TnA-directed Deletion of the *trp* Operon from RSF2124-*trp* in *Escherichia coli*

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Plasmid pMT-*trp* was constructed by digestion of RSF2124-*trp* with restriction endonuclease *Pst*I and ligation with T4 ligase. In pMT-*trp* about 78 % of the DNA of transposon TnA from RSF2124-*trp* was deleted, and hence the gene for ampicillin resistance was lost. All Trp[−] segregants from pMT-*trp* carriers in *Escherichia coli* W3110 and its derivatives were found to have lost the entire plasmid. On the other hand, deletion plasmids which had lost the *trp* operon were found among Trp[−] segregants from RSF2124-*trp* carriers, particularly from the mutant strain *trpAE1 trpR tnaA*. The experimental fact that deletion occurred exclusively in RSF2124-*trp* suggests that the presence of TnA in the plasmid (RSF2124-*trp*) was responsible for the deletion.

INTRODUCTION

'Transposons' have been recognized as the translocatable elements that carry drug-resistance gene(s) (Kleckner, 1977). It has been reported that transposons such as TnA (ampicillin resistance), Tn10 (tetracycline resistance), etc. promote deletion of DNA in their immediate vicinity (Nisen *et al.*, 1977; Holmans *et al.*, 1978; Noel & Ames, 1978; Tu & Cohen, 1980).

Our previous work on the stability of the *trp* operon recombinant plasmid RSF2124-*trp*, which carries TnA, revealed many deletion plasmids amongst Trp[−] segregants (Imanaka *et al.*, 1980). However, there are no reports in the literature on whether such deletion occurs if the transposon has been removed artificially from the plasmid. The purpose of this paper is to study the effect on plasmid stability of absence or presence of a transposon. Plasmids with deletions were examined by using restriction endonucleases to ascertain the whereabouts of the deletion.

METHODS

Materials. Ampicillin (Ap), chloramphenicol, agarose, lysozyme and RNAase A were purchased from Sigma. Pronase E was from Kaken Chemical Co., Tokyo, Japan. Restriction endonucleases (*Eco*RI, *Bam*I, *Hind*III, and *Pst*I), DNA of phage λ and T4 ligase were purchased from Miles Laboratories, Elkhart, Ind., U.S.A. Bacto-tryptone was from Difco. All other reagents were purchased from Wako Pure Chemical Industries, Osaka, Japan.

Media and solutions. The complete medium (PBB) and the minimal medium (MM) used throughout were exactly the same as those described elsewhere (Imanaka *et al.*, 1980). However, for the culture of pMT-*trp* carriers (for the preparation of pMT-*trp*, see below), Casamino acids in MM were adjusted to 5 g l^{−1} instead of 0.5 g l^{−1}. The MM agar, Tryptone broth, TS buffer, TES buffer and Tris/borate buffer used were also as described previously (Imanaka *et al.*, 1980).

Bacterial strains, plasmids and phages. Strains, plasmids and phages used in this work are listed in Table 1.

Table 1. *Strains of E. coli and plasmids and phages used*

<i>E. coli</i> strain	Relevant properties	Plasmid	Reference
C600-1	$r_k^- m_k^- leu6 thr1 thi1 supE44 lacY1 tonA21 Trp^-$	(RSF2124-trp)	Imanaka <i>et al.</i> (1980)
W3110	<i>trpAE1</i> *†	(RSF2124-trp)	Imanaka <i>et al.</i> (1980)
W3110	<i>trpAE1 trpRam27</i> *‡	(RSF2124-trp)	Imanaka <i>et al.</i> (1980)
W3110	<i>trpAE1 trpR tnaA</i> *§	(RSF2124-trp)	Imanaka <i>et al.</i> (1980)
KM605	<i>str his recA1 su⁻ thy gal trp</i>	($\lambda dv1$)	Matsubara (1972)
A745	<i>met thy</i>	(ColE1)	Sakakibara & Tomizawa (1974)
Phage	Reference		
$\lambda C190$	Kaiser (1957)		
$\lambda_{C_{12}, C_{13}}$	Ptashne & Hopkins (1968)		

*Isolated originally by C. Yanofsky.

† Deletion mutant of *trpA-E*, designated AE1.

‡ *trp* repressor amber mutant, designated Ram.

§ Tryptophanase-deficient mutant, designated Tna.

