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# Stages in the Nuclear Association of the Herpes Simplex Virus Transcriptional Activator Protein ICP4

DAVID M. KNIPE,* DAVID SENECHEK, STEPHEN A. RICE, AND JANET L. SMITH

*Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts 02115*

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**The nuclear localization of the herpes simplex virus transcriptional activator protein ICP4 was studied by indirect immunofluorescence. At early times after viral infection, ICP4 quickly localized to a diffuse intranuclear distribution. ICP4 later concentrated in globular compartments within the nucleus. The redistribution to the compartments was dependent on viral DNA replication. Double staining for ICP4 and ICP8, the early major DNA-binding protein, revealed that both were found in the same intranuclear globular compartments at late times. These were previously named "replication compartments" (M. P. Quinlan, L. B. Chen, and D. M. Knipe, Cell 36:857–868, 1984). Because ICP4 and ICP8 are known to function in transcriptional activation and DNA replication, respectively, both DNA replication and late transcription may occur in these compartments. The association of ICP4 and ICP8 with the replication compartments appeared to be independent in that the retention of ICP8 in the compartments required ongoing viral DNA synthesis, while the association of ICP4 was independent of viral DNA synthesis once the compartments were formed. Because ICP4 shows a different distribution at early and late times, stimulation of transcription by ICP4 may involve different molecular events or contacts during these two periods of the replicative cycle.**

Several viral gene products have been shown to function as *trans*-acting transcriptional activators. These include the adenovirus E1A gene product (5, 25), the herpes simplex virus (HSV) ICP4 (10, 28, 37, 49), the pseudorabies immediate early protein (4, 16, 21, 23), and the simian virus 40 large-T antigen (7, 26). The mechanism by which they activate transcription remains to be elucidated. In addition, the nature of their interactions with nuclear structures and macromolecules is not defined. To understand the nature of the association of the HSV ICP4 protein with the cell nucleus, we initiated studies on the maturation of this protein.

Viral gene expression during lytic infection of cells by HSV occurs in a coordinately regulated sequence (reviewed in references 43 and 48). Immediately after infection, a set of viral gene products requiring no prior viral protein synthesis, named alpha or immediate early gene products, is expressed. The beta or delayed early gene products are then expressed. These proteins are largely responsible for viral DNA replication. After DNA replication, the gamma or late gene products are expressed at optimal levels, and they are involved in assembling the progeny virus.

The alpha protein ICP4 is required for beta and gamma gene transcription (20, 37, 49). ICP4 is synthesized as a polypeptide with an apparent molecular weight of 155,000 to 160,000, and it matures to three forms with apparent molecular weights of 163,000, 165,000, and 170,000 (33). This change in electrophoretic mobility is apparently due to phosphorylation (50) and possibly poly(ADP ribosyl)ation (39). ICP4 from crude extracts will bind to DNA-cellulose (2, 18), but one report indicates that purified ICP4 has lost its ability to bind to DNA-cellulose (18). ICP4 quickly localizes to the cell nucleus after its synthesis (35), and a portion of it may associate with the nuclear matrix (6). ICP4 expressed in stable cell lines (36) or transfected cells (15, 19, 34, 41) is capable of stimulating expression of beta genes introduced into the cells. Some reports have suggested that stimulation of beta gene expression may occur by direct interaction of ICP4 with the beta gene promoter sequences (3, 13), while others have argued that ICP4 acts indirectly by affecting the level or activity of cellular transcriptional factors (11, 24). ICP4 is required for early and late viral transcription (10, 49), and these two functions of ICP4 may be genetically distinct (9).

We had previously shown that the HSV beta major DNA-binding protein ICP8 showed a series of stages in its association with the cell nucleus (27, 40). ICP8 initially associated with the nuclear matrix upon entry into the nucleus, and it demonstrated a punctate staining pattern in this form. As viral DNA replication occurred, ICP8 showed a redistribution onto viral DNA, and it showed a globular distribution in this form. Alteration of the status of viral DNA replication caused the protein to move back and forth between these two distributions. The studies reported in this paper were initiated to determine whether the transcriptional activator protein ICP4 showed a similar series of stages in its maturation.

## MATERIALS AND METHODS

**Cells and viruses.** Virus stocks were prepared and titrated in Vero cells. Vero cells were infected for immunofluorescence studies. The original references and sources of the viruses were as follows: HSV-1 strain KOS1.1 wild type (*ts*$^+$; 22) was provided by M. Levine, University of Michigan; HSV-1 strain 17 *ts*$^+$ and 17 *ts*K (37) viruses were provided by J. Subak-Sharpe, MRC Virology Institute, Glasgow.

**Indirect immunofluorescence.** Cells were grown and infected on glass cover slips as described previously (29). Unless stated otherwise, cells were infected at a multiplicity of infection (MOI) of 20. The fixation and staining procedures described previously (40) were used for this work.

Microscopy was performed with a Zeiss standard microscope equipped for phase-contrast and fluorescence microscopy utilizing a Plan Neofluar 63X objective. For detection of fluorescence from fluorescein isothiocyanate-conjugated

* Corresponding author.

antibodies, a filter set (green filter) consisting of an excitation filter for light for wavelength from 450 to 490 nm, a mirror for less than 510 nm, and a final band filter for 515 to 565 nm was used. For detection of fluorescence from rhodamine isothiocyanate-conjugated antibodies, a filter set (red filter) consisting of an excitation filter for 546 nm, a mirror for greater than 580 nm, and a final filter for greater than 590 nm was used. These two filter sets showed almost complete discrimination of the fluorescence from the two fluorochromes (see Fig. 3).

The 58S (anti-ICP4) and 39S (anti-ICP8) mouse monoclonal antibodies (46) were kindly provided by M. Zweig. The H1083 (anti-ICP0) and H1113 (anti-ICP27) mouse monoclonal antibodies (1) were kindly provided by L. Pereira, University of California, San Francisco. The rabbit polyclonal anti-ICP4 serum (32) was kindly provided by K. Wilcox, Medical College of Wisconsin. The 3-83 polyclonal anti-ICP8 serum was prepared by immunization of a rabbit with purified ICP8 (D. Daniels, M. Levin, and D. Knipe, unpublished data). ICP8 was purified from infected-cell nuclei (27) which were sonicated and incubated with DNase and 1 M NaCl. The solubilized proteins were fractionated by ammonium sulfate precipitation, phosphocellulose chromatography, and single-stranded DNA-cellulose chromatography. The protein used for immunization was a single band of approximately 127,000 molecular weight. The rhodamine isothiocyanate-conjugated goat anti-rabbit and -mouse immunoglobulin (immunoglobulin G [IgG] plus IgM plus IgA) antibody and the fluorescein isothiocyanate-conjugated goat anti-rabbit and -mouse immunoglobulin (IgG plus IgM plus IgA) antibody were purchased from Cooper Biomedical, Inc., West Chester, Pa.

