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Electron Microscope Heteroduplex Studies of Sequence Relations Among Plasmids of *Escherichia coli*: Structure of F100, F152, and F8 and Mapping of the *Escherichia coli* Chromosomal Region *fep-supE-gal-attλ-uvrB*

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The genetic and physical structures of commonly used F-prime factors carrying the galactose region of the *Escherichia coli* chromosome were analyzed. Deletions in the chromosomal DNA sequences in the F-prime factors were found to be frequent events. A genetic method was developed to reconstruct the original F-prime factors from deletion variants. Heteroduplex analysis of the reconstructed F-prime factors confirmed the derivation of the F-prime factors F100 and F152, from the same Hfr, and finally determined the normal *E. coli* chromosomal sequence in the region between *fep* and *uvrB*, containing about 5 min in genetic units and about 246.5 in kilobase units (kb). This sequence could be connected with the DNA sequences of the *lac-purE* region, which had been physically determined previously. Together they constituted a total of 528.6 kb. From these combined sequences, the distance from *lacPO* to *galK* was calculated to be 412.9 kb, which corresponds to 8.8 min in genetic units.

F-prime factors are covalently closed circular DNA molecules containing defined sequences of the *Escherichia coli* chromosome in addition to the sequences of the fertility factor F. They have been useful for genetic study of defined segments of the bacterial chromosome.

As reported previously (9, 16, 29, 34), this laboratory has applied the electron microscope heteroduplex technique (11) to the study of the physical structures of several F-prime factors. In the present communication, we present more detailed analysis of the chromosomal sequences of the *gal* region, as well as F sequences, carried on the F-prime factors F100 (F1*gal*), F152 (F2*gal*), and F8 (14, 19). These F-prime factors together contain a region of *E. coli* chromosome spanning from *fep* to *uvrB*, or about 5% of the *E. coli* genome (see *E. coli* genetic map [5]).

The bacteria carrying the F-prime factors studied in this paper have been widely distributed to many laboratories and have been extensively used in genetic and biochemical studies. We found that plasmids isolated from strains from different laboratories differ in genetic and physical properties; most of them contain deletions in the chromosomal DNA sequences. These deletion derivatives are useful for mapping the physical locations of the genetic markers of the *E. coli* chromosome in the region between *fep* and *uvrB*.

To establish the deletion map, we developed a method to construct from the deletion derivatives F-prime factors containing the wild-type chromosomal DNA sequence. This method is also very useful for study of mutations and sequence rearrangements in the *E. coli* chromosome.

From the physical mapping results on the region *fep-gal-uvrB* reported in the present paper and on the region *lac-purE* reported previously (16), we discuss the relation of genetic map units in minutes to physical units in kilobases.

MATERIALS AND METHODS

Bacterial strains and F-prime factors studied. Bacterial strains used in this study are listed in Table 1, and the F-prime factors studied are depicted in Fig. 1.

Media. Penassay broth (Difco) was routinely used for growing bacterial cultures for genetic experiments. Bacteria were grown in tryptone broth containing 1 μg of thiamine per ml for extraction of plasmid DNA. L plates (22) were used as a complete solid medium. Davis minimum agar plates (10) were used for genetic analysis. They were supplemented with appropriate amino acids, vitamins, and 0.2% glucose (or 1% galactose), depending on the genetic marker to be tested. Eosin-methylene blue (EMB) plates were used for *gal* and *mal* markers.

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TABLE 1. *E. coli K-12 strains*

Strain	Plasmid	Chromosomal genotype	Source and reference
B583	F100-11 (F450)	*Δ(gal-attλ-chlA) str* λ^-	D. Freifelder (34)
MR44	F100-12 (F_{1S})	*galE galT recA spc*	E. R. Signer (34)
JE5606	F153 (F_3*gal*)	*gal lac met arg his leu recA str*	E. Ohtsubo (28 and this paper)
F152/KL253	F152 (F_2*gal*)	*galK recA pyrD trp his tryA thi mal xyl mtl str* λ^-	B. Low (23)
ND6	F152-1	*galK recA pyrD trp his tyr thi mal xyl mtl str* λ^-	This paper
JE5303	F8	*recA str galT trp arg* λ^-	E. Ohtsubo (8 and 34)
JE3100	F8-12	*thr leu gal-2 lac-52 pil fla str*	E. Ohtsubo (34)
ND8	F8-13	*met*	F. Fukasawa and this paper
JE3513	F8-33	*thr leu gal-2 lac-52 pil fla str*	E. Ohtsubo (31, 34)
W3747	F13	*metB relA*	Y. Hirota (14)
N23-76	F^-	*recA str galT trp arg* λ^-	H. Ogawa (27)
PL225	F^-	*recA str thi Δ(gal-nadA)* λ^-	T. Fukasawa (25)
W3350	F^-	*galT galK* λ^-	J. Lederberg (3)
PB314	F^-	*lac purE thi str tsx*	P. Broda (36)
KL231	F^-	*leuS* (Ts) *thyA str*	B. Low, (24)
AB1325 *lip-13*	F^-	*tsx proA lacY galK purB his str mtl xyl thi lip-13* λ^-	J. R. Guest (13)
FRAG-5	F^-	*lacZX82* (Am) *gal rha thi Δ(kdpABC)* λ^-	W. Epstein (12)
AN102	F^-	*leu proC trpE thi fep mtl xyl ara lac tonA tsx strA azi* λ^-	I. Young
AN146	F^-	*ubi-411 gal pyr*	I. Young
W620	F^-	*gltA galK suc pyrD str thi* λ^-	J. R. Guest (13)
FRAGG-1	F^-	*lacZX82 gal rha thi gltA* λ^-	W. Epstein (12)
WGA *suc-23*	F^-	*gal trpA suc-23* (*sucA*) λ^-	J. R. Guest (13)
W3110 *suc-17*	F^-	*suc-17* (*sucB*) λ^-	J. R. Guest (13)
NO712	F^-	*gal tolB thr leu proA his arg thi lac xyl ara mtl str*	M. Nomura (26)
NO570	F^-	*gal tolA thr leu lac str*	M. Nomura (26)
W3102	F^-	*galK* λ^-	J. Lederberg (3)
W3995	F^-	*galE str*	J. Lederberg (3)
SA291	F^-	*his str Δ(gal-chlA) relA* λ^-	A. Campbell (32)
KL229	F^-	*serS thyA35 str*	B. Low (24)
KRO	F^-	*Δlac-4680 trp8*(Am) *str recA*	E. Signer (35)
X7026	F^-	*Δ(pro-lac) supE thi relA* λ^-	W. Epstein (12)
NO52	ColE2	*str*	M. Nomura (26)

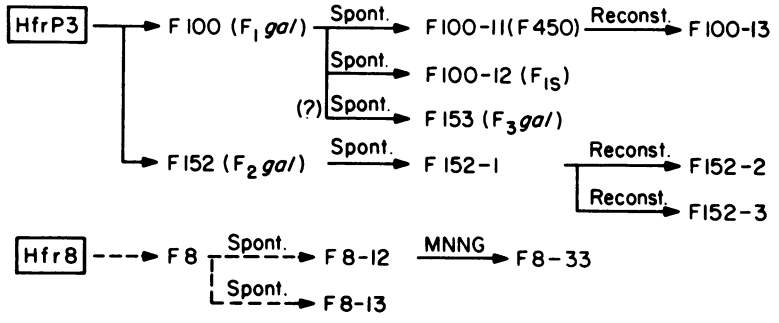

FIG. 1. *Pedigree of the F-prime factors studied, constructed according to the genetic and physical results obtained in the present paper. We follow the nomenclature of F-prime factors suggested by Low (23). F100 and F152 are equivalent to* F_1*gal and* F_2*gal, respectively (23). F153 was previously called* F_3*gal by Ohki and Tomizawa (28). F450 and* F_{1S} *(34) are renamed F100-11 and F100-12 because they are shown to be deletion derivatives of F100 (see text).* F_8*(1),* F_8*(2) and* F_8*(N33) studied by Sharp et al. (34) are renamed F8, F8-12, and F8-33, respectively. An F8 carried in ND8, which was sent to us as* F_8 *by Fukasawa, was named by us F8-13, since it was found to carry a deletion in the bacterial DNA of F8. Other F-prime factors were newly isolated by the genetic reconstruction experiments presented in the text.*

Transfer of F'*gal*'s. In transferring F'*gal*'s, we avoided the use of EMB galactose as a differential medium. Variants carrying smaller F'*gal*'s resulting from spontaneous deletion are usually more recognizable as Gal$^+$ colonies on EMB galactose medium and therefore may preferentially be picked for analysis.

Consequently, the following procedure was used. All transfers of F'*gal*'s were done by using N23-76 (F$^-$ *gal trp arg str recA*) (27) as either a donor or a recipient. This strain has an extremely slow growth rate and therefore can easily be identified in a mixed culture. Donor and recipient bacteria were grown in Penassay broth at 37°C overnight. They were mixed in a ratio of about 5:1 and incubated for another 2 h at 37°C. The mating mixture was then plated with appropriate dilution on an L plate, which allows every bacterium plated to grow and form a colony. Tiny colonies were picked up for examination when N23-76 was used as recipient, whereas large colonies were chosen when N23-76 was used as donor. Over 80% of bacteria selected by this method receive F'*gal*'s with the original characteristics. The conditions for the mating used in reconstruction experiments are described in the text.

Testing of bacterial markers on F'*gal*'s. N23-76 derivatives, carrying various F-prime factors, were usually used as donors in testing the transferability of exogenote markers. Since N23-76 is RecA$^-$, the levels of chromosome mobilization can be neglected.

