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 EcoRI site of RP4 (Jacob & Grinter, 1975; Pastrana, 1976; Pastrana & Brammar, 1979). The recombinant plasmid can integrate into att 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 Adri/D18 inserted at att (Fig. 1b). This phage makes a dominant rifampicin-resistant (rifD) RNA polymerase β subunit (Kirschbaum & Konrad, 1973) and a temperature-sensitive phage repressor (cI857) (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 1a and b).
Fig. 1. For legend see opposite.
2. THE STRUCTURES OF $pZD_{23}$ AND $pZD_{44}$

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 $pZD_{23}$ has lost and $pZD_{44}$ 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 $pZD_{23}$ 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 $pZD_{44}$ comes from $Adr/D18$. It appears (Plate 16) as the 10-7 and 13-7 kb HindIII fragments, the 7-6 kb BamHI fragment and the 10-7, 7-7, 5-2 and 1-6 kb fragments from HindIII/BamHI double digests. About half of the $Adr/D18$ DNA present in $pZD_{100}$ (data not shown) is absent from $pZD_{23}$ and $pZD_{44}$ (Plate 1a). The 10-8, 6-8, 5-5 and 4-2 kb HindIII fragments are missing from both plasmids. In confirmation of our genetic results, the restriction analysis shows that the BamHI site located in the Ap r gene is absent from $pZD_{23}$ but present in $pZD_{44}$ (Plate 1a, track 2).

The restriction results can be simply combined to give two mutually consistent plasmid maps (Fig. 1c), which can be derived by single deletions from a common parent plasmid, $pZD_{100}$ (Fig. 1b). The structure inferred for $pZD_{100}$ is precisely that predicted for the product of insertion of $Adr/D18$ into RP4 $att$ (Fig. 1a). It also shows us that the phase fragment is inserted in the orientation ...KmR(\text{xis int att P'OP}) Ap r...

It will be recalled that the phase fragment in the RP4 $att$ Hfr strain is transferred in the order – $\text{xis int att P'}$ – (Watson & Scaife, 1978). This fact, together with the finding reported here leads us to conclude that RP4 transfers its markers anticlockwise (Fig. 1a). 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 $Adr/D18$. (a) Postulated mechanism confirmed by our results. (b) The inferred structure of the temperature-sensitive plasmids $pZD_{100}$. $\Delta 23$ and $\Delta 44$ represent the two deletions in $pZD_{23}$ and $pZD_{44}$ respectively. (c) The two temperature-resistant plasmids $pZD_{23}$ and $pZD_{44}$, showing the HindIII (\text{o}, outer circle) and BamHI (\text{a}, 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).
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**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 \( \mu g/ml \)) (McDonell et al. 1977). Unlabelled fragments of \( \lambda \)rifD18 are from the contaminating DNA of helper phage \( \lambda \). (a) pZD23 DNA digestion with (1) HindIII and (2) BamHI. Track 3 shows pZD44 digested with BamHI. (b) A HindIII digest of pZD44 (2) compared with HindIII digests of RP4 \( \lambda \)att (1) and \( \lambda \)rifD18 (3) (c) HindIII, BamHI double digest of pZD44. Controls – HindIII: (1) \( \lambda \)rifD18, (2) pZD44. BamHI: (3) RP4 \( \lambda \)att (4) \( \lambda \)rifD18. Double digest: (6) RP4 \( \lambda \)att, (7) \( \lambda \)rifD18, (8) pZD44.