JOURNAL OF VIROLOGY, Sept. 1976, p. 1006-1011
Vol. 19, No. 3
Copyright © 1976 American Society for Microbiology
Printed in U.S.A.

Cleavage of λ DNA by a Site-Specific Endonuclease from *Serratia marcescens*

REGINALD H. McPARLAND, LYLE R. BROWN, AND GEORGE D. PEARSON*

Department of Biochemistry and Biophysics and Department of Microbiology, Oregon State University, Corvallis, Oregon 97331*

Received for publication 10 May 1976

Three sites recognized by *Sma*I endonuclease, purified from *Serratia marcescens* SB, have been located on λ DNA at 0.406, 0.656, and 0.825 fractional lengths from the left end of the DNA molecule.

The genetic and physical structure of the λ bacteriophage chromosome is known in considerable detail (8). Recently, the sites recognized by *Eco*RI endonuclease have been mapped on λ DNA (1, 7, 21). We report here the locations on λ DNA of sites recognized by *Sma*I endonuclease purified from *Serratia marcescens* SB. We discuss the significance of the *Sma*I cleavage map with respect to the genetic map and to the construction of transducing vehicles (14, 17, 20).

MATERIALS AND METHODS

Phage and DNA. λcI857S7 was induced from a lysogen, *Escherichia coli* strain CSH45 (13). The cells were grown, the prophage were induced, the phage were purified, and the DNA was extracted as previously described (13). The purification of adenovirus DNA has also been described (15). R. T. Kovacic provided *Hae*III fragments of PM2 DNA (22).

Enzymes and assays. *Eco*RI endonuclease was purified by the method of Greene et al. (5). *Eco*RI endonuclease reactions were usually carried out at 37°C for 1 h in 10 mM Tris (pH 7.2), 100 mM KCl, 1 mM EDTA, and 10 mM $MgCl_2$. Reactions were stopped by adjusting the solutions to 20 mM EDTA and 10% glycerol. *Sma*I endonuclease was purified from *S. marcescens* SB, using a modification of a procedure developed by C. Mulder (personal communication). A detailed account will be published (McParland, McLean, Brown, and Pearson, manuscript in preparation). *Sma*I endonuclease reactions were carried out as described above except that the solutions contained 5 mM $MgCl_2$. Reactions with *Eco*RI and *Sma*I together also only contained 5 mM $MgCl_2$. In general, 2 μl of *Eco*RI or *Sma*I endonuclease was sufficient to cleave 1 μg of λ DNA in a total volume of 50 μl. Before electrophoresis, digested DNA was heated to 70°C for 3 min to melt hydrogen-bonded (cohesive) ends of λ molecules and then cooled on ice.

Gel electrophoresis. Agarose (Bio-Rad Laboratories) was dissolved in E buffer (12) containing 0.5 μg of ethidium bromide per ml by boiling at 100°C. Slab gels were cast with the following dimensions: 20 cm high, 22 cm wide, and 0.3 cm thick. The running buffer, E buffer, contained 0.4 μg of ethidium bromide per ml. The gel compositions, voltages, and durations of electrophoresis are detailed in the appropriate figure legends. DNA bands were visualized by the fluorescence from bound ethidium bromide under long-wave UV irradiation. The gels were photographed with a Polaroid camera equipped with Tiffen Photar orange and haze 2-A filters using type 107 film. The photographic images have been reversed (i.e., the bright bands against a dark background appear as dark bands against a light background.)

