JOURNAL OF VIROLOGY, Jan. 1983, p. 397–407
0022-538X/83/010397-11$02.00/0

Vol. 45, No. 1

Molecular Genetics of Herpes Simplex Virus

VIII. Further Characterization of a Temperature-Sensitive Mutant Defective in Release of Viral DNA and in Other Stages of the Viral Reproductive Cycle

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Received 12 July 1982/Accepted 6 October 1982

Previous studies have shown that cells infected with the herpes simplex virus 1(HFEM) mutant *ts*B7 and maintained at the nonpermissive temperature fail to accumulate viral polypeptides. Analyses of intertypic recombinants generated by marker rescue of *ts*B7 with herpes simplex virus 2 DNA fragments localized the mutation between 0.46 and 0.52 map units on the viral genome (Knipe et al., J. Virol. **38**:539–547, 1981). In this paper we report that the mutation in *ts*B7 affects several aspects of the reproductive cycle of the virus at the nonpermissive temperature. Thus, (i) viral capsids accumulate at the nuclear pores and do not release viral DNA for at least 6 h postinfection at 39°C. The DNA was released within 30 min after a shift to the permissive temperature. (ii) Experiments involving shifts from the permissive to the nonpermissive temperature indicated that viral protein synthesis was not sustained in cells maintained at the permissive temperature for less than 4 h. (iii) Viral DNA synthesis was delayed at the permissive temperature for as long as 8 h. Once initiated, it continued at 39°C. (iv) Marker rescue of *ts*B7 by transfection with herpes simplex virus 1(F) DNA fragments localized the mutation to between 0.501 and 0.503 map units on the viral genome. These results are consistent with the *ts*B7 lesion being in a gene coding for a virion component which affects release of viral DNA from capsids and onset of viral DNA synthesis.

Herpes simplex virus 1 (human herpesvirus 1; HSV-1) mutant *ts*B7 of strain HSV-1(HFEM) was reported to complement other temperature-sensitive mutants in doubly infected cells (8, 14). However, viral gene products were not detected in cells infected and maintained at the nonpermissive temperature. The mutation was mapped by marker rescue between 0.46 and 0.52 map units on the prototype arrangement of the DNA (8).

In this paper, we report evidence that *ts*B7 is defective in several functions, such as release of viral DNA from capsids, viral DNA synthesis, and late gene expression, even though fine mapping of the lesion indicates that it is contained within a 300-base-pair region of the DNA.

MATERIALS AND METHODS

Viruses and cells. HSV-1(HFEM) *ts*B7 *syn*$^+$ was isolated by bromodeoxyuridine mutagenesis of HSV-1(HFEM)-infected cells (4), and the properties of this mutant were described elsewhere (8). Virus stocks were prepared and titrated on Vero or HEp-2 cells as previously described (12). Rabbit skin cells were originally obtained from J. McLaren. Human embryonic lung (HEL) cells were originally obtained from Flow Laboratories, Inc., Rockville, Md.

Enzymes and radioisotopes. The restriction endonucleases *Bam*HI, *Sal*I, and *Xho*I were obtained from New England Biolabs (Waverly, Mass.) and Bethesda Research Laboratories (Rockville, Md.). T4 DNA ligase was purchased from P-L Biochemicals, Inc., Milwaukee, Wis. The radioisotopes [*methyl*-^{3}H]thymidine (59 Ci/mmol) and [^{14}C]leucine, [^{14}C]sioleucine, and [^{14}C]valine (each >250 mCi/mmol) were purchased from New England Nuclear Corp., Boston, Mass.

Electron microscopy. Infected cells were harvested by scraping into ice-cold phosphate-buffered saline. Cells were pelleted and fixed in 2% glutaraldehyde for 1 h and 1% osmium tetroxide for 1 h. The fixed cells were dehydrated through graded alcohols and embedded in Epon. Thin sections were cut and stained in 2% uranyl acetate. Sections were viewed in a Siemens 102 electron microscope at an accelerating voltage of 80 kV and with 200- and 50-μm condenser and objective apertures, respectively.

Preparation of viral DNA, restriction endonuclease digestion, and construction of plasmids containing HSV-1 DNA. Intact viral DNA was purified by NaI density gradient centrifugation (10) of DNA extracted from cytoplasmic nucleocapsids. Digestion of viral DNA, preparative agarose gel electrophoresis, and isolation

of individual restriction endonuclease-derived fragments were described elsewhere (11). The isolation and procedures for purification of plasmid pRB101 containing the *Bam*HI-D fragment of HSV-1(F) DNA cloned in pBR322 were previously described (11). The relevant HSV-1(F) *Bam*HI/*Sal*I (*Sal*I-ΔB, pRB609; *Sal*I-ΔM, pRB610); *Bam*HI/*Xho*I (*Xho*I-ΔAD, pRB611); and *Xho*I (*Xho*I-B, pRB612; *Xho*I-C, pRB613) restriction endonuclease fragments of pRB601 were cloned into the *Bam*HI and *Sal*I sites of pBR322 and the *Xho*I site in pACYC177 (1–3), respectively.

Marker rescue. Intertypic marker rescues were done by transfection of rabbit skin cells with mixtures of intact mutant DNA and cloned HSV-1(F) DNA fragments as described elsewhere (9, 13). The HSV-1 fragments used for marker rescue were (i) *Bam*HI-D, cloned in pBR322 (pRB101) and mapping between coordinates 0.46 and 0.515 in the prototype arrangement, and (ii) the *Sal*I and *Xho*I fragments derived by digestion of the *Bam*HI-D fragment. The progeny obtained after transfection of rabbit skin cells were titrated at 33.5 and 39°C on Vero cells, and the plating efficiency was determined.

Synthesis of viral DNA in infected cells. Confluent monolayer cultures of Vero cells were infected and labeled with [*methyl*-^{3}H]thymidine at 20 μCi/ml. DNA was extracted from infected-cell monolayers as described previously (6). The DNA was banded in CsCl equilibrium density gradients in a Beckman 75 Ti rotor at 40,000 rpm and 20°C for 60 h. Fractions of equal volume were collected from the gradients, and radioactivity was measured as described previously (6).

