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Molecular cloning of DNA from F sex factor of *Escherichia coli* K-12

(*Eco*RI restriction endonuclease/*tra* operon/surface exclusion/bacteriophage T7 inhibition/F pili)

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ABSTRACT **We describe the molecular cloning of various DNA segments generated by partial *Eco*RI endonuclease digestion of the sex factor F. These segments have been analyzed by agarose gel electrophoresis of *Eco*RI digests and were arranged in a series of overlapping fragments using the *Eco*RI fragment map of F established by H. Ohtsubo and E. Ohtsubo. The clones isolated demonstrate one or more of the following F-specified functions: inhibition of female-specific phage (T7) multiplication, formation of F pili, surface exclusion, or immunity to lethal zygosis. These properties are discussed in terms of the *Eco*RI fragments of F that specify them.**

Detailed genetic and physical analyses (1–5) have enabled spectacular advances in our understanding of the F sex factor of *Escherichia coli*. Twelve of the transfer (*tra*) genes (*traA* thru *traI;* see Fig. 2) are known to constitute a single operon which is under the positive control of *traJ* (1, 3). Nine of the genes in the *tra* operon, *traA, traL, traE, traK, traB, traC, traF, traH,* and *traG,* are required for the formation of F pili and hence for the adsorption of male-specific phage. All of these nine cistrons and an additional two cistrons, *traD* and *traI,* are necessary for transfer proficiency (Tra^+) (6). Whereas, $traD^-$ strains make F pili and are sensitive to fd DNA phage they remain unable to support infection by f2 RNA phage (6).* The twelfth known gene within the *tra* operon, *traS,* is required for the expression of surface exclusion (Sfx) (5). Surface exclusion operates to reduce DNA transfer from one F-factor-carrying cell to another (10) and is manifested against both F transfer *per se* and chromosomal transfer as with Hfr strains (11). This phenomenon should be distinguished from incompatibility, which provides a second barrier to the establishment of more than one F factor in the same cell (5, 11).

traJ, which provides positive control over the *tra* operon, constitutes part of a second operon (1, 3) and is in turn subject to negative control by plasmids specifying the FinOP system of fertility inhibition (Fin) (12). This negative control is exerted at a site *traO,* located immediately to the left of, or within, *traJ* (N. S. Willetts, personal communication), and requires, in addition, an F-specified component, the product of the *finP* cistron (12). Other sites or functions specified on, or by, the F sex factor include *ori* (13), a sequence required for initiation of transfer, replication regions (ref. 14; M. A. Lovett and D. R. Helinski, personal communication), immunity to lethal zygosis (Ilz) (ref. 15; R. A. Skurray, M. Achtman, N. S. Willetts, and P. Reeves, in preparation), and the inhibition of multiplication of female-specific bacteriophage such as T7 (16, 17), T3, or ϕII (18).

Molecular cloning (19, 20) now offers an additional approach to the understanding of plasmid-specified functions. In this paper we describe the cloning of various segments of DNA from the sex factor F.

