Hfr FORMATION DIRECTED BY Tn10

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ABSTRACT

The transposable drug-resistance element, Tn10, can serve as a region of homology to direct the insertion of an F'ts114 *lac* plasmid into the chromosome of *Salmonella typhimurium*. Derivatives of F'ts114 *lac* were constructed that carry Tn10 insertions; these plasmids were transferred to strains having a Tn10 insertion in the chromosome. Under these circumstances, Hfr formation requires homologous recombination between plasmid-borne and chromosomal Tn10 elements. The process is dependent on *recA* function and on the presence of both Tn10 elements. All Hfr's isolated from a given merodiploid show the same direction of transfer. Depending on the orientation of Tn10 in the F' plasmid, Hfr's transferring in either direction can be obtained from any chromosomal Tn10 insertion. Since Tn10 insertions can be generated in any region of the chromosome, this method permits the isolation of Hfr's with either direction of transfer having their origin at almost any predetermined site. The Hfr's constructed by this method are sufficiently stable for standard genetic mapping crosses, and they have also been used to generate new F' plasmids. Implicit in the results above is the possibility of determining the orientation of any chromosomal Tn10 insertion by constructing an Hfr using a standard F' Tn10 plasmid and determining the direction of chromosome transfer. The general approaches described here are applicable to other transposable elements and other bacterial systems.

THE lack of an appropriate Hfr has occasionally limited the utility of bacterial conjugation in genetic mapping and has made it difficult to derive F' plasmids carrying certain genes. We have developed a method using the transposable drug resistance element, Tn10 (KLECKNER *et al.* 1975; KLECKNER, ROTH and BOTSTEIN 1977), which permits the isolation of an Hfr with an origin at essentially any selected site on the chromosome. The method also permits selection of the direction of chromosome transfer. This method should be valuable for *E. coli* and Salmonella, but it should be particularly useful in genetic systems for which few Hfr's have been characterized. We have used newly formed Hfr's with origins near the genes for proline utilization (*put*) in the derivation of F' plasmids that carry the *put* region.

The method for Hfr formation involves the directed insertion of an F'ts114 *lac* plasmid into the chromosome by recombination between a Tn10 sequence carried on the plasmid and a second Tn10 sequence located on the chromosome, as diagrammed in Figure 1. This is an extension of the method described by CUZIN and JACOB (1964) and by BECKWITH, SIGNER and EPSTEIN (1966) for the transposition of the lactose operon in *E. coli*. Wild-type *S. typhimurium* lacks the

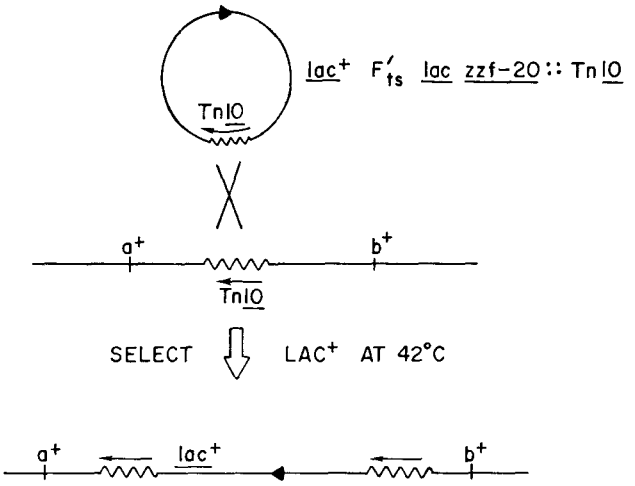

FIGURE 1.—A diagrammatic representation of Hfr formation. Recombination between Tn10 sequences carried by both the plasmid and the chromosome integrates F'ts114 *lac* (JACOB, BRENNER and CUZIN 1963; CUZIN and JACOB 1967) into the chromosome. The Tn10 elements are represented by wavy lines, and an arrow indicates the orientation of each insertion. For the Hfr formed, the origin of transfer is between the markers *a* and *b*; marker *b* will be transferred early. If the orientation of either Tn10 insertion were reversed, the resulting Hfr would transfer *a* early, instead of *b*.

genes for the utilization of lactose, but it is Lac$^+$ when it harbors a plasmid carrying the *lac* operon of *E. coli*, such as F'ts114 *lac*. This particular plasmid is temperature sensitive for replication (JACOB, BRENNER and CUZIN 1963; CUZIN and JACOB 1967), and it is readily lost during growth at 40°, conferring a temperature-sensitive Lac$^+$ phenotype on the Salmonella host cell. When Lac$^+$ survivors are selected at 40°, the most common class results from integration of the F'ts114 *lac* plasmid into the chromosome and its passive replication along with the chromosomal DNA (BECKWITH, SIGNER and EPSTEIN 1966). The point of F′ insertion determines the origin of an Hfr; the orientation of the homologous chromosomal and episomal regions during the insertion event determines the direction of chromosome transfer.

The Tn10 element is a 9,200 base-pair DNA sequence that determines resistance to the antibiotic, tetracycline. The entire element can transpose as a unit in a process that does not depend on homologous recombination (KLECKNER *et al.* 1975). Tn10 insertion mutations within or near any genes of interest can be recovered by rather simple procedures (KLECKNER, ROTH and BOTSTEIN 1977). We have isolated and characterized a number of these insertions in the *his* and *put* regions, in addition to a number of insertions into the F'128 *pro lac* plasmid. Among the insertions into F'128, some are located in F-specific sequences, and some are located in bacterial chromosomal sequences. Tn10 can insert in either of two orientations, and our method for Hfr isolation permits us to determine the relative orientation of any individual insertion, simply by observing the direction of chromosome transfer of Hfr's formed by recombina-

tion between a given chromosomal Tn10 element and a Tn10 element carried by the F′ plasmid. The orientation of the chromosomal Tn10 insertion can be confirmed by using a series of F′ plasmids carrying Tn10 in different orientations. All the Hfr's we have isolated from any given merodiploid transfer the chromosome in the same preferred direction.

Although this work has been done in Salmonella, the procedures should be applicable to other transposable elements and other bacterial systems, where they might facilitate otherwise difficult genetic analyses.

MATERIALS AND METHODS

Bacterial strains: The strains used for this study are derivatives of *Salmonella typhimurium* LT2. Table 1 lists the numerical designations and full genotypes of multiply marked strains. Unless otherwise indicated, the isolation or construction of strains is described in this paper. Strains bearing the prefix "TT" either contain the transposable tetracycline resistance element, Tn10, or they were derived from parental strains that contained Tn10.

