# Intramolecular amplification of the tetracycline resistance determinant of transposon Tn1771 in *Escherichia coli*

By F. SCHÖFFL and A. PÜHLER

*Institut für Mikrobiologie und Biochemie, Lehrstuhl für Mikrobiologie
der Universität Erlangen-Nürnberg, D 8520 Erlangen, Egerlandstr. 7, Germany*

## SUMMARY

Strains of *Escherichia coli* harbouring a conjugative plasmid that carries a transposon for tetracycline resistance (Tn1771) were found to be adaptable to very high tetracycline concentrations. The molecular analysis of plasmids isolated from strains with enhanced levels of tetracycline resistance revealed an intramolecular amplification of the resistance determinant of the tetracycline transposon.

A model for the molecular structure of the transposon is presented, which suggests that there are three repetitive DNA segments on Tn1771. This accounts for the properties both of transposition and of gene amplification.

## 1. INTRODUCTION

Several plasmid-mediated drug resistance determinants have been found to reside within discrete translocatable DNA segments (Hedges & Jacob, 1974; Kopecko & Cohen, 1975; Berg *et al.*, 1975; Kleckner *et al.*, 1975; Heffron, Rubens & Falkow, 1975). These genetic elements, termed transposons, are defined by their *recA*⁺-independent transposition to other cellular replicons (Goebel *et al.*, 1977; Kleckner, Roth & Botstein, 1977).

We recently reported the existence of a complex plasmid system in a multiply drug resistant clinical isolate of *Escherichia coli* (Schöffl, Pühler & Heumann, 1977). In this bacterial strain, UR12644, a new transposable genetic element conferring tetracycline resistance was identified. This transposon, designated Tn1771, has a molecular weight of 7·1 Mdal (megadalton), and two fragments of 3·5 Mdal and 3·6 Mdal are generated from it by the restriction endonuclease *Eco*RI (Schöffl & Pühler, 1979).

The topic of this paper is the intramolecular amplification in *E. coli* of that part of the transposon DNA sequence specifying tetracycline resistance.

17-2

## 2. MATERIAL AND METHODS

(i) *Bacterial strains and plasmids*. Wild type *E. coli* C (provided by Dr W. H. Traub, Erlangen) was used as a host strain for plasmid pFS202. pFS202 was constructed by inserting Tn1771 into pFS2. Plasmid pFS2 is a naturally occurring self-transmissible (Tra⁺) plasmid that originated from the clinically isolated *E. coli* strain UR12644 (Schöffl *et al.* 1977). pFS203 is a spontaneously formed tetracycline-sensitive (Tcˢ) derivative of pFS202. The properties of these plasmids are listed in Table 1.

Table 1. *Molecular and genetic properties of plasmids used for the study of transposon* Tn1771

| Plasmid | Phenotype | Molecular weight* (Mdal) | Derivation |
|---|---|---|---|
| pFS2 | Tra⁺ | 18·9 | Naturally occurring (Schöffl *et al.* 1977) |
| pFS202 | Tra⁺, Tcʳ | 26 | By insertion of Tn1771 into pFS2 (Schöffl & Pühler, 1979) |
| pFS203 | Tra⁺, Tcˢ | 22·4 | Spontaneously formed tetracycline-sensitive mutant of pFS202 (this paper) |

\* Determined by electron microscopic length measurements according to Lang (1970) and/or by agarose gel electrophoresis of restriction fragments using λDNA-fragments as molecular weight markers (Helling, Goodman & Boyer, 1974).

(ii) *Media and growth conditions*. Bacterial strains were cultured in Penassay Broth (Difco, antibiotic medium 3) with continuous aeration at 37 °C. Solid media contained 1·8 % Difco bacto agar. Tetracycline was routinely added to a final concentration of 3 $\mu$g/ml for the induction of tetracycline resistance of *E. coli* strains harbouring pFS202. Enhanced tetracycline resistance of *E. coli* C (pFS202) was selected by growth in the presence of 185 $\mu$g/ml tetracycline.

(iii) *Isolation 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 (ccc) plasmid DNA was purified by caesium chloride-ethidium bromide centrifugation (Radloff, Bauer & Vinigrad, 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, 1 mM EDTA, pH 8).

