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Assemblons: Nuclear Structures Defined by Aggregation of Immature Capsids and Some Tegument Proteins of Herpes Simplex Virus 1

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Received 5 February 1996/Accepted 25 March 1996

In cells infected with herpes simplex virus 1 (HSV-1), the viral proteins ICP5 (infected-cell protein 5) and VP19c (the product of UL38) are associated with mature capsids, whereas the same proteins, along with ICP35, are components of immature capsids. Here we report that ICP35, ICP5, and UL38 (VP19c) coalesce at late times postinfection and form antigenically dense structures located at the periphery of nuclei, close to but not abutting nuclear membranes. These structures were formed in cells infected with a virus carrying a temperature-sensitive mutation in the UL15 gene at nonpermissive temperatures. Since at these temperatures viral DNA is made but not packaged, these structures must contain the proteins for immature-capsid assembly and were therefore designated assemblons. These assemblons are located at the periphery of a diffuse structure composed of proteins involved in DNA synthesis. This structure overlaps only minimally with the assemblons. In contrast, tegument proteins were located in asymmetrically distributed structures also partially overlapping with assemblons but frequently located nearer to nuclear membranes. Of particular interest is the finding that the UL15 protein colocalized with the proteins associated with viral DNA synthesis rather than with assemblons, suggesting that the association with DNA may take place during its synthesis and precedes the involvement of this protein in packaging of the viral DNA into capsids. The formation of three different compartments consisting of proteins involved in viral DNA synthesis, the capsid proteins, and tegument proteins suggests that there exists a viral machinery which enables aggregation and coalescence of specific viral protein groups on the basis of their function.

The herpes simplex virus 1 and 2 (HSV-1 and HSV-2) particles consist of four concentric structural elements, i.e., a central DNA core; a capsid consisting of the products of the genes UL18 (VP23), UL19 (VP5), UL26 (VP21, VP22a, and VP24), UL35 (VP26), and UL38 (VP19c); an amorphous protein structure called the tegument, which surrounds the capsid; and an envelope containing viral glycoproteins (4, 6, 8, 14, 21, 22, 30, 34, 36). The HSV genomes are both transcribed and replicated in a central nuclear compartment, which is also the site of immature viral capsid assembly. The studies described in this report were initiated following the observation that the product of a newly discovered open reading frame designated UL43.5 and mapping antisense to UL43 colocalized with capsid proteins in dense structures located at the periphery of the nucleus late in infection (44). Spurred by these observations, we began a systematic study of the localization of several classes of structural and nonstructural viral proteins involved in the various stages of mature capsid assembly.

Viral DNA is synthesized via a rolling-circle mechanism (reviewed in reference 36) in a central nuclear compartment that has been defined by the presence of the single-stranded viral DNA-binding protein ICP8 (9). The newly replicated DNA is cleaved from its concatemeric form and packaged into the preformed, immature capsids, which then acquire an envelope derived from the inner nuclear membrane upon exit from the nucleus. In this report, we compare the distribution of capsid proteins with both ICP8 and the viral DNA polymerase accessory protein encoded by the UL42 gene, as well as the UL15 gene product, which has been associated with cleavage and packaging of viral DNA on the basis of analyses of a spontaneously arising mutant virus carrying a temperature-sensitive (ts) mutation in the UL15 gene. At the nonpermissive temperature, cells infected with this mutant accumulate uncleaved concatemeric viral DNA, and packaging into preformed capsids does not ensue (1, 26). Several other viruses which contain ts mutations in genes whose products are either capsid proteins or proteins known to be required for viral DNA synthesis accumulate uncleaved viral DNA (36).

