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Transduction mechanisms of bacteriophage ϵ^{15}

I. General properties of the system

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SUMMARY

Bacteriophage ϵ^{γ} is capable of transduction both by replacement of a genetic segment of the recipient by the homologous genetic material from the donor strain and by the formation of defective transducing particles capable of lysogenizing the recipient strain of *S. anatum*.

The isolation of strains carrying such prophages, which have incorporated the lactose or arabinose operons, is reported. Lysogenic strains, carrying both normal and defective transducing prophage, form high-frequency transducing lysates. Other strains, carrying only defective prophage, show evidence that the association of prophage genes and transduced materials is stable since the loss of one frequently entails loss of the other.

1. INTRODUCTION

Bacteriophage ϵ^{15} is a generalized transducing phage (Iseki & Sakai, 1954) which adsorbs to the 3:10 somatic antigen of Salmonellae of the E1 subgenus. Cells lysogenic for the ϵ^{15} prophage have somatic antigens 3:15, typical of the E2 subgenus (Sakai & Iseki, 1953; Iseki & Sakai, 1953) and these cells are unable to adsorb superinfecting ϵ^{15} particles. A strain of ϵ^{15} carrying two mutations was used in the experiments described in this paper. This phage, known as ϵ^{γ}, has little qualitative influence on the somatic antigen and lysogens are, therefore, capable of being superinfected (Uetake & Uchida, 1959; Robbins & Uchida, 1965). The presence of ϵ^{γ} does, however, reduce the amount of somatic antigen present in the lipopolysaccharide layer (Robbins & Uchida, 1965).

As well as bringing about generalized (low-frequency) transduction, phage ϵ^{15} can effect specialized (high-frequency) transduction of a tetracycline resistance marker from an R factor (Harada *et al.* 1963; Kameda *et al.* 1965). In this case the DNA coding for the transduced genetic material is apparently integrated by recombination into a phage genome, replacing some of the genetic material of the phage to produce a structure analogous in organization (though different in the mechanisms that produced it) with λd*g* genomes (Morse, Lederberg & Lederberg, 1956; Campbell, 1969).

Ikeda & Tomizawa (1965) studied transduction by phage P 1 and concluded that 'most of the transducing particles lack phage genome and carry only fragments

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of the bacterial chromosome'. Similarly, Okubo *et al.* (1963) and Mahler, Cahoon & Marmur (1964) found that the bacterial DNA isolated from lysates of transducing phages of *Bacillus subtilis* was not covalently bonded to phage DNA. Yet other workers have clearly demonstrated that phage P 1 can produce transducing particles in which a segment of DNA from the bacterial chromosome becomes integrated into the genome of the transducing phage. (Adams & Luria, 1958.)

Thus, it seems that there are two mechanisms of generalized transduction. So far as present information allows generalization, all phages capable of generalized transduction by either mechanism can make use of both. The case of P1 is the best understood, but the generalization seems to hold for P22 (Dubnau & Stocker, 1964), ϵ^{15} (Kameda *et al.* 1965). *Pseudomonas* phage Pf 16 (Chakrabaty & Gunsalus, 1969) and *Staphylococcus* phage P 11 (Novick, 1967). Other phages, such as λ (Campbell, 1969) and P2 (Bertani, 1958), are incapable of generalized transduction by either mechanism.

It seems that most transducing particles of generalized transducing phages are incapable of establishing lysogeny. For example, a lysate of P1 is capable of transducing the lactose operon with an efficiency of about 10^{-5} her plaque-forming unit (p.f.u.) but establishes P1d*lac*-containing lysogens with an efficiency of about 10^{-10} per p.f.u. (Adams & Luria, 1958). Thus, transductants of the latter type form only an insignificant fraction of total transductants and will only be detectable in an experimental system in which transductants formed by recombination with a homologous region of host chromosome or plasmid DNA are not produced.

In order to isolate modified transducing prophage genomes it therefore seemed desirable to study the transduction of a genetic marker into a strain having no homologous genetic material. Transductants under these conditions are likely to be produced by integration of modified prophage which may be subjected to genetic analysis.

Strategies available to achieve this end are, for example, transduction into strains that have suffered large deletions, or into strains unable to undergo general recombination, though able to be lysogenized (Brooks & Clark, 1967). Both of these techniques have been attempted (Hedges, unpublished). In each case the efficiency of transduction was reduced from the level typical of generalized transduction (approximately 10^{-5} per p.f.u.) to below 10^{-9} per normal phage but the transductants produced showed no evidence of containing modified phage genomes.

