The Restriction of Bacteriophage λ in *Escherichia coli* Strain w

By G. KERSZMAN*, S. W. GLOVER AND J. ARONOVITCH†

*Medical Research Council, Microbial Genetics Research Unit,
Hammersmith Hospital, Ducane Road, London, W. 12.*

*(Accepted 15 March 1967)*

**SUMMARY**

*Escherichia coli* strain w adsorbs phage λ very efficiently but the phage does not form plaques on this strain. In a very small fraction (10⁻⁴) of the infected cells the phage grows and produces small bursts of progeny phage also unable to form plaques on strain w. *E. coli* strain w is lysogenic for a temperate phage, *wφ*, related to phage *φ2*. Non-restricting hosts for phage λ became restricting hosts when made lysogenic for *wφ*. When *32P*-labelled λ adsorbed to restricting *wφ* lysogenic hosts, > 20% of the *32P* become acid-soluble shortly after infection. No *wφ* specific modification was carried by the small number of λ phages which escaped this restriction process. It is concluded that *wφ* controls a host-restriction mechanism but not a host-modification process, and in parallel with other examples of host-controlled restriction and modification can be represented as *r+m* or *r+m°*. λw mutants have been isolated which escape this restriction and which form plaques on strain w and *wφ* lysogenic strains with an efficiency of 1-0. With these mutants a *w*-specific host modification controlled by the genome of strain w was demonstrated. Mixed infection experiments with restricted λ and unrestricted λw showed that that restricted phage did not block the growth of the unrestricted mutant nor did the mutant permit the restricted phage to grow. In addition it was shown that λ obtained from bacteria mixedly infected with λ and λw was still unable to grow in restricting hosts and λw similarly obtained from mixedly infected bacteria still retained its ability to grow on restricting hosts. It is concluded that there is a nucleotide sequence in the DNA of phage λ which, when λ infects a restricting host, is specifically recognized by the restriction mechanism controlled by the *wφ*. The mutation to λw involves an alteration to this sequence such that it is no longer recognized by the restriction mechanism of the *wφ*.

Mutants of *wφ* were isolated not restrictive for phage λ.

**INTRODUCTION**

In recent years many aspects of the processes of restriction and modification which can occur when phage λ infects *Escherichia coli* have been clarified (reviewed by Arber, 1965a). The process of modification acts directly on DNA and probably takes the form of specific alteration of certain base sequences, perhaps by methylation (Arber, 1965b; Klein & Sauerbier, 1965; Arber & Smith, 1966). Therefore DNA synthesized in a particular strain may be provided with a characteristic modification pattern. Restriction is a process which can occur when foreign DNA enters a cell.

* Permanent address: Department of Microbiology, University of Lodz, Nowotki 18, Lodz, Poland.
† Permanent address: Department of Bacteriology, Hadassah Medical School, The Hebrew University, Jerusalem, Israel.
If this foreign DNA does not bear a modification acceptable by the recipient cell it may be rapidly degraded shortly after entry at a site near the cell surface (Dussoix & Arber, 1962; Schell & Glover, 1966a).

In normal wild type strains which exert host-controlled modification the processes of restriction and modification are such that these strains accept DNA bearing their own modification pattern. In several laboratories mutant strains have been isolated which either have lost the ability to restrict but are still able to modify DNA, or have lost both the ability to restrict and to modify DNA. If the phenotype of wild type strains is represented as r+m, indicating their ability to restrict and to modify DNA, then the phenotypes of these mutants can be represented as r−m+ and r−m− (Glover et al. 1963; Wood, 1966; Lederberg, 1966). An interesting point about all these investigations is that among the mutants of phage P1, Eschericha coli K and E. coli B selected for their r− phenotypes there are roughly equal numbers which are m+ and m−. That is, the mutations leading to the double phenotypic change r−m− occur as frequently as the mutations leading to the single change r−m+. The genetic location of both these mutations has been determined in E. coli K (Colson et al. 1965). Another possible class of mutants would have the phenotype r+m−. It can be argued that these would be lethals because they would be able to degrade their own DNA. However, in the light of recent experiments which locate the site of action of the restricting enzymes near the cell surface this does not appear to be a very convincing argument (Fukasawa, 1964a; Molholt & Fraser, 1965; Schell & Glover, 1966c). No such mutants have been reported in phage P1, E. coli K or E. coli B, although it should be noted that there is no obvious selective system available for isolating m− phenotypes. Attempts to isolate such mutants by a simple screening method without selection have been unsuccessful (Glover, unpublished). Therefore the occurrence in nature of strains which, superficially at any rate, behave like r+m− is of considerable interest. An obvious characteristic of such strains, provided that they are not lethal, is that they will not propagate phage. The small fraction of bacteria which yield phage after the first cycle of infection will be unable to exert host modification so that subsequent infections will be unsuccessful. If sufficient phage is tested on such a strain the bacteria may be killed but dilutions of the phage will produce no plaques. It has been observed that phage λ behaves in this way when plated on E. coli w. In this paper we describe the characteristics of the restriction of phage λ in this strain which is controlled by a temperate phage wφ (Pizer, Miovic & Pylkas, 1967; Glover & Kerszman, 1967); and give an account of the properties of some λ mutants which have been isolated which are able to form plaques on E. coli w.

METHODS

Bacteria. Escherichia coli w (Davis, 1950); E. coli c (Bertani & Weigle, 1953); E. coli K is strain c 600 (Appleyard, 1954); E. coli K r−m− (Colson et al. 1965); E. coli c (p2) strains kindly supplied by Dr B. Kelly; Hfr derivatives of E. coli c kindly supplied by Dr G. Bertani.

Bacteriophages. Phage λ, a clear plaque mutant λc and a virulent mutant λv (Jacob & Wollman, 1954); phage P1 (Lennox, 1955); phage P2 kindly supplied by Dr G. Bertani; phage wφ isolated from Escherichia coli w (Glover & Kerszman, 1967); phage λsus mutants kindly supplied by Dr R. Thomas.

Media. (a) Media for phage λ (Arber & Dussoix, 1962). (b) H medium (Stent
Restriction of phage A in E. coli w & Fuerst, 1955) with phosphorus supplied only by 0.05% casamino acids, pH 7.2.

(c) Other media and buffers (Glover, 1962).

Phage techniques. The general phage techniques used were described by Adams (1950). Special techniques relating to phage λ were described by Arber (1960).