Protein labeling and gel electrophoresis. Labeling of HSV-1-infected cultures with [^{14}C]leucine, [^{14}C]isoleucine, and [^{14}C]valine, polyacrylamide gel electrophoresis of the cell lysates, and autoradiography were performed as previously described (10).

RESULTS

Accumulation of viral capsids at nuclear pores in cells infected with HSV-1(HFEM) *ts*B7 and maintained at nonpermissive temperatures. The experiments described in this section show that in cells infected and maintained at 39°C, *ts*B7 filled nucleocapsids accumulated at nuclear pores. These capsids released their DNA after infected cells were shifted to the permissive temperature. Two series of experiments were done.

In the first, Vero cells were infected with HSV-1(HFEM) *ts*B7 (multiplicity of infection = 200) or with the parent virus HSV-1(HFEM) (multiplicity of infection = 200) and incubated at either the permissive (33.5°C) or the nonpermissive temperature (39°C). At 2 h postinfection (p.i.), the cells were harvested, and thin sections, prepared as described above, were examined by electron microscopy. The viral capsids within the infected cells were classified on the basis of appearance as either empty or devoid of cores, as full or intact capsids containing discrete cores, or as intermediates, i.e., capsids containing partial cores or from which cores were protruding. The results of this study are summarized in Table 1, and representative electron micrographs are presented in Fig. 1. The striking observation was that in cells maintained at 39°C, there were no or very few empty capsids; the vast majority of capsids contained distinct cores. In contrast, in cells maintained at 33.5°C, the majority of capsids were either empty or of the intermediate type. At 39°C, the majority of parental HFEM capsids were empty. These results indicate that at the nonpermissive temperature, 39°C, *ts*B7 capsids do not release their DNA cores into the nucleus, and hence, viral replication is blocked very early in infection.

The second series of experiments was designed to determine whether the capsids accumulated at the nuclear pores released their DNA when the infected cultures were shifted to the permissive temperature. Two cultures of Vero cells were infected with *ts*B7 (multiplicity of infection = 100) and incubated at the nonpermissive temperature for 6 h. At 6 h p.i., one culture was harvested, and the cells were fixed for thin sectioning, while the second culture was transferred to the permissive temperature. After 30 min at the permissive temperature, the cells were harvested and fixed. The results of the experiment are summarized in Table 1, and representative electron micrographs are presented in Fig. 2. The number of empty capsids found in cells infected with *ts*B7 and maintained at 39°C was low. However, *ts*B7-infected cells shifted to the permissive temperature for 30 min contained a large percentage of empty capsids and capsids at intermediate stages of disassembly abutting the nuclear membrane. These results indicate that a brief exposure to the permissive temperature is sufficient to allow *ts*B7 capsids to release their DNA cores.

TABLE 1. Distribution of capsid types in cells infected with HSV-1(HFEM) *ts*B7 and HSV-1(HFEM)[a]

Virus	Temp (°C)	Time p.i.(h)	Capsid type (%)		
			Empty	Intermediate	Full
HFEM	39	2	70	28	2
*ts*B7	33	2	22	48	30
	39	2	0	20	80
	39	6	0	11.8	88.2
	39	6	12.3	44.6	43.1
	33	0.5			

[a] For each virus strain and time point, approximately 2,000 capsids contained in 200 to 250 thin sections of infected Vero cells were examined and scored as described in the text.

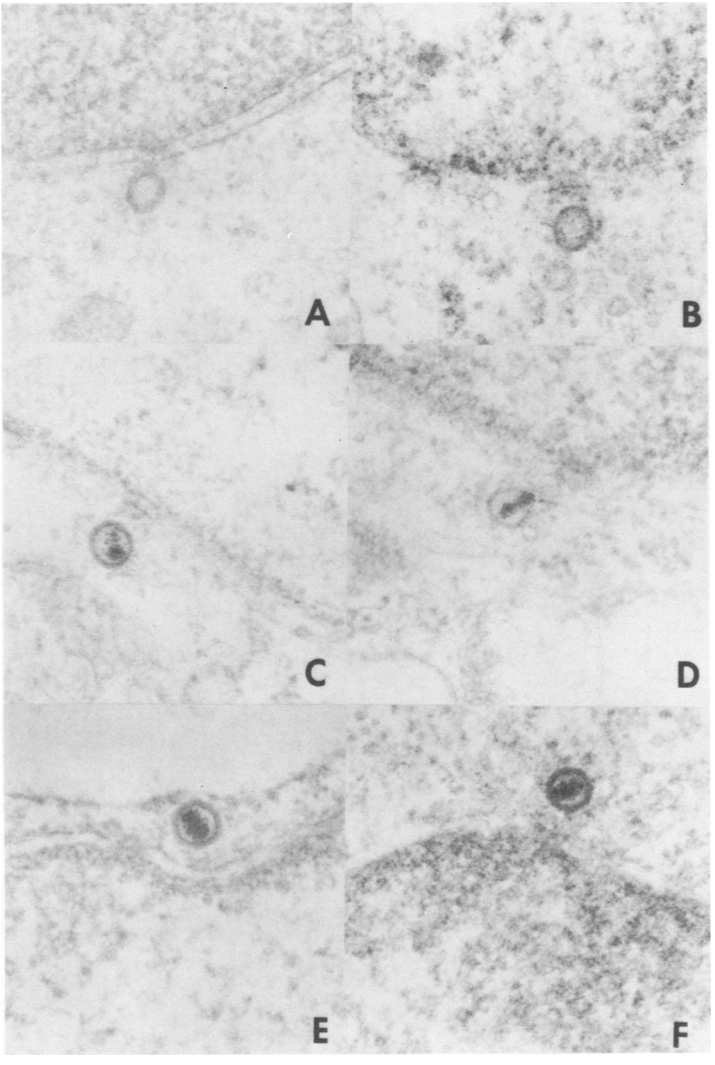

FIG. 1. Thin sections of infected Vero cells. (A) An empty capsid in the cytoplasm of a Vero cell 2 h after infection with 10 PFU of HFEM at 39°C. The capsid abuts a nuclear pore and has lost its core. (B, C, and D) Capsids in the cytoplasm of Vero cells 2 h after infection with 100 PFU of *ts*B7 at 33°C. Panel (B) shows an empty capsid near the nuclear membrane. Panels (C) and (D) show capsids at intermediate stages of disassembly near the nuclear membrane. (E and F) Capsids in the cytoplasm of Vero cells 2 h after infection with 100 PFU of *ts*B7 at 39°C. In panel (E), a full capsid abuts a nuclear pore. Panel (F) shows a full capsid near the nuclear membrane.

