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# Use of a Restriction Endonuclease in Analyzing the Genomes from Two Different Strains of Vaccinia Virus

J. DAVID GANGEMI[1] AND D. GORDON SHARP

*Department of Bacteriology and Immunology, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27514*

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A restriction endonuclease from *Haemophilus influenzae* (*Hind* III) specifically cleaved vaccinia DNA into 14 fragments. The molecular weights of these fragments were determined by gel electrophoresis and ranged from $0.5 \times 10^6$ to $30 \times 10^6$. *Hind* III digestion of the DNA from the WR and CV-1 strains of vaccinia revealed a small molecular weight difference in one of the resulting fragments. The average molecular weight of the entire vaccinia genome was calculated to be $125 \times 10^6$.

Bacterial restriction endonucleases have been used extensively in the biophysical characterization of small DNA molecules, i.e., simian virus 40 (4, 5, 10, 14, 16), polyoma (3, 11, 13), $\phi$X174 (9), lambda (1), and adenovirus (17) DNA. The increased availability of these enzymes has made it possible to construct genome maps (3, 5, 13), determine the sites for initiation and termination of DNA replication (10, 14), and determine the location of missing segments in defective DNA molecules (11). Comparable studies with larger DNA molecules, like those found in the poxvirus, have not been attempted due to the excessive number of digestion fragments produced by most endonucleases. In this communication, we present data showing that vaccinia DNA is specifically cleaved into a limited number of fragments by the restriction endonuclease *Hind* III from *Haemophilus influenzae*. This observation has made it possible to compare and characterize the genomes from three vaccinia host range varieties.

Cloned populations of virions that plaque on mouse L but not on rabbit kidney cells ($L^+RK^-$) and virions that plaque on both mouse L and rabbit kidney cells ($L^+RK^+$) were isolated from the WR strain (ATCC) of vaccinia, whereas virions that plaque on rabbit kidney cells but not on mouse L cells ($L^-RK^+$) were isolated from the CV-1 strain of vaccinia. The CV-1 strain was the Rivers attenuated strain that originated from the calf lymph strain of vaccinia (New York City Board of Health). Its passage history included 4 passages in rabbit testicles, 34 passages in chicken embryo explants, and a further 6 passages in rabbit testicles; 59 further passages were made in chicken embryo explants, followed by 19 passages on the chorioallantoic membrane. Wyeth laboratories provided our virus seed (CV-1-78), and this virus was passaged once again on the chorioallantoic membrane. All host range variants used in this study were cloned three times and passaged at a multiplicity of 50 virions per cell.

Gentle extraction procedures were used to isolate the DNA from these three varieties. Briefly, purified virions were suspended in 8.0 ml of 27% (wt/wt) sucrose containing 1% deoxycholate (Difco), 0.001 M disodium EDTA, and 0.01 M Tris (pH 7.2), and were sonicated for 2 min at 20 kHz to eliminate virus aggregation. To each of the three virus suspensions, 500 mg of Pronase (B grade, Calbiochem; predigested at 37°C for 1 h) per ml was added, and the suspensions were incubated at 37°C for 18 h. The DNA was then phenol extracted, layered over preformed CsCl gradients (1.4 to 1.7 g/cm$^3$), and isopycnically banded in a Beckman SW27 rotor (22,000 rpm for 18 h at 4°C). The 280 to 260 nm ratio of adsorbance was 0.5 to 0.55 for all three DNA preparations.

Purified preparations of endonuclease *Hind* III from *H. influenzae* was a gift from June H. Middleton. The isolation and purification of endonuclease *Eco*RI from *Escherichia coli* was essentially the same as that described by Mulder and Delius (16). Digestion of DNA extracted from the bacterial virus lambda with either *Eco*RI or *Hind* III resulted in specific fragments that were used as molecular weight markers. The molecular weights of the lambda *Hind* III fragments were determined by com-

[1] Present address: United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD 21701.

