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Molecular Genetics of Herpes Simplex Virus

VI. Characterization of a Temperature-Sensitive Mutant Defective in the Expression of All Early Viral Gene Products

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The herpes simplex virus 1 (HFEM) mutant *ts*B7 failed to express any detectable viral polypeptides and did not significantly inhibit host cell protein synthesis in infected cells maintained at the nonpermissive temperature. The mutant could complement the growth of a coinfecting temperature-sensitive mutant virus differing in plaque phenotype and thus appeared capable of penetrating doubly infected cells. The yield of *ts*B7 was enhanced by the coinfecting virus but not to the extent that the coinfecting virus was enhanced. Coinfection studies suggested that the *ts*B7 defect was complemented in *trans*, but poorly, by the wild-type parent and other viruses. Marker rescue of *ts*B7 by transfection with herpes simplex virus 2 *Xba*I DNA fragments mapped the mutation between 0.45 and 0.70 map units. Analysis of the DNA structure of the ts^+ intertypic recombinants generated by this rescue showed that the herpes simplex virus 2 DNA substitutions all contained the region between 0.46 and 0.52 map units, thus further defining the map position of the mutation. Analyses of the polypeptides expressed by these intertypic recombinants defined the genome location of the genes specifying polypeptides 2, 6, 10, 32, 43, and 44 and indicated that the mutation maps in or near genes coding for virion structural polypeptides. This region of the genome is represented as stable transcripts and cytoplasmic mRNA only after viral DNA replication (P. C. Jones and B. Roizman, J. Virol. 31:299-314, 1979), and thus this gene appears to be a late function. These results are consistent with the *ts* mutation in *ts*B7 being in a gene coding for a virion component which functions before expression of the alpha genes early in infection. The most likely explanation is that the mutant is blocked at a stage of uncoating and the defect is complemented, although poorly, by a coinfecting virus gene product.

In this paper, we report on the characteristics of the herpes simplex virus 1 (HSV-1) temperature-sensitive mutant *ts*B7. This mutant fails to produce any detectable viral polypeptides and has no appreciable long-term effect on the synthesis of host proteins at the nonpermissive temperature. Pertinent to this report are the following.

(i) HSV-1 and herpes simplex virus 2 (HSV-2) are genetically closely related (14). Both HSV-1 and HSV-2 specify approximately 50 polypeptides (12, 23) which form at least three groups whose synthesis is coordinately regulated and sequentially ordered in a cascade fashion (12, 25). The α polypeptides made immediately after infection of permissive cells with HSV-1 attain peak rates of synthesis between 2 and 4 h postinfection and require no viral protein synthesis for the synthesis and processing of their mRNA. These polypeptides are required for the synthesis of β polypeptides, and these in turn shut off the synthesis of α polypeptides; the β polypeptides are involved in the synthesis of viral DNA and induce the synthesis of structural γ polypeptides.

(ii) In the course of their replication, herpes simplex viruses shut off the synthesis of host proteins and DNA and reduce the synthesis of host RNA (22, 29, 30). The shutoff of the synthesis of host proteins appears to occur in two stages. The first occurs immediately after infection and is mediated by a structural protein inasmuch as it does not require the transcription of the viral genome (6). The second stage requires the expression of viral protein and appears to coincide with the synthesis of β poly-

peptides (12, 21). In general, HSV-2 isolates inhibit host protein synthesis more rapidly and more completely than HSV-1 during the first stage of inhibition of host macromolecular synthesis (2, 22). Analysis of HSV-1 × HSV-2 intertypic recombinants showed that the HSV-2 gene(s) responsible for the accelerated shutoff of host protein synthesis maps between 0.52 and 0.59 map units (5, 20).