The cross-streaking method (21) was generally used. The markers tested by this method are shown in Table 2. Donor strains carrying various F'*gal*'s were cross-streaked against recipients on appropriate selective plates described in the reference for each marker. Genetic analysis of *supE* is complicated and is described in the text. The procedures for other markers were as follows. (i) *leuS* and *serS* (24): the recipients used were KL231 and KL229, respectively. They carry temperature-sensitive mutations in these two genes. The recipients were grown at 30°C, cross-streaked with N23-76 donors on Davis minimal plates, and then incubated at 42°C. Growth of recipients in the cross-streaked area at the restrictive temperature indicates the presence of these markers in the F-prime factor tested. (ii) *tolAB* (26): the F'*gal* to be tested was transferred into NO712 and NO570, and the F-ductants were tested for sensitivity (Tol$^+$) to colicin E2 spontaneously excreted from NO52. (iii) *pgl* (2): F'*gal*'s in N23-76 were transferred into SA291, and the *gal*$^+$ F-ductants were examined for a "blue" character (Pgl$^+$) on EMB maltose plate. (iv) *uvrB*: F-ductants from (iii) and control strains were streaked onto an L plate. These were exposed to UV light from a GE germicidal lamp (G8T5) by sliding a flat glass shield along the streak at 5-s intervals. (The lamp-to-plate distance is about 30 cm; maximum exposure time is about 30 s.) No or poor growth of bacteria in the UV-exposed region indicates the absence of a *uvrB*$^+$ allele. (v) *att*λ: the same F-ductants used in the previous test were cross-streaked against a phage λ lysate on an EMB maltose plate. A clearer lysis zone on the cross-streaked region indicates the absence of *att*λ in F'*gal*'s. (vi) *lip* and *gal* on the F-prime factor harbored in PL225 and KRO: the donor colonies to be tested were replica plated onto *lip* selective succinate plates (13) or *gal* selective plates that contained 25 μg of chloramphenicol per ml and were seeded with the recipient AB1325 *lip-13* R100-31$^+$. R100-31 is a transfer-defective R factor that cannot be complemented by the F transfer system and carries *str chl sul* drug resistance markers. The recipient used above was prepared by P1 transduction of R100-31 (31) into AB1325 *lip-13*.

TABLE 2. *Recipient strains used for marker transfer test*

Genetic marker	Recipient	Reference
purE	PB314	36
fep	AN102	8
lip	AB1325 *lip-13*	13
ubiF	AN146	39
kdp	FRAG-5	12
sucA	WGA *suc-23*	13
sucB	W3110 *suc-17*	13
nadA	PL225	37
aroG	PL225	38
gal	W3102, W3995	3
bio	SA291	32

Isolation of F-prime DNA. F-prime DNA was prepared essentially as described by Sharp et al. (34). Additions and modifications of the previous method were as follows: The lysozyme-treated cells were lysed by the addition of sodium lauroyl sarcosine to a final concentration of 0.6% and incubation at 37°C for several minutes until the solution became clear. The DNA was then sheared gently, to avoid breakage of large F-prime DNA, by one or two passages through a syringe without a needle.

After the denaturation and neutralization steps, the Na$^+$ concentration in the DNA solution was adjusted to 0.3 M. The extract was then mixed with 60 g (rather than the previous value of 100 g) of nitrocellulose that had been ground by pestle and mortar and washed twice with the buffer solution.

After removal of the nitrocellulose by centrifugation, the DNA solution was filtered through glass wool. This was also done with a smaller glass wool plug after the DNA pelleting step. These filtrations remove particulate materials that interfere with CsCl-ethidium bromide banding.

Electron microscope analysis of heteroduplexes. The basic techniques for spreading DNA molecules in aqueous or formamide solution have been described (11, 34). Heteroduplexes between two plasmid DNA molecules were prepared under the conditions reported by Sharp et al. (34).

Length measurements are reported in units of kilobases (1,000 bases or base pairs). We take F to be 62.6×10^6 daltons or 94.5 kilobases (kb) (34). The molecular weights of F-primes studied were usually determined against F DNA as an internal standard, using the aqueous spreading technique.

The following procedure was used to determine the size of features in heteroduplex molecules. The lengths of single-strand DNA in heteroduplex molecules and of circular single-strand F-prime DNA observed in the same grid were measured against single-strand φX viral DNA as an intermediate standard, present at adjoining regions of the same grid square. Renatured

homoduplex circles of F-prime factors examined were used as double-strand standards. Sometimes replicative double-strand DNA of φX174 was also used as an intermediate-length standard. The kilobase units of the heteroduplex features were then calculated by taking φX units of an F-prime factor, the size of which in kilobases had already been determined.

Note that single-strand viral DNA and replicative double-strand DNA of φX174 are used as intermediate standards in the procedure for the length determination mentioned above, but not as an absolute standard. We have previously reported that the length of F is 17.95 φX-RF (replicative form) units. We find, however, that by direct comparison under our usual formamide spreading conditions, the ratio of the length of F to that of φX is about 18.5 for duplex molecules and 17.5 for intact single strands. (Furthermore, the variability in single-strand lengths is greater than that of double strands.) Thus, φX molecules are not good length standards for very long DNA segments. (This may be due to differential stretching effects on large and small molecules or to other unknown effects.)

The standard procedure for identifying the F and bacterial sequences in an F-prime is to analyze the structure of heteroduplexes with F and with F8-33. A heteroduplex with F is useful for measuring the size of the F and bacterial components in an F-prime. F8-33 carries two characteristic small insertions at coordinates 91.0 and 35.2, respectively, on the physical map of F (29, 34) as shown in Fig. 2a. They are named *c* and ζε, respectively. These two insertions are used as physical markers to map the F or the bacterial sequences in the F-prime factors. In the present studies, F8-33 is also useful for determining the relationships within the bacterial sequences of each of the F'*gal*'s. The positions of *nadA*, *aroG*, and *gal* have actually been mapped on F8-33 (34) and are indicated in Fig. 2a. A linear representation of the heteroduplex molecule is used in this paper. Figure 2b shows such a depiction of F8-33/F heteroduplex.

The coordinates shown here are modifications of those determined previously (34). This is due to a modified method of length measurements and to the treatment of a large collection of relevant data with a least-squares analysis (R. Deonier and N. Davidson, private communication).

There are sometimes several possible measurements available to assign the coordinate of a special junction point in an F-prime factor, for instance, the end points of deletions found in some of the F-prime factors. In such cases, we principally choose to measure the shorter segment, since the measurements of shorter DNAs are more accurate.

RESULTS

Molecular weights of F'*gal*'s. The molecular weights of the F-prime factors studied, as calculated from measured duplex lengths, are shown in Table 3. All the DNA preparations contained various kinds of small circular DNA species. The fraction of these unknown plasmid DNA is about 10% or less, by number, of the total closed circular DNA isolated.

Analysis of bacterial markers carried by F'*gal*'s. The presence of a marker on an F-prime factor was tested as described in Materials and Methods. A number of markers between *purE* and *uvrB* on the *E. coli* chromosome were scored (Table 4).

Physical structures of F'*gal*'s. The sequence relationships among the several F-primes studied are rather complex. It will be

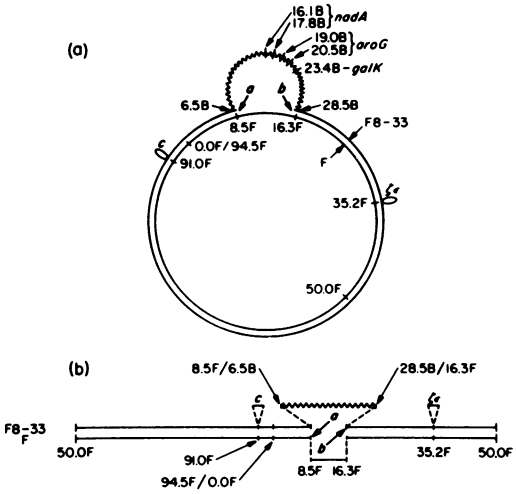

FIG. 2. *Heteroduplex of F8-33/F. (a) A circular representation previously described by Sharp et al. (34). (b) An alternative linear representation used throughout the present paper. Solid lines are F DNA; the sawtooth line is bacterial DNA. Coordinates labeled with F and B are distances in kilobases from the selected origin on F and on bacterial DNA, respectively. The coordinates shown here are modifications of those reported previously (34), due to a modified method of length measurements as discussed in Materials and Methods and to the treatment of a large collection of relevant data. a and b are the junction points of the substitution loop due to nonhomology between F DNA and bacterial DNA of F8-33. The two insertions indicated by c and ζε are characteristic of F8-33 DNA and are used as position markers for mapping F and bacterial sequences in other F-prime factors. F8-33 carries the chromosomal markers nadA, aroG, and gal (34). Their modified positions are indicated in the representation (a).*

TABLE 3. *Molecular weights of F-prime DNA molecules*

DNA	Mol wt (×10⁶)	kb
F152	205.1 ± 5.0	310
F152-1	79.6 ± 1.3	120.2
F100-11	114.1 ± 2.7[a]	171.1[a]
F100-12	151.9 ± 2.3	229.5
F100-13	225.5 ± 4.0	340.6
F	62.6 ± 1.6[a]	94.5[a]

[a] Sharp et al. (34).

TABLE 4. *Genetic markers carried by F-prime factors*

F-prime factor	Marker																	
	purE	*fep*	*leuS*	*lip*	*supE*	*ubiF*	*kdp*	*gltA*	*sucA, B*	*tolA, B*	*nadA*	*aroG*	*gal*	*pgl*	*attλ*	*bio*	*uvrB*	*serS*
F152 (F_2*gal*)	−	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	
F152-1	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	−	−	
F152-2	−		+	+			+	+	+	+	+	+	+	−	−	−	−	
F152-3	−		+	+			−[a]	+	+				+	−	−	−	−	
F100-11 (F450)	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	−
F100-12 (F_{1S})	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	−
F100-13	−			+	+[b]			+	+				+					
F8	−	−	−	−	−	−	−	−	−	+	+	+	+	−	−	−	−	−
F8-12				−					−	+	+	+	+					
F8-13				−					−	−	−	−	+					
F153(F_3*gal*)	−			−	+		+	+	+		+	+	+	+	+	+	+	
F13	+			−						−	−	−	−	−	−		−	

[a] The F152-3 carries a *kdpABC* deletion (see text).
[b] The F-prime factor carries the mutant allele *supE* (suII$^+$).

conducive to clarity, we believe, first to present the conclusions and then to give the evidence that leads to these interpretations.

