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The plasmid system of *Escherichia coli* strain UR12644:

Identification and molecular characteristics of transposons involved in the generation of endogenous R-plasmids

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SUMMARY

Two spontaneously formed R-plasmids (pFS401 and pFS402) originating from the multiple drug-resistant *Escherichia coli* strain UR12644 were found to carry transposable drug-resistance elements. Incompatibility between these two plasmids was used to select for transposition. An ampicillin transposon (Tn1781) residing on pFS401 and a tetracycline transposon (Tn1771) present on pFS402 were independently translocated to the endogenous RTF-plasmid pFS2. Molecular weight determinations of pFS2::Tn1781(Ap) and pFS2::Tn1771(Tc) revealed a value of 2·9 Mdal for Tn1781 and 7·1 Mdal for Tn1771. The arrangement of 3 *Pst*I and 1 *Bam*HI restriction endonuclease sites was found to be characteristic for the ampicillin transposon whereas the restriction map of Tn1771 features a nearly symmetrical location of 3 *Eco*RI cleavage sites, two of them close to the termini and one in the middle of the transposon. A model is presented suggesting the existence of repetitive DNA-segments at these positions which represent the structural preconditions for the genetic properties of Tn1771. The role of a cryptic plasmid involved in the generation of the endogenous R-plasmids pFS401 and pFS402 is discussed.

1. INTRODUCTION

We have previously shown (Schöffl, Pühler & Heumann, 1977) that a multiple-drug-resistant *E. coli.* strain isolated from a human urine specimen from the Erlangen clinics harbours a complex plasmid system characterized by the following components: (i) a R-plasmid pFS1 (65 Mdal) carrying determinants for multiple resistance against 11 antimicrobial drugs, (ii) an unmarked RTF-plasmid pFS2 (18·9 Mdal) representing a transfer factor capable of co-integration with pFS1, (iii) two small cryptic plasmids pFS4 (5·8 Mdal) and pFS5 (3·7 Mdal) originally not involved in the transfer of drug resistance genes and (iv) a spontaneously formed non-self-transmissible plasmid pFS401 (8·7 Mdal) conferring ampicillin resistance which can be mobilized by pFS2.

As reported earlier by Traub & Kleber (1975), several but not all drug resistances of strain UR12644 could be simultaneously 'cured' by ethidium bromide

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treatment. Non-curable determinants for resistance against tetracycline, sulfonamide and streptomycin were still present. This partial curing might reflect either a partial elimination of the multi-R-determinant plasmid pFS1 or, in the case of a complete elimination of this plasmid, a chromosomal location of the remaining resistance genes.

This paper deals with the physical characterization of plasmids of the cured strain UR12644-cu1 and the identification of a spontaneously generated plasmid (pFS402) conferring the originally 'non-curable' tetracycline resistance on its hosts. The involvement of transposable drug resistance elements in the formation of endogenous R-plasmids was investigated.

2. MATERIAL AND METHODS

(i) *Bacterial strains and plasmids*

E. coli strains used in this study are listed in Table 1, the properties of plasmids are depicted in Table 2. Strain UR12644 is prototrophic with the drug resistant phenotype: Ap, Cb, Cp, Cm, Gm, Km, Nm, Sm, Tc, Tm, Su; strain UR12644-cu1: prototrophic, Tc, Sm, Su. The nomenclature of plasmids and resistance markers follows the proposal of Novick *et al.* (1976). Other chromosal markers were abbreviated according to Demerec *et al.* (1966). These markers are for strain NC22: (F$^-$), *met*, *leu*, *thy*, *rif*, *nal*; strain HB101: (F$^-$), *thi*, *leu*, *pro*, *lac*, *gal*, *his*, *hsr*, *hsm*, *rec*A, *str*B; strain C600 and its derivatives: (F$^-$), *lac*Y, *thr*, *leu*, *thi*, *ton*A, *sup*E.

(ii) *Media and growth conditions*

Bacteria were usually cultured in Penassay Broth (Difco, antibiotic medium 3) with continuous aeration at 37 °C. Solid media contained 1·8% Difco agar. Antimicrobial drugs were used at final concentrations of 100 μg/ml ampicillin (Hoechst, Frankurt); 25 μg/ml kanamycin (Grünenthal, Stolberg); 10 μg/ml tetracycline (Hoechst, Frankfurt); 100 μg/ml nalidixic acid (Serva, Heidelberg); 200 μg/ml streptomycin (Serva, Heidelberg).

Antibiotic susceptibility tests (antibiograms) were performed according to the method of Bauer *et al.* (1966).

The curing procedure used for the elimination of R-plasmids was carried out essentially as described by Traub & Kleber (1974, 1975).

