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Plasmid Cointegrates of F*lac* and Lambda Prophage

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Fifteen cointegrates of the plasmid F*lac* and prophage lambda that had suffered no detectable change in plasmid phenotype were isolated and characterized. The locations of the prophage insertions were determined by genetic analysis of deletion mutants obtained from each cointegrate as survivors of growth at 42°C. In 11 cointegrates, the prophage was inserted between *traI* and *lac*, although probably in more than one location; in 3 others, it was on one side or the other of *lac*; and in 1 it was between *lac* and *pif*. Deletions covering all or part of the transfer region, as well as of *lac* and of *pif*, were obtained in the course of this analysis. Deletion mutants that had lost all known transfer genes were also *oriT*, but they retained the capacity to recircularize after transfer. Attempts were made to isolate lambda transducing phages for nearby plasmid genes from the cointegrates, and λp*traGD*, λp*traD*, λp*traI*, and λd*traDI* phages were obtained.

Genetic and physical studies of the *Escherichia coli* K-12 sex factor F have identified numerous functions specified by this plasmid, the two major ones being the capacities for autonomous replication and for conjugal transfer to a recipient cell. The replication region is relatively small, ≤9 kilobases (kb; 1 kb = 1,000 base pairs of DNA), even though this contains all the genes necessary for autonomous replication and its control and for incompatibility, as well as the origin of vegetative replication, *oriV* (21, 29).

In contrast, about 30 kb of DNA, equivalent to one-third of the F molecule, is required for conjugal transfer. The major part of this is taken up by the *tra* cistrons that are concerned with pilus formation, surface exclusion, and DNA metabolism during conjugation (15, 19). The conjugation system that the *tra* genes determine initiates transfer from an origin (*oriT*) that is distinct in location and function from *oriV* (27, 33). Expression of the transfer genes is controlled in two stages by two further F genes, *traJ* and *finP* (11, 12, 35), which are located near *oriT* (19, 32). F lacks a *finO* gene such as is present on most other F-like plasmids.

Other functions specified by F include immunity to lethal zygosis, *ilz* (28), and inhibition of the growth of female-specific bacteriophages, *pif* (5, 6). An additional feature of F, important with regard to its recombinational abilities, is the presence of several insertion sequences (17, 18).

As a further tool for the genetic and physical analysis of F, we have used a modified version (8) of the technique of Shimada et al. (26) to isolate cointegrates of F*lac* and prophage lambda. Such cointegrates are useful in several ways: first, for the physical location of plasmid genes into which the prophage is inserted, via electron microscope heteroduplex analysis; second, for isolating F deletion mutants as survivors to induction of the prophage; and third, for cloning DNA fragments containing F genes and sites of interest on λ transducing phages.

We report here the isolation and characterization of F*lac*(λ) cointegrates that have suffered no phenotypic change and the deletion mutants and transducing phages derived from them. Transfer-defective F*lac*(λ) cointegrates will be described in a separate publication.

MATERIALS AND METHODS

Bacterial strains and plasmids. The F*lac* plasmid is JCFLO (2). F100 is an F*gal* plasmid (30), and F57 is an F*his* plasmid (3). The suppressible and nonsuppressible F*lac tra* mutants used in complementation tests have been described (3, 32). Phage insertions were generated in ED2160, a JCFLO derivative of the *att*λ-deleted strain ED2149. The derivations of ED2149, as well as those of ED2144, ED2145, ED395, and ED3814, were given by Dempsey and Willetts (8). ED24, JC3272, JC6256, and JC6255 have been described elsewhere (3, 11). JC6310 is a *lys*$^+$ *recA56* derivative of JC3272; JC6455 is a Gal$^-$ derivative of JC5455 (3); and M174 is a Gal$^-$ derivative of JC6256.

Phage strains. The λ strain inserted into F*lac* was EDλ4, λ*cI857Sam7b515b519*. Construction of EDλ4, hereafter called λ, has been described (8). Lysogens were selected with a mixture of λ*b2c*$^-$ and λ*h80c*$^-$Δ9, both obtained from R. A. Weisberg via J. Gross (26). Stocks of EDλ4, λ*b2c*$^-$, and λ*h80c*$^-$Δ9 were made by a confluent plate lysis technique as described (8).

Media. Most of the media have been described

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previously (8). Giemsa indicator agar (22; R. Hull, personal communication) contained, per liter: 10 g of tryptone, 10 g of yeast extract, 8 g of NaCl, and 15 g of Difco agar. After sterilization by autoclaving, the following were added: 4 ml of 0.5 M $CaCl_2$, 50 ml of 20% glucose, and 12.5 ml of Giemsa "R66" (George T. Gurr Ltd., London).

Phage methodology. The procedures of Shimada et al. (26) were followed for determining burst sizes, heat-pulse curing, and spontaneous curing frequencies of the lysogens, except that lactose-tetrazolium medium was used and the lysogens were not made λ*vir* resistant.

