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Herpesvirus Infection Modifies Adenovirus RNA Metabolism in Adenovirus Type 5-Transformed Cells

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The effect of herpes simplex virus (HSV) infection on mRNA metabolism was examined in a system where the fate of a specific RNA sequence can be assayed. Adenovirus type 5-transformed rat embryo cell line 107 synthesizes adenovirus-specific RNA (ad-RNA), which functions in the cytoplasm as mRNA. We have utilized ad-RNA as a model for mRNA metabolism, and in a preliminary study we characterized ad-RNA in the nucleus and cytoplasm by hybridization to filter-bound adenovirus DNA. The results indicated that ad-RNA accumulates in the nucleus and that cytoplasmic polyadenylic acid [poly(A)]-containing ad-RNA turns over with a half-life of a few hours. Pulse-chase experiments confirmed these observations, and a half-life of about 5 h was determined for the poly(A)-containing cytoplasmic ad-RNA. A second class of ad-RNA remains in the nucleus, where it turns over with a longer half-life (about 24 h). The infection of 107 cells by HSV was restricted at 37°C, giving a burst size of 5 PFU per cell and allowing continued host DNA synthesis. Protein synthesis was inhibited greater than 50% by 7 h after infection, and total RNA synthesis was 50% inhibited by 4 h after infection. During the first 8 h after infection, HSV has little effect on the rate of synthesis of ad-RNA as determined by hybridization of nuclear RNA samples, but, during the same period, HSV inhibits the accumulation of poly(A)-containing ad-RNA in the cytoplasm. The degree of this inhibition increases steadily throughout this period and reaches 60% by 6.5 to 8 h after infection. No significant effect was seen on the accumulation of total cellular poly(A)-containing RNA. It was concluded from these experiments that HSV infection alters the metabolism of ad-RNA so as to prevent the normal appearance of the poly(A)-containing mRNA in the cytoplasm. The result for ad-RNA may not represent the behavior of total cellular poly(A)-containing RNA under conditions where infection is restricted.

Infection with herpes simplex virus (HSV) or pseudorabies virus results in the reduced incorporation of radioactive uridine into RNA (14, 19, 22, 37, 39, 48). It has been established for some time that the accumulation of rRNA is inhibited by HSV infection (48). However, the effect of the virus on cellular mRNA metabolism has been more difficult to define, partly because of problems in assaying cellular mRNA sequences. The observation that eucaryotic mRNA's may contain polyadenylic acid [poly(A)] sequences at their 3′ ends has permitted a more direct assay of mRNA molecules in HSV-infected cells. Studies have shown that infection inhibits the incorporation of uridine into nonviral adenylated RNA in both the nucleus and the cytoplasm, thus implying that production of cellular mRNA was inhibited by HSV type 1 (HSV-1) (44).

Previous work in our laboratory established that HSV-1 infection reduces both the rate of synthesis and the steady-state level of polyoma-specific RNA in polyoma virus-transformed BHK cells (36). Such studies have employed the transformed cell message as a model for a specific cellular mRNA. To extend these observations, we report here on the metabolism of adenovirus-specific RNA (ad-RNA) in adenovirus-transformed rat embryo cell line 107. The characteristics of ad-RNA in transformed cells suggest that it is metabolized in a fashion similar to cellular mRNA's (42, 47, 49). We thought that this system might prove useful for studying mRNA metabolism in HSV-1-infected cells. This approach has also revealed a model for the

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metabolism of ad-RNA sequences in the uninfected transformed cell.

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MATERIALS AND METHODS

Cells and virus. 107, a continuous line derived from the transformation of Fisher rat embryo cells by adenovirus 5 temperature-sensitive mutant H5ts107, was a generous gift of H. S. Ginsberg. Cells were grown as monolayers in Eagle minimal essential medium (MEM) without calcium and supplemented with 5% fetal bovine serum and 0.1 mM nonessential amino acids (15). Cultures were screened periodically for mycoplasma by their ability to incorporate radioactive uridine and uracil (16). KB cells were grown in Eagle MEM with 10% calf serum. BHK cells were grown in Eagle MEM with 5% fetal bovine serum.

The prototype strain of adenovirus 5 was obtained from H. S. Ginsberg. Stocks of virus were prepared by infecting suspension cultures of KB cells. Cells were harvested 36 to 40 h after infection. After six cycles of freezing and thawing, cellular debris was removed by centrifugation.

HSV-1 strain HF was grown in KB cells (13). Stocks were prepared by infecting confluent monolayers with 0.1 PFU per cell for 48 to 72 h. Virus was released from the cells by homogenization. High-titer stocks of HSV-1 were prepared by infecting monolayers or suspension cultures of KB cells at 5 to 20 PFU per cell. After 24 h, infected cells were collected and suspensions were disrupted by sonic treatment. All media for HSV-1-infected cultures were supplemented with 0.1 mM arginine. Indirect immune fluorescence using antisera against HSV-1 antigens was employed to demonstrate that all 107 cells were infected under the experimental conditions used.

Assay of viruses. Adenovirus was assayed by a fluorescent-focus procedure (35, 46, 50). The plaque assay for HSV was a modification of the assay described by Flanagan (14). Confluent monolayers of BHK cells on 35-mm-diameter plates were infected with 0.25 ml of sonically treated viral suspensions which had been diluted appropriately. At 2 h, cells were overlaid with 3 ml of medium. After 24 h, the medium was poured off and the plates were allowed to air dry. Plaques appeared as syncytia.

Purification of viral DNAs. Adenovirus was purified from infected cells by the method of Lawrence and Ginsberg (24). Herpesvirus nucleocapsids were prepared according to Kieff et al. (23). DNA was extracted from virus by a modification of the technique of Bello and Ginsberg (8). Samples were dialyzed against 0.15 M NaCl–0.015 M sodium citrate–0.01 M phosphate (pH 7.2). Viral DNA was released by adding sodium dodecyl sulfate (SDS) to 1% for 30 min followed by incubation for 4 h in the presence of 0.5 mg of Pronase per ml, all at 37°C. All Pronase solutions were freshly made and predigested for 30 min at 37°C to inactivate contaminating nucleases. The DNA was kept at room temperature overnight and then warmed to 37°C before being extracted twice with water-saturated phenol. The phenol was removed from the aqueous phase by ether treatment, and DNA was dialyzed against 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate). DNA concentrations were determined by measuring absorbance at 260 nm (A_{260}). DNA with an A_{260}/A_{280} ratio greater than 1.9 was used for hybridization.

DNA-RNA hybridization. The filter hybridization technique used is a modification of the procedures of Gillespie and Spiegelman (17) and Stubbs and Hall (45), using formamide (26). All procedures were carried out with sterile glassware and solutions.

Hybridizations were performed in 1.5-ml plastic conical centrifuge tubes (Eppendorf). For adenovirus, 6.5-mm filters containing specific and nonspecific DNA were immersed in 200 μl of RNA solution containing 50% formamide (Eastman, Spectrograde), 2× SSC, 0.1% SDS, and 0.2 M triethanolamine (TEA)-hydrochloride, pH 7.5. Incubation was at 37°C for 90 to 96 h. Filters were washed twice with 2× SSC and then treated for 60 min at room temperature with 2× SSC containing 10 μg of pancreatic RNase per ml (Worthington, heated to 90°C for 10 min) and 2 units of T1 RNase (Worthington) per ml. Filters were washed extensively with 2× SSC, dried and counted for times sufficient to record at least 1,000 total counts.

For HSV-specific RNA, hybridization was done at 66°C for 44 to 48 h in a solution containing 0.9 M NaCl, 0.2 M TEA-hydrochloride, pH 7.5, and 0.1% SDS. Filters were washed and treated as described above.

