

NOTES

Analysis of Human Cytomegalovirus Nucleoprotein Complexes

STEPHEN ST. JEOR,* CLAIRE HALL, CATHY MCGAW, AND MARK HALL

Department of Microbiology, School of Medicine, University of Nevada, Reno, Nevada 89557

Received 6 April 1981/Accepted 24 July 1981

When chromatin was isolated from cells infected with human cytomegalovirus, the virus DNA remained with the chromatin fraction. If deproteinized virus DNA was added to either isolated nuclei or chromatin, the DNA was lost during the chromatin isolation. When isolated chromatin from cytomegalovirus-infected cells was banded in isopycnic metrizamide gradients, a single peak with a density of 1.18 g/cm^3 was present. Analysis of this peak in isopycnic neutral CsCl gradients indicated that it contained both human cytomegalovirus and human embryonic lung cell DNAs. When infected nuclei were treated with micrococcal nuclease, 11S subunit particles which cosedimented with cell nucleosomes and contained virus DNA were isolated.

DNA from eucaryotic cells and from a number of animal viruses occurs as a protein-DNA complex rather than as free DNA (4, 5, 7, 8, 10-12, 16). The DNA-protein structures in eucaryotic cells as well as in certain papovaviruses are based on repeating subunits containing 150 to 200 base pairs of DNA associated with histone proteins (7, 8, 13). These repeating structures are referred to as nucleosomes.

In contrast to eucaryotic DNA, little is known concerning protein-DNA interactions and intracellular herpesvirus DNA. However, there are several reports indicating that both Epstein-Barr virus and herpes simplex virus intracellular DNAs occur associated with proteins (10-12, 14). Furthermore, it has been reported that integrated Epstein-Barr virus DNA occurs in a nucleosome-like structure (14).

The studies reported here were undertaken to determine whether intracellular human cytomegalovirus (HCMV) DNA occurs in association with proteins, and if so, whether the DNA-protein complex is present as a nucleosome-like structure.

(A report of the findings described in this article was presented at the Fifth Cold Spring Harbor Meeting on Herpesviruses, August 1980, at Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.)

Isolation of nucleoprotein by extraction of chromatin. If intracellular HCMV DNA occurs as a nucleoprotein complex, then procedures developed to isolate chromatin from eucaryotic cells (2) would be expected to result in the isolation of

HCMV nucleoprotein. To test this proposal, we initiated the following study.

Confluent monolayers of human embryonic lung (HEL) cells were propagated as described previously (15) and either mock infected or infected with HCMV (strain AD169) at a multiplicity of infection of 1. After infection, the cells were labeled from 12 to 36 h postinfection with [^3H]thymidine ([^3H]TdR; $10 \mu\text{Ci/ml}$). After the labeling period, approximately 2×10^7 cells were obtained from each culture, and chromatin was isolated as described by Axel (2), using buffers of decreasing ionic strength. A sample of nuclei was removed for DNA analysis before the chromatin isolation. After the chromatin extraction, both the nuclei and chromatin were deproteinized and analyzed by isopycnic centrifugation in neutral CsCl gradients as described previously (15). The results obtained from the infected nuclei and chromatin are shown in Fig. 1. Since both HCMV and *Klebsiella pneumoniae* have identical densities (1.716 g/cm^3), the *K. pneumoniae* marker facilitates the location of HCMV DNA in the gradient. The results of this preliminary study indicate that when the amounts of virus and cell DNAs in the nuclei and chromatin preparations were compared, the chromatin preparation was enriched in virus DNA.

If [^3H]TdR-labeled deproteinized HCMV DNA was added to both nuclei and chromatin preparations, all of the trichloroacetic acid-precipitable counts were removed by the isolation procedure, indicating that virus DNA in infected

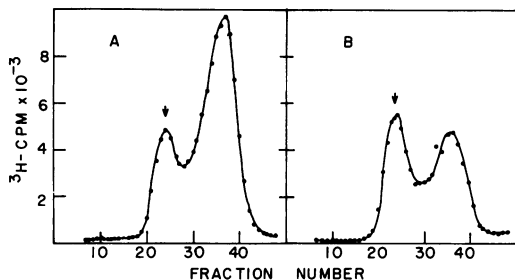


FIG. 1. Presence of HCMV DNA in cell chromatin. Isopycnic CsCl gradients were run on pronase-digested nuclei (A) and chromatin after extraction (B). Gradients were adjusted to an initial density of 1.716 g/cm^3 and analyzed in a Beckman type 40 rotor at 18°C for 60 h at 32,000 rpm in a Beckman L5-75 ultracentrifuge. Arrows indicate the position of the *K. pneumoniae* density marker (1.716 g/cm^3) (1).

cells was not present as deproteinized DNA (data not shown).

