# DEFECTIVE LAMBDOID PROPHAGES IN E.COLI K12

by

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A thesis presented for the degree of
Doctor of Philosophy
at the University of Edinburgh.

Department of Molecular Biology
University of Edinburgh, September 1979.

## ABSTRACT

This thesis confirms the hypothesis of Low (1973) that many *E.coli* K12 strains contain a prophage (the Rac prophage) located a few minutes clockwise of the *trp* operon on the genetic map. Restriction endonucleases and $^{32}$P-labelled phage DNA probes were used to investigate several *E.coli* K12 DNAs and hence construct a physical map of this prophage. Some *E.coli* K12 strains have lost the entire prophage by a specific deletion. This is consistent with prophage excision by site-specific deletion.

λ reverse (λ*rev*) phages are recombination proficient derivatives of phage λ in which the phage recombination functions have been replaced by analogous functions (RecE) derived from the host chromosome. The data of this thesis support the origin of λ*rev* phages by recombination between λ and the Rac prophage following excision of the Rac genome from the E.coli K12 chromosome.

*E.coli* K12 strains which carry *sbcA*$^-$ mutations express the Rac exonuclease gene (*recE*) constitutively. Investigation of the DNAs of several such strains showed them to fall into more than one class. In *sbcA8* strains a large section of the Rac genome (including a hybrid attachment site and probably the prophage repressor gene) is deleted. Several other *sbcA*$^-$ strains carry multiple (and probably tandemly repeated) copies of the prophage genome.

λ*qsr'* phages are characterised by the replacement of the region of the λ genome that contains *Q*, *S*, *R*, and the late gene promotor, $P'_R$, with host-derived DNA that codes for functions analogous to those deleted. This thesis shows the substitutions in λ*qsr'* phages to be derived from a second (and as yet unlocalised) lambdoid prophage (the *qsr'*$P$prophage) in *E.coli* K12

FOREWORD.

With the exception of the isolation and genetic characterisation of λ891, which was carried out by Noreen Murray, this thesis and the experiments described in it are my own work. The original ideas for this work were suggested by my supervisors, Noreen and Ken Murray, and by Ed Southern of the Mammalian Genome Unit, University of Edinburgh. These ideas sprang from observations made by participants in the EMBO Restriction Endonuclease Course, Basel, 1976. In addition to the above I would like to thank Noreen Murray for her interest and advice throughout the course of this work; Keith Cartwright, Keith Peden, David Morris, and Mitch Smith for help with practical and/or analytical problems; Pam Beattie for expert help with electron microscopy; Sandra Bruce and Karen Browne for providing many important enzymes; Jo Rennie for photography; Anna Nowosielska and my mother for typing; the staff of the media and washing-up rooms; Richard Ambler, David Finnegan, Ed Southern, and Neil Willetts for critical reading of parts of the manuscript; and Ramon Diaz, Pat Barnsley and Bob Pritchard for making their data available prior to publication.

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CONTENTS



continued overleaf

ABBREVIATION AND CONVENTIONS.

| | |
|---|---|
| bp | base-pairs |
| kb | kilo base-pairs |
| Md | mega-dalton |
| UV | ultraviolet light |
| $O.D._x$ | represents the absorbance of a solution for light of wavelength x nm over a path length of 1cm. |
| m.o.i. | multiplicity of infection |
| p.f.u. | plaque forming unit |
| Ap | ampicillin |
| Tc | tetracycline |
| Cm | chloramphenicol |

Restriction endonuclease nomenclature is according to Smith and Nathans (1973).

# CHAPTER I

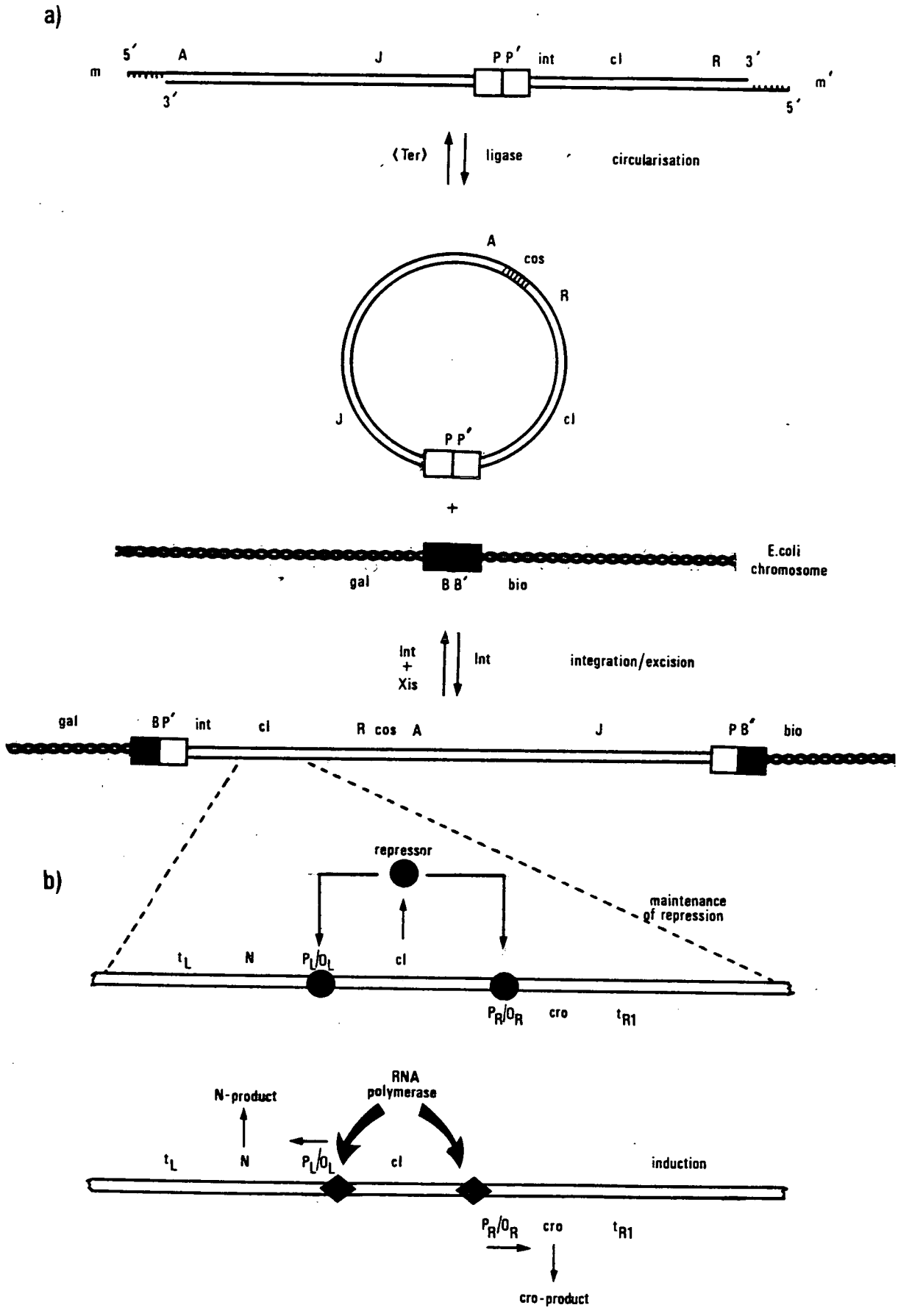
## Introduction

The original isolate of Escherichia coli K12 (Gray and Tatum, 1944; Lederberg and Tatum, 1946) was used as an experimental organism for a number of years before it was shown to be lysogenic for bacteriophage lambda (λ), the detection of which depended upon a chance curing of a subculture of the original isolate in order to provide bacteria sensitive to infection by λ (Lederberg, 1951). No other phages have been isolated from E.coli K12 or its derivatives during many years of investigation. However, several different lines of genetic evidence (see Introduction to Chapter 3) suggest that E.coli K12 does harbour another lambdoid prophage(s), the presence of which is not normally detected, either because of its defective nature or for the lack of a sensitive host. For the purposes of this thesis, lambdoid prophages in E.coli K12 were identified as regions of the chromosome which code for functions that may substitute for analogous functions carried by λ and which share homology with the DNA of λ or related phages. Using these criteria I have shown that at least two lambdoid prophages are associated with the E.coli K12 ($\lambda^-$) chromosome. In the absence of any plaque forming phages from E.coli K12 ($\lambda^-$) strains, and since the new prophages appear to be deleted for essential late genes, I believe them to be defective.

(a) Bacteriophage Lambda

Several aspects of λ biology relevant to the subject matter of this thesis are described here. For a more complete overview of λ the Cold Spring Harbour monograph "The Bacteriophage Lambda" (ed. A.D. Hershey, 1971a) is invaluable reading.

λ phage particles consist of protein and DNA in approximately equal proportions (Hershey and Dove, 1971). λ DNA is a linear duplex molecule, 49 kb long (Philippsen and Davis, 1978) which at both 5' ends carries short (12 bases), complementary, single-stranded projections (Wu and Taylor, 1971). These cohesive ends (Fig. 1.1(a))


a)
m
5′
A
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int
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ligase
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J
cI
P P′
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gal
B B′
bio
E.coli
chromosome
Int
+
Xis
Int
integration/excision
gal
B P′
int
cI
R cos A
J
P B′
bio
b)
repressor
maintenance
of repression
$t_L$
N
$P_L/O_L$
cI
$P_R/O_R$
cro
$t_{R1}$
N-product
RNA
polymerase
$t_L$
N
$P_L/O_L$
cI
induction
$P_R/O_R$
cro
$t_{R1}$
cro-product

Fig. 1.1.

(a) Model for site specific insertion or excision of a λ genome (after Campbell, 1962). ═══ is phage DNA; ●●●●●●● is bacterial DNA; P and P' are elements of the phage attachment site; B and B' are elements of the bacterial attachment site. The symbols representing phage markers are described in the legend to Fig. 1.3. The bacterial genes *gal* and *bio* which flank BB' are required for the utilisation of galactose and the synthesis of biotin respectively. The distance between markers is arbitrary.

(b) Repression and induction of the λ prophage. Repressor protein switches off the expression of all phage genes other than the repressor gene (*cI*) itself. Maintenance of *cI* expression in a lysogen is promoted by binding of repressor at $O_R$ (reviewed in Weisberg *et al*, 1976 ). Induction occurs when repressor fails to bind to the operators $O_L$ And $O_R$. RNA polymerase is then able to bind to $P_L$ and $P_R$ and transcribe the immediate early genes (*N* and *cro*). Transcription is initially terminated at sites $t_L$ and $t_{R1}$ at the ends of the *N* and *cro* genes respectively.

allow the linear DNA molecule to form rings and chains (Hershey et al, 1963). λ DNA consists of several segments differing markedly in nucleotide composition (Skalka, et al, (1968).

In the λ phage particle the DNA is enclosed within a protein coat consisting of two morphologically distinct regions: the head and the tail. During infection of sensitive E.coli by λ, phage tails attach to the bacterial cell wall and the linear DNA molecule is extruded into the bacterial cytoplasm. Once inside the cell the cohesive ends anneal, E.coli DNA ligase seals the single-strand nicks (Fig. 1.1(a)), phage "early" functions are expressed, and the now circular DNA molecule begins to replicate. Two alternative modes of development (lytic and lysogenic) are then open to a temperate phage such as λ. If the lytic pathway is followed, replication continues, late genes which code for structural proteins and their assembly functions are expressed, the DNA is packaged into phage heads and tails, and the cell is lysed to release approximately 100 phage progeny. Lytic development and its regulation have been summarised by Echols (1971).

Alternatively the lysogenic pathway may be followed, in which case the phage genome becomes integrated into the bacterial chromosome (Fig. 1.1(a)). The resulting prophage is then inherited in the same way as a normal bacterial gene. Integration of the λ genome is mediated by the product of the λ int gene which catalyses a site specific recombination between unique attachment sites on the λ and E.coli chromosomes (Gottesman and Weisberg, 1971). These are known as PP' and BB' respectively and, as the nomenclature implies, are essentially bipartite structures. Integration generates two new hybrid attachment sites (BP' and PB') at either end of the prophage genome. The hybrid attachment sites, although differing in structure from both PP' and BB', are able to serve as substrates for insertion of a λ genome (Gingery and Echols, 1968). Since PP' is not coincident with the cohered ends (cos) of the circular phage genome (in fact it is almost diametrically opposite), the prophage genetic map is a cyclic permutation of the

phage map (Fig. 1.1(a)).

BB' is the primary attachment site for λ in E.coli K12. Much less frequently λ will lysogenise at other sites on the chromosome (Shimada et al, 1972). Integration at these secondary attachment sites, like normal integration, requires the int product and occurs by a cross-over at the normal attachment site (PP') on the phage chromosome, thus generating a prophage with the same permutation of genetic markers as a prophage at the primary attachment site. Extensive DNA homology between the λ and E.coli attachment sites does not appear to be a prerequisite for Int mediated recombination (Gottesman and Weisberg, 1971).

The lysogenic state is maintained by synthesis of a repressor protein (the product of the cI gene) which binds specifically to the leftward ($O_L$) and rightward ($O_R$) operators, thus blocking transcription from the early promoters, $P_L$ and $P_R$ (Steinberg and Ptashne, 1971; see Fig. 1.1(b)). Similarly, λ lysogens are immune to superinfection by λ since the incoming phage genome cannot initiate expression of either the lytic or integration functions in the presence of repressor.

A λ prophage may be replicated indefinitely as a part of the bacterial chromosome. However, λ prophages may be induced to enter the lytic cycle (Fig. 1.1(b)). Induction occurs spontaneously at low frequency but may be stimulated by treatments such as ultraviolet irradiation which inactivate repressor. Zygotic induction occurs on transfer of a λ prophage by Hfr mating into an $F^-(\lambda^-)$, and hence repressor-free, recipient. Induction usually results in precise excision of the prophage genome from the bacterial chromosome by a reversal of the recombination event which leads to integration. However, in addition to the int product, excision from either a primary or a secondary attachment site requires expression of the xis gene (Guaneros and Echols, 1970; Kaiser and Masuda, 1970; Shimada et al, 1972).

Specialised transducing phages appear at low frequency in lysates prepared by induction of a λ lysogen. The genomes of these phages are hybrids in which part of the λ genome is replaced by a segment of bacterial DNA that

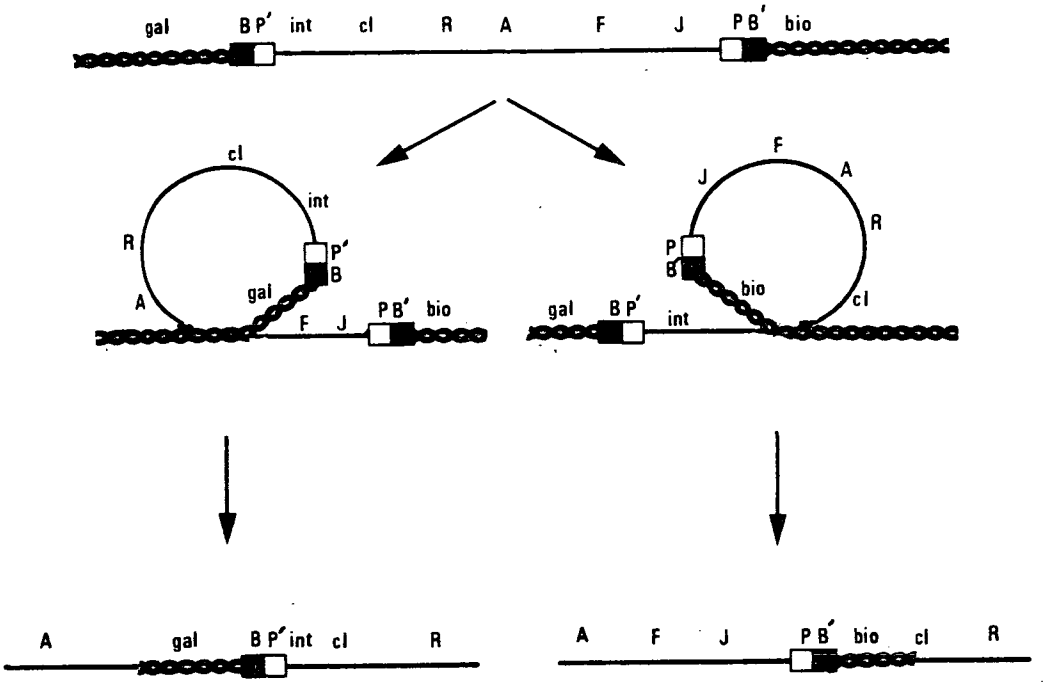



Fig. 1.2. The origin of transducing phage lines (after Campbell, 1962). The symbols are described in the legend to Fig. 1.3. Recombination is assumed to take place at the bottom of the circular loops. The formation of a λ*gal* (left) and a λ*bio* (right) phage is shown. The distance between markers is arbitrary.

was originally located adjacent to BB' (Campbell, 1962; see Fig. 1.2). Upon infection with these phages, bacteria may acquire genes (e.g. gal and bio) from the original lysogen. This process is called transduction. Specialised transducing phage genomes are generated by "illegitimate" recombination processes (reviewed by Franklin, 1971) that do not depend upon any known bacterial or phage recombination functions or on the presence of extensive phage/host DNA homology at the site of recombination. Since the generation of transducing phage genomes involves deletion of λ genes, transductants and their progeny are defective lysogens that cannot (in the absence of a helper or a hetero-immune superinfecting phage) be induced to produce phage particles.

Similarly a defective prophage may be generated by aberrant excision, random deletion, or other mutations that remove essential genes or a hybrid attachment site from a previously intact prophage. Defective lysogeny may take several forms depending upon the type of mutation or extent of deletion involved. Some defective prophages remain inducible (at least in the presence of a hetero-immune superinfecting phage). However, a prophage which has lost a hybrid attachment site cannot readily be induced to leave the chromosome and may thus be considered a relatively permanent acquisition for the host bacterium. Except on very rare occasions (e.g. back mutation), plaque forming phages do not arise spontaneously from defective lysogens.

The λ genes are clustered on the genetic map according to function (Fig. 1.3). Lytic development can be divided into different stages (immediate-early, delayed-early, and late) according to which groups of genes are being expressed (Echols, 1971). The immediate-early genes (N and cro) are transcribed (from $P_L$ and $P_R$ respectively) by the host RNA polymerase immediately upon infection or induction. The N product is a positive regulator of λ development. Its function is to antagonise the rho dependent termination of mRNA transcripts initiated at $P_L$ and $P_R$ in order to allow expression of the delayed-early genes. These include

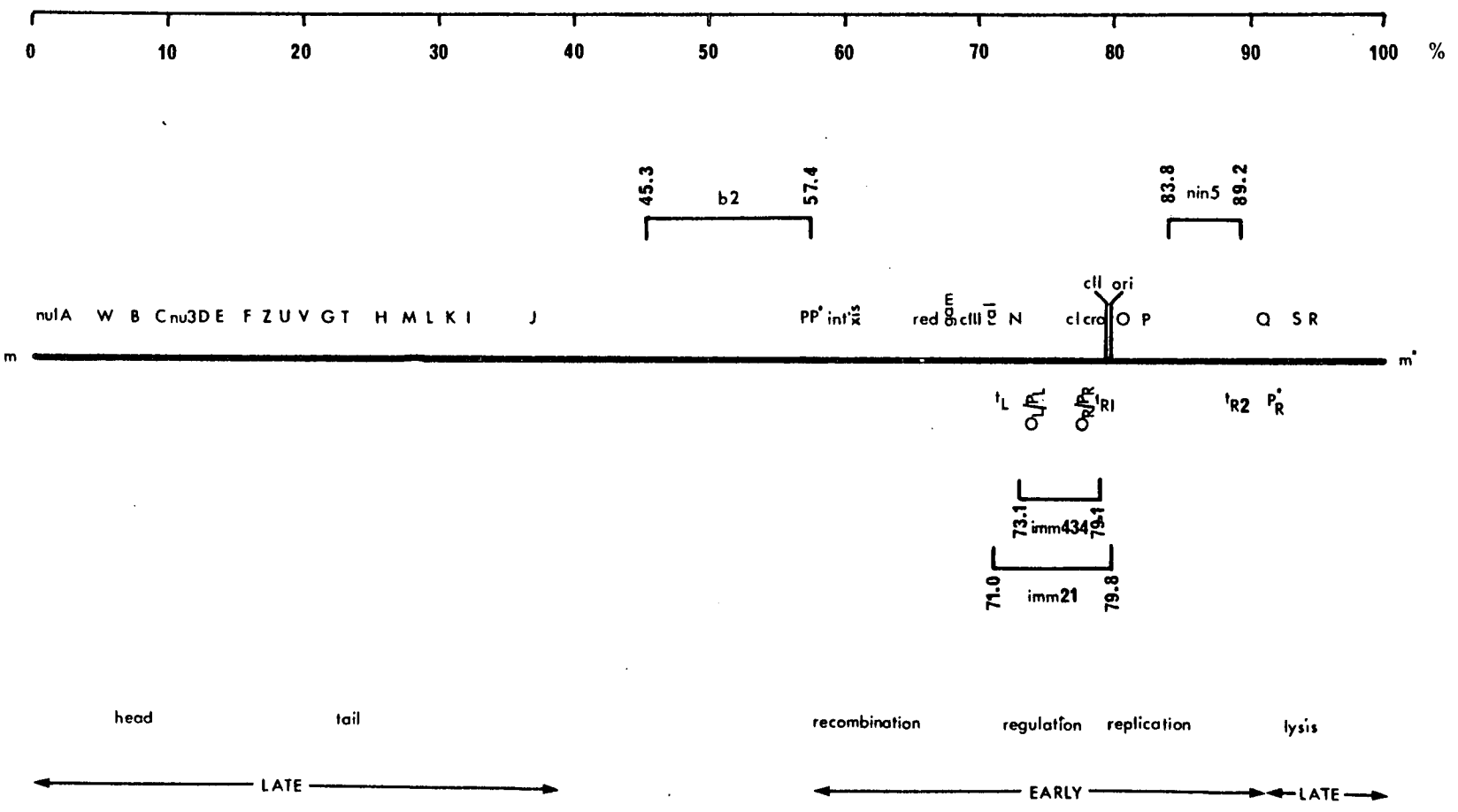

0
10
20
30
40
50
60
70
80
90
100
%
45.3
b2
57.4
83.8
nin5
89.2
cII ori
nulA W B Cnu3D E F Z U V G T H M L K I J
PP' int xis
red gam cIII cI N cI cro O P Q S R
m
m'
tL POL
OR PR tRI
tR2 PR'
73.1
imm434
79.1
71.0
imm21
79.8
head
tail
recombination
regulation
replication
lysis
LATE
EARLY
LATE

Fig. 1.3. The λ genetic map. The following is adapted from Szybalski (1974) and Gottesman and Adhya (1977) where a full set of references can be found.

| | |
|---|---|
| m and m' | The cohesive termini (mature ends) of λ DNA extracted from phage particles. The ends are generated by endonucleolytic cleavage (by the Ter function) at the cohesive end recognition sites (cos). Concatameric λ DNA produced by the "rolling circle" mode of replication can be matured. Monomeric circles can not. |
| nuI, A, W, B, C, nu3, D, E, and F | code for DNA maturation and phage head proteins. Gene A codes for a protein that has Ter activity in vitro but which is not a part of the completed head. |
| Z, U, V, G, T, H, M, L, K, I, and J | code for phage tail proteins. Gene J determines host specificity. |
| b2 | This region, which includes the left component (P) of the phage attachment site, is deleted in the λb2 mutant and codes for several proteins, the functions of which are largely unknown, and that are inessential for λ growth. λb2 phages are unable to lysogenise efficiently. |
| PP' (attP) | Specific attachment site at which integrative recombination with the E.coliK12 chromosome occurs during lysogenisation. The site of integration in the E.coliK12 chromosome is called BB' or attB. |
| int | codes for an enzyme that promotes phage integration (int alone) and excision (int+xis) at the attachment site. |
| xis | codes for an enzyme that promotes excision in conjunction with the int product. |
| red | Composed of redα (exo) and redβ, the products of which promote general recombination and growth in recA⁻ and Feb⁻ (e.g. polA⁻) hosts and are weakly stimulated by the gam product. The Red system is also involved in λ DNA replication. |
| gam | codes for a protein that inhibits the degradative effects on λ DNA of Exonuclease V (Exo V), the product of the E.coliK12 recBC genes. |

Fig. 1.3 (cont).

| | |
|---|---|
| Fec$^-$ phenotype | Single *red*$^-$ or *gam*$^-$ mutants grow well on both *rec*$^+$ and *rec*$^-$ hosts. *red*$^-$*gam*$^-$ double mutants are unable to grow on *recA*$^-$ hosts (Fec$^+$ phenotype; Zissler *et al*, 1971) due to degradation of λ DNA by the *recBC* nuclease (Exo V) in the absence of the inhibitory effects of the *gam* (or *recA*) product. A functional *red* gene either prevents this degradation or is able to rescue λ DNA that has been attacked. |
| Spi$^+$ phenotype | (susceptibility to P2 interference): inability to grow on a P2 lysogen. The *old* product of P2 kills host cells that are either truly *recBC*$^-$ (Exo V$^-$) or are phenotypically RecBC$^-$ due to the inhibitory effects of the *gam* product (Lindahl *et al*, 1970; Sironi *et al*, 1971; Zissler *et al*, 1971). *red*$^-$*gam*$^-$ phages are able to grow on a P2 lysogen (Spi$^-$ phenotype, full expression of which requires both *red* and *gam* to be *deleted*). The role of the *red* product in the Spi effect is not clear. |
| *cIII* | The *cIII* product acts together with the *cII* product in turning on expression of the *cI* (prophage repressor) gene. |
| *ral* | The *ral* product alleviates the restriction of non-modified λ DNA in *E.coli*K12 and B hosts. |
| $t_L$ | See under *N*. |
| *N* | The *N* product antagonises rho-dependent termination of transcription at sites immediately to the left ($t_L$) and the right ($t_{R1}$) of the *N* and *cro* genes respectively and at a site ($t_{R2}$) between *P* and *Q*. rho is a protein factor that can be isolated from uninfected *E.coli*. |
| $P_L$ | Promoter for initiation of transcription of the *N*-operon (*N-int*). |
| $O_L$ | See under *cI*. |
| *cI* | codes for the λ repressor which binds to the leftward ($O_L$) and rightward ($O_R$) operators, thus preventing initiation of transcription from $P_L$ and $P_R$ respectively. |

Fig. 1.3 (cont.).

| | |
|---|---|
| $P_R$ | Promoter for initiation of transcription of the cro-operon (cro-Q). |
| $O_R$ | See under cI. |
| cro | The cro product depresses transcription from $P_L$, and probably also from $P_R$, and therefore indirectly inhibits repressor synthesis by turning off the cII and cIII genes. |
| $t_{R1}$ | See under N. |
| cII | See under cIII. |
| ori | Origin for bidirectional replication of λ DNA. |
| O, and P | The O and P products control λ DNA synthesis. |
| $t_{R2}$ | See under N. |
| nin5 | (N-independent): a deletion which removes $t_{R2}$ and relieves the requirement of the N product for phage growth. |
| Q | Positive regulator for transcription of the phage late genes (S-J) from the $P'_R$ promoter. |
| $P'_R$ | See under Q. |
| S | The S product is required for cell (membrane) lysis. |
| R | codes for an endolysin (endopeptidase) that cleaves components of the cell wall. |
| $imm^{434}$ | Substitution of the λ immunity region by the corresponding region of the phage 434 genome. |
| $imm^{21}$ | Substitution of the λ immunity region by the corresponding region of the phage 21 genome. |

cIII, gam, red, xis and int (in the N-operon) to the left and cII, O, P and Q (in the cro-operon) to the right of the immunity region. The delayed-early genes are transcribed from the same promoters ($P_L$ and $P_R$) as are N and cro. The cro product also has a regulatory function, which acting more slowly than the N product, turns off expression of the early genes and allows the smooth transition to late gene expression. cII and cIII are regulatory genes, the products of which act to turn on the transcription of the cI (prophage repressor) gene. If the cro product turns off expression of cII and cIII before sufficient repressor protein has been synthesised, the phage may enter the lytic cycle; if not, lysogeny may ensue. gam, red, xis and int code for general and site specific recombination functions that are inessential for lytic development in a wild type host. Genes O and P are concerned with DNA replication. The product of gene Q regulates transcription of the late genes, probably by permitting extension of a short transcript originating from $P_R'$ in a manner analogous to the anti-terminating effects of the N product on transcription from $P_L$ and $P_R$ (Roberts, 1975). The group of late genes forms a single transcriptional unit when the ends of the linear phage DNA molecule cohere. S and R are concerned with bacterial lysis; A-F with the structural components and assembly of the phage head and DNA maturation; Z-J with the structural components and assembly of the phage tail.

Although λ is the best studied phage of its type, it is in fact only one member of a large family of related temperate coliphages (lambdoid phages) which are alike in basic morphology and are able to form hybrid recombinants with each other. These include the phages λ, 434 and 21. Lambdoid phages are usually found to have cohesive ends identical to those of λ (Murray and Murray, 1973); they possess repressors which are sensitive to ultraviolet irradiation although these may have an immunity specificity different from that of λ (as do 434 and 21); their DNA shares a variable degree of homology with λ DNA as shown by DNA/DNA hybridisation (see below) and electron microscopy of DNA heteroduplexes (Simon et al, 1971); their genes are

clustered in a similar fashion to those of λ and in some cases may be interchangeable with analogous genes or groups of genes carried by λ.

Lambdoid phages integrate at specific sites on the E.coli chromosome. The same site is used by λ and 434 and is different to that used by phage 21. E.coli K12 carries attachment sites for these and other lambdoid phages. If two lambdoid phages have the same immunity specificity, then it is not possible to superinfect a lysogen of one of them with the other (homo-immune) phage. However, lambdoid phages are able to superinfect hetero-immune lysogens. Genetic recombination occurs between pairs of lambdoid phages upon mixed infection of a sensitive E.coli strain, allowing one to isolate hybrid recombinants between different phages. In this manner, recombinants have been isolated which carry the repressor gene and immunity specificity of another lambdoid phage (e.g. λimm$^{21}$, λimm$^{434}$; see Fig. 1.3).

Hybrid recombinants have also been formed between λ and the temperate bacteriophage P22 of Salmonella typhimurium (Botstein and Herskowitz, 1975). λ and P22 are similar in functional organisation (Dove, 1971; Botstein et al, 1972) and share DNA sequence homology as measured by DNA/DNA hybridisation (Cowie and Szafranski, 1967). On the other hand, there are profound differences between the two phages, not least of which is that the P22 genome is circularly permuted and terminally repititous whereas the λ genome is not permuted and has cohesive ends. Botstein and Herskowitz (1975) isolated a hybrid recombinant which carries the immunity, early control, and DNA replication genes of P22 in place of the analogous λ genes. The immunity of the hybrid phage was identical to that of coliphage 21 both in function and, at least to the resolution afforded by heteroduplex mapping, in nucleotide sequence. Botstein and Herskowitz therefore felt justified in categorising P22 as a lambdoid phage even though it lacks several characteristics (e.g. cohesive ends) normally associated with this group of phages.

