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Electron Microscope Heteroduplex Studies of Sequence Relations Among Plasmids of *Escherichia coli*: Isolation of a New F-Prime Factor, F80, and Its Implication for the Mechanism of F Integration into the Chromosome

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A new F-prime factor, F80, was isolated from an *Escherichia coli* strain harboring the F-prime factor F8 by selecting for transfer of the *supE* marker to a RecA⁻ recipient. Genetic analysis shows that F80 carries a segment of the chromosomal DNA between *lip* and *suc* in addition to the *tol-gal* region normally in F8. Physical analysis by the electron microscope heteroduplex method suggests that the formation of F80 from F8 involves recombination between the $\alpha\beta$ segment of F, which is present in F8, and the homologous sequence of F present in the *E. coli* chromosome at the site where F is supposed to integrate to form HfrP3. The implications of this result for the general mechanisms of F integration to form Hfr's are discussed.

The integration of F-factor DNA into the *Escherichia coli* chromosome giving rise to an Hfr has interesting properties. Unlike λ and φ80 phages, there are many F integration sites in the *E. coli* chromosome (see review by Low [10]).

Several recent studies have offered evidence that there are special sequences of F that are active in F-related recombination events (1, 7). It is believed that these sequences are also resident on the *E. coli* chromosome, and that some cases of Hfr formation are due to reciprocal recombination between one of these special sequences on F and the corresponding sequence on the bacterial chromosome.

In this paper, we present evidence supporting the presence of one of these sequences of F on the *E. coli* chromosome and evidence for recombination events between duplicate copies of the sequence. The particular F sequence involved is called $\alpha\beta$ and has a length of 1.3 kilobases (kb). The evidence is derived from the structure of a new F-prime, F80. This plasmid was formed, starting with the smaller plasmid, F8, by a mechanism involving integration of F8 into the *E. coli* chromosome and subsequent excision of F80.

MATERIALS AND METHODS

Bacterial strains. Bacterial strains used are listed in Table 1.

Conjugation. Conjugation between X7026F8⁺ and KRO was performed in Penassay broth (Difco) as described in the preceding paper (14). Trp⁺ Str^r colonies were selected on a Davis minimal medium plate (2) containing 100 μg of streptomycin per ml.

Testing of bacterial markers of F-prime factors. All the F-prime factors to be examined were first transferred to the F⁻ strain N23-76, and then the resulting F-prime strains were used as donors for testing bacterial markers on the F-prime factors by a cross-streaking technique. The procedures for mating were described in the preceding paper (14). As recipients, we used F⁻ strains of PB314, AB1325 *lip-13*, W3110 *suc-17*, SA291, and FRAG-5 in testing for transfer of *purE*⁺, *lip*⁺, *suc*⁺, *bio*⁺, and *kdp*⁺, respectively.

All other aspects of our experimental technique were also described in previous papers (13, 14, 17).

RESULTS

The F-prime factor F8 carries a segment of the *E. coli* chromosome in the region from *tol* to *gal* (6, 14). It does not carry any genes and DNA sequences in the region *purE-lip-supE-suc*, which is adjacent to the *tol-gal* region (14). However, when X7026, which harbors F8 and carries *supE*, an amber suppressor previously called suII⁺, was crossed with a recipient strain, KRO [*trp*(Am) *str^r recA*], Trp⁺ Str^r colonies could be obtained at a low frequency of 6.5×10^{-7} per donor cell.

