Modification of discrete nuclear domains induced by herpes simplex virus type 1 immediate early gene 1 product (ICP0)

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The outcome of herpes simplex virus type 1 (HSV-1) infection depends upon the interplay of both host and viral factors. During lytic infection, HSV-1 causes a loss of immunofluorescent staining of discrete nuclear domains (ND10). This elimination of the host's ND10 staining occurs under conditions that allow only HSV-1 immediate early viral gene expression. Western blot analysis indicates that the loss of ND10 staining is due to ND10 redistribution, rather than protein degradation or turnover. When deletion mutants of all of the HSV-1 immediate early genes were tested, only infection with an immediate early gene 1 product (ICP0) deletion mutant, dl1403, was unable to eliminate ND10 antigen staining. Also, ICP0 transiently colocalized with ND10 antigens, after which ND10 antigens became undetectable. At late times during infection with dl1403, the host ND10 antigens were retained in virus-induced structures which were never observed during wild-type HSV-1 infection. These results suggested that ICP0 may be directly involved in the modification of the host nuclear domain. Infection with an adenovirus recombinant that expressed ICP0 demonstrated that in the absence of other HSV-1 proteins ICP0 was sufficient for the change in nuclear distribution of host antigens located at ND10. We postulate that the trans-activation function of ICP0 during viral replication may be mediated by replacing, modifying or reorganizing nuclear host factors.

Introduction

Many nuclear processes occur in specific domains. Recent immunohistochemical and in situ hybridization studies have identified several nuclear domains, including splicing islands (Lerner et al., 1981), chromosomal areas (Mannuelidis, 1990), centromeres (Moroi et al., 1980), telomeres (Rawlins et al., 1991), coiled bodies (Andrade et al., 1991) and transcription tracks (Lawrence et al., 1989). We have recently characterized a nuclear domain designated ND10 for the approximate number of loci per nucleus. Despite their frequent paired appearance, ND10 are not present in telomeres, centromeres or splicing islands (Ascoli & Maul, 1991). These sites, however, are upregulated by interferon (Guldner et al., 1992) and respond to stress by a great increase in number from the average of 10 per nucleus in cultured cells (G. G. Maul & Z. F. Fu, unpublished).

Early during herpes simplex virus type 1 (HSV-1) replication in cultured cells, ICP8, a protein essential in DNA replication, aggregates in nuclear domains referred to as prereplication sites (deBruyn-Kops & Knipe, 1988; Quinlan et al., 1984). At early times following infection, they have the approximate number and apparent distribution of ND10. The HSV-1 immediate early protein ICP0 is also distributed in discrete nuclear sites when expressed from transfected plasmids (Everett, 1987, 1988; Gelman & Silverstein, 1986) or in cells infected with recombinant adenovirus 5/ICP0 viruses, which express ICP0 (Zhu et al., 1988). We therefore investigated by immunofluorescence whether ND10 represented a nuclear domain where ICP8-positive prereplication sites were initiated or represented host sites where ICP0 localized.

In the course of our investigation, we found that infection by several herpesviruses [HSV-1, human cytomegalovirus (HCMV) and Epstein–Barr virus (EBV)] caused elimination of immunofluorescent staining of ND10. However, this is not a general feature of all DNA viruses that replicate in the nucleus, because adenovirus infection did not eliminate ND10 antigen recognition. In HSV-1-infected cells, ND10 staining was lost early in the replication cycle, and under conditions that permitted the expression of only immediate early (IE) or z gene products. Analysis of HSV-1 IE gene mutants indicated that ICP0 was the only IE gene product necessary for the loss of ND10 staining and redistribution of the protein components of ND10. Infection with an adenovirus 5/ICP0 recombinant (Zhu et al., 1988) was used to demonstrate that ICP0 was sufficient to produce a
reorganization of ND10. These results suggest that trans-activation by ICP0 may be mediated through an interaction with protein components of the nuclear structure called ND10.