MATERIALS AND METHODS

All bacterial strains were derivatives of *E. coli* K-12. The F^- strain AB1157 (21), the HfrH strains AB259 (22) and JC158 (23), and the F^+ strain W1485 (24) have been described. The $Fhis^+$ strain JC6535 (M. Achtman, Ph.D. Dissertation, University of California, Berkeley, 1969) carrying F57 (25) and the $Fthr^+leu^+$ strain JC7221 (M. Guyer, Ph.D. Dissertation, University of California, Berkeley, 1974) carrying pJC36 were isolated in this laboratory. The tetracycline-resistant (Tc^r) plasmid pSC101 (19) in the C600 background was kindly supplied by S. N. Cohen. pSC101 DNA was used to transform AB1157 to provide an F^-/Tc^r strain (JC10101). The $Fhis^+$ plasmid from JC6535 was transferred to JC10101 to give an $Fhis^+/Tc^r$ strain (JC10112). The R factor, R1 (26), which specifies resistance to sulfonamide, streptomycin, ampicillin, kanamycin, and chloramphenicol, was kindly supplied by V. Hershfield; R1 was introduced into the cointegrate plasmid strains by conjugation, selecting for chloramphenicol resistance. T7 bacteriophage was kindly supplied by M. Chamberlin; f2, fd, and ϕII phage were from laboratory stocks. λ DNA was isolated from a λcI857s7 lysogen (kindly supplied by R. Fischer) by the method of Wu *et al.* (27). The procedure employed for isolation of covalently closed circular (CCC) plasmid DNA has been described (28). W1485 (kindly supplied by M. Guyer) was the source of F DNA. *Eco*RI restriction endonuclease digestion, agarose slab gel electrophoresis, ligation, and transformation were essentially as described (20). Electrophoresis on 1.58% and 0.65% agarose gels has been used by H. Ohtsubo and E. Ohtsubo (personal communication) for the resolution of low and high molecular weight (M_r) F DNA fragments, respectively. Tetracycline was employed at 5 μg/ml throughout. *Eco*RI restriction endonuclease and *E. coli* DNA ligase were generously supplied by H. Boyer and P. Modrich, respectively. Tc^r transformants were stored in Microtitre trays (Cooke Engineering Co., Alexandria, Va.) as described (29); these trays were also employed in microtests for F-specified functions (30). Acridine orange (AO) sensitivity (50 μg/ml) was determined as described (31).

RESULTS

Construction and Selection of pRS Plasmids. The F factor is 94.5 kilobases (kb) in length (28), of which approximately 30 kb are occupied by the *tra* operon (2). Since the largest fragment generated by complete *Eco*RI digestion of

Abbreviations: AO, acridine orange; CCC, covalently closed circular; *Eco*RI, restriction endonuclease RI from *E. coli*; Fin, fertility inhibition; Ilz, immunity to lethal zygosis; kb, kilobase; M_r, molecular weight; Sfx, surface exclusion; Tc^r, tetracycline resistance; Tra^+, transfer proficient; Thr, threonine; Leu, leucine.

* It should be noted that Ohtsubo *et al.* (7) have described six transfer gene complementation groups (A, B, C, D, E, and F) which were mapped on the F-prime, F_8 (8). These groups were found by N. S. Willetts and M. Achtman (9) to correspond to the *tra* genes *I, D, G, F, C,* and *E,* respectively.

F DNA has a mobility, on agarose gel electrophoresis, equivalent to a length of 13.2 kb (Fig. 1A and H. Ohtsubo and E. Ohtsubo, personal communication), it was expected that a cloned segment with *tra* operon function would arise only if a partial digest of F DNA was employed. On this basis, CCC F factor DNA was treated with *Eco*RI by incubating for 2 min at 0° and the reaction was terminated by incubating for 5 min at 65°. The resulting DNA products, when analyzed by electrophoresis in agarose gels, showed a range of bands with mobilities corresponding to fragments up to 50 kb long.

pSC101, an *E. coli* Tc^r plasmid, was employed for the cloning of F segments. This plasmid is cleaved by *Eco*RI at a single site, resulting in the formation of linear fragments of M_r 5.8 × 10^6 (19). The products of a complete *Eco*RI digest (37° for 5 min) of pSC101 CCC DNA and of the partial digest of F DNA (see above) were mixed (molar ratios of approximately 2 pSC101:1 F ensured an excess of F fragments per pSC101 fragment), ligated, and used to transform the F^- strain AB1157. Since no direct selection was available for F:pSC101 cointegrate plasmids the initial selection was for Tc^r. A number of these Tc^r clones were subsequently subjected to a series of microtests designed to detect those exhibiting one or more F-specified functions, such as surface exclusion, incompatibility, self-transmissibility, or sensitivity to the male-specific bacteriophage f2, in addition to Tc^r. Of some 2000 transformants tested, 15 demonstrated reduced recipient ability, to some degree, with either Hfr or F*his*$^+$ donors, or with both, suggesting the presence of F genes coding for surface exclusion or incompatibility. No other F-specified properties tested were observed among the transformants examined in these microtests.

The CCC plasmid DNA from these 15 suspected F:pSC101 clones has now been analyzed by agarose gel electrophoresis of *Eco*RI digests (see below) and, in addition to a fragment corresponded to pSC101, 13 of these clones yielded DNA with fragments corresponding to one or more F-factor fragments. The phenotypic properties of strains bearing these plasmids, which are designated the pRS plasmid series, have also been analyzed; results with six representative pRS plasmids are presented in this preliminary communication.

