be greater than that for 15 x 7 (0.044%); 8 x 17 (0.048%) should be greater than 15 x 17 (0.050%) and 8 x 19 (0.019%) should be greater than 16 x 19 (0.032%). The frequency of 8 x 61 (0.036%) is greater than that for 16 x 61 (0.033%), as it should be if the above order is correct. It would appear that the results obtained with mutant 8 are smaller in all cases than they ought to be. However a position between mutations *Pam*16 and *Wam*15 is still the most likely.

*Lam*21 appears to map close to *Mam*19 (recombination frequency = 0.0035%). The results for this mutant, assuming the most likely order of mutations derived above, are as follows.




As can be seen, these results are quite inconsistent. Most of them are the average of two experiments only and perhaps more crosses would improve the frequencies. All that can be said about *Lam*21 from the above results is that it appears to map close to *Mam*19.

When *Jam*41 was crossed with the other mutants the recombination frequencies obtained for the various crosses can be arranged in the following order:

61 x 41 > 15 x 41, 16 x 41 > 21 x 41 > 7 x 41, 17 x 41 > 19 x 41 > 8 x 41

By reference to the most likely order of mutations it can be seen that it is not possible to assign a position to the mutant *Jam*41.

In summary, the most likely order for the 186 mutations derived from the results of Table 5.7 is:

*O*61 - *M*19 - *P*16 - *T*8 - *W*15 - *E*7 - *B*17
(*L*21)

The map obtained is unsatisfactory for several reasons:

(1) Although this is the most likely order there is a considerable number of inconsistencies.

(2) It was not possible to assign a position to *Jam*41.

(3) Once again, as for the results in the absence of $\lambda$, the order obtained involves the separation of genes with related functions. Genes involved in 186 head synthesis (*T* and *W*) map between genes involved in 186 tail synthesis (*M* and *L* on one side, *E* on the other).

In most of the crosses described in Table 5.7 the product of the recombinants and the phage added was greater than 3 x $10^7$ and

specks were a problem. Avoiding counting specks may have increased the variability of the results but since the order obtained is, where comparable, identical to the order obtained in the absence of λ (where, due to dilution, recombination on the plate was not a problem) the failure of these crosses to produce a unique order was probably not due to recombination on the plate. Again, since the order with and without λ is the same, the presence of λ in the cross does not appear to be perturbing the order obtained for the 186 mutations.

## 5.4 186 TWO-FACTOR CROSSES AFTER PREIRRADIATION WITH ULTRAVIOLET LIGHT

The presence of phage λ in a 186 two-factor cross increased the frequency of recombination by two to three fold, but this increase was not large enough to solve the problem of recombination on the plate. A more efficient way of increasing 186 recombination was found to be the UV irradiation of the parental phage prior to infection.

The frequency of 186 recombination was found to depend on the dose of UV irradiation applied to the parental phage. For UV exposures of up to about 2½ minutes (equivalent to 2,280 ergs/$mm^2$) an increase in recombination frequency corresponded to an increase in UV exposure (see Table 5.9). For exposure times greater than 2½ minutes the recombination values obtained were unreliable due to the low numbers of recombinants scored. Table 5.9 also shows that an increase in UV dose caused a decrease in phage viability. After 60 seconds irradiation the *eop* of the phage was reduced to one third. For UV exposures of up to 60 seconds the actual numbers of recombinants increased, but beyond 60 seconds (for example, 90 seconds), even though the recombination frequency increased, both the number of recombinants and the number of progeny phage decreased. For this reason 60 seconds seemed the most suitable exposure time and was used for all future experiments.

TABLE 5.9

EFFECT OF DURATION OF UV IRRADIATION ON RECOMBINATION FREQUENCY AND PHAGE VIABILITY IN THE CROSS *Bam*17 x *Mam*19

| UV irradiation | | | Recombination frequency (%) | Phage viability *(eop)* |
|---|---|---|---|---|
| Time | | Dose | | |
| (mins) | (secs) | (ergs/mm$^2$) | | |
| | 0 | 0 | 0.013 | 1 |
| | 10 | 152 | 0.052 | 0.57 |
| | 20 | 304 | 0.082 | 0.64 |
| | 30 | 456 | 0.20 | 0.55 |
| | 40 | 608 | 0.30 | 0.56 |
| | 50 | 760 | 0.33 | 0.40 |
| | 60 | 912 | 0.49 | 0.28 |
| 0 | | 0 | 0.010 | 1 |
| 1 | | 912 | 0.35 | 0.37 |
| 1.5 | | 1368 | 0.45 | 0.26 |
| 2 | | 1824 | 0.44 | 0.089 |
| 2.5 | | 2280 | 0.92 | 0.047 |
| 0 | | 0 | 0.010 | 1 |
| 1 | | 912 | 0.17 | 0.29 |
| 2 | | 1824 | 0.43 | 0.054 |
| 3 | | 2736 | 0.38[a] | 0.017 |
| 4 | | 3648 | 0.63[a] | 0.0036 |
| 5 | | 4560 | 0.42[a] | 0.00045 |

[a] These recombination frequencies are unreliable due to the low numbers of recombinants scored.

Table 5.10 shows the results of four experiments involving two-factor crosses with the mutants *Bam*17 and *Mam*19. In the absence of any UV irradiation the recombination frequency was 0.010% while after 60 seconds irradiation the frequency was 0.36%. This represents a 36 fold increase in recombination frequency. For the cross 17 x 19 in the presence of λ the average recombination frequency obtained was 0.091%, which represents only a 9 fold increase. Therefore UV irradiation seems to have more potential than λ as a method for increasing the recombination frequency in phage 186.

In Section 5.3, when calculating the increase in frequency due to the presence of λ, the value used for the cross 17 x 19 in the absence of λ was 0.043%. If this value is used to calculate the increase in recombination frequency, then the presence of λ produced a two fold increase while 60 seconds UV irradiation produced an 8.4 fold increase. However a 36 fold increase with 60 seconds UV is probably more accurate since the values 0.010% without UV and 0.36% with UV were obtained under the same conditions.

Table 5.11 records the results of a set of two-factor crosses, involving mutants in genes *B*, *M* and *P*, following 60 seconds of UV irradiation. In Section 5.3 the recombination frequency obtained for the cross 16 x 17 was 0.030%. In the presence of λ the frequency obtained was 0.061% (a two fold increase). In Table 5.11 the frequency obtained for this cross after UV irradiation was 0.34% (an eleven fold increase). For the cross 16 x 19 the recombination frequencies were 0.0086%, in a standard two-factor cross, 0.032% in the presence of λ (a four-fold increase) and 0.12% after UV irradiation (a 14 fold increase). Therefore, once again, UV irradiation has produced larger recombination frequencies than those obtained in the presence of phage λ.

The results recorded in Table 5.11 for the single set of

TABLE 5.10

RECOMBINATION FREQUENCIES FOR THE CROSS *Bam*17 x *Mam*19 IN THE PRESENCE AND ABSENCE OF 60 SECONDS UV IRRADIATION

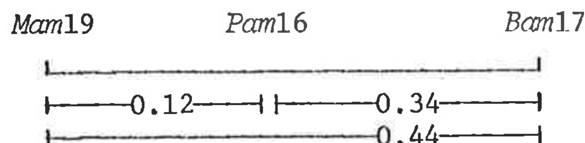
| UV irradiation | | Recombination frequency (%) | Average recomb. freq.(%) |
|---|---|---|---|
| Time (secs) | Dose (ergs/mm$^2$) | | |
| 0 | 0 | 0.013, 0.010, 0.010, 0.0096 | 0.010 |
| 60 | 912 | 0.49, 0.35, 0.17, 0.44 | 0.36 |

TABLE 5.11

TWO-FACTOR CROSSES WITH ALLELES OF GENES *B*, *M* AND *P* FOLLOWING 60 SECONDS UV IRRADIATION

| Cross | UV irradiation | | Recombination frequency (%) | Phage viability (*eop*) |
|---|---|---|---|---|
| | Time (secs) | Dose (ergs/mm$^2$) | | |
| *Bam*17 x *Mam*19 | 0 | 0 | 0.0096 | 1 |
| *Bam*17 x *Mam*19 | 60 | 912 | 0.44 | 0.33 |
| *Pam*16 x *Bam*17 | 60 | 912 | 0.34 | |
| *Pam*16 x *Mam*19 | 60 | 912 | 0.12 | |

crosses with the mutants 16, 17 and 19 suggest the following map order.




This is also the order obtained for these mutants both in standard two-factor crosses and in two-factor crosses involving phage λ, and later experiments have shown that this order is incorrect (see Section 7). Therefore although UV shows a greater potential than λ for increasing the frequency of 186 recombination there is no indication that its use would lead to a different, and correct order being obtained.

The large increase in recombination due to UV irradiation almost eliminated problems due to recombination on the plate. With this high recombination frequency it was no longer necessary to use a large number of plates to measure the recombinants, in order to keep "Lindahl's product" below 3 x $10^7$.

## 5.5 186 THREE-FACTOR CROSSES WITH THE DOUBLE MUTANT *Mam*60*Ets*7

To check the gene order suggested by the results of two-factor crosses, two experiments involving three-factor crosses were done. In these experiments the double mutant *Mam*60*Ets*7 was crossed with several other *am* mutants. $Am^+$ recombinants were scored on an $Su^-$ indicator at 30°C while $am^+ts^+$ recombinants were scored on an $Su^-$ indicator at 40°C or 38°C (both of which are restrictive temperatures for the mutant *Ets*7).

For the cross *Mam*60*Ets*7 x *am*α, three positions for the mutation α can be distinguished. If the mutation lies within the interval between *am*60 and *ts*7 (as in Fig. 5.1a) then:

(1) The number of $am^+ts^+$ recombinants should be much lower for

*Figure 5.1* THREE-FACTOR CROSSES WITH THE DOUBLE MUTANT *Mam60Ets7*

The figure shows the recombination events required to form an $am^{+}ts^{+}$ recombinant in a cross between the double mutant *Mam60Ets7* and a single mutant *amα*.

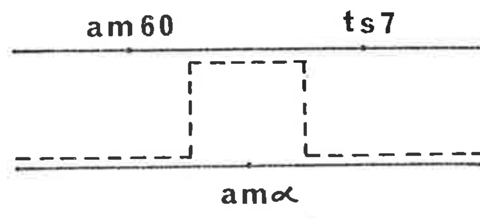
(a) Recombination between the double mutant and a mutant located between *am60* and *Ets7*.

(b) Recombination between the double mutant and a mutant located to the left of *am60*.

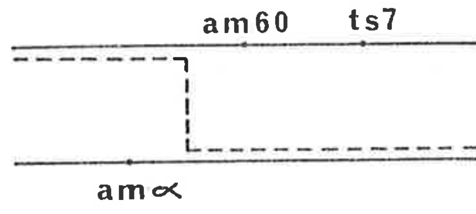
(c) Recombination between the double mutant and a mutant located to the right of *ts7*.

The broken lines represent $am^{+}ts^{+}$ recombinants.

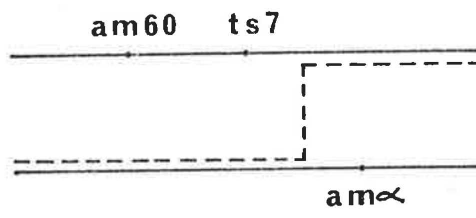
(a)




(b)




(c)



the cross *am*60*ts*7 x *am*α than for either *am*60 x *am*α or *ts*7 x *am*α. (In the absence of negative interference the former frequency should be the product of the two latter frequencies.)

(2) For the cross *am*60*ts*7 x *am*α, the number of $am^{+}ts^{+}$ recombinants (38°C) should be much less than the number of $am^{+}$ recombinants (30°C).

If *am*α lies to the left of *am*60 (as in Fig. 5.1b) then:

(1) The number of $am^{+}ts^{+}$ recombinants for the cross *am*60*ts*7 x *am*α should be the same as for the cross *am*60 x *am*α.

(2) For the cross *am*60*ts*7 x *am*α the number of $am^{+}ts^{+}$ recombinants (38°C) should be equivalent to the number of $am^{+}$ recombinants (30°C).

If *am*α lies to the right of *ts*7 (Fig. 5.1c) then:

(1) The number of $am^{+}ts^{+}$ recombinants for the cross *am*60*ts*7 x *am*α should be the same as for the cross *ts*7 x *am*α.

(2) For the cross *am*60*ts*7 x *am*α the number of $am^{+}ts^{+}$ recombinants (38°C) should be less than the number of $am^{+}$ recombinants (30°C).

The results of such an experiment in which three different "α" mutations were tested are shown in Table 5.12.

For the crosses not involving the *ts* allele, that is *Mam*60 x *Wam*15, *Mam*60 x *Bam*17 and *Mam*60 x *Oam*61, the recombination frequency was larger at 30°C than at 40°C. Since the same estimate of total progeny (determined at 30°C) was used in the calculation of both frequencies, the difference is due to the different numbers of recombinants appearing at 30°C and 40°C and this is a reflection of the low *eop* of 186 at 40°C when compared with its *eop* at 30°C.

In the experiments involving *Wam*15 the number of $am^{+}ts^{+}$

TABLE 5.12

THREE-FACTOR CROSSES WITH THE DOUBLE MUTANT *Mam60Ets7* AND THE MUTANTS *Wam15*, *Bam17* AND *Oam61*

| Cross | Recombination frequency (%) at 30°C | Recombination frequency (%) at 40°C | Ratio[a] |
|---|---|---|---|
| *Wam15* x *Ets7* | | 0.00066 | |
| *Wam15* x *Mam60* | 0.0017 | 0.00063 | 0.37 |
| *Wam15* x *Mam60Ets7* | 0.0047 | 0.0011 | 0.22 |
| *Bam17* x *Ets7* | | 0.00011 | |
| *Bam17* x *Mam60* | 0.0072 | 0.00088 | 0.12 |
| *Bam17* x *Mam60Ets7* | 0.0060 | 0.00017 | 0.028 |
| *Oam61* x *Ets7* | | 0.0016 | |
| *Oam61* x *Mam60* | 0.0017 | 0.00043 | 0.25 |
| *Oam61* x *Mam60Ets7* | 0.0022 | 0.00044 | 0.20 |

*a* Ratio of the recombination frequency measured at 40°C to the recombination frequency measured at 30°C.

TABLE 5.13

THREE-FACTOR CROSS: *Wam15* x *Mam60Ets7*

| Cross | Recombination frequency (%) at 30°C | Recombination frequency (%) at 38°C | Ratio[a] |
|---|---|---|---|
| *Wam15* x *Ets7* | | 0.061 | |
| *Wam15* x *Mam60* | 0.0081 | 0.0045 | 0.56 |
| *Wam15* x *Mam60Ets7* | 0.034 | 0.016 | 0.47 |

*a* Ratio of the recombination frequency measured at 38°C to the recombination frequency measured at 30°C.

Reversion frequencies for the mutants *Ets7*, *Wam15*, *Mam60* and *Mam60Ets7* were determined following single infections. In all cases the frequencies obtained (whether measured at 30°C or 38°C) were less than 0.0001%. (The reversion frequency for *Ets7* was determined at 38°C only.)

recombinants (40°C) for the cross *Wam*15 by the double mutant is of the same order as the numbers obtained when *Wam*15 was crossed with the component single mutations and not much less as would have been expected if *am*15 was located between the mutations *am*60 and *ts*7. This was also found to be true for crosses involving the mutants *Bam*17 and *Oam*61 and suggests that all three mutations tested lie outside the interval defined by the mutations *am*60 and *ts*7.

For the crosses involving *am*15 the frequency obtained with the double mutant was not significantly closer to the frequency obtained with one of the single mutants than to the frequency obtained with the other. Therefore, from this, it is not possible to determine which side of the *am*60 - *ts*7 interval *am*15 lies. With *am*17 the frequency with the double mutant was closer to the frequency of *ts*7 x *am*17 than to that of *am*60 x *am*17. This suggests that *am*17 lies to the right of *ts*7. With *am*61 the frequency with the double mutant was closer to that of *am*60 x *am*61 than to that of *ts*7 x *am*61, suggesting that *am*61 lies to the left of *am*60.

From the ratio of the recombination frequencies at 40°C and 30°C it can be seen that for mutants 15 and 61 there is only a small loss of recombinants at 40°C (other than those accounted for by the reduction in *eop* at this temperature). By contrast, with mutant 17 there is quite a marked drop. These findings are consistent with positions of *am*15 and *am*61 to the left of *am*60 and a position of *am*17 to the right of *ts*7.

The results for the mutant *Wam*15 were repeated (Table 5.13), the recombinants being measured at 30°C and 38°C. There is still a reduction in *eop* at 38°C but not as severe as the reduction at 40°C. Once again the frequency for the cross *Wam*15 by the double mutant was much greater than the product of the frequencies with the two single mutants. In this experiment the frequency with the double mutant was

closer to that of *am*60 x *am*15 than to that of *ts*7 x *am*15 suggesting that *am*15 lies to the left of *am*60. Again there is very little loss of recombinants at 38°C (other than of those due to the drop in *eop*) which also agrees with a position to the left of *am*60 rather than to the right of *ts*7.

In summary the three-factor crosses show that the three mutants *Wam*15, *Bam*17 and *Oam*61 lie outside the 60 - 7 interval. *Wam*15 and *Oam*61 probably lie to the left of *Mam*60 and *Bam*17 probably lies to the right of *Ets*7. The positions for *Bam*17 and *Oam*61 agree with the positions obtained from two-factor crosses, but the results with *Wam*15 show that its position as determined by two-factor crosses is incorrect.

The results obtained using a different mapping method (described in Section 7) confirm that the two-factor crosses gave the wrong order. In the new order all 186 genes involved in tail synthesis map together, as do the genes involved in head synthesis.

## 5.6 MAPPING OF THE GENES *c*I AND *c*II AND OF THE MUTATION *vir*2 WITH RESPECT TO THE LONG BLANK REGION

The 186 mutants *Ram*51 and *Wam*52 each contain a clear plaque mutation in addition to the *cIts* mutation of the phage from which they were derived. Complementation studies with *c*I and *c*II mutants showed that for phage *Ram*51 and *Wam*52 the additional clear plaque mutations lie in the *c*I and *c*II genes respectively. From crosses involving these two mutants and the mutant *Aam*5 it is possible to determine which side of the long blank region the *c*I and *c*II mutations lie.

Genes *R* and *W* are separated from gene *A* by the long blank region, gene *A* lying to the right of this region and genes *R* and *W* to the left. (The orientation of the 186 map is defined by its relation to the genetic map of phage P2 (see Section 5.8) and by the heteroduplex

analysis of P2-186 hybrid phage (Younghusband *et al.* 1975).) If the *c*I and *c*II genes lie to the left of the long blank region then a large majority of the *am*$^+$ recombinants in the crosses *Aam*5 x *Ram*51 and *Aam*5 x *Wam*52 should have turbid plaque morphologies at 30°C. If instead the *c*I and *c*II genes lie to the right of the long blank region then most of the *am*$^+$ recombinants should give clear plaques at 30°C. The results of Table 5.14 show that both genes *c*I and *c*II must lie to the right of the long blank region since for both the crosses *Aam*5 x *Ram*51 and *Aam*5 x *Wam*52 a large majority of the *am*$^+$ recombinants (97% and 96% respectively) have clear plaque morphologies.

Similar considerations apply to the mapping of the clear plaque mutant *vir*2. For the crosses *Aam*5 x *Eam*7*vir*2 and *Aam*5 x *Bam*17*vir*2 a majority of the *am*$^+$ recombinants gave clear plaques (97% and 96% respectively, see Table 5.14), suggesting that as with the *c*I and *c*II genes, the *vir*2 mutation lies to the right of the long blank region. The 186 *vir*2 mutant is able to grow on 186 lysogens and by analogy with phage λ it is likely that this mutant is defective in the operator(s) to which the 186 repressor protein binds. One hundred clear plaque recombinants from each of the last two crosses of Table 5.14 were tested for virulence, that is for their ability to grow on a 186 lysogen, and all 200 plaques were found to be virulent. Therefore all sites required for 186 virulence map to the right of the long blank region. That is, the binding site, or sites, of the 186 repressor must lie to the right of the long blank region.

## 5.7 THREE-FACTOR CROSSES WITH THE 186*c*I*ts* MUTATION

The mutants *Dam*48, *Ham*50 and *Qam*49 were derived from the phage 186*c*I$^+$, whereas most of the other amber mutants were derived from 186*c*I*ts*. Therefore, in two-factor crosses involving any of these three

TABLE 5.14

MAPPING OF THE GENES *c*I AND *c*II AND OF THE MUTATION *vir*2 WITH RESPECT TO THE LONG BLANK REGION

| Cross | Plaque morphology of the $am^+$ recombinants at 30°C | | | |
|---|---|---|---|---|
| | Clear | | Turbid | |
| | Number scored | % of total | Number scored | % of total |
| *Aam*5*c*I*ts* x *Ram*51*c*I*ts* *c*I | 663 | 97 | 21 | 3 |
| *Aam*5*c*I*ts* x *Wam*52*c*I*ts* *c*II | 229 | 96 | 9 | 4 |
| *Aam*5*c*I*ts* x *Eam*7*c*I*ts* *vir*2 | 843 | 97 | 25 | 3 |
| *Aam*5*c*I*ts* x *Bam*17*c*I*ts* *vir*2 | 640 | 96 | 30 | 4 |

mutants and any mutant derived from 186*c*I*ts*, observations of the plaque morphology of the $am^+$ recombinants (at 37°C) should lead to some information on the relative positions of the *am* mutants with respect to the *c*I gene.

In Section 5.2 the results of two-factor crosses suggested the order *Qam*49 - *Ham*50 - *Dam*48 - *Aam*5, with *Aam*5 mapping to the right of the long blank region. In Section 5.6 it was shown that the *c*I gene maps to the right of the long blank region and thus to the right of genes *Q*, *H* and *D*. That is, the order of this set of genes from left to right on the genetic map is *Q*-*H*-*D*-*c*I. If the mutant *Ham*50$c$I$^+$ is crossed with a mutant *Xam*α*c*I*ts* and the majority of $am^+$ recombinant plaques are turbid, then the mutation *Xam*α should lie to the right of *Ham*50 (see Fig. 5.2a). Conversely, if most recombinant plaques are clear then *Xam*α maps to the left of *Ham*50 (see Fig. 5.2b). Positions to the left or right of mutations *Dam*48 and *Qam*49 can be determined in the same manner.

The distribution of clear and turbid plaques in two-factor crosses involving parental phage differing at the *c*I locus are recorded in Table 5.15. Only crosses for which at least 40 recombinant plaques were scored have been included. For the first six crosses of Table 5.15 the majority of $am^+$ recombinants produced turbid plaques. This suggests that the mutations *Bam*17, *Eam*7 and *Fam*20 all lie to the right of mutations *Qam*49 and *Ham*50.

In the next set of crosses *Lam*21 and *Mam*19 appear to map to the left of *Ham*50 since the majority of $am^+$ recombinants produced clear plaques. Similarly *Pam*16 appears to map to the left of *Dam*48. For the cross *Pam*16 x *Ham*50 a slight majority of clear plaques was obtained, however the figures are too close to be sure that the difference is significant. The remaining five crosses in this second

*Figure 5.2* THREE-FACTOR CROSSES WITH THE *cIts* MUTATION

This figure illustrates the results of single recombination events between two *am* mutant phage carrying different *cI* alleles. At 37°C phage carrying the *cIts* allele form clear plaques while phage carrying the $cI^+$ allele form turbid plaques. The plaque morphology of the recombinant phage depends on the relative positions of the two *am* mutations.

(a) Recombination between the $Ham50cI^+$ phage and an *Xamα cIts* phage in which the mutation *Xamα* lies to the right of the mutation *Ham*50. Most of the $am^+$ recombinant phage should form turbid plaques at 37°C.

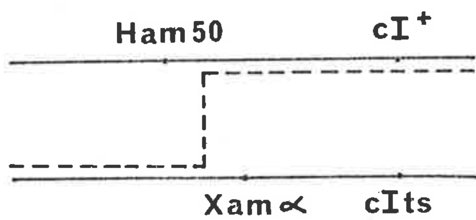
(b) Recombination between the $Ham50cI^+$ phage and an *Xamα cIts* phage in which the mutation *Xamα* lies to the left of the mutation *Ham*50. Most of the $am^+$ recombinant phage should form clear plaques at 37°C.

(c) Recombination between $Ham50cI^+$ and *Aam*5*cIts*. Most of the $am^+$ recombinant phage form turbid plaques at 37°C.

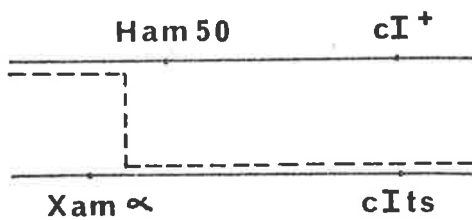
(d) Recombination between $Qam49cI^+$ and *Iam*40a*Aam*40b*cIts*. Most of the $am^+$ recombinant phage form turbid plaques at 37°C.

The broken lines represent $am^+$ recombinants.

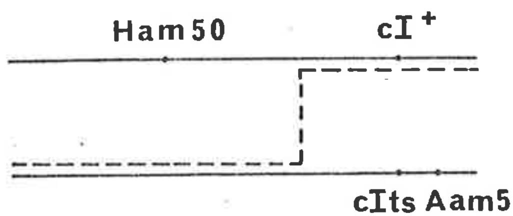
(a)




(b)




(c)




(d)

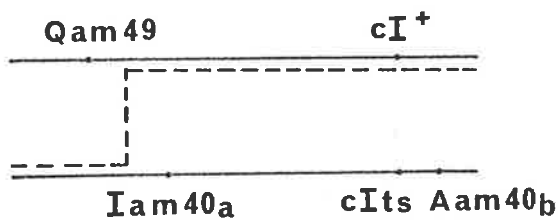


TABLE 5.15

THREE-FACTOR CROSSES WITH THE *cIts* MUTATION

| Cross | | $am^+$ recombinants | | | |
|---|---|---|---|---|---|
| *cIts* phage | $cI^+$ phage | Turbid plaques | Clear plaques | Total plaques | % Turbid plaques |
| *Bam*17 | x *Ham*50 | 43 | 19 | 62 | 69 |
| *Bam*17 | x *Qam*49 | 55 | 9 | 64 | 86 |
| *Eam*7 | x *Ham*50 | 39 | 17 | 56 | 70 |
| *Eam*7 | x *Qam*49 | 175 | 21 | 196 | 89 |
| *Fam*20 | x *Ham*50 | 103 | 27 | 130 | 79 |
| *Fam*20 | x *Qam*49 | 214 | 43 | 257 | 83 |
| | | | | | |
| *Lam*21 | x *Ham*50 | 18 | 39 | 57 | 32 |
| *Mam*19 | x *Ham*50 | 14 | 35 | 49 | 29 |
| *Pam*16 | x *Dam*48 | 6 | 41 | 47 | 13 |
| *Pam*16 | x *Ham*50 | 44 | 53 | 97 | 45 |
| *Qam*1 | x *Ham*50 | 20 | 44 | 64 | 31 |
| *Tam*8 | x *Dam*48 | 12 | 39 | 51 | 24 |
| *Tam*8 | x *Ham*50 | 52 | 99 | 151 | 34 |
| *Tam*8 | x *Qam*49 | 40 | 70 | 110 | 36 |
| *Wam*15 | x *Ham*50 | 15 | 29 | 44 | 34 |
| | | | | | |
| *Aam*5 | x *Dam*48 | 82 | 2 | 84 | 98 |
| *Aam*5 | x *Ham*50 | 1178 | 56 | 1234 | 95 |
| *Aam*5 | x *Qam*49 | 1444 | 67 | 1511 | 96 |
| | | | | | |
| *A,Iam*40 | x *Qam*49 | 76 | 0 | 76 | 100 |

group show that *Qam*1 maps to the left of *Ham*50, *Tam*8 maps to the left of all mutants *Dam*48, *Ham*50 and *Qam*49, and *Wam*15 maps to the left of *Ham*50.

The next set of crosses involves the mutant *Aam*5 and in all cases a very large majority of turbid plaques was obtained. The relative position of the genes *A* and *c*I is not known but these results suggest that *A* lies either to the left of *c*I or if to the right, then very close to the *c*I gene (see Fig. 5.2c).

The final cross involved the double mutant *am*40 (*Iam*40a*Aam*40b). As can be seen from Figure 5.2d, for either orientation of the mutations *Iam*40a and *Qam*49, a majority of turbid $am^+$ recombinants should result. Therefore, this cross is not useful for the mapping of the mutation *Iam*40a.

In summary, the results of Table 5.15 suggest that the mutation *Ham*50 lies between the mutations *Bam*17, *Eam*7, *Fam*20 mapping to the right and the mutations *Lam*21, *Mam*19, *Qam*1, *Tam*8 and *Wam*15 mapping to the left. In addition *Tam*8 appears to map to the left of *Qam*49.

Using the three mutants *Qam*49, *Ham*50 and *Dam*48, derived from 186$c$I$^+$, it should be possible, by the method described above, to assign the amber mutation of any phage derived from 186*c*I*ts* to one of four regions. These regions are: to the left of *Qam*49, between *Qam*49 and *Ham*50, between *Ham*50 and *Dam*48, and to the right of *Dam*48. Finer mapping would require other amber mutants derived from 186$c$I$^+$. Assuming the availability of these mutants this method appears more promising than two-factor crosses for the construction of a 186 map.

A feature of the results of Table 5.15 is the very high frequency of double recombinants. In view of the extremely low frequency of recombination observed for phage 186, it might be expected

that the frequency of double recombinants would be negligible. However, from the results of Table 5.15 it can be seen that as many as 45% of the *am*$^{+}$ recombinants may be due to double recombination events. The frequency of formation of double recombinants is greater than would be expected from the product of the recombination frequencies in the two component regions, and this phenomenon is termed negative interference. A measure of the level of this negative interference can be determined by calculating the interference index, *i*, which is the factor by which the proportion of double recombinants exceeds that to be expected for independently occurring exchanges (Hershey, 1958; Amati and Meselson, 1965).

For the cross *Tam8c*I*ts* x *Ham*50*c*I$^{+}$, 34% of the *am*$^{+}$ recombinants gave turbid plaques and are therefore double recombinants (see Fig. 5.2b). The interference index, *i*, for this cross can be determined by dividing the above proportion of double recombinants by the recombination frequency between the mutations *Ham*50 and *c*I*ts*. This last recombination frequency is not known. However, since the results of the third group of crosses in Table 5.15 show that the mutants *Aam*5 and *c*I*ts* lie very close together, the recombination frequency between the mutants *Ham*50 and *Aam*5 has been used instead. From Table 5.5 this frequency is 0.026%. Therefore $i = \frac{X \times 34}{X \times 0.026}$ where X is the recombination frequency between *Tam*8 and *Ham*50. For this cross, *i* is approximately equal to $1.3 \times 10^{3}$. The *i* values for the other crosses in the second group in Table 5.15 vary between about $8 \times 10^{2}$ and about $1.7 \times 10^{3}$.

These *i* values are very much greater than those obtained for phage λ. For λ the *i* values varied from about 3 for distant markers (separated by more than a quarter of the genome) to a maximum of about 70 for markers mapping close to each other (Amati and Meselson,

1965). The negative interference present in 186 recombination is much closer to that observed for phage P2. The *i* values for two P2 three-factor crosses reported by Lindahl (1969a) were $7.7 \times 10^3$ and $5.6 \times 10^3$.

From the results of Amati and Meselson (1965) it seems that the proportion of double recombinants in the $am^+$ (or $sus^+$) recombinant class generally lies between about 8% and about 30%. Similar frequencies were obtained for 186. For the first two groups of crosses in Table 5.15 the proportion of double recombinants among the $am^+$ recominants varied between 11% and 45% although most frequencies were around 30%. It therefore appears that once a single recombination has taken place (giving rise to an $am^+$ recombinant) the chance of a second cross-over is just as great in 186 as it is in λ. The difference in the *i* values for 186 and λ must therefore be due to the low chance of the initial recombination event in 186. For phage P2 the proportion of double recombinants in the $am^+$ recombinant class is also similar to those obtained for 186 and λ (see Lindahl, 1969a).

## 5.8 DISCUSSION

The results of the 186 two-factor crosses described in this section show that the recombination properties of phage 186 are very similar to those of the related phage P2. Both these phage show extremely low general recombination frequencies (of the order of 0.01%) when compared with frequencies obtained for phage λ, which are approximately a hundred-fold higher.

The most striking feature of the P2 genetic map constructed by Lindahl (1969a) is the "long blank region", which is devoid of mutations and covers about 80% of the map. Lindahl (1969b) has shown that the appearance of this blank region is due to the presence of the product of the *int* gene, which catalyses recombination at the phage

attachment site. In phage P2 this site-specific recombination is much more efficient than general recombination, the result being that crosses with mutants spanning the *att* site give much greater frequencies of recombination than crosses with mutants on the same side of the *att* site. When both parental phage in the P2 two-factor crosses were also mutant in the *int* gene, recombination between markers spanning the *att* site was reduced by about 100 fold, thus eliminating the apparent "long blank region" (Lindahl, 1969b).

In phage 186 a similar long blank region was also found. The frequency of recombination in this region (0.1 to 0.5%) was 10 to 50 fold higher than the frequency of general recombination found in other regions. It is probable, by analogy with the results for phage P2, that the relatively large frequency of recombination in this region is due to site-specific recombination catalysed by the product of the 186 *int* gene and occurring at the 186 *att* site. However this has not been proved since the necessary *int* derivatives of 186 *am* mutants have not as yet been constructed.

In phage P2 all the genes involved in the formation of the phage particle map to the left of the long blank region while genes *A* and *B*, required for DNA replication, and the repressor gene lie to the right. In phage 186 all the genes involved in the formation of the phage particle map together at one end of the long blank region and this has been defined as the left end to correspond with the P2 map. Only one known essential gene of phage 186, gene *A*, lies to the right of the long blank region. The phage attachment site should therefore lie between this gene and the remaining essential genes. Three-factor crosses showed that the *c*I and *c*II genes and the *vir*2 mutation all lie to the right of this presumed *att* site. The *int* gene has not been mapped with respect to the *att* site, and the relative positions of the *c*I, *c*II and *A* genes are not known (but see

general discussion for probable locations).

In two-factor crosses involving mutant *Aam*5 and the mutants *Dam*48, *Ham*50 or *Qam*49 (all three of which were derived from the phage 186*c*I$^+$), it was found that the frequency of recombination across the *att* site was greatly reduced. A similar result was obtained when an attempt was made to cross the allele *c*I*ts* into the phage 186*Ham*56*c*II. This phage, although mutant in the *c*II gene, was derived from wild-type 186 and has a wild-type *c*I gene. It was crossed with the phage 186*Gam*27*c*I*ts* and the frequency of turbid plaques (at 30°C) on C600 determined. The allele *am*27 is not suppressed by the suppressor (*supE*) of C600, and all turbid plaques obtianed will therefore be recombinants, with the *am*27$^+$ allele from 186*Ham*56*c*II and the *c*I*ts* allele from 186*Gam*27*c*I*ts*. The frequency of turbid plaques obtained in this cross was only 0.0018%, very much smaller than the expected frequency across the 186 *att* site.

