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Electron Microscope Studies of Temperature-Sensitive Mutants of Herpes Simplex Virus Type 2

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Nine temperature-sensitive mutants of herpes simplex virus type 2 representing eight complementation groups were assigned to two classes as a consequence of the virion forms and virus-specific cellular alterations observed in thin sections of mutant-infected human embryonic lung cells grown at the nonpermissive temperature. Mutants in class A, one DNA^- and one DNA^+, failed to synthesize detectable virus particles. Mutants in class B, 4 DNA^- and 3 DNA^+, produced moderate to large numbers of empty nucleocapsids. Dense-cored nucleocapsids were not observed in thin sections of cells infected with any of the nine mutants at this temperature. Virus-specific cellular alterations consisted primarily of margination of chromatin and nuclear membrane thickening and duplication.

The application of temperature-sensitive (ts) mutants with defects in various stages of replication as tools for the elucidation of the sequence of events in viral assembly has been well established. Previous reports from this laboratory have described the isolation of ts mutants of herpes simplex virus type 1 (HSV-1) and the classification of 22 of these mutants into 15 complementation groups (12). An ultrastructural description of defects of virion morphology, assembly, and maturation exhibited by mutants representing each of these complementation groups in thin sections at the nonpermissive temperature has been published (13). This study demonstrated that the mutants varied in their ability to synthesize viral nucleocapsids, in the kinds of nucleocapsids synthesized, and in their ability to produce mature enveloped virions. Thus, these mutants are apparently blocked in various stages of virus assembly and maturation at the nonpermissive temperature.

Further investigations have dealt with the isolation, complementation, and phenotypic characterization of ts mutants of herpes simplex virus type 2 (HSV-2), in which nine ts mutants were classified into eight complementation groups (1, 4).

In the present report we describe the results of thin-section analysis of HSV-2 ts mutant-infected cells maintained at the nonpermissive temperature. This study demonstrates that mutants representing eight complementation groups differ markedly from each other, from the wild-type virus, and from ts mutants of HSV-1 with regard to the products of morphogenesis at this temperature.

MATERIALS AND METHODS

Cell cultures and media. Human embryonic lung fibroblasts, strain 670, in passage 9, were grown in 8-ounce (ca. 0.24-liter) prescription bottles or 60-mm petri dishes using Eagle medium supplemented with 10% fetal bovine serum and 0.075 or 0.225% $NaHCO_3$ for stoppered vessels or plates, respectively. They were maintained in the same medium containing 5% fetal bovine serum and 0.150 or 0.225% $NaHCO_3$ for bottles or plates.

Virus and virus assays. HSV-2, strain 186 (11), and nine HSV-2 ts mutants derived from strain 186 were used in this investigation. The mutants, assigned to eight complementation groups, were isolated and characterized as described previously at permissive (pT°) and nonpermissive (npT°) temperatures of 34 and 38 C, respectively (1, 4). Preparation of virus stocks (2, 13) and infectivity assays (3) were conducted in accordance with previous reports. Dilution of virus suspensions and washing of monolayers were performed using Tris phosphate buffer at pH 7.4 containing 1% fetal bovine serum. Cultures in bottles were incubated in water baths (Blue M. Electric Co., Blue Island, Ill.) having temperature variations of ±0.1 C, whereas petri dishes were incubated in humidified CO_2 (5%) incubators (Wedco Inc., Silver Spring, Md.) with temperature variations of ±0.2 C.

Infection of monolayers. Subconfluent monolayers in 8-ounce prescription bottles containing approximately 3×10^6 cells were inoculated with 1 ml of cell-free wild-type (WT) or ts mutant virus containing 5 PFU/cell. Replicate cultures were incubated for 1 h at 37 C to allow for virus adsorption. Cultures were then washed twice and incubated with 10 ml of fresh maintenance medium at 34 and 38 C in water baths. At the indicated times, cells were scraped into the medium. For infectivity assay a 1.5-ml sample of infected cell suspension was frozen, thawed, and then sonicated for 1 min at 10 kcycles/s with a

Raytheon sonic oscillator. After clarification of the suspension by low-speed centrifugation at 4 C, the supernatant fluid was assayed at 34 C to determine the total yield of infectious virus and at 38 C to determine the yield of ts$^+$ revertant virus as previously described (13). The remaining cell suspension was processed for electron microscopy as described below.

Electron microscopy. Cell suspensions were centrifuged at 1,500 rpm at 4 C, resuspended in cold (4 C) Tris, and centrifuged again. The resultant pellets were fixed in cold 2.5% glutaraldehyde buffered with Sorensen solution, pH 7.2. Pellets were washed, postfixed, dehydrated, and embedded as previously described (5). Thin sections were examined in a Hitachi HU-11B electron microscope operating at an accelerating voltage of 75 kV.

RESULTS

Growth of WT virus at pT° and npT°. The replicative cycle of the WT virus in human embryonic lung cells grown at the pT° and npT° was examined before ultrastructural studies of ts mutants were initiated. For this purpose, WT virus-infected and control, mock-infected cultures were harvested, processed, assayed for infectious virus, and examined by thin-section analysis at intervals from 0 through 50 h post-infection (p.i.). Conditions of one-step infection were achieved when cultures were inoculated with 5 PFU/cell (Fig. 1). Progeny virus was first evident at approximately 6 p.i. and continued to be synthesized through 24 h at 38 C and through 50 h at 34 C. Titers of virus at 24 h were 6×10^7 PFU/ml at 38 C and 8×10^7 PFU/ml at 34 C. The growth curves of WT HSV-2 at the two temperatures differed in two respects. (i) Virus was produced more rapidly at 38 C between 7 and 13 h p.i. as compared to 34 C; and (ii) at 38 C production of infectious virus decreased after 24 h p.i.—presumably due to inactivation at this temperature—whereas virus production at 34 C continued to increase slightly.

