Evidence that the Immediate-Early Gene Product ICP4 Is Necessary for the Genome of the Herpes Simplex Virus Type 1 ICP4 Deletion Mutant Strain d120 To Circularize in Infected Cells

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Following infection, the physical state of linear herpes simplex virus (HSV) genomes may change into an “endless” or circular form. In this study, using Southern blot analysis of the HSV genome, we provide evidence that immediate-early protein ICP4 is involved in the process of converting the linear HSV-1 ICP4-deleted mutant strain d120 genome into its endless form. Under conditions where de novo viral DNA synthesis was inhibited, the genome of the ICP4 deletion mutant d120 failed to assume an endless conformation following infection of Vero cells (compared with the ability of wild-type strain KOS). This defect was reversed in the Vero-derived cell line E5, which produces the ICP4 protein, suggesting that ICP4 is necessary and sufficient to complement the d120 defect. When ICP4 protein was provided by the replication-defective DNA polymerase mutant HP66, the genomes of mutant d120 could assume an endless conformation in Vero cells. Western blot analysis using antibody specific to the ICP4 protein showed that although the d120 virions contained ICP4 protein, the majority of that ICP4 protein was in a 40-kDa truncated form, with only a small fraction present as a full-length 175-kDa protein. When expression of ICP4 protein from E5 cells was inhibited by cycloheximide, the d120 virion-associated ICP4 protein was unable to mediate endless formation after infection of E5 cells. Collectively, these data suggest that ICP4 protein has an important role in mediating the endless formation of the HSV-1 genome upon infection and that this function can be provided in trans.

During the life cycle of herpes simplex virus (HSV), the viral genome exists in several different physical states, which may influence the outcome of infection. Following entry into a cell, the virion linear double-stranded herpes simplex virus type 1 (HSV-1) genome undergoes a change in physical state in which it assumes a nonlinear, presumably circular form that has been referred to as “endless” (12, 20, 31). Consistent with the nonlinear, circular form of the input genomes, Strang and Stow (26) demonstrated that the circularization of the HSV genome occurs early in lytic infection. This structural change occurs in nonneuronal and neuronal-like cells (29). The circular form predominates in latently infected cells in humans and experimental animals (9, 18, 22, 26). However, the kinetics of the state change is not known.

The cellular and viral factors involved in this transformation are not well understood, but the transformation from linear to circular form has generally been assumed to be essential for replication (12, 20) until the recent challenge by Jackson and Deluca (14). These investigators assert that the circular form of the viral genome is not the template for DNA replication but is the structure that is preferred for the establishment of latency.

HSV-1 ICP4 is a major regulatory protein that is synthesized immediately after the infection of permissive cells in tissue culture and is essential for the virus life cycle (6, 15, 24, 33). Although not all modifications of the ICP4 protein have been fully characterized, it is modified by phosphorylation (3, 23) and nucleotidylation (4, 19). Both positive and negative regulation of viral functions are achieved by binding of the ICP4 protein to different consensus and nonconsensus sites throughout the genome. These interactions are regulated by the degree of phosphorylation of different sites on the protein (1, 34). In addition, the ICP4 protein has been suggested to be involved in the initial stage of formation of viral DNA replication complexes (2, 30), which appear to occur adjacent to ND10 sites at prereplicative domains (10, 11, 17, 27, 32). This suggests an important role of the ICP4 protein in initiating viral DNA replication, possibly by mediating the maturation of prereplicative domains.

In the course of studying the stability of the herpes simplex virus type 1 viral genome in neuronal-like cell cultures, we observed a surprising property associated with ICP4-defective virus: the genome of ICP4 deletion mutant d120 remained linear up to 30 days postinfection (Y.-H. Su, A. Ng, and T. M. Block, unpublished observation). To explore the possibility that the ICP4 protein is necessary for viral genome circularization, we studied the genomic structure of an ICP4-deleted mutant, d120, in Vero cells and its Vero-derived complementary E5 cells (from which mutant d120 virus stock was prepared).

Here we report that the ICP4-deleted mutant d120 genome failed to assume an endless state upon infection in Vero cells. The inability of the d120 mutant genome to form the endless state can be rescued by infecting (i) Vero-derived ICP4 ex-
pressing E5 cells or (ii) Vero cells preinfected with an HSV-1 DNA polymerase mutant, HP66. Furthermore, the d120 virus-on-associated ICP4 protein was found to be mainly in a truncated 40-kDa form and thus failed to mediate the endless structure formation of the d120 genome in the infected E5 cells when de novo protein synthesis was inhibited. Our results strongly suggest that the failure of the d120 genome to form endless genomes upon infection of Vero cells is due to its lack of functional ICP4 protein.

