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Control of Expression of the Herpes Simplex Virus-Induced Deoxypyrimidine Triphosphatase in Cells Infected with Mutants of Herpes Simplex Virus Types 1 and 2 and Intertypic Recombinants

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Infection of cells with herpes simplex virus type 1 (HSV-1) induces high levels of deoxypyrimidine triphosphatase. The majority of the enzyme activity is found in infected cell nuclei. A similar activity is induced by HSV type 2 (HSV-2) which, in contrast to the HSV-1 enzyme, fractionates to more than 99% in the soluble cytoplasmic extract. Of a series of temperature-sensitive mutants of HSV-1 studied, only the immediate-early mutants in complementation group 1-2 (strain 17 mutants *ts*D and *ts*K and strain KOS mutant *ts*B2) induced reduced levels of triphosphatase at nonpermissive temperature. Of a series of temperature-sensitive mutants of HSV-2 strain HG52, *ts*9 and *ts*13 failed to induce wild-type levels of the enzyme at nonpermissive temperature; *ts*9 was the most defective mutant with regard to triphosphatase expression of both herpes simplex virus serotypes. After shift-up from permissive to nonpermissive temperature, triphosphatase activity in cells infected with *ts*9 decreased rapidly, whereas all other mutants continued to exhibit enzyme levels comparable with controls kept at the permissive temperature. The type 1-specific nuclear expression of the triphosphatase was mapped physically by the use of HSV-1 × HSV-2 intertypic recombinants, based on enzyme levels different by more than two orders of magnitude found in nuclei of HSV-1- and HSV-2-infected cells. The locus for the type-specific expression maps between 0.67 and 0.68 fractional length on the HSV genome.

Recently, we reported on the presence of a virus-specific deoxypyrimidine triphosphatase (dPyTPase) in nuclei from herpes simplex virus type 1 (HSV-1)-infected cells (22). The enzyme catalyzes the degradation of dTTP, dCTP, and dUTP to their respective monophosphates and PP_i and requires Mg^{2+} or Mn^{2+}; the dPyTPase is active at 4°C and, under these conditions, specific for virus-infected cells. Although dUTP is the best substrate, the HSV-1 enzyme will, in contrast to deoxyuridine triphosphatases (dUTPases) from other systems, also degrade dCTP and dTTP, though at a lower rate. In its extended substrate specificity it thus resembles the viral deoxypyrimidine kinase.

In our previous report (22) we mentioned that nuclei from HSV type 2 (HSV-2)-infected cells lack a comparable activity. In this paper we demonstrate that HSV-2 does induce a dPyTPase which, in contrast to the type 1 enzyme, fractionates into the cytoplasmic (soluble) extract. The studies presented here bear on the difference between the two serotypes of HSV with respect to the expression of the dPyTPase.

The difference in the distribution of the triphosphatase made it seem possible to define the physical map position of the locus controlling the type specificity of dPyTPase expression by analyzing the enzyme levels induced by HSV-1 × HSV-2 intertypic recombinants. Such recombinants have been useful to map viral gene products physically, based on differences in electrophoretic mobility or differential physicochemical and immunological properties of homologous virus-specific proteins (see, for example, references 8, 13, and 15).

Data obtained with two laboratory strains each of HSV-1 and HSV-2, several temperature-sensitive mutants of both serotypes, and intertypic recombinants substantiate the following points: (i) the dPyTPase of type 1 is predominantly nuclear, whereas that of type 2 is exclusively cytoplasmic; (ii) several genetic loci control the full expression of dPyTPase activity, in particular mutants in complementation group 1-2, but only one of them (complementation group 2-4) is continuously required; (iii) the type-specific expression of the dPyTPase is controlled by

a locus between 0.67 and 0.68 fractional length on the viral genome.

MATERIALS AND METHODS

Cells and viruses. BHK-21 clone 13 cells (11) were grown in Dulbecco modified Eagle medium complemented with 10% (vol/vol) calf serum (Colorado Serum Co.) and 10% (vol/vol) tryptose phosphate broth (Difco Laboratories) on 9-cm plastic dishes as previously described (5). Virus strains used were: HSV-1 Glasgow strain 17 [HSV-1(17)] ts^+ syn^+; temperature-sensitive DNA$^-$ mutants of the same strain, namely, *ts*B, *ts*D, *ts*E, *ts*H, *ts*J, *ts*K, *ts*S, and *ts*U; HSV-1 strain KOS [HSV-1(KOS)] ts^+; mutants derived from this strain, namely, *ts*A1, *ts*B2, *ts*C4, and *ts*D9; HSV-1 strain HG52 [HSV-2(HG52)] ts^+ and the derived mutants *ts*1, *ts*6, *ts*9, and *ts*13; the *ts*13 revertant *ts*13R(4-8) (16); and HSV-2 strain 186 [HSV-2(186)] ts^+. The HSV-1 × HSV-2 intertypic recombinants were obtained from the Institute of Virology, University of Glasgow, Glasgow, Scotland. Their genesis and genome structures have been described elsewhere (2–4, 13, 18). Virus stocks were prepared by infection of BHK cells and titrated on monolayers of BHK cells under an agarose overlay as described (5).

