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Heteroduplex Analysis of *traΔ* F′ Plasmids and the Mechanism of Their Formation

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Four *traΔ* mutant F*argG*$^+$ plasmids, derived from matings between Hfr AB312 and a *recA* recipient, have been shown to have deletions of at least 50% of the F genome, including the region in which the *tra* genes map. The mutant plasmids do contain the F genes required for plasmid maintenance. Correlations can be made between, on the one hand, the F genes present on the *traΔ* F′ plasmids and the F genes transferred early by an Hfr donor, and, on the other hand, the F genes deleted from the *traΔ* F′ plasmids and the F genes transferred late by an Hfr donor. A biased representation of proximally and distally transferred chromosomal markers among the *traΔ* F′ elements was also demonstrated. Taken together, the asymmetrical representation of Hfr genes and the *cis* dominance of the Tra phenotype of these mutants can best be explained by the hypothesis that the *traΔ* F′ plasmids are formed by repliconation of the transferred exogenote in a *recA* recipient.

We recently characterized a novel class of mutant F′ plasmids (11, 18-20), found in *Escherichia coli* as the major product of conjugal matings between an Hfr donor and a *recA* F$^-$ recipient (11). These plasmids are Tra, being unable to promote their own transfer by conjugation. Genetic analysis indicated that the mutants are unable to complement point mutations in any of nine of the known *tra* genes (1, 30) including cistrons located in two different operons (14). Furthermore, the Tra phenotype of the mutants is *cis* dominant, implying that the *tra* mutation they carry also affects a structural determinant required for conjugal activity (31), or a gene whose product is required in the *cis* configuration for donor ability. As the most economical hypothesis, we concluded that the lesion affecting the Hfr-derived mutant plasmids is a deletion (termed *traΔ*). We now report the results of heteroduplex mapping of these plasmids that confirm this conclusion.

The formation of F′ plasmids is generally held to occur by aberrant excision of F from the chromosome of an Hfr cell, the Campbell model (5, 26). Novick, however, has presented (22) an alternative model to explain F′ formation in the special case of F′ plasmids, carrying a proximal donor marker, which are recovered from Hfr × Rec F$^-$ matings. The salient difference between the two hypotheses, in terms of the generation and properties of the *traΔ* F′ plasmids, is that the formation of the autonomous replicon occurs in the donor cell prior to transfer in the case of the Campbell model but after conjugal transfer, in the recipient cell, according to the Novick proposal. We have examined the adequacy of each of these hypotheses to account for the properties of the *traΔ* F′ plasmids.

MATERIALS AND METHODS

Bacterial and phage strains. The bacterial strains used are described in Table 1. T6, f2, fd, and ΦII phages were prepared as previously described (11).

Nomenclature. In general, the recommendations of Demerec et al. (8) and Novick et al. (23) were followed. The following abbreviations are used: Ura, uracil; Lac, lactose; Leu, leucine; Str, streptomycin; Spc, spectinomycin. When used with these abbreviations, "−" stands for deficient, dependent, or nonutilizing, and "+" stands for proficient, independent, or utilizing. Superscript "s" stands for sensitive and superscript "r" stands for resistant.

The system of coordinates used on the genetic map of F has been described in detail (16, 24). In brief, locations are described in the unit of the kilobase (kb; 1,000 nucleotide base pairs) from an arbitrary origin at "0/94.5F" from which the units increase in a clockwise direction. To define a specific sequence, the end points of that sequence are listed in such a way that moving along the circular map of F in a clockwise direction from the first end point

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TABLE 1. *Bacterial strains used*

Strain	Plasmid	Genotype																		Reference[a]
		tra	*thr*	*ara*	*leu*	*tonA*	*tsx*	*proA*	*lac*	*gal*	*his*	*recA*	*argG*	*rpsL*	*rpsE*	*malA*	*xyl*	*mtl*	*pyrB*	Other	
JC7105	Hfr	+	+	+	+	+	+	+	I23	+	+	+	+	+	+	+	+	+	+	*serA6*; P01	11
JC7102	F⁻	−	1	14	6	+	33	2	Y1	K2	4	+	+	31	307	1	5	1	+		11
JC7103	F⁻	−	+	+	6	2	+	+	−	6	1	+	+	104	309	1	7	+	−	*metB1*	This paper
JC7107	F⁻	−	+	+	6	2	+	+	−	6	+	56	+	104	309	1	7	+	−	*metB1*	This paper
JC7108	F⁻	−	1	14	6	+	33	2	Y1	K2	+	56	+	31	307	1	5	1	+		This paper
JC7133	F⁻	−	+	+	6	2	315	+	Y1 or Z4	+	+	56	6	104	+	1	7	2	−		11
AT3155	F42-1	+	+	+	+	+	+	+	+/Δ175	+	+	+	+	−	+	+	+	+	+		16
EC1000	F42-114	+	+	+	+	+	+	+	+/−	+	+	+	+	+	+	+	+	+	+		
JC7247	pJC60	Δ446	+	+	6	2	315	+	Y1 or Z4	+	+	56	+/6	104	+	2	7	2	−		11
JC7249	pJC61	Δ447	+	+	6	2	315	+	Y1 or Z4	+	+	56	+/6	104	+	2	7	2	−		11
JC7250	pJC62	Δ448	+	+	6	2	315	+	Y1 or Z4	+	+	56	+/6	104	+	2	7	2	−		11
JC7251	pJC63	Δ449	+	+	6	2	315	+	Y1 or Z4	+	+	56	+/6	104	+	2	7	2	−		11
JC7253	pJC65	+	+	+	6	2	315	+	Y1 or Z4	+	+	56	+/6	104	+	2	7	2	−		11
JE513	F13-4	+	+	+	+	+	+	+	+/−	+	+	+	+	+	+	+	+	+	+	*purB*	16
W1655	FΔ(33-43)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*metB* λ[r]	2

[a] The derivations of strains isolated in this experiment are as follows: JC7108 is a His⁺ Rec [Sm[r]] recombinant isolated from a mating between JC5088 (3) and JC7102. DG291 is a *pyrB* mutant of JC411 (3) and was obtained from B. Wolff. JC7073 is a spontaneous Spc[r] mutant of DG291. JC7103 was isolated as an Arg⁺ [Spc[r]] recombinant from a mating between AB261 (3) and JC7073. JC7107 is a His⁺ Rec [Sm[r]] recombinant isolated from a mating between JC5088 and JC7103.

listed to the second will describe an arc representing the sequence.