Methods

Antibodies. Human sera 455 and 1569 (Ascoli & Maul, 1991) and three monoclonal antibodies (MAbs) with reactivity to ND10 were used. Prior to use in immunofluorescence double-labelling experiments with HSV-1-infected cells, serum 455 was preabsorbed overnight with a lysate from HSV-1-infected Hep-2 cells in order to remove HSV-1 antibodies. Owing to the high levels of ND10 antibodies and the very low amount of cellular ND10 antigen, HSV-1 antibodies were preferentially adsorbed. MAb 1150 was derived from a human lymphocyte antigen, and recognizes a 65K protein in activated Raji cells (Epstein, 1984). MAb 138 was made against a cloned β-galactosidase fusion protein that had been obtained by screening a human cDNA expression library with autoantibodies (AAbs) (D. Ziembicka-Kotula & G. G. Maul, unpublished data); it recognizes a 55K protein from Hep-2 cells (Ascoli & Maul, 1991). MAb 5El0 was derived from mice immunized with an isolated nuclear matrix preparation from rat liver nuclei, and recognizes a 126K antigen (Stuurman, 1991). Human autoimmune sera that recognize ND10 react with a 100K protein (Szostecki et al., 1987, 1990, 1992) in 0.14 M NaCl nuclear extracts, and with the 55K protein in nuclear matrix preparations (Ascoli & Maul, 1991). The ND10-specific rabbit anti-spl00 antibodies were generated from a bacterially expressed spl00 fragment (Szostecki et al., 1990). MAbs against ICP8 were provided by M. Zweig (Showalter et al., 1981), MAb H1083 against ICP0 by L. Pereira, University of California, San Francisco, Calif., U.S.A. (Ackerman et al., 1984), and hybridomas producing ICP4 antibodies were purchased from the ATCC. Human antiserum reactive with HSV-1, from our stock of control sera that did not label any uninfected cellular structures by immunofluorescence, was used to test for production of viral proteins.

Cell culture. Human epidermoid carcinoma Hep-2 cells and baby hamster kidney (BHK) cells were used because they are very flat, which allowed us to focus nearly throughout the cell at high magnification. These cells were maintained in modified Eagle’s medium supplemented with 10% fetal calf serum at 37 °C in a humidified atmosphere containing 5% CO₂. To inhibit protein, RNA or viral DNA synthesis, 100 μg/ml cycloheximide, 200 μg/ml actinomycin D (Act-D) or 400 μg/ml phosphonoacetic acid (PAA) was added to HSV-1-infected and control cultures for 6 h. Similar results were obtained when inhibitors were added at the time of infection or 1 h prior to infection. For immunofluorescence, cells were grown on round cover slips in 24-well plates (Corning). For quantification, 400 cells in random fields were counted for the absence or presence of the respective antibody staining.

Immunofluorescence microscopy. Cells were fixed at room temperature for 5 min with freshly prepared 1% (w/v) paraformaldehyde in PBS pH 7.4, washed with PBS, and permeabilized for 20 min on ice with 0.2% (v/v) Triton X-100 (Sigma) in PBS. Nuclear antigen localization was determined after incubation of permeabilized cells with human autoimmune serum or MAb diluted in PBS for 1 h at room temperature. Avidin-fluorescein or avidin-Texas Red was complexed with 0-2% (v/v) Triton X-100 (Sigma) in PBS. Nuclear antigen with primary antibodies through biotinylated secondary antibodies (Vector Laboratories). Goat anti-human or anti-rabbit immunoglobulin G conjugated with Texas Red was used in double-label immunofluorescence experiments. Cells were stained for DNA with 0.5 mg/ml of bis-benzimide (Hoechst 33258; Hoechst Celanese Corporation) in PBS mounted with Fluoromount G (Fisher Scientific). Fluorescence images were recorded using a Nikon Optiphot with a Plan 100 × objective using blue excitation for fluorescein, green excitation for Texas Red and violet excitation for bis-benzimide. TMAX P3200 black and white print film or EES800/1600 colour reversal film (Eastman Kodak) was used for photography.