Enzymes. (i) Alkaline DNase and DNA polymerase. Alkaline DNase was assayed by the method of Morrison and Keir (14) as described by Francke et al. (7). Viral DNA polymerase was measured as described by Weissbach et al. (21).

(ii) dPyTPase. A total of 2×10^7 cells were washed on the dish with cold Tris-buffered saline and then hypotonically disrupted in 20 mM 4-(2-hydroxethyl)-1-piperazine ethanesulfonic acid buffer (HEPES) (pH 7.8)–1 mM dithiothreitol–1 mM $MgCl_2$. Hypotonic lysates obtained as described (5), containing 2×10^7 cells in 150 μl, were diluted eightfold in the same buffer. The resulting suspension was treated with Nonidet P-40 (final concentration 0.2%) for 20 min and centrifuged at 500 × *g* for 10 min. The supernatant was made 80 mM in potassium acetate and cleared by centrifugation at 20,000 × *g* for 30 min; this constituted the soluble (cytoplasmic) fraction. The nuclear pellet was washed with 20 mM HEPES (pH 7.8)–1 mM dithiothreitol–1 mM $MgCl_2$ and suspended in the same buffer containing 80 mM potassium acetate at 2×10^7 nuclei per 300 μl if not stated otherwise. dPyTPase assay mixtures contained in a total volume of 50 μl: 45 μl of enzyme fraction, 10 mM $MgCl_2$, 1 mM dithiothreitol, 1 mM ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′,-tetraacetic acid (EGTA), 2 mM ATP, and 1 mM [^{3}H]dUTP (0.1 Ci/mmol). For the assay of HSV-2 nuclear dUTPase the [^{3}H]dUTP concentration was 50 μM (2 Ci/mmol). Deoxycytidine triphosphatase (dCTPase) and deoxythymidine triphosphatase (dTTPase) activities were assayed under the same conditions, except that the [^{3}H]dUTP was replaced by 50 μM [^{3}H]dCTP (2 Ci/mmol) or 50 μM [^{3}H]dTTP (2 Ci/mmol), respectively. The reaction was carried out at 4°C and was terminated by addition of 20 μl of 100 mM EDTA and 105 μl of methanol. Aliquots of the resulting mixture were applied to prewashed polyethyleneimine-cellulose strips together with unlabeled marker nucleotides. The strips were developed at room temperature with 1 M HCOOH–0.5 M LiCl and dried, and the position of deoxynucleotides was determined by UV light. Formation of ^{3}H-labeled deoxypyrimidine monophosphates was monitored by liquid scintillation counting of the corresponding spots as described (22). Under these standard conditions the generation of deoxynucleoside monophosphate was linear with time up to 30 min and proportional to the number of cells between 2×10^5 and 1×10^7 cells per assay.

Viral DNA synthesis. Incorporation of [^{3}H]thymidine into viral DNA during infection was measured by extraction of total DNA and subsequent equilibrium centrifugation in CsCl gradients as previously described (1).

Radioisotopes. [^{3}H]thymidine (*methyl*-^{3}H; 45 Ci/mmol), [^{3}H]dTTP (*methyl*-^{3}H; 50 Ci/mmol), [^{3}H]dCTP (5-^{3}H; 20 Ci/mmol), and [^{3}H]dUTP (5-^{3}H; 13.4 Ci/mmol) were purchased from Amersham.

RESULTS

In an earlier paper, we reported that nuclei from cells infected with HSV-1 contain a virus-specific dPyTPase which is virtually absent from nuclei of HSV-2-infected cells (22). Table 1 shows that HSV-2-infected cells do contain a dPyTPase, but that more than 99% of the activity fractionates into the soluble (cytoplasmic) extract, whereas the majority of the HSV-1-induced enzyme is insoluble and appears to be firmly bound to the nucleus of the infected cell. Different extraction conditions, such as NaCl concentrations up to 100 mM, treatment with 1% Nonidet P-40 instead of 0.2%, or varying the $MgCl_2$ concentration (between 1 and 10 mM) did not affect the distribution of the enzyme (data not shown). Since the HSV-1-specific nuclear enzyme was consistently insoluble under the various extraction conditions used, it is likely that the differences in the distribution of dPyTPase induced by the two HSV serotypes are not preparation artifacts. That part of the type 1 activity appears in the cytoplasmic fraction is probably a result of the increased fragility of the nuclei late in infection.