The present paper describes the results obtained from transduction of episome-borne markers into strains lacking homologous DNA sequences. The episomes used were F*lac*, F*ara* and R220. If *S. anatum* is typical of its genus the recipient has no lactose operon comparable with that of *Escherichia coli* (Brenner *et al.* 1969; Sanderson, 1967). This assumption was confirmed in the experiments described below.

The arabinose operon of the F*ara* episome was derived from *E. coli* B (Sheppard & Englesberg, 1967). It was introduced into a strain of *S. anatum* that had

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suffered an *ara*$^-$ mutation. Phage grown on this strain was used to transduce an F$^-$ strain (carrying the same *ara*$^-$ mutation) to ara$^+$. Since the *ara* operon of the donor has the base sequence specificity of *E. coli* whilst the recipient carries an *ara* operon of *S. anatum* specificity it was hoped that the imperfection of homology would be sufficiently marked to reduce the efficiency of recombination to a level far below that found in homologous (intraspecific) transduction. Genetic divergence between *Salmonella* and *Escherichia* leading to reduced levels of inter-generic recombination has been reported for several genes (Demerec & Ohta, 1964; Eisenstark, 1965).

R 220 is an R factor carrying resistance to ampicillin and four other antibacterial compounds (streptomycin, tetracycline, kanamycin and sulfa drugs).

2. MATERIALS AND METHODS

Bacterial strains and bacteriophages. These are listed in Table 1. From the prototrophic *S. anatum* A1 were isolated:

(a) An arabinose non-utilizing mutant induced by ICR 191 and stimulated to revert by that compound. This mutant, presumably a frame-shift (Ames & Whitfield, 1967), had a spontaneous reversion rate $< 10^{-10}$.

Table 1

Bacteria	Genotype	Obtained from:
S. anatum A1	Prototrophic	H. Uetake
E. coli 108 F*lac*	K12 *met*, *pro*, *try*, *lac*/F*lac* ($i^- z^+ y^+$)	R. H. Pritchard
E. coli J5-3 (R220)	K12 F$^-$, *pro*, *met* (R220)	N. Datta
F*ara* donor	K12, *try*, *araA*, *araC*/F*ara*$^+$	E. Englesberg
Bacteriophages		
ϵ^{15}	—	H. Uetake
ϵ^{γ}	—	H. Uetake
ϵ^{34}	—	H. Uetake
C341	—	T. Uchida

(b) A quadruple auxotrophic strain requiring tryptophane (or indole), histidine, leucine and arginine (specifically). All were spontaneous mutations isolated after penicillin screening. The strain also carried a spontaneous mutation conferring high-level streptomycin resistance (strA).

Media. Minimal media were M9 liquid or agar (Clowes & Hayes, 1968). Nutrient agar was that specified by Clowes & Hayes (1968). Liquid nutrient medium was LB broth (Hercules, Knacht & Zubay, 1968). Indicator media were EMB agar (Clowes & Hayes, 1968) and tetrazolium agar (Zamenhoff, 1961).

Propagation of phage. The agar layer method (Adams, 1959) was used.

Heat shock. A brief period at high temperature has been shown to reduce the ability of bacteria to recognize the 'foreignness' of DNA synthesized in cells with different modification patterns (Uetake, Toyama & Hagiwara, 1964). Since it is probable that *S. anatum* A1 and *E. coli* K12 have different modification

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patterns the recipient population was subjected to heat shock before intergeneric transfer of episomes. Log-phase cultures, containing about 10^8 cells per ml., in shallow broth in thin-walled flasks, were plunged into a shaking water-bath at 51 °C and left for 3 min, immediately before use.

Transfer of genetic material. Episomes were transferred from *E. coli* to heat-shocked *S. anatum* by conjugation. Equal quantities of log-phase cultures of donor and recipient strains were mixed and incubated at 37 °C for 1 h. The mixtures were plated on appropriated selective media.

For transduction, fully grown broth cultures of recipient were used. Three to five phage, p.f.u. per recipient cell were introduced. The phage-treated culture was plated on appropriate selective medium.

For transduction of episomal genes, episomes were transferred by conjugation to *S. anatum*. ϵ^{γ} lysates of the recipients were prepared and applied to episome-free cultures of *S. anatum*.

