A NON-TRANSMISSIBLE VARIANT OF RP4 SUITABLE AS CLONING VEHICLE FOR GENETIC ENGINEERING

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1. Introduction

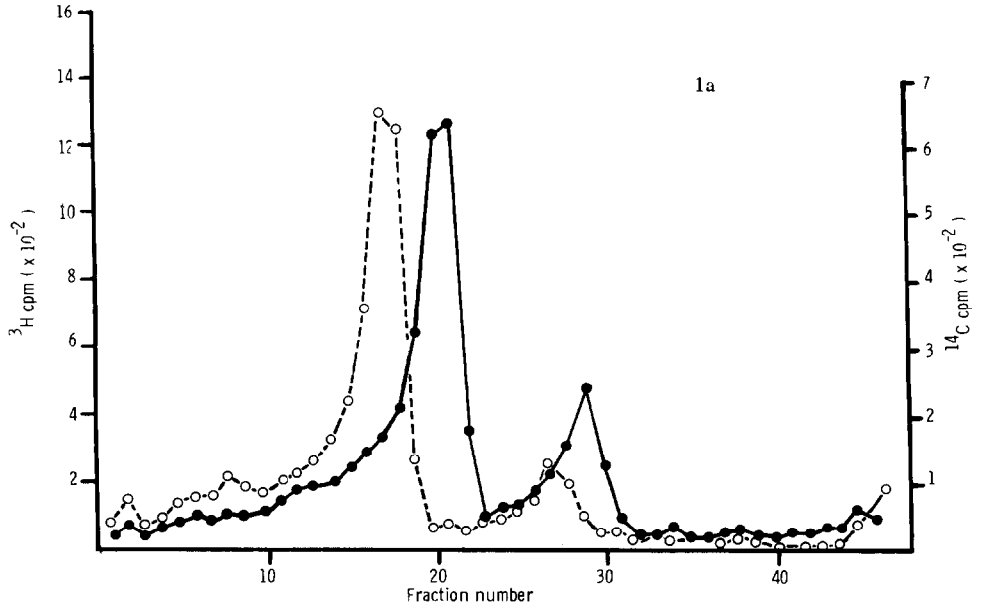
RP4, a plasmid of compatibility group P, determines resistance to ampicillin, tetracycline and kana mycin [1]. It has a molecular weight of 36×10^6 (P. T. Barth, personal communication) and a single site susceptible to cutting by the *Eco*RI restriction nuclease [2].

We have been able to insert segments of bacterial chromosomal DNA into RP4 producing plasmids capable of replication and conjugal transmission (AEJ, JMC and RWH, unpublished). The uniquely wide host range of P group plasmids implies that the incorporated genetic material could be tested in a great variety of hosts.

It has, however, been proposed that only non-self-transmissible plasmids should be used as cloning vehicles. Non-self-transmissible mutants of RP4 and other P plasmids have been isolated by their resistance to the pilus phage PRRI [3,5], but most of these are revertible point mutants.

2. Experimental and results

Following transfection, we have isolated a spontaneous deletion mutant of plasmid RP4 (in *E. coli* C