(iv) *Agarose gel electrophoresis of plasmid DNA*. ccc-plasmid DNA was analysed using 1–3 $\mu$g DNA loaded on a 0·5 % agarose gel in a buffer containing 36 mM Tris-HCl, pH 7·8, 30 mM-$NaH_2PO_4$ and 1 mM EDTA. Electrophoresis was carried out at 1·5 V/cm for 15 h at room temperature.

Restriction endonuclease 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 6 V/cm for 14 h at room temperature.
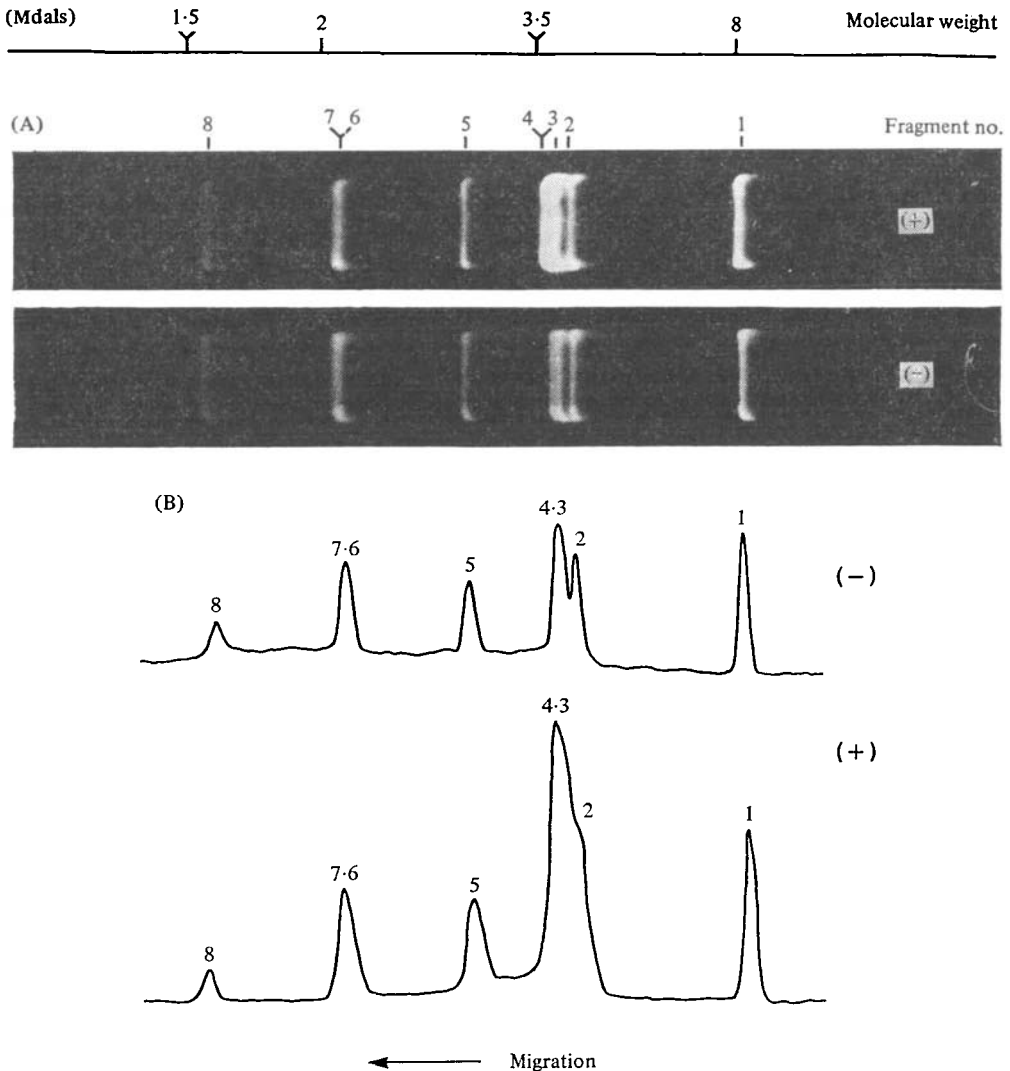
Fig. 2. Agarose gel electrophoresis of *Eco*RI digested plasmid DNA isolated from *E.coli* C (pFS202) grown in the presence and absence of high tetracycline concentrations.

