Herpes Simplex Virus-Induced Changes in Cellular and Adenovirus RNA Metabolism in an Adenovirus Type 5-Transformed Human Cell Line

RICHARD M. STENBERG[†] AND LEWIS I. PIZER*

Department of Microbiology and Immunology, University of Colorado Medical Center, Denver, Colorado 80262

Received 28 September 1981/Accepted 15 January 1982

We used the viral transcripts (designated Ad-RNA) that accumulated in the cytoplasm of adenovirus type 5-transformed human embryonic kidney cells (cell line 293-31) as models for cellular RNAs to examine how herpes simplex virus modifies cellular RNA metabolism. Infection of 293-31 cells with herpes simplex virus type 1 strain 17 led to extensive inhibition of Ad-RNA accumulation by 4 h postinfection. The major part of this inhibition was due to an immediate early or alpha gene function, which reduced the rate of transcription of Ad-RNA within the nuclei of the infected cells. In addition, host polyadenylic acid-containing RNA accumulation and rRNA accumulation were affected, but to a lesser extent and at a slower rate than Ad-RNA accumulation. In conjunction with previous data, our experimental data allowed us to propose a general scheme for how herpes simplex virus type 1 alters the metabolism of cellular RNA, the possible mechanisms for these changes, and how they correlate with the regulation of herpes simplex virus gene expression.

Herpes simplex virus (HSV) is a human virus which is thought to have a wide host range (9). Infection of permissive cells in culture with HSV leads to extensive cytopathic effects and the subsequent release of infectious progeny within the first 10 h of infection (44). Although the molecular aspects of HSV replication have been studied in great detail (41), little is known concerning the mechanisms involved in HSV-induced inhibition of host macromolecular synthesis.

Early studies showed that infection with HSV leads to disruption of host DNA, RNA, and protein syntheses (18, 36, 47). Studies with intertypic recombinants have succeeded in mapping the "rapid host shutoff" function of HSV type 2 (HSV-2) to a defined region of the viral genome (11, 27). Fenwick and Walker (12) showed that a virion component of HSV-2 is responsible for the shutoff of host DNA and protein syntheses since these inhibitory actions take place in the absence of de novo viral protein synthesis. Nishioka and Silverstein showed that infection with HSV-1 inhibits the synthesis of globin in induced Friend erythroleukemia cells (28). In addition, these authors showed that the

steady-state levels of globin mRNA fell rapidly after infection (28) and that this degradation required viral de novo protein synthesis (29). Pizer and Beard (31) showed that the synthesis and steady state levels of polyoma RNA in polyoma-transformed BHK cells were reduced after infection with HSV-1. Spector and Pizer (42) showed that in adenovirus-transformed rat cells the transport of adenovirus RNA from the nucleus of the cytoplasm was inhibited by infection with HSV-1. These studies, which used specific mRNAs, suggested that HSV-1 reduces the level of cellular protein synthesis at least in part by modulating the availability of mRNA.

In our studies, we have used a human cell line (cell line 293-31) transformed by adenovirus type 5 (14) to examine the effects of HSV-1 on the synthesis and accumulation of adenovirus-specific RNA (designated Ad-RNA) and cellular mRNA. The cells of this line contain both the left 12% and the right 9% of the adenovirus genome, although only the left end is expressed (1). They offer the following distinct advantages over the cells which have been used in previous studies: (i) they are totally permissive for HSV replication (44); (ii) they contain specific assayable mRNAs that accumulate to high levels and have been well characterized in terms of physical structure (5, 43); and (iii) the cells are of human origin (14), the natural host of HSV.

[†] Present address: Department of Microbiology, University of Iowa School of Medicine, Iowa City, IA 52242.

Our studies with this system have indicated that infection with HSV alters the accumulation of cytoplasmic RNA at two levels. The first is that the rates of synthesis of both Ad-RNA and cellular RNA are inhibited after infection. This transcriptional effect is due to an immediate early or alpha gene function and is responsible for the majority of the inhibition of cytoplasmic RNA accumulation. In addition to this effect, a second gene function, which occurs either early or late in infection, contributes to the inhibition of cytoplasmic Ad-RNA. We speculate that this second effect is due to the HSV-induced function which is responsible for reducing the steady-state levels of cellular mRNAs (28, 29, 31; Stenberg and Pizer, manuscript in preparation).

MATERIALS AND METHODS

Cells and virus. Cell line 293-31 cells (from D. Spector) and rabbit skin cells (from J. Gerdes) were grown as monolayers in Eagle minimal essential medium (Flow Laboratories, Inc.) supplemented with 5% fetal calf serum (Flow) or 7% newborn calf serum (Kansas City Biological). HSV-1 strain 17 and its temperature-sensitive mutants tsK, tsD, and tsT (from H. Marsden and J. Subak-Sharpe) were propagated by infecting rabbit skin cells at a multiplicity of 0.01 to 0.1 PFU/cell for 48 to 72 h or until generalized cytopathic effects occurred. Cells were collected by centrifugation at $800 \times g$, suspended in 1 ml of maintenance medium (Eagle minimal essential medium supplemented with 1% fetal calf serum and 0.1 mM arginine) per 4×10^7 cells, and frozen at -70°C . Immediately before use the virus was released from the cells by sonic treatment with a Branson Sonifier cell disruptor.

Virus progeny production was determined by plaque assay on rabbit skin cells, using an overlay of 0.75% methylcellulose (10). For the temperature-sensitive mutants, the assay was carried out at both permissive (32°C) and nonpermissive (39°C) temperatures; the relative efficiency of plating at the two temperatures (39 and 32°C) was always less than 0.0005.

Preparation of HSV DNA. HSV DNA was prepared by infecting rabbit skin cells in monolayers at a multiplicity of 10 PFU/cell. After 20 h the cells were scraped into the medium and collected by centrifugation at $800 \times g$. The cells were suspended at a concentration of 10^8 cells per ml in 10 mM sodium phosphate (pH 7.2) containing 0.5% sodium deoxycholate (Sigma Chemical Co.) and 1% Nonidet P-40 (Shell Chemical Co.) and homogenized 10 times with a tight-fitting Dounce homogenizer. The nuclei were removed by centrifugation at 1,500 rpm for 15 min and the supernatant was layered over a preformed CsCl gradient (1.2 to 1.4 g/cm^3) and centrifuged for 2 h at 20,000 rpm in an SW27.1 rotor (Beckman Instruments, Inc.). The nucleocapsid band was removed and diluted 1:4 in TE buffer (10 mM Tris, pH 7.5, 1 mM EDTA), and HSV DNA was purified as previously described (42, 44).

Growth and purification of bacterial plasmids. *SmaI* fragment F of adenovirus type 5 DNA (2.8 to 10.7 map units) inserted into the *EcoRI* site of bacterial plasmid

pBR322 with *EcoRI* linkers was a gift from F. Laski. *HpaI* fragment E of adenovirus type 2 DNA (0 to 4.4 map units) inserted by G-C tailing into the *PstI* site of pBR322 was a gift from J. Darnell.