Media. The compositions of L broth and buffer 56 (11) as well as tryptone broth (27) have been described.

Isolation and characterization of progeny from Hfr × Rec F⁻ matings. Progeny that inherited terminal donor markers were isolated from previously reported matings (11). All techniques for characterizing the donor ability and phage sensitivity of such isolates, their content of plasmid deoxyribonucleic acid (DNA), and the stability, acridine orange sensitivity, and incompatibility properties of their plasmids have been described (11).

Heteroduplex mapping. A 25-ml portion of an overnight culture of an F*argG*⁺ strain in arginine-free minimal medium was used to inoculate 2 liters of tryptone broth. The inocula for other strains were stationary-phase tryptone broth cultures. The cultures were inoculated at 37°C with aeration until the cells were in late log phase (optical density at 590 nm of about 0.7 to 0.8). Plasmid DNA was extracted from all strains as described (27; E. Ohtsubo, and M.-T. Hsu, manuscript in preparation). For heteroduplex analysis, DNAs from two different plasmids were X-ray nicked, mixed, denatured, renatured, and mounted for electron microscopy as described (27; Ohtsubo and Hsu, manuscript in preparation). Samples were examined in a Phillips 300 electron microscope. Lengths were measured with a Hewlett-Packard 9280A calculator interfaced with a Hewlett-Packard 9864A digitizer.

RESULTS

On the basis of genetic characterization, the *traΔ* F′ plasmids appear to be a uniform group of genetic elements whose properties depend only in a minor way on the particular Hfr strain from which they were derived. Those *traΔ* F′ plasmids derived from Hfr JC7105, an HfrH derivative, and from Hfr AB312 have been found to have almost identical properties (11); and *traΔ* plasmids derived from Hfr strains ED1009 (M. S. Guyer and A. J. Clark, submitted for publication), JC182, and B7 (unpublished data) also show a high degree of similarity with the more well-characterized isolates. Preliminary experiments showed that useful quantities of supercoiled DNA could much more easily be isolated from strains carrying F*argG*⁺ plasmids derived from AB312 than from strains derived from JC7105 that harbor F*leu*⁺ plasmids (unpublished observations; because of the distance between F and *leu* in HfrH, about 7 min, the F*leu*⁺ plasmids are expected to be very large and, consequently, fragile). Thus, the F*argG*⁺ plasmids were selected, as representatives of the *traΔ* mutants, for analysis. Four *traΔ* F*argG*⁺ plasmids and one *tra*⁺ F*argG*⁺ plasmid were characterized (Fig. 1, Table 2). All five plasmids were very large, ranging in size from 172 to 300 kb (Table 2); by comparison, F is 94.5 kb in size. In every case, a portion of the F genome was found to be absent from the F*argG*⁺ plasmids. Fifty to 60% of the sequences of the wild-type F plasmid are deleted from the *traΔ* mutants (Fig. 1, Table 2). The deleted region includes the sites of all of the mapped *tra* genes, confirming our earlier genetic analysis (11). The evidence upon which these conclusions are based is summarized in Fig. 2, which shows the structures of the heteroduplexes of a representative *traΔ* mutant, pJC61, with each of the reference plasmids used for analysis.

Heteroduplex analysis of pJC61. FΔ(33-43) is a deletion mutant of F; it is missing the sequence 32.6 to 42.9F (2). Figures 2a and 3 show that the pJC61/FΔ(33-43) heteroduplex consists of a single-stranded deletion loop, a duplex region, and one very large substitution loop. The deletion loop is 10.6 ± 0.6 kb (13 molecules) in size, which corresponds to the amount of DNA deleted from the reference plasmid. Since the deletion carried by FΔ(33-43)

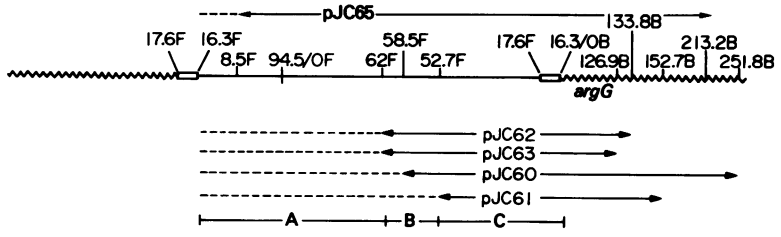

FIG. 1. *Diagram (not to scale) of the proposed structure of the Hfr AB312 chromosome in the region of the integrated F plasmid. F sequences are indicated by a solid line, and chromosomal sequences by a sawtooth line. Duplicated IS2 sequence at the F-chromosomal junction is represented by a box. Presence of only the right-hand copy of IS2 has been demonstrated; existence of the duplication is based upon the hypothesis of Davidson et al. (7) for the mechanism of Hfr formation. F sequences present in each of the plasmids described in the text are also indicated by solid lines bounded by arrow heads. Dashed lines represent the F sequences deleted from each plasmid. A, Region of deletion common to all of the traΔ mutants; C, region present in all of the traΔ plasmids; B, region present to different extents among the mutants.*

