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Genetics

Excision of F plasmid sequences by recombination at directly repeated insertion sequence 2 elements: Involvement of *recA*

(episome/transposon/integration/R-factor/F')

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ABSTRACT **The DNA of the F plasmid is joined to bacterial DNA sequences in the F' ORF203 by directly repeated insertion sequence 2 (IS2) elements. The rate of excision of the F plasmid from this F' (presumably by recombination at the directly repeated IS2s) has been estimated in both *recA*$^+$ and *recA*$^-$ strains. Normal F is produced in the *recA*$^+$ strain, but is not detected in *recA*$^-$. The autonomous plasmids produced in the *recA*$^-$ background were F's having deletions. F excision in this particular *recA*$^+$ case is specific in the sense that the directly repeated IS2s appear to be more active in recombination than similarly disposed IS3 direct repetitions in this F'.**

The fertility plasmid F can exist either autonomously in the cell or covalently inserted into the bacterial chromosome (see refs. 1 and 2 for discussion). When it became evident that both F and the *Escherichia coli* K-12 chromosome contain insertion sequence 2 (IS2), Saedler and Heiss (3) proposed that integration of F into the bacterial DNA might be attributed to recombination between IS elements present on F and on the bacterial chromosome. Subsequent physical mapping of IS2 and IS3 on F and on the bacterial chromosome provided evidence that F does indeed sometimes integrate by recombination at IS2 and IS3 elements on the bacterial chromosome (4–6). Direct physical mapping of the integrated F sequences in two independent Hfr strains showed that in two separate instances, F had integrated at an identical chromosomal IS3 (7).

A fundamental aspect of any episomal system is the genetic control of the integration and excision processes. For the bacteriophage λ, phage-specified gene products (*int* and *xis*) mediate integration or excision, independent of the bacterial recombination system (8). For the F system, the genetic determinants specifying integration and excision have not been defined. Because some F integration and excision events occur by recombination at IS2 or IS3, there are two genetic mechanisms that might be postulated: *recA*-independent processes known to be available to some IS elements (9); and mechanisms that depend upon the homology between IS elements located on the bacterial chromosome and on F, and thus upon the bacterial *recA* gene.

The present study explores the dependence of F excision upon *recA*, by examining the excision of F from the F' ORF203, a 334,000 base pair (334 kb) plasmid which carries the bacterial genes *lac*, *proC*, and *purE* (10). Physical studies have shown that all of the normal F DNA sequences are included in this F', and that F is probably physically linked to the bacterial sequences in this F' in the same way as F is linked to bacterial DNA in the parental Hfr. The structure of this F' is indicated in Fig. 1*B*. There is a duplication of IS2 at the two junctions between F and the bacterial DNA in ORF203. Recombination between these two IS2 elements would produce wild-type F and a circular piece of bacterial DNA that would presumably lack the ability to replicate autonomously. There are also directly repeated IS3 elements, denoted as $\alpha_1\beta_1$, $\alpha_2\beta_2$, and $\alpha_5\beta_5$. Recombination of $\alpha_5\beta_5$ with either of the other $\alpha\beta$ elements would lead to excision of an F-type plasmid, again with loss of *lac*, *proC*, and *purE*. As shown by heteroduplex mapping, the directly repeated IS3 elements have approximately the same length and degree of homology as the directly repeated IS2s (5, 10).

Loss of bacterial genes from ORF203 in a host with a deletion provides a genetic way for monitoring excision of F DNA sequences, and we here report experiments that exploit this property to measure rates of F excision associated with IS elements in *recA*$^+$ and *recA*$^-$ genetic backgrounds. The specificity of the excision process is determined by isolation of primary excision products, which are identified by restriction enzyme analysis.