Previously, we observed that ICP35 protein was distributed in discrete patches at the periphery of the nucleus late in infection (44). We asked whether these discrete patches also contained proteins associated with mature capsids, thereby suggesting that these sites could be sites of capsid assembly rather than represent a concentration of soluble proteins. We therefore compared the distribution of ICP35 with that of ICP5 and UL38 (VP19c). Relevant to this are the following points. (i) The UL26 gene encodes a protease precursor, Prn (19). A transcriptional unit designated UL26.5, 3′ coterminal with the UL26 gene, yields ICP35cd (18). Both the protease precursor Prn and the ICP35cd protein are cleaved by the protease to yield several sets of products. ICP35cd is cleaved to yield ICP35ef and a small carboxy-terminal peptide (19). Prn is cleaved to yield the mature protease, a polypeptide designated ICP35ab, and the same small carboxy-terminal peptide as that cleaved from ICP35cd (10, 12, 20). ICP35ab, ICP35ef, and Prn (the amino-terminal cleavage product of Prn) form the scaffolding of the capsid. Upon packaging of viral DNA, only Prn (VP21) remains in the capsid (14, 25). Monoclonal antibody H725 reacts with an epitope present in Prn, ICP35ab, ICP35cd, and ICP35ef but not in Prn (3, 4, 18). This antibody therefore...
identifies accumulations of U15 and U43.5 proteins in rabbit ts (27). HSV-1(mP) with 5% newborn calf serum. done in Verocells grown in Dulbecco’s modified Eagle’s medium supplemented mers and is not packaged into preformed capsids (1, 26). All experiments were nonpermissive temperatures, viral DNA is synthesized but remains in concate- 
lation of soluble protein as well as the accumulation of both immature and mature capsids, and antibody to ICP5 identifies the accumu-
lation of soluble protein as well as the accumulation of both immature and mature capsids (36). (iii) The U138 gene encodes a capsid protein designated VP19c (5, 16, 21). This protein has been previously shown to bind viral DNA in a nonspecific fashion and may play a role in anchoring the DNA to the capsid (5, 45).

The site of acquisition of the tegument is uncertain. The observations that purified capsids from nuclei do not contain tegument proteins (14) and that thickened patches of membrane form at the site of envelopment at the inner nuclear membrane have led to the suggestion that tegument proteins are part of the envelopment complex at the nuclear membrane (35). The localization of two tegument proteins has been in- vestigated in this study. The product of the U11 gene is an abundant tegument protein, which binds RNA in a sequence- and conformation-specific fashion, binds to ribosomes, and also localizes in the nucleus (11, 37–39). The alpha-trans-inducing factor (αTIF) (24, 43), also known as virion protein 16 (42), transactivates the transcription of the α genes—the first set of genes expressed in productively infected cells (28, 36). αTIF is also an essential tegument protein. Virions produced in cells infected with ts mutant viruses generated by substitu-
tion of cysteines in the αTIF protein for glycines fail to mature at the nonpermissive temperature (27).

In this report we show the following. (i) Viral proteins in-
volved in the synthesis of viral DNA are dispersed throughout a large portion of the nucleus. (ii) The proteins associated with immature capsids, i.e., ICP5, ICP35, and U138 (VP19c), ag-
gregate in defined, antigenically dense nuclear structures frequently located at the edges of the large, diffuse nuclear do-
 mains defined by the proteins involved in DNA synthesis. (iii) The U15 protein, which is known to be required for packaging of viral DNA into capsids but is not itself a capsid protein, localizes with proteins associated with viral DNA synthesis and surprisingly not with the aggregates of capsid proteins. We conclude from studies presented here that late in infection at the time of heightened assembly of virions, components of the capsid and some tegument proteins assemble in discrete nu-
clear structures on the fringes of the space occupied by pro-
tiens involved in DNA synthesis. We have designated these structures assemblies to show that they include proteins of both mature and immature capsids and also some tegument proteins.

**MATERIALS AND METHODS**

**Cells and viruses.** HSV-1 strain F [HSV-1(F)] is the prototype strain used in our laboratories (13). HSV-1(F)/R7032 lacks the entire U138 gene which encodes glycoprotein E (23); it was used in immunofluorescence studies to avoid non-
specific immunofluorescence caused by binding of immunoglobulin G (IgG) to the Fc receptor expressed by glycoprotein E. HSV-1(F)/R2063 carries two mutations in the UL48 (αTIF) gene, and its lesion in the α4 gene is repaired (27). HSV-1(F)/R606-4 carries a ts mutation in the U15 gene (26). At the nonpermissive temperatures, viral DNA is synthesized but remains in concate-
mers and is not packaged into preformed capsids (1, 26). All experiments were done in Vero cells grown in Dulbecco’s modified Eagle’s medium supplemented with 10% newborn calf serum.