Banding of chromatin from virus-infected cells in metrizamide. To determine whether chromatin from infected cells banded at a density similar to chromatin from eucaryotic cells, infected cells (labeled with [^3H]TdR from 24 to 40 h postinfection) were extracted by the chromatin extraction method of Axel (2). The supernatants were layered onto a preformed 10 to 40% metrizamide gradient and centrifuged to equilibrium (3). The gradient was collected by bottom puncture into tubes, and fractions were sampled for density determinations (using a Bausch & Lomb refractometer) and trichloroacetic acid-precipitable counts. The radioactive material was present in a single peak banding at a density of 1.18 g/cm^3 . This value is what has been previously reported for eucaryotic chromatin banded in metrizamide gradients (3). The peak fractions banding at a density of 1.18 g/cm^3 were then pooled, deproteinized, and centrifuged to equilibrium in a neutral CsCl gradient. Figure 2A represents the profile from the metrizamide gradient, and Fig. 2B represents the gradient profile from the neutral CsCl gradient. The metrizamide gradient contained a single principal peak of radioactivity, whereas the CsCl gradient contained a mixture of virus (as indicated by the arrow denoting the position of the *K. pneumoniae* marker) and cell DNA.

Determination of whether HCMV DNA is present in virus-infected cells in a nucleosome structure. We used a dual radioactive label to determine whether nucleosomes from infected cells contain virus DNA. This was necessary since HCMV DNA contains guanine plus cytosine-rich regions (6); it is difficult to analyze nucleosome size pieces on CsCl gradients since the DNA pieces are heterogeneous in density. Unin-

fectected HEL cells were pulse-labeled with ^{32}P (2-h pulse) after the labeling period, the isotope was removed and chased, and the cells were infected with HCMV. The infected cells were then labeled from 26 to 30 h postinfection with [^3H]TdR. After the final radioactive label, the cells were harvested and nuclei were isolated. After removal of a sample for DNA analysis, nuclei were treated with micrococcal nuclease as previously described by Shaw et al. (14), and the supernatants were analyzed on 5 to 24.7% isokinetic sucrose gradients. The nuclei, which had been sampled before micrococcal nuclease treatment, were deproteinized and centrifuged to equilibrium in neutral CsCl gradients. The results obtained from the CsCl gradients are shown in Fig. 3A, and those obtained from isokinetic gradients are shown in Fig. 3B. The majority of the [^3H]TdR counts were found at a density of 1.716 g/cm^3 (as determined by the *K. pneumoniae* marker), whereas the ^{32}P counts were associated with cell DNA (density, 1.700 g/cm^3) (Fig. 3A). When the supernatants were analyzed on isokinetic gradients for radioactive counts (Fig. 3B), the [^3H]TdR and ^{32}P counts cosedimented, with the majority of the counts banding at a sedimentation coefficient of 11S (as determined from the S value of form I simian virus 40 DNA). The more rapidly sedimenting material (fractions 14 and 15) presumably was dimers or trimers of nucleosomes or both, and the more slowly sedimenting material (fractions 36 and 37) was less than unit length or core pieces.