For the purposes of this thesis it was felt sufficient

to define as lambdoid prophages, regions of the E.coli K12 chromosome which code for functions that may substitute for analogous functions carried by λ and which share significant homology with the DNA of λ or related phages.

(b) The investigation of λ/E.coli DNA homology.

Investigation of λ/E.coli DNA homology began in the early 1960's almost as soon as appropriate nucleic acid hybridisation techniques became available. The initial impetus for these experiments was a desire to explain the (then) poorly understood phenomena of site specific integration and the generation of specialised transducing phages (see for example Green, 1963; Cowie and Brenner, 1968), processes which might well have been expected to require large scale phage/host DNA homology, although this is now known not to be the case (see above). In particular, since λ phages deleted for the b2 region are unable to lysogenise efficiently (Kellenberger et al, 1961), this region was suspected of being homologous with the site of integration for λ in E.coli. The first evidence for complementarity between λ and E.coli DNA sequences was provided by Cowie and McCarthy (1963) using the DNA-agar hybridisation procedure (Bolton and McCarthy, 1962). In this technique, high molecular weight single-stranded DNA is immobilised in agar, radioactive single-stranded DNA fragments or molecules of RNA are allowed to interact with the DNA-agar preparation and, after washing off unbound radioactivity, the extent of interaction between the two components is determined. Cowie and McCarthy (1963) estimated that 33% of in vivo labelled $^{32}$P-λDNA fragments were retained by a large excess of non-lysogenic E.coli (K12 or B) DNA-agar after hybridisation under conditions (2xSSC at 60°C) that had proved to be roughly optimal for the annealing of complementary nucleic acid sequences (SSC is 0.15M NaCl, 0.015M Na citrate). They found that E.coli related bacterial DNAs would bind λ DNA fragments to a slightly lesser degree.

Nitrocellulose membranes have adsorption characteristics which can be used to separate free RNA from RNA bound to

DNA (Nygaard and Hall, 1963). They adsorb denatured DNA or DNA/RNA complexes but do not adsorb free RNA. This method of assaying hybrid formation was found to be more suitable than the DNA/agar method for detailed kinetic studies and was also more quantitative and faster. Green (1963) used the nitrocellulose membrane method to measure the fraction of *in vitro* synthesised $^{32}$P-λRNA remaining bound to non-lysogenic *E.coli* K12 DNA after hybridisation in 2xSSC at 61 °C and subsequent digestion with pancreatic RNase. Under these conditions only 8% of the λ RNA remained bound and at least 80% of the observed homology was found to lie outside the *b*2 region of λ.

Comparison of thermal denaturation profiles showed that the complexes formed by λ RNA (Kiger and Green, 1964) or λ DNA (Cowie and Hershey, 1965) with *E.coli* DNA have high melting temperatures similar to those of λRNA/λDNA or λDNA/λDNA complexes indicating some degree of authentic matching of the sequences. Cowie and Hershey (1965) separated out fragments from the left and right arms of λ DNA by density gradient centrifugation and purified DNA from the *b*2 region by absorbing out cross-reacting material from $^{32}$P-λ*b*$^{+}$ DNA fragments on λ*b*2 DNA-agar. All three classes of fragment were capable of some degree of interaction with *E.coli* DNA sequences. Ingraham, Ehring and Hershey (1966) confirmed these findings and demonstrated that the strongest reaction occurred with fragments containing 46% guanine + cytosine (G+C) derived from the right half of the DNA molecule.

These studies were extended to include a larger range of phage and bacterial DNAs (Cowie and Szafranski, 1967; Cowie and Brenner, 1968). In general, lambdoid coliphages and *Salmonella* (P22) phages were found to be closely related both to each other and to their hosts. In contrast little or no interaction was observed between *E.coli* DNA or λ DNA and the DNAs of virulent coliphages (e.g. T4) which are unable to form lysogens.

The study of relationships between lambdoid phage DNAs soon received a necessary boost with the introduction of the electron microscope to the visualisation of DNA heteroduplexes

(see Davidson and Szybalski, 1971; Simon et al, 1971). In contrast, the significance of phage/host DNA homology remained unclear, especially with the demonstration that heteroduplexes constructed from the DNAs of λ and a λ derivative carrying BB' shared no homology in the region of the attachment sites(Davis and Parkinson, 1971). A better understanding of the nature of λ/E.coli DNA homology demanded a new approach.

(c) Southern's gel transfer method.

In the nitrocellulose membrane method (Nygaard and Hall, 1963) annealing of DNA and RNA was carried out in solution. The disadvantage of this method is the competition between hybrid formation and the reformation of DNA/DNA duplexes. By thoroughly drying the membranes after addition of denatured DNA, Gillespie and Spiegelman (1965) were able to fix the DNA irreversibly to the nitrocellulose. Annealing of DNA and radioactive RNA could then be carried out under conditions in which unwanted interaction between the DNA strands was eliminated. Denhardt (1966) extended this technique to the detection of DNA/DNA complementarity which had not previously been achieved on nitrocellulose membranes due to non-specific binding of single-stranded DNA during hybridisation. After addition of denatured DNA and thorough drying, Denhardt incubated the membranes, prior to hybridisation, in a solution containing albumen, Ficoll and polyvinylpyrrolidone. Such pre-incubation almost totally eliminated non-specific binding of radioactive single-stranded DNA.

The introduction of restriction endonucleases to the analysis of genome organisation,and to the in vitro construction of hybrid plasmids or phages containing cloned genomic DNA fragments, generated new interest in the use of hybridisation methods as a means of demonstrating sequence complementarity and genetic relationships between different nucleic acid species. Since agarose gel electrophoresis is a commonly used technique for separating DNA fragments by size (for example, those generated with restriction endonucleases), and is capable of high

resolution, the method described by Southern (1975) for transferring fragments of DNA from agarose gels to nitrocellulose membranes has found wide application. In this method, which has been extensively used in the experiments described below, DNA fragments denatured by alkali *in situ* are eluted from an agarose gel with a concentrated salt solution and are transferred to a nitrocellulose membrane, held in contact with the surface of the gel, where their specific electrophoretic pattern is retained. The membrane is thoroughly dried, pre-incubated and used for hybridisation with a radioactive RNA or DNA probe. After washing off unbound probe, bands of radioactivity are detected by autoradiography or autofluorography. Whereas the hybridisation methods previously used were largely quantitative in their estimation of nucleic acid complementarity, the transfer method allows comparison of hybrids formed between different nucleic acid species to be made on a more qualitative basis, since the position of a band of hybridisation represents a DNA fragment of a well defined size and may be directly compared with the positions of DNA fragments in a photographic reproduction of the original (ethidium bromide stained) gel.

# CHAPTER 2

## Materials and Methods

(a) Bacterial strains, phage strains, and bacterial plasmids.

These are listed in Tables 1.1, 1.2 and 1.3 respectively.

(b) Enzymes and chemicals.

Pancreatic DNase and RNase were purchased from Worthington Biochemical Corporation. The restriction endonucleases were made in this laboratory. R.EcoRI and R.BamHI were prepared by Karen Brown; R.HindIII by myself. T4 polynucleotide ligase was made by Sandra Bruce. DNA polymerase I was purchased from Boehringer Mannheim; DNase I (type 1), lysosyme and protease (pronase P) from Sigma; HAP (DNA grade Bio-gel HTP) from Bio-Rad; PEG 6000 from BDH; agarose from Miles Laboratories; Ficoll 400 from Pharmacia; nitrocellulose filters from Schleicher and Schull; Fast Tungstate intensifying screens from Ilford; and X-ray film (X-Omat H) from Kodak. $\alpha$-$^{32}$P-dGTP (specific activity approx. 350 Ci/mmol) was purchased from the Radiochemical Centre, Amersham. Where appropriate chemicals of A.R. grade were used.

(c) Media.

Where appropriate these were sterilised by autoclaving at 15lb/sq.in. for 15 min. before use.

L broth: Difco Bacto Tryptone, 10g; Difco Bacto yeast extract, 5g; NaCl, 10g; water to 1 litre.

L broth agar: L broth + 1.5% Difco agar.

BBL agar: Baltimore Biological Laboratories tryp-ticase, 10g; Difco agar, 10g; NaCl, 5g; water to 1 litre.

BBL top agar: As for BBL agar but only 6.5g. agar/l.

Minimal agar: Davis New Zealand agar, 16g; water to 800ml. After autoclaving, 0.25vol. of 4xM9 salts were added. The final solution was supplemented with 0.2% w/v glucose; 300µg/ml $MgSO_4.7H_2O$;

TABLE 1.1. BACTERIAL STRAINS.

| Strain | Relevant features | Source | Reference |
|---|---|---|---|
| (a) *E.coli* K12 derivatives: | | | |
| AB1157 | $Rac^-$ | Neil Willetts | Low (1973) |
| C600 | $Rac^+$ | F.W. Stahl | Appleyard (1954) |
| C600(λ) | $Rac^+$ | Noreen Murray | |
| CR63 | $Rac^+$ | N.C. Franklin | Appleyard *et al* (1965) |
| CS520 | $Rac^-$ (λ lysogen) | J. Carbon | Clarke & Carbon (1976) |
| ED8659 | $Rac^+$ | Bill Brammar | Kelley *et al* (1977) |
| KL181/F123 | F123 extends from *rac* to *trp* | John Guest | Low (1972) |
| QR47 | *supE* | Noreen Murray | Signer & Weil (1968) |
| *ligts* $sup^0$ | | " | Wilson *et al* (1977) |
| *ligts* $sup^0$ ($\lambda imm^{21}$) | | " | |
| KB8 | *endA polA1* $sup^0$ | J.P. Brockes | Kelley *et al* (1977) |
| W3101 | $sup^0$ | N.C. Franklin | Campbell (1961) |
| W3101 *recA* | *recA13* $sup^0$ | " | |
| W3101 (P2) | $sup^0$ (P2) | Bill Brammar | |
| C600 (P2) | *supE* (P2) | Noreen Murray | |
| C600 ($\lambda imm^{21}$) | | " | |
| LE450 | $Rac^+$, *recA*$^-$ derivative of C600 | Ramon Diaz | Diaz *et al* (1979) |
| LE451 | $Rac^-$ derivative of LE450 | " | " |
| LE452 | " | " | " |

(continued overleaf)

TABLE 1.1 (continued).

| Strain | Relevant features | Source | Reference |
|---|---|---|---|
| JC5174 | *sbcA1* | Neil Willetts | Barbour *et al* (1970) |
| JC5412 | Hfr *sbcA8* | " | Lloyd & Barbour (1974) |
| S583*sbcA8* | *sbcA8* in AB1157 background | Bob Lloyd | Lloyd (1974) |
| JC5491 | Hfr *recB*$^-$*recC*$^+$ Rac$^+$ | John Clark | Willetts & Clark (1969) |
| JC7661 | Hfr *sbcA23*, derivative of JC5491 | " | Gillen *et al* (1977) |
| JC8679 | *sbcA23* in AB1157 background | Neil Willetts | " |
| PE108 | *sbcA50 polA1* | Peter Emerson | Strike & Emerson (1974) |
| PE138 | *sbcA51* | " | " |
| (b) Other strains: | | | |
| *E.coli* B | | Richard Hayward | Studier (1969) |
| *E.coli* C-1a | | G. Bertani | L.E. Bertani (1968) |

A variety of wild type *E.coli* isolates was provided by Dr. Ian Sutherland of the Microbiology Department, Edinburgh University, and by Dr. Keith Cartwright of the Western General Hospital, Edinburgh.

| | | |
|---|---|---|
| *Shigella boydii* | | Keith Cartwright |
| " | *dysenteriae* | " |
| " | *flexneri* | " |
| " | *sonnei* | " |
| *Salmonella typhimurium* LT/2 | | Zainab Al-Doori |

TABLE 1.2. PHAGE STRAINS.

| Strain | Relevant features | Source | Reference |
|---|---|---|---|
| λcI857 Sam7 | Source of λ DNA | Noreen Murray | Goldberg & Howe (1969) |
| λrev | immλ cI857 | " | Gottesman et al (1974) |
| λ891* | red⁻ imm$^{21}$ nin5 | This thesis | |
| λb508 Nam7am53 cI857 nin5 chiD123 | | D.F. Ward | |
| λimm$^{434}$ qinA3 | | I. Herskowitz | Fiandt et al (1971) |
| λimm$^{21}$ b2 p4 | | " | " |
| λcI26 | | Noreen Murray | |
| λh$^{82}$ b522 cI skλ1 | | " | |
| λb2 imm$^{21}$ clear | | " | |
| λh$^{82}$ imm$^{21}$ clear | | " | |
| Ø21 | Related to λ by DNA homology | R.L. Baldwin | Simon et al (1971) |
| 434 | " " | " | " |
| Øm61 | " " , Spi⁻ | | Tsygankov et al (1976) |
| Øm151 | " " , " | | " |

* 891 is the stock number of this phage in this laboratory.

TABLE 1.3. BACTERIAL PLASMIDS.

(a) Plasmid Vectors:

| Plasmid | Confers resistance to | Source | Reference |
|---|---|---|---|
| pBR313 | Ap, Tc. | Noreen Murray | Bolivar *et al* (1977a) |
| pBR322 | Ap, Tc. | John Watson | Bolivar *et al* (1977b) |
| pBR325 | Ap, Tc, Cm. | " | Bolivar (1978) |
| ColE1 | colicin E1 | | |

(b) Recombinant Plasmids:

| Plasmid | Vector/host | Source of insert | Insert generated by | Reference |
|---|---|---|---|---|
| pK1[1] | pBR313/C600 | AB1157 | R.*Hin*dIII | This thesis. |
| pK2[1] | pBR322/C600 | " | R.*Eco*RI + R.*Hin*dIII | " |
| pK3[1] | pBR325/C600 | C600 | R.*Eco*RI | " |
| pK4[1] | " | " | " | " |
| pK5[3] | " | " | " | " |
| pK8[2] | " | F123 | " | " |
| pK9[2] | " | " | " | " |
| pK12[2] | pBR322/*E.coli* C-1a | " | R.*Hin*dIII | " |
| 605[1] | ColE1/C600*recA* | CS520 | Shearing | Clarke & Carbon (1976) |
| 1105[1] | " | " | " | " |

[1] derived from *qsr'* prophage; [2] from Rac prophage; [3] from third prophage in *E.coli* K12.

thiamine (B1), 0.2µg/ml; and where necessary, 20µg/ml of the appropriate amino acids, and 200µg/ml streptomycin (Sm).

M9 salt mix: $Na_2HPO_4$, 28g; $KH_2PO_4$, 11g; NaCl, 2g; $NH_4Cl$, 4g; water to 1 litre.

EMB agar: Difco agar, 10g; Difco Bacto tryptone, 4g; Difco Bacto yeast extract, 1g; NaCl, 2.5g; water to 450ml. Adjusted to pH6.5 before addition of 5ml EMB dye mix.

EMB dye mix: $K_2HPO_4$, 20g; eosin, 4g; methylene blue, 0.65g; water to 100ml.

Antibiotics: Used at the following concentrations and added to the molten medium immediately before pouring. Tetracycline (Tc), 25-50µg/ml; Ampicillin (Ap), 50-100µg/ml; chloramphenicol base (Cm), 100µg/ml.

Phage buffer: $KH_2PO_4$, 3g; $Na_2HPO_4$ (anhydrous), 7g; NaCl, 5g; 100mM $MgSO_4$, 10ml; 10mM $CaCl_2$, 10ml; 1% (w/v) gelatin solution, 1ml; water to 1 litre.

DNA buffer: 10mM Tris/HCl pH8.0; 1mM EDTA.

TES buffer: DNA buffer with 50mM NaCl.

Restriction endonuclease buffers (x10): 100mM Tris/HCl pH7.5; 100mM $MgCl_2$; 100mM β-mercaptoethanol; 0.05% Triton X-100; + 1M NaCl (R.EcoRI)
+ 500mM NaCl (other enzymes).

T4 polynucleotide ligase buffer (x10): 1M Tris/HCl pH7.5, 660µl; 400mM EDTA pH9.0, 25µl; 1M $MgCl_2$, 100µl; 1M β-mercaptoethanol, 100µl; 100mM ATP, 10µl; water to 1ml.

SSC: 150mM NaCl; 15mM Na citrate.

Agarose gel loading buffer: (final concentration) Ficoll 400, 10% (w/v); bromophenol blue, 0.025% (w/v); in water.

Phenol: Phenol, redistilled under $N_2$ and stored frozen at -20°C, was equilibrated with an equal

volume of 500mM Tris/HCl pH7.5 before use.

(d) Plating cells.

A fresh overnight culture was diluted 1:20 in L broth, grown with aeration at 37°C for two hours, and the cells were resuspended twice in half the initial volume of 10mM $MgSO_4$.

(e) Phage titration.

0.1ml of an appropriately diluted phage solution was mixed with 0.2ml of plating cells and the mixture was left at room temperature for 15min. to allow phage adsorption. Following addition of 2.5ml of molten BBL top agar, the mixture was poured on to a dry BBL agar plate and incubated overnight at the appropriate temperature ( usually 37°C).

(f) Phage stocks.

Phages from a single plaque were transferred with a sterile toothpick into 1ml of phage buffer, 1 drop of chloroform was added, and the solution was vortexed. After separation of the chloroform by low speed centrifugation, 0.1 to 0.2ml of this solution was mixed with 0.1ml of fresh plating cells. After 10 minutes for adsorption, 2.5ml BBL top agar was added and the mixture was poured onto a fresh L agar plate. Confluent lysis was observed after 6-10 hours at 37°C. The top layer was then harvested in 2.5ml of L broth to which 2 drops of chloroform had been added and the solution was vortexed. Following low speed centrifugation the supernatant was decanted, titred, and stored at 4°C. Titres varied from $5x10^9$ to $5x10^{11}$ /ml depending on the phage and bacterial host used.

(g) Phage crosses.

These were performed in the E.coli K12 strain QR47 which is $sup^+$ and $rec^+$ and is sensitive to both $h^{80}$ and $h^{\lambda}$ phages (h indicates the host range specificity). Freshly prepared QR47 plating cells were coinfected at a multiplicity of infection (m.o.i.) of 5 with the

two parent phages. After 15min. adsorption at room temperature the infected cells were diluted 1:20 into pre-warmed L broth and grown at 37°C for two hours. 3 drops of chloroform were added and after low speed centrifugation the supernatant was titred on a permissive host for total progeny and on a selective host for the required recombinant.

(h) Construction of lysogens.

Overnight cultures, starved by incubation in 10mM $MgSO_4$ for 1 hour, were infected with the appropriate phage(s) at a m.o.i. of 5-10. After 15min. pre-adsorption at room temperature, the cells were diluted 1:20 in L broth and were grown for 3 hours at the appropriate temperature (usually 33°C). Dilutions of this culture were spread onto dry EMB plates together with $10^9$ plaque forming units (p.f.u.) each of two homo-immune phages that have different host ranges and that carry clear mutations in their repressor genes (phages with such mutations are unable to form lysogens). In experiments designed to measure the frequency of lysogenisation, the dilution/3 hour growth step was omitted. Lysogens (and λ-resistant bacteria) survived this selection procedure and appear as pink staining colonies after about 20 hours of incubation at 33°C. To eliminate the λ-resistant clones, selected colonies were cross-streaked at 33°C against a homo- and a hetero-immune phage, both of which carry clear mutations in their repressor genes. Clones which were resistant to both phages were discarded.

(i) Test for red and gam genes.

Serial dilutions of the phage were spotted (approx. 10µl) on to a lawn of either lig-ts or polA1 cells on BBL agar plates. Wild type (red$^+$ gam$^+$) phages give normal size plaques whereas phages that are either red$^-$ or gam$^-$ do not grow or give very small plaques.

(j) Test for the Spi phenotype.

red$^-$ gam$^-$ phages are able to grow on a P2 lysogen.

Phages were tested by spotting dilutions onto a P2 lysogenic host.

(k) Construction of a plaque forming $N^-$ derivative of λ891. The putative λrecE phage (λ891), isolated following the insertion of fragments of E.coli K12 DNA (ED8659) into a $red^-$ $gam^+$ phage vector, was detected as a phage that was able to form plaques on both polA and lig-ts bacteria. $red^-$ phages do not normally form plaques on either of these hosts (Zissler et al, 1971). To test whether a $Red^-$ $Gam^-$ derivative of λ891 would have the $Spi^-$ $Fec^+$ phenotype diagnostic of λrev (Zissler et al, 1971: and see Section 3.2(c)), $N^-$nin5 derivatives of this phage were isolated. Such recombinants were selected on lig-ts $sup^o$ ($\lambda imm^{21}$) from a cross of λ891 with λb508 Nam7am53 cI857 nin5 chiD123. All $imm^{\lambda}$ recombinants must be $N^{\lambda}$ and hence Nam. λNam nin5 on infection of a suppressor-free host remains $N^-$ and consequently phenotypically $Red^-$ $Gam^-$. Nam nin5 derivatives of λ891 were analysed for their Fec and Spi phenotypes in suppressor-free hosts.

(l) Phage liquid lysates.
The host strain, typically C600, was diluted 1:20 from a fresh overnight culture into L broth containing 1mM $MgSO_4$, and grown to $O.D._{650}$ of approximately 0.5. Phage was added at a m.o.i. of 1 and shaking was continued until the $O.D._{650}$ reached a minimum (usually 2-4 hours after infection). 1ml/l of chloroform was added and after 15 min. shaking the lysate was clarified by centrifugation at 15,000g for 10min. in an MSE HS18 centrifuge. The supernatant was decanted and titred.

(m) Preparation of phage DNA.
Phages from a liquid lysate were pelleted by centrifugation at 21,000rpm (45,000g) for 3h at 6°C in a 10x100ml Al rotor. The phages were re-suspended overnight in a small volume of phage buffer by gentle shaking in the cold; any remaining bacterial debris

was pelleted at 10,000rpm for 10min. at 4°C in an MSE HS18 centrifuge; DNase and RNase (10µg/ml of each) were added to the decanted supernatant; and the mixture was incubated for 1h at room temperature. The phages were then purified on a CsCl step gradient as follows.

A stock solution of saturated CsCl (at 20°C) was diluted with phage buffer to give 66%, 50%, and 33% v/v solutions. 0.5ml of saturated CsCl was placed in the bottom of a 14ml centrifuge tube followed (gently) by 0.5ml of the 66% solution, 1.0ml of the 50% solution, 1.5ml of the 33% solution and approximately 9ml of the concentrated phage solution. The gradients were centrifuged in a 6x14ml Ti swing-out rotor at 33,000rpm (140,000g) for 2h at 20°C and phage bands were collected through the side of the tube with a sterile syringe and needle. Phages intended to provide DNA for <u>in vitro</u> recombination experiments were further purified by equilibrium centrifugation. The recovered phage bands from step gradients were mixed with an appropriate volume of pre-clarified CsCl solution (41.5% w/w) and centrifuged in a 6x5ml Ti swing-out rotor at 33,000rpm (110,000g) for 36h at 4°C. Phage bands were collected as above.

Recovered phage bands were dialysed against DNA buffer for at least 1h to remove CsCl (dialysis tubing was boiled in 2mM EDTA for 20min. before use) and DNA was released from the phage particles by gentle mixing with an equal volume of pre-equilibrated phenol followed by low speed centrifugation to separate the two phases. Two more phenol extraction steps were performed and the aqueous phase was dialysed in the cold against three changes of DNA buffer over a period of 36 hours.

DNA concentration was determined by measurement of the $O.D._{260}$. An $O.D._{260} = 1$ indicates a DNA concentration of approx. 50µg/ml. The ratio of

absorbance at 260nm and 280nm is indicative of the amount of contaminating protein present in the solution. For clean preparations this ratio was in the range 1.7-2.0:1. Absorbance at 320nm was also measured. This should be zero for a solution free from particulate matter.

(n) F123 DNA isolation.

Covalently closed circular (CCC) F123 DNA was prepared by the method of Guerry et al (1973) from 1 litre cultures of KL181/F123 grown to mid-exponential phase in L broth. The cells were harvested by centrifugation at 5,000rpm in a 6x250ml A1 rotor in an MSE HS18 centrifuge and the pellets were resuspended in 34ml of 25% sucrose, 50mM Tris/HCl pH8.0 at room temperature. Lysozyme (2.5ml of a 10mg/ml solution in 250mM Tris/HCl pH8.0) was added and the mixture was gently inverted 4 times and placed on ice. After 5 min., 12.5ml of a 20% (w/v) SDS solution in DNA buffer was added and the mixture was heat pulsed 8 times for 15sec. at 55°C with 5 inversions lasting 3sec. each in between each heat pulse. 25ml of TES and 16ml of a 20% (w/v) SDS solution was added followed by 31ml of 5M NaCl. The mixture was inverted 20 times during 1min. at room temperature and placed on ice overnight. Following centrifugation (13,000rpm for 30min. in a 6x250ml A1 rotor in an MSE HS18 centrifuge), the supernatant was decanted and the plasmid DNA was concentrated by PEG precipitation (Humphreys et al, 1975). 0.25vol. of a 50% PEG solution in DNA buffer was added and the mixture was placed on ice overnight. The PEG/DNA complex was precipitated by low speed centrifugation and the pellet was resuspended in 10ml of TES. Final purification was by ethidium bromide-CsCl gradient centrifugation at 38,000rpm for 40h in a Ti 50 rotor in a Beckman L2-65B ultra-centrifuge.

(o) Preparation of DNA from plasmids with a ColE1 replicon.

Overnight cultures of cells that contain such multi-

copy plasmids were diluted 1:20 into L broth and were grown with shaking at 37°C to mid-exponential phase. Chloramphenical was added, 250µg/ml, and the mixture was shaken overnight. This treatment inhibits bacterial replication but does not affect plasmid replication as quickly and therefore maximises the yield of plasmid from a culture. Cells were harvested by low speed centrifugation and lysates were prepared by the Triton lysis procedure (Katz et al, 1973). Cells from a 500ml culture were re-suspended in 6ml of 25% (w/v) sucrose, 50mM Tris/HCl pH8.0 and were placed on ice. 1ml of a 10mg/ml lysosyme solution in 250mM Tris/HCl pH8.0 was added and the mixture was gently shaken and placed on ice. After 5min., 1ml of 500mM EDTA pH8.0 was added, the mixture was gently shaken and again placed on ice. After 5min., 15ml of Triton mix (2ml of 10% Triton X-100; 25ml of 500mM EDTA pH8.0; 10ml of 1M Tris/HCl pH8.0; water to 200ml) was added and the mixture was gently shaken and allowed to stand at room temperature for 20min. The cells lyse during this period. The lysate was spun in an ultra-centrifuge at 25,000rpm for 25min. in a 8x50 Al rotor at 4°C and the super-natant was decanted. 0.95g of CsCl and 0.1ml of 5mg/ml ethidium bromide was added per ml of super-natant and the refractive index of the solution was measured in an Abbé refractometer and was adjusted to 1.392. This mixture was spun in the ultra-centrifuge for 60h at 38,000rpm in 10ml tubes in an 8x40 Ti rotor and at 20°C. Bands of DNA were visualised using long wavelength UV light and the lower (supercoiled plasmid) band was collected with a syringe and needle through the side of the tube. Ethidium bromide was removed by extracting three times with iso-propanol (pre-equilibrated with CsCl) and the aqueous phase containing plasmid DNA was dialysed in the cold against DNA buffer for 36h with three changes. DNA concentrations were measured as described for phage DNA.

(p) Bacterial DNA isolation.

Lysates of bacterial DNA prepared from 250ml cultures of exponentially growing cells by the Triton lysis procedure (Katz et al, 1973; described in (o) above) were incubated at 37°C for 3-6h with 100µg/ml pronase (autolysed for 1h at 37°C in distilled water prior to use). After gently extracting the lysates 3 times with an equal volume of phenol the aqueous phase was placed on ice and overlayed with 2 volumes of cold ethanol. After gentle inversion of the mixture (5-10 times) the precipitated DNA was collected on the end of a pasteur pipette and redissolved in 5ml of TES by gently shaking in the cold. After treatment with pancreatic RNase (pre-incubated in TES buffer for 10min. at 80°C) at a concentration of 20µg/ml for 3h at 37°C and gentle extraction with an equal volume of phenol the aqueous phase was overlayed with cold ethanol, precipitated and collected as described above. The precipitate was placed inside a dialysis tube with 1.5ml of TES buffer and dialysed for 36h in the cold against DNA buffer.

(q) Restriction endonuclease digestion of DNA.

DNA was digested with restriction endonucleases and 0.1 volumes of 10x restriction endonuclease buffer in a total reaction volume of 10-100µl. Digestions were performed at 37°C and were stopped by heating the solutions at 70°C for 10min.

(r) Ligation of DNA.

DNA cleaved with restriction endonucleases was diluted to 5-30µg/ml with 10mM Tris/HCl pH7.5, 100mM NaCl. 0.1 volumes of 10x ligase buffer and T4 DNA ligase was added, and the mixture was incubated at 10°C for 3-6h and then placed on ice for at least 24 h. before use.

(s) Transfection/transformation.

Recombinant phage or plasmid DNA molecules were recovered by transfection or transformation of a suitable bacterial host made competent for the uptake

of DNA by the method of Lederberg and Cohen (1974). Fresh overnight cultures were diluted 1:50 in L broth and were grown with shaking for 90min. at 37°C. Cells were harvested in the cold by low speed centrifugation, washed in 0.5 volumes of ice cold 100mM $MgCl_2$, resuspended in 0.05 volumes of ice cold $CaCl_2$, and kept on ice for at least 30min. prior to use.

Recombinant DNA molecules were diluted to approx. 0.5µg/ml in 0.1ml of SSC:$CaCl_2$ (SSC and 100mM $CaCl_2$ in the ratio 3:4) and 0.2ml of competent cells were added. The mixture was left on ice for 30min. and then heat shocked at 42°C for 2min. In the case of transfection, this mixture was placed on ice for 30min., serially diluted, and 0.1ml samples were mixed with 2.5ml of BBL top agar (supplemented with 10mM $MgSO_4$) and poured on to BBL agar plates. Transfection efficiency (for intact λ DNA) is usually in the range $10^5$-$10^6$ p.f.u./µg.

In the case of transformation, the DNA/recipient mixture was diluted with 1ml of L broth after the heat shock step and was incubated for 20min.-1h at 37°C before plating on selective (i.e. antibiotic containing) medium. $10^5$-$10^6$ transformants/µg may be obtained with intact plasmids.

(t) Agarose gel electrophoresis of DNA.

