Cotransduction of Morganocinogenic Plasmid 174 and R Factor R772

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SUMMARY

Attempts were made to obtain recombinants between the morganocinogenic factor Mor174 and R plasmid R772 by transductional techniques. Transduction frequencies of the kanamycin resistance marker of R772 to *Proteus mirabilis* PM5006 or Providence P29 carrying Mor174 by phages 5006M and PL25, respectively, were 1000-fold higher than to the corresponding Mor174⁻ strains. The frequency of phage P1-mediated transduction of the same marker to *Escherichia coli* J62 was high and was not affected by the presence of Mor174 in the recipient. Transduction of the kanamycin resistance marker to PM5006 yielded heterogenote-like transductants. Transduction of the same marker to PM5006 with Mor174 as resident resulted in transductants which not only yielded low frequency kanamycin resistance transducing particles on induction but could transfer Mor174 and the transduced marker independently by conjugation. It was suggested that Mor174 exerted some function in establishing a transductionally shortened R factor. Mor174 also possibly played a role in the separation of the phage component of the transducing particle from the R factor portion. The severed phage genes could then integrate in tandem to a cryptic prophage to render transductants inducible. A phage 5006M lysate of PM5006(Mor174R772) produced a cotransductant. This transductant could not, possibly due to more extensive transductional shortening, transfer markers by conjugation. Induction yielded some particles which could cotransduce the markers to PM5006(P-lac). With assistance of the latter conjugative plasmid, markers of Mor174 and R772 were transferred as a unit by conjugation to strain J62. Phage P1 reared on the latter transconjugant cotransduced the genes for Mor174 activity and kanamycin resistance. Morganocin production by the recombinant equalled that of Mor174.

INTRODUCTION

Proteus morganii 174 carries a bacteriocinogenic plasmid (Mor174) which codes for the synthesis of morganocin 174, but although morganocin synthesis can be induced, the yield of morganocin is low (Williams, 1977). The plasmid is non self-transmissible but may be mobilized by R factor R772 (Williams, 1977; and see below). In an attempt to obtain higher yields of morganocin by altering the structure of the morganocinogenic plasmid, we tried to isolate a recombinant between the latter and R772 by using transduction techniques. To select bacteriocinogenic transductants of a sensitive strain, use may be made of the fact that a bacteriocinogenic cell accumulates immunity substance. However, lack of an organism sensitive to both morganocin and a transducing phage precluded direct selection for Mor174⁺ transductants. Consequently R772 was transduced into Mor174-containing organisms in the hope that, amongst other events, possible transductional shortening (Shipley & Olsen,

