Nuclear Matrix Modifications at Different Stages of Infection by Herpes Simplex Virus Type 1

By Viviane Bibor-Hardy,* Monique Bernard and René Simard

Institut du Cancer de Montréal, 1560 Est, rue Sherbrooke, Montréal, Québec H2L 4M1, Québec, Canada

(Accepted 9 January 1985)

SUMMARY

In BHK-21 cells infected with herpes simplex virus type 1 many virus-induced proteins were found attached to the nuclear matrix. To understand the role of this cell fraction during virogenesis, matrix-associated proteins were analysed at different stages of infection. All the immediate-early protein species were bound to the nuclear matrix and their association with this structure was stable. During the first few hours of infection, the pattern of virus-induced proteins attached to the nuclear matrix remained identical, indicating that polypeptides from the early group are not associated with this cell fraction. Among the late proteins, which are generally structural proteins, 60% of the nuclear proteins were tightly bound to the nuclear matrix. This suggests that the nuclear matrix is involved in at least two different events during virogenesis, regulation of viral infection and assembly of viral capsids.

INTRODUCTION

The study of the structural and functional organization of the nucleus has led to the discovery of a nuclear skeleton in all the eukaryotic systems examined to date. Currently, this nuclear skeleton, which will be referred to as the nuclear matrix, is only defined on an operational basis as the structure remaining after sequential treatment of purified nuclei with non-ionic detergent, low-magnesium buffer, high-salt buffer and extensive digestion with nucleases (Aaronson & Blobel, 1975; Berezney & Coffey, 1974). Examination by electron microscopy reveals that it is composed of a lamina with nuclear pores, residual nucleoli and an internal fibrogranular network (Berezney & Coffey, 1977; Kaufmann et al., 1981, 1983). In normal cells, the nuclear matrix is highly enriched in newly replicated DNA and unprocessed RNA. This has led to an increasingly strong tendency to view the matrix as the active structure of the nucleus where cellular DNA replication (McCready et al., 1980; Pardoll et al., 1980; Berezney & Buchholtz, 1981) and RNA processing take place (Ciejek et al., 1982; Robinson et al., 1982; van Eekelen & van Venrooji, 1982).

The interaction of herpes simplex virus (HSV) with eukaryotic cells can result in productive, abortive, transforming, persistent or latent infection. Viral DNA replication and encapsidation occur in the nucleus. Previous work with HSV type 1 (HSV-1) has demonstrated that during a productive infection, several virus-induced proteins become part of the nuclear matrix; many viral capsids, mostly empty, remain attached to the internal fibrogranular network (Bibor-Hardy et al., 1982a, b). The presence of viral proteins in the nuclear matrix has been confirmed for HSV-2 (Tsutsui et al., 1983) and observed with several other viruses such as adenovirus (Chin & Maizel, 1977), polyomavirus (Buckler-White et al., 1980) and simian virus 40 (Ben-Ze'ev et al., 1981; Jones & Su, 1982).

These findings raised the question of the role of the nuclear matrix during virogenesis. This structure may be the site of viral capsid formation and/or where viral DNA is synthesized (as is the case for cellular DNA) and stored before encapsidation. However, before assigning a
function to the nuclear matrix during HSV infection, it is necessary to characterize the proteins associated with this structure at the different stages of virogenesis. HSV-1 gene expression is tightly regulated during the reproductive cycle. Specifically, the HSV-1 genes form three groups, immediate-early, early and late, whose synthesis is coordinately regulated and sequentially ordered in a cascade fashion (Honess & Roizman, 1974, 1975). The five known immediate-early genes are expressed first and at least one, ICP4, is required for the expression of early genes (Dixon & Schaffer, 1980; Watson & Clements, 1980). The early genes turn off the synthesis of immediate-early genes and enable the expression of late genes which are mostly structural virion proteins.

In this paper, we have examined the protein composition of the nuclear matrix during the course of infection by HSV-1.

