Ultrastructural Characterization of Herpes Simplex Virus Type 1 (Strain 17) Temperature-sensitive Mutants

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SUMMARY

We report the ultrastructural features associated with wild-type and temperature-sensitive (ts) mutant infection at non-permissive temperature for each of 18 herpes simplex virus type 1 (HSV-1) (strain 17) ts mutants. The mutants were classified by their ability to induce nucleocapsid-related structures: class I failed to induce any, class II induced empty and partially filled structures while class III induced all of the identified structures including those containing an electron-dense core. The time when expression of the ts lesion blocked virus replication was estimated for most mutants; this allows both mutant gene expression and the resulting ultrastructural features to be correlated with the sequence of virus replicative events. Three ts mutant-triggered features not previously described in HSV-1-infected cells were also recognized: a modification of rough endoplasmic reticulum, intranuclear accumulation of enveloped virus particles and cytoplasmic accumulation of novel doughnut-shaped particles having a concentric double ring appearance in thin section.

INTRODUCTION

Previous electron microscope (EM) studies of herpes simplex virus type 1 (HSV-1) strain KOS and HSV-2 (strains 186 and HG52) temperature-sensitive (ts) mutant-infected cells (Schaffer et al., 1974; Cabral & Schaffer, 1976; Atkinson et al., 1978) have led to the classification of mutants by the ultrastructural alterations they induce in infected cells at non-permissive temperature (NPT). The present report extends the classification to HSV-1 (strain 17) ts mutants and correlates the EM observations with the estimated times when virus replication becomes blocked by the expression of the mutant gene. Thus, the time of expression of the ts mutant gene is related firstly to associated ultrastructural changes and secondly to the replicative sequence of events. Using transmission electron microscopy, the HSV-1 (strain 17) ts mutants were characterized with respect to their ability to induce in infected cells at non-permissive temperature the various components of the wild-type pattern of morphological alteration. The morphological alterations that are consequences of HSV wild-type replication, assembly or maturation have been well described (Morgan et al., 1968; Nii et al., 1968a, b, c; Schwartz & Roizman, 1969; Nii, 1971a, b) and are separable into features which are observed in the infected nuclei, i.e. margination of host chromatin, accumulations of small regular and large globular (granular) aggregates, distortion and reduplication of nuclear membrane, the accumulation of empty, partially filled or dense-cored nucleocapsids, and features which are confined exclusively to the cytoplasm of infected cells, i.e. reduplication of cytoplasmic membranes, the presence of enveloped virus particles and virions. Below, we report three additional features induced by only some ts mutants: the presence of intranuclear enveloped virus particles, cytoplasmic ring particles and modified (denuded) rough endoplasmic reticulum.

The mutant classes given are those defined by Schaffer et al. (1974), Cabral & Schaffer (1976), and Atkinson et al. (1978). Class I (or A) mutants fail to induce at NPT the synthesis of any nucleocapsid-related structures (NRS), class II (or B) mutants induce only empty or partially filled structures, while class III (or C) mutants induce the synthesis of all identified NRS (including those with an electron-dense core).
METHODS

Cells. BHK-21 C13 cells were grown in 80 oz roller bottles using Eagle's medium (Glasgow modification) supplemented with 10% (v/v) calf serum (EC10).

Virus. The wild-type used was HSV-1 strain 17 syn. The temperature-sensitive mutants had all been isolated from this stock following bromodeoxyuridine mutagenesis. The isolation, characterization, assembly into complementation groups and physical mapping of many of these mutants have been reported elsewhere (Brown et al., 1973; Crombie, 1975; Marsden et al., 1976; Stow et al., 1978).

Temperature downshifts. Near-confluent monolayers (4 x 10⁶ cells) were infected at a multiplicity of infection (m.o.i.) of 5. Following 1 h adsorption at the non-permissive temperature (38.5 °C), the plates were washed and overlaid with 4 ml EC10 and incubated at the permissive temperature (PT, 31 °C) or NPT as appropriate. At various times post-adsorption, appropriate plates were downshifted from NPT to PT, while other plates were harvested according to the experimental time schedule. Cells were harvested by scraping into the medium, followed by ultrasonic disruption and titration of the released virus at 31 °C.

