High Frequency Cotransduction of a Morganocinogenic Plasmid and Markers of R Plasmids

By J. N. COETZEE

Department of Microbiology, University of Pretoria, South Africa

(Received 13 June 1977)

A converting phage for ampicillin resistance – phage 5006Mpa – was used to transduce *Proteus mirabilis* strain PM5006 to ampicillin resistance. The infecting phage carried Tn1 as the source of its phenotype. The recipient had the conjugative plasmid P-lac as well as a non-self-transmissible plasmid as residents. The latter was a recombinant between a morganocinogenic plasmid Mor174 and R plasmid R772 which coded for kanamycin resistance. This recombinant plasmid had possibly undergone transductional shortening as a result of previous uptake by transducing phage 5006M. From these transductants, three transducing systems for transfer of morganocinogeny were obtained. The first consisted of the complete transduction of a plasmid expressing markers of Mor174 and ampicillin resistance at frequencies of about 1×10^{-5} per phage particle adsorbed. This frequency could have equalled that of the adsorption of transducing phage to recipient cells. The transduced plasmid was formed by translocation of Tn1 to the Mor174R772 complex with inactivation of the kanamycin resistance marker of the latter. The transducing phage was named phage 5006Mmora. The second – a high frequency transducing system for morganocinogeny, kanamycin and ampicillin resistances – was the result of markers of the Mor174R772 complex inserting contiguously to Tn1 in the 5006Mpa—PM5006 cryptic prophage sequence. The insertion converted the terminally redundant, circularly permuted phages into deficient phages lacking some (not always the same) genes and being unable to circularize (and hence lysogenize) due to lack of terminal repetition. The recombination of two defective genomes resulted in doublets which could reduce to the prophage state. This mutual aid explained the phage multiplicity dependence phenomenon encountered with this system. The phage was named phage 5006MHFTmorka. The third system of transduction resulted from deletion of non-essential genes from the oversized genomes just described. This restored terminal redundancy and consequently allowed individual genomes to circularize and thus transduce the three markers. This phage was named phage 5006Mdpmorka. These phages also transduced their markers to *P. mirabilis* PMOXX, a strain to which only slight and variable adsorption of the phage could be demonstrated. Properties of the systems are described.

INTRODUCTION

Bacteriocin-positive transductants have been selected as a result of their lethal effect on indicator strains (Lovett, Duvall & Keggings, 1976; Bramucci & Lovett, 1976; and see Threlfall & Holland, 1970). Provided the recipient is also sensitive to the bacteriocin produced by the transduced bacteriocinogenic material, the bacteriocin immunity phenomenon may also be used to select transductants (Tagg, Skjold & Wannamaker, 1976; Fukumaki, Shimada & Takagi, 1976). A third method is to select for the products of genes cotransduced with the bacteriocinogenic material in question (Fredericq & Delhalle, 1972). Coetzee, Krizsanovich-Williams & Williams (1977) reported the cotransduction of a non-self-transmissible morganocinogenic plasmid (Mor174) derived from *Proteus morganii* 174 and

the kanamycin resistance marker of R plasmid R772 by phage 5006M to *P. mirabilis* strain PM5006. The system was interesting in the sense that transductants were inducible, often lost the markers independently and, in the presence of *P-lac* (Falkow *et al.*, 1964), could transfer transduced markers by conjugation. It probably meant that incoming recombinant transduced particles split into phage and, in some bacteria, separate Mor174 and R772 components. A product of Mor174 was possibly responsible for the cleavage. The phage portion would then integrate in tandem to the cryptic prophage of PM5006 to render transductants inducible. The R plasmid portion had probably undergone transductional shortening (Shipley & Olsen, 1975) and this rendered it, and combined (or severed) Mor174, non-transferable by conjugation but still capable of independent replication. Induction of these transductants would yield some cotransducing phage particles. On transduction to a host which carried a resident conjugative plasmid like *P-lac*, these cotransduced particles, after severance of phage genes, would again be transmissible to other hosts. Transduction frequencies were low. In experiments reported here, attempts were made to increase the frequency. The strategy used involved attempts to add a transposon to the complex in the hope that possible accompanying deletions (Ahmed & Johansen, 1975; Cabezón *et al.*, 1975; Dempsey & Willetts, 1976; Kopecko & Cohen, 1975; Lee *et al.*, 1974; Manly, Signer & Radding, 1969; Reif & Saedler, 1975, 1976; Starlinger & Saedler, 1972, 1976; Kleckner *et al.*, 1975) would shorten the material to an extent which would allow phage genes and the covalently linked Mor174 complex to be contained in a single converting phage capsid (see Coetzee, 1977). The experiments were not successful, but three new systems for Mor174 transduction were obtained. Two of these involved defective high frequency transducing phage for the markers concerned.

METHODS

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

Media. Nutrient broth was Oxoid no. 2, code CM67. Nutrient agar was the same broth solidified with 1.2 % (w/v) Difco agar. MacConkey agar was from Difco, and minimal medium was that of Grabow & Smit (1967). When lactose utilization was the selected marker in transduction experiments, the minimal medium contained 0.3 % (w/v) lactose and 0.01 % (w/v) tetrazolium chloride (Grabow, 1972) instead of glucose. Buffered saline was 0.85 % (w/v) NaCl in 0.066 M-phosphate buffer (pH 7.1). Incubation temperature was 37 °C.

Drugs and antibiotics. Ampicillin, kanamycin and nalidixic acid were used at 50 µg ml⁻¹. Streptomycin was used at 1 mg ml⁻¹.

General phage techniques. These were described by Adams (1956) and Coetzee (1974*b*). With transduction experiments, the multiplicities of phage infection was usually not determined as the multiplicity of input (m.i.) of phages to bacteria was found to be a reasonable reflexion of the multiplicity of infection (m.o.i.) of host PM5006. This resulted in a negligible underestimation of transduction frequencies. Freeze-dried phage 34 antiserum (Coetzee & Sacks, 1960) with a neutralization constant (*K*) of 180 min⁻¹ against phage 5006M was used.

Phage adsorption. This was as described by Coetzee (1976*a*).

Ultraviolet irradiation of phage and phage induction. These were done by the methods of Coetzee (1974*b*).

Purification and concentration of phage lysates. The caesium chloride method of Botstein (1968) was used. Lysates often contained many defective phage particles which did not register in ordinary plaque assay. To obtain a true estimate of the total particle titre of some lysates, the absorption of purified lysates was determined at 260 nm in a Beckman spectrophotometer. This was then compared with the absorption of a similar lysate of the wild-type phage 5006M of known plaque-forming titre. The plaque-forming titre of many lysates had to be multiplied by factors of 10, 14 or 16 to obtain total concentrations of phage particles.

Conjugal transfer of plasmids. The standard method was that described by Coetzee *et al.* (1973). When many potential donor cultures had to be screened, 0.3 ml of mating mixtures and controls, constituted as above, were plated individually on very dry nutrient agar plates and incubated overnight. The growth was then replicated to selective media.

Detection of morganocinogeny. The replica-plating method of Williams (1977) was used.

Selection of segregants. Overnight broth cultures were plated on MacConkey agar to give about 50 colonies per plate after incubation. The plates were then replicated, by means of velveteen pads, to antibiotic-containing agar or to plates inoculated with the Mor174 indicator strain.