Genetic and physical analysis of these colonies showed that they harbored new F-prime factors different from the original F8 by the following properties. (i) All of them (24 colonies were tested) could transfer the *E. coli* chromosome markers between *lip*⁺ and *suc*⁺, markers that are not present in F8, at a high frequency, but not

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TABLE 1. *Bacterial strains used*

Strain	Plasmid	Chromosomal genotype	Source or reference
X7026 F8$^+$	F8	Δ(*lac-pro*) *supE thi* λ$^-$	14
KRO	F$^-$	Δ(*lac4680*) *trp8*(Am) *str recA*	18
N23-76	F$^-$	*recA str galT trp arg* λ$^-$	12
AB1325 *lip-13*	F$^-$	*tsx proA lacY galK purB his str mtl xyl thi lip-13* λ$^-$	5
W3110 *suc-17*	F$^-$	*suc-17* (*sucB*) λ$^-$	5
PB314	F$^-$	*lac purE thi str txs*	P. Broda
SA291	F$^-$	*his str* Δ(*gal-bio-chlA*) *relA* λ$^-$	16
FRAG-5	F$^-$	*lac8X82*(Am) *gal rha thi* λ$^-$ Δ(*kdpABC*)	3
N23-76F80$^+$	F80	Same as N23-76	14
N23-76 F152-3$^+$	F152-3	Same as N23-76	14
N23-76 F100-12$^+$	F100-12	Same as N23-76	14
N23-76 F8-33$^+$	F8-33	Same as N23-76	14

purE$^+$, *bio*$^+$, and *trp*$^+$. This result indicates that KRO, which is *recA*, gave rise to new F-prime plasmids different from the parent F8. (ii) Closed circular plasmid DNA isolated from one such strain was 195.5 × 10^6 (± 1.1) daltons and was 2.5 times as large as F8. We call this plasmid F80.

The final structure of F80 (Fig. 1) shows that this plasmid contains additional sequences of 118.7 kb in length of the *E. coli* chromosome to the righthand terminus of the *E. coli* chromosomal sequence carried by the parental F8. F80 is missing 8.5 kb more of F sequences compared with F8.

Figure 2 shows further interpretations of the structures of F8 and F80, using the coordinate system for the *E. coli* chromosome and F sequences that was used by Ohtsubo and Hsu (14): F80 carries the *E. coli* chromosome sequence from 0B to 217.2B. This sequence substitutes for the F sequence from 0F to 16.3F. The two junc-

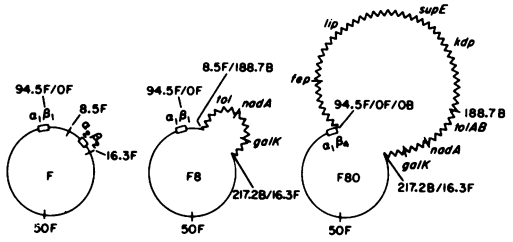

FIG. 1. *Circular structures of F, F8, and F80 DNA molecules. See also the linear representation of these structures shown in Fig. 2.*