Preparation of ³²P-labelled phage λ. Approximately 2 mc of ³²P as Na₂HPO₄ (specific activity 219 mc per mm) were added to 100 ml of H medium. A sample of the medium was inoculated with Escherichia coli c and aerated until the culture reached 4 x 10⁷ bacteria per ml. Phage λv.c was added at a multiplicity of 0.05 and aeration continued until lysis occurred. The phage was purified by alternate low-speed (5000 g for 15 min.) and high-speed (70,000 g for 90 min.) centrifugation using unlabelled carrier phage.

RESULTS

The growth of phage λ in Escherichia coli strain w

It was initially observed that although phage λ adsorbs very efficiently to Escherichia coli w it cannot form plaques on this strain. If sufficient phage is spotted on to a lawn of E. coli w the bacteria may be killed but serial dilutions of the phage do not give plaques. To find out more precisely what was happening in this system it was necessary to follow a single cycle of growth of phage λ in strain w. The results of initial experiments with this strain were obscured by the presence of a second phage

Table 1. Single cycle growth of phage λv.c in Escherichia coli strain w and E. coli c(wφ)

<table>
<thead>
<tr>
<th>Host strain</th>
<th>Multiplicity of infection</th>
<th>Unadsorbed phage</th>
<th>Free phage after antisera</th>
<th>Number of infective centres assayed on:</th>
</tr>
</thead>
<tbody>
<tr>
<td>w</td>
<td>1.1</td>
<td>1.2%</td>
<td>5 x 10⁴</td>
<td>cwφ&lt;sup&gt;+&lt;/sup&gt; 3.1 x 10⁴</td>
</tr>
<tr>
<td>c(wφ)</td>
<td>0.85</td>
<td>2.4%</td>
<td>1.5 x 10⁵</td>
<td>w 2.7 x 10⁴</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>c(wφ) 2.8 x 10⁵</td>
</tr>
</tbody>
</table>

Phage λv.c was adsorbed to 10⁶ starved bacteria for 15 min. The mixture was diluted with fresh growth medium to assure injection of the phage genomes. Unadsorbed phage was assayed after killing the infected bacteria with chloroform both before and after the addition of antiserum. Infective centres were assayed at 25 min. on E. coli w, E. coli c made resistant to wφ and E. coli c lysogenic for wφ.

*The fraction of infected bacteria which produce infective centres on cwφ<sup>+</sup>.

in lysates of E. coli w. This was a temperate phage wφ for which strain w is lysogenic. It forms plaques on E. coli c with an efficiency which, in the absence of a better indicator, we arbitrarily call 1.0; its properties have been described elsewhere (Glover & Kerszman, 1967; Pizer, Miovic & Pykajas, 1967). To overcome this complication a strain of E. coli c was isolated resistant to phage wφ. This cwφ<sup>+</sup> strain was used as an indicator for phage λ in a single cycle growth experiment in E. coli w. Phage λ adsorbed efficiently to w bacteria but only 10⁻⁵ of the infected cells yielded a burst (Table 1). This fraction was not influenced by altering the host-specificity of the infecting phage; λ, K, λ, β and λ, c (p1) all gave the same result and phage λ in the burst from the infected cells formed plaques on E. coli c but not on E. coli w. Thus, although the growth of λ in strain w was restricted λ was not host-modified. A single-cycle
growth experiment was made of phage \( \lambda \) in \( E. coli \) c made lysogenic for \( w\phi \) and the results were similar to the results of a single cycle of phage \( \lambda \) in strain \( w \). Namely, \( \lambda \) adsorbed to \( E. coli \) c (\( w\phi \)) but only a small fraction of the infected bacteria yielded a burst and this burst formed plaques on \( E. coli \) c but not \( E. coli \) c (\( w\phi \)) (Table 1).

The mechanism of the restriction process in Escherichia coli \( w \) and \( E. coli \) c (\( w\phi \))

The \( \lambda s u s \) class of mutants are unable to grow in non-permissive hosts but are able to grow in permissive strains, in which the \( \lambda s u s \) mutations are suppressed. To discover therefore whether, in parallel with the block to the development of \( \lambda s u s \) in non-permissive hosts, there was a block to the development of phage \( \lambda \) in \( E. coli \) c (\( w\phi \)) the double lysogen \( c(\lambda)(w\phi) \) was prepared. Phage \( \lambda \) does not form plaques on \( E. coli \) c (\( w\phi \)) but phage \( w\phi \) readily formed plaques on \( E. coli \) c(\( \lambda \)) and from the centre of turbid plaques on \( c(\lambda) \) double lysogens \( c(\lambda)(w\phi) \) could be isolated. The supernatants after centrifugation of cultures of \( E. coli \) c(\( \lambda \)) and c(\( \lambda)(w\phi) \) contained about the same number of \( \lambda \) particles and furthermore ultraviolet irradiation of suspensions of \( E. coli \) c lysogenic for both \( \lambda \) and \( w\phi \) induced \( \lambda \) as efficiently as u.v.-irradiation of \( E. coli \) c lysogenic for phage \( \lambda \) alone. We conclude therefore that there is no genetic block to the development of \( \lambda \) caused by the presence of the \( w\phi \) prophage. Since \( \lambda \) adsorbs to \( E. coli \) w and to c(\( w\phi \)) and apparently develops normally after u.v.-induction of \( E. coli \) c(\( \lambda)(w\phi) \), what steps remain which could be blocked? Obvious candidates are the injection and successful penetration of \( \lambda \) DNA. To test these possibilities the fate of infecting phage DNA was followed using \( ^{32}P \)-labelled \( \lambda \). c in a series of experiments with different bacterial hosts according to the techniques described by Dussoix & Arber (1962). A period of 15 min. adsorption to starved bacteria was followed by a period of 15 min. growth in aerated tryptone broth to provide conditions favourable to injection and the onset of phage growth. At the end of this period samples were treated with cold 3% perchloric acid and the \( ^{32}P \) counts in the soluble and insoluble fractions were determined (Table 2). More than 98% of the \( ^{32}P \) was found in the acid-insoluble fraction after infection of \( E. coli \) c. The amount of acid-soluble \( ^{32}P \) corresponded to the amount of soluble \( ^{32}P \) found in the \( ^{32}P \)-labelled \( \lambda \). c preparation after purification and represents residual impurities. About 27% of the \( ^{32}P \) was found in the acid-soluble fraction after infection of \( E. coli \) w and 21% in the acid-soluble fraction after infection of \( E. coli \) c(\( w\phi \)). These figures compare well with our results for \( \lambda \). c infection of \( E. coli \) k in which 22% of the \( ^{32}P \) was found in the acid-soluble fraction, though a somewhat more efficient breakdown was demonstrated by Arber, Hattman & Dussoix (1963).