Table 1. *Bacteria, plasmids and phages*

Bacteria	Properties*	Reference
<i>Proteus mirabilis</i>		
PM5006	Host for phages. Cryptically lysogenic for phage 5006M. Sensitive to A, K. Not susceptible to morganocin 174	Coetzee & Smit (1970), Krizsanovich (1973), Coetzee (1974a, 1975a), Coetzee <i>et al.</i> (1977)
PM5006 <i>str-r</i>	Spontaneous mutant resistant to 1 mg streptomycin ml ⁻¹	Coetzee (1976b)
PMOxK	Susceptible to many temperate and virulent Proteus phages. Adsorption of phage 5006M and its derivatives is slight (< 20 %) and variable (for no apparent reason) and plaque formation could not be demonstrated. Sensitive to A, K. Not known to be lysogenic	Brandis & Schwarzrock (1956), Vieu & Ducrest (1961), Vieu & Capponi (1965), Coetzee (1975a, b), Coetzee (1976a, b), Coetzee (unpublished)
<i>Proteus morganii</i> 163 <i>str-r</i>	Wild-type strain, resistant to morganocin 174. Spontaneous mutant resistant to 1 mg streptomycin ml ⁻¹ . Morganocin 174 indicator	Williams (1977)
<i>Escherichia coli</i> J62-1	Nalidixic acid-resistant mutant of J62, an F ⁻ <i>lac pro his trp</i> mutant of <i>E. coli</i> K12. Not susceptible to morganocin 174	Clowes & Hayes (1968), Coetzee (1974a), Coetzee <i>et al.</i> (1977)
Plasmids		
PM5006(P- <i>lac</i>)	P- <i>lac</i> plasmid confers the property of lactose fermentation and Su resistance on hosts. Introduced by conjugation	Falkow <i>et al.</i> (1964), Hedges (1975), Coetzee (unpublished)
PMOxK(P- <i>lac</i>)	As above	Coetzee (unpublished)
PM5006(P- <i>lac</i> Mori174K)†	Markers of morganocinogenic plasmid Mori174 (3 × 10 ⁸ dalton) and R plasmid R772 (27 × 10 ⁸ dalton) were cotransduced with phage 5006M to PM5006 carrying P- <i>lac</i> as resident to produce a transductant PM5006(P- <i>lac</i> Mori174R772). With the help of P- <i>lac</i> , the transduced recombinant was transferred by conjugation to J62-1. The latter transconjugant (again with aid of P- <i>lac</i>) could transfer the recombinant plasmid back to PM5006. P- <i>lac</i> did not always accompany the recombinant. Although this is a transconjugant, the superior line is retained to indicate the derivation of markers. Because the constitution of the recombinant plasmid is not known, R772 has been replaced with its selected marker K	Coetzee <i>et al.</i> (1977), Hedges (1975)
Bacteriophages		
5006M.PM5006	Cryptic prophage of PM5006. Produced by lytic infection of PM5006. Generalized transducing for PM5006. Serologically identical to phages 34 and 5006Mpa. Linear DNA approx. 23 × 10 ⁶ dalton, circularly permuted, terminally redundant. Lysogens of PM5006 are converted to homologous phage non-adsorption	Coetzee & Sacks (1960), Coetzee (1961), Krizsanovich (1973), Coetzee (1977), Coetzee <i>et al.</i> (unpublished)
5006Mpa	Carries TnI and is converting for A	Hedges & Jacob (1974), Cohen (1976), Coetzee (1977)

* Symbols designate resistance to: A, ampicillin; K, kanamycin; Su, sulphonamide.

† Throughout this paper, superior lines indicate that markers were transduced.

Table 2. *Properties of 212 transductants of transconjugant PM5006(P-lacMor174K) by phage 5006Mpa*

Ultraviolet induction and transfer of markers to *E. coli* J62-1 was done as outlined in Methods. Plate matings were first done and those transductants which failed to transfer markers were then tested by the standard fluid method. Non-selected markers in purified transductants were detected by replication to MacConkey agar containing antibiotics and to agar which had been seeded with the Mor174 indicator strain.

Transductant nos	Plaque-forming titre of lysate on induction*	Transfer of markers to J62-1 by conjugation		
		Selected marker	Transfer frequency per donor cell	Non-selected markers†
1-157, 159-208, 210-212	$5-8 \times 10^9$	K	8×10^{-4}	<i>lac</i> ⁺ , Mor174 (20/20)
		A	$< 1 \times 10^{-8}$	—
		<i>lac</i> ⁺	5×10^{-2}	K, Mor174 (8/12)
158, 209	5×10^7 ‡	K	$< 1 \times 10^{-8}$	—
		A	$< 1 \times 10^{-8}$	—
		<i>lac</i> ⁺	8×10^{-2}	0

* p.f.u. ml⁻¹ on PM5006.

† Numbers in parentheses denote the fraction of transductants with markers.

‡ This titre had subsequently to be multiplied by a factor of 10 to yield the total particle concentration of the lysate.

Standard and replica-plate transduction experiments. These were done according to the Millipore membrane filter technique (Coetzee, 1974*b*). In transductions which involved markers of antibiotic resistance, the membranes with impinged cells, after filtering, were incubated on nutrient agar for 2 h before transfer to selective media. Direct selection for markers of morganocinogeny was not attempted.

RESULTS

Derivation of phage 5006Mmorka and 5006Mmora

Transduction of ampicillin resistance marker by phage 5006Mpa to transconjugant PM5006(P-lacMor174K). Transduction of the marker (not shown) proceeded at a frequency greater than unity at the 2.5 multiplicity of infection used in attempts to secure entry of more than one phage genome per recipient (Coetzee, 1977). All transductants retained resident markers of the recipient.

Transfer of markers of transductants by conjugation. Two hundred and twelve transductants were screened for the ability to transmit markers to J62-1 by conjugation. All but two of these transferred markers for morganocinogeny (Mor174), kanamycin resistance (K) and lactose fermentation (*lac*⁺) at frequencies of about 1×10^{-3} per donor (Table 2, lines 1 to 3). The transconjugants from this cross segregated the former two markers independently of *lac*⁺ (not shown) and it was assumed that P-*lac* had merely acted as a conjugative plasmid (Coetzee *et al.*, 1977). The remaining two transductants (nos 158 and 209) only transmitted the *lac*⁺ marker at the high frequencies (Table 2, lines 4 to 6) previously encountered (Coetzee, 1974*a*).

Induction of transductants. Transductants which transferred the markers Mor174, K and *lac*⁺ to J62-1 yielded lysates with plaque-forming titres of about 5×10^9 ml⁻¹ on PM5006 (Table 2, line 1). Only about 5% of plaques resembled the wild type. The remainder were hazy and about 1 mm in diameter and resembled those produced by phage 5006Mpa (Coetzee, 1977). Replication of these titration plates to kanamycin or ampicillin agar showed that the growth in all the small plaques and in a variable proportion of the large plaques replicated to the ampicillin agar while none of the plaques replicated to kanamycin agar. These replica transductants (Coetzee, 1977) were all Mor174⁻. The two transductants (nos 158 and 209) which only transferred the resident *lac*⁺ marker, consistently produced lysates

Table 3. *Transduction of markers by phage in induced lysates of PM5006(P-lacMori174KA) to strains of P. mirabilis*

Transductions were done as outlined in Methods. Adsorption was for 20 min. Membranes were incubated on nutrient agar for 2 h before transfer to MacConkey agar containing ampicillin and/or kanamycin at 50 µg ml⁻¹. Non-selected markers were detected by replication of purified transductant clones to other antibiotic-containing agar and to plates inoculated with the Mori174 indicator strain. Conjugations were done as outlined in Methods.

