Translocation of the Tetracycline Resistance Determinant from R100-1 to the Escherichia coli K-12 Chromosome

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Pairs of normally incompatible derivatives of R100-1 (one Chl\(^+\) Tet\(^R\), the other Chl\(^R\) Tet\(^R\)) were forced to coexist in a recA host by selection for Chl\(^R\) Tet\(^R\) cells. After many generations stable derivatives were isolated. The analysis of nine independent stabilization experiments showed that in each case Tet\(^R\) was translocated from the plasmid to the chromosome of the host. No evidence for the joint integration of other plasmid genes (those controlling transfer, antibiotic resistance, incompatibility, or origin of transfer replication) was obtained. One of the chromosomal Tet\(^R\) determinants was mapped close to metE.

Closely related plasmids often fail to replicate stably when harbored in the same cell, one of the plasmids being lost at cell division (incompatibility; 17). Several reports have appeared of attempts to isolate stabilized derivatives by selection for properties specified by two incompatible plasmids. Willetts and Bastarrachea (30) and Palchauhuri et al. (18) isolated stable fused F' elements from F'/F' heterozygotes at a low frequency in recA cells. Escherichia coli mutants which can harbor stably two F' factors have also been described (19, 22). Compatible (Inc\(^-\)) mutants have been isolated from F/Hfr heterozygotes: in some (14) the stabilized derivatives contained Inc\(^-\) Tra\(^-\) deletions of the integrated F plasmid, but more recently (3) Inc\(^-\) Tra\(^+\) strains have been obtained; the Inc\(^-\) phenotype, however, was expressed only when the F plasmid was integrated into the chromosome.

It is possible that incompatibility is a manifestation of the control of replication of autonomous plasmids and that Inc\(^-\) plasmids may show altered patterns of replication. We have therefore isolated and analyzed stable derivatives of strains carrying differently marked derivatives (Tet\(^R\) Chl\(^R\) and Tet\(^R\) Chl\(^R\)) of the Tet\(^R\) Chl\(^R\) plasmid R100-1. Since recombination between incompatible R plasmids occurs at high frequency in rec\(^+\) bacteria, but not detectably in recA (6), the experiments were carried out in a recA host. Stable Tet\(^R\) Chl\(^R\) derivatives were isolated after prolonged selection on Tet + Chl-containing agar; in all derivatives the Tet\(^R\) Chl\(^R\) plasmid remained in the extrachromosomal state, whereas the Tet\(^R\) marker was translocated to the bacterial chromosome, the other markers on the plasmid being lost. Some properties of these derivatives are described.

MATERIALS AND METHODS

Media. The composition of the nutrient broth and nutrient agar has been described by Foster and Walsh (8), and the minimal agar is described by Foster and Willetts (manuscript in preparation).

Antibiotics were used at the following concentrations: tetracycline (Tet; Lederle; 20 μg/ml), chloramphenicol (Chl; Parke-Davis; 20 μg/ml), rifampin (Rif; Ciba; 50 μg/ml), streptomycin (Str; Glaxo; 10 μg/ml for testing for plasmid-determined resistance, 500 μg/ml for chromosomal resistance). Sulfonamide (Sul) resistance was tested on agar containing 3% lysed horse blood and 500 μg of sulfathiazole per ml (Abbott Laboratories).

Bacterial strains and plasmids. Bacterial strains and plasmids are listed in Table 1.

Plasmid transfer. For strains, construction of 0.2 ml of exponential-phase donor and recipient cultures were mixed and incubated for 60 min. Exconjugants were selected by streaking on selective agar. Transfer frequency measurements were performed by mixing 0.5 ml of exponential donor culture with 4.5 ml of recipient and incubating for 30 min before spreading suitable dilutions on agar to select for exconjugants. Transfer frequencies are expressed as exconjugants per 100 donors.

Transduction. Transduction was performed with bacteriophage P1 (13, 27). P1vir was used in transduction from recA strains since other derivatives of P1 will not grow on Rec\(^-\) bacteria (31).

