JOURNAL OF VIROLOGY, Nov. 1974, p. 1235-1244 Vol. 14, No. 5
Copyright © 1974 American Society for Microbiology Printed in U.S.A.

Analysis of Endonuclease R·*Eco*RI Fragments of DNA from Lambdoid Bacteriophages and Other Viruses by Agarose-Gel Electrophoresis

ROBERT B. HELLING, HOWARD M. GOODMAN, AND HERBERT W. BOYER

Departments of Microbiology, and Biochemistry and Biophysics, University of California, San Francisco, California 94143, and Division of Biological Sciences, University of Michigan, Ann Arbor, Michigan 48104

Received for publication 3 June 1974

By means of agarose-gel electrophoresis, endonuclease R·*Eco*RI-generated fragments of DNA from various viruses were separated, their molecular weights were determined, and complete or partial fragment maps for lambda, ϕ80, and hybrid phages were constructed.

Agarose was used as a support medium for electrophoresis of DNA several years ago (30, 31), but it was not until a set of DNA fragments of defined size in an an appropriate molecular weight range became available that the excellence of agarose-gel electrophoresis for separation of single- (15) or double-stranded (1, 29) DNA molecules became obvious. The use of ethidium bromide to stain double-stranded DNA has greatly simplified the identification of DNA bands in the gel (1, 29).

We were interested in using gel electrophoresis to separate and identify specific DNA fragments produced by restricting nucleases. In the experiments reported in this paper we used low-voltage agarose-gel electrophoresis in order to maximize the separation of large molecules of DNA (14). The method was used to determine molecular weights and to map endonuclease R·*Eco*RI-derived fragments of DNA from various viruses.

MATERIALS AND METHODS

DNA. Phage DNA was prepared from purified phage particles produced on thermal induction of a temperature-sensitive lysogen or after lytic infection (16). All preparations involved extraction with phenol, ethanol precipitation, and dialysis against 1/10 SSC (0.15 M sodium citrate, 0.015 M NaCl, pH 7.0) or 2×10^{-3} M Tris-hydrochloride, 10^{-4} M EDTA, pH 7.85. A small plaque strain of *Povavirus simiae* (SV40) was propagated on CV-1 cells (11). The intracellular DNA was purified from a Hirt lysate (11). Other sources of virus or DNA are as follows: ϕ80 and ϕ80 *trp* hybrids, C. Yanofsky; P22, E. Jackson; lambda *bio*1, J. Siegel; Mu-1, E. Daniell; lambda p*lac*5, A. Riggs; ϕ80 p*lac*1 and ϕ80 p*lac*1 Δ20, R. Dickson and W. Barnes; polyoma (710 Wt plaque/S3, a plaque-purified stock passaged at low multiplicity), M. Vogt; herpes simplex type 1 (a high passage stock), W. Steinhart, J. Alwine, and C. W. Hill; *Escherichia coli* plasmid JA 5a and *Staphylococcus* plasmid CM, S. Cohen.

Endonuclease R.EcoRI. Purification of the enzyme (through phosphocellulose and hydroxylapatite) and digestion of DNA were essentially as described (P. J. Greene, M. D. Betlach, H. M. Goodman, and H. W. Boyer, *Methods in Molecular Biology*, vol. 9, in press). Generally the digested DNA was treated with phenol, ethanol precipitated, and dialyzed before electrophoresis. Specific fragments of DNA generated from larger DNA molecules by this enzyme will be referred to as *Eco*RI fragments.

Agarose-gel electrophoresis. Gels (15 by 0.6 cm) were formed in 16-cm glass tubing with a slight constriction at one end or with nylon netting (panty hose) across one end to retain the gel. Agarose (SeaKem) in TEA (0.05 M Tris, 0.02 M sodium acetate, 2×10^{-3} M Na_2 EDTA; pH adjusted to 8.05 with glacial acetic acid) plus 0.018 M Nacl was melted by autoclaving. A small amount was used to seal the bottom of the tube. Then additional agarose (cooled below 60 C) was added to fill the column. After hardening, the upper end of the gel was extruded and sliced evenly to form a 15-cm gel. The sample was heated for 5 min at 65 C in order to separate loosely associated DNA molecules and quenched on ice. Bromophenol blue and sucrose were added (to 20% sucrose) to give a final sample volume of 15 to 100 μliters. The sample was run into the gel for 5 min at 100 V and thereafter at 1.5 V/cm of gel (22.5 V). Buffer chambers contained TEA + NaCl. After about 18 h at room temperature (approximately 22 C), the dye marker was at the gel tip. With 0.7% agarose, the dye mobility is equivalent to that of DNA of 10^6 to 3×10^6 mol wt under these conditions. All gels shown in the figures in this paper contain 0.7% agarose. In order to visualize the DNA, the gels were extruded into 1 μg of ethidium bromide per ml (1, 29). After 0.5 h the stained bands were visualized by fluorescence over long wavelength ultraviolet light (C50 Trans-illuminator, Ultraviolet Products, San Gabriel, Calif.). Gels were photographed using a yellow filter (Kodak no. 9 Wratten gelatin filter), and measurements were taken from the prints (Polaroid 55 P/N or 52).