Figure 3 is a summary of the genetic and physical structures of the various F-prime factors studied. The structures of the bacterial sequences of each F-prime factor are shown in Fig. 3a. Dotted lines indicate deleted sequences. For example, F100-11 and F100-12 are both deletion mutants of the original F100. The bacterial sequence between coordinates 29.0 and 196.5 kb, which includes the markers between *fep* and *suc*, is deleted in F100-11. In F100-12 the sequence deleted lies between 6.9 and 117.0 kb and includes the markers *fep*, *leuS*, and *lip*.

The coordinate system we have chosen to use for the bacterial DNA of F100 and F152 and their derivatives is shown in the top line of Fig. 3a. For F8 and its derivatives, the coordinate system we use is different from that of F100 (bottom line in Fig. 3a). We have taken the counterclockwise junction of bacterial DNA with F DNA in an undeleted F-prime factor of any one series as the origin of coordinates for the bacterial DNA.

The coordinates of the *E. coli* chromosome and the F sequences in an F-prime factor are identified by the letters B and F, respectively, after the coordinate number. For example, in Fig. 3, the deletions of F100-11 are between 29.0B and 196.5B in the *E. coli* chromosome DNA and between 0.0F and 2.8F in the F DNA.

The arrangements of the F sequences in the F-prime factors studied are shown in Fig. 3b. F100, F152, and their derivatives are all missing the F sequence with coordinates from 0.0F to 2.8F; the junctions with bacterial DNA occur at these two points. Similarly, in the F8 episomes, the F sequence 8.5F to 16.3F has been substituted by bacterial DNA. (The small insertion loops, $\zeta\epsilon$ and *c* at coordinates 35.2F and 91.0F that occur in some F8 derivatives, are not shown in Fig. 3.)

The evidence leading to the structures shown in Fig. 3 is described below. All of the quantitative data obtained in the analysis of heteroduplexes involving F152 and F152-1 are presented in the legend for Fig. 4, to illustrate the experimental accuracy and the consistency of different measurements of the same feature. In other sections we give final coordinates without presenting all the data.

(i) F152 (F_2*gal*). F152 was originally isolated by Jacob and Wollman (19) (see also reference 4). Its genetic properties have been intensively studied by Herbert and Guest (13). It transfers *gal* as a proximal marker and *lip* as a distal marker.

Previous efforts to isolate this F-prime from W3101(F152) and PA106(F152) were unsuccessful, yielding only a deleted F (34). From a new source, F152/KL253, kindly supplied by B. Low, two different F-prime factors were isolated.

F152. A large F-prime factor about 310 kb in length, was isolated from F152/KL253. Genetic analysis of this F-prime factor (see Table 4) showed that it carries all the markers tested between *fep* and *gal*. We believe that this is the original F152 plasmid.

F152 is deleted for an F sequence of length 2.8 kb (Fig. 3b), which is substituted by a segment of bacterial DNA of length 218.4B. These results were obtained by analyzing the structure of F152/F and F152/F8-33 heteroduplexes.

Figures 4a and 4b display the observed structures of these heteroduplexes, as well as our assignment of the several duplex and single-strand regions to bacterial and F sequences. An electron micrograph of the F152/F heteroduplex is shown in Fig. 5. Application of the methods

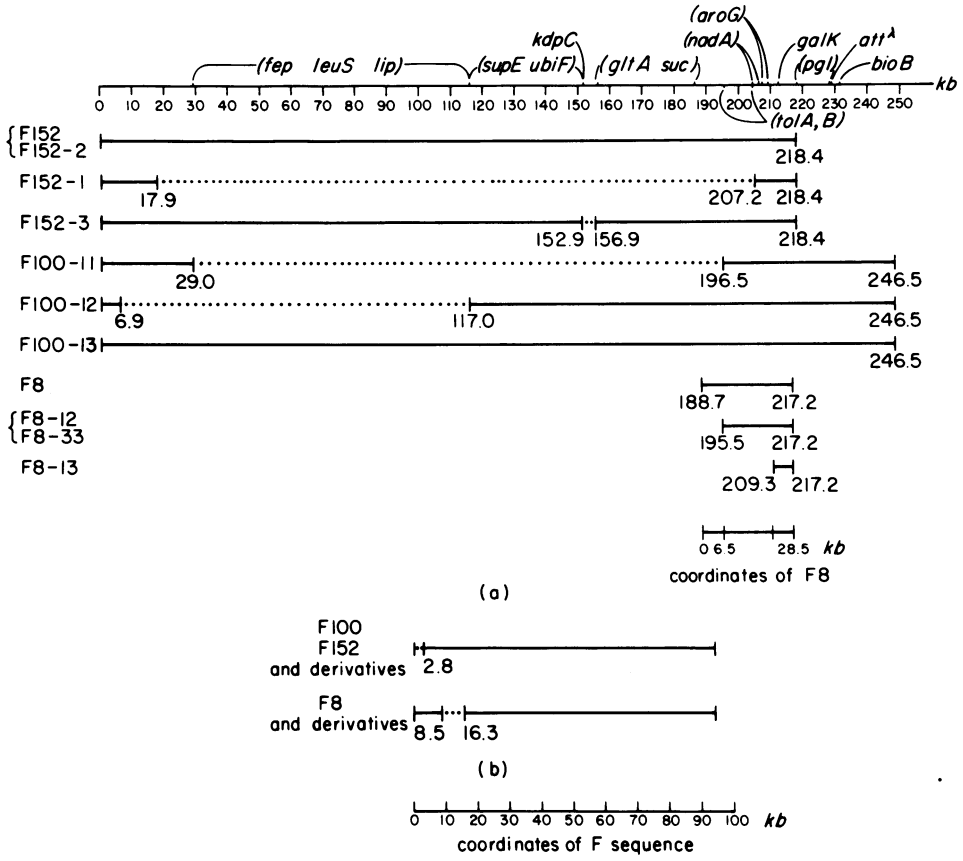

FIG. 3. *Summary of the genetic and physical structures of F-prime factors studied. (a) The bacterial DNA of F-prime factors. The deletions are indicated by the dotted lines. F100-13 carries a small I-D loop of 0.7 kb in length that is not shown in this figure. The top line is the coordinate system of F100, F152, and their derivatives. The bottom line is the coordinate system of F8 and its derivatives. (b) The F DNA sequence of the F-prime factors. The sequence deleted is indicated by the dotted line. Bacterial DNA sequence is substituted for the F sequence deleted.*

that have been discussed previously (34) for interpreting these structures leads to the structure of F152 given in Fig. 3. It should be noted that the F152/F8-33 heteroduplex is useful not only to determine the position of the F sequences missing in F152 but also to map the regions of homology between the *E. coli* chromosome sequences of F152 and F8-33. The results show that all of the 21.7 ± 0.8 kb of the chromosome sequences of F8-33 are contained in F152.

F152-1, a deleted F152. The first stab of F152/KL253 we received from B. Low was found to contain mostly Gal$^-$ bacteria when streaked on EMB *gal* plates. One of the few Gal$^+$ colonies was picked, and its genetic properties were examined. The presence of an F-prime factor capable of transferring the *gal*$^+$ marker was confirmed. However, several bacterial markers that are supposed to be present in F152 were missing (see Table 4). The plasmid isolated had a length of about 120.2 kb, considerably shorter than F152. We named it F152-1.

Evidence indicating that F152-1 is a derivative of F152 that arose by deletion of bacterial DNA was obtained by analyzing heteroduplexes, F152-1/F152, F152-1/F, and F152-1/F8-33.

The F152-1/F152 heteroduplex (Fig. 4c) has a simple structure, consisting of a duplex circle with a single large deletion loop. This suggests that F152-1 is derived from F152 by a single deletion. The end points of the deletion are determined by comparing the heteroduplexes F152-1/F8-33 (Fig. 4e and Fig. 6) and F152/F8-33 (Fig. 4b). This leads to the structure of F152-1 given in Fig. 3.

