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Effect of Cycloheximide on RNA Metabolism Early in Productive Infection with Adenovirus 2

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The presence of cycloheximide during the early phase of adenovirus 2 replication causes an increase in the virus-specific content of newly synthesized mRNA. The total cytoplasmic RNA from control cultures labeled 2 to 5 h after infection hybridized to viral DNA 0.8%, whereas RNA synthesized in the presence of cycloheximide annealed 6%. Cytosine arabinoside, an inhibitor of DNA synthesis, did not affect the percent hybridization to viral DNA. Oligo(dT)-cellulose chromatography was used to purify the portion of cytoplasmic RNA containing poly(A). The poly(A)-containing RNA from cultures labeled in the presence of cycloheximide hybridized to viral DNA 32% as compared to 2.2% for RNA from control cultures. Hybridization-inhibition experiments between RNAs from control- and cycloheximide-treated cultures demonstrated that the cultures treated with cycloheximide did not have an increased content of viral RNA or a new class of viral RNA sequences. Therefore, the increased hybridization appears to be caused by a reduction in synthesis of cellular cytoplasmic mRNA. Nucleoplasmic RNAs lacking and containing poly(A) were annealed to viral DNA. For both classes, RNA from cultures treated with cycloheximide hybridized 5- to 10-fold more than RNA from control-infected cultures. Therefore, the increased hybridization of cytoplasmic RNA synthesized in the presence of cycloheximide is caused either by reduced transcription of the cellular genome or by greatly increased instability of cellular heterogeneous nuclear RNA.

The productive replication of adenovirus 2 in cultured human (KB) cells can be divided into two distinct phases, an early period which precedes the onset of DNA replication, and the late period which follows. RNA-DNA hybridization studies have demonstrated that the functional viral RNA synthesized in the early period represents a limited portion of the viral genome (6, 10, 22). In productive infections with adenovirus 2, viral DNA replication begins approximately 6 to 7 h after infection (21). Since the onset of DNA replication may not be synchronous, most studies of early RNA synthesis (10, 14, 22, 23) have used inhibitors of DNA (5, 7) or protein synthesis (8) to insure the absence of viral DNA replication and thus late RNA sequences.

We have investigated the effect of a DNA synthesis inhibitor, cytosine arabinoside, and a protein synthesis inhibitor, cycloheximide (CH), on viral and host RNA synthesis. Parsons and Green (11) initially reported a stimulation of viral RNA synthesis in the presence of cycloheximide. The experiments presented here demonstrate that cycloheximide has little effect on the amount of viral RNA synthesized, but seems to cause a considerable decrease in the synthesis of host mRNA. In contrast, cytosine arabinoside does not alter the relative amounts of viral and host mRNA synthesized early in infection.

MATERIALS AND METHODS

Cell culture and viral infection. Exponentially growing KB suspension cultures were concentrated to 1.2×10^7 cells per ml and infected with adenovirus 2 at a multiplicity of 100 PFU per cell as previously described (16). After a 1-h adsorption period the cells were diluted to 9×10^5 cells per ml. In many experiments, 25 μg of CH per ml (Sigma Chemical) or 10 μg of cytosine arabinoside per ml (CA; Calbiochemical) were added at the time of dilution. Cultures were labeled with 12.5 μCi of [^{3}H]uridine per ml (40 Ci/mmol, New England Nuclear Corp.) from 2 to 5 h after infection.