Properties of Strains Carrying pRS Plasmids. Transformants selected on the basis of a reduced recipient ability in qualitative tests were compared, in a series of quantitative tests, with AB1157 transformed for pSC101 alone, which acted as a convenient Tc^rF^- control, and with a pSC101/F*his*$^+$ double plasmid strain, which provided wild-type F function in a Tc^rAB1157 background (Table 1). On the basis of these results, transformants were arbitrarily divided into two groups; those with marked reduction (carrying pRS26, pRS15, or pRS31) and those with marginal reduction (carrying pRS5, pRS21, or pRS30) in recipient ability. The first group, like the F*his*$^+$ control strain, formed fewer progeny than the pSC101 strain with both Hfr and F-prime donors, suggesting that surface exclusion (Sfx) was responsible for this property. It was also possible to determine an aspect of the control of this property since surface exclusion is temporarily reduced in aerated stationary-phase cultures of F-factor-carrying strains (33). Likewise, surface exclusion was reduced when stationary-phase cultures of strains carrying pRS26 or pRS31 were employed as indicated by an increased recipient ability (Table 1). Of the second group of transformants, strains with pRS5 or pRS21 showed fewer progeny only with a F*thr*$^+$*leu*$^+$ donor, whereas the strain carrying pRS30 showed slightly fewer progeny with both Hfr and F-prime types. Such results suggest that these strains are deficient in surface exclusion (Sfx^-).

Lethal zygosis is the phenomenon whereby the number of viable F^- cells is reduced after mating with a large excess of Hfr cells (32). There is, associated with cells carrying an F

Table 1. Phenotypic characteristics of strain AB1157 carrying pRS plasmids

Plasmid	Efficiency of plating female- and male-specific bacteriophages*				Recipient efficiency† × JC158		Recipient efficiency† × JC7221	Sensitivity to lethal zygosis with HfrH (AB-259)‡
	T7	φII	fd	f2	Exponential	Stationary	Exponential	
pSC101	1.0	1.0	$<3 \times 10^{-9}$	$<1 \times 10^{-7}$	100	100	100	++
pSC101/F*his*$^+$	1.5×10^{-1} §	1.3×10^{-2} §	1.0	1.0	0.2	12.2	0.3	—
pRS5	2×10^{-3} §	$<10^{-5}$	$<3 \times 10^{-9}$	$<1 \times 10^{-7}$	180	160	60	++
pRS21	1.1	1.0	$<3 \times 10^{-9}$	$<1 \times 10^{-7}$	170	125	50	++
pRS30	1.0	7×10^{-1}	0.2	$<1 \times 10^{-7}$	90	85	65	++
pRS26	6×10^{-1}	3×10^{-1}	$<3 \times 10^{-9}$	$<1 \times 10^{-7}$	0.005	11	0.1	—
pRS15	5×10^{-1}	4×10^{-1}	$<3 \times 10^{-9}$	$<1 \times 10^{-7}$	0.4	0.7	0.7	—
pRS31	6×10^{-1}	2×10^{-1}	$<3 \times 10^{-9}$	$<1 \times 10^{-7}$	0.07	6	0.1	—

* Efficiency of plating bacteriophages was determined as previously described (6). Values with female-specific (T7, φII) or male-specific (fd, f2) bacteriophages were calculated relative to strain AB1157 carrying pSC101 or both pSC101 and F*his*$^+$, respectively.

† Recipient efficiencies, expressed as percent relative to strain AB1157 carrying pSC101, were determined by liquid medium matings at 37°. Either an exponential- or an aerated stationary-phase culture, of the plasmid-bearing strain under test as a recipient, was mixed with an exponential-phase culture of an HfrH (JC158) or F*thr*$^+$*leu*$^+$ (JC7221) donor strain to give a ratio of 1:1, and a final culture density of 2 to 3 × 10^8 cells per ml. After 60 min, samples were blended, suitably diluted, and plated, by the overlay technique, on media selective for Thr^+-Leu^+[Tc^r] progeny. Absolute values for progeny obtained with AB1157 carrying pSC101 were: × JC158 exponential, 4.2 × 10^6/ml; × JC158 stationary, 4.4 × 10^6/ml; × JC7221, 5 × 10^7/ml.