Total dPyTPase levels induced by HSV-1 were consistently higher than those found in HSV-2-infected cells. These values were obtained with two laboratory strains of each type (the HSV-1 strains 17 and KOS and the HSV-2 strains HG52 and 186) as well as with a number of clinical isolates (F. Wohlrab, D. R. Mayo, G. D. Hsiung, and B. Francke, unpublished data). In the isolated nuclei from infected cells, the difference between the enzyme levels induced by the two serotypes of herpes simplex virus was greater than two orders of magnitude. This type specificity was observed for all three substrates. Because dUTP is the optimal substrate, only the values for dUMP production are listed in the following experiments. It should be mentioned, though, that dCTPase and dTTPase

TABLE 1. Conversion of deoxypyrimidine triphosphate into deoxypyrimidine monophosphate (dPyMP) by nuclear and cytoplasmic fractions from HSV-infected and mock-infected BHK cells[a]

Virus	pmol of dPyMP formed per min per 10^7 cells from:					
	dUTP		dCTP		dTTP	
	Nuclei	Cytoplasm	Nuclei	Cytoplasm	Nuclei	Cytoplasm
HSV-1(17)	22,000 (54)	19,400 (46)	36 (60)	24 (40)	17 (45)	20 (55)
HSV-1(KOS)	29,400 (63)	16,800 (37)	41 (69)	19 (31)	21 (56)	16 (44)
HSV-2(HG52)	21 (0.3)	6,946 (99.7)	0.7 (7.8)	8.2 (92.2)	0.4 (6.9)	5.4 (93.1)
HSV-2(186)	20 (0.3)	6,870 (99.7)	0.8 (8.9)	8.2 (92.1)	0.3 (5.1)	5.6 (94.9)
Mock-infected	6 (4.5)	127 (95.5)	0.4 (7.8)	4.7 (92.2)	<0.1	<0.1

[a] BHK cells were infected at a multiplicity of 10 with the respective virus and harvested 18 h after infection at 31.5°C. Reaction conditions were as described in the text; time points were taken at 5-min intervals between 0 and 60 min at 4°C. Rates were calculated from the linear portion of the kinetics. The numbers in parentheses give the percentage of the total activity found in the infected cell present in either the nuclear or the cytoplasmic fractions.

activities were also monitored and gave in every case qualitatively identical results.

Table 2 shows dUTPase levels in the nuclear and the cytoplasmic fractions of cells infected with a series of temperature-sensitive mutants of HSV-1 at both the permissive and the nonpermissive temperature. To compensate for the temperature effect on the timing of the replication cycle, cultures infected at 31.5°C were harvested after 18 h, and those infected at 39°C were harvested after 11 h. As previously reported (22), the HSV-1(17) mutants *ts*D and *ts*K were deficient in expressing wild-type levels of dPyTPase at the nonpermissive temperature. In addition, the KOS mutant *ts*B2 underproduced the enzyme at 39°C. All three mutants fall into the same complementation group, 1-2 (19), and code for an immediate-early polypeptide believed to be necessary for the activation of viral transcription (12, 17). It is worth noticing that these mutants were not completely deficient in inducing dUTPase at nonpermissive temperature and expressed under these conditions still considerably more nuclear enzyme than either uninfected or HSV-2-infected cells.

Shift-up of cells infected with mutants in com-

TABLE 2. dUTPase levels induced by temperature-sensitive mutants of HSV-1[a]

Virus	dUTPase[b] (nmol of dUMP formed per min per 10^7 cells)				HSV-specific DNA polymerase[c] (39°C)	HSV-specific alkaline nuclease[c] (39°C)	Viral DNA[c] (39°C)
	Nuclei		Cytoplasm				
	31.5°C	39°C	31.5°C	39°C			
HSV-1(17) *syn*$^+$							
ts$^+$	4.27	4.23	3.19	2.81	+	+	+
*ts*B	3.39	3.03	2.30	1.90	+	+	−
*ts*D	3.14	1.05	1.64	0.87	−	−	−
*ts*E	3.86	3.00	1.15	1.15	+	+	−
*ts*H	3.86	2.71	0.93	1.82	−	+	−
*ts*J	1.74	0.69	1.55	0.60	−	+	−
*ts*K	3.91	0.64	2.28	0.71	−	−	−
*ts*S	2.50	2.09	1.13	1.40	ND	ND	−
*ts*U	1.98	1.35	0.93	0.88	ND	ND	−
HSV-1(KOS)							
ts$^+$	4.02	6.14	1.92	2.27	+	ND	+
*ts*A1	3.86	4.49	1.72	1.88	+	ND	−
*ts*B2	3.21	0.60	2.39	0.64	−	ND	−
*ts*C4	1.29	1.25	1.20	1.20	−	ND	−
*ts*D9	4.50	3.91	1.74	1.37	−	ND	−

[a] BHK cells were infected at a multiplicity of 5 and harvested 18 h after infection at 31.5°C or 11 h after infection at 39°C. Nuclear and cytoplasmic fractions for the dUTPase assay were prepared as described in the text.