## RESULTS

We had previously shown that the HSV early DNA-binding protein, ICP8, showed a series of stages in its maturation into and within the infected cell nucleus (27, 40). These studies employed both cell fractionation and indirect immunofluorescence to characterize the nuclear interactions of ICP8. To examine the nuclear interactions of ICP4 and how they might result in activation of viral transcription, we attempted to study the nuclear localization of ICP4 by cell fractionation and indirect immunofluorescence. We were able to separate infected Vero cells into the nuclear, cytoplasmic, and detergent wash fractions as described previously (27), with the complete recovery of ICP4. However, further fractionation of nuclei led to a loss of ICP4 (data not shown) which could not be prevented by several inhibitors of proteolysis in Vero cell extracts (32, 51). For this reason, our studies presented here utilized immunofluorescence to localize ICP4 in the infected cell.

**Localization of ICP4 at various times after infection.** To examine the time course of ICP4 localization to the infected-cell nucleus, we examined the intracellular distribution of ICP4 at different times after infection. For these experiments, we employed indirect immunofluorescence using the 58S monoclonal antibody which recognizes ICP4 (46; C. Lee and D. Knipe, unpublished data). At 2 h postinfection (p.i.), ICP4 was distributed throughout the infected cell with some concentration in the nucleus but exclusion from the nucleolus (Fig. 1C and D). At 3 h p.i., ICP4 was localized specifically in the cell nucleus and it showed a diffuse pattern of staining with a few foci of intranuclear staining (Fig. 1E and F). By 4 to 5 h p.i., ICP4 was concentrated in globular structures in the cell nucleus and in a few cytoplasmic granules (Fig. 1G and H). Therefore, ICP4 showed a change in nuclear distribution between early and later times after infection.

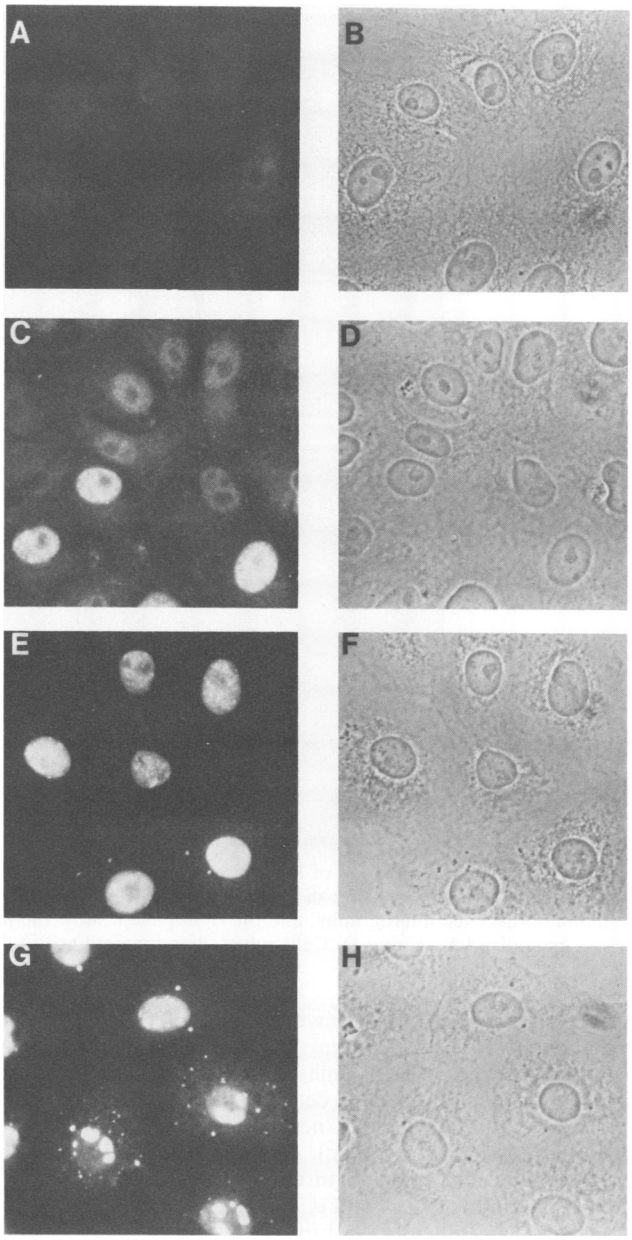


FIG. 1. Nuclear localization of ICP4 at different times after infection. At various times after infection with KOS1.1 virus, Vero cells were fixed and reacted with 58S (anti-ICP4) monoclonal antibody and rhodamine-conjugated goat anti-mouse immunoglobulin antibody. The left panels show immunofluorescence micrographs, and the right panels show the phase-contrast micrographs of the same fields. (A and B) Mock-infected cells, 5 h p.i. (C and D) 2 h p.i. (E and F) 3 h p.i. (G and H) 5 h p.i.

**Effect of viral DNA replication on ICP4 distribution.** The change in ICP4 distribution was reminiscent of the change in distribution of ICP8 that was dependent on viral DNA replication (40). To examine the effect of viral DNA replication on ICP4 location, we examined the ICP4 distribution in cells infected without or with phosphonoacetate (PAA), a specific inhibitor of the viral DNA polymerase (30, 31). At 5 h p.i., ICP4 showed the globular pattern of staining de-

