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

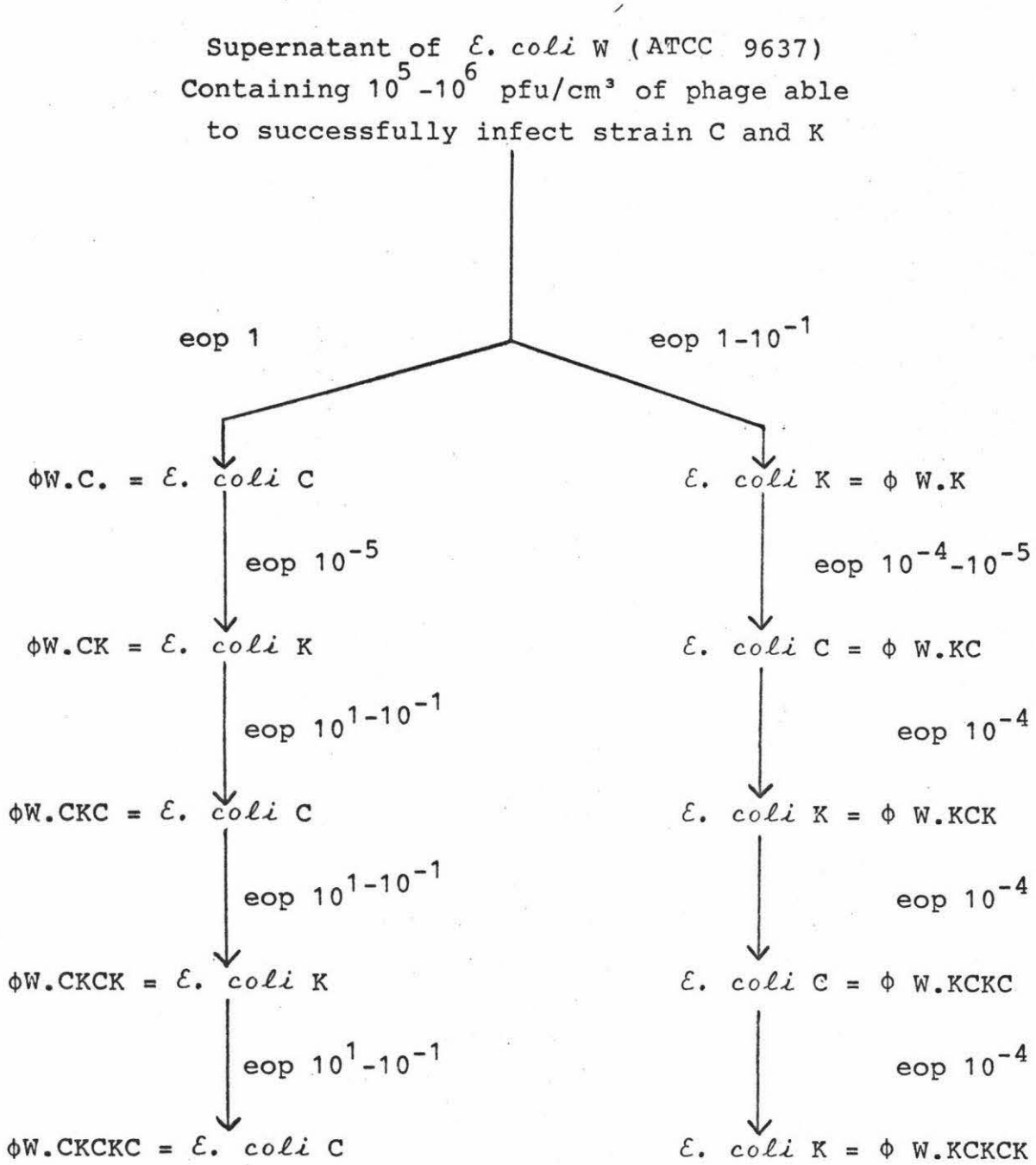

FIGURE 1: Plating efficiencies of phage from the supernatant of *E. coli* W on *E. coli* strains C and K.

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.

