



PROPHAGE INDUCTION OF "NON-INDUCIBLE" COLIPHAGE 186

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Doctor of Philosophy

by

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SUMMARY

The thesis describes studies on the induction of "non-inducible" coliphage 186, its genetic map and the location of the prophage on the host chromosome.

Suppressor-sensitive mutants of phage 186 were isolated and preliminary experiments toward obtaining a genetic map for this phage performed.

Prophage 186 gives comparable yields to prophage λ when subjected to ultraviolet irradiation. However, the ultimate decrease in the optical density of the induced culture is slight for 186, and delayed, compared to that for λ . That other "non-inducible" phage do not show ultraviolet induction was confirmed. Induction by nalidixic acid, mitomycin C and the rate of spontaneous phage production itself, are similar for 186 and λ . A recA⁺ host is required for induction.

A larger dose of UV radiation is necessary for 186 than for λ to yield a comparable degree of induction. Survival curves of irradiated 186 lysogens are identical to those for non-lysogens, in contrast to λ . As the irradiated lysogens also give rise to infectious centres, it appears that one cell can give rise to both a surviving colony and an infectious centre. The latent period of 186 ultraviolet induction is over twice as long as that for its heat induction, or following infection, whereas that for λ is only slightly increased. Phage 186 infection of irradiated cells has a delayed burst, whereas the latent period for

either λ or P2 infection is the same in irradiated and un-irradiated hosts.

The 186 attachment site (attl86) is located between the origins of Hfr's KL16 and KL98, and enters just before pheA (50 min) in interrupted matings. Two factor P1 transductions put attl86 at 51.1 min. Three factor P1 transductions involving nalB, attl86 and pheA or cysC locate nalB at 51.5 min and attl86 between nalB and pheA. Phage 186 is integrated between nalB and pheA causing loss of linkage between these markers.

Coliphage 186 is not zygotically inducible as evidenced by, (i) lack of effect on the gradient of transfer of adjacent markers; (ii) lack of effect on the transfer of distal markers; and (iii) no increase in phage titre relative to the transfer of an adjacent marker. These results are obtained regardless of the direction of transfer of the 186 prophage. Transfer of a 186 prophage carrying a suppressible clear plaque mutation of the cI gene from an su⁺ male to an su⁻ female does not show zygotic induction. Phage 186 is not induced to display an infectious centre on ultraviolet irradiation of a conjugating female, whether present as prophage or introduced by conjugation. This result is obtained with either Hfr or F' mediated conjugation. Phage 186 shows neither zygotic nor indirect induction in F' mediated conjugation.

Phage 186 forms an ultraviolet inducible prophage. However, after irradiation, or conjugation, the bacterial cell is transiently refractory to 186 expression, which leads on the one hand

to a delayed lysis in ultraviolet induction, and on the other hand to the absence of zygotic induction with a prophage that is normally inducible.

STATEMENT

This thesis contains no material previously submitted for any other degree or diploma in any University, and, to the best of my knowledge and belief, no material previously published or written by any other person, except where due reference is made in the text.

W.H. WOODS.

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ABBREVIATIONS

The abbreviations used for bacterial genes are those of Taylor (1970). The abbreviations used for phage λ genes are summarised by Szybalski (1971), and the nomenclature adopted for phage 186 is based on these. Other abbreviations are listed below.

Where reference is made to another part of this thesis, this will be simply indicated by the designation of the particular section in parenthesis, for example, (3.2.a).

CAP	catabolite activator protein
cAMP	cyclic 3'5' adenosine monophosphate
DNA	deoxyribionucleic acid
MMS	methyl methane sulphonate
NNG	N-methyl-N'-nitro-N-nitrosoguanidine
OD	optical density
pfu/ml	plaque forming units per millilitre
UV	ultraviolet (radiation)

CHAPTER 1.

GENERAL INTRODUCTION

1.1 INTRODUCTION

This chapter will serve to introduce the problem of prophage induction. Throughout this thesis the term induction will be used to indicate derepression of the phage genome and the following phage production. In view of the recent comprehensive reviews of the genetics of the inducible phage λ ("The Bacteriophage Lambda", A.D. Hershey, ed., Cold Spring Harbour Laboratory, New York, 1971) and the non-inducible P2-related phages (Bertani and Bertani, 1971) only the stimuli causing induction will be described here. Thus this chapter will, in the main, deal with inducible phages. Relevant behaviour of non-inducible phages will be described in the introductions to succeeding chapters.

1.2 ULTRAVIOLET INDUCTION

Ultraviolet irradiation was the first treatment identified as causing prophage induction (Lwoff, Siminovitch and Kjeldgaard, 1950) in particular of coliphage λ (Weigle and Delbruck, 1951). Subsequently, many other treatments have been shown to cause a similar induction; thymine starvation of a thymine requiring lysogen (Korn and Weissbach, 1962), treatment with mitomycin C (Otsuji, Sekiguchi, Iijima and Takagi, 1959), nalidixic acid (Cowlshaw and Ginoza, 1970), streptonigrin (Levine and Borthwick, 1963), 5-fluoro-uracil (Zgaga and Novak, 1967), lysozyme/ethylene diamine tetra-acetic acid (Novak, Tkalac and Zgaga, 1967), colicin E2 (Endo, Kamiya and Ishizawa, 1963), panfuran (Kato,

Sugino and Endo, 1966), organic peroxides, epoxides and ethylene imines (Lwoff and Jacob, 1952), nitrogen mustard (Jacob, 1952a), hydrogen peroxide (Lwoff and Siminovitch, 1952), hydroxyurea (Gado and Horvath, 1968), 1-methyl-3-nitro-1-nitrosoguanidine (Zampieri, 1966a), nitrous acid (Zampieri, 1966b), 2'3'-dideoxyadenosine (Geissler, Scherneck and Theile, 1972), ethyl methane sulphonate (Theile, Scherneck and Geissler, 1972), iyomycin (Yajima, Tago and Hata, 1963), irradiation with soft X rays (Latarjet, 1951), visible irradiation after pre-incubation with acridine orange or methylene blue (Freifelder, 1966) and irradiation with α particles emitted from polonium 210 (Devoret, 1963). Where tested, these treatments also interfere with host DNA metabolism, are mutagenic, or, in higher organisms carcinogenic (Jacob and Wollman, 1953; Levine, 1961; Stuy, 1959; above references). In particular, where a related series of treatments was tested (the colicins, Kato et al, 1966; the iyomycins, Yajima et al, 1963) those members found to have inducing properties also inhibited host DNA synthesis (Nomura, 1963; Yajima et al, 1963).

Such induction is not at all unique to phage λ or Escherichia coli. UV irradiation and mitomycin C, in particular, have been used to induce prophage from Bacillus megatherium (Lwoff et al, 1950), Pseudomonas pyocyanea (Jacob and Wollman, 1953), Pseudomonas aeruginosa (Yamamoto and Chow, 1968), Caulobacter sp (Driggers and Schmidt, 1970), Bacillus licheniformis (Huang and Marmur, 1970), Clostridium perfringens

(Mahoney and Kalz, 1968) and Corynebacterium renale (Yanagawa, Morikazu and Nerome, 1968). The temperate coliphage P1, which is maintained as a stable plasmid (Ikeda and Tomizawa, 1968) rather than integrated into the host chromosome, is also subject to induction by, for example, thymine starvation of a thymine auxotrophic host (Melechen and Skaar, 1962) or by mitomycin C (Seno and Melechen, 1964).

That the effect of inducers such as UV irradiation is not directly upon the prophage, or its repressor, has long been suggested and is now indicated by many independent lines of argument. Jacob and Wollman (1953) argued that the effect was indirect since low UV doses on a P. pyocyanea strain lysogenic for two different phage (one more inducible at low doses than the other, in single lysogens) induced either or both phage in separate cells (Jacob, 1952b). In addition, photorestitution up to 15 min after irradiation, could reverse the inducing effect of that radiation (Jacob, 1950). Immunity to superinfection also persisted till 15 min after irradiation.

Particularly strong evidence for the indirect effect of inducers is provided by indirect induction, where the inducing stimulus is applied to a non-lysogenic cell which is subsequently mated with a lysogenic recipient. This will be described in detail later (1,3).

Tomizawa and Ogawa (1967) used infection by a λ mutant unable to integrate, to produce daughter segregants containing λ

repressor, but not the phage DNA. UV irradiation of such segregants destroyed immunity to superinfection, that is, caused inactivation of the repressor. Thus the presence of the prophage is not required for the induction process.

Additional evidence that the inducing effect is not directly upon the prophage is provided by the involvement of some host functions in induction by, for example, UV, but not in infection or heat induction. In particular the primary host recombination system is involved, in that recA lysogens only produce a low level of phage either spontaneously or following UV induction, and the majority of these are due to clear plaque mutations (Brooks and Clark, 1967; Fuerst and Siminovitch, 1965). The Salmonella phage P22 behaves similarly in a recombination deficient host. In E. coli, the effect appears limited to recA mutants of the host recombination system (Van de Putte, Zwenk and Rorsch, 1966; Clark, 1967), although all are more sensitive to UV irradiation than the wild type. Hertman and Luria (1967) found that a recA lysogen could be irradiated and, provided the recA⁺ gene was introduced (by P1 transduction) within 2 hours, induction would result. Thus it appears that recA mediated repair of damaged DNA may result in prophage induction. Monk and Gross (1971) have found that prophage induction can result from irradiation damage in chromosomes that are unable to replicate, but their results do not rule out some DNA turnover as might occur in repair of damaged DNA.

Donch, Greenberg and Green (1970) have found that exrA

(UV sensitive) mutants of E. coli B show no additional sensitivity to UV radiation when lysogenised by phage λ . The spontaneous phage production is only 10% of that from a wild type lysogen. After UV irradiation, phage production is slight and delayed $2\frac{1}{2}$ to 4 hours compared to the burst from a wild type lysogen (Donch, Green and Greenberg, 1971). Devoret and Blanco (1970) isolated, from a thymine auxotroph, bacterial mutants that did not show prophage induction upon thymine deprivation. Of these, 50% had recA phenotype, and the remainder could be divided into two groups (on the basis of susceptibility to curing); suggesting that further host functions may be involved in induction. Host mutants showing low levels of spontaneous phage production have also been found in Bacillus megaterium (Fadeeva, Rautenshtein and Baikova, 1968).

Another class of bacterial mutants more sensitive to UV irradiation than the wild type are the uvr mutants, which are unable to excise thymine dimers formed on irradiation (Howard-Flanders, Boyce and Theriot, 1966). In this case, however, the induction of prophage λ also occurs at much smaller UV doses (Mattern, van Winden and Rorsch, 1965). If the dose is too large, phage are not recovered (Howard-Flanders and Boyce, 1966) unless the culture is exposed to visible light to allow photoreactivation. The case of mutants deficient in DNA polymerase I is similar, in that an increased sensitivity to UV radiation is displayed by the polA host and by prophage induction of polA lysogens (Monk, Peacey and Gross, 1971).

The extent of involvement of the λ prophage in lifting immunity seems to be limited to the repressor coded for by the cI gene. Phage mutants not inducible by UV irradiation or mitomycin C are mapped in this gene (Jacob and Campbell, 1959; Eshima, Fujii and Horiuchi, 1972). These mutants do not necessarily produce more repressor than wild type, but rather an altered repressor (Eshima et al, 1972; Ptashne, 1971). Such mutants are also not induced by thymine deprivation (Korn, 1964). Thus the phage repressor may be considered as incapable of recognising an inducing signal produced by induction treatments applied to the host.

A temperature-sensitive host mutation, T44, isolated by Jacob and Goldthwait (1964), appeared to indicate the type of inducing signal finally produced. Raised temperature induced T44, when lysogenic for wild type phage λ , and, more significantly, the addition of guanosine or cytidine protected against induction, whereas addition of adenine resulted in induction even at low temperatures. Non-lysogenic T44 was observed to be defective in cell division at elevated temperatures - that is filaments were formed. This too was favoured by addition of adenine, and reduced by addition of cytosine and guanine (Kirby, Jacob and Goldthwait, 1967). Thus it was suggested that a low molecular weight compound, possibly an adenine derivative, was an effector molecule in regulation of both cell division and prophage repression.

Attempts to identify the effector molecule have followed

two lines. The first was to determine nucleotide levels in T44 and its parent strain (Ruff, Kirby and Goldthwait, 1971). No significant differences were found except in the level of inosine and this has no effect when added to T44 cultures. The second line of investigation was on the effects of addition of a wide range of ribosides, pantoyl lactone and furfural derivatives to the T44 mutant (Kirby, Ruff and Goldthwait, 1972). The results indicate that the effector molecule probably has a five-membered oxygen-containing ring as part of its structure.

The relationship between prophage induction and filament formation had been recognised before the work with T44. Two phage inducers, 5-fluoro-uracil (Tomasz and Borek, 1960) and lysozyme/ethylene diamine tetra-acetic acid treatment (Novak, Tkalac and Zgaga, 1967), interfere with the cell wall. Witkin (1967) proposed a relationship between prophage induction and filament formation because; (i) both occur in every member of a population exposed to low doses of UV radiation, (ii) both are initiated by a variety of agents other than UV irradiation, but which are all able to cause DNA damage in treated cells, (iii) both occur in "old" cultures, and (iv) both are photoreversible. The link between the processes would appear to be that bacterial DNA is associated with the cell membrane (Jacob, Ryter and Cuzin, 1966) and thus inducers may affect both host DNA and membrane metabolism.

The nature of the inducing signal and its derivation have been the subjects of considerable postulation. From the nature

of inducers, Jacob and Wollman suggested, as long ago as 1953, that "an alteration of the bacterial nucleic acid balance is the primary effect of the inducing shock." The work with the T44 mutant, described above, led to the suggestion that induction was caused by the accumulation of a DNA precursor which specifically inactivates the (inducible) phage repressor (Goldthwait and Jacob, 1964). Likhoded, Padalko, Poverennyi, Saenko and Tolcheev (1969) suggested that inducers cause damage to the DNA secondary structure, and that phage repressors are lost by non-specific linkage with the damaged regions. Geissler (1970) has advanced a similar hypothesis, although it involves specific binding. He suggests that repressors bind to oligonucleotides produced during repair of damaged DNA or with single stranded DNA fragments released during post replication repair. The binding is said to occur because the oligonucleotides and polynucleotide fragments contain base sequences which are identical with base sequences of the phage operators. Cowlshaw and Ginoza (1970) found a temporal heterogeneity in the production of induced cells by nalidixic acid. They propose this may reflect the heterogeneity in DNA metabolism of an unsynchronised population of cells. A preliminary report (Waggener, Shalek and Smith, 1967) has appeared of cyclic sensitivity to UV induction in synchronised lysogens. Cowlshaw and Ginoza (1970) propose that DNA synthesis inhibition triggers the set of functions that are normally activated upon chromosome replication, including the signals for cell division. However,

they suggest that premature triggering of these functions results in filament formation and prophage induction.

These problems do not appear capable of resolution until systems established for in vitro induction (L.H. Johnston, pers. comm.) are perfected.

1.3 INDIRECT INDUCTION

In attempting to show that UV induction of a prophage was an indirect process, Borek and Ryan (1958) found that it was possible to transfer the UV-stimulus from UV irradiated non-lysogenic F^+ males to lysogenic females, during conjugation. This resulted in the (indirect) induction of the prophage in the female. Transfer of the inducing stimulus was unidirectional, occurring only from F^+ to F^- cells. They found that the inducing stimulus was stable in the cold, unstable at 37° , and subject to photoreactivation (Borek and Ryan, 1960).

Devoret and George (1964a) found the efficiency of indirect induction to be about 50% that of direct induction at a particular UV dose. Both forms of induction showed similar dependence on UV dose at low doses, and if both were applied to the same prophage, a synergistic effect was observed. They confirmed the polarity of indirect induction (Devoret and George, 1964b), and found that Hfr males did not show transfer of the inducing stimulus. The same workers (Devoret and George, 1967) showed that indirect induction occurs even if the donor is lysogenic for λ_{ind}^- , that is, a non-inducible prophage in the donor cannot

prevent transfer of the inducing stimulus. Another inducible phage, 434, did exhibit indirect induction from a lysogenic female, whereas λ_{ind}^- did not. Heat induction of a temperature-inducible male prophage did not result in an inducing stimulus capable of transfer to a lysogenic female. Likewise, neither X irradiation nor mitomycin C treatment of the F^+ donor resulted in indirect induction.

Indirect induction is not limited to prophage, but has also been demonstrated for the colicin I factor (Devoret, Monk and George, 1965). In fact, indirect induction of λ is possible through col I mediated conjugation. In addition, the col I episome itself, in a recipient, may be induced indirectly by F mediated conjugation from an irradiated donor.

Since indirect induction did not occur when the recipient was capable of restricting the DNA entering from the donor, George (1966) concluded that the inducing signal was transmitted by the incoming DNA. Hfr mediated conjugation did not exhibit indirect induction, therefore she suggested that the signal was contained in the irradiated F factor itself. It was proposed that the important property of this DNA was that it constituted a replicon, that is, it is capable of self replication (Devoret, et al, 1965).

Monk (1967) found that indirect induction did not require prolonged contact between the irradiated donor and the lysogenic recipient. Significantly, similar contact with a non-lysogenic recipient resulted in inhibition of cell division and formation

of filaments by that recipient. Although there was a substantial delay to transfer of functional col I factor from an irradiated donor, the effects of indirect induction were apparent after only 2 min contact. Assay of col I factor transfer by autoradiography (Monk, 1969) showed this was not delayed by irradiation, that is, damaged non-functional col I factor was transferred within 2 min of contact, and caused indirect induction.

Indirect induction may also be caused by infection of a λ lysogen with irradiated phage P1 (Rosner, Kass and Yarmolinsky, 1968). Phage P1 is similar to F and col I factors in that it forms stable lysogens without being integrated into the host chromosome (Ikeda and Tomizawa, 1968). This supports the conclusion that indirect induction is caused by the entry into a lysogenic cell of a UV-damaged replicon. However, Kanter and Harriman (1972) have shown indirect induction with UV irradiated phage Mu-1, which, in all cases studied, has been found to have a chromosomal location.

When the lysogenic recipient was immune to the irradiated DNA (e.g. lysogenic for P1) no indirect induction occurred (Rosner *et al*, 1968). Since the bacterial recA function is required for indirect induction (George and Devoret, 1971), and recA mediated repair is thought to be associated with replication of the irradiated DNA (Rupp and Howard-Flanders, 1968), Monk and Gross (1971) proposed that indirect induction was not observed in this case because the incoming replicon is repressed

and unable to replicate. However, they were able to demonstrate direct UV induction in a host where replication of the damaged chromosome did not occur. Their results did not rule out slight DNA turnover as may occur during recA dependent repair.

Another form of indirect induction has been reported by Zgaga and Novak (1967), in that contact between a 5-fluoro-uracil treated male or female and a lysogen of the opposite sex caused indirect induction. Similarly, lysozyme/ethylene diamine tetraacetic acid treatment may cause indirect induction (Novak, Tkalac and Zgaga, 1967), again independently of the polarity of conjugation. It is proposed that contact of the non-treated lysogen with the altered cell walls of treated cells causes this induction.

1.4 ZYGOTIC INDUCTION

Two forms of induction do not require a recA⁺ host. These are heat induction of a temperature-sensitive clear plaque mutant (Brooks and Clark, 1967) and zygotic induction (Clark and Margulies, 1965). Both these inductions are postulated to have a direct effect on the repressor; the first by denaturing it, and the second by transfer of the prophage DNA away from the repressor.

Zygotic induction of a phage may occur following transfer by conjugation of the prophage from a lysogenic male to a non-lysogenic female. It was first demonstrated for phage λ by Jacob and Wollman (1954a). They found a great increase in infectious

centres from such a cross, compared to those from the lysogenic male alone. The female used was resistant to infection by λ , but the phage replication could be shown to occur in the female. It was sensitive to streptomycin only if the female was sensitive to this drug, and if an E. coli C female was used, then the phage produced plated at only low efficiency on E. coli K12. Thus the prophage was transferred to the female where it then multiplied. The increase in plaque activity on mixing the male and female cells showed a two step increase with time; the first increase due to induction of the prophage on transfer into the female, showing λ anti-serum resistant infectious centres, followed by a second increase in titre on the lysis of these infectious centres. A secondary effect of the induction was to reduce the number of bacterial recombinants detected from the cross, and few of these recombinants carried the prophage. This result explained earlier difficulties with F^+ conjugation, where it had not been possible to show transfer of prophage λ from $F^+(\lambda)$ to $F^-\lambda^-$, although the inverse transfer could be demonstrated (Appleyard, 1953; Wollman, 1953).

Jacob and Wollman (1954b) went on to report the effects of zygotic induction on conjugation experiments. Markers close to att λ were most affected, leading to a steepening of the gradient of transfer, and the few recombinants obtained for these markers were only rarely lysogenic. When the female carried a defective λ prophage no zygotic induction was found on conjugation with a lysogenic male (Jacob and Wollman, 1956). If a similar defective

λ prophage was present in a male conjugated with a non-lysogenic female, although there was no increase in plaque activity, zygotic induction was indicated by a steepening of the gradient of transfer of bacterial markers and a low frequency of recombinants for markers near att λ (Jacob and Wollman, 1957). That only transfer of the prophage carried by the male chromosome into a non-immune recipient results in transition of the phage to the vegetative state, indicates that the cytoplasm of the zygote is important and led to the idea of the phage-coded repressor (Jacob and Monod, 1961). Thus, if the female is lysogenic, the incoming male prophage is repressed.

Zygotic induction is not unique to phage λ . The group of phages isolated by Jacob and Wollman (1956) as UV inducible, also showed zygotic induction. The degree of the induction on conjugation from HfrH lysogens was correlated with the map positions of the various prophages and thus their degree of transfer by HfrH. Transfer of these prophages to non-lysogenic females affected the gradient of transfer of bacterial markers, as was found with λ , but with variations appropriate to the map position of each phage (Jacob and Wollman, 1958). In particular, for λ , 21 and 424, there was almost no measurable transfer of markers distal to the prophage, and recombinants selected for proximal markers were only rarely lysogenic (c.1%). The zygotic induction of another inducible phage, ϕ 80, has not been described, but Matsushiro (1963) in locating att ϕ 80 by conjugation was careful to avoid crosses from a lysogenic donor to a non-

lysogenic female. Pekhov and Polukhina (1967) have found zygotically inducible phage among lysogens of the OIII:B⁴ serotype. Matsuyama and Uetake (1972) have reported that two UV inducible phage of Salmonella anatum, E¹⁵ and g₃₄₁, are also zygotically inducible. Transfer of either phage to a non-lysogenic recipient greatly decreased the level of recombinants obtained for adjacent markers, when compared to crosses with lysogenic recipients. Unfortunately, "infective center formation is not adequate" in their system, so there is no evidence for an increase in plaque activity due to zygotic induction.

There is direct evidence for a high efficiency of λ zygotic induction (Jacob and Wollman, 1956), in that UV irradiation after conjugation does not increase the number of infectious centres; that is, all prophages transferred are induced. Sussman (1970) has pointed out that there is no such evidence for the other inducible phage. However, like λ , these phage almost never appear as prophage in recombinants selected in a cross between lysogenic males and non-lysogenic females (Jacob and Wollman, 1958).

All the above work in E. coli utilised clockwise transferring HfrH, but Jacob and Wollman (1961) have shown that phage λ and 424 are zygotically induced when transferred (as late markers) by anti-clockwise transferring Hfr4, although here there is no data as to the extent of induction compared to transfer. George and Devoret (1971) have, incidentally, shown zygotic induction of greater than 50% with an F'gal donor, lysogenic

for λ , that transfers in an anti-clockwise direction.

The more or less absolute expression of a prophage upon conjugal transfer in zygotic induction has been used to determine the coefficient of integration for normal bacterial markers (Jacob and Wollman, 1961; DeHaan and Gross, 1962). Similarly, zygotic induction has been used as an index of the efficiency of conjugation as follows; phage λ in Hfr matings by Curtiss *et al* (1968), phage 434 in F' matings by Rosner, Kass and Yarmolinsky (1968), and phage λ in F' matings by George and Devoret (1971). Ptashne (1965) and Prell (1965) have used zygotic induction of λ to show that the male prophage, and thus DNA, is replicated during transfer to the female. Rupp and Ihler (1968) have used λ zygotic induction from the clockwise transferring HfrH and an anti-clockwise transferring F'gal, to show that it is always the 5' to 3' strand that is injected from the male.

Phage λ zygotic induction occurs by a different mechanism to its UV induction since λ ind⁻, the non-inducible mutant, is zygotically inducible (Jacob and Campbell, 1959). Further, neither the donor (Hertman, 1967) nor the recipient (Clark and Margulies, 1965) need be recA⁺. The level of zygotic induction may be somewhat lower in the recA recipient (Itoh and Tomizawa, 1971) or not (Willettts and Clark, 1969), and is about 50% that in a rec⁺ recipient in the case of recB, recC, recBC, recAB and recABC recipients (Willettts and Clark, 1969). Whether the donor or recipient is uvr⁺ or uvr⁻ also has little effect on zygotic

induction (George and Devoret, 1971).

Thus it is seen that zygotic induction and UV-type induction have little in common, and that neither is fully understood. In view of the dissimilarity between these forms of induction, it is surprising that they remain linked in the literature (e.g. Dove, 1971).

1.5 AIM OF PROJECT

The aim of this project was to investigate prophage induction by determining why prophage 186 is not inducible. During the course of the work it was found that phage 186 had been incorrectly classified as non-UV-inducible. However, its UV induction does not result in 186 lysogens being more sensitive to UV irradiation than are non-lysogens. Phage 186 was confirmed as not zygotically inducible, and possible reasons for this investigated.

CHAPTER 2

MATERIALS AND METHODS

2.1 MATERIALS

a. Chemicals - In general, chemicals were of analytical reagent grade. N-methyl-N'-nitro-N-nitrosoguanidine was obtained from Aldrich Chemical Co., Milwaukee, U.S.A. Streptomycin sulphate was from Drug Houses of Australia Ltd., Adelaide, South Australia and mitomycin C, the gift of W.H. Elliott, from Kyowa Hakko Ltd., Tokyo, Japan. Methyl methane sulphonate, the gift of B.W. Holloway, was from Eastman Kodak Co., Rochester, U.S.A. Nalidixic acid was the gift of Winthrop Laboratories, Ermington, New South Wales. Adenine, guanine, thymine, galactose, benzyl penicillin and tris(hydroxymethyl) aminomethane (Reagent Grade) were obtained from Sigma Chemical Co., St. Louis, U.S.A. British Drug Houses Ltd., Poole, England provided uracil, nicotinic acid and thiamine. Amino acids were from Mann Research Laboratories Inc., New York, U.S.A. G.T. Gurr Ltd., London, England supplied eosin Y and methylene blue. The Difco Laboratories, Detroit, U.S.A. provided agar, tryptone, yeast extract, beef extract, peptone and maltose.

Anti-sera against phage λ and against phage 186 were prepared from rabbits immunised against the particular phage by a series of subcutaneous injections of filtered (Millipore, pore size 0.42μ) high titre stocks into the interscapular space. Anti-serum preparations were produced with neutralisation constants, K , of 54 min^{-1} for phage λ , and 570 min^{-1} for phage 186. Anti-serum against phage 299 ($K=10 \text{ min}^{-1}$), was prepared similarly by Roger Harlow.

b. Media - The amounts of components detailed below were dissolved in 1 litre glass distilled water. Media were sterilised by autoclaving for 25 min at 120° under a pressure of 15 lbs/inch².

- i. Broths - The complete medium used, L broth (LB), contained 10g tryptone, 5g yeast extract and 10g NaCl; it was supplemented with glucose (0.1%) for conjugation experiments (LGB) and also with CaCl_2 (2mM) for use with P1 or P2 (LGCB). 10xLGB contained the above components at ten times the concentrations listed. Tryptone broth (TB), containing 10g tryptone and 5g NaCl, was used in early work and, supplemented with CaCl_2 (2mM), with P2. Nutrient broth (NB) contained 3g beef extract and 5g peptone.
- ii. Buffer and salt solutions - TM was 0.01M tris(hydroxymethyl) aminomethane, pH7.0, 0.01M MgSO_4 . H-1 (Kaiser and Hogness, 1960) consisted of 0.1M potassium phosphate buffer, pH7.0, 0.015M $(\text{NH}_4)_2\text{SO}_4$, 0.001M MgSO_4 and 1.8×10^{-6} M FeSO_4 . Medium C was 0.1M sodium citrate buffer, pH6.0. The minimal medium used was half-strength medium 56 of Monod et al (1951). It was supplemented with glucose (0.2%) as carbon source and other appropriate growth requirements in the concentrations ($\mu\text{g/ml}$) listed below, as suggested by A.J. Pittard. Amino acids; arginine (140), cysteine (25), glycine (80), histidine (40), isoleucine-valine (40 each), leucine (40), lysine (30), methionine (25), proline (70),

phenylalanine (40), threonine (40), tryptophan (20), and tyrosine (40). Bases; adenine (20), guanine (20), thymine (50) and uracil (20). Vitamins; nicotinic acid (5) and thiamine (1). When required for male counter-selection, streptomycin (200) was included.

iii. Agars - The above broths and minimal medium were solidified with 15g agar for plates. EMB-gal plates contained 10g tryptone, 1g yeast extract, 5g NaCl, 2g $K_2HPO_4 \cdot 3H_2O$, 0.065g methylene blue, 0.4g eosin Y, 15g agar, and 4g galactose.

Soft agars for overlays were solidified with 7g agar per litre. Stabs and slants contained 3g yeast extract, 9g beef extract, 15g peptone and 7 and 15g agar respectively.

c. Phage Strains - The phage strains utilised in this work are described in Table 2-I.

The strain $\lambda_i^{434} \underline{h80} \underline{att\lambda}$ was isolated as a recombinant from a cross between λ_i^{434} and $\lambda_{cIts857h80} \underline{att\lambda}$. The parent phage were added at a multiplicity of 10 each to log phase 594 (2×10^8 colony formers per ml) resuspended from LGB into 0.01M potassium phosphate buffer pH7.0, 0.01M $MgSO_4$. After 20 min at 37° , the mixture was subjected to 50 sec UV irradiation (dose, 760 ergs/mm^2), diluted into LGB and shaken at 37° for 90 min. The culture was chilled, treated with chloroform and recombinants (0.4%) selected on KL19, which is λ lysogenic and resistant to λ .

TABLE 2-I: LIST OF PHAGE STRAINS

Strain	Description	Source ^a	Reference or derivation
18	non-inducible phage	C600(18)R.L. Baldwin	Jacob & Wollman (1956)
186	non-inducible phage	CR34(186)R.L. Baldwin	Jacob & Wollman (1956)
186 <u>cIts</u> ^b	temperature-inducible mutant	W3350(186 <u>cIts</u>)R.L. Baldwin	Baldwin <u>et al</u> (1966)
186 <u>cI</u>	clear plaque mutant	V. Huddleston	Huddleston(1970); 186
186 <u>suscI</u> 53	suppressible clear plaque mutant	this work (3.3a)	186
186 <u>suscI</u> 54	suppressible clear plaque mutant	this work (3.3.a)	186
186 <u>cII</u>	clear plaque mutant	V. Huddleston	Huddleston(1970); 186
186 <u>cIts</u> <u>suscII</u> 55	clear plaque mutant suppressible at 30°	this work (3.3.a)	186 <u>cIts</u>
186 <u>vir</u>	virulent mutant	this work (3.3.a)	186 <u>suscI</u> 53
186 <u>cIts</u> <u>susI</u> -47, 51 & 52	suppressor sensitive mutants	this work (3.3.a)	186 <u>cIts</u>
186 <u>susK</u> 4, K6&K9 ^c	suppressor sensitive mutants	A.D. Kaiser	186

TABLE 2-I Cont'd.

Strain	Description	Source ^a	Reference or derivation
299	non-inducible phage	C600(299) R.L. Baldwin	Jacob & Wollman (1956)
299R ⁺ 11	temperature-inducible mutant	R.W.H. Harlow	Harlow (1969)
434	UV-inducible phage	C600(434) D.S. Hogness	Jacob & Wollman (1956)
Pl _{vir}	generalised transducing phage	A.J. Pittard	
P2k	non-inducible phage grown on <u>E. coli</u> K12	K-40(P2)6 B. Kelly	Kelly (1963)
T6	virulent phage	P. Reeves	
W	non-inducible phage	CR34(W) R.L. Baldwin	Jacob & Wollman (1956)
λ	UV-inducible phage	C600(λ) D.S. Hogness	Lederberg (1951)
λ _{vir}	virulent mutant	D.S. Hogness	Jacob & Wollman (1954c)
λ _{ind} ⁻	non-UV-inducible mutant	A.J. Pittard	Jacob & Campbell (1959)
λ _{ind} ⁻ cIts857	temperature-inducible mutant	C600(λ _{ind} ⁻ cIts857) D.S. Hogness	Sussman & Jacob (1962)

TABLE 2-I Cont'd.

Strain	Description	Source ^a	Reference or derivation
λ <u>cIts</u> 857h80 <u>att</u> λ	host range of ϕ 80, but attachment and immunity of λ	N. Franklin	Franklin, Dove & Yanofsky (1965)
λ <u>i</u> ⁴³⁴	λ with immunity only of 434	C690(λ <u>i</u> ⁴³⁴)D.S.Hogness	Kaiser & Jacob (1957)
λ <u>i</u> ⁴³⁴ h80 <u>att</u> λ	immunity of 434, host range of ϕ 80 and attachment of λ	this work-derivation in text (2.1.c)	
ϕ 80	UV-inducible phage	P. Reeves	Matsushiro (1961)
ϕ 80 <u>sup</u> F	specialised transducing(plaque forming) phage	S. Altman	Smith <u>et al</u> (1966)

^aIf obtained as lysogens, the designation of the lysogen is quoted.

^bThis is the temperature-sensitive clear plaque mutant of 186 isolated by Baldwin et al (1966), designated 186p by them. It is described here as 186cIts, so that it may conform to the nomenclature used for the clear plaque mutants of 186 (Huddleston, 1970) and other phage.

^cThese mutants have been allocated numbers 48,49 and 50 respectively in the series of 186 sus mutants, but will be referred to in this thesis as K4, K6 and K9 to aid comparisons.

d. Bacterial Strains - The bacterial strains utilised in this work are detailed in Table 2-II. All listed are derivatives of E. coli K12. Also used was an E. coli C derivative, C1055 (F^+ , thr leu xan his str^r; Wiman, Bertani, Kelly and Sasaki, 1970) obtained from M. Mandel.

The strain 594 his (E828) was constructed by transduction of 594 with Plvir grown on JC2926(186). After 20 min incubation of the mixture of log phase 594 grown in LGCB and Plvir, added at a multiplicity of 0.05, it was diluted into H-1 supplemented with glucose (0.2%) and histidine (40 μ g/ml) and incubated overnight. This culture was washed twice with H-1 and resuspended in ten times the volume of H-1 plus glucose. After 3 $\frac{1}{2}$ hours at 37° penicillin (1.2 mg/ml) was added to the thinly grown culture. Incubation at 37° for a further 60 min was followed by another series of washings and final resuspension in H-1 with glucose and histidine. After overnight growth the penicillin enrichment procedure was repeated and the washed culture plated on minimal plates supplemented with glucose and histidine. Of the surviving colonies, 19/20 required histidine for growth.

TABLE 2-II: LIST OF E. coli K12 DERIVATIVES

Strain	Sex	Relevant genotype ^a	Source	Reference or derivation
152	F ⁻	<u>recA</u> 56 <u>gal</u> <u>str</u> ^r <u>su</u> ⁻	D.S.Hogness	Gottesman & Yarmolinsky (1968)
152(<u>λ</u> <u>ind</u> ⁻)			this work	152
152(<u>λ</u> <u>ind</u> ⁻ <u>cIts</u> 857)			L.M.Pilarski	Pilarski (1972)
152(186 <u>cIts</u>)			this work	152
152(P2K)			this work	152
594	F ⁻	<u>galK</u> <u>galT</u> <u>str</u> ^r <u>su</u> ⁻	D.S.Hogness	Campbell (1965)
594(<u>λ</u> <u>ind</u> ⁻ <u>cIts</u> 857)			this work	594
594(186)			this work	594
594(186 <u>cIts</u>)			this work	594
AB259	HfrH	<u>thi</u> <u>str</u> ^s <u>λ</u> ⁻	P. Reeves	Hayes (1953)
AB259(<u>λ</u>)			this work	AB259
AB259(<u>λ</u> <u>ind</u> ⁻)			this work	AB259
AB1133	F ⁻	<u>thr</u> <u>leu</u> <u>pro</u> <u>his</u> <u>argA</u> <u>thi</u> <u>str</u> ^r	P. Reeves	

TABLE 2-II Cont'd.