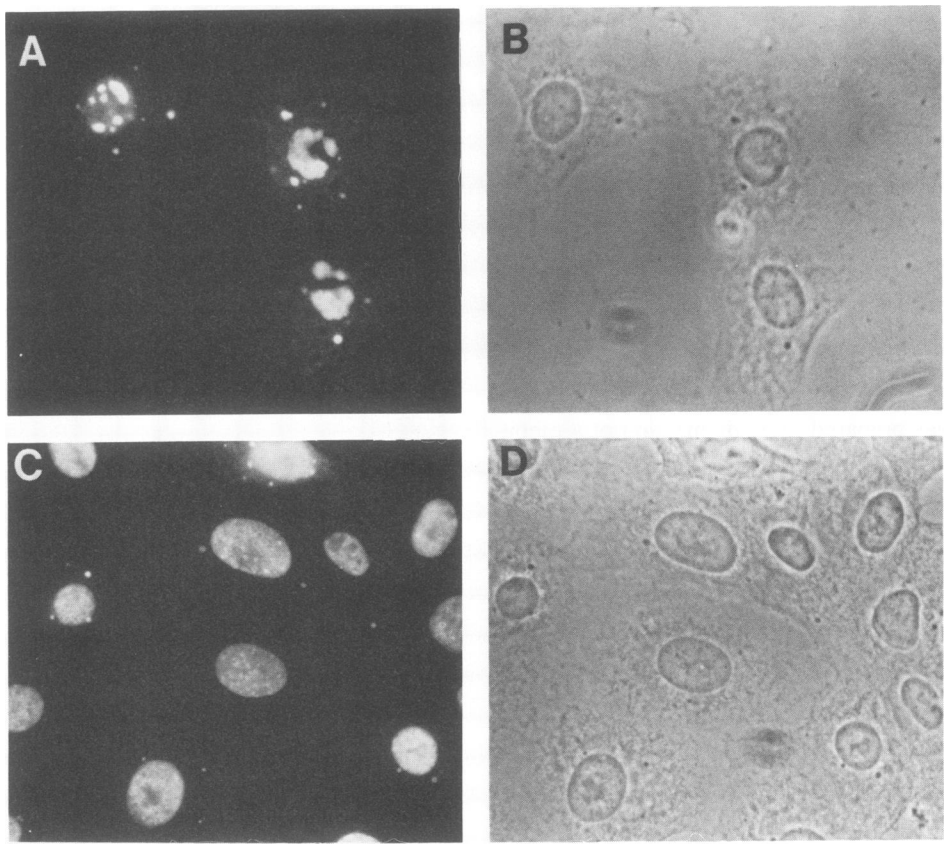


FIG. 2. Nuclear localization of ICP4 in the presence or absence of viral DNA replication. Cells were infected with KOS1.1 virus in the absence or presence of 400 μg of PAA per ml. At 5 h p.i., the cells were fixed and reacted with 58S antibody. The left panels show immunofluorescence micrographs, and the right panels show the phase-contrast micrographs of the same fields. (A and B) Cells infected in the absence of PAA. (C and D) Cells infected and maintained in the presence of PAA.

scribed above (Fig. 2A). However, in cells infected in the presence of PAA, ICP4 remained in the diffuse nuclear pattern observed at early times (Fig. 2C). A similar diffuse distribution was observed in cells infected at the nonpermissive temperature with DNA-negative temperature-sensitive (*ts*) mutants (data not shown). Therefore, the movement to the globular distribution required viral DNA replication.

**Codistribution of ICP4 and ICP8 in globular compartments.** ICP4 and ICP8 both exhibited staining of globular intranuclear structures late in the replication cycle, and the formation of these structures was dependent on viral DNA replication. To determine whether these two proteins were located in the same structures, we performed double-label immunofluorescence to localize ICP4 and ICP8 simultaneously. For these studies we employed the 39S monoclonal antibody specific for ICP8 and a rabbit polyclonal serum specific for ICP4 (32). At 5 h p.i., the 39S antibody stained globular compartments in the nuclei of infected cells (Fig. 3A), as described previously (40). The ICP4 antibody stained the same intranuclear globular structures as the ICP8 antibody and a few cytoplasmic structures not shared by ICP8 (Fig. 3B). When cells were singly stained for ICP4 or ICP8 and the appropriate second antibody, little fluorescence was observed with the other filter (Fig. 3D and F). Therefore, there was little crossover between the two fluorochromes with the filters used. Similarly, there was little staining when the second antibody used was not specific for the first antibody or antiserum (data not shown). Therefore, the intranuclear globular compartments contained both ICP4 and ICP8. Results similar to those shown in Fig. 3 were obtained when ICP4 was stained with the 58S monoclonal antibody and ICP8 was stained with the 3-83 rabbit polyclonal antiserum prepared against purified ICP8 (data not shown).

We also examined cells at early times of infection by double staining for ICP4 and ICP8 in cells infected and maintained in PAA. In these cells we observed a punctate nuclear staining pattern for ICP8 (Fig. 4A), while in the same nucleus, we observed a diffuse pattern of ICP4 staining (Fig. 4B). At early times ICP4 and ICP8 showed distinct distributions in the infected-cell nucleus. Therefore, their staining patterns did not always completely overlap.

**Nuclear localization of a mutant ICP4 molecule.** Nearly all the *ts* mutant ICP4 molecules fail to localize to the infected-cell nucleus at the nonpermissive temperature, as judged by immunofluorescence (29). However, we observed that at least one type of mutant ICP4 molecule can localize into the nucleus of Vero cells at the nonpermissive temperature. ICP4 encoded by the mutant 17 *ts*K cannot stimulate the expression of delayed early gene products (37), but the protein could localize into the nucleus of Vero cells infected at the nonpermissive temperature at an MOI of 10 to 20 (Fig. 5B). The nuclear distribution of the mutant protein looked much like the distribution of the wild-type strain 17 ICP4 at early times during infection (Fig. 5A). Both showed diffuse patterns of staining with a few intranuclear foci of staining.

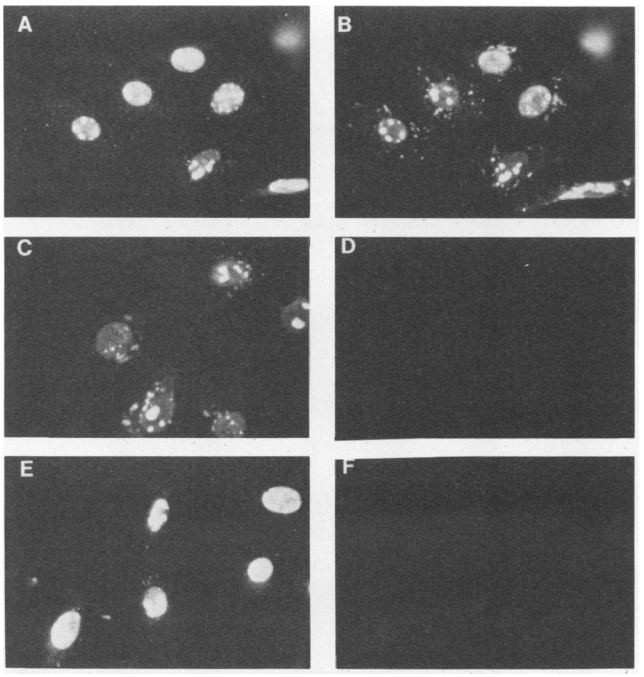


FIG. 3. Colocalization of ICP4 and ICP8 in replication compartments. At 5 h p.i., cells were fixed and reacted with 39S (anti-ICP8) monoclonal antibody or a rabbit anti-ICP4 serum or both and the appropriate second antibodies. (A) Infected cells reacted with 39S (anti-ICP8) monoclonal antibody, anti-ICP4 serum, rhodamine-conjugated goat anti-mouse immunoglobulin antibody, and fluorescein-conjugated goat anti-mouse immunoglobulin antibody. The ICP8 distribution shown was visualized with the red filter. (B) The same field as in panel A is shown, except that the green filter was used to determine the ICP4 distribution. (C) Infected cells reacted with rabbit anti-ICP4 serum and fluorescein-conjugated goat anti-rabbit immunoglobulin. The fluorescence micrograph shown was made with the green filter. (D) The same field as in panel C except that the red filter was used. Exposure time was the same as panel A. (E) Infected cells reacted with 39S (anti-ICP8) monoclonal antibody and rhodamine-conjugated goat anti-mouse immunoglobulin antibody. The red filter was used to record the fluorescent image. (F) The same field as in panel E except that the green filter was used. Exposure time was the same as panel B.

