***Proteus mirabilis* Chromosome Mobilization by Plasmid D: a Physical Characterization**

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Plasmid D, a hybrid of plasmids P-*lac* and R1*drd*19, mediates polarized chromosome mobilization from one origin in *Proteus mirabilis* strain PM5006, while the parental plasmids neither individually nor combined mobilize this chromosome. To elucidate its acquired mobilizing ability plasmid D was characterized physically in relation to P-*lac* and R1*drd*19. Restriction patterns of these plasmids were compared and it was shown that D consists of P-*lac* and only the r-determinant (r-det) of R1*drd*19. A mechanism for the formation of plasmid D, via transduction of the r-det and subsequent transposon-like integration into P-*lac*, involving insertion sequence IS1, was suggested. Evidence for aberration in plasmid D DNA as a result of r-det integration into P-*lac* was attributed to IS1 elements which flank the r-det. Recombination regions of parental plasmid DNA were located on *Hind*III fragments α and β of plasmid D and were subsequently inserted *in vitro* into IncP-1 plasmid RP4 that fails to mobilize the *P. mirabilis* chromosome. RP4:: *Hind*III α plasmids did not mobilize the latter chromosome, but rendered the *Proteus* host *lac*⁺. RP4:: *Hind*III β plasmids pMC1 and pMC17, containing the fragment in opposite orientations, mobilized the *P. mirabilis* chromosome. For pMC17, mobilization was indistinguishable from that of plasmid D, i.e. having the same orientation and the same single origin. However, mobilization promoted by pMC1 was from two distinctly different origins, different from that of pMC17. This apparently deviates from known examples where inversion of homologous DNA inserted into plasmids leads to mobilization from the same origin but in reverse direction.

INTRODUCTION

Plasmid D is a hybrid of plasmids P-*lac* (Wohlhieter *et al.*, 1964) and R1*drd*19 (Meynell & Datta, 1967) and was originally named P-*lac*R1*drd*19 (Coetzee, 1974). It mediates polarized chromosome mobilization from a single origin in *Proteus mirabilis* strain PM5006 (Coetzee, 1975). The parental plasmids, although capable of chromosome mobilization in *Escherichia coli* (Falkow *et al.*, 1964; Pearce & Meynell, 1968), neither individually nor combined mobilize the chromosome of *P. mirabilis* (Coetzee, 1975).

Plasmid D contains the Tra functions and *lac* marker of P-*lac* (Hedges, 1975) and at least the r-determinant (r-det) of R1*drd*19, as suggested by genetic evidence (Coetzee, 1974). However, plasmid D has not been physically analysed in terms of the parental plasmids. Except for a molecular mass determination of 101 MDal (Coetzee *et al.*, 1975) P-*lac* has also not been physically characterized, whereas restriction maps and a molecular mass of 62.5 MDal have been established for R1*drd*19 (Blohm & Goebel, 1978; Clerget *et al.*, 1981).

This paper deals with a comparative analysis of plasmids P-*lac*, R1*drd*19 and D by restriction fragmentation, with a view to establishing the composition of plasmid D, characterizing the recombination sites of P-*lac* and R1*drd*19 in the hybrid, and elucidating its chromosome mobilizing ability (Cma).

Table 1. *Bacterial strains and plasmids*

Bacterial strain	Relevant properties	Derivation	Reference/Source
<i>Proteus mirabilis</i>			
PM5006	Wild-type Tc ^R	NTG*-induced mutants of PM5006	J. N. Coetzee
UP101	<i>his-1 nalA1 str-2</i>		J. N. Coetzee (unpublished)
UP102	<i>trpA nalA1 str-2</i>		
UP103	<i>ilv-2 nalA1 str-2</i>		
UP104	<i>pur-1 nalA1 str-2</i>		
UP105	<i>cysC nalA1 str-2</i>		
UP106	<i>metF nalA1 str-2</i>		
UP107	<i>argE nalA1 str-2</i>		
<i>Escherichia coli</i> K12			
C600 r ⁻ m ⁻	<i>thr-1 leuB6 thi-1</i> <i>hsdS1 lacY1 tonA21</i> λ^- <i>supE44</i>		<i>E. coli</i> Genetic Stock Centre
Plasmid†			
P-lac	IncA-C Tra <i>lac</i> ⁺ Su ^R		Hedges (1975)
R1drd19	IncFII Tra Ap ^R Cm ^R Km ^R Sm ^R Su ^R		Meynell & Datta (1967)
D	IncA-C Tra <i>lac</i> ⁺ Ap ^R Cm ^R Km ^R Sm ^R Su ^R		Coetzee (1978)
RP4	IncP-1 Tra Ap ^R Km ^R Tc ^R		Datta <i>et al.</i> (1971) Holloway & Richmond (1973)
pMC1 } pMC17 } pMC41 } pMC52 }	IncP-1 Tra Ap ^R Km ^S Tc ^R	<i>In vitro</i> recombi- nants of RP4 and <i>Hind</i> III fragments of plasmid D	This work