Media: Difco nutrient broth (8 g/l), with 5 g per l NaCl added, was used as rich medium. The E medium of VOGEL and BONNER (1956), containing 2% glucose, was routinely used as minimal medium. To select for growth on lactose as the sole carbon source, the NCE (modified E) medium described by BERKOWITZ *et al.* (1968) was used, supplemented with 0.2% lactose, final concentration. To select for growth on 0.2% proline as the sole nitrogen source, strains were grown on the NCN-succinate medium described by RATZKIN, GRABNAR and ROTH (1978). For use in plates, medium was solidified by the addition of Difco agar to 1.5%.

When required, tetracycline (Sigma) was added to a final concentration of 25 μg per ml in rich medium or 10 μg per ml in minimal medium.

Lactose tetrazolium indicator plates (LEDERBERG 1948) contained, per liter, 23 g Difco nutrient agar, 1 g NaCl, 50 mg Difco Bacto-TTC, and, finally, 50 ml of a filter-sterilized 20% lactose solution added after autoclaving the other ingredients.

Transductional methods: Bacteriophage P22 containing the mutations HT105/1 (SCHMIEGER 1971), which causes an increased frequency of generalized transduction, and *int-201* (ANDERSON and ROTH 1978), which prevents stable lysogen formation, was used for all transductions. Phage were grown on donor strains as described by SCOTT, ROTH and ARTZ (1975). In most crosses, phage and bacteria were mixed directly on selective media. When selection was made for inheritance of a donor Tn10, phage and bacteria were mixed and preincubated for 30 min in a nonselective liquid medium before plating on tetracycline-containing media. Transductant clones were purified and made phage-free by streaking alternately on rich and selective media.

Construction of merodiploids containing chromosomal and plasmid-borne Tn10 insertions: F′ts114 *lac* plasmids carrying various Tn10 insertions were transferred from *pyr*⁻ strains TT627, TT628, and TT629 into recipients containing Tn10 insertions in the *put* or *his* regions and a counter-selective auxotrophic marker. The donor strains were grown selectively at 30° in NCE medium containing lactose and uracil, and Lac+ transconjugants were selected at 30° by cross-streaking donor and recipient on lactose plates supplemented as required by the recipients, but containing no uracil. These merodiploids were purified by streaking on the same selective medium.

In all these merodiploids, the Lac+ character was stable at 30°, but was lost at high frequency when the strains were grown in nutrient broth at 42°. Stability of Lac+ was determined by growing the strains at 30° or 42° in nutrient broth from a 10^{-3} dilution of a dense, selectively grown culture. The broth culture was then plated for single colonies on lactose tetrazolium indicator plates.

Isolation of Hfr's from merodiploids: Hfr's were isolated from merodiploids containing chromosomal and episomal Tn10 insertions by the following general procedure. In each experiment, the merodiploid was diluted 10^{-3} from an overnight culture grown in NCE lactose medium

TABLE 1

Strains of Salmonella typhimurium

TR	35	= *hisDE712 ser-821 arg-501*/F'T80 *his*+ (J. ROTH)
	132	= *strA1 adeC7 proA46 ile-405 gal-501* Azaserine^R/F'128 *pro*+ *lac*+ (J. ROTH)
	2246	= HfrB2 *recA1 strA1 metA22* (J. WYCHE)
	2278	= *proAB47 argK539 recA1 strA1* (R. KAYE)
	2647	= *pyrC7 strA1*/F'ts114 *lac*+ (B. RATZKIN)
	4178	= *hisOG203 srl-201* (R. P. ANDERSON)
	5050	= *hisIE640 srl-201 proAB47*
	5295	= *proAB47 recA1 put-544*
	5297	= *hisOG203 put-544 recA1*
NK	337	= *leu-414* SuII+ (P22 *c2ts29 12amN11 13amH101 int-3* TC10) (N. KLECKNER)
	397	= *edd*$^-$ *zee-1*::Tn10 (N. KLECKNER)
TT	28	= *hisC8579*::Tn10
	46	= *hisC8556*::Tn10
	429	= *hisC8571*::Tn10
	513	= *zee-2*::Tn10
	515	= *zee-4*::Tn10
	616	= *hisOE3050*/F'128 *pro*+ *lac*+ *zzf-24*::Tn10
	617	= *hisOE3050*/F'128 *pro*+ *lac*+ *zzf-25*::Tn10
	618	= *hisOE3050*/F'128 *pro*+ *lac*+ *zzf-20*::Tn10
	619	= *hisOE3050*/F'128 *pro*+ *lac*+ *zzf-21*::Tn10
	620	= *hisOE3050*/F'128 *pro*+ *lac*+ *zzf-22*::Tn10
	621	= *hisOE3050*/F'128 *pro*+ *lac*+ *zzf-23*::Tn10
	622	= *proAB47 argI539 strA1 recA1*/F'128 *pro*+ *lac*+ *zzf-24*::Tn10
TT	623	= *proAB47 argI539 strA1 recA1*/F'128 *pro*+ *lac*+*zzf-25*::Tn10
	624	= *proAB47 argI539 strA1 recA1*/F'128 *pro*+ *lac*+ *zzf-20*::Tn10
	625	= *proAB47 argI539 strA1 recA1*/F'128 *pro*+ *lac*+ *zzf-21*::Tn10
	626	= *proAB47 argI539 strA1 recA1*/F'128 *pro*+ *lac*+ *zzf-22*::Tn10
	627	= *strA1 pyrC7*/F'ts114 *lac*+ *zzf-20*::Tn10
	628	= *strA1 pyrC7*/F'ts114 *lac*+ *zzf-21*::Tn10
	629	= *strA1 pyrC7*/F'ts114 *lac*+ *zzf-22*::Tn10
	776	= *hisC8556*::Tn10 *proAB47 srl-201*
	1788	= *zcc-4*::Tn10
	1791	= *zcc-5*::Tn10
	1794	= *zcc-6*::Tn10
	1804	= *hisOE3050 zcc-4*::Tn10
	1805	= *hisOE3050 zcc-5*::Tn10
	1806	= *hisOE3050 zcc-6*::Tn10
	1809	= *hisOG203 srl-201 zcc-4*::Tn10
	1810	= *hisOG203 srl-201 zcc-6*::Tn10
	1811	= *hisOG203 recA1 zcc-4*::Tn10
	1812	= *hisOG203 recA1 zcc-6*::Tn10
	1813	= *hisOE3050 zcc-4*::Tn10/F'ts114 *lac*+ *zzf-20*::Tn10
	1814	= *hisOE3050 zcc-4*::Tn10/F'ts114 *lac*+ *zzf-21*::Tn10
	1816	= *hisOE3050 zcc-5*::Tn10/F'ts114 *lac*+
	1817	= *hisOE3050 zcc-5*::Tn10/F'ts114 *lac*+ *zzf-20*::Tn10
	1820	= *hisOE3050 zcc-5*::Tn10/F'ts114 *lac*+
	1825	= *hisOG203 srl-201 zcc-4*::Tn10/F'ts114 *lac*+ *zzf-20*::Tn10