**Antibodies.** Rabbit polyclonal antibodies to the UL10 (glycoprotein M) (2), U15 (1), and U138 (44) proteins have been described previously. Rabbit polyclonal antibody to the capsid protein U138 was generated by immunization of rabbits with a bacterial fusion protein as described below. The preparation of the monoclonal antibody to U113 protein has been described elsewhere (39). Monoclonal antibodies to the capsid proteins ICP5 and ICP35 were obtained from Goodwin Biotechnology Inst., Plantation, Fla. Monoclonal antibody LP1, which reacts with αTIF protein, was the kind gift of Dan Tenney, Bristol-Meyers Squibb. The goat anti-rabbit fluorescein isothiocyanate (FITC)-conjugated antibody was purchased from Sigma Chemical Co., St. Louis, Mo. The goat anti-mouse Texas red-conjugated antibody was purchased from Molecular Probes, Inc., Eugene, Ore.

**Preparation of anti-U138 polyclonal antiserum.** Figure 1 illustrates the con-
struction of plasmid pRB4956 encoding the U138-glutathione-3-transferase (U138-GST) fusion protein. Line 1 shows the sequence arrangement of the HSV-1 genome, and line 2 shows the sequence arrangement of HSV-1(F) HindIII-K cloned into the HindIII site of pBR322. The resultant plasmid, designated pRB210, contains the U137, U138, and U139 open reading frames (ORFs). The 3.1-kbp SalI-KpnI fragment of pRB210 was ligated into the SalI and KpnI sites in pGM3Z (Promega, Madison, Wis.) to yield pRB4954 (Figure 1, line 3). The 864-bp Avai fragment of pRB4954 encoding the amino-terminal 277 amino acids of the U138 ORF was ligated into the Avai site of pGEX2T (Phar-
macia). The resultant plasmid, designated pRB4955 (Fig. 1, line 4), was pre-
dicted to encode the bacterial GST in frame with the amino-terminal region of U138. pRB4955 was digested with pPU81 and EcoRI (the EcoRI site is in the vector polylinker), and the ends were made blunt and religated, reducing the U138 portion of the GST–U138 fusion protein to 54 amino acids (a.a.). A, Avai; E, EcoRI; H, HindIII; K, KpnI; P, pPU81; S, SalI.

**FIG. 1. Schematic representation of the sequence arrangements of HSV-1 (F) DNA and of the plasmids used to construct the GST fusion with the U138 ORF. Line 1, sequence arrangement of HSV-1(F) DNA. Open boxes, internal repeat sequences flanking the unique long (U1) and unique short (U0) regions; line 2, sequence arrangement of pRB210 containing the U137, U138, and U139 ORFs located within the HindIII K fragment of HSV-1(F) DNA; line 3, se-
quence arrangement of pRB4954 containing the SalI-KpnI fragment from pRB210 cloned into the pGM3Z vector and encompassing the entire U138 ORF and a portion of the U137 ORF; line 4, 864-bp Avai fragment from pRB4954 encoding the N-terminal 277 amino acids of U138 cloned into the pGEX-KpnI site of vector pGEX-2T, creating pRB4955; line 5, pRB4955 digested with pPU81 and EcoRI (site present in vector polylinker), with the ends made blunt and religated, reducing the U138 portion of the GST–U138 fusion protein to 54 amino acids (a.a.). A, Avai; E, EcoRI; H, HindIII; K, KpnI; P, pPU81; S, SalI.
**RESULTS**

**Specificity of the U₃₈ rabbit polyclonal antiserum.** We generated a rabbit polyclonal antiserum to U₃₈ protein for use in colocalization studies with mouse monoclonal antibodies to ICP5 and ICP35. The antibody produced following inoculation of rabbits with U₃₈-GST fusion protein specifically reacted with a protein of approximately 53 kDa present in HSV-1(F)-infected cell lysates (Fig. 2, right panel). The antibody did not react with any proteins present in mock-infected cell lysates (right panel), nor did the preimmune sera react with any proteins present in either uninfected or infected cell lysates (left panel).