* *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.

† Plasmids were contained in *P. mirabilis* PM5006. Symbols designate resistance to: Ap, ampicillin; Cm, chloramphenicol; Km, kanamycin; Sm, streptomycin; Su, sulfonamide; Tc, tetracycline. Tra, transfer proficient.

METHODS

Bacterial strains and plasmids. These are listed in Table 1.

Plasmid isolation. This was done according to Prakash *et al.* (1981), as modified in this laboratory (Beck *et al.*, 1982).

Media and antibiotics. These were as described by Beck *et al.* (1982) except that the minimal medium was not supplemented with methionine and calcium pantothenate.

Restriction analysis of plasmids. Plasmids were physically characterized by restriction fragmentation. Restriction endonucleases *Eco*RI, *Hind*III, *Sma*I and *Pst*I as well as T4 DNA ligase were obtained from Boehringer Mannheim. Reaction buffers for these were as recommended by the manufacturers. Double as well as some single digests were performed in a universal restriction buffer (Ruvkun & Ausubel, 1981). Digests were electrophoresed according to McDonnell *et al.* (1977), with buffer circulation. Gels usually contained 1 µg ethidium bromide ml⁻¹ and were frequently stained further in water or electrophoresis buffer containing 1 µg ethidium bromide ml⁻¹ followed by a brief de-staining rinse. Electrophoresis patterns were photographed on Polaroid 665 film under far-UV illumination and negatives were densitometrically scanned with the scanning attachment of a Varian Cary 210 spectrophotometer. Nomenclature and molecular weights of R1drd19 restriction fragments were determined according to Blohm & Goebel (1978). Molecular weights of other fragments were calculated relative to these or the known values for RP4 restriction fragments (Depicker *et al.*, 1977). Restriction fragments of preparative gel runs were eluted using the NaClO₄-glass fibre filter technique of Chen & Thomas (1980). Incubation of the filters in TE (1 mM-Tris, pH 8 plus 0.4 mM-EDTA) was extended to between 2 h and overnight at 4 °C to obtain yields of about 80% after two or three washes.

Construction of RP4 recombinants. The single *Hind*III restriction site in the kanamycin resistance marker (Km) of plasmid RP4 (Barth & Grinter, 1977) was utilized to construct and select recombinant clones of RP4 with specific *Hind*III fragments of plasmid D. *In vitro* ligation of 0.2 µg of fragment with 0.2 µg of *Hind*III-restricted RP4, transformation of *E. coli* C600 r⁻ m⁻ with the ligated mixture and selection and purification of transformants were as described by Beck *et al.* (1982). The Km^S clones were cultivated and plasmid DNA was

extracted using the method of Clewell & Helinski (1969). Inserted fragments were analysed for size and orientation by restriction fragmentation of the recombinant plasmids with *Hind*III, *Pst*I and *Sma*I.

Plate matings. These were done as described previously (Beck *et al.*, 1982; Coetzee, 1978). Briefly, recombinant plasmids were transferred by conjugation to PM5006 on Millipore membranes placed on NB2 agar. Transconjugants were selected by ampicillin ($30 \mu\text{g ml}^{-1}$) on minimal media for donor counterselection. Screening for Cma was done by overnight matings of donors and auxotrophs (Table 1) on Hershey's agar. Recombinants were selected by replication to minimal media containing streptomycin (1 mg ml^{-1}) and nalidixic acid ($75 \mu\text{g ml}^{-1}$). In control experiments either donor or recipient was omitted.

