Properties of R Plasmid R772 and the Corresponding Pilus-specific Phage PR772

By J. N. COETZEE, G. LECATSAS, W. F. COETZEE

*Department of Microbiology, University of Pretoria, and
MRC Unit for Microbial Genetics, Pretoria, South Africa*

AND R. W. HEDGES

Department of Bacteriology, Royal Postgraduate Medical School, London

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R plasmid R772 was isolated from a strain of *Proteus mirabilis* and is a self-transmissible P-1 incompatibility group plasmid having a molecular weight of about 27×10^6 . It renders bacterial hosts resistant to kanamycin. Phage PR772 was isolated as a phage dependent on the presence of R772 in bacterial hosts. It is hexagonal-shaped with a diameter of 53 nm, has a thick inner membrane and no tail. Vaguely defined appendages are sometimes apparent at some vertices and the phage possesses double-stranded DNA. The DNA has a guanine plus cytosine molar content of 48%. The phage is sensitive to chloroform and has a buoyant density of 1.26 g cm^{-3} . These observations suggested that the inner membrane of the phage could contain lipid. Phage PR772 differs in morphology from the double-stranded DNA plasmid-specific phages PR4 and PRR1 which adsorb to tips and sides, respectively, of sex pili coded for by P-1 incompatibility group plasmids. Phage PR772 formed clear plaques which varied in diameter. Serologically, phages PR772 and PR4 are possibly related though very distantly, but the two phages have identical host ranges. Phage PR772 adsorbed by one of its apices to tips of sex pili coded for by plasmid R772 in *Escherichia coli*. It also formed plaques on *Salmonella typhimurium*, *Proteus morganii* and Providence strains harbouring this plasmid as well as strains of *E. coli* carrying plasmids of incompatibility groups N or W. The phage produced areas of partial clearing on lawns of *P. mirabilis* PM5006 harbouring plasmid R772, the P-1 incompatibility group plasmid RP4, the W group plasmid RSa or the N group plasmid N3, and on lawns of Providence strain P29 carrying plasmid RP4.

INTRODUCTION

Hedges (1975) described a series of plasmids conferring resistance to kanamycin, all derived from strains of *Proteus mirabilis* from a hospital in Boston, U.S.A. R plasmid R772, a member of this series, was found to be self-transmissible at frequencies of 5×10^{-3} per donor cell and able to mobilize Mor174, a non self-transmissible *Proteus morganii* bacteriocinogenic plasmid (Williams, 1977; Coetzee *et al.*, 1977). It can also mobilize the *P. mirabilis* chromosome (Coetzee, 1978). Because the incompatibility group of R772 had not been determined and because the relatively high frequency of conjugal transfer made it feasible to attempt to isolate a phage specific for the transfer apparatus of the plasmid (Meynell & Lawn, 1968; Khatoon *et al.*, 1972), we decided to do both simultaneously. A specific phage was isolated and R772 proved to be a P-1 incompatibility group plasmid. Properties of the system are described in this paper.