Molecular weights. The six endonuclease R.*Eco*-RI-generated fragments of lambda DNA were used as standards in estimating molecular weights of other DNA species in the same gel. Size estimates of the six fragments as determined by electron microscopy were obtained from R. Davis (manuscript in preparation). These estimates of the fractional equivalents of total lambda DNA are 0.445, 0.154, 0.121, 0.113, 0.098, and 0.069 ± 2%, corresponding to mol wts of 13.7, 4.74, 3.73, 3.48, 3.02, and 2.13 million based on a total mol wt of 30.8×10^6 (8). Independent estimates of the sizes of the six fragments (2, 3) do not differ significantly from those given above (their estimates: 13.7, 4.49, 3.54, 3.54, 3.04, and 2.31 million).

RESULTS

Six *Eco*Ri endonuclease-generated lambda DNA fragments ranging in mol wt from 2.13×10^6 to 13.7×10^6 can be separated on a 0.7% agarose gel (Fig. 1A). A straight-line relationship is approximated when the log molecular weight of each of the smaller fragments is plotted against its relative mobility (Fig. 2). The resolution of lambda fragments 3 and 4 as two distinct bands shows the excellent separation of large DNA molecules on agarose gels. Using the ethidium bromide-staining procedure, 10 ng or less of DNA can be detected in a single fluorescing band. We estimate that approximately 40 ng each are in bands 3 and 4 (Fig. 1A). These bands separate when up to 1.8 μg of *Eco*RI lambda fragments (220 and 210 ng in the two bands) are loaded on a single gel.

Base composition can affect mobility on acrylamide gels (34). Fragments of DNA from different regions of the lambda genome differ considerably in their base composition (8). However, the plot of log molecular weight versus mobility of the lambda DNA fragments (Fig. 2) does not deviate markedly from linearity (in the region of proportionality), suggesting that base composition does not grossly affect mobility under our conditions. Presumably as a result of the differences in base composition, relative mobilities of the *Eco*RI lambda DNA fragments in acrylamide gels are altered greatly by running them at different temperatures (2). However, in these 0.7% agarose gels the relative mobilities of the lambda DNA fragments were the same at 3 as at 23 C.

Fragment separation on gels of different agarose concentration is shown in Fig. 3. The upper limit of the range of proportionality decreases progressively as the gel concentration is increased; however, separation within this range is better at higher gel concentrations. The bands of DNA of lower molecular weight are diffuse rather than sharp, and this blurring appears to be unaffected by increasing the gel concentration. By increasing the voltage, electrophoresis can be completed rapidly enough that fragments of small molecular weight are resolved as narrow bands (not shown). However, the separation of high-molecular-weight DNA becomes progressively poorer as the voltage is raised.

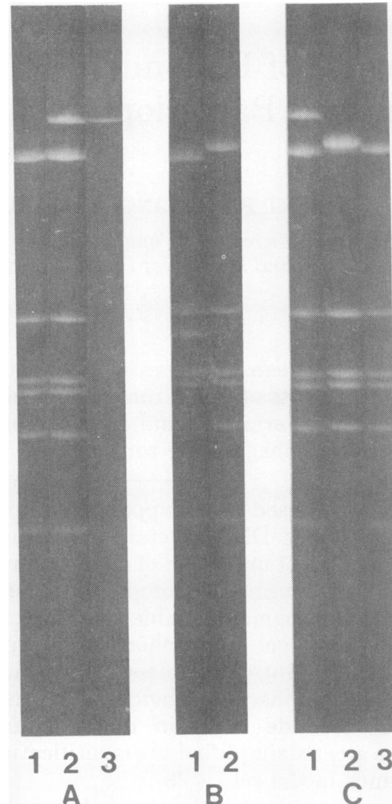

FIG. 1. *Electrophoresis of DNA in 0.7% agarose gels. In all gels migration was from top (cathode) to bottom (anode). (A) Gel 1, EcoRI fragments of lambda DNA; gel 2, intact lambda and EcoRI lambda fragments; gel 3, intact lambda DNA. (B) Gel 1, EcoRI fragments of lambda DNA; gel 2, EcoRI fragments of lambda plac5 DNA. (C) Gel 1, intact lambda and EcoRI fragments; gel 3, EcoRI lambda fragments; gel 2, as gel 3 except the sample was not heated and NaCl was added to the DNA to 0.1 M before loading on the gel.*

Just as with polyacrylamide gels (10), the mobility of DNA on agarose gels is a function of molecular conformation (1), as shown in Fig. 4 (gels 1 and 2) by the shift in band position when superhelical polyoma DNA is cleaved to produce noncircular DNA of the same molecular weight. The circular DNAs from SV40 and polyoma are each cleaved once by the *Eco*RI endonuclease (11, 22, 24), producing a single band of noncircular DNA (Fig. 4). From the

