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# SHORT PAPERS

## The orientation of transfer of the plasmid RP4

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The probably identical broad host range plasmids RP4, RP1 and RK2 (Datta *et al.* 1971; Grinsted *et al.* 1972; Olsen & Shipley, 1973; Meyer *et al.* 1975; Beringer, 1974; Towner & Vivian, 1976) have been extensively studied as vectors for *in vitro* recombination and mediators of conjugative transfer of DNA between species (Olsen & Gonzalez, 1974; Jacob *et al.* 1976; Dixon *et al.* 1976; Stepanov *et al.* 1976; Meyer *et al.* 1977; Nagahari *et al.* 1977). Studies of the conjugation system have led to the identification of transfer (*tra*) genes and have mapped the drug resistance determinants (Barth & Grinter, 1977; Grinsted *et al.* 1977; Thomas *et al.* 1979). We present here a deletion analysis which shows in which direction the plasmid is transferred during conjugation.

A short (5·7 kb) λ fragment (Szybalski & Szybalski, 1979; Daniels *et al.* 1980) containing *att* and *int* was inserted into the single *Eco*RI site of RP4 (Jacob & Grinter, 1975; Pastrana, 1976; Pastrana & Brammar, 1979). The recombinant plasmid can integrate into $att_\lambda$ on the *Escherichia coli* chromosome to form a stable Hfr strain (Watson & Scaife, 1978). It transfers the chromosome in the orientation: O-*lac-leu-thr*...*trp*. We have recently reported (Watson & Scaife, 1980) that plasmid excision specifically depends on the *xis* function of λ, confirming that Hfr formation is mediated by *int*-directed, site-specific recombination and, by implication, uses *att* in the same orientation as the parent phage. Chromosome transfer by the Hfr thus allows us to establish the orientation of the λ*att* fragment relative to the chromosome. Here, we present deletion studies with an RP4 λ*att* derivative, pZD100 (Al-Doori and Scaife, in preparation) which establish the orientation of the phage fragment in the original plasmid.

The plasmid pZD100 carries λ*drif*$^{D}$18 inserted at *att* (Fig. 1*b*). This phage makes a dominant rifampicin-resistant (*rif*$^{D}$) RNA polymerase β subunit (Kirschbaum & Konrad, 1973) and a temperature-sensitive phage repressor (*c*I857) (Sussman & Jacob, 1962). Electron microscope studies on pZD100 DNA (date not shown) indicate that it occurs in several non-multimeric sizes. For this reason we have preferred to analyse deletion mutants of pZD100 which exist in a single form and were made as follows.

Bacteria carrying pZD100 cannot form colonies on rifampicin medium at 42 °C since the phage is induced and is either excised from the plasmid or interferes with its replication. However, rare mutants do grow on this medium. They have deletions extending through most of the prophage into the plasmid DNA (Fig. 1). Two such deletion plasmids, pZD23 and pZD44 have been analysed in detail (Plate 1*a* and *b*).

