New Translocation Sequence Mediating Tetracycline Resistance Found in Escherichia coli Pathogenic for Piglets

SIGRID TUE JØRGENSEN,† BRUNELLO OLIVA,‡ JOHN GRINSTED, AND PETER M. BENNETT

Department of Bacteriology, The Medical School, University of Bristol, Bristol BS8 1TD, England

A discrete piece of deoxyribonucleic acid coding for tetracycline (Tc) resistance was found to move from one R plasmid to another in an Escherichia coli strain which is pathogenic for piglets. Since this phenomenon took place also in rec strains, the Tc segment was classified as a transposon and called Tn804. Restriction enzyme analysis with EcoRI, BglII, and HindIII indicated that Tn804 is related to Tn10, a well-known transposon that codes for resistance to tetracycline.

Hybridization between plasmids carrying the two transposons provided proof of homology between Tn10 and part of Tn804. Electron microscopic studies showed a transposon-like structure composed of one loop-stem structure with inverted repetitions of approximately 0.9 megadaltons inserted into the loop of a second loop-stem structure. It is suggested that Tn804 is composed of Tn10 plus another transposable sequence.

Genes encoding resistance to tetracyclines are commonly found on a wide range of resistance (R) plasmids (2), and in some cases these genes comprise part of a transposon (10, 18).

We are currently studying a plasmid-borne tetracycline (Tc) resistance determinant that was found in a strain of Escherichia coli pathogenic for piglets. This strain, 1324, contains several plasmids among which are two R plasmids as described in a previous publication (9). Genetic experiments reported there show that a deoxyribonucleic acid (DNA) segment coding for tetracycline resistance moves from one R plasmid to the other in the absence of the recA function. The hypothesis was therefore put forward that the Tc marker might form part of a transposon. This hypothesis is confirmed in the present paper, which describes the experiments that led to the identification of the Tc marker as part of a transposon. Furthermore, data are presented which indicate that the transposon is a novel derivative of the tetracycline resistance-encoding transposon Tn10 (10).

MATERIALS AND METHODS

Strains, plasmids, and bacteriophages. Strain 1324, antigen type O8:K87 with resistance markers Tc, Cm (chloramphenicol), Sm (streptomycin), and Su (sulfonamide), was isolated at Statens Veterinære Serumlaboratorium, Copenhagen, from a piglet that had died from E. coli colitis. The reference strains and bacteriophages were those mentioned in a previous publication (9). Plasmids are listed in Table 1.

† Present address: Department of Animal Genetics, Royal Veterinary and Agricultural University, Copenhagen, Denmark.
‡ Present address: Institute of Microbiology, Faculty of Medicine and Surgery, University of Rome, Rome, Italy.

Media and antibiotics were those described by Jørgensen (9). The conjugation technique was also described in that paper. Transduction and transformation were done according to Lee and Nathans (13) and Cohen et al. (3), respectively.

Plasmid DNA was isolated as described by Petrocheilou et al. (16), with the exception that the dialysis step was replaced by precipitation with 4 N sodium acetate and ice-cold ethanol (S. T. Jørgensen, J. Grinsted, P. Bennett, and M. H. Richmond, Plasmid, in press).

Restriction enzyme analysis was carried out as described by Grinsted et al. (6).

Electron microscopy was performed according to Bennett and Richmond (1), and heteroduplex analysis was performed as described by Lai and Nathans (12).

Transfer of DNA to nitrocellulose filters (Schleicher and Schuell, BAS8) was carried out as described by Southern (20), as modified by Hutchison (7).

Plasmid DNA was labeled in vitro by nick translation using the technique of Maniatis et al. (14). DNA polymerase I was purchased from Boehringer, and deoxyadenosine 5′-[α-32P]triphosphate was obtained from Amersham. Labeled DNA was separated from unincorporated nucleotides by precipitation with isopropanol.

Before hybridization, the nitrocellulose membranes were soaked in Denhardt’s mixture (5) for at least 2 h at 60°C. The membranes were cut into strips corresponding to the channels containing DNA in the original gel and inserted into dialysis tubing (Visking size 1, 8/32 in. [ca. 0.64 cm]). Labeled probe DNA, which had been precipitated to separate it from unincorporated nucleotides, was suspended in 100 µl of 10 mM tris (hydroxymethyl) aminoethanehydrochloride (pH 7.5) and 0.1 mM ethylene diaminetetraacetic acid at 37°C, heat denatured (100°C, 10 min) together with 30 µg of calf thymus DNA, and quickly mixed with ice-cold hybridization buffer (4× SSC [1× SSC = 0.15 M NaCl plus 0.015 M sodium citrate], 40% formamide, and 0.1% sodium dodecyl sulfate) to a final volume of
were resistant other gave plasmid of transformants gave to croscope, of gants. Two latter class strain, inferred sufficiently i.e., irrespective fomamide with only marker transfer the that amide. Further and all streptomycin, were inferred from to E. coli. 