Phage \( \lambda \) therefore adsorbed to \( E. coli \) c(\( w\phi \)) and \( E. coli \) w and \( > 20 \% \) of the \( ^{32}P \) became acid-soluble shortly after infection. The block to \( \lambda \) development in these strains could not be due to a failure of the injection mechanism; rather they indicate that \( \lambda \) development was blocked because the \( \lambda \) DNA was degraded shortly after injection. We may therefore conclude that in \( E. coli \) c(\( w\phi \)) the \( w\phi \) prophage controls a restriction process which operated against phage \( \lambda \) like that controlled by prophage \( P1 \) (Dussoix & Arber, 1962), but that unlike that controlled by prophage \( P1 \) the \( \lambda \) which escaped restriction is not host-modified. Systems of host-controlled modification like that controlled by phage \( P1 \) have been represented phenotypically as \( r^+m^+ \), on this basis then the system controlled by \( w\phi \) can be represented as \( r^+m^- \),
or perhaps better as $r^+m^-$ to indicate that there is restriction but no modification. In 
_E. coli_ \(c(w\phi)\) most \(\lambda\) infections probably fail because the \(\lambda\) DNA is degraded, but 
when \(\lambda\) escapes this degradation or is able to overcome it, normal phage development 
proceeds with the production of \(\lambda c\) with, apparently, no host-modification of the 
phage.

<table>
<thead>
<tr>
<th>Host strains</th>
<th>C</th>
<th>W</th>
<th>c(w\phi)</th>
<th>K</th>
<th>Control*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Efficiency of plating of (\lambda c)</td>
<td>1.0</td>
<td>&lt; 10^{-8}</td>
<td>&lt; 10^{-8}</td>
<td>1 \times 10^{-4}</td>
<td>—</td>
</tr>
<tr>
<td>Adsorption 15 min. + growth</td>
<td>10,800</td>
<td>8,400</td>
<td>7,650</td>
<td>7,600</td>
<td>10,110</td>
</tr>
<tr>
<td>15 min. cold PA insoluble (^{32}P) counts/min.</td>
<td>140</td>
<td>3,210</td>
<td>2,010</td>
<td>2,200</td>
<td>80</td>
</tr>
<tr>
<td>Cold PA soluble (^{32}P) counts/min.</td>
<td>1.3%</td>
<td>27.6%</td>
<td>20.8%</td>
<td>22.5%</td>
<td>0.79%</td>
</tr>
<tr>
<td>% cold PA soluble (^{32}P)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Bacteria were grown in broth to about 10^8 cells/ml. and starved in 0.01M MgSO_4. Samples of 
starved bacteria were infected to give a final multiplicity of infection of 1.0 with highly purified \(\lambda c\)
labelled with \(^{32}P\). After 15 min. adsorption fresh broth was added and a further 15 min. allowed for 
growth. After the addition of cold carrier DNA \(^{32}P\) counts were determined by treatment of samples 
with cold 3% perchloric acid (PA).

* Control. A sample of highly purified \(\lambda c\) labelled with \(^{32}P\) was treated in the same way as the 
adsorption mixtures except for the addition of host bacteria. The cold PA soluble \(^{32}P\) represented 
impurities in the stock suspension. The counts of \(^{32}P\) were all adjusted to the initial concentration and 
expressed as \(^{32}P\) counts per min. per ml. The coefficient of variation for the estimations of cold PA 
soluble \(^{32}P\) in the control and with the host bacteria c was 20%. For the other estimations of \(^{32}P\) it 
did not exceed 3%.

The isolation and properties of \(\lambda\) mutants able to grow on the restricting 
host Escherichia coli strain \(w\)

The efficiency of plating of phage \(\lambda\) on _Escherichia coli_ \(w\) is extremely low. When 
large quantities of phage \(\lambda\) or \(\lambda\nu\) (more than 10^8 particles) are inoculated on lawns of \(w\) 
bacteria the phage is adsorbed and the bacteria are lysed. Phage was harvested from 
these lytic areas and re-inoculated on \(w\) bacteria. Again the bacteria were lysed by 
high concentrations of phage, but at lower dilutions few, if any, plaques were pro-
duced and the efficiency of plating was very little improved. After several such enrich-
ment cycles the efficiency of plating increased and dilutions of the phage grown on 
strain \(w\) produced individual plaques on strain \(w\). These plaques contained mutants 
of \(\lambda\) which, quite unlike the wild type, were able to plate on _E. coli_ \(w\) with an efficiency 
of plating of 1.0. These mutants we called \(\lambda w\); they are defined solely by their ability 
to form plaques on _E. coli_ \(w\). Rarely, when more than 10^10 particles of phage \(\lambda c\) were 
mixed with an excess of \(w\) bacteria and plated out a few plaques were obtained. These 
plaques contained \(\lambda w\) phage. This does not give an accurate measure of the frequency 
of the mutation from \(\lambda\) to \(\lambda w\) because \(\lambda w\) grown in _E. coli c_ does not form plaques on 
_E. coli_ \(w\) with an efficiency of 1.0 but at a much lower efficiency, about 10^{-8}. We 
therefore estimated that the frequency of \(\lambda w\) mutants is about 10^{-7} (Glover & Arono-
vitch, 1967).
The properties of these mutants were investigated in some detail and compared with the properties of wild-type λ and phage wφ (Table 3). Clearly, λw is identical with λ in most respects and is not a contaminant. Nor is it at all likely that λw is a recombinant between phage λ and phage wφ because the buoyant densities of phage λ and λw are identical while the buoyant density of phage wφ is quite different. Secondly λw mutants can be isolated from phage λ grown in *Escherichia coli* c by indirect selection on *E. coli* w.

Among the λw mutants isolated, three different types can be recognized by their efficiencies of plating on *Escherichia coli* c and *E. coli* kr-m-. λw-1 forms plaques on *E. coli* w and on *E. coli* kr-m- but not on *E. coli* c; λw-2 forms plaques on all three indicators and λw-3 forms plaques on *E. coli* w and *E. coli* c with an efficiency of 10⁶, and on *E. coli* kr-m- with an efficiency of about 10⁻⁶. The reason why λw-1 does not form plaques on *E. coli* kr-m- has not been investigated; however, when more than 10⁹ particles of this phage are inoculated on lawns of c bacteria a few plaques appear. If these plaques were due to back-mutations to wild type, phage from them should not plate on *E. coli* w. Phage from six such plaques was isolated and tested on the indicators w, c and kr-m-. Phage from one of them formed plaques on *E. coli* c but not *E. coli* w and was thus a reversion to wild-type λ. Phage from the other five formed plaques on all three indicators and thus behaved like λw-2. This established the mutation pattern:

\[
\begin{align*}
\lambda & \xrightarrow{\phi} \lambda w\-1 \\
\lambda & \xrightarrow{\phi} \lambda w\-2 \\
\lambda & \xrightarrow{\phi} \lambda w\-3
\end{align*}
\]

Selective indicators were not available to test the other mutation frequencies.

