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Replication Region Fragments Cloned from F*lac*$^+$ Are Identical to *Eco*RI Fragment f5 of F

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The replication region fragments from F*lac*$^+$ cloned in plasmids pSC138 and pML31 are identical with each other and with *Eco*RI fragment 5 of plasmid F.

Deoxyribonucleic acid (DNA) fragments which specify replication functions have recently been isolated from *Eco*RI endonuclease digests of F*lac*$^+$ by two groups (13, 17). The experimental approach by each group was similar and involved selection of a recombinant plasmid comprised of a nonreplicating DNA fragment carrying a gene determining antibiotic resistance and one (or more) F*lac*$^+$ *Eco*RI fragments. Timmis et al. (17), using an *Eco*RI fragment which specifies ampicillin (Ap) resist-

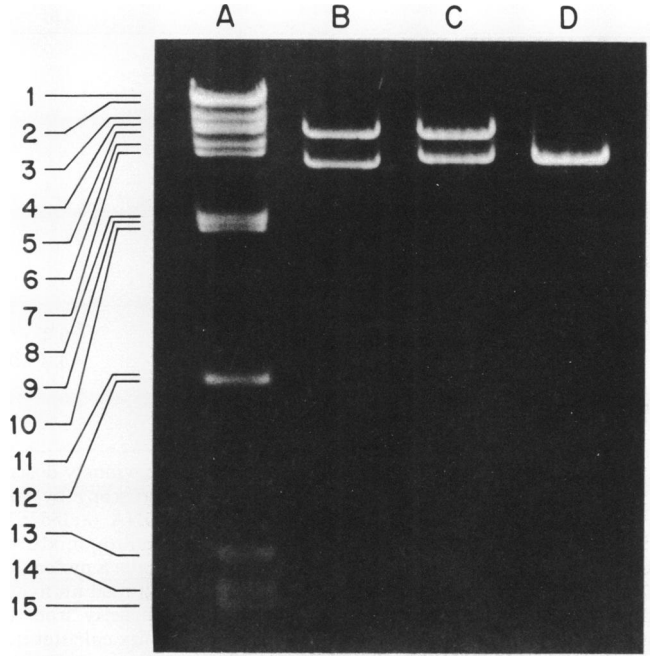

FIG. 1. *Analysis of pSC138 and pML31 DNA by agarose gel electrophoresis. DNA was digested to completion with EcoRI endonuclease and subjected to electrophoresis on 0.7% agarose slab gels (9, 16). DNAs are: A, F; B, pML31; C, pSC138; D, EcoRI Ap fragment. The pML31, pSC138, and F DNAs were isolated by the method of Sharp et al. (15) with modifications as noted previously (14). The ampicillin fragment DNA was isolated as described previously (17). The kanamycin fragment in pML31 (lower band, well B) has a mobility which corresponds to its previously determined molecular weight of 4.5 × 10^6 (9). Only 15 (numbered 1-15 in order of decreasing molecular weight) of the 19 EcoRI fragments of F (16; H. Ohtsubo and E. Ohtsubo, personal communication) are shown on this diagram.*

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ance, found that a unique F*lac*$^+$ fragment (f6) was recovered in 10 independently isolated recombinant plasmids; pSC138 is typical of the plasmids found by this group. A unique fragment, such as in pML31, was also found in three recombinants isolated by Lovett and Helinski (13), who employed a fragment determining kanamycin (Km) resistance as a selection vehicle.

Whereas functional analyses (copy number regulation, acridine orange sensitivity, incompatibility with F*lac*$^+$) of strains carrying pSC138 and pML31 indicated that the replicative fragments cloned in these plasmids were genetically similar, initial molecular weight estimations indicated that the fragments were different. The F*lac*$^+$ fragment of pSC138 was calculated to have a molecular weight of 5.2×10^6 (17), relative to PM2 double-stranded DNA, which was taken to have a molecular weight of 5.91×10^6 as originally reported (5). In contrast, the F*lac*$^+$ fragment of pML31 was reported to have a molecular weight of 6.0×10^6, based on its electrophoretic mobility in agarose gels and its sedimentation coefficient (13). Values of 5.2×10^6 and 6.0×10^6 are similar to the molecular weights of F *Eco*RI fragments f6 and f5, respectively (H. Ohtsubo and E. Ohtsubo, personal communication; Skurray, Nagaishi and Clark, unpublished data), both of which might be expected to determine replication functions: f5 overlaps the region 42.9 to 52 F