Lysate	Recipient	M.i.*	Selected marker	Phage 5006M M.i.*	Transduction frequency† or no. of transductants‡	Non-selected markers in transductants	Lysogenic transductants	Conjugal transfer of transduced markers§
1	PM5006(P-lac)	0.01	K	—	< 1 × 10 ⁻⁷	—	—	—
	PM5006(P-lac)	0.01	A	—	1.5	0	10/10	3/10¶
	PM5006(P-lac)	1.1	AK	—	< 5 × 10 ⁻⁹	—	—	—
	PMOXX(P-lac)	1.2	AK	—	< 1 × 10 ⁻⁸	—	—	—
	PMOXX(P-lac)	3.1	A	—	56‡	0	8/8	0/8
209	PM5006(P-lac)	0.001	K	—	5 × 10 ^{-2**}	A, Mori174	ND	0/3
	PM5006(P-lac)	0.001	A	—	1 × 10 ^{-1**}	K, Mori174	ND	0/5
	PM5006(P-lac)	0.001	AK	3.1	1 × 10 ⁻¹	Mori174	10/10	0/5
	PM5006(P-lac)	3.1	AK	—	8 × 10 ⁻²	Mori174	10/10	0/3
	PMOXX(P-lac)	3.0	K	—	0‡	—	—	—
	PMOXX(P-lac)	3.0	A	—	3‡	Mori174, K	3/3	0/3
	PMOXX(P-lac)	3.0	AK	—	4‡	Mori174	4/4	0/4
	PMOXX(P-lac)	3.0	AK	—	4‡	Mori174	4/4	0/4

ND, Not done.

* M.i., Multiplicity of input calculated on total particle concentration of lysate.

† Transduction frequency per input phage particle.

§ The denominator indicates the number of clones tested for ability to transfer transduced markers by conjugation to *E. coli* J62-1.

|| Three clones in about 10000 tested by replica plating were Mori174⁺.

¶ The three transductants which could transfer were those which were Mori174⁺.

** Minute transductants.

with titres 100-fold lower (Table 2, line 4). The plaque-forming titre of these two lysates had to be multiplied by a factor of 10 to yield their total particle content. Most of the plaques were small and hazy and the remainder resembled the wild type. The growth in all the small plaques replicated to kanamycin plus ampicillin agar while that in the large plaques failed to replicate to either agar. All the replica transductants were Mori174⁺.

Transduction of markers by phage in induced lysates. The lysate derived from transductant no. 1 (Table 2, line 1) (and nos 2, 100 and 200, not shown) transduced only the A marker to PM5006(P-lac) at frequencies greater than unity (Table 3, lines 1 to 3); it also yielded some transductants with PMOXX(P-lac) as recipient at a high multiplicity of input (Table 3, lines 4, 5). Three out of about 10000 transductants (including those from PMOXX), screened by the replica-plating method, were morganocinogenic and were named PM5006(P-lacMori174A) (Table 3, line 2, column 7). These three were able to transfer the markers for *lac*⁺, morganocinogeny and ampicillin resistance to J62-1 by conjugation (Table 3, line 2, column 9). The transducing activity of phage in lysate no. 209 was dependent on the multiplicity of input. At very low multiplicities, only tiny transductant clones were observed after 48 h incubation, and transduction frequencies were high but variable (Table 3, lines 6, 7). At higher m.i. (Table 3, line 9) large transductant colonies had more or less completely replaced the tiny clones and transduction frequencies were high. A similar effect was produced by co-infection of cells with transducing phage at low m.o.i. and phage 5006M at high m.i. (Table 3, line 8). All transductants were Mori174⁺ and lysogenic. These transductants were named PM5006(P-lacMori174KA). Phage in lysate no. 209 was named 5006MHFTmorka. The same lysate also transduced the three markers to PMOXX(P-lac) in small numbers at high

Table 4. *Loss of transduced markers*

Overnight cultures grown in non-selective broth were plated on MacConkey agar to yield about 50 clones per plate. Between 4000 and 7000 clones of each transductant were replicated to ampicillin agar, kanamycin agar or plates seeded with morganocin 174 indicator. Sensitive clones or morganocin-negative colonies were subsequently replicated to the other selective plates. Lysozyme was tested by treating purified segregants with phage antiserum and then submitting them to ultraviolet induction. The plating efficiency of phage 5006M was also tested and phage adsorption was studied in those strains which failed to plate the phage.

Transductant	Reference	Phenotype of segregants (%) [*]				Lysogenic status of segregants	
		Mori174 ⁻ A ^S K ^S	Mori174 ⁻ A ^R K ^S	Mori174 ⁺ A ^S K ^S	Mori174 ⁺ A ^R K ^S	Phage liberation	Phage 5006M adsorption
PM5006(P- <i>lac</i> Mori174A)	Table 3, line 2	0.64†	0†	0†	0†	+	—
PM5006(Mori174A)	Table 5, line 1	0.54†	0†	0†	0†	+	—
PM0XX(P- <i>lac</i> Mori174KA)	Table 3, line 12	3.1	0.35	0.25	0.06	+	‡
PM5006(P- <i>lac</i> Mori174KA)	Table 3, line 9	2.2	0.07	0.22	0.04	—	—
PM5006(Mori174KA)	Table 5, line 12	4.1	0.10	0.28	0.06	—	—
PM0XX(Mori174KA)	Table 5, line 15	2.8	0.51	0.36	0.11	+	‡

^{*} All segregants of *lac*⁺ transductants retained *lac*⁺. Superscripts R and S denote resistance and sensitivity respectively.

† Parent transductants K^S.

‡ Not determined as phage adsorption to PM0XX was slight and variable.

multiplicities of input (Table 3, lines 10 to 12). Transduction of antibiotic resistance markers by high frequency transducing variants of phage 5006M to *Proteus* strains, to which only slight and variable adsorption of phage, or none at all, could be demonstrated, has been described (Coetzee, 1975*b*, 1976*a*, 1977). As adsorption of phage to PMOXX(P-*lac*) could not be measured the number of phage genomes involved was unknown. The latter transductants were labelled PMOXX(P-*lac*Mori74KA) and were lysogenic (Table 3, lines 11, 12). None of these transductants could transfer transduced markers to J62-1 by conjugation. The lysate of transductant no. 158 (Table 2, line 4) gave similar results (not shown).

Segregation of markers of transductants PM5006(P-*lac*Mori74A), PM5006(P-*lac*Mori74KA) and PMOXX(P-*lac*Mori74KA). About 0.6% of colony-forming bacteria of transductant PM5006(P-*lac*Mori74A) lost both transduced markers simultaneously and segregants were still lysogenic (Table 4, line 1). All three transduced markers segregated at higher frequencies from the other two transductants followed by loss of pairs of markers or a single marker at low frequencies (Table 4, lines 3, 4). No matter which marker was selected, all segregants had lost the kanamycin resistance marker. The possible inactivation of a marker by integration of Tn1 in the corresponding gene (Coetzee, 1977) was not investigated. Segregants of PMOXX still liberated phage while those of PM5006 did not liberate phage but were still lysogenically converted to homologous phage non-adsorption (Table 4, lines 3, 4, columns 7, 8).