RESULTS

Restriction patterns of plasmids *P-lac*, *D* and *R1drd19*

Physical characterization of plasmid D was performed by comparing its restriction pattern to those of the parental plasmids *P-lac* and *R1drd19* (Fig. 1). *Hind*III fragments of plasmids *P-lac* and *R1drd19* (Fig. 1*a,c*) had their equivalents in the patterns produced for plasmid D (Fig. 1*b*) except for *P-lac* fragment *Hind*III C (Fig. 1*a*) and *R1drd19* fragments *Hind*III A and D. This suggested that in the formation of plasmid D, a region of *R1drd19*, encompassing at least the *Hind*III B, C, E and F fragments, had been inserted within the *Hind*III C fragment of *P-lac*. In addition, two new *Hind*III fragments, noted as *Hind*III α and β (Fig. 1*b*), which had no equivalent in either *P-lac* or *R1drd19*, emerged for plasmid D. These may represent the recombination regions between the two DNA molecules.

This analysis was carried further by digestion with *Eco*RI which produced a more detailed pattern (Fig. 1*d,e,f*). The observed *Eco*RI pattern of *R1drd19* (Fig. 1*f*) deviated in one respect

Fig. 1. Restriction patterns of plasmids *P-lac* (*a, d*), *D* (*b, e*) and *R1drd19* (*c, f*) for *Hind*III (*a, b, c*) and *Eco*RI (*d, e, f*). Nomenclature and molecular weights of *R1drd19* fragments are according to Blohm & Goebel (1978) except for fragments *Hind*III A and *Eco*RI E (see text). Agarose gels of 0.4% (not shown) were used to resolve *Eco*RI D and E (*f*), *Hind*III α and C (*a, b*) and fragments > 12 MDal. These facilitated establishment of indicated molecular weights. Fragments < 1.15 MDal were observed on 2% agarose gels (not shown), in agreement with Blohm & Goebel (1978). *Hind*III-restricted phage λ (Philippsen *et al.*, 1978) was used as molecular weight markers.

Fig. 2. (a) *Eco*RI and (b) *Hind*III restriction maps of R1drd19 (Clerget *et al.*, 1981) amended with respect to size difference in *Eco*RI E and *Hind*III A (Fig. 1). (c) Location of r-det, RTF and IS1 sequences (Clerget *et al.*, 1981) with respect to restriction maps. (d) Segment of plasmid D containing the r-det insert of R1drd19 (see text). Recombination regions with P-lac DNA (broken line) on fragments *Hind*III α and β of plasmid D are indicated (see text).

from that described by Blohm & Goebel (1978). Fragment E was found to be larger (6.5 MDal) than that observed before (5.1 MDal). This difference is not due to experimental conditions and was not further studied but may perhaps be attributed to a transposable element that had entered the plasmid. Consequently, revised restriction maps (Clerget *et al.*, 1981) were amended (Fig. 2a, b) to accommodate this difference, and the molecular size of R1drd19 was taken as 63.9 MDal.

Figures 1(d, e and f) show that the *Eco*RI fragments of P-lac and R1drd19 (Fig. 1d, f) had their equivalents in the pattern for D (Fig. 1e) with the exception of P-lac fragments *Eco*RI D and F (Fig. 1d) and R1drd19 fragments *Eco*RI A, B, C, E, F and K (Fig. 1f) as well as M (as visualized on a 2%, w/v, agarose gel; not shown). Moreover, the only fragments common to D and R1drd19 were *Hind*III B, C, E and F (see above) and *Eco*RI, D, G, H, I, J and L (Fig. 1e, f) as well as N and O (as revealed on a 2% agarose gel; not shown), which stem from the R1drd19 r-det (Clerget *et al.*, 1981; see also Fig. 2a, b). This suggested that in the formation of plasmid D only the r-det of R1drd19 was inserted into P-lac.

The lack of two *Eco*RI fragments of P-lac in the pattern for D (Fig. 1d, e) is curious and leads to the speculation that the insertion events in the formation of plasmid D were complex and may have involved deletions and/or rearrangements. This is supported by the finding of three rather than two non-P-lac or R1drd19 fragments, noted as *Eco*RI α , β and γ , in the *Eco*RI digest of plasmid D (Fig. 1e).