Strain	Sex	Relevant genotype ^a	Source	Reference or derivation
AB1133(λ)			this work	AB1133
AB2102	F ⁻	<u>thr</u> <u>leu</u> <u>pro</u> <u>trp</u> <u>his</u> <u>thy</u> <u>thi</u> <u>str</u> ^r λ ^r	P. Reeves	Bouck & Adelberg (1970)
AB2463	F ⁻	<u>thr</u> <u>leu</u> <u>pro</u> <u>his</u> <u>thi</u> <u>arg</u> <u>recA13</u> <u>str</u> ^r	J. Davison	Howard-Flanders & Theriot(1966)
AB2463(186cIts)			this work	AB2463
AB2463(P2k)			this work	AB2463
AB2528	Hfr 0-13	<u>leu</u> <u>thi</u> <u>ilv</u> <u>supM</u> <u>str</u> ^r <u>tsx</u> ^s (λ)	A.J.Pittard	Pittard (1965)
AB2528(186)			this work	AB2528
AT713	F ⁻	<u>lysA</u> <u>argA</u> <u>cysC</u> <u>str</u> ^r	A.J.Pittard	Taylor & Trotter (1967)
AT713(186)			this work	AT713
AT2092	F ⁻	<u>his</u> <u>purF</u> <u>pheA</u> <u>thi</u> <u>str</u> ^r <u>tsx</u> ^r (Mul)	A.J.Pittard	Willettts,Clark & Low (1969)
AT2092(186)			this work	AT2092
AT2457	HfrH	<u>glyA</u> <u>thi</u>	T.S.Matney	Bukhari &Taylor (1971)

TABLE 2-II:Cont'd.

Strain	Sex	Relevant genotype ^a	Source	Reference or derivation
AT2457(186)			this work	AT2457
C600	F ⁻	<u>thr</u> <u>leu</u> <u>thi</u> <u>tonA</u> <u>supE</u>	D.S.Hogness	Appleyard (1954)
C600(λ)			D.S.Hogness	
C600(λ <u>ind</u> ⁻)			this work	C600
C600(λ <u>ind</u> ⁻ <u>cIts</u> 857)			D.S.Hogness	
C600(λ <u>i</u> ⁴³⁴)			D.S.Hogness	
C600(186)			this work	C600
C600(186 <u>cIts</u>)			this work	C600
C600(186 <u>cIts</u> <u>sus</u> 16)			this work	C600
C600(186 <u>sus</u> K4)			this work	C600
C600(186 <u>sus</u> K6)			this work	C600
C600(186 <u>sus</u> K9)			this work	C600
C600(18)			R.L.Baldwin	

TABLE 2-II Con'td.

Strain	Sex	Relevant genotype ^a	Source	Reference or derivation
C600(299)			R.L.Baldwin	
C600(P2k)			this work	C600
CGSC4291	F ⁺	<u>thy</u> ⁺ <u>recA</u> ⁺ <u>tyrA</u> ⁺ / <u>tyrA</u> <u>pyrD</u> <u>thi</u> <u>his</u> <u>trp</u> <u>thy</u> <u>str</u> ^r <u>recA</u> 1	B. Bachmann	K.B. Low (pers. comm.)
CGSC4292	F ⁺	<u>thy</u> ⁺ <u>recA</u> 1 <u>tyrA</u> ⁺ / <u>tyrA</u> <u>pyrD</u> <u>thi</u> <u>his</u> <u>trp</u> <u>thy</u> <u>str</u> ^r <u>recA</u> 1	B. Bachmann	K.B. Low (pers. comm.)
CR34(W)		<u>thy</u>	R.L.Baldwin	
DM455	F ⁻	<u>galK</u> <u>galT</u> <u>recA</u> 99 <u>str</u> ^r <u>su</u> ⁻	B. Rolfe	Mount (1971)
DM455(186)			this work	DM455
E784 ^b	Hfr KL19 $\lambda^{-}\lambda^{\text{r}}$		this work	KL19
E784(186)			this work	E784
E817	Hfr KL16 <u>thi</u> (ϕ 80 _{supF})		this work	KL16
E817(186)			this work	E817

TABLE 2-II Cont'd.

Strain	Sex	Relevant Genotype ^a	Source	Reference or derivation
E817(186 <u>susc</u> I53)			this work	E817
E817(186 <u>sus</u> K4)			this work	E817
E817(186 <u>sus</u> K9)			this work	E817
E828 ^b	F ⁻	<u>gal</u> K <u>gal</u> T <u>his</u> <u>str</u> ^r <u>su</u> ⁻	this work	594
E828(ø80)			this work	E828
E828(ø80 <u>sup</u> F)			this work	E828
E828ø80 ^r	F ⁻	<u>gal</u> K <u>gal</u> T <u>his</u> <u>str</u> ^r <u>su</u> ⁻ ø80 ^r	this work	E828
E828(186)ø80 ^r			this work	E828ø80 ^r
E847 ^b	Hfr 0-13	<u>leu</u> <u>thi</u> <u>ilv</u> <u>str</u> ^r <u>tsx</u> ^s λ ⁻	this work	AB2528
E847(186)			this work	E847
E873 ^b	F ⁺	<u>gal</u> ⁺ / <u>met</u> λ ⁻ λ ^r	this work	P601
H-724		<u>his</u> <u>tyr</u> A <u>trp</u> <u>pur</u> C <u>gua</u> B <u>thi</u>	T.S.Matney	Tritz, Matney & Gholson (1970)

TABLE 2-II Cont'd.

Strain	Sex	Relevant Genotype ^a	Source	Reference or derivation
H-724(186)			this work	H-724
H-888		<u>his</u> <u>trp</u> <u>purG</u> <u>thi</u>	T.S.Matney	Tritz, Matney & Gholson (1970)
H-888(186)			this work	H-888
JC2918		<u>thr</u> <u>leu</u> <u>pro</u> <u>his</u> <u>arg</u> <u>supE</u> <u>str</u> ^r	A.J.Clark	
JC2926		<u>thr</u> <u>leu</u> <u>pro</u> <u>his</u> <u>arg</u> <u>recA13</u> <u>supE</u> <u>str</u> ^r	A.J.Clark	
JC2926(186)			this work	JC2926
JP190	F ⁻	<u>tyrA</u> <u>thi</u> <u>ilvD</u> <u>metB</u> <u>argH</u> <u>gal</u> <u>str</u> ^r <u>tsx</u> ^r λ ^r	A.J.Pittard	
JP190(186)			this work	JP190
JP264	F ⁻	<u>thi</u> <u>ilvD</u> <u>metB</u> <u>argH</u> <u>his</u> <u>gal</u> <u>str</u> ^r <u>tsx</u> ^r <u>nal</u> ^r λ ^r	A.J.Pittard	
JP264(186)			this work	JP264
KL16	Hfr	<u>thi</u> <u>str</u> ^s λ ⁻	P.Reeves	Low (1968)

TABLE 2-II Cont'd.

Strain	Sex	Relevant Genotype ^a	Source	Reference or derivation
KL16(186)			this work	KL16
KL16(186 <u>cIts</u>)			this work	KL16
KL16(186 <u>cIts</u> <u>sus</u> 15)			this work	KL16
KL16(186 <u>sus</u> K4)			this work	KL16
KL16(186 <u>sus</u> K6)			this work	KL16
KL16(186 <u>sus</u> K9)			this work	KL16
KL19	Hfr	<u>str</u> ^S (λ) λ ^r	B.Bachmann	Low (1968)
KL98	Hfr	<u>str</u> ^S (λ)	A.J.Pittard	Low (1968)
KL98 (186)			this work	KL98
KL164	Hfr KL16	<u>thi</u> <u>thyA</u> <u>nalB</u> <u>str</u> ^S λ ⁻	B.Bachmann	Hane & Wood (1969)
KL164(186 <u>cIts</u>)			this work	KL164
P601	F'	<u>gal</u> ⁺ / <u>met</u> (λ) λ ^r	P.Reeves	Skurray & Reeves (1973)
P980	HfrH	<u>thi</u> <u>str</u> ^S <u>tra</u> ⁻ λ ⁻	P.Reeves	Skurray & Reeves (1973), AB259

TABLE 2-II Cont'd.

Strain	Sex	Relevant Genotype ^a	Source	Reference or derivation
PA3306	F ⁻	<u>nadB</u> <u>purI</u> <u>argH</u>	T.S.Matney	Bukhari & Taylor (1971)
PA3306(186)			this work	PA3306
S26 <u>su</u> ⁻		<u>su</u> ⁻ λ ⁻	D.S.Hogness	Garen, Garen & Wilhelm (1965)
S26 <u>suI</u> ⁺		<u>supD</u> λ ⁻	D.S.Hogness	" " "
S26 <u>suII</u> ⁺		<u>supE</u> λ ⁻	D.S.Hogness	" " "
S26 <u>suIII</u> ⁺		<u>supF</u> λ ⁻	D.S.Hogness	" " "
X478	F ⁻	<u>thi</u> <u>metE</u> <u>lysA</u> <u>trpE</u> <u>purE</u> <u>proC</u> <u>leu</u> <u>str</u> ^r	B.Bachmann	Berg & Curtiss (1967)
X478(186)			this work	X478
W3805		<u>galE</u> <u>su</u> ⁻	A.D.Kaiser	Kayajanian (1971)
W3805(186)			this work	W3805
W3805(299)			this work	W3805

^aThe genotypes of lysogens constructed for this work are not repeated. The abbreviations are those of Taylor (1970).

^bDerivation described in text (2.1.d).

AB2528 was cured of its λ prophage by heteroimmune super-infection with λ_{i}^{434} to yield E847. The technique was modified from that of Kaiser and Masuda (1970). To log phase AB2528 grown in TB supplemented with maltose (0.1%), λ_{i}^{434} was added at a multiplicity of 10, and the mixture incubated at 37° for 20 min. It was then diluted into TB and shaken for 120 min at 37°. Dilutions were spread on LG plates and grown overnight. Single colonies were tested for absence of λ and λ_{i}^{434} prophages by toothpicking into a plate seeded with C600 and then into one seeded with C600(λ_{i}^{434}). Of the survivors, 13% were cured of the λ prophage, although 40% of these now carried a λ_{i}^{434} prophage. Several non-lysogenic survivors were purified and tested.

KL19 and P601 are both λ lysogenic and λ resistant. A triple hybrid phage, λ_{i}^{434} h80 att λ , was constructed (2.1.c) to cure these, yielding E784 and E873 respectively. To a log phase culture of the particular strain grown in LGB, the phage was added at a multiplicity of 10. The mixture was incubated for 20 min at 37°, diluted into LGB and shaken at 37° for 120 min. Survivors were spread on L plates and, after overnight growth, tested for absence of the various prophage by toothpicking into a plate seeded with 594. Several non-lysogenic survivors of each strain were purified and tested.

2.2 METHODS

This section contains general techniques and common experimental procedures. Techniques specific to a particular chapter are described in the Materials and Methods section of that chapter.

a. Storage of Phage and Bacterial Stocks - Phage stocks were stored over chloroform at 4°. Bacterial stocks were stored in stabs at 4°. Slants, stored at 4°, were maintained for strains in current usage. Fresh slants were prepared from the previous slants, or from stabs, when the slants were nearing exhaustion, but generally every two months. The F' strains CGSC4291 and CGSC4292 were stored in stabs of minimal agar supplemented with glucose, uracil, thiamine, histidine and tryptophan, but lacking thymine and tyrosine.

b. Growth of Cultures - A loop was used to inoculate broth cultures from the appropriate slant. Cultures were shaken overnight in a New Brunswick gyrotory water bath shaker at 37°, or 30° if temperature-sensitive. Such overnight cultures were diluted one part into ten or one hundred parts fresh broth and shaken as before. The optical density at 600mμ (OD) was followed, samples being measured with a Zeiss PMQII spectrophotometer. Cultures were generally used at an OD of 0.8-1.0 ($2-3 \times 10^8$ colony formers/ml) although indicator bacteria were grown to OD 1.5. Usually the LB series of broths were used for growth of bacteria, although indicator bacteria such as 594 and C600, with their lesser growth requirements, were grown in TB. The F' strains CGSC4291 and CGSC4292 were grown overnight in minimal medium supplemented with glucose, uracil, thiamine, histidine and tryptophan, but grown to log phase immediately before use in LGB.

c. Preparation of Phage Stocks - Phage 186 stocks were prepared by a confluent plate lysis technique. A single plaque was

taken into 1 ml TM with a sterile toothpick. A 0.1 ml sample of a 10^{-1} dilution of this solution was plated with indicator bacteria using TB soft agar and TB plates. Following $3\frac{1}{2}$ hours incubation at 30° the plates were flooded with 3 ml TM and chilled overnight at 4° . The phage solution was removed, filtered (Millipore, pore size 0.42μ) and stored over chloroform. Phage 186 titres of $1-5 \times 10^{10}$ pfu/ml were attained. Where other phage were involved, the plates were incubated for at least 6 hours at 37° before flooding. The procedure for preparation of P1 phage stocks will be detailed later (5.2.a).

Alternately, phage stocks were prepared by UV or heat induction of lysogens, as appropriate.

d. Assay of Phage and Bacteria - Bacteria were diluted in H-1 and spread, generally, on L plates to assay colony formers per ml. Phage were diluted in TM (LGCB for P1 and P2), or if infectious centres were involved in H-1, and 0.1 ml samples mixed with 0.2 ml log phase (OD 1.5) indicator bacteria. Strains 594 and C600 were standard indicators for phage 186. Preadsorption was allowed by incubation of the mixture at 37° for 15 min, before plating in a layer of TB soft agar (supplemented with 4mM CaCl_2 for P1 and P2) on TB plates (TB with 4mM CaCl_2 for P2, LGC plates for P1). After overnight incubation at 37° , or 30° if appropriate, plaques were counted using a Gallenkamp colony counter. Where adsorption was being assayed, the first dilution was into chilled TM saturated with chloroform. After 10 min at 0° , further dilutions were made and assayed as above.

e. Resuspension of Bacteria - Bacteria were pelleted by

centrifugation at 7,600g for 5 min at 4°, using an MSE High Speed 18 centrifuge. When the culture was to be used for conjugation, the centrifugation was at 20°. The supernatant was removed and the pellet gently resuspended in the appropriate medium.

f. Preparation of Lysogens - Lysogens were prepared from the turbid centres of single plaques of the phage on the particular indicator. A small platinum ball, formed by melting the end of a platinum wire, was used to take off such bacteria and streak them for single colonies on L plates. After growth, these single colonies were tested for lysogeny, usually by toothpicking into an L plate master, a plate seeded with sensitive bacteria, and finally one seeded with a lysogen immune to the phage under test. For a few lysogens that did not produce phage [e.g. recA (186)] the single colonies were tested for immunity by streaking with a toothpick across lines of phage on an L plate. These lines of phage were applied with a 0.1 ml pipette (about 0.01 ml of a high titre phage stock-c. 10^{10} pfu/ml-per line). For example, a suspected 186 lysogen would be streaked across two phage lines, the first 186cI, the second 186vir. A 186 lysogen should be immune to the former, but sensitive to the latter. Phage producing (or immune) colonies were restreaked on L plates and the procedure repeated till the lysogen had been purified through three single colony isolations or, if still not showing 100% lysogeny (20/20 single colonies), until this was achieved. Finally the new lysogen was grown overnight in liquid culture and tested for appropriate bacterial and phage markers. In

addition, this culture was streaked for single colonies and 50 of these tested for the new prophage as above. When CGSC4292 lysogens of 186 were constructed, the appropriate minimal medium was used throughout.

g. UV Irradiation - Ultraviolet irradiation was at 50 cm from a General Electric 15 watt germicidal lamp. The sample was never more than 1mm deep and was swirled on a rotating table during the irradiation. The dose rate was measured with a UV dosimeter supplied by Oliphant Bros., Springbank, South Australia and consistently found to be $15.2 \text{ ergs/mm}^2/\text{sec}$.

h. Induction of Phage Lysogens - All phage lysates were treated with chloroform before assay and, if to be kept, were clarified by pelleting bacterial debris and/or filtering (Millipore, pore size 0.42μ).

i. Heat induction - Lysogens of temperature-inducible phage mutants were grown to OD 1.0 at 30° , shaken by hand at 45° for 10 min (or 15 min if a large volume was involved) and then shaken at 37° till lysis.

ii. UV induction - Lysogens grown in LGB to OD 0.8-1.0 were resuspended in H-1, UV irradiated for 50 sec, a one tenth volume of 10xLGB added, and the resultant culture shaken at 37° or 30° till lysis (180 min at 37° , or 250 min at 30° , for 186 lysogens). In some experiments the UV dose was varied and the culture assayed for colony forming bacteria and for plaque

forming units (infectious centres) immediately after irradiation.

iii. Mitomycin C induction - Lysogens grown in LGB to OD 1.0 were resuspended in H-1, then mitomycin C (1 or 5 $\mu\text{g}/\text{ml}$) and one tenth volume of 10xLGB added, and the culture shaken at 30° for 240 min.

iv. Nalidixic acid induction - Lysogens were grown in LGB to OD 0.5 and resuspended in H-1. One tenth volume of 10xLGB and nalidixic acid (12.5 $\mu\text{g}/\text{ml}$) were added and the cultures shaken at 37° for 10 min. Then they were diluted one hundred fold into fresh LGB and shaken at 37° for 180 min.

i. Conjugation.

i. Patch test - Male cultures were periodically checked and purified on the basis of donor ability as assayed by a patch test on solid medium. Single colonies of the male strain were patched out onto an L plate (three short parallel streaks with the flat end of a sterile toothpick, covering about 1 cm^2). The patches were grown to log phase (6 hours at 37°, or 9 hours at 30°) and then replicated with a velvet pad onto a series of plates; firstly onto an L plate master, and then onto two selective minimal plates, the second of these thickly spread with an overnight culture of a female, chosen as mutant in a selectable

marker transferred early by the particular male, which had been washed and resuspended in one tenth volume of H-1. The selective plates were as appropriate to select recombinants for the marker transferred early. On incubation, Hfr colonies produced full patches of recombinants, whereas F^+ donors only produced isolated single colonies.

ii. Conjugation technique - Mating conditions were similar to those described by Adelberg and Burns (1960) save that lysogenic donors and/or recipients were involved. Cultures were grown in LGB to OD 0.8 then washed and resuspended at 20° in fresh LGB to reduce the free phage concentration. Males and females were mixed in a ratio of 1:10 to 1:20 respectively, in the presence of anti-serum to the phage concerned, and swirled very gently in a flask with a capacity at least 20 times the volume of the mating mixture. Male and female titres were assayed at mixing. After the required mating period, samples were diluted into H-1 containing streptomycin ($200\mu\text{g/ml}$) or added to phage T6 as described by Hayes (1957) to kill the donor cells. Dilutions were spread on minimal plates to select recombinants, or plated immediately with indicator bacteria (resistant to streptomycin or T6, as appropriate) in a layer of TB soft agar on TB plates (both containing streptomycin, if appropriate)

to assay phage activity.

iii. Interrupted matings - Where interruption of mating was required, a sample from the mating mixture (as described above) was diluted into 2 ml H-1 in a large test tube (150mm long by 13mm diameter), the top of the tube covered with Parafilm, and the tube violently agitated for 15 sec in a vibratory mixer of the type described by Low and Wood (1965), generously loaned by Peter Reeves and modified in this laboratory. Following blending, samples were further diluted, if required, and plated immediately with soft minimal agar on selective minimal plates for recombinants, or with indicator bacteria and TB soft agar on TB plates (both containing 200 μ g/ml streptomycin) to assay phage activity.

CHAPTER 3

THE GENETIC MAP OF COLIPHAGE 186.

3.1 INTRODUCTION

The availability of detailed genetic maps for phages λ (Campbell, 1971) and P2 (Lindahl, 1969a) has enabled comparison (Calendar, 1970; Dove, 1971; Lindahl, 1971; Bertani and Bertani, 1971) of their organisation into clusters of genes with related functions, of the locations of other genes involved in the lytic cycle, and of the primary control regions determining the activity of the genome. The spatial arrangement of the structural genes and those involving DNA synthesis in both phage and prophage maps (that is with respect to the cohesive ends and the attachment region) is similar for λ and P2. Genes concerned with cell lysis are located at the far right of the λ phage map but in the left half for P2. The primary control regions are dissimilar, although the genes specifying immunity and prophage insertion appear on the right halves of the phage maps. A number of λ functions do not, as yet, have P2 counterparts, the more significant being N, Q, red, cII and cIII.

The similarities between the λ and P2 genetic maps have been used to draw more general conclusions about the benefits of certain genetic organisations and the dissimilarities to explain where λ and P2 differ. For example, (i) organisation into clusters of related genes may simplify coordination of their activity, and (ii) the low frequency of general phage recombination for P2 is due to the absence of the genes found between int and cI in λ , which includes the phage specific recombination

system. Of particular interest is the arrangement for transcription of the int gene which Bertani (1970) proposes is severed by P2 integration such that the P2 prophage is not inducible. This contrasts with λ where integration does not sever the int gene from its promoter since λ int gene is transcribed leftward (Gottesman and Weisberg, 1971).

The production of a 186 genetic map is seen as useful in reinforcing or possibly modifying conclusions drawn from similarities in phage genetic maps. More importantly, since the different induction potentials of λ and P2 have been related to their genetic organisation, it is necessary to consider this in studying induction of prophage 186. The present author has initiated studies in this direction. This work will be described and then discussed having regard to results obtained by others in this laboratory.

3.2 MATERIALS AND METHODS

a. Isolation of Suppressor Sensitive Conditional Lethal Mutants.

A technique similar to those of Thomas et al (1967) and Parkinson (1968) was employed. A stock of 186cIts was diluted to 6×10^7 pfu/ml in 0.01M MgSO_4 and then UV irradiated ($9,600 \text{ ergs/mm}^2$) in a swirled glass petri dish, the depth of solution being less than 1mm. This gave a survival of 0.005%. Samples were plated in a layer of UV irradiated (Weigle, 1953) S26su⁺ bacteria ($1,500 \text{ ergs/mm}^2$ in TB, 20% survival- 3×10^6 viable bacteria/plate) overlaying S26su⁻ bacteria (6×10^7 /plate). Plates

were incubated at 37° overnight. Any turbid or unusual plaques, and all the small plaques, were stabbed with a sterile toothpick into a plate seeded with S26su⁻ bacteria and then into one seeded with S26su⁺. Ninety such stabbings can be comfortably fitted on each plate. Those phage showing more activity after incubation on the permissive host than on the non-permissive host were sampled with a toothpick into 1ml of TM. A 0.1ml aliquot from a 10⁻² dilution of this was plated on S26su⁺ bacteria to obtain single plaques. Ten plaques for each suspected mutant were retested by toothpicking as above, one selected, plated again for single plaques and a stock made from one of these.

b. Isolation of Suppressor Sensitive Clear Plaque Mutants

A log phase culture of 594(186) was washed and resuspended in medium C, an equal volume of NNG solution (200µg/ml in medium C) added, and shaken at 37° for 30 min. This was washed twice with medium C, then resuspended in 10x volume LGB and shaken at 37° for 270 min. The bacteria were pelleted and the supernatant filtered through a Millipore filter (pore size 0.42µ) before being plated for single plaques on 594 as indicator. Plaques clearer than wild type were selected and purified as above (3.2.a) though being tested for clear activity on su⁻(594) and turbid activity on su⁺(AB2528).

c. Complementation Tests

- i. Spot tests - Plates were seeded with S26su⁻ and 5x10⁷ pfu of one sus mutant, chilled, and other sus

mutants spotted on (0.005ml) from stocks of 1×10^8 pfu/ml. Overnight incubation was at 37° . Use of less phage in plate or spot gave negative results, and more phage resulted in single plaques, possibly indicative of recombination, for mutants in the same cistron.

ii. Cross streak tests - Plates were seeded with S26 $\underline{\text{su}}^-$ and chilled. A line of one sus mutant was streaked across the plate with a sterile platinum loop from a phage stock of 1×10^9 pfu/ml, and then various other sus mutants streaked across perpendicular to the original streak and intersecting it once. After incubation overnight at 37° these intersections were examined for lysis.

d. Recombination of 186 $\underline{\text{sus}}$ Mutants

This technique was modified from that used in this laboratory for phage λ recombination - in particular with respect to the different adsorption properties of 186. Phage were added at a multiplicity of 10 each to log phase bacteria in LGB (2×10^8 colony formers/ml) and incubated without aeration for 20 min at 37° . This gave 40 to 60% adsorption. The infected bacteria were diluted into LGB containing 186 anti-serum ($K=1 \text{ min}^{-1}$) and incubated a further 5 min at 37° (free phage concentration reduced 100 fold). This was diluted 10^{-3} into LGB and shaken at 37° for 90 min before assaying for progeny

and recombinants. Each parental phage was carried alone through the procedure and revertants found insignificant.

3.3 RESULTS

a. Isolation of Mutants

Suppressor sensitive or sus mutants (Campbell, 1961) were isolated from 186cIts using the mixed indicator technique described above (3.2.a). Few turbid plaques were found at 37° and most of these were 186c⁺ revertants. Thus the majority of sus mutants were initially detected as small plaques on the mixed indicator plates. Four mutagenesis runs, all employing UV irradiation of the same 186cIts plate stock produced from a single plaque, were performed using S26suI⁺ (2 runs), S26suII⁺ (1 run) or S26suIII⁺ (1 run) as the permissive host. From about 30,000 plaques screened 2,500 plaques were initially selected and finally 48 suppressible conditional lethal (186cIts sus_{1,2,4-47,51 and 52}) and two suppressible plaque morphology mutants were purified. One of the plaque morphology mutants (186cIts sus₃) produces larger plaques on the permissive than the non-permissive host, and the other (186cIts suscII₅₅) gives clear plaques on su⁻ at 30°, turbid plaques on su⁺ at 30° and clear plaques on both indicators at 37°. Lyso-gens of all these sus mutants except sus₁, sus₅₁ and sus₅₂ which had acquired absolute clear plaque mutations during the isolation procedure, were prepared, generally in S26suII⁺ or, if the particular mutant grew poorly in this host, in the preferred permissive strain.

Suppressor sensitive clear plaque mutants were isolated from a wild type 186 lysogen as described in Section 3.2.b. From 8000 plaques screened, 200 clearer plaques were tested and two susc mutants purified; being 186suscI53 and 186suscI54. These mutants were selected as giving clear plaques on 594 and turbid plaques on AB2528 (which carries a weak suppressor). They also give turbid plaques on S26suI,II or III⁺. Lysogens were prepared in S26suIII⁺ for storage.

Two virulent mutants of 186 were isolated and purified during the above mutant isolations, one derived from 186cIts sus16, the other from 186suscI53. The former shows activity only on permissive hosts (although plating at an efficiency of 3×10^{-4} on a non-permissive lysogen, presumably through rescue of the sus16 function from the prophage), while the latter is virulent on both permissive and non-permissive lysogens. Although detected as "spontaneous" changes during these isolations both the virulent mutants arose in mutagenised stocks, and both where the CI gene was not wild type.

b. Some Properties of the 186 sus Mutants

To prepare stocks of the sus phage it was found necessary to increase the time of incubation before flooding in the standard confluent plate lysis technique (Chapter 2) up to 9 hours at 30°. Again some mutants required a specific permissive host. A few mutants still gave poor titres by the plate technique, so stocks of these were prepared by liquid infection.

Finally titres of 10^{10} pfu/ml or more were attained for each mutant. Reversion frequencies measured on S26su⁻ ranged from less than 3×10^{-9} to 5×10^{-5} . These frequencies, however, were not always characteristic of the particular mutants since different stocks of the same mutant could show quite different reversion frequencies.

The various sus mutants retain their parental induction behaviour. For example 186cIts sus16 as prophage in C600 is inducible by heat, UV irradiation and mitomycin C (results shown in Table 4.III). Lysogens of the susc mutants also were still UV inducible, and the 186cIts suscII55 prophage heat inducible.

As mentioned above various of the sus mutants have preferred permissive hosts. In particular sus18 and sus47 require suI⁺, sus16 suII⁺, sus27 suIII⁺ and sus21 either suI⁺ or III⁺. Other mutants have not all been rigorously tested on all permissive hosts.

c. Complementation of the 186 sus mutants

Complementation studies of 43 of the 186sus mutants were performed on S26su⁻ at 37° by the spot test (3.2.c.i) in all possible combinations. Complementation was evidenced by a clear spot and non-complementation by lack of phage activity or a few single plaques due to recombination. Various intermediate conditions were found. The results were unsatisfactory in that many of the mutants did not complement well. However, as preliminary results, they did suggest three large complementing

groups, designated (for the purpose of this thesis only); group I of 7 members, group II of 12 definite and several possible members, and group III of 7 members. The remainder of the mutants could not be allocated to groups and one, sus40, did not complement with any of the other mutants. These results have since been superseded in this laboratory and therefore will not be described in more detail.

In view of the disappointing results obtained with the spot complementation test, 9 of the mutants were investigated by the cross streak test (3.2.c.ii). The results confirmed 3 members of group I (sus5,11 and 24) and gave promise of much better resolution than possible with the spot test. This has been pursued further by another student in this laboratory.

The susc mutants were tested by the cross streak method against known 186cI and cII mutants (Huddleston, 1970) where cI is defined by 186cIts as responsible for the maintenance of repression. The 186 derived mutants were assigned to the cI gene (186suscI53 and 54) and the 186cIts derived mutant to the cII gene (186cIts suscII55).

d. Recombination between 186 sus Mutants

Frequencies of recombination between 186 sus mutants 5,11,15 and 16 were determined and found to be independent of whether or not (i) phage anti-serum was used to inactivate unadsorbed phage, (ii) the incubation of infected bacteria was extended from 90 to 120 min, (iii) chloroform was used after

this incubation to ensure complete lysis, or (iv) the host was defective in recA. The effect of these variations on recombination between 186cIts sus11 and 16 is shown in Table 3-I.

However the permissive character of the host for recombination may have a substantial effect as shown in Table 3-II. The primary effect is on the number of progeny phage. This is relatively constant in the su⁺ host but varies markedly in the su⁻ host. The percentage progeny phage produced in an su⁻ host by two sus mutants of that produced by sus⁺ phage may be used as a measure of complementation between the mutants (Lindahl, 1969a). It is seen that sus5 and sus11 (which are in the same group) complement poorly with sus15 and only slightly better with sus16. However sus15 and sus16 complement quite well. Although the progeny level may be depressed the number of recombinants is similar in the su⁻ host, which has the secondary effect of inflating the recombination frequencies in this host. This maintained level of recombinants in face of a decrease in total progeny suggests that in an su⁻ host, cells which give rise to wild type recombinants contribute more to the burst than those lacking a sus⁺ phage. Nevertheless the same trends are apparent. Group I mutants are comparatively loosely linked to sus15 and sus16, which are themselves tightly linked. Preliminary experiments in conjunction with Linda Pilariski in this laboratory have shown that phage λ helps recombination in an su⁺ host between 186sus15 and sus16, and within Group I, increasing the number of recombinants up to fifty fold whilst

TABLE 3-I: EFFECT OF VARIATION IN TECHNIQUE UPON RECOMBINATION BETWEEN 186cIts sus11
AND 186cIts sus16.

Variation from Standard Technique ^a	Host	Progeny pfu/ml on C600	Recombinants pfu/ml on 594	Percentage Recombination ^b
None	C600	2.4×10^{10}	8.5×10^7	0.71
Without anti-serum after adsorption	C600	3.6×10^{10}	1.1×10^8	0.62
With chloroform after 90 min	C600	2.1×10^{10}	8.6×10^7	0.82
120 min incubation of infected cells	C600	2.6×10^{10}	8.2×10^7	0.63
Host- <u>su</u> II ⁺ <u>rec</u> ⁺	JC2918 ^c	2.3×10^{10}	9.2×10^7	0.80
Host- <u>su</u> II ⁺ <u>rec</u> A13	JC2926 ^c	2.4×10^{10}	9.9×10^7	0.82

^aSection 3.2.d. Briefly, 20 min adsorption, 5 min treatment with anti-serum, 90 min incubation before assays.

^bRecombination frequency expressed as a percentage, = $200 \times \frac{\text{recombinant titre}}{\text{progeny titre}}$

^cApart from the recA gene, isogenic hosts.

TABLE 3-II: EFFECT OF PERMISSIVE CHARACTER OF HOST ON RECOMBINATION BETWEEN 186 sus MUTANTS^a

Parents ^b	C600 <u>suII</u> ⁺			594 <u>su</u> ⁻			
	Progeny	<u>sus</u> ⁺ Re-combinants	% Recombination	Progeny	<u>sus</u> ⁺ Re-combinants	% Recombination	% Complementa-tion ^c
<u>sus</u> 5 x <u>sus</u> 11	2.5x10 ¹⁰	2.0x10 ⁵	0.002	1.1x10 ⁸	1.0x10 ⁵	0.2	0.5
<u>sus</u> 5 x <u>sus</u> 15	2.5x10 ¹⁰	3.0x10 ⁷	0.24	3.0x10 ⁸	4.5x10 ⁶	3.0	1.4
<u>sus</u> 11x <u>sus</u> 15	2.5x10 ¹⁰	3.6x10 ⁷	0.29	4.4x10 ⁸	8.7x10 ⁶	4.0	2.0
<u>sus</u> 5 x <u>sus</u> 16	2.0x10 ¹⁰	6.9x10 ⁷	0.68	1.4x10 ⁹	3.7x10 ⁷	5.5	6.4
<u>sus</u> 11x <u>sus</u> 16	2.3x10 ¹⁰	7.5x10 ⁷	0.66	2.1x10 ⁹	5.4x10 ⁷	5.2	9.5
<u>sus</u> 15x <u>sus</u> 16	1.3x10 ¹⁰	1.0x10 ⁶	0.016	1.0x10 ¹⁰	1.1x10 ⁶	0.021	45.
<u>sus</u> ⁺	2.9x10 ¹⁰			2.2x10 ¹⁰			

^aData expressed as in Table 3-I. All crosses using standard technique (3.2.d).

^bAll phage carried 186cIts.

^cIn the su⁻ host, progeny from two sus parental phage as a percentage of that from sus⁺ parental phage.

only halving the number of 186 progeny. However there was little effect on recombination between Group I and sus15 or sus16. Further a λ red3 helper had no effect on recombination frequency.

3.4 DISCUSSION

a. The mutants

Stephanie Hocking in this laboratory has vigorously pursued the testing of the conditional lethal sus mutants. Complementation tests using the cross streak method have confirmed Groups I, II and III (although two members of Group II and one of Group III were wrongly assigned) and revealed eight further groups of fewer members from amongst the mutants I could not classify or did not test. Further testing on a polarity suppressor strain (suA, Morse and Primakoff, 1970) has split groups II and III and one of the new groups, but not group I, giving a total of twenty complementing groups.

These results are most reassuring. The sus mutants isolated fall into many genes with no groupings of a particular gene in a particular mutagenesis run. Different mutants within the same gene show different su⁺ host preferences. Thus it can be inferred that the mutants represent independent isolations. Although nominally using the mixed indicator selection method, in the main small plaques were picked making the technique more equivalent to that of Campbell (1961) with the advantage that sus mutants efficiently suppressed by the su⁺ host still may form smaller plaques than the wild type because of the additional

presence of su⁻ bacteria. Thus it is not surprising that a broad spectrum of mutants is found, rather than the more limited one (for λ) from the true mixed indicator technique (Thomas et al, 1967). The isolation of 20 essential complementing groups for 186 compared to 18 for P2, 16 for P22 and 24 for λ (Dove, 1971) has defined most of the essential 186 functions considering that 186 DNA has a molecular weight of 20×10^6 (Wang, 1967) compared to 22×10^6 , 26×10^6 and 31×10^6 daltons respectively for the other phages (Dove, 1971).

The use of the three different su⁺ hosts in separate runs has clearly resulted in the isolation of mutants, though not necessarily genes, which would have been missed had selection only been made on suII⁺ as has been done for λ (Campbell, 1961; Thomas et al, 1967; Parkinson, 1968). That is 186 mutants were found that grow only on suI⁺, only on suII⁺, or only on suIII⁺. These mutants provide an ideal set for distinguishing these three suppressors (especially if lysogenic for λ) but have not been tested on weak suppressors. The set of λ mutants proposed by Van Montagu, Leurs, Brachet and Thomas (1967) also allows distinction of weak suppressors.

The isolation of two complementing groups of clear plaque mutants (Huddleston, 1970) and, in particular, susc mutants (3.3.c) implies two genes coding for protein products that are concerned with producing turbid plaques of 186 on E. coli. Thus 186 is, so far, different from P2 (1 gene) and λ (3 genes).

b. Recombination

Stephanie Hocking has made a start on producing a linkage map for phage 186. Mutants in Group I give higher recombination frequencies with other groups than are observed between these other groups, confirming the results in Table 3-II. From the results reported above it is clear that 186 is behaving very similarly to P2. Recombination frequencies between 186 sus mutants are similar to those for P2ts mutants (Lindahl, 1969a) and P2am mutants in an su⁺ host (Lindahl, 1971). It also appears that "int" catalysed recombination across the episite could be responsible for the larger frequencies between group I and any other, if Group I is the only group so far identified on one side of that episite. Again like P2 the recombination frequency is independent of the major recombination system of the host (Lindahl, 1969b).