Results

ND10 staining disappears early after infection with HSV-1

The AAb 445 and autoantibodies from a primary biliary cirrhosis patient recognize precisely circumscribed nuclear domains, ND10, which are present in replicating virus infected nuclei. These results suggest that trans-activation by ICP0 may be mediated through an interaction with protein components of the nuclear structure called ND10.
ICP8-positive prereplicative sites in HSV-1-infected cultured cells have been described as precisely circumscribed small nuclear domains that upon viral DNA synthesis expand to larger replication domains (deBruyn-Kops & Knipe, 1988; Quinlan et al., 1984). In the early stages of HSV-1 infection (3 to 4 h p.i.) of HEp-2 and BHK cells, MAbs against ICP8 detected a number of nuclear sites that appeared similar to ND10. Therefore, we tested the effect of HSV-1 infection on ND10 and found that ND10 disappeared early during infection. Fig. 2(a) shows that there are numerous cells, all of which contain ND10 except the mitotic cells or pairs of early G1 cells (arrows; cells were judged to be in early G1 phase by their condensed DNA pattern and connection by the Flemming body). Fig. 2(b) shows the same field of cells stained for DNA to outline the nuclei. At 5 h p.i., most cells have no ND10 in their nuclei as shown in Fig. 2(c). A field had been chosen that contains two cells that retain ND10 so as to allow placement of nuclei within the region stained for DNA (Fig. 2d). This image demonstrates that ND10 become undetectable after infection with HSV-1. When parallel infected cultures were tested at 5 h p.i. for the presence of ICP4, the number of cells positive for ICP4 (Fig. 2e, f) equalled the number that were negative for ND10, which was 92% in this particular experiment. Similar results were obtained with the rabbit anti-sp100 antibody and the MAb 5E10. Thus, it is the HSV-1-infected cells that lose ND10 reactivity and more than one protein is affected. To determine whether this response applied to other herpesviruses, we tested HCMV-infected HEp-2 cells and EBV-transformed Raji cells after induction with TPA. Both herpesviruses reduced ND10 staining (Table 1). However, it was 24 h before HCMV-infected cells reached the maximum number of ND10-negative cells. Variable numbers of Raji cells treated with TPA were found to be without ND10 in separate experiments (Laux et al., 1988). Similar analysis with adenovirus 5, a DNA virus which also has a short lytic cycle and encodes trans-activating proteins during replication in the nucleus, did not eliminate ND10 over a 14 h test period. Therefore, the elimination of ND10 is not a general feature of DNA virus infection.

\[ ND10 \text{ sites disappear before the appearance of prereplication sites}\]