Restriction analysis of fragments *Hind*III α and β of plasmid D

*Eco*RI restriction of *Hind*III β (4.5 MDal; Fig. 1b) gave rise to three fragments of 1.85, 1.75 and 0.85 MDal (not shown). The first and last corresponded with *Eco*RI fragments I and N, respectively, of R1drd19 (Fig. 2a). Hence, *Hind*III β may be interpreted as containing the right-hand terminal region of the R1drd19 r-det (Fig. 2c, d). The 1.75 MDal fragment which had no equivalent in either P-lac or R1drd19 would then contain the right-hand recombination region of these plasmids (Fig. 2d).

*Hind*III α (Fig. 1b) gave rise to four detectable *Eco*RI fragments of 3.7, 2.2, 1.2 and 0.9 MDal (not shown). The sum of the four fragments amounted to 8.0 MDal as compared to 8.8 MDal for *Hind*III α , suggesting that additional *Eco*RI fragments (smaller than 0.6 MDal) must have been generated, which could not be visualized under the conditions of these experiments. The

Table 2. Chromosome mobilization in *Proteus mirabilis* strain PM5006 by different plasmids

Values are from one of two independent but mutually consistent experiments. Map locations are from the circular chromosome map of Coetzee *et al.* (1982).

Donor	Map location (min) ...	No. of recombinants per 10 ⁸ donor cells						
		<i>his-1</i> 7	<i>trpA</i> 21	<i>ilv-2</i> 32	<i>pur-1</i> ~32	<i>cysC</i> 42	<i>metF</i> 55	<i>argE</i> 85
PM5006(RP4)		<1	<1	<1	<1	<1	<1	<1
PM5006(D)		2 × 10 ³	120	200	34	30	42	4
PM5006(pMC17)		2 × 10 ³	100	100	12	50	75	<1
PM5006(pMC1)		16	1.5 × 10 ³	9	150	1.2 × 10 ³	<1	<1
PM5006(pMC41)		<1	<1	<1	<1	<1	<1	<1
PM5006(pMC52)		<1	<1	<1	<1	<1	<1	<1

2.2 MDal fragment corresponded to *EcoRI* γ (Fig. 1e) of plasmid D, supporting the notion that *HindIII* α of plasmid D contains the other (left-hand) recombination region of P-*lac* and R1*drd19*. As no R1*drd19* fragment was obtained from *HindIII* α the location of the left-hand recombination region could only be inferred in *HindIII* D of R1*drd19* (Fig. 2b, d).

Construction of RP4 recombinant plasmids

Upon ligation of *HindIII*-restricted plasmid RP4 with purified *HindIII* fragments α and β of plasmid D, approximately 5% (11 clones) of transformants for *HindIII* α and 2.5% (27 clones) for *HindIII* β , were sensitive to kanamycin but resistant to ampicillin and tetracycline. This may suggest that the relevant fragments have been inserted into RP4.

Physical characterization of RP4 recombinant plasmids

HindIII restriction of plasmid DNA from the 11 transformants for RP4:: α yielded fragment *HindIII* α in seven while four were not restricted. The latter was attributed to improper ligation leading to loss of both kanamycin resistance and the *HindIII* restriction site. Five of the seven plasmids containing *HindIII* α yielded non-RP4 fragments of 15.4 and 7.2 MDal when restricted with *SmaI* and two gave fragments of 20.2 and 2.4 MDal (not shown), as compared with the known restriction patterns of RP4 (Depicker *et al.*, 1977). This, together with the observation that *SmaI* cleaved *HindIII* α into fragments of 6.8 and 2.0 MDal (not shown), is consistent with the notion that the two populations of recombinants named pMC41 and pMC52, respectively, contained *HindIII* α in reverse orientation. Similarly, *HindIII* β , which gave fragments of 3.2 and 1.3 MDal when restricted with *PstI* (not shown), proved to be in reverse orientation in plasmids pMC1 and pMC17 from the 27 clones obtained by transformation with RP4:: β .