MATERIALS AND METHODS

Viruses. The isolation and properties of HSV-2 (G) and HSV-1 (HFEM) ts^+ *syn* are described in references 4 and 9, respectively. HSV-1 (HFEM) *ts*B7 syn^+ was isolated by bromodeoxyuridine mutagenesis of HSV-1 (HFEM)-infected cells. HFEM originally contained a mixture of *syn* and syn^+ viruses, and the *ts*B7 mutant apparently arose from a syn^+ virus. HSV-1 (F) is a primary isolate used extensively in the Chicago laboratory and has been passaged no more than four times in cell culture at 34°C (4, 11). We have found that this strain replicates poorly at 39°C and that the efficiency of plating at 39°C relative to 34°C is less than 10^{-5}. It does replicate well at 37°C, as its efficiency of plating at 37°C relative to 34°C is 0.5. This phenotype is not unusual for fresh isolates of HSV-1, since many other isolates tested showed similar properties (D. M. Knipe and B. Roizman, unpublished observations). The temperature-sensitive marker in HSV-1 (F) maps in the repeated sequences of the S component, 0.83 to 0.865 and 0.965 to 1.0 map units (P. J. Godowski and D. M. Knipe, unpublished data). The virus expresses largely α polypeptides at 39°C, and therefore the wild-type HSV-1 (F) strain produces a temperature-sensitive α product similar to those of *ts* mutants in complementation group 1-2 (28).

HSV-1 (F) *ts*502 *syn*1,2 is the designation of a recombinant produced by marker transfer of the *syn* loci from HSV-1 (1061) to HSV-1 (F) (27). At the permissive temperature it fuses both HEp-2 and Vero cells.

Virus stocks were prepared and titrated in Vero cell cultures. The infected cells were maintained in medium 199 supplemented with 1% inactivated calf serum.

Complementation tests. Complementation tests were performed by a modification of the procedure of Schaffer et al. (28). After addition of virus to the cultures, the flasks were submerged in a 39°C water bath or a 39°C convection incubator. After the 1-h absorption period, the virus inoculum was removed and 5 ml of medium 199–1% inactivated calf serum was added. After 18 h of incubation, the virus was harvested and titrated. Equivalent results were obtained in experiments in which the cells were or were not washed at the end of the absorption period.

Marker rescue mapping. Mapping of the *ts* lesion in *ts*B7 was performed by the cotransfection of mutant DNA and individual fragments of HSV-2 (G) DNA generated by cleavage of the DNA with *Xba*I restriction endonuclease (6, 15). The flasks were incubated for 3 to 4 days at 34°C, and the progeny virus was harvested and titrated at 34 and 39°C. The rescued ts^+ clones were isolated by four cycles of plaque purification under agarose overlay at 39°C (19).

Purification of viral DNA and analysis of recombinant genomes. Viral DNA for the marker rescue studies and for analysis of recombinant genomes was purified by NaI density gradient centrifugation of infected cell extracts (31). *Hsu*I, *Bgl*II, *Xba*I, and *Hpa*I restriction endonucleases were prepared as described previously (19). *Bam*HI and *Kpn*I restriction endonucleases were purchased from New England Biolabs.

Analysis of polypeptides in infected and mock-infected cells. Cells were labeled with L-*U*-^{14}C-labeled amino acids (leucine, isoleucine, and valine) at times specified in the text and under conditions described by Morse et al. (20). At the end of the labeling interval, the cells were harvested, disrupted with sodium dodecyl sulfate, and subjected to electrophoresis in polyacrylamide slab gels as described (20).

RESULTS

Replication of HSV-1 (HFEM) *ts*B7. The efficiency of plaque formation by *ts*B7 was 1×10^5- to 3×10^5-fold lower at 39 than at 34°C. The yield of infectious virus from cultures infected at a multiplicity of 5 PFU/cell and maintained at 39°C was 10^2- to 10^3-fold lower than that obtained from replicate cultures maintained at 34°C. The viral progeny obtained from the infected cultures incubated at 39°C were still temperature sensitive and did not represent revertants. Therefore, at high multiplicities of infection the mutant showed some leakiness. At 34°C the virus formed plaques which were much smaller than those of its parent, HFEM, and which increased in size slowly.

Transient temperature fluctuations below 39°C allowed the formation of very small plaques and expression of viral polypeptides; thus, the phenotype of *ts*B7 was very sensitive to temperature fluctuation. For this reason all experiments were done in flasks submerged in a water bath maintained at 39°C or in a convection-heated incubator with strict temperature control.

