JOURNAL OF VIROLOGY, June 1982, p. 1131–1134
0022-538X/82/061131-04$02.00/0

Vol. 42, No. 3

Herpesvirus Infection Alters the Steady-State Levels of Cellular Polyadenylated RNA in Polyoma Virus-Transformed BHK Cells

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Received 10 December 1981/Accepted 16 February 1982

Polyoma-transformed BHK cells are permissive for the replication of herpes simplex virus type 1. The effect of herpes infection on the steady-state levels of bulk mRNA sequences in these cells was studied by using cDNA to polyadenylated cytoplasmic RNA from uninfected cells. The principal findings were: (i) herpes simplex virus type 1 infection caused a pronounced reduction in the cytoplasmic levels of moderately abundant mRNAs; (ii) after infection, increased amounts of RNA complementary to the cDNA were isolated as part of the nonadenylated cytoplasmic RNA fraction.

The effect of herpes simplex virus type 1 (HSV-1) infection on the synthesis and steady-state levels of cellular RNA is not completely understood. Nishioka and Silverstein (8–10) have attributed the shutdown of cellular protein synthesis in Friend erythroleukemia cells to the dissociation of cellular mRNA from ribosomes and its subsequent degradation. To quantitate the extent of degradation of cellular RNA sequences, they examined the metabolism of globin mRNA as a representative species of cellular mRNA. Similarly, Pizer and Beard (11) studied the effect of HSV-1 infection on steady-state levels of polyoma-specific RNA in polyoma-transformed BHK (PyBHK) cells (2, 6). They showed that after infection with HSV-1, the level of polyoma-specific cytoplasmic RNA is 20% of the level found in uninfected controls. Because polyoma-specific mRNA represents only a small fraction of the total mRNA in PyBHK cells (6), changes in its level may not be typical of cellular mRNA. We have therefore extended these studies by investigating the effect of HSV-1 infection on bulk cellular mRNA.

Cytoplasmic RNA was isolated from uninfected cultures of PyBHK cells or from cultures infected for 5 h with HSV-1 (strain HF) at 50 PFU per cell. The RNA was enriched for polyadenylated sequences by oligodeoxythymidylic acid-cellulose chromatography as described by Aviv and Leder (1). The RNA not retained by oligodeoxythymidylic acid-cellulose after two passages through the column was taken to be the nonadenylated fraction. Radioactive (^{3}H-labeled) cDNA was synthesized by avian myeloblastosis virus reverse transcriptase in the presence of sodium pyrophosphate (4, 5), with polyadenylated RNA from uninfected cells as the template and oligodeoxythymidylic acid$_{10}$ as the primer. The size of this cDNA, estimated by alkaline agarose electrophoresis, ranged from 250 to 2,500 nucleotides, with the peak of distribution at 750 nucleotides. To compare steady-state levels of cellular mRNA in infected and uninfected cells, we examined the hybridization kinetics of the cDNA with polyadenylated, nonadenylated, and total cytoplasmic RNA isolated from these cells. The kinetic measurements indicate the abundance of the RNA sequences that are complementary to the cDNA probe in each RNA fraction. When these RNA fractions are used to drive the hybridization, the RNA molecules need not be intact, provided that they are large enough to form RNA-DNA hybrids stable enough to protect the cDNA from digestion by S1 nuclease. We will therefore refer to all RNAs complementary to the cDNA probe as "messenger sequences," although not all these sequences may represent intact, functional mRNA molecules (see below).

Figure 1a shows the kinetics of hybridization of the cDNA driven by its own template and by polyadenylated cytoplasmic RNA from infected cells. At least 85% protection of the cDNA from S1 nuclease digestion was attained in both cases. Hybridization of most messenger sequences occurred at a faster rate with RNA from the uninfected cells than with RNA from the infected cells. These data indicate that cellular polyadenylated RNA sequences were reduced in concentration, probably by the viral polyadenylated RNA present in the infected cells. Figure 1b shows the kinetics of hybridization of the cDNA with nonadenylated cytoplasmic RNA.