TABLE 2. *Physical characteristics of traΔ F' plasmids*

Plasmid	Genotype	Mol wt[a]	Coordinates of F sequences present[b]	Length of F sequences present (kb)[c]	Length of chromosomal sequence (kb)[d]
pJC60	*traΔ446*	295.5 ± 7.1 (12)	16.3-58.5	42.2	243.3
pJC61	*traΔ447*	188.5 ± 2.8 (12)	16.3-52.2	35.9	152.6
pJC62	*traΔ448*	180.7 ± 2.2 (14)	16.3-62.2	45.9	134.8
pJC63	*traΔ449*	172.6 ± 3.5 (15)	16.3-62.0	45.7	126.9
pJC65	*tra*$^+$	299.9 ± 8.9 (9)	16.3-8.5	86.7	213.2

[a] The molecular weight was determined by comparing the length of the plasmid with the length of a reference plasmid of comparable size and known molecular weight. The reference plasmids for the molecular weight determinations were FΔ(33-43) for pJC60, pJC61, and pJC65 and F152-1 (16) for pJC62 and pJC63. The number of molecules measured is indicated in parentheses.

[b] Determined by heteroduplex analysis. The coordinates of the end points of the sequences present are based upon a kilobase scale relative to an arbitrary origin at 0/94.5 on the map of F (7, 24). The convention used is described in the text.

[c] Calculated from the coordinates of the F sequences present on the plasmid.

[d] Calculated as the difference between the length of the plasmid and the length of the F sequences present.

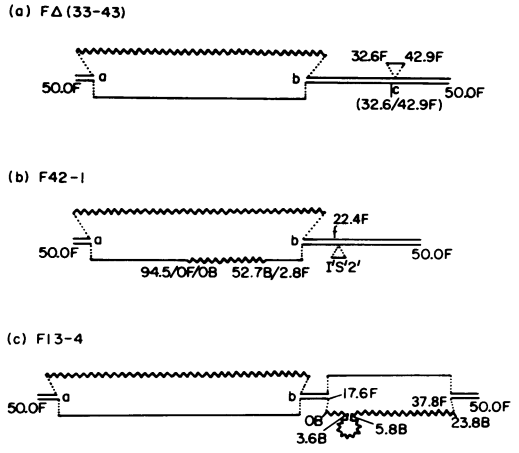

FIG. 2. *Diagrams of pJC61 heteroduplexes with each of the reference plasmids, (a) FΔ(33-43), (b) F42-1, and (c) F13-4. Circular structures are shown in linear form, with a break arbitrarily introduced at the point 50F. F sequences are represented with solid lines and E. coli chromosomal sequences with sawtooth lines. In each case, pJC61 is represented by the upper line and the reference plasmid by the lower line. The letters a,b,c, and f denote the same features on these diagrams as the same letters do in Fig. 3 and 4. The system of map coordinates is described in the text and in (16, 24). Lengths are not to scale.*

is the sequence 32.6 to 42.9F, the point at which the single-stranded loop emerges from the duplex can be identified as 32.6/42.9F. Two duplex arms extend from the 32.6/42.9F site; these are 9.8 ± 0.1 kb (ac, 12 molecules) and 16.8 ± 0.2 kb (bc, 12 molecules) in length. The duplex arms are composed of F sequences present on both pJC61 and FΔ(33-43). The sum of the lengths of these arms and the length of the deletion loop is 36.6 ± 0.4 kb. The smaller single-stranded arm of the substitution loop is 57.4 ± 0.4 kb (four molecules) in size. This is the size expected for the remaining sequences of FΔ(33-43). As this arm is uninterrupted by any other duplex region, there must be no sequences on pJC61 homologous to any portion of this region. Therefore, pJC61 contains a total of only 36.6 kb of F DNA (about 40% of the F genome) and this sequence is continuous on the map of F.

The forked structures at the ends of the duplex region in the pJC61/FΔ(33-43) heteroduplex represent the junctions between chromosomal sequences and F sequences in pJC61. The coordinates of these two sites cannot be determined from this heteroduplex alone because of the ambiguity involved in orienting a sequence on a circular molecule relative to a single reference feature. Relative to the deletion loop, the coordinates of the forks may be either 22.8F and 59.7F or 15.8F and 52.7F. The ambiguity was resolved by analysis of the heteroduplex of pJC61 with F42-1.

F42-1 is an F*lac*$^+$ plasmid that carries an insertion of IS2 (10, 15) at 22.4F (16). Figures 2b and 4 show that the insertion appears as a single-stranded loop 5.6 ± 0.2 kb (11 molecules) from one end of the duplex region of the pJC61/F42-1 heteroduplex. One of the end points of the *traΔ447* deletion carried by pJC61 is located therefore at approximately 16.8F. This conclusion is more consistent with the second of the interpretations of the pJC61/FΔ(33-43) heteroduplex, which places that end point at 15.8F. The other end point of *traΔ447* must then be between 52 and 53F.