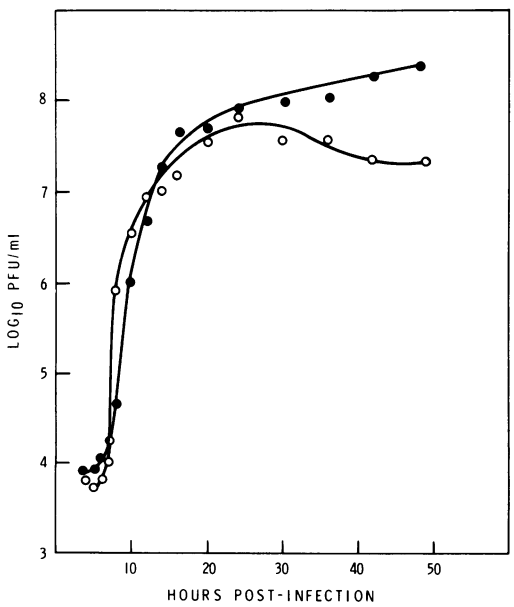

FIG. 1. *Growth curve of HSV-2 (strain 186) WT virus in monolayer cultures of human embryonic lung fibroblasts. Cells were infected at a multiplicity of 5 PFU/cell, and virus infectivity was assayed as described in Materials and Methods. Symbols: ●, infectivity at the permissive temperature (34 C); ○, infectivity at the nonpermissive temperature (38 C).*

Thin-section analysis of cells infected with WT virus and maintained at 34 C demonstrated the presence of naked and enveloped viral particles from 7 through 30 h p.i. (Table 1). The relative number of virus particles per nucleus was maximum from 12 through 16 h p.i., although large numbers continued to be observed after that period. Sections of WT virus-infected cells incubated at 38 C, on the other hand, contained naked viral particles in 25% of nuclear profiles examined as early as 5 h p.i., whereas enveloped particles were not observed until 3 h later. The maximum relative number of virus particles per nucleus at 38 C preceded that at 34 C by 2 to 4 h. Large numbers of intranuclear particles were produced, nevertheless, through 20 h p.i. Prominent clearing of nuclei and occasional aberrant nucleocapsids were observed in sections of cells cultured after 20 h p.i.; consequently, accurate quantification of virus particles was not possible at either temperature after this time.

On the basis of these data, an incubation period of 18 h was selected for examination of ts mutants, since approximately equal infectivity titers were produced at both 34 and 38 C at this time and because thin-section analysis demonstrated that all cells infected with the WT virus and incubated at 34 or 38 C contained both naked and enveloped viral particles in large and nearly equal numbers (Table 1).

Furthermore, growth curves of the WT virus and the mutants at 34 C have been shown to be similar in both the time of first appearance of infectious virus and in the time at which maximum yields were reached (unpublished data). No adjustments in the time of harvest were, therefore, made for any of the mutants.

Yields of infectious WT and ts mutant virus after 18 h at pT° and npT°. Yields of infectious virus from human embryonic lung cell cultures infected with the WT virus and with the nine ts mutants at the pT° and npT° after incubation for 18 h are shown in Table 2. At 34 C yields of four of five DNA$^-$ mutants and one DNA$^+$ mutant (tsF3) approximated those of the WT virus, whereas three of four DNA$^+$ mutants produced

TABLE 1. *Quantification of virus particles at different time intervals in thin sections of cells infected with the WT virus at 34 and 38 C*

Time p.i. (h)	% Cells with virus particles		% Cells with naked viral particles[a]		% Cells with enveloped viral particles[a]		Relative no. of virus particles per nucleus[b]	
	34 C	38 C	34 C	38 C	34 C	38 C	34 C	38 C
4	0	0	0	0	0	0	0	0
5	0	25	0	25	0	0	0	1+
6	0	50	0	50	0	0	0	1+
7	100	100	100	100	33	0	2+	2+
8	100	100	100	100	50	40	3+	4+
10	100	100	100	100	100	100	3+	4+
12	100	100	100	100	100	100	4+	3+
14	100	100	100	100	100	100	4+	3+
16	100	100	100	100	100	100	4+	3+
20	100	100	100	100	100	100	3+	3+
24	100	100	100	100	100	100	3+	ND[c]
30	100	100	100	100	100	100	3+	ND

[a] Percentage of positive cells observed in 50 profiles examined.
[b] Numbers of particles per nucleus were graded as follows: 4+, >50; 3+, 20-50; 2+, 10-20; 1+, 1-9.
[c] ND, Not done.

TABLE 2. *Yields of infectious virus from cultures infected with the WT virus and ts mutants at 34 and 38 C*

Mutant class	Virus	Viral DNA phenotype[a] (38 C)	Infectious virus[b] (PFU/ml)	
			34 C	38 C
A	WT	+	6×10^7	1×10^8
	tsC2	−	4×10^7	2×10^3
	tsD6	+	2×10^7	2×10^3
B	tsA1	−	5×10^7	6×10^3
	tsA8	−	5×10^7	6×10^3
	tsB5	−	8×10^7	1×10^4
	tsH9	−	2×10^7	3×10^3
	tsE7	+	1×10^7	7×10^3
	tsF3	+	4×10^7	1×10^4
	tsG4	+	1×10^7	2×10^4[c]

[a] Viral DNA phenotypes from Esparza et al. (4).
[b] Mutants were grown at 34 and 38 C; assays were performed at 34 C.
[c] 10^3 PFU of ts$^+$ revertant virus per ml was present in the yield of tsG4 at 38 C.