The male-specific phages, f1, f2, Qβ, and μ2 and the female-specific phage φII were laboratory stocks. Lysates were made on appropriate F^+ or F^- strains by a confluent plate lysis technique, using LC top agar (31) and Oxoid nutrient agar plates. Response to these phages was determined by spotting 0.01 ml of a lysate containing approximately 10^6 plaque-forming units per ml on LC top agar lawns of strains to be tested.

For screening large numbers of colonies for response to μ2 or φII, single colonies were patched on Oxoid medium, incubated for 6 to 8 h, and replica plated to lawns of phage (10^8 to 10^{10} plaque-forming units per plate) on Giemsa medium. After overnight incubation, sensitive patches were a deep purple-red color and resistant patches were a light mauve color.

Isolation of lysogens. The method of Shimada et al. (26) was adapted as described by Dempsey and Willetts (8) with the following modifications: 0.1 ml of λ-treated culture of ED2160 was plated at 10^{-2} and 10^{-3} dilutions with 10^9 plaque-forming units of λ*b2c*$^-$ and 10^9 plaque-forming units of λ*h80c*$^-$Δ9 on lactose-tetrazolium agar to select λ lysogens. The plates were incubated at 33°C.

The next day, 200 Lac^+ colonies from each original clone were patched onto Oxoid nutrient agar and incubated for 8 to 10 h at 33°C. The nutrient plates were replica-plate mated (7) with $Lac^-\lambda^r\phi80^rStr^r$ λ-lysogenic (ED2145) and nonlysogenic (ED2144) strains to identify clones carrying F*lac*(λ) cointegrates (see Results).

Isolation of deletions. Single colonies of each F*lac*(λ) cointegrate were inoculated into L broth for overnight growth at 33°C. Portions of 0.1 ml of each overnight culture were spread on lactose-tetrazolium medium, and the plates were incubated overnight at 42°C. The *Sam*7 mutation in EDλ4 prevents lysis of the induced cells and consequently minimizes the isolation of λ^r survivors.

In the first instance, ten Lac^+ survivors from each original clone were patched onto Oxoid nutrient agar, incubated for 6 to 8 h at 37°C, and replica-plate mated to ED2145 to identify transfer-deficient survivors.

In later experiments, both Lac^+ and Lac^- 42°C-resistant survivors were patched and examined, the Lac^+ survivors for transfer of F*lac* to ED2145, and both Lac^+ and Lac^- derivatives for their response to phage μ2 on Giemsa plates. Tests for sensitivity to φII were also carried out on Giemsa plates, although the results were not so reproducible as with phage μ2. All mutant phage response phenotypes were confirmed by spot tests as described above.

Mating procedures. Quantitative donor ability was measured as described (8). Donor ability is expressed as the number of Lac^+ progeny per 100 Lac^+ donor cells (determined by plating dilutions of the donor cultures on lactose-tetrazolium agar).

The Tra phenotype of Lac^- male-specific phage-sensitive deletion plasmids was tested by mixing 1 ml of an exponential-phase culture with 1 ml of a similar culture of JC3272 (Str^r) and incubating at 37°C for 60 min. Dilutions were plated on minimal medium containing streptomycin to contraselect the donor strains. Twenty to fifty of the resultant Str^r colonies were patched on Oxoid medium and replicated to Giemsa plates spread with μ2 as described above. Under these conditions, 10 to 50% of the Str^r colonies had received a Tra^+ Lac^- plasmid, making them sensitive to μ2.

Semiquantitative conjugational complementation tests using a microtiter system were carried out as described previously (1). Quantitative complementation tests at 37°C were as described (11) except that the F*lac tra* plasmid-carrying strain being tested was the intermediate host, and 0.6 ml of L broth was added following T6 treatment. The donor ability of the resultant heterozygous cells was then measured by adding 1.0 ml of an exponential culture of the Lac^- Str^r strain JC3272 and incubating for 40 min. Dilutions were plated selecting Lac^+ [Str^r] transconjugants. Appropriate controls, using strains carrying JCFL201 (F*lac* with an amber-suppressible *lacZ* mutation; 3), or broth, in place of donor or intermediate F*lac tra* cultures, were always included.

Transductional complementation tests, using non-suppressible F*lac traL* and *traK* donors, were carried out using phage P1*vir* as has been described (19, 31).