<table>
<thead>
<tr>
<th>Plasmid designation</th>
<th>Relevant markers*</th>
<th>Size (Mdal)</th>
<th>Source (reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pHG24</td>
<td>Cm Sm Su</td>
<td>52</td>
<td>Strain 1324 (9)</td>
</tr>
<tr>
<td>pHG27</td>
<td>Tc Sm</td>
<td>82</td>
<td>Strain 1324 (9)</td>
</tr>
<tr>
<td>pHG31-1</td>
<td>Tc Cm Sm Su</td>
<td>64.5</td>
<td>This paper</td>
</tr>
<tr>
<td>pHG31-2</td>
<td>Tc Cm Sm Su</td>
<td>64.0</td>
<td>This paper</td>
</tr>
<tr>
<td>R388:Tn10</td>
<td>Tc Tp Su</td>
<td>ND*</td>
<td>Ward (unpublished)</td>
</tr>
<tr>
<td>R388</td>
<td>Tp Su</td>
<td>22</td>
<td>(4)</td>
</tr>
</tbody>
</table>

Abbreviations used are those recommended by Novick et al. (15). Tp, Trimethoprim resistance.

ND, Not determined.

3 ml. This was placed in a dialysis bag and hybridized for 20 to 48 h at 50 ± 2°C. These conditions comply with Tm = 20°C for a DNA of 50 mol% guanine plus cytosine content (J. G. Wetmur, Ph.D. thesis, California Institute of Technology, Pasadena, 1967). After hybridization, filters were removed from the dialysis bags, washed three times in 2x SSC at room temperature, dried, and autoradiographed for 1 to 4 days at room temperature using Kodak X-ray film (Kodirex EP).

RESULTS

Separation of linkage groups and origin of Tc marker. Conjugal transfer of the resistance markers Tc, Cm, Sm, and Su of strain 1324 to E. coli K-12 initially indicated that the markers were present on two replicons. This was inferred from the following. When selection was with chloramphenicol or streptomycin, transconjugants with the phenotype Cm Sm Su were found in very high numbers, but when tetracycline was used only a few transconjugants appeared, and all were resistant to tetracycline, chloramphenicol, streptomycin, and sulfonamide. Further experiments showed, however, that the tetracycline-resistant transconjugants were of two classes: one (4/13 clones) could transfer the complete marker pattern such that all transconjugants were resistant to tetracycline, chloramphenicol, streptomycin, and sulfonamide irrespective of the marker selected; the other class (9/13 clones) could transfer the four-marker group Tc, Cm, Sm and Su rarely, and only with selection with tetracycline, but transferred the three-marker group irrespective of marker selection, Cm, Sm, and Su very efficiently i.e., behaved like the original donor strain, 1324, in transfer of markers.

Plasmid DNA was isolated from one of the latter class of tetracycline-resistant transconjugants. Two plasmids were seen by electron microscope, one of 52 megadaltons (Mdal) and the other of 82 Mdal. Transformation of E. coli C600 to chloramphenicol resistance with this DNA gave transformants containing a single plasmid of 52 Mdal; transformation to tetracycline resistance gave transformants containing a single plasmid of 82 Mdal. The former transformants were resistant to chloramphenicol, streptomycin, and sulfonamides, and the latter were resistant to tetracycline and streptomycin. Thus, the transconjugant from which the plasmid DNA was isolated contained two plasmids: one, called pHG24 (52 Mdal), codes for resistance to chloramphenicol, streptomycin, and sulfonamide, and the other, pHG27 (82 Mdal), codes for resistance to tetracycline and streptomycin. It is probable that this is also the case with the original strain, 1324.

The data suggest that pHG24 is tra* and that pHG27 is tra, but that pHG24 can mobilize pHG27. Thus, both plasmids would have been transferred to the tetracycline-resistant transconjugants that behaved like the original strain. However, when plasmid DNA was isolated from the tetracycline-resistant transconjugants that transferred their marker pattern as a single unit, only one plasmid was seen in agarose gels and in the electron microscope. Its molecular mass was about 64 Mdal, as determined by contour length measurements. A heteroduplex between one such plasmid, pHG31-1, and pHG24 shows an insertion loop indicating that pHG31-1 is a recombinant of pHG24 with DNA from pHG27 coding for tetracycline resistance (Fig. 1). The insertion of DNA in pHG24, about 12 Mdal, is, however, more complex than expected: instead of a single loop-stem structure, the element consists of one loop with a stem, i.e., inverted repetitions, of about 0.9 Mdal inserted into another loop-stem structure in which the inverted repeat is short. Similar structures have been found in other recombinant plasmids (pHG31-2 and pHG31-3), where, according to restriction data, the 12-Mdal element has inserted into other sites in pHG24.