TABLE 3. *Quantification of virus particles in thin sections of cells infected for 18 h with the WT virus and ts mutants at 38 C*

Mutant class	Virus	Viral DNA phenotype (38 C)	% Cells with naked virus particles[a]	% Cells with enveloped virus particles[a]	Relative no. of virus particles per nucleus[b]
A	WT	+	100	100	4+
	tsC2	−	0	0	0
	tsD6	+	0	0	0
B	tsA1	−	80	0	2+
	tsA8	−	75	2[c]	2+
	tsB5	−	60	0	2+
	tsH9	−	30	0	1+
	tsE7	+	90	0	3+
	tsF3	+	90	3[c]	3+
	tsG4	+	80	0	2+

[a] Percentage of positive cells observed in 75 to 100 profiles examined.
[b] Numbers of particles per nucleus were graded as follows: 4+, >100; 3+, 20-50; 2+, 10-20; 1+, 1-9.
[c] No enveloped particles were seen at the cell surface of these cells. Enveloped forms were observed in the cytoplasm and at the nuclear membrane.

slightly less infectious virus. At the higher temperature, however, all mutants produced significantly less virus, with titers approximately 10^{-4} that of the WT virus. Leaky (ts) virus was produced by all mutants, and one mutant (tsG4) yielded 10^3 PFU of revertant (ts$^+$) virus per ml. The absence of ts$^+$ revertant virus in the progeny of eight of nine mutants and the low revertant yield found with tsG4 substantiate that ultrastructural observations were indicative of replicative events of ts mutant virus and not of ts$^+$ revertant virus.

Electron microscope observations. Quantification of virus particles and evaluation of virus forms and specific cellular alterations in thin sections were performed after examination of 75 to 100 cell profiles of each sample.

Cells infected for 18 h with the WT virus and incubated at 34 and 38 C and cells infected with ts mutants at 34 C demonstrated little qualitative difference.

(i) WT virus. The relative number of virus particles per nucleus in WT virus-infected cells grown at 18 h at the npT° was significantly higher than that of ts mutant-infected cells (Table 3). Characteristic features of WT virus-infected cells in thin sections included thickening and duplication of the nuclear membrane (Fig. 2A and B) and condensation and margination of chromatin (Fig. 2A and C). (A better representation of the pattern of marginated chromatin

observed in WT virus-infected cells is demonstrated in Fig. 4A, which illustrates a ts mutant-infected cell.) Intranuclear fibrillar bundles (Fig. 2D) were also observed in WT virus-infected cells (Table 4) but not in mock-infected cells. Fine-grained nuclear inclusions were also present, distributed either as single electron-dense round masses or as irregular anastomosing arrays (Fig. 3A). All nuclear profiles contained coreless or empty nucleocapsids, capsids surrounding a 45- to 50-nm, ring-shaped core (partial capsids), and capsids containing dense cores (i.e., presumably containing viral DNA) (Fig. 3B). Occasionally, particles without dense