Polypeptide expression by *ts*B7. To examine the proteins specified by the mutant virus at the permissive and nonpermissive temperatures, we infected cultures of cells with HSV-1 (HFEM) and *ts*B7, incubated the cultures at 39 or 33°C, and labeled them with [^{35}S]methionine. The labeled cell extracts were then subjected to gel electrophoresis (Fig. 1). At 33°C, *ts*B7 expressed a pattern of polypeptides similar to that of HFEM except that certain early proteins (infected cell polypeptides [ICP]6, -8, and -38) were overrepresented and certain late proteins (ICP10, -15, -19, and -39) were underrepresented in the mutant profile, presumably because the mutant infection had not progressed as far as

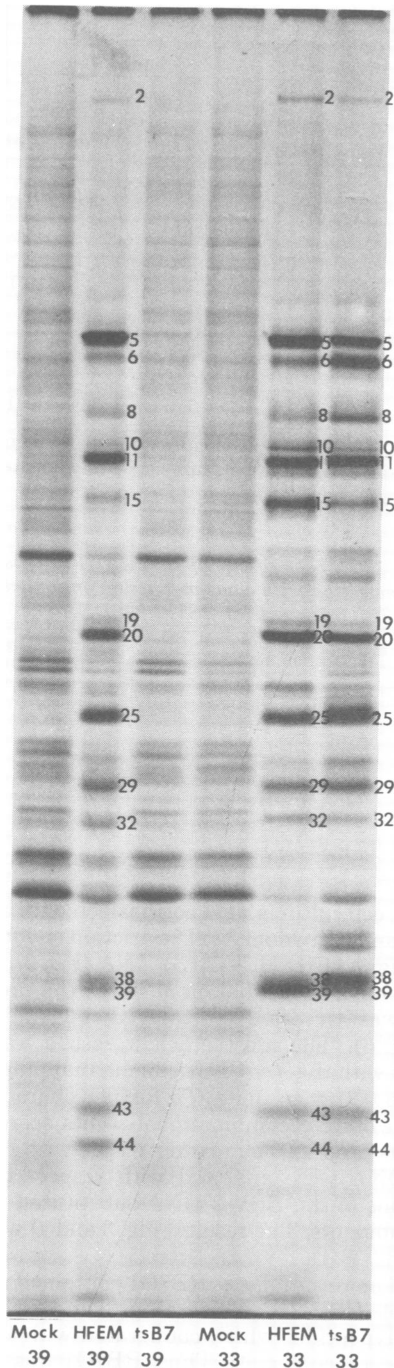

FIG. 1. *Autoradiographic images of polypeptides extracted from HEp-2 cells infected with HSV-1 (HFEM) tsB7 or HSV-1 (HFEM) or mock infected and electrophoretically separated in sodium dodecyl sulfate-polyacrylamide gels. The infected cells were incubated at temperatures shown and were labeled with ^{14}C-amino acids from 18 to 20 h postinfection.*

the wild-type infection. In all infections performed at 33°C, the infections initiated by *ts*B7 progressed more slowly than those initiated by HFEM. This is consistent with the slow plaque formation by the mutant virus.

At 39°C, HFEM expressed a pattern of polypeptides equivalent to that at 33°C, except that some late proteins (ICP10, -19, and -39) were made at lower levels at 39°C. However, at 39°C *ts*B7 did not express any detectable viral polypeptides. The pattern of proteins labeled in cells infected with *ts*B7 at 39°C was very similar to the pattern of polypeptides from mock-infected cells at 39°C, with at most a very slight decrease in intensity of each cellular polypeptide band. Thus, *ts*B7 did not significantly inhibit host cell protein synthesis at 39°C.

Complementation of other mutants by *ts*B7. Schaffer et al. (28) previously reported that *ts*B7 could complement other temperature-sensitive mutants, but in those studies the viruses were absorbed for 1 h at 37°C. We have found that, under these conditions of infection, *ts*B7 will express many of the late viral proteins and is therefore leaky (W. Batterson and B. Roizman, unpublished data). We therefore reexamined the question of the ability of *ts*B7 to perform complementation under conditions where it expresses no proteins, i.e., continual maintenance at 39°C.

In this series of experiments, cultures of Vero cells were individually or doubly infected with *ts*B7, *ts*502, or HFEM. Cells coinfected with *ts*B7 showed a 100-fold increase in the yield of *syn* plaques (*ts*502 marker) and a twofold increase in the *syn*$^+$ plaques (*ts*B7 marker) relative to the singly infected cultures (Table 1). The increase in yield was not due to recombination, since *ts*$^+$recombinants constituted no more than 1% of the virus yield from the doubly infected cells. It should be noted that most of the *ts*$^+$ recombinants were *syn*$^+$, like *ts*B7. A high frequency of the *ts*$^+$ recombinants would be expected to be *syn*$^+$ in that the *syn*1,2 mutations (0.70 to 0.83 map units) are closer to *ts*502 (0.83 to 0.865 and 0.965 to 1.0 map units) than to the map location for *ts*B7, shown below to be 0.46 to 0.52 map units.