**Redistribution of ICP35 and ICP5 during HSV-1 infection.** The localization of two proteins associated with immature (ICP35) and mature (ICP5) capsids (33, 36) was examined at early (6 h) and late (16 h) times postinfection. At 6 h postinfection, both ICP5 (Fig. 3A) and ICP35 (Fig. 3D) proteins are diffusely distributed in an irregular pattern throughout the nucleus. In some cells, some aggregation of capsid proteins can be seen (Fig. 3A, compare cells labeled a and b), which progresses to form discrete, brightly fluorescent structures by 16 h postinfection (Fig. 3B [ICP5], cell labeled a; Fig. 3C [ICP5]; Fig. 3E and F [ICP35]). The localization of the tegument protein, αTIF (Fig. 3G to I), is discussed below.

**Colocalization of U₃₈, ICP35α-f, and ICP5.** As previously reported (44), the U₃₈ protein colocalizes with ICP35 in dense, strongly fluorescent nuclear structures (Fig. 4a to c). In this series of experiments, we asked whether these structures contained exclusively proteins associated with immature capsids by comparing the localization of three proteins associated with mature (ICP5 and U₃₈) and immature (ICP5, U₃₈, and ICP35) capsids. The U₃₈ protein was detected with FITC-conjugated antibody against rabbit IgG. ICP5 and ICP35 were detected with Texas red-conjugated antibody against mouse IgG. The results of these studies show that in some cells, ICP35 and ICP5 (Fig. 4g, i, and m) aggregated in both diffuse and highly dense, strongly fluorescent nuclear structures. Colocalization of these proteins with the U₃₈ protein is visualized by yellow fluorescence (Fig. 4, right-hand column). U₃₈ protein colocalized both with ICP35 and ICP5 in the dense, strongly fluorescent nuclear structures (Fig. 4k, l, n, o, q, and r) and with the diffuse, less strongly fluorescent ICP5 (Fig. 4n and o) but not with the diffuse, distributed ICP35 (Fig. 4h and i). In Fig. 4, we have illustrated the dominant pattern consisting of a small number of dense structures (three to eight) prevalent in the infected-cell nuclei late in infection. In some cells, particularly in those in which infection was retarded, the nuclei contained a large number of relatively smaller dense, fluorescent structures. This is illustrated in Fig. 5b and c. To define the localization of these dense structures more precisely, infected cells were reacted simultaneously with rabbit polyclonal antibody to glycoprotein M and with monoclonal antibody to ICP35. As illustrated in Fig. 6g to i, the dense, strongly fluorescent nuclear structures containing ICP35 were separated from the uniform shell formed by glycoprotein M in the nuclear membrane.

**Tegument proteins partially overlap the dense, strongly fluorescent nuclear structures containing capsid proteins.** We have examined the localization of two tegument proteins, U₃₈ and αTIF. U₃₈ protein accumulated in diffuse nuclear regions at or near nuclear membranes in most of the infected cells, in addition to nucleoli as previously reported (39). Even in cells in which U₃₈ protein is largely in the dense, strongly fluorescent nuclear structures, the U₃₈ protein overlapped only in part with these structures containing capsid proteins (Fig. 4d to f).

