Transduction mechanisms of bacteriophage ϵ^{15}

II. Isolation of a bacteriophage related to ϵ^{15}

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SUMMARY

A bacteriophage was isolated from a strain of *Salmonella manilla*. This phage, v^{15}, was found to have adsorption and conversion properties similar to phage ϵ^{15}. The two phages show similar transduction efficiencies but phage v^{15} lysogenizes more efficiently. Phage v^{15} is not subject to repression by ϵ^{15} type prophage. The densities of the two phages are indistinguishable.

Phage v^{15} can 'cure' ϵ^{15} type prophages and recombination is possible between genomes of the two phages. It seems probable that they are heteroimmune representatives of a single bacteriophage family.

1. INTRODUCTION

Several cases have been reported in which a group of temperate bacteriophages share a number of properties, in particular to show extensive genetic homology and ability to undergo recombination, and yet to differ among themselves in respect of the immunity specificity of the prophage repression system. Examples are the lamboid phages (Dove, 1968), P2, P2dis, 186 and others (Mandel & Berg, 1968; Calendar, Lindquist, Sironi & Clark, 1970) of *Escherichia coli* and the A1 and A2 phages of *Salmonella typhimurium* (Boyd & Bidwell, 1957).

In attempts to analyse the structure of ε*dlac* genomes (or other transducing derivatives of phage ϵ^{15}) a phage genetically homologous to ϵ^{15} but not susceptible to its prophage repressor would be useful. Uetake (1957) concluded 'Different strains of phage ϵ^{15}, each of which was isolated from group E2 and E3 *Salmonellae*, were compared with each other with respect to their various properties such as plaque form, host range, pH range, heat-resistance, ultraviolet sensitivity and serological characters. However, no significant difference was noted between or among different phage strains.'

2. MATERIALS AND METHODS

Bacteria: Salmonella anatum A1 were obtained from Dr H. Uetake. The E2 *Salmonella* strains *S. binza* 313, *S. drypool* 2840/67, *S. kinshasa* 2623/67, *S. manila* 1021, *S. newbrunswick* 86, *S. newington* 2872/67 were obtained from Dr J. Taylor.

Bacteriophages: ϵ^{15}, ϵ^γ and ϵ^{34} were supplied by Dr H. Uetake (ϵ^{15}c is a spontaneous clear-plaque mutant of phage ϵ^{15}). C 341 was obtained from Dr T. Uchida.

Methods are described by Hedges (1971).

3. RESULTS AND DISCUSSION

Lysates of six E 2 strains of *Salmonella* were prepared, adsorbed to heat-shocked cells of *S. anatum* A 1 (Uetake, Toyama & Hagiwara, 1964) and plated on a lawn of bacteria of that species. All six strains produced plaques though none did so on unshocked cells. Phage from isolated plaques were tested for ability to grow on a strain of *S. anatum* lysogenic for phage ϵ^γ. Those that formed plaques on this strain must be insensitive to the prophage repressor of ϵ^γ.

Those phage isolates which grew on the strain lysogenic for ϵ^γ were tested on a similar strain lysogenic for ϵ^{15}. Since the only differences between the two prophages are two mutational defects on the ϵ^γ genome abolishing steps in the somatic antigen conversion pathway, any phage which failed to grow on the ϵ^{15} lysogen must adsorb on the somatic antigen and recognise the 3:10 but not the 3:15 pattern, i.e. the specificity of adsorption must be similar to that of phage ϵ^{15}.

Strains of *S. anatum* A 1 lysogenic for each of the newly isolated phages were constructed. The sensitivity of these lysogens to phages ϵ^{15} and ϵ^{34} was tested. Strains converted by their prophage from the 3:10 to the 3:15 antigen are sensitive to phage ϵ^{34} but unable to adsorb ϵ^{15} (Uetake, Luria & Burrous, 1958).

