Determination of Minimum Herpes Simplex Virus Type 1 Components Necessary To Localize Transcriptionally Active DNA to ND10

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DNA viruses such as herpes simplex virus type 1 (HSV-1) appear to start their replicative processes at specific nuclear domains known as ND10. In analyses to determine the minimum viral components needed for transcript accumulation at ND10, we find that a specific viral DNA sequence, OriS, and the viral immediate-early proteins ICP4 and ICP27 are sufficient for a reporter gene placed in cis to the OriS sequence to transcribe at ND10. A chromatin immunoprecipitation assay demonstrated expected critical intermediates in retaining the minimal genome at ND10 for the HSV-1 replication origin through direct or indirect binding to the host protein Daxx. Coimmunoprecipitation assays with antibodies to Daxx and ICP4, ICP27, and ICP8 showed that the respective proteins interact, possibly forming a complex. A potential complex between the origin, early viral DNA-binding protein ICP8 and Daxx did not result in transcription at ND10. Thus, the deposition of transcriptionally active HSV-1 genomes at ND10 is most likely a consequence of retention at ND10 through the interaction of viral genome-bound ICP4 and ICP27 with Daxx. Such a complex might be more likely immobilized at the outside of ND10 by the PML-interacting Daxx than at other nuclear sites.

Most DNA viruses enter the nucleus by facilitated transport through the nuclear pore complex (49). Once inside the nucleus, some viral genomes make their way to nuclear domains called ND10, PML bodies, or PODs (18, 33). It is not clear whether viral genomes diffuse through the nuclear spaces or are deposited by an active mechanism at ND10. Apparently, only the few viral genomes arriving at this nuclear domain begin transcription and later, presumably at the same site, replication (20), suggesting a specifically advantageous environment for the virus at ND10. On the other hand, the dominant proteins of ND10 are interferon upregulated and have repressive properties (26, 51). Moreover, most DNA viruses encode an immediate-early (IE) protein that induces the degradation of ND10-associated proteins (12, 18, 22, 32) and, in the absence of these viral proteins, replication is severely retarded (35, 46). These findings have led to the hypothesis that ND10 represent sites of a nuclear defense mechanism (34).

ND10 are nuclear accumulations of various proteins, and PML is essential for their recruitment (19, 23, 52). These nuclear domains appear to function as nuclear depots, since several proteins, when increased in abundance by induced transcription or reduced turnover, accumulate at these sites (38). The capacity of the depots for protein recruitment is increased by the interferon upregulation of PML, Sp100, and Daxx (7, 8, 14–16, 19, 24, 45), and the protein recruitment is regulated by the sumofication of PML (19). The release of proteins from ND10 is regulated by the SENP-1 desumofication of PML and by p38 MAPK/ERK1/2 phosphorylation pathways, depending on the presence of an external signal such as hyperthermia or heavy metal exposure (37). The recruitment and segregation of undesirable components such as viruses to ND10 suggests that ND10 may also function as a disposal site. Evidence for such a function comes from the deposition of overexpressed proteins such as BRCA-1 or hGCN5 (34), the accumulation of ubiquitinated proteins (11) and proteosomes in ND10 (13), and the segregation of antigenomic hepatitis delta virus RNA at these sites (2). Identification of the viral components relevant for such deposition may help to determine how the cell protects itself against viral infection.

We have shown that viruses start their transcription and replication at or beside ND10 (3, 18, 20, 33). Since the large ND10 do not move substantially (36, 40), we reasoned that viral genomes are retained at ND10 through a mechanism that involves the viral DNA (31). A search for this mechanism in the small DNA genome virus simian virus 40 (SV40) showed that the minimum sequence leading to ND10 deposition contained the core origin of replication, although the origin was necessary but not sufficient. Large T antigen (T-ag) was also required, suggesting that viral transcription of T-ag precedes SV40-ND10 association (47). In the present study, we examined the minimum components of herpes simplex virus type 1 (HSV-1) that lead to an association of transcriptionally active DNA with ND10. We made use of an ampiclon containing only the HSV-1 origin of replication, the “a” sequence required for cleavage and packaging, and a transgene useful as a reporter for the localization of new transcripts. The ampiclon can be replicated and packaged in the presence of helper virus. Essentially helper-free ampiclon stocks can be produced and used to infect cells, as do intact viruses. This allows delivery of individual genomes in capsules surrounded by an apparently normal set of tegument proteins (28).