Additional experiments (data not shown) demonstrated that early gene products, such as ICP8, were not expressed in cells infected with *ts*K at 39.7°C. Therefore, this mutation separates the ability to localize to the nucleus from transcriptional activation in that the protein was localized into the nucleus but could not function normally or interact with the proper molecules to stimulate delayed early gene expression. An additional phenotype shared by the other ICP4 mutants is a ts block in ICP0 nuclear localization (29). The ICP0 encoded by *ts*K was capable of nuclear localization (Fig. 5D) and showed a nuclear distribution similar to that of the wild-type ICP0 (Fig. 5C). Therefore, there appears to be a correlation between the abilities of ICP4 and ICP0 to localize to the cell nucleus.

Preston (38) previously reported that the *ts*K ICP4 protein was defective in its association with the cell nucleus. Although no defect is apparent in Fig. 5, we have observed that, at higher MOIs (e.g., at MOI = 40), ICP4 was blocked for nuclear localization in a small proportion of Vero cells (data not shown). Therefore, the ability of *ts*K ICP4 to localize to the nucleus may be cell type or multiplicity dependent or both. Nevertheless, it appears that in Vero cells infected at MOIs of 20 or less, the inability of *ts*K ICP4 to stimulate beta gene expression is not due to its inability to enter the nucleus.

**Double staining for ICP4 and ICP0.** ICP4 and ICP0 can independently stimulate expression of HSV delayed early gene products in transfected cells (15, 19, 34, 41). When introduced together, they demonstrated a synergistic response (15, 19, 41). Therefore, it was conceivable that the two proteins interacted in the infected cell. Double staining for ICP4 and ICP0 was performed with a rabbit polyclonal serum and the H1083 mouse monoclonal antibody (1). These experiments showed that some of the ICP0 molecules localized to nuclear structures similar to those containing ICP4 (Fig. 6A and B) but that some of the ICP0 was located in areas where ICP4 was not concentrated. Thus, there appeared to be a partial overlap in the distributions of the two proteins.

**Double staining for ICP4 and ICP27.** ICP4 is required for late gene expression, and ICP27 is believed to affect the levels of late gene expression (44). To determine whether ICP4 and ICP27 were localized to similar locations at late times, we infected cells and double stained for ICP4 and ICP27 using a rabbit polyclonal anti-ICP4 serum and the H1113 mouse anti-ICP27 monoclonal antibody (1). These experiments showed that when ICP4 was concentrated in nuclear compartments (Fig. 6C), ICP27 showed a diffuse distribution throughout the entire nucleus (Fig. 6D). Although some ICP4 and ICP27 may interact with each other or with similar structures, there was no evidence that ICP27 concentrates in the same structures as ICP4.

**Relationship between ICP4 and ICP8 in globular compartments.** To determine whether ICP4 and ICP8 show an interdependent association with the replication compartments, we determined the distribution of the two proteins after inhibition of DNA replication, protein synthesis, or transcription. We had shown previously that ICP8 redistributed to the prereplicative punctate staining pattern when viral DNA synthesis was inhibited (40). Before inhibition of viral DNA synthesis, both ICP4 and ICP8 were localized in globular compartments in the nucleus (Fig. 7A and B). When we inhibited protein synthesis, both ICP4 and ICP8 remained in the replication compartments (Fig. 7C and D). When we inhibited viral DNA synthesis with PAA in the presence of cycloheximide to prevent any new protein synthesis, we observed that preexisting ICP8 redistributed in most cells to the prereplicative sites (Fig. 7E). However, in these cells ICP4 was observed mostly in the compartments in the nucleus (Fig. 7F). Therefore, ICP4 maintained its association with the globular compartments even when most of the ICP8 had redistributed. The retention of the two proteins within the replication compartments appeared to be independent. Both ICP8 and ICP4 remained in compartments when infected cells were incubated in medium containing actinomycin D (Fig. 7G and H). Control experiments (data not shown) indicated that the actinomycin inhibited viral transcription. Thus, inhibition of transcription did not appear to alter the intranuclear location of ICP4.

## DISCUSSION

We showed that a progression of stages can be defined for the nuclear association of the HSV transcriptional activator protein, ICP4. At early times after infection ICP4 is distributed throughout the infected cells, but it quickly localizes into the nucleus where it shows a diffuse distribution. When

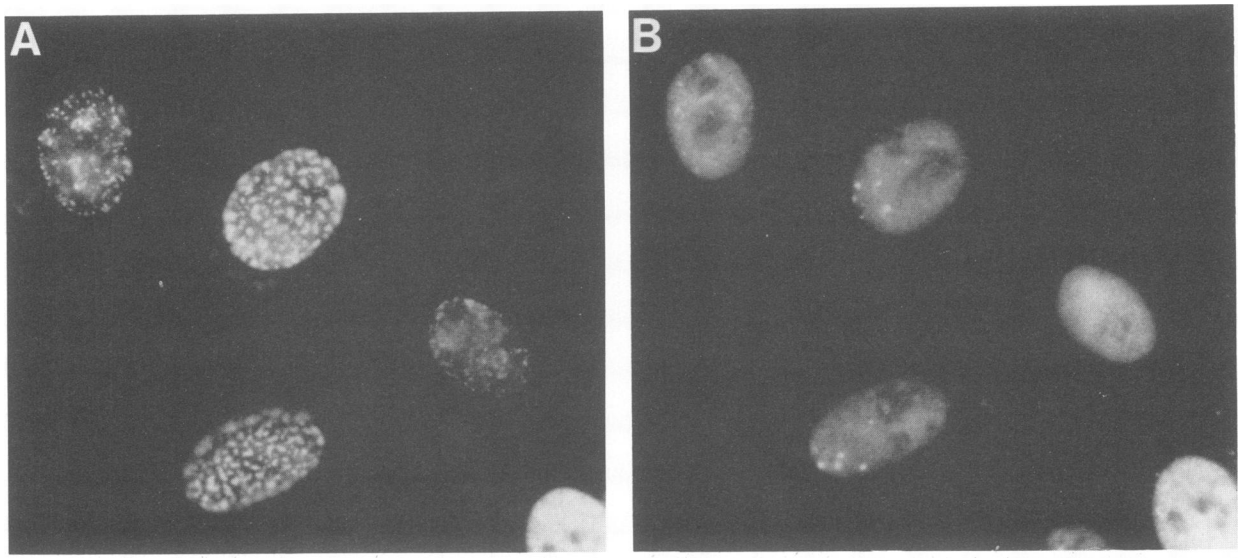


FIG. 4. Distributions of ICP4 and ICP8 at early times of infection. At 4 h p.i., infected cells maintained in 400 μg of PAA per ml were fixed and reacted with antibodies to stain both ICP4 and ICP8 as described in the legend to Fig. 3A and B. (A) ICP8 distribution. (B) ICP4 distribution.

viral DNA replication occurs, ICP4 is redistributed into globular compartments. We will focus on each of these stages separately.

