High Frequency Transduction of Kanamycin Resistance in *Proteus mirabilis*

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SUMMARY

Properties of a transducing system with a phage able to transduce a kanamycin resistance marker of the T compatibility group R factor R394 at high frequency are described. The phage was detected in *Proteus mirabilis* strain PM5006 transduced to kanamycin resistance by phage 34 grown on *P. mirabilis* strain PM13 carrying the R factor. The PM5006 transductants, specially selected at the lowest multiplicities of infection (m.o.i.) of the high frequency transducing (h.f.t.) phage, are normal lysogens. They liberate h.f.t. phage spontaneously and high-titre phage may be obtained by u.v. induction. The phage is serologically identical to phage 34 and is defective in that, at low m.o.i., transduction frequency is increased by the simultaneous presence of homologous non-transducing phage. Ultraviolet irradiation of the h.f.t. lysate produces an exponential fall in transduction frequency. It is concluded that the defective phage transduces by lysogenization. Phage present in h.f.t. lysates can also transduce various chromosomal markers of PM5006 at low frequencies. These low-frequency transductants are usually not kanamycin resistant. Transductants do not transfer the kanamycin resistance marker conjugally and produce kanamycin-sensitive normal lysogenic segregants at a high rate. Strain PM5006 is cryptically lysogenic for a phage which is morphologically and serologically identical to phage 34 and many of the particular features of this transduction system are explicable in terms of complementation or recombination between genes of the phages involved.

INTRODUCTION

High frequency transduction (h.f.t.) by phage P22 of the tetracycline resistance marker of an R factor was described by Dubnau & Stocker (1964). The phage was defective in that simultaneous infection of the recipient with a non-transducing helper phage greatly increased the efficiency of transduction. The tetracycline resistance marker of the R factor had been incorporated in the phage genome and transductants were lysogenic for a defective P22 phage carrying this marker. Watanabe, Ogata, Chan & Botstein (1972) and Chan, Botstein, Watanabe & Ogata (1972) described a similar situation where a h.f.t. lysate for tetracycline resistance was obtained by induction of a particular tetracycline resistant P22 lysogen. The lysogen was obtained by phage P22-mediated transduction of R factor R222 to *Salmonella typhimurium* LT2. The lysate was composed of particles that could not replicate or lysogenize on single infection but could co-operate with one another for these functions. Kameda, Harada, Suzuki & Mitsuhashi (1965) described high-frequency transduction of the tetracycline resistance marker from an R factor by a defective phage, epsilon. The lysate was obtained by u.v. induction of a tetracycline resistant transductant which had been lysogenized by wild-type phage. The resulting h.f.t. lysate contained many plaque-forming particles. Transduction efficiency was independent of the m.o.i. and could not be increased

Table 1. *Bacteria, plasmids and phages*

Bacteria	Properties	Reference
<i>Proteus mirabilis</i> PM13	Recipient for general transducing phage 34	Coetzee & Sacks (1960)
<i>P. mirabilis</i> PM5006	Recipient for general transducing phage 34	Coetzee & Smit (1970)
<i>P. mirabilis</i> PM5006nal-r	Spontaneous mutant resistant to 100 µg nalidixic acid/ml	Coetzee (unpublished)
<i>P. mirabilis</i> PM5006str-r	Spontaneous mutant resistant to 1000 µg streptomycin/ml	Coetzee (unpublished)
<i>P. mirabilis</i> PM5006trp-1	Auxotrophic mutants produced by nitroso-guanidine treatment	Coetzee (unpublished)
<i>P. mirabilis</i> PM5006arg-1		
<i>P. mirabilis</i> PM5006lys-1		
Providencia P29	Providencia strain P29	Coetzee (1972, 1974)
Providencia P29nal-r	Spontaneous mutant resistant to 100 µg nalidixic acid/ml	Coetzee (unpublished)
<i>Escherichia coli</i> J53-1	Nalidixic acid-resistant mutant of J53, an F ⁻ lac ⁺ , pro, met mutant of <i>E. coli</i> K12	Clowes & Hayes (1968) Coetzee <i>et al.</i> (1972)
<i>E. coli</i> J62-1	Nalidixic acid-resistant mutant of J62, an F ⁻ lac, pro, his, trp mutant of <i>E. coli</i> K12	Clowes & Hayes (1968) Coetzee (1974)
Plasmids and hosts		
PM13 (R394)	R394 introduced by conjugation. R394 is a T compatibility group R factor with markers A, K*	Coetzee <i>et al.</i> (1972) Coetzee <i>et al.</i> (1973)
PM5006(R394)-O	Original transductant clone. The superior line indicates that the R factor was introduced by transduction	Coetzee (unpublished)
J53-1 (R401)	R401 introduced by conjugation. R401 is a T compatibility group R factor with markers A, S*	Coetzee <i>et al.</i> (1972)
P29(P-lac)	P-lac confers property of lactose fermentation on hosts. Introduced by conjugation	Coetzee (1974) Coetzee <i>et al.</i> (1973) Coetzee (unpublished)
Bacteriophage		
34	General transducing phage for PM13 and PM5006	Coetzee & Sacks (1960)