Test for *oriT*. The method used to determine whether Tra^- deletion plasmids carried the origin of transfer (*oriT*) was based on the procedure of Willetts (33). A 0.1-ml volume of an exponential culture of the F100 derivative of M174 ($T6^s$ Str^s) was mixed with 0.1 ml of a 1:5 dilution in L broth of a stationary-phase culture of JC6310 ($RecA^-$ $T6^r$ Str^r) carrying the Tra^- deletion plasmid at 37°C for 40 min. The donor cells were killed by the addition of 0.2 ml of T6 (10^{11} particles per ml, treated with 3,000 ergs of UV light per mm^2) and incubation for 20 min. Then, following the addition of 0.6 ml of L broth, the mixture was incubated for 40 min to allow expression of the F*lac* and F100 transfer genes in the intermediate strain. A 0.1-ml volume of a 10^{-4} dilution was spread in duplicate on lactose-tetrazolium plates containing streptomycin; heterozygous (F*lac* F*gal*) cells gave sectored colonies. The culture was also mated for 40 min with an equal volume of an exponential culture of the Lac^- Gal^- spectinomycin (Spc)-resistant strain JC6455. Dilutions were plated on media selective for either Lac^+ [Spc^r] or Gal^+ [Spc^r] transconjugants. Transfer was expressed as the number of Lac^+ or Gal^+ progeny per 100 (F*lac* F*gal*)$^+$ heterozygotes.

Test for recircularization ability. The mating procedure was the same as that for the *oriT* test described above except that (i) the primary donor was an F57 (F*his*) derivative of JC6256 ($T6^s$); (ii) cultures of the deletion plasmids, in ED2149 ($T6^r$ Str^s), were diluted 1:10 in L broth before use; (iii) quantitation of the number of (F*lac* F*his*)$^+$ heterozygotes was by spreading on lactose-tetrazolium agar; and (iv) two

final recipients were used, JC3272 (Lac⁻ Str^r His⁻ RecA⁺) and JC6310 (Lac⁻ Str^r His⁻ RecA⁻). Dilutions were plated on medium selective for Lac⁺ [Str^r] transconjugants. Transfer was expressed as the number of Lac⁺ progeny per 100 (*Flac Fhis*)⁺ heterozygotes. The Lac⁺ RecA⁻ progeny were examined by replica-plating techniques for the presence of the *his*⁺ marker of F57 and for their ability to transfer *lac*⁺ to ED24 (Lac⁻ Spc^r).

RESULTS

Detection of F*lac*(λ) cointegrates. Lysogenic survivors of the F*lac* derivative of ED2149 (Δ*att*λ) treated with λ and challenged with λ*b2c*⁻ and λ*h80c*⁻Δ9 could have λ integrated either into F*lac* or into the bacterial chromosome. Our procedure for detecting the F*lac*(λ) cointegrates among the whole population of lysogens was based on the phenomenon of zygotic induction, in which an incoming prophage is induced, killing the cell, in a mating with a nonlysogenic recipient (20). Single colonies of the presumptive lysogens were patched and replica-plate mated with nonlysogenic and lysogenic Lac⁻ recipients, expecting that F*lac*(λ) cointegrates would apparently transfer *lac*⁺ only to the lysogenic recipient. Chromosomal lysogens should transfer F*lac* to both recipients. This mating procedure allowed a further distinction to be made, since lysogens with λ inserted into a gene required for transfer should transfer to neither recipient.

By this technique, the frequency of lysogens with λ inserted into F*lac* was 16 out of 3,000 lysogens examined (0.5%). Of the 16 F*lac*(λ) strains, 15 were Tra⁺ and one was Tra⁻. This Tra⁻ strain and others isolated subsequently with a male-specific phage selection procedure will be discussed in a separate publication. No insertions into *finP* were found, and attempts to select for these as high-level donors after lysogenization in the presence of a *finO*⁺ R plasmid were unsuccessful.

All the transfer-proficient F*lac*(λ) strains were temperature sensitive, as expected, due to the λ*c*I857 mutation. The cointegrates were further characterized as shown in Table 1. In quantitative donor ability tests, their transfer to the nonlysogenic strain ED2144 was greatly reduced because of zygotic induction and the consequent death of the transconjugant cells, whereas transfer to the lysogenic strain ED2145 was unimpaired.

The burst sizes separated the cointegrates into two groups, those liberating less than 1 phage per cell and those liberating 200 to 400 phages per cell. Based on the original observations of Shimada et al. (26) and the correlation of burst size with molecular weight for R100 (λ) cointegrates (8), these two classes represent single and multiple lysogens, respectively.

As with abnormal chromosomal λ integration (26), the frequencies of spontaneous curing and heat-pulse curing of the F*lac*(λ) cointegrates varied over a wide range. In each case, however, the values for heat-pulse curing were lower than