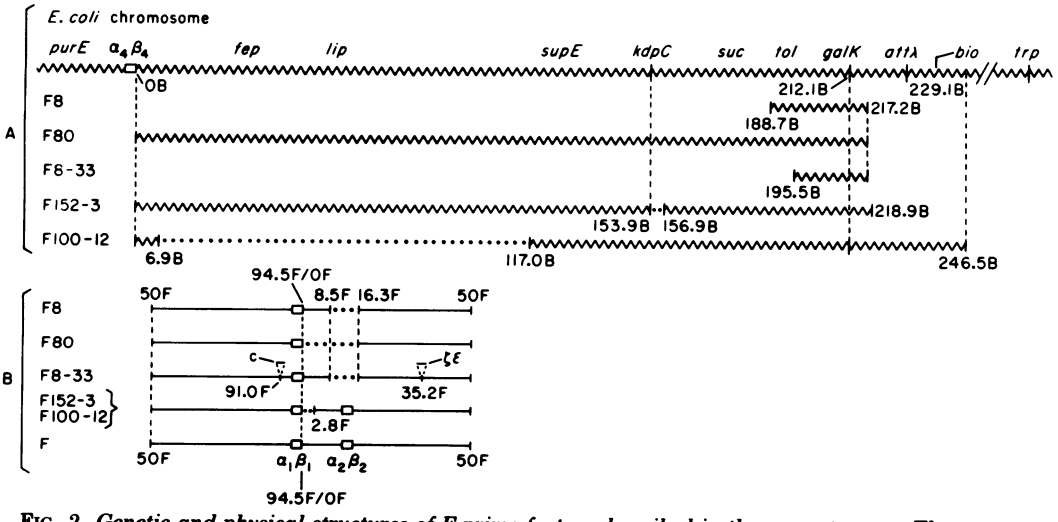

FIG. 2. *Genetic and physical structures of F-prime factors described in the present paper. The structures are linear representations of circular molecules. The E. coli chromosomal DNA (sawtooth line) and F sequences in the F-prime factors are separately shown. The chromosomal DNA is actually substituted to the deleted region of F. The coordinates for chromosome and F sequences are labeled with B and F, respectively. The final structure of a new F-prime F80 and of the parental F-prime F8 are shown. F8-33, F152-3, and F100-12 are reference DNA molecules, the physical structures of which have been analyzed by the electron microscope heteroduplex method (14).*

tions of F DNA with chromosomal DNA occur at 0F/0B and 217.2B/16.3F.

The evidence leading to this structure for F80 was derived from the following analysis of heteroduplex molecules of F80 with F and reference F-prime factors F8-33, F152-3, and F100-12.

As schematically shown in Fig. 3a, a heteroduplex of F80 with F has a simple substitution loop that is used to measure the size of the deletion in the F sequence and the size of the *E. coli* chromosome sequences as well. Figure 3b shows the heteroduplex of F80 with F8-33. F8-33 carries a *gal* sequence (195.5B to 212.2B) that substitutes for the F sequence (8.5F to 16.3F) and has, in addition, the two characteristic insertion loops *c* and ζ∈ at positions of 91.0F and 35.2F, respectively (13, 14; see also Fig. 2). The two characteristic loops seen in the F80/F8-33 heteroduplex serve as useful markers to map the positions of the F-chromosomal DNA junctions. Results obtained from this heteroduplex and the F80/F heteroduplex show the deletion of the F sequence in F80 in the region 0F to 16.3F. The junction regions between the *E. coli* chromosome and F sequences around 16.3F in both F-prime factors are completely homologous. An electron micrograph of the F80/F8-33 heteroduplex is shown in Fig. 4.

The chromosomal DNA of F80 is much larger than that of F8. The evidence that this region contains chromosomal DNA between *fep* and *gal* was derived from the analysis of the heteroduplex of F80 with F152-3. F152-3 is a derivative of F152 that carries the sequence of the *E. coli* chromosome from *fep* to *gal*, but deletes a small region containing the *kdp* gene (14; see also Fig. 2). This deletion was used to orient the DNA sequence in the heteroduplex. As shown