A low but significant population of ampiclon have been shown by direct observation by using time-lapse photography to attach at the periphery of ND10. Only ampiclon that attached replicated, and the proportion that did bind to ND10 was increased by the presence of additional viral promoters.
We show here that a specific viral sequence containing an ICP4 consensus-binding site can, in conjunction with ICP4 and ICP27, result in the emergence of reporter transcripts at ND10. Complex formation of ICP4 and ICP27 with the PML-RINGdominant domain is critical for viral packaging, and ori sequences, derived from pASK/E after deletion of ICP4-binding site and ori sequences, cells were incubated overnight at 65°C for 5 min, the beads were removed by centrifugation, and the supernatants were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis and immunoblotting.

**MATERIALS AND METHODS**

**Cell lines and tissue culture.** The Vero cell line-derived E5 cells, which expresses ICP4 of HSV-1, was kindly provided by P. A. Schaffer (10) and was maintained in Dulbecco modified Eagle medium supplemented with 10% fetal calf serum, 1% penicillin-streptomycin, and 400 μg of Geneticin/mL. Infections and transfections were performed in Geneticin-free medium. Mouse embryonic fibroblasts (MEF) were immortalized by transformation with T-ag (19). Daxx-negative MEF (Daxx-/-MEF 4227; 21), HEp-2 cells, human foreskin fibroblasts (FF 242F), and Vero cells were maintained in Dulbecco modified Eagle medium supplemented with 10% fetal calf serum and 1% penicillin-streptomycin. For immunohistochemical staining and for in situ hybridization, cells were grown on round coverslips (Corning Glass, Inc., Corning, N.Y.) in 24-well plates.

**Antibodies.** ND10-associated proteins were visualized using the following antibodies: a polyvalent rabbit serum produced against the N-terminal half of PML (unpublished); MAbs 14, 14 produced against the Daxx (43) and rabbit antibodies against murine Daxx (Santa Cruz Biotechnology, Inc., Santa Cruz, Calif.) Monoclonal antibodies against HSV-1 ICP4, ICP27 (H1119) were kindly provided by R. D. Everett (MRC, Glasgow, United Kingdom).

**Coomassie precipitation.** Antibodies were coupled to paramagnetic beads (Dynabeads M-450 coated with anti-rabbit or anti-mouse immunoglobulin G; Dynal, Oslo, Norway) according to the manufacturer’s instructions. After a washing step with phosphate-buffered saline (PBS) containing 0.1% bovine serum albumin (BSA), the beads were incubated overnight at 4°C with nuclear extracts prepared as described previously (1). Beads were washed again in PBS: 0.1% BSA and then resuspended in a mixture of 20 μl of PBS and 20 μl of 2× Laemmi buffer. After being heated at 95°C for 5 min, the beads were removed by centrifugation, and the supernatants were analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and immunoblotting.

**HSV-1 strains and amplicons.** Wild-type HSV-1 17, ICPO ring mutant HSV-1 strain FXE, and ICPO-deleted HSV-1 strain D0b were obtained from R. D. Everett. HSV-1 deletion mutant d07, lacking ICP0, ICP4, and ICP27, and HSV-1 d109, deleted of all IE genes (ICP0, ICP4, ICP27, ICP22, and ICP47), have been described (42) and were obtained from N. DeLuca (Pittsburgh, Pa.). Amplicons were generated from plasmids containing the HSV-1 origin of replication (oriS) and packaging sequences (Amplicons were generated from plasmids containing the HSV-1 oriS sequence by partial digestion with NotI, which lacks both the lacZ sequence, derived from pASK/E after deletion of NotI, which are critical for viral packaging, and ori sequences, cells were incubated overnight at 37°C with 55% formamide in 2× SSC (twice for 15 min each), 2× SSC (10 min), and 0.25× SSC (twice for 5 min each). Hybridized probes were labeled with FITC-avidin (Vector Laboratories; 1:50 in 4× SSC plus 0.5% BSA), and signals were amplified by using biotinylated anti-avidin (Vector Laboratories; 1:250), followed by another round of FITC-avidin staining. Finally, cells were equilibrated in PBS, stained for DNA with Hoechst 33258 (0.5 μg/ml), and mounted in Fluoromount G (Fisher Scientific, Newark, Del.).