Cell fractionation. Cytoplasmic extracts were prepared by resuspending the cells in hypotonic buffer (reticulocyte standard buffer, RSB; 0.01 M Tris-hydrochloride pH 7.4, 0.01 M NaCl, 0.0015 M $MgCl_2$)

(12) for 15 min followed by the addition of 0.5% Nonidet P-40 (NP-40) (Shell Chemical Co.), and subsequent disruption with several strokes of a stainless steel Dounce homogenizer. Cell breakage was monitored by microscopy. Nuclei were removed by centrifugation at 800 × g for 10 min, and the cytoplasmic extract was clarified by centrifugation at 18,000 × g for 20 min. Cytoplasmic tabs were removed from nuclei by washing two times with RSB containing 0.5% NP-40 and 0.5% sodium deoxycholate, as described by Penman (12). The nucleoplasm was prepared by lysing the nuclei in high salt buffer (HSB; 0.5 M NaCl, 0.05 M $MgCl_2$, 0.05 M Tris-hydrochloride, pH 7.4), followed by treatment with 100 μg of DNase per ml (electrophoretically purified, Worthington Biochemical Co.) at room temperature until the viscosity was reduced (approximately 10 min). The extract was then centrifuged at 10,000 × g for 5 min to remove the nucleoli (13).

RNA purification and fractionation. Cytoplasmic extracts were purified by the addition of 0.5% sodium dodecyl sulfate (SDS) and was followed by three extractions with equal volumes of water-saturated phenol and chloroform-isoamyl alcohol (at a ratio of 24:1) at room temperature. After the first extraction, interphase material was re-extracted according to the following procedure. The aqueous and organic phases above and below the interphase were removed with a Pasteur pipette. RSB, phenol, and chloroform-isoamyl alcohol were then added in the same volume as originally present. The interphase was then extracted and the resultant aqueous portion combined with the aqueous phase obtained from the first extraction. After two more extractions, the RNA was precipitated by the addition of two volumes of 95% ethanol in the presence of 0.15 M NaCl.

Total nuclear RNA and nucleoplasmic RNA were extracted in the same manner. Nuclei were treated with DNase as described above and then diluted fivefold with TES buffer (0.05 M Tris-hydrochloride, pH 7.4; 0.02 M EDTA; 0.5% SDS). After dilution, the preparations were extracted with water-saturated phenol and chloroform-isoamyl alcohol as for cytoplasmic extracts. The RNA was collected by alcohol precipitation, resuspended in HSB, and incubated with 25 μg of DNase per ml for 30 min at room temperature. After DNase treatment the RNA was diluted fivefold with TES buffer, extracted two times with water-saturated phenol, and precipitated with ethanol. To obtain whole cell RNA, cell pellets were resuspended in HSB and extracted as described for total nuclear RNA.

Molecules containing poly(A) were separated from molecules lacking poly(A) by selective retention on oligo(dT)-cellulose (Collaborative Research, Inc.) (2). RNA was applied to an oligo(dT)-cellulose column (0.5 by 1 cm) in 0.5 M KCl, 0.01 M Tris-hydrochloride, pH 7.5. The column was then washed with 15 ml of the same buffer. Ninety to 100% of the poly(A)-containing RNA was eluted in 2 ml of 0.01 M Tris-hydrochloride, pH 7.5.

RNA-DNA hybridization. All hybridizations were performed with adenovirus 2 DNA immobilized on 6.5-mm cellulose nitrate filters (Schleicher and Schuell, type B6). Single-step hybridizations of [^{3}H]RNA were incubated for 20 h at 66 C in a volume of 100 μliters of 6× SSC (1× SSC is 0.15 M NaCl, 0.015 M sodium citrate) containing 0.1% SDS. Filters were then washed with 2× SSC, treated with pancreatic RNase (20 μg/ml in 2× SSC) for 1 h at room temperature, and washed again with 2× SSC. The percentage of viral RNA in a preparation was determined by hybridization to increasing amounts of DNA (0.5, 1, 2, and 4 μg). To obtain the amount of [^{3}H]RNA binding at infinite DNA concentration, the counts per minute hybridized were plotted against the reciprocal of the micrograms of DNA used. The maximal amount of RNA capable of hybridization was determined by extrapolating to the [^{3}H]RNA hybridized at infinite DNA concentration. To avoid errors introduced by extrapolation, the [^{3}H]RNA input was adjusted so that the difference in hybridization to 2 and 4 μg of DNA was less than 20%.