Induction of transductants PM5006(P-*lac*Mori74A), PM5006(P-*lac*Mori74KA) and PMOXX(P-*lac*Mori74KA). The transductant PM5006(P-*lac*Mori74A) (Table 3, line 2) yielded a lysate with a titre of 3×10^9 plaque-forming units (p.f.u.) ml⁻¹. These plaques resembled wild-type 5006M plaques and did not yield transductants when replicated to ampicillin or kanamycin agar. Induction of PM5006(P-*lac* Mori74KA) (Table 3, line 9) produced a lysate with a titre of 9×10^5 p.f.u. ml⁻¹ on PM5006 and 80% of the plaques were small and hazy. The remainder resembled the wild-type plaque. This lysate contained many defective particles and the plaque-forming titre had to be multiplied by a factor of 14 to yield the total phage content. All small plaques replicated to ampicillin plus kanamycin agar and all replica transductants were Mori74⁺. Four PMOXX(P-*lac*Mori74KA) (Table 3, line 12) transductants (nos. 5 to 8) were induced. They produced lysates which plated on PM5006 to titres of 1×10^5 p.f.u. ml⁻¹ but had no action on PMOXX. Lysates nos. 6 to 8 also produced about 80% small plaques while no. 5 mainly produced small plaques – only about 1% of plaques were large. The plaque-forming titre of lysates nos. 5 and 6 to 8 had to be multiplied by factors of 16 and 14 respectively to obtain an estimate of the total particle content. All small plaques replicated to kanamycin plus ampicillin agar and these replica transductants were all Mori74⁺.

Transduction of markers by phage in induced lysates of PM5006(P-*lac*Mori74A), PM5006(P-*lac*Mori74KA) and PMOXX(P-*lac*Mori74KA) nos 5 and 8. Phage in PM5006(P-*lac*Mori74A) lysate cotransduced the A and Mori74 markers at low m.i. at frequencies of about 1×10^{-5} per p.f.u. adsorbed to PM5006. Co-infection with phage 5006M did not influence the transduction frequency (Table 5, lines 1 to 3). The markers were also transduced to PMOXX in low numbers (Table 5, line 4). None of the PM5006 or PMOXX transductants were capable of transferring markers to J62-1 by conjugation (Table 5, lines 1 to 4, column 9) which probably meant that the same markers were previously transferred (Table 3, line 2, column 9) by the conjugative plasmid P-*lac*. The transducing phage present in the PM5006(P-*lac*Mori74A) lysate was named phage 5006M*mora*. Phage in lysate PM5006(P-*lac*Mori74KA) behaved like phage 5006MHFT*morka* in that it produced only minute transductant clones at low m.i., but in the presence of helper phage or at higher input ratios transductant clones were of normal size and also arose at high frequency (Table 5, line 5 to 7). All markers were present and transductants were lysogenic, showed conversion to phage 5006M non-adsorption and could not transfer markers by conjugation to J62-1. Lysate PMOXX(P-*lac*Mori74KA) no. 8 had exactly the same properties as described

Table 5. *Transduction of markers by phage in induced lysates of PM5006(P-lacMori174A), PM5006(P-lacMori174KA) and PMOXX(P-lacMori174KA) to strains of P. mirabilis*

Lysate	Recipient	M.i.*	Phage 5006M M.i.*	Selected marker	Transduction frequency† or no. of transductants‡	Non-selected markers in transductants§	Lysogenic transductants	Conjugal transfer of selected marker¶
PM5006(P-lacMori174A)	PM5006	0.001	—	A	1×10^{-5}	Mori174 (10/10)	20/20	0/10
	PM5006	3.4	—	A	9×10^{-6}	Mori174 (10/10)	5/5	0/3
	PM5006	3.4	4	A	9×10^{-6}	Mori174 (10/10)	3/3	0/5
	PMOXX	3.1	—	A	17‡	Mori174 (10/10)	20/20	0/5
PM5006(P-lacMori174KA)	PM5006	0.001	—	K	8×10^{-2} **	Mori174A	ND	ND
	PM5006	0.001	3	K	1×10^{-1}	Mori174A	10/10	0/4
	PM5006	3.0	—	K	1×10^{-1}	Mori174A	10/10	0/4
PMOXX(P-lacMori174KA) no. 8	PM5006	0.001	—	A	3×10^{-2} **	Mori174K	ND	ND
	PM5006	3.1	—	A	5×10^{-2}	Mori174K	10/10	0/4
	PM5006	0.001	3	K	3×10^{-2}	Mori174A	10/10	0/4
	PMOXX	3.0	—	K	27‡	Mori174A	10/10	0/4
	PM5006	0.001	—	K	1×10^{-1}	Mori174A	10/10	0/5
PMOXX(P-lacMori174KA) no. 5	PM5006	0.001	3	A	8×10^{-2}	Mori174K	10/10	0/5
	PM5006	3.6	—	A	8×10^{-2}	Mori174K	10/10	0/5
	PMOXX	3.6	—	A	12‡	Mori174K	10/10	0/3

ND, Not done.

* M.i., Multiplicity of input calculated on total particle concentration of lysate.

† Transduction frequency per input phage particle.

‡ Numbers in parentheses indicate the fraction of transductants with markers.

|| The denominator indicates the number of clones tested for phage liberation on ultraviolet induction and plating efficiency of phage 5006M in the case of PM5006 transductants.

¶ The denominator indicates the number of clones tested for ability to transfer selected markers by conjugation to *E. coli* 162-1.

** Minute transductants.

Transductions were done as outlined in Methods. Adsorption was for 20 min. Membranes were incubated on nutrient agar for 2 h before transfer to MacConkey agar containing ampicillin and/or kanamycin at $50 \mu\text{g ml}^{-1}$. Non-selected markers were detected by replication of purified transductant clones to other antibiotic-containing agar and to plates inoculated with the Mori174 indicator strain. The lysogenic status of transductants was tested by ultraviolet induction of purified transductant clones which had been treated with phage antiserum. Clones were also tested for phage 5006M plating efficiency and in cases where the phage did not plate, for phage 5006M adsorption. Conjugations were done by the standard method outlined in Methods.

Table 6. Transduction of markers to PM5006 by phage in lysates of induced PM5006 transductants

Phage lysates were obtained by ultraviolet induction of corresponding transductants. Transductions were done as outlined in Methods. Adsorption was for 20 min. Membranes were incubated on nutrient agar for 2 h before transfer to MacConkey agar containing ampicillin or kanamycin at $50 \mu\text{g ml}^{-1}$. Non-selected markers were detected by replication of purified transductant clones to other antibiotic-containing agar and to plates inoculated with the Mori74 indicator strain.

Lysate	Table 5 reference	M.i.*	Selected marker	Transduction frequency†	Non-selected markers‡
PM5006(Mori74A)	line 1	0.005	A	8×10^{-6}	Mori74 (10/10)
PM5006(Mori74KA) no. 3	line 7	0.001	A	1.7	0
PM5006(Mori74KA) no. 2	line 7	3	K	1×10^{-1}	Mori74, A (10/10)
PM5006(Mori74KA) no. 100	line 9	2.5	A	1.2	0
PM5006(Mori74KA) no. 105	line 9	2.2	K	$< 5 \times 10^{-9}$	—
PM5006(Mori74KA) no. 105	line 9	2.2	A	$< 5 \times 10^{-9}$	—
PM5006(Mori74KA) no. 500	line 14	1.1	A	2.1	0
PM5006(Mori74KA) no. 509	line 14	2.1	A	$< 5 \times 10^{-9}$	—
PM5006(Mori74KA) no. 509	line 14	2.1	K	1×10^{-6}	Mori74 (10/10)

* M.i., Multiplicity of input.

† Transduction frequency per input phage particle.

‡ Numbers in parentheses indicate the fraction of transductants with markers.

above (Table 5, lines 8 to 11), but lysate no. 5 produced complete transductants even at low m.i. and transduction frequencies were not affected by helper phage (Table 5, lines 12 to 15). Phage present in lysate no. 5 was named 5006Mdp*morka*, indicating defective plaque-forming transducing phage for the markers concerned.

Properties of transductants

Minute transductants. These were thought to be abortive transductants (Stocker, 1956) because similar colonies did not develop when uninfected cells were plated on selective media. This is consistent with the observation that co-infection with phage 5006M (Table 3, line 8; Table 5, lines 6, 10) decreased the number of minute transductants by converting them to complete large transductants. The minute transductants were not further investigated.