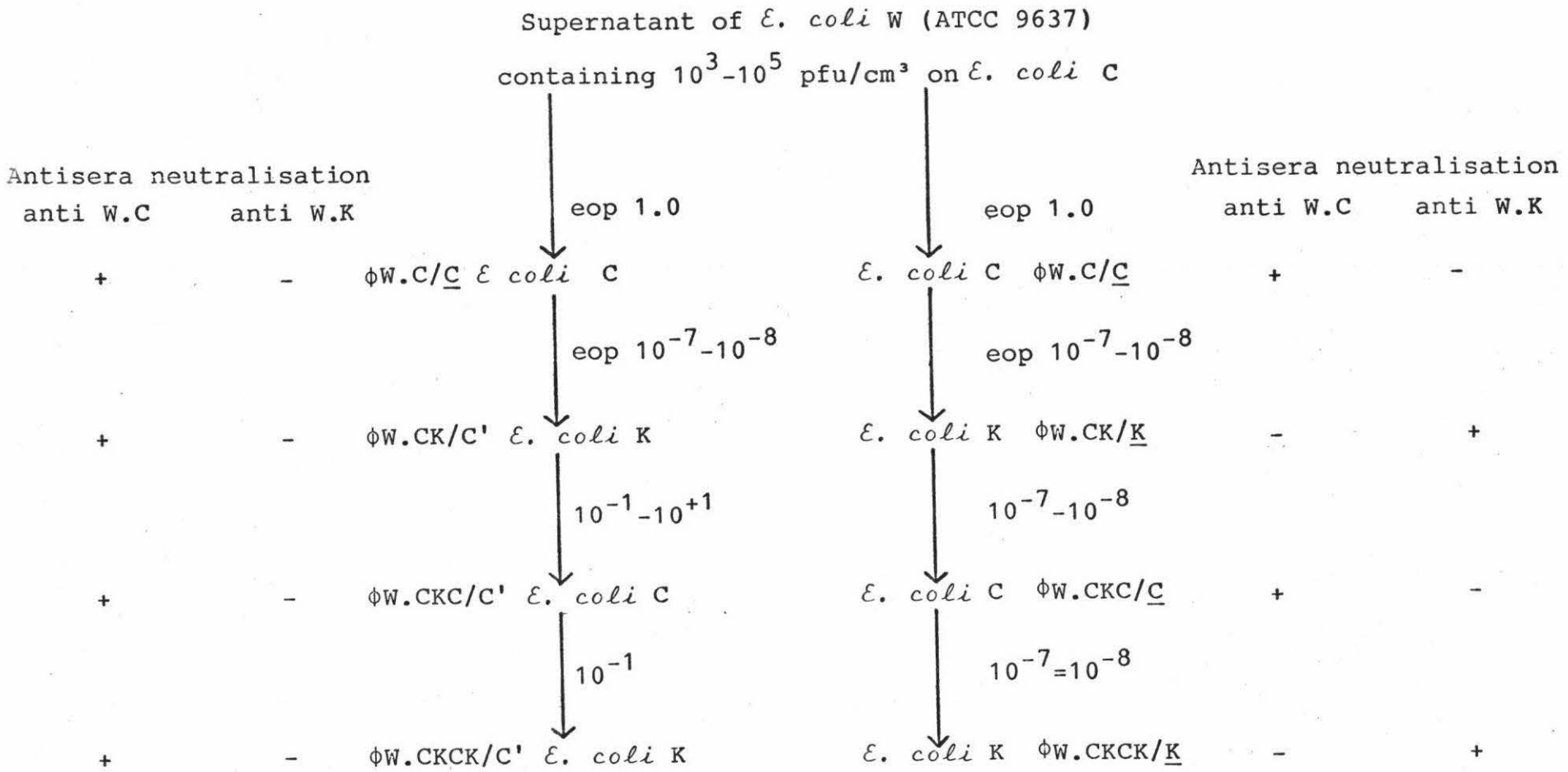

FIGURE 2: The plating efficiency and adsorption of φ W.C on *E. coli* strains C and K. To the left exhibiting the host range mutation plating behaviour, and to the right exhibiting alternate host specificity plating behaviour, as observed by Rice (1980).