To test whether the prereplicative sites and the similarly distributed ND10 overlap, we used a MAb against ICP8 and AAb 455 in double-labelling experiments at 3 h after HSV-1 infection of HEp-2 cells. No overlap of antibody staining was seen. Cells in Fig. 3 were stained for ND10, ICP8 and DNA. A cell positive for ND10 is shown in Fig. 3(a). The same cell is negative for ICP8 (Fig. 3b). The DNA staining (Fig. 3c) shows that there are additional nuclei that did not react with either AAb 455 or antibody to ICP8. In the same preparation, we found a nucleus with ICP8 staining (Fig. 3e) but not ND10 staining (Fig. 3d). Again the DNA staining (Fig. 3f) shows additional nuclei that do not react with antibodies to either antigen. No nucleus ever showed both ND10 and prereplicative sites, but many nuclei were negative for both although presumed to be infected by HSV-1 as judged from a parallel infected culture stained for ICP4 in which approximately 85% of the cells were reactive in this experiment. To determine the relationship between ICP8 and ND10 expression, ND10-negative and ICP8-positive cells with prereplicative or replication sites were counted in a double-labelling time course experiment. The percentage of cells without ND10 and those showing prereplication or replication sites are plotted in Fig. 4. From the difference between the two curves, it appears that aggregation of ICP8 into prereplication sites lags behind ND10 disappearance by approximately 2 h in this experiment. ICP8 staining was recognized in many cells as a diffuse staining before the prereplication sites became apparent. The rate of disappearance of ND10 staining and the appearance of prereplication sites was dependent on the amount of HSV-1 used for infection. We have observed 85% ND10-negative cells by 3 h p.i. and 80% cells with prereplicative sites by 5 h p.i. using 2 p.f.u./cell. ND10 staining disappears in the time frame of maximal immediate early protein synthesis, and at the beginning of β protein synthesis.
Fig. 2. Effect of HSV-1 infection on HEP-2 cells. (a) Uninfected cells reacted with MAb 138 show that most cells contain ND10. (b) Same field as (a) except stained with Hoechst 33258 to show nuclear distribution. Arrows point to two early G1 cells without ND10 staining. (c) In HSV-1-infected cells at 5 h p.i., ND10 are not recognized by MAb 138 in most cells. (d) Same field as (c) depicting the nuclear distribution of the cells without ND10. Arrows point to two cells that have retained ND10 and are probably not infected. (e) Field of cells infected as in (c) but reacted with anti-ICP4 MAb demonstrating that most cells are infected. Arrows point to two antigen-negative cells and a mitotic one. Some of the cells show ICP4 only lightly. (f) Nuclear staining of cells in (e). The bar marker represents 10 μm.
Virion proteins are not sufficient for ND10 disappearance

To determine whether the ND10 response after HSV-1 infection was due to the introduction of input virion proteins, ND10 levels in cells infected with u.v.-inactivated HSV-1 were assayed; ND10 remained at the same levels as in uninfected cells. Moreover, ND10 staining did not disappear in HSV-1-infected cells treated with Act-D (98% cells with ND10) or cycloheximide (96% cells with ND10) at the time of infection, whereas in an infected culture without inhibition only 5% of the cells retained ND10. Also, in uninfected cells, these inhibitors have no effect on ND10 distribution within 6 h (97% cells with ND10). The reason why less than 100% of uninfected cells had ND10 is that cells in mitosis or very early G1 phase do not show ND10 staining (see Fig. 2a, b). Both inhibitors allow the cytoplasmic release and activity of virion proteins, but prevent new viral protein synthesis. The results indicate that HSV-1 protein synthesis was essential for the modification of ND10. When viral DNA synthesis was blocked with PAA, ND10 staining was still eliminated as only 7% of cells retained ND10. Thus, viral DNA synthesis with its potential for disruption of nuclear structures is not a requirement for elimination of ND10 staining.

The disappearance of ND10 depends on the synthesis of α proteins

To determine the kinetic class of the HSV-1 gene(s) involved in ND10 reorganization, cells were infected under conditions that permit only HSV-1 IE gene expression. Protein synthesis was blocked with cycloheximide at the time of infection, and α mRNA was allowed to accumulate for 4 h. At this point, ND10 staining was the same as in uninfected cells (Fig. 5, time zero). Elimination of ND10 staining was then assayed over time after a shift from cycloheximide block to Act-D block, which permits the synthesis of α proteins but blocks the synthesis of β mRNAs. ND10 immunofluorescence disappeared rapidly within 30 min after the shift (Fig. 5), suggesting that one or more of the α proteins are responsible. The slope of the curve suggests a very rapid disappearance of ND10 after the α protein synthesis block was released. Considering the time required for cycloheximide reversal, α protein synthesis, nuclear transport and accumulation, and elimination of ND10, the reaction is indeed very rapid.