Viral protein synthesis in *ts*B7-infected cells. If uncoating were the only *ts*B7 defect, a transient exposure to the permissive temperature in excess of 30 min should allow normal viral gene expression to proceed at the nonpermissive temperature. The experiments described in this section show that this is not the case. Viral gene expression in *ts*B7-infected cells at 39°C did in fact require a transient incubation at 33.5°C, and viral protein synthesis was not required during this brief incubation at the permissive temperature. However, viral protein synthesis initiated early in infection was not sustained in infected cultures shifted to 39°C after less than 4 h at the permissive temperature.

Two series of experiments illustrate these points. A schematic representation of the experimental design for the first series is shown at the top of Fig. 3. Cells were infected with *ts*B7, incubated at the temperatures indicated, and harvested immediately after labeling with ^{14}C-amino acids for 1 or 2 h at the times shown. The autoradiographic images of the electrophoretically separated polypeptides (Fig. 3) indicate the following.

(i) At the permissive temperature, *ts*B7-infect-

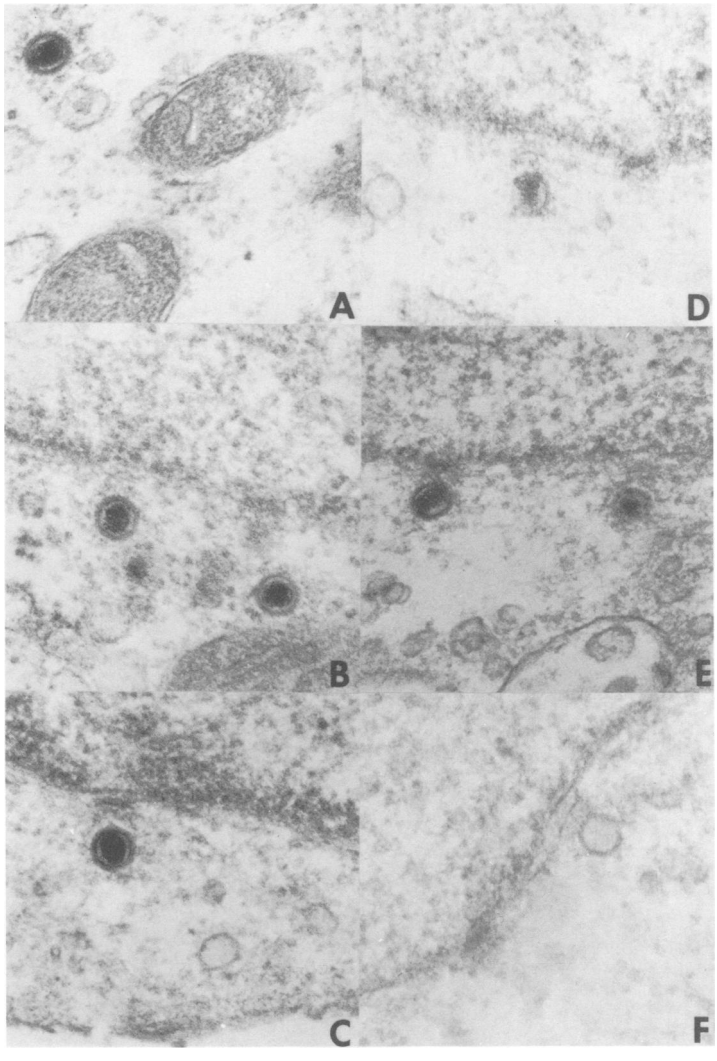

FIG. 2. Thin sections of Vero cells infected with *ts*B7 virus. Replicate Vero cell cultures were infected with 100 PFU of *ts*B7 and incubated for 6 h at 39°C. One culture was harvested and fixed immediately, whereas the other was incubated for an additional 30 min at 33.5°C before harvesting. (A, B, and C) Representative full capsids in the cytoplasm of cells after 6 h of incubation at 39°C. (D, E, and F) Capsids in intermediate stages of disassembly (D and E) or empty capsids (F).

ed cells synthesized normal amounts of virus-induced polypeptides at the expected times (lanes A, B, and C).

(ii) *ts*B7-infected cells incubated at the nonpermissive temperature did not synthesize any detectable viral polypeptides (lanes D and E).

(iii) A transient 1-h incubation at the permissive temperature was sufficient to allow expression of viral proteins at the nonpermissive temperature at 0 to 2 h p.i. (lane F) and at 5 to 6 h p.i. (lanes G, H, I, and J) in amounts equivalent to those that would have been expressed had the cultures been maintained at the permissive temperature.

(iv) The ability of *ts*B7-infected cultures to synthesize γ infected-cell polypeptides at the nonpermissive temperature at 18 to 20 h p.i. was clearly related to the length of time that the infected cultures remained at the permissive temperature. Most of the γ infected-cell polypeptides (infected-cell polypeptide-5, -11, -20, -25, -29, -32, -35, -38, -39, -40, -43, -44) were not synthesized at 18 to 20 h p.i. in amounts comparable to the control (lane C) unless the cultures were incubated at the permissive temperature for at least 4 h (lanes K, L, M, and N).

