

## Transposition of a Plasmid Deoxyribonucleic Acid Sequence That Mediates Ampicillin Resistance: Identity of Laboratory-Constructed Plasmids and Clinical Isolates

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The structural gene for ampicillin resistance resides upon a  $3.2 \times 10^6$ -dalton sequence of deoxyribonucleic acid, TnA, that can be transposed from replicon to replicon in laboratory experiments. TnA was transposed from a large conjugative plasmid to a small nonconjugative plasmid, RSF1010. Several RSF1010::TnA plasmids isolated in these laboratory experiments have been shown to be identical to plasmids found in clinical isolates. These data provide direct support to the theory that transposition of drug resistance genes play a key role in the evolution of R plasmids.

Small nonconjugative R plasmids conferring resistance to sulfonamide (Su), streptomycin (Sm), and ampicillin (Ap) are relatively common in clinical isolates of enteric bacilli. The prototypes of these plasmids are the Ap(NTP1) and Su Sm(NTP2) plasmids initially described by Anderson and his colleagues (1-3). Smith et al. (16) showed that 19 of 26 Su Sm plasmid species from various *Salmonella* serotypes belonged to the same compatibility group as did an Su Sm Ap plasmid. Barth and Grinter (4) examined a number of Su Sm plasmids that were representative of a broad host and geographical range. They found that 10 of 12 of these plasmids possessed an identical molecular mass and had many of the same nucleotide sequences. On this basis, it was suggested that a single plasmid evolved once and has spread efficiently with relatively few modifications around the world. The prototype Ap plasmid has been shown to be compatible with, and unrelated to, Su Sm plasmids, but highly related to other Ap plasmids of independent origin (16; Crosa, Matta, Olarte, and Falkow, unpublished observations). Nevertheless, a strain of *Salmonella typhimurium* carrying an independent AP and Su Sm plasmid produced a single Su Ap plasmid after ultraviolet irradiation; this was interpreted as the insertion of at least a part of the Ap determinant into Su Sm with loss or inactivation of the streptomycin resistance gene (16).

There is now clear laboratory evidence that the structural gene for ampicillin resistance resides upon a  $3.2 \times 10^6$ -dalton sequence of

deoxyribonucleic acid (DNA), TnA, that is capable of transposition from replicon to replicon (5, 10 to 13). Insertion of TnA is mutagenic when it occurs within structural genes and polar when it occurs within an operon (11). We have shown that TnA from two large conjugative R plasmids, R1*drd*-19 and R64-1, can insert in the plasmid RSF1010 (RSF1010 is identical to the prototype Su Sm plasmid of Anderson and Lewis [2, 3]). TnA insertion can occur at a minimum of 19 distinct sites on the RSF1010 genome (11, 15). Depending upon the site and orientation of TnA insertion, the phenotype of the recombinant plasmid may be Su Sm Ap; Su Ap; Sm Ap; and Ap only (11, 15). A number of Su Ap plasmids as well as Su Sm Ap nonconjugative plasmids have been identified in clinical isolates of *Escherichia coli* (Meyers and Falkow, unpublished observations), *S. typhimurium* (16), and *Proteus mirabilis* (Naomi Datta, personal communication; 14). We have examined two such nonconjugative plasmids, R645 and R870 (14), to determine their molecular nature and their relationship to naturally occurring Su Sm plasmids and TnA-containing RSF1010 plasmids isolated in laboratory experiments. The properties of RSF1010, R645, R870, and other plasmids used in this study are summarized in Table 1.

R645 and R860 were isolated as covalently closed circular molecules as described previously (11, 12) and transformed (8) into *E. coli* strain C600. Both plasmid species contained a single site susceptible to cleavage by the *Eco*RI restriction endonuclease and possessed a molec-

ular mass of  $8.7 \times 10^6$  daltons by sedimentation in neutral sucrose gradients and by contour length measurements of open circular DNA in the electron microscope. TnA was transposed from the  $63 \times 10^6$ -dalton conjugative plasmid R1drd-19 to RSF1010 by methods described previously (11). RSF1010 carrying TnA (recombinant plasmids) were likewise uniformly  $8.7 \times 10^6$  in molecular weight and contained a single *EcoRI* site (11). When sedimented together in neutral sucrose gradients, the clinical isolates and laboratory-constructed plasmids precisely cosedimented (Fig. 1). DNA-DNA hybridizations between an RSF1010::TnA(232) and the clinical isolates showed that they had over 90% of their DNA sequences in common. These data suggested, therefore, that the two naturally occurring plasmids, R645 and R870, had evolved by transposition of TnA into an Su Sm plasmid.