TABLE 1—Continued

Strains of Salmonella typhimurium

TT1826 = *hisOG203 srl-201 zcc-4*::Tn10/F'ts114 *lac*+ *zzf-21*::Tn10
1831 = *hisOG203 srl-201 zcc-6*::Tn10/F'ts114 *lac*+ *zzf-20*::Tn10
1832 = *hisOG203 srl-201 zcc-6*::Tn10/F'ts114 *lac*+ *zzf-21*::Tn10
1836 = *hisOG203 srl-201*/F'ts114 *lac*+ *zzf-20*::Tn10
1837 = *hisOG203 srl-201*/F'ts114 *lac*+ *zzf-21*::Tn10
1847 = *proAB47 recA1 putPA544*/F'ts601 *put*+
1848 = *proAB47 recA1 putPA544*/F'ts602 *put*+
1849 = *proAB47 recA1 putPA544*/F'ts603 *put*+
1850 = *proAB47 recA1 putPA544*/F'ts604 *put*+
1851 = *proAB47 recA1 putPA544*/F'ts605 *put*+
1864 = *proAB47 recA1 putPA544*/F'ts603 *put*+
1922 = *zee-2*::Tn10 *proAB47 srl-201*
1923 = *zee-4*::Tn10 *proAB47 srl-201*
1924 = *zee-1*::Tn10 *proAB47 srl-201*
1928 = *zee-4*::Tn10 *proAB47 srl-201*/F'ts114 *lac*+ *zzf-20*::Tn10
1929 = *zee-4*::Tn10 *proAB47 srl-201*/F'ts114 *lac*+ *zzf-21*::Tn10
1934 = *hisC8556*::Tn10 *proAB47 srl-201*/F'ts114 *lac*+ *zzf-20*::Tn10
1935 = *hisC8556*::Tn10 *proAB47 srl-201*/F'ts114 *lac*+ *zzf-21*::Tn10
1939 = *zee-1*::Tn10 *proAB47 strA1 recA1*/F'ts114 *lac*+ *zzf-20*::Tn10
1940 = *zee-1*::Tn10 *proAB47 strA1 srl-201*/F'ts114 *lac*+ *zzf-20*::Tn10
1941 = *hisIE640 strA1 recA1 proAB47*/F'ts114 *lac*+ *zzf-20*::Tn10
1942 = *hisIE640 strA1 srl-201 proAB47*/F'ts114 *lac*+ *zzf-20*::Tn10

Multiply marked strains of *Salmonella typhimurium* LT2 used in this study. The source of strains not isolated or constructed in the course of this work is indicated in parentheses following the genotype. The isolation or construction of all other strains is described in detail in relevant sections of the text of this paper.

(supplemented as required) at 30° into the same medium and then grown to full density at 42°. From the same starting inoculum, these strains require 48 hr to grow to full density in lactose medium at 42°, compared to 12 hr at 30°. During incubation at high temperature, there is a selection for individuals in the population that can maintain the temperature-sensitive F'ts114 *lac* plasmid. These 42° cultures were then plated at 42° for single colonies, also on supplemented minimal lactose medium. This procedure was varied slightly in the case of strains TT1940, TT1941, and TT1942, which were grown nonselectively in nutrient broth at 42° and then plated at 42° on lactose tetrazolium indicator agar, from which Lac+ colonies were chosen.

Four individual Lac+ clones (putative Hfr strains) were then selected from each merodiploid and purified by streaking selectively at 42°. These were grown overnight in lactose medium at 42°, and then diluted 10^{-1} into nutrient broth and incubated with shaking at 37° for two to four hr just prior to mating. For conjugation, 0.1 ml of these donor cultures (either undiluted or diluted 10^{-2}) was plated directly onto minimal medium along with 0.1 ml of a nutrient broth culture of the appropriate auxotrophic recipient, and prototrophic recombinants were counted after a 48-hr incubation at 37°.

Isolation of strains containing F' put plasmids: To isolate F' plasmids harboring the *put* genes, 2×10^9 exponentially growing cells of a donor Hfr with a Tn10-directed origin near *put* (TT1825, TT1826, TT1831, or TT1832) were mixed with 10^{10} exponentially growing *put*$^-$ *recA*$^-$ recipient cells (TR5295). The cell mixture was collected on a Millipore filter and incubated for five hr on a prewarmed nutrient broth plate. The filter was removed to 20 ml of liquid nutrient broth and incubated for 12 hr with vigorous shaking. Aliquots from the liquid culture were then spread on selective NCN-proline-succinate plates. All operations were done at 30° since the plasmids were expected to have the original temperature-sensitive character of the

F'ts114 *lac* plasmid used to form the donor Hfr's. After four days' growth at 30°, the selective plates showed a heavy lawn of growth with several thousand papillae. Replica printing to a new NCN-proline-succinate plate allowed the identification of papillae that were true Put+ colonies. The need for the "second selection" by replica printing to the original selective medium is most likely due to the leaky nature of the proline utilization selection and the large number of cells plated (1 to 5×10^9 per plate). In each experiment summarized in Table 6, the full 20 ml volume of the donor/recipient mixed culture was examined on 20 selective plates.

Ultraviolet light irradiation of strains: A nutrient broth culture was grown overnight from a single colony taken from a rich plate. A few ml of this culture was placed in the lid of a sterile petri dish and exposed to two General Electric 15W germicidal lamps at a distance of 75 cm. At 20-second intervals, the culture was thoroughly mixed and 0.2 ml aliquots were removed in order to determine the viable count and inoculate a subculture. Samples were kept in the dark for initial handling and growth. Killing was an exponentially increasing function of the time of exposure, with 1% survival of the starting population after about 140 seconds.

Nomenclature: Insertion mutations made by Tn10 have been named according to the convention of CAMPBELL *et al.* (1977). According to this convention alleles are designated normally but followed by a double colon and Tn10 (*i.e.*, *hisC8556*::Tn10). Allele numbers are assigned as suggested by DEMEREC *et al.* (1966).

