SHORT COMMUNICATION

Recombinant Plasmids Formed *in vivo* Carrying and Expressing Two Incompatibility Regions

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The formation *in vivo* of recombinants between a plasmid of incompatibility group N (R1010-10) and plasmids of groups P (R751) and W (R388) is described. From examination of the molecular weights of these recombinant plasmids, they appear to be cointegrates. These cointegrates have the incompatibility properties of both 'parent' plasmids.

Classification of bacterial plasmids by their incompatibility interactions has been proposed (Datta & Hedges, 1971; Chabbert *et al.*, 1972; Grindley *et al.*, 1972) and numerous studies have confirmed the utility of this system (Jacob *et al.*, 1977). An unambiguous classification can be produced only if each plasmid manifests the properties of a single incompatibility group and the large majority of plasmids found in nature do behave in this way. Although certain anomalies have been reported (see, for example, Smith *et al.*, 1973; Grant & Pittard, 1974; Monti-Bragadin & Samer, 1975), it is almost always possible to assign a plasmid to one incompatibility group. Plasmids have been constructed by fusion *in vitro* of DNA molecules from unrelated plasmids and these retain incompatibility properties of both parental plasmids (Cabello *et al.*, 1976). In these cases, at least one of the parental plasmids was of a type whose incompatibility properties were well-characterized. Here we describe the construction *in vivo* and incompatibility properties of recombinants between a plasmid of group N and plasmids of groups P and W. There is no incompatibility or significant DNA homology between plasmids of these three groups (Falkow *et al.*, 1974; Jacob *et al.*, 1977).

R1010 is a plasmid of group N (Matthew *et al.*, 1979). R1010-6 is a spontaneous segregant which has lost transmissibility and all resistance determinants except the ability to produce β -lactamase (Ap^{R}). R1010-9 is a recombinant between R1010-6 and R702, a plasmid of group P, in which approximately 3 megadaltons (Mdal) of DNA of the latter carrying resistance to streptomycin (Sm^{R}) and sulphonamides (Su^{R}) has been inserted into R1010-6. R1010-10 is a spontaneous deletion derived from R1010-9 which has lost approximately 4 Mdal of DNA and the resistances derived from R702. R1010-6, R1010-9 and R1010-10 are all members of group N, incompatible with N3T (Hedges, 1972) and completely compatible with plasmids of group P (Table 1).

In the present work, the plasmid R1010-10 was chosen for study. When *Escherichia coli* K12 strain J62 (*pro his trp lac*) carrying R1010-10 and the P group plasmid R751 (conferring resistance to trimethoprim, Tp^{R} ; Jobanputra & Datta, 1974) was mated with *E. coli* J53 (*pro met*), Tp^{R} was transferred with an efficiency of 10^{-3} to 10^{-4} per donor per hour and Ap^{R} at about 10^{-6} . All Ap^{R} transciipients (30/30) were also Tp^{R} . The plasmids present in the $\text{Ap}^{\text{R}}\text{Tp}^{\text{R}}$ transciipients were examined using a single colony lysis technique with subsequent electrophoresis of plasmid species into an agarose gel (Eckhardt, 1978). These transciipients were shown to contain a single plasmid of 70 Mdal; this plasmid was designated

Table 1. *Plasmid characters*

Plasmid	Resistance*	Trans- missibility	Com- patibility	$10^{-6} \times$ Mol. wt†
R1010	ApSmCmKmSu	+	N	53
R1010-6	Ap	—	N	37
R1010-9	ApSmSu	—	N	40
R1010-10	Ap	—	N	36
N3T	Tc	+	N	34
Sa	CmGmKmSmSuTp	+	W	25
R702	KmSmSuTc	+	P	46
R751	Tp	+	P	30
R751-1010	TpAp	+	P and N	70
R388	SuTp	+	W	21
R388-1010	TpAp	+	W and N	60
R388-1010 sul ^R ‡	SuTp	+	W	21
R388-1010-N3T	TpApTc	+	NT	80
R388-1010-Sa	TpApCmKmSmSu	+	NT	80
R751-1010-702	TpApKmSmSuTc	+	NT	90
R751-1010-N3T	TpApTc	+	NT	95

NT, Not tested.