Transfer of recombinant plasmids to strain PM5006

Transfer frequencies of plasmids pMC1, pMC17, pMC41 and pMC52 from *E. coli* C600 $r^- m^-$ to *P. mirabilis* PM5006 by conjugation were 10^{-4} – 10^{-3} per donor h^{-1} . pMC41 and pMC52 rendered PM5006 Lac⁺, showing that *HindIII* α of plasmid D carried the essential genes of the P-*lac* *lac* operon.

Chromosome mobilization in PM5006

Transfer frequencies of various chromosomal markers for recombinant plasmids were compared with those for plasmids RP4 and D (Table 2). Since recipients were single auxotrophs, effects of strain variations can not be excluded and may explain slight modulation in transfer frequency gradients, e.g. for plasmid D (Table 2, line 2). These experiments (Table 2) indicate that only plasmids which contained the *HindIII* β fragment of plasmid D could mobilize the PM5006 chromosome. Mobilization by pMC17 was indistinguishable from that of plasmid D, whereas that of pMC1 (with *HindIII* β in reverse orientation) was from two different origins (Table 2). This suggested that mobilization by plasmid D could have been due to a new base sequence on *HindIII* β as a result of recombination of P-*lac* and R1*drd19* DNA.

DISCUSSION

Insertion of one DNA molecule into another can be achieved by homologous recombination (Dressler & Potter, 1982; Eisenstark, 1977) or by site-specific insertion events such as transposition (Kleckner, 1981; Starlinger, 1980) or chi-sequence-mediated recombination (Stahl, 1979). The present data showed that only the r-det of R1drd19 was present in plasmid D. This together with the fact that the r-det is flanked by direct repeats of insertion sequence IS1 (Clerget *et al.*, 1981) favours a transposon-like insertion event of the R1drd19 r-det into P-lac as a mechanism for the formation of plasmid D. This is supported by a number of findings. First, in transducing R1drd19 by *Providencia* phage PL25 into *Providencia* strain P29 (P-lac) no transductants were found with recombinant plasmids other than containing all antibiotic resistance markers of the R1drd19 r-det (Coetzee, 1974). Second, stable variants of R1drd19 containing only the RTF and kanamycin marker, e.g. R1drd16 (Meynell & Datta, 1967), or all but the kanamycin marker, e.g. pSC50 (Kopecko & Cohen, 1975), or only the RTF (Cohen & Miller, 1970; Silver & Falkow, 1970) have been described. These structures may be interpreted in terms of the presence of IS1 copies on R1drd19, analogous to plasmid R100.1 (Hu *et al.*, 1975; Ptashne & Cohen, 1975) where IS1 elements gave rise (Chandler *et al.*, 1977; Hu *et al.*, 1975) to poly-r-det R-factors, multimeric and monomeric autonomous r-dets and an autonomous RTF under different antibiotic selection pressure conditions (Perlman & Rownd, 1975; Perlman *et al.*, 1975; Rownd & Mickel, 1971). Third, the packaging capacity of phage PL25 is 24.7 MDal of linear DNA (Van Rensburg, 1970) and is therefore capable of transducing a section of R1drd19 DNA containing the 23 MDal r-det (Clerget *et al.*, 1981). Further, if DNA rearrangements and/or deletions have occurred in the immediate vicinity of the recombination regions in plasmid D, as suggested by the complexity of the *EcoRI* restriction patterns of plasmid D and its *HindIII* α fragment, these could be attributed to similar IS1 activities (Kleckner, 1981; Kopecko, 1980; Nevers & Saedler, 1978; Reif & Saedler, 1977; Saedler, 1977; Starlinger & Saedler, 1976; Starlinger, 1977, 1978), and correspondingly point towards a transposon-like insertion event of the r-det which was circularized by virtue of IS1 after being transduced by phage PL25.