TABLE 1. *Some properties of Flac(λ) cointegrates*

Plasmid no.	Donor ability (%)[a] to:		Burst size (phage/cell)	Spontaneous curing frequency[b]	Heat-pulse curing frequency[b]
	ED2144	ED2145			
JCFLO	20	30			
EDFL170	3×10^{-4}	43	0.03	5×10^{-4}	8×10^{-4}
EDFL180	1×10^{-4}	22	0.10	4×10^{-4}	2×10^{-3}
EDFL211	3×10^{-4}	29	0.01	$<5 \times 10^{-6}$	$<2 \times 10^{-4}$
EDFL212	2×10^{-4}	47	0.20	4×10^{-4}	7×10^{-3}
EDFL213	2×10^{-4}	33	0.001	4×10^{-4}	2×10^{-2}
EDFL214[c]	1×10^{-3}	38	300	4×10^{-4}	3×10^{-3}
EDFL215	3×10^{-4}	18	0.004	$<5 \times 10^{-8}$	3×10^{-4}
EDFL216[c]	4×10^{-4}	31	200	8×10^{-4}	2×10^{-2}
EDFL217	7×10^{-4}	45	0.30	6×10^{-4}	9×10^{-2}
EDFL218	4×10^{-4}	23	0.50	8×10^{-4}	5×10^{-2}
EDFL219	5×10^{-4}	29	400	1×10^{-3}	5×10^{-2}
EDFL231	3×10^{-4}	35	200	6×10^{-4}	8×10^{-4}
EDFL234	4×10^{-4}	40	0.30	5×10^{-4}	5×10^{-3}
EDFL235	9×10^{-4}	32	400	4×10^{-4}	8×10^{-3}
EDFL236	7×10^{-4}	45	0.05	3×10^{-4}	3×10^{-3}

[a] (%), The number of recombinants per 100 donor cells added in a 90-min mating at 33°C. ED2144 and ED2145 are the non-λ-lysogenic and λ-lysogenic recipients, respectively.

[b] A normal lysogen, ED395 (EDλ4), was cured spontaneously at a frequency of 2×10^{-5}, and, after heat pulse, at a frequency of 0.22.

[c] Heat-pulse treatment (26) was used to isolate single lysogens (with low burst size) from EDFL214 and EDFL216. These derivative plasmids were numbered EDFL288 and EDFL279.

for a normal EDλ4 lysogen, reflecting the reduced efficiency of site-specific recombination at an abnormal *att*λ site (26).

Deletion analysis and prophage location. We next determined the approximate sites of λ integration. For this, clones surviving a 42°C treatment were examined; these had lost lethal λ functions and, in some cases, adjacent plasmid genes. A summary map of F*lac* showing the extents of some of the plasmid deletions is presented in Fig. 1.

Initially, 42°C-resistant survivors were selected from 10 single clones of each cointegrate on lactose-tetrazolium agar, and the Lac$^+$ colonies were screened to identify Tra$^-$ clones (see Materials and Methods). Each Tra$^-$ clone was purified and tested for complementation with F*lac* *traJ*, *traA*, and *traD* point mutants by using a semiquantitative microtiter technique (1). The majority of the Lac$^+$ Tra$^-$ survivors did not complement any of the three point mutants, so that either the whole transfer region or the promoter region for the transfer operon together with the control gene *traJ* must have been deleted. In such cases, the λ prophage could have been located at either end of the transfer region, between *rep* (expected to be a forbidden region for deletion) and *traA*, or *traD* and *lac*. Representative deletion plasmids of this type are listed in Tables 2 and 3.

However, deletions ending within the transfer region were obtained for one group of five F*lac*(λ) cointegrates, which were in fact similar to each other and to a sixth coinegrate, EDFL214, in giving about 1% Lac$^+$ clones, about 5% of which were Tra$^-$, among the 42°C survivors. Deletions from these cointegrates are described in Table 2. Two of the deletion plasmids, EDFL177 and EDFL178, retained *traJ* but had lost the entire transfer operon, since neither the first (*traA*) nor the last (*traD*) gene of this was present. Two other deletion plasmids, EDFL172 and EDFL181, had lost the transfer operon genes *traG* and *traD*. Three others, EDFL171, EDFL179, and EDFL184, were Tra$^-$ but were still sensitive to the three F-specific phages f1, f2, and Qβ and so probably carried deletions of *traI*. The ability of the λp*traDI* transducing phage EDλ105 to complement the transfer defect of these plasmids supports this explanation. Furthermore, none of these seven *traJ*$^+$ partial *tra* deletion plasmids could be complemented by R100-1, indicating that in all cases a plasmid-specific gene, probably *traI*, had been lost.

The genotypes of these deletion plasmids suggest that, for all five of the parental F*lac*(λ) cointegrates, the λ prophage is located between *traI* and *lac* or possibly between *traD* and *traI*. To distinguish between these alternatives, a search was made for 42°C survivors that had lost *lac* but retained all the genes necessary for pilus formation and consequent sensitivity to the F-specific phage μ2. Such deletion mutants were obtained from all five cointegrates and also from EDFL214, and the numbers of representative Lac$^-$ μ2^s plasmids are given in Table 2. The abilities of these plasmids to transfer were then tested as described in Materials and Methods, and all were found to be Tra$^+$. This showed that neither *traI* nor any other transfer gene had

