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Tn*2301*, a Transposon Construct Carrying the Entire Transfer Region of the F Plasmid

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The largest R·*Bam*HI fragment of the plasmid F, which carries the entire F conjugation system, has been cloned into the single R·*Bam*HI site of the ampicillin (Ap) resistance transposon Tn*1*. pDS1106 (ColE1 *mob*::Tn*1*) was the vector plasmid, and the resultant conjugative plasmid, pED830, was characterized both genetically and by restriction enzyme analysis. The transposon construct, denoted Tn*2301*, was transposable at frequencies similar to Tn*1* to small nonconjugative plasmids or to the *Escherichia coli* host chromosome. In the former case, Ap^r conjugative plasmids were obtained, whereas in the latter case, Hfr strains resulted. Representative Hfr strains were characterized by quantitative and interrupted mating experiments. Extension of this technique for Hfr formation should aid chromosome mapping both in *E. coli* and in other bacterial genera.

One of the common and important properties determined by bacterial plasmids is conjugation, that is, the ability to promote their own intercellular transfer by a process involving cell-to-cell contact. For this purpose, conjugative plasmids carry a set of genes encoding a transfer system, and a specific site or sequence, denoted *oriT*, from which DNA transfer is initiated (1, 37).

If a conjugative plasmid becomes covalently joined to the bacterial host chromosome, the latter can be transferred as an "extension" of the plasmid molecule, allowing the formation of merozygotes, and ultimately recombinants, in the recipient cell. The best known example of this is of course Hfr formation by integration of the sex factor F into the *Escherichia coli* chromosome. This occurs mainly via infrequent homologous recombination between the insertion sequences carried both by F and the chromosome (11, 12, 25), but any other recombination process would give a similar result. These Hfr strains have been of fundamental importance in mapping the genes on the *E. coli* chromosome, and other plasmid-determined conjugation systems have played similar roles in other organisms (reviewed by Holloway [24]).

Since a specific plasmid-chromosome interaction is usually involved in Hfr formation, this normally limits the range of chromosomal origins of transfer obtainable in *E. coli* or other bacterial genera to which F is transmissible to the relatively small number of sites in the chromosome where there is an insertion sequence (7). It occurred to us, therefore, that it would be convenient to be able to transpose an entire transfer system into the bacterial chromosome, indeed into any other replicon (such as a nonconjugative plasmid). This would be expected to allow the formation of Hfr strains with new chromosomal origins in *E. coli* and facilitate their formation in other enterobacterial species. Specificity would be limited only by that of the transposition process.

We have therefore cloned the entire F transfer system into the Tn*1* transposon carried by the ColE1::Tn*1* plasmid pDS1106. Tn*A* transposition has low specificity (23), and cotransposition of the transfer genes with ampicillin resistance facilitates selection for this process. This paper describes the basic characteristics of the new transposon, Tn*2301*, including investigations of the effect of increasing the size of Tn*1* 10-fold on the frequency of transposition. A preliminary account of this work has been presented elsewhere (D. A. Johnson and N. S. Willetts, *in* C. Stuttard and K. R. Rozee, ed., *Plasmids and Transposons: Environmental Effects and Maintenance Mechanisms*, in press).

MATERIALS AND METHODS

Bacterial strains and plasmids. The *E. coli* strains and plasmids used in this study are listed in Table 1. ED410 was constructed by P1 transduction of kanamycin resistance (Km^r) from DB1447-109 (a *lacZ*::Tn*5* derivative of DB1447 constructed by D. E. Berg essentially as described before [4]) to JC-5422 (38).

