THE HOST SPECIFICITIES OF THE LYSOGENIC PHAGE OF *ESCHERICHIA COLI* STRAIN W AND OF PHAGE Mu.

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A B S T R A C T

Investigation of the bacteriophages present in the supernant of broth cultures of E.coli strain W have demonstrated a phage which plates on E.coli strain C, designated W.C, and another phage which plates on E.coli strain K, designated W.K. Both phages are very closely related. Differences were observed in the ability of their respective lysogens to exclude phage Pl_v. By available criteria, the phage W.K was found to be indistinguishable from phage Mu. As such, E.coli W may be a natural reservoir for phage Mu.

Antisera were prepared against the phage W.C, W.K and Mu.K respectively. Neutralisation tests have demonstrated that the W phages and Mu phage may express one of several tail forms with respect to their tail components associated with host adsorption and therefore host specificity; the 'C' form is recognised by neutralising antibodies in the W.C antiserum, and the 'K' form is recognised by neutralising antibodies in the W.K and Mu.K antisera. Failure of any of these antisera to neutralise phage Mu propogated on Citrobacter freundii support the proposal for a third tail form 'F'.

Observation of the plating behaviour of these phages, W.C, W.K and Mu.K, together with their neutralisation characteristics established that the phages can vary their host range specificities in two different ways: either they behave as host range mutants, plating on E.coli strains C and K with approximately equal frequencies - extended host range mutants; or they exhibit an alternate host-specificity type of behaviour, plating efficiently, only on the host in which they were last propogated. In this latter mode, a distinct event, or possibly two events, are required at the genome level to permit the observed change in the host range phenotype. As phages W.K and Mu.K appear identical, and phage W.C is very closely related to phage W.K, the events required for this

phenotypic change to occur may as postulated include inversion of the G region of the phage DNA.

Recent studies overseas, and as presented here, demonstrate that induced phage Mu (or phage W.K) are of two type, a G(+) form and a G(-) form, which differ in the orientation of their G region. The G(+) form is the form of the phage adsorbing to, and propogating lytically on the host bacterium E.coli K, the G(-) form has been found to adsorb to and propogate on a restrictionless strain of C. freundii. The G(-) form of phage Mu was not neutralised by the W.C or the Mu.K antisera. As such, it represents a third tail form that the phage Mu is capable of expressing. Evidence is also presented that all these phage, W.C, W.K and Mu.K, and their derivatives may express a mom-like function.

A C K N O W L E D G E M E N T S

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INTRODUCTION

In order for a DNA containing bacteriophage to replicate, it must infect a susceptible bacterial host. Typically, the infectious cycle occurs in two stages: the phage adsorbs via its tail components to a specific receptor site on the host bacterium's cell surface and then releases its DNA into the host cell. When the infecting bacteriophage is a temperate phage, one of two possible courses of events may follow. Normally, the lytic response is initiated. This involves full expression of the phage genome, leading to multiplication of the phage, and the eventual death of the host cell by lysis, thus releasing the progeny phage to initiate further rounds of infection and replication. The alternative is the lysogenic response. In these instances, following release of the phage DNA into the infected cell, the phage genome is incorporated into the host's chromosomal DNA as a prophage. In this latent state only a limited number of phage genes are expressed, but significantly these include a gene that codes for a cytoplasmic repressor protein which prevents expression of genes responsible for initiation of the lytic cycle.

The prophage remains dormant, and is replicated along with the bacterial host DNA, and is thus transmitted from generation to generation. At some point, the process of induction occurs. This may be spontaneous or induced by external stimuli, e.g. U/V light, heat shock. During induction, the prophage is normally excisied from the host DNA, and then initiates a lytic response leading eventually to lysis of the cell and release of progeny phage.

There are a number of mechanisms that may prevent a phage from carrying out a successful infection. The most straightforward situation is when the phage fails to adsorb to the host cell due to the bacterium not carrying the specific

receptor sites required by that particular phage. Such a bacterium is termed resistant. Once the phage has adsorbed to the cell surface, and released its DNA, there are still two basic mechanisms whose operation may cause the infection to be non-productive or abortive.

Super-infection immunity applies to temperate bacteriophages and their associated immune system. The presence of a resident prophage will prevent expression and therefore also replication of the superinfecting phage genome if both phages share the same immune system and therefore the same cytoplasmic repressor protein being synthesied under control of the resident prophage. Such closely related phage are said to be co-immune, and the lysogenic bacterium immune.

Most bacterial strains possess their own host-controlled restriction and modification systems which provide the bacteria with the ability to recognise and break-down (restrict) incoming foreign DNA, whether it be viral, plasmid, episomal or bacterial DNA. Recognition depends upon specificity of the restriction endo-nuclease of the recipient cell and upon certain nonheritable modification characteristics imparted to the incoming DNA by its previous host.(1) Restriction and modification systems may also be coded for by a plasmid, or by a prophage carried by the host. The modification process usually involves methylation of specific bases in a DNA sequence. Restriction is due to a cleavage of the introduced DNA by specific endonucleases, which recognise specific sites (sequences) on the target DNA unless these sequences have been methylated. Thus, foreign DNA if not suitably modified is rapidly degraded. In most cases the restriction system present is balanced by the corresponding modification system.

E. coli W (ATCC9637) - the Waksman strain - was established as a laboratory strain at an early date and was a popular organism for metabolic studies in the late 1940's and 1950's. An unusual characteristic of this strain which came to

light, is the inability of E.coli W to plate many of the standard laboratory phages: T1 to T7, P1, Lambda, even though these phages adsorb efficiently to strain W (with the single exception of phage T4). During a course of experiments in which the growth of phage Lambda on E.coli W was being studied (2), it became apparent that strain W was lysogenic for a hitherto undescribed bacteriophage, Wø, which played an important role in the restriction of Lambda (3).

Jamieson (4) set about trying to clear up the confusion surrounding the existence of the Wø and the reasons why E.coli W would not plate a large number and variety of bacteriophages. The results he obtained strongly suggested that E.coli W was in fact lysogenised by two very closely related phage, rather than by just the Wø. These two phage, designated W.C and W.K were shown to exhibit different patterns of plating behaviour. From overnight broth cultures of E.coli W, the supernants were demonstrated to plate approximately 10^6 pfu (plaque forming units) /cm^3 on E.coli strain C, and 10^5 pfu/cm^3 on E.coli strain K. The phage which plated on strain C was designated W.C, the phage which plated on strain K, W.K.

The phage W.C was propogated on E.coli C. The resultant lysate of phage W.C was then tested for the ability of the phage to adsorb to E.coli strains C and K, and the e.o.p. (efficiency of plating) of the phage on E.coli K compared with the plating on E.coli C. It was found that phage W.C readily adsorbed to E.coli C, but did not adsorb to E.coli K, and the e.o.p. on strain K was approximately 10^{-5}. Once plage W.C had plated on E.coli K, the resulting phage, designated W.CK (to denote its propagating host history) was then able to adsorb to both E.coli strains C and K, and plated back on strain C with an e.o.p. of $1\text{-}10^{-1}$, thus acting as a typical host range mutant of phage W.C.

The phage W.K was tested in the same manner. A lysate of phage W.K propogated on E.coli K was used. It was shown that phage W.K would adsorb only to E.coli K, but not E.coli C, and that the e.o.p. of phage W.K on E.coli C was in the order of 10^{-4} - 10^{-5}. The resultant phage, W.KC however, was able to adsorb only to E.coli strain C, but not strain K and the e.o.p. back on to strain K was approximately 10^{-4} - the phage W.K was acting very differently from the phage W.C, plating best on the host in which it was last propogated. This pattern was described as an alternating host specificity type behaviour. See figure 1 for a summary.

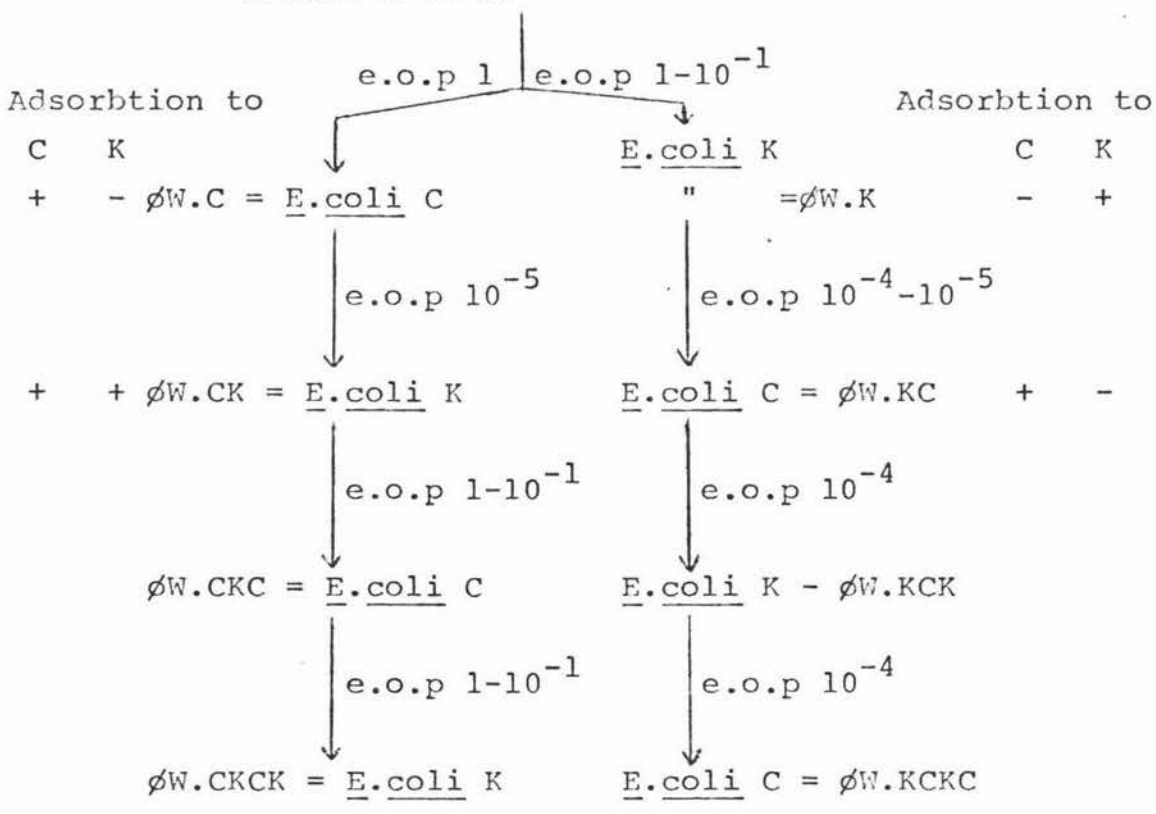


Figure 1: Relative efficiencies of plating and adsorption characteristics of phage W.C and phage W.K as observed by Jamieson.

Jamieson's work went on to map preliminarly the tentative positions of the two prophages on the E.coli W chromosome. See figure 2

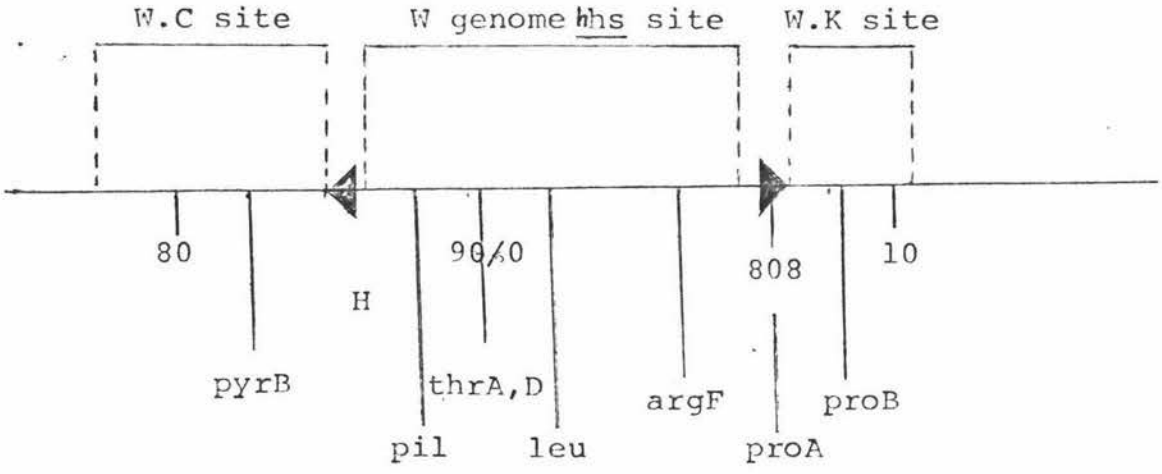


Figure 2: The tentative locations on the chromosome of E.coli W of the W genome hhs site, and the integration sites of phage W.K and phage W.C. [Jamieson (4)]

By employing conjugal crosses, Jamieson was able to eliminate phage W.K from E.coli W, thus producing a derivative of E.coli strain W, F-2-3-27, whose supernant contained only phage W.C. Repeated attempts were made to cure strain W of phage W.C, but were unsuccessful. This result, together with other observations gave rise to the possibility that phage W.C may occupy more than one integrative site on the W chromosome.

The phages W.C and W.K are co-immune, share identical heat sensitivity and morphology (4), and are not inducible by U/V light (5). Electron - micrographs depict a tadpole-like structure very similar to phage P2, T1 or Mu. The head appears spherical (icosahedral?) about 60nm in diameter, the tail 120nm long and 16nm in diameter. The tail is attached to the head of the phage via a short constricted region. In particles with contracted tails, the tail exhibits thick and thin regions. The thick region is approximately 26nm in diameter, and represents the outer contracted sheath. The thin region is the exposed core, and measures 8nm in diameter. Phage particles with non-contracted tails are seen to possess a base plate.

Serologically, the phage W.CKC (= host range mutant of phage W.C) and the phage W.KC were demonstrated to be the same, using antisera prepared against phage W.CKC and phage W.KC in neutralisation tests (4).

Glover et al (5), did work on E.coli strain W, specifically with respect to attempts to propogate phage Lambda on the bacterium. The presence of a temperate phage in the supernant from an overnight broth culture of E.coli W was demonstrated. The phage seen however, was only observed to plate on E.coli C, producing about 10^6 pfu/cm^3. No reports were given of a phage from the supernant of strain W able to plate on E.coli K. The phage present was designated the Wø. In Jamieson's, and the nomenclature employed in this study, it is presumed that the Wø described is the same as the phage W.C. Glover also detailed that

the Wø, W.C, plated on E.coli K with an e.o.p. of 10^{-6}, and that this phage, W.CK, plated back onto E.coli C with an e.o.p. of 1, thus acting as a host range mutant of phage W.C.