MATERIALS AND METHODS

Virus and cells. HSV-1 wild-type strain KOS was prepared in Vero cells, and HSV-1 mutant HP66, containing a 2.3-kb deletion in the locus of DNA polymerase (16), kindly provided by Don Coen (Harvard Medical School, Boston, MA), was prepared in the complementing cell line polB3 (13) (kindly provided by Charles Hwang, SUNY Health Science Center, Syracuse, NY). HSV-1 mutant d120, containing a 4.1-kb deletion in both copies of the ICP4 gene (8), and the complementary cell line E5 (7) were kindly provided by Priscilla Schaffer (Harvard Medical School, Boston, MA). The stock of mutant d120 virus was prepared in E5 cells. Every stock of d120 cells was examined for its infectivity on Vero cells. This was done by infecting 10^6 PFU of viruses from the stock in Vero 120 virus was examined for its infectivity on Vero cells. This was done by infecting 10^6 PFU of viruses from the stock in Vero monolayer, harvesting the culture medium, and then determining the existence of any infectious progeny by plaque assay on Vero cells.

Nuclear DNA isolation. To isolate nuclear DNA, cells were scraped into medium and collected by centrifugation at 1,000 rpm for 10 min at 4°C. The cell pellet was resuspended into lysis buffer (1 mM CaCl2, 60 mM KCl, 15 mM NaCl, 3 mM MgCl2, 10 mM Tris, pH 7.5, 5% sucrose) containing 0.5% NP-40, homogenized with a Dounce homogenizer, and washed in nuclei lysis buffer containing 0.1% sodium deoxycholate. The nuclei were then collected by centrifugation at 2,000 rpm for 10 min at 4°C. The pelleted nuclei were subjected to DNA isolation by sodium dodecyl sulfate (SDS)/proteinase K digestion, phenol-chloroform extraction, and ethanol precipitation.

Viron DNA preparation. The method to isolate HSV virion DNA was described previously (29). Briefly, HSV-1-injected CV-1 cells were scraped into medium and collected by centrifugation at 1,500 rpm for 10 min at 4°C. The cells were lysed by sonication at 40% power (Heat System Ultrasonicator, Farmington, NY) for 1 min. Cell debris was separated from virions by centrifugation at 2,000 rpm for 15 min at 4°C. HSV-1 virions were pelleted by centrifugation at 28,000 rpm using an SW41 rotor for 1 h at 10°C. Isolated virions were then lysed in lysis buffer (0.25% Triton X-100, 10 mM EDTA, 10 mM Tris, pH 8.0), and viral capsids were isolated by centrifugation through a 20% sucrose cushion at 28,000 rpm using an SW55 rotor for 50 min at 4°C. DNA was isolated by SDS/proteinase K digestion, phenol-chloroform extraction, and ethanol precipitation.

DNA structure analysis by Southern blot hybridization. To determine the structure of HSV-1 DNA following infection, Vero or E5 cells were infected with wild-type KOS or the ICP4-deleted mutant d120 at a multiplicity of infection (MOI) of 5 in the presence or absence of the DNA replication inhibitor phosphonoacetic acid (PAA; 400 μg/ml) or protein synthesis inhibitor cycloheximide (CHX; 50 μg/ml). Three hours postinfection (hpi), nuclear DNA was isolated as described above. To control for the effectiveness of PAA on viral DNA replication inhibition, KOS DNA in infected Vero cells after 8 hpi was determined in the presence of PAA and showed no detectable increase in the amount of HSV-1 KOS DNA after 8 hpi compared to the input DNA.

Infected or uninfected nuclear DNA was digested with the restriction endonuclease BamHI. The digested DNA was resolved on a 1% agarose gel and transferred to a nylon membrane by capillary transfer. The probes, BamHI B and P5 fragments, were isolated from cloned plasmids PRB112 (21) and pBamSP (5), respectively. The probes were prepared by labeling with [α-32P]dCTP using the random primer labeling kit (Rapidprime DNA labeling system; Invitrogen, Carlsbad, CA) per the manufacturer’s specification. The membrane was hybridized with the selected 32P-radiolabeled probe of interest. The autoradiographic image was generated and quantified by a PhosphorImager (Bio-Rad Laboratories, Inc., Hercules, CA).