Both the above results suggest that the presence of a wild-type *c*I allele may inhibit *int* promoted recombination. It is possible that the *int* gene is under repressor control and the presence of repressor (coded for by the *c*I$^+$ gene) may be inhibiting its transcription, thus reducing recombination dependent on the *int* protein. In this respect the results for 186 differ markedly from those obtained for P2. For P2 Lindahl (1969b) found that recombination across the long blank region was actually greater in the presence of the $c^+$ allele. For example the cross *ts*4 x *ts*40 gave a frequency of 0.30% while *ts*4*vir*$_1$ x *ts*40*vir*$_1$ gave a frequency of 0.10% (Lindahl, 1969b). If the reduced *int* promoted recombination for phage 186 in the presence of the *c*I$^+$ allele is due to repressor control of the 186 *int* gene, then the above difference between P2 and 186 would be expected since the P2 *int* gene is not under repressor control (Bertani, 1970).

Although in phage 186 and P2 *int* promoted recombination is much more efficient than general recombination, it is still less efficient than *int* promoted recombination in phage λ. For λ, in the absence of its own recombination system *red* and the major recombination system of the host (the *rec* system), recombination in the region between genes *J* and *c*I (which includes the *att* site) is still 2.0%. This recombination depends on the presence of the *int* gene (Signer and Weil, 1968a). By contrast, with phage 186 and P2, the frequency of *int* promoted recombination is only one tenth to, at most one quarter of the frequency obtained for λ.

General recombination is also much lower in P2 and 186 than in λ. In P2 Lindahl (1969b) found similar recombination frequencies in two-factor crosses whether the bacterial host had an intact or defective recombination system, and concluded that P2 was unable to use the major recombination system of the host. In phage 186, a single two-factor cross in a *recA* strain showed a 20 fold reduction in recombination frequency when compared with that obtained in a $rec^+$ strain. This suggests that two-factor recombination in 186 does depend to a considerable extent on the host *rec* system. However the strains used in this experiment (C600 and QR48) were not isogenic and so further experiments in isogenic strains would be required to confirm this.

If 186 does use the host *rec* system it is at a much reduced efficiency compared with that of λ since general recombination in 186 is about 100 fold less than in λ (even in the absence of the λ *red* and *int* proteins (see Signer and Weil, 1968a)). Similarly, although the presence of the λ *red* product does stimulate 186 recombination two to three fold, the frequencies obtained are still very much lower than the *red* promoted recombination frequencies obtained in phage λ. Perhaps some difference in DNA replication or phage maturation makes the 186 DNA

relatively unavailable to the products of the *rec* and *red* genes.

Lindahl (1969a) showed that the low frequency of recombination in P2 was not due to the inability of more than one type of parental phage to replicate in a cell. He suggested that this low frequency might be due to the separation into "compartments" of the replicating phage genomes in a bacterial cell, thus reducing the chance of an initial recombination event. The high negative interference obtained for both 186 and P2 (Table 5.15 and Lindahl, 1969a) shows that once the initial recombination event has occurred the chance of a second cross-over is quite high. Whatever the explanation for the low recombination frequencies in P2 and 186 it is probable that the mechanism operating is the same for both phage.

The values for *i* obtained for P2 (approximately 7 x $10^3$) are somewhat higher than those obtained for 186 (approximately 1 x $10^3$) but these results are not directly comparable since different regions of the genomes are involved. For P2 Lindahl (1969b) showed that negative interference across the long blank region was about four fold lower than in other regions. The 186 results of Table 5.15 involve mutants on either side of the long blank region but the situation is complicated by the inhibiting effect of the 186 repressor protein present in these crosses. Negative interference in P2 and 186 may not be significantly different.

In two-factor recombination experiments with phage 186, recombination occurred on two different occasions. Firstly, during the initial coinfection in the liquid medium, and secondly, on the Su⁻ indicator plates used to measure the wild-type recombinants formed. Recombination occurring in the liquid medium should lead to a true indication of the recombination frequency since the *moi* was such that every cell should have been infected by at least one of each parental

phage type. On the other hand "recombination on the plate" can not be used to measure recombination frequencies since the *moi* is low and only a minority of infected cells will receive both parental phage types, and so have the potential to give rise to recombinants.

As a method for mapping the 186 genome two-factor crosses proved unsuccessful. Recombination on the plate was a major problem in most of these crosses, but even when conditions which almost eliminated this problem were used there were still too many inconsistencies to allow the construction of a genetic map. Part of the problem in obtaining a consistent map is probably the different levels of suppression obtained for the various mutants on an $Su^+$ host.

For some of the mutants quite a large variation in titre could be obtained by assaying the mutant on various suppressor strains. It appears that both the number and size of plaques depends on the suitability of the amino acid incorporated by the suppressor strain. For example *Jam*41 forms small plaques on *supE* strains, but a larger number of larger plaques on *supF*. Presumably tyrosine is a more suitable amino acid than glutamine at the particular site of the protein coded for by the codon mutant in *Jam*41. *Gam*27 and *Nam*47 both fail to show any plaques (frequency $< 10^{-6}$) on *supE* strains. *Gam*27 grows well on *supF* and *Nam*47 is suppressed best by *supD*. If, on the particular suppressor strain used to measure the total phage output in a two-factor cross, some of the mutants are more efficiently suppressed than others, then this must lead to variations in recombination frequencies which are not dependent on the distance between mutations.

Although the variation in suppression levels must severely affect the reliability of the results of two-factor crosses, it is not the only problem present. For example, mutant *Tam*8 produces very small plaques on C600, the strain used in the two-factor crosses. This

suggests that it is poorly suppressed by this strain and that consequently the number of progeny phage in crosses involving this mutant may be underestimated. This should lead to artifactually high recombination frequencies but, in practice, the recombination frequencies obtained with this mutant appear to be abnormally small.

Of the variations in two-factor recombination methods tried for 186 the most useful appears to be the irradiation of the parental phage with ultraviolet light. After 900 ergs/mm$^2$ of UV irradiation a 36 fold increase in 186 recombination was obtained. This compares unfavourably with the results for P2 in which Lindahl (1969a) obtained a 100 fold increase in recombination after only 400 ergs/mm$^2$. At a dose of 400 ergs/mm$^2$ 186 recombination was only increased about 12 fold. That is, at the same dose, UV irradiation produces an increase in P2 recombination about 8 times greater than that obtained for 186.

The few three-factor crosses described in Section 5.6 were useful in determining the positions of several essential genes. The order obtained in these crosses appeared more likely than that suggested by the results of two-factor crosses. It would be possible to obtain a map of 186 from three-factor crosses but in practice this would be difficult due to the technical problems involved in constructing the necessary double mutants. Due to the low frequency of recombination in 186 the formation of a double mutant would be a rare event and for double amber mutants the absence of a simple screening technique makes the isolation of these mutants impractical. In Section 2 (Materials and Methods) a possible technique for the production of double amber mutants, not involving recombination, is described. However this was not pursued as the marker rescue mapping methods described in Sections 6 and 7 allowed a simple and reliable determination of the 186 gene order.

# SECTION 6

# MARKER RESCUE FROM P2-186 HYBRID PROPHAGE

## 6.1 INTRODUCTION

Although two-factor crosses are often used in the mapping of phage genomes, an alternative method which has proved effective in many genetic systems is deletion mapping. Unfortunately there are no known deletion mutants of 186 lacking essential genes, but there are several P2-186 hybrid phage which can be used instead. These hybrid phage are 186 immune and have the plating characteristics of phage P2, indicating, by reference to the P2 genetic map (Lindahl, 1969a), that they contain the right-hand section of the 186 genome and the left-hand section of the P2 genome. This has been shown to be the case for hybrids Hy2 and Hy5 (Younghusband *et al.*, 1975) and should also be true for the other ten hybrid phage. The hybrid phage were selected as recombinants between phage 186 and P2, appearing at a frequency of about 5 x $10^{-8}$ (Bradley *et al.*, 1975). This is well below the frequency of 186-186 recombination (approximately $10^{-4}$) and so P2-186 recombination should not interfere with the use of the hybrids in marker rescue experiments.

In marker rescue experiments the rescue of a marker, β, was indicated by the appearance of plaques when a 186*am vir* double mutant was plated on an $Su^-$ host lysogenic for one of the hybrid phage. The *vir* mutation in the superinfecting phage was required because the hybrids have the immunity region of phage 186. From this cross plaques could be formed either by complementation, the prophage supplying the product of the gene mutant in the super-infecting phage, or by recombination between the prophage and the superinfecting phage to produce $am^+$, wild-type, recombinants. If marker rescue is the result of recombination then the *eop* of an *am* mutant on a hybrid lysogen should be related to the location of the mutation on the genetic map. However if the appearance of plaques

is due to complementation, then the presence or absence of a gene on the hybrid can still be determined, but no mapping data will be obtained.

Consider the frequency of marker rescue for a 186 *am* mutant from a 186 prophage (see Fig. 6.1a). If marker rescue is due to recombination, then the frequency of recovery of the $\beta^+$ allele from the 186 prophage will be a function of the product of distances b and c-b. That is, the frequency y can be expressed as $y = kb(c-b)$ where k is a constant. This is the equation of a parabola and the recombination frequency should reach a maximum when the mutation, $\beta$, lies equidistant between the left-hand cohesive end and the phage attachment site, that is when $b = c-b$. The assumptions being made in this and the following examples are (1) that the frequency of recombination is proportional to the distance between markers and (2) that the presence of part of the phage attachment site is not affecting the frequency of recombination.

If the prophage in the marker rescue experiment is a P2-186 hybrid phage rather than a 186 phage, the equation describing the frequency of recombination becomes $y = kb(a-b)$ (see Fig. 6.1b).

To be able to compare the marker rescue frequencies for two different amber mutants, the number of plaques appearing on a particular lysogen must be related to the number of phage added. For amber mutants an estimate of this last figure can be obtained by determining their titre on an $Su^+$ strain, but such an estimate of phage numbers is unreliable since an $Su^+$ strain can show different levels of suppression for different amber mutants. A better way to express marker rescue from a hybrid prophage is to divide it by the marker rescue frequency for the same amber mutant from a 186 prophage. This provides an internal control and eliminates the necessity of relying on a phage titre as determined on $Su^+$. Expressed in this way the

*Figure 6.1* RECOMBINATION BETWEEN AN *am* MUTANT SUPERINFECTING PHAGE AND VARIOUS PROPHAGE

(a) Recombination between a 186*am*β*vir* superinfecting phage and a 186 prophage.

(b) Recombination between a 186*am*β*vir* superinfecting phage and a P2-186 hybrid prophage.

(c) Recombination between a 186*am*β superinfecting phage and a λp186 prophage.

(d) Relationship between the relative marker rescue frequency (y) and the distance (x) between a β mutation and various hybrid cross-over points.

(e) Relationship between the relative marker rescue frequency (y) and the distance (x) between a hybrid cross-over point and various β mutations.

*vir* a virulent mutation in the superinfecting phage

β an amber mutation in an essential gene of the super-infecting phage

α a P2-186 hybrid cross-over point (Figure 6.1b)

α,δ λ-186 junctions in the λp186 phage (Figure 6.1c)

a distance of α from the 186 *att* site

b distance of β from the 186 *att* site

c distance of the left-hand cohesive end from the 186 *att* site

d distance of δ from the 186 *att* site

x distance between the hybrid end point and the β mutation (i.e. x = a-b)

y relative marker rescue frequency (i.e. marker rescue from a P2-186 hybrid prophage expressed as a fraction of marker rescue from a 186 prophage)

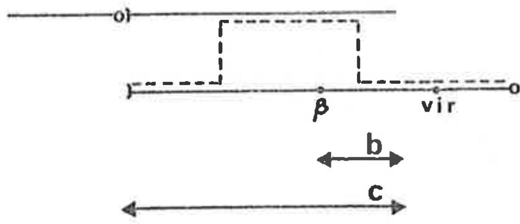
—o)— a prophage

)—— a superinfecting phage

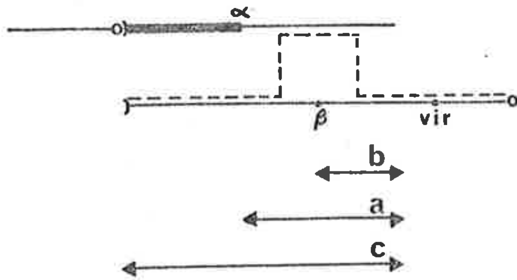
- - - - an $am^+$ recombinant

▬▬ non-186 DNA

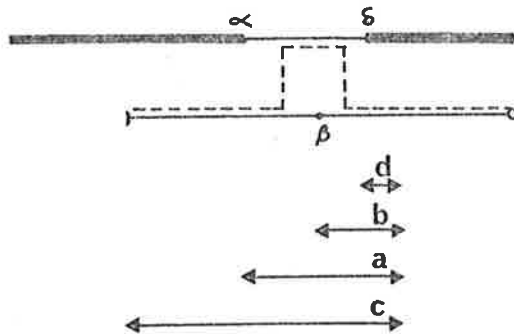
(a)



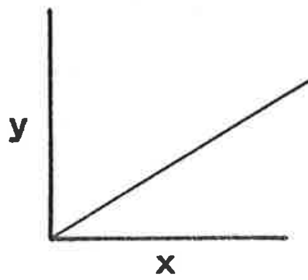

(b)



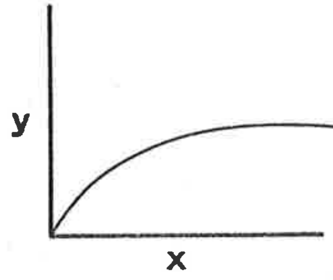

(c)




(d)




(e)



marker rescue frequency (y) from a hybrid lysogen becomes:

$$y = \frac{kb(a-b)}{kb(c-b)} = \frac{a-b}{c-b}$$

The frequency described by this equation will be referred to as the relative marker rescue frequency (or relative *mrf*).

Consider an experiment involving several different hybrid prophage but only one superinfecting phage mutant. In this case both b and c are constants and y is directly proportional to a-b, which equals x, the distance between the hybrid cross-over point and the amber mutation (see Fig. 6.1d).

Consider an experiment involving only one hybrid prophage but several different 186 superinfecting mutants. In this case a and c are constants and b is variable. The equation $y = \frac{a-b}{c-b}$ can be rewritten as $y = \frac{x}{x-a+c}$ (where x = a-b), and this is the equation of a rectangular hyperbola with centre (a-c, 1) and asymptotes y = 1 and x = a-c. The section of the hyperbola relevant to the marker rescue experiments is shown in Fig. 6.1e.

In conclusion, if one mutant phage is used as the superinfecting phage in marker rescue experiments with several different hybrid prophage, then the marker rescue frequency should increase linearly with the distance of the mutation from the hybrid cross-over point. If several different mutant superinfecting phage are used but only the one hybrid prophage, then once again the marker rescue frequency should increase with the distance of the mutation from the hybrid cross-over point. However in this case the increase is not linear, and resolution will be poor when the distance between the $\alpha$ and $\beta$ positions is large.

The final situation is that encountered for marker rescue experiments involving the λp186 transducing phage described in

section 8. In this case marker rescue from the λp186 phage (y=k(a-b)(b-d)) shares no common factor with marker rescue from a 186 prophage (y=kb(c-b)) (see Fig. 6.1c). It is therefore necessary to express it, not as a percent of marker rescue from a 186 lysogen, but as a percent of the number of phage present as measured on an $Su^+$ strain. However this means that the marker rescue frequencies for different mutants cannot be compared since, due to different levels of suppression, the titre measured on $Su^+$ is not a reliable estimate of the number of phage present. Therefore only the presence or absence of a particular allele on the 186 DNA of the λp186 phage can be determined.

## 6.2 186 MARKER RESCUE FROM P2-186 HYBRID PROPHAGE

Table 6.1 records the results of plating *vir* derivatives of eighteen 186 amber mutants on $Su^-$ lysogens of the twelve P2-186 hybrid phage, and on an $Su^-$ nonlysogen. It also records, in the final column, the frequency at which the wild-type alleles of the amber mutants can be recovered from a $186^+$ prophage.* The results in this column show that the wild-type alleles of *Bam*17 and *Bam*57 were recovered from a $186^+$ lysogen at a very high frequency (10 to 20%) compared with the recovery of *am* alleles for all the other mutants (0.1 to 0.3%). This suggests that there is a different mechanism acting for gene *B* alleles.

This difference is again seen when marker rescue, from the hybrid lysogens, of gene *B* mutants and other mutants are compared.

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* Since the hybrids are derivatives of 186*c*I*ts* rather than of the wild-type $186c^+$, some of the tests were repeated using a lysogen of 186*c*I*ts* in the control. This had no effect on the frequency of marker rescue.

TABLE 6.1

186 MARKER RESCUE FROM THE PROPHAGE OF TWELVE P2-186 HYBRID PHAGE[a]

| Super-infecting phage[b] | Prophage[c] | | | | | | | | | | | | | %*mrf*[d] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Hy10 | Hy9 | Hy11 | Hy1 | Hy2 | Hy3 | Hy4 | Hy12 | Hy8 | Hy5 | Hy6 | Hy7 | None | |
| *Bam*17 | 100 | 98 | 87 | 120 | 110 | 120 | 100 | 110 | 100 | 96 | 95 | 100 | 0.0012 | 19 |
| *Bam*57 | 130 | 140 | 100 | 180 | 150 | 130 | 120 | 96 | 150 | 120 | 100 | 130 | 0.0005 | 13 |
| *Dam*14 | 4.1 | 3.6 | 2.7 | 0.66 | 0.71 | 0.76 | 0.83 | 0.65 | 0.26 | 0.20 | 0.21 | 0.18 | 0.0029 | 0.22 |
| *Dam*23 | 4.6 | 4.6 | 2.8 | 0.65 | 0.65 | 0.70 | 0.74 | 0.71 | 0.35 | 0.20 | 0.21 | 0.19 | 0.0028 | 0.17 |
| *Dam*26 | 2.7 | 2.7 | 1.3 | 0.26 | 0.27 | 0.27 | 0.18 | 0.17 | 0.078 | 0.068 | 0.064 | 0.061 | <0.0017 | 0.23 |
| *Dam*48 | 5.2 | 4.4 | 2.4 | 0.33 | 0.43 | 0.37 | 0.39 | 0.33 | 0.095 | 0.090 | 0.089 | 0.073 | 0.0026 | 0.21 |
| *Eam*35 | 2.8 | 2.3 | 1.2 | 0.20 | 0.18 | 0.19 | 0.21 | 0.20 | 0.0089 | 0.0022 | 0.0041 | 0.0038 | 0.0010 | 0.15 |
| *Eam*46 | 5.2 | 4.6 | 3.5 | 0.076 | 0.049 | 0.084 | 0.068 | 0.0021 | 0.0006 | 0.0002 | 0.0005 | 0.0002 | 0.0015 | 0.24 |
| *Eam*7 | 2.8 | 2.7 | 1.4 | <0.0015 | <0.0015 | 0.0015 | 0.0015 | 0.0015 | <0.0015 | <0.0015 | 0.0015 | <0.0015 | 0.0015 | 0.20 |
| *Fam*20 | 3.0 | 2.2 | 1.1 | <0.0023 | <0.0023 | <0.0023 | <0.0023 | 0.0046 | <0.0023 | <0.0023 | <0.0023 | 0.0023 | 0.0023 | 0.15 |
| *Gam*9 | 5.5 | 5.0 | 2.0 | 0.0018 | 0.0018 | <0.0018 | 0.0035 | <0.0018 | <0.0018 | <0.0018 | 0.0035 | 0.0018 | 0.0035 | 0.10 |
| *Gam*27 | 2.0 | 1.4 | 0.63 | 0.0073 | <0.0037 | <0.0037 | <0.0037 | 0.0037 | <0.0037 | 0.0037 | 0.0037 | 0.0073 | 0.0037 | 0.11 |
| *Gam*25 | 2.2 | 2.5 | 0.24 | <0.0005 | 0.0010 | 0.0010 | 0.0010 | 0.0020 | 0.0015 | 0.0020 | 0.0015 | 0.0020 | 0.0064 | 0.078 |
| *Gam*29 | 1.4 | 1.4 | 0.076 | <0.0024 | 0.0024 | <0.0024 | <0.0024 | <0.0024 | <0.0024 | <0.0024 | <0.0024 | <0.0024 | 0.0024 | 0.12 |
| *Ham*50 | 3.9 | 3.4 | 0.0012 | 0.0012 | 0.0012 | 0.0017 | 0.0012 | 0.0002 | 0.0014 | 0.0009 | 0.0012 | 0.0017 | 0.0024 | 0.30 |
| *Ham*56 | 0.80 | 0.65 | 0.0018 | 0.0010 | 0.0007 | 0.0010 | 0.0010 | 0.0009 | 0.0008 | 0.0006 | 0.0010 | 0.0007 | 0.0025 | 0.30 |
| *Iam*40a | 2.8 | 2.6 | <0.0061 | <0.0061 | <0.0061 | 0.0061 | <0.0061 | <0.0061 | <0.0061 | <0.0061 | <0.0061 | <0.0061 | <0.0061 | 0.080 |
| *Jam*41 | 0.82 | 0.31 | 0.0023 | <0.0023 | <0.0023 | 0.0023 | <0.0023 | 0.0023 | <0.0023 | 0.0023 | 0.0023 | <0.0023 | 0.0094 | 0.19 |

[a] The figures given are the number of plaques produced when an *am vir* double mutant was assayed on one of the hybrid lysogens (or the non-lysogen) expressed as a percent of the number of plaques produced when the same *am vir* mutant was assayed on a $186^+$ lysogen.

[b] All mutants also contain the *vir*2 mutation.

[c] All bacterial strains used are derived from lysogenisation of strain 594.

[d] The figures given in this column are the number of plaques obtained on an $Su^-(186^+)$ lysogen (E699) expressed as a percent of the number of plaques obtained on an $Su^+$ strain (strain H12R8A for *Gam*27 and strain C600 for all other mutants).

*Am*17 and *am*57 are recovered from the hybrid prophage at 100% of the efficiency of their recovery from a 186$^+$ prophage, whereas the values obtained for the other mutants are much lower (less than 6% even from the hybrid prophage with the longest section of the 186 genome).

These results could be explained if marker rescue was due to complementation for the alleles of gene *B*, and recombination for the alleles of all other genes. This would also explain why there is no significant difference between the levels of recovery of the *B* alleles from the different hybrid prophage. By contrast, the level of recovery for all other mutants varies depending on which hybrid prophage is used.

From the results shown in Table 6.1, it appears that the wild-type alleles of the two known mutants in gene *B* (*am*17 and *am*57) and the wild-type alleles of all known amber mutants in gene *D*, are each present on all twelve hybrid phage. The section of 186 DNA in each of the twelve hybrid phage must therefore extend to the left of the 186 genes *B* and *D*.

The allele 35$^+$ of gene *E* was recovered from hybrids 10, 9, 11, 1, 2, 3, 4 and 12. The values for its recovery from hybrids 5, 6, 7 and 8 were very low, but marginally greater than the recovery from the nonlysogen. Allele 35$^+$ is therefore probably present on all hybrids. Allele 46$^+$ of gene *E* was recovered from hybrids 10, 9, 11, 1, 2, 3 and 4 but not from 5, 6, 7 or 8. The figure for hybrid 12 was only marginally greater than the control figure and it is uncertain whether allele 46 is on hybrid 12. The other allele of gene *E*, *am*7$^+$, was recovered only from hybrids 10, 9 and 11. Therefore the order of the alleles of gene *E*, from left to right on the genetic map, must be 7-46-35.

Since *Eam*35$^+$ is present on all hybrids whereas *Eam*7$^+$ could only be recovered from hybrids 9, 10 and 11, the three groups

of hybrids (1, 2, 3, 4), (12) and (5, 6, 7, 8) must all cross-over within gene *E*. Since every *B* and *D* allele could be recovered from all hybrids these two genes must lie to the right of gene *E*. Genes *F*, *G*, *H*, *I* and *J*, none of whose alleles are present on hybrids 1 to 8 or 12, must lie to the left of gene *E*.

The *F* allele $20^+$ and *G* alleles $9^+$, $27^+$, $25^+$ and $29^+$ could all be recovered from hybrids 9, 10 and 11, whereas the *H* alleles $50^+$ and $56^+$, the *I* allele $40a^+$ and the *J* allele $41^+$ could only be recovered from hybrids 9 and 10. Genes *F* and *G* must therefore lie to the right of genes *H*, *I* and *J*, and hybrid 11 must cross-over between these two groups of genes. Hybrids 9 and 10 must both cross-over to the left of genes *H*, *I* and *J*. The information derived above is summarised in Fig. 6.2.

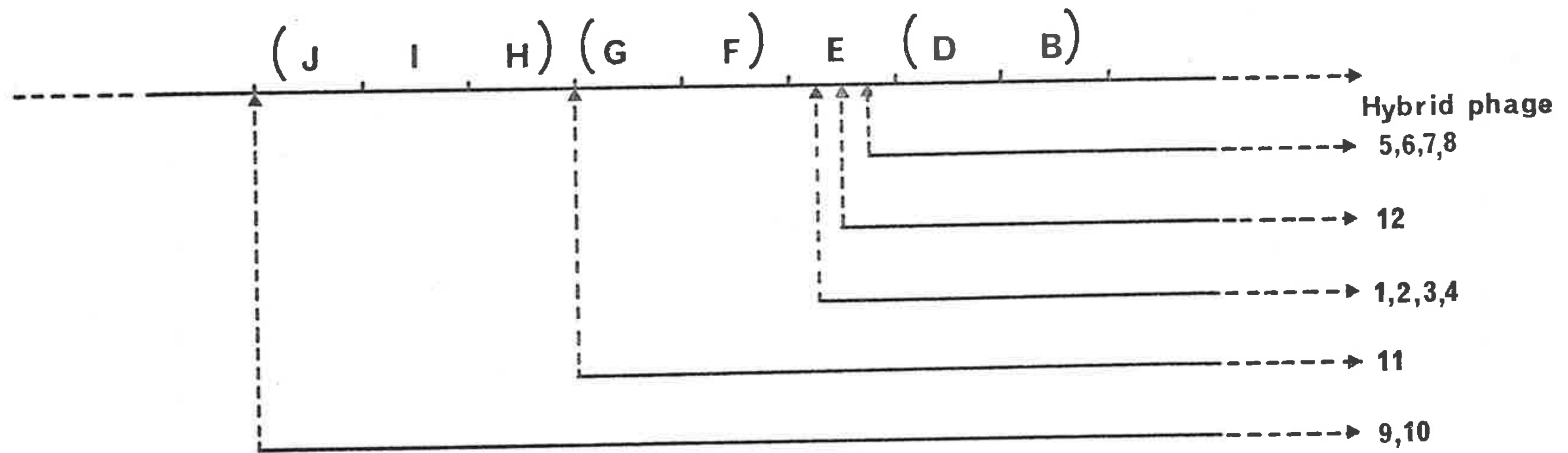
Mutants from several other genes were also tested for marker rescue from the hybrid prophage. For mutants *Mam*19, *Nam*47, *Oam*61, *Pam*16 and *Wam*15 no marker rescue could be demonstrated from lysogens of any of the 12 hybrid phage (data not shown). Mutants *Kam*42, *Kam*22, *Kam*58, *Lam*2, *Lam*21, *Mam*31 and *Mam*60 were tested for marker rescue from a Hy10 prophage but once again no marker rescue could be demonstrated (data not shown).

Although all alleles of genes *A* to *J* are present on both hybrids 9 and 10, the frequency of recovery of a particular mutant was almost always greater on hybrid 10 than on hybrid 9. The exceptions to this were the mutants 23, 26 and 29, for which the frequency of recovery from both Hy9 and Hy10 was the same, and mutant 25 for which recovery from Hy9 was actually greater than from Hy10.

In Table 6.1 the results shown for a particular amber mutant were all obtained on the same day. This allows a more

*Figure 6.2* PRELIMINARY ORDER FOR SEVERAL GROUPS OF 186 GENES AS DETERMINED FROM RESULTS RECORDED IN TABLE 6.1

This figure shows the relative locations of four groups of genes. Genes within brackets are not ordered with respect to each other. The upper horizontal bar represents a section of the 186 genome. The lower horizontal bars represent the 186 portions of the various P2-186 hybrid phage. The vertical broken lines indicate the lcoations of the hybrid cross-over points.



J
I
H
G
F
E
D
B
Hybrid phage
5,6,7,8
12
1,2,3,4
11
9,10

reliable comparison of the frequencies of recovery from the different hybrid prophage, since the same value for the recovery from the 186 prophage is used in all calculations. However most of the tests have been repeated giving the same qualitative results. The results for some of these repeat experiments with hybrids 9, 10 and 11 are shown in Table 6.2.

Again results for a particular mutant were obtained on the same day and again it can be seen that in almost all cases (including those of 23, 26, 29 and 25 this time) the marker rescue frequency on hybrid 10 was greater than that on hybrid 9. The only exception is now mutant 40a. These results therefore strongly suggest that hybrid 10 has a greater proportion of the 186 genome than has hybrid 9.

Similarly, the recovery figures for hybrid 11 were invariably lower than for hybrids 9 and 10. The length difference between hybrids 9 and 11 is probably greater than that between 9 and 10.

In Table 6.1 a further drop in the frequency of recovery can be seen for hybrids 1, 2, 3, 4 and 12. The recovery figures for hybrids 1, 2, 3 and 4 do not vary significantly and the lengths of 186 DNA in these hybrids must be very close and possibly identical. Hybrids 1, 2, 3 and 4 were all isolated (together with hybrid 5) from the same recombination experiment. It is quite possible therefore, that 1, 2, 3 and 4 represent re-isolates of the same hybrid. The results of Table 6.1 provide no evidence to suggest that these hybrids are not identical. The recovery frequency of *Eam*46$^+$ from hybrid 12 compared with its recovery frequency from hybrids 1, 2, 3 and 4, suggests that hybrid 12 has less 186 DNA than do hybrids 1, 2, 3 and 4.

Considering the results obtained with mutants 14, 23, 26

TABLE 6.2

186 MARKER RESCUE FROM THE PROPHAGE OF Hy9, Hy10 AND Hy11[a]

| Superinfecting phage[b] | Prophage[c] | | | |
|---|---|---|---|---|
| | Hy10 | Hy9 | Hy11 | None |
| *Dam*23 | 4.5 | 4.2 | | 0.21[d] |
| *Dam*26 | 4.2 | 3.5 | 1.7 | 0.0032 |
| *Dam*48 | 3.1 | 2.1 | | < 0.0019 |
| *Eam*35 | 2.2 | 2.0 | 0.98 | 0.0014 |
| *Eam*46 | 4.8 | 3.6 | 3.3 | 0.0037 |
| *Eam*7 | 3.3 | 2.5 | 1.8 | < 0.0015 |
| *Fam*20 | 5.7 | 4.0 | 1.7 | < 0.0046 |
| *Gam*27 | 2.9 | 2.7 | 1.5 | 0.0056 |
| *Gam*25 | 2.8 | 2.2 | | < 0.016 |
| *Gam*29 | 0.99 | 0.83 | | < 0.018 |
| *Ham*50 | 1.6 | 1.4 | | 0.0025 |
| *Iam*40a | 3.9 | 4.4 | 0.0011 | 0.0046 |
| *Jam*41 | 1.0 | 0.35 | 0.0023 | 0.016 |

*a* For an explanation of the figures given see Table 6.1.

*b* All mutants also contain the *vir*2 mutation.

*c* All bacterial strains used are derived from lysogenisation of strain 594.

*d* The stock of *Dam*23*vir*2 used in this experiment contained a large number of revertants.

and 35, a further drop in recovery was observed with hybrids 5, 6, 7 and 8 when compared with the frequencies obtained for hybrids 1, 2, 3, 4 and 12. Hybrids 5, 6, 7 and 8 were all independently isolated. The results might suggest that hybrid 8 is marginally longer and hybrid 7 marginally shorter than hybrids 5 or 6. However the figures are too close to be confident of this.

It is apparent from the recovery figures on the nonlysogen (594) that this strain is not the best control. Where the figures on the hybrid lysogens are much greater than on the nonlysogen there is no doubt about the presence of the allele on the hybrid. However with mutant 35 the question arises of whether or not the allele is present on the hybrid. Most of the negative results given are shown as less than or equal to a low figure. This usually means that recovery was too low to be measured, or was measured by the presence of only a few plaques. However in a case such as *Eam*46 the number of plaques on the control (594) was significantly greater than the number of plaques on lysogens of hybrids 5, 6, 7 and 8, on which the wild-type allele of *am*46 is not present. This is probably due to the fact that 186*vir* plates less well on a 186 lysogen than on a nonlysogen (the *eop* is usually down by 50%). A better control would therefore be to use a 594 lysogen of the mutant being tested rather than 594 itself. For example, to determine whether the allele $35^+$ is on hybrids 5, 6, 7 and 8, the recovery frequency should be compared with that for 594 lysogenic for *am*35 and not with that for 594 itself. The 594 must be lysogenic for *am*35 and not for $186^+$ in order to avoid marker rescue.

Repeat experiments for mutants 35 and 46 on some of the hybrids, incorporating this better control, are recorded in Table 6.3. Since marker rescue is so very low with these particular mutants and hybrids, it was necessary to score several plates for recombinants

TABLE 6.3

186 MARKER RESCUE WITH MUTANTS *Eam*35 AND *Eam*46[a]

| Prophage[b] | Superinfecting phage[c] | | | |
|---|---|---|---|---|
| | *Eam*46 | *Eam*46 | *Eam*35 | *Eam*35 |
| Hy1 | 0.032 | | | |
| Hy2 | 0.032 | | | |
| Hy3 | 0.034 | | | |
| Hy4 | 0.036 | | | |
| Hy5 | 0.0009 | | 0.0031 | 0.0045 |
| Hy6 | 0.0012 | | 0.0052 | 0.0047 |
| Hy7 | 0.0009 | | 0.0034 | 0.0044 |
| Hy8 | 0.0012 | | 0.0085 | 0.0096 |
| Hy12 | 0.0019 | 0.0022 | 0.082 | |
| 186*Eam*46 | 0.0009 | 0.0010 | | |
| 186*Eam*35 | | | 0.0017 | 0.0017 |
| None | 0.0034 | 0.0017 | 0.0041 | 0.0061 |

*a* For an explanation of the figures given see Table 6.1.

*b* All bacterial strains used are derived from lysogenisation of strain 594.

*c* Both mutants also contain the *vir*2 mutation.

in order to obtain a significant number of plaques. The marker rescue frequencies for *am*35 on hybrids 5, 6 and 7 are now similar to or somewhat less than the reversion frequency measured on 594. However marker rescue frequencies on all four hybrids (5, 6, 7 and 8) are significantly higher than on the control lysogen, 594(*Eam*35). This suggests that the wild-type allele of *am*35 is present on all four hybrids. The frequencies obtained on hybrids 5, 6 and 7 do not appear to differ significantly but the frequency on hybrid 8 is elevated, suggesting that hybrid 8 has a longer portion of the 186 genome than do hybrids 5, 6 or 7.

The wild-type allele of *Eam*46 can clearly be rescued from hybrids 1, 2, 3 and 4. The recovery frequencies for hybrids 5, 6, 7 and 8 are similar to that for 594(*Eam*46) suggesting that the allele $46^+$ is not on this set of hybrids. For hybrid 12 recovery is lower than for 594 but about twofold greater than for the control 594(*Eam*46). This suggests that allele $46^+$ is present on hybrid 12 and that hybrid 12 contains a portion of the 186 genome intermediate in length between those of the two groups of hybrids (1, 2, 3, 4) and (5, 6, 7, 8).

To date, all the results discussed have been derived from considering the frequencies obtained with a particular amber mutant and several different hybrid prophage. However, more mapping information can be obtained by considering the relative marker rescue frequencies of different amber mutants from a single hybrid prophage. In this case the resolution in the relative *mrfs* obtained for different mutants should be much worse, the further the mutations are located from the hybrid cross-over points (see Fig. 6.1e). The "longer" hybrids, 9, 10 and 11 are on the whole of no use in mapping the alleles by this method, but it appears that some mapping data can be obtained from the "shorter" hybrids.