**ICP4 in diffuse nuclear distribution.** At early times of infection, ICP4 appeared in a diffuse nuclear distribution, while the beta ICP8 protein showed a punctate distribution. Thus, it appears that the two proteins have a distinct distribution in these cells, although they codistribute at later

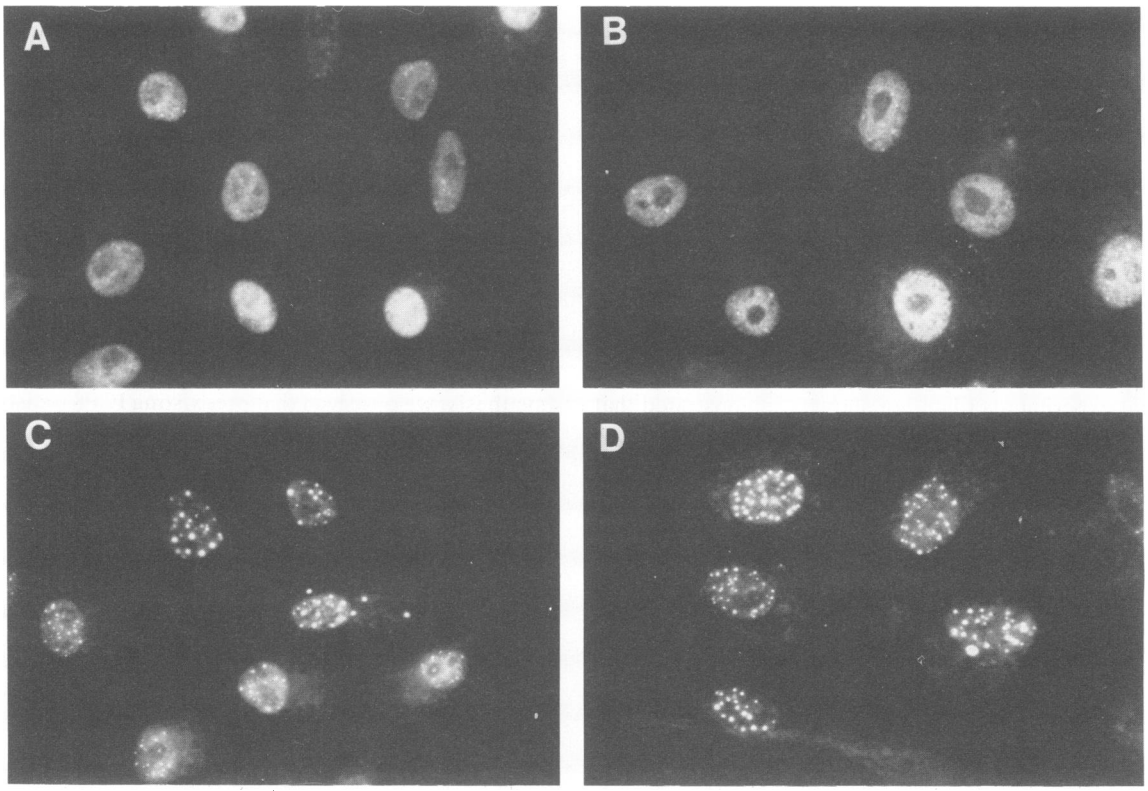


FIG. 5. Nuclear localization of ICP4 and ICP0 in cells infected with $ts^+$ and *ts*K viruses. Cells infected at 39.7°C with HSV-1 17 $ts^+$ or *ts*K were fixed at 4 h p.i. and reacted with the indicated first antibody and the appropriate second antibody. For the experiment shown, the MOI was 10. Similar results were observed with an MOI of 20 (data not shown). (A) Cells infected with 17 $ts^+$ virus and reacted with 58S (anti-ICP4) antibody. (B) Cells infected with 17 *ts*K virus at 39.7°C and reacted with 58S (anti-ICP4) antibody. (C) Cells infected with 17 $ts^+$ virus at 39.7°C and reacted with H1083 (anti-ICP0) monoclonal antibody. (D) Cells infected with 17 *ts*K virus at 39.7°C and reacted with H1083 (anti-ICP0) monoclonal antibody.

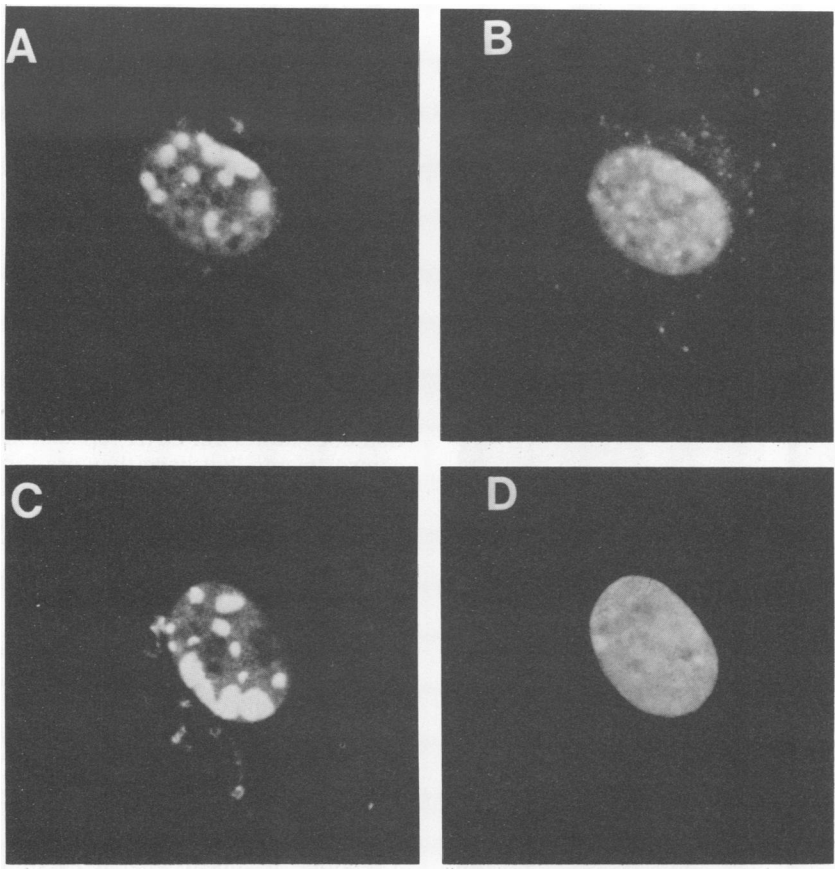


FIG. 6. Double staining for ICP4 and other alpha proteins. Cells infected with KOS1.1 $ts^+$ virus were fixed at 5 h p.i. and reacted with the indicated antibodies. (A) Cells reacted with rabbit anti-ICP4 serum and mouse H1083 anti-ICP0 monoclonal antibody followed by fluorescein-conjugated goat anti-rabbit immunoglobulin antibody and rhodamine-conjugated goat anti-mouse immunoglobulin antibody. The ICP4 distribution was photographed with the green filter. (B) The same field as in panel A photographed through the red filter to determine the ICP0 distribution. (C) Cells reacted with rabbit anti-ICP4 serum and mouse H1113 anti-ICP27 monoclonal antibody followed by the same antibody conjugates described in the legend to panel A. The ICP4 distribution was photographed with the green filter. (D) The ICP27 distribution in the same field as in panel C was photographed through the red filter.