* Symbols designate resistance to: A, ampicillin; S, streptomycin; K, kanamycin.

by helper phage. A converting variant of phage P1 has been described (Kondo & Mitsuhashi, 1964) which, on lysogenization, converted the *Escherichia coli* host to chloramphenicol resistance. A converting phage for ampicillin resistance in *E. coli* has also been described (Williams Smith, 1972).

Phage 34 (Coetzee & Sacks, 1960) grown on *Proteus mirabilis* strain PM13 carrying the T compatibility R factor R394 (Coetzee, Datta & Hedges, 1972) transduced this R factor to strain PM13 at a frequency of 8×10^{-6} /plaque-forming unit (p.f.u.) adsorbed (Coetzee, Datta, Hedges & Appelbaum, 1973). Transductants inherited both antibiotic resistance markers of the R factor and could transfer these by conjugation. With the same donor lysate and a closely related *P. mirabilis* strain, PM5006 (Coetzee & Smit, 1970; Coetzee, 1974), as recipient among many complete transductants, a number were obtained that expressed only the kanamycin resistance marker of R394. One of these, named PM5006(R394)-O was selected for further study.

METHODS

Bacteria, plasmids and phages. These are listed in Table 1.

Media. Media were as described previously (Coetzee *et al.* 1973), except that, when lactose utilization was the selected marker in transduction experiments, the minimal medium con-

tained 0.3 % (w/v) lactose and 0.01 % (w/v) tetrazolium chloride (Grabow, 1972) instead of glucose.

Selection for drug resistance was made on MacConkey agar containing one or more of the following: ampicillin, nalidixic acid, streptomycin or kanamycin, at 50 µg/ml unless otherwise indicated. Incubation temperature was 30 °C.

Conjugal transfer of plasmids and preparation of lytic phage lysates. Methods were as described by Coetzee *et al.* (1973).

Coexistence of plasmids and selection of bacteria in which segregation of markers had occurred. Dilutions of overnight broth cultures were plated on MacConkey agar with or without antibiotics. Alternatively colonies on MacConkey agar were replicated to agar containing antibiotics. About 5000 colonies were examined. To determine the stability of the kanamycin-resistance marker in PM5006(R394), 1 ml of an overnight broth culture was centrifuged and the cells were resuspended in 10 ml saline (0.85 %, w/v, NaCl). The suspension was diluted 1:100 in broth containing phage 34 antiserum ($K_m = 40$) to prevent infection of phage-sensitive segregants. Dilutions were plated on MacConkey agar. After 2 h incubation further dilutions were plated on agar which had immediately previously been spread with the same antiserum. The plates were incubated overnight, and then replicated to kanamycin agar.

Phage neutralization. This was done according to the method of Adams (1956). Freeze-dried phage 34 rabbit antiserum (Coetzee & Sacks, 1960) with a K_m value of 180/min was used.

Ultraviolet irradiation of phage. The source was a G.E.C. sterilamp which, at a distance of 44 cm, had an output of 13.5 erg/mm²/s measured by the inactivation of phage T2. The phage was irradiated in saline in thin layers for various periods.

Phage induction. An overnight culture of the organism was diluted 1:10 in warm broth and incubated for 90 min. Bacteria were then sedimented, resuspended in 5 ml saline and irradiated for 40 s in the dark. The suspension was added to 10 ml broth and incubated for 3 h, after which the cells were sedimented and the supernate was sterilized with 0.1 vol. chloroform.

