Mobilization of the *Proteus mirabilis* Chromosome by R Plasmid R772

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The P-1 incompatibility group plasmid R772 can mobilize the chromosome of *Proteus mirabilis* strain PM5006. The decreasing gradient of recombinant recovery frequencies found for markers which were increasingly distal to 0 min with plasmid D donors was not found with R772. Instead, it produced recombinants for all markers at frequencies of about 5×10^{-5} per donor. This is about 10-fold lower than the plasmid transfer frequency. Recombinants were stable and recombination was only detected over short segments of the chromosome which corresponded to about 10 min on the D plasmid map of the chromosome. All recombinants had inherited R772 and expressed all properties of the plasmid. Attempts to isolate variant plasmids with increased frequencies of recombinant formation were unsuccessful.

INTRODUCTION

The recombinant plasmid P-lacR1drd19 (Coetzee, 1974) can mobilize the *Proteus mirabilis* chromosome (Coetzee, 1975, 1978). This plasmid (now named D) causes polarized transfer of a group of chromosomal markers with origin near *his-1* (Fig. 1). Genetic circularity of the chromosome has not been demonstrated.

R plasmid R772 was identified in a strain of *P. mirabilis* isolated from clinical material (Hedges, 1975). It confers kanamycin resistance on bacterial hosts. Subsequently it was used to mobilize a non self-transmissible bacteriocinogenic plasmid Mor174 (Coetzee *et al.*, 1977; Williams, 1977), and found to be a P-1 incompatibility group plasmid (unpublished). Since R772 could mobilize the Mor174 plasmid it was decided to test the chromosome mobilizing ability of R772. Coetzee (1978) reported an increased frequency of chromosomal recombinant recovery, with sex plasmid D, when mating occurred on non-selective solid minimal medium at 30 °C. Because R772 belongs to the incompatibility group P-1, the mating conditions chosen were those mentioned above (see Dennison & Baumberg, 1975; Towner & Vivian, 1977; Haas & Holloway, 1976). In preliminary investigations (Coetzee, 1978) R772 was found to possess sex factor activity in *P. mirabilis* and this aspect of the plasmid is reported here.

METHODS

Bacteria and plasmids. 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. Minimal medium (MM) was that of Grabow & Smit (1967). For auxotrophic strains this medium was supplemented with amino acids or uracil (each at 20 µg ml⁻¹) when necessary. Incubation temperature was 30 °C and media were brought to that temperature before inoculation.

Antibacterial drugs. Nalidixic acid (500 µg ml⁻¹) was added to MM to counterselect donors in interrupted

Table 1. *Bacteria and plasmids*

	Relevant properties	Reference(s)
Bacteria		
<i>Proteus mirabilis</i>		
PM5006	Wild-type strain resistant to tetracycline	Coetzee & Smit (1970)
UP3	<i>his-1</i> mutant of PM5006 <i>nalA1</i>	Coetzee (1975)
UP4	<i>pyrB1</i> mutant of PM5006 <i>nalA1</i>	Coetzee (1975)
UP14	<i>ura-1</i> mutant of PM5006 <i>str-1</i>	Coetzee (1975)
UP19	<i>ura-3</i> mutant of UP18 (<i>ser-2 str-2 his-1 nalA1</i>)	Coetzee (1975)
UP21	<i>trp-3</i> mutant of UP16 (<i>str-2 his-1 nalA1</i>)	Coetzee (1975)
UP37	<i>leuB3</i> mutant of UP3	<i>N</i> -methyl- <i>N'</i> -nitro- <i>N</i> -nitrosoguanidine (NTG) induced mutation (unpublished)
UP39	<i>argE3</i> mutant of UP3	NTG induced mutation (unpublished)
UP47	<i>leuB2</i> mutant of UP4	Coetzee (1978)
UP49	<i>argE1</i> mutant of UP4	Coetzee (1978)
UP77	<i>argE2</i> mutant of UP7 (<i>tyr-1 str-1</i>)	Coetzee (1978)
UP177	<i>cysA12 gly-17 argA7</i> mutant derived from UP17 (<i>pyrB2 str-2 his-1 nalA1</i>)	NTG induced mutations (Coetzee, 1975, and unpublished)
UP433	<i>cysC1 nalA1</i>	Coetzee (1978)
UP466	<i>metF2 nalA1</i>	Coetzee (1978)
UP476	<i>metF6</i> mutant of UP477	NTG induced mutation (unpublished)
UP477	<i>leuB2 nalA1</i>	Coetzee (1978)
UP500	<i>rif-1</i> mutant of UP4	Spontaneous mutant (unpublished)
<i>Escherichia coli</i>		
J62-1	Nalidixic acid resistant mutant of J62, an F ⁻ <i>lac pro his trp</i> κ 12 strain	Clowes & Hayes (1968)
Plasmids		
D	D is the symbol for plasmid P- <i>lacRI</i> $\overline{drd19}$ *. Carries markers <i>lac</i> ⁺ ACKSSu†	Coetzee (1974, 1975, 1978)
R 772	P-1 incompatibility group plasmid. Molecular weight about 27 $\times 10^6$. Confers kanamycin resistance on hosts	(unpublished)

* Superior line indicates transduced R plasmid.