(iii) *Plasmid transfer, genetic transformation, selected translocation*

Standard bacterial crosses selecting for R-plasmid transfer were performed using filter-disk matings. From exponentially growing parental cultures (in Penassay Broth) 10^8 donor cells and 2×10^8 recipient cells were spotted on to Millipore filters (0·45 μm pore size) on a Penassay plate. After 2–3 h incubation at 37 °C, cells were resuspended in 10 ml PS-buffer (0·85% sodium chloride) and harvested by centrifugation. After resuspension of the pellet in 1 ml fresh PS-buffer, serial dilutions were spread onto selective media containing appropriate drug combina-

Table 1. E. coli *strains and plasmids*

Strains	Plasmids*	Derivation
UR12644	pFS1, pFS2, pFS4, pFS5	Schöffl *et al.* (1977); Traub & Kleber (1975)
UR12644-cu1	pFS2, pFS4, pFS5	cured variant of UR12644 (this paper)
NC22	—	Saunders and Grinsted (1972)
FS2201	pFS1, pFS2	UR12644 × NC22, Schöffl *et al.* (1977)
FS2202	pFS2, pFS401	UR12644 × NC22, Schöffl *et al.* (1977)
FS2203	pFS2, pFS402	UR12644–cu1 × NC22 (this paper)
HB101	—	Lovett & Helinski (1976)
FS1011	pFS401	transformation of HB101 with pFS401, Schöffl *et al.* (1977)
FS1012	pFS402	transformation of HB101 with pFS402, (this paper)
FS1013	pFS403	transformation of HB101 with *in vitro* constructed pFS403, (this paper)
FS1014†	pFS201, pFS402	FS2203 × FS1011 (this paper)
FS1015†	pFS202, pFS401	FS2203 × FS1011 (this paper)
C	—	Wild type, supplied by W. H. Traub, Erlangen
FS01	pFS201	FS1014 × C (this paper)
FS02	pFS202	FS1015 × C (this paper)
S100	—	Nx^r mutant of C600, provided by R. Simon, Erlangen
S200	—	Sm^r mutant of C600, provided by R. Simon, Erlangen

* In a previous publication (Schöffl *et al.* 1977) plasmids were assigned as ER1, (pFS1), no. 2(pFS2), a (pFS5), b (pFS4), no. 4 (pFS401).

† The plasmid character of these strains is deduced from genetic experiments.

tions. Nalidixic acid was used for counterselection of UR12644 and UR12644-cu1 whereas NC22 derivatives were counterselected by streptomycin. pFS401 and pFS201 transconjugants were selected by ampicillin, pFS402 and pFS202 transconjugants by tetracycline. Plasmid transconjugants resulting from crosses between the donor strains FS1014 and FS1015 and the recipient strain *E. coli* C (prototrophic) were isolated on minimal medium containing appropriate drugs. Viable counts of donor and recipient strains were used for the determination of transfer frequencies (transconjugants/donor cell).

Genetic transformation of strain HB101 by plasmid DNA was performed according to the method described by Cohen, Chang & Hsu (1972). Transposition of drug-resistance elements from pFS401 and pFS402 to plasmid pFS2 was selected by plasmid incompatibility in a *rec*A$^-$ background (Kleckner *et al.* 1977). Incompatibility of pFS401 (Ap) and pFS402 (Tc) was tested after pFS402 transformation into HB101 (pFS401).

Tc^R transformants were selected and purified on tetracycline-/streptomycin-containing plates followed by examination of the ampicillin resistance marker. Transposition was selected on ampicillin-/tetracycline-containing plates in crosses between NC22 (pFS2, pFS402) and HB101 (pFS401). In this experiment pFS402

Table 2. *Physical and genetic properties of plasmids*

Plasmid	Phenotype	Molecular weight (Mdal)	Buoyant density (g/ml)
pFS1	Tra+, Ap, Cb, Cp, Cm, Gm, Km, Nm, Sm, Tc, Tm, Su	65*	1.710
pFS2	Tra+	18·9*	1.700
pFS4	Cryptic	5·8*	Unknown
pFS5	Cryptic	3·7*	Unknown
pFS401 (pFS4::Tn1781)	Tra−, Ap	8·8*	1·706
pFS402 (pFS4::Tn1771)	Tra−, Tc	12·9*	1·712
pFS403	Tra−, Tc	9·3†	Unknown
pFS201 (pFS2::Tn1781)	Tra+ Ap	21·8*	1·701
pFS202 (pFS2::Tn1771)	Tra+, Tc	26*	1·705

* Molecular weight calculated from EM length measurements.

† Molecular weight determined by agarose-gel electrophoresis using molecular-weight standards.

was mobilized by pFS2. Double resistant HB101 transconjugants were purified on streptomycin/tetracycline and streptomycin/ampicillin plates, followed by tests for the stable inheritence of the unselected resistance marker. Stable inheritence of both markers by the transconjugants FS1014 and FS1015 indicated transposition.

(iv) *Isolation and physical characterization of plasmid DNA*

Plasmid DNA was isolated from cells grown in Penassay Broth. Cell lysates were prepared according to the sarcosyl method (Bazaral & Helinski, 1968). Covalently closed circular plasmid DNA was purified by CsCl-ethidium bromide centrifugation (Radloff, Bauer & Vinograd 1967). Ethidium bromide was removed from DNA samples by repeated extraction with iso-amyl alcohol. The DNA-solutions so obtained were extensively dialysed against TE-buffer (10 mM Tris-HCL, pH 8, 1 mM EDTA).

Length measurements and molecular-weight determinations of plasmids were performed according to the procedures described by Burkardt *et al.* (1978) for electron microscopy of plasmid DNA.