Considering the results for the alleles of gene *D*, recorded in Table 6.1, it can be seen that for the hybrids 1, 2, 3, 4, 5, 6, 7, 8 and 12 the recovery frequency for the alleles *am*14 and *am*23 is invariably greater than for the alleles *am*26 and *am*48. This suggests that mutants 14 and 23 lie further to the right than mutants 26 and 48. No significant difference for the alleles 14 and 23 is observable. Allele $48^+$ does appear to be recoverable at a slightly higher frequency than $26^+$ but this is probably not significant since recombination experiments suggest that these two alleles are probably identical.

The order of genes *B* and *D* can not be determined by this method since the recovery frequencies for the *B* alleles are not dependent on the distance between the mutation and the hybrid cross-over point. However gene *D* belongs to the same polarity group as genes *E*, *F* and *G* while gene *B* does not. This suggests that gene *D* lies to the left of gene *B*.

As discussed previously the presence or absence of the gene *E* alleles, 7, 35 and 46, on the various hybrids suggested the order 7 - 46 - 35 for these alleles. In agreement with this order is the observation that $35^+$ can be recovered at a greater frequency from hybrids 1, 2, 3, 4 and 12 than can $46^+$.

The marker rescue frequencies obtained for *Fam*20 and *Gam*9 from hybrid 11 are too large to be useful in mapping. However a much lower frequency of recovery was obtained for the *G* allele *am*29. This suggests that gene *G* lies closer to the Hy11 cross-over point than does gene *F*, that is, gene *G* must lie to the left of gene *F*. The relative frequencies of recovery for the other *G* alleles from hybrid 11 suggest that the order of alleles from left to right within this gene is 29 - 25 - 27 - 9.

Marker rescue frequencies for the alleles of genes *H*, *I* and *J* on hybrids 9 and 10 are anomalous. Alleles 50 and 56 of gene *H* show very different frequencies and *Iam*40a gives a frequency intermediate between those of the two *H* alleles. Perhaps the most likely order for these alleles is, left to right, *Jam*41 - *Ham*56 - *Ham*50 - *Iam*40a.

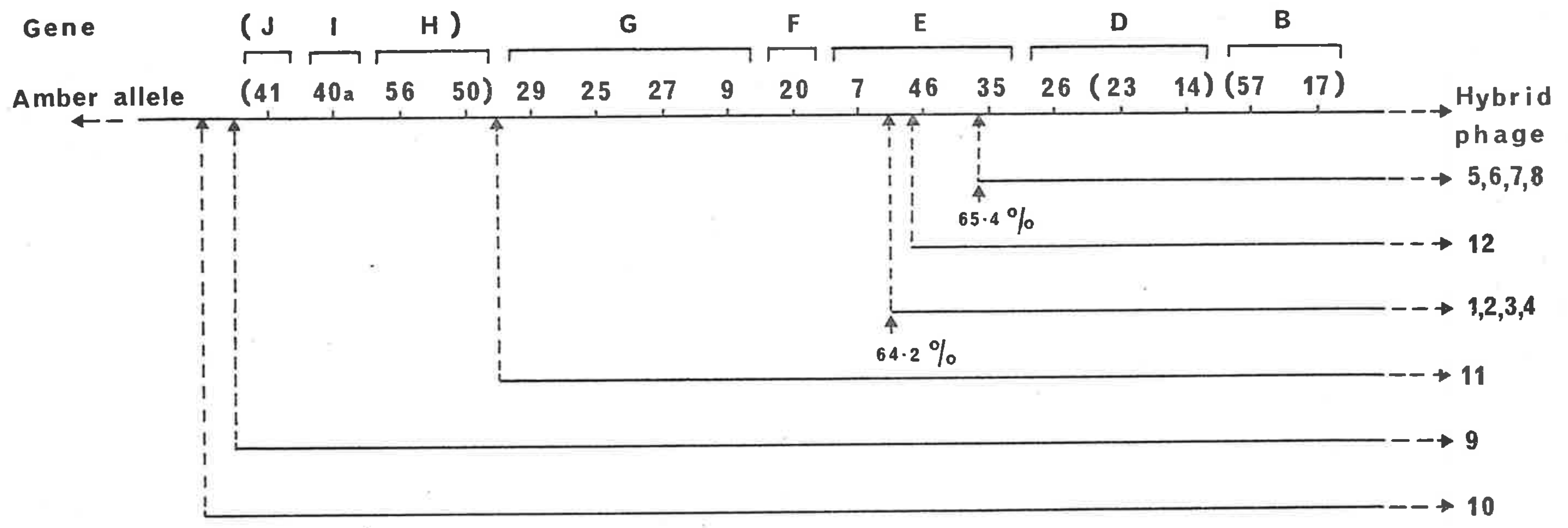
Figure 6.3 summarises the information on the genetic map of 186 obtained by marker rescue experiments with the twelve P2-186 hybrid phage.

## 6.3 P2 MARKER RESCUE FROM P2-186 HYBRID PROPHAGE

To characterise the hybrids further, the contribution of the P2 genome to each of them was determined by marker rescue experiments involving one amber allele in each of six P2 genes. The P2 mutants used are derivatives of P2$vir_1$, but unlike the 186*vir* mutants they are immunity sensitive. This does not affect the detection of marker rescue as the hybrids have the immunity region of 186, not that of P2, but it does mean that the control involving recovery from a $P2^+$ lysogen cannot be done. The results of marker rescue with P2 cannot therefore be expressed in the same manner as were those with 186. The results, recorded in Table 6.4, are expressed directly as the titre (in *pfu*/ml) obtained on each of the hybrid lysogens, and this is dependent on the number of phage added. For example, the fact that $amJ_{214}$ gave $49 \times 10^5$ *pfu* on a lysogen of hybrid 1 and $amF_4$ only $7.8 \times 10^5$, does not necessarily mean that *J* was recovered at a greater frequency but rather that the phage stock of *J* used could have had a higher titre. The results for different mutants are therefore not comparable. The results for a particular mutant on the different hybrid lysogens are comparable as they were obtained on the same day

*Figure 6.3* GENE AND ALLELE ORDER FOR A SECTION OF THE 186 GENETIC MAP AS DETERMINED BY MARKER RESCUE EXPERIMENTS WITH TWELVE P2-186 HYBRID PHAGE

This figure shows the relative locations of 186 genes and alleles, but it is not drawn to scale and does not show either genetic or physical distances. The figure also shows which 186 genes and alleles are present on the 186 sections of the hybrid genomes. The upper horizontal bar represents a section of the 186 genome. The lower horizontal bars represent the 186 portions of the various P2-186 hybrid phage. The vertical broken lines indicate the locations of the hybrid cross-over points. The cross-over points for Hy2 and Hy5 are located 64.2% and 65.4% respectively from the left end of the 186 genome (Younghusband *et al.* 1975). The physical locations of the other hybrid cross-over points are unknown. The relative locations of the genes and alleles in brackets could not be determined.



Gene
(J
I
H)
G
F
E
D
B
Amber allele
(41
40a
56
50)
29
25
27
9
20
7
46
35
26
(23
14)
(57
17)
Hybrid phage
5,6,7,8
65·4 %
12
1,2,3,4
64·2 %
11
9
10

TABLE 6.4

P2 MARKER RESCUE FROM THE PROPHAGE OF TWELVE P2-186 HYBRID PHAGE[a]

| Prophage[b] | Superinfecting P2 phage | | | | | |
|---|---|---|---|---|---|---|
| | *J* *am*214 | *F* *am*4 | *E* *am*30 | *T* *am*5 | *U* *am*25 | *D* *am*6 |
| Hy1 | 49 | 7.8 | 26 | 1.2 | 11 | 6.2 |
| Hy2 | 48 | 6.6 | 21 | 1.6 | 13 | 6.8 |
| Hy3 | 44 | 6.2 | 18 | 0.5 | 10 | 5.7 |
| Hy4 | 51 | 6.8 | 24 | 0.8 | 13 | 7.1 |
| Hy5 | 36 | 3.6 | 17 | 0.48 | 5.7 | 0.055 |
| Hy6 | 16 | 1.8 | 6.0 | 0.14 | 1.9 | 0.043 |
| Hy7 | 15 | 2.1 | 7.5 | 0.45 | 2.8 | 0.11 |
| Hy8 | 16 | 1.7 | 5.4 | 0.30 | 1.6 | 0.049 |
| Hy12 | 12 | 2.0 | 4.8 | 0.28 | 1.8 | 0.017 |
| Hy11 | 14 | 2.8 | 0.32 | < 0.001 | 0.001 | 0.003 |
| Hy9 | 9.3 | 0.20 | 0.002 | < 0.001 | 0.004 | 0.002 |
| Hy10 | 8.5 | 0.20 | 0.001 | < 0.001 | 0.002 | 0.006 |
| 186 | 0.001 | < 0.001 | 0.002 | < 0.001 | 0.001 | 0.002 |

[a] The figures given are *pfu*/ml x $10^{-5}$.

[b] All bacterial strains used are derived from lysogenisation of strain 594.

using the same phage stock. The relative number of plaques on the different hybrid lysogens should reflect the frequency of recovery.

Allele 214 of gene *J* was recoverable from all the hybrids. Therefore the P2 portion of all the hybrids must extend to the right of this allele. The frequency of recovery from hybrids 1, 2, 3, 4 and 5 was greater than that for the other hybrids, suggesting that these five hybrids contain more P2 DNA than do the others.

Allele 4 of gene *F* was also recoverable from all the hybrids. Again hybrids 1, 2, 3 and 4 gave the largest frequencies. Hy5 gave an intermediate frequency and the others a lower frequency. Of the other hybrids, Hy9 and Hy10 gave a very low frequency and should therefore contain the least amount of P2. From these results the hybrids can be arranged in the following order, corresponding to the length of the P2 genome that they contain:

Hy9, Hy10<Hy6, Hy7, Hy8, Hy11, Hy12<Hy5<Hy1, Hy2, Hy3, Hy4.

With $amE_{30}$ no recovery was observed with hybrids 9 or 10 but recovery occurred with all the other hybrids. This result confirms the fact that 9 and 10 contain the shortest length of the P2 genome, and suggests that the cross-over points for these two hybrids lie to the left of the mutation $amE_{30}$. Recovery from hybrids 1, 2, 3, 4 and 5 was greater than from hybrids 6, 7, 8, 11 and 12. Of this last group the value obtained from hybrid 11 was very much lower than that obtained for the others, suggesting that, with the exception of hybrids 9 and 10, hybrid 11 contains the least P2 DNA.

Allele 25 of gene *U* was not recoverable from any of the hybrids 11, 9 or 10 but could be recovered from all the other hybrids. This result confirms that Hy11 has less P2 DNA than do hybrids 1 to 8 and 12, as was suggested by the results of marker rescue with the mutant $amE_{30}$. It also shows that the P2 cross-over point in hybrid

11 lies to the left of the mutation *am*$U_{25}$. Recovery values were greater for hybrids 1, 2, 3 and 4 than for hybrid 5 which was greater than for hybrids 6, 7, 8 and 12.

The wild-type allele of *am*$D_6$ is clearly on hybrids 1, 2, 3 and 4 and clearly not on hybrids 9, 10 and 11. With hybrids 5, 6, 7 and 8 a low, but consistently above background, level of recovery was obtained. It appears that allele $D_6$ is present on these hybrids. A very low frequency was obtained with Hy12 but it is hard to say whether this represents extremely low recovery or not.

The mutant *am*$T_5$ was also used in these experiments, but the plaques obtained were very faint and hard to count. The results therefore are somewhat doubtful. However the wild-type allele of *am*$T_5$ could definitely be recovered from hybrids 1 to 8 and 12. It appeared not to be present on hybrids 9, 10 and 11.

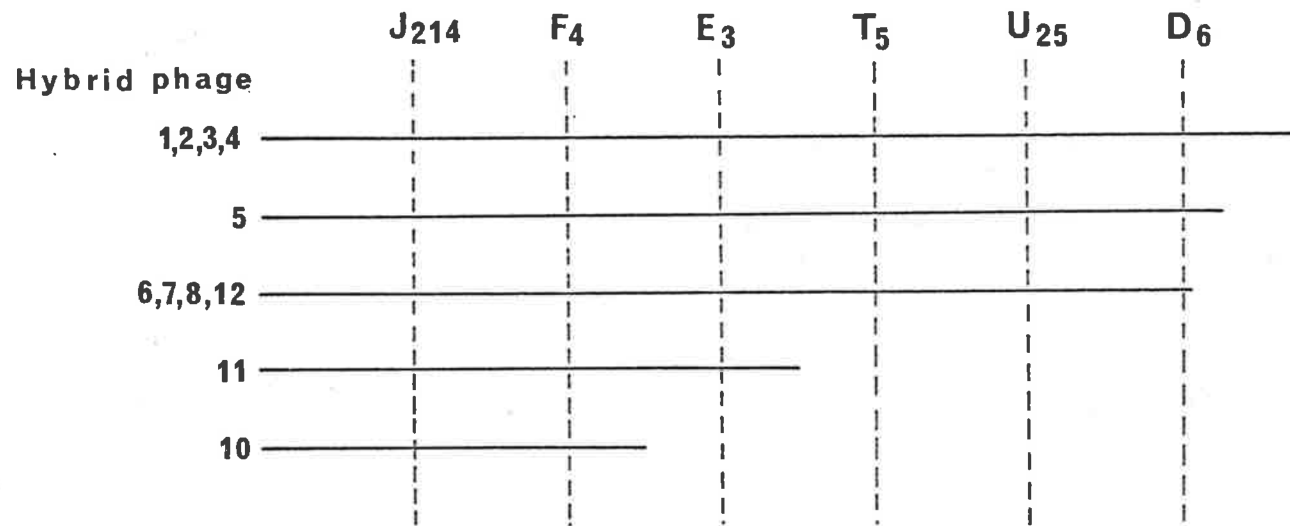
The location, on the P2 genetic map, of the cross-over points of the various hybrids, suggested by the above results, are shown in Figure 6.4.

Several anomalies appear in the results recorded in Table 6.4. Firstly, in view of the fact that the P2 portion of Hy11 is shorter than that of all the other hybrids (except hybrids 9 and 10), the frequency of recovery of *am*$F_4$ should be less on this hybrid than on hybrids 6, 7, 8 and 12. In this case it was not. However in a repeat experiment it did show a value ($1.5x10^5$) intermediate between those for hybrids 6, 7, 8 and 12 (2.3, 2.2, 2.3, $2.0x10^5$) and those for hybrids 9 and 10 (0.15, $0.25x10^5$).

Secondly, for mutants of genes *J*, *F*, *E* and *U*, hybrid 5 appeared to have a longer section of the P2 genome than did hybrids 6, 7, 8 and 12. However when *am*$D_6$ was used Hy5 appeared to have a P2 length similar to those of hybrids 6, 7, 8 and 12. This was

*Figure 6.4* CONTRIBUTION BY P2 TO THE GENOMES OF THE P2-186 HYBRID PHAGE AS DETERMINED FROM THE RESULTS RECORDED IN TABLE 6.4

This figure records the presence or absence of the wild-type alleles of six P2 *am* mutants on the P2 sections of the hybrid genomes. The horizontal bars represent the portions of the P2 genome and the vertical broken lines indicate the relative locations of the P2 mutants. The figure is not drawn to scale and does not show either genetic or physical distances.



J214
F4
E3
T5
U25
D6
Hybrid phage
1,2,3,4
5
6,7,8,12
11
10

unexpected since generally resolution improves as the phage mutation approaches the hybrid cross-over point. A repeat experiment again showed recovery frequencies similar to those for hybrids 6, 7, 8 and 12. The reason for this is not known.

With *am* mutants in genes *E*, *U* and *D*, Hy7 showed higher recovery frequencies than did hybrids 6, 8 and 12. A repeat experiment gave the same result. This may suggest that the P2 portion of Hy7 is marginally longer than that of the other hybrids. However in view of the anomaly observed with Hy5 this difference may not be significant.

From this work the hybrids can be arranged in an ascending order, corresponding to the length of the P2 genome that they contain. The order is:

Hy9, Hy10<Hy11<Hy6, Hy7, Hy8, Hy12<Hy5<Hy1, Hy2, Hy3, Hy4.

## 6.4 186 MARKER RESCUE FROM $\lambda$p186 PROPHAGE

The $\lambda$p186 phage, described in Section 8, can be used in marker rescue experiments in the same way that the P2-186 hybrids were. Three different $\lambda$p186 phage were available for this work, $\lambda$p186*shn*1-2, $\lambda$p186*srI*1-2 and $\lambda$p186*srI*3-1. The results of marker rescue experiments involving these last two phage are recorded in Table 6.5. The *pfu* obtained on each lysogen, for each mutant, is expressed both as a percentage of the *pfu* added (i.e. of the titre determined on Su$^{+}$) and as a percentage of the *pfu* obtained on a 186 lysogen. However, as discussed in Section 6.1, neither of these representations allows a comparison of frequencies for different *am* mutants to be made.

The phage $\lambda$p186*srI*3-1 contains the 186 cohesive ends together with 0.7 kb (2.4%) from the left end of the 186 genome and 2.5 kb (8.4%) from the right end (R. B. Saint, personal communication).

TABLE 6.5

186 MARKER RESCUE FROM THE PROPHAGE OF λp186*srI*1-2 AND λp186*srI*3-1[a]

| Super-infecting phage[b] | Prophage[c] | | | | | |
|---|---|---|---|---|---|---|
| | % *mrf* | | | Relative % *mrf* | | |
| | λp186*srI*1-2 | λp186*srI*3-1 | None | λp186*srI*1-2 | λp186*srI*3-1 | None |
| *Uam*37 | $1.7 \times 10^{-6}$ | $1.1 \times 10^{-6}$ | $2.3 \times 10^{-6}$ | 0.0014 | 0.0009 | 0.0018 |
| *Uam*64 | $4.4 \times 10^{-6}$ | $2.2 \times 10^{-6}$ | $3.1 \times 10^{-6}$ | 0.0013 | 0.0007 | 0.0009 |
| *Uam*63 | $6.4 \times 10^{-6}$ | $3.9 \times 10^{-6}$ | $6.9 \times 10^{-6}$ | 0.0021 | 0.0013 | 0.0023 |
| *Vam*38 | $7.3 \times 10^{-3}$ | $2.6 \times 10^{-7}$ | $1.6 \times 10^{-6}$ | 1.7 | $< 0.0001$ | 0.0004 |
| *Wam*15 | $6.4 \times 10^{-3}$ | $4.4 \times 10^{-6}$ | $9.7 \times 10^{-6}$ | 3.0 | 0.0021 | 0.0046 |
| *Wam*39 | $3.4 \times 10^{-3}$ | $1.2 \times 10^{-5}$ | $2.2 \times 10^{-5}$ | 1.5 | 0.0057 | 0.0098 |
| *Wam*52 | $6.9 \times 10^{-3}$ | $1.2 \times 10^{-5}$ | $2.1 \times 10^{-5}$ | | | |

*a* Marker rescue frequencies are expressed as a % of the *pfu* obtained on $Su^+$ (columns 2, 3 and 4) and as a % of the *pfu* obtained on an $Su^-$ lysogen of 186*c*I*ts* (columns 5, 6 and 7). The $Su^+$ strain used was C600 for all mutants except *Vam*38 and *Wam*52 for which H12R8A was used.

*b* All mutants except *Wam*52 also contain the *vir*2 mutation.

*c* All lysogens used are derived from lysogenisation of strain 594.

The results in Table 6.5 show that none of the wild-type alleles of mutants in genes *U*, *V* or *W* could be recovered from this phage. Other tests with *Tam*8, *Sam*4, *Wts*68 and *Wts*69 also failed to produce any increase in phage activity (data not shown). These five genes map at the left-hand end of 186 (see Section 7) and so none of the known mutations of 186 is present in the left-hand 2.4% of the genome. There is only one known essential gene (gene *A*) mapping to the right of the 186 *att* site (see Section 5). One allele of this gene, *Aam*5, was used in marker rescue experiments with this λp186 phage, but once again no increase in phage activity was observed (data not shown). Therefore at least this allele of gene *A* (and probably the entire gene) is not present in the right-hand 8.4% of the 186 genome.

The phage λp186*srI*1-2 contains the piece of DNA lying next to the 0.7 kb fragment mapping at the left end of the genome. This fragment of 186 is 3.5 kb in length (R. B. Saint, personal communication) and therefore long enough to code for three to four proteins. The results in Table 6.5 show that the wild-type alleles of all known amber mutants in genes *V* and *W* could be recovered from this λp186 phage. (The frequency of marker rescue was at least 150 fold greater than the reversion frequency.) The wild-type alleles of the two other known mutations in gene *W*, *ts*68 and *ts*69 could also be recovered from this λp186 phage (data not shown). However no allele of gene *U* could be recovered (Table 6.5), nor could the wild-type alleles of *Tam*8 and *Sam*4 (data not shown). In summary, the marker rescue results with these two λp186 phage place genes *V* and *W* at the left end of the 186 map, between positions located at 2.4% and 14.2% from the left end of the genome. All other known genes in 186 lie to the right of the 14.2% position. No known allele of any gene maps in the left-most 2.4% or the right-most 8.4% of the genome.

The third λp186 phage, λp186*shn*1-2, contains a 6.6 kb fragment from the centre of the 186 genome (R. B. Saint, personal communication). The results recorded in Table 6.6 show that all alleles of genes *G*, *H*, *I*, *J* and *K* can be recovered from this phage, but the alleles of genes *F* and *L* cannot. Table 6.6 also shows that the alleles *Eam*7$^+$ and *Mam*19$^+$ cannot be recovered, and spot tests have shown that neither the remaining two alleles of gene *M* nor the single allele of gene *N* can be recovered. Genes *G*, *H*, *I* and *J* were present on the P2-186 hybrid phage Hy9 and Hy10 whereas gene *K* was not. Gene *K* must therefore map between this group of genes and the remaining genes, *L* to *W*. The piece of 186 incorporated in the phage λp186*shn*1-3, is located between positions 40.5% and 62.8% from the left end of the 186 genome. Therefore genes *L* to *W* must lie in the left 40.5% of the genome.

The results with mutant *Jam*41 shown in Table 6.6 are peculiar in that the *eop* of this phage in this particular experiment was 90 fold higher on the λp186 prophage than on the wild-type 186 prophage. However, when the recovery from the λp186 prophage is expressed as a percent of the titre on Su$^+$, the value obtained for *Jam*41 is comparable with those obtained for the other mutants tested. It therefore appears that the problem is an abnormally small recovery from the wild-type 186 lysogen. In fact, in this experiment, when expressed as a percent of the Su$^+$ titre, the recovery of *Jam*41 from a wild-type 186 lysogen was only 0.004% (cf. the much higher figure, 0.19%, of Table 6.1). A repeat experiment again showed higher recovery from a λp186 prophage than from a wild-type 186 prophage and the reason for this behaviour is unknown.

## 6.5 EFFECT OF *recA* ON 186 MARKER RESCUE

The effect of the product of the bacterial gene *recA* on

TABLE 6.6

186 MARKER RESCUE FROM A λp186*shn*1-2 PROPHAGE[a]

| Super-infecting phage[b] | Prophage[c] | | | |
|---|---|---|---|---|
| | % *mrf* | | Relative % *mrf* | |
| | λp186*shn*1-2 | None | λp186*shn*1-2 | None |
| *Eam*7 | $8.4 \times 10^{-6}$ | $2.5 \times 10^{-5}$ | 0.0042 | 0.013 |
| *Fam*20 | $1.6 \times 10^{-5}$ | $1.1 \times 10^{-5}$ | 0.038 | 0.025 |
| *Gam*9 | $1.1 \times 10^{-2}$ | $3.0 \times 10^{-5}$ | 7.8 | 0.021 |
| *Gam*27 | $1.4 \times 10^{-2}$ | $2.7 \times 10^{-6}$ | 19 | 0.0035 |
| *Gam*25 | $3.0 \times 10^{-2}$ | $< 2.1 \times 10^{-5}$ | 19 | < 0.013 |
| *Gam*29 | $5.3 \times 10^{-3}$ | $8.0 \times 10^{-6}$ | 9.4 | 0.014 |
| *Ham*50 | $1.8 \times 10^{-1}$ | $< 6.8 \times 10^{-5}$ | 58 | < 0.022 |
| *Ham*56 | $7.5 \times 10^{-2}$ | $< 2.8 \times 10^{-5}$ | 35 | < 0.013 |
| *Iam*40a | $3.0 \times 10^{-2}$ | $1.7 \times 10^{-4}$ | 19 | 0.11 |
| *Jam*41 | $1.3 \times 10^{-2}$ | $< 2.6 \times 10^{-4}$ | * | * |
| *Kam*42 | $3.7 \times 10^{-2}$ | $5.1 \times 10^{-6}$ | 13 | 0.0022 |
| *Kam*22 | $4.3 \times 10^{-2}$ | $5.5 \times 10^{-6}$ | 20 | 0.0026 |
| *Kam*58 | $1.9 \times 10^{-2}$ | $3.4 \times 10^{-6}$ | 11 | 0.0022 |
| *Lam*2 | $< 3.1 \times 10^{-6}$ | $3.1 \times 10^{-6}$ | < 0.0016 | 0.0016 |
| *Lam*21 | $< 3.8 \times 10^{-6}$ | $< 3.8 \times 10^{-6}$ | < 0.0017 | < 0.0017 |
| *Mam*19 | $4.5 \times 10^{-6}$ | $9.1 \times 10^{-6}$ | 0.0011 | 0.0022 |

*a* Marker rescue frequencies are expressed as a % of the *pfu* obtained on Su⁺ (columns 2 and 3) and as a % of the *pfu* obtained on an Su⁻ lysogen of 186*cIts*(columns 4 and 5). The Su⁺ strain used was C600 for all mutants except *Gam*27 and *Jam*41 for which H12R8A was used.

*b* All mutants also contain the *vir*2 mutation.

*c* All lysogens used are derived from lysogenisation of strain 594.

* The *eop* of *Jam*41 on 594(λp186*shn*1-2) was 90 fold greater than on 594(186*cIts*). That is, the relative % *mrf* was 9,000. The relative % *mrf* of *Jam*41 on 594 was less than 2.5.

the frequency of marker rescue in 186 was tested in an experiment involving mutants *Dam*26 and *Bam*17. It was found that with the mutant *Dam*26 the recovery frequency from a 186*cIts* prophage in a *recA* cell was about one third the recovery from the same prophage in a $rec^+$ cell (the *mrfs* obtained were 0.13% in E772 (*recA*) and 0.32% in E701 ($rec^+$)). With the mutant *Bam*17 there was no difference in the frequency of marker rescue in *recA* and $rec^+$ cells (the *mrf* was 20% in both cases).

From the results with the mutant *Dam*26 it appears that the product of the *recA* gene is involved in 186 marker rescue to some extent, although a considerable proportion of the recombination obtained was not dependent on this product. Even in the absence of the *recA* product the residual recombination frequency was still very much larger than those observed in two-factor crosses with mutants to the left of *att*. The failure of the *recA* product to affect marker rescue with the mutant *Bam*17 lends further support to the suggestion that the 186 *B* gene alleles are recovered by complementation rather than by recombination.

## 6.6 DISCUSSION

As a method for mapping the 186 genome the deletion mapping described in this section has proved much more reliable than two-factor crosses. It also has an added advantage in that the appearance of recombinant phage occurred at a much higher frequency than that obtained in a two-factor cross. For the mutants described in Table 6.1, recombination with a 186 prophage produced wild-type recombinants at a frequency of 0.1 to 0.3% of the phage added. This frequency is closer to the 0.2 to 0.5% recombination frequency found in two-factor crosses for mutants on different sides of the *att* region, rather than to the 0.01% frequency obtained for most pairs of mutants.

It is possible that this elevated frequency is partly due to some *int* promoted recombination, occurring at the mixed phage-bacterial *att* site located at the right-hand end of the prophage. However, the marker rescue frequencies obtained for the λp186 phage, none of which include any part of the 186 *att* site, are similar to those obtained for the P2-186 hybrid phage. This suggests that *int* promoted recombination is not contributing to the frequency of marker rescue. Similarly, the 186 cohesive ends do not appear to be involved in marker rescue since they are not present in the phage λp186*shn*1-2 and λp186*srI*1-2. It is possible however that the frequencies of marker rescue with the λp186 prophage are abnormally high due to the presence of the λ*red* gene. The λ vector used to make these transducing phage is itself *red*$^-$ but the helper phage, λ*imm*$^{434}$, used in the formation of the dilysogen, is *red*$^+$. However, this possibility is unlikely since the *red* gene is under repressor control (Luzzati, 1970) and so the *red* protein should not be present in lysogenic cells.

The marker rescue frequencies obtained for the P2 mutants were also much higher than the frequencies encountered in two-factor crosses. For example, for marker rescue with the mutant *amJ*214 from Su$^-$ lysogens of hybrids 1 to 4, *mrfs* of around 0.2% were obtained. This compares with a frequency of about 0.01% for an average two-factor cross (Lindahl, 1969a).

The frequency of 186 marker rescue was stimulated in the presence of the product of the bacterial gene *recA*. However a considerable proportion of the recombination was not dependent on the *recA* product. This contrasts with results obtained by J. B. Egan (personal communication) for marker rescue with phage P2. He found that, while he could recover the wild-type allele of the P2 mutant *amJ*214 from *rec*$^+$ lysogens of the twelve P2-186 hybrid phage, he could

not recover this allele from *recA* lysogens of the same phage.

Marker rescue in phage 186 appears to be occurring by two different mechanisms: complementation for gene *B* mutants and recombination for mutants in all other genes. Recombination as a mechanism for the recovery of most mutants is suggested by the following observations. Marker rescue frequencies were relatively low, (0.1 to 0.3%), were dependent on the distance between the mutation and the hybrid cross-over point, and were influenced by the presence of the bacterial *recA* gene. By contrast, marker rescue frequencies with gene *B* alleles were much higher (10 to 20%), were independent of the distance between the mutation and the hybrid cross-over point, and were not affected by the presence or absence of the bacterial *recA* gene.

Recovery by complementation rather than by recombination may mean that the product of gene *B* is present in a normal lysogenic cell, that is, that gene *B* is not under repressor control. Alternatively it may mean that superinfection with a 186*vir* mutant is inducing the transcription of the prophage gene *B*. This second case may be equivalent to the transactivation of late λ genes, occurring upon the superinfection of a λ lysogen with a heteroimmune phage (Thomas, 1971). By analogy, it may be that gene *B* is not directly under repressor control, but its expression may be dependent on the product of another 186 gene which is under repressor control. This second gene product could then be supplied by the superinfecting 186*vir* phage, thus allowing transcription of the prophage gene *B*. Since the other 186 genes are recovered by recombination rather than by complementation it appears that, unlike λ late genes, 186 late genes cannot be transactivated.

In this section marker rescue has been considered as if occurring between a prophage and a linear superinfecting phage molecule (see Fig. 6.1). Another possibility is recombination between the

prophage and a circular superinfecting phage molecule. If this were the case it should be possible to obtain a recombinant between a 186 superinfecting phage mutant and a hybrid prophage in which the corresponding 186 gene is absent (see Fig. 6.5). The resultant recombinant would be a hybrid phage but should be viable. The fact that this does not occur (see Table 6.1, for example) can be interpreted as evidence for recombination occurring with a linear superinfecting molecule.

It is also possible that the presence of the superinfecting phage causes excision of the prophage so that marker rescue, in fact, may occur between two vegetative phage. However, this is most unlikely since it would mean that, for P2 marker rescue from hybrid prophage, a P2 infecting phage could cause the excision of a prophage integrated at the 186 *att* site and possessing the 186 *int* gene.

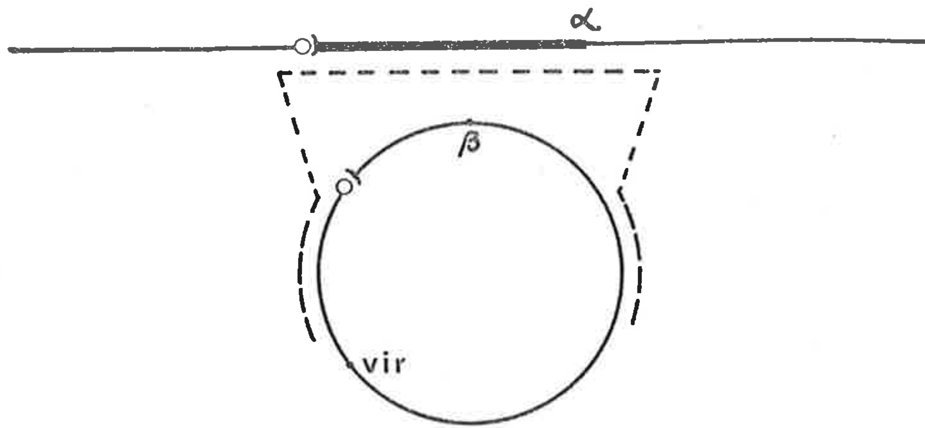
The marker rescue frequencies recorded in Table 6.1 can be analysed in either of two ways. Firstly, frequencies obtained with one superinfecting phage mutant and various hybrid prophage can be compared. Secondly, frequencies obtained with several superinfecting phage mutants but only the one hybrid prophage can be compared. In the first case the relative marker rescue frequency will increase linearly with the distance between the hybrid cross-over point and the superinfecting phage mutation, whereas in the second case the increase will be hyperbolic (see Section 6.1).

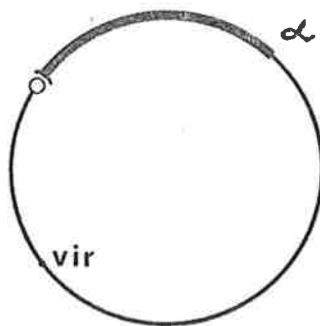
This difference may account in part for the better results obtained in experiments of the first type. In the second type of experiment, resolution of marker rescue frequencies is rapidly lost the further the mutations are from the hybrid cross-over point. An example of this can be seen in Table 6.1 if the recovery frequencies for *am* mutants 26 and 35 are compared. On lysogens of hybrids 5, 6, 7 and 8, in which the hybrid cross-over point is very close to *am*35,

*Figure 6.5* RECOMBINATION BETWEEN A CIRCULAR SUPERINFECTING PHAGE AND A HYBRID PROPHAGE

This figure illustrates the formation of viable *am*$^+$ phage from a double recombination event between a circular molecule of a 186 *am vir* superinfecting phage and a P2-186 hybrid prophage lacking the corresponding 186 *am*$^+$ allele. The two cross-overs (one to the left of the cohesive ends of the prophage but to the right of the *vir*$^+$ allele of the prophage, and the other to the right of the P2-186 hybrid cross-over point) occur between homologous 186 DNA segments and give rise to a viable hybrid phage carrying the *vir* mutation.

| | |
|---|---|
| *vir* | a virulent mutation |
| β | an amber mutation in an essential gene |
| α | a P2-186 hybrid cross-over point |
| —o)— | cohesive ends |
| ▬▬▬ | P2 DNA |
| – – – – | an *am*$^+$ recombinant |


α
β
vir


α
vir

the frequency of recovery for *am*26 is tenfold higher than for *am*35. However for hybrids 1, 2, 3 and 4 which cross-over within the same gene and about 400 base pairs away (Younghusband *et al.*, 1975), *am*26 and *am*35 show very little difference in recovery frequency. For mutations further than about one gene length from the hybrid cross-over point no mapping information can be obtained. (For example, see recovery frequencies obtained for different mutants on lysogens of hybrids 9 or 10).