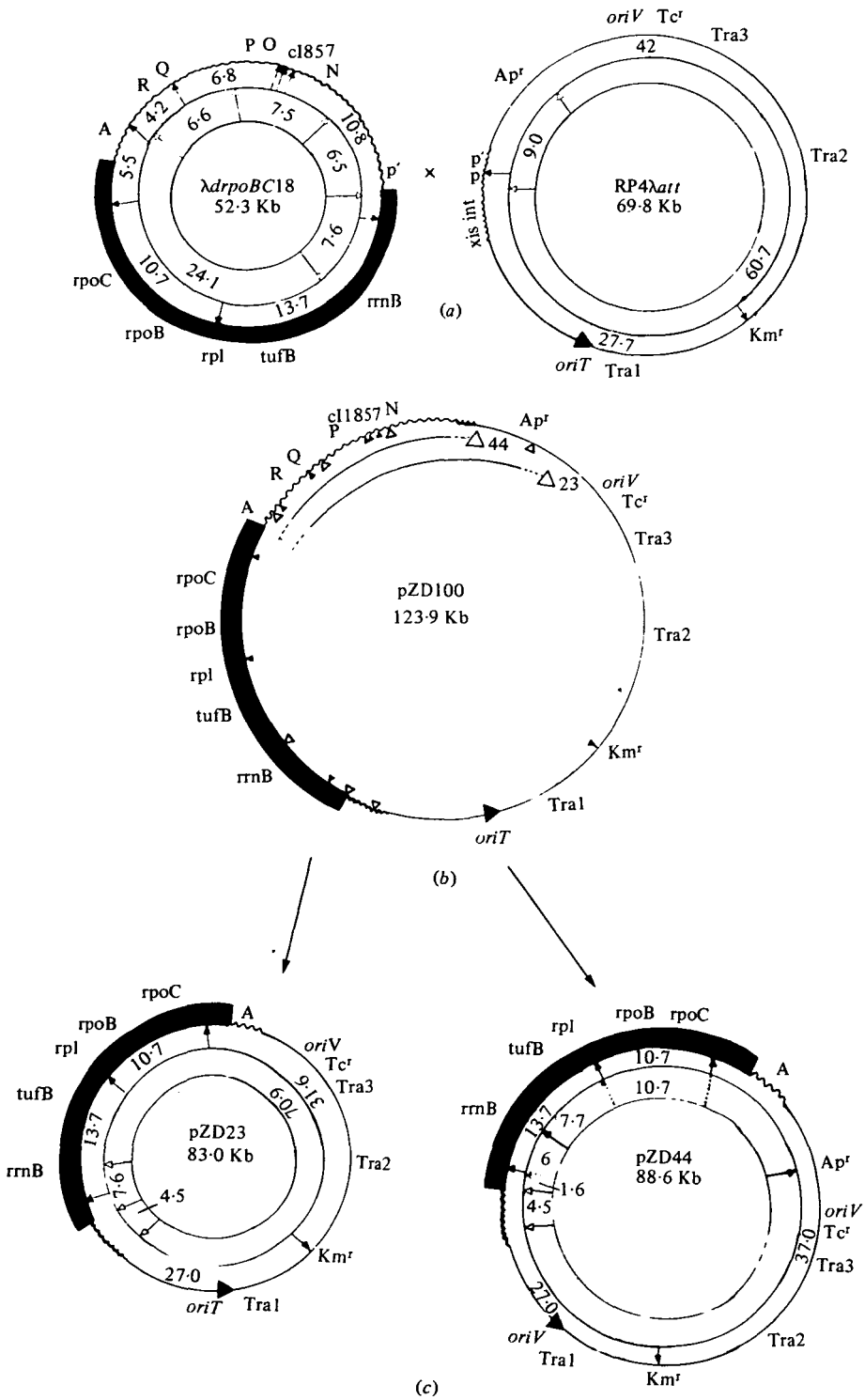


Fig. 1. For legend see opposite.

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## 2. THE STRUCTURES OF pZD23 AND pZD44

Genetic tests show that both of these plasmids retain determinants for resistance to rifampicin (100 $\mu$g/ml), tetracycline (10 $\mu$g/ml) and kanamycin (25 $\mu$g/ml). The plasmids differ in that pZD23 has lost and pZD44 retains $Ap^r$. Both plasmids have lost $\lambda$ immunity (tested according to Miller, 1972) but still produce pili since they are still sensitive to PRR1 phage (Olsen & Shipley, 1973; Olsen & Thomas, 1973). However, only pZD23 is $Tra^+$, a point which will be considered elsewhere (Al-Doori and Scaife, in preparation). Restriction patterns of the two, independently isolated plasmids can be simply interpreted as the result of single deletions arising in the parent plasmid.

Some of the DNA in pZD44 comes from $\lambda drif^D18$. It appears (Plate 1*b*) as the 10·7 and 13·7 kb *Hin*dIII fragments, the 7·6 kb *Bam*HI fragment and the 10·7, 7·7, 5·2 and 1·6 kb fragments from *Hin*dIII/*Bam*HI double digests. About half of the $\lambda drif^D18$ DNA present in pZD100 (data not shown) is absent from pZD23 and pZD44 (Plate 1*a*). The 10·8, 6·8, 5·5 and 4·2 kb *Hin*dIII fragments are missing from both plasmids. In confirmation of our genetic results, the restriction analysis shows that the *Bam*HI site located in the $Ap^r$ gene is absent from pZD23 but present in pZD44 (Plate 1*a*, track 2).