TABLE 1. *Molecular weights and lengths from contour measurements*[a]

DNA	Lambda standard 30.8×10^6	PM2 standard[b]	
		5.91×10^6	6.58×10^6
PM2	$6.58 \pm 0.18 \times 10^6$ (55) 9.9 kb		
pSC122 (pSC101 + *Eco*RIAp fragment)	$10.56 \pm 0.25 \times 10^6$ (62) 16.0 kb		
pSC101	$6.02 \pm 0.37 \times 10^6$ (82) 9.1kb		
*Eco*RI Ap fragment[c]	4.54×10^6 6.9 kb		
pSC138	$10.66 \pm 0.43 \times 10^6$ (58) 16.1 kb	9.4×10^6 14.2 kb	10.47×10^6 15.8 kb
*Eco*RI replication fragment of F*lac*$^+$[d]	6.1×10^6 9.2 kb		5.9×10^6 8.9 kb

[a] The pSC101 (1), pSC122, and pSC138 (17) DNAs were isolated as previously described. PM2 viral DNA (5) was a gift of R. T. Espejo. DNA was prepared for electron microscopy by the aqueous method of Kleinschmidt (11) as described by Davis et al. (4). Intracellular λ DNA (λcI857S7), provided by D. A. Clayton, originally obtained from M. Thomas, was included in the spreading mixtures as a standard. Only circular λ DNA molecules were measured, and these were assumed to have a molecular weight of 30.8×10^6 and a contour length of 46.5 kb (3, 15); calculation of kilobase pairs assumed an average base pair residue weight of 662 daltons. DNA molecular measurements were made directly from 35-mm film that was projected onto a sonic digitizer board linked to a programmable Tektronix calculator. Direct comparison of measured lengths of plasmid or PM-2 DNA molecules to λ DNA molecules present in the same frame of photographic film permitted the calculation of molecular weight and kilobase pair values. The first value represented for each DNA is the molecular weight and its standard deviation; the second value is its contour length in kilobase pairs. The number in parentheses indicates the number of measurements.

[b] The molecular weight for the DNA of plasmid pSC138 originally reported as 9.4×10^6 (17) was based on a published molecular weight of 5.91×10^6 for PM2 viral DNA (5), which was used as a reference standard. However, using a λ DNA standard in the present studies, the molecular weight of PM2 was determined to be 6.58×10^6 and the contour length, 9.9 kb, in agreement with the values reported by Sharp et al. (15) and Kriegstein and Hogness (12). Using this alternative molecular weight value for PM2, adjustment of the previously published molecular weight value of pSC138 results in a value of 10.5×10^6. This value is in close agreement with a molecular weight of 10.7×10^6 obtained by direct comparison of pSC138 DNA with λ DNA.

[c] The molecular weight and contour length of the *Eco*RI Ap fragment were obtained from the differences between the values for pSC122 and pSC101.

[d] The molecular weight and contour length of the *Eco*RI replication fragment of F*lac*$^+$ in pSC138 were obtained from the differences between the values for pSC138 and the *Eco*RI AP fragment.

which is considered to contain the genes for autonomous maintenance of F (2), and f6 is thought to include *oriT*, a sequence necessary for the initiation of conjugal transfer replication (7, 18; Clark, Crisona, Nagaishi, and Skurray, in press; Guyer, Davidson, and Clark, manuscript in preparation). Thus, one possibility was that pSC138 and pML31 contain f6 and f5, respectively, of F. However, in view of the fact that PM2 DNA has recently been assigned molecular weights greater than that originally reported (6, 12, 15), an alternative possibility was that pSC138 and pML31 both contain f5 of F.