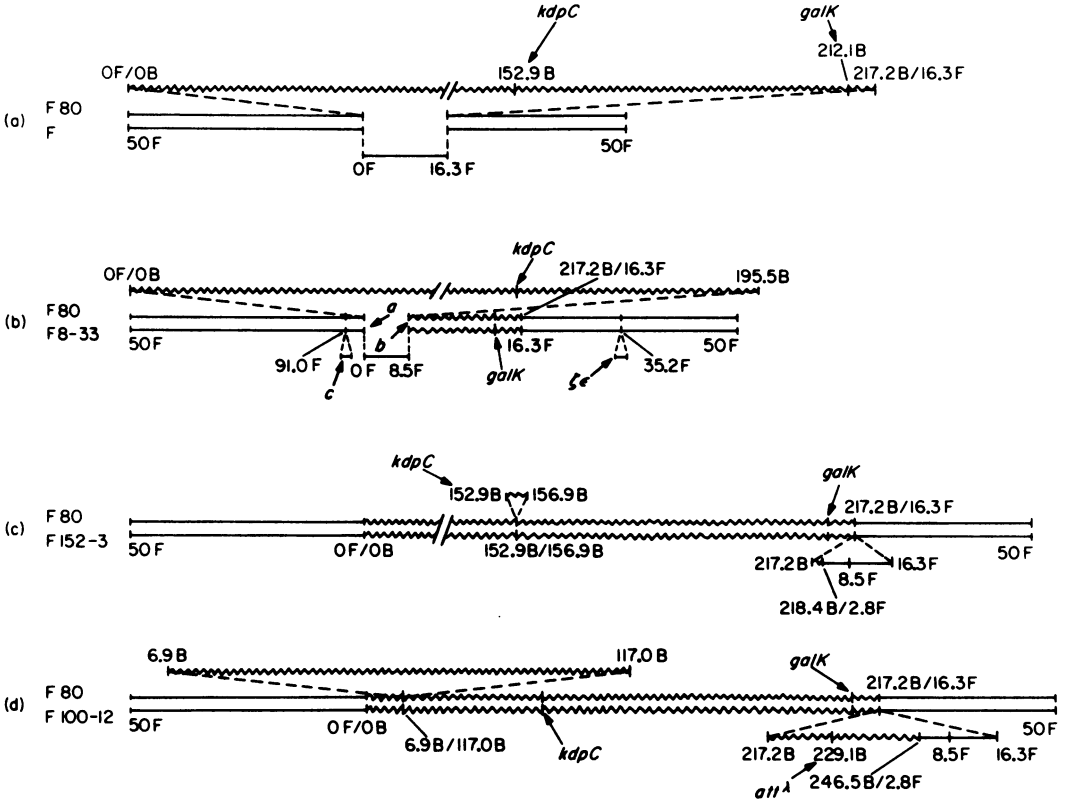

FIG. 3. *Schematic drawing of heteroduplexes F80. The sawtooth line is E. coli chromosome DNA. Numbers are distances in kilobases from the selected origins of F and chromosome DNA. The coordinates for the E. coli chromosome and F sequences are labeled with B and F, respectively. (a) F80/F heteroduplex. A simple substitution loop is seen. (b) F80/F8-33 heteroduplex. An electron micrograph is shown in Fig. 4. The two insertion loops of F8-33, indicated by c and ζ∈, respectively, are used to orient the F sequence in the heteroduplex. Note that the F and the chromosome DNA sequences around the junction of F8-N33, 28.5B/16.3F are exactly the same as those of F80. (c) F80/F152-3 heteroduplex. The loop a corresponding to the kdp deletion orients the DNA sequences in the heteroduplex. Note that 0F/0B in both F80 and F152 are exactly the same. (d) F80/F100-12 heteroduplex.*

in Fig. 3c, the F80/152-3 heteroduplex showed two loops; the small loop corresponds to the *kdp* deletion in F152-3, and the other loop is composed of (i) a segment of chromosomal DNA not present in F80 and (ii) an F sequence deleted in F80. The important information obtained from this heteroduplex is that the bacterial sequences of F80 and F152 are completely homologous and that both the F and bacterial DNA of two F-prime factors in the region of 0.0F are the same.