METHODS

Cell culture, viral infection and labelling. BHK-21 cells were grown at 37 °C in α-medium (Gibco) supplemented with 10% foetal calf serum (Flow Laboratories) and 1% kanamycin (Gibco). The tsG8 mutant strain KOS (from Dr P. Schaffer, Harvard Medical School and Sidney Farber Cancer Institute, Boston, Mass., U.S.A.) used in previous studies on involvement of the nuclear matrix during HSV virogenesis (Bibor-Hardy et al., 1982a, b) was propagated and assayed on BHK cells.

For labelling of early and late viral proteins, confluent monolayers of BHK cells were infected at an m.o.i. of 20 p.f.u./cell and incubated at 37 °C for 45 min. After infection, the cells were washed 3 times with α-medium containing 2% foetal calf serum and 1% kanamycin and further incubated at 34 °C, the permissive temperature. Virus-induced proteins (ICP) were labelled 4 h post-infection with 10 μCi/ml [35S]methionine (1000 Ci/mmol, Amersham) in α-medium containing one-tenth the usual concentration of methionine and 2% foetal calf serum.

For labelling of immediate-early viral proteins, exponentially growing BHK cells were infected at an m.o.i. of 5 p.f.u./cell in α-medium supplemented with 8% foetal calf serum, 1% kanamycin, 50 μg/ml cycloheximide, and incubated for 1 h at 37 °C. After a 16 h incubation at 34 °C in the same medium, the cycloheximide block was released by washing the cells 4 times with α-medium (without methionine) containing 2% foetal calf serum and 1% kanamycin. The proteins were labelled for 30 min in this medium with 40 μCi/ml [35S]methionine.

For pulse and chase experiments, the infected cells, labelled as previously described, were washed 4 times at 34 °C in the same medium without radioactive isotope and further incubated at 34 °C.

Purification of nuclei. Nuclei were purified with an Ultra-Turrax (Janke & Kunkel) in detergent–collagenase buffer as previously described (Bibor-Hardy et al., 1982a) which can lead to some degradation of the cytoplasmic proteins or in TECK buffer (10 mM-Tris–HCl pH 7.8, 3 mM-CaCl2, 1 mM-EDTA, 10 mM-KCl; Dagenais et al., 1984). At this stage, the nuclei were clean and devoid of any cytoplasmic contamination as monitored by phase-contrast or electron microscopy.

Purification of nuclear matrix. The sequential treatment of purified nuclei with low-magnesium buffer, DNase and high-salt (2 M-NaCl) buffer for nuclear matrix purification of BHK cells has been described elsewhere (Bibor-Hardy et al., 1982a; Dagenais et al., 1984) except that 0.1 mM-vanadyl ribonucleoside complex (Bethesda Research Laboratories) was present in all the buffers to improve preservation of the fragile structure after infection. A better recovery of nuclear matrices was obtained by increasing the length of the centrifugations (10 min after low-salt and DNase treatments, and 20 min after high-salt treatment). Supernatants were kept after each step, centrifuged at high speed to remove all aggregates, and TCA-precipitated.

Protein analysis. Samples were dissolved in 2° SDS, 10° glycerol and 5° 2-mercaptoethanol and boiled for 5 min. SDS-PAGE (Laemmli, 1970) was performed using a 5 to 12° gradient separating gel and a 4° stacking gel. After treatment with Enhance (New England Nuclear) the gels were fluorographed on X-Omat RP X-ray films at −70 °C. The HSV-induced polypeptides were designated according to Honess & Roizman (1973) or using molecular weight markers (from Bio-Rad and Pharmacia) ranging from 200 000 (myosin) to 14 400 (α-lactalbumin) mol. wt. The absorbance of bands on the fluorograms was measured with a microdensitometer (Hoeffer) and the proteins quantified by calculation of the area under the recorded peak with respect to the total surface, using an MOP-3 (Zeiss).