Pizer et al (6) in their work relating to E.coli W demonstrated that the supernant from an overnight culture of strain W would produce plaques on both E.coli strains C and K with an e.o.p. of 1. Also, the phage which plated on E.coli C, phage W.C, then plated with an e.o.p. of 10^{-5} on E.coli K, and the phage which initially plated on E.coli K, would plate on E.coli C with an e.o.p of 10^{-3}, both results approximating Jamieson's.

Attempts to propogate phage Lambda on E.coli W, and on E.coli C lysogenised by phage W.C, revealed an unusual characteristic of the prophage, namely, that it codes for a system of genetic restriction expressed in the lysogenic host but fails to endow the host with any corresponding system of modification.

Phage Lambda readily adsorbs to E.coli strain W, strain C, and substrains of C.W.C. The e.o.p. of Lambda, propogated on strain C i.e Lambda.C, on E.coli C is 1. The e.o.p. of the same phage on E.coli strain W, or substrain C.W.C was less than 10^{-8} (5). With a low efficiency (10^{-4}-10^{-5}), phage Lambda was able to grow and replicate in E.coli W and E.coli C.W.C, and produced a burst of progeny phage, but these phage were unable to form plaques on E.coli strain W or substrain C.W.C. Kerszman et al (3) demonstrated that Lambda DNA was degraded in E. coli W and E.coli C.W.C. This leads to the conclusion that phage W.C controls a host restriction mechanism, but not a modification process, represented as r+m-, compared with the normal host controlled restriction and modification, r+m+, thus the progeny phage produced are not host-modified with respect to the phage controlled restriction system, and therefore subsequent infections are unsuccessful. Although a r+m- system might appear lethal to its own genome,

this does not necessarily follow. The genome itself may lack the specific recognition site of the restriction endonuclease, or degredation may occur in an area remote from chromosomal DNA, for e.g. in the periplasm.

Further investigations of different strains of E.coli C lysogenised by phage W.C revealed that the ability of the lysogens to degrade phage Lambda varied greatly. This was potentially explained by the hypothesis that phage W.C may integrate at several different sites within the E.coli C genome, similar to phage P2 or Mu (if extended to the W genome this may also explain the inability to derive an E.coli W strain lacking phage W.C), and that the site of integration may in some manner affect expression of the restriction activity of the prophage. Jamieson (4) also noted a similar phenomenon. He reported that substrains of E.coli C lysogenisied by phage W.CKC (a host range mutant of phage W.C) varied markedly in their ability to exclude phage P1. Lysogens of E.coli K - K.WCK (a host range mutant of phage W.C), K.W.K, and K.W.KCK - have never been reported to exclude phage Lambda or phage P1 (4,5).

A paper by Martuscelli et al (7) states: "The mutagenic phage used in this investigation was isolated from the American Type Culture Collection (ATCC) strain 9637 by plating cell-free culture medium with indicator cells on E.coli K-12 (strain AB259). Since the phage from ATCC9637 is indistinguishable from the phage Mu-1 originally isolated from a chance lysogen of strain K-12 in respect of its morphology, lysogenic immunity, bacterial host range, and ability to induce mutations, we presume that the two phages are closely related and that ATCC9637 may be the natural reservoir of phage Mu-1". The method reported for isolation of the phage Mu-1 is the same procedure used to isolate the phage designated W.K, i.e. the plating of the E.coli W supernant on E.coli K.

This report, taken together with the known morphology of phage W.K, and its limited host range, suggest that

phage W.K and phage Mu-1 may be very closely related, if not identical. Since direct comparisons will be made during the course of this study, a basic review of phage Mu properties would be in order.

The temperate bacteriophage Mu can be propogated on E.coli K. The phage's genome consists of a linear, double-stranded DNA molecule, of 36-38 kilobases (Kb) in length, a base composition of approximately 50% G-C, and a molecular weight of 25×10^6 daltons. Figure 3 outlines the Mu DNA structure.

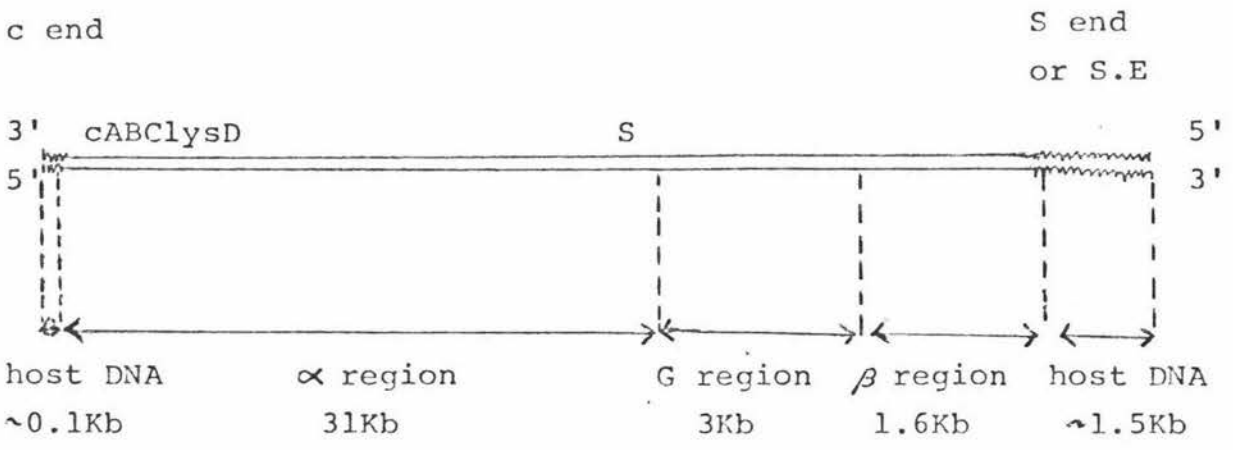


Figure 3: Structure of Mu DNA (segments not drawn to scale) showing its division into three regions, ∝, G and β, and the attached bacterial host DNA at each end. The known essential genes of Mu (A to S) are located in the ∝ region. The c gene at the end is the immunity gene. [Adapted from Chow (8)].

Two special features of phage Mu DNA are the associated host sequences at either end, and the G region. It is believed that the host sequences fulfill a role in the headful packaging of phage Mu DNA (9). The G region of Mu is an invertible segment of phage DNA. It is 2.9 $\pm$ 0.4 Kb long, and is flanked on both sides by an inverse repeat sequence of less than 50 base pairs. The inversion of the G region is thought to occur by recombination between the inverse repeats at the ends of G (8). This inversion can be demonstrated by observation using an electron-microscope after the DNA extracted from mature phage particles has undergone denaturation and renaturation. The G bubble which is observed is a direct consequence of the two single DNA strands carrying the G region in opposite orientations; thus it appears as a non-renaturable bubble. The frequency with which the bubble (and therefore G region inversion) is observed is dependent upon the method of phage propogation. A lytic preparation of phage Mu will have the G region inverted in less than 0.5% of cases. However, when an E.coli K lysogenised with Mu is induced, and the resultant phage preparation examined, approximately 50% of the phage have the G region inverted. See figure 4.

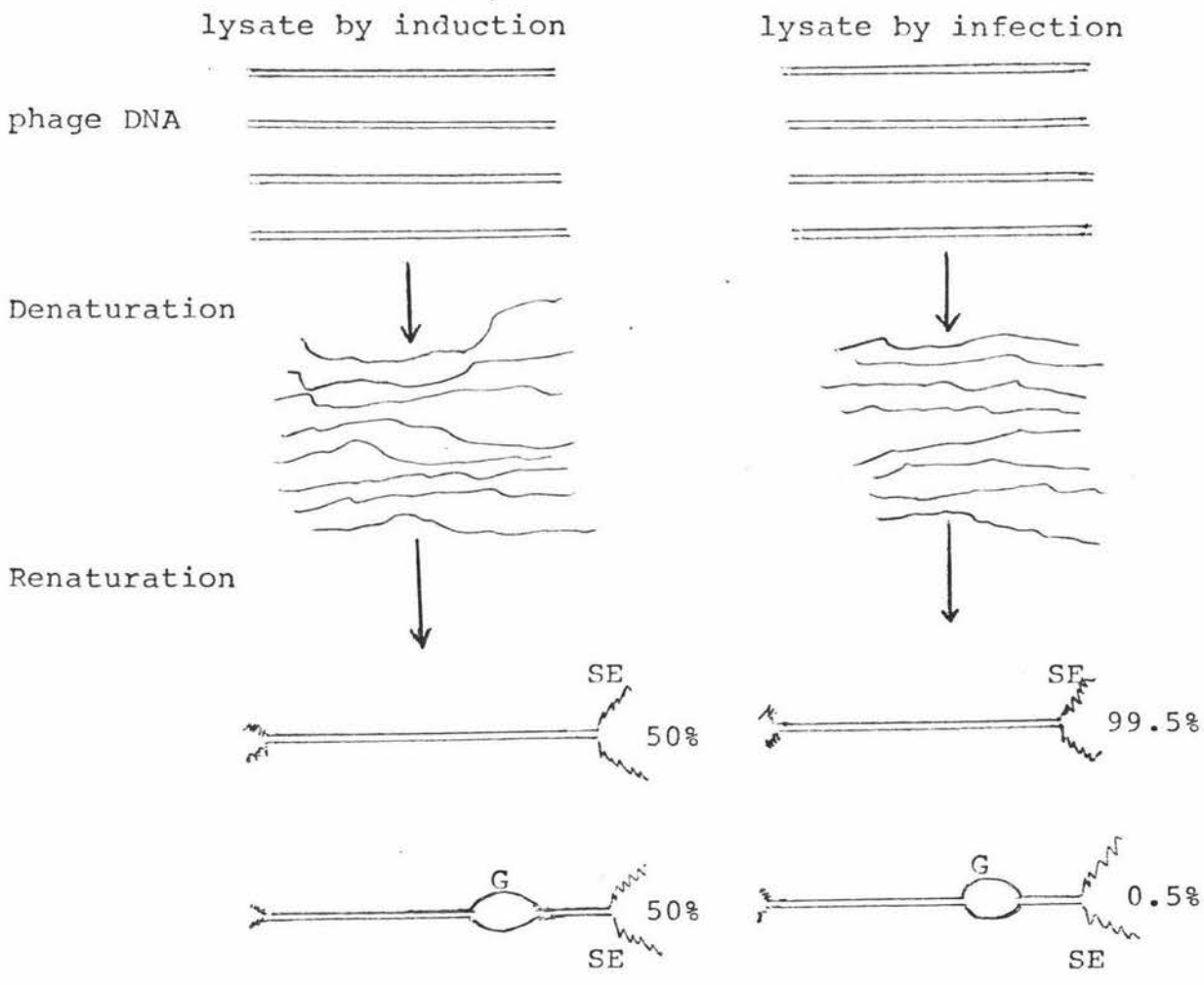


Figure 4: Structures observed in the electron - microscope after denaturation and renaturation of Mu DNA extracted from mature particles. [Adapted from Bukhari (10)].

Of the phage generated by induction of a Mu lysogen, only about 50% are able to adsorb and propogate on E.coli K. The remainder, presumably with the G region inverted (G(-) forms) as compared with the state it is in during lytic propogation (G(+) forms), are unable to form plaques on E.coli K.

Within the G region, there is another segment of DNA about 1Kb in length and flanked by inverse repeats, that may itself invert, though no evidence has been put forward to date to demonstrate this. See figure 5.

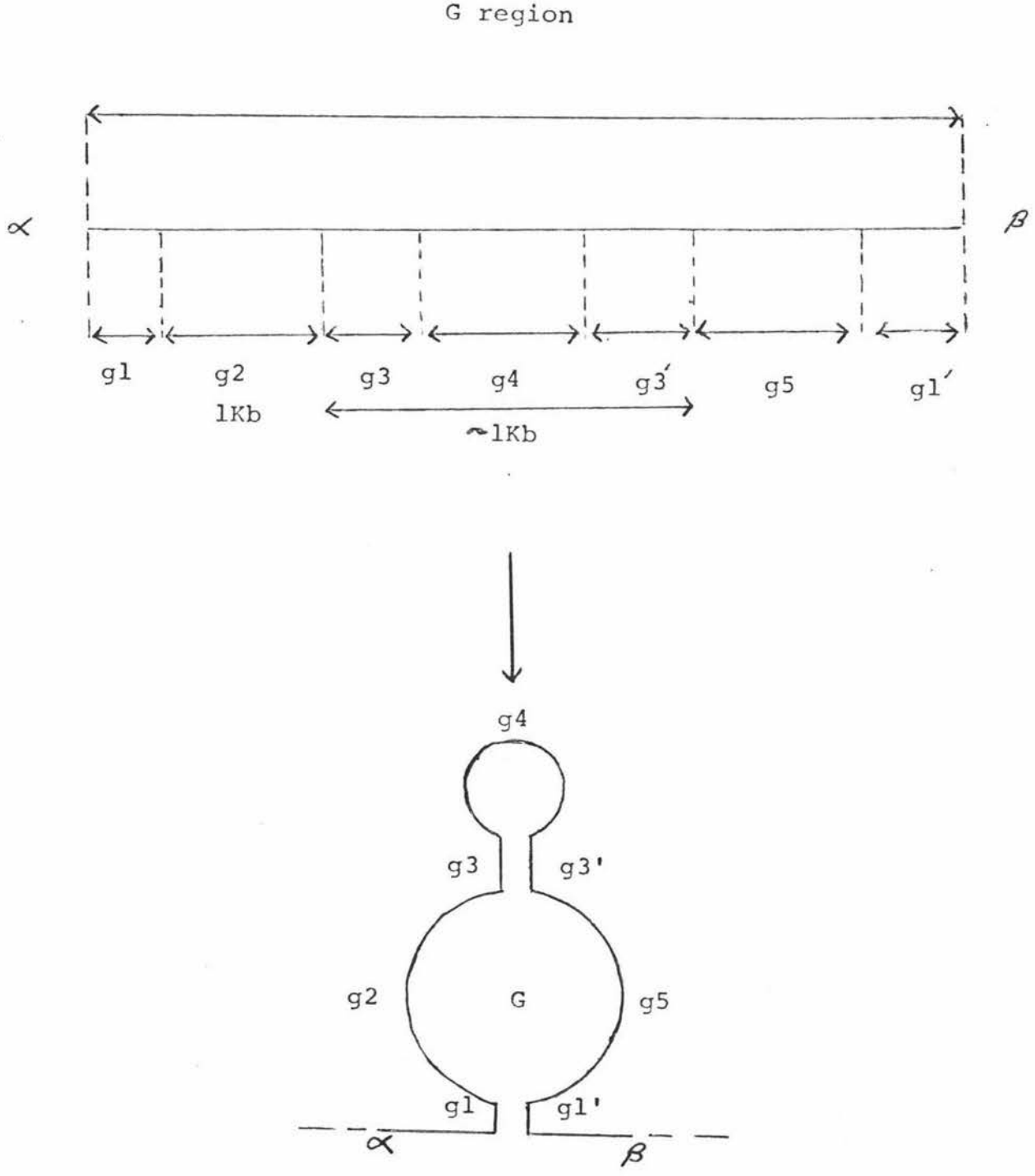


Figure 5: Structure of the G region (segments not to scale). A single-stranded DNA structure is shown. g1 and g1' are inverted repeats, as are g3 and g3'. [Adapted from Chow and Bukhari (11)].