RESULTS

Failure of the HSV-1 strain KOS ICP4 mutant d120 genome to assume an endless structure in Vero cells after infection. To determine if the ICP4 protein is involved in a genomic structural change after infection, the ICP4-deleted mutant virus, d120, was used to infect Vero cells as well as the Vero-derived ICP4-expressing E5 cells at an MOI of 5. Mutant d120, as described below, is derived from strain KOS and contains a 4.1-kb deletion in both ICP4 loci (8), as illustrated in Fig. 1A and B, which also shows two conformations of the viral genome: linear and circular.

The physical state of the viral genomes following infection of Vero cells was determined by comparing the relative abundance of the terminal (BamHI S and P) and penultimate (BamHI E and B) restriction fragments of the viral genome present in the virion and after infection, generally as described previously (26). Infection was performed in the presence of the DNA replication inhibitor PAA (400 μg/ml), which prevents all detectable viral DNA replication (Su et al., unpublished; see also Materials and Methods). DNA was isolated from virions and from infected cells 3 hpi and was digested to completion with BamHI, resolved through agarose gels, transferred to nylon membranes, and hybridized with radioactive probes specific for BamHI SP and B/E.

The results presented in Fig. 1C show that terminal fragments (S and P) derived from KOS-infected Vero cells are almost undetectable, whereas they exist in amounts approximately equimolar to those of the internal joint SP fragments.
present in virion DNA (Fig. 1C; Table 1). This result is not surprising, since circularization, or “endless” DNA formation, is expected to occur shortly after infection, even in the absence of DNA synthesis. Circularization would result in joining of the terminal S and P fragments and a loss of their detection (as illustrated in Fig. 1A and B).

The situation with $d_{120}$ is dramatically different. There is no detectable reduction of $d_{120}$ genomic terminal S and P fragments following infection of Vero cells. The restriction pattern (Fig. 1C) and the molar ratio of PS/P+S (Table 1) of the digests of genomes derived from infected Vero cells and virions are almost indistinguishable. This is consistent with an absence of genomic “state change” or “endless formation” following infection.

Note that due to the 4.1-kb deletion in both ICP4 loci of the $d_{120}$ genome, the BamHI P fragment no longer existed when the $d_{120}$ genome was digested with restriction endonuclease BamHI. The 500-nucleotide ladder of $d_{120}$ genome-derived
BamHI S and PS fragments is presumably due to multiple insertions of the “a” sequence with a BamHI restriction site and not the presence of multiple isolates within the virus stock, since plaque-purified viral clones (n = 3) bred true with respect to this restriction pattern (data not shown). In any event, d120 genomes did not “circularize” or assume the “endless” DNA formation (presumably circularization) of the viral genome in Vero cells in any of the experiments (0% for all the experiments performed), while 33% to 78%, with a median of 51%, of the infected d120 genomes in E5 cells were shown to be in a circular form shortly after infection. In d120-infected E5 cells, the percentage of d120 DNA that circularized was not statistically different from that of KOS in Vero cells (p = 0.156). Thus, the ICP4 protein provided from d120-infected E5 cells was able to compensate for the defect of d120 in the formation of the endless genome structure to the level of the wild type.

Circularization of the d120 genome in Vero cells preinfected with an ICP4-producing DNA replication-incompetent HSV-1 mutant. Since d120 did not form a genomic endless state in Vero cells but did so in ICP4-producing E5 cells, it was reasoned that ICP4, provided by the E5 cells, was mediating the genome state change. However, it was possible that factors other than ICP4, present in E5 cells (but not in Vero cells), were responsible for the complementation. Given that the ICP4 protein is a pleiotropic phosphoprotein with multiple phosphorylation sites that possess both DNA and protein binding domains, it was possible that the physiology of E5 cells was altered after being stably transfected with the ICP4 gene, and these secondary effects were responsible for causing the d120 genome to assume an endless form in the E5 cells.

To determine whether or not the endless formation of the d120 genome is E5 specific, Vero cells were first infected with another DNA replication-incompetent mutant to provide ICP4 protein and then to provide the degree to which the d120 genome circularized was determined. To prevent viral DNA replication due to the complementation between these two mutants, the infections were performed in the presence of PAA, as before.