Two-step hybridization-inhibition experiments were performed in 30% formamide, 3× SSC, 0.1% SDS, 0.01 M Tes [N-tris(hydroxymethyl) methyl-2-aminoethane sulfonic acid], pH 7.4, for 24 h at 45 C. In the first step, increasing concentrations of unlabeled RNA were incubated for 20 h with filters containing 0.02 μg of adenovirus DNA (10). After incubation the filter was washed three times with 2× SSC and treated with 20 μg of pancreatic RNase per ml in 2× SSC for 45 min at room temperature. The filter was then treated with 0.15 M iodoacetic acid to inhibit RNase activity (20, 23) and then washed again with 2× SSC. ^{3}H-labeled RNA was then added for a second incubation of 20 h, after which the filter was washed three times at 60 C with 2× SSC and treated with pancreatic RNase as before. The amount of [^{3}H]RNA required to saturate 0.02 μg of DNA was determined before the hybridization-inhibition experiments were performed. Saturating amounts were used in all experiments.

RESULTS

Hybridization of cytoplasmic RNA synthesized in the presence or inhibitors of protein and DNA synthesis. Cytoplasmic RNA synthesized early after infection with adenovirus 2 was hybridized to viral DNA to determine the fraction virus specified. Total cytoplasmic RNA was purified from cultures labeled with [^{3}H]uridine from 2 to 5 h after infection. When annealed to viral DNA, this RNA was found to be 0.8% virus specified (Table 1). The RNA prepared from infections performed in the presence of an inhibitor of DNA synthesis, cytosine arabinoside, also hybridized 0.8% (Table 1). In contrast, RNA from cultures treated with the protein synthesis inhibitor, cycloheximide, hybridized 6%, 7.5-fold more than RNA from the control culture.

Since cycloheximide is known to inhibit the synthesis of 28 and 18*S* rRNA (4, 24), the enhancement of viral RNA in cycloheximide-

treated cultures might be accounted for by the decrease in the synthesis of rRNA. Since at least 65% of early cytoplasmic viral RNA contains poly(A) (9), an increase in viral RNA content caused solely by the reduction of rRNA synthesis should be accompanied by a concurrent increase in binding to oligo(dT)-cellulose. As shown in Table 2, the percentage of poly(A)-containing RNA in CH-treated cultures was 2.7 times higher than the control.

To examine the possibility that cycloheximide treatment alters the virus/cell ratio in the mRNA fraction which contains poly(A), the RNA binding to oligo(dT)-cellulose was hybridized to adenovirus 2 DNA (Table 1). RNA from the control culture annealed 2.2%, and the RNA from a cytosine arabinoside-treated culture hybridized 2.7%. The poly(A)-containing RNA from the cycloheximide-treated culture hybridized to viral DNA 32%.

Early viral RNA sequences synthesized in the presence and absence of cycloheximide. Hybridization-inhibition experiments were performed to determine the relationship between early viral RNA sequences transcribed in the presence and absence of cycloheximide. Such hybridization-inhibition experiments are based on the ability of nonradioactive RNA to inhibit the hybridization of saturating amounts of radioactive viral RNA. Prior to performing the inhibition experiments, the amount of radioactive RNA needed to saturate 0.02 μg of DNA was determined for both types of RNA preparations (Fig. 1A). When RNA from cyclohexi-

TABLE 1. *Hybridization of cytoplasmic RNA synthesized in absence and presence of inhibitors*[a]

Cytoplasmic RNA	Total cytoplasmic RNA				Poly(A)-containing cytoplasmic RNA			
	DNA (μg)	Counts/min hybridized	Hybridized (%)	Calculated % hybridized	DNA (μg)	Counts/min hybridized	Hybridized (%)	Calculated % hybridized
Plus cycloheximide ..	1	14,866	4.3		1	2,867	24.2	
	2	17,221	4.9	6	2	3,477	29.2	32
	4	20,677	5.9		4	3,687	31.2	
Plus cytosine arabinoside	1	922	0.38		1	313	1.96	
	2	1,563	0.65	0.81	2	379	2.41	2.7
	4	1,949	0.78		4	420	2.62	
Control	1	1,071	0.43		1	454	2.0	
	2	1,866	0.74	0.8	2	533	2.2	2.2
	4	1,982	0.79		4	538	2.2	