3. RESULTS

The F*lac* episome of *E. coli* (strain 108/F*lac*) or an R factor (R 220) from *E. coli* J 5-3 was transferred to *S. anatum* by conjugation. That the F*lac* episome was replicating as a plasmid was demonstrated by its instability (Pritchard, 1969) and by the low efficiency with which the strain transferred the *S. anatum* genes relative to the efficiency of transfer of the lactose genes. Transfer of the lactose genes to *S. anatum* A1 (wild type) occurred with an efficiency of about 10^{-2} per donor cell whilst the transfer of individual chromosmal markers to the quadruple strain never exceeded 10^{-5}.

Phage ϵ^{15} (and ϵ^{γ}) grew equally well on the original bacteria and on strains carrying F*lac* or R 220. Since phage lysates were prepared by the agar-layer method, almost all the particles were produced as a result of a lytic infection. These stocks, which had titres of about 10^{10} p.f.u. per ml. effected homologous transduction of chromsomal (*S. anatum*) markers (e.g. tryptophan genes) with an efficiency of about one transductant per 10^5 p.f.u.

DESCRIPTION OF PLATE

Fig. 1. Colonies of lactose-positive transductant T 25 growing on lactose tetrazolium agar. The strain shows lactose-negative sectors and the peculiar 'rough' phenotype. (In order to obtain maximum expression of roughness rather dry plates were used and incubated at 37 °C for about 36 h.)

Fig. 2. Transductant T 5 grown overnight (approximately 20 generations) in lactose minimal medium and spread on lactose-tetrazolium indicator agar. Note that the lactose marker must have replicated so that the instability cannot be due to abortive transduction.

Fig. 3. Colonies of an arabinose-positive transductant on arabinose tetrazolium agar. The pink colonies show pale sectors (marked *a*) and dark red (arabinose-negative) sectors (marked *b*). To provide a clearer picture a smooth segregant of the transductant was isolated and this strain is shown in the photograph.

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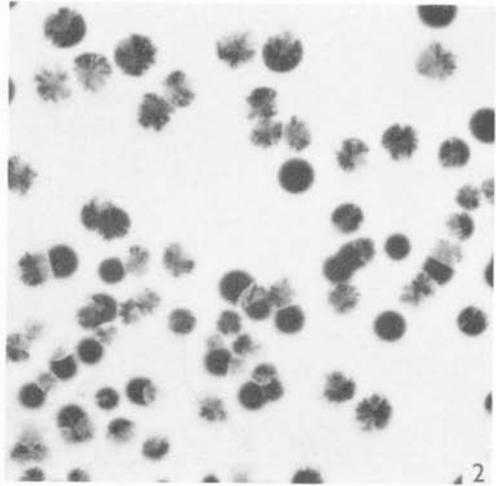

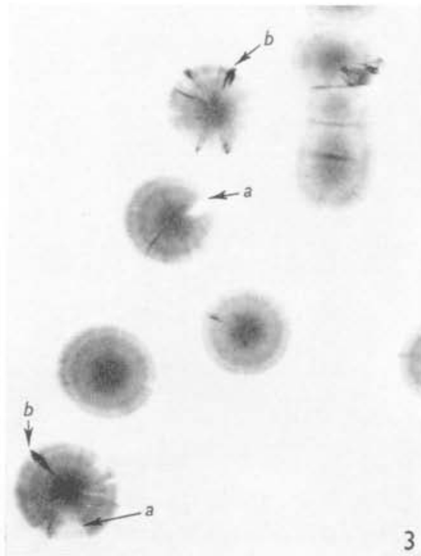

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(i) *Transduction of lactose genes*

Lysates of phage ϵ^{γ} from *S. anatum* (F*lac*) transduced wild type *S. anatum* recipients with an efficiency of approximately one transductant per 10^9 p.f.u. The transductants were all unstable. With one exception, all showed a peculiar colonial morphology when plated on lactose-tetrazolium indicator agar (Fig. 1). This effect is apparently due to the partial roughness produced by lysogenization with phage ϵ^{γ} (Hedges, 1971*b*).

Transductants could be divided into three classes: (A) those capable of producing plaque-forming phage particles; (B) those producing no plaque-forming particles but immune to homologous superinfection; (C) those producing no plaque-forming particles and susceptible to homologous superinfection.