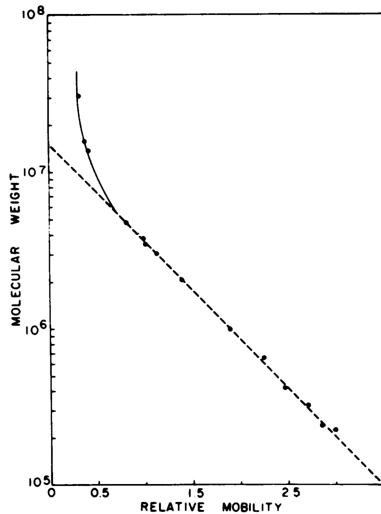

FIG. 2. *Relative electrophoretic mobility of noncircular double-stranded DNA in 0.7% agarose gel. Points represent the mobility relative to EcoRI lambda fragment 4 of DNA from intact lambda, EcoRI lambda fragments, head and tail EcoRI fragment dimers, and SV40 fragments generated by the EcoRI and HpaI restriction endonucleases. The ranges of relative mobility for the five smaller lambda fragments from 18 gels independently run at approximately 22 C fall within the points plotted (the mobility of fragment 4 was arbitrarily set at 1.0). The range in relative mobility of the largest fragment varied from 0.38 to 0.43. The relative mobilities of the six lambda fragments in gels run at 3 C fall within the above ranges.*

positions of these bands relative to the lambda *Eco*RI fragments, their molecular weights can be calculated (Table 1). The DNA of the small plaque strain of SV40 used in this laboratory appears to be slightly smaller than that of polyoma, as shown by the difference in mobility of the noncircular polyoma and SV40 DNAs (Fig. 4) and equivalent differences in mobilities of the superhelical and nicked circular forms of the same DNA (not shown). We believe the difference in mobility is unlikely to be due to the difference in base composition between polyoma (49% guanine plus cytocine [G+C] and SV40 (41% G+C) (32), because we already showed that base composition did not grossly affect mobility of the lambda DNA fragments, and because in one study the mobility of DNA on acrylamide has been shown to increase with G+C content (10).

The molecular weights of *Eco*RI fragments from various other DNA sources have been determined from their mobilities relative to λ*Eco*RI fragments included in the same gel (Fig. 5 and 6, Table 1). The molecular weight of intact DNA, from viruses producing more than one cleavage product, has been estimated by summing the weights of the fragments (Table 1). The generally good agreement with literature values suggests that this may be a useful procedure for determining total molecular weights of up to 10^8 or higher. Sometimes two fragments are so close in molecular weight as to run as a single band. This is usually obvious from the relative ethidium bromide staining intensities (e.g., ϕ80 fragments 3 and 4, Fig. 6; P1 fragments 4 and 5, Fig. 5), and, when less DNA is electrophoresed for a longer time, we have usually been able to resolve the separate bands. In some cases radioactive labeling (6, 12, 25) has been used to directly quantitate the amount of DNA per band (Table 1).

Complex DNA. Most of the *Eco*RI DNA fragments from *E. coli* can be separated by electrophoresis on 0.7% agarose (Fig. 5C). By examining the gel patterns from deletion mutants and using individual fragments in transformation it should be possible to identify the genes associated with each fragment. The *Eco*-RI fragments of eukaryotic DNA from various sources were also fractionated by this procedure

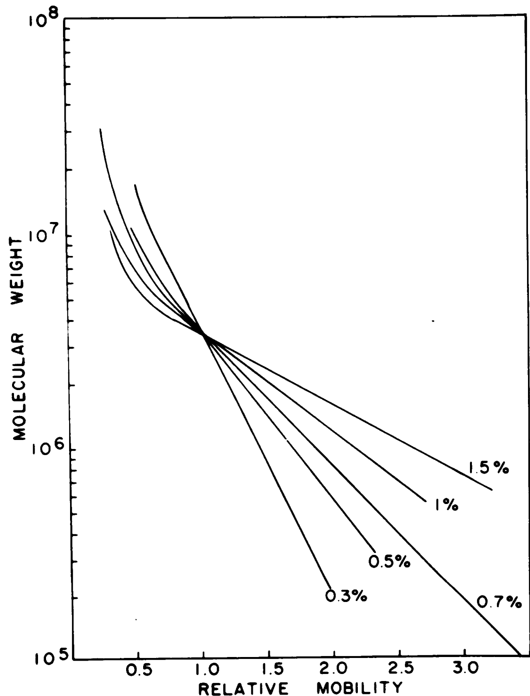

FIG. 3. *Relative electrophoretic mobilities of noncircular double-stranded DNA as a function of agarose concentration. Mobilities were determined and curves were plotted as in Fig. 2.*