Labeling and extraction of RNA. The infection and labeling of 107 cells for DNA were done in maintenance media (MM) (Eagle MEM without calcium supplemented with 1% fetal bovine serum, nonessential amino acids, and 0.1 mM arginine). Cells growing on 100-mm-diameter plates were infected with 2 ml of virus suspension in MM. After a 1.5- to 2-h absorption period, the infecting medium was removed and the plates were washed twice with 5 ml of MM. Each plate then received 10 ml of MM until isotope was added. A 2-ml portion of [5-^{3}H]uridine (25 to 30 Ci/mmol; New England Nuclear Corp.), at 100 μCi/ml, and 50 μM uridine were added to each plate. Incorporation was stopped by rapidly chilling the plates. Labeled medium was removed and the cells were washed with 5 ml of ice-cold lysis buffer (0.25 M sucrose–0.025 M NaCl–5 mM $MgCl_2$–10 mM TEA-hydrochloride, pH 7.5).

All subsequent procedures were carried out with sterile solutions and glassware. All manipulations up to the phenol extractions were performed rapidly at 0°C. Nuclear and cytoplasmic fractions were prepared according to Buetti (12) and Beard et al. (7). For each set of three to five plates, the buffer was removed and 1 ml of fresh buffer containing 1% Nonidet P-40 (NP-40) was added to the first plate. The cells were scraped from the plates sequentially and removed to a centrifuge tube. Plates were washed in the same sequence with 0.5 ml of lysis buffer containing 0.5% NP-40, and the cells were disrupted by aspiration. Nuclei were pelleted at 2,000 rpm for 10 min and washed once with 0.5% NP-40 in lysis buffer. The cytoplasmic supernatants were pooled and clarified at 10,000 rpm for 20 min.

Nuclei were treated with 10 μg of RNase-free DNase (Worthington) at 37°C for 1 min. Cellular fractions were quick frozen in a dry ice-ethanol bath and stored at −20°C until RNA extractions could be performed.

Pulse-chase of RNA. Cells labeled for 3 h as described above were washed twice with medium containing no uridine and fed a 10-fold excess of cold uridine (500 μM) in MM. RNA was extracted from nuclear and cytoplasmic samples. To normalize the recovery of radioactivity, total counts per minute were corrected for variations in yield and for the growth of the cells.

As a control for the ability to separate nuclei from cytoplasmic RNA contamination, components of the nuclear and cytoplasmic RNA present after an 18-h chase were examined. RNA was fractionated by electrophoresis in 2.8% polyacrylamide gels. As expected, the major component accounting for over 60% of the radioactivity in the stable cytoplasmic RNA was 18S and 28S RNA. There was also appreciable rRNA in the nuclear preparation; this was presumed to be contaminated by ribosomes associated with the outer nuclear membrane. There were also a significant number of counts distributed throughout the nuclear gel which were absent from the cytoplasm. An estimate of the amount of cytoplasmic RNA contaminating a typical nuclear RNA preparation was calculated from the amount of rRNA in several gels by assuming that all of the rRNA in the cell is cytoplasmic in origin. About 65% of the cytoplasmic counts per minute were in rRNA (2.7×10^7 to 3.0×10^7 cpm). For nuclear RNA, 40% of the counts per minute were in 18 or 28S (3.0×10^6 cpm). Thus about 10% of the total rRNA was retained in the nuclear sample.

Extraction of nuclear RNA. Nuclear RNA was extracted by the hot phenol-SDS procedure (2, 41). Frozen nuclei were thawed in 4 ml of extraction buffer (10 mM sodium acetate, pH 5.0) and made 1% in SDS. RNA was extracted three times at 65°C with 90% distilled phenol in extraction buffer, and a final extraction was performed at room temperature. Nucleic acids were precipitated from the aqueous phase by adding NaCl to 0.25 M and 2 volumes of ice-cold ethanol. Precipitation was at −20°C for at least 3 h. RNA was precipitated again and the second precipitate was dried in vacuo and dissolved in 1 ml of buffer (0.5 M NaCl–5 mM $MgCl_2$–10 mM $CaCl_2$–10 mM TEA-hydrochloride, pH 7.5). A 10-μg/ml amount of RNase-free DNase was added for 30 min, and the digestion was repeated (9). In the experiment described in Fig. 5, RNA was further deproteinized by digestion with 0.5 mg of freshly made, predigested Pronase per ml for 1 h at 37°C. Otherwise, EDTA was added to 3 mM, and RNA was extracted with an equal volume of 90% phenol at room temperature and precipitated twice. The RNA pellet was dried and dissolved in extraction buffer. A small aliquot was taken for determinations of total counts and A_{260}. Pure RNA preparations gave an A_{260}/A_{280} of greater than 2.1. The remainder of the RNA was divided in half. One portion was precipitated for hybridization, and the second was used for oligodeoxythymidylic acid [oligo(dT)]-cellulose chromatography.

Extraction of cytoplasmic RNA. The procedure of Perry et al. was used (34). Cytoplasm was diluted with buffer (0.05 M NaCl–6 mM EDTA–10 mM TEA-hydrochloride, pH 7.5), made 1% in SDS, and extracted with an equal volume of phenol-chloroform-isoamyl alcohol (50:50:1). The aqueous phase and interface were reextracted first with an equal volume of the mixture and then with just chloroform. After two precipitations, RNA was dried and quantitated as described above. Cytoplasmic RNA solutions from infected cells were found to require DNase, Pronase, and phenol treatment as described above. The final dried precipitate was dissolved in nuclear extraction buffer and apportioned as described for nuclear RNA.

Oligo(dT)-cellulose chromatography. Oligo-(dT)-cellulose (Collaborative Research, Inc.) was packed into columns (0.5 by 1.5 cm), and poly(A)-containing RNA was selected by the procedure of Aviv and Leder (3). RNA was bound with 0.5 M NaCl–0.1% SDS–10 mM TEA-hydrochloride, and the column was washed extensively with 0.1 M NaCl–10 mM TEA-hydrochloride, pH 7.5. Poly(A)-RNA was eluted in 10 mM TEA-hydrochloride, pH 7.5. Unbound RNA was directly precipitated with ethanol. Bound RNA was precipitated by adding NaCl to 0.25 M, 100 μg of *Escherichia coli* tRNA per ml, and ethanol.

Polyacrylamide gel electrophoresis of RNA. The procedure of Loening (25) was used except that ethylene diacrylate was used as cross-linking agent (10). The buffer system was 40 mM Tris-acetate (pH 7.5)–0.2 mM EDTA–10% glycerol. RNA samples were run on 3% gels of 12 cm in length for 3 h, or on 2.8% gels for 4 h, at 5 mA per gel. Gel slices (2 mm) were solubilized with 0.3 ml of concentrated NH_4OH and counted.

Growth of HSV in the presence of neutral red. The use of neutral red to inactivate HSV-1 was described by Rapp et al. (38). Concentrated neutral red-grown HSV-1 was prepared in aluminum foil-covered glass bottles. Cells were pretreated with 25 μg of neutral red per ml for 2 h. The excess dye was removed by four washes with warm phosphate-buffered saline before infection. Infected cells were harvested and stored in the dark. Virus was inactivated by exposure to direct fluorescent light for 1 h, a treatment which completely prevents plaque formation by HSV (38; M. Factor and G. H. Cohen, personal communication). All infections of 107 cells with neutral red virus (treated or untreated) were done in the dark.

Cells growing on 35-mm plates were infected with 0.2 ml of virus suspension for 1 to 2 h, and the infecting medium was removed. Plates were washed twice with 0.5 ml of MM, and 1.0 ml of the same medium was added until labeling was to begin. Labeling was done at 6 h with 5 μCi of [^{3}H]uridine and 2 μCi of [^{35}S]-methionine per ml in 0.5 to 1.0 ml of MM. Incorporation was stopped by rapid chilling. Trichloroacetic acid-insoluble radioactivity was determined. Spillover of ^{35}S into the ^{3}H channel was 6% with a 10% counting efficiency.