Adjacent to the G region, there are two interesting genes; the mom gene and the gin gene, both located in the β region of the Mu genome. The gin gene function is necessary for the inversion of the G region (12). The mom gene codes for the modification of Mu phage DNA. This modification is non-specific, acting on Mu DNA, and on any other DNA present in the host cell at the time, and provides protection against many restricting nucleolytic enzymes (1). During lytic propogation of phage Mu, mom is expressed only at a very low level late in the infection process. It is not expressed during the prophage state, but after induction of a Mu lysogen, the mom gene is expressed at a much higher level (1). A correlation between the expression of mom and the inversion of the G region has been suggested.

A recent paper by Van de Putte et al (13) has demonstrated that the G (-) forms of Mu are able to adsorb and propogate on a Citrobacter freundii strain that lacks the normal host restriction system, i.e. it is r-m+.

AIMS OF THE INVESTIGATION

1. To observe the behaviour of the bacteriophages present in the supernant of broth cultures of E.coli strain W, one of which plates on E.coli strain C, and another which plates on E.coli strain K.

2. To observe the behaviour of the bacteriophage Mu which plates on E.coli strain K, and the subsequent plating behaviour of this phage on E.coli strains C and K.

3. To investigate the relatedness of phage W.C to phage W.K, and phage W.K to phage Mu.

4. To observe the restricting or non-restricting abilities of E.coli strains C and K lysogenised by phages W.C, W.K and Mu respectively.

5. To extend recent overseas observations on phage Mu G (-) particles.

B A C T E R I A

Strain	Sub-strain	Geneotype	Source
Escherichia coli W	ATCC9637	wild-type	D.F. Bacon
	F-2-3-27	met^- his^- Hfr_{808}	D.F. Bacon
Escherichia coli C	518C		Arber
Escherichia coli K	T186	wild-type, λ^s	D.F. Bacon
	AB266	thr^- leu^- B_1^- pro^- gal^- lac^- Sm^R sup E_{44} F^-	D.F. Bacon
	PB1395	r_K^- m_K^+ (trp^- met^-?) sup E_{44} sup F F^-	D.F. Bacon
	RS54	ara D^- : Mu^{c+} derived from Hfr_H	M.M. Howe
	Q_1	thr^- leu^- ton A lac^- Su II^+ F^- : $Mu^{cts}61$	M.M. Howe
	Q_1	thr^- leu^- ton A lac^- Su II^+ F^- : $Mu^{cts}62$	M.M. Howe
	CSH55	$Mu^R\Delta$ (lac pro) nal A thi sup E / F' pro^+ lac^+ : $Mu^{cts}62$	Coclho
	Z (Pizer)	$r_{K_s}^-$ m_K^- thr^- leu^- lac^- Sm^s Sup E_{44}	) Referred
	G461	gal_1 $gal_2 \rho m^-$ $Sm^R{}_1$ F^-	) to only
Citrobacter freundii	G331	r_F^- m_F^+ arg-1 trp-1 nal-1	P. van de Putte

Table 1: Bacterial strains

B A C T E R I O P H A G E S

Phage	Source
$P1_v$	Cold Spring Harbor
λ_v	J. Zissler

Table 2: Bacteriophages

MEDIA and SOLUTIONS

Lennox broth (LB)

Bacto-tryptone	10g
Bacto-yeast extract	5g
NaCl	10g

The medium was made up to 1 litre with de-ionised distilled water, and then adjusted to pH 7.5 with 6N NaOH before autoclaving.

Modified Lennox broth

LBM = LB + $MgSO_4$ (2×10^{-3}M)

LBMC = LB + $MgSO_4$ (2×10^{-3}M) + $CaCl_2$ (2×10^{-3}M)

To every 2.5cm^3 of LB, 0.1cm^3 of the required salt solution was added from 5×10^{-2}M solutions, to give the required final concentration of 2×10^{-3}M.

Lennox broth - double strength (LB.XX)

Bacto-tryptone	20g
Bacto-yeast extract	10g
NaCl	20g

The medium was made up to 1 litre with de-ionised distilled water, and then adjusted to pH 7.5 with 6N NaOH before autoclaving.

Lennox plate agar (L agar)

LB.XX	250cm^3
Distilled de-ionised water	250cm^3
Agar (N.Z. Davis)	5g

Thirty cm^3 of L agar was dispensed per plate to form the basal layer.

Tetrazolium solution .XX

Triphenyl Tetrazolium chloride salts	0.6g
NaCl	1.6g
KCl	0.08g
Distilled de-ionised water	100cm^3

Solution stored in the absence of light.

Modified Lennox soft agar (ML soft agar)

LB	100cm^3
Agar (N.Z. Davis)	0.5g
2.5cm^3 was dispensed per tube to which was added	
$MgSO_4$ (5 x 10^{-2}M)	0.1cm^3
$CaCl_2$ (5 x 10^{-2}M)	0.1cm^3
Tetrazolium solution .XX	0.5cm^3

S buffer

NaCl	5g
Tris. HCl	2.7g
Trizma Base	0.32g
De-ionised distilled water	1 litre

Modified S buffer

SM = S + $MgSO_4$ (2 x 10^{-3}M)

SMC = S + $MgSO_4$ (2 x 10^{-3}M) + $CaCl_2$ (2 x 10^{-3}M)

Saline 0.9%

NaCl	9g
De-ionised distilled water	1 litre

S broth (SB)

Bacto-tryptone	32g
Bacto-yeast extract	20g
NaCl	5g

The medium was made up to 1 litre with de-ionised distilled water and then adjusted to pH 7.3 with 6N NaOH before autoclaving.

Modified S broth

SBPM = SB + Pb $(CH_3COO)_2$ (2×10^{-3}M) + $MgSO_4$ (2×10^{-3}M)

METHODS

(i) Tetrazolium agar overlay technique for phage titrations

A soft agar overlay consisting of ML soft agar, 3 drops of seed bacteria (from an overnight culture grown in LB), and 0.1cm^3 of an appropriately diluted phage lysate was poured onto a plate of L agar. The plates were incubated overnight at 37^{o}C. The tetrazolium in the overlay is initially colourless but is reduced to the red formazan by growth of the seed bacteria. This enabled easier and more accurate plaque counting.

(ii) Preparation of phage lysate stocks

Lytic propogation of the phage was carried out using a tube method. A plaque was picked, and added to 0.3cm^3 of fresh bacterial host cells. (Fresh cells were prepared by diluting an overnight culture of the bacteria (in LB) 1:1 with fresh medium (LBMC) and incubating for 1 hour at 37^{o}C.) The mixture was vortexed and then allowed to stand for 15 minutes at 37^{o}C to allow pre-adsorption to occur. Then 6.0cm^3 of fresh medium (LBMC), pre-warmed to 37^{o}C, was added. Incubation was continued at 37^{o}C under gentle aeration until clearing (lysis) of the culture occured, generally in 2-4 hours.

A few drops of chloroform were added, the culture vortexed and then allowed to stand for a further 15 minutes at 37^{o}C. After vortexing again, the culture was transferred to a tube for centrifugation. (Sorvall type A bench centrifuge, held for 1 minute at 3,400 x g.) The supernant (lysate) was removed and stored at 4^{o}C.

Prior to use, the sample of the lysate required was aerated for 15 minutes at 37^{o}C to remove dissolved chloroform. The lysates usually contained 10^7 - 10^9 plaque forming units per cm^3 (pfu/cm^3).

(iii) Preparation of phage lysate stocks for antigen use.

Procedure (ii) was followed. Once the supernant was harvested, it was immediately transferred to tubes for ultra-centrifugation. Samples ($6.0cm^3$) of lysate were spun in the Beckman model L5-75 ultra-centrifuge at 30,000 r.p.m. for 2 hours (55,000Xg). This pelleted the phage. The supernants were removed, and each phage pellet resuspended in $2.0cm^3$ of S buffer. The lysates were ultra-centrifuged once more, their supernants removed, and each phage pellet resuspended in $2.0cm^3$ of S buffer, and stored at -20^oC. These preparations of phage were used as antigens in the immunization of rabbits.

(iv) Preparation of Antisera.

From procedure (iii), $0.4cm^3$ of phage suspension was added to $0.6cm^3$ of Freund's complete adjuvant, emulsified, and injected intra-muscularly into a rabbit. After 4 weeks, a second injection was made of $0.4cm^3$ of antigen (phage lysate) alone. This injection was repeated after one more week. Five days later, the rabbit was bled from the ear veins, yeilding about $20cm^3$ of blood, which was allowed to coagulate, and the serum harvested ($\sim 10\ cm^3$) and stored at -20^oC in $2.5cm^3$ aliquots.

(v) Phage neutralisation test.

The antiserum was diluted in LB to give 90-95% neutralisation of the reference phage in 10 minutes at 37^oC. To $0.9cm^3$ of the diluted antiserum, $0.1cm^3$ of the phage lysate at a concentration of approximately 10^6 pfu/cm^3 was added. Note, both the antiserum and the phage lysate were pre-warmed to 37^oC. A $0.1cm^3$ sample was removed from the mixture at 0 minutes and again at 10 minutes. These samples were immediately diluted in $9.9cm^3$ of LB at room temperature to stop any further neutralisation occuring. The tubes were then titrated at appropriate dilutions.

(vi) Determination of phage neutralisation kinetics

a) Procedure (v) was followed. In addition to 0 and 10 minute samples, samples were also taken at 2, 5, 20 and 30 minutes, and the results plotted.

b) Procedure (v) was followed with the exception that the antiserum was used at double its normal strength.

(vii) Isolation of lysogens

a) A plaque was stabbed with a sterile needle and this was then used to innoculate a L agar plate, which was streaked for single colonies. A number of single colonies were picked, and re-streaked for single colonies. Single colonies from this second plate were picked and spotted onto a third L agar plate to act as a reference plate.

Each isolate was then grown up overnight in LB and then tested for the presence of the lysogenic phage by plating the supernant to obtain a titre of spontaneously induced phage, and plating the isolate cells with the same phage, testing for insensitivity. Isolates were designated lysogenic if they produced the phage in their supernant, and were immune to infection by the same phage.

b) A plate of L agar was covered with a ML soft agar lawn of sensitive seed bacteria. Once set, approximately 10^7-10^8 phage were spread on top using a glass spreader. The plate was incubated overnight at $37^{\circ}C$. Most of the bacterial lawn is killed due to a lytic response by the phage. The surviving bacteria form distinct isolated colonies. Presumably the colonies seen are due to bacteria that have mutated and become resistant to the phage, or bacteria which have become lysogenised by the infecting phage.

Colonies were picked, and grown overnight in LBM. Each isolate was then streaked out on a L agar plate for single

colonies. A single colony of each isolate was then picked and spotted onto a reference L agar plate, and then tested as in part (a) for lysogeny.

(viii) Heat induction of temperature sensitive lysogens

The bacterial cells were grown for 6 hours at 30°C in 2.5cm^3 of SBPM. An equal volume of SBPM at 55°C was then added to the culture of young cells, and incubated for 1 minute at 55°C. Then the culture was incubated at 44°C for 25 minutes, and finally at 37°C under gentle aeration until lysis occured (1-3 hours). Chloroform was added and the supernant harvested; this was titred in the normal manner.

R E S U L T S

I PLATING BEHAVIOUR OF PHAGE W.C ON E. COLI STRAINS C AND K

The supernants from overnight cultures of E.coli W were shown to contain plaque forming units varying from 10^3-10^5 per cm^3 (pfu/cm^3) able to form plaques on E.coli C (518C); this phage was designated W.C. The plaques formed by phage W.C on E.coli C were typical of temperate phages, being relatively large in diameter 2-3mm, turbid with the growth of lysogenised cells. Phage W.C adsorbs to, and propogates on E.coli C with an arbitrary e.o.p. of 1. The phage W.C does not adsorb to E.coli K (4).

Lysates of phage W.C were prepared from single plaques of this phage plated on E.coli C (518C). This W.C lysate was then plated on strains C and K (T186) to obtain a relative e.o.p. From single plaques on K, lysates were again prepared (W.CK lysates) and plated on K and C. Further lysates were prepared from single plaques of W.CK on C (W.CKC), propogated on C and plated again on E.coli strains C and K to obtain the relative plating efficiencies. It soon became apparent that the lysates prepared in the above manner showed either one or other of two very different plating patterns.

Initially, the pattern seen was consistent with the earlier work done by Glover (5) and again later by Jamieson (4), that is, consistent with the derivative phages of W.C, viz W.CK and W.CKC, acting as host range mutants of the phage W.C. See table 3 and figure 6.

The letters immediately following W denote the propogating host history of the phage; the letter after the bar express the host specificity of the phage and thus reflect the phage's tail component form:

/C = 'C' tail form as recognised by the W.C antiserum, able to adsorb and plate efficiently on E.coli strain C only.

/C' = 'C' tail form as recognised by the W.C antiserum, but in a modified form able to adsorb and plate efficiently on E.coli strains C and K.

Phage origin	e.o.p. on C	e.o.p. on K
E.coli W supernant	1.0	
W.C/C	1.0	10^{-7}-10^{-8}
W.CK/C'	10^{-1}-10^{+1}	1.0
W.CKC/C'	1.0	10^{-1}

Table 3: The relative efficiencies of plating of phage W.C and its derivatives on E.coli strains C and K, demonstrating the host-range mutation type behaviour.