The α protein responsible for ND10 disappearance is ICP0

Deletion mutants of the α genes were used to determine which of the five HSV-1 α proteins were essential for the disappearance of ND10 staining (Table 2). Viruses with deletion in the genes encoding ICP22 [R325TK+ (Sears et al., 1985)] or ICP47 [R3631 (Mavromara-Nazos et al., 1986)] replicate as efficiently in some cell types as wild-type virus. Infection of HEp-2 cells with the ICP22 and ICP47 deletion mutants resulted in ND10 disappearance, demonstrating that these proteins are not involved in the interaction with ND10. The ICP27 deletion mutant [d27-1 (Rice & Knipe, 1990)] also caused elimination of ND10 immunofluorescence, even though ICP27 is essential for viral replication (Sacks et al., 1985). Similarly, an ICP4 deletion mutant (dl10 (DeLuca et al., 1985)) had the same effect as wild-type HSV-1 on ND10, even though ICP4 is also essential for HSV-1 replication. There was no significant difference in the ability of each of the respective parental HSV strains (17+, KOS, F) to eliminate ND10 staining, although minor experimental variation was found.

Each of the mutant viruses has the capacity to express the other α genes in infected cells. In contrast, in HEp-2 cells infected with the ICP0 mutant dl1403 (Stow & Stow, 1986), no change in ND10 was recognizable at 6 h.p.i. when ND10 staining has disappeared in wild-type HSV-1-infected cells. This result indicates that ICP0 is essential for the disappearance of ND10 antigen from specific nuclear sites. A similar finding was made when cells were infected with up to 10 p.f.u./cell of dl1403, except that in a few cells the compact structure of ND10 became looser and tracks formed. At 14 to 16 h.p.i., long antibody-labelled tracks were observed in approximately 30% of the BHK cell nuclei (Fig. 6a to c). These tracks were localized over areas devoid of cellular DNA in nuclei that had obvious structural changes, as seen by the stained DNA (Fig. 6b, c). The DNA distribution in nuclei with tracks was indistinguishable from that in nuclei without tracks, but considerably different from control cells or cells at early stages of infection (Fig. 3c, f). The tracks induced by the mutant virus do not correspond to any known nuclear structure. Cells without nuclear tracks exhibited many small antigen-positive dots (Fig. 6d), similar to the heat shock-induced redistribution of antigen (G. G. Maul & Z. F. Fu, unpublished), or large apparently hollow antigen-surfaced structures (Fig. 6e), which appeared to be made up of many small subunits contrasting with the mock-infected cells that show the average size of ND10, as in Fig. 1(a). This demonstrates a reorganization and redistribution of ND10 antigen during the later stages of infection with the ICP0 mutant dl1403.

To determine whether ICP0 alone was sufficient to cause the reorganization of ND10, we utilized the ICP0 recombinant adenovirus MLP-O developed by Zhu et al. (1988). MLP-O expresses ICP0 under the control of the
Fig. 4. Comparison of ND10 elimination and ICP8 staining in HSV-1-infected cells. Four-hundred randomly selected cells of an HSV-1-infected HEp-2 cell culture per time point were evaluated after double-labelling with ICP8-reactive MAb and ND10-reactive AAb 455. Each cell was checked for the absence of ND10 (■) and the presence of ICP8 (■); no cell had both. The low percentage of cells without ND10 at 0 h of infection reflects the number of cells in mitosis and very early G1 phase as judged from their chromosome condensation and presence of Flemming body.

Fig. 5. Effect of the HSV-1 infection on ND10 under conditions that allow only IE protein synthesis. Four-hundred cells of an HSV-1-infected HEp-2 cell culture per time point were evaluated for the presence of ND10 using MAb 138 at different times after a shift from cycloheximide block to Act-D block at 4 h p.i.