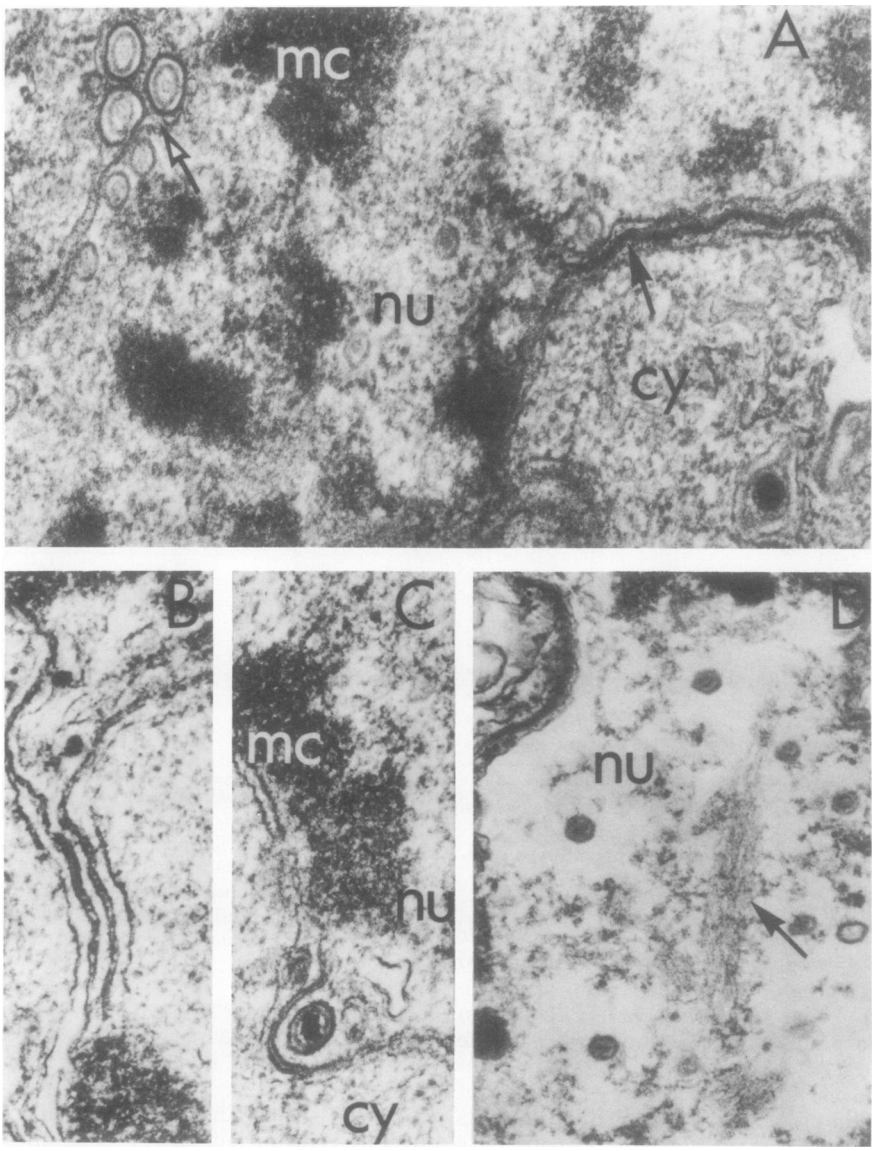

FIG. 2. *Human embryonic lung cells infected with the WT virus after 18 h at 38 C. (A) Nucleus (nu) and cytoplasm (cy) of infected cell demonstrating margination of chromatin (mc) and thickening of nuclear membrane (closed arrow). Note the presence of partial nucleocapsids adjacent to and budding from the nuclear membrane (open arrow). An enveloped nucleocapsid with a dense core is seen in the cytoplasm. (B) Duplication of the nuclear membrane. (C) Nucleus (nu) and cytoplasm (cy) of infected cell showing partially enveloped dense-cored capsid and margination of chromatin (mc). (D) Nucleus (nu) of infected cell showing fibrillar bundle (arrow). ×59,800.*

TABLE 4. *Virion forms and virus-specific alterations in cells infected with WT virus and ts mutants at 38 C*[a]

MUTANT CLASS	VIRUS	VIRAL DNA PHENOTYPE 38°	NUCLEUS: FIBRILLAR BUNDLES	NUCLEUS: RING-LIKE COMPONENTS	NUCLEUS: MEMBRANE THICKENING	NUCLEUS: MEMBRANE DUPLICATION	NUCLEUS: "PERICHROMATIN-LIKE" GRANULES	NUCLEUS: NUCLEOCAPSIDS EMPTY/PARTIAL	NUCLEUS: NUCLEOCAPSIDS DENSE	CYTOPLASM AND/OR EXTRACELLULAR SPACE: ENVELOPED NUCLEOCAPSIDS EMPTY/PARTIAL	CYTOPLASM AND/OR EXTRACELLULAR SPACE: ENVELOPED NUCLEOCAPSIDS DENSE
	WT	+	+	+	+	+	+	+ +	+	±	+
A	ts C2	-	-	-	+	-	+ +	-	-	-	-
	ts D6	+	-	-	±	-	+	-	-	-	-
B	ts A1	-	-	-	+	±	+	+	-	±	-
	ts A8	-	-	-	+	-	+	+	-	±	-
	ts B5	-	-	-	+	-	+ +	+	-	-	-
	ts H9	-	-	-	-	-	+	+	-	-	-
	ts E7	+	-	-	-	-	-	+ +	-	-	-
	ts F3	+	-	-	+	±	-	+ +	-	±	-
	ts G4	+	-	-	-	-	-	+	-	-	-

[a] Evaluation based on the frequency of virion forms and the degree of alteration observed in virus-positive cells only: −, absent; ±, present in small amounts or numbers; +, present in moderate amounts or numbers; + +, present in large amounts or numbers.

cores were observed budding from the inner nuclear membrane (Fig. 2A). Ring-like intranuclear components 25 to 27 nm in diameter, similar to those described previously for HSV-1 (13), were also observed. Cytoplasmic alterations included vacuolization and formation of concentric arrays of membranous cisternae. Numerous enveloped dense-cored particles were seen in most WT-infected cells (Fig. 3C). In addition, moderate numbers of virions were localized at the cell surface and in the extracellular space (Fig. 3D).

(ii) Ts mutants. The nine mutants examined were subdivided into two classes as a consequence of (i) the cellular alterations they induced and (ii) their ability to synthesize viral particles at 38 C (Tables 3 and 4). Class A mutants included one DNA^- mutant, tsC2, and one DNA^+ mutant, tsD6. These mutants were maximally defective, at least at 18 hours p.i., since cells infected with them and maintained at 38 C exhibited few virus-induced alterations (Table 4; Fig. 4A). Cell profiles of class A mutant-infected cultures contained no detectable viral particles. Although nuclear membrane thickening was present to a moderate degree in tsC2-infected cells (Fig. 4A) and, to a lesser extent, in tsD6-infected cells, nuclear membrane duplication and intranuclear fibrillar bundles were not observed. Margination of chromatin was observed in nuclei of cells infected with both mutants.

The remaining seven mutants, four DNA^- (tsA1, tsA8, tsB5, and tsH9) and three DNA^+ (tsE7, tsF3 and tsG4), were assigned to class B. The incidence of class B mutant-infected cells containing virus particles varied from 30% for tsH9 to 90% for tsE7 and tsF3 (Table 3). Generally speaking, the greater the number of cells containing virus particles, the greater the number of particles observed per nucleus. Cells in which 90% of nuclear profiles contained naked viral particles (i.e., tsE7- and tsF3-infected cells) contained 20 to 50 particles per nucleus, and cells in which 60 and 75% of nuclear profiles contained naked viral particles (i.e., tsB5- and tsA8-infected cells, respectively) contained 10 to 20 particles per nucleus. In comparison, cells infected with tsH9 contained naked particles in only 30% of nuclei and each nucleus contained fewer than 10 particles (Table 3).