Purification of *Eco*RI and *Sma*I fragments. λ DNA was digested with either *Eco*RI or *Sma*I endonuclease. *Sma*I fragments were separated on 0.5% agarose-ethidium bromide tube gels (6 mm by 30 cm) by electrophoresis at 5 mA/gel for 6 h. *Eco*RI fragments were separated on 0.7% agarose-ethidium bromide tube gels under the same conditions. DNA bands were sliced from the gels. The slices were frozen at −20°C, mascerated with a glass rod, and centrifuged at 12,000 rpm for 10 min in a Sorvall SS-34 rotor. The liquid was retained, and the pellet was twice extracted with 2 ml of digestion buffer (10 mM Tris, 100 mM KCl, and 1 mM EDTA, pH 7.2). The DNA in the combined solutions was precipitated with ethanol and dissolved in digestion buffer at a concentration of 0.5 μg/μl. The purity of each fragment was checked by gel electrophoresis. All fragments except *Eco*RI fragments 5 and 6 (and one preparation of *Sma*I-D) were contaminated with trace amounts of other fragments. They were used without further purification.

RESULTS

Digestion of λ DNA with *Sma*I endonuclease produced four limit fragments, lettered A through D (Fig. 1, slot 1; the largest fragment in this slot is a partial digestion product). By comparison to *Eco*RI fragments of λ DNA, numbered 1 through 6 (Fig. 1, slots 3 and 4), the *Sma*I fragments were smaller than *Eco*RI-1 (13.7×10^6 daltons) but larger than *Eco*RI-2 (4.67×10^6 daltons). The combined action of *Sma*I and *Eco*RI endonucleases is shown in Fig. 1, slot 2. The following fragments were gener-