The only phage which grew on *S. anatum* lysogenic for ϵ^γ but not on strains lysogenic for ϵ^{15}, and which converted *S. anatum* to the 3:15 antigen was a phage isolated from *S. manila* and designated phage v^{15} (upsilon 15). Like phage ϵ^{15} it transduced arabinose fermentation and tryptophan independence with an efficiency of about 10^{-5} per plaque-forming unit (p.f.u.). The efficiency of transduction of the lactose marker from a strain carrying *Flac* was about 10^{-9} per p.f.u.

A difference between the phages was the efficiency of lysogenization. That of phage ϵ^{15} was low (Uetake *et al.* 1958). Thus, in intraspecific transduction, where the cells were challenged with an input multiplicity of about three ϵ^{15} particles (and subject to unknown but probably numerous subsequent infections on the plate) the proportion of transductants which were lysogenic rarely exceeded 50 % (averaging about 35 %). After transduction by phage v^{15} at least 80 % of the transductants were lysogenic. A parallel to this difference between ϵ^{15} and v^{15} is provided by the data for the curing of a $\epsilon dlac$ prophage from strain T 31 (Table 1). T 31 is not immune to superinfection by phage ϵ^{15}, which facilitates comparison of the efficiencies of curing by the two phages. v^{15} is the more effective curing agent. Since curing of prophages is catalysed by recombination enzymes of the phage integration system (Gottesman & Yarmolinsky, 1968) it seems that the integration-specific recombination enzymes of phage v^{15} are more efficient than those of ϵ^{15}.

(i) *Double lysogens*

Strains of *E. coli* lysogenic for two homologous prophages can segregate singly lysogenic strains (Appleyard, 1954). Lac$^+$ transductants which were active lysogens (presumably double lysogens of the ϵ^γ:$\epsilon d lac$ type) segregated lac$^-$ progeny at a higher rate than did transductants carrying only an $\epsilon d lac$ prophage (Table 1). The presence of an ϵ^γ prophage can thus destabilize an $\epsilon d lac$ prophage. If v^{15} is homologous with ϵ^{15}, an v^{15} prophage should have the same effect.

Table 1. *Segregation of lactose-positive transductants of* Salmonella anatum

| | Spontaneous | | | ϵ^{15} curing | | | v^{15} curing | | |
| | | Lac$^-$ | | | Lac$^-$ | | | Lac$^-$ | |
Strain	No. tested	No.	%	No. tested	No.	%	No. tested	No.	%
T 31	3904	2	0·05	1465	137	9	444	129	29
T 23	2180	3	0·2	Not done	—	—	1354	127	9
T 24	2007	4	0·2	Not done	—	—	1429	54	4
T 25	795	51	6	Not done	—	—	Not done	—	—

Transductants T 23 and T 24 are defective lysogenic strains carrying $\epsilon d lac$ prophages. Transductant T 25 is a double lysogen carrying a defective $\epsilon d lac$ and a non-defective ϵ^γ prophage.

Table 2. *The instability of strain S 59 after lysogenization with bacteriophage* v^{15}

A. Instability of the lactose genes

Strain	Lac$^+$ (and Lac^v)	Lac$^-$
S 59	979	2
S 59 (v^{15})	120	237

B. Instability of the bacteriophage-producing potential

	Bacteriophage producers	Bacteriophage non-producers
S 59 (v^{15}) lac$^+$	3	31
S 59 (v^{15}) lac$^-$	37	2

The lysogenic strain was grown to saturation in LB broth and appropriate dilutions were spread on lactose tetrazolium indicator plates. The colonies developing on these plates were counted and the results are presented in the upper part of the table.

Lactose-positive and lactose-negative colonies were then stabbed and streaked against a lawn of wild-type *S. anatum* to test for the ability to produce phage.