Pool size determinations. Labeling and the preparation of extracts were carried out according to Roller and Cohen (40). Forty-eight hours before the experiment cells were transferred to media containing 2.5 μCi of $^{32}PO_4$ per ml (carrier free; New England Nuclear Corp.). All infections and ^{3}H labelings were done in MM with 2.5 μCi of $^{32}PO_4$ per ml. Cells were harvested,

and soluble extracts were prepared as previously described (40).

Ribonucleoside triphosphates were separated from all other phosphate-containing small molecules on thin-layer plates of polyethyleneimine-cellulose (Brinkman MN PEI-cellulose 300) according to M. Cashel (personal communication). Plates were prepared by developing with an ascending water wash before use. Samples (10 μl) were spotted and fan dried. Plates were then immersed in absolute methanol for 5 min and fan dried again. Chromatograms were developed in the first dimension with 3.3 M ammonium formate containing 4.2% boric acid adjusted to pH 7.0 with NH_4OH. After air drying, the solvent front was cut off and the plates were washed with 90% methanol for 15 min. After fan drying, the plates were rotated 90° and developed in the second dimension with 0.75 M KH_2PO_4, pH 3.4. Plates were dried in a warm circulating oven, and ^{32}P-containing ribonucleoside triphosphates were localized by overnight exposure of the chromatograms to Kodak medical X-ray film. Radioactive areas were cut out of the chromatograms and counted. The spillover of ^{32}P into the ^{3}H channel was 3% with a 30% ^{3}H counting efficiency. The identity of spots was determined by chromatography of known standards. To standardize counting and to measure the specific activity of phosphate solutions, known fractions of ^{32}P media were spotted onto polyethyleneimine-cellulose plates, and the radioactivity was measured. Inorganic phosphate was assayed according to Bartlett (6).

RESULTS

Metabolism of ad-RNA in 107 cells. To interpret any experiments on the synthesis of ad-RNA in HSV-1-infected 107 cells, it was necessary to examine the metabolism of this RNA in uninfected cells. As a first step, the accumulation of radioactive uridine into ad-RNA was followed. Monolayers were labeled with [^{3}H]uridine as described in Table 1, and RNA extracted from nuclear and cytoplasmic samples was hybridized to adenovirus DNA under conditions of DNA excess. Unexpectedly, ad-RNA accumulated in the nucleus at a faster rate than polyadenylated ad-RNA accumulated in the cytoplasm. Since most of the newly labeled cytoplasmic ad-RNA is adenylated (Table 2), this result suggested that more than half of the ad-RNA is retained in the nucleus and builds up as a stable population.

To examine this phenomenon more carefully the decay of prelabeled RNA was studied. The 107 cells were labeled with [^{3}H]uridine for 3 h, and the radioactivity was chased by washing the cells with media without added nucleoside followed by incubation in the presence of excess unlabeled uridine. RNA was extracted from nuclear and cytoplasmic samples, and from these fractions poly(A)-containing RNA was isolated. To normalize the recovery of radioactivity, total counts were corrected for variations in RNA yield and for the growth of the cells. An effective chase should result in an increase in the quantity of stable cellular RNA proportional to the cell number, but the amount of radioactivity in this RNA should not increase. The radioactivity in total RNA and in the polyadenylated RNA in samples taken after the chase is shown in Fig. 1. An efficient chase is suggested because accumulation of radioactivity into total cytoplasmic RNA, mostly stable rRNA, ceased by 4 to 6 h after the chase, and 80% of the adenylated nuclear RNA chased during the first 4 h. These two results suggest that there is a low level, if any, of incorporation of isotope into stable and unstable species after 4 h, and that the chase was sufficient to permit the determination of the stabilities of all but the most rapidly turning over RNAs.

Figure 1 also shows that the cytoplasmic

TABLE 1. *Accumulation of ad-RNA sequences in 107 cells*[a]

Source of RNA	Duration of labeling (h)	Hybridization			Radioactivity in sample[b] (cpm × 10^{-3})	Ad-RNA (cpm)
		RNA added (cpm × 10^{-4})	Hybridized			
			cpm	%		
Nucleus	1.0	80	60	0.008	160	1,280
	1.5	110	70	0.006	230	1,380
	3.0	150	150	0.010	250	2,500
	6.0	200	210	0.010	400	4,000
	9.0	230	280	0.012	470	5,640
Cytoplasm [poly(A)-containing]	1.0	1.4	34	0.24	3.8	910
	1.5	3.0	40	0.13	8.6	1,150
	3.0	5.8	60	0.10	13	1,300
	6.0	12	140	0.12	26	3,120
	9.0	20	160	0.08	43	3,440

[a] Cells at 4.3×10^{7} per plate were labeled with [^{3}H]uridine at a concentration of 50 μM (2 μCi/nmol). Monolayers were fed with fresh labeled medium at 3 and 6 h. At the times indicated samples were harvested and RNA was extracted from the nucleus and cytoplasm. Samples were hybridized to 8 μg of adenovirus DNA and the background hybridizing to T4 DNA filters (3 to 8 cpm) was subtracted.

[b] Normalized for RNA yield from 1-h sample by A_{260} measurements.

TABLE 2. *Turnover of cytoplasmic ad-RNA*[a]

Source of RNA	Duration of chase (h)	Hybridization			Radioactivity in sample (cpm × 10^{-5})	Ad-RNA (cpm)	% Adenylated
		RNA added (cpm × 10^{-4})	Hybridized				
			cpm	%			
Cytoplasm (total)	0	110	101	0.009	250	2,250	75
	4	170	85	0.005	420	2,100	51
	6	210	84	0.004	450	1,800	43
	10	200	55	0.003	450	1,350	34
	18	190	56	0.003	460	1,380	16
Cytoplasm [poly(A)-containing]	0	12	150	0.12	14	1,680	
	4	9.5	53	0.09	12	1,080	
	6	7.2	48	0.07	11	770	
	10	4.4	27	0.06	7.6	460	
	18	3.2	12	0.04	5.4	220	

[a] Samples prepared as in Fig. 1 were hybridized to 16 μg of adenovirus DNA. The background and radioactivity hybridizing to T4 DNA filters (average of 8 cpm) were subtracted.

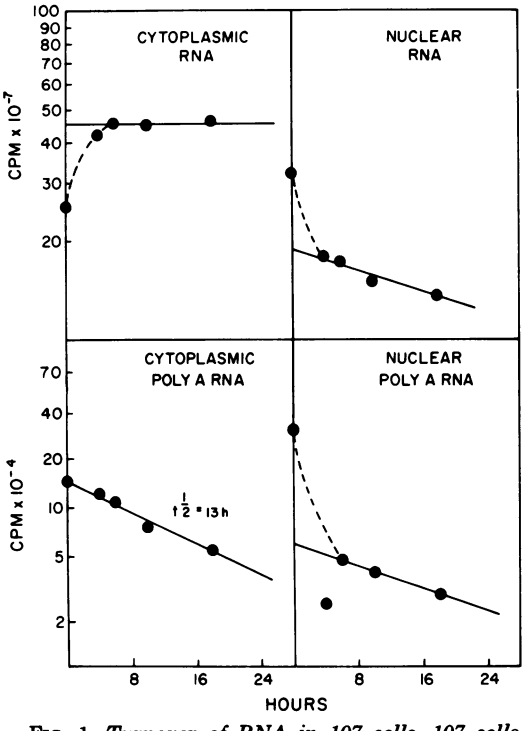

FIG. 1. *Turnover of RNA in 107 cells. 107 cells were labeled for 3 h as described in Table 1, and the labeling medium was removed. Plates were washed twice with medium containing 0.5 mM cold uridine. At the times indicated RNA was extracted from nucleus and cytoplasm, and poly(A)-containing RNA was isolated. Recoveries of RNA were corrected for RNA yields by A_{260} measurements and normalized to the zero time point. Recoveries were also corrected for the growth of the cells by counting the cells on the plates at the beginning and end of the chase.*

poly(A)-containing RNA has an average half-life of about 13 h. Nuclear RNA has a rapidly decaying component, which includes the adenylated sequences, and a stable component, much of which may arise from contamination with rRNA (see Materials and Methods).