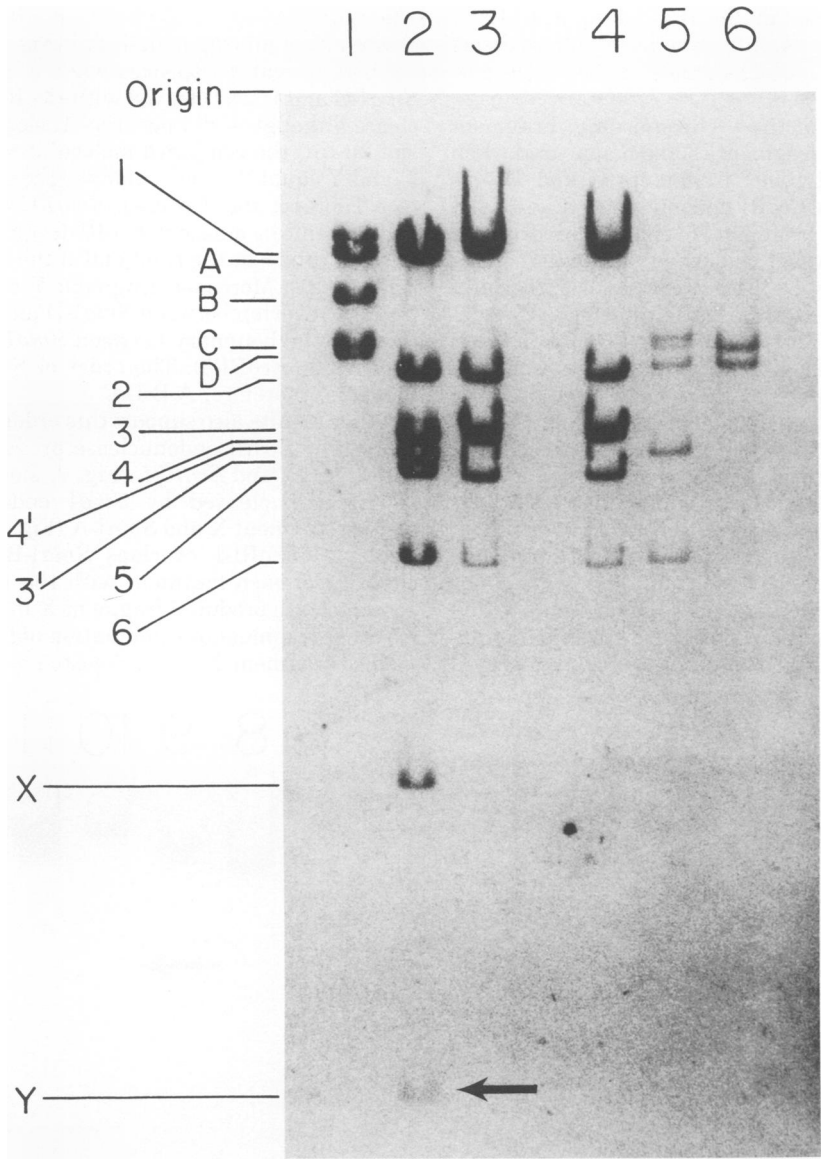

FIG. 1. *Agarose gel electrophoresis of λ DNA after digestion with restriction endonucleases. Migration was from top (cathode) to bottom (anode). Electrophoresis was for 15 h at 50 V in a 1% agarose-ethidium bromide slab gel. SmaI fragments are labeled A through D. EcoRI fragments are numbered 1 through 6 (reference 7). Fragments 3', 4', X, and Y are produced by digestion with both SmaI and EcoRI endonucleases. (1) SmaI digest of λ DNA. The top band is a partial digestion product. (2) SmaI and EcoRI double digest of λ DNA. The arrow indicates fragment Y. (3 and 4) EcoRI digests of λ DNA. (5) EcoRI digest of a mixture of SmaI fragments C and D. The largest bands are intact fragments C and D. (6) EcoRI digest of SmaI fragment D. The largest fragment is intact D.*

ated: *Sma*I-A, *Eco*RI fragments 2, 5, and 6, and at least four new fragments designated 3', 4', X, and Y.

Left end of the λ chromosome. The left and right ends of the λ chromosome have already been identified as *Eco*RI fragments 1 and 6, respectively (7, 21). *Sma*I-A (12.5×10^6 daltons, see Table 1 below) is also the left end since: (i) it was not cleaved by *Eco*RI endonuclease (i.e., it must be contained entirely within an *Eco*RI fragment); (ii) only *Eco*RI-1 is large enough to contain it; (iii) if it were not, there would be an

*Sma*I fragment smaller than or equal to 1.2×10^6 daltons (we would have been able to detect fragments at least as small as 0.4×10^6 daltons).

Right end of the λ chromosome. Fragment 3′ and *Eco*RI fragments 2 and 6 appeared when a mixture of *Sma*I fragments C and D was digested with *Eco*RI endonuclease (Fig. 1, slot 5; the largest fragments in this slot are intact C and D). However, cleavage of *Sma*I-D alone produced only *Eco*RI-2 (Fig. 1, slot 6; the largest fragment in this slot is intact D). We conclude that *Sma*I-C contains *Eco*RI-6 and is, therefore, the right end of the λ chromosome.

Order of internal fragments. In addition to the fragments listed above, cleavage of combined *Sma*I fragments C and D with *Eco*RI endonuclease yielded fragment Y, which could be detected when a greater amount of DNA was electrophoresed (Fig. 2, slot 2). Fragments 3′, 4′, and Y appeared when a mixture of *Eco*RI fragments 3 and 4 was digested with *Sma*I endonuclease (Fig. 2, slot 8). Fragments 3′ and Y most likely represent the entire *Eco*RI fragment 3 because: (i) neither 3′ nor Y appeared when *Sma*I-B was cleaved with *Eco*RI endonuclease although fragment 4′ did (Fig. 2, slot 3); (ii) both 3′ and Y appeared when a mixture of *Sma*I-C and D was cleaved with *Eco*RI endonuclease although 4′ did not (Fig. 1, slot 5; Fig. 2, slot 2); (iii) the combined molecular weights of 3′ and Y equal the molecular weight of *Eco*RI-3 (see Tables 2 and 3 below). *Sma*I-C and D together contain adjacent *Eco*RI fragments 2, 3 (3′ + Y), and 6 at the right end of the λ chromosome (7, 21). Moreover, fragment Y must arise from the overlap between *Sma*I-D and *Eco*RI-3 (that is, the boundary between *Sma*I-C and D falls inside *Eco*RI-3). The order of *Sma*I fragments is, therefore, A-B-D-C.