(v) The synthesis of α and β polypeptides was shut off late in infection in cultures shifted to the

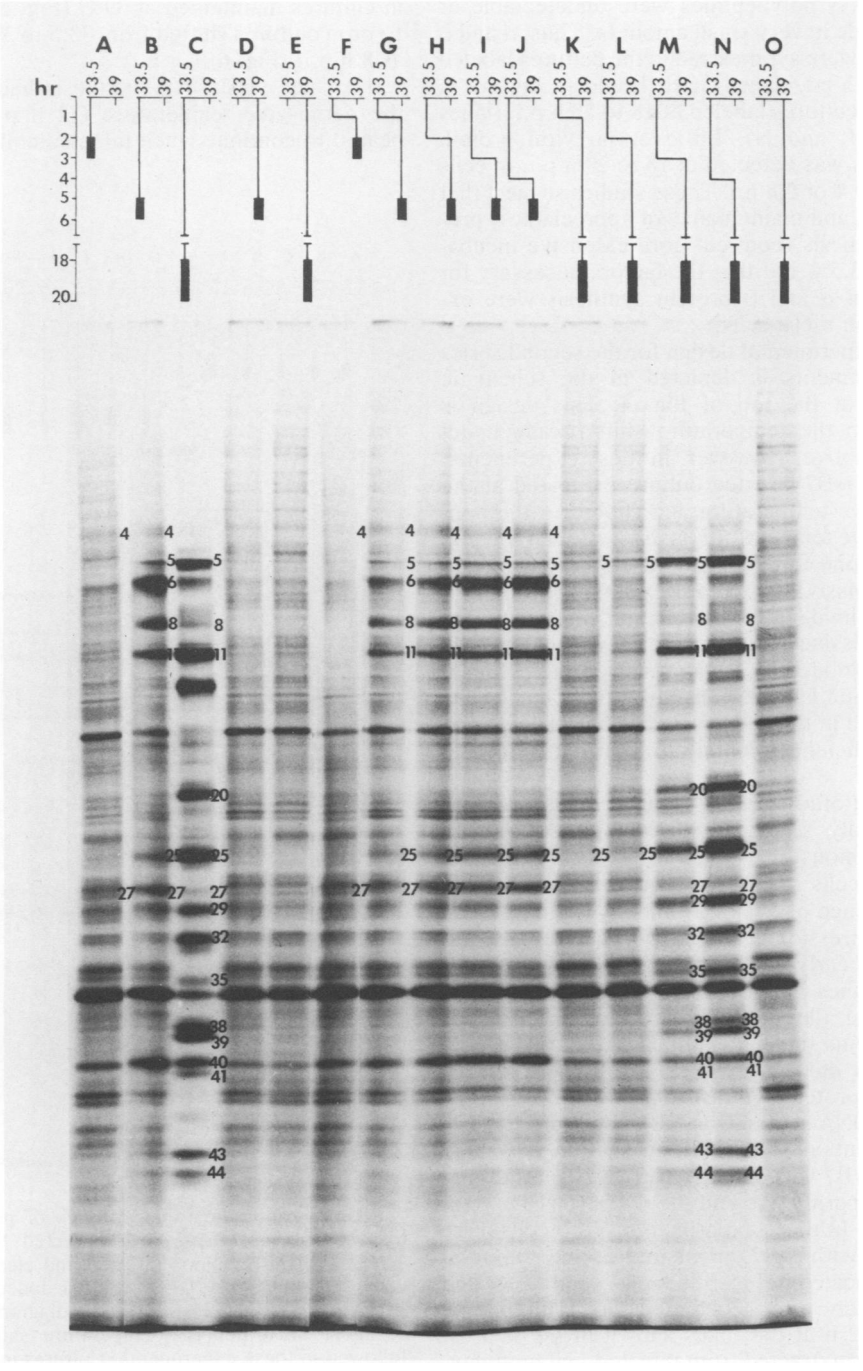

FIG. 3. Autoradiographic images of polypeptides extracted from HEp-2 cells infected with HSV-1(HFEM) *ts*B7 or mock infected and electrophoretically separated in a 9.25% sodium dodecyl sulfate–polyacrylamide gel. The schematic diagram at the top of the figure graphically illustrates the temperature shift protocol followed in these experiments. Cultures in lanes A to N were infected with *ts*B7 (10 PFU per cell). The culture in lane O was mock infected. Cultures were treated as depicted in the schematic diagram and were labeled with ^{14}C-amino acids at the times shown, as described in the text. The heavy black lines indicate the labeling intervals.

nonpermissive temperature even though in some instances γ polypeptides were undetectable or were made in very small amounts. Thus, α and β proteins were synthesized in the cultures labeled at 5 to 6 h p.i. (lanes G, H, I, and J), but not in infected cultures labeled at 18 to 20 h p.i. (lanes K, L, M, and N). Little or no viral protein synthesis was detected at 18 to 20 h p.i. in cells shifted at 1 or 2 h p.i. These studies suggest that initiation and maintenance of appreciable γ protein synthesis required more extensive incubation at 33.5°C but that the factors necessary for shutoff of α and β protein synthesis were expressed in these cells.

The experimental design for the second series of experiments is depicted in the schematic diagram at the top of Fig. 4. This design is similar to the temperature shift paradigm described above. However, in these experiments, for each *ts*B7-infected culture incubated at the permissive temperature and shifted to the nonpermissive temperature (lanes A, C, E, G, I, and K), a second equivalent culture was incubated at the permissive temperature in the presence of cycloheximide (200 μg/ml) and shifted to the nonpermissive temperature, at which time the cycloheximide block was reversed (lanes B, D, F, H, J, and L). The concentration of cycloheximide used in these experiments has been shown to be sufficient to inhibit viral protein synthesis (7).

The autoradiographic images of the electrophoretically separated polypeptides shown in Fig. 4 demonstrate that a 1-h exposure of *ts*B7-infected cells at the permissive temperature in the presence of cycloheximide was sufficient to allow expression at the nonpermissive temperature of all early viral proteins (cf. lanes A, C, and E with lanes B, D, and F). These experiments reinforced the conclusion drawn in previous experiments that the shutoff of α and β protein synthesis did not require extensive and sustained γ protein synthesis.