Such recombination in strains containing pHG24 and pHG27 generates tra plasmids carrying the complete marker series (Tc, Cm, Sm, and Su); transfer of these plasmids would then produce transconjugants that could themselves transfer the whole series of markers. The generation of such recombinants from the two plasmids was observed to occur also in rec strains, so there is prima facie evidence that pHG27 contains a transposable element that encodes tetracycline resistance, has a molecular
mass of about 12 Mdal, and can transpose to pHG24. We call this element Tn804.

Molecular analysis of the transposable element. Plasmids from two transconjugants that each contained one such recombinant plasmid (called pHG31-1 and pHG31-2) were analyzed with restriction enzymes. The patterns observed were only slightly different from those seen after digestion of pHG24: for instance, in the case of EcoRI, both pHG31-1 and pHG31-

![Fig. 1. Heteroduplex formed between DNA of plasmids pHG24 and pHG31-1.](https://journals.asm.org/journal/aac)
2 had lost one of the parental fragments and gained two extra fragments, but the actual fragments lost and gained were different in the two recombinants (Fig. 2a). This is consistent with the insertion at different points in pHG24 of a DNA segment containing a single EcoRI site. Formation of a heteroduplex between the two recombinant plasmids showed two apparently identical insertions that are separated by about 1.5 Mdal (Fig. 3).

**Comparison of Tn804 with Tn10.** Tn10 is a well-known transposon that codes for resistance to tetracycline (10). It was therefore of interest to see whether there is any similarity between Tn10 and Tn804.

Data from restriction enzyme analyses show that pHG31-1 and pHG31-2 each have three BgIII fragments which are not present in pHG24 (Fig. 2b, lanes 1, 2, and 4), and which are consequently generated by cutting with BgIII at sites inside Tn804. The smallest of these is 1.6 Mdal (Fig. 2b, arrow), i.e., the same size as a BgIII fragment in Tn10 (Fig. 2b, lane 3; (8)). Furthermore, pHG31-1 and pHG31-2 each have one HindIII fragment of 2.9 Mdal, as has Tn10 (data not shown); pHG24 lacks this fragment, which therefore must be part of Tn804.

These data encouraged us to look for homology between the DNA of Tn804 and that of Tn10. Consequently, a BgII digest of pHG31-1 was hybridized against a 32P-labeled R388::Tn10 probe (Fig. 4). It is obvious (Fig. 4) that there is strong homology between the 1.6-Mdal fragment of Tn804 and sequences on R388::Tn10, while two other pHG31-1 fragments blacken the film to a lesser extent. A control hybridization between pHG31-1 and R388 (data not shown) did not show any significant homology between these plasmids. Reversing the procedure and using 32P-labeled pHG31-1 confirmed that this plasmid carries sequences homologous to those on R388::Tn10, but missing from R388 (data not shown). We conclude, therefore, that Tn804 has DNA sequences in common with Tn10.

**DISCUSSION**

A transposon is defined as a discrete piece of DNA which can move from one site in a replicon to another site in the same, or a different, replicon by a mechanism which is independent of the host cell's recombination system (10). Tn804, a nucleotide sequence that encodes tetracycline resistance, fulfills this condition. Furthermore, it integrates into several distinct sites on pHG24, as expected of transposition-mediated recombination (10).

The physical appearance of Tn804 in heteroduplex studies is one of a double loop-stem structure (Fig. 3) in which the terminal loop-stem structure is similar in size to that of another tetracycline resistance transposon, Tn10 (17, 19). Restriction enzyme analysis with EcoRI, BgIII, and HindIII indicates similarities between Tn804 and Tn10. Both have a single EcoRI site, both are cut by BgIII to give a fragment of 1.6 Mdal, and both yield a HindIII fragment of 2.9
Fig. 3. Heteroduplex formed between DNA of plasmids pHG31-1 and pHG31-2. The arrows indicate the two Tn804 structures.
Mdal. Not only are these fragments the same size in the two transposons, but the homology between their base sequences is also extensive, as shown by hybridization experiments (Fig. 4).

Consequently, Tn8O4 contains an element which is Tn10-like, but the size of Tn8O4, as well as its appearance in the electron microscope as a double loop-structure, show that it is not Tn10. It is tempting to suggest, on the basis of the electron microscope data (Figs. 1 and 3), that Tn8O4 is Tn10 inserted into another transposable sequence, very like the relationship between Tn3 and Tn4 on R1 (formerly TnA and TnS, respectively [11]).

As we have not yet succeeded in transposing Tn8O4 to anything but pHG24, we do not know whether the double loop-structure is now a permanent arrangement or whether one structure can still transpose independently of the other. Nor do we know whether the non-Tn10-like part of Tn8O4 carries any markers. However, experiments are in progress to determine this as well as to construct a complete map of Tn8O4.

ACKNOWLEDGMENTS

S.T.J. was in receipt of a Carlsberg-Wellcome travelling research fellowship, which is gratefully acknowledged. B.O. was the recipient of a grant from Fondazione Cerchi-Bolognetti.

LITERATURE CITED