Table 1. *Bacteria, bacteriophages and plasmids*

Bacteria	Properties*	Reference	
<i>Proteus mirabilis</i> PM5006	Host for generalized transducing phage 5006M. T, PMX	Coetzee & Smit (1970); Coetzee (unpublished)	
PM5006nal-r	Spontaneous Nal mutant	Coetzee (1974)	
<i>Proteus morgani</i> 2815nal-r	NCTC strain 2815. Host for generalized transducing phage M. Spontaneous Nal mutant	Coetzee (1966); Coetzee <i>et al.</i> (1966)	
Providence P29nal-r	Providence strain NCTC 9211. Biogroup A. Recipient for phage PL25. Spontaneous Nal mutant. A	Coetzee <i>et al.</i> (1966)	
<i>Salmonella typhimurium</i> LT2 trpA8	Tryptophan A auxotroph of strain LT2	N. Datta	
<i>Escherichia coli</i> J53	F ⁻ pro, met mutant of <i>E. coli</i> K12	Clowes & Hayes (1968)	
J53-1	Spontaneous Nal mutant	Clowes & Hayes (1968)	
J62	F ⁻ pro, his, trp mutant of <i>E. coli</i> K12	Clowes & Hayes (1968)	
J62-1	Spontaneous Nal mutant	Coetzee <i>et al.</i> (1972)	
J62str-r	Spontaneous S mutant	Coetzee (unpublished)	
<i>Pseudomonas phaseolicola</i> HB10Y	Host for phage ϕ 6	Vidaver <i>et al.</i> (1973); supplied by W. Snipes	
Bacteriophages	Properties	Reference/source	
ϕ 6	Buoyant density 1.27 g cm ⁻³	Vidaver <i>et al.</i> (1973); supplied by W. Snipes	
PRR1	RNA phage. Adsorbs to sides of pili coded for by P-1 group plasmids	Olsen & Thomas (1973); supplied by D. E. Bradley	
PR4	Lipid-containing double-stranded DNA phage. Adsorbs to tips of pili coded for by N, P and W group plasmids	Stanisich (1974); supplied by D. E. Bradley	
Plasmids	Properties*	Incompatibility group	Reference
R772	Circular DNA approx. 27 $\times$ 10 ⁶ dalton. K	P-1	Hedges (1975); this paper and unpublished
R1drd19	A, C, K, S, Su	FII	Meynell & Cooke (1969)
R64drd11	A, T	I	Meynell & Datta (1967)
R144drd3	K, T	I	
ColIldrd16	Colicin I producing	I	N. Datta
R391	K	J	Coetzee <i>et al.</i> (1972)
R392	K	?J	Coetzee <i>et al.</i> (1972)
R397	K	?J	Coetzee <i>et al.</i> (1972)
N3	S, Su, T	N	Hedges (1972)
R378	A, C, S, Sp, Su, T	N	Coetzee <i>et al.</i> (1972)
RP4	A, K, T	P-1	Datta <i>et al.</i> (1971)
R751	Tp	P-1	Jobanputra & Datta (1974)
R68.45	A, K, T	P-1	Haas & Holloway (1976)
P-lac	Lactose utilization, Su	A-C	Hedges (1975)
RSa	C, K, S, Su	W	Hedges & Datta (1971)

* Symbols designate resistance to: A, ampicillin; C, chloramphenicol; K, kanamycin; Nal, nalidixic acid; PMX, polymyxin; S, streptomycin; Sp, spectinomycin; Su, sulphonamide; T, tetracycline; Tp, trimethoprim.

METHODS

Bacteria, bacteriophages and plasmids. 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. NBY media (Sands *et al.*, 1974) were used for the growth of *Pseudomonas phaseolicola*, the host of phage ϕ 6. The incubation temperature was 37 °C, apart from work with phage ϕ 6 where it was 25 °C. Ampicillin, chloramphenicol, tetracycline, nalidixic acid, streptomycin, kanamycin and polymyxin B were used at 30 µg ml⁻¹. Streptomycin was also used at 1 mg ml⁻¹.

Production of phage lysates and general phage techniques. The methods described by Adams (1956) and Coetzee (1974) were used.

Phage buffer. TNTC buffer (Olsen *et al.*, 1974) was used; this consisted of 0.01 M-Tris, 1.17 M-NaCl, 0.5 % (w/v) Tryptone and 0.001 M-CaCl₂, at pH 7.

Isolation of bacteriophage specific for bacteria harbouring plasmid R772. The method of Meynell & Lawn (1968) was used. Briefly, attempts were made to enrich the phage population of a sample of sewage by overnight incubation with either PM5006*nal-r*(R772) or J62-1(R772). The mixture was then filtered through a 0.45 µm pore-size Millipore membrane and drops of the filtrate were spotted on lawns of the other organism and both the wild-type parent organisms lacking the plasmid. Five daily samples from the main Pretoria sewage works and three specimens from the sewage of a Pretoria hospital were tested independently.

Phage adsorption. A sample of exponential phase bacteria in broth (3 ml, about 5×10^8 viable organisms ml⁻¹) was mixed with 0.1 ml of a phage suspension [1×10^9 plaque-forming units (p.f.u.) ml⁻¹] and incubated for 20 min. The bacteria were then deposited by centrifugation at 8000 g for 10 min and the supernatant was titrated for unadsorbed phage. As a control, an isogenic strain which lacked the plasmid was substituted for the test strain and processed simultaneously. The percentage of phage adsorbed was calculated from the difference in plaque-forming titres between the test and control suspensions.