RESULTS

Presence of immediate-early proteins in the nuclear matrix

BHK-21 cells were infected at 5 p.f.u./cell in the presence of cycloheximide to enable accumulation of immediate-early mRNAs. The virus-induced polypeptides synthesized following the withdrawal of cycloheximide were labelled for 30 min. In these conditions, the pattern of nuclear matrix proteins synthesized from normal BHK cells is similar to the one of
Fig. 1. Distribution of virus-induced immediate-early proteins in HSV-infected BHK cells. Cells were infected at 5 p.f.u./cell and incubated for 15 h in the presence of cycloheximide. The infected cell proteins were labelled between 0 and 0.5 h after the removal of the inhibitor. Nuclei were purified using a TECK buffer and sequentially extracted with low-salt, DNase and high-salt. Proteins from each fraction were analysed by SDS-PAGE and fluorography. Ideally, for each cellular fraction, an equal amount of radioactive material was loaded to each well (10000 c.p.m.). This was not always possible, since in the low-salt and the DNase supernatants only very small amounts of radioactivity were often recovered. In this case, all the material available was analysed. Lane 1, total cell extract; lane 2, cytoplasmic fraction; lane 3, nuclear fraction; lane 4, low-salt supernatant; lane 5, DNase supernatant; lane 6, high-salt supernatant; lane 7, nuclear matrix; lane 8, HSV-infected cell nuclear matrix protein labelled from 4 to 8 h post-infection used as a control.

untreated cells (results not shown). Treated cells were first separated into cytoplasmic and nuclear fractions, and the purified nuclei were sequentially extracted with detergent, low-magnesium buffer, DNase and high-salt to obtain nuclear matrices. Labelled proteins in each fraction were analysed by SDS-PAGE and fluorography. Fig. 1 shows the presence of ICP4, ICP0, ICP22 and ICP27 in the total cell extract where they represented respectively about 1%, 3%, 1.5% and 5% of the labelled proteins as quantified by densitometry. All these proteins were found in the cytoplasmic fraction although to a lesser extent than in the nucleus where they represented 15% of the labelled proteins. ICP4 and ICP27 were partially released from the nucleus after the high-salt extraction but a considerable amount of these proteins remained firmly attached to the nuclear matrix. ICP0 and ICP22 were mostly located in this subnuclear fraction. At this time post-infection, the immediate-early proteins represented more than 25% of the labelled nuclear matrix proteins (3% ICP4, 14% ICP0, 4% ICP22 and 5% ICP27).
In order to determine whether or not the immediate-early proteins and their association with the nuclear matrix are transient during the early stages of infection, the same experiment was repeated but with a chase of 2 or 4 h after labelling. Proteins from total cell extracts, purified nuclei or nuclear matrices were analysed by SDS-PAGE and fluorography. Fig. 2 demonstrates that the immediate-early proteins synthesized during the first 30 min were still present in the cells after a 4 h chase, indicating the great stability of these proteins. The percentage of immediate-early proteins bound to the nuclear matrix remained stable during the chase (Fig. 2, lanes 7, 8 and 9). In this fraction, the relative amount measured for ICP4 was 2% after the pulse-labelling and 4% after the 4 h chase; for ICP0, 11% and 14% respectively; for ICP22, 4% and 4%; for ICP27, 8% and 6%.