The observation that in the complementation test the increase in titer of *ts*502 was greater than the increase in the *ts*B7 titer raised the possibility that *ts*B7 contained a *cis*-acting mutation that causes it to replicate poorly. To examine this question, we first coinfected cells with *ts*B7 and *ts*502 at 34°C and examined the prog-

The infected cell polypeptides were numbered according to the nomenclature published elsewhere (12, 20).

TABLE 1. *Tests for complementation between tsB7 and ts502*[a]

Complementation test									
39°C						34°C			
Virus (PFU/cell)		Yield (10^5)				Virus (PFU/cell)		Yield (10^6), 34°C	
		34°C		39°C					
*ts*502	*ts*B7	*ts*502	*ts*B7	*ts*502	*ts*B7	*ts*502	*ts*B7	*ts*502	*ts*B7
5		0.64		10^{-5}		0	1		49
10		1.33		10^{-5}		0	5		150
	5		11.7		1.4×10^{-2}	1	0	96	
	10		16.0		1.5×10^{-2}	5	0	153	
						5	1	278	20
5	5	207	32.0	0.19	1.85	5	5	172	62
						1	5	56	117

[a] Cells were infected with each mutant alone or with mixtures of both at multiplicities shown and incubated at 39 and 34°C. The viral progeny produced in these cells was titrated at temperatures shown. The titers for *ts*502 and *ts*B7 are based on the plaque morphology types of the progeny.

eny at 34°C. Again the *syn* or *ts*502 progeny were favored, although by a lower ratio than from the cultures infected at 39°C. Thus, the poor growth of *ts*B7, even at the permissive temperature, could explain in part the poor yield of *ts*B7 from the complementation experiments.

We also examined the ability of HFEM ts^+ *syn* to complement *ts*B7 syn^+ at 39°C. The yield of syn^+ viruses was significantly greater in cultures coinfected with HFEM ts^+ *syn* than in singly infected cultures (Table 2). Thus, HFEM could complement the replication of *ts*B7 at 39°C. However, the yield of syn^+ virus was approximately 10% of the yield of the *syn* virus, even though all cells were coinfected with both. Therefore, even the wild-type parent of *ts*B7 could only partially complement *ts*B7. This is in contrast with the situation when HFEM and *ts*502 coinfected cells. The progeny virus from cells infected with these two mutants showed an efficiency of plating (39/34°C) of approximately one-half that of the progeny from a culture infected with HFEM alone (Table 2). Thus, approximately one-half of the progeny from the doubly infected cells were temperature sensitive. Under these conditions of infection, the progeny contained approximately equal amounts of the two infecting viruses.

The mutant *ts*B7 appeared to have a partial *cis*-acting phenotype, although it could apparently be complemented by coinfecting viruses. As discussed further below, a partial complementation of an uncoating mutant would give a partial *cis*-acting mutant phenotype.

Marker rescue of *ts*B7 with HSV-2 DNA fragments. The genome location of the *ts* lesion in *ts*B7 was determined by the cotransfection method of marker rescue (16). In these experiments, cell cultures were cotransfected with *ts*B7 DNA and individual *Xba*I restriction endonuclease fragments of HSV-2 (G) DNA (Fig. 2). After 3 to 4 days of incubation at 34°C, the progeny virus was harvested and assayed at 34 and 39°C (Table 3). The most efficient rescue was obtained with the *Xba*I-D fragment, mapping from 0.45 to 0.71 map units. To further characterize the site of the recombinational events, six recombinants produced by marker rescue with HSV-2 (G) DNA were analyzed with respect to the domains of the HSV-2 DNA substituted in the recombinants. The results (Fig. 3 and 4) were as follows.