TABLE 1. *Calculation of molecular weights of EcoRI fragments*

Viral DNA	Mol wts of *Eco*RI fragments ($\times 10^{-6}$)										Lower limit detectable[a]	Summation of mol wts	Reported mol wt of DNA
	1	2	3	4	5	6	7	8	9	10			
λ[b]	13.7[c]	4.67	3.71	3.57	3.04	2.11[d]						30.8	30.8 (8)
λ	14 (45.2 ± 1.6)[e]	4.8 (15.7 ± 0.8)	7.3 (23.8 ± 0.8)		2.9 (9.3 ± 0.5)	1.9 (6.0 ± 0.9)							
λ*plac*5	12[c]	4.67 (λ-2)	4.35	3.71 (λ-3)	3.57 (λ-4)	2.11[d] (λ-6)						30.4	30.8 (21)
λ*plac*5	12 (39.8 ± 1.4)[e]	4.9 (15.9 ± 0.9)	4.4 (14.2 ± 1.2)	6.5 (21.2 ± 0.6)		1.9 (6.2 ± 0.7)							
λ*bio*1	13.7[c] (λ-1)	6.7	3.71 (λ-3)	3.04 (λ-6)	2.11[d]							29.2	29.2 (18)
λ*dara*	16–18[c]	4.67 (λ-2)	3.71 (λ-3)	2.11[d] (λ-6)							10^6	26.5–28.5	26.7 (28)
λ*h*80	5.5	4.67 (λ-2)	4.05[c] (φ80-3)	3.95 (φ80-4)	3.71 (λ-3)	3.35 (φ80-5)	2.11[d] (λ-6)				8×10^5	27.3	27.7 (33)
φ80	9.2 (11) 0.13	5.30[d]	4.05[c]	3.95	3.35	1.66	0.39	0.38	0.32	0.13	10^5	28.9	28.3 (8)
φ80 p*lac* 1	9	5.3[d] (φ80-2)	4.05[c] (φ80-3)	3.95 (φ80-4)	3.35 (φ80-5)	1.66 (φ80-6)	1.2	0.39 (φ80-7)	0.38 (φ80-8)	0.32 (φ80-9)	3×10^5	29.6	
φ80 p*lac* 1 ∇20	As 80 p*lac* 1 except fragment 1 reduced in size to 7.3×10^6 mol wt											27.9	
φ80 *hpt*190 (EA)	10	4.5[d]		In addition contains all φ80 fragments except 1 and 2							10^5	28.9	(9)
φ80*trp* CB15	9.5	6.5[d]		In addition contains all φ80 fragments except 1, 2, and 6							10^5	28.7	(9)
Mu-1	11	7.9–8.5[f]	3.85	3.3							5×10^5	26.1–26.7	24.7 (8)
Plbt	9.6 (11) 1.6	6.5 (12) 1.0	6.2	4.3 (13) 0.87	4.2 (14) 0.78	3.8	3.5 (15) 0.67	2.1	2.0	1.8	5×10^5	48.9	58 (19)
P22	12[g]	5.6	4.5	2.5[h]	1.4	0.63	0.60	0.45				27.7	27 (26)
SV40	3.25[i]											3.25	3.6 (32)
Polyoma	3.39[i]											3.39	3.1–3.7 (32)

Herpes simplex type 1	14 (11) 1.8	12[j] (12)	11 1.45	10.7 (13) 0.47	10 (14)	9 0.2	8.4[j]	3.54	3.28	2.54	2×10^6	88.4	99 (32)
Plasmid DNA JA 5a	20+	1.25									8×10^6		
Plasmid DNA Staph CM	2.75[i]										4×10^6		

[a] Not checked for fragments smaller than the molecular weight listed. Where no value is given, no additional fragment present in equimolar amount with other identified fragments is likely to exist.

[b] In order to determine molecular weight of DNA by electrophoresis one assumes that mobility is a linear function of the log molecular weight, even though this assumption may not be strictly valid (34). For this reason the listed molecular weights of the five smaller lanbda fragments were derived by making the best fit (by the method of least squares) of the logs of the measured molecular weights (see Materials and Methods) to their relative mobilities (as determined from 18 independently run gels). (They do not differ significantly from the measured sizes.) This makes explicit what is otherwise implicit in the use of the lambda fragments as molecular weight standards.

[c] Left end.

[d] Right end.

[e] Molecular weights estimated from the distribution of radioactivity (shown in parentheses as percent ± 2 standard deviations) from ^{32}P-labeled lambda or lambda p*lac*5 DNA fragments separated by agarose-gel electrophoresis. Amount of radioactivity was determined by Cerenkov counting as described (25). Number of counts in lambda bands 3 and 4 were added together because the bands overlapped.

[f] Disperse band. In addition, two faint bands corresponding to mol wts greater than 20×10^6 and 16×10^6 were observed several times, but are believed to result from incomplete digestion by the nuclease.

[g] Additional disperse band from 10 to 11.8 million mol wt.

[h] Staining intensity shows this band contains less than equimolar equivalent of DNA.

[i] Single restriction site converting circular DNA to noncircular DNA.

[j] Faint bands.

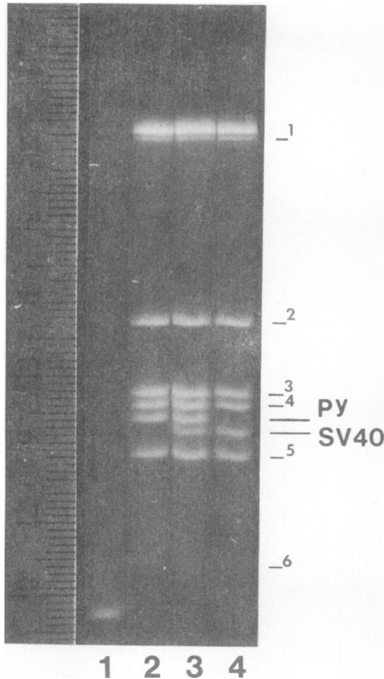

FIG. 4. *Agarose-gel electrophoresis of polyoma and SV40 DNA, Gel 1: Intact superhelical polyoma DNA. Nicked circular DNA of the same size has a mobility slightly less than that of the noncircular DNA, neither of which is visible in this preparation. Gel 2: EcoRI-digested polyoma and lambda DNA. Gel 3: EcoRI-digested polyma, SV40, and lambda DNA. Gel 4: EcoRI-digested SV40 and lambda DNA. Because of the salt in the reaction mixtures, lambda head-tail dimers are present.*

(not shown). Few individual bands could be seen. However, the mobilities of the *Eco*RI fragments of lambda were not altered by a large excess of eukaryotic DNA fragments in the same gel, suggesting that even these complex mixtures of many sizes of DNA molecules separate according to molecular weight (as expected), even though individual bands could not be identified.

