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Formation of Recombinants Between Nontransmissible Drug-Resistance Determinants and Transfer Factors

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Noninfectious drug-resistance determinants acquired conjugal transmissibility by the formation of recombinants with transfer factors, suggesting the origin of R factors.

It was reported previously that nontransferable drug-resistance determinants, obtained by transduction of R factors with phages ϵ or P1 (1, 6), acquired the ability to transfer by conjugation after recombination with the sex factor, F, or wild-type R factors (2-4, 7, 8). Since known sex factors apparently can recombine with nontransferable R-factor derivatives to form functional, infectious R factors, it was of interest to study the distribution of genetic elements (termed T factors) among clinical isolates capable of conferring conjugal ability on noninfectious drug-resistance determinants.

Two noninfectious R factors harbored in *Escherichia coli* K-12 were employed. The R_{21} determinant confers resistance to tetracycline (TC) and is integrated between the *lac* and *pro* loci of the *E. coli* chromosome (5). The R_{530} factor, which also confers TC resistance, differs from the R_{21} factor in being cured by acriflavine from which we infer an extrachromosomal existence in the *E. coli* host strain. A series of bacterial strains were isolated from clinical sources in order to survey the distribution of such T factors in nature. Numerous enteric strains were tested for the presence of T factors by mixed cultivation experiments employing *E. coli* strains harboring R_{530} factor. Strains carrying T factors (40%) were most frequently observed in *E. coli* and *Shigella flexneri* isolates (Table 1). *E. coli* K-12 T_{95}^+ was found to be resistant to male phages, and chromosome transfer by F factor was inhibited by the presence of T_{95} factor in donor strain. Only one (less than 1%) T_{95} factor was cured by treatment with acridine dyes, although 70% or more of F factor was lost from the host strain by the same treatment. These results suggest that T_{95} factor is a different episome from F factor. Detailed results of the comparison of T factors with F factor will be described elsewhere.

Further investigations on the transmission of otherwise nontransferable TC resistance were carried out by using a T factor termed T_{95}. *E. coli* T_{95}^+ was mixed with *E. coli* W1177 r_{21}^+ and incubated at 37 C. After overnight incubation, the cultures were mixed with *E. coli* 58-161 F^-NA^r (resistant to nalidixic acid), the final recipient of TC resistance. After 18 hr of incubation, the mixed culture was spread on selective plates containing both TC and nalidixic acid. After 18 hr of incubation at 37 C, the resistant colonies were purified and investigated for their TC donor ability. Two classes of TC^r recipients were isolated: one class capable of transferring TC^r and a second class unable to conjugally transfer TC^r.

It was found that the transferable TC resistance among the conjugants $(58\text{-}161)r_{21}^+$ were all transmissible by conjugation even when transferred to new recipient W3110.

Among the TC^r conjugants W3110 obtained in the experiments described above, the strains carrying transferable TC^r were selected and their genetic characters were investigated by transductional analysis with P1*kc* (9). When a TC^r conjugant W3110, to which TC^r was transmitted from $(58\text{-}161)r_{21}^+$ in conjunction with T factor, was used as a donor of TC resistance, the TC^r in transductants was found to be all transmissible by conjugation. These results indicate that the acquisition of transmissibility of nontransmissible TC^r of r_{21} determinant that is integrated into host chromosome is accounted for by the formation of recombinant T-*tet* factor following infection with T_{95} factor.

Nontransmissible R factors, which are defective in transfer function, regain this property when they recombine with functional sex factors such as F *TC*, F'*TC*, R *TC*, and P1*CM* (2, 4, 7). These results suggest that an R factor consists

TABLE 1. *Distribution of T factors*[a]

Strain	No. of strains tested	No. of strains which carry T factors
E. coli	56	25
E. freundii	1	0
Shigella flexneri	11	9
S. sonnei	15	1
Salmonella	3	1
Arizona	1	0
Klebsiella	5	2
Proteus	12	4
Serratia	1	0
Total no.	105	42 (40%)

[a] A T_{95} factor was isolated from *E. coli* GN12 of clinical origin.

of resistance determinants plus genetic determinants responsible for autonomy and transfer.

Our findings indicate that recombinant formation is one of the mechanisms involved in the acquisition of transferability by nontransferable R factors.

There are other possibilities to consider for the acquisition of nontransferable *r* determinant in cooperation with other episomic elements: (i) transfer of *r* determinant integrated into host chromosome accompanying the transfer of host chromosome by bacterial episomes, i.e., by R-mating (10) and by T-mating; (ii) transfer of extrachromosomal *r* determinant by complementation with bacterial episomes, i.e., F, R, and T factors (Kameda et al., *unpublished observation*). The details will be published elsewhere.

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