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Origin of *Escherichia coli* K-12 Hfr B7

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Several F′ plasmids encoding resistance to tetracycline have been derived from a *trg*::Tn*10* Hfr B7 strain of *Escherichia coli* K-12. One of these plasmids, JGF312, was analyzed by restriction endonuclease digestion and Southern blot hybridization to cloned chromosomal fragments. This analysis revealed that JGF312 was formed by Tn*10*-promoted deletion from the Tn*10* insertion (31.4 min) to within the prophage *rac* at 30.1 min. Hfr B7 was shown to result from recombination between IS*2* of FΔ(33-43) and a chromosomal IS*2* located within the *rac–man* region at 30.9 min on the genetic map.

Until the recent construction, by transposon insertion, of strains containing markers at cotransducible distances from each other (4, 11), mapping of genes in the region of the terminus of replication of the *Escherichia coli* K-12 chromosome had been restricted to conjugational analysis. Several genes were mapped with respect to the only available stable Hfr in that region, Hfr B7. This Hfr, due to integration of the deletion mutant of F, F Δ(33-43) (1), transfers counterclockwise from the point of origin (PO 43) (7). F′123 (KLF23 [17]), which contains the genes from (PO 43) to *galU* (27.3 min), has also been used for gene localization in this region. The information from such analyses, however, has been restricted by the absence of a precise mapping of (PO 43). Harayama et al. (13) established the order *pyrF-rac*-(PO 43)-*trg-manA*. On the restriction map of the terminus region, Bouché et al. (6) localized *rac* between 29.6 and 30.1 min and *trg* at 31.4 min. Kaiser and Murray (16) found that restriction fragments extending 12.7 kilobase pairs (kb) clockwise on the genetic map from the end of *rac* were present on F′123. According to our relation between physical and genetic distances in that region (6), this limits the position of (PO 43) to between 30.4 and 31.4 min.

Fouts and Barbour (11) mapped the kasugamycin resistance gene *ksgD* at 30.4 min, a position recently confirmed by cotransduction with a transposon marker (12). The marker *ksgD* was reported to be transferred late by a derivative of Hfr B7. Thus, a position of (PO 43) at or very close to 30.4 min would be in full agreement with all of these data.

In this paper we report the analysis of an F′ plasmid derived from Hfr B7 containing all of the chromosomal DNA from *rac* to *trg*. We conclude that the actual position of (PO 43) is 30.9 min and that Hfr B7 was formed by IS*2*-IS*2* recombination.

MATERIALS AND METHODS

Bacterial strains and plasmids. The following strains were used in this study: UH43A (Hfr[PO 43] *trg2*::Tn*10 rel*) (13), LN536 (F⁻ *recA rpsL700 manA4 thi argE3 galK2 lacY1*) (our collection), and LN762 (LN536 *trp*::Mu *gyrA*) (this work). The plasmids used in this work and their origin are listed in Table 1.

Map of F Δ(33-43). The map of F Δ(33-43) was generated from data in the literature. The size of F, the origin of coordinates, and the positions of the *Eco*RI and *Hin*dIII sites in the *tra* region were as given by Willetts and Skurray (23). The positions and sizes of Tn*1000*, IS*2*, and IS*3* and of the deletion Δ(33-43) were taken from the electron microscopy data of Ohtsubo and Ohtsubo (19) and Palchaudhuri and Maas (20) and expanded by a factor of 1.03 (2). Finally, the positions of the *Eco*RI and *Hin*dIII sites in the *tra* region, taken from Guyer (12), Skurray et al. (22), and Manis and Kline (18), were adjusted into this frame.

Methods. F′ DNA was purified following essentially the method described by Deonier and Mirels (9). Methods for the preparation of amplifiable plasmid DNA, nick translation, and hybridizations were as previously reported (6).