(A) *Eco*RI restriction patterns of pFS202(−), non-amplified by tetracycline treatment, pFS202(+), amplified by treatment with 185 $\mu$g/ml tetracycline during cultivation.

(B) Densitometer tracing of the restriction patterns shown in A. For this experiment a laser scanning densitometer model 504 (Helen Instruments/Biomed Chicago) was used. Molecular weight estimates of fragments were based on migrates relative to *Eco*R generated fragments of λcI857 (Helling, Goodman & Boyer, 1974).

determinant is carried by the 7·1 mdals Tn1771 element, only a part of the DNA sequence of the transposon can be involved in the amplification process.

This amplification model was confirmed by further examination of the amplified pFS202 plasmid DNA, using the restriction endonucleases *Sma*I, *Hind*III and *Pst*I. For the non-amplified pFS202 plasmid it is known that the restriction sites of these enzymes are located only on the transposon segment of the molecule (Schöffl & Pühler, 1979). The relative positions of these cleavage sites on Tn1771 are shown on the physical map in Fig. 3(A). The Tn1771 DNA is cut by *Eco*RI enzyme into two fragments having molecular weights of 3·5 and 3·6 mdal. The Tn1771 DNA is cut by *Eco*RI enzyme into two fragments having molecular weights of 3·5 and 3·6 Mdal. The 3·6 Mdal fragment carries one *Hind*III and one *Pst*I site, whereas the 3·5 Mdal fragment contains two *Sma*I sites. The analysis of amplified pFS202 DNA with these restriction enzymes (results not shown) indicated one part of TN1771 DNA, probably the 3·5 Mdal *Eco*RI fragment, is involved in the amplification process. The resultant restriction map of the amplified structure of the transposon is depicted in Fig. 3(B). This structure differs from the normal transposon by a tandem repetition of the smaller *Eco*RI fragment carrying the two *Sma*I sites. The tandem arrangement of this fragment generates a characteristic new *Sma*I fragment, not present in the *Sma*I digest of non-amplified pFS202. It should be noted that Fig. 3(B) only shows the first step in the amplification process. Further amplification leads to an increase in the number of the directly repeated 3·5 Mdal fragments. This model is in accordance with the results shown in Fig. 1, where eight classes of plasmids isolated from cells with enhanced tetracycline resistance (Fig. 1, lane A) are shown.

Following the models developed for the amplification of R-determinants of certain R-plasmids in *Proteus mirabilis* (Rownd, Perlman & Goto, 1975) and *Streptococcus faecalis* (Yagi & Clewell, 1977) we suggest that the intramolecular amplification in pFS202 is due to small directly repeated DNA sequences flanking an amplifiable DNA region of Tn1771. Recombinational events occurring at these sites of homology probably result in the gene amplification process. Similar structures, termed recombination sequences, have been identified by electron microscopic heteroduplex studies on the *Streptococcus faecalis* plasmid pAMα1 carrying an amplifiable tetracycline resistance determinant (Yagi & Clewell, 1977).

The existence of the proposed direct repeats on Th1771 is also indicated by a spontaneous formation of tetracycline-sensitive derivatives of plasmid pFS202. It was found that tetracycline-sensitive cells are generated at frequencies between $10^{-2}$ and $10^{-3}$ in *E. coli* C (pFS202) cultures grown in tetracycline-free medium. From such Tc$^s$ strains, plasmid variants of pFS202 were isolated; all were found to lack the 3·5 Mdal *Eco*RI fragment of Tn1771. One plasmid variant, called pFS203, was further characterized. The molecular properties of this plasmid are summarized in Table 1. The restriction map of the residual part of Tn1771 on pFS203 is shown in Fig. 3(C). This structure of the reduced transposon suggests that the small direct repeats flanking the 3·5 Mdal fragment carry an *Eco*RI site.