Orientated, plasmid-mediated transfer of bacterial chromosome may result from interaction of homologous regions on plasmid and host chromosome (Pittard & Adelburg, 1963). Whatever the nature of the homology, whether due to insertion of common sequences into both plasmid and chromosome (Dénarié *et al.*, 1977; Grinter, 1981; Johnson & Romig, 1979) or to production of R-prime plasmids (Barth, 1979; Beck *et al.*, 1982; Juliot & Boistard, 1979), the altered plasmids without exception exhibited Cma, the extent and polarity of which corresponded to the extent and orientation of the homologous regions. The possibility that recombination of R1drd19 with P-lac in the formation of plasmid D produced a region of homology with the PM5006 chromosome is unlikely on grounds of the fact that plasmids pMC1 and pMC17 with *HindIII* β in opposite orientation did not produce reversed mobilization trajectories. This may indicate that more than simple homology between plasmid D and the chromosome was involved, e.g. a new sequence on plasmid D fragment *HindIII* β due to insertion and/or rearrangement events. Duplications in *HindIII* β as a source of new sequences may be ruled out, since restriction mapping of *HindIII* β with 15 restriction enzymes did not reveal any duplicated segment (J. H. Hauman, this laboratory, unpublished results).

Possible roles for IS1 and the chloramphenicol resistance marker which were identified (J. H. Hauman, unpublished results) on *HindIII* β as well as for IS8, adjacent to the *HindIII* insertion site on RP4 (Depicker *et al.*, 1980) are being investigated.

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REFERENCES

- BARTH, P. T. (1979). Plasmid RP4, with *Escherichia coli* DNA inserted *in vitro*, mediated chromosome transfer. *Plasmid* **2**, 130-136.
- BARTH, P. T. & GRINTER, N. J. (1977). Map of plasmid RP4 derived by insertion of transposon C. *Journal of Molecular Biology* **113**, 455-474.