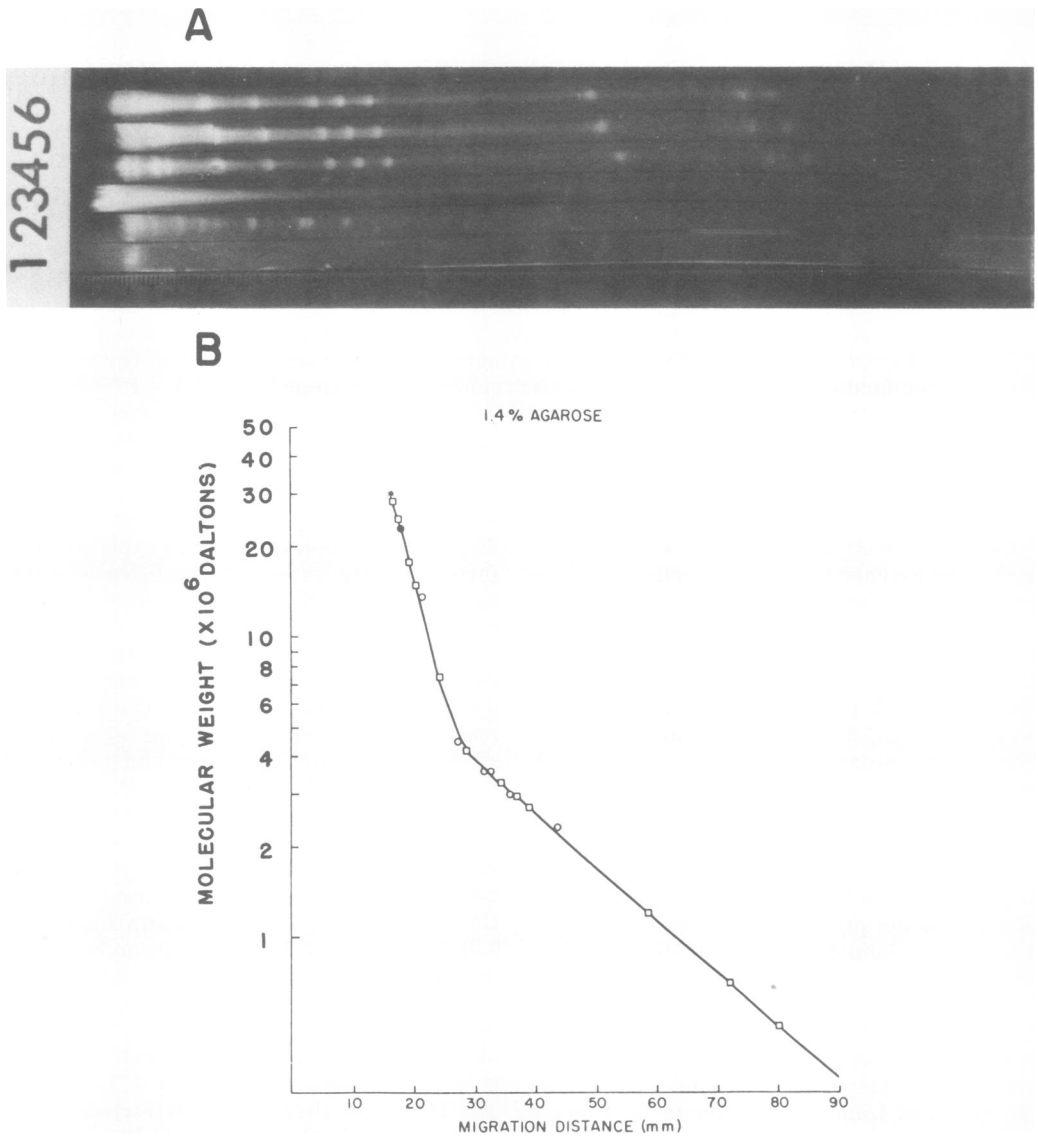


FIG. 1. *(A) Electrophoresis of vaccinia fragments produced after endonuclease digestion. (1) Undigested lambda DNA; (2) 3 μg of lambda DNA digested with 30 μl of EcoRI; (3) mixture of lambda DNA (3 μg) and vaccinia DNA (2 μg) digested with EcoRI; (4) $L^+RK^-$ vaccinia DNA (3 μg) digested with 10 μl of endonuclease Hind III; (5) $L^+RK^+$ DNA (3 μg) digested with 10 μl of HindIII; (6) $L^-RK^+$ DNA (3 μg) digested with 10 μl of Hind III. Electrophoresis was in 1.4% agarose gels for 12 h at 4 mA per gel. Direction of migration is from top to bottom toward the anode. Gels were stained with 1 μg of ethidium bromide per ml for 3 h, illuminated with a UV light, and then photographed with a Polaroid camera through a UV filter screen. (B) Mass-versus-mobility plot of endonuclease Hind III vaccinia DNA digests. Symbols: (○) Lambda RI marker fragments; (□) vaccinia Hind III fragments; (●) vaccinia Hind III $L^-RK^+$ fragment 2 (second fragment from top of gel).*

paring their migration profiles with those of the previously characterized (1) lambda *Eco*RI fragments.

Endonuclease RI did not cleave vaccinia DNA into specific fragments, and in a mixture of both vaccinia and lambda DNA, lambda DNA was not cleaved. Vaccinia DNA was, however, cleaved by endonuclease *Hin*d III. Figure 1A illustrates the fragments produced by *Hin*d III digestion of the DNA from three host range varieties of vaccinia after electrophoresis in 1.4% agarose gels. No fragments smaller than the last visible fragments were found in more concentrated gels, and no changes in these patterns were observed as a result of adding more enzyme or prolonging the incubation time. Electron microscopic examination of the substrate DNA (6) used in these experiments revealed that most of the molecules were in a rosette rather than extended configuration; however, little mechanical fragmentation was apparent. At least 12 DNA fragments were detected from each of the three host range genomes after digestion with *Hin*d III and electrophoresis in 1.4% agarose. Estimates of their molecular weights were determined by comparing fragment migration distances with those appearing in parallel lambda RI digests (Fig. 1B). These molecular weight estimates are presented in Table 1.