The end point of the deletion in pJC61, which lies between 15.8 and 16.8F, probably repre-

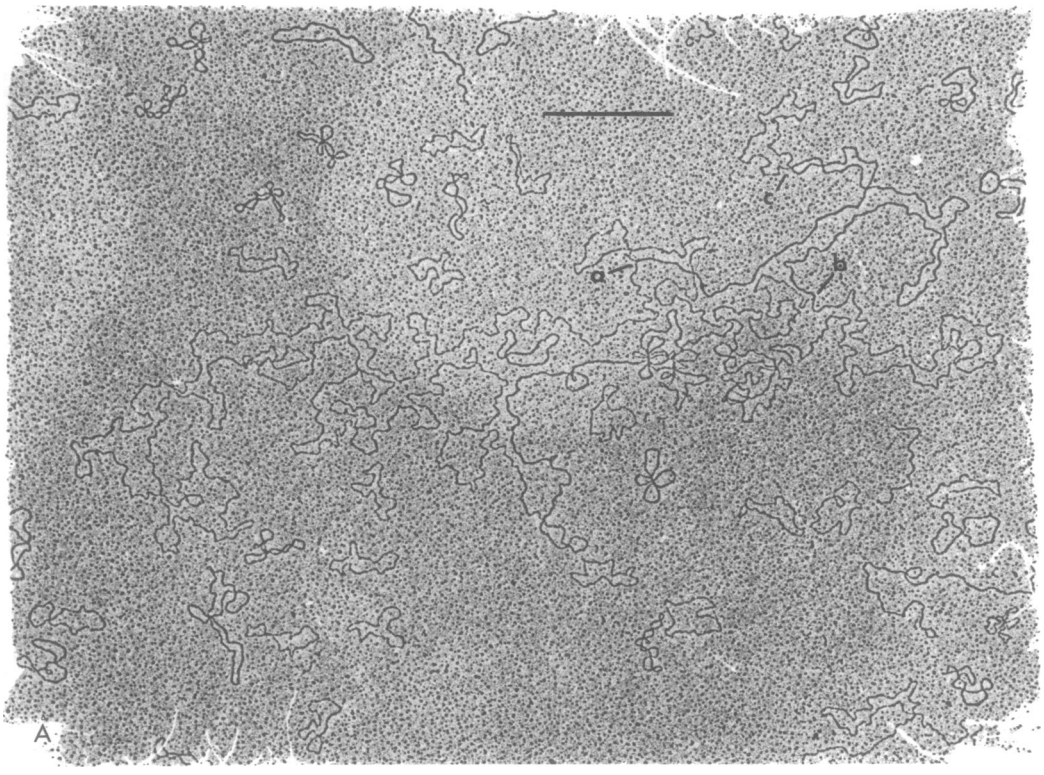

FIG. 3. *Electron micrograph of the pJC61/FΔ(33-43) heteroduplex. (A) Entire heteroduplex is shown; bar represents 3.1 kb of double-stranded DNA and 3.9 kb of single-stranded DNA. (B) Detail of the heteroduplex shown in (A); bar represents 5 kb of double-stranded DNA and 6 kb of single-stranded DNA. (C) Tracing of the duplex region. a and b (arrows), Junctions of the duplex region with each end of the long substitution loop; c, point on duplex (32.6/42.9F) at which deletion loop emerges.*

sents the integration site of F in the Hfr AB312 from which pJC61 was derived (Table 2 and below). An IS2 sequence located at 16.3 to 17.6F is known to function as an attachment site for Hfr formation (16). To map the end point of the deletion of pJC61 precisely, in order to compare it with the location of that IS2 sequence, the heteroduplex pJC61/F13-4 was examined.

F13-4 is an F*lac*$^+$ plasmid that contains a substitution of 23.8 kb of bacterial DNA for the F sequences 17.6 to 37.8F (16). The bacterial DNA contains a small inverted repetition (termed jj') that forms an inversion loop located 3.6 kb from the 17.6 site on F. Figure 2c shows that the pJC61/F13-4 heteroduplex shows a duplex region, 1.2 ± 0.2 kb (four molecules) in length, which is 3.0 ± 0.1 kb from the inversion loop. This duplex region is the sequence 16.3 to 17.6F, which therefore must be carried by pJC61. Therefore, the end point of *traΔ447* in question occurs at 16.3F, and the IS2 sequence of F forms the boundary between F and chromosomal sequences in this plasmid.

Heteroduplex analysis of other F*argG*$^+$ plasmids. The structure of the heteroduplex of each of the other *traΔ* F*argG*$^+$ plasmids with FΔ(33-43) is qualitatively similar to that of pJC61/FΔ(33-43). These results indicate that the other *traΔ* mutants have suffered deletions of 50 to 55% of the F genome (Table 2) and confirm the hypothesis that the *traΔ* F′ plasmids carry large deletions of F. In contrast, the *tra*$^+$ plasmid pJC65 contains 92% of the wild-type F sequences (Table 2).

Heteroduplexes between FΔ(33-43) and the three other *traΔ* F*arg*$^+$ plasmids each have one duplex arm of the same size as that which extends to 16.3F in pJC61/FΔ(33-43) (Fig. 2a, arm bc). Therefore, we conclude that the deletions in all four *traΔ* F′ mutants have a common end point at 16.3F. 16.3F is also a junction between plasmid and chromosomal sequences in pJC65 (Table 2). Because the F-chromosomal junction at 16.3F is fixed in several F′ plasmids derived from Hfr AB312 and because *argG* is transferred early by this donor, we conclude