[a] One hour after infection with adenovirus 2, a culture of KB cells was diluted into three portions (100 ml each): one containing 10 μg of cytosine arabinoside per ml, one containing 25 μg of cycloheximide per ml, and a control with no addition. From 2 to 5 hours after infection the culture was incubated with 12.5 μCi of [^{3}H]uridine per ml. At 5 h, the cytoplasmic RNA was purified, and the poly(A)-containing RNA was separated from the non-poly(A)-containing RNA by oligo(dT)-cellulose chromatography. Samples of total cytoplasmic RNA and poly(A)-containing RNA were hybridized to 1, 2, and 4 μg of adenovirus DNA, and the percentage of the [^{3}H] RNA which was viral in each sample was calculated as described in Materials and Methods.

TABLE 2. *Oligo(dT)-cellulose chromatography of cytoplasmic RNA synthesized in the absence or presence of cycloheximide*[a]

Exp	Control			RNA plus cycloheximide		
	Total counts/min $\times 10^{-5}$	Counts/min bound $\times 10^{-5}$	Binding (%)	Total counts/min $\times 10^{-5}$	Counts/min bound $\times 10^{-5}$	Binding (%)
1	36.7	2.0	5.5	17.2	2.4	13.9
2	125	5.2	4.2	28.1	3.7	13.2
3	159	5.9	3.7	46.8	5.8	12.3

[a] One hour after infection with adenovirus 2, a culture of KB cells was diluted into two portions; 25 μg of cycloheximide per ml was added to one portion. At 2 h after infection, [^{3}H]uridine was added, and at 5 h the cells were harvested. The cytoplasmic RNA was purified and applied to an oligo(dT)-cellulose column as described in Materials and Methods. The total counts per minute recovered from the column and the ^{3}H-counts per minute bound and eluted at low salt were used to calculate the percentage of binding.

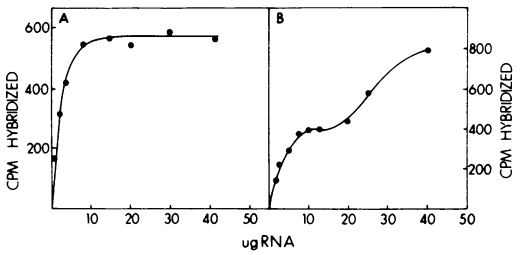

FIG. 1. *Saturation hybridization of cytoplasmic viral RNA synthesized in the presence and absence of cycloheximide. One hour after infection, a 2-liter infection was divided into two portions: 25 μg of cycloheximide per ml was added to one portion. At 2 h after infection, [^{3}H]uridine was added to both cultures. At 5 h, the cultures were harvested and the cycloplasmic RNA purified. Increasing amounts of RNA from cycloheximide-treated (A) and control (B) cultures were hybridized to 0.02 μg of adenovirus DNA.*

mide-treated cultures was utilized, 7.5 to 10 μg of RNA saturated the DNA. The plateau (counts per minute hybridized per microgram of DNA) remained constant when as much as 40 μg of [^{3}H]RNA was added. Using RNA from control cultures harvested 5 h after infection, a plateau of hybridization was reached at about 7 to 10 μg of RNA. However, when more than 20 μg was added, increasing hybridization of [^{3}H]RNA could be detected (Fig. 1B). The additional hybridization with increasing amounts of RNA from control cultures may reflect the presence of low levels of "late" RNA at 5 h after infection, for some cells in the control culture may have already begun to replicate viral DNA by that time.