Media and genetic techniques. Media have been described by Finnegan and Willetts (17). Antibiotics

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TABLE 1. *Bacterial strains and plasmids*

Strain/ plasmid	Relevant characteristics	Source/reference
Bacteria		
JC3272	*his trp lys Δlac-74 str*	(2)
ED2196	*his trp Δlac-74 nal*	(19)
ED8654	*met hspR*	(6)
JC5422	*thr leu proA his thyA argE str*	(38)
ED410	Km^r derivative of JC5422	See text
Plasmids		
pBR325	Ap^r Cm^r Tc^r	(5)
pDS1106	ColEl::Tn*1*	(14, 15)
pSC101	Tc^r	(10)
pED712	Tc^r	Watson and Willetts, in preparation
pED851	pBR322 carrying the largest R·*Bam*HI fragment of F	Johnson and Willetts, in press; in preparation
pED815	Ap^s pBR322	See text
pED855	pBR325 carrying the largest R·*Bam*HI fragment of F	See text
pED830	pDS1106::Tn*2301*	See text
pED831	pSC101::Tn*2301*	See text

were added to media at the following concentrations: ampicillin (Ap), 50 μg/ml; tetracycline (Tc), 10 μg/ml; chloramphenicol succinate (Cm), 50 μg/ml.

Bacterial matings to measure plasmid transfer efficiencies were carried out as described by Finnegan and Willetts (18). Interrupted matings with Hfr donor strains used a mechanical shaker of the type described by Low and Wood (31). F-specific phage techniques were described by McIntire and Willetts (33). Transformation was by the method of Lederberg and Cohen (30). Colicin E1 production was tested by exposing colonies to chloroform vapor and overlaying with a colicin E1-sensitive strain in top agar.

Transposition frequencies were estimated from the ability of Tn*2301* to mobilize a nonconjugative plasmid such as pSC101 or pED712 (J. Watson and N. S. Willetts, manuscript in preparation). pED830 was introduced by conjugation into JC3272 carrying either pSC101 or pED712. Such strains were mated (18) with ED2196, and an apparent transposition frequency was calculated from the ratio of Tc^r::Ap^r transconjugants.

Physicochemical techniques. Plasmid DNA was prepared by the cleared-lysate polyethylene glycol precipitation and ethidium bromide-cesium chloride density gradient techniques described by Clewell and Helinski (8) and Humphries et al. (26) except that ethidium bromide was removed by butanol extraction.

Restriction enzymes were purchased from Miles Laboratories, Stoke Poges, Slough, England, and Boehringer Mannheim GmBH, Brighton, England, and used under the conditions recommended by the supplier. T4 ligase was a gift of K. Murray. Conditions for agarose gel electrophoresis were as described by Willetts and McIntire (39) except that a horizontal gel apparatus was used.

Construction of pED815. A 2.5-μg sample of pBR325 DNA was restricted with 5 U of R·*Pst*I for 2 h at 37°C. After heating for 10 min at 70°C, the linear DNA, which had been cleaved within the Ap^r gene, was used to transform ED8654. Cm^r transformants were selected, and these were then screened by a replica-plate technique to identify Ap^s clones. One such colony, carrying a plasmid designated pED815, was purified and used to prepare plasmid DNA. Restriction analysis confirmed that the site of cleavage of R·*Pst*I had indeed been lost while the R·*Eco*RI, R·*Hin*dIII, R·*Bam*HI, and R·*Sal*I sites had been retained. When pED815 DNA was used to transform JC3272, 50 of 50 Tc^r transformants were Cm^r Ap^s.

Construction of pED855. A 1.5-μg sample of pED815 DNA and 6.2 μg of pED851 DNA were digested for 2 h at 37°C with 10 U of R·*Bam*HI in a volume of 30 μl. pED851 is an Ap^r Cm^s plasmid and served as a convenient source of the largest F R·*Bam*HI fragment carrying the entire transfer region (Johnson and Willetts, in press; manuscript in preparation). After heating for 10 min at 70°C, the mixture was diluted to 100 μl with T4 ligase buffer, and the appropriate amount of T4 ligase was added. Ligation was allowed to proceed for 20 h at 12°C, and the DNA was then used to transform ED8654. Cm^r transformants were selected by overnight growth in L-broth containing chloramphenicol. Exponential cultures were grown from these and mated with exponential cultures of JC3272 for 1 h at 37°C, and Cm^r [Str^r] transconjugants were selected. These clones were tested to identify those which were Tc^s Ap^s, sensitive to F-specific phages, and transfer proficient. One such solony was purified and used for plasmid DNA preparation to confirm that the large F R·*Bam*HI fragment had indeed been cloned into pED815 (see Fig. 2a, tracks 2 to 4). This plasmid was designated pED855.