RESULTS

Analysis of F′ plasmid JGF312. Twenty-three merodiploid strains were isolated from conjugation of UH43A with LN536 by selection for tetracycline (Tet) and streptomycin resistance. In all instances the resistance to tetracycline was transferable with high efficiency to a secondary recipient strain, LN762, indicating that Tn*10* was carried by an F′ plasmid which is stable in the *recA* background of LN536. Five of the merodiploid strains were Man⁺ and formed mucoid colonies on minimal medium plates, whereas the remainder had neither of these characters. One of the Man⁻ isolates was randomly chosen,

TABLE 1. Plasmids

Plasmid	Vector	Insert[a]	Reference or source
pBS1	pBR325	H36*b*	3
pBS2	pBR325	P5657*v*	3
pBS3	pBR325	P49*v*::IS*1*	3
pBS4	pBR325	P32*r*	3
pJPB3	pCR1	P3839*v*, *trg*::Tn*10*	6
pJPB6	pBR322	P3839*v*::IS*50*	S. Harayama[b]
pMJVI	pCR1	See text	This work
p*galOP*::IS*2*#1	pBR322	See text	J. Fritz
pHD009	pBR322	See text	J. Fritz
JGF312			This work

[a] Names of inserts refer to the fragment nomenclature in the region of the terminus (5).

[b] Plasmid pTH51 (13), which contains fragment P3839*v* inserted into pBR322, was kindly supplied by G. L. Hazelbauer in a *trg*::Tn*5* background. Upon subcloning, the plasmid (pJPB6) had acquired IS*50* within the insert.

and its plasmid, JGF312, was studied in detail.

JGF312 DNA digestions by *Eco*RI and *Hin*dIII yielded the patterns shown in Fig. 1. The size of the plasmid, estimated from the sizes of the fragments, was 155 to 160 kb. Since JGF312 was Tra$^+$ and Tet^r, it was likely to contain both the F Δ(33-43) from Hfr B7 (90 kb) and Tn*10* (9.2 kb).

The restriction maps of F Δ(33-43) (see Materials and Methods), Tn*10* (15), and the region of the terminus (5) are known. The site of insertion and the orientation of Tn*10* in strain UH43A have been established (6). The present problem was thus to determine how these constituent elements are linked in JGF312. Figure 2, which summarizes our results, can be used as a guide to what follows.

The identification of the F′ fragments containing chromosomal sequences was performed by Southern blot hybridization to various plasmids carrying sequences from the replication termination region: pJPB3 (6), pBS1, pBS2, and pBS4 (3).

Fragments of JGF312 DNA hybridizing to pBS2 and pBS4 had the same sizes as the fragments located on the map of the terminus region between the *Hin*dIII site at position 223 kb and the *Eco*RI site of Tn*10* inserted in *trg* at position 247 kb (Fig. 3). Hybridization of pJPB3 DNA, which carries the Tn*10* insertion in *trg*, revealed the presence of the fragments internal to Tn*10* and those located to the left of IS*10*-right, up to the *Hin*dIII site at position 233 kb (Fig. 3), in agreement with the data provided by hybridization to plasmid pBS4. In contrast, all fragments containing the IS*10*-left boundary were replaced by new ones. The interpretation of these data is given in Fig. 4. In JGF312, in contrast to UH43A, the first *Eco*RI and *Pst*I sites beyond the limit of IS*10*-left are almost coincident, whereas the first *Hin*dIII site is about 12 kb more distant. On the map of the terminus region (5), this arrangement of sites can only correspond to that of the *Eco*RI, *Pst*I, and *Hin*dIII sites located near prophage *rac* at positions 189, 189, and 201 kb, respectively. The linkage of IS*10*-left at a position near 186 kb would indicate that JGF312 contains 247 − 186 (= 61) kb from the chromosome, a value in agreement with our preliminary analysis.

To better define the exchange points on the chromosome, the *Pst*I fragment of JGF312 that