TABLE 2. *Marker rescue of HSV-1 (HFEM) tsB7 by cotransfection with XbaI restriction endonuclease fragments of HSV-2 (G) DNA*

Transfection mixture	Ratio of titers, 39/34°C (10^3)
*ts*B7 DNA alone	0.01
+ *Xba*I-A-C	1
+ *Xba*I-D	25
+ *Xba*I-G	1.8
+ *Xba*I-H	0.3
+ *Xba*I-I	0.1
+ *Xba*I-J	0.5

(i) The recombinant RB7G1 contained no detectable HSV-2 sequences (Fig. 3) and expressed no polypeptides which comigrated with HSV-2 polypeptides (Fig. 4). Thus, RB7G1 was either a revertant or a recombinant containing too small a replacement of HSV-1 sequences to be detected.

(ii) The recombinant RB7G2 retained the HSV-1 *Eco*RI-M-O and the *Hpa*I-F-E cleavage sites but lost all intervening HSV-1 cleavage sites. Therefore, the maximal left boundary of

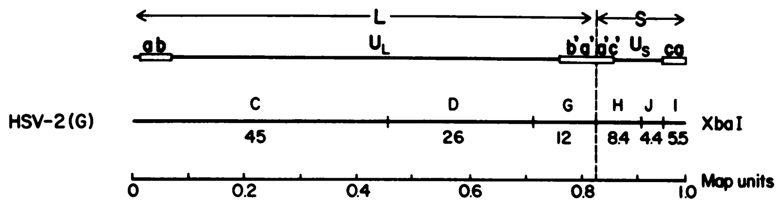

FIG. 2. *Schematic diagram of the sequence arrangement and XbaI restriction endonuclease maps of DNA fragments tested in marker rescue studies. L and S represent the long and short covalently linked components of HSV-DNA, and U_L and U_S represent the unique sequences of each component, respectively; ab and b'a' represent the inverted repeated sequences bounding the L component, whereas a'c' and ca represent the inverted repeated sequences bounding the S component. The letters above the line denote the name of the fragment; the number below the line is the molecular weight of the DNA fragment in millions.*

TABLE 3. *Coinfection of tsB7 and HFEM ts^+ syn*[a]

Infecting virus	34°C titer		39°C titer		39/34°C ratio
	syn	*syn*$^+$	*syn*	*syn*$^+$	
*ts*B7		$<10^3$		$<10^3$	
HFEM *syn*	5×10^6		3.5×10^6		0.7
*ts*502	10^5		$<10^3$		10^{-2}
*ts*B7 + HFEM	6×10^6	6×10^5	3.5×10^6		0.58
*ts*502 + HFEM	6.3×10^6		2.5×10^6		0.39

[a] Cultures were infected with the indicated viruses at 39°C. After 18 h of incubation, the progeny virus was harvested and titrated at 34 and 39°C.

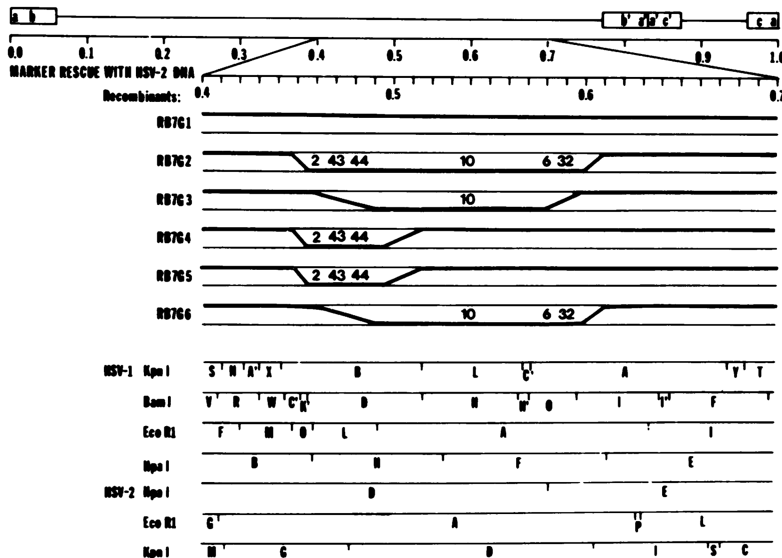

FIG. 3. *Summary of the rescue of the HSV-1 (HFEM) tsB7 with HSV-2 DNA. Diagram shows the location of the HSV-2 sequences replacing HSV-1 sequences in recombinants produced by marker rescue of tsB7 with HSV-2 DNA. The top and bottom lines next to the designation of each recombinant represent HSV-1 and HSV-2 DNA, respectively. The heavy line represents the sequences identified in each recombinant. The numbers above the heavy line identify the HSV-2 ICPs produced by the recombinants and shown in Fig. 4. The mapping of the DNA sequences in the recombinants is based on the restriction endonuclease maps for HSV-1 and HSV-2 shown on the bottom. The HSV-1 KpnI and BamHI of HSV-1 (HFEM) tsB7 were determined from the maps for HSV-1 (F) produced by Locker and Frenkel (18).*

