Transduction of R Factors in *Proteus mirabilis* and *P. rettgeri*

By J. N. COETZEE, NAOMI DATTA, R. W. HEDGES
AND P. C. APPELBAUM

Department of Microbiology, University of Pretoria, Republic of South Africa and
Department of Bacteriology, Royal Postgraduate Medical School,
Hammersmith Hospital, London

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SUMMARY

Phage 34 transduces some R factors to *Proteus mirabilis* strain 13. These transductants can transmit the R factor by conjugation. Transduction frequencies vary from 1×10^{-5} /plaque-forming unit (p.f.u.) adsorbed (higher than chromosomal marker transduction) to 5×10^{-8} /p.f.u. adsorbed. Transduced drug resistance markers of other R factors were detected only in the presence of resident *fi*⁻ factors in recipients and recombination between the R factors concerned could account for the fact that these transductants were able to transmit hybrid R factors by conjugation. Transduction of portions of R factors, including an *fi*⁺ factor occurred and some transductants were unable to transmit transduced markers by cell-to-cell contact despite the presence of resident *fi*⁻ R factors. In transductants which were conjugally infectious the transduced R factor did not appear to be associated with the genome of the phage. Incompatibility had no demonstrable effect on transduction rates. The presence of an *fi*⁻ resident factor reduced transduction rates when these were measurable in the absence of the resident. In two cases which involved N group resident plasmids this reduction was possibly due to restriction and host modification of transduced R factor DNA. Because of the natural resistance of the *P. rettgeri* host only R factors which bore the marker for kanamycin resistance could be selected in transductions with phage 7. R49. Only a T group factor was transduced. The transduction frequency was similar to that of a chromosomal marker but transductants were non-infectious.

INTRODUCTION

R factors or their component genes may be transduced by a number of general transducing phages. In the P1-*Escherichia coli* system (Nakaya, Nakamura & Murata, 1960; Watanabe & Fukasawa, 1960, 1961; Watanabe, Ogata & Sato, 1964; Watanabe, Furuse & Sakaizumi, 1968; Iyobe, Hashimoto & Mitsuhashi, 1969; Kameda, Harada, Suzuki & Mitsuhashi, 1969) the phage transfers the entire R factor so that transductants can transmit the plasmid by conjugation. There have also been reports of incomplete R factor transduction by this phage (Bannister, 1970; see also Romero & Meynell, 1969). P22 and ϵ 15 often only transmit portions of R factors to recipient *Salmonella typhimurium* LT-2, *S. anatum* and *S. newington* respectively (Watanabe & Fukasawa, 1960, 1961; Harada, Kameda, Suzuki & Mitsuhashi, 1963; Watanabe, Furuse & Sakaizumi, 1968). These transductants are usually unable to transmit their resistance traits by conjugation. This is because the P22-transduced defective R factor fragments are integrated in the recipient chromosome (Harada *et al.* 1963; Watanabe, Furuse & Sakaizumi, 1968; Watanabe & Ogata, 1970*a*) by mediation of the phage genome-transduction by lysogenization (Luria, Adams & Ting, 1960). There are exceptions.

In the P22-*S. typhimurium* system Watanabe, Furuse & Sakaizumi (1968) found all drug-resistant transductants with factor Rs-a could transfer their drug resistance markers by cell-to-cell contact. Okada & Watanabe (1968) found that some rough mutants of *S. typhimurium* LT-2 were sensitive to phage P1. The pattern of transduction of R factors by P1 in these strains was similar to that of P1 in *Escherichia coli*. This indicates that the pattern of R factor transduction was dependant on the transducing phage rather than on the species of bacterium involved. Drabble & Stocker (1968) reported that P22 reared on some *fi*⁻ R factor carrying strains could often transmit the entire R factor to strain LT-2. They also found, for particular *fi*⁺ R factors, that transductants which had received segregated genes of R factors via P22 could be rendered conjugally infective by infecting transductants with F'-13*lac* (Harada, Kameda, Suzuki & Mitsuhashi, 1964). Watanabe & Ogata (1970*b*) investigated the use of *S. typhimurium* recipients carrying a transfer factor or R factors which were phenotypically distinguishable (but of the same origin) as the incoming R factor genes brought by phage P22. They found that the transduction rate was increased and the majority of transductants now acquired conjugal transferability. Transduced R factors may thus be rescued from an abortive fate by recombination with replicons of the recipient and possibly be rendered transmissible by the same event. Nakaya & Rownd (1971) described R factor transduction by ϕ m in *Proteus mirabilis*. The recipient had to carry a resident R factor. The reason given is that ϕ m transduces only a portion of the R factor contained in the donor. Transductant R factors were recombinants of donor and resident R factors. The transduction rate was low but transductants were conjugally infective.