Table 1. Effect of virus infection on ND10

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cells negative for ND10 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninfected HEp-2 cells</td>
<td>6</td>
</tr>
<tr>
<td>HCMV-infected HEp-2 cells 24 h p.i.</td>
<td>85</td>
</tr>
<tr>
<td>Raji cells uninduced</td>
<td>6</td>
</tr>
<tr>
<td>Raji cells after 16 h TPA treatment</td>
<td>15–37</td>
</tr>
<tr>
<td>Adenovirus 5-infected HEp-2 cells 14 h p.i.</td>
<td>4</td>
</tr>
<tr>
<td>Adenovirus E1A-mutant (dl312) virus-infected</td>
<td>7</td>
</tr>
<tr>
<td>ICP0 recombinant adenovirus (MLP-O)-infected</td>
<td>95</td>
</tr>
</tbody>
</table>

Table 2. Effect of HSV-1 IE mutants on ND10

<table>
<thead>
<tr>
<th>Virus strain (gene deleted)</th>
<th>HEp-2 cells negative for ND10 (%) (6 h p.i.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV-1 wt* strain 17</td>
<td>91</td>
</tr>
<tr>
<td>HSV-1 wt strain KOS</td>
<td>88</td>
</tr>
<tr>
<td>HSV-1 wt strain F</td>
<td>93</td>
</tr>
<tr>
<td>R325 TK* (ICP22)</td>
<td>88</td>
</tr>
<tr>
<td>R3631 (ICP47)</td>
<td>92</td>
</tr>
<tr>
<td>d27-1 (ICP27)</td>
<td>87</td>
</tr>
<tr>
<td>dl20 (ICP4)</td>
<td>89</td>
</tr>
<tr>
<td>dl1403 (ICP0)</td>
<td>5</td>
</tr>
<tr>
<td>Uninfected HEp-2 cells</td>
<td>4</td>
</tr>
</tbody>
</table>

* wt, Wild-type.

though MLP-O replicates only in 293 cells that express E1A, the amounts of ICP0 produced in MLP-O-infected 293 and HeLa cells are comparable to the levels in HSV-1-infected cells (Zhu et al., 1988). HEp-2 cells were infected with the MLP-O virus and assayed at 14 h p.i. with MAb 138. The nuclear appearance is slightly different from that in other experiments because adenovirus infection causes cell rounding. As shown in the double labelling experiment, cells that contained ICP0 (Fig. 7a) did not contain ND10 (Fig. 7b) (although a certain number of cells contained both; see below). In the image selected one cell does not contain ICP0 and retained ND10. Fig. 7(c) shows the combined image. Both fluorescein isothiocyanate (FITC) and Texas Red were simultaneously scanned using the Leica confocal scanning microscope. This finding demonstrates that ICP0 is capable of affecting ND10 in the absence of any other HSV-1 gene products. The wild-type adenovirus 5 strain and the E1A mutant (dl312) used to construct MLP-O had no effect on ND10 staining (Fig. 7d shows the dl312; Table 1), even though adenovirus 5 can replicate in HEp-2 cells whereas MLP-O is replication-defective.

adenovirus major late promoter. This promoter is expressed at low levels under IE conditions of protein synthesis inhibition (Lewis & Matthews, 1980). Even
ICP0 binds transiently to ND10 sites

To determine how ICP0 may affect ND10, we analysed the relative locations of ND10 and ICP0 in the nucleus. At a low multiplicity of 0.1 p.f.u., punctate staining of ICP0 in infected cells was observed over longer time periods than at higher m.o.i. Cells double-labelled at 4 h after HSV-1 infection with ICP0 MAb H1083 and AAb 455 revealed distinct colocalization of ICP0 at some ND10 sites. The cells in Fig. 8(a) and (c) are stained for ND10, and Fig. 8(b) and (d) show MAb H1083 reacting with ICP0. The cell in the lower half of
HSV-1 ICPO modifies a nuclear domain

Fig. 8 Double labelling of ICPO and ND10 in HSV-1-infected cells probed with preabsorbed AAb 455 (a and c) and MAb against ICPO (b and d). Double labelling shows that ND10 correspond to ICPO-positive sites in the lower cell of (a) and (b). The arrows in the upper cells of (b) point to closely spaced (paired) ICPO-positive sites. Three cells are shown in (c) and (d). The lower nucleus has no detectable ICPO and is only recognizable by the outline of its ND10 in (c). The middle cell shows both ND10 and ICPO, and arrows mark the corresponding antigen-positive sites. The upper cell shows mostly ICPO-specific sites (d) with only faint ND10 (c). The bar marker represents 10 μm.