Construction of pED830. A 2.7-μg sample each of pDS1106 (ColE1::Tn*1*) and pED855 DNA were restricted with 10 U of R·*Bam*HI for 2 h at 37°C. The reaction was terminated by heating at 70°C, and ligation was carried out as described for pED855. Ap^r transformants of ED8654 were selected by growth overnight in L-broth supplemented with ampicillin. Exponential cultures were grown and mated with similar cultures of JC3272, and Ap^r [Str^r] (streptomycin-resistant) transconjugants were selected.

The transconjugants were screened for the phenotypes expected if the large R·*Bam*HI fragment of F had been cloned into pDS1106, i.e., Cm^s, colicin E1 production, sensitivity to F-specific phages, and the ability to act as a high-frequency donor of Ap^r. One such clone, carrying a plasmid designated pED830, was purified; plasmid DNA was isolated, and its structure was confirmed by restriction analysis (see below).

RESULTS

Construction of a Tn*A* derivative carrying the F transfer system. The basis for our construction of a transposon carrying the entire F transfer region lies in two previous observations. First, Tn*3* has a unique R·*Bam*HI site, and interruption of Tn*3* at this point by cloning in a DNA fragment (21) did not prevent either expression of the β-lactamase gene or transpo-

sition of the mutant Tn*3* sequence (21). Second, the entire F transfer system is carried on a single 44.5-kilobase (kb) R·*Bam*HI fragment (Fig. 1) (34, 36). We therefore decided to clone this large F R·*Bam*HI fragment into the R·*Bam*HI site of Tn*1*, which is 85% homologous to Tn*3* (14).

The source of the F R·*Bam*HI fragment was not F itself, but pED851. This plasmid already contained the F R·*Bam*H1 fragment cloned into the R·*Bam*HI site of pBR322, and with a copy number of about 40 should provide a ready source of the fragment (Johnson and Willetts, in press; in preparation). The vector chosen was pDS1106, which is a ColE1::Tn*1* derivative (15). Its advantages are that it has only the single R·*Bam*H1 site in Tn*1*, and that as a result of the Tn*1* insertion it has lost the ability to be mobilized by the F transfer system. Hence by cloning the large R·*Bam*HI fragment of F (carrying the F transfer system) into pDS1106, the required recombinants could be selected on the basis of their ability to transfer Ap^r to recipient cells.

However, pED851 was itself Ap^r and transfer proficient, and attempts to purify sufficient amounts of the F R·*Bam*HI fragment by agarose gel electrophoresis or sucrose gradient centrifugation proved unsuccessful. It was therefore decided to first clone the F R·*Bam*HI fragment into the Ap^s Cm^r Tc^r vector pED815, which was made for the purpose by in vitro deletion of the R·*Pst*I site within the Ap^r gene of pBR325 (see above). This gave the plasmid pED855.

Finally, pED855 and pDS1106 DNAs were mixed, restricted with R·*Bam*HI, ligated, and used to transform the Str^s strain ED8654 as detailed above. Cultures containing Ap^r transformants were mated with cultures of the Str^r recipient strain JC3272, and Ap^r [Str^r] transconjugants were selected. Of these, about 4% were Cm^s and produced colicin E1, characteristics of pDS1106 but not of the pED815 vector. One of these, carrying a plasmid denoted pED830, was chosen for further study. It was sensitive to F-specific bacteriophages and transferred Ap^r with a two- to threefold higher efficiency (Table 2) than is observed for F.