The buoyant density of plasmid DNA was determined by analytical ultracentrifugation. CsCl-gradients, containing 0·5–1 μg plasmid DNA, were run in double sector cells in a Model E (Beckman) ultracentrifuge at 44×10^3 rev/min. The density-profiles obtained by photoelectric scanning at 262 nm were recorded with an external 10 in recorder (Beckman). Buoyant densities and base compositions of DNA were calculated according to Schildkraut, Marmur & Doty (1962). Chromosomal DNA of *Micrococcus lysodeikticus* (ρ = 1·731 g/ml) was used as a density marker.

(v) *Restriction analysis of plasmids*

Usually 0·5–3 μg plasmid DNA was used for restriction endonuclease digestion. The enzymes *Hin*dIII, *Bam*HI, *Pst*I (purchased from Boehringer Mannheim) *Sal*I (supplied by W. Kaschka, Erlangen), *Eco*RI (supplied by A. Rösch, Erlangen) were applied for 45 min at 37 °C, in a buffer containing 10 mM Tris-HCl, pH 7·6, 50 mM-NaCl, 10 mM-$MgCl_2$. *Sma*I (supplied by A. Rösch, Erlangen) was applied for 45 min at 25 °C, in a buffer containing 15 mM Tris-HCl, pH 8·5, 15 mM-KCl, 6 mM-$MgCl_2$. The digests of plasmid DNA were analysed in 1% agarose gels containing 40 mM Tris-HCl, pH 8·2, 20 mM sodium acetate, 1 mM EDTA at 100 V for 2·5 h at room temperature. λ-DNA, digested by *Eco*RI (Helling, Goodman & Boyer, 1974) and *Hin*dIII (Rosenvold & Honigman, 1977) respectively was used as a standard for molecular-weight determinations of DNA-fragments.

Self-ligation of DNA-fragments generated by *Eco*RI digestion of pFS402 (5 μg) was tested by treatment with T_4-ligase, according to the cloning-prodecure described by Pühler, Burkardt & Klipp (1979). The ligation mixture was used for transformation of HB101 selecting for tetracycline resistance.

3. RESULTS

(i) *Characterization of plasmids involved in conjugational transfer of non-curable* Tc^R *of* E. coli *UR12644-cu1*

According to the experiments described by Traub & Kleber (1975), drug-resistance genes of strain UR12644 were eliminated using the curing reagent ethidium bromide. This treatment was successful in producing a neomycin-sensitive variant, designated UR12644-cu1. The cured strain was tested for additional loss of drug resistances by disk antibiograms according to the method of Bauer *et al.*, (1966). Compared with the original strian UR12644 the cured variant has lost resistance against ampicillin, carbenicillin, cephalothin, chloramphenicol, gentamycin, kanamycin, neomycin and tobramycin. The remaining drug resistances against tetracycline, streptomycin and sulfonamides were tested for conjugational transferability.

In order to identify a plasmid replicon carrying the non-curable drug resistance determinants, we selected for infectious transfer of individual resistance markers from UR12644-cu1 (relevant phenotype: Tc^R, Sm^R, Su^R, Nx^S) to recipient strain NC22 (relevant phenotype: Tc^S, Sm^S, Su^S, Nx^R). Only the tetracycline resistance determinant could be mobilized in this cross whereas streptomycin-resistance and sulfonamide-resistance remained non-transmissible. The frequency of 3×10^{-6}, obtained for the production of Tc^R transconjugants was very low compared to the transmission of curable Tc^R specified by pFS1, which occurs at a frequency of 3×10^{-3}. However, the low transfer frequency of non-curable tetracycline resistance is valid only for the initial mating. Retransfer of this resistance gene revealed an enhanced transfer frequency of 10^{-2} in crosses between the primary selected transconjugant strain FS2203: Tc^R, Nx^R, Sm^S with recipient strain S200: Tc^S,

Nx^S, Sm^R. Similar differences of the transfer frequencies between primary and secondary matings were previously observed for a spontaneously formed individual Ap^R-determinant, originating from strain UR12644 (Schöffl *et al.* 1977).

In order to examine the molecular basis of the transferable Tc^R of the cured strain UR12644-cu1, plasmid DNA was isolated from this strain as well as from the transconjugant strain FS2203. The plasmid molecules were physically characterized by electron-microscopic length measurements and by CsCl-buoyant density determination, using the analytical ultracentrifuge. The results of these measurements are depicted in Table 3. The donor strain UR12644-cu1 carries three different plasmids with average lengths of 1·8, 2·8 and 9·4 μm. These plasmids form two DNA peaks at 1·700 and 1·709 g/ml respectively. It is obvious that the multi-r-determinant plasmid pFS1 with the physical properties of 31·5 μm length and 1·71 g/ml density has been lost.

Table 3. *Physical characterization of plasmids involved in the transfer and transposition of translocatable drug-resistance elements.*

Strain	Plasmid	Length (μm) ± S.D.	No. of molecules measured	Density (g/ml)
UR12644-cu1	pFS2	9·4 ± 0·5	40	1·700
	pFS4	2·8 ± 0·22	43	1·709†
	pFS5	1·8 ± 0·18	53	
FS2203	pFS2	9·6 ± 0·32	7	1·700
	pFS402	6·2 ± 0·35	5	1·712
FS1012	pFS402	6·3 ± 0·13	11	1·712
FS01 (Ap^R)	pFS201	10·5 ± 0·4	43	1·701
		20·5 ± 0·4*	4	
FS02 (Tc^R)	pFS202	12·5 ± 0·2	36	1·705
		24·8 ± 1·1*	4	

* These molecules represent probably dimers of the monomeric pFS201 (pFS202).