This report describes results of R factor transduction in the phage 34-*Proteus mirabilis* (Coetzee & Sacks, 1960) and phage 7-*P. rettgeri* (Krizsanovich, De Klerk & Smit, 1969) systems.

METHODS

Bacteria, phages and plasmids are listed in Table 1.

Media. Nutrient broth was Oxoid no. 2 code CM67; nutrient agar was the same solidified with 1.2 % Difco agar. MacConkey agar was Difco. Minimal medium was that of Grabow & Smit (1967). Incubation temperature was 30 °C.

Antibacterial drugs. Bacterial sensitivity was tested by inoculation on MacConkey agar containing one of the following: ampicillin, chloramphenicol, tetracycline, nalidixic acid, spectinomycin, streptomycin and kanamycin at 50 µg/ml. Kanamycin was also used at 15 µg/ml. Polymyxin B sulphate (Burroughs Wellcome and Co.) was used at 25 µg/ml to prevent growth of *Escherichia coli*. Sulphonamide resistance was tested according to the method of Datta & Hedges (1972).

Conjugal transfer of plasmids. Methods were those of Datta, Hedges, Shaw, Sykes & Richmond (1971). A mixture of 1.0 ml of a broth culture containing about 3×10^8 colony-forming organisms/ml of the donor, 4.5 ml of a late log-phase shaken broth culture of recipient and 4.5 ml broth was incubated for 1 h when 0.1 ml was plated on selective media. For the transfer of P-*lac* from strain 13 to *Proteus rettgeri* strain R49*trp*-1 the method of Wohlhieter, Falkow, Citarella & Baron (1964) was used. The presence of R factors in exconjugants was verified by their ability to transmit drug-resistance markers to *Escherichia coli* E27*nal-r*. When resistance markers were transduced into an organism possessing a resident plasmid with distinguishable markers, the purified 'double' transductant (Coetzee, Datta & Hedges, 1972), if conjugally infectious for transduced markers, was tested to see whether the introduced marker(s) was transferred concomitantly with the resident plasmid. A clone was grown in broth and used in a mating with *E. coli* E27*nal-r* or PM13*nal-r*(34) as recipient.

Table 1. *Bacteria, plasmids and phages*

Bacteria	Properties	Reference or source
<i>Escherichia coli</i> E27nal-r	Spontaneous mutant selected for nalidixic acid resistance. Sensitive to other drugs used. E27 is not an <i>E. coli</i> K12 strain	Maré & Coetzee (1965)
<i>E. coli</i> J62-1	Nalidixic acid-resistant mutant of J62 a F ⁻ lac, pro, his, trp mutant of <i>E. coli</i> K12	Coetzee <i>et al.</i> (1972) Clowes & Hayes, (1968)
<i>Proteus rettgeri</i> R49	Recipient for general transducing phage 7.R49. Sensitive to kanamycin and nalidixic acid. Resistant to other drugs used. GC%, 44	Krizsanovich <i>et al.</i> (1969)
<i>P. rettgeri</i> R49trp-1	Tryptophan-requiring mutant isolated by treatment with manganese chloride	Krizsanovich <i>et al.</i> (1969)
<i>P. mirabilis</i> PM13	Recipient for general transducing phage 34. Chromosomally resistant to tetracycline. GC%, 39	Coetzee & Sacks (1960), Coetzee & Smit (unpublished)
PM13nal-r	Spontaneous mutant selected for nalidixic acid resistance	Coetzee (unpublished)
PM13argH2	Arginine-requiring mutant isolated by treatment with manganese chloride	Prozesky (1967)
PM13nal-r(34)	Strain lysogenic for phage 34. Does not adsorb homologous phage	Coetzee (1961)

Host strain	Properties*	Compati- bility group	Reference or source
PM13(P-lac)	PM13 with P-lac introduced by conjugation. GC%, 50.	P	Professor S. Falkow, Falkow <i>et al.</i> (1964), Wohlhieter <i>et al.</i> (1964), Hedges & Datta (unpublished)
PM13(R1drd19)	ASCKSu Three replicons GC%, 48, 51, 56	F-like	Datta & Hedges (1972), Haapala & Falkow (1971)
PM13(Rs-a)	SCK† Su	W	Datta & Hedges (1972)
PM13(RP4)	ATK	P	Datta <i>et al.</i> (1971), Datta & Hedges (1972)
PM13(R438)	ATK	?P	Dr N. Datta
PM13(R447b)	AK	N	Hedges, Datta, Coetzee & Dennison (unpublished)
PM13(R6886)	ATK	P	Dr N. Datta, Stanisich & Holloway (1971)
J62-1 (R391)	K	J	Coetzee <i>et al.</i> (1972)
J62-1(R394)	AK	T	
J62-1(R395)	ASSpTCSu	N	
J62-1(R397)	K	?J	
J62-1(R398)	ASSpTCSu	N	
J62-1(R401)	AS	T	
J62-1(R402)	ASSp	T	
J62-1(R468)	K	fi-‡	Dr N. Datta. Present in strains of <i>P. mirabilis</i> isolated in London
J62-1(R469)	K	fi-‡	
J62-1(R492)	A	fi-‡	
J62-1(R493)	A	fi-‡	