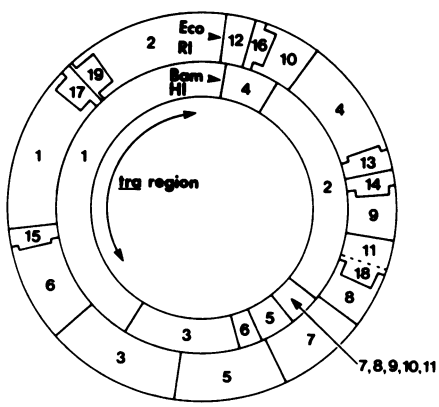

FIG. 1. *A map showing the R·EcoRI and R·BamHI sites on the plasmid F with the extent of the transfer region outlined. This drawing has been adapted from a map published by Skurray et al. (36).*

The parent replicon of pED830 was plasmid ColE1 (14, 15), and the increased transfer efficiency observed for pED830 might be expected to result from cloning of the transfer genes into the multicopy vector because of a gene dosage effect. The copy number of pED830 was measured by assaying β-lactamase specific activity (D. A. Johnson and N. S. Willetts, manuscript in preparation) and estimated to be 37 per chromosome when F was assigned a value of 1 per chromosome. Cloning of the large R·*Bam*HI fragment into pDS1106 did not therefore change the multicopy nature of the replicon, but the increased gene dosage did not proportionately increase the expression of the transfer genes as measured by frequency of transfer. This relationship was also observed upon cloning the F transfer region into pBR322 (Johnson and Willetts, in preparation) and is discussed further there.

Restriction enzyme analysis of pED830. pED855, pED830, pDS1106, and F DNA were restricted with R·*Bam*HI, and the fragment patterns were compared after agarose gel electrophoresis (Fig. 2, tracks 4 to 7). It was clear that, as expected from its genetic properties, pED830 was indeed composed of pDS1106 together with the largest F R·*Bam*HI fragment. The size of Tn*1* had therefore been increased 10-fold from 4.9 kb to 49.4 kb, to give the putative transposon Tn*2301*.

To deduce the orientation of the F R·*Bam*HI fragment in pED830, it was first necessary to determine the orientation of the Tn*1* insertion in pDS1106. Use was made of the enzymes R·*Bam*HI, which cleaves Tn*1* once (21),

TABLE 2. *Transfer and transposition properties of Tn2301*[a]

Plasmid	% Transfer frequency of			Apparent transposition frequency
	Ap^r	Tc^r	Lac$^+$	
pED830	173	—	—	—
pED830/pSC101	197	0.0009	—	4.5×10^{-6}
	295	0.009	—	3×10^{-5}
pED830/pED712	75	0.012	—	1.6×10^{-4}
pED831 (pSC101::Tn*2301*)	120	65		

[a] In each experiment, 25 transconjugants were tested for the predicted antibiotic resistance phenotype and found to be identical by this criterion.