MATERIALS AND METHODS

Bacterial Strains. RD20 [X7026(ORF203)] is a derivative of X7026 [which is K-12 F$^-$ λ$^-$ *thi-1 rel-1*? Δ(*proB lac*)$_{XIII}$ *supE44*] that has been described previously (10). To provide an isogenic *recA*$^-$ background, a *thyA*$^-$ derivative of X7026 was constructed by selection on plates containing trimethoprim, and the resultant strain (RD15) was mated with Hfr JC5088 (which is *thr-300 ilv-318 spc-300 recA56*) to generate a *thyA*$^+$ *recA*$^-$ recombinant, RD17. The *recA*$^-$ character of this strain was confirmed by demonstrating its inability to support growth of λ *bio10* bacteriophage, which has the Fec phenotype (11). The *proB–lac* deletion and the ability to suppress amber mutations were retained. RD22 [RD17(ORF203)] was constructed by transferring ORF203 from RD20 into RD17.

Recipient strains for tests of marker transfer were AB1157 (which is F$^-$ λ$^-$ *thi-1 argE3 his-4 proA2 leu-6 thr-1 lacY1 galK2 ara-14 mtl-1 xyl-5 str-31 tsx-33 sup-37*) (obtained from B. Bachmann); and χ478 (which is F$^-$ *ara leu lacZ proC tsx purE trp lys str mtl xyl metE thi*), PB314 (which is F$^-$ *lac purE thi tsx str*), and ED1111 (which is PB314, *recA*$^-$) [obtained from N. Davidson (5)].

Media. Minimal medium (12) supplemented with thiamine at 1 μg/ml and proline at 20 μg/ml and containing 0.2% lactose was used for fluctuation tests. For routine genetic testing of various isolates, growth was either in L-broth (13) or in tryptone broth containing tryptone at 10 g/liter, NaCl at 5 g/liter, and thiamine at 1 μg/ml, pH 7.4. Tryptone broth was employed for growth of cultures from which DNA was to be isolated.

MacConkey agar (Difco) was used in screening for colonies unable to ferment lactose (Lac$^-$). Eosin/methylene blue agar (Difco) supplemented with glucose was employed as solid

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Abbreviations: kb, 10^3 base pairs; IS, insertion sequence; $\alpha_i\beta_i$, the *i*th designated IS3 element (mixed subscripts indicate presumptive recombinant sequences); $\alpha_i'\beta_i'$, an IS3 inverted with respect to the polarity defined by another IS3; Lac$^-$, unable to ferment lactose.