Multiplication of phage in liquid medium. A sample of exponential phase bacteria in broth (1 ml, 10^7 to 10^8 bacteria ml⁻¹) was diluted 1:3 in pre-warmed broth and 0.1 ml of a phage PR772 suspension (5×10^9 p.f.u. ml⁻¹) was added. Phage suspension was also added to 3 ml broth as a control. The mixtures were incubated overnight, then centrifuged and the phage titre of the supernatant was determined.

Preparation of phage antiserum. Eight injections of phage PR772 (1.0 ml, 5×10^{10} p.f.u. ml⁻¹) were given intravenously at 3 d intervals to each of three rabbits. After resting the animals for 10 d, blood was collected and the serum was heated at 56 °C for 30 min to inactivate complement. The serum was then absorbed with J62-1(R772).

Purification of phage. Cell debris was removed from 250 ml PR772 lysate by centrifugation at 10000 g for 15 min at 4 °C in a Sorvall SS34 rotor. Phage was then sedimented by centrifugation at 90000 g for 2 h at 4 °C, using an SW27 rotor. Pellets were resuspended overnight in TNTC buffer and then centrifuged at 10000 g for 15 min at 4 °C. CsCl gradients were prepared by adding CsCl to a mean density of 1.3 g cm⁻³, as determined refractometrically. Centrifugation was carried out at 90000 g in a SW65 rotor for 47 h at 4 °C. An intense band was observed in each gradient. The bands were collected through the sides of the tubes by means of syringes and were pooled. The pooled materials were centrifuged in separate CsCl gradients as above and yielded one well-defined band. The band was collected as above and dialysed against TNTC buffer. The phage yield was 5×10^{12} p.f.u. ml⁻¹.

For PR4, the same procedure was followed. Phage ϕ6 was prepared using *Pseudomonas phaseolicola* strain HB10Y as host bacterium.

Nucleic acid melting. Phage PR772 nucleic acid was extracted as described by Olsen *et al.* (1974). A melting profile was obtained by the method of Mandel & Marmur (1968).

Conjugal transfer of plasmids. Methods were as described by Datta *et al.* (1971) and Datta & Hedges (1972). Conjugation mixtures were incubated for 1 h.

Compatibility of R factors and transfer from 'doubles'. These methods were described by Coetzee *et al.* (1972).

Electron microscopy. When necessary, specimens were concentrated by centrifugation. A small portion of the pellet was taken up in distilled water and the suspension was mixed with an equal volume of 3 % (w/v) phosphotungstic acid. A drop of this mixture was then applied to a Formvar-carbon coated grid, allowed to dry and examined in a Philips EM300 electron microscope at an instrumental magnification of 42000.

RESULTS

Interspecific plasmid transfer and compatibility properties

Plasmid R772 was transferred between strains of *E. coli* K12 with an efficiency of about 1×10^{-3} per donor which is similar to that shown by P-1 incompatibility group plasmids like RP4 and R751 (Hedges & Jacob, 1974).

When R772 was transferred to a strain carrying R751 there was efficient elimination of the trimethoprim resistance caused by R751. When the reciprocal transfer was made, however, elimination of kanamycin resistance was usually delayed, and if strains showing both resistances were grown in drug-free medium and then plated on agar, most colonies had lost trimethoprim resistance. However, prolonged growth in $\bar{\mu}$ medium with trimethoprim led eventually to the loss of kanamycin resistance.

Isolation of an R772-dependent bacteriophage

From only one of the sewage samples (from the main sewage works) incubated with PM5006*nal-r*(R772), a phage was isolated which plated on J62-1(R772) and not on J62-1 or PM5006*nal-r*. The phage also did not form plaques on the organism ostensibly used for phage enrichment – PM5006*nal-r*(R772) (see below). The phage was named PR772 and was propagated on J62-1(R772).