Bacterial plasmids were grown in *Escherichia coli* HB101 and amplified with 200 μg of chloramphenicol (Sigma) per ml by a modification of the method of Klein et al. (19). Cells in mid-log phase were amplified with chloramphenicol for 16 to 18 h and collected by centrifugation at 5,000 rpm. Cells were suspended in 50 mM Tris-hydrochloride (pH 8) and treated with 1 mg of lysozyme (Sigma) per ml at room temperature for 15 min. An equal volume of water-saturated phenol-chloroform-isoamyl alcohol (50:50:1) was added, and the spheroplasts were gently extracted by inverting the tube 10 times. The suspension was centrifuged at $10,000 \times g$, and the aqueous phase was removed, made 200 mM in NaCl and 2 mM in EDTA, reextracted with phenol-chloroform-isoamyl alcohol, and finally extracted with 100% chloroform. Nucleic acid was precipitated with 2 volumes of absolute ethanol, collected by centrifugation, and subjected to equilibrium centrifugation on cesium chloride-ethidium bromide (1.56 g/cm^3 ; 200 $\mu\text{g/ml}$) gradients. Supercoiled DNA was removed through the side of the tube with a syringe and immediately passed over a 2-ml Dowex Ag-50 column equilibrated with TE buffer to remove the ethidium bromide. Plasmid DNA was collected by ethanol precipitation.

DNA-RNA hybridization. Hybridization of RNA to excess filter-bound DNA (0.25 cm square; 1 to 4 μg of DNA per filter) occurred at 44°C using 50% formamide as previously described (42), except that in the washing of the filters, the RNase treatment occurred overnight at room temperature. Before loading onto nitrocellulose, plasmid DNAs were linearized by digestion with *BamHI* restriction endonuclease (Bethesda Research Laboratories) under the conditions suggested by the supplier. After digestion, the DNA was purified by extraction with phenol-chloroform-isoamyl alcohol, extraction with chloroform, and ethanol precipitation. Ad-DNA filters contained both region 1A (0 to 4.4 map units) and region 1B (2.8 to 10.7 map units) plasmid DNAs at equal molar concentrations. In all cases, hybridization was carried out by using one, two, or three viral or plasmid DNA filters in the hybridization reaction in order to determine true DNA excess. Filters were counted with a Searle liquid scintillation counter for a period of time sufficient to obtain 1,000 counts over background. Blank filters either had no DNA added or contained T4 DNA (a gift from C. Trotter). Hybridization to blank filters was always less than 0.001% of the input radioactivity.

Labeling and extraction of RNA. All infections were performed in maintenance medium. Cell line 293-31 cells on 150-mm plates (10^7 cells per plate at 50% confluence) were infected at a multiplicity of 20 PFU/cell or mock infected with 2 ml of maintenance medium for 90 min at 37 or 39°C , as indicated below. After the 90-min adsorption the inoculum was removed, and the cells were overlaid with 10 ml of maintenance medium. At the appropriate time, the cells were labeled with 5 ml of a [^3H]uridine preparation (25 Ci/mmol; 200 $\mu\text{Ci/ml}$). Incorporation was stopped by washing the cells in ice-cold TSM buffer (30 mM Tris-hydrochloride, pH 7.5, 100 mM NaCl, 1.5 mM MgCl_2). The cells were then immediately scraped into 1 ml of

lysis buffer (TSM buffer containing 0.2% Nonidet P-40), and the plates were washed with an additional 1 ml of lysis buffer.

RNA was prepared by using modifications of previously described procedures (13, 15). For cytoplasmic RNA, the extract was removed quickly to a Corex centrifuge tube equilibrated on ice, aspirated gently to assist in the disruption of the plasma membranes, and centrifuged at 10,000 rpm for 20 min. The resulting supernatant was added to 6 ml of extraction buffer (10 mM Tris-hydrochloride, pH 7.5, 350 mM NaCl, 1 mM EDTA, 2% sodium dodecyl sulfate, 7 M urea) and then extracted twice with equal volumes of phenol-chloroform-isoamyl alcohol and once with chloroform. Nucleic acid was collected by ethanol precipitation. Nuclei for RNA isolation were prepared by a modification of the method of Weinmann et al. (49), as previously described (26). Cell extracts were layered over an equal volume of TGME buffer (10 mM Tris-hydrochloride, pH 7.5, 25% glycerol, 5 mM MgCl₂, 1 mM EDTA) and centrifuged at 1,500 × *g* for 15 min. The cytoplasm and TGME buffer were removed, and the nuclei were suspended in an additional 1 ml of TGME buffer. The nuclei were added to 6 ml of extraction buffer, and the centrifuge tube was washed with an additional 1 ml of TGME buffer. After the wash was added to the extraction buffer, the nucleic acid was extracted as described above for cytoplasmic RNA.

DNase (Sigma) was freed of contaminating RNase by treatment with sodium iodoacetate (52). To remove contaminating DNA, 100 µg of RNA was reacted with 10 µg of DNase in 2 ml of DNase buffer (10 mM Tris-hydrochloride, pH 7.5, 0.5 M NaCl, 10 mM MgCl₂) for 20 min at room temperature. An equal volume of "stop buffer" (1% sodium dodecyl sulfate, 20 mM EDTA, 10 mM Tris-hydrochloride, pH 7.5) was added, and the nucleic acid was extracted with phenol-chloroform-isoamyl alcohol as described above.

Polyadenylic acid [poly(A)]-containing RNA was selected by oligodeoxythymidylic acid cellulose (Boehringer Mannheim) chromatography, using the method of Aviv and Leder (3) as previously described (42), except that Tris-hydrochloride (pH 7.5) was used as the buffer and the column was washed with buffer containing 0.5 M NaCl.

Quantitation of rRNA. A modification of the method of Thomas (46) for transferring RNA to nitrocellulose was used to quantitate the amount of 28S and 18S rRNA that accumulated after HSV infection. poly(A)⁺ RNA (the RNA which failed to bind to oligodeoxythymidylic acid cellulose) labeled with [³H]uridine was reacted with 1 M glyoxal (Sigma) in 50% dimethyl sulfoxide (Sigma)-10 mM sodium phosphate (pH 6.8) at 50°C for 60 min (25). The RNA was electrophoresed on 1.5% agarose gels in 10 mM sodium phosphate (pH 6.8) by using a two-chamber apparatus to maintain the proper pH for the gel. The RNA was transferred to nitrocellulose (Schleicher & Schuell Co.) and baked at 80°C for 2 h. The nitrocellulose sheet was then washed twice with 1 × SSC (0.15 M NaCl plus 0.015 M sodium citrate), air dried, and placed into Liquifluor toluene (New England Nuclear Corp.) for 2 h. The sheet was air dried and subjected to autoradiography at -70°C on Kodak XR-5 film, using Du Pont Cronex intensifying screens. The nitrocellulose sheet was then lined up with the autoradiogram, and the rRNA bands were excised from the nitrocellulose. The radioactive bands

were then placed in Liquifluor toluene and counted with a Searle liquid scintillation counter.