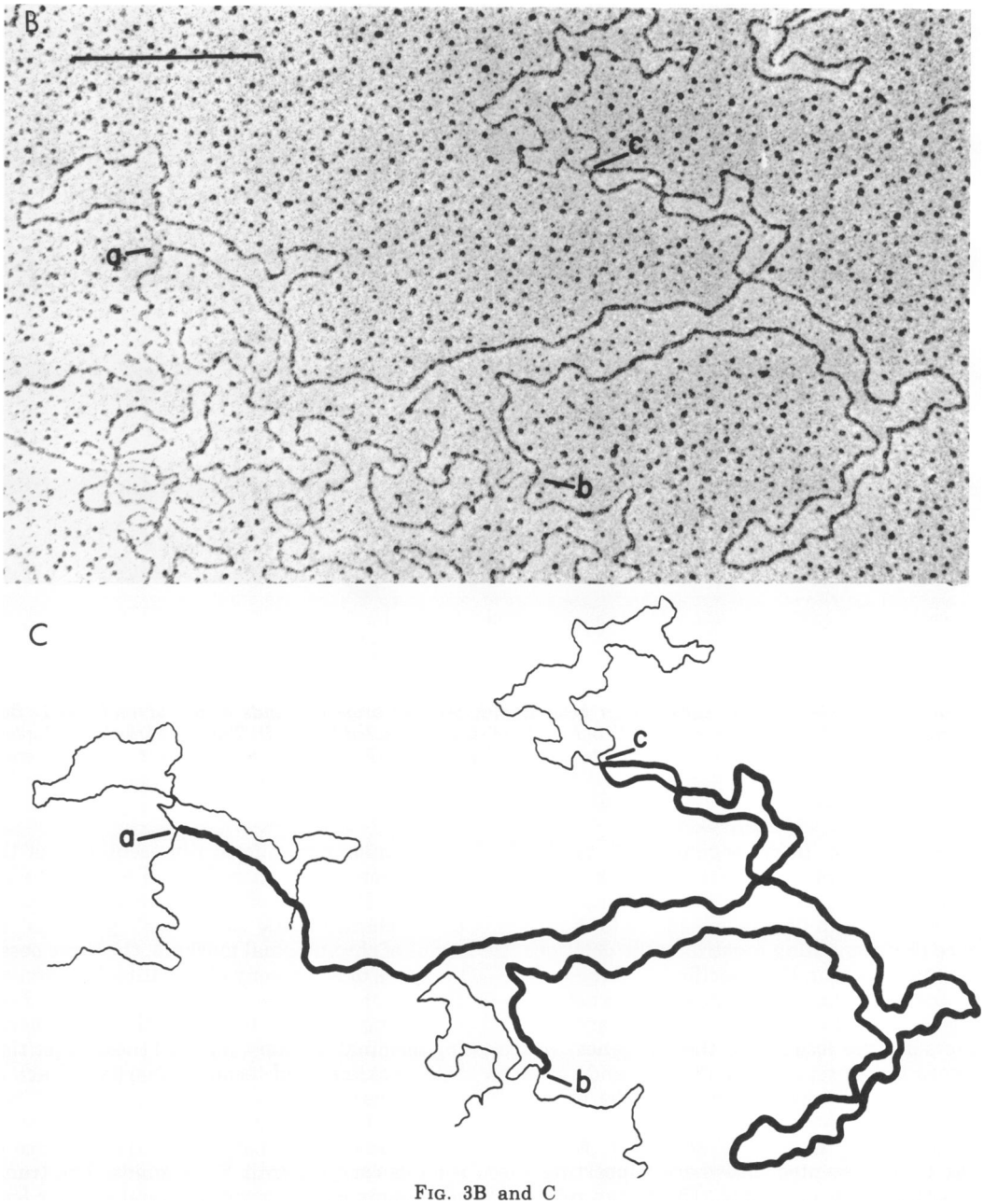

FIG. 3B and C

that IS2 is probably the sequence at which recombination between F and the *E. coli* chromosome occurred to produce the Hfr AB312. This conclusion is consistent with the hypothesis of Davidson et al. (7) that IS sequences are the sites of integrative recombination between F and *E. coli* chromosome.

Implications of the heteroduplex analysis. From the above evidence, we can draw a map of the chromosome of AB312 in the vicinity of the integrated F factor (Fig. 1). The sequences of the F genome retained by each of the *traΔ* plasmids as well as the sequences deleted are indicated. Three regions are obvious. All the plasmids have lost the sequence 62.2 to 16.3F; this is the common deleted region. All the mutants retain the sequence 16.3 to 52.7F; this is the common region retained. There remains an