For a determination of the sensitivity of deproteinized DNA to digestion with micrococcal nuclease, deproteinized and [^3H]TdR-labeled

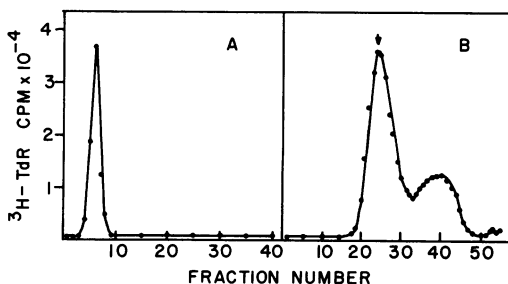


FIG. 2. Analysis of infected-cell chromatin on metrizamide and CsCl gradients. Chromatin extracted by the chromatin extraction method of Axel (2) was analyzed on a 10 to 40% preformed metrizamide gradient (48 h at 32,000 rpm at 10°C). The peak (A) corresponds to a density of 1.18 g/cm^3 , equivalent to cell chromatin (3). (B) CsCl gradient of the metrizamide peak. Fraction numbers 4 to 8 of the metrizamide gradient were pooled, digested with pronase, and run on a CsCl density gradient for 60 h at 32,000 rpm at 18°C in a Beckman type 40 rotor.

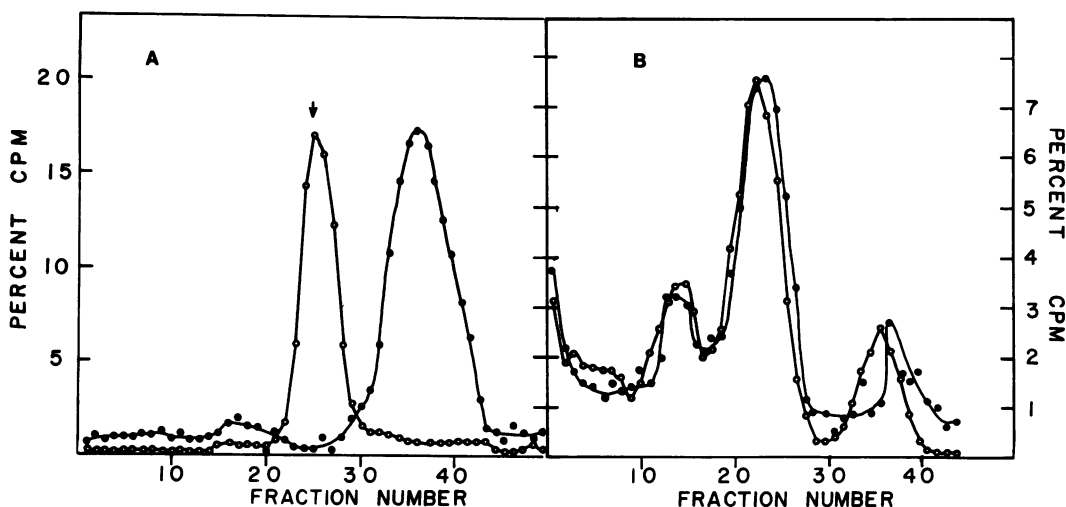


FIG. 3. Presence of HCMV DNA in nucleosomes. (A) Isopycnic centrifugation analysis in neutral CsCl of nuclear DNA from HEL cells prelabeled with ^{32}P (\bullet), infected with HCMV, and pulse-labeled (26 to 30 h postinfection) with $[^3\text{H}]\text{TdR}$ (\circ). The arrow indicates the position of the *K. pneumoniae* marker. (B) Profile of micrococcal nuclease-treated nuclei described in (A) and analyzed on 5 to 24% isokinetic sucrose gradients (9) (type 50.1 Beckman rotor, 30,000 rpm, 20 h at 4°C). Symbols: \bullet , ^{32}P label; \circ , $[^3\text{H}]\text{TdR}$ label.

HCMV DNAs were added to a reaction mixture containing HEL cell nuclei and micrococcal nuclease. The reaction mixture was sampled periodically for trichloroacetic acid-precipitable counts. Deproteinized labeled virus DNA was digested within 15 min by the micrococcal nuclease treatment (data not shown).

The results of these studies indicated that during chromatin isolation HCMV DNA remains with the chromatin fraction. In fact, the ratio of virus to cell DNA increased in the chromatin fraction as compared with the ratio of virus to cell DNA present in the nuclear preparation before chromatin isolation. When deproteinized virus DNA was added to the mixture either before or during chromatin isolation, it was rapidly diluted and removed during the chromatin isolation procedure.