Other results also support this order. *Sma*I-B cleaved by *Eco*RI endonuclease produced fragments 4′, X, and *Eco*RI-5 (Fig. 2, slot 3; Table 1). *Eco*RI-1 cleaved by *Sma*I endonuclease yielded fragment X and *Sma*I-A (Fig. 2, slot 10; Table 2). *Eco*RI-1 overlaps *Sma*I-B because cleavage of each fragment with the reciprocal endonuclease produced fragment X in common. No other fragment or combination of fragments yielded fragment X when digested with either

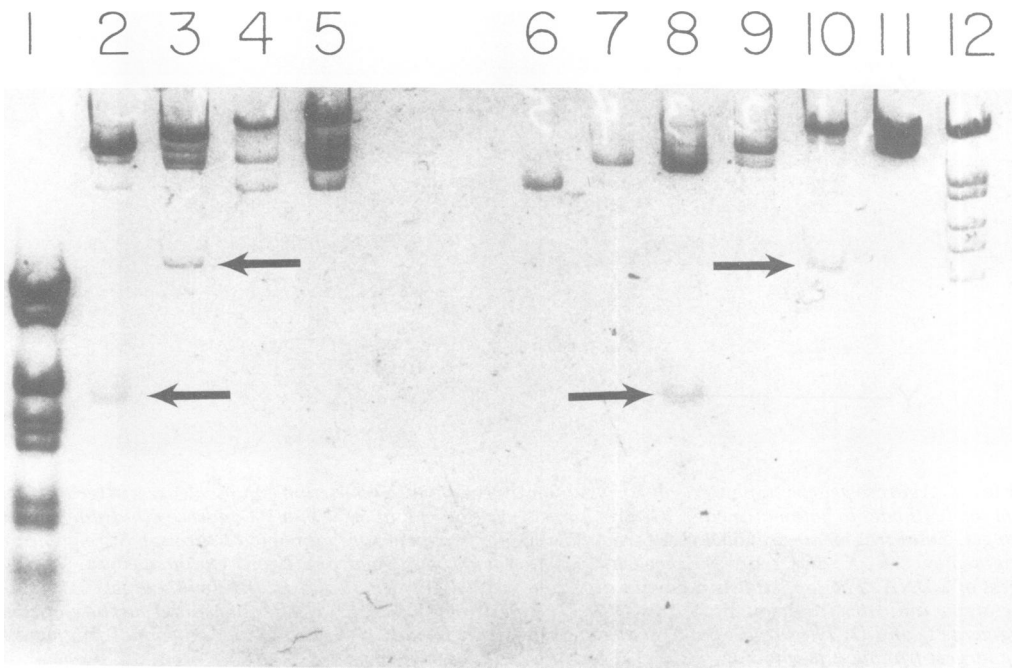

FIG. 2. *Redigestion of isolated SmaI and EcoRI fragments of λ DNA. Migration was from top to bottom. Electrophoresis was for 3 h at 40 V in a 1.5% agarose-ethidium bromide slab gel. (1) HaeIII digest of PM2 DNA (reference 22). (2) EcoRI digest of a mixture of SmaI fragments C and D. The arrow indicates fragment Y. (3) EcoRI digest of SmaI fragment B. The arrow indicates fragment X. (4) EcoRI digest of SmaI fragment A. (5) EcoRI digest of intact λ DNA. (6) SmaI digest of EcoRI fragment 6. (7) SmaI digest of EcoRI fragment 5. (8) SmaI digest of a mixture of EcoRI fragments 3 and 4. The arrow indicates fragment Y. (9) SmaI digest of EcoRI fragment 2. (10) SmaI digest of EcoRI fragment 1. The arrow indicates fragment X. (11) SmaI digest of intact λ DNA. (12) EcoRI digest of adenovirus type 2 DNA (reference 16).*