Nuclear membrane modifications were prominent features of cells infected with different mutants of class B. Thickening of the nuclear membrane occurred in cells infected with three DNA^- mutants (i.e., tsA1, tsA8, and tsB5) (Table 4) and with one DNA^+ mutant, tsF3 (Table 4). Whereas tsD6 (class A)-, tsA1-, tsA8-, and tsB5-infected cells exhibited only short segments of membrane thickening, in cells infected with tsF3 the thickening exceeded that of any of the other mutant-infected cells and resembled changes observed in WT virus-infected cells (Fig. 4B). Membrane duplication, a prominent feature of cells infected with WT virus, was limited to small segments of the nuclear membrane in cells infected with tsA1 and tsF3. Like mutants in class A, intranuclear fibrillar bundles were not observed in any of the cells infected with class B mutants.

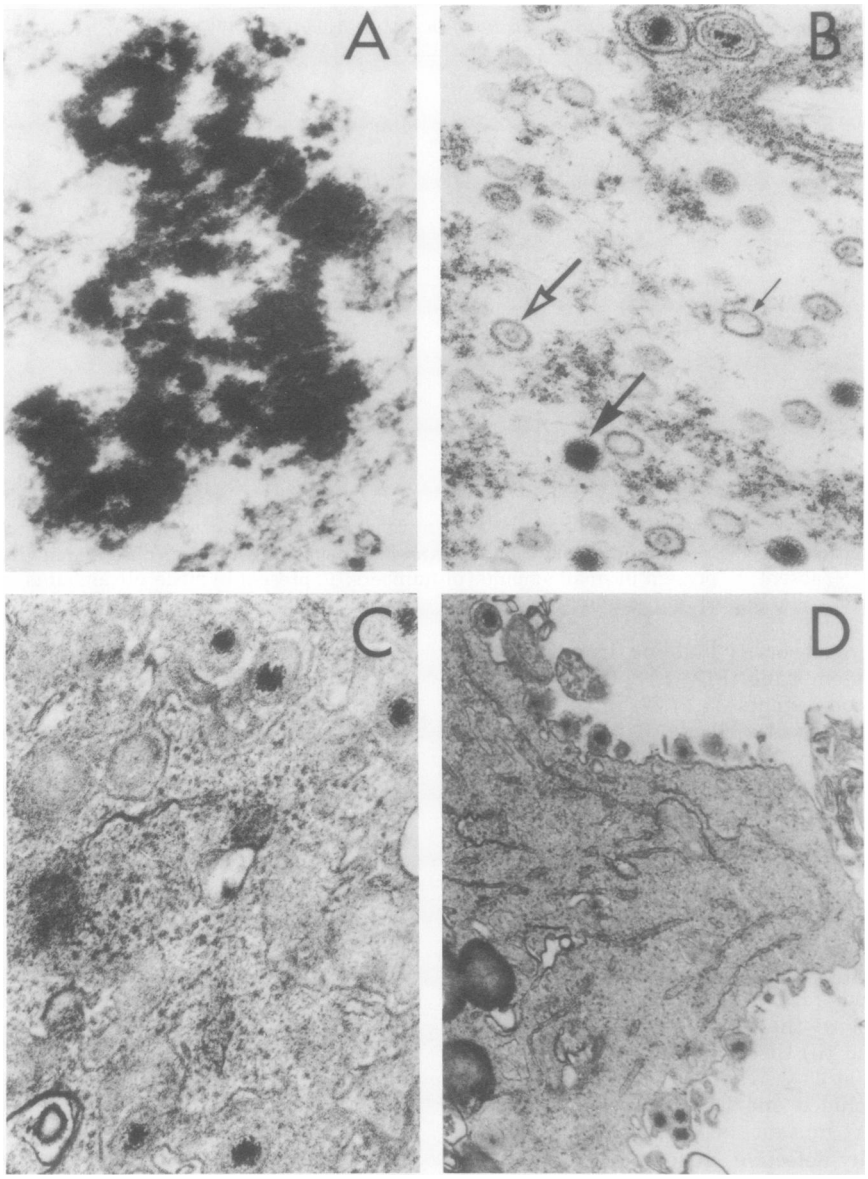

FIG. 3. *Human embryonic lung cells infected with WT virus after 18 h at 38 C. (A) Irregular anastomising inclusion body (ar) found within the nucleus (nu). Note the presence of marginated chromatin (mc). (B) Nucleocapsids with dense cores (long arrow), partial cores (short arrow), and empty capsids (open arrow) in the nucleus. (C) Enveloped nucleocapsids with dense cores present in the cytoplasm. (D) Enveloped nucleocapsids with dense cores are seen at the cell surface and in the extracellular space. (A–C) ×59,800; (D) ×27,000.*

Nuclei of cells infected with five of the class B mutants contained small to moderate numbers of empty and partial nucleocapsids (Table 4). The nuclei of tsF3 and tsE7 mutant-infected cells, however, included large numbers of predominantly partial nucleocapsids (Fig. 4C). In no case were nucleocapsids containing dense cores observed. In instances where capsids containing partial cores were adjacent to the nuclear membrane (e.g., tsA8) thickening of the membrane and budding of particles were occasionally observed (Fig. 5A). Cytoplasmic alterations, in comparision to WT-infected cells, were minor. In three cases (tsA1-, tsA8-, and tsF3-infected cultures) enveloped particles containing empty nucleocapsids were located in