F152-1 carries the bacterial markers *aroG* and *gal*, but deletes those from *fep* to *nadA* (Table 4). The locations of *nadA* and *aroG* have been

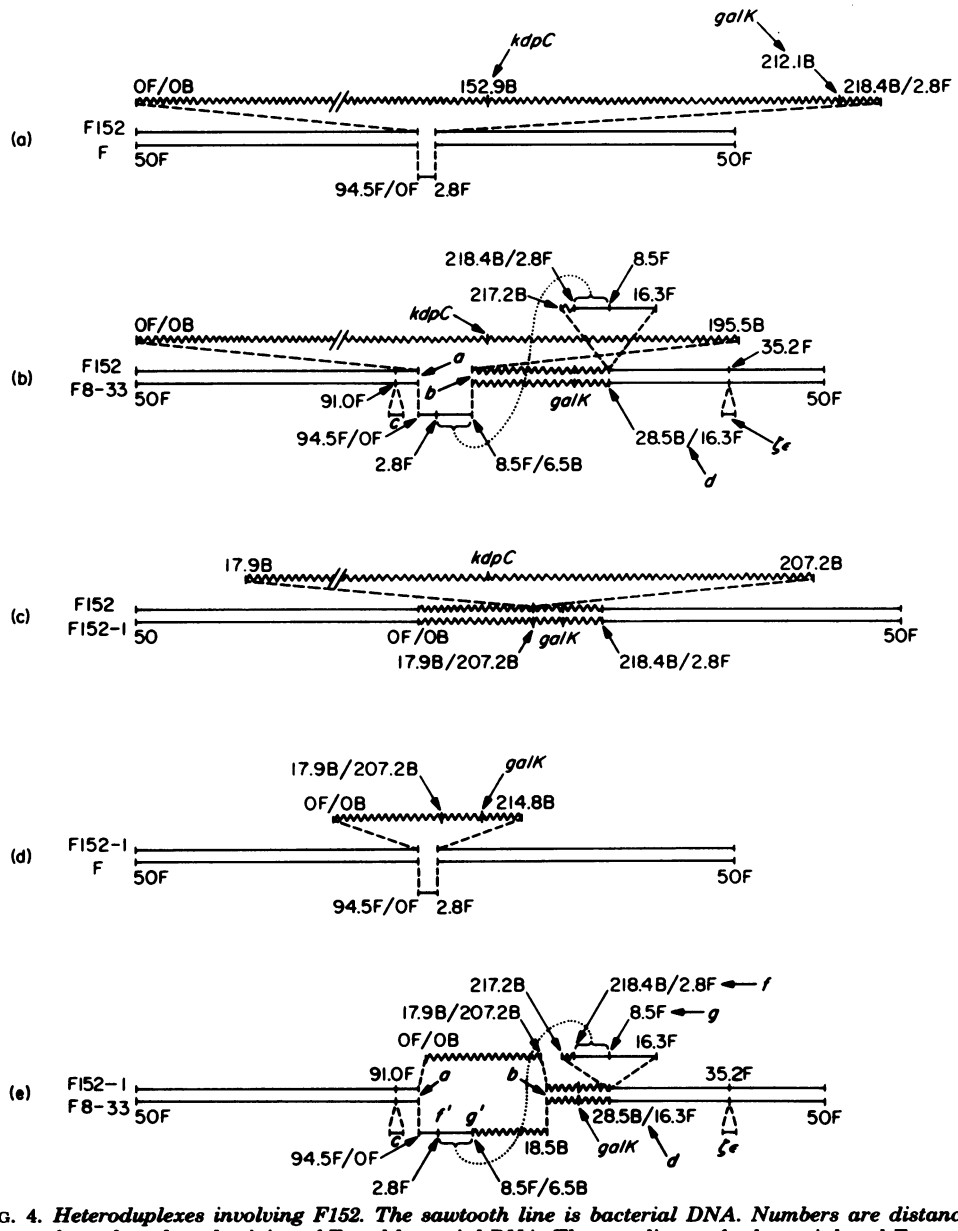

FIG. 4. *Heteroduplexes involving F152. The sawtooth line is bacterial DNA. Numbers are distances in kilobases from the selected origins of F and bacterial DNA. The coordinates for bacterial and F sequences are labeled with B and F, respectively. (a) F152/F heteroduplex. The electronmicrograph is shown in Fig. 5. (b) F152/F8-33 heteroduplex. The two insertion loops of F8-33 are indicated as c and ζε. Sequences connected by the dotted line sometimes form base pairs that cause tangled heteroduplexes. (c) F152/F152-1 heteroduplex. (d) F152-1/F heteroduplex. (e) F152-1/F8-33 heteroduplex. The micrograph is shown in Fig. 6. Sequences fg and f'g', connected by the dotted line, sometimes form base pairs. This is mostly seen in heteroduplexes between two incomplete strands. The length measurements in kilobase units, their standard deviations, and number of samples for the several segments are the following: (a) 0F to 2.8F, 2.9 ± 0.3 (5); 0B to 218.4B, 212.1 ± 3.2 (3). (b) 91.0F to 94.5/0F, 3.7 ± 0.2 (9); 0F to 8.5F, 817 ± 0.7 (4); 0B to 195.5B, 195.7 (1); 195.5B to 217.2B, 21.7 ± 0.5 (4); 217.2B to 218.4B/2.8F to 16.3F, 14.3 ± 1.0 (6); 16.3F to 35.2F, 19.5 ± 0.8 (3). (c) 17.9B to 207.2B, 190.0 ± 4.4 (5). (d) 0F to 2.8F, 2.6 ± 0.3 (13); 0F to 17.9F/207.2B to 214.8B, 27.2 ± 1.3 (13). (e) 91.0B to 94.5B/0F, 3.5 ± 0.3 (39); 0F to 8.5F/6.5B to 18.5B, 19.6 ± 0.5 (9); 0B to 17.9B, 17.9 ± 0.8 (17); 207.2B to 217.2B, 10.0 ± 0.6 (17); 217.2B to 218.4B/2.8F to 16.3F, 13.4 ± 0.7 (5); 16.3F to 35.2F, 17.9 ± 0.7 (8); 0F to 2.8F, 2.8 ± 0.3 (13); 2.8F to 8.5F, 5.6 ± 0.3 (8); 6.5B to 18.5B, 11.6 ± 0.4 (6); 217.2B to 218.4B, 1.2 ± 0.1 (5); 8.5F to 16.3F, 7.9 ± 0.9 (3).*

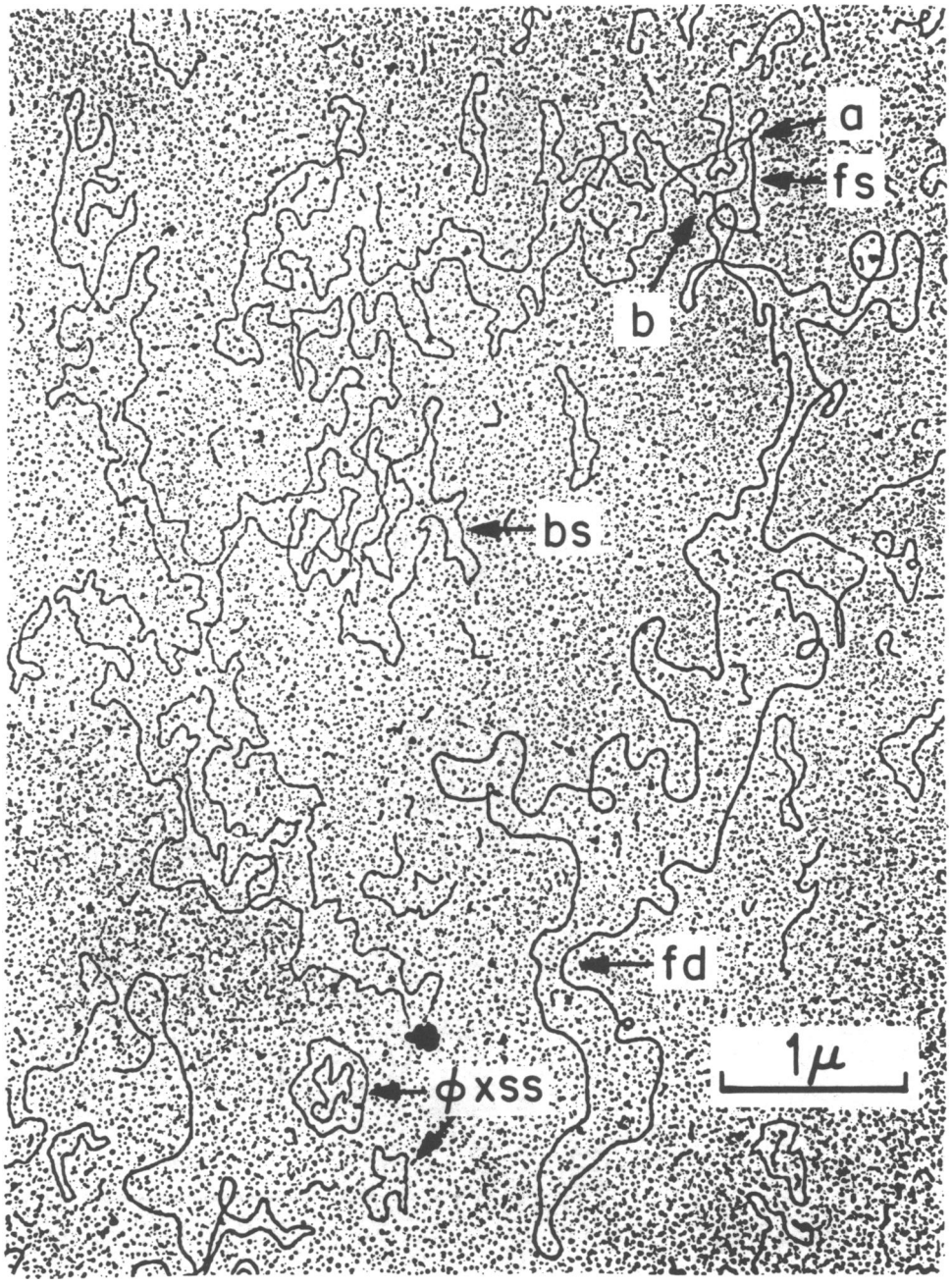

FIG. 5. *F152/F heteroduplex. The schematic representation is shown in Fig. 4a. Letters a and b point to the junctions of duplex and single-stranded DNA at 0F/0B and 2.8F/218.4B, respectively. Duplex F DNA, single-stranded F DNA, and single-stranded bacterial DNA are identified as fd, fs, and bs respectively. Several φX single-stranded DNAs are also marked.*

mapped in the regions 16.1B to 17.8B and 19.05B to 20.5B of F8, respectively (see Fig. 2a). The deletion of F152-1 in the 0B-to-18.5B region in the F8 coordinate system is consistent with the genetic analysis that *nadA* is missing in F152-1.