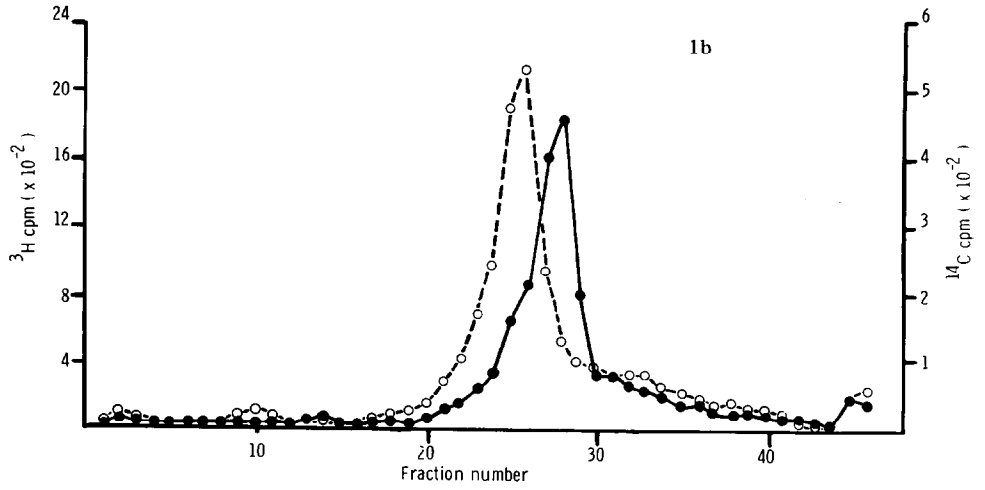


Fig.1. Neutral sucrose gradient analysis of RP4-δ1 DNA. The covalently closed circular DNA form of ^{3}H-labelled RP4-δ1 and ^{14}C-labelled RP4 plasmids was isolated using caesium chloride–ethidium bromide density gradient centrifugation [7,11]. DNA samples were mixed and analysed by sedimentation through a 5 to 20% sucrose gradient at 100 000 *g* and 20°C [11]. Sedimentation was from right to left. Fractions (0.1 ml) were collected directly onto glass fibre disks and, after drying, washing and redrying, were assayed for radioactivity: (•) ^{3}H; (○) ^{14}C. (a) ^{3}H-labelled RP4-δ1 DNA plus ^{14}C-labelled RP4 DNA, 90 min sedimentation. (b) 60 μl plasmid mixture (in 100 mM Tris–HCl, 50 mM NaCl, 5 mM $MgCl_2$, pH 7.5) was incubated with 3 μl *Eco*RI endonuclease for 30 min at 37°C, before sedimentation for 120 min.

host) which has lost kanamycin resistance and transferability, but retained the site susceptible to *Eco*RI endonuclease. We suggest that this mutant could be used as a cloning vehicle.

We have designated the deletion mutant RP4-δ1. By comparing the rate of sedimentation of ^{3}H-labelled RP4-δ1 DNA with ^{14}C-labelled RP4 DNA through a 5–20% neutral sucrose gradient (fig.1a) we have calculated the mol. wt. of RP4-δ1 as 28×10^6. The DNA bands with peaks at fractions 17 and 21 are the covalently closed circular DNA tertiary forms of RP4 and RP4-δ1 respectively, and those with peaks at fractions 27 and 29 are the open circular DNA forms. By comparing the rates of sedimentation of *Eco*RI digested RP4 and RP4-δ1 DNAs we have shown that RP4-δ1 has retained the *Eco*RI susceptible site (fig.1b).

RP4-δ1 is stably inherited and retains the P group compatibility of RP4 (i.e. is eliminable by R751 [6]). Thus, the replication functions of the plasmid are not altered by the deletion.

No conjugal transfer of RP4-δ1 was observed and it was shown that the efficiency of transfer was reduced (relative to that of RP4) by a factor of more than 10^5. Since the mutation leading to loss of transferability was a large deletion (of sufficient DNA for about 20 genes) we suggest that the loss of transferability is likely to be complete and irreversible.

RP4-δ1 differs from RP4 in being unable to exclude entry of other P group plasmids. The presence of RP4 in the recipient strain reduced the efficiency of transfer of R751 a hundred fold [7] but an RP4-δ1$^+$ culture is almost as efficient a recipient as the homologous R$^-$ strain. When RP4-8 (a mutant of RP4 lacking tetracycline resistance [7] was transferred to an RP4-δ1$^+$ clone, the resident plasmid was usually eliminated, but a minority of clones retained all resistances (ampicillin, tetracycline and kanamycin). They were found to contain self transmissible plasmids indistinguishable from wild type RP4.

Whereas RP4 confers sensitivity to phages PRR1 and PR4 [3,8,9], RP4-δ1 does not. The receptor for PRR1 is the RP4-determined pilus, but that for PR4 is unknown.

3. Discussion

RP4-δ1 is defective in a variety of transfer associated properties and appears to conform with the

characteristics for a cloning vehicle suggested by the Asilomar Conference [4]. Genetic material from any source could be incorporated into this plasmid and recombinant inserted into an appropriate host by transformation [10]. In that host it may be tested for pathogenic or other undesirable properties.

If no unacceptable characteristics are detected, the recombinant plasmid may be permitted to recombine with a transmissible P group plasmid. If RP4-8 is transferred into a strain carrying a recombinant plasmid derived from RP4-δ1 and selection imposed for resistance to both tetracycline and kanamycin recombination will be forced leading to the formation of plasmids carrying the kanamycin resistance gene (and hence the transfer genes) of RP4-8 and the tetracycline resistance gene of RP4-δ1. A proportion will carry the inserted DNA sequence which can then be transferred into all the species capable of permitting replication of a P plasmid.

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