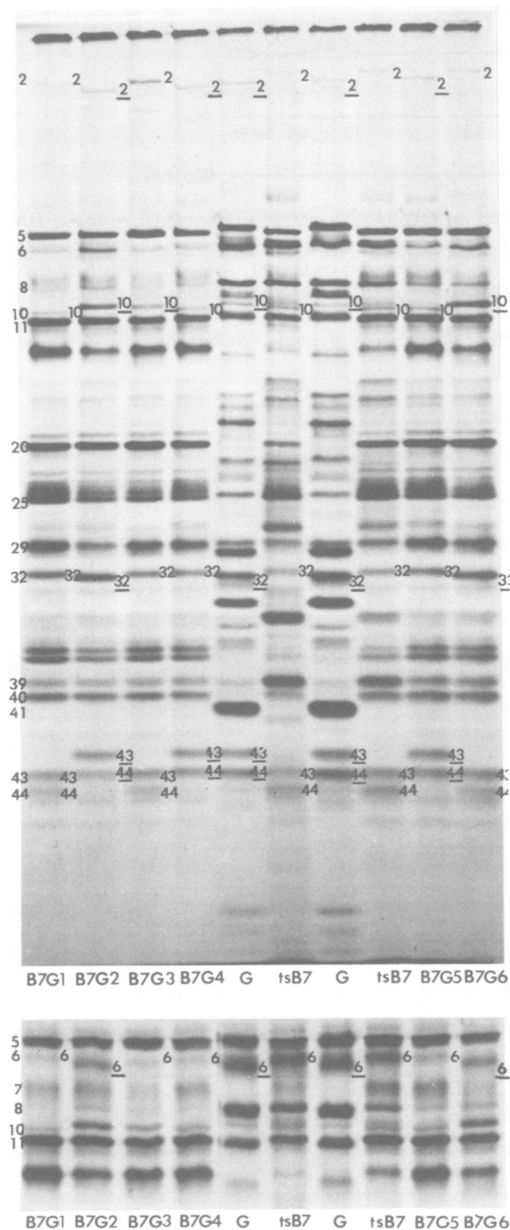

FIG. 4. *Autoradiographic images of the polypeptides extracted from HEp-2 cells infected with HSV-2 (G), HSV-1 (HFEM) tsB7, and recombinants produced by marker rescue of tsB7 with HSV-2 (G) DNA and electrophoretically separated in sodium dodecyl sulfate-polyacrylamide gels. The cells were labeled with ^{14}C-amino acids from 10 to 12 h postinfection. The polypeptides are identified by number according to the nomenclature of Honess and Roizman (12) and Morse et al. (20). The HSV-2 ICPs are identified by a dash under the number. The insert on the bottom illustrates a portion of a gel showing a better separation of HSV-1 and HSV-2 ICP6.*

the recombinational event was the HSV-1 *Eco*RI-M-O cleavage site, and the minimal left boundary was the HSV-1 *Hpa*I-B-H cleavage site. The maximal right boundary was the HSV-1 *Hpa*I-E-F cleavage site, and the minimal right boundary was the *Bam*HI-O-I cleavage site (Fig. 3). This recombinant expressed the ICP2, -6, -43, -44, -10, and -32 of HSV-2 (Fig. 4).

(iii) The recombinant RB7G3 retained the HSV-1 *Eco*RI-O-L cleavage site and the *Bam*HI-O-I cleavage site but lost all intervening HSV-1 cleavage sites. The maximal left boundary was the HSV-1 *Eco*RI-O-L cleavage site, and the minimal left boundary was the *Eco*RI-L-A cleavage site. The maximal right boundary was the HSV-1 *Bam*HI-O-I cleavage site, and the minimal right boundary was the HSV-2 *Hpa*I-D-E cleavage site. This recombinant specified the HSV-2 ICP10 (Fig. 4).