A series of genetic crosses was made between λw-1 and λsus mutants (Table 4). In these crosses the inability of λsus mutants and λw-1 to form plaques on *Escherichia coli* c was utilized to select λ wild-type recombinants which would be able to grow on c bacteria. The selective indicator was made resistant to wφ because suspensions of λw may contain small amounts of wφ which is able to infect *E. coli* c. The crosses were made in a permissive kr-m- strain in which λsus and λw-1 were able to grow and the burst was assayed on kr-m- to determine the total number of phage progeny and on cwφ' to determine the number of wild-type recombinants. Control experiments showed that the λsus mutants formed plaques on the selective indicator at a
Restriction of phage $\lambda$ in *E. coli* w

frequency of $< 10^{-5}$ and that $\lambda w-I$ formed plaques on the selective indicator at a frequency of $< 10^{-6}$. Control crosses between the $\lambda sus$ mutants yielded $sus^+$ recombinants at a frequency in agreement with the location assigned to these mutants by Eisen *et al.* (1966). The number of plaques obtained on the selective indicator when the progeny of the $\lambda w-I \times \lambda sus$ crosses were tested exceeded by a factor of several hundred the number that could be accounted for by the plating efficiency of either parent alone. We conclude therefore that these plaques were produced by $\lambda sus^+ w-I^+$ recombinants. The frequency of recombination was low and on the basis of these preliminary crosses it was not possible to assign a location to the $\lambda w-I$ mutation.

**Table 4. Crosses between $\lambda w-I$ and $\lambda sus$ N213, $\lambda sus$ P207 and $\lambda sus$ Q203**

| Mutant prophage | Infecting prophage | Titre of burst on $K\gamma m^-$ | Titre of burst on $c w \phi^\prime$ | $sus^+ w-I^+$ recombinations (%)
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda sus$ Q203</td>
<td>$\lambda sus$ Q203</td>
<td>$3.5 \times 10^2$</td>
<td>$1.0 \times 10^2$</td>
<td>—</td>
</tr>
<tr>
<td>$\lambda sus$ P207</td>
<td>$\lambda sus$ Q203</td>
<td>$5.5 \times 10^2$</td>
<td>$4.5 \times 10^2$</td>
<td>—</td>
</tr>
<tr>
<td>$\lambda sus$ N213</td>
<td>$\lambda sus$ N213</td>
<td>$4.0 \times 10^2$</td>
<td>$1.4 \times 10^2$</td>
<td>—</td>
</tr>
<tr>
<td>$\lambda sus$ Q203</td>
<td>$\lambda sus$ P207</td>
<td>$4.2 \times 10^2$</td>
<td>$1.7 \times 10^2$</td>
<td>0.4</td>
</tr>
<tr>
<td>$\lambda sus$ Q203</td>
<td>$\lambda sus$ P207</td>
<td>$6.5 \times 10^2$</td>
<td>$4.2 \times 10^2$</td>
<td>0.65</td>
</tr>
<tr>
<td>$\lambda sus$ P207</td>
<td>$\lambda sus$ N213</td>
<td>$6.0 \times 10^2$</td>
<td>$3.0 \times 10^2$</td>
<td>0.65</td>
</tr>
<tr>
<td>$\lambda sus$ P207</td>
<td>$\lambda sus$ N213</td>
<td>$6.0 \times 10^2$</td>
<td>$3.0 \times 10^2$</td>
<td>0.85</td>
</tr>
<tr>
<td>$\lambda sus$ Q203</td>
<td>$\lambda sus$ N213</td>
<td>$3.7 \times 10^2$</td>
<td>$7.0 \times 10^2$</td>
<td>1.9</td>
</tr>
<tr>
<td>$\lambda sus$ P207</td>
<td>$\lambda sus$ Q203</td>
<td>$4.2 \times 10^2$</td>
<td>$4.0 \times 10^2$</td>
<td>0.95</td>
</tr>
<tr>
<td>$\lambda sus$ Q203</td>
<td>$\lambda w-I$</td>
<td>$1.6 \times 10^2$</td>
<td>$7.0 \times 10^2$</td>
<td>0.044</td>
</tr>
<tr>
<td>$\lambda sus$ P207</td>
<td>$\lambda w-I$</td>
<td>$3.0 \times 10^2$</td>
<td>$2.7 \times 10^2$</td>
<td>0.09</td>
</tr>
<tr>
<td>$\lambda sus$ N213</td>
<td>$\lambda w-I$</td>
<td>$4.5 \times 10^2$</td>
<td>$2.6 \times 10^2$</td>
<td>0.058</td>
</tr>
<tr>
<td>$\lambda sus$ N213</td>
<td>$\lambda w-I$</td>
<td>$4.5 \times 10^2$</td>
<td>$2.6 \times 10^2$</td>
<td></td>
</tr>
</tbody>
</table>

In each cross a log phage culture of *E. coli* Κ carrying the mutant prophage was centrifuged and resuspended in 0.01 M-MgSO₄. This suspension was irradiated with u.v. and immediately afterwards superinfected with the second phage previously grown on Κ at a multiplicity of 3.0. After 15 min. adsorption the infected cells were diluted into fresh broth and aerated for a further 90 min. The lysate was then treated with chloroform and suitable dilutions plated on *E. coli* $K\gamma m^-$ to assay the total number of progeny phage and on *E. coli* $c w \phi^\prime$ to assay the number of $sus^+ w-I^+$ recombinants.