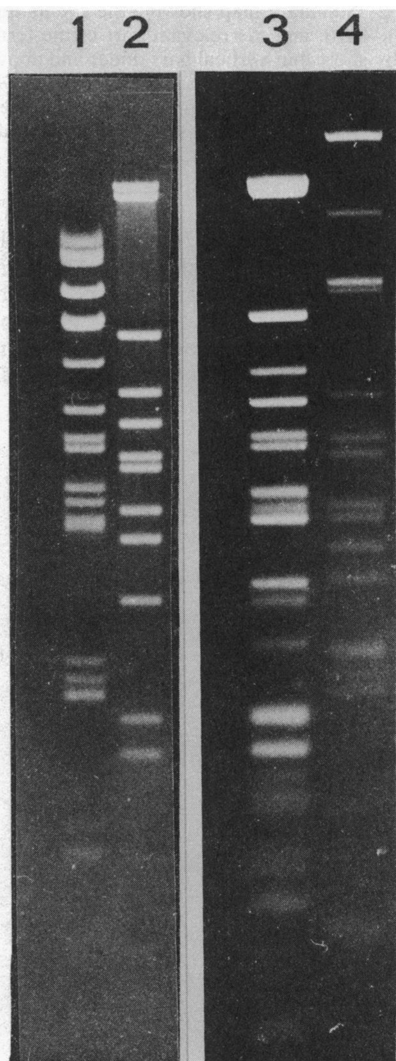

FIG. 1. Restriction endonuclease digestion of JGF312 DNA. Lanes 1 and 4, Digestion of JGF312 DNA by *Eco*RI and *Hin*dIII, respectively; lane 2, mixture of *Eco*RI- and *Hin*dIII-digested λ *c*I857 *S7* DNA; lane 3, mixture of *Eco*RI-, *Hin*dIII-, and *Hin*cII-digested λ *c*I857 *S7* DNA.

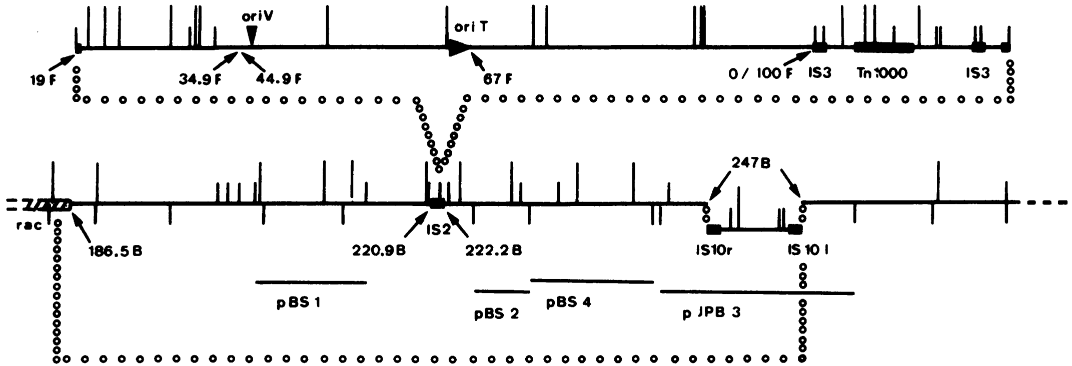

FIG. 2. Summary map showing the events involved in the formation of F′ plasmid JGF312. The maps of F Δ(33-43), Tn*10*, and the relevant part of the terminus region are shown separately. *Pst*I and *Hin*dIII sites are shown by short thin vertical bars under and above these lines, respectively, and *Eco*RI sites are shown by long thin vertical bars. Insertion sequences and Tn*1000* DNA (thick lines) and *rac* DNA (hatched lines) are shown. Open circles indicate the three events involved in JGF312 formation: insertion of F Δ(33-43) by IS*2*-IS*2* recombination, Tn*10* transposition-insertion, and Tn*10* transposition-recombination within *rac*.

contains Tn*10* was cloned into plasmid pCR1. The size of the *Hin*dIII-*Pst*I fragment at the IS*10*-left side of the resulting plasmid, pMJV1, measured more accurately than from the hybridization data, was 5.4 kb instead of 6.8 kb for plasmid pJPB3 (Fig. 4), confirming the interpretation of the hybridization data. Plasmids pJPB3 and pMJV1 were cut by *Eco*RI and *Pst*I, thus separating the sequences on either side of Tn*10*. The digested DNAs were fractionated on an agarose gel and hybridized to the labeled DNA of pJPB6, which carries the same chromosomal sequences as pJPB3 but not Tn*10*. Although both the IS*10*-left- and IS*10*-right-containing