Fig. 8(a) and (b) illustrates that ND10 sites also have ICPO reactivity, although one of the ND10 sites does not (arrow in Fig. 8a); the upper cell has more diffuse ND10 staining and substantially more ICPO-positive sites. Only some of the ND10 sites coincide with ICPO labelling. In addition, ICPO is also localized at several closely paired sites (Fig. 8b, arrows) that have a centre to centre spacing only slightly larger than paired centromeres (0.69 versus 0.57 μm; average of 40 measurements) each using a centromere-binding AAb from a patient with limited systemic sclerosis: Ascoli & Maul, 1991) in G2 or prophase. Fig. 8(c) and (d) show the double labelling of three cells to illustrate the transient nature of colocalization of ND10 antigen and ICPO. These figures show cells in different stages of relative ICPO and ND10 antigen staining. The lower cell shows ND10 staining (Fig. 8c) with no obvious labelling of ICPO (Fig. 8d) and may be uninfected. The middle cell is reactive with both antibodies (arrows) that colocalize although some of the ICPO-derived signal is weak. The upper cell has only ICPO staining and little, if any, reactivity with ND10 (Fig. 8c). The apparent successive changeover from ND10 antigen in uninfected cells, to colocalization of ND10 and ICPO, to the exclusive presence of ICPO in infected cells suggests that ICPO may directly interact with some component(s) of ND10. The images also demonstrate that antibody staining for ND10 and ICPO do not cross-react, and that there is little spillover of Texas Red and fluorescein into each other’s channel. Over time, the number of ICPO sites increases beyond that of ND10, and is comparable with the number of sites induced by heat-shock. Later during infection, ICPO is present in a diffuse nuclear distribution with local areas of higher intensity in cells lacking ND10 staining.

The amount of ND10 protein does not change even though ND10 ‘disappears’ during infection. ND10 protein localization clearly shows a change in distribution upon HSV-1 infection. This change could be due to elimination of the ND10 proteins, masking of the epitopes, or dilution of solubilized antigen. Western blotting of ND10 protein 6 h after infection, when most ND10 were absent (Fig. 9c), shows that one of the ND10 antigens is present in similar amounts in mock-infected cultures (Fig. 9c, lane 1), wild-type HSV-1 177-infected cells (Fig. 9c, lane 2), and HSV-1 dl1403-infected cells (Fig. 9c, lane 3). Antigen sp100 has been chosen for the Western blot, as it is the most completely characterized of the very low abundance proteins of ND10. The question of where the ND10 antigens localize after removal from ND10 is presently being investigated.

Discussion

We have described a new clearly circumscribed nuclear domain, ND10 (Ascoli & Maul, 1991; Freundlich et al., 1988; Szostek et al., 1987). These sites superficially resemble, in number and position, HSV-1 prereplicative sites as defined by the localization of ICP8 (Quinlan et al., 1984). However, during attempts to determine
whether the prereplicative sites and ND10 colocalize, we discovered that HSV-1 infection eliminated ND10 staining about 2 h before ICP8 nuclear aggregation. It proved, therefore, impossible either to establish or to disprove the hypothesis that prereplication sites are induced at ND10 loci. However, the modification of ND10 by HSV-1 represented a very early nuclear host–virus interaction and was, therefore, further investigated.