To determine the turnover characteristics of ad-RNA, the RNA samples shown in Fig. 1 were hybridized to adenovirus DNA. The results for cytoplasmic ad-RNA (Table 2 and Fig. 2) led to the following conclusions about ad-RNA in 107 cells. (i) The polyadenylated ad-RNA in the cytoplasm has a short half-life of about 5 h. (ii) The nuclear ad-RNA consists of two metabolic classes. One is transported rapidly to the cytoplasm, or decays, within the first 4 h of the chase. The second is quite stable and is probably responsible for the accumulation of ad-RNA in the nucleus. (iii) There appear to be nonadenylated ad-RNA sequences in the cytoplasm whose absolute amount does not change appreciably during the chase. This ad-RNA becomes a larger and larger fraction of the total cytoplasmic ad-RNA sequences during the chase since the adenylated ad-RNA is decaying.

Replication of HSV-1 in 107 cells. Figure 3 shows the growth cycle of HSV-1 in 107 cells. After a latent period of at least 6 h, progeny virus was detected at 9 h, and the yield of 5 to 10 PFU per cell reached a peak at 15 to 24 h. This time course agrees with that usually observed for HSV-1, and we chose the latent period to study the effect of HSV-1 on cellular RNA synthesis. Indirect immune fluorescence with anti-herpes serum demonstrated that HSV antigens were present in all the cells. From the intensity of fluorescence it appeared that the amount of antigen increased with time postinfection.

RNA and protein synthesis in infected 107 cells. To measure the rate of RNA and protein synthesis at different times after HSV infection, monolayers of 107 cells were infected or mock infected and labeled for 30-min periods with [^{3}H]uridine and [^{35}S]methionine. The data in Fig. 4 show that the rate of RNA synthesis is

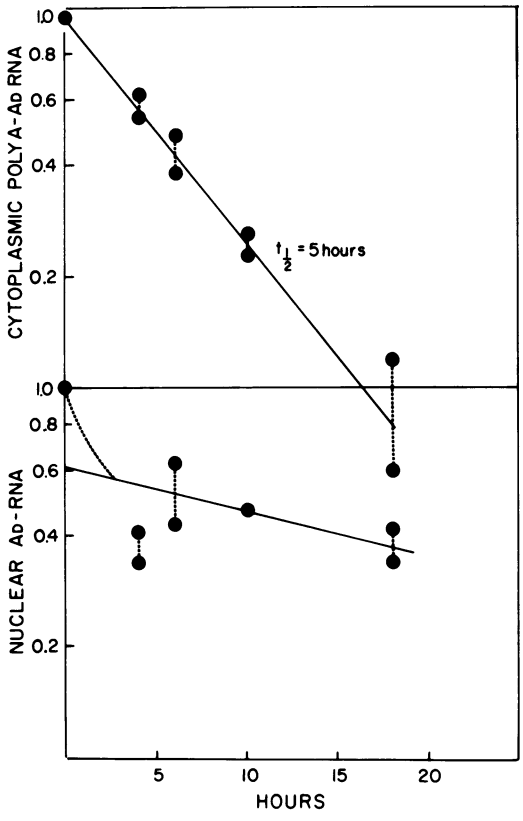

FIG. 2. *Turnover of ad-RNA in 107 cells. Samples prepared as in Fig. 1 were hybridized to excess adenovirus DNA and the backgrounds and counts per minute hybridizing to T4 DNA filters (1 to 5 cpm) were subtracted. The ad-RNA was calculated from the percent hybridization and total radioactivity in the samples as in Table 2. Results are shown as fraction remaining for two separate determinations. Top, Cytoplasmic poly(A)-containing RNA; bottom, nuclear RNA.*

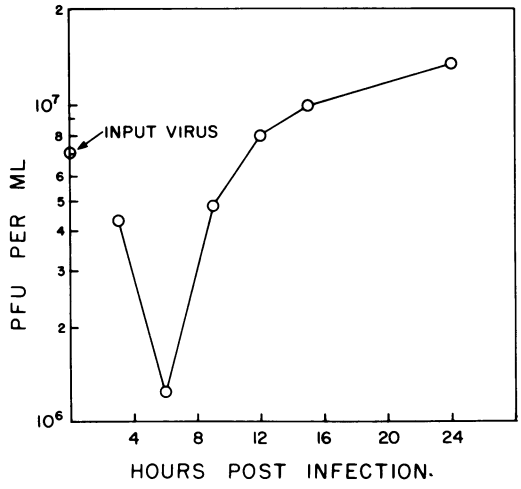

FIG. 3. *Growth curve of HSV-1 in 107 cells. 107 cells in suspension culture at 10^6 cells per ml were infected with 7 PFU of HSV-1 per cell. At 2 h after infection, cells were centrifuged to remove the infecting medium and resuspended in fresh warm medium containing 0.1 mM arginine. Duplicate samples were withdrawn at the times indicated and assayed for infectious virus.*

inhibited by 4 h after infection to about 50% of that in uninfected controls, and the inhibition increases to a maximum of 80% by 10 h. The inhibition of protein synthesis is not apparent at 4 h but also reaches nearly 80% by 10 h.

To determine whether the inhibition of RNA and protein synthesis by HSV-1 is multiplicity dependent, 107 cells were infected at different multiplicities and assayed for rates of RNA and protein synthesis as described above. Somewhat greater inhibitions were obtained by increasing the multiplicity from 25 PFU per cell to 50 or 100. However, no further inhibition was obtained at multiplicities greater than 100 (data not shown).

The virus effect on host synthesis might be due to an HSV-coded function or to components of the infecting fluid. To test these possibilities, conditions were sought under which expression of the viral genome was inhibited but infection of the cells was unaffected. Cycloheximide could not be used with the 107 cells to study the inhibition of RNA synthesis by HSV-1 because it had too great an effect on RNA synthesis in the uninfected cell. An alternate approach was to use neutral red-inactivated virus. HSV-1 grown in the presence of the intercalating dye will replicate normally in the absence of light. Exposure of the virus stock to light activates the dye and blocks viral replication upon further passage (38). To test whether neutral red-inactivated virus inhibits RNA and protein synthesis, infections were done with neutral red-grown virus and cells were labeled at 6 h after infection to measure rates of RNA and protein synthesis. The results in Table 3 show that the neutral red virus exposed to light did not inhibit RNA or protein synthesis. The variation in the level of inhibition in protein synthesis between normal HSV and unirradiated neutral red HSV was not considered significant (see Fig. 4 for degree of variation). We conclude that expression of the viral genome is responsible for the inhibition of both RNA and protein synthesis in HSV-infected 107 cells. This interpretation is dependent upon the premise that light exposure only affects the genome of the virus and does not have any secondary effects such as altering the virus' ability to penetrate and infect the cells or interfering with some inhibitory component in the infecting fluid.