Transduction. Overnight broth cultures of recipient bacteria were diluted 1:5 in broth and incubated for 90 min. A dilution of the phage suspension in saline (0.1 ml) was added to 2 ml of the culture and the mixture incubated for 10 min. The adsorption mixture was then diluted in saline and samples filtered through membrane filters (Coetzee, Smit & Prozesky, 1966). Membranes were incubated on nutrient agar for 1 h for kanamycin and 4 h for chromosomal streptomycin resistance transductions (Coetzee & Sacks, 1960) before transfer to selective media. Plates were incubated overnight. In experiments where prototrophy or lactose utilization were selected, bacteria in the adsorption mixture were washed in saline and plated on minimal medium or minimal lactose medium respectively. Plates were incubated for 48 h. Broth was substituted for phage suspension in control experiments and sterility of the phage was checked by inoculation into broth. The adsorption rate of phage to bacteria was determined by removing samples from the mixture at the beginning and end of the adsorption period. These were pipetted into chloroform broth and titrated on PM5006. A simple screening technique for detecting transducing lysates capable of high frequency transduction of antibiotic resistance was to replicate plaques on a plate from a phage titration by means of velvet pads to antibiotic-containing agar (see also Matsushiro, 1963; Kondo & Mitshuhashi, 1964). When attempts were made to isolate non-lysogenic transductants, the lowest m.o.i. that still yielded a few transductants were used. With most u.v.-induced lysates this was a dilution of 10^{-5} to 10^{-8} . After the 10 min adsorption the mixture was rapidly diluted in phage antiserum ($K_m = 20$), filtered, and the membrane placed on

agar which had just been spread with the same antiserum. Only membranes with a few well-separated transductants were finally examined. The unpublished observation (see Coetzee & Sacks, 1960) that phage 34 is dependent on Ca^{2+} for adsorption was not confirmed.

RESULTS

Properties of transductant PM5006($\overline{\text{R394}}$)-O

This transductant inherited only one of the two drug resistance markers of the donor, strain PM13, and was resistant to 500 μg kanamycin/ml and sensitive to 5 μg ampicillin/ml. Conjugal transfer of the kanamycin resistance marker was not observed. The strain was lysogenic and spontaneously released a phage which was serologically identical to phage 34, the phage used to transduce PM5006. Replication of plaques on titration plates from high dilutions to kanamycin agar yielded no colonies, whereas low dilution plates, with many plaques, produced a disproportionate number of resistant clones. Thus replication of a plate with 153 plaques from a 10^{-4} dilution of the lysate yielded three resistant colonies, whereas a plate from a 10^{-2} dilution produced nearly confluent growth on kanamycin agar. Transduction experiments with this phage, and PM5006 or PM5006*str-r* as recipients, showed that it transduced kanamycin resistance at a frequency of 2×10^{-1} /p.f.u. adsorbed. These transductants, named PM5006($\overline{\text{R394}}$) and PM5006*str-r*($\overline{\text{R394}}$), were chosen for further study.

*Properties of PM5006 ($\overline{\text{R394}}$) and PM5006*str-r*($\overline{\text{R394}}$)*

Both transductants resembled the donor, PM5006($\overline{\text{R394}}$)-O, in that they were resistant only to kanamycin and released a phage, both spontaneously and after u.v. induction, that was able to transduce kanamycin resistance with a high frequency (3×10^{-1} /p.f.u. adsorbed). Transductants were fairly uniform in size and no minute colonies suggestive of abortive transduction were encountered.

Eighty-four transductants were tested for their ability to adsorb the transducing phage, phage 34. None was found to do so. This is in agreement with the findings that phage 34 converts its host to non-adsorption of homologous phage (Coetzee, 1961). All 84 liberated transducing phage.

Thirty of the 84 transductants were examined for stability of kanamycin resistance under non-selective conditions. The marker was lost from all clones at a high frequency (2.5×10^{-3} /bacterium/generation). This equals the frequency with which the *gal* character is lost from transductional heterogenotes of λdg (Campbell, 1957). All of 300 segregants examined spontaneously, liberated phage 34, which was unable to transduce kanamycin resistance.

Conjugal transfer of the kanamycin resistance marker carried by PM5006($\overline{\text{R394}}$) was attempted to strains P29*nal-r* and J62-I without success. In a control experiment, both resistance markers from PM5006($\overline{\text{R394}}$) were transferred to these recipients at a frequency of about 4×10^{-5} /donor cell.