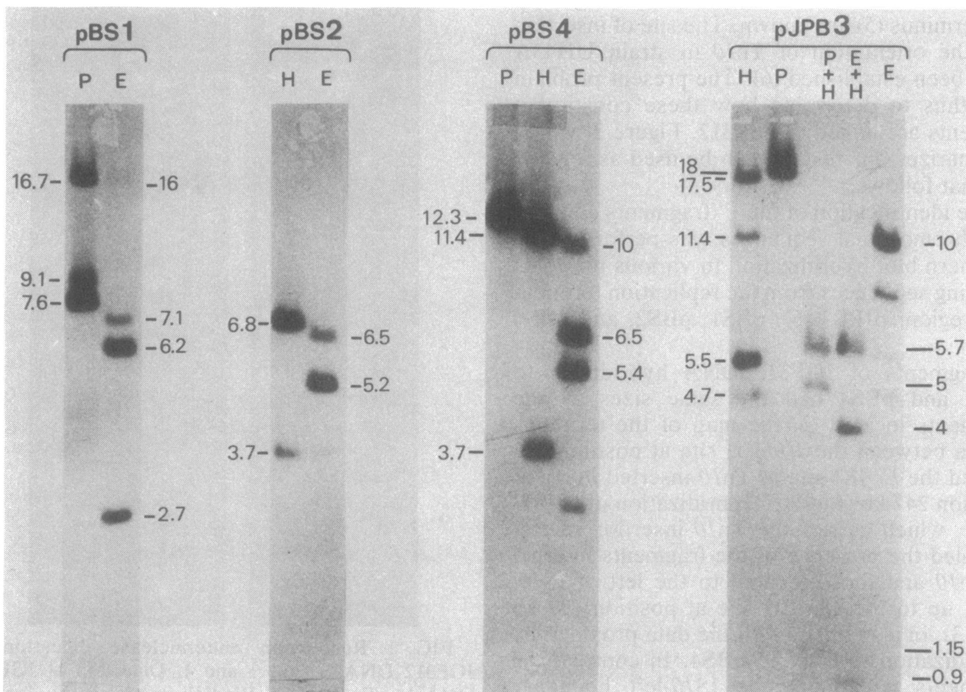

FIG. 3. Hybridization of JGF312 DNA to probes carrying fragments of the terminus region. E, H, and P stand for *Eco*RI, *Hin*dIII, and *Pst*I, respectively. Some additional fragments can be seen in the *Eco*RI lanes; they result from partial *Eco*RI* activity.

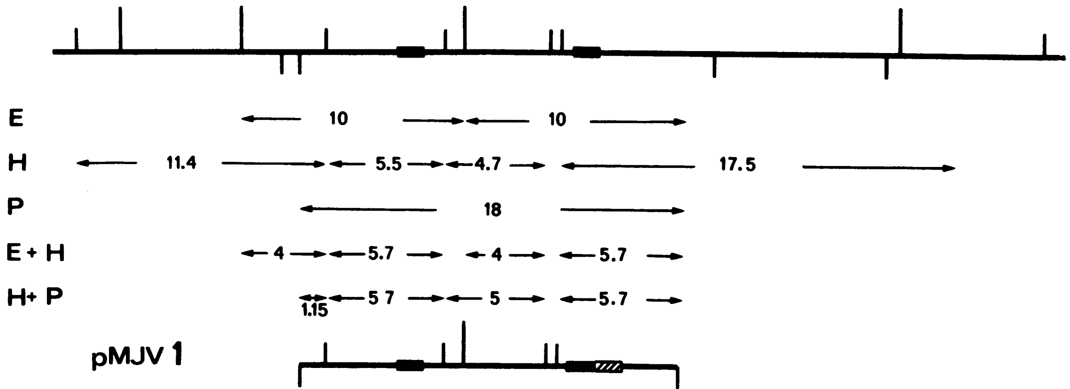

FIG. 4. Interpretation of the hybridization data of JGF312 to pJPB3. (Upper line) Restriction map of the chromosomal segment of strain UH43A. (Lower line) Restriction map of the inserted *Pst*I fragment in pMJV1. (Middle lines) Location of the fragments of JGF312 showing homology with plasmid pJPB3 DNA. Symbols are as in the legend to Fig. 2.