1µg DNA samples to which loading buffer had been added were separated by electrophoresis in 0.7% or 1% (w/v) agarose gels containing 40mM Tris/HCl pH8.2, 20mM Na acetate, 1mM EDTA, 0.5µg/ml ethidium bromide. Electrophoresis was usually carried out overnight at 1V/cm. The gels were photographed on a black background under UV light on Ilford FP4 film with a 4x red filter. Exposed film was developed in Microphen developer.

Gels were calibrated using DNA fragments of known sizes present in a mixture of λcI857 DNA digested with R.EcoRI, R.HindIII and with both enzymes. The sizes of these fragments have been estimated by Philippsen and Davis (1978).

(u) Isolation of DNA fragments from agarose gels.
Fragments of DNA were isolated from agarose gels by electrophoresis of the required band into a trough containing hydroxylapatite (HAP) essentially as described by Tabak and Flavell (1978). A narrow trough, just wider than the band, was cut with a scalpel immediately in front of the band and was filled with HAP in electrophoresis buffer. Electrophoresis was continued for approx. 30min. by which time the band of interest had become adsorbed by the HAP. The HAP was removed, placed in a small plastic tube, and subjected to centrifugation. The supernatant was removed and replaced by 0.2ml of 1M Na phosphate, 1mM EDTA pH6.8, and the mixture was vortexed. After centrifugation the (DNA containing) supernatant was removed and dialysed overnight in the cold against DNA buffer with two changes. This solution (approx. 0.3ml) was used for "nick translation" without further concentration.

(v) In vitro labelling of DNA (nick translation).
DNA was labelled *in vitro* with $^{32}P$ by "nick translation" and denatured by heating at 100°C for 5min. prior to use (Maniatis et al, 1975).

Reaction was usually carried out in 100µl containing the DNA to be labelled (approx. 1µg); 10-20 µCi of $^{32}P$-dGTP, specific activity approx. 350 Ci/mmol (dried down before use); 180pmol each of the unlabelled dNTPs (dATP, dCTP, TTP); 50mM Tris/HCl pH7.8; 5mM $MgCl_2$; 10mM β-mercaptoethanol; 0.5µl (approx. 1 unit) of DNA polymerase I; and 1µl of $10^{-7}$g/ml DNaseI (freshly diluted). This solution was incubated for 2h at 10°C after which time 1 drop of phenol was added. The mixture was vortexed and applied to the top of a Sephadex G-50 (fine) column (approx. dimensions 8cmx0.5cm) together with a small amount of Orange G marker dye. TES was used as an elution buffer. Labelled DNA elutes well ahead of the dye which runs with the unincorporated nucleotides.

Small fractions (2-10 drops) were collected and their activity measured in a scintillation counter. Peak fractions were selected for hybridisation experiments. Specific activities were in the range $1-2 \times 10^7$ d.p.m./µg. of DNA.

(w) Southern's gel transfer method and DNA:DNA hybridisation.

DNA fragments, separated by electrophoresis in agarose gels, were transferred overnight to nitrocellulose filters (Southern, 1975). In this method the fragments are denatured in the gel by immersion of the gel in 0.5M NaOH, 1.5M NaCl. After 30min., the alkali is neutralised by immersion of the gel in 1M Tris, 3M NaCl pH6.0, for 45min. and the gel is laid onto a bed made of several sheets of blotting paper on top of a glass plate. The blotting paper is allowed to overlap the glass plate so that two of its edges may hang down into a trough containing 20x SSC. Two strips of plastic the same thickness as the gel (3-4mm) are placed parallel with the long edges of the gel and separated by 0.5cm from it. A sheet of nitrocellulose filter (pre-wetted in water) is placed over the gel such that it covers the whole gel and rests on the plastic spaces on either side. A wad of blotting paper (approx. 2cm deep) cut to the size of the nitrocellulose sheet is placed on top. Transfer of 20x SSC occurs by capillarity and DNA is eluted out of the gel and on to the nitrocellulose filter where it binds. The set up is usually left overnight after which time the nitrocellulose filter is removed, washed in 2x SSC, dried at room temperature and baked at 80°C for two hours.

Before hybridisation, nitrocellulose membranes prepared as above were pre-incubated for 3-6h at 65°C in 6x SSC, 0.2% SDS, 0.1% bovine serum albumen (BSA), 0.1% Ficoll 400, 0.1% polyvinylpyrollidone (PVP) (adapted from Denhardt, 1966). Hybridisation was carried out with $10^5$-$10^6$ cpm of $^{32}$P-labelled DNA per filter in 6x SSCP, 0.1% BSA, 0.2% SDS, 0.1% Ficoll 400, 0.1% PVP at 65°C for 24-36h in sealed plastic bags. SSCP is 120mM NaCl, 15mM Na citrate,

13mM $KH_2PO_4$, 1mM EDTA pH7.2. Unless otherwise stated filters were washed in 4x SSC, 0.5% SDS (four times, 60min. each at 65°C), and rinsed in 2x SSC at room temperature. After drying in air at room temperature the filters were placed under pre-sensitised X-ray film and a phosphotungstate intensification screen was placed over both (Laskey and Mills, 197). Exposure times ranging from overnight to 3 weeks at -70°C were used.

(x) Colony hybridisation.

Transformant colonies whose pattern of antibiotic resistance showed them to contain recombinant plasmids were screened with radioactive DNA probes by the Lis/Prestidge modification of the Grunstein and Hogness (1975) colony hybridisation procedure, in order to select for plasmids of interest. Insertion of a foreign DNA fragment into the R.HindIII site of pBR322 or pBR313 (both $Ap^R$, $Tc^R$) and subsequent transformation may yield an $Ap^R$, $Tc^S$ bacterium since the unique R.HindIII site in these plasmids lies within the promoter for the $Tc^R$ determinant. Similarly, insertion at the R.EcoRI site of pBR325 ($Ap^R$, $Tc^R$, $Cm^R$) and subsequent transformation yields an $Ap^R$, $Tc^R$, $Cm^S$ bacterium since the unique R.EcoRI site in this plasmid lies within the $Cm^R$ determinant.

Appropriate colonies were picked with sterile toothpicks into an orderly array on L agar plates containing Ap (the master plates) and were grown overnight at 37°C. The masters were then replica plated onto nitrocellulose filters (cut to the size of a Petri dish) on the surface of L agar plates containing Ap. These plates were incubated overnight at 37°C and the master plates were stored at 4°C. The filters, now carrying an orderly array of bacterial colonies, were taken through the following treatments by placing them colony side up on blotting paper saturated with the appropriate solutions:

(i) 0.5M NaOH for 7min.

(ii) 1M Tris/HCl pH7.4 for 2min (twice).

(iii) 1.5M NaCl, 0.5M Tris/HCl pH7.4 for 4min.

The filters were blotted dry on filter paper and were baked under vacuum at 80°C for two hours. Pre-incubation, hybridisation (approx. $5 \times 10^4$cpm of the appropriate radioactive DNA per filter), washing and autoradiography were performed as described in (w) above.

In the case of libraries representing the whole E.coli K12 genome, approx. 3,000 colonies were screened at a time in order to give a reasonable chance of finding the required recombinant. For libraries of F123 DNA fragments, only 200-300 colonies were screened. Colonies showing significant hybridisation were picked from the master plate, restreaked, and large cultures were prepared from which the plasmid of interest was isolated.

(y) Electron microscopy and preparation of heteroduplexes. Heteroduplexes of phage DNAs were prepared for electron microscopy as described by Davis et al (1971). Solutions of heteroduplex DNA, cytochrome C, Tris, EDTA and formamide were mixed to give final concentrations of 100mM Tris, 10mM EDTA, 48% formamide, and 0.18mg/ml of cytochrome C. These solutions were made from a stock solution of Tris and EDTA adjusted to pH8.6. The molecules were mounted on parlodion films, stained with uranyl acetate and rotary shadowed with platinum. Pictures were taken with a Siemens Elmiskop 101 electron microscope operated at 80kv and x20,000 magnification. Images of the molecules were projected at x5 magnification onto paper using a photographic enlarger and were traced with a pen. Measurements were made with a map measurer.

# CHAPTER 3

## The Rac Prophage in *E.coli* K 12

## 3.1. INTRODUCTION.

The presence of a defective prophage in many E.coli K12 strains is supported by a number of different lines of genetic evidence. Low (1973) first suggested such a prophage as a result of his investigation of the rac (recombination activation) locus, situated a few minutes clockwise of the trp operon on the genetic map. DNA introduced into a $Rec^+$ (recombination proficient) recipient by Hfr mating can recombine with the bacterial chromosome. Recipients that carry mutations in the recB and/or recC genes (the products of which form a single enzyme, exonuclease V) yield less than 1% of the $Rec^+$ level of recombinants. Low (1973) found that transfer of the $rac^+$ locus by Hfr mating into $rac^-$ $recB^-$ or $rac^-$ $recC^-$ cells almost totally restored the recombination proficiency of the merozygotes. The recombinant progeny, however, retained the $Rec^-$ phenotype and were almost always $rac^-$. To account for this result Low proposed that the rac locus is carried on a defective prophage (the Rac prophage) whose functions are normally repressed but which may be zygotically induced by transfer into a $rac^-$ cell in which the prophage and hence the prophage repressor is absent. Segregation of $rac^-$ progeny is thus consistent with the loss of the hypothetical prophage from the cell line. Low performed analagous experiments with a defective λ prophage (described by Lloyd, 1974). He found that if a $\lambda P^-$ prophage was introduced into a $recB^-\lambda^-$ recipient, the yield of recombinants for markers distal to the prophage approached that obtained with $Rec^+$ recipients. This effect was not observed if the prophage was also $red^-$ (red codes for the λ exonuclease). These results suggested that the Rac effect might also be mediated by induction of a prophage encoded exonuclease function.

Recombination proficiency is also restored to $recB^-$ or $recC^-$ strains by acquisition of the sbcA mutation (Barbour et al, 1970; Templin et al, 1972). $sbcA^-$ appeared to be a regulatory mutation resulting in derepression of the recE gene which codes for the ATP-

independent exonuclease VIII (ExoVIII) (see also Clark, 1973; Kushner et al, 1974). As well as being able to replace the recBC product (Exo V) in bacterial crosses, Exo VIII can replace λ-exonuclease in phage crosses (Unger et al, 1972). Since sbcA mutants cannot be isolated from the rac⁻ strain AB1157 (Templin et al, 1972) and sbcA maps close to rac (Lloyd and Barbour, 1974), it seems possible that recE is equivalent to (or a component of) rac.

Evidence supporting Low's hypothesis was provided by characterisation of λ reverse (λrev), a derivative of phage λ in which the λ recombination functions have been replaced by new recombination functions (Der) derived from the host chromosome (Gottesman et al, 1974). These functions include a general recombination system and probably a site specific recombination system analogous to Int but with different site specificity, lysogens of λrev having the prophage located close to rac. λrev could not be derived from, nor could it lysogenise, the rac⁻ strain AB1157. Gillen et al (1977) have compared the ATP-independent exonuclease produced by λrev with ExoVIII, the recE product, and have found them to be indistinguishable by several biochemical criteria.

The generation of λrev can therefore be most simply explained as the result of recombination between λ and the Rac prophage. Considerable evidence based on cross-hybridisation studies is available to suggest that sequences homologous to regions of the λ genome are present in the DNA of E.coli K12 (see Chapter 1(b)).

The following functions have also been attributed to the Rac prophage. (a) Penny Toothman (personal communication) has found a Ral (restriction alleviation) activity in λrev which she has called Lar. A similar activity is associated with sbcA mutants (Simmon and Lederberg, 1972). The recE gene may be the determinant for both the Der and Lar activities (Gillen et al, 1977). (b) the λ derivatives qin (Q-independent) (Sato and Campbell, 1970), p4 (Jacob and Wollman, 1954) and p41 (Henderson and Weil, 1975) contain host derived DNA coding for functions analogous to the λ

functions Q, S and R. As with λ*rev*, these λ*qsr'* phages cannot be derived from the *rac*⁻ strain AB1157 (Strathern and Herskowitz, 1975; Henderson and Weil, 1975).
(c) Diaz and Pritchard (1978) have isolated as a plasmid a region of the *E.coli* K12 chromosome that contains a replication origin (*oriJ*), the behaviour of which led them to propose that it is derived from the Rac prophage (see Section 3.2(g)).

The experiments described in this chapter demonstrate the presence of λ homologous sequences within that region of the *E.coli* K12 chromosome thought to contain the Rac prophage. Restriction endonucleases were used to construct a physical map of this region, and the relative positions of the *recE* gene (*der* and possibly *lar*), the replication origin *oriJ*, and the regions homologous to λ DNA were ascertained. In contrast, host DNA present in λ*qsr'* phage is not derived from the Rac prophage. Cleavage mapping with restriction endonucleases, cross-hybridisation, and electron microscopy were used to compare the DNA of λ*rev* with that of a similar phage generated *in vitro* by ligation of R.HindIII cleaved *E.coli* K12 DNA and a λ replacement vector. These experiments show that the λ*rev* substitution contains sequences present at two non-adjacent sites within the Rac prophage. Low (1973) has suggested that the Rac prophage may be excised from the bacterial chromosome following derepression. It seems probable that this process occurs spontaneously at a low frequency by a process analogous to excision of the λ prophage, and that recombination between λ DNA and the circular DNA molecule so generated is necessary for the formation of λ*rev*.

## 3.2. RESULTS.

### (a) Detection of E.coli DNA sequences homologous to λ DNA.

Phage λ DNA, labelled with $^{32}P$ by 'nick translation', was used as a probe to detect homologous sequences in the DNAs of several different E.coli strains (Fig. 3.1, see legend for details). The hybridisation pattern observed in tracks (a) and (b) appears to be typical of E.coli K12 strains in general and is different from that observed in the DNA of E.coli B and E.coli C strains (tracks (d) and (e)). DNA prepared from a C600 (λ) lysogen has the pattern of the non-lysogen superimposed on bands which derive from the λ prophage (data not shown). Thus it is unlikely that any of the observed homologies in the non-lysogenic E.coli K12 strains lie close to or within attB, the bacterial attachment site for λ.

DNA from the rac$^-$ strain AB1157 also contains restriction endonuclease fragments homologous to λ DNA (track (c)), several of which are of the same size and have the same relative intensities as the bands seen in tracks (a) and (b). On the other hand, several bands present in tracks (a) and (b) are either missing completely or changed in size in AB1157 DNA. In the light of Low's hypothesis discussed above, it seemed likely that these differences reflect the presence in most E.coli K12 strains of a defective prophage(s), related to λ, which has undergone change in AB1157, possibly by deletion. However, on the sole basis of hybridisation with λ DNA, it was not possible to make a simple and unambiguous interpretation of the observed differences between C600 and AB1157 DNAs.

### (b) A specific subset of λ homologous sequences is carried by F123.

In view of the difficulties in interpreting the λ homologies in C600 and AB1157 DNAs, and the possibility that more than one defective prophage is present in these strains, investigations were confined to the small section of the E.coli K12 chromosome carried by F123 (Fig. 3.2(a)).

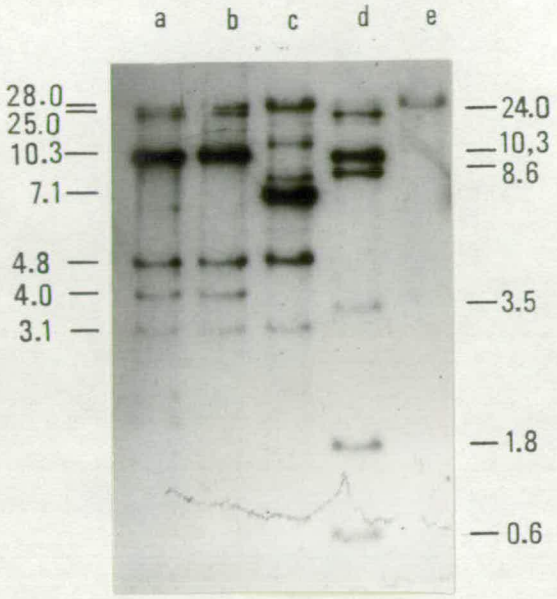


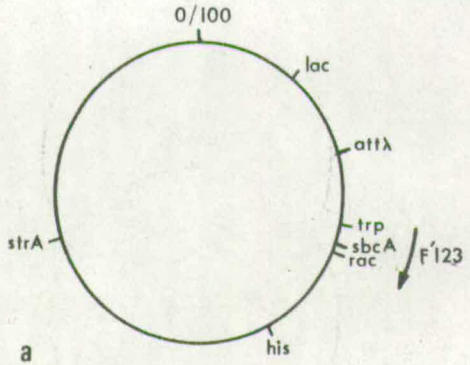

Fig. 3.1. Autoradiograph showing those R.HindIII fragments in several E.coli DNAs which share homology with λ DNA. Electrophoresis was carried out in a 1% agarose gel. Sizes of identified bands are given in kilobase pairs (kb). Tracks (a) and (b): CR63 and C600 DNAs respectively. This pattern is typical of E.coli K12 strains in general. Track (c): AB1157 DNA. Three bands (28kb, 4.8kb, 3.1kb) are of the same size and relative intensity as bands in C600 DNA digests. Two C600 DNA fragments (25kb and 4.0kb) are not found in AB1157 DNA digests. A 10.3kb fragment in C600 DNA digests appears to have been altered to give a 7.1kb fragment in AB1157 DNA. The two unidentified bands in AB1157 DNA are due to incomplete digestion of this DNA with the enzyme. Tracks (d) and (e): E.coli B and E.coli C DNAs respectively.

F123 is an F' factor capable of lysogenisation by λrev and known to carry the rac and sbcA loci (Gottesman et al, 1974). Fig. 3.2(b) shows that a specific subset of the λ homologous fragments present in C600 DNA is represented in the region of the chromosome carried by F123 (see legend to figure for details). This result provides additional evidence relating λ homologies in E.coli K12 strains to the Rac phenomenon, especially since the only two λ-homologous R.HindIII fragments present in both C600 and F123 DNAs (25kb and 4.0kb) are missing in AB1157 DNA (see Fig. 3.1). The set of λ-homologous fragments present in C600 and AB1157 DNAs, but not carried by F123, are derived from a defective prophage(s) other than the Rac prophage (see below and Chapter 5).

(c) In vitro construction of a λrecE phage (λ891).

Wild type λ will grow on recombination deficient (recA$^-$) E.coli but not on E.coli lysogenic for phage P2 (Spi$^+$ phenotype: susceptibility to P2 interference, see legend to Fig. 1.3). λ phages deficient in the phage genes red and gam are unable to grow on recA$^-$ hosts (Fec$^-$ phenotype, see legend to Fig. 1.3) but are able to grow on P2 lysogens (Spi$^-$ phenotype). Phage λ mutant in either the red or gam genes is unable to grow on Feb$^-$ hosts (e.g. polA$^-$, ligts) (Zissler et al, 1971).

The Fec$^+$ and Spi$^-$ phenotypes appear to be mutually exclusive, yet the λ derivative λrev is able to grow on both recA$^-$ and P2 lysogenic hosts (Zissler et al, 1971). Gottesman et al (1974) have shown that λrev contains host derived recombination genes which share no homology with those deleted from λ and which are probably derived from the Rac prophage. Since three independent isolates of λrev appear, by heteroduplex mapping, to result from identical substitutions and deletions (Gottesman et al, 1974), it seems possible that λrev is generated by recombination at specific sites of homology between λ and the Rac prophage. Gillen et al (1977) who have shown that λrev codes for ExoVIII, the recE product found in sbcA$^-$ strains, suggest that ExoVIII (M.W. $1.4 \times 10^5$) may be equivalent to a gene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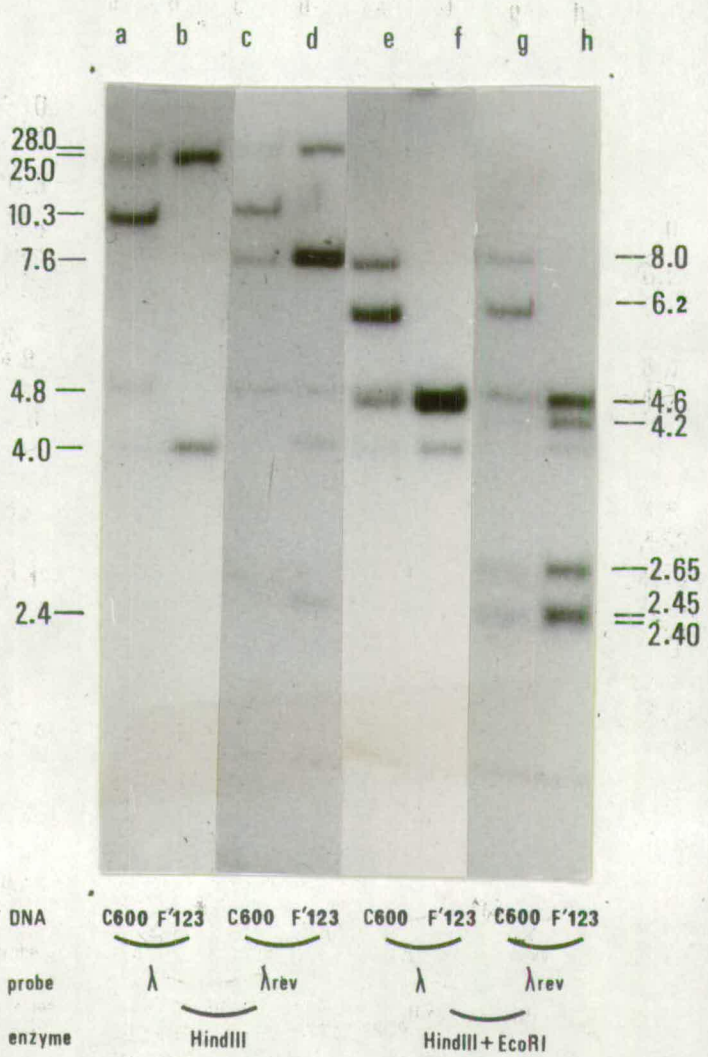
lac
attλ
trp
sbcA
rac
F'123
strA
his

a


a b c d e f g h
28.0
25.0
10.3
7.6
4.8
4.0
2.4
8.0
6.2
4.6
4.2
2.65
2.45
2.40
DNA C600 F'123 C600 F'123 C600 F'123 C600 F'123
probe λ λrev λ λrev
enzyme HindIII HindIII + EcoRI

b


a b c
17.5
15.2
13.6
8.4
6.5
4.6
4.2
2.65
DNA F'123 F'123 C600

c

Fig. 3.2. A specific subset of λ-homologous sequences present in C600 DNA is carried by F123. F123 and C600 DNA also share considerable homology with substitutions present in the DNAs of λ891 and λ*rev*.

(a) Genetic map of *E.coli* K12 after Bachmann *et al* (1976). The section of the chromosome carried by F123 is shown (Low, 1972).

(b) Autoradiographs showing fragments in digests of C600 and F123 DNA which share homology with λ and λ*rev* DNA. Electrophoresis was carried out in a 1% agarose gel. Comparison of tracks (a) and (b) and of tracks (e) and (f) shows that a specific subset of λ-homologous fragments is carried by F123. In R.*Hin*dIII digests, this subset consists of those fragments (25kb and 4.0kb) which are not seen in AB1157 DNA digests (see Fig. 3.1). The 4.0kb R.*Hin*dIII fragment is not easily visible in C600 DNA digests in this autoradiograph. λ-homology in the 25kb R.*Hin*dIII fragment is contained within a 4.6kb R.*Eco*RI + R.*Hin*dIII fragment (tracks (e) and (f)). The 4.6kb fragment is seen in C600 DNA digested only with R.*Eco*RI (see Fig. 3.9) and is thus internal to the 25kb R.*Hin*dIII fragment.

The hybridisation pattern in C600 (tracks (c) and (g)) and F123 (tracks (d) and (h)) DNAs digests seen with a λ*rev* DNA probe contains all fragments observed with a λ DNA probe due to interaction between the two arms of λ*rev* DNA (see Fig. 3.3) and those fragments. New bands are also seen (7.6kb and 2.4kb in the case of R.*Hin*dIII digests). The 7.6kb fragment is cleaved to three fragments by R.*Eco*RI (2.65kb, 2.45kb, 2.40kb) (track h). The 2.65kb fragment is an internal R.*Eco*RI fragment (see Fig. 3.2(c)). A 4.2kb R.*Eco*RI fragment (tracks (g) and (h) and Fig. 3.2(c)) which is internal to the λ-homologous, 25kb R.*Hin*dIII fragment is also seen. DNA from the λ*rev* substitution is therefore closely associated with λ-homologous sequences in the C600 chromosome (see section 3.2(e).

(c) Autoradiograph showing R.*Eco*RI fragments in F123 and C600 DNAs which share homology with λ*rev* DNA and with the λ891 substitution. Electrophoresis was carried out in a 0.7% agarose gel. Track (a): fragments in F123 DNA that share homology with the purified 7.6kb R.*Hin*dIII fragment which comprises the λ891 substitution. Tracks (b) and (c): fragments in the DNAs of F123 and C600 respectively which share homology with λ*rev* DNA. Comparison of 3.9. shows that in addition to λ-homologous sequences, several new bands are seen. A 17.5kb fragment is peculiar to C600 DNA. Three new fragments (8.4kb, 4.2kb, 2.65kb) are common to C600 and F123 DNAs. The latter two are not cleaved by R.*Hin*dIII (see Fig. 3.2(b)). A common λ-homologous 13.6kb fragment (see also Fig. 3.9) hybridises strongly with the λ*rev* substitution. The 13.6kb, 8.4kb and 2.65kb fragments are the same size as fragments which hybridise with the λ891 substitution (track (a)). The 7.6kb substitution in λ891 DNA (see Fig. 3.3(b)) is thus equivalent to the same size fragment present in R.*Hin*dIII digests of C600 and F123 DNAs and which hybridises with the λ*rev* substitution (see Fig. 3.2(b), tracks (c) and (d)).

fusion product possessing some, if not all, of the activities of the λ proteins it replaces in λ*rev*.

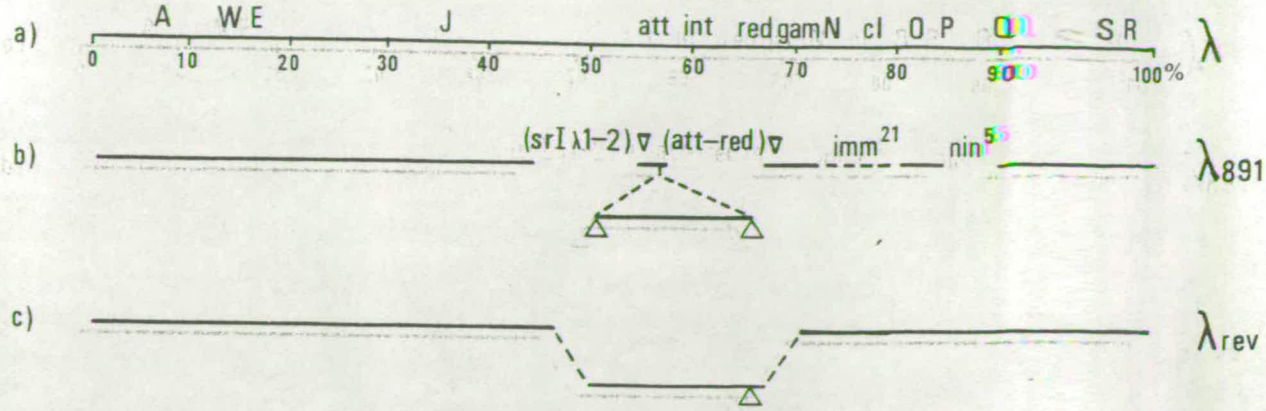
Phages including the *recE* gene which it was hoped would contain host derived DNA in addition to that carried by λ*rev*, were sought among the population of *in vitro* recombinants formed by inserting into a Red$^-$ vector, the R.*Hin*dIII digestion products of *E.coli* K12 DNA isolated from the strain ED8659 (Kelley *et al*, 1977). Red$^-$ phages able to form plaques on both *polA*$^-$ and *ligts* hosts were potential candidates. Such a phage (λ891) was isolated and *Nam nin*5 derivatives were made. The *nin*5 deletion permits the *Nam* phages to form plaques in the absence of a suppressor (Court and Sato, 1969). In the absence of a suppressor these *N*$^-$, and hence phenotypically Red$^-$ Gam$^-$, phages were shown to resemble the classical λ*rev* in that they were both Spi$^-$ and Fec$^+$. Subsequently it was found that when gene *N* of the *Nam nin*5 derivatives of λ891 was suppressed these phages remained Spi$^-$ and Fec$^+$ and that λ891 itself, despite the presence of the *gam* gene, had the λ*rev* phenotype. λ891 contains a 7.6kb R.*Hin*dIII fragment (Fig. 3.3) derived from *E.coli* K12 DNA and is described in more detail below.