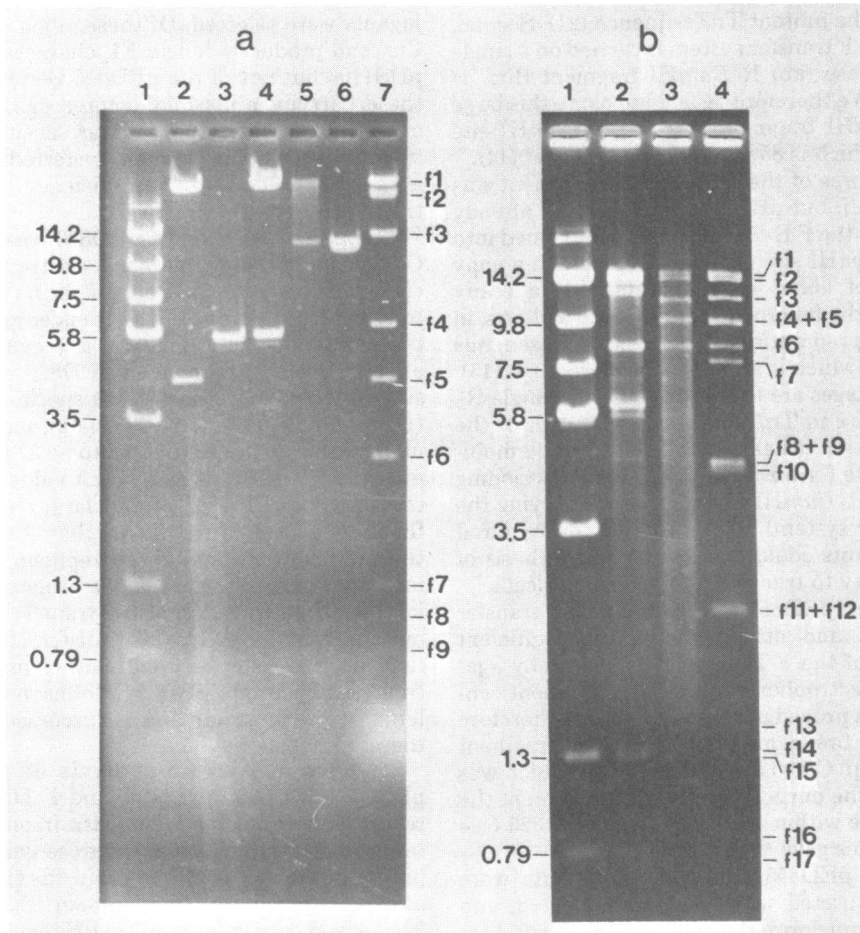

FIG. 2. *Restriction cleavage patterns of plasmid DNA after electrophoresis through agarose gels, staining with ethidium bromide, and photographing during illumination with UV light (39). (a) Construction of pED830: (1) EDλ97 DNA cleaved with R·EcoRI (fragment sizes are from reference 39); (2) pED851 DNA cleaved with R·BamHI; (3) pED815 DNA cleaved with R·BamHI; (4) pED855 DNA cleaved with R·BamHI; (5) pED830 DNA cleaved with R·BamHI; (6) pDS1106 (vector for construction of pED830) DNA cleaved with R·BamHI; (7), F DNA cleaved with R·BamHI, with the fragments numbered according to size. (b) Orientation of F DNA in pED830: (1) EDλ97 DNA cleaved with R·EcoRI; (2) pED830 DNA cleaved with R·EcoRI; (3) pED831 DNA cleaved with R·EcoRI; (4) F DNA cleaved with R·EcoRI. The F R·EcoRI fragments are numbered as in Fig. 1.*

and R·*Sma*I, which cleaves ColE1 at a unique site (14). Of the two possible orientations, restriction analysis allowed unambiguous assignment of the orientation shown in Fig. 3, with the gene coding for β-lactamase proximal to the gene for colicin E1 production (14).

Restriction of pED830 with R·*Eco*RI (Fig. 2b, track 2) was then utilized in a similar fashion to demonstrate that the orientation of the F R·*Bam*HI fragment cloned into pDS1106 is as illustrated in Fig. 3, which summarizes the genetic and physical constitution of the plasmid. Comparison with the R·*Eco*RI digest of F DNA (Fig. 2b, track 4) shows that, as expected, the f1, f2, f6, f15 (f19 is too small to be seen on this gel) fragments of F are produced on digestion of pED830. The remaining two fragments were hybrid, consisting of parts of ColE1 and of Tn*A* linked to parts of f3 and f12, respectively.

Transposition of Tn*2301*. To demonstrate that the putative transposon Tn*2301* was indeed transposable, and to determine the frequency of this transposition process, we made use of two small nonconjugative and non-mobilizable Tc^r plasmids, pSC101 and pED712. The latter is a 7.0-kb Tra$^-$ Ap^s Km^s mini-RP1 obtained by R·*Hae*II "scrambling" of RP1 (Watson and Willetts, in preparation). The rationale was that

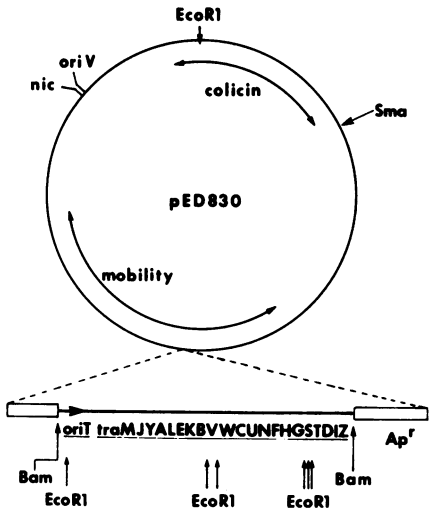

FIG. 3. *A genetic and physical map of pED830. ColE1 DNA is shown as the circle, Tn1 DNA is shown by the broad parallel lines, and F DNA is shown as a single thick line. Shown are the R·SmaI, R·EcoRI, and R·BamHI sites used in the determination of the orientation of the F DNA carrying the transfer genes. This diagram is not to scale.*

after transposition of Tn*2301* to the Tc^r recipient replicon, this would become transfer proficient with an efficiency of transfer of approximately 1; thus the frequency of transposition could be measured as the frequency of Tc^r transconjugants given by donor cells carrying both pED830 and one of the Tc^r plasmids.