Bacteriophage	Properties	Reference or source
34. 13	General transducing for PM13 GC%, 40	Coetzee & Sacks (1960), Coetzee & Smit (unpublished)
7.R49	General transducing for <i>P. rettgeri</i> strain R49. GC%, 47	Krizsanovich <i>et al.</i> (1969)

* Symbols designate resistance to: A, ampicillin; S, streptomycin; Sp, spectinomycin; T, tetracycline; C, chloramphenicol; K, kanamycin; Su, sulphonamide.

† Resistance to 15 µg kanamycin/ml.

‡ Compatibility group not determined.

GC% is the guanine plus cytosine molar percentage of the DNA.

Table 2. *Frequencies and types of transductants following transduction of R factors by phage 34 to Proteus mirabilis strain 13*

Experiment no.	Donor		Recipient	Recipient resistance	Selected marker	Resistance of transductants*	Transduction frequency† (/adsorbed p.f.u.)	Conjugal transfer of selected marker‡
	Lysate	Resistance						
1	PM13(R1drd19)	ASCKSu	PM13argH2	—	S	SC	5×10^{-8}	0/20
2			PM13argH2	—	C	SC	5×10^{-8}	0/20
3			PM13argH2	—	A	—	0	—
4			PM13argH2	—	K	—	0	—
5			PM13(R401)	AS	C	ASC	1×10^{-8}	0/20
6			PM13(R447b)	AK	C	ASCK	8×10^{-9}	0/20
7			PM13(R469)	K	S	SCK	1×10^{-8}	0/20
8	PM13(Rs-a)	SCKSu	PM13argH2	—	S	SCKSu	5×10^{-7}	30/30
9			PM13(R492)	A	K§	ASCKSu	5×10^{-8}	30/30
10	PM13(RP4)	AK	PM13argH2	—	A	AK	7×10^{-7}	20/20
11			PM13(R395)	ASSpCSu	K	ASSpCKSu	1×10^{-8}	20/20
12	PM13(R438)	AK	PM13argH2	—	A	AK	5×10^{-8}	31/31
13			PM13(P-lac)	—	A	AK	5×10^{-8}	10/10
14			PM13(R493)	A	K	AK	1×10^{-8}	35/35
15	PM13(R447b)	AK	PM13argH2	—	A	A	5×10^{-7}	25/25
16			PM13argH2	—	K	K	1×10^{-7}	0/50
17			PM13(P-lac)	—	A	A	5×10^{-7}	15/15
18			PM13(P-lac)	—	K	K	1×10^{-7}	0/35
19			PM13(R395)	ASSpCSu	K	ASSpCKSu	1×10^{-8}	25/25
20			PM13(R402)	ASSp	K	ASSpK	1×10^{-8}	25/25
21			PM13argH2	—	A	AK	5×10^{-7}	14/14
22	PM13(R6886)	AK	PM13(R492)	A	K	AK	1×10^{-8}	14/14
23	PM13(R391)	K	PM13argH2	—	K	—	0	—
24			PM13(R395)	ASSpCSu	K	ASSpCKSu	8×10^{-9}	20/20

Table 2 (cont.)