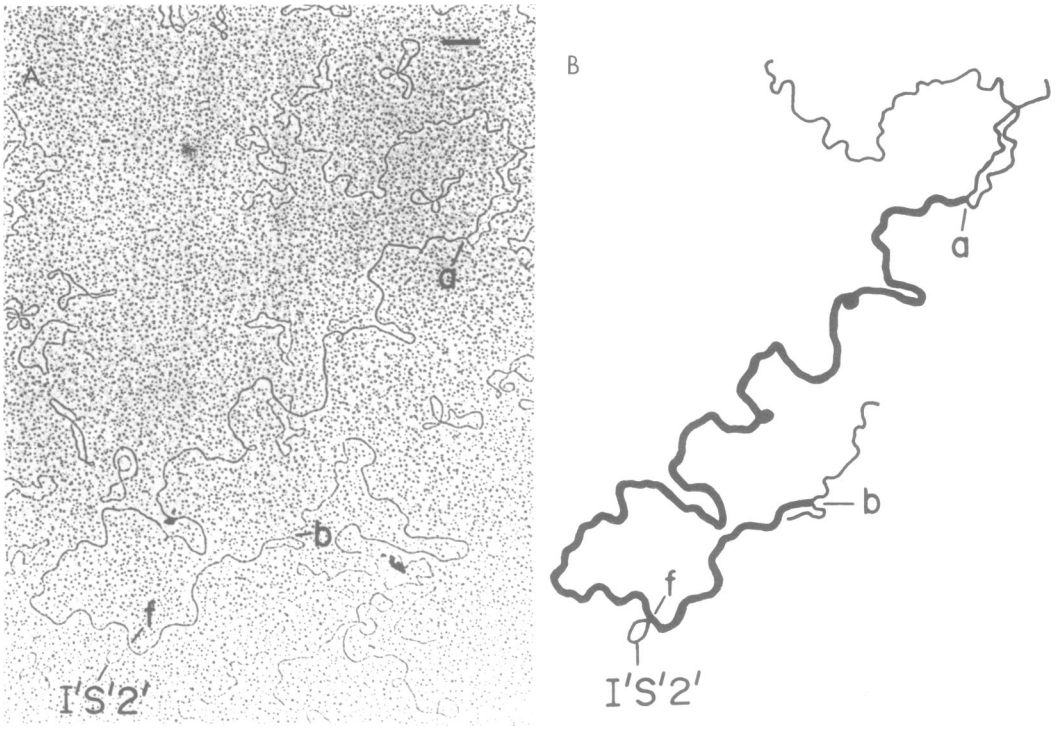

FIG. 4. *(A) Electron micrograph of a heteroduplex between broken strands of pJC61 and F42-1. Bar represents 1 kb of double-stranded DNA and 1.2 kb of single-stranded DNA. (B) Tracing of the heteroduplex. a and b, Junctions at the ends of the duplex region; f, point (22.4F) at which the IS2 insertion loop emerges from the duplex. The inserted sequence is denoted as I'S'2' because the orientation of that sequence in F42-1 is opposite that of the IS2 sequence of F (16).*

approximately 10-kb region, 52.7 to 62.2F, which is present to a greater or lesser extent in individual *traΔ* mutants. This organization of the several *traΔ* deletions leads us to the inference that something constrains the deletions to terminate within this specific 10-kb region. We propose that the boundary conditions determining the extent of the *traΔ* deletions are, on the one side, the location of the *frp* genes, which determine F replication (7, 28), and, on the other side, the location of *oriT*, the origin of transfer replication.

Asymmetry in *traΔ* F′ structure. Evidence has been presented elsewhere supporting the hypothesis just presented. The region retained by all of the *traΔ* F′ plasmids includes the sequence 42.9 to 49.3F, which is where the genes essential to F replication have been mapped (12, 28). One of these genes has been shown to be transferred proximally by an Hfr donor (Guyer and Clark, submitted for publication). Conversely, at least a portion of the F region that is absent from all of the *traΔ* F′ plasmids (the *tra* region) is transferred distally by Hfr donors (4; Guyer and Clark, submitted for publication).

A similar nonuniform representation of the chromosomal sequences located around the integrated F genome of an Hfr can also be shown to be characteristic of *traΔ* F′ plasmids. In terms of chromosomal markers, the three possible types of F′ progeny of a mating between an Hfr donor and a *recA* recipient are: (i) those inheriting proximal markers; (ii) those inheriting terminal markers; and (iii) those inheriting both proximal and terminal markers. Each of these classes was recovered from each of two matings between Hfr JC7105 and the Rec F⁻ JC7133 and were analyzed for the presence of isolates carrying *traΔ* F′ plasmids. The transconjugants carrying the proximal marker *leu*⁺ have been described (11). The terminal marker used for selection was *pyrB*⁺, which determines a Ura⁺ phenotype.

A total of 75 Ura⁺ [Str^r] progeny, which had inherited the *pyrB*⁺ allele from JC7105, were recovered from the two matings. Fifty-one of these (68%) were Tra⁺, being able to act as donors of Ura⁺ equally well to both Rec and Rec⁺ recipients. They must each carry a *tra*⁺ F*pyrB*⁺. The properties of a representative isolate, JC7185, are shown in Table 3.

TABLE 3. *Properties of representative transconjugants from mating between JC7105 and JC7133*

Strain	Original selection	Tra phenotype	Donor ability[a] (% transconjugants)				Phage response[b]			Stability[c]				Compatibility[d] (% Lac⁺ Ura⁺)	Plasmid DNA[e]	Plasmid no.	*tra* genotype
			Leu⁺		Ura⁺		Male specific		Female specific	Leu colonies		Ura colonies					
			recA⁺ F⁻	*recA* F⁻	*recA*⁺ F⁻	*recA* F⁻	f2	fd	ΦII	+AO[f]	−AO	+AO	−AO				
JC7185	Ura⁺[Str^r]	+	−	−	3×10^{-1}	2×10^{-1}	s	s	r	−	−	>98	<2	<1	+	pJC90	*tra*⁺
JC7299	Ura⁺[Str^r]	−	−	−	$<6 \times 10^{-5}$	$<6 \times 10^{-5}$	r	r	s	−	−	<2	<2	>99	−	None	−
JC7193	Leu⁺[Str^r]	−	$<10^{-3}$	$<10^{-3}$	−	−	r	r	sr	>98	2	−	−	NT[g]	+	pJC30	*traΔ423*
JC7293	Leu⁺[Str^r]	−	$<10^{-4}$	$<10^{-4}$	−	−	r	r	s	<2	<2	−	−	NT	−	None	−
JC7196	Leu⁺Ura⁺[Str^r]	+	3×10^{1}	10^{1}	5×10^{1}	10^{1}	s	s	r	>98	<2	>98	<2	<1	NT	pJC91	*tra*⁺

[a] The number of Leu⁺ [Arg⁺ Spc^r] colonies per 100 donor cells or the number of Ura⁺ [Spc^r] colonies per donor cells. The *leu recA*⁺ recipient was JC7102; the *leu recA* strain was JC7108; the *pyrB recA*⁺ recipient was JC7103; the *pyrB recA* recipient was JC7107.

[b] The phage response was assayed by plaque formation. s, Plaques were formed at the same frequency as on the positive standard; r, no plaques were observed. In the case of ΦII, sr indicates that the number of plaques formed was 2- to 100-fold fewer than on the F⁻ standard, but 10- to 50-fold higher than on the F-containing standard and that the plaques were intermediate in size between those on the two standards (11).

[c] The percentage of colonies auxotrophic for the indicated nutrient after growth under nonselective conditions.