Isolation of Tet\(^R\) translocation strains. The recA strains ED2030 and JC6310 carrying pDU8 and pDU100 or pUB201 and pUB231 were plated on Chl + Tet agar. Sample hetero-R colonies were grown over-
night in broth and spotted onto Chl + Tet agar to select for a population of hetero-R cells. The spots were replica plated eight times alternately on drug-free and Chl + Tet agar to select for faster growing (presumably stabilized) Chl" Tet" derivatives. Growth on drug-free medium presumably allows segregation of the unstable plasmid heterozygotes without the extensive re mating that occurs in liquid culture. The final replicas were purified on Chl + Tet agar. Sometimes a mixture of small and large colonies was observed after incubation for 24 h. Subsequent analysis showed that the small colonies harbored the two markers unstably, whereas the large colonies inherited stably the Chl" and Tet" markers. This procedure allowed stabilized derivatives to be isolated in nine of nine independent experiments.

Conjugational complementation. Conjugational complementation tests between the transposed Tet" derivatives of JC372 and Flac Tra- mutants were performed as described by Foster and Willetts (in preparation). The amber-suppressible Flac Tra- elements were transferred into the Lac": Tet" JC372 strains by mobilization from the amber-suppressing host JC6255 selecting for Lac" [Tet" Str] progeny. After purification, these were mated with DU1000 selecting for Lac- [Nal"] exconjugants. Flac transfer-deficient mutants in non-plasmid-specific genes traA through traD are complemented by R100-1 Tra" and the appropriate R100-1 Tra- deletion mutants; the order of the genes in the Tet" region of R100-1 is probably -inc-ori?-traJ-traA-traC-traD (Foster and Willetts, in preparation).

Plasmid incompatibility. The presence of the plasmid incompatibility determinant (Inc") in the
JC3272 Tet^r translocation strains was tested by introducing a Chi^R Tet^r R100-1 mutant (pDU8) by conjugation and selecting for Chi^R Tet^r progeny. Colonies were picked without further purification, grown overnight in drug-free broth, and plated on drug-free agar for single colonies. Between 100 to 500 colonies were replica plated onto Chi + Tet agar. Inc^- derivatives were >99% stable, whereas incompatible pairs showed 20 to 50% segregation of one or another of the markers.

**Mobilization of tetracycline resistance.** R100-1 Tet^r mutants pDU8 and pDU17 were transferred into the JC3272 Tet^r strains by conjugation. These derivatives were mated with DU1004, and the frequency of transfer of Tet^r and Chi^R was determined as described above.

**Mapping experiments.** Chromosomal Tet^r markers were transduced to Hfr strains, and preliminary mating experiments were performed to determine the approximate map position of the markers. The Tet^r determinant from one translocation strain (DU3083) was mapped more precisely by interrupted mating experiments with P4-X and KL14 as donors and CSH57 as the recipient. Transduction experiments with the recipient strains LC607 and CSH57 were performed to demonstrate linkage of Tet^r with a chromosomal gene. ilu^r, met^r, and Tet^r transductants were selected in the appropriate strain. These were scored for inheritance of the unselected marker (Table 3).

**Isolation of a recA derivative of DU3083.** A thyA'^- mutant of DU3083 was selected by the trimethoprim Procedure (15); after purification, this was mated for 30 min with JC5088, an Hfr thyA'^- recA56 strain that transfers the thyA recA region early, and Thy'* recombinants were selected. These were screened for ultraviolet sensitivity, and putative recA^- derivatives were purified and tested for recombination deficiency.

**RESULTS**

**Isolation of stable E. coli strains initially harboring two derivatives of R100-1.** Two differently marked derivatives of R100-1 (one Chi^R Tet^r, the other Chi^R Tet^r) were forced to coexist in the same recA host as described above. After several alternate replica platings on drug-free and selective (Chi + Tet) agar, single Chi^R Tet^r colonies were isolated and tested for the stability of the two markers. Altogether, nine independently isolated stable Chi^R Tet^r strains have been investigated.

P1vir was propagated on the stabilized strains, and Chi^R and Tet^r transductants were isolated. All the Chi^R transductants were found to contain the parental Chi^R R factor and were not investigated further. The JC3272 Tet^r strains (see Table 1) were tested to determine the nature of the Tet^r determinant.

All the JC3272 Tet^r strains were Chi^R and Sul^r and were also transfer deficient in 30-min matings with DU1000 (<10^-4%). In addition, no evidence was obtained for the presence of any R100-1 tra genes. No conjugational complementation was obtained with traA'^-, traC'^-, and traD'^- mutants of Flac. It has been found that R100-1 Tra^+ and the appropriate R100-1 Tra^- mutants will complement Flac mutants in these non-plasmid-specific genes (Foster and Willets, in preparation). It was not possible to determine whether traJ was present in the JC3272 Tet^r strains, as R100-1 traJ point mutants are not known and complementation of Flac traJ mutants would not occur because traJ is a plasmid-specific gene.