† Correlation between this DNA-density with one of the cryptic plasmids (pFS4, pFS5) is assumed.

The plasmids of the transconjugant strain FS2203 belong to two size classes of 6·4 and 9·6 μm with the DNA densities of 1·700 and 1·712 g/ml. The fact that two plasmids are involved in the transfer of Tc^R-genes from UR12644-cu1 to NC22 shows a striking similarity to the mobilization of the Ap^R-plasmid pFS401 by the conjugative plasmid pFS2 (Schöffl *et al.* 1977). The identification of the Tc^R-plasmid pFS402 with the physical properties of 6·2 μm length and 1·712 g/ml density resulted from DNA transformation of *E. coli* HB101 using a mixture of pFS2/pFS402 DNA, isolated from strain FS2203. Tc^R transformants were obtained at a frequency of $10^5/\mu g$ DNA, all of them carrying pFS402. The results of the physical characterization of pFS402, which has been isolated from transformant strain FS1012, are depicted in Table 3. They show a length of 6·3 μm and a density of 1·712 g/ml. Self-transmission of pFS402 from strain FS1012 to *E. coli* S100 was not observed in filter crosses (detectable frequency 10^{-7}).

The infectious transfer of pFS402 seems to be due to a mobilizing ability of the conjugative plasmid pFS2 present in the transconjugant strain FS2203 and in strain UR12644-cu1. On the other hand, pFS402 is obviously not a representative plasmid of the cured strain. Neither UR12644-cu1 nor its parental strain UR12644 harbour plasmids with the physical properties of pFS402. Its appearance suggests spontaneous formation in UR12644-cu1 due to transposition of a tetracycline transposon from a chromosomal origin to one of the cryptic plasmids. Mobilization of the cryptic plasmids pFS4 and pFS5 in the presence of pFS2 could not be detected because no genetic markers are known residing on these plasmids. The mobilizing ability of pFS2 is not only restricted to pFS401 and pFS402. The hybrid plasmids ColE1:pSC101 and ColE1:Kan (Timmis, Cabello & Cohen, 1974) can also be efficiently mobilized in the presence of the pFS2-derivatives pFS201 and pFS202 (unpublished results).

(ii) *Translocation and physical characterization of the transposons Tn1771(Tc) and Tn1781(Ap)*

The translocation-ability of DNA-segments encoding Ap^R of pFS401 and Tc^R of pFS402 was tested using incompatibiliy between these two plasmids for selection of transposition events according to the report of Kleckner *et al.* (1977). This incompatibility phenomenon, preventing stable coexistence of both plasmids, was indicated in transformation experiments, introducing pFS402 into strain FS1011 (pFS401). Selection for the maintenance of the incoming plasmid resulted in a 93–95% loss of pFS401, indicated by ampicillin sensitivity of Tc^R transformants.

In order to examine the transposition of resistance determinants from plasmids pFS401 (Ap^R) and pFS402 (Tc^R) to the conjugative plasmid pFS2, the following experiments were conducted:

(*a*) *Construction of strains which initially carry the plasmids pFS2, pFS401, pFS402.* Matings between the plasmid donor strain FS2203 (pFS2, pFS402) and the $RecA^-$ recipient strain FS1011 (pFS401) were performed selecting the doubly resistant FS1011 transconjugants. The low frequency (2×10^{-6}) observed for the production of these transconjugants was probably due to the strong incompatibility between pFS401(Ap) and pFS402(Tc). Stable coexistence of both resistance markers (tested under non-selective conditions) presumably requires transposition of drug resistance elements accompanied by the loss of pFS401 or pFS402 respectively. The presence of the transfer plasmid pFS2 was deduced from the transferability of resistance markers using the transconjugant strains (FS1014, FS1015) as new donors in crosses with *E. coli* C (see below).

(*b*) *Isolation of strains carrying derivatives of pFS2 which have picked up translocatable drug resistance elements.* Secondary matings were performed using the doubly resistant transconjugants (FS1014, FS1015) as new plasmid donors in crosses with the recipient strain *E. coli* C. The criterion for the identification of pFS2 derivatives conferring Ap^R or Tc^R respectively, was the appearance of individual drug-resistant transconjugants among double-resistant ones. In fact, the cross FS1014 × C produced individual Ap^R transconjugants whereas the cross

FS1015 × C resulted in individual Tc^R transconjugants. The proportion of doubly resistant transconjugants appearing in both matings was about 60%.