The localization of αTIF was examined under five conditions, i.e., in cells infected with HSV-1(F) or with viruses carrying ts mutations in U₃₈ [HSV-1(mPr66-4)] or αTIF (R2603) and maintained at either permissive or nonpermissive temperatures. The results of these studies were as follows. (i) Abrogation of DNA cleavage and packaging did not affect the appearance of any of the compartments defined by the presence of capsid proteins or proteins associated with DNA synthesis in cells infected with this mutant virus at the nonpermissive temperature. (ii) As a general rule, αTIF was an abundant nuclear protein that was present either diffusely throughout the nucleus (Fig. 5a) with accumulation of protein in nuclear membranes (Fig. 3G and H) or aggregated and only partially filling the nucleus (Fig. 3I). The compartment occupied by αTIF was considerably more diffuse and only partially colocal-
ized with UL38 protein and, by extension, with other capsid proteins (Fig. 5atoc).(iii) At the nonpermissive temperature for the UL15 mutant protein, the dominant features of the infected cells were that both aggregates of αTIF localized within the nucleus and that αTIF accumulated at the margins of the nucleus in the proximity of the nuclear membranes (Fig. 5d). In this instance, there was little or no overlap in the localization of αTIF and the dense, fluorescent nuclear structures containing UL38 protein and, by extension (as indicated below), other capsid proteins (Fig. 5f). We found no change in distribution of capsid or tegument proteins in cells infected with R2603 (αTIF ts mutant) at the nonpermissive temperature (data not shown).

The localization of capsid proteins differs from that of proteins associated with viral DNA synthesis. Viral proteins associated with DNA replication accumulate in a specific nuclear compartment that is defined by the presence of the viral single-stranded-DNA-binding protein ICP8 (9). We examined the distribution of another protein associated with viral DNA synthesis, the polymerase accessory protein encoded by UL42. Our observations, supported by other colocalization studies described below, are that the viral DNA replication compartment abuts but does not overlap the dense nuclear fluorescent structures containing capsid proteins (Fig. 6a to c). Studies with monoclonal antibody to ICP8 yielded similar results (not shown).

Colocalization of UL15 protein with proteins involved in viral DNA synthesis. Earlier studies have shown that UL15 protein is redistributed from the cytoplasm to the nucleus between 6 and 12 h after infection (1). In this study, the

FIG. 3. Confocal, digital images of HSV-1(F)-infected (G to I) or R7032(gE-)-infected (A to F) Vero cells fixed at 6 h (A and D) or 16 h (B, C, and E to I) postinfection and reacted with antibodies to ICP5 (A to C), to ICP35 (D to F), or to αTIF protein (G to I) and to anti-mouse IgG conjugated to Texas red. The images were captured with software provided by Zeiss with the instrument and printed by a Codonics CP210 printer.