Infection of cells for EM analysis. BHK-21 monolayers (1.5 x 10⁶ cells) were infected at an m.o.i. of 5 with wild-type or ts mutant virus using a 0.1 ml inoculum. After adsorption at NPT, the plates were washed and overlaid with 2 ml EC10. Following incubation for 24 h at PT or NPT selected plates were either harvested for the infectivity assay or processed for EM analysis. A 0 h control sample was also taken.

Processing of EM samples. After removal of growth medium, the infected monolayers were fixed with 2.5% glutaraldehyde in phosphate-buffered saline (PBS) for 30 min, washed three times with PBS and then post-fixed with 1% osmium tetroxide in PBS for 15 min. After further washing in PBS, the monolayers were harvested by scraping into 1 ml PBS and the cells were pelleted by low-speed centrifugation. The cell pellet was dehydrated through a series of increasing alcohol concentrations and finally in propylene oxide. Cell pellets were infiltrated and embedded with Araldite resin. Gold sections were cut with a glass knife, stained for 30 min with saturated uranyl acetate in 50% (v/v) ethanol, rinsed in distilled water and stained with lead citrate for 10 min (Reynolds, 1963). Thin sections were examined in a Siemens 101 electron microscope operating at an accelerating voltage of 80 kV.

RESULTS

Concurrent with EM analysis, infectious virus yields from wild-type and ts mutant-infected cells were assayed at 31 °C at 0 h and 24 h post-adsorption from duplicate plates maintained at PT and NPT. The relative infectious virus yields per cell (NPT/PT) were as follows: wild-type syn 0.58, ts A syn+ 0.0007, ts B syn+ 0.0005, ts D syn 0.0005, ts E syn+ 0.0002, ts F syn 0.0006, ts G syn 0.0007, ts H syn 0.00002, ts I syn 0.00008, ts J syn 0.0005, ts K syn 0.0002, ts L syn 0.0025, ts M syn 0.0025, ts N syn 0.0159, ts O syn 0.0002, ts P syn 0.0002, ts Q syn 0.0025, ts R syn+ 0.0159, ts S syn 0.0002, ts T syn 0.00002, ts U syn 0.00016 and ts X syn 0.0008.

The difference in the efficiency of plating for most ts mutants was between 3 and 4 log₁₀ units, except for ts R syn+ where it was only about 1.8 log₁₀ units. Progeny analysis of the pinhead-sized plaques induced by ts R syn+ at NPT failed to show any ts+ virus and it is thought that such plaques reflect the leakiness of the ts R mutation. Thus, with the possible exception of ts R syn+, the ts mutants used in this study were 'tight' and relatively free of temperature-resistant revertants. Several morphological differences from the typical non-infected cell were observed at NPT in the thin sections of infected cells. It follows that these differences can be attributed to expression of HSV genes up to the block due to the ts lesion.

Ultrastructural features of wild-type infection

Wild-type virus-infected cells exhibited the following HSV-characteristic ultrastructural features at either PT or NPT: marginated chromatin, intranuclear accumulation of small regular and large globular (granular) aggregates, accumulation of empty, partially filled and electron-dense cored virus particles, distortion and reduplication of both nuclear and cytoplasmic membranes and the presence of virions in the infected cytoplasm (Fig. 1a, Fig. 2a). Aberrant nucleocapsids and intranuclear ring-like components were not found in this study, although they are a reported feature of HSV-1 (KOS) wild-type and some ts mutant infections (Schaffer et al., 1974).
EM observations on ts mutants of HSV-1

Fig. 1. Electron micrographs of BHK-21 cells infected with wild-type virus for 24 h at 38.5 °C. (a) Arrows indicate (A) margined chromatin, (B) spherical bodies which are probably fragments of disorganized nucleolus, (C) an invaginated distortion of nuclear membrane, (D) cytoplasmic (Ct) enveloped virus particle and (E) non-enveloped cytoplasmic virus particle. Many nucleocapsid-related structures of different types are present in the infected nucleus (Nc). The insert shows reduplicated nuclear membrane. Scale bars representing the indicated sizes are shown. (b) The spectrum of observed nucleocapsid-related structures. Magnifications are not standard, but each structure has a diameter of 100 nm.
Fig. 2. Electron micrographs of BHK-21 cells infected for 24 h at NPT. (a) Wild-type virus showing (A) reduplicated membranes within the cytoplasm and (B) typical rough endoplasmic reticulum (ER). (b) ts T syn (class I) showing as evidence of HSV infection large globular intranuclear accumulations (A), cytoplasmic accumulations of unknown origin (B) and an invaginated distortion of nuclear membrane (C).
(c) ts D syn (class I) showing the differences in appearance and granular structure between large globular (A) and small regular (B) intranuclear accumulations and that of host cell chromatin (C). (d) ts X syn [class II(2)] showing a typical group of about 20 doughnut-shaped cytoplasmic ring particles which are apparently contained within the lumen of endoplasmic reticulum.
**Ultrastructural features of ts mutant infection at NPT**