Mapping. By comparing the fragment patterns produced from various lambda and $\phi 80$ hybrid phage DNAs by the *Eco*RI endonuclease, the order of lambda fragments determined by R. Davis (personal communication), and shown in Fig. 7, was confirmed, and partial fragment maps of $\phi 80$ and the hybrids were obtained (Fig. 7). The map of lambda fragments (and of lambda p*lac*5 fragments) has been independently determined (2, 3) and, as revised (3), does not differ from our map. As shown in Fig. 1B, lambda p*lac*5, a lambda derivative in which part of the *lac* operon is substituted for lambda DNA (21), has four *Eco*RI fragments which appear identical to those from wild-type lambda. However, the positions of two of the bands are different. These contain the two fragments on the left of the genetic map, as expected if part of the lambda DNA containing the left-most *Eco*RI cleavage pcint has been deleted and replaced by new (*lac*) DNA with a new cleavage point closer to the left end of the intact DNA. The *lac* operator is in the smaller of the two new lambda p*lac*5 *Eco*RI fragments, as shown by repressor binding experiments with the DNA eluted from each band (unpublished experiments, with A. Riggs).

Lambda p*bio*1 has a deletion of 10.5% of the lambda DNA to the right of *att* and a substitution of *bio* DNA equivalent to 5.2% of lambda (18). The deletion should remove the cleavage site between fragments 4 and 2 and, (unless a new *Eco*RI restriction site is introduced with

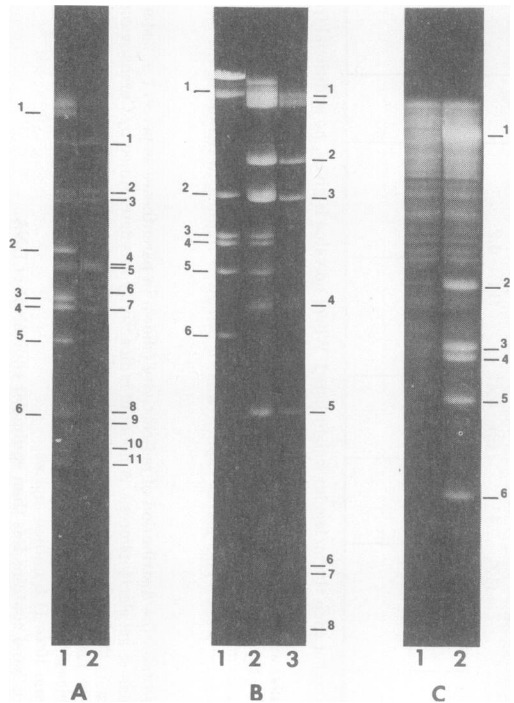

FIG. 5. *Separation of EcoRI DNA fragments in agarose gels. (A) Gel 1, EcoRI-digested P1 and lambda DNA; gel 2, EcoRI-digested P1 DNA. (B) Gel 1, intact P22 DNA and EcoRI-digested lambda DNA; gel 2, EcoRi-digested P22 and lambda DNA; gel 3, EcoRI-digested P22 DNA. (C) Gel 1, EcoRI-digested DNA from E. coli; gel 2, EcoRI-digested DNA from lambda and E. coli; The E. coli DNA was from strain HB 129 (27). In gels A1, B1, and B2, lambda head-tail dimers are present because of salt in the reaction mixtures.*

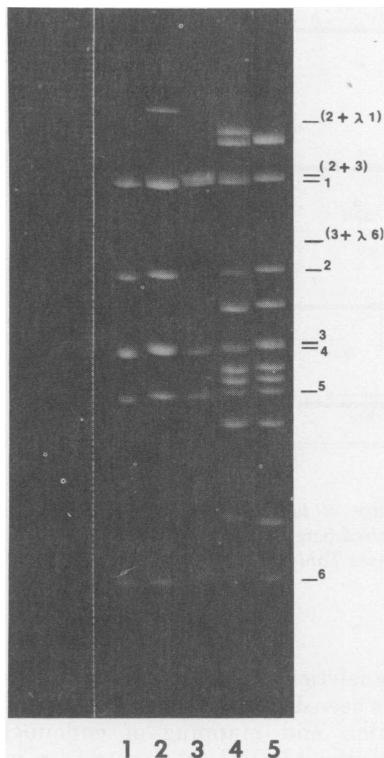

FIG. 6. *Separation of φ80 EcoRI fragments in agarose gels. Gel 1: EcoRI-digested φ80 DNA; Gel 2: as gel 1 plus intact φ80 DNA; Gel 3: as gel 1 but NaCl added to 0.1 M; Gel 4: as gel 5 but NaCl added to 0.1 M; Gel 5: EcoRI-digested lambda and φ80 DNA. All samples were heated to 65 C for 5 min (after adding NaCl to samples 3 and 4) and were then kept at room temperature for 30 min before loading on gels. In repeated experiments, lambda-φ80 dimers (gel 4) were less frequent than lambda-lambda and φ80-φ80 dimers, suggesting preferential association because of inexact homology of the ends or possibly an influence of fragment length on probability of association.*

bio), result in the loss of the corresponding gel bands and the appearance of a single new fragment of mol wt 6.7×10^6. The observed gel pattern was exactly as predicted (Table 1).