- BECK, Y., COETZEE, W. F. & COETZEE, J. N. (1982). *In vitro* constructed RP4-prime plasmids mediate oriented mobilization of the *Proteus morganii* chromosome. *Journal of General Microbiology* **128**, 1163–1169.
- BLOHM, D. & GOEBEL, W. (1978). Restriction map of the antibiotic resistance plasmid R1drd-19 and its derivatives pKN102 (R1drd-19B2) and R1drd-16 for the enzymes *Bam*HI, *Hind*III, *Eco*RI and *Sal*I. *Molecular and General Genetics* **167**, 119–127.
- CHANDLER, M., SILVER, L., FREY, J. & CARO, L. (1977). Suppression of an *Escherichia coli* *dnaA* mutation by the integrated R-factor R100.1: generation of small plasmids after integration. *Journal of Bacteriology* **130**, 303–311.
- CHEN, C. W. & THOMAS, C. A. (1980). Recovery of DNA segments from agarose gels. *Analytical Biochemistry* **101**, 339–341.
- CLERGET, M., CHANDLER, M. & CARO, L. (1981). The structure of R1drd19: a revised physical map of the plasmid. *Molecular and General Genetics* **181**, 183–191.
- CLEWELL, D. B. & HELINSKI, D. R. (1969). Supercoiled circular DNA–protein complex in *Escherichia coli*: purification and induced conversion to an open circular DNA form. *Proceedings of the National Academy of Sciences of the United States of America* **62**, 1159–1166.
- COETZEE, J. N. (1974). Properties of *Proteus* and *Providencia* strains harbouring recombinant plasmids between P-lac, R1drd19 or R447b. *Journal of General Microbiology* **80**, 119–130.
- COETZEE, J. N. (1975). Chromosome transfer in *Proteus mirabilis* mediated by a hybrid plasmid. *Journal of General Microbiology* **86**, 133–146.
- COETZEE, J. N. (1978). Extension of a chromosome linkage group of *Proteus mirabilis*. *Journal of General Microbiology* **107**, 155–163.
- COETZEE, J. N., JACOB, A. E. & HEDGES, R. W. (1975). Susceptibility of a hybrid plasmid to excision of genetic material. *Molecular and General Genetics* **140**, 7–14.
- COETZEE, J. N., VAN DIJKEN, M. C. & COETZEE, W. F. (1982). *Proteus mirabilis*. In *Genetic Maps Vol. II*, pp. 152–157. Edited by S. J. O'Brien. Bethesda, Maryland: National Cancer Institute, National Institutes of Health.
- COHEN, S. N. & MILLER, C. A. (1970). Non-chromosomal antibiotic resistance in bacteria. III. Isolation of the discrete transfer unit of the R-factor R1. *Proceedings of the National Academy of Sciences of the United States of America* **67**, 510–516.
- DATTA, N., HEDGES, R. W., SHAW, E. J., SYKES, R. B. & RICHMOND, M. H. (1971). Properties of an R-factor from *Pseudomonas aeruginosa*. *Journal of Bacteriology* **108**, 1244–1249.
- DÉNARIÉ, J., ROSENBERG, C., BERGERON, B., BOUCHER, C., MICHEL, M. & BARATE DE BERTALMIO, M. (1977). Potential of RP4::Mu plasmids for *in vivo* genetic engineering of Gram-negative bacteria. In *DNA Insertion Elements, Plasmids and Episomes*, pp. 507–520. Edited by A. I. Bukhari, J. A. Shapiro & S. L. Adhya. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory.
- DEPICKER, A., VAN MONTAGU, M. & SCHELL, J. (1977). Physical map of RP4. In *DNA Insertion Elements, Plasmids and Episomes*, pp. 678–679. Edited by A. I. Bukhari, J. A. Shapiro & S. L. Adhya. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory.
- DEPICKER, A., DE BLOCK, M., INZE, D., VAN MONTAGU, M. & SCHELL, J. (1980). IS-like element IS8 in RP4 plasmid and its involvement in cointegration. *Gene* **10**, 329–338.
- DRESSLER, D. & POTTER, H. (1982). Molecular mechanisms in genetic recombination. *Annual Review of Biochemistry* **51**, 727–761.
- EISENSTARK, A. (1977). Genetic recombination in bacteria. *Annual Review of Genetics* **11**, 369–396.
- FALKOW, S., WOHLHIETER, J. A., CITARELLA, R. V. & BARON, L. S. (1964). Transfer of episomic elements to *Proteus*. II. Nature of *lac*⁺ *Proteus* strains isolated from clinical specimens. *Journal of Bacteriology* **88**, 1598–1601.
- GRINTER, N. J. (1981). Analysis of chromosome mobilization using hybrids between plasmid RP4 and a fragment of bacteriophage λ carrying IS1. *Plasmid* **5**, 267–276.
- HEDGES, R. W. (1975). R factors from *Proteus mirabilis* and *P. vulgaris*. *Journal of General Microbiology* **87**, 301–311.
- HOLLOWAY, B. W. & RICHMOND, M. H. (1973). R factors used for genetic studies in strains of *Pseudomonas aeruginosa* and their origin. *Genetical Research* **21**, 103–105.
- HU, S., OHTSUBO, E., DAVIDSON, N. & SAEDLER, H. (1975). Electron microscope heteroduplex studies of sequence relations among bacterial plasmids: identification and mapping of the insertion sequences IS1 and IS2 in F and R plasmids. *Journal of Bacteriology* **122**, 746–755.
- JOHNSON, S. R. & ROMIG, W. R. (1979). Transposon-facilitated recombination in *Vibrio cholerae*. *Molecular and General Genetics* **170**, 93–101.
- JULIOT, J. S. & BOISTARD, P. (1979). Use of RP4-prime plasmids constructed *in vitro* to promote a polarized transfer of the chromosome in *Escherichia coli* and *Rhizobium meliloti*. *Molecular and General Genetics* **173**, 289–298.
- KLECKNER, N. (1981). Transposable elements in prokaryotes. *Annual Review of Genetics* **15**, 341–404.
- KOPECKO, D. J. (1980). Specialized genetic recombination systems in bacteria: their involvement in gene expression and evolution. *Progress in Molecular and Subcellular Biology* **7**, 135–234.
- KOPECKO, D. J. & COHEN, S. N. (1975). Site-specific *recA*-independent recombination between bacterial plasmids: involvement of palindromes at the recombinational loci. *Proceedings of the National Academy of Sciences of the United States of America* **72**, 1373–1377.
- MCDONELL, M. W., SIMON, M. N. & STUDIER, F. W. (1977). Analysis of restriction fragments of T7 DNA and determination of molecular weights by electrophoresis in neutral and alkaline gels. *Journal of Molecular Biology* **110**, 119–146.
- MEYNELL, E. & DATTA, N. (1967). Mutant drug resistance factors of high transmissibility. *Nature, London* **214**, 885–887.
- NEVERS, R. & SAEDLER, H. (1978). Mapping and characterization of an *E. coli* mutant defective in