Thin sections of cells infected at NPT with wild-type or each of 18 ts mutants were examined. At least 50 nucleated cell profiles, chosen at random, were scored for the various morphological features. The results for each ts mutant shown in Table 1 were confirmed by at least two independent experiments. The mutants were characterized in terms of occurrence of NRS and consequently assigned to one of the three classes described earlier.

The spectrum of NRS is shown in Fig. 1(b). NRS type A is devoid of a core structure and is considered to be empty. NRS types B, C and D contain some form of core. Since many C and D structures were found in cells infected with DNA-negative ts mutants, it is thought that such structures do not contain DNA. The unusual punctate structure represented by NRS B is rare and may possibly represent a short-lived stage in the maturation of the HSV core. Punctate-cored nucleocapsids, similar but not identical to NRS B, have previously been reported for HSV (Friedmann et al., 1975), herpesvirus of turkeys (Nazerian et al., 1971; Okada et al., 1974), tree shrew herpesvirus (McCombs et al., 1971), human cytomegalovirus (Nii, 1976) and equine herpesvirus, type 1 (EHV-1) (Perdue et al., 1976). That the punctate-cored nucleocapsid was a developmental stage in EHV-1 nucleocapsid maturation was clearly shown by Perdue et al. (1976). The NRS types E, F, G, H, I and J all contain an electron-dense core. Differences in the shapes of the cores are probably due to the plane of section.

**Class I mutants**

This class contains the DNA-negative mutants ts K, T and D. All three are members of the same complementation group and all have their ts lesion within the diploid gene encoding the Vmw IE 175K polypeptide (Preston, 1981). Although no NRS were produced by these mutants at NPT, the cells nevertheless did show evidence of HSV infection (Fig. 2b, c). Thus, host chromatin was characteristically marginated, the nuclear membrane was distorted by invaginations of cytoplasm and aggregates of intranuclear granular material were found. In addition, in some of these ts mutant-infected cells, cytoplasmic accumulations of an unknown nature were occasionally observed.

**Class II mutants**

This class contains the DNA-negative mutants ts E, B, S, H, U, L, X, R, J and P. Since no enveloped particles were found in the cytoplasm of cells infected with class II mutants, it appears that the empty and partially filled NRS which were produced were retained at NPT in the infected nucleus. Class II can be subdivided into three subgroups. The four mutants composing subgroup II(1) (ts E, B, S and H) induce a pattern of morphological alterations which strongly resembles that of class I mutants, except for the presence of empty and partially filled NRS. The four mutants composing subgroup II(2) (ts L, U, X and R) induced in most cases, in addition to the class II(1) alterations, nuclear membrane reduplication and the modification of host endoplasmic reticulum. They also induced the synthesis of cytoplasmic ring particles. Neither altered endoplasmic reticulum nor cytoplasmic ring particles have been observed in wild-type infections and are consequently considered novel mutant-induced features. The two mutants (ts J and ts P) which compose subgroup II(3) generate the morphological alterations produced by subgroup II(2) mutants but never induce cytoplasmic ring particles (Table 1).