Another contributing factor to the poor results obtained in the second type of experiment may be the fact that a greater number of experimental estimates are required for the calculation of each result. In the first type of experiment three experimental results are required for the comparison of two marker rescue frequencies. These are the *pfu* obtained on each of two hybrid lysogens and the *pfu* obtained on a 186 lysogen. This is true if all the results to be compared have been obtained in the same experiment (as they were in Table 6.1). If two experiments are involved then there are four experimental estimates, since the *pfu* on a 186 lysogen must be re-measured in the second experiment. This situation applies when the results in Table 6.1 are compared with those in Table 6.2. For a single cross it can be seen that the frequency of marker rescue does vary, although generally the variation is less than twofold and occurs for all lysogens tested that day. That is, the major part of the variation is due to the extra measurement involved, namely the second determination of *pfu* on a 186 lysogen. The second type of experiment always involves four estimations whether or not the two crosses, whose frequencies are to be compared, form part of the same experiment. This probably contributes to the greater variations obtained in this type of experiment.

The deletion mapping described in this section is very sensitive and recombination can be demonstrated even in very short sections of the genome. For example, mutants 14, 23, 26, 48, 35 and 46 all showed significant differences in their recovery frequencies from hybrids 2 and 5 and this can be attributed to the extra 400 base pairs of 186 DNA present in hybrid 2. The alleles of gene *D* were recovered from hybrids 1, 2, 3 and 4 at a frequency three- to four-fold higher than from hybrids 5, 6, 7 and 8 and so their distance from the Hy5 cross-over point should be about one third their distance from the Hy2 cross-over point, that is roughly 200 base pairs. With *Eam*35, recovery frequencies with hybrids 1 to 4 were 40 fold higher than with hybrids 5 to 8, suggesting that *am*35 lies about 10 base pairs from the Hy5 cross-over point. These figures are probably not reliable but it appears that marker rescue can be detected for mutants lying well within a distance of 400 base pairs from a hybrid cross-over point. However it is very doubtful that an allele lying within a few codons from the cross-over point could be recovered. Therefore, for all the results recorded in this section the possibility remains that an allele present on a hybrid, but very close to the cross-over point, would be recorded as not present on the hybrid.

The results of marker rescue with P2 mutants showed that hybrids 1 to 8 and 12 all cross-over to the right of the P2 allele $amD_6$. It is possible therefore that all these hybrids contain a complete set of P2 structural genes, and although each of these hybrids also contains one complete 186 structural gene, it is probable that the 186 protein is not used in morphogenesis and that the proteins of the phage particle are all P2 gene products. However, hybrid 11 lacks P2 genes *U*, *D* and probably *T*, while hybrids 9 and 10 lack P2 genes *E*, *T*, *U* and *D*. Therefore in thse hybrids 186 proteins must

be substituting for the missing P2 proteins.

The 186 tail genes *D*, *E*, *F* and *G* are present on hybrid 11 while the 186 tail genes *D*, *E*, *F*, *G*, *H*, *I* and *J* are present on hybrids 9 and 10. The products of some of these genes may not be required for the formation of the hybrid phage tail, and those that are may be involved in its assembly rather than being part of the structure itself. However results suggesting that the product of the P2 gene *T* forms part of the P2 tail structure have been presented by Lengyel *et al.* (1974). If this is so then at least one of the 186 proteins should appear in the tail structures of the hybrid phage Hy9 and Hy10 (and perhaps Hy11). The mixing of the P2 and 186 tail functions in hybrids 9, 10 and 11 does lead to phage capable of infection but the phage particles appear to be somewhat unstable since two DNA containing bands were obtained for each of these hybrids when phage stocks were purified on a CsCl gradient. Although not checked by electron microscopy it was assumed (by analogy with the results for the mutant *Lam*21 presented in Section 3) that the upper band contained whole phage particles while the lower band contained phage heads only.

The P2 genes *E*, *T*, *U* and *D* are absent from hybrids 9 and 10 and it appears therefore that they are not involved in the adsorption process since these hybrids can still adsorb to *E. coli* strain C. (186 is not able to adsorb to this strain (Woods and Egan, 1974).)

Hybrids 1 to 5 were constructed from phage 186*cIts* and $P2^+$. The only restriction on the location of the cross-over point was that it should lie to the left of the 186 immunity region, but to the right of the P2 gene required for adsorption to an *E. coli* C strain. However hybrids 6 to 12 were derived from phage 186*cIts* and P2$vir_1amD_6$

and so the cross-over point should lie to the left of the $amD_6$ allele since the selective indicator was Su$^-$ and hybrid phage carrying the $amD_6$ mutation should not plate. Hybrids 9, 10 and 11 do indeed cross-over to the left of the allele $D_6$ but hybrids 6 to 8 (and perhaps 12) appear to have the $D_6^+$ allele. These phage may be the result of an initial recombination event giving rise to a hybrid phage carrying the P2$amD_6$ allele. This hybrid phage would not itself be able to plate on the Su$^-$(P2) selective indicator, but could recombine with the P2 prophage, producing a hybrid phage carrying the $D_6^+$ allele which would be able to plate on this indicator. To avoid this, and to ensure that the hybrids formed cross-over to the left of $D_6$, the selective indicator should be lysogenic for an $amD_6$ mutant. This technique could be used to produce hybrids with cross-over points to the left of other P2 genes by choosing the appropriate selective indicator, but the technique is limited to cross-overs occurring to the right of the P2 tail gene required for adsorption to *E. coli* C.

## 6.7 SUMMARY

The results in this section have established the following gene order for phage 186:

$(W,V) - (U,T,S,R,Q,P,O,N,M,L) - K - (J,I,H) - G - F - E - D - B - att - A$

The segments of the 186 genome in which these genes are to be found are recorded in Table 6.7. The order of the hybrids arranged in an ascending order of the length of the 186 genome is:

Hy5, Hy6, Hy7, Hy8<Hy12<Hy1, Hy2, Hy3, Hy4<Hy11<Hy9<Hy10.

In ascending order of the length of P2 the order is:

Hy9, Hy10<Hy11<Hy6, Hy7, Hy8, Hy12<Hy5<Hy1, Hy2, Hy3, Hy4.

TABLE 6.7

PHYSICAL LOCATION OF THE GENES OF PHAGE 186

| Gene | Segment of genome[a] | | |
|---|---|---|---|
| *W*,*V* | 2.4% | to | 14.2% |
| *U*,*T*,*S*,*R*,*Q*,*P*,*O*,*N*,*M*,*L* | 14.2% | " | 40.5% |
| *K*,*J*,*I*,*H*,*G* | 40.5% | " | 62.8% |
| *F*, part of *E* | 62.8% | " | 64.2% |
| part of *E* | 64.2% | " | 65.4% |
| part of *E*,*D*,*B*, part of *int* | 65.4% | " | 70.3% |
| part of *int*, part of *c*I | 70.3% | " | 73.5% |
| part of *c*I | 73.5% | " | 73.7% |
| *A* | 73.7% | " | 91.6% |

[a] Measured in percent of the 186 genome from the left-hand end. Figures 64.2%, 65.4%, 70.3%, 73.5% and 73.7% are from Younghusband *et al.* (1975). Other figures are from R. B. Saint (personal communication).

## SECTION 7

## MARKER RESCUE FROM 186 *am* MUTANT PROPHAGE

## 7.1 INTRODUCTION

The P2-186 hybrid phage were only useful in the mapping of a limited region of the 186 genome, and to map the rest of the genome by this method would require hybrids with cross-over points in almost every 186 gene. A more general method is therefore needed to complete the 186 map, and such a method is provided by the use of 186 *am* mutant prophage, instead of hybrids, in marker rescue experiments. In these experiments the amber mutation in the prophage is acting in a way similar to the cross-over point of a hybrid.

Consider an experiment which involves several different *am* prophage but only one *am* superinfecting phage. If the prophage mutations all lie to the left of the infecting phage mutation, as in Figure 7.1a, the situation is identical to that encountered in the use of the P2-186 hybrid prophage. As in that case the frequency of recovery, when expressed as a proportion of the frequency of recovery of the same allele from a wild-type 186 prophage, can be described by the equation:

$$y = \frac{kb(a-b)}{kb(c-b)} = \frac{a-b}{c-b} = \frac{x}{c-b} \quad \text{..Equation 7.1}$$

Since only one superinfecting phage is being used, b and c are constants. Y is therefore directly proportional to a-b (or x), that is, to the distance between the prophage mutation and the superinfecting phage mutation (see Fig. 7.1c).

If, however, the prophage mutations lie to the right of the superinfecting phage mutation, as in Figure 7.1b, then there is a different relationship between marker rescue frequency and the distance between the $\alpha$ and $\beta$ mutations. This relationship can be described by the equation:

$$y = \frac{k(b-a)(c-b)}{kb(c-b)} = \frac{b-a}{b} = \frac{-x}{b} \quad \text{..Equation 7.2}$$

*Figure 7.1* RECOMBINATION BETWEEN AN *am* MUTANT SUPERINFECTING PHAGE AND AN *am* MUTANT PROPHAGE

(a) Recombination between a 186*am*α prophage and a 186*am*β*vir* superinfecting phage in which the α mutation is located to the left ot the β mutation.

(b) As in (a) except that the α mutation lies to the right of the β mutation.

(c) Relationship between the relative marker rescue frequency (y) and the distance (x) between a β mutation and various α mutations. The line with the positive slope describes the relative *mrf* for α mutations to the left of the β mutation. The line with the negative slope describes the relative *mrf* for α mutations to the right of the β mutation.

(d) Relationship between the relative marker rescue frequency (y) and the distance (x) between an α mutation and various β mutations. The hyperbola with the positive slope describes the relative *mrf* for β mutations to the right of the α mutation. The hyperbola with the negative slope describes the relative *mrf* for β mutations to the left of the α mutation.

*vir* a virulent mutation in the superinfecting phage

α an amber mutation in an essential gene of the prophage

β an amber mutation in an essential gene of the superinfecting phage

a distance of α from the 186 *att* site

b distance of β from the 186 *att* site

c distance of the left-hand cohesive end from the 186 *att* site

x distance between α and β mutations (i.e. x = a-b)

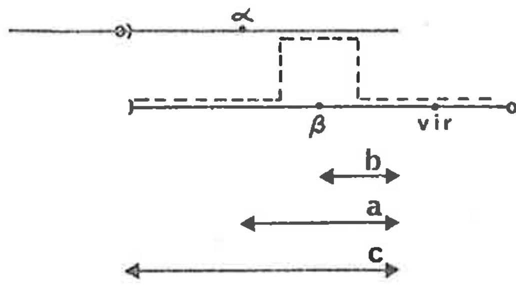
y relative marker rescue frequency (i.e. marker rescue from a 186*am*α prophage expressed as a fraction of marker rescue from a 186 wild-type prophage)

—o)— a prophage

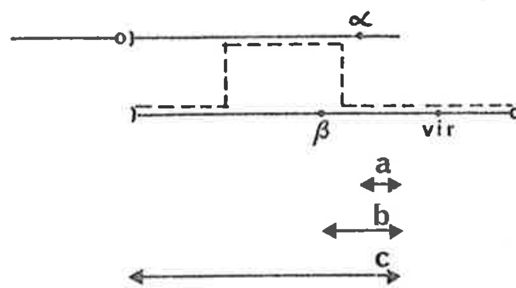
)—— a superinfecting phage

---- an $am^+$ recombinant

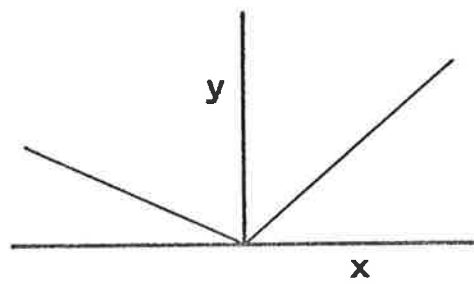
(a)



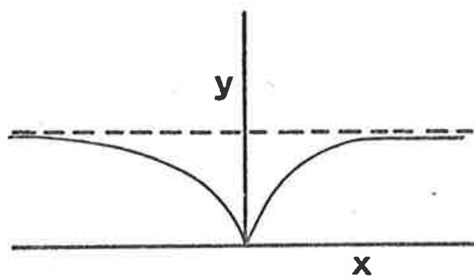

(b)




(c)




(d)



Once again b is a constant and y is directly proportional to a-b, or x, the distance between mutations $\alpha$ and $\beta$ (Fig. 7.1c).

However the two straight lines described by equations 7.1 and 7.2 have different slopes. If y is plotted against x the straight line obtained for equation 7.1 has a slope of 1/c-b, whereas that obtained for equation 7.2 has a slope of -1/b. This means that for two prophage mutations ($\alpha$) on the same side of the infecting phage mutation ($\beta$) a higher frequency of recombination implies a greater distance between the $\alpha$ and $\beta$ mutations, whereas for two $\alpha$ mutations, one each side of the $\beta$ mutation, a higher frequency of recovery may not mean that the $\alpha$ to $\beta$ distance is greater but merely that the slope of the marker rescue function produced by that particular $\beta$ mutation is steeper on one side than the other (see Fig. 7.1c).

For an experiment involving only one prophage mutation ($\alpha$) but several superinfecting phage mutations ($\beta$) the equations are once again:

$$y = \frac{a-b}{c-b} \quad \text{if } \beta \text{ is to the right of } \alpha$$

and

$$y = \frac{b-a}{b} \quad \text{if } \beta \text{ is to the left of } \alpha$$

In this case a and c are the constants and b the variable and the equations can be rewritten as:

$$y = \frac{x}{x-a+c} \quad \text{(for } \beta \text{ to the right of } \alpha\text{)} \qquad \text{..Equation 7.3}$$

and

$$y = \frac{x}{x-a} \quad \text{(for } \beta \text{ to the left of } \alpha\text{)} \qquad \text{..Equation 7.4}$$

Under these conditions equation 7.3 represents the same rectangular hyperbola derived in Section 6. That is, a rectangular hyperbola with centre (a-c, 1) and asymptotes y=1 and x=a-c. Equation 7.4 represents a different rectangular hyperbola, namely one with centre (a, 1) and asymptotes y=1 and x=a. The slopes of these two hyperbolas, given by their derivatives, are $(c-a)/(x-a+c)^2$ for equation 7.3 and

$-a/(x-a)^2$ for equation 7.4. These two slopes could only be the same if c were equal to zero, which is not the case for the marker rescue experiments. So, once again, the frequency of marker rescue depends not only on the distance between the $\alpha$ and $\beta$ mutations but also on whether the $\beta$ mutation lies to the right or to the left of the $\alpha$ mutation (see Figs. 7.1c and d). Therefore, whether the experiment involves one $\alpha$ and several $\beta$ mutations, or one $\beta$ and several $\alpha$ mutations, it is essential that the group of mutations (whether $\alpha$ or $\beta$) all lie to one side of the single test mutation. Only then can a comparison of marker rescue frequencies be used to obtain the order of mutations.

For a group of unmapped mutants the first step is to identify the mutation at either end of the group, so that this mutant can then be used as the test mutant to determine the order of the other mutations. An end mutation can be identified by a consideration of the results of the marker rescue tests themselves. For example, if a superinfecting phage containing one of the mutations from the group to be mapped is crossed with different prophage, each containing one of the other mutations from the group, then the largest marker rescue frequency obtained will be for an $\alpha$ mutation which lies at one end of the group. This $\alpha$ mutation may not be the mutation furthest from the $\beta$ mutation since there may be another, further away, on the other side of the $\beta$ mutation but giving a lower marker rescue frequency. However it must be further away than any other mutation on the same side of the $\alpha$ mutation, and is therefore by definition one of the end mutations. The order of mutations in a particular group can then be determined from the marker rescue frequencies obtained with this end mutant. However in order to minimise the risk of a wrong order being obtained due to experimental error, and to maximise the information obtained for a particular set of mutants, the results

for all mutations in the β position crossed with all mutations in the α position are used to determine the order. For each β mutation, the relative *mrfs* obtained with the various α mutations are plotted against the positions of the α mutations, which are arranged in an order such that an increase in % *mrf* corresponds to an increase in the distance between the α and β mutations. The most likely order for the mutations is the one suggested by a majority of the above graphs.

In Section 6 it was found that results of experiments which involved a single superinfecting phage mutant but various hybrid prophage were more reliable than the results of experiments involving only one hybrid prophage but several superinfecting phage mutants. This difference in the reliability of results for the two types of experiment was also observed in this section for *am* mutant prophage. For this reason the analysis of results in this section has been confined to comparisons of marker rescue frequencies obtained with a single superinfecting phage mutant on different mutant prophage (that is, to results obtained with a single β mutation but various α mutations).

## 7.2 186 MARKER RESCUE FROM A 186*cIts* PROPHAGE

The frequencies of marker rescue from a 186*cIts* prophage for the various amber alleles of phage 186, are shown in Table 7.1. Excluding the results obtained for the *B* alleles 17 and 57, it can be seen that the frequencies obtained were generally between 0.1% and 0.7%. These are quite high frequencies when compared with a normal two factor recombination frequency (approximately 0.01%) and are closer to the frequency of *int* promoted recombination, observed in two-factor crosses, for mutants spanning the *att* site (see Section 5). This method of mapping therefore has two immediate advantages over two-factor crosses. Firstly, the frequency is much higher and secondly,

## TABLE 7.1

### MARKER RESCUE WITH 186 *am* MUTANTS FROM A 186*cIts* PROPHAGE

| Mutant[a] | % *mrf*[b] | Mutant[a] | % *mrf*[b] |
|---|---|---|---|
| *Bam*17 | $23^{7}$ | *Mam*60 | $0.20^{5}$ |
| *Bam*57 | $16^{1}$ | *Nam*47 | $0.33^{3}$ |
| *Dam*14 | $0.09^{4}$ | *Oam*61 | $0.50^{3}$ |
| *Dam*23 | $0.12^{3}$ | *Oam*62 | $0.52^{3}$ |
| *Dam*26 | $0.18^{8}$ | *Pam*36 | $0.61^{2}$ |
| *Dam*48 | $0.25^{4}$ | *Pam*45 | $0.61^{2}$ |
| *Eam*35 | $0.22^{5}$ | *Pam*65 | $0.51^{1}$ |
| *Eam*46 | $0.35^{10}$ | *Pam*16 | $0.82^{5}$ |
| *Eam*7 | $0.26^{8}$ | *Pam*66 | $0.29^{2}$ |
| *Fam*20 | $0.12^{6}$ | *Pam*67 | $0.66^{5}$ |
| *Gam*9 | $0.21^{5}$ | *Qam*49 | $0.58^{3}$ |
| *Gam*27 | $0.17^{3}$ | *Qam*1 | $0.48^{2}$ |
| *Gam*25 | $0.12^{4}$ | *Ram*6 | $0.37^{4}$ |
| *Gam*29 | $0.10^{5}$ | *Ram*51 | $0.23^{2}$ |
| *Ham*50 | $0.32^{6}$ | *Sam*34 | $0.42^{3}$ |
| *Ham*56 | $0.29^{4}$ | *Sam*4 | $0.55^{2}$ |
| *Iam*40a | $0.18^{7}$ | *Sam*18 | $0.88^{1}$ |
| *Jam*41 | $0.12^{8}$ | *Tam*8 | $0.11^{3}$ |
| *Kam*42 | $0.19^{6}$ | *Uam*37 | $0.16^{4}$ |
| *Kam*22 | $0.19^{8}$ | *Uam*64 | $0.36^{5}$ |
| *Kam*58 | $0.17^{4}$ | *Uam*63 | $0.37^{4}$ |
| *Lam*2 | $0.18^{6}$ | *Vam*38 | $0.38^{4}$ |
| *Lam*21 | $0.19^{5}$ | *Wam*15 | $0.21^{4}$ |
| *Mam*19 | $0.35^{4}$ | *Wam*39 | $0.25^{2}$ |
| *Mam*31 | $0.24^{3}$ | | |

[a] Each mutant listed has, in addition, a *vir*2 mutation.

[b] Calculated as: $$\% \, mrf = \frac{pfu \text{ on } Su^-(186cIts)}{pfu \text{ on } Su^+} \times 100$$

The $Su^-$ strain used was a 186*cIts* lysogen of 594.
The $Su^+$ strain was C600 for all mutants except *Gam*27 and *Nam*47 for which H12R8A and S26R1e were used respectively. The superscript indicates the number of experiments used to calculate the average frequency given.

since there are no problems arising due to recombination on the plate (for discussion see Section 7.4), it is not necessary to keep the total number of phage added to the plate to a minimum. The high frequencies obtained for marker rescue with the *B* alleles are probably due to complementation rather than to recombination, as discussed in Section 6.

The alleles listed in Table 7.1 are arranged in the order (as determined in this section) of their positions on the 186 map. Ideally, the marker rescue frequency should increase as the mutant approaches the half-way mark between the left-hand cohesive end and the phage *att* site, and decrease on either side. However the frequencies recorded do not show any particular trend although perhaps a general maximum occurs around the region of the lysozyme (gene *P*) alleles. The main reason for this variation is probably the different levels of suppression obtained for different mutants on an $Su^+$ host. In all other tables in this section the frequency of wild-type recombinants is recorded as a percentage of the wild-type recombinants obtained with the same superinfecting phage on a lysogen of 186*cIts*. The marker rescue frequency, thus expressed, is no longer dependent on the phage titre as measured on $Su^+$. The variability in Table 7.1 may also be due in part to the comparison of recoveries for different superinfecting phage from the 186*cIts* prophage. As was found in Section 6, such variation makes it very difficult to determine an order by comparison of marker rescue frequencies in experiments involving a single mutant prophage but several superinfecting phage mutants. Comparisons of this type were not used in the determination of the order of the 186 mutations described in this section.

## 7.3 186 MARKER RESCUE FROM 186 *am* MUTANT PROPHAGE

In this section the frequency of marker rescue was used to map all the known essential genes of phage 186 which lie between the left-hand cohesive end and the phage *att* site. Only one known essential gene of 186 (gene *A*) lies outside this region (to the right of the *att* site). Mapping was commenced at the right-hand end of the above region with a group of alleles for which some mapping data was already available from the results of experiments recorded in Section 6. Mapping then proceeded leftward through the remaining tail genes to the lysozyme gene and then into the head region, concluding with the head genes *V* and *W* which are known to be located at the left end of the genome from the results of marker rescue experiments with the λp186 transducing phage (see Section 6).

The hybrids with the shortest section of 186 DNA, hybrids 5, 6, 7 and 8, have the wild-type alleles of all the *B* and *D* mutations as well as that of mutation 35 in gene *E*. The results recorded in Table 7.2 show that the order of the *D* alleles must be 26-23-14. However these could be in either orientation with respect to the *att* site, namely, 26-23-14-*att* or 14-23-26-*att*. The results using the *B* mutants as superinfecting phage were not helpful since the marker rescue frequency is not dependent on the distance between mutations. However when the *B* alleles were used in the prophage form it can be seen that frequencies with *am*57 were smaller than with *am*17. This means either that 57 lies closer to the *D* alleles than does 17, or that 57 and 17 lie on opposite sides of the *D* alleles. Since 57 and 17 belong to the same gene they should lie on the same side of the *D* alleles. Since *D* belongs to the same polarity group as genes *E*, *F*, and *G*, whereas *B* does not, the *B* alleles must lie to the right of the *D* alleles. Two orders are therefore possible: 26-23-14-57-17-*att*

TABLE 7.2

MARKER RESCUE WITH ALLELES OF GENES *B* AND *D*[a]

| Super-infecting phage[b] | Prophage[c] | | | | | Experiment number |
|---|---|---|---|---|---|---|
| | *D* *am*26 | *D* *am*23 | *D* *am*14 | *B* *am*57 | *B* *am*17 | |
| *Dam*26 | < 0.009 | 2.4 | 3.5 | 5.1 | 6.3 | 1 |
| " | 0.001 | 1.2 | 1.6 | | 2.7 | 2 |
| *Dam*23 | 0.20 | 0.002 | 0.49 | 1.2 | 1.6 | 1 |
| " | 0.31 | 0.001 | 0.55 | | 2.2 | 2 |
| *Dam*14 | 0.74 | 0.64 | < 0.001 | 2.7 | 4.0 | 1 |
| " | 0.38 | 0.24 | < 0.001 | | 1.4 | 2 |
| *Bam*57 | 110 | 120 | 130 | < 0.001 | 0.37 | 1 |
| *Bam*17 | 97 | 110 | 110 | 0.024 | < 0.001 | 1 |

[a] The frequencies given are the *pfu* obtained for each superinfecting phage when plated on Su$^-$ strains carrying the various *am* mutant prophage, expressed as a percentage of the *pfu* obtained for the same superinfecting phage on an Su$^-$ lysogen of 186*c*I*ts*.

[b] All mutants also contain the *vir*2 mutation.

[c] All bacterial strains used are derived from lysogenisation of strain 594.

TABLE 7.3

MARKER RESCUE WITH ALLELES OF GENES *B*, *D* AND *E*[a]

| Superinfecting phage | Prophage | | | | | | |
|---|---|---|---|---|---|---|---|
| | *E* *am*7 | *E* *am*46 | *D* *am*26 | *D* *am*23 | *D* *am*14 | *B* *am*57 | *B* *am*17 |
| *Eam*7 | < 0.001 | 0.32 | 0.74 | 1.0 | 1.3 | 1.3 | 1.6 |
| *Eam*46 | 0.13 | 0.001 | 0.44 | 1.0 | 1.4 | 1.6 | 1.9 |

[a] For an explanation of the figures given, the bacterial indicators and the superinfecting phage, see Table 7.2.

or 14-23-26-57-17- *att*.

The results in Table 7.3 distinguished between these two possibilities. The results with hybrid prophage (Section 6) showed that the *E* alleles 7 and 46 lie to the left of the *B* and *D* alleles. Since these two alleles therefore lie at one end of this group of mutations the order can be read directly from the marker rescue frequencies obtained. With both mutants the order was found to be:

(*E*7, *E*46) - *D*26 - *D*23 - *D*14 - *B*57 - *B*17 - *att*,

confirming that the *B* alleles lie together and to the right of the *D* alleles and showing that the first of the two orders suggested by the results of Table 7.2 is the correct one. The results with hybrid prophage also showed that *E*7 lies to the left of *E*46 so the complete order for this set of mutants is:

*E*7 - *E*46 - *D*26 - *D*23 - *D*14 - *B*57 - *B*17 - *att* ...Order 7.1

Table 7.4 shows the results of two marker rescue experiments involving alleles of genes *D*, *E* and *G*. From these results it would appear that the *D* alleles *am*26 and *am*48 occur at the same site. If they are not the same mutation then they map so close to each other that recombination cannot be measured by marker rescue. These two mutants also failed to show any recombination in two-factor crosses and for the rest of this work they are considered to have the same mutation.

Excluding the results with *Gam*9, the order which fits these results best is 46-7-35-(26,48). This can be more easily seen in Figure 7.2 where the results of Table 7.4 are presented in a graphical form. The frequencies have been plotted assuming the order 7-46-35-(26,48) and the results of both experiments conform fairly well with this order. However an improvement in the shape of the curves can be obtained by switching the positions of mutants 7 and 46. If

TABLE 7.4

MARKER RESCUE WITH ALLELES OF GENES *D*, *E* AND *G*[a]

| Superinfecting phage | Prophage | | | | | | Experiment number |
|---|---|---|---|---|---|---|---|
| | *G* *am*9 | *E* *am*7 | *E* *am*46 | *E* *am*35 | *D* *am*26 | *D* *am*48 | |
| *Gam*9 | 0.003 | 2.5 | 3.8 | 1.9 | 3.5 | 3.3 | 1 |
| " | 0.006 | 2.5 | 3.6 | 2.4 | 3.1 | | 2 |
| *Eam*7 | 3.1 | 0.001 | 0.57 | 0.78 | 1.3 | 1.3 | 1 |
| " | 2.1 | 0.001 | 0.46 | 0.63 | 0.94 | | 2 |
| *Eam*46 | 2.2 | 0.22 | 0.002 | 0.30 | 0.84 | 0.82 | 1 |
| " | 1.1 | 0.15 | 0.002 | 0.28 | 0.62 | | 2 |
| *Eam*35 | 1.5 | 0.27 | 0.28 | < 0.001 | 0.25 | 0.24 | 1 |
| " | 1.2 | 0.17 | 0.23 | 0.005 | 0.17 | | 2 |
| *Dam*26 | 1.4 | 0.38 | 0.42 | 0.18 | < 0.001 | < 0.001 | 1 |
| " | 1.0 | 0.29 | 0.30 | 0.20 | < 0.001 | | 2 |
| *Dam*48 | 1.7 | 0.48 | 0.58 | 0.27 | < 0.001 | < 0.001 | 1 |
| " | 1.2 | 0.26 | 0.38 | 0.14 | < 0.001 | | 2 |

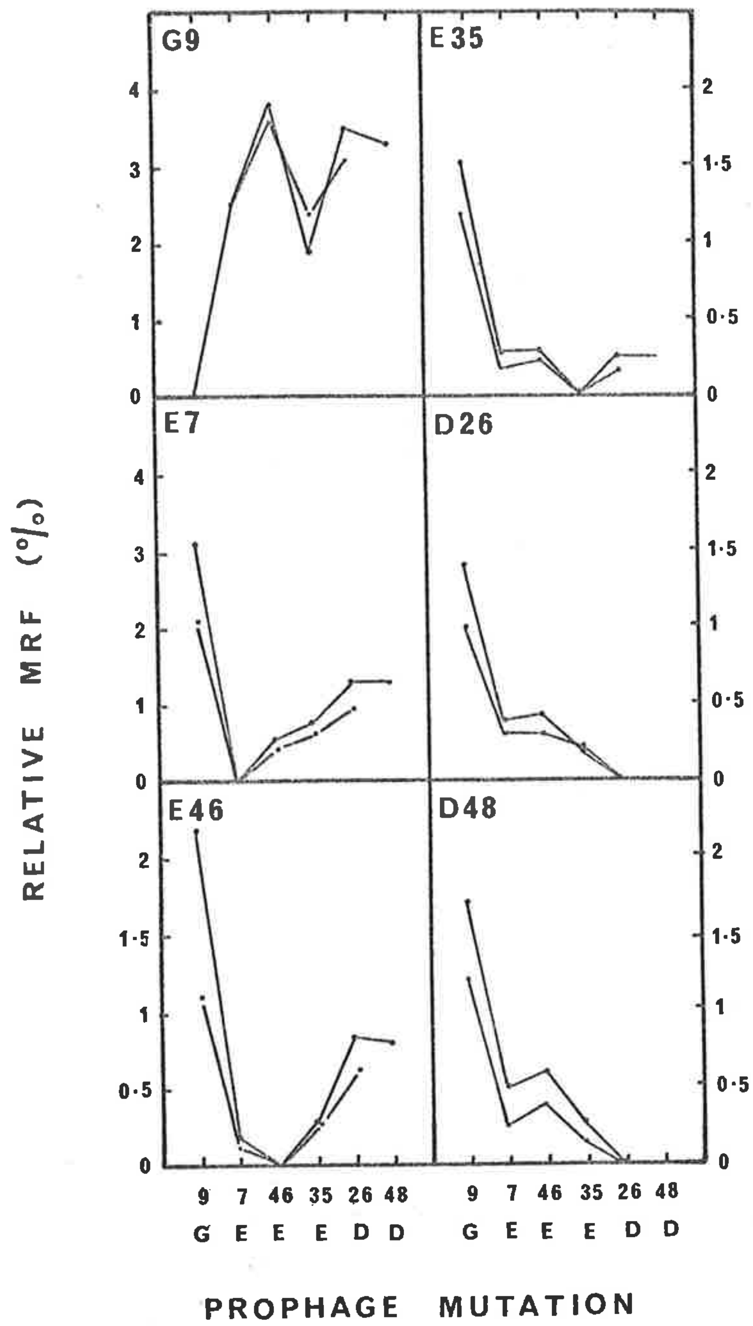
[a] For an explanation of the figures given, the bacterial indicators and the superinfecting phage, see Table 7.2.

*Figure 7.2* GRAPHS OF RELATIVE MARKER RESCUE FREQUENCIES WITH ALLELES OF GENES *D*, *E* AND *G*.

Each box records the results of marker rescue for a particular superinfecting phage mutant from various mutant prophage. The superinfecting phage used is listed in the top left-hand corner of each box; for example, the top left-hand box labelled G9 shows the results obtained with the superinfecting phage 186*c*I*tsGam*9*vir*2. The relative marker rescue frequencies obtained with the various mutant superinfecting phage are plotted against the most likely positions of the prophage mutations, these being arranged in an order such that, wherever possible, an increase in the relative marker rescue frequency corresponds to an increase in the distance between the mutations of the prophage and the superinfecting phage. The allele numbers and gene letters of the prophage mutations used are given below the diagram.

—●— Results from Experiment 1, Table 7.4

—■— Results from Experiment 2, Table 7.4


G9
E35
E7
D26
E46
D48
RELATIVE MRF (%)
PROPHAGE MUTATION
9 G
7 E
46 E
35 E
26 D
48 D

this were done the shapes of the curves would fit exactly the expectations from the theoretical curves. However the results in Section 6 show that *am*7 lies to the left of *am*46 and both lie to the left of *am*35 and *am*26. The best order therefore for these mutants is the one shown in Figure 7.2.