FIG. 4. Confocal, digital images of R7032(gE-)-infected Vero cells fixed and stained 16 h postinfection and double labeled with combinations of antibodies to viral proteins and to either anti-mouse IgG conjugated to Texas red (red fluorescence) or anti-rabbit IgG conjugated to FITC (green fluorescence). Single-color images were captured separately and are shown in the left and middle columns; the two colors were then captured simultaneously and are shown in the right column. The yellow color visualized in the overlaid images (right column) represents colocalization of red and green fluorescence. (a to c) ICP35 (red) and UL143.5 (green); (d to f) UL11 (red) and UL38 (green); (g to i) ICP35 (red) and UL38 (green); (j to l) ICP35 (red) and UL38 (green); (m to o) ICP3 (red) and UL38 (green); (p to r) ICP5 (red) and UL38 (green). The images were captured with software provided by Zeiss with the instrument and printed by a Codonics CP210 printer. The abundance of UL143.5 protein is lower than that of ICP35. Therefore, the image of UL143.5 was digitally enhanced to match that of ICP35.
FIG. 5. Confocal, digital images of HSV-1(mPl66-4 (α lesion in the U15 gene)-infected Vero cells incubated at the permissive (a to c and g to l) or nonpermissive (d to f) temperatures. The cells were fixed at 16 h postinfection and double labeled with combinations of antibodies to viral proteins and with the appropriate secondary antibody conjugated to either Texas red (red fluorescence) or FITC (green fluorescence). Single-color images are represented in the left and middle columns; the right column represents simultaneous acquisition of both colors. The yellow color visualized in the overlaid images (right column) represents colocalization of red and green fluorescence. (a to c and d to f) αIF (red) and U15 (green), respectively; (g to i and j to l) ICP5 (red) and U15 (green), respectively. The images were captured with software provided by Zeiss with the instrument and printed by a Codonics CP210 printer. The fluorescence signal obtained for the individual proteins shown in this figure was adequate and did not require digital enhancement.
FIG. 6. Confocal, digital images of R7032(gE)-infected Vero cells fixed at 16 h postinfection and double labeled with combinations of antibodies to viral proteins and with the appropriate secondary antibody conjugated to either Texas red (red fluorescence) or FITC (green fluorescence). Single-color images are represented in the left and middle columns; the right column represents simultaneous acquisition of both colors. The yellow color in the overlay images (right column) represents colocalization of red and green fluorescence. (a to c) UL42 (red) and UL38 (green); (d to f) ICP35 (red) and UL15 (green); (g to i) ICP35 (red) and gM (green); (j to l) UL42 (red) and UL15 (green). The images were captured with software provided by Zeiss with the instrument and printed by a Codonics CP210 printer. The fluorescence signal obtained for the individual proteins shown in this figure was adequate and did not require digital enhancement.
localization of the U₅₅ protein in cells infected with HSV-1(F) and in cells infected with HSV-1(mP66-4) and maintained at either permissive or nonpermissive temperatures was investigated. We noted two patterns of distribution of U₅₅ protein in the nucleus that appear to be independent of the temperature of incubation. In one pattern, relatively large amounts of protein reactive with the anti-U₅₅ antibody occupied the center of the nucleus [HSV-1(F), Fig. 6e; HSV-1(mP66-4 at 37°C, Fig. 5k)]. In the other pattern, the distribution of U₅₅ protein appears to be more diffuse (e.g., Fig. 5h). In cells in which ICP5 did not localize to the discrete peripheral structures but accumulated in large amounts, filling the nucleus, its distribution overlapped the distribution of the U₅₅ protein (Fig. 5j to l). The more common image is that of localization of the dense nuclear fluorescent structures containing capsid proteins at the periphery and only partially overlapping the compartment containing the U₅₅ protein (e.g., Fig. 5g to i and Fig. 6d to f). The most striking and unexpected observation was that U₅₅ protein colocalized with the proteins associated with viral DNA synthesis (e.g., U₄₂ [Fig. 6j to l]).

**DISCUSSION**

In a recent paper, we reported that the product of U₅₅, a newly discovered open reading frame, colocalizes with the capsid protein ICP35 (Fig. 4a to e) in nuclear compartments which we have described as dense fluorescent nuclear structures (44). A central and intriguing question was whether this association was unique to the proteins tested or, rather, reflected a nuclear compartmentalization of the various functions associated with viral morphogenesis: DNA synthesis, capsid assembly, packaging of DNA, and envelopment. To investigate the possibility that a functional compartmentalization exists, we prepared antibody to one additional capsid protein, VP19C (15, 42), encoded by U₃₈ (5, 21). We then analyzed the localization of three capsid proteins (ICP35, ICP5, and U₃₈), two tegument proteins (U₄₂ and αTIF), proteins involved in viral DNA synthesis (ICP8 [results not shown] and the product of the U₄₂ gene) and packaging (U₅₅ [1, 26]), and a component of the virion envelope glycoprotein M, the product of the U₁₀ gene (2). We also asked whether this compartmentalization occurred in the absence of certain viral functions, i.e., DNA packaging and virion maturation, by examining cells infected with mutant viruses carrying ts mutations in either the U₅₅ (Fig. 5) or the αTIF (data not shown) genes and maintained at nonpermissive temperatures.

We have identified compartments of proteins in which three distinct functions involved in viral assembly appear to occur. These compartments contain capsid proteins, proteins involved in DNA synthesis, and tegument proteins. We have recently shown that after the onset of DNA synthesis, ICP4 aggregates into dense structures (17), which form a compartment different from those described here. The salient features of our studies which merit discussion are as follows. (i) The capsid compartment is defined by the coalescence of ICP5, ICP35, and VP19C (U₃₈) into the dense nuclear structures, which occurs late in infection in most cells. These structures were located at the periphery of the nucleus separate from nuclear membranes and did not significantly overlap the compartments containing proteins involved in viral DNA synthesis. (ii) U₅₅ protein, generally associated with viral DNA cleavage and packaging, colocalized with the DNA polymerase accessory protein U₄₂ and ICP8 (not shown) but not with the dense nuclear bodies containing capsid proteins. (iii) The predominant distribution of the tegument proteins αTIF and U₅₅ was in asymmetrically arranged masses near the periphery of the nucleus, adjacent to or partially overlapping with the dense nuclear structures containing capsid proteins, although the distribution of the two proteins varied somewhat from cell to cell. We did not observe significant differences in the compartmentalization of the proteins studied here in cells in which either DNA packaging was abrogated (U₅₅ ts mutant) or virions did not mature (αTIF mutant).