One of these proposed direct repeats borders the Tn1771 element at the left end (Fig. 3A). Since Tn1771 carries a further *Eco*RI site close to the right end, one can speculate that a third repetitive sequence bonds Tn1771 at the right end.
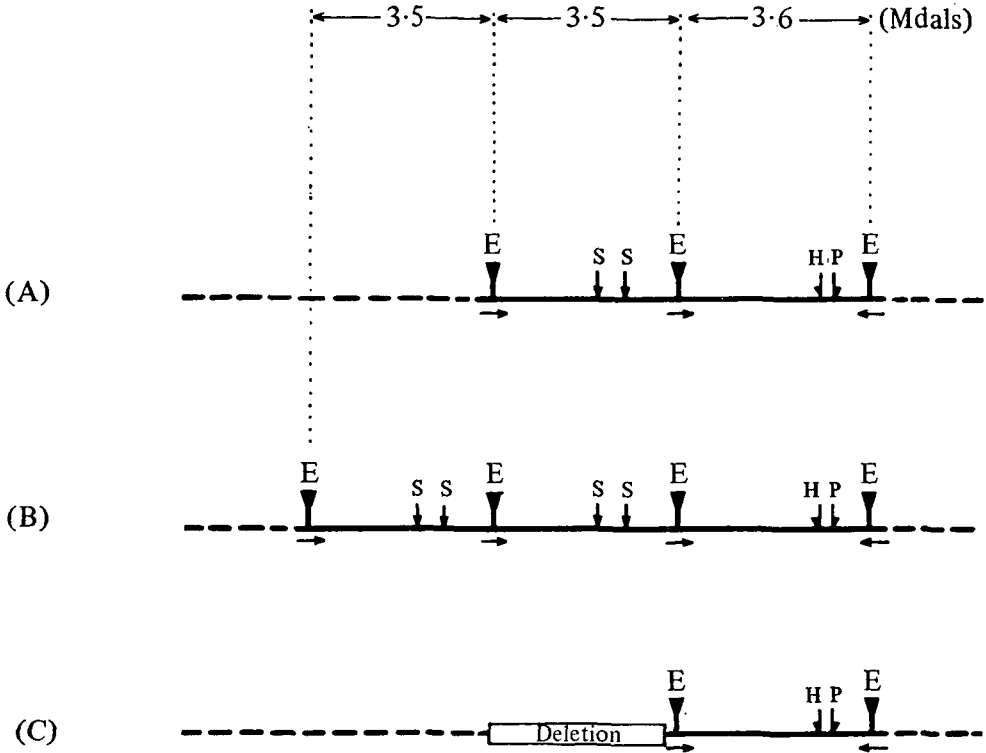


Fig. 3. Endonuclease cleavage maps of the transposon Tn1771. Restriction endonuclease cleavage sites on Tn1771 DNA are shown as E for *Eco*RI, S for *Sma*I, H for *Hind*III and P for *Pst*I. The arrows underlining the *Eco*RI sites indicate the proposed positions and orientations of small repetitive DNA sequences.

(A) Shows the normal structure of Tn1771, present on non-amplified pFS202 plasmids.

(B) Shows the amplified structure of Tn1771, deduced from the analysis of tetracycline amplified pFS202 molecules.

(C) Shows the residual part of the transposon Tn1771 found on the tetracycline-sensitive plasmid pFS203.

Horizontal dashed lines mark the connexions to the host plasmid DNA.

Taking into consideration that in general transposons are flanked by inverted repeats (Kopecko & Cohen, 1975; Berg *et al.* 1975; Heffron *et al.* 1975), we propose the following model (Fig. 3*a*). The two directly repeated sequences at the left end and in the middle are responsible for the amplification process whereas the inverted repeats at the ends are involved in the transposition process. This model accounts for the gene amplification and transposition of Tn1771, and also allows certain predictions that can be tested experimentally: first, it should be possible to detect the translocation of only the right half of Tn1771 (see Fig. 3*c*) which

is also flanked by inverted repeats, and, secondly, the left half of the transposon should allow the amplification of foreign DNA inserted it.

*Note added in proof.* During the preparation of this manuscript we were informed by R. Schmitt (University of Regensburg) of the amplification of a tetracycline resistance determinant located on a transposable genetic element on a plasmid which originated from a chicken in India. Both the physical and genetical properties of this element seem to be very similar to those of Tn1771.