Fig. 2. Cellular distribution of pulse-labelled and chased immediate-early proteins in HSV-infected cells. Cells prepared as described in Fig. 1 were chased either for 2 h or 4 h after the pulse-labelling. These cells were then harvested, nuclei were purified and nuclear matrices extracted. Proteins from total cells (lanes 1 to 3), nuclei (lanes 4 to 6) or nuclear matrices (lanes 7 to 9) were analysed by SDS-PAGE and fluorography. Lanes 1, 4 and 7, 30 min pulse-labelled proteins without chase; lanes 2, 5 and 8, after a 2 h chase; lanes 3, 6 and 9, after a 4 h chase.
Fig. 3. Distribution of virus-induced proteins synthesized at different times following the removal of a cycloheximide block. BHK cells were infected at 5 p.f.u./cell and incubated for 15 h in the presence of cycloheximide. The infected cells were labelled for 30 min at different times following the removal of the inhibitor. Cells were harvested after the labelling and processed to obtain the nuclear matrices. Proteins from total cells (lanes 1 to 4) or nuclear matrices (lanes 6 to 9) were analysed by SDS-PAGE and fluorography. Lanes 1, 6, labelling from 0 to 0.5 h; lanes 2, 7, labelling from 1 to 1.5 h; lanes 3, 8, labelling from 2 to 2.5 h; lanes 4, 9, labelling from 3 to 3.5 h; lane 5, labelling of proteins prior to the removal of cycloheximide.

From these experiments we can conclude that a portion of all the immediate-early protein species is associated with the nuclear matrix in HSV-infected cells and remains as such over an extended period of time.

Assocation of the virus-induced early proteins

We next looked for the presence of viral proteins from the early group. The experiment described in Fig. 1 was repeated except that after the cycloheximide was removed, the synthesis of polypeptides was allowed to continue. In these conditions, early polypeptide synthesis soon follows and replaces synthesis of the immediate-early polypeptides. Newly synthesized proteins were labelled for 30 min at different times after the removal of cycloheximide and their association with the nuclear matrix was examined. Fig. 3 shows that ICP4, ICP22 and ICP27 were still synthesized in the infected cells 3 h after the removal of the inhibitor, while in the same conditions ICP0 synthesis was no longer detectable. The synthesis of ICP6, ICP8, ICP10 and ICP17 began 1 h after the removal of the block, but none of them was localized in the nuclear matrix subfraction. Analysis of the proteins released at each step during the preparation of
Fig. 4. Distribution of virus-induced early and late proteins in HSV-infected BHK cells. Cells were infected at 20 p.f.u./cell at 34 °C and were labelled from 4 to 8 h post-infection. Nuclei were prepared in a detergent-collagenase buffer and fractionated as described in Fig. 1. Proteins from each fraction were analysed by SDS-PAGE and fluorography. Lane 1, total cell extract; lane 2, insoluble cytoplasmic fraction; lane 3, soluble cytoplasmic fraction; lane 4, nuclei with nuclear membranes; lane 5, nuclei without nuclear membranes; lane 6, low-salt supernatant; lane 7, DNase supernatant; lane 8, high-salt supernatant; lane 9, nuclear matrices.

nuclear matrices indicates that ICP6 is located in the cytoplasm and that at this stage of infection, ICP8 is mainly extracted during the high-salt treatment (results not shown). At the nuclear matrix level, the patterns of viral proteins labelled in these conditions between 3 and 3.5 h or 0 and 0.5 h post-treatment were nearly identical, meaning that only the immediate-early proteins are bound to the nuclear matrix.

**Accumulation of the late proteins**

In the previous experiment, some synthesis of ICP5 (from the late group) was detectable as early as 3 h post-treatment, but this protein was not associated with the nuclear matrix (Fig. 3, lanes 4 and 9). However, it has been shown that among the viral proteins associated with the nuclear matrix 15 h after the infection, ICP5 is one of the most abundant species (Bibor-Hardy et al., 1982a, b). To verify the behaviour of late proteins, BHK-21 cells were infected at an m.o.i. of 20 p.f.u./cell and [35S]methionine was added 4 h after infection. Viral proteins from different cellular compartments were analysed at three times post-infection: 6 h, when according to Honess & Roizman (1975) early proteins are at their maximum rate of synthesis and late protein...
Table 1. Accumulation of the virus-induced proteins (ICP) in different cellular compartments during infection with HSV-1.