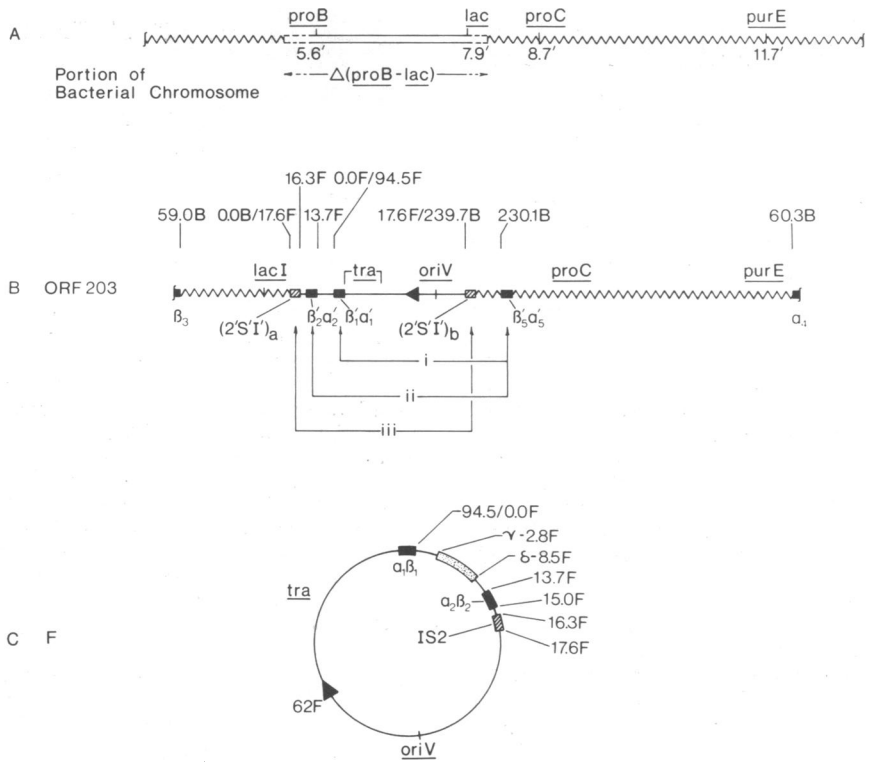

FIG. 1. Correlation of genetic and physical structures. (*A*) The genetic map of the *proB–purE* region of the chromosome of the bacterial strains in which ORF203 was maintained. Map units are minutes of transfer time. The physical end-points of the deletion (open box) are not known, and in particular, it is not known whether the deletion includes the 2′S′I′ or $\beta_5'\alpha_5'$ (IS3) elements that are believed to be present on the bacterial chromosome. (*B*) The physical map of ORF203. The IS2 (hatched boxes) and IS3 ($\alpha\beta$) elements (solid boxes) and neighboring regions are drawn on an expanded scale for clarity. F DNA is represented by a straight line, and bacterial DNA is indicated by a serrated line. Lengths are given in kb, with suffixes F and B denoting F sequences and bacterial sequences, respectively. The solid triangle on the F region represents the F transfer origin. Correlation with the genetic map is based on physical mapping of *lacI* (5, 27) and the approximate location of *purE* (5, 10). Arrows *i*, *ii*, and *iii* connect pairs of duplicated IS elements. Recombination between members of any of the indicated pairs would lead to F-like plasmids containing the transfer operon (*tra*) of F and the origin for vegetative replication of F, *oriV*. Recombination involving repeated IS2s (*iii*) would yield wild-type F. (*C*) An abbreviated map of the F plasmid. Scale is approximate.

medium for tests of sensitivity to bacteriophage f2 by the cross-streak method (14). Tryptone agar plates were used for overlays.

Fluctuation Tests. The rate of production of Lac$^-$ segregants was determined by performing Luria–Delbrück fluctuation tests (15). A summary of procedures and the formula for calculating rates are given in the legend to Table 1.

Genetic Testing. All Lac$^-$ isolates were tested to verify retention of the X7026 (or RD17) genetic backgrounds, and also were tested for sensitivity to bacteriophage f2 (see legend, Table 2). A number of Lac$^+$ clones were also tested to check for retention of ORF203 in the initial inoculum. Plate matings were conducted by the cross-streak method (14). Routine tests for the *recA*$^-$ character were performed by exposing streaks of culture on L-plates to ultraviolet light at 257 nm for several different times (*recA*$^-$ bacteria are ultraviolet sensitive).

Preparation of Plasmid DNA. Twenty-five milliliter T-broth cultures were grown to 10^9 cells per ml with shaking at 150 rpm in a waterbath shaker and were harvested and lysed as described by Sharp *et al.* (16). Immediately after lysis, the lysate was sheared by passage through a 5-ml plastic pipette 5–10 times at 1 ml/sec. It was then transferred to a polyallomer centrifuge tube and adjusted with TES (0.05 M Tris·HCl/0.05 M NaCl/0.005 M EDTA, pH 8.5) to 2.8 g, and 0.4 g of TES containing ethidium bromide at 5 mg/ml was added. After mixing, 3 g of solid CsCl was dissolved in the lysate, and the solutions were then centrifuged at 44,000 rpm in a Beckman type 50 Ti rotor for 36 hr at 15°, as described by Clewell and Helinski for preparation of ColE1 DNA (17). After centrifugation, the fluorescent upper band was removed from the top and discarded. The lower band was further purified by an additional equilibrium centrifugation in CsCl/ethidium bromide. Final yields were 1–3 μg of covalently closed circular plasmid DNA. Even the large ORF203 plasmid (334 kb) could be purified in this way.