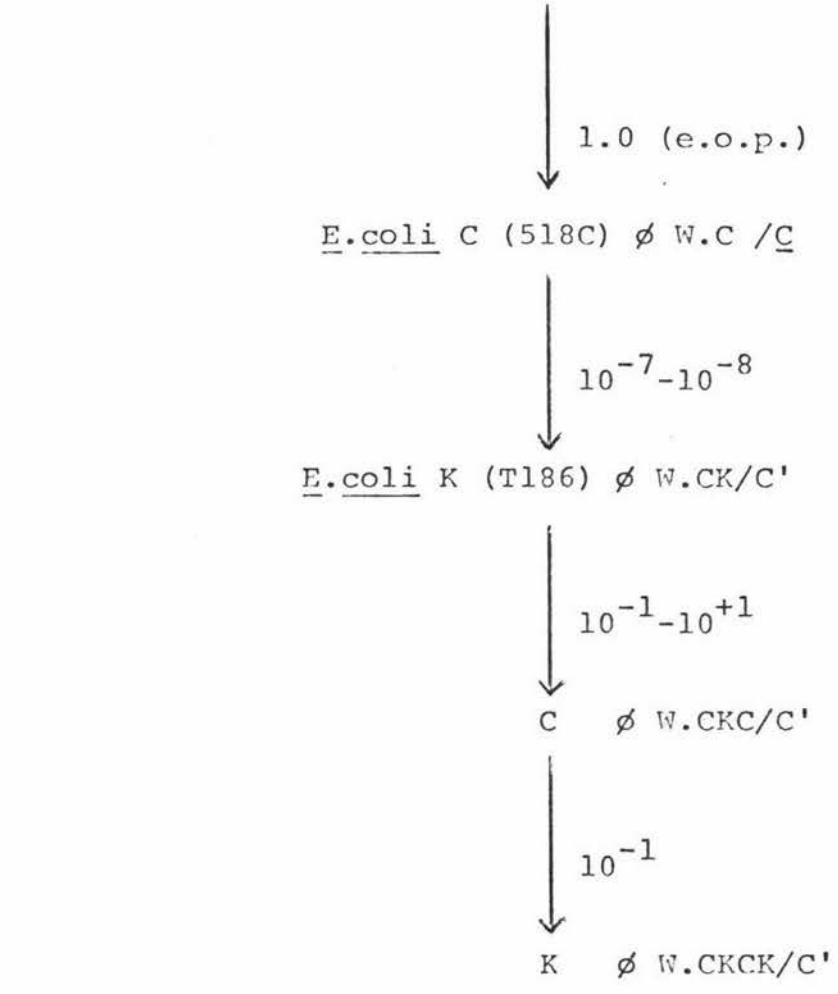


Figure 6: Plating efficiencies of phage W.C on E.coli strains C and K demonstrating the host-range mutation pattern of behaviour.

To check on the observed behaviour, the phage lysates were run against antisera in neutralisation tests. The results are set out in table 4.

Phage lysate	Antisera (i)			Host Plated on
	anti W.C	anti W.K	anti Mu.K	
W.C/C	+	-	-	E.coli C (518C)
W.CK/C'	+	-	-	E.coli K (T186)
W.CKC/C'	+	-	-	E.coli C (518C)

Note: (i) $\geqslant$80% neutralisation = +ve result
$\leqslant$10% neutralisation = -ve result

(ii) The result obtained using Mu.K antiserum is to be compared to the results obtained using W.K antiserum.

(iii) The dilution at which each antiserum preparation was used is given in Appendix A.

Table 4: The neutralisation of phage W.C and its derivatives by antisera prepared against the phages W.C, W.K and Mu.K.

From these results, it is proposed that the W.C phage derivative, phage W.CK/C', has the W.C tail configuration as recognised by the W.C antiserum preparation, and not the 'K' configuration. However, the 'C' form has been modified by mutation, allowing the phage to adsorb to, and propogate efficiently on both E.coli strains C and K. The phage W.CKC/C' has the 'C' tail configuration, but in a modified form as it is also capable of plating efficiently on both E.coli strains C and K.

When phage W.CK/C' is plated back onto strain C, the progeny phage, W.CKC/C' will be unmodified, and when plated back onto strain K (r^+) should have been restricted, but the e.o.p. obtained of approximately 10^{-1} indicates that the phage is apparently unaffected by the hsk host restriction system. At a later point, the interrelationships of phages W.C, W.K and Mu will be discussed; it is possible that phage W.C carries a mom gene as does phage Mu, and that in the above lytic propogation gene mom is being expressed giving protection against restriction systems, in this case against the Eco K system.

The second distinct pattern of behaviour is consistent with an alternating host-specificity, where the phage exhibits a very low e.o.p. on the alternate host, and adsorbs efficiently only to the host on which the phage was last propogated. The plating pattern is outlined in table 5 and figure 7.

Note: /K = 'K' tail form as recognised by the W.K or Mu.K antisera, able to adsorb and plate efficiently on E.coli strain K only.

Phage origin	e.o.p. on K	e.o.p. on K
E.coli W supernant	1.0	
W.C/C	1.0	10^{-7}-10^{-8}
W.CK/K	10^{-7}-10^{-8}	1.0
W.CKC/C	1.0	10^{-7}-10^{-8}

Table 5: The relative efficiencies of plating of phage W.C and its derivatives on E.coli strains C and K demonstrating the alternating host specificity.

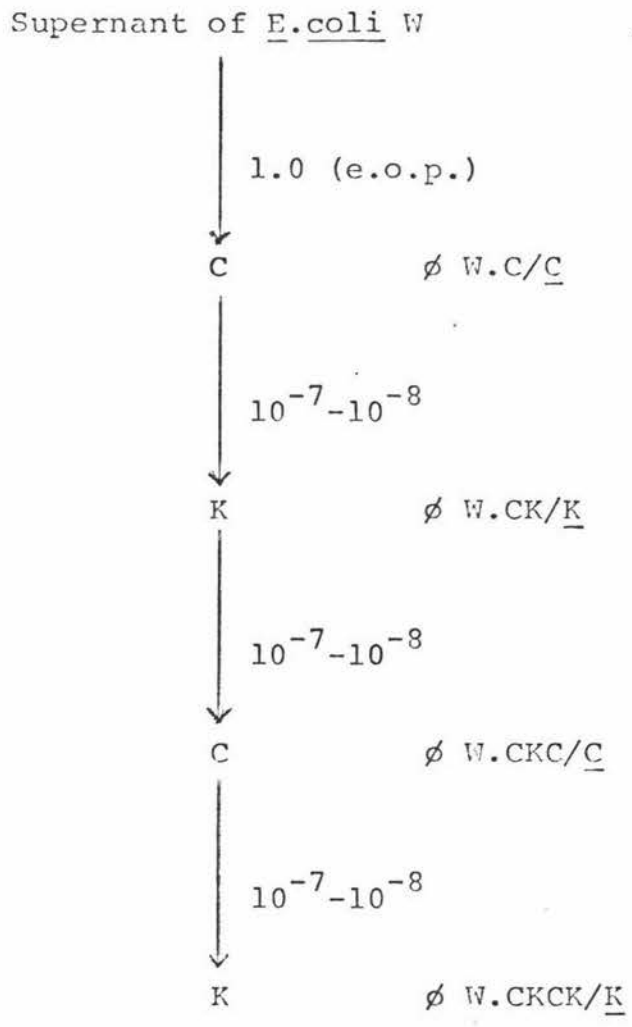


Figure 7: Plating efficiencies of phage W.C on E.coli strains C and K demonstrating the alternating host specificity.

The antisera were used again to identify the tail configurations of the phages. The results are set out in table 6.

Phage lysate	Antisera			Host Plated on
	anti W.C	anti W.K	anti Mu.K	
W.C/C	+	-	-	E.coli C (518C)
W.CK/K	30-40*	+	+	E.coli K (T186)
W.CKC/C	+	-	-	E.coli C (518C)

* The degree of neutralisation seen between the phage W.CK/K and the W.C antiserum was further investigated. Kinetic studies of the neutralisation confirmed a cross reaction between the phage and the antiserum. See figure 8.

Table 6: The neutralisation of phage W.C and its derivatives demonstrating the alternating host specificity.

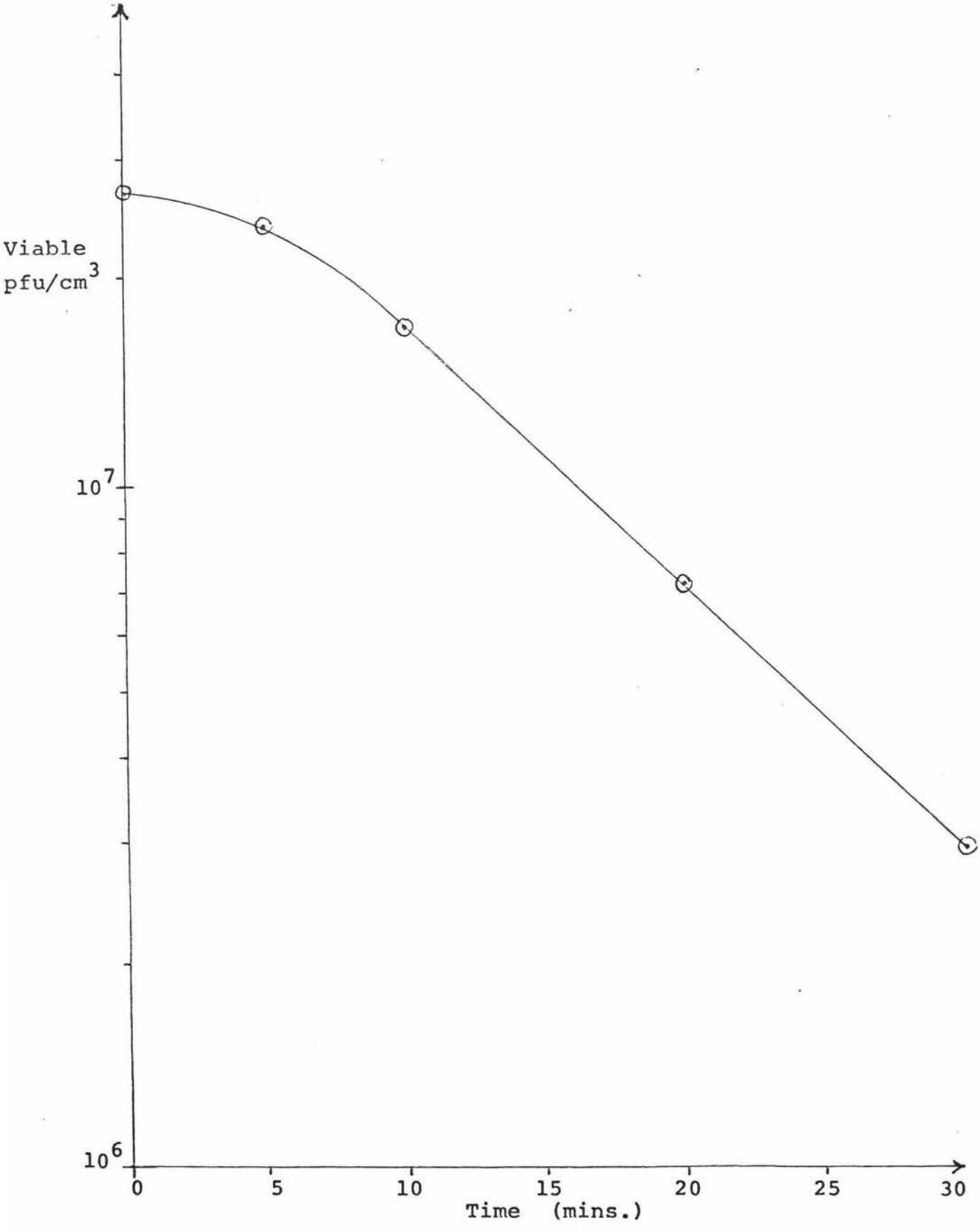


Figure 8: The kinetics of neutralisation of phage W.CK/K by antiserum prepared against phage W.C.

The results obtained with the antisera, taken together with the relative efficiencies of plating, illustrate that in this second mode, a complete change in the tail components can be observed. It is either in the 'C' form as with phage W.C/C and W.CKC/C and is able to adsorb to strain C and not to strain K, or in the 'K' form as with phage W.CK/K and is able to adsorb to strain K and not to strain C.

The phage W.C and its derivatives, on being plated from strain C to strain K, and strain K to strain C, gave approximately the same e.o.p. (10^{-7}-10^{-8}). In addition, phage W.C was plated onto an E.coli strain K r^+ (T186) and a strain K r^-m^+ (PB1395) for a comparative e.o.p. In the former, the result was approximately 10^{-7}-10^{-8}, while in the latter approximately 10^{-6}-10^{-7}. This result taken together with those on the previously reported host range mutants, demonstrate that phage W.C and its derivatives are apparently unaffected, or affected only to a small degree, by the hs K host restriction system. Two possible explanations for this are:

(i) the phage DNA does not possess the specific site recognised by the hs K restriction endonuclease, and therefore escapes restriction,

OR

(ii) considering the phage's relatedness to phage W.K (and Mu.K) it may be protected by the operation of a system of non-specific modification of the phage DNA in the same manner as the mom function of phage Mu as described by Toussaint (1).

When Mu is modified in such a way, it becomes very less sensitive to a number of host restriction systems e.g. hs A, hs B, hs K, hs P (due to P_1 lysogeny).

Specifically, Toussaint demonstrated that Mu.O (i.e. unmodified) grown lytically would plate on the hs K restriction system with an e.o.p. of approximately 10^{-2} (compared with Mu.O mom^- mutants at 10^{-4}-10^{-5}) and on hs P at approximately

$1\text{-}10^{-1}$ (compared with 10^{-5} for *mom*$^-$ mutants).

Glover (5) in his early work investigating *E.coli* W and its phages, lysogenised strain C with P_1, and then proceed to plate phage W.C on it. In doing so, he obtained an e.o.p. of 1 (as compared to phage W.C on strain C). This can indicate two possibilities again:

(i) either the W.C phage does not possess the specific site recognised by the *hs* P restriction endonuclease, or

(ii) the W.C phage is in fact susceptible to the *hs* P restriction system but is being protected from it by expression of the *mom* or a similar gene.

Clearly then, there are two very different events which can occur to phage W.C that will allow it to plate on strain K. The simplest to explain is the host-range mutation type where the 'C' form of the tail component becomes modified (it is still recognised as 'C' by the W.C antiserum), thus enabling it to adsorb efficiently to both strains C and K. The other event which leads to an alternating host specificity involves a change in the tail form of the phage; it changes from the 'C' form to the 'K' form, and is now not recognised by the W.C antiserum, but is by the W.K (and the Mu.K) antiserum. This proposes that the phage W.CK/*K* is able to adsorb to K, but not to C. The fact that it is then capable of switching back to the 'C' form once again as is seen with the phage W.CKC/*C* with approximately the same frequency as the initial switch lends support to the possibility of an inversion taking place within the phage genome, similar in principle to the G region inversion of Mu DNA.

II PLATING BEHAVIOUR OF THE PHAGES W.K AND MU.K ON E.COLI STRAINS C AND K

Throughout this section, the phage W.K and the phage Mu.K are run in parallel through many of the experiments. From the results obtained, it is apparent that both phages are very closely related, or, as seems very likely, are in fact the same phage. Evidence for such a statement is presented here and in later sections.

The supernant from an overnight culture of E.coli W was shown to contain approximately 10^5 pfu/cm^3 able to form plaques on E.coli K (T186); this phage is designated W.K.