<table>
<thead>
<tr>
<th></th>
<th>Radioactivity (% of total labelling)*</th>
<th>6 h</th>
<th>8 h</th>
<th>12 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>100 (1.95 x 10^8)</td>
<td>100 (4.97 x 10^8)</td>
<td>100 (4.74 x 10^8)</td>
</tr>
<tr>
<td>Cell</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytoplasm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soluble</td>
<td>21·0</td>
<td>19·3</td>
<td>20·3</td>
<td></td>
</tr>
<tr>
<td>Insoluble</td>
<td>28·7</td>
<td>30·8</td>
<td>22·6</td>
<td></td>
</tr>
<tr>
<td>Nucleus + nuclear matrix</td>
<td>50·2</td>
<td>49·9</td>
<td>57·2</td>
<td></td>
</tr>
<tr>
<td>Nucleus–nuclear matrix</td>
<td>100 (3.1 x 10^7)</td>
<td>100 (8.08 x 10^7)</td>
<td>100 (10.38 x 10^7)</td>
<td></td>
</tr>
<tr>
<td>Low-salt</td>
<td>2·6</td>
<td>12·7</td>
<td>8·7</td>
<td></td>
</tr>
<tr>
<td>DNase</td>
<td>12·5</td>
<td>14·2</td>
<td>13·5</td>
<td></td>
</tr>
<tr>
<td>High-salt</td>
<td>20·3</td>
<td>17·3</td>
<td>13·3</td>
<td></td>
</tr>
<tr>
<td>Matrix</td>
<td>64·6</td>
<td>55·7</td>
<td>64·5</td>
<td></td>
</tr>
</tbody>
</table>

* The total radioactivity incorporated into each fraction is expressed as a percentage of the total cell labelling or the total nuclear labelling.
† Total c.p.m.

synthesis is well underway, 8 h, when early and late proteins are synthesized at about half their maximum rate, and finally 12 h post-infection, when late proteins have attained their maximal rate of synthesis. The results obtained for 8 h are presented in Fig. 4. In the infected cells, ICP5 was detectable 6 h after the infection and already present at the nuclear level in association with the nuclear matrix. As the infection continued, ICP5 remained attached to this subnuclear structure (Fig. 4, lane 9). The detergent extraction removed many proteins between 100000 and 155000 mol. wt.; ICP5 and a large band at 130000 mol. wt., most probably ICP8, remained attached to these detergent-treated nuclei (Fig. 4, lane 5). At this stage ICP8 was now partially released during the DNase digestion (Fig. 4, lane 7), although the most important portion was still extracted during the 2 M-NaCl treatment (Fig. 4, lane 8). The DNase digestion also consistently removed a 63000 mol. wt. protein.

For each of these fractions, TCA-precipitable total radioactivity was calculated and reported as a percentage of the radioactivity of the cells. As shown in Table 1, half of the radioactivity incorporated into proteins was located in the nucleus. To differentiate further between the different localizations in the nucleus, purified nuclei were sequentially extracted with detergent, low-magnesium buffer, DNase and high-salt buffer, leaving a nuclear matrix. This sequential treatment left 60% of the infected cell proteins initially found in the nucleus associated with the nuclear matrix (Table 1).