Properties of phage PR772

Plaque morphology. Plaques on lawns of all hosts (see below) were always clear and varied from 1 to 3 mm in diameter. Single plaque isolates of small or large plaques again produced plaques within this diameter range.

Chloroform sensitivity. A phage suspension was gently shaken with 0.1 vol. chloroform for 10 min. A control suspension, to which nothing had been added, was also shaken. After 15 min to allow phase separation, the plaque-forming titre of the two suspensions was determined. The chloroform treatment reduced the titre by 99%.

Morphology. Electron microscopy of negatively-stained preparations showed that the phage particle had a clear hexagonal form suggesting icosahedral symmetry. Some intact particles showed poorly-defined appendages at some vertices (Fig. 1) which could represent the structures reported by Olsen *et al.* (1974) for phage PRD1. The diameter was 53 ± 3 nm and there was no tail (Fig. 1). An internal layer was visible in presumed 'empty' particles and this core had a diameter of 36 ± 2.2 nm (Fig. 2). The appearance of the particles inactivated with chloroform suggested that the internal layer had collapsed, although untreated suspensions also contained some particles with a partially disrupted internal component. Chloroform treatment had no effect on the external layer of the phage particle. About 10% of particles in preparations with the phage added to suspensions of J62-1(R772) were attached to the end of rigid, hollow tubular rods with lengths of 40 to 400 nm (Figs 3, 4). The appearance of these free complexes did not allow a decision as to whether the phage was attached to a particular end. The rods had a diameter of 9 ± 0.75 nm and were attached to one of the corners of the icosahedral-shaped phage capsid. Phage PR772 was never seen attached to the sides of the rods. The rods resembled the bacterial pili coded for by P-1 incompatibility group plasmids (Bradley, 1974, 1975).

Serology. The three rabbits produced antisera with phage PR772 inactivation constants (K) of 6.76, 4.86 and 8.32 min⁻¹. The plaque-forming titre of phage PR4 was neutralized at K values of 0.68, 0.20 and 0.34 min⁻¹ by the three sera, respectively. These are very low values for PR4 and the phages may possibly be distantly related serologically.

Host range. Phage PR772 formed plaques on strains of *E. coli*, *Salmonella typhimurium*, *P.morganii* and Providence which harboured plasmid R772. It also lytically infected strains of *E. coli* carrying plasmids of incompatibility groups P-1, N or W (Table 2) but had no

Bar markers represent 100 nm in Figs 1 to 6, 200 nm in Fig. 7.

All preparations were stained with 3% (w/v) phosphotungstic acid.

Fig. 1. Typical appearance of phage PR772. Note the vaguely defined appendages at some vertices on the lower particles.

Fig. 2. 'Empty' phage particle (arrowed) showing the inner layer.

Fig. 3. Phage particle attached to a long pilus fragment [J62-1(R772) preparation]. Note the length of the pilus relative to the phage diameter.

Fig. 4. Phage particle attached to a short piece of pilus [J62-1(R772) preparation].

Figs 5, 6. Preparations of J62-1(R772) showing attachment of phage PR772 to the sides of pili and phage PR772 to the tips of the same structures.

Fig. 7. Phage PR772 adsorbed to a cell of strain PM5006*nal-r*(R772). Closely packed phage particles cover the cell surface.

Table 2. *Host range of phages*

[Drops of phage suspension (titre 5×10^9 p.f.u. ml⁻¹) were spotted on lawns of organisms contained in top-layer agar.

Host strain	Plasmid incompatibility group	PR772	PR4	PRR1
J62-1(R772)	P-1	+	+	+
P29nal-r(R772)	P-1	+	+	+
2815nal-r(R772)	P-1	+	+	+
LT2(R772)	P-1	+	+	+
J62(RP4)	P-1	+	+	+
J62(R68.45)	P-1	+	+	+
J53-1(RSa)	W	+	+	—
J53(N3)	N	+	+	—
J62-1	.	—	—	—
J53-1	.	—	—	—
P29nal-r	.	—	—	—
2815nal-r	.	—	—	—

+, Plaque formation; —, no visible action.