Plasmid construction and preparation. For construction of the specific plasmid designated pMT-trp, RSF2124-trp DNA in TS buffer was digested with restriction endonuclease *Pst*I (for details of digestion, see below), followed by an elevation in temperature to 65 °C for 10 min to inactivate *Pst*I, and ligation with T4 ligase by the procedure of Tanaka & Weisblum (1975). *Escherichia coli* W3110 *trpAE1* cells were then transformed with the DNA solution by the method of Tanaka & Weisblum (1975). Transformants (Trp⁺, Ap^s) were selected by the procedure described previously (Imanaka *et al.*, 1980).

Transformation of other *E. coli* cells also followed the method of Tanaka & Weisblum (1975). DNAs of plasmids were purified by the method of Clewell (1972).

Phenotypic stability of plasmids. Phenotypic stability of RSF2124-trp and pMT-trp was tested by the procedure used previously (Imanaka *et al.*, 1980), except that no drug was added to the preculture and subsequent culture media of pMT-trp carriers. Colicin E1 immunity and/or λ immunity were tested, where necessary, as described by Imanaka *et al.* (1980).

Digestion of plasmid DNA with restriction endonucleases. Plasmid DNA (about 1 µg) in TS buffer was digested at 37 °C for 1 h in each reaction mixture (total vol. 25 µl). The composition of the mixtures was as follows: (i) 1 µl *Eco*RI, 10 mM-MgCl₂, 50 mM-NaCl, 10 mM-Tris, pH 7.5; (ii) 2 to 3 µl *Bam*I, 10 mM-MgCl₂, 100 mM-Tris, pH 7.5; (iii) 2 to 3 µl *Hind*III, 6 mM-MgCl₂, 50 mM-NaCl, 2.5 µg bovine serum albumin, 6 mM-Tris, pH 7.5; (iv) 2 to 3 µl *Pst*I, 10 mM-MgSO₄, 90 mM-Tris, pH 7.5 (Nagahari, 1978); (v) 1 µl *Eco*RI and 3 µl *Bam*I, 10 mM-MgCl₂, 100 mM-Tris, pH 7.5.

Agarose gel electrophoresis of DNA. DNA samples were subjected to electrophoresis in agarose (10 g l⁻¹) dissolved in Tris/borate buffer as previously described (Imanaka *et al.*, 1980). λ DNA digested with *Eco*RI and/or *Hind*III was used as an internal standard.

Preparation of crude extracts and assay of tryptophan synthase activity. Cells carrying RSF2124-trp and/or pMT-trp were grown until late exponential phase at 37 °C in MM. Preparation of crude extract and assay of tryptophan synthase activity are described elsewhere (Smith & Yanofsky, 1962; Imanaka *et al.*, 1980). In parallel with the assay of tryptophan synthase activity, the phenotypic stability of RSF2124-trp and/or pMT-trp was assessed. The specific activity of tryptophan synthase was corrected by dividing by the fraction of Trp⁺ cells in the stability test (Imanaka *et al.*, 1980).

RESULTS

Restriction map of RSF2124-trp

The restriction map of the recombinant plasmid RSF2124-trp is shown in Fig. 1. The arrows inside and outside the circle indicate the cleavage sites, which were determined by reference to the results of other workers as well as our own work on each restriction endonuclease, i.e. *Eco*RI (E), *Hind*III (H), *Bam*I (B) and *Pst*I (P). The molecular sizes of the DNA fragments obtained with each restriction endonuclease are listed in Table 2.

Fig. 1. Restriction map of RSF2124-trp. The arrows inside and outside the circle indicate the cleavage sites for restriction endonucleases *EcoRI* (E), *HindIII* (H), *BamI* (B) and *PstI* (P). Different cleavage sites for the same restriction endonuclease are distinguished by subscripts. The sizes of the DNA fragments are listed in Table 2. *ori*, replication origin; *immCol*, colicin E1 immunity; A, B, C, D, E, structural genes of *E. coli* tryptophan (*trp*) operon; *P_T*, *trp* operon promoter; N, N gene; *P_L*, leftward promoter; *CI*, λ immunity; *P_R*, rightward promoter; *Ap'*, ampicillin resistance gene.

Table 2. Sizes of DNA fragments obtained by restriction endonuclease cleavage of RSF2124-trp

The fragments are those shown in Fig. 1 (read clockwise).