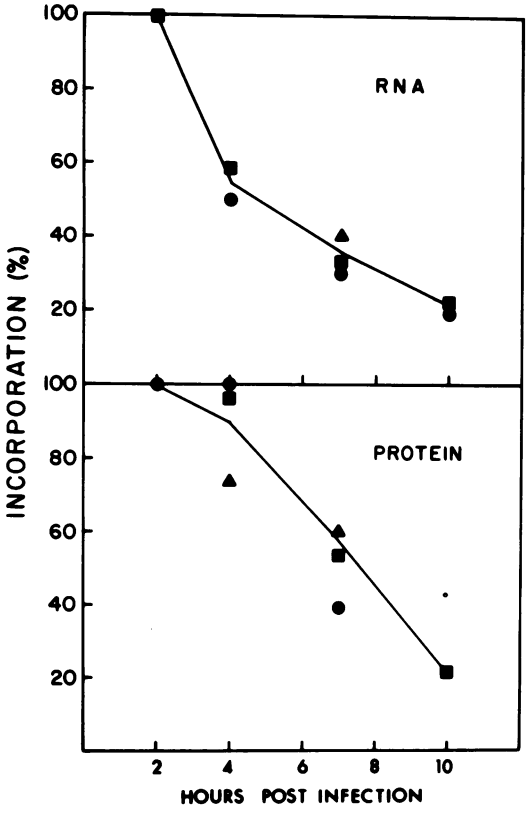

FIG. 4. *Inhibition of RNA and protein synthesis in 107 cells infected by HSV-1. Monolayers of 107 cells (5 × 10⁶ cells per plate) were either infected with 20 to 25 PFU per cell or mock infected. At the times indicated, cells were labeled with 100 μCi of [³H]uridine per ml for 30 min to measure RNA or 2 μCi of [³⁵S]methionine per ml for 60 min to measure protein. Cells were harvested at the times indicated, and acid-insoluble radioactivity was determined. Results were expressed as percent incorporation of mock-infected cultures at each time point. Duplicate plates were assayed at each time, and the results from three different experiments are shown.*

TABLE 3. *Inhibition of RNA and protein synthesis in 107 cells infected with neutral red-inactivated HSV-1*[a]

Virus	Treatment	% Inhibition	
		RNA	Protein
Normal HSV-1	None	49	37
Neutral red HSV-1	None	61	73
	Light	2	7

[a] Virus grown in neutral red-treated cells was inactivated by exposure to direct fluorescent light for 60 min. 107 cells were infected in the dark with light-exposed virus, virus maintained in the dark, or normal virus, or mock infected. At 6 h after infection, plates received [³H]uridine and [³⁵S]methionine for 30 min, and acid-insoluble radioactivity was determined for duplicate plates. The level of inhibition is expressed as percentage of mock-infected cultures.

TABLE 4. *Ribonucleoside triphosphate pools in HSV-1-infected and mock-infected 107 cells*[a]

Time of harvesting (h) of:	Nucleotides (nmol)			
	GTP	ATP	CTP	UTP
Uninfected (0)	0.27	1.44	0.29	1.33
Uninfected (2)	0.53	2.50	0.46	1.87
Infected (2)	0.58	2.27	0.48	2.01
Uninfected (10)	0.75	3.61	0.75	3.63
Infected (10)	1.07	3.87	1.30	4.43

[a] 107 cells were grown in monolayers and maintained in medium containing $^{32}PO_4$ and 10% fetal calf serum. 9.6 × 10⁶ cells per plate were infected with 100 PFU of HSV-1 per cell or mock infected. At the times indicated, acid-soluble extracts were prepared and nucleoside triphosphates were separated and quantitated. Results are expressed per 10⁶ cells.

Rate of RNA synthesis in 107 cells infected with HSV-1. To interpret our measurements of uridine incorporation into RNA classes after HSV-1 infection, it was necessary that the specific radioactivity of the immediate precursor to RNA (UTP) be monitored. Corrections may then be made for any large fluctuations in precursor specific activities which might be reflected in the rate of accumulation of isotope into RNA.

To see if infection alters the pools of ribonucleoside triphosphates in 107 cells, the experiment described in Table 4 was performed. There was no evidence for altered pools in infected cells. The incorporation of exogenous radioactive uridine (³H labeled) into the intracellular UTP pool was then followed to see if RNA was being synthesized from a pool of constant specific activity throughout the infection. Table 5 shows that the specific radioactivity of the UTP pool does not vary greatly in infected 107 cells and cannot account for the inhibition of RNA synthesis observed, assuming that compartmentalization of the UTP pool has not obscured such changes. To the contrary, the slight increase in the specific activity of the UTP pool could only result in an underestimation of the degree of inhibition of RNA synthesis. The pool expansion seen in both experiments was not a consequence of infection but rather of the media change.

To determine more accurately the rate of RNA synthesis in infected cells, infected or mock-infected monolayers were labeled for 30 min at different times, and RNA was extracted from nuclei. In a 30-min label, 90% of the radioactivity in RNA is present in the nucleus. The

TABLE 5. *Size and specific activity of the UTP pool in HSV-1-infected 107 cells*[a]

Time of labeling after infection (h)	Analysis of UTP pool			Relative concn (infected/uninfected)
	nmol	^{3}H cpm $\times 10^{-3}$	^{3}H cpm/pmol	
Uninfected	1.2	35	29	1.0
2.0–2.5	1.1	45	41	1.4
3.5–4.0	1.6	54	34	1.2
5.0–5.5	1.7	64	38	1.3
6.5–7.0	2.2	85	39	1.3

[a] 107 cells were grown in layers and maintained in medium containing $^{32}PO_4$ as described in Materials and Methods. 2.5×10^7 cells per plate were infected with 100 PFU of HSV-1 per cell and labeled with 100 μCi of [^{3}H]uridine per ml (2 μCi/nmol) at the times shown. Acid-soluble extracts were prepared and nucleoside triphosphates were separated and quantitated. The ^{32}P and ^{3}H present in UTP were determined. Results are expressed per 10^6 cells. The radioactivity shown above represents the [^{3}H]uridine incorporated into UTP, and the quantities were calculated utilizing measurements of both ^{32}P (endogenous pool size) and [^{3}H]uridine (exogenous uridine incorporation).

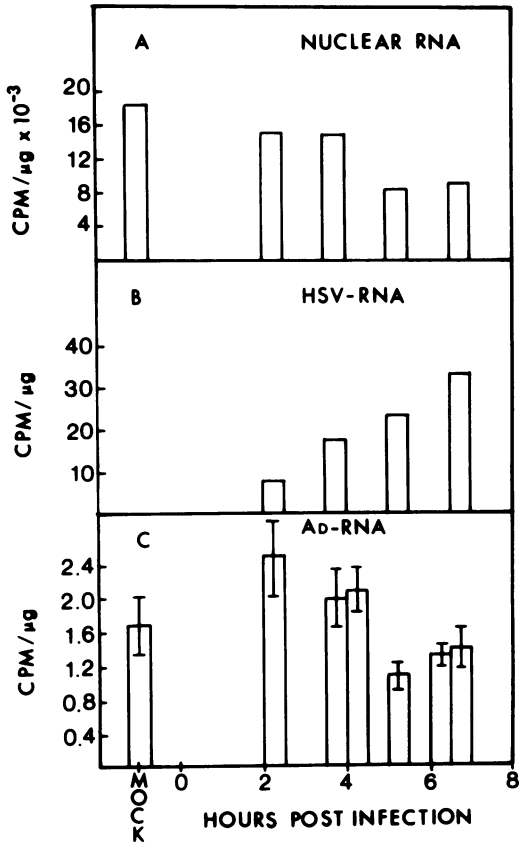

FIG. 5. *Rates of RNA synthesis in HSV-1-infected 107 cells. For each sample 7.5×10^7 cells (three plates) were infected with 100 PFU of HSV-1 per cell or mock infected. At the time shown [^{3}H]uridine was added for 30 min at 100 μCi/ml (2 μCi/nmol). Cells were harvested and RNA was extracted from nuclei. (A) Yields of RNA were normalized to the RNA from the uninfected culture on the basis of A_{260}. (B) Samples were hybridized to 4 μg of HSV-1 DNA, and RNase-resistant radioactivity was determined. (C) Samples were hybridized to adenovirus DNA. The results of two separate experiments were averaged and the variation from the plotted mean values is indicated.*

results in Fig. 5 have been corrected for the minor changes in the specific activity of the UTP pools. They indicate that infection causes a gradual shut off of RNA synthesis, reaching greater than 50% by 5 to 5.5 h after infection (Fig. 5A). When samples were hybridized to HSV-1 DNA, it could be seen that the rate of herpes-specific RNA synthesis steadily increases during this time period (Fig. 5B). Thus the inhibition of RNA synthesis is specific for host RNA.