**Class III mutants**

This class comprises only DNA-positive mutants ts G, Q, F, I and A. Cells infected with these mutants exhibited essentially the complete set of wild-type-induced morphological alterations, except that ts Q, F, I and A additionally modified the endoplasmic reticulum. Although class III mutants produced all the NRS at NPT, in general fewer cytoplasmic virions were seen than with wild-type virus. Mutants ts G and ts Q also accumulated enveloped virus particles within the nucleus of infected cells.
Table 1. The patterns of morphological alterations induced in infected cells by ts mutants at NPT*

<table>
<thead>
<tr>
<th>Virus</th>
<th>Virus DNA</th>
<th>Granular accumulation</th>
<th>Nucleus</th>
<th>Cytoplasm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Small regular</td>
<td>Large globular</td>
<td>Margination chromatin</td>
<td>Empty or partial</td>
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<tr>
<td>Wild-type</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>++</td>
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<tr>
<td>ts K</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>++</td>
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<tr>
<td>ts D</td>
<td>-</td>
<td>+</td>
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<td>-</td>
<td>±</td>
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<td>-</td>
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<tr>
<td>ts B⁺</td>
<td>-</td>
<td>±</td>
<td>+</td>
<td>++</td>
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<td>ts S</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>++</td>
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<td>-</td>
<td>±</td>
<td>-</td>
<td>++</td>
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<td>ts U</td>
<td>-</td>
<td>±</td>
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<td>++</td>
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<tr>
<td>ts A⁺</td>
<td>+</td>
<td>±</td>
<td>±</td>
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</tr>
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</table>

* Based on the frequency and degree of morphological alterations: -, not observed; ±, rarely observed; +, seen in 10 to 50% of cells; ++, seen in almost all cells.

† syn⁺ plaque morphology; all other mutants were syn. Wild-type syn and syn⁺ controls showed no differences in EM morphology.
Fig. 3. Electron micrographs of BHK-21 cells infected for 24 h. (a) ts X syn [class II(2)] at PT. Several incomplete cytoplasmic ring particles (A) lie close to the nuclear membrane and in the proximity of a budding virus particle (B). A further enveloped virus particle (C) is present in the cytoplasm; mitochondrion (m), chromatin (ch) and nucleocapsid-related structures (NRS) are also labelled. (b) ts Q syn (class III) at NPT showing that several membranes of the endoplasmic reticulum (ER) system have been modified locally resulting in a structure with a lamellate appearance. A contiguous portion of ER is indicated by an arrow.
(c) is D syn (class I) at PT; much of the ER around the periphery of the nucleus has been modified but some unmodified membrane is also present (arrow). (d) is G syn (class III) at NPT, showing several enveloped dense-cored virus particles bounded by either reduplicated (A) or single (B) nuclear membrane. These particles are probably contained within an invagination of the nucleus. The enveloped dense-cored virus particle (C) is possibly contained within the lumen of the nuclear membrane. The group of enveloped dense-cored virus particles (D) is close to the nuclear membrane but is apparently free in the nuclear matrix and not associated with membranous material.
Ultrastructural features of wild-type and ts mutant infections at PT

Wild-type virus-infected cells exhibit the same pattern of morphological alterations at PT and at NPT. In permissive infection all the ts mutants displayed essentially wild-type patterns of morphological changes, except that mutants ts K, D, T, B, R, J, P, Q, F, I and A induced (at least in low frequency) modification to the rough endoplasmic reticulum. In addition, ts X was found to induce a small number of cytoplasmic ring particles in a proportion of cells.

Mutant-induced features

Cytoplasmic ring particles

These appeared in thin section as concentric double-ring structures with an outer diameter of about 75 nm and an inner one of about 55 nm. Ring particles were characteristically found in chain-like groups containing about 20 particles and were located exclusively in the cytoplasm. No similar or apparent precursor structures could be identified in the infected nucleus, nor were ring particles observed in the extracellular spaces. Although not obviously associated with any particular organelle, ring particles were frequently observed within the lumen of the rough endoplasmic reticulum (Fig. 2d). Where incomplete ring particles were found, it was not clear whether they were in the process of synthesis or degradation (Fig. 3a).

The time course of ring particle synthesis at both PT and NPT was determined using ts X, the most efficient ring particle-inducing mutant (Fig. 4). At PT a small number of ring particles were observed at all times between 16 h and 26 h after adsorption. At NPT ring particles first appeared at some time prior to 16 h, attaining maximum numbers at 19 h, after which their number gradually declined. This implies either that their rate of disintegration exceeds that of synthesis after 19 h or that ring particles are released from the infected cells, perhaps via the reticuloendothelial system in the same manner as HSV particles (Schwartz & Roizman, 1969). Unfortunately, we cannot support either possibility since incomplete or apparently damaged ring particles were only rarely found, while extracellular ring particles were never seen.

Despite numerous attempts we have not yet succeeded in isolating ring particles from cell extracts: a prerequisite for their characterization.