(iv) The recombinants RB7G4 and RB7G5 retained the HSV-1 *Eco*RI-M-O cleavage site and the HSV-1 *Bam*HI-D-H cleavage site but lost all intervening HSV-1 cleavage sites. Therefore, the maximal left boundary of the recombinational events was the *Eco*RI-O-L cleavage site, and the minimal left boundary was the HSV-1 *Hpa*I B-H cleavage site. The maximal right boundary was the *Bam*HI cleavage site, and the minimal right boundary was the HSV-1 *Eco*RI-L-A cleavage site. These recombinants gained one new *Kpn*I cleavage site consistent with the position of the HSV-2 *Kpn*I-G-D cleavage site. These recombinants expressed the HSV-2 ICP2, -43, and -44 (Fig. 4).

(v) The recombinant RB7G6 retained the HSV-1 *Hpa*I-B-H and *Hpa*I-F-E cleavage sites but lost all intervening HSV-1 cleavage sites. The maximal left boundary was the HSV-1 *Hpa*I-B-H cleavage site, and the minimal left boundary was the HSV-1 *Eco*RI-L-A cleavage site. The maximal right boundary was the *Hpa*I-F-E cleavage site, and the minimal right boundary was the HSV-1 *Bam*HI-O-I cleavage site. This recombinant expressed the HSV-2 ICP6, -10, and -32 (Fig. 4).

In summary, the region of HSV-2 DNA replacement common to all recombinants was the region between the HSV-1 *Eco*RI-O-L cleavage site and the *Bam*HI-D-H cleavage site, or map positions 0.46 to 0.52. This further defines the map position of the *ts* mutation in *ts*B7. The maximal boundaries for the type-specific determinants of the polypeptides were ICP2, -43, and -44 (the *Eco*RI-M-O cleavage site to the *Eco*RI-L-A cleavage site); ICP10 (the *Eco*RI-L-A cleavage site to the *Bam*HI-O-I cleavage site); and ICP6 and -32 (the HSV-2 *Hpa*I-D-E cleavage site to the HSV-1 *Hpa*I-F-E cleavage site). The *ts* mutation in *ts*B7 was also closely linked

to the ICP10 type-specific determinant (three of seven recombinants) and the ICP2, -43, and -44 type-specific determinants (three of seven recombinants).

DISCUSSION

We have presented evidence that the mutant HSV-1 (HFEM) *ts*B7 expresses no detectable viral polypeptides and does not significantly inhibit host cell protein synthesis in infected cells maintained at the nonpermissive temperature. Furthermore, the mutant can complement the growth of a coinfecting virus. Thus, the mutant viral genome can penetrate doubly infected cells. The block at the nonpermissive temperature appears to occur after entry of the virus into the host cell and before the expression of the alpha gene products.

Complementation studies with *ts*B7. The mutant *ts*B7 was clearly shown to be capable of complementing the replication of a coinfecting temperature-sensitive mutant which maps within the region of the genome encoding ICP4 (Godowski and Knipe, unpublished data) and which expresses largely alpha proteins at the nonpermissive temperature (L. Pereira and B. Roizman, unpublished data). Therefore, in order for *ts*B7 to complement *ts*502, *ts*B7 must express ICP4. Because *ts*B7 does not express ICP4 in singly infected cells at any detectable level, it appears that *ts*502 must supply the defective gene function in order for *ts*B7 to express ICP4. The mutated *ts*B7 gene function must be (at least in part) a *trans*-acting gene product. However, we observed that the yield of *ts*B7 was always lower than the yield of the coinfecting virus when cells were infected at 39°C. Therefore, it appears that the mutation in *ts*B7 is partially *cis*-acting in nature; i.e., the mutation cannot be fully complemented by a coinfecting virus. It should be noted that a defect in uncoating which is partially complemented by a coinfecting virus would give reduced yields of the mutant virus defective in uncoating because the effective number of genomes participating in replication would be less for the uncoating mutant. Because of the time of the block in replication in *ts*B7-infected cells, a defect in uncoating of the *ts*B7 genome inside infected cells is a possible explanation for the mutant defect. An alternative explanation that we cannot rule out completely at the present time is that a very small, undetectable amount of early proteins is made from the *ts*B7 genome which can complement the growth of *ts*502. If the block in replication of *ts*B7 were in uncoating, this explanation would also lead to higher yields of *ts*502 than *ts*B7.