† Resistance to ampicillin (A), chloramphenicol (C), kanamycin (K), streptomycin (S), sulphonamide (Su).

mating experiments. Kanamycin, tetracycline (each at 50 $\mu\text{g ml}^{-1}$) or rifampicin (500 $\mu\text{g ml}^{-1}$) were used in MM or MacConkey agar when necessary.

Plate matings. These were done by the method of Coetzee (1978). Briefly, mating mixtures were constituted on non-selective MM (i.e. supplemented with growth requirements of recipient and donor) and incubated for 6 h before harvesting in saline. Control experiments lacked either donor or recipient. After washing, suitable dilutions were plated on selective MM and incubated for 48 h.

Construction of P. mirabilis UP strains containing plasmid R772 or D. The liquid mating technique of Coetzee *et al.* (1973) was used. Donors, J62-1(R772) or J62-1(D), were counterselected with tetracycline and transfer of both plasmids was selected with kanamycin. Plate mating was also employed for R772 transfer, with the donor counterselective agent as well as kanamycin incorporated in MM. Transfer of kanamycin resistance of plasmid R772 to various UP strains occurred in broth and on non-selective MM at frequencies of about 5×10^{-4} per donor cell. Similarly plasmid D was readily transferred without affecting chromosomal markers of the strains (Coetzee, 1975). All donor strains were maintained on nutrient agar slopes containing kanamycin at 10 °C. They were subcultured at intervals of about 2 months.

Interrupted mating. The solid medium method of Haas & Holloway (1976) was used.

Unselected marker analysis and recombinant stability. Recombinant clones were purified on agar similar to that used for selection. Single colonies were then suspended in 0.5 ml saline and loopfuls were spotted on selective media and on the medium used for selection of the particular recombinant. Stability was tested for by plating a suitable dilution of an overnight broth culture of a purified recombinant clone on MacConkey agar. After overnight incubation the growth was replicated to selective MM.

R plasmid characterization in chromosomal recombinants. Kanamycin resistance was tested by replication of patched colonies on MM to the same medium containing kanamycin. Ability to transfer kanamycin resistance to UP500 was tested by the liquid mating technique mentioned above using rifampicin for donor counterselection on MacConkey agar also containing kanamycin. The sensitivity of recombinants to phage

Table 2. *Recombination frequencies of markers using plasmids D and R772 as donors*

Plate matings were done as described in Methods. Selective MM was supplemented, where necessary, with growth requirements for unselected recipient markers.

Cross	Selected marker	Map location	Plasmid	Recombination frequency*
1: UP19 × UP477(D) or (R772)	<i>his-1</i>	7 min	D R772	3×10^{-4} 4×10^{-5}
2: UP4 × UP3(D) or (R772)	<i>pyrB2</i>	20 min	D R772	4×10^{-5} 5×10^{-5}
3: UP433 × UP3(D) or (R772)	<i>cysC1</i>	40 min	D R772	8×10^{-7} 4×10^{-5}
4: UP47 × UP21(D) or (R772)	<i>leuB2</i>	Most distal marker	D R772	1×10^{-7} 5×10^{-5}
5: UP49 × UP21(D) or (R772)	<i>argE1</i>	Unknown	D R772	$< 5 \times 10^{-9}$ 8×10^{-5}

* Recovery of recombinants per donor cell in mating mixture.

Fig. 1. The order of markers on the chromosome map of *P. mirabilis*. Where known, the approximate location relative to the plasmid D origin is given (in min). Where the time of entry of markers is not known the spacing is arbitrary. A bar across markers indicates that their relative positions have not been determined (Coetzee, 1975, 1978, and unpublished).

PR772 was not tested, because phage sensitivity of *P. mirabilis* recombinants is not easy to determine (unpublished).

Chromosome donor ability (Cda) (Haas & Holloway, 1976) was measured by replication of patched auxotrophic recombinant colonies on suitably supplemented MM to MM recently surface-seeded with UP4. Selection was for *pyrB*⁺ recombinants.