Restriction Enzyme Digestion and Electrophoretic Analysis. Ethidium bromide was removed from DNA samples by extraction of the CsCl solution with isoamyl alcohol. Digestion with *Eco*RI was conducted by using the standard conditions (18). A 2-fold excess of enzyme was used to ensure complete digestion. Digested samples were analyzed electrophoretically (19, 20), using 0.7% agarose slab gels and Tris/acetate buffer (19). Mobility standards were λ*cI857S7* DNA and F DNA (from *E. coli* strain W1485) digested with *Eco*RI. When appropriate, band intensities were monitored by scanning photographic negatives with a Joyce–Loebl microdensitometer.

RESULTS

Segregation of Lac$^-$ Clones from *recA*$^+$ *E. coli* carrying ORF203. The rates of generation of Lac$^-$ clones in the *recA*$^+$ and *recA*$^-$ backgrounds were determined by performing Luria–Delbrück fluctuation tests. Lac$^-$ clones could arise in a number of ways, including spontaneous loss (curing) of the ORF203 plasmid, spontaneous mutation of the *lac* operon present on the plasmid, or loss of some or all of the bacterial DNA sequences carried by ORF203. Clones of the latter category, which would include any events in which F was excised

Table 1. Determination of rate of production of Lac$^-$ segregants: summary of fluctuation test data

Strain	No. of experiments	Total no. of clones screened	Average rate of generating Lac$^-$ clones, (events/cell-generation)
RD20 (*recA*$^+$)	16	161,000	4.2×10^{-4}
RD22 (*recA*$^-$)	10	213,000	6.2×10^{-4}

All experiments were conducted by growth in minimal medium supplemented with proline and lactose. For starting each experiment, a single, fresh Lac$^+$ colony from a MacConkey plate was subcultured into supplemented minimal medium and diluted so that each of approximately 20 sample cultures contained 5–20 cells in 0.1- or 0.2-ml culture volume. Equal culture volumes were dispensed with plastic disposable pipettes into the wells of a microtiter tray (0.4-ml capacity per well). After incubation at 37° for 8–12 hr, the entire contents of each well were removed by using an automatic pipetter with disposable plastic tips and were plated on MacConkey-lactose agar plates, which were then incubated at 37° for 12–16 hr. To score for Lac$^-$ colonies, which on MacConkey plates are white instead of red, plates were examined at least twice with the aid of a 10× magnifier, using both transmitted and oblique lighting. The few cultures found to have abnormally high numbers of Lac$^-$ clones were discarded because they probably received *lac*$^-$ cells in the initial inoculum. Lac$^-$ colonies were picked and restreaked on MacConkey-lactose agar to verify the Lac$^-$ character and to ensure purity of each clone. Sectored colonies, which appeared at a lower frequency than pure Lac$^-$ colonies, were not used for calculation of rates. Rates for each experiment were calculated according to the following formula: rate $= -(\ln P_0)\cdot(\ln 2)/N$, in which N is the average number of bacteria per sample culture at the end of growth, and P_0 is the proportion of sample cultures having no Lac$^-$ clones. Average values for the rate corresponding to each strain were calculated by using the total number of colonies examined in each experiment as a weighting factor for the rate measured in that experiment.

from ORF203, were identified genetically, and the fraction of the Lac$^-$ clones that this type represents was multiplied by the appropriate rate constant to determine the influence of *recA* on F excision.

A summary of the results of the fluctuation tests is presented in Table 1. It is seen that the rate of generating Lac$^-$ clones is comparable for both the *recA*$^+$ and *recA*$^-$ strains, with the rate in *recA*$^-$ being somewhat higher. All experiments were performed while the bacteria were in the logarithmic phase of growth and at concentrations of less than 5×10^4 cells per ml. Under these conditions, superinfection of F′ bacteria by F will be inhibited because surface exclusion is being expressed, and spread of F′s to F$^-$ bacteria will be minimized, because dilution markedly lowers mating efficiency (21). Rate calculations are based on the number of sample cultures having no mutants (i.e., no Lac$^-$ clones), so that the rates measured should be independent of any differences in generation times of Lac$^+$ and Lac$^-$ bacteria (22). Control experiments indicated that *lac*$^+$ and *lac*$^-$ bacteria in both the *recA*$^+$ and *recA*$^-$ genetic backgrounds have the same plating efficiencies on MacConkey plates.