‡ By lethal zygosis solid media tests as previously described (32). Cells of F^- strains are sensitive (++); cells of F-factor-carrying strains are insensitive (−), due to immunity to lethal zygosis (15).

§ Marked reduction in plaque size compared to pSC101 alone.

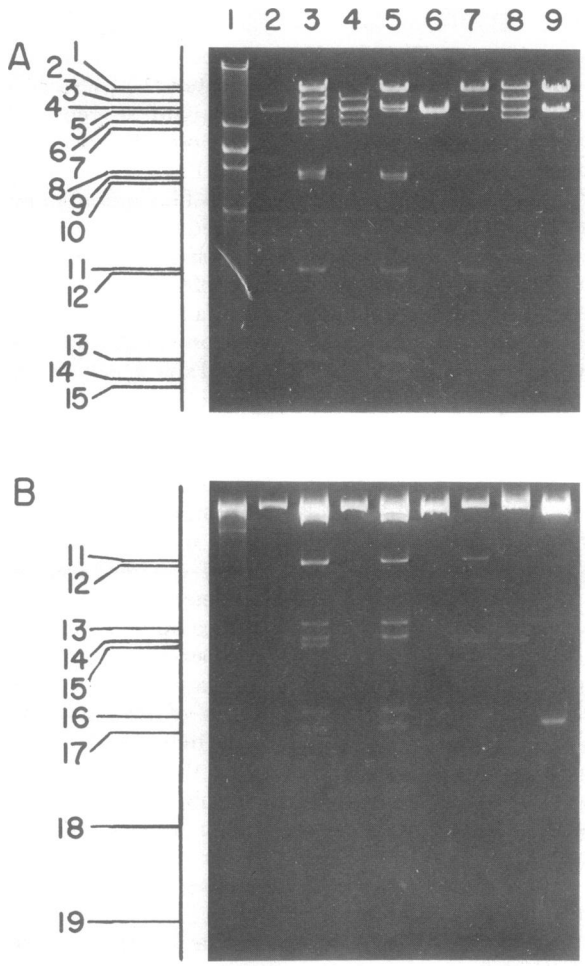

FIG. 1. Analysis of pRS plasmids by agarose gel electrophoresis. CCC plasmid DNA was digested to completion with *Eco*RI endonuclease and subjected to electrophoresis on 0.68% (A) and 1.58% (B) agarose slab gels. Plasmid DNAs in both A and B are 1, λ; 2, pSC101; 3, F; 4, pRS5; 5, pRS15; 6, pRS21; 7, pRS26; 8, pRS30; 9, pRS31. The distribution of F DNA fragments (well 3), numbered 1–19 in order of decreasing molecular weight, is diagramed at the side of each gel. Our interpretation of the data from these gels is based on the observation (37) that fragments of nearly the same molecular weight run as what appears to be a single band under some conditions. The duplicity of these bands is signalled by their relatively greater width and intensity of fluorescence (e.g., in well 3, fragments 1 and 2, 4 and 5, 8 and 9, and 11 and 12). Note also that pSC101 and fragment 5 appear as a single band in wells 4 and 6. We have resolved these fragments, in most cases, by varying the conditions of electrophoresis.

factor, an immunity to lethal zygosis (Ilz) which is independent of surface exclusion (ref. 15; R. A. Skurray, M. Achtman, N. S. Willetts, and P. Reeves, in preparation). Results from solid media tests showed the Sfx^+ strains, carrying the plasmids pRS26, pRS15, pRS31, to be insensitive to an excess of Hfr cells, indicating that they are immune. Apart from these two properties, this group of plasmids did not exhibit any of the other F-specified functions tested, including male-specific phage sensitivity, an indicator of F pili presence, or inhibition of female-specific phage (Table 1). In addition, none were self-transmissible (Tra^+) or sensitive to acridine orange (AO^s), as was the wild-type $Fhis^+$.