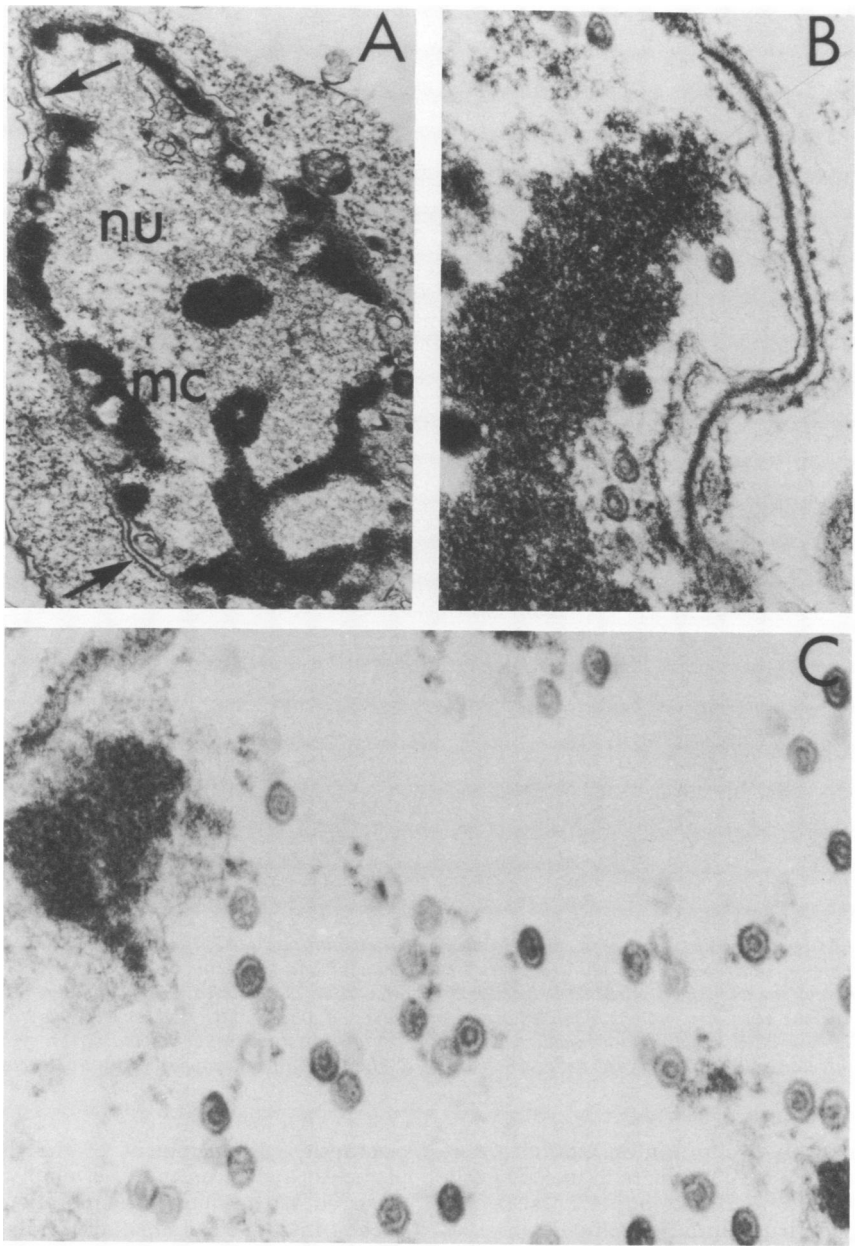

FIG. 4. *(A) Human embryonic lung (HEL) cell infected with class A mutant tsC2 after 18 h at 38 C. Note the presence of marginated chromatin (mc) and thickened segments of nuclear membrane (arrows) and the absence of viral nucleocapsids in the nucleus. ×10,000. (B) HEL cell infected with class B mutant tsF3 after 18 h at 38 C showing extensive thickening of the nuclear membrane. ×59,800. (C) HEL cell infected with class B mutants tsF3 after 18 h at 38 C. Note nucleus containing relatively large amounts of nucleocapsids containing partial cores. ×59,800.*

the cytoplasm of cells exhibiting intact nuclear membranes (Fig. 5B and C).

A prominent feature of cells infected with DNA$^-$ mutants in both class A and class B was the presence of round, 50- to 60-nm "perichromatin-like" intranuclear granules (Table 4; Fig. 6). These were especially numerous in cells infected with tsB5, a class B mutant, and tsC2, a class A mutant. Nuclei of cells infected with class B mutants tsA1, tsA8, and tsH9 contained