times. The nature of the nuclear structures with which ICP4 interacts at early times is not well defined. Bibor-Hardy et al. (6) have reported that some of each of the HSV alpha proteins is attached to the nuclear matrix within 30 min after their synthesis. Furthermore, this association was stable during a chase period. However, the authors did not quantitate the recovery of ICP4 in the different subcellular fractions, and it is difficult to determine whether a significant fraction of ICP4 remains in the nuclear matrix fraction. As described above, our own efforts to fractionate nuclei were hampered by proteolysis of ICP4.

The adenovirus E1A protein and the *myc* protein also show diffuse nuclear staining patterns and have been reported to fractionate with the nuclear matrix (12, 17). However, one report indicates that the tight association of the *myc* protein with the nuclear matrix is dependent on incubation of nuclei at temperatures of 37°C or higher (14). Thus, the association of at least some proteins with the nuclear matrix may be dependent on the extraction procedure.

At the nonpermissive temperature the *ts*K ICP4 protein localized into the nucleus but could not stimulate the expression of beta genes. These data are somewhat different from those of Preston (38) who reported that *ts*K ICP4 was defective in its association with the nucleus of BHK cells. We also observed that the nuclear localization of the *ts*K ICP4 was somewhat inhibited in Vero cells at the nonpermissive temperature at higher MOIs. It is conceivable that there are cell-type- or multiplicity-dependent differences in the nuclear localization of the *ts*K ICP4. It appears clear that in Vero cells infected with *ts*K at MOIs of 20 or less that ICP4 is largely nuclear but cannot effect beta gene transcription. These results separate the ability of ICP4 to localize to the cell nucleus from the ability to activate early gene transcription.

**ICP4 in globular nuclear distribution.** After viral DNA replication commences, ICP4 localizes to large globular structures in the nucleus. These structures also contain ICP8 and have been previously called "replication compartments" (40). Because ICP4 and ICP8 promote transcription and DNA replication, respectively, it is likely that these compartments serve as the sites for viral DNA replication and late gene transcription. Although more processes than viral DNA replication may occur in these compartments, the name replication compartment is still appropriate in that they are formed as replication proceeds. Pulse-labeled DNA accumulates in globular structures such as these (42), but no information is available regarding the intranuclear location of viral transcription at late times.

The replication compartments visualized at 4 to 5 h p.i. by immunofluorescence detection of viral proteins are not ap-

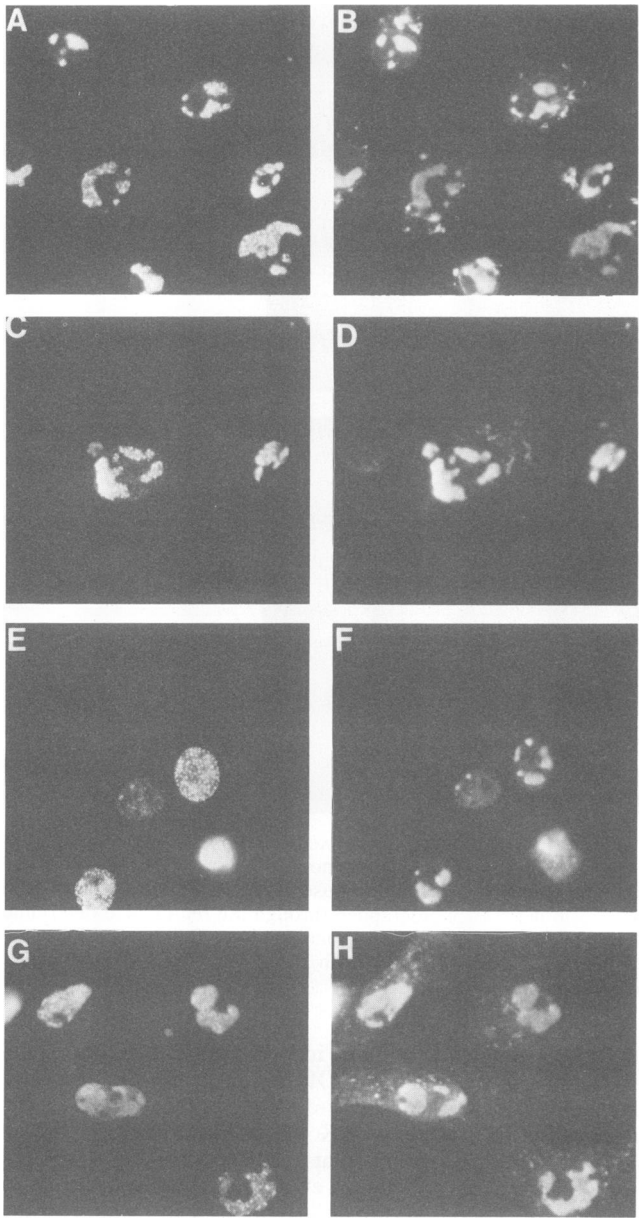


FIG. 7. Distribution of ICP4 and ICP8 after inhibition of protein synthesis, viral DNA replication, or transcription. Vero cells were infected with KOS1.1 $ts^+$ virus and incubated at 33°C for 4.5 h. Two cultures were fixed at this time. To two of the cultures, cycloheximide was added to 50 μg/ml. To two other cultures, cycloheximide was added to 50 μg/ml, and PAA was added to 400 μg/ml. To two other cultures, cycloheximide was added to 50 μg/ml, and actinomycin D was added to 10 μg/ml. The cultures were incubated for an additional 2 h. The cells were stained to determine the ICP8 and ICP4 distributions as described in the legend to Fig. 4A and B. (A) ICP8 distribution, 4.5 h p.i. (B) ICP4 distribution in the same field shown in panel A. (C) ICP8 distribution, 6.5 h p.i., 2 h after addition of cycloheximide. (D) ICP4 distribution of the same field shown in panel C. (E) ICP8 distribution, 6.5 h p.i., 2 h after addition of cycloheximide and PAA. (F) ICP4 distribution of the same field shown in panel E. (G) ICP8 distribution, 6.5 h p.i., 2 h after addition of cycloheximide and actinomycin D. (H) ICP4 distribution of the same field shown in panel G.

parent by phase-contrast microscopy. These are probably distinct from or early precursors to the classical inclusion bodies seen in HSV-infected cells and visible by hematoxylin and eosin staining (8, 45). The inclusion bodies are usually not apparent until approximately 10 h p.i. (8, 47) and may contain, in addition to viral DNA, a deposition of viral capsids in various stages of assembly. The replication compartments are probably similar to the "perichromatin-like granules" previously documented by Smith and deHarven (47) to appear at about 3 h p.i.