The introduction of a Fin^+ plasmid of the FinOP fertility inhibition system, such as R1, into an F-factor-carrying strain reduces not only the transfer of the F factor but also the expression of the *traS* gene and hence surface exclusion (34). Unlike the $Fhis^+$ strain, whose recipient ability increased approximately 300-fold, the Sfx^+ pRS plasmid strains retained their low levels of recipient ability in the presence of the FinOP system exerted by the R factor; R1 (R. A. Skurray and A. J. Clark, unpublished data). Ilz^+, which is independent of *traJ* control and of all known genes within the *tra* operon (R. A. Skurray, M. Achtman, N. S. Willets, and P. Reeves, in preparation), was found to be also independent of the FinOP system of R1, in the strains carrying $Fhis^+$ or any of the pRS plasmids.

The Sfx^- strains carrying the plasmids, pRS5, pRS21, or pRS30 were all Ilz^- (Table 1), Tra^-, and AO^r. However, two of these plasmids conferred other F-specified functions: one (pRS5), determined female-specific phage inhibition and another (pRS30), male-specific phage sensitivity (Table 1). As expected, the introduction of the Fin^+ plasmid, R1, did not affect the inhibition of T7 observed with strains carrying either $Fhis^+$ or pRS5, while it did abolish sensitivity to fd phage in both $Fhis^+$ and pRS30 strains (R. A. Skurray and A. J. Clark, unpublished data).

Electrophoresis of *Eco*RI Digest of pRS Plasmid DNA. Analysis of *Eco*RI fragments from F (35) and from F_8 (P17) (36) DNA has previously been described. In our experiments, the products from *Eco*RI digestion of CCC DNA isolated from the F^+ strain W1485 were separated into 19 bands on agarose gel electrophoresis (Fig. 1). The values for molecular weights of these fragments, determined from their mobilities in these gels relative to the six λ *Eco*RI fragments of known M_r (37), are in close agreement with values obtained by H. Ohtsubo and F. Ohtsubo (personal communication), who also determined that F produces 19 *Eco*RI fragments. The sum of the M_rs of these fragments is 64.8×10^6, which is in accord with the independently determined value of 62.5×10^6 for the M_r of F (28). As briefly stated earlier, CCC DNA isolated from each of the pRS plasmids yielded one or more fragments, in addition to a fragment corresponding to linear pSC101 DNA (M_r 5.8×10^6), when digested to completion with *Eco*RI endonuclease (Fig. 1). By comparison with the fragments of the parental F^+ plasmid, the pRS plasmid fragments could be assigned an F fragment number, from f1–f19, and hence the composition of each pRS plasmid could be determined (Table 2).

DISCUSSION

In this paper we have described the cloning of segments of DNA from the sex factor F. These segments, composed of one or more *Eco*RI fragments, could be employed to order, partially, the fragments of F. However, the complete *Eco*RI fragment order has already been established by H. Ohtsubo and E. Ohtsubo (personal communication) and we have employed their map as our standard of reference. One advantage of having this standard is that it serves to assess the degree to which random assortment of fragments occurred in the ligation step prior to transformation. The F fragments from each of the pRS plasmids could be arranged contiguously (Fig. 2) by comparison with the map of H. Ohtsubo and E. Ohtsubo. The same has been found for the *Eco*RI fragments of the seven pRS plasmids not discussed here. The absence of noncontiguous fragments indicates that random assortment of fragments was not frequent. We therefore presume these composite segments are derived from a partial digest fragment, rather than from limit digest fragments, although the latter possibility cannot be dismissed. F-specified properties of each pRS plasmid were also analyzed and these properties can now be considered in terms of the F segments specifying them.

Table 2. Composition of pRS plasmids*

Plasmid	Number of fragments	Fragments identified†
pSC101	1	pSC101
F	19	f1–f19
pRS5	5	f3, f5, pSC101, f6, f7
pRS21	2	f5, pSC101
pRS30	5	f1, f3, pSC101, f6, f15
pRS26	7‡	f1, f2, pSC101, f12, f15, f17 (f19)‡
pRS15	12‡	f1, f2, f4, pSC101, f9, f10, f12, f13, f14, f16, f17, (f19)‡
pRS31	4	f2, pSC101, f17, f19

* Fragment numbers and identities are from Fig. 1.