When radioactive cytoplasmic RNA from control cultures was used in hybridization-inhibition experiments, greater than 90% of the sequences were prevented from hybridizing to adenovirus DNA by unlabeled RNA synthesized either in the absence or presence of cycloheximide (Fig. 2A). In reciprocal experments with [^{3}H]RNA labeled 2 to 5 h after infection in the presence of cycloheximide, the hybridization of early viral RNA was inhibited more than 90% by unlabeled RNA from cells infected either in the absence or presence of cycloheximide (Fig. 2B). In both cases the inhibition curves with the two types of nonradioactive RNA are almost superimposable; complete inhibition was obtained with equal amounts of RNA from the two types of preparations.

Hybridization of nuclear RNAs synthesized early in infection. Nuclear RNA radioactively labeled from 2 to 5 h after infection was hybridized to determine if the relative enhancement of viral RNA found in the cytoplasm was also present in the nucleus. Using oligo(dT)-cellulose the nuclear RNA was separated into fractions containing and lacking poly(A), and then each fraction was hybridized to viral DNA. In the experiment presented in Table 3, poly(A)-containing molecules synthesized in the presence of cycloheximide hybridized 17%, whereas the poly(A)-containing molecules of the control culture hybridized 1.7%. The non-poly(A)-containing molecules hybridized 3.3 and 0.5%, respectively. Thus, an enrichment of viral RNA was found in both the poly(A) and non-poly(A)-containing RNA.

The increased hybridization of non-polyadenylated RNA synthesized in the presence of cycloheximide could be due to the selective inhibition of ribosomal RNA synthesis by the drug. Indeed, the percentage of total nuclear RNA binding to oligo(dT)-cellulose was

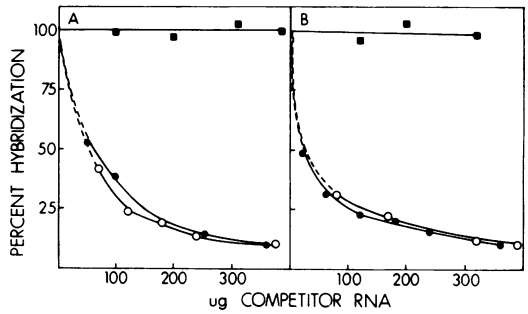

FIG. 2. *Sequence relationships between viral RNAs synthesized in the presence and absence of cycloheximide. One hour after infection, a 300-ml culture of KB cells was divided into two portions; cycloheximide (25 μg/ml) was added to one of the two. [^{3}H]uridine was added to both portions 2 h after infection. At 5 h after infection, the cultures were harvested, and cytoplasmic RNA was purified. As shown in Fig. 1, increasing amounts of each RNA preparation were hybridized to adenovirus 2 DNA to determine the amount needed to reach saturation. In this experiment, 10 μg of RNA from a cycloheximide-treated culture (specific activity of 40,000 counts per min per μg) and 10 μg of RNA from a control culture (specific activity 116,000 counts per min per μg) were required for saturation. A two-step hybridization-inhibition experiment was performed as described in Materials and Methods. The unlabeled RNAs used as inhibitors were purified from whole cell pellets harvested 5 h after infections performed in the presence (●) or absence (○) of cycloheximide. Whole cell RNA from uninfected KB cultures (■) was used as a control. These unlabeled RNAs were tested for the ability to inhibit the hybridization of ^{3}H-labeled RNA synthesized in the presence (A) and absence (B) of cycloheximide.*

TABLE 3. *Hybridization of nuclear RNA synthesized in the presence and absence of cycloheximide*[a]

Nuclear RNA	Non-poly(A)-containing RNA				Poly(A)-containing RNA			
	DNA (μg)	Hybridized (counts/min)	Hybridized (%)	Calculated % hybridize	DNA (μg)	Hybridized (counts/min)	Hybridized (%)	Calculated % hybridized
Control	1	13,500	0.26		1	4,229	1.1	
	2	20,800	0.40	0.5	2	6,208	1.6	1.7
	4	25,000	0.48		4	6,483	1.7	
Plus cycloheximide	1	4,888	2.5		1	24,300	12.7	
	2	5,556	2.9	3.3	2	28,678	15.0	17.3
	4	6,270	3.2		4	31,800	16.7	