(d) *Physical characterisation of λ891 and λrev phages.*

The DNAs of λ891 and the classical λ*rev* were compared by heteroduplex mapping, digestion with restriction endonucleases, and cross-hybridisation:

(i) *Heteroduplex mapping*:

λ/λ*rev* heteroduplexes reveal that the λ*rev* substitution shares no homology with the λ DNA it replaces (Gottesman *et al*, 1974). λ891/λ and λ891/λ*rev* heteroduplexes were constructed and analysed. Their interpretation was aided by the fact that λ891 is *imm*$^{21}$ and carries the *nin*5 deletion. As with λ*rev*, the bacterial DNA substitution in λ891 shares no homology with λ DNA (data not shown). In λ891/λ*rev* heteroduplexes however (Fig. 3.4), a region of homology between the two substitutions extending for 5.9± 0.2kb can be detected. This region of homology


a)
A
W E
J
att int red gam N cI O P
S R
λ
0
10
20
30
40
50
60
70
80
90
100%
b)
(srI λ1–2) ∇ (att–red) ∇
imm21
nin5
λ891
c)
λrev

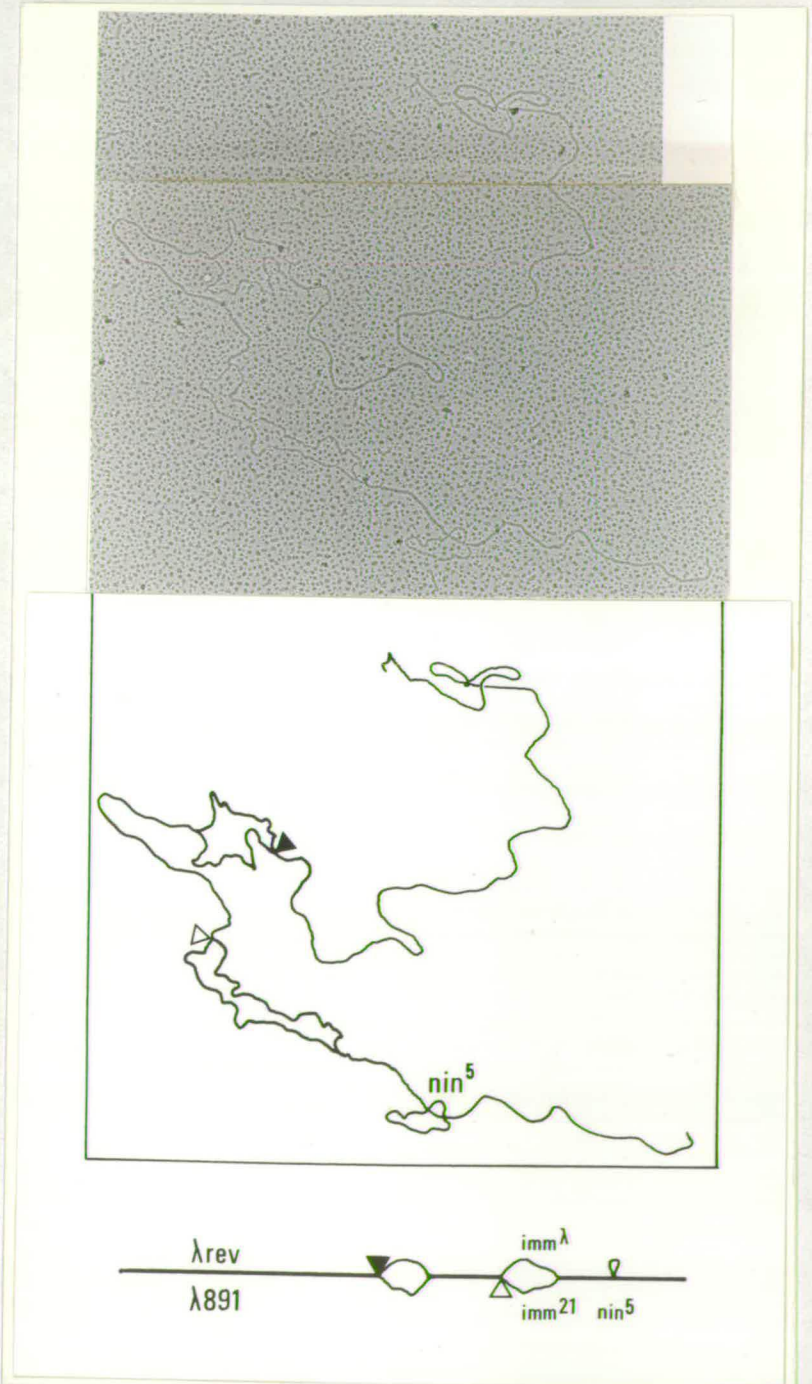

nin5
λrev
λ891
immλ
imm21
nin5

Fig. 3.3. Physical characterisation of λ891 and λ*rev* DNA.

(a) Genetic and physical map of the wild type λ genome. The scale 0 to 100 represents the length of $\lambda^+$ DNA. 100 λ units = 49kb (Philippsen and Davis, 1978).

(b) The λ891 genome (see text for details). The left end of the srIλ1-2 deletion is an R.*Eco*RI site at 44.5 units (21.8kb). λ891 contains a 7.6kb R.*Hin*dIII fragment derived from *E.coli* K12 DNA.

(c) The λ*rev* genome is generated by substitution of an *E.coli* K12 DNA fragment for the region of the λ genome shown deleted. The left end of the λ DNA deletion lies between *sbam*λ2 (46.6 units) and *shin*λ1 (47.2 units). The right end lies at approximately 71 units and the length of the λ*rev* substitution is approximately 18 λ units or 8.8kb (Gottesman *et al*, 1974).

△ indicates the positions of R.*Hin*dIII sites within the λ891 and λ*rev* substitutions (see Fig. 3.5).

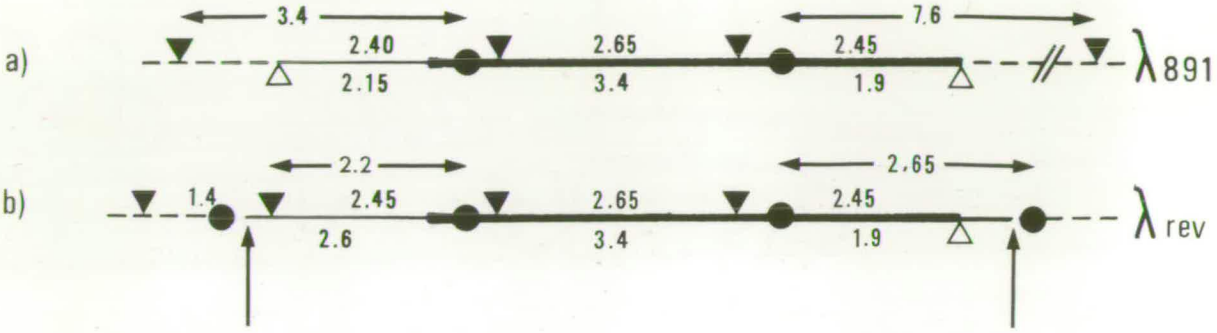
Fig. 3.4. An electron micrograph of a λ891/λ*rev* DNA heteroduplex. The left arms of λ and λ*rev* DNAs are identical up to the R.*Eco*RI site ( ▼ ) at 44.5 units (21.8kb) (see legend to Fig. 3.3). The position of the *nin*5 deletion with respect to the right end of λ891 DNA is also known (10.8 λ units = 5.2kb) (Fiandt *et al*, 1971). These positions serve as internal standards. A schematic diagram of the heteroduplex is shown. A region of homology between the λ891 and λ*rev* substitutions lies to the left of the immunity regions and measures 5.9± 0.2kb (n = 15), considerably shorter than either substitution. The R.*Hin*dIII site ( △ ) defining the right hand end of the λ891 substitution is shown (see Fig. 3.5). Heteroduplexes were made and analysed by Pam Beattie.

lies to the right hand side of the substitutions in both phages and is significantly shorter than both substitutions (Fig. 3.5).

(ii) Restriction endonuclease mapping and cross-hybridisation. The DNAs of λ891 and λrev were digested with R.EcoRI, R.HindIII and R.BamHI, and fragment sizes were estimated by comparison with digests of λcI857 DNA following separation by electrophoresis through 1% agarose gels (data not shown). The positions of the cleavage sites for these enzymes in both substitutions are shown in Fig. 3.5. Comparison of these maps shows that in the regions indicated by the heavy lines, cleavage sites occur in equivalent positions in both substitutions. To the left of these regions there is no such equivalence. A radioactive probe made to the purified 7.6kb R.HindIII fragment comprising the λ891 substitution hybridises strongly to restriction endonuclease fragments contained within the heavy line in the λrev substitution and weakly, or not at all, to those overlapping with or those totally excluded from the heavy line (Fig. 3.6). These results are in agreement with the λ891/λrev heteroduplex observations.

That the λ891 substitution isolated from ED8659 is also present in the other E.coli K12 strains studied was ascertained by hybridising the purified, $^{32}$P-labelled, 7.6kb R.HindIII fragment from this phage with R.HindIII digests of C600, CR63 and F123 DNAs. A single 7.6kb fragment was seen in all three cases (data not shown). Since this fragment must then represent a continuous segment of the E.coli K12 chromosome, the substitution in λrev would appear to contain DNA from two non-adjacent regions of the chromosome.

Low(1973) hypothesised that the induction event leading to expression of the rac locus after transfer by Hfr mating into the rac$^-$ strain AB1157 is accompanied by excision of the Rac prophage from the donor chromosome. By analogy with the λ prophage, excision would require a site specific


a)
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λ891
b)
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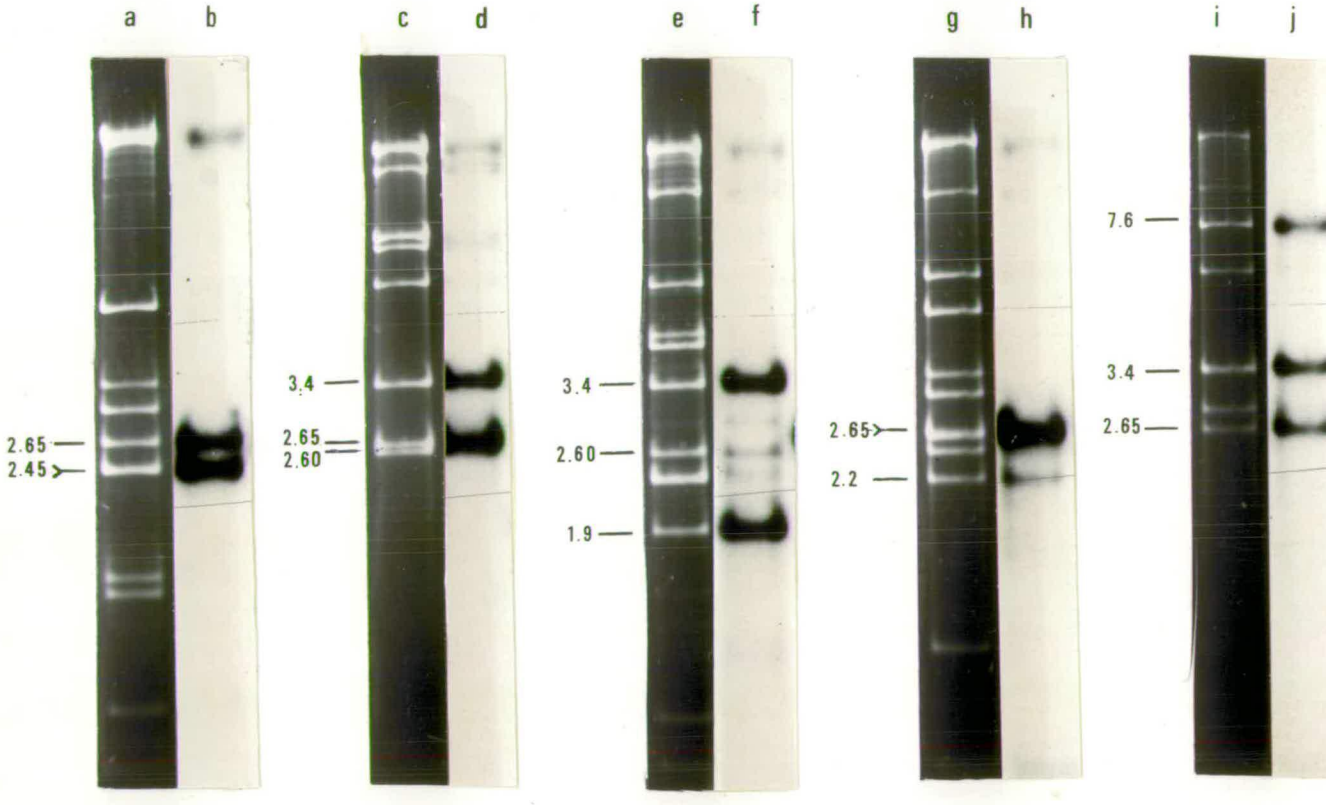
λ rev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3.4
2.65

Fig. 3.5. A comparative analysis of the DNA substitutions in λ891 (a) and λrev (b) using the restriction endonucleases R.EcoRI (▼), R.HindIII (△), and R.BamHI (●). Sizes (in kb) of fragments generated by double digestion with R.BamHI + R.HindIII (below the line) and R.BamHI + R.EcoRI (immediately above the line) are indicated. Sizes of some other fragments are shown.

---- indicates λ DNA. ↑ indicates the approximate end points of the λrev substitution. ▬▬▬ indicates the region of homology between the substitutions as measured in λ891/λrev heteroduplexes (5.9kb) (see legend to Fig. 3.4). A 2.65kb R.EcoRI fragment and a 3.4kb R. BamHI fragment are common to both substitutions and lie to the left of a common R.HindIII site (which defines the right end of the λ891 substitution). Thus, all cleavage sites within the heavy lines are at equivalent positions in both substitutions. No such equivalence is evident to the left of the heavy lines. DNA from this region of the λrev substitution fails to cross-hybridise with the λ891 substitution (see Fig. 3.6) suggesting that the attachment sites of λ891 and λrev are located at the left ends of the heavy lines (see text).

Fig. 3.6. Restriction endonuclease fragments in digests of λrev DNA which cross-hybridise with the purified, $^{32}$P-labelled, λ891 substitution (compare with Fig. 3.5). Electrophoresis was carried out in a 1% agarose gel. ⊱ indicates an unresolved doublet. Track (a): R.EcoRI + R.HindIII digest of λrev DNA. Track (b): Fragments in (a) which hybridise with the λ891 substitution. Both 2.45kb fragments cross-hybridise. Hybridisation to R.EcoRI digests of λrev (data not shown) shows that the leftmost 2.45kb fragment in the λrev substitution (see Fig. 3.5) shares a relatively small degree of homology with the λ891 substitution. Track (c): R.BamHI digest of λrev DNA. Track (d): fragments in (c) which hybridise with the λ891 substitution. Both the 2.65kb and 2.60kb fragments cross-hybridise, the latter only weakly however (see track (f)). Track (e): R.BamHI + R.HindIII digest of λrev DNA. Track (f): fragments in (e) which hybridise with the λ891 substitution. The 2.60kb fragment hybridises very weakly. Track (g): R.BamHI + R.EcoRI digest of λrev DNA. Track (h): fragments in (g) which hybridise with the λ891 substitution. Both 2.65kb fragments hybridise strongly (see tracks (b) and (d)). The 2.2kb band is relatively weak. Track (i): R.BamHI + R.HindIII digest of λ891 DNA. Track (j): fragments in (i) which hybridise with the λ891 substitution.

Cross-hybridisation between the λ891 and λrev substitutions takes place only within the regions indicated by heavy lines in Fig. 3.5.

recombination between two flanking hybrid attachment sites, i.e. between BP' and PB' (Fig. 3.7). If the Rac prophage is flanked by two such attachment sites, induction and circularisation may occur spontaneously, and specifically, albeit at a low frequency. It seemed likely that such a circular molecule is involved in the recombination event leading to the formation of λrev as this would bring together, within the λrev substitution, two previously non-adjacent regions of the chromosome (Fig. 3.7).

According to this model, λ891 and λrev may be distinguished on the basis of the type of attachment site they carry. λrev should have a pure phage attachment site, PP', flanked by prophage DNA sequences on both sides, while λ891 should have a hybrid attachment site, BP' (see Section 3.3 and Fig. 3.12(a)) flanked on the right by prophage DNA and on the left by host DNA. Approximate positions for these postulated attachment sites in λ891 and λrev can be deduced since they will lie **at** the left end of the region of homology between the substitutions in these two phages (i.e. at the left ends of the heavy lines in Fig. 3.5).

(e) Confirmation of the above model.

On the basis of the above model, several testable predictions can be made:

(i) Restriction endonuclease fragments spanning BP' cross-hybridise relatively weakly with the λrev substitution

λ891 and λrev have identical R.EcoRI fragments (2.65kb) internal to their substitutions, and lying to the right of their hypothesised attachment sites (Figs. 3.5 and 3.12(a)). Thus the substitutions in both these phages should hybridise not only with this common fragment but also with the same flanking chromosomal fragments (in both C600 and F123 DNAs). The chromosomal fragment to the left spans the hybrid attachment site BP' and

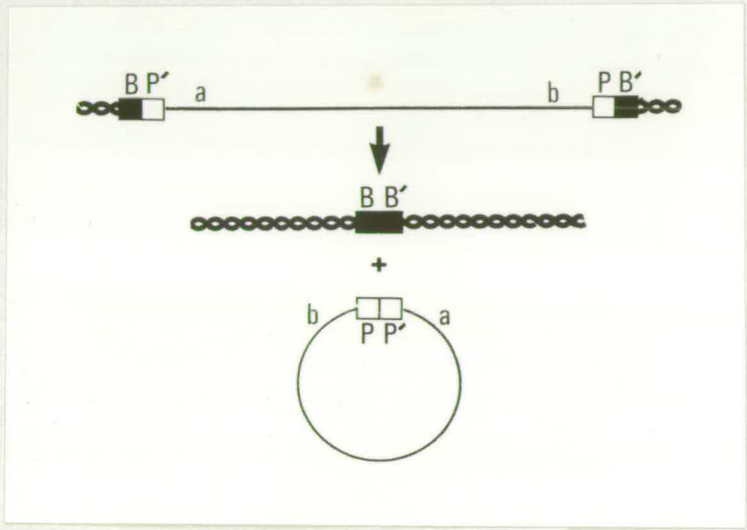



Fig. 3.7. Diagram showing a mechanism by which two non-adjacent regions of the E.coli K12 chromosome (both ends of a prophage) may be juxtaposed within a circular DNA molecule (formed by excision at the prophage attachment sites). Recombination between this circular DNA molecule and λ DNA could generate the λrev genome. B and B' are elements of the bacterial attachment site, P and P', elements of the phage attachment site; ●●●●●●●●●● is bacterial DNA; ———— is prophage DNA.

should therefore hybridise relatively weakly with the λrev substitution. The λ891 substitution hybridises with three R.EcoRI generated fragments in F123 (Fig.3.2(c)) and C600 (Fig.3.8) DNAs (13.6kb, 8.4kb and 2.65kb respectively). λrev DNA cross-hybridises with three R.EcoRI fragments of identical size in digests of F123 and C600 DNA (Fig. 3.2(c)). Since the λrev substitution hybridises relatively weakly with the 8.4kb R.EcoRI fragment however, this fragment should lie to the left of the central 2.65kb fragment, while the 13.6kb fragment should therefore lie to the right of the central 2.65kb fragment and thus be internal to the prophage.

The 13.6kb fragment also hybridises with a λ DNA probe (Fig.3.9). Only two R.EcoRI fragments (13.6kb and 4.6kb - data not shown) and two R.HindIII fragments (25kb and 4.0kb - Fig. 3.2(b)) in F123 DNA cross-hybridise with λ DNA. Equivalent fragments are found in digests of C600 but not AB1157 DNA (Figs. 3.1 and 3.9). The 4.6kb R.EcoRI fragment is internal to the 25kb R.HindIII fragment (see legend to Fig. 3.2(b)). Since the 4.0kb R.HindIII fragment is not cleaved by R.EcoRI (Fig. 3.2(b), tracks (b) and (f)) it must therefore be internal to the 13.6kb R.EcoRI fragment (see Fig. 3.12(a)).

(ii) Restriction endonuclease fragments close to or spanning PB' cross-hybridise uniquely with the λrev substitution

λrev DNA has an R.EcoRI site internal to its substitution and lying to the left of its hypothesised attachment site at the left end of the heavy line in Fig. 3.5(b). Thus λrev DNA should hybridise with two R.EcoRI fragments situated at the end of the prophage remote from the fragments discussed in (i) above. The right hand of these two fragments should contain the hybrid attachment site PB' and will only be seen with a λrev probe. The left hand fragment will

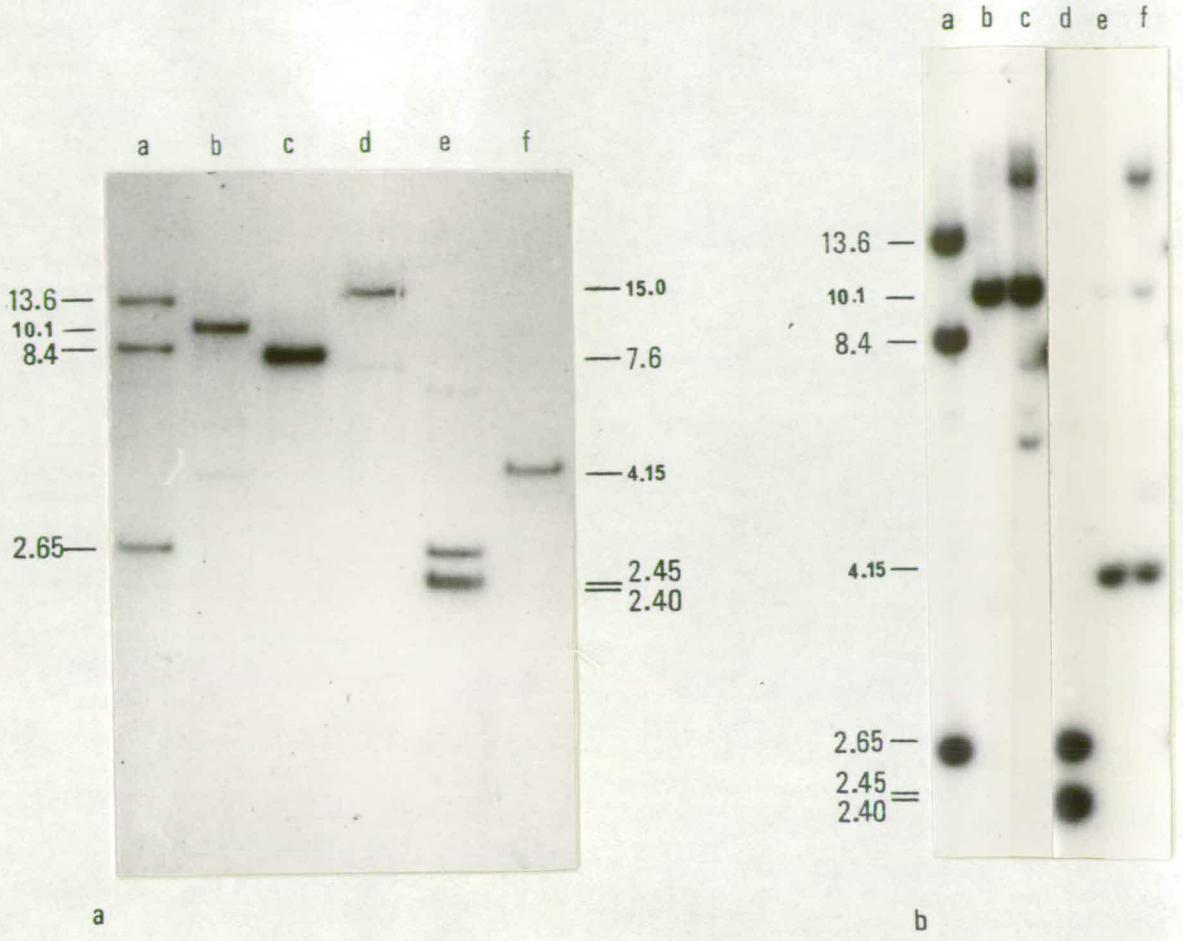



Fig. 3.8.

(a) Fragments in C600 and AB1157 DNA which share homology with the purified λ891 substitution. Electrophoresis was carried out in a 1% agarose gel. Tracks (a) and (b): R.EcoRI digests of C600 and AB1157 DNA respectively. Tracks (c) and (d): R.HindIII digests of C600 and AB1157 DNA respectively. Tracks (e) and (f): R.EcoRI + R.HindIII digests of C600 and AB1157 DNA respectively.

(b) DNA from the E.coli K12 strain CS520 resembles AB1157 DNA in its hybridisation with the λ891 substitution. Electrophoresis was carried out in a 0.7% agarose gel. Digests of C600 DNA are included for comparison. Tracks (a), (b) and (c): R.EcoRI digests of C600, AB1157 and CS520 DNAs respectively. Tracks (d), (e) and (f): R.EcoRI + R.HindIII digests of C600, AB1157 and CS520 DNAs respectively. CS520 is a λ lysogen. Secondary bands in tracks (c) and (f) result from hybridisation between contaminating λ DNA in the probe and the DNA fragments which derive from the λ prophage (data not shown).

share only slight homology with the λrev substitution but may also hybridise with λ DNA (Fig. 3.12(a)).

A 4.2kb R.EcoRI fragment which hybridises uniquely with the λrev substitution is present in both F123 (Fig. 3.2(c)) and C600 (Fig. 3.9) DNAs (but not in AB1157 DNA). This 4.2kb fragment must therefore be the right hand of the two fragments discussed above (see Fig. 3.12(a)). All but one of the R.EcoRI generated DNA fragments of F123 which cross-hybridise with λrev DNA (Fig. 3.2(c)) have now been identified. The remaining 4.6kb fragment must therefore be the left hand of the two fragments discussed above (see Fig. 3.12(a)). This fragment shares considerable homology with λ DNA. Both the 4.2kb and the 4.6kb fragments are contained within a 25kb R.HindIII fragment (see legend to Fig. 3.2(b)).

(iii) AB1157 DNA is specifically deleted for Rac prophage DNA sequences

In addition to the R.EcoRI fragments discussed above, the λrev (but not the λ891) substitution hybridises with a 17.5kb R.EcoRI fragment in C600 and AB1157 DNA (Figs. 3.8 and 3.9). No fragment of this size is detected in F123 DNA (Fig. 3.2(c)). This homology represents cross-hybridisation between the Rac prophage sequences carried by λrev (but not λ891) DNA and another defective prophage, located elsewhere on the E.coli K12 chromosome (see Chapter 5). The 17.5kb fragment is associated with a 28kb R.HindIII fragment which shares homology with λ DNA and is present in both C600 and AB1157 (but not F123) DNA (data not shown).

Other than the 17.5kb R.EcoRI fragment discussed above, no R.EcoRI fragments which share homology with the λrev substitution can be found in the DNA of AB1157 (Fig. 3.9), suggesting that this strain is deleted for the Rac prophage. It seems probable that a precise excision event occurred in one of the

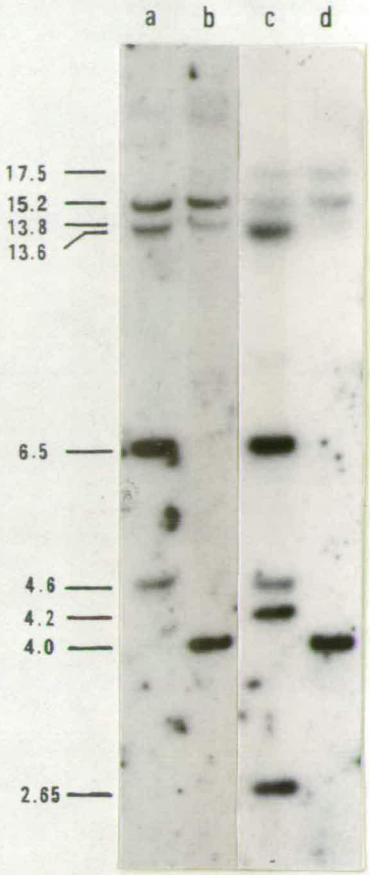



Fig. 3.9. Autoradiograph showing hybridisation of λ and λrev DNA probes with R.EcoRI digests of C600 and AB1157 DNAs. Electrophoresis was carried out in a 0.7% agarose gel. Tracks (a) and (b): λ-homologous fragments in C600 and AB1157 DNA digests respectively; the filter was washed in 4 x SSC at 65°C subsequent to hybridisation in 6 x SSCP at 65°C for 36 hours. A 13.6kb and a 4.6kb fragment are present in (a) but not in (b). A 6.5kb fragment has been replaced by a 4.0kb AB1157 DNA fragment. Tracks (c) and (d): λrev-homologous fragments in C600 and AB1157 DNA digests respectively. The filter was hybridised in 6 x SSCP at 65°C for 36 hours and washed in a 2 x SSC at 65°C in order to reduce relatively non-specific hybridisation. Track (c): in addition to all fragments seen with a λ DNA probe, with a λrev DNA probe a 17.5kb, a 4.2kb and a 2.65kb fragment have been detected and the relative intensity of the 13.6kb band has been greatly increased. Track (d): only a 17.5kb fragment is detected due to homology with the λrev substitution (c.f. track (b)).

ancestors of AB1157, regenerating the bacterial attachment site BB'. If this view is correct, AB1157 DNA should retain homology with the λ891 substitution (Fig. 3.12(a)) as this DNA fragment carries bacterial DNA in addition to prophage DNA. Furthermore it should be possible to find E.coli K12 strains derived independently of AB1157 which are also deleted for the Rac prophage and, if the excision event is specific, these strains will show the same homology with the λ891 substitution as does AB1157 DNA. CS520 is such a strain (Fig. 3.8(b)). In this E.coli K12 strain no DNA sequences derived from the Rac prophage can be found (data not shown). The λ891 substitution hybridises with the same size fragments in both AB1157 and CS520 DNAs.

(f) A physical map of the Rac prophage.

The λrev substitution contains a small region of DNA to the right of the R.HindIII site which defines the right end of the λ891 substitution (Fig. 3.5). Thus, one would expect an R.HindIII fragment in C600 but not AB1157 DNA to hybridise with the λrev but not the λ891 substitution. Comparison of Fig.3.2(b) (tracks c and d) with Fig.3.8(a) (track c) shows that only the λrev substitution shares homology with a 2.4kb R.HindIII generated fragment which must therefore be within the 13.6kb λ-homologous R.EcoRI generated fragment discussed in Section 3.2(e) (see Fig.12(a)).

This 13.6kb R.EcoRI fragment in C600 and F123 DNAs which shares homology with λ DNA (via an internal 4.0kb R.HindIII fragment), with the λ891 substitution (via a 2.45 kb R.EcoRI + R.HindIII fragment) and with the λrev substitution (via a 2.45kb R.EcoRI + R.HindIII fragment and an internal 2.4kb R.HindIII fragment) lies internal to the Rac prophage (see above and Fig. 3.12(a)) and has been shown to contain the replication origin oriJ (Diaz et al, 1979; and see Section 3.2(g)).The cleavage map of this fragment was kindly made available to me prior to publication and is in agreement with my data. Their map includes two internal R.HindIII

fragments (4.0kb and 2.4kb), the smaller of which overlaps slightly with the right hand end of the λrev substitution. The larger internal R.HindIII fragment (4.0kb) is the only region of the 13.6kb R.EcoRI fragment which shares homology with λ DNA (see above and Fig. 3.2(b)). One end of the 13.6kb fragment overlaps with a large R.HindIII fragment, present in C600 DNA but not in AB1157 DNA (Diaz et al, 1979). Diaz et al estimate the size of this fragment as 14Md (approximately 22kb). Since estimates of fragment sizes in this range are open to considerable error this fragment could be the 25kb, λ-homologous, R.HindIII fragment described above and which carries the hybrid attachment site PB' and DNA sequences homologous to the λrev substitution. The following evidence supports this.

Libraries of bacterial colonies containing fragments of F123 DNA linked to plasmid vectors were screened with λ891 and λrev DNA probes by the colony hybridisation procedure (Grunstein and Hogness, 1975). In this manner, several recombinant plasmids which contain F123 DNA fragments derived from the Rac prophage were isolated (Table 1.3 ). Of particular interest were the plasmids pK9 (pBR325oriJ) and pK8 (see Section 3.2(h)) which contain a 13.6kb and a 8.4kb R.EcoRI generated fragment respectively, and pK12 which contains a 25kb, R.HindIII generated, DNA fragment. Mapping of these plasmids with the enzymes R.EcoRI and R.HindIII and cross-hybridisation studies between the cleaved plasmids and λ or λrev DNAs (data not shown) showed the cloned fragments to be equivalent to those previously recognised as belonging to the Rac genome (see Fig. 3.12(a)).