With *am*9 as a prophage mutation the results clearly show that it must lie at one end of the group of mutations 7, 46, 35, 26 and 48. A position at either end would be consistent with the results. However since $9^+$ is not present on hybrids 1 to 8 and 12 whereas $35^+$, $26^+$ and $48^+$ are, *am*9 must lie at the left end of this group of mutations. The results with *am*9 as the superinfecting phage are anomalous as there is a large dip in frequency at the position of *am*35. The position of *am*35 is fixed by the hybrid experiments and agrees with the other results of Table 7.4, so the dip in the curve occurring when *am*9 is the infecting phage must be an artifact. However it is not due to random variation since the two independent experiments showed exactly the same sort of dip at this position. Despite this dip and the small discrepancy in the results for mutants 7 and 46, the most likely order for this set of mutants is:

*G*9 - *E*7 - *E*46 - *E*35 - *D*26,48 ...Order 7.2

Table 7.5 and Figure 7.3 show the results of marker rescue experiments involving alleles of the genes *E*, *F*, *G* and *H*. If once again the results for *am*9 are excluded then it is clear that for this set of mutants there is only one possible order, namely

*H*50 - *G*29 - *G*25 - *G*27 - *F*20 - *E*7 - *E*46

When *am*9 is used as the infecting phage mutation (or β mutation) an obvious discrepancy occurs with one of the mutants 25 or 29. (A dip also appears at position 29 when *am*46 is used as the β

TABLE 7.5

MARKER RESCUE WITH ALLELES OF GENES *E*, *F*, *G* AND *H*[a]

| Superinfecting phage | Prophage | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *H* *am*50 | *G* *am*29 | *G* *am*25 | *G* *am*27 | *G* *am*9 | *F* *am*20 | *E* *am*7 | *E* *am*46 |
| *Ham*50 | < 0.001 | 1.0 | 1.1 | 1.7 | 4.4 | 4.9 | 5.3 | 5.7 |
| *Gam*29 | 0.87 | 0.006 | 0.23 | 0.62 | 1.1 | 1.4 | 1.4 | 1.6 |
| *Gam*25 | 1.1 | 0.20 | 0.009 | 0.92 | 1.3 | 1.6 | 2.2 | 2.8 |
| *Gam*27 | 2.9 | 1.5 | 1.0 | 0.001 | 0.89 | 1.4 | 1.7 | 2.9 |
| *Gam*9 | 3.4 | 0.92 | 1.1 | 0.43 | 0.003 | 1.4 | 2.4 | 3.3 |
| *Fam*20 | 7.5 | 3.7 | 2.9 | 2.1 | 3.3 | < 0.002 | 2.0 | 2.5 |
| *Eam*7 | 3.6 | 2.1 | 1.7 | 1.3 | 1.8 | 0.52 | 0.002 | 0.43 |
| *Eam*46 | 3.4 | 0.92 | 1.7 | 1.6 | 1.6 | 0.50 | 0.18 | < 0.001 |

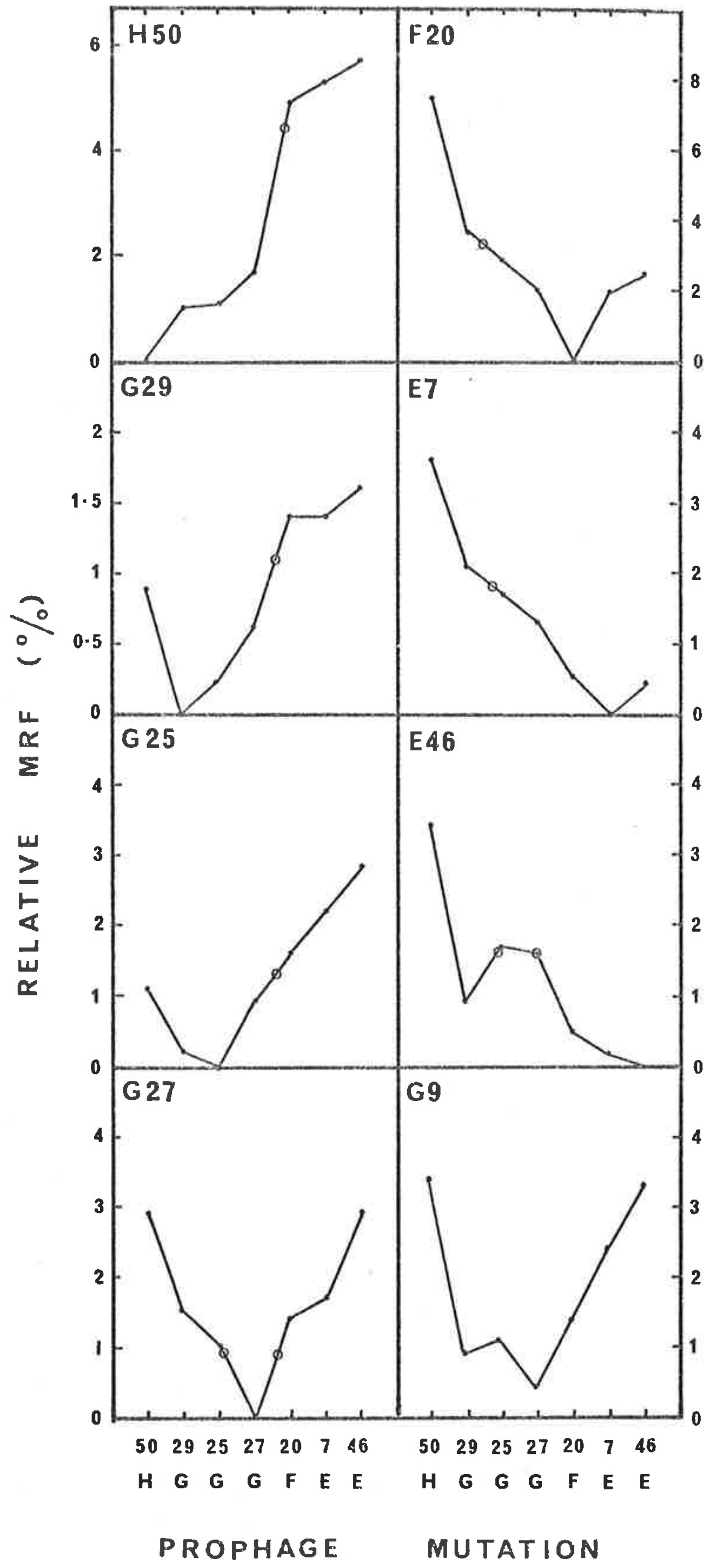
[a] For an explanation of the figures given, the bacterial indicators and the superinfecting phage, see Table 7.2.

*Figure 7.3* GRAPHS OF RELATIVE MARKER RESCUE FREQUENCIES WITH ALLELES OF GENES *E*, *F*, *G* AND *H*

For an explanation of the form of the diagram see Figure 7.2.

—●— Results from Table 7.5

Open circles represent the relative marker rescue frequencies obtained with various superinfecting phage mutants from a *Gam*9 mutant prophage. The positions on the x axis corresponding to these frequencies give the possible locations for the mutation *Gam*9.


H50
F20
G29
E7
G25
E46
G27
G9
RELATIVE MRF (%)
0
2
4
6
8
0.5
1
1·5
0·5
3
50 29 25 27 20 7 46
H G G G F E E
PROPHAGE MUTATION

mutation, and this suggests that the result with 29 may be too low rather than the result with 25 being too high.) With *am*9 in the β position the lowest point in the curve occurs with *am*27 as the prophage mutation. This suggests that *am*9 may lie between mutants 25 and 27 or between mutants 27 and 20. With *am*9 as the prophage mutation and 7 or 20 as the β mutation a position for *am*9 between the mutants 29 and 25 is suggested. With 9 as the α mutation and 25, 29 or 50 as β, a position between 27 and 20 is suggested. With 27 or 46 as the β mutation a position between 25 and 27 or between 27 and 20 would be consistent with the results. All three locations suggested for *am*9, that is between *G*29 and *G*25, between *G*25 and *G*27 or between *G*27 and *F*20, are possible since *am*9 is an allele of the gene *G*, but the weight of evidence suggests that the most likely location is between *G*27 and *F*20. If this were the correct position only two results would be in error, those with 7 and 20 as the β mutations, which place *am*9 much too far to the left. The order for this set of mutants, with 9 marked as doubtful, is as follows:

*H*50 - *G*29 - *G*25 - *G*27 - ?*G*9 - *F*20 - *E*7 - *E*46 ...Order 7.3

The results for the next set of mutants are shown in Table 7.6 and Figure 7.4. The experiments with hybrid phage in Section 6 showed that *am*22 lies at the left end of this group of mutants and *am*29 at the right end, that is, the order is 22-(41, 40a, 56, 50)-29. Once again the shapes of the curves obtained for two independent experiments are very reproducible, but there are some discrepancies present. Results with *am*41 in the α position are consistently too high (or the results with *am*22 as the α mutation are too low). The curves would be greatly improved if 41 and 22 changed positions. However this is not possible since the hybrid experiments have shown unequivocally that 22 must lie to the left of 41.

TABLE 7.6

MARKER RESCUE WITH ALLELES OF GENES *G*, *H*, *I*, *J* AND *K*[a]

| Superinfecting phage | Prophage | | | | | | Experiment number |
|---|---|---|---|---|---|---|---|
| | *K* *am*22 | *J* *am*41 | *I* *am*40a | *H* *am*56 | *H* *am*50 | *G* *am*29 | |
| *Kam*22 | < 0.002 | 5.3 | 6.2 | 5.9 | 7.7 | 8.6 | 1 |
| " | < 0.002 | 2.2 | 2.2 | | 3.4 | 3.7 | 2 |
| *Jam*41 | 9.1 | < 0.059 | 15 | 13 | 17 | 22 | 1 |
| " | 4.4 | < 0.005 | 5.3 | | 9.7 | 9.3 | 2 |
| *Iam*40a | 2.4 | 3.7 | 0.039 | 1.3 | 2.5 | 2.2 | 1 |
| " | 2.7 | 3.9 | 0.024 | | 2.4 | 2.4 | 2 |
| *Ham*56 | 1.3 | 1.4 | 0.60 | 0.002 | 0.10 | 0.48 | 1 |
| " | 1.8 | 1.5 | 0.54 | | 0.091 | 0.33 | 2 |
| *Ham*50 | 2.8 | 4.2 | 2.2 | 0.18 | 0.002 | 1.9 | 1 |
| " | 2.6 | 3.9 | 1.9 | | < 0.002 | 1.2 | 2 |
| *Gam*29 | 2.3 | 3.5 | 1.9 | 0.90 | 1.3 | < 0.008 | 1 |
| " | 2.1 | 2.5 | 1.4 | | 0.83 | < 0.006 | 2 |

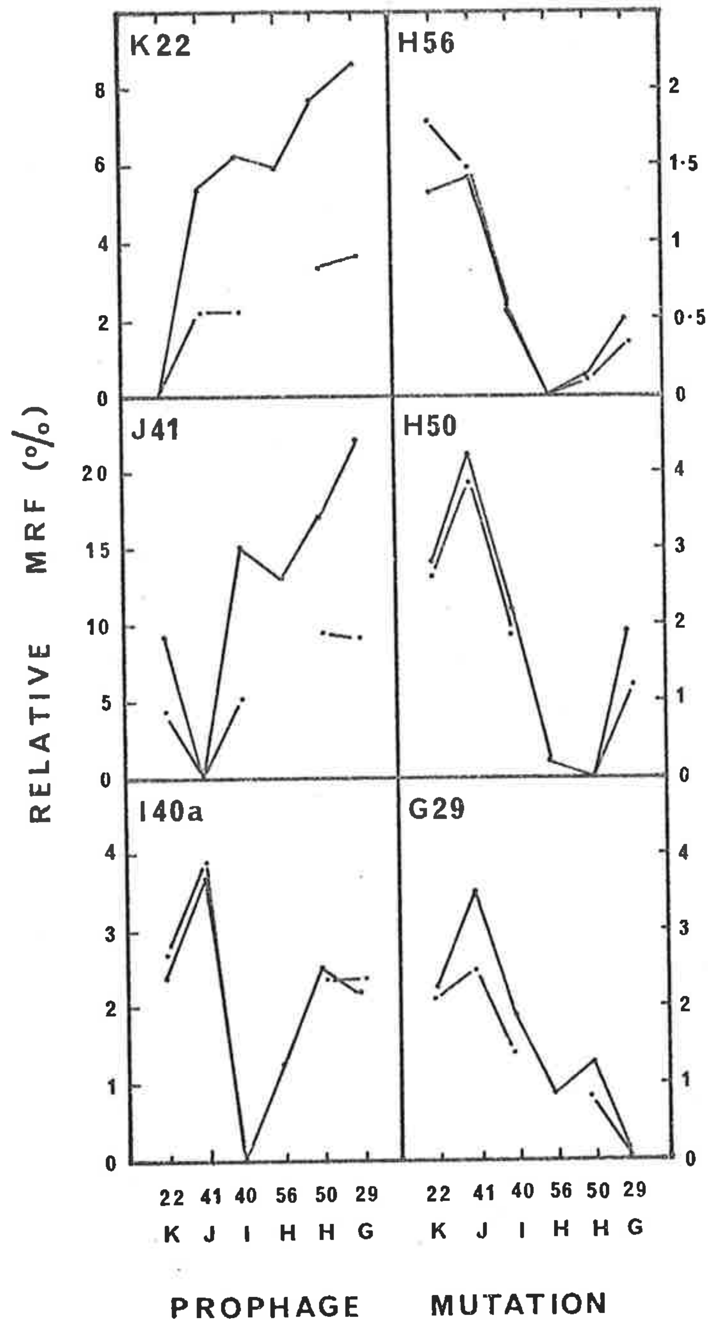
[a] For an explanation of the figures given, the bacterial indicators and the superinfecting phage, see Table 7.2.

*Figure 7.4* GRAPHS OF RELATIVE MARKER RESCUE FREQUENCIES WITH ALLELES OF GENES *G*, *H*, *I*, *J* AND *K*

For an explanation of the form of the diagram see Figure 7.2.

—●— Results from Experiment 1, Table 7.6

—■— Results from Experiment 2, Table 7.6


K22
H56
J41
H50
I40a
G29
RELATIVE MRF (%)
22 41 40 56 50 29
K J I H H G
PROPHAGE MUTATION

Results for 41 as the β mutation are also unusual in that the relative marker rescue frequencies obtained were much higher than those obtained for other β mutations. This could be due to an abnormally small recovery of $41^{+}$ from the 186*c*I*ts* prophage (such as was observed in Section 6), which would lower the denominator in the calculation of the relative *mrf*.

Ignoring the results with 56 as the α mutation and bearing in mind that 41 cannot lie to the left of 22, the most likely order is:

*K*22 - *J*41 - *I*40a - *H*50 - *G*29

A position for *Jam*41 to the right of *Kam*22 rather than to the left is again suggested by the fact that *Jam*41, *Iam*40a and *Ham*50 all belong to a single polarity group to which *Kam*22 does not belong.

With mutant 56 in the α position, β mutants 22 and 41 suggest that it lies between the mutations 41 and 40a. The β mutant 40a suggests either the 41 to 40a interval or the 40a to 50 interval. β mutants 56 and 50 suggest the 40a to 50 interval or the 50 to 29 interval. Finally, the β mutant 29 suggests the 50 to 29 interval. Since there is no common interval suggested by all these β mutations there will always be dips in the curves wherever 56 is placed. Since mutations 56 and 50 both lie in gene *H* whereas 40a and 41 belong to different genes, 56 cannot lie between mutants 41 and 40a. It must lie either between 40a and 50 or between 50 and 29. The results are not good enough to distinguish between these two so it is not possible to map the mutations 50 and 56 with respect to each other. The order obtained for this set of mutants is therefore

*K*22 - *J*41 - *I*40a - (*H*56, *H*50) - *G*29 ...Order 7.4

The results for marker rescue experiments with the alleles of the genes *I*, *J*, *K* and *L* are shown in Table 7.7. Considering only the results for allesles of genes *K* and *L* (Fig. 7.5) it can be seen

TABLE 7.7

MARKER RESCUE WITH ALLELES OF GENES *I*, *J*, *K* AND *L*[a]

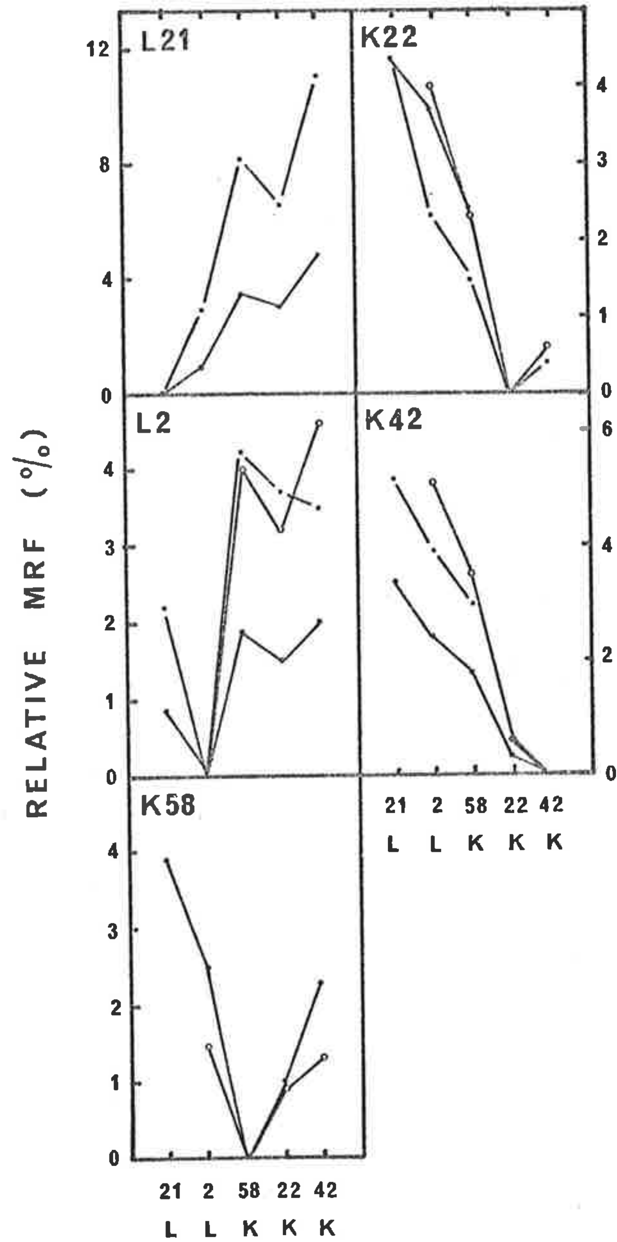
| Superinfecting phage | Prophage | | | | | | | Experiment number |
|---|---|---|---|---|---|---|---|---|
| | *L* *am*21 | *L* *am*2 | *K* *am*58 | *K* *am*22 | *K* *am*42 | *J* *am*41 | *I* *am*40a | |
| *Lam*21 | < 0.002 | 0.93 | 3.4 | 3.0 | 4.8 | 10.3 | 6.7 | 1 |
| " | 0.001 | 2.9 | 8.2 | 6.5 | 11 | | | 3 |
| *Lam*2 | 0.86 | < 0.001 | 1.9 | 1.5 | 2.0 | 6.3 | 5.9 | 1 |
| " | | 0.023 | 4.0 | 3.2 | 4.6 | | | 2 |
| " | 2.2 | 0.012 | 4.2 | 3.7 | 3.5 | | | 3 |
| *Kam*58 | 3.9 | 2.5 | < 0.002 | 1.0 | 2.3 | 6.1 | 5.7 | 1 |
| " | | 1.5 | < 0.001 | 0.88 | 1.3 | | | 2 |
| *Kam*22 | 4.4 | 3.7 | 2.4 | < 0.002 | 0.41 | 5.3 | 5.7 | 1 |
| " | | 4.0 | 2.3 | < 0.002 | 0.58 | | | 2 |
| " | 4.4 | 2.3 | 1.5 | 0.002 | 0.40 | | | 3 |
| *Kam*42 | 3.4 | 2.4 | 1.8 | 0.33 | 0.001 | 3.5 | 4.4 | 1 |
| " | | 5.1 | 3.5 | 0.67 | < 0.002 | | | 2 |
| " | 5.2 | 3.9 | 3.0 | 0.64 | < 0.002 | | | 3 |
| *Jam*41 | 21 | 18 | 8.7 | 6.0 | 8.7 | < 0.11 | 9.8 | 1 |
| *Iam*40a | 13 | 13 | 4.5 | 4.3 | 8.1 | 5.1 | 0.034 | 1 |

[a] For an explanation of the figures given, the bacterial indicators and the superinfecting phage, see Table 7.2.

*Figure 7.5* GRAPHS OF RELATIVE MARKER RESCUE FREQUENCIES WITH ALLELES OF GENES *K* AND *L*

For an explanation of the form of the diagram see Figure 7.2.

—●— Results from Experiment 1, Table 7.7
—○— Results from Experiment 2, Table 7.7
—■— Results from Experiment 3, Table 7.7



L21
K22
L2
K42
K58
RELATIVE MRF (%)
12
8
4
0
4
3
2
1
0
6
21 2 58 22 42
L L K K K
PROPHAGE MUTATION

that there is no one order which fits all the results obtained. If the results for mutant 58 are not included then there in only one order possible for the remaining mutants, namely, 21-2-22-42. Similarly if the results for 22 are excluded then the only possible order is 21-2-58-42. This suggests that both mutants 58 and 22 lie between mutants *Lam*2 and *Kam*42. As both 58 and 22 belong to gene *K* either order of these two mutants is possible. The order 2-22-58 is suggested by crosses involving β mutants 21 and 2 while the order 2-58-22 is suggested by crosses involving β mutants 22 and 42. As can be seen in Figure 7.5, when 22 and 42 were crossed with each other a very low marker rescue frequency was observed. If 58 lay between these two mutants the last two curves in Figure 7.5 would show very large dips. It seems more likely then that 58 lies to the left of 22, that is, that the order is 21-2-58-22-42. However since these results are somewhat doubtful the order of these mutants has been left undefined.

Mutants 40a and 41 in genes *I* and *J* are known from the hybrid experiments to lie to the right of the alleles of genes *K* and *L*. These two mutants gave larger marker rescue frequencies with α mutants 21 and 2 than with α mutants 58, 22 and 42 suggesting that the order is 21-2-(58,22)-42-(41,40a) rather than 42-(22,58)-2-21-(41,40a). This is consistent with the finding in Section 6 that 186 genes *I*, *J* and *K* are present on the phage λp186*shn*1-2 whereas gene *L* is not. Therefore the most likely order for this set of mutants is:

*L*21 - *L*2 - (*K*58, *K*22) - *K*42 - (*J*41, *I*40a) ...Order 7.5

Table 7.8 and Figure 7.6 record the results of marker rescue experiments with alleles of the genes *L*, *M* and *N*. Four of the six graphs shown in Figure 7.6 have dips and once again the results of two independent experiments show that these dips are highly

TABLE 7.8

MARKER RESCUE WITH ALLELES OF GENES *L*, *M* AND *N*[a]

| Superinfecting phage | Prophage | | | | | | Experiment number |
|---|---|---|---|---|---|---|---|
| | *N* *am*47 | *M* *am*60 | *M* *am*31 | *M* *am*19 | *L* *am*21 | *L* *am*2 | |
| *Nam*47 | < 0.001 | 2.1 | 1.8 | 4.1 | 5.0 | 4.1 | 1 |
| " | < 0.002 | 3.4 | 2.9 | 6.6 | 8.4 | 6.6 | 2 |
| *Mam*60 | 1.4 | < 0.003 | 0.75 | 1.4 | 1.9 | 1.5 | 1 |
| " | 3.9 | < 0.001 | 1.3 | 3.4 | 3.5 | 2.7 | 2 |
| *Mam*31 | 1.4 | 0.62 | 0.004 | 0.21 | 0.85 | 1.2 | 1 |
| " | 3.4 | 1.3 | < 0.003 | 0.39 | 1.3 | 1.6 | 2 |
| *Mam*19 | 4.5 | 2.1 | 0.52 | < 0.001 | 1.1 | 1.5 | 1 |
| " | 3.6 | 2.0 | 0.51 | < 0.001 | 1.3 | 1.9 | 2 |
| *Lam*21 | 4.9 | 3.6 | 1.1 | 1.1 | < 0.002 | 1.4 | 1 |
| " | 9.0 | 5.3 | 2.1 | 2.4 | 0.001[b] | 2.9[b] | 2 |
| *Lam*2 | 6.8 | 3.8 | 2.4 | 3.1 | 2.6 | < 0.001 | 1 |
| " | 6.2 | 3.6 | 2.1 | 2.8 | 2.2[b] | 0.012[b] | 2 |

[a] For an explanation of the figures given, the bacterial indicators and the superinfecting phage, see Table 7.2.

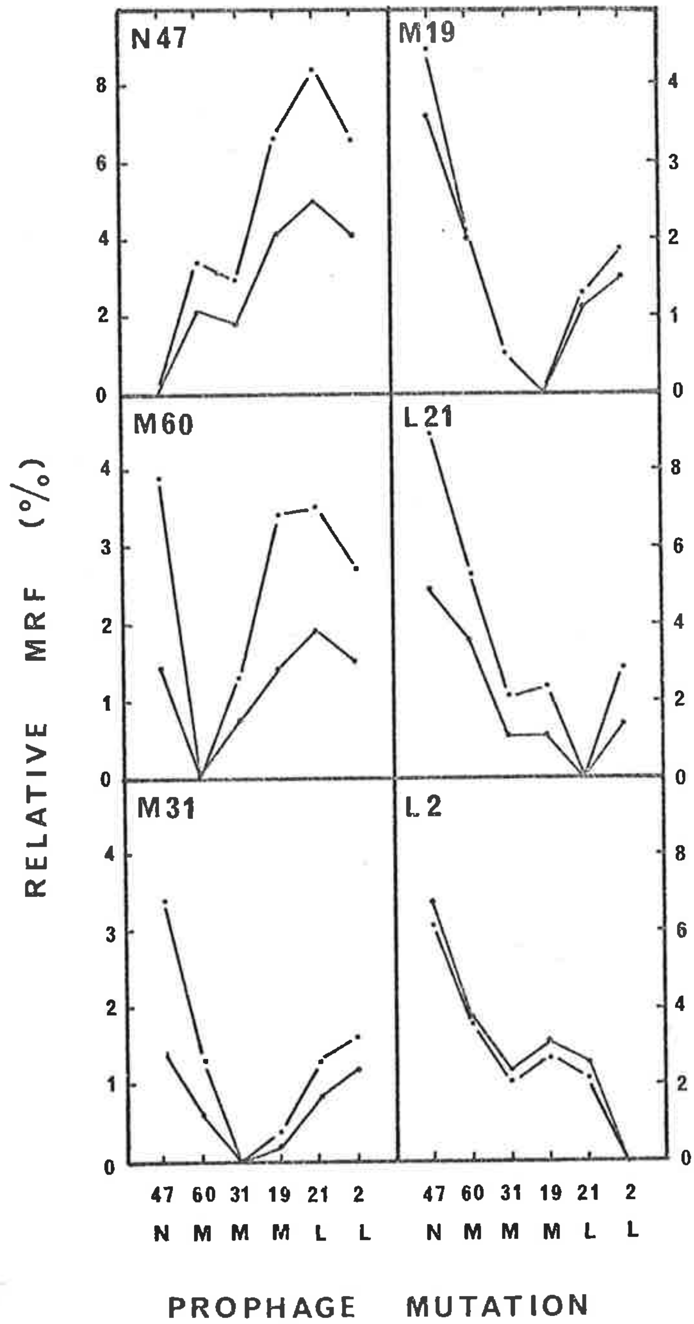
[b] These four results also appear in Table 7.7.

*Figure 7.6* GRAPHS OF RELATIVE MARKER RESCUE FREQUENCIES WITH ALLELES OF GENES *L*, *M* AND *N*.

For an explanation of the form of the diagram see Figure 7.2.

—●— Results from Experiment 1, Table 7.8

—■— Results from Experiment 2, Table 7.8



N 47
M19
M60
L 21
M 31
L 2
RELATIVE MRF (%)
47 60 31 19 21 2
N M M M L L
PROPHAGE MUTATION

reproducible. A dip in a particular graph can be eliminated by changing the order of mutations, but if this is done for the results in Figure 7.6 dips appear in other graphs previously unaffected. If the positions of 47 and 60 are exchanged one curve is improved but five are spoilt. If 60 and 31 are exchanged one curve is improved, two good curves are spoilt and two curves, although already possessing dips, are now worse than before. The shape of one curve is not affected. If 31 and 19 are exchanged, one curve is improved, one is spoilt, one becomes worse than before and three are unaffected. Changing 19 and 21 improves one curve, ruins four and leaves one unaffected. If 21 and 2 are exchanged two curves are improved but three are spoilt. One is unaffected. Therefore, despite the discrepancies in this set of data the order given in Figure 7.6 appears to be the one best fitting the results. Any exchange of mutations leads to more discrepancies than it cures. The only doubtful position is that of 31, it may be incorrectly placed with respect to 19. The order 21 to the left of 2, suggested here, was also obtained in Table 7.7. The most likely order for this set of mutants is therefore:

*N*47 - *M*60 - *M*31 - *M*19 - *L*21 - *L*2 ...Order 7.6

Marker rescue frequencies obtained with mutants in genes *M*, *N*, *O* and *P* are recorded in Table 7.9. These results, together with several more taken from Table 7.10, are shown graphically in Figure 7.7. Although reproducible, the mapping results obtained with these mutants are unsatisfactory. The previous experiment (Table 7.8, Fig. 7.6) showed that 47 maps to the left of 60. This is to be expected since 60 belongs to the same polarity group as the alleles of genes *K*, *L* and *M*, whereas 47 does not. For the same reason alleles of the lysozyme gene (*P*) cannot lie to the right of 60. The

TABLE 7.9

MARKER RESCUE WITH ALLELES OF GENES *M*, *N*, *O* AND *P*[a]

| Super-infecting phage | Prophage | | | | | Experiment number |
|---|---|---|---|---|---|---|
| | *P* *am*16 | *O* *am*61 | *O* *am*62 | *N* *am*47 | *M* *am*60 | |
| *Pam*16 | < 0.001 | 2.2 | 3.2 | 2.9 | 3.0 | 1 |
| " | < 0.006 | | | 6.6 | 6.5 | 4 |
| *Oam*61 | 1.2 | < 0.002 | 0.17 | 0.46 | 0.83 | 1 |
| " | | 0.001 | 0.082 | 0.22 | 0.86 | 2 |
| *Oam*62 | 1.2 | 0.11 | < 0.001 | 0.44 | 0.72 | 1 |
| " | | 0.71 | 0.001 | 2.1 | 2.8 | 2 |
| " | 0.66 | | < 0.001 | 0.19 | 0.48 | 3 |
| *Nam*47 | 6.7 | 3.1 | 3.3 | < 0.012 | 6.8 | 1 |
| " | | 0.86 | 0.60 | < 0.001[b] | 2.1[b] | 2 |
| " | 4.2 | | 2.0 | < 0.014 | 4.9 | 3 |
| " | 3.2 | | | < 0.002[b] | 3.4[b] | 4 |
| *Mam*60 | 4.6 | 1.8 | 2.9 | 2.9 | < 0.007 | 1 |
| " | | 0.66 | 1.3 | 1.4[b] | < 0.003[b] | 2 |
| " | 3.2 | | 2.2 | 2.5 | < 0.006 | 3 |
| " | 4.6 | | | 3.9[b] | < 0.001[b] | 4 |

[a] For an explanation of the figures given, the bacterial indicators and the superinfecting phage, see Table 7.2.

[b] These results also appear in Table 7.8.

*Figure 7.7* GRAPHS OF RELATIVE MARKER RESCUE FREQUENCIES WITH ALLELES OF GENES *M*, *N*, *O* AND *P*

For an explanation of the form of the diagram see Figure 7.2.

First column

—●— Results from Experiment 1, Table 7.9

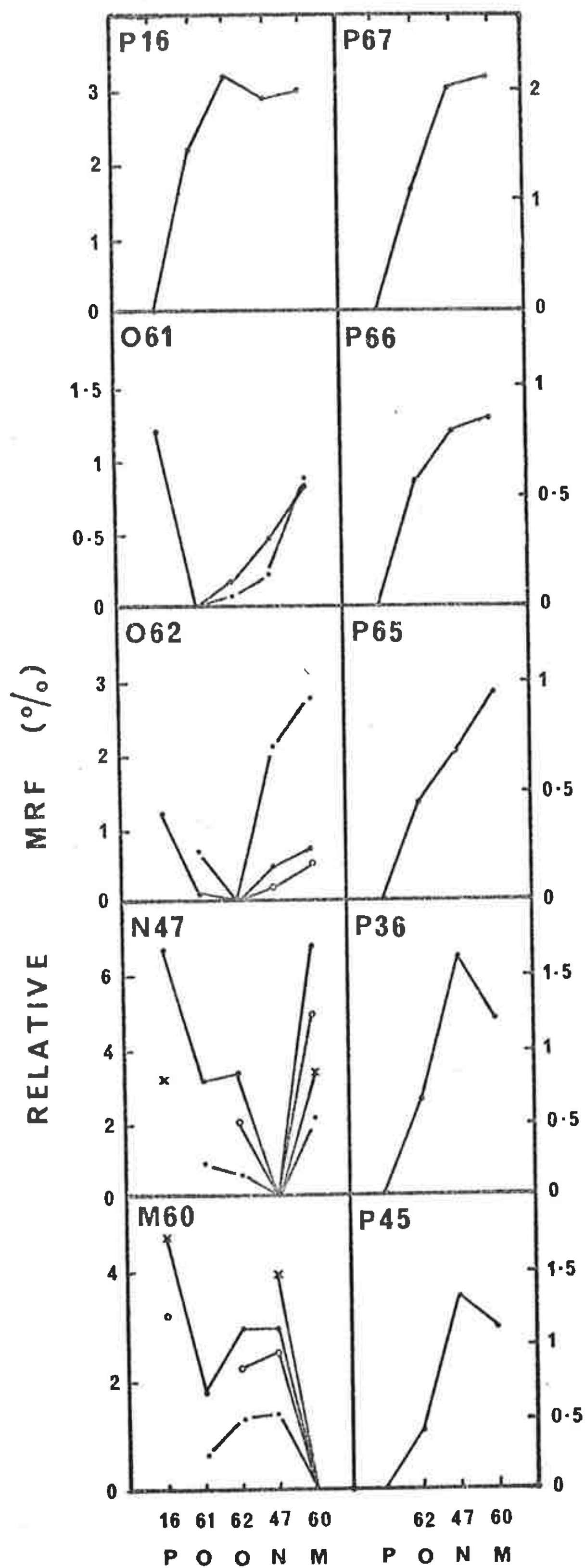
—■— Results from Experiment 2, Table 7.9

—○— Results from Experiment 3, Table 7.9

—×— Results from Experiment 4, Table 7.9

Second column

—●— Results from Experiment 1, Table 7.10


P16
P67
O61
P66
O62
P65
N47
P36
M60
P45
RELATIVE MRF (%)
16 61 62 47 60
P O O N M
62 47 60
P O N M
PROPHAGE MUTATION

curves produced by β mutants 61, 62 and 60 all suggest that 16 is at one end of the group of mutations 16, 61, 62, 47 and 60. It must therefore lie at the left-hand end, the order being 16-47-60. When any of the gene *P* alleles are used as a β mutation, marker rescue frequencies with α mutations *Oam*62, *Nam*47 or *Mam*60 are always greater than those when the α mutation is another gene *P* allele (Table 7.10). This shows that all the gene *P* alleles map together. When approached from the left by the gene *P* alleles 67, 66, 65, 36 or 45 the α mutant 62 appears closer than either of the α mutants 47 or 60 (Fig. 7.7). The only exception occurs with the β mutant *P*16. In this case both 47 and 60 appear closer than 62. (This is not possible since 62 does not belong to the same polarity group as 60.) The order must therefore be: gene *P* alleles - 62-47-60.

*Am*61 and *am*62, the alleles of gene *O*, appear to map fairly close to each other. 61 appears closer to 16 (which maps to the left) than does 62, but it also appears closer to 60 (which maps to the right) than does 62. Therefore it is not possible to map these two mutations with respect to each other. The most likely order for the mutations in this region is:

gene *P* alleles (67,66,65,36,45,16) - (*O*61, *O*62) - *N*47 - *M*60...Order 7.7

The results for the mapping of the lysozyme (gene *P*) alleles with respect to each other (see Table 7.10) are unfortunately totally inconsistent and no allelic order can be derived within this gene. Mutations 66 and 67 appear to be the same and seem to be located at one end of the gene *P* alleles (Fig. 7.8). Perhaps the most consistent order for these alleles is (66,67)-16-65-45-36.