That the λ red phage recombination system could help 186 recombination was suggested by Mandel (pers. comm.) and has been reported (Mandel and Kornreich, 1972). Their result is difficult to interpret in terms of recombination frequencies. They were using "two 186 sus mutants (gift of A.D.Kaiser)" and an su⁺ host. We have shown that 186susK4, K6 and K9 (from A.D. Kaiser) are probably all on the same side of the episite and that the λ red system can indeed increase the recombination frequency between 186 sus mutants on the same side of the episite, in an su⁺ host. Also the λ red system has little effect on the already better recombination postulated as being 186 "int"

promoted across the episite. In fact this difference in λ red effect reinforces the idea of a 186 specific recombination between Group I and the other groups. The effect of the λ red system on P2 recombination could be tested but would require use of P2 strains carrying old mutations to avoid interaction between $P2_{old}^+$ and λ_{spi}^+ (Lindahl *et al.*, 1970).

Like P2 (Lindahl, 1969a), 186 shows poor complementation, which is so poor for some mutants to perhaps lead to the suggestion of cis-acting products (Lindahl, 1970). Group I product could act in this way. Even in su⁺ hosts some combinations of 186 mutants may produce less progeny than others, particularly if a mutant is suppressed and/or complements poorly. This will perturb the recombination frequency calculated and will need to be borne in mind when drawing up a linkage map for phage 186.

Even at this early stage in work towards the 186 genetic map it is apparent that many parallels will be found between phages 186 and P2.

CHAPTER 4

ULTRAVIOLET INDUCTION OF COLIPHAGE 186

4.1 INTRODUCTION

The division of temperate coliphages by Jacob and Wollman (1956) into two groups the one, the " λ -related" phages showing zygotic induction, UV induction and recombination with λ , and the other, the "non-inducible" group showing none of these properties, has led to the idea of non-inducible phages that display none of the induction properties typical of λ . Phage 186, isolated by Jacob and Wollman (1956), was placed in this latter group as it neither recombined with λ , nor exhibited zygotic induction though UV induction was not tested.

Phage P2 has been described as the prototype of a "non-inducible" phage (Bertani, G., 1968). Repression is not lifted when P2 lysogens are UV irradiated (Bertani and Bertani, 1971) and even when repression is lifted either by heating a temperature-sensitive clear plaque mutant (Bertani, L.E., 1968) or as may occur during conjugation from a lysogenic male to a non-lysogenic female (Kelly, 1963), an abortive induction results in that no phage are produced. Similar results have been reported for a temperature-sensitive clear plaque mutant of the related phage 299 (Golub and Zvenigorodsky, 1969) and for a similar mutant of the hetero-immune phage 18 (Golub and Reshetnikova, 1970). That no phage are produced following lifting of immunity has been attributed to lack of excision of the prophage. It has been proposed that the int function of P2, required for this excision, belongs to a constitutive operon

that is physically disrupted by prophage integration (Bertani, 1970). Furthermore mutants have been isolated which allow heat induction of a temperature-sensitive clear plaque mutant of P2 (Calendar et al, 1972) and which also may alter the control of int expression (Sunshine, 1972) and thereby bypass the block caused by integration.

Coliphage 186 appears able to excise once immunity has been lifted as an inducible temperature-sensitive clear plaque mutant has been described (Baldwin et al, 1966). Also, as will be described in this chapter, it is UV inducible (Woods and Egan, 1969). The effects of ultraviolet irradiation on prophage 186 will be compared and contrasted to those on λ and various "non-inducible" phages W, 18, 299, P2 and the non-inducible mutant λ_{ind}^- (Jacob and Campbell, 1959).

4.2 MATERIALS AND METHODS

a. Determination of Latent Periods

i. Infection - Log phase bacteria, C600 in all cases, were grown in LGB at 37° to 2.5×10^8 viable cells/ml. If required the bacteria were resuspended in H-1 and subjected to UV irradiation for 10 or 30 sec (90% and 50% survival respectively) under the standard conditions, then a tenth volume of 10xLGB added. Phage were added at a multiplicity of 0.6 and the mixture incubated at 37° without aeration for 5 min (for P2k, 10 min incubation in presence, for adsorption only, of

4mM CaCl_2). Adsorption under these conditions was only 10 to 15% giving a multiplicity of infection of less than or equal to 0.1. The mixture was diluted ten fold into LGB containing anti-serum to the phage concerned ($K=1.5 \text{ min}^{-1}$, antiserum prepared against phage 299 was used against P2k) and incubated 5 min at 37° (reduced free phage 1000 fold), before dilution 10^{-3} and 10^{-5} into LGB. These dilutions were shaken at 37° and assayed periodically for phage activity on C600 (P2k on C1055) without pre-adsorption.

ii. UV induction. Log phase C600 lysogens of λ and 186 were grown in LGB at 37° to 2×10^8 and 3×10^8 viable cells/ml respectively, resuspended in H-1 and UV irradiated for 10, 20 or 30 sec. Immediately these were diluted 10^{-5} and 10^{-7} into LGB and shaken at 37° . Samples were assayed periodically for phage activity on C600 without preadsorption. Free phage (that is, chloroform resistant plaque forming units) were assayed immediately after the irradiation and found insignificant.

iii. Heat induction. Log phase C600, 594 or 152 lysogens of λ ind⁻cIts857 and 186cIts were grown in LGB at 30° to 3×10^8 viable cells/ml. These were heat induced by shaking at 45° for 10 min before being diluted 10^{-5} and 10^{-7} into LGB. The dilutions were shaken at 37° and assayed periodically for phage activity on C600

without pre-adsorption. Free phage (assayed as above) were insignificant immediately after the heat treatment.

4.3 RESULTS

a. Prophage 186 is UV inducible. The data in this subsection (4.3.a) only are selected from my B.Sc. honours thesis (Woods, 1968). Since this data has not been published it is included here to introduce the problem of 186 induction.

Figure 4-I shows the changes in optical density (O.D. at 600m μ) following UV induction of C600 lysogens of λ , 186 and 186cIts where the post-irradiation incubation was at 37°. Tryptone broth was used in these experiments. There is only a slight drop in O.D. after irradiating the 186 lysogen compared to that for the λ lysogen, and the decrease occurs later. Incubation at 37° alone is sufficient to induce the 186cIts lysogen, but when this is in addition irradiated the drop in O.D. is much less and more resembles that after irradiation of the 186 lysogen.

The phage titres attained after 250 min of post-irradiation incubation of various lysogens at 30° are shown in Table 4-I. For prophages 18, 299, W and λ ind⁻cIts857 cultures show no increase in phage titre after UV irradiation, while lysogens of λ , 186 and 186cIts show a 10³ to 10⁴ fold increase and are thus UV inducible. The somewhat lower titres in the non-irradiated cultures for prophages which are unaffected by the irradiation

FIGURE 4-1: The change in optical density at 600m μ following ultraviolet induction (closed symbols) of C600 lysogens of various coliphages over 180 min of post-irradiation incubation at 37°.

The open symbols represent the non-irradiated controls.

- ○ C600(λ)
- ▲ △ C600(186)
- □ C600(186cIts)

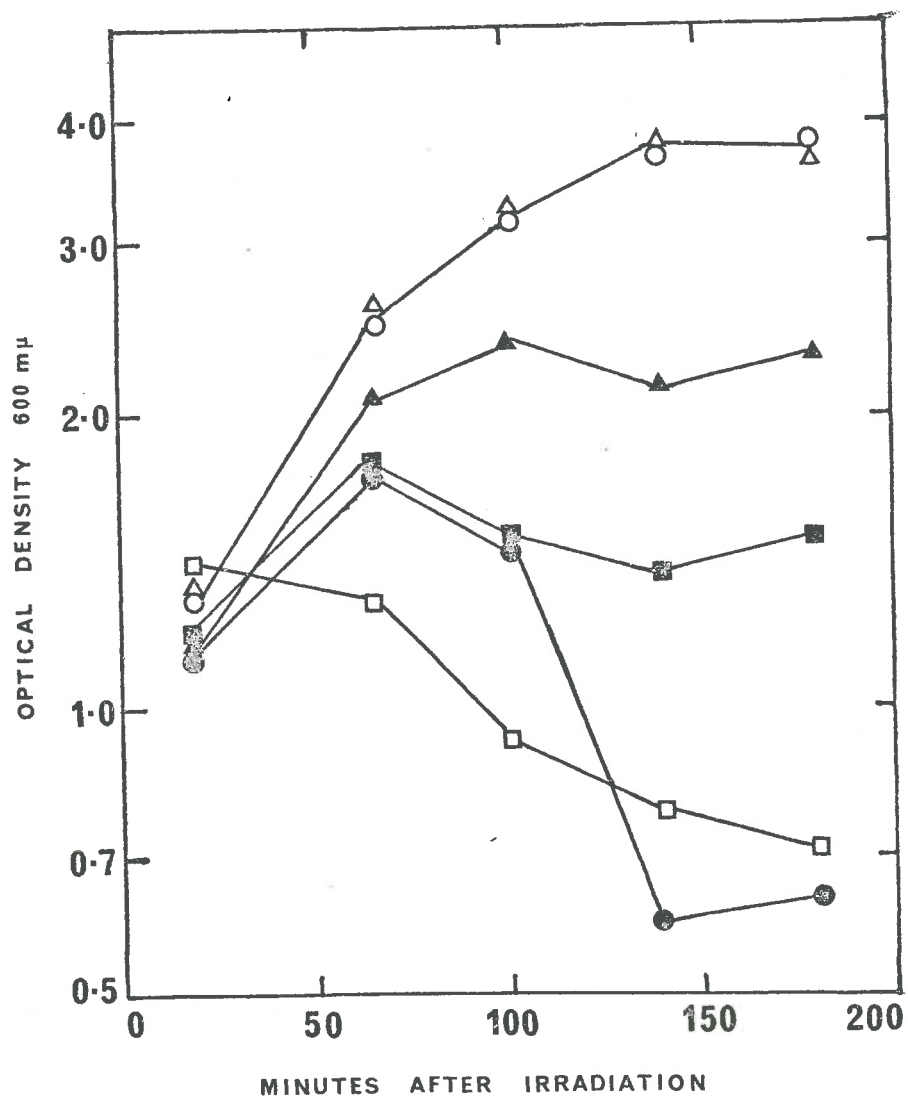


TABLE 4-I: PHAGE TITRES (pfu/ml) OF UV IRRADIATED AND NON-IRRADIATED CULTURES FOLLOWING 250 min POST-IRRADIATION INCUBATION AT 30°.^a

Lysogen	Non-irradiated culture	Irradiated culture
C600(18)	1.0×10^4	1.5×10^4
C600(299)	2.0×10^4	3.2×10^4
CR34(W)	5.2×10^5	3.4×10^5
C600(λ <u>ind</u> ⁻ <u>cIts</u> 857)	1.0×10^4	5.0×10^4
C600(λ)	1.0×10^6	2.3×10^{10}
C600(186)	1.5×10^7	3.2×10^{10}
C600(186 <u>cIts</u>)	9.6×10^6	1.7×10^{10}

^aInduction technique as described in Chapter 2. Tryptone broth was used for this experiment.

indicate a lower frequency of spontaneous phage production. As shall be seen later (4.3.e) it is fortuitous that this frequency is similar for λ_{ind}^- and phages of the "non-inducible" group. Phage P2 will be confirmed later as not UV inducible (4.3.f).

b. Prophage 186 is Induced by Nalidixic Acid. Induction by nalidixic acid was studied by the technique described in Chapter 2 following a preliminary report of such induction of λ (Cowlshaw and Ginoza, 1968). From this report and my own trials nalidixic acid was used at a concentration of 12.5 ug/ml for 10 min at 37°. Results appear in Table 4-II for lysogens of λ , λ_{ind}^- and 186. Both λ and 186 show about a five fold increase in titre which is similar to that found by Cowlshaw and Ginoza (1970) under such conditions. Prophage 186 is not induced by nalidixic acid in a $nalA^r$ strain (JP264; resistant to 50µg/ml) but it is UV inducible in this strain (Table 4-III). Further the non-UV-inducible mutant λ_{ind}^- is not induced by nalidixic acid, confirming the suggestion of Cowlshaw and Ginoza (1968, 1970) that nalidixic acid induction probably occurs by (indirectly) inactivating the repressor similarly to UV induction.

c. Prophage 186 is Induced by Mitomycin C. Mitomycin C (1µg/ml) added to a culture of C600(186) as described in Chapter 2 caused no change in the optical density relative to a non-treated control over 240 min incubation at 30°, but phage titres

TABLE 4-II: PHAGE TITRES (pfu/ml) OF NALIDIXIC ACID TREATED AND UN-TREATED CULTURES FOLLOWING 180 min POST-TREATMENT INCUBATION AT 37°.^a

Lysogen	Untreated culture	Nalidixic acid treated culture
C600(λ)	8.6×10^7	6.4×10^8
C600(186)	9.3×10^7	6.9×10^8
JP190(186)	1.5×10^8	7.4×10^8
JP264(186)	3.2×10^8	3.9×10^8
AB259(λ_{ind}^-)	2.8×10^6	3.2×10^6

^aAs described in Chapter 2 these cultures were diluted after induction with nalidixic acid and then shaken at 37°. The above titres include the dilution factor.

at 240 min were 8.1×10^6 pfu/ml for the untreated culture and 1.5×10^{10} pfu/ml for the mitomycin treated culture. A concentration of $5 \mu\text{g/ml}$ did slightly depress the O.D. relative to the control after 140 min and the phage titre at 240 min was 2.4×10^{10} pfu/ml.

An alternate method of treatment with mitomycin C allowed assessment of the degree of induction. Here resuspended log phase cultures of C600(λ) and C600(186) were diluted and plated on TB plates with C600 indicator bacteria using 0.7% TB agar containing mitomycin C at $1 \mu\text{g/ml}$ (this concentration in the soft agar layer only). Under these conditions both λ and 186 showed similar degrees of induction - 77% of the λ lysogenic bacteria scoring as infectious centres, whilst 62% of the 186 lysogens did so.

d. Generality of 186 Induction. In view of the surprising inducibility of prophage 186 a number of different host strains were investigated. Prophage 186 was UV inducible in C600, W3350 and CR34 (Woods, 1968). Its induction has been confirmed in a number of other strains (Table 4-III). Lysogens obtained in later experiments (6.3.c.i) as a result of conjugation between AB2528(186) and JP264; that is JP264 his⁺(186), are equally inducible. Lysogens of 186 and 299 in W3805 are included because the results of Kayajanian (1971) indicate similar titres of λ , 186cIts and 299 following UV irradiation of double lysogens of λ dgal and λ ,186cIts or 299 in this strain. It can be seen that 186 is UV inducible in W3805 but 299 remains

TABLE 4-III: PHAGE TITRES (pfu/ml) OF INDUCED AND NON-INDUCED CULTURES FOLLOWING
180 min INCUBATION AT 37° (OR 250 min AT 30°)

Lysogen	Inducer ^a (Incubation Temp.)	Non-induced culture	Induced culture
1. JP190(186)	UV (37°)	3.9×10^7	1.5×10^{10}
2. JP264(186)	UV (37°)	4.9×10^7	2.8×10^{10}
3. JP264 <u>his</u> ⁺ (186) - four isolates	UV (37°)	$1.1-1.3 \times 10^7$	$2.1-2.7 \times 10^{10}$
4. W3805(186)	UV (37°)	3.9×10^7	1.4×10^{10}
5. W3805(299)	UV (37°)	1.2×10^5	1.5×10^5
6. C600(186 <u>cIts</u> <u>sus</u> 16)	UV (30°)	1.1×10^7	1.6×10^{10}
7. C600(186 <u>cIts</u> <u>sus</u> 16)	mitomycin C ^b (30°)	1.1×10^7	1.4×10^{10}
8. C600(186 <u>cIts</u> <u>sus</u> 16)	heat	1.1×10^7 (30°)	5.4×10^{10} (37°)
9. C600(186 <u>sus</u> K4)	UV (37°)	1.9×10^8	3.2×10^{10}
10. C600(186 <u>sus</u> K6)	UV (37°)	1.5×10^8	5.0×10^{10}
11. C600(186 <u>sus</u> K9)	UV (37°)	2.5×10^8	4.7×10^{10}

^aInduction techniques as described in Chapter 2, save that C600(186susK4, K6 and K9) cultures (lines 9-11) were diluted 10^{-2} after induction; the titres shown include this dilution factor.

^b1 μ g/ml.

non-inducible even in this strain. As already described (4.3.b) prophage 186 is induced by nalidixic acid in C600 and JP190.

In addition, a number of 186 mutants have been tested. Prophage 186~~cIts~~ is UV inducible (Table 4-I), and 186~~cIts~~.sus16 is induced by UV or mitomycin C (Table 4-III). Of particular significance is the UV induction of C600(186~~sus~~K4, K6 and K9) since these mutants were obtained from a different source. Finally, a P2-186 hybrid phage, constructed in this laboratory by Cliff Bradley as having 186 immunity and P2 adsorption properties, is UV inducible in both E. coli K12 594 and E. coli C1055.

The only cases where UV-type induction of prophage 186 has been found lacking are (i) in a conjugating female (Chapter 6) and (ii) in a purB host starved of adenine (results not shown).

e. Behaviour of Phage 186 in a recA Host. As found for λ (Brooks and Clark, 1967) and P2 (Lindahl, Sironi, Bialy and Calendar, 1970) 186 is capable of lysogenising recA host strains. (Unlike P2, 186 gives turbid plaques on recB or recAB hosts. Also λ has the same plating efficiency on C600 and C600(186) and vice versa, so 186 does not display a phenotype similar to P2 old⁺). Lysogens of 186 in recA hosts were detected by immunity to superinfection and, as for such λ lysogens (Brooks and Clark, 1967; Fuerst and Siminovitch, 1965), produced low levels of free phage either spontaneously or following UV irradiation. No evidence of the stable host conversion to rec⁺ phenotype

described for λ by Erskine (1969) was found here with 186. Table 4-IV shows the phage yields from various recA lysogens of phage 186. The phage titres are much less than those from rec⁺ hosts, and the majority of phage produced spontaneously are clear plaque mutants. Irradiation greatly reduces the number of clear plaques, presumably through killing many of the cells which are to provide these mutants, and causes a 5-6 fold increase in the number of turbid plaques which can be attributed to a small amount of induction or low level of rec⁺ revertants. A larger dose of UV reduces both clear and turbid phage titres. However heat induction of 186cIts prophage is successful in the recA host, the latent period being similar in isogenic rec⁺ and recA strains 594 and 152 as is also shown for λ (Figure 4-2). Similar results were obtained for λ by Brooks and Clark (1967). A low level of infective phage production by a recA strain carrying 186 and defective λ prophages was noted by Fuerst and Siminovitch (1965).

Non-inducible phages were also investigated in recA hosts. Phage P2 gives the same spontaneous titres in 152, AB2463 and C600 and the plaques are similarly turbid. This has also been found by others (Calendar; Laffler and Luria; as cited in Bertani and Bertani, 1971) and indicates that the recA⁺ gene product is not required for spontaneous P2 production. Nevertheless spontaneous production of the non-inducible mutant λ_{ind}^- does require this product. A culture of 152(λ_{ind}^-) had a supernatant titre of 5.9×10^2 pfu/ml, and 49/59 plaques were

TABLE 4-IV: PHAGE TITRES (pfu/ml) OF INDUCED AND NON-INDUCED CULTURES OF *recA* LYSOGENS OF PHAGE 186 FOLLOWING 180 min INCUBATION AT 37° (OR 250 min at 30°).

Lysogen	Inducer ^a (Incubation Temp.)	Phage titre	
		turbid plaques	clear plaques
DM455(186)	none (30°)	3. x10 ²	4.5x10 ³
AB2463(186 <i>cIts</i>)	heat (37°)	1.0x10 ⁹ ^b	-
JC2926(186) ^c	none (37°)	1.3x10 ³	3.3x10 ³
JC2926(186) ^c	UV 76ergs/mm ² (37°)	3.8x10 ³	5.8x10 ²
JC2926(186) ^c	UV 152ergs/mm ² (37°)	7.6x10 ³	5.1x10 ²
JC2926(186) ^c	UV 304ergs/mm ² (37°)	8.7x10 ³	3.5x10 ²
JC2926(186) ^c	UV 760ergs/mm ² (37°)	2.4x10 ³	6. x10 ¹

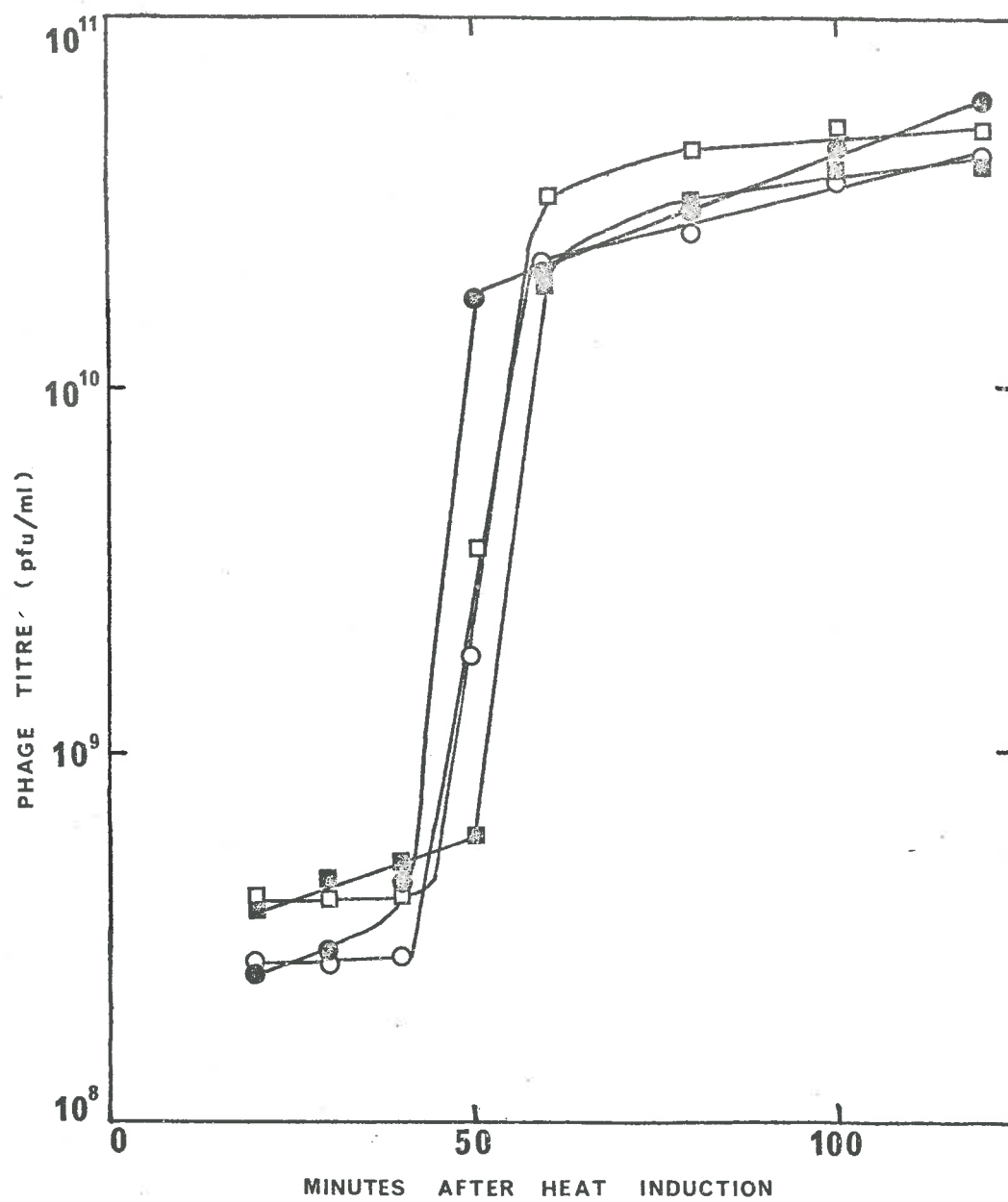
^aInduction techniques as described in Chapter 2.

^bAssayed at 30°.

^cInduced at 2.9x10⁸ viable cells/ml. All doses of UV gave a bacterial survival of less than 10⁻⁴.

FIGURE 4-2: Latent period following heat induction
of various lysogens as described in Section 4.2.a.iii.
Transfer to 45° at zero min.

- 594(186cIts)
- 152(186cIts)
- 594(λind⁻cIts857)
- 152(ind⁻cIts857)



clear, which is similar to that found for λ . This confirms that the recA⁺ gene product is required for even spontaneous production of λ . Thus it is purely fortuitous that the rates of spontaneous phage production are similar for P2 and λ ind⁻ since different mechanisms are involved at least as far as recA⁺ is concerned.

During the search for a 186recA⁺ transducing particle a strange 152 lysogen was obtained (5.3.e). It remained quite sensitive to UV radiation, was immune to 186cI and sensitive to 186vir, but produced no phage at all spontaneously in contrast with recA(186) lysogens above, which produced a low level of clear plaques. However, upon introduction of recA⁺ into the "defective" lysogen by the F143 episome (CGSC4291), a normal level of phage activity was obtained. In rec⁺ hosts those phage liberated from the heterogenote formed lysogens that liberated normal levels of phage but, in recA hosts, immune lysogens again produced no phage at all. Thus the defect is in the phage and since the mutant plates equally well on rec⁺ and recA hosts, the defect would appear to be inability to excise the prophage from the recA host. Therefore the recA⁺ function is possibly involved in excision of this mutant 186 prophage.

f. Susceptibility of Prophage 186 to UV Induction. The susceptibility of prophage 186 to induction, and of the 186 lysogen to killing, as a result of UV irradiation was investigated as described in Chapter 2 by plating immediately after the UV treatment for bacterial survivors and infectious centres at 30°.

The induction of infectious centres from C600(λ), C600(186) and C600(186cIts) is shown in Figure 4-3. The same results appear in Figure 4-4 together with the bacterial survival of these lysogens and for comparison, non-lysogenic C600, C600(λ ind⁻), C600(P2) and C600(299). The latter three lysogens produced less than one infectious centre per hundred initial cells, confirming the lack of UV induction, in particular of P2. The UV-inducible phages λ and 186 showed a background of 5-20 infectious centres per hundred cells without irradiation, presumably due to spontaneous phage production, but these plaques were poor and easily distinguished from those due to the irradiation.

Prophage 186 requires a greater dose of irradiation for maximal expression of induction than does prophage λ . As the dose is further increased the number of infectious centres falls as cells are damaged to the extent that they cannot support the phage lytic cycle. More interestingly, the bacterial survivals of C600(186) and C600(186cIts) follow that of the non-lysogen and lysogens of non-inducible phages, whilst the λ lysogen alone has a much greater sensitivity to the irradiation. The survivors of irradiation of C600(186) and C600(186cIts) remained lysogenic (greater than 99% immune to 186cI).

g. Latent Period of 186 Phage Production. The latent periods for phage production by infection, heat induction and UV induction were determined as described in Section 4.2.a. C600 was the host for all these experiments. Figure 4.5 shows the

FIGURE 4-3: Percentage of lysogenic cells scoring as infectious centres at 30° following UV induction as described in Chapter 2 as a function of UV dose administered. Standard conditions used for irradiation (15.2ergs/mm²/sec).

- C600(λ)
- ▲ C600(186)
- C600(186cIts)

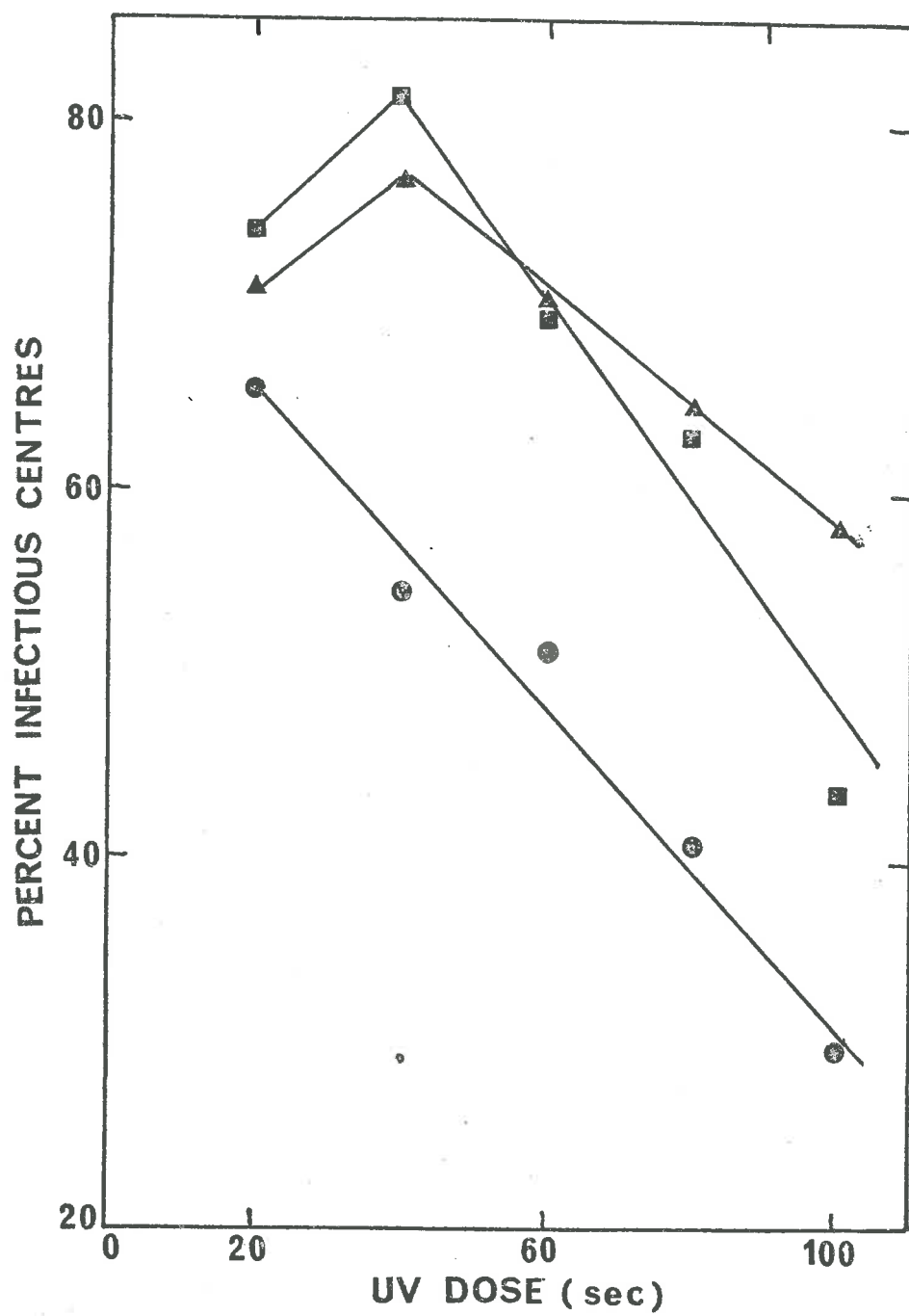
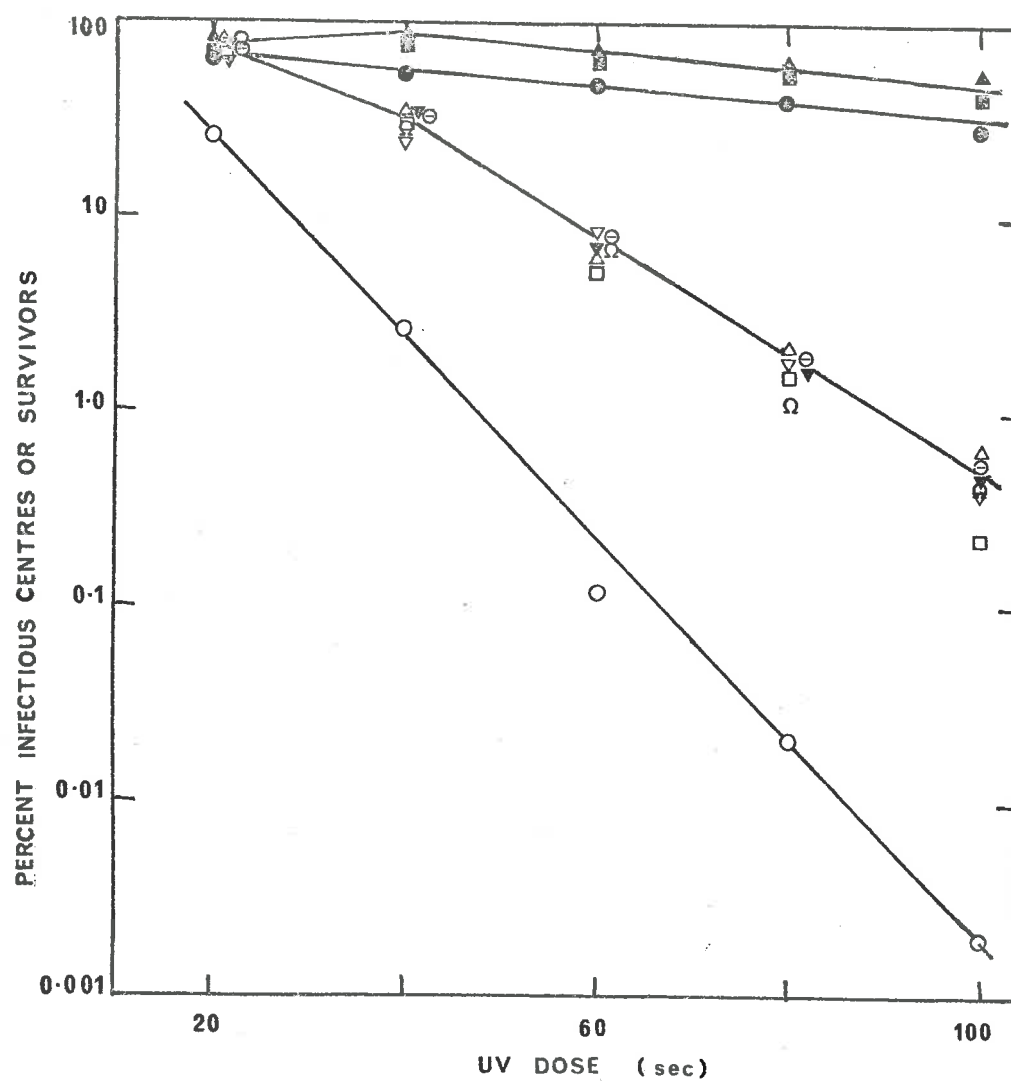


FIGURE 4-4: Percentage of lysogenic cells scoring as infectious centres or surviving colonies at 30° following UV induction.

Details as for Figure 4-3.

	<u>infectious centres</u>	<u>surviving colonies</u>
C600(λ)	●	○
C600(186)	▲	△
C600(186 <u>cIts</u>)	■	□
C600(P2)		▼
C600(299)		▽
C600(λ_{ind}^-)		⊖
C600		Ω



results for heat induction of, and infection by, 186cIts and for comparison λ ind⁻cIts857. The latent period of λ induction is similar here to that found before, but the 186 latent period is shorter in the su⁺ C600 host than in the su⁻ hosts 152 and 594 (Figure 4-2). Results for UV induction of prophages 186 and λ following 10, 20 and 30 sec of UV irradiation appear in Figure 4-6. Infection and heat induction show similar graphs allowing the difference in initial level due to the low multiplicity of infection, but the latent period for UV induction of 186 is much delayed compared to that for λ . A low dose of radiation (10 sec) fails to maximally induce 186, but it does not delay the burst as much as larger doses. That 186 and 186cIts infection of bacteria irradiated for 30 sec also exhibit this delay, while λ infection does not, is shown in Figure 4-7. Artificial lysis by treatment with chloroform did not shorten the delay of the 186 burst. Figure 4-8 shows that increasing the dose of radiation administered to the host increases the latent period of 186 infection. Finally Figure 4-9 indicates that the latent period of P2 infection is unaffected by similar irradiation of the host.

The average latent periods (taken as the time from induction or infection till half the final increase in phage titre on a log scale is achieved) from the above graphs are listed in Table 4-V. For λ the latent period following infection or heat induction is about 50 min, and following UV induction 15 min greater irrespective of UV dose. Phage 186 shows a latent period

FIGURE 4-5: Latent period following infection
(4.2.a.i) or heat induction (4.2.a.iii) for phages
 $\lambda_{\text{ind}}^{-}\text{cIts857}$ and 186cIts. Phage added at zero min
for infection. Transfer to 45° at zero min for heat
induction.