Kinetics of the expression of kanamycin resistance in transductants

Phenotypic expression of resistance to 50 μg kanamycin/ml was present immediately after the adsorption period but there was a delay of about 1 h for complete expression of resistance to 500 μg of the antibiotic/ml (Table 2). Watanabe & Fukasawa (1961) found that with phage P1-mediated transduction of an R factor, chloramphenicol and tetracycline resistance

Table 2. *Kinetics of transduction and expression of kanamycin resistance*

Transduction conditions are described in text. Time 0 coincides with the end of the adsorption period. Membranes were prepared in triplicate. For the period indicated, membranes were kept on nutrient agar before transfer to kanamycin agar.

Incubation period (min)	No. resistant transductants/plate with kanamycin ($\mu\text{g/ml}$)		
	50	200	500
0	156	110	16
30	153	124	87
60	161	158	140
120	170	163	186

were expressed rapidly whereas resistance to streptomycin was not fully expressed in transductants until 90 min had elapsed.

Transduction of other markers

An u.v.-induced lysate of $\text{PM5006str-r}(\overline{\text{R394}})$ transduced various auxotrophs of PM5006 to prototrophy at frequencies of 5×10^{-9} to 9×10^{-9} /p.f.u. adsorbed. It also transduced these strains to streptomycin resistance with about the same frequency. Transductants produced by single plaque-forming particle infection were usually kanamycin sensitive (Table 3). The u.v.-induced lysate was thus not only capable of h.f.t. of kanamycin resistance but also mediated general transduction. The u.v.-induced lysate grown in lytic cycle on strain PM5006str-r , transduced the *str-r* marker and prototrophy to auxotrophs at a frequency about tenfold higher than that of the induced lysate. It retained some transducing ability for kanamycin resistance (not shown) which was however lost when, in turn, it was used to prepare a further lysate of PM5006str-r .

The effect of helper phage

Dilutions of the h.f.t. lysate were made in a constant dilution of the lysate of PM5006str-r which transduces the *str-r* marker (but not kanamycin resistance) so that the m.o.i. of the latter phage in transduction mixtures was constant at about 3 p.f.u. The effect of this phage on the efficiency of kanamycin resistance transduction is illustrated in Fig. 1. The non-transducing phage (for kanamycin resistance) exerted a helper effect by increasing the transduction frequency of kanamycin resistance from 3×10^{-2} /p.f.u. adsorbed for low m.o.i. of the h.f.t. phage to 3×10^{-1} /p.f.u. adsorbed. The tenfold aid is less than the helper effect of phage lambda on transductions by λdg (Arber, Kellenberger & Weigle, 1957). The rapid fall of the slope between a m.o.i. of 10^0 and 10^{-1} does not favour mutual aid among transducing particles as encountered by Chan *et al.* (1972) for a specialized transducing lysate of phage P22. The helper effect demonstrated here affords an explanation for the discrepancy noted with the replica-plate method of detecting transductants mentioned earlier.

Conjugal transfer of plasmids P-lac and R401 to $\text{PM5006str-r}(\overline{\text{R394}})$

P-lac had previously shown a propensity for recombination with R factors (Coetzee, 1974). Since it also transfers at a high rate (Coetzee, 1974), it was of interest to see what effect it would have on the kanamycin marker of $\text{PM5006str-r}(\overline{\text{R394}})$. Both R394 and R401 belong to

Table 3. *Transduction of markers by an u.v.-induced lysate of PM5006str-r(R394) and by phage produced by lytic infection of PM5006str-r with the u.v.-induced lysate*

Lysate	Titre on PM5006 (p.f.u./ml)	Recipient	Transduction frequency/p.f.u. adsorbed				
			Kanamycin resistance Frequency	Prototrophy		Streptomycin resistance	
				Frequency	No. K ^r *	Frequency	No. K ^r *
U.v. induced PM5006str-r(R394)	1×10^{10}	PM5006/ys-I	5×10^{-2}	5×10^{-9}	1/16	1×10^{-8}	0/18
		PM5006arg-I	5×10^{-2}	6×10^{-9}	3/20	9×10^{-9}	2/17
		PM5006trp-I	5×10^{-2}	9×10^{-9}	3/30	9×10^{-9}	3/29
Lytic infection of PM5006str-r by above lysate	6×10^8	PM5006/ys-I	0	9×10^{-8}	0/54	2×10^{-7}	0/24
		PM5006trp-I	0	1×10^{-7}	0/21	2×10^{-7}	0/31

Transductions were done at a m.o.i. of 10^{-2} . Protrophic and streptomycin-resistant transductants were selected on minimal medium and IccO μ g streptomycin agar respectively. Prototrophic and streptomycin resistant transductants were replicated to kanamycin agar.