All of our results are fully consistent with the map developed by Davis and by Allet et al. (3). Independent of the evidence from the fragment patterns of hybrid DNA, we have confirmed that the largest and smallest lambda fragments contain the lambda ends. At high ionic strength the cohesive ends found in intact lambda DNA pair to form circles (17); the same pairing of the two terminal *Eco*RI fragments results in the loss of the largest and smallest fragments and the appearance of a new band corresponding to the joined fragments (Fig. 1C).

The end fragments from phage φ80 were identified by the same procedure. These correspond to bands 2 (mol wt 5.30×10^6) and 3 (mol wt 4.05×10^6) which join to band at a position expected of DNA of mol wt approximately 9×10^6 (Fig. 6, gel 3). Because φ80 DNA and lambda DNA can pair through their cohesive ends, the φ80 and lambda end fragments should also pair appropriately. From the molecular weights of the individual fragments we can predict the positions expected of the φ80-lambda dimers. When lambda and φ80 *Eco*RI fragments were mixed and end-joining was allowed, two new bands were seen after electrophoresis (Fig. 6, gel 4). One band appeared at the position expected of a combination of φ80 fragment 3 and the lambda right-end fragment. The second was of high molecular weight and could have resulted from the combination of the lambda left end with either φ80 fragment 2 or 3. No band corresponding to φ80 fragment 2 combined with the right-end fragment of lambda was apparent. Thus, we conclude that band 2 corresponds to the right-end fragment of φ80 and band 3 corresponds to the left end of φ80.

This assignment of φ80 ends is consistent with the *Eco*RI fragment patterns of φ80 hybrids. A φ80-lambda hybrid (lambda *h*80) containing the lambda fragment 6 (right end) lacks φ80 fragment 2, but still has φ80 fragment 3 (Table 1). The position of fragment 2 from φ80*h pt*EA, known to have a deletion near the right end (and, in addition, containing the entire *trp* operon), is shifted appropriately, but fragment 3 is unaffected (Table 1).

Neither fragment 2 nor 3 contains the attachment region. Study of six different φ80 *trp* hybrids showed that the *att* region is in fragment 1 because in each hybrid this band is altered in mobility (in two of the hybrids, φ80*h pt*190 (EA) and CB15 bands 2 or 6 were altered also, presumably as the result of a short deletion [13], Table 1). φ80 p*lac*1 Δ20 differs from φ80 p*lac*1 only by a deletion of part of the *lac* insertion (R. Dickson and W. Barnes, personal communication); the gel patterns differ only by a reduction in size of the largest fragment (Table 1), consistant with the location of *att* in fragment 1 of φ80. φ80 fragment 6 is adjacent to fragments 1 and 2 because in a lambda-φ80 hybrid (lambda *h*80) containing the rightmost three fragments of lambda, φ80 bands 1, 2, and 6 disappear but the other φ80 bands remain (with the possible exception of those below 2×10^6 mol wt). Therefore, fragments 4, 5, 7, 8, and 9 must be right of 3 (left end) and left of 1, 2,

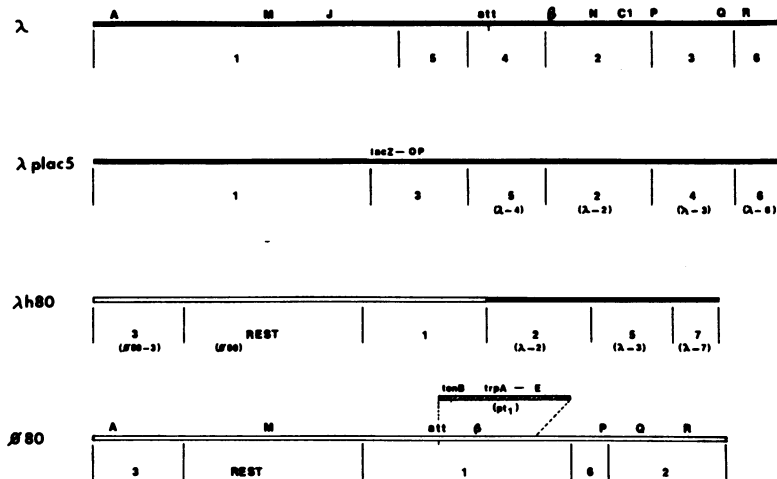

FIG. 7. *Endonuclease R·EcoRI-generated DNA fragment maps of lamboid phages. Solid bars indicate DNA derived from lambda; open bars, φ80 DNA; and cross-hatched bars, E. coli DNA. Sizes of intact DNAs and location and sizes of hybrid regions are as reported previously (see Table 1).*

and 6 (because they are present in lambda *h*80). Comparison of the fragment maps of lambda *h*80 and φ80 (Fig. 7) shows that, if φ80 fragment 6 were to the left of fragment 1, then it should be present in lambda *h*80. Because it is not, we conclude that it maps between φ80 fragments 1 and 2. The map locations of such genetic markers as *att* and all our subsequent work are consistent with the maps shown in Fig. 7.