Table 2. *Segregation of lactose-negative colonies by lactose-metabolizing transductants of* S. anatum

Strain	Lac⁻ colonies	Total colonies	Segregants (%)
T 31 (class C)	2	3904	0·05
T 23 (class B)	3	2180	0·2
T 24 (class B)	4	2007	0·2
T 25 (class A)	51	795	6

Colonies that appeared uniformly lactose-positive on indicator plates were stabbed and grown overnight in LB broth. Appropriate dilutions were then plated on lactose tetrazolium indicator agar.

Table 3. *Production of phage ϵ^{γ} by strains of* S. anatum

Strain	Bacterial cells/ml	Phage particles/ml	Ratio
A 1 (ϵ^{γ})	$3{\cdot}8 \times 10^9$	$7{\cdot}1 \times 10^7$	10^{-2}
T 25	$3{\cdot}3 \times 10^9$	$2{\cdot}0 \times 10^6$	10^{-3}
T 25 Lac⁻ segregant 1	$5{\cdot}2 \times 10^9$	$9{\cdot}4 \times 10^6$	10^{-3}
T 25 Lac⁻ segregant 2	$4{\cdot}2 \times 10^9$	$4{\cdot}5 \times 10^6$	10^{-3}
T 5	$3{\cdot}7 \times 10^9$	$2{\cdot}0 \times 10^4$	10^{-5}
T 5 Lac⁻ segregant 1	$3{\cdot}2 \times 10^9$	$2{\cdot}6 \times 10^4$	10^{-5}
T 5 Lac⁻ segregant 2	$5{\cdot}8 \times 10^9$	$1{\cdot}2 \times 10^4$	10^{-5}

Cultures were grown overnight in nutrient broth and the total number of bacterial cells assayed by dilution and plating. Phage assays were performed after the bacteria in the cultures had been killed by chloroform. The chloroform was removed by incubation at 37 °C before phage estimation.

(*a*) *Phage-producing transductants* (*class A*)

This class, which constituted about half the total lactose-positive transductants, fell into two apparently distinct groups. One group, of which T5 is a typical example, was extremely unstable (Fig. 2) whilst members of the other group (typified by strain T25) were relatively stable (Table 2).

Both produced less phage than a standard lysogen: the extremely unstable group produced very low levels of phage, while strains of the more stable type

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produced almost as much as standard lysogens (Table 3). Both groups were stably lysogenic. Loss of the ability to use lactose did not markedly affect the level of phage production.

Spontaneous lysates from transductants of the T5 type were never found to transduce lactose genes, but lysates from strain T25 (and similar strains) were very efficient transducing agents. In spontaneous lysates (from log phase cultures) have an average titre of 5×10^4 p.f.u. per ml the transducing efficiency averaged 7×10^3 transducing particles per ml and never fell below 2×10^3.

All the lactose-positive transductants produced by phage from T25 (using a multiplicity of infection of about 10^{-3} p.f.u. per bacterium) were immune but unable to produce phage particles.

To test whether any of the phage-producing lactose-positive transductants carried a prophage genome with both the lactose genes and the genetic information necessary for plaque formation, plaques formed by phages from the high frequency transducing (HFT) lysates were picked up in glass tubes and the bacteria spread on EMB lactose plates. None of 100 plaques tested showed any lactose-positive transductants. When HFT lysates were titrated on EMB lactose plates, both lactose-positive transductants and plaques were seen. They did not coincide more frequently than might be expected by chance. Thus it seems that few, if any, of the plaque-forming particles carried the lactose genes.

(*b*) *Non-phage-producing transductants* (*classes B and C*)

These fall into three subclasses: B, Immune – rough colonies (type examples, T23 and T24); Ci, non-immune – rough colonies (type example, S59); Cii, non-immune – smooth colonies (sole example, T31).

Among all non-phage-producing transductants the first class was the most frequent (102 of 169). All but one of the remainder (i.e. 66) were of the second class and only one representative of the third class was observed. No transductant which was immune to superinfection but did not show the rough colonial form was ever observed.

Spontaneous delysogenization and curing. The lactose genes were unstable in all transductants. The stablest were those which produced no phage. To find whether the phage was lost with the lactose genes, eight lactose-negative segregants of strains T23 and seven of T24 were isolated. All the lactose-negative segregants of strain T23 and four of those from strain T24 were sensitive to phage ϵ^{15}. Of the three lactose-negative segregants of strain T24 that retained immunity, two were unstable, segregating lactose-positive revertants with a frequency approaching 10^{-3}. As controls, fifty lactose-positive colonies each of strains T23 and T24 were tested. All remained immune.

