THE HOST SPECIFICITIES OF PHAGE MU

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Thus says the Lord,

"Let not a wise man boast in his wisdom, and let not the mighty man boast of his might, let not a rich man boast of his riches; but let him who boasts boast of this, that he understands and knows Me, that I am the Lord who exercises loving kindness, justice and righteousness on earth; for I delight in these things," declares the Lord.

Jer 9 vs 23, 24.

ABSTRACT

Phage Mu, a temperate phage, possesses an invertible region of DNA 3kb in length, the G region.

The orientation of the G region determines the host specificities of phage Mu (Van de Putte *et al.*, 1980). The G region codes for tail polypeptides, the products of the S and U genes.

In one orientation (+) the phage adsorbs to and infects *E. coli* K12 (Mu.K). In the opposite orientation (-) the phage adsorbs to and infects a strain of *C. freundii* (Mu.F). However Mu also plates on *E. coli* C, *Serratia marcescens*, *E. cloacae* and several other hosts.

Rice (1980) was able to raise specific antisera to the Mu.K form and the W.C (Mu.C equivalent) form of Mu. However no antisera was raised to the Mu.F form.

We attempted to isolate *gin*$^-$ mutants, in which the G region is not inverted; such a mutant would provide a specific antigen for the G(-) Mu.F phage. Presumptive *gin*$^-$ mutants were identified as phage that could plate only on one host, *E. coli* K12 or *C. freundii*.

A specific antisera was raised to the MH_{4400} *gin*$^-$ G(-) phage.

Attempts were made to isolate the Mu.KC phage as seen by Jamieson (1971) and Rice (1980). For this purpose, heat induced lysogens of *E. coli* K12 were used, as opposed to lytic Mu.K lysates; the former give high titre lysates of G(-) and G(+) phage. The resulting Mu.KC lysogens were examined for their plating behaviour and neutralisation by the anti W.C serum.

Two classes of phage were isolated, Mu.KC'' and Mu.KC'''. These phage differed from the Mu.KC phage seen by Jamieson and Rice as judged from their limited neutralisation by the anti W.C serum, and they also differed from each other with respect to their plating on *C. freundii* and the extent to which they are neutralised by the anti W.C serum.

The Mu.KC" forms do not plate on *C. freundii* , e.o.p. $<8 \times 10^{-9}$, and are not neutralised by the anti W.C serum. Whereas the Mu.KC''' forms do plate on *C. freundii*, e.o.p. 10^{-1} -10^{-2}, and exhibit a low level of neutralisation with the anti W.C serum.

E. coli C lysogens of phage Mu.KFC were also isolated. These also fell into two distinct classes as judged by their plating ability on *C. freundii*; Mu.KFC'' forms do not plate on this host, e.o.p. $<5 \times 10^{-8}$, while the Mu.KFC''' forms do plate on *C. freundii*, with an e.o.p. 1×10^{-1} (comparable to the Mu.KC'' and Mu.KC''' mentioned above).

During the study, differences in the plating ability of lytic and induced lysates propagated on the same host were observed. For example, compare the *E. coli* K12 system; lytic Mu.K phage plate on *C. freundii* with an e.o.p. of $<10^{-9}$, while induced Mu.K plate on this host with an e.o.p. of 1. Again, lytic Mu.KF lysates plate on *E. coli* K12, e.o.p. 10^{-1}, and an *E. coli* C, e.o.p. 2×10^{-5}. However, induced Mu.KF lysates plate on *E. coli* K12, e.o.p. of 10^{1}, but these lysates were not observed to plate on *E. coli* C, e.o.p. $<2.5 \times 10^{-9}$. And yet again, lytic Mu.KC'' and Mu.KC''' plate on *E. coli* K12 with an e.o.p. of 10^{-2}-10^{-3}, however induced Mu.KF'' and Mu.KFC''' lysates plate on this host with a e.o.p. of 2.0 - 8.5.

The MH_{4400} strain, *gin*$^{-}$ (albeit leaky), enabled the detection of the role of G inversion during plating Mu onto different hosts. This phage plates with a reduced frequency

when G inversion is involved, e.g. induced MH_{4400}.K plated on *C. freundii* with an e.o.p. of 10^{-3}.

The e.o.p. of induced MH_{4400}.KFC on *E. coli* K12 (4.5×10^{-4}), was observed to be significantly lower than the wild type Mu (Mu*cts*61) on this host (e.o.p. 2.0 - 8.5).

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CHAPTER 1

INTRODUCTION

In the late 1940's and early 1950's *Escherichia coli*, strain W, the Waksman strain, was a widely used organism for metabolic studies. It was an established laboratory strain and was deposited with the American Type Culture Collection (ATCC) as ATCC 9637.

An unusual feature of the Waksman strain became apparent when various laboratory phage failed to propagate freely on this strain. (D.F. Bacon *pers. commun.*) Phages T1-T7, λ and P1 all adsorbed efficiently (with the exception of T4), but plaques did not develop in agar overlays seeded with this *E. coli* strain (Jamieson 1971, Glover and Aronovitch, 1967).

Glover was able to obtain mutants of λ which would propagate efficiently on *E. coli* W. In the course of these experiments, Glover (Glover and Kerszman, 1967) observed that *E. coli* W was lysogenised by a temperate phage, which he designated Wϕ. This phage plates on *E. coli* strain C, and forms λ like plaques, 2-3mm in diameter with turbid centres. The Wϕ phage resembles phages P2 and T1 morphologically (Glover and Kerszman, 1967).

It was demonstrated (Pizer *et al.*, 1968) that phage Wϕ coded for a restriction system, but not for a corresponding modification of infecting phage. This system would be represented either as r^+m° or r^+m^-. This situation was previously thought to be a lethal one; it has often been assumed that a bacterium which can not modify, and thus protect, its own resident DNA will be sensitive to any restriction endonucleases which are encoded by the genome. If however, the genome of *E. coli* strain W does not have

the nucleotide sequence recognised by the endonuclease(s) or, again, if the endonuclease(s) is confined strictly to the periplasm and is absent from the cytoplasm, then the $r^+ m^-$ or $r^+ m^\circ$ genotype may not represent a lethal situation; localisation of the endonuclease(s) to the periplasm may well account for the observations in *E. coli*, strain W.

The observed genetic restriction operating in *E. coli* W is not to be accounted for solely by that coded for by the W prophage (Pizer *et al.*, 1967). Jamieson (1971) in this laboratory provided evidence for two prophages (carried by *E. coli* W) coding for restriction of phage P1 and, in addition for a restriction modification system ($r_w m_w$) coded for by the *E. coli* W genome. This latter system corresponds to the restriction-modification systems carried by other strains of *E. coli* eg. $r_k m_k$ in *E. coli*, strain K12, and $r_B m_B$ in *E. coli*, strain B.

There was however some confusion regarding the plating of the W phage on different hosts. Jamieson set about examining this situation; two distinctive plating patterns emerged during his work.

He plated the supernatant from *E. coli* strain W on *E. coli* strain C and on *E. coli* strain K12, and obtained titres of 10^6 plaque forming units (pfu)/cm³ and 10^5 pfu/cm³ respectively.

The phage that propagates on *E. coli* C, Jamieson designated W.C, the equivalent of Glovers Wϕ. W.C readily adsorbed to *E. coli* C but did not adsorb at a significant level to *E. coli* K12, on which it propagated with an efficiency of plating (e.o.p.) of 10^{-5}.

Once the W.C phage had adsorbed to *E. coli* K12 (now designated W.CK), it was then able to adsorb and propagate

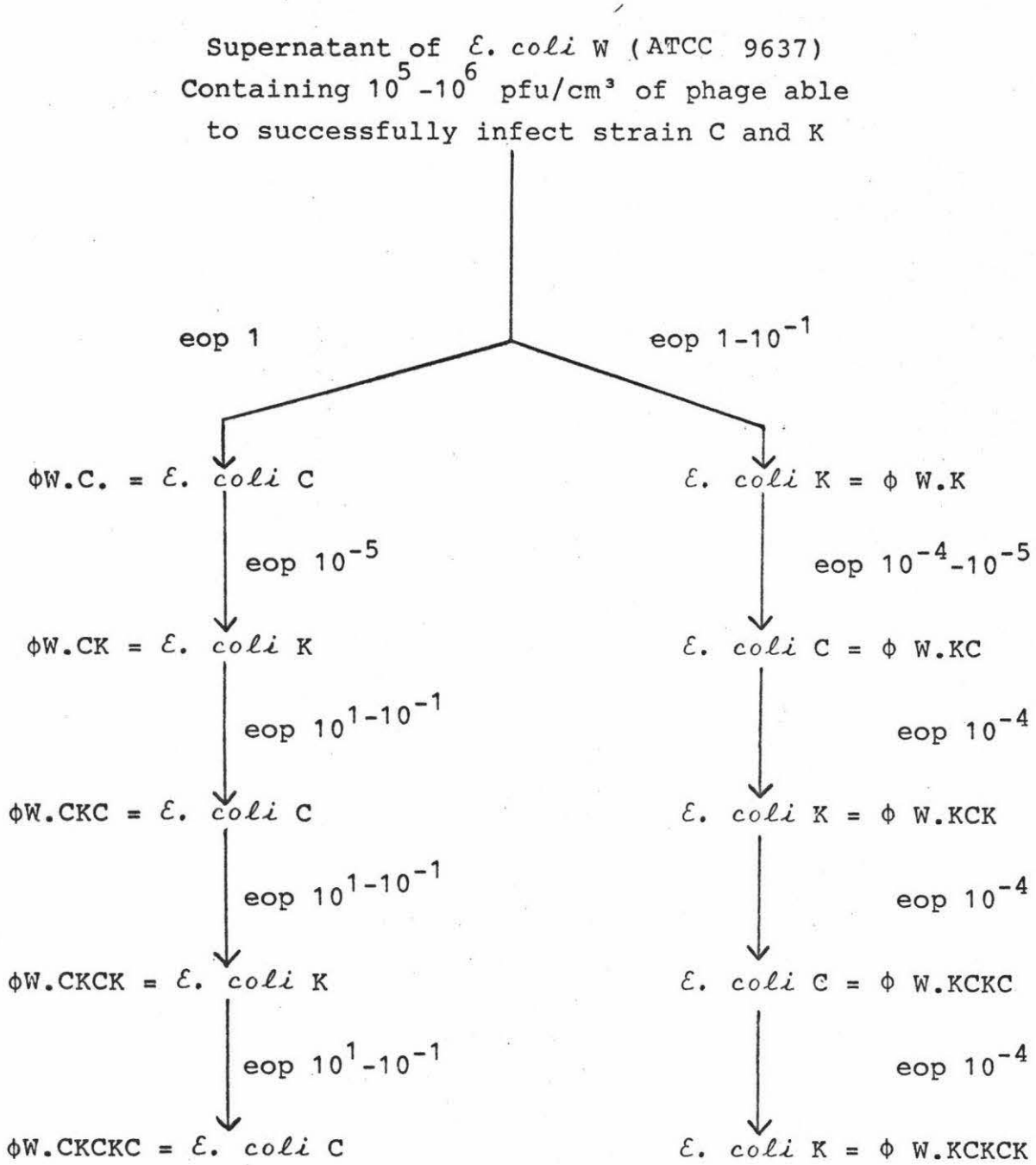


FIGURE 1: Plating efficiencies of phage from the supernatant of *E. coli* W on *E. coli* strains C and K.

Adapted from Jamieson (1971).

on *E. coli* strains C and K12. The e.o.p. for W.CK plating back on *E. coli* C was $1\text{-}10^{-1}$. Here the phage is acting as a typical host range mutant of phage W.C (see Figure 1).

Phage W.K is the phage isolated from the *E. coli* strain W supernatant by plating on *E. coli* strain K12. Glover had at no time plated the *E. coli* W supernatant on *E. coli* K12, and for this reason there is no equivalent to the phage W.K in Glover's work.

The phage W.K was tested in the same manner as for phage W.C; it was propagated on *E. coli* K12, and then the two hosts, *E. coli* K12 and *E. coli* C were challenged. This phage adsorbed only to *E. coli* K12 and not to *E. coli* C. However, it gave plaques on overlays incorporating *E. coli* C with an e.o.p. of $10^{-4}\text{-}10^{-5}$.

The resulting phage, W.KC, was able to adsorb to *E. coli* strain C, but <u>not</u> to *E. coli* strain K12. The e.o.p. for phage W.KC plating on *E. coli* K was approximately 10^{-4} (Jamieson, 1971). (see Figure 1).

In these studies phage W.K acted in a very different manner from phage W.C. Phage W.K adsorbed efficiently and plated on the host on which it was last propagated, but not on the alternate host. This pattern of plating has been referred to as alternating host specificity type behaviour, and is not to be accounted for by two systems of restriction and modification. This led to the proposal that W.C and W.K are two closely related but distinct phage.

The previous plating of W.C seen by Pizer *et al.* (1968) and Glover (Glover and Kerszman, 1967) also fits the patterns observed by Jamieson, and approximate his results.

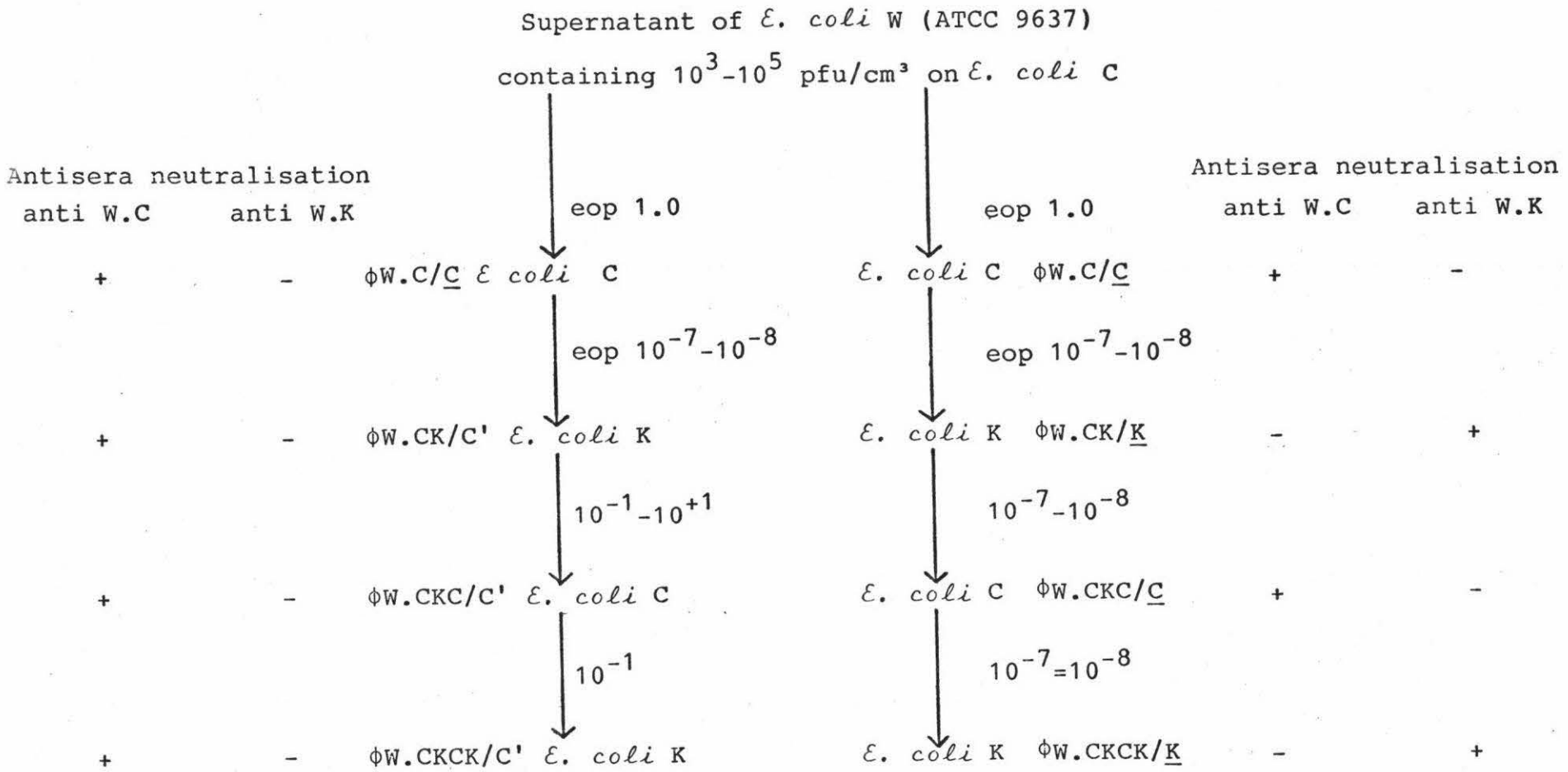


FIGURE 2: The plating efficiency and adsorption of φ W.C on *E. coli* strains C and K. To the left exhibiting the host range mutation plating behaviour, and to the right exhibiting alternate host specificity plating behaviour, as observed by Rice (1980).

The phages W.C and W.K are co-immune, share identical heat sensitivities and morphology, (Jamieson, 1971) and are not inducible by u.v. light (Glover and Kerszman, 1967). The electron micrographs revealed tailed phage resembling phages P1 and T1. The head was spherical, about 60 nm in diameter, the tail approximately 120 nm long and 16 nm in diameter. The sheath when contracted was 26 nm in diameter, and the exposed core 8 nm in diameter. Jamieson went on to map the tentative position of W.C and W.K prophages on the *E. coli* W chromosome.

The plating behaviour of phages W.K and W.C were observed again by Rice (Rice, 1980). The results again approximated those seen by Jamieson. However, it should be noted that the efficiency with which phage W.K would plate on alternate hosts, *E. coli* K and *E. coli* C, was lower than that observed by Jamieson, often 10^{-7} rather than the 10^{-4}-10^{-5} in earlier studies. Van de Putte *et al.*,1980) have also reported variability in e.o.p. values. The reason for this is not clear.

Rice (1980) developed antisera to phage W.C and W.K, in an attempt to identify which tail type specificities ('C' or 'K') the phage were carrying; this was based on neutralisation studies.

When phage W.C exhibited the host range mutant pattern, the phage could only be neutralised by anti W.C serum (not anti W.K serum); this is consistent with the tails being either in the 'C' form or the mutant 'C′' form; the 'C′' form represented the host range mutant (see Figure 2).

However when the phage W.C underwent the alternate host specificity change, then the nature of the tail components changed from C to K and the phage was neutralised by the corresponding K antiserum (see Figure 2). A low level of neutralisation was seen between the K tail of phage W.CK and the anti W.C serum.

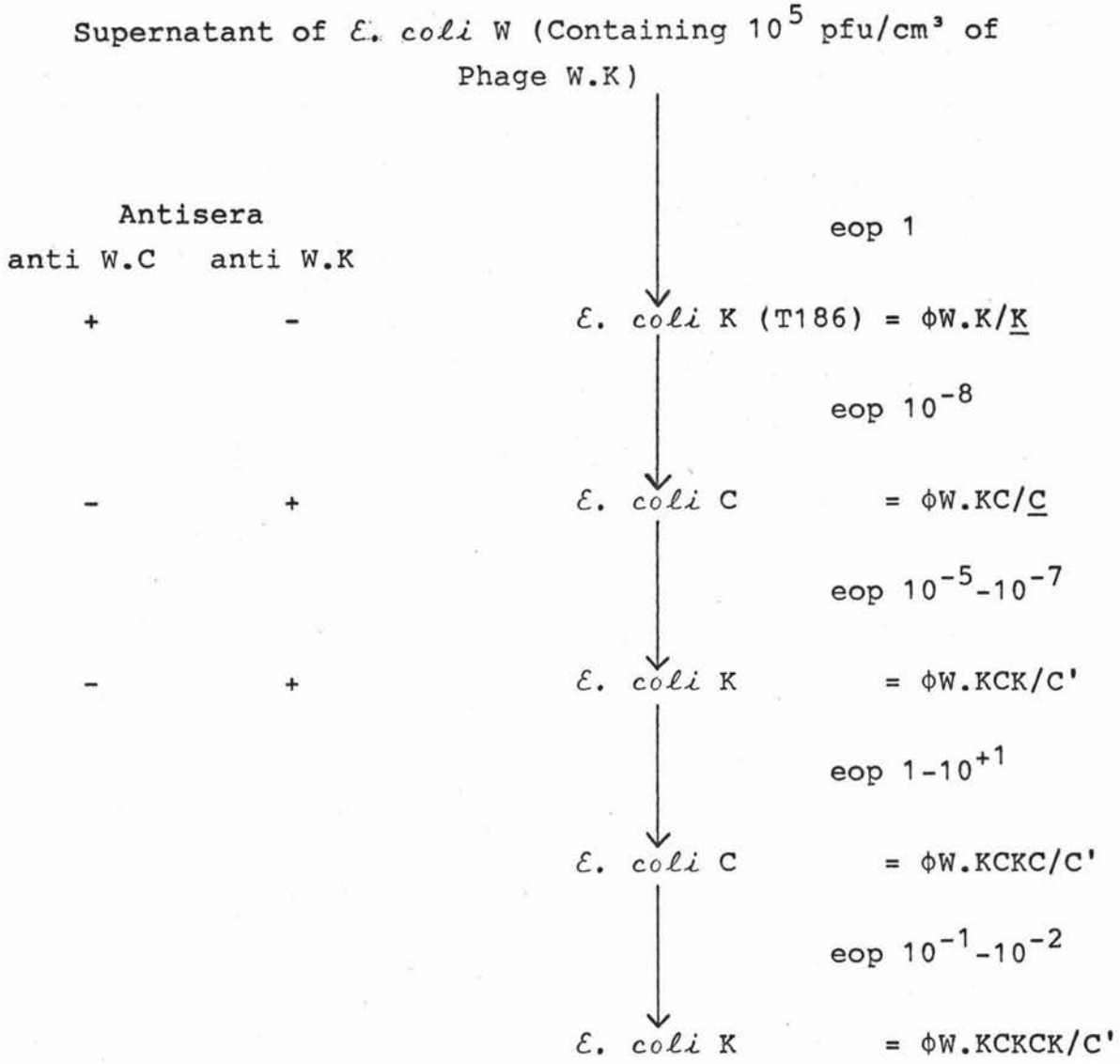


FIGURE 3: The efficiency and adsorbtion of ϕW.K to *E. coli* strains K and C, as observed by Rice (1980)

For the phage W.K, Rice obtained an extended host range mutant with corresponding host range, as well as the previously seen alternating host specificity. The host range mutant isolated from phage W.K was only produced while it was carrying a 'C' tail i.e. W.KCK/C'. This resulted in 'C'' tails being able to propagate on *E. coli* strains C and K12. No isolate was made of a 'K' ' tail type that could propagate on both hosts (see Figure 3).