ICP4 and ICP8 have different requirements for retention in the replication compartments. Inhibition of viral DNA replication caused ICP8 to redistribute to the prereplicative sites, while ICP4 remained in structures resembling replication compartments. Thus, retention of ICP8 in the compartments requires on-going DNA replication, while ICP4 retention does not. Viral DNA replication is needed to form the compartments, but once they are formed, ICP4 association is independent of viral DNA replication. This also indicates that the compartments do not disappear when viral DNA replication is inhibited. Because of the differences in requirements for their association, ICP4 and ICP8 may be totally independent in their association with the replication compartments.

The replication compartments may also contain part of the ICP0 present in the cell nucleus, but ICP27 does not specifically accumulate in these nuclear structures. It will be of interest to determine what viral and cellular proteins are localized to these structures and what other aspects of replication occur within them.

**ICP4 localization and stimulation of transcription.** ICP4 is required for both early and late viral transcription (10, 49). Therefore, it appears that ICP4 stimulates transcription at early times while in a diffuse distribution but that it stimulates late transcription while in the replication compartments. It is likely that, like ICP8, ICP4 initially localizes to sites in the nucleus defined by cellular structures and later localizes to intranuclear structures assembled as viral DNA replication proceeds. Therefore, the molecular events or contacts involved in transcriptional stimulation may be different at early or late stages. DeLuca et al. (9) have reported genetic evidence that the required functions of ICP4 for early and late gene expression can be separated. These mutants express early viral gene products but not late gene products at the nonpermissive temperature. It will be of interest to determine whether these ICP4 molecules are defective for localization to the replication compartments or for their activity therein.

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## LITERATURE CITED

1. **Ackermann, M., D. K. Braun, L. Pereira, and B. Roizman.** 1984. Characterization of herpes simplex virus type 1 proteins 0, 4, and 27 with monoclonal antibodies. J. Virol. **52**:108–118.
2. **Bayliss, G. J., H. S. Marsden, and J. Hay.** 1975. Herpes simplex virus proteins: DNA-binding proteins in infected cells and in the