Segregation of markers of transductants PM5006(Mori74A), PM5006(Mori74KA) and PMOXX(Mori74KA). The pattern and frequencies of marker segregants was similar to that previously found, with simultaneous loss of markers from PM5006(Mori74A) (Table 4, line 2) being less frequent than loss of markers from the other two transductants (Table 4, lines 5, 6). The lysogenic status of the segregants was also the same as previously found (Table 4, lines 1, 3, 4 and 2, 5, 6, columns 7, 8).

Ultraviolet light induction. Transductants PM5006(Mori74A) were lysogenic (Table 5, lines 1 to 3) and yielded phage lysates with titres of 1×10^9 to 4×10^9 p.f.u. ml^{-1} , similar to those produced by PM5006(P-lacMori74A) (see above). Phage in these lysates cotransduced the markers at similar frequencies (Table 6, line 1) to those listed in Table 5, lines 1 to 3. Induction of three PM5006 transductants (nos. 1 to 3) by lysate PM5006(P-lacMori74KA) – phage 5006MHFT*morka* – (Table 5, line 7) yielded lysates with plaque-forming titres of about $1 \times 10^6 \text{ ml}^{-1}$ with the same distribution of plaque-types as phage 5006MHFT*morka* lysates. However, growth in the small plaques produced by phage in lysates nos 1 and 3 replicated only to ampicillin agar and the replica growth was not morganocinogenic (also see Table 6, line 2). Phage in lysate no. 2 (Table 6, line 3) had properties similar to phage 5006MHFT*morka*. Induction of seven PM5006 transductants produced by lysate PMOXX

(P-lacMori174KA) no. 8 (Table 5, line 9) produced lysates with different transducing potentials. Two of the lysates (nos 100 and 102) plated on PM5006 yielding mostly small hazy plaques. Growth in these plaques only replicated to ampicillin agar and replica transductants were not morganocinogenic (see Table 6, line 4). Another lysate (no. 105) yielded only large plaques which did not transduce resistance to kanamycin or ampicillin (morganocinogeny was not selected for) (Table 6, lines 5, 6). Four other lysates (nos. 109, 112, 114 and 120) contained particles with properties similar to phage 5006MHFTmorka (not shown). Induction of the 27 PMOXK transductants produced by phage in the lysate PMOXK (Plac-Mori174KA) no. 8 (Table 5, line 11) produced lysates with properties similar to the latter lysate, i.e. phage 5006MHFTmorka (not shown). Induction of 10 PM5006 transductants by phage in lysate PMOXK(P-lacMori174KA) no. 5 – phage 5006Mdpmorka – (Table 5, line 14) again yielded an array of transducing lysates. One (no. 500) only transduced ampicillin resistance to PM5006 (Table 6, line 7). Another (no. 509) transduced kanamycin resistance and morganocinogeny (but not ampicillin resistance) to PM5006 at frequencies of 1×10^{-6} per input phage (Table 6, lines 8, 9). Both the latter lysates only formed large plaques on PM5006 and, in common with all lysates, had no visible action on PMOXK. The remaining eight transductants yielded lysates containing phage with properties identical to that in lysate PMOXK(P-lacMori174KA) no. 5, i.e. phage 5006Mdpmorka (not shown). Again, phage in all 12 lysates of PMOXK transductants by PMOXK(P-lacMori174KA) no. 5 lysate – phage 5006Mdpmorka – (Table 5, line 15) behaved in the same way as the producing lysate (not shown).

Properties of phages

Neutralization by antiserum. The plaque-forming titres of phages 5006M, 5006Mmora, 5006MHFTmorka and 5006Mdpmorka were neutralized at frequencies of 170 min^{-1} and were considered to be serologically identical.

Concentration dependence of plaque assay. The formation of small plaques by phage 5006MHFTmorka was multiplicity dependent and the lysate plated as a function of the square of concentration (Fig. 1). Large plaques produced by this lysate, as well as plaques by phages 5006Mdpmorka and 5006Mmora, showed a linear relationship to dilution plated (Fig. 1).

Transduction frequencies and multiplicities of phage infection. The frequency of phage 5006Mdpmorka transduction of the ampicillin and kanamycin markers to PM5006 remained constant over a large range of multiplicities of input (Fig. 2). Transductions by phage 5006MHFTmorka, on the other hand, were multiplicity dependent. At multiplicities of phage input below one particle per cell, the number of ampicillin and kanamycin resistant transductants (which were all Mori174⁺) decreased as the square of the particle concentration (Fig. 2). However, in the presence of multiple infection by non-defective helper phage, the transduction frequency of phage 5006MHFTmorka was restored to previous levels irrespective of the multiplicity of input (Fig. 2).

Ultraviolet irradiation of phage lysates. Attempts were made to determine the position of transduced markers relative to the chromosome of the recipient by studying the effect which ultraviolet irradiation of transducing lysates had on transduction frequencies of markers (Arber, 1960; Luria, Adams & Ting, 1960). The progressive decrease in transduction frequencies of markers of all phages with increasing exposure (Fig. 3), favoured either an extrachromosomal location of markers or the possibility that markers were part of a phage genome which had integrated in the host chromosome by a single crossover event (Campbell, 1962).

Fig. 1

Fig. 2

Fig. 1. Concentration dependence of plaque assay. Serial doubling dilutions of purified phage lysates were titrated with constant volumes of a buffered saline suspension of PM5006. To obtain statistically significant numbers of the large plaque-forming particles in the phage 5006MHFT*morka* lysate, more than one plate per dilution was prepared. □, Phage 5006*Mmora*; ●, phage 5006MHFT*morka* small plaques; ■, phage 5006MHFT*morka* large plaques; ○, phage 5006Mdp*morka*.

Fig. 2. Effect of multiplicity of input of phage on the transduction of ampicillin and kanamycin resistances. Transductions were done as outlined in Methods and suitable dilutions of the adsorption mixture were filtered to determine the number of transductants. The results show the total numbers of ampicillin and kanamycin resistant transductants per ml of adsorption mixture. The total particle titre of phage 5006MHFT*morka* was 14 times, and that of phage 5006Mdp*morka* was 16 times the plaque-forming titre on PM5006. ○, Phage 5006MHFT*morka* alone; ■, phage 5006MHFT*morka* plus phage 5006M at m.i. 4; ●, phage 5006Mdp*morka*.

Fig. 3. Effect of ultraviolet irradiation on phage lysates. Quantitative transduction experiments were done with phage in the irradiated lysates at m.i. of unirradiated phage of 3. Selection was for ampicillin resistance with phage 5006*Mmora* and for ampicillin and kanamycin resistance with phages 5006*Mmorka* and 5006Mdp*morka*. □, Transducing activity of phage 5006*Mmora*; ■, transducing activity of phage 5006*Mmorka*; ○, transducing activity of phage 5006Mdp*morka*; ●, plaque formation by all lysates on PM5006.