We have made a slight addition to these conventions. Many Tn10 insertions have been isolated that are not known to be mutations of any particular gene. Most are isolated as insertions near a gene of interest. We have modified a suggestion of HONG and AMES (1971) to permit naming Tn10 insertions according to map position when the insertion is not within a particular gene. All such insertions are designated by a three-letter symbol starting with *z*; the second and third letters designate approximate map position in minutes. The second position designates 10-minute map segments numbered clockwise from minute 0 ($a = 0$–10; $b = 10$–20; $c = 20$–30, etc.); the third letter similarly designates minutes within any 10 minute segment. For example, a Tn10 insertion located between minutes 2 and 3 would be designated *zac-1*::Tn10; a Tn10 insertion near *his* at minute 44 is designated *zee-2*::Tn10. Allele numbers are assigned serially to such insertions regardless of the letters appearing in positions 2 and 3. Thus, if more refined mapping data warrants a new three-letter symbol, the numerical identity of the insertion mutation is not lost. This convention uses designations *zaa-zjj*. We have designated insertion mutations on extra-chromosomal elements with *zz*, followed by a letter denoting the element used. In this paper, only *zzf* is used; it denotes insertion mutations on an F' plasmid.

RESULT

Isolation and characterization of Tn10 insertions in the put *and* his *regions:* Strains carrying Tn10 insertions near the *put* and *his* genes were isolated by the following general procedure. Tn10 was allowed to insert randomly into the chromosome of *S. typhimurium* LT2 (KLECKNER *et al.* 1975; KLECKNER, ROTH and BOTSTEIN 1977), and approximately 3000 independent insertion mutants were then grown in mixed culture. Phage P22 was grown on this pool, and the lysate was used to transduce *put-557* or *hisOG203* (selecting Put+ or His+, respectively). Among 12,000 Put+ transductants, 32 proved to be tetracycline resistant, while among 10,000 His+ transductants, 27 were tetracycline resistant. The clones that had simultaneously acquired Put+ or His+ and tetracycline resistance were then examined for transductional linkage of Tn10 to the structural genes in question. Among the 32 potential Tn10 insertions near *put*, one (*zcc-4*::Tn10) was 20% linked, and two (*zcc-5*::Tn10 and *zcc-6*::Tn10) were 50% linked in P22 transductional crosses. The other 28 transductants showed no linkage (less than 5%) of tetracycline resistance and *put*; these clones prob-

ably resulted from double transduction events in the original cross. Among 12 potential Tn10 insertions near *his*, one was less than 3% linked to *hisDC2236*, and the other 11 were from 50–85% linked. Three-factor crosses and deletion mapping crosses were then performed to yield the maps shown in Figure 2.

Tn10 insertions into the *hisC* gene were also used in some experiments, and these were obtained by Tn10 mutagenesis of *S. typhimurium* LT2 as described by KLECKNER *et al.* (1975).

Isolation and characterization of Tn10 insertions in F' pro lac: An essential requirement for Hfr formation in the procedure we have outlined is the isolation of Tn10 insertions on an F' plasmid. To accomplish this, Tn10 was transposed from the P22 vector obtained by induction of NK337 into strain TR132, which

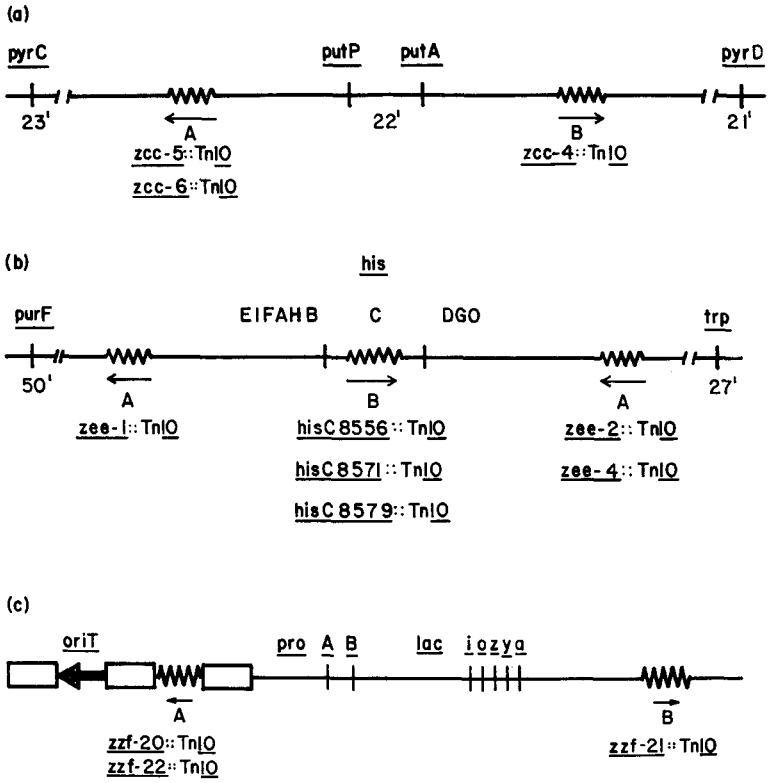

FIGURE 2.—Genetic maps showing the sites of Tn10 insertions used in Hfr isolation and the positions of chromosomal markers scored in conjugational crosses. Figure 2a shows the *put* and Figure 2b the *his* region; these map at 22 min and 44 min on the *S. typhimurium* chromosome, respectively. Figure 2c is a map of F'128 *pro lac*. The plasmid is represented in linear fashion, where the box indicates F-DNA and the straight line indicates bacterial DNA. The relative orientation of the Tn10 insertions is also shown. For the chromosomal insertions, orientation "A" indicates a Tn10 insertion yielding Hfr's that transfer counterclockwise when an episome integrates which carries the insertion *zzf-20*::Tn10; orientation "B" indicates clockwise transfer. For the F' Tn10 insertions, orientation "A" indicates the same orientation as *zzf-20*::Tn10; orientation "B" indicates the opposite orientation.

contains F'128 *pro lac*, using the procedure of KLECKNER *et al.* (1975). Following the initial transposition, 215 independent tetracycline-resistant (Tet^R) derivatives of TR132 were grown in nutrient broth and used as donors in a conjugational cross with *hisOE3050* as a recipient. Among these 215 isolates, six were able to transfer the Tet^R characteristic along with Lac^+ in the conjugational crosses. The extrachromosomal location of the Tet^R determinant was confirmed by a second co-transfer of Lac^+ and Tet^R from the *hisOE3050*/F'128 *pro lac* Tet^R transconjugants above to the Rec^- strain TR2278. A summary of the strains with Tn10 on the plasmid (TT616 to TT629) is given in Table 1.