We compared pSC138 and pML31 directly. Figure 1 shows the electrophoretic patterns of the products of an *Eco*RI digestion of pSC138, pML31, and F DNA. Both pSC138 and pML31 DNA contain a fragment corresponding in mobility to f5 of F (molecular weight of 6.15×10^6 relative to λ DNA under these conditions (Skurray, Nagaishi, and Clark, unpublished data), in addition to the Ap or Km fragment, respectively. In light of this result, we reestimated the molecular weight of the F*lac*$^+$ fragment of pSC138. For this purpose, the well-characterized DNA of bacteriophage lambda was used as an independent molecular weight standard for electron microscopy. A value of 6.1×10^6 was obtained for the molecular weight of the pSC138 F fragment (Table 1). Also, by comparison with lambda DNA, the molecular weight of PM2 DNA was calculated to be 6.6×10^6, in agreement with the value recently obtained by Sharp et al. (15) and Kriegstein and Hogness (12). Adjustment of the originally pub-

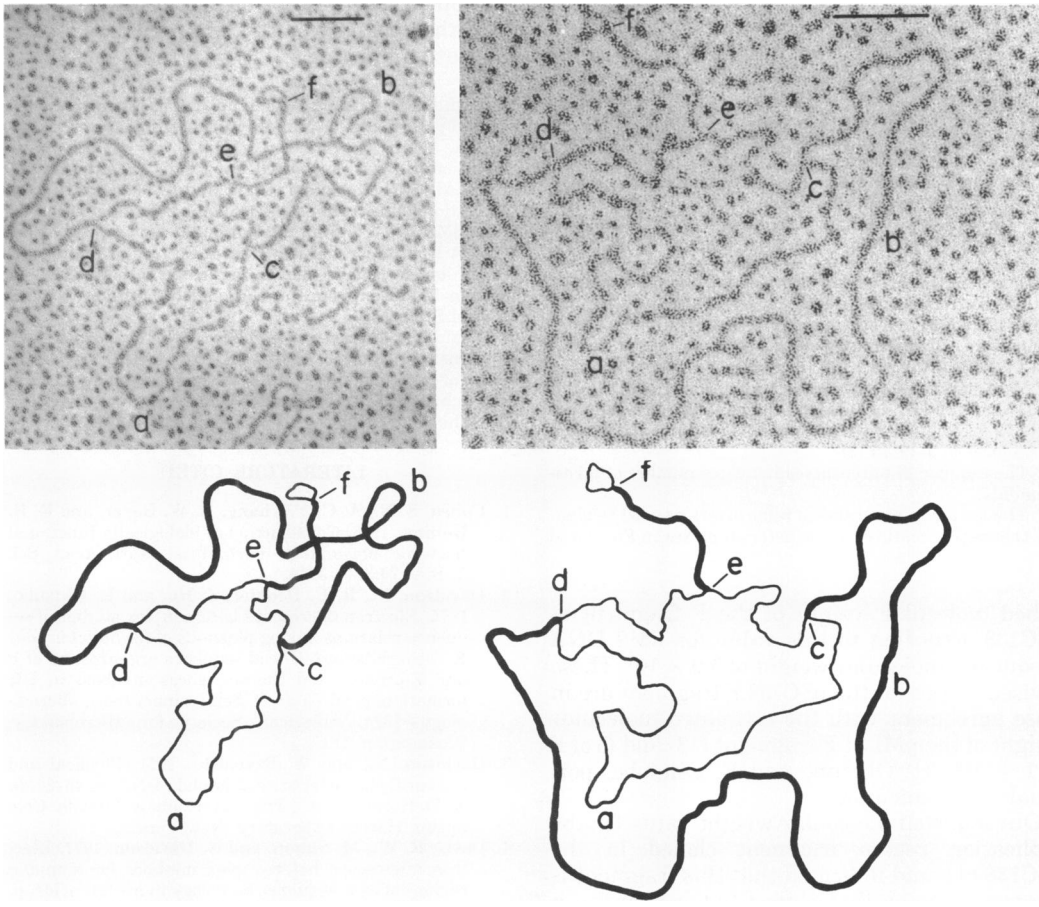

FIG. 2. *Electron micrographs of two heteroduplexes between single strands of pML31 and pSC138 DNA. DNA was mixed, denatured, renatured, and mounted for electron microscopy by the formamide technique (14). Micrographs were analyzed as previously described (8); tracings of each heteroduplex are shown. The arrows e and f denote the ends of the inverted repeat of pML31 used as the reference feature. The arrows c, b, and d denote the region of homology between pML31 and pSC138. The bar represents a length of 0.5 kb.*