The *ts*B7 mutation differs from the two other classes of viral mutants that show a complete lack of viral gene expression under nonpermissive conditions, i.e., the *ts*3 mutant of polyoma virus (3), the *ts*D mutants of simian virus 40 (24), and the group I host range mutants of adenovirus (1, 10). The simian virus 40 and polyoma mutants map in the late region of the genome (8, 17) and appear to be defective in uncoating of the viral DNA (3, 24). Therefore, the DNA of these mutants cannot be transcribed to yield early mRNA. These mutants are unable to complement other coinfecting mutants, and thus this uncoating function is a *cis*-acting function (3).

The group I host range mutants of adenovirus map in the early region I of the viral genome, and only the mRNA's for that specific region are expressed from the mutant genome in nonpermissive cells (1, 7). This appears to be a "pre-early" gene function whose synthesis is needed for expression of the early genes. The mutation of *ts*B7 differs from these because it can be complemented by other viruses, and the existing evidence strongly argues that the α gene products require no prior viral protein synthesis (12, 13). Several different inhibitors of alpha protein synthesis have been used to allow accumulation of α mRNA. There is no evidence to suggest that a "pre-early" protein gene product must be synthesized before expression of the α genes.

Map position of the *ts* mutation of *ts*B7. The marker rescue mapping of the *ts* mutation of *ts*B7 by analysis of intertypic recombinants determined the map position of the lesion to be within 0.46 to 0.52 map units. This location has been confirmed by fine-structure mapping using cloned HSV-1 (F) DNA fragments (Batterson and Roizman, work in progress). Therefore, the *ts* mutation seems likely to map in this region of the viral DNA. This region of the genome is represented in stable cytoplasmic mRNA only after viral DNA replication (13). It therefore seems likely that the *ts*B7 mutation is within a gene expressed after DNA replication. Furthermore, because most of the genes expressed after DNA replication code for virion structural polypeptides, it also seems likely that the *ts*B7 lesion is within a structural polypeptide gene.

We have used the intertypic recombinants generated by rescue of *ts*B7 with HSV-2 DNA to determine the viral polypeptides encoded near the *ts*B7 lesion. None of the viral polypeptides which show type-specific mobilities on polyacrylamide gels correlated exactly with the *ts*B7 lesion; thus, none can be said to contain the lesion. However, the type-specific determinants for ICP2, -10, -43, and -44 were transferred along with the rescuing sequences in three of the seven recombinants analyzed. Thus, the genes for

these proteins are closely linked to the *ts*B7 locus.

As indicated in the beginning of this paper, the shutoff of host protein and DNA synthesis occurs in two stages. The mutant *ts*B7 did not significantly inhibit host cell protein synthesis as assayed by the pattern of host cell proteins displayed on polyacrylamide gels. In addition to the experiments cited in the text, other experiments involving infection of sparsely seeded cell cultures incubated at the nonpermissive temperature indicated that the infected cells were able to multiply for at least 3 days, but that subsequent shiftdown to the permissive temperature resulted in the destruction of the cells (Batterson and Roizman, work in progress). Thus, the effect on the cells of infection with *ts*B7 at the nonpermissive temperature seems to be minimal. Previous studies have mapped the accelerated shutoff by HSV-2 virions to the region between 0.52 and 0.79 map units (5, 20) or near the *ts*B7 lesion. Therefore, it is conceivable that the two functions are encoded within the same gene. It is still unclear whether the lack of host shutoff by *ts*B7 is due to the lack of expression of viral gene products in cells infected at the nonpermissive temperature. Thus, we cannot determine at this time whether the two functions are within the same gene, whether they are in different genes and both are lost by *ts*B7, or whether the two functions are in different genes and only one is lost by *ts*B7. Further studies are in progress to discriminate among these possibilities.

Nature of the *ts*B7 defective gene product. The current evidence suggests that the defective gene product in *ts*B7 is a virion component that can be complemented by a gene product contributed by another coinfecting virus. This gene product could act upon the *ts*B7 capsid to uncoat the viral DNA or to allow it to express the α genes. Alternatively, it could alter the host RNA polymerase so that it can transcribe the viral genome efficiently. These functions would be required only for infections initiated by virions because it is known that rigorously deproteinized HSV is infectious, albeit at lower efficiencies than virions. Therefore the *ts*B7 gene function appears to be a late viral gene product that functions early in the viral replication cycle.

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