DNA fragment	Size (Mdal)	Reference
E ₁ -E ₂	10.8	Nagahari <i>et al.</i> (1977)
E ₂ -E ₁	7.4	This work (Fig. 5k)
H ₁ -H ₂	1.9	Nagahari (1978)
H ₂ -H ₃	1.8	Nagahari (1978)
H ₃ -H ₄	2.8	Nagahari (1978)
H ₄ -H ₅	0.2	Nagahari (1978)
H ₅ -H ₁	11.5	Nagahari <i>et al.</i> (1977); Nagahari (1978)
B ₁ -B ₂	4.1	Heffron <i>et al.</i> (1977); Nagahari (1978)
B ₂ -B ₁	14.1	Heffron <i>et al.</i> (1977); Nagahari (1978)
P ₁ -P ₂	7.8	This work (Fig. 2a, 5a); Nagahari (1978)
P ₂ -P ₃	1.5	This work (Fig. 2a, 5a); Dougan <i>et al.</i> (1978); Nagahari (1978)
P ₃ -P ₄	0.8	This work (Fig. 2a, 5a); Dougan <i>et al.</i> (1978)
P ₄ -P ₅	2.4	This work (Fig. 2a, 5a); Collins & Brüning (1978); Dougan <i>et al.</i> (1978)
P ₅ -P ₆	1.8	This work (Fig. 2a, 5a); Collins & Brüning (1978)
P ₆ -P ₇	0.4	This work (Fig. 2a, 5a); Bukhari <i>et al.</i> (1977); Collins & Brüning (1978)
P ₇ -P ₈	2.6	This work (Fig. 2a, 5a); Bukhari <i>et al.</i> (1977); Smith <i>et al.</i> (1976)
P ₈ -P ₁	0.9	This work (Fig. 2a, 5a); Nagahari (1978); Smith <i>et al.</i> (1976)
B ₁ -P ₆	1.1	Collins & Brüning (1978)
E ₁ -B ₂	0.7	This work (Fig. 5k)
B ₂ -E ₂	10.1	This work (Fig. 5k)
E ₂ -B ₁	4.0	This work (Fig. 5k)
B ₁ -E ₁	3.4	This work (Fig. 5k)
TnA · R-E ₁	1.1	Dougan <i>et al.</i> (1978)
TnA · L-E ₁	4.3	Dougan <i>et al.</i> (1978)
TnA · L-B ₁	0.9	Heffron <i>et al.</i> (1977); Holmans <i>et al.</i> (1978)
TnA	3.2	Heffron <i>et al.</i> (1977); So <i>et al.</i> (1975)

Fig. 2. Agarose gel electrophoresis of pMT-trp digested with restriction endonucleases. (a) *Pst*I digest of RSF2124-trp (control); (b) *Pst*I digest; (c) *Eco*RI digest; (d) *Hind*III digest; (e) *Hind*III digest of λ as reference. The sizes of the fragments in (e) are (from top) 14.9, 6.07, 4.13, 2.71, 1.42, 1.14 and 0.34 Mdal (Bukhari *et al.*, 1977) (the 0.34 Mdal fragment is not visible in the figure).

Properties of pMT-trp

The principal motive for constructing pMT-trp from RSF2124-trp, by digestion with *Pst*I followed by ligation with T4 ligase (see Methods and Fig. 1), was to obtain a plasmid to serve as a 'control' in this work. This plasmid, in which most of TnA is deleted, was needed because TnA has been confirmed by previous workers to promote the deletion of the plasmid carrying it (Nisen *et al.*, 1977; Holmans *et al.*, 1978; Tu & Cohen, 1980). Plasmid pMT-trp was prepared such that the ampicillin resistance gene *Ap*^r was deleted without impairing the *trp* operon on RSF2124-trp. *Esche: ichia coli* cells carrying pMT-trp were found to be imm λ ⁺ and immCol⁺.

Plasmid pMT-trp digested with *Pst*I was analysed by agarose gel electrophoresis, with RSF2124-trp digested with the same restriction endonuclease included for reference (Fig. 2a, b). It is evident from Fig. 2(b) that three fragments of DNA, of 7.8, 2.6 and 1.5 megadaltons (Mdal), constitute pMT-trp. The largest fragment, 7.8 Mdal, originates from P₁ to P₂ on the restriction map of RSF2124-trp (see Fig. 1 and Table 2) and thus includes the *trp* operon. The second fragment, 2.6 Mdal, extends from P₇ to P₈, and contains the replication origin (*ori*) and immCol. The last fragment, 1.5 Mdal, encompasses the region from P₂ to P₃, which carries the λ CI gene (*imm* λ).