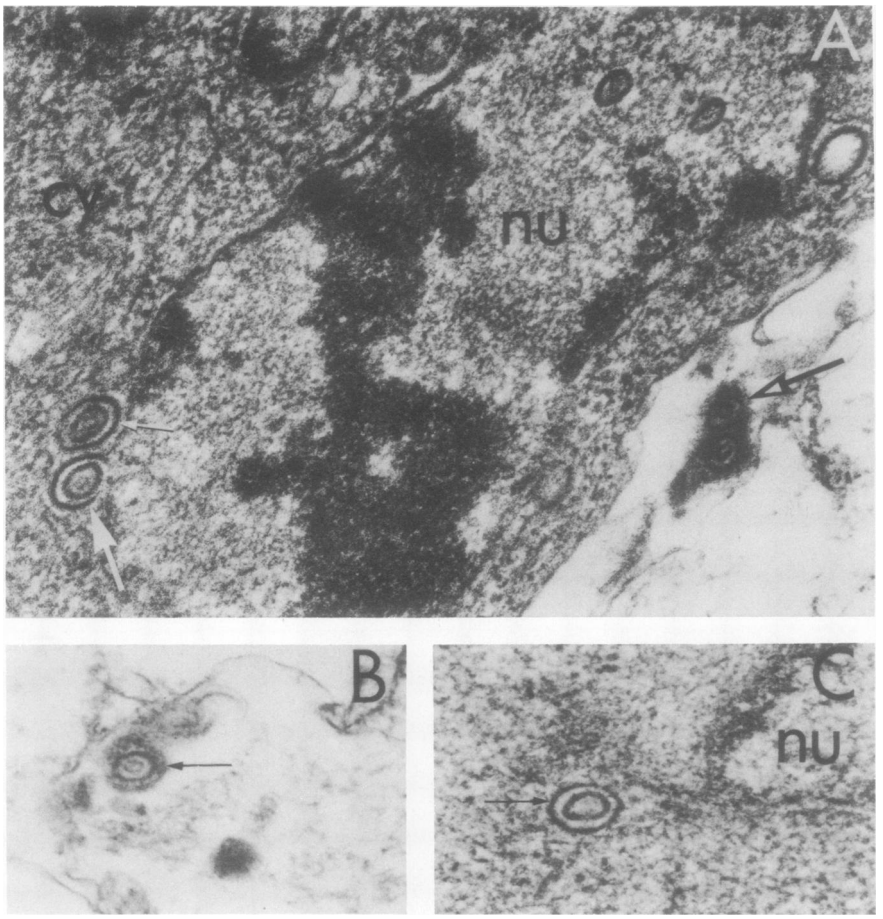

FIG. 5. *(A) Human embryonic lung (HEL) cell infected with mutant tsA8 after 18 h at 38 C. Nucleus (nu) and cytoplasm (cy) of infected cell show a partial nucleocapsid budding from the nuclear membrane (long arrow), whereas an enveloped partial nucleocapsid (short arrow) is localized within the perinuclear space. Two extranuclear enveloped naked particles are also seen (open arrow). (B) HEL cell infected with mutant tsA8 after 18 h at 38 C showing enveloped nucleocapsid containing a partial core localized in cytoplasm. (C) HEL cell infected with mutant tsA1 after 18 h at 38 C showing an enveloped empty nucleocapsid in the cytoplasm. ×59,800.*

moderate numbers of granules. Granules were also present in small numbers in nuclei of cells infected with one DNA$^+$ mutant, tsD6, and with the WT virus. Granules were not observed in nuclei of cells infected with three DNA$^+$ mutants (i.e., tsE7, tsF3, or tsG4) or in nuclei of mock-infected cells.

DISCUSSION

The sequence of events observed in HSV-infected cells has been reported in detail (6–9, 16). In cells infected in this study with strain 186 of HSV-2 and maintained at both pT° and npT°, thickening and duplication of nuclear membrane were accompanied by the intranuclear appearance of empty and dense-cored nucleocapsids. Envelopment of the dense-cored particles, and occasionally of empty particles, occurred at the nuclear membrane. The presence of intranuclear fibrillar bundles was also observed.

Although they resembled WT virus-infected cells at the pT°, the HSV-2 mutants examined in the present investigation exhibited all forms of nuclear virus at the npT° except that of dense-cored particles (i.e., those containing DNA). Unlike the study with ts mutants of HSV-1 (13), neither previously undescribed intranuclear forms nor aberrant nucleocapsids were seen in cells infected with the HSV-2 mutants at the npT°. (It should be pointed out, however, that thin-section analysis was per-