The independence of the roles of prophage $w \phi$ and the genome of *Escherichia coli* w in the restriction and modification of phage $\lambda$

Single-cycle growth experiments have shown that phage $\lambda$ grows in only a small fraction of the *Escherichia coli* w and *E. coli* c($w \phi$) which it infects. The vast majority of such infections probably fail because the $\lambda$ DNA is degraded in the restricting hosts. Furthermore, it has been demonstrated that phage $\lambda$ which escapes restriction in these hosts and the $\lambda$ phage which can be obtained from the double lysogen *E. coli* c($\lambda$($w \phi$)) is not host-modified by them. It may be concluded therefore that prophage $w \phi$ controls the restriction of phage $\lambda$ but does not host-modify it. $\lambda w$ mutants escape this restriction process in *E. coli* w, so one can ask whether the genome of $w$ bacteria if not the $w \phi$ prophage, imposes a specific modification upon the $\lambda w$ growing in this strain. To test this possibility $\lambda w-2$ was grown on *E. coli* c, c($w \phi$) and $w$ and the efficiency of plating of the phage suspensions was tested on all three indicator strains. It was about $10^{-3}$ for $\lambda w-2$.c and $\lambda w-2$.c($w \phi$) on *E. coli* w, and 1.0 for $\lambda w-2$. w, on all three strains. These results define a $w$-specific host-induced modification which must be carried by $\lambda w$ for it to form plaques on *E. coli* w
efficiently and which is not conferred upon \( \lambda w \) when it is grown in \( E. coli \) or \( c(\psi \phi) \). Furthermore, it appears that \( E. coli w \) exercises two restriction processes; one controlled by the prophage which restricts the growth of \( \lambda \), and a second controlled by the \( w \) genome which restricts the growth of \( \lambda w. c \). The fact that \( \lambda w. c \) forms plaques on \( E. coli w \) with an efficiency of \( 10^{-3} \) explains the difficulty encountered isolating \( \lambda w \) mutants from suspensions of phage \( \lambda. c \) since only \( 10^{-3} \) of spontaneously occurring mutants can form a plaque on \( E. coli w \).

### Mixed infections with restricted and unrestricted phage \( \lambda \)

The \( \lambda w \) mutants of phage \( \lambda \) are defined by their ability to form plaques on the restricting host strain \( Escherichia coli w \). The \( w \) mutation enables phage \( \lambda \) to escape the restriction exercised by the prophage \( \psi \phi \) and in addition permits the detection of a \( w \)-specific host-induced modification not acquired by wild type \( \lambda \). This difference between \( \lambda \) and \( \lambda w \) can be accounted for in three quite distinct ways. First, it may be supposed that \( \lambda w \) makes a product which protects it against the degradation process controlled by \( \psi \phi \) and that \( \lambda \) is unable to make this product. This assigns an active positive role to \( \lambda w \). This activity of \( \lambda w \) is not likely to be the synthesis of an inhibitor of the restriction enzyme(s) responsible for the degradation of \( \lambda \) DNA since the evidence suggests that the degradation of \( \lambda \) DNA happens rapidly, probably before the phage could express such a function in the restricting host cell. Rather, we may suppose that \( \lambda w \) synthesizes a product, in any host cell which it infects, which affords protection to its DNA against the restriction enzyme(s). Thus \( \lambda w \) could be described as self-modifying and therefore its DNA would be protected before injection into the restricting host cell. If this were so, \( \lambda w \) should protect the DNA of wild type \( \lambda \) when both are grown together in the same cell. Secondly, one may assign an active negative role to \( \lambda \) and argue that \( \lambda \) makes a product which renders its DNA sensitive to degradation by the restriction enzyme(s) while \( \lambda w \) does not make this product. This activity of \( \lambda \) is not likely to be the synthesis of a co-factor required to ensure the degradation of \( \lambda \) DNA since the evidence suggests that degradation takes place before the phage could express such a function in the restricting host. Alternatively, the active negative role of \( \lambda \) could be to synthesize a product, in any host cell which it infects, which renders its DNA susceptible to the restriction enzyme(s). Thus \( \lambda \) would be self-sensitizing and therefore its DNA would be susceptible to degradation before injection into the restricting host cell. If this were so, \( \lambda w \) should protect the DNA of wild type \( \lambda \) when both are grown together in the same cell. Thirdly, we may suppose that there is normally in \( \lambda \) DNA a nucleotide sequence or sequences susceptible to degradation by enzyme(s) synthesized under the control of prophage \( \psi \phi \) in restricting hosts and that \( \lambda w \) mutations involve alterations to this sequence which make it resistant to degradation or alter it so that it is no longer recognized by the restriction enzymes. In contrast to the two previous models the difference between \( \lambda \) and \( \lambda w \) is described as passive rather than active since no function other than DNA replication is necessary to maintain the \( \lambda w \) condition. In mixed infection therefore this model predicts that \( \lambda \) and \( \lambda w \) will not affect one another and that phage grown under these conditions will not differ from phage grown in singly infected hosts.

These predictions were tested experimentally and the transmission of the phages \( \lambda w. z \) and \( \lambda \) was measured (Table 5). The restricted phage \( \lambda. c \) does not interfere with the growth of the unrestricted phage \( \lambda w. z. w \). The transmission of \( \lambda w. z. w \) through
Restriction of phage λ in E. coli w

Escherichia coli w remained unchanged despite the presence of a considerable excess of λ.c (Expts 3 and 4). Similarly, λw-I.w did not apparently increase the transmission of λ.c through E. coli w although there appeared to be a multiplicity effect with λ.c in strain w similar to that described for λ.c in E. coli K1 by Paigen & Weinfeld (1963). Since we already know that λ.c DNA is degraded in a majority of the infected bacteria this result is hardly surprising. But the models make a further prediction. The active positive model predicts that λ grown in the presence of λw would acquire from the active product produced by λw some protection against restriction upon subsequent infection of a restricting host. The active negative model predicts the λw grown in the presence of λ would become sensitized to the restriction enzymes as a result of the activity of λ. The passive model predicts that λ and λw will have no influence on one another in mixed infection. To test these further predictions the bursts from the mixed infections with λ and λw were adsorbed to restricting hosts and the transmission of the phages measured (Table 6). After mixed infection λw did not confer upon λ.c an ability to escape restrictions in E. coli c(wφ) since the transmission of λ.c in E. coli c(wφ) was the same in Expt 1 (λ.c stock suspension) and in Expt 4 (λ.c obtained from mixed infection with λw-I.w). Similarly, growth in the presence of λ.c does not enhance the restriction of λw-I.w in E. coli w since the transmission of λw-I is the same in Expt 2 (λw-I.w stock suspension) and in Expt 3 (λw-I.w obtained from mixed infection with a large excess of λ.c). Models 1 and 2 can, therefore, be rejected and the conclusion drawn that λw mutations represent changes in the nucleotide sequence of λ DNA which render it insensitive to the restriction process controlled by phage wφ, most probably by altering the sequence or sequences normally recognized by the restriction enzymes synthesized by prophage wφ.