(ii) F100. F100 and F152 were said to be independently derived from the same Hfr, HfrP3 (4, 19). F100 differs from F152 in that it carries the markers *attλ*, *bio*, and *uvrB* in addition to those present in F152 (23). We have collected

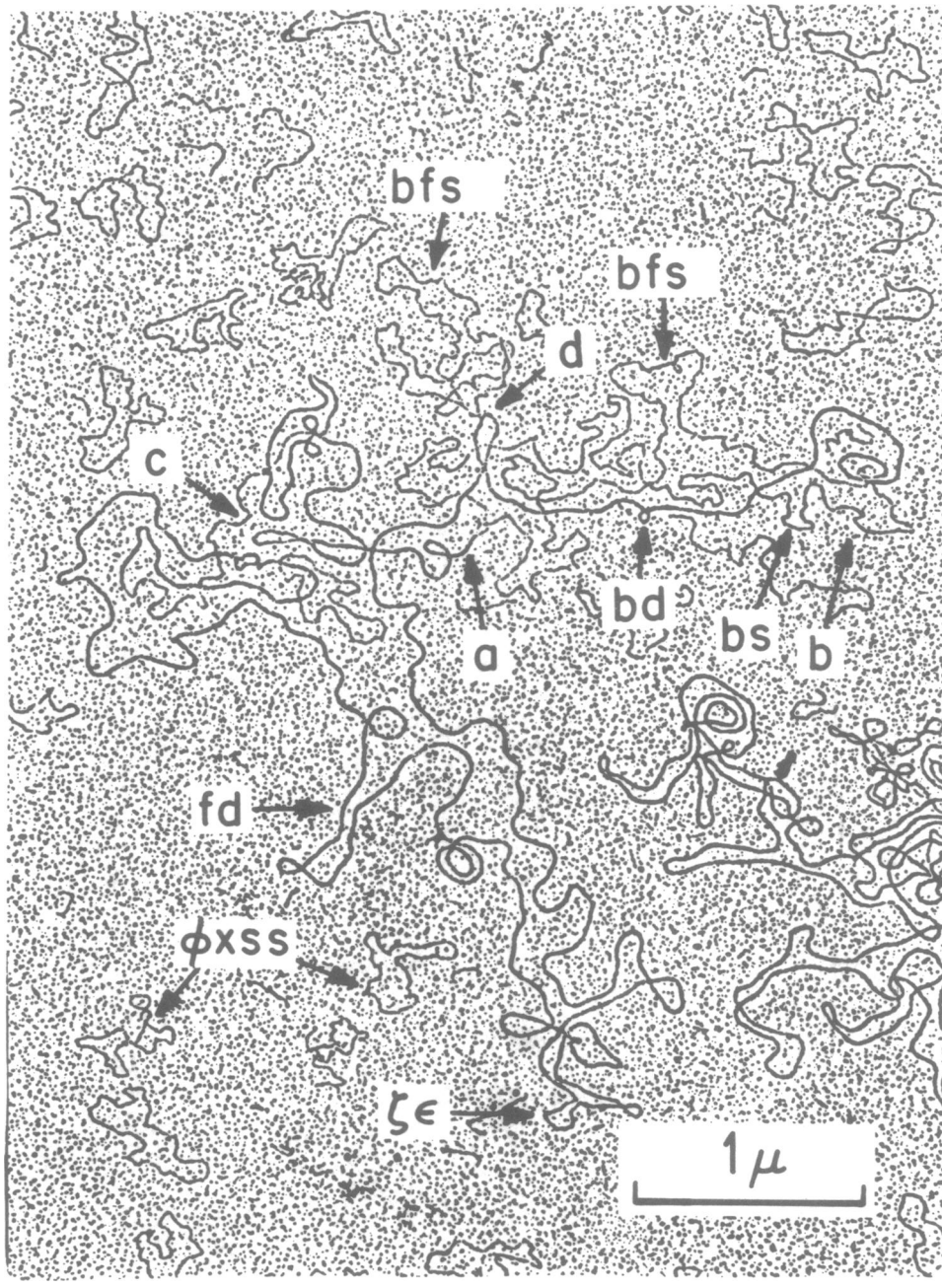

FIG. 6. *Structure of an F152-1/F8-33 heteroduplex. The structure is illustrated in Fig. 4e. The junctions of the substitution loop are marked by the letters a and b. The letter d points to the place where the I-D loop of F152-1, bfs, comes out of the duplex region. The two insertion loops of F8-33 are labeled by c and ζϵ. bfs indicates that the single-strand DNA is composed of both bacterial and F sequences. fd, fs, bd, and bs indicate duplex F DNA, single-strand F DNA, duplex bacterial DNA, and single-strand bacterial DNA, respectively.*

two F-prime factors, F450 and F_{1S}, which carry *gal* and *attλ*. The structures of F sequences in those two F-prime factors have been reported by Sharp et al. (34).

The structures of the bacterial sequences of F450 and F_{1S} (we now call them F100-11 and F100-12) as shown in Fig. 3 suggest that these two episomes are independent deletion variants

of the original F100 episome, which contained all of the bacterial sequences from 0.0B to 246.5B (see Fig. 3). This interpretation is based on the structures of F100-11 and F100-12 as analyzed by heteroduplexes with F152 and F152-1 (see Fig. 7 and 8).

The positions of the deletions as mapped by heteroduplex analysis are used to assign the physical positions of the genetic markers missing in these two plasmids. For example, the markers *fep*, *leuS*, and *lip* are not present in F100-11 and F100-12 (see Table 4). Therefore their positions must be inside the common sequences deleted in these two plasmids, i.e., from 29.0B to 117.0B (see Fig. 3). Similar arguments are used to assign the physical positions of various genetic markers shown in Fig. 3.

(iii) F8, F8-12, and F8-13. The F8 plasmid isolated by Hirota and Sneath (14) is very frequently used for genetic analysis. In this section, three F8 plasmids derived from different sources were analyzed. The structures of F8 [which was called F8(1) in reference 34] and F8-12 [which was called F8(2) in reference 34] were reported previously (see also Fig. 9). From the analysis of F8/F8-33 and F8/F100-12 heteroduplexes, we conclude that F8-12 is a derivative of F8 with a deletion in the bacterial sequence from 0.0B to 6.5B on the F8 coordinate system (or 188.7B to 195.5B on the F100 coordinate system). F8-13 is a plasmid that was also called F8 (25). We found, however, that it does not carry the markers *tolAB*, *nadA*, and *aroG*, which are present in F8 (Table 4). Heteroduplex analysis of F8-33/F8-13 (Fig. 9) shows that its bacterial sequence is deleted from 0.0B to 20.5B on F8 coordinates (or 188.7B to 209.3B on F100 coordinates). This allows us to assign the physical locations of the *tolAB*, *nadA*, and *aroG* markers.

Reconstruction of F152 and F100. The in-

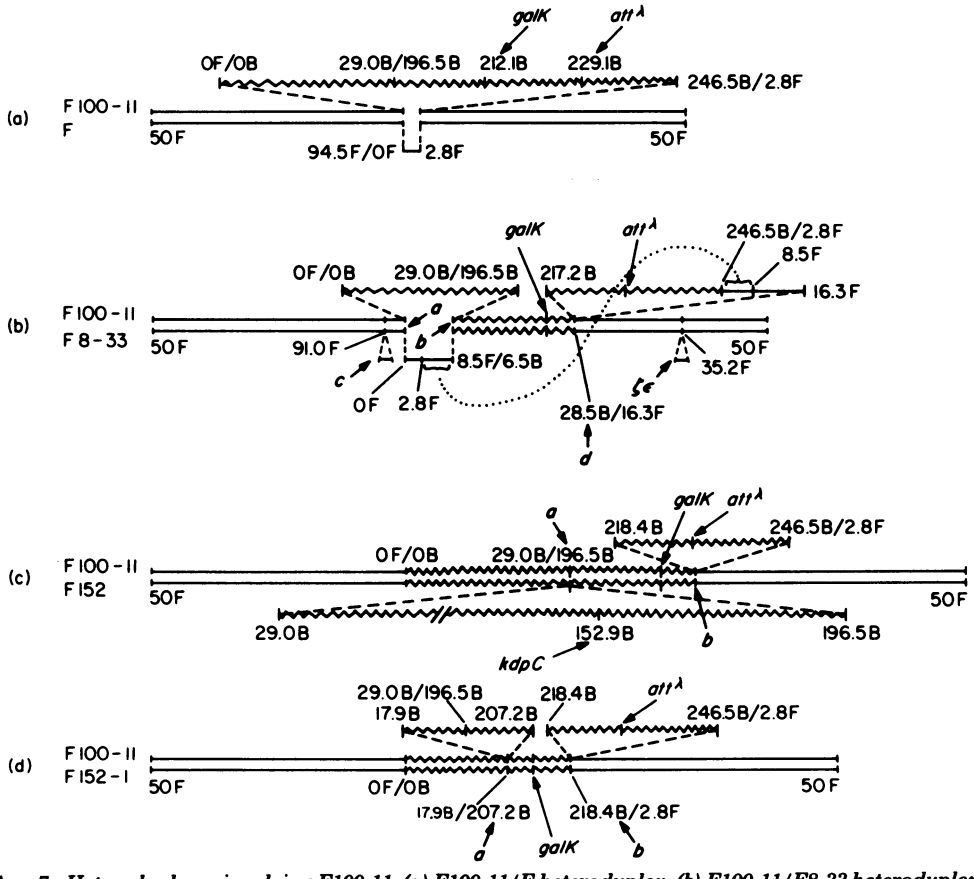

FIG. 7. *Heteroduplexes involving F100-11. (a) F100-11/F heteroduplex. (b) F100-11/F8-33 heteroduplex. The sequences connected by the dotted line sometimes form pairs. The heteroduplexes (a) and (b) have been reported by Sharp et al. (34) and are cited here with new coordinates established in this paper. The positions of markers gal and attλ are indicated in the figures. (c) F100-11/F152 heteroduplex. (d) F100-11/F152-1 heteroduplex. Note that 0F/0B in both F100-11 and F152-1 (or F152) are exactly the same.*