- IS1-mediated deletion formation. *Molecular and General Genetics* **160**, 209–214.
- PEARCE, L. E. & MBEYNELL, E. (1968). Specific chromosomal affinity of a resistance factor. *Journal of General Microbiology* **50**, 159–172.
- PERLMAN, D. & ROWND, R. H. (1975). Transition of R factor NR1 in *Proteus mirabilis*: molecular structure and replication of NR1 deoxyribonucleic acid. *Journal of Bacteriology* **123**, 1013–1034.
- PERLMAN, D., TWISE, T. M., HOLLAND, M. J. & ROWND, R. H. (1975). Denaturation mapping of R factor deoxyribonucleic acid. *Journal of Bacteriology* **123**, 1035–1042.
- PHILIPPSEN, P., KRAMER, R. A. & DAVIS, R. W. (1978). Cloning of the yeast ribosomal DNA repeat unit in *Sst*I and *Hind*III lambda vectors using genetic and physical size selections. *Journal of Molecular Biology* **123**, 371–386.
- PITTARD, A. J. & ADELBURG, E. A. (1963). Gene transfer by F' strains of *Escherichia coli* K12. II. Interaction between F-merogenote and chromosome during transfer. *Journal of Bacteriology* **85**, 1402–1408.
- PRAKASH, R. K., SCHILPEROORT, R. A. & NUTI, M. P. (1981). Large plasmids of fast-growing rhizobia: homology studies and location of structural nitrogen fixation (*nif*) genes. *Journal of Bacteriology* **145**, 1129–1136.
- PTASHNE, K. & COHEN, S. N. (1975). Occurrence of insertion sequence (IS) regions on plasmid deoxyribonucleic acid as direct and inverted nucleotide sequence duplications. *Journal of Bacteriology* **122**, 776–781.
- REIF, H.-J. & SADLER, H. (1977). Chromosomal rearrangements in the *gal* region of *E. coli* K12 after integration of IS1. In *DNA Insertion Elements, Plasmids and Episomes*, pp. 81–91. Edited by A. I. Bukhari, J. A. Shapiro & S. L. Adhya. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory.
- ROWND, R. & MICKEL, S. (1971). Dissociation and reassociation of RTF and r-determinants of R factor NR1 in *Proteus mirabilis*. *Nature New Biology* **234**, 40–43.
- RUVKUN, G. B. & AUSUBEL, F. M. (1981). A general method for site-directed mutagenesis in procaryotes. *Nature, London* **289**, 85–88.
- SAEDLER, H. (1977). IS1 and IS2 in *E. coli*: Implications for the evolution of the chromosome and some plasmids. In *DNA Insertion Elements, Plasmids and Episomes*, pp. 65–72. Edited by A. I. Bukhari, J. A. Shapiro & S. L. Adhya. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory.
- SILVER, R. P. & FALKOW, S. (1970). Studies on resistance transfer factor deoxyribonucleic acid in *Escherichia coli*. *Journal of Bacteriology* **104**, 340–344.
- STAHL, F. W. (1979). Special sites in generalized recombination. *Annual Review of Genetics* **13**, 7–24.
- STARLINGER, P. (1977). DNA rearrangements in procaryotes. *Annual Review in Genetics* **11**, 103–126.
- STARLINGER, P. (1978). *In vivo* formation of recombinant DNA molecules by IS-elements and transposons. In *Genetic Engineering*, pp. 123–143. Edited by A. M. Chakrabarty. West Palm Beach, Florida: C.R.C. Press.
- STARLINGER, P. (1980). IS-elements and transposons. *Plasmid* **3**, 241–259.
- STARLINGER, P. & SAEDLER, H. (1976). IS-elements in microorganisms. *Current Topics in Microbiology and Immunology* **75**, 111–152.
- VAN RENSBURG, A. J. (1970). *Studies on Proteus and Providence spheroplasts, I-forms and bacteriophage nucleic acids*. M.D. thesis, University of Pretoria, Republic of South Africa.
- WOHLHIETER, J. A., FALKOW, S., CITARELLA, R. V. & BARON, L. S. (1964). Characterization of DNA from a *Proteus* strain harboring an episome. *Journal of Molecular Biology* **9**, 576–588.