(*c*) *Physical characterization of plasmids carrying transposons.* One Ap^R transconjugant, strain FS01, and one Tc^R transconjugant, strain FS02, were chosen for molecular analysis of their plasmids. The results of contour length measurements and DNA buoyant density determinations are depicted in Table 3. The plasmid pFS201 with a length of 10·5 μm and a density of 1·701 g/ml was isolated from the Ap^R transconjugant strain FS01. The formation of pFS201 suggests translocation of an ampicillin transposon, Tn1781 (1·4 μm), from pFS401 to pFS2 (length: 9·1 μm; density: 1·700 g/ml). The plasmid pFS202, which was isolated from the Tc^R transconjugant strain FS02, shows a contour length of 12·5 μm and a density of 1·705 g/ml. The generation of this plasmid suggests transposition of a tetracycline transposon (Tn1771) from pFS402 to pFS2. The physical properties of Tn1771 (length: 3·4 μm; density: 1·72 g/ml) were deduced from the differences of the molecular parameter between pFS2 and pFS202.

The appearance of doubly resistant transconjugants (Ap^R, Tc^R) in cross FS1014 × *E. coli* C is probably due to mobilization of pFS402 by pFS201 whereas in the cross FS1015 × *E. coli* C, transfer of pFS202 and mobilization of pFS401 determines the double resistant character of the transconjugants.

The genetic relationship between pFS201(Ap^R) and pFS202(Tc^R) is demonstrated by strong incompatibility between them. In experiments testing the stable coexistence of these plasmids, 100% loss of the recipient plasmid was observed following selection for acquisition of the incoming plasmid. The molecular relationship between pFS201 and pFS202 was examined by restriction analysis and this is reported in the next section.

(iii) *The restriction map of Tn1771(Tc) and Tn1781(Ap).*

Genetical and physical evidence suggested a molecular relationship between the plasmids pFS401 and pFS402 as well as between pFS201 and pFS202. On the other hand pFS401 and pFS201 had been expected to share common DNA-sequences due to their ampicillin transposon Tn1781. A similar relationship exists between the plasmids pFS402 and pFS202 due to the tetracycline transposon Tn1771. These relationships were examined using agarose gel electrophoresis analysis of restriction endonuclease digestions of plasmid DNA. Fig. 1 shows the fragmentation patterns of plasmids obtained after single or double digestion using the enzymes *Eco*RI, *Hin*dIII, *Pst*I, *Bam*HI, *Sal*I and *Sma*I.

Restriction sites mapping on the Tn1781-elements of pFS401 and pFS201 are 3 for *Pst*I and 1 for *Bam*HI as indicated by the comigration of fragments resulting from *Pst*I digests and *Pst*I/*Bam*HI double-digests (data not shown). It should be noted that these restriction sites map exclusively on the Tn1781-part of the plasmids. Compared to RP4, a plasmid carrying the ampicillin transposon Tn1, an identical fragment pattern of transposon-specific bands was observed.

The relationship between pFS202 and pFS402, resulting from the Tn1771-insertion, is featured by the appearance of two *Eco*RI-fragments banding at