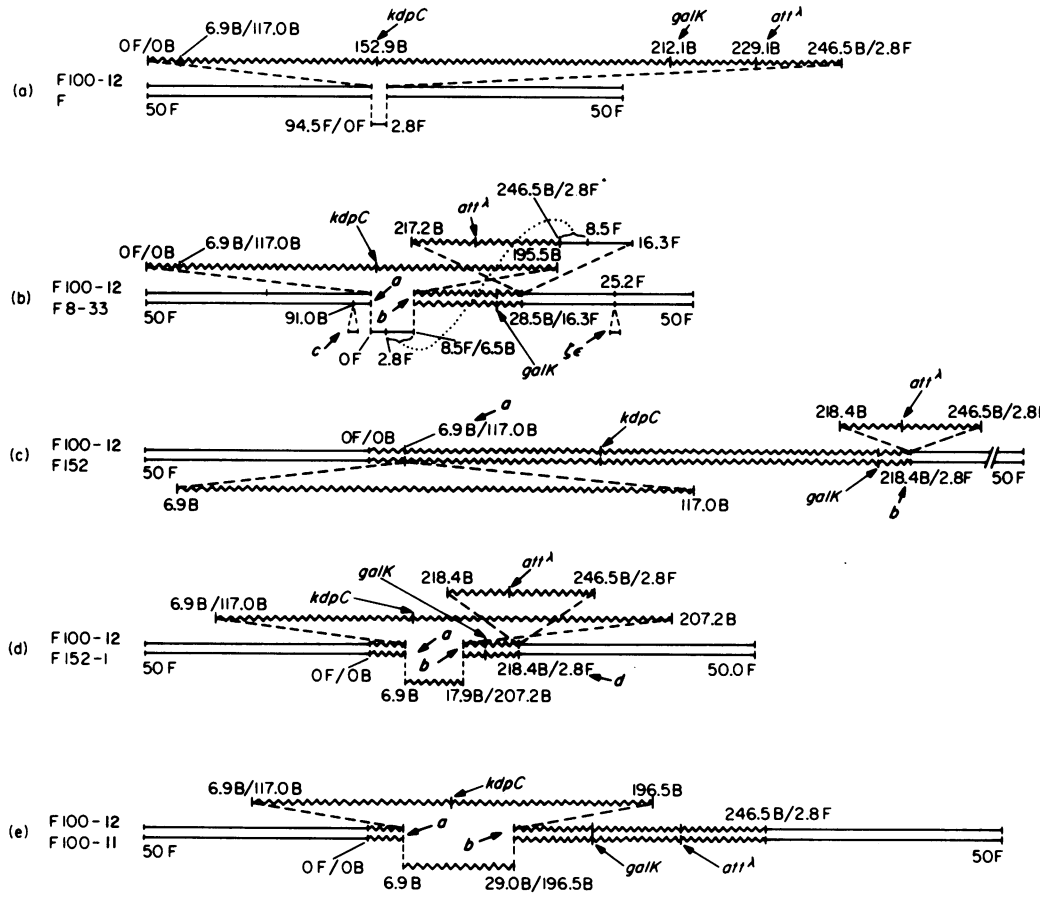

FIG. 8. *Heteroduplexes involving F100-12. (a) F100-12/F heteroduplex. (b) F100-12/F8-33 heteroduplex. The sequences connected by the dotted line sometimes form base pairs. (c) F100-12/F152 heteroduplex. (d) F100-12/F152-1 heteroduplex. (e) F100-12/F100-11 heteroduplex.*

terpretation of the electron microscope studies in the previous section depends on the assumption that the F152 plasmid isolated from KL253 (F152) carries the normal bacterial sequence of the *E. coli* chromosome between *fep* and *gal* and is the same as that of the original F152. To test this assumption, we compared F152 from F152/KL253 with an "F152" or "F100" that had genetically been reconstructed from the deletion derivatives. The reconstruction was accomplished by rescuing the deleted markers by recombination with the wild-type bacterial chromosomes. The results to be presented below support the assumption that F152 from F152/KL253 is the same as the original F152.

(i) Reconstruction of F152 from F152-1: F152-2 and F152-3. The process for reconstructing F152 from F152-1 is illustrated in Fig. 10. F152-1, which carries a large deletion of bacterial DNA, was transferred into W3350, which carries double point mutations in *gal* but is otherwise normal. Recombination between F152-1 and the W3350 chromosome in regions 1 and 2 or in regions 1 and 3 will result in formation of an F-prime factor carrying the sequence deleted in F152-1. Such an F-prime factor can be selected by transferring into a *recA* recipient containing a mutation in one of the markers deleted in F152-1. In the present experiments, we used the recipient strain PL225 [F^- *recA str thi* Δ(*nadA-gal*)], which has a deletion from *nadA* to *gal*, to select *nadA*$^+$.

W3350/F152-1$^+$ was mixed with PL225 in Penassay broth. The ratio between donor and recipient cells was 1:3. After standing incubation of the mixture at 37°C for 1 h, it was plated on a minimal medium containing 2 μg of thiamine and 100 μg of streptomycin per ml to select NadA$^+$ [Str^r] colonies. The frequency of formation of such colonies was about 5×10^{-5} per donor cell. The NadA$^+$ [Str^r] colonies were tested for the ability to transfer *lip*, which is also

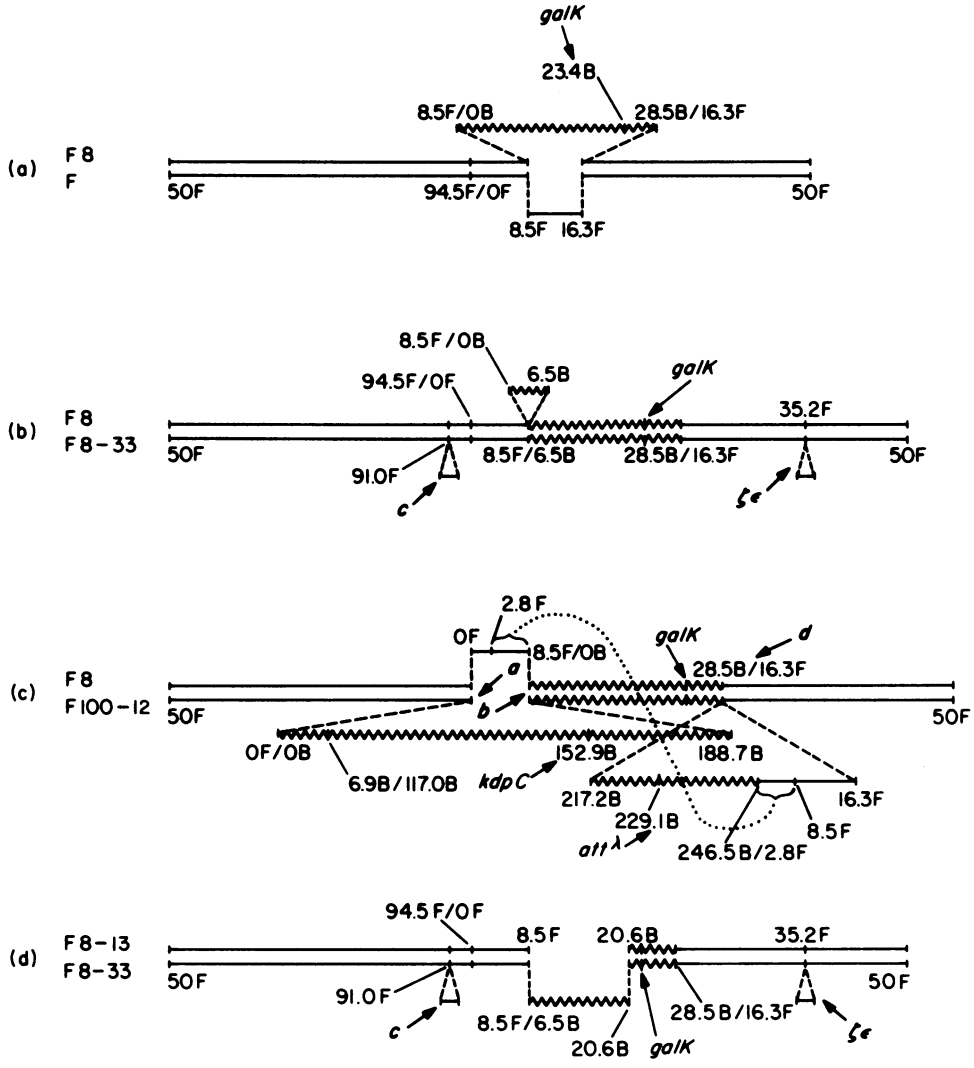

FIG. 9. *Heteroduplexes involving F8 and F8-13. (a) F8/F heteroduplex. (b) F8/F8-33 heteroduplexes. The heteroduplexes (a) and (b) have been reported by Sharp et al. (34) and are cited here with new coordinates established in this paper. (c) F8/F100-12 heteroduplex. The sequences connected by the dotted line sometimes form base pairs. (d) F8-13/F8-33 heteroduplex.*

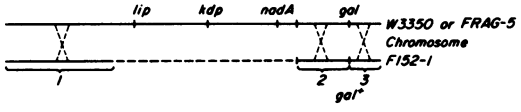

FIG. 10. *Reconstruction of original F152 from F152-1. Only the bacterial sequence of F152-1 is shown in the figure. A host strain W3350 carries the gal-1,2 mutations; FRAG-5 carries the gal mutation and also a deletion mutation in kdp sequences (12).*

deleted from F152-1, but was not directly selected for use in the reconstruction experiment. All were capable of transferring *lip*. Of the 135 NadA$^+$ [Str^r] colonies isolated, 25% were found to be Gal$^+$, presumably resulting from recombination in regions 1 and 2, whereas the rest of them were Gal$^-$, resulting from recombination in regions 1 and 3 (see Fig. 10). One of the *gal*$^+$ F-prime factors was analyzed further. We called it F152-2.