To determine the location of the plasmid-borne Tn10 insertions, P22 phage were grown on strains TT622, TT623, TT624, TT625, and TT626. The phage were used as donors of Tet^R in crosses with recipients carrying various F' plasmids (see Table 2). If a particular phage preparation can efficiently transduce Tet^R into a strain, the donor and recipient strains must share homology in the region where the Tn10 insertion is located. The results in Table 2 show that the Tn10 insertions can be assigned to particular regions of F'128 *pro lac*. Insertions *zzf-20*::Tn10 and *zzf-22*::Tn10 seem to be located in F sequences. Insertion *zzf-21*::Tn10 seems to be located outside of F in sequences common to F'128 *pro lac* and F'ts114 *lac*. Insertions *zzf-24*::Tn10 and *zzf-25*::Tn10 appear to be located in sequences unique to F'128 *pro lac*. The nature of the rare transductants seen in crosses with *hisOE3050*, which is F^-, and with other strains not carrying regions of homology is not clear, but may represent secondary transpositions. Transductants inheriting Tn10 in the F'ts114 *lac* plasmid of TR2647 were purified and kept for use in later experiments (TT627, TT628, and TT629, listed in

TABLE 2

Transduction of plasmid-borne Tn10 insertions

Donor	Number of Tet^R transductants with indicated recipient: *hisOE3050* (no F')	TR132 (F' *pro lac*)	TR2647 (F'ts *lac*)	TR35 (F'T80 *his*)	Inferred location of Tn10
TT622 (*zzf-24*::Tn10)	2	~ 500	4	2	Bacterial region not shared by F' *pro lac* and F'ts *lac*
TT623 (*zzf-25*::Tn10)	12	~ 500	14	3	Bacterial region not shared by F' *pro lac* and F'ts *lac*
TT624 (*zzf-20*::Tn10)	3	~ 500	~ 500	~ 500	F-genes
TT625 (*zzf-21*::Tn10)	3	~ 500	~ 500	12	Bacterial region shared by F' *pro lac* and F'ts *lac*
TT626 (*zzf-22*::Tn10)	5	~ 500	~ 500	~ 500	F-genes

Transductions were performed by mixing about 2×10^8 recipient cells with transducing phage (about 10^{10} pfu) in liquid at 30° and incubating for 30 min. The mixture was then plated on nutrient broth containing tetracycline. The number of transductants per plate was scored after a 24-hr incubation at 30°. When either transducing phage or recipient bacteria were plated alone, no Tet^R colonies were observed.

Table 1). The plasmid location of Tn10 in these strains was confirmed by both co-segregation of Lac+ and Tet^R at 42° and co-transfer of Lac+ and Tet^R in conjugational crosses with Rec$^-$ recipients.

Chromosome transfer from merodiploids grown at high temperature in bulk culture: A series of merodiploids was constructed carrying the F'ts114 *lac* plasmid and various Tn10 insertions in the F' plasmid and the *put* region of the chromosome, as described above in MATERIALS AND METHODS. These strains were tested for their ability to transfer the chromosomal *pyrC* and *pyrD* genes, which flank the *put* region. Transfer was tested before and after selection for insertion of the F' plasmid. The results are presented in Table 3.

From Table 3 it is evident that prior growth at 42° greatly enhances the ability of these merodiploids to mobilize the chromosome, although there is no such enhancement if the F'ts114 *lac* episome does not carry a Tn10 insertion. Chromosome transfer from these merodiploids is highly directional; any single merodiploid shows widely different efficiency in the transfer of *pyrC* and *pyrD* genes. The direction of transfer is evidently determined by the orientation of the Tn10 insertions relative to the transfer origin on F; the chromosomal insertions *zcc-4*::Tn10 and *zcc-5*::Tn10 must be in opposite orientations, because merodiploids containing these insertions and F'ts114 *lac* *zzf-20*::Tn10 transfer in opposite directions. Similarly, the F' insertions *zzf-20*::Tn10 and *zzf-21*::Tn10 must be in opposite orientations, because merodiploids containing these insertions in F'ts114 *lac* and the chromosomal insertion *zcc-4*::Tn10 transfer in opposite directions.

Isolation of Hfr's from merodiploids containing chromosomal and episomal Tn10 insertions: The results presented in Tables 4 and 5 demonstrate that individual Hfr isolates from a single merodiploid all behave like identical Hfr's, with the origin of transfer at the site of the chromosomal Tn10 insertion; all

TABLE 3

Chromosome mobilization from merodiploids containing chromosomal and plasmid-borne Tn10 insertions

Donor Strain	Genotype	Number of prototrophic recombinant colonies with *pyrC7* no pretreatment	with *pyrC7* pretreatment	with *pyrD121* no pretreatment	with *pyrD121* pretreatment
TT1813	*hisOE3050 zcc-4*::Tn10/F'ts *lac*+ *zzf-20*::Tn10	50	2000	4	6
TT1814	*hisOE3050 zcc-4*::Tn10/F'ts *lac*+ *zzf-21*::Tn10	14	15	12	150
TT1816	*hisOE3050 zcc-4*::Tn10/F'ts *lac*+ (no Tn10)	10	10	2	6
TT1817	*hisOE3050 zcc-5*::Tn10/F'ts *lac*+ *zzf-20*::Tn10	9	12	50	2000
TT1818	*hisOE3050 zcc-5*::Tn10/F'ts *lac*+ *zzf-21*::Tn10	20	500	5	10
TT1820	*hisOE3050 zcc-5*::Tn10/F'ts *lac*+ (no Tn10)	10	10	2	3

The donors were grown at 30° in medium containing lactose and histidine. They were then grown for mating by diluting 10^{-3} into the same medium either at 30° ("no pretreatment") or at 42° ("pretreatment"). Matings were performed by spreading 0.1 ml of a donor culture in mid-exponential phase and 0.1 ml of a mid-exponential phase recipient culture [either TR5711(*pyrC7*) or TR5712(*pyrD121*)] on minimal medium at 37°. Data are presented as the number of prototrophic recombinants per mating, counted after 48 hr of incubation.

TABLE 4

Chromosome transfer from Hfr's formed in the put *region*

Strain	Genotype	Isolate number	Number of prototrophic recombinant colonies with: *pyrD121* (ccw transfer)	*pyrC7* (cw transfer)	Direction of transfer
TT1825	*hisOG203 srl-201 zcc-4*::Tn10/F'ts *lac*+ *zzf-20*::Tn10	1	24	440	cw
		2	12	460	cw
		3	23	560	cw
		4	10	270	cw
TT1831	*hisOG203 srl-201 zcc-6*::Tn10/F'ts *lac*+ *zzf-20*::Tn10	1	1000	50	ccw
		2	1400	40	ccw
		3	1500	40	ccw
		4	1500	75	ccw
TT1836	*hisOG203 srl-201* (no Tn10 insertion)/ F'ts *lac*+ *zzf-21*::Tn10	1	12	9	none
		2	8	8	none
		3	3	2	none
		4	6	4	none
TT1826	*hisOG203 srl-201 zcc-4*::Tn10/F'ts *lac*+ *zzf-21*::Tn10	1	600	17	ccw
		2	370	10	ccw
		3	380	10	ccw
		4	1500	130	ccw
TT1832	*hisOG203 srl-201 zcc-6*::Tn10/F'ts *lac*+ *zzf-21*::Tn10	1	9	290	cw
		2	4	180	cw
		3	10	270	cw
		4	12	480	cw
TT1837	*hisOG203 srl-201* (no Tn10 insertion)/ F'ts *lac*+ *zzf-21*::Tn10	1	3	1	none
		2	16	13	none
		3	2	3	none
		4	1	2	none