[d] The percentage of Lac⁺ Ura⁺ colonies among Lac⁺ colonies recovered from a mating between an F42 donor and a Ura⁺ recipient.

[e] +, Presence of a satellite peak in a Sarkosyl extract, as determined by dye-buoyant density centrifugation.

[f] AO, Acridine orange.

[g] NT, Not tested.

The remaining 24 Tra Ura$^+$ [Str^r] isolates were analyzed in several ways for the presence of *traΔ* F*pyrB*$^+$ plasmids. The behavior of a typical isolate, JC7299, is shown in Table 3. In addition to being Tra, JC7299 is f2^r, fd^r, and ΦII^s. The Ura$^+$ character of JC7299 is fully stable under nonselective conditions (growth in complex medium), during growth in the presence of acridine orange, or when the inheritance of F42 is selected (Table 3). Furthermore, JC7299 did not contain any (<0.8%) supercoiled DNA (Fig. 5). These properties are those of an F$^-$ strain, such as JC7293 (Table 3), rather than the properties of a strain containing a *traΔ* F*leu*$^+$ plasmid, such as JC7193 (Table 3). The other 23 Tra Ura$^+$ [Str^r] isolates had the same properties as JC7299 and were also F$^-$. Thus, 68% of the progeny that had inherited a terminal Hfr marker carried *tra*$^+$ plasmids, and 32% carried no plasmid at all. None (<1.3%) carried a *traΔ* F*pyrB*$^+$ plasmid.

Four isolates that had inherited both proximal and terminal donor markers (*pyrB*$^+$ *leu*$^+$) were recovered from the two matings between JC7105 and JC7133. All four of these isolates were Tra$^+$, and the properties of a representative, JC7196, are included in Table 3.

Table 4 summarizes the results of the cross between JC7105 and JC7133. The frequency of progeny inheriting a proximal marker was nine times the frequency of progeny inheriting a terminal marker. This difference was attributable solely to the occurrence of *traΔ* plasmids among the proximal marker-inheriting progeny and their absence from the progeny inheriting terminal markers. Thus, it appears that the formation of *traΔ* plasmids is completely asymmetrical and is similar to the asymmetry evident in the unidirectional transfer from Hfr cells.

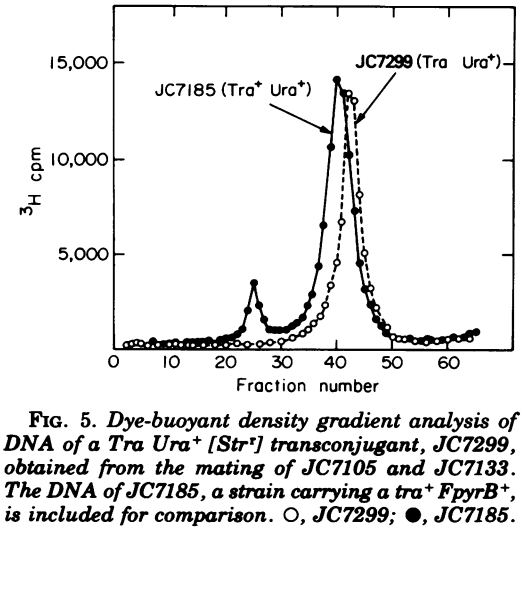

FIG. 5. *Dye-buoyant density gradient analysis of DNA of a Tra Ura$^+$ [Str^r] transconjugant, JC7299, obtained from the mating of JC7105 and JC7133. The DNA of JC7185, a strain carrying a tra$^+$ FpyrB$^+$, is included for comparison.* ○, *JC7299;* ●, *JC7185.*

TABLE 4. *Frequency of tra$^+$ and traΔ plasmids among F′ transconjugants from matings between JC7105 and JC7133 as a function of the marker selected*

Selected marker	Transconjugant frequency[a]	No. of F′ transconjugants tested	% which were:	
			tra$^+$	*tra*
leu$^+$	4.1×10^{-5}	37	3	97
pyrB$^+$	4.9×10^{-6}	51	100	<2
leu$^+$ *pyrB*$^+$	6×10^{-7}	4	100	<25

[a] Number of transconjugants per 100 Hfr donor cells; average of two experiments.

DISCUSSION

Physical analysis of *traΔ* F′ plasmids has confirmed the hypothesis that these mutants have suffered extensive deletions of F sequences. When the sequences deleted from, and those retained by, four *traΔ* F*arg*$^+$ plasmids are compared with F, it is clear that the *traΔ* deletions have one common end point, 16.3F, and one variable end point. The shortest deletion (62.2 to 16.3F, *traΔ448* in pJC62) removes the functions of all the *tra* genes tested (11) as well as those of *finP* and *traO* (32; Skurray et al., *in* Proc. Second Int. Pili Conference, in press). Clark et al. (6) have found that the *Eco*RI fragment f6 of F carries the genes *traJ, A, L, E, K,* and *B*. Fragment f6 maps between 62.2 and 68.1F. Taken together with the coordinates of *traΔ448*, these results indicate that those six *tra* genes, as well as *finP* and *traO*, map between 62.2 and 68.1F.

There is one other gene that also must be located in the 62.2 to 68.1 interval, the gene whose absence is responsible for the *cis* dominance of the Tra phenotype of the *traΔ* mutations. Willetts (31) has defined a gene *ori* as the site on F at which transfer is initiated. He has hypothesized that the *cis* dominance of certain *tra* deletions of an Hfr chromosome is due to the deletion of *ori*. This gene has been termed *oriT* to differentiate it from the origin of the vegetative replication of F, which in turn has been called *oriV* (6). Since strains carrying the *traΔ* plasmid pJC62 display a *cis*-dominant Tra phenotype (11) similar to that of Willetts' Hfr deletion strains (31), we can propose that the deletion in pJC62 has also removed *oriT*. For further discussion, we will assume this is true, although it cannot be ruled out at present that the *cis*-dominant Tra phenotype stems from the deletion of a gene that determines a *cis*-acting protein (11).