Experiment no.	Donor		Recipient	Recipient resistance	Selected marker	Resistance of transductants*	Transduction frequency† (/adsorbed p.f.u.)	Conjugal transfer of selected marker‡
	Lysate	Resistance						
25	PM13(R394)	AK	PM13argH2	—	K	AK	8×10^{-6}	20/20
26	PM13(R395)	ASSpCSu	PM13argH2	—	C	ASSpCSu	7×10^{-7}	20/20
27			PM13(RP4)	AK	C	ASSpCKSu	1×10^{-8}	20/20
28	PM13(R397)	K	PM13argH2	—	K	—	0	—
29			PM13(R395)	ASSpCSu	K	ASSpCKSu	7×10^{-9}	20/20
30	PM13(R398)	ASSpCSu	PM13argH2	—	A	—	0	—
31			PM13argH2	—	C	—	0	—
32			PM13argH2	—	S	—	0	—
33			PM13(R394)	AK	C	ASSpCKSu	1×10^{-7}	20/20
34	PM13(R401)	AS	PM13argH2	—	A	—	0	—
35			PM13argH2	—	S	—	0	—
36			PM13(Rs-a)	SCKSu	A	ASCKSu	7×10^{-9}	30/30
37	PM13(R402)	ASSp	PM13argH2	—	S	ASSp	5×10^{-7}	20/20
38			PM13(R447b)	AK	S	ASSpK	6×10^{-9}	20/20
39	PM13(R492)	A	PM13argH2	—	prototrophy (argH2 ⁺)	—	5×10^{-6}	—
40			PM13argH2	—	A	A	1×10^{-5}	28/28
41	PM13(R493)	A	PM13argH2	—	A	A	7×10^{-6}	15/15
42			PM13(R469)	K	A	AK	5×10^{-7}	15/15

* Suspensions of purified transductants were spotted on selective media to determine pattern of resistance. Tetracycline was ignored as recipient is naturally resistant. Symbols for drugs as in Table 1.

† When average no. drug-resistant recipient colonies was no higher than mutants on control plates the transduction frequency is listed as zero.

‡ Recipient was *Escherichia coli* E27nal-r. Denominator refers to no. clones tested.

§ 15 µg/ml.

Mixtures were plated on selective media to measure frequency of transfer of the original marker(s) used in selection of the transductant and on media selective for the resident plasmid. Exconjugant clones (30 or more from each selection medium) were purified and tested for the presence of selected and unselected markers.

Preparation of phage lysates. This was done by a soft-agar layer method (Prozesky, De Klerk & Coetzee, 1965). The R⁺ donor organism was grown overnight in broth containing one or more drugs to which it was resistant, washed and suspended in broth immediately before use. Lysates were sterilized by shaking with 0.1 vol. chloroform. Before storage the lysate was decanted. Remaining traces of chloroform were removed by bubbling with sterile air at 37 °C. Sterility of phage lysates was tested by incubating 5 ml with 100 ml broth overnight.

Phage 34 reared on R⁺ strains of PM13 had titres of 1 to 5×10^9 p.f.u./ml. Phage 7 propagated on R⁺ strains of R49 had titres of 1 to 5×10^{10} p.f.u./ml. All lysates were sterile.

Transduction. Recipient bacteria were grown overnight (in the presence of a selective drug when they carried a resident R factor). The culture was diluted 1:5 in broth and incubated for 90 min. The bacteria in 3 ml were harvested by centrifugation and resuspended in the phage suspension to yield a multiplicity of phage input of about 5. A sample was removed immediately into chloroform broth and titrated to estimate input phage. The mixture was then incubated for 15 min when another sample was pipetted into chloroform broth for estimation of free phage. For selection of prototrophs, bacteria in the adsorption mixture were washed in saline and plated on minimal medium (Prozesky, 1968). For selection of drug-resistant organisms the mixture was filtered through a membrane (Coetzee, Smit & Prozesky, 1966). The membrane was then incubated on nutrient agar for 90 min before transfer to the selective medium. In controls of prototroph transductions broth was substituted for phage suspension. In R factor transduction controls the phage lysate was prepared on the corresponding R⁻ organism.

RESULTS

Conjugal transfer of plasmids to Proteus mirabilis strain 13 and Proteus rettgeri strain R49. The plasmid P-lac was readily transferred to R49trp-1 and all R factors were transferred from *Escherichia coli* J62-1 to PM13. Tetracycline resistance could not be selected as PM13 is naturally resistant to this drug. Because of the resistance pattern of *P. rettgeri* R49, only R factors which transmit kanamycin resistance could be selected. Factor Rs-a could however not be selected for in R49 as the average number of drug-resistant recipient colonies was no higher than the frequency of spontaneous mutation to 15 µg/ml kanamycin. All selected exconjugants could transmit their drug-resistance markers to *E. coli* E27nal-r.

Transduction of R factors by phage 34. Phage 34 can transduce all markers (complete transduction) of some R factors. These transduction rates vary from 1×10^{-5} (greater than chromosomal marker transduction) to 5×10^{-8} /p.f.u. adsorbed (Table 2). The compatibility groups of all *fi*⁻ factors have not been determined. Others include members of groups N, P, T, W. Only C and S markers were detected in transduction experiments with the *fi*⁺ factor R1drd19 (experiments 1 to 7, Table 2). Transductants could not transfer these markers by conjugation and attempts to establish transfer by means of resident plasmids R401, R447b, R469 were unsuccessful. Markers of factors R391, R397, R398, R401 (experiments 24, 29, 33, 36, Table 2; lines 9 to 12, Table 4) were only detected in the presence of a distinguishable plasmid in recipient. All combinations of resident and donor R factors were not tried.