The restriction results can be simply combined to give two mutually consistent plasmid maps (Fig. 1*c*), which can be derived by single deletions from a common parent plasmid, pZD100 (Fig. 1*b*). The structure inferred for pZD100 is precisely that predicted for the product of insertion of $\lambda drif^D18$ into RP4 $\lambda att$ (Fig. 1*a*). It also shows us that the phage fragment is inserted in the orientation... $Km^R$(*xis int att P′OP*) $Ap^r$...

It will be recalled that the phage fragment in the RP4 $\lambda att$ Hfr strain is transferred in the order – *xis int att P′* – (Watson & Scaife, 1978). This fact, together with the finding reported here leads us to conclude that RP4 transfers its markers anticlockwise (Fig. 1*a*). The origin of transfer, *oriT*, has recently been located on the RK2 map (Thomas *et al.* 1979). Assuming structural identity of these plasmids we conclude that during mating the plasmid transfer genes enter the recipient last. This property is also shown by the sex factor, F, of *E. coli* (Willetts, 1972; Guyer & Clark, 1977).

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Fig. 1. The lysogenisation of RP4 *att* with $\lambda drif^D18$. (*a*) Postulated mechanism confirmed by our results. (*b*) The inferred structure of the temperature-sensitive plasmids pZD100. Δ23 and Δ44 represent the two deletions in pZD23 and pZD44 respectively. (*c*) The two temperature-resistant plasmids pZD23 and pZD44, showing the *Hin*dIII (▲, outer circle) and *Bam*HI (△, inner circle) sites established in this study. The thick, thin and wavy lines represent *E. coli*, RP4 and $\lambda$ DNA respectively. *oriT* is sited according to its position in RK2 (Thomas *et al.* 1979).

## REFERENCES


BARTH, P. T. & GRINTER, N. J. (1977). Map of plasmid RP4 derived by insertion of transposon C. *Journal of Molecular Biology* **113**, 455–474.

BERINGER, J. E. (1974). R factor transfer in *Rhizobium leguminosarum*. *Journal of General Microbiology* **84**, 188–198.

DANIELS, D. L., DE WET, J. R. & BLATTNER, F. R. (1980). New map of bacteriophage lambda DNA. *Journal of Virology* **33**, 390–400.

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.

DIXON, R., CANNON, F. & KONDOROSI, A. (1976). Construction of a P plasmid carrying nitrogen fixation genes from *Klebsiella pneumoniae*. *Nature* **260**, 268–271.

GRINSTED, J., SAUNDERS, J. R., INGRAM, L. C., SYKES, R. B. & RICHMOND, M. H. (1972). Properties of an R factor which originated in *Pseudomonas aeruginosa* 1822. *Journal of Bacteriology* **110**, 529–537.

GRINSTED, J., BENNETT, P. M. & RICHMOND, M. H. (1977). A restriction enzyme map of R-plasmid RP1. *Plasmid* **1**, 34–37.

GUYER, M. S. & CLARK, A. J. (1977). Early and late transfer of F genes by Hfr donors of *E. coli* K12. *Molecular and General Genetics* **157**, 215–222.

JACOB, A. E. & GRINTER, N. J. (1975). Plasmid RP4 as a vector replicon in genetic engineering. *Nature* **255**, 504–506.

JACOB, A. E., CRESSWELL, J. M., HEDGES, R. W., COETZEE, J. N. & BERINGER, J. E. (1976). Properties of plasmids constructed by the *in vitro* insertion of DNA from *Rhizobium leguminosarium* or *Proteus mirabilis* into RP4. *Molecular and General Genetics* **147**, 315–323.

KIRSCHBAUM, J. B. & KONRAD, E. B. (1973). Isolation of a specialized lambda transducing bacteriophage carrying the $\beta$ subunit gene for *E. coli* ribonucleic acid polymerase. *Journal of Bacteriology* **116**, 517–526.

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.

MEYER, R., FIGURSKI, D. & HELINSKI, D. R. (1975). Molecular vehicle properties of the broad host range plasmid RK2. *Science* **190**, 1226–1229.

MILLER, J. H. (1972). *Experiments in Molecular Genetics*, p. 466. Cold Spring Harbor Laboratory, New York.

MEYER, R. J., FIGURSKI, D. & HELINSKI, D. R. (1977). Properties of the plasmid RK2 as cloning vehicle. In *DNA Insertion Elements, Plasmids and Episomes* (ed. A. I. Bukhari, J. A. Shapiro and S. L. Adhya), p. 559. Cold Spring Harbor Laboratory, New York.