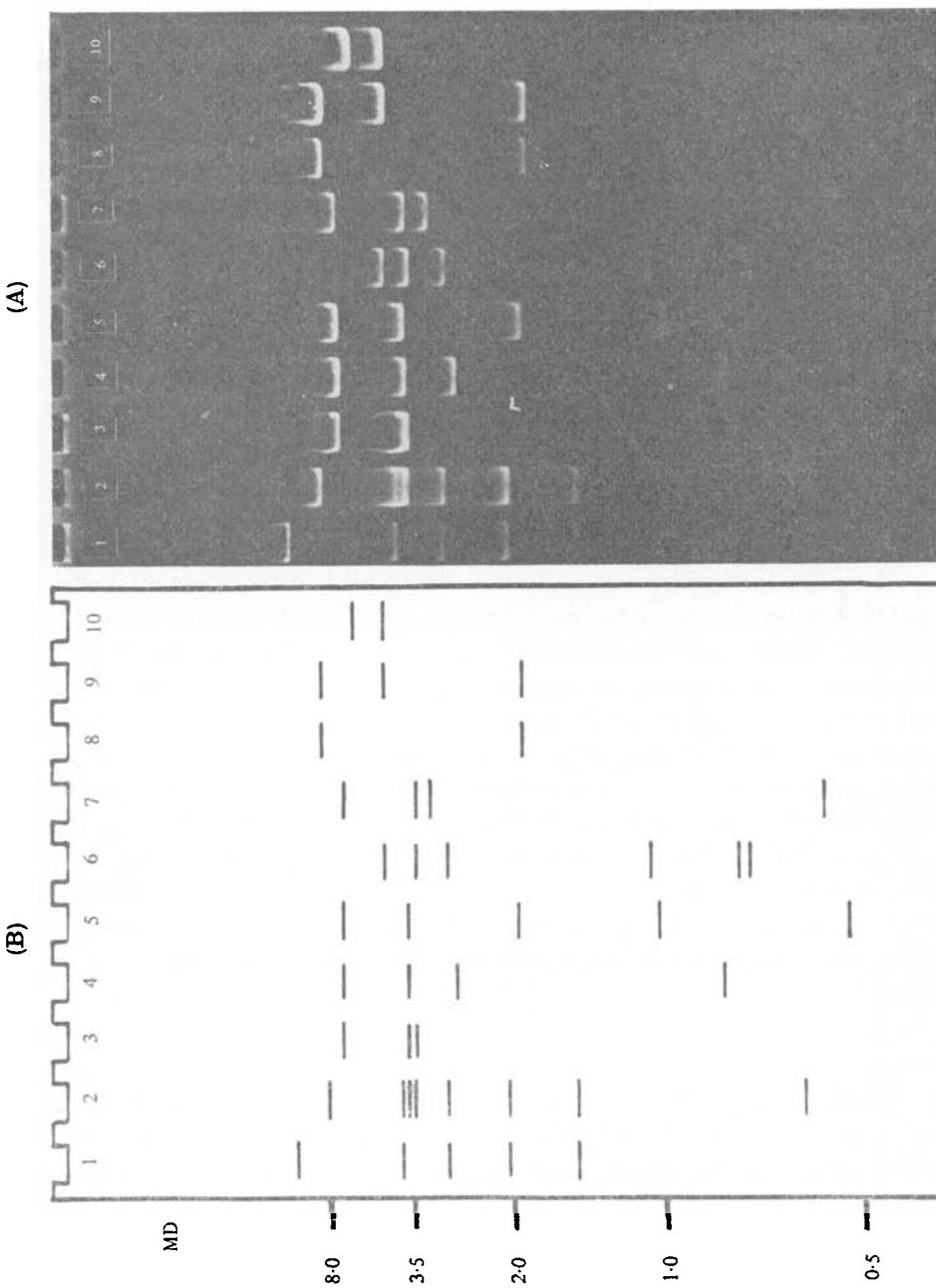

Fig. 1. (A) Restriction patterns of plasmids involved in the transposition of Tn1781 (Ap) and Tn1771(Tc). Lanes 1–2 show the fragment patterns obtained by *Eco*RI digestion of pFS201 and pFS202; lanes 3–7 show the patterns of pFS402 digested by *Eco*RI (3), *Eco*RI/*Sal*I (4), *Eco*RI/*Sma*I (5), *Eco*RI/*Hind*III (6) and *Eco*RI/*Pst*I (7); lanes 8–10 show *Hind*III generated fragment patterns of pFS401 (8), pFS402 (9) and pFS403 (10) (B) Diagram of the fragmentation patterns of (A) fitted with a scale of molecular weights given in Mdal.

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positions of 3·5 Mdal and 3·6 Mdal in the gel (not completely resolved in Fig. 1 lanes 2 and 3). The 3·5 Mdal-fragment is cut once by *Sal*I and twice by *Sma*I (lanes 4 and 5), whereas the 3·6 Mdal fragment carries additional restriction sites, one for *Hin*dIII and one for *Pst*I (lanes 6 and 7).

Leaving out the restriction sites of the transposon-specific DNA, one can deduce a common 'core region' for pFS401 and pFS402. This 'core plasmid' is characterized by two *Hin*dIII sites as indicated by the fragmentation patterns of pFS401 and pFS402 (Fig, 1, lanes 8 and 9).

Tn1781 is inserted within the larger *Hin*dIII fragment of pFS401 whereas the tetracycline transposon of pFS402 maps on the smaller *Hin*dIII-fragment (see Fig. 2). Plasmid pFS403, which is a derivative of pFS402, has lost one *Hin*dIII site along with the 3·6 Mdal *Eco*RI fragment of Tn1771 (see Fig. 1, lane 10). This plasmid was constructed *in vitro* by self-ligation of *Eco*RI-generated fragments of pFS402. Transformation of *E. coli* HB101 cells using the ligation mixture resulted in Tc^R transformants. Most of them have lost the 3·6 Mdal *Eco*RI fragment of pFS402. pFS403 represents one of these plasmids carrying the 3·5 Mdal fragment of Tn1771 in its original orientation. This experiment demonstrates that the genetic determinant for Tc^R maps on the remaining half of the transposon in pFS403. Further restriction analysis of this plasmid proved also the arrangement of *Hin*dIII sites mapping on the 'core plasmid' (Fig. 1, lane 10).

The molecular relationship between pFS201 and pFS202 is demonstrated by the similarities of their fragment patterns generated by *Eco*RI digestion. The migration rates of four different fragments are identical as shown in Fig. 1, lanes 1 and 2. The differences between these two fragmentation patterns result from the insertion of transposon DNA at different sites of these plasmids. Both elements are inserted within the largest *Eco*RI fragment of pFS2. The arrangement of the restriction sites mapping on these transposons was deduced from additional double digestion experiments (data not shown) using appropriate enzyme combinations. The results are summarized in Fig. 2, showing diagrams of pFS401, pFS402 and pFS403. The restriction maps of pFS201 and pFS202 are not completely known since only the *Eco*RI-enzyme cuts the plasmid-specific DNA. All the other restriction enzymes used in this study recognize only sequences within the transposon-segments of both plasmids.

4. DISCUSSION

The two transposable genetic elements described in this study were derived from different replicons of the originally mutiple-drug-resistant *E. coli* strain UR12644. The ampicillin transposon Tn1781 residing on pFS401 must have been previously located on the multiresistant R-plasmid pFS1, since transfer and curing of pFS1 are associated with an Ap^R and Ap^S phenotype, respectively. The generation of pFS401 suggests the involvement of the small cryptic plasmid pFS4 as a new substrate for the ampicillin transposon Tn1781 (see Fig. 3).