To determine which of the Lac$^-$ clones represented F$^+$ segregants, all Lac$^-$ isolates were subjected to a battery of genetic tests, as summarized in Table 2. Those Lac$^-$ clones carrying either integrated or autonomous F DNA with the proximal portion of the transfer operon (*tra*) were identified by testing for sensitivity to the bacteriophage f2. Both excisions involving the IS3 elements, as shown in Fig. 1 (*i* and *iii*), would have produced clones that retain the *tra* operon, and these would presumably have been detected by this method, had they appeared. If excision events were to delete portions of the *tra* operon, F-like autonomous plasmids that would be resistant to f2 might arise. Clones containing such plasmids would not have been scored as F$^+$ or F′$^+$ in these experiments.

Table 2. Genetic characterization of Lac$^-$ segregants

Strain	No. of independent Lac$^-$ clones tested	Lac$^-$ clones that were f2-sensitive, %	Lac$^-$ clones that were Hfr, %	Lac$^-$ clones that were F$^+$, %
RD20 (*recA*$^+$)	104 (182)	70	55	15.4
RD22 (*recA*$^-$)	126 (255)	2	0	0

The numbers in parentheses refer to the total number of clones actually tested. In this table, "independent" clones are those derived from separate sample cultures. For an estimate of the actual number of independent clones, see *text*. Sensitivity to bacteriophage f2 was tested by cross-streaking on eosin/methylene blue/glucose plates for clones derived from RD20. Those appearing to be resistant by this method were rechecked by the overlay technique. RD22 shows f2 sensitivity by the overlay method, but not by cross-streaking. Therefore, all Lac$^-$ clones derived from RD22 were tested for f2 sensitivity by the overlay technique. Clones that were sensitive to f2 were tested for their ability to transfer *proC*, *purE*, and *his* into the *recA*$^+$ recipients χ478, PB314, and AB1157, respectively, and for ability to transfer *purE* to ED1111 (*recA*$^-$), to allow discrimination of Hfr types.

It is seen that in both *recA*$^+$ and *recA*$^-$ backgrounds F$^-$ clones were detected, which suggests spontaneous curing of the F′, a phenomenon that has been described previously (23). In the *recA*$^-$ strain RD22, these represent the majority of Lac$^-$ isolates: only two clones sensitive to f2 were detected, and in both cases, these were found to carry large F′s, as will be described below. It is seen from the data of Table 1 and Table 2 that F′$^-$ (F$^-$) segregants appear at a higher rate in *recA*$^-$ background than in *recA*$^+$ background, a phenomenon that we did not explore further. Control experiments indicated that strain RD17 (the F$^-$ *recA*$^-$ strain from which RD22 was constructed) carrying wild-type F is sensitive to f2; hence, had F been generated in RD22 by segregation of F from ORF203, it would have been detected.

Lac$^-$ clones that were sensitive to f2 were tested for their ability to transfer *proC* and *purE*, which are carried by ORF203, as well as for ability to transfer the chromosomal marker *his*. Fifty-five percent of the Lac$^-$ clones isolated from RD20 were found to be Hfr. A number of these clones were further tested for transfer of *argE* and *leu*, and all were of the same type: *purE* was transferred more efficiently than *his*, while *argE* and *leu* were transferred at barely detectable levels. The transfer properties of these Hfrs are similar to those of Hfr OR21, from which ORF203 was derived. The Hfr character of these clones is also indicated by their inability to produce *purE*$^+$ recombinants when mated with a *recA*$^-$ *purE*$^-$ recipient (ED1111), whereas they can produce *purE*$^+$ recombinants when mated with the *recA*$^+$ *purE*$^-$ recipient PB314. If the transferred genes were on an F′, transconjugants would be expected from a mating with a *recA*$^-$ recipient (24). The formation of Hfrs with the concomitant loss of *lac* could possibly have occurred by nonreciprocal recombination between the bacterial sequences on ORF203 and the bacterial DNA of the host strain, which has a deletion that includes *lac*. Such processes are known to constitute a significant portion of recombination events in *E. coli* (25). Because F′ integration *via* the homology between the bacterial chromosome and the bacterial DNA on the F′ should be depressed in *recA*$^-$ backgrounds, few Hfr derivatives of RD22 would be expected. None were detected.