[b] dUTPase was determined under the standard conditions as described.

[c] Enzymes and viral DNA synthesis were measured as described. −, Values indistinguishable from mock-infected controls; +, 30% or more of wild-type activity; ND, not determined.

plementation group 1-2 from permissive to nonpermissive temperature 15 h after infection did not result in a drop of dUTPase activity below the enzyme levels present in controls kept at 31.5°C (Fig. 1).

The *ts*J mutant of HSV-1(17) consistently induced the lowest levels of all mutants tested even at permissive temperature (Table 2 and other results not shown). The difference between enzyme activities induced at permissive and nonpermissive temperature, though, was less than that for the 1-2 mutants, and no temperature sensitivity was observed for the *ts*J dPyTPase after temperature shift-up (data not shown).

dUTPase activities expressed by temperature-sensitive mutants of HSV-2(HG52) after infection at both the permissive and nonpermissive temperature are listed in Table 3. In cells infected with *ts*13 (complementation group 2-14), total activity at nonpermissive temperature was 4.2-fold lower than at permissive temperature. This mutant has been reported to contain two lesions, one affecting virion stability and the other one resulting in a thermolabile alkaline nuclease (16). HSV-2(HG52) *ts*13R(4-8), a revertant of *ts*13 selected for growth at nonpermissive temperature but still containing a temperature-sensitive nuclease (*nuc*$^-$), induced dUTPase levels comparable to *ts*13, suggesting that the *nuc*$^-$ mutation is responsible for the reduced triphosphatase expression.

It is important to note that both *ts*13 and the revertant after infection at nonpermissive temperature still produced significant increases of dPyTPase activity over the one found in mock-infected cells (3.4-fold and 2.6-fold, respectively). In contrast to this, the HSV-2 mutant *ts*9 (complementation group 2-4) was extremely deficient in induction of dUTPase activity at 39°C. The enzyme activity found in *ts*9-infected cells at nonpermissive temperature was only slightly higher than the activity in uninfected cells. Among all the mutants tested, *ts*9 was the most defective one for the expression of dPyTPase. The mutant has been reported to be deficient in the synthesis of a number of viral polypeptides at nonpermissive temperature (3), including the virus-coded DNA polymerase (9). The *ts*9 mutation has been physically mapped by marker rescue to a locus between 0.44 and 0.52 units on the HSV-2 genome, distinct from the DNA polymerase locus (3). In contrast to *ts*9, the mutant *ts*6 (complementation group 2-3), which

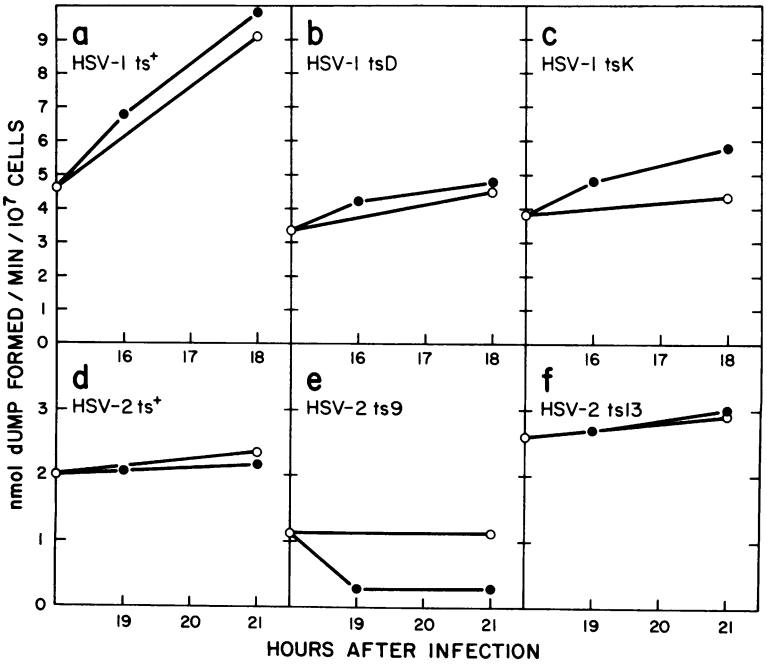

FIG. 1. dUTPase levels in cells infected with temperature-sensitive mutants of HSV-1(17) and HSV-2(HG52) after shift-up from permissive to nonpermissive temperature late in infection. Cells were infected at a multiplicity of 5 with (a) HSV-1(17) *ts*$^+$, (b) HSV-1(17) *ts*D, (c) HSV-1(17) *ts*K, (d) HSV-2(HG52) *ts*$^+$, (e) HSV-2(HG52) *ts*9, and (f) HSV-2(HG52) *ts*13, at 31.5°C. At 15 h (a, b, c) or 18 h (d, e, f) after infection, a set of cultures was shifted to 39°C. At the times indicated, cultures were harvested and assayed as described in the text. Symbols: ○, cultures kept at 31.5°C; ●, cultures shifted to 39°C.