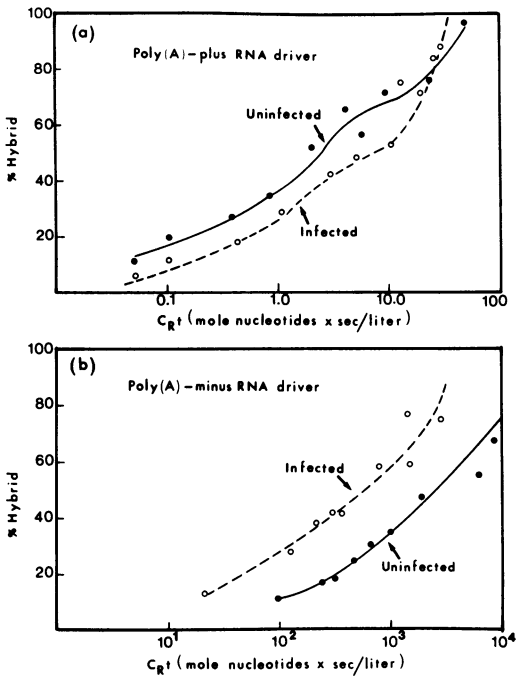

FIG. 1. Kinetics of cDNA hybridization with fractionated RNA. cDNA hybridization was driven by polyadenylated (a) and nonadenylated (b) cytoplasmic RNA from uninfected (●) and HSV-1–infected (5 h postinfection; ○) PyBHK cells. The cytoplasmic RNA was extracted by lysis of cells with 0.15% Nonidet P-40 in 10 mM Tris-hydrochloride (pH 8.0), 0.14 M NaCl, and 10 mM $MgCl_2$, with nuclei removed by centrifugation. The cytoplasmic extract was first deproteinized by phenol-chloroform-sodium dodecyl sulfate (SDS) extraction and then by proteinase K digestion. The phenol-chloroform-SDS extraction was repeated after the proteinase K digestion, and any trace quantity of DNA in the RNA solution was digested with DNase I treated with iodoacetate (14). The DNase I was subsequently removed by a third phenol-chloroform-SDS extraction. The reaction mixtures for hybridization contained 0.24 M phosphate buffer (equimolar disodium and monosodium phosphates), 1.25 mM EDTA, 0.1% SDS, approximately 1,500 cpm of [^{3}H]cDNA per μl, 1 mg of *Escherichia coli* tRNA per ml, and 200 to 5,000 μg of RNA driver per ml. Aliquots (1.0 μl) of this mixture were sealed in capillary tubes and incubated at 64°C for appropriate times to attain the desired C_rt. The percentage of cDNA that was resistant to S1 nuclease was determined by the method of Maxwell et al. (7). The separation of the cytoplasmic RNA into the polyadenylated and nonadenylated fractions is described in the text.

Because more than 80% of the cDNA can hybridize at a high C_rt, we conclude that most messenger sequences are present in the nonadenylated RNA fractions of infected and uninfected cells. These RNA molecules may be functional mRNAs that have short polyadenylate tails or mRNAs that have been partially degraded. Protection of 50% of the cDNA from S1 nuclease was observed at C_rt values of 2,500 and 620 with RNA from uninfected and infected cells, respectively; that is, the nonadenylated RNA of infected cells drove the hybridization at a faster overall rate than the nonadenylated RNA of uninfected cells. These data indicate that, on average, cellular mRNA sequences are fourfold more abundant in the nonadenylated RNA fraction of the infected cells than in the same fraction of the uninfected cells.

To determine how these shifts in the abundance of messenger sequences in the polyadenylated and nonadenylated RNA fractions are reflected in steady-state levels of hybridizable messenger sequences in the cytoplasm, total cytoplasmic RNA was extracted from uninfected cells and from HSV-1–infected cells at 5 h postinfection. These RNAs were used to drive hybridization reactions with the radioactive cDNA. The kinetics of hybridization (Fig. 2) indicate that the percentage of messenger sequences that hybridized at C_rt values of 100 to 1,000 (where C_r = total cytoplasmic RNA concentration) was particularly affected in infected cells. These sequences appear to be present at a four-to fivefold-higher level in uninfected cells.