**Probe preparation.** The plasmid prSVZ to detect lacZ RNA (47) was labeled with biotin-11-dUTP by nick translation. The DNAse concentration for nick translation was adjusted to yield probe DNA 200 to 500 bp in length (25). Probe DNA was dissolved at 10 ng/ml and hybridized to the probe DNA at 94°C for 5 min. After hybridization, samples were washed at 37°C with 55% formamide in 2× SSC (twice for 15 min each), 2× SSC (10 min), and 0.25× SSC (twice for 5 min each). Hybridized probes were labeled with FITC-avidin (Vector Laboratories; 1:50 in 4× SSC plus 0.5% BSA), and signals were amplified by using biotinylated anti-avidin (Vector Laboratories; 1:250), followed by another round of FITC-avidin staining. Finally, cells were equilibrated in PBS, stained for DNA with Hoechst 33258 (0.5 μg/ml), and mounted in Fluoromount G (Fisher Scientific, Newark, Del.).

**Microscopy.** Confocal images of cells were obtained by using a Leica TCS SP2 confocal laser scanning system. Two channels were recorded sequentially or separately, and the third channel was added subsequently. Data acquisition was controlled for possible breakthrough between the FITC and Texas red signals and between Texas red and Cy5.

**RESULTS**

The HSV-1 origin of replication and viral proteins are required for ND10 association. Most DNA viruses start their replication and apparently their transcription at ND10. Preliminary experiments had shown that the HSV-1 amplicon replicates at ND10 in the presence of helper virus and therefore contains HSV-1 DNA that can arrive at ND10. Amplicons have a highly reduced HSV-1 DNA sequence of 1.6 kb versus 153 kb for the full HSV-1 genome and contain 15 U of this sequence, essentially the replication origin (oriS), the packaging sequence “a,” and an IE4 promoter driving the β-galactosidase (β-Gal) transgene, and are packaged like infectious HSV-1 (27). Importantly, amplicons are enveloped like HSV-1 and enter cells and nuclei by infection. The relative positions of the transcripts and thus presumably the transcribing genomes were localized by fluorescence in situ hybridization combined with immunohistochemistry. At 3 h postinfection (p.i.) neither amplicon DNA (Fig. 1A) nor β-Gal transcripts were found at ND10 (Fig. 1B). Transcripts were dispersed throughout the nucleus and most often at a substantially lower concentration.
than that seen in Fig. 1B, which was selected to most clearly show the dominant distribution. About 20% of infected cells showed aggregates of transcripts, but these were not associated with ND10 (data not shown, but see below and Fig. 5). In contrast, coinfection of amplicons with the HSV-1 FXE mutant (RING finger mutant of ICP0) or the ICP0 deletion mutant 1403, led to β-Gal transcript accumulations at ND10 (Fig. 1C). Although it does not prove that transcriptionally active viral DNA is only located at ND10, we assume from the location of the β-Gal transcripts that most transcribing amplicon genomes were located at ND10 and that the IE protein ICP0 is not required for the amplicon transcription at ND10. This experiment indicates that an HSV-1 DNA sequence necessary for ND10 deposition is present in the short amplicon sequence but is not sufficient for transcripts to appear at ND10. A protein of the helper virus other than ICP0 must be synthesized for transcripts to appear at ND10. The location of amplicons at sites other than ND10 and the diffuse distribution of β-Gal transcripts in the absence of helper virus demonstrates that amplicons can transcribe at other sites.

Transfection-based assays mimic directly observable properties of infecting viral genomes inside the nucleus, as demonstrated for SV40 (47). To determine the minimal DNA sequence of the amplicon necessary for transcript association with ND10, we transfected cells with various amplicon sequences deleted in the packaging sequence, the origin, or both. We introduced the HCMV IE promoter to driving the β-Gal transgene, since the IE4 promoter is damaged when the origin of replication is deleted. Like amplicon infection, transfection of the amplicon sequence did not result in transcript accumulation at ND10 (not shown). Infection of human fibroblasts with the ICP0 deletion mutant virus at 3 h after transfection with the various amplicon plasmid constructs was used to supplement the necessary viral proteins. Cells were fixed 6 h after infection with the helper virus. The control plasmid containing the “a” sequence and the origin of replication showed ND10-associated β-Gal transcripts (Fig. 2B). Plasmids without the origin (Fig. 2D and F) showed a transcript distribution predominantly like that in Fig. 1B for amplicons without the helper virus. A smaller proportion showed the transcripts highly aggregated but not associated with ND10 (Fig. 2E). The reason for the transcript aggregation in a subpopulation of cells is not clear at present but could be due to a higher rate than in other cells. The results indicate that a DNA segment of the virus, here identified as the origin of replication, is essential but not sufficient for transcript accumulation at ND10.