By using specific antiserum to the 'C' tail form, Rice observed that phage W.KCK/C ' (the host range mutant of phage W.KC) phage W.CK/C' (the host range mutant of phage W.C), and phage W.C. were serologically the same.

In 1963 Taylor reported (Taylor, 1963) a new temperate phage, Mu-1 (now known as Mu). Mu was discovered by chance, during tests for lysogeny in a *E. coli* strain that had been exposed to P1, and surprisingly the plaques that were recovered did not show the morphological characteristics of P1. The phage, Mu, was not neutralised by a P1 antiserum, and was not co-immune to P1. It was noticed that when Mu lysogenised a host cell, the lysogen frequently exhibited mutations. This was thought to be due to Mu integrating into a given gene and rendering it non-functional.

In a research paper by Martuscelli, Taylor *et al.*,(1971) the source of phage Mu is listed as follows:
"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, *E. coli* K12 (strain AB259). Since the phage from ATCC9637 is indistinguishable from the phage Mu-1 originally isolated from a chance lysogen of strain K12, in respect to its morphology, lysogenic immunity, bacterial host range, and ability to induce mutations, we presume that the two phage are closely related and that ATCC 9637 may be the natural reservoir of phage Mu-1".

Phage Mu.K and Phage W.K properties: similarities.

- Temperate phage
- Non-inducible by u/v light
- Propagate lytically on *E. coli* K
- Identical morphology (by E.M.)
- Co-immune
- Exhibit same alternating host specificities and Host range mutation
- *mom* gene function
- Plaque morphology e.g. W.K/$\underline{K}$ = MuK/$\underline{K}$
  W.KC/$\underline{C}$ = MuKC/$\underline{C}$
- Prophage coded restriction
  lysogens *E. coli* C/W.KC and *E. coli* C/Mu.KC are both restricting for phage P_1 v whereas lysogens *E. coli* K/W.K and *E. coli* KC/Mu.K are both non restricting for P_1V.
- Induced phage preparations (from *E. coli* W and *E. coli* K lysogens) can propagate on *E. coli* K and *C. freundii* r^- in approximately equal numbers i.e. G region inversion.

FIGURE 4: A list of properties expressed and shared by phage W.K and phage Mu.K (adapted from Rice, 1980)

The method reported by Martuscelli *et al.* for the isolation of a phage that is, or is closely related to Mu, is the same method followed by Jamieson and Rice to obtain phage W.K (Jamieson 1971, Rice 1980).

This report, taken with the knowledge then known of the morphology of phage W.K and its limited host range, suggested that phage W.K and phage Mu may be closely related if not identical.

This prompted a direct comparison of the two phage, Mu and W.K, to be compiled by Rice (Rice 1980). This also indicated that the two phage are closely related if not identical (see Figure 4).

Phage Mu has been studied by many workers and has proven to be very interesting. This phage resembles P1 and T1 in morphology; the virus is composed of an isocohedral head about 54 nm in diameter, a contractile tail sheath 100 nm long and 18 nm wide, a base plate and at least three tail spikes (To *et al.*, 1966, Martuscelli *et al.*, 1971).

The phage genome appears as a linear double stranded (ds) DNA molecule, 36-38 kilobases (kb) in length corresponding to a molecular weight of 25 x 10^6 Daltons (Martuscelli *et al.*, 1971, Daniell *et al.*, 1973a), and has a base composition of 50% G-C.

The phage DNA integrates into many sites around the host chromosome, often integrating into functional genes causing a mutational effect. Thus Mu integration can induce a bacterial prototroph to become auxotrophic, and require a growth factor.

Two distinctive features of the Mu genome are;

1) host DNA sequences at each end, and
2) the G region.

The terminal host DNA sequences are variable and as a result appear as non-homologous DNA; this generates split ends of Mu DNA in heteroduplex electron micrographs. The host sequences at each end of Mu are shed as the prophage integrates into a new host site. At the C end of the Mu chromosome the host sequence is 0.1 kb in length, and is barely visible in electron micrographs. The c end host sequence does not vary in length. At the S end the host sequence is approximately 1.5 kb, although this varies slightly in length in DNA isolated from wild type phage; but is reduced or increased significantly when a Mu phage genome carries an insertion or deletion respectively.

The host DNA at the S end is thought to reflect packaging of a complete headful, thus ensuring that all the Mu DNA is packaged (Chow and Bukhari, 1977).

The G region is an invertible region of DNA (Daniell *et al.*, 1973a), 2.9 ± .4kb in length and 31kb away from the right (S) end of the phage genome. It is flanked on both sides by an inverse repeat sequence of less than 50 base pairs (bp). The G region codes for S and U gene products (M. Howe, Schumm, Taylor, 1979); these are tail polypeptides of the Mu virion (Giphant-Gassler *et al.*, 1981). The S and U gene products are modified by inversion of the G region. (For a map of Mu see Figures 5 and 6).

The G region is also homologous to the invertible DNA segment in phage P1, the C region (Chow and Bukhari, 1976), although there may be a low level of mismatched base pairs not detected in heteroduplex structures. However, the invertible region of P1 has terminal inverse repeat sequences of 650bp, whereas the G region of Mu has only 50bp terminal inverse repeat sequences.

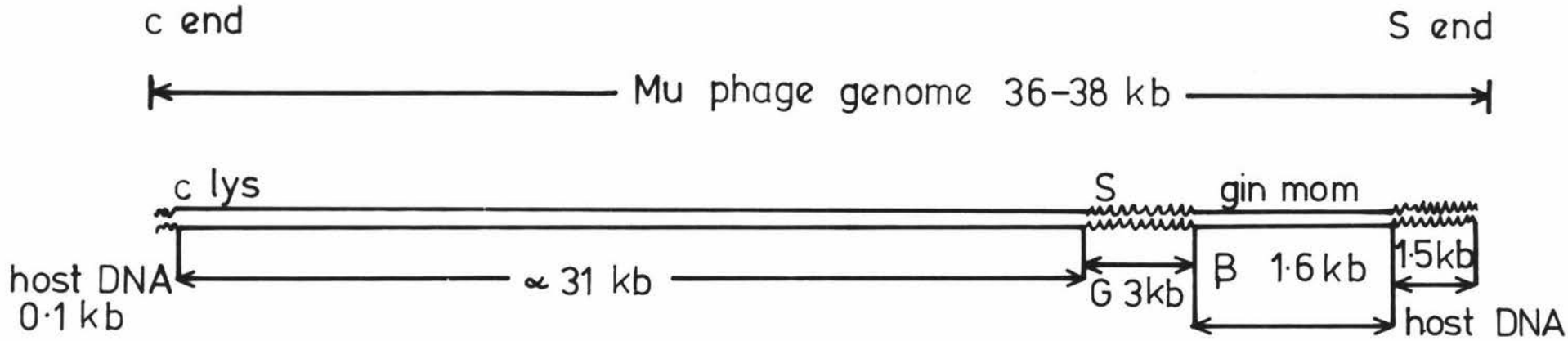
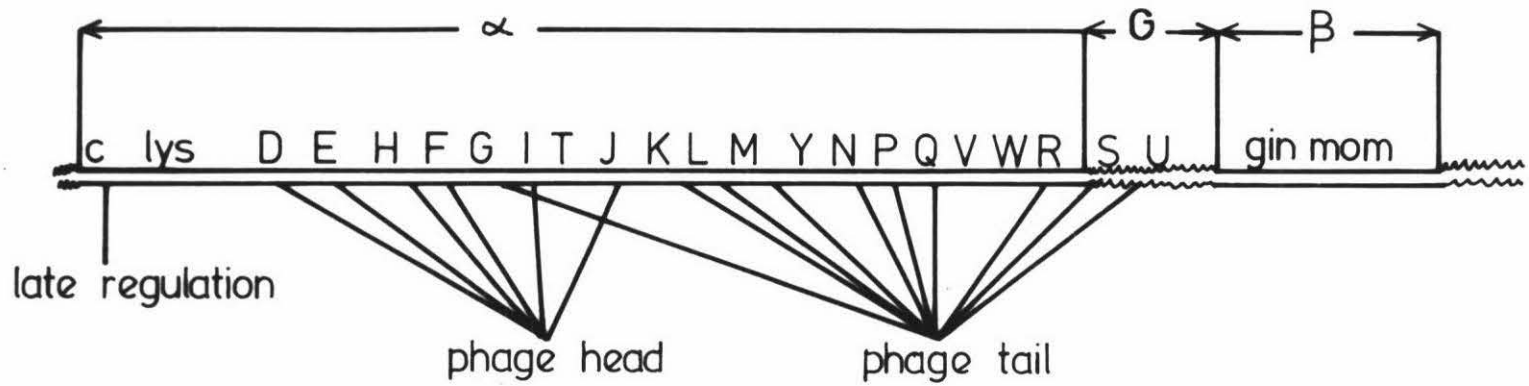


FIGURE 5: Structural map of Mu, adapted from Chow & Bukhari (1977).

c = immunity gene lys = gene for cell lysis

NOT DRAWN TO SCALE



Major head polypeptides

53 54 (F)

Major tail polypeptides

55 (L) Mr 12·5 (Y) m

Molecular weight of minor head polypeptides 64 (M) 30, Mr

units = Mr 29, 25, 21, 14, 8, Mr

Minor tail polypeptides

64, 60 (N), 56 (S), 41·5, 20·5 (U) Mr

All essential genes are in the α region, except S and U.

NOT DRAWN TO SCALE

FIGURE 6: Map of Mu with gene position.
Adapted from Giphart-Gassler, *et al.* (1981).

The inversion of G is thought to occur by recombination between the terminal inverse repeats at either end of the G region (Hsu and Davidson, 1974). The inversion is controlled by a protein, MW 21,500 (Kwoh and Zipser, 1981), the product of the *gin* gene (G inversion); the *gin* gene is located immediately to the right of the G region. Inversion of the G region is thought to be mediated by a phage specific enzyme system, as G inversion of a gin^- mutant of a Mu prophage (with G unable to be inverted) can be mediated by a gin^+ gene carried on a helper prophage (in the trans position) within the same cell (Kamp *et al.*, 1978).

Inversion of the G region can be demonstrated in heteroduplex studies., by the presence of an inversion loop at the G region in place of ds DNA (see Figure 7).

Also, within the G region there is another segment of DNA flanked by inverse repeats (Hsu and Davidson 1974). This inner region is 1 kb in length, including the inverse repeat sequences, although the individual lengths may vary depending on conditions used for spreading the DNA. These 'inner', inverse repeats have a slightly lower stability than the 50bp inverse repeats that flank the ends of the G segment. The inverted repeat sequences (both terminal and 'inner') give rise to inter and intra-strand secondary structures, when single strand or self renatured DNA is under mildly denaturing conditions, as seen in electron micrographs (see Figure 8). However, no evidence has been observed of the g4 (the 'inner') segment undergoing inversion.

The frequency with which the G bubble is observed is therefore a measure of the frequency with which the G region inversion takes place, and is dependent upon the method of phage propagation.

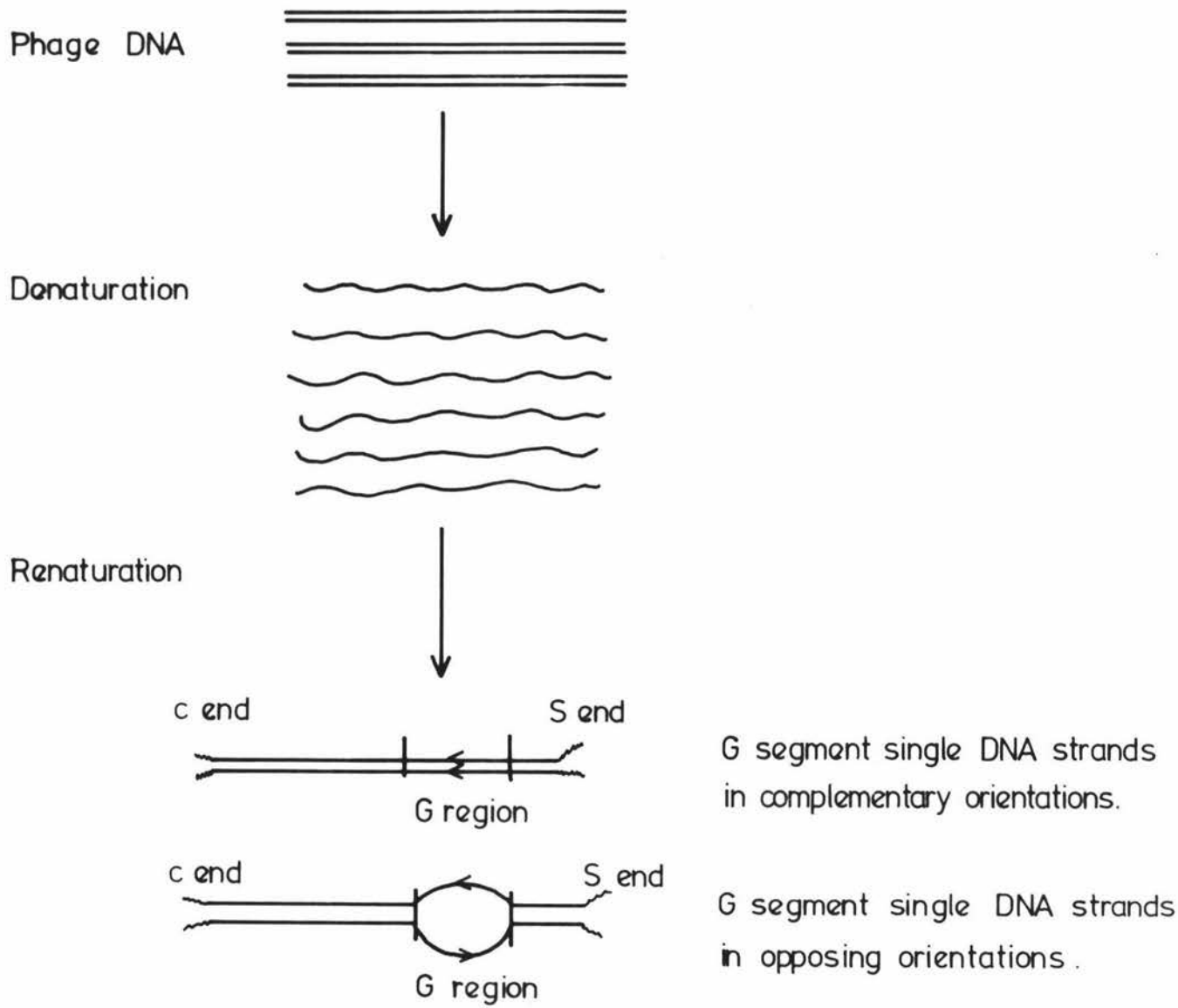


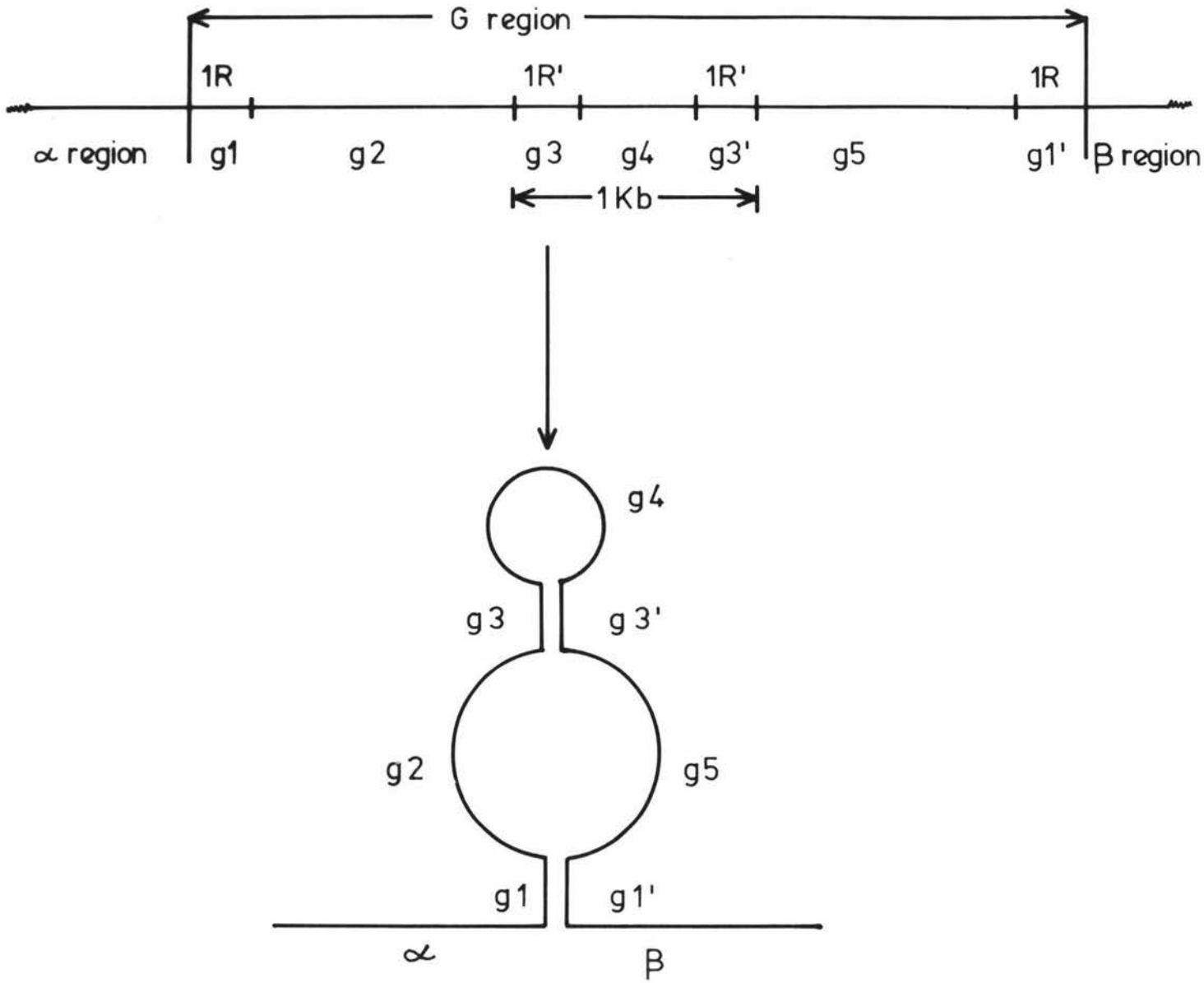
FIGURE 7: The formation of the G bubble in Mu DNA extracts.

FIGURE 8: Structure of the G region, and showing the structure seen under EM when inverse repeat sequences self renature.

g1 and g', g3 and g3' are inverse repeats (IR) sequences

g2, g5, and g3 + g4 + g3' are all approximately 1kb in length.

(Adapted from Chow and Bukhari, 1977)


G region
1R
1R'
1R'
1R
α region
g1
g2
g3
g4
g3'
g5
g1'
β region
1Kb
g4
g3
g3'
g2
g5
g1
g1'
α
β

DNA prepared from phage grown lytically on *E. coli* K12 has a very low frequency of G region inversion. In this case less than 1% of G 'bubbles' will be seen, and therefore in less than 0.5% of the chromosomal DNA has a G region inversion taken place. But when *E. coli* K12, lysogenised by Mu, is induced and the phage examined as before approximately 50% of the phage DNA molecules have the G region inverted (Daniell *et al.*,1973b). (see Figure 9).

Expression of the *gin* gene is no doubt implicated in these two different rates of G inversion. It has been suggested that the promoter for the *gin* gene in the lytic phase may differ from the promoter which is functioning in the prophage phase, and hence a different rate of inversion may be observed (Van de Putte, *et al.*, 1980).