The formation of pFS402, carrying the new tetracycline transposon Tn1771 is due to very similar events. However, the original location of Tn1771 in UR12644

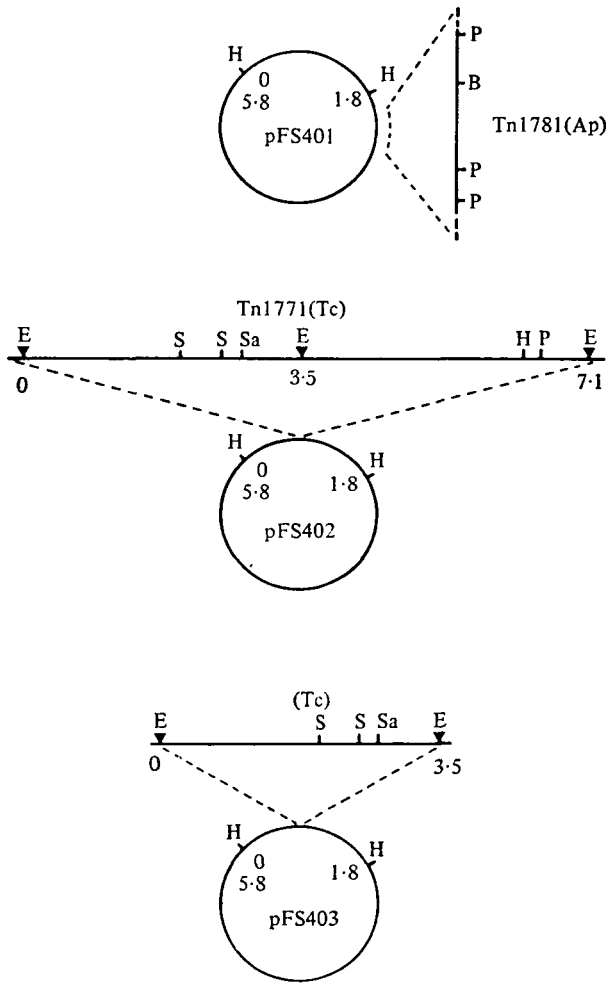

Fig. 2. Schematic diagrams of plasmids carrying insertions of transposon DNA sequences. Restriction endonuclease sites are: H for *Hin*dIII, P for *Pst*I, B for *Bam*HI, E *for Eco*RI, S for *Sma*I and Sa for *Sal*I. The distances of certain restriction sites on plasmids and transposon sequences are given in Mdal. The exact insertion of Tn1781(Ap) on pFS401 and the arrangement of its restriction sites is not yet ascertained accurately. pFS401 and pFS402 are naturally isolated plasmids, pFS-403 is a derivative of pFS402, constructed *in vitro* by self-ligation of *Eco*RI fragments.

is probably not the multi-resistance plasmid pFS1, since curing of this plasmid restores tetracycline resistance genes in strain UR12644-cu1. A possible location of the 'non-curable' Tc^R-genes of UR12644-cu1 might be the bacterial chromosome. Transposition of these genes corresponds with the generation of pFS402 which is not a representative plasmid of the cured strain (see Fig. 3).

The incompatibility of pFS401 and pFS402 and the arrangement of *Hin*dIII cutting-sites on these plasmids (see Fig. 2) suggests in both cases the usage of pFS4 as a common 'core plasmid' for the acquisition of Tn1781 and Tn1771. The

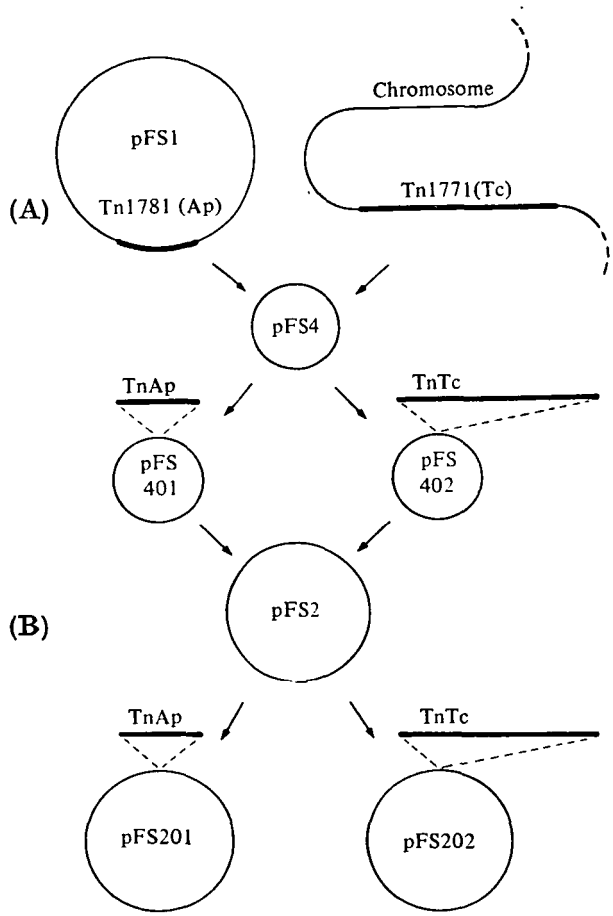

Fig. 3. Scheme of transposition of Tn1781(Ap) and Tn1771(Tc). (A) Transposition events generating pFS401 and pFS402 in *E. coli* UR12644 and UR12644-cu1 respectively. (B) Selected transposition generating pFS201 ond pFS202 in the *E. coli* HB101 derivatives FS1014 and FS1015 respectively. pFS1: R-plasmid conferring multiple drug resistance; pFS4: cryptic plasmid; pFS2: transfer plasmid present in the original donor strains mobilizes pFS401 and pFS402.

molecular weights deduced for the 'core plasmids' coincide in both cases with the molecular weight of pFS4 (2·8 Mdals).