[a] A culture of KB cells was diluted into two portions (150 ml each) 1 h after infection with adenovirus 2; 25 μg of cycloheximide per ml was added to one of the cultures. An hour later, 12.5 μCi of [^{3}H]uridine was added per ml, and at 5 h, the cells were harvested and nuclear RNA was purified. The poly(A)-containing RNA was separated from non-poly(A)-containing RNA on oligo(dT)-cellulose as described in Table 1. The poly(A) (+) and poly(A) (−) RNAs were hybridized to viral DNA, and the percentage of the RNA which was viral was calculated as in Table 1 and Materials and Methods.

twofold higher for the RNA labeled in the presence of cycloheximide (Table 4), presumably reflecting the inhibition of synthesis of rRNA which does not bind to oligo(dT)-cellulose.

To eliminate labeled rRNA from [^{3}H]RNA preparations the nucleoli were removed from nuclear preparations prior to the RNA purification. This procedure reduced the amount of labeled rRNA precursors at least 80% (data not shown). Moreover, the percentage of [^{3}H]RNA containing poly(A) increased accordingly (Table 4), from 11.3 to 55% in the cylohexiimide-treated cultures and from 6.7 to 39% in the control cultures. When the non-poly(A)-containing RNA from the nucleoplasm of CH-treated and control cultures was annealed to viral DNA, 4.8 and 0.9% hybridized (Table 5), again demonstrating increased hybridization of RNA synthesized in the presence of cycloheximide.

DISCUSSION

It is well known that inhibition of protein synthesis interferes with viral DNA synthesis and "late" RNA synthesis in many animal virus (18) and bacteriophage systems (17). For example, in the T4 bacteriophage system, inhibition of protein synthesis prevents the synthesis of a class of early RNA sequences, "delayed early" RNAs, which are physical extensions of "immediate early" RNA molecules (3, 17). Furthermore, in this system chloramphenicol treatment interferes with the normal shut-off of host mRNA synthesis (1). In the adenovirus system, inhibitors of protein and DNA synthesis restrict replication and transcription of the adenovirus 2 genome, but the detailed effects of these different classes of inhibitors are not yet known.

The studies presented here demonstrate that cycloheximide, an inhibitor of protein synthesis, has a novel effect on mRNA metabolism in adenovirus 2-infected KB cells. As analyzed by hybridization to viral DNA, the poly(A)-containing RNA synthesized during cycloheximide treatment is enriched about 10-fold in viral sequences. As much as one third of the cytoplasmic poly(A)-containing RNA synthesized during cycloheximide treatment is viral specific as compared to 2 to 3% of the RNA from control-infected cultures. This alteration of mRNA metabolism is not induced by cytosine arabinoside, an inhibitor of DNA synthesis.

Although viral RNA comprises a large portion of the mRNA synthesized in the presence of cycloheximide, hybridization-inhibition experiments (Fig. 2) demonstrated that the same sequences are synthesized in the presence or absence of cycloheximide. Results consistent with the hybridization-inhibition experiments have been obtained by using liquid hybridization of radioactive viral DNA with cytoplasmic or whole cell RNAs prepared from cultures 5 h after infection (E. Craig et al., manuscript in preparation); RNA prepared from cultures infected in the absence or presence of cycloheximide converted the same portion of viral DNA to double-stranded form. Moreover, the kinetics of both types of hybridization studies indicate that viral sequences are present in the same amounts (number of molecules per cell) when the RNA is prepared from cultures infected in the absence or presence of cycloheximide. Therefore, the enrichment of viral RNA must be caused by a relative decrease in the appearance of new poly(A)-containing host RNA in the cytoplasm.