### Table 5. Mixed infection of Escherichia coli w with λw-I.w and λ.c

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of bacteria</td>
<td>1.11 x 10^8</td>
<td>1.11 x 10^8</td>
<td>1.11 x 10^8</td>
<td>1.11 x 10^8</td>
</tr>
<tr>
<td>No. of phage particles</td>
<td>λ.c 1.4 x 10^9</td>
<td>λw-I.w 1.2 x 10^7</td>
<td>λw-I.w 1.92 x 10^6</td>
<td>λw-I.w 1.5 x 10^6</td>
</tr>
<tr>
<td>Unadsorbed phage</td>
<td>λ.c 1.31 x 10^9</td>
<td>λw-I.w 7.7 x 10^9</td>
<td>λw-I.w 3 x 10^9</td>
<td>λw-I.w 3 x 10^9</td>
</tr>
<tr>
<td>Free phage after anti-serum treatment</td>
<td>λ.w 1.62 x 10^6</td>
<td>λ.w 4 x 10^6</td>
<td>λ.w 1 x 10^6</td>
<td>λ.w 6 x 10^4</td>
</tr>
<tr>
<td>Infective centres assayed on</td>
<td>w(wφ) 2.28 x 10^6</td>
<td>w(wφ) 2.82 x 10^6</td>
<td>w(wφ) 1.5 x 10^7</td>
<td>w(wφ) 1.5 x 10^7</td>
</tr>
<tr>
<td>Transmission*</td>
<td>λ.c 2 x 10^{-3}</td>
<td>λ.w 1 x 10^{-3}</td>
<td>λ.c 1 x 10^{-3}</td>
<td>λ.w 1 x 10^{-3}</td>
</tr>
<tr>
<td></td>
<td>λw-I.w 1.0</td>
<td>λw-I.w 1.0</td>
<td>λw-I.w 1.0</td>
<td>λw-I.w 1.0</td>
</tr>
</tbody>
</table>

Phage was adsorbed to starved bacteria in 0.01 M-MgSO_4 for 15 min. The adsorption mixtures were diluted with fresh tryptone and a further 15 min. allowed for injection. A sample was removed for the assay of free phage by centrifugation followed by chloroform treatment to kill the infected bacteria. After treatment with anti-λ serum infective centres were assayed on the appropriate indicator bacteria. A sample of the infective centres was allowed to burst and the progeny phage used in the experiment shown in Table 5.

*The fraction of infected bacteria which produce infective centres on the appropriate indicator bacteria.
The properties of \( w \phi \) lysogens

*Escherichia coli* \( w \) is lysogenic for a temperate phage \( w \phi \) which, antigenically and in a number of other respects, is similar to phage \( \tau \). This \( w \phi \) forms plaques on *E. coli* \( c \) but not on strains \( b \), \( K \) or \( K r^{-}m^{-} \). The few plaques which appear on lawns of *E. coli* \( K \) when concentrated suspensions of phage \( w \phi \) are plated are due to mutants designated \( w \phi k \) (Glover & Kerszman, 1967). These mutants form plaques on *E. coli* strains \( \kappa \) and \( c \). The \( w \phi \) and its mutant \( w \phi k \) were used to prepare the following lysogenic strains \( c(w \phi), c(w \phi k) \) and \( K(w \phi k) \). Phage \( \lambda \) does not form plaques on strain \( w \) because of the restriction mechanism controlled by the \( w \phi \) prophage so it was of obvious interest to test the efficiency of plating of \( \lambda \) on these new \( w \phi \) lysogenic strains.

Table 6. *Infection of restricting* *Escherichia coli* *hosts with progeny phage from the mixed infections shown in Table 5*

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of bacteria</td>
<td>( c(w \phi) )</td>
<td>( 1.1 \times 10^{8} )</td>
<td>( - )</td>
<td>( - )</td>
</tr>
<tr>
<td>No. of phage particles</td>
<td>( \lambda . c )</td>
<td>( 1.4 \times 10^{7} )</td>
<td>( - )</td>
<td>( - )</td>
</tr>
<tr>
<td>No. of unadsorbed phage particles</td>
<td>( \lambda . c )</td>
<td>( 1.5 \times 10^{8} )</td>
<td>( - )</td>
<td>( - )</td>
</tr>
<tr>
<td>No. of phage particles after anti-( \lambda ) treatment</td>
<td>( \lambda . c )</td>
<td>( 5 \times 10^{8} )</td>
<td>( - )</td>
<td>( - )</td>
</tr>
<tr>
<td>Infective centres assayed on</td>
<td>( c \phi )</td>
<td>( 3 \times 10^{8} )</td>
<td>( - )</td>
<td>( - )</td>
</tr>
<tr>
<td>Transmission</td>
<td>( \lambda . c )</td>
<td>( 3 \times 10^{-3} )</td>
<td>( - )</td>
<td>( - )</td>
</tr>
</tbody>
</table>

Phage \( \lambda . c \) had an efficiency close to 1.0 on strain \( c(w \phi k) \) and phage \( \lambda . k \) had an efficiency of almost 1.0 on strain \( \kappa (w \phi k) \). Therefore \( w \phi k \) can be regarded as a mutant of \( w \phi \) which has lost the ability to restrict \( \lambda \) and is analogous to the \( r^{-} \) mutants of phage \( \phi 1 \) which were isolated by Glover *et al.* (1963). However, this explanation is rendered less plausible by the fact that not all *E. coli* \( c(w \phi) \) isolates restrict the growth of phage \( \lambda \). Among the *E. coli* \( c(w \phi) \) isolates obtained by picking the growth from the centre of turbid plaques produced by phage \( w \phi \) on strain \( c \), some do not accept \( \lambda \) at all, others accept it at an efficiency approaching 1.0 and the remainder at efficiencies between \( 10^{-2} \) and \( 10^{-5} \). The frequency of these three different classes among *E. coli* \( c(w \phi) \) isolates varies depending upon the strain of *E. coli* \( c \) which is made lysogenic. The reasons for these differences is not known. However, phage \( w \phi \) is, in many respects, like phage \( \phi 2 \) and may, like \( \phi 2 \), occupy any one of several alternate locations on the chromosome of *E. coli* (Bertani, 1962; Kelly, 1963). If the expression of restriction is dependent upon chromosome location it should be possible to demonstrate differences between the location of phage \( w \phi \) in \( c(w \phi) \) bacteria which restrict \( \lambda \) and those which do not. Experiments to test this using \( \text{Hfr} \) derivatives of *E. coli* \( c \) are in progress.
The isolation of mutants of Escherichia coli \( w \) which accept phage \( \lambda \)