Table 1. *Bacteria, bacteriophages and plasmids*

Bacteria	Properties*	Reference	
<i>Proteus mirabilis</i> PM5006	Host for phage 5006M. Cryptically lysogenic for phage 5006M. Not susceptible to morganocin 174. Resistant to T and PMX	Coetzee & Smit (1970) Krizsanovich (1973) Coetzee (unpublished)	
PM5006nal-r	Spontaneous mutant resistant to 100 µg Nal ml ⁻¹	Coetzee (1974b)	
<i>Proteus morganii</i> <i>P. morganii</i> 174	Has morganocinogenic plasmid 174 (Mor174) as resident. Resistant to A. Mor174 circular DNA approximately 3 × 10 ⁶ dalton	Williams (1977) Williams, Krizsanovich-Williams & Harley (unpublished)	
<i>P. morganii</i> 163str-r	Wild-type strain, sensitive to morganocin 174. Spontaneous mutant resistant to 1 mg S ml ⁻¹ . Morganocin 174 indicator	Williams (1977)	
Providence P29	Providence strain NCTC9211. Biogroup I. Recipient for general transducing phage PL25. Resistant to A. Not susceptible to morganocin 174	Coetzee, Smit & Prozesky (1966)	
P29nal-r	Spontaneous mutant resistant to 100 µg Nal ml ⁻¹	Coetzee (1974b)	
<i>Escherichia coli</i> J62	F ⁻ , lac, pro, his, trp mutant of <i>E. coli</i> K12. Not susceptible to morganocin 174	Clowes & Hayes (1968)	
J62-I	Spontaneous mutant resistant to 100 µg Nal ml ⁻¹	Coetzee, Datta & Hedges (1972)	
Bacteriophages	Properties	Reference	
5006M	Cryptic prophage of PM5006. Produced by lytic infection of PM5006. Generalized transducing for PM5006. Linear DNA approx. 23 × 10 ⁶ dalton	Krizsanovich (1973) Coetzee (1974b) Coetzee, Lecatsas & Coetzee (unpublished)	
PL25	Generalized transducing for P29	Coetzee <i>et al.</i> (1966)	
P1	Generalized transducing for <i>E. coli</i> K12 strains. Linear DNA 60 × 10 ⁶ dalton	Ikeda & Tomizawa (1965, 1968)	
Plasmids and hosts	Properties*	Incompatibility group	Reference
J62-I(R772)	K. Circular DNA approx. 27 × 10 ⁶ dalton	Undetermined	Hedges (1975) Williams & Krizsanovich-Williams (unpublished), Hedges <i>et al.</i> (1973)
J62-I(R447b)	A, K	N	
PM5006(R772)	as above	as above	
PM5006(R447b)	as above	as above	
PM5006(R392)	K	?J	Coetzee <i>et al.</i> (1972)
PM5006(R397)	K	?J	Hedges <i>et al.</i> (1975)
PM5006(R753)	A, C, S, Su	V	Hedges (1975)
PM5006(R755)	A, C, S, Su	V	Hedges (1975)
PM5006(R905)	C, S, Su	V	Hedges (1975)
PM5006(RS-a)	C, K, S, Su	W	Hedges & Datta (1971)
PM5006(R192drd7)	C, S, Su, T	FII	Meynell & Datta (1966)
PM5006(P-lac)	Su, lactose utilization	A-C	Hedges (1975)
<i>P. morganii</i> 174(R1drd19)	A, C, K, S, Su	FII	Meynell & Datta (1967) Coetzee (unpublished)
<i>P. morganii</i> 174(R772)	as above	as above	Coetzee (unpublished)
<i>P. morganii</i> 174(R447b)	as above	as above	Coetzee (unpublished)
<i>P. morganii</i> 174(P-lac)	as above	as above	Coetzee (unpublished)

* Symbols signify resistance to: A, ampicillin; C, chloramphenicol; K, kanamycin; Nal, nalidixic acid; PMX, polymyxin B sulphate; S, streptomycin; Su, sulphonamide; T, tetracycline.

1975) would result in a recombinant. When R772 was similarly transduced into the corresponding Mori174⁻ organisms the transduction frequency was strikingly lower. This paper describes investigations into the nature of the interaction between R772 and Mori174.

METHODS

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

Media and antibiotics. 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. The incubation temperature was 37 °C. Ampicillin, chloramphenicol, tetracycline, nalidixic acid, streptomycin, kanamycin and polymyxin B were used at 50 µg ml⁻¹.

Production of phage lysates and general phage techniques. The methods described by Adams (1956) and Coetzee (1974*b*) were used. Lysates of phages 5006M.PM5006(R772), 5006M.PM5006(Mori174R772), PL25.P29(R772) and PL25.P29(Mori174R772) had titres of 7×10^{10} to 9×10^{10} plaque-forming units (p.f.u.) ml⁻¹. Phage lysates were sterile.

Transduction. The Millipore membrane filter technique (Coetzee, 1974*b*) was used. A superior line indicates that the marker was transduced.

Phage induction. Ultraviolet (u.v.) light induction was according to Coetzee (1974*b*). Transductants PM5006(Mori174R772) and P29(Mori174R772) yielded plaque-forming titres of 1×10^9 and 5×10^8 p.f.u. ml⁻¹ respectively.

Concentration of phage lysates. The method described for phage P22 by Yamamoto & Alberts (1970) was used.

Detection of morganocinogeny and assay of morganocin. This was done as described by Williams (1977).

Conjugal transfer of plasmids. This was done according to Coetzee *et al.* (1973).

Selection of segregants. The methods of Coetzee (1975) and Williams (1977) were used.

RESULTS

Mobilization of Mori174

Mori174 was non self-transmissible within the limits of the methods used but it transferred to various hosts in the presence of R plasmid R772 (but see Hedges, 1975) and all of a small number of other self-transmissible plasmids subsequently tested (Table 2). Transconjugants of some of the crosses which had received markers of both the conjugative plasmid and the bacteriocinogenic factor were designated PM5006(Mori174R772), P29nal-r(Mori174R772), J62-1(Mori174R772) and PM5006(Mori174R447*b*).