TABLE 1. *Cleavage of SmaI fragments with EcoRI endonuclease*

Fragment	Digestion products[a]	Measured molecular weight[b] ($\times 10^{-6}$)	Calculated molecular weight[c] ($\times 10^{-6}$)
A	Not cleaved	12.8 ± 0.5[d]	12.5[e]
B	4', 5, X	7.85 ± 0.3	7.7
C	3', 6	5.5 ± 0.2	5.3 (5.4)[f]
D	2, Y, (Z)[g]	5.25 ± 0.1	5.2
		Σ31.4	30.7 (30.8)

[a] See Fig. 1 for labeling of fragments. The data are from Fig. 1 and 2 and other unpublished gels.
[b] See Fig. 3. The molecular weight of each fragment was calculated from measurements on at least four separate gels.
[c] Sum of *Eco*RI products from each *Sma*I fragment.
[d] Standard error of the mean.
[e] *Eco*RI fragment 1 minus fragment X.
[f] Sum of *Eco*RI fragments 3 and 6 minus fragment Y.
[g] Not observed on gels.

TABLE 2. *Cleavage of EcoRI fragments with SmaI endonuclease*

Fragment	Molecular weight[a] ($\times 10^{-6}$)	Digestion products[b]
1	13.7	A, X
2	4.67	Not cleaved
3	3.71	3', Y
4	3.57	4', (Z)[c]
5	3.04	Not cleaved
6	2.11	Not cleaved

[a] From Helling et al. (7). These values do not differ significantly from other estimates of the molecular weights (2, 4).
[b] See Fig. 1 for labeling of fragments. The data are from Fig. 1 and 2 and other unpublished gels.
[c] Not observed on gels.

endonuclease. Thus, *Sma*I-A (contained in *Eco*RI-1) is adjacent to *Sma*I-B (which contained *Eco*RI-5, the fragment adjacent to *Eco*RI-1 [7, 21]), and the order of *Sma*I fragments is again A-B-D-C.

Sizes of fragments. Fragments were sized by electrophoresis on agarose gels using *Eco*RI fragments of type 2 adenovirus DNA (16) or λ DNA (7, 21) or *Hae*III fragments of PM2 DNA (22) as molecular weight standards. These overlapping sets of fragments span a size range from 55 base pairs to greater than 20,000 base pairs. In Fig. 3 the logarithm of the molecular weight of each marker fragment is plotted against the distance migrated. The molecular weights of fragments generated by *Sma*I endonuclease alone (Fig. 3B; Table 1) or in combination with *Eco*RI endonuclease (Fig. 3A and B; Table 3) were determined from the fragment positions relative to standards in the same gel. The sum of the molecular weights of the four *Sma*I fragments is 31.4 × 10^6, which is within 2% of 30.8 × 10^6 (3), the molecular weight of the λ chromosome. We believe there is another double digestion product, designated fragment Z, that has not yet been detected. Together with fragment 4', it ought to have been produced from *Eco*RI-4 after digestion with *Sma*I endonuclease (Fig. 2, slot 8). From the difference in molecular weight between *Eco*RI-4 and fragment 4', we estimated Z to be 0.1 × 10^6 daltons or 150 base pairs (Table 3). Since the large *Sma*I fragments migrated in a region of the gel where the standard curve was not linear (Fig. 3B), their sizes were also calculated from the molecular weights of their *Eco*RI cleavage products (Table 1).

Cleavage map. *Sma*I endonuclease cleaved *Eco*RI fragments 1, 3, and 4 (Table 2). The distances between adjacent *Sma*I and *Eco*RI sites (and, therefore, the positions of *Sma*I sites) can be measured directly from the sizes of