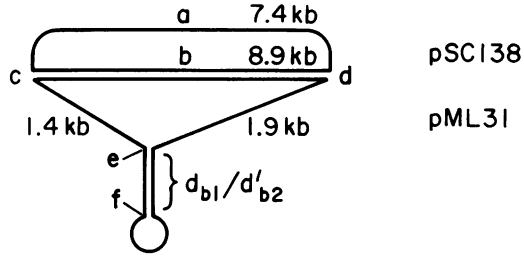

FIG. 3. *Diagrammatic representation of the pML31/pSC138 heteroduplex from Fig. 2. The symbols a through f denote the same points on the heteroduplex as they do in Fig. 2 and Table 2. The inverted duplication of pML31 is labeled "d_{b_1}/d'_{b_2}" in accordance with established convention (8, 10).*

TABLE 2. *Molecular lengths from heteroduplex analysis*

Feature	Molecular length (kb)[a]	
	pML31/pSC138	pML31/F8-33[b]
Loop of inverted repeat	0.9 ± 0.2 (21)[c]	1.0 ± 0.1
Stem of inverted repeat (ef)[d]	1.0 ± 0.1 (21)	1.0 ± 0.1
Shorter single-stranded arm from inverted repeat (fc)	1.4 ± 0.2 (21)	1.5 ± 0.1
Longer single-stranded arm from inverted repeat (fd)	1.9 ± 0.2 (20)	2.3 ± 0.2
Long duplex (cbd)	8.9 ± 0.8 (23)	9.0 ± 0.2
Long single strand (cad)	7.4 ± 0.6 (20)	–

[a] The lengths of double-stranded and single-stranded features were measured relative to φX174 RFII and φX174 single-stranded circles, respectively. The relative measurements were converted to absolute lengths by taking the size of φX174 DNA to be 5.1 kb.

[b] Values from heteroduplex analysis between pML31 and F8-33 DNA (8) are included for convenience. The long duplex of 9.0 kb between pML31 and F8-33 is equivalent to the F sequence 40.3–49.3 F(8).

[c] The number in parentheses indicates number of measurements.

[d] The letters in parentheses refer to letters used to identify the specific features of the heteroduplexes in Fig. 2 and 3.

lished molecular weight of the F fragment of pSC138 according to this value for PM2 DNA produces a molecular weight of 5.9×10^6. These revised values for the pSC138 F fragment are in close agreement with the estimated molecular weight of the pML31 F fragment (13) and f5 of F (6.1×10^6, H. Ohtsubo and E. Ohtsubo, personal communication).

Our adjusted molecular weight value for the replication region fragment cloned in the pSC138 plasmid indicated that this fragment is the same one as that cloned independently in pML31. To determine directly whether pSC138 and pML31 contain the same polynucleotide sequence of F*lac*$^+$, the plasmids were examined by heteroduplex analysis. The heteroduplex between pSC138 and pML31 (Fig. 2, 3) contains duplex regions of 8.9 kb in length (Table 2), a value similar to that determined for the length of f5 of F (8; H. Ohtsubo and E. Ohtsubo, personal communication). The location, on pML31, of the sequence homologous with pSC138 was determined relative to the inverted repeat structure d_{b_1}/d'_{b_2} (Fig. 2, 3). This location (Table 2) is identical to the location of the sequence on pML31 which is homologous to the 40.3 to 49.3 sequence of F (8). Thus, both pSC138 and pML31 contain the F sequence 40.3 to 49.3 F which has been shown to be equivalent to *Eco*RI fragment f5 (H. Ohtsubo and E. Ohtsubo, personal communication).

*Eco*RI f5 of F, which from this study and from the work of R. Thompson (personal communication) corresponds to *Eco*RI f6 of F*lac*$^+$, is thus, to date, the only *Eco*RI F fragment identified which is capable of supporting autonomous replication. These results do not preclude, however, the existence of a second region on F with replicative function.

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LITERATURE CITED

1. **Cohen, S. N., A. C. Y. Chang, H. W. Boyer, and R. B. Helling.** 1973. Construction of biologically functional bacterial plasmids *in vitro*. Proc. Natl. Acad. Sci. U.S.A. 70:3240–3244.
2. **Davidson, N., R. C. Deonier, S. Hu, and E. Ohtsubo.** 1975. Electron microscope heteroduplex studies of sequence relations among plasmids of *Escherichia coli*. X. Deoxyribonucleic acid sequence organization of F and F-primes, and the sequences involved in Hfr formation, p. 56–65. *In* D. Schlessinger (ed.), Microbiology–1974. American Society for Microbiology, Washington, D.C.
3. **Davidson, N., and W. Szybalski.** 1971. Physical and chemical characteristics of lambda DNA, p. 45–82. *In* A. D. Hershey (ed.), The bacteriophage lambda, Cold Spring Harbor Laboratory, New York.
4. **Davis, R. W., M. Simon, and N. Davidson.** 1971. Electron microscope heteroduplex methods for mapping regions of base sequence homology in nucleic acids, p. 413–428. *In* L. Grossman and K. Moldave (ed.), Methods in enzymology, vol. 21. Academic Press Inc., New York.
5. **Espejo, R. T., E. S. Canelo, and R. L. Sinsheimer.** 1969. DNA of bacteriophage PM2: a closed circular double-stranded molecule. Proc. Natl. Acad. Sci. U.S.A. 63:1164–1168.

6. **Gabain, A. von, G. S. Hayward, and H. Bujard.** 1976. Physical mapping of the *Hind* III, *Eco*RI, *Sal*, and *Sma* restriction endonuclease cleavage fragments from bacteriophage T5 DNA. Mol. Gen. Genet. 143:279-290.
7. **Guyer, M. S., and A. J. Clark.** 1976. *cis*-Dominant, transfer deficient mutants of the *Escherichia coli* K-12 sex factor. J. Bacteriol. 125:233-247.
8. **Guyer, M. S., D. Figurski, and N. Davidson.** 1976. Electron microscope study of a plasmid chimera containing the replication region of the *Escherichia coli* F plasmid. J. Bacteriol. 127:988-997.
9. **Hershfield, V., H. W. Boyer, C. Yanofsky, M. A. Lovett, and D. R. Helinski.** 1974. Plasmid ColE1 as a molecular vehicle for cloning and amplication of DNA. Proc. Natl. Acad. Sci. U.S.A. 71:3455-3459.
10. **Hu, S., E. Ohtsubo, N. Davidson, and H. Saedler.** 1975. Electron microscope heteroduplex studies of sequence relations among bacterial plasmids: identification and mapping of the insertion sequences IS1 and IS2 in F and R plasmids. J. Bacteriol. 122:764-775.
11. **Kleinschmidt, A. K.** 1968. Monolayer techniques in electron microscopy of nucleic acid molecules, p. 361-377. *In* L. Grossman and K. Moldave (ed.), Methods in enzymology, vol. 12. Academic Press Inc., New York.
12. **Kriegstein, H. J., and D. S. Hogness.** 1974. Mechanism of DNA replication in *Drosophila* chromosomes: structure of replication forks and evidence for bidirectionality. Proc. Natl. Acad. Sci. U.S.A. 71:135-139.
13. **Lovett, M. A., and D. R. Helinski.** 1976. Method for the isolation of the replication region of a bacterial replicon: construction of mini-F' *km* plasmid. J. Bacteriol. 127:982-987.
14. **Ohtsubo, E., R. C. Deonier, H. J. Lee, and N. Davidson.** 1974. Electron microscope heteroduplex studies of sequence relations among plasmids of *Escherichia coli*. IV. The F sequence of F 14. J. Mol. Biol. 89:565-583.
15. **Sharp, P. A., M-T Hsu, E. Ohtsubo, and N. Davidson.** 1972. Electron microscope heteroduplex studies of sequence relations among plasmids of *Escherichia coli* I. Structure of F-prime factors. J. Mol. Biol. 71:471-497.
16. **Skurray, R. A., H. Nagaishi, and A. J. Clark.** 1976. Molecular cloning of DNA from F sex factor of *Escherichia coli* K-12. Proc. Natl. Acad. Sci. U.S.A. 73:64-68.
17. **Timmis, K., F. Cabello, and S. N. Cohen.** 1975. Cloning, isolation, and characterization of replication regions of complex plasmid genomes. Proc. Natl. Acad. Sci. U.S.A. 72:2242-2246.
18. **Willetts, N. S.** 1972. Location of the origin of transfer of the sex factor F. J. Bacteriol. 112:773-778.