Lac+ isolates selected at 42° from the merodiploids indicated were tested for the ability to transfer *pyrC*+ or *pyrD*+ markers in conjugation with TR5712(*pyrD121*) and TR5711(*pyrC7*), as described in MATERIALS AND METHODS. The data are presented as the number of prototrophic recombinants per mating, using 0.1 ml of a 10^{-2} dilution of the donor culture and 0.1 ml of undiluted recipient culture. The donor strains plated alone yielded no colonies on minimal medium, while the recipients yielded from zero to ten Pyr+ revertants per plate.

transfer the chromosome in the same preferential direction. In the case of Hfr's originating in the *put* region, this direction of transfer agrees with the direction observed in the bulk culture experiments described above. Tables 4 and 5 also show that the chromosomal Tn10 insertion is essential for directed Hfr formation, as no directed Hfr's could be recovered from strains containing only a Tn10 insertion in F'ts114 *lac*. Table 5 also shows that a strain carrying both a plasmid-borne and a chromosomal Tn10 insertion does not give rise to Hfr's with origins at the chromosomal Tn10 insertion if it lacks *recA* function.

From the data presented in Tables 4 and 5 and other similar results, it was apparent that all the Hfr's isolated from a given merodiploid mobilize the chromosome in the same preferred direction. Two large experiments were performed

TABLE 5

Chromosome transfer from Hfr's formed in the his *region*

Strain	Genotype	Isolate number	Number of prototrophic recombinant colonies with: *purF145* (cw transfer)	*trp-101* (ccw transfer)	Direction of transfer
TT1928	*proAB47 srl-201 zee-4*::Tn10/F'ts114	1	16	500	ccw
	lac+ *zzf-20*::Tn10	2	4	500	ccw
		3	100	1200	ccw
		4	34	500	ccw
TT1929	*proAB47 srl-201 zee-4*::Tn10/F'ts114	1	150	7	cw
	lac+ *zzf-21*::Tn10	2	300	27	cw
		3	200	26	cw
		4	200	25	cw
TT1934	*proAB47 srl-201 hisC8556*::Tn10/F'ts114	1	500	80	cw
	lac+ *zzf-20*::Tn10	2	500	150	cw
		3	400	80	cw
		4	300	16	cw
TT1935	*proAB47 srl-201 hisC8556*::Tn10/F'ts114	1	3	400	ccw
	lac+ *zzf-21*::Tn10	2	11	500	ccw
		3	0	250	ccw
		4	1	200	ccw
TT1939	*proAB47 srl-201 zee-1*::Tn10/F'ts114	1	2	250	ccw
	lac+ *zzf-20*::Tn10	2	11	250	ccw
		3	4	400	ccw
		4	0	300	ccw
TT1940	*proAB47 srl-201 zee-1*::Tn10 *recA1*/F'ts114	1	0	0	none
	lac+ *zzf-20*::Tn10	2	1	0	none
		3	0	0	none
		4	0	0	none
TT1941	*proAB47 srl-201* (no Tn10 insertion)/	1	0	0	none
	F'ts114 *lac*+ *zzf-20*::Tn10	2	0	0	none
		3	0	0	none
		4	0	0	none
TT1942	*proAB47 srl-201* (no Tn10 insertion)/	1	0	0	none
	F'ts114 *lac*+ *zzf-20*::Tn10	2	0	0	none
		3	0	0	none
		4	0	0	none

Lac+ isolates selected at 42° from the merodiploids indicated were tested for the ability to transfer *trp*+ or *purF*+ markers in conjunction with TR5713(*trp-101*) or TR5714(*purF145*), as described in MATERIALS AND METHODS. The data are presented as the number of prototrophic recombinants per mating, using 0.1 ml each of the undiluted donor and recipient cultures. When the donors were plated alone, no colonies grew up on minimal medium, and there were no Trp+ revertants from TR5713. When TR5714 was plated alone, from zero to five Pur+ revertants were observed per plate.

to test this rule. First, TT1809, containing the *put*-region Tn10 insertion, *zcc-4*:: Tn10, was plated for single colonies. F'ts114 *lac* carrying the insertion *zzf-21*:: Tn10 was transferred into each of these isolates from strain TT628, and merodiploids were selected and purified at 30°. A single isolate retaining the Lac$^+$ character at 42° was selected from each merodiploid and purified, and 99 of these were then mated in crosses with *pyrC7* and *pyrD121*. Among the 99 potential donors, 97 transferred preferentially in the *pyrD* direction (ten- to 50-fold more *pyrD*$^+$ than *pyrC*$^+$ recombinants), while two failed to transfer either gene with high frequency.

A second experiment was then undertaken to try to detect inversion of the Tn10 insertion in TT1809. We reasoned that exposure to ultraviolet light, which is known to stimulate recombination (CLARK *et al.* 1950), might promote an exchange between the inverted terminal repeats of Tn10, thereby reversing the orientation of the intervening sequences. Strains carrying such inversions should be identifiable because they will generate Tn10-directed Hfr's with an inverse direction of transfer. A culture of TT1809 was exposed to UV until 1% of the cells were surviving, as described in MATERIALS AND METHODS. Following UV exposure, the irradiated culture was diluted into fresh nutrient broth, grown to full density, and plated for single colonies at 42° on E medium containing tetracycline. Merodiploids were then constructed and Lac$^+$ survivors were isolated in the usual manner. Among 126 potential Hfr isolates tested, 123 transferred the *pyrC* or *pyrD* genes with high frequency, while three showed no transfer of either gene. The preferred direction of transfer in 117 of the fertile isolates agreed with previous observations. None of the fertile isolates transferred preferentially in the opposite direction; thus none seems to be a simple case of Tn10 inversion. The six unusual isolates transferred the *pyrC* and *pyrD* markers with roughly equal frequency and with a ten- to 100-fold increased efficiency (recombinants per donor cell), compared to the major class of Hfr's. The behavior of these six isolates is consistent with their containing F′ plasmids that include both *pyrC* and *pyrD* genes. It is also possible that chromosomal rearrangements have occurred in these strains, placing both *pyr* markers on the same side of the origin. We have not pursued these exceptional observations further. In summary, none of our experiments have yielded strains that could be accounted for by simple inversion of a Tn10 element.