Even the orientation of the gene *P* alleles with respect to outside markers is in doubt. Assuming the order above, the slope of the curve using the β mutation 38 suggests that 36 lies at the left-

## TABLE 7.10

## MARKER RESCUE WITH ALLELES OF GENES *M*, *N*, *O*, *P* AND *V*[a]

| Super-infecting phage | Prophage | | | | | | | | | | Experiment number |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *V* *am*38 | *P* *am*67 | *P* *am*66 | *P* *am*65 | *P* *am*36 | *P* *am*45 | *P* *am*16 | *O* *am*62 | *N* *am*47 | *M* *am*60 | |
| *Vam*38 | < 0.001 | 2.9 | 3.1 | 2.7 | 1.2 | 2.5 | 3.0 | 2.4 | 5.1 | 4.6 | 1 |
| *Pam*67 | 2.1 | < 0.001 | < 0.001 | 0.20 | 0.24 | 0.34 | 0.27 | 1.1 | 2.0 | 2.1 | 1 |
| " | | < 0.001 | 0.001 | 0.49 | 0.52 | 0.62 | 0.60 | | | | 2 |
| *Pam*66 | 1.8 | < 0.001 | < 0.001 | 0.23 | 0.21 | 0.30 | 0.27 | 0.56 | 0.78 | 0.84 | 1 |
| " | | 0.001 | < 0.002 | 0.52 | 0.85 | 0.75 | 0.66 | | | | 2 |
| *Pam*65 | 0.73 | 0.13 | 0.11 | < 0.001 | 0.053 | 0.056 | 0.073 | 0.44 | 0.67 | 0.94 | 1 |
| " | | 0.29 | 0.35 | < 0.001 | 0.17 | 0.10 | 0.17 | | | | 2 |
| *Pam*36 | 0.83 | 0.23 | 0.22 | 0.13 | < 0.001 | 0.092 | 0.15 | 0.65 | 1.6 | 1.2 | 1 |
| " | | 0.54 | 0.57 | 0.34 | < 0.001 | 0.25 | 0.43 | | | | 2 |
| *Pam*45 | 0.89 | 0.16 | 0.13 | 0.064 | 0.023 | < 0.001 | 0.085 | 0.40 | 1.3 | 1.1 | 1 |
| " | | 0.72 | 0.70 | 0.28 | 0.22 | < 0.001 | 0.53 | | | | 2 |
| *Pam*16 | | 1.7 | 1.6 | 0.95 | 1.1 | 0.89 | < 0.001 | | | | 2 |
| *Oam*62 | 1.7 | 0.69 | 0.62 | 0.34 | 0.35 | 0.54 | 0.66[b] | < 0.001[b] | 0.19[b] | 0.48[b] | 1 |
| *Nam*47 | 13 | 5.2 | 4.1 | 1.5 | 2.9 | 4.2 | 4.2[b] | 2.0[b] | < 0.014[b] | 4.9[b] | 1 |
| *Mam*60 | 11 | 4.1 | 3.2 | 1.9 | 2.1 | 2.8 | 3.2[b] | 2.2[b] | 2.5[b] | < 0.006[b] | 1 |

*a* For an explanation of the figures given, the bacterial indicators and the superinfecting phage, see Table 7.2.

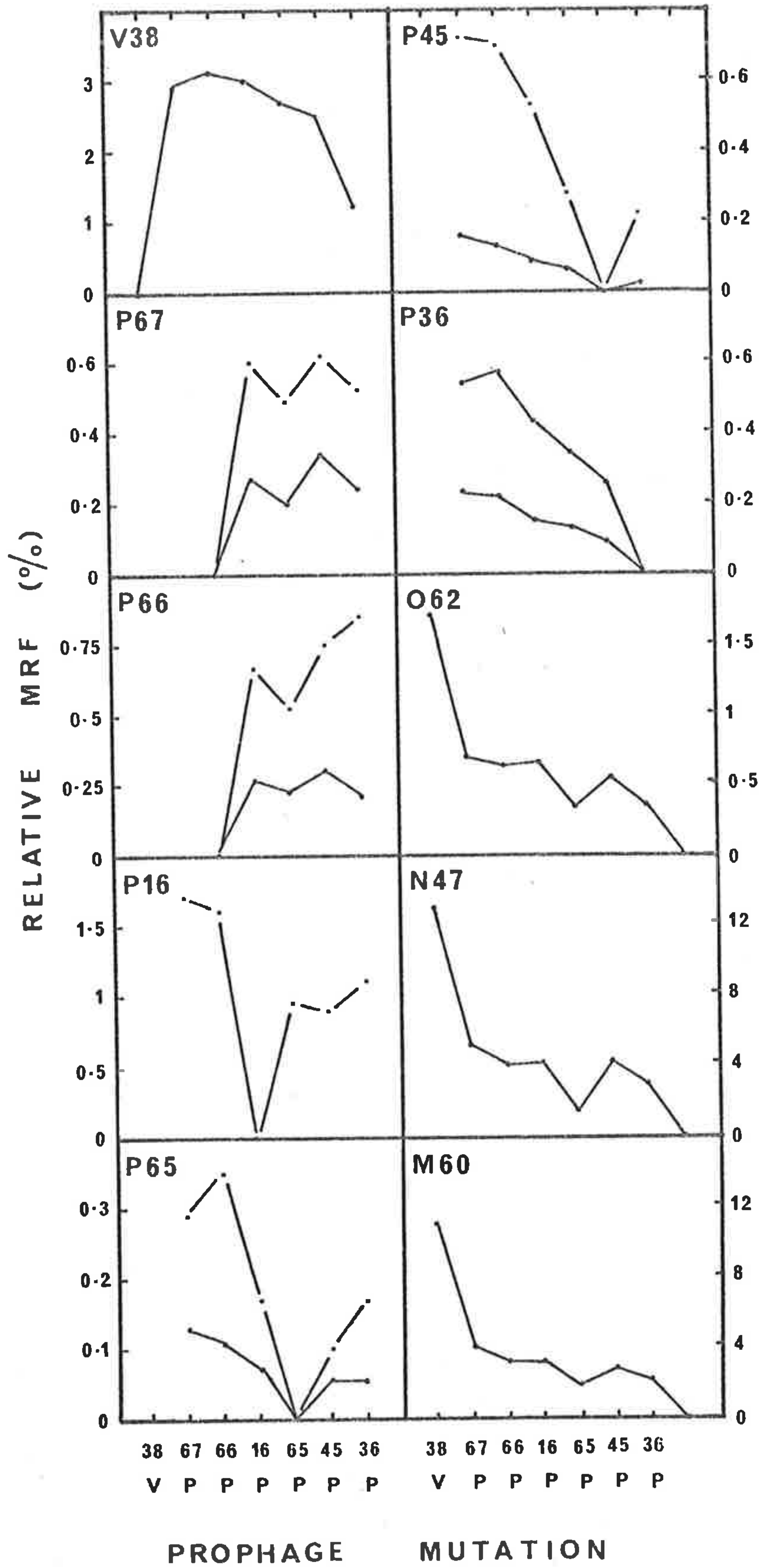
*b* These results also appear in Table 7.9.

*Figure 7.8* GRAPHS OF RELATIVE MARKER RESCUE FREQUENCIES WITH ALLELES OF GENES *M*, *N*, *O*, *P* AND *V*

For an explanation of the form of the diagram see Figure 7.2.

—●— Results from Experiment 1, Table 7.10

—■— Results from Experiment 2, Table 7.10



V38
P45
P67
P36
P66
O62
P16
N47
P65
M60
RELATIVE MRF (%)
38 V
67 P
66 P
16 P
65 P
45 P
36 P
PROPHAGE MUTATION

hand end and 66, 67 at the right-hand end. However the slopes obtained with 62, 47 or 60 as the β mutations suggest the opposite orientation. In Table 7.14, crosses involving β mutations *am*6, *am*34, *am*1 and *am*49, which all map to the left of the gene *P* alleles, suggest that 67 lies further right than 16. That is, all β mutants tested which lie to the left of the gene *P* alleles suggest the orientation 36-45-65-16-(66,67) whereas β mutations lying to the right of the gene *P* alleles suggest the orientation (66, 67)-16-65-45-36. Therefore neither the order or the gene *P* alleles nor their orientation with respect to outside alleles can be determined from these marker rescue experiments.

Results of marker rescue experiments for mutants in the 186 head genes *Q*, *R*, *S*, *T*, *U*, *V* and *W* are shown in Table 7.11 and Figure 7.9. From these results only one order is possible, namely

*V*38 - *U*63 - *U*64 - *S*4 - *R*6 - *Q*49 - *P*67

All the above mutations, with the exception of the lysozyme allele *P*67, belong to the same polarity group. *Wam*15 and *Wam*39 do not belong to this polarity group and so must lie at one end of this set of head mutations. The results for *am*15 in the β position (Table 7.11) suggest that it lies to the left of *V*38 rather than to the right of *Q*49 or *P*67 (a conclusion supported by the results of marker rescue with the phage λp186*srI*1-2, see Section 6). The above order therefore becomes:

(*W*39,*W*15) - *V*38 - *U*63 - *U*64 - *S*4 - *R*6 - *Q*49 - *P*67 ...Order 7.8

An unusual feature of the results recorded in Table 7.11 is the extremely high marker rescue from prophage carrying the *Wam*15 mutation. The only β mutation which failed to show this high recovery from an *am*15 prophage was *am*39, another gene *W* mutation. However when *am*39 was used as the prophage mutation, low (that is,

TABLE 7.11

MARKER RESCUE WITH ALLELES OF GENES *P*, *Q*, *R*, *S*, *T*, *U*, *V* AND *W*[a]

| Superinfecting phage | Prophage | | | | | | | | | Experiment number |
|---|---|---|---|---|---|---|---|---|---|---|
| | *W* *am*39 | *W* *am*15 | *V* *am*38 | *U* *am*63 | *U* *am*64 | *S* *am*4 | *R* *am*6 | *Q* *am*49 | *P* *am*67 | |
| *Wam*39 | 0.005 | 1.1 | 9.0 | 8.9 | | | | | | 3 |
| *Wam*15 | | 0.008 | 5.3 | 8.1 | 5.9 | | 11 | 8.1 | 11 | 2 |
| " | 0.39 | 0.006 | 9.2 | 9.4 | | | | | | 3 |
| *Vam*38 | | | < 0.011 | 2.9 | 3.6 | 5.8 | 6.9 | 7.7 | 8.7 | 1 |
| " | 4.8 | 59 | < 0.001 | 2.1 | | | | | | 3 |
| *Uam*63 | | | 1.3 | 0.001 | 0.89 | 2.6 | 2.5 | 2.9 | 3.8 | 1 |
| " | | > 11 | 0.64 | 0.001 | 0.51 | | 1.5 | 1.6 | 2.2 | 2 |
| " | 5.1 | 54 | 3.6 | < 0.002 | | | | | | 3 |
| *Uam*64 | | | 3.0 | 1.3 | < 0.001 | 3.6 | 4.9 | 4.3 | 6.8 | 1 |
| " | | > 17 | 0.97 | 0.38 | < 0.001 | | 1.5 | 1.7 | 2.4 | 2 |
| *Tam*8 | | | 5.4 | 4.1 | 3.7 | 3.4 | 4.5 | 5.2 | 6.8 | 1 |
| *Sam*4 | | | 2.4 | 2.0 | 1.2 | < 0.001 | 0.46 | 1.9 | 3.4 | 1 |
| *Sam*18 | | | 1.9 | 1.8 | 0.99 | < 0.001 | 0.41 | 1.0 | 2.3 | 1 |
| *Sam*34 | | | 2.0 | 1.6 | 1.4 | 0.56 | 0.56 | 0.81 | 1.8 | 1 |
| *Ram*6 | | | 2.2 | 1.7 | 1.5 | 0.89 | 0.001 | 1.9 | 3.0 | 1 |
| " | | > 14 | 1.4 | 1.5 | 1.3 | | 0.001 | 1.3 | 2.1 | 2 |
| *Qam*49 | | | 1.5 | 1.3 | 1.0 | 0.81 | 0.54 | < 0.001 | 1.5 | 1 |
| " | | > 8.4 | 1.2 | 1.3 | 1.0 | | 0.62 | < 0.001 | 1.3 | 2 |
| *Pam*67 | | | 3.9 | 3.8 | 3.6 | 3.0 | 2.9 | 2.4 | < 0.001 | 1 |
| " | | > 16 | 3.4 | 3.0 | 3.2 | | 2.2 | 1.7 | < 0.001 | 2 |

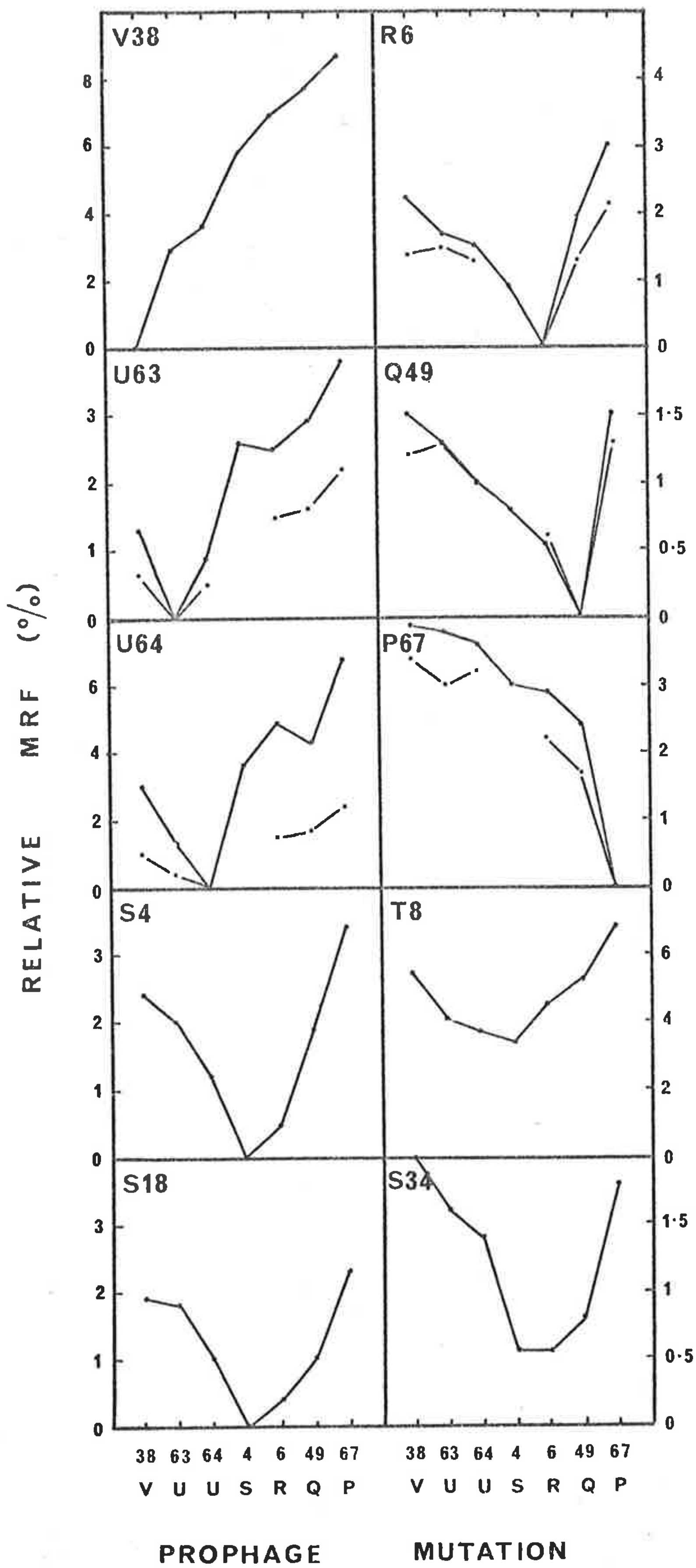
[a] For an explanation of the figures given, the bacterial indicators and the superinfecting phage, see Table 7.2.

*Figure 7.9* GRAPHS OF RELATIVE MARKER RESCUE FREQUENCIES WITH ALLELES OF GENES *P*, *Q*, *R*, *S*, *T*, *U* AND *V*

For an explanation of the form of the diagram see Figure 7.2.

—●— Results from Experiment 1, Table 7.11

—■— Results from Experiment 2, Table 7.11



V38
R6
U63
Q49
U64
P67
S4
T8
S18
S34
RELATIVE MRF (%)
38 63 64 4 6 49 67
V U U S R Q P
38 63 64 4 6 49 67
V U U S R Q P
PROPHAGE MUTATION

normal) marker rescue frequencies were obtained whether the β mutation was in gene *W* or in another gene. The reason for the different behaviour of these two alleles of gene *W* is not known.

The results of Table 7.11 (Fig. 7.9) also suggest possible positions for the mutations *Tam*8 and *Sam*34. When *am*8 is used as the β mutation the lowest point in the curve occurs when *am*4 is the α mutation. This suggests that *am*8 lies in either the 64-4 or the 4-6 interval. (In fact the 64-4 is the correct interval, see later in Table 7.13, Fig. 7.10.) With *am*34 in the β position low points in the curve correspond to mutants 4 and 6 being in the α positions. This suggests that *am*34 lies between mutants 4 and 6 although positions between 64 and 4 and between 6 and 49 are also possible. Since *am*34 lies in the same gene (gene *S*) as *am*4 a position between 6 and 49 is not likely. (The interval 4-6 appears to be the correct one. The 4-64 interval is ruled out by the results shown in Figure 7.10.) *Am*18 also lies in gene *S* but it appears to be identical with the allele *am*4 since no marker rescue could be obtained with an *am*18*vir*2 infecting phage and an *am*4 mutant prophage.

The results of Table 7.12 suggest possible positions for the mutations *Qam*1, *Uam*37 and *Ram*51. The mutation *Ram*51 appears identical with the mutation *Ram*6 (no marker rescue with 51 in the β position and 6 in the α position). The next lowest point in the curve produced by *Ram*51 as the β mutation occurs for mutants 4 and 18. This suggests that *Ram*51 lies adjacent to these mutations, which agrees with the position suggested for *Ram*6 by the results of Table 7.11. The lowest point in the curve produced by the mutation *Qam*1 occurs at the position of mutation *Qam*49, suggesting that *Qam*1 lies adjacent to *Qam*49, either to the left between *Ram*6 and *Qam*49 or to the right between *Qam*49 and the lysozyme alleles. This result is to be expected since *am*1 and *am*49

TABLE 7.12

MARKER RESCUE WITH ALLELES OF GENES *P*, *Q*, *R*, *S*, *T*, *U* AND *V*[a]

| Superinfecting phage | Prophage | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *V* *am*38 | *U* *am*63 | *U* *am*64 | *T* *am*8 | *S* *am*4 | *S* *am*18 | *R* *am*6 | *Q* *am*49 | *P* *am*67 |
| *Qam*1 | 1.4 | 1.4 | 1.1 | 0.89 | 0.84 | 0.74 | 0.81 | 0.28 | 1.4 |
| *Uam*37 | 1.5 | 0.95 | 1.0 | 0.65 | 1.4 | 1.1 | 1.3 | 1.6 | 2.5 |
| *Ram*51 | 2.2 | 1.8 | 2.1 | 1.8 | 1.4 | 0.96 | $< 0.011$ | 2.0 | 2.8 |

[a] For an explanation of the figures given, the bacterial indicators and the superinfecting phage, see Table 7.2.

belong to the same gene. (Later results (Table 7.14, Fig. 7.11) suggest that the correct position is to the left of *Qam*49.) The mutant *Uam*37 shows its lowest marker rescue frequency with the prophage mutation *am*8 suggesting a position either between 64 and 8 or between 8 and 4. (The results of Table 7.13, Fig. 7.10, suggest that 64-8 is the correct interval.)

Even though mutations 4 and 18 appear to be identical, the results for marker rescue with the prophage mutation *am*18 are lower than those obtained with the prophage mutation *am*4 (see Table 7.12). The reason for this is not known.

The results of further marker rescue experiments with alleles of the genes *R*, *S*, *T* and *U* are shown in Table 7.13 and Figure 7.10. From these results the most likely order for this set of mutations appears to be

*U*63 - *U*64 - *U*37 - *T*8 - *S*4 - *S*34 - *R*6 ...Order 7.9

Changing the positions of mutations 64 and 37 would improve the shape of the curve obtained with the β mutant *am*4 but would create dips in the curves produced by β mutants 63, 37 and 8. The change would have little effect on the shapes of the other curves. It seems most likely then that 37 is correctly placed between mutants 64 and 8. Mutants 63, 64 and 37 were assigned to gene *U* by complementation tests, but the results of these tests were somewhat unsatisfactory. The above three mutants all belong to the same polarity group but the use of the polarityity suppressing strain, XA7007, did not lead to any improvement in complementation. However on a normal $Su^-$ host (594) mutants 37 and 64 did produce some increase in phage activity on coinfection and it is quite possible that these two mutations belong to different genes.

In Table 7.13 the results involving the β mutant *Tam*8 are abnormally large. As was found in experiments using *Jam*41*vir*2 this

TABLE 7.13

MARKER RESCUE WITH ALLELES OF GENES *R*, *S*, *T* AND *U*[a]

| Superinfecting phage | Prophage | | | | | | | | Experiment number |
|---|---|---|---|---|---|---|---|---|---|
| | *U* *am*63 | *U* *am*64 | *U* *am*37 | *T* *am*8 | *S* *am*4 | *S* *am*34 | *R* *am*6 | *R* *am*51 | |
| *Uam*63 | < 0.002 | 3.8 | 4.5 | 5.1 | | | | | 2 |
| *Uam*64 | | < 0.002 | 4.2 | 5.0 | 5.4 | 5.5 | 6.8 | 29 | 1 |
| " | 2.4 | < 0.002 | 4.1 | 4.9 | | | | | 2 |
| *Uam*37 | | 4.4 | < 0.002 | 3.5 | 5.6 | 6.3 | 6.8 | 40 | 1 |
| " | 5.7 | 4.8 | < 0.006 | 4.2 | | | | | 2 |
| *Tam*8 | | 12 | 11 | < 0.11 | 14 | 15 | 20 | 5200 | 1 |
| *Sam*4 | | 3.9 | 5.2 | 3.4 | < 0.002 | 2.4 | 2.2 | 20 | 1 |
| *Sam*34 | | 6.4 | 6.5 | 5.6 | 4.0 | < 0.004 | 4.1 | 47 | 1 |
| *Ram*6 | | 10.3 | 12.1 | 11.5 | 10.7 | 10.4 | 0.001 | 0.013 | 1 |
| *Ram*51 | | 7.5 | 7.8 | 7.6 | 6.3 | 7.5 | < 0.005 | 0.005 | 1 |

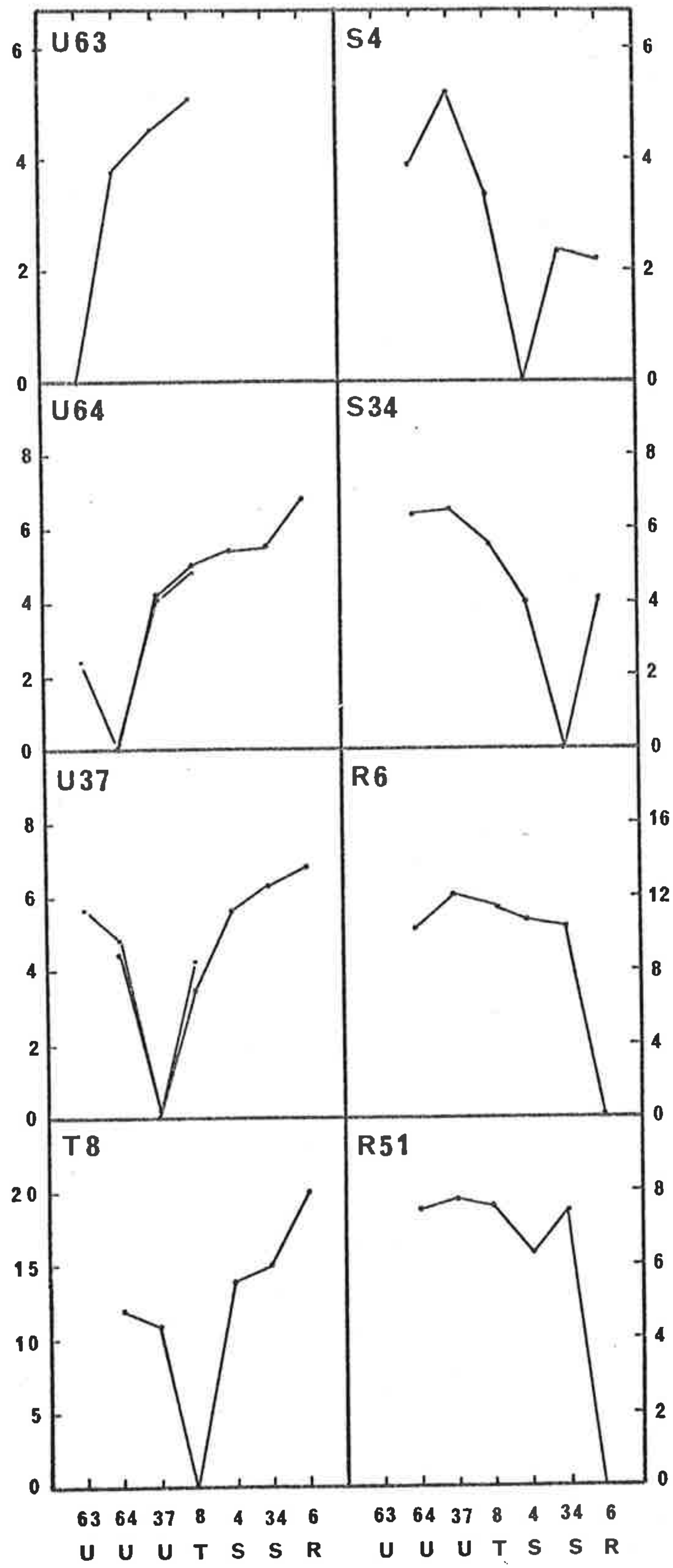
[a] For an explanation of the figures given, the bacterial indicators and the superinfecting phage, see Table 7.2.

*Figure 7.10* GRAPHS OF RELATIVE MARKER RESCUE FREQUENCIES WITH ALLELES OF GENES *R*, *S*, *T* AND *U*

For an explanation of the form of the diagram see Figure 7.2.

—●— Results of Experiment 1, Table 7.13

—■— Results of Experiment 2, Table 7.13


U63
S4
U64
S34
U37
R6
T8
R51
RELATIVE MRF (%)
63 64 37 8 4 34 6
U U U T S S R
63 64 37 8 4 34 6
U U U T S S R
PROPHAGE MUTATION

is probably due to a low recovery frequency from the 186*cIts* prophage (affecting the denominator in the calculation of the relative *mrf*). In this particular experiment the recovery of *Tam*8 from the wild-type lysogen was only 0.004% of the *eop* of this mutant on C600 (cf. results in Table 7.1). Results involving the β mutant *Ram*6 also appear somewhat large and this is even more pronounced for the results with this mutant recorded in Table 7.14. However in this case a normal recovery frequency from the 186 lysogen was obtained (approximately 0.5% of the *eop* on C600).

A further abnormal result observed in both Tables 7.13 and 7.14 is the very high recovery frequencies obtained with the prophage mutation *Ram*51. This is reminiscent of the results obtained with prophage *Wam*15 (see Table 7.11). As in that case the same effect is not observed with other alleles of the same gene (*Wam*39 in Table 7.11 and *Ram*6 in Tables 7.13 and 7.14). Since *Ram*6 and *Ram*51 differ in this respect it appears that they are, in fact, different mutations.

The final table, Table 7.14, shows marker rescue results for alleles of the genes *P*, *Q*, *R* and *S*. Graphs of these results are shown in Figure 7.11 and from them it appears that only one order is possible for this set of mutations, namely, 6-34-1-49-16-67. This order does not agree with that obtained previously from the results of Table 7.13 in which *am*34 mapped to the left of *am*6. When approached from the left by mutants 64, 37 or 8, 34 appears to map to the left of 6. When approached from the right by mutants 67, 16, 49 or 1, 34 appears to map to the right of 6. Since 34 belongs to the same gene as 4 it should map adjacent to it, that is to the left of 6 as in Figure 7.10 not to the right of 6 as in Figure 7.11. Mutant *Sam*4 clearly lies to the left of *Ram*6 whether it is approached from the left (Figs. 7.9 and 7.10) or from the right (Fig. 7.9). Ignoring

TABLE 7.14

MARKER RESCUE WITH ALLELES OF GENES *P*, *Q*, *R* AND *S*[a]

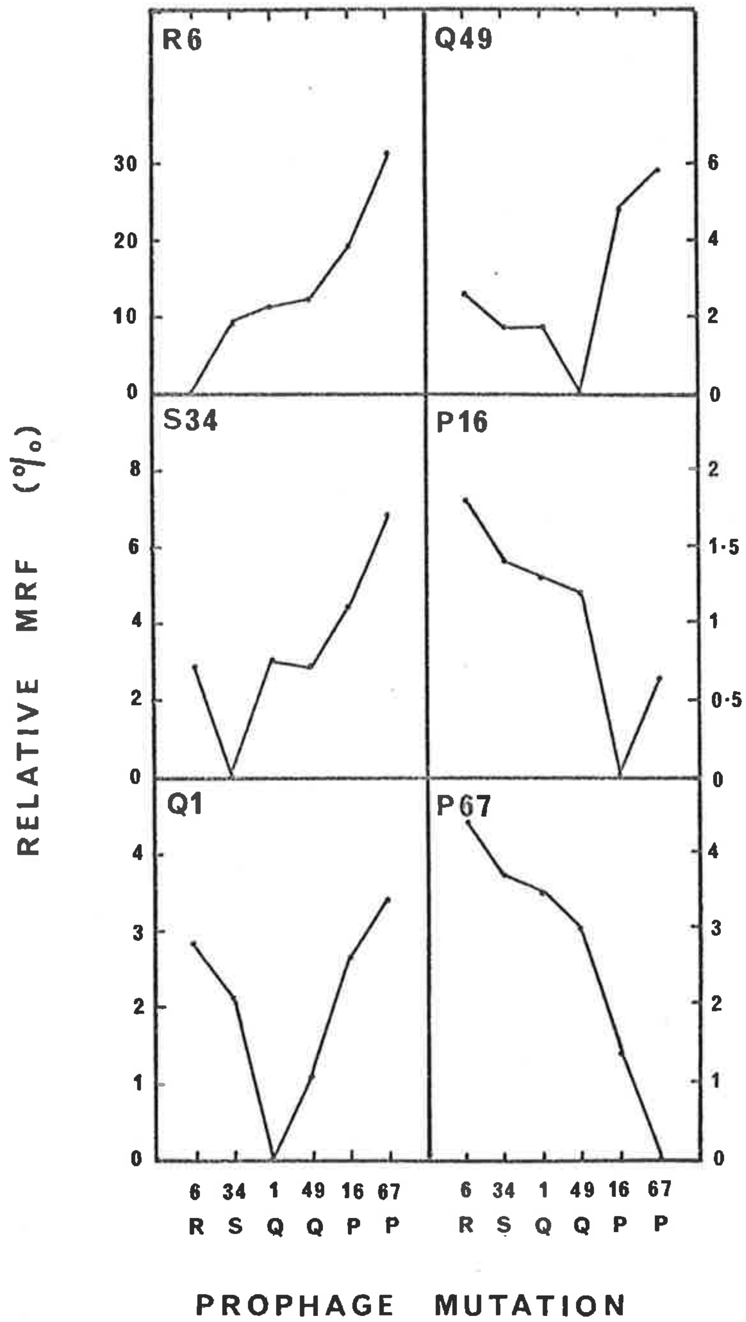
| Superinfecting phage | Prophage | | | | | | |
|---|---|---|---|---|---|---|---|
| | *R* *am*6 | *S* *am*34 | *Q* *am*1 | *Q* *am*49 | *P* *am*16 | *P* *am*67 | *R* *am*51 |
| *Ram*6 | < 0.009 | 9.0 | 11 | 12 | 19 | 31 | < 0.088 |
| *Sam*34 | 2.9 | < 0.003 | 3.0 | 2.9 | 4.4 | 6.8 | ∿30 |
| *Qam*1 | 2.8 | 2.1 | < 0.001 | 1.1 | 2.6 | 3.4 | ∿19 |
| *Qam*49 | 2.6 | 1.7 | 1.7 | < 0.001 | 4.8 | 5.8 | ∿23 |
| *Pam*16 | 1.8 | 1.4 | 1.3 | 1.2 | < 0.001 | 0.62 | ∿40 |
| *Pam*67 | 4.4 | 3.7 | 3.5 | 3.0 | 1.4 | < 0.002 | ∿38 |

[a] For an explanation of the figures given, the bacterial indicators and the superinfecting phage, see Table 7.2.

*Figure 7.11* GRAPHS OF RELATIVE MARKER RESCUE FREQUENCIES WITH ALLELES OF GENES *P*, *Q*, *R* AND *S*

For an explanation of the form of the diagram see Figure 7.2.

—◆— Results from Table 7.14


R6
Q49
S34
P16
Q1
P67
RELATIVE MRF (%)
PROPHAGE MUTATION
6 R
34 S
1 Q
49 Q
16 P
67 P
0
10
20
30
2
4
6
8
1
3
0.5
1
1.5
2
0·5
1·5

the position of *am*34, Figure 7.11 suggests the following order for the remaining mutants:

*R*6 - *Q*1 - *Q*49 - *P*16 - *P*67 ...Order 7.10

The three amber alleles of gene *W* could not be mapped with respect to each other. One of these mutants, *Wam*52, also contains a *c*I mutation (apart from the *c*I*ts* mutation) and it would be necessary to remove this mutation by recombination before *Wam*52 could be used in mapping experiments. The other two gene *W* mutations, *am*15 and *am*39 could not be mapped with respect to each other due to the abnormally large marker rescue frequencies obtained in crosses involving an *am*15 mutant prophage.

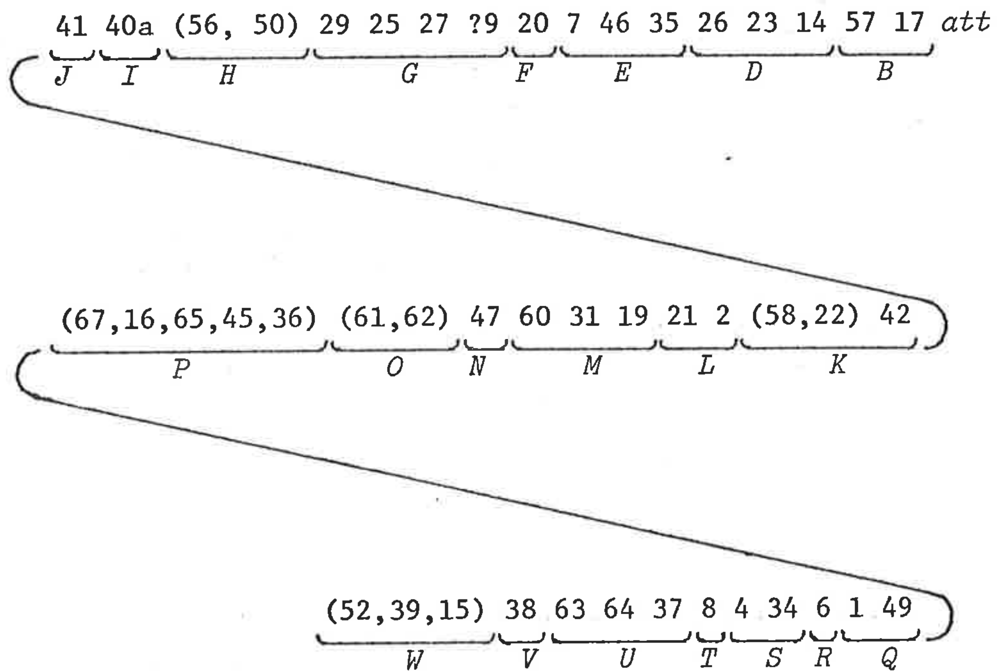
The final order for the alleles of genes *B* to *W* of phage 186, obtained by combining the orders 7.1 to 7.10, is shown in Figure 7.12.