Table 3. *Adsorption and multiplication of phage PR772 on different bacterial strains*

Host strain	Resident plasmid	Plasmid incompatibility group	Phage action								
			Plaque formation			Adsorption (%)			Multiplication		
			PR772	PR4	PRR1	PR772	PR4	PRR1	PR772	PR4	PRR1
PM5006	.	.	—	—	—	< 4	< 4	< 4	—	—	—
P29nal-r	.	.	—	—	—	< 4	< 4	< 4	—	—	—
PM5006nal-r	R772	P-1	*	*	*	3-6	3-6	3-6	—	—	—
PM5006	RP4	P-1	*	*	*	3-6	3-6	3-6	—	—	—
P29nal-r	R772	P-1	+	+	+	81	72	52	+	+	+
P29nal-r	RP4	P-1	*	*	*	3-6	3-6	3-6	—	—	—

* No plaque formation but areas of partial clearing; +, plaque formation or increase in titre of about 5×10^2 p.f.u. ml⁻¹; —, no action or no increase in titre detected.

Table 4. *Transfer frequency of plasmid R772*

Donor	Recipient	Selection*	Frequency of transfer (per donor cell)
J62-1(R772)	PM5006	T K	1×10^{-3}
PM5006nal-r(R772)	J62str-r	S K	8×10^{-3}
PM5006(RP4)	P29nal-r	Nal K	1×10^{-3}
P29nal-r(RP4)	J62str-r	S K	3×10^{-3}

* Symbols as in Table 1.

action on strains of *E. coli* with resident plasmids of the other groups tested (see Table 1; results not shown). The host range of phage PR4 appeared to be identical but that of phage PRR1 was confined to strains carrying the P-1 plasmids RP4, R68.45 or R772 (Table 2). Although the phage suspensions were made on J62-1 carrying R772, a presumed P-1 plasmid (see above and Discussion), the efficiency of plating (not shown) was slightly higher on J53-1 carrying the W group plasmid RSa. Only a few other combinations of bacterial host, plasmid and phage were tested (Table 3). The remarkable feature was that although undiluted suspensions (about 1×10^9 p.f.u. ml⁻¹) of all three phages formed areas of partial clearing when spotted on lawns of PM5006nal-r(R772), PM5006(RP4) or P29nal-r(RP4), plaque formation was never detected. The areas of clearing were specific in the sense that no such effect was detected on isogenic organisms lacking the plasmids

Fig. 8. (a) Coseimentation to equilibrium of PR772 and $\phi 6$ in CsCl. CsCl was added to the mixture in TNTC buffer to give a mean density of 1.26 g cm^{-3} . Centrifugation was at 90000 g for 48 h at 4°C in an SW65 rotor. Fractions were collected dropwise from the bottom of the tube. Samples of $20 \mu\text{l}$ were taken from alternate fractions for refractometrical measurements and all fractions were diluted serially. Spot titrations were followed by duplicate plating on strains J62-1(R772) and HB10Y of the fractions shown. $\circ$, Phage $\phi 6$; $\bullet$, phage PR772; $\square$, density. (b) Coseimentation to equilibrium of PR4 and PR772 in CsCl. Conditions were as in (a). Strain J62-1(R772) was used as indicator.

(Table 3). In other experiments phage adsorption to these strains was slight and often doubtful and phage multiplication in liquid medium could not be demonstrated (Table 3). These strains could, however, transfer plasmids R772 or RP4 to other recipients (Table 4).

Adsorption of phage PR772 to plasmid-specific pili. In preparations of J62-1(R772) to which phages PRR1 and PR772 had both been added, many tubular rods, with dimensions similar to those described above, were seen with PRR1 adsorbed to their sides and PR772 adsorbed to one tip (Figs 5, 6). With PM5006*nal-r*(R772) similar structures were not seen. Instead phage PR772 adsorbed to the latter in closely packed virions uniformly distributed over the cell surface (Fig. 7). In these preparations free phage was purely of the hexagonal type. No adsorption of phage PRR1 to this organism could be demonstrated (but see Table 3). In preparations of PR772 added to J53(N3) the phage was distributed over the surface of the organism, much like that seen with PM5006*nal-r*(R772) preparations. Here again free phage was purely of the hexagonal type and no 'tails' or tubes were seen.