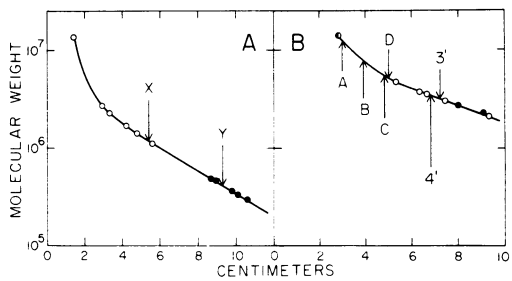

FIG. 3. *Molecular weights of restriction fragments determined by agarose gel electrophoresis. Migration is from left to right. (A) Electrophoresis was for 7 h at 40 V in a 1.5% agarose-ethidium bromide slab gel. Symbols: ○, EcoRI fragments of adenovirus type 2 DNA (reference 16); ●, HaeIII fragments D, E, F, G, and H of PM2 DNA (reference 22). (B) Electrophoresis was for 18 h at 50 V in a 1% agarose-ethidium bromide slab gel. Symbols: ○, EcoRI fragments of λ DNA (reference 7); ●, EcoRI fragments A, B, and C of adenovirus type 2 DNA.*

TABLE 3. *Fragments produced by double digestion*

Fragment[a]	Molecular weight[b] ($\times 10^{-6}$)
3'	3.17 ± 0.05[c]
4'	3.47 ± 0.05
X	1.17 ± 0.03
Y	0.42 ± 0.02
(Z)[d]	(0.1)[e]

[a] See Fig. 1 for labeling of fragments.
[b] See Fig. 3. The molecular weight of each fragment was calculated from measurements on at least four separate gels.
[c] Standard error of the mean.
[d] Not observed on gels.
[e] *Eco*RI fragment 4 minus fragment 4'.

the digestion products. Since the absolute error in determining the molecular weights of fragments on agarose gels decreases logarithmically with increasing distance migrated, we used the small fragments X, Y, and Z (actually 4', see above). Thus, the first *Sma*I site from the left end is located at 0.445 (the right coordinate of *Eco*RI-1) − 0.039 (the size of fragment X) = 0.406 fractional lengths. Similarly, the remaining *Sma*I sites were mapped at 0.656 and 0.825 fractional lengths. Fig. 4 shows the cleavage map of λ DNA.

DISCUSSION

*Sma*I endonuclease cleaved λ DNA into four fragments. The fragments were ordered by analyzing the products generated by the reciprocal cleavage of purified *Sma*I and *Eco*RI fragments. The fragments were sized by agarose electrophoresis. Since the relationship between the logarithm of the molecular weight and the distance migrated was not linear for fragments larger than 5×10^6 daltons, the molecular weights of *Sma*I fragments were also calculated from the sizes of their *Eco*RI cleavage products. The results are summarized in Fig. 4, which compares the *Sma*I and *Eco*RI cleavage maps. During the preparation of this manuscript, we learned that James et al. (9) had independently mapped the *Sma*I cleavage sites on λ DNA. Both maps agree.

When compared with the physical gene map of λ (3), several features of the *Sma*I cleavage map are noteworthy. *Sma*I-A contains all of the late structural genes. *Sma*I-B spans a dispensible region including the λ attachment site: for example, the nonlethal deletion *b*221 extends from 0.406 to 0.629. *Sma*I-D contains all of the early control genes as well as *O* and *P*, genes essential for λ DNA replication. *Sma*I-C contains the late control gene *Q* and the lysis functions *S* and *R*.

Unlike *Eco*RI endonuclease (6, 12), *Sma*I endonuclease does not create cohesive ends (9). Recently, the sequence of the *Sma*I site was found to be

5'-CCC|GGG-3'
3'-GGG|CCC-5'

(S. Endow and R. J. Roberts, manuscript in preparation). However, an isoschizomer purified from *Xanthomonas malvacearum*, *Xma*I endonuclease, produces cohesive ends with the sequence

5'-C|CCGG G-3'
3'-G GGCC|C-5'

(S. Endow and R. J. Roberts, manuscript in preparation). Thus, a mutant of λ lacking the *Xma*I (*Sma*I) site at 0.825 could be developed as a vehicle to transduce foreign DNA inserted in place of *Xma*I-B (*Sma*I-B). Transducing vehicles lacking *Eco*RI sites at 0.811 and 0.932 have already been constructed (14, 17, 20) to accommodate foreign DNA inserted in place of *Eco*RI fragments 4 and 5 (2, 20).