Similarly, from an overnight culture of strain RS54 (an E.coli K lysogenised by phage Mu), the harvested supernant was shown to contain approximately 10^4 pfu/cm^3 that were able to form plaques on E.coli K (T186) due to the phage designated Mu.K. In both cases, the plaques formed by the phage on E.coli K were medium to small in diameter, ~1mm, and relatively clear.

Attempts were made to plate phage W.K on RS54, and phage Mu.K on T186 lysogenised by phage W.K. In neither case were any plaques seen. The failure of the phages to propogate cannot be due to lack of adsorption to E.coli K, or restriction by the bacterial host. It is most probably a result of superinfection immunity (i.e. the phage being co-immune).

The phage W.K and the phage Mu.K will adsorb to, and propogate on E.coli K with an arbitrary e.o.p. of 1. Neither phage adsorbs readily to E.coli C (4).

Lysates of the phage W.K and the phage Mu.K were prepared from single plaques of the phage plated on E.coli K (T186). These W.K and Mu.K lysates were then plated on strains

K (T186) and C (518C) to obtain relative e.o.p's. Repeated attempts failed to yield any plaques of W.K or Mu.K on E.coli C, the e.o.p. being less than 10^{-8}.

A different method was therefore employed to obtain W.KC and Mu.KC phage. The W.K and Mu.K lysates were propogated individually on a 1:1 mixture (by volume) of E.coli strains C and K. These propogating systems were then plated on E.coli C for plaques. Single plaques were picked and propogated on C, producing W.KC and Mu.KC lysates. The phage were then plated on C and K for their relative e.o.p. From single plaques on K, lysates were again prepared (W.KCK and Mu.KCK lysates) and plated on K and C. Further lysates were prepared from single plaques of W.KCK on C (W.KCKC) and plated on C and K. The plating efficiencies obtained from such a series is outlined in tables 7 & 8 and figure 9.

7. Phage origin	e.o.p. on K	e.o.p. on C
E.coli W supernant	1.0	
W.K/K	1.0	$< 10^{-8}$
W.KC/C	10^{-5}-10^{-7}	1.0
W.KCK/C'	1.0	$1\text{-}10^{+1}$
W.KCKC/C'	10^{-1}-10^{-2}	1.0

8. Phage origin	e.o.p. on K	e.o.p. on C
RS54 Supernant	1.0	
Mu.K/K	1.0	$< 10^{-8}$
Mu.KC/C	10^{-7}	1.0
Mu.KCK/C'	1.0	$1\text{-}10^{+1}$

Table 7 & 8: The relative efficiencies of plating of phage W.K and phage Mu.K and their derivatives on E.coli strains C and K.

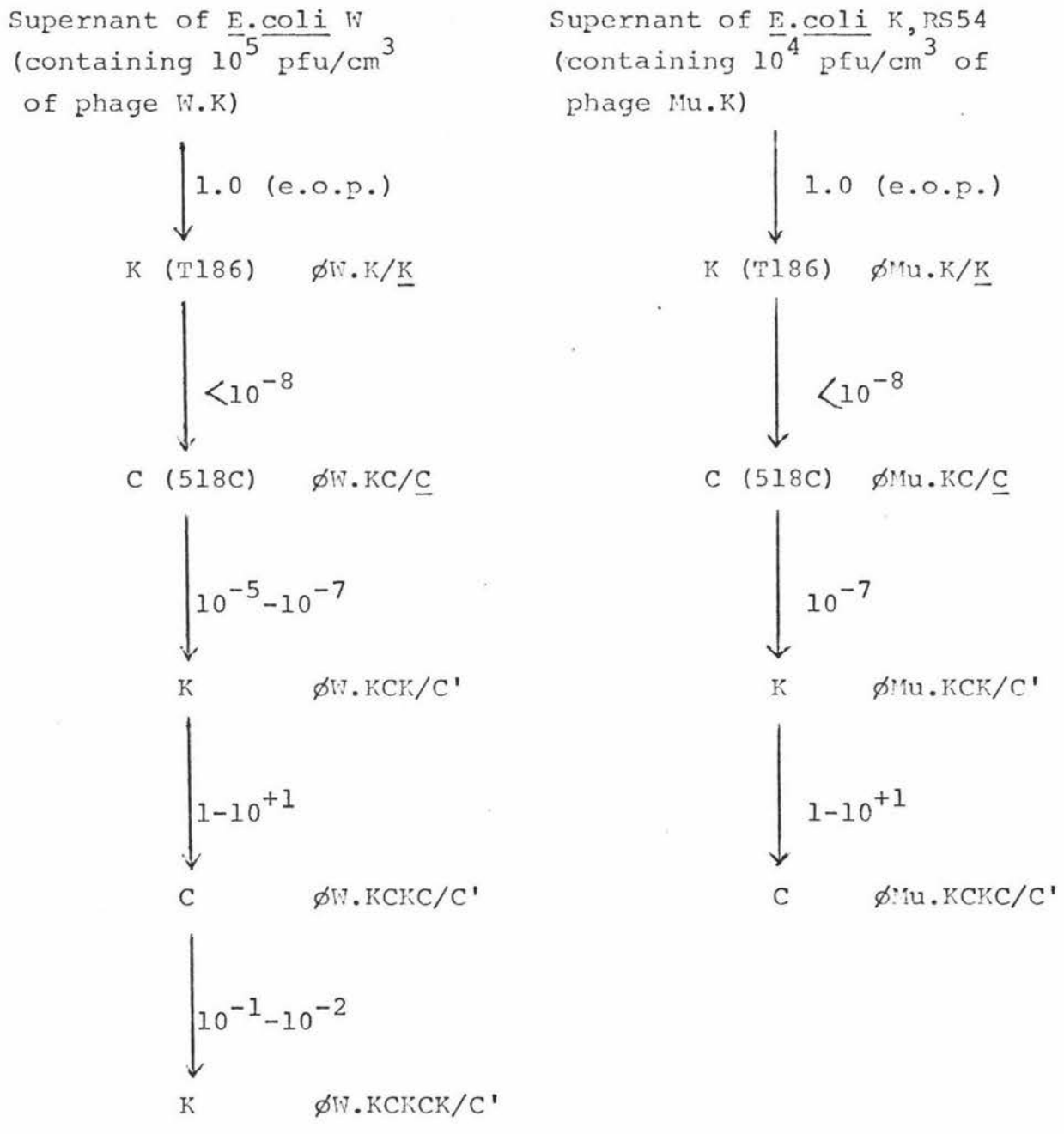


Figure 9: Plating efficiencies of the phage W.K and the phage Mu.K on E.coli strains C and K.

Each phage lysate was then run against antisera in neutralisation tests. The results are set out in tables 9 & 10.

9.

Phage lysate	Antisera: Anti W.C	Anti W.K	Anti Mu.K	Host Plated on
W.K/K	-	+	+	E.coli K (T186)
W.KC/C	+	-	-	E.coli C (518C)
W.KCK/C'	+	-	-	E.coli K (T186)
W.KCK/C'	+	-	-	E.coli C (518C)

10.

Phage lysate	Antisera: Anti W.C	Anti W.K.	Anti Mu.K	Host Plated on
Mu.K/K	-	+	+	E.coli K (T186)
Mu.KC/C	+	-	-	E.coli C (518C)
Mu.KCK/C'	+	-	-	E.coli K (T186)
Mu.KCK/C'	+	-	-	E.coli C (518C)

Tables 9 & 10: The neutralisation of phage W.K, and phage Mu.K, and their derivatives by antisera prepared against the phages W.C, W.K and Mu.K.

The plating efficiencies, together with the antisera neutralisation results demonstrate that both the phage W.K and the phage Mu.K are behaving in a parallel manner.

With the first host change, when the phages are made to propogate on E.coli C, the pattern of plating and neutralisation of the progeny phages, W.KC/C and Mu.KC/C, are consistent with an alternate host specificity. The phages will plate efficiently only on E.coli strain C, but not on strain K (presumably they do not adsorb to K (4)). The tail configuration of each is recognised only by the W.C antiserum, but not by the W.K or Mu.K antisera.

When the phages are plated back onto, and propogated on the K strain again, the phage obtained, W.KCK/C' and Mu.KCK/C', are now found to exhibit an extended host range, i.e. a host range mutation type of behaviour. The phages will adsorb to, and propogate readily on both E.coli strains C and K, and are neutralised only by the W.C antiserum, but not the W.K or Mu.K antisera, demonstrating that the phage now have the /C' tail form, as seen previously with the phage W.CK/C'.

When the phage W.KCK/C' is plated back onto C, and propogated up on C, the resultant W.KCKC/C' progeny phage will be unmodified, and when plated back onto K, should have been restricted by the hsK host restriction system. The e.o.p. obtained for this, of 10^{-1}-10^{-2} would indicate that the phage is unrestricted, or restricted only at a very low level. A similar result to that obtained with the phage W.C, again suggesting the possibility that the phage DNA is being protected by a mom-like function (1).

The results obtained differ from those seen by Jamieson (4) when he did the initial study of the phage W.K and its derivatives. The pattern of plating behaviour which he observed was consistent with phage W.K and its derivatives

exhibiting only the alternating host specificity; he did not see any host-range mutants of phage W.K.

Waddell (14), using the supernant from an E.coli C lysogenised with the phage W.KC/C, originally isolated by Jamieson (4), obtained a set of results for plating efficiencies on E.coli strains C and K which imitated the pattern set out in table 7.

III THE PROPERTIES OF E.COLI STRAINS C AND K LYSOGENS

Substrains of E.coli C and K were lysogenised with a range of phages, and their ability to support propogation of phage Lambda$_v$ and P1$_v$ investigated. E.coli K (T186) was lysogenised with phage W.K/K. An E.coli K strain lysogenised with phage Mu.K/K was already available - RS54. E.coli C (518C) was lysogenised with phage W.C/C, and phages W.KC/C and Mu.KC/C.

The results obtained for lysogens of E.coli K demonstrated that the phages P1$_v$.K and Lambda$_v$.K were both able to propogate efficiently on their host bacteria, unaffected by the presence of the phage W.K/K or the phage Mu.K/K. See table 11. This is in agreement with the work carried out by Jamieson (4) and suggests that apart from the normal hhs K host restriction and modification process, the prophage W.K or Mu.K are not expressing a system of restriction in E.coli K.

Lysogen	No. Screened	e.o.p. of Pl_v.K	e.o.p. of $Lambda_v$.K
E.coli K			
T186.WK/*K*	2	1	1
RS54 (K12.Mu.K/*K*)	1	1	1

Table 11: The ability of *E.coli* K lysogenised with phage W.K/*K* or phage Mu.K/*K* to support the propogation of phages Pl_v and $Lambda_v$.

Substrains of E.coli C (518C) were lysogenised with phage W.C/C, while others were lysogenised by phage W.KC/C or phage Mu.KC/C. See table 12.

Lysogen	No. Screened	e.o.p. of Pl_v.C	e.o.p. of $Lambda_v$.C
E.coli C			
518C.WC/C	12	$< 10^{-3}$	$1-10^{-1}$
518C.WKC/C	3	$< 10^{-5}$	$1-10^{-1}$
518C.MuKC/C	4	$< 10^{-6}$	$1-10^{-1}$

Table 12: The ability of E.coli C lysogenised with phage W.C/C, W.KC/C or Mu.KC/C to support the propogation of phages Pl_v and $Lambda_v$.

All the isolates of 518C.WC/C allowed phage $Lambda_v$.C to propogate with high efficiency. This result contrasts with that seen by previous workers. Glover (3, 5) working with phage Lambda.K (not the virulent mutant $Lambda_v$ or Lambda propogated on C previously as the host bacterium), obtained a relative e.o.p. of less than 10^{-8}. He also reported that Lambda was restricted to varying degrees in different isolates of C.WC. Jamieson (4) had an e.o.p. of less than 10^{-7} for Lambda on C.WC.

The phage $P1_v$.C however was found to have a low e.o.p. on E.coli C lysogenised by phage W.C/C. This is consistent with Jamieson's results (4). The low e.o.p. is presumed to be a direct result of restriction of phage P1 by the lysogenised host (3,4,5).

The results obtained from the 518C.WKC and 518C.Mu.KC lysogens parallel each other, as well as the 518C.WC lysogens, in that $P1_v$.C is restricted, but $Lambda_v$.C is unrestricted, and propogates readily on the lysogenic hosts. The restriction occuring must be due to the system operated by phage W.C/C, W.KC/C and Mu.KC/C, as E.coli C lacks its own host controlled restriction and modification system.

IV PLATING BEHAVIOUR OF THE PHAGES W.C, W.K AND Mu.K ON CITROBACTER FREUNDII

During lytic propogation of phage Mu on E.coli K, the invertible G region of the phage DNA, which codes for components of the phage tail, appears fixed in the G(+) orientation. In this form, phage Mu adsorbs to and propogates efficiently only on E.coli K.

In contrast, when Mu stocks are prepared by induction of an E.coli K lysogenised by phage Mu, the lysate contains two forms of phage Mu. The G(+) form, and in approximately equivalent numbers, the G(-) form. The G(-) form of Mu does not propogate on E.coli K. Until recently, the host specificity of the G(-) particles was unknown. But it has recently been reported by Van de Putte (13) that a strain of Citrobacter freundii, deficient in its own host-controlled restriction system, but still mediating its host-controlled modification system i.e. $r^{-}m^{+}$, can serve as a host for these phage particles. See table 13.

Phage Preparation	e.o.p. on hosts		
	E.coli K (T186)	*E.coli* C (518C)	*C. freundii* r^-m^+ (G331)
Mu (lytic)	1	$< 10^{-8}$	10^{-9}
Mu (induced)	1	$< 10^{-8}$	1

Table 13: Plating efficiencies of phage Mu lysates, lytic and induced, on different hosts.

The results demonstrate that during lytic propogation, the G region of phage Mu is fixed rigidly in the G(+) orientation; the progeny phage propogate only on E.coli K. However, after induction, approximately equal numbers of G(+) and G(-) forms of Mu are present, thus giving an e.o.p. of 1 on both E.coli K and C.freundii r^-.

The behaviour of phage W.C was investigated, looking specifically for any signs of a G-like inversion occuring during lytic propogation, or being present after induction of a W.C lysogen. The supernant from an overnight culture of E.coli W (F-2-3-27) which contains the phage W.C, but not the phage W.K, was tested as well as the supernant of an E.coli C (518C.W.C) lysogen and a lytic preparation of phage W.C. The observed e.o.p's are set out in table 14.

Phage Preparation	e.o.p. on hosts		
	E.coli K (T186)	*E.coli* C (518C)	*C. freundii* r^-m^+ (G331)
W.C (lytic)	10^{-7}-10^{-8}	1	$<10^{-6}$
W.C (induced)	$<10^{-4}$	1	$<10^{-4}$

Table 14: Plating efficiencies of phage W.C lysates, lytic and induced, on different hosts.

When phage W.C was induced, no evidence was seen for the appearance of phage able to propogate on E.coli K or C. freundii r^-.