Close examination of the second-largest fragment from $L^-RK^+$ DNA digests revealed that it migrated faster in 1.4% gels than did the second-largest fragment from either $L^+RK^-$ or $L^+RK^+$ digest. We therefore tried to obtain better resolution of this difference and more accurate measurements of the molecular weights of the larger DNA fragments from each of the three digests by electrophoresing in "softer" gels (0.85% agarose). Figure 2A more clearly illustrates the migration differences between the second-largest fragment in $L^-RK^+$ ($23.0 \times 10^6$ daltons) digests and those of the other two host range varieties ($25.0 \times 10^6$ daltons); two additional fragments from each digest were also resolved (the three smallest fragments are not shown). Molecular weight estimates of the two additional fragments and recalculation of the larger fragments were determined by comparing migration distances with those of lambda RI or *Hin*d III fragments (Fig. 2B). Table 2 lists the molecular weights of the smaller fragments, calculated from 1.4% gels, and the larger fragments, calculated from 0.85% gels. No additional vaccinia DNA fragments were resolved when *Hin*d III digests were electrophoresed in 0.5% gels.

TABLE 1. *Molecular weight determination of vaccinia Hind III fragments in 1.4% agarose*

| Electrophoresis band | Sizes from λ/RI migration distance comparisons (mol wt × $10^6$) | |
|---|---|---|
| | $L^+RK^-$, $L^+RK^+$ | $L^-RK^+$ |
| 1 | 30.0 | 30.0 |
| 2 | 25.0 | 23.0 |
| 3 | 18.0 | 18.0 |
| 4 | 15.0 | 15.0 |
| 5 | 7.4 | 7.4 |
| 6 | 4.2 | 4.2 |
| 7 | 3.25 | 3.25 |
| 8 | 2.95 | 2.95 |
| 9 | 2.70 | 2.70 |
| 10 | 1.40 | 1.40 |
| 11 | 0.70 | 0.70 |
| 12 | 0.50 | 0.50 |
| Total mol wt | 111.10 | 109.10 |