Nuclear RNA samples were hybridized to adenovirus DNA. Figure 5C shows that infection by HSV-1 had a small effect on the rate of RNA synthesis at least during the first 6 h postinfection. It should be noted that since the rate of total RNA synthesis is declining, nuclear RNA from infected cells actually contains a higher proportion of radioactivity in ad-RNA. This experiment suggested that early after infection, herpesvirus may only inhibit slightly the transcription of ad-RNA in 107 cells.

Accumulation of RNA in the cytoplasm of infected cells. If 107 cells are labeled with uridine for 90 min, almost 30% of the label in RNA is present in the cytoplasm. To examine the short-term accumulation of RNA classes in the cytoplasm, infected or mock-infected 107 cells were labeled for 90-min intervals and RNA was extracted from the cytoplasmic samples. Although there is some effect of HSV-1 infection on the appearance of total cytoplasmic RNA (Table 6), this decrease did not reflect the rate of nuclear RNA synthesis (Fig. 5A). This is especially evident at 5 and 6.5 h after infection when the rate of synthesis is inhibited by more than 50% while the accumulation in the cytoplasm is inhibited by 20 to 30%. Because the appearance of rRNA in the cytoplasm is reduced to 25% of that in uninfected cells (Table 6), the transport of non-rRNA including polyadenylated RNA (last two columns of Table 6 and Fig. 6A) must be maintained.

To determine if HSV-specific RNA is made in

TABLE 6. *Accumulation of classes of cytoplasmic RNA in HSV-1-infected 107 cells*[a]

Time of labeling after infection (h)	Total RNA		rRNA		Poly(A)-RNA	
	Sp act (cpm/ng)	% Inhibition	% of total	Sp act (cpm/ng)	% of total	Sp act (cpm/ng)
Uninfected	10		15	1.5	6.0	0.60
2.0–3.5	8.4	16	23	1.9	7.8	0.66
3.5–5.0	10	0	24	2.4	8.0	0.80
5.0–6.5	8.3	17	10	0.8	8.3	0.69
6.5–8.0	6.9	31	6	0.4	10	0.69

[a] Cytoplasmic RNA was extracted from samples labeled for 90-min periods under conditions described in the legend to Fig. 5. Quantities of RNA were determined by A_{260}. For quantitation of rRNA, samples were electrophoresed for 3 h on 3% polyacrylamide gels. ^{14}C-marker rRNA was used to locate the 18S and 28S peaks in the gels. The percentage of rRNA was calculated from the radioactivity in the slices. For the determination of poly(A)-containing RNA, samples were fractionated on oligo(dT)-cellulose columns, and the percentage of the input radioactivity that bound to the column was determined.

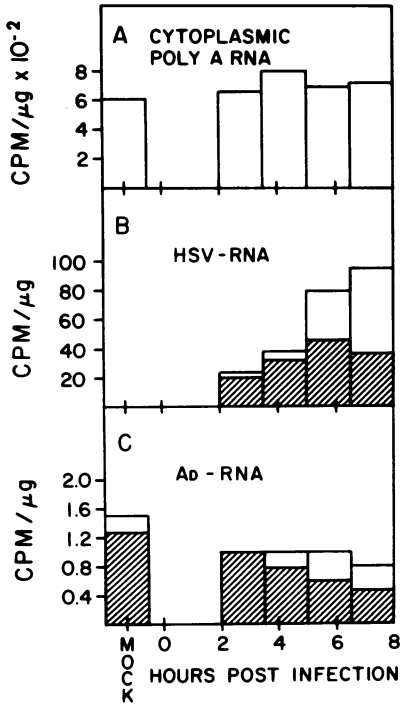

FIG. 6. *Accumulation of RNA in the cytoplasm of HSV-1-infected cells. (A) Poly(A)-containing RNA plotted from Table 6. (B) Samples were hybridized to 4 μg of HSV-1 DNA. (C) Samples were hybridized to 8 or 16 μg of adenovirus DNA. Poly(A)-containing: ▨; total: □.*

sufficient amounts to mask a drop in the level of cellular polyadenylated RNA, samples were hybridized to HSV DNA. The results in Fig. 6B show that HSV-specific RNA accounts for at least 6% of the radioactivity in the 5- to 6.5-h poly(A)-containing RNA sample. This assumes 100% efficiency of hybridization and hence is a minimum value. The amount of HSV RNA could be higher, in which case the quantity of cellular poly(A)-containing RNA would be lower in infected cells.

The fraction of HSV-specific RNA that contains poly(A) declined after infection (Fig. 6B). It has been reported that 40% of the HSV RNA in the cytoplasm of infected HEp-2 cells is adenylated, as indicated by its binding to nitrocellulose filters (5), and a more careful study showed that perhaps one-third of the RNA which did not bind to nitrocellulose contains small poly(A) tracts (43). Stringer et al. also noted a substantial fraction of polysomal HSV RNA that was not adenylated (44).

To determine if HSV-1 infection has an effect on the appearance of ad-RNA in the cytoplasm of 107 cells, samples were hybridized to adenovirus DNA. When the poly(A)-containing ad-RNA was assayed, it was found that the appearance of this material dropped steadily throughout the infection (Fig. 6C). The proportion of adenylated ad-RNA declined from 0.21% in mock-infected cells to 0.07% at 6.5 to 8 h post-infection. The total ad-RNA in the cytoplasm does not change as markedly during infection.

Kinetic experiments (Table 1) indicated that ad-RNA accumulated in both the nucleus and cytoplasm of transformed cells. To see whether HSV infection altered this phenomenon, we also examined cells labeled for longer time periods. Infected or mock-infected cells were labeled from 2 to 6 h with [^{3}H]uridine, and RNA was extracted from the nucleus and cytoplasm. The data in Table 7 indicate that even during a 4-h labeling period, ad-RNA accumulates both in the nucleus and cytoplasm of infected cells about as well as in mock-infected cells. However, there is a substantial decrease in the adenylated ad-RNA in the cytoplasm of infected cells.

DISCUSSION

There are many cited examples where HSV inhibits cellular macromolecular synthesis, and

TABLE 7. *Accumulation of ad-RNA in infected and uninfected 107 cells*[a]

Source of RNA	Sample	Hybridization results			Sp act of sample (cpm/ng)	Sp act of ad-RNA (cpm/μg)	% Adenylated
		RNA added (cpm × 10^{-5})	Hybridization				
			cpm	%			
Nucleus (total)	Infected	23	605	0.026	56	15	
	Uninfected	26	520	0.020	73	15	
Cytoplasm (total)	Infected	14	130	0.009	47	4.2	36
	Uninfected	17	135	0.008	74	5.9	61
Cytoplasm [poly(A)-containing]	Infected	0.63	60	0.095	1.6	2.8	
	Uninfected	0.90	115	0.13	2.8	3.6	

[a] Cells were infected or mock infected and labeled from 2 to 6 h after infection with [^{3}H]uridine. RNA was extracted from the nucleus and cytoplasm, and cytoplasmic samples were fractionated on oligo(dT)-cellulose columns to separate poly(A)-containing RNA. Total nuclear and cytoplasmic RNA and cytoplasmic poly(A)-containing RNA were hybridized to 8 μg of adenovirus DNA on filters.

it was our objective to study the manner in which the virus might alter a specific class of cellular RNA. The selection of an adenovirus-transformed cell was based on the ability to measure defined RNA transcripts. However, in the course of these studies, we found it necessary to gather additional information on both the total cellular RNA and that arising from the adenovirus sequences.