In a similar manner, the properties of a lytic preparation of phage W.K and an induced preparation of the phage from an E.coli K (T186.W.K) lysogen were tested. See table 15.

Phage Preparation	e.o.p. on hosts		
	E.coli K (T186)	*E.coli* C (518C)	*C. freundii* r^-m^+ (G331)
W.K (lytic)	1	$< 10^{-8}$	$< 10^{-8}$
W.K (induced)	1		1

Table 15: Plating efficiencies of phage W.K lysates, lytic and induced, on different hosts.

The plating pattern seen for phage W.K is identical to that of phage Mu.K in that during lytic propogation of the phage, there is no evidence of a G-like inversion, however, after induction of a W.K lysogen, phage W.K exhibits the same plating efficiency of 1 on E.coli K (T186) as it does on C. freundii r^- (G331), indicating strongly that phage W.K has a G region which can invert during bacterial (and thus prophage) replication. To test this hypothesis further, the supernant from an overnight culture of E.coli W (ATCC9637) was examined, and the plating efficiencies on E.coli K and C. freundii r^- recorded. See table 16.

Phage Preparation	e.o.p. on hosts	
	E.coli K	*C. freundii* r^-m^+
E.coli W supernant (ATCC9637)	1	$1\text{-}10^{-1}$

Table 16: Plating efficiencies of phage W.K from the supernant of *E.coli* W on *E.coli* K and *C. freundii* r^-.

Although the supernant from E.coli W (ATCC9637) contains phage W.K and phage W.C, previous results (table 14) demonstrated that the phage W.C was not plating on E.coli K or C. freundii r^- after induction of E.coli W (F-2-3-27). As such, the plating seen on C. freundii r^- would be due to a proportion of the phage W.K progeny being in the G(-) form. This is further support for the hypothesis that phage W.K and phage Mu.K are in fact the same.

To this point it has been demonstrated that induced phage W.K and induced phage Mu.K (from various sources) are able to propogate on C. freundii r^- with an e.o.p. of approximately 1 relative to the plating efficiency on E.coli K, and further that this is due to the progeny phage being of two types, the G(+) form and the G(-) form. No evidence was seen of the lytic phages W.K or Mu.K plating readily on C. freundii r^-.

Van de Putte (13) gives the e.o.p. of lytic Mu.K on C. freundii r^- as 2.5×10^{-9} (Mu propogated on E.coli K) and 10^{-6} (Mu propogated on E.coli K sup. A) indicating that inversion of the G region can occur, but only at a very low frequency during lytic propogation. This contrasts with the high frequency of G region inversion observed from an induced lysogen, presumably due to inversion of the G region occuring during bacterial and thus prophage replication.

Observations so far were confirmed by running the phage lysates, lytic and induced, with antisera in phage neutralisation tests. See table 17.

Phage Preparation	Antisera			Host Plated on
	anti W.C	anti W.K	anti Mu.K	
Mu.K (lytic)	-	+	+	E.coli K (T186)
Mu.K (induced)	-		50-65%	E.coli K (T186)
			-	C. freundii r (G331)

Table 17: The neutralisation of phage Mu.K from lytic and induced preparations by antisera prepared against the phages W.C, W.K and Mu.K.

The Mu.K antiserum was prepared using a lytic lysate of phage Mu.K i.e. the G(+) form of Mu. Thus, the 50-65% neutralisation of the induced Mu lysate when plated on E.coli K after neutralisation represents only the neutralisation of phage Mu.K G(+) forms. The failure of the Mu.K antiserum to neutralise the Mu induced lysate when it was plated on C. freundii r^- supports the idea that the G(-) form of Mu is unaffected by the G(+) form of Mu antiserum.

A plaque of Mu.K on C. freundii r^- was picked, and propogated up on C. freundii r^-. The resultant phage lysate, of phage Mu.KF, was then tested against the W.C and Mu.K antisera. See table 18.

Phage Preparation	Antisera		Host Plated on
	anti W.C	anti Mu.K	
Mu.KF (lytic)	-	-	C. freundii r^- (G331)

Table 18: The neutralisation of phage Mu.KF by antisera prepared against the phages W.C and Mu.K.

From these results it appeared that the Mu.KF lysate consisted of Mu.KF phage, corresponding to the G(-) form of Mu which is unaffected by W.C or Mu.K antisera. With phage Mu now in the G(-) form, it was of interest to see how readily it would plate back on E.coli K, presumably due to the G(-) form inverting back to the G(+) form. A lytic preparation of phage Mu.KF was then plated on E.coli K (T186) and E.coli K $r^{-}m^{+}$ (PB1395). See table 19.

Phage Preparation	e.o.p. on hosts			
	C. freundii $r^- m^+$ (G331)	E.coli K (T186)	E.coli K $r^- m^+$ (PB1395)	E.coli C (518C)
Mu.KF (lytic)	1	10^{-6}-10^{-7}	10^{-2}-10^{-3}	10^{-5}

Table 19: The relative efficiencies of plating of phage Mu.KF on various hosts.

The relatively high e.o.p. of phage Mu.KF on E.coli K r^-m^+ suggests that the G region of phage Mu does undergo inversion during lytic propogation of the phage in C. freundii r^-, although not to the same degree seen in E.coli strain W and K lysogens. As a check that the phage Mu has now inverted back to the G(+) form, a plaque from the plating of phage Mu.KF on E.coli K r^-m^+ was picked, and propogated up on E.coli K r^-m^+, and the resultant lysate, of phage Mu.KFK, then tested with the antisera preparations. See table 20.

Phage Preparation	Antisera		Host Plated on
	anti W.C	anti Mu.K	
Mu.KFK (lytic)	-	+	*E.coli* K r^-m^+ (PB1395)

Table 20: The neutralisation of phage Mu.KFK by antisera prepared against the phage W.C and Mu.K.

The neutralisation pattern seen clearly demonstrates that the phage Mu is in fact in the G(+) form again, and also has the 'K' tail form. Table 19 results also suggest that phage Mu.KF is being restricted when it is plated back onto E.coli K as compared with E.coli K r^-m^+. This indicates that the G(-) form of Mu, when propogated lytically, may not have the mom function operating.

The Mu.KFK/K lysate was plated back onto E.coli C and C. freundii r^-. The plating efficiency in each case was less than 10^{-5}, which was the result expected as phage Mu.KFK/K should behave in the same manner as phage Mu.K. See table 21.

Phage Preparation	e.o.p. on hosts			
	E.coli K (T186)	*E.coli* K $r^- m^+$ (PB1395)	*E.coli* C (518C)	*C. freundii* $r^- m^+$ (G331)
Mu.K/*K* (lytic)	1		$<10^{-8}$	10^{-9}
Mu.KFK/*K* (lytic)		1	$<10^{-5}$	$<10^{-5}$

Table 21: The relative efficiencies of phage Mu.K/*K* and phage Mu.KFK/*K* on various hosts.

The Mu.KF lysate was plated on E.coli C, a plaque picked, and propogated up on E.coli C, thus giving a Mu.KFC lysate. The plating efficiency of this phage, Mu.KFC, was then checked on E.coli K r^+ and r^- strains and on C. freundii r^-; the ability of the W.C antiserum to neutralise this phage was also examined.
See tables 22 & 23.

Phage Preparation	e.o.p. on hosts			
	E.coli C (518C)	*E.coli* K (T186)	*E.coli* K r^-m^+ (PB1395)	*C. freundii* r^-m^+ (G331)
Mu.KFC (lytic)	1	10^{-7}	10^{-1}–10^{-3}	10^{-1}–10^{-2}

Table 22: The relative efficiencies of plating of phage Mu.KFC on various hosts.

Phage Preparation	Antiserum anti W.C	Host Plated On
Mu.KFC (lytic)	20-25%	E.coli C (518C)

Table 23: The neutralisation of phage Mu.KFC by antiserum prepared against phage W.C.

A kinetic study of the neutralisation of phage Mu.KFC by W.C antiserum (results not shown) indicated that the neutralisation seen was not a cross-reaction between the phage and the antiserum, but was in fact due to the Mu.KFC lysate being a mixture of phage types with approximately 20-25% having the 'C' tail form.

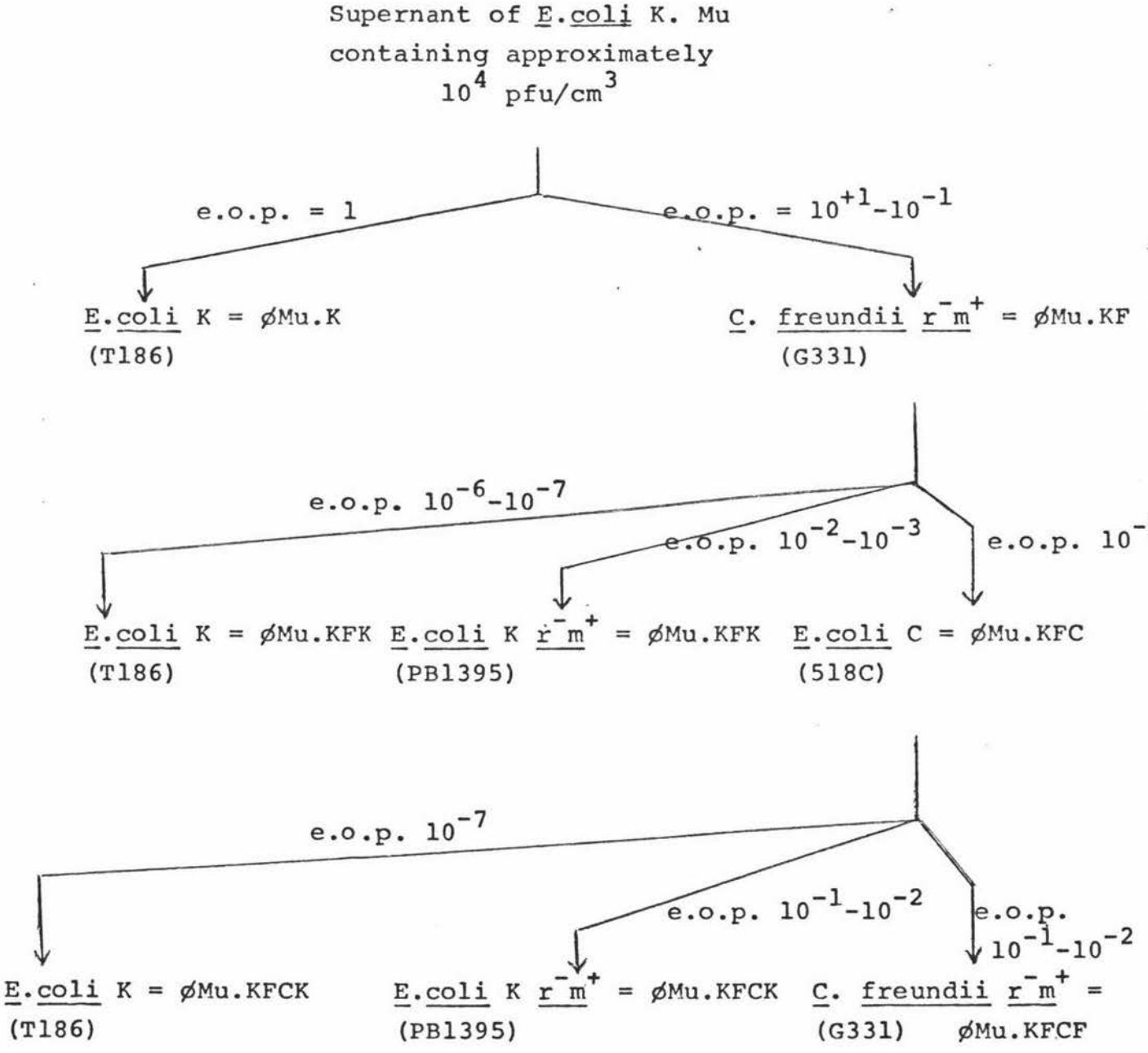


Figure 10: Diagramatic summary of the relative efficiencies of plating of phage Mu.K (induced), and its derivative phages on various hosts.

D I S C U S S I O N

During the course of this study, it was observed that phage W.C was capable of exhibiting two very different plating patterns; initially it was seen to act as a host range mutant, able to adsorb and propogate efficiently on both E.coli strain C and strain K. Subsequently, it was observed to exhibit an alternating host specificity pattern, able to adsorb and propogate only on one host strain of E.coli at a time, for example, if the phage was propogated on E.coli C, then it would not adsorb or propogate readily on E.coli K, and vice versa.

Jamieson's work (4) only observed the first pattern of behaviour, i.e. the host range mutations for phage W.C, and only the second pattern of behaviour, i.e. the alternating host specificity for phage W.K. As such, he proposed that the different plating behaviours of the phages W.C and W.K, reflected a major discernable difference between the two phages, presumably at the level of their genomes. This is no longer applicable as a difference.

Other experiments concerned with the restriction of phages Pl_v and $Lambda_v$ on E.coli strain C and K lysogens however, still support a notable difference between phage W.C and phage W.K. Table 24 summarises the results of section III.

	Lysogen	Ability to exclude phage	
		Pl_v	$Lambda_v$
E.coli K	T186.WK/K	-	-
	K12.MuK/K	-	-
E.coli C	518C.WC/C	+	-
	518C.WKC/C	+	-
	518C.MuKC/C	+	-

Table 24: Summary of the exclusion of phages Pl_v and $Lambda_v$ by E.coli strain C & K lysogens.

E.coli C, lysogenised with phage W.C, clearly excludes phage Pl_v.C. The bacterium itself lacks a host controlled restriction and modification process, therefore the exclusion of the phage is most probably due to the W.C phage mediated restriction system (3,4). Phage Pl_v.K was unrestricted and propogated readily on E.coli K lysogens of phage W.K and Mu.K, implying that these phage do not control a restriction system when present in E.coli K. However, the Pl_v.K phage would not propogate on E.coli C lysogenised by the phage W.KC and Mu.KC. This suggests that the W.K and Mu.K phage are in fact expressing a restriction system when they are present in E.coli C. Thus, with the host change from E.coli K to E.coli C, the phages have changed from a non-restricting form to a restricting form.

Waddell (14) in her work attempting to derive a mutant Pl_v able to propogate on E.coli C lysogenised with the W prophages, derived the virulent phage W.KC_v. This mutant phage was able to propogate on a restricting E.coli C.W.KC lysogen. This is evidence that the W.KC_v phage was apparently unaffected by its own restriction system (mediated by the W.KC prophage). This could be a consequence of the W.KC_v phage not possessing the specific recognition site for the endonuclease, or being protected by a mom-like function. The phage W.KC_v was able to propogate on E.coli C.W.C non-restricting lysogens, but not on E.coli C.W.C restricting lysogens. Since phage W.KC and phage W.C are co-immune, this provides evidence that phages W.K and W.C mediate two different restriction systems. As such, the different restriction systems mediated by the phages W.C and W.K (Mu.K) can be used to support the hypothesis that the phages, though very closely related, are to be differentiated.