The 13.6kb fragment carried by pK9 is indistinguishable from that isolated by Diaz and Pritchard (Diaz and Pritchard, 1978; Diaz et al, 1979). However in contrast with the oriJ containing plasmid constructed by Diaz and Pritchard (Diaz et al, 1979; see also Section 3.3) pK9 is capable of autonomous replication in both $rac^+$ and $rac^-$ bacteria since it retains the pBR325 origin of replication.

Figs. 3.10(a) and 3.10(b) show R.EcoRI and R.HindIII fragments respectively in C600 DNA which cross-hybridise with the

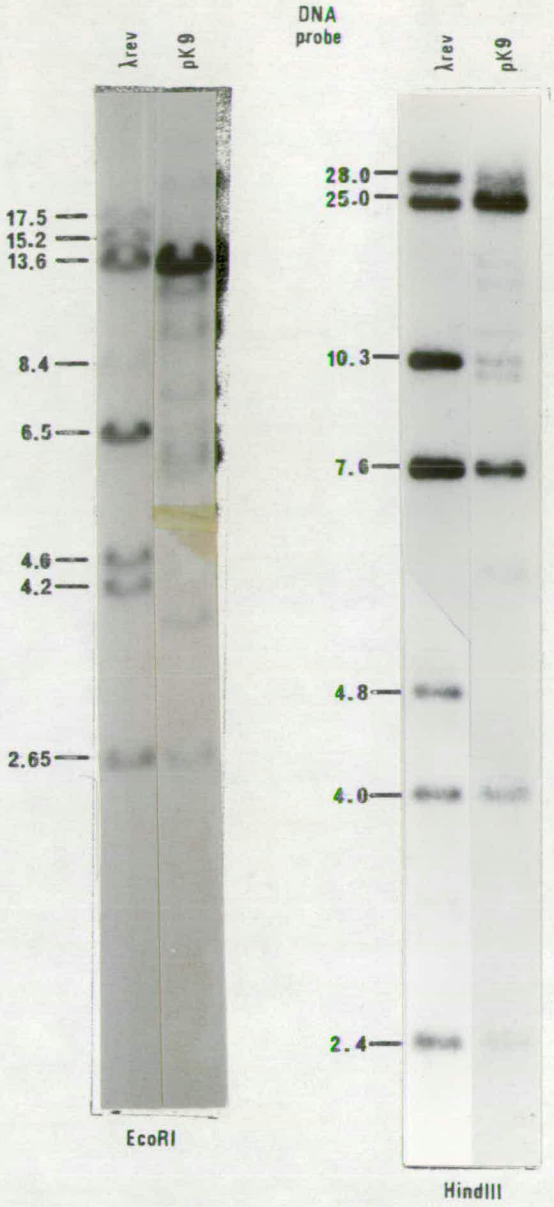



Fig. 3.10. R.EcoRI and R.HindIII fragments in the DNA of C600 which share homology with λrev and pK9 DNAs. Electrophoresis was carried out in a 0.7% agarose gel.

(a) R.EcoRI digests of C600 DNA. Both λrev and pK9 DNAs cross-hybridise with a 13.6kb fragment in C600 DNA.

(b) R.HindIII digests of C600 DNA. Both λrev and pK9 DNAs cross-hybridise with a 25kb R.HindIII fragment in C600 DNA. In the case of the λrev probe, an eight hour exposure (upper half) and a four day exposure (lower half) have been combined.

DNAs of λrev and pK9. The pBR325 vector itself shares no homology with E.coliK12 DNA. Fig. 3.10(a) shows that the 13.6kb fragment identified above by cross-hybridisation with λ and λrev DNAs in digests of C600 and F123 DNAs (Fig.3.2(c) and Fig.3.9)is indeed the same size as the R.EcoRI fragment in C600 DNA with which pK9 cross-hybridises. Fig. 3.10(b) shows that the 13.6kb R.EcoRI fragment in pK9 cross-hybridises with a large R.HindIII fragment (25kb) in C600 DNA which is equal in size to the λ-homologous fragment described in Section 3.2(e) and which carries the hybrid attachment site PB'. R.HindIII fragments internal to the 13.6kb fragment (2.4kb and 4.0kb) and the 7.6kb R.HindIII fragment which comprises the λ891 substitution and contains the hybrid attachment site BP' are also observed (see also Fig. 3.12(a)). In both R.EcoRI (Fig. 3.10(a)) and R.HindIII (Fig. 3.10(b)) digests of C600 DNA, several additional fragments are identified by a pK9 probe. Their origin is discussed in Section 3.3.

The insert in pK12 is the 25kb R.HindIII fragment which spans the Rac prophage hybrid attachment site PB'. Only two fragments are generated from within the 25kb fragment by digestion with R.EcoRI (data not shown), these being the 4.6kb and the 4.2kb fragments described above and which share homology with the λrev substitution. Cross-hybridisation studies show that thecloned fragments in pK9 and pK12 share homology via a 4.7kb R.EcoRI + R.HindIII fragment as expected (data not shown but see Fig. 3.12(a) and legend to Fig. 3.11).

Fig. 3.11 shows restriction endonuclease generated fragments in F123, C600 and AB1157 DNAs which cross-hybridise with pK12 DNA. As expected a 4.2kb, a 4.6kb and a 13.6kb fragment are identified in R.EcoRI digested F123 and C600 DNAs but not in AB1157 DNA. A 15.0kb fragment is seen in all these DNAs and must therefore overlap with the end of the 25kb DNA fragment not contained within the Rac prophage (Fig. 3.12(a)). As with a pK9 probe,several additional and relatively less intense bands are identified with a pK12 probe in E.coliK12 DNA (see Section 3.3).

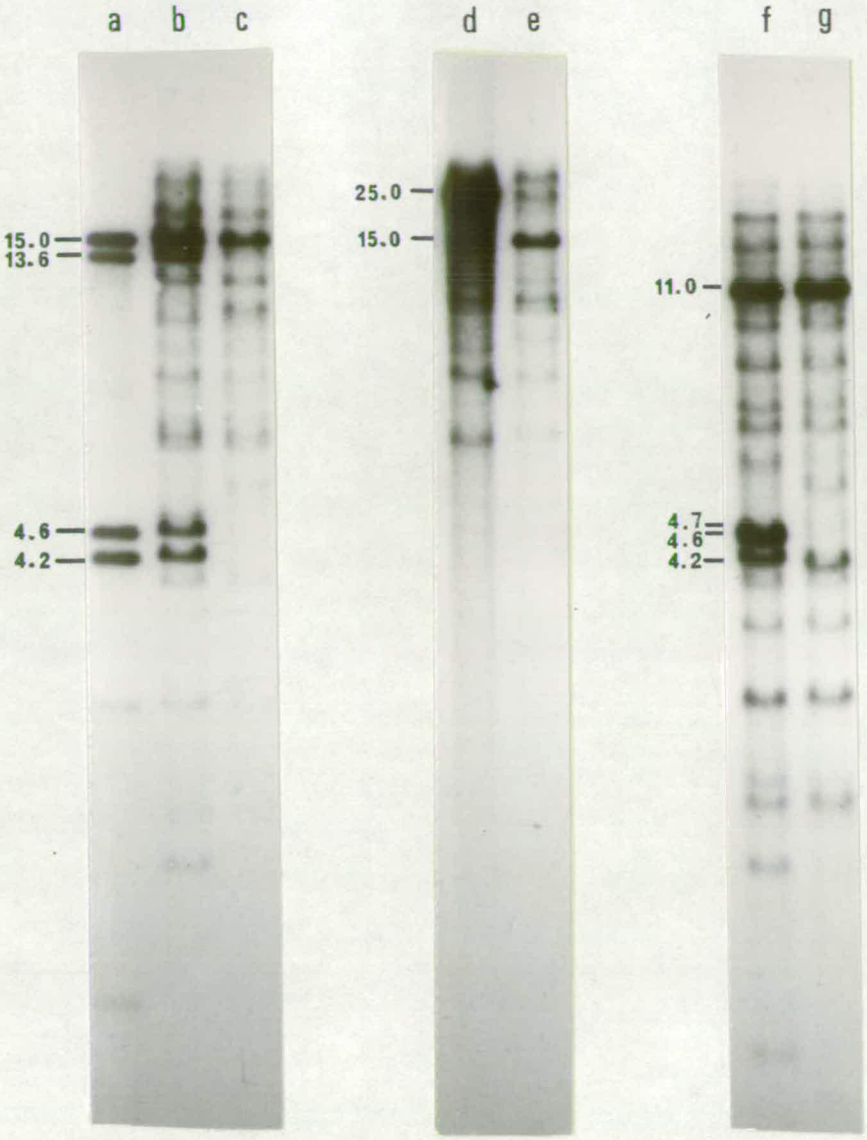


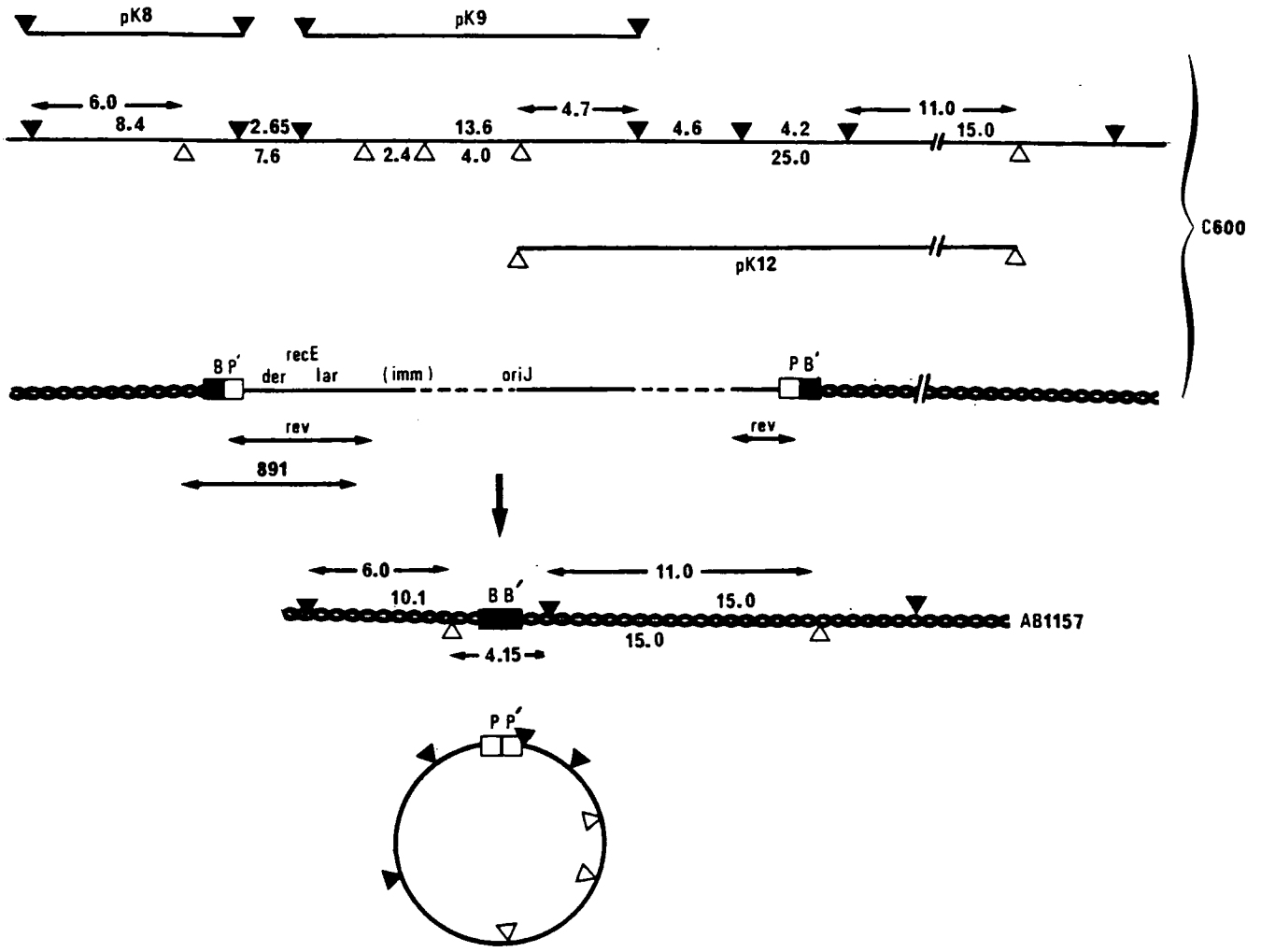

Fig. 3.11. Autoradiograph showing fragments in F123, C600 and AB1157 DNAs which cross-hybridise with pK12 DNA (compare with Fig. 3.12(a)). Electrophoresis was carried out in a 0.7% agarose gel. Tracks (a), (b) and (c): R.EcoRI generated DNA fragments in F123, C600 and AB1157 DNAs respectively. A 4.2kb, a 4.6kb and a 13.6kb fragment are unique to F123 and C600 DNAs. A 15.0kb fragment is observed in all three DNAs. Tracks (d) and (e): R.HindIII generated fragments in C600 and AB1157 DNAs respectively. The 25kb insert in pK12 cross-hybridises with an equivalent fragment in C600 but not in AB1157 DNA. Tracks (f) and (g): R.EcoRI + R.HindIII generated fragments in C600 and AB1157 DNAs respectively. The 4.7kb fragment in C600 DNA digest is the region of overlap between the 13.6kb and 25kb fragments.

Since cloned DNA fragments (in either phage or plasmid vectors) are now available which cover the entire Rac genome, it is possible to construct a complete physical map of the Rac prophage (Fig. 3.12(a)). Similarly it is possible to deduce the positions of R.HindIII and R.EcoRI targets flanking the postulated attachment site BB' in AB1157 DNA. These latter positions are in agreement with the data of Fig. 3.8. The map of the Rac prophage (Fig. 3.12(a)) shows the approximate position of the recE gene (der and possibly lar), the hybrid attachment sites BP' and PB', the regions of homology with λ, and the replication origin oriJ. This map is consistent with all of the results presented above and with those of Diaz et al (1979).

(g) Rare derivatives of C600 have been specifically deleted for the Rac prophage.

The 13.6kb, R.EcoRI generated DNA fragment described by Diaz and Pritchard (1978), and which carries the Rac origin of replication (oriJ) was isolated as a recombinant plasmid (oriJ-Ap), the second component of which is a DNA fragment (the Ap fragment) coding for ampicillin resistance but incapable of self-replication. Transformation of a suitable bacterial strain and selection for ampicillin resistance yielded colonies containing the oriJ-Ap plasmid. Fortuitously, the recipient strain used was AB2463, a recA$^-$ derivative of the rac$^-$ strain AB1157. Subsequent attempts to transform a number of other E.coli K12 strains (all of which were probably rac$^+$) with the oriJ-Ap plasmid were unsuccessful. Consequently it seemed possible that oriJ replication functions are repressed in rac$^+$ hosts (Diaz and Pritchard, 1978; Diaz et al, 1979). The simplest explanation of these results is that the 13.6kb DNA fragment carries an origin of replication, other essential functions for autonomous replication not provided by the host, and a repressor binding site. Introduction of the oriJ-Ap plasmid into a rac$^+$ host in which the prophage repressor is present would therefore

a)


pK8
pK9
6.0
8.4
2.65
7.6
2.4
13.6
4.0
4.7
4.6
4.2
25.0
11.0
15.0
C600
pK12
B P'
recE
der
lar
(imm)
oriJ
P B'
rev
rev
891
6.0
10.1
B B'
11.0
15.0
15.0
AB1157
4.15
P P'

b)

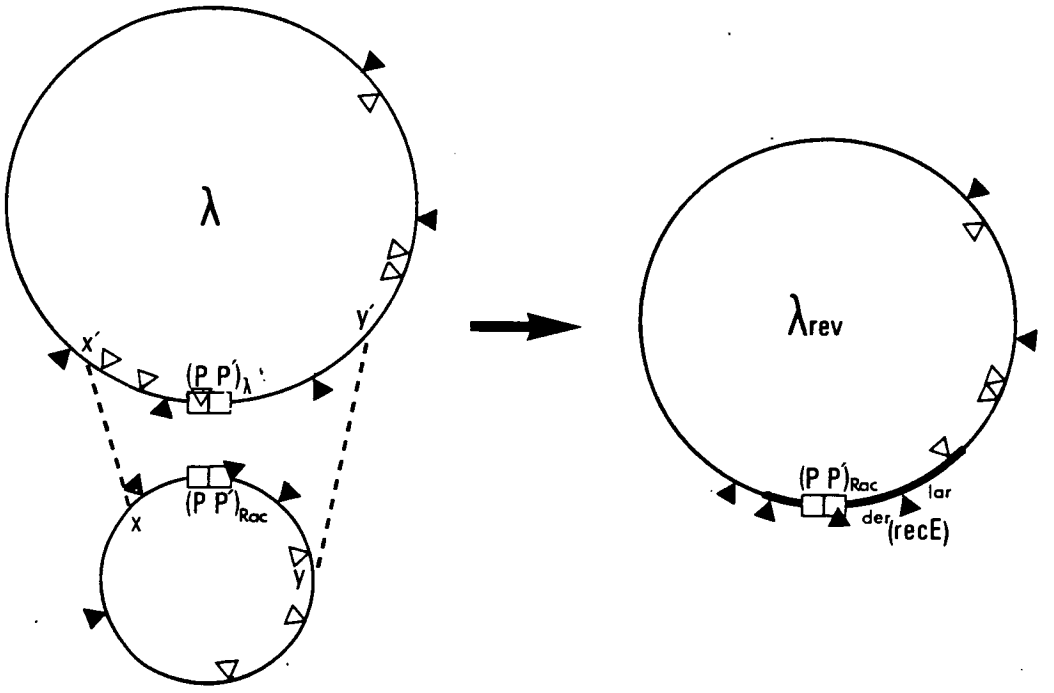

λ
x'
y'
(P P')λ
x
(P P')Rac
y
λrev
(P P')Rac
lar
der
(recE)

Fig. 3.12.

(a) A physical map of the region of the C600 chromosome which contains the Rac prophage. Excision of the prophage leads to regeneration of the bacterial attachment site and production of a circular DNA molecule. The symbols used are as follows: ▼, R.*Eco*RI site; Δ , R.*Hin*dIII site; B and B', elements of the bacterial attachment site; P and P', elements of the phage attachment site; ▬▬, bacterial DNA; - - - - -, prophage restriction endonuclease fragments which share detectable homology with λ DNA. Sizes of R.*Eco*RI and R.*Hin*dIII fragments are shown immediately above and below the line respectively. Sizes of some other DNA fragments are indicated. Regions of DNA contained within the λ891 and λ*rev* substitutions and within the recombinant plasmids described in this chapter are shown. The *recE* gene which is carried by λ*rev*, and by inference by λ891, must lie to the right of BP' and in the region of the prophage these phages have in common. Only the approximate relative loci of *oriJ* (see Diaz *et al*, 1979) and *recE* can be shown. Prophage excision at the hybrid attachment sites generates a circular DNA molecule containing the phage attachment site (PP'), the *recE* gene and *oriJ*. The bacterial attachment site (BB') is regenerated within the bacterial chromosome (as in AB1157). The orientation of the prophage with respect to external markers remains unknown.

(b) Proposed model for generation of λ*rev* phages by recombination between λ DNA and the circular DNA molecule formed by excision of the Rac prophage (i.e. at xx' and yy'). The heavy line denotes the region of λ*rev* DNA derived from the Rac prophage. R.*Eco*RI sites (▼) and R.*Hin*dIII sites (Δ) are shown.

inhibit replication of the plasmid. On the other hand, replication would proceed normally in derivatives of AB1157 in which there is no prophage repressor. On this hypothesis the oriJ-Ap plasmid would have no repressor gene of its own or one which was poorly expressed. Since by analogy with λ one would expect the prophage repressor gene to lie to the left of oriJ, to the right of the region of the prophage common to the λrev and λ891 substitution, close to the repressor binding site (probably analogous to $O_R$) which controls expression of replication functions, and therefore within the 13.6kb oriJ fragment (Fig. 3.12(a)), the latter hypothesis seems the most probable.

Diaz et al (1979) showed that rare oriJ-Ap transformants of rac$^+$ strains do arise at approx. $10^{-4}$ of the transformation frequency for rac$^-$ strains. It was found that Ap$^S$ segregants of these rare transformants arose with high frequency and they were presumed to have lost the plasmid. When these segregants were in turn transformed with oriJ-Ap they showed transformation frequencies close to those obtained for AB1157 and its derivatives. LE451 and LE452 are two such segregants derived from the strain LE450, a recA$^-$ derivative of C600 (Diaz et al, 1979). Hybridisation analysis showed LE451 and LE452 DNAs not to contain sequences homologous with the oriJ-Ap plasmid (Diaz et al, 1979). It therefore seemed probable that these strains, like AB1157 and its derivatives, are specifically deleted for the Rac prophage. It is possible that such cells arise spontaneously but at low frequency in a culture of rac$^+$ bacteria.

In order to test whether LE451 and LE452 are specifically deleted for the Rac prophage, DNA was isolated from these strains and from their progenitor, LE450, and the hybridisation behaviour of these DNAs with λrev and λ891 DNA probes was compared with that of AB1157 (and E.coliC) DNAs with the same probes. LE450, LE451, and LE452 were provided by Ramon Diaz. The hybridisation patterns in C600 and LE450 DNAs with a λ or λrev probe are indistinguishable, indicating that LE450, like C600, carries an intact Rac prophage (data not shown).

Fig. 3.13(a) shows those R.EcoRI generated fragments in these DNAs which cross-hybridise with λrev DNA. Fragments for which sizes are indicated derive from the Rac prophage in LE450 DNA. Unidentified fragments are derived from other lambdoid prophages in E.coli K12 strains (see Chapter 5). Like AB1157 DNA, LE451 and LE452 DNAs appear to be deleted for Rac prophage DNA sequences. The hybridisation pattern seen in E.coliC DNA digests does not appear to be related to that seen in E.coli K12 DNA.

Fig. 3.13(b) shows those R.EcoRI generated fragments in the same DNAs which cross-hybridise with λ891 DNA. As described in Section 3.2(e), strains such as AB1157 that are specifically deleted for the Rac prophage retain homology with the λ891 substitution. This homology resides within a 10.1kb, R.EcoRI generated fragments in AB1157 DNA (see Fig. 3.12(a)). Fig. 3.13(b) shows that an indistinguishable fragment is carried by LE451, LE452 and E.coliC DNAs. Thus, LE451 and LE452 DNAs, like AB1157 DNA would appear to be specifically deleted for the Rac prophage. Since E.coliC DNA also carries a 10.1kb fragment that shares homology with λ891 DNA, E.coliC may contain an attachment site for Rac.

(h) Lysogenisation of rac⁻ strains by λrev and λ891.

Specific excision of the Rac prophage would lead to the regeneration of the bacterial attachment site, BB', as appears to have occurred in an ancestor of AB1157. If BB' were a primary attachment site for the Rac prophage, then by analogy with λ, lysogenisation of AB1157 by λrev should occur at high frequency since the λrev phage carries the phage attachment site PP'. However, λrev does not lysogenise AB1157 efficiently although it integrates quite efficiently close to the rac locus in a recA⁻rac⁺ strain (Gottesman et al, 1974). Site specific integration into one of the hybrid attachment sites of the Rac prophage would be consistent with the latter observation. BB' does not therefore appear to behave as a primary attachment site for λrev. It is possible that BB' is equivalent to a

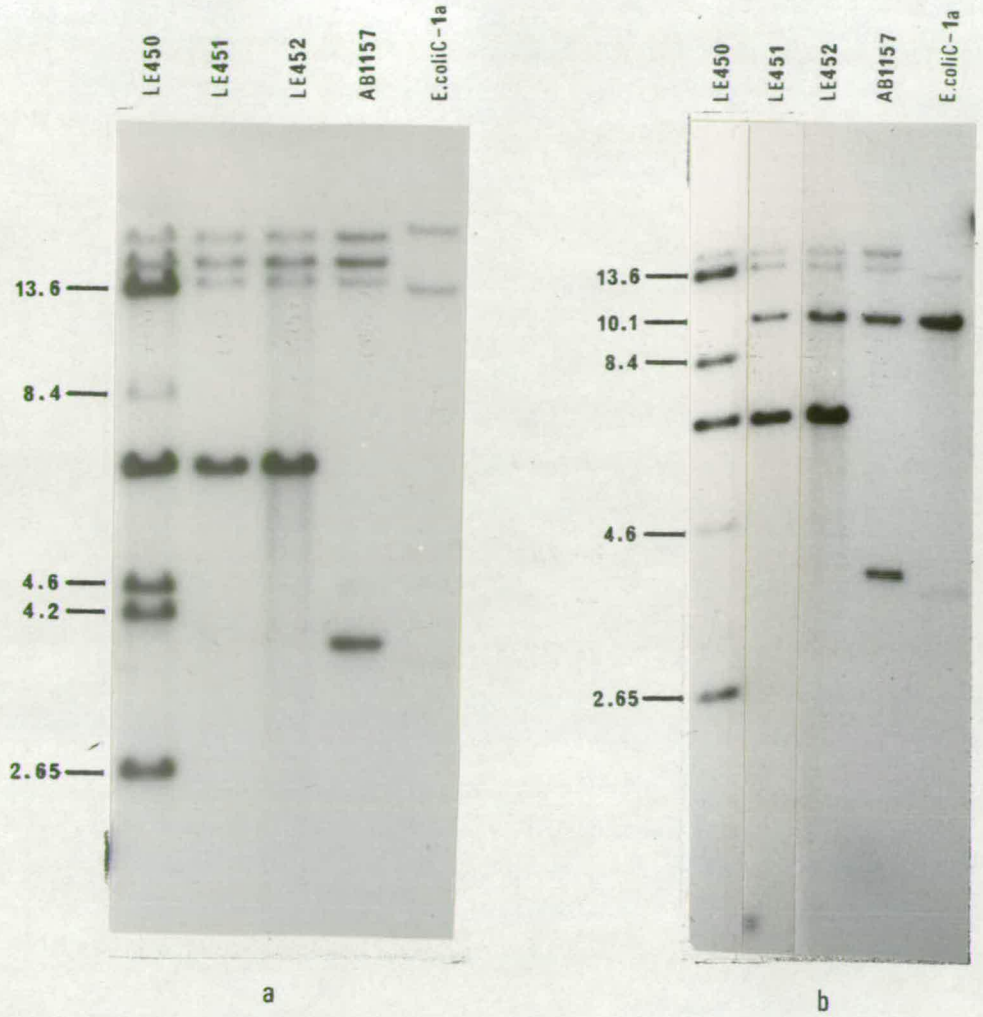



Fig. 3.13. Electrophoresis was carried out in 0.7% agarose gels.

(a) R.EcoRI generated fragments which cross-hybridise with λrev DNA in DNAs prepared from the E.coli strains discussed in Section 3.2(g). Fragments for which sizes are indicated derive from the Rac prophage in LE450 (C600recA) DNA (see Fig. 3.12(a)). Unidentified fragments in the E.coli K12 DNAs are derived from other lambdoid prophages (see Chapter 5). LE451 and LE452 DNAs, like AB1157 DNA, are deleted for Rac prophage sequences.

(b) R.EcoRI generated fragments which cross-hybridise with λ891 DNA in the same DNAs as are described in Fig. 3.13(a). LE451 and LE452 DNAs, like AB1157 DNA, carry a 10.1kb fragment characteristic of the region surrounding the bacterial attachment site for Rac in E.coli K12, and possibly E.coli C-1a, DNAs (see Fig. 3.12(a)).

secondary attachment site for λrev, and that no primary attachment site for this phage is present in E.coli K12 strains. Alternative interpretations of the low rate of lysogenisation of AB1157 by λrev are that AB1157 has a mutated attachment site, or even that the integration behaviour of λrev does not resemble that of λ.

In an attempt to distinguish these hypotheses, the frequency of lysogenisation of C600, AB1157, LE450, LE451 and LE452 by λrev has been measured and DNA prepared from representative lysogens has been examined by hybridisation methods as a check on the specificity of integration.

Table 3.1 shows the frequency of lysogenisation by λrev and λ891 in the above strains relative to the lysogenisation frequency for λ in those strains. As shown by Gottesman et al (1974) the frequency of lysogenisation by λrev is relatively independent of the recA genotype of the host. However, the frequencies shown in Table 3.1 for lysogenisation by λrev in C600 (or LE450) and AB1157 are somewhat higher (x10 and $x10^2$ respectively) than those recorded by Gottesman et al (1974) in similar, and in the case of AB1157, identical strains. This feature remains unexplained. Nevertheless, as shown by Gottesman et al, (1974), λrev forms lysogens far less readily in AB1157 than it does in $Rac^+$ strains. Table 3.1 reveals no significant differences between the frequencies of lysogenisation by λrev in AB1157, LE451 and LE452 and therefore if the integration of λrev in these strains is specific (as it seems to be; see below), the attachment sites (BB') carried by AB1157, LE451 and LE452 are indistinguishable. Thus, the low frequency of lysogenisation by λrev in $Rac^-$ strains (c.f. the high frequency of lysogenisation by λ in non-lysogenic strains) is not peculiar to AB1157 and would therefore appear to be a genuine feature of λrev (and hence Rac) integration behaviour.

Table 3.1 also shows the lysogenisation frequencies of λ891 in the above strains. λ891 carries the prophage hybrid attachment site BP' (see Fig. 3.12(a) and might have been expected (by analogy with λ) to behave like a

| Strain | RecA Phenotype | Lysogenisation frequency | | |
|---|---|---|---|---|
| | | λ | λrev | λ891 |
| C600(Rac$^+$) | + | 1 (8/10) | $1.3\times10^{-1}$ (2/20) | $0.8\times10^{-1}$ (1/20) |
| AB1157(Rac$^-$) | + | 1 (10/10) | $0.8\times10^{-2}$ (18/20) | $0.8\times10^{-3}$ (10/10) |
| LE450(Rac$^+$) | - | 1 (9/10) | $0.8\times10^{-1}$ (20/20) | $0.4\times10^{-1}$ (20/20) |
| LE451(Rac$^-$) | - | 1 (10/10) | $0.3\times10^{-2}$ (20/20) | n.t. |
| LE452(Rac$^-$) | - | 1 (10/10) | $0.7\times10^{-2}$ (20/20) | $0.4\times10^{-4}$ (2/20) |

TABLE 3.1. Lysogenisation by λrev and λ891 in Rac$^+$ and Rac$^-$ strains.