## 7.4 DISCUSSION

The method described in this section, involving the frequency of marker rescue from *am* mutant prophage, has allowed the mapping of most of the known genes and alleles of phage 186. This method has several advantages over other mapping methods. Firstly, it is a general method which can be used for any conditional lethal mutation and does not depend on the availability of suitable deletions, as does deletion mapping. Secondly, the frequencies obtained are not dependent on the titre of the phage as determined on an $Su^+$ strain (as they are in a two-factor cross). This means that the different levels of suppression for the various amber alleles should not contribute to the variability of the results. Thirdly, the method is technically very simple, involving only phage assays on various bacterial strains. The only requirements for this mapping method are $Su^-$ lysogens of the

FIGURE 7.12

GENE AND ALLELE ORDER IN PHAGE 186




The letters represent the genes of phage 186 while the numbers refer to the amber alleles of these genes.

*att* represents the phage attachment site.

Mutants within brackets could not be ordered with respect to each other and a question mark next to an allele means the position of this mutation is doubtful.

various phage mutants and the presence on the superinfecting phage of a *vir* mutation, in addition to the mutation to be mapped.

For phage 186 another advantage in mapping by marker rescue rather than by two-factor crosses was the much larger frequency of recombination obtained. This allowed the mapping of mutations located very close together, even within a single gene. Perhaps the biggest advantage for phage 186 provided by this mapping method was the avoidance of the problem of "recombination on the plate" encountered in two-factor crosses. The frequency of this type of recombination is very dependent on the number of indicator bacterial cells coinfected by two different phage mutants. It was therefore necessary in two-factor crosses to eliminate this type of recombination by reducing the number of phage added to the plate. This problem does not occur in the marker rescue method since every indicator cell carries one of the "parental" phage and all cells infected will have the potential to form recombinants. The frequency of recombination is therefore not dependent on the actual number of phage added to the plate and so this does not have to be kept to a minimum as it does in two-factor crosses.

The size of the plaques formed in marker rescue experiments was smaller than the average size of wild-type 186 plaques. This is probably due in part to the requirement for recombination, and in part to the smaller size of a 186*vir* plaque on a 186 lysogen when compared with its size on a nonlysogen. Larger plaques were observed in Section 6 for marker rescue from the heteroimmune λp186 transducing phage. However the smaller plaque size was no problem since the plaques were large enough to be scored easily.

A potential problem with the use of marker rescue as a mapping method is the possibility of recovery by complementation rather than by recombination. Fortunately, for phage 186, the alleles of

only one gene, gene *B*, appeared to be recovered by this mechanism. In practice, even these two alleles were able to be mapped by marker rescue, from the results of crosses in which they were present as prophage mutations.

There were, however, several problems which did occur with this method. For example, extremely large marker rescue frequencies were obtained in experiments involving prophage mutations *Wam*15 and *Ram*51. This cannot be accounted for by complementation and appears to be a property of these two mutations rather than of the genes in which they are located. Other alleles in the same genes did not show the same behaviour and the fact that *Ram*51 and *Ram*6 differed in this respect probably means that these two mutations are not the same even though little, if any, recombination could be demonstrated between them. The reason for the high recoveries with prophage mutants *Wam*15 and *Ram*51 is not known.

Larger than normal marker rescue frequencies were also obtained with several superinfecting phage mutants. This was probably due to lower than normal recovery from the wild-type 186 lysogen, and while it is hard to understand why this should occur, it does not seem to affect the order of prophage mutations derived with these mutants.

Finally the most serious problem encountered was the misleading, although highly reproducible, frequencies obtained for certain pairs of mutations. In some cases the mutant or combination of mutants at fault were obvious. For example in Table 7.5 when the results for the mutant *Gam*9 were excluded a unique order was obtained for the remaining seven mutations. However in certain other cases in which more than one mutant or pair of mutants was giving misleading frequencies the order of alleles could not be determined. For example

no order could be obtained for the alleles of gene *P*. The reason for these systematic errors in recombination frequency is again not known.

Of the known essential genes of phage P2 the one mapping at the left-hand end of the genome is gene *Q*. When mapping this gene Lindahl (1969a) noted that "In most, but not all, cases, the recombination frequencies obtained with the mutants in this cistron are unexpectedly high, however, when the frequencies are compared with the other data. The reason for this is not known". This behaviour is similar to that described in this section for the alleles of gene *W*, the 186 gene mapping at the left end of the genome. (Problems were also encountered with 186*Wam*15 in two-factor crosses. However, in this case the reverse problem arose, recombination frequencies being too small, with the result that *Wam*15, a head mutant, appeared to map within the tail region.) It may be that many of the frequency problems encountered in the marker rescue experiments described in this section are not peculiar to this method.

Despite the problems listed above the frequency of marker rescue proved a successful method for the mapping of the 186 genome. Although the position of several of the alleles is somewhat doubtful there appears to be little doubt about the gene order. The position of gene *J*, represented by the single mutant, *am*41, is probably the only doubtful assignment.

# SECTION 8

## λp186 TRANSDUCING PHAGE AND P2-186 HYBRID PHAGE

## 8.1 INTRODUCTION

As a method for mapping the genes of phage 186 two-factor crosses were found to be very unsatisfactory (see Section 5). The method described in Section 7, based on the frequency of marker rescue with various 186 *am* mutants from *am* mutant prophage, was found to be much more reliable. It is a general method and can be used for any of the genes of 186. However the best method of all was found to be deletion mapping (see Section 6) but unfortunately this is not a general method and is limited by the number and the location of deletion mutants available.

The only "deletions" of 186 that were available were the P2-186 hybrid phage made by Cliff Bradley. P2 and 186 are sufficiently different for marker rescue with 186 *am* mutants to occur only from the 186 portion of the prophage. However the P2-186 cross-over points in all these hybrids are located within the tail region, and since these hybrids can only be used in the mapping of 186 genes located to the right of the cross-over points (and therefore present on the hybrid genomes) they could not be used in the mapping of the head region and part of the tail region which are located to the left of the cross-over points. Several unsuccessful attempts to make more hybrids are described in this section.

The availability of restriction enzymes and λ phage and plasmid vectors has provided another and a more general method for the production of 186 deletions. Any section of the 186 genome can be separated from other sections provided it is bounded by two restriction sites for which a suitable phage or plasmid vector is available. In this section the construction of two λ phage carrying different pieces of 186, generated by the restriction enzyme *Eco*RI, is described. These phage are referred to in this thesis as λp186 phage since they

resemble other λ transducing phage (for example λp*bio*), the difference being that these phage carry a piece of 186 DNA rather than a piece of *E. coli* DNA.

## 8.2 CONSTRUCTION OF λp186 PHAGE

The restriction endonuclease *Eco*RI cuts 186 DNA in three places producing fragments of size 0.7 kb, 2.5 kb, 3.5 kb and 23 kb (R. B. Saint, personal communication). The order of these fragments from left to right on the 186 map is: 0.7 kb - 3.5 kb - 23 kb - 2.5 kb (R. B. Saint, personal communication). The two end fragments, each containing the 19 base pairs of complementary single stranded DNA of a 186 cohesive end, can cohere to form a fifth fragment of size 3.2 kb. The action of the enzyme *Eco*RI itself produces single stranded cohesive ends and of the above five fragments the 3.2 kb, 3.5 kb and 23 kb fragments will have two, 4 base cohesive ends produced by the *Eco*RI enzyme. The remaining two fragments of size 0.7 kb and 2.5 kb will only have one of these cohesive ends, the other end being the 19 base single stranded region of the 186 cohesive end. Fragments with two "*Eco*RI ends" can be inserted into vector molecules (which have also been restricted by the enzyme *Eco*RI) by virtue of the complementarity of the cohesive ends.

The λ vector used in the construction of the λp186 phage was an immunity insertion vector, λ*imm*$^{434}$ stock number 641 (Murray *et al.*, 1977). This phage has only one *Eco*RI restriction site and this lies within the *c*I gene. In the formation of a λp186 phage the piece of 186 DNA will be inserted into the λ*imm*$^{434}$ *c*I gene and the resulting phage will therefore have a clear plaque phenotype. Any λ DNA molecules which have escaped restriction will form turbid plaques after transfection, as will molecules formed by the rejoining of the

two λ fragments, and so these phage can be easily distinguished from λ phage carrying 186 fragments.

The λ vector used also has several DNA deletions, the net deletion being equivalent to 20% of a λ DNA molecule. It can accommodate an extra fragment of DNA with molecular weight up to about 6 x $10^6$ daltons (Murray *et al.*, 1977), that is up to a length of about 9 kb. Therefore of the 186 fragments bounded by two *Eco*RI sites only two are small enough to be incorporated into the vector. These are the 3.5 kb piece and the 3.2 kb combination of the two end fragments. (Incorporation of both these fragments together as a 6.7 kb piece would also be possible in the event of incomplete digestion.)

In the preparation of the λp186 phage a mixture of λ641 DNA and 186*c*I*ts* DNA was restricted with the enzyme *Eco*RI and then ligated with T4 polynucleotide ligase. The ligated mixture was used in a transfection experiment and the morphology of plaques recorded.

Two restriction mixtures were prepared and each one used in a ligation reaction. Samples of all four mixtures, each containing 0.1μg of λ DNA, were used to transfect strain ED8654. 0.001μg of unrestricted λ641 DNA was also tested by transfection. The plaques obtained after transfection were tested for phage immunity by stabbing into lawns of E513 and E573 (C600 lysogens of phage λ*imm*$^{434}$ and $186^+$ respectively). The results of these transfections are shown in Table 8.1.

The restriction efficiency in this experiment appeared to be satisfactory as only one in every 2,300 of the λ molecules remained intact after restriction. Two clear plaques with the immunity of λ*imm*$^{434}$ were also obtained after transfection of the restriction mixes and these were subsequently shown to be due to λp186 phage. Therefore some ligation has occurred even in the absence of added ligase.

## TABLE 8.1

## TRANSFECTION WITH RESTRICTED AND RESTRICTED/LIGATED MIXTURES OF DNA FROM PHAGE λ641 AND 186*cIts*[a]

| DNA | Treatment | Transfectants | | | |
|---|---|---|---|---|---|
| | | Total *pfu* (per 0.1μg λ DNA) | Plaque types | | |
| | | | 186 | λ (turbid) | λ (clear) |
| λ641 | | $1.2 \times 10^5$ | | | |
| λ641 + 186*cIts* (1) | restriction | 84 | 25 | 57 | 2 |
| λ641 + 186*cIts* (2) | restriction | 182 | 134 | 48 | 0 |
| λ641 + 186*cIts* (1) | restriction/ligation | 109 | 7 | 96 | 6 |
| λ641 + 186*cIts* (2) | restriction/ligation | 258 | 200 | 56 | 2 |

[a] DNA mixtures (1) and (2) were restricted with the restriction endonuclease *Eco*RI, a sample of each ligated with T4 polynucleotide ligase and all four mixtures used to transfect strain ED8654.

Presumably this is due to the *E. coli* polynucleotide ligase acting during transfection. Some of the $\lambda imm^{434}$ turbid plaques appearing after transfection may also be produced by ligation events (rather than being due to non-restricted molecules) especially since in this case only one ligation is required compared with the two necessary to form a λp186 phage.

Unfortunately the transfections for the ligation mixtures and restriction mixtures were not done on the same day and so the transfection frequencies cannot really be compared since the competency of the cells in transfection may have differed on the two days. However it does not appear as though the ligation reaction was very successful. A large increase in *pfu* after ligation was not obtained. Only one day elapsed between the preparation of the ligation mixture and its use in transfection and better ligation may perhaps have been obtained by extending this period. Murray *et al.* 1977 reported that a maximum yield of phage was usually obtained after a period of 4-6 days. However, despite this, an additional 8 clear plaques with the immunity of $\lambda imm^{434}$ were obtained. A repeat transfection experiment with all the restriction and ligation mixtures as well as with untreated λ641 DNA was done a week after the initial transfection. In this experiment there was no increase in *pfu* after ligation; however one more clear plaque with $\lambda imm^{434}$ immunity was obtained. Therefore, despite the relatively poor ligation obtained in these experiments a total of 11 presumptive λp186 phage were isolated.

It is possible that these 11 clear plaque isolates are the result of point mutations in the $\lambda imm^{434}$ *c*I gene, rather than being the result of insertions of 186 DNA. Alternatively they may have been produced by incorrect rejoining of the two λ fragments, causing a small deletion within the *c*I gene and thus the loss of repressor activity.

In this case the single *Eco*RI restriction site of the vector molecule will also be lost, whereas if the isolates are due to point mutations the restriction site should be unaffected. In either case the number of restriction sites will be less than the number in a genuine λp186 phage which should have two sites.

The number of *Eco*RI restriction sites in a phage molecule can be readily determined by a simple genetic test (Murray and Murray, 1974). The greater the number of restriction sites per DNA molecule the fewer such molecules will escape restriction. The sensitivities of different DNA molecules to the restriction enzyme *Eco*RI can be compared by reference to their restriction coefficients, the restriction coefficient (RC) being defined as the titre obtained on a non-restricting strain (for example strain 5K) divided by the titre on a restricting strain (for example strain 5KR1).

When restriction coefficients were determined for the eleven clear plaque isolates using strains 5K and 5KR1 two distinct classes were obtained. One class, containing six of the isolates, had an average restriction coefficient of 30. The other class, containing the five remaining isolates, was restricted four to five times more efficiently (average RC = 140). However both these restriction coefficients appear to be consistent with the presence of two *Eco*RI sites. Murray and Murray (1974) obtained RCs of 30 and 80 for phage with 2 sites, an RC of 400 for a phage with 3 sites and an RC of 10 or less for phage with only one site. The restriction coefficient of a turbid plaque isolate obtained by transfection with the ligation mixture was determined and found to be 10, in agreement with the expectation of only one site for such a phage. The 11 clear plaque isolates therefore appear to be genuine λp186 phage rather than spontaneous clear plaque mutants, or *c*I deletion mutants of λ641.

As described earlier there are only two pieces of 186 DNA which could be incorporated into the vector λ641. It is possible that the two RC classes obtained correspond to the two possible λp186 phage. However it may also be that only one of the two possible 186 pieces is being inserted, although in two different orientations. The difference in the RC could then be due to the different combination of half sites produced in each of these orientations.

However when dilysogens of each of the eleven isolates of λp186 were tested by marker rescue it was found that the wild-type allele of 186*c*I*ts*W*am*15 could be recovered from all the six isolates belonging to the class with an RC of 30 but could not be recovered from any of the five isolates belonging to the other class (RC = 140), that is the two RC classes do seem to correspond to the two different 186 pieces. Confirmation of this was provided by R. B. Saint when he digested the DNA of two λp186s (one from each RC class) with the enzyme *Eco*RI and compared the fragments obtained on a gel with those of 186*c*I*ts*. He found that the λp186 with an RC of 30 had a fragment corresponding to the 3.5 kb fragment of 186 while the λp186 with an RC of 140 contained a fragment of 3.2 kb corresponding to the combination of the two end fragments of 186.

The three *Eco*RI sites of 186 are labelled 1, 2 and 3 from left to right on the 186 map. The λp186 phage with RCs of 30 contain the DNA of 186 lying between the *Eco*RI sites 1 and 2 and have been named λp186*srI*1-2. The other isolates (RC = 140) contain the DNA to the right of site 3 and to the left of site 1, including the 186 cohesive ends. These phage are named λp186*srI*3-1.

No significant differences were obtained in the RCs of the isolates belonging to the same class. This suggests that the orientation of the 186 DNA is not affecting the restriction coefficient. The

right-hand half site of site 3 together with the left-hand half site of site 1 are four to five times more sensitive to *Eco*RI than the right-hand half site of site 1 together with the left-hand half site of site 2, but no conclusion can be drawn about the relative sensitivities of the whole sites 1, 2 and 3.

## 8.3 PREPARATION OF DILYSOGENS

The eleven λp186 isolates described in the previous section are all unable to form lysogens since the 186 DNA has been inserted into the λ*imm*$^{434}$ repressor gene. However to be of use in marker rescue experiments lysogens are required. These lysogens can be prepared by means of co-lysogenization with a helper phage λ*imm*$^{434}$*Nam*7 which can provide the repressor product that the λp186 phage is unable to produce. Since the helper phage is defective in gene *N* it should not produce the *int* protein and so should not be able to form a lysogen in the absence of the λp186 phage. Therefore the only lysogen produced should be the dilysogen, in which case the λp186 would provide the *int* protein and the helper phage λ*imm*$^{434}$*Nam*7 would provide the repressor.

Such dilysogens were prepared by spotting a mixture of the two phage ($10^7$ *pfu*/ml of each phage) onto a lawn of strain 594. After overnight incubation at 37°C bacteria from the centre of the spot were streaked for single colonies. These colonies were tested by stabbing into lawns of 594 and C600. A monolysogen of the helper phage should show phage activity on C600 but not on 594 (due to the *Nam*7 mutation), whereas a dilysogen should show phage activity on both strains. Single colonies showing phage activity on both strains were purified through 2 or 3 more single colony isolations with testing for phage production at each step. Dilysogens were prepared in this way for all 11 isolates of the λp186 phage made with the restriction enzyme *Eco*RI as well as for 5

isolates of a λp186 phage made by R. B. Saint with the restriction enzyme *Hind*III.

The frequency of dilysogen formation was low; only 3.7% of the colonies tested turned out to be dilysogens. The frequency of helper phage monolysogens was even lower, 0.7% of the total colonies, and since these were not tested further they may not in fact be true monolysogens but rather be due to carry-over of free phage.

The dilysogens formed appeared to be more unstable than a normal monolysogen. Even after purification through four single colony isolations there were usually one or two non-lysogenic colonies to be found in every 30 to 40 tested. Several very tiny plaques were also sometimes noticeable on lawns of the dilysogens and this may also indicate instability. However despite this the dilysogens were completely satisfactory in marker rescue experiments (see Section 6).

## 8.4 ATTEMPTS TO MAKE P2-186 HYBRID PHAGE

### a. "Reverse" hybrids

Cliff Bradley isolated P2-186 hybrid phage by selection of recombinants on an *E. coli* C strain lysogenic for phage P2. Neither parental phage was able to grow on this strain since 186 cannot adsorb to *E. coli* C strains (Woods and Egan, 1974) and P2 cannot grow on a P2 lysogen. The only phage able to grow would be a hybrid, with phage P2 tails (and presumably adsorption properties) and a 186 immunity region. The reverse type hybrid having 186 tail proteins and a P2 immunity region would not of course plate on the selective indicator.

To make such a "reverse" hybrid a strain resistant to P2 but not to 186 is required, but no such strain has been isolated. However P2 shows a requirement for $Ca^{++}$ in adsorption not shown by 186. Bertani *et al.* (1969) found that the addition of 0.04M sodium citrate

to an LG plate prevented the growth of P2 wild-type phage while 0.03M sodium citrate had very little effect. However under the conditions used here it was found that the addition of 0.01M sodium citrate to an LG plate was sufficient to prevent any growth of P2 (the *eop* was less than 1 in 4 x $10^8$). This concentration had no effect on the plating efficiency of 186*cIts* (*eop* = 1). However higher concentrations, 0.03M or more, completely eliminated 186 and 0.02M severely reduced its *eop*.

The calcium dependency of P2 can therefore be used as a selection technique for reverse hybrids. Such a hybrid should be able to grow when plated on a 186 lysogen on an LG plate supplemented with 0.01M sodium citrate, but neither parental phage should grow. The lack of $Ca^{++}$ should prevent P2 growth and 186 immunity should prevent growth of 186. A "forward" hybrid (P2 tails, 186 immunity) should also fail to grow.

Table 8.2 records the results of a recombination experiment between P2 wild-type and 186*cIts* phage. Also shown are results for single infections with each of the parental mutants. In the mixed infection P2 appears to be produced at a normal frequency but the production of 186 is depressed when compared with the titre obtained in a single infection.

The selection technique used in this experiment was satisfactory since neither parental phage grew on the selective indicator. However no hybrid phage were obtained suggesting that the frequency of "reverse" hybrid formation is less than 1 in 5 x $10^8$. Cliff Bradley obtained "forward" hybrids in this same region at a frequency of about 1 in 2 x $10^7$ and this suggests that the failure to obtain "reverse" hybrids is not due to an inability of the parental DNAs to recombine but rather to the inviability of the hybrid formed. (Unless, of course, the requirement for $Ca^{++}$ is not due to a single gene product

TABLE 8.2

ATTEMPTED ISOLATION OF "REVERSE" HYBRIDS BY RECOMBINATION BETWEEN 186*cIts* AND $P2^+$

| Indicator | Plate | Measure of: | Titre (*pfu*/ml) in phage lysates | | |
|---|---|---|---|---|---|
| | | | Single infections | | Co-infection |
| | | | 186*cIts* | $P2^+$ | 186*cIts* + $P2^+$ |
| C600 | LGC | Total progeny | $2.7 \times 10^{10}$ | $4.0 \times 10^{9}$ | $5.0 \times 10^{9}$ |
| C600 | LGcit | 186 progeny | $2.7 \times 10^{10}$ | $< 1 \times 10^{1}$ | $1.7 \times 10^{9}$ |
| C600($186^+$) | LGC | P2 progeny | $< 1 \times 10^{1}$ | $3.1 \times 10^{9}$ | $2.1 \times 10^{9}$ |
| C600($186^+$) | LGcit | Hybrid phage | $< 1 \times 10^{1}$ | $< 1 \times 10^{1}$ | $< 1 \times 10^{1}$ |

mapping to the left of the P2 *att* site.)

Viable hybrid phage exist which consist of the early gene region of the 186 genome and the late, or structural gene region of the P2 genome. Some of these viable hybrids even have mixed P2 and 186 structural genes (hybrids 9, 10 and 11). However the reverse situation in which 186 structural genes would be combined with P2 early genes could be inviable for several reasons. For example the products of the P2 early genes may be unable to act on the late region of 186, or perhaps the length of DNA generated may be too long to be packaged in a 186 head particle (which is probably smaller than a P2 head particle (Ackermann, 1974)). K. Murray has also attempted to isolate reverse hybrids by the above procedure. He used a P2 phage containing the *del*1 deletion which is missing approximately 7% of the P2 genome from the extreme right-hand end (Chattoraj and Inman, 1972; Chattoraj *et al.*, 1975). However he did not obtain any hybrids and it appears that reverse hybrids are probably inviable *per se*.

b. "Forward" hybrids

Attempts to produce forward hybrids with cross-overs in regions other than those obtained by Cliff Bradley were equally unsuccessful. In these attempts amber mutants of P2 and 186 were recombined and wild-type recombinants selected on an $Su^-$ indicator (strain 594). Two crosses were tried, 186*cItsWam*15 x P2$vir_1 amE_{30}$ and 186*cItsOam*61 x P2$vir_1 amE_{30}$. Whether a forward or a reverse hybrid would result from these crosses depends on the relative locations of the 186 and P2 mutations. In both the above crosses the 186 mutations should lie to the left of the P2 mutation and so a "forward" hybrid (that is a 186 immune hybrid) should result.

The main problem in these two experiments was the reversion of the amber mutations. In no case was there any suggestion of an

increase in *pfu* on the selective indicator for the co-infection above the level of reversion obtained for the single infections. The P2*amE* mutation reverted at a frequency of about 1 in 5 x $10^6$. This problem can be eliminated by selecting the hybrid on E811 (594 lysogenic for P2) but the reversion of the 186 amber mutation (~1 in 3 x $10^7$) although lower than for the P2 amber mutations is still too high to permit isolation of hybrids.

For future attempts of this kind the reversion frequency must be reduced, for example by the use of a double mutant, or the recombination frequency increased above the level of reversion, for example by UV irradiation of the parental P2 and 186 phage. It is known that UV irradiation enhances both 186-186 recombination (Section 5) and P2-P2 recombination (Lindahl, 1969a) and so it is possible that it would also increase P2-186 recombination.

## 8.5 HETERODUPLEX ANALYSIS OF P2-186 HYBRIDS

Heteroduplex analysis of P2-186 hybrids Hy2 and Hy5 and of the 186 insertion mutants *ins*1, *ins*2 and *ins*3, (Younghusband *et al.*, 1975), when combined with the results of marker rescue experiments, provided information about the physical positions of both 186 and P2 genes on their respective genomes. Heteroduplex analysis with the other hybrid phage could provide more information about physical positions and this section describes some preliminary data obtained for the hybrids Hy9 and Hy11 as well as for the 186 *int* mutant, *hr*22.

Homoduplex lengths for molecules of Hy11 and *hr*22 were determined and are recorded in Table 8.3. An internal ϕX174 RF standard was present in the *hr*22 and Hy11 preparations but not in the Hy9 preparation and the length of hybrid 9 was determined approximately by using the internal standard of the *hr*22 preparation. The *hr*22 and

TABLE 8.3

MOLECULAR LENGTH MEASUREMENTS OF DNA FROM P2-186 HYBRID PHAGE, Hy9 AND Hy11 AND FROM A 186 *int* MUTANT *hr*22

| DNA | No. of molecules | Mean length measured (cm)[a] | Length (base pairs)[b] |
|---|---|---|---|
| 186*hr*22*ins*5 | 50 | 39.53 ± 1.55 | 32,700 ± 1300 |
| ϕX174 RF | 79 | 6.28 ± 0.41 | |
| Hy9 | 70 | 40.64 ± 1.52 | 33,700 ± 1300 |
| Hy11 | 33 | 54.24 ± 1.34 | 34.000 ± 800 |
| ϕX174 RF | 55 | 8.30 ± 0.49 | |

[a] This column records the actual lengths measured. The larger values for the Hy11 preparation are due to a higher EM magnification setting.

[b] Relative to an internal standard of 5,200 base pairs for ϕX174 RF (Wu *et al.*, 1972). (Standard not present in the Hy9 preparation.)

Hy9 homoduplex grids were photographed on the same film, with the same EM settings and the molecules were measured consecutively and under identical magnification conditions. The ϕX174 RF molecules present in the *hr*22 prepration are therefore acting as a calibration standard for the electron microscope in the measurement of the Hy9 molecules. However the results for Hy9 are less reliable than those for *hr*22 and Hy11 due to the absence of the internal standard.

From the length measurements it appears that the DNA of *hr*22 is 10% longer than that of wild-type 186. (The length of 186 is 29,600 ± 600 base pairs (Younghusband *et al.*, 1975).) This is considerably larger than the 4.2% additions obtained for other 186 *hr* mutants (Younghusband *et al.*, 1975). Some 186/*hr*22 heteroduplexes were prepared but there were insufficient molecules for significant measurements to be obtained. However, on inspection, the size of the insertion loop appeared to be less than 10% of the length of a 186 molecule. The size of the loop in two heteroduplexes measured was approximately 5% of the molecular length. Measurements of a much larger sample of heteroduplexes would be required to determine the exact length of the insertion loop but it appears that it is greater than for the other known insertion mutants. Measurements of more heteroduplexes should also provide information on the location of the insertion. It is likely that the addition of DNA is responsible for the $int^-$ phenotype and thus the location of the inserted DNA will provide more mapping data for the 186 *int* gene. The location of the single stranded loop in the few heteroduplexes examined was consistent with the position of the *int* gene as determined by heteroduplex analysis with the mutant *hr*19*ins*3 (Younghusband *et al.*, 1975).

The lengths of the hybrid 9 and hybrid 11 homoduplexes were found to be 33,700 ± 1300 and 34,000 ± 800 base pairs, respectively.

These lengths are similar to those obtained by Younghusband *et al.* (1975) for hybrids 5 and 2 (32,900 ± 300 and 34,300 ± 600 base pairs respectively). The DNA length of all four hybrids is greater than that present in either parental phage. Several Hy11/P2 and Hy9/Hy11 heteroduplexes were prepared but the cross-over points could not be determined since too few were obtained to allow accurate measurements. However a consideration of the marker rescue results of Section 6 allows a rough estimation of the region in which the cross-over must have occurred.

The results of Table 6.4 show that the P2 DNA in both hybrids 9 and 11 extends to the right of the P2 gene *F*. The DNA of hybrid 11 extends even further right to include gene *E*. Lindahl (1971) mapped the non-essential P2 gene *fun* between genes *G* and *F*, that is to the left of gene *F*. The *del*2 deletion which is genetically *fun* (Bertani, 1975) is viable and so cannot overlap either genes *G* or *F*. This deletion mutant is lacking the DNA located between 45.5% and 51.6% from the left end of the P2 genome. Therefore P2 gene *F* must lie to the right of the 51.6% position and hybrids 9 and 11 which have the P2 gene *F* must have at least 51.6% (or 16.6 kb) of the P2 genome. This does not allow for gene *F* and there must be a minimum of at least 17 kb of P2 DNA in these two hybrids.

The results of Table 6.1 show that both hybrids 9 and 11 have the 186 gene *G* and the results of Table 6.5 show that this gene must lie to the left of the site 186*shn*2, located 37.2% (or 11 kb) from the right-hand end of 186. Hybrid 11 has one 186 gene (gene *G*) to the left of this site and hybrid 9 has four (genes *G*, *H*, *I* and *J*). Therefore the minimum length of 186 DNA present in these hybrids is 11½ - 12 kb for hybrid 11 and approximately 13 - 15 kb for hybrid 9.

The homology maps constructed by Younghusband and Inman (1974) show that there is a region of high homology between the DNA of

phage P2 and 186 located 17 to 21 kilobases from the left end of P2 and 14.5 to 17.5 kilobases from the left end of 186 (or 12 to 15 kb from the right end of 186). The marker rescue results discussed above provide minimum estimates of the lengths of P2 and 186 DNA in the hybrid phage. By subtracting these lengths from the lengths of the Hy9 and Hy11 molecules (Table 8.3) maximum estimates of the length of the P2 and 186 contributions can be obtained. This shows that Hy9 should cross-over between 17 and 21 kb from the left end of P2 and between 13 and 17 kb from the right end of 186. Hy11 should cross-over 18 to 22 kb from the left end of P2 and approximately 12 to 16 kb from the right end of 186. For both phage it seems likely that the cross-over has occurred within the region of high P2-186 homology, although for Hy9 a position just to the left of the region in the 186 DNA is possible. However both hybrid phage should have at least part of the homology region to the right of the cross-over point.

The presence of homology to the right of the cross-over point will create problems in heteroduplex analysis since the location of the single stranded regions may reflect the end of a region of homology rather than the hybrid cross-over point. Two Hy9/P2 heteroduplexes observed showed a long region of duplex DNA at one end followed by an unequal substitution loop, then a small duplex region and finally a long single stranded region at the other end. This is consistent with a cross-over within or just to the left of the high homology region. This region contains alternating sections of high and partial homology (Younghusband and Inman, 1974) and the conditions used in the preparation of these heteroduplexes were such that the partial homology should appear single stranded while the high homology regions would be duplex. The measurement of many heteroduplexes should allow a determination of the location of the substitution loop and this should help in locating

the hybrid cross-over point. However if this cross-over point occurs in a region of high homology it is unlikely that its exact position could be determined by heteroduplex analysis. A few Hy9/Hy11 heteroduplexes were also prepared and showed a small substitution loop but once again analysis of these would be complicated by the presence of P2-186 homology.

A better method for determining the cross-over points in these hybrids would probably be to compare their restriction maps with those of the parental phage P2 and 186. Although in heteroduplex analysis P2 and 186 show a high degree of homology this is not reflected in the location of restriction sites. R. B. Saint has shown that there is no similarity in the restriction maps of phage 186 and P2 obtained with the restriction enzymes *Eco*RI, *Hind*III, *Bgl*II, *Bam*HI, *Sal*I or *Pst*I. He has digested DNA of Hy10 (which is genetically very similar to Hy9) with the restriction enzyme *Sal*I and has found that a restriction site present 14.6 kb from the right end of 186 is still present in the hybrid DNA. The cross-over point in the hybrid must therefore lie to the left of this position. It is probable that this restriction site would not be present in hybrid 11 and if this were so it would limit the amount of 186 DNA present in this hybrid to less than 14.6 kilobases. Similar experiments with other restriction enzymes and with P2 as well as with 186 should lead to a fairly accurate location for the hybrid cross-over points.

## 8.6 DISCUSSION

In phage 186 the production of deletion phage, for use in mapping experiments, has been a problem. In phage λ the generation of defective phage carrying nearby bacterial genes is an effective way of producing deletions of essential λ genes. However this method could

not be used for phage 186 since no known bacterial gene is situated near enough to the 186 attachment site to be incorporated in a 186 defective phage. Another possibility would be to lysogenize 186 at an unusual site in the *E. coli* chromosome, nearby to a selective bacterial marker, as has been done for phage λ (Shimada *et al.*, 1972, 1973). It should then be possible to isolate defective transducing phage from these lysogens. However the lysogenization of a phage at an unusual site requires a deletion of the normal phage attachment site and no such deletion of the 186 *att* site is known.

P2-186 hybrid phage have been very useful as a form of 186 "deletion" but the formation of such hybrids is limited by the requirement that a viable phage be produced from a combination of P2 and 186 genes. Such a limitation does not exist for the production of λp186 phage since the presence or absence of the 186 DNA has no effect on the viability of the λ vector. A very large range of 186 "deletions" can therefore be made by this method, the only limitation being the number of restriction enzymes and vectors available. So far 186 fragments produced by the enzymes *Eco*RI and *Hind*III have been "cloned" but there are many more fragments which could be cloned with the restriction enzymes and vectors already available. For example the restriction enzymes *Sal*I and *Bam*HI produce useful fragments of 186 and plasmid vectors for these enzymes are available. Fragments bounded by different restriction sites can also be cloned. For example there are both phage and plasmid vectors available which could be used to clone pieces of DNA lying between *Eco*RI and *Hind*III sites.

Such λp186 phage could have many uses. They could be used, as in this thesis, both for the genetic mapping of the 186 genes and for the determination of the physical positions of these genes on the 186 genome. They could also be used for selecting mRNA from particular

regions of the 186 genome for the purpose of transcription studies. They could even be used for studying the proteins of phage 186 which could not be studied, as were the proteins of phage λ (Hendrix, 1971; Murialdo and Siminovitch, 1971) in UV irradiated bacterial cells because UV irradiation inhibits 186 production (Woods, 1972). However as part of a λp186 molecule they could be studied in UV irradiated cells, so long as the 186 fragment incorporated had its own promoter or was under the control of a λ promoter. λp186 phage carrying amber mutations could then be used to identify, on gels, the proteins coded for by each gene.

## 8.7 SUMMARY

1. Two λp186 phage have been constructed. λp186*srI*3-1 contains the left 0.7 kb and right 2.5 kb fragments of 186 (including the cohesive ends) and λp186*srI*1-2 contains a 3.5 kb fragment from the 186 head gene region.

2. "Reverse" hybrids (186 structural proteins, P2 immunity) occur at a frequency of less than 1 in 5 x $10^8$. They are probably inviable.

3. Preliminary results suggest that Hy11 is 34,000 ± 800 base pairs in length, Hy9 33,700 ± 1300 base pairs and *hr*22 32,700 ± 1300 base pairs.

# SECTION 9

# GENERAL DISCUSSION

In this thesis each section has included a discussion of the results recorded therein and this discussion will be confined to some general comments on the function and location of the 186 genes. Where possible these will be compared with those of the closely related phage P2.

For phage 186 22 essential genes have been defined, and of these 18 are involved in phage morphogenesis; 11 in the formation of the phage tail and 7 in the formation of the phage head. Of the 4 remaining essential genes the product of only one, gene *A*, is required for DNA replication. The product of gene *B* is not required for DNA replication but probably controls the synthesis of late mRNA (E. J. Finnegan, personal communication). The product of gene *P* is required for host cell lysis and the function of gene *O* is unknown. Gene *O* does not appear to be involved in morphogenesis.