The digestion pattern of pMT-trp with *Eco*RI shows two fragments of DNA, of 10.9 and 1.0 Mdal (Fig. 2c). The DNA fragments from the digestion of pMT-trp with *Hind*III were of 5.2, 2.8, 1.9, 1.8 and 0.2 Mdal [Fig. 2d; the 0.2 Mdal fragment is not clear, but this molecular size was taken from Nagahari (1978)]. The specific DNA fragment of 1.0 Mdal appearing from the digestion with *Eco*RI, and the five fragments emerging from that with *Hind*III, yielded the restriction map of pMT-trp shown in Fig. 3. Note that *trp* genes A to E on the *trp* operon are reversed with reference to *ori* in comparison with their sequence in the RSF2124-trp restriction map (Fig. 1).

Fig. 3. Restriction map of pMT-trp. The arrows indicate the cleavage sites for restriction endonucleases *EcoRI* (E), *HindIII* (H), *BamI* (B) and *PstI* (P). Reading from *ori* clockwise on the map, the first P site represents ligation of P₈ and P₃ in Fig. 1; the second P site is P₂ in situ; the third P site represents ligation of P₇ and P₁. The abbreviations are explained in the legend to Fig. 1.

Fig. 4. Stability of recombinant plasmids and tryptophan synthase activity in mutants of *E. coli* W3110: *trpAE1* (AE1), *trpAE1 trpRam27* (Ram) and *trpAE1 trpR tnaA* (Tna). The fraction of colonies (100 scored for each experiment) exhibiting Trp⁺ phenotype is shown in the histograms, for bacteria grown on each of three media (see text for details). Tryptophan synthase activity (tabulated above figure) was assayed with extracts from sonicated cells grown in MM. All the data for RSF2124-trp are reproduced from Imanaka *et al.* (1980). ■, RSF2124-trp; □, pMT-trp.

Phenotypic stability of RSF2124-trp and pMT-trp

To study the phenotypic stability of RSF2124-trp and pMT-trp, *E. coli* W3110 *trpAE1* (designated AE1), *E. coli* W3110 *trpAE1 trpRam27* (designated Ram) and *E. coli* W3110 *trpAE1 trpR tnaA* (designated Tna), each harbouring one of the plasmids, were grown for 20 to 25 generations in PBB, MM or MM plus tryptophan (50 µg ml⁻¹) and then plated on PBB agar. Colonies were then transferred to MM to score for Trp⁺ phenotype. The percentages of

colonies showing Trp⁺ phenotype, and the tryptophan synthase activities of host cells carrying each plasmid, are shown in Fig. 4. (Since all the Trp⁺ colonies of RSF2124-trp carriers were Ap^r, drug resistance is not shown on the ordinate of Fig. 4.) The tryptophan synthase activities of the bacteria carrying pMT-trp were 4 to 13 times greater than those of RSF2124-trp carriers. This high activity of tryptophan synthase seems plausible, since because there was no deletion of the *trp* operon in pMT-trp, whereas there was an appreciable deletion in RSF2124-trp (see below), the number of *trp* operons per chromosome may have been larger in pMT-trp carriers than in RSF2124-trp carriers. Our previous observation that phenotypic stability was inversely related to tryptophan synthase activity (Imanaka *et al.*, 1980) was confirmed in the present study (see open bars in Fig. 4).

Immunity tests revealed that all Trp⁻ segregants from pMT-trp carriers were immλ⁻ immCol⁻, indicating that the plasmid had probably been lost; this was confirmed by the absence of plasmid covalently closed circular (CCC) DNA in CsCl-ethidium bromide density-gradient centrifugation analyses. Trp⁻ segregants from RSF2124-trp carriers were either Ap^r or Ap^s. Immunity tests of the Trp⁻ Ap^s segregants showed them to be immλ⁻ immCol⁻; the disappearance of the plasmid was confirmed by CsCl-ethidium bromide density-gradient centrifugation. Immunity tests of the Trp⁻ Ap^r segregants (about 300 in total) showed them to be almost exclusively immλ⁻ immCol⁺, but exceptionally, one segregant was immλ⁺ immCol⁺ and another was immλ⁻ immCol⁻. Plasmid CCC DNA could be extracted from all the Trp⁻ Ap^r immλ⁺ immCol⁺ or Trp⁻ Ap^r immλ⁻ immCol⁺ segregants, but not from the specific segregant Trp⁻ Ap^r immλ⁻ immCol⁻. This segregant, harbouring no plasmid, may have had the TnA element translocated on to the bacterial chromosome.