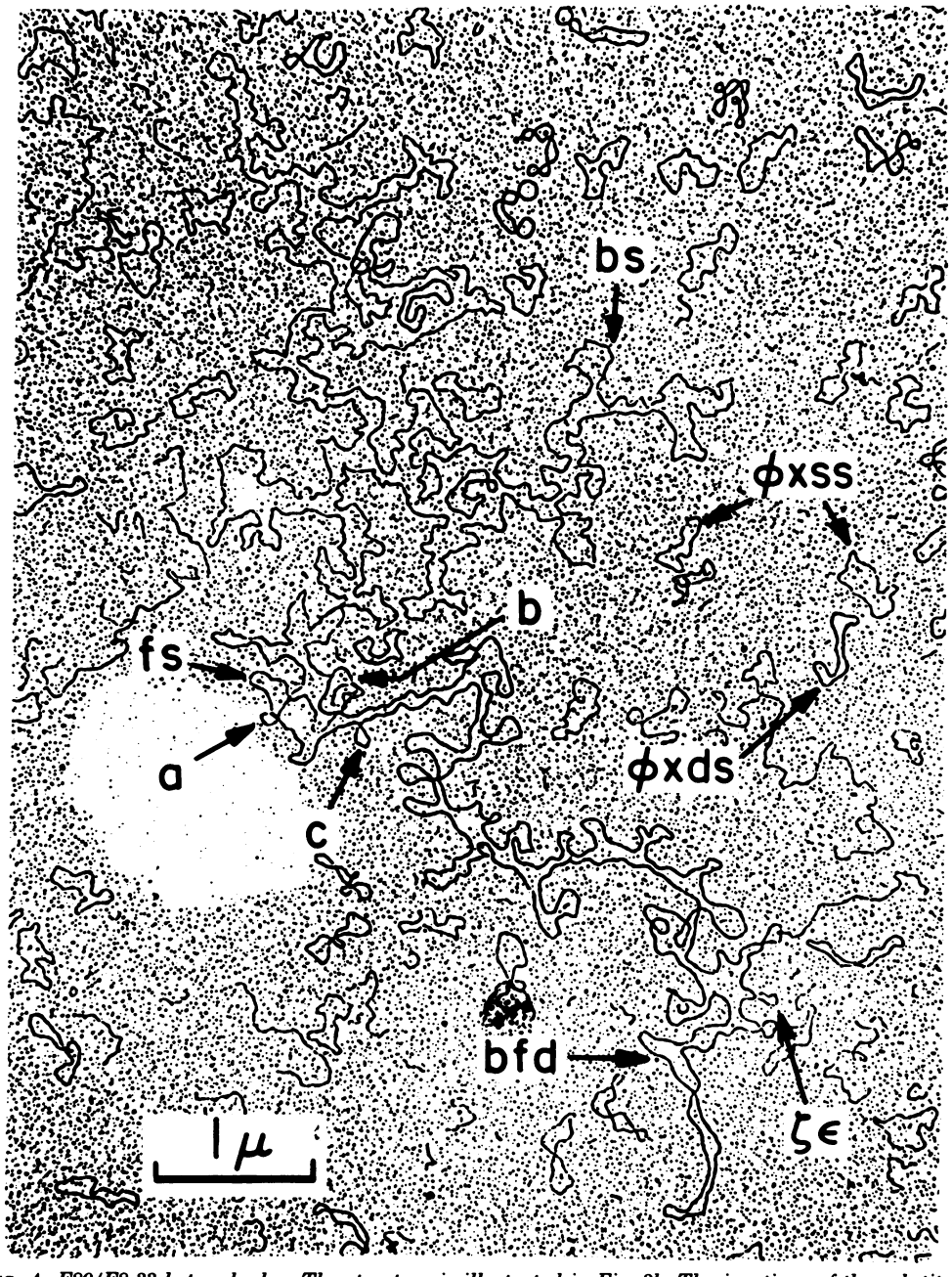

FIG. 4. *F80/F8-33 heteroduplex. The structure is illustrated in Fig. 3b. The junctions of the substitution loop are marked by the letters a and b. The two small insertions of F8-33 are labeled with c and ζϵ. fs and bs indicate F and bacterial single strands, respectively. bfd indicates that the double-strand DNA is composed of both bacterial and F sequences. Several φX single-strand and double-strand DNA are also marked.*

The same conclusion is derived from the analysis of the heteroduplex, F80/F100-12. F100-12 is a derivative of an F-prime F100 with a large deletion in the chromosomal sequence (14; see also Fig. 2). As shown in Fig. 3d, two loops are seen in an otherwise duplex molecule. From the lengths of these two loops, a large loop is identified as the deletion of F100-12 and a small loop is composed of a segment of (i) bacterial DNA not present in F80 or F152 and (ii) F sequences between 0.0F and 16.3F deleted in F80. The heteroduplex indicates that the junctions 0F/0B in both F80 and F100-12 (or F100) are exactly the same.