Lysogenisation frequencies were determined as described in Section 2(h) and are expressed as a fraction of the lysogenisation frequency for λ in a particular strain. λ and λrev lysogens were selected in the presence of $10^9$ p.f.u. each per plate of λcI26 and λ$h^{82}$cI; λ891 lysogens in the presence of $10^9$ p.f.u. each per plate of λ$imm^{21}$ clear and λ$h^{82}imm^{21}$ clear. cI and other clear mutants cannot establish repression and so do not form lysogens. Selection with such phages ensures that most non-lysogenic cells are killed. $h^{82}$ phages have the host specificity of the lambdoid phage ø82. Selection with both $h^{82}$ and $h^{\lambda}$ phages ensures that resistant bacteria, the majority of which are resistant to only one of these phages, will arise relatively infrequently.

Numbers in parentheses indicate the fraction of non-resistant clones after approx. 20h. growth at 33°C. Where this fraction is low, estimates of lysogenisation frequency can only be taken as approximate.

n.t. not tested.

λgal transducing phage which carries the λ prophage hybrid attachment site BP' (see Fig. 1.2). λgal does not integrate efficiently into a non-lysogenic host (BP' x BB') although it does integrate efficiently into a heteroimmune lysogen (BP' x BP') (Gottesman and Weisberg, 1971). As Table 3.1 shows, λ891 integrates almost as efficiently as does λrev into a Rac$^+$ host (C600 or LE450). Integration is probably of the BP' x BP' variety but the specificity of integration has not been checked. λ891 integrates very inefficiently (and at least one order of magnitude less efficiently than λrev) in Rac$^-$ strains (AB1157 and LE452). Thus, λ891 would seem to behave in a similar fashion to λgal although, since the integration behaviour of λrev does not appear to be analogous to that of λ, this similarity may be superficial.

DNA was prepared from representative AB1157(λrev) and LE452(λrev) lysogens and radioactive probes were used to check on the specificity of integration of λrev in Rac$^-$ strains. The probes used were λrev DNA and the recombinant plasmid pK8. The isolation of pK8 is described in Section 3.2(f). This plasmid carries an 8.4kb, R.EcoRI generated DNA fragment which spans the hybrid attachment site BP' of the Rac prophage (see Fig. 3.12(a)). Fig. 3.14 shows R.EcoRI generated fragments in the DNAs of AB1157(λrev) and LE452(λrev) lysogens which cross-hybridise with these probes. As explained in the legend to Fig. 3.14, λrev appears to integrate at the correct attachment site for Rac (and hence λrev) in Rac$^-$ strains.

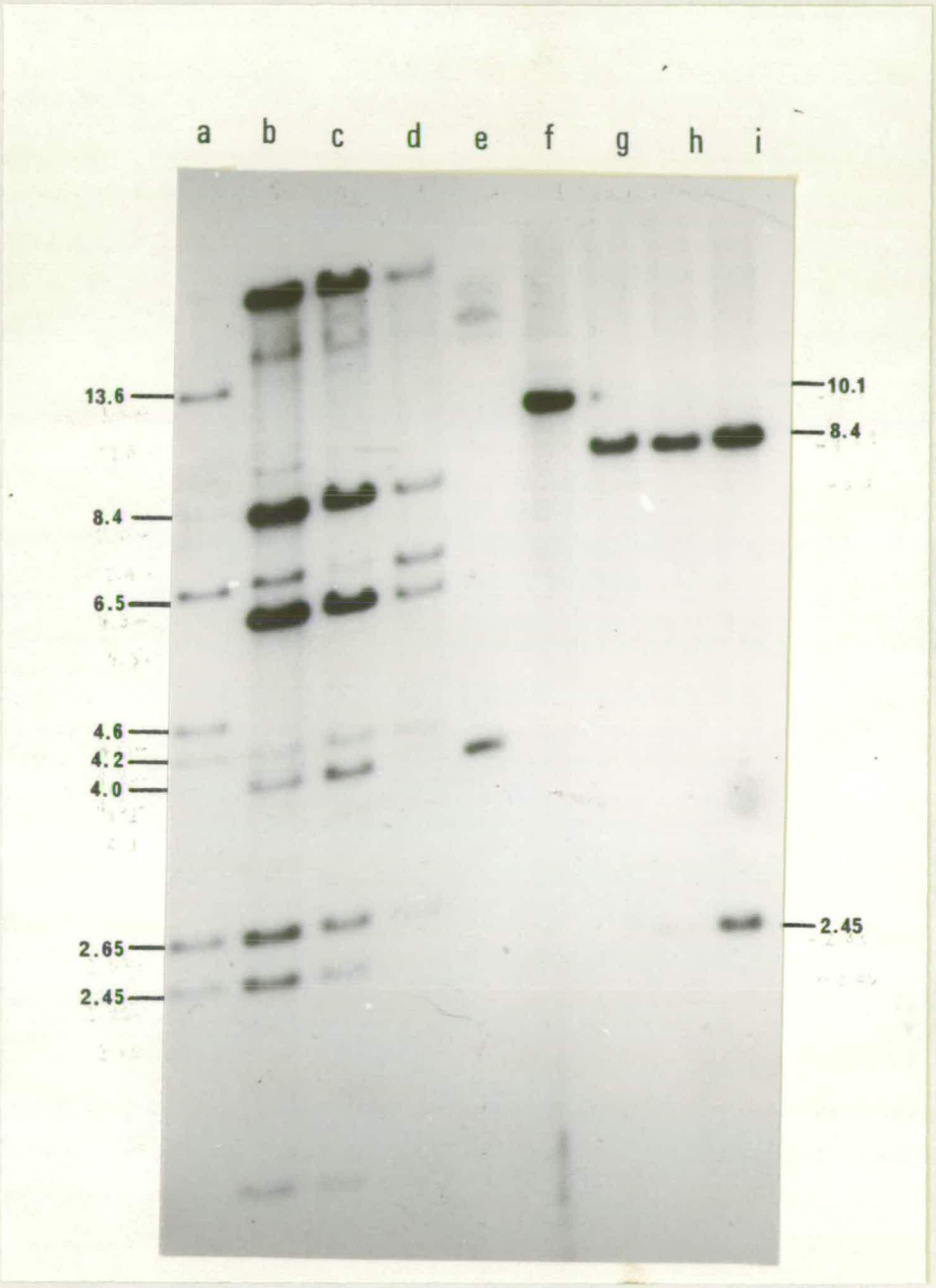

a
b
c
d
e
f
g
h
i
13.6
8.4
6.5
4.6
4.2
4.0
2.65
2.45
10.1
8.4
2.45

Fig. 3.14. The λrev genome integrates site specifically at the attachment site for Rac (BB') in Rac$^-$ strains. All DNA fragments were generated by cleavage with R.EcoRI. Electrophoresis was carried out in a 0.7% agarose gel.

Tracks (a) to (e): λrev DNA probe. Track (a); JC7661 DNA. JC7661 carries three, probably tandemly repeated, copies of the Rac genome (see Section 4.2(b)). The 2.45kb fragment in this DNA derives from the junction between two such prophages and is thus equivalent to the same size fragment which spans the attachment site PP' in λrev (see Fig. 4.4(b)). Tracks (b) and (c): DNAs prepared from two independently isolated AB1157 (λrev) lysogens. The presence of a 2.45kb fragment in these DNAs suggests that both carry tandemly repeated copies of the λrev genome. Track (d): LE452(λrev) DNA. By the above criterion, this strain carries only a single copy of λrev. Track (e): AB1157 DNA. The presence of a 4.2kb and an 8.4kb band in tracks (b), (c) and (d) (the latter is largely obscured in these tracks by a slightly smaller fragment internal to the λrev genome, but see tracks (g) and (h)) suggests that λrev has integrated at the attachment site for Rac (BB') in AB1157 and LE452 DNAs, thus regenerating those DNA fragments which span the hybrid attachment sites in Rac$^+$ strains (see Fig. 3.12(a)). The 4.0kb (AB1157 derivatives) and the 6.5kb (other strains) fragments are derived from a second lambdoid prophage in E.coli K12 strains (see Chapter 5).

Tracks (f) to (i): pK8 DNA probe. Track (f): AB1157 DNA. The 10.1kb fragment spans the attachment site BB' in Rac$^-$ strains (see Fig. 3.12(a)). Track (g): LE452(λrev) DNA. Tracks (h) and (i): the same AB1157(λrev) lysogen DNAs as in tracks (c) and (b) respectively. The 8.4kb fragment is seen in all three lysogen DNAs and is exactly equivalent to the cloned fragment in pK8 which spans the hybrid attachment site BP' in Rac$^+$ strains (see Fig. 3.12(a)). The 2.45kb fragment observed in tracks (b) and (c) shares homology with pK8 via the small region of prophage (rather than bacterial) DNA that the insert in this plasmid carries (see Fig. 3.12(a)) and is therefore also seen in tracks (h) and (i).

These results indicate that λrev integrates site specifically, and in the correct orientation, at the attachment site for Rac (and hence λrev) in Rac$^-$ strains.

## 3.3. DISCUSSION.

The experiments described in this chapter confirm the hypothesis of Low (1973) that the chromosome of many *E.coli* K12 strains contains a prophage (the Rac prophage) located close to the *trp* operon.

The Rac prophage has the following properties. It may be induced and lost from the chromosome, probably by precise excision of the prophage. Such an event must have occurred in one of the ancestors of AB1157 and has also been observed in rare *oriJ* transformants obtained from *rac*$^+$ strains (LE451, LE452). The data suggest that the prophage, by analogy with the λ prophage, retains functional hybrid attachment sites (BP' and PB') flanking the prophage genome. Evidence for a recombination function, Der (Gottesman *et al*, 1974), a restriction alleviation function, Lar (Toothman, personal communication), and probably an integration function is available (Gottesman *et al*, 1974). Der and Lar are thought to be functions of the *recE* gene product (Gillen *et al*, 1977). Since the ancestor of the Rac prophage is unknown, the maintenance of a specific immunity pattern is not readily observed. That such an immunity pattern exists however, is suggested by the apparent zygotic induction of the *rac* locus after transfer by mating into a *rac*$^-$ (AB1157) cell (Low, 1973) and by the low efficiency of transformation of *rac*$^+$ bacteria by a plasmid whose replication is driven by the prophage replication origin, *oriJ* (Diaz and Pritchard, 1978; Diaz *et al*, 1979). This latter result is explained if repressor binds to a site in the *oriJ* plasmid, thereby preventing expression of genes whose products are necessary for replication. By analogy with λ one would expect to find the prophage repressor gene to the left of *oriJ* (Fig. 3.12(a)) and close to the repressor binding site.

Those λ derivatives characterised by replacement of the genes *Q*, *S* and *R* with analogous genes (*q'*, *s'* and *r'*) derived from the *E.coli* K12 chromosome cannot be isolated from the *rac*$^-$ strain AB1157 (Strathern and Herskowitz, 1975;

Henderson and Weil , 1975) and might therefore be expected to include a region of the Rac prophage. This is not the case. The positions of the cleavage sites for R.EcoRI and R.HindIII within the substitutions in two λqsr' phages (λb2 p4 and λqinA3) were determined. Radioactive probes made from the DNA of these phages reveals a region equivalent to the qsr' substitutions in the DNA of C600. This region, which is closely associated with λ-homologous sequences, is not carried by F123 and is altered in AB1157 DNA. It seems probable that qsr' phages are generated by recombination between λ and a defective lambdoid prophage, distinct from the Rac prophage, and present at an, as yet, unidentified site on the E.coliK12 chromosome. The origin of λqsr' phages is discussed more fully in Chapter 5.

The size of the Rac prophage genome (approximately 25kb) is in keeping with that of a lambdoid phage extensively deleted for the late genes. Two regions of the prophage share homology with λ DNA. However, these regions probably amount to no more than a few per cent of the prophage genome. The extent of homology between the Rac prophage and the DNAs of Øm 61, Øm 151, Ø 21 and 434 which are related to λ by DNA homology (K. Kaiser, data not shown; Simon et al, 1971) was investigated. Øm 61 and Øm 151 are Spi$^-$ (Tsygankov et al, 1976) but do not share homology with the Rac prophage sequences in λrev DNA (K. Kaiser, unpublished observation). None of these phages appears to be significantly more closely related to the Rac prophage than does λ.

DNA from both ends of the Rac prophage is brought together within the λrev substitution. A model for the generation of λrev phages which is consistent with this observation is shown (Fig. 3.12(b)). This model involves recombination between λ DNA and the circular molecule produced as a consequence of excision of the Rac prophage and, in its simplest form, would involve recombination between sequences common to λ DNA and the Rac prophage (i.e. at xx' and yy' in Fig. 3.12(b)). Such homology is observed within the R.EcoRI fragment spanning x and may thus define the end point of the substituted DNA. Homology

between the y and y' regions remains to be demonstrated however, and is inferred only because the recombination event which generates λ*rev* appears to be specific, independent λ*rev* isolates being identical on the basis of heteroduplex mapping (Gottesman *et al*, 1974). λ*rev* phages can be generated in the absence of *recA*, *recB* and λ recombination functions (Zissler et al, 1971). However it is possible that the *recE* product, which is able to catalyse generalised phage recombination in *recA*$^-$ hosts (Zissler *et al*, 1971) or illegitimate recombination is involved.

Gottesman *et al* (1974) found that the frequency of lysogenisation by λ*rev* in the Rac$^-$ strain AB1157 is very low, although as shown above integration in this strain, when it does occur, is site specific. Thus, by analogy with λ, BB' does not appear to behave as a primary attachment site for λ*rev* (and hence Rac). As discussed in Section 3.2(h), this could be so for several reasons, one of which is that AB1157 has a mutated attachment site. Since the frequency of lysogenisation by λ*rev* in other independently isolated Rac$^-$ strains (LE451 and LE452) is essentially the same as that in AB1157, it seems more probable that the observed integration behaviour in Rac$^-$ strains is a normal state of affairs, at least as far as the *E.coli* K12/Rac system is concerned. It is conceivably possible that BB' is equivalent to a secondary attachment site for λ*rev* (and hence Rac), and that no primary attachment site for this phage is present in *E.coli* K12 strains.

Diaz *et al* (1979) observed several minor homologies between the R.*Eco*RI fragment containing *oriJ* and *E.coli* K12 DNA. Similar homologies are apparent in Fig. 3.10. The additional fragments appear to represent a class of repeated DNA sequences in the *E.coli* K12 chromosome, one member of which lies within the Rac prophage. All *E.coli* K12 DNAs tested contain such a set of fragments (data not shown). However, a comparison of different E.coli K12 DNAs usually reveals differences in the position of one or two of these fragments, consistent with them containing a transposable DNA sequence, repeated about 8-10 times in the *E.coli* K12 chromosome.

Although this thesis is primarily concerned with the investigation of λ-homologous sequences in the DNAs of E.coli K12 and its derivatives, several other E.coli and closely related enterobacterial DNAs have been investigated for the presence of λ-homologous sequences and also for the presence of DNA fragments diagnostic of the Rac prophage or a derivative of it. In all, 19 independently isolated E.coli strains (K12, B, C-1a and 16 more recent clinical isolates), 4 different subspecies of Shigella (boydii, dysenteriae, flexneri and sonnei), and Salmonella typhimurium LT/2 were investigated. The non-E.coli strains all share considerable DNA homology with E.coli, have similar chromosome organisation, and in several other respects appear closely related to E.coli (Sanderson, 1976). Cross-hybridisation studies between DNAs isolated from these strains and λ DNA showed all except one clinical E.coli isolate to share considerable homology with λ DNA. In no two cases was an identical pattern of hybridisation observed. The clinical isolates were not tested for the presence of inducible lambdoid phages though in some cases it may have been these that were observed. However, less than 5% of independently isolated E.coli strains appear to carry inducible lambdoid prophages (Jacob and Wollman, 1956; Bertani and Bertani, 1971; Krylov and Tsygankov, 1976). Thus, in the majority of cases the observed homology probably represents the presence of defective lambdoid prophages in these strains.

The same DNAs were then investigated with a λ891 DNA probe. In 3 of the clinical E.coli isolates and also in E.coliB an 8.4kb and a 2.65kb R.EcoRI generated fragment were observed. These fragments did not cross-hybridise with λ DNA and are therefore diagnostic of a Rac prophage (or a derivative of it) in these strains (see Fig. 3.12(a)). The use of a λ891 DNA probe only allows one end of the Rac genome to be investigated (i.e. that which surrounds the hybrid attachment site BP'). However, this probe cross-hybridises with the DNA of strains (such as AB1157) which carry an attachment site for the Rac prophage, in which case a 10.1kb, R.EcoRI generated fragment, which shares no

homology with λ DNA, is observed. By this criterion, 6 of the clinical E.coli isolates, E.coli C-1a, and S. typhimurium LT/2 may carry an attachment site for the Rac prophage.

# CHAPTER 4

## The Nature of *sbcA* Mutations in *E.coli* K12

## 4.1. INTRODUCTION.

Conjugational transfer of the *rac*$^+$ locus into a *rac*$^+$*recB*$^-$ cell provides transient recombination proficiency in the recipient but very rarely results in the formation of *rac*$^+$ recombinants (Low, 1973). By analogy with zygotic induction of the λ prophage, Low argued that recombination activation might be caused by induction of a prophage exonuclease gene on entering a (*rac*$^-$) cell in which the prophage, and hence the prophage repressor, is absent. The low frequency of *rac*$^+$ recombinants suggests that the (presumably defective) prophage genome is excised from the donor chromosome as a result of induction but is readily lost from the exconjugant progeny (Low, 1973). Further evidence for a prophage repressor in *rac*$^+$ strains is given in Chapter 3 (see also Diaz *et al*, 1979). The data in Chapter 3 suggest that induction results in transient expression of the Rac exonuclease gene (*recE*) accompanied by a specific excision of Rac by recombination at the hybrid attachment sites.

This chapter concerns the nature of *sbcA*$^-$ (suppressor of *recB* and *recC*; Barbour *et al*, 1970) mutations which result in constitutive expression of *recE* (Templin *et al*, 1972; Clark, 1973; Kushner *et al*, 1974) and which restore an exonuclease activity (ExoVIII), resistance to ultraviolet radiation, and recombination proficiency to RecB and RecC mutants. *sbcA*$^-$ mutations cannot be generated in AB1157 (Templin *et al*, 1972) which is deleted for Rac and which does not therefore possess *recE*. Two independent *sbcA*$^-$ mutations (*sbcA6* and *sbcA8*) map close to *rac* and are recessive to *sbcA*$^+$ in *sbcA*$^+$/*sbcA*$^-$ merozygotes (Lloyd and Barbour, 1974) suggesting that SbcA$^+$ cells contain a regulatory protein which interferes with expression of the *recE* gene and that SbcA$^-$ cells retain a binding site for this protein. Since the exonuclease activity (ExoVIII) found in SbcA$^-$ strains is a prophage encoded function, the SbcA$^-$ phenotype would not appear to be accompanied by prophage excision. Thus, Low (1973) argued that the *sbcA*$^+$ product cannot be the prophage repressor, mutations

in which would be expected to promote prophage excision with consequent loss of recE. In contrast to inheritance of rac, Lloyd (1974) found that the frequency of inheritance of the sbcA allele in Hfr recB$^-$sbcA8 x F$^-$recB$^-$rac$^-$ (a recB$^-$ derivative of AB1157) matings is consistent with the segregation of sbcA as a conventional genetic locus rather than as a component of an inducible prophage, as was the apparent frequency of inheritance of the sbcA$^+$ allele in the reverse (Hfr rac$^+$sbcA$^+$ x F$^-$sbcA8) cross. Moreover, the majority of the apparently sbcA$^+$ (i.e. Rec$^-$) progeny of the latter cross were also found to be Rac$^-$. Since in these experiments, sbcA and rac appeared to segregate as independent genetic loci, Lloyd (1974) concluded that the sbcA gene is not carried by the Rac prophage.

The experiments described below do not wholly support the above explanations of the SbcA$^-$ phenotype. Rather, it appears that such a phenotype may arise from more than one type of mutational change. In particular, the sbcA8 mutation appears to be a deletion of part of the Rac prophage genome in which the hybrid attachment site, PB', and probably the prophage repressor gene, have been removed. By analogy with the generation of λ transducing phages it seems probable that the sbcA8 mutation occurred by aberrant excision of the Rac prophage.

## 4.2 RESULTS.

### (a) The JC5412 (sbcA8) chromosome contains a partially deleted Rac prophage.

Fig. 4.1(a) shows fragments which cross-hybridise with λ*rev* DNA in the DNAs of JC5412 (*sbcA*8) and C600 (*rac*$^+$). Sizes of those R.*Eco*RI generated fragments in C600 DNA which derive from the Rac prophage are indicated. These fragments cross-hybridise with the DNA substitution in λ*rev* (see Chapter 3 and Fig. 4.4(a)). The fragments observed in both C600 and JC5412 DNAs, and for which sizes are not given, result from cross-hybridisation between λ*rev* DNA and another lambdoid prophage(s) located elsewhere in the *E.coli*K12 chromosome (see Chapter 5). R.*Eco*RI digested JC5412 DNA also contains fragments derived from the Rac prophage. Two of these fragments (8.4kb and 2.65kb) are the same size as fragments present in C600 DNA and are associated with the prophage hybrid attachment site BP' in that DNA (Fig. 4.4(a)). This region of the Rac prophage contains the exonuclease gene, *recE* (see Chapter 3), the product of which (Exo VIII) is found in SbcA$^-$ strains.

Other R.*Eco*RI generated fragments (13.6kb, 4.6kb, 4.2kb) which are seen in C600 DNA digests, and which derive from the Rac prophage, are not observed in JC5412 DNA digests. However, a new fragment (1.9kb) is seen in R.*Eco*RI digested JC5412 DNA with both a λ*rev* (Fig. 4.1(a)) and a λ891 (Fig. 4.1(b)) DNA probe. This fragment does not cross-hybridise with λ DNA (Fig. 4.1(c)) and must therefore derive from that region of the Rac prophage common to both λ*rev* and λ891.

The simplest interpretation of these observations is that the JC5412 chromosome is deleted for a substantial portion of the Rac genome. R.*Eco*RI generated fragments which are associated with the hybrid attachment site PB' in *rac*$^+$ strains (4.6kb and 4.2kb) are not observed in JC5412 DNA, nor is the 13.6kb fragment which contains the prophage replication origin, *oriJ*, and at least one repressor binding site, if not the prophage repressor gene itself

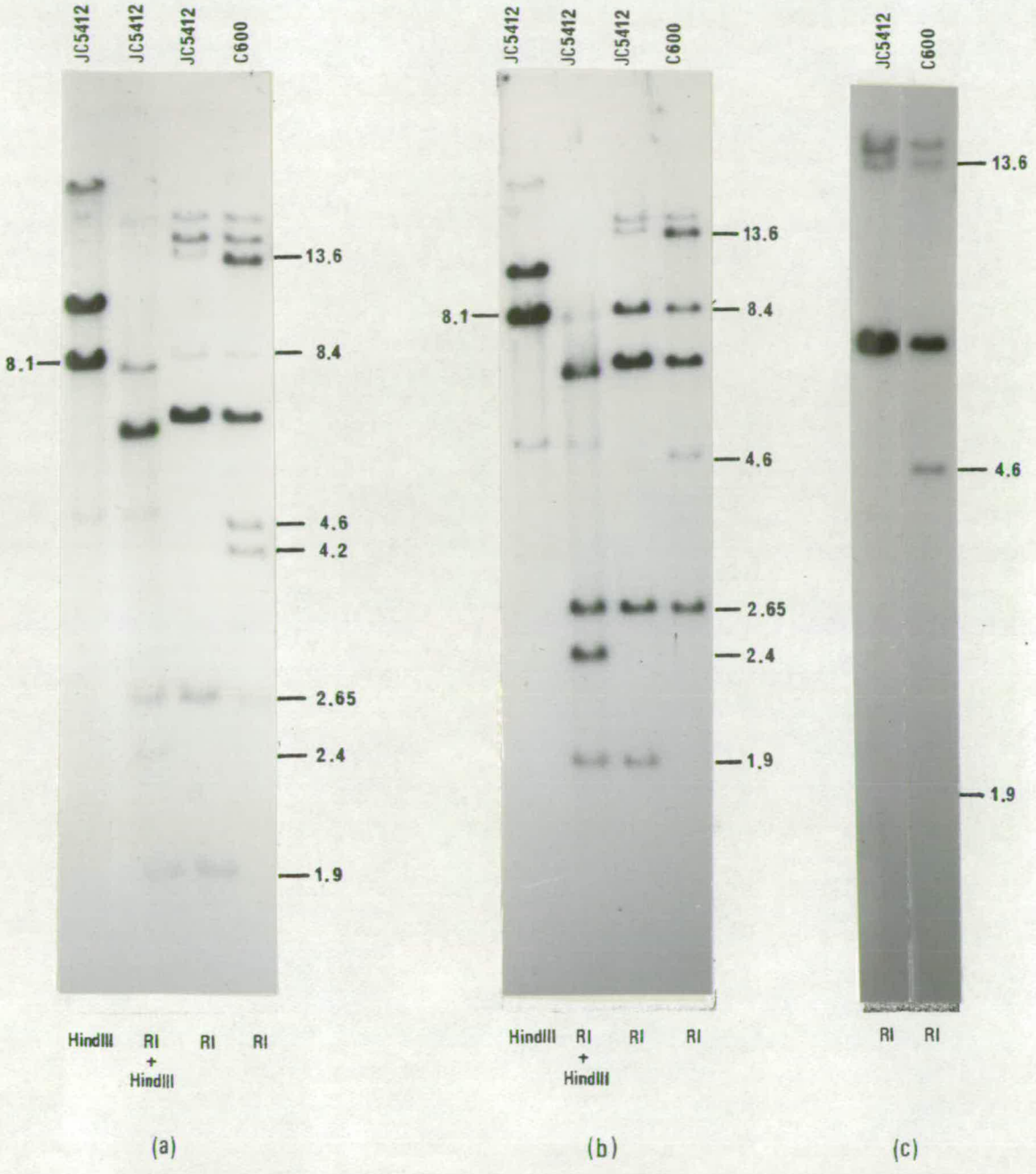



Fig. 4.1 Restriction endonuclease generated fragments in JC5412 (sbcA8) and C600 DNAs which cross-hybridise with (a) λrev DNA; (b) λ891 DNA; and (c) λ DNA. Electrophoresis was carried out in 0.7% agarose gels. Fragments for which sizes (in kb) are given contain Rac prophage DNA sequences. The 8.1kb fragment seen in R.HindIII digested JC5412 DNA with either a λrev or a λ891 probe contains all of the Rac prophage DNA sequences retained by JC5412 (see Fig. 4.4(a)). For other details see text.

(Diaz and Pritchard, 1978; Diaz et al, 1979). A map of the R.EcoRI and R.HindIII targets around the partially deleted Rac prophage in JC5412 DNA can be constructed from the data of Fig. 4.1 (Fig. 4.4(a)). Thus, the deletion in JC5412 extends from the right of the prophage and ends within the 13.6kb R.EcoRI generated fragment found in $rac^+$ strains. Undeleted DNA from the left end of the 13.6kb fragment shares homology with both the λrev and λ891 substitutions so that a new R.EcoRI generated fragment (1.9kb) is observed in JC5412 DNA digests when either a λrev or λ891 probe is employed.

Lloyd (1974) transferred the sbcA8 allele from HfrJC5412 into a $recB^-$ derivative of AB1157 (S583) and used the resulting strain (S583sbcA8) in his experiments on the inheritance of the sbcA8 (see Section 4.1). DNA prepared from S583sbcA8 shows the same pattern of hybridisation with λrev DNA as does JC5412 DNA (data not shown).

(b) Other $SbcA^-$ mutants.

Fig. 4.2 shows R.EcoRI generated fragments which cross-hybridise with λrev DNA in DNAs prepared from several other $SbcA^-$ strains: JC5174 (sbcA1), JC7661 (sbcA23); JC8679 (sbcA23); PE108 (sbcA50) and PE138 (sbcA51). JC5491 is the $recB^-recC^-sbcA^+$ progenitor of JC7661. The hybridisation pattern seen in JC5491 DNA digests is the same as that seen in C600 DNA digests.

The hybridisation patterns observed in JC7661, PE108 and PE138 DNAs (Fig. 4.2(a)) have several features in common with each other. These DNAs all contain R.EcoRI generated fragments of the sizes expected from an intact Rac prophage (compare with R.EcoRI digested C600 DNA) but in addition they each contain a new fragment (2.45kb) which hybridises strongly with a λrev DNA probe. This 2.45kb fragment also cross-hybridises weakly with λ891 DNA but not at all with λ DNA (Fig. 4.3), and is therefore indistinguishable, both in size and hybridisation behaviour, from the R.EcoRI generated fragment which spans the attachment site (PP') in λrev DNA (Fig. 4.4(b)). Densitometer

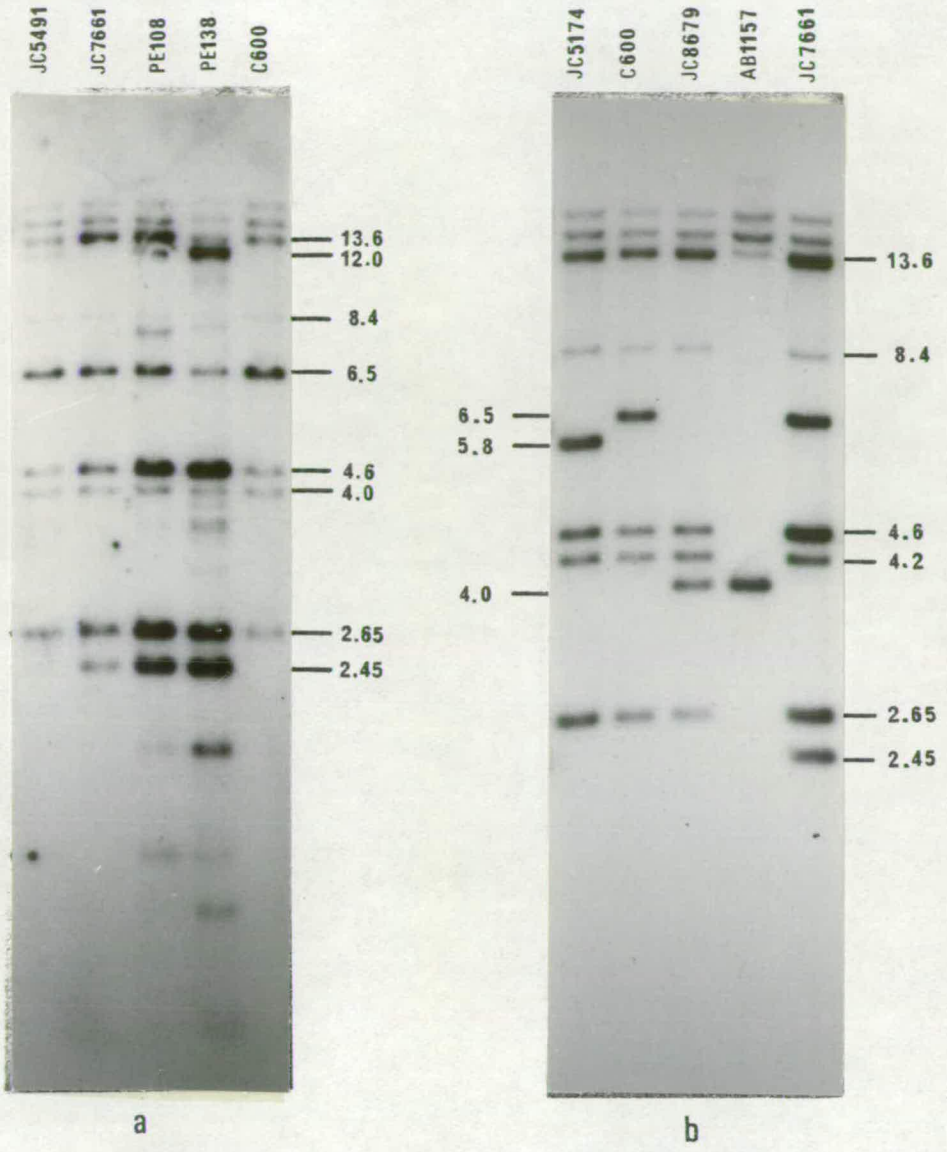



Fig. 4.2 R.EcoRI generated fragments which cross-hybridise with λrev DNA in the DNAs of several sbcA strains. Electrophoresis was carried out in 0.7% agarose gels. Band sizes on the right in (b) are for fragments which contain Rac prophage DNA sequences. Band sizes on the left are for fragments which derive from a second lambdoid prophage in E.coliK12 strains (see Chap. 5). For other details see text.