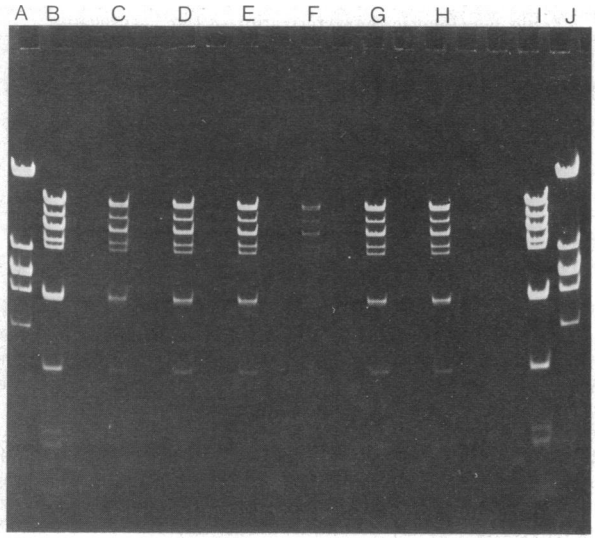

FIG. 2. Electrophoretic analysis of *Eco*RI digests of F-like plasmids isolated from RD20. Lanes A and J contain λ*cI857S7* and lanes B and I are F plasmid from W1485F$^+$. The plasmids run in lanes F and G came from the same sample culture. The others (C, D, E, and H) were each isolated from independent sample cultures. It is seen that for five independent clones, standard F was produced. Similar results were obtained from ten other independent isolates from RD20.

The remaining Lac$^-$ f2-sensitive clones obtained from RD20 showed low levels of *purE* and *his* transfer, suggestive of autonomous F. The two Lac$^-$ f2-sensitive clones obtained from RD22 were able to transfer *proC* into χ478 and to transfer *purE* to both *recA*$^+$ and *recA*$^-$ recipients, indicating that they carried F's. Covalently closed circular DNA could be extracted from both of these clones, confirming the presence of autonomous plasmids.

Influence of *recA* on the Generation of F Plasmid Types. Table 2 indicates that the percentage of *recA*$^+$ (RD20) sample cultures containing Lac$^-$ segregants that also contained F$^+$ clones was 15.4% in a sample of 104 cultures. The percentage in the *recA*$^-$ (RD22) case was 0% out of 126 sample cultures. From the former figure and the measured rate of formation of Lac$^-$ clones (4.2×10^{-4} events per cell-generation), we calculate that the rate of F excision in the *recA*$^+$ case is 6.5×10^{-5} events per cell-generation.

From the latter figure, we can calculate an upper bound for the rate at a given statistical confidence level for the *recA*$^-$ case. First, the probable number of *independent* Lac$^-$ clones is estimated for each fluctuation experiment by using P_0 for that experiment. We estimate that 180 independent Lac$^-$ clones were generated in the fluctuation tests for RD22. To this can be added 20 other independent Lac$^-$ isolates grown in a similar fashion, to give a total of 200 independent Lac$^-$ clones. (Note that 255 Lac$^-$ clones were actually tested.) None of these contained standard F. At 95% confidence, the fraction of F$^+$ clones among the independent Lac$^-$ clones is less than $^3/_{200}$, which indicates that the rate of F$^+$ production from ORF203 in RD22 is less than ($^3/_{200}$) × (6.2×10^{-4} events/cell-generation), i.e., less than 9.3×10^{-6} events per cell-generation.