DISCUSSION

We propose one of two alternative and basically equivalent mechanisms for the formation of F80 (Fig. 5 and 6). It should first be noted that F contains two copies of a sequence of length 1.3 kb that we call $\alpha\beta$, with coordinates 93.2F to 94.5/0F and 13.7F to 15.0F (7, 13; see Fig. 1 and 2). The former, which we call $\alpha_1\beta_1$, is present on F8. A previous study of the structure of F13 suggested that an $\alpha\beta$ sequence, denoted $\alpha_4\beta_4$, is resident in the *E. coli* chromosome at the point which has coordinates 0B in our present notation. This point lies between *purE* and *fep* and is the point of origin for HfrP3 (7; see Fig. 2).

We previously proposed that HfrP3 was formed by reciprocal recombination between $\alpha_1\beta_1$ of F and the $\alpha_4\beta_4$ sequence on the *E. coli* chromosome.

The two alternative mechanisms that we propose for the formation of F80 (Fig. 5 and 6) consist of the same two steps in opposite order: (i) integration of F8 into the *E. coli* chromosome by reciprocal recombination of the homologous *gal* sequences, followed by excision of F80 by reciprocal recombination between $\alpha_1\beta_1$ and $\alpha_4\beta_4$ and (ii) integration of F8 into the *E. coli* chromosome by reciprocal recombination between $\alpha_1\beta_1$ and $\alpha_4\beta_4$, followed by excision of F80 by reciprocal recombination between the two homologous *gal* sequences.

The same structure for F80 would be produced by either mechanism. The only way to distinguish between them would be to study the properties of the Hfr's. The important points are that either mechanism is completely consistent with the observed structure for F80, and this provides independent evidence for the proposition that

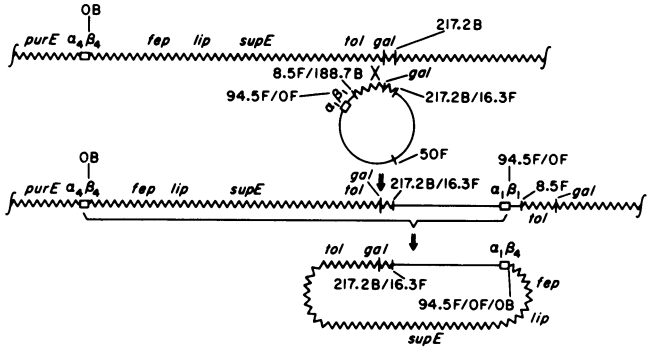

FIG. 5. *Proposed mechanism for the formation of F80 from F8. Sawtooth lines represent the E. coli chromosomal sequences.*

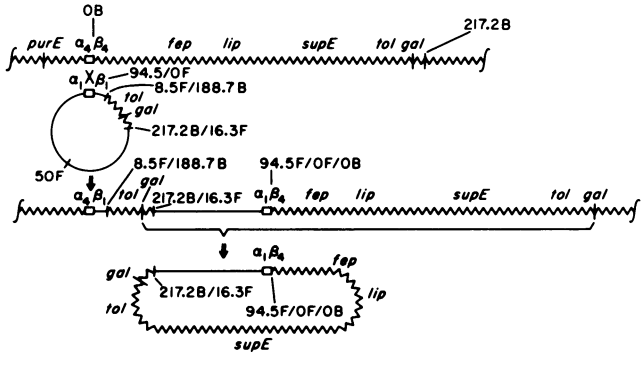

FIG. 6. *Alternative mechanism for the formation of F80 from F8. See Fig. 5.*

there is an $\alpha_4\beta_4$ sequence at the chromosomal site that is the point of origin for HfrP3, and that this sequence is active in F-related recombination events.

We note that the processes carried out here suggest a technique for reconstructing entire regions of the *E. coli* genome on F-prime factors and for exploring the structure of other known hot spots for Hfr formation. It should also be noted that recent evidence (9) shows that $\alpha\beta$ is homologous to the insertion sequence IS3 (4, 11). Thus the present communication adds to evidence previously offered that insertion sequences play a role in plasmid-related recombination phenomena (8, 15).

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