RESULTS

HSV-induced changes in Ad-RNA accumulation. Previous studies have shown that the accumulation of Ad-RNA in the cytoplasm of 293-31 cells is linear for 4 to 5 h (D. Spector, personal communication). Our initial studies were designed to determine how HSV affects this accumulation of Ad-RNA. To examine this question, 293-31 cells were infected with HSV-1 strain 17 at 39°C and at different times after infection were labeled for 90 min with 200 µCi of [³H]uridine per ml. Poly(A)⁺ RNA was prepared and analyzed by hybridization to Ad-RNA. Table 1 shows that the accumulation of Ad-RNA was inhibited by 91% by 2.0 to 3.5 h postinfection (p.i.) and by virtually 100% by 4.0 to 5.5 h p.i. (97% compared with control values). In addition, we performed similar studies with HSV-1 strains HF and KOS and HSV-2 strain 186 and in all cases obtained the same degree of inhibition of cytoplasmic Ad-RNA accumulation. Infection with HSV-1 strain 17 at 32°C caused inhibition, but at the lower temperature the effect was less pronounced at early times after infection (data not shown). The rapid and extensive inhibition of Ad-RNA accumulation provided the basis for our examination of HSV-induced changes in Ad-RNA metabolism.

Ad-RNA accumulation in 293-31 cells infected with temperature-sensitive mutants of HSV. The use of temperature-sensitive mutants allows investigators to restrict HSV replication to specific points in the replication cycle. We used three mutants of HSV-1 strain 17 (tsK, tsD, and tsT), all of which contain lesions in HSV immediate early protein V_{MW}175 (ICP4) (32, 35), which is a regulatory protein that appears to be responsible for the initiation and maintenance of early and late transcription (33, 47). All three of these mutants synthesize defined classes of HSV polypeptides. However, tsK causes a more restricted infection, making only immediate early or alpha polypeptides at the nonpermissive temperature. Mutants tsD and tsT are early DNA-minus mutants which allow the synthesis of immediate early proteins and a discrete class of early proteins, including thymidine kinase (33, 35). In two separate experiments, we examined the ability of these mutants to inhibit Ad-RNA accumulation at the nonpermissive temperature (39°C). The time period of 4.0 to 5.5 h was chosen because inhibition of Ad-RNA accumulation by HSV-1 strain 17 is practically complete by this time. In addition, during this time the HSV polypeptides which are synthesized at the nonpermissive temperature accumulate at maximal rates (data not shown).

Table 1 shows that with all three mutants the

TABLE 1. Cytoplasmic poly(A)⁺ RNA accumulation in HSV-1-infected 293-31 cells at 39°C

Expt	Source of RNA	Hybridization			RNA in 10 ⁷ cells ^a		
		Input radioactivity (cpm, ×10 ⁵)	Ad-RNA radioactivity (cpm)	Ad-RNA hybridized (%)	Total poly(A) ⁺ RNA radioactivity (cpm, ×10 ⁵)	Ad-RNA radioactivity (cpm)	Inhibition of Ad-RNA accumulation (%)
1	Mock-infected cells	3.13	1,655	0.53	3.13	16,550	
	HSV-1 strain 17-infected cells						
	2-3.5 h p.i.	4.87	147	0.03	4.84	1,460	91
	4-5.5 h p.i.	4.46	44	0.01	4.28	420	97
2 ^b	Mock-infected cells	3.38	1,247	0.370	3.38	12,470	
	HSV tsK-infected cells	2.78	258	0.093	2.82	2,620	79
3 ^b	Mock-infected cells	4.44	1,166	0.263	4.44	11,660	
	HSV tsK-infected cells	3.06	168	0.055	2.38	1,310	89
	HSV tsD-infected cells	2.78	170	0.061	2.55	1,560	87
	HSV tsT-infected cells	2.88	188	0.065	2.61	1,700	85

^a Corrected for yield of total RNA. RNA was quantitated by measuring optical density at 260 nm, using an extinction coefficient of 40 U of optical density at 260 nm equaling 1 mg of RNA per ml.

^b Infected cell RNAs were labeled from 4.0 to 5.5 h p.i.

accumulation of Ad-RNA was inhibited. However, with these mutants the extent of inhibition was somewhat less pronounced than when the cells were infected with the parent strain (HSV-1 strain 17). The levels of inhibition of Ad-RNA accumulation in mutant-infected cells ranged from 80 to 90% depending on the individual experiment, whereas with strain 17 the inhibition was more than 97% in several experiments. Because the inhibitory effects occurred in tsK-infected cells, we conclude that an HSV immediate early protein(s) is responsible for the major part of the inhibition of Ad-RNA accumulation.

Because of the precision of the hybridization assay and the reproducibility of the results, we have confidence that the quantity of Ad-RNA accumulating in cells infected with the mutants is higher than the quantity accumulating in cells infected with the wild-type virus.

Accumulation of rRNA in HSV-infected cells. In previous experiments, incorporation of [³H]uridine into poly(A)⁺ cytoplasmic RNA was equal to or only slightly reduced from control values (data not shown). Previous reports have shown that rRNA is reduced after infection with HSV (42, 47). To determine whether rRNA is reduced in 293-31 cells after HSV infection, poly(A)⁺ RNAs from the previous experiment were analyzed by a modification of the method of Thomas (46), as described above. Table 2 shows that the rate of inhibition of rRNA synthesis was similar to the rate of inhibition observed for host poly(A)⁺ RNA accumulation. At 2.0 to 3.5 h p.i. very little inhibition of either 28S or 18S rRNA accumulation was found. However, by 4.0 to 5.5 h p.i. the accumulation of 28S and 18S rRNAs was inhibited by 92 and 80%, respectively, compared with controls.

These results with the stable species of rRNA are in accord with those described above for total host poly(A)⁺ RNA and Ad-RNA and are consistent with the data reported previously. In addition, these data provide the basis for further studies designed to identify the viral functions responsible and the mechanisms by which the viral functions induce changes in RNA metabolism.

rRNA accumulation in 293-31 cells infected with temperature-sensitive mutants of HSV. To determine how the accumulation of rRNA was affected by infection with temperature-sensitive mutants, the poly(A)⁺ RNAs from the experiments shown in Table 1 were analyzed (Table 2). Table 2 shows that the inhibition of rRNA accumulation in temperature-sensitive mutant-infected cells ranged from 40 to 70% compared with uninfected controls. These values are considerably lower than the 80 to 90% inhibition found in wild-type virus-infected cells (see above).

TABLE 2. rRNA accumulation in HSV-1-infected cells

Expt	Source of RNA ^a	28S RNA		18S RNA	
		Radioactivity (cpm, $\times 10^4$)	% Inhibition	Radioactivity (cpm, $\times 10^4$)	% Inhibition
1	Mock-infected cells	9.6		60.0	
	HSV-1 strain 17-infected cells				
	2-3.5 h p.i.	11.4	0	50.9	15
2	4-5.5 h p.i.	0.8	92	12.1	80
	Mock-infected cells	14.6		44.9	
	HSV tsK-infected cells	9.2	37	25.1	44
3	Mock-infected cells	25.8		68.1	
	HSV tsK-infected cells	8.9	66	26.0	62
	HSV tsD-infected cells	8.2	68	16.4	66
	HSV tsT-infected cells	7.7	70	19.0	72

^a Infected cells were labeled 4.0 to 5.5 h p.i.