To define the helper HSV-1 protein necessary to rescue the amplicon’s ability to transcribe at ND10, transcript localization was analyzed in cells infected with specific HSV-1 IE deletion mutants and transfected with plasmids expressing individual IE proteins. The appearance of β-Gal transcripts from the amplicon relative to ND10 was categorized as “ND10-association” (see Fig. 1C) or “diffuse” (see Fig. 1B). Of the five IE proteins, ICP0 was already shown not to be necessary for transcription at ND10 (Fig. 1C). Table 1 summarizes the results of the complementation experiment. In both HEp-2 and Vero cells, no transcriptional activity from amplicons was found at ND10 when the helper virus used was deleted of all IE proteins. The ICP4- and ICP27-deleted helper virus was also ineffective, i.e., the remaining ICP22 and 47 could not reconstitute transcription at ND10. Because the amplicon carries the major activator VP16 in its tegument, we were able to use Vero E5 cells, which contain a Vp16-inducible ICP4 insert; induction of ICP4 was also not sufficient to complement the ND10 association phenotype. Finally, transfection of HEp-2 cells with ICP27, followed by infection with the amplicon, also did not result in transcript accumulation at ND10. On the other hand, β-Gal transcripts were found at ND10 in Vero cells which can be upregulated to produce ICP4 by Vp16 from the tegument and induced to express ICP27 by transient transfection (see Table 1). Together, the results point to the origin of replication and ICP4 and ICP27 as the minimum requirement for transcripts to appear at ND10. Surprisingly, HSV-1 amplicons require two IE viral proteins in order to transcribe at ND10.

Identification of potential protein intermediates between viral DNA and ND10. The question arose as to whether the viral transcription at ND10 could be due to binding of ND10-associated proteins to the incoming viral origin of replication, followed by collision with ND10 and stabilization by interaction with ND10 proteins bound to the viral complex. The critical intermediates of such a scenario would be an origin of replication/viral protein complex bound to a cellular protein that binds at ND10. Since Daxx reportedly binds directly or indirectly to specific DNA sequences (21a), and since Daxx is necessary in establishing the HCMV immediate transcript environment at ND10 (21), we tested whether Daxx might facilitate the association of the origin of replications with ND10. Single viral DNA/protein complexes preclude the direct visual
recognition, since the number of host molecules binding is likely insufficient for immunofluorescence detection or recognition over the nuclear distribution of the protein. We therefore used ChIP analysis to test whether binding of Daxx to the origin of replication can be demonstrated. We developed a Daxx knockout mouse (embryonal lethal; A. M. Ishov and G. G. Maul, unpublished data) and derived cells from the Daxx knockout mouse embryos that serve as a control (20). MEF and Daxx+/H11001/H11001 MEF were infected with amplicons containing helper virus and fixed with paraformaldehyde at 3 or 16 h p.i., and the sonicated supernatant was immunoprecipitated with Daxx antibodies. The different fractions were PCR amplified with primers spanning the origin. Origins from infected Daxx+/H11001/H11001 cells were immunoprecipitated before replication at 3 h p.i. and, to a somewhat greater extent, after 16 h of incubation when replication has taken place (Fig. 3). When identically infected Daxx+/− cells were used to serve as an additional control for the possibility that Daxx antibodies precipitate a nonspecific protein in the origin/protein complex, no origins were precipitated at either 3 or 16 h p.i., indicating that Daxx can bind to the origin or an origin/protein complex (Fig. 3). However, the requirement for the viral proteins ICP4 and ICP27 suggests that Daxx binds indirectly to an origin/protein complex.

We asked with which of the viral proteins essential for the transcript localization at ND10 Daxx may bind. Coimmunoprecipitation assays and Western blots with anti-Daxx antibodies and infected and mock-infected cells revealed that Daxx antibodies immunoprecipitated ICP4, ICP27, and ICP8 in Daxx+/H11001/H11001 cells (Fig. 4A, lane 9), but none of these proteins precipitated from infected Daxx+/−/H11002 cells (Fig. 4A, lane 10). Daxx+/− cells can be productively infected as shown by using anti-ICP8 antibodies. ICP8 is precipitated and coprecipitates ICP4 and ICP27, suggesting that these proteins can exist as a complex. The reason for the inability of ICP8 to precipitate ICP4 in Daxx+/− cells is not clear. We could confirm the