DISCUSSION

With the vast majority of phage 5006Mpa transductants it is suggested that the phage had merely lysogenized recipients leaving the Mor174R772 complex untouched and still susceptible to transfer by the resident P-lac plasmid (Table 2, lines 1 to 3). On induction of transductant no. 1, the ampicillin marker of the vegetative phage in one or a small number of cells transposed to the Mor174R772 plasmid with inactivation of the kanamycin resistance marker of the latter. This new plasmid was then taken up in a phage capsid and this could account for the three morganocinogenic ampicillin-resistant transductants produced by this lysate (Table 3, line 2, column 7) with recipient PM5006(P-lac). The fact that the markers could be transferred by conjugation (Table 3, line 2, column 9) meant that the plasmid was deposited intact and extrachromosomally by the phage. Induction of these transductants would result in further chance-envelopment by capsids with subsequent transfer to new recipients. Results of segregation studies on these transductants (Table 4, lines 1, 2) are compatible with loss of a single-copy plasmid (see below) bearing both markers. The relatively high transduction frequency could result from the extrachromosomal nature of events and be a measure of the adsorption frequency of the particles involved (see Arber, 1960; Iordănescu, 1975; Shipley & Olsen, 1975). This course of events was supported by the steady decrease in transduction frequencies with increasing irradiation of the transducing lysate (Fig. 3), the fact that the frequencies were not affected by helper phage (Table 5, line 3, column 4) and that the particles in transducing lysates plated linearly (Fig. 1). The fact that at low m.i. transductants were lysogenic (Table 5, line 1) could mean that transducing particles contained some phage genes (Schmieger, 1970) which, on integration in tandem to the cryptic prophage, rendered the latter inducible (Krizsanovich, 1973; Coetzee, 1974b). The addition of TnI to the Mor174R772 complex (already shortened for accommodation in the phage 5006M capsid – see Coetzee *et al.*, 1977) could be in the kanamycin resistance gene (thus accounting for inactivation of the latter) (Heffron, Rubens & Falkow, 1975; Hedges & Jacob, 1974; Berg *et al.*, 1975; Kleckner *et al.*, 1975; and see Freeman, Bibb & Hopwood, 1977) or to another portion of the plasmid accompanied by a deletion in the latter gene (see Lee *et al.*, 1974; Kleckner *et al.*, 1975; Reif & Saedler, 1975, 1976). The last explanation is favoured as it need not involve an increase in mass. The fact that markers of the plasmid were non-transferable by conjugation in the absence of P-lac (Table 5, lines 1 to 4, column 9) could imply that, just like the parent (Coetzee *et al.*, 1977), this recombinant required a conjugative plasmid for transfer.

A different course of events must have followed entry of phage 5006Mpa genomes into PM5006(P-lacMor174K) to form transductants nos 158 and 209 as only P-lac was transferable to J62-1 by conjugation (Table 2, lines 4 to 6). This could mean that the Mor174R772 complex had integrated in the chromosome, or possibly that a gene on this plasmid, necessary for its mobilization by P-lac, had been inactivated by addition of TnI. The latter phenotype would correspond to a Mob⁻ ColE1 (Warren & Sherratt, 1977; Hershfield *et al.*, 1976). Mor174 is a multicopy plasmid maintaining 30 to 40 copies per genome equivalent in *P. morganii* 174 (Williams, 1977), like low molecular weight colicinogenic factors (Hardy, 1975). It is not known how many copies of it or the Mor174R772 complex are present in host PM5006. Recombinant plasmids have been described (Hershfield *et al.*, 1976) which have different copy numbers from parent replicons. It is also undetermined whether an incompatibility situation exists between integrated and extrachromosomal states of the complex as encountered with the F factor (Scaife & Gross, 1962; Maas, 1963). In any case, for the phenotype to have been detected, unaffected copies of plasmid Mor174R772 (if they existed) must have been removed.

The thesis put forward here is that markers of the Mor174R772 plasmid inserted contiguously to the ampicillin resistance gene in the prophage 5006Mpa—cryptic prophage 5006M sequence on the PM5006 chromosome. Whether this was facilitated or even mediated

by TnI (Cohen, 1976; Robinson, Bennett & Richmond, 1977) is not known. However with excision of the oversized prophage(s) on induction, the insertion caused derangement of the encapsidated genomes. Instead of being terminally redundant with each particle carrying a complete (permuted) set of genes like the wild type, these genomes lacked terminal repetition and some (not always the same) genes. Such populations of phage, while possessing all genetic information as a group, were individually defective and depended on mutual aid for propagation. The insertion model has been meticulously elucidated (Chan *et al.*, 1972; Tye, Chan & Botstein, 1974; Chan & Botstein, 1976) for the circularly permuted, terminally redundant phage P22 and the size of inserted DNA could approach that of the phage headful (Tye *et al.*, 1974). Two linear defective genomes would recombine to form a terminally repetitious doublet which could then circularize and integrate by a single crossover in the chromosome. This mutual aid is reflected both in the multiplicity dependence of phage 5006MHFT*morka* plaque formation (Fig. 1) and the multiplicity of infection necessary for complete transduction of markers (Fig. 2).

The abortive transductants encountered with single infection by phage 5006MHFT*morka* did not influence the steady decline in transduction frequencies with increasing dose of ultraviolet irradiation to the phage lysate (Fig. 3). When good homology exists between markers and chromosome, small doses of irradiation often stimulate double crossover events and result in an initial increase in transduction frequencies (Luria *et al.*, 1960; Lacey, 1973; Drexler & Kylberg, 1975; Tagg *et al.*, 1976; Pemberton & Tucker, 1977). Here it is assumed that no such homology existed (Jessop, 1976) and the only way of getting transduced markers attached to the chromosome was by doublet formation. The properties of phage 5006Mdp*morka* are compatible with an origin as a deletion of some genetic material of prophage 5006MHFT*morka* in PMOXK. Deletion has been described in association with transpositions and insertions (see Kopecko & Cohen, 1975; Kleckner *et al.*, 1975; Ahmed & Johansen, 1975; Reif & Saedler, 1976) and as a result of ultraviolet irradiation of lysogens (Chan *et al.*, 1972). On being excised this phage genome would now have its terminal redundancy restored and could function independently provided the material lost was expendable. That this was so is shown by the linear relationships existing between plaque formation (Fig. 1), transduction frequency (Fig. 2) and multiplicity of infection. Obviously these phages were defective in some function(s) associated with plaque formation, possibly in the ease with which infecting genomes were reduced to prophage. On the other hand, loss of the inserted piece would completely restore the genome and this is given as an explanation for the presence of a variable proportion of wild-type phage in the lysates. The fact that transduced markers were usually lost *en bloc* at an appreciable frequency (Table 4, lines 3 to 6) and that transductants were lysogenic usually yielding transducing phage, suggested that the entire transducing phage had been integrated in the bacterial chromosome and that transductants were heterogenote-like (Luria *et al.*, 1960; Jessop, 1972).

The fragmentation or rearrangement of incoming genomes of phages 5006MHFT*morka* and 5006Mdp*morka* often encountered in PM5006 (see above and Table 6, lines 2 to 9) was a unique event not associated with any of the other *Proteus* high frequency transducing systems (Coetzee, 1974*b*, 1975*a*, 1976*b*, 1977). This phenomenon was mostly (but not exclusively) encountered with PM5006 transductants by phage assembled in PMOXK. In PMOXK the phages bred true and PMOXK transductants form the only reliable source of these phages at present. Strain PM5006 possesses a host modification system (Coetzee & Smit, 1970). This system has not yet been studied in relation to PMOXK, one encumbrance being that none of the phages investigated plates on the latter organism. Transduction frequencies were high, however, and extensive degradation of incoming DNA by PM5006 was unlikely (Coetzee & Smit, 1969). A further indication of possible derangement of prophage genes in PM5006 was the altered lysogenic status of segregants from lysogenic transductants of only the latter strain. These segregants for one or more markers failed to liberate phage on induction but retained the property of lysogenic conversion to homologous phage non-adsorption

(Table 4, lines 4, 5). Although the mechanism is not understood, there is possibly a nice distinction between genes involved in induction of prophage and those responsible for lysogenic conversion to homologous phage non-adsorption (see Coetzee, 1961, 1974*b*; Krizsanovich, 1973). It is unlikely that translocation of Tn1 played a role as it was also present in transductants of PMOXK. Apart from not knowing the number of phage genomes involved in PMOXK transductant formation (see above), another factor which could have contributed to differences between the PMOXK and PM5006 transductants was the presence of the cryptic prophage in PM5006 (Krizsanovich, 1973). The cryptic prophage remains an enigma. It could apparently not substitute for a wild-type (or another defective) genome in the transduction by lysogenization (Luria *et al.*, 1960) described here for phage 5006MHFT-*morka*. This form of cooperation is well known between prophage and infecting genomes in the P22 system (Chan & Botstein, 1976; Jessop, 1976; see also Rosner, 1975). However the cryptic prophage may have played a part in the rearrangement of markers of incoming transducing particles mentioned above. No form of lysogeny has been demonstrated in PMOXK (unpublished; Vieu & Capponi, 1965) and the sensitivity of this strain to many *Proteus* phages (Brandis & Schwarrock, 1965; Vieu & Ducrest, 1961) would support the contention that it carries no prophage.