F152-2 was transferred into N23-76 (F$^-$*gal recA*) and tested for the bacterial markers carried. By this criterion, F152-2 is indistinguishable from the F152 isolated from F152/KL253 (Table 4). F-prime DNA was extracted from PL225 (F152-2$^+$) and hybridized with F152. No heteroduplex of any sort can be observed in the electron microscope. The structure of the F152-

2/F100-12 heteroduplex is essentially the same as that of the F152/F100-12 heteroduplex (Fig. 11a). Therefore F152-2 and F152 are the same by both genetic and heteroduplex criteria. Consequently it can be concluded that the F152 in F152/KL253 is the same as the original F152 F-prime factor.

The recombination process leading to the reconstruction of F152 from F152-1 is more dramatically demonstrated by reconstructing a new F152-type plasmid carrying a deletion originally located in the bacterial chromosome (see Fig. 10). F152-1 was transferred into FRAG-5, which is known to carry a deletion in the *kdp* gene (12). A newly constructed episome was then transferred into PL225 by selecting NadA$^+$ [Str^r] transconjugants. As in the case of the F152-2 episome, both Gal$^+$ and Gal$^-$ transconjugants were obtained. The frequency of Gal$^-$ bacteria was 83% (43/52). All of the transconjugants could transfer *lip* with high efficiency. Genetic analysis of these new F-prime factors in N23-76 showed that the *kdp* marker was missing. The physical structure of one of the new F-prime factors, which we named F152-3, was analyzed. The sole nonhomology feature observed in the F152-3/F152 heteroduplex was an I-D loop 3.9 ± 0.3 kb long (Fig. 11b). From the heteroduplex F152-3/F100-12 (Fig. 11c and Fig. 12), the position of this I-D loop was mapped to be between coordinates 152.9B and 156.9B, assuming that it is a deletion. The *kdp* gene is known to be comprised of four cistrons, *kdpDCBA*, in this order along the chromosome. Therefore the point 152.9B must be within *kdpC*, since *kdp* in FRAG-5 is deleted in part of *kdpC* and all the *kdpBA* (12).

The results of the heteroduplex analysis of F152-3 again confirm the previous conclusion that the bacterial DNA carried in F152 is the normal bacterial sequence.

The high frequency of recovery of reconstructed F-prime factors indicates that recombination frequently occurs at homologous bacterial sequences between F-prime factor and host chromosome. Such genetic association and dissociation of genetic materials have been reported by Adelberg and Pittard (1).

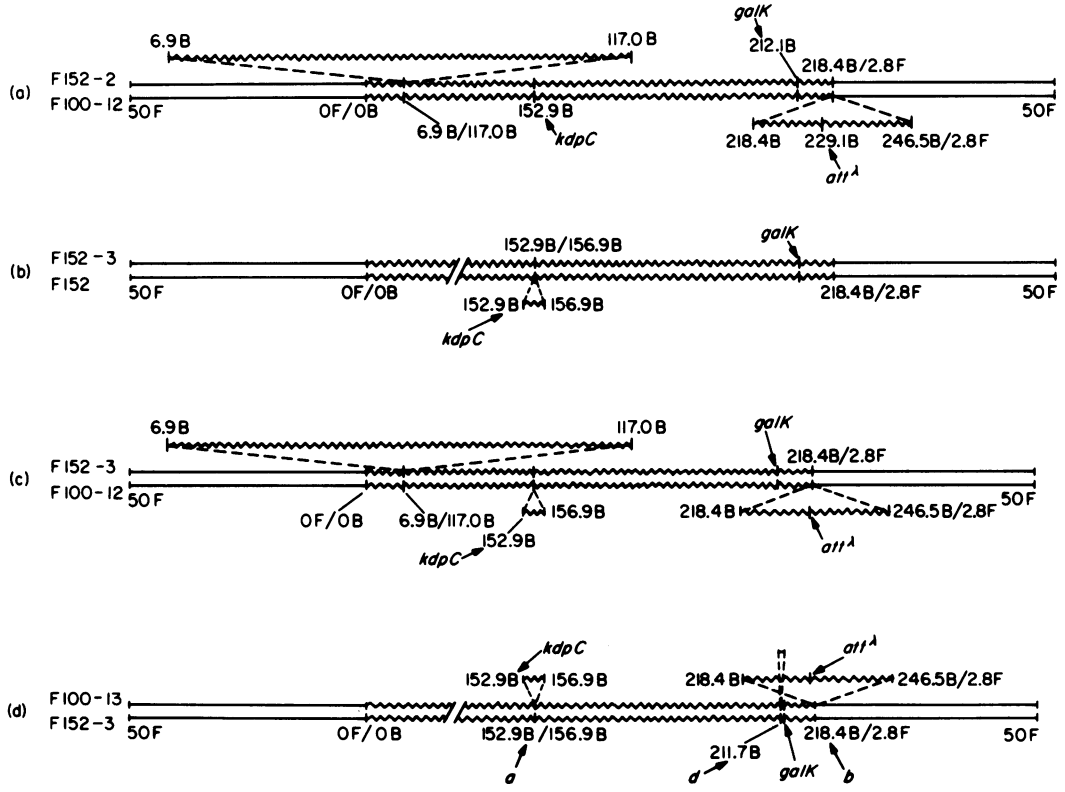

FIG. 11. *Heteroduplexes involving F152-2, F152-3, and F100-13. (a) F152-2/F100-12 heteroduplex. (b) F152-3/F152 heteroduplex. (c) F152-3/F100-12 heteroduplex. A characteristic small loop observed in the heteroduplex (b) can be identified in this heteroduplex. The electron micrograph is shown in Fig. 12. (d) F100-13/F152-3. An unexpected small I-D loop was observed at d.*

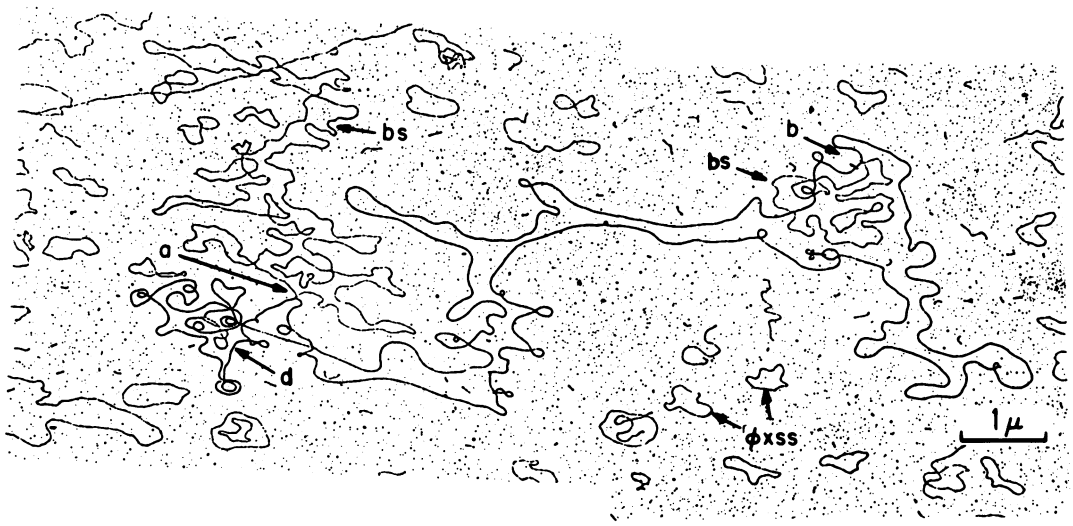

FIG. 12. *Heteroduplex between F100-12 and F152-3 showing three I-D loops. The structure is illustrated in Fig. 11c. I-D loop a is the bacterial sequence deleted in F100-12. I-D loop b is the sequence containing attλ, bio, and uvrB. I-D loop d is the kdp deletion of F152-3.*

(ii) Reconstruction of F100 from F100-11 and F100-12: F100-13. As an independent check of the previous physical results, the original F100 was isolated and compared with F152. The hypothetical F100 can be constructed from F100-11 or F100-12. The process for the reconstruction is closely related to the genetic analysis of the *supE* marker in the F-prime factors studied. Thus we first describe the genetic tests for *supE* on the F-prime factors.

supE is located between *lip* and *kdp*. The *supE* mutation is known to be an amber suppressor (previously called suII$^+$); it is dominant to the wild-type allele (35). It is thus difficult to determine whether the *supE*$^+$ allele is present on the F-prime factors by the simple marker transfer tests described in Materials and Methods.

However, F152 can be shown to carry the *supE* allele by the use of the same marker rescue experiment described in the previous section. X7026 is an F$^-$ strain carrying *supE* (suII$^+$). X7026 carrying F152-1, which is deleted from *fep* to *nadA*, was prepared and mated with PL225. All the NadA$^+$ [Str^r] colonies selected showed the ability to transfer *lip*, as expected, and at the same time *supE*. The transfer of *supE* can be readily checked by mating with strains having an amber-suppressible mutation, such as FRAGG-1 [*lac*(Am)] and KRO (*trp* amber). Such a joint rescue of *lip* and *supE* with *nadA* indicates that F152-1 was initially deleted for at least part of *supE* as well as *lip*$^+$. Thus the *supE* sequence is somewhere in the region 0B to 218.4B in F152. This conclusion is supported by the genetic and physical results of the reconstruction of F152 described in the previous section.