Viral DNA synthesis in *ts*B7-infected cells. The experiments described in this section show that mutant *ts*B7 was defective in DNA synthesis at both the permissive and the nonpermissive temperature. In these experiments, Vero cells were infected with *ts*B7, incubated at the temperatures indicated in Fig. 5, 6, and 7, and harvested immediately after labeling with [^{3}H]thymidine for 1 or 2 h at the times shown in the figures. DNA was extracted from infected-cell monolayers and banded in CsCl equilibrium density gradients. The distribution of [^{3}H]thymidine label in the gradients indicated the following.

(i) In cells maintained at 33.5°C, the onset of *ts*B7 viral DNA synthesis was delayed as compared with that in cells infected with the parental HFEM virus (Fig. 5A, B, and C).

(ii) *ts*B7 viral DNA synthesis was not detected in cultures maintained at 39°C (Fig. 5D, E, and F) or in cultures shifted from 33.5 to 39°C as late as 8 h p.i. (Fig. 6).

(iii) Once viral DNA synthesis had begun at the permissive temperature (12 h p.i.), it appeared to continue when infected cultures were

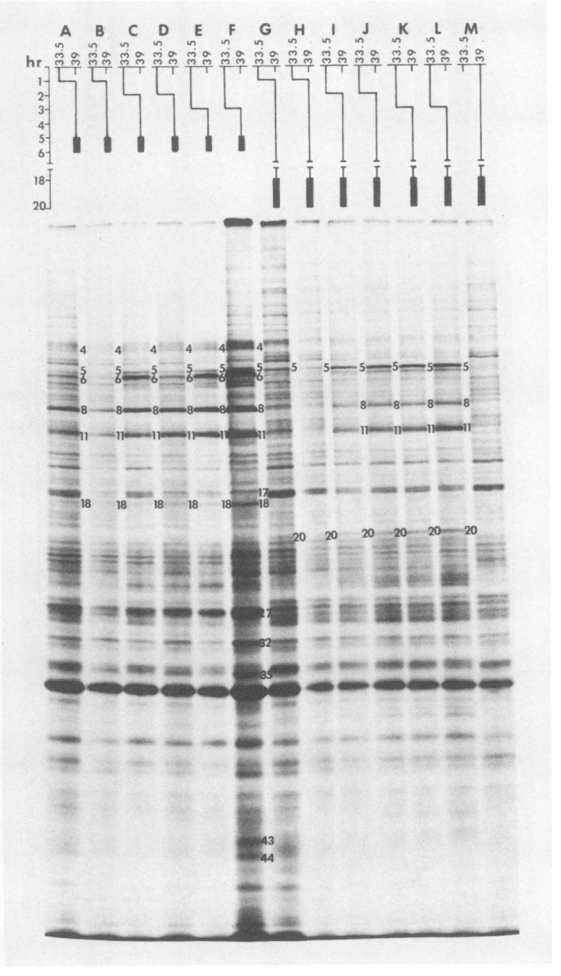

FIG. 4. Autoradiographic images of polypeptides extracted from HEp-2 cells infected with HSV-1(HFEM) *ts*B7 or mock infected and electrophoretically separated in a 9.25% sodium dodecyl sulfate–polyacrylamide gel. The schematic diagram at the top of figure illustrates the temperature shift protocol followed in these experiments. Cultures in lanes A to L were infected with *ts*B7 (10 PFU per cell); the culture in lane M was mock infected. Cultures were treated as depicted in the schematic diagram except that the cultures in lanes B, D, F, H, J, and L were treated with cycloheximide (200 μg/ml) while at the permissive temperature, as described in the text. Cultures were labeled with ^{14}C-amino acids at the times indicated by the heavy black lines.