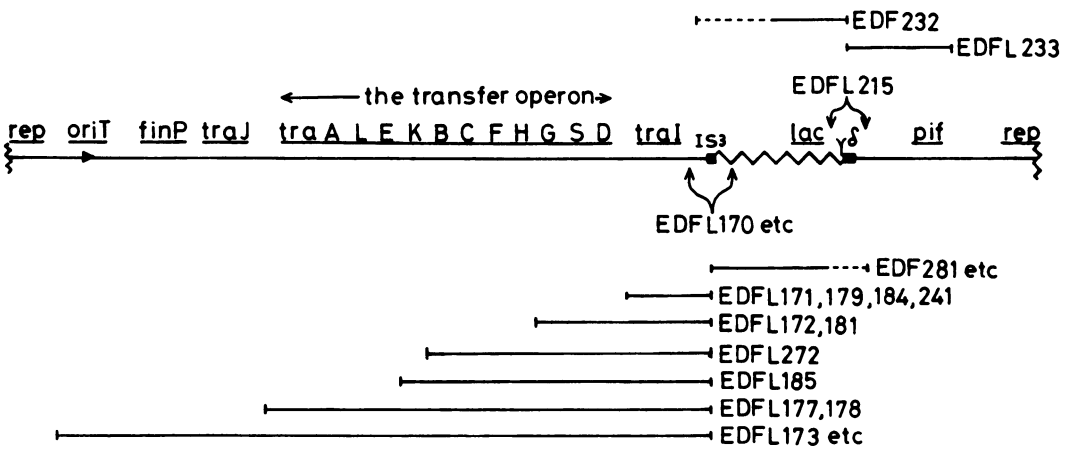

FIG. 1. *Map of λ insertion points and deletions. This map summarizes the data presented in Tables 2 and 3, where other examples of insertions between traI and lac and of lac and total tra deletions are listed. The double arrows indicate that λ integration may be into either F or chromosomal DNA sequences. Dotted lines indicate that deletion end points are not known.*

TABLE 2. *Deletions from one group of Tra⁺ Flac(λ) cointegrates*

Cointegrate plasmid no.	Deletion phenotypes						No. of representative deletion plasmid	Lac⁻ μ2ˢ deletion plasmid[a]
	Phage sensitivity			Complementation				
	f1	f2	Qβ	*traJ*	*traA*	*traD*		
EDFL170	R	R	R	−	−	−	EDFL173	EDF281
	R	R	R	+	−	−	EDFL177[b]	
	S	R	S	+	+	−	EDFL172[b, c]	
	S	S	S	+	+	+	EDFL171[b, d]	
EDFL180	R	R	R	−	−	−	EDFL182	EDF282
	R	R	R	+	+	−	EDFL181[b, c]	
EDFL211	R	R	R	−	−	−	EDFL188	EDF283
	R	R	R	+	−	−	EDFL178[b]	
EDFL212	R	R	R	−	−	−	EDFL186	EDF276
	S	S	S	+	+	+	EDFL179[b, d]	
EDFL213	R	R	R	−	−	−	EDFL189	EDF277
	S	S	S	+	+	+	EDFL184[b, d]	
EDFL214	R	R	R	−	−	−	EDFL274	EDF284

[a] All the Lac⁻ μ2ˢ plasmids were Tra⁺ Pif⁺.

[b] These deletion mutants had lost a plasmid-specific gene, since they were transferred at only low frequencies (0.2 to 2%) when R100-1 (which was transferred at 25 to 75%) was present in the same cell.

[c] These plasmids were *traH⁺ traG traD* as determined in further quantitative complementation tests. There was no complementation by the λd*traCFHGD* phage EDλ97, showing that *traI* had also been lost.

[d] These deletion mutants were complemented by the λp*traDI* phage EDλ105, which was constructed in vitro by J. Beggs.

been deleted and confirmed that in all six parental cointegrates the prophage location was between *traI* and *lac*.

For the cointegrates not initially giving Lac⁺ deletions ending within the transfer region, we next examined a larger number of both Lac⁺ and Lac⁻ 42°C-resistant survivors, screening these for their response to both the male-specific phage μ2 and the female-specific phage φII. The Lac⁺ survivors were also replica-plate mated to test their transfer abilities, and, where appropriate, complementation tests were carried out to determine the end points of *tra* deletions (Table 3).

From these experiments, plasmid deletions were obtained defining the prophage location in the cointegrates EDFL231, EDFL234, EDFL235, and EDFL236. These cointegrates were similar to each other in giving about 0.5% Lac⁺ survivors of which about 70% were Tra⁻, after the 42°C treatment. From each, both a Lac⁺ Tra⁻ and a Lac⁻ μ2ˢ Tra⁺ deletion was obtained, showing that the prophage location was again between *traI* and *lac*.

EDFL215 produced Lac⁺ Tra⁺ μ2ˢ φIIˢ and Lac⁻ Tra⁺ μ2ˢ φIIʳ survivors to the 42°C treatment. The first type has lost only *pif*, the gene(s) responsible for resistance to female-specific phages (5, 6), and the second has lost only *lac*. We therefore deduce that the λ integration site in EDFL215 is between *lac* and *pif*.