Modified (denuded) endoplasmic reticulum

This novel feature is seen as a reduction of membrane width and of the number of ribosomes attached to affected portions of membrane. Two morphologically distinct forms were recognized. The first consists of membranes packed together to form a localized structure with a lamellate appearance (Fig. 3b). The second is more common and is seen as a generalized reduction of membrane thickness and associated ribosome number in the vicinity of the nucleus (Fig. 3c). The modified membrane is rough endoplasmic reticulum, since a portion of affected membrane can occasionally be traced to a contiguous portion of typical endoplasmic reticulum (Fig. 3b).

Intranuclear enveloped virus

Envelopment of HSV has generally been accepted to occur by budding of virus through the inner nuclear membrane (Darlington & Moss, 1968). However, at NPT two mutants (ts G and ts Q) regularly gave rise to intranuclear enveloped virus particles (Fig. 3d). Such particles frequently occurred in small groups sometimes surrounded by thickened or reduplicated nuclear membrane. While in some cases enveloped particles clearly lay within finger-like invaginations of nuclear membrane (A and possibly B in Fig. 3d), other enveloped particles appeared not associated with any membranous structure (D in Fig. 3d), though always near the nuclear membrane. This suggests that envelope may be acquired by dense-cored nucleocapsids in the vicinity of the nuclear membrane but without necessarily budding through it. Similar observations have also been reported for HSV-2 (Atkinson et al., 1978) and herpesvirus saimiri (Heine et al., 1971).
EM observations on ts mutants of HSV-1

Temperature downshifts

By implication, temperature-sensitive mutations become effective at those times during the replicative cycle when their essential gene products have to be functional. It follows that ts mutations in different genes will block virus replication at different times (block-times) during the normal replicative cycle. Observations made in the EM on ts mutant-infected cells must represent the, possibly complex, end result of mutant-induced blocks at different stages in the normal sequence of replicative events. The block-times for several of the HSV-1 ts mutants have been ascertained by temperature downshift.

In these experiments, ts mutant-infected cells were shifted down from 38.5 °C (NPT) to 31 °C (PT) at set times after adsorption. From the resulting series of growth curves the delay in virus growth due to incubation at the restrictive temperature can be estimated. If the delay is plotted as a function of the incubation period at NPT prior to downshift, this allows estimation of the time when the effects of the ts lesion interrupt the replicative cycle: this is the mutant’s block-time. Fig. 5 shows the curves obtained in temperature downshifts performed with the wild-type virus and ts T, J and A, which are representative of very early, early and late ts mutations.

The delay in virus growth due to the period of incubation at the NPT was estimated from the curves provided by temperature downshifts at 2, 4, 6, 8 and 12 h post-adsorption by measuring the displacement of the apparent points of inflection on each curve. Block-times were calculated by extrapolation of the curve produced from a plot of the delay in virus growth against the incubation period at NPT (Fig. 6).

Fig. 4. Time course of cytoplasmic ring particle synthesis with ts X ring particle numbers at PT (□), ring particle numbers at NPT (■), virus particle numbers at PT (●) and virus particle numbers at NPT (〇). BHK-21 cells infected with ts X syn at NPT were harvested and processed for EM analysis at the indicated times after adsorption. The numbers of virus particles and cytoplasmic ring structures were scored from the same randomly chosen nucleated 40-cell profile.
Fig. 5. Temperature downshifts. The vertical lines in the graphs indicate the point of infection at PT; horizontal lines indicate the delay observed on downshift after the indicated number of hours at NPT. Coincident curves demonstrate that no delay was suffered by incubation at NPT after various periods, which can be identified by the spur point from the NPT control curve.
EM observations on ts mutants of HSV-1

Fig. 6. Estimation of mutant block-time.