In addition to these 22 essential genes there are probably others which have not, as yet, been identified. In particular the results of Section 4 suggest the possible presence of two more genes, one an "early" gene and the other a "late" gene. The early gene would be responsible for the inhibition of host DNA synthesis occurring after induction of phage 186. This inhibition occurred for all mutants tested including gene *A* and gene *B* mutants. The unidentified gene is not, therefore, under the control of either gene *A* or gene *B*. It is an early gene since inhibition commences within 10 minutes after induction, well before the onset of 186 DNA replication. An unidentified late gene could be responsible for the termination of 186 DNA replication occurring at the end of the lytic cycle. This termination of DNA replication is not the result of host cell lysis since it occurs even after induction of gene *P* mutants which are unable to lyse the host cell. The λ late gene *S* is responsible for the termination

of DNA synthesis at the end of the λ lytic cycle and an unidentified 186 gene may have a function analogous to this gene. However there are other possible explanations for the termination of DNA synthesis (see Section 4) and 186 may not have a λ "S" type gene. For phage P2 the presence of an unidentified gene of the λ "S" type has also been suggested in order to account for the termination of phage DNA synthesis (Lindqvist, 1971) and phage protein synthesis (Lengyel and Calendar, 1974) occurring at the normal lysis time, even after infection of cells by lysis defective mutants.

The marker rescue results described in this thesis have allowed the construction of a genetic map of phage 186 and this is shown in Figure 9.1. From the map it can be seen that 186 shows the typical clustering of genes with related functions found for other bacteriophage. It can also be seen that there are extensive similarities between the 186 map and that of the related phage P2. For both phage all the morphogenetic genes are located to the left of the phage attachment site and they are arranged in the same order on the genome, namely genes concerned with phage head synthesis at the left and genes concerned with phage tail synthesis further right, the two groups being separated by a gene involved in host cell lysis. In phage 186 there is a second non-morphogenetic gene (gene *O*)located between the head and tail gene groups but the function of this gene is unknown.

In preliminary investigations of the 7 known head genes of phage 186, mutants in only two, genes *T* and *U*, were found not to accumulate head related structures in defective lysates. Mutants in the head genes *R*, *S*, *V* and *W* did produce head-like structures but these were invariably "empty". Several apparently full heads were observed in a lysate produced by a mutant in the head gene *Q*. For

*Figure 9.1* GENETIC MAP OF COLIPHAGE 186

This map shows the order of the 186 genes as determined in this thesis and is drawn to scale with genes being spaced equally within the limits defined by the known physical locations of restriction sites and hybrid cross-over points.

The functions of the genes are recorded below the map.

The horizontal lines above the genes refer to transcription units as defined by polarity groups.

The numbers at the top of the map record the physical locations on the 186 genome of restriction sites, hybrid cross-over points, the genes *c*I and *int* and the site *ori*, in percentages of the 186 genome from the left-hand end. (Figures for the *c*I and *int* genes and the Hy2 and Hy5 cross-over points are from Younghusband *et al.*, 1975. Figures for the *Eco*RI and *Hin*dIII restriction sites are from R. B. Saint, personal communication. The figure for *ori* is from Chattoraj and Inman, 1973.)

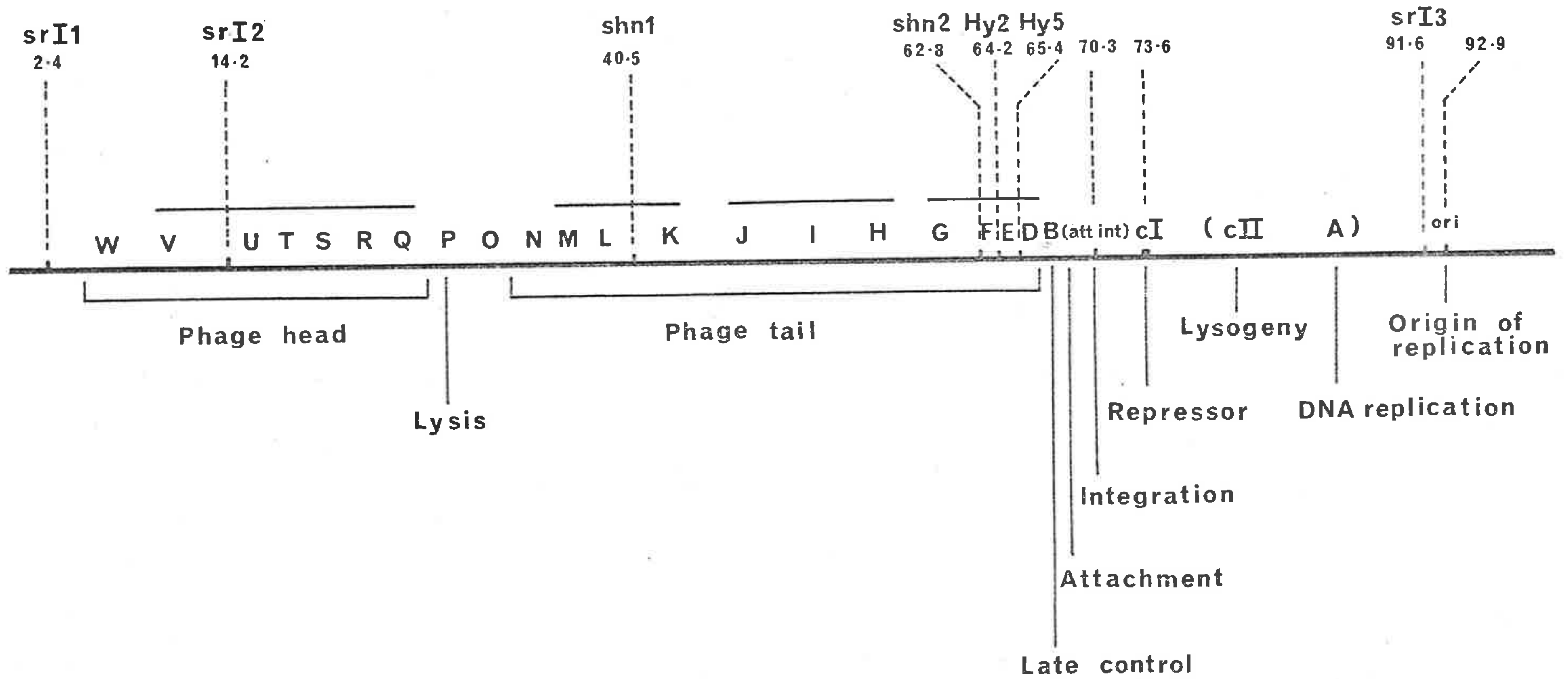
Brackets indicate that the order of genes or sites within them is not known.

The function of gene *O* is unknown.

Gene *V* is involved in phage morphogenesis but the exact nature of its function is uncertain.

The *vir*2 mutation has been mapped to the right of the *att* site but its location with respect to the other genes in this region is unknown.

The origin of replication is located close to the *Eco*RI restriction site *srI*3 but the order of these two sites on the genetic map is not known.


srI1
2·4
srI2
14·2
shn1
40·5
shn2
62·8
Hy2
64·2
Hy5
65·4
70·3
73·6
srI3
91·6
92·9
W V U T S R Q P O N M L K J I H G F E D B (att int) cI ( cII A ) ori
Phage head
Lysis
Phage tail
Late control
Attachment
Integration
Repressor
Lysogeny
DNA replication
Origin of replication

phage P2 there are six known head genes (Lindahl, 1974) and of these only two (genes *N* and *O*) fail to show any head related structures in mutant infected cells (Lengyel *et al.*, 1973). These two genes may perhaps be equivalent in function to the 186 genes *T* and *U*. The P2 *N* gene codes for a precursor of the major capsid protein (Lengyel *et al.*, 1973).

Of the 186 head genes all but gene *W*, which maps at the left-hand end of the genome, belong to a single polarity group. The two genes mapping at the left end of the P2 genome (genes *P* and *Q*) also belong to a separate polarity group and the 186 gene *W* may perhaps be equivalent in function to one of these genes. In addition to defective heads, the mutant in the 186 gene *V* also showed abnormally long tails, although this mutant is an efficient tail donor in *in vitro* reconstitution. No equivalent gene in phage P2 is known.

There is a possible analogy between the functions of gene *Q* of 186 and gene *L* of P2, both of which are involved in phage head formation. Full heads were observed in the lysate produced by 186*Qam*1 and a relatively high proportion of full heads has been found in P2 *L* mutant infected cells (Lengyel *et al.*, 1973). The work of Pruss (1977) also shows that P2 *L* mutants are capable of producing full heads. The locations of genes *Q* of 186 and *L* of P2 are also similar, both mapping to the right of all the other known head genes. DNA containing, but defective heads have also been found to be intermediates in the assembly of the head particles of both λ and T4. For λ, mutants in two head genes, *W* and $F_{II}$, produce heads which are morphologically indistinguishable from normal heads (Casjens *et al.*, 1972). For T4 there are several head genes whose mutants produce DNA containing heads (King, 1968).

When examined by electron microscopy the defective lysates

produced by mutants in most of the 186 tail genes showed no tail-related structures. One exception to this was the defective lysates produced by a mutant in gene *N*. Electron micrographs of these lysates showed that tail structures were present but were abnormal in morphology, varying in length and often showing the core projecting from the sheath. Almost identical results have been reported for a mutant in the P2 gene *R* (Lengyel *et al.*, 1974). Both genes *N* of 186 and *R* of P2 map at the extreme left of the tail regions in their respective genomes, and it would appear that both function to control the length of the phage tail. λ mutants, in gene *U*, unable to control the length of the phage tail have also been described (Mount *et al.*, 1968; Kemp *et al.*, 1968).

The 186 tail gene *N* also differed from all the other tail genes in its reconstitution properties. The lysate produced by a gene *N* mutant showed reconstitution with lysates produced by mutants in all the other tail genes although the level of this reconstitution was very much lower than that obtained for head-tail joining. Efficient intra-tail reonstitution has been demonstrated for phage λ, when special conditions were used for the preparation of the defective lysates (Kühl and Katsura, 1975). These authors demonstrated that many of the steps involved in tail assembly could occur *in vitro*. The results with the 186 gene *N* suggest that intra-tail *in vitro* reconstitution could also be used to study 186 tail morphogenesis. The lysates used here were of the same type used by Weigle (1966) who did not obtain any intra-tail reconstitution for λ. Using the methods of Kühl and Katsura (1975) more information might be obtained about the assembly of the 186 phage tail.

Intra-tail reconstitution for the P2 gene *R* mutant has not been reported and it is doubtful that low levels of intra-tail reconstitution such as those found for the 186 gene *N* mutant could be

detected in the presence of the large number of viable phage present in the P2 mutant lysates. These phage may be unadsorbed phage or be the result of leaky mutations. In either case their presence reduces the usefulness of *in vitro* reconstitution for the study of P2 morphogenesis. No such problem was encountered for 186 since the *am* mutants available are not at all leaky and the inducibility of 186 allows the preparation of lysates by induction, eliminating the problem of unadsorbed phage.

In addition to gene *N* mutants, mutants in two other tail genes, *H* and *J* showed tail structures in electron micrographs of defective lysates. These tail particles appeared to be normal and this behaviour has not been observed for any of the known P2 tail genes (Lengyel *et al.*, 1974).

The 186 morphogenetic genes are arranged in four known polarity groups. One group contains 6 of the 7 known head genes, the other three groups each contain 3 or 4 known tail genes. Since only *am* mutants of 186 have been studied the direction of transcription of these genes is unknown.

To the right of the 186 tail region but still to the left of the *att* site lies the essential gene *B*. This gene is not involved in phage morphogenesis or in phage DNA replication but appears to be involved in controlling late mRNA synthesis. The failure of gene *B* mutants to terminate DNA synthesis at the normal lysis time may be due to the product of gene *B* itself or, more likely, to the product of one (or more) of the late genes under gene *B* control.

In phage P2 a non-morphogenetic gene mapping between the tail region and the phage attachment site has also been identified (Sunshine and Sauer, 1975). A mutation in a bacterial gene, *gro*, has been found to prevent P2 wild-type infection by preventing the synthesis

of late mRNA (Sunshine and Sauer, 1975). A P2 mutant, *ogr*, has been identified which overcomes this block, allowing formation of late mRNA. That is, the *ogr* gene controls production of late mRNA in bacterial *gro* mutants and Sunshine and Sauer (1975) suggest that the wild-type form of the *ogr* gene probably controls late mRNA production in a wild-type P2 infection. Gene *ogr* therefore would be analogous both in function and in location to the 186 gene *B*.

Only one known essential gene of phage 186, gene *A*, is located to the right of the *att* site. The product of this gene is required for DNA synthesis. To the right of the P2 *att* site two essential genes have been found, genes *A* and *B*, both of which are required for P2 DNA synthesis. However other properties of the 186 gene *A* strongly suggest that its function is analogous to that of the P2 gene *A* rather than the P2 gene *B*. No known 186 gene has yet been found with a function equivalent to that of the P2 gene *B*. The P2 *A* gene and the 186 *A* gene seem very similar in that both are essential genes required for DNA replication, both show very poor complementation suggesting both *cis*-action and a requirement of their products for the expression of other genes, and both map in a similar position on the genome, to the right of the *att* site.

Only three non-essential genes have been identified in phage 186. These are the genes *int*, *c*I and *c*II. The *int* and *c*I genes have been located on the genome by heteroduplex analysis (Younghusband *et al.*, 1975) and found to lie 70.3% and 73.6% from the left end of the genome respectively. The cross-over point in the hybrid phage Hy5 occurs 65.4% from the left end of the 186 genome. Between the *c*I gene and the Hy5 cross-over point lie the genes *int*, *B* and *D* as well as part of gene *E*. Therefore, in a distance of only 8.2% (or 2.4 kb) there are known to be three complete genes (*int*, *B* and *D*) and parts of

two others (*E* and *c*I). It is therefore most unlikely that any other unidentified genes lie in this region. Genes *c*II and *A* (both of which map to the right of *att*) have therefore been placed to the right of the *c*I gene. The relative order of these two genes is not known.

Genes *A*, *c*I and *c*II have all been shown to map to the right of the phage attachment site while gene *B* maps to the left of it. The *int* gene has not been mapped relative to *att* and there are therefore two possible locations for the phage *att* site, namely, between genes *B* and *int* or between genes *int* and *c*I. For future work it will be important to determine the location of the *int* gene with respect to the *att* site since both the location of the *int* gene and its direction of transcription are basic to any comparison of the induction properties of 186 and P2.

The P2 *int* gene lies to the right of the P2 *att* site as does the P2 gene *C*. Gene *C* of P2 appears to be equivalent to gene *c*I of 186 in coding for the repressor protein. A P2 gene corresponding to gene *c*II of 186 has not as yet been found. The location of the P2 operator as defined by "strong virulent" mutations is to the right of the P2 *att* site, between genes *C* and *B* (Lindahl, 1971). The location of the 186 operator (again as defined by virulent mutations) is also to the right of the phage *att* site but its exact location is not known.

Other P2 non-essential genes which have been mapped include genes *cox*, *fun*, *old* and *Z*. The product of the *cox* gene is required for the excision of a P2 prophage but is not required for integration (Lindahl and Sunshine, 1972), whereas the product of the *int* gene is required for both processes (Choe, 1969). For phage 186 the product of the *int* gene is also required for both integration and excision (Bradley *et al.*, 1975) but it is not known whether 186 requires

the product of any other gene (such as the *cox* gene of P2 or the *xis* gene of λ) since excision-deficient mutants have not been looked for.

The presence of prophage P2 converts *E. coli* C to sensitivity to 5-fluoro-deoxyuridine and failure to cause this conversion is due to a mutation in the gene *fun*. The effect of the presence of a 186 prophage on sensitivity of the host to 5-fluoro-deoxyuridine has not been tested and so it is not known whether or not 186 has a *fun* gene. Phage λ is unable to grow on a P2 lysogen and this phenomenon is due to the product of the P2 gene *old*. Since λ can grow on a 186 lysogen it appears that 186 does not have an *old* gene. No gene analogous to the P2 gene Z has yet been found for 186.

Marker rescue experiments have allowed the mapping of many of the 186 essential genes in relation to known physical positions on the genome, such as restriction sites and hybrid cross-over points. From Figure 9.1 it can be seen that the left-hand 75% of the genome is well represented with identified genes. There are 23 known genes in the left hand 22 kb (75%) of the 186 genome. The distribution of these genes, however, is not uniform, the regions 14.2% - 40.5% and 62.8% - 73.6% being heavily occupied while the regions 0% - 14.2% and 40.5% - 62.8% are less well represented.

Contrasting with the relative gene saturation found in the left-hand 75% of the genome is the right-hand 25% of the genome in which only two known genes (*c*II and *A*) are located. A similarly blank region is found in the P2 genome, only two genes, *A* and *old*, mapping in the right-hand 20% of the genome. As mentioned in the introduction, 7% of the DNA from the extreme right-hand end (excluding the cohesive end) can be removed without affecting the viability of the phage. Therefore much of the DNA in the right-hand section of the P2 genome either codes for non-essential proteins or does not code for proteins

at all. This probably also applies to the right-hand section of the 186 genome.

Apart from genes *c*II and *A* only one other known feature lies in the right-hand 25% of the genome. This is the origin of replication which is located 92.9 ± 1.8% from the left end (Chattoraj and Inman, 1973). This is relatively close to the restriction site *srI*3 (91.6%) but these figures are not accurate enough to determine the relative positions of these two sites. The allele *am*5 of gene *A* could not be recovered from the phage λp186*srI*3-1 (see Section 6) and so this allele lies either to the left of the site *srI*3 or if to the right then so close that marker rescue could not be detected. These results do not allow a determination of the location of gene *A* with respect to the origin of replication. For phage ϕX174 there is a gene, gene *A*, which is similar to the 186 and P2 genes *A* in that it is both *cis*-acting and required for DNA replication (Sinsheimer, 1968). It has been found that the ϕX174 origin of replication is located within this gene (Weisbeek and Van Arkel, 1976). This may be a general feature of *cis*-acting proteins required for DNA replication and in this connection it would be of interest to determine the relative locations of the 186 origin of replication and the 186 gene *A*. A more accurate determination of the location of the 186 gene *A* could be obtained by marker rescue from λp186 phage (or plasmid-186 *in vitro* recombinants) generated by other restriction enzymes. For phage P2 the location of gene *A* with respect to the origin of replication is similarly unknown. The origin of replication is located 89% from the left end of the genome (Schnös and Inman, 1971) while gene *B* is located 80% from the left end (Chattoraj and Inman, 1974). The location of the P2 gene *A* is not known other than that it lies to the right of gene *B*.

The map shown in Figure 9.1 shows only the relative locations of the 186 genes but the method developed in Section 7 has allowed finer mapping of the alleles within these genes. (For a map of the allele order see Figure 7.12.) This method is particularly useful because of its generality and it can be used for all 186 essential genes. This method could also be used for phage P2 since the results of Section 6 show that rescue of P2 functions is by recombination not complementation. However it could not be used to map the genes of phage λ due to the phenomenon of late gene transactivation.

For phage λ late gene functions can be recovered from a prophage, after heteroimmune superinfection, in the absence of phage excision and recombination (Thomas, 1970). This suggests that the late genes are only under indirect immunity control and that some λ product (presumably the *Q* gene product) produced by the superinfecting phage is able to turn on (or transactivate) the λ late proteins. These products can then be used by the superinfecting phage (that is, complementation can occur).

Similar transactivation of late genes does not occur for phage 186, as shown by the dependence of marker rescue frequencies on the distances between mutations. Transactivation of the P2 late genes in the hybrid phage is similarly absent (see Section 6) and L. E. Bertani and E. Six (personal communications, cited by Lindahl (1970)) have suggested that transactivation may not occur for phage P2. It would therefore appear that either the late proteins of 186 and P2 are under direct immunity control or else the superinfecting phage is unable to supply the protein necessary to turn on late genes. Direct immunity control of the late genes seems unlikely since these genes are found in several apparently independent transcription units, while there appears to be only one repressor binding site (as judged by the

relative ease with which *vir* mutations are obtained).

As suggested by Lindahl (1970) a lack of transactivation of P2 late genes could be due to a requirement for the P2 *A* gene product, which is required for the expression of all late P2 genes (Lindahl, 1970). This product is *cis*-acting and so *A* protein made by the superinfecting phage could not be used by the prophage for the expression of its late genes. Since 186 gene *A* also appears to be *cis*-acting the same explanation could account for the lack of transactivation in this phage.

The question now arises of why the product of the 186 gene *B* can be recovered by complementation (see Tables 6.1 and 7.1). It would appear that either gene *A* product is not required for gene *B* product synthesis and that *B* protein is turned on by the superinfecting phage as the result of the action of another phage protein, or that gene *B* product is made constitutively in a 186 lysogen. In either case it would appear that the gene *A* product is not required for gene *B* protein synthesis. However the absence of complementation between these two mutants suggests that *A* product is required for the expression of gene *B*. This contradiction is hard to resolve. It may suggest a different control of *B* protein synthesis in vegetative phage and prophage. Such a difference in the expression of the P2 *int* gene led Bertani (1970) to propose a split operon model. Complicated split operon models could also be devised to explain 186 *B* protein synthesis. For example, the product of an unidentified gene *X* may repress *B* protein synthesis until the product of gene *A* releases this inhibition. If gene *X* and its promoter are separated by the *att* site then no *X* protein will be made by the prophage and gene *B* could be expressed constitutively. Gene *B* would therefore only be indirectly under *A* gene control in contrast to the other essential genes which would be

under direct control. However this complicated system seems a bit unlikely and it would appear to be more useful at this stage to obtain more information about gene *B* before proposing any models. Such information should include a determination of the direction of transcription of this gene and a determination of whether *B* mRNA is made constitutively by the prophage or whether its synthesis is activated by the superinfecting phage. This could be done using the multiple-step hybridization method described by Bøvre and Szybalski (1971).

Since the gene *B* product is either present in the lysogenic state or transactivated by the superinfecting phage it is either not under repressor control or only indirectly under repressor control. A determination of whether *B* mRNA is present in a lysogen would distinguish between these alternatives. The product of gene *B* is required for late mRNA synthesis (E. J. Finnegan, personal communication) but the absence of transactivation, and the poor complementation of *A* gene mutants show that the product of gene *A* is also required.

Although the method described in Section 7 cannot be used for mapping λ genes because of the presence of transactivation of the late genes, it could still be used to map mutations occurring within a single gene. In this case no recovery of phage by complementation should be possible and the number of recombinant phage should reflect the distance between alleles. Such mapping has been successful for the alleles 17 and 57 of the 186 gene *B* despite the fact that gene *B* is recovered by complementation.

# BIBLIOGRAPHY

Adhya, S., Sen, A. and Mitra, S. (1971). In *The Bacteriophage Lambda* (A.D.Hershey, ed.), pp. 743-746. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Akermann, H. W. (1974). In *Handbook of Microbiology* (A.I.Laskin and H.A.Lechevalier, eds.), vol. 1, pp. 579-607. CRC Press, Cleveland, Ohio.

Amati, P. and Meselson, M. (1965). *Genetics* 51, 369-379.

Anderson, T. F. (1960). *Proc. Eur. Reg. Conf. Electron Microscopy (Delft)*, vol. 2, pp. 1008-1011.

Appleyard, R. K. (1954). *Genetics* 39, 440-452.

Baldwin, R. L., Barrand, P., Fritsch, A., Goldthwait, D. A. and Jacob, F. (1966). *J. molec. Biol.* 17, 343-357.

Beckwith, J. (1963). *Biochim. biophys. Acta (Amst.)* 76, 162-164.

Bertani, G. (1968). In *Molecular Genetics* (H.Wittman and N.Schuster, eds.), pp. 180-186. Springer-Verlag, Berlin.

Bertani, G. (1975). *Molec. gen. Genet.* 136, 107-137.

Bertani, G. and Bertani, L. E. (1974). *Proc. nat. Acad. Sci. (Wash.)* 71, 315-319.

Bertani, G., Choe, B. K. and Lindahl, G. (1969). *J. gen. Virol.* 5, 97-104.

Bertani, L. E. (1960). *Virology* 12, 553-569.

Bertani, L. E. (1964). *Biochim. biophys. Acta (Amst.)* 87, 631-640.

Bertani, L. E. (1968). *Virology* 36, 87-103.

Bertani, L. E. (1970). *Proc. nat. Acad. Sci. (Wash.)* 65, 331-336.

Bertani, L. E. (1976). *Virology* 71, 85-96.

Bertani, L. E. and Levy, J. A. (1964). *Virology* 22, 634-640.

Bode, V. C. (1971). *J. Virol.* 8, 349-351.

Bøvre, K. and Szybalski, W. (1971). *Methods in Enzymology* 21, 350-383.

Bradley, C. (1972). B.Sc.(Honours) Thesis, University of Adelaide.

Bradley, C., Ong, P. L. and Egan, J. B. (1975). *Molec. gen. Genet.* 140, 123-135.

Brooks, K. (1965). *Virology* 26, 489-499.

Buchwald, M. and Siminovitch, L. (1969). *Virology* 38, 1-7.

Campbell, A. (1959). *Virology* 9, 293-305.

Campbell, A. (1961). *Virology* 14, 22-32.

Campbell, A. (1965). *Virology* 27, 329-339.

Campbell, A. and del Campillo-Campbell, A. (1963). *J. Bact.* 85, 1202-1207.

Casjens, S. (1971). In *The Bacteriophage Lambda* (A.D.Hershey, ed.), pp. 725-738. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Casjens, S., Hohn, T. and Kaiser, A. D. (1972). *J. molec. Biol.* 64, 551-563.

Chattoraj, D. K. and Inman, R. B. (1972). *J. molec. Biol.* 66, 423-434.

Chattoraj, D. K. and Inman, R. B. (1973). *Proc. nat. Acad. Sci. (Wash.)* 70, 1768-1771.

Chattoraj, D. K. and Inman, R. B. (1974). *Proc. nat. Acad. Sci. (Wash.)* 71, 311-314.

Chattoraj, D. K., Younghusband, H. B. and Inman, R. B. (1975). *Molec. gen. Genet.* 136, 139-149.

Choe, B. K. (1969). *Molec. gen. Genet.* 105, 275-284.

Cohen, S. N. and Chang, A. C. Y. (1970). *J. molec. Biol.* 49, 557-575.

Davis, R. W. and Parkinson, J. S. (1971). *J. molec. Biol.* 56, 403-423.

Davis, R. W., Simon, M. and Davidson, N. (1971). *Methods in Enzymology* 21, 413-428.

Dove, W. F. (1966). *J. molec. Biol.* 19, 187-201.

Edgar, R. S. and Wood, W. B. (1966). *Proc. nat. Acad. Sci. (Wash.)* 55, 498-505.

Epstein, R. H., Bolle, A., Steinberg, C. M., Kellenberger, E., Boy de la Tour, E., Chevalley, R., Edgar, R. S., Susman, M., Denhardt, G. H. and Lielausis, A. (1963). *Cold Spring Harbor Symp. quant. Biol.* 28, 375-394.

Garen, A., Garen, S. and Wilhelm, R. C. (1965). *J. molec. Biol.* 14, 167-178.

Gottesman, M. E. and Yarmolinsky, M. B. (1968). *J. molec. Biol.* 31, 487-505.

Harris, A. W., Mount, D. W. A., Fuerst, C. R. and Siminovitch, L. (1967). *Virology* 32, 553-569.

Hendrix, R. W. (1971). In *The Bacteriophage Lambda* (A.D.Hershey, ed.), pp. 355-370. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Hershey, A. D. (1958). *Cold Spring Harbor Symp. quant. Biol.* 23, 19-46.

Hocking, S. M. (1972). B.Sc.(Honours) Thesis, University of Adelaide.

Hohn, B. and Hohn, T. (1974). *Proc. nat. Acad. Sci. (Wash.)* 71, 2372-2376.

Hohn, T. and Hohn, B. (1973). *J. molec. Biol.* 79, 649-662.

Hubacek, J. and Glover, S. W. (1970). *J. molec. Biol.* 50, 111-127.

Huddleston, V. (1970). B.Sc.(Honours) Thesis, University of Adelaide.

Inman, R. B. and Bertani, G. (1969). *J. molec. Biol.* 44, 533-549.

Inman, R. B., Schnös, M., Simon, L. D., Six, E. W. and Walker, D. H. (1971). *Virology* 44, 67-72.

Jacob, F. and Wollman, E. L. (1956). *Ann. Inst. Pasteur* 91, 486-510.

Jacob, F. and Wollman, E. L. (1961). *Sexuality and the Genetics of Bacteria.* Academic Press, New York.

Joyner, A., Isaacs, L. N., Echols, H. and Sly, W. S. (1966). *J. molec. Biol.* 19, 174-186.

Kaiser, D. and Masuda, T. (1973). *Proc. nat. Acad. Sci. (Wash.)* 70, 260-264.

Katsura, I. and Kühl, P. W. (1975a). *J. molec. Biol.* 91, 257-273.

Katsura, I. and Kühl, P. W. (1975b). *Virology* 63, 238-251.

Kemp, C. L., Howatson, A. F. and Siminovitch, L. (1968). *Virology* 36, 490-502.

King, J. (1968). *J. molec. Biol.* 32, 231-262.

Kretschmer, P. J. and Egan, J. B. (1975). *J. Virol.* 16, 642-651.

Kühl, P. W. and Katsura, I. (1975). *Virology* 63, 221-237.

Lengyel, J. A. and Calendar, R. (1974). *Virology* 57, 305-313.

Lengyel, J. A., Goldstein, R. N., Marsh, M., Sunshine, M. G. and Calendar, R. (1973). *Virology* 53, 1-23.

Lengyel, J. A., Goldstein, R. N., Marsh, M. and Calendar, R. (1974). *Virology* 62, 161-174.

Lindahl, G. (1969a). *Virology* 39, 839-860.

Lindahl, G. (1969b). *Virology* 39, 861-866.

Lindahl, G. (1970). *Virology* 42, 522-533.

Lindahl, G. (1971). *Virology* 46, 620-633.

Lindahl, G. (1974). *Molec. gen. Genet.* 128, 249-260.

Lindahl, G., Sironi, G., Bialy, H. and Calendar, R. (1970). *Proc. nat. Acad. Sci. (Wash.)* 66, 587-594.

Lindahl, G. and Sunshine, M. (1972). *Virology* 49, 180-187.

Lindqvist, B. H. (1971). *Molec. gen. Genet.* 110, 178-196.

Lindqvist, B. H. and Sinsheimer, R. L. (1967). *J. molec. Biol.* 30, 69-80.

Luzzati, D. (1970). *J. molec. Biol.* 49, 515-519.

Mandel, M. and Berg, A. (1968). *Proc. nat. Acad. Sci. (Wash.)* 60, 265-268.

Mandel, M. and Kornreich, B. (1972). *Virology* 49, 300-301.

Morse, D. E. and Primakoff, P. (1970). *Nature (London)* 226, 28-31.

Mount, D. W. A., Harris, A. W., Fuerst, C. R. and Siminovitch, L. (1968). *Virology* 35, 134-149.

Murialdo, H. and Siminovitch, L. (1971). In *The Bacteriophage Lambda* (A.D.Hershey, ed.), pp. 711-723. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Murialdo, H. and Siminovitch, L. (1972). *Virology* 48, 785-823.

Murray, N. E., Brammar, W. J. and Murray, K. (1977). *Molec. gen. Genet.* 150, 53-61.

Murray, K. and Murray, N. E. (1973). *Nature (London)* 243, 134-139.

Murray, N. E. and Murray, K. (1974). *Nature (London)* 251, 476-481.

Ong, P. L. (1973). B.Sc.(Honours) Thesis, University of Adelaide.

Padmanabhan, R. and Wu, R. (1972). *J. molec. Biol.* 65, 447-467.

Parkinson, J. S. (1968). *Genetics* 59, 311-325.

Protass, J. J. and Korn, D. (1966). *Proc. nat. Acad. Sci. (Wash.)* 55, 1089-1095.

Pruss, G. J. (1977). Ph.D. Thesis, University of California.

Ptashne, M. (1967). *Proc. nat. Acad. Sci. (Wash.)* 57, 306-313.

Rachmeler, M., Gerhart, J. and Rosner, J. (1961). *Biochim. biophys. Acta (Amst.)* 49, 222-225.

Reader, R. W. and Siminovitch, L. (1971a). *Virology* 43, 607-622.

Reader, R. W. and Siminovitch, L. (1971b). *Virology* 43, 623-637.

Schnös, M. and Inman, R. B. (1971). *J. molec. Biol.* 55, 31-38.

Séchaud, J. (1960). *Arch. Sci. (Genève)* 13, 427-474.

Shimada, K., Weisberg, R. A. and Gottesman, M. E. (1972). *J. molec. Biol.* 63, 483-503.

Shimada, K., Weisberg, R. A. and Gottesman, M. E. (1973). *J. molec. Biol.* 80, 297-314.

Signer, E. R. and Weil, J. (1968a). *Cold Spring Harbor Symp. quant. Biol.* 33, 715-719.

Signer, E. R. and Weil, J. (1968b). *J. molec. Biol.* 34, 261-271.

Sinsheimer, R. L. (1968). *Progr. Nucleic Acid Res. molec. Biol.* 8, 115-169.

Sironi, G. (1969). *Virology* 37, 163-176.

Skalka, A. and Hanson, P. (1972). *J. Virol.* 9, 583-593.

Smith, H. O. and Levine, M. (1964). *Proc. nat. Acad. Sci. (Wash.)* 52, 356-363.

Sunshine, M. G. and Sauer, B. (1975). *Proc. nat. Acad. Sci. (Wash.)* 72, 2770-2774.

Sunshine, M. G., Thorn, M., Gibbs, W., Calendar, R. and Kelly, B. (1971). *Virology* 46, 691-702.

Sussman, R. and Jacob, F. (1962). *Compt. Rend. Acad. Sci. (Paris)* 254, 1517-1519.

Szybalski, W., Kubinski, H., Hradecna, Z. and Summers, W. C. (1971). *Methods in Enzymology* 21, 383-413.

Thomas, R. (1970). *J. molec. Biol.* 49, 393-404.

Thomas, R. (1971). In *The Bacteriophage Lambda* (A.D.Hershey, ed.), pp. 211-220. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Waggoner, B. T., Gonzales, N. S. and Taylor, A. L. (1974). *Proc. nat. Acad. Sci. (Wash.)* 71, 1255-1259.

Waites, W. M. and Fry, B. A. (1964). *J. gen. Microbiol.* 34, 413-426.

Wang, J. C. (1967). *J. molec. Biol.* 28, 403-411.

Wang, J. C. and Schwartz, H. (1967). *Biopolymers* 5, 953-966.

Weigle, J. (1966). *Proc. nat. Acad. Sci. (Wash.)* 55, 1462-1466.

Weisbeek, P. J. and Van Arkel, G. A. (1976). *Virology* 72, 72-79.

Woods, W. H. (1972). Ph.D. Thesis, University of Adelaide.

Woods, W. H. and Egan, J. B. (1974). *J. Virol.* 14, 1349-1356.

Wu, M., Davidson, N., Attardi, G. and Aloni, Y. (1972). *J. molec. Biol.* 71, 81-93.

Yamagishi, H., Nakamura, K. and Ozeki, H. (1965). *Biochem. biophys. Res. Comm.* 20, 727-732.

Yamamoto, K. R., Alberts, B. M., Benzinger, R., Lawhorne, L. and Treiber, G. (1970). *Virology* 40, 734-744.

Young, E. T. and Sinsheimer, R. L. (1967). *J. molec. Biol.* 30, 165-200.

Younghusband, H. B., Egan, J. B. and Inman, R. B. (1975). *Molec. gen. Genet.* 140, 101-110.

Younghusband, H. B. and Inman, R. B. (1974). *Virology* 62, 530-538.