Properties of Mori174 transconjugants. Markers of the two plasmids carried by these hosts were lost spontaneously and independently at low frequencies (not shown) and those strains which had lost the kanamycin resistance marker of R772 were designated PM5006(Mori174R772)K^s etc. to indicate that loss of expression of the resistance marker did not necessarily imply defective segregation of the plasmid. Subsequently spontaneous one-step kanamycin- and ampicillin-sensitive derivatives of PM5006(Mori174R447*b*) were also obtained. Transfer of Mori174 by these antibiotic-sensitive derivatives of Mori174 transconjugants was not detected (transfer frequency $< 1 \times 10^{-5}$ per donor cell). Kanamycin-sensitive derivatives of transconjugants designated PM5006(R772)K^s etc. which had only received R772 (Table 2) were also obtained. None of the antibiotic-sensitive derivatives mentioned above exhibited surface exclusion towards the entry of homologous plasmids (Table 3.)

Table 2. *Mobilization of Mor174*

Stationary-phase recipient cultures were mixed with exponentially growing donor cultures at a ratio of 4:1, v/v. Mixtures were incubated for 1 h before samples were plated on selective media and incubated for 48 h. For details, see Methods.

Donor	Recipient	Donor counter-selection	Selected marker	Selected marker transfer*	Cotransfer of morganocinogeny†
<i>P. morganii</i> 174	PM5006	T	Mor174‡	$< 3.3 \times 10^{-5}$	—
<i>P. morganii</i> 174(R772)	PM5006	T	K	1×10^{-6}	9/20
<i>P. morganii</i> 174(R772)	P29nal-r	Nal	K	1×10^{-5}	4/20
<i>P. morganii</i> 174(R772)	J62-1	Nal	K	5×10^{-5}	6/20
<i>P. morganii</i> 174(R447b)	PM5006	T	K	1×10^{-6}	5/10
<i>P. morganii</i> 174(R1drd19)	PM5006	T	K	1×10^{-3}	8/10
<i>P. morganii</i> 174(P-lac)	PM5006	T	lac	1×10^{-3}	8/20

* Frequency per donor cell.

† Denominator indicates the number of transconjugants tested.

‡ No selection for Mor174 was possible.

Table 3. *Transfer of R772 to kanamycin-sensitive derivatives of R772 transconjugants*

Conjugation experiments were done as described in Table 2. Donors were counterselected with polymyxin B sulphate and selection was for kanamycin resistance. Morganocinogeny was tested by replication of transconjugant clones to streptomycin agar seeded with a lawn of morganocin indicator bacteria.

Donor	Recipient	Selected marker transfer*	Mor174 ⁺ transconjugants†
J62-1(R772)	PM5006	5×10^{-6}	0/10
J62-1(R772)	PM5006(Mor174R772)K ^s	6×10^{-6}	10/10
J62-1(R772)	PM5006(R772)K ^s	5×10^{-6}	0/10
J62-1(R772)	P29nal-r(Mor174R772)K ^s	1×10^{-6}	10/10
J62-1(R772)	P29nal-r(R772)K ^s	1×10^{-6}	0/10
J62-1(R772)	P29	1×10^{-6}	0/10
J62-1(R447b)	PM5006(Mor174R447b)A ^s K ^s	4×10^{-6}	10/10
J62-1(R447b)	PM5006	5×10^{-6}	0/10

* Frequency per donor cell.

† Denominator indicates the number of clones tested.

Transduction of R772 to Mor174⁺ and Mor174⁻ recipients

Phage lysates 5006M.PM5006(R772) and PL25.P29(R772) transduced the kanamycin resistance marker of R factor R772 to strains PM5006(Mor174R772)K^s and P29nal-r(Mor174R772)K^s at frequencies of 7×10^{-7} and 5×10^{-7} per p.f.u. adsorbed, respectively. When recipient organisms did not have Mor174 as resident, these frequencies were about 1000-fold lower. This was irrespective of whether or not Mor174 was resident in the organism on which the lysate had been made (Table 4, lines 1 to 11). Recipients PM5006 and P29 were transduced to kanamycin resistance at the same low frequencies as PM5006(R772)K^s and P29nal-r(R772)K^s, respectively (Table 4, lines 2, 5 and 8, 11). When R772 was transduced into J62 by phage P1, the presence of Mor174 in the recipient was immaterial (Table 4, lines 12, 13). These results indicated that the resident Mor174 fulfilled a function in increasing the transduction efficiency of R772 by phages 5006M and PL25. This function appeared to be specific to Mor174 since the presence of other plasmids in the recipient, viz. P-lac, R192-7, R753 (Table 4, lines 14 to 16) and R755 and R905 (not shown), did not affect the