Buoyant density. Phage PR772 was centrifuged together with $\phi 6$ in the same CsCl gradient, starting with a mean density of 1.26 g cm^{-3} (Fig. 8a). Using the relation $\rho^{25} = 10.2402 \eta_D^{25} - 12.6483$ (Bruner & Vinograd, 1965) and the density of 1.27 g cm^{-3} for $\phi 6$ (Vidaver *et al.*, 1973), a buoyant density of 1.26 g cm^{-3} was obtained for phage PR772.

Phages PR772 and PR4 were also centrifuged together, each at $10^8 \text{ p.f.u. ml}^{-1}$, in one CsCl gradient. The buoyant densities of these phages were indistinguishable (Fig. 8b), as expected since the buoyant density of PR4 is 1.265 g cm^{-3} (Bradley & Rutherford, 1975).

Fig. 9. Melting profile of PR772 DNA obtained by the method of Mandel & Marmur (1968).

Nucleic acid melting. The melting profile of PR772 DNA is shown in Fig. 9. *Escherichia coli* DNA was used as a reference standard. Assuming 50% G+C for the *E. coli* DNA (Marmur & Doty, 1962), the G+C content of PR772 DNA was calculated from the observed T_m (73.6 °C) as 48%.

DISCUSSION

Results of incompatibility studies reported here assigned R772 to the P-1 group (Stanisich, 1974; Bradley & Rutherford, 1975; Shahrabadi *et al.*, 1975; Bryan *et al.*, 1973; Jacoby, 1974; Hedges & Jacob, 1975) although its incompatibility interactions with R751 are not typical of members of this group. Plasmids RP4, R702 or R1033, for example, interact reciprocally with R751, eliminating and being eliminated by it (Hedges & Jacob, 1974; Smith *et al.*, 1975). Plasmid R772 eliminates R751 efficiently but is subject to elimination only after a long delay and, in the absence of selection, strains carrying both plasmids segregate far more R751⁻ progeny than R772⁻ progeny. Such asymmetric segregation patterns have been previously reported for some pairs of incompatible plasmids but this is the first instance involving R751.

The diameter of the head of phage PR772 is smaller (53 nm versus 65 nm) than that of phages PR4 and PRD1 (Bradley & Cohen, 1977; Bradley, 1976; Olsen *et al.*, 1974) and it does not have the elusive tail (Stanisich, 1974; Bradley, 1974; Olsen *et al.*, 1974) which the last two phages possess (Bradley & Cohen, 1977; Bradley & Rutherford, 1975). The chloroform sensitivity of PR772 together with a buoyant density of 1.26 g cm⁻³ suggest that the inner membrane of the phage (Fig. 2) contains lipid (Bradley & Rutherford, 1975).

The hollow tubular rods attached to a small proportion of particles could be misinterpreted as tails. However, their diameter was similar to that of sex pili coded for by incompatibility group P-1 plasmids (Bradley, 1974, 1975) and phage PRR1 adsorbed to the sides of these structures (Figs 5, 6). The fact that various lengths of these structures were found attached to the particles (Figs 3, 4) also suggested that the former were broken pili to which the phage was attached at one end. This view was strengthened by the fact that with phage PR772-J53(N3) added to J53(N3), no phage 'tails' were observed. Plasmids of the N group are thought not to code for 'conventional' sex pili (see below).

The presence of large numbers of phage attached to the periphery of PM5006^{nal-r}(R772) was puzzling since no particles in these preparations were attached to free pili. It could be argued that the particles had been drawn to the surface of the cell by retractile pili (Bradley, 1972a, b, 1973, 1976). The situation is reminiscent of that for phages PRD1 (Olsen *et al.*,

1974) and PR4 (Bradley & Rutherford, 1975; Bradley, 1974) before they were found to attach to the tips of pili (Bradley, 1976; Bradley & Cohen, 1977). If it is further postulated that sex pili must attain a certain mean length before retraction (or sometimes shedding) and that these events are strongly host-dependent, the hypothesis could accommodate most features of the odd behaviour of PM5006*nal-r*(R772). Another explanation could be that R772 codes for pili in J62-1 but that in PM5006*nal-r* the pili are only expressed as somatic receptors (Bradley, 1974).