*Eco*RI-*Pst*I fragments of pJPB3 hybridized to the probe, the IS*10*-left-containing fragment from pMJV1 did not (data not shown). Thus, no chromosomal sequence located beyond the IS*10*-left end in UH43A occurs in JGF312.

As indicated previously, the size of the *Hin*dIII-*Pst*I fragment at the IS*10*-left end of the pMJV1 insert is 5.4 kb. This fragment contains the DNA from the leftmost *Hin*dIII site of Tn*10* to the proximal IS*10* (0.3 kb [15]), some non-Tn*10* DNA, and possibly the IS*10*-left itself. If IS*10*-left was absent, the amount of non-Tn*10* DNA would be 5.1 kb. Since the map of the terminus region (5) shows an *Eco*RI site at 4.2 kb and a *Pst*I site at 4.6 kb from the *Pst*I site of pMJV1, and since pMJV1 does not contain these sites, IS*10*-left (1.4 kb) must be present in the fragment, thus shortening the amount of non-Tn*10* DNA to about 3.7 kb. Therefore, Tn*10* is intact in JGF312 and a plausible mechanism for its formation is a transposition in direct repeat promoted from the IS*10*-left external end, followed by a recombination between the duplicated sequences during or after transposition.

Kaiser and Murray (16) carried out a heteroduplex analysis showing that the attachement site of *rac* is at 2.5 kb from the *Eco*RI site at position 189 kb. Therefore, approximately 1 kb of *rac* DNA is present in the F′ (see Fig. 2 and 4).

When ^{3}H-labeled pBS1 DNA was hybridized to JGF312 DNA, all of the bands expected from the chromosome between positions 189 and 221 kb were found. On the contrary, the 12.3-kb *Pst*I fragment (position, 214 to 226 kb) was absent and replaced by a 16.7-kb fragment (Fig. 3). Combined with the result obtained from the hybridization to plasmid pBS2, it indicated that F insertion had occured between positions 221 and 223 kb, within the row of three *Hin*dIII sites separated from each other by 1,050 base pairs.

Examination of the digestion pattern of JGF312 by *Hin*dIII (Fig. 1) showed that all visible bands could be attributed to either F Δ(33-43) or the chromosomal moiety. This suggested the involvement of IS*2* or IS*3* in the integration process since both contain *Hin*dIII sites. A priori, four possibilities of insertion of F existed: recombination of an IS*3* sequence spanning the 222- and 223-kb *Hin*dIII sites with either IS*3* element of F Δ(33-43), or recombination of IS*2* carried by F Δ(33-43) with an IS*2* at position 222 or 223 kb. Taking into account the orientation of F once inserted in HfrB7, the sizes of the insertion sequence-carrying *Eco*RI fragments can be predicted for each of these possibilities. An analysis of the *Eco*RI fragments of JGF312 (Fig. 1) showed that the chromosomal fragment of 3.15 kb (position, 221 to 224 kb) was absent. Instead, a new 2.8-kb fragment was present. Only recombination of F Δ(33-43) IS*2* with an IS*2* located at position 222 kb could result in such a short junction fragment.

This hypothesis was confirmed by hybridization of p*galOP*::IS*2*#1 and pHD009 DNAs to JGF312 DNA. These plasmids carry, respectively, the largest (0.8 kb) and the shortest (0.4 kb) parts of IS*2* on either side of its *Hin*dIII site, subcloned into pBR322 from the transducing phage λ p*gal8*::IS*2* (21). The three hybridizations shown in Fig. 5 revealed two bands containing IS*2*-homologous material, confirming the presence of a chromosomal IS*2*. The two *Eco*RI junction fragments had sizes of 2.8 and 10 kb. Plasmids p*galOP*::IS*2*#1 and pHD009 revealed the two *Hin*dIII fragments flanking IS*2* of F Δ(33-43); fH9 (2.35 kb) and fH2 (11 kb), respectively. In addition, both probes hybridized to