Anomalies in gel patterns. With several sources of DNA (herpes simplex virus type 1, Mu-1, P22 [Fig. 5B]), we have observed an anomalous spreading or blurring of one of the *Eco*RI bands and a less than quantitative intensity of staining of one of the other bands (P22 and herpes). The heterogeneity revealed in the population of DNA molecules of herpes may be a consequence of passaging the virus at high multiplicity. Such stocks of certain viruses (including SV40 and polyoma) contain segments of heterogeneous DNA derived from the host (32). Daniell et al. (7) have shown that one end of the Mu-1 DNA is from the host, and the host region varies in length and in sequence from molecule to molecule. Thus, we expect that the length of the host DNA attached to the nearest *Eco*RI restriction site in the Mu-1 phage will also vary. We have not determined the cause of the anomalous P22 gel pattern but it may reflect cyclic permutations among the DNA molecules found in the intact phage DNA (26). In addition, the gel patterns have revealed contaminating host DNA in some phage DNA preparations. Generally the procedure has been useful for identifying heterogeneity in DNA preparations.

DISCUSSION

The analytical use of agarose-gel electrophoresis has been demonstrated in this paper by the separation and mapping of endonuclease-R·*Eco*RI-derived DNA fragments from various sources. The nice resolution of mixtures of DNA fragments with molecular weights in excess of a million as distinct bands results from the use of agarose instead of acrylamide and from the use of much lower voltage than is customary with proteins and RNA. The agarose-gel procedure is an extremely useful one for estimating molecular weights, provided that one has standards of known size which can be run in the same gel. Nevertheless, molecular weight estimates of DNA molecules often differ depending on whether they were obtained by means of electron microscopy, relative distribution of radioactivity in gels, relative electrophoretic mobility, or otherwise (e.g., 6, 12, 23, 25), and so this procedure should not be relied upon for sole size determination.

The construction of maps of the DNA fragments produced by the *Eco*RI or other endonuclease makes it possible to predict which genes are associated with each fragment and whether specific genes are cleaved by the enzyme. For example, a restriction site cleaved by the *Eco*RI enzyme is found in the *lac* operon, probably at the distal end of the *lac*Z gene, as inferred from the altered fragment pattern of lambda p*lac*5. However, the *trp*, *ara*, and *bio* (and *gal* [20]) operons are not cut by this enzyme, because the only variation in the gel patterns of DNA fragments from hybrid phage containing these

operons is a change in the mobility of a single band corresponding to an increase or decrease in the size of a single DNA fragment (and by a decrease in the total number of fragments in cases where a restriction site is deleted).

The purification of a specific fragment containing a known gene can be achieved by elution of the appropriate band from a gel segment or by using other standard procedures in conjunction with size determination in gels. This should be highly useful for studies of transcription and translation in vitro, for sequencing, and for construction of new plasmids containing specific combinations of genes (4, 5, 23).

ACKNOWLEDGMENTS

We are grateful to those who gave us samples of phage or DNA and to Ron Davis for giving us his unpublished data. We thank J. Sambrook and P. Sharp for their suggestions.

This investigation was supported by U.S. Public Health Service grants CA 14026 from the National Cancer Institute and GM 14378 and GM 21287 from the National Institute of General Medical Sciences and by a grant from the University of Michigan Cancer Research Institute. R. B. H. was a Special Fellow of the National Institutes of Health.

