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Evidence of a Repetitive Sequence in Vaccinia Virus DNA

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Analysis of vaccinia DNA by reassociation kinetics revealed that 7% of the genome contains a sequence repeated 10 times. This sequence does not contain any host cell DNA, is viral specific, and is found in virions passed at either high or low multiplicities of infection.

Berns and Silverman (1) have shown that the vaccinia DNA genome is a double-stranded DNA of 1.2×10^8 daltons. This DNA is unique in having a cross-link at or near each end (1, 7). Analyses by DeFilippes (6) of the rapidly renaturing fragments of vaccinia virus DNA with restriction enzymes are also consistent with the Bern model of vaccinia DNA structure. Denaturation studies of vaccinia DNA with an electron microscope (7) also suggested that ca. 95% of the viral genome is present in unique sequences. As part of other studies, we examined the reassociation kinetics of vaccinia DNA by using S_1 nuclease and found that a surprisingly high percentage (7%) of the vaccinia genome is repetitious and consists of a sequence(s) repeated 10 times.

Vaccinia virus, strain WR, was grown in HeLa S-3 cells as previously described (5) and purified by the method of Joklik (8). The primary virus stocks, which were used for subsequent infections, were prepared in one of two ways. In method 1, HeLa S-3 cells were infected with 3 PFU/cell, and the virus was collected after one growth cycle at 24 h after infection. In method 2, HeLa cells were infected with 0.01 PFU/cell, and growth of the virus was carried out for two presumptive cycles (48 h). The latter method was used to minimize the number of defective virus particles that often appear in virus stocks passed at high multiplicities of infection (9). For the preparation of ^{3}H-labeled vaccinia virus, HeLa cells were infected with virus prepared by method 1 at 2 PFU/cell or with virus prepared by method 2 at 1 PFU/cell. The media contained 5 to 10 μCi of [^{3}H]thymidine per ml, and the virus was harvested 24 h after infection. Vaccinia DNA of specific activity 3.5×10^6 to 5×10^6 cpm/μg can be obtained by this procedure. DNA concentrations were determined by the diphenylamine method (4).

The DNA used in the reassociation kinetics was sheared to 500 to 600 nucleotides in length (measured by sedimentation analysis) by heating it in 500 μl of 0.3 M NaOH at 100°C for 15 min. After cooling to 0°C, the solution was neutralized with 185 μl of 1 M KH_2PO_4 to a final pH of 7.3 to 7.4. The solution was dialyzed overnight against 5 mM Tris-hydrochloride (pH 7.7) containing 0.5 M NaCl and 1 mM EDTA. The resultant DNA solutions were used for reassociation kinetic analyses (3) as described in the legend to Fig. 1.

The kinetics of ^{3}H-labeled vaccinia DNA reassociation is shown in Fig. 1A. There are two distinctly different species of DNA. The main species which comprises at least 85% of the DNA, reassociated with a $C_0t_{1/2}$ of 0.074 (or a rate of 13.44 M^{-1} s^{-1}). This rate, under our conditions, indicates a molecular complexity of 1.8×10^5 to 2.0×10^5 nucleotide pairs for vaccinia DNA. A minor, faster reassociation component was detected at a C_0t value below 10^{-3} and is clearly visible. The reassociation kinetic data of this minor component, representing about 7% of the vaccinia DNA, is shown in magnified form in Fig. 1B. The minor component reassociated at 135 M^{-1} s^{-1} or 10 times the rate of the main vaccinia DNA species. The same results were found for ^{3}H-labeled vaccinia DNA prepared from virus derived from cells infected with stock virus prepared at high multiplicities (method 1) or low multiplicities (method 2). We also found that 0.6% of the vaccinia DNA was already reassociated by C_0t 1×10^{-5}, and this instantly reassociating DNA presumably included the covalently joined ends of the vaccinia DNA remaining after the shearing procedure.

The rapidly reassociating component of vaccinia DNA in the C_0t range 10^{-5} to 10^{-3} could be caused by: (i) naturally occurring repeated viral sequences, (ii) repeated viral sequences arising from defective virus in the preparations, or (iii) contamination of the vaccinia DNA with repeated sequences of HeLa DNA. The second alternative can probably be eliminated, since we observed the same percentage of rapidly reassociating viral DNA components