TABLE 3. dUTPase levels induced by temperature-sensitive mutants of HSV-2

Virus	dUTPase[a] (pmol of dUMP formed per min per 10^7 cells)				DNA polymerase[b] (39°C)	Alkaline DNase[b] (39°C)	Viral DNA[b] (39°C)
	Nuclei		Cytoplasm				
	31.5°C	39°C	31.5°C	39°C			
HSV-2(HG52)							
ts$^+$	34.2	36.7	2,070	1,540	+	+	+
*ts*1	27.5	23.9	2,410	1,510	+	+	−
*ts*6	34.4	24.7	2,360	1,490	−	+	−
*ts*9	19.3	5.8	1,050	196	−	+/−	−
*ts*13	48.2	65.0	2,800	615	+/−	−	+/−
*ts*13R(4–8)	23.0	50.0	2,620	459	+/−	−	+/−
Mock-infected	2.1	4.6	124	181			

[a] BHK cells were infected at a multiplicity of 5 with the respective virus and harvested 18 h after infection at 31.5°C or 12 h after infection at 39°C. Extraction procedure and dUTPase assay were as described in the text, except that for determination of nuclear dPyTPase levels the concentration of the nuclei in the reaction mixture was raised to 8×10^7 nuclei per 150 μl. Under these conditions the reaction was linear for at least 30 min.

[b] DNA polymerase, alkaline nuclease, and total viral DNA synthesis were measured as described. +/−, Values below 30% of wild type, yet clearly higher than mock-infected controls. Other symbols are as detailed in Table 2, footnote *c*.

induces a thermolabile DNA polymerase (9), expressed wild-type levels of dPyTPase at 39°C, indicating that the polymerase is not involved in control of dPyTPase expression. This compares with the results obtained with the HSV-1 DNA polymerase mutants 17 *ts*H and KOS *ts*D9 (complementation group 1-4), which induced approximately equal amounts of triphosphatase at 31.5 and 39°C. In addition to this, HSV-2 *ts*1 (complementation group 2-1), which overproduces DNA polymerase at 39°C (9), expressed normal dPyTPase activity under nonpermissive conditions.

If cells infected with *ts*13 were shifted from permissive to nonpermissive temperature 18 h after infection, the dUTPase levels remained comparable to those found in controls kept at the permissive temperature (Fig. 1). In contrast to this, dUTPase levels dropped sharply in cells infected with *ts*9 after temperature shift-up. It therefore seems that the gene mutated in *ts*9 is continuously required for dPyTPase expression.

The different phenotypes of HSV-1 and HSV-2 with regard to the dPyTPase, and in particular the large differences in nuclear enzyme activities, can be exploited to physically map the locus of the type specificity by analyzing dPyTPase induction in cells infected with HSV-1 × HSV-2 intertypic recombinants. Table 4 summarizes the dUTPase levels in nuclei of cells infected with 24 such recombinants (obtained from the Institute of Virology, Glasgow). The data presented in the table were obtained after one passage of the recombinant stocks in our laboratory. With lower multiplicities of infection and longer incubation times (0.04 PFU/cell and 72 h), qualitatively identical results were obtained with the unpassaged original stocks. All recombinants produced normal amounts of viral DNA polymerase, alkaline nuclease, and viral DNA (data not included in the table). In contrast, the levels of nuclear dPyTPase varied drastically and could be used to classify each recombinant as type 1 or type 2 with respect to the enzyme expression. The reason for the relatively low levels induced by D×1(34-2) is not understood, but the distribution of dPyTPase (less than 50% cytoplasmic, not shown) allowed its classification as type 1. Similarly, R12-3, which induces relatively high nuclear enzyme levels, was classified as type 2, since >95% of the total dPyTPase fractionated in the cytoplasm.

The physical maps of the cross-over points of the recombinants have been published (see references in Table 4). Cross-over maps of selected recombinants are shown in Fig. 2. Analysis of the results can be exemplified by two recombinants. B×6(17-1) contains a single cross-over site at 0.67 units of the prototype map. The region from 0.00 to 0.67 is represented by HSV-1 DNA sequences, and the one from 0.67 to 1.00 is represented by HSV-2 sequences. The low nuclear dPyTPase level classifies the recombinant as type 2. The type specificity of dPyTPase expression must therefore map to the right of 0.67. In contrast, $17^+ \times 11^r$(1-A) contains only a single piece of HSV-1 DNA between maximally 0.56 and 0.68 units, yet expresses HSV-1 dPyTPase. The locus must therefore lie between those boundaries. Taken together, these two recombinants permit us to narrow the region

TABLE 4. Nuclear dUTPase levels of cells infected with HSV-1 × HSV-2 intertypic recombinants[a]

Virus	Reference	Nuclear dUTPase activity[b]	Classification[c] (virus type)
HSV-1 *ts*$^+$		100	1
HSV-2 *ts*$^+$		0.4	2
Mock		0.02	
B × 1 (28-1-29)	4	101	1
B × 1 (31-2)	13	0.3	2
B × 5 (7-2)	13	0.3	2
B × 5 (10-7)	13	111	1
B × 6 (17-1)	13	0.9	2
RA-5	13	89	1
RE-6	13	98	1
RS-1	13	72	1
RS-6	13	88	1
B × 1 (24)	13	0.2	2
D × 1 (34-2)	13	32	1
D × 1 (32)	18	0.4	2
D × 1 (48)	18	88	1
D × 1 (31)	18	99	1
D × 1 (43)	18	79	1
D × 1 (51)	18	86	1
D × 1 (53)	18	89	1
D × 1 (57)	18	91	1
R1-1	3	97	1
R1-2	3	93	1
R6-29	2	0.2	2
R12-3	3	1.8	2
R13-2	3	92	1
17$^+$ × 11^r (1-A)	13	101	1

[a] For sources and references to the intertypic recombinants used, see the text. Cells were infected at a multiplicity of 1 with the respective recombinant and harvested 18 h after infection at 31.5°C.

[b] Assays were under standard conditions for 15 min at 4°C; the nuclear dUTPase activity for HSV-1 *ts*$^+$ was 3.24 nmol/min per 10^7 nuclei and was taken as 100% value.

[c] Nuclear dPyTPase values of >30% and <2% compared to the HSV-1 *ts*$^+$ control were used to classify the recombinants as "type 1" or "type 2," respectively. All recombinants classified as type 2 did induce significant amounts (>10% of HSV-2 *ts*$^+$) of cytoplasmic dPyTPase, as determined in a separate experiment.

that determines the type specificity of dPyTPase expression to 0.67 to 0.68 units. All other recombinants are consistent with this assignment. There are two exceptions, B×1(31-2) and B×1(24). Both are derived from crosses between HSV-1(17) *ts*B and HSV-2(HG52) *ts*1 and contain type 1 genetic material from 0.48 to 1.00 and 0.56 to 0.79, respectively, but do not induce a nuclear dPyTPase. We have at the moment no clear explanation for the failure of B×1(31-2) and B×1(24) to express nuclear dPyTPase, except that a cross-over or a deletion might have occurred within the region of interest which was too small to be detected by restriction enzyme mapping. With 22 recombinants giving consistent results, we feel confident in placing the locus for the type specificity of the dPyTPase between 0.67 and 0.68 fractional length on the viral genome within the long unique region.

DISCUSSION

The results of the experiments described in this communication allow the following conclusions.

(i) The HSV-1-specific dPyTPase is predominantly nuclear, whereas the HSV-2 enzyme fractionates into the cytoplasm. The reasons for this difference remain unclear. One possibility is the association of the HSV-1-specific dPyTPase with a nuclear structure such as DNA or with nuclear proteins. Alternatively, if the HSV-2 enzyme were also confined to the nucleus in vivo, the effect might be caused by a differential ability of the HSV-1 and HSV-2 enzymes to cross the nuclear membrane after disruption of the cells. Experiments to investigate this question are currently under way.

(ii) Although the mutants in complementation group 1-2 are deficient in dPyTPase expression at nonpermissive temperature, the shift-up experiment shown in Fig. 1 indicates that the protein specified by these mutants (175,000 molecular weight) (17) is not identical with the

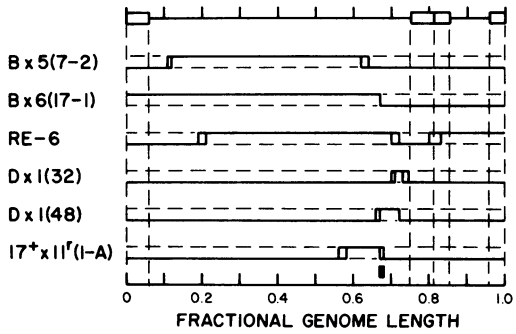

FIG. 2. Cross-over maps of some of the HSV-1 × HSV-2 intertypic recombinants used in the experiment described in Table 4. For the establishment of these maps see the references given in the text and in Table 4. The sequences in a recombinant derived from the HSV-1 or HSV-2 parent are shown by thick continuous lines superimposed on the upper and lower, respectively, of the two horizontal dashed lines representing the viral genome. The vertical dotted lines delimit the TR_L, U_L, IR_L, IR_S, U_S, and TR_S regions of the genome. Regions in which cross-overs have occurred are shown by two vertical lines between the thick horizontal lines. The black bar at the bottom of the Figure indicates the map position of the locus for the type specificity of expression of the viral dPyTPase.

dPyTPase. It follows that the triphosphatase is under control of the 1-2 gene, which has been shown to map in the short repeat region of the HSV-1 genome (20).

(iii) The *ts*J mutant of HSV-1(17) has not been assigned to a complementation group (19). At nonpermissive temperature, the synthesis of at least 13 polypeptides is reduced (12). In addition to the low levels of dPyTPase expression, this mutant shows generally somewhat reduced levels of DNA synthesis, DNA polymerase, and alkaline DNase even at permissive temperature and is an extremely tight DNA^- mutant at nonpermissive temperature (1). The relative lack of temperature sensitivity with respect to the triphosphatase makes it seem unlikely that the lethal mutation of *ts*J is in the structural gene for the dPyTPase. It is possible, though, that this mutant carries multiple lesions, one or several of which may result in its general defectiveness.

(iv) The viral DNA polymerase mutants in complementation groups 1-4 and 2-3 produced normal amounts of dPyTPase at nonpermissive temperature. This argues against the dPyTPase activity being a part of the viral DNA polymerase enzyme, though it does not exclude a complex of both enzymes in vivo.

(v) The type 2 mutants *ts*13 and *ts*13R(4-8) (nuc^-) showed temperature-sensitive expression of triphosphatase. In addition to the decreased levels of dPyTPase in cells infected with both *ts*13 and *ts*13R(4-8), viral DNA polymerase activity and total viral DNA synthesis are greatly reduced at nonpermissive temperature (6). It therefore appears that the nuc^- mutation affects a variety of viral functions, including the dPyTPase. The level at which the interference occurs is not known. Analogous to the mutants in complementation group 1-2, the temperature-shift results argue against the gene(s) mutated in *ts*13 as the structural gene for dPyTPase. The properties of *ts*6 and *ts*13 also argue against the possibility that the observed triphosphatase activity in HSV-2 may result from the combined activities of the viral DNA polymerase and the alkaline nuclease and support the idea that the triphosphatase activity is carried by a separate polypeptide.

(vi) The gene for this polypeptide is most likely specified by the *ts*9 mutation, as demonstrated by the temperature-shift experiment shown in Fig. 1. In vivo and in vitro heat-inactivation studies on the *ts*9-induced dPyTPase, currently in progress, have substantiated this finding (manuscript in preparation).

(vii) The type specificity of dPyTPase expression is controlled by a locus mapping between 0.67 and 0.68 on the prototype map. It is interesting that this map position lies in a region in which HSV-1 and HSV-2 share the least sequence homology (10). This map location in the L_U segment of the genome is clearly distinct from the S-repeat region, which contains the gene specified by complementation group 1-2. It is also distinct from the map position of the *ts*9 mutation of HSV-2 (0.44 to 0.52).

Taken together, our data show that for expression of wild-type levels of dPyTPase in infected cells the products of the genes of complementation group 1-2, located in the S-repeat region, and in the case of HSV-2 at least two other functions, the *ts*13 and *ts*9 genes, are required. In addition, the locus at 0.67 to 0.68 units seems to determine the type-specific subcellular fractionation of the enzyme. The *ts*9 gene product is remarkable in that it appears to be required continuously for expression of the triphosphatase, indicating that the complementation group 2-4 may represent the structural gene for the dPyTPase, at least for HSV-2.

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LITERATURE CITED

1. **Bone, D. R., M. Brown, I. Crombie, and B. Francke.** 1978. Viral DNA synthesis in cells infected with temperature-sensitive mutants of herpes simplex virus type 1. J. Virol. **28**:14–19.
2. **Chartrand, P., N. D. Stow, M. C. Timbury, and N. M. Wilkie.** 1979. Physical mapping of *paa*^{r} mutations of herpes simplex virus type 1 and type 2 by intertypic marker rescue. J. Virol. **31**:265–276.
3. **Chartrand, P., N. M. Wilkie, and M. C. Timbury.** 1981. Physical mapping of temperature-sensitive mutations of herpes simplex virus type 2 by marker rescue. J. Gen. Virol. **52**:121–133.
4. **Davison, A. J., H. S. Marsden, and N. M. Wilkie.** 1981. One functional copy of the long terminal repeat gene specifying the immediate-early polypeptide IE 110 suffices for a productive infection of human foetal lung cells by herpes simplex virus. J. Gen. Virol. **55**:179–191.
5. **Francke, B.** 1977. Cell-free synthesis of herpes simplex virus DNA: conditions for optimal synthesis. Biochemistry **16**:5655–5664.
6. **Francke, B., and B. Garrett.** 1982. The effect of a temperature-sensitive lesion in the alkaline DNase of herpes simplex virus type 2 on the synthesis of viral DNA. Virology **116**:116–127.
7. **Francke, B., H. Moss, M. C. Timbury, and J. Hay.** 1978. Alkaline DNase activity in cells infected with a temperature-sensitive mutant of herpes simplex virus type 2. J. Virol. **26**:209–213.
8. **Halliburton, I. W., L. S. Morse, B. Roizman, and K. E. Quinn.** 1980. Mapping of the thymidine kinase genes for type 1 and type 2 herpes simplex viruses using intertypic recombinants. J. Gen. Virol. **49**:235–253.
9. **Hay, J., H. Moss, A. T. Jamieson, and M. C. Timbury.**

1976. Herpesvirus proteins: DNA polymerase and pyrimidine deoxynucleoside kinase activities in temperature-sensitive mutants of herpes simplex virus type 2. J. Gen. Virol. 31:65–73.

10. **Knipe, D. M., W. T. Ruyechan, B. Roizman, and I. W. Halliburton.** 1978. Molecular genetics of herpes simplex virus. Demonstration of regions of obligatory and non-obligatory identity within diploid regions of the genome. Proc. Natl. Acad. Sci. U.S.A. 75:3896–3900.
11. **MacPherson, I. A., and M. G. P. Stoker.** 1962. Polyoma transformation of hamster cell clones—an investigation of genetic factors affecting cell competence. Virology 16:147–151.
12. **Marsden, H., I. K. Crombie, and J. H. Subak-Sharpe.** 1976. Control of protein synthesis in herpes virus-infected cells: analysis of polypeptides induced by wild type and sixteen temperature-sensitive mutants of HSV strain 17. J. Gen. Virol. 31:347–351.
13. **Marsden, H., N. D. Stow, V. G. Preston, M. C. Timbury, and N. M. Wilkie.** 1978. Physical mapping of herpes simplex virus-induced polypeptides. J. Virol. 28:624–655.
14. **Morrison, J. M., and H. M. Kier.** 1968. A new DNA exonuclease in cells infected with herpes virus: partial purification and properties of the enzyme. J. Gen. Virol. 3:337–347.
15. **Morse, L. S., L. Pereira, B. Roizman, and P. A. Schaffer.** 1978. Anatomy of herpes simplex virus (HSV) DNA. X. Mapping of viral genes by analysis of polypeptides and functions specified by HSV-1 × HSV-2 recombinants. J. Virol. 26:389–410.
16. **Moss, H., P. Chartrand, M. C. Timbury, and J. Hay.** 1979. Mutant of herpes simplex virus type 2 with temperature-sensitive lesions affecting virion thermostability and DNase activity: identification of the lethal mutation and physical mapping of the *nuc*$^-$ lesion. J. Virol. 32:140–146.
17. **Preston, C. M.** 1979. Abnormal properties of an immediate early polypeptide in cells infected with the herpes simplex virus type 1 mutant *tsK*. J. Virol. 32:357–369.
18. **Preston, V., A. J. Davison, H. S. Marsden, M. C. Timbury, J. H. Subak-Sharpe, and N. M. Wilkie.** 1978. Recombinants between herpes simplex virus types 1 and 2: analysis of genome structure and expression of immediate early polypeptides. J. Virol. 28:499–517.
19. **Schaffer, P. A., V. C. Carter, and M. C. Timbury.** 1978. Collaborative complementation study of temperature-sensitive mutants of herpes simplex virus types 1 and 2. J. Virol. 27:490–504.
20. **Stow, N. D., J. H. Subak-Sharpe, and N. M. Wilkie.** 1978. Physical mapping of herpes simplex virus type 1 mutations by marker rescue. J. Virol. 28:182–192.
21. **Weissbach, A., S.-C. L. Hong, J. Aucker, and R. Muller.** 1973. Characterization of herpes simplex virus-induced deoxyribonucleic acid polymerase. J. Biol. Chem. 248:6270–6277.
22. **Wohlrab, F., and B. Francke.** 1980. Deoxypyrimidine triphosphatase activity specific for cells infected with herpes simplex virus type 1. Proc. Natl. Acad. Sci. U.S.A. 77:1872–1876.