A lac$^+$ transductant, S 59 which carried an $\epsilon d lac$ prophage not conferring immunity, was infected with phage v^{15}. Ten strains lysogenic for v^{15} (and hence resistant to $\epsilon^{15}c$) yet able to grow with lactose as carbon source were isolated and found to be very unstable, segregating single lysogens at high frequency. Table 2 shows the pattern of segregation of one of these strains. The instability of this double lysogen indicates homology between the $\epsilon d lac$ and v^{15} prophages. Why these double lysogens should be markedly more unstable than the ϵ^γ:$\epsilon d lac$ double

lysogens is not obvious. Possibly an excision enzyme normally repressed in lysogens is expressed constitutively (Erskine, 1969).

(ii) *Complementation of defective ϵ^γ genome by phage v^{15}*

Strains T23 and T24 are believed to carry defective *edlac* prophages (Hedges, 1971). Tests on cultures of these strains failed to detect the presence of any plaque-forming phage particles. Thus, these strains carry prophages that have suffered loss of some essential genetic information. Since neither treatment of cultures with chloroform nor growth in the presence of penicillin leads to the liberation of infective particles, the genes lost do not code merely for the production of enzymes involved in the release of phage particles (comparable with the S and R genes of phage λ – Harris *et al.* 1967) but included information required for the assembly of ϵ^γ particles.

When a log phase culture of strain T24 or T23 at approximately 10^8 cells per ml was infected with phage v^{15} (input multiplicity $= 0 \cdot 2$) grown for 90 min, treated with chloroform and centrifuged to remove the debris, a final phage titre of about 10^9 p.f.u./ml. was normally found. In either case the frequency of transducing particles was between 3×10^4 and 10^5 per ml. Remembering the low efficiency of lysogenization of bacteriophage ϵ^{15} (Uetake *et al.* 1958) and the fact that transducing phage genomes may lysogenize less efficiently than normal particles (Kayajanian, 1968), the proportion of transducing particles to plaque formers can be taken as not less than one in 10^4. This may be compared with the efficiency of transduction of lactose genes from an F*lac*-carrying strain of about one in 10^9. Thus phage v^{15} can complement these defective ϵ^γ genomes permitting the production of transducing phage particles.

(iii) *Recombination between phage v^{15} and defective ϵ^γ genomes*

The genomes carried by the transducing particles in the lysates produced by superinfection of strains T23 and T24 could be identical with the prophages or recombinants carrying information derived from both the prophage and the superinfecting v^{15}.

These lysates were used to transduce wild-type *S. anatum* to lac$^+$ (input multiplicity about 10^{-3}). From these experiments a large number of transductants was isolated. None was capable of producing phage, so each of these transductants probably contains a single defective prophage.

More than thirty transductants derived from T23 and T24 were tested for phage sensitivity. The results are presented in Table 3.

It is clear that recombination between the prophages and the superinfecting phages occurred. The transductants fell into four classes. Type B, the most abundant class, resembled the primary transductants (strains T23 and T24). They presumably carried prophages having conversion properties similar to those of bacteriophage ϵ^γ. (This is a double mutant strain of ϵ^{15} which has lost the ability to make β-polymerase and the repressor of the bacterial transacetylase (Robbins & Uchida, 1965).)

Transductants of class A are sensitive to phage ϵ^{34} but resistant to all other phages. They resemble strains lysogenic for phage v^{15} (or ϵ^{15}). Apparently, they carry prophages arising by recombination between the $\epsilon dlac$ prophage and the superinfecting v^{15}, and having both the conversion genes of the latter.

The genetic constitution of the other two classes of transductant is less well established. Probably they represent strains containing prophages having recombinant conversion specificity. Since it is known that phage G341 will not adsorb to a strain of *S. anatum* in which the galactosyl residues are not acetylated but that phage ϵ^{15} can do so (Uetake & Hagiwara, 1969) it is probable that transductants of type C (sensitive to infection by phage v^{15} but on which phage C341 failed to form plaques) carry prophages which have the immunity of phage