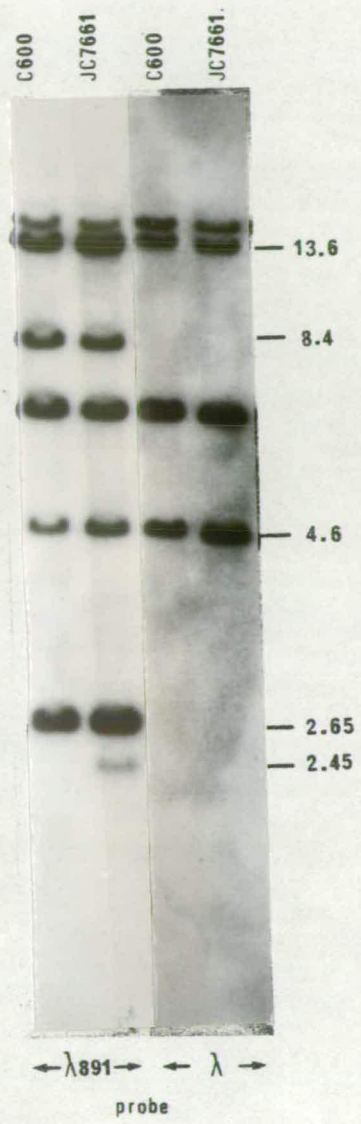



Fig. 4.3 R.EcoRI generated fragments in C600 and JC7661 (sbcA23) DNAs which cross-hybridise with λ891 and λ DNAs. Electrophoresis was carried out in a 0.7% agarose gel (see text for other details).

tracings of the tracks containing C600, JC7661, PE108 and PE138 DNAs (data not shown), or even a comparison of those tracks by eye, show that in the three sbcA⁻ DNAs, bands due to R.EcoRI generated fragments which are internal to the Rac prophage (e.g. 2.65kb, 4.6kb) are raised in intensity relative to bands due to the two fragments (8.4kb, 4.2kb) which span the hybrid attachment sites, and relative to bands (e.g. 6.5kb) which derive from other lambdoid prophages. This observation is consistent with the presence of multiple copies of Rac in JC7661, PE108 and PE138. The copies could be present as tandemly repeated units within the bacterial chromosome; as a concatameric, extra-chromosomal molecule in a cell which carries one or more copies of the prophage within the chromosome; or as a number of monomeric, circular, copies of the prophage genome in such a cell. For simplicity and since the prophage genome appears to be segregated at high frequency from the progeny of a cell in which the prophage has been induced (Low, 1973), only the former model is considered below (Fig. 4.4(b)). Thus the 2.45kb, R.EcoRI generated fragments observed in JC7661, PE108 and PE138 DNAs with a λrev DNA probe contains PP', spans the junction between two Rac prophages, and is indeed equivalent to the 2.45kb R.EcoRI generated fragment carried by the λrev substitution (Fig. 4.4(b)). This DNA fragment shares homology with the λ891 substitution via the small region of DNA carried to the right of PP'. The relative intensities of the 4.6kb and 2.65kb (internal to prophage), the 8.4kb and 4.2kb (span hybrid attachment sites) and the 6.5kb (from second prophage) bands (in Fig. 4.2(a)) as measured from densitometer tracings suggest that JC7661 is a tri-lysogen and that PE108 and PE138 contain at least eight and twelve copies of the Rac prophage respectively (data not shown). In R.EcoRI digested PE108 and PE138 DNAs several new bands, other than the 2.45kb band, are observed with a λrev DNA probe. These may result from mutations within a fraction of the repeated units in these DNAs. In particular R.EcoRI digested PE138 DNA contains a prominent new band (12.0kb) which almost completely replaces the 13.6kb band in

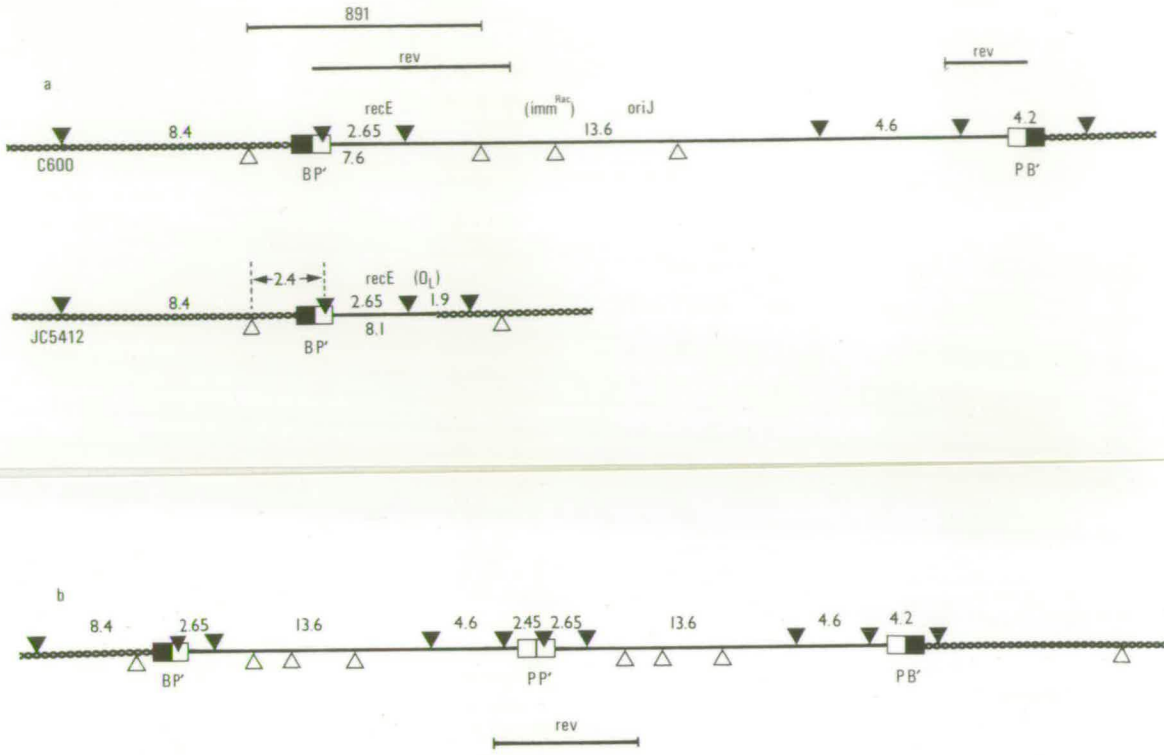



Fig. 4.4.

(a) Physical maps of the region of the C600 chromosome which carries the Rac prophage, and its derivative in JC5412 (sbcA8). Regions of the Rac genome carried by the λrev and λ891 substitutions are shown. Sizes of R.EcoRI and R.HindIII generated DNA fragments are shown immediately above and below the lines respectively. Only the approximate relative positions of phage markers are known. $imm^{Rac}$ indicates the postulated position of the prophage repressor gene and associated regulatory functions. $O_L$ indicates the hypothetical repressor binding site which controls expression of the recE gene. Other symbols are described in the legend to Fig. 3.12(a).

(b) Tandem lysogeny. A di-lysogen is shown. Sizes of R.EcoRI generated DNA fragments are indicated above the line. If there are n tandemly repeated units of the Rac genome, DNA fragments totally contained within the prophage (2.65kb, 13.6kb, 4.6kb) are present as n copies; fragments which span the junction between two prophages (2.45kb) as (n-1) copies; fragments which span the hybrid attachment site (8.4kb and 4.2kb) as one copy. The region which spans the junction between two prophages is equivalent to the λrev substitution.

intensity. The simplest interpretation of this observation is that many of the repeated prophage units in PE138 carry a small deletion (approx. 1.5kb) within the 13.6kb fragment. An unexplained feature of the hybridisation patterns in JC7661, PE108 and PE138 DNAs is that in each case the 13.6kb band, or its derivative in PE138 DNA, is less intense than expected from the predicted number of prophages (but still considerably more intense than expected from only a single copy of the prophage).

JC8679 (sbcA23) is an Exo VIII$^+$ derivative of AB1157. JC8679 was derived from one of the progeny of a mating between HfrJC7661 (sbcA23) and JC5519 (a recBC$^-$ derivative of AB1157) (Gillen and Clark, 1977).
AB1157 and its derivatives are specifically deleted for the Rac prophage (see Chapter 3) and carry an internal deletion within a second lambdoid prophage such that a 4.0kb R.EcoRI generated fragment is identified with a λ (or λrev) DNA probe in place of the 6.5kb fragment identified in C600 DNA digests (see Chapter 5). By the criteria established above JC8679 has acquired one intact copy of Rac from JC7661 (Fig. 4.2(b)). This copy appears to be located at the correct attachment site (BB') for Rac in AB1157 since the 8.4kb and 4.2kb R.EcoRI generated fragments which span the hybrid attachment site in rac$^+$ strains are also observed in JC8679 DNA digests. If, as appears likely, the JC7661 chromosome contains three tandemly repeated copies of the Rac prophage it would not be possible for the JC8679 chromosome to have gained only a single copy of Rac as the result of a simple recombination event. More likely zygotic induction of the Rac prophages carried by HfrJC7661 occurred on transfer into JC5519, followed by a site specific integration of one (now circular) copy of the prophage at the bacterial attachment site for Rac (BB') in JC5519.

JC5174 (sbcA1) DNA also contains all R.EcoRI generated fragments expected from a single copy of the Rac prophage integrated at BB' (Fig. 4.2(b)). However, this DNA contains a 5.8kb R.EcoRI generated fragment in place of the

6.5kb fragment observed in C600 DNA, suggesting that JC5174 also carries a mutation in the region of the E.coliK12 chromosome which contains a second lambdoid prophage.

## 4.3. DISCUSSION.

It appears that more than one type of mutation may result in the constitutive expresion of *recE* characteristic of SbcA$^-$ strains. Cross-hybridisation studies between λ*rev* DNA and DNA prepared from several SbcA$^-$ strains reveal at least three classes of SbcA$^-$ mutant.

The *sbcA8* mutation carried by JC5412 appears to be a deletion of part of the Rac prophage genome. By analogy with the generation of λ transducing phages, it seems probable that the *sbcA8* mutation arose by aberrant excision of Rac from the host chromosome. The region of the prophage deleted in JC5412 includes the hybrid attachment site PB' and probably the prophage repressor gene which by analogy with λ would lie within the 13.6kb R.*Eco*RI generated DNA fragment found in *rac*$^+$ strains (see Section 3.2(g)). The *recE* gene resides in that region of the Rac prophage common to λ*rev* and λ891: i.e. the region adjacent to BP' (Fig. 4.4(a)). This is the region of the prophage retained by JC5412. Derepression of *recE* caused by deletion of the prophage repressor gene could thus account for the presence of ExoVIII in JC5412. By analogy with λ, the putative *int*$^{Rac}$ and *xis*$^{Rac}$ genes will lie to the left of *recE* and one would expect them also to be expressed in JC5412. However, since one hybrid attachment site (PB') is deleted in JC5412, excision of the prophage cannot occur.

Such an interpretation of the SbcA8 phenotype is in agreement with the conclusions of Lloyd and Barbour (1974) as to the location of the *sbcA8* mutation (close to *rac*) and also with the finding by Lloyd (1974) that the frequency of inheritance of *sbcA8* in Hfr matings is consistent with *sbcA8* being segregated as a conventional genetic locus rather than as a component of an inducible prophage. Thus on the basis of experiments involving JC5412, or its derivatives, it does not seem necessary to attribute any regulatory function to the *sbcA*$^+$ product other than that of the prophage repressor itself.

Lloyd and Barbour (1974) found that the *sbcA8* mutation

is recessive to *sbcA*$^+$ in *sbcA*8/*sbcA*$^+$ merozygotes. In this experiment the sbcA$^+$ allele in the merozygotes was provided by the F' factor F123 which carries an intact Rac prophage (see Chapter 3). It therefore seems possible that the JC5412 chromosome retains a binding site for the prophage repressor such that in the presence of repressor (provided by the prophage carried by F123), expression of *recE* from the JC5412 chromosome is switched off and the cell becomes SbcA$^+$ (Rec$^-$). By analogy with λ, one would expect this site (probably equivalent to $O_L$) to lie just to the right of the *recE* gene (Fig. 4.4(a)). If this hypothesis is correct it should also be possible to turn off expression of the *recE* gene in JC5412 by introduction of a recombinant plasmid or phage into which the 13.6kb, R.*Eco*RI generated DNA fragment that is thought to contain the prophage repressor gene (see Section 3.2(g)) has been inserted.

pK9 (see Section 3.2(f) and Fig. 3.12(a)) is such a plasmid. For an as yet unexplained reason it has not proved possible to transform JC5412 with this plasmid. However, the 13.6kb fragment from pK9 has been transferred (in both orientations) into a λ replacement vector and derivatives of JC5412 have been constructed that are lysogenic for these recombinant phages. Experiments are in progress at the time of writing to determine the Rec and hence SbcA phenotypes of these strains.

Lloyd and Barbour (1974) observed that transfer of the *sbcA*8 region of HfrJC5412 into an F$^-$*rac*$^+$ recipient caused a sharp increase in the frequency of recombinants for genetic markers that were proximal to *trp* on the Hfr chromosome. Although this appears very similar to the Rac effect observed by Low (1973), one would not expect such stimulation of recombinant recovery to have occurred since the recipient was *rac*$^+$. It is possible that transient expression of *recE* from the donor (JC5412) chromosome may occur prior to the establishment of repression by repressor present in the recipient.

Three independently isolated SbcA$^-$ strains (JC7661, *sbcA*23; PE108, *sbcA*50; and PE138, *sbcA*51) contain

multiple copies of the Rac prophage which are probably present as tandemly repeated units within the bacterial chromosome. In one strain (PE138) at least twelve copies of the prophage appear to be present. Since the Rac prophage genome consists of approximately 25kb of DNA (see Section 3.3) while the E.coli chromosome has about $5 \times 10^3$kb of DNA, PE138 must have in excess of 6% of its genome in the form of prophage DNA. How multiple lysogeny could facilitate constitutive expression of recE remains unclear. Secondary mutations within one or more of the prophage units may be involved (see below).

Two SbcA$^-$ strains (JC5174, sbcA1; and JC8679, sbcA23) harbour a Rac prophage that is indistinguishable in its hybridisation behaviour from the prophage carried by rac$^+$ strains (e.g. C600). The sbcA23 allele appeared to have been introduced into an immediate ancestor of JC8679 by conjugational transfer from HfrJC7661 (sbcA23). JC7661 appears to carry three copies of the prophage genome, only one of which can have been inherited by JC8679. It is thus possible that tandem lysogeny is not directly associated with the SbcA$^-$ phenotype in JC7661 but that one or more copies of the prophage in this strain (one of which has been inherited by JC8679) carry mutations allowing expression of recE in the absence of prophage excision. This may also be true of the other strains (PE108 and PE138) exhibiting multiple lysogeny. It is also possible that the SbcA phenotype of JC5174 and JC8679 is a result of mutations in a regulatory gene external to the Rac prophage as suggested by Lloyd (1974) to explain the behaviour of the sbcA8 locus. However, such a gene need not now be restricted to the vicinity of rac. It is interesting to note that both JC5174 and JC8679 carry mutations in a second lambdoid prophage.

In addition to ExoVIII, a Ral (restriction alleviation) activity has been observed in SbcA$^-$ strains (Simmon and Lederberg, 1972). Penny Toothman (personal communication) has found a Ral activity in λrev which she has called Lar. A similar function is specified by λ. The λ ral gene lies to the right of red and gam and to the left of cIII and $O_L$.

Thus we may expect *lar* to occupy a similar position in the Rac prophage (i.e. between *recE* and $O_L$; see Fig. 4.4(a)). Gillen and Clark (1977) have shown that the *recE* product (ExoVIII) is a single polypeptide with a M.W. of $1.4 \times 10^5$ and which therefore requires approximately 4.2kb of coding DNA. In JC5412 *sbcA8*, intact *recE* and *lar* genes will be present if the repressor binding site ($O_L$) is present. The maximum amount of DNA available in JC5412 to code for the RecE function is approximately 4.75kb (i.e. the distance between BP' and the right end of the 1.9kb, R.*Eco*RI generated, fragment) and since the intensity of the 1.9kb band is weak compared with that expected if the 1.9kb fragment were predominantly prophage DNA, the actual amount of coding DNA may be rather less. This result bears out the suggestion of Gillen and Clark (1977) that ExoVIII may be equivalent to a gene fusion product of the λ proteins it replaces in λ*rev* (*redα*, *redβ*, *gam*, *ral*, *cIII*) and raises the possibility that the putative Rac prophage Int and Xis functions (which in λ require approximately 1.5kb of coding DNA) are also provided by ExoVIII.

# CHAPTER 5

## The Origin of Q-independent Derivatives of Phage λ

5.1 INTRODUCTION.

Expression of the late λ functions depends on the presence of gene Q, the product of which allows transcription to proceed into the late region of the λ genome in a manner analogous to the anti-terminating effect of the gene N product (Roberts, 1975). Since λQ⁻ phages cannot express their late genes they do not form active virions. However, several independent bypass mutants have been characterised which are able to complete lytic development in the absence of gene Q. The best studied are the p4 (Jacob and Wollman, 1954), qinA3 (Q-independent; Court and Sato, 1969; Sato and Campbell, 1970) and p41 (Henderson and Weil, 1975) mutants which are characterised by the replacement of the region of the λ genome which contains Q, S, R and the late gene promoter, $P'_R$, with foreign DNA, thought to be derived from the host (E.coli K12) chromosome (Fiandt et al, 1971; Henderson and Weil, 1975). λp4 and λqinA3 contain indistinguishable substitutions which nowhere share homology with the λDNA they replace (Fiandt et al, 1971) while λp41 has a larger but related substitution including a small region (approx. 190bp) of homology with λDNA (Henderson and Weil, 1975). These substitutions appear to code for a function analogous to Q, but with a different specificity, since hetero-immune λp4 superinfecting phage can trans-activate the late genes of a $\lambda imm^{434}$ p4 or $\lambda imm^{434}$ qinA3 prophage but not those of a $\lambda imm^{434}$ prophage (Herskowitz and Signer, 1974). Since the Q-independent mutants can lyse cells it seems reasonable to assume that they also code for functions analogous to S and R.

These observations have led several authors to suggest that the new functions (qsr') normally reside in a prophage, or more likely a defective prophage, present in the E.coli K12 chromosome, and that Q-independent (λqsr') phages are the product of recombination between λ and the hypothetical prophage (Fiandt et al, 1971; Szybalski and Szybalski, 1974; Herskowitz and Signer, 1974; Henderson and Weil, 1975; Strathern and Herskowitz, 1975). Henderson and Weil (1975) and Strathern and Herskowitz (1975) tried unsuccessfully to isolate λqsr' mutants from the rac⁻ strain

AB1157 and its derivatives suggesting that the qsr' genes also reside in the Rac prophage.

In the experiments described below, restriction endonuclease analysis and DNA/DNA hybridisation methods were used to investigate the origin of λqsr' phages. It appears that the λqsr' substitutions are derived from a region of the E.coli K12 genome which, although distinct from the Rac prophage, shares considerable homology with λDNA and probably represents a second lambdoid prophage located at an as yet unidentified site on the chromosome (the qsr' prophage). In AB1157, from which λqsr' phages cannot be generated, this new prophage has suffered an internal deletion.

5.2. RESULTS.

(a) Sequences in E.coli K12 DNAs which share homology with λ DNA.

At least two regions of the E.coli K12 chromosome (one of which is the Rac prophage) share significant homology with λDNA (see Chapter 3). It is from the Rac genome that the substitution in λrev DNA is derived. AB1157, in which neither λqsr' nor λrev phages can be generated, is specifically deleted for the Rac prophage. Fig. 5.1 shows the sizes of R.EcoRI and R.HindIII generated fragments which cross-hybridise with λDNA in the DNAs of C600(rac$^+$) and AB1157(rac$^-$). The pattern observed in C600 DNA is typical of E.coli K12 strains in general. Those fragments which derive from the Rac prophage (and which are therefore not present in AB1157 DNA) are indicated. Several additional fragments are apparent in both C600 and AB1157 DNAs. Most of these are the same size in both DNAs. However, in each type of digest an intense band in C600 DNA (e.g. 10.3kb with R.HindIII)is replaced by a smaller, but almost equally intense band in AB1157 DNA (e.g. 7.1kb with R.HindIII) indicating that AB1157 is mutated in the region of the bacterial chromosome which carries these fragments.

Of the λ-homologous fragments observed in Fig. 5.1, only those which derive from the Rac genome are represented in the region of the bacterial chromosome carried by the F' factor, F123 (see Chapter 3).

(b) Restriction endonuclease analysis of the substitutions in λqsr' phages.

In order to determine whether the substitutions in λqsr' phages are associated with any of the λ-homologous fragments observed in C600 and AB1157 DNAs it was first necessary to construct a map of the R.EcoRI and R.HindIII targets within such a substitution. Fig. 5.2 shows a physical map of the λimm$^{21}$p4 genome. Analysis of λ/λp4 DNA heteroduplexes shows the substitution in λp4 DNA, which nowhere shares homology with the λ DNA it replaces, to have the approximate endpoints and length shown (Fiandt et al,

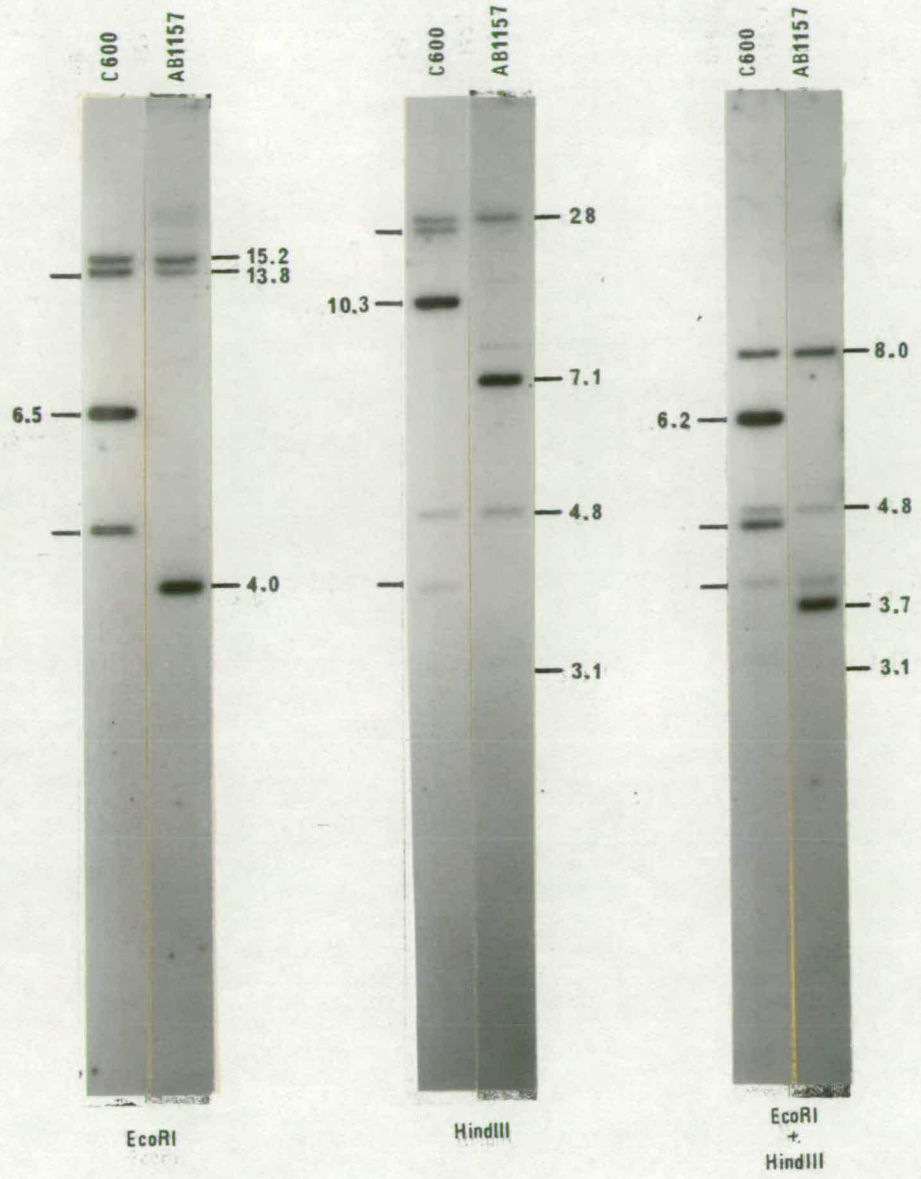



Fig. 5.1 R.EcoRI and R.HindIII generated fragments in C600 and AB1157 DNAs which cross-hybridise with λ DNA. Electrophoresis was carried out in a 1% agarose gel. Identified fragments for which sizes are not given are derived from Rac prophage in C600 DNA (see Chapter 3) and are not present in AB1157 DNA. The 3.1kb, R.HindIII generated, fragment is present in both C600 and AB1157 DNAs but is not clearly visible in this autoradiograph (but is visible in Fig. 3.1). The weak 4.0kb fragment seen in R.EcoRI + R.HindIII digested AB1157 DNA is the result of incomplete digestion of the 4.0kb R.EcoRI generated fragment from this DNA by R.HindIII.

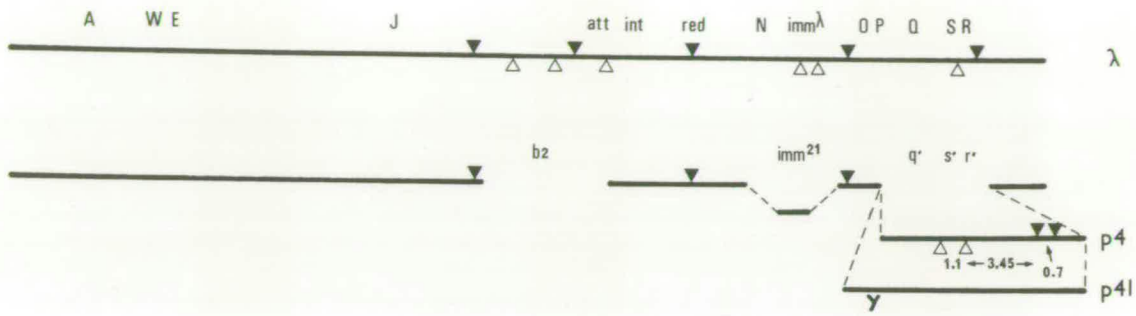



Fig. 5.2. Physical characterisation of λ*qsr'* phages.

Above: Genetic and physical map of the wild type λ genome. The length of $\lambda^{+}$ DNA is taken as 100 units = 49kb (Phillippsen & Davis, 1978).

Below: The λ*imm*$^{21}$ *b2 p4* genome. The end points of the *b2* deletion and of the *imm*$^{21}$ substitutions are known (Fig. 1.3). The λ*b2 p4* genome was generated by substitution of an *E.coli* K12 DNA fragment (19 units or approx. 8.3kb) for the region of the λ genome that contains the *Q*, *S* and *R* genes (83.8 to 94 units) (Fiandt *et al*, 1971). The positions of R.*Eco*RI and R.*Hind*III targets are shown above and below the line respectively. The *p4* substitution is indistinguishable from that carried by λ*qinA3* (see text).

The substitution carried by λ*p41* (22 units or approx. 9.8kb) has the same right hand end point as the *p4* substitution and contains all of the DNA present in the *p4* substitution, and in addition, a further 1.5kb at its left end (Henderson and Weil, 1975). The region of the λ*p41* substitution denoted y is a small segment (approx. 190bp) homologous to the region of the λ genome between 83.4 and 83.8 units. This segment in the *p41* substitution lies immediately to the left of the region of this substitution carried by the *p4* (and *qinA3*) substitutions. Except for this small region, the *p41* substitution shares no homology with λ DNA.

1971). The sizes of R.EcoRI and R.HindIII generated λimm$^{21}$ p4 DNA fragments were determined by comparison with digests of λcI857 DNA following their separation by electrophoresis through agarose gels (data not shown). The positions of the targets for these enzymes in λimm$^{21}$ p4 are shown in Fig. 5.2. In confirmation of the heteroduplex data, a λDNA probe fails to cross-hybridise with restriction endonuclease generated DNA fragments totally contained within the p4 substitution (data not shown). Heteroduplex analysis shows λqinA3 DNA to contain a substitution which is indistinguishable from that carried by λp4 DNA (Fiandt et al, 1971). The positions of R.EcoRI and R.HindIII targets within the qsr' substitutions of λp4 and λqinA3 are equivalent (data not shown) and are not consistent with these substitutions being derived from the Rac genome (see Fig. 3.12(a)).

(c) Fragments in E.coli K12 DNAs which cross-hybridise with λp4 DNA.

Fig. 5.3 shows the sizes of fragments in LE451 and AB1157 DNAs which share homology with λp4 DNA. LE451 is a derivative of C600 in which (as in AB1157) the Rac prophage has been specifically deleted (see Chapter 3). However, in all other respects the pattern of λ-homologous fragments in LE451 DNA digests is identical to that observed in C600 DNA digests. Fig. 5.3 is not simple to interpret since only a few of the observed fragments can be attributed to the region of the bacterial chromosome associated with the qsr' genes. However, several relevant features of the hybridisation patterns in Fig. 5.3 can be discerned:

(i) Fragments equivalent in size to those generated from within the λp4 substitution (0.7kb, 1.1kb, 3.45 kb) are present in digests of LE451 DNA. In AB1157 DNA the R.EcoRI generated, 0.7kb, fragment is not observed.