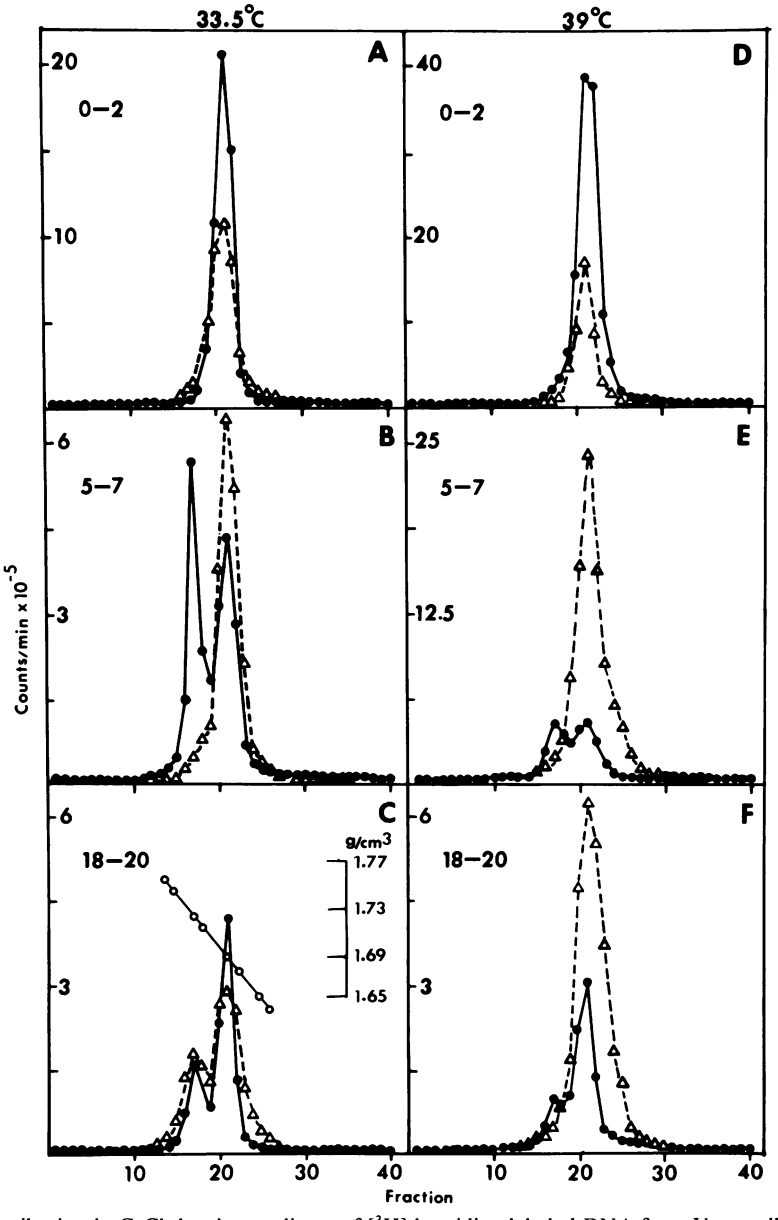

FIG. 5. Distribution in CsCl density gradients of [^{3}H]thymidine-labeled DNA from Vero cells infected with parent (HFEM) or mutant (*ts*B7) virus and incubated at the permissive temperatures. Symbols: ●, HSV-1(HFEM); △, HSV-1(HFEM) *ts*B7. (A) 33.5°C and labeled at 0 to 2 h p.i. (B) 33.5°C and labeled at 5 to 7 h p.i. (C) 33.5°C and labeled at 18 to 20 h p.i. (D) 39°C and labeled at 0 to 2 h p.i. (E) 39°C and labeled at 5 to 7 h p.i. (F) 39°C and labeled at 18 to 20 h p.i.

shifted to the nonpermissive temperature (Fig. 7).

Fine mapping of the *ts*B7 temperature-sensitive lesion by marker rescue with cloned fragments of HSV-1 DNA. Previous mapping of the HSV-2 sequences in recombinants produced by marker rescue of HSV-1(HFEM) *ts*B7 with HSV-2(G) DNA fragments localized the *ts*B7 lesions between 0.46 and 0.52 map units on the viral genome (8). In the experiments reported here, the location of the *ts*B7 lesion was mapped more precisely by marker rescue with HSV-1(F) DNA fragment *Bam*HI-D cloned as pRB101 in pBR322 and with *Sal*I and *Xho*I fragments derived by cleavage of the *Bam*HI-D fragment as shown in Fig. 8. The results of the marker rescue

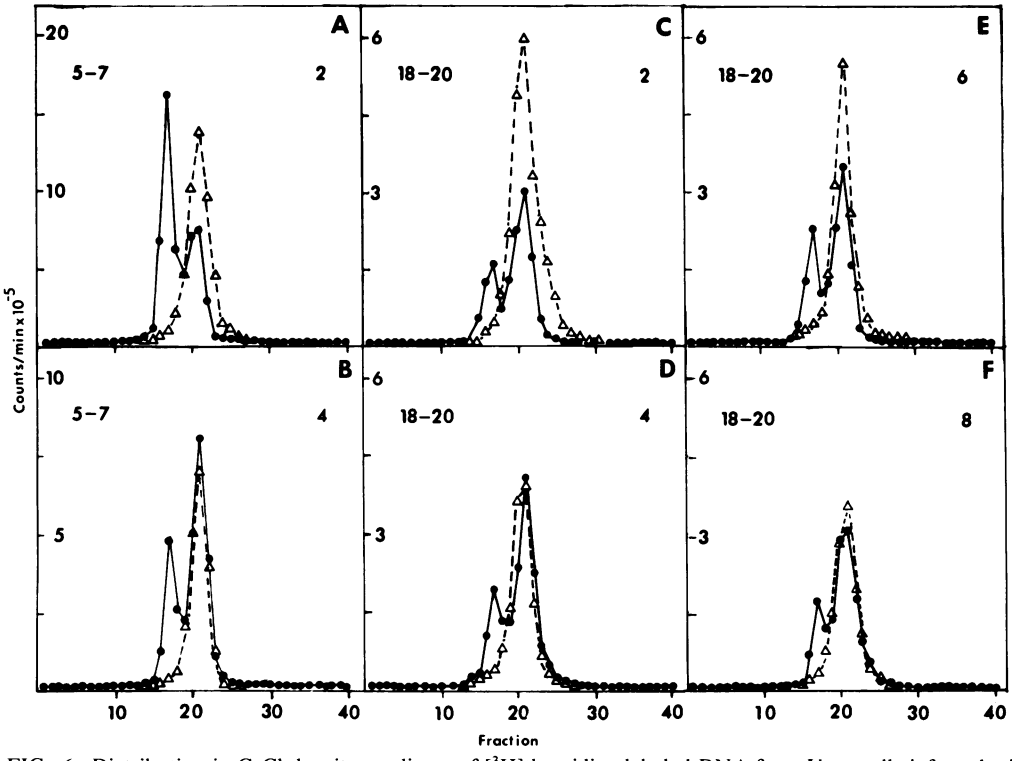

FIG. 6. Distribution in CsCl density gradients of [^{3}H]thymidine-labeled DNA from Vero cells infected with HFEM or *ts*B7 and shifted from 33.5 to 39°C at different times p.i. Symbols: ●, HSV-1(HFEM); △, HSV-1(HFEM) *ts*B7. (A) Shifted 2 h p.i.; labeled at 5 to 7 h p.i. (B) Shifted 4 h p.i.; labeled at 5 to 7 h p.i. (C) Shifted 2 h p.i.; labeled at 18 to 20 h p.i. (D) Shifted 4 h p.i.; labeled at 18 to 20 h p.i. (E) Shifted 6 h p.i.; labeled at 18 to 20 h p.i. (F) Shifted 8 h p.i.; labeled at 18 to 20 h p.i.

experiments, summarized in Table 2, indicate the following.