Ten lactose-negative segregants were isolated from strains T5 and T25. All retained the ability to produce phage at approximately the same rate as the original transductant (Table 3).

The defective lysogens T23, T24 and T31 were curable by superinfection with phage υ^{15}, a phage believed to be a hetero-immune relative of phage ϵ^{15} (Hedges,

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1971*a*). Strain T31, non-immune to superinfection with phage ϵ^{15}, could be cured by either phage.

(ii) *Transduction of arabinose genes*

Efficiency of transduction for arabinose utilization by ϵ^{γ} was approximately 10^{-8} per p.f.u. Many of the transductants gave dark pink colonies on arabinose-tetrazolium indicator agar easily distinguishable from wild type or the arabinose-negative mutant strain. Pale (pink to white) sectors, with colour typical of wild type (arabinose-positive) bacteria, were frequently observed (Fig. 3).

Among 183 arabinose-positive transductants (not all of which were shown to be of independent origin) three were clearly unstable. All three were immune but none produced plaque-forming particles. Twenty independently occurring arabinose-negative segregants were isolated from one of these strains. Six had lost superinfection immunity. None of 50 control (arabinose-positive) colonies had lost immunity.

(iii) *Transduction of penicillin resistance*

Study of transductants of R factor genes led to the isolation of a penicillin-resistant transductant apparently analogous to the T5 group of Lac^{+} transductants. The penicillinase determinant was inherited extremely unstably, and only a very low level of spontaneous ϵ^{γ} phage production could be detected. A remarkable feature of this strain was that the ϵ^{γ} particles produced from it were clear plaque formers with very little (if any) ability to lysogenize new hosts.

4. DISCUSSION

This paper presents various classes of transductants isolated when recipient bacteria lacked genetic homology with the transduced DNA.

The subclass exemplified by strain T25 seems to be most easily explained as carrying two prophage genomes: one normal, non-defective ϵ^{γ}, and the other defective transducing genome, ϵd*lac*. That there are two separate prophages rather than a single plaque-forming, transducing genome comparable with ϕ80p*t* genomes (Matsushiro, Sato & Kida, 1964) is indicated, since secondary transductants were not capable of producing phage, and cells lysogenized by phage from the primary transductants were not lactose-positive.

The class represented by strains T23 and T24 were apparently carriers of defective prophages containing the gene(s) conferring immunity and the gene(s) leading to the rough colonial form (presumably the gene making the inhibitor of the bacterial α-polymerase (Robbins & Uchida, 1965; Losick, 1969)), but lacking one or more of the genes required to produce complete phage particles.

The fact that the lactose genes and the phage immunity are often lost together favours the view that the former are stably integrated into the defective prophage genome. The fact that this genome can be 'cured' by superinfection with a phage apparently related to ϵ^{15} indicates that the ϵd*lac* genome is integrated at the normal site.

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Transductants such as strains T 31 and S 59 are apparently very similar to strains T 23 and T 24, differing only in that the segment deleted from the prophage includes the immunity gene(s) and, in the case of strain T 31, the gene(s) causing the rough phenotype. The curing of these prophages by phage ϵ^{15} confirms the view that, even in the case of strain T 31 (in which no phage genes were identified), the lactose genes exist as part of a defective ϵ^{γ} prophage.

Sixty-seven transducing prophages lacking the immunity region were identified but only one of these was incapable of inducing the rough phenotype. This suggests, very strongly, that although both the immunity function and the conversion genes are expressed in the lysogen, they are not closely linked and hence do not form an operon. It is possible that phage ϵ^{15} resembles phage ϵ^{34} in having its conversion genes closely linked to the attachment site, so that most deletions of the conversion genes extend into the attachment site and so abolish the ability to transduce the lactose genes. Possibly, in the integrated prophage, the converting genes are no longer associated with a phage operon but are transcribed from a promoter on the bacterial chromosome (cf. Bertani, 1970).