We wished to know whether the HSV-1–induced effects on RNA metabolism altered the level of functional cellular mRNA as measured by protein synthesis. To answer this question, cultures of PyBHK were infected with HSV-1 at a multiplicity of 20 PFU per cell, and protein synthesis was monitored by labeling with [^{35}S]methionine during 2-h periods at different times postinfection. The results (Fig. 3) indicated that although the total acid-precipitable radio-

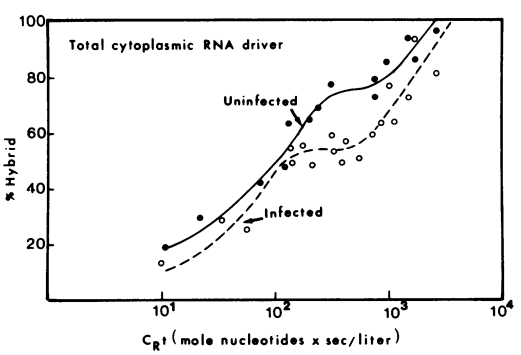

FIG. 2. Kinetics of cDNA hybridization to total cytoplasmic RNA. cDNA hybridization was driven by total cytoplasmic RNA from uninfected (●) and HSV-1–infected (5 h postinfection; ○) PyBHK cells. The extraction and hybridization procedures were as described in the legend to Fig. 1.

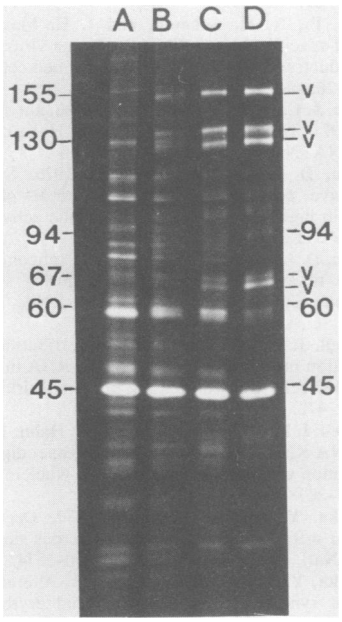

FIG. 3. SDS-polyacrylamide gel electrophoresis of [^{35}S]methionine-labeled infected cell extract. Plates containing about 10^5 cells were mock infected or infected with 20 PFU of HSV per cell. After 90 min of virus adsorption, the cells were overlayed with medium containing 0.1 the normal concentration of methionine, and at the designated times, [^{35}S]methionine was added (10 μCi/ml) for 2-h periods. After being labeled, the cells were washed with buffer and lysed with 0.3 ml of 50 mM Tris-hydrochloride buffer (pH 7.5) containing 1% SDS. The radioactivity present in samples of the lysates was measured, and 20-μl samples were subjected to electrophoresis on a 12.5% polyacrylamide gel as previously described (12). A radioautogram of the dried gel is shown. Lane A, uninfected cell extract which contained 6.2×10^5 cpm; Lane B, cell extract labeled 2 to 4 h postinfection which contained 7.7×10^5 cpm; Lane C, cell extract labeled 4 to 6 h postinfection which contained 3.4×10^5 cpm; Lane D, cell extract labeled 6 to 8 h postinfection which contained 4.0×10^5 cpm. The mobility of molecular weight standards ($\times 10^3$) are indicated by the numbers on the side of the gel, and predominate viral proteins, e.g., the major capsid protein of 155,000 molecular weight, are marked with the symbol V.

activity incorporated at 4 to 6 and at 6 to 8 h postinfection was approximately 50% of that incorporated by the uninfected control, cellular polypeptides were still being synthesized at these times. Clearly, some cellular mRNA remains functional in PyBHK cells late in the viral replication cycle.