Table 4. *Effect of Mori174 on transduction frequency of plasmid markers*

Phage at an m.o.i. of about 0.5 was added to 1 ml of an exponentially growing culture of the recipient strain and incubated for 20 min. The suspension was then filtered through a Millipore membrane, and the membrane with impinged cells was kept on nutrient agar for 2 h before transfer to selective medium. Where appropriate, transductants were replicated to morganocin 174 indicator plates. For recipient controls, phages 5006M.PM5006, PL25.P29 and P1.J62 were used.

Expt no.	Phage lysate	Recipient	Selected marker	Transduction frequency per p.f.u. adsorbed
1	5006M.PM5006(R772)	PM5006(Mori174R772)K ^a	K	7×10^{-7}
2	5006M.PM5006(R772)	PM5006	K	8×10^{-10}
3	5006M.PM5006(Mori174R772)	PM5006(Mori174R772)K ^a	K	8×10^{-7}
4	5006M.PM5006(Mori174R772)	PM5006	K	$1 \times 10^{-9*}$
5	5006M.PM5006(R772)	PM5006(R772)K ^a	K	1×10^{-9}
6	5006M.PM5006(R772)	PM5006(Mori174R447b)A ^a K ^a	K	9×10^{-7}
7	PL25.P29nal-r(R772)	P29nal-r(Mori174R772)K ^a	K	5×10^{-7}
8	PL25.P29nal-r(R772)	P29	K	3×10^{-10}
9	PL25.P29nal-r(Mori174R772)	P29nal-r(Mori174R772)K ^a	K	5×10^{-8}
10	PL25.P29nal-r(Mori174R772)	P29	K	1×10^{-10}
11	PL25.P29nal-r(R772)	P29nal-r(R772)K ^a	K	1×10^{-10}
12	P1.J62-1(R772)	J62-1(Mori174R772)K ^a	K	1×10^{-6}
13	P1.J62-1(R772)	J62	K	1×10^{-6}
14	5006M.PM5006(R772)	PM5006(P-lac)	K	8×10^{-10}
15	5006M.PM5006(R772)	PM5006(R192drd7)	K	7×10^{-10}
16	5006M.PM5006(R772)	PM5006(R753)	K	5×10^{-10}
17	5006M.PM5006(RS-a)	PM5006(Mori174R772)K ^a	S	8×10^{-7}
18	5006M.PM5006(RS-a)	PM5006	S	8×10^{-7}
19	5006M.PM5006(R392)	PM5006(Mori174R772)K ^a	K	2×10^{-9}
20	5006M.PM5006(R392)	PM5006	K	2×10^{-9}
21	5006M.PM5006(R397)	PM5006(Mori174R772)K ^a	K	2×10^{-9}
22	5006M.PM5006(R397)	PM5006	K	2×10^{-9}

* One transductant was Mori174⁺.

transduction frequency. The function of Mori174 appeared to be specific for the transduction of R772 since plasmids RS-a, R392 and R397 were transduced with the same frequency to recipients with or without Mori174 (Table 4, lines 17 to 22). It was not possible to investigate whether lysates 5006M.PM5006(Mori174R772) and PL25.P29(Mori174R772) could transduce the morganocin plasmid due to lack of selection, but kanamycin-resistant transductants (Table 4, lines 3, 4) were screened for concomitant acquisition of Mori174. One of three transductants of PM5006 transduced with lysate 5006M.PM5006(Mori174R772) was Mori174⁺. Properties of this transductant are described below. Phages 5006M and PL25 showed the same dependence of transduced R772 on resident Mori174 and further experiments were done only with PM5006 transductants.

Properties of PM5006(Mori174R772) and PM5006(R772)

Segregation pattern. Resident Mori174 was not displaced by transduced R772, neither did it play a role in maintenance of the transduced marker since markers of the two plasmids were lost independently (Table 5, line 1). The kanamycin resistance marker was lost at a higher frequency from PM5006(R772) than from PM5006(Mori174R772) (Table 5, lines 1, 2).