The data thus indicate that the rate of excision of F from ORF203 is at least 7-fold higher in *recA*$^+$ background than in *recA*$^-$ background. The most probable ratio of rates is much greater than this.

Physical Analysis of Independently Derived Plasmids. F$^+$ clones obtained from independent sample cultures descended from small inocula necessarily represent independent excision events. The precision of the excision event can be deduced by determining the physical structure of the excision products. This was achieved by preparing 1–2 μg of plasmid DNA as described in *Materials and Methods*, and digesting with *Eco*RI. Because the *Eco*RI cleavage pattern of F is known (26), any changes in the electrophoretic pattern will be diagnostic for a modified F-type plasmid.

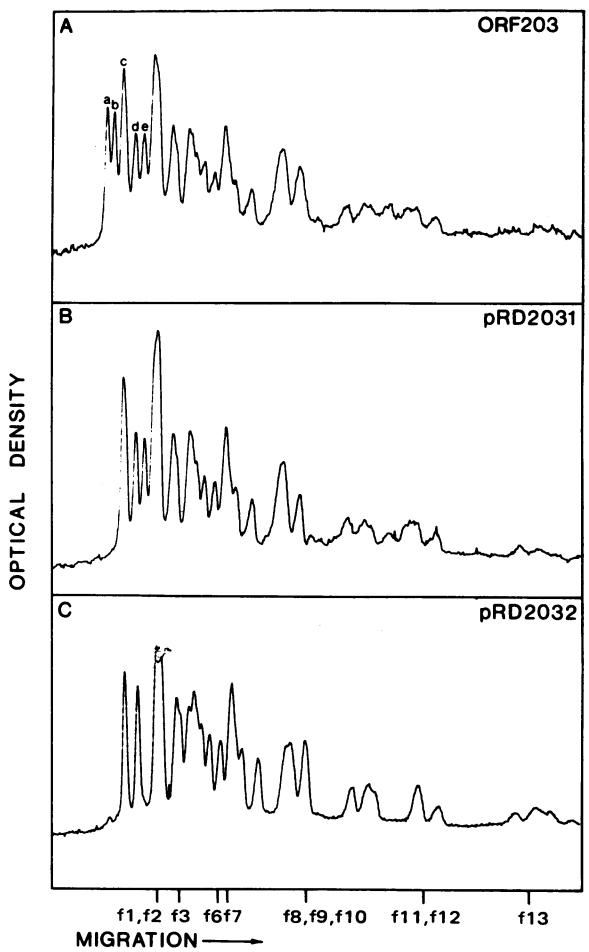

FIG. 3. Densitometer traces of gel patterns obtained after *Eco*RI digestion of plasmids ORF203, pRD2031, and pRD2032. The latter two plasmids are *lac*$^-$ deletion mutants of ORF203 obtained in the *recA*$^-$ strain RD22. The distances of migration for some of the *Eco*RI cleavage products of F are indicated at the bottom. Electrophoresis was for 3.5 hr in 0.7% agarose. It will be noted that pRD2031 and pRD2032 both appear to have lost bands *a* and *b* of ORF203, and that pRD2032 has lost in addition band *e* and at least one other small band. Exact enumeration of differences in these plasmids is complicated by superposition of bands having similar mobilities.

The electrophoretic patterns of digested plasmid DNA from fifteen independent F-type plasmids derived from ORF203 in the *recA*$^+$ background were indistinguishable from those for standard F from W1485 (see Fig. 2). The conclusion is that in 15 separate instances, complete, normal F was excised from ORF203. In the *recA*$^-$ background only two *lac*$^-$ F′ plasmids were obtained from the fluctuation tests: pRD2031 and pRD2032. As shown in Fig. 3, *Eco*RI digests of these F's yielded many bands, which is expected of F′ plasmids large enough to carry *proC* and *purE*, as these F's do. By analyzing the densitometer traces of these two plasmids (taking into account fragments that migrate at similar mobilities), we estimate the molecular sizes for pRD2031 and pRD2032 to be in the 240 to 280-kb size range—some 50–90 kb smaller than ORF203. These F's were not further characterized.