We have previously shown that the site of TnA insertion into RSF1010 can be accurately mapped by electron microscope heteroduplex analysis of *EcoRI*-cleaved RSF1010 recombinant plasmids with similarly cleaved DNA of the plasmid R684 (11). R684 was identical to RSF1010 except for the presence of an additional  $0.8 \times 10^6$  daltons of DNA. Hence, the heteroduplex molecule formed between RSF1010 and R684 showed complete homology except for a single insertion loop. This insertion loop acted as a molecular marker to delineate the "right" and "left" ends of linear *EcoRI*-cleaved molecules. A heteroduplex between an RSF1010 recombinant plasmid and R684 showed the marker loop and a single-stranded  $3.2 \times 10^6$ -dalton loop corresponding to TnA. Figure 2 shows the appearance of an RSF1010::TnA(133/R684 heteroduplex as well as R645/R684 and R870/R684 heteroduplex mol-

ecules. It may be seen that R645 and R870 were homologous with R684 except for the small marker insertion and the presence of a single-stranded loop corresponding to TnA. The identity of this loop with TnA was determined separately by heteroduplexing with RSF1030, a plasmid initially used to define the molecular nature of TnA (12). The site of the TnA loop in R645 was measured at some 20% from the left-hand *EcoRI* end of the heteroduplex molecule with R684, whereas the site of TnA insertion in R870 was measured some 89% from the left-hand end of the molecule. As noted earlier, we

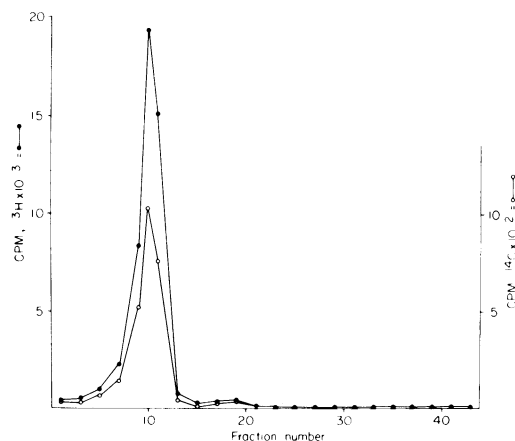


FIG. 1. Cosedimentation of  $^3\text{H}$ -labeled R870 DNA with  $^{14}\text{C}$ -labeled RSF1010::TnA(133) in a neutral sucrose gradient.  $^3\text{H}$ -labeled R870 and  $^{14}\text{C}$ -labeled RSF1010::TnA DNAs were prepared as described (10) and mixed together before sedimentation on a 5 to 20% neutral sucrose gradient. The DNAs were sedimented for 4 h at 39,000 rpm ( $15^\circ\text{C}$ ) in the Beckman SW40.1 rotor, and 6-drop fractions (about 0.15 ml) were collected and counted (10) in a Packard Tricarb scintillation counter.

TABLE 1. Bacterial plasmids

| Plasmid            | Mol wt <sup>a</sup><br>( $\times 10^6$ ) | Phenotype <sup>b</sup>   | Derivative                                    | Reference |
|--------------------|--|--------------------------|---|-----------|
| R1drd-19           | 63                                       | IncFII Su Sm<br>Cm Km Ap | Derepressed conjugative plasmid               | 9         |
| RSF1010            | 5.5                                      | Su Sm                    | Naturally occurring nonconjugative plasmid    | 2; 11     |
| R684               | 6.3                                      | Su Sm                    | Naturally occurring nonconjugative plasmid    | 4         |
| R645               | 8.7                                      | Su Sm Ap                 | Naturally occurring nonconjugative plasmid    | 14        |
| R870               | 8.7                                      | Su Sm Ap                 | Naturally occurring nonconjugative plasmid    | 14        |
| RSF1010::TnA(232)  | 8.7                                      | Su Sm Ap                 | Transposition of TnA from R1drd-19 to RSF1010 | 12        |
| RSF1010::TnA:(113) | 8.7                                      | Su Sm Ap                 | Transposition of TnA from R1drd-19 to RSF1010 | 12        |

<sup>a</sup> Determined by measurement of contour lengths of open circular molecules seen in the electron microscope as described by Heffron et al. (11).

<sup>b</sup> Abbreviations: IncFII, incompatibility specificity FII; Su, Sm, Ap, Cm, and Km, resistance to sulfonamide, streptomycin, ampicillin, chloramphenicol, and kanamycin, respectively.