* Symbol as in Table 1. Denominator equals no. clones tested.

Fig. 1. Effect of helper phage on transduction frequency. Dilutions of the h.f.t. lysate were made in a constant concentration of a lysate of PM5006*str-r* produced by lytic infection with the h.f.t. lysate. The m.o.i. of the non-kanamycin resistance transducing phage was 3 p.f.u. ●, H.f.t. lysate alone; ×, with helper phage.

the T compatibility group of plasmids (Coetzee *et al.* 1972), and should R401 transfer to PM5006*str-r*(R394) and reside stably, it might be argued that R394 exists in PM5006 in a state which does not allow it to express compatibility freely, or that the relevant compatibility genes are not present in the cell. Results are presented in Table 4. *P-lac* transferred at a characteristic high rate to PM5006*str-r*(R394), but exconjugants transmitted only *P-lac* at unusually low rates (Coetzee, 1974). Kanamycin resistance was not transferred. PM5006(R394 *P-lac*) still liberated transducing phage with all the features of the h.f.t. lysates mentioned above. *P-lac* was not transduced by these lysates. It was concluded that *P-lac* had not recombined with the kanamycin resistance marker, but the reason for low transfer rate from PM5006*str-r*(R394 *P-lac*) is not understood. In the original communication about *P-lac*, Falkow, Wohlietier, Citarella & Baron (1964) observed that the transfer of *P-lac* from cells that also harboured sex factor F was greatly diminished (see also Coetzee, 1974). R401, transferred to PM5006*str-r*(R394), existed stably in this strain and retained the property of independent transfer from it. The kanamycin resistance marker segregated from these doubles at the same frequency as from PM5006(R394).

Effect of u.v. irradiation of lysates on transduction frequency

Kinetics of the transduction of PM5006 by an irradiated h.f.t. lysate are shown in Fig. 2. The curve shows a simple exponential decline with a slope about half that for inactivation of phage 34. This suggests that some phage functions are either unnecessary for transduction or are provided by other phage by a process of complementation or recombination. This result differed from that obtained for phage 34 when streptomycin resistance transduction was measured as a function of irradiation (Coetzee & Sacks, 1960) where an increase in transductants at low doses was followed by slow inactivation. The increase has been attributed to an increase in the frequency of recombination between the chromosome and the exogenate (Arber, 1958). An exponential decline in transductants with increased doses of irradiation was encountered by Arber (1960) for the transduction of various episomes. Luria, Adams & Ting (1960) ascribed the simple decline in numbers of *lac*⁺ *Shigella* transductants, produced by irradiation of a PI lysate of *Escherichia coli*, to the failure of the transduced element to persist as prophage. A similar interpretation may be given to results presented here.

Table 4. *Conjugal transfer of plasmids to PM5006str-r(R394)*

Donor	Donor markers	Recipient	Recipient markers	Selected markers	Transfer frequency of donor markers	Exconjugant clones tested for presence of each plasmid	Transfer frequency from 'doubles', Selection for transfer of	
							R394	Other
P29(P-lac)	lac	PM5006str-r(R394)	K	lac ⁺	5 × 10 ⁻²	20/20 both present	0	2 × 10 ^{-4*}
J53-1(R401)	A, S	PM5006str-r(R394)	K	A	5 × 10 ⁻⁴	20/20 both present	0	1 × 10 ⁻⁴

Mating conditions are described in text. Streptomycin was used to contra-select donors. Frequency of transfer was per donor cell.
* J62-1 was recipient.
Symbols as in Table 1.

Fig. 2. The effect of ultraviolet irradiation on a h.f.t. lysate. Quantitative transduction experiments were done with the irradiated lysates at a m.o.i. of 2 p.f.u. of the unirradiated phage. ○, Transducing activity; ●, plaque formation.