Vero cells were infected first with the replication-defective mutant HP66 at an MOI of 5. HP66 is defective in the polymerase gene but produces a fully functional ICP4 protein. Three hours after HP66 infection, cultures were superinfected with mutant d120 at an MOI of 5. The infected cells were harvested 3 h after d120 infection. Nuclear DNA was isolated and analyzed for the formation of the endless viral genome, measured by the reduction of terminal fragments as described in Table 1.
Based upon the reduction in the relative amount of terminal to internal BamHI restriction fragments (Fig. 2), approximately 40% of the input HP66 genomes formed the endless structure following infection. This is in contrast to the situation with monoinfection of Vero cells with \( d_{120} \), where there was little, if any, evidence of circularization of the viral genome. As expected for the controls, an increased amount of the joint fragment accompanying a decreased amount of terminal fragments was observed in the BamHI-digested KOS-infected Vero DNA, suggesting the circularization of the wild-type KOS genome was evident in Vero cells after wild-type KOS infection.

As mentioned above, the joint and terminal fragments of a complete BamHI-digested viral genome as revealed by the BamHI PS fragment hybridization are different in size for \( d_{120} \) and HP66, since HP66 has no deletion in those regions of the genome. Thus, we can determine the percent of circularization of each virus in the HP66 and \( d_{120} \) mix-infected nuclear DNA.

There was a clear loss of the detectable \( d_{120} \) terminal fragment, suggesting that the circularization had occurred in mixed infection. Thus, the capability of the \( d_{120} \) genome to form the endless structure in the E5 cells was not due to the effect of the permanently transfected ICP4 gene on the physiology of the E5 cells. The ICP4 protein provided by mutant HP66 in \textit{trans} was capable of mediating the endless formation event of the \( d_{120} \) genome in Vero cells.

\textbf{ICP4 protein expression in \( d_{120} \)-infected Vero and E5 cells.}

Previous studies reported that the viral DNA “state change” or circularization can occur in the absence of de novo protein synthesis (12, 14, 20). Since the ICP4 protein is present in the virion (35), \( d_{120} \) progeny propagated in the ICP4-complementary E5 cells might have been expected to acquire sufficient ICP4 protein to mediate the circularization event.

However, our results suggest that, to the extent that ICP4 protein was associated with the \( d_{120} \) virion, it failed to mediate the circularization event following infection of Vero cells. It seemed possible, therefore, that the ICP4 associated with \( d_{120} \) derived from E5 cells was of a different quality or quantity than that associated with wild-type KOS.

To help resolve this issue, we compared the virion-associated ICP4 protein in \( d_{120} \) and wild-type KOS by Western blot analysis. Western blot analysis was first performed on infected cell lysates from the \( d_{120} \)-infected Vero cells and the \( d_{120} \)-infected E5 cells that produce ICP4 polypeptide upon infection of HSV-1 (25), as detailed in Materials and Methods. Briefly, Vero and E5 cells were infected with mutant \( d_{120} \) at an MOI of 5. Total cell lysate was harvested 6 hpi and analyzed, as shown in Fig. 3A. The antibody to the ICP4 protein recognizes...
the full-length 175-kDa ICP4 protein in both KOS-infected Vero cells and d120-infected E5 cell lysates. No band was detected in mock-infected Vero cell lysates, showing the specificity of the antibody, although a similar amount of Vero cell lysate was loaded, as indicated by reprobing the blot with antibody against actin.

The amount of ICP4 protein made in uninfected E5 cells was below the level of detection by Western blot analysis. Note that the expression of ICP4 protein in E5 cells is under the control of the ICP4 promoter, thus, its expression is significantly increased only following HSV infection, presumably as a consequence of activation of the promoter by the trans-activator virion-associated VP16 protein.

Consistent with a previous report (8), the 40-kDa amino-terminal portion of the ICP4 is detected in d120-infected cells. Interestingly, we detected the truncated 40-kDa ICP4 protein in three different size forms on an SDS-polyacrylamide gel in d120-infected Vero cells and d120-infected E5 cells. No full-length ICP4 protein was detected in Vero cells infected with d120 virus. The different-sized forms of the truncated ICP4 protein are likely due to heterogeneity of phosphorylation (34). Further studies are needed to confirm whether these different forms were due to the difference in phosphorylation.