† The 19 F factor *Eco*RI fragments are designated f1-f19. Fragments are listed in order of decreasing molecular weight.

‡ f19 was undetected in pRS26 and pRS15; however, we assume these plasmids carry the contiguous set f17, f19, f2, as does pRS31 (see Fig. 2).

Plasmid-carrying strains were arbitrarily divided into two groups: (*i*) those carrying pRS26, pRS15, and pRS31 which were surface exclusion proficient (Sfx^+) and (*ii*) those carrying pRS5, pRS21, and pRS30 which were deficient in surface exclusion (Sfx^-). The Sfx^+ strains were also Ilz^+, $f2^r$, AO^r, Tra^-, $T7^s$, and fd^r while the Sfx^- strains were Ilz^-, $f2^r$, AO^r, and Tra^-. One of the latter strains was $T7^r$ fd^r while another was $T7^s$ fd^s.

Neither pRS21 nor pRS30, whose segments overlap to some extent with pRS5, possessed fragment f7 (Fig. 2) and it can be concluded that genes for female-specific phage inhibition (*fex*) (17) are encoded either on this fragment or at the *Eco*RI cleavage site between f7 and f5. This location is in agreement with the map position of 32.6–42.9 kb obtained by heteroduplex analysis (18). pRS30, in common with pRS5, carried f3 and f6; however, it also carried f15 and f1 in common with pRS26. Since strains carrying either pRS5 or pRS26 were insensitive to fd phage and presumably lacked F pili, then F pilus formation requires, at least, a segment with fragments f6, f15, f1 adjacent to one another (Fig. 2). This conclusion is in agreement with the kb locations (2) for the *tra* genes required for pilus formation and is further supported by complementation analysis. pRS30 was able to complement $F lac^+$ strains with point mutations in *traJ*, *traA*, *traL*, *traE*, *traB*, *traC*, *traF*, *traH*, or *traG* (R. A. Skurray and A. J. Clark, unpublished data). The control exerted by the FinOP system of the R factor, R1 (12), on fd sensitivity of the strain carrying pRS30 indicates that both *finP* and *traO* are encoded on pRS30, presumably on f6 to the left of *traJ* in accordance with the unpublished observations of Willetts *et al.*

The pRS plasmids carried in surface-exclusion-proficient strains each have the fragments f17, f19, and f2 in common, a segment which occupies the coordinates 82.3–1.71 kb (see Fig. 2). *traS*, whose expression is responsible for surface exclusion, has been mapped by Willetts (5) between *traG* and *traD*, which are located between 80.4 and 88.9 kb (2), and we can therefore place *traS* more accurately between 82.3 and 88.9 kb. Furthermore, each of these plasmids which conferred surface exclusion, and is presumably $traS^+$, has a DNA segment which terminates either to the right of, or at, 68.1 kb (Fig. 2), yet *traJ*, the positive control element necessary for the expression of *traS* (1, 3), is located to the left of 68.1 kb on the F map. One explanation for this seemingly anomalous expression is that pSC101 possesses a promotor near the *Eco*RI cleavage site and that inserted F segments would come under the control of that element. The failure of the FinOP system of R1 to inhibit surface exclusion in the pRS plasmids supports the conclusion that expression of *traS* in these plasmids is subject to control other than that of *traJ*.

Immunity to lethal zygosis is thought to be genetically complex, since one gene, *ilzA*, is located between *traG* and *traS* while a second gene, *ilzB*, lies outside of the *tra* operon to the right of *traI;* the presence of either gene confers the