When a Mu lysogen of *E. coli* K12 is induced and the phage plated on *E. coli* K12, not all of the phage are able to initiate plaques. Further, not all of the phage adsorb to *E. coli* K12, and only those that do are able to initiate successful infection, resulting in plaque formation (Kamp *et al.*, 1978).

It was also found that the proportion of Mu with the G(+) orientation was equivalent to the proportion of plaques formed on *E. coli* K12 as compared to the number of phage plated. That is, those phage with G in the (+) orientation were the phage producing infective centres on *E. coli* K12, while the phage with the G(-) orientation did not infect this bacterium, due to non-adsorption (Symonds and Coelho, 1978, Bukhari and Ambrosio, 1978). If the orientation of the G region affects the viability of the phage, it appears to be at a biological disadvantage to have a mechanism that allows only 50% of the phage to be viable i.e., G(+) phage.

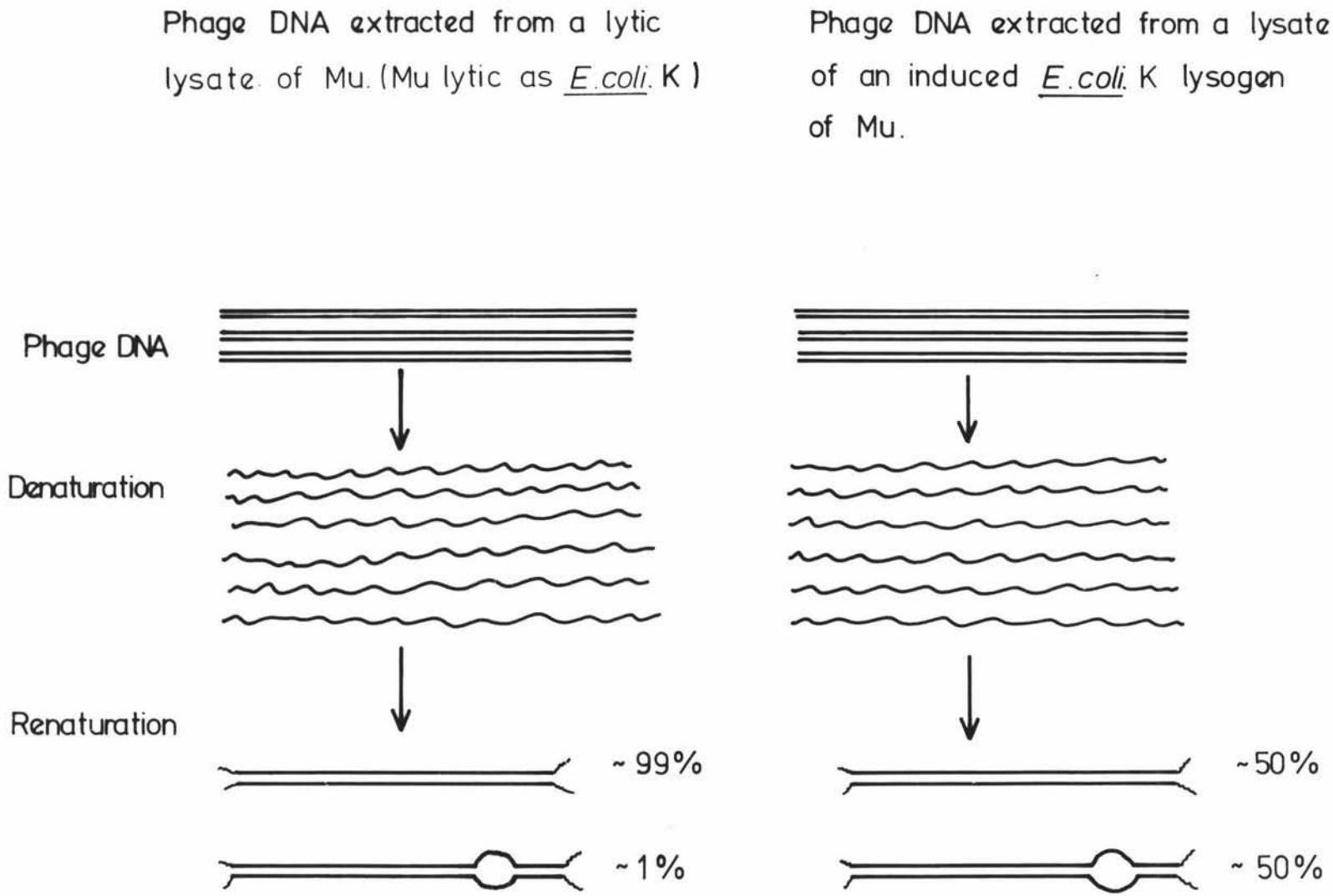


FIGURE 9: The G inversion rate due to mode of phage propagation. Adapted from Chow and Bukhari, 1977.

	Phage	Mode of Propagation	Host	Efficiencies of Plating on				
				E. coli K12(r^-)	*E. coli* K12(r^+)	*C. freundii* (r^-)	*S. sonnei* (r^+)	*E. coli* C(r^-)
1)	Muc2000	lytic	*E. coli*	1	1	2.5×10^{-9}	$< 10^{-9}$	$< 10^{-9}$
2)	Muc*ts*62	induced	*E. coli* K12	1	1	1	$(10^{-6})^+$	$(4 \times 10^{-5})^+$
3)	Muc*ts*62*gin*$^-$	induced	*E. coli* K12	1	1	$< 10^{-9}$	$< 10^{-9}$	$< 10^{-9}$
4)	Muc2000	lytic	*C. freundii*	2.6×10^{-3}	2.0×10^{-6}	1	8.5×10^{-3}	7.5×10^{-2}

TABLE I: Plating efficiencies of Mu phage lysates on different hosts.
Adapted from Van de Putte, Cramer, Giphart-Gassler (1980)

\+ variable efficiencies

Van de Putte (1980) suggested that the orientation of G determines the host specificity, i.e., the host to which Mu can adsorb, and not the viability of the phage. Thus the Mu phage with the G(-) orientation are not defective and non-viable, but have a host specificity other than *E. coli* K12; they are able to adsorb to and propagate on an alternative host. Van de Putte established that G(-) Mu phage could, in fact, propagate on a restriction-less (r-) strain of *Citrobacter freundii* (G331), and that this host strain is not sensitive to G(+) Mu phage; this form is the predominant product of lytic growth on *E. coli* K12. Mu phage propagated lytically on *E. coli* K12 plated on *C. freundii* with and e.o.p. of 2.5×10^{-9}. However when a Mu lysogen of *E. coli* K12 was induced and the resultant phage plated on *C. freundii* r^-, the e.o.p. is now approximately 1, consistent with a 1:1 ratio of G(+) and G(-) phage types (see Table I lines 1 and 2).

Van de Putte also induced a Mu*cts* phage that was defective in the *gin* gene, i.e., stable in the G(+) state. If the G region failed to invert, no G(-) phage would be produced and therefore no phage would be expected to propagate on *C. freundii*. The phage obtained by induction of a Mu*ctsgin*- lysogen plated on *E. coli* K12 with an e.o.p. of 1, and plated on *C. freundii* (r-) with an e.o.p. $<10^{-9}$ (line 3), thus again supporting Van de Putte's model.

Other bacterial species and *E. coli* strains were tested for their sensitivity to G(+) and G(-) phage. (Van de Putte *et al.*, 1980, Kamp, 1981). Van de Putte found that *S. sonnei* and *E. coli* C(see line 5, Table I) were sensitive to Mu propagated lytically on *Citrobacter freundii*, and therefore were sensitive to G(-) phage. Kamp found *Serratia marcescens* and *Enterobacter cloacae* allowed plaque formation of G(-) phage, but are insensitive to infection by Mu G(+). Kamp also noted that purified lipopoly-saccharide of *E. coli* K12 caused G(+) phage particles to lose their infectivity, (that is, the G(+) are able

Phage	Antisera		Host plated on
	Anti W.C	Anti W.K (MuK)	
MuKF.(lytic)	-	-	*C. freundii*
Phage MuK lytic	-	+	*E. coli* K-12 (PB1395) r^-
Phage Mu.KC/C (lytic)	+	-	*E. coli* C
Phage MuKFK (lytic)	-	+	*E. coli* K-12 r^+(PB1395)

TABLE II: Phage neutralisation of the 'K', 'F' and 'C' tail types with anti W.C and anti W.K sera (Rice 1980)

to adsorb to the lipopolysaccharide of *E. coli* K12), while the G(-) phage are unaffected. Conversely, the lipopolysaccharide of a strain of *E. cloacae* inactivated G(-) phage. This supports the view that the G(-) phage adsorb to this strain of *E. cloacae* and not to *E. coli* K12.

However, the plating of G(-) phage on these hosts is not well defined. The e.o.p. of Mu G(-) on *E. cloacae* (10^{-3}), *S. marcescens* (5×10^{-3}), *S. sonnei* (8.5×10^{-3}) and *E. coli* C (7.5×10^{-2} Van de Putte, 10^{-5} Rice) are relatively low compared to the e.o.p. on *C. freundii* (e.o.p. 1).

The lower e.o.p. on *E. coli* C than on *C. freundii* cannot be due to restriction, as *E. coli* C is naturally restrictionless. This implies that there may be another mechanism involved that contributes to the plating of Mu on yet other hosts that is still not defined.

Using antisera to W.K (Mu.K) and W.C. phage, Rice (1980) presented evidence that the 'F' tail form (the tail form that adsorbed to *C. freundii*), was substantially different from the 'C' and 'K' tail forms. He reported that the 'F' tail was not affected by the anti WC or anti W.K (Mu.K) sera, where as Mu with the 'K' tail form was neutralised by anti W.K (Mu.K) sera, and Mu with the 'C' tail form was neutralised by the anti W.C. serum (see Table II).

Rice also demonstrated that a complete tail change was associated with the acquired ability of Mu.KF to propagate on *E. coli* K12, i.e. Mu.KFK phage; the 'K' tail on these phage was totally different from the F tail (see Table II).

These observations led to the proposal that a change from G(+) to G(-) is not, in itself, sufficient to permit propagation on *E. coli* C.

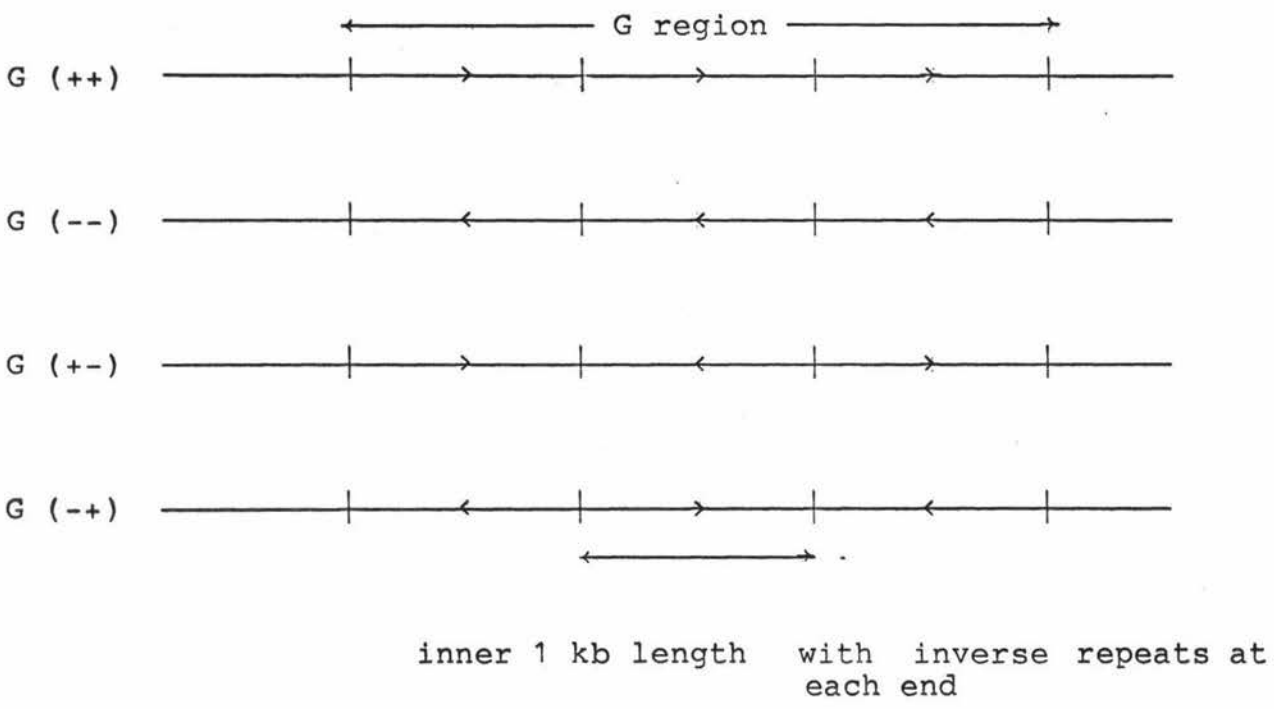


FIGURE 10: The possible orientation of the G region and the inner 1 kb region flanked by inverse repeat sequences (as proposed by Rice 1980)

It has been assumed, as a first assumption, that tail changes observed in phage Mu, i.e., between the 'K', 'F' and 'C' forms, are all somehow related to the G region. The changes from the 'K' tail to the 'F' tail form and vice versa are effected by inversion of the active G region.

Rice noted phage Mu coding for the 'F' tail structure was able to plate on *E. coli* C at a higher frequency (e.o.p. 10^{-5}) than Mu.K phage coding for the K tail. Based on these observations, Rice developed a model involving the 1kb region in the middle of the G region that is flanked by inverse repeat sequences. If this region inverted independently of the external G region, this might account for a third and possibly a fourth tail type of phage Mu thus enabling Mu to adsorb to 3 or 4 different hosts, one for each tail type (see Figure 10).

In the further development of this (Rice's) model, phage Mu.K adsorbing to *E. coli* K12 can be represented as G(++) and, as the phage Mu.KF (G(--)) adsorbs to *E. coli* C at a greater frequency than the phage Mu.K (G(++)), Rice proposed that a further event is required to plate the Mu.KF phage G(--) on *E. coli* C; this, according to Rice, could involve the 1kb central region inverting to give a G orientation of G(-+). This then raises the possibility of a fourth host sensitive to Mu phage with the G orientation G(+-). However, to date there is no evidence for the inversion of the 1kb region within phage Mu.

Rice examined the level of neutralisation of a Mu.KFC lytic preparation of his isolate using the anti W.C serum. Surprisingly only 20-25% of the phage were neutralised in 10 minutes at 37° against the expected 90%; this is equivalent to a relative neutralising activity of 0.1-0.12 (see Table III).

Phage	Anti W.C. serum	Host plated on
MuKFC	20-25% (1.0-1.2 relative neutralising activity)	*E. coli* C (518c)

TABLE III: Neutralisation of phage MuKFC with anti W.C serum (Rice 1980)

The orientation of the G region may have more than just an effect on the tail types of the Mu phage (Toussaint, 1976, Van de Putte *et al.*,1980). As lytic Mu.KF phage lysates (propagated on *C. freundii*), plate on the restrictionless *E. coli* K12 with a high e.o.p. (2.6×10^{-3}) compared to lytic phage Mu.K plating on *C. freundii* r^- (e.o.p. 2.5×10^{-9}). Thus in contrast to the lytic propagation of Mu G(+) phage on *E. coli* k12, the G region is inverting more frequently when Mu is propagated as a G(-) phage on *C. freundii* (see p. Table I, lines 1 and 5).

The function of the *mom* gene (modification of Mu) does not operate efficiently when Mu is grown lytically on *C. freundii* . This results in the lytic phage Mu.KF (propagated on *C. freundii*) plating on *E. coli* K12 r^+ with an e.o.p. of 2.0×10^{-7} , and this phage plating on *E. coli* K12 r^- with an e.o.p. of 2.6×10^{-3} (see p Table I line 5). Thereby indicating that the general modification of Mu has not been expressed (Van de Putte *et al.*,1980). The observations reported by Rice (1980) approximate those observed by Van de Putte.

Aims of the Investigation

From the observations made by Van de Putte (1980), there are at least 2 different host specificity types controlled by the G region; these are;

1) G(+) that adsorbs to and infects *E. coli* K12, and

2) G(-) that adsorbs to and infects a strain of *C. freundii*.

However earlier work by Jamieson (1971), and more recently by Rice (1980), has indicated that phage Mu adsorbing to and infecting *E. coli* C has a different host specificity from those Mu phage adsorbing to *E. coli* K12 or *C. freundii*.

It was the aim of this study to investigate the feasibility that there exists at least three host specificity phenotypes; these being:

1) G(+) that adsorbs to and infects *E. coli* K12,

2) G(-) that adsorbs to and infects *C. freundii* and

3) a third host specificity type that adsorbs to and infects *E. coli* C.

In doing so, our objectives were;

1) To isolate a Mu*gin*$^-$ G(-) phage for use as an antigen for the production of a specific anti Mu.F G(-) serum.

   This would allow phage Mu to be neutralised by specific antisera to the three tail types K, F and C. (The anti Mu.K serum and the anti W.C serum had been produced by Rice).

2) To isolate Mu.KC phage as observed by Rice and Jamieson, not by the use of lytic Mu.K lysates but by the use of heat inducible *E. coli* K12 (Mu) lysogens;

this was prompted by the ready production of high titre lysates of G(+) phage by induction of lysogens.

3) To isolate phage Mu.KFC, as observed by Rice (1980), by plating Mu.KF lysates onto *E. coli* C.

4) To study phage Mu.KC and Mu.KFC, on the basis of their plating ability on various hosts and the neutralisation of the phage by specific antisera (anti Mu.K, anti Mu.F and anti W.C sera).

5) To attempt to gain insight into the functions of the G region in plating phage Mu onto *E. coli* C.

CHAPTER 2

MATERIALS AND METHODS

2.1 Media and Solutions

Lennox Broth (L.B.)

Bacto-tryptone	10g
Bacto-yeast extract	5g
NaCl	10g
Distilled water	1 litre

The medium was adjusted to pH 7.5 before autoclaving.

Modified Lennox Broth

$$LBMC = LB + Mg60_4(2 \times 10^{-3}M) + CaCl_2\ (2 \times 10^{-3}M)$$

Lennox Plate Agar

L.B.	1 litre
Agar (N.Z. Davis)	10g

20 cm^3 of Lennox plate agar was dispensed per plate to form the basal layer for a bacterial overlay.

Lenox Soft Agar

L.B. XX (double strength) 50cm^3

1.0% Agar (Gibco diagnostic agar, purified Driform) in 50 cm^3 distilled water

The broth warmed to 55°C, the agar melted, the broth and the agar combined aseptically. Final concentration of agar 0.5%.

BHI (Brain Heart Infusion) Broth

Bacto-Brain Heart Infusion	37g
Distilled water	1 litre

Dispense as required and autoclave

BHI Agar

BHI broth	1 litre
Agar (N.Z. Davis)	10g

Autoclave and dispense 20cm^3 per plate.

Minimal Medium

K_2HPO_4	10.5g
KH_2PO_4	4.5g
$(NH_4)_2SO_4$	1 g
Sodium Citrate.$2H_2O$	0.5g
Distilled water	1 litre

Mix above compounds and autoclave

Glucose	4cm^3 of sterile 50% w/v solution added after autoclaving
$MgSO_4.7H_2O$	1cm^3 of sterile 20% w/v solution added after autoclaving

Mineral Agar

Minimal Media	1 litre$^+$
Agar (NZ Davis)	12g

+ Glucose and Mg $SO_4.7H_2O$ were added after autoclaving, double strength minimal broth and double strength agar in distilled water were autoclaved separately. Equal volumes of broth and agar were mixed while hot, and 20 cm^3 dispensed per plate.

If required further additions were either rubbed into the plates, or added aseptically after autoclaving and before dispensing the medium.

Further additions	Final concentration
Streptomycin	100 µg/cm^3
Naladixic acid	50 µg/cm^3
Vitamins (B_1 etc)	1 µg/cm^3
Amino acids	50 µg/cm^3

Tetrazolium Solution

Triphenyl tetrazolium chloride	0.3g
NaCl	0.8g
KCl	0.04g
Distilled water	100cm^3

This solution was autoclaved at10 lb/10 minutes, and stored in the absence of light; The solution was incorporated into media as detailed elsewhere (Method 1).

S Buffer

NaCl	5 g
Tris. HCl	2.7g
Trizma base	0.32g
Deionised water	1 litre

Citrate Buffer (0.1M Na Citrate pH 5.5)

Citric acid	10.5g
NaOH	4.4g
distilled water	500cm³

adjusted to pH 5.5 with 2N NaOH

Phosphate Buffer (0.1M phosphate buffer pH 7.0)

KH_2PO_4	6.8g
NaOH	1.16g
distilled water	500cm³

adjusted to pH 7.0 with 2N NaOH.

Saturated Nitrosoguanide solution

1 crystal of N-methyl-N'-nitro-N-Nitrosoguanide+ (NG)

Citrate buffer (0.1M Na citrate pH 5.5) 5 mls

The crystal of NG was left overnight in the citrate buffer in absence of light, to dissolve. This gives a saturated solution of approximately 1mg NG/cm³

+ a strong carcinogen: - gloves must be worn, no mouth pipetting.

.85% Saline solution (Physiological saline)

NaCl	8.5g
distilled water	1 litre

Dispense and autoclave.