The physical properties of Tn1781, transposed from pFS401 to pFS2, are very similar to that of the other well-known TnA elements Tn1, Tn2 and Tn3 (Hedges & Jacob, 1974; Heffron, Rubens & Falkow, 1975; Kopecko, Brevet & Cohen, 1976). The molecular weight of about 3 Mdal and the arrangement of *Pst*I and *Bam*HI restriction sites (data not shown) are identical with those of Tn1, present on the RP4 plasmid (DePicker, Van Montagu & Schell, 1977). The question whether the TnA element of pFS401 specifies TEM-1 or TEM-2 type of β-lactamase was not further investigated.

The tetracycline transposon Tn1771 represents a new transposable genetic element. Its transposition from pFS402 to the previously unmarked transfer plasmid

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pFS2 occurred in a *rec*A background, indicating a mechanism of illegitime recombination as proposed for several other translocatable elements (Rubens, Heffron & Falkow, 1976; Kleckner *et al.* 1978). The physical properties of Tn1771 (MW: 7·1 Mdal; ρ = 1·72 g/ml) in addition to the arrangement of *Eco*RI sites mapping on this DNA-segment, show some interesting features. The molecular weight of Tn1771, calculated from the size of its *Eco*RI fragments (3·5 plus 3·6 Mdal), coincides exactly with the molecular weight calculated from contour length measurements. This result suggests a nearly complete removal of the transposon from its insertion sites on the host replicons by *Eco*RI treatment (see Fig. 2). Considering the fact that transposable genetic elements are generally flanked by short inverted DNA segments at their termini (Bukhari, Shapiro & Adhyda, 1977; Kleckner, Roth & Botstein, 1977) it is suggested that the *Eco*RI sites map within the presumed DNA-repeats of Tn1771. Gene amplification studies on the r-determinant region of the transposon suggest the existence of a third DNA-repeat mapping at the position of the *Eco*RI site in the middle of Tn1771 (Schöffl & Pühler, 1979). The extension and the relative orientation of these short DNA-repeats is currently being investigated by DNA sequencing experiments. The unusual density of 1·72 g/ml calculated for the Tn1771 DNA may account for a foreign origin of this element outside *E. coli*.

The r-determinant region of Tn1771 (MW: 7·1 Mdal) was found to be restricted to only about one half (3·5 Mdal*Eco*RI fragment) of the transposon-DNA. Positive evidence resulted from the analysis of the Tc^R plasmid pFS403, which was constructed *in vitro* by self-ligation of *Eco*RI digested pFS402 (see Fig. 2). The resulting plasmid pFS403 has lost the 3·6 Mdal *Eco*RI fragment but still expresses Tc^R in its host.

Additional evidence resulted from an *in vivo* production of spontaneously formed Tc^R derivatives of pFS202, which all lack the 3·5 Mdal *Eco*RI fragment of the transposon but retain the 3·6 Mdal fragment (Schöffl and Pühler, 1979). The genetic properties encoded by the 3·6 Mdal fragment of Tn1771 are not yet known. The question of whether this fragment specifies functions for tranposition of the element can be tested experimentally in a suitable transcomplementation system.

In contrast to the transposability of the intact Tn1771 element, transposition of the r-determinnat region from pFS403 to the genome of a suitable λ vehicle: b_{515} b_{519}, int29, cI857, nin5 (Kleckner *et al.* 1979) could not be observed (unpublished results).

Compared to the two other tetracycline transposons characterized so far, Tn1771 shows striking resemblance to Tn1721, an element detected on plasmid pRSD1 (Schmitt, Bernhard & Mattes, 1979). Both the physical characteristics and the properties of selective gene amplification are very similar for both elements. The molecular relationship between Tn1771 and Tn1721 is currently under investigation by DNA hybridization studies. The Tn1771 and Tn10 element are not identical since the restriction map of Tn10 (Kleckner *et al.* 1978) differs significantly from the Tn1771 map (see Fig. 2). These two elements differ also with respect to the extension and arrangement of inverted repeats at their ends. In

contrast to the 1400 bp repeats of Tn10 (Ptashne & Cohen, 1975), Tn1771 contains three relatively short repeats (less than 100 base pairs long) flanking the entire transposon as inverted and the amplifiable r-determinant region as direct repeats (Schöffl and Pühler, 1979; Schöffl & Burkardt, 1979). The analysis of these DNA repeats acting as target sites in *rec*A-independent tranposition and *rec*A-dependent gene-amplification (Schöffl, 1979) may offer new insights into these processes and into the evolution of this element.

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