The hypothesis that the dense, strongly fluorescent structures contain both protein forming immature capsids and the immature capsids themselves, in addition to any mature capsids which may also be present, is supported by two observations: (i) the presence of large amounts of ICP35 protein within these structures and (ii) the formation of these structures in infected cells under conditions in which immature capsids (lacking viral DNA) are made, viral DNA accumulates in the form of concatemers, but the DNA is not cleaved from concatemers and packaged into the preformed capsids (1, 26). At the time points investigated in this report, infectious virus accumulates exponentially, and we assume that this represents the time of maximum assembly of capsids. “Dense fluorescent nuclear structures,” albeit descriptive, is an unsatisfactory term for these structures, and we propose to designate them assemblons, i.e., bodies in which proteins involved in capsid assembly aggregate and most probably assemble.

The arrangement of assemblons in the periphery of the nucleus surrounding and abutting the compartment containing the proteins involved in viral DNA synthesis suggests the existence of a machinery which feeds upon (i.e., captures, packages, and cleaves) viral DNA generated within the DNA synthesis compartment. The distribution of U₅₅ protein raises some interesting issues. U₅₅ bears partial homology to the bacteriophage terminase involved in cleavage and packaging of phage DNA (7, 29, 32), and analyses of a ts mutant virus revealed the requirement for functional U₅₅ protein for cleavage and packaging of HSV-1 DNA into capsids (1, 26). Because it is generally thought that cleavage of newly synthesized viral DNA occurs concurrently with its packaging (reviewed in reference 36), it could have been predicted that U₅₅ would colocalize with immature capsids or with structures associated with capsid assembly. Thus, the colocalization of U₅₅ protein with proteins involved in viral DNA synthesis and, conversely, the virtually complete exclusion of U₅₅ protein from the assemblons were unexpected. Our results suggest that U₅₅ becomes associated with DNA-protein complexes prior to packaging and that it may even be an accessory component of the machinery which synthesizes viral DNA.

In contrast, and with few exceptions, the two tegument proteins investigated in this study (e.g., Fig. 5d to f) were localized closer to nuclear membranes than were the assemblons. We have not resolved the question of whether tegument proteins bind to capsids during capsid assembly or at the time of envelopment. Both U₅₅ and αTIF are abundant virion proteins; however, our results suggest that the assemblons do not contain large amounts of U₅₅ or αTIF as might be expected if tegument proteins bound to capsids within these structures.

The conclusions drawn from these studies have generated a number of questions. In principle, we could have expected that assembly of capsids in the nucleus would occur at random but that the capsids would move radially to the nuclear membrane. The fact that the capsid proteins accumulate in assemblons suggests that assembly is facilitated by and is dependent on protein concentration. This raises the question of the mechanisms which result both in the coalescence of the capsid proteins into assemblons and in the segregation of proteins involved in DNA synthesis and those forming the tegument.
An additional, equally intriguing question concerns the relationship between the various viral compartments and the electron-dense bodies which are readily apparent upon electron-microscopic examination of thin sections of infected cells (31, 40). Progress in identification of the component of these bodies will require the development of methods which enable penetration of antibodies into cells without distortion of the nuclear architecture.

ACKNOWLEDGMENTS

We thank Lindsay Smith for excellent technical assistance and A. Minson and D. Tenney for gifts of the anti-α-TIF and anti-U142 antibodies, respectively. These studies were aided by grants from the National Cancer Institute (CA47541) and the National Institute for Allergy and Infectious Diseases (AI124009). U.S. Public Health Service.

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