There are precedents for the behaviour of PM5006: N incompatibility group plasmids may not code for structures resembling conventional sex pili and the specific phage IKE apparently adsorbs directly to the cell surface (Brodt *et al.*, 1974; Dennison & Baumberg, 1975*a, b*) although the phenomenon of pilus retraction, mentioned above, appears to have been ignored. Baumberg & Dennison (1975) and Morgan & Kaplan (1977) found that F pili are poorly expressed in *P. mirabilis*. Also *P.morganii* strain 2815 carrying N group plasmids does not propagate phage IKE although N-linked IKE sensitivity is transferrable from the strain (Dennison & Baumberg, 1975*a*). The phenomenon of plasmid-specific phage not forming plaques on certain strains of organisms harbouring the plasmid has also been noted by Olsen & Thomas (1973) and Olsen *et al.* (1974). In the cases presented here the refractory strains were capable of transferring their plasmids (Table 4). Evidently the maintenance of an R plasmid permitting the observed degree of donor activity in these strains was not sufficient in itself to allow phage development. In this regard, a nice distinction can be made between the incompatibility group P-1 plasmids RP4 and R772: in Providence strain P29*nal-r* only the latter allows plaque formation by phage PR772.

The low neutralization constants of antisera prepared with phage PR772 were unsatisfactory for demonstrating serological relatedness between phages, but low K values are a feature of this type of phage (Franklin, 1974; Stanisich, 1974; Bradley & Rutherford, 1975). Nevertheless all three sera neutralized phage PR4 at much lower efficiencies than homologous phage and the two phages may possibly be only distantly related antigenically. One implication may be that tips of pili to which the phages adsorb possess more than one kind of adsorption site. This may be possible, as tips of F pili have at least three similar sites for adsorption of f1 phage (Caro & Schnös, 1966).

Plasmids of compatibility groups P, N and W determine production of receptors for phages PR772 and PR4. DNA sequences of these plasmid groups show no homology by hydroxyapatite or single-strand S1 nuclease methods (Falkow *et al.*, 1974) although it is unlikely that similarity limited to one gene (e.g. that for a pilus subunit) could be reliably detected by these methods. Pili determined by P and W plasmids are distinct morphologically and unrelated serologically (Bradley, 1975, 1976; Bradley & Cohen, 1977) and the transfer apparatus determined by N pili cannot be detected by the techniques which have been used to characterize W and P pili. Yet, the phages adsorb to the tips of P and W pili and presumably to analogous sites on the hypothetical N pili.

We propose that (as implied by the morphological and serological differences of the pili and by the unrelatedness of the DNA sequences) the transfer apparatus determined by P, W and N pili may be phylogenetically unrelated but that, presumably due to the need to bind to a common component in the cell walls of recipient bacteria, the pilus tips have undergone convergent evolution leading to stereospecific similarities great enough to permit them to act as common receptors for tip-binding phages. There would be no comparable selective forces leading to convergence of other aspects of pilus structure and thus phages (such as PRR1) binding to the sides of pili have narrow host specificities adsorbing only on to the pili coded by plasmids with closely related transfer apparatus.

This is not the only example of evolutionary convergence of pilus tips; at least two other cases seem well established. Grindley *et al.* (1973) showed that phage If1 (Meynell & Lawn, 1968; Meynell, 1972) which adsorbs to the tips of I pili can also be bound by cells carrying the plasmid TP114 which has no detectable DNA homology with the I group plasmids.

Also F pili act as receptors for RNA phages (which adsorb to the sides) and for filamentous DNA phages (which adsorb to the pilus tips). The former phages are absolutely specific, binding only to pili of F-like plasmids, whilst phages of the latter group adsorb to serologically unrelated pili produced by a variety of other plasmid types (Bradley, 1977).

We therefore conclude that, in these several cases, groups of unrelated transmissible plasmids have undergone evolutionary convergence of the stereospecificity of the pilus tips, presumably as a result of the requirement for recognition of common cell wall receptors for conjugation and, as a consequence, provide common receptors for phages which bind to the pilus tips.

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