RESULTS

Inheritance of chromosomal markers

Transfer. Marked differences in frequencies of recombinant recovery were noted using plasmids D and R772 as donors (Table 2). With plasmid D the recovery of recombinants for four markers, dispersed over the entire linkage group (Fig. 1), showed typical polarization with frequencies per donor cell which varied between 3×10^{-4} and 1×10^{-7} . With plasmid R772 recombinant recovery frequencies were similar (at about 5×10^{-5}) for the four markers concerned. A fifth marker, *argE1*, which had failed to produce recombinants in plasmid D-mediated transfer (Coetzee, 1978), again did not produce recombinants under promotion of this plasmid, but recombinants were produced when the donor carried R772. All progeny were stable and behaved like haploid recombinants (Meade & Signer, 1977).

Table 3. *Segregation of unselected markers in crosses*

Plate matings were done as described in Methods. Selective MM was supplemented with growth requirements for unselected recipient markers. For each selected marker, the first row of results is for the cross UP177 × UP476(R772) and the second row is for the cross UP177 × UP433(R772).

Selected marker	Co-inheritance of unselected chromosomal markers (%) [*]				
	<i>his</i> ⁺	<i>pyr</i> ⁺	<i>cys</i> ⁺	<i>gly</i> ⁺	<i>arg</i> ⁺
<i>his</i> -1 ⁺	—	7	0	0	0
	—	8	0	0	0
<i>pyr</i> B2 ⁺	8	—	4	1	0
	11	—	6	0	1
<i>cys</i> A12 ⁺	6	25	—	3	0
	6	19	—	6	0
<i>gly</i> -17 ⁺	0	6	31	—	1
	0	0	27	—	7
<i>arg</i> A7 ⁺	0	0	3	46	—
	0	0	6	39	—

* One hundred recombinants for each selected marker were tested for the presence of unselected markers by the procedure described in Methods.

Segregation of unselected markers. Using strain UP177 (*his*-1 *pyr*B2 *cys*A12 *gly*-17 *arg*A7) as recipient and donor strains UP476(R772) and UP433(R772), with contraselection markers *leu*B2, *met*F6 and *cys*C1 respectively, sets of linkage data were obtained (Table 3). These results are consistent with there being only one chromosomal transfer event per recombinant and with the marker order derived from the use of plasmid D. Although data for the time of marker entry is not satisfactory (Coetzee 1975, 1978), it is evident that recombinants may seldom inherit lengths of donor chromosome longer than about 10 min on the D plasmid map.

To demonstrate genetic circularity of the chromosome, attempts were made to show co-inheritance of the proximal marker *his*-1 with *leu*B3. The latter marker is an allele of the most distal marker *leu*B2 on the D plasmid map and is carried by strain UP37. Strain UP39 was also used; this strain carries an allele of *arg*E1, a marker which did not yield recombinants (Coetzee, 1978) and is presumably located distally to *leu*B2. Strain UP466-(R772) was used as donor in the former cross, and UP14(R772) in the latter. Both crosses were sterile.

Interrupted mating. Using some recipient strains employed before (Coetzee, 1978) and the same donor now carrying R772, a marked difference was noted between times of entry of chromosomal markers and the times previously recorded for plasmid D-mediated chromosomal transfer (Coetzee, 1975, 1978). With plasmid D, individual chromosomal markers entered between 7 and 42 min. Under promotion of R772, recombinants for all markers registered between 4 and 7 min. The above findings were confirmed by crossing the multiply marked recipient strain UP177 and donor UP77(R772). Recombinants of each individual marker selected registered between 4 and 7 min (not shown). These results, as well as those presented in Table 2, indicate that plasmid R772 has many sites on the chromosome from which chromosome mobilization may be initiated. Plasmid R772 transfer, like that of D (Coetzee, 1975, 1978), was early and occurred at high frequency.

Properties of plasmid R772 in chromosomal recombinants

All of hundreds of purified recombinants derived from the experiments presented in Table 3 were kanamycin resistant, could transfer the marker and possessed Cda. To test for enhanced Cda, 10 auxotrophic recombinants from each of crosses 1, 4, 5 (Table 2) and

all crosses of Table 3 were mated with UP4 with selection for *pyrB*⁺ recombinants. Recombinants were recovered from all crosses at frequencies similar to those recorded in Table 2.