Conjugal ability of R772. R772 transduced to organisms containing Mori174 could be transferred by conjugation with the same frequency as the original R772 (Table 6, lines 1, 2) and could mobilize Mori174. The Mori174⁻ segregant, PM5006(Mori174R772)Mori174⁻, had

Table 5. *Loss of transduced markers of Mor174 and R772*

Overnight cultures grown in non-selective broth were plated on MacConkey agar to yield about 80 clones per plate. These were replicated to kanamycin agar or plates seeded with morganocin 174 indicator. Kanamycin-sensitive and morganocin-negative clones were subsequently replicated to morganocin indicator and kanamycin agar plates respectively.

Transductant	Segregants (%)			
	Mor174 ⁺ R772 ⁻	Mor174 ⁻ R772 ⁺	Mor174 ⁻ R772 ⁻	R772 ⁻
PM5006(Mor174R772)	0.75	2.0	0.06	
PM5006(R772)				2.5
PM5006(Mor174R772)	1.5	0.01	0.64	

Table 6. *Conjugal ability of transduced markers of R772*

Mating experiments were done as in Table 2. Kanamycin resistance was the selected marker and donors were counterselected with nalidixic acid. Where appropriate, transconjugants were replicated to morganocin 174 indicator plates.

Expt no.	Donor	Recipient	Selected marker transfer*
1	PM5006(Mor174R772)	J62-I	1×10^{-6} (35)†
2	PM5006(Mor174R772)	J62-I	1×10^{-6} (33)
3	PM5006(Mor174R772)Mor174 ⁻	J62-I	1×10^{-6}
4	PM5006(R772)	J62-I	$< 1 \times 10^{-9}$
5	PM5006(R772)	J62-I(Mor174R772)K ^s	$< 1 \times 10^{-9}$
6	PM5006(Mor174R772)	J62-I(Mor174R772)K ^s	1×10^{-6}
7	PM5006(Mor174R772)	J62-I	$< 1 \times 10^{-9}$
8	PM5006(Mor174R772)	P29nal-r	$< 1 \times 10^{-9}$

* Frequency per donor cell.

† Numbers in parentheses indicate the percentage cotransfer of morganocinogeny.

a similar transfer frequency (Table 6, line 3). In contrast, R772 transduced into PM5006 lacking a resident Mor174 factor could not transmit the kanamycin resistance marker (Table 6, line 4). The presence of Mor174 in the recipient did not influence the transfer frequency (Table 6, lines 5, 6).

Transduction by induced lysates. Lysates of phage 5006M induced from transductants PM5006(Mor174R772) again transduced kanamycin resistance to Mor174⁺ recipients at a frequency about 1000-fold higher than to Mor174⁻ recipients (Table 7, lines 1, 2). Phage induced from rare transductants, PM5006(R772), transduced kanamycin resistance at high frequency (Table 7, lines 3, 4).

Properties of PM5006(Mor174R772)

Segregation pattern. The transductant which had received markers of Mor174 and R772 simultaneously (Table 4, line 4) lost the markers independently (Table 5, line 3).

Conjugal ability. This transductant could not transfer kanamycin resistance or morganocinogeny to P29nal-r or J62-I (Table 6, lines 7, 8).

Transduction by induced lysates. Phage induced from PM5006(Mor174R772) transduced kanamycin resistance to PM5006 and PM5006(P-lac) at frequencies of 5×10^{-8} per p.f.u. adsorbed. About 70 % of transductants were Mor174⁺ (Table 7, lines 5, 6). Transductants of PM5006, PM5006(Mor174R772), could not transfer markers by conjugation (Table 6,

Table 7. *Transduction of markers by u.v.-induced lysates of transductants*

Transductions were done as in Table 5; kanamycin resistance was the selected marker.

Expt no.	Phage lysate	M.o.i.	Recipient	Transduction frequency per p.f.u. adsorbed	Conjugal transfer of selected marker*
1	5006M.PM5006(Mori74R772)	0.2	PM5006	1×10^{-9}	0/10
2	5006M.PM5006(Mori74R772)	0.2	PM5006(Mori74R772)K ^a	8×10^{-7}	0/20
3	5006M.PM5006(R772)	0.1	PM5006	2×10^{-1}	0/10
4	5006M.PM5006(R772)	0.1	PM5006(Mori74R772)K ^a	4×10^{-2}	0/10
5	5006M.PM5006(Mori74R772)	0.2	PM5006	5×10^{-8} (69)†	0/15
6	5006M.PM5006(Mori74R772)	0.1	PM5006(P-lac)	5×10^{-8} (71)	4/4

* Denominator indicates the number of clones tested for transfer to J62-1.