When these data were examined in terms of the amounts of rRNA that accumulated, it appeared that appreciably more rRNA was present in cells infected with the temperature-sensitive mutants than in cells infected with the wild-type virus. This differential effect on rRNA accumulation observed 4 to 5.5 h after infection suggests that in addition to the immediate early viral function found in tsK-infected cells that inhibits rRNA accumulation, a second function not present in tsK-infected cells also contributes to the extensive inhibition of rRNA accumulation characteristic of a wild-type infection.

Accumulation of HSV-specific and total cellular poly(A)⁺ RNAs after infection. In contrast to the inhibition of Ad-RNA, which occurred rapidly after infection, total cellular poly(A)⁺ RNA did not appear to be inhibited but increased in infected cells (Table 1). This increase in [³H]uridine incorporated into total cytoplasmic poly(A)⁺ RNA could be explained by the production of large quantities of HSV RNA after infection. In a previous study, Stringer et al. (45) showed by exhaustive hybridization that the majority of the polysomal poly(A)⁺ RNA was HSV specific at both early and late times after infection. Based on this study, it is likely that the majority of the total cytoplasmic poly(A)⁺ RNA accumulating in the cytoplasm of HSV-infected 293-31 cells is HSV specific. To investigate this possibility, we attempted to measure accurately the quantity of HSV RNA accumulating in the cytoplasm of infected cells at early and late times. The quantitation of HSV RNA is difficult. At late times in infection specific regions of the HSV genome are transcribed into RNA classes of high abundance (17), making it difficult to obtain true DNA excess for these species in a hybridization reaction. However, by hybridizing HSV RNA to increasing quantities of HSV DNA, it is possible to obtain a saturation curve in which the percentage of RNA hybridized increases with the DNA concentration. A dou-

ble-reciprocal plot of the data allows extrapolation to the Y axis, which represents the hybridization level at an infinite HSV DNA concentration. This type of analysis, which has been used successfully to quantitate the level of viral RNA accumulating early in an adenovirus infection (24), permits an estimation of the quantity of HSV RNA accumulating in the cytoplasm of infected cells.

Cytoplasmic poly(A)⁺ RNA from a previous experiment with HSV-1 strain 17 was hybridized to increasing quantities of HSV DNA as described above and in Table 3. Figure 1 shows the reciprocal plot of such a set of hybridizations. Extrapolation to the Y axis enabled the calculation of the fraction of HSV RNA present at an infinite HSV DNA concentration (Table 3). Our data show that 37 and 62% of the poly(A)⁺ RNA accumulating in the cytoplasm from 2.0 to 3.5 h p.i. and from 4.0 to 5.5 h p.i., respectively, were HSV specific. From these values we computed the quantity of HSV RNA present, subtracted these values from the total cytoplasmic poly(A)⁺ RNA, and calculated the quantity of host poly(A)⁺ RNA (Table 4). Our data also show that host poly(A)⁺ RNA continued to accumulate at approximately control levels as late as 2.0 to 3.5 h p.i. However, by 4.0 to 5.5 h p.i. the accumulation of host RNA was inhibited by 53% compared with the controls. These data are consistent with previous data which indicated that the accumulation of total host poly(A)⁺ RNA is inhibited after infection, and they show that the effect on total poly(A)⁺ RNA is less pronounced than the effect on Ad-RNA.

Effect of cycloheximide on HSV-induced inhibition of cytoplasmic Ad-RNA accumulation. The data in Tables 1 and 2 suggest that an immediate early gene product of HSV inhibits the accumulation of Ad-RNA in infected cells. However, it is possible that a virion polypeptide(s) which enters the cells with the infecting virus might be responsible for the observed effect. Previous

TABLE 3. Quantitation of HSV RNA in HSV-1 strain 17-infected 293-31 cells

Time p.i. (h)	Hybridization			Extrapolation		
	Input radio- activity (cpm) ^a	Amt of HSV DNA (μ g)	HSV RNA in hybrid	% of HSV RNA hybridized	1/% of HSV RNA hybridized	1/Y intercept ^c
2-3.5	2,590	1	279	10.8	0.093	0.027
		2	437	16.9	0.059	
		3	518	20.0	0.050	
	4,779	1	466	9.8	0.102	
		2	720	15.1	0.066	
		3	977	20.4	0.049	
4-5.5	2,472	1	422	17.1	0.059	0.016
		2	686	27.8	0.036	
		3	846	34.2	0.029	
	4,883	1	800	18.3	0.055	
		2	1,193	27.2	0.037	
		3	1,441	32.9	0.030	

^a Hybridization was performed by using two concentrations of RNA in separate reactions.^b The Y intercept was determined by inspection of Fig. 1.^c 1/Y intercept represents the percentage of HSV RNA present in the hybrid at an infinite HSV DNA concentration.

FIG. 1. Hybridization of HSV RNA to HSV DNA. Cytoplasmic poly(A)⁺ RNA from the experiment described in Table 1 was hybridized to 1, 2, or 3 μ g of HSV DNA as described in the text and Table 3. The data represent the reciprocal of the percentage of HSV RNA hybridized versus the reciprocal of the quantity of HSV DNA in the hybridization reactions presented in Table 3. Symbols: ○, HSV RNA from 2.0 to 3.5 h p.i.; ●, HSV RNA from 4.0 to 5.5 h p.i.

studies have shown that virion polypeptides of both HSV-1 and HSV-2 disrupt cellular functions (11, 29). To test for this possibility, cells were infected in the presence and absence of cycloheximide, and the effects on Ad-RNA accumulation were measured in the absence of de novo viral protein synthesis.

In the first experiment, cells were infected with either HSV-1 strain 17 or tsK at 39°C in the presence or absence of 200 μ g of cycloheximide per ml. At 4 h p.i., the cells were labeled with 200 μ Ci of [³H]uridine per ml for 90 min. Cytoplasmic poly(A)⁺ RNA was prepared and analyzed as described above and in Table 1. The data indicate (Table 5) that in the presence of cycloheximide, Ad-RNA accumulation was inhibited by 60% compared with the mock-infected control sample. Despite the inhibition produced by cycloheximide alone, the interpretation of the data is clear. In the presence of cycloheximide, neither virus inhibited Ad-RNA

TABLE 4. Accumulation of HSV and host cytoplasmic poly(A)⁺ RNAs in HSV-infected 293-31 cells

Source of RNA	Total poly(A) ⁺ RNA radioactivity (cpm, $\times 10^5$)	% of HSV RNA ^a	HSV RNA radioactivity (cpm, $\times 10^5$)	Host RNA radioactivity (cpm, $\times 10^5$) ^b	Inhibition of host RNA (%) ^c
Mock-infected cells	31.3			31.3	
HSV-infected cells					
2-3.5 h p.i.	48.4	37	18	30.4	3
4-5.5 h p.i.	42.8	62	28	14.8	53

^a Data from Table 2.^b Total poly(A)⁺ RNA minus calculated amount of HSV RNA.^c Calculated by comparing the values for host RNA radioactivity with 31.3×10^5 cpm as the uninhibited value.

accumulation, and the Ad-RNA levels were greater than those found in the mock-infected cycloheximide-treated control sample. These data reinforce the proposal that an immediate early polypeptide and not a virion component is responsible for the observed inhibitory effect. The inhibition of Ad-RNA accumulation in untreated infected cells was 98% for HSV-1 strain 17-infected cells and 90% when tsK was used. As described above, because of the precision of the assay the differences in the extent of inhibition are meaningful, and as was the case in previous experiments, the quantity of Ad-RNA in tsK-infected cells was significantly greater than the quantity found in HSV-1 strain 17-infected cells.