Although the above procedure gave rise to Lac⁻ μ2ˢ φIIʳ Tra⁺ deletion mutants from the cointegrates EDFL216, EDFL217, EDFL218, and EDFL219, no Tra⁻ or Pif⁻ clones were found among 150 to 300 Lac⁺ 42°C survivors tested. In a further effort to determine whether the location of the inserted prophage was between *traI* and *lac* and could therefore give rise, albeit at a low frequency, to Lac⁺ Tra⁻ survivors, we incorporated a selection for μ2 resistance into the procedure for isolating deletions. Because 99% of the 42°C survivors of EDFL216 were Lac⁺, we continued to plate on lactose-tetrazolium agar at 42°C, but prespread these plates with 10^9 plaque-forming units of μ2 phage. The other three cointegrates were plated with μ2 phage on lactose-minimal medium at 42°C, since only 1 to 3% of the 42°C survivors were Lac⁺.

From EDFL216, both partial and total *tra* deletions were obtained (Table 3), fixing the prophage location between *traI* and *lac*. However, the Lac⁺ μ2ʳ Tra⁻ survivors obtained from EDFL217, EDFL218, and EDFL219 were very leaky for transfer and/or appeared to carry point mutations in a variety of *tra* genes such as *traA, traH,* or *traG* (data not shown). They presumably result from a double event: loss of the

TABLE 3. *Deletions from other Tra⁺ Flac(λ) cointegrates*

Cointegrate plasmid no.	Deletion phenotype				No. of representative deletion plasmid[a]
	Lac	Tra	μ2	φII	
EDFL215	+	+	S	S	EDFL233
	−	+	S	R	EDFL232
EDFL216	+	−	R	R	EDFL242[b]
	+	−	R	R	EDFL272[c, d, e]
	−	+	S	R	EDF268
EDFL217	−	+	S	R	EDF269
EDFL218	−	+	S	R	EDF270
EDFL219	−	+	S	R	EDF271
EDFL231	+	−	R	R	EDFL237[b]
	+	−	R	R	EDFL185[c, f]
	−	+	S	R	EDF265
EDFL234	+	−	R	R	EDFL240[b]
	+	−	S	R	EDFL241[c, g]
	−	+	S	R	EDF285
EDFL235	+	−	R	R	EDFL238[b]
	−	+	S	R	EDF266
EDFL236	+	−	R	R	EDFL239[b]
	−	+	S	R	EDF267

[a] As for Table 2, footnote *a*.
[b] The deletion removed *traJ*, the entire transfer operon, and *traI*.
[c] As for Table 2, footnote *b*.
[d] The deletion end point was *traK⁺ traB*.
[e] Made from the single lysogen EDFL279, rather than from EDFL216 itself.
[f] The deletion end point was *traE⁺ traK*.
[g] As for Table 2, footnote *d*.

TABLE 4. *oriT: F100-promoted transfer from RecA⁻ host*[a]

Plasmid no.	Transfer (%)	
	gal⁺	*lac⁺*
EDFL173	43	0.003
EDFL186	34	0.02
EDFL181	35	33

[a] Matings to JC6455 (Gal⁻ Lac⁻) were performed as described in the text. The results represent the average of three experiments.

prophage to give 42°C resistance and spontaneous mutation in a *tra* gene to give μ2 resistance. Since, therefore, it proved impossible to isolate either Lac⁺ Tra⁻ or Lac⁺ Pif⁻ deletions from these three cointegrates, we are unable to say on which side of *lac* the λ prophage is inserted.

The origin of transfer and the site for recircularization. Since the majority of the Lac⁺ Tra⁻ 42°C survivors from prophage insertions between *traI* and *lac* were total *tra* deletions ("F*lac* Δ*tra*"), it was of interest to determine whether the deletions extended through *oriT*, the locus required in *cis* for plasmid transfer (33). If the F*lac* Δ*tra* plasmid retained *oriT*, an incoming F*gal tra⁺* plasmid (F100) should be able to provide all the *tra* products in *trans* and allow transfer of the F*lac* Δ*tra*. If, however, the *tra* deletion extended through *oriT*, then F*lac* Δ*tra* transfer can only occur via recombinational mobilization as as a covalently linked F*lac*-F*gal* structure, using the *oriT* as well as the *tra* genes of F*gal*. This is demonstrated by the low level of mobilization from RecA⁻, compared to RecA⁺, strains (Tables 4 and 5). To prevent such linkage, matings were performed in a RecA⁻ host, as described in Materials and Methods. F itself has no system for homologous recombination (34). Representative data are shown in Table 4. EDFL181 was chosen as an *oriT⁺* control plasmid, since the deletion end point was known to be in or near *traG* (Table 2); as expected, the incoming F*gal* plasmid allowed its transfer. EDFL173 and EDFL186 were chosen as representatives of the mutants with deletions known to cover the *tra* genes *traJ* through *traI*. Transfer of neither of these plasmids was restored by F*gal*, and we therefore conclude that both deletions extend through *oriT*. As a consequence, the two genes known to lie between *oriT* and *traJ*, *finP* (32) and *traM* (N. S. Willetts, unpublished data), must also have been deleted.