Table 3. Frequencies of transduction of R factors by phage 7 in *Proteus rettgeri* strain R49

Experiment no.	Donor lysate	Recipient	Selected marker	Transduction frequency (/adsorbed p.f.u.)	Conjugal transfer of marker
1	R49(R394)	R49 <i>trp</i> -I	Prototrophy (<i>trp</i> ⁺)	5 × 10 ⁻⁷	—
2	R49(R394)	R49 <i>trp</i> -I	K	7 × 10 ⁻⁷	0/30
3	R49(R394)	R49 <i>trp</i> -I (P- <i>lac</i>)	K	7 × 10 ⁻⁷	0/30
4	R49(R1 <i>drd</i> 19)	R49 <i>trp</i> -I (P- <i>lac</i>)	K	0	—
5	R49(R397)	R49 <i>trp</i> -I (P- <i>lac</i>)	K	0	—
6	R49(RP4)	R49 <i>trp</i> -I	K	0	—
7	R49(R468)	R49 <i>trp</i> -I	K	0	—
8	R49(R469)	R49 <i>trp</i> -I	K	0	—
9	R49(R6886)	R49 <i>trp</i> -I	K	0	—

Transduction of R factors by phage 7. R49. Because only R factors which carry the K trait could be selected, the number studied was limited to 7 factors (Table 3).

Factor Rs-a which gives low levels of K resistance in *Proteus mirabilis* strain 13 (unpublished observations) and in *Escherichia coli* (Hedges & Datta, 1971), and would have been useful as a resident in the recipient, could not be transferred conjugally to donor R49. Only a member of the T group (R394) was transduced. The transduction frequency was at least as high as for a chromosomal marker but transductants were non-infectious. The presence of the P-lac plasmid in recipients had no effect. Effects of resident R factors could not be studied for the reason mentioned. R394 is transduced completely by phage 34 (experiment 25, Table 2).

Drug resistance patterns of R factor transductants selected in the presence of resident factors in recipients. Conjugation experiments to determine whether transductants, which were conjugally infectious for transduced markers, carried hybrid R factors resulting from recombination between donor and resident plasmids are summarized in Table 4. The first six lines of Table 4 show that when the original plasmid was transduced *in toto* as judged from corresponding experiments with R⁻ organisms (experiments 8, 12, 15, 17, 21, 26, 41, Table 2), markers of both plasmids were frequently transferred together. Whether they are transmitted as one molecule is doubtful because they are not transmitted at the same frequency nor are both present in all exconjugants. Experiments 15, 17 (Table 2) are exceptions. When the A marker was selected the K trait was not present in transductants, but the latter were conjugally infective for A. When K was selected (experiments 16, 18, Table 2) A was not detected and transductants were non-infectious for the K marker. In the presence of resident factors R395 or R402 (experiments 19, 20, Table 2) with selection for K, transduction occurred at a lower rate and again only K was expressed but transductants were now conjugally infective. Lines 7 to 12 (Table 4) represent results where the whole plasmid was not transferred as judged from corresponding experiments with R⁻ recipients (experiments 18, 23, 28, 32, 34, Table 2). The latter results suggest that possible recombination with a resident plasmid offered the transduced particle a selective advantage (Watanabe, Sakaizumi & Furuse, 1968) as markers of the two plasmids travelled together at the same frequency.

Modification and restriction of R factors. Where comparison was possible, the presence of a resident R factor did not increase the transduction rate of markers above that for R⁻ recipients and usually lowered it by a factor of about 10. This reduction suggested the possibility of host-controlled restriction exerted by one *fi*⁻ factor on the other. Two cases