- C600 infection by $\lambda_{\text{ind}}^{-}\text{cIts857}$
- C600 infection by 186cIts
- ▲ heat induction of C600($\lambda_{\text{ind}}^{-}\text{cIts857}$)
- △ heat induction of C600(186cIts)

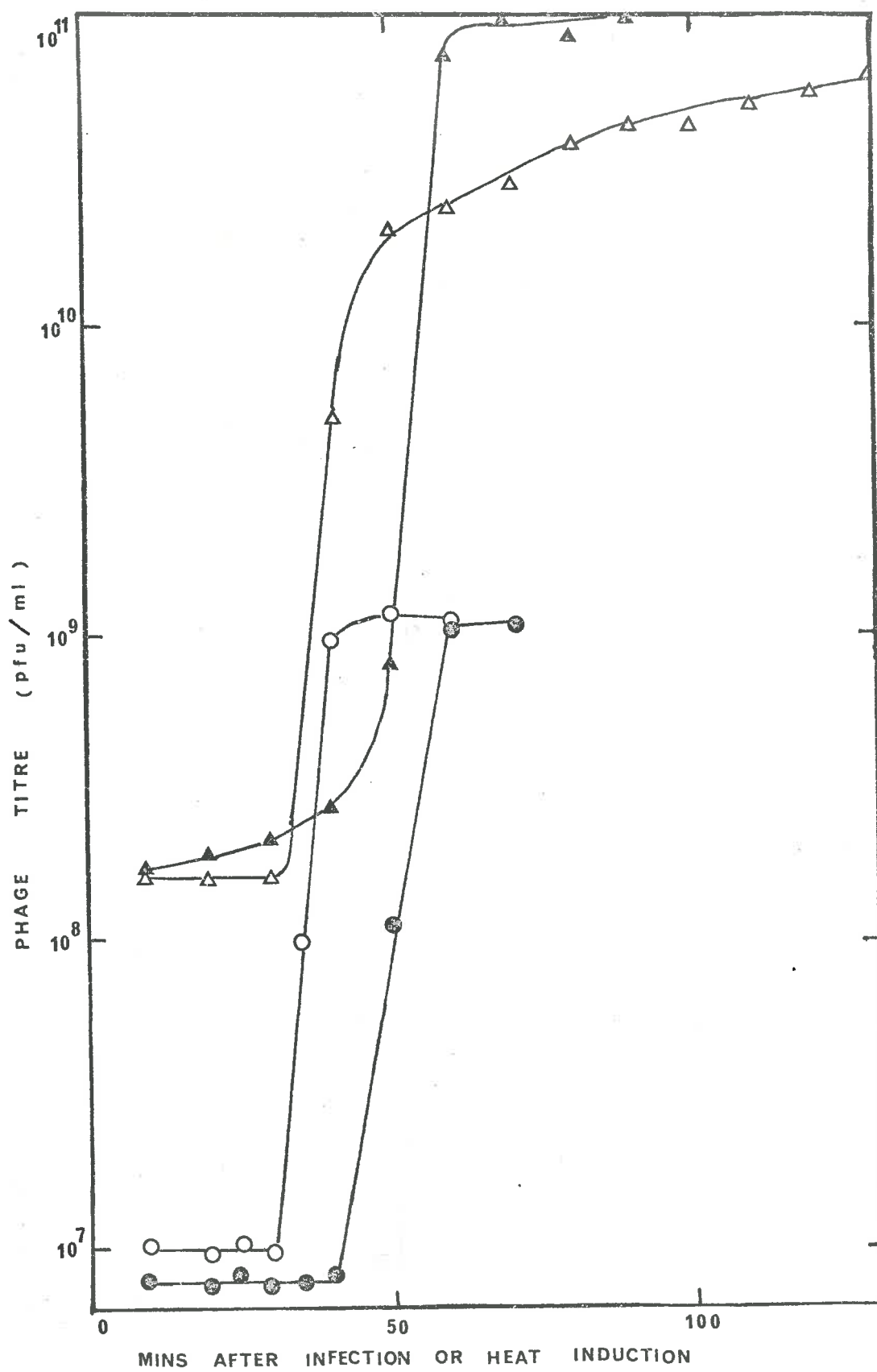


FIGURE 4-6: Latent period following UV induction of C600(λ) and C600(186) as described in Section 4.2.a.ii. Dilution into LGB at zero min, immediately following irradiation.

- C600(λ) 10 sec irradiation
- ▲ C600(λ) 20 sec irradiation
- C600(λ) 30 sec irradiation
- C600(186) 10 sec irradiation
- △ C600(186) 20 sec irradiation
- C600(186) 30 sec irradiation

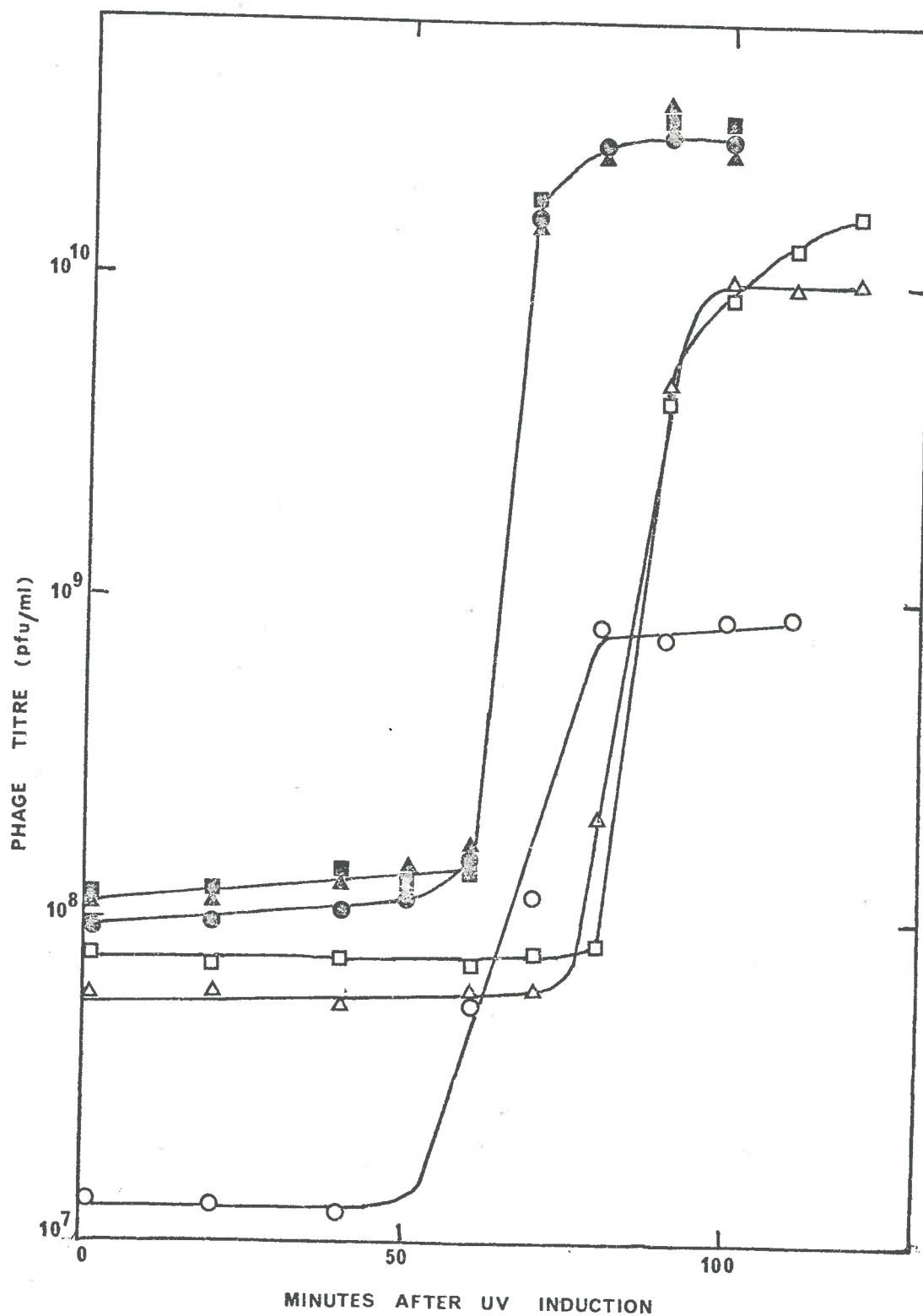


FIGURE 4-7: Latent period following infection of C600, UV irradiated for 30 sec, by phage λ ind⁻cIts857, 186 or 186cIts as described in Section 4.2.a.i. Infection at zero min.

- C600UV infection by λ ind⁻cIts857
- △ C600UV infection by 186
- C600UV infection by 186cIts

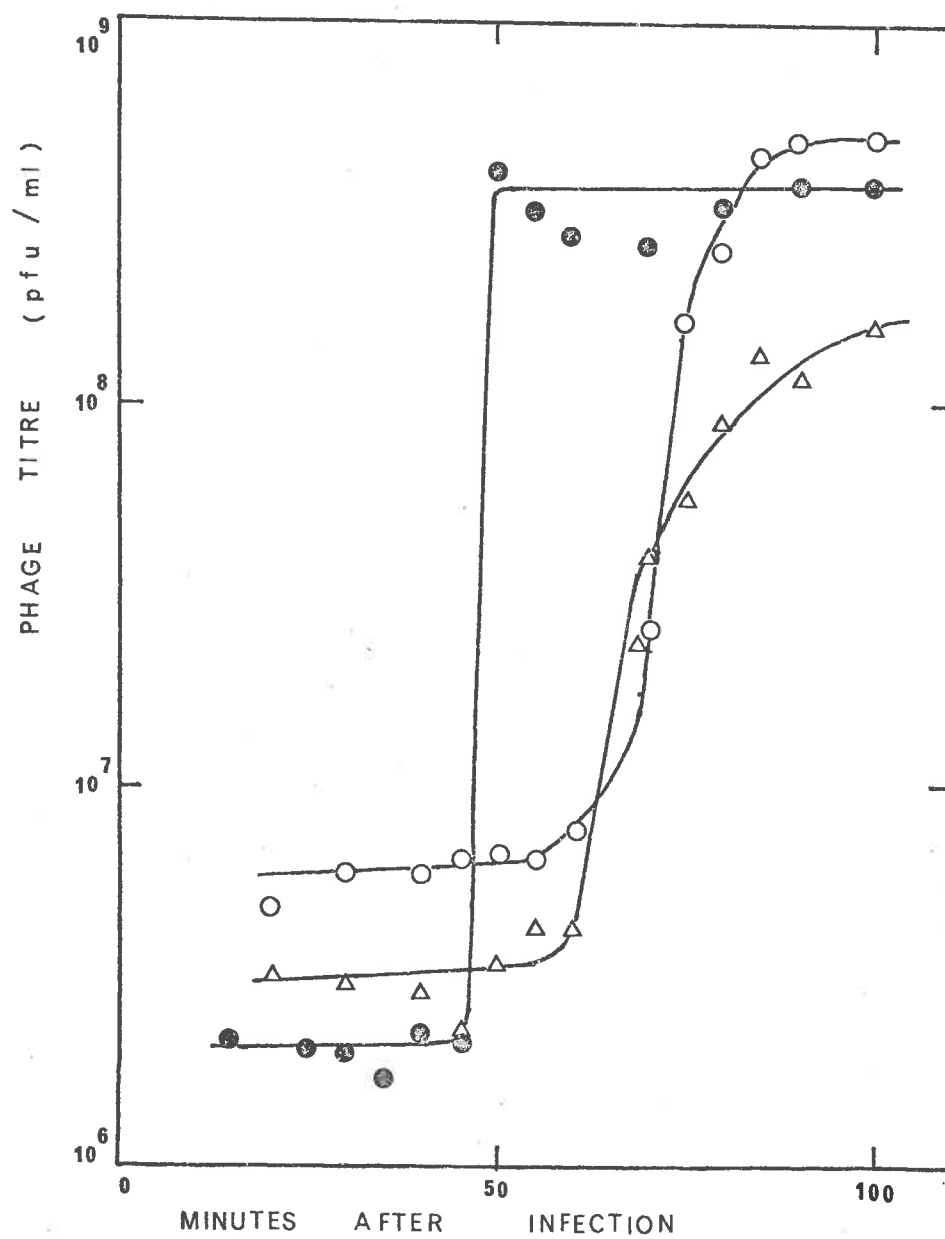


FIGURE 4-8: Latent period following infection of C600, UV irradiated for 10, 20 and 30 sec, by phage 186 as described in Section 4.2.a.i. Infection at zero min.

- C600UV 10 sec, infection by 186
- C600UV 20 sec, infection by 186
- △ C600UV 30 sec, infection by 186

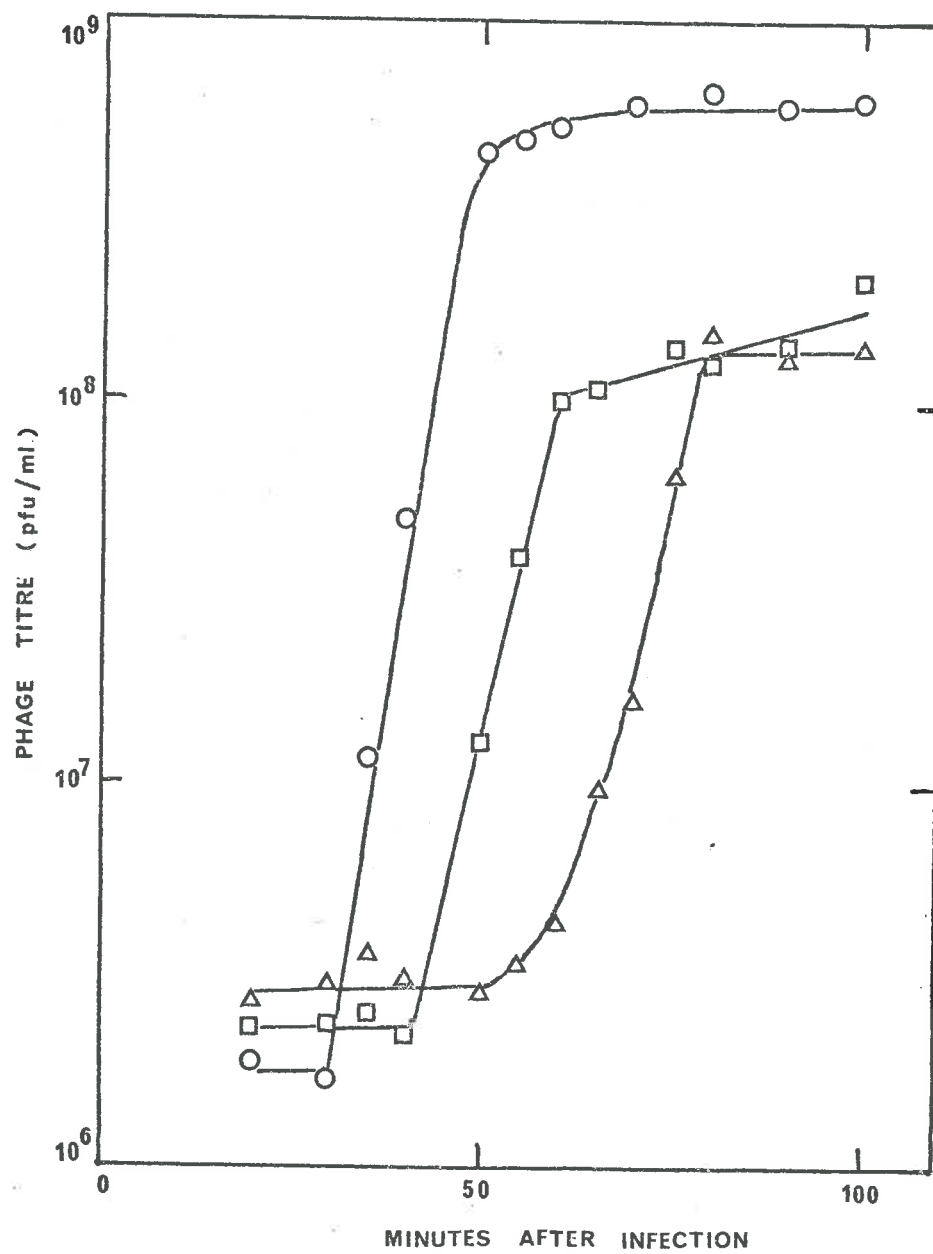


FIGURE 4-9: Latent period following infection of C600, UV irradiated for 0, 10 and 30 sec, by phage P2k as described in Section 4.2.a.i. Infection at zero min.

- C600UV 0 sec, infection by P2k
- C600UV 10 sec, infection by P2k
- △ C600UV 30 sec, infection by P2k

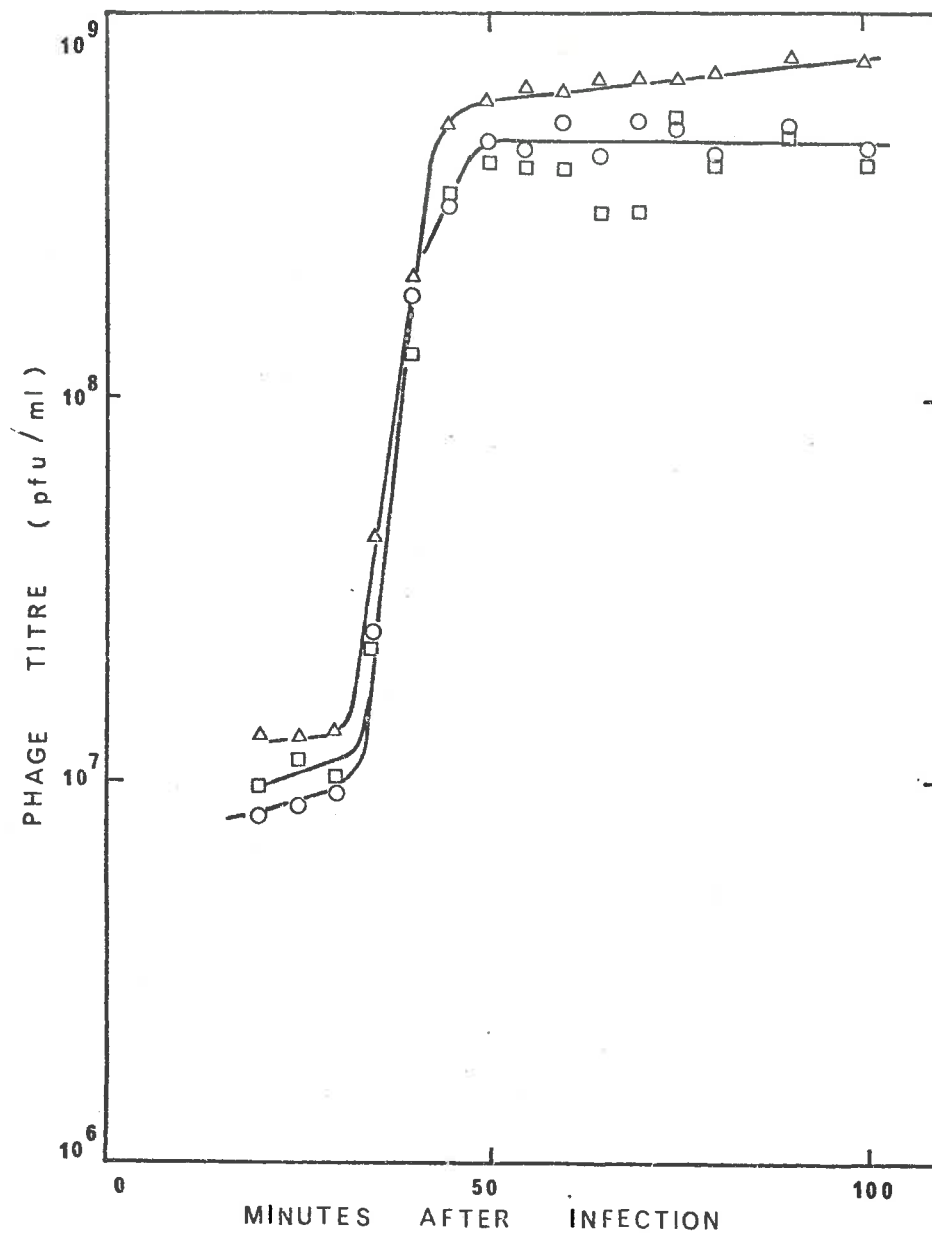


TABLE 4-V: AVERAGE LATENT PERIODS FOLLOWING INDUCTION OR INFECTION FOR PHAGES λ , 186 and P2 WITH C600 AS HOST UNLESS NOTED OTHERWISE.

Phage	Method of Production	UV dose ^a (sec)	Average Latent Period (min)
λ <u>ind</u> <u>cIts</u> 857	infection	0	50
λ <u>ind</u> <u>cIts</u> 857	infection	30	48
λ <u>ind</u> <u>cIts</u> 857	heat induction	0	53
λ <u>ind</u> <u>cIts</u> 857	heat induction	0	52 host 594
λ <u>ind</u> <u>cIts</u> 857	heat induction	0	56 host 152
λ	UV induction	10	65
λ	UV induction	20	65
λ	UV induction	30	65
186	infection	0	39
186 <u>cIts</u>	infection	0	35
186	infection	10	51
186	infection	30	70, 68
186 <u>cIts</u>	infection	30	71
186 <u>cIts</u>	heat induction	0	40
186 <u>cIts</u>	heat induction	0	49 host 594
186 <u>cIts</u>	heat induction	0	46 host 152
186	UV induction	10	66
186	UV induction	20	85
186	UV induction	30	87
P2k	infection	0	48
P2k	infection	10	48
P2k	infection	30	48

TABLE 4-V Cont'd.

^aUV irradiation administered under standard conditions to lysogen or C600 immediately before infection.

following infection or heat induction of about 40 min which is increased by about 10 min or 30 min when the host cells are UV irradiated for 10 or 30 sec respectively. UV induction shows latent periods 15 min greater again, depending on dose; for 10 sec UV induction the latent period is $40+10+15$ (c.66min) and for 30 sec UV induction $40+30+15$ (c.87min). Thus the exceptional delay following 186 UV induction is due to a dose dependent effect common to 186 infection and UV induction (10 or 30 min delay due to 10 or 30 sec UV) and a dose independent effect unique to UV induction (15 min increase in latent period for both λ and 186).

4.4 DISCUSSION

The results above show that prophage 186 is truly induced to enter its lytic cycle by UV radiation. It is so inducible in many E. coli strains and various mutant derivatives of 186 still exhibit UV induction. There was no search for a non-inducible mutant of 186 but this could be simply pursued as for λ (Eshima, Fujii and Horiuchi, 1972) since 186 produces clearish plaques on plates containing mitomycin C whereas ind⁻ mutants would produce turbid plaques. Prophage 186 behaves similarly to λ in a recA host, and also shares with λ sensitivity to induction by nalidixic acid and mitomycin C. Thus 186 falls in neither of Jacob and Wollman's groups but allows the proposition of four groups of temperate coliphages (Woods and Egan, 1969). These are;

- (i) Group UZ, the members of which exhibit both ultraviolet and zygotic induction, e.g. the λ -related phages,
 - (ii) Group U, which exhibits ultraviolet but not zygotic induction, defined by phage 186,
 - (iii) Group Z, which exhibits zygotic but not ultraviolet induction, e.g. λ_{ind}^- (Jacob and Campbell, 1959),
- and (iv) Group NUZ, which exhibits neither induction, e.g. phages P2, W, 18 and 299.

More simply, it appears that there is no correlation between sensitivity to UV induction and ability to induce zygotically. Also, it is clear that phage 186 is not only capable of heat induction (Baldwin *et al*, 1966) but of induction following other treatments. Thus in the division on this basis by Golub and Reshetnikova (1970) λ_{ind}^- now remains the only example of a non-inducible phage relying solely on repressor stability for lack of induction.

Weigle and Delbruck (1951) found that prophage λ was maximally induced at a dose of 300 ergs/mm² and that higher doses decreased the number of infectious centres just as is shown in Figure 4-3. That prophage 186 requires a greater UV dose to exhibit maximal induction could reflect that a greater dose is required to (indirectly) inactivate the repressor or that 186 is better able to replicate in a UV-damaged cell than is λ . However the 186 latent period is found delayed in irradiated cells whilst λ is unaffected suggesting that the greater dose is required for repressor inactivation.

The latent period following UV induction of λ was determined by Weigle and Delbruck (1951) with results identical to those shown in Figure 4-6 for similar doses. They did find, as did Marcovich (1956), that a dose much greater than used in this work did delay the λ burst. As already discussed (4.3.g) the latent periods of λ and 186 seem increased by 15 min where UV is used to lift immunity and in addition 186 infection and induction are delayed in a dose dependent manner in an irradiated cell. However λ infection of a UV irradiated cell does not result in an increased latent period. Similar results were obtained by Jacob and Wollman (1953) with Pseudomonas pyocyanea phage p8 where the latent period of UV induction was 20 min longer than that of infection of irradiated or non-irradiated cells. For 186, lysis by chloroform did not cause premature release of the delayed phage, so the type of delayed lysis found for mutants of λ (Groman, 1962) and P22 (Cohen, 1969) is not involved. The delay to 186 phage production is also reflected in the optical density profile following incubation of an irradiated 186cIts lysogen at 37° (Figure 4-1), that is heat induction is also delayed. Maintenance at high temperature also appears able to delay 186cIts phage production. Single colonies of a 186cIts lysogen spread on plates all survive, and remain lysogenic, if incubated at 45° for longer than 60 min before transferring to 39°, whereas incubation at 45° for less than 30 min before the transfer allows killing of the lysogens.

It is interesting to compare the fates of 186 and λ

lysogens following irradiation. Table 4-VI shows the effect of irradiation on the sum of infectious centres and bacterial survivors from C600 and its lysogens. As also is evident from the data of Weigle and Delbruck (1951) the λ lysogen never produces more derivatives than parental colonies, but does show an increase in derivatives compared to the non-lysogen. This can be attributed to the ability of phage λ to produce an infectious centre in a UV-damaged cell which, even in the absence of λ , would not be able to score as a colony. However at low UV doses there are $1\frac{1}{2}$ times the number of 186 lysogen derivatives than parents.

Non-destructive phage 186 production by a bacterium is not supported by the bacterial lysis and death following heat induction of prophage 186cIts and by the identification of a gene involved in bacterial lysis. (Lysogens of this mutant in a non-permissive host do not lyse after induction, but artificial lysis with chloroform liberates intact infectious phage; S. Hocking, pers. comm.) Therefore the irradiated 186 lysogen divides before lysis and in some cases, one daughter cell survives and remains lysogenic whereas the other lyses displaying an infectious centre. If this asymmetric production did not occur, no increase in derivatives would be seen, since by this stage the colonies have been localised on plates and two daughters behaving in a like manner would only score once. It is significant that for phage λ , where this effect is not seen, the irradiated cell doubles in size, but does not divide before lysis (Weigle and Delbruck, 1951). Microscopic observation of irradiated 186 lysogens might resolve this problem. Since all survivors of the 186 induction

TABLE 4-VI: SUMMATION OF INFECTIOUS CENTRES AND BACTERIAL SURVIVORS ASSAYED AT 30° FOLLOWING UV IRRADIATION OF C600, C600(λ), C600(186) and C600(186cIts) EXPRESSED AS A PERCENTAGE OF THE NUMBER OF CELLS BEFORE IRRADIATION^a

UV dose (sec)	C600	C600(λ)	C600(186)	C600(186 <u>cIts</u>)
20	80	91	154	148
40	30	57	112	114
60	7.0	52	77	75
80	1.9	41	68	65
100	0.5	30	59	44

^aData from Figure 4-4.

remain lysogenic it would appear that excision has not occurred before the cell division, that is that excision is delayed. This may be supported by the requirement for a larger dose of UV to induce 186 if the repressor has to be inactivated for a considerable period of time before excision and phage production occur. It is not clear if the delay to excision allows the cell division to occur, or if excision is delayed because it requires a cell division.

All this is reminiscent of the work of Calef on the immune and anti-immune phases of defective λ lysogens carrying mutations in genes N and O or P. Such lysogens are not killed by UV irradiation and, allowing similar irradiation conditions, follow the survival of the non-lysogen (Calef, Avitabile, del Guidice, Marchelli, Menna, Neubauer and Soller, 1971). However the irradiation does result in a shift to the anti-immune phase, and the surviving colonies are mixed in that from 70-90% of individual cells from these colonies are found to be immune to superinfection whilst the remainder are sensitive. Thus a λ prophage unable to excise and replicate gives results comparable to those obtained for 186. In the case of 186 the immune phase cells are represented by the lysogenic survivors and the non-immune phase cells by the infectious centres. Because a cell displayed as an infectious centre is lost it has not been possible to assay for mixed clones amongst the survivors of the irradiation as was done for λ . Attempts to develop techniques for displaying 186 infectious centres and surviving colonies on the same plate were unsuccessful.

The similarities in the dose dependent delay to 186 infection and UV induction in the irradiated cell, suggest that a common step is involved. If the above postulates are accepted it becomes necessary to involve a step of the infectious cycle in prophage excision. A candidate would appear to be phage DNA replication which, for B. subtilis phage ϕ 105, has been found to occur as an alternative to prophage excision (Armentrout and Rutberg, 1971). Thus 186 DNA replication may be delayed in an irradiated cell via an effect on either a bacterial or a phage system, and this in turn delays both infection and induction. The bacterial function rep⁺ which is required for growth of phages P2 and 186, but not λ (Calendar, Lindqvist, Sironi and Clark, 1970) may not be the system delayed in an irradiated cell because P2, in contrast to 186, has its usual latent period in an irradiated cell. The rep⁺ function was an attractive candidate for the factor responsible for delaying the 186 latent period in an irradiated cell, since Matsubara, Shimada and Takagi (1967) have shown that a heavily UV irradiated cell is phenotypically rep. However, unless P2 and 186 require different levels of rep⁺ product, it is not involved here.

Moreover, it seems unlikely that phage 186 DNA which is capable of circularisation (Baldwin et al, 1966), whereas DNA circles have not been observed for ϕ 105 (Armentrout and Rutberg, 1971), should need to depend on phage DNA replication for its escape from the host chromosome. It is more likely that the formation of covalent circles itself may involve limited DNA synthesis or repair following infection or induction of phage

186. Gene recA⁺ activity, although implicated in the excision of the mutant described in Section 4.3.c, is not required for 186cIts heat induction which exhibits a normal latent period in a recA host. Thus it is unlikely that recA⁺ is the function lacking in the irradiated cell. Rather than require direct involvement, a (phage) function controlling both excision and a step of the infectious cycle, such as replication, may be the one affected by irradiation. If a phage function is involved, the effect of irradiation on it must be indirect since the effect is expressed in non-lysogenic cells infected after irradiation.

Thus it appears that phage 186 is intermediate between P2 and λ , in its induction patterns (the four groups defined above) and in its ability to excise from the host chromosome. Phage λ has no difficulty, P2 is unable to excise and phage 186 has its excision perturbed by UV irradiation. The parallel between the behaviour following UV induction of (i) a λ lysogen defective in genes N and O or P, and (ii) a 186 lysogen, is a particularly striking one. It may be surmised that the 186 prophage behaves similarly but that the defect is merely temporary, finally allowing phage multiplication and the consequent killing of the host in those cells converted to the anti-immune phase.

CHAPTER 5

THE INTEGRATION SITE OF COLIPHAGE 186

5.1 INTRODUCTION

The loci of the attachment sites for a number of coliphages are known with varying degrees of accuracy. Jacob and Wollman (1956) isolated a number of temperate coliphages and mapped the attachment sites of seven inducible phages (Jacob and Wollman, 1958) by conjugation using HfrH. Matsushira (1963) found the inducible prophage ϕ 80 located close to trp, in the general region where these other inducible phages are located. The attachment sites of their group of non-inducible phages were not transferred by this Hfr, but they have stated (Jacob and Wollman, 1956, 1957a, 1959, 1961) that, using a different Hfr, the non-inducible prophages were situated on that segment of the bacterial linkage group "carrying the maltose, mannitol, and xylose characters." No data has been published (Jacob, pers. comm.) save for prophage 18 (Jacob and Wollman, 1961) which is strongly linked to metB (77 min). The non-inducible phage P2 can attach at a number of different locations in both E. coli C and K, the various observations having been summarised by Bertani and Bertani (1971). During the course of this work Abe and Tomizawa (1971) reported linkage of prophage 186 to pheA using P1 transduction.

For a number of phages integration into the continuity of the bacterial chromosome has been established through reduction in linkage between bacterial markers flanking the prophage. Among the inducible phages, λ , 434 and 82 have been shown integrated between gal and bio (Rothman, 1965) and ϕ 80 between trp and supC (Signer, 1966). Non-inducible P2 at site II is

integrated between metE and rha (Calendar and Lindahl, 1969). Jacob and Wollman (1959) have suggested that another non-inducible phage, phage 18, may be not integrated but rather synapsed with the bacterial chromosome and overlapping the metB gene, because the prophage appeared to enter after metB by clockwise or counter-clockwise conjugation.

Integration has been further indicated for λ (Shapiro and Adhya, 1969) and $\phi 80$ (Franklin, Dove and Yanofsky, 1965) through simultaneous loss by deletion of some prophage markers and contiguous bacterial markers. Direct physical evidence for the insertion of prophage λ into a bacterial sex factor carrying a λ attachment site has been provided by Freifelder and Meselson (1970). The insertion increases the X-ray target size for conversion of the sex factor twisted DNA circles to more slowly sedimenting forms exactly as would be predicted from the Campbell (1962) model of prophage integration.

Part of the initial evidence for the Campbell model was the existence of a specialised transducing particle λ_{dgal} (Morse, Lederberg and Lederberg, 1956). Such transducing particles have proved most useful and have been isolated similarly using; (i) λ for the following bacterial genes, bio (Wollman, 1963), narD and narF (Venables and Guest, 1968), aroG (Simpson *et al*, 1971), hut of Salmonella typhimurium (Smith, 1971) and phr (Sutherland, Court and Chamberlin, 1972); (ii) $\phi 80$ for trp (Matsushiro, 1963) and supF (Smith *et al*, 1966); and (iii) 434 and 82 for gal (Reissig and Wollman, 1963). Where a gene is not located

sufficiently close to a phage attachment site it may be moved there by directed transposition using an F' episome carrying the gene and temperature-sensitive in its own replication, and (preferably) a recipient deleted for the chromosomal regions carried by the episome (Beckwith and Signer, 1966). This has been done to construct, among others, $\phi 80\text{dara}$ (Gottesman and Beckwith, 1969), λlac (Ippen, Shapiro and Beckwith, 1971), $\phi 80\text{argF}$ (James and Gorini, 1972) and $\phi 80\text{gnd}$ (Wolf and Fraenkel, 1972). Alternately one may force, for example, λ to attach at less favoured locations by deleting its normal bacterial attachment site (Shimada, Weisberg and Gottesman, 1972) so bringing further genes within range for transduction.

This chapter presents evidence for the location of the 186 attachment site (att186), the integration of the prophage and its ability to perhaps transduce an adjacent marker. The primary stimulus for this work was a need to locate att186 for the interpretation of the results of zygotic induction experiments.

5.2 MATERIALS AND METHODS

a. Pl Transductions - Plvir lysates were prepared on the donor strain by a confluent plate lysis technique. Approximately 10^5 phage were added to 6×10^7 bacteria which had been grown to log phase in LGCB. The mixture was spread on LGC plates with TB soft agar containing 2mM CaCl_2 . Plates were incubated for 6 hours at 37° or 9 hours at 30° before flooding with 3ml LGCB. They were chilled overnight, the elutate removed, filtered through

a Millipore filter (0.42 μ pore size) and assayed on the recipient strain using the above plating conditions. All lysates were recycled on the same host before use. Titres ranged from 8×10^9 to 6×10^{10} pfu/ml. In all transductions, except those involving nalB and lysogenic donors, the same lysate from 594 was used. Lysates prepared on 186 lysogens were treated with 186 anti-serum by dilution into LGCB containing 186 anti-serum ($K=6.5 \text{ min}^{-1}$) for 10 min at 37° immediately before use. This did not affect the Pl titre.