The other *traΔ* plasmids analyzed all have deletions longer than that carried by pJC62. The longest deletion begins at 52.7 kb, the next longest at 58.5 kb, and the third is at 62.0 kb, indistinguishably close to the end point in pJC62. All of these deletions also remove the *tra* genes and *oriT*. The region retained by the four *traΔ* plasmids includes the sequence 40.3 to 49.3F. This sequence probably contains the *frp* genes (12, 28), *oriV* (9), and the F genes responsible for incompatibility (25). Finally, *traΔ* plasmids are found exclusively among those F′ progeny of a *recA* F⁻ by Hfr cross that inherit a proximal donor marker and not among the F′ progeny that inherit a terminal marker. The asymmetrical distribution of both chromosomal and F sequences in the *traΔ* F′ plasmids, as well as the deletion of a functional *oriT* locus, can be accounted for by a single hypothesis to explain the origin of these mutant plasmids.

This hypothesis was originally outlined by Novick (22). It holds that F′ plasmids can be generated by the repliconation of the conjugational exogenote in a *recA* recipient. The exogenote is formed by transfer of the Hfr chromosome. Transfer is assumed to begin within the integrated F element at *oriT* and progress unidirectionally toward the host chromosomal sequences. Since the *frp* genes are transferred early (Guyer and Clark, submitted for publication), they would be included in the exogenote. Subsequent inclusion of *frp* in a circular DNA molecule formed by recombination would permit that molecule to replicate and segregate as a plasmid. The extent of any deletion formed by this mechanism alone is strictly limited. No deletion could be any longer than from about 49.3F to *att* because a more extensive deletion would remove part of the *frp* genes required for plasmid maintenance. Similarly, no deletion could be any shorter than about 62.2F to *att* because DNA transfer begins at *oriT* (at or near 62.2F) and proceeds unidirectionally, leaving the region 62.2F to *att* to be transferred terminally.

We have considered many hypotheses other than that of Novick's that can be offered to explain the formation of the *traΔ* plasmids. Basically, these fall into two groups: (i) *traΔ* F′ plasmids are formed by aberrant excision in Hfr cells (29) in a manner similar to the formation of *tra*⁺ F′ plasmids (5, 26) and are transferred to recipients preformed; and (ii) *traΔ* plasmids are formed by deletions of sequences from *tra*⁺ F′ plasmids that have been transferred to *recA* cells. Given the *cis* dominance of the *traΔ* mutations, the first class of hypotheses would require an unreasonably high frequency of occurrence of *traΔ* F′ plasmids in the Hfr population in order to account for the frequency of transconjugants carrying *traΔ* F′ mutants obtained from the Hfr × *recA* F⁻ mating. The frequency with which each of the characterized *traΔ* F′ mutants is transferred from transient *tra*⁺/*traΔ* heterozygotes is $<10^{-3}$ per heterozygote cell (11; this figure includes any available recombinational modes of transmission). The frequency with which the *traΔ* mutants are recovered from Hfr × Rec F⁻ matings is about 10^{-6} per Hfr cell. Therefore, the frequency of Hfr cells in the donor populations tested that contained *traΔ* F′ would have to be about 1 per 10^3 cells. In comparison, the transfer frequency of *tra*⁺ F′ plasmids is about 0.1 to 1 per cell, whereas the frequency with which *tra*⁺ plasmids were recovered from the same mating mixtures as the *traΔ* plasmids was about 10^{-7} per Hfr cell. Therefore, the frequency of Hfr cells in the same donor population containing *tra*⁺ F′ plasmids was about 1 per 10^6 to 10^7 cells. It is implausible to expect that there would be 10^3 to 10^4 as many excision events leading to the formation of *traΔ* F′ plasmids as there are similar excisions that result in the formation of *tra*⁺ F′ plasmids. The second class of hypotheses also seems unsatisfactory because we were unable to produce any evidence for the production of *traΔ* mutations in plasmids carrying terminal, or terminal and proximal, markers that had been transferred to *recA* cells. Such F′ plasmids carrying terminal markers are thought to be formed by excision in the donor cells prior to their transfer (17). We conclude that the *traΔ* F′ plasmids are not generated, directly or indirectly, by excision from the Hfr chromosome by the mechanism proposed by Campbell (5).

We are left then with the hypothesis that recircularization of the exogenote by illegitimate recombination is the mechanism of plasmid formation. It is likely that this repliconation event occurs in the zygote after DNA transfer as originally proposed (22). Another possibility is that recircularization occurs in the donor cell sometime after the initiation of conjugation. This seems less likely, however, since all *cis*-acting events required for transfer would have to be completed prior to plasmid formation.

The only thing not explained specifically by the Novick hypothesis is the variability of one end point of the *traΔ* deletions. According to the hypothesis, one end point would be fixed for any Hfr source of *traΔ* plasmids by the attachment site on F used in the integration event that produced that Hfr. The other end point might also be expected to be fixed at *oriT*, but this was not observed. At present we are con-

sidering three explanations for the variability: (i) the recombination producing the circular plasmid occurs between different sequences on the exogenote; (ii) a variable amount of degradation occurs at the 5′ end of the exogenote prior to repliconation; and (iii) *oriT* is actually only a recognition site for an endonuclease that can then cleave F at different sites. An analogy for the third alternative is the class of restriction endonucleases (type I) that recognize a specific DNA sequence but introduce breaks at other, random sites nearby (21). An analogy also exists for either of the first two possibilities, namely, the ability of linear plasmid DNA to repliconate after transformation (13).

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