A second experiment was designed to examine the possibility that after treatment with cycloheximide, cells could be released from the drug, thereby allowing inhibition of Ad-RNA accumulation. To accomplish this, cells were infected with tsK at 39°C in the presence and absence of cycloheximide, as described above. At 5.5 h p.i., drug-treated infected and mock-infected cultures were released from the cycloheximide block, and infection was allowed to proceed for 4 h. At this time the cells were labeled with [³H]uridine for 90 min, and cytoplasmic poly(A)⁺ RNA was isolated and analyzed.

In this experiment (Table 5, experiment 2), cycloheximide inhibited Ad-RNA accumulation by 40%. Again, interpretation of the data is clear. Infection with tsK inhibited Ad-RNA accumulation by 88%, with Ad-RNA continuing to accumulate at low but significant levels. In the presence of cycloheximide, inhibition of Ad-RNA did not occur in tsK-infected cells. After the drug was removed, accumulation of Ad-RNA was inhibited, but low levels of Ad-RNA continued to accumulate as late as 11 h p.i. These data support our earlier findings that an immediate early protein is largely responsible for the inhibition of Ad-RNA accumulation.

Rates of RNA synthesis in HSV-infected cells. The HSV-induced inhibition of cytoplasmic RNA accumulation could be due to inhibition of synthesis or nuclear-cytoplasmic transport, or alternatively, the RNA could be degraded rapidly after synthesis. To distinguish among these possibilities, we examined the rates of synthesis of both Ad-RNA and total nuclear RNA in HSV-infected cells.

293-31 cells were infected with HSV-1 strain 17 at 37°C, and at 3 h p.i. infected and uninfected cells were labeled with 200 μ Ci of [³H]uridine per ml for 5, 10, 15, and 30 min. Total nuclear RNA was prepared and hybridized to filter-bound adenovirus DNA under conditions of DNA excess. Table 6 and Fig. 2A show that

TABLE 5. Effect of cycloheximide on HSV-induced inhibition of cytoplasmic Ad-RNA accumulation

Expt	Source of RNA	Hybridization					RNA in 10 ⁷ cells ^a		Inhibition of Ad-RNA accumulation (%) ^b
		Input radio-activity (cpm, ×10 ³)	Ad-RNA radio-activity (cpm)	Ad-RNA hybridized (%)	Total poly(A) ⁺ RNA radioactivity (cpm, ×10 ⁶)	Ad-RNA radio-activity (cpm)			
1	Mock-infected cells	4.03	1,596	0.396	4.03	15,960			
	Mock-infected cells, cycloheximide treated	2.95	553	0.187	3.30	6,200			
	HSV-1 strain 17-infected cells	6.11	25	0.004	7.15	290			98
	HSV-1 strain 17-infected cells, cycloheximide treated	2.99	716	0.239	2.96	7,090			0
	HSV tsK-infected cells	2.56	157	0.061	2.66	1,630			90
2	HSV tsK-infected cells, cycloheximide treated	1.90	796	0.419	1.92	8,040			0
	Mock-infected cells ^c	4.44	1,166	0.263	4.44	11,660			
	Mock-infected cells, cycloheximide treated	3.43	848	0.247	2.80	6,930			
	Mock-infected cells, cycloheximide treated, reversal	3.51	1,848	0.526	3.09	16,290			
	HSV tsK-infected cells ^c	3.06	168	0.055	2.38	1,310			89
	HSV tsK-infected cells, cycloheximide treated	2.19	679	0.310	2.07	6,410			8
	HSV tsK-infected cells, cycloheximide treated, reversal	0.80	94	0.118	0.96	1,130			93

^a Corrected for yield of total RNA.^b Inhibition of Ad-RNA accumulation was determined by comparing mock-infected cultures with virus-infected cultures and mock-infected, cycloheximide-treated cultures with virus-infected, cycloheximide-treated cultures.^c The data shown were generated at the same time as the data in Table 1, experiment 2.

TABLE 6. RNA synthesis in HSV-1 infected 293-31 cells

Expt	Temp (°C)	Source of RNA ^a	Length of labeling (min)	Hybridization			RNA in 10 ⁷ cells ^b		Inhibition of Ad-RNA accumulation (%)
				Input radioactivity (cpm, ×10 ⁵)	Ad-RNA radioactivity (cpm)	Ad-RNA hybridized (%)	Total RNA radioactivity (cpm, ×10 ⁵)	Ad-RNA radioactivity (cpm)	
1	37	Mock-infected cells	5	7.98	85	0.011	46.9	500	71
			10	29.74	235	0.008	131.6	1,040	
			15	47.09	341	0.007	215.9	1,560	
			30	90.76	534	0.006	453.8	2,670	
			5	5.37	30	0.006	25.9	140	
2	39	HSV-1 strain 17-infected cells	10	15.78	62	0.004	75.1	300	72
			15	25.32	85	0.003	107.2	360	77
			30	46.86	102	0.002	292.9	640	76
			5	21.9	325	0.015	109.5	1,630	
			10	36.1	614	0.017	209.4	3,580	
		Mock-infected cells	15	63.8	869	0.014	341.5	4,630	
			5	10.2	98	0.009	48.9	470	71
			10	26.2	172	0.006	118.4	780	78
			15	42.8	205	0.004	186.0	890	81

^a Infected and uninfected cells were labeled with [³H]uridine at 3 h p.i. at 39°C.^b Corrected for yield of total RNA.

FIG. 2. RNA synthesis in HSV-1 strain 17-infected 293-31 cells at 37°C. Cells were mock infected or infected at a multiplicity of 20 PFU/cell and at 3 h p.i. were labeled for 5, 10, 15, or 30 min with 200 μ Ci of [3 H]uridine per ml. Nuclear RNA was prepared and analyzed as described in the text and Table 3. Symbols: ●, mock-infected cells; ○, virus-infected cells.

there was a lag of approximately 2.25 min until uridine appeared in the bulk of the nuclear RNA. Incorporation into total nuclear RNA was linear for at least 15 min and probably continued for 30 min. Infected cell RNA also showed linear uridine incorporation, but the rate was inhibited by 47% compared with the uninfected control values.

The incorporation of [3 H]uridine into adenovirus-specific RNA began within approximately 30 s after the start of labeling and appeared to be linear for 15 min at 37°C (Fig. 2B and Table 6). The rate of incorporation of uridine into Ad-RNA was inhibited by 72% compared with the uninfected control cells after infection with HSV. These data show that HSV primarily alters the accumulation of Ad-RNA at the level of synthesis. However, the inhibition of transcription alone cannot account for the extensive inhibition of cytoplasmic Ad-RNA accumulation (97 to 98% by 4 h p.i.), suggesting that another virus-specific function must be involved. One possibility is an HSV-induced cytoplasmic RNA

degradation effect, which has been described previously (28, 29, 31).