![FIG. 2. Individual amplicon sequences were deleted as shown. Amplicon DNA was transfected, and after 3 h the cells were infected with HSV-1 1403. (A) The location of β-Gal transcripts relative to ND10 was determined by in situ hybridization. A “+” indicates transcript association with ND10. (B) pASK/E-derived transcripts are associated with ND10 (Daxx). (C) Transcripts from pASK/E, deleted in the packaging signal “a” were found adjacent to ND10 (Daxx). (D and E) Transcripts from pASK/E deleted in the origin were located throughout the nucleoplasm (Daxx), but in fewer cells in aggregates not associated with ND10 (Daxx) (E). (F) Transcripts from pASK/E, deleted in both origin and the packaging signal, did not associate with ND10 (Daxx).](http://jvi.asm.org/)}
interactions of the viral proteins by using anti-ICP4 and anti-ICP27, i.e., anti-ICP4 coprecipitates ICP27 and ICP8, whereas anti-ICP27 coprecipitates ICP8 and ICP4 (Fig. 4B). The protein interactions, as demonstrated by immunoprecipitation, are outlined in Fig. 4C, where the arrowheads point to the protein that binds the one from which the arrow originates.

ICP8 is not necessary for transcript accumulation at ND10. If Daxx interacts with ICP8 and if ICP8 interacts with the viral DNA, the minimal viral components leading to viral transcription at ND10 would be the origin and ICP8, with ICP4 and ICP27 necessary only to transactivate ICP8 from the helper virus. To test whether ICP8 alone leads to the transcription of amplicons at ND10, we transfected cells to express ICP8, followed by infection with the amplicons and assay for the location of β-Gal transcript location relative to ND10. We used triple labeling for ICP8 to locate the transfected cells (red), for Daxx to identify ND10 (blue), and for β-Gal transcripts (green) (Fig. 5). ICP8 is found in different distributions. As shown in Fig. 5A and color separated for Daxx and the transcript (Fig. 5B and C), transcripts were not located at ND10 when present at higher local aggregations. However, ICP8 can be seen aggregated in several instances adjacent to ND10. Also, small dots of ICP8 were found in association with β-Gal transcript aggregates. The absence of Golgi apparatus-associated labeling of Fc receptor-binding viral proteins indicates that this cell had only amplicons and that no helper virus was present. This excludes the presence of helper virus-derived ICP8, ICP4, and ICP27. The same non-ND10-associated transcript location was observed in cells that showed a diffuse ICP8 distribution (Fig. 5D). In such cells, the size and number of ND10 were substantially reduced. As in cells infected with amplicons alone (Fig. 1B), β-Gal transcripts were dispersed in most cells (not shown). Together, the results suggest that a viral DNA/ICP8/Daxx complex is not sufficient to result in the appearance of β-Gal transcripts at ND10. Also, it demonstrates that ICP4 and ICP27 are not essential to produce transcript accumulations but that they are required to produce transcript accumulations at ND10.

DISCUSSION

A certain percentage of infecting DNA viruses from several families are deposited at specific nuclear domains and start their replication at these nuclear sites (31). HSV-1 amplicons appear to follow a random path through the nucleus and become attached adjacent to ND10 as shown by time-lapse cinematography; a statistically significant number of amplicons also started their replication at this site (4, 44), indicating that some currently unknown consequence of this association leads to replication. ND10 are also the site of IE transcript accumulation. For HCMV, these IE transcript environments coincide with the replication compartments formed (20). To address the question of how viral genomes that can transcribe are deposited at ND10, we searched for the minimal components of HSV-1 that are necessary for such deposition. The initial finding that HSV amplicons containing only the origin of replication, packaging sequences, and a viral promoter transcribed and replicated at ND10 when complemented with a helper virus appeared to narrow the search for a DNA sequence necessary for ND10 deposition. This approach seemed justified based on our previous finding that only 64 bp of the SV40 core
amplicon containing oriS required protein synthesis of the mid transcript accumulation at ND10. Like SV40, the HSV-1 origin in the amplicon, but the presence of Daxx and the N-terminal domain, which interacts with the viral protein (17, 21), making Daxx a reasonable candidate as an adapter protein of the virus DNA to an ND10-associated protein. The viral strand DNA-binding protein ICP8 appears to be a likely candidate for amplicon origin complex formation. ICP8 has also been shown to form prereplication domains (6, 9, 30, 33, 41, 50) and to associate with PML (5). Thus, when bound to the viral genome, ICP8 might allow immobilization on ND10 by its binding to PML or Daxx. However, when cells were transfected with ICP8, followed by infection with amplicons, most transcript accumulations were not located adjacent to ND10. This result eliminates a simple Daxx/ICP8/oriS complex as the basis for virus immobilization on the PML-based ND10.