Table 2. Block-times and class of 18 HSV-1 (strain 17) ts mutants

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Block-time (h post-adsorption)</th>
<th>Mutant class</th>
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<tbody>
<tr>
<td>K</td>
<td>0-1*</td>
<td>I</td>
</tr>
<tr>
<td>D</td>
<td>ND†</td>
<td>I</td>
</tr>
<tr>
<td>T</td>
<td>0-1</td>
<td>I</td>
</tr>
<tr>
<td>E</td>
<td>0-1†</td>
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<td>U</td>
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<td>II(1)</td>
</tr>
<tr>
<td>L</td>
<td>ND</td>
<td>II(2)</td>
</tr>
<tr>
<td>X</td>
<td>4</td>
<td>II(2)</td>
</tr>
<tr>
<td>R</td>
<td>3*</td>
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</tr>
<tr>
<td>J</td>
<td>3†</td>
<td>II(3)</td>
</tr>
<tr>
<td>P</td>
<td>ND</td>
<td>II(3)</td>
</tr>
<tr>
<td>G§</td>
<td>1.5†</td>
<td>III</td>
</tr>
<tr>
<td>Q</td>
<td>ND</td>
<td>III</td>
</tr>
<tr>
<td>F</td>
<td>3-8‡</td>
<td>III</td>
</tr>
<tr>
<td>I</td>
<td>5-3</td>
<td>III</td>
</tr>
<tr>
<td>A</td>
<td>7</td>
<td>III</td>
</tr>
</tbody>
</table>

* M. Brown (unpublished data).
† ND, No data.
‡ M. Mechie (unpublished data).
§ This is TK− at either temperature.

The block-times for all ts mutants used in the EM study are given in Table 2. Under restrictive conditions class I mutants are capable of only a short period of normal growth, between 0 and 1 h post-adsorption. The class II mutants exhibit block-times ranging from within the first hour until 4 h, while the class III mutants, with the exception of ts G, have block-times between 3-8 and 7 h. The block-time of 1-5 h obtained for ts G is unaccountably early for a DNA-positive mutant; however, there is increasing evidence to suggest that the ts G stock used contains at least two mutations, which may have been contributory to this result. The block-times of the mutants ts E and ts B are also puzzling. They appear to belong to the same complementation group (Brown et al., 1973) but have different block-times and the patterns of morphological alterations obtained for ts B and ts E also differed slightly (Table 1). Two lines of preliminary evidence, a study of non-ts reversion (D. Dargan & J. H. Subak-Sharpe, unpublished results) and marker rescue (B. Matz, personal communication), suggest that ts B possesses at least two mutations. Although ts E appears to revert to wild-type, it was found to be rescued with HSV-1 DNA fragments from two separate locations within U₄ (Stow, 1978), which implies that this mutant may also contain two or more mutations.
The class I \( ts \) mutants reported here, \( ts \) K, T and D, all have their \( ts \) lesions located within the \( V\text{mw IE 175} \) gene (Preston, 1981). The very early block-times obtained for these mutants are consistent with \( ts \) lesions in an immediate-early (IE) function. A fully functional \( V\text{mw IE 175} \) polypeptide is required for the 'switch-on' of early gene transcription and is also needed at all times for subsequent transcription (Preston, 1979; Watson & Clements, 1978, 1980). The infected cell polypeptide profiles obtained on SDS-polyacrylamide gels for \( ts \) K, T and D were demonstrably different (Marsden et al., 1976; Gerdes et al., 1979) although these mutants are due to three different mutations within the same gene. At the level of electron microscope morphology no differences could be detected. The infected cell polypeptide profiles of \( ts \) T and D were very similar, but that of \( ts \) K was much more restricted. Only IE polypeptides were induced at NPT by \( ts \) K, except that some major capsid protein and some precursor glycoprotein B are also made (MacDonald, 1980). These two exceptions could account respectively for the intranuclear granular accumulations and nuclear membrane distortion found in all class I \( ts \) mutant-infected cells.

All of the class I mutants overproduce the \( V\text{mw IE 175} \) polypeptide at NPT, which in \( ts \) K was found to be incorrectly processed and to accumulate in large quantities in the cytoplasm of infected cells (Preston, 1979). We have no evidence to suggest that any \( V\text{mw IE 175} \) polypeptide is involved in the cytoplasmic accumulations found after some class I infections. However, this seems likely as similar accumulations were found by Cabral et al. (1980) and identified as \( V\text{mw IE 175} \)-related. These authors used a monospecific antiserum and an immunoperoxidase procedure in cells infected with the class I mutant \( ts \) B2 of HSV-1 (KOS) which is known to overproduce \( V\text{mw IE 175} \) polypeptide at NPT.