HSV-1 transcription and protein synthesis were required for the loss of ND10 staining. Input virions were not sufficient to eliminate ND10 in the absence of protein synthesis. The loss of ND10 staining under conditions that permitted only HSV-1 IE gene expression demonstrated that IE gene functions were sufficient and allowed the use of HSV-1 mutants with deletions in the genes encoding IE proteins to show that ICP0 was implicated. It is unlikely that the persistence of ND10 following infection with the HSV-1 ICP0 deletion mutant dll1403 was due to reduced synthesis of the other viral IE proteins for two reasons; the effect was specific to dll1403, whereas the other IE mutants behaved the same as wild-type virus, and increasing the m.o.i. of dll1403 from 1.0 to 5 or 10 p.f.u./cell did not alter the outcome at 6 h.p.i. Late in dll1403 infection (14 to 16 h) ND10 antigens were seen in most cells as numerous small dots, reminiscent of the heat-shock response with this antigen (G. G. Maul & Z. F. Fu, unpublished), and about 30% of the BHK cells exhibited long nuclear tracks. The proportion of nuclear tracks in HEp-2 cells was lower, and also the tracks were shorter. ND10 antigen-positive nuclear tracks in HSV-1 dll1403-infected cells do not resemble any known nuclear structure. Such tracks were present in the virus-occupied nuclear space which is devoid of cellular DNA. It remains to be determined whether these tracks contain HSV-1 components. Direct evidence that ICP0 is sufficient to reorganize the components of ND10 is based upon the loss of ND10 staining following infection of HEp-2 cells with an ICP0 recombinant adenovirus, MLP-O (Zhu et al., 1988).

A temporal colocalization of ND10 antigen and ICP0 was observed early in the infectious cycle at low m.o.i. This may be due to the necessity to reach a threshold amount of ICP0 in order to redistribute ND10 antigen. Larger quantities of ICP0 eliminated ND10 recognition very quickly, as shown by the translation of accumulated IE mRNA after release from a protein synthesis block. All of the immunofluorescence results and their interpretation rest on the recognition of ND10 antigen by specific antibodies. ND10 disappearance could be due to the dispersion of antigen, a change in epitopes caused by a change in protein folding, secondary modification such as phosphorylation, or physical interference. However, the possibility that epitopes located on three different proteins (p55, sp100 and 126K) known to reside at ND10 (Ascoli & Maul, 1991; Stuurman, 1991; Szostecki et al., 1990) are covered or blocked by ICP0 is considered unlikely, particularly in such a large structure (0.3 μm). Redistribution or dispersion of the antigens may be more likely and is suggested by the diffuse staining of the nucleus with ND10 antibodies before their complete disappearance, and the finding by Western blotting that the amount of the antigen tested (sp100) is similar in HSV-1-infected and in uninfected cells.

The ICP0 gene is one of five HSV-1 IE genes, and all except ICP47 trans-activate HSV-1 genes in transfection assays. Yet only ICP0 caused an effect on the organization and distribution of ND10. Several features dis-
tistinguish ICP0 from the other HSV-1 IE gene products and it is tempting to speculate that the interaction with ND10 might be relevant to these differences. In contrast to the other IE gene products, ICP0 is a promiscuous trans-activator that can stimulate a wide variety of cellular and viral promoters in transient transfection assays (Everett et al., 1991). A zinc finger region is required for trans-activation (Chen et al., 1991; Everett, 1987, 1988, 1989; Everett et al., 1991), but it is not known whether ICP0 acts at a transcriptional or post-transcriptional level, or both. During tissue culture infection ICP0 is dispensable at high m.o.i., but plays an important role at low m.o.i. A cellular functional homologue of ICP0 that can stimulate HSV-1 ICP0 mutant replication has been identified and exhibits a cell cycle dependence (Cai & Schaffer, 1991). Although studies of ICP0 mutants are complicated by an overlap with the gene encoding the latency-associated transcripts (Fraser et al., 1992; Spivack & Fraser, 1987; Spivack et al., 1991; Stevens et al., 1987), it is evidence that ICP0 plays a role in the reactivation of latent infection in an in vitro system (Harris et al., 1989), and that ICP0 alone is sufficient (Zhu et al., 1990). The search for the mechanism of ICP0 trans-activation (or its equivalent in other herpesviruses), and the host function localized to these discrete nuclear sites, may profitably take place in this host–virus interaction defined by ICP0 and proteins that make up ND10.


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