We expected that parameters determined for RNA in 107 would follow those observed in other cultured cell lines, particularly other rodent cells. For example, the nuclear RNA of 107 cells has a substantial component which includes most of the poly(A)-containing RNA in the nucleus and which turns over very rapidly. From accumulation studies, other investigators have determined a nuclear half-life of about 25 min for this RNA (11, 33). The poly(A)-containing RNA in the cytoplasm of 107 cells, which includes much of the mRNA, decays with an average half-life of about 13 h. L cells and 3T6 cells have one major class of mRNA with an average half-life on the order of that in 107 (1, 18). Thus, our data indicate that 107 cell RNA behaves in the manner expected of cultured rodent cells.

Investigation of the ad-RNA indicated that stable adenovirus-specific transcripts are present in the nucleus (Fig. 2). From the estimate of the limits of contamination of nuclear RNA samples by cytoplasmic RNA (see Materials and Methods), it is unlikely that the stable nuclear ad-RNA is an artifact of preparation. Further evidence against this explanation is provided by the fact that nuclear ad-RNA accumulates at a much faster rate than cytoplasmic (Table 1). Maxwell has described the accumulation of polyoma-specific RNA in the nucleus of polyoma-transformed cells and the distribution of virus-specific RNA between the nucleus and cytoplasm was similar to that reported in this paper (27). The presence of a stable class of ad-RNA restricted to the nucleus complicates the interpretation of ad-mRNA metabolism after HSV infection.

The ad-mRNA in the cytoplasm as defined by poly(A)-ad-RNA (80% polysome associated—data not shown) has a relatively short half-life, being more unstable than other mRNA's that have been assayed specifically (4, 21, 30–32). Because the accumulation of ad-RNA in transformed cells may be cell cycle dependent (20), the amount of messenger in the cytoplasm could be regulated by altering the rate of synthesis, the rate of decay, or the rate at which the cells traverse the cell cycle. In addition, total ad-RNA in the cytoplasm contained a stable nonadenylated fraction (Table 2). The presence of this RNA in the cytoplasm necessitates that we focus our attention on the poly(A)-containing class of ad-RNA.

Taking the above observations into consideration, we have studied ad-RNA as our model for cellular mRNA in HSV-infected cells. By 5 h postinfection, we observe a marked decrease in the poly(A)-ad-RNA in the cytoplasm. This class of RNA presumably acts as mRNA for adenovirus proteins such as T antigens and has a definite biological function. In contrast, we find that HSV infection did not inhibit the production of nuclear and nonadenylated cytoplasmic classes. These RNAs cannot be assigned unambiguous roles in the life cycle of mRNA. These results indicate that HSV infection may lead to a selective effect on the different classes of RNAs transcribed from the integrated adenovirus DNA sequences. Further investigation of this possibility requires detailed analysis of the nature of the ad-RNA in the nucleus of 107 cells and the relationship between these classes and those in the cytoplasm. For example, the level of reduction in total nuclear ad-RNA may

account completely for the drop in cytoplasmic poly(A)-ad-RNA provided that one class of nuclear transcripts (i.e., unstable species) is the true messenger precursor. With this information, we could evaluate whether HSV-1 disrupts the production of ad-mRNA primarily at the processing and/or transport steps, as may be the case for abundant RNA sequences in pseudorabies virus infection (37).

In other cases where the levels of specific mRNA's have been measured after HSV infection there were considerable decreases. In a system analogous to the one described in this paper, the amount of polyoma-specific RNA in the cytoplasm of polyoma-transformed BHK cells declined by more than 80% after HSV-1 infection (36). At the same time after infection, the polyoma RNA in the nucleus was reduced by 50% (unpublished observations). For Friend leukemia virus-transformed cells expressing the globin gene, infection by HSV caused a marked drop in globin message as measured by either complementary DNA protection experiments or in vitro protein synthesis (29).

These measurements of mRNA are consistent with our measurements of poly(A)-ad-RNA but differ from our data with total cytoplasmic ad-RNA. The amount of cellular poly(A)-RNA accumulating in the cytoplasm of infected 107 cells did not appear to change (Fig. 6). This result suggests that under the experimental conditions utilized, the observed effect on ad-RNA might not extend to the major fraction of the cellular mRNA. This is in contrast to results in HSV-1-infected HeLa cells where there was an apparent inhibition of the accumulation of nonviral adenylated RNA on polyribosomes at 5 to 6 h after infection (44). The difference in the two systems may be related to the semipermissive character of the 107 cells for HSV at the temperature (36°C) used for these experiments. Even though all the cells in the culture are infected as shown by indirect immune fluorescence, the yield of virus is low compared with nontransformed rat embryo cells (data not shown). In addition, the shut off of host DNA and protein synthesis is both delayed and incomplete (manuscript in preparation). The replication of HSV-1 and its effect on ad-RNA metabolism also can be studied at 32°C, which is a more permissive temperature, and experiments under these conditions might indicate a connection between the extent of HSV-1 replication and viral control of host cell functions.

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LITERATURE CITED

1. **Abelson, H. T., L. F. Johnson, S. Penman, and H. Green.** 1974. Changes in RNA in relation to growth of the fibroblast. II. The lifetime of mRNA, rRNA and tRNA in resting and growing cells. Cell **1**:161-165.
2. **Acheson, N. H., E. Buetti, K. Scherrer, and R. Weil.** 1971. Transcription of the polyoma virus genome: synthesis and cleavage of giant late polyoma-specific RNA. Proc. Natl. Acad. Sci. U.S.A. **68**:2231-2235.
3. **Aviv, H., and P. Leder.** 1972. Purification of biologically active globin messenger RNA by chromatography on oligothymidylic acid-cellulose. Proc. Natl. Acad. Sci. U.S.A. **69**:1408-1412.
4. **Aviv, H., Z. Voloch, R. Bastos, and S. Levy.** 1976. Biosynthesis and stability of globin mRNA in cultured erythroleukemic Friend cells. Cell **8**:495-503.
5. **Bachenheimer, S. L., and B. Roizman.** 1972. Ribonucleic acid synthesis in cells infected with herpes simplex virus. VI. Polyadenylic acid sequences in viral messenger ribonucleic acid. J. Virol. **10**:875-879.
6. **Bartlett, G. P.** 1959. Phosphorus assay in column chromatography. J. Biol. Chem. **234**:466-468.
7. **Beard, P., N. A. Acheson, and I. H. Maxwell.** 1976. Strand-specific transcription of polyoma virus DNA early in productive infection and in transformed cells. J. Virol. **17**:20-26.
8. **Bello, L. J., and H. S. Ginsberg.** 1969. Relationship between deoxyribonucleic acid-like ribonucleic acid synthesis and inhibition of host protein synthesis in type 5 adenovirus-infected KB cells. J. Virol. **3**:106-113.
9. **Birnie, G. D., J. Delcour, D. Angus, G. Threlfall, and J. Paul.** 1974. Distribution of nuclear RNAs in growing and nongrowing cells, p. 757-768. *In* B. Clarkson and R. Baserga (ed.), Control of proliferation in animal cells. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
10. **Bishop, D. H. L., J. R. Claybrook, and S. Spiegelman.** 1967. Electrophoretic separation of viral nucleic acids on polyacrylamide gels. J. Mol. Biol. **26**:373-387.
11. **Brandhorst, B. P., and E. H. McConkey.** 1974. Stability of nuclear RNA in mammalian cells. J. Mol. Biol. **85**:451-463.
12. **Buetti, E.** 1974. Characterization of late polyoma mRNA. J. Virol. **14**:249-260.
13. **Cohen, G. H., R. K. Vaughan, and W. C. Lawrence.** 1971. Deoxyribonucleic acid synthesis in synchronized mammalian KB cells infected with herpes simplex virus. J. Virol. **7**:783-791.
14. **Flanagan, J. F.** 1967. Virus-specific ribonucleic acid synthesis in KB cells infected with herpes simplex virus. J. Virol. **1**:583-590.
15. **Freeman, A. E., P. H. Black, R. Wolford, and R. J. Heubner.** 1967. Adenovirus type 12-rat embryo transformation system. J. Virol. **1**:362-367.
16. **Garrett, A. J., and D. E. Reeson.** 1975. Rapid screening of tissue culture cells for mycoplasmal contamination. J. Biol. Stand. **3**:181-184.
17. **Gillespie, D., and S. Spiegelman.** 1965. A quantitative assay for DNA-RNA hybrids with DNA immobilized in a membrane. J. Mol. Biol. **12**:829-842.
18. **Greenberg, J. R.** 1972. High stability of messenger RNA in growing cultured cells. Nature (London) **240**:102-104.
19. **Hay, J., G. J. Koteles, H. M. Keir, and J. Subak-Sharpe.** 1966. Herpesvirus specified ribonucleic acids. Nature (London) **210**:387-390.
20. **Hoffman, P. R., and J. E. Darnell, Jr.** 1975. Differential