Digestion of deletion plasmids with restriction endonucleases

Agarose gel electrophoresis showed that plasmid CCC DNAs extracted from Trp⁻ Ap^r immCol⁺ segregants were smaller than RSF2124-trp (results not shown). Accordingly, the plasmids were deletion mutants of RSF2124-trp. In contrast, no deletion plasmids were obtained from pMT-trp, from which most of the TnA element has been deleted (Fig. 3). It thus appears that the presence of TnA in RSF2124-trp was the main factor responsible for the appearance of deletion plasmids.

Several deletion plasmids were prepared from cultures of RSF2124-trp carriers in PBB medium in exactly the same fashion as in the stability test. Segregants (Trp⁻ Ap^r immλ⁻ immCol⁺—with one exception: Trp⁻ Ap^r immλ⁺ immCol⁺) gave various deletion plasmids. The results of agarose gel electrophoresis of RSF2124-trp and several deletion plasmids, all of which were digested with *Pst*I, are shown in Fig. 5 (*a* to *i*). Deletion plasmids were obtained from host bacteria of Tna, Ram and AE1 and designated ΔT, ΔR and ΔA, respectively. The numbers in the ΔT and ΔR designations are the serial numbers of plasmids obtained from the same host strain. The deletion plasmid designated ΔC was obtained from *E. coli* C600-1 carrying RSF2124-trp.

It can be seen from Fig. 5 that DNA fragments P₅–P₆ (1.8 Mdal) and P₆–P₇ (0.4 Mdal) are common to all the deletion plasmids. Except for ΔC (Fig. 5*i*), another DNA fragment, P₇–P₈ (2.6 Mdal) could also be found in all the deletion plasmids. On the other hand, P₁–P₂ (7.8 Mdal) was apparently deleted in the deletion plasmids. Except for ΔR3 (Fig. 5*g*), DNA fragments P₂–P₃ (1.5 Mdal), P₃–P₄ (0.8 Mdal) and P₄–P₅ (2.4 Mdal) were also deleted. It is difficult to distinguish P₇–P₈ (2.6 Mdal) from P₄–P₅ (2.4 Mdal) in Fig. 5, but the deletion of P₄–P₅ was confirmed by the fact that all the deletion plasmids possessed the E₁ cleavage site (ΔR3 also possessed the E₂ cleavage site), and thus contained fragment P₇–P₈.

These observations on DNA fragments of deletion plasmids digested with *Pst*I can be summarized as follows: fragments P₅–P₆ and P₆–P₇ were present, while fragments P₅–P₄, P₄–P₃, P₃–P₂ and P₂–P₁ were absent (except for ΔR3) (see Fig. 1 and Table 2). Consequently, one of the deletion termini of the plasmids other than ΔR3 might have been between P₄ and P₅ sites, and that for ΔR3 between sites P₁ and P₂.

Fig. 5. Agarose gel electrophoresis of restriction endonuclease digests of RSF2124-trp and deletion plasmids derived from it. (a) *Pst*I digest of RSF2124-trp. The sizes of the fragments are (from top) 7.8, 2.6, 2.4 (appearing as doublet), 1.8, 1.5, 0.9, 0.8 and 0.4 Mdal. (b) to (i) *Pst*I digests of deletion plasmids Δ T1 (b), Δ T2 (c), Δ T3 (d), Δ R1 (e), Δ R2 (f), Δ R3 (g), Δ A (h) and Δ C (i). (Deletion plasmids designated Δ T, Δ R, Δ A and Δ C were obtained from host bacteria of *E. coli* Tna, Ram, AE1 and C600-1, respectively. The numbers in the Δ T and Δ R designations are the serial numbers of plasmids from the same host strain.) (j) *Hind*III digest of λ (control). (k) *Eco*RI and *Bam*I double digest of RSF2124-trp (used for Fig. 1 and Table 2 – the 7.4 Mdal fragment E_2-E_1 between B_2-E_2 and E_2-B_1 on the gel is not visible on the Figure).