For transduction, phage were added at a multiplicity of 0.05 to a log phase LGCB culture of the recipient at 3×10^8 viable cells/ml and the mixture incubated for 30 min at 37°. The cells were then washed and resuspended in H-1 before plating on selective medium in a layer of soft minimal agar. Plates were incubated at 37° for 2 days, counted and the transductants analysed. The three factor transductions, some of which involve 186cIts, were performed at 30° throughout.

b. Analysis of Transductants - Care was taken to analyse all the separate colonies from a given plate, or from an arbitrary sector of that plate, rather than involve any choice of colonies. Transductants were purified through short streaks on selective medium before testing for 186 lysogeny by toothpicking into a plate seeded with 594, then into one seeded with 594(186). Where a particular recipient was already lysogenic for some other phage, it was used itself as indicator in this test, with its 186 lysogen for the control plate (e.g. AT2092 carries phage

Mul). This was necessary to avoid spurious lysis of 594. A greater problem was the activity of Pl_{vir} phage carried over from the transduction step. Attempts were made to isolate Pl resistant indicators of 186 activity from 594 and other strains among the survivors of massive infection by Pl_{vir}. However, all survivors that resisted Pl were also resistant to 186vir (200 tested), and therefore unsuitable. Thus it was necessary to further purify many transductants away from Pl_{vir} [as judged necessary by phage activity on 594(186)] by streaking for single colonies on selective medium. In such cases four single colonies were tested for each transductant and in all cases all four showed similar 186 activity. Where argA and nalB were involved, transductants were initially patched out on selective medium and the thickly grown patches replicated to medium selective for arg⁺ or an L plate containing nalidixic acid (6µg/ml) for assay of nalB. Induction by nalidixic acid (4.3.b) did not cause loss of nalB^r lysogenic patches as tested with KL164(186cIts). In these cases the patches were used in lieu of short streaks as preliminary purification before testing 186 lysogeny.

c. Specialised Transduction - The potential low frequency transducing lysate was added at various multiplicities from 1 to 10 to the recA recipient strain 152 grown to 3×10^8 viable cells/ml in LGB. The mixture was incubated still for 30 min at 37° (or 30° where 186cIts was involved) and then shaken at the same temperature. Samples were taken at various times, concentrated ten-fold in H-1 and spread on plates selective for rec⁺. These

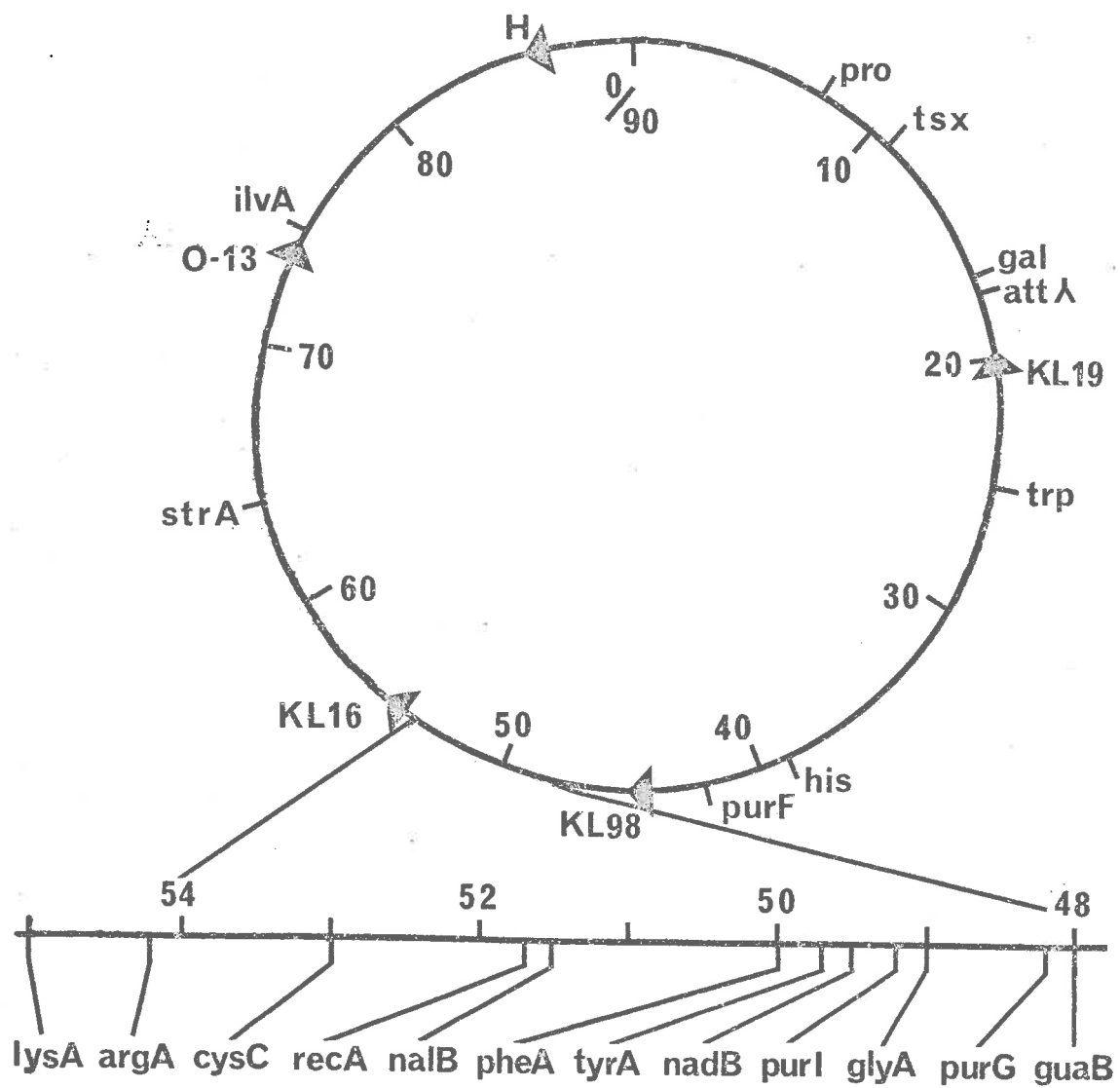
were of nutrient agar base with 0.01% methyl methanesulphonate (MMS; Howard-Flanders and Boyce, 1966) and streptomycin (200 μ g/ml), the latter to help suppress contaminants. It was necessary to prepare these plates fresh, adding the MMS immediately before pouring the cooled agar, followed by drying for 1 hour at 37° with lids off. After 2 or 3 days any rec⁺ cells formed small colonies. The phage lysate was checked for bacterial sterility on similar plates. On these plates 594(186) displayed a similar number of colonies (98%) as formed on nutrient agar alone, indicating that the presence of a 186 prophage does not prejudice a colony's ability to score on MMS plates. Alternately, samples from the transduction mix were resuspended in H-1, 30 min after addition of the phage, subjected to UV irradiation (456 ergs/mm²), one tenth volume of 10xLGB added and then survival of the cells followed during incubation at 30° (186cIts used) by assaying colony formers on L plates.

5.3 RESULTS

Throughout this section it will be assumed that prophage 186 is not induced during conjugation from a lysogenic male to a non-lysogenic female - this will be substantiated in Chapter 6. The E. coli linkage map appears in Figure 5-1 and shows the origins of transfer of Hfr strains and location of genes referred to below.

a. Uninterrupted Matings - Since Jacob and Wollman (1956) located att186 in the lower left quadrant of the E. coli linkage

FIGURE 5-1: Escherichia coli linkage map, based on
that of Taylor (1970), showing origins of transfer of
Hfr strains and detail of the region 48-54 min.



map, linkage studies were initiated with Hfr strain AB2528(186) which transfers this whole region. As shown in Table 5-1 this male transferred prophage 186 to his⁺ and tyrA⁺ recombinants. The tight linkage to tyrA prompted use of Hfr KL16 which gave similar results (Table 5-1). Linkage of attl86 to tyrA is similar whether male, or both male and female, are lysogenic. Prophage 186cIts shows similar linkage as the wild type prophage. Although Hfr KL16 transferred attl86, Hfr KL98(186) did not transfer the prophage (less than 1% linkage) to his⁺ or purF⁺ recombinants of AT2092 in 60 min. Thus attl86 is located between the origins of KL16 and KL98 and close to pheA.

b. Interrupted Matings - The results of an interrupted mating experiment (as described in Chapter 2) between KL16(186) and JP190 are shown in Figure 5-2. Prophage 186 enters close to tyrA⁺ and about 1.2 min before it. The lesser slope of the (186) entry compared to tyrA⁺, reflects the efficiency of assay for the (186) marker. The method depends upon the appearance of a plaque on indicator bacteria by spontaneous phage production in a recombinant cell, or its daughters, before the indicator bacteria are no longer capable, due to growth, of displaying such a plaque. In reconstruction experiments only 20% of bacteria lysogenic for 186 scored as plaques under these conditions, and the plaques were poorly defined. This 20% plating efficiency explains why the (186) entry slope is 1/5th that of the tyrA⁺ slope.

A similar mating between KL16(186) and AT2092 is depicted

TABLE 5-I: LINKAGE IN CONJUGATION OF att186 TO his, purF, tyrA and pheA.^a

Donor	Recipient	Recombinants selected	Analysis of recombinants ^b			% Linkage of <u>att186</u> to marker
			186 ⁻	(186)	(186 <u>cIts</u>)	
1.AB2528(186)	JP264	<u>his</u> ⁺ <u>tsx</u> ^r	55	65	-	55
2.AB2528(186)	JP190	<u>tyr</u> ⁺ <u>tsx</u> ^r	4	69	-	94
3.E847(186)	JP190	<u>tyr</u> ⁺ <u>tsx</u> ^r	6	68	-	92
4.KL16(186)	JP190	<u>tyr</u> ⁺ <u>str</u> ^r	25	160	-	87
5.KL16(186 <u>cIts</u>) ^c	JP190	<u>tyr</u> ⁺ <u>str</u> ^r	12	-	153	93
6.KL16(186 <u>cIts</u>) ^c	JP190(186)	<u>tyr</u> ⁺ <u>str</u> ^r	-	21	164	89
7.KL16(186)	AT2092	<u>pur</u> ⁺ <u>str</u> ^r	47	153	-	77
8.KL16(186)	AT2092	<u>phe</u> ⁺ <u>str</u> ^r	12	188	-	94

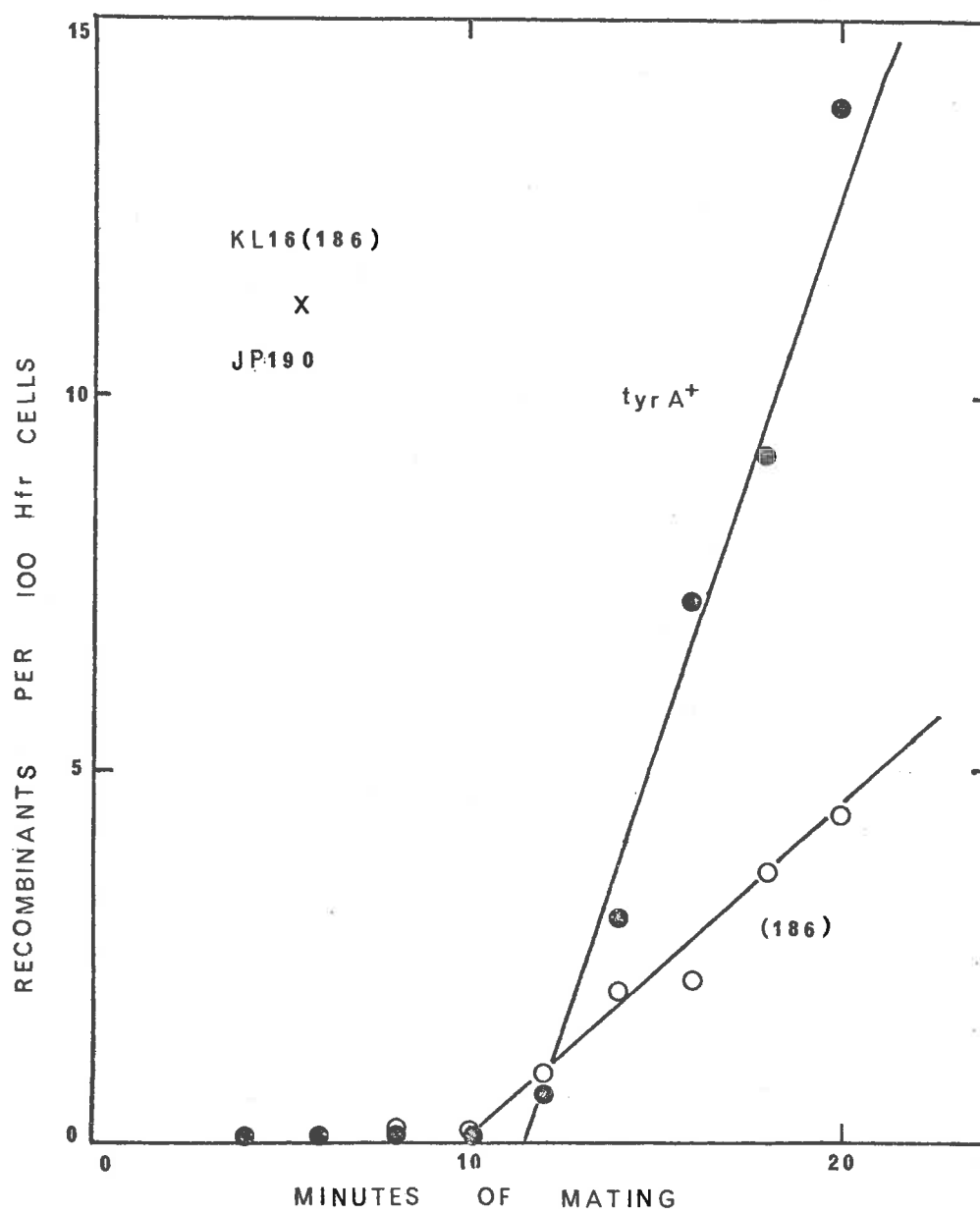
^aConjugation as described in Chapter 2.

^bThe prophage states are indicated as; 186⁻, non-lysogenic for 186, (186), lysogenic for 186, and (186cIts) lysogenic for 186cIts.

^cThese experiments at 30° throughout.

FIGURE 5-2: Interrupted mating between Hfr KL16(186) and F⁻ JP190 showing marker entry curves for tyrA⁺ and (186). Conditions as described in Chapter 2.

- tyrA⁺
- (186)



in Figure 5-3. Prophage 186 enters just before pheA⁺ and again the slope of its entry is $1/5^{\text{th}}$ that of the adjacent bacterial marker. The entry time of the his⁺ marker varied from run to run in this series of experiments, and was always delayed, compared to literature values (Taylor and Trotter, 1967), in the presence of the high concentration of 186 anti-serum used ($K=3.6\text{min}^{-1}$). In later experiments (6.3.d) where the mating mixture was diluted after 5 min contact into broth with less 186 anti-serum ($K=1.3\text{min}^{-1}$) the his marker entered at 22-23 min.

c. Two Factor P1 Transductions - To more closely locate attl86 cotransduction of this locus with various other bacterial markers by phage P1vir was investigated as described in Section 5.2.a. The donor was 594, non-lysogenic for 186, and prototrophic. Transductants were analysed as described in Section 5.2.b, and the results appear in Table 5-II. Two important controls shown in the table must be mentioned. Firstly, for each recipient strain transduction of markers unlinked to attl86 invariably gave lysogenic transductants (c.200/200), so that the transduction procedure does not of itself cause curing of the lysogenic recipients. Secondly, in each instance, transduction to a non-lysogenic recipient gives a similar transduction frequency as to the lysogenic recipient, so the recipient prophage does not affect the production of transductants. The linkage displayed indicates that attl86 is located at about 51 min between pheA and cysC.

The percentage cotransduction between pheA and attl86 is

FIGURE 5-3: Interrupted mating between Hfr KL16(186) and F⁻ AT2092 showing marker entry curves for (186), pheA⁺, purF⁺ and his⁺. Conditions as described in Chapter 2.

- (186)
- pheA⁺
- ▲ purF⁺
- his⁺

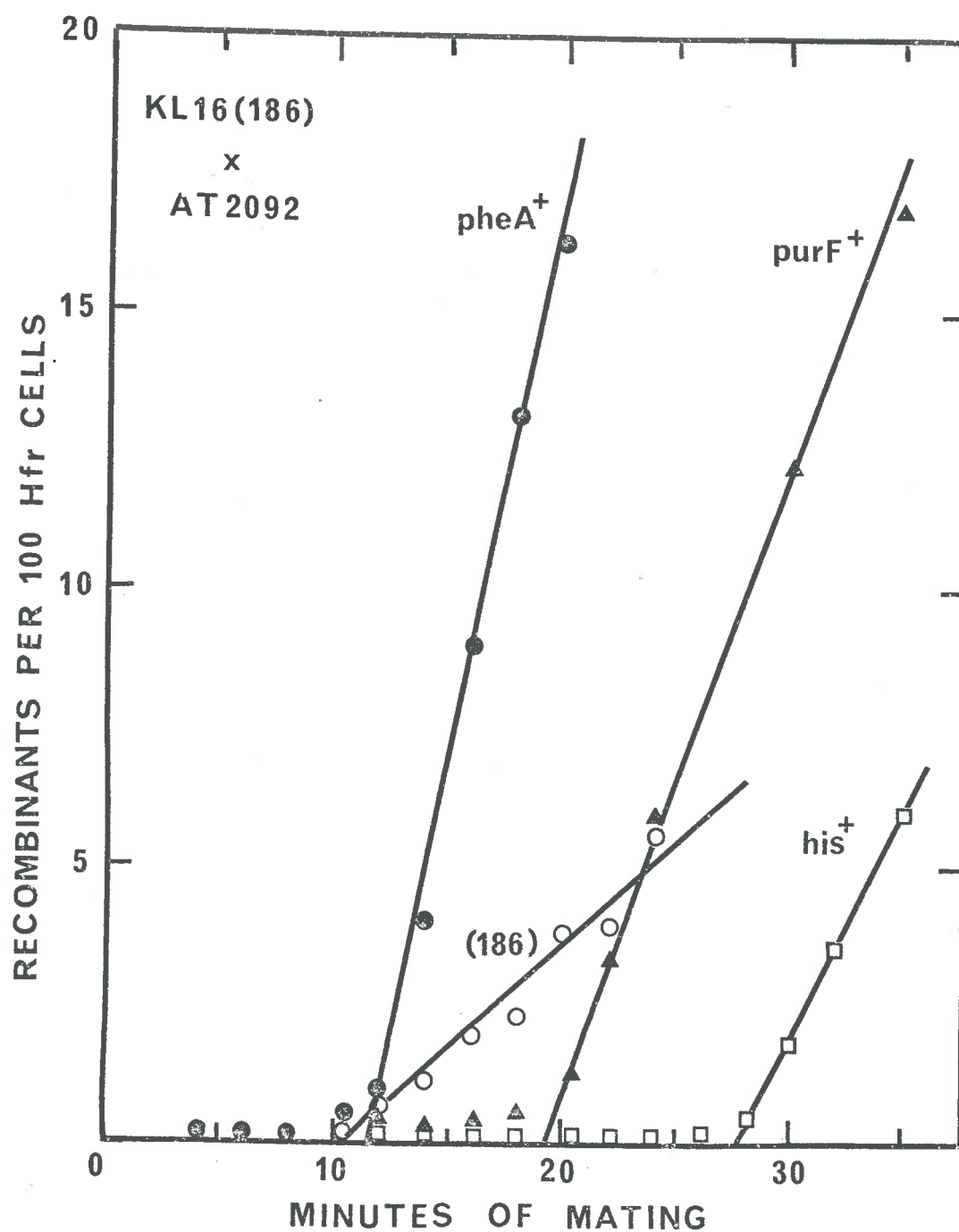


TABLE 5-II: TWO FACTOR P1 TRANSDUCTIONS FROM DONOR 594 TO 186 LYSOGENIC RECIPIENTS,
SHOWING COTRANSDUCTION OF att186 AND VARIOUS MARKERS^a

Recipient strain	Marker ^c selected	Transductants per 10 ⁶ P1 pfu	Analysis of transductants for (186) ^b		
			Fraction non-lysogenic	% Cotransduction	Separation mind ^d
JP190	<u>ilvA</u> ⁺	76.5			
JP190(186)	<u>ilvA</u> ⁺	85.3	0/200	<0.5	
AT713	<u>argA</u> ⁺	2.8			
AT713(186)	<u>argA</u> ⁺	3.2	0/206	<0.5	
AT713	<u>cysC</u> ⁺	2.9			
AT713(186)	<u>cysC</u> ⁺	3.0	0/508	<0.2	
AT2092	<u>pheA</u> ⁺	6.5			
AT2092(186)	<u>pheA</u> ⁺	6.4	97/968	10.0	1.1
JP190	<u>tyrA</u> ⁺	4.5			
JP190(186)	<u>tyrA</u> ⁺	5.1	18/672	2.7	1.4
PA3306	<u>nadB</u> ⁺	3.4			
PA3306(186)	<u>nadB</u> ⁺	3.1	8/348	2.3	1.4
PA3306	<u>purI</u> ⁺	22.7			
PA3306(186)	<u>purI</u> ⁺	22.6	5/392	1.3	1.5

Cont'd.

TABLE 5-II Cont'd.

Recipient	Marker ^c selected	Transductants per 10 ⁶ Pl pfu	Analysis of transductants for (186) ^b		
			Fraction non- lysogenic	% Cotransduc- tion	Separation min ^d
AT2457	<u>gly</u> A ⁺	26.0			
AT2457(186)	<u>gly</u> A ⁺	20.2	0/542	<0.2	
H-888	<u>pur</u> G ⁺	6.3			
H-888(186)	<u>pur</u> G ⁺	8.4	0/201	<0.5	
H-724	<u>gua</u> B ⁺	13.8			
H-724(186)	<u>gua</u> B ⁺	15.0	0/174	<0.6	
AT2092	<u>pur</u> F ⁺	7.5			
AT2092(186)	<u>pur</u> F ⁺	7.2	0/241	<0.4	
AT2092	<u>his</u> ⁺	5.8			
AT2092(186)	<u>his</u> ⁺	5.3	0/200	<0.5	
H-888	<u>trp</u> ⁺	7.4			
H-888(186)	<u>trp</u> ⁺	11.0	0/205	<0.5	
H-724	<u>trp</u> ⁺	16.2			
H-724(186)	<u>trp</u> ⁺	14.0	0/204	<0.5	

Cont'd.

TABLE 5-II Cont'd.

^aMethod as described in Section 5.2.a.

^bAs described in Section 5.2.b.

^cThe markers are listed in anti-clockwise order on the E. coli linkage map (Figure 5-1).

^dCalculated according to Wu (1966).

similar whether the recipient [Table 5-II: donor 594, recipient AT2092(186), cotransduction 97/968=10.0%; Table 5-IV line 2: donor KL164, recipient AT2092(186), cotransduction 54/526=10.3%] or the donor [Table 5-IV line 3: donor KL164(186cIts), recipient AT2092, cotransduction 59/524=11.3%] or both [Table 5-IV line 4: donor KL164(186cIts), recipient AT2092(186), cotransduction 55/528=10.4%] are lysogenic. However when the donor is lysogenic it is necessary to treat the P1 lysate with 186 anti-serum (5.2.a) otherwise all transductants selected from a non-lysogenic recipient are found lysogenic [donor KL164(186cIts), recipient AT2092, "cotransduction" 123/123]. That this is due to free 186 phage in the lysate can be confirmed since transduction from a recA(186) donor gives meaningful cotransduction of att186 and pheA without the antiserum treatment [donor JC2926(186), recipient AT2092, cotransduction 33/287=11.5%]. The level of free 186 phage released by such a strain has been shown to be one thousand fold less than from a rec⁺ strain (4.3.e).

d. Three Factor P1 Transductions - Finer mapping within the cysC-pheA region was possible using the nalB marker. Interrupted matings by Hane and Wood (1969) placed nalB at 51[±]1 min. Three factor P1 transductions involving argA, cysC, nalB (and att186) are detailed in Table 5-III. It is seen that the prophage has no effect on the other markers, so that the data may be pooled to emphasise that argA and nalB are not cotransduced together, with cysC. As the order lysA-argA-cysC has been

TABLE 5-III: ORDER OF THE argA, cysC AND nalB LOCI DETERMINED BY P1 TRANSDUCTION^a

P1 DONOR : KL164 (argA⁺cysC⁺nalB^r)

RECIPIENT: AT713 (argA⁻cysC⁻nalB^s)

Prophage state ^b		Analysis of <u>cys</u> ⁺ Transductants			
Donor	Recipient	<u>arg</u> ⁺ <u>nal</u> ^s	<u>arg</u> ⁻ <u>nal</u> ^r	<u>arg</u> ⁻ <u>nal</u> ^s	<u>arg</u> ⁺ <u>nal</u> ^r
186 ⁻	186 ⁻	34	25	469	0
186 ⁻	(186)	32	28	468	0
(186 <u>cIts</u>)	186 ⁻	36	29	459	0
(186 <u>cIts</u>)	(186)	37	26	484	0
Totals:		139	108	1880	0

^aTransduction and analysis, as described in Sections 5.2.a and b, at 30° throughout. The data is from the same transductions reported in Table 5-IV.

^bThe prophage states are indicated as 186⁻, non-lysogenic for 186, (186), lysogenic for 186, and (186cIts), lysogenic for 186cIts.

previously established (Taylor and Trotter, 1967), the order inferred is (lysA)-argA-cysC-nalB-(pheA).

The results of three factor transductions involving attl86, nalB and pheA or cysC are shown in Table 5-IV. For nalB these show cotransduction at 5.5% with pheA (line 1) and at 4.7% with cysC (line 5), frequencies which correspond to 1.2 and 1.3 min respectively. This cysC-pheA distance of 2.5 min compares favourably with 2.7 min reported by Willetts, Clark and Low (1969) from P1 transduction data, and with 3.0 min shown on the E. coli linkage map from conjugation data. Therefore nalB is approximately equidistant from cysC and pheA at about 51.5 min.

With relation to attl86, there is no cotransduction of this locus with cysC and the cysC-nalB cotransduction is unaffected by prophage in donor (line 7), recipient (line 6) or both (line 8), indicating that phage 186 does not integrate between cysC and nalB. However attl86 is cotransducible with pheA independent of whether it is the donor (line 3), the recipient (line 2) or both which are lysogenic, as was discussed previously (5.3.c). More importantly, the presence of 186cIts prophage in the donor (lines 3 and 4) destroys the cotransduction of nalB with pheA, indicating that the prophage is integrated between these genes. Although prophage in the recipient only (line 2) has little, if any, effect on the cotransduction of nalB with pheA, the frequency of the recombinant class representing quadruple exchanges [nal^r(186) phe⁺: 10/526] is high compared with that representing double exchanges [nal^r186⁻ phe⁺: 16/526] suggesting that recombination is

TABLE 5-IV: THREE FACTOR TRANSDUCTIONS INVOLVING att186, nalB and pheA or cysC^a

Donor ^{b,c} markers			Recipient ^{b,d} markers		Analysis of <u>phe</u> ⁺ transductants				Cotransduction frequency
A	B		a	b	AB	Ab	aB	ab	<u>nalB</u> - <u>pheA</u>
1.	<u>nal</u> ^r 186 ⁻	<u>phe</u> ⁺	<u>nal</u> ^s 186 ⁻	<u>phe</u> ⁻	29		499		29/528
2.	<u>nal</u> ^r 186 ⁻	<u>phe</u> ⁺	<u>nal</u> ^s (186)	<u>phe</u> ⁻	16	10	38	462	26/526
3.	<u>nal</u> ^r (186 <u>cIts</u>)	<u>phe</u> ⁺	<u>nal</u> ^s 186 ⁻	<u>phe</u> ⁻	0	0	59	465	0/524
4.	<u>nal</u> ^r (186 <u>cIts</u>)	<u>phe</u> ⁺	<u>nal</u> ^s (186)	<u>phe</u> ⁻	0	0	55	473	0/528
					Analysis of <u>cys</u> ⁺ transductants				<u>cysC</u> - <u>nalB</u>
5.	<u>cys</u> ⁺ <u>nal</u> ^r 186 ⁻		<u>cys</u> ⁻ <u>nal</u> ^s 186 ⁻		25		503		25/528
6.	<u>cys</u> ⁺ <u>nal</u> ^r 186 ⁻		<u>cys</u> ⁻ <u>nal</u> ^s (186)		0	28	0	500	28/528
7.	<u>cys</u> ⁺ <u>nal</u> ^r (186 <u>cIts</u>)		<u>cys</u> ⁻ <u>nal</u> ^s 186 ⁻		0	29	0	495	29/524
8.	<u>cys</u> ⁺ <u>nal</u> ^r (186 <u>cIts</u>)		<u>cys</u> ⁻ <u>nal</u> ^s (186)		0	26	0	521	26/547

^aTransduction and analysis, as described in Sections 5.2.a and b, at 30° throughout.

^bThe prophage states are indicated as 186⁻, non-lysogenic for 186, (186), lysogenic for 186, and (186cIts), lysogenic for 186cIts.

^cKL164 was used as donor.

^dAT2092 and AT713 were used as recipients for pheA and cysC selections respectively.

affected in this region.

e. Specialised Transduction by Phage 186 - Specialised transduction of recA⁺ by phage 186, produced by UV induction of C600(186), was sought using the technique described in Section 5.2.c. From over 10^{11} pfu of phage 186 screened by variations on the MMS technique, no transducing particles were found. Few survivors were found on the MMS plates and these were not resistant to UV irradiation. (It should be emphasised that 186 lysogens do not show the extensive killing on UV irradiation typical of other UV inducible phages; Section 4.3.f.) All but one were capable of producing phage, but these lysates showed no capability for specialised transduction of recA⁺ as assayed by the MMS technique. The one defective lysogen found proved to be a phage mutant unable to excise in a recA host (4.3.e). Revertants of this mutation were found among larger colonies which produced low levels of mainly clear plaque mutants from a recA host (as for 186 wild type; 4.3.e) so a point mutation is involved. Similarly no transducing particles were detected among 4×10^{10} pfu of 186cIts screened by the MMS technique.

Rather than apply continuous selection for rec⁺ as with the MMS technique, a 30 sec dose of UV radiation was applied 30 min after addition of phage to the recA strain 152 (5.2.c). In this experiment phage 186cIts was used, at high (10) and low (2.5) multiplicities of addition, from two sources. Firstly, UV induced from rec⁺ C600(186cIts) at 30° and as control, heat induced from recA AB2463(186cIts). Both lysates contained no bacterial

contaminants. The survival of the infected cultures is shown in Figure 5-4 as assayed on L plates. Addition of either phage lysate at high multiplicity depresses the survival of the culture following UV presumably through phage lytic activity. Phage from recA(186cIts) has little effect at low multiplicity, but phage from rec⁺(186cIts) shows a slight protection of 152 from the effects of UV radiation. However, similar samples plated on MMS plates showed no survivors, indicating no permanent conversion to rec⁺. Likewise, colonies detected as survivors on the L plates proved to be UV sensitive.

f. Other 186 Attachment Sites - Throughout this work many different strains have been lysogenised with phage 186, and used in transduction and conjugation experiments. No evidence of any permanent association of the prophage with other than the "pheA-linked" site has been found. Prophage 186cIts behaves similarly to the wild type as is evident in this chapter. In addition, 186susK4, K6 and K9 from a different source and 186cIts sus15 show the standard linkages to pheA in conjugation and P1 transduction (Table 5-V).

5.4 DISCUSSION

With uninterrupted matings it was first established that att186 was between the origins of Hfr's KL16 at 55 min and KL98 at 44 min. The interrupted matings showed att186 entering just before pheA and tyrA. It is interesting that Hane and Wood (1969), in similar matings to locate nalB, found it necessary to multiply

FIGURE 5-4: Survival at 30° of strain 152 following infection with 186cIts and UV irradiation (30 sec), as described in Section 5.2.c.

Source of phage:

- △ none
- C600(186cIts) 2.5 phage/bacterium
- C600(186cIts) 10 phage/bacterium
- AB2463(186cIts)2.5 phage/bacterium
- AB2463(186cIts)10 phage/bacterium

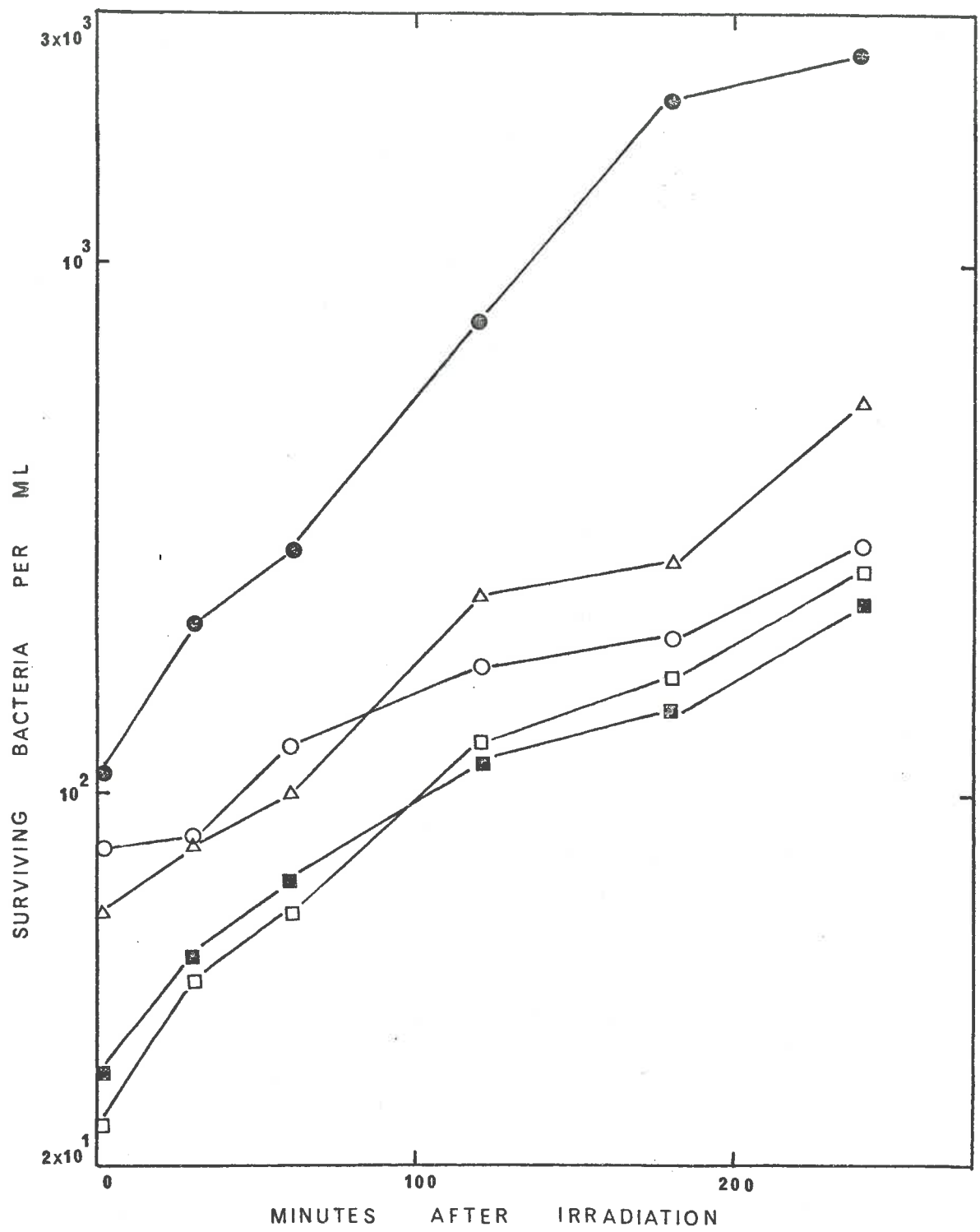


TABLE 5-V: ATTACHMENT SITE OF PHAGE 186 DERIVATIVES ASSAYED BY LINKAGE TO pheA

186 derivative	Method ^a	Donor	Recipient	Cotransfer	
				Fraction	%
186 <u>sus</u> K4	conjugation	KL16(186 <u>sus</u> K4)	AT2092	113/127	89
186 <u>sus</u> K4	conjugation	E817(186 <u>sus</u> K4)	AT2092	71/76	93
186 <u>sus</u> K4	conjugation	E817(186 <u>sus</u> K4)	AT2092(186)	43/50	86
186 <u>sus</u> K6	conjugation	KL16(186 <u>sus</u> K6)	AT2092	118/130	91
186 <u>sus</u> K6	transduction	C600(186 <u>sus</u> K6)	AT2092	16/135	11.9
186 <u>sus</u> K9	conjugation	KL16(186 <u>sus</u> K9)	AT2092	123/134	92
186 <u>sus</u> K9	conjugation	E817(186 <u>sus</u> K9)	AT2092	55/62	89
186 <u>sus</u> K9	conjugation	E817(186 <u>sus</u> K9)	AT2092(186)	46/50	92
186 <u>cIts sus</u> 15	conjugation ^b	KL16(186 <u>cIts sus</u> 15)	AT2092	108/122	88

^aAs described in Chapter 2 (conjugation) and Section 5.2.a (P1 transduction).

^bAt 30°.

their data for nalB by a factor of five to avoid intersecting marker entry curves, just as could be done for the entry of the adjacent 186 prophage here (Figures 5-2, 5-3). Two factor transduction data put att186 at 51.1 min, 1.1 min anticlockwise from pheA. The limited P1 transduction data reported by Abe and Tomizawa (1971) are similar to the results shown in Table 5-II, although they reported 2% (3/157) cotransduction with glyA. No details were given as to the prophage state of the donor or recipient used, or to the controls employed. This cotransfer with glyA could well represent free phage infection from a lysogenic donor. In the present study it was found necessary to treat lysates from lysogenic donors with a high concentration of 186 anti-serum to avoid such infection (5.3.c). Blumenthal (1972) found that P1 transduction from a P2 lysogenic donor was satisfactory without such treatment in that, in almost all cases, transductants that carried P2 were those in which it entered by transduction. He attributes this to the low level of infection of E. coli K12 by P2 in either liquid medium, or on minimal plates. This is not true for phage 186, but a more important factor is most probably the higher rate of spontaneous phage production by a 186 lysogen compared to a P2 lysogen. In addition, no evidence for heterogenotic transductants following transfer of prophage 186 was found, although this has been demonstrated following transfer of P2 prophage (Blumenthal, 1972).