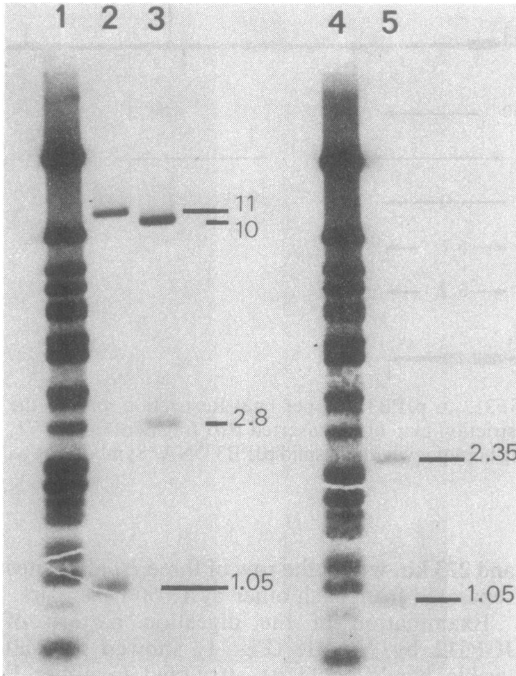

FIG. 5. Hybridization of JGF312 DNA to IS*2* probes. Lanes 1 and 4, Mixture of λ *c*I857 *S7* DNA digested by *Eco*RI, *Hin*dIII, or *Hin*cII; lanes 2 and 3, hybridization of p*galOP*::IS*2*#1 DNA to Southern blots of *Hin*dIII- and *Eco*RI-digested JGF312 DNA, respectively; lane 5, hybridization of pHD009 DNA to *Hin*dIII-digested DNA from JGF312.

1.05-kb *Hin*dIII fragments. Therefore, the chromosomal IS*2* is located at the level of the central *Hin*dIII site at position 222 kb. Figure 2 summarizes the three events leading to the formation of JGF312: insertion of F Δ(33-43) by recombination with a chromosomal IS*2* at position 222 kb, transposition of Tn*10* within gene *trg*, and Tn*10*-promoted deletion of the *rac-trg* region.

DISCUSSION

Our results localize the site of insertion (PO 43) in Hfr B7 at 222 kb on the map of the terminus region. When converted into genetic units, this corresponds to 30.9 min. This result is in agreement with all previous observations, except one. Fouts and Barbour (10, 11) mapped gene *ksgD* at 30.4 min by cotransduction with *sbcA* (29.7 min) and with an independently mapped IS*10*-Cam^r-IS*10* insertion. They also reported a l .e transfer of *ksgD* (after about 70 min) during conjugation, using a derivative of Hfr B7. According to our mapping, *ksgD* should be transferred as an early marker, and we have no explanation for this discrepancy.

The present study identifies one of the chromosomal IS*2* elements within a 3.15-kb *Eco*RI fragment. In their studies on the number of insertion sequence elements in various *E. coli* strains, Deonier et al. (8) and Hu and Deonier (14) found no IS*3*-carrying fragment smaller than 6 kb. On the other hand, a 3-kb *Eco*RI fragment containing IS*2* was found in all laboratory strains tested, and it was concluded that only one IS*2* element per genome was present in a fragment of that size. It is therefore probable that the IS*2* element located at 30.9 min is one representative of the "basic" set of insertion sequences present in the original isolate of *E. coli* K-12.