Previous attempts to specifically cleave the entire vaccinia DNA molecule with the restriction enzymes from *Haemophilus parainfluenzae* (*Hpa* I and *Hpa* II) resulted in a large number of fragments that were difficult to resolve by gel electrophoresis (7). We have observed that cleavage with the restriction enzyme *Hin*d III from *H. influenzae* results in a smaller number of fragments, which can be easily analyzed. The inability of endonuclease RI to cleave vaccinia DNA alone or to cleave lambda DNA in an equal mixture with vaccinia DNA suggests that vaccinia DNA either contains RI recognition sites but is not completely cleaved or contains an RI inhibitory substance.

*Hin*d III digestion of the DNA from three vaccinia host range varieties revealed a minor difference between $L^-RK^+$ genomes from the CV-1 strain and $L^+RK^+$, $L^+RK^-$ genomes from the WR strain. Whether or not this difference was related to the virus strain, host range characteristics, or particle defectiveness has not been determined. It is interesting to note, however, that the plaquing efficiency (plaques/virions) of $L^+RK^-$ virions on mouse L cells was 1:10, whereas that of $L^-RK^+$ virions on rabbit kidney cells was 1:100.

Our estimates on the genome weight of vaccinia based on the cleavage fragments produced by *Hin*d III were lower than previous molecular weight estimates ($170 \times 10^6$) derived from sedimentation and contour length analysis (8, 15, 18), but agreed with the more recent molecular weight analysis of Geshelin and Berns (12). Underestimation of the actual genome weight of vaccinia by our methods could have resulted from repetitive DNA sequences or small fragments that were not detected. However, since vaccinia DNA has been shown not to contain