2.2 Methods

1) Phage Titration

i) Tetrazolium soft agar overlay

The soft agar overlay, consisting of the following, was prepared at 55°C:

Lennox soft agar (.5%)	2.5cm^3
tetrazolium solution	0.5cm^3
$MgSO_4$ (5 x 10^{-2}M)	0.1cm^3
$CaCl_2$ (5 x 10^{-2}M)	0.1cm^3

When used approximately 0.1cm^3 (2-3 drops from a pasteur pipette) of fresh seed bacteria and 0.1cm^3 of an appropriately diluted phage lysate was added, mixed by vortex, and poured into a fresh Lennox Broth plate. The fresh plate ensures good bacterial lawn and plaque formation.

Fresh seed bacteria are prepared by dilution of an overnight culture grown in BHI broth with an equal volume of fresh BHI broth, and incubated 1 hour at 37°C before use.

The tetrazolium is colourless initially but is reduced to the red formazan form by the growth of the seed bacteria in the overlay. The plaques are visible as pale areas surrounded by a red bacterial lawn. This method allows for small plaques to be resolved which would normally not be seen, or seen with great difficulty.

ii) Phage titration that allows 0.8cm^3 of phage lysate to be sampled

When phage Mu*cts*62 was grown at 42°C we found the plaques to be easily seen and thus easily countable. This permitted us to omit the tetrazolium solution and increase the volume of the phage lysate without destroying the soft agar overlay.

The same basic method as for the tetrazolium soft agar overlay was used except that the 0.5cm³ of tetrazolium solution was omitted, and 0.8cm³ of undiluted phage lysate was incorporated.

2) Preparation of Phage Lysate Stocks by Lytic Propagation

i) Tube method

A plaque was picked, or 0.1-0.4 cm³ of lysate containing 10^7-10^8 pfu/cm³ was used, and added to 0.3cm³ of fresh bacterial cells. The latter was prepared by diluting an overnight culture of bacteria grown in LB 1:1 with fresh medium, LBMC, and incubating for 1 hour at 37°C.

The phage/bacterial cell mixture was vortexed and then held for 15 minutes at 37°C to allow adsorption to occur, 6.0cm³ of fresh medium (BHI broth) prewarmed to 37°C was added. Incubation continued at 37°C under gentle aeration until clearing (lysis) occurred, generally in 2-4 hours.

A few drops of chloroform was added, the culture vortexed, after vortexing again, the culture was transferred to a tube for centrifugation (sorvall type A bench centrifuge) and held 1 minute at 3,400 x g. The supernatant was removed and stored at 4°C.

Prior to use, the sample of lysate required was aerated for 10 minutes at 37°C to remove dissolved chloroform.

ii) Plate method

A plaque was picked by pasteur pipette, and added to .3cm³ of BHI broth, vortexed, and spotted by pasteur pipette onto a soft agar overlay containing the appropriate seed bacteria. The plate was then incubated (lid uppermost to avoid the spot running) overnight at 42°C.

The next day this was repeated, sampling from the area of phage propagation. This was repeated until an area of confluent lysis was obtained. From this area of confluent lysis a large amount of the soft agar overlay was removed by pasteur pipette and added to .5cm³ of BHI broth and vortexed.

This suspension was diluted, and from each dilution 0.1cm³ was added to a soft agar overlay containing the appropriate seed bacteria and poured onto a fresh LB plate. This was done in duplicate or triplicate.

To the plates that gave confluent lysis, usually within 5-6 hrs., 2.5cm³ of BHI broth was added. The soft agar overlay was broken up, and the resultant suspension removed, vortexed, then centrifuged at 3,400 x g (sorvall type A bench centrifuge) for 10 minutes.

The supernatant (lysate) was removed and used immediately (the lysate usually contained 10^8 -10^{10} pfu/cm³). Alternatively a few drops of chloroform was added. The lysate was then vortexed and allowed to stand at 37°C for 10 minutes before storing at 4°C.

Before use the dissolved chloroform was removed from a sample by aeration at 37°C for 10 minutes, or by dilution.

3) Heat Induction of Temperature Sensitive Lysogens

One loopful of a temperature sensitive lysogenic culture was added to 5cm³ of BHI broth. The broth was then vortexed and from this one loopful was used to inoculate 1cm³ volumes of BHI broth. These 1cm³ volumes were then incubated overnight at 33°C.

The following day, each 1cm³ volume of broth was added to 1cm³ of fresh BHI broth, prewarmed to 55°C, shaken and moved immediately to a 44°C water bath for 20 minutes (The tube now contains 2cm³ of broth culture). The cultures were then transferred to a 37°C water bath and diluted 1:1 with fresh BHI broth, prewarmed to 37°C (giving 4cm³ in total).

The tubes were shaken occassionally for aeration and watched for lysis. Lysis within 1 hour usually gave a titre of 10^8 -10^{10} pfu/cm³.

The lysate was either used immediately, or transferred to a tube for centrifugation (sorvall type A bench centrifuge) and held at 3,400 x g for 10 minutes. The supernatant (lysate) was removed and a few drops of chloroform added; the lysate was then vortexed and allowed to stand at 37°C for 10 minutes before being stored at 4°C.

Prior to use the sample was aerated for 10 minutes at 37°C, or diluted to remove dissolved chloroform.

4) N-methyl-N'-nitro-N-nitrosoguanide (NG) Treatment of Q1 (Mu*cts*61) and Q1 (Mu *cts*62)

An overnight culture of either Q1 (Mu*cts*62) or Q1 (Mu*cts*62), grown in BHI broth was diluted 1:1 in fresh BHI broth, and incubated for 1 hour at 30°C.

The culture was spun down (Sorvall type A bench centrifuge) at 3,400 x g for 10 minutes and washed twice in .5M Na citrate buffer pH 5.5 (equal volumes) and resuspended in an equal volume of Na citrate buffer. From this cell suspension 0.05cm³ was taken and added to 2cm³ of fresh .5mg/cm³ NG solution (this is the concentration of NG that gave 90% kill of Q1 (Mu*cts*61) in 30 minutes).

This NG solution with cells was incubated at 30°C for 30 minutes. Following this the cells were spun down (Sorvall type A bench centrifuge) at 3,400 x g for 10 minutes washed once, and resuspended in an equal volume of phosphate buffer pH 7.0; this eliminates the NG. 0.2cm³ of the treated cell suspension was added to a series of test tubes each containing 1.8cm³ of BHI broth. These cultures were grown overnight at 30°C. The overnight cultures were diluted and plated for single colonies.

5) Preparation of Phage Lysate Stocks for Antigens Use (M. Rice)

The procedure for lytic propagation by the plate method was followed to obtain lytic high titre phage lysates. The Mu phage, $MH_{4400}gin^{-}G(-)$ propagated on *Citrobacter freundii* with a titre of 7.5×10^{8}, and was not observed to plate on *E. coli* K12 r^{-}, and thus had an e.o.p. on this host of $< 1.3 \times 10^{-9}$. The Mu phage JP_2 had a titre on *C. freundii* of 4×10^{9} and did not plate on *E. coli* K12 r^{-}, in keeping with the JP_2 phenotype.

Once the supernatant was harvested it was immediately transferred to tubes for ultracentrifugation. A sample of lysate, 6.0cm³ was spun in the Beckman model L2-6SB ultracentrifuge at 30,000 rpm for 2 hours (55,000 x g). This pelleted the phage.

The supernatants were removed and each phage pellet resuspended in 2.0cm³ of S buffer.

The lysates were centrifuged once more, their supernatants removed and each phage pellet resuspended in 2.0cm³ of S buffer, and stored at -20°C. These preparations were used as antigens in the immunization of rabbits.

6) Preparation of Antisera (M. Rice)

From the above procedure (5) 0.4 cm^3 of phage suspension was added to 0.6 cm^3 of Freunds complete adjuvant, emulsified and injected intra-muscularly into a rabbit.

After 4 weeks, a second injection was made of 0.4 cm^3 of antigen (phage lysate alone). This injection was repeated after one more week. Five days later the rabbits were bleed from the ear veins, yielding between 5 to 15 cm^3 of blood. The blood was allowed to coagulate, and the serum harvested (about 3-10 cm^3) and stored at -20°C in 2.5 cm^3 aliquots.

The rabbit was again injected with 0.4 cm^3 antigen phage lysate alone, one week later. Five days after this the rabbit was bled again, for a second bleeding, and again the blood was allowed to coagulate, the serum harvested, and stored at -20°C in 2.5 cm^3 aliquots.

7) Phage Neutralisation Test (M. Rice)

The antisera were diluted in BHI broth to yield serum dilutions which gave 90-95% neutralisation of the reference homologous phage in 10 minutes at 37°C.

To .9 cm^3 of the diluted antiserum 0.1 cm^3 of the phage lysate (concentration approximately 10^6 pfu/cm^3) was added. Both the antiserum and the phage lysate were prewarmed to 37°C. A 0.1 cm^3 sample was removed at 0 minutes and again at 10 minutes. These samples were immediately diluted in 9.9 cm^3 of BHI broth, at room temperature, to prevent further neutralization occurring. The tubes were then titrated.

8) Determination of Phage Neutralisation Kinetics

The procedure above (7) was followed. In addition to the 0 and 10 minute samples, samples were also taken at 5, 15, 20, 25, 30 minutes and 1 hour, often using 0, 10, 20 and 30 minutes only. The results were plotted on a semilog graph as percentage survival versus time. Depending upon the circumstances, the serum dilution was sometimes increased or decreased by a factor of two.

9) Isolation of Lysogens (Muc*ts*61, Muc*ts*62 and MH_{4400})

A plaque was picked, and added to 0.3 cm^3 of BHI broth. This was then vortexed, and spotted by pasteur pipette onto a soft agar overlay containing the appropriate seed bacteria, these plates were incubated overnight at 30°C (lid uppermost to avoid the spot running).

From the area within the spot, a loopfull of the soft agar overlay was taken and streaked onto a BHI plate, which was then incubated at 30°C. Single colonies were tested for the presence of phage, on the basis of their heat sensitivity; in these instances the phage carried a c temperature sensitive mutation.

A colony was assumed to be lysogenic if growth was observed at 30°C, while at 42°C no growth or very little growth was observed.

10) Acridine Orange Treatment (Curing of F')

1 cm^3 of acridine orange was added to 50 cm^3 of LB (pH 7.6 before autoclaving) to give 100 µg/cm^3. 5cm^3 of the 100 µg/cm^3 acridine orange containing broth medium was inoculated with the appropriate F' plasmid bearing bacter-

ium and incubated overnight in the absence of light. The inoculum being 0.1 cm^3 of a 10^{-4} dilution of an overnight culture.

The following day the acridine orange cultures were checked for growth, if good growth was present the broth was diluted 10^{-6} and 0.2 mls of this dilution was plated onto BHI agar.

2.3 Bacteria and Bacteriophage

Bacteria

Substrain		Genotype	Source
Escherichia coli Strain C	518c	*wild type*	Arber
Escherichia coli Strain K12	PB1395	$r^-_k m^+_k$ *Sup* ε_{44} *Sup F* F^-	P. Bergquist
	PB1390	F^- *his* Δ*gal* str^r (*P1 chl*^{r}) Cm^r *at 32°* CM^S *at 42°*	P. Bergquist
	Q1	F^- *thr*$^-$ *leu*$^-$ *tonA lac*$^-$ *SuII*$^+$ (*Mucts61*)	M.M. Howe
	Q1	F^- *thr*$^-$ *leu*$^-$ *tonA lac*$^-$ *SuII*$^+$ (*Mucts62*)	M.M. Howe
	CSH50	*ana*$^-$ Δ(*lac pro*) B_1^- sm^r	J.H. Miller (Cold Spring Harbor)
	CSH55	Δ(*lac pro*) *SupE NalA* B_1^-	Coelho
	Bu260F'	*F' pro*$^+$ *lac z::Mucts62*/ Δ(*pro lac*) *thy*$^-$ Mu^r	Bukhari

CSH55F'	*F' pro⁺ lacz::Mucts62/*	Coelho
	Δ(pro lac) SupE Nal^r	
	B1⁻ Mu^r	
MH_{4400}	*F⁻ thy⁻ A λ^r mal⁻*	
	(mucts62 G⁺ gin^r II)	M.M. Howe

Escherichia coli

Strain W F-2-3-27	*met⁻ his⁻ Hfr (W.C)*	D.F. Bacon
	derivative of E. coli W,	
	(ATCC9637)	
	lacking the prophage W.K	

Citrobacter freundii

G331	$r^-_F M^+_F$ *arg-1 trp-1 Nal-1*	P. Van de Putte

Bacteriophage

Mu*cts*61 and Mu*cts*62 isolated from *E. coli* strain K12 (Q_1, see above) heat induced lysates plating on *E. coli* K12.

Mu*cts*62*gin*⁻ isolated from *E. coli* strain K12 (MH_{4400}, see above) heat induced lysates plating on *E. coli* k12.
(This phage is referred to as the Mu mutant MH_{4400} in the text.)

W.C isolated from *E. coli* strain W (F-2-3-27, a derivative of ATCC9637, see above) plating the supernatant on *E. coli* strain C.

P1 (chl^r) isolated from *E. coli* strain K12 (PB1390, see above) plating the supernatant on *E. coli* strain K12.

CHAPTER 3

THE PREPARATION OF A Mu.KF ANTISERUM

3.1 Isolation of Mutants of Phage Mu *cts*61 with the G Region Fixed in the (+) or (-) Orientation

3.1a Introduction

In this laboratory (Rice, 1980) had initiated a serological study of phage Mu and phage W.K host range types: he had produced antisera against phages Mu.K and W.K and also against phage W.C; this latter antiserum neutralised Mu.KC and W.KC forms as efficiently as it did the W.C. phage.

Lacking in this work was an antiserum specific for either the Mu.KF or the W.KF phage, i.e. for the phenotype corresponding to the G(-) genotype.

In this study and also in Rice's work evidence has been obtained that, whereas in lytic propagation of phage Mu.K or W.K on *E. coli* K-12, the phage produced are almost entirely of the G(+) phenotype and therefore an appropriate antigen in the production of a specific antiserum, in contrast, lytic propagation of Mu.KF and W.KF phage (G(-)) on *C. freundii* generate lysates which plate on *E. coli* K-12 with an e.o.p. of 10^{-1}-10^{-3}.

These and other results were consistent with the conclusion that, in lytic propagation of phage Mu on *C. freundii* the G region of the phage is undergoing inversion at a low frequency (Van de Putte *et al.*, 1980, Rice, 1980) and generating some Mu.K G(+) and W.K G(+) forms in the otherwise Mu.KF G(-) or W.KF G(-) populations, i.e., that these G(-) lysates were, in fact, mixed for these two phage types and therefore not suitable as an antigen for the production

of specific antisera against the Mu.KF (G-) or W.KF (G-) phage.

Presumably the *gin* gene is not expressed during lytic propagation on *E. coli* K-12, but is expressed, albeit at a low level, in the *C. freundii* host to which the G(-) phage phenotype adsorbs and infects.

An alternative source of a homogenous G(-) phage antigen would be a lysate on *C. freundii* of a phage Mu mutant in which the G region of the phage genome is "frozen" in the G(-) orientation.

The following experiment was designed to isolate mutants of phage Mu in which the G region is frozen in one orientation. Mutants were identified as phage which failed to propagate on one or other of the alternative hosts. Such a phenotype could result from a mutation in the *gin* gene, (*gin*$^-$), or again, a mutation in one or other of the terminal sequences of the G region; in either case these mutations could prevent inversion of the G region. Finally, mutations which affect the specific tail component for either the G(+) or the G(-) phage forms could also prevent adsorption to one or other of the two corresponding hosts.

M. Howe (*personal communication*) isolated a *gin*$^-$ point mutant; $MH_{4400'}$ which is unable to complement *Hin*-mutants of *Salmonella typhimurium*; inversion of the H segment in *Salmonella* is controlled by the *Hin* gene. Howe offered as evidence in support of the *gin*$^-$ genotype the observation that the mutant phage failed to propagate on *E. coli* C, but did propagate on *E. coli* K-12. However the results to be reported in this study would lead us to question the validity of using the host, *E. coli* C, in this way, i.e. as a host for Mu in the G(-) form.

In this laboratory we observed that heat induced lysates of the mutant (MH_{4400}) plated on *E. coli* K12 (G+ host) with an e.o.p. of 1 but on *C. freundii* (G- host) with an e.o.p. of 10^{-3}. This latter figure is to be compared with a figure of approximately 1 when heat induced lysate of Mu*cts*61 are plated on *C. freundii*. These observations are consistent with a mutation in the *gin* gene, but one which is leaky. It is postulated that in such a mutant the G region is inverting with significantly reduced frequency in *E. coli* K12 lysogens.

3.1b Isolation of Mutants of phage Mu*cts*61

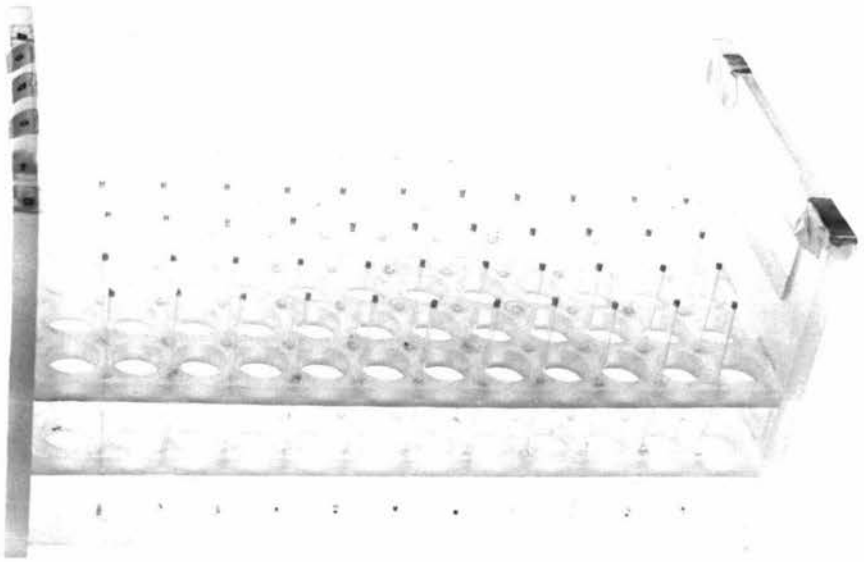
Single colonies of (Mu*cts*61) arising after the NG treatment (see method 4) were sampled as follows; each colony was picked with the tip of a sterile capillary tube (micro hematocrit tube), the tip of the capillary tube was introduced into 0.3 cm^3 of BHI held in individual wells of the microtitre plate; The broth was drawn up into the capillary tube by capillary action, washing cells into the tube and leaving a residue of cells in the well. The capillary tube was removed from the well, the broth in the capillary tube was tapped along to the middle of the tube and the end of the tube was sealed, to give a miniature tube culture effect.

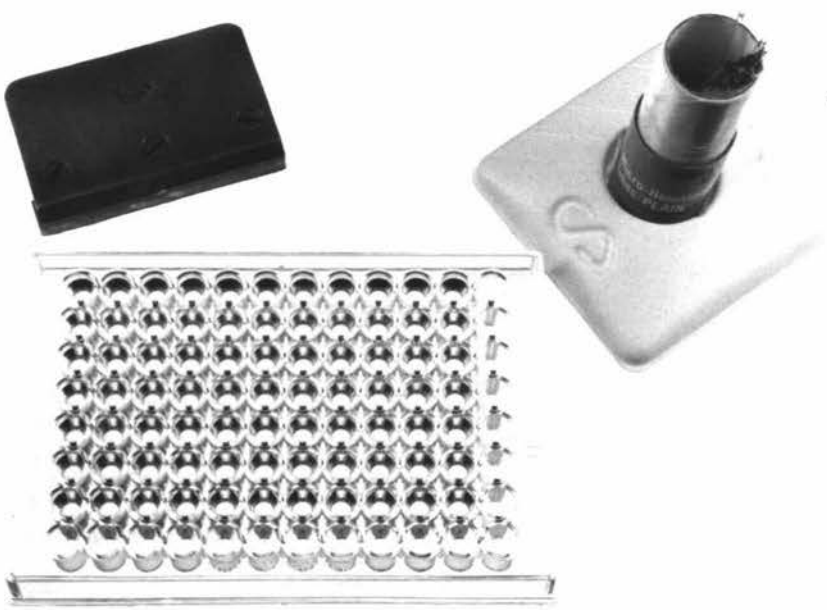
These micro cultures were placed in plastic racks, in each case the position in the rack corresponding with the position of the well in the microtitre plate. Two mm diameter holes were drilled in Bel Art plastic racks to accomodate the capillary tube cultures. The racks held a maximum of seventy-seven hemacrit capillary tubes (see plates 1 and 2 for the equipment used in this section).

Racks holding the tube cultures were passed through a modified heat induction process. First the rack was placed in a 55°C waterbath for 1 minute, transferred to a 44°C

PLATE 1: The plastic Bel Art rack adapted for accomodating the capillary tube cultures during heat induction.

PLATE 2: Equipment used in screening colonies by the capillary spot method; a glass cutter, a microtitre plate and micro-hematocrit capillary tubes.





waterbath for 25 minutes, and finally into a 37°C waterbath for 50 minutes.