A specific DNA fragment, not originally observed in RSF2124-trp when digested with *Pst*I, emerged in the digests shown in Fig. 5 (c, d, e, g, h, i). No new fragments seemed to appear in *Pst*I digests of Δ T1 (Fig. 5b) or Δ R2 (Fig. 5f). However, in the Δ T1 digest, the 1.8 Mdal DNA fragment appeared as a doublet; the original fragment, P_5-P_6 , was superimposed on another fragment of 1.8 Mdal that had been generated due to the deletion of DNA. In the Δ R2 digest (Fig. 5f), the fragment corresponding to P_3-P_4 was different from that in the original plasmid RSF2124-trp (Fig. 5a). The difference was checked by digestion with *Bam*I (results not shown).

To recapitulate, the deletion plasmids (Ap^r) studied in this work had *Pst*I sites (P_5 , P_6 and P_7) (see Fig. 5) and an *Eco*RI site (E_1) in common. These deletion plasmids ranged in size from 4.3 Mdal (Δ C) to 11.7 Mdal (Δ R3). Since the DNA fragment from E_1 to TnA·L has been shown to be 4.3 Mdal in size (Table 2), the fact that Δ C was 4.3 Mdal in size suggests that one of the deletion termini for Δ C is located next to TnA·L.

DISCUSSION

Recently, keen attention has been paid to translocatable elements in prokaryotes (Kleckner, 1977). Holmans *et al.* (1978) observed that one of these translocatable elements,

Fig. 6. Restriction maps of RSF2124-trp and deletion plasmids derived from it. The maps were constructed from digestions with *EcoRI*, *BamI* and *PstI*, followed by agarose gel electrophoresis. The deletions (indicated by the dashed lines), except for $\Delta R3$, were postulated to occur at or next to the left terminus of TnA. The size of each plasmid (Mdal) is shown on the right of the figure.

TnA, which was harboured in a natural plasmid, R6K, could trigger the deletion of R6K. In addition, Nisen *et al.* (1977) reported that TnA caused the disappearance of a tetracycline resistance gene from pSC105::TnA.

We also observed the deletion of the *trp* operon from the recombinant plasmid RSF2124-trp. This deletion can be attributed to the effect of TnA, because such deletion did not occur in pMT-trp, which was prepared such that the TnA element was almost missing. All Trp^- segregants from pMT-trp carriers (about 500 in total) were always accompanied by the $\text{imm}\lambda^- \text{immCol}^-$ phenotype and no plasmid CCC DNA was detected in these segregants by CsCl-ethidium bromide density-gradient centrifugation.

The frequency of deletion from the plasmid DNA of Trp^- segregants was affected by the RSF2124-trp carrier host, being nearly 100% for Tna, 30% for Ram and 2% for AE1 and C600-1. It is significant that the frequency of deletion showed a positive relationship with the tryptophan synthase activity of the host bacteria. As was discussed in our previous paper (Imanaka *et al.*, 1980), the higher the activity of tryptophan synthase in the host bacteria, the more marked was the difference in growth rate between the cells carrying the Trp^+ recombinant plasmid and those carrying the vector plasmid. In other words, a sort of 'enrichment' (Hershfield *et al.*, 1974) took place during our experimental procedure. This is one of the reasons for the high percentage of deletions, especially in Tna, which exhibited high tryptophan synthase activity. To reiterate, two factors must be taken into account to clarify our experimental results: one, the presence of the TnA element in RSF2124-trp, and the other, the 'enrichment' of the segregant population that carries deletion plasmids. Gelfand *et al.* (1978) proposed a working hypothesis, in this connection, that deletion formation might be caused by a 'transposase' coded on TnA.

Referring to Figs 1 and 5, restriction maps of RSF2124-trp and the deletion plasmids derived from it were prepared (Fig. 6). In view of the fact that one of the deletion termini of these plasmids (except for $\Delta R3$) was between the P_4 and P_5 sites, and also in the light of previous work on the possible deletion at the termini of TnA (Ohtsubo *et al.*, 1979; Takeya *et al.*, 1979; Tu & Cohen, 1980), the deletion was postulated here to occur at or next

to the left terminus of TnA. The distinction between 'at' and 'next to' the terminus was beyond the scope of this work. DNA sequence analyses of various deletion plasmids to examine precisely the position of deletion are in progress.

It is interesting to reconfirm from Fig. 6 that several deletion plasmids appeared. However, $\Delta T2$ and $\Delta T3$ taken from the same batch of cultivation of (Tna) bacteria were exactly the same size (6.2 Mdal). $\Delta R3$ (Trp⁻ Ap^r imm λ ⁺ immCol⁺) was exceptional among nearly 300 segregants: the other segregants were of Trp⁻ Ap^r imm λ ⁻ immCol⁺ phenotype.

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