The truncated form is the major ICP4 polypeptide in d120 virions grown in E5 cells. To compare the virion-associated ICP4 protein in d120 and wild-type KOS virions, virions were purified by centrifuging through a 20% sucrose gradient and then followed by a 20% to 60% sucrose gradient, as detailed in Materials and Methods. Purified virions were lysed and analyzed for ICP4 protein by Western blot analysis.

To examine the purity of the virion preparation, two approaches were used. First, the fractions above (Fig. 3A, upper) and below (Fig. 3A, lower) the virion band, between 45% to 50% sucrose concentration, were collected at the end of a 20% to 60% sucrose gradient purification and concentrated through a microconcentrator-10 column.

The quantity of protein in the concentrates of the upper and lower fractions was below the level of detection (absorption at 280 nm) by the spectrophotometer. This was further confirmed by the Western blot analysis. While the viral VP16 protein was clearly detected in 1/10th of the virion preparation (30 ng of virion lysate), no detectable VP16 protein was found in the upper and lower fractions (Fig. 3B).

Second, since ICP27 protein is not associated with virions (36), the ICP27 protein can be used to control for cellular contamination during virion purification. Seventy micrograms of protein lysates from Vero cells, E5 cells, KOS-infected Vero cells, d120-infected E5 cells, KOS virions, and d120 virions were resolved in a 9% SDS-polyacrylamide gel, transferred, and Western blotted with the antibodies to viral ICP4, VP16, glycoprotein D (gD), and ICP27 proteins (Fig. 3C). In agreement with previous studies (36) that the ICP27 protein is not a virion-associated protein, no detectable ICP27 protein was found in both KOS and d120 virion lysates by Western blot analysis. This further confirmed the purity of virion preparations.

As shown in Fig. 3C, all the viral proteins tested were readily detected in both the KOS-infected Vero cell lysate and the d120-infected E5 cell lysate but not in uninfected Vero or E5 cell lysates. When the membrane was probed with the anti-ICP4 antibody, a full-length 175-kDa ICP4 protein was readily detected in wild-type KOS virion lysate. Interestingly, the ICP4 protein detected in d120 virion lysate was predominately the truncated 40-kDa form, with only a small fraction of the ICP4
The amounts of other known virion-associated proteins, VP16 and gD, were similar in both KOS and d120 virion preparations, suggesting that similar quantities of virion lysates were loaded in each lane. Since an expected amount of full-length ICP4 protein was readily detected in KOS virion, the lesser amount of full-length ICP4 detected in d120 virion was not due to the inefficiency of transferring the protein to the membrane. It is thus concluded that the truncated ICP4 protein is the major species of the ICP4 proteins found in the d120 virion.

The d120 virion-associated ICP4 protein is insufficient to mediate the circularization of the infecting viral genome in the E5 cells. The results of the Western blot study indicate that the major species of ICP4 protein present in assembled d120 virions is a truncated, defective protein. As shown in Fig. 3C, only a minority of the virion-associated ICP4 protein is full length. It seemed likely that this small amount of full-length ICP4 protein is not sufficient to mediate the genomic structure change. Thus, the ICP4 protein that mediates endless viral genome formation (as seen in d120-infected E5 cells) should be the result of de novo-synthesized ICP4 protein from E5 cells following d120 infection.

To test this hypothesis, we infected E5 cells with d120 mutant viruses in the presence or absence of a protein synthesis inhibitor, cycloheximide (CHX; 50 μg/ml), and analyzed the structure of the viral genome 3 hpi. As controls, infections of wild-type KOS in the presence or absence of CHX were performed in both Vero and E5 cells in the presence of PAA. The infected DNA was harvested 3 hpi and subjected to DNA structure analysis.

As shown in Fig. 4A, terminal fragments of the d120 genome were reduced significantly in the E5 cells by 3 hpi in the absence of CHX, suggesting that the structure of the genome changed from linear to endless, as expected. In contrast, when de novo protein synthesis was inhibited (by the presence of CHX), no detectable difference were seen in the relative molarity of terminal S, joint PS, and B and E fragments between linear virion DNA and the DNA harvested 3 hpi in the E5-infected cultures. As a reference, the d120 genomes present in infected Vero cells are also shown.