The transductants most difficult to explain are those exemplified by strain T 5. In such strains the lactose genes are inherited very unstably whilst the prophage is inherited much more stably. Perhaps the two elements are not physically associated in the transduced cells. But no very unstable transductant in non-lysogenic strains, nor in normally productive lysogens, has been observed. This may indicate that the lactose genes are dependent upon the prophage – perhaps integrated into it but for some reason extremely susceptible to deletion. The failure to effect transduction using phage from strain T 5 argues against this possibility, but not decisively in view of the small number of phage particles and the fact that many of them must have been produced by cells that had already lost the lactose genes.

A possible explanation of these transductants is that a phage coat may enclose two separate DNA molecules, a fragment of the F*lac* and an incomplete phage genome. In the recipient cell the lactose genes replicate as part of a plasmid (a fragment of the original F*lac* episome). The partial defectiveness of the prophage may be explicable in terms of loss of part of the normal prophage DNA, not essential for phage production but involved in the normal excision machinery (Dove, 1967). The fact that a penicillinase transductant was obtained from the R 220^{+} donor may be significant since in that case a recognizable phage function has been lost. Most plausibly, the clear-phage phenotype arises from the loss of a gene necessary for the establishment but not for the maintenance of lysogeny. In the hope of proving the loss of a part of the phage genome, the buoyant densities of the phages produced by strain T 5, the penicillinase transductant and a normal lysogen were compared. No differences could be detected. All had a density of about 1·492–1·493. It is known, however, that certain phages (such as T 4, where the chromosome has permuted terminal duplications) can compensate for deletions, producing genomes of normal length but with longer terminal duplications (Nomura & Benzer, 1961). Thomas (1967) postulated that all phages

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capable of generalized transduction have these permuted terminal redundancies. This conclusion seems to have been refuted by the behaviour of phage *chi* which is capable of generalized transduction (Iino & Lederberg, 1964) but does not show double stranded terminal redundancies, having single stranded chromosome termini (Thomas, Kelly & Rhodes, 1969). Thus, no sure deductions can be drawn about the genetic organization of phage ϵ^{15} on the basis of its transducing properties.

Experiments with the arabinose genes were designed to test whether the production of ϵd*lac* genomes required any special and peculiar properties of the lactose region or whether the phage could form similar recombinant transducing genomes with other genetic material of *E. coli*. The lactose operon was chosen as the first transduction marker because it was believed that the *Salmonella* chromosome had no genetic material homologous with these genes (Miyake, 1962). It seems, however, that there may be genetic information in *Salmonella* DNA at least partially homologous with that of the *E. coli* lactose genes (Brenner *et al.* 1969). Therefore, it is surprising that not one of the lactose transductants showed any sign of having integrated the lactose genes into the 'homologous' genetic region of the *S. anatum* chromosome. Presumably, these two genetic regions, though showing gross homologies, have diverged sufficiently (through evolutionary drift and selection) to prevent recombination. It was therefore of interest to test whether a similar divergence of the two arabinose operons had occurred.

Transduction of the *E. coli* arabinose genes into the *S. anatum* chromosome occurred with a frequency approximately a thousandth of the rate of homologous transduction of *S. anatum* genes. This is evidence for genetic divergence (Demerec & Ohta, 1964).

The frequency of transduction of the *E. coli* arabinose genes was about ten times that of the lactose genes so that, if the formation of ϵd*ara* particles and the integration of their genomes occurred with the same efficiency as that of ϵd*lac* particles, about one-tenth of the transductants should carry ϵd*ara* prophages. The rest of the transductants presumably resulted from cells in which part or all of the *E. coli* arabinose operon had replaced the 'homologous' *S. anatum* genetic material.

In fact, about 2% of the transductants were shown to carry such prophages: this was a minimum figure since screening only picked out obviously unstable transductants. The finding of these defective, prophage-carrying transductants suggests that phage ϵ^{15} is capable of forming defective, transducing prophages containing almost any genetic marker.

The dark pink colonies formed by the transductants on arabinose tetrazolium indicator agar may indicate that the *E. coli* and *S. anatum* components of these strains are not perfectly compatible (so that the transductants metabolise arabinose at a slow rate). The pale sectors (which have the phenotype typical of wild-type (arabinose-positive) strains) arise too frequently to be explicable as mutations, unless that rate is unusually high. The primary transductants and the derived 'pale' strains produce arabinose-negative sectors at about the same rate (Fig. 4).

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Thus, the 'pale' sectors are still heterogenetic, and are, presumably, not produced by recombination between the arabinose operons of host and prophage.

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