**DISCUSSION**

In this paper, we present evidence that several virus-induced proteins are associated with the nuclear matrix throughout the productive cycle of HSV-1. During infection, 60% of the nuclear ICP were bound to this structure. The importance of this figure becomes evident when we compare this situation to a normal cell where only 12% of the nuclear proteins are part of the nuclear matrix (Dagenais et al., 1984). If we look at each step of the extraction, the greatest discrepancy between the normal and the pathological situation occurred at the high-salt step, where 54% of the nuclear BHK cell proteins but only 20% of the viral proteins were extracted. These results emphasize the involvement of the nuclear matrix in HSV virogenesis but give no clue as to the biological significance of this involvement. In normal cells, the nuclear matrix is a structure involved in the synthesis of DNA and its transcription, but in cells infected with viruses no functional relationship has been identified so far. The association of viral proteins with the nuclear matrix was studied with the hope that such a study could help in the understanding of nuclear matrix function and shed light on the process of virogenesis as well.
HSV-1 virogenesis is tightly regulated and the proteins synthesized can be classified in three groups, immediate-early, early and late. Functions are known for relatively few of the 50 or more polypeptides synthesized. However, functional immediate-early proteins are required for the expression of the two subsequent kinetic classes of viral genes, and that requirement is at the transcriptional level (Honess & Roizman, 1975; Preston, 1979a, b; Watson & Clements, 1978). Only ICP4 and ICP22 have been studied with regard to their function and ICP22 appears to be dispensable (Post & Roizman, 1981). ICP4 is involved in the transition from immediate-early to early and late, and is required continuously during the infection to maintain mRNA synthesis (Preston, 1979b; Dixon & Schaffer, 1980). In our experiments, all the immediate-early protein species detectable in the total cell extract were found in the nuclear matrix, where their association was stable as judged by pulse and chase experiments. The fact that proteins implicated in the regulation of viral protein synthesis are associated with the nuclear matrix and stay attached long after their synthesis would imply that this structure is involved in the control of mRNA transcription during virogenesis. Up to now, only two eukaryotic proteins have been associated unequivocally with the positive regulation of gene expression: the E1A protein of adenovirus and ICP4 (Imperiale et al., 1983). Recently, E1A was also found attached to the nuclear matrix (Feldman & Nevin's, 1983). From these observations, it is tempting to postulate that viral gene expression is regulated at the nuclear matrix level; the regulated proteins would first bind to the nuclear matrix and, once associated, mediate attachment of viral DNA to stimulate transcription. Clearly, more work must be done in order to understand the relation between viral DNA, immediate-early proteins and nuclear matrix.

Among the proteins of the early group, many are implicated in viral DNA synthesis or have regulatory functions similar to ICP4, allowing transcription of late mRNA and inhibiting transcription of immediate-early mRNA. The early protein(s) responsible for this regulatory function are undetermined. Since viral DNA is firmly bound to the nuclear matrix (D. Villard, unpublished results), we were expecting to find proteins involved in DNA replication to be associated with the nuclear matrix. In our preparations, no early protein seemed attached to the nuclear matrix. However, in nuclear matrix labelled from 2 to 2.5 h after removal of cycloheximide, many proteins were found in the 90,000 to 100,000 mol. wt. region (ICP0, ICP10, ICP17) and were poorly resolved in SDS–PAGE. Presently, we are constructing two-dimensional gels to characterize these proteins better. Faint bands were also present in the 30,000 mol. wt. region in our gels and must be better characterized. Before ruling out any involvement of the nuclear matrix in herpesvirus DNA replication, DNA-binding proteins attached to the nuclear matrix will be examined.

Late proteins are mostly structural components of the virion and when present in the nucleus, more than 60% were bound to the nuclear matrix. The major capsid protein was found almost exclusively in this structure, and we now have evidence that immediately after its transport into the nucleus, ICP5 is incorporated into capsids. These results confirm our previous hypothesis that formation of viral capsids occurs within the nuclear matrix (Bibor-Hardy et al., 1982a).

In conclusion, our results suggest that the nuclear matrix is implicated in at least two different events during virogenesis, regulation of viral infection and assembly of viral capsids.

We would like to thank Lucie Brouillette for her excellent technical assistance. This work was supported by the National Cancer Institute and the Medical Research Council of Canada.

REFERENCES


HSV and the nuclear matrix


PRESTON, C. M. (1979b). Control of herpes simplex virus type 1 mRNA synthesis in cells infected with wild-type virus or the temperature-sensitive mutant tsK. Journal of Virology 29, 275-284.


(Received 5 October 1984)