In view of the serological relationship between PMOXK and *Rickettsia tsutsugamushi* (Wilson & Miles, 1975), it may now be possible to attempt the introduction of markers carried by various *Proteus* high frequency transducing phages into this organism.

The author is in receipt of grants from the South African Medical Research Council.

REFERENCES

- ADAMS, M. H. (1956). Methods of study of bacterial viruses. *Methods in Medical Research* **2**, 1-73.
- AHMED, A. & JOHANSEN, E. (1975). Reversion of the *gal3* mutation of *Escherichia coli*: partial deletion of the insertion sequence. *Molecular and General Genetics* **142**, 263-275.
- ARBER, W. (1960). Transduction of chromosomal genes and episomes in *Escherichia coli*. *Virology* **11**, 273-288.
- BERG, D. E., DAVIES, J., ALLET, B. & ROCHAIX, J. D. (1975). Transposition of R factor genes to bacteriophage λ . *Proceedings of the National Academy of Sciences of the United States of America* **72**, 3628-3632.
- BOTSTEIN, D. (1968). Synthesis and maturation of phage P22 DNA. 1. Identification of intermediates. *Journal of Molecular Biology* **34**, 621-641.
- BRAMUCCI, M. G. & LOVETT, P. S. (1976). Low-frequency, PBS1-mediated plasmid transduction in *Bacillus pumilus*. *Journal of Bacteriology* **127**, 829-831.
- BRANDIS, H. & SCHWARZROCK, A. (1956). Über *Proteus*-Bakteriophagen. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene (Abteilung I, Originale)* **165**, 226-232.
- CABEZÓN, T., FAELÉN, M., DE WILDE, M., BOLLEN, A. & THOMAS, R. (1975). Expression of ribosomal protein genes in *Escherichia coli*. *Molecular and General Genetics* **137**, 125-129.
- CAMPBELL, A. (1962). Episomes. *Advances in Genetics* **12**, 101-145.
- CHAN, R. K. & BOTSTEIN, D. (1976). Specialized transduction by bacteriophage P22 in *Salmonella typhimurium*: genetic and physical structure of the transducing genomes and the prophage attachment site. *Genetics* **83**, 433-458.
- CHAN, R. K., BOTSTEIN, D., WATANABE, T. & OGATA, Y. (1972). Specialized transduction of tetracycline resistance by phage P22 in *Salmonella typhimurium*. *Virology* **50**, 883-898.
- CLOWES, R. C. & HAYES, W. (1968). *Experiments in Microbial Genetics*, p. 227. Oxford and Edinburgh: Blackwell Scientific Publications.
- COETZEE, J. N. (1961). Lysogenic conversion in the genus *Proteus*. *Nature, London* **189**, 946-947.
- COETZEE, J. N. (1974*a*). Properties of *Proteus* and *Providencia* strains harbouring recombinant plasmids between P-lac and R1dr19 or R447b. *Journal of General Microbiology* **80**, 119-130.
- COETZEE, J. N. (1974*b*). High frequency transduction of kanamycin resistance in *Proteus mirabilis*. *Journal of General Microbiology* **84**, 285-296.
- COETZEE, J. N. (1975*a*). High frequency transduction of ampicillin and kanamycin resistance in *Proteus mirabilis*. *Journal of General Microbiology* **87**, 173-176.
- COETZEE, J. N. (1975*b*). Transduction of a *Proteus vulgaris* strain by a *P. mirabilis* bacteriophage. *Journal of General Microbiology* **89**, 299-309.
- COETZEE, J. N. (1976*a*). Intra-species transduction with *Proteus mirabilis* high frequency transducing phages. *Journal of General Microbiology* **93**, 153-165.
- COETZEE, J. N. (1976*b*). Derivation and properties of *Proteus mirabilis* systems for high frequency transduction of streptomycin-sulphonamide and streptomycin-sulphonamide-kanamycin resistances. *Journal of General Microbiology* **96**, 95-107.