Broth-grown log. phage cultures of *Escherichia coli* \( w \) were washed by centrifugation and treated with 100 \( \mu g/\)ml. of nitrosoguanidine in phosphate buffer at pH 6·0 according to the method of Adelberg, Mandel & Chen (1965). Broth cultures were prepared from the surviving colonies and screened for the following properties: (1) the ability to accept phage \( \lambda \); (2) the efficiency of plating of phage \( \lambda w-2.c \); (3) the ability to confer \( w \) host specificity upon \( \lambda w-2 \). About 2 % of the colonies proved to be mutants in respect of one or more of these properties. Only one of these, strain \( w 122 \), was examined in any detail. This strain accepts \( \lambda.c \) at an efficiency of \( 10^{-1} \) compared with \( r-o \) on *E. coli* \( c \), it accepted \( \lambda w-2.c \) more efficiently than does the wild type and it does not confer \( w \) host specificity to \( \lambda w-2 \). The \( w\phi \) was isolated from this strain and a lysogenic strain of *E. coli* \( c \) was prepared from it. The properties of this strain were identical with those of a c\((w\phi)\) strain prepared from the \( w\phi \) obtained from wild type strain \( w \). The conclusion may be drawn therefore that strain \( w 122 \) does not carry a mutant of \( w\phi \) but that it is a complex mutant affecting the expression of the restriction process normally controlled by the \( w\phi \) prophage and the restriction and modification processes normally controlled by the \( w \) genome. The properties of the other mutants have not yet been investigated but preliminary experiments indicate that they are less complex than strain \( w 122 \).

**DISCUSSION**

Recent experiments on the genetics of host-controlled modification of phage \( \lambda \) in *Escherichia coli* have shown that the genetic control of the process of restriction is separable from the genetic control of modification. The phenotype of wild type strains which exert the processes of restriction and modification may be represented as \( r+m^+ \). In several laboratories mutant strains have been isolated which can be represented as \( r^-m^+ \) or \( r^-m^- \) according to the behaviour of their phenotypes (Glover *et al.* 1963; Wood, 1966; Lederberg, 1966). Another possible class of mutant would have the phenotype \( r^-m^- \); however, no such mutants have been reported for the host-controlled modifications controlled by phage \( \Phi^0 \) and *E. coli* strains \( K \) or \( B \) which have been the subject of genetic investigations. There is no obvious selective system available for isolating \( m^- \) phenotypes but attempts to isolate such mutants by a simple screening method without selection have been unsuccessful (Glover, unpublished). Therefore the occurrence in nature of systems which, superficially at any rate, behave like \( r^+m^- \) is of considerable interest.

The growth of phage \( \lambda \) in *Escherichia coli* strain \( w \) which is lysogenic for a temperate phage \( \omega\phi \) and in strain \( c \) made lysogenic for this phage is characteristic of the behaviour predictable for \( r^+m^- \) systems. These strains do not propagate phage \( \lambda \). Only a small fraction of the infected bacteria yield phage after the first cycle of infection and subsequent infections are unsuccessful because this phage is not host-modified. Consequently \( \lambda \) is unable to form plaques on strains \( w \) and \( c(w\phi) \). The restriction process which prevents all but a small fraction of initial infections from proceeding to completion involves the degradation of the infecting \( \lambda \) DNA. Some 15 min. after infection more than 20 % of the \( ^{32}P \) label from the infecting phage is present as acid-soluble \( ^{32}P \) in the medium. The small fraction of \( \lambda \) particles which escape this degradation and grow in the restricting host bacteria yield bursts of phage which is not host-
modified and this phage does not form plaques on the restricting strains. An interesting parallel has been observed when T2 infects E. coli strain w. Progeny phage is not produced and by 3 to 5 min. after infection with 32P-labelled phage 35 to 40% of the 32P becomes acid-soluble. This value subsequently drops to 20% unless the infected strain is a starved auxotroph, presumably because the degradation products are reutilized by the infected bacteria (Smith & Pizer, 1966).

It might be supposed that r+m− or r+m° strains would be auto-lethal since they apparently possess the capacity to degrade their own unmodified DNA. This is obviously not the case for the system controlled by phage wφ. It could be that the bacterial chromosome escapes degradation because there is no wφ-specific sensitive site on its DNA, or alternatively that degradation takes place at a site remote from the chromosomal DNA. The fact that the yield of λ.c obtained from strain c(λ) and from the double lysogenic strain c(λ)(wφ), which restricts the growth of λv.c, is the same shows that λ DNA can replicate in the cytoplasm of such a cell so the restriction process must operate outside the region of λ DNA synthesis and therefore most probably takes place at a site near the cell surface. Recently several independent lines of evidence have been reported which indicate that in r+m+ systems the restriction process also operates at a site near the cell surface (Schell & Glover 1966a-c). Fukasawa (1964a, b) reported evidence that non-glucosylated T2 DNA can replicate in the cytoplasm of a cell which is able to degrade DNA in this form when it enters the cell from infecting phage particles, and concluded that degradation is exerted by an enzyme system located outside the cytoplasmic membrane and presumably not available inside the cell.

Mutants of λ have been isolated which form plaques on strain w lysogenic for wφ with an efficiency of 1.0. With these λw mutants it has been possible to separate the roles which the w bacterial genome and the wφ prophage play in the restriction of phage λ. About a 10⁻³ fraction of strain c(wφ) bacteria infected with λ.c form infective centres when tested on strain cwφ+. But an additional restriction can be demonstrated in strain w since the efficiency of plating of λw.c on strain c(wφ) is 1.0, while on strain w it is c. 10⁻³. So that in strain c(wφ) there is a restriction process controlled by phage wφ which operates against λ.c but not against λw, while in strain w there is an additional restriction which operates against λw phage grown on Escherichia coli c. The transmission of λc through strain w is c. 10⁻⁴, which is considerably higher than would be predicted if the two restriction processes were additive. Not uncommonly, however, when two restriction processes operate in the same cell the reduction in the plating efficiency of restricted phage is less than the value that can be calculated from the results observed when they operate separately (see review by Klein, 1965). λw mutants are defined by their ability to form plaques on the restricting strain w. This mutation enables λ to escape the restriction process controlled by prophage wφ. With λw mutants it has also been possible to detect a w-specific host-induced modification. This modification is conferred upon λw phages when they are grown in strain w but not when they are grown in strain c(wφ). We may therefore summarize the situation in the following manner. The genome of strain w controls a restriction process which acts upon λw.c and also controls a modification process which acts upon λw. The wφ prophage controls a restriction process which acts upon phage λ but not upon λw mutants and it does not confer a wφ-specific host-induced modification upon λ since the λ phage obtainable from strain c(λ)(wφ) is.
Restriction of phage \( \lambda \) in E. coli \( w \) is unable to form plaques on the restricting host strain \( c(w\phi) \). Three general models can be constructed to explain the behaviour of \( \lambda w \) mutants:

1. **Active positive model.** This supposes that \( \lambda w \) mutants make a product which protects them against the degradation process controlled by \( w\phi \). Thus, \( \lambda w \) could be described as self-modifying and therefore its DNA would be protected before injection into the restricting host cell. If this were so \( \lambda w \) should protect the DNA of wild type \( \lambda \) when both are grown together in the same cell.