* Resistances: Ap, ampicillin; Sm, streptomycin; Cm, chloramphenicol; Gm, gentamicin; Km, kanamycin; Su, sulphonamides; Tp, trimethoprim; Tc, tetracycline.

† Molecular weights were determined by lysis of single colonies on the top of agarose gels, followed by electrophoresis of the released plasmids into the gel. Agarose gels were calibrated using plasmids of known molecular weight. The procedure is described by Datta *et al.* (1979).

‡ A variant of R388-1010 which regained sulphonamide resistance and lost ampicillin resistance.

R751-1010 (Table 1). The molecular weight of R751-1010 was that expected if R1010-10 had fused with R751 without any loss of genetic material (cointegrate formation).

When the W group plasmid R388 (Datta & Hedges, 1972; Gorai *et al.*, 1979) was used to mobilize R1010-10, a rather different result was obtained. R388 was transferred to J53-2 with an efficiency of 10^{-3} to 10^{-4} but the rate of transfer of Ap^R was less than 10^{-9} . In fact only one Ap^R transcient was observed in three experiments. This Tp^RAp^RSu^S transcient was shown to contain a single plasmid of 60 Mdal, which was designated R388-1010 (Table 1). As with R751-1010, the molecular weight of this plasmid was that expected if R1010-10 had formed a cointegrate plasmid with R388. The loss of Su^R in this cointegrate suggested that the Su^R gene had been broken by insertion of the DNA molecule carrying the Ap^R gene. This conclusion was confirmed when it was shown that the clone could regain sulphonamide resistance and that this reversion was always (10/10) accompanied by a loss of ampicillin resistance and, in the one case studied, by a deletion such that the Su^RTp^RAp^R plasmid had precisely regained the molecular weight of R388 (Table 1).

When the fusion plasmids R751-1010 or R388-1010 were transferred between *E. coli* strains, Tp^R and Ap^R were cotransferred in more than 95% of cases. Among the occasional breakdown products observed were plasmids which in molecular weight, resistance determinants and compatibility properties were indistinguishable from the parental plasmids. Thus, we believe that in each case both parental plasmids are incorporated complete into the fusion plasmid.

The incompatibility properties of the fusion plasmids are shown in Table 2. In almost every case, entry of R751-1010 led to loss of N or P plasmids from a recipient clone. Thus the fusion plasmid has the eliminating power of both parental plasmids. When an N or P plasmid was introduced into an R751-1010⁺ strain (and maintenance of the incoming plasmid was demanded by presence of a selective antibiotic), half the transcipts lost the resident plasmid but half retained all resistance determinants of both the incoming and the resident plasmids. These clones were shown to carry a single plasmid whose molecular weight was rather less than the sum of the incoming and resident molecules (Table 1).