Table 4. *Conjugal transfer of transductant markers*

Transductant 'double'	Table 2 experi- ment no.	Recipient	Transfer frequency* selection for		Markers of both plasmids present in exconjugant clones derived from columns†	
			Resident plasmid	Transduced marker	4	5
PM13(R469R493)‡	42	<i>E27nal-r</i>	$1 \times 10^{-4}(K)$	$1 \times 10^{-3}(A)$	22/30 both present	19/30 both present
PM13(R493R438)	14	<i>E27nal-r</i>	$8 \times 10^{-3}(A)$	$5 \times 10^{-4}(ATK)$	12/30	20/20
PM13(P-lacR447b)	17	13 <i>nal-r</i> (34)	$1 \times 10^{-4}(P-lac)$	$1 \times 10^{-3}(AK)$	21/30 §	20/30§
PM13(R492Rs-a)	9	<i>E27nal-r</i>	$5 \times 10^{-2}(A)$	$5 \times 10^{-3}(SCKSu)$	11/20	21/30
PM13(R492R6886)	22		$5 \times 10^{-2}(A)$	$3 \times 10^{-3}(ATK)$	17/30	20/20
PM13(RP4R395)	27		$5 \times 10^{-2}(ATK)$	$1 \times 10^{-3}(ASSpTCSu)$	22/30	15/30
PM13(R395R447b)	19		$5 \times 10^{-3}(ASSpTCSu)$	$5 \times 10^{-3}(AK)$	30/30	30/30
PM13(R402R447b)	20		$1 \times 10^{-4}(ASSp)$	$1 \times 10^{-4}(AK)$	20/20	20/20
PM13(R395R391)	24		$1 \times 10^{-3}(ASSpTCSu)$	$1 \times 10^{-3}(K)$	30/30	30/30
PM13(R395R397)	29		$1 \times 10^{-3}(ASSpTCSu)$	$1 \times 10^{-3}(K)$	30/30	30/30
PM13(R394R398)	33		$5 \times 10^{-4}(AK)$	$5 \times 10^{-4}(ASSpTCSu)$	30/30	30/30
PM13(Rs-aR401)	36		$5 \times 10^{-3}(SCKSu)$	$5 \times 10^{-3}(AS)$	30/30	30/30

* Transfer frequency/donor cell.

† Denominator equals no. clones tested.

‡ Superior line indicates transduced R factor but does not necessarily imply presence of entire R factor.

§ Lactose fermenting clones.

Symbols in parentheses in columns 4, 5 denote drug resistance markers of plasmids. The italicized symbol in each column is the marker used for selection. When only one of the two markers was present in an exconjugant clone the remainder normally associated with it were detected by replication to appropriate media. The K marker of experiment 17 was never detected.

Table 5. Host controlled modification of factors R402 and RP4

Factor R402 was transferred conjugally to PM13(R447b) and R395 was transferred in the same way to PM13(RP4). Lysates of phage 34 were made on these double R factor containing strains and used in transduction experiments to listed recipients. Results of experiments 10, 11, 37, 38 (Table 2) are included.

Donor	Table 2 experiment no.	Recipient	Selection	Phage 34 transduction frequency (adsorbed p.f.u.)
PM13(RP4)	10	PM13argH2	A	7×10^{-7}
PM13(RP4)	11	PM13argH2(R395)	K	1×10^{-8}
PM13(RP4 R395)	—	PM13argH2(R395)	K	7×10^{-7}
PM13(R402)	37	PM13argH2	S	5×10^{-7}
PM13(R402)	38	PM13(R447b)	S	1×10^{-8}
PM13(R402 R447b)	—	PM13(R447b)	S	6×10^{-7}

(experiments 11, 38, Table 2) were investigated. Factor R402 was transferred conjugally to PM13(R447b) and R395 was transferred in the same way to PM13(RP4). Each pair of factors exists stably together without recombining (Hedges & Datta, 1971; Coetzee *et al.* 1972). Phage 34 was then prepared on each of the double R factor containing strains and lysates were used in transduction experiments to PM13(R447b) and PM13(R395) respectively. Results shown in Table 5 reveal that the transduction frequency of R⁺ recipients now equalled that to R⁻ recipients and suggest that factors R402 and RP4 probably undergo host-controlled modification in cells which carry R447b and R395 respectively.

Immunity of transductants. All transductants were immune to homologous phage. Some transductants which possess conjugal transferability of R factors were examined to determine whether immunity to phage 34 was associated with the transduced R factor. Transductants from experiments 9, 19, 22, 36, 40 (Table 2) were mated with *Escherichia coli* E27nal-r and R⁺ clones of E27 purified. The R factors were then transferred back to *Proteus mirabilis* 13 by conjugation and R⁺ clones challenged with phage 34. All were sensitive to the phage. This suggests that immunity of the original transductants to phage 34 was due to secondary infection resulting in lysogeny rather than to an association of phage genome with transduced R factor. As precautions were not taken to avoid secondary infection of transductants (Coetzee & Sacks, 1960) this explanation does not necessarily apply to those which did not possess conjugal transferability (experiments 1, 2, 5 to 7, 16, 18, Table 2; no. 2, 3, Table 3).