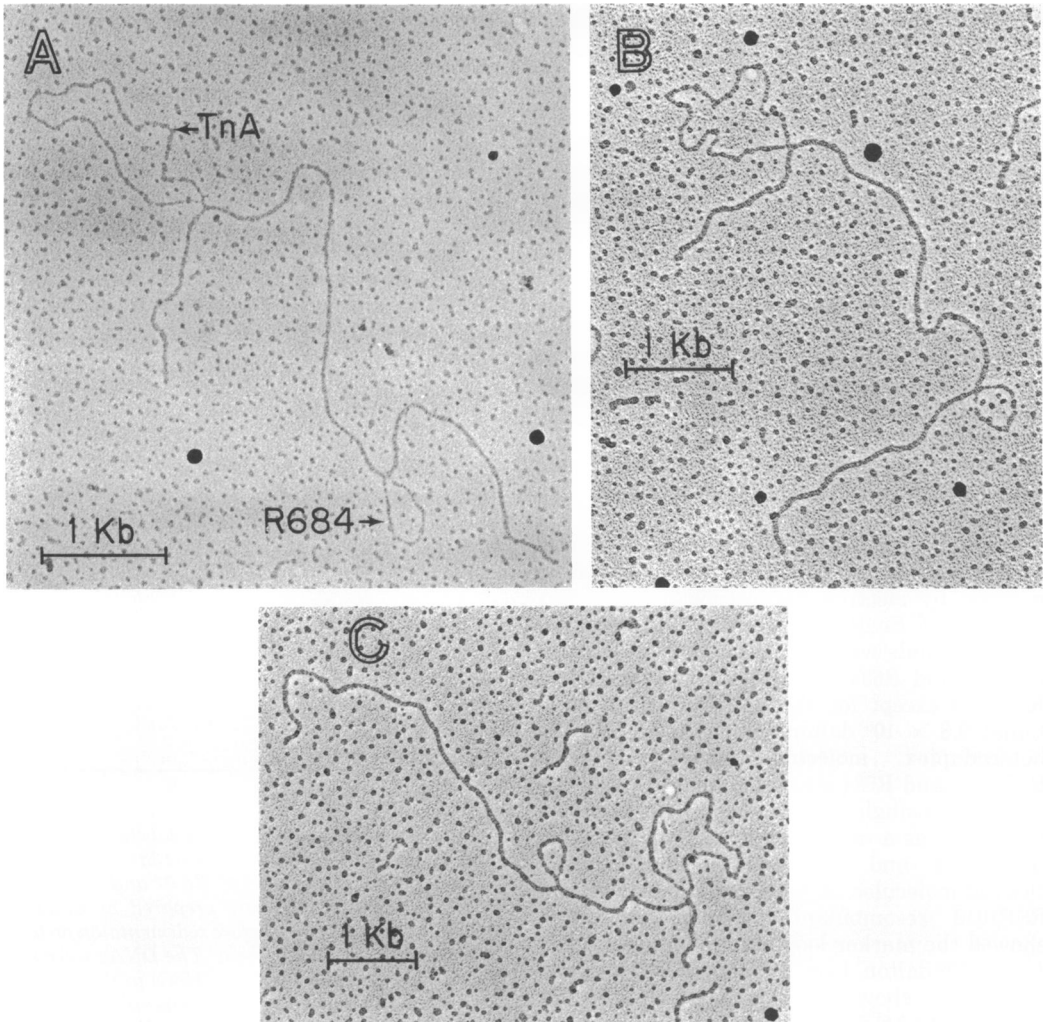


FIG. 2. Demonstration of the similarity of clinically isolated plasmids to laboratory-constructed plasmids. A DNA-DNA heteroduplex between *EcoRI*-cleaved RSF1010::TnA(133) and R684 is shown in A. The small loop from R684 serves as the marker in distinguishing right from left. In B, a similar heteroduplex has been prepared between R645 and R684, and in C, a heteroduplex has been prepared between R870 and R684.

had shown that TnA can insert into RSF1010 at a minimum of 19 distinct sites (11, 15). We have not observed any TnA insertion into RSF1010 corresponding to that observed for R870. However, the site of TnA insertion for plasmid R645, 20% from the left-hand end of the molecule, was encountered (3 of 38 laboratory isolates examined) in our laboratory transposition experiments. Consequently, we heteroduplexed R645 with RSF1010::TnA(113), in which TnA had apparently been inserted at the same site as R645. These plasmids showed complete homology when viewed under the electron microscope. Thus, we have isolated several plasmids in lab-

oratory experiments that are identical to those found in clinical isolates.

Several theories for the origin of R plasmids and the origin of antibiotic resistance genes have been proposed (6, 7, 17). The discovery of the transposability of antibiotic resistance genes provides a mechanism by which they might be easily interchanged between replicons and accounts for several long-standing observations about the epidemiology of R plasmids (9, 11). R plasmid-encoded antibiotic resistance enzymes show, by and large, no relationship to chromosome-encoded enzymes of the enteric bacteria, suggesting that they have not been

picked up from the chromosome as in the formation of F's. Many R plasmids show little DNA homology with each other, but possess the same phenotype and apparently specify identical antibiotic resistance enzymes. On the other hand, plasmids of the same compatibility group show a high degree of homology with each other and possess identical transfer and replication genes, but may specify quite different antibiotic resistance patterns. Thus, it appears that the R plasmid antibiotic resistance genes have arisen independently from the transfer and replication genes. The data presented in this paper add direct support to the theory that transposition *in vivo* plays a key role in both the rapid evolution of resistance plasmids and the reassortment of resistance determinants among plasmids within a wide variety of bacterial hosts.

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