2. **Active negative model.** This supposes that \( \lambda \) makes a product which renders its DNA sensitive to degradation in restricting host cells and \( \lambda w \) does not make this product. Thus, \( \lambda \) would be self-sensitizing and therefore its DNA would be susceptible to degradation before injection into the restricting host. On this assumption \( \lambda \) should render \( \lambda w \) susceptible to the restriction process when both are grown together in the same cell.

The results of mixed infection experiments show that \( \lambda \) does not render \( \lambda w \) susceptible to restriction nor does \( \lambda w \) protect the DNA of wild type \( \lambda \) so we are forced to reject both these models. However, the behaviour of \( \tau 3 \) is quite different. Cells infected with \( \tau 3 \) synthesize an enzyme S-adenosyl-methioninease which splits S-adenosyl-methionine and this compound normally acts as the methyl donor for the methylation of DNA (Gold et al. 1964), and in mixed infection \( \tau 3 \) prevents the effective modification of \( \tau 1 \) by preventing the methylation of \( \tau 1 \) DNA (Klein & Sauerbier, 1965).

3. **Passive model.** This supposes that in \( \lambda \) DNA there is normally a nucleotide sequence which is susceptible to degradation in restricting hosts, and that the \( \lambda w \) mutations represent structural changes in this nucleotide sequence which make it resistant to degradation. The results obtained in mixed infection experiments are consistent with the prediction from this model that \( \lambda \) and \( \lambda w \) will not influence one another when grown together in the same cell.

There are two additional observations which are of some interest. First, the mutation from \( \lambda \) to \( \lambda w \) permits the detection of a \( w \)-specific host-induced modification. The nucleotide sequence which is recognized and degraded in \( w\phi \) lysogenic bacteria could therefore be the same as the sequence in \( \lambda w \) which is recognized and can be host-modified by the genome of strain \( w \). The second observation of note is that \( \lambda w \) like \( \lambda \) can be host-modified in strains \( b \) and \( k(\phi \text{S}) \) so that the change from \( \lambda \) to \( \lambda w \) does not interfere with the host-specific sites involved in these systems.

Three different classes of \( \lambda w \) mutant can be distinguished by their abilities to form plaques on the indicator strains \( w \), \( c \), and \( k\phi \text{m}^- \). \( \lambda w-2 \) grows normally in all the strains so far tested, thus the \( \lambda w-2 \) mutation appears to affect the host-specific sequence of the DNA alone. \( \lambda w-1 \) does not form plaques on strain \( c \) and \( \lambda w-3 \) does not form plaques on strain \( k\phi \text{m}^- \). The reasons for this have not been investigated in detail but the DNA of these phages is apparently not broken down since there is a transmission of almost 1.0 with the production of extremely small bursts. If this interpretation is correct then the \( \lambda w-1 \) and \( \lambda w-3 \) mutations do not make \( \lambda \) sensitive to a restriction process in strains of \( c \) and \( k\phi \text{m}^- \) but rather affect some other function necessary for normal growth in these hosts. The region where these mutations are located may in addition to being the specific site recognized and degraded in \( w\phi \) lysogenic bacteria also be concerned with some function necessary for growth in certain host strains but not in others. Alternatively, they may be amber-like mutations suppressible in one
host but not in another, or \( \lambda w-2 \) could possess a point mutation while \( \lambda w-1 \) and \( \lambda w-3 \) have gross mutations such as deletions. It is not possible on the available evidence to discriminate between these possibilities. But preliminary experiments indicate that in mixed infection \( \lambda .c \) is not able to complement some function which \( \lambda w-1 \) lacks.

Mutants of \( w \phi \) have been isolated which will lysogenize both *Escherichia coli* c and *E. coli* K but these new lysogenic strains propagate \( \lambda \) normally. It appears therefore that these mutants have lost the ability to degrade \( \lambda \) DNA and are equivalent to \( P1r^- \) mutants isolated by Glover et al. (1963). This conclusion is, however, only tentative since not all newly isolated \( c(w\phi) \) lysogenic strains restrict \( \lambda \) as efficiently as *E. coli* w. It is possible that \( w \phi \) may occupy any one of several alternative chromosomal locations and that the location of the prophage influences the expression of the restricting function. However, \( \lambda \) is unable to form plaques on the \( P2 \) lysogenic strains \( C34, C86 \) and \( C77 \) in which \( P2 \) occupies locations I, II and III respectively (Kelly, 1963). In this respect it is also interesting to note that in spite of the parallel between the restriction of phage \( \lambda \) by \( w \phi \) prophage and the inability of \( \lambda \) to form plaques on \( P2 \) lysogenic strains and the close relatedness of phage \( P2 \) and \( w \phi \) the \( \lambda w \) mutants are not able to form plaques on \( P2 \) lysogenic strains.

An interesting interaction between the host cell genome and the expression of the restricting function of the \( w \phi \) prophage is revealed by the behaviour of a nitroso- guanidine induced mutant of strain \( w \) which propagates \( w \phi \) normally in spite of the fact that it carries a \( w \phi \) prophage which when transferred to *Escherichia coli* c converts the c strain into a restricting host.

J. Aronovitch is grateful to the British Council for a scholarship during the academic year 1964–65; G. Kerszman is grateful to the British Council for a scholarship during the academic year 1965–66. During the course of this work we learned that a phage had also been isolated from *Escherichia coli* w by Dr Lewis Pizer and we are grateful to him for a copy of a manuscript before publication. We wish to thank Mrs Lyn Howden and Miss Sally Macdonald for excellent technical assistance.

REFERENCES


Restriction of phage $\lambda$ in $E. coli$ $w$


LENNOX, E. S. (1955). Transduction of linked genetic characters of the host by bacteriophage $\phi$1. *Virology* 1, 190.


(Received 28 January 1967)