Rates of RNA synthesis in HSV tsK-infected 293-31 cells. The experiments described above show that an immediate early protein of HSV produces the majority of the inhibition of cellular RNA accumulation. This inhibition appears to occur at the level of RNA synthesis. To prove conclusively that an immediate early protein is responsible for the inhibition of transcription, it was necessary to examine the rates of synthesis in HSV-infected cells under conditions where only immediate early proteins were expressed.

To accomplish this, 293-31 cells were infected with HSV tsK at 39°C. Under these conditions, HSV gene expression was restricted to the immediate early class of HSV proteins. At 3 h p.i. infected and uninfected cells were labeled with 200 μ Ci of [3 H]uridine per ml for 5, 10, and 15 min. Total nuclear RNA was prepared and hybridized to filter-bound adenovirus DNA under conditions of DNA excess. Figure 3A and Table 6 (experiment 2) show that total nuclear RNA synthesis was inhibited by approximately 49% in the tsK-infected cells. Ad-RNA synthesis (Fig. 3B and Table 6) was inhibited by approximately 75% during the first 10 min of incorporation. These data are identical to the data obtained with wild-type HSV-1 strain 17-infected cells. It should be noted that the rates of transcription were linear for only 10 min at 39°C and that the levels of incorporation were higher than those at 37°C. These observations are consistent with the fact that RNA synthesis occurs at a faster rate at higher temperatures. Collectively, these data indicate that the effect of HSV on transcription is due to an immediate early gene function(s).

DISCUSSION

In this study we used a permissive adenovirus-transformed cell system to provide insight into the mechanisms by which HSV alters the metabolism of cellular RNAs. Our data indicate that very soon after infection, an HSV immediate early gene function is expressed, which inhibits the transcription of Ad-RNA in the nuclei of infected cells. This function is the primary reason for the extensive inhibition of accumulation of Ad-RNA in the cytoplasm. It should be pointed out that not all RNAs are affected equally. Incorporation into total poly(A)⁺ RNA remains high after infection, indicating that RNA polymerase is still active and that depletion of the ribonucleotide triphosphate pools probably does not play a role in restricting RNA synthesis. A large part of the poly(A)⁺ RNA synthesized after HSV infection is viral, and this fact must be taken into account when calculating the extent to which cellular RNA accumulation is inhibited. When this is

FIG. 3. RNA synthesis in HSV-1 tsK-infected 293-31 cells at 39°C. Cells were mock infected or infected at a multiplicity of 20 PFU/cell and at 3 h p.i. were labeled for 5, 10, and 15 min with 200 μ Ci of [3 H]uridine per ml. Nuclear RNA was prepared and analyzed as described in the text and Tables 5 and 6. Symbols: ●, mock-infected cells; ○, virus-infected cells.

done, we find that cellular poly(A)⁺ RNA and rRNA accumulation is inhibited, but to a lesser extent and at a slower rate than adenovirus-specific RNA. These data indicate that Ad-RNA is behaving as a distinct class of RNA. With the availability of cloned human cDNA probes, our approach can be extended to other human cellular mRNAs to determine whether after HSV infection individual species or restricted classes of cellular RNAs have a characteristic metabolism distinct from the population as a whole. We expect that the effect of HSV on these different populations of cellular RNA probably varies as far as the rate and extent of inhibition but not in the mechanism by which inhibition is produced.

We chose to use adenovirus-transformed cells for our study because the DNA corresponding to Ad-RNA was readily available and the metabolism of the mRNA transcribed from the integrated adenovirus DNA has been the subject of intensive investigation (51). Some of the characteristics of the Ad-RNA transcripts are shown in Fig. 4. The two segments of the adenovirus

genome in transformed cells (IA and IB) are transcribed with different kinetics, accumulate to different levels, and decay with different half-lives. The half-lives of the Ad-RNAs in transformed cells are longer than the half-lives in lytically infected cells, but these RNAs still turn over rapidly compared with other cellular mRNAs. Although differences in Ad-RNA metabolism are likely in each transformed cell line, we expect that the Ad-RNA in 293-31 cells in general behaves as previously reported for other cells. Preliminary experiments (data not shown) showed linear uridine incorporation into nuclear RNA from the IB DNA region for at least 120 min. Transcripts from the IA region were not detected. Analyses of the cytoplasmic Ad-RNA after the 120-min labeling period showed 1,930 and 13,100 cpm hybridizing to IA DNA and IB DNA, respectively. These data indicate that transcripts from the IB region constitute the major fraction of the Ad-RNA (87%) and that the transcripts which originate from region IA must be transported to the cytoplasm very rapidly. In a typical experiment performed at 37°C, about 9,000 cpm was present in cytoplasmic poly(A)⁺ Ad-RNA after a 90-min labeling period. It appears that in uninfected 293-31 cells uridine incorporation into cytoplasmic Ad-RNA is essentially linear for at least 120 min and reflects accumulation after synthesis, processing, and transport (Fig. 4, steps 1 and 2). Before infection degradation is not extensive during the 90-min labeling period, but HSV infection alters this situation.

The accumulation of Ad-RNA in the cytoplasm of wild-type HSV-infected 293-31 cells is inhibited by 97 to 98% compared with control cells by 4 h p.i. Figure 4 is our model for how HSV alters this accumulation. An examination of the rates of synthesis of Ad-RNA in uninfected and infected cells revealed that infection reduced the incorporation of [3 H]uridine into total nuclear Ad-RNA by 70 to 75% compared with control cells. This result indicates that the HSV-induced inhibition of accumulation of cytoplasmic Ad-RNA occurs primarily at the level of transcription. The experiment with the temperature-sensitive mutants of HSV showed that an immediate early gene function is responsible for this inhibition. The difference between the inhibition of transcription of Ad-RNA (75%) and the inhibition of accumulation of cytoplasmic Ad-RNA (~95%) indicates that an additional factor(s) affects accumulation. Because Ad-RNA accumulates to significantly higher levels in the cytoplasm of tsK-, tsD-, and tsT-infected cells, we suggest that a second function expressed in wild-type virus-infected cells but not in temperature-sensitive mutant-infected cells is responsible for the second effect on accumula-

tion. Although theoretically any posttranscriptional step could be altered by infection and thereby inhibit accumulation, additional measurements of the steady-state levels of cytoplasmic Ad-RNA have indicated that an HSV gene function causes active degradation of cytoplasmic Ad-RNA (Stenberg and Pizer, submitted for publication). This second function or degradation function could account for the extensive reduction in cytoplasmic Ad-RNA levels in wild-type virus-infected cells and provide an explanation for previous reports (28, 29, 31) which described reduced levels of cellular RNAs after HSV infection.

At the same time after infection, when Ad-RNA synthesis is strongly inhibited, total cellular RNA synthesis is inhibited by only approximately 50% compared with the control cells. This level of inhibition agrees with the inhibition of cytoplasmic host poly(A)-containing RNA accumulating in the cytoplasm. However, the accumulation of rRNA was more strongly affected (80 to 90%). As with Ad-RNA, steady-state measurements have indicated that the normally stable 18S and 28S RNAs are degraded after infection (data not shown). It appears that after infection, different RNA classes have different susceptibilities to the degradation process, and kinetic measurements with a cDNA probe for total cellular mRNA are consistent with this conclusion (H. Nakai, I. Maxwell, and L. I. Pizer, *J. Virol.*, in press).