A Tet^r Chi^R derivative of R100-1 (pDU8) was introduced into the Tet^r JC3272 strains by conjugation. This plasmid was always inherited stably after growth in drug-free medium, indicating that the Tet^r marker was not associated with the plasmid incompatibility gene(s).

Phage P1 was propagated on the JC3272 Tet^r strains, and the frequency of transduction of Tet^r to rec^- and recA^- strains was measured. Tet^r was always transducible to rec^+ strains, but never to recA^- strains. This suggests that the Tet^r markers of pUB201 and pDU100 have been translocated to the E. coli chromosome in the absence of any other R-factor genes.

The tetracycline resistance determined by R100-1 is inducible. Growth and challenge tests in broth (8) showed that the translocated Tet^r marker was inducible to the same level as the plasmid-borne gene, and the genes that control expression of Tet^r are therefore closely linked to the structural gene(s).

The majority (ca. 90%) of spontaneous Tet^r mutants of R100-1 are deletions that have lost the whole tet region (frequency ca. 10^-4/cell generation; T. J. F., unpublished data). This instability may be conferred by the inverted repeat sequence of deoxyribonucleic acid thought to surround the Tet^r marker (23). The Tet^r marker of one JC3272 derivative (DU3083) was investigated. Eight Tet^r mutants were isolated from independent nonmutagenized cultures as previously described (5). Only one of eight mutants yielded detectable wild-type Tet^r revertants, which suggests that the translocated Tet^r marker retains the deoxyribonucleic acid sequences that predispose it to be deleted.

One further R factor marker that is closely linked to tet on R100-1, ori (the origin of transfer replication), was investigated. R100-1 Tet^- Tra^+ plasmids were transferred into the Tet^r translocation strains. The strains bearing pDU8 (a single-site revertible Tet^r mutant) that were constructed for the incompatibility tests
described above were mated with DU1004, and Tet\textsuperscript{R} [Rif\textsuperscript{R}] and Chi\textsuperscript{R} [Rif\textsuperscript{R}] progeny were selected. The Tet\textsuperscript{R} marker was transferred at a 5,000- to 8,000-fold lower rate than the plasmid Chi\textsuperscript{R} marker (Table 2). But since the Tet\textsuperscript{R} lesion carried by pDU8 is a point mutation, there may be considerable homology between this allele and the translocated Tet\textsuperscript{R} marker, and recombination might occur to repair the R-factor defect. A Tet\textsuperscript{R} Tra\textsuperscript{+} deletion mutant of R100-1, which retains all other R100-1 properties but has lost the whole of the Tet\textsuperscript{R} structural gene region (5), was introduced into the JC3272 Tet\textsuperscript{R} strains and similar matings with DU10004 were performed. The rate of transfer of Tet\textsuperscript{R} was barely detectable (50- to 100-fold lower than with pDU8). This suggests that the transfer of Tet\textsuperscript{R} by pDU8 resulted from recombination between regions of homology on the chromosome and plasmid, which repaired the R-factor Tet\textsuperscript{R} lesion. In support of this, Tet\textsuperscript{R} transfer was abolished in recA derivatives of the donor strain. Also, 50 of 50 Tet\textsuperscript{R} exconjugants from one experiment were linked to the transferable Chi\textsuperscript{R} marker and are presumed to be plasmid borne. A further conclusion is that the origin of transfer replication (ori) must either be missing in the translocation strains, or, if present, it must have a similar orientation to ori on the integrated Flac element analyzed by Willetts (29) in which the tra genes are transferred last. Transfer of chromosome markers adjacent to the tet gene in a recA\textsuperscript{-} derivative of DU3083 was not, however, detected, indicating that ori is not present in this Tet\textsuperscript{R} strain.