After this process the tubes were opened, with a glass cutter, and a drop of broth was spotted first onto a soft agar overlay containing *C. freundii*, and then onto a soft agar overlay containing *E. coli* K-12. This was done in a grid formation, and numbered so that the original cells could be recovered from the correct microtitre plate well if needed.

The 'spotting' is a preliminary trial to detect colonies which produce phage that plate only on either *E. coli* K-12 or on *C. freundii*. (see Plates 3 and 4).

The presumptive mutants were recovered from the corresponding wells of the microtitre plate; they were heat induced by the normal procedure (method 3) and plated on *E. coli* K-12 and *C. freundii* to confirm the mutant phenotype, i.e. that the heat induced phage plated on only one of the two hosts.

From Q1 (Mu *cts*61) we derived a total of four mutants, JP_1, JP_2, JP_{5a} and JP_8. The use of replicate cultures following NG treatment (see method 4) and the retention of no more than one mutant from any culture ensured that all isolates represented independent mutational events.

Mutants JP_1, JP_{5a} and JP_8 have phenotypes which would correspond to the G region being frozen in the G(+) orientation; heat induced lysates of these mutants plate with an e.o.p. of 1 on *E. coli* K-12, but with an e.o.p. on *C. freundii* which is less than 10^{-9}.

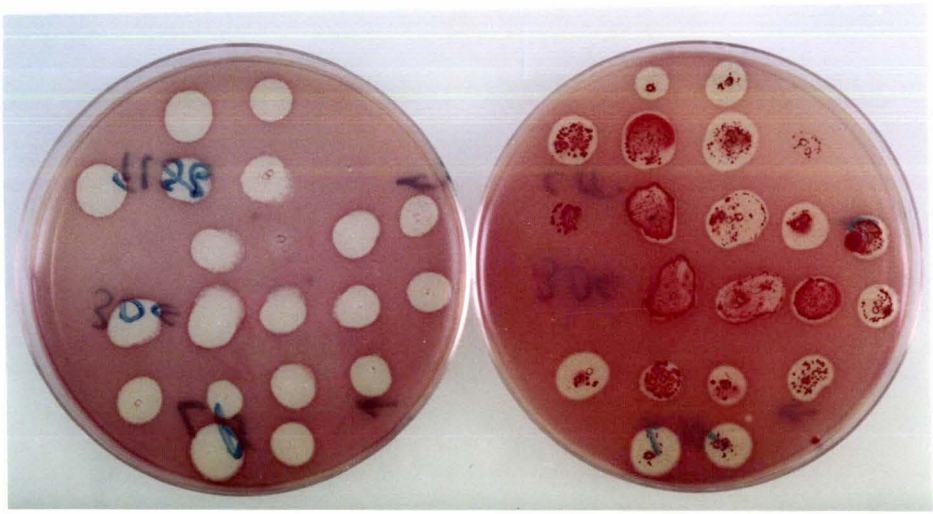


Plate 3

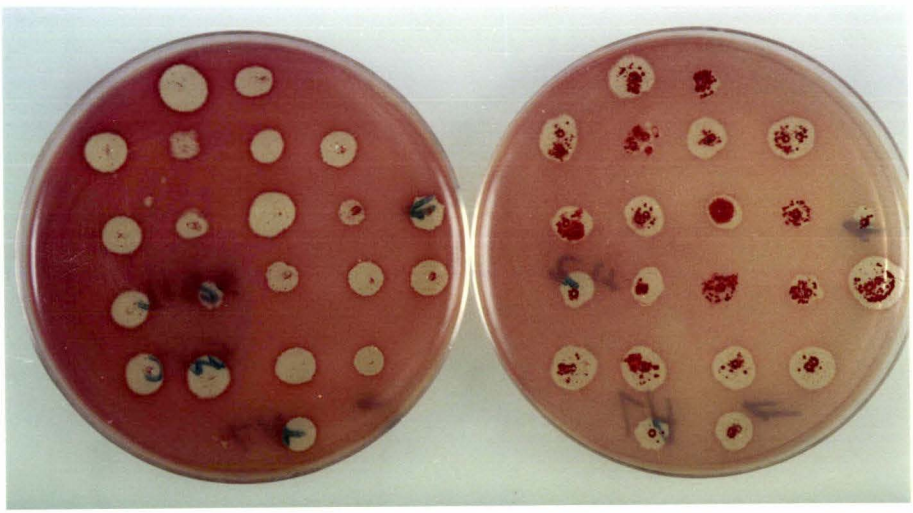


Plate 4

PLATES 3 and 4: Examples of 'spotting' results using capillary tubes. Plates on the left have an *E. coli* K-12 overlay, while the plates on the right have a *C. freundii* overlay. Presumptive mutants are seen in Plate 3, 4th row and 1st column, in Plate 4 in the 4th row and 3rd column.

Mutant JP_2, on the other hand, has a phenotype which would correspond to the G(-) region being frozen in the G(-) orientation. The e.o.p. of heat induced lysates of JP on *C. freundii* is 1, while the e.o.p. on *E. coli* K-12 is less than 10^{-9}. Although mutant JP_2 was isolated as a mutant prophage, carried in *E. coli* K-12, this phage is unable to infect that host.

3.2 Inversion of the G Region of the Phage Mutants JP_1, JP_{5a}, JP_8 and MH_{4400} using a F' Plasmid Carrying a Mu Prophage, Wild Type with Respect to the *gin* Gene (*gin*$^+$)

3.2a Introduction

G region inversion is the result of a specific recombinant type event involving the terminal sequences of the G region (Hsu and Davidson, 1974). The *gin* gene (G inversion) of phage Mu codes for a protein, MW 21,500 (Kwoh and Zipser, 1981) which mediates a specific intramolecular recombination event leading to the inversion of the phage Mu G region. The recombination involved is not dependent on the bacterial Rec A, B and C system (Daniel *et al.*, 1973b).

The G region of a phage Mu genome defective in the *gin* gene (*gin*$^-$) can be inverted with a 'helper' Mu *gin*$^+$ prophage (Kamp *et al.*, 1978); i.e. a Mu prophage introduced into the bacterial cell on a F' plasmid; the *gin* gene product coded by the introduced prophage is able to activate the inversion of the G region of the mutant prophage. However if the Mu mutant is not of the *gin*$^-$ type but plates on only one host for some other reason, then the helper, Mu *gin*$^+$, will have no effect.

When the helper Mu *gin*$^+$ is removed from the bacterial cells, the G region of a resident Mu *gin*$^-$ prophage will once again be 'frozen' in one orientation, and the resulting lysogens are of two types: cells in which the Mu prophage has the G region 'frozen' in the (-) orientation or in the (+) orientation.

In the following experiment we attempted to invert the G region of the Mu mutants JP_1, JP_{5a} and JP_8. The aim was to obtain isolates of Mu with the G region 'frozen' in the (-) orientation. These might then be used to raise specific antisera to the Mu.KF G(-) phage.

The JP_2 mutant phenotype, G(-), for technical reasons was not included in this experiment, as the *E. coli* K-12 strain which carries the mutant prophages cannot serve conveniently as a receptor of the F' plasmid. The *E. coli* K-12 strains, CSH50 and CSH55, which are more appropriate recipients were therefore lysogenized with the mutant phages. However, mutant phage JP_2, which does not infect *E. coli* K-12 could not be included in this series.

3.2b Inversion of the G Region by a 'Helper' Mu*gin*$^+$ Phage (Experiment)

The mutants JP_1, JP_{5a}, JP_8 were used to lysogenise the *E. coli* K-12 strains CSH50 Δ(*lac pro*, sm^r) and CSH55 Δ(*lac pro*, Nal^r). CSH50 was also lysogenised by MH_{4400}, a *gin*$^-$ mutant with G in the (+) orientation. It should be noted that this phage has a phenotype consistent with the *gin*$^-$ mutation being leaky; as heat induced lysates of K12 lysogens plate on *C. freundii* with an e.o.p. 10^{-3}.

Each of the lysogenised derivatives of CSH55 and CSH50 were crossed either with CSH55F' or Bu260F'; these are Mu resitant strains with an F' plasmid carrying the *pro lac* region and the Mu*cts*62 genome, inserted into the lac_z gene (CSH55 Δ(*pro lac*) Mu^r/F' *pro*$^+$ *lac*z::Mu*cts*62, and Bu260 Δ(*pro lac*) *thy*$^-$ Mu^r/F' *pro*$^+$ *lac*z::Mu*cts*62).

Exconjugants were selected using contraselecting markers *pro*$^+$ and *sm*^{r} or *pro*$^+$ and *Nal*^{r}. The F' recipients were then tested following heat induction for the presence of the two phage types, G(+) and G(-); this confirmed the transmission of the F' plasmid carrying the Mu*cts* 62 prophage with a functional *gin* gene into the recipients.

The curing of these derivates was achieved by acridine orange treatment (see method 10). The strains were grown overnight in acridine orange broth, plated out the following day, and then screened for the appropriate markers (CSH50 *pro*$^-$*sm*^{r}, CSH55 *pro*$^-$ Nal^r). Loss of the *pro*$^+$ marker was inferred to be associated with loss of the F' *pro lac* plasmid.

The lysogens cured of the F' plasmid were heat induced and the phage released checked for propagation on *E. coli* K-12 (G+) and/or on *C. freundii* (G-).

Across the JP_1, JP_{5a} and JP_8 mutants 37 cured lysogens were screened, but there were no instances in which the phage plated solely on *C. freundii*. The G region appeared not to be inverted; all isolates were in the (+) orientation. The MH_{4400} strain however yielded one isolate among 14 screened that plated on *C. freundii* and not on *E. coli* K-12, i.e. a Mu*gin*$^-$ G(-) phage.

These results indicate that in the mutants, JP_1, JP_{5a} and JP_8 the observed phenotypes may be due to events other than mutations in the *gin* gene, as complementation of the *gin* gene should have become evident following exposure to the *gin*$^+$ helper phage, but inversion of the G region in the mutant phage JP_1, JP_{5a} and JP_8 was not observed. We would note however that the MH_{4400} *gin*$^-$ mutant did yield a G(-) form i.e. a *gin*$^-$ G(-) Mu KF form, and lysates of this phage were investigated as a possible source of a Mu.KF antigen.

3.3 Mu.KF Antisera

3.3a Specificity of the Mu.KF Antisera

With the aim of producing specific antisera to the Mu.KF phage form, the JP_2 mutant, a mutant plating only on *C. freundii*, and a MH_{4400} *gin*⁻ G(-) derivative were used to immunize rabbits; the rabbits were bled and the sera harvested.

Neutralisation tests (see method section) were run against the homologous phage using a range of serum dilutions to determine the dilution, which at 10 minutes and 37°C inactivation neutralised approximately 90% of the homologous phage.

The anti JP_2 serum neutralised the MH_{4400} *gin*⁻ G(-) lytic phage to the same extent as the homologous JP_2 phage. While the anti MH_{4400} serum neutralised the JP_2 lytic phage on *C. freundii* to the same extent as the homologous MH_{4400} *gin*⁻ G(-) phage.

3.3b Determination of the Neutralising Activity of an Antiserum

In subsequent neutralisation tests the neutralising activity of an antiserum against a phage under standard conditions (normally 10 minutes at 37°C) and at a constant dilution has been expressed as the log of the plaque forming units (pfu) at time x (normally 10 minutes) subtracted from the log of the pfu at zero time.

The relative activity of an antiserum against a heterologous phage has been calculated by relating the activity obtained against the heterologous phage to the activity obtained for the homologous phage under the same conditions normally a constant dilution for 10 minutes at 37°C.

Phage Neutralised	Method of Propagation	Relative Neutralising Activity	Host Plated on
MH_{4400} *gin*⁻ G(-)	lytic on *C. freundii* G331	1	*C. freundii* G331
Mu G(-)	Heat induced *E. coli* K12 lysogen	1	*C. freundii* G331
Mu G(+)	Heat induced *E. coli* K12 lysogen	0.27	*E. coli* K12 PB1395

TABLE IV: The Neutralisation of Phage by the Anti JP_2G(-) Serum

Phage Neutralised	Method of Propagation	Relative Neutralising Activity	Host Plated on
JP_2	lytic on *C. freundii*	1	*C. freundii* G331
Mu G(-)	Heat induced *E. coli* K12 lysogen	1	*C. freundii* G331
Mu G(+)	Heat induced *E. coli* K12 lysogen	0.1	*E. coli* K12 PB1395
W.C	lytic on *E. coli* C (518c)	0	*E. coli* C (518c)

TABLE V: The Neutralisation of Phage by the Anti MH_{4400} G(-) Serum

In summary:

$$\frac{\text{log pfu heterol. phage at zero time - log pfu heterol. phage at 10 min.}}{\text{log pfu homol. phage at zero time - log pfu homol. phage at 10 min.}}$$

The anti JP_2 and MH_{4400} sera were run against Mu.K G(+) phage to test the specificity of these antisera.

An *E. coli* K12 lysogen of Mu*cts*61 was heat induced and the lysate, of approximately equal proportions of the G(-) and the G(+) phage, was then used in the neutralisation test. In this test the Mu G(-) phage (plating on *C. freundii*) was neutralised to the same extent as the homologous phage by both antisera, relative activities of 1 (see Tables IV and V).

The MH_{4400}.KF G(-) antiserum gave a relative activity against the Mu.K, G(+) phage of 0.1. However the anti JP_2 serum neutralised the Mu.G(+) phage with a relative neutralising activity of 0.27 (see Tables IV and V). This is a relatively high level of cross neutralisation and limits the use of this antiserum. However the anti MH_{4400}.KF G(-) serum is far more specific.

The anti MH_{4400} G(-) serum was used to neutralise the lytic W.C phage, derived from *E. coli* strain W, F-2-3-27, a strain that carries only the W.C phage.

The lytic W.C phage was not neutralised by the anti MH_{4400} G(-) serum, relative neutralising activity of 0 (see Table V).

Thus the anti MH_{4400} serum is specific towards the Mu.KF phage, to an extent that enables this serum to be useful for further neturalisation tests.

CHAPTER 4

THE PLATING OF PHAGE Mu.K ONTO *E. coli*, STRAIN C, AND THE BEHAVIOUR OF THE DERIVED Mu.KC PHAGE AND *E. coli* C LYSOGENS

4.1 The Plating of Heat Induced Mu.K onto *E. coli* C

4.1a Introduction

The lytic phage Mu.K has been observed to plate on *E. coli* C with a low e.o.p., but the observed values have varied; Rice (1980) reported the e.o.p. to be less than 10^{-8}, and Van de Putte *et al* 1980 less than 10^{-9}. Jamieson (1971) using phage W.K., a phage identical or closely related to phage Mu.K, reported an e.o.p. on *E. coli* C of 10^{-4} -10^{-5}.

Rice observed that when phage Mu was propagated lytically on *C. freundii* (phage Mu.KF), these lysates plated with an increased e.o.p. on *E. coli* C, viz., with an e.o.p. of 10^{-5}. When plating lytic Mu.KF onto *E. coli* C, Van de Putte *et al*. (1980) also observed a higher e.o.p. (7.5×10^{-2}) than that observed when lytic Mu.K was plated on this strain ($< 10^{-9}$).

Van de Putte had established that Mu phage with the G region in the (-) orientation can propagate on *C. freundii* (1980). Following these observations Rice proposed that phage Mu requires the G region in the (-) orientation, plus an additional event in order to plate on *E. coli* C, thereby accounting for the higher e.o.p. he observed when plating Mu.KF, G(-), as this host strain.

If this hypothesis is correct, then Mu phage which are present in heat induced lysates of *E. coli* K12 lysogens should also plate with a higher e.o.p. on *E. coli* C, as

approximately half of the phage particles in these lysates are G(-), whereas lytic propagation of Mu on *E. coli* K12 yields lysates in which the phage are almost entirely in the G(+) orientation.

Thus, according to Rice (1980) one event is required to enable heat induced Mu.K G(-) phage to plate on to *E. coli* C as compared to the two events needed to plate lytic Mu.K G(+) phage on this host strain. One would also assume that if the G region is unable to invert and the G remains fixed in the G(+) orientation, then such a strain of Mu.K would not yield derivatives which are able to plate on *E. coli* C.

4.1b The Plating of Mu.K (Mu *cts*61) Heat Induced Lysates on to *E. coli* C

To test the above hypothesis, viz., that the heat induced Mu.K phage lysates plate on *E. coli* C with a higher e.o.p. than lytic Mu.K lysates, eighty-five *E. coli* K12 lysogens of Mu were heat induced and sampled. Twenty of these lysates were titrated for plaque forming units (pfu) on both *E. coli* K-12 (PB1395) and *C. freundii* (G331). The mean titre on *E. coli* K12 (PB 1395) was 4.4×10^9 pfu/cm^3 with confidence limits ±1.7 and with a probability of <0.05. The mean titre on *C. freundii* (G331) (and hence the titre of G(-) Mu) was 2.1×10^9 pfu/cm^3 with confidence limits ± 1.3 and with a probability of <0.05.

All eighty-five lysates were plated on *E. coli* C; from each lysate, three 0.8cm^3 aliquots were incorporated into soft agar overlays seeded with *E. coli* C. That is, 2.4cm^3 from a total lysate volume of 4cm^3 was sampled. Of the eighty-five lysates sampled, fifteen developed one or more plaques per 2.4cm^3, and of these ten had less than five plaques per 2.4cm^3 of lysate, and five samples had only one plaque per 2.4cm^3 of lysate (see Figure 11).

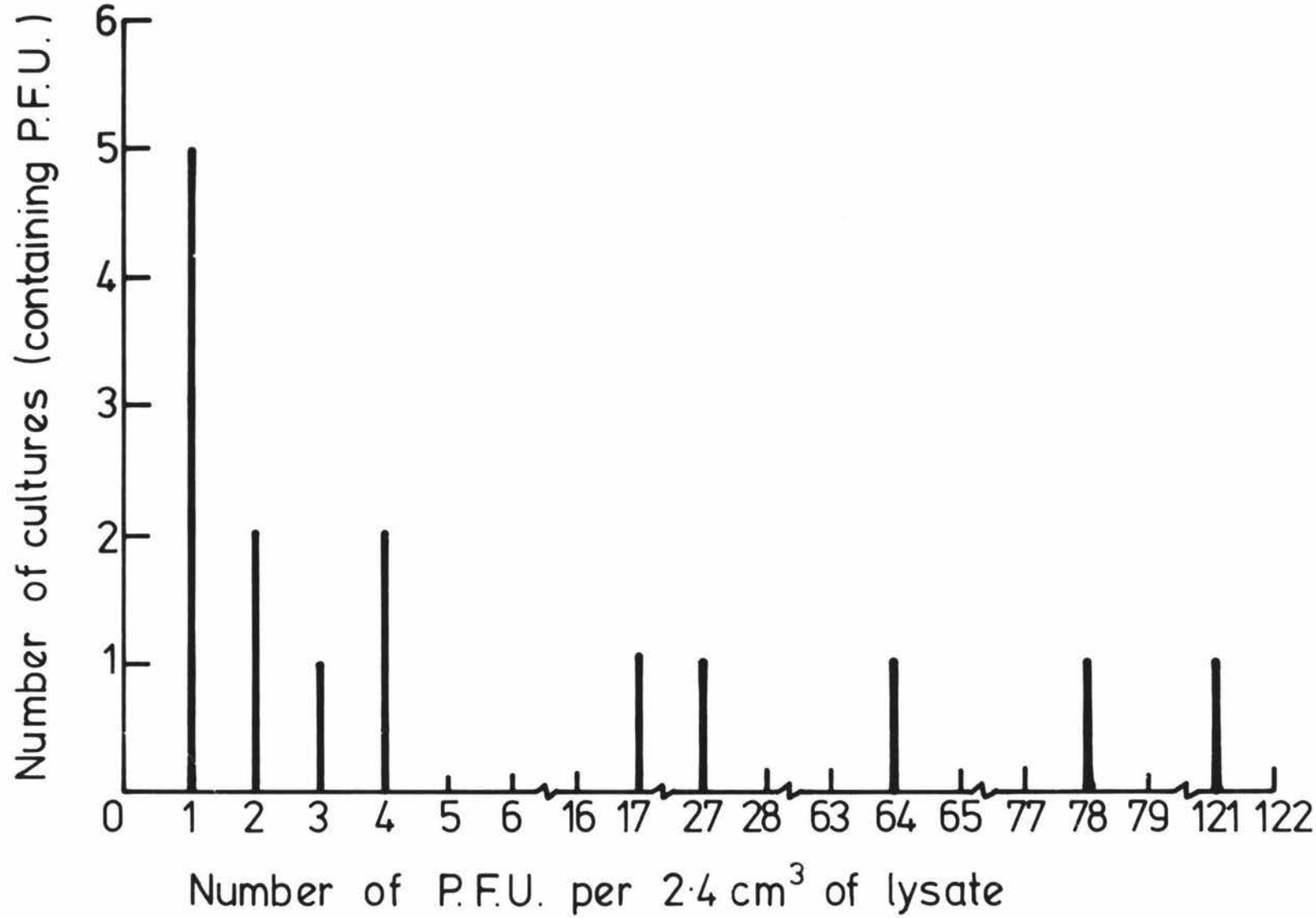


FIGURE 11: The distribution of the number of P.F.U. When heat induced MuK is plated on *E. coli* C.