Other F-prime factors that have been shown by heteroduplex analysis to have deletions were then tested to determine whether the *supE* allele is present on them. X7026 carrying F100-12, for example, was prepared. F100-12 has a deletion within the 0B-to-218.4B region. F100-12 must therefore have sequences homologous to the host chromosome in the region between *purE* and *lip* and in the region of *gal*. If *supE* is directly rescued in X7026 (F100-12$^+$) onto the F-prime factor, there are two possible cases, which depend on the structure of the deletion in the F-prime factor. In the case that the *supE* sequence is deleted in F100-12, an F-prime carrying *supE* resulting from F-prime–host recombination will invariably acquire another gene, for instance, *lip*, which is deleted in F100-12 (Fig. 13a). In the other case, that F100-12 carries the *supE* sequence, some of the F-prime factors carrying *supE* will still carry the *lip* deletion due to two crossovers in regions 2 and 3 (Fig. 13b).

The experiments were done as follows: KRO is an F$^-$*recA* strain having an amber-suppressible *trp* mutation and a *str*^{r} mutation. Therefore, if Trp$^+$ [Str^r] colonies are selected from the mating of X7026 (F$^-$*supE str*^{s}) carrying an F-prime with KRO, bacteria harboring a *supE* F-prime factor can be isolated.

The results of the *supE* test for several F′*gal*′s are given in Table 5. Analysis of unselected

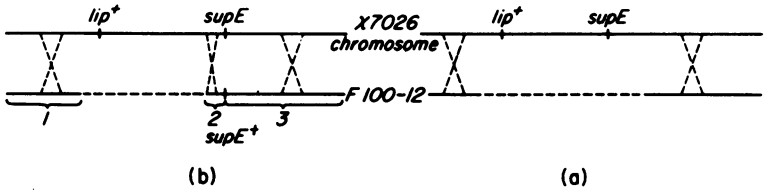

FIG. 13. *Probable processes of rescue of the supE (suII+) marker by (a) F100-12, which deletes supE sequence, or (b) F100-12, which does carry supE sequence through recombination with the host chromosome. Only the bacterial sequence of F100-12 is shown.*

markers *lip*+ and *gal*+ (see Materials and Methods) suggests that F152-1 and F100-11 do not carry *supE*, whereas F100-12 and F153 do. F8 was actually used as a control in Table 5, since it has been shown not to have any bacterial sequences between *purE* and *suc*. Unexpectedly, however, F8 was able to rescue *supE* with a very low frequency. Moreover, all the Trp+ [Str^r] colonies harboring F-prime factors were found to carry *lip*+. The formation of such F-prime factors from F8 is discussed in the accompanying paper (30).

The results of F100-12 are consistent with those obtained by Signer et al. (35). We also analyzed a closely related F-prime factor, F153, obtained from J. Tomizawa, who received the bacterial strain from F. Jacob (Tomizawa, private communication). Genetically, it is very similar to F100-12 (Tables 4 and 5).

The F-prime factors that are derived from F100-11 and F100-12 and carry *lip*+ as well as *supE* must be similar to the original F100 if the model presented in Fig. 13 is correct. One of the reconstructed "F100's" (called F100-13) that was derived from F100-11 was transferred to N23-76 and was tested for the markers carried. F100-13 carried all the markers between *fep* and *uvrB* (Table 4). The episomal DNA was prepared. It was almost twice as large as F100-11 (Table 3). The structure of the F100-13/F152-3 heteroduplex is shown in Fig. 11d. Three I-D loops were observed: the I-D loop at point *a* with a length of 4.0 to 0.5 kb is the *kdp* deletion characteristic of F152-3 heteroduplex as shown in Fig. 11d. The I-D loop at point *b* with a length of 17.7 ± 1.5 kb is identified as the sequence containing *attλ*, *bio*, and *uvrB*, which is missing in F152 (see reference 34); the I-D loop at point *d* mapping at coordinate 211.7B is the unexpected structure. Its length, 0.68 ± 0.13 kb, is similar to that of the IS1 insertion found in some strong polar mutations of bacterial genes (15, 17). Disregarding the small I-D loop at point *d*, the structure of F100-13 is that expected for the hypothetical F100 F-prime factor. It carries *attλ*, *bio*, and *uvrB* sequences in addition to those present in F152. The sequence near the counterclockwise F-chromosomal DNA junction in F100-13 is homologous with F152, indicating that they were derived from the same Hfr. This result also reaffirms our previous conclusion that F152 carries the normal bacterial sequence and is the same as the original F152.

TABLE 5. *Frequency of supE transfer (Trp+ [Str^r]) and analysis of transfer of lip+*

F-prime factor in X7026	Trp+ [Str^r] colonies/donor cell	No. of colonies tested	No. of colonies capable of transferring *lip*+
F100-11	5.8×10^{-4}	84[a]	84
F100-12	1.3×10^{-3}	85[a]	8
F152	1.2×10^{-3}		
F152-1	4.9×10^{-5}	40[a]	40
F153	1.0×10^{-3}	40[a]	4
F8	8.6×10^{-7}	16[b]	12

[a] All bacteria were able to transfer *gal*+.

[b] Four of the *trp*+ bacteria were found unable to transfer both *lip*+ and *gal*+. They were also resistant to a male-specific phage, M13. The formation of these plasmids is described in the accompanying paper (30).

DISCUSSION

The results presented show that deletions have occurred in several of the frequently used F-prime factors such as F100, F152, and F8. The occurrence of these deletions is useful for localizing the physical positions of several bacterial markers between *fep* and *uvrB*. These are shown in the top line of Fig. 3a. In general, these positions are in good agreement with the *E. coli* linkage map (5).

Figure 14 displays the physical map of the *fep*-to-*uvrB* region and the adjacent region *lac-proC-purE* analyzed recently by Hu et al. (16). Together they constitute a total of about 11 min in genetic units of the *E. coli* chromosome (5, 6). From the relative physical positions of *lac* and *galK* in Fig. 14, we can calculate the approximate physical length corresponding to the genetic unit, the minute of conjugational transfer time, as 47 kb. Based on this value, the molecular weight of the *E. coli* chromosome would be 3.1×10^9. By autoradiography, Cairns (7) estimates the length of the *E. coli* chromosome as 22 times

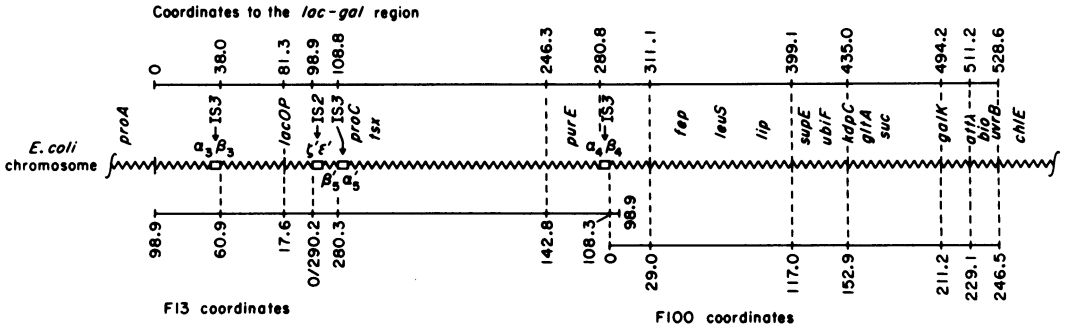

FIG. 14. *Summary of physical and genetic maps in the region of lac-purE-gal-uvrB. The E. coli chromosome sequences on the F13 F-prime has been reported previously (9, 16). The relations of the DNA sequences of the E. coli chromosome on F13 and F100 (or F152) have also been described (9, 16). The positions of lacOP and attλ are computed from the results obtained by Hu et al. (18) and Sharp et al. (33). αβ and εζ are special sequences of F and have been identified as insertion sequences IS3 and IS2, respectively (see references 9, 16, 17, 18, 29).*

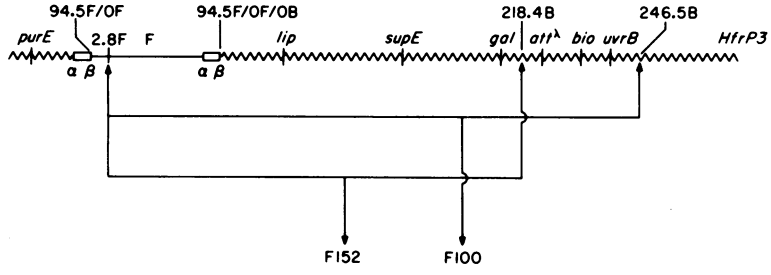

FIG. 15. *Probable processes for the formation of F100 and F152 from HfrP3. Note that the excision point on F is the same for both F-prime factors.*

that of T2 bacteriophage. Kim and Davidson (20) give a revised value of the molecular weight of unglucosylated T2 DNA as 1.12×10^8. This gives the molecular weight of *E. coli* chromosome as 2.5×10^9. Our estimation is therefore in good agreement with this value.

Generalized transduction crosses mediated by bacteriophage P1 have been used to comprise the principal source of data for genetic mapping of the *E. coli* chromosome. In our hands, phage P1 DNA was measured to be 2.09 ± 0.10 times phage λ DNA (37 molecules were measured). Therefore P1 DNA can be calculated to be 97.2 ± 4.7 kb in length. From this value, we assume that phage P1 transduces an *E. coli* DNA segment of about 2.1 min in genetic map units.

The physical structures of F152 and F100 suggest that they are derived from the same Hfr, HfrP3, by different excision processes (see Fig. 15). The structures of these two F-prime factors also provide a clue as to how HfrP3 was formed. By comparing the sequences of these two F-prime factors and a classical F-prime factor, F13 (16), it is implied that the *E. coli* chromosome contains a copy of a 1.3-kb-long DNA sequence homologous to the αβ sequence of F DNA at the integration site of HfrP3. We believe, therefore, that HfrP3 was formed by recombination between the homologous sequences of F and the *E. coli* chromosome between *purE* and *lip*. A more general discussion of the formation of Hfr's using this type of mechanism is given by Davidson et al. (9).

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