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LITERATURE CITED

1. **Anthony, W. M., R. C. Deonier, H. J. Lee, E. Ohtsubo, and N. Davidson.** 1974. Electron microscope studies of sequence relations among plasmids of *Escherichia coli*. IX. Note on the deletion mutant of F, F Δ(33-43). J. Mol. Biol. **89:**647–650.
2. **Bachmann, B. J., and K. B. Low.** 1980. Linkage map of *Escherichia coli* K-12, edition 6. Microbiol. Rev. **44:**1–56.
3. **Bejar, S., and J.-P. Bouché.** 1983. Molecular cloning of the region of the terminus of *Escherichia coli* K-12 DNA replication. J. Bacteriol. **153:**604–609.
4. **Bitner, R. M., and P. L. Kuempel.** 1981. P1 transduction map spanning the replication terminus of *Escherichia coli* K-12. Mol. Gen. Genet. **184:**208–212.
5. **Bouché, J. P.** 1982. Physical map of a 470×10^3 base-pair region flanking the terminus of DNA replication in the *Escherichia coli* K-12 genome. J. Mol. Biol. **154:**1–20.
6. **Bouché, J. P., J. P. Gélugne, J. Louarn, J. M. Louarn, and K. Kaiser.** 1982. Relationships between the physical and genetic maps of a 470×10^3 base-pair region around the terminus of *Escherichia coli* K-12 DNA replication. J. Mol. Biol. **154:**21–32.
7. **Broda, P.** 1967. The formation of Hfr strains in *Escherichia coli* K-12. Genet. Res. **9:**35–47.
8. **Deonier, R. C., R. G. Hadley, and M. Hu.** 1979. Enumeration and identification of IS*2* elements in *Escherichia coli* strains. J. Bacteriol. **137:**1421–1424.
9. **Deonier, R. C., and L. Mirels.** 1977. Excision of F plasmid sequences by recombination at directly repeated insertion sequence 2 elements: involvement of *rec*A. Proc. Natl. Acad. Sci. U.S.A. **74:**3965–3969.
10. **Fouts, K. E., and S. D. Barbour.** 1981. Transductional mapping of *ksgB* and a new Tn*5*-induced kasugamycin resistance gene, *ksgD*, in *Escherichia coli* K-12. J. Bacteriol. **145:**914–919.
11. **Fouts, K. E., and S. D. Barbour.** 1982. Insertion of transposons through the major cotransduction gap of *Escherichia coli* K-12. J. Bacteriol. **149:**106–113.
12. **Guyer, M. S.** 1978. The γδ sequence of F is an insertion sequence. J. Mol. Biol. **126:**347–365.
13. **Harayama, S., E. T. Palva, and G. L. Hazelbauer.** 1979. Transposon-insertion mutants of *Escherichia coli* K-12 defective in a component common to galactose and ribose chemotaxis. Mol. Gen. Genet. **171:**193–204.
14. **Hu, M., and R. C. Deonier.** 1981. Comparison of IS*1*, IS*2*, and IS*3* copy number in *Escherichia coli* strains K-12, B and C. Gene **16:**161–170.
15. **Jorgensen, R. A., and W. S. Reznikoff.** 1979. Organization

of structural and regulatory genes that mediate tetracycline resistance in transposon Tn*10*. J. Bacteriol. **138**:705–714.

16. **Kaiser, K., and N. E. Murray.** 1979. Physical characterization of the "rac prophage" in *E. coli* K-12. Mol. Gen. Genet. **175**:159–174.
17. **Low, K. B.** 1972. *Escherichia coli* K-12 F-prime factors, old and new. Bacteriol. Rev. **36**:587–607.
18. **Manis, J., and B. Kline.** 1977. Restriction endonuclease mapping and mutagenesis of the F sex factor replication region. Mol. Gen. Genet. **152**:175–182.
19. **Ohtsubo, H., and E. Ohtsubo.** 1977. Repeated DNA sequences in plasmids, phages and bacterial chromosomes, p. 49–63. *In* A. I. Bukhari, J. A. Shapiro, and S. L. Adhya (ed.), DNA insertion elements, plasmids and episomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
20. **Palchaudhuri, S., and W. K. Maas.** 1977. Physical mapping of a DNA sequence common to plasmids of incompatibility group F1. Proc. Natl. Acad. Sci. U.S.A. **74**:1190–1194.
21. **Saint-Girons, I., H. J. Fritz, C. Shaw, E. Tillman, and P. Starlinger.** 1981. Integration specificity of an artificial kanamycin transposon constructed by the *in vitro* insertion of an internal Tn*5* fragment into IS*2*. Mol. Gen. Genet. **183**:45–50.
22. **Skurray, R. A., H. Nagaishi, and A. J. Clark.** 1978. Construction and *Bam*H1 analysis of chimeric plasmids containing *Eco*R1 DNA fragments of the F sex factor. Plasmid **1**:174–186.
23. **Willetts, N., and R. A. Skurray.** 1980. The conjugation system of F-like plasmids. Annu. Rev. Genet. **14**:41–76.