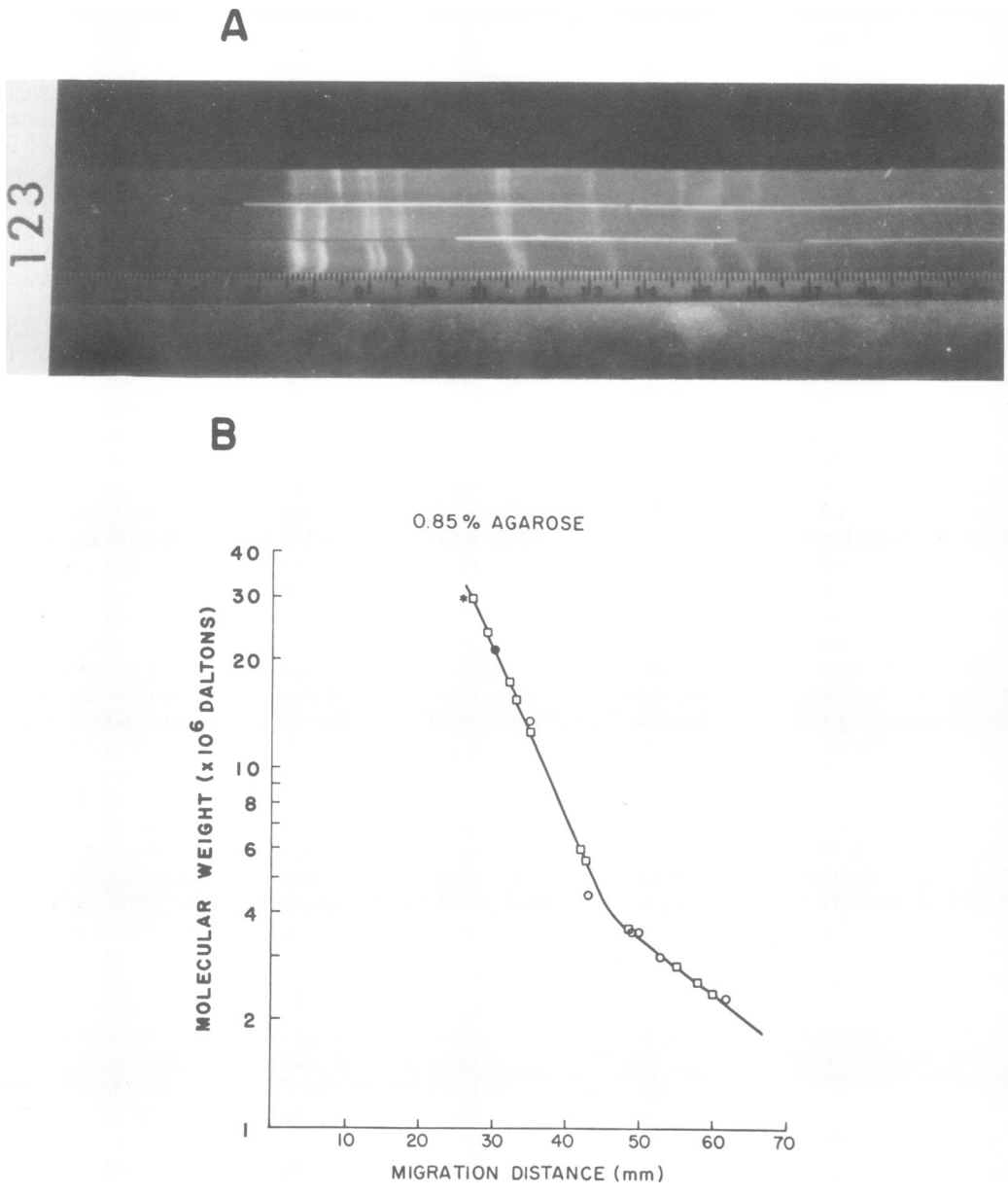


FIG. 2. *(A) Electrophoresis of vaccinia fragments in 0.85% agarose gels. (1) $L^+RK^-$ vaccinia DNA (3 μg) digested with endonuclease Hind III; (2) $L^+RK^+$ DNA (3 μg) digested with endonuclease Hind III; (3) $L^-RK^+$ DNA (3 μg) digested with endonuclease Hind III. Electrophoresis was for 9 h at 4 mA per gel. Direction of migration is from top to bottom toward the anode. Gels were stained and photographed as described in Fig. 1. (B) Mass-versus-mobility plot of endonuclease Hind III vaccinia DNA digests. Symbols: (○) Lambda RI marker fragments; (□) vaccinia Hind III fragments; (*) whole lambda DNA; (●) vaccinia Hind III $L^-RK^+$ fragment 2 (second fragment from top of gel).*

TABLE 2. *Molecular weight determination of vaccinia Hind III fragments in 0.85 and 1.4% agarose gels*

| Electrophoresis band[a] | Sizes from λ/RI and λ/*Hin*d III migration distance comparisons (mol wt × $10^6$) | |
|---|---|---|
| | $L^+RK^-$, $L^+RK^+$ | $L^-RK^+$ |
| 1 | 30.0 | 30.0 |
| 2 | 24.0 | 21.5 |
| 3 | 17.5 | 17.5 |
| 4 | 15.75 | 15.75 |
| 5[b] | 12.75 | 12.75 |
| 6 | 6.0 | 6.0 |
| 7[b] | 5.6 | 5.6 |
| 8 | 3.6 | 3.6 |
| 9 | 2.85 | 2.85 |
| 10 | 2.55 | 2.55 |
| 11 | 2.40 | 2.40 |
| 12 | 1.40 | 1.40 |
| 13 | 0.70 | 0.70 |
| 14 | 0.50 | 0.50 |
| Total mol wt | 125.60 | 123.10 |

[a] Bands 1 through 11 are from 0.85% agarose. Bands 12, 13, and 14 are from 1.4% agarose.

[b] Additional bands resolved in 0.85% agarose.

repetitive sequences (2) and small fragments were not observed after electrophoresis in higher concentrations of agarose, we believe that our data support the recent molecular weight estimates (122 ± 2.2 × $10^6$) of Geshelin and Berns (12).

We would like to thank Clark Huang for his help in preparing endonuclease *Eco*RI and June Middleton for her generous gift of endonuclease *Hin*d III. We would also like to thank Clyde Hutchison and Marshall Edgell for their encouragement and support of this study.

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