Table 2. *Transfer and incompatibility properties of R751-1010 and R388-1010*

Donor	Recipient	Selective agent	No. of transipients of each character
J53 (R751-1010)	J62	Ap Tp	30/30 (ApTp) ^R 30/34 (ApTp) ^R
J62 (R751-1010)	J53 (R702)†	Ap Tp	10/10 (ApTp) ^R Km ^{S*} 10/10 (ApTp) ^R Km ^{S*}
J53 (R702)	J62 (R751-1010)	Km	$\begin{cases} 13 & \text{Km}^R(\text{ApTp})^S \\ 12 & (\text{KmApTp})^R \\ 1 & (\text{KmAp})^R\text{Tp}^S \end{cases}$
J62 (R751-1010)	J53 (N3T)†	Ap Tp	$\begin{cases} 29/29 & (\text{ApTp})^R\text{Tc}^S \\ 29 & (\text{ApTp})^R\text{Tc}^S \\ 2 & (\text{ApTcTp})^R \end{cases}$
J53 (N3T)	J62 (R751-1010)	Tc	$\begin{cases} 11 & (\text{ApTcTp})^R \\ 5 & \text{Tc}^R(\text{ApTp})^S \\ 6 & (\text{TcTp})^R\text{Ap}^S \end{cases}$
J53 (R388-1010)	J62	Ap Tp	$\begin{cases} 12/12 & (\text{ApTp})^R \\ 15 & (\text{ApTp})^R \\ 1 & \text{Tp}^R\text{Ap}^S \end{cases}$
J62 (R388-1010)	J53 (Sa)†	Ap Tp	$\begin{cases} 32/32 & (\text{ApTp})^R\text{Cm}^S \\ 31 & (\text{ApTp})^R\text{Cm}^S \\ 1 & \text{Tp}^R(\text{ApCm})^S \end{cases}$
J53 (Sa)	J62 (R388-1010)	Cm	$\begin{cases} 29 & \text{Cm}^R(\text{ApTp})^S \\ 2 & (\text{ApTpCm})^R \\ 1 & (\text{ApCm})^R\text{Tp}^S \end{cases}$
J62 (R388-1010)	J53 (N3T)	Ap Tp	$\begin{cases} 32/32 & (\text{ApTp})^R\text{Tc}^S \\ 14 & (\text{ApTp})^R\text{Tc}^S \\ 1 & \text{Tp}^R(\text{ApTc})^S \end{cases}$
J53 (N3T)	J62 (R388-1010)	Tc	$\begin{cases} 17 & (\text{ApTpTc})^R \\ 11 & \text{Tc}^R(\text{ApTp})^S \end{cases}$

* Several of these clones retained the resistances of R702 throughout the usual purification procedure. All, however, proved unstable (losing R702 from at least 80% of cells after 6 h exponential growth in drug-free broth). This prolonged retention of the resident plasmid suggests that R751-1010 does not produce as much P repressor as does R751, but this has not been clearly established.

† See Table 1 for further details.

We interpret these results to imply that R751-1010 produces repressors of replication (incompatibility effectors) specific for both P and N replication initiators and thus its entry into a plasmid-carrying recipient can lead to elimination of resident N or P plasmids. It can replicate in either the N or P mode. [N and P plasmids produce approximately equal numbers of plasmid molecules per chromosome (Falkow *et al.*, 1974) so that an N-P fusion plasmid would be expected to determine normal levels of both repressors.] When an N or P plasmid enters an R751-1010⁺ recipient, the incoming plasmid is unstable unless it can cause the elimination of the resident plasmid or can fuse with it. The mechanism of elimination is obscure but it may be that if an N plasmid enters a cell in which the resident plasmid is replicating in the N mode, the latter may not be able to switch into P mode replication rapidly enough to escape elimination. (An exactly analogous argument would hold for elimination by a P plasmid.)

The results for R388-1010 were similar (Table 2) but differed slightly in that the fusion plasmid was very efficiently eliminated by entry of the W plasmid Sa. We suggest that as W plasmids have a slightly higher copy number than do N plasmids (Falkow *et al.*, 1974) R388-1010 tends to replicate in the W mode and the N initiator is blocked by excess repressor. However, the fact that R388-1010 is fairly efficiently eliminated by entry of an N plasmid suggests that the W initiator is not invariably used.

During vegetative growth R751-1010-702, R751-1010-N3T, R388-1010-N3T and R388-1010-Sa proved fairly stable. More than 95% of cells carried all resistances after 6 h exponential growth in drug-free broth at 37 °C, but during transfer they tended to break down producing a variety of segregants.

The fact that the triparental fusion plasmids have lower molecular weights than would be expected from the simple addition of molecular weights of the three parental plasmids is probably due to the elimination of homologous segments. Thus, for example, R388 (and hence R388-1010) has extensive DNA homology with Sa (Falkow *et al.*, 1974, Gorai *et al.*, 1979). The shortening may either be due to random occurrence of internal recombination events, or the deletion of one of the duplicated incompatibility regions (the W specificity site in the case of the R388-1010-Sa fusion plasmid) may be required to permit the plasmid's replication. We hope that analysis of the triparental fusion plasmids and of their segregation products will resolve this matter.

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