- COETZEE, J. N. (1977). Derivation of a *Proteus mirabilis* converting phage for ampicillin resistance. *Journal of General Microbiology* **99**, 127-138.
- COETZEE, J. N. & SACKS, T. G. (1960). Transduction of streptomycin resistance in *Proteus mirabilis*. *Journal of General Microbiology* **23**, 445-455.
- COETZEE, J. N. & SMIT, J. A. (1969). Restriction of a transducing bacteriophage in a strain of *Proteus mirabilis*. *Journal of General Virology* **4**, 593-607.
- COETZEE, J. N. & SMIT, J. A. (1970). Properties of *Proteus mirabilis* phage 13vir. *Journal of General Virology* **9**, 247-249.
- COETZEE, J. N., DATTA, N., HEDGES, R. W. & APPELBAUM, P. C. (1973). Transduction of R factors in *Proteus mirabilis* and *P. rettgeri*. *Journal of General Microbiology* **76**, 355-368.
- COETZEE, J. N., KRIZSANOVICH-WILLIAMS, K. & WILLIAMS, J. A. (1977). Cotransduction of morganocinogenic plasmid 174 and R factor R772. *Journal of General Microbiology* **100**, 299-308.
- COHEN, S. N. (1976). Transposable genetic elements and plasmid evolution. *Nature, London*, **263**, 731-738.
- DEMPSEY, W. B. & WILLETTS, N. S. (1976). Plasmid co-integrates of prophage lambda and R factor 100. *Journal of Bacteriology* **126**, 166-176.
- DREXLER, H. & KYLBERG, K. J. (1975). Effect of UV irradiation on transduction by coliphage T1. *Journal of Virology* **16**, 263-266.
- FALKOW, W., WOHLHIETER, J. A., CITARELLA, R. V. & BARON, L. S. (1964). Transfer of episomic elements to *Proteus*. *Journal of Bacteriology* **88**, 1598-1601.
- FREDERICO, P. & DELHALLE, E. (1972). Recombinaison entre facteurs R et facteurs colicinogènes chez *Escherichia coli* K12. *Annales de l'Institut Pasteur* **122**, 909-921.
- FREEMAN, R. F., BIBB, M. J. & HOPWOOD, D. A. (1977). Chloramphenicol acetyltransferase-independent chloramphenicol resistance in *Streptomyces coelicolor* A3(2). *Journal of General Microbiology* **98**, 453-465.
- FUKUMAKI, Y., SHIMADA, K. & TAKAGI, Y. (1976). Specialized transduction of colicin E1 DNA in *Escherichia coli* K-12 by phage lambda. *Proceedings of the National Academy of Sciences of the United States of America* **73**, 3238-3242.
- GRABOW, W. O. K. (1972). Growth-inhibiting metabolites of *Proteus mirabilis*. *Journal of Medical Microbiology* **5**, 191-196.
- GRABOW, W. O. K. & SMIT, J. A. (1967). Methionine synthesis in *Proteus mirabilis*. *Journal of General Microbiology* **46**, 47-57.
- HARDY, K. G. (1975). Colicinogeny and related phenomena. *Bacteriological Reviews* **39**, 464-515.
- HEDGES, R. W. (1975). R factors from *Proteus mirabilis* and *P. vulgaris*. *Journal of General Microbiology* **87**, 301-311.
- HEDGES, R. W. & JACOB, A. E. (1974). Transposition of ampicillin resistance from RP4 to other replicons. *Molecular and General Genetics* **132**, 31-40.
- HEFFRON, F., RUBENS, C. & FALKOW, S. (1975). Translocation of a plasmid DNA sequence which mediates ampicillin resistance: molecular nature and specificity of insertion. *Proceedings of the National Academy of Sciences of the United States of America* **72**, 3623-3672.
- HERSHFIELD, V., BOYER, H. W., CHOW, L. & HELINSKI, D. R. (1976). Characterization of a mini-ColEI plasmid. *Journal of Bacteriology* **126**, 447-453.
- IORDĂNESCU, S. (1975). Recombinant plasmid obtained from two different compatible staphylococcal plasmids. *Journal of Bacteriology* **124**, 597-601.
- JESSOP, A. P. (1972). A specialized transducing phage of P22 for which the ability to form plaques is associated with transduction of the *proAB* region. *Molecular and General Genetics* **114**, 214-222.
- JESSOP, A. P. (1976). Specialized transducing phages derived from phage P22 that carry the *proAB* region of the host, *Salmonella typhimurium*: genetic evidence for their structure and mode of transduction. *Genetics* **83**, 459-475.
- KLECKNER, N., CHAN, R. K., TYE, B. K. & BOTSTEIN, D. (1975). Mutagenesis by insertion of a drug-resistance element carrying an inverted repetition. *Journal of Molecular Biology* **97**, 561-575.
- KOPECKO, D. J. & COHEN, S. N. (1975). Site specific *recA*-independent recombination between bacterial plasmids: involvement of palindromes at the recombinational loci. *Proceedings of the National Academy of Sciences of the United States of America* **72**, 1373-1377.
- KRIZSANOVICH, K. (1973). Cryptic lysogeny in *Proteus mirabilis*. *Journal of General Virology* **19**, 311-320.
- LACEY, R. W. (1973). Genetic basis, epidemiology, and future significance of antibiotic resistance in *Staphylococcus aureus*: a review. *Journal of Clinical Pathology* **26**, 899-913.
- LEE, H. J., OHTSUBO, E., DEONIER, R. C. & DAVIDSON, N. (1974). Electron microscope heteroduplex studies of sequence relations among plasmids of *Escherichia coli*. V. *ihv*⁺ deletion mutants of F14. *Journal of Molecular Biology* **89**, 585-597.
- LOVETT, P. S., DUVAL, E. J. & KEGGINGS, K. M. (1976). *Bacillus pumilus* plasmid pPL10: properties and insertion into *Bacillus subtilis* 168 by transformation. *Journal of Bacteriology* **127**, 817-828.
- LURIA, S. E., ADAMS, J. N. & TING, R. C. (1960). Transduction of lactose-utilizing ability among strains of *E. coli* and *S. dysenteriae* and the properties of the transducing phage particles. *Virology* **12**, 348-390.
- MAAS, R. (1963). Exclusion of an *F-lac* episome by an HFR gene. *Proceedings of the National Academy of Sciences of the United States of America* **50**, 1051-1055.
- MANLY, K. F., SIGNER, E. R. & RADDING, C. M. (1969). Nonessential functions of bacteriophage λ . *Virology* **37**, 177-188.
- PEMBERTON, J. M. & TUCKER, W. T. (1977). Naturally occurring viral R plasmid with a circular supercoiled genome in the extracellular state. *Nature, London* **266**, 50-51.
- REIF, H. J. & SAEDLER, H. (1975). ISI is involved in deletion formation in the *gal* region of *E. coli* K12. *Molecular and General Genetics* **137**, 17-28.

- REIF, H. J. & SAEDLER, H. (1976). IS1-dependent deletion formation in the *gal* region of the *E. coli* chromosome. *Genetics* **83**, S61-S62.
- ROBINSON, M. K., BENNETT, P. M. & RICHMOND, M. H. (1977). Inhibition of TnA translocation by TnA. *Journal of Bacteriology* **129**, 407-414.
- ROSNER, J. L. (1975). Specialized transduction of *pro* genes by coliphage P1. Structure of a partly diploid P1-*pro* prophage. *Virology* **66**, 42-55.
- SCAIFE, J. & GROSS, J. D. (1962). Inhibition of multiplication of an F-*lac* factor in HFR cells of *Escherichia coli* K-12. *Biochemical and Biophysical Research Communications* **7**, 403-407.
- SCHMIEGER, H. (1970). The molecular structure of the transducing particles of *Salmonella* phage P22. II. Density gradient analysis of DNA. *Molecular and General Genetics* **109**, 323-337.
- SHIPLEY, P. L. & OLSEN, R. H. (1975). Isolation of a nontransmissible antibiotic resistance plasmid by transductional shortening of R factor RPI. *Journal of Bacteriology* **123**, 20-27.
- STARLINGER, P. & SAEDLER, H. (1972). Insertion mutations in micro-organisms. *Biochimie* **54**, 177-185.
- STARLINGER, P. & SAEDLER, H. (1976). IS-elements in micro-organisms. *Current Topics in Microbiology and Immunology* **75**, 111-152.
- STOCKER, B. A. D. (1956). Abortive transduction of motility in *Salmonella*: a non-replicated gene transmitted through many generations to a single descendant. *Journal of General Microbiology* **15**, 575-598.
- TAGG, J. R., SKJOLD, S. & WANNAMAKER, L. W. (1976). Transduction of bacteriocin determinants in Group A streptococci. *Journal of Experimental Medicine* **143**, 1540-1544.
- THRELFALL, E. J. & HOLLAND, I. B. (1970). Co-transduction with *serB* of a pleiotropic mutation affecting colicin E2 refractivity, ultraviolet sensitivity, recombination proficiency and surface properties of *Escherichia coli* K12. *Journal of General Microbiology* **62**, 383-398.
- TYE, B. K., CHAN, R. K. & BOTSTEIN, D. (1974). Packaging of an oversize transducing genome by *Salmonella* phage P22. *Journal of Molecular Biology* **85**, 485-500.
- VIEU, J. F. & CAPPONI, M. (1965). Lysotypie des *Proteus* OX19, OXK, OX2 et OXL. *Annales de l'Institut Pasteur* **108**, 103-106.
- VIEU, J. F. & DUCREST, P. (1961). Notions nouvelles sur la lysotypie de *Proteus hauseri*: le lysotype 1. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene (Abteilung I, Originale)* **182**, 49-56.
- WARREN, G. & SHERRATT, D. (1977). Complementation of transfer deficient ColE1 mutants. *Molecular and General Genetics* **151**, 197-201.
- WILLIAMS, J. A. (1977). Mobilization of morganocin 174 plasmid and kinetics of morganocin production in *Proteus* and *Escherichia coli* hosts. *Antimicrobial Agents and Chemotherapy* **11**, 514-520.
- WILSON, G. S. & MILES, A. (1975). *Topley and Wilson's Principles of Bacteriology, Virology and Immunity*, 6th edn, pp. 2351-2352. London: Edward Arnold.