(i) The ratios of titers at 33.5 and 39°C of the viral progeny obtained from cultures cotransfected with both *ts*B7 DNA and the *Bam*HI-D fragment cloned in pBR322, the *Sal*I-E' frag-

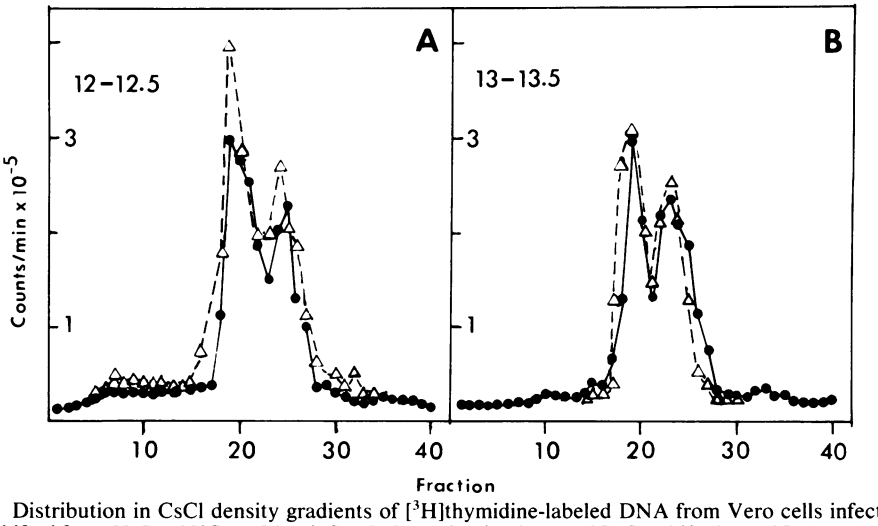

FIG. 7. Distribution in CsCl density gradients of [^{3}H]thymidine-labeled DNA from Vero cells infected with *ts*B7 and shifted from 33.5 to 39°C at 12 h p.i. Symbols: maintained at 33.5°C (●); shifted to 39°C (△). (A) Labeled at 12 to 12.5 h p.i. (B) Labeled at 13 to 13.5 h p.i.

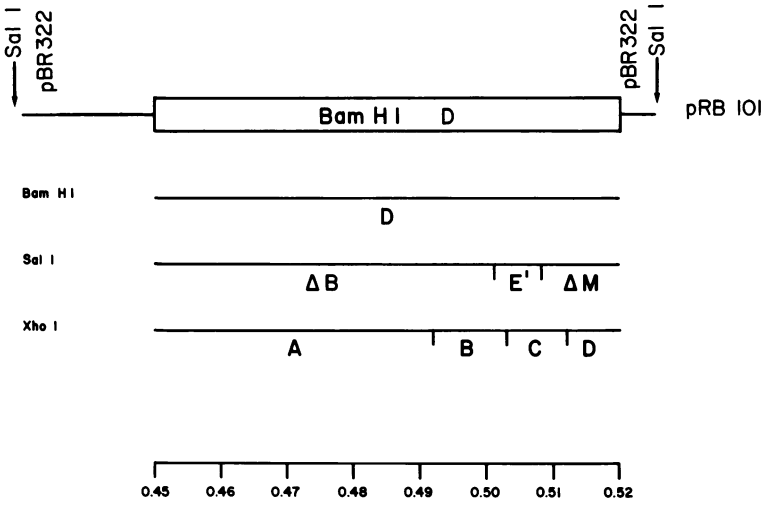

FIG. 8. *Bam*HI, *Sal*I, and *Xho*I maps of the HSV-1(F) *Bam*HI fragment D contained within the plasmid pRB101. The plasmid DNA is shown in linear form, produced by cleavage at the unique *Sal*I site within the pBR322 sequences. The numbers below the lower line refer to map coordinates on the prototype arrangement of HSV-1(F) DNA (10). The symbol Δ indicates that only a portion of the designated *Sal*I or *Xho*I fragment is contained within the *Bam*HI fragment D. Other recombinant plasmids, not shown in this figure, contained the *Sal*I-ΔB, *Sal*I-ΔM, *Xho*I-ΔAD, *Xho*I-B, and *Xho*I-C fragments. The *Xho*I fragments were provisionally designated as *Bam*HI-D/*Xho*I-A, -B, -C, and -D until a complete HSV-1 *Xho*I restriction endonuclease map is constructed.

ment, or the *Xho*I-B fragment cloned in pACYC177 were significantly greater than the ratio of titers of the viral progeny produced when cells were transfected with *ts*B7 DNA alone.

(ii) Cotransfection with *ts*B7 DNA and the cloned fragments *Sal*I-ΔB, *Sal*I-ΔM, *Xho*I-ΔAD, or *Xho*I-C gave rise to viral progeny with plating efficiencies similar to that of the virus produced by transfection of *ts*B7 DNA alone.

From the map positions of these fragments presented in Fig. 8, it is concluded that the *ts*B7 mutation lies within the *Sal*I-E' fragment (map units 0.501 to 0.5085) and maps between the *Sal*I site at 0.501 and the *Xho*I site at 0.503. The *Sal*I-E' and *Xho*I-B fragments overlap by approximately 300 base pairs.

DISCUSSION

Phenotype of HSV-1(HFEM) *ts*B7. In this and a preceding paper (8), it was shown that *ts*B7 possesses several unusual phenotypic properties at the nonpermissive (39°C) temperature. They are specifically the following.

(i) In cells infected and maintained at 39°C, viral capsids containing DNA cores accumulate at the nuclear pores. Consistent with the conclusion that viral DNA is not released from these capsids, viral protein synthesis has not been detected at the nonpermissive temperature. Nevertheless, *ts*B7 was shown to complement other mutants at the nonpermissive temperature in both this and other laboratories (8, 14).

(ii) A shift down from the nonpermissive to the permissive temperature for 30 min at 6 h p.i. resulted in the release of viral DNA cores from a large fraction of the capsids accumulating at the nuclear pores. Consistent with the conclusion that the defect in *ts*B7 is in the release of DNA

TABLE 2. Rescue of HSV-1(HFEM) *ts*B7 with HSV-1(F) DNA fragments

HSV-1 fragment[a]	HSV-1 map position	Ratio of titers[b]	
		Exp 1	Exp 2
None		1.80×10^{-5}	5.20×10^{-5}
*Bam*HI-D	0.495–0.515	2.15×10^{-1}	3.05×10^{-1}
*Sal*I-ΔB	0.455–0.501	1.10×10^{-4}	4.30×10^{-5}
*Sal*I-E'	0.501–0.508	8.9×10^{-2}	ND[c]
*Sal*I-ΔM	0.508–0.515	1.5×10^{-4}	9.35×10^{-5}
*Xho*I-ΔAD	0.455–0.492 0.5115–0.515	4.0×10^{-5}	3.10×10^{-5}
*Xho*I-B	0.492–0.502	6.3×10^{-3}	5.8×10^{-3}
*Xho*I-C	0.502–0.5115	3.75×10^{-5}	2.15×10^{-5}

[a] Marker rescues were done as described in the text. Fragment *Bam*HI-D cloned as pRB101 was used in both experiments. All other fragments were obtained by cleavage of cloned *Bam*HI-D (see Fig. 8) and were either purified from agarose gels (experiment 1) or were cloned in pBR322 or pACYC177 (experiment 2).