## REFERENCES

BAZARAL, M. & HELINSKI, D. R. (1968). Circular DNA forms of colicinogenic factors E1, E2 and E3 from *Escherichia cgli*. *Journal of Molecular Biology* **36**, 185–194.

BERG, D. E., DAVIES, J., ALLET, B. & ROCHAIX, J.-D. (1975). Transposition of R factor genes to bacteriophage λ. *Proceedings of the National Academy of Sciences, U.S.A.* **72**, 3628–3632.

GOEBEL, W., LINDENMAIER, W., PFEIFER, F., SCHREMPF, H. & SCHELLE, B. (1977). Transposition and insertion of intact, deleted and enlarged ampicillin transposon Tn3 from mini-R1 (Rsc) plasmids into transfer factors. *Molecular and General Genetics* **157**, 119–129.

HANSEN, J. B. & OLSEN, R. H. (1978). Isolation of large bacterial plasmids and characterization of the P2 incompatibility group plasmids pMG1 and pMG5. *Journal of Bacteriology* **135**, 227–238.

HEDGES, R. W. & JACOB, A. E. (1974). Transposition of ampicillin resistance from RP4 to other replicons. *Molecular and General Genetics* **132**, 31–40.

HEFFRON, F., RUBENS, C. & FALKOW, S. (1975). Translocation of a plasmid DNA sequence which mediates ampicillin resistance: Molecular nature and specificity of insertion. *Proceedings of the National Academy of Sciences, U.S.A.* **72**, 3623–3627.

HELLING, R. B., GOODMAN, H. M. & BOYER, H. W. (1974). Analysis of endonuclease R.EcoRI fragments of DNA from lamboid bacteriophages and other viruses by agarose-gel electrophoresis. *Journal of Virology* **14**, 1235–1244.

KLECKNER, N., CHAN, R., TYE, B. & BOTSTEIN, D. (1975). Mutagenesis by insertion of a drug-resistance element carrying an inverted repetition. *Journal of Molecular Biology* **97**, 561–575.

KLECKNER, N., ROTH, J. & BOTSTEIN, D. (1977). Genetic engineering *in vivo* using translocatable drug-resistance elements: New Methods in Bacterial Genetics. *Journal of Molecular Biology* **116**, 125–159.

KOPECKO, D. J. & COHEN, S. N. (1975). Site-specific recA-independent recombination between bacterial plasmids: Involvement of palindromes at the recombinational loci. *Proceedings of the National Academy of Sciences, U.S.A.* **72**, 1373–1377.

LANG, D. (1970). Molecular weights of coliphages and coliphage DNA. III. Contour length and molecular weight of DNA from bacteriophages T4, T5 and T7 from bovine papillon virus. *Journal of Molecular Biology* **54**, 557–565.

RADLOFF, R., BAUER, W. & VINOGRAD, J. (1967). A dye-buoyant-density method for the detection and isolation of closed circular duplex DNA. The closed circular DNA in Hela cells. *Proceedings of the National Academy of Sciences, U.S.A.* **57**, 1514–1520.

ROWND, R. H., PERLMAN, D. & GOTO, N. (1975). Structure and replication of R-factor deoxyribonucleic acid in *Proteus mirabilis*. In *Microbiology–1974* (ed. D. Schlessinger), pp. 76–94. Washington, D.C.: American Society.

SCHÖFFL, F., PÜHLER, A. & HEUMANN, W. (1977). Identification and characterization of a new resistance transfer factor isolated from *Escherichia coli*. *Plasmids*, Medical and Theoretical Aspects (ed. S. Mitsuhashi *et al.*), pp. 161–170. Berlin: Springer-Verlag.

Schöfel, F. & Pühler, A. (1979). The plasmid system of the clinically isolated *Escherichia coli* strain UR12644: Identification and molecular characterization of the tetracycline transposon Tn1771. *Genetical Research* (sumitted for publication).

Yagi, Y. & Clewell, D. B. (1977). Identification and characterization of a small sequence located at two sites on the amplifiable tetracycline resistance plasmid pAMα1 in *Streptococcus faecalis. Journal of Bacteriology* **129**, 400–406.