Matsubara and Kaiser (11) isolated λdv, an autonomously replicating fragment of λ DNA. Carrier cells contain 50 to 100 copies of the plasmid. λdv extends from 0.734 to 0.873 (3) and includes the *Xma*I site at 0.825. λdv would be useful to clone very large *Xma*I fragments. Moreover, cells carrying the plasmid (or chimeric plasmids) could be selected by their immunity to λ (11). *Xma*I-D (*Sma*I-D) shares 65% of the sequences found in λdv, including all of the genes essential for λ DNA replication and the origin of replication (19). It remains to be seen whether the *Xma*I-D fragment of λvir can exist as a plasmid.

*Sma*I cleavage sites have also been mapped on φ80 DNA (9), T5 DNA (23), and types 2 and 5 adenovirus DNA (cited in reference 18). *Sma*I endonuclease does not cleave simian virus 40 DNA (C. Mulder, personal communication) nor PM2 DNA (R. T. Kovacic, personal communication). Since *Sma*I endonuclease recognizes a subset of the sequences cleaved by *Hpa*II endonuclease (4), it will be useful in mapping *Hpa*II fragments.

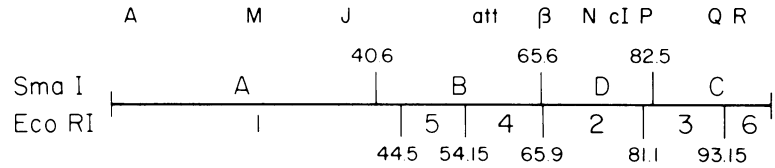

FIG. 4. *Map of SmaI and EcoRI cleavage sites in λ DNA. Coordinates are percentage of the molecular weight of λ DNA (30.8 × 10⁶; reference 3). The symbols above the cleavage map indicate the positions of λ genes (3).*

ACKNOWLEDGMENTS

We thank Marda Brown and Colleen McLean for excellent technical help. We also thank Carel Mulder for supplying *S. marcescens* SB and for information about the purification of *Sma*I endonuclease. R. T. Kovacic generously provided *Hae*III fragments of PM2 DNA. Jon Beaty purified *Eco*RI endonuclease.

This research was supported by grants NP-67B and NP-67C from the American Cancer Society and Public Health Service grant CA 17699-01 from the National Cancer Institute to G.D.P. and by Public Health Service grant 2R1 GM 19617-03 from the National Institute of General Medical Sciences to L.R.B. L.R.B. is the recipient of Career Development Award 5K04 GM 70495-04 from the National Institute of General Medical Sciences.