Thus att186 was placed in a region of the E. coli linkage map devoid of selectable markers. The mutation to ability to

grow on 1,2-propanediol (prd) shown in this region of the current linkage map (Taylor, 1970) is in fact located between argA and cysC (B. Konrad, pers. comm; R.R.B. Russell, pers. comm.). Fortunately nalB is cotransducible with both pheA and cysC (Table 5-IV) thus enabling the location of attI86 by three factor transductions (5.3.d.). That cotransduction of the spanning markers pheA and nalB is unaffected when the donor is non-lysogenic and the recipient lysogenic, but abolished when the situation is reversed, suggests that the prophage in the recipient can "loop out" such that the pheA-nalB distance is similar in both the recipient and the transduced section of DNA. This allows similar integration of the transduced section whether the recipient be lysogenic or not. It has been shown that λ (Rothman, 1965) and P2 at location II (Calendar and Lindahl, 1969) behave similarly.

This "looping out" would probably occur as envisaged by the Campbell model preparatory to excision. However there is no turn on of the genes required for excision so the prophage is not necessarily cured. For example, the attI86-pheA linkage is the same whether the recipient is lysogenic or not. Where the episome involved is an F factor integrated in a Hfr strain similar results are obtained (Pittard, 1965), except that there is a high rate of conversion from Hfr to F^+ on cotransduction of spanning markers from F^- to Hfr. This suggests that excision of the F factor may not require expression of extra genes, but utilises existing functions. Thus the formation of the F factor loop forced or favoured by the mispairing during such transduction may be all that is required for F factor excision.

The search for specialised transduction by 186 did not produce stable conversion of a recA strain to rec⁺. This is not surprising since recA is mapped at 51.7 min (Willetts, Clark and Low, 1969) and attl86 at 51.1 min (5.3.c). Thus the distance between recA and attl86 is similar to the length of the 186 DNA (19.7×10^6 daltons, Wang, 1967). However, phage 186cIts induced from a rec⁺ host did slightly protect a recA recipient against UV radiation, whereas the same phage induced from a recA host had no effect (Figure 5-4). Although this result is of a preliminary nature only, and could even reflect that the phage from the rec⁺ host were produced by UV rather than heat induction, I shall consider the possibility that the protection is due to transfer of recA⁺ by the phage. The protection is only of a transient nature, since no survivors were rec⁺.

This may be explained if the 186recA⁺ transducing DNA is defective, in particular such that it cannot integrate or replicate and is lost by dilution. That is, the formation of the 186recA⁺ DNA has deleted the phage genes responsible for these functions. Indeed it may have lost one of the phage vegetative ends. This has been proposed for some λdgal particles by Kayajanian (1970), and would simultaneously preclude integration and replication. Why then does such a 186recA particle not form transductants by marker rescue, that is by recombination between the recA chromosome and the rec⁺ transducing DNA? It could be that the level of recA⁺ product in such a situation is insufficient to promote this recombination, or alternately to protect

the linear fragment from recB⁺ exonuclease, if the transducing DNA is incapable of circularisation (Pilarski and Egan, 1972). Hertman and Luria (1967) did find that P1 transduction of recA⁺ was abortive in 80 to 90% of recA recipients initially protected against UV radiation by the transduction. In the case of 186recA⁺ also, the level of recA⁺ product is sufficient to display protection against UV radiation. The fact that high multiplicity of infection, equivalent to use of wild type helper phage, abolishes the protection of 152 by 186recA⁺ may be attributed to lysis if the decision in the cell is for the lytic cycle. Alternately if the cell becomes lysogenic this may well turn off expression of the 186recA⁺ DNA. It is not known if phage 186 can form tandem lysogens.

This all appears to indicate that the distance between attl86 and recA is so long that if any transducing particles are produced, they are defective, at least, in integration. This is most probably through a physical defect of the DNA rather than loss of a complementable function since wild type helper phage were of no benefit. Such a suggestion does not augur well for the production of high frequency transducing lysates, which are required if the 186recA⁺ particle is to be of any use in elucidating recA⁺ function. Fortunately Gottesman and Beckwith (1969) have found that, if the selection technique is good enough and sufficient phage are screened, deletions and inversions may be forced in making transducing particles for genes somewhat distant from the attachment site.

This chapter has established the location of att186 at 51.1 min, and demonstrated the integration of prophage 186 between pheA and nalB. The 186 prophage may be capable of specialised transduction of the recA⁺ gene, but if so, the transductants are unstable.

CHAPTER 6

ZYGOTIC INDUCTION OF COLIPHAGE 186.

6.1 INTRODUCTION

Jacob and Wollman (1956) have identified two groups of temperate coliphages, the " λ -related" group displaying zygotic and UV inductions, and the "non-inducible" group showing neither induction. The zygotic induction of, for example, phage λ occurs during conjugal transfer of the prophage from a lysogenic male to a non-lysogenic female and is manifest in three ways. Firstly, there is a great increase in infectious centres over the level obtained from the lysogenic male alone. This increase depends on the degree of transfer of the prophage by the male concerned, and production of infectious centres representing up to 68% of the male cells has been reported under appropriate conditions (Jacob and Wollman, 1961). In particular, the percentage infectious centres is from twice (Jacob and Wollman, 1961, Table XXV) to fifteen times (Jacob and Wollman, 1957b) the recovery of recombinants for an adjacent bacterial marker, as determined in control experiments without prophage or with both participants lysogenic, so that zygotic induction does not occur.

The second manifestation of zygotic induction is in the nature of the bacterial recombinants obtained. Recombinants for markers proximal to the prophage are decreased in frequency to varying degrees by zygotic induction. Markers are more affected the closer they are to the prophage since it is more likely that the prophage will be transferred to such recombinants resulting in their death through zygotic induction. This has the effect of steepening the gradient of transfer (Jacob and

Wollman, 1954b). Recombinants for markers distal to the prophage are only found at very low frequency (Jacob and Wollman, 1958) as such recombinants must have received the male prophage and thus be liable to killing by zygotic induction.

Thirdly, amongst the lower levels of recombinants from a cross involving zygotic induction, lysogenic clones are only found very rarely (Jacob and Wollman, 1958).

The "non-inducible" group of phages are stated to show none of these properties (Jacob and Wollman, 1956) and data has been published for phage 18 (Jacob and Wollman, 1961). This indicates that prophage 18 behaves as a normal bacterial marker, having no effect on the gradient of transfer. Att18 has the same distribution among recombinants whether it is the male or the female that is lysogenic.

The other non-inducible phage studied, P2, shows an abortive zygotic induction. Kelly (1963), in studying the P2 attachment sites in E. coli K12 and C (location II), found different linkage of P2 with met depending on whether the male or the female was lysogenic. When the female only was lysogenic (or both strains lysogenic for distinguishable P2 mutants) about 70% of met⁺ recombinants were non-lysogenic (or carried the male prophage). When the male was lysogenic and the female not, only about 30% of met⁺ recombinants carried the prophage. Thus it appeared that, in the latter case, where zygotic induction was possible, many met⁺ recombinants that should have received the male prophage were not scored. Since no production of P2 by

the female cells could be demonstrated (Kelly, 1963), Bertani (1968) termed the effect abortive zygotic induction.

However, the data of Kelly (1963) is in terms of the linkage of attP2 to met, no absolute description of the frequency of the met⁺ recombinants being given, so it is not clear if an actual loss of met⁺ recombinants occurs. Wiman, Bertani, Kelly and Sasaki (1970) have shown that transfer of prophage P2 at location I in E. coli C has no effect "on the shape of the entry curves" of adjacent markers, comparing lysogenic and non-lysogenic recipients. A perturbation is apparent though in that, of arg⁺ recombinants selected at 70 min, 0/50 carried phage when the male was lysogenic, whereas 14/99 were non-lysogenic when it was the female that was lysogenic. Thus it may be suggested that the abortive zygotic induction of P2 is a type of curing which does not result in the death of females receiving the prophage. However, it must be noted that Kelly (1963) and Wiman et al (1970) do find reciprocal linkage between P2 at location H in E. coli K12 and his, and between P2 at location III in E. coli C and Tl,try respectively, in crosses where either parent is lysogenic. The same is true for phage 18 and arg (Jacob and Wollman, 1961). That is, the effects of abortive zygotic induction have only been noted for phage P2 at locations I and II.

If abortive zygotic induction of phage P2 does not lead to death of the host, then it is at variance with the abortive heat induction of P2 which does lead to the death of the host.

(Bertani, 1968). This killing appears to be unique among the non-inducible phages. Temperature-sensitive clear plaque mutants of phages 299 (Golub and Zvenigorodsky, 1969) and 18 (Golub and Reshetnikova, 1970) undergo abortive heat induction but the host survives and remains lysogenic. There is no evidence for killing following abortive zygotic induction of P2, and it appears that at some attachment sites prophage P2 is not even abortively induced during conjugation, as discussed above.

This chapter serves to confirm that phage 186 is not zygotically inducible, especially following the discovery that it is UV inducible (Chapter 4), and to examine possible reasons for the lack of zygotic induction.

6.2 MATERIALS AND METHODS

a. Interrupted Matings - The interrupted matings reported in this chapter were performed as described in Chapter 2, but with some variations. In crosses involving Hfr E784, the mating mixture was gently diluted 10 fold into fresh LGB containing 186 anti-serum ($K=1.9\text{min}^{-1}$) after 5 min of mating. After interruption, trp⁺, lys⁺ and trp⁺lys⁺ recombinants were selected directly on appropriate minimal media. Phage 186 activity was measured with indicator bacteria 594, as before, on TB plates containing streptomycin (200 $\mu\text{g/ml}$). The trp⁺ recombinants obtained were assayed for presence of the 186 prophage by toothpicking at least 500 colonies for each time sample into indicator bacteria 594.

In the interrupted matings involving Hfr E817, the mating mixture was gently diluted 100 fold into fresh LGB containing

186 anti-serum ($K=1.3\text{min}^{-1}$) after 5 min of mating. After interruption, his⁺ recombinants were selected on minimal medium, and phage 186 activity measured by plating with E828 ϕ 80^r indicator bacteria on TB plates containing streptomycin (200 $\mu\text{g}/\text{ml}$).

b. UV Induction of Prophage 186 Following Transfer by Conjugation - This was tested in interrupted matings involving Hfr E817. For the interruption step samples are diluted 10 fold or more into H-1 before blending. This solution is now suitable for UV irradiation, which was carried out for 50 sec under the standard conditions (dose; 760 erg/mm^2). Suitable dilutions were assayed for his⁺ recombinants, or phage activity with E828 ϕ 80^r indicator bacteria on TB plates containing streptomycin (200 $\mu\text{g}/\text{ml}$), with and without the irradiation.

c. Recovery of UV Inducibility after Conjugation - A normal conjugation between Hfr E817(186) and F⁻E828 ϕ 80^r (Hfr:F⁻ ratio = 1:10) was interrupted after 30 min at 37° (transfer 0.96 186 plaques/100 Hfr assayed immediately) and diluted into LGB containing 186 anti-serum ($K=1.9\text{min}^{-1}$) and streptomycin (200 $\mu\text{g}/\text{ml}$). The dilution was shaken at 37°. At various times samples were diluted 20 fold into H-1 and assayed for 186 phage activity with E828 ϕ 80^r indicator on TB plates containing streptomycin (200 $\mu\text{g}/\text{ml}$) before or after a 50 sec dose of UV irradiation (dose; 760 erg/mm^2).

d. Latent Period of 186cIts Heat Induction Following Transfer by Conjugation - A normal conjugation between Hfr KL164

(186cIts) and F^- E828 ϕ 80 r (Hfr: F^- ratio=1:10) was interrupted after 60 min at 30°. This was diluted into LGB containing streptomycin (200 μ g/ml) and shaken at 39°. At various times samples were assayed for 186cIts phage activity at 30° with E828 ϕ 80 r indicator on TB plates containing streptomycin (200 μ g/ml). A control culture of 594(186cIts) at a similar initial concentration to that of KL164(186cIts) in the mating mixture, was carried throughout.

e. UV Induction of Prophage 186 in a Female Lysogen Following Conjugation - These conjugations involved Hfr: F^- ratios of 1:1 in the presence of 186 anti-serum ($K=3.6\text{min}^{-1}$) as usual. After 10 min conjugation at 37°, mating was interrupted. This involved a 40 fold dilution into H-1 before blending. The solution was then assayed for 186 phage activity with E828 ϕ 80 r indicator on TB plates containing streptomycin (200 μ g/ml) before and after UV irradiation (dose; 760 ergs/mm 2). For ϕ 80 and λ female lysogens the same technique was employed, but the indicator was 594.

f. Indirect Induction - These experiments utilised F_{gal}^+ carrying strain P601 cured of its λ prophage (E873) and F^- strains E828 and JP190 lysogenic for 186 or ϕ 80. Conjugation was at $F':F^-$ ratio of 1:1 at 37°. Before conjugation, all cultures were grown to log phase in LGB, resuspended in H-1, a portion of the F' culture UV irradiated (760 ergs/mm 2), and then all resuspended in LGB. After 20 min conjugation samples were diluted in H-1 and assayed for, (i) gal^+str^r recombinants on

EMB-gal plates containing streptomycin (200 μ g/ml), and (ii) phage activity before and after 50 sec UV irradiation (760 ergs/mm²) with 594 indicator bacteria on TB plates containing streptomycin (200 μ g/ml).

6.3 RESULTS

a. Bacterial Mutants Resistant to Phage 186 - In zygotic induction experiments it has been standard practice (Jacob and Wollman, 1961) to wash and resuspend lysogenic cultures before mating to reduce the level of free phage activity and to use females unable to adsorb the phage. The male is selectively killed, for example, with streptomycin. This all ensures that phage activity scored is due to phage entering the female by conjugation. Jacob and Wollman (1956) have shown that vegetative phage in the male are not transferred during conjugation. Therefore it was intended to isolate 186 resistant derivatives of female strains. However, Calendar, Lindqvist, Sironi and Clark (1970) have reported that P2, 186 and other non-inducible phages will not plate on the rep mutants isolated as resistant to ϕ X174 by Denhardt, Dressler and Hathaway (1967). The defect is in phage DNA replication rather than adsorption, at least for ϕ X174 and P2. Thus it is important that such a "resistant" mutant should not be used as a female in zygotic induction experiments.

Phage 186 resistant derivatives of JP264 were selected as the survivors of massive infection by 186cIts at 37°. Of 30 survivors which proved to be resistant to 186, 5 were also resistant to phage 299. Three members of each class of resistant

mutant were tested against other phage by spotting. The results appear in Table 6-I. Apart from phages 18 and P2k, the same results were obtained at 30° and 37°. Class I mutants will not plate 186 or W, and only plate P2k at 37°. Class II mutants plate none of the non-inducible phage. Phage 18 only shows activity at 37° on all E. coli K12 indicators tested, as had been noted before (Woods, 1968). Similar groups of 186 resistant mutants were obtained from 594 and AB1133. Class I mutants show a similar pattern of phage activity as a bacterial mutant resistant to 186 isolated by Jacob and Wollman (1956). Class II mutants show activity with none of the non-inducible phages as is found for rep mutants (Calendar et al, 1970) and for a mutant isolated as resistant to phage 62 (Jacob and Wollman, 1956). Sample mutants from each class, and the parent JP264, showed the same rates of adsorption of phage 186, so the defect in these mutants is in a post-adsorption step. This precludes their use as females for zygotic induction experiments. Therefore, phage anti-serum was used to prevent free phage infection of the female.

b. Zygotic Induction of Phage λ - To establish appropriate conditions, especially with regard to the use of phage sensitive females, zygotic induction of λ was investigated. Zygotic induction could be demonstrated with λ resistant or sensitive females, all crosses being carried out in the presence of λ anti-serum ($K=1.5\text{min}^{-1}$). The results are shown in Table 6-II. A substantial increase in phage titre (representing infectious

TABLE 6-I: SENSITIVITY OF JP264 AND 186 RESISTANT DERIVATIVES
OF JP264 TO OTHER COLIPHAGE^a

Phage Spot	Indicator Bacteria			
	JP264	JP264 186 ^r class I	JP264 186 ^r class II	
λ b	- ^c	-	-	
T6 ^b	-	-	-	
λ cIts857h80	+	+	+	
434	+	+	+	
186	+	-	-	
186cIts	+	-	-	
W	+	-	-	
18	+	+	-	
299	+	+	-	
299Rt11	+	+	-	
P2k	+	+	-	
18 at 30°	-	-	-	
P2k at 30°	+	-	-	

^aTested by spotting phage stocks (10^7 pfu/ml) on indicator lawns and incubating overnight at 37°.

^bJP264 is itself resistant to λ and T6.

^c+ indicates phage activity, - no activity.

TABLE 6-II: ZYGOTIC INDUCTION OF PHAGE λ IN CROSSES BETWEEN
HfrH(λ) AND F $^{-}\lambda^{-}$ ^a

Donor	Recipient	$\frac{\text{pro}^{+}\text{str}^{r}}{\text{per 100 Hfr cells}}$ recombinants	λ plaques ^c per 100 Hfr cells
1. AB259(λ)	alone	< 0.01	0.2
2. AB259(λ)	AB2102	-	54
3. AB259(λ)	AB1133	2.5	64
4. AB259(λ)	AB1133(λ)	6.2	-
5. AB259(λ_{ind}^{-})	alone	< 0.01	0.5
6. AB259(λ_{ind}^{-})	AB2102	-	57
7. AB259(λ_{ind}^{-})	AB1133(λ)	4.0	-
8. AB259(λ_{nzi1}) ^b	alone	-	0.8
9. AB259(λ_{nzi1}) ^b	AB2102	-	52
10. AB259(λ_{nzi2}) ^b	alone	-	1.1
11. AB259(λ_{nzi2}) ^b	AB2102	-	50

^aAll crosses as described in Chapter 2. Mating mixtures contained λ anti-serum ($K=1.5\text{min}^{-1}$). Plated for recombinants or phage activity after 60 min mating.

^bThese lysogens carry prophage obtained from $\text{trp}^{+}\text{str}^{r}$ recombinants of the cross described in line 2 of this table, which are thus potential non-zygotically inducible (nzi) mutants.

^cThe plaques are from infectious centres and are not due to spontaneous phage production from λ lysogens, which results in few very poorly defined plaques.

centres) is seen upon conjugation of AB259(λ) with either AB1133 or AB2102 (lines 2 and 3). With the λ sensitive female, AB1133, zygotic induction reduces the recovery of pro⁺ recombinants (line 3). The appearance of infectious centres with this recipient was followed in a crude interrupted mating experiment using 1 min agitation on a Vortex Genie for interruption. The increase started at about 30 min as found by Jacob and Wollman (1961), and plaque activity was chloroform sensitive till at least 60 min. This indicates that the increase is due to the formation of infectious centres from 30 min, and not to lysis at 30 min following free phage infection of the λ sensitive females. Thus it is apparent that λ anti-serum alone is sufficient to prevent free phage infection and allow the demonstration of zygotic induction. Curtiss et al (1968) have also used λ anti-serum to protect λ sensitive females in zygotic induction experiments.

It was found important to keep everything at 37° throughout a zygotic induction experiment, most simply done by performing this in a 37° room. Plates to be used to assay phage activity were well dried and the soft agar layer encouraged to set at 37° by leaving the lids off such plates for 20 min. In addition to phage λ , zygotic induction was confirmed for ind⁻ in both AB2102 (Table 6-II, lines 5,6 and 7) and 594. A search was made for a non-zygotically inducible mutant of λ amongst trp⁺str^r recombinants from the mating of AB259(λ) with AB2102. These recombinants were only found at a low level (0.2/100Hfr cells) and only 2/120

were lysogenic. Lysogens of these two potential mutant phage were constructed in AB259, and zygotic induction tested. However, both proved to be zygotically inducible (Table 6-II, lines 8-11). Jacob and Wollman (1961) have suggested that these rare lysogenic recombinants may be produced by free phage infection following a perturbation of the phage resistance of the female. This seems unlikely in the present case where λ anti-serum was present in the mating mixture. The lysogenic trp⁺str^r recombinants were still resistant to λ vir.

c. Zygotic Induction of Phage 186 -

i. Uninterrupted matings - Initial experiments were performed with the O-13 Hfr, AB2528(186), using 186 anti-serum to protect the females from free phage infection. The conditions used were exactly similar to those found necessary for successful zygotic induction of phage λ (Chapter 2, and above). Similar levels of his⁺ recombinants were obtained whether the female JP264 was lysogenic for 186 or not (Table 6-III, lines 2,3) and there was only a slight increase in plaque activity on conjugation to the sensitive female compared with that from the male alone (Table 6-III, lines 1,2). The his⁺ recombinants of JP264 were tested for the inheritance of the male prophage (Table 5-I, line 1) which 55% of them had received. Four of these lysogenic recombinants were shown to be capable of displaying UV induction of the prophage (Table 4-III, line 3) indicating that the lack of zygotic induction is not due

TABLE 6-III: LACK OF ZYGOTIC INDUCTION OF PHAGE 186 IN UNINTERRUPTED MATINGS^a

Donor	Recipient	Recombinants selected	Recombinants ^b /100 Hfr cells	186 plaques ^b /100 Hfr cells
1. AB2528(186)	alone	<u>his</u> ⁺ <u>tsx</u> ^r	<0.01	0.9
2. AB2528(186)	JP264	<u>his</u> ⁺ <u>tsx</u> ^r	1.2	1.4
3. AB2528(186)	JP264(186)	<u>his</u> ⁺ <u>tsx</u> ^r	1.5	-
4. AB2528(186)	alone	<u>tyr</u> ⁺ <u>tsx</u> ^r	<0.01	0.8
5. AB2528(186)	JP190	<u>tyr</u> ⁺ <u>tsx</u> ^r	2.6	1.4
6. AB2528(186)	JP190(186)	<u>tyr</u> ⁺ <u>tsx</u> ^r	2.7	-
7. KL16(186)	alone	<u>tyr</u> ⁺ <u>str</u> ^r	<0.01	0.9
8. KL16(186)	JP190	<u>tyr</u> ⁺ <u>str</u> ^r	19.1	5.6
9. KL16(186)	JP190(186)	<u>tyr</u> ⁺ <u>str</u> ^r	17.9	-
10. KL16(186 <u>cIts</u>) ^c	alone	<u>tyr</u> ⁺ <u>str</u> ^r	<0.01	0.5
11. KL16(186 <u>cIts</u>) ^c	JP190	<u>tyr</u> ⁺ <u>str</u> ^r	5.5	2.3
12. KL16(186 <u>cIts</u>) ^c	JP190(186)	<u>tyr</u> ⁺ <u>str</u> ^r	5.9	-

^aAll crosses as described in Chapter 2. Mating mixtures contained 186 anti-serum ($K=3.6\text{min}^{-1}$).

^bAssayed after 90 min mating for lines 1-6, and after 60 min for lines 7-12.

^cExperiment performed at 30°, all others at 37°.

to a permanent inability of the recombinants to allow 186 induction.

Similar results are found with the same male and female JP190 when tyr⁺ recombinants are selected (Table 6-III, lines 4-6). The plaque activity obtained with the sensitive female (line 5) is only slightly above background (line 4) and below the transfer of the adjacent marker tyrA (lines 5,6). The tyr⁺ recombinants of JP190 were tested for inheritance of the male prophage (Table 5-I, line 2) which 94% of them had received. Such a high cotransfer indicates that these markers are closely linked and is a good indication that zygotic induction, which normally leads to the recovery of few lysogenic recombinants, has not occurred. Similar results were obtained with E847(186), that is AB2528(186) cured of its λ prophage, and JP190 (which, anyway, is λ resistant) indicating that the presence of the λ prophage is not affecting the results. In any case, AB2528 transfers λ as a late marker. However all these results are not entirely satisfactory due to the poor transfer of att186 and adjacent markers by the O-13 male.

Similar experiments using the male KL16(186), which transfers the 50 min region earlier than does AB2528, confirm that the level of tyr⁺ recombinants is the same for JP190 and JP190(186) [Table 6-III, lines 8,9], and again the plaque activity with JP190 (line 8) is less

than the transfer of tyrA, although it is substantially above the background titre (line 7). This increase in plaque activity will be explained later (6.3.c.ii).

Again JP190tyr⁺ recombinants show high cotransfer of the male prophage with tyrA (Table 5-I, line 4).

Prophage 186cIts is also not zygotically inducible, as it behaves exactly as 186 above, allowing the difference in efficiency of transfer since the experiment was performed at 30° (Table 6-III, lines 10-12). The analysis of the tyr⁺ recombinants from this experiment (Table 5-I, lines 5,6) shows that the cotransfer of 186cIts with tyrA is the same whether the recipient is lysogenic or not. Thus there is no loss of tyr⁺(186cIts) recombinants when the female is not lysogenic, that is, there is no abortive zygotic induction of 186 as described by Kelly (1963) for P2. In addition 186susK4, K6 and K9 show similar high linkage to tyrA in conjugations, again whether the recipient is lysogenic or not (Table 5-V), indicating that these 186 mutants obtained from a different source also do not show abortive zygotic induction.

Another manifestation of zygotic induction is disturbance of the gradient of transfer of bacterial markers. The gradient of transfer of pheA, purF, and his from donor KL16(186) is the same whether the recipient is lysogenic or not (Table 6-IV). As well as the recovery of phe⁺ recombinants, their composition is the same.

TABLE 6-IV: LACK OF EFFECT OF PROPHAGE 186 ON THE GRADIENT OF TRANSFER OF pheA, purF and his BY Hfr KL16 to F⁻ AT2092^a

Donor	Recipient	<u>phe</u> ⁺ <u>str</u> ^r recombinants ^b /100 Hfr cells	Analysis of <u>phe</u> ⁺ <u>str</u> ^r recombs			
			<u>purF</u> ⁺		<u>his</u> ⁺	
			fraction %		fraction %	
KL16	AT2092	33	52/89	58	25/89	28
KL16(186)	AT2092(186)	26	74/147	50	48/147	33
KL16(186)	AT2092	30	121/231	52	72/231	31

^aData obtained in conjunction with undergraduate practical class.

All crosses as described in Chapter 2. Mating mixtures contained 186 anti-serum ($K=3.6\text{min}^{-1}$).

^bAfter 60 min mating at 37°.

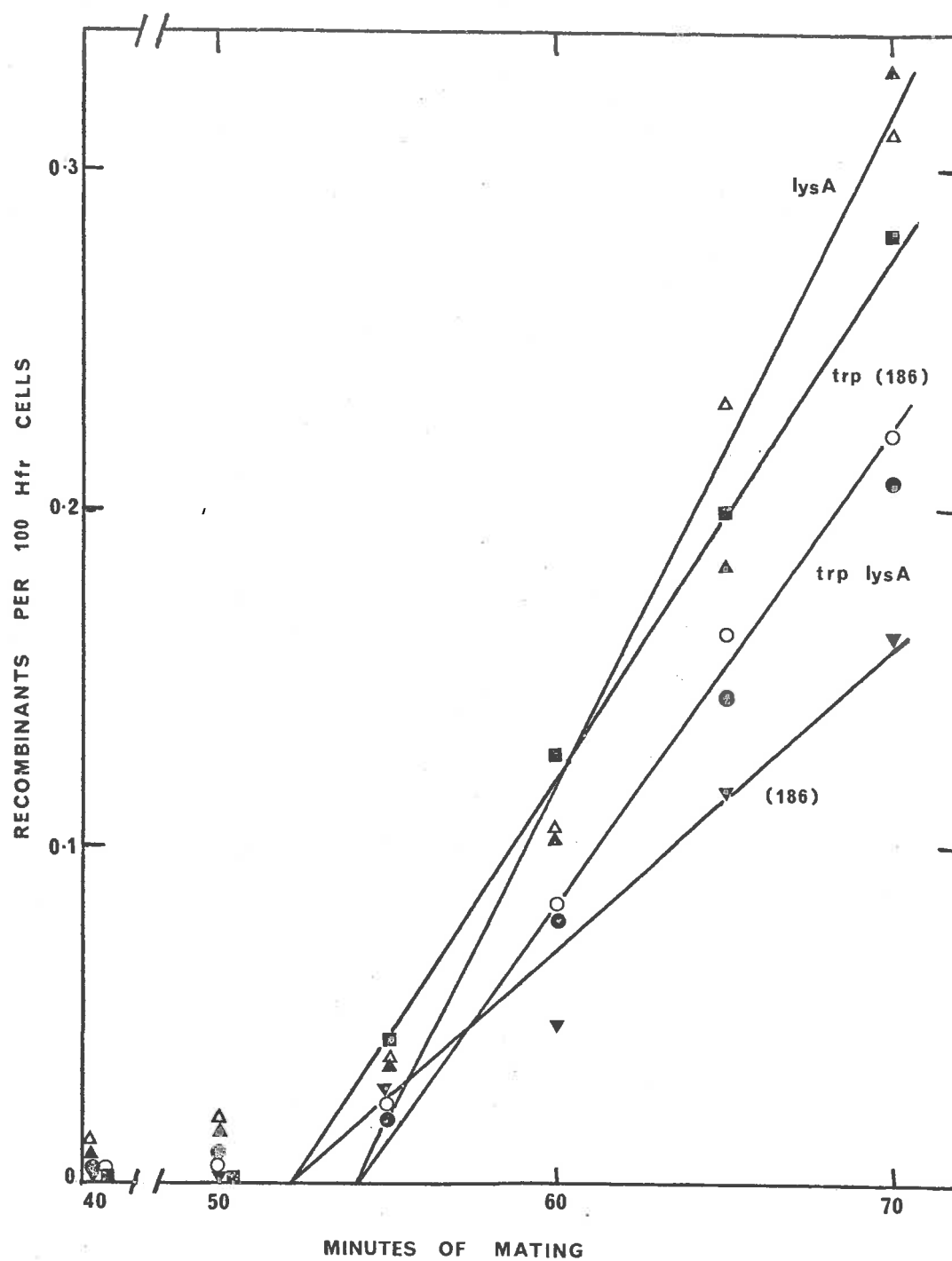
ii. Interrupted matings - The interrupted matings with Hfr KL16(186) as donor already described (5.3.b) also serve to illustrate that phage 186 is not zygotically inducible. For the bacterial markers involved (tyrA, pheA, purF and his) the same entry curves were obtained whether the female was lysogenic (not shown) or not. Where the female is not lysogenic the slope of the (186) entry curve is about one fifth that for the adjacent markers tyrA (Figure 5-2) and pheA (Figure 5-3). This is due to the method of assay for the (186) marker as previously described (5.3.b). Even when the 186 anti-serum was omitted from the mating mixture, although the basal level of 186 activity was much higher, the entry of (186) could be followed and had the same slope as in former experiments. Only one fifth of the 186 lysogenic recombinants from conjugation score as poor plaques, and do so by spontaneous phage production on the indicator plates. That is, the apparent increase in plaque activity on conjugation is due to plating lysogenic recombinants so they may score as infectious centres by spontaneous phage production. The plates used contain streptomycin, thus the male lysogen is unable to contribute to this expression. It must be stressed that the level of plaque activity seen (related to the concentration of Hfr cells initially present in the mating mixture) is much lower than the level of recombinants obtained for an adjacent marker.

Were 186 zygotically inducible, the level of plaque activity would be at least twice the level of recombinants found for an adjacent marker in an analogous cross where zygotic induction did not occur.

It might be argued that the direction of conjugation is important in zygotic induction - perhaps as the sense strand for some lytic phage functions enters before that for other phage functions. For this reason, zygotic induction of 186 was investigated in crosses involving the Hfr KL19 (cured of its λ prophage = E784) which transfers the male chromosome clockwise, that is in the opposite direction to KL16. The female X478 allows use of trp as an early marker and att186 and lysA as late and adjacent markers. In this experiment the mating mixture was diluted 1 part in 10 in fresh LGB containing 186 anti-serum after 5 min (6.2.a) in an endeavour to synchronise the mating. A greater dilution was not used since only low levels of lys⁺ recombinants were obtained. The entry curve for trp was virtually identical for donors E784 and E784(186). The entry curves for (186), lys, trp(186) and trp lys appear in Figure 6-1. The entry curves for lys and trp lys are virtually identical for both males, so 186 prophage in the male is not perturbing the transfer of a distal marker. The trp lys entry slope is much less than that for lys⁺ selection alone, since not all lys⁺ recombinants

FIGURE 6-1: Marker entry curves for lysA and trp lysA in an interrupted mating between E784 and X478 and for lysA, trp lysA, (186) and trp (186) in an interrupted mating between E784(186) and X478. Method as described in Section 6.2.a.

	<u>E784 x X478</u>	<u>E784(186) x X478</u>
<u>lysA</u> ⁺	Δ	▲
<u>trp</u> ⁺ <u>lysA</u> ⁺	○	●
(186)		▼
<u>trp</u> ⁺ (186)		■



are trp⁺. The prophage (186) enters about 4 min before lysA, as expected from their respective map positions (51 and 55min), and its slope is much less than that of lysA entry. As before this is due to the assay of (186) by spontaneous phage production. In this case, however, an absolute measure of (186) entry is available from the assay of trp⁺ recombinant colonies for 186 lysogeny. A comparison of the trp lysA and trp (186) entry curves shows them to have similar slope. That is, (186) behaves as a normal bacterial marker. It is not zygotically induced in either anti-clockwise (KL16) or clockwise (KL19) transfer.

d. Potential Zygotically Inducible Mutants of Phage 186 - These experiments were designed to test the alternatives suggested by Echols and Joyner (1968), that a phage may be not zygotically inducible through (i) transfer of the repressor during conjugation due to tight binding of it to the DNA, or (ii) rapid resynthesis of the repressor in the zygote. It was reasoned that conjugation of a prophage carrying a suppressible mutation in the cI gene from an su⁺ male into an su⁻ female should result in zygotic induction if repressor resynthesis is required immediately in the female. That is, every prophage transferred should show phage activity.

The male strains used were all derived from KL16. This su⁻ strain was made su⁺ by lysogenisation with the specialised transducing phage ϕ 80^{sup}F to yield strain E817. Prophage 186 or

186suscI53 (isolation, section 3.3.a) was then introduced. The female strains were all derived from 594, into which was transduced the his marker to yield strain E828. Again this su⁻ strain was made either, (i) su⁺ by lysogenisation with $\phi 80_{\text{supF}}$, or (ii) $\phi 80$ resistant by selection of such a spontaneous mutant, or (iii) $\phi 80$ immune by lysogenisation with $\phi 80$, or (iv) 186 immune by lysogenisation with 186, or various combinations of these. The derivation of these various strains is described in Chapter 2. Phage 186suscI53 makes clear plaques on E828, and turbid plaques on E817 and E828($\phi 80_{\text{supF}}$).

The occurrence of zygotic induction was checked in various crosses between E817 and E828 derivatives using the interrupted mating technique (6.2.a) as was done for the wild type phage (6.3.c.ii). A one hundred fold dilution after 5 min served to synchronise the mating pairs. The results appear in Figures 6-2, 6-3, and 6-4 for the entry of his and (186). An analysis for 186 prophage carried by his⁺ recombinant colonies selected after 60 min mating is shown in Table 6-V. A quick inspection shows that in all crosses his enters at 22-23 min, in agreement with literature values (Taylor and Trotter, 1967). As before [Figure 5-3], attl186 enters at 10 min with this male. Two levels of his⁺ recombinants are found, which for convenience shall be termed high and low, the low value being about one half the high value. The initial rate of (186) or (186suscI53) entry is the same in all cases and about one third that of the high level his entry, as before (Figure 5-3). The final level of plaque activity is similar in all cases and about one fifth that of

FIGURE 6-2: Marker entry curves for his and (186suscI53) in interrupted mating experiments between E817 and E828 ϕ 80^r, and between E817(186suscI53) and E828(ϕ 80). Method as described in Section 6.2.a.