It should be noted that the distribution of positive lysates throughout the eighty-five which were sampled, did not appear to be random; positive lysates appeared in clusters; they tended to be associated with batches of lysates induced on particular days. The reason for this non-random distribution is not known.

4.1c The Plating of Mu.K (Mucts61) Heat Induced Lysates onto *E. coli* C; Statistical Analysis of the Results

As five lysates had only one p.f.u. when slightly more than half the lysate was sampled, the probability is that in five additional lysates, the volume unsampled contained one p.f.u.

Therefore it would be reasonable to assume that twenty lysates contained one or more p.f.u.

Applying the poisson distribution and the derived equation, $m = -\ln Po$ where m equals the average frequency of rare chance events (mutations) in a series of cultures (see Luria and Delbrück, 1952) and Po represents the frequency of cultures in which none of these chance events has occurred, then, if the rare event (mutational) is the change in the genotype associated with the phenotypic change Mu.K to Mu.KC, then the above equation allows an estimate of the frequency of this genotypic change.

$$m = -\ln Po$$
$$\therefore m = -2.3 \log_{10} Po$$

as there were an estimated 20 lysates which contained Mu.KC phage, therefore there were an estimated sixty-five lysates without phage Mu.KC, in a total of eighty-five lysates.

Therefore the zero incidence Po = 0.764 solving for M

where $m = -2.3 \log_{10} 0.764$

$m = 0.3$

Therefore the chance of obtaining an event (mutational) conventing Mu.K to the Mu.KC form in any one lysate is 0.3 or 1 in 2.8×10^{10} G(-) Mu phage, and an e.o.p. of $1.7\ 10^{-11}$.

4.1d The Plating of Mu.K (MH_{4400} and JP_1) Heat Induced Lysates onto *E. coli* C

If as proposed by Rice (1980) it is necessary for the G region to be in the (-) orientation in order to express a Mu.KC phenotype, then phage Mu.K with G frozen in the G(+) orientation would not be expected to generate Mu.KC forms which plate on *E. coli* C. To test this hypothesis we heat induced 36 *E. coli* K12 lysogens carrying the Mu mutant, MH_{4400}. These were plated onto *E. coli* as were the heat induced Mu.K lysogens.

The MH_{4400} strain of Mu is a *gin*$^-$ mutant, albeit leaky; heat induced G(+) lysates of Mu MH_{4400} plate on *C. freundii* with an e.o.p. of 10^{-3}.

The thirty six heat induced lysates of MH_{4400} produced no plaques on *E. coli* C, e.o.p. $< 1.0 \times 10^{-11}$ (see Table VI).

The JP_1 mutant, of Mu *cts*61, a mutant carried as a prophage in an *E. coli* K-12 lysogen and which, when induced plates only on *E. coli* K12 and not on *C. freundii*, was also sampled in the same manner.

The mutations carried by the JP_1 mutant could be the result of a modified terminus to the G region and thus unable to be inverted, or, a mutation in a region coding for a

lysates by heat induction of *E. coli* K12 lysogens.	Relative Plating Efficiency on hosts		
	E. coli K12 (PB1395)	*C. freundii* (G331)	*E. coli* C (518c)
Mucts61	1	5×10^{-1}	1.7×10^{-11}
JP_1	1	1.3×10^{-12} [+]	1.3×10^{-12}
MH_{4400}	1	10^{-3}	$<1.0 \times 10^{-11}$
JP_2	1.8×10^{-11} [++]	1	1.8×10^{-11}

TABLE VI: Efficiency of plating (e.o.p.) of heat induced lysates of Mucts61, JP_2, MH_{4400} and JP, lysogens of *E. coli* K12: hosts *E. coli* C, *C. freundii* and *E. coli* K12

[+] revertant

[++] unable to recover phage from plaques to test for revertant phenotype

specific tail protein such that, in the Mu.K G(-) state the phage is no longer able to adsorb to *C. freundii*. A mutation in the *gin* gene (*gin*$^-$) of JP_1 is considered unlikely, as the G region of this mutant was not observed to invert in the presence of a 'helper' Mu *gin*$^+$ phage.

Sixty-four cultures of JP_1 were heat induced and the lysates sampled. From these sixty-four lysates, one plaque was observed on *E. coli*C, e.o.p. 1.3×10^{-12}, and one plaque on *C. freundii*, e.o.p. 1.3×10^{-12} (see Table VI). The plaque that plated on *C. freundii* proved capable of plating on both *E. coli* K12 and *C. freundii* just as the parental Mu*cts*61 does, and thus has the phenotype of a revertant.

Consistant with the above hypothesis, the heat induced lysates of the *gin*$^-$ MH_{4400} phage did not plate on *E. coli* C, and to that extent supports the model that G inversion from (+) to (-) is a necessary and associated event but is not in itself sufficient to effect the phenotypic change Mu.K to Mu.KC.

It should be noted that induced lysates of the JP_1 lysogen plated on *E. coli* C with a very low frequency, one plaque observed from sixty-four lysates sampled, e.o.p. 1.3×10^{-12}. Either the hypothesis is not supported, or that JP_1 does not have the G region frozen in the G(+) orientation but rather carries a mutation in a gene coding for a F tail specific protein. Or, finally, that the single plaque was an artifact and the results are not inconsistent with the hypothesis.

4.1e The Plating of Mu.K (JP_2) Heat Induced Lysates onto *E. coli* C

If as proposed by rice (1980) the Mu phage requires the G region to be in the (-) orientation to plate on *E. coli* C, then induced K-12 lysogens carrying a Mu.K prophage with

the G region frozen in the (-) orientation would be expected to plate on *E. coli* C with a frequency similar to the induced Mu.K, wild type.

To confirm the above, the JP_2 mutant of the Mu *cts*61 was heat induced and sampled as before. The JP_2 mutant of Mu *cts*61 is carried as a prophage in an *E. coli* K12 lysogen; heat induced lysates of this lysogen do not plate on *E. coli* K12. The mutation carried by the JP_2 mutant could be the result of;

1) a mutation in the *gin* gene (*gin*$^-$), or
2) a modified terminus of the G region and thus unable to be inverted, or
3) a mutation in a region coding for a specific tail protein, here a 'K' tail protein, such that, in the Mu.K G(+) state the phage no longer adsorbs to *E. coli* K12.

Eighteen heat induced lysates of JP_2 were sampled. The mean titre of JP_2 on *C. freundii* was 1.8×10^9 with confidence levels ± 1.6 with a probability of < 0.05.

On *E. coli* K-12, two plaques were observed, originating from different JP_2 lysates (e.o.p. 1.8×10^{-11}). We were not successful in recovering phage from these plaques, to test for the presence of revertant plating behaviour.

From these JP_2 lysates, two lysates plated Mu.KC phage. Solving for m, where $\bar{m}$ is the chance of a rare event occurring, $m = 2.3 \log_{10} Po$.

And Po, the zero fraction = 0.11

$$\therefore m = -2.3 \log_{10} 0.11$$
$$= 0.13$$

The chance of any one lysate of heat induced JP_2 containing a Mu.KC phage is estimated to be 0.13, or 1 lysate in 7.7. This is equivalent to 1 Mu.KC phage in 5.6×10^{10} Mu JP_2 G(-) phage or an e.o.p. of 1.8×10^{-11} (see Table VI), a frequency similar to that obtained when plating heat induced Mu.K (Mu *cts*61) onto *E. coli* C (e.o.p. 1.7×10^{-11}) where 1 Mu.KC phage is derived in $2.8\ 10^{10}$ G(-) Mu phage (e.o.p. 1.8×10^{-11}).

4.1f Discussion

The results presented in this section are not inconsistent with the hypothesis presented by Rice (1980), namely, that the G region of Mu is required to be in the (-) orientation and that an additional event is required to generate a Mu.KC phenotype. However, if this is so one would expect the heat induced Mu.K phage, Mu *cts*61, to plate on *E. coli* C with an e.o.p. comparable to the lytic Mu.KF phage; the heat induced Mu.K lysate has a high titre of G(-) phage as does the lytic Mu.KF lysate. While the e.o.p. of lytic Mu.KF on *E. coli* C is 10^{-5}, the plating of heat induced Mu.K (Mu *cts*61) on *E. coli* C is very low, with a calculated frequency of 1.7×10^{-11} i.e. 1 Mu.KC phage in 2.8×10^{10} G(-) Mu phage.

Van de Putte *et al.* (1980) plated heat induced Mu.K (using the strain Mu *cts*62) onto *E. coli* C and they reported an e.o.p. of 4×10^{-5}. They also commented that this frequency was "extremely variable and dependant on plating conditions". These workers also plated a heat induced Mu *gin*$^-$, Mu *cts*62 *gin*$^-$, and reported as e.o.p. which was less than 10^{-9}.

The *gin*$^-$ mutant (MH_{4400}) and JP_1 support the model that the G(+) inversion to G(-) is a requirement to plate Mu.K onto *E. coli* C. While our results indicate Mu.K (and JP_2) plate higher than the *gin*$^-$ MH_{4400} and JP_1 strains, the freuqency is not comparable with the frequency, 10^{-4}-10^{-5}, when lytic Mu.KF is plated on *E. coli* C, and is therefore far lower than would be expected. These results prompt several questions: are two events required, first that the G region be in the (-) orientation, and second, that an additional event (mutation) must occur, and finally, whether this second event is occurring more frequently during lytic propagation on phage Mu in *C. freundii* than it is during the growth of the *E. coli* K12 lysogens and subsequent phage replication following heat induction?

A similar discrepancy was observed when the frequency of phage Mu plating on *E. coli* C was determined;

1) among G(-) phage generated by lytic propagation on *C. freundii*, and
2) by heat induction of a G(-) lysogen of *C. freundii*; the lytic phage plated on *E. coli* C with an e.o.p., as we have reported above of 10^{-4} -10^{-5}, while the induced phage plated on *E. coli* C with an e.o.p. less than 10^{-8}. In both instances the phage are in the G(-) form. Does a second event occur with much higher frequency during lytic propagation in *C. freundii* than during the growth of lysogen and in the subsequent heat induced phage replication? It should be noted that the *gin* gene is expressed in *E. coli* K12 lysogens but <u>not</u> during lytic growth of phage Mu in *E. coli* K-12.

Phage	Relative Plating Efficiency on hosts		
	E. coli C (518c)	*C. freundii* (G331)	*E. coli* K12 (PB1395
induced Mu.KC no 1	1	$<8 \times 10^{-9}$	10^{1}
induced Mu.KC no 5	1	$10^{-1} - 10^{-2}$	8×10^{0}
lytic Mu.KC no 1	1	$<2.4 \times 10^{-7}$	10^{-2}
lytic Mu.KC no 5	1	5.0×10^{-1}	10^{-3}
induced JP_2.KC, 1, 2, 3,	1	0.2 - 2.0	$<2 \times 10^{-8}$

TABLE VII: The plating of heat induced and lytic Mu.KC, and heat induced JP_2.KC onto *E. coli* C *E. coli* K12 and *C. freundii*.

4.2 The Plating of Mu.KC Phage onto Various Hosts

Plaques of Mu.KC forms arising when heat induced lysates of Mu.K were plated on *E. coli* C were picked and propagated and streaked for lysogen isolation (see method 9). In all, eight Mu.KC lysogens in *E. coli* C were isolated.

For further studies two types of lysates were prepared from these lysogens;

1) heat induced lysates by heat induction of the lysogens and
2) lysates by propagation on *E. coli* C.

In both cases the lysates were plated on: *E. coli* C (518c), *C. freundii* (G331) and *E. coli* K-12 (PB1395).

All heat induced lysates plated on *E. coli* C (arbituary given an e.o.p. 1) and on *E. coli* K-12 with an e.o.p. of 8×10^{0}. However, when plating these lysogens on *C. freundii* the phage fell into two groups, either the e.o.p. was very low, less than 8×10^{-9} (lysogens 1, 2, 3, 4 and 8), or the e.o.p. was relatively high, 10^{-1}-10^{-2} (lysogens 5, 6 and 7), (see Table VII).

The corresponding Mu.KC phage prepared as lytic lysates on *E. coli* C plated on *E. coli* C (arbituary e.o.p. 1) and on *E. coli* k-12 with an e.o.p. of 10^{-2}-10^{-3}, (approximately 10^{3}-10^{4} lower than the corresponding e.o.p. when induced lysates were used). However the e.o.p. on *C. freundii* again divided these phage into two distinct classes; either they gave low values of, less than 2.4×10^{-7} (MuKC 1), or relatively high values of 5×10^{-1} (MuKC 5) (see Table VII). These lysogens all originated from different plaques on *E. coli* C.

It is assumed that the differences observed between the lytic and induced MuKC lysates plated on *E. coli* K-12 (PB1395) could represent differences in the frequency of G inversion with the frequency of the inversions and hence the titre, depending on the method of phage propagation, lytic or induced, compare the *E. coli* K-12 host system.

Rice (1980) observed when plating the W.C phage, propagated lytically or induced, that the e.o.p. on *C. freundii* was low, less than 10^{-6} for lytic W.C on *C. freundii*, and less than 10^{-4} for induced W.C. on *C. freundii*.

One might expect the phage Mu.KC to behave as the phage W.C, as the serology of W.C and Mu.KC, as reported by Rice (1980) is identical, thus indicating that the tail components are the same.

However, while plating the lytic or induced phage Mu.KC, isolated in this study, onto *C. freundii*, two Mu.KC forms have been observed; a group consisting of the lysogens 1, 2, 3, 4 and 8, which gave an expected low e.o.p. on *C. freundii* and a second group consisting of lysogenes 5, 6 and 7, which plated on *C. freundii* with a relatively high e.o.p.

From a heat induced lysate of the *E. coli* K12 (JP_2) lysogen, that was plated on *E. coli* C, three *E. coli* C (JP_2) Mu.KC lysogens were isolated. These lysogens 1, 2 and 3 were heat induced and the lysates of phage JP_2 KC, plated back onto *E. coli* K12 and *C. freundii*.

The JP_2KC phage did not plate on *E. coli* K12 (e.o.p. less than 2×10^{-8}) consistent with its expected phenotype, G(-), but plated on *C. freundii* with an e.o.p. of 0.2-2.0 (see Table VII, p.68). This high e.o.p. on *C. freundii* was again not expected and is similar to the plating of Mu.KC derivates 5, 6 and 7, see above.

Phage	antiserum	Host plated on	Relative Neutralising Activity
lytic Mu.KC no 1	anti W.C.	*E. coli* C	0
lytic Mu.KC no 5	anti W.C.	*E. coli* C	0.1
lytic Mu.KC (Rice)	anti W.C.	*E. coli* C	1

TABLE VIII: Relative neutralising activity of the W.C. antiserum against lytic Mu.KC phage number 1, number 5, and lytic Mu.KC Phage as isolated by Rice (1980).

4.3 The Lytic Mu.KC Phage Verse the Anti W.C Serum

The Mu.KC number 1 and Mu.KC number 5 lytic phage lysates, propagated an *E. coli* C, were tested for neutralisation with the anti W.C. serum.

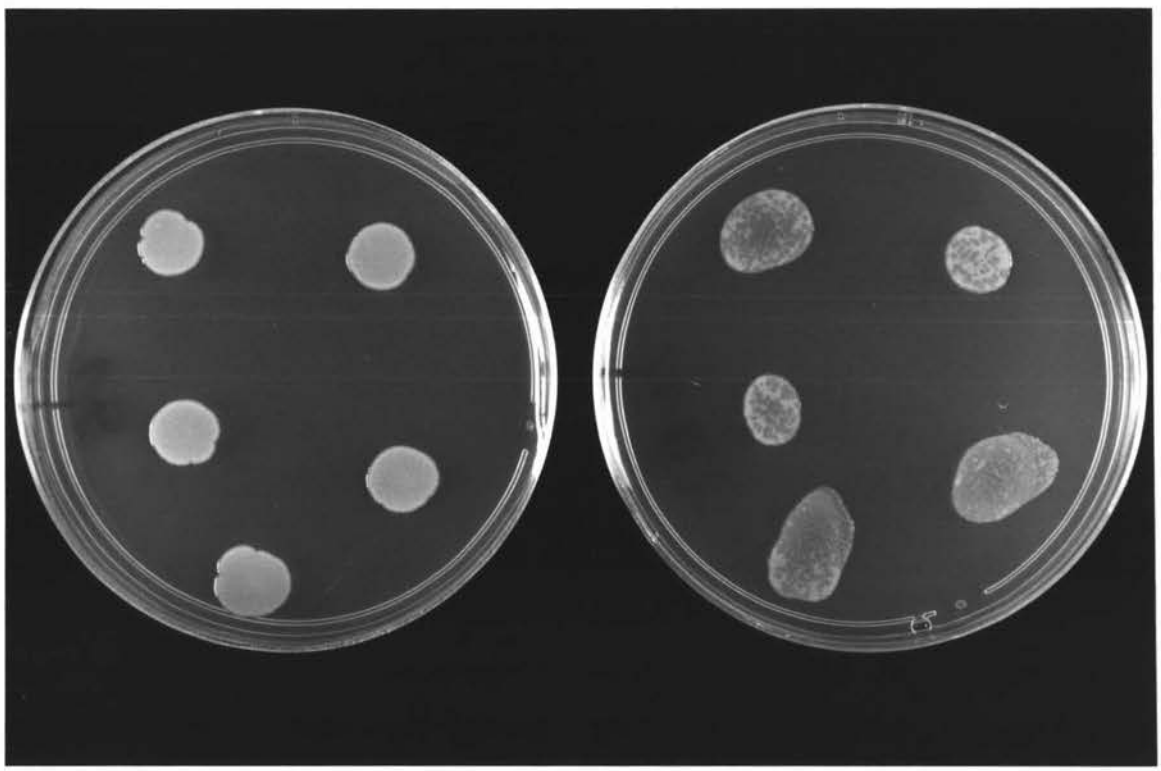
The Mu.KC number 1 lysate was not neutralised at all by the anti W.C serum, while the Mu.KC number 5 lysate was neutralised at a reduced rate, the relative neutralising activity was 0.1 (see Table VIII). A high rate of neutralisation was not observed with either of the Mu.KC phage lysates by the anti W.C serum as reported by Rice (1980); Rice observed that his lytic Mu.KC was neutralised by the anti W.C serum at the same rate as the homologous phage, (a relative neutralising activity of 1).

4.4 The Ability of *E. coli* C Lysogenised with Phage Mu.KC to Support the Propagation of Phage P_1

Jamieson (1971), while plating P_1 onto various phage W lysogens of *E. coli* C and *E. coli* K12 found that phage P_1 was restricted on *E. coli* C (W.KC) lysogens. The plating efficiency of P_1 on these lysogens was less than 10^{-7}.

The phage Mu equivalent of this lysogen, *E. coli* C (Mu.KC) was challenged with the P_1 (chl^r) phage. However, the eight *E. coli* C (Mu.KC) lysogens and the wild type *E. coli* C (518c) showed no restriction of the P_1 (chl^r) phage; the P_1 phage appeared to propagate on these lysogens with the same e.o.p. as on the *E. coli* C wild type. And, further more, no distinction was observed between *E. coli* C (Mu.KC) lysogens, numbers 1, 2, 3, 4 and 8 in which the associated Mu.KC derivates plate with a low e.o.p. on *C. freundii* and the *E. coli* C (Mu.KC) lysogens, numbers 5, 6, and 7, in which the Mu.KC plate with a high e.o.p. on *C. freundii* (please see Plate 5).

PLATE 5: On the left *E. coli* C (Mu.KC) lysogens without the P_1 (chl^r) challenge, on the right *E. coli* C (Mu.KC) lysogens challenged with P_1 (chl^r). The lysogens are, top, left to right, wild type and number 7, middle left to right, numbers 6 and 8, and bottom left is number 5.



Source	DF	SS	MS	F
Treatment	1	4.98×10^{19}	4.98×10^{19}	4.70
Error	38	4.03×10^{20}	1.06×10^{19}	
Total	39	4.52×10^{20}		

TABLE IX: The analysis of variance on the titres obtained from the plating of Mu.K heat induced lysates on *E. coli* K12 and *C. freundii* . The two treatments are significantly different, $p<0.05$. A completely randomised block was used to carry out the analysis of variance, and a variance ratio (F) test was used to test the significance of the observed differences between the two treatments.

4.5 Analysis of Variance for Plating Mu.K Heat Induced Phage *E. coli* K12 and *C. freundii*

Throughout the heat induction and plating of *E. coli* K12 (Muc*ts*61) lysogens in this study it has been observed that the titre on *C. freundii* ($2.1 \times 10^9 \pm 1.3$ $p<0.05$) is frequently lower than the titre on *E. coli* K12 ($4.4 \times 10^9 \pm 1.7$ $p<0.05$). This difference can be as great as a factor of 10 less on *C. freundii* than it is on *E. coli* K12.

To determine whether the titre on *E. coli* K12 and *C. freundii* are significantly different an analysis of variance was carried out.

This was accomplished by using a completely randomised block to carry out an analysis of variance and a variance ratio (F) test to test the statistical significance of the difference between the treatments, i.e., the plating of Mu.K heat induced phage onto *E. coli* K12 or *C. freundii* (see Table IX).

The variance between the plating of this phage onto these two host strains is significantly different, $p<0.05$. However we do not know if this is a real difference in the frequency with which G(+) or G(-) phage particles were released in heat induced lysogenic cultures, or whether the plating conditions used were less suitable for phage adsorption to *C. freundii*, than they were for adsorption of phage to *E. coli* K12.