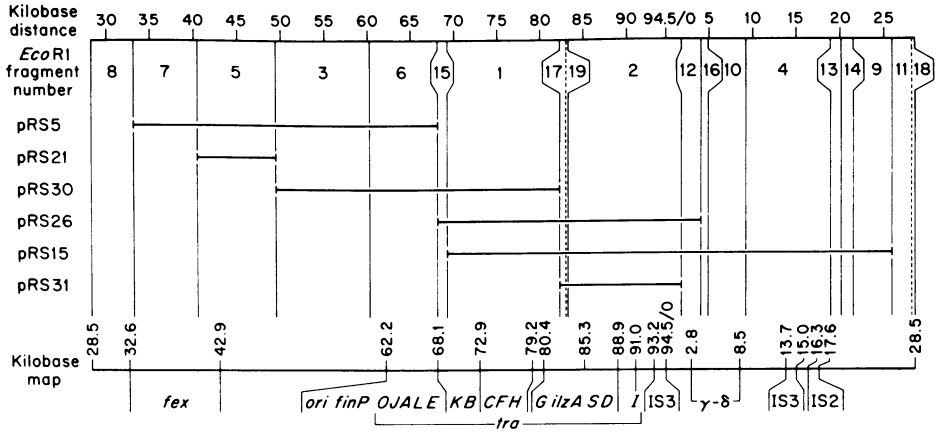

FIG. 2. F DNA segments of the pRS plasmids. *Eco*RI fragments from each pRS plasmid were arranged in a contiguous set by comparison with the *Eco*RI fragment map of F (H. Ohtsubo and E. Ohtsubo, personal communication). The lengths (supplied by Ohtsubo and Ohtsubo) of the 19 F fragments were adjusted to provide a summed length of 94.5 kb, in accordance with the previously determined value for F (28). The order of two adjacent fragments separated by a broken line on the *Eco*RI F map has not yet been determined (Ohtsubo and Ohtsubo). The kilobase map at the bottom provides a direct comparison of the *Eco*RI fragments with the known genes and sequences of F, derived from the following sources. The kb coordinates of the *tra* genes *A, L, E, K, B, C, F, H, G, D,* and *I* as well as the IS2, IS3 (38) and $\gamma\delta$ sequences are according to Davidson *et al.* (2). The coordinates of *traJ* and *ori* have recently been determined (M. Guyer, A. J. Clark, and N. Davidson, in preparation). *traS* is located between *traG* and *traD* (5) whereas *ilzA* is thought to be between *traG* and *traS* (R. A. Skurray, M. Achtman, N. S. Willetts, and P. Reeves, in preparation). *traO* lies within or to the immediate left of *traJ* while *finP* lies between *ori* and *traJ* (N. S. Willetts, J. Maule and S. MacIntire, personal communication). A strain carrying an F factor deleted from 32.6 to 42.9 kb is unable to inhibit female-specific phage multiplication (18). The gene(s) thought to be responsible for this inhibition have recently been designated *fex* (17).

Ilz^+ phenotype (R. A. Skurray, M. Achtman, N. S. Willetts, and P. Reeves, in preparation). Since all the plasmids that confer Ilz^+ (pRS26, pRS15, and pRS31) possess the segment 82.3–1.71 kb, at least *ilzA* and perhaps *ilzB* would be encoded on these plasmids.

Recently a DNA fragment carrying regions for replication and incompatibility has been cloned from $F lac^+$ by Timmis *et al.* (14) and by M. A. Lovett and D. R. Helinski (personal communication). The fragment that each group has cloned has a mobility corresponding to fragment f5 of F on agarose gel electrophoresis (R. A. Skurray, H. Nagaishi, and A. J. Clark, unpublished observations). Fragment f5 is present on pRS5 and pRS21 and the small reduction in recipient ability observed with strains carrying these plasmids, in crosses with an $F thr^+ leu^+$ donor, might be attributable to incompatibility encoded on this fragment. It could therefore be further reasoned that full expression of incompatibility requires the plasmid to be under the control of the F replicator rather than the pSC101 replicator, as is probably the case with these plasmids.

Data from physical and functional analyses of the other seven pRS plasmids, not discussed in this communication, support the locations of the F-specified properties discussed above.

We thank Dr. Eiichi Ohtsubo for providing us with the *Eco*RI fragment map of F which he presented at the ICN-UCLA Squaw Valley Symposium on Bacterial Plasmids, March 14–16, 1975, and for providing us with a revised map shortly before submission of this paper. We also thank Dr. Stan Cohen for pSC101, Dr. Herb Boyer for *Eco*RI, Dr. Paul Modrich for DNA ligase, and Linda Margossian for skillful assistance with the microtests. This investigation was supported by Public Health Service Research Grant no. AI-05371 from the National Institute of Allergy and Infectious Diseases to A.J.C. and by a California Division-American Cancer Society Fellowship no. J-275 to R.A.S.

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