virus structure. Virology **60**:124–134.
3. **Beard, P., S. Faber, K. W. Wilcox, and L. I. Pizer.** 1986. Herpes simplex virus immediate early infected cell polypeptide 4 binds to DNA and promotes transcription. Proc. Natl. Acad. Sci. USA **83**:4016–4020.
4. **Ben-Porat, T., P. Hoffman, L. Brown, L. Feldman, and M. L. Blankenship.** 1982. Partial characterization of temperature-sensitive mutants of pseudorabies virus. Virology **122**:251–267.
5. **Berk, A. J., F. Lee, T. Harrison, J. Williams, and P. A. Sharp.** 1979. Pre-early adenovirus 5 gene product regulates synthesis of early viral messenger RNAs. Cell **17**:935–944.
6. **Bibor-Hardy, V., M. Bernard, and R. Simard.** 1985. Nuclear matrix modifications at different stages of infection by herpes simplex virus type 1. J. Gen. Virol. **66**:1095–1103.
7. **Brady, J., J. B. Bolen, M. Radonovich, N. Salzman, and G. Khoury.** 1984. Stimulation of simian virus 40 late gene expression by simian virus 40 tumor antigen. Proc. Natl. Acad. Sci. USA **81**:2040–2044.
8. **Crouse, H. V., L. L. Coriell, H. Blank, and T. F. M. Scott.** 1950. Cytochemical studies on the intranuclear inclusions of herpes simplex. J. Immunol. **65**:119–128.
9. **DeLuca, N. A., M. A. Courtney, and P. A. Schaffer.** 1984. Temperature-sensitive mutants in herpes simplex virus type 1 ICP4 permissive for early gene expression. J. Virol. **52**:767–776.
10. **Dixon, R. A. F., and P. A. Schaffer.** 1980. Fine-structure mapping and functional analysis of temperature-sensitive mutants in the gene encoding the herpes simplex virus type 1 immediate early protein VP175. J. Virol. **36**:189–203.
11. **Eisenberg, S. P., D. M. Coen, and S. L. McKnight.** 1985. Promoter domains required for expression of plasmid-borne copies of the herpes simplex virus thymidine kinase gene in virus-infected fibroblasts and micro-injected frog oocytes. Mol. Cell. Biol. **5**:1940–1947.
12. **Eisenman, R. N., C. V. Tachibana, C. Y. Abrams, and S. R. Hann.** 1985. v-*myc* and c-*myc* proteins are associated with the nuclear matrix. Mol. Cell. Biol. **5**:114–126.
13. **Elkareh, A., A. J. M. Murphy, T. Fichter, A. Efstratiadis, and S. Silverstein.** 1985. "Transactivation" control signals in the promoter of the herpes simplex virus thymidine kinase gene. Proc. Natl. Acad. Sci. USA **82**:1002–1006.
14. **Evan, G. I., and D. C. Hancock.** 1985. Studies on the interaction of the human c-*myc* protein with cell nuclei: p62 c-*myc* as a member of a discrete subset of nuclear proteins. Cell **43**: 253–261.
15. **Everett, R. D.** 1984. Transactivation of transcription by herpes virus products: requirements for two HSV-1 immediate early polypeptides for maximum activity. EMBO J. **3**:3135–3141.
16. **Feldman, L. T., M. J. Imperiale, and J. R. Nevins.** 1982. Activation of early adenovirus transcription by the herpes virus immediate early gene: evidence for a common cellular control factor. Proc. Natl. Acad. Sci. USA **79**:4952–4956.
17. **Feldman, L. T., and J. R. Nevins.** 1983. Localization of the adenovirus E1A$_a$ protein, a positive-acting transcriptional factor, in infected cells. Mo. Cell. Biol. **3**:829–838.
18. **Freeman, M. J., and K. L. Powell.** 1982. DNA-binding properties of a herpes simplex virus immediate early protein. J. Virol. **44**:1084–1087.
19. **Gelman, I. H., and S. Silverstein.** 1985. Identification of immediate early genes from herpes simplex virus that transactivate the virus thymidine kinase. Proc. Natl. Acad. Sci. USA **82**:5265–5269.
20. **Godowski, P. J., and D. M. Knipe.** 1986. Transcriptional control of herpes virus gene expression: gene functions required for positive and negative regulation. Proc. Natl. Acad. Sci. USA **83**:256–260.
21. **Green, M. R., R. Treisman, and T. Maniatis.** 1983. Transcriptional activation of cloned human β-globin genes by viral immediate-early genes. Cell **35**:137–148.
22. **Hughes, R. G., and W. H. Munyon.** 1975. Temperature-sensitive mutants of herpes simplex virus type 1 defective in lysis but not in transformation. J. Virol. **16**:275–283.
23. **Imperiale, M. J., L. T. Feldman, and J. R. Nevins.** 1983. Activation of gene expression by adenovirus and herpesvirus regulatory genes acting in *trans* and by a *cis*-acting adenovirus enhancer element. Cell **35**:127–136.
24. **Jones, K. A., K. R. Yamamoto, and R. Tjian.** 1985. Two distinct transcription factors bind to the HSV thymidine kinase promoter *in vitro*. Cell **42**:559–572.
25. **Jones, N., and T. Shenk.** 1979. An adenovirus type 5 early gene function regulates expression of other viral genes. Proc. Natl. Acad. Sci. USA **76**:3665–3669.
26. **Keller, J. M., and J. C. Alwine.** 1984. Activation of the SV40 late promoter: direct effects of T antigen in the absence of viral DNA replication. Cell **36**:381–389.
27. **Knipe, D., and A. Spang.** 1982. Definition of a series of stages in the association of two herpes viral proteins with the cell nucleus. J. Virol. **43**:314–324.
28. **Knipe, D. M., W. T. Ruyechan, R. W. Honess, and B. Roizman.** 1979. Molecular genetics of herpes simplex virus. IV. The terminal a sequences of the L and S components are obligatorily identical and constitute a part of the structural gene mapping predominantly in the S component. Proc. Natl. Acad. Sci. USA **76**:4534–4538.
29. **Knipe, D. M., and J. L. Smith.** 1986. A mutant herpes virus protein leads to a block in nuclear localization of other viral proteins. Mol. Cell. Biol. **6**:2371–2381.
30. **Leinbach, S. S., J. M. Reno, L. F. Lee, A. F. Isbell, and J. A. Boezi.** 1976. Mechanism of phosphonoacetate inhibition of herpes virus-induced DNA polymerase. Biochemistry **15**:426–430.
31. **Mao, J. G.-H., and E. E. Robishaw.** 1975. Mode of inhibition of herpes simplex virus DNA polymerase by phosphonoacetate. Biochemistry **14**:5475–5479.
32. **Metzler, D. W., and K. Wilcox.** 1985. Isolation of herpes simplex virus regulatory protein ICP4 as a homodimeric complex. J. Virol. **55**:329–337.
33. **Morse, L. S., L. Pereira, B. Roizman, and P. A. Schaffer.** 1978. Anatomy of herpes simplex virus (HSV) DNA. Mapping of viral genes by analysis of polypeptides and functions specified by HSV-1 × HSV-2 recombinants. J. Virol. **26**:389–410.
34. **O'Hare, P., and G. S. Hayward.** 1985. Evidence for a direct role for both the 175,000- and 110,000-molecular weight immediate early proteins of herpes simplex virus in the transactivation of delayed-early promoters. J. Virol. **53**:751–760.
35. **Pereira, L., M. H. Wolff, M. Fenwick, and B. Roizman.** 1977. Regulation of herpesvirus macromolecular synthesis. X. Properties of polypeptides made in HSV-1 and HSV-2 infected cells. Virology **77**:733–749.
36. **Persson, R. H., S. Bacchetti, and J. R. Smiley.** 1985. Cells that constitutively express the herpes simplex virus immediate early protein ICP4 allow efficient activation of viral delayed-early genes in *trans*. J. Virol. **54**:414–421.
37. **Preston, C. M.** 1979. Control of herpes simplex virus type 1 mRNA synthesis in cells infected with wild-type virus or the temperature-sensitive mutant *ts*K. J. Virol. **29**:275–284.
38. **Preston, C. M.** 1979. Abnormal properties of an immediate early polypeptide in cells infected with herpes simplex virus type 1 mutant *ts*k. J. Virol. **32**:357–369.
39. **Preston, C. M., and E. L. Notarianni.** 1983. Poly (ADP ribosyl)ation of a herpes simplex virus immediate early polypeptide. Virology **131**:492–501.
40. **Quinlan, M. P., L. B. Chen, and D. M. Knipe.** 1984. The intranuclear location of a herpes simplex virus DNA-binding protein is determined by the status of viral DNA replication. Cell **36**:857–868.
41. **Quinlan, M. P., and D. M. Knipe.** 1985. Stimulation of expression of a herpes simplex virus DNA-binding protein by two viral functions. Mol. Cell. Biol. **5**:957–963.
42. **Rixon, F. J., M. A. Atkinson, and J. Hay.** 1983. Intranuclear distribution of herpes simplex virus type 2 DNA synthesis: examination by light and electron microscopy. J. Gen. Virol. **64**:2087–2092.
43. **Roizman, B., and W. Batterson.** 1986. Herpesviruses and their replication, p. 607–636. *In* B. N. Fields and D. M. Knipe (ed.), Fundamental virology. Raven Press, New York.
44. **Sacks, W. R., C. C. Greene, D. P. Aschman, and P. A. Schaffer.**

1985. Herpes simplex virus type 1 ICP27 is an essential regulatory protein. J. Virol. **55**:796–805.

45. **Scott, T. F. M., C. F. Burgoen, L. L. Coriell, and H. Blank.** 1983. The growth curve of the virus of herpes simplex in rabbit corneal cells grown in tissue culture with parallel observations on the development of the intranuclear inclusion body. J. Immunol. **71**:385–396.
46. **Showalter, S. D., M. Zweig, and B. Hampar.** 1981. Monoclonal antibodies to herpes simplex virus type 1 proteins including the immediate-early protein ICP4. Infect. Immun. **34**:684–692.
47. **Smith, J. D., and E. deHarven.** 1973. Herpes simplex virus and human cytomegalovirus replication in WI-38 cells. J. Virol. **12**:919–930.
48. **Wagner, E. K.** 1984. Individual HSV transcripts: characterization of specific genes, p. 65–104. *In* B. Roizman (ed.), The herpesviruses, vol. 3. Plenum Publishing Corp., New York.
49. **Watson, R. J., and J. B. Clements.** 1980. A herpes simplex virus type 1 function continuously required for early and late virus RNA synthesis. Nature (London) **285**:329–330.
50. **Wilcox, K. W., A. Kohn, E. Sklyanskaya, and B. Roizman.** 1980. Herpes simplex virus phosphoproteins. I. Phosphate cycles on and off some viral polypeptides and can alter their affinity for DNA. J. Virol. **33**:167–182.
51. **Zezulak, K. M., and P. G. Spear.** 1984. Limited proteolysis of herpes simplex virus glycoproteins that occurs during their extraction from Vero cells. J. Virol. **50**:258–262.