TABLE 4. *Oligo(dT)-cellulose chromatography of nuclear and nucleoplasmic RNA synthesized in the absence or presence of cycloheximide*[a]

RNA	Control			RNA plus cycloheximide		
	Total counts/min × 10^{-5}	Counts/min bound × 10^{-5}	Binding (%)	Total counts/min × 10^{-5}	Counts/min bound × 10^{-5}	Binding (%)
Nuclear	461.2	31.1	6.7	147.1	16.6	11.3
Nucleoplasmic	28.3	11.1	39	10.5	5.8	55

[a] One hour after infection, cultures of KB cells were diluted into two portions, one containing 25 μg of cycloheximide per ml. Two hours after infection, 12.5 μCi of [^{3}H]uridine was added per ml, and at 5 h, the cells were harvested. The total nuclear and nucleoplasmic RNA were purified and applied to an oligo(dT)-cellulose column as described in Materials and Methods. The total counts per minute recovered from the column and the ^{3}H-counts per minute bound and eluted at low salt were used to calculate the percentage of binding.

TABLE 5. *Hybridization of nucleoplasmic RNA synthesized in the presence and absence of cycloheximide*[a]

RNA	Non-poly(A)-containing RNA				Poly(A)-containing RNA			
	DNA (μg)	Hybridized (counts/min)	Hybridized (%)	Calculated % hybridized	DNA (μg)	Hybridized (counts/min)	Hybridized (%)	Calculated % hybridized
Control	1	1,031	0.47		1	1,961	1.5	
	2	1,831	0.82	0.9	2	2,451	1.9	2.3
	4	2,001	0.89		4	2,831	2.2	
Plus cycloheximide	1	1,603	3.6		1	7,155	12.5	
	2	2,110	4.7	4.8	2	9,934	17.4	18.5
	4	2,124	4.8		4	10,453	18.3	

[a] KB cells were infected, treated with cycloheximide, and labeled with [^{3}H]uridine as described in the legend to Table 4. The RNA from the nucleoplasm was extracted and separated into poly(A) (+) and poly(A) (−) RNA on oligo(dT)-cellulose. The two classes of RNA were hybridized, and the percentage of the labeled RNA which was viral was calculated.

Nuclear viral RNA metabolism was studied in order to determine at what level of metabolism the alteration induced by cycloheximide occurs, at the time of transcription, polyadenylation, or transport from nucleus to cytoplasm. The enhancement of viral RNA by cycloheximide was also seen in the nuclear RNA containing poly(A). Although the percentage of hybridization of such nuclear RNA was about 50% lower than that of cytoplasmic RNA, the ratio of hybridization of +CH:−CH RNA was about 10:1 in both cases. If the enhancement of viral RNA were due to the selective inhibition of host mRNA transport from nucleus to cytoplasm, this ratio should be lower. Further experiments (Table 5) demonstrated that the cycloheximide effect cannot be explained by the selective inability of treated cells to polyadenylate host mRNA. When nucleoplasmic RNA lacking poly(A) was hybridized, RNA from the CH-treated cultures hybridized 6.5-fold more than the comparable fraction from untreated cultures.

Since the hybridization-inhibition studies demonstrated that cycloheximide does not cause an increased stability of viral RNA, the simplest explanation for the increased hybridization of RNA synthesized during cycloheximide treatment is a dramatic decrease in the synthesis of host mRNA. Alternatively, there may be a greatly increased instability of host pre-mRNA which prevents accumulation of labeled cellular RNA in cultures labeled for 3 h.

Infection with adenovirus 2 may have a unique effect on cellular metabolism which causes the inhibition of host mRNA synthesis when infected cells are treated with cycloheximide. Alternatively, cycloheximide may have a similar effect on host mRNA synthesis in uninfected cells. Perhaps continued synthesis of certain nuclear proteins, such as the non-acidic chromosomal proteins (19), is required for cellular but not viral RNA synthesis. Since the amount of mRNA synthesis is difficult to monitor in uninfected cells, such a general effect could be detected more easily in infected cells which allow quantitative measurements of viral RNA molecules.

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