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.

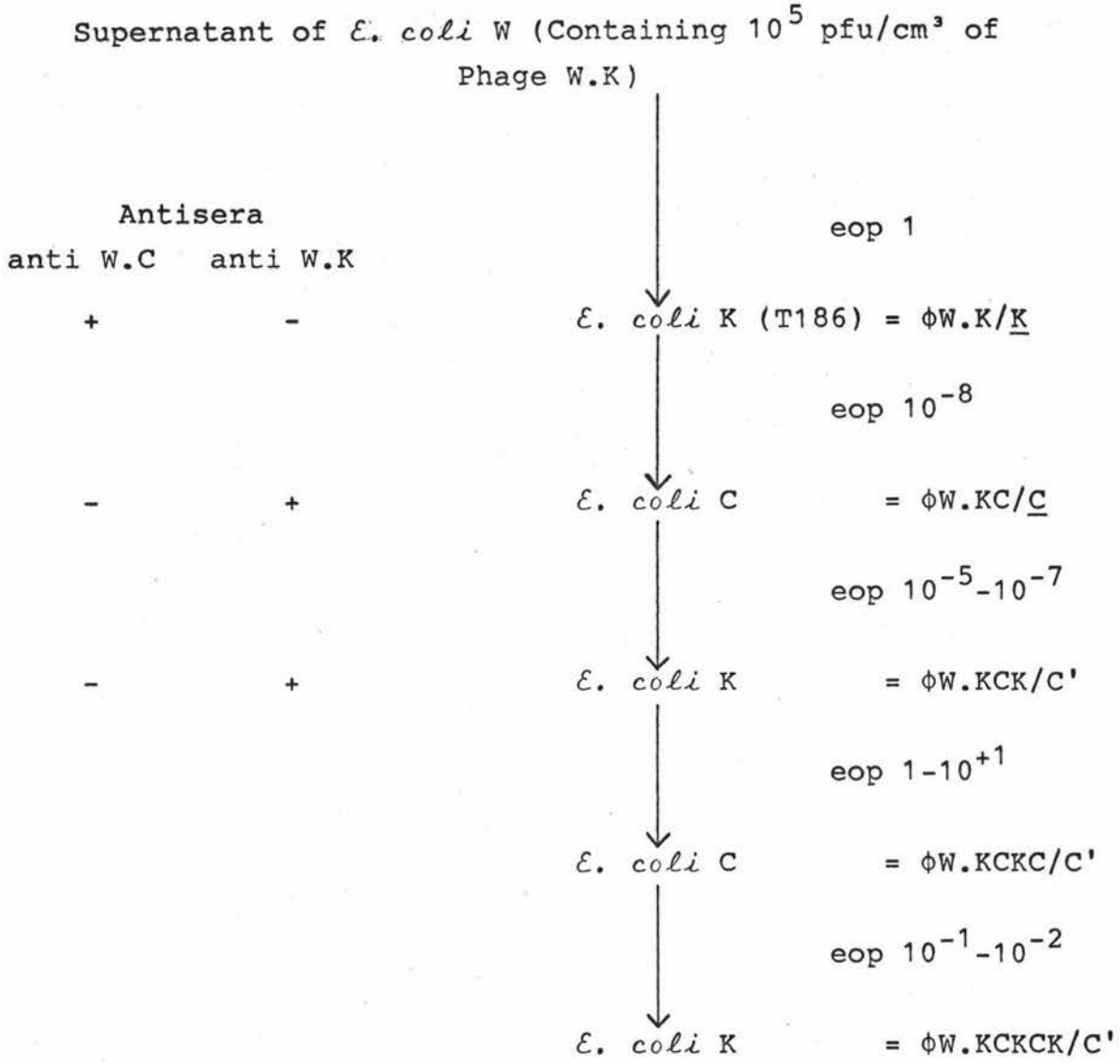

FIGURE 3: The efficiency and adsorbtion of ϕW.K to *E. coli* strains K and C, as observed by Rice (1980)

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.