Besides EDFL173 and EDFL186, 44 other deletion mutants that had lost the genes *traJ* through *traI* were isolated in the course of the work described above. These included from one to six such mutants from each of 11 F*lac*(λ)

TABLE 5. *Recircularization after F57-promoted transfer to a RecA⁻ recipient*[a]

Plasmid no.	*oriT*	Transfer (%) to		Lac⁺ progeny of RecA⁻ (%)	
		RecA⁺	RecA⁻	Lac⁺ His⁺	Tra⁺
EDFL173	−	4.1	0.4	1	1
EDFL186	−	4.5	0.5	<0.5	<0.5
EDFL181	+	482	138	<0.7	<0.7

[a] Matings to recipient strains JC3272 (RecA⁺) and JC6310 (RecA⁻) were performed as described in the text. The results represent the average of three experiments. The apparently high levels of EDFL181 transfer may derive from underestimates of the number of sectored colonies representing heterozygote donors.

cointegrates. From this total of 46, including EDFL173 and EDFL186, 43 were tested (32) for the presence of *oriT* in complementation experiments carried out with F100 in a Rec$^+$ host, and all had lost this locus.

The F*lac* Δ*tra* plasmids were further examined to determine whether they carried a site or region at which the transferred linear plasmid DNA molecule could be recircularized in the recipient cell. If unit- or near unit-length plasmid molecules are transferred (9, 10), recircularization must take place at or near to *oriT*, whereas if multi-unit plasmid molecules are transferred, for example by a rolling circle mechanism (13), recircularization at a site or region located elsewhere could occur.

Although the F*lac* Δ*tra* plasmids are incapable of autonomous transfer, introduction of an F*his tra*$^+$ plasmid into a Rec$^+$ (F*lac* Δ*tra*)$^+$ cell produced a transient heterozygote capable of low-level F*lac* transfer (Table 5). This transfer presumably occurred by recombinational mobilization via a covalently linked F*his*-F*lac* Δ*tra* structure (diagramed in Fig. 2a), using the *tra* products and *oriT* of F*his*. Both Rec$^+$ and RecA$^-$ recipients were used in these crosses, and transfer to the Lac$^-$ His$^-$ RecA$^-$ recipient was of particular interest, since under these conditions all recircularization must take place by a mechanism other than recombination between repeated homologous plasmid DNA regions promoted by the host's recombination system. If such plasmid-determined recircularization requires a site or region, perhaps including *oriT*, that has been deleted in F*lac* Δ*tra*, then the Lac$^+$ progeny should contain only a covalently linked F*lac* Δ*tra*-F*his* plasmid (Fig. 2b) and consequently be both His$^+$ and Tra$^+$. If a recircularization site or region is still present in F*lac* Δ*tra*, however, Lac$^+$ progeny that are His$^-$ and Tra$^-$ should be found (Fig. 2c).

The results (Table 5) showed that an *oriT*$^+$ control plasmid and two *oriT* F*lac* Δ*tra* plasmids gave rise to Lac$^+$ progeny the majority of which were both His$^-$ and Tra$^-$. This indicated that the F*lac* Δ*tra* plasmids had been reformed in the RecA$^-$ recipient strain. Further evidence that this was the case was obtained by comparing the sizes of the F*lac* Δ*tra* plasmid DNA molecules in the Rec$^+$ ED2149 parental and RecA$^-$ JC6310 progeny strains. The methodology was essentially similar to that described by Willetts and Bastarrachea (36). The Rec$^+$ strain was labeled with [^{3}H]thymidine, and the RecA$^-$ strain with [^{14}C]thymidine, the cells were mixed and lysed, and plasmid DNA was separated by a cleared lysate-dye CsCl gradient technique. Sucrose gradient analysis then confirmed that the ^{3}H- and ^{14}C-labeled plasmids had identical sedimentation velocities (data not shown).

We interpret these data to mean that the two F*lac* Δ*tra* plasmids still retain a recircularization site or region, which must therefore be distinct from *oriT*.