**Table 2.** Transfer of tetracycline resistance from plasmid-bearing derivatives of Tet\textsuperscript{R} translocation strains

<table>
<thead>
<tr>
<th>Tet\textsuperscript{R} strain</th>
<th>pDU8</th>
<th>pDU17</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tet\textsuperscript{R}</td>
<td>Chi\textsuperscript{R}</td>
</tr>
<tr>
<td>DU3091 rec\textsuperscript{+}</td>
<td>0.0085</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>(5294)</td>
<td>(10\textsuperscript{*})</td>
</tr>
<tr>
<td>DU3083 rec\textsuperscript{+}</td>
<td>0.0053</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>(8490)</td>
<td>(5 \times 10\textsuperscript{4})</td>
</tr>
<tr>
<td>DU3099 recA\textsuperscript{+}</td>
<td>&lt;10\textsuperscript{-6}</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>(&gt;10\textsuperscript{7})</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{*} The transfer frequency (per 100 donors) of the Tet\textsuperscript{R} and Chi\textsuperscript{R} markers in matings with DU1004 was measured as described in Materials and Methods. The figures in parentheses are the ratios of the transfer frequencies of the Chi\textsuperscript{R} and Tet\textsuperscript{R} markers.

\textsuperscript{a} The frequency of transfer of the R factors pDU8 and pDU17 from the recA donor strain is reduced.

**Mapping experiments.** The Tet\textsuperscript{R} marker of DU3083 was transduced into Hfr trains. Preliminary interrupted mating experiments with KL14 and P4-X and the recipient CSH57 indicated that the Tet\textsuperscript{R} marker is located between mtl and metB. The linkage of Tet\textsuperscript{R} to the chromosomal marker metE was also demonstrated by P1 transduction experiments (Table 3). The co-transduction frequencies of metE and Tet\textsuperscript{R} were 10 to 20\%, but they were <1\% for Tet\textsuperscript{R} and ilv. This suggests that Tet\textsuperscript{R} is located to the right of metE (ca. 75 min on the map of Taylor and Trotter [24]) and not between ilv and metE.

**DISCUSSION**

Pairs of derivatives of R100-1, which are normally incompatible, were forced to coexist for many generations in E. coli recA cells. In a rec\textsuperscript{+} host the markers selective for the hetero-R state (Chi\textsuperscript{R} and Tet\textsuperscript{R}) would readily become stabilized by recombination between the plasmids. Since recombination cannot occur between F-like R factors in recA hosts (6), it was expected that some stabilized derivatives of these plasmids might carry one plasmid mutant deficient in incompatibility. Other possibilities were that R fusion might occur, analogous to the F' fusion detected at a low frequency in recA cells by Willetts and Bastarrachea (30), or that E. coli host mutants capable of harboring the two R factors might be isolated (analogous to mutants capable of carrying two F' elements [18, 22]). However, the result of stabilizing the two R-factor markers Chi\textsuperscript{R} and Tet\textsuperscript{R} in the nine experiments reported here was that the Tet\textsuperscript{R} determinant was translocated to the chromosome of the recA host. The Tet\textsuperscript{R} marker was transduced to a rec\textsuperscript{+} recipient, and subsequent analysis showed that no other R-factor markers were inherited. Tet\textsuperscript{R} is located close to traA on

**Table 3.** Co-transduction of Tet\textsuperscript{R} and chromosomal markers

<table>
<thead>
<tr>
<th>Recipient strain</th>
<th>Marker selected</th>
<th>No. of transductants tested</th>
<th>Inheritance of unselected markers\textsuperscript{*}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>met\textsuperscript{+}</td>
<td>423</td>
<td>43 (10.1)</td>
</tr>
<tr>
<td></td>
<td>Tet\textsuperscript{R}</td>
<td>171</td>
<td>46 (26.9)</td>
</tr>
<tr>
<td>CSH57 ilv\textsuperscript{-}</td>
<td>194</td>
<td>0 (&lt;0.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tet\textsuperscript{R}</td>
<td>177</td>
<td>1 (0.5)</td>
</tr>
</tbody>
</table>

\textsuperscript{*} Figures in parentheses are the percentage of inheritance of the unselected marker.

\textsuperscript{+} — Not done or not applicable.
R100-1 (Foster and Willetts, in preparation). There was no evidence for insertion of traA or ori, the nearest R-factor markers to tet that could be tested.