NAGHARI, K., SANO, Y. & SAKAGUCHI, K. (1977). Derepression of *E. coli trp* operon on interfamilial transfer. *Nature* **226**, 745–746.

OLSEN, R. H. & SHIPLEY, P. (1973). Host range and properties of the *Pseudomonas aeruginosa* R factor R1822. *Journal of Bacteriology* **113**, 772–798.

OLSEN, R. H. & THOMAS, D. D. (1973). Characteristics and purification of PRR1, an RNA phage specific for the broad host range Pseudomonas R1822 drug resistance plasmid. *Journal of Virology* **12**, 1560–1567.

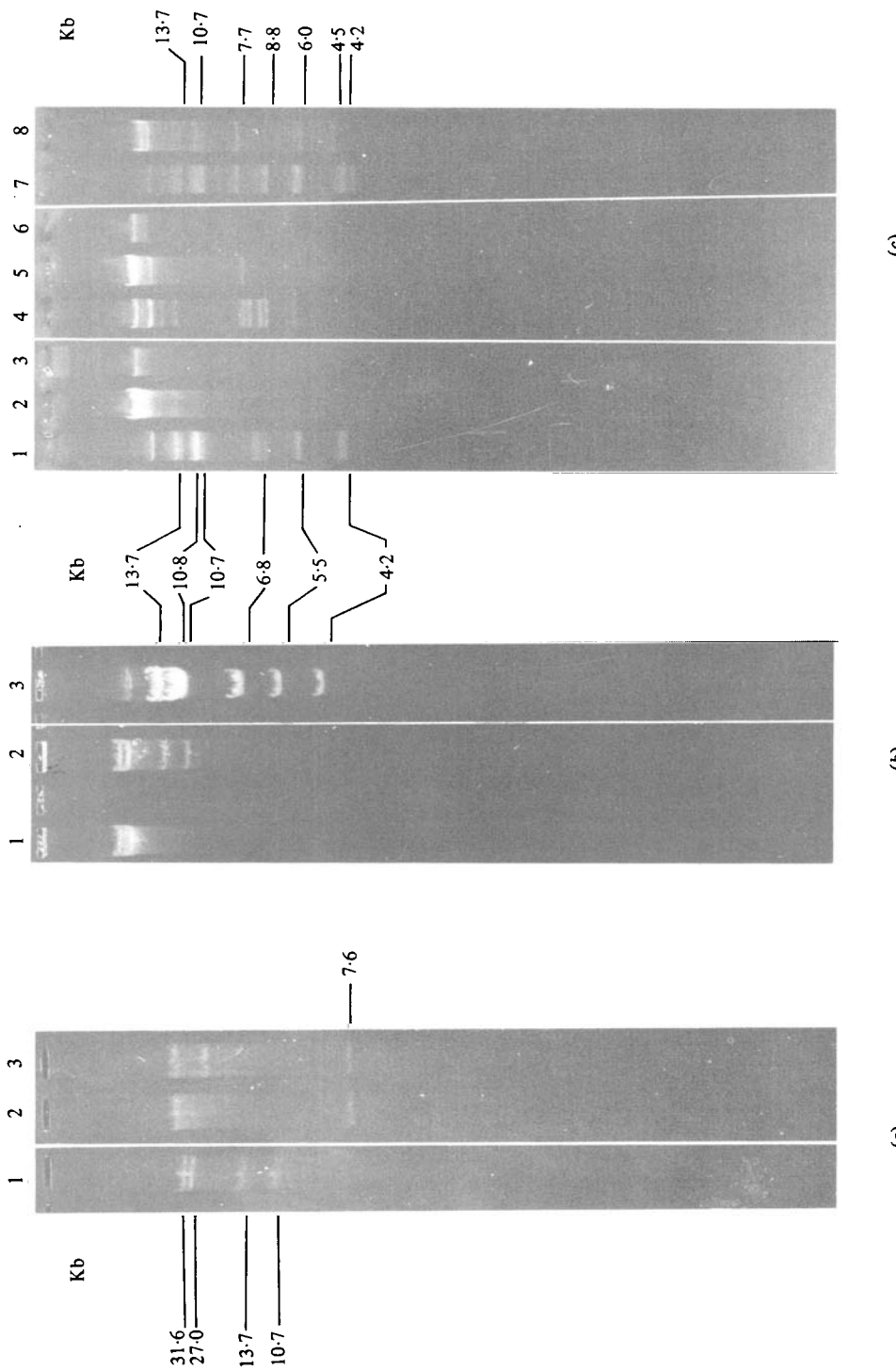
OLSEN, R. H. & GONZALEZ, C. (1974). *E. coli* gene transfer to unrelated bacteria by a histidine operon-RP1 drug resistance plasmid complex. *Biochemical and Biophysical Research Communications* **59**, 337–385.