[b] Ratio of titers obtained at 39 and 33.5°C.

[c] ND, Not done.

from capsids, incubation for 1 h at the permissive temperature both in the presence and in the absence of inhibitors of protein synthesis resulted in the expression of α and β genes when the cells were shifted to the nonpermissive temperature. Nevertheless, significant and sustained γ protein synthesis did not occur unless the cells were maintained at the permissive temperature for at least 4 h before the shift to 39°C. In accord with this observation, at the permissive temperature, a significant amount of viral DNA synthesis was detected much later in the reproductive cycle of *ts*B7 than in that of the parent virus. However, once viral DNA synthesis began, it was continued at the nonpermissive temperature concomitant with the maintenance of synthesis of γ proteins.

(iii) A significant property of the macromolecular metabolism of *ts*B7 at the nonpermissive temperature is the apparent dissociation of the shutoff of synthesis of α and β proteins from significant and sustained synthesis of γ proteins.

(iv) A characteristic of *ts*B7 is that even at the permissive temperature the plaques are small and retarded relative to the size and time of formation of plaques by the wild-type parent. Both intertypic marker rescue with fragmented HSV-2 DNA and intratypic marker rescue with cloned fragments of HSV-1 DNA yielded virus indistinguishable from wild type with respect to growth at the nonpermissive temperature and plaquing properties. The marker rescue experiments presented here verified and extended the results of intertypic rescues published previously (8) and mapped the lesion in an approximately 300-base-pair region of HSV-1 DNA.

It is of interest to note that a temperature-sensitive mutant of pseudorabies virus was recently reported (5) to be defective in uncoating of the DNA at the nonpermissive temperature. The available data of the phenotypic properties of that mutant are not sufficient to determine whether it corresponds to *ts*B7 with respect to the function of the mutated gene.

Incongruity between the failure of *ts*B7 capsids to release the DNA cores and the complementation of other temperature-sensitive mutants by *ts*B7. The results presented in this paper indicated that a major defect in *ts*B7 expressed at 39°C is due to its failure to release viral DNA cores from nucleocapsids. These data support the hypothesis that gene expression is unlikely at 39°C and that *ts*B7 should not complement or be complemented by other mutants. The complementation data obtained by us and others (8, 14) are therefore puzzling. One conceivable explanation is that a small amount of leakage of *ts*B7 at 39°C permitted complementation of other mutants. This explanation is consistent with the observation that complementation was unidirectional; whereas *ts*B7 complemented the paired mutants, it was itself poorly complemented.

Relation between the defect in DNA release from capsids and the failure to synthesize γ proteins and DNA in cultures shifted to 39°C after less than 4 h at the nonpermissive temperature. A necessary and significant conclusion of the results is that the reproductive cycle of *ts*B7 contains a second, temperature-sensitive event which occurs after the release of HSV DNA but before the onset of viral DNA synthesis. The nature of the event or the viral gene product whose defect it reflects is uncertain. Even though marker rescue studies place the location of the lesion within a small region of the viral genome, we cannot differentiate between the hypothesis that the mutations are in overlapping genes involved in both packaging and release of DNA from capsids and in initiation of viral DNA synthesis and the alternate hypothesis that the mutation is in a single gene responsible for both events. It is conceivable, for example, that release of viral DNA is actuated by proteins bound to viral DNA and that because of the mutation in these proteins, initiation of DNA synthesis upon the release of viral DNA is retarded at the permissive temperature and does not occur at the nonpermissive temperature.

Synthesis and interrelation between γ proteins and other protein groups in *ts*B7-infected cells shifted to the nonpermissive temperature at different times after infection. Two phenomena observed in the course of these studies are of interest and are not entirely explainable by the available data. First, the failure of infected cells to make significant amounts of γ proteins following a shift to the nonpermissive temperature after less than 4 h at the permissive temperature could be explained by the observation that γ protein synthesis is dependent on the availability of competent α and β proteins and on initiation of DNA synthesis. It is less clear why γ protein synthesis was not sustained upon prolonged incubation at 39°C. One hypothesis which could explain the results is that in the absence of DNA synthesis, the structure of *ts*B7 DNA becomes altered, blocking the synthesis of γ proteins. It is likely that the change involves a function of the mutated gene product and does not involve destruction of the viral DNA inasmuch as the infected cells resume viral replication upon being shifted to the permissive temperature after 12 h at the nonpermissive temperature.

The second phenomenon is the apparent dissociation of the shutoff of α and β protein synthesis from synthesis of γ proteins. One simple hypothesis consistent with available data on regulation of HSV gene expression is that the transient expression of the γ proteins resulted in the shutoff of gene expression of genes ex-

pressed early in infection. Our results cannot exclude the hypothesis that trace amounts of γ genes produced even in the absence of significant DNA synthesis are sufficient to shut off β genes or even that the β genes are autoregulatory.

Little is known of the function of the gene carrying the *ts*B7 lesion other than the observation that the region in which it resides appears to be expressed after the onset of DNA synthesis (7). Identification of the gene product and the nature of its interaction with viral DNA should help elucidate its functions in the reproductive cycle.

ACKNOWLEDGMENTS

These studies were aided by Public Health Service grants CA-08494 and CA-19264 from the National Cancer Institute and by American Cancer Society grant MV-2Q. W.B. and D.F. were Public Health Service postdoctoral trainees (grant 5 T32 PHS AI 07812 from the National Institutes of Health).

We thank Shu-Fen Chou for excellent assistance with electron microscopy.

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