DISCUSSION

Phage 34 and P22 resemble one another morphologically (Prozesky *et al.* 1965; Anderson, 1960). The phages contain double-stranded DNA with mol. wt of 29.5×10^6 (Van Rensburg, 1970) and 27×10^6 (Rhoades, MacHattie & Thomas, 1968) daltons respectively. The mol. wt of the double-stranded DNA from øm is 24×10^6 daltons (Nakaya & Rownd, 1971) while that of phage 7. R49 is not known. The only R factor used in this investigation which has been employed for similar purposes is Rs-a (Watanabe, Furuse & Sakaizumi, 1968). The mol. wt of its double-stranded DNA is 17×10^6 daltons (S. Falkow, personal communication). It is transduced completely by phage 34 and P22 into *Proteus mirabilis* 13 and *Salmonella typhimurium* LT-2 respectively. The mol. weights of the other *fi*⁻ R factors used are not known but the many complete transductions reported here may be because *fi*⁻ R factors

often exist largely as composite molecules in *Proteus mirabilis* which may be small enough to be accommodated in the phage vector. We have no explanation for the great variation in the transduction frequencies but Watanabe, Furuse & Sakaizumi (1968) also encountered the phenomenon in complete transductions of R factors with P1. The high transduction frequency of sex factor F by P1 (Arber, 1960) was explained on grounds that F need not be integrated into the chromosome of the recipient and transduction frequencies actually represented frequencies of transfer. Size of the plasmid is important. If markers are more than the phage DNA length from replication or maintenance loci the markers may be conveyed by the vector but cannot maintain themselves in the recipient. Unless these markers integrate into the chromosome they are diluted out. This may account for experiments (3, 4, 23, 28, 30 to 32, 34, 35, Table 2; no. 4 to 9, Table 3) where no R⁻ transductants were encountered. Transduction of the same markers in experiments where the recipient is R⁺ (no. 24, 29, 33, Table 2; lines 9 to 11, Table 4) may mean that recombination occurred between markers and resident plasmid. Frequencies of transduction may be a reflexion of the relative frequency of recombination. The partial transduction of R447b (experiments 16, 18, Table 2) may also be interpreted on a size basis. Dissociation of genes for K resistance from those determining transfer leads to transductants with the K marker borne on a plasmid lacking transfer genes. Conjugal transfer of this marker can be achieved (experiments 19, 20, Table 2; lines 7, 8, Table 4) with the use of certain resident R factors. This probably implies recombination between plasmids concerned. It is interesting that P-lac (compatibility group P) is not suitable. The presence of possibly only the S and C markers of R1drd19 in non-infectious transductants (experiments 1, 2, 5 to 7, Table 2) may mean that these markers are integrated in the bacterial chromosome or are on a plasmid which lacks transfer genes. The composite R1 DNA molecule has a weight of 65×10^6 daltons (Cohen & Miller, 1970). This is much more than that of phage 34.

Other factors may play a role in determining transduction frequencies. One of these, stressed by Nakaya & Rownd (1971) and proffered as an explanation of differences in frequencies of transduction of various R factors (Watanabe, Furuse & Sakaizumi, 1968) is ability of the transducing phage to enclose other DNA in its coat. A parameter used was the guanine plus cytosine (G+C) molar content (GC%) of phage and R factor DNA. This is unlikely to be the sole criterion. For example: P1 DNA which has a GC% of 46 (Ikeda & Tomizawa, 1965) can transduce the λ prophage (Arber, 1960) with a GC% which ranges from 42 to 57 (Davidson & Szybalski, 1971). The GC% of phages 34.13, 7.49, their hosts, factor R1drd19 and P-lac are known (Table 1) but not that of the ϕ ⁻ factors used here. Where transductants were non-infectious (experiments 1, 2, 5 to 7, 16, 18, Table 2; no. 2, 3, Table 3) a possibility is that R factor genes are transduced in close association with phage genes and may be saved from an abortive fate by this association. The prophage of P22 may attach to the host chromosome (Luria *et al.* 1960; Dubnau & Stocker, 1964), while that of P1 may persist as a plasmid (Boice & Luria, 1963; Ikeda & Tomizawa, 1968). The relationship of prophages 34 and 7 to the host chromosome is unknown. Iyobe *et al.* (1969) demonstrated that a transduced R factor gene may also integrate independently of the phage into the bacterial chromosome. No direct correlation may exist between ability of R factor genes to integrate into DNA of phage, host chromosome, other R factors and their DNA base ratios. Recombination is restricted to relatively small segments and the chromosomal integration of λ , for example, is accomplished by mechanisms apparently independent of sequence homologies (Gottesman & Weisberg, 1971). R factor genes may exist in transductants as non-infectious replicons. Where transductants were conjugally infective or rendered so by combination of the transduced particle with a resident plasmid the R factors were not associated

with the genome of prophage 34. Nakaya & Rownd (1971) had similar results with their system. These findings may differ from those of Coetzee & Sacks (1960) who transduced chromosomal *str-r* with very low multiplicities of phage 34 under conditions where secondary infection could be excluded. Most transductants were lysogenic and showed lysogenic conversion.

