Role of the Nuclear Matrix in the Growth of Herpes Simplex Virus Type 2

By

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With 5 Figures

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Summary

Nuclear matrix was prepared from Vero cells infected with herpes simplex virus type 2. In the early stage of infection, both 155K and 110K viral proteins were associated with the nuclear matrix, while in the late stage, 155K protein, presumably a viral capsid protein, was predominantly associated with the matrix. Electron microscopic study showed that empty capsids were bound to the filamentous networks of the nuclear matrix of the late stage. Neither viral DNA nor viral DNA polymerase activity was associated with the nuclear matrix. These results may indicate that the nuclear matrix plays some role in the growth of herpes simplex virus, especially during the morphogenesis.

Introduction

Herpes simplex virus (HSV) is a large DNA virus which replicates its DNA and forms part of virus particle in the nucleus of the host cell. The viral genome has been shown to encode more than forty polypeptides. Because of the complexity of this virus, the process of DNA replication and morphogenesis is still not fully elucidated.

Chin and Maizel (1977) reported that E3, which is an intranuclear adenovirus type 2 (Ad2)-induced early protein (11,000 daltons), was enriched in the nuclear matrix fraction. Recently, Buckler-White et al. (1980) suggested that the nuclear matrix might play an important role in DNA replication and encapsidation of polyoma virus. The nuclear matrix is a nuclear framework structure which resists a wide variety of chemical treatments and extractions.
and contains a residual peripheral lamina with nuclear pore complexes, residual nucleoli and an internal fibrogranular network (Berezney and Coffey, 1976; Comings and Okada, 1976; Hodge et al., 1977; Berezney and Coffey, 1977). Considerable evidence has been accumulated to indicate that the nuclear matrix is involved in the physiology of nuclear functions, including a preferential association of newly synthesized DNA, and the tenacious binding of a kind of RNA and protein (Shaper et al., 1978). It was also reported that nuclear matrix from actively replicating liver cells contains significant DNA polymerase activity (Smith and Berezney, 1980). It would be of interest whether the nuclear matrix plays some role in DNA replication and morphogenesis of herpes viruses which, unlike polyoma virus, use virus-induced DNA polymerases for their DNA replication.

In the present study, we isolated a nuclear matrix from Vero cells infected with HSV-2 and examined the nuclear matrix using both biochemical and morphological investigations.

Materials and Methods

Cells and Viruses

Vero cells, a continuous line of African green monkey cells, which were kindly supplied by Dr. K. Yamanishi, Osaka University, Japan, were grown in Eagle’s minimal essential medium (MEM) supplemented with 10 per cent foetal calf serum, 100 units of penicillin, and 100 μg/ml of streptomycin. HSV-2 strain 186 derived from single plaque was propagated in Vero cells by infecting at a low multiplicity (0.01 PFU/cell). Infected cells were harvested when almost all cells exhibited cytopathic effects (CPE). After freezing and thawing three times and eliminating cell debris at 3000 r.p.m. for 10 minutes, virus was stored at −80 °C (Nishiyama and Rapp, 1981).

Preparation of Nuclear Matrix

Nuclei were isolated from cells according to the procedure of Shimada et al. (1972). The cell sheet was washed with phosphate-buffered saline (PBS) three times. The cells were suspended in 10 ml of RSB buffer [10 mM Tris-HCl, 10 mM NaCl, 1.5 mM MgCl₂, pH 7.4], and left for 5 minutes on ice. After adding Nonidet p-40 (NP-40) to a final concentration of 0.5 per cent, the cells were homogenized by 10 strokes with a glass homogenizer (Kontes Glass Co., Vineland, N. J.). The homogenate was layered over an equal volume of 0.5 M sucrose in RSB buffer, and centrifuged at 1500 r.p.m. for 5 minutes. The nuclear pellet was then washed again with TM sucrose buffer (0.25 M sucrose, 5 mM MgCl₂, 50 mM Tris-HCl, pH 7.4).

Nuclear matrix was prepared according to the method of Buckler-White et al. (1980). Nuclei isolated from 5 × 10⁷ cells were resuspended in 10 ml with TM sucrose. Triton X-100 was added to a final concentration of 1 per cent. The nuclei were centrifuged at 1500 r.p.m. for 10 minutes and then resuspended in TM sucrose to yield Triton nuclei. After sedimentation, the nuclei were resuspended in 5 ml of TM sucrose and incubated with 20 μg/ml DNase I (Worthington, Freehold, N. J.) for 20 minutes at room temperature under continuous shaking, followed by three washes in 10 ml of LM buffer (0.2 mM MgCl₂, 10 mM Tris-HCl, pH 7.4). The final LM pellet was extracted for 15 minutes in 5 ml of HS buffer (2 mM NaCl, 0.2 mM MgCl₂, 10 mM Tris-HCl, pH 7.4) on ice and then pelleted at 3000 r.p.m. for 15 minutes. This high salt extraction was repeated. The nuclear matrix was obtained after a final wash with 10 ml of LM buffer.

Electrophoresis in SDS-Polyacrylamide Gels

Infected or uninfected Vero cells were labeled with 5 μCi/ml of ³⁵S-methionine under the conditions described in the text. Aliquots of samples in each extraction