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Anatomy of Herpes Simplex Virus DNA

XI. Apparent Clustering of Functions Effecting Rapid Inhibition of Host DNA and Protein Synthesis

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Herpes simplex virus type 2 (HSV-2) strains inhibit the synthesis of both DNA and protein of the host cell more rapidly than HSV-1 strains. Several intertypic HSV-1 × HSV-2 recombinants and parental strains were examined for their ability to inhibit rapidly the synthesis of host protein and DNA. The two functions cosegregated in all of eight recombinants tested and are therefore controlled by the same gene or by different genes in the same region of the viral DNA.

A characteristic consequence of herpes simplex virus (HSV) infections is the suppression of host DNA and protein synthesis (7-9). Recent studies with HSV type 2 (HSV-2) (2) suggested that this effect is mediated by a component of the virion. In an earlier paper from this laboratory (4), it was reported that one or more genes whose products are involved in the inhibition of host protein synthesis lie between 0.52 and 0.59 map units on the prototype arrangement (3) of HSV-2 DNA. The mapping of this function was based on the observation that a number of HSV-2 strains suppress host protein synthesis more rapidly than HSV-1 strains (5, 6). It was accomplished by comparing the structure and provenance of the DNAs of HSV-1 × HSV-2 intertypic recombinants with their effects on host protein synthesis. In this paper we report that HSV-2 also inhibits cell DNA synthesis more rapidly than HSV-1 and that the accelerated inhibition of host DNA synthesis by HSV-2 maps in the same region.

We have measured suppression of host protein and DNA synthesis in cells infected with HSV-1, HSV-2, or intertypic recombinants. The simple technique that we have used is based on the observation that rapid suppression of host synthesis by HSV-2 does not require detectable viral protein or DNA synthesis (2). Therefore, cells were either (i) infected in the presence of actinomycin D to prevent transcription of viral mRNA, and the rate of incorporation of ^{14}C-amino acids into acid-precipitable material was measured 1.5 h later, or (ii) infected with UV light-irradiated virus, and the rate of incorporation of [^{3}H]thymidine measured 1.5 h later.

Figure 1 shows the structure and provenance of the DNA sequences of the intertypic recombinants tested in this study (3). Recombinant A8E was derived by a cross between HSV-1(17)*ts*J and HSV-2(GP6). Recombinant B1E was derived by a cross between HSV-1(17)*ts*J and HSV-2(186). Series C recombinants were derived by crossing HSV-1(HFEM)*ts*N102 with HSV-2(186). In addition to the parental strains identified in greater detail elsewhere (3, 4), we also tested the prototype strains used in this laboratory [HSV-1(F), and HSV-2(G)] (1). All plaque assays for infectivity were done in monolayer cultures of Vero cells.

To measure suppression of cellular protein synthesis, confluent monolayers of Vero cells (2.5×10^6 cells) were mock infected or infected with 20 PFU/cell. After 20 min at 20°C, the inoculum was replaced by 2 ml of cell growth medium containing actinomycin D (1 μg/ml) and incubated at 37°C for 1.5 h. The medium was then replaced by 1.5 ml of medium lacking leucine, isoleucine, and valine and containing [^{14}C]leucine, [^{14}C]isoleucine, and [^{14}C]valine (0.2 μCi/ml, total), and the cultures were reincubated at 37°C for an additional 30 min. The cells were washed and lysed in 1 ml of 1% sodium dodecyl sulfate. After brief ultrasonic disruption to reduce the viscosity, duplicate 0.2-ml samples were mixed with 10% trichloroacetic acid and filtered on filters (Millipore Corp.). The radioactivity of acid-precipitable materials on the washed and dried filters was measured in a scintillation counter.

To measure suppression of cellular DNA synthesis, virus was diluted in phosphate-buffered saline containing 1% glucose to give a concentration of 5×10^7 PFU/1.5 ml. A 2-ml amount of diluted virus in a 9-cm-diameter petri dish was exposed to a germicidal lamp (wavelength, 254

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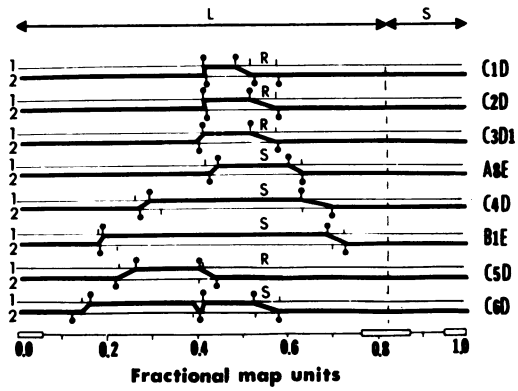

FIG. 1. *HSV-1 and HSV-2 DNA sequences in the intertypic recombinants (3) tested for their effects on host DNA and protein synthesis. The upper and lower lines in each duplet marked at the left with 1 and 2, respectively, represent HSV-1 and HSV-2 DNA sequences. The heavy line represents the DNA sequences present in each recombinant. The mapping of the DNA of the recombinants was done on the basis of presence or absence of Hsu I, EcoRI, Bgl II, Xba I, and Hpa I restriction enzyme cleavage sites characteristic of HSV-1 and HSV-2 parental DNAs as reported by Morse et al. (3). The dash intersecting the line representing each DNA indicates the restriction enzyme cleavage sites defining the crossover site. The dash ending in a knob indicates the presence of a restriction enzyme cleavage site characteristic of the parental DNA. The dash lacking the knob represents the absence of a cleavage site characteristic of that parental DNA. The L and S components of HSV-DNA are shown at the top of the figure and are demarcated by the dashed line intersecting all lines at 0.83 map units. The letters R and S on top of each doublet refer to rapid and slow inhibition, respectively, of host DNA and protein synthesis by the recombinants according to the results presented in Table 2.*

nm). The total dose delivered to the surface of the fluid was 1,000 ergs/mm^2. A 1.5-ml amount of irradiated virus was used to infect a culture of Vero cells as before. After 1.5 h in growth medium at 37°C [^{3}H]thymidine was added (2.5 μCi/ml), and the cells were lysed 30 min later. Acid-precipitable radioactivity was measured.

Preliminary experiments confirmed that no virus-specific proteins were labeled in the cells treated with actinomycin and that no viral DNA was labeled in the cells infected with UV-irradiated virus. Thus, rates of acid-precipitable radioactivity could be used as a measure of the overall rates of cellular protein or DNA synthesis.

HSV-1 causes a more gradual inhibition of host synthesis than HSV-2. To be able to distinguish reliably between HSV-1 and HSV-2 effects, it was necessary to ensure that all the cells were infected and to show that variations in degree of inhibition did not merely reflect differences in multiplicity of infection.

Table 1 shows the effect of the multiplicity of infection by recombinant A8E and by its two parental strains, HSV-1(17) and HSV-2(GP6), on the suppression of cellular protein synthesis. It can be seen that increasing the multiplicity did not significantly enhance the inhibition of cell protein synthesis. The differences between the effects of HSV-1 and HSV-2 are still evident at an added multiplicity of 80 PFU/cell, and the recombinant A8E resembles its type 1 parent.

The effects on cell protein and DNA synthesis of various virus strains and intertypic recombinants at a multiplicity of 20 PFU/cell are shown in Table 2. The three strains of HSV-2 caused substantially greater suppression of both protein and DNA synthesis than the three HSV-1 strains. The pattern of inhibition of protein synthesis observed in this study was identical to

TABLE 1. *Effect of increasing multiplicity of infection on inhibition of cellular protein synthesis*

Virus	% Reduction in amino acid incorporation in cells infected with:		
	20 PFU/cell	40 PFU/cell	80 PFU/cell
HSV-1 (17)	67	75	74
HSV-2 (GP6)	94	97	98
Recombinant A8E	71	68	75

TABLE 2. *Inhibition of protein and DNA synthesis*

Virus	% Reduction in[a]:	
	^{14}C-amino acid incorporation	[^{3}H]thymidine incorporation
Exp 1		
HSV-1(F)	30(−)	29(−)
HSV-2(G)	96(+)	93(+)
HSV-1(17)	67(−)	46(−)
HSV-2(GP6)	95(+)	94(+)
A8E	71(−)	29(−)
HSV-2(186)	83(+)	74(+)
B1E	60(−)	21(−)
Exp 2		
HSV-1(HFEM)	34(−)	35(−)
HSV-2(186)	85(+)	75(+)
C1D	87(+)	80(+)
C2D	94(+)	90(+)
C3D1	93(+)	85(+)
C4D	53(−)	48(−)
C5D	85(+)	72(+)
C6D	37(−)	35(−)

[a] (+), Rapid inhibition; (−), slow inhibition. Rapid inhibition was defined as greater than 80% reduction of ^{14}C-amino acid incorporation and greater than 70% reduction in [^{3}H]thymidine incorporation.

that reported previously (4) with the exception that recombinant C7D was shown to resemble its HSV-2 parent and not HSV-1 parent as previously reported. In general the recombinants resembled one or other parent in both inhibitory functions, although A8E and B1E appeared to be even less active than their HSV-1(17) parent in inhibiting DNA synthesis.

The data in Table 2 show that all those recombinants (and only those) that caused rapid suppression of host protein synthesis also caused rapid suppression of DNA synthesis. By examining the crossover sites in the DNAs of recombinants C3D1, A8E, and C6D (Fig. 1), we conclude that the gene or genes whose products effect the rapid inhibition of host DNA synthesis and protein synthesis lie in the region between 0.52 and 0.59 map units. We do not know whether the inhibitions result from the pleiotropic effect of one gene or the simultaneous effects of independent genes. This region of the DNA has a molecular weight of about 7×10^6, and the structural genes of at least six polypeptides [ICP 2, 7, 10, 25, 32, and glycoprotein VP8(C2)] have been mapped within or close to it (4), but only in the case of ICP 10 was the HSV-2 form of the polypeptide consistently associated with the rapid suppression of host synthesis in all of the recombinants examined.

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