Determination of the orientation of Tn10 insertion mutations: Chromosome transfer in Hfr's derived from merodiploids containing various chromosomal and plasmid-borne Tn10 insertions suggests that the observed direction of mobilization is a property of the orientation of the Tn10 insertions with respect to the origin of transfer on F, as noted above. Based on the data presented in Tables 3, 4, and 5, we have assigned an unambiguous relative orientation to a number of Tn10 insertions in the *his* and *put* regions, as well as in the F′128 *pro lac* episome. Figure 2 summarizes these determinations.

F′ plasmid formation: Low (1968) has demonstrated that F′ plasmids can be isolated by selective transfer from an appropriate Hfr donor strain into a Rec$^-$ recipient. We have been able to show that Hfr's formed by Tn10-directed F′

insertion can be used to form F′ plasmids carrying genes near the origin of transfer. This provides further evidence for the Hfr nature of the Tn10-directed chromosome mobilization and should provide a general procedure for the isolation of plasmids carrying genes of interest in bacteria. F′ *put* plasmids were constructed by mating one of the Tn10-directed Hfr's (described above in Table 4) with a Put⁻ Pro⁻ Rec⁻ recipient (TR5295), selecting for growth on proline as the

TABLE 6

Characteristics of Put+ colonies potentially harboring F'ts put *plasmids*

Experiment	Genotype of donor prior to Hfr selection	*Put*+ transconjugants in each class A	B	C1	C2	D1	D2	E
Part A								
I	(TT1825) *hisOG203 zcc-4*::Tn10 *srl-201*/ F'ts *lac zzf-20*::Tn10	0	0	1	0	0	1	1
II	(TT1831) *hisOG203 zcc-6*::Tn10 *srl-201*/ F'ts *lac zzf-20*::Tn10	2	0	0	0	0	0	0
III	(TT1832) *hisOG203 zcc-6*::Tn10 *srl-201*/ F'ts *lac zzf-21*::Tn10	2	0	0	0	0	0	0
IV–VIII	(TT1826) *hisOG203 zcc-4*::Tn10 *srl-201*/ F'ts *lac zzf-21*::Tn10	2	2	2	2	2	0	4

Part A: These are the results of eight separate experiments designed to isolate F'ts *put* plasmids. Line 4 shows the pooled results of experiments IV to VIII. The experiments are described in MATERIALS AND METHODS. The nature of each class of transconjugants is defined in Part B of this figure. The actual Hfr strains derived from TT1825, TT1826, TT1831 and TT1832 were maintained only for the course of these experiments as they are unstable and they can easily be regenerated from the parental merodiploids.

Class	Phenotype of Put+ transconjugants	Total number observed	Induced segregation at 42°*	Ability to transfer marker to *recA*⁻ recipient†
Part B				
A	Pro+ Rec+ Lac⁻ Tet^S	4	not tested	not tested
B	Pro+ Rec+ Lac+ Tet^R	2	not tested	not tested
C1	Pro⁻ Rec⁻ Lac+ Tet^R	3	Put+, Lac+ and Tet^R all cosegregate	Put+, Lac+, Tet^R all cotransfer
C2	Pro⁻ Rec⁻ Lac+ Tet^R	2	Lac+ and Tet^R cosegregate	Lac+ and Tet^R cotransfer
D1	Pro⁻ Rec⁻ Lac⁻ Tet^R	2	Put+ and Tet^R cosegregate	Put+ and Tet^R cotransfer
D2	Pro⁻ Rec⁻ Lac⁻ Tet^R	1	no segregation	no transfer observed
E	Pro⁻ Rec⁻ Lac⁻ Tet^S	7	no segregation	no transfer observed

Part B:

* Induced segregation refers to nonselective growth at 42° for 10 to 15 generations. Where segregation is noted, 20 to 90% of the colonies tested had lost the phenotypes indicated.

† Strain TR5297 was used as a potential recipient for classes C and D (Pro⁻ counterselected, Tet^R Lac+ selected). Strain TT1846 was used as a potential recipient for class E (Tet^S counterselected, Put+ selected). Crosses were performed on nutrient broth plates at 30° and then printed to selective plates at 30° after 12 hours.

sole nitrogen source (Put+). Procedural details are presented in MATERIALS AND METHODS. The resulting Put+ transconjugants were scored for inheritance of Lac+, Tet^R, and Rec function. The results of eight separate experiments are given in Table 6, part A. The table shows that two classes (C1 and D1) have properties consistent with the possession of a plasmid carrying the *put* genes; the Put+ phenotype can be transferred to a new Rec$^-$ host, and it shows the temperature sensitivity characteristic of the parental F'ts114 lac episome. The other classes of Put+ transconjugants can be explained as true *put*+ recombinants that arose following transfer and expression of the *recA*+ gene. This can occur with stable integration of the *rec* function (classes A and B) or with only transient expression of *rec* (classes C2, D2, & E). In these *put*+ recombinants, the parental plasmid may (classes B and C2) or may not be inherited (classes A, D2, and E).

The plasmids of classes C1 and D1 were analyzed for the possession of other chromosomal markers near *put*. In these experiments the plasmids were transferred into an auxotrophic strain, selecting either Tet^R or Lac+. The transconjugants were then purified and tested for their auxotrophy. The results given in Table 7 show that F'ts601 and F'ts602 carry none of the additional markers tested. F'ts603 carries the *pyrD* gene, and F'ts604 and F'ts605 carry both *pyrC* and *purB*.

Figure 3 presents the structure of the Hfr origin and events leading to F'-formation. These conclusions were based on the composition of the plasmid, direction of Hfr transfer and the location of the Tn10 insertions involved. All the plasmids can be accounted for by either simple type 1 excision events (F'ts601, 602, 604, and 605 or by type 2 excision events (F'ts603) (DAVIDSON *et al.* 1974). The placement of genes in Figure 3 is made assuming that plasmid formation occurs without complex rearrangements. Although rearrangements cannot be ruled out, we choose to present the simplest hypothesis consistent with our observations. We postulate that plasmid F'ts603 (Figure 3b) contains two Tn10 elements, since it shows considerable instability; from 1 to 5% of the cells retain *lac* but lose the *pyrD* and *put* genes after 10 to 15 generations of nonselective growth at 30°. This instability is expected due to homologous recombination

TABLE 7

Identification of markers carried by F'ts put *plasmids*

Plasmid	Original strain designation following formation in TT5295	Ability of plasmid to complement indicated mutation following transfer				
		aroG	*pyrD*	*pyrC*	*purB*	*aroA*
F'ts601 (Lac$^-$ Tet^R)	TT1847	no	no	no	no	no
F'ts602 (Lac$^-$ Tet^R)	TT1848	no	no	no	no	no
F'ts603 (Lac+ Tet^R)	TT1849	no	yes	no	no	no
F'ts604 (Lac+ Tet^R)	TT1850	no	no	yes	yes	no
F'ts605 (Lac+ Tet^R)	TT1851	no	no	yes	yes	no

F'ts *put* plasmids were transferred into a variety of auxotrophic strains selecting either Lac+ or Tet^R. The ability of the plasmids to complement the indicated auxotrophs was scored following purification of the transconjugants. Recipient mutations are placed in the Table in map order from left to right.