Our data also suggest an additional site of regulation at the ribosome level. The experiments of Stringer et al. clearly demonstrate that late in infection, about 90% of the poly(A)⁺

RNA in polysomes is HSV specific (45), but we found that approximately 50% of the poly(A)⁺ accumulating in the cytoplasm is cellular. Apparently, after infection the cellular RNAs are excluded from the ribosomes and fail to act as mRNAs for protein synthesis.

Whatever additional factors alter RNA metabolism after HSV infection, the primary effect, which is mediated by an immediate early protein, is a reduction in the rate of transcription. When one considers the mechanisms by which an immediate early protein might inhibit transcription, two alternatives can be considered. The first is that an immediate early protein(s) can modify the DNA template. However, the levels of the immediate early proteins in HSV-infected cells are low, and these proteins are synthesized for only a short period of time immediately after infection, after which their synthesis becomes reduced (16, 50). Therefore, it is not likely that they could physically bind to the entire host DNA template and prevent transcription.

A more attractive alternative is that an immediate early polypeptide interacts with a cellular regulatory component or enzyme, thus altering its function. The types of regulatory components which can be considered initially are the cellular RNA polymerases and the factors which regulate the activity of these polymerases. It has been shown previously that host RNA polymerase II is responsible for the transcription of HSV RNA (2, 4). To accomplish a change in transcriptional activity as typified by the reduction in Ad-RNA synthesis, an immediate early protein could alter the specificity of either the

FIG. 4. Schematic representation of Ad-RNA metabolism in HSV-infected 293-31 cells. The size and location of Ad-RNA transcripts are as previously reported (5, 43). The numbers in the cell correspond to the description concerning Ad-RNA metabolism in HSV-infected cells.

polymerase or one of its activating factors. The search for new or altered polymerase activities has been unsuccessful (23, 26, 34, 39). However, most of these studies have involved the purification of polymerases under conditions where any HSV-specific regulatory subunits could have become dissociated from the enzyme complex. To examine how these proteins might be interacting within the nuclei of infected cells, alternative approaches must be used. The use of mono-specific antisera against the different cellular polymerases and their activating factors or the immediate early HSV proteins would permit investigators to look for interactions which are readily disrupted by standard procedures.

It is possible that the specificity of RNA polymerase is not altered after infection by interaction with a viral protein, but the polymerase is enzymatically modified. A number of workers have proposed that RNA polymerases are partially regulated by a phosphorylation-dephosphorylation mechanism (7, 8, 20, 21, 37, 40). Phosphorylation of proteins also appears to play a role in the control of HSV infections (6, 22, 30, 50). There is a protein kinase activity associated with purified virions (22, 38) and an HSV-specific protein kinase activity which appears within the first hour of infection (6). Virtually all of the immediate early proteins are phosphorylated (30), and several of these proteins (i.e., V_{MW175} [ICP4], V_{MW68} [ICP22], and V_{MW63} [ICP27]) are phosphorylated and dephosphorylated in a coordinate manner throughout the course of the replicative cycle (50). Although these observations taken together indicate a role for phosphorylation in regulating transcriptional specificity, proof of the hypothesis will require detailed analyses of changes in the phosphorylation patterns of the polymerase enzyme within infected cells and subsequent analyses of whether the specificity of the polymerase can be altered by *in vitro* phosphorylation.

ACKNOWLEDGMENTS

We thank Donald MacDonald, Margaret Miovic, David Spector, Luis Villarreal, and Roberto Weinmann for helpful discussions and Meredith Peake and Linda Yamamoto for excellent technical assistance. We are grateful for the gift of mutant virus stocks from Howard Marsden and John Subak-Sharpe.

This work was supported by grant NP-209 from the American Cancer Society and by Public Health Service grants 5T32 AI07209 and 1R01 AI17873 from the National Institutes of Health.

LITERATURE CITED

1. Aiello, L., R. Guilfoyle, K. Huebner, and R. Weinmann. 1979. Adenovirus 5 DNA sequences present and RNA sequences transcribed in transformed human embryo kidney cells (HEK-Ad-5 or 293). *Virology* 94:460-469.
2. Alwine, J. C., W. L. Steinhart, and W. C. Hill. 1974. Transcription of herpes simplex type 1 DNA in nuclei isolated from infected HEP-2 and KB cells. *Virology* 60:302-307.
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. Ben-Zeer, A., Y. Asher, and Y. Becker. 1976. Synthesis of herpes simplex virus-specified RNA by an RNA polymerase II in isolated nuclei. *Virology* 71:302-311.
5. Berk, A. J., F. Lee, T. Harrison, J. Williams, and P. A. Sharp. 1979. Pre-early adenovirus 5 gene product regulates synthesis of early viral messenger RNAs. *Cell* 17:935-944.
6. Blue, W. T., and D. G. Stobbs. 1981. Isolation of a protein kinase induced by herpes simplex virus type 1. *J. Virol.* 38:383-388.
7. Dahmus, M. E. 1976. Stimulation of ascites tumor RNA polymerase II by protein kinase. *Biochemistry* 15:1821-1829.
8. Dahmus, M. E. 1981. Phosphorylation of eukaryotic DNA-dependent RNA polymerase. *J. Biol. Chem.* 256:3332-3339.
9. Darlington, R. W., and A. Granoff. 1973. Replication—biological aspects, p. 93-132. *In* A. S. Kaplan (ed.), *The herpes viruses*. Academic Press, Inc., London.
10. Dreesman, G. R., and M. Benyesh-Melnick. 1967. Spectrum of human cytomegalovirus complement-fixing antigens. *J. Immunol.* 99:1106-1114.
11. Fenwick, M., L. S. Morse, and B. Roizman. 1979. Anatomy of herpes simplex virus DNA. XI. Apparent clustering of functions effecting rapid inhibition of host DNA and protein synthesis. *J. Virol.* 29:825-827.
12. Fenwick, M. L., and M. J. Walker. 1978. Suppression of synthesis of cellular macromolecules by herpes simplex virus. *J. Gen. Virol.* 41:37-51.
13. Goldenberg, C. J., and H. J. Raskas. 1979. Splicing patterns of nuclear precursors to the mRNA for adenovirus 2 DNA binding protein. *Cell* 16:131-138.
14. Graham, F. L., J. Smiley, W. C. Russell, and R. Nairn. 1977. Characteristics of a human cell line transformed by DNA from human adenovirus type 5. *J. Gen. Virol.* 36:59-72.
15. Holmes, D. S., and J. Bonner. 1973. Preparation, molecular weight, base composition and secondary structure of giant nuclear ribonucleic acid. *Biochemistry* 12:2330-2337.
16. Honess, R. W., and B. Roizman. 1974. Regulation of herpes virus macromolecular synthesis. I. Cascade regulation of the synthesis of three groups of viral polypeptides. *J. Virol.* 14:8-19.
17. Jacquemont, B., and B. Roizman. 1975. Ribonucleic acid synthesis in cells infected with herpes simplex virus. XI. Characterization of viral high molecular weight nuclear RNA. *J. Gen. Virol.* 29:155-165.
18. Kay, J., G. J. Koteles, H. M. Keir, and J. Subak-Sharpe. 1966. Herpes virus specified ribonucleic acids. *Nature (London)* 210:387-390.
19. Klein, R. D., E. Selsing, and R. D. Wells. 1980. A rapid microscale technique for isolation of recombinant plasmid DNA suitable for restriction enzyme analysis. *Plasmid* 3:88-91.
20. Kranias, E. G., and R. A. Jungmann. 1978. Phosphorylation of calf thymus RNA polymerase II by nuclear cyclic 3',5' AMP-independent protein kinase. *Biochim. Biophys. Acta* 517:439-446.
21. Kranias, E. G., J. S. Schweppe, and R. A. Jungmann. 1977. Phosphorylation and functional modifications of nucleoplasmic RNA polymerase II by homologous adenosine 3':5' monophosphate dependent protein kinase from calf thymus and by heterologous phosphatase. *J. Biol. Chem.* 252:6750-6758.
22. Lemaster, S., and B. Roizman. 1980. Herpes simplex virus phosphoproteins. II. Characterization of the virion protein kinase and of the polypeptides phosphorylated in the virion. *J. Virol.* 35:798-811.