† Numbers in parentheses indicate the percentage cotransduction of morganocinogeny.

Table 8. *Production of morganocin by Mori74⁺ organisms*

Exponentially growing organisms were induced with mitomycin C. At intervals samples were centrifuged and the supernatant fluids were assayed for morganocin 174 activity by spotting dilutions on lawns seeded with morganocin indicator bacteria. The morganocin titres are expressed as the reciprocal of the highest dilution which gave a clear zone of inhibition.

Organism	Time after induction (h) . . .	Morganocin titre						
		0	2	4	8	24	48	72
PM5006(Mori74R772)		0	0	0	4	16	16	32
PM5006(Mori74R772)		0	0	0	4	8	16	32
J62-1(Mori74R772)		0	8	16	2	0	0	0
J62-1(Mori74R772)		0	4	16	4	0	0	0
J62-1(P-lacMori74R772)		6	8	16	4	6	6	6

lines 7, 8 and Table 7, line 5) but all four PM5006(P-lacMori74R772) transductants could transfer markers to J62-1 (Table 7, line 6) and transconjugants selected for kanamycin resistance were usually *lac*⁺ and always Mori74⁺. These transconjugants, J62-1(P-lacMori74R772), in turn transferred their markers to PM5006. All transconjugants from the latter cross selected for kanamycin resistance were Mori74⁺ but only about 50 % had also acquired the *lac* marker (results not shown). Lysates of phage 5006M prepared lytically on one of the transconjugants from this cross, PM5006(Mori74R772), and of phage P1 prepared on the parent J62-1(P-lacMori74R772), showed 100 % cotransduction of the kanamycin resistance marker and morganocinogeny in 200 transductants of each phage lysate.

Morganocin synthesis by PM5006(Mori74R772) and J62-1(Mori74R772)

The morganocin titres produced by PM5006(Mori74R772) and J62-1(Mori74R772) did not exceed those of the corresponding organisms which had received Mori74 by conjugation (Table 8).

DISCUSSION

Mori74 could only be introduced into the hosts of transducing phages through the mediation of R plasmid R772 or other plasmids tested (Table 2). To obtain these hosts with only the Mori74 marker, spontaneous loss of the single r-determinant of R772 had to be

subsequently selected. We consider that the kanamycin-sensitive organisms so obtained had segregated the entire plasmid because they could no longer transfer the *Mor174* plasmid to other strains and surface exclusion, to entry of the homologous plasmid, was lacking (Table 3) (Meynell, 1969; see also Meynell, Meynell & Datta, 1968). Should the plasmid R772 in the *Mor174*⁺ host merely have undergone some form of inactivation of the kanamycin resistance gene, recombination of incoming transduced R772 with the remaining homologous fragment might possibly account for the increased transduction frequency to *Mor174*⁺ organisms compared with that to the corresponding *Mor174*⁻ hosts (Watanabe, Sakaizumi & Furuse, 1968; Watanabe & Ogata, 1970; and see Coetzee *et al.*, 1973). A counter argument is that transduction of kanamycin resistance to *Mor174*⁻ hosts proceeded at the same low frequency as it did to kanamycin-sensitive derivatives of R772 transconjugants of the same strains (Table 4, lines 2, 5 and 8, 11). These kanamycin-sensitive derivatives, which had never been in contact with *Mor174*, had presumably arisen through segregation of the R772 plasmid. Finally, transduction of the kanamycin resistance marker of R772 also exhibited the same dependence on a resident *Mor174* plasmid in a one-step ampicillin- and kanamycin-sensitive derivative of PM5006(*Mor174*R447*b*) (Table 4, lines 2, 6). This derivative had probably segregated R447*b*.

Transduced R772 in transductants PM5006(*Mor174* $\overline{\text{R772}}$) was self-transmissible, could mobilize *Mor174* and was stable in *Mor174*⁻ segregants (Table 6, lines 2, 3). The transduced R plasmid markers thus possessed the properties by which the R factor is known and it may be concluded that the phage transduced all essential functions of the plasmid. The ability of *Mor174* to increase the transduction frequency (Table 4, lines 1 to 13) could be regarded as providing a function for establishment of the transduced markers of R772. Resident *Mor174* in the donor or recipient did not influence the transfer frequency of R772 markers by conjugation (Table 3; Table 6, lines 2, 6).