Attempts to prepare plasmid DNA from Lac$^-$ clones that

displayed transfer properties characteristic of Hfrs failed to yield any covalently closed circular DNA. This property would be expected of Hfr strains.

DISCUSSION

The results presented here indicate that the rate of excision of F from ORF203 in *recA*$^-$ background is significantly lower than in a nearly isogenic *recA*$^+$ genetic background. Out of 161,000 Lac$^+$ clones screened in fluctuation tests using the *recA*$^+$ strain RD20, 15 F$^+$ Lac$^-$ clones were obtained, while no F$^+$ Lac$^-$ clones were detected by screening 213,000 Lac$^+$ *recA*$^-$ RD22 clones. Statistical analysis indicates that, at the 95% confidence level, the rate of F excision from ORF203 is depressed by at least a factor of seven in the *recA*$^-$ strain. This indicates that the host recombination system is involved in the majority of F excision events in this particular system; however, the data do *not* exclude the possibility that *recA*-independent mechanisms leading to F excision from ORF203 may operate at a lower rate. Unpublished observations by R. Curtiss III and J. Renshaw indicate that integration of F to form Hfrs is depressed in *recA*$^-$ strains (Roy Curtiss III, personal communication), an observation that parallels our data for F excision.

Even though *recA* influences the rate of F excision from ORF203, specificity is still observed. As shown in Fig. 1, recombination involving three different combinations of directly repeated IS elements would produce F-type plasmids containing all of the F genes required for replication and conjugation: (*i*) $\alpha_1\beta_1$–$\alpha_5\beta_5$, (*ii*) $\alpha_2\beta_2$–$\alpha_5\beta_5$, and (*iii*) IS2–IS2. In all of these cases, the directly repeated elements are separated by similar distances (85.5 kb, 100.5 kb, and 93.2 kb, respectively), they have approximately the same length (1.4 kb), and by electron microscope-heteroduplex criteria they have the same degree of homology (10). In fifteen separate isolates, the products that were obtained from RD20 (*recA*$^+$) were normal F, with no other autonomous plasmids being detected. This indicates that in the F′ ORF203 recombination events between the directly repeated IS2 elements occur at a higher rate than recombination between the directly repeated IS3 elements. This result agrees with our previous observations based on examination of plasmids prepared from a large culture of RD20(ORF203) (10). In contrast to the *recA*$^+$ situation, the autonomous plasmids obtained in the *recA*$^-$ background were F′s with deletions, which appeared at a rate that is lower than the rate of F$^+$ production in *recA*$^+$.

There are a number of possible explanations for the specificity of the *recA*-mediated F excision from ORF203 (10). Some trivial explanations are: (*a*) that inclusion of bacterial sequences between IS2 and $\alpha_5\beta_5$ in an F-like plasmid (see Fig. 1) might for some reason yield strains that are resistant to the bacteriophage f2 (which therefore would have been overlooked), or (*b*) that bacterial DNA adjacent to $\alpha_5\beta_5$ influences this element somehow to make it inactive in recombination. We consider the former possibility to be unlikely, because strains carrying ORF203 show the expected sensitivity to f2. We cannot exclude possibility *b* at the present time.

Other more interesting explanations for the observed specificity of the F excision are: (*c*) that IS2 direct repetitions are intrinsically more recombinationally active than IS3 direct repetitions in a *recA*$^+$ background; (*d*) that one or more of the three directly repeated IS3 elements is itself recombinationally defective; and (*e*) that F regulates the excision process to ensure restoration of the complete F plasmid. Explanations *c* and *d* would both appear to suggest an interplay between the host recombination system (*recA*) and some specific properties of directly repeated IS elements.

Examination of the separation of F from any F′s in which F is inserted at $\alpha_5\beta_5$ would help clarify what sort of mechanism is operating. If it should turn out that F is able to control specificity of recombination between directly repeated IS elements, then studies of F excision may provide further insight into other recombinational processes associated with IS2 and IS3.

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