The studies dealing with nucleosomes in infected cells indicated that the nucleosomes isolated from infected cells were similar to those of uninfected cells in their sedimentation coefficients (as determined with form I simian virus 40 DNA). Although this does not in itself indicate that HCMV DNA is present as a nucleosome, when we examine the data from the double-label experiment, the majority of the ^{32}P counts represented cell DNA and the $[^3\text{H}]\text{TdR}$ counts represented virus DNA (Fig. 3). When the sedimentation coefficient was determined from the supernatant material, the gradient profiles of $[^3\text{H}]\text{TdR}$ and ^{32}P counts were superimposable, indicating that virus DNA was present in a nucleosome-like structure.

In light of the paucity of information about the intracellular interactions of HCMV DNA with viral and cellular proteins during the virus replicative cycle, it would be of interest to further examine virus or cellular proteins or both associated with intracellular HCMV DNA. These experiments should provide additional information concerning the role of histone and nonhistone proteins in control of DNA replication and gene expression.

This investigation was supported by Public Health Service grant CA-28089 awarded by the National Cancer Institute and a grant from the Reno Cancer Society.

LITERATURE CITED

- Andersson, M., and T. Lindahl. 1976. Epstein-Barr virus DNA in human lymphoid cell lines. In vitro conversion. *Virology* 73:96-105.
- Axel, R. 1978. Dissection of the eucaryotic chromosome with deoxyribonucleases. *Methods Cell Biol.* 18:41-54.
- Birnle, G. D. 1978. Fractionation of chromatin by buoyant density gradient sedimentation in metrizamide. *Methods Cell Biol.* 18:23-39.
- Green, M. H., H. I. Miller, and S. Hendler. 1971. Isolation of a polyoma-nucleoprotein complex from infected mouse-cell cultures. *Proc. Natl. Acad. Sci. U.S.A.* 68:1032-1036.
- Hall, M. R., W. Meinke, and D. A. Goldstein. 1973. Nucleoprotein complexes containing replicating simian virus 40 DNA: comparison with polyoma nucleoprotein complexes. *J. Virol.* 12:901-908.
- Kilpatrick, B. A., and E.-S. Huang. 1977. Human cytomegalovirus: partial denaturation map and organization of genome sequences. *J. Virol.* 24:261-276.
- Kornberg, R. D. 1974. Chromatin structure: a repeating unit of histones in DNA. *Science* 184:868-871.
- Kornberg, R. D. 1977. Structure of chromatin. *Annu. Rev.*

- Biochem. 46:931-954.
9. **McCarty, K. S., Jr., R. T. Yollmer, and K. S. McCarty.** 1974. Improved computer program data for the resolution and fractionation of macromolecules by isokinetic sucrose density gradient sedimentation. *Anal. Biochem.* 61:105-183.
 10. **Pignatti, P. F., and E. Cassai.** 1980. Analysis of herpes simplex virus nucleoprotein complexes extracted from infected cells. *J. Virol.* 36:816-828.
 11. **Pignatti, P. F., E. Cassai, and U. Bertazzoni.** 1979. Herpes simplex virus DNA synthesis in a partially purified soluble extract from infected cells. *J. Virol.* 32:1033-1036.
 12. **Pignatti, P. F., E. Cassai, G. Meneguzzi, N. Chenciner, and G. Milanesi.** 1979. Herpes simplex virus DNA isolation from infected cells with a novel procedure. *Virology* 93:260-264.
 13. **Ponder, B. A. J., and L. V. Crawford.** 1977. The arrangement of nucleosomes in nucleoprotein complexes from polyoma virus and SV40. *Cell* 11:35-49.
 14. **Shaw, J. E., L. F. Levinger, and C. W. Carter, Jr.** 1979. Nucleosomal structure of Epstein-Barr virus DNA in transformed cell lines. *J. Virol.* 29:657-665.
 15. **St. Jeor, S., and R. Hutt.** 1977. Cell DNA replication as a function in the synthesis of human cytomegalovirus. *J. Gen. Virol.* 37:65-73.
 16. **Tate, V. E., and L. Philipson.** 1979. Parental adenovirus DNA accumulates in nucleosome-like structures in infected cells. *Nucleic Acids Res.* 6:2769-2786.