In these systems, then Tn*2301* had an apparent transposition frequency of 1.6×10^{-4} to 4.5×10^{-6} per cell (Table 2). These values are comparable to those previously reported for the transposition of Tn*1* itself from the *E. coli* chromosome to various R factors (1×10^{-2} to 2×10^{-6}; 3). Similar values have also been found for transposition of the similar if not identical Tn*A* variant Tn*3* from pMB8::Tn*3* to a Km^r derivative of F, RSF2001 (7×10^{-4}; 22). We conclude that cloning the 44.5-kb F R·*Bam*HI fragment into Tn*1* and increasing the molecular weight 10-fold did not substantially reduce the frequency of the transposition event. However, we cannot rule out the possibility that a decreased frequency due to the enlarged size of the transposon was being counteracted by either the multicopy nature of pED830 or inactivation, as a result of insertion into the Tn*1* R·*Bam*HI site, of the 19,000-dalton protein that normally reduces the frequency of transposition by about 10-fold (13, 20), or both. Our results incidentally confirm that this 19,000-dalton protein is not also required for transposition per se.

Another effect of transposition caused by deletions of the region around but not including the R·*Bam*HI site of Tn*3* was that cointegrates of the donor and recipient plasmids, thought normally to be intermediates in the transposition process, were observed and were only slowly resolved in Rec$^+$ cells (20). In our experiments, however, the Tc^r Ap^r transconjugants from matings from donor cells carrying both pED830 and one of the Tc^r plasmids did not produce colicin E1, and therefore did not carry pED830 either in the free form or as a cointegrate with pSC101::Tn*2301* or pED712::Tn*2301*. The hypothesized cointegrate intermediates must therefore have been resolved either by a retained function of the transposon system or by the host's recombination system. Even so, frequent simultaneous transfer of pED830 to recipient cells together with pSC101::Tn*2301* or pED712::Tn*2301* was expected, since the F R·*Bam*HI fragment cloned into Tn*2301* has no known incompatibility or replication functions (see reference 29), and pED830 was fully compatible with both pSC101 and pED712. We therefore suspect that the reason why no recipient cell carried both pED830 and pSC101::Tn*2301* or pED712::Tn*2301* must relate to the fact that both of them express the F transfer system; further investigations of this phenomenon are in progress. This phenomenon may hinder the utilization of these conjugative Tc^r plasmids for studies of conjugation and its control by preventing the formation of cells stably diploid for the entire F transfer region.

The properties of a representative transposition derivative of pSC101, called pED831, are shown in Table 2; similar results were observed for two other independently derived pSC101::Tn*2301* isolates and one pED712::Tn*2301* isolate. As expected, cells carrying this plasmid now transferred both Ap^r and Tc^r at similar high frequencies, and there was 100% coinheritance of the two antibiotic resistance markers. The apparent slightly reduced level of Tc^r transfer can be explained by its delayed expression in transconjugants. Restriction analysis of pED831 DNA confirmed that it had the expected size and R·*Eco*RI fragments for a pSC101::Tn*2301* plasmid (Fig. 2b, track 3), but did not allow an unambiguous assignment of the location or orientation of the insertion.

Formation of Hfr strains by Tn*2301* transposition. Transposition of Tn*2301* to the *E. coli* host chromosome should result in linkage of the latter to the *oriT* sequence and transfer genes carried by the transposon, and thus in the formation of Hfr strains capable of efficient and oriented transfer of chromosomal markers.