	Cross 1 E817xE828 ϕ 80 ^r	Cross 5 E817(186 <u>suscI53</u>)xE828(ϕ 80)
<u>his</u> ⁺	●	○
(186 <u>suscI53</u>)		△

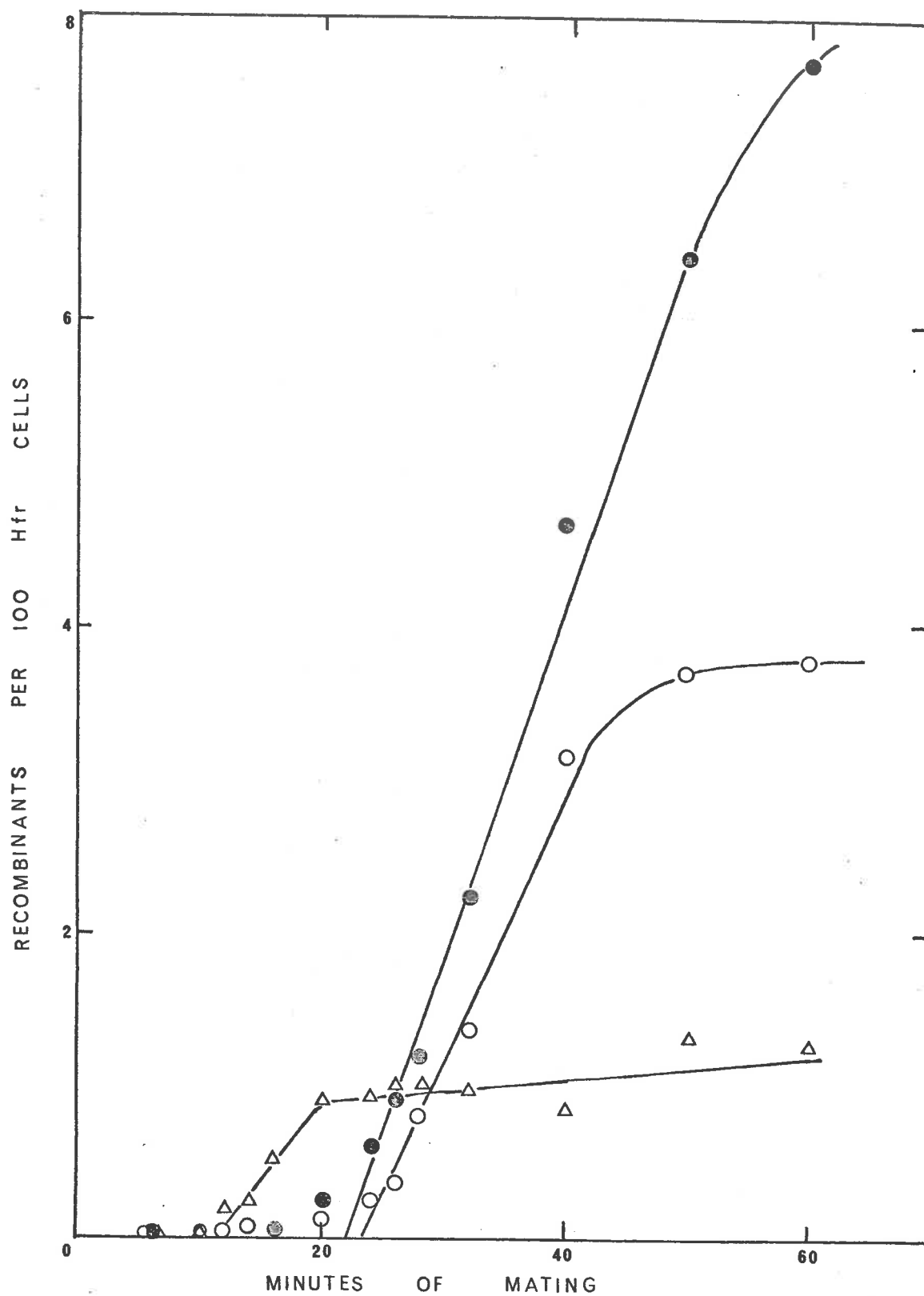


FIGURE 6-3: Marker entry curves for his and (186) in interrupted mating experiments between E817(186) and E828 ϕ 80^r, and between E817(186suscI53) and E828(186) ϕ 80^r. Method as described in Section 6.2.a.

	Cross 2 E817(186)xE828 ϕ 80 ^r	Cross 6 E817(186 <u>suscI53</u>) x E828(186) ϕ 80 ^r
<u>his</u> ⁺	○	●
(186)	Δ	

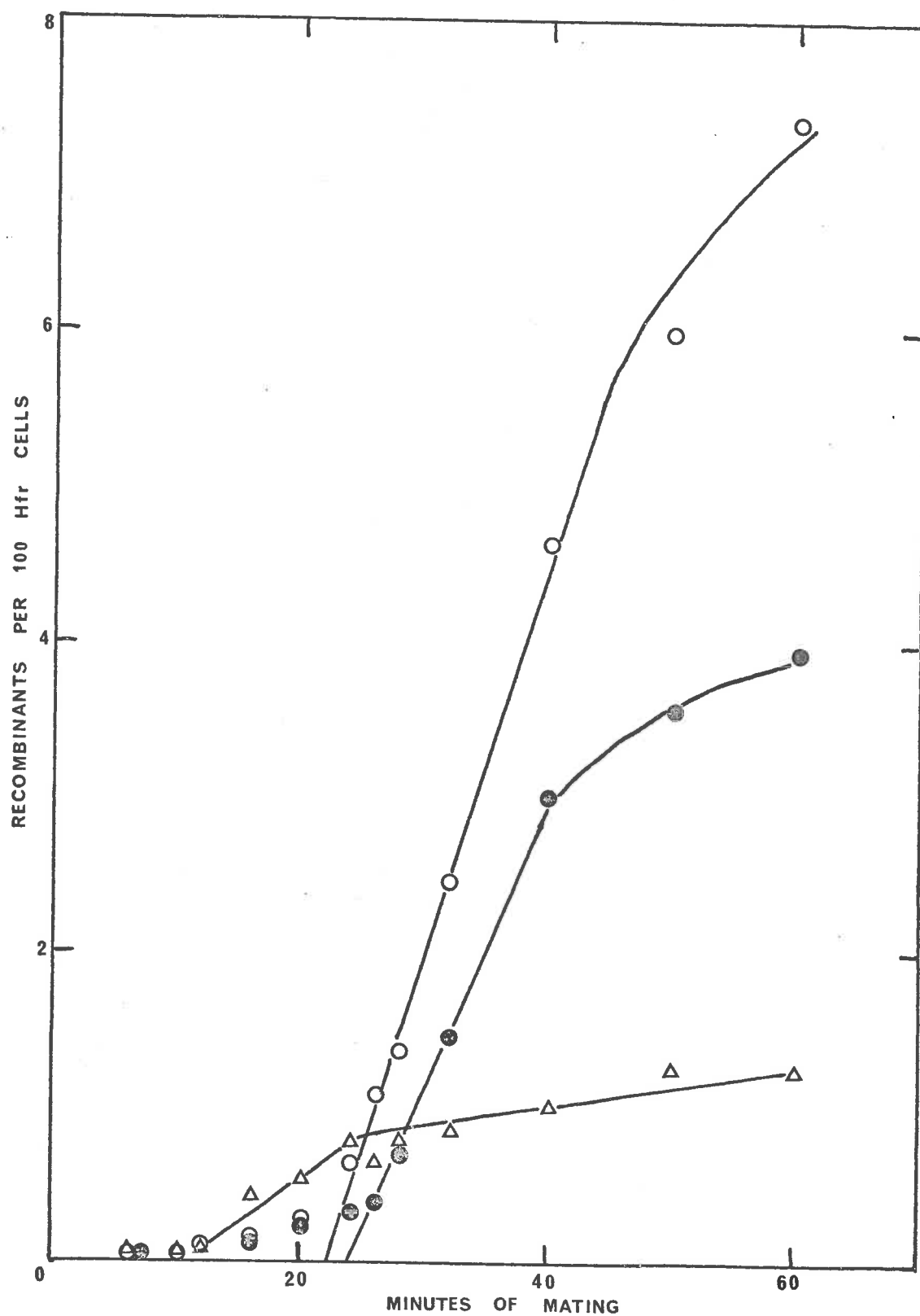


FIGURE 6-4: Marker entry curves for his and
 (186suscI53) in interrupted mating experiments be-
 tween E817(186suscI53) and E828 ϕ 80^r, or E828(ϕ 80supF).
 Method as described in Section 6.2.a.

	Cross 3	Cross 4
	E817(186 <u>suscI53</u>)	E817(186 <u>suscI53</u>)
	^x	^x
	E828(ϕ 80 <u>supF</u>)	E828 ϕ 80 ^r
<u>his</u> ⁺	●	○
(186 <u>suscI53</u>)	▲	△

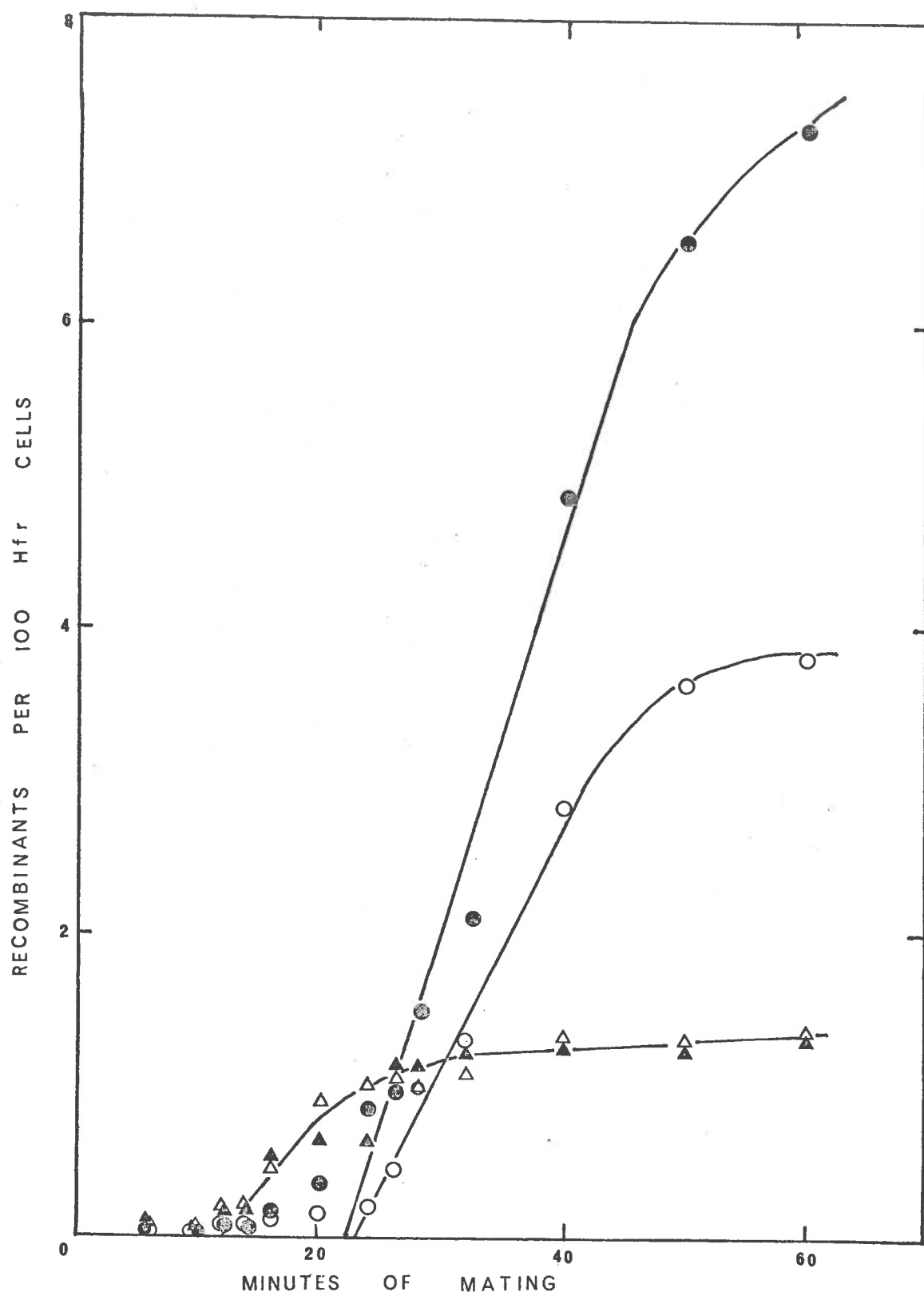


TABLE 6-V: ANALYSIS OF his^+ RECOMBINANT COLONIES SELECTED AT 60 MIN FROM INTERRUPTED MATINGS BETWEEN E817 and E828 DERIVATIVES^a

Donor	Recipient	Analysis of his^+ recombinant colonies ^b			
		Level ^c (186)	(186 <u>suscI53</u>)	186 ⁻	% donor prophage
1.E817	E828 ϕ 80 ^r	high	-	-	-
2.E817(186)	E828 ϕ 80 ^r	high	48	52	48
3.E817(186 <u>suscI53</u>)	E828(ϕ 80 ^{supF})	high	-	43	57
4.E817(186 <u>suscI53</u>)	E828 ϕ 80 ^r	low	-	0	100
5.E817(186 <u>suscI53</u>)	E828(ϕ 80)	low	-	0	100
6.E817(186 <u>suscI53</u>)	E828(186) ϕ 80 ^r	low	100	0	0

^aThe interrupted matings are shown in Figures 6-2, 6-3 and 6-4.

^bThe prophage states are indicated; (186), lysogenic for 186, (186suscI53), lysogenic for 186suscI53, and 186⁻, non-lysogenic for 186.

^cRefers to the level of his^+ recombinants found in the interrupted mating, as discussed in the text.

the high level his⁺ recombinants. Now each cross will be considered in turn.

The cross between E817 and E828 ϕ 80^r (Figure 6-2, cross 1), where no 186 prophage is involved, shows the high level of his⁺ recombinants, so this is the unperturbed level. When the male is lysogenic for 186, that is E817(186) x E828 ϕ 80^r (Figure 6-3, cross 2), the level of his⁺ recombinants is unaffected and high, and 48% of these recombinants carry the male 186 prophage (Table 6-V, line 2). Thus the male 186 prophage has not affected the transfer of a distal marker and the rise in phage activity is much less than the transfer of even this distal marker. As discussed before (6.3.c.ii) these type of results indicate that zygotic induction has not occurred. Exactly similar results are obtained in the cross E817(186suscI53) x E828(ϕ 80^{supF}) [Figure 6-4, cross 3; and Table 6-V, line 3] indicating that where the 186suscI53 male prophage can synthesise repressor in the su⁺ female, no zygotic induction occurs.

However, when the male prophage is unable to synthesise repressor in the female different results are obtained. In the cross E817(186suscI53) x E828 ϕ 80^r, only the low level of his⁺ recombinants is obtained (Figure 6-4, cross 4) and none of them carry the male prophage (Table 6-V, line 4). Nevertheless, there is no dramatic increase in the plaque activity (Figure 6-4). It is clear that half the his⁺ recombinants, being that half which are also recombinant at att186 and thus carry the male prophage, cannot score as colonies. They also do not score as infectious centres. Exactly the same results are obtained where the su⁻

female is immune to $\phi 80$, in the cross E817(186suscI53) x E828($\phi 80$) [Figure 6-2, cross 5 and Table 6-V, line 5] indicating that the $\phi 80$ supF male prophage is not affecting the results by transfer and zygotic induction.

These results are explained by the cross E817(186suscI53) x E828(186) $\phi 80^r$ where, even when the female is immune to 186, the same perturbations are found. That is, the low level of his⁺ recombinants is obtained (Figure 6-3, cross 6) and none of these carry the donor prophage (Table 6-V, line 6). Thus the half of the his⁺ recombinants also recombining the male attI186, and thus prophage 186suscI53, into their chromosomes are killed by subsequent expression of this phage which is unable to direct the synthesis of functional repressor in the su⁻ cell. This expression must not occur immediately since there is no increase in infectious centres scoring on indicator bacteria - that is, it occurs later when the indicator is no longer able to display a plaque. The delay in expression of the 186suscI53 prophage in the su⁻ female could be caused by transfer of repressor from the male during conjugation, and this is subsequently diluted out by division. The transfer of repressor would explain why not every his⁺ recombinant is lost, although these all must have received the 186suscI53 prophage from the male, whether or not it is subsequently recombined into the female chromosome. It would also explain the lack of increase in plaque activity.

Alternately, phage 186 may be unable to perform an essential function of its lytic cycle in a zygote immediately following or during conjugation. This would explain lack of activity from

186suscI53 transferred but not recombined into the su⁻ female's chromosome, and a delay to activity from (186suscI53) recombinants, whether or not the repressor is transferred. As shall be seen in the following sections, prophage 186 is unable to induce immediately following conjugation.

e. Induction of Prophage 186 Following Transfer by Conjugation - The effect of UV irradiation, immediately after interruption, on the recovery of his⁺ recombinants or plaque activity in the above matings was investigated, using a dose of 760 ergs/mm² under the standard conditions. The results are shown in Table 6-VI. The his⁺ recombinants are all similarly sensitive (lines 1, 2 and 3) and no more sensitive than non-conjugating cells (e.g. Figure 4-4). Since the 186 infectious centres are ascribed to spontaneous phage production by 20% of the 186 lysogenic recombinants, a dose of 50 sec irradiation should increase the plaque activity by 3 to 4 times, since a 50 sec dose of UV displays infectious centres from 75% of 186 lysogens present (Figure 4-3). To the contrary, it is decreased by the irradiation even more severely than is the level of his⁺ recombinants (Table 6-VI, lines 2 and 3). The decrease is apparent from early after (186) entry till at least 50 min after its transfer. Thus prophage 186 is unable to be induced by UV irradiation to score as an infectious centre up to 50 min after its transfer by conjugation. The level of his⁺ recombinants is not abnormally sensitive to the irradiation, so that this is not a general effect on cell viability, but appears to be limited to an affect on the ability of prophage 186 to score as an infectious centre following UV irradiation, in this situation.

TABLE 6-VI: EFFECT OF UV IRRADIATION ON his⁺ AND (186) SCORED
IN INTERRUPTED MATINGS BETWEEN E817 AND E828
DERIVATIVES^a

Donor	Recipient	min of mating	Recombinants surviving 50 sec UV ^b			
			<u>his</u> ⁺		(186) ^c	
			fraction	%	fraction	%
1.E817	E828 ϕ 80 ^r	28	33/110	30	-	-
		60	149/704	21	-	-
2.E817(186)	E828 ϕ 80 ^r	28	37/292	13	14/167	8
		40	271/1135	24	16/215	7
		50	300/1460	21	18/268	7
		60	299/1576	19	17/270	6
3.E817(186 _{susc} I53)	E828 ϕ 80 ^r	28	21/86	24	6/86	7
		60	104/338	31	7/121	6

^aMethod described in Section 6.2.b. Results of interrupted matings shown in Figures 6-2, 6-3 and 6-4.

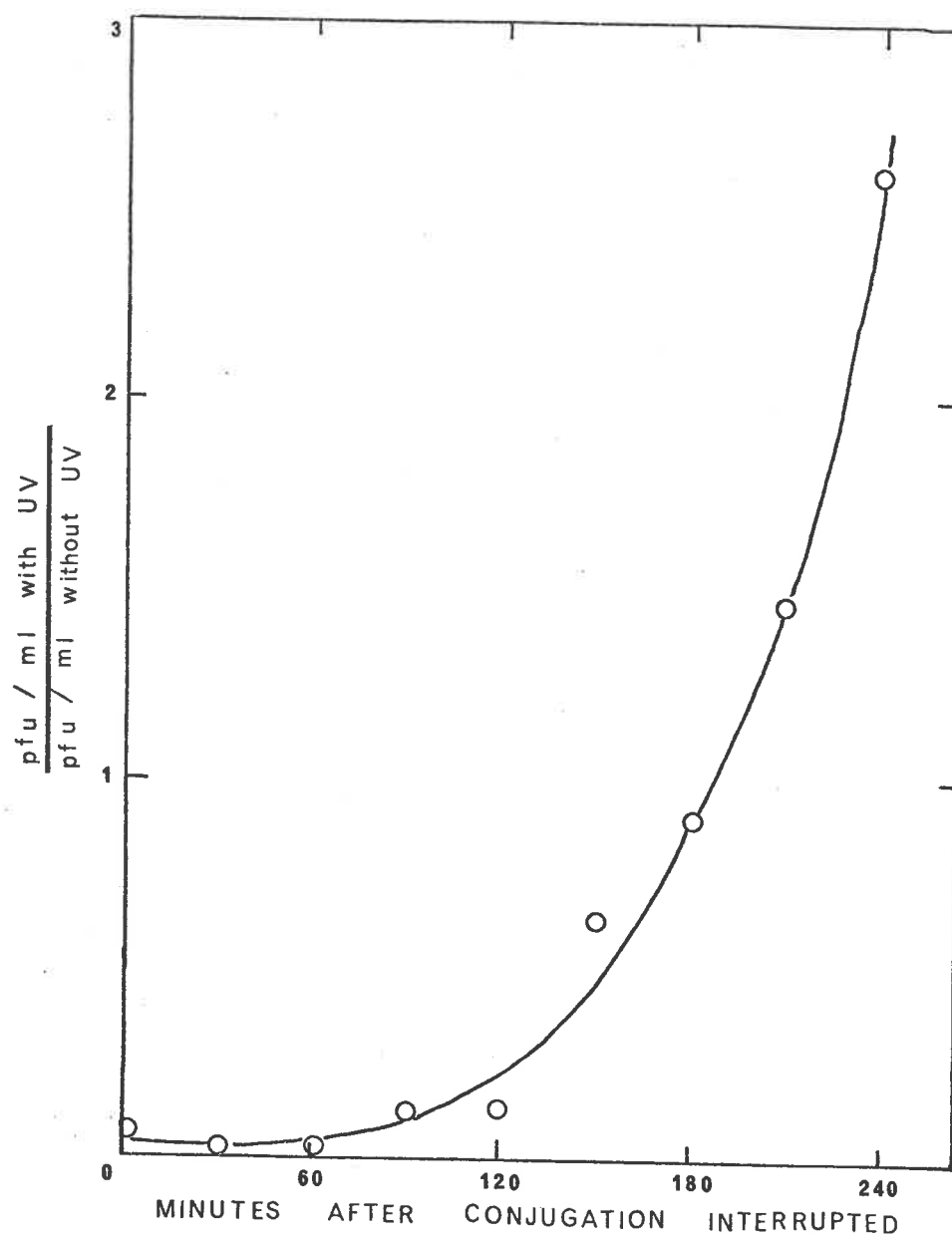
^bFigures are from duplicate platings, one sample being irradiated before plating. The results are expressed as the titre obtained with irradiation over the titre without irradiation. The same dilution was used for all his⁺ and (186) assays, thus the numbers are directly comparable within each cross. They have not been normalised from cross to cross as to the number of Hfr donor cells.

^c(186) assayed only by plating for infectious centres.

Previously it was found that purified lysogenic recombinants that had received their prophage via conjugation were capable of displaying UV induction (6.3.c) so the defect is not a permanent one. The recovery of capability to display 186 infectious centres, following irradiation, by females made lysogenic by 30 min conjugation between E817(186) and E828/80^r is shown in Figure 6-5. Until 150 min after conjugation is interrupted, the irradiation causes a drop in the number of infectious centres. From this time the recovery is slow until, at 240 min, a more normal increase in plaque activity on irradiation (UV induction) is seen.

Since it has already been shown that UV irradiation delays the 186 latent period (4.3.g) it was important to check the susceptibility of a prophage, recently transferred by conjugation, to another form of induction. As described in Section 6.2.d, the latent period of heat induction of 186cIts following transfer from KL164(186cIts) to E828 during 60 min conjugation at 30°, was determined. The mating mixture was interrupted and diluted into fresh broth at 39°, which is sufficient to induce 186cIts, then assayed with time for plaque activity. The use of E828 (=594 his) as female allowed assay of conjugation (3% transfer of his⁺ at 90 min uninterrupted). At interruption the transfer of (186cIts) as assayed by infectious centres at 30° was 2.5%. A control culture of broth plus 594(186cIts) at a similar initial concentration (colony formers/ml) to the donor in the mating mixture was carried through the whole procedure. The results of

FIGURE 6-5: Recovery of UV inducibility after transfer of prophage 186 from Hfr E817(186) to F⁻ E828/80^r by 30 min conjugation at 37°. Method as described in Section 6.2.c.



phage assays after the interruption and dilution are shown in Figure 6-6. It is apparent that the control culture exhibits a latent period similar to that found before in this strain (Figure 4-2). However the latent period of 186cIts heat induction is increased by about 80 min following transfer by conjugation.

f. Induction of Prophage 186 in a Female Lysogen Following Conjugation - The above section dealt with prophage 186 transferred into the female by conjugation. The same lack of demonstrable UV induction occurs when the prophage is carried by the female. These conjugations involved mating ratios of 1 male to 1 female to ensure that as many as possible of the female lysogens were involved in conjugation. A higher ratio of males to females would lead to killing of females by lethal zygotis, as described by Jacob and Wollman (1961). After 10 min conjugation, the matings were interrupted and the phage titre (representing infectious centres) determined immediately with and without UV irradiation as described in Section 6.2.e. The results appear in Table 6-VII. It is clear that in all crosses with an active male involving 186 prophage that there is only a slight increase in phage titre on irradiation (lines 1-4), most probably arising from female lysogens that were not involved in conjugation. This occurs even when Hfr AB259 is involved, where there is clearly no transfer of att186 in the 10 min mating allowed. When a male killed with streptomycin, a tra⁻ (Skurray and Reeves, 1973) male (that is a male incapable of forming recombinants; no recombinants found at 10⁻⁵ level obtained with AB259) or no male is used, a

FIGURE 6-6: Latent period of heat induction for 186cIts following transfer during conjugation at 30° between Hfr KL164(186cIts) and E828. At 60 min conjugation was interrupted, the mixture diluted, and incubated at 39°. Method described in Section 6.2.d.

- 594(186cIts) + broth, control
- KL164(186cIts) x E828

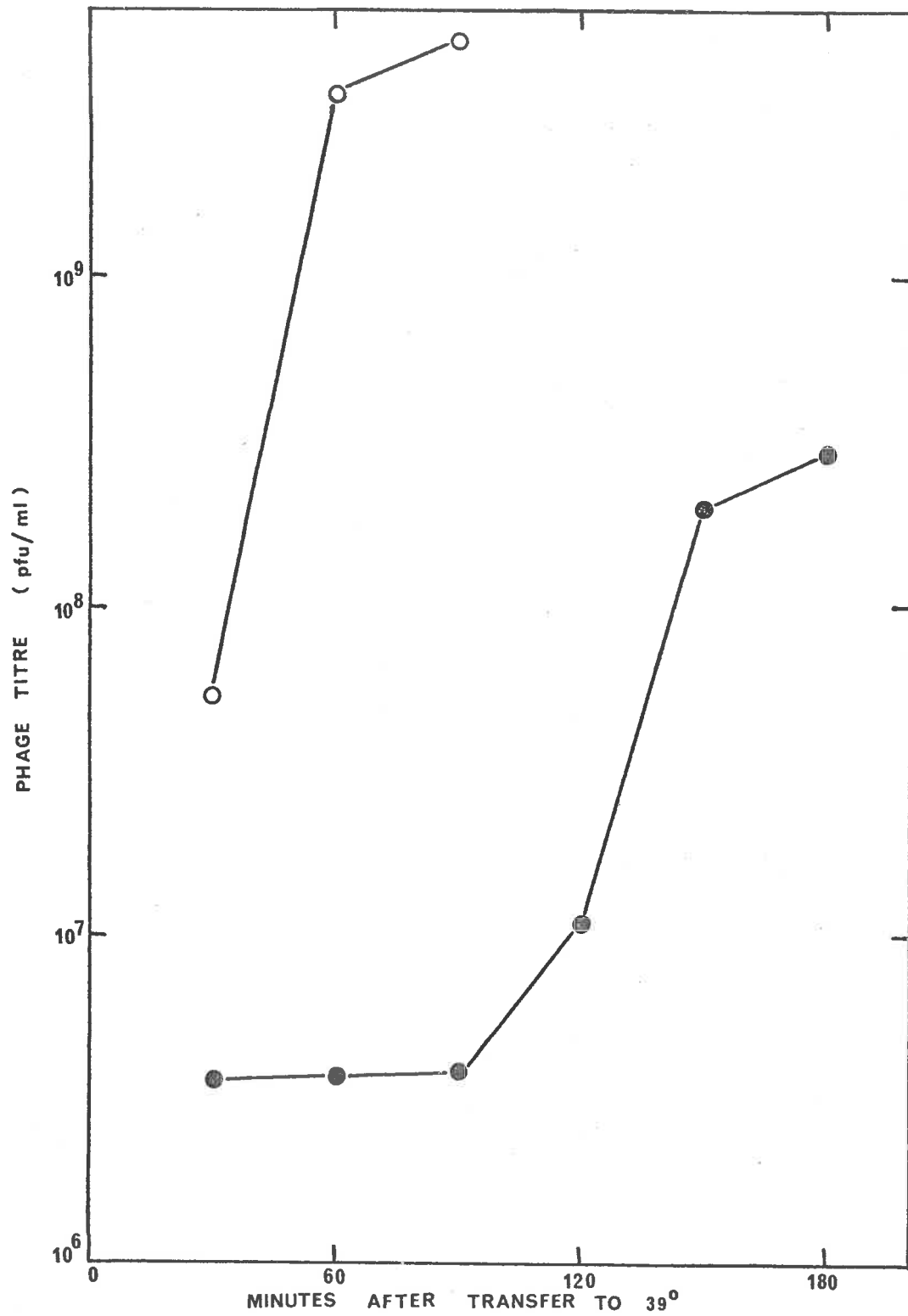


TABLE 6-VII: INDUCTION OF λ , ϕ 80 AND 186 IN FEMALE LYSOGENS
RECENTLY INVOLVED IN CONJUGATION^a

Donor	Recipient	Phage titre (pfu/ml) ^b		Titre with UV/titre without UV
		without UV	with UV ^c	
1. E817(186)	E828(186) ϕ 80 ^r	2.4×10^7	3.8×10^7	1.6
E817(186)+strep ^d	E828(186) ϕ 80 ^r	2.3×10^7	8.7×10^7	3.8
broth	E828(186) ϕ 80 ^r	2.5×10^7	9.2×10^7	3.7
2. AB259	X478(186)	3.2×10^7	4.8×10^7	1.5
P980	X478(186)	1.7×10^7	7.4×10^7	4.4
broth	X478(186)	2.4×10^7	7.3×10^7	3.0
3. AB259	JP190(186)	1.6×10^7	2.8×10^7	1.8
P980	JP190(186)	1.3×10^7	4.9×10^7	3.8
broth	JP190(186)	1.2×10^7	5.4×10^7	4.5
4. AB259	C600(186)	4.2×10^7	6.6×10^7	1.6
P980	C600(186)	2.5×10^7	9.0×10^7	3.6
broth	C600(186)	2.5×10^7	1.0×10^8	4.0
5. AB259	C600(λ)	7.3×10^7	3.0×10^8	4.1
P980	C600(λ)	4.8×10^7	2.3×10^8	4.8
broth	C600(λ)	3.5×10^7	1.7×10^8	4.9
6. AB259	E828(ϕ 80)	2.4×10^7	1.2×10^8	5.0
AB259+strep ^d	E828(ϕ 80)	1.3×10^7	7.5×10^7	5.8
P980	E828(ϕ 80)	1.7×10^7	9.4×10^7	5.5
broth	E828(ϕ 80)	1.4×10^7	8.5×10^7	6.1

^aMethod as described in Section 6.2.e.

^bAssayed immediately after interruption of 10 min mating at 37°. Lines 1-4, 186 phage activity; line 5, phage λ activity; and line 6, ϕ 80 activity.

^cDose 760 ergs/mm², immediately after interruption of mating.

^d10 min pretreatment of male with streptomycin (200 μ g/ml).

much greater increase is seen. For phages λ and $\phi 80$ (lines 5 and 6) the increase in titre is similar whether the females have been involved in conjugation, or not. Thus, a 186 prophage in a female involved in conjugation is not capable of forming an infectious centre on UV irradiation. This effect is unique to 186, since λ and $\phi 80$ are perfectly capable of UV induction in these circumstances. The effect on 186 activity is seen in all four females tested. The four strains used were chosen as typical of the range of 186 indicators that have been used in this work.

g. Behaviour of Prophage 186 in F'-mediated Conjugation.

i. Zygotic induction - Phage 186 lysogens were prepared in the F' strain CGSC4292 which carries the Fl43-1 episome. This episome covers att186, tyrA and recA. In this particular strain both episome and host are mutant in recA. Conjugation of CGSC4292 and its 186 lysogens were carried out with JP190 under the standard conjugation conditions described in Chapter 2. On minimal plates to assay tyr⁺ recombinants the male could be selected against since it requires tryptophan and uracil for growth, whereas JP190 does not. It was not necessary to kill the male when assaying 186 plaque activity because CGSC4292 is phenotypically recA and thus lysogens only produce a low level of phage spontaneously (4.3.e). The results are shown in Table 6-VIII. The transfer of tyrA⁺ is similar in

TABLE 6-VIII: LACK OF ZYGOTIC INDUCTION OF PHAGE 186 IN CROSSES
BETWEEN F⁺ CGSC4292 AND F⁻JP190^a

Donor	Recipient	$\frac{\text{tyr}^+}{100}$	recombinants Hfr cells ^b	186 Hfr cells ^b	plaques/100 cells ^b
1.CGSC4292	JP190		1.4		-
2.CGSC4292(186)-3	JP190		1.5		0.34
3.CGSC4292(186)-3	alone		<0.02		<0.02
4.CGSC4292(186)-4	JP190		1.4		<0.02
5.CGSC4292(186)-4	alone		<0.02		<0.02

^aConjugation conditions as described in Chapter 2.

^bAssayed after 30 min mating at 37°.

all cases (lines 1, 2 and 4), so there is no loss of recombinants due to zygotic induction. The tyr⁺ recombinants from isolate 3 were all found to be lyso-genic for 186 (167/167) whereas those from isolate 4 were all sensitive to 186 (129/129). It is apparent that in isolate 4 the prophage is carried at the chromosomal attachment site and is not transferred by conjugation. In isolate 3 a 186 prophage is carried at the episomal 186 attachment site and is transferred to all tyr⁺ recombinants, as is expected through the transfer of the F' episome. The transfer leads to a level of expression of 186 activity (Table 6-VIII, line 2) about one fifth that of the transfer of the adjacent marker tyrA. This is just as was found in Hfr conjugations (6.3.c) and can again be attributed to spontaneous phage production from the recombinants. Thus 186 is not zygotically induced upon transfer by an F' episome into a sensitive female.

ii. Induction of prophage 186 in a female lysogen following conjugation - The male used was P601, cured of its λ prophage (=E873), which harbours an F'gal⁺. This experiment was done concurrently with the indirect induction experiment to be described in the following section. The method is described in Section 6.2.f. The male was mated with various gal⁻str^r recipients for 20 min at 37° and the mating mixture assayed for plaque activity with and without UV irradiation. The

results appear in Table 6-IX. The irradiation does not greatly increase the plaque titres for 186 lyso-gens (lines 1 and 3), about 20% only of the F⁻ lyso-gens scoring as plaques. That is, 186 is not UV inducible in females involved in F'-mediated conjugation. To the contrary, ϕ 80 is capable of UV induction in such a host (line 5). Similar results are seen when the F' has been irradiated before conjugation (lines 2, 4 and 6). Although this decreases the colony forming ability of the F' cells (17% survival) and the ability of the F' to transfer its F particle, as judged by recovery of gal⁺str^r recombinants (down at least 4 fold), the F' cells are still able to interfere with 186 expression in the female.

iii. Indirect induction of phage 186 - This is the same experiment as described above. The comparison to be drawn from the data in Table 6-IX is between the level of phage activity where the male is UV irradiated and the level where the UV stimulus is not supplied via the transfer of a UV irradiated Fgal⁺ episome. For prophage 186, irradiating the male has little effect on the level of plaque activity (lines 1 and 2; 3 and 4), whereas the λ -related prophage ϕ 80 does display a 2.5 fold increase in activity where the male is irradiated (lines 5 and 6). That is, ϕ 80 is indirectly inducible, and 186 is not to any marked extent. This is perfectly in accord with the finding above that 186 cannot display induction

TABLE 6-IX: LACK OF INDIRECT INDUCTION OF PHAGE 186 IN CROSSES
BETWEEN F⁺ E873 AND VARIOUS RECIPIENTS^a

Donor	Recipient	gal ⁺ str ^r recombs ^b /100 F ⁻ cells	plaques ^b /100 F ⁻ cells	plaques ^b /100 F ⁻ cells with UV ^d
1.E873	E828(186)ø80 ^r	43	16	20
2.E873+UV ^c	E828(186)ø80 ^r	11	21	24
3.E873	JP190(186)	53	16	21
4.E873+UV ^c	JP190(186)	7	19	27
5.E873	E828(ø80)	45	15	60
6.E873+UV ^c	E828(ø80)	10	37	57

^aMethod as described in Section 6.2.f.

^bAssayed after 20 min mating at 37°. Lines 1-4, 186 plaques;
 lines 5 and 6, ø80 plaques.

^c760 ergs/mm² under the standard conditions, before mating
 (survival 17%).

^d760 ergs/mm² under the standard conditions, after 20 min
 mating.

in a female involved in F'-mediated conjugation. For $\phi 80$, the increase in plaque activity due to indirect induction can be augmented by direct UV induction (line 6).