accumulation of virus-specific RNA during the cell cycle of adenovirus-transformed rat embryo cells. J. Virol. **15**:806–811.

21. **Hunt, J. A.** 1974. Rate of synthesis and half-life of globin messenger ribonucleic acid: rate of synthesis of globin messenger ribonucleic acid calculated from data of cell haemoglobin content. Biochem. J. **138**:499–510.
22. **Kaplan, A. S., and T. Ben-Porat.** 1960. The incorporation of ^{14}C-labeled nucleosides into rabbit kidney cells infected with pseudorabies virus. Virology **11**:12–27.
23. **Kieff, E. D., S. L. Bachenheimer, and B. Roizman.** 1971. Size, composition, and structure of the deoxyribonucleic acid of herpes simplex virus subtypes 1 and 2. J. Virol. **8**:125–132.
24. **Lawrence, W. C., and H. S. Ginsberg.** 1967. Intracellular uncoating of type 5 adenovirus deoxyribonucleic acid. J. Virol. **1**:851–867.
25. **Loening, V. E.** 1967. The fractionation of high-molecular-weight ribonucleic acid by polyacrylamide-gel electrophoresis. Biochem. J. **102**:251–257.
26. **McConaughy, B. L., C. D. Laird, and B. J. McCarthy.** 1969. Nucleic acid reassociation in formamide. Biochemistry **8**:3289–3295.
27. **Maxwell, I. H.** 1976. Intracellular distribution and sedimentation properties of virus-specific RNA in two clones of BHK cells transformed by polyoma virus. J. Virol. **18**:461–472.
28. **Milcarek, C., R. Price, and S. Penman.** 1974. The metabolism of a poly(A) minus mRNA fraction in HeLa cells. Cell **4**:189–197.
29. **Nishioka, Y., and S. Silverstein.** 1977. Degradation of cellular mRNA during infection by herpes simplex virus. Proc. Natl. Acad. Sci. U.S.A. **74**:2370–2374.
30. **Palmiter, R. D.** 1975. Quantitation of parameters that determine the rate of ovalbumin synthesis. Cell **4**:189–197.
31. **Palmiter, R. D.** 1973. Rate of ovalbumin messenger ribonucleic acid synthesis in the oviduct of estrogen-primed chicks. J. Biol. Chem. **248**:8260–8270.
32. **Perry, R. P., and D. E. Kelley.** 1973. Messenger RNA turnover in mouse L cells. J. Mol. Biol. **79**:681–696.
33. **Perry, R. P., D. E. Kelley, and J. LaTorre.** 1974. Synthesis and turnover of nuclear and cytoplasmic polyadenylic acid in mouse L cells. J. Mol. Biol. **82**:315–331.
34. **Perry, R. P., J. LaTorre, D. E. Kelley, and J. R. Greenberg.** 1972. On the lability of poly A sequences during extraction of messenger RNA from polyribosomes. Biochim. Biophys. Acta **262**:220–226.
35. **Philipson, L.** 1961. Adenovirus assay by fluorescent cell-counting procedure. Virology **15**:263–268.
36. **Pizer, L. I., and P. Beard.** 1976. The effect of herpes simplex virus infection on mRNA in polyoma virus-transformed cells. Virology **75**:477–480.
37. **Rakusanova, T., T. Ben-Porat, and A. S. Kaplan.** 1972. Effect of herpesvirus infection on the synthesis of cell-specific RNA. Virology **49**:537–548.
38. **Rapp, F., J. H. Li, and M. A. Jerkofsky.** 1973. Transformation of mammalian cells by DNA-containing viruses following photodynamic inactivation. Virology **55**:339–346.
39. **Roizman, B., G. S. Borman, and M. Kamali-Rousta.** 1965. Macromolecular synthesis in cells infected with herpes simplex virus. Nature (London) **206**:1374–1375.
40. **Roller, B., and G. H. Cohen.** 1976. Deoxyribonucleoside triphosphate pools in synchronized human cells infected with herpes simplex virus types 1 and 2. J. Virol. **18**:58–64.
41. **Scherrer, K.** 1969. Isolation and sucrose gradient analysis of RNA, p. 413–432. *In* K. Habel and N. P. Salzman (ed.), Fundamental techniques in virology. Academic Press, Inc., New York.
42. **Shimada, K., K. Fujinaga, S. Hama, K. Sekikawa, and Y. Ito.** 1972. Virus-specific ribonucleic acid in the nucleus and cytoplasm of rat embryo cells transformed by adenovirus type 2. J. Virol. **10**:648–652.
43. **Silverstein, S., R. Millette, P. Jones, and B. Roizman.** 1976. RNA synthesis in cells infected with herpes simplex virus. XII. Sequence complexity and properties of RNA differing in extent of adenylation. J. Virol. **18**:977–991.
44. **Stringer, J. R., L. E. Holland, R. I. Swanstrom, K. Pivo, and E. K. Wagner.** 1977. Quantitation of herpes simplex virus type 1 RNA in infected HeLa cells. J. Virol. **21**:889–901.
45. **Stubbs, J. D., and B. D. Hall.** 1968. Level of tryptophan messenger RNA in *Escherichia coli*. J. Mol. Biol. **37**:289–302.
46. **Thiel, J. F., and K. O. Smith.** 1967. Fluorescent focus assay for viruses in cell monolayers in plastic petri dishes. Proc. Soc. Exp. Biol. Med. **125**:892–895.
47. **Tsuei, D., K. Fujinaga, and M. Green.** 1972. The mechanism of viral carcinogenesis by DNA mammalian viruses: RNA transcripts containing viral and highly reiterated cellular base sequences in adenovirus-transformed cells. Proc. Natl. Acad. Sci. U.S.A. **69**:427–430.
48. **Wagner, E. K., and B. Roizman.** 1969. Ribonucleic acid synthesis in cells infected with herpes simplex virus. I. Patterns of ribonucleic acid synthesis in productively infected cells. J. Virol. **3**:36–46.
49. **Wall, R., J. Weber, Z. Gage, and J. E. Darnell.** 1973. Production of viral mRNA in adenovirus-transformed cells by the post-transcriptional processing of heterogeneous nuclear RNA containing viral and cell sequences. J. Virol. **11**:953–960.
50. **Wheelock, E. F., and I. Tamm.** 1961. Enumeration of cell-infecting particles of Newcastle disease virus by the fluorescent antibody technique. J. Exp. Med. **113**:301–315.