In agreement with previous studies (12, 14, 20) reporting that the HSV-1 wild-type genome circularization could occur in the absence of de novo protein synthesis, a noticeable amount of input KOS genomes circularized in the presence of CHX in both Vero and E5 cells (Fig. 4B). Thus, the de novo protein synthesis is not needed for the wild-type HSV-1 genome to circularize following infection. In contrast, the structural change of the d120 genome from linear to endless (or circular) seen in infected E5 cells was mediated by de novo-synthesized ICP4 protein from the E5 cells after d120 infection. The d120 virion-associated ICP4 protein alone was not sufficient to mediate the circularization of the d120 genome.
DISCUSSION

This study provides evidence that one of the HSV-1 immediate-early gene products, the ICP4 protein, is essential for endless formation, presumably circularization, of the mutant d120 viral genome following infection. To our knowledge, this is the first report of a role for ICP4 in viral genome circularization.

ICP4 protein provided by the ICP4-expressing E5 cell line, or by another replication-incompetent HSV-1 polymerase mutant (HP66), was sufficient to complement the defect of the ICP4-deleted mutant virus, d120. However, the ICP4 present within the d120 virion was apparently insufficient to mediate circularization. These data suggest, for the first time, an important role for the virion-associated ICP4 protein.

The inadequacy of the ICP4 present in the d120 virion to mediate circularization of the infecting genome was probably due to the fact that the majority of d120 virion-associated ICP4 was present as a truncated form (40 kDa). Only a small amount of the ICP4 present in the virions derived from the ICP4-producing E5 cells was full-length ICP4 protein. This raises questions regarding the mechanism whereby ICP4 polypeptide associates with progeny virions.

It has been shown previously that viral genome circularization does not require de novo protein synthesis following infection, since it occurs in the cells treated with inhibitors of protein synthesis at the time of infection, as demonstrated by previous studies (12, 14, 20). This suggests that the ICP4 protein associated with wild-type virions is sufficient to mediate circularization. However, the ICP4 proteins associated with the infectious d120 virion were unable to mediate the circularization of the d120 genome. Western blot analysis using the antibody to the ICP4 protein showed that the d120 virion-associated ICP4 protein was predominately in a truncated form with only a small fraction of the ICP4 protein being full length. Interestingly, although an amount of the full-length ICP4 protein sufficient to support the productive viral life cycle was made in E5 cells after d120 infection, it seems that during the virion assembly the truncated form was preferentially packed into the virion. The circularization of the linear d120 genome observed in d120-infected E5 cells thus required de novo synthesis of the ICP4 protein from E5 cells after d120 infection, as demonstrated in Fig. 4. Therefore, we reason that the amount of full-length ICP4 protein present in d120 virion is insufficient to mediate the circularization.

ICP4 is a multifunctional protein characterized by its role as a trans-activator as well as a repressor. In addition to its role in transcription regulation, ICP4 has been previously associated with the formation of viral DNA replication compartments (2, 30). After infection, ICP4 protein was colocalized with the viral genome at a site next to ND10 structures. The DNA replication compartment was formed next to ND10 early in the infection, and ND10 structures were destroyed soon after by ICP0 protein (10, 11). Interestingly, the formation of the ICP4 foci early in the infection was dependent on the correct folding of its DNA binding domain, and this required the input of viral genomes. In this study, our data indicate an important role for virion-associated ICP4 protein in the event of viral DNA circularization following infection, and it is possible that this event occurs at the site where DNA replication compartments later develop.

The failure of d120 to circularize in Vero cells is in contrast to the behavior of d109, another ICP4-defective mutant, which apparently remains circularization competent (14). However, d120 and d109 differ in several important ways. For example, d109 contains deletions in other immediate-early genes, including ICP27, ICP0, ICP22, and ICP47. This study also reports that ICP0 inhibits viral genomic circularization (14). Perhaps d109 virions, derived from their ICP4-complementing cells, contain a sufficient amount of virion-associated ICP4 to mediate the circularization. This may or may not be due to the lack of the circularization inhibitor (ICP0) in d109-infected Vero cells. The genotype of d109 is complicated, and work is needed to resolve the differences between the phenotypes of d120 and d109 with respect to virion-associated proteins and circularization.

The inability of d120 to circularize in Vero cells and the ability of ICP4 provided in trans to complement this defect suggest that ICP4 has a role in the circularization process. To our knowledge, this is the first report of a role for the virion-associated ICP4 protein in mediating the circularization of the viral genome. The management of the physical state of the viral genome is likely to be of central importance to the virus life cycle, given how important genome structure is to infection, replication, latency, or encapsidation. Thus, it is important to study the mechanism whereby ICP4 protein achieves this function.

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