Transductions of factors R391, R397, R398 and R401 by phage 34 (experiments 24, 29, 33, 36, Table 2) resemble those of NR1 and NR84 by ϕ m (Nakaya & Rownd, 1971) as the presence of a resident R factor is mandatory and transductants are conjugally infectious for factors which are inferred to be recombinant for the original donor and resident R factors. With phage 34 the transduction frequencies were generally higher by a factor of 5 than those reported by the latter workers. This low rate is surprising because in some of their experiments, Nakaya & Rownd used distinguishable derivatives of the same R factor as donor and resident. Watanabe, Sakaizumi & Furuse (1968) and Watanabe & Ogata (1970*b*), in similar experiments, found a tenfold increase in transduction frequencies when donor and resident were distinguishable variants of the same R factor. They ascribed the increase to the greater homology existing in the latter case. Only in experiment 19 (Table 2) is it known that donor and recipient organisms carry plasmids of the same compatibility group. In this experiment resident and transduced factors belonged to group N and the incompatibility aspect of superinfection immunity would be expected to play a role in determining transduction frequencies. Watanabe, Sakaizumi & Furuse (1968) found no superinfection immunity in transduction of an f_i^+ R factor to recipients which carried f_i^+ or f_i^- R factors. N factors may co-exist for a few generations before incompatibility finally dislodges the resident factor (Datta & Hedges, 1971). The relative unimportance of incompatibility in transductions reported here is confirmed by experiment 20 (Table 2) where the resident R factor was replaced by one of a different compatibility group. The low transduction frequency is not affected. Watanabe, Sakaizumi & Furuse (1968) found that recombination takes place readily between a transduced f_i^+ factor and f_i^+ or f_i^- resident R factors in *Salmonella typhimurium* or *Escherichia coli* recipients. We could not demonstrate recombination between transduced markers of f_i^+ factor R1*drd*19 and various f_i^- factors (experiments 5 to 7, Table 2). Recombination has not been tested between transduced f_i^- plasmids and resident f_i^- plasmids (Watanabe, Sakaizumi & Furuse, 1968) although this has possibly been encountered here.

Apart from hazards mentioned, restriction and host modification is another obstacle in the way of incoming R factor DNA. Some f_i^- R factors are known to determine restriction and modification of phages (Watanabe, Nishida, Ogata, Arai & Sato, 1964; Bannister & Glover, 1968) and Watanabe, Sakaizumi & Furuse (1968) have shown that a f_i^+ factor is modified by the presence of an f_i^- R factor in the same cell so that transfer of the former (by transduction) is no longer restricted by the presence of the latter in recipient cells. Most work has been done on R factors of group N (Bannister, 1970; Hedges, 1972) and our results (Table 5) may also implicate group N factors. Possible restriction by members of other f_i^- groups in Table 2 were not investigated. Hedges (1972) and Coetzee *et al.* (1972) have shown that the P group factor RP4 and the W compatibility factor Rs-a as well as J and group T factors do not restrict phage λ DNA and diminished transduction frequencies in the presence of these factors may require other explanations.

R factors may exist as multiple copies of transfer and resistance-determinant molecules as well as composites in *Proteus mirabilis* (Cohen & Miller, 1970; Rownd, Watanabe, Mickel, Nakaya, & Gargan, 1970; Coetzee 1972). A problem which Nakaya & Rownd (1971) encountered was how transductant cells, which initially harboured only one recombinant R

factor in a multicopy pool of resident R factors, could be converted to a state in which all R factors are recombinant during purification of the R⁺ transductant. We encountered a similar situation when R factors from transductant clones of *P. mirabilis* were transferred by mating to *Escherichia coli* E27nal-r. All *E. coli* clones had the same drug resistance pattern as the R⁺ transductants used as donors irrespective of whether a marker of donor or resident *fi*⁻ R factors in transduction experiments (no. 19, 20, 24, 29, 33, 36, Table 2; lines 7 to 12, Table 4) was used to select *E. coli* R⁺ clones in mating experiments. R factors of the *fi*⁻ type can persist in the composite single molecular form in *P. mirabilis* (Nisioka, Mitani & Clowes, 1969; Kontomichalou, Mitani & Clowes, 1970; Silver & Falkow 1970; Datta & Hedges, 1972) but may exist as multiple copies in this organism (Rownd 1967; Rownd *et al.* 1970). A possible explanation is that the transduced marker can only replicate in a recombinant plasmid. Selection for this marker would favour the recombinant which is probably incompatible with the resident factor.

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