The data presented indicate that margination of chromatin, intranuclear granular accumulations and nuclear membrane distortion are very early events following HSV-1 infection (0 to 1 h after adsorption) and must be due either to infecting particle components or more likely due to IE polypeptide expression. Nucleocapsid-related structures do not appear in class I mutant infections at NPT and are genetically under control of the \( V\text{mw IE 175} \) gene, which begins to operate within the first hour after adsorption. The functions required to induce nuclear membrane reduplication are not only under control of the \( V\text{mw IE 175} \) gene but are also dependent on the function of the \( E, B, H, S \) and \( X \) genes. The functions required to induce cytoplasmic membrane reduplication depend additionally on the function of the \( L, R \) and \( J \) genes. The observed modification to endoplasmic reticulum is dependent on normal function of the \( K, D, T, E, B, S, H, U, L, X, R \) and \( G \) genes. In this context the rare positive observations with \( ts \) D, \( ts \) B, \( ts \) X and \( ts \) R are believed to be due to some leakiness. All the functions required for virus DNA synthesis are needed for the production of electron-dense cored virus particles. The accumulation of cytoplasmic ring particles is confined to mutants \( ts \) U, \( ts \) L, \( ts \) X and \( ts \) R, which are known from other studies (D. Dargan & J. H. Subak-Sharpe, unpublished observations) to fall into two complementation groups. These ring particles are thought to be due to mutant by-products peculiar to the class II(2) \( ts \) mutations. Lastly, \( ts \) G and \( ts \) Q mutations are regularly associated with the formation of intranuclear enveloped virus particles.

The results presented here for \( ts \) mutants, I, G, J and D are in general agreement with those published by Gerdes et al. (1979), although these authors reported only a few of the features scored here. Interestingly, Gerdes et al. (1979) also demonstrated that the ultrastructural changes associated with HSV-1 wild-type infection were identical in BHK, neuroblastoma and neuron cells, while those associated with \( ts \) I, \( ts \) G and \( ts \) D varied with cell type. This was most marked for \( ts \) G, which failed to package the virus DNA synthesized in neuroblastoma cells, so that \( ts \) G would be reclassified as a class II mutant in these cells.

The processes which give rise to the modification to endoplasmic reticulum are not clear: disaggregation and rearrangement of host polysomes (Sydiskis & Roizman, 1966, 1967, 1968), the insertion of HSV-coded polypeptides into host membrane (Miyamoto et al., 1971; Roizman & Spear, 1971) or even de novo synthesis of membrane including HSV antigens may be involved. We do not believe that the observed modification to endoplasmic reticulum can be explained as a simple consequence of cell morbidity.
ATKINSON, M. A., BARR, S. & TIMBURY, M. C. (1978). The fine structure of cells infected with temperature-sensitive mutants of herpes simplex virus type 2 (HG 52) ts mutant infections with certain mutants of all three classes: ts 1 (class II); ts 2 (class I); ts 6 (class II); ts 11 (class III). In contrast, the HSV-1 (strain 17) mutants inducing these structures reported here (ts L, ts U, ts X, ts R) are confined to class II. However, ts 2 (class I) only induced cytoplasmic ring particles after preincubation for 1 h at below the restrictive temperature. Mutant ts 11 (class III) has been shown by Halliburton & Timbury (1976) to have a DNA-negative phenotype at NPT. The DNA-containing virus particles found in ts 11-infected cells at NPT by Atkinson et al. (1978) were probably due either to leakiness under their experimental conditions or to reversion. The ring particles induced by ts mutants of HSV-1 and HSV-2 appear morphologically indistinguishable, having similar dimensions, cytoplasmic location and occurring in characteristic chain-like aggregations. The nature and origin of these unusual particles are under study; despite considerable effort we could obtain no evidence for the hypothesis that ring particles might be associated with some unidentified contaminant replicating in the cytoplasm.

The mutant classification reported here reflects a sequence of ultrastructural changes occurring during HSV-1 replication. The estimation of mutant block-times has allowed some correlation between the time when the ts mutant function is needed and the ultrastructural features observed as a consequence of the ts mutation. Thus, marginated chromatin, nuclear membrane distortion, intranuclear granular accumulations, nucleocapsid-related structures, nuclear membrane reduplication, modified endoplasmic reticulum, cytoplasmic membrane reduplication and the appearance of electron-dense cored virus particles probably reflect successive virus gene-controlled manifestations of the infectious process.

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