Transfer of Tet\(^{a}\) from a donor strain bearing both the chromosomal Tet\(^{d}\) determinant and a Tet\(^{a}\) point mutant of R100-1 (pDU8) occurred at a 10\(^{-4}\) to 10\(^{-5}\) lower frequency than the transfer of the R-factor Chi\(^{a}\) marker. It seems that this Tet\(^{a}\) transfer resulted from a recA-dependent general recombination process in which the lesion in the plasmid tet gene was repaired. Fifty of 50 Tet\(^{a}\) exconjugants from one experiment were Chi\(^{a}\), and were retransferable at the same high frequency. Also, Tet\(^{a}\) transfer was abolished when a recA\(^{-}\) derivative of the donor strain was used.

A similar experiment was performed with pDU17, a Tet\(^{a}\) Tra\(^{+}\) deletion mutant of R100-1 which has probably lost the whole tet region. The transfer of Tet\(^{a}\) was reduced to a barely detectable level. A similar result was obtained with all nine translocation strains, and also with seven independently isolated R100-1 deletion mutants. This experiment measured either the recombination between the chromosomal Tet\(^{a}\) marker and any residual homology on the plasmid mutant or retranslocation of the marker from chromosome to plasmid (or both). It has been shown that translocation is a recA-independent process, and it might be expected that some Tet\(^{a}\) transfer would occur from recA\(^{-}\) donor strains. The absence of such transfer in this study might reflect the reduced efficiency of recA strains as conjugational donors in these short-term matings (30 min; 1:10 donor-recipient ratio). This possibility is currently being investigated.

Translocation of the Tet\(^{d}\) marker has already been reported between the R100-like factor 222, phage P22, and the chromosome of Salmonella typhimurium (2, 4, 26, 28). The Tet\(^{d}\) marker of R100-1 is flanked by two sequences of deoxyribonucleic acid which are inverted repetitions (23); these sequences have been shown to be the insertion sequence IS3 (20). It is likely that these sequences are the determinants of translocation of Tet\(^{d}\) in the experiments we describe; in addition, they probably confer on this marker the predisposition to be lost by deletion, either by an accurate process giving Tet\(^{a}\) Tra\(^{+}\) mutants or by an inaccurate process giving Tet\(^{a}\) Tra\(^{-}\) mutants (5). It has been reported that one of these sequences is capable of tandem duplication and insertion into the Tet\(^{d}\) structural gene region, causing a Tet\(^{a}\) mutation (23), and it thus fulfils one of the criteria of an insertion sequence. Insertion of IS1 into the gal region of E. coli promotes a high frequency of deletion mutation in the region (21). Also, it is worth speculating that translocation of Tet\(^{d}\) occurs by a similar process to the site-specific recA-independent recombination between two plasmids, which involves repetitions at the site of recombination (12).

The Amp\(^{a}\) determinant of RP4 has also been translocated to other replicons (10). The translocated Amp\(^{a}\) marker contains the genetic determinants of translocation. We have not yet tested the translocated Tet\(^{a}\) marker for retranslocation, but it did retain the genetic property of predisposition to loss by deletion. The translocatable deoxyribonucleic acid sequence that includes the TEM beta-lactamase structural gene has been termed TnA (= transposon A [10]; translocon A [10a]); a sequence of deoxyribonucleic acid has been found to be common to a number of plasmids specifying this enzyme (11). We propose that the translocatable Tet\(^{a}\) region be termed translocon D (TnD; TnB and TnC have already been assigned to other sequences in work not yet published [Hedges, personal communication]). Translocation of TnD does not require a functional recA\(^{+}\) gene in E. coli, an observation that accords with the finding of Chan (see reference 11) that the translocation of Tet\(^{a}\) from the R factor 222 to phage P22 and then to the S. typhimurium chromosome is independent of Salmonella and P22 rec functions. Translocation of TnA in E. coli has been reported as requiring recA\(^{+}\) (11), however, and Tschäpe (25) has found that mobilization of Tet\(^{a}\) from Proteus mirabilis by an introduced F plasmid requires the Proteus rec\(^{+}\) function. Experiments to elucidate this are now in progress.

Reports of translocation of antibiotic resistance genes, together with data on the physical structures involved, have recently been submitted by other laboratories (tetracycline: Kleckner, Chan, Tye, and Botstein, J. Mol. Biol., in press; penicillin: Heffron et al. [10a] and Bennett and Richmond, submitted for publication; kanamycin: Berg et al. [1a]; chloramphenicol: Rosner and Gottesman). The technique of forcing for coexistence of incompatible plasmids is a further method for the isolation and study of such elements.

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