CHAPTER 5

THE PLATING OF PHAGE Mu.KF ONTO *E. coli*, STRAIN C, AND THE PLATING ABILITY OF THE DERIVED Mu.KFC PHAGE

5.1 The Plating of Phage Mu.KF onto *E. coli* C

5.1a The Plating of Lytic Phage Mu.KF (Muc*ts*61) onto *E. coli* C

Rice observed that when phage Mu.KF, which had been propagated lytically on *C. freundii*, was plated onto *C. freundii* (G331), *E. coli* C (518c) and *E. coli* K12 r^+ (T186) and r^- (B1395), a different plating pattern was observed as compared to that obtained with the lytic Mu.K phage, viz., a high titre on *C. freundii*, a relatively high titre on *E. coli* K12 (r^-) and a low but significant titre on *E. coli* C. The results reported by Van de Putte *et al.* (1980) approximate those observed by Rice (see Table X).

These observations have prompted Van de Putte to suggest that the relatively high e.o.p. (10^{-3}) on *E. coli* K12 (r^-) is due to the G region undergoing inversion during lytic propagation on *C. freundii*, in contrast to the extremely low frequency of inversion during lytic propagation on *E. coli*, K12.

Secondly, the difference between the e.o.p.'s of phage Mu.KF on the K12, r^+ and r^-, host strains indicates that modification of Mu is not taking place.

The final difference is the higher e.o.p. obtained when plating lytic Mu.KF phage onto *E. coli* C (10^{-5}) as compared to the e.o.p. Rice (1980) observed for lytic Mu.K phage on *E. coli* C (less than 10^{-8}).

Phage preparation	The Relative Plating Efficiency (e.o.p.) on hosts			
	C. freundii r^- (G331)	*E. coli* K-12 r^- (PB1395)	*E. coli* K-12 r^+)T186)	*E. coli* C (518c)
Mu.KF (lytic) Rice 1980	1	10^{-2} - 10^{-3}	10^{-6} -10^{-7}	10^{-5}
Mu.KF (lytic) the present study	1	10^{-1}		2×10^{-5}
Muc2000 (lytic) Van de Putte *et al.*	1	2.6×10^{-3}	2.0×10^{-6}	7.2×10^{-2}

TABLE X: The relative efficiencies of plating lytic phage Mu.KF on various hosts.

This latter result indicated that once the Mu.K phage had propagated on *C. freundii*, (Mu.KF phage), it was now able to plate on *E. coli* C with a higher e.o.p. This suggests that the orientation of the G region is involved, i.e., that phage Mu with the G region in the (-) orientation is able to plate on *E. coli* C more readily than Mu with a G(+) orientation.

Rice proposed that, in order to plate on *E. coli* C, the G region of Mu must be in the (-) orientation and that an additional event is required, thereby accounting for the low e.o.p. (less than 10^{-8}) when plating lytic Mu.K phage or *E. coli* C.

In this present study lytic phage Mu.KF was again plated on these various hosts and the results obtained approximated those reported by Rice (1980) (see Table X). Indicating again evidence of G inversion, such that lytic phage Mu.KF plated on *E. coli* K-12 r^- with an e.o.p. 10^{-1}. Also a higher e.o.p. was observed when plating this phage (lytic Mu.KF) onto *E. coli* C as compared to the e.o.p. of Mu.K on this host.

5.1b The Plating of Lytic Mu.KF Phage (MH_{4400} and JP_2) onto *E. coli* C

If the high titres of lytic Mu.KF on *E. coli* K-12 is due to G inversion, then Mu unable to invert the G region or able only to do so at a reduced frequency would not be expected to give a similar high titre on *E. coli* K-12.

To confirm this the *C. freundii* lysogens of MH_{4400} *gin*$^-$ and JP_2, were heat induced and the resultant phage propagated lytically on *C. freundii*. These lysates were plated onto *C. freundii*, *E. coli* K-12 and *E. coli* C.

Phage propagation	The Relative Plating Efficiency (e.o.p.) on hosts		
	C. freundii r⁻ (G331)	*E. coli* K-12 r⁻ (PB1395)	*E. coli* C (518c)
Mu.KF lytic (Mu*cts*61)	1	10^{-1}	2×10^{-5}
Mu.KF lytic (MH_{4400})	1	1.4×10^{-7}	1.6×10^{-7}
Mu.KF lytic (JP_2)	1	$<2.5 \times 10^{-10}$	1.8×10^{-7}

TABLE XI: The relative efficiencies of plating lytic Mu.KF phage (Mu*cts*61, JP_2 and MH_{4400} *gin*⁻) onto various hosts.

The MH_{4400} strain is a *gin*$^-$ mutant of phage Mu, albeit leaky, while the JP_2 Mu mutant can not plate on *E. coli* K12. The mutation in JP_2 is unidentified.

The lytic Mu.KF phage, MH_{4400}, plated on *C. freundii* (e.o.p. 1) and as expected plated with a lower titre on *E. coli* K12 (e.o.p. 1.4×10^{-7}), as a consequence of this Mu phage possessing a reduced frequency of a G inversion. However a decrease in the plating on *E. coli* C (e.o.p. 1.6×10^{-7}) was also observed with this phage (see Table XI).

With the lytic Mu.KF phage, JP_2, (e.o.p. of 1 on *C. freundii*) no plaques were observed on *E. coli* K-12 in keeping with the JP_2 mutant phenotype. However, once again an unexpected drop in titre was observed on *E. coli* C; the e.o.p. on this host strain was 1.8×10^{-7} (see Table XI).

The lytic propagation of mutant MH_{4400} *gin*$^-$ on *C. freundii* (i.e. phage Mu.KF) provided evidence for G segment inversion during lytic propagation of Mu on *C. freundii*. However, for some unknown reason the *gin*$^-$ mutation of MH_{4400} and the mutation carried by JP_2 appear to affect the efficiency of plating of these lytic Mu.KF phage on *E. coli* C.

5.1c The Plating of Induced Mu.KF (Mu*cts*61) onto *E. coli* C

The Mu lysogens of *C. freundii* were heat induced and plated, to observe the plating ability of these lysates. This allows a comparison to the mode of lytic and induced lysates of phage Mu.KF; it is then possible to establish whether, as in the Mu.K lysates, there is a difference in plating behaviour associated with the mode of phage propagation.

Phage propagation	The Relative Plating Efficiency (e.o.p.) on hosts		
	C. freundii (G331)	*E. coli* K-12r$^-$ (PB1395)	*E. coli* C (518)
Mu.KF induced (Mu*cts*61)	1	10^1	$<2.5 \times 10^{-9}$
Mu.KF induced (MH_{4400})	1	$10^{-2} - 10^{-3}$	$< 10^{-9}$
Mu.KF induced (JP_2)	1	6×10^{-10}	$<6.0 \times 10^{-10}$

TABLE XII: The plating of heat induced Mu.KF lysogens (Mu*cts*61, MH_{4400} *gin*$^-$ and JP_2) onto various hosts.

The heat induced Mu.KF (Mu*cts*61) plates on *C. freundii* with an arbituary e.o.p. of 1. Plating of this page on *E. coli* K-12 is high, e.o.p. 10^{1}, i.e., higher than the e.o.p. observed for the host on which it was propagated, and higher than the lytic Mu.KF titre on *E. coli* K-12, e.o.p. 10^{-1}. (see Table XII)

It is possible that this could result from an increased frequency of the G inversion, G(-) → G(+) taking place such that an increase proportion of the phage released are in the G(+) form and thereby accounting for a higher titre on *E. coli* K-12 than on *C. freundii*. Or, that a high frequency of G inversion is taking place, and approximately equal proportions of the G(-) and G(+) phage are produced, however, with the G(-) phage being unable to adsorb as efficiently to the host strain (*C. freundii*).

The induced Mu.KF phage plated on *E. coli* C with an e.o.p. less than 2.5×10^{-9} and this is to be compared to an e.o.p. of 2×10^{-5} for the lytic Mu.KF plating on *E. coli* C (see Table XII and XI).

5.1d The Plating of Induced *Citrobacter freundii* (Mu; JP_2 and and MH_{4400}) Lysogens

The *C. freundii* lysogens of MH_{4400} and JP_2 were heat induced and plated on *C. freundii*, *E. coli* C and *E. coli* K-12.

The MH_{4400} (*gin*$^-$) induced Mu.KF (e.o.p. on *C. freundii* 1) showed a decrease in e.o.p. (10^{-2} -10^{-3}) when plated on *E. coli* K-12 (see Table XII). This supports the earlier proposal that G inversion is taking place during induced phage propagation on *C. freundii* as the MH_{4400} strain is *gin*$^-$, albeit leaky, and this resulted in a decreased frequency of G inversion, and a consequent reduction in the e.o.p. on *E. coli* K-12.

Further, the induced MH_{4400} Mu.KF phage was not observed to plate on *E. coli* C (e.o.p. $<10^{-9}$).

From the heat induced Mu.KF JP_2 lysate (e.o.p. on *C. freundii* 1) one plaque was observed on *E. coli* K-12 (e.o.p. 6×10^{-10}), presumably a revertant of the JP_2 phenotype.

As with the induced Mu *cts*61 Mu.KF and MH_{4400} Mu.KF lysates, no plaques derived from the induced JP_2 Mu.KF were observed to plate on *E. coli* C (see Table XII).

5.1e Discussion

The mode of phage Mu propagation (lytic or induced) on *C. freundii* does affect the plating pattern of the phage lysates.

The induced Mu.KF phage plates on *E. coli* K-12 with a high e.o.p. (10^{1}) i.e. with an efficiency above that on *C. freundii*, the host on which the phage was propagated. The lytic Mu.KF phage plates on *E. coli* K-12 with a high e.o.p. (10^{-1}), although not as high as the induced Mu.KF (see Tables XI and XII, p 81 and 83).

In both lysates (lytic and induced) the high titre on *E. coli* K-12 is a result of G inversion. Support for this is provided by the decreased e.o.p. on *E. coli* K-12 when the MH_{4400} *gin*$^-$ Mu.KF is plated on this host strain. The MH_{4400} is *gin*$^-$, albeit leaky, resulting in a reduced frequency of G segment inversion in both lytic and induced lysates.

The e.o.p. of Mu.KF plating on *E. coli* C also varies with the mode of propagation. The lytic Mu.KF phage (Mu *cts*61) plates on *E. coli* C with an e.o.p. 2×10^{-5}. The lytic JP_2

and MH_{4400} phage plate on this host with a slightly lower efficiency; the lytic JP_2 (Mu.KF) e.o.p. on *E. coli* C is 1.8×10^{-7}, and the lytic MH_{4400} (Mu.KF) plates on this host strain with an e.o.p. of 1.6×10^{-7}. However, no induced Mu.KF phage (Mu*cts*61, JP_2 or MH_{4400}) were observed to plate on *E. coli* C. This suggests that a necessary event does not occur readily during heat induction of lysogens and thus prevents the induced Mu.KF phage from plating on *E. coli* C.

The *gin*$^-$ mutation of MH_{4400} and the mutation carried by the JP_2 phage appear to have an additional effect on the plating of the lytic phage Mu.KF phage such that, these two lysates plate on the *E. coli* C host strain with an e.o.p. decreased by a factor of 10^{-2} (see Table XI, p 81).

5.2 The Plating of Heat Induced Mu.KFC Onto Various Host Strains

The lytic preparation of phage Mu.KF (Mu*cts*61, MH_{4400} and JP_2) were plated onto *E. coli* C for the purposes of isolating *E. coli* C lysogens. These lysogens were heat induced and plated onto the following hosts: *E. coli* C, *E. coli* K12 and *C. freundii*

Two lysogens of *E. coli* C (Mu *cts*61) number 1 and number 2 were isolated from a single plaque. Surprisingly, as outlined below, the phage released by these two lysogens differed in their phenotype. Heat induced lysates from both Mu*cts*61 KFC lysogens plated on *E. coli* C, e.o.p. 1 and on *E. coli* K12, e.o.p. 2.0 - 8.5. However, lysates derived from *E. coli*C lysogen number 1 plated on *C. freundii* with an e.o.p. of 1×10^{-1}, while lysates from *E. coli* C lysogen number 2 did not plate on *C. freundii* (e.o.p. $< 5 \times 10^{-8}$), (see Table XIII). Previously we have reported that *E. coli* C lysogens of Mu*cts*61 also exhibited two classes of phage with regard to their plating behaviour on *C. freundii* . The Mu.KFC lysogen number 1 as described above exhibits the same plating behaviour reported for phage Mu.KC (Mu*cts* 61) lysogen numbers 5, 6 and 7. While the Mu.KFC lysogen number 2 exhibits a plating behaviour similar to that observed with lysates of phage Mu.KC numbers 1, 2, 3, 4 and 8.

Rice isolated a single lysogen of *E. coli* C (Mu.KFC). Lytic lysates derived from this lysogen also plated in a similar manner to Mu.KFC number 1 and Mu.KC numbers 5, 6 and 7, in possessing the ability to plate on *C. freundii* (e.o.p. 10^{-1} -10^{-2}). Rice neutralised this Mu.KFC phage with the anti W.C serum and plated the phage on *E. coli* C. The serum neutralised the phage with a relative activity of 0.1 - 0.12 (20-25%) instead of an expected relative

Lysates by heat induction of *E. coli* C lysogens	Efficiency of plating (e.o.p.) on hosts		
	E. coli C (518c)	*C. freundii* (G331)	*E. coli* K-12 (PB1395)
Muc*ts*61.KFC number 1	1	1×10^{-1}	2.0 - 8.5
Muc*ts*61.KFC number 2	1	$<5 \times 10^{-8}$	3.0 - 5.0
MH_{4400}.KFC numbers 2 & 3	1	$<2 \times 10^{-8}$	4.5×10^{-4}
JP_2.KFC numbers 1, 2 & 3	1	3×10^{-8}	$<2 \times 10^{-8}$

TABLE XIII: The plating of heat induced Mu.KFC lysogens (Muc*ts*61, MH_{4400} and JP_2) on various host strains.

neutralising activity of 1. The Mu.KC lysogen number 5 also showed a similar neutralising activity (0.1) as well as a similar plating pattern on *C. freundii* as Rice observed with his Mu.KFC lysate.

The heat induced MH_{4400} *gin*$^-$, KFC phage plated on *E. coli* C (e.o.p. 1) but on *E. coli* K12 with an e.o.p. 4×10^{-4}. The reduced e.o.p. of this phage on *E. coli* K12 as compared to that for Mu*cts*61.KFC is presumably due to the *gin*$^-$ mutation of MH_{4400}; the fact that it is as high as it is, is no doubt due to the leakiness of the *gin*$^-$ mutations, but nevertheless accounts for the reduced frequency of G inversion as compared to the wild type phage (here Mu*cts*61). The two lysates of heat induced MH_{4400}.KFC do not plate on *C. freundii* (e.o.p. $< 2 \times 10^{-8}$), these lysates are similar in this respect to Mu.KC lysogens numbers 1, 2, 3, 4 and 8 (see Table XII, and Table VII, p 68).

Two *E. coli* C JP_2 lysogens (Mu.KFC) were isolated. The heat induced lysate from these lysogens plated on *E. coli* C (e.o.p. 1), but these phage did not plate on *E. coli* k12 in keeping with the JP_2 phenotype; a low e.o.p. (3×10^{-8}) was observed on *C. freundii* (see Table XIII). The JP_2.KFC lysates exhibited the plating pattern associated with Mu.KC lysogens 1, 2, 3, 4 and 8, in not plating on *C. freundi*. When heat induced JP_2.KC lysates were plated on *C. freundii* the e.o.p. was 0.2 - 2.0, i.e., similar to the alternate group of lysogens that do plate on *C. freundii* (see Table XIII, and Table VII, p 68).

CHAPTER 6

NEUTRALISATION OF NONHOMOLOGOUS PHAGE BY ANTISERA; AND THE KINETICS OF THE NEUTRALISATION BY ANTI G(-) MH_{4400} SERUM OF THE HOMOLOGOUS PHAGE

6.1 Reactions between Antisera and Nonhomologous Phage

In determining the specificity of the antisera used by Rice and those used in this study, some cross reactions were observed between the antisera and nonhomologous phage. The anti W.C serum and the anti G(+) Mu.K serum used in this study were prepared and characterised by Rice. Rice observed that the anti G(+) Mu.K serum did not neutralise the W.C lytic phage (plated on *E. coli* C), that is, that it had a neutralising activity 0. The anti W.C serum, however neutralised the G(+) Mu.K lytic phage, (plated on *E. coli* K12) with a relative neutralising activity 0.05, i.e. with a relatively low rate of neutralisation (see Table XIV).

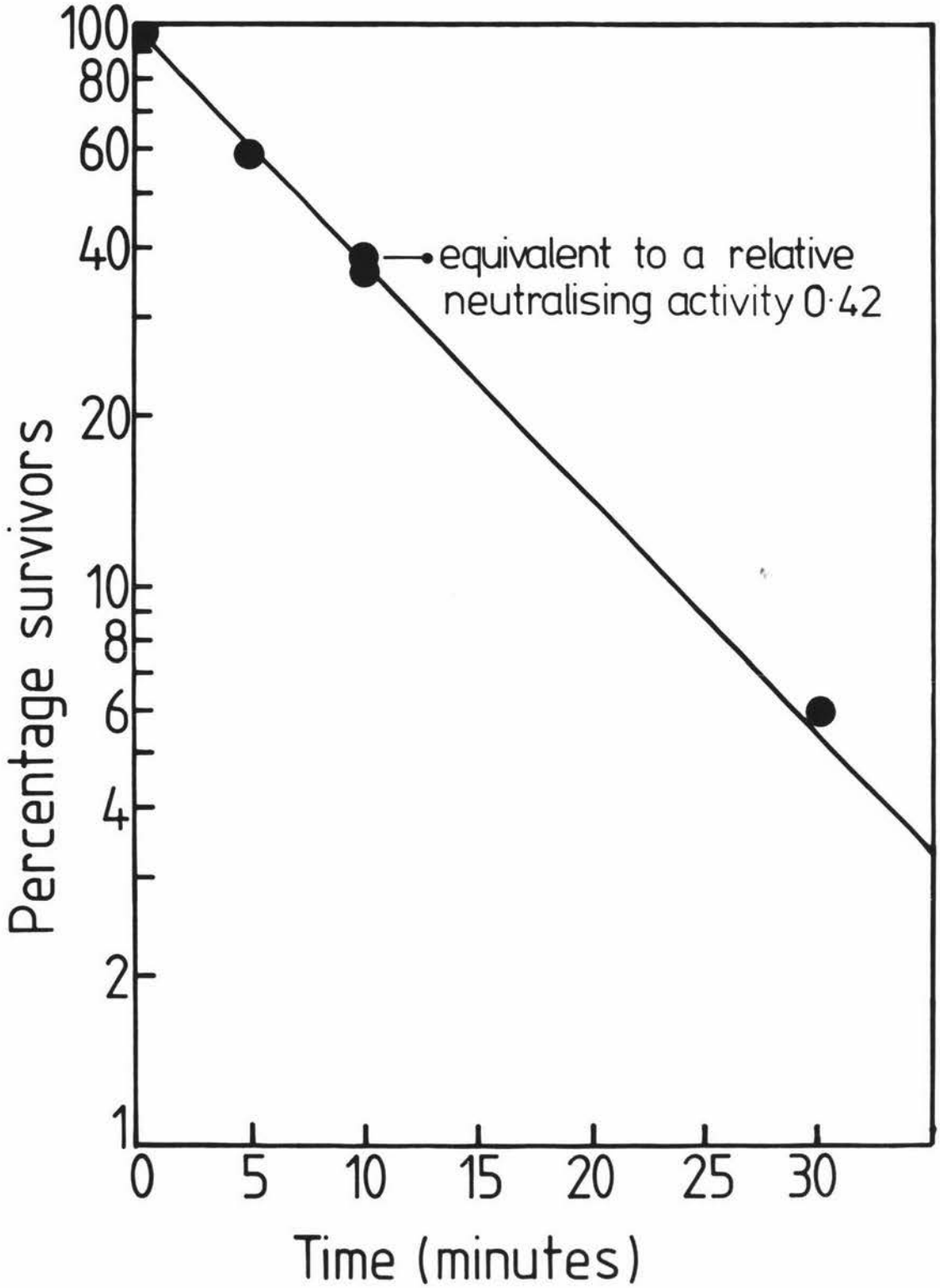
The anti G(-) MH_{4400} serum neturalises the Mu.K G(+) induced phage (plated on *E. coli* K12) with a relative neturalising activity of 0.1. In the reciprocal neutralisation, the anti G(+) Mu.K serum neutralised the G(-) MH_{4400} lytic phage (plated on *C. freundii*) with a relative neutralising activity of 0.2. However, the anti G(+) W.K serum neutralised this phage at a greater rate, with a relative neturalising activity of 0.42 (see Table XIV and Figure 12). The W.K. phage is thought to be identical or closely related to Mu.K phage.