PASTRANA, R. (1976). Lysogeny of lambdoid phages studies with genetic fusions made *in vitro*. Ph.D. Thesis, University of Edinburgh.

PASTRANA, R. & BRAMMAR, W. J. (1979). *In vitro* insertion of the $\lambda$ attachment site into the plasmid RP4. *Molecular and General Genetics* **177**, 162–168.

STEPANOV, A. I., ZIMINA, M. S. KHLEBALINA, O. I., RABINOVICH, P. M., BEBUROV, M. YU. & DEBABOV, V. G. (1976). The transmissible hybrid plasmid RP4 ColE1. *Genetika* **12**, 162–164.

SUSSMAN, R. & JACOB, F. (1962). Sur un Système de répression thermosensible chez le

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bacteriophage λ d'*Escherichia coli*. *Comptes rendus hebdomadaires des séances de l'Académie des sciences* (*Paris*) **254**, 1517–1519.

SZYBALSKI, E. & SZYBALSKI, W. (1979). A comprehensive molecular map of bacteriophage lambda. *Gene* **7**, 217–270.

TAYLOR, W. E. & BURGESS, R. R. (1979). *Escherichia coli* RNA polymerase binding and initiation of transcription on fragments of λ*rif*$^{d}$18 DNA containing promoters for λ genes and for *rrnB*, *tufB*, *rplK*, *A*, *rplJ*, *L* and *rpoB*, *C* genes. *Gene* **6**, 331–365.

THOMAS, C. M., STALKER, D., GUINEY, D. & HELINSKI, D. R. (1979). Essential regions of the replication and conjugal transfer of the broad host range plasmid RK2. In *Plasmids of Medical, Environmental and Commercial Importance* (ed. K. N. Timmis and A. Pühler), pp. 375–385. Elsevier: North-Holland Biomedical press.

TOWNER, K. J. & VIVIAN, A. (1976). RP4-mediated conjugation in *Acinetobacter calcoaceticus*. *Journal of General Microbiology* **93**, 355–360.

WATSON, M. D. & SCAIFE, J. G. (1978). Chromosomal transfer promoted by the promiscuous plasmid RP4. *Plasmid* **1**, 226–237.

WATSON, M. D. & SCAIFE, J. G. (1980). Integrative compatibility: stable coexistence of chromosomally integrated and autonomous derivatives of plasmid RP4. *Journal of Bacteriology* **142**, 462–466.

WILLETTS, N. S. (1972). Location of the origin of transfer of the sex factor, F. *Journal of Bacteriology* **112**, 773–778.

PLATE 1

Restriction analysis of pZD23 and pZD44. Plasmid digests were electrophoresed in tris-acetate buffer on 0·7 % agarose gels containing ethidium bromide (1·5 μg/ml) (McDonell *et al.* 1977). Unlabelled fragments of λd*rif*$^{D}$18 are from the contaminating DNA of helper phage λ. (*a*) pZD23 DNA digestion with (1) *Hin*dIII and (2) *Bam*HI. Track 3 shows pZD44 digested with *Bam*HI. (*b*) A *Hin*dIII digest of pZD44 (2) compared with *Hin*dIII digests of RP4 λ*att* (1) and λd*rif*$^{D}$18 (3) (*c*) *Hin*dIII, *Bam*HI double digest of pZD44. Controls – *Hin*dIII: (1) λd*rif*$^{D}$18, (2) pZD44. *Bam*HI: (3) RP4 λ*att* (4) λd*rif*$^{D}$18. Double digest: (6) RP4 λ*att*, (7) λd*rif*$^{D}$18, (8) pZD44.