The reason for the requirement of ICP4 and ICP27 for the transcript accumulation at ND10 after infection with HSV-1 amplicons is not obvious. The requirement for these two proteins is most readily rationalized by their ability to form a complex (39). Our finding that these proteins also bind Daxx suggests a complex between viral DNA, ICP4, ICP27 and Daxx. oriS contains a consensus-binding site for ICP4 (TCGTC), which might bind complexes such as those anticipated from the communoprecipitation analysis of ICP4, ICP27, and Daxx. With the full viral genome containing many such ICP4 consensus sequences, the number of such complexes may be numerous, increasing the chance that such complexes attach to the surface of ND10 via the known Daxx-PML interaction (19). This argument and the finding that the presence of the gD promoter with its ICP4 binding sites in the amplicon increases the chance of its attachment to ND10 suggest that the origin may not be the only stretch of viral DNA that can be immobilized at ND10 and has been deposited or retained there and that helper virus functions were necessary. The presence of a promoter in addition to the origin was also found to enhance the likelihood that an amplicons containing an operon/repressor complex were immobilized at ND10 (44). Since the IE4 promoter is partly overlapping with oriS, we may claim only that a certain viral DNA sequence has been identified as necessary to enhance the probability that transcripts accumulate at ND10. The time lapse-based demonstration that the amplicons move through the nucleus until they become immobilized at the much larger ND10 and start to replicate there (44) is also strong evidence against the notion that ND10 move to the viral genomes or that they induce ND10 formation.

Immobilization of the viral DNA at ND10 requires that at least one ND10-associated protein binds to oriS. ChIP analysis with Daxx antibodies served to detect direct or indirect binding of the virus DNA to an ND10-associated protein. The viral origin of replication necessary for ND10 deposition was precipitated from Daxx+/+, but not Daxx−/− cells, indicating that the precipitation was Daxx specific. Thus, Daxx binding to the origin seems to be one of the critical components necessary for ND10 deposition. Daxx is also required for HCMV transcripts to be accumulated at ND10. Daxx interacts with pp71 and p71 deletion mutants accumulate IE transcripts at sites other than ND10 (21). Daxx also has two protein interaction sites, the C-terminal domain, which interacts with PML (19), and a more N-terminal domain, which interacts with the viral protein (17, 21), making Daxx a reasonable candidate as an adapter protein between the viral genome and the surface of ND10.

Control cells infected with amplicons contained Daxx and the origin in the amplicon, but the presence of Daxx and the origin of replication alone did not result in amplicon or plasmid transcript accumulation at ND10. Like SV40, the HSV-1 amplicon containing oriS required protein synthesis of the helper virus to transcribe at ND10. Our complementation analysis revealed the requirement for the IE proteins ICP4 and ICP27 of the helper virus for amplicon transcription at ND10. Both proteins are required in transactivating early protein gene transcription of the helper virus, and the early single-strand DNA-binding protein ICP8 appears to be a likely candidate for amplicon origin complex formation. ICP8 has also been shown to form prereplication domains (6, 9, 30, 33, 41, 50) and to associate with PML (5). Thus, when bound to the viral genome, ICP8 might allow immobilization on ND10 by its binding to PML or Daxx. However, when cells were transfected with ICP8, followed by infection with amplicons, most transcript accumulations were not located adjacent to ND10. This result eliminates a simple Daxx/ICP8/oriS complex as the basis for virus immobilization on the PML-based ND10.

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Our search for the minimal viral components necessary for viral transcript accumulation at ND10 yielded a specific viral DNA sequence and the IE proteins ICP4 and ICP27. We hypothesize that a cellular protein such as Daxx interacting...
with the viral proteins acts as the adapter to PML, the matrix protein of ND10, and that the viral genome complexed with such interacting proteins is retained at the highest PML concentrations (ND10) to a significantly higher degree than at other sites. Further refinement of the mechanism by which viral genes are deposited at ND10 may help in determining why competent viral genomes can be suppressed within the cell, as shown recently for mouse cytomeglovirus (48) and why genomes initially based at ND10 have a higher probability of replication.

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