DISCUSSION

Plasmid R772 is the latest addition to the P-1 incompatibility group plasmids (Bryan *et al.*, 1973; Haas & Holloway, 1976) which can promote chromosome transfer in particular bacterial species. Chromosomal transfer by P plasmids may be strain specific (Stanisich & Holloway, 1971; Stanisich & Richmond, 1975). With other P-1 plasmids the requirements for donor ability are less strict but still very specific. Thus RP4 does not mobilize the *Pseudomonas aeruginosa* chromosome (Stanisich & Holloway, 1971; Haas & Holloway, 1976), has only marginal activity in *Rhizobium leguminosarum* (Beringer & Hopwood, 1976) but promotes chromosome transfer in *R. meliloti* (Meade & Signer, 1977) and *Acinetobacter calcoaceticus* (Towner & Vivian, 1976*a, b*, 1977). Another member of the P-1 group, R68.45, promotes chromosome transfer in strains of *Ps. aeruginosa*, *Ps. putida* (Haas & Holloway, 1976), *R. leguminosarum*, *E. coli* (Beringer & Hopwood, 1976) and *R. meliloti* (Kondorosi *et al.*, 1977), but fails to do so in *P. mirabilis* (unpublished observations). Specificity is thought (Jacob *et al.*, 1976, 1977) to depend on the presence of homologous insertion sequences (ISs) on chromosome and plasmid, respectively, in much the same manner as the F factor of *E. coli* mobilizes that chromosome (Hu *et al.*, 1975). Jacob *et al.* (1977) presented evidence that R68.45, a variant of plasmid R68 which shows enhanced Cda, has a molecular weight that is 1.4×10^6 higher. This is consistent with the acquisition of an IS.

The present failure to isolate variants of R772 with Cda greater than about 5×10^{-5} per donor could mean that the wild-type R772 already possesses the optimal number of suitable homologies required for *P. mirabilis* chromosome mobilization. These homologous areas must be stable, since, although R772 was first detected in *P. mirabilis* (Hedges, 1975), it was transferred to an *E. coli* strain which served as the source of R772 for the present investigation. Plasmid R772 has multiple sites of origin of chromosome transfer on the *Proteus* chromosome (Table 2) and apparently mobilizes fairly short segments of the donor chromosome (Table 3; Fig. 1). Both these plasmid properties also apply to R68.45-mediated chromosome transfer in *Ps. aeruginosa* (Haas & Holloway, 1976), but it has not been determined whether the limited chromosome transfer is due to fragile mating pair formation or to some inherent property of the sex factors concerned. In *R. leguminosarum* R68.45 also transfers the chromosome from multiple sites but the size of transferred fragments varies and some may encompass one-seventh of the chromosome (Beringer *et al.*, 1978). With *R. meliloti* (Meade & Signer, 1977; Kondorosi *et al.*, 1977) there are also multiple sites of origin for chromosomal transfer by R plasmids RP4 and R68.45, but large segments of chromosome are transferred. This could point to defects in mating pair formation in the *P. mirabilis*, *Ps. aeruginosa* and, possibly, *R. leguminosarum* systems mentioned above. A phenomenon that may be related to the above is the fact that markers distal to the selected marker on the D plasmid map of *P. mirabilis* rarely yielded recombinants (Table 3). Pearce & Meynell (1968) encountered a similar phenomenon with plasmid R1-mediated chromosome transfer in *E. coli* and Coetzee (1975) also noted it with D-promoted recombination in *P. mirabilis*.

Failure to demonstrate polarized transfer of chromosome markers in R772-promoted transfer may be attributed to multiple origins of chromosome transfer. The polarized transfer mediated by a single plasmid is then obscured (and levelled out) in the population as a whole by similar events occurring independently and concurrently from other origins. Should there be only one preferred site of plasmid chromosome interaction, donor efforts tend to become synchronized with resulting polarized transfer of genetic markers. This latter state may apply to *P. mirabilis* chromosome transfer by plasmid D (Coetzee,

1975) and RP4-mediated chromosomal transfer in *A. calcoaceticus* (Towner & Vivian, 1976a, b).

R772 was inherited apparently unaltered by all chromosomal recombinants. A similar situation exists for R68.45-promoted chromosomal recombinants of *R. leguminosarum* where the R plasmid does not appear to lose any of its functions in recombinants (Beringer *et al.*, 1978). This is unlike the fate of R68.45 in *Ps. aeruginosa* (Haas & Holloway, 1976) or *R. meliloti* (Kondorosi *et al.*, 1977) recombinants produced by mediation of this plasmid; in many of these recombinants plasmid function was either completely lost or only partially present. A similar situation could exist in RP4-promoted chromosomal recombinants of *A. calcoaceticus* (Towner & Vivian, 1976b). A reason given for this instability (Jacob *et al.*, 1977) is that ISs may promote deletions in genes near insertion sites (Weisberg & Adhya, 1977). On these grounds it may be argued that if insertions and deletions do occur in R772 (but see Bennett *et al.*, 1977) they take place at sites which do not affect the phenotype of the plasmid.

The isolation of a further sex factor for *P. mirabilis* PM5006 adds to the genetic versatility of the strain. The ability of R772 to mobilize distal markers on the D plasmid map of that organism may provide proof of the genetic circularity of the chromosome although the limited segments of chromosome over which recombination takes place could be a temporary limitation.

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