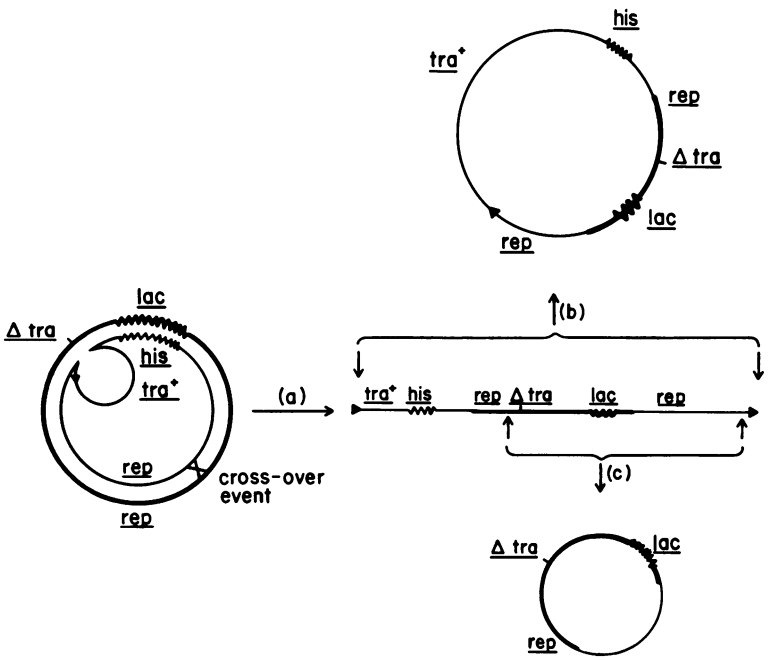

FIG. 2. *Recircularization of F*his*-mobilized F*lac* Δ*tra* plasmids. (a) Transient heterozygote F*his*-F*lac* Δ*tra* donor. (b) Recircularization requires oriT. (c) Recircularization does not require oriT.*

λ transducing phages carrying nearby regions of F*lac*. Efforts were made to isolate transducing phages carrying transfer genes from cointegrates with the prophage inserted between *traI* and *lac*. For this, lysates (10 to 1,000 times concentrated by high-speed centrifugation) were made from strains carrying representative cointegrates and used to infect ED2149 carrying either F*lac* or JCFL41 (F*lac traI41*). Lysogens able to transfer the F*lac* plasmid to the lysogenic strain ED2145, but not to the nonlysogenic strain ED2144, were isolated as described in Materials and Methods for the isolation of EDλ4 secondary site lysogens. In the present case, however, the lysogenic clones were expected to include ones arising by recombinational insertion of λ transducing phages, carrying regions of F*lac* DNA, into the homologous region of the F*lac* plasmid. Such lysogens were induced with and without infection by EDλ4 helper phages, and the lysates were used in spot complementation tests to find which, if any, transfer genes were carried.

By these means, a λd*traDI* phage (EDλ107) was isolated from EDFL170, and λp*traGD* (EDλ108), λp*traD* (EDλ110), and λp*traI* (EDλ109) phages were isolated from EDFL217. The λp*traGD* and λp*traD* phages presumably carry internal deletions of the F region between these transfer genes and the prophage in EDFL217. All four will be described further in future publications. The other F*lac*(λ) cointegrates tested (EDFL212, EDFL279, and EDFL234) gave no phages carrying recognizable transfer genes.

Efforts to isolate λ*lac* phages by transducing ED2149 carrying JCFL201 (F*lac*$^-$) and selecting Lac$^+$ derivatives were unsuccessful, as were attempts to select λ*pif* phages from EDFL215 (in which the λ prophage is inserted between *lac* and *pif*). Probably, the distances of the *lac* and *pif* genes from the sites of λ insertion are too great.

DISCUSSION

The molecular length of JCFLO (= F42) is 143.3 kb (25), and that of the *E. coli* chromosome is 4.1×10^3 kb (4). Therefore, if λ integration into secondary attachment sites occurred randomly, we would expect 3.5% of the lysogenic survivors of infection to be plasmid-lambda cointegrates. We found fewer than this (0.5%), in agreement with the reports of Shimada et al. (26) that there are preferred secondary sites on the bacterial chromosome. In fact the majority (11, and possibly 14, out of 15) of our Tra$^+$ cointegrates had λ inserted between *traI* and *lac*. This initially suggested that there is a unique secondary attachment site in this region, but the varying patterns of 42°C survivors from these cointegrates later indicated that several secondary attachment sites were being utilized. One or more of these sites could be in the 37.1 to 52.7 kb of bacterial DNA sequences that lie between *traI* and *lac* (16); however, there is no frequently used secondary chromosomal integration site for λ in this region (26).

The F*lac*(λ) cointegrates served as good sources of transfer deletion mutants. Many had lost the entire transfer region (the reasons for this preponderance are not presently understood), but others had deletion end points within the transfer region. Such deletion mutants have already proven useful for the mapping of *finP* (32) and *ilz* (28), and, together with the *lac* and *pif* deletion mutants, they may help in the mapping of any further new F genes. The deletions that had removed the entire transfer region had also removed *oriT*; these deletion mutations were therefore *cis*-dominant, like the mutations described by Guyer and Clark (14). Two of these F*lac* Δ*tra* plasmids retained the capacity to recircularize in a RecA$^-$ recipient cell, indicating that this can take place at a site or region separable from *oriT* and lying outside the entire *oriT-traI* region. Such a mechanism would allow recircularization after transfer of a greater than unit length of F DNA (23, 24) to a RecA$^-$ strain (7). The high efficiency of *recA*-independent recircularization that was observed may indicate the existence of a special F-encoded recircularization system, rather than interaction between the insertion sequences on the F*his* and F*lac* Δ*tra* plasmids.

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