DISCUSSION

Two features of this transducing system merit attention. Firstly, all transductants are normal lysogens and liberate h.f.t. phage spontaneously. Secondly, the h.f.t. lysate is also capable of general transduction. In most specialized transduction systems – e.g. *λdg* (Arber *et al.* 1957; Campbell, 1957), *P1dl* (Luria *et al.* 1960) and *φ80dt* (Matsushiro, 1963) – transductants obtained at low m.o.i. are defective lysogens capable of transducing only certain genes. Similarly, single infection with phage epsilon, producing high-frequency transduction of tetracycline resistance (Kameda *et al.* 1965), results in a defective lysogen. High-frequency transducing lysates of phage P22 for the proline region of *Salmonella typhimurium* transduce only that region of the chromosome and transducing particles are defective (Smith-Keary, 1966; Wing, 1968). However, Jessop (1972) described a non-defective, plaque-forming, specialized transducing phage, P22, that was capable of high-frequency transduction of proline genes and general transduction of other markers; the converting phage P1CM for chloramphenicol resistance (Kondo & Mitsuhashi, 1964) is also capable of general transduction.

An explanation of features of the present system may be that *Proteus mirabilis* strain PM5006 is cryptically lysogenic for a phage which is morphologically and serologically identical to phage 34 (Krizsanovich, 1973). This phage is non-inducible but phage is liberated sporadically. The phage does not convert strains to non-adsorption of phage 34, nor does it impose superinfection immunity on the strain. Krizsanovich explains absence of superinfection immunity and non-inducibility of the prophage on the grounds that the episite for integration of this permuted phage (Van Rensburg, 1970) into the bacterial chromosome may lie in the repressor gene which, on integration, is split and so inactivated (Bertani, 1970). On superinfection, the incoming homologous phage may have another episite for integration at a secondary locus on the prophage, so maintaining its repressor gene intact, or tandem lysogenization may occur with reconstitution of the gene. Lysogenic conversion to failure of adsorption of homologous phage makes it impossible to determine whether repressor is synthesized in these double lysogens. What has been claimed for the repressor

gene may instead apply to the gene for lysogenic conversion which is expressed by double but not cryptic, lysogens.

The presence of the cryptic prophage may explain failure to isolate defective lysogenic transductants, since defective functions of the transducing particles may be rectified by complementation or recombination with the cryptic prophage. Complementation may also lead to excision of the cryptic prophage by mechanisms which are not understood (Ozeki & Ikeda, 1968). The capsid protein of this excised phage may become associated with bacterial genes during vegetative growth, possibly by the wrapping-choice model proposed by these authors, as may happen with phage produced by spontaneous lysis (Smith-Keary, 1966). This may account for the general transducing property of the h.f.t. lysate, albeit at a lower rate than with phage produced by lytic infection (see also Coetzee & Sacks, 1960).

Gratia (1973) demonstrated recombination of the vegetative genome of a lambdoid phage with the tryptophan region of the bacterial chromosome, and Schmieger (1970) found that transducing particles of phage P22 may be formed by a recombination event between newly synthesized phage DNA and the host chromosome. The suggestion here is that a portion of the R factor consisting of at least the kanamycin resistance locus, but possibly not that of compatibility expression, recombined with the phage 34 chromosome during vegetative growth of the latter in the original PM13(R394) (Coetzee *et al.* 1973). The resulting hybrid phage genome is defective in certain functions, one of which is recognized by the assistance which helper phage can render with regard to efficiency of transduction (Fig. 1). As for λ dg (Arber *et al.* 1957) it is possible that the phage must pass through a pre-lysogenic stage before establishing itself as prophage. It may be that the defective phage cannot always pass through this stage and the presence of a vegetative helper phage can supply the necessary function. This function may not be supplied, or may be supplied less well, by the cryptic prophage.

The linear decrease in transduction frequency with increasing doses of u.v. irradiation suggests that the genes from the R factor do not have a homologue in the recipient. This supports a mechanism of transduction by lysogenization by the defective phage particle (Luria *et al.* 1960; Ozeki & Ikeda, 1968) analogous to heterogenote formation by λ dg (Morse, Lederberg & Lederberg, 1956). The latter term has been avoided here because of lack of knowledge regarding the homology of the genes involved.

The omnipresence of the cryptic prophage renders it difficult to pin-point other deficiencies of the transducing phage. A defect in a maturation function corresponding to that of λ dg (Arber *et al.* 1957) may account for the fact that kanamycin-sensitive normal lysogenic segregants occur. It may be that the excised defective transducing phage cannot always be complemented (or permitted) to multiply by the resident cryptic prophage or produce a h.f.t. lysate. This may be due to the particular mode of excision of the transducing prophage which restores cryptic prophage genes to functional integrity. This is a system where a probability exists of detecting non-defective plaque-forming transducing phage (Ozeki & Ikeda, 1968). No such phage has yet been encountered.

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