It should also be noted that although exclusion of phage Pl_v was seen by lysogens of E.coli C tested, none were able to exclude $Lambda_v$ effectively, which plated with an e.o.p. of 10^{-1}-1. Because this latter result contrasts with those of previous workers (3, 4, 5), verification that the λ_v stock used was in fact in order would have been helpful, and should be undertaken in any further studies that may be initiated.

Another apparent difference between phage W.C and phage W.K, is that substrains of E.coli W have been derived with phage W.K eliminated by recombination, but as yet, attempts to remove phage W.C have failed. This could be explained by phage W.C being able to occupy more than one integrative site on the W chromosome. The variable restriction shown by E.coli C lysogens of phage W.C/C and phage W.CKC/C' may also be accommodated by this theory.

The alternating host specificity type behaviour exhibited by phage W.C, where it can be observed to switch from the 'C' tail form (phage W.C/C) as recognised by the antiserum prepared against phage W.C, to the 'K' tail form (phage W.CK/K) as recognised by the antiserum prepared against the phage W.K on phage Mu.K, and then back to the 'C' tail form again (phage W.CKC/C) could potentially be explained by a G region type inversion as seen with phage Mu: one orientation provides the phage with the 'C' tail form; the alternate orientation provides the phage with the 'K' tail form.

Another feature of phage W.C which was observed was the apparent operation of a mom-like function. The phage W.CKC/C', propogated on E.coli C, would be unmodified. When it was then plated on E.coli K r^+ (T186), the e.o.p. obtained was approximately 10^{-1}, which was much higher than expected in the presence of the hs K restriction system. Similarly, when phage W.CKC/C was propogated on E.coli C, the resultant unmodified phage progeny when plated on the restricting host E.coli K r^+ (T186) gave an e.o.p. of 10^{-7}-10^{-8}, the same e.o.p. obtained for plating phage W.CK/K on the non-restricting host E.coli C. The phage W.C and its derivatives, when plated on E.coli K r^+ were apparently being protected from the action of the hs K restriction system. The alternative, that the phage was insensitive to the hs K restriction system (due to lack of the specific site recognised by the restriction endonuclease) can be disregarded assuming the very close relationship between phage W.C and phage W.K, and thus phage

Mu.K, as Toussaint (1) has demonstrated that Mu mom$^-$ mutants are sensitive, and restricted by the hs K system.

Throughout this investigation, no evidence was seen to distinguish phage W.K from phage Mu.K. In all respects, these two phages acted in an identical manner, and exhibited the same traits. As well as being co-immune, possessing the same host, E.coli K, identical plaque morphology, and structural morphology, both phages proved to be indistinguishable serologically, the antiserum prepared against phage W.K gave the same results as those obtained with the antiserum prepared against phage Mu.K.

The phages were unable to plate directly on E.coli C, the e.o.p. being less than 10^{-8}. However, after the switch to E.coli C, the derived phage, W.KC/C and Mu.KC/C both exhibited the 'C' tail form as recognised by the W.C antiserum, compared to the 'K' tail form that they had previously expressed. As with phage W.C, this change in host specificity may be linked with a G region type inversion.

Observation of the results obtained with E.coli K lysogens of phage W.K/K, or phage Mu.K/K, indicate that the prophage in strain K is non-restricting for phage Pl_v. However, the phages W.KC/C or Mu.KC/C, when present in E.coli C as prophages, endowed the host bacterium with the ability to restrict phage Pl_v. Thus, in the 'K' form, phage W.K and Mu.K appear non-restricting, but in the 'C' form, they appear restricting. When the host range mutant phage W.KCKC/C' was plated back onto E.coli K r^+ (T186), the e.o.p. obtained was 10^{-1}-10^{-2}. This again was higher than expected if phage W.K is sensitive to the hs K restriction system, but could be explained by the operation of the mom function.

The ability of the antisera to identify the particular tail form a phage was expressing proved to be a great asset during this study. Taken together with the adsorption characteristics and plating pattern of the phage, conclusive statements could be made as to whether the phage in question

was showing an alternating host specificity, changing from the 'C' tail form to the 'K' tail form, or vice-versa, or whether the phage was acting as an extended host range mutant, possessing the 'C' tail form (note: no extended host range mutants with a 'K' tail form were observed).

When strains Q, E.coli K lysogenised by phage $Mu^{cts}61$ or $Mu^{cts}62$, were heat induced, and the resultant lysates tested for neutralisation by the Mu.K antiserum and plated on E.coli K (T186), the subsequent 50-65% reduction in the titre compared with the control titre (table 17) was an unexpected result. Closer examination of the specific lysates involved showed that Mu titres on C. freundii r^- were 10^1-10^2 higher than the titres on E.coli K. This contrasts with the more usual ratio of approximately 1:1. It may reasonably be assumed that in the neutralisation tests, >80% of the G(+) forms of Mu (Mu.K) have been neutralised. The remaining 35-50% of the plaques seen on the plates of E.coli K after neutralisation with the Mu.K antiserum cannot readily be accounted for by the un-neutralised portion of the Mu.K phage alone. It has been demonstrated that the G(-) form of Mu is not neutralised by the Mu.K antiserum. This leaves open the possibility that the above neutralisation result may be explained by Mu G(-) phage (which would be expected to have the hhs K modification) expressing an ability to plate on E.coli K with a low efficiency. As such, they normally have little effect on the phage titres obtained on E.coli K, but in the event that the majority of the G(+) forms of Mu have been neutralised, the residual plating of the G(-) phage may now become significant.

More experiments need to be conducted to understand fully what is happening. A kinetic study of the neutralisation behaviour may be helpful. Also, a Mu G(-) antiserum would be very valuable. To this point, it has not been feasible

to prepare such an antiserum as phage Mu.KF, G(-), propogated lytically on C. freundii r^- was demonstrated to plate back onto E.coli K r^- with an e.o.p. of 10^{-2}-10^{-3}, presumably due to the G region re-inverting to produce the G(+) form of Mu again, thus a pure G(-) antiserum would not be possible. It would be desirable to obtain a more stable form of the Mu G(-) phage. Two potential means to accomplish this are:

(i) Using a C. freundii Mu.KF lysogen to derive a gin$^-$ mutant of the same, which will thus have the G region frozen in the G(-) orientation,

OR

(ii) To use an existing E.coli K.Mu^{cts}62 gin$^-$ lysogen. These have already been isolated, with the G region fixed in the G(+) orientation (1, 12). If an F plasmid carrying a wild-type Mu can be introduced into the lysogen, then the wild type Mu on the F plasmid will allow the G region of the prophage on the bacterial chromosome to invert. If the F plasmid is then removed, the lysogenic cells will once more have the G region of their Mu prophage fixed in one or other orientation; this could be either the G(+), or the G(-). Clones might then be derived and tested for lysogens whose supernant produced plaques on C. freundii r^-, but not on E.coli K, presumably due to the Mu prophage being G(-) and gin$^-$. (Note: The use of the temperature sensitive Mu, Mu^{cts}62, has the added advantage of allowing heat induction of the lysogen to readily obtain a high titre of phage.)

Either of the outlined procedures could thus be used as a pure source of Mu G(-) phage to serve as the antigen for the preparation of a specific Mu G(-) antiserum.

As stated previously, after the induction of E.coli K lysogenised by phage Mu, approximately equal numbers of the G(+) and the G(-) forms of Mu are normally present. The results seen with respect to phage W.K suggest it too behaves in the same manner, with approximately equal numbers of the induced phage progeny from E.coli K.WK or E.coli W plating on E.coli K and C. freundii r^-. The change in the phage's host specificity is attributed to inversion of the G region. This confers on the phage a different tail form. The phage Mu.KF, the G(-) form of Mu which propogated on C. freundii, is not neutralisied by either the W.C antiserum, or the Mu.K antiserum (prepared with a lytic Mu stock (i.e. the G(+) form of Mu)). This implies that phage Mu may in fact express three distinctive tail forms: the 'K' form (or G(+) form) as recognised by the Mu.K/W.K antisera; the 'C' form as recognised by the W.C antiserum, and a third 'F' form (or G(-) form), which is not recognised by either the W.C or the Mu.K/W.K antisera.

As stated earlier, the switch from the 'K' (G(+)) form of Mu to the 'F' (G(-)) form of Mu is achieved by inversion of the G region of the phage genome. Presumably, the 'C' tail form of phage Mu is to some degree controlled by the G region. No definite function has been assigned to the invertible region found within the G region itself (see figure 5). A model can be proposed to explain the 'C' tail form of Mu which takes into account this second invertible region.

The G region of phage Mu can be divided into three main areas consisting of g2, g4 and g5. Areas g1 and g1', and g3 and g3' represent inverted repeats. See figure 11.

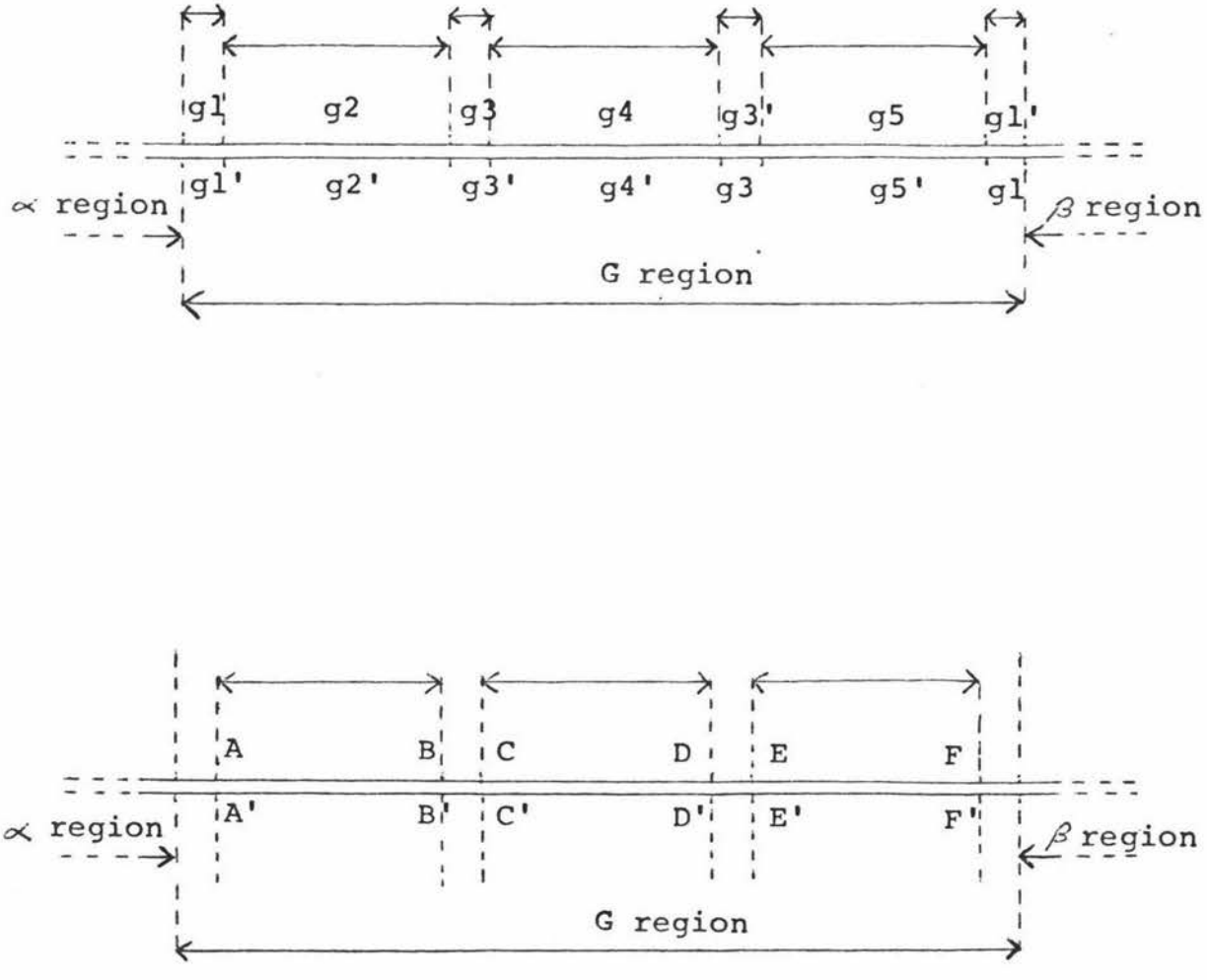
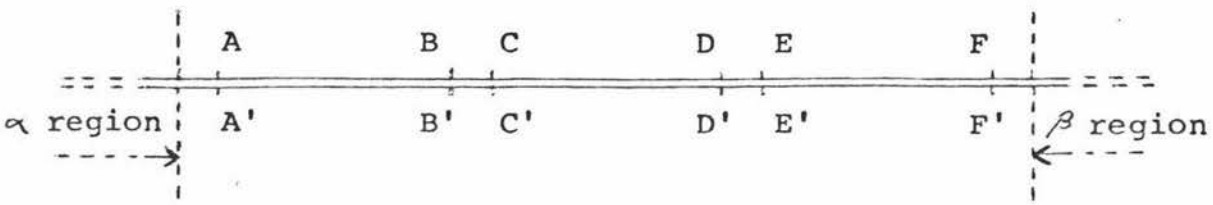


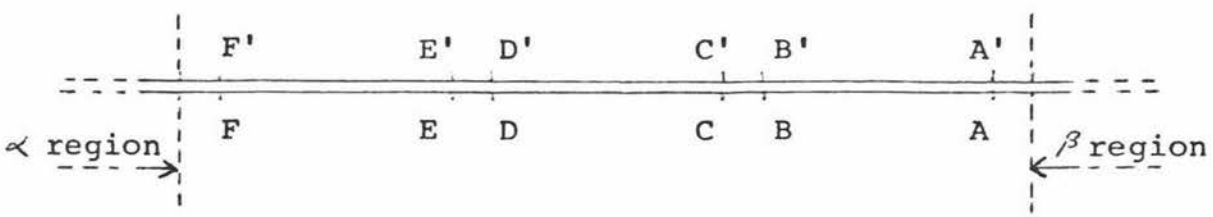
Figure 11: Diagramatic representation of the double-stranded G region of phage Mu.K. The letters A B C D E F and A' B' C' D' E' F' were assigned arbitrarily to make interpretation of the following figures simpler.

Phage Mu.K has the G region in the (+) form. This is better represented now as G (++), with the second plus relating to the orientation of the inner invertible segment of the G region i.e. g4. Phage Mu.KF, which has the G region in the opposite orientation, can thus be represented as G (--). See figure 12 (b). There are now two other possible orientations of the G region, giving rise to a G (-+) and a G (+-) form of Mu. See figure 12 (c) & (d).

