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Inhibition of RNA Synthesis in Mouse Myeloma Cells Infected with Vesicular Stomatitis Virus

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Infection of mouse myeloma cells (MPC-11) with vesicular stomatitis (VS) virus resulted in rapid and marked reduction in cellular RNA synthesis considerably before cell viability was compromised. Mouse myeloma cells responded maximally to viral infection at a multiplicity of 1 and were considerably more sensitive to shut-off of RNA synthesis than were mouse L cells or BHK-21 cells. This inhibition of cellular RNA synthesis was shown not to be caused by differential membrane permeability of infected and uninfected MPC-11 cells to [^{3}H]uridine, nor was it due to greater degradation of previously synthesized RNA. VS viral infection appeared not to impede transport of newly synthesized nuclear RNA to the cytoplasm; moreover, infected cells accumulated polyadenylated mRNA at the same rate as did uninfected cells. Polyacrylamide gel electrophoresis of newly synthesized nuclear RNA demonstrated that the polydisperse nature and size distribution were not affected by VS viral infection. Isolated nuclei of infected MPC-11 cells also exhibited greatly impaired capacity to synthesize RNA despite the absence of cytoplasmic factors. Infected-cell cytosol did not inhibit transcription by uninfected-cell nuclei, nor did uninfected-cell cytosol reverse viral inhibition of nuclear transcription. Studies with α-amanitin revealed that VS viral infection inhibited the activity of polymerases I, II, and III, but only polymerase II was affected progressively throughout infection and to a much greater extent. These data suggest that, even at low multiplicities of infection, VS virus rapidly shuts off cellular RNA synthesis at the level of nuclear transcription.

Infection of various cell types with infectious vesicular stomatitis (VS) virus, or possibly defective interfering particles, leads to a relatively rapid and extensive inhibition of host macromolecular synthesis (3, 32, 38) and eventually to cell death (18, 19). This disruption of normal cellular functions can affect synthesis of RNA (13, 37), DNA (39), and protein (17, 23) in the infected cell. Although it is well documented that viral infection shuts off overall cellular RNA synthesis, it remains to be determined which of the multiple steps involved in RNA production are altered during VS viral infection.

The production of functional RNA molecules in the eukaryotic cell involves a number of complex processes that include post-transcriptional events as well as the primary event of transcription (7). Thus, it seems possible that VS viral infection could alter any one or all of the processes that take place during the metabolism of RNA. Viral infection could cause alterations in the rates of synthesis, turnover, processing, and/or transport of RNA from the nucleus to the cytoplasm. Slight alterations in all of these steps of RNA synthesis could result in a significant reduction in the overall production of cellular RNA. Alternatively, shut-off at the level of transcription not only would reduce synthesis of RNA but also could cause concomitant and similar degrees of inhibition of the events that occur after transcription. Studies involving various types of virus and host cell systems have demonstrated that the rate of degradation of RNA remains unaltered after viral infection (6, 15) and that the inhibition of RNA synthesis in vivo possibly occurs at the level of transcription initiