THE SYNTHESIS OF HERPES SIMPLEX VIRUS PROTEINS IN THE ABSENCE OF VIRUS DNA SYNTHESIS*

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Summary. When herpes simplex virus infected cells were blocked from synthesizing DNA, alterations in the production of certain classes of herpes simplex virus specific proteins were detected. Some proteins were made in excess, some in normal amounts, some in reduced amounts and some were not made. An example of each type was chosen, and its kinetics of synthesis studied. Possible explanations of the phenomena are discussed.

While many bacteriophage and animal viruses have been shown to produce late proteins, which by definition are made only after DNA is synthesized, this has not been shown to be the case for the herpesvirus group. Bone and Courtney (1) and Honess and Roizman (2) have shown that capsid protein can be produced in the absence of DNA synthesis. Recently, however, Ward and Stevens (3) questioned these findings and suggested that capsid protein synthesis did not occur under those conditions.

In this study we examined the production of herpesvirus proteins in cells infected in the presence or absence of an inhibitor of DNA synthesis -- cytosine arabinoside -- or in cells infected with temperature-sensitive mutants which do not make DNA. We chose to examine in detail the synthesis of one protein from each of Honess and Roizman's

α, β, and γ protein groups of herpes simplex virus type 1* (2). Briefly, α and β proteins are made early in infection but only α proteins are made immediately after removal of a cycloheximide block added at the time of infection; γ proteins (including capsid protein) are made later. We also studied corresponding proteins produced by a herpes simplex virus type 2* strain.

Materials and Methods. Most of the methods, including tissue culture, virus propagation, infection, radiolabelling and polyacrylamide gel electrophoresis procedures, were described recently (4). The wild-type viruses used in this study were the KOS strain of HSV-1 and the 186 strain of HSV-2. The temperature-sensitive mutants of HSV-1, their DNA synthesis, and the methods required to examine viral DNA synthesis have been described previously (5, 6). Cytosine arabinoside (Calbiochem, La Jolla, California) was used at a concentration of 50 μg/ml.

The scheme of experiments was simple. Approximately 6 X 10^6 cells (grown in 100-mm diameter plastic petri dishes) were infected with the wild-type viruses, or with the mutants, and the virus was allowed to adsorb for 1 hour at 37°. At the end of the adsorption period, fresh medium was added and incubation was continued at 37° (wild-type), 34 or 39° (mutants). At various times after infection cells were labelled for 1 hour with [^{14}C] amino-acid mixture (5 μCi/ml, specific activity 57 mCi/m Atom carbon) (Amersham-Searle, Inc., Arlington Heights, Chicago, Illinois), and with [^{3}H] thymidine (10 μCi/ml, specific activity 52 Ci/mmol) (Schwarz-Mann, Inc., Orangeburg, New York). Cells were harvested at the end of the labelling period and the whole-cell fractions were processed as described previously for either polyacrylamide gel electrophoresis (4) or caesium chloride equilibrium density gradient centrifugation (6).

RESULTS

Inhibition of DNA synthesis. Figure 1 shows caesium chloride equilibrium density gradient analysis of the [^{3}H] thymidine labelled DNA synthesized in virus infected cells 8 to 9 hours post-infection. Infected cells were maintained in the presence or absence of cytosine arabinoside for wild-type virus, or at permissive and nonpermissive temperatures for tsD9. There was no viral DNA synthesis detected by this technique using either method of viral DNA synthesis inhibition.

* Abbreviations: HSV-1 and HSV-2, herpes simplex virus types 1 and 2, respectively.

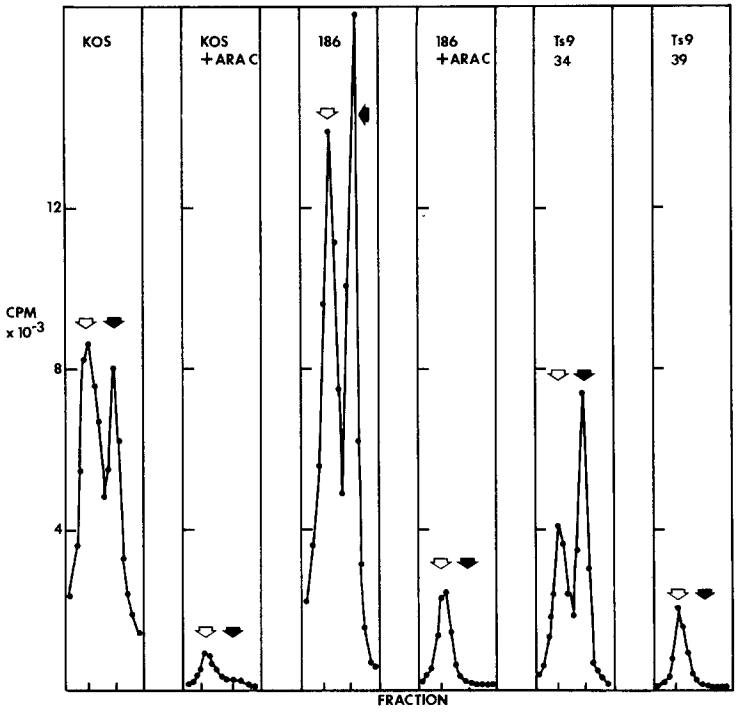

FIGURE 1: DNA synthesis 8 to 9 hours post-infection under normal and inhibitory conditions. Caesium chloride equilibrium density gradients are shown of the DNA synthesized in herpes simplex virus type 1 (KOS) and type 2 (186) infected cells in the presence and absence of cytosine arabinoside, and in cells infected with the mutant of KOS (tsD9) at permissive and nonpermissive temperature. DNA of cell density (1.690 to 1.700 g/cm^3) is indicated thus ⇩ and of viral density (1.725 to 1.727 g/cm^3), thus ⇩.

Polypeptides studied. The polypeptides were selected for study on the basis of their kinetic group and rate of synthesis after the withdrawal of a cycloheximide block (2, 4, 7). The polypeptides we chose to study are listed in Table I and are also indicated on the electrophoretic profiles of polypeptides made early and late in the replicative cycles of both types of herpesvirus shown in Figure 2. As a representative of the α class, we chose a high molecular weight, nonstructural polypeptide of HSV-1, designated ICP4 by Honess and Roizman

TABLE I. POLYPEPTIDES STUDIED

Class	Time of Synthesis	HSV-1*		HSV-2*	
		Number	M.W.†	Number	M.W.†
α	Early only	ICP4 (VP175)‡	184	ICSP5-8	182-186
β	Slightly later than α	ICP6 (VP134)	146	ICSP10	153
γ	Early and late	ICP5 (VP154)	155	ICSP9	157
γ_2	Late only	ICP17	103	ICSP16	119

* HSV-1 data from Honess and Roizman (2, 7); HSV-2 data from Powell and Courtney (4).

† The differences in molecular weight (M.W.) of type 1 and type 2 virus polypeptides are real, and are obvious upon parallel electrophoresis of samples.

‡ Nomenclature in parentheses is that used by Courtney and Benyesh-Melnick (8) and Bone and Courtney (1). ICP6 is one component of the VP134 region.

(2) and VP175 by Courtney and Benyesh-Melnick (8), and a corresponding protein ICSP5-8 for HSV-2 (4). As a representative of the β class, we chose a protein made early in infection, but not made immediately after withdrawal of a cycloheximide block; this was ICP6 for HSV-1 (2) and ICSP10 for HSV-2 (4). As a representative of the γ class made at a maximum rate late during infection, we chose the major nucleocapsid protein of both virus types (ICP5 or VP154 for HSV-1, and ICSP9 for HSV-2).

In preliminary studies we have observed an unusual subgroup of γ proteins, which we call γ_2. These polypeptides are made only late in infection and undergo post-translational modification. We chose ICSP16 as a representative of γ_2 for HSV-2 (4) and a previously unidentified polypeptide of type 1, which migrates close to or may be ICP17 of

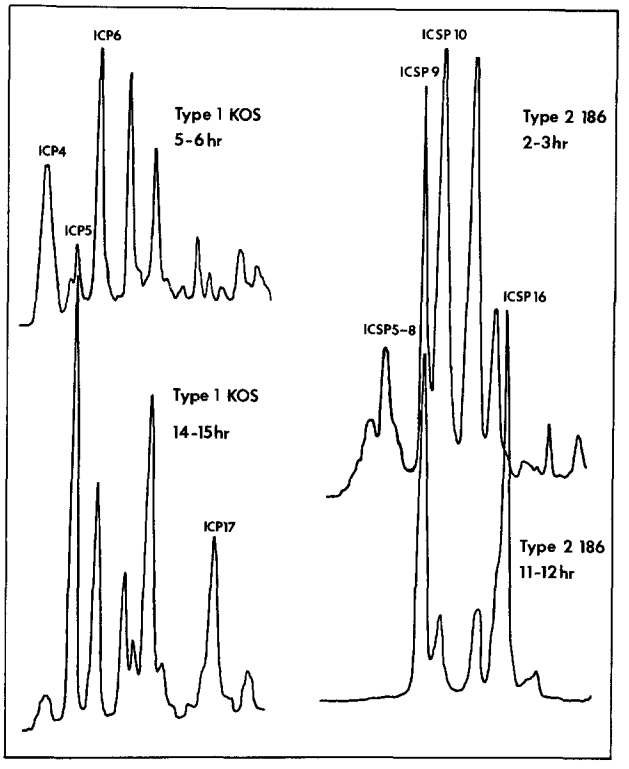

FIGURE 2: Polypeptides of high molecular weight made early and late in infection of cells by herpes simplex virus type 1 (KOS) and type 2 (186). Densitometer scans of x-ray film autoradiograms of the 100,000 to 200,000 molecular weight region of 7% slab polyacrylamide gels are shown. Polypeptides studied in this communication are indicated above the peak concerned.

Honess and Roizman (7). These polypeptides appear only after about 4 hours of infection in HSV-2 infected cells or after 6 hours in HSV-1 infected cells.

Polypeptide synthesis in the absence of DNA synthesis. The effect of inhibiting DNA synthesis on the kinetics of synthesis of each of the four chosen groups of polypeptides is shown in Figure 3. The synthesis of α proteins (ICP4 or ICSP5-8) was stimulated by the absence of DNA synthesis; the β proteins (ICP6 or ICSP10) were made in the

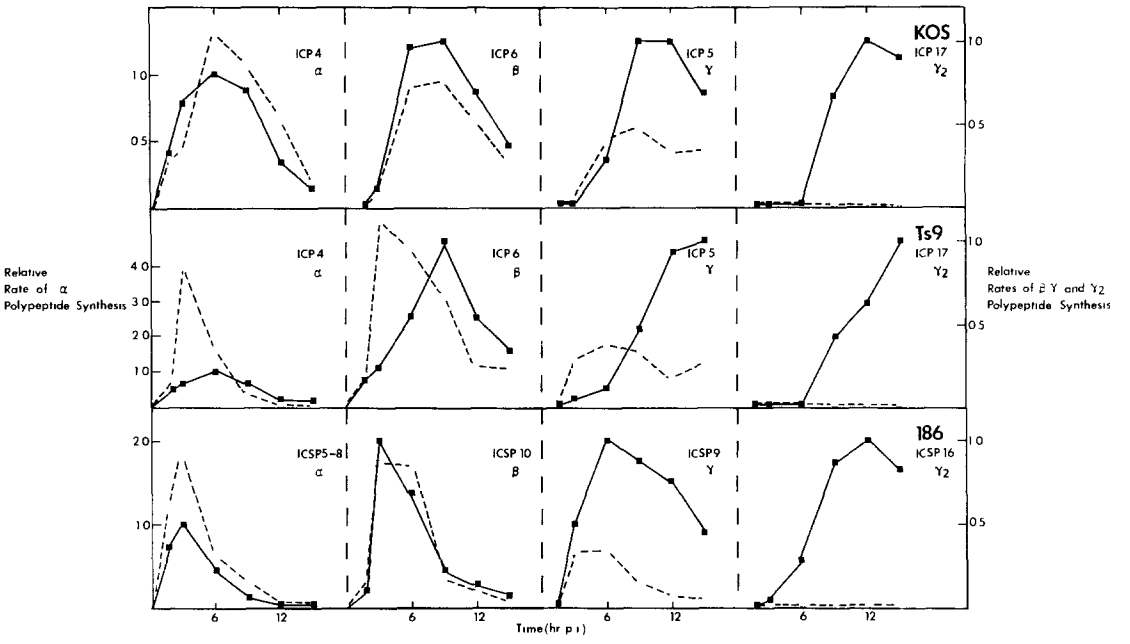

FIGURE 3: Relative rates of polypeptide synthesis at various times post-infection. The rate of a particular α (ICP4 [VP175], ICSP5-8), β (ICP6, ICSP10), γ (ICP5 [VP154], ICSP9) or γ_2 (ICP17, ICSP16) polypeptide synthesized in the presence (■——■) or absence (□----□) of DNA synthesis is plotted as a function of time post-infection. The amount of a particular protein synthesized, as a percentage of the total protein at a particular time after infection, was calculated from an autoradiograph of a polyacrylamide gel on which proteins labelled at this time had been separated. The autoradiogram was scanned using a densitometer, and each peak converted to a percentage of the total using a curve resolver. From this data the amount of a polypeptide synthesized at a particular time (t) was calculated, according to the formulae:

relative rate of polypeptide synthesis =

$$K \left(\frac{\text{absorbance of polypeptide band}}{\text{total absorbance of polypeptide bands in sample}} \times 100 \right)$$

$$\text{where } K = \frac{\text{total } [^{14}C] \text{ amino acid incorporation at time t}}{\text{highest observed } [^{14}C] \text{ amino acid incorporation when DNA was synthesized}}$$

and was then plotted relative to the maximum rate observed when DNA was synthesized normally. A more detailed description of this procedure has been given previously (4).

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usual amounts with or without DNA synthesis. The synthesis of γ proteins (ICP5 or ICSP9) was inhibited about $\frac{1}{3}$ to $\frac{1}{2}$ of the usual amount,

in agreement with Honess and Roizman (2). Lastly, the fourth group of proteins represented by ICP17 or ICSP16 was not detected in the absence of DNA synthesis.

This situation was observed whenever DNA synthesis was inhibited, either by the addition of cytosine arabinoside or by incubation of DNA-negative, temperature-sensitive mutants at the nonpermissive temperature. It should be noted that the rate of synthesis of γ polypeptide ICP5 closely follows that of DNA synthesis. Figure 4 shows the kinetics of synthesis of virus DNA by the mutant tsD9 at 34 and 39°. At 34° DNA synthesis is slightly slower than in wild-type virus infected cells and it peaks at 11 to 12 hours post-infection. The ICP5 polypeptide is only made at maximum rate after this time (Fig. 3).

Similar results with respect to these four polypeptides were

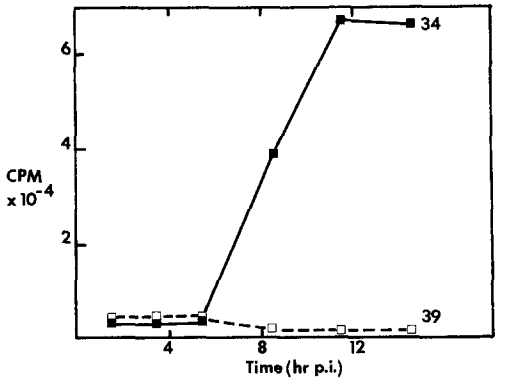

FIGURE 4: Kinetics of DNA synthesis at 34° (■——■) and 39° (□----□) in cells infected with the tsD9 mutant of herpes simplex virus type 1. The rate of viral DNA synthesis was calculated from the quantity of DNA of virus density labelled during a 1-hour period at various times after infection. This was determined using caesium chloride equilibrium density gradient profiles similar to those shown in Figure 1.

found with mutants of all four DNA-negative complementation groups of type 1 mutants isolated in this laboratory (Table II). In those experiments infected cells were labelled from 4 to 24 hours after infection. From Figure 5 it is clear that the overall effect of cytosine arabinoside on protein synthesis reflects the patterns seen with individual polypeptides. This is especially clear when observing cells infected with HSV-2 (strain 186). In this situation after 4 to 5 hours of infection γ and γ_2 proteins are the predominant polypeptides being synthesized and a large reduction is apparent in overall protein synthesis. The situation observed in HSV-1 (strain KOS) infected cells is less clear cut; here host polypeptide synthesis is inefficiently shut off (Honess and Roizman, 1973; Powell and Courtney, 1975) and total protein synthesis is a less clear indication of virus protein synthesis. However, even in this situation a reduction in total protein synthesis is observed in the presence of cytosine arabinoside.

TABLE 2. RATIO OF POLYPEPTIDE SYNTHESIZED AT 39°/ POLYPEPTIDE SYNTHESIZED AT 34°, BY VARIOUS DNA-NEGATIVE MUTANTS OF HERPES SIMPLEX VIRUS TYPE 1

Polypeptide	Mutant				Wild Type
	A_1	B_2	C_4	D_9	
α ICP4 (VP175)	2.730	8.085*	1.778	2.360	1.005
β ICP6 (VP134)	1.466	1.254	0.998	1.090	1.011
γ ICP5 (VP154)	0.631	0.508	0.325	0.489	0.893

* Courtney and Benyesh-Melnick (8) have described this overproduction of VP175.

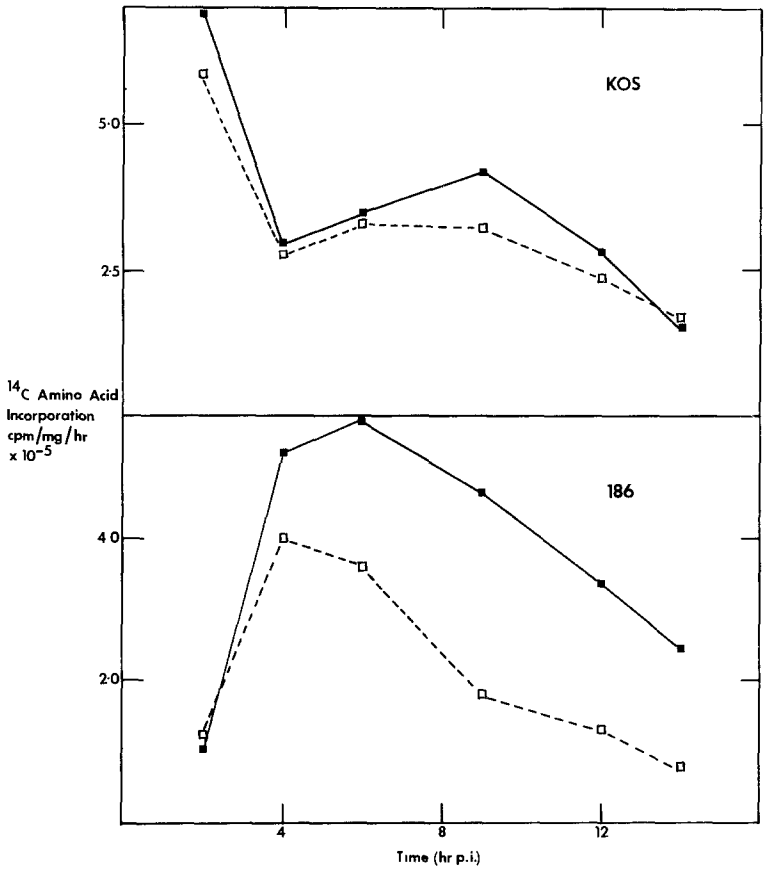

FIGURE 5: Total protein synthesis in cells infected by herpes simplex virus in the presence and absence of cytosine arabinoside. Cytosine arabinoside was added at the time of infection of HEp-2 cells with herpes simplex virus type 1 (strain KOS) or herpes simplex virus type 2 (strain 186). At various times after infection [^{14}C] labelled amino acid mixture was added to cytosine arabinoside treated (□----□) and control (■——■) cultures. After 1 hour of labelling the cultures were harvested and incorporation of label into cold TCA precipitable material determined.

DISCUSSION

We observed three major changes in virus protein synthesis when herpes simplex virus infected cells were prevented from making viral DNA. These were (a) an increased amount of α proteins, (b) a decreased amount of γ proteins, and (c) the failure to synthesize a novel, previously undescribed group of proteins which we have called γ_2 proteins.

It is clear that parental DNA can serve as template for messenger RNA for α, β, and γ polypeptides. The observed stimulation of α protein synthesis may be due to more parental DNA being available for transcription than is usually the case. If that were so, one might expect a stimulation in β protein synthesis also -- this may in fact occur but could be masked by a corresponding decrease in β protein synthesis due to lack of progeny DNA, available for transcription. We feel that the lack of progeny DNA was responsible for the decrease in γ protein synthesis. This decrease can probably be detected because a greater proportion of γ protein is made from progeny DNA than is the case with β proteins. It is important to note, however, that the γ proteins were made in the absence of DNA synthesis, presumably from parental DNA.

There are two possible explanations for the missing proteins when DNA synthesis is inhibited. Firstly, they may be true late polypeptides made only from progeny DNA. Alternatively, the missing proteins which undergo post-translational modification may be used up faster than they are synthesized when their synthesis is inhibited.

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