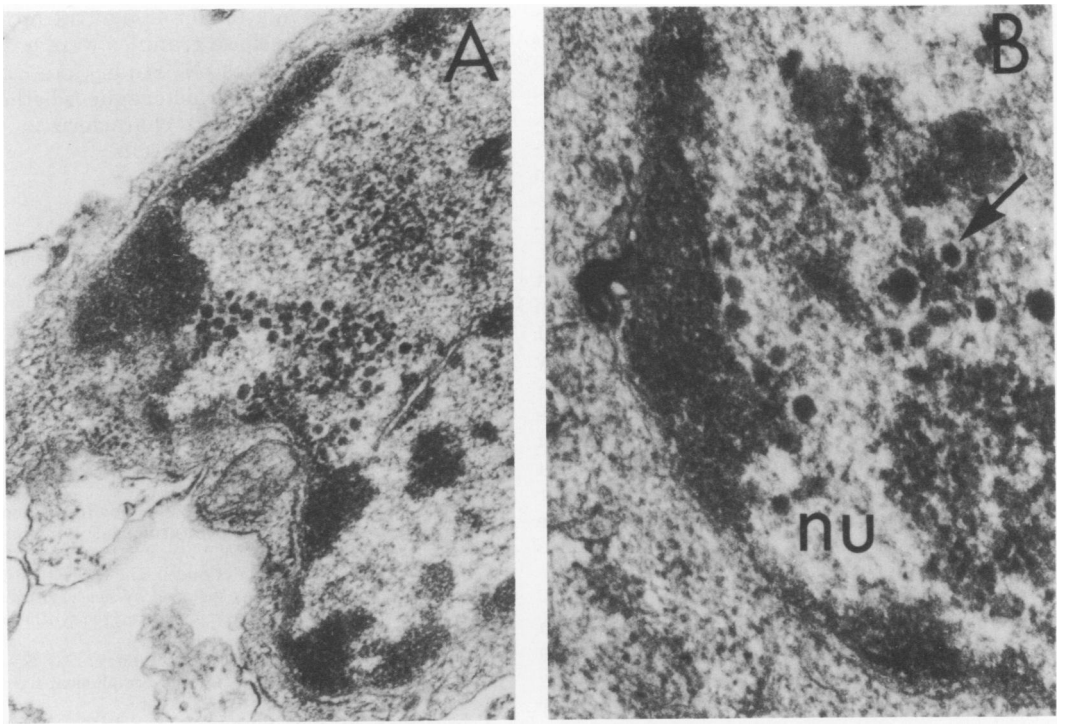

FIG. 6. *Human embryonic lung cells infected with DNA⁻ mutant tsB5 after 18 h at 38 C. (A) Infected cell containing numerous "perichromatin-like" granules within the nucleus (nu; arrow). ×25,600. (B) Nucleus of infected cell demonstrating the presence of a clear "halo" (arrow) separating the granule from the surrounding nucleoplasm. ×76,500.*

formed at a later time in the HSV-1 study.) Furthermore, all cellular changes and virus particle forms were also observed in WT virus-infected cells. These observations suggest that the virus forms observed at the npT° represent actual intermediates in the replicative cycle of HSV-2 or possibly defective particles resulting from aberrant assembly.

No apparent correlation was observed between the viral DNA phenotype and the presence of physical particles in HSV-2 mutant-infected cells grown at 38 C. Although large numbers of empty and partial nucleocapsids were found in nuclei of cells infected with two DNA^+ mutants (tsE7 and tsF3), those of cells infected with one DNA^+ mutant (tsG4) and four DNA^- mutants (tsA1, tsA8, tsB5, and tsH9) contained moderate numbers of particles. In addition, the two class A mutants, one DNA^+ and one DNA^-, failed to produce observable virus particles. All of the mutants, however, induced the synthesis of viral-specific antigens at the npT° as determined by immunofluorescence (4) and polyacrylamide gel electrophoretic analysis (10).

Therefore, synthesis of viral proteins and their subsequent assembly does not appear to depend upon viral DNA synthesis. These findings are in agreement with those of Nii et al. (9) and of Schaffer et al. (13), for HSV-1, who suggested that the information required for the synthesis of HSV structural proteins is read from the parental genome when viral DNA synthesis is inhibited.

Dense-cored particles were not observed in any of the mutant-infected cells at the npT°. The absence of dense-cored nucleocapsids in DNA^+ mutant-infected cells suggests that DNA encapsidation did not occur and that these mutants are blocked in an early step in maturation preceding encapsidation of the viral genome. In addition, envelopment of empty and partial nucelocapsids, observed previously by Smith and De Harven (15) and Schaffer et al. (13), appears not to depend on DNA encapsidation, since enveloped empty core particles were localized in the cytoplasm of cells infected with tsF3, a DNA^+ mutant, and with tsA1 and tsA8, DNA^- mutants.

Moderate to large accumulations of "perichromatin-like" granules were present in nuclei of cells infected with each of the DNA^-

mutants and grown at 38 C. These inclusions were also present in small numbers in cells infected with WT virus and with one DNA$^+$ mutant, tsD6. The granules have been regarded by others as precursors to viral nucleoids (14). The presence of a clear "halo" surrounding these densities and their 50- to 60-nm diameter have led some investigators to suggest that they may be identical to the perichromatin granules found in uninfected cells (16). That these inclusions were especially abundant in DNA$^-$ mutant-infected cells and absent from mock-infected cells at the npT° suggests that they are not viral DNA and that they may represent accumulations of viral or cellular proteins, or extranuclear RNA. Since each of the four DNA$^-$ mutants is defective in a different cistron, the absence of viral DNA itself and not a defect in a specific viral gene may account for the appearance of these granular clusters. The nature and composition of the inclusions are currently being investigated.

Similarities and differences between cells infected at the npT° with ts mutants of HSV-2 in these studies and with ts mutants of HSV-1 (13) are noteworthy. Certain mutants of both types exhibited thickening and duplication of nuclear membranes and the presence of empty and partial nucleocapsids in nuclei. The relative numbers of capsids per nucleus profile were higher in HSV-1 mutant-infected cells. Dense-cored capsids, however, were observed only in cells infected with six DNA$^+$ mutants of HSV-1. Consequently, all the HSV-2 mutants used in this study were apparently blocked at 38 C in a stage of assembly that precedes encapsidation of the viral genome. In addition, aberrant nucleocapsids present in cells infected with five different mutants of HSV-1 were not observed in any of the HSV-2 WT- or mutant-infected cells. Although mutants in four HSV-2 complementation groups possess thermolabile virion structural components, these components are either (i) not associated with the capsid or (ii), if they do represent capsid components, their assembly is not visibly defective at 38 C.

Nucleocapsids were present in nuclei of HSV-1 DNA$^-$ mutant-infected cells, a finding in agreement with the observations presented in this communication. The presence of capsids in both HSV-1 and HSV-2 DNA$^-$ mutant-infected cells suggests that DNA synthesis is not required for nucleocapsid assembly. With regard to "perichromatin-like" granules, it was not possible to determine accurately whether they were present in nuclei of HSV-1 mutant-infected cells since that investigation was conducted using cells infected for 48 h at the npT°; in the present study these granules were present only at earlier times p.i. Studies are currently being conducted to determine whether these granules are indeed characteristic of HSV-1 DNA$^-$ mutant-infected cells.

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