Because the shutoff of cellular polypeptide synthesis was not as dramatic as that previously observed in other cells, the question was raised of how permissive the PyBHK cells are for HSV-1. Growth curves of HSV-1 in PyBHK cells indicated a mean burst size of 30 to 50 PFU per cell (Nystrom and Pizer, unpublished data). This value is somewhat lower than that for other permissive cell lines such as the parent BHK 21/C13, which has a mean burst size of over 200 PFU per cell (12). The reduced level of cellular protein synthesis and the appearance of viral polypeptides correlate well with the changes in RNA levels, and the incomplete shutoff of cellular protein synthesis might account for the relatively low yield of virus.

When results of the present experiments are compared with other reports on RNA metabolism in HSV-1–infected cells, the effects in PyBHK cells appear to be less pronounced. The slower rate of hybridization with polyadenylated RNA from the infected cells, compared with that from the uninfected cells, was probably due to dilution of cellular sequences with HSV-1 mRNA. HSV-1 mRNA can, in some cell lines, constitute more than 70% of the polyadenylated RNA (13; Stenberg and Pizer, unpublished data). The dilution of cellular polyadenylated sequences may be enhanced by HSV-1–induced degradation or deadenylation of messenger sequences or by the reduced capacity of HSV-1–infected cells to synthesize or process cellular mRNA. The faster rate of cDNA hybridization driven by the nonadenylated RNA from infected cells may also be an indication of degradation of cellular polyadenylated mRNA or improper processing of nuclear transcripts in infected cells.

The kinetics of cDNA hybridization driven by total cytoplasmic RNA suggest that HSV-1 infection causes a selective reduction in the steady-state level of moderately abundant messenger sequences. Since earlier experiments indicated that the polyoma-specific RNA in these cells was markedly reduced by HSV-1 infection, we calculated whether the polyoma-specific mRNA belongs to the abundancy class that shows this marked effect. Taking the average molecular weight of polyoma transcripts in the cytoplasm to be 1×10^6 (6) and the $C_rt_{1/2}$ (pure) of hemoglobin mRNA (M_W 200,000) to be 3×10^{-4} mol·s/liter (3), we estimated that the $C_rt_{1/2}$ (pure) of polyoma mRNA hybridizing with its cDNA would be approximately 1.5×10^{-3} mol·s/liter. From RNA labeling experiments, it has been estimated that approximately 0.0012% of the steady-state cytoplasmic RNA of PyBHK cells is polyoma specific (6). Using these data, we calculate that the polyoma mRNA hybridizes with its cDNA at a $C_rt_{1/2}$ (mixture) of 125 mol·s/liter (where C_r = total cytoplasmic RNA concentration). This value is consistent with the proposal that polyoma transcripts belong to the abundancy class of mRNA (which hybridizes at

cytoplasmic C_rt values of 100 to 1,000) that shows the greatest change after infection.

It is interesting to note that while increased amounts of most classes of messenger sequences were isolated in the nonadenylated cytoplasmic RNA fraction of infected cells, the largest reduction in the level of messenger sequences in the total cytoplasmic RNA fraction occurs in a subset. It is possible that infection with HSV-1 has two effects: one effect, such as degradation or deadenylation, which causes most messenger sequences to be isolated in the nonadenylated RNA fraction; and a second effect, such as reduced transcription, which reduces the appearance of a certain class of mRNA in the cytoplasm. The synthesis of polyoma-specific RNA is reduced after infection (11), and this RNA could be an example of the latter class of RNA described above. The results of this paper demonstrate that the levels of polyoma-specific mRNA in the cytoplasm of PyBHK cells cannot be representative of all cellular mRNA sequences. Other individual mRNA species should be examined independently to describe precisely the range of effects of HSV-1 on host RNA metabolism.

This research was supported by grant NP-209 from the American Cancer Society and Public Health Service grant 5 T32 AI07209 from the National Institutes of Health.

We acknowledge helpful discussions with Richard Stenberg that contributed to this research and thank Meredith Peak for performing the electrophoretic analyses of the radioactive proteins. L.I.P. thanks Heidi Diggelmann and the members of ISREC for their help in initiating these studies.

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