23. Lowe, P. 1978. Levels of DNA-dependent RNA polymerases in herpes simplex virus-infected BHK 21 C13 cells. *Virology* 86:577-580.
24. Lucas, J. J., and H. S. Ginsberg. 1971. Synthesis of virus-specific ribonucleic acid in KB cells infected with type 2 adenovirus. *J. Virol.* 8:203-213.
25. McMaster, G. K., and G. C. Carmichael. 1977. Analysis of single- and double-stranded nucleic acids on polyacrylamide and agarose gels by using glyoxal and acridine orange. *Proc. Natl. Acad. Sci. U.S.A.* 74:4835-4838.
26. Mlovic, M. L., and L. I. Pizer. 1979. Characterization of RNA synthesized in isolated nuclei of herpes simplex virus type 1-infected KB cells. *J. Virol.* 33:567-571.
27. Morse, L. S., L. Pereira, B. Roizman, and P. A. Schaffer. 1978. Anatomy of herpes simplex virus DNA. XI. Mapping of viral genes by analysis of polypeptides and functions specified by HSV-1 $\times$ HSV-2 recombinants. *J. Virol.* 26:389-410.
28. 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.
29. Nishioka, Y., and S. Silverstein. 1978. Requirement of protein synthesis for the degradation of host mRNA in Friend erythroleukemia cells infected with herpes simplex virus type 1. *J. Virol.* 27:619-627.
30. Pereira, L., M. Wolff, M. Fenwick, and B. Roizman. 1977. Regulation of herpes virus synthesis. V. Properties of α polypeptides specified by HSV-1 and HSV-2. *Virology* 77:733-749.
31. Pizer, L. I., and P. Beard. 1976. The effect of herpes virus infection on mRNA in polyoma virus-transformed cells. *Virology* 75:477-480.
32. Preston, C. M. 1979. Abnormal properties of an immediate early polypeptide in cells infected with the herpes simplex virus type 1 mutant tsK. *J. Virol.* 32:357-369.
33. Preston, C. M. 1979. Control of herpes simplex virus type 1 mRNA synthesis in cells infected with wild-type virus or the temperature-sensitive mutant tsK. *J. Virol.* 29:275-284.
34. Preston, C. M., and A. A. Newton. 1976. The effects of herpes simplex virus type 1 on cellular DNA-dependent RNA polymerase activities. *J. Gen. Virol.* 33:471-482.
35. Preston, V. G. 1981. Fine structure mapping of herpes simplex virus type 1 temperature-sensitive mutations within the short repeat region of the genome. *J. Virol.* 39:150-161.
36. Roizman, B., G. S. Borman, and M. K. Roust. 1965. Macromolecular synthesis in cells infected with herpes simplex virus. *Nature (London)* 206:1374-1375.
37. Rose, K. M., D. A. Stetler, and S. T. Jacob. 1981. Protein kinase activity of RNA polymerase I purified from a rat hepatoma: probable function of M_r 42,000 and 24,600 polypeptides. *Proc. Natl. Acad. Sci. U.S.A.* 78:2833-2837.
38. Rubenstein, D. S., M. Granell, and R. Darlington. 1972. Protein kinase in enveloped herpes simplex virions. *Virology* 50:287-290.
39. Sasaki, Y., R. Sasaki, G. H. Cohen, and L. I. Pizer. 1974. RNA polymerase activity and inhibition in herpes virus-infected cells. *Intervirology* 3:147-161.
40. Sekimizu, K., Y. Kubo, K. Segawa, and S. Natori. 1981. Difference in phosphorylation of two factors stimulating RNA polymerase II of Ehrlich ascites tumor cells. *Biochemistry* 20:2287-2292.
41. Spear, P. G., and B. Roizman. 1980. Herpes simplex viruses, p. 615-745. *In* J. Tooze (ed.), *Molecular biology of tumor viruses*, 2nd ed., part 2. DNA tumor viruses. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
42. Spector, D., and L. I. Pizer. 1978. Herpes virus infection modifies adenovirus RNA metabolism in adenovirus-transformed cells. *J. Virol.* 27:1-12.
43. Spector, D. J., D. N. Halbert, and H. J. Raskas. 1980. Regulation of integrated adenovirus sequences during adenovirus infection of transformed cells. *J. Virol.* 36:860-871.
44. Stenberg, R. M., D. J. Spector, and L. I. Pizer. 1979. Modulation of herpes simplex virus replication in adenovirus transformed cells. *J. Gen. Virol.* 44:297-309.
45. Stringer, J. R., L. E. Holland, R. I. Swanson, K. Pivo, and E. K. Wagner. 1977. Quantitation of herpes simplex virus type 1 RNA in infected HeLa cells. *J. Virol.* 21:889-901.
46. Thomas, P. S. 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. *Proc. Natl. Acad. Sci. U.S.A.* 77:5201-5205.
47. Wagner, E. K., and B. Roizman. 1969. RNA synthesis in cells infected with herpes simplex virus. I. The patterns of RNA synthesis in productively infected cells. *J. Virol.* 4:36-46.
48. Watson, R. J., and J. B. Clements. 1980. A herpes simplex virus type 1 function continuously required for early and late virus RNA synthesis. *Nature (London)* 285:329-330.
49. Weinmann, R., H. J. Raskas, and R. G. Roeder. 1974. Role of DNA-dependent RNA polymerases II and III in transcription of the adenovirus genome late in productive infection. *Proc. Natl. Acad. Sci. U.S.A.* 71:3426-3430.
50. Wilcox, K. W., A. Kohn, E. Sklyanskaya, and B. Roizman. 1980. Herpes simplex virus phosphoproteins. I. Phosphate cycles on and off some viral polypeptides and can alter their affinity for DNA. *J. Virol.* 33:167-182.
51. Wilson, M. C., J. R. Nevins, J.-M. Blanchard, H. S. Ginsberg, and J. E. Darnell, Jr. 1979. The metabolism of mRNA from the transforming region of adenovirus type 2. *Cold Spring Harbor Symp. Quant. Biol.* 44:447-455.
52. Zimmerman, S. B., and G. Sandeen. 1966. The ribonuclease activity of crystallized pancreatic deoxyribonuclease. *Anal. Biochem.* 14:269-277.