Table 3. *Transductants from strains T23 and T24*

No. of transductants	Type	Phage production	Sensitivity to			
			ϵ^{15}	v^{15}	C 341	ϵ^{34}
(1) Transductants from strain T 23						
8	A	−	−	−	−	+
15	B	−	−	+	+	−
2	C	−	−	+	−	−
10	D	−	−	−	−	−
Total 35						
(2) Transductants from strain T 24						
6	A	−	−	−	−	+
14	B	−	−	+	+	−
3	C	−	−	+	−	−
11	D	−	−	−	−	−
Total 34						

Lactose-positive transductant strains T 23 and T 24 were infected with phage ϵ^{15}. Phage from these lysates at low multiplicity (10^{-3}) was used to transduce cells of *S. anatum* to lactose-positive phenotype. The phage sensitivities of these transductants are presented in this table.

ϵ^{15}, the ability to repress the production of the transacetylase (derived from phage v^{15}) but are unable to make the β-polymerase (contain the allele from phage ϵ^{γ}). Transductants of class D may contain prophages capable of making β-polymerase but unable to repress the formation of transacetylase, but this is still uncertain.

That the pattern of phage sensitivity is determined by the prophage was established by selecting lac⁻ segregants of strains of transductants of both types. Two transductants of each class were tested. Most such segregants from all strains were found to have regained the phage sensitivities of the original strain (i.e. sensitive to phages ϵ^{15}, v^{15} and C341, resistant to phage ϵ^{34}). This confirms that the lactose genes and the genes determining the pattern of phage sensitivity are physically linked.

(iv) *Density determination*

The buoyant densities of phages ϵ^{15} and v^{15} were determined by the CsCl equilibrium density-gradient technique. Both gave identical values of 1·492–1·493. When roughly equal concentrations of phage v^{15} and a spontaneous clear-plaque mutant of phage ϵ^{15} were centrifuged together, the peaks for clear and turbid plaques coincided precisely. Thus it seems clear that the densities of the two phages are closely similar.

(v) *Evolutionary aspects*

The previously reported cases of groups of related heteroimmune phage types have been concerned either with phages unable to effect conversion (Dove, 1968; Mandel & Berg, 1968) or with phages carrying out conversion of the somatic antigen not leading to resistance to homologous superinfection (Boyd & Bidwell, 1957).

Thus it seems that all the reported cases refer to situations in which homologous superinfection is possible (though often restricted, e.g. Rao, 1968). In such cases selective advantage can be postulated for the possession of polymorphism in respect of immunity specificities. Firstly, it increases the chances for genetic exchange. If phage recombination were limited to lytic crosses it would presumably be a very rare event occurring only when two genetically dissimilar particles happened to infect a bacterial cell simultaneously. If, however, superinfection of a lysogenic strain can lead to the production of progeny phage (i.e. the lysogenic strain is not immune to the infecting phage) then among the progeny may be phages carrying genetic markers from both the prophage and the superinfecting genome. If a significant proportion of bacteria are lysogenic such events may be quite frequent.

Stahl & Murray (1966) have used the logic of population genetics to point out the advantages of a circular genetic linkage map. Since it is clear that a temperate phage (such as phage λ) can adopt a circular form and even replicate in this configuration (Carter, Shaw & Smith, 1969) it is paradoxical that the genetic map, derived from lytic crosses, is linear (Dove, 1968). A consequence of recombination between a superinfecting phage and an established prophage may be that, considering all types of crosses (lytic × lytic, lytic × prophage and prophage × prophage), the genetic map may be circular. Prophage × prophage crosses are presumably rare and this 'circularization' of the genetic map may depend upon the lytic × prophage crosses which require that the infecting phage is not repressed by the immunity system of the prophage. Thus, the existence of a number of different immunity patterns within a group of genetically related phages may influence the pattern of recombination within that group.

None of these arguments seems to apply to the present situation, since any bacterium lysogenic for either ϵ^{15} or v^{15} is completely resistant to infection by the other. If the arguments do apply to this polymorphism we must assume that the phage group of which ϵ^{15} and v^{15} are members includes phages which do not effect conversion and hence can be superinfected or which can infect converted bacterial cells.

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