As a means to select for cells in which such a transposition event had occurred, the incompatibility between pED830 (Ap^r) and pED815 (Cm^r

Tc^r), which are both derived from the ColE1 plasmid, was exploited. pED830 was transferred into strain ED8654 carrying pED815, and cells initially carrying both plasmids were selected on medium supplemented with both ampicillin and chloramphenicol. These colonies were then subcultured in medium containing only chloramphenicol, and single colonies grown from these cultures were found by a replica-plating technique to include a variable proportion that were still Ap^r. Such Ap^r clones were purified and shown to carry the Cm^r and Tc^r markers of pED815, but not the colicin E1 gene of pED830. Further tests showed that the colonies were sensitive to F-specific phages, but did not transfer the Cm^r marker to a recipient strain; i.e., in none of those tested had Tn*2301* integrated into pED815 to give a Tra$^+$ pED815::Tn*2301* plasmid. Representative clones were therefore chosen as putative Hfr strains.

As control for the above tests, the pED830 derivative of ED8654 was constructed. This had the expected phenotype, but unexpectedly grew more slowly than ED8654 itself; furthermore, among the majority of small colonies on a nutrient agar plate containing ampicillin were a minority of larger, faster growing clones. After purification, such colonies were found to be Ap^r and F-specific phage sensitive, but unable to produce colicin E1. They therefore did not carry pED830 and were also putative Hfr strains. Presumably, the presence of the large multicopy plasmid pED830 reduced the rate of growth of its host cell, whereas transposition of Tn*2301* to the chromosome, followed by loss of pED830, gave Ap^r cells without this hindrance. The amount of ampicillin added to supplement these plates was insufficient to reduce the rate of growth of cells with chromosomal insertions relative to those containing pED830, even though in the latter type a much increased β-lactamase-specific activity has been observed.

To test the putative Hfr strains for their ability to transfer the host chromosome, crosses were performed with the recipient strain ED410. This carries auxotrophic markers (*thr*, *leu*, *proA*, *his*, *thyA*, and *argE*) located at suitable intervals around the chromosome and, to allow contraselection appropriate for the chromosomal region transferred, is both Str^r (*rpsL*) and Km^r (*lacZ*::Tn*5*) (see Fig. 4D). Initially, replica plate matings were used to determine which strains transferred which markers most efficiently, allowing representative strains to be chosen for quantitative matings. In these tests, some strains apparently did not transfer any of the markers tested efficiently; these were not further characterized.

Ultimately, four strains were chosen including one (ED5061) from the "large-colony" selection technique. Their quantitative donor abilities for various chromosomal markers are given in Table 3. One or more markers were transferred at very high frequency, compared to the parental strain ED8654(pED830), whereas others were transferred less efficiently. These are the results expected for oriented chromosome transfer by Hfr

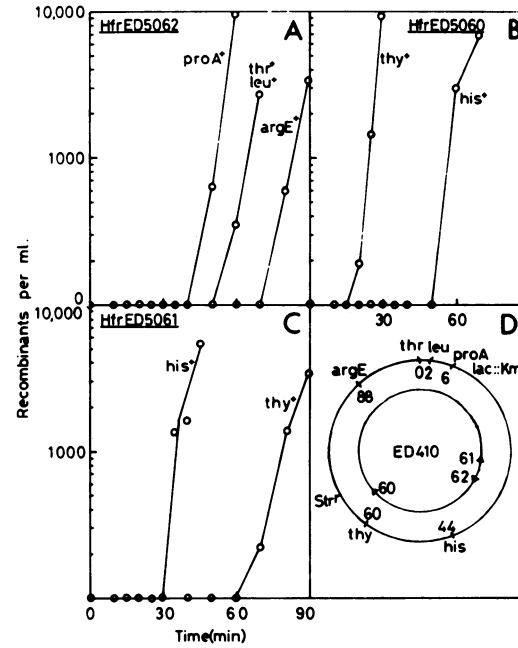

FIG. 4. *Times of entry of chromosomal markers in interrupted matings between Hfr strains formed by Tn2301 transposition and the recipient strain ED410. The deduced points of the origin of transfer are shown in (D). That of ED5063 was similar to that of ED5061.*

TABLE 3. *Hfr strains obtained by Tn2301 transposition to the chromosome*[a]