6.4 DISCUSSION

a. Phage λ - The conjugations involving phage λ (6.3.b) served to establish conditions for zygotic induction experiments. In view of the inability to select a 186 resistant mutant where the defect was due to lack of adsorption (6.3.a), it was also necessary to establish conditions for zygotic induction experiments using phage sensitive females. This was done with the Hfr AB259(λ) x F⁻ AB1133 conjugations. A similar increase in phage titre was found here to that when F⁻ AB2102, which is λ resistant, was used. In addition the increase in phage titre with F⁻ AB1133 was followed in an interrupted mating and found to start at about 30 min - the time that att λ is transferred by HfrH (Jacob and Wollman, 1961) - and to be chloroform sensitive at that time. Thus the increase represents newly formed infectious centres, and not free phage liberated from infection of the λ sensitive female by free phage from the male culture on mixing the males and females.

Zygotic induction was confirmed for λ and λ_{ind}^- by, (i) a great increase in phage titre with the non-immune female, relative to the transfer of pro⁺ in a cross where both partners were lysogenic, and (ii) a decrease in pro⁺ recombinants where zygotic induction was possible.

The low level of trp⁺ λ lysogenic recombinants from a cross where zygotic induction was possible also confirms the high efficiency of λ zygotic induction. However the two such prophages found were able to zygotically induce, and there had been no permanent breakdown in the λ resistance of the recipient. Thus it appears that these recombinants are in no way unique, but could represent a low level of lysogeny following transfer of the λ DNA into the non-immune recipient. Nevertheless, λ DNA entering a sensitive female by conjugation is much less likely to enter the lysogenic cycle than is λ DNA entering by infection (frequency of λ lysogenisation c.20%). Why this is so is not clear. However, cyclic AMP (cAMP) and the catabolite activator protein (CAP) do aid in establishing λ lysogeny (Grodzicker, Arditti and Eisen, 1972). If either of these components was lacking or in short supply in a conjugating female, the frequency of lysogenisation of the incoming phage DNA would be reduced. That is, most prophage conjugated into a non-immune female would enter the lytic pathway and show zygotic induction. In this context it is interesting that Dubnau and Maas (1969) found a variable lag in the induction of β -galactosidase, by addition of the inducer thiomethyl- β -galactoside, following transfer of the lacZ⁺ gene into a lacZ female by Hfr mediated conjugation. This lag could be shortened by incubating the zygotes for 30 or 60 min after conjugation had been interrupted before adding the inducer. Since cAMP and CAP are also required for transcription of the lac operon (deCrombrugge *et al*, 1971) it may be lack of one or both of these that causes the delay in

β -galactosidase induction in the recently conjugating female. This temporary lack of either cAMP or CAP does not affect the rate of β -galactosidase synthesis achieved later (in a rec⁺ host; Dubnau and Maas, 1969), but would be responsible for channelling phage λ into the lytic rather than the lysogenic cycle. This model can be tested by determining the frequency of lysogenisation of phage λ infecting a conjugating female in relation to the ability of that female to provide cAMP and CAP.

b. Phage 186 - The results presented in Section 6.3.c indicate that phage 186 does not display zygotic induction, on any of the three counts mentioned in the introduction to this chapter. Firstly, the percentage transfer of 186 to a sensitive female, as measured by infectious centres (as zygotic induction of λ is assayed) is much less than the recovery of recombinants for an adjacent bacterial marker in experiments where zygotic induction is not possible. Secondly, recombinants for adjacent and, in particular, distal markers are recovered at similar frequencies whether only the male, or the male and the female, or neither, is lysogenic for phage 186. In addition these recombinants are identical in their composition with respect to unselected bacterial markers. Thirdly, lysogeny is inherited by recombinants just as any other bacterial marker. Thus a potential 186 zygotic induction situation has no effect on the number or nature of bacterial recombinants.

c. Why is Phage 186 Not Zygotically Induced? Several theories have been advanced concerning non-inducible phages.

Jacob and Wollman (1958) suggested that the location of the inducible phages on the right side of the E. coli map, with the non-inducible phages in a separate group on the left side, may be significant. However, the non-inducible phage P2 can attach on the right side of the map (site II in E. coli K12; Calendar and Lindahl, 1969) between the attachment sites of inducible phages 424 and 466 (Jacob and Wollman, 1961). Signer (1966) proposed that the method of attachment of the prophage to the host chromosome could be important. In this context, the type of synapsis proposed for phage 18 by Jacob and Wollman (1959) does not occur for phage 186, the integration of which has similar effects on the linkage of spanning markers as does integration of an inducible phage (5.3.d).

Phage 186 does not display abortive zygotic induction as reported for P2 (Kelly, 1963) in that bacterial recombinants carry the donor 186 prophage with equal frequency whether or not the female is immune to phage 186 (6.3.c.i and Table 5-I, lines 5 and 6). Thus there is no evidence for loss of prophage 186 following its transfer by conjugation. That both clockwise and anti-clockwise conjugation fail to zygotically induce prophage 186 (6.3.c.ii) indicates that the order of entry of the 186 prophage genes cannot influence the expression of that prophage. In fact it appears that prophage 186 cannot display expression of the lytic cycle in a female immediately following conjugation.

The inability of prophage 186 to display infectious centres induced by UV irradiation of a recently conjugating female applies

to both a prophage transferred into the female by conjugation (6.3.e) and a prophage already present in the female (6.3.f). That is, the state of the female is important, not just the derivation of the prophage. The lack of induction could be due to extensive killing of the female ex-conjugants by the irradiation. However, there is no more severe loss of his⁺ recombinants (Table 6-VI) than expected from the dose of radiation used. This lack of any peculiar sensitivity has been reported before for both UV (Wollman, Jacob and Hayes, 1956) and X irradiation (Johansen and Ustaheim, 1968) of conjugating females. In addition, prophages λ and ϕ 80 are capable of displaying UV induction under the same conditions (Table 6-VII) that prophage 186 cannot do so. Itoh and Tomizawa (1971) have also shown that prophage λ cIts1 is as capable of heat induction in a conjugating female as in the unmated lysogen. The use of the isogenic tra⁻ male or a male pretreated with streptomycin, does not affect the UV inducibility of the female 186 prophage (Table 6-VII). Thus the lack of inducibility when a normal male is used is due to the actual conjugation. The tra⁻ male lacks an F-pilus (R.A. Skurray, pers. comm.) so is unable to form mating pairs. It will be interesting to try the effects of male mutants which are able to form mating pairs, but unable to transfer donor markers. This may be the case with a proportion of the irradiated F' donors (6.3.g.ii).

The inability of 186 to display infectious centres following UV irradiation of the conjugating female is only temporary, being gradually recovered over 4 hours growth (Figure 6-5).

This effect of irradiation on the conjugating female is more severe than on a normal lysogen, where it only increases the λ 86 latent period by 30-45 min (4.3.g). The effect of conjugation alone is to increase the latent period of λ 86 cIts heat induction by 80 min (Figure 6-6). Thus the effect of conjugation plus UV, if additive, would be to delay the latent period about 120 min. In fact, as mentioned above, it takes till about 240 min after conjugation is interrupted before λ 86 lysogens are even capable of displaying infectious centres on UV irradiation. Thus it appears that the delays mediated by UV irradiation and conjugation are not independent, but probably involve the same factor or interacting factors.

The results obtained with F'-mediated conjugation (6.3.g) serve to confirm those involving Hfr donors. In a female recently involved in F'-mediated conjugation, prophage λ 86 is not capable of displaying an infectious centre on UV irradiation (Table 6-IX) whereas prophage ϕ 80 is capable of this. Thus it is not surprising that phage λ 86 introduced into a sensitive female by F'-mediated conjugation does not display zygotic induction (Table 6-VIII). In addition, prophage λ 86 does not display indirect induction in the system tested involving an F' donor, whereas prophage ϕ 80 is indirectly inducible (Table 6-IX). It will be interesting to see if prophage λ 86 may be indirectly induced by infection with UV irradiated phage P1, where conjugation is not involved, as reported for prophage λ (Rosner, Kass and Yarmolinsky, 1968).

The experiments described in Section 6.3.d were designed to determine if repressor resynthesis was required in the female to avoid 186 zygotic induction. The results obtained are now complicated by, and explicable in terms of, the lack of ability of phage 186 to express its lytic cycle immediately in an ex-conjugant female. As already discussed (6.3.d) the lack of zygotic induction of 186^{suscI53} on transfer to an su⁻ recipient could be explained sufficiently by either transfer of repressor from the male, or by the inability of 186 to express its lytic cycle in an ex-conjugant female (or both). Since the latter defect has been demonstrated it seems unlikely that repressor is transferred. Fisher (1962) has shown that the λ repressor is not transferred during conjugations involving Hfr donors, thus a non-specific transfer may be ruled out. To allow transfer of the 186 repressor during conjugation it is thus necessary to postulate that it remains tightly bound to its operator DNA during conjugation (even if single-stranded). In this way only one or two repressor molecules (depending on the number of repressor binding sites on the 186 DNA) could be transferred. It is unlikely that so few repressors could alone stop zygotic induction and, more particularly, maintain the 50% linkage between his and att186 in an su⁻ recipient. Consider the case of a his⁺ recombinant which also has recombined into its chromosome the donor att186, and thus 186^{suscI53} prophage with one or two repressor molecules bound. On division the prophage is replicated but not the repressor molecules. Thus control would be lost. If excision of the prophage occurred before the cell was

capable of displaying a 186 infectious centre, it too could be diluted out and lost, leaving a cured surviving recombinant. This does not happen; all the his⁺ recombinants that are deduced to have "recombined in" the 186suscI53 prophage (i.e. c.50%) are lost. In addition where the su⁻ recipient carries a 186 prophage, no cured recombinants are found. Thus the prophage is not excised soon after conjugation. Transferred repressor alone may be insufficient to stop excision, so the defect in an ex-conjugant female is most probably concerned with prophage excision. Such lack of excision explains why prophage 186 has behaved as a normal bacterial marker in all crosses studied. It also explains the lack of, or delay to, 186 induction in an ex-conjugant female. This model requires that de-repression of 186 DNA without excision from the host chromosome does not kill the host cell. This is true for other non-inducible phages, except the abortive heat induction of some P2 mutants, as discussed in the introduction to this chapter.

This chapter has demonstrated that phage 186 does not display zygotic induction since the prophage is unable to express its lytic cycle in a female after conjugation with an Hfr or F' donor. It is suggested that the prophage is unable to excise in such an host.

CHAPTER 7

GENERAL DISCUSSION.

The results presented in this thesis indicate that coliphage 186 has been incorrectly classified as non-inducible. It is capable of exhibiting induction upon UV irradiation of a 186 lysogen and is inducible by other inducers tested. This is surprising because of the close relationship between 186 and the non-inducible phage, P2. As summarised by Bertani and Bertani (1971), P2 and 186 phage particles have similar morphologies, their DNA molecules have similar molecular weight, and similar cohesive ends, and they are serologically related. Skalka and Hanson (1972) have shown by DNA hybridisation experiments that there are more homologous sequences between the DNA species of coliphage 186 and P2, than between those of 186 and λ .

Although prophage 186 is subject to UV induction, the latent period of 186 phage production is increased by UV irradiation. This is true for both infection of an irradiated cell and UV induction of a 186 lysogen. The effect is unique to 186, of the phage tested, since P2 and λ both have normal latent periods on infection of irradiated cells.

The identity of the function or functions causing the delay to the latent period of 186 in an irradiated cell is not known. The recA⁺ gene product is not involved in 186 phage production since heat induction of 186cIts has the same latent period in recA and recA⁺ hosts. It also seems unlikely that the rep⁺ function, required for growth of both 186 and P2 but not λ (Calendar et al, 1970), is disturbed in cells subjected to the low doses of UV radiation (456 ergs/mm²) used in this work, since

the latent period following P2 infection is unaffected. The report of irradiated cells being phenotypically rep (Matsubara et al, 1967) was concerned with much greater UV doses (12,000 ergs/mm²). However, it is possible that some function common to production of phage 186 and P2 following induction is perturbed. This is indicated by preliminary experiments showing that the P2 heat-inducible mutant (P2cts nip; Calendar et al, 1972) has a longer latent period after heat induction if the lysogen is subjected to UV irradiation (L.M. Pilarski and W.H. Woods, unpublished). Thus induction of P2cts nip, and induction of or infection by 186, all have longer latent periods in irradiated cells. A host function common to P2 and 186 production may be indicated by the class II 186 resistant bacterial mutants (6.3.a). Similarly, class I mutants may define a host function required by 186 but not P2.

The surprising facet of 186 UV induction is that the survival following UV irradiation of bacteria lysogenic for the phage is similar to that of non-lysogens. This is also found for λ lysogens of the UV sensitive exrA mutant of E. coli B (Donch, Greenberg and Green, 1970) although UV induction is slight in this strain and delayed 2½ to 4 hours compared to the wild type strain (Donch, Green and Greenberg, 1971). However, since Witkin (1967) has proposed a relationship between prophage induction and filament formation, it is worth noting that the exrA or recA mutations suppress filament formation following UV irradiation of non-lysogenic strains in which this normally occurs (lon strains; Donch, Green and Greenberg, 1968; Green,

Greenberg and Donch, 1969).

UV induction of phage $\phi 80$ in the UV sensitive strain, E. coli B_{s-1} is even more similar to UV induction of 186 in wild type E. coli K12. On lysogenisation with $\phi 80$, B_{s-1} shows no additional sensitivity to UV radiation, although this might be expected through induction of the prophage (Iwo, 1968). In addition, although sensitivity to UV induction in this strain is increased by a degree similar to the increased UV sensitivity of the non-lysogen, the appearance of free phage is delayed, at least allowing one post-induction division. This is exactly as has been proposed for 186 UV induction, but in the case of B_{s-1}($\phi 80$), better evidence exists. This is the observation of, (i) "colony centred plaques", that is a surviving colony and coincident infectious centre derived from the same irradiated cell (Iwo, 1968), and (ii) irradiated cells under the microscope (Takebe, Ichikawa, Iwo and Kondo, 1967). In the latter experiment, some cells formed filaments which later lysed completely, whereas other cells divided, only some of the resultant progeny suffering lysis.

This leads to the suggestion of an interaction between the 186 prophage and the host cell such that the lysogen or the irradiated lysogen is similar in phenotype to E. coli B_{s-1}. The important consequence of the interaction may be that 186 lysogens do not form filaments on UV irradiation. An interaction between phage and host, particularly of genes concerned with repair of damaged DNA as is exrA, is not unreasonable in light of the somewhat complex relationship between P2 old⁺, λ Spi⁺ and E. coli

recB⁺ (Sironi, Bialy, Lozeron and Calendar, 1971).

There also appears to be a delay to 186 phage production in a female cell during and immediately following conjugation. This is probably best exemplified by the 80 min increase in the latent period of 186cIts heat induction soon after transfer by conjugation, compared to its heat induction in a cell not involved in conjugation. Although the production of phage by heat induction is merely delayed, such a delay may also be applied to other methods of 186 induction by the physiology of the ex-conjugant female. In the case of a 186 prophage entering a non-lysogenic female by conjugation, a delay to 186 phage production, especially as this may involve delayed excision, may be sufficient to allow establishment of repression. In this context, it must be mentioned that phage 186 appears to have a similar dependence to phage λ on cAMP and CAP for the establishment of lysogeny (W.H. Woods, unpublished). Therefore, if as suggested to explain λ zygotic induction (6.4.a), cAMP and/or CAP are low in a conjugating female, this will favour the 186 lytic cycle. It is obvious that the final outcome depends on the rate of recovery of the female from these various disturbances. The latent period of 186cIts heat induction is increased by 80 min by conjugation, which is considerably longer than the delay to induction of β -galactosidase (15-40 min) found by Dubnau and Maas (1969), so it may be suggested that the delay to the 186 lytic cycle is more severe than the possible delay to establishment of lysogeny. This would result in a lack of zygotic induction for phage 186.

A delay to 186 phage production in an ex-conjugant female may be sufficient to explain lack of zygotic induction, therefore it is not surprising that 186^{susc} shows no induction on transfer into an su⁻ female. Consequently this experiment fails to indicate if the repressor is transferred from the male or if rapid resynthesis of repressor occurs in the female. If the latter were true, phage 186 might be expected to show a higher frequency of lysogenisation upon infection than does phage λ . However, these two phage show similar lysogenisation frequencies in non-conjugating cells (Moore, 1971). Therefore it is unlikely that a high rate of repressor production causes lack of zygotic induction. Similarly, it seems unlikely that limited repressor transfer could alone be sufficient to control the transferred 186 prophage, as already discussed (6.4.c).

That Hfr and F' mediated conjugation both delay 186 phage production may indicate that the delay is caused by conjugal cell contact, rather than some consequence of the amount of DNA transferred into the recipient. Although UV irradiated F' donors gave many fewer gal⁺ recombinants they had similar effect to un-irradiated donors upon 186 induction in the female. However, this cannot be taken as evidence that DNA transfer is not important in causing the delay to phage 186, because irradiated and un-irradiated donors have been shown to transfer similar amounts of DNA, even though resulting in different levels of recombinants (e.g. Monk, 1969). Whatever its mechanism, the delay to 186 phage production stands as a most significant indication of a change in the physiology of a female during conjugation. That

the effect may be specific to conjugation, rather than to processes involving recombinant formation, is indicated by transfer induction; but first, this form of induction must be described.

Jacob (1955) found that transfer of prophage λ by the specialised transducing phage 363 to a non-lysogenic recipient could result in λ plaque activity that was insensitive to λ anti-serum, but sensitive to 363 anti-serum. He did not report any effect on the transduction frequency of the neighbouring gal locus at 37°. There was no loss of expected gal⁺ transductants at 20°, but λ does not multiply at this temperature. Arber (1960) using Phage P1, showed that λ transfer induction was more frequent than the transduction of stable lysogeny to a non-lysogenic recipient, but attributed this to λ plaque activity from abortive transductants. Calef, Marchelli and Guerrini (1965) also transduced phage λ to sensitive recipients and found lysogenic transductants at a frequency similar to that obtained for other markers. It appears that induction of λ on transfer by P1 or 363 to a non-lysogenic recipient does not perturb the transduction of neighbouring markers. Thus the result reported in Chapter 5 that presence or absence of 186 prophage had little effect on transduction frequencies, is quite compatible with the recent report by Harriman (1972) that phage 21, 186, and λ show similar frequencies of transduction by P1, as measured by infectious centres due to transfer induction.

Now, the conclusion that may be drawn from the occurrence of transfer induction for phage 186 is that plaque production

by 186 is not significantly delayed in this case. That is, a 186 prophage entering a non-immune cell via generalised transduction is capable of forming an infectious centre. The delay seen following transfer mediated by conjugation does not occur. Thus it is predicted that indirect induction of prophage 186 will be possible by infection of a 186 lysogen with irradiated phage P1, as is possible for prophage λ (Rosner et al, 1968), because conjugation is not involved in this case.

Both conjugation and UV irradiation act on the 186 latent period to increase it. However in both cases, infectious centres may be displayed immediately upon induction. When an ex-conjugant female lysogen is UV irradiated, that is, both delaying treatments applied, infectious centres cannot be demonstrated until 3-4 hours after interruption of conjugation. If independent factors were involved, the delay exhibited by the combined treatments would be expected to be the greater of the separate delays. That the delay is substantially increased indicates that both conjugation and UV irradiation are affecting a common factor or inter-related factors responsible for delaying 186 phage production. It is most significant that in these cases, it appears that excision does not occur, since survivors are still lysogenic. However prophage 186 is perfectly capable of excision in other situations and so is intermediate between phage λ and phage P2 in this respect in particular.

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Integration Site of Noninducible Coliphage 186

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From conjugational data, the attachment site for noninducible coliphage 186 (*att186*) was located between the origins of Hfr strains KL16 and KL98, and close to the *pheA* gene in *Escherichia coli* K-12. P1 transductions indicated that *att186* lies at 51 min on the standard genetic map of *E. coli*, with the order *cysC-nalB-att186-pheA*. The presence of prophage 186 in the donor destroyed linkage between *nalB* and *pheA*, which is taken as evidence for the integration of the 186 prophage between these genes.

We have found that the prophage of coliphage 186 is induced by ultraviolet light but is not zygotically induced (23; Woods and Egan, *in preparation*). To explain this apparently contradictory behavior is a major aim of our program to study prophage induction, but our immediate interest here is to map the attachment site for this temperate phage (*att186*).

The loci of the attachment sites for a number of coliphages are known with various degrees of accuracy (9, 13, 18). For the inducible phages λ , 434, 82 (15), and ϕ 80 (16), and noninducible phage P2 (location II; 4), the attachment sites are known accurately, integration of these prophages into the continuity of the bacterial chromosome being indicated by reduction in linkage between bacterial markers flanking the prophage. Further evidence demonstrating actual integration of the prophage, as proposed in the Campbell model of lysogeny (5), has been reviewed by Signer (17) and Gottesman and Weisberg (6).

Evidence is presented here for the location and integration of coliphage 186 at 51 min on the *Escherichia coli* K-12 linkage map (19). Jacob and Wollman in their original description of coliphage 186 placed *att186* "on the segment of the bacterial linkage group carrying the maltose, mannitol and xylose characters" (10). During the course of this work, Abe and Tomizawa (1) reported limited P1 transduction data locating *att186* close to 50 min.

MATERIALS AND METHODS

Bacterial strains. The strains used in this work are all derivatives of *E. coli* K-12 and are described in Table 1. Phage 186 lysogens, indicated (186), were isolated from the turbid centers of single plaques, and were purified by repeated single-colony isolation.

Bacteriophage strains. Phage 186 and the temperature-sensitive clear plaque mutant 186p (3) were obtained as lysogens from R. L. Baldwin, P1vir was obtained from A. J. Pittard, and T6 from P. Reeves.

Media. The complete medium used, L broth, contained (per liter) 10 g of tryptone (Difco), 5 g of yeast extract (Difco), and 10 g of NaCl; it was supplemented with glucose (0.1%) for conjugation experiments and with CaCl_2 (2 mM) for use with P1. The minimal medium used was half-strength medium 56 (56/2) described by Monod et al. (14) supplemented with 0.2% glucose as carbon source and with vitamin and amino acid requirements. H-1 buffer-salts solution (11) was used for diluting and resuspending. Streptomycin sulfate (Drug Houses of Australia Ltd., Adelaide, South Australia) and nalidixic acid (a gift of Winthrop Laboratories, Ermington, New South Wales) were used at 200 $\mu\text{g}/\text{ml}$ and 6 $\mu\text{g}/\text{ml}$, respectively.

Conjugation experiments. Conditions were as described by Adelberg and Burns (2), with the exception that lysogenic males were washed and resuspended in fresh broth to reduce the free phage concentration. Phage 186 antiserum (1 part in 150 of rabbit serum with $K = 550 \text{ min}^{-1}$) was added to neutralize the remaining free phage. If required, conjugation was interrupted by use of a vibratory device of the type described by Low and Wood (12), generously made available by P. Reeves. The male population was counterselected with streptomycin or, in the case of AB2528, phage T6 (8). Samples were plated on selective media for recombinants, or with indicator bacteria to assay for 186 prophage. To test isolated recombinants for 186 lysogeny, colonies were placed with a toothpick first onto plates seeded with 594, and then onto plates of 594 (186) as a control. When the female strain used was lysogenic for phage Mu1, the indicator bacteria used to score lysogeny were also lysogenic for Mu1 (AT2092).

P1 transductions. P1vir lysates were prepared on the donor strain by a confluent plate lysis technique. All lysates were recycled on the same host once before use. In all transductions except those involving *nalB*, the same lysate from 594 was used throughout.

TABLE 1. *E. coli* K-12 strains utilized^a

Strain	Mating type ^b	Relevant genotype	Source
AB2528	Hfr	<i>ilvD leu thi str^r (λ)</i>	A. J. Pittard; same origin as AB313
KL16	Hfr	<i>thi str^a</i>	P. Reeves
KL98	Hfr	<i>str^a (λ)</i>	A. J. Pittard
KL164	Hfr	<i>thyA thi nalB^r</i>	CGSC 4304; B. Low
JP190	F ⁻	<i>tyrA ilvD argH metB thi str^r tsx^r</i>	A. J. Pittard
JP264	F ⁻	<i>his ilvD argH metB thi str^r tsx^r</i>	A. J. Pittard
AT713	F ⁻	<i>lysB argA cysC str^r</i>	A. J. Pittard
AT2092	F ⁻	<i>pheA purF thi his str^r tsx^r (Mu1)</i>	A. J. Pittard
AT2457		<i>glyA thi</i>	UTH 4133 ^c
H-724		<i>his tyrA trp purC guaB thi</i>	UTH 4069 ^c
H-888		<i>his trp purG thi</i>	UTH 4105 ^c
PA3306		<i>nadB purI argH</i>	UTH 4664 ^c
594	F ⁻	<i>galK galT str^r</i>	D. S. Hogness; H-111

^a The genetic symbols are those used by Taylor (19).

^b The origins of the Hfr strains are shown in Fig. 1. In addition, 186 or 186p lysogens of many of the above strains were prepared.

^c Obtained from T. S. Matney.

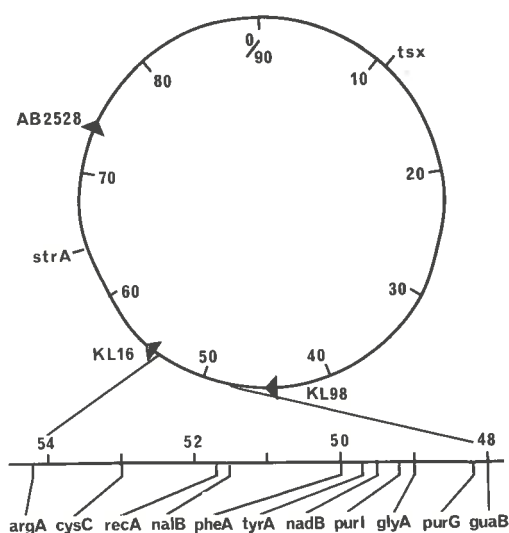


FIG. 1. *E. coli* linkage map, based on that of Taylor (19), showing origins of transfer of Hfr strains and detail of the region 48 to 54 min.

Lysates prepared on 186 lysogens were treated with 186 antiserum. For transduction, phage were added at a multiplicity of 0.05 to an exponential-phase culture of the recipient at 3×10^8 viable cells/ml, and the mixture was incubated for 30 min at 37 C. Then the cells were washed and resuspended in H-1 before being plated on selective medium. The three-factor transductions, some of which involved 186p, were performed at 30 C throughout.

Analysis of transductants. Transductants were purified through short streaks on selective medium before being tested for 186 lysogeny as above. A P1-resistant 186 indicator strain could not be isolated, necessitating further streaking away from P1 on

selective medium before retesting many transductants. Where *nalB* was involved, transductants were patched out on selective medium, and the thickly grown patches were replicated to an L plate containing nalidixic acid. In this case, the patches were used in lieu of short streaks as preliminary purification before testing for 186 lysogeny.

RESULTS

Uninterrupted matings. Since Jacob and Wollman located *att186* in the lower left quadrant of the *E. coli* linkage map (9), we commenced with the Hfr strain AB2528 (186). Recombinants for the *his* marker (39 min) were selected after 90 min of conjugation with JP264, and 55% (65 of 120) of these were found to be lysogenic for 186, compatible with Jacob and Wollman's assignment. Conjugations (Table 2) involving Hfr KL16 (186) showed linkage of (186) to *tyrA* (50 min), *purF* (44 min) and *pheA* (50 min), whereas Hfr KL98 (186) did not transfer (186) to *his⁺* or *purF⁺* recombinants of AT2092 in 60 min. Thus, *att186* is located between the origins of KL16 and KL98, and close to *pheA*. The recovery of markers distal to (186) confirms the absence of zygotic induction with this prophage (Woods and Egan, *in preparation*).

Interrupted mating. The results of an interrupted-mating experiment between KL16 (186) and AT2092 are shown in Fig. 2. Prophage 186 enters close to and probably before *pheA*. The scatter of points for (186), and the lesser slope of its entry compared with *pheA*, reflect the efficiency of assay for the (186) marker. This method depends upon the appearance of a plaque on indicator bacteria due

TABLE 2. Linkage of *att186* to various genes in crosses of *KL16* (186) \times *F*^{-a}

<i>F</i> ⁻ auxotroph	Marker	Fraction of recombinants lysogenic for 186
JP190	<i>tyrA</i>	313/350
AT2092	<i>purF</i>	153/200
AT2092	<i>pheA</i>	188/200

^a Recombinants for the markers in column 2 were selected after 60-min conjugation between *KL16* (186) and the nonlysogenic *F*⁻ shown in column 1.

to spontaneous phage production in a recombinant. In reconstruction experiments, only 20% of bacteria lysogenic for 186 plated with indicator bacteria scored as plaques, and these plaques were poorly defined. This 20% plating efficiency would explain the (186) slope being one-fifth of the *pheA* slope.

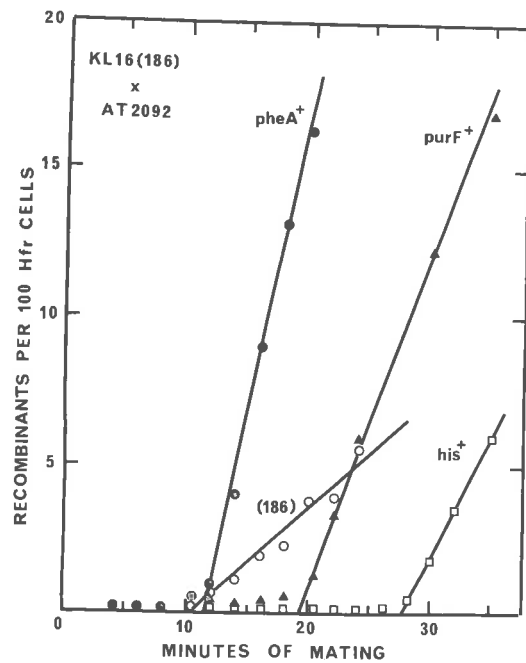
The entry time of the *his* marker varied from experiment to experiment, and was always delayed in the presence of 186 antiserum compared with literature values (20). However, (186) always entered with *pheA*.

Similar matings were performed with JP190, and in these (186) entered 0.8 to 1.2 min before *tyrA*.

Two-factor P1 transductions. These transductions can be represented as *X*⁺ donor and *X*⁻ (186) recipient. *X*⁺ transductants were selected, and linkage of *X* to prophage 186 was determined by checking these transductants for the absence of 186 lysogeny. In each instance, transduction to a nonlysogenic *X*⁻ recipient was performed, and the transduction frequency (number of transductants/plaque-forming unit) for the *X* marker was found to be the same, regardless of the lysogenic state of the recipient. For each recipient strain, transduction of markers unlinked to (186) invariably gave lysogenic transductants (200 of 200), so that the absence of prophage among transductants for some markers reflects linkage of the prophage to those markers. Markers checked in this manner were *trp* (25 min), *his* (39 min) *purF* (44 min), *guaB* (48 min) *purG* (48 min), *argA* (54 min), and *ilvA* (74 min).

For these two-factor transductions, P1vir propagated on 594 was used as donor, and the results recorded in Table 3 place *att186* at about 51 min, between *pheA* and *cysC*.

Three-factor P1 transductions. Finer mapping within the *cysC-pheA* region was possible by use of the *nalB* marker, which we found to be about halfway between *cysC* and *pheA* (Table 4, lines 1 and 5). (Since an accu-

FIG. 2. Marker entry curves for (186), *pheA*, *purF*, and *his* for an interrupted mating between *KL16* (186) and AT2092.TABLE 3. Two-factor P1 transductions from donor *X*⁺ to recipient *X*⁻ (186)^a

<i>X</i>	Nonlysogenic <i>X</i> ⁺ /total <i>X</i> ⁺	Per cent co-transduction	Separation (min)
<i>cysC</i>	0/508	<0.2	
<i>pheA</i>	97/968	10.0	1.1
<i>nalB</i>	8/348	2.3	1.4
<i>purI</i>	5/392	1.3	1.5
<i>glyA</i>	0/542	<0.2	

^a The markers *X* are shown in their order on the *E. coli* genetic map. The separation *X* from *att186* is calculated according to Wu (24).

rate locus for *nalB* has not been reported in the literature, we confirmed the order *argA-cysC-nalB* by three-factor P1 transduction.) Three-factor crosses were set up both to determine the relative order of *cysC*-(*nalB*, *att186*)-*pheA* and to seek evidence of disturbed linkage relationships indicative of actual integration of the prophage into the continuity of the bacterial chromosome. The results are recorded in Table 4.

DISCUSSION

With uninterrupted matings, it was first established that *att186* was between the origins of *KL16* at 55 min and *KL98* at 44 min. After

TABLE 4. Three-factor transductions involving *att186*, *nalB*, and *pheA* or *cysC*

Donor ^{a,b} markers		Recipient ^{a,c} markers		Analysis of <i>phe</i> ⁺ or <i>cys</i> ⁺ transductants ^d				Cotransduction frequency
A	B	a	b	AB	Ab	aB	ab	<i>nalB</i> - <i>pheA</i>
1.	<i>nal</i> ^r 186 ⁻ <i>phe</i> ⁺	<i>nal</i> ^s 186 ⁻	<i>phe</i> ⁻	29		499		29/528
2.	<i>nal</i> ^r 186 ⁻ <i>phe</i> ⁺	<i>nal</i> ^s (186)	<i>phe</i> ⁻	16	10	38	462	26/526
3.	<i>nal</i> ^r (186p) <i>phe</i> ⁺	<i>nal</i> ^s 186 ⁻	<i>phe</i> ⁻	0	0	59	465	0/524
4.	<i>nal</i> ^r (186p) <i>phe</i> ⁺	<i>nal</i> ^s (186)	<i>phe</i> ⁻	0	0	55	473	0/528
<i>cysC</i> - <i>nalB</i>								
5.	<i>cys</i> ⁺ <i>nal</i> ^r 186 ⁻	<i>cys</i> ⁻ <i>nal</i> ^s 186 ⁻		25		503		25/528
6.	<i>cys</i> ⁺ <i>nal</i> ^r 186 ⁻	<i>cys</i> ⁻ <i>nal</i> ^s (186)		0	28	0	500	28/528
7.	<i>cys</i> ⁺ <i>nal</i> ^r (186p)	<i>cys</i> ⁻ <i>nal</i> ^s 186 ⁻		0	29	0	495	29/524
8.	<i>cys</i> ⁺ <i>nal</i> ^r (186p)	<i>cys</i> ⁻ <i>nal</i> ^s (186)		0	26	0	521	26/547

^a The prophage states are indicated as: 186⁻, nonlysogenic for 186; (186), lysogenic for 186; and (186p), lysogenic for 186p.

^b KL164 was used as donor.

^c AT2092 and AT713 were used as recipients for *pheA* and *cysC* selections, respectively.

^d Lines 1-4, *phe*⁺; lines 5-8, *cys*⁺.

interrupted matings, *att186* could be placed close to *pheA*, and two-factor P1 transduction data put *att186* at about 51.1 min, 1.1 min to the left of *pheA* on the *E. coli* K-12 map (19). This statement assumes that the presence of the prophage in the recipient chromosome but not in the donor chromosome does not seriously disturb transduction of that region, an assumption for which we can offer some justification later in the discussion.

The three-factor transductions performed concerned the relationship of *att186* to *nalB*, a marker which has been placed in this region from interrupted mating (7). The results of these transductions (Table 4) show *nalB* to cotransduce at 5.5% with *pheA* and at 4.7% with *cysC*, frequencies which correspond to 1.2 min and 1.3 min, respectively. This *cysC*-*pheA* distance of 2.5 min compares favorably with 2.7 min reported by Willetts, Clark, and Low (22) from P1 transduction data, and with 3.0 min shown on the *E. coli* linkage map from conjugation data (20). We therefore place *nalB* equidistant from *cysC* and *pheA* at about 51.5 min.

With relation to *att186*, the important entries to note are numbers 4 and 8, in which both donor and recipient chromosomes carry the 186 prophage. When these are compared with transductions in the absence of prophage (entries 1 and 5, respectively) one notes that the *nalB*-*pheA* linkage drops from 5.5% to <0.2% because of the prophage, whereas the *cysC*-*nalB* linkage remains constant at 4.7%. Therefore, *att186* lies between *nalB* and *pheA*, and the prophage is probably integrated into the continuity of the bacterial chromosome.

A further point to note from Table 4 is that, in transduction from the nonlysogenic donor to the lysogenic recipient (entry 2), the presence of the prophage in the recipient chromosome has marginal, if any, effect on the cotransduction frequency of *nalB* with *pheA* (a drop of 5.5 to 4.9%), and justifies our assumption of this fact earlier in this discussion. However, it should also be noted that the frequency of the recombinant class representing quadruple exchanges [*nal*^r (186) *phe*⁺: 10 of 526] is not much less than that representing double exchanges (*nal*^r 186⁻ *phe*⁺: 16 of 526), suggesting that recombination is affected in this region.

The marker *recA* is mapped at 51.7 min (22), and an exciting prospect is the possibility of recovering a specialized transducing particle 186 d *recA*. The molecular weight of phage 186 deoxyribonucleic acid is 19.7×10^6 (21), and therefore its length is similar to the distance between *recA* and *att186*. Although it seems unlikely that a gene so far away could be recovered, the value of such a particle justifies our current attempts to isolate it.

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