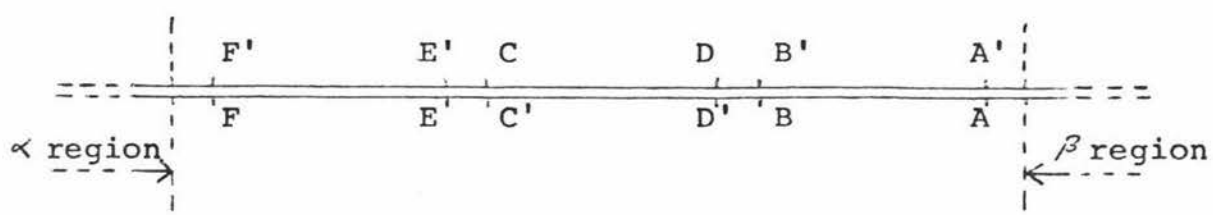
a) Mu.K (++)



b) Mu.KF (--)



c) Mu (-+)



d) Mu (+-)

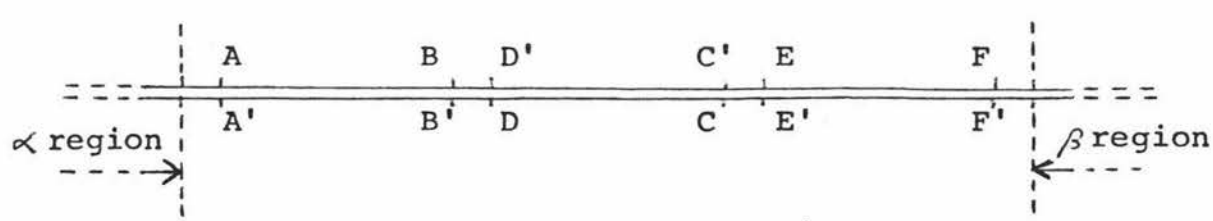


Figure 12: Diagramatic representation of possible G region orientations for various forms of phage Mu.

The 'C' form of Mu could thus be depicted as either Mu G (-+) or Mu G (+-). There is also the possibility that a fourth form (and subsequently a fourth host) of Mu can be present.

To test the presented model, a series of heteroduplexing studies would be very helpful. The proposed configurations which might be seen are illustrated in figure 13.

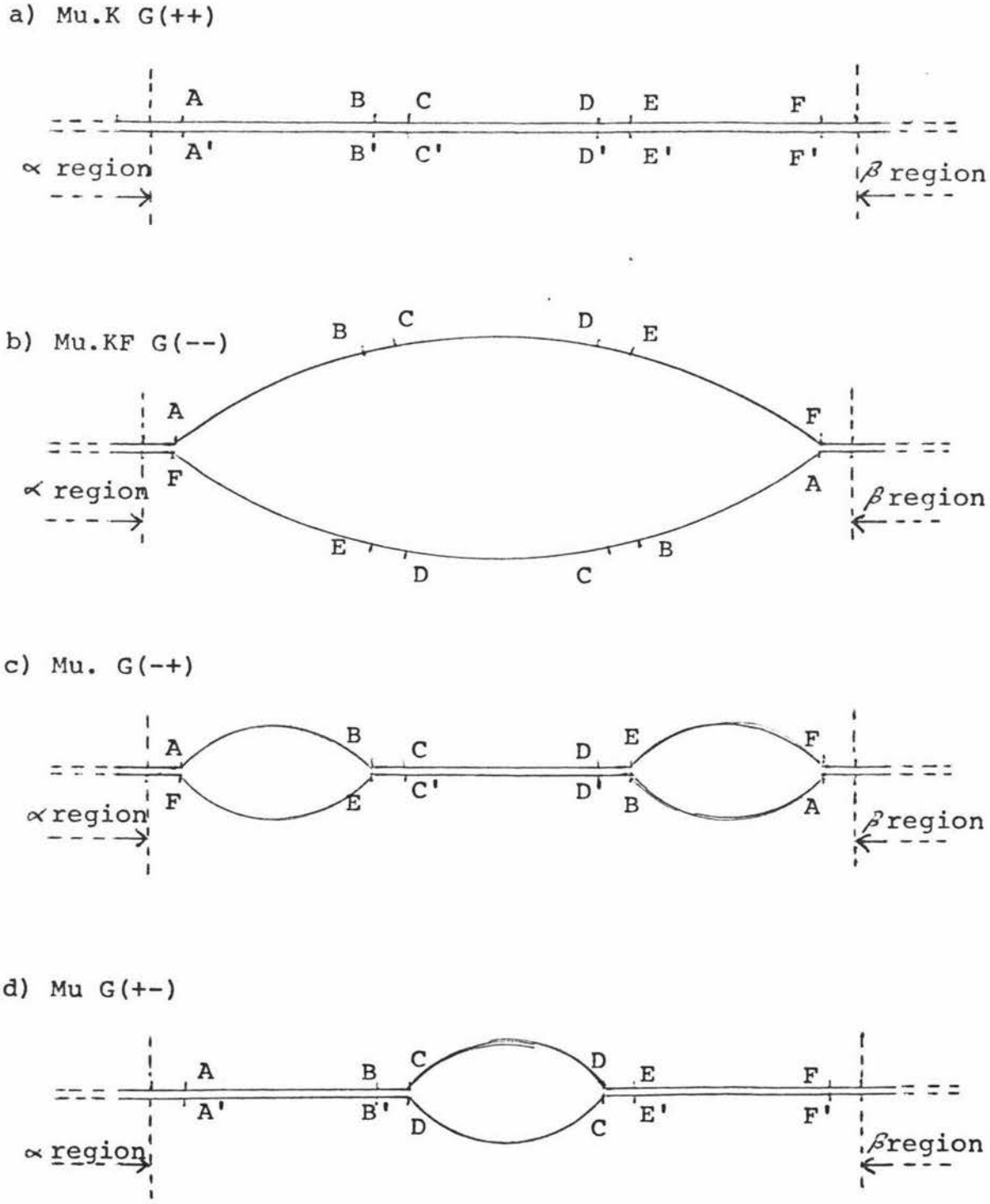


Figure 13: Proposed heteroduplex structures between a single strand of phage Mu.K (G++) and a single strand of the four possible G region orientations.

The expression of the mom gene and the gin gene of phage Mu is believed to be related to the orientation of the G region (1, 13). During lytic propogation of phage Mu.K on E.coli K, the frequency of G region inversion is very low (approximately 10^{-9} as reported by Van de Putte (13) for the plating efficiency of phage Mu.K on C. freundii r^-). The frequency of inversion, and the e.o.p. on C. freundii r^- increased to approximately 10^{-6} when phage Mu.K was propogated lytically on an E.coli K sup A strain, which is defective in the transcription terminator factor ρ. Since the transcription of phage Mu is reported to be unidirectional, starting at the c end (15), it has been suggested by Van de Putte (13) that the (+) orientation of the G region might act as a transcription barrier for gin expression if the gin promoter is located in the α region of Mu. In the (-) orientation of the G region, the barrier no longer exists, and thus gin can be expressed more frequently. This is supported by observing the apparent increased frequency of inversion occuring when phage Mu.KF i.e. G (-) is propogated lytically on C. freundii r^-. The phage will plate back on E.coli K r^- with an e.o.p. of 10^{-2}-10^{-3}. As a prophage in E.coli K, the gin gene would appear to be expressed from a different promoter site which is not functioning during lytic propogation.

From the e.o.p's obtained by plating phage W.K (Mu.K) on E.coli C ($<10^{-8}$), and phage Mu.KF on E.coli C (10^{-5}), it appears that once phage Mu has switched from the G(+) form to the G(-) form, it is then much easier for the phage to plate on E.coli C. This leads to the proposition that for phage Mu to plate on E.coli C, two events are needed. Firstly, the G region must invert to the G(-) form (this has also been suggested by Van de Putte (13)), and that a second event is necessary in order that the Mu.KF phage will be able to plate on E.coli C. This second event may be the inversion of the inner G region, g4, from the (-) form to the (+) form,

and thus ending up with the phage Mu.KFC as G (-+) as represented in figure 12 (c). If this is in fact happening, then presumably, if phage W.K (Mu.K) is grown in a sub-strain of E.coli K possessing a suppressor, a higher e.o.p. on E.coli C would be expected in comparison to phage W.K (Mu.K) grown on an E.coli K lacking a suppressor. Information from various sources suggest that this is the case. See table 25.

Sub-strain of E.coli K used as the propogating host	Reference	e.o.p. of phage W.K/K on E.coli C	Host genotype
T186	Rice	$< 10^{-8}$	wild-type
G461	Van de Putte (13)	$< 10^{-9}$	lacks sup
AB266	Jamieson (4)	10^{-4}-10^{-5}	sup E_{44}
Z *	Pizer (6)	10^{-3}	sup E44

* Note: Z is an arbitary designation. For fuller genotypes see table 1.

Table 25: Various sub-strains of E.coli K, their genotype, and the plating efficiency of their progeny phage on E.coli C.

The difficulties encountered in this study and also in previous work in this department (14), may in part be due to adopting E.coli K strain T186 as the 'standard' E.coli K for the experiments, without realising the apparent importance of using a strain of K possessing a suppressor. As a consequence, the frequency of gin expression, and therefore G region inversion during the lytic propogation of phage Mu.K would be extremely low (of the order 10^{-9} or less as compared to 10^{-6} or higher). The possibility also exists, that the use of E.coli K, strain T186, was instrumental in the expression by phage W.K (Mu.K) of the host range mutation type behaviour; the alternative course of behaviour (a G region type inversion) might have been effectively blocked.

Specific areas which might profitably be further investigated are listed below:

(i) The role of the G region inversion as it affects and is affected by the expression of the mom and gin gene functions.

(ii) The derivation of a stable form of phage Mu G(-) to act as a pure antigen source to prepare a specific Mu G(-) antiserum.

(iii) The behaviour of phage Mu.KFC, especially in comparison to phage Mu.KC.

(iv) Does phage W.C, like phage Mu possess a G region, have host DNA fragments at its ends, and cause mutations in its host?

(v) The restricting or non-restricting abilities of various W phage lysogens. Can this be related to orientation of the G region, and/or the location site of the prophage in the host genome?

(vi) Can evidence be found to support the hypothesis that the inner portion of the G region, g4, can invert?

(vii) Can phage Mu express a fourth tail form, and subsequently a fourth bacterial host?

Phage W.K and Mu.K appear identical to each other, and also very closely related to phage W.C. As such, it seems feasible to claim that phage W.C also possesses a G region. Many of the properties of these phage seem related, directly (e.g. tail form), or indirectly (e.g. mom and gin expression) to the G region. Phage P1 also possesses the same G region as phage Mu (11), and thus constitutes another system in which this very fascinating phenomenum of gene expression being controlled or modulated by an inverting DNA sequence can be studied.

C O N C L U S I O N

The bacterium Escherichia coli strain W has been shown to be lysogenic for two very closely related phage, phage W.C and phage W.K. The phage W.K, which has not been distinguishable from phage Mu.K, has also been demonstrated to be present in two forms following induction of E.coli W. These two forms of phage W.K/Mu.K differ primarily in the orientation of the G region on their genome: the G(+) form adsorbs to and propogates readily on E.coli K; the G(-) form adsorbs to and propogates readily on C. freundii r^-.

Phage W.C and phage W.K/Mu.K are both capable of giving expression to a 'C' tail form or a 'K' tail form. The phage W.K/Mu.K is also capable of expressing a third tail form 'F' (one which allows the phage to adsorb to and propogate readily on C. freundii i.e. corresponds to the G(-) form of phage W.K/Mu.K). No evidence was seen for phage W.C expressing the 'F' tail form, but this aspect has not been fully investigated.

Phage W.C and phage W.K/Mu.K have the potential to express two distinct plating patterns: one which consists of an alternating host specificity type behaviour; the other of an extended host range mutation type behaviour. The former may involve inversion of the G region in the phage genome and/or an area g4 defined within the G region itself. The plating pattern exhibited by a particular phage preparation may depend heavily on the conditions, and especially the specific sub-strain of bacteria used as the propogating host.

The W phages, when in the lysogenic state, were seen to endow the host bacterium with a system of genetic restriction but without the complementary modification system, i.e. the prophages code for a r^+m^- system. The system coded for by phage W.C appears to differ from that coded for by phage W.K/Mu.K, and as such constitutes a

discernable difference between the phages.

The phages all seem capable of expressing a mom gene function, though this was observed not to be operating in all cases (specifically when Mu G(-) is propogated lytically on C. freundii) and may be controlled/modulated to a degree depending upon the orientation of the G region of the phage.

Summaries are presented in the form of tables 26 and 27 outlining the similarities observed between phage W.C and phage W.K, and between phage W.K and phage Mu.K.

Phage W.C and Phage W.K Properties: Similarities
- Temperate phage
- Non-inducible by U/V light
- Morphology (by E.M.)
- Co-immune
- Heat sensitivity
- Alternate host specificity type behaviour
- Extended host range mutation type behaviour
- mom gene function
- Plaque morphology e.g. W.C/C = W.KC/C W.CK/K = W.K/K
- Serology e.g. W.C/C = W.KC/C W.CK/K = W.K/K

Table 26: A list of the properties expressed and shared by phage W.C and phage W.K.

Phage W.K and Phage Mu.K Properties:Similarities

- Temperate phage
- Present in the supernant from overnight broth cultures of E.coli W (ATCC9637)
- Non-inducible by U/V light
- Propogate lytically on E.coli K
- Able to lysogenise E.coli K
- Morphology (by E.M.)
- Co-immune
- Alternate host specificity type behaviour
- Extended host range mutation type behaviour
- mom gene function
- Plaque morphology e.g. W.K/K = Mu.K/K
  W.KC/C = Mu.KC/C
- Serology e.g. W.K/K = Mu.K/K
  W.KC/C = Mu.KC/C
- Lysogen behaviour
  i.e. K.W.K and K.Mu.K are non-restricting for Pl_v
  C.W.KC and C.Mu.KC are restricting for Pl_v
- Induced phage preparations (from E.coli W or E.coli K lysogens) can plate on E.coli K and C. freundii r^- in approximately equal numbers i.e. G region inversion occuring.

Table 27: A list of the properties expressed and shared by phage W.K and phage Mu.K.

A P P E N D I X A

Antisera	Dilution Used	Reference Phage	% Neutralisation
anti W.C	1/20	W.C/C	95
anti W.K	1/320	W.K/K	91
anti Mu.K	1/160	Mu.K/K	91

Table 28: The dilution of antisera used in neutralisation tests which produces 90-95% neutralisation of the reference phage in 10 minutes at 37°C.

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