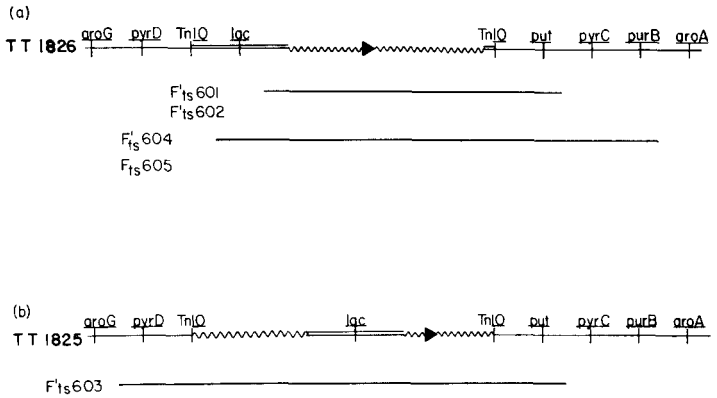

FIGURE 3.—The inferred structure of the parental Hfr's and derived F′ plasmids. Part (a) is a map of an Hfr strain derived from TT1826. The horizontal bars below the map indicate the extent of chromosome which circularized to form F′ts601, 602, 604, and 605. Part (b) is a map of an Hfr strain derived from TT1825; the horizontal bar below the map indicates the composition of F′ts603. In both parts, (a) and (b), the wavy line indicates F-sequences, and the double line indicates the *E. coli* chromosomal sequences carried by F′ts114 *lac*. Tn10 insertions are indicated by a single hatch mark.

between the two Tn10 elements. Other work in our laboratory has shown that when genes are flanked by Tn10 insertions having the same orientation, these genes are lost with considerable frequency (F. CHUMLEY and M. SCHMID, unpublished results). If F′ts604 or F′ts605 contained a second Tn10 element, we would expect similar instability for *put*, *pyrC*, and *purB*: we do not observe this, and we infer that only one Tn10 element is included in each of these plasmids. Episomes F′ts601, F′ts602, F′ts603, F′ts604, F′ts605 all behave as normal stable episomes at 30°. Any marker losses can be explained by simple episome segregation, since all markers were lost as a unit.

The plasmid F′ts601 is presently being used in complementation studies of the *put* genes. It behaves well in homogenotization experiments, and *put* mutations can be transduced to and from the plasmid. The temperature sensitivity of the plasmid requires that experiments be done at 30°, but does allow convenient isolation of F^- segregants following growth at 42°.

DISCUSSION

The experiments reported in this paper demonstrate that homology between a Tn10 insertion on an F′ plasmid and a chromosomal Tn10 insertion can be used to direct F′ insertion, thereby generating an Hfr with an origin at the site of the chromosomal insertion. The direction of transfer of such an Hfr is determined by the orientations of the two Tn10 insertions.

The involvement of homologous recombination in the formation of Hfr's by the scheme above is implied by the dependence on both plasmid-borne and chromosomal Tn10 insertions, as well as a functional *recA* gene. That insertion of the F′ plasmid is involved can be inferred by the enhancement of directional

chromosome mobilization following the selection for F′ insertion (Table 3). Plasmid insertion is also implied by the observation that the Hfr's made by this procedure can serve as a source of new F′ plasmids carrying markers located in the region near the site of the chromosomal Tn10 insertion.

The possibility of isolating an Hfr with an origin at a desired chromosomal site with either orientation should aid in both chromosome mapping and F′ plasmid isolation. This should be particularly useful in *S. typhimurium* where the number of available Hfr's and F′ plasmids is limited. This method should be applicable to any bacterium in which both F′ plasmids and Tn10 elements can be used.

Tn10 insertions in F-factor sequences (*e.g., zzf-20*) have been isolated that do not interfere with either replication or transfer of F. Such insertions can be transduced onto any F′ plasmid and may provide a convenient marker for selecting plasmid transfer and maintenance.

Since the direction of transfer of a Tn10-generated Hfr is dependent on the orientation of both the plasmid-borne and chromosomal Tn10 insertions, the orientation of all chromosomal Tn10 insertions can be established by the direction of transfer of the resultant Tn10-generated Hfr's formed with a single F′-plasmid Tn10 insertion. We have applied this method to a number of chromosomal Tn10 insertions and have in each case been able to assign an unambiguous orientation. The utility of this method is not limited to Tn10 insertions as we have been able to orient Tn5 insertions by Tn5-directed Hfr's made in a strictly analogous fashion (R. MENZEL, unpublished observations). Directed Hfr formation may permit determination of the orientation of any transposable drug resistance insertion.

Experiments presented here suggest that there may be some restriction on recombination between the 1400-base-pair inverted terminal repeats (IS10 sequences) found in the Tn10 element. Two types of recombination events involving the IS10 sequences would give rise to exceptional Tn10-generated Hfr's that transfer the chromosome in the opposite direction from the majority of the isolates derived from a particular merodiploid. First, recombination between the terminal regions of a Tn10 insertion would reverse the orientation of the intervening sequences. If the orientation of a chromosomal or episomal Tn10 insertion were reversed in this manner, any Hfr's subsequently formed would transfer in the opposite direction from Hfr's formed using the original Tn10 insertion. The second type of recombination event that would give rise to such exceptional Hfr's could take place during insertion of the F′ts114 *lac* plasmid. The existence of inverted repeats of IS10 sequences in the Tn10 elements should occasionally permit integration events that lead to insertion of the F′ plasmid in an orientation oposite to that which occurs most frequently. These recombination events would involve only IS10 sequences. Simply based on the lengths of homology available for recombination, one would expect 25% of the Hfr isolates to carry the F′ plasmid integrated in this manner. We have now examined some 328 Hfr's independently derived from 27 different merodiploids without finding any exceptional isolates with regard to the direction of chromosome mobilization. It is apparent, therefore, that integration events involving only the IS10

sequences must occur at a considerably lower frequency than might be expected. Similarly, our data have not revealed evidence that IS10 sequences can recombine to invert the Tn10 element, even following stimulation of recombination by exposure to UV.

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