Strain no.	% Recombinants			
	proA$^+$	*his*$^+$	*thyA*$^+$	*argE*$^+$
ED8654(pED830)	<0.0001	0.001	0.0001	<0.0001
ED5060	0.033	**1.2**	**9.8**	0.009
ED5061	0.001	**0.72**	0.02	0.0007
ED5062	**0.22**	0.0008	0.0006	0.007
ED5063	0.003	**0.15**	0.019	0.001

[a] Matings between exponential cultures (2×10^8 cells per ml) of the donor strains and the recipient strain ED410 mixed in the ratio 1:10 were for 2 h at 37°C. Str^r or Km^r, as appropriate, was used to contraselect the donor strains, and methionine (the donor strains are *met*) was included in all plates. Markers transferred with the highest frequencies are shown in bold face. As expected from the constitution of Tn*2301* shown in Fig. 3, Ap^r transconjugants of ED410 were never obtained (<10^{-5}%).

strains. As a further proof of this, interrupted matings were carried out (Fig. 4A to C). The observed times of entry were consistent with the previously observed transfer efficiencies for the various markers, and the approximate locations of the points of origin (and therefore of Tn*2301*) derived from these data are shown in Fig. 4D.

DISCUSSION

Using an in vitro recombination technique, we introduced the entire F transfer region into Tn*1* to construct the derivative transposon Tn*2301*. This was able to transpose both ampicillin resistance and transfer proficiency to a recipient replicon at frequencies similar to those for Tn*1* transposition. The recipient replicon could be either a bacterial plasmid or the host chromosome. In the latter case, Hfr strains able to transfer chromosomal genes with high frequency were formed, and we believe that this could be an important use for this transposon. Tn*1* has a relatively low specificity for sequences into which it can transpose (23), so that generation of an extensive range of Hfr strains with different points of origin and orientations of transfer should be possible.

In *E. coli*, a wider range of Hfr strains should be obtainable, since the usual requirement for a small region of homology on the chromosome (i.e., an IS sequence) to allow F plasmid integration (7, 32) is avoided. In fact, it should be possible to select for Tn*2301* insertion at any required chromosomal location. One technique would be transposition with simultaneous cycloserine selection for auxotrophy to give an insertion into a given gene or set of genes. Although the Hfr strains described in the present study had no additional growth requirements, Cys and Ile^- strains have more recently been found after transposition. A technique of wider applicability would be an extension of that described by Kleckner et al. (28, section 3[c] [i]): P1 cotransduction from a mixed "pool" of Hfr strains to obtain one with ampicillin resistance and transfer proficiency linked to any given gene.

In *E. coli*, transposition of Tn*2301* to the bacterial chromosome was easy to select for by using either the incompatibility or large-colony techniques described. An alternative technique would be selection of 42°C-resistant Ap^r derivatives of a strain carrying a plasmid mutant, temperature sensitive for replication, to which Tn*2301* had been transposed. Yet another possible technique might be based upon the inability of ColE1 to replicate in a *polA*(Ts) cell (27) at the nonpermissive temperature; however, we were unable to use this because for reasons not presently understood pDS1106 itself was not lost under these conditions.

In other bacterial genera it should also be possible to select for Tn*2301* transposition to the chromosome, giving the Hfr strains useful for mapping studies. Techniques for this, similar to those described above and starting with either pDS1106 or the wide host-range plasmid pED712 as vector for Tn*2301*, can easily be devised. Two essential requirements that need to be tested beforehand for any new genus are that cells carrying the plasmid with Tn*2301* should express the F transfer genes and give a functional transfer system, and that the surface of plasmid-free cells should be recognized by this system so that they can function as recipients. These requirements are likely to be met among enterobacteria related to *E. coli*, but preliminary experiments indicate that this is not the case for the more distantly related *Pseudomonas aeruginosa*.

Finally, extension of the techniques described here and those used before by Goebel et al. (21) could prove useful in the creation of new transposable elements, allowing manipulation of important DNA sequences between bacterial species. As an example, linking of the genes for nitrogen fixation (35) into a transposon would facilitate their movement between chromosomal DNA and plasmids, and even into plant cells (16).

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