LITERATURE CITED

1. Aaij, C., and P. Borst. 1972. The gel electrophoresis of DNA. Biochim. Biophys. Acta 269:192-200.
2. Allet, B., P. G. N. Jeppesen, K. J. Katagiri, and H. Delius. 1973. Mapping the DNA fragments produced by cleavage of λ DNA with endonuclease RI. Nature (London) 241:120-123.
3. 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.
4. Chang, A. C. Y., and S. N. Cohen. 1974. Genome construction between bacterial species *in vitro*: replication and expression of *Staphylococcus* plasmid genes in *Escherichia coli*. Proc. Nat. Acad. Sci. U.S.A. 71:1030-1034.
5. Cohen, S. N., A. C. Y. Chang, H. W. Boyer, and R. B. Helling. 1973. Construction of biologically functional bacterial plasmids *in vitro*. Proc. Nat. Acad. Sci. U.S.A. 70:3240-3244.
6. Dana, K., and D. Nathans. 1971. Specific cleavage of simian virus 40 DNA by restriction endonuclease of *Hemophilus influenzae*. Proc. Nat. Acad. Sci. U.S.A. 68:2913-2917.
7. Daniell, E., J. Abelson, J. S. Kim, and N. Davidson. 1973. Heteroduplex structures of bacteriophage Mu DNA. Virology 51:237-239.
8. Davidson, N., and W. Szybalski. 1971. Physical and chemical characteristics of lambda DNA, page 45-82. *In* A. D. Hershey (ed.), The bacteriophage lambda. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
9. Deeb, S. S., K. Okamoto, and B. D. Hall. 1967. Isolation and characterization of nondefective transducing elements of bacteriophage φ80. Virology 31:289-295.
10. Dingman, C. W., M. P. Fisher, and T. Kakefuda. 1972. Role of molecular conformation in determining the electrophoretic properties of polynucleotides in agarose-acrylamide gels. II. Biochemistry 11:1242-1250.
11. Dugaiczyk, A., J. Hedgpeth, H. W. Boyer, and H. M. Goodman. 1974. Physical identity of the SV40 deoxyribonucleic acid sequence recognized by the *Eco*RI restriction endonuclease and modification methylase. Biochemistry 13:503-512.
12. Edgell, M. H., C. A. Hutchison III, and M. Sclair. 1972. Specific endonuclease R fragments of bacteriophage φX174 deoxyribonucleic acid. J. Virol. 9:574-582.
13. Fiandt, M., A. Hradecna, H. A. Lozeron, and W. Szybalski. 1971. Electron micrographic mapping of deletions, insertions, inversions, and homologies in the DNAs of coliphages lambda and phi80, p. 329-354. *In* A. D. Hershey (ed.), The bacteriophage lambda. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
14. Fisher, M. P., and C. W. Dingman. 1971. Role of molecular conformation in determining the electrophoretic properties of polynucleotides in agarose-acrylamide composite gels. Biochemistry 10:1895-1899.
15. Hayward, G. S., and M. G. Smith. 1972. The chromosome of bacteriophage T5. I. Analysis of the single-stranded DNA fragments by agarose gel electrophoresis. J. Mol. Biol. 63:383-396.
16. Hedgpeth, J., H. M. Goodman, and H. W. Boyer. 1972. DNA nucleotide sequence restricted by the RI endonuclease. Proc. Nat. Acad. Sci. U.S.A. 69:3448-3452.
17. Hershey, A. D., E. Burgi, and L. Ingraham. 1963. Cohesion of DNA molecules isolated from phage lambda. Proc. Nat. Acad. Sci. U.S.A. 49:748-755.
18. Hradecna, Z., and W. Szybalski. 1969. Electron micrographic maps of deletions and substitutions in the genomes of transducing coliphages λdg and λ*bio*. Virology 38:473-477.
19. Ikeda, H., and J. Tomizawa. 1968. Prophage P1, an extrachromosomal replication unit. Cold Spring Harbor Symp. Quant. Biol. 33:791-798.
20. Jackson, D. A., R. H. Symons, and P. Berg. 1972. Biochemical method for inserting new genetic information into DNA of simian virus 40: circular SV40 DNA molecules containing lambda phage genes and the galactose operon of *Escherichia coli*. Proc. Nat. Acad. Sci. U.S.A. 69:2904-2909.
21. Malamy, M. H., M. Fiandt, and W. Szybalski. 1972. Electron microscopy of polar insertions in the *lac* operon of *Escherichia coli*. Mol. Genet. 119:207-222.
22. Morrow, J. F., and P. Berg. 1972. Cleavage of simian virus 40 DNA at a unique site by a bacterial restriction enzyme. Proc. Nat. Acad. Sci. U.S.A. 69:3365-3369.
23. Morrow, J. F., S. N. Cohen, A. C. Y. Chang, H. W. Boyer, H. M. Goodman, and R. B. Helling. 1974. Replication and transcription of eukaryotic DNA in *E. coli*. Proc. Nat. Acad. Sci. U.S.A. 71:1743-1747.
24. Mulder, C., and H. Delius. 1972. Specificity of the break produced by restricting endonuclease RI in SV40 DNA as revealed by partial denaturation. Proc. Nat. Acad. Sci. U.S.A. 69:3215-3219.
25. Pettersson, V., C. Mulder, H. Delius, and P. A. Sharp. 1973. Cleavage of adenovirus Type 2 DNA into six unique fragments by endonuclease R·RI. Proc. Nat. Acad. Sci. U.S.A. 70:200-204.
26. Rhoades, M., L. A. MacHattie, and C. A. Thomas, Jr. 1968. The P22 Bacteriophage DNA molecule. I. The mature form. J. Mol. Biol. 37:21-40.
27. Roulland-Dussoix, D., and H. W. Boyer. 1969. The *E. coli* B restriction endonuclease. Biochim. Biophys. Acta 195:219-229.
28. Schleif, R., J. Greenblatt, and R. W. Davis. 1971. Dual control of arabinose genes on transducing phage λ *dara*. J. Mol. Biol. 59:127-150.
29. Sharp, P. A., B. Sugden, and J. Sambrook. 1973. Detection of two restriction endonuclease activities in *H. parainfluenzae* using analytical agarose-ethidium bromide electrophoresis. Biochemistry 12:3055-3063.
30. Takahashi, M., T. Ogino, and K. Baba. 1969. Estimation of relative molecular length of DNA by electrophoresis

in agarose gel. Biochim. Biophys. Acta **174**:183-187.
31. Thorne, H. V. 1966. Electrophoretic separation of polyoma virus DNA from host cell DNA. Virology **29**:234-239.
32. Tooze, J. (ed.). 1973. The molecular biology of tumour viruses. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
33. Wu, M., N. Davidson, and J. Carbon. 1973. Physical mapping of the transfer RNA genes on λ*h*80d *glyTsu*$^{+}_{36}$. J. Mol. Biol. **78**:23-34.
34. Zeiger, R. S., R. Salomon, C. W. Dingman, and A. C. Peacock. 1972. Role of base composition in the electrophoresis of microbial and crab DNA in polyacrylamide gels. Nature N. Biol. **238**:65-69.