The anti G(-) MH_{4400} serum did not neturalise the W.C lytic phage (plated on *E. coli* C). In the reverse neutralisation, anti W.C serum against the G(-) MH_{4400} lytic phage, plated

Antisera	Phage	Relative neutralising activity	Host plated on
anti G(+) Mu.K serum	W.C lytic	0	*E. coli* C (518c)
anti W.C serum	G(+) Mu.K lytic	0.05	*E. coli* K12 (PB1395)
anti G(-) MH_{4400} serum	G(+) Mu.K induced	0.1	*E. coli* K12 (PB1395)
anti Mu.K G(+) serum	G(-) MH_{4400} lytic	0.2	*C. freundii* (G331)
anti W.K. (G+) serum	G(-) MH_{4400} lytic	0.42	*C. freundii* G(331
anti G(-) MH_{4400} serum	W.C lytic	0	*E. coli* C (518c)
anti W.C serum	G(-) MH_{4400} lytic	0.1	*C. freundii* (G331)

TABLE XIV: The relative neutralising activity of the cross neutralisation between antisera and non homologous phage.

Fig 12. Anti G(+) W.K. serum against lytic G(−)MH_{4400}.KF, plated on *C.freundii* (G 331)



on *C. freundii*, the phage was neutralised slowly with a relative neutralising activity of 0.1 (see Table XIV).

From these results, it appears that the 'K' tail and the 'C' tail forms are serologically distinct and that the 'F' tail and 'C' tail forms are also serologically distinct. However, from the observed cross neutralisations it is also clear that while the 'F' tail and the 'K' tail forms are distinct they are serologically related.

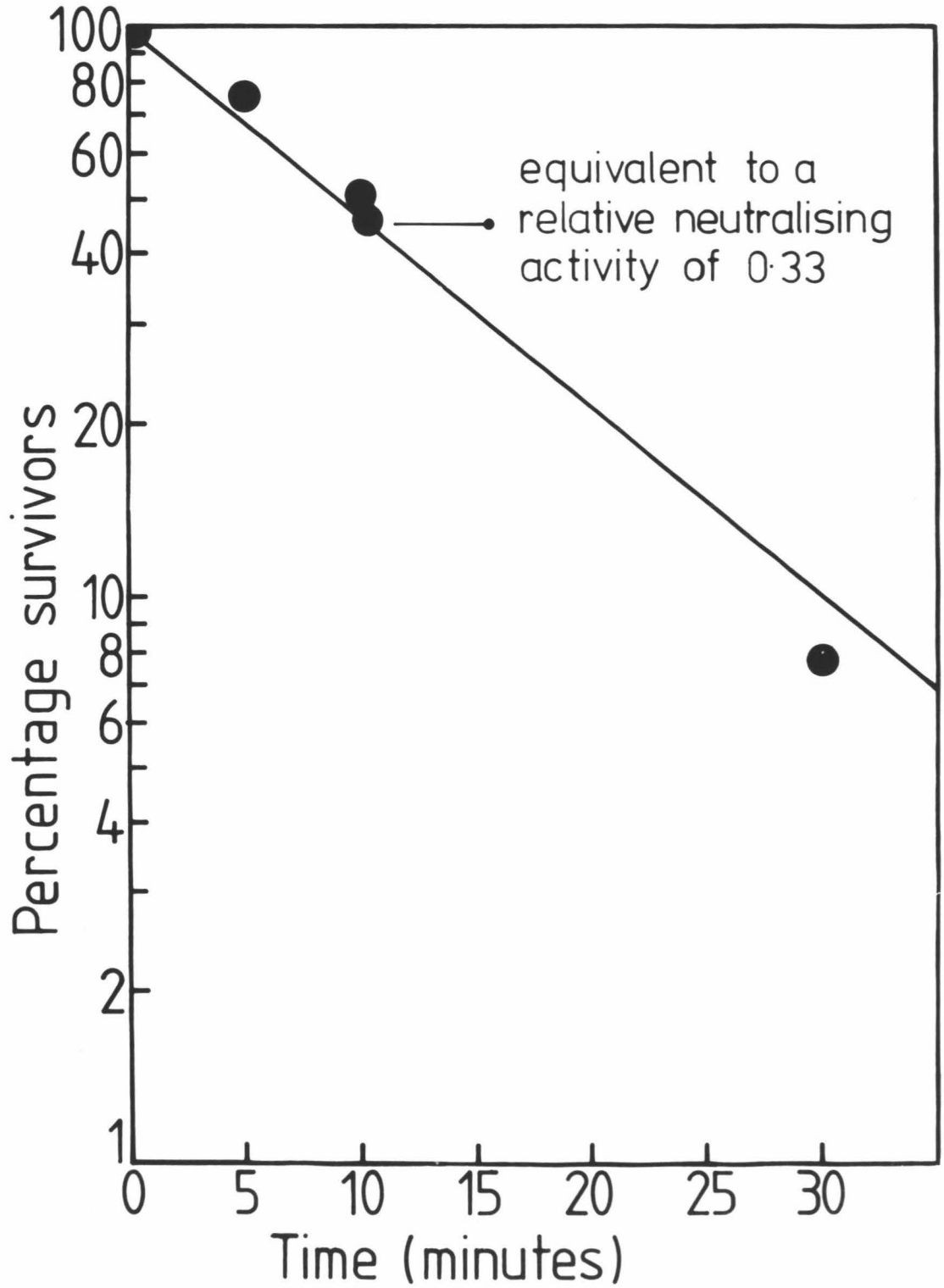
The low levels of cross reaction between the various antisera and the nonhomologous phages may result from antisera recognising common phage tail antigenic determinants shared by the different tail types ('K', 'F' and 'C'). In this context, it has been established (Howe *et al*, 1979, Van de Putte *et al*., 1980 and Giphart-Gassler *et al*., 1981) that, in phage Mu G(+), two genes S and U code for two tail proteins, S and U; inversion of the G region leads to the expression of two different genes, S' and U'. This change in components is correlated with the change in host specificity associated with the G region inversion from G(+) to G(-). More recently Van de Putte's group (1982)* reported on his genetic studies which lead him to propose a model in which the S gene lies partially outside the G region, an Sc gene fragment, and partially inside the G region, an Sv gene fragment. When the G region inverts the Sc fragment becomes associated with an Sv' gene fragment. In this proposed "split genes" the Sc fragment carries the promoter for transcription. In summary the S gene can be represented as Sc + Sv (G(+) orientation) and the S' gene as Sc + Sv' (G(-) orientation). That is, that the constant S region, Sc, is joined by G inversion to one of two variable gene portions (Sv or Sv'); this results in the synthesis of either a tail protein S or S' of the 'K' or 'F' tail. If this is so, the Sc polypeptide fragment would be common to both the 'F' and 'K' tails. The cross neutralisation between these two tail forms, 'K' and 'F', may result from the antisera recognising antigenic determinants coded by the shared Sc portion of the S and S' genes.

6.2 The Kinetics of Neutralisation by the Anti G(-) MH_{4400} Serum at One Third Standard Dilution

The anti G(-) MH_{4400} serum, normally diluted 1/320, neutralises 90% of the homologous phage in 10 minutes, relative neutralising activity 1. One would expect that if the serum was neutralising the phage normally that when the standard dilution of serum (1/320) was diluted a further 3 fold, then the relative neturalising activity would be equivalent to a third, i.e., (0.33); and that the phage would be neutralised to the same level as with the standard dilution of antisera in three times the normal time i.e. 90% in 30 minutes.

The anti G(-) MH_{4400} serum was diluted 1/960 and titrated against the G(-) MH_{4400} lytic phage; the relative neutralising activity was equivalent to 0.33, and 90% of the phage was neutralised in 30 minutes (see Figure 13). Thus indicating that the neutralisation of phage by this serum is not affected, beyond what would be expected, by dilution.

Fig 13. Anti G(-) MH_{4400}.KF serum at 1/3 standard dilution against lytic G(-) MH_{4400}.KF, plated on *C. freundii* (G 331)



CHAPTER 7

GENERAL DISCUSSION

7.1a Specific Antisera Against the Different Phage Mu Host Types:

With the aim of obtaining a specific antiserum to the Mu.KF G(-) phage, mutants of phage Mu plating solely on *E. coli* K12 i.e. G(+) (JP_1, JP_{5a} and JP_8) or on *C. freundii* i.e. G(-) (JP_2) were isolated.

An attempt was made to invert the G region of the mutants with the G(+) phenotype i.e., JP_1, JP_{5a} and JP_8, and also the MH_{4400} *gin*$^-$ G(+) phage. This was to be accomplished with a 'helper' Mu *gin*$^+$ prophage carried on a F' plasmid. Inversion of the G region was not observed in the JP_1, JP_{5a} and JP_8 isolates. However, the G region of a MH_{4400} *gin*$^-$ lysogen isolated carried an inverted G region yielding a MH_{4400} *gin*$^-$ G(-) phage.

In order to raise an antiserum to the Mu.KF tail configuration, lytic phage preparations of JP_2 and the MH_{4400} *gin*$^-$ G(-) phage (on *C. freundii*) were used as antigens in the immunization of rabbits. One of the resulting sera, the anti MH_{4400} Mu.KF G(-) serum proved to be specific to the Mu.KF phage. This serum did not neutralise the W.C phage, relative neutralising activity 0, and showed a low rate of neutralisation of the Mu.K phage, relative neutralising activity 0.1.

The mutants of phage Mu plating solely on *E. coli* K12 or solely on *C. freundii* could represent three possible genotypes: -

1) carrying a mutation in the *gin* gene, *gin*$^-$, such that the *gin* gene product is defective and the G region is not inverted, i.e., it remains 'fixed' in the G(+) or G(-) orientation; only G(+) or G(-) phage are released from heat induced lysogens;

2) in which the terminus of the G region has been modified and although the *gin* gene is functional the *gin* gene product does not invert the G region due to the modified terminus; and

3) one which carries a defective 'F' tail gene, such that, although the G region is undergoing inversion and both Mu G(+) and Mu G(-) phage are produced, the 'F' tail is defective and no longer adsorbs to its host strain, *C. freundii*.

The *gin*$^-$ genotype (see number 1 above) can be identified: in this case the G region will be inverted by a helper Mu *gin*$^+$ prophage; once the helper Mu prophage is removed, the isolates recovered will have the G region fixed in one orientation, or the other, i.e., both Mu *gin*$^-$ G(+) phage and Mu *gin*$^-$ G(-) phage will be recovered. Heteroduplex studies of the *gin*$^-$ mutants would show an absence of G inversion loops i.e., G inversion is not taking place.

Identification of the second genotype with a modified terminus which prevents the G region from undergoing inversion, is also possible. In this case orientation of the G region will not be altered in the presence of 'helper' Mu *gin*$^+$ prophage and heteroduplex studies of these mutants would indicate that no inversion of the G region is occurring.

The third genotype, one that produces Mu G(-) phage with defective tails such that the phage no longer adsorbs to its G(-) host strain, could also be identified. Presence

of the Mu *gin*$^+$ helper phage will not alter the host range phenotype of these mutants; these mutants already carry a functional *gin* gene. However, hetroduplex studies of a heat induced lysate of these mutants would provide evidence that the G region is undergoing inversion.

In tests for neutralisation of nonhomologous phage by the different antisera, low levels of cross neutralisation were observed in some instances. The level of cross neutralisation varied, depending on the serum used. In particular cross neutralisation was observed in the case of the anti Mu.KF G(-) serum versus the Mu.K phage and again in the case of the anti Mu.K G(+) serum versus the Mu.KF phage. These observations may be due to common antigenic determinants in the tail region of both these phage forms.

It can be concluded that the three tail forms, Mu.K, Mu.F and Mu.C, which may be expressed by phage Mu are serologically distinct, although the Mu.K and Mu.F forms are serologically related and this latter observation may not be unexpected in view of the evidence, which Van de Putte's group (1982)* have presented for a "split gene" coding for a tail component present in both of these latter phage forms.

7.1.b Plating of Phage Mu on *E. coli* Strain C

A model was proposed by Rice (1980) in which the G region of phage Mu was required to be in the G(-) orientation as a first but not in itself sufficient requirement for phage Mu to plate on *E. coli* C. This model was supported by the low efficiency of plating of Mu.K lysates (predominately G(+) phage) on *E. coli* C, e.o.p. 10^{-8}, compared to the efficiency of plating of lytic Mu.KF (predominately G(-) phage) on *E. coli* C, e.o.p. 10^{-4}-10^{-5}. The observations made by Van de Putte *et al.* (1980) also supported this model;

* see Giphart-Gassler *et al* (1982)

these workers reported that the heat induced Mu.K lysates (G(-) and G(+) phage particles) plated on *E. coli* C, e.o.p. 4×10^{-5}, and that the heat induced Mu.K *gin*$^-$ (G(+)) lysates failed to plate on this host.

It should be noted, however, that the titre of the heat induced Mu.K lysates on *E. coli* C was observed to be highly variable.

During the present study we observed that heat induced lysates of Mu.K plated on *E. coli* C with a very low e.o.p., 1.7×10^{-11} and also it was noted that the heat induced lysates of phage Mu.KF (Mu*cts* 61, JP_2 and MH_{4400}) with predominantly G(-) phage, did not plate on *E. coli* C at the levels examined.

From the above observations we are proposing that, in order to propagate on *E. coli* C it is necessary for the G region of phage Mu to be in the (-) orientation and that an additional event must take place during lytic propagation of the phage. This second event does not appear to occur, or does so with very low frequency, during the propagation of heat induced phage in *E. coli* K12 and *C. freundii*.

Van de Putte (1980) observed that during lytic phage propagation of Mu on *C. freundii* inversion of the G region appears to be taking place with higher frequency as compared to the frequency during lytic propagation of Mu on *E. coli* K12. Evidence for this was provided by the relatively high efficiency of plating of lytic Mu.KF lysates on *E. coli* K12, e.o.p. 2.6×10^{-3}. In plating lytic Mu.KF lysates on *E. coli* K12 we observed an even higher e.o.p. 10^{-1}. To further support this conclusion we observed that the lytic and induced lysates of MH_{4400} *gin*$^-$ Mu.KF showed a decrease in e.o.p. on *E. coli* K12 as compared to the wild type Mu *cts* 61 i.e., wild type with regard to the *gin* gene.

However, when plating the lytic mutant Mu.KF phage MH_{4400} and JP_2, onto *E. coli* C, a decreased e.o.p. was observed, 1.6×10^{-7}, as compared to the wild type Mu.KF. The mutations carried by these two phage appear to affect the efficiency of plating of Mu on this host strain, by some unknown mechanism.

Rice (1980) isolated Mu.KC phage from lytic Mu.K lysates by plating these lysates on *E. coli* C. These Mu.KC phage were neutralised by the anti W.C serum to the same extent as the homologous phage (relative neutralising activity 1), but were not neutralised by the anti W.K or anti Mu.K sera. (The phage isolated by Rice will be referred to as Mu.KC.) However, the Mu.KC phage isolated in the present study, from heat induced Mu.K lysates were observed to differ significantly from the Mu.KC phage isolated by Rice. The Mu.KC phage isolated in this study fell into two distinct classes. Firstly, those phage (lytic or induced) that did not plate on *C. freundii*, e.o.p. $<2.4 \times 10^{-7}$; this case includes Mu.KC numbers 1, 2, 3, 4 and 8, and will be referred to as Mu.KC''. Secondly, those phage (lytic or induced) that do plate on *C. freundii*, e.o.p. $10^{-2} - 5 \times 10^{-1}$; this class includes the Mu.KC numbers 5, 6 and 7, and will be referred to as Mu.KC'''.

The lytic Mu.KC number 1 phage from the Mu.KC'' class is not neutralised by the anti W.C serum, relative neutralising activity 0. The lytic Mu.KC number 5 phage, from the Mu.KC''' class is neutralised by the anti W.C serum with a relative neutralising activity of 0.1.

Both classes Mu.KC'' and mu.KC''' plated on *E. coli* K12 (PB1395) with an e.o.p. for lytic lysates of 10^{-2}-10^{-3}, and for induced lysates of 2.0 - 8.5.

In summary: we attempted to isolate the Mu.KC phage that had been isolated by Jamieson and again by Rice. However, by the selection procedure applied we derived two additional classes of Mu.KC phage; these differed from each other on the basis of their plating ability on *C. freundii*, and differed from the Mu.KC phage on the basis of the neutralisation reaction with the anti W.C serum.

The Mu.KFC phage isolated in this study also formed two distinct groups, on the basis of their plating on *C. freundii*. Firstly, a Mu.KFC phage that does not plate on *C. freundii*; this group is represented by the Mu.KFC phage isolate number 2, and MH_{4400}.KFC isolates numbers 2 and 3. This class is referred to as the Mu.KFC'' and should be compared with the Mu.KC'' class above. Secondly, a Mu.KFC phage that does plate on *C. freundii*; this group is represented by the Mu.KFC phage isolate number 1 and the JP_2 KFC phage isolates, numbers 2 and 3. This class is referred to as Mu.KFC''' and should be compared to the Mu.KC''' class above.

These two classes of Mu.KFC phage appear to resemble the two classes of Mu.KC phage, MuKC'' and Mu.KC'''. However no neutralisation tests on the Mu.KFC phage with the anti W.C serum were carried out.

Rice towards the end of his work isolated one Mu.KFC isolate. He found that lytic lysates of this phage plated on *C. freundii* (e.o.p. 10^{-1} -10^{-3}), and that this phage was not fully neutralised with the anti W.C. serum, relative neutralising activity of 0.1. There is a strong possibility that the Mu.KFC Rice isolated was part of the Mu.KFC''' series; the Mu.KC''' and Mu.KFC''' parallel each other as judged by their plating ability on *C. freundii*.

It appears that there are three or more events (mutational) which yield phage plating on *E. coli* C, formally phage

Mu.KC (or Mu.KFC). Some of these derivates, particularly Mu.KC'' and Mu.KC''' may be host range mutants of either Mu.K G(+) phage or Mu.K G(-) phage respectively. This last possibility may now be substantiated, by the use of the anti Mu.K G(+), anti W.C and anti Mu.KF G(-) sera; the specific anti Mu.KF G(-) serum was prepared in this study.

In the above we have maintained that the different plating patterns of the Mu.KC (and Mu.KFC) phage on *C. freundii* has resulted from a single event which, at the outset, permitted the phage to propagate on *E. coli* C. However it should be noted that lysogens Mu.KFC number 1 and Mu.KFC number 2 (e.o.p of induced lysates on *C. freundii* 1×10^{-1} and $<5 \times 10^{-8}$, respectively) were derived from a single plaque (see p.), and as such we must assume that the initial event which permitted propagation on *E. coli* C was the same for <u>both</u> of these isolates. This raises the question whether the difference in the observed phenotype represents a second event in one or other of these isolates.

The induced Mu.KFC lysates, reported in this study plate on *E. coli* K12 with an e.o.p. of 2.0 - 8.5, similar to the Mu.KC'' and Mu.KC''' lysates. The lytic Mu.KC lysates, i.e., Mu.KC'' and Mu.KC''' plate on *E. coli* K12 with an e.o.p. of 10^{-2}-10^{-3}. The plating of the Mu.KC and Mu.KFC lysates on *E. coli* K12 could be the result of a G or G like inversion, comparable to that seen in Mu lysogens of *E. coli* K12, that is, with induced lysates plating with a higher efficiency on the second host, here *E. coli* K12, than do lytic lysates.

The plating of the Mu.KFC (MH_{4400} *gin*$^-$) lysates support this proposal. The e.o.p. of the induced lysates of this phage on *E. coli* K12 is 4.5×10^{-4}, a decrease in plating efficiency presumably due to a reduced frequency of G inversions. The Mu.KFC JP_2 phage does not plate on *E. coli*

K12, in keeping with the JP_2 phenotype.

The specific antisera used in this study, the anti W.C, anti Mu.K and the anti Mu.KF sera, could in future be used to further classify the phage isolates and their plating behaviour. For example, the use of specific antisera would permit differentiation between host range mutants and alternating host specificity.

Finally, throughout this study it has been observed that lytic and induced Mu lysates, propagated on the same host, vary in their plating ability on alternative bacterial hosts.

It has been noted by Van de Putte *et al.*, (1980), observed by Rice (1980) and again in the present study that lysates of phage Mu propagated lytically on *E. coli* K12 plate on *C. freundii* with a low e.o.p., 2.5×10^{-9} in this study, and Mu.K induced lysates plate on *E. coli* K12 and *C. freundii* with approximately equal e.o.p. of 1.

We have observed that the mode of phage propagation, lytic or induced, of phage Mu on *E. coli* C and *C. freundii* affects the plating ability of these lysates. The Mu.KF lytic lysates plate on *E. coli* K12 with an e.o.p. of 10^{-1} and on *E. coli* C an e.o.p. of 2×10^{-5}. However the Mu.KF induced lysates plate on *E. coli* K12 with an e.o.p. of 10^{1}, but these lysates have not been observed to plate on *E. coli* C, e.o.p. $<2.5 \times 10^{-9}$.

Also in this study, phage Mu propagated lytically on *E. coli* C i.e., Mu.KC'' and Mu.KC''', plate on *E. coli* K12 with an e.o.p. of 10^{-2}-10^{-3}, the induced Mu.KC'' and Mu.KC''' lysates plate on *E. coli* K12 with an e.o.p. of 2.0 - 8.5. However both lytic and induced lysates of phage class Mu.KC''' plate on *C. freundii* with approximately the same e.o.p., 10^{-1} -10^{-2}. While the Mu.KC'' phage lysates, lytic or induced, were not observed to plate on *C. freundii*.

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