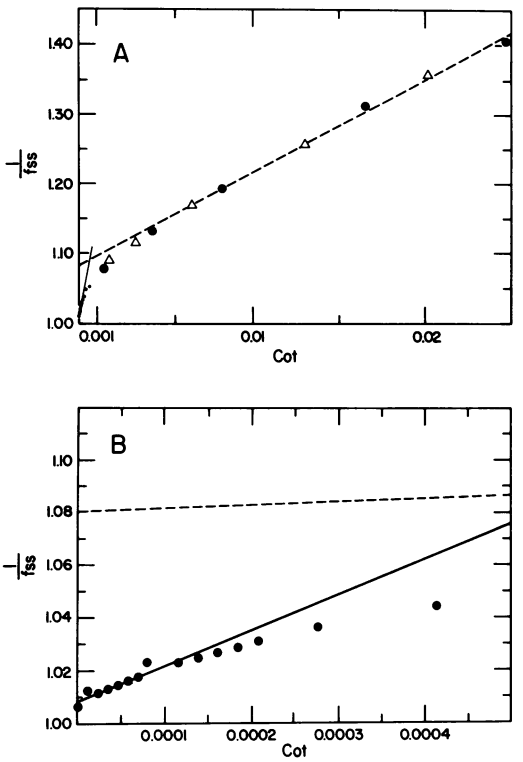

FIG. 1. *Reassociation kinetics of vaccinia DNA. Reassociation kinetic analysis was carried out at 65°C in a solution containing 5 mM Tris-hydrochloride (pH 7.7), 0.5 M NaCl, and 1 mM EDTA. Each sample of DNA (25 μl; containing 5.5 × 10⁴ to 9.5 × 10⁴ cpm) was sealed in glass capillary tube. At appropriate times, the amount of reassociated DNA was determined by the S_1 nuclease method; 100 μl of a reaction mixture containing 60 mM succinate buffer, (pH 4.9), 1.5 mM $ZnSO_4$, 4 μg of native salmon sperm DNA, and 7.4 U of S_1 nuclease (10) was added to the 25-μl sample. After incubation at 37°C for 30 min, 1 μg of denatured salmon sperm DNA was added to the reaction mixture, and the incubation continued for an additional 30 min at 37°C. The reaction was terminated by chilling to 0°C, and 60 μl from each sample was placed on GF/C filter disk and batch-washed as described by Bollum (2) to determine the acid-soluble radioactivity. Another 30 μl was placed on a GF/C filter to determine the total counts per minute in the sample. (A) The reassociation mixtures contained (●) ^{3}H-labeled vaccinia virus DNA at 0.75 μg/ml with 7 μg of carrier salmon sperm DNA per ml; (△) ^{3}H-labeled vaccinia virus DNA at 17.5 μg/ml. Both preparations of [^{3}H]DNA were obtained from virus produced after infection of cells with stock virus obtained by method 1. Reassociation was carried out until 85% of the vaccinia DNA was in a double-stranded form. (B) Enlargement of (A) showing the C_0t range from 1 × 10⁻⁵ to 5 × 10⁻⁴. (-----) Extrapolation of analogous line in (A); (——) ^{3}H-labeled vaccinia virus DNA at 0.75 μg/ml with 7 μg of carrier salmon sperm DNA per ml.*

and the same reassociation kinetics when virus was obtained from infections with vaccinia virus derived from either low multiplicities of infection (method 2) or high multiplicities of infection (method 1). Control experiments with a 100-fold excess of HeLa DNA showed that the rapidly reassociating fragment of vaccinia DNA contains no detectable HeLa DNA sequences.

Further experiments were carried out with a 20-fold excess of either polydeoxyadenylate or polydeoxy(adenylate-thymidylate) in the hybridization mixtures with vaccinia DNA. These synthetic polymers caused no change in the reassociation kinetics observed with vaccinia DNA and indicated that there are no detectable stretches of polydeoxythymidylate in the viral DNA.

We conclude, therefore, that 7% of vaccinia virus DNA contains repeated sequences. Assuming that there are 1.82 × 10⁵ base pairs in the vaccinia genome (7), the repeated sequences represent 1.3 × 10⁴ nucleotide pairs or 10 copies of a repeated sequence of 1.3 × 10³ nucleotide pairs. This calculation assumes that the thymidine content of the rapidly reassociating fragment is the same as that of the whole vaccinia DNA. It will be of interest to locate this sequence on the vaccinia genome and determine its function.

LITERATURE CITED

1. **Berns, K. I., and C. Silverman.** 1970. Natural occurrence of cross-linked vaccinia virus deoxyribonucleic acid. J. Virol. 5:299-304.
2. **Bollum, F. J.** 1959. Thermal conversion of nonpriming deoxyribonucleic acid to primer. J. Biol. Chem. 234:2733-2734.
3. **Britten, R. J., and D. E. Kohne.** 1968. Repeated sequences in DNA. Science 161:529-540.
4. **Burton, K.** 1956. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem. J. 62:315-323.
5. **Citarella, R. V., R. Muller, A. Schlabach, and A. Weissbach.** 1972. Studies on vaccinia virus-directed deoxyribonucleic acid polymerase. J. Virol. 10:721-729.
6. **DeFilippes, F. M.** 1976. Restriction enzyme digests of rapidly renaturing fragments of vaccinia virus DNA. J. Virol. 17:227-238.
7. **Geshelin, P., and K. I. Berns.** 1974. Characterization and localization of the naturally occurring cross-link in vaccinia virus DNA. J. Mol. Biol. 88:785-796.
8. **Joklik, W. K.** 1962. The preparation and characteristics of highly purified radioactivity labeled poxvirus. Biochim. Biophys. Acta 61:240-301.
9. **Schröder, C. H., B. Stegmann, H. F. Lauppe, and H. C. Kaerner.** 1975/76. An unusual defective genotype derived from herpes simplex virus strain ANG. Intervirology 6:270-284.
10. **Wiegand, R. C., G. N. Godson, and C. M. Radding.** 1975. Specificity of the S_1 nuclease from *Aspergillus oryzae*. J. Biol. Chem. 250:8848-8855.