Due to a small disparity in molecular weights between the DNA of R772 and that of phage 5006M, accommodation of the former within the phage would be expected to involve transductional shortening (Shipley & Olsen, 1975). In this particular case it could involve excision of a DNA sequence necessary for establishment after transduction. This function could possibly be provided by *Mor174* resident in the recipient.

Arber (1960) reported that the transfer by phage P1 of plasmids which could readily be accommodated and which did not require integration in a resident genome of the recipient proceeded at the high frequencies of 1×10^{-5} to 5×10^{-4} per p.f.u. adsorbed (but see Ozeki & Stocker, 1958). The low frequencies of phage 5006M-mediated transduction of R772 to wild-type hosts could be a reflexion of the processes involved in transductional shortening. In this connexion it is interesting to note that phage P1 transduction of R772 was not influenced by a resident *Mor174* in the recipient and proceeded at much higher frequencies than corresponding transductions by phages 5006M and PL25 which have smaller genomes (Table 4, lines 1 to 13).

Transduction of the kanamycin resistance marker of R772 with phage 5006M to PM5006 produced typical heterogenote-like transductants (Luria, Adams & Ting, 1960; Coetzee, 1974*b*): they yielded high frequency kanamycin resistance transducing phage on u.v. induction (Table 7, lines 3, 4) and could not transfer the marker by conjugation (Table 6, line 5). In contrast, transduction of R772 to PM5006(*Mor174*R772)K^s resulted in the paradoxical situation of having single transductant clones which could yield kanamycin resistance transducing phage on u.v. induction (Table 7, lines 1, 2) and transfer the same marker by conjugation, using phage-resistant bacteria as recipients (Table 6, line 2). Phage 5006M did not transduce the kanamycin marker at high frequency as would have been expected if

the phage components and marker had been induced from the chromosome as a single unit (Luria *et al.*, 1960). An explanation could be that, in the original transduction, incoming transduced R772 DNA was severed from the phage component (Schmieger, 1970). The former then established itself as an independent plasmid while the latter integrated in tandem to the cryptic prophage of PM5006 (Krizsanovich, 1973; Coetzee, 1974*b*) and in this way rendered the latter inducible. Mori174 could function in connexion with the severance mentioned above and/or with establishment of dissociated DNA fragments.

Wild-type R factors, in addition to carrying the genes for various r-determinants, may also code for colicin I (see Meynell & Cooke, 1969), and a recombinant between a non self-transmissible plasmid coding for *EcoRI*, colicin E1 and the ampicillin resistance gene of R factor R1 has been described (Smith *et al.*, 1976). Fredericq & Delhalle (1972) obtained an array of recombinants between r-determinants of R factors R45 or R50 and ColV and ColB by using phage P1 as a transducing vehicle. Also the kanamycin resistance marker of plasmid pSC105 was recombined *in vitro* with ColE1 (Hershfield *et al.*, 1974) or a mini ColE1 plasmid (Lovett & Helinski, 1976). The recombinant described here was selected by transductional techniques. Joint transduction of markers is an accepted method of demonstrating linkage between the loci concerned (Clowes, 1972; and see Coetzee, 1974*a*). Recombination had probably occurred during conjugal cotransfer of markers from the *P. morganii* host to PM5006 (Table 2, line 2). However, ability to recombine stably is not necessarily a requirement for conjugal cotransfer (see Kameda *et al.*, 1969; Cooper, 1971). This is borne out by the fact that phage raised on the transconjugant clone also transduced kanamycin resistance independently (Table 4, line 4). The finding that the cotransductant lost the markers independently (Table 5, line 3) probably meant that, as above, the incoming recombinant transduced particle split into two or, in some cells, all three components. The phage portion was always detached and integrated in the host chromosome, as mentioned above, to render the system inducible (Table 7). The R plasmid portion had probably undergone transductional shortening (Shipley & Olsen, 1975) to render it, and combined (or severed) Mori174, non-transferrable by conjugation (Table 6, lines 7, 8) but still capable of independent replication. Induction of these transductants would again yield some cotransducing phage particles. On transduction to a host possessing a resident conjugative plasmid like P-*lac* (Table 2, line 7) these cotransduced particles, after the usual severance of phage genes, would be transmissible by conjugation to other hosts. Phage prepared lytically on the latter would be expected to cotransduce the markers at low frequency.

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