(ii) A 13.8kb and a 3.1kb fragment are identified with a λ probe in both LE451 and AB1157 DNAs after digestion

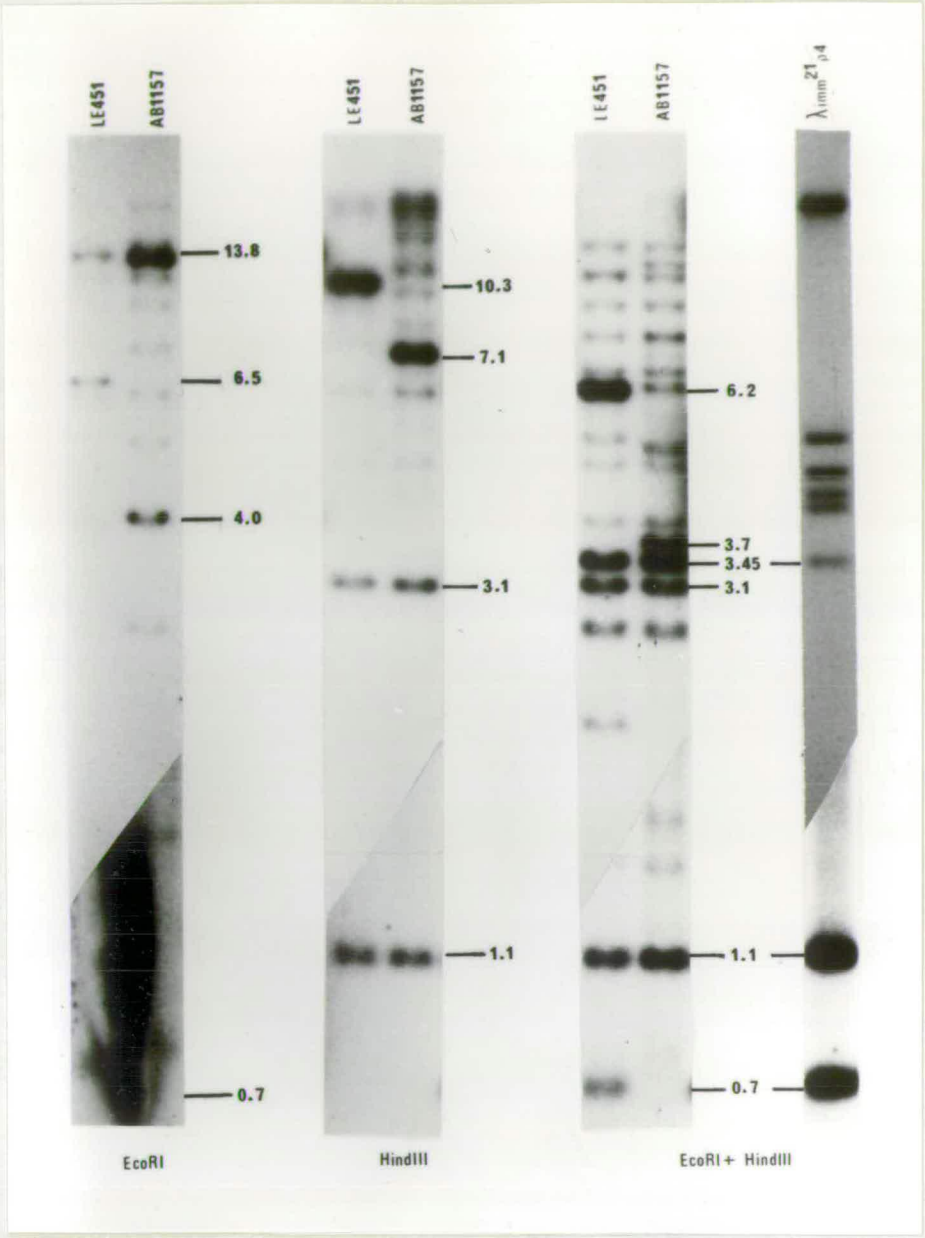



Fig. 5.3.

R.EcoRI and R.HindIII generated fragments in LE451, AB1157 and λimm$^{21}$ b2 p4 DNAs which cross-hybridise with λimm$^{21}$ b2 p4 DNA. Electrophoresis was carried out in a 0.7% agarose gel. Upper section, overnight exposure; Lower section, 1 week exposure. Fragments in the E.coli K12 DNA for which sizes are not indicated are thought to represent a repeated (transposon-like) sequence in the E.coli K12 chromosome, one member of which lies within the p4 substitution (see text). For other details see text. In R.EcoRI digested LE451 DNA, the 0.7kb fragment is obscured.

with R.EcoRI and R.HindIII respectively. Fragments of the same size but of higher relative intensity are identified with a λp4 DNA probe in these DNAs.

(iii) Other prominent fragments in Fig. 5.3 are those of the sizes identified with a λ⁻ DNA probe and which are derived from a region of the E.coli K12 chromosome in which AB1157 is mutated (see 5.2(a)). In R.EcoRI digested LE451 and AB1157 DNAs, one of these fragments (6.5kb and 4.0kb respectively) is seen in addition to the intense 13.8kb fragment while in the same DNAs digested with R.HindIII, only one intense band (10.3kb and 7.1kb respectively) is seen.

Thus, it seems that the qsr' block is associated with λ-homologous DNA sequences in the E.coli K12 chromosome. Moreover, the inability of AB1157 to generate λqsr' phages may be because AB1157 is mutated in the region of the bacterial chromosome which carries these sequences.

The many weak, and unidentified, bands in Fig. 5.3 represent a class of repeated DNA sequences in the E.coli K12 genome, one member of which lies within the region of the chromosome which comprises the λp4 substitution. All E.coli K12 DNAs tested contain such a set of fragments. However, when any two different E.coli K12 DNAs are compared, differences in the sizes of one or two of these fragments are often apparent (as in Fig. 5.3). These findings are consistent with the λp4 substitution carrying a transposable DNA sequence repeated several times in the E.coli K12 chromosome. A second copy of this sequence has been identified within the Rac genome (see Chapter 3).

(d) Isolation and characterisation of recombinant plasmids containing qsr' associated DNA.

A preliminary map of the qsr' region of the LE451 (or C600) chromosome and its derivative in AB1157 can be constructed from the data of Figures 5.2 and 5.3. However, in order to better characterise this region, libraries of bacterial colonies containing fragments of the E.coli K12 genome linked to plasmid vectors were screened with λ DNA

and λp4 DNA probes by the colony hybridisation procedure (Grunstein and Hogness, 1975). In this manner, several recombinant plasmids which contain E.coli K12 DNA fragments from the qsr' region were isolated (Table 1.3). The plasmids were mapped with the enzymes R.EcoRI and R.HindIII and were used as hybridisation probes to recognise homologous sequences in C600 and AB1157 DNAs. 605 and 1105 were isolated from a library ofsheared DNA fragments which had been inserted by poly-dAT tailing into the R.EcoRI site of ColE1 (Clarke and Carbon, 1976). Only the approximate end points of the insertions in these plasmids have been determined. None of the plasmid vectors used share detectable homology with E.coli K12 DNA.

The cloned fragments all share homology with at least part of the λp4 substitution and also with λ DNA (data not shown). Moreover, when the plasmids are cleaved with R.EcoRI and R.HindIII, or are used as hybridisation probes against C600 or AB1157 DNA digests, they behave in the manner expected (see Section 5.2(c)) of fragments derived from the qsr' region of the E.coli K12 chromosome or its derivative in AB1157. Regions of the C600 and AB1157 genomes spanned by the cloned fragments are shown in Fig. 5.4(b).

The simplest interpretation of these results is that the qsr' genes are carried by a previously unrecognised, and probably defective, lambdoid prophage (the qsr' prophage) located at an as yet unidentified site on the E.coli K12 chromosome. In AB1157 DNA, the R.EcoRI and R.HindIII targets which define the left and right ends respectively of the insertion carried by pK2 (Fig. 5.4(b)) are equivalent to R.EcoRI and R.HindIII targets carried by C600 DNA. Moreover, pK2 hybridises significantly with only the 0.7kb and 6.5kb R.EcoRI generated fragments in C600 DNA. Thus it appears that in AB1157 the qsr' prophage has suffered an internal deletion (Fig. 5.4(b) which has removed the region of the prophage genome that spans the end point (x) common to the λp4, λqinA3 and λp41 substitutions and probably part of the qsr' block

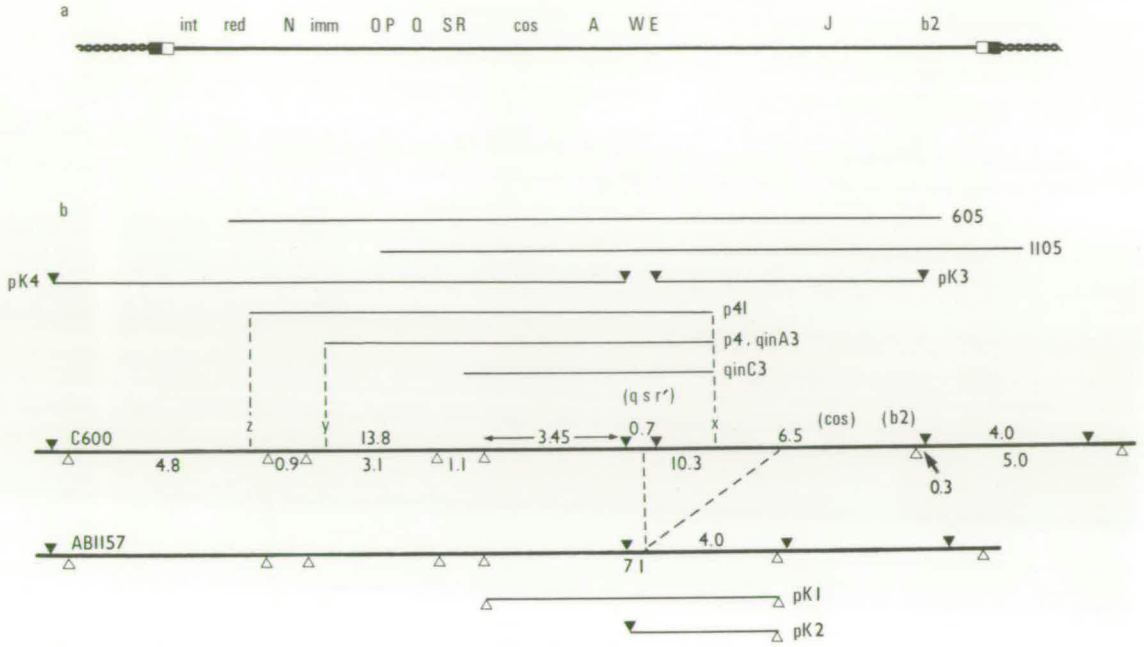



Fig. 5.4.

(a) Genetic and physical map of the λ prophage. Symbols are described in the legend to Fig. 1.3.

(b) A physical map of the region of the C600 chromosome which carries the qsr' prophage, and its derivative in AB1157. The AB1157 map is equivalent to the C600 map with an internal deletion. Sizes of R.EcoRI and R.HindIII generated fragments are indicated above and below the lines respectively. Sizes of some other fragments are shown. Regions of the C600 and AB1157 genomes carried by the λqsr' substitutions and by the recombinant plasmids discussed in Section 5.2(d) are indicated. x, y and z represent end points of the λqsr' substitutions discussed in this chapter. y is the small region of homology with λ DNA (approx. 190bp) discussed in the legend to Fig. 5.2. DNA fragments from C600 and AB1157 which share detectable homology with λ are those spanning x, y and z. The approximate positions of the q', s' and r' genes are shown, as are the regions of the prophage genome which cross-hybridise with the ends of the linear λ DNA molecule (cos) and with the b2 region of λ. Neither the location on the E.coli K12 chromosome nor the orientation of the qsr' prophage on the chromosome are known.

N.B. (a) and (b) are not to the same scale.

of genes (see discussion). Fragments which share detectable homology with λ DNA are those spanning x, y and z (see Fig. 5.1). pK2 and pK3 cross-hybridise with both ends of the mature, linear, λ DNA molecule (data not shown) suggesting that the cohered ends (cos) of the qsr' prophage are at the approximate position shown. These plasmids also share homology with the b2 region of the λ genome (see discussion).

(e) A third lambdoid prophage in E.coliK12?

The majority of the fragments in C600 and AB1157 DNAs which share homology with λ DNA (Fig. 5.1) have now been accounted for. However, one R.EcoRI (15.2kb) and one R.HindIII (28kb) generated fragment common to both DNAs does not appear to derive from either the Rac or qsr' prophages (at least to the extent that the latter has been mapped). These two DNA fragments overlap so as to generate a 8.0kb fragment in R.EcoRI + R.HindIII digests (Fig.5.1). A 17.5kb fragment is observed in R.EcoRI digested C600 or AB1157 DNAs with a λrev DNA probe but not with a λ DNA probe. This 17.5kb fragment hybridises specifically with the λrev substitution but is physically associated with the 28kb, λ-homologous, fragment rather than with the Rac prophage in the E.coliK12 chromosome (see Section 3.2(e)). Since the 17.5kb fragment is not cleaved by R.HindIII (data not shown) it must be totally contained within the 28kb fragment. No fragments with these characteristics can be detected in F123 DNA (see Chapter 3).

pK5 (Table 1.3 ) contains a 15.2kb, λ-homologous, DNA fragment derived from R.EcoRI digested C600 DNA. A map of the targets for R.HindIII within the pK5 insert was determined and the plasmid was used as a probe to detect complementary sequences in C600 and AB1157 DNAs (data not shown). Fig. 5.5 shows a composite map of the region of the E.coliK12 chromosome from which the 15.2kb and associated fragments are generated. This region is the same in all E.coliK12 strains tested.

It is at least possible that the region of the E.coliK12 chromosome described in this section is a part of the qsr'

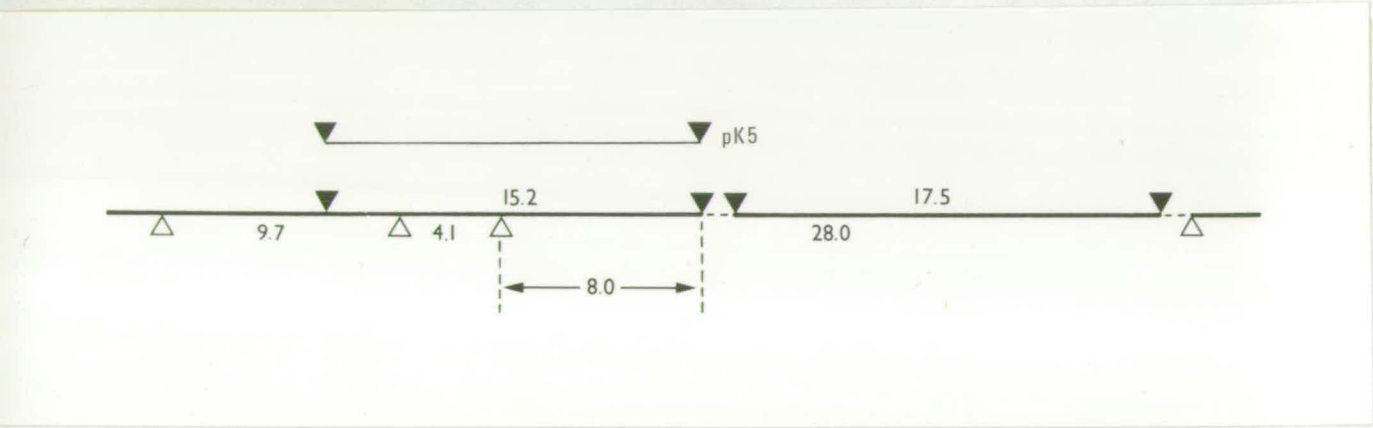



Fig. 5.5. Physical map of the region of the E.coli K12 chromosome suspected of being a third lambdoid prophage. Sizes of R.EcoRI and R.HindIII generated fragments are indicated immediately above and below the line respectively. The region carried by the recombinant plasmid pK5 is shown. The regions denoted ----- have not been investigated due to the lack of a suitable probe.

prophage. More probably however, since no overlaps between the two regions are apparent, although some homology between them has been observed (data not shown), the new region is a third defective lambdoid prophage.

## 5.3. DISCUSSION.

In this chapter two new regions of the E.coli K12 genome which share significant homology with λ DNA, and which are probably defective lambdoid prophages, have been described. In particular, one of these regions (the qsr' prophage) rather than the Rac prophage (as had been previously supposed) is the source of foreign DNA carried by the Q-independent (λqsr') derivatives of λ. In AB1157, from which λqsr' phages cannot be generated, the qsr' prophage has suffered an internal deletion.

Fig. 5.4 (b) shows a physical map of the region of the E.coli K12 chromosome which carries the presumed qsr' prophage and its derivative in AB1157. The sites denoted x, y and z represent the endpoints of the substitutions in λqsr' phages as determined from a comparison of λ/λqsr' DNA heteroduplexes (Fiandt, et al, 1971; Henderson and Weil, 1975) together with the calculated position of R.EcoRI and R.HindIII targets within the substitutions (Fig. 5.2). x, y and z all lie within R.EcoRI or R.HindIII generated DNA fragments which share homology with λ DNA. In particular, analysis of λ/λp41 DNA heteroduplexes (Henderson and Weil, 1975) has shown y to be a short (approx. 190bp) region of homology with λ DNA. Thus, the generation of λqsr' phages, which requires either host (Rec) or phage (Red) recombination functions (Strathern and Herskowitz, 1975) appears to be the result of recombination between λ DNA and homologous sequences within the qsr' prophage.

The region of the prophage deleted in AB1157 spans the endpoint (x) common the the λp4, λginA3 and λp41 substitutions. However, this feature alone is not sufficient to explain the inability of AB1157 to generate λqsr' phages since undeleted DNA to the right of x (contained in a 4.0kb R.EcoRI generated fragment in AB1157 DNA - Fig. 5.4 (b)) shares considerable homology with λ DNA (Fig. 5.1) and could therefore still facilitate recombination between the prophage and λ DNA. Thus, it seems probable that at least part of the qsr' block of

genes, which should therefore lie in the approximate position shown in Fig. 5.4 (b), is deleted in AB1157. The λ*qsr'* phage, λ*qin*C3 (Fiandt, et al, 1971) carries only that region of the *qsr'* prophage shown in Fig. 5.4 (b) consistent with the approximate position of the *qsr'* block deduced above.

Cross-hybridisation studies between cloned fragments of the *qsr'* prophage and λ DNA suggest that the prophage has its cohered ends (cos) located in the approximate position shown in Fig. 5.4 (b). In λ and related phages, the region of the *prophage* genome which codes for the structural (head and tail) proteins (equivalent to the left arm of the linear λ DNA molecule (Fig. 5.2)) lies between *cos* and the "*b2* region" (Fig. 5.4 (a) and require approximately 15kb of coding DNA. The 6.5kb fragment generated in most *E.coli* K12 DNAs by digestion with R.*Eco*RI (and carried by pK3) cross-hybridises with the *b2* region of the λ genome as well as with both ends of the linear λ DNA molecule. Thus, if the permutation of genes in the *qsr'* prophage is similar to that found in the λ prophage there would not appear to be sufficient DNA available to code for a full complement of structural proteins. This feature would explain the apparently defective nature of the *qsr'* prophage.

In order to determine whether possession of the *qsr'* prophage or a derivative of it is a common feature of *E.coli* strains, the same set of *E.coli* and related enterobacterial DNAs that was investigated for the presence of λ-homologous and Rac associated DNA sequences (see Section 3.3) was screened with a λ*p4* DNA probe. Comparison of the resulting autoradiographs with autoradiographs showing λ-homologous sequences in the same DNAs (data not shown) revealed only one other bacterial DNA (that of the laboratory strain *E.coli*B) that carried sequences related to the *qsr'* prophage. In particular, *E.coli*B DNA appears to carry a 6.5kb, R.*Eco*RI generated DNA fragment equivalent to that thought to carry the *cos* region of the *qsr'* prophage in *E.coli* K12 strains (see Fig. 4.4(a)). *E.coli*B DNA also

carries a fragment that though slightly smaller than the 13.8kb R.*Eco*RI generated fragment carried by the *asr'* prophage (Fig. 4.4(a)), would appear to be derived from it (data not shown).

The autoradiographs discussed in the last paragraph showed in addition that the "transposon-like" sequence thought to be contained within the λ*p*4 substitution (and also within the Rac prophage; see Section 3.3) and observed in approximately 10 copies with a λ*p*4 DNA probe in digests of *E.coli* K12 DNAs is in fact unique to *E.coli* K12.

# CHAPTER 6

# Discussion

Regions of the E.coli K12 ($\lambda^-$) chromosome which share homology with λ and related phage DNAs fall into three linkage groups. At least two of these (the rac and qsr' regions) contain blocks of genes that may functionally replace analogous genes carried by λ and are therefore thought to be lambdoid prophages. The genomes of the Rac and qsr' prophages are apparently organised in a similar fashion to the λ prophage but, by analogy with λ, are probably deleted for structural (head and tail) genes suggesting that both prophages are defective. The Rac prophage retains functional hybrid attachment sites at either end of the prophage genome and, as was shown by the experiments of Low (1973), is inducible. For this and other reasons the Rac genome is believed to code for a repressor protein which, under normal circumstances, blocks expression of genes that would promote prophage excision. No such information is available with regard to the presence or otherwise of attachment sites and/or repressor genes in the qsr' prophage.

Little is known about the third λ-homologous region of the E.coli K12 chromosome. However, since this region shares homology at non-adjacent sites with both λ DNA and with the region of the Rac genome carried by λrev (which itself shares no homology with λ DNA), it too is suspected of being a lambdoid prophage, and probably (like the Rac and qsr' prophages) a defective lambdoid prophage. In any case, no viable phages have ever been isolated from E.coli K12 ($\lambda^-$) strains during many years of investigation. The original isolate of E.coli K12 ($\lambda^+$) was therefore lysogenic for at least three, and probably four, lambdoid prophages, only one of which (λ) has been found able to produce an infective virion.

Although examples of multiple and/or defective lysogeny have not been previously established for lambdoid phages among naturally occurring E.coli strains, they are not uncommon features of several other phage/host systems (see

for example Holloway & Krishnapillai, 1975; Bradley, 1967; Garro and Marmur, 1970; Reeves, 1972; Campbell, 1977). One multiply lysogenic Pseudomonas aeruginosa strain has been described that harbours at least 8 different prophages (Shionoya et al, 1967). In several instances defective bacteriophages have been implicated in the production of inducible bacteriocidal agents. For example, the 'colicin' produced by E.coli15 (Endo et al, 1965) is apparently an inducible but defective phage as are bacteriocidal agents produced by several P.aeruginosa strains (Reeves, 1972), by Vibrio cholerae (Gerdes and Romig, 1975) and by Bacillus subtilis (Garro and Marmur, 1970). In the case of the PBSX phage of B.subtilis, a chromosomal location for the PBSX prophage has been established (Thurm and Garro, 1975). Induction of these defective phages generally leads to the production of phage-like structures (in some cases complete with phage DNA) that, although not able to productively inject, retain sufficient functions to kill their host or other bacteria.

In a comparatively small number of cases, bacterial strains not known to produce phage-like particles on treatment with inducing agents have been shown to harbour phage genes that may recombine with and functionally replace those of an active phage. For example, E.coliB is known to contain a non-inducible, P2-like, prophage, the immunity region of which is able to substitute for the equivalent (but hetero-immune) region of P2 (Chattoraj and Inman, 1975). Similarly, the presence of a defective lambdoid prophage(s) in E.coli K12 was inferred from the characteristics of the λgsr' and λrev phages. However, with only a few exceptions, the detection of defective prophages has depended on them being able to produce (after treatment of their hosts with UV light or other inducing agents) an observable effect on their hosts or on other bacteria. It seems probable therefore that many defective lysogenic strains exist in nature without their lysogenic nature being realised.

For E.coli, the limited available evidence suggests that less than 10% of independently isolated strains carry

inducible prophages, approximately one third to one half of which are of the lambdoid variety (Jacob and Wollman, Bertani and Bertani, 1971; Krylov and Tsygankov, 1976). However, in the light of experiments performed with E.coli K12, it seemed probable that many apparently non-lysogenic E.coli strains (and possibly also related enterobacterial strains such as Salmonella typhimurium and the various subspecies of Shigella) harbour defective lambdoid prophages. Almost without exception (as described in the discussions to Chapters 3 and 5), the DNAs of such strains were found to contain sequences which share homology with λ DNA, suggesting that the vast majority of enterobacterial strains isolated from the wild do harbour defective lambdoid prophages. In no case was the same pattern of hybridisation observed in two independent isolates even though 13 of the E.coli strains were isolated from patients in the same Edinburgh hospital. These results suggest either that the different strains had diverse origins or that defective variants of lambdoid prophages arise frequently (and are probably lost equally frequently) in the wild. In this respect the Rac prophage (or derivatives of it) may be an exception since evidence for at least the recE region of this prophage was found in approximately 25% of the E.coli strains investigated (but not in any other enterobacterial strains).

Thus, defective (and probably multiple) lysogeny would appear to be a common feature of E.coli and related enterobacteria as well as of other phage/host systems. It seems reasonable to suppose that hybridisation methods will eventually reveal defective prophages in many bacterial strains presently thought to be non-lysogenic. It is even possible that other, non-lambdoid, prophages await discovery in the E.coli K12 chromosome.

The apparent ubiquity of defective prophages has several implications for the evolution of bacteria and their phages. It has often been proposed that the exchange of genetic material between bacteria and their phages might be a common process in their evolution, since

it may lead in some cases to the assimilation and stable control of a particular new function, or set of functions, by one or other of the participants in the exchange (see for example Lindahl *et al*, 1971; Szybalski and Szybalski, 1974). The *sbcA8* derivative of *E.coli* K12, JC5412 (see Section 4.2(a) and Fig. 4.4(a)), in which a hybrid attachment site and probably the prophage repressor gene have been deleted from the Rac prophage, could be considered an intermediate stage in such a process. According to this interpretation, the host strain has gained a potentially useful gene (*recE*), allowing its expression to take place in the absence of prophage excision which would remove *recE* from the cell line. The *recE* gene of JC5412 is therefore, to all intents and purposes, a permanent acquisition for this cell line.

The situation with respect to the *sbcA8* mutation in *E.coli* K12 is not dissimilar to the phenomenon of lysogenic conversion as the phage mediated acquisition of new bacterial traits is known. The description "lysogenic conversion" has usually been used to describe expression from a prophage of a gene that plays no essential role in the phage lifecycle. However, this need not be an absolute requirement. In several instances, lysogenic conversion is known to be the cause of bacterial pathogenicity. For example, the toxigenicity of *Corynebacterium diphtheriae*, the causative organism of diphtheria, has been shown to be dependent on the bacterium being lysogenic for *coryne-bacteriophage* β (Groman, 1953; Barksdale and Pappenheimer, 1954). Similarly, surface antigens carried by certain *Salmonella* strains are known to be elaborated by prophages in these strains (Uetake *et al*, 1958; Uetake and Hagiwara, 1961). The detection of lysogenic conversion has usually depended upon the prophage being inducible so that phage can be isolated and the phenotype of lysogens and non-lysogens can be directly compared. Thus, it is not unreasonable to assume that particular phenotypes exhibited by many bacterial strains will prove to be the result of lysogenic conversions in which the phages are defective. As noted by Hayes (1968), it is

surprising that more attention has not been paid to the possible role of bacteriophages in the conferment of bacterial pathogenicity. Hopefully, recombinant DNA/DNA hybridisation methods will make the investigation of this possibility less intractable than has previously been the case.

Lysogenic conversion may, therefore, eventually be invoked to explain the presence of certain types of defective prophage in particular strains since permanent acquisition of a phage-encoded function may provide a selective advantage for the bacteria which carries it, and thus ensure perpetuation of the defective prophage in the cell line. It is probable, however, that the vast majority of defective prophages (at least in the enterobacterial strains described above) are little more than chromosome debris.

It is apparent from studies of hybrids formed between different lambdoid phages, and of heteroduplexes formed between the DNAs of such phages, that much of the existing variation between lambdoid phages can be explained on the basis of a modular hypothesis of phage evolution (Hershey, 1971b; Simon et al, 1971; Botstein and Herskowitz, 1974; Szybalski and Szybalski, 1974; Campbell, 1977). This hypothesis views a phage such as λ as having evolved by the association of smaller elements derived from diverse sources. Several of the functions elaborated by a phage such as λ (which include replication, regulation, virion formation, lysis, integrative and general recombination) are also those required by the host cell and by non-viral replicons. The clustering by function of related genes and control elements might therefore reflect the origin of a lambdoid phage genome from its component segments rather than design for optimum efficiency (Campbell, 1977). Implicit in this type of hypothesis is the suggestion that interstrain recombination, and hence reassortment between more or less distantly related phage genomes, might be a regular occurrence in the wild. If this be the case, classification of phages into distinct families will become extremely difficult.

The simplest (and most extreme) alternative to the modular hypothesis is that "---- groups of related viruses have developed by linear evolutionary divergence from a common viral progenitor that contained base sequences ancestral to all those present in every member of the group, and that recombination between distant relatives, though possible in the laboratory, is inconsequential in nature." (Campbell, 1977). This hypothesis requires that mixed infection, and hence interstrain recombination, takes place rarely, if at all, in the wild.

Too few comparative studies have been done, even among the relatively well studied group of lambdoid phages, to satisfactorily resolve this issue, although it is likely that the true state of affairs lies somewhere between these two opposing hypotheses. Certainly, the discovery of hybrid formation and extensive DNA and functional homology between such outwardly dissimilar phages as λ and P22 of Salmonella typhimurium (Botstein and Herskowitz, 1974; discussed in Section 1(b)) makes it difficult to accept the common ancestor hypothesis in its most extreme form.

The ubiquity of defective lambdoid prophages in E.coli and related entero-bacterial strains also lends support to at least some type of modular hypothesis for phage evolution since it implies that the majority of phage infections in these strains are, in effect, mixed infections (one component of which is a defective prophage) and may therefore result in the formation, at a significant frequency, of viable hybrid recombinants between more or less distantly related phages. λrev and λgsr' derivatives are examples of the formation of hybrid recombinants between viable and defective phages, and it may be reasonably supposed that such recombinants are generated, if only rarely, in the wild. If able to propagate successfully such phages and their descendants may enter the pool of wild type lambdoid phages on equal terms. Thus, reassortment between phage genomes may be a major source of phage variation rather than the extremely rare exception postulated on the basis of the common ancestor hypothesis.

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