LITERATURE CITED

1. **Allet, B., K. J. Katagiri, and R. F. Gesteland.** 1973. Characterization of polypeptides made *in vitro* from bacteriophage lambda DNA. J. Mol. Biol. 78:589–600.
2. **Cameron, J. R., S. M. Panasenko, I. R. Lehman, and R. W. Davis.** 1975. *In vitro* construction of bacteriophage λ carrying segments of the *Escherichia coli* chromosome: selection of hybrids containing the gene for DNA ligase. Proc. Natl. Acad. Sci. U.S.A. 72:3416–3420.
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, Cold Spring Harbor, N.Y.
4. **Garfin, D. E., and H. M. Goodman.** 1974. Nucleotide sequences at the cleavage sites of two restriction endonucleases from *Hemophilus parainfluenzae*. Biochem. Biophys. Res. Commun. 59:108–116.
5. **Greene, P. J., M. C. Betlach, H. M. Goodman, and H. W. Boyer.** 1974. The *Eco*RI restriction endonuclease, p. 87–111. *In* R. B. Wickner (ed.), Methods in molecular biology series: DNA replication and biosynthesis, vol. 7. Marcel Dekker, Inc., New York.
6. **Hedgpeth, J., H. M. Goodman, and H. W. Boyer.** 1972. DNA nucleotide sequence restricted by the RI endonuclease. Proc. Natl. Acad. Sci. U.S.A. 69:3448–3452.
7. **Helling, R. B., H. M. Goodman, and H. W. Boyer.** 1974. Analysis of endonuclease R·*Eco*RI fragments of DNA from lambdoid bacteriophages and other viruses by agarose-gel electrophoresis. J. Virol. 14:1235–1244.
8. **Hershey, A. D. (ed.).** 1971. The bacteriophage lambda, p. 792. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
9. **James, P. M., D. Sens, W. Natter, S. K. Moore, and E. James.** 1976. Isolation and characterization of the specialized transducing bacteriophages *ϕ80dargF* and *λh80cI857dargF*: specific cleavage of arginine transducing deoxyribonucleic acid by the endonucleases *Eco*RI and *Sma*R. J. Bacteriol. 126:487–500.
10. **Loening, U. E.** 1967. The fractionation of high-molecular-weight ribonucleic acid by polyacrylamide-gel electrophoresis. Biochem. J. 102:251–257.
11. **Matsubara, K., and A. D. Kaiser.** 1968. λdv: an autonomously replicating DNA fragment. Cold Spring Harbor Symp. Quant. Biol. 33:769–775.
12. **Mertz, J. E., and R. W. Davis.** 1972. Cleavage of DNA by R_1 restriction endonuclease generates cohesive ends. Proc. Natl. Acad. Sci. U.S.A. 69:3370–3374.
13. **Miller, J. H.** 1972. Experiments in molecular genetics, p. 331–337. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
14. **Murray, N. E., and K. Murray.** 1974. Manipulation of restriction targets in phage λ to form receptor chromosomes for DNA fragments. Nature (London) 251:476–481.
15. **Pearson, G. D.** 1975. Intermediate in adenovirus type 2 replication. J. Virol. 16:17–26.
16. **Pettersson, U., C. Mulder, H. Delius, and P. A. Sharp.** 1973. Cleavage of adenovirus type 2 DNA into six unique fragments by endonuclease R·RI. Proc. Natl. Acad. Sci. U.S.A. 70:200–204.
17. **Rambach, A., and T. Tiollais.** 1974. Bacteriophage λ having *Eco*RI endonuclease sites only in the nonessential region of the genome. Proc. Natl. Acad. Sci. U.S.A. 71:3927–3930.
18. **Sambrook, J., J. Williams, P. A. Sharp, and T. Grodzicker.** 1975. Physical mapping of temperature-sensitive mutations of adenovirus. J. Mol. Biol. 97:369–390.
19. **Stevens, W. F., S. Adhya, and W. Szybalski.** 1971. Origin and bidirectional orientation of DNA replication in coliphage lambda, p. 515–533. *In* A. D. Hershey (ed.), The bacteriophage lambda. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
20. **Thomas, M., J. R. Cameron, and R. W. Davis.** 1974. Viable molecular hybrids of bacteriophage lambda and eukaryotic DNA. Proc. Natl. Acad. Sci. U.S.A. 71:4579–4583.
21. **Thomas, M., and R. W. Davis.** 1975. Studies on the cleavage of bacteriophage lambda DNA with *Eco*RI restriction endonuclease. J. Mol. Biol. 91:315–328.
22. **Van Holde, K. E., B. R. Shaw, D. Lohr, T. M. Herman, and R. T. Kovacic.** 1975. Subunit structure of chromatins, p. 57–72. *In* G. Bernardi and F. Gros (ed.), Organization and expression of the eukaryotic genome. Proceedings of the 10th FEBS Meeting, vol. 38. North-Holland/American Elsevier, New York.
23. **von Gabain, A., G. S. Hayward, and H. Bujard.** 1976. Physical mapping of the *Hin*dIII, *Eco*RI, *Sal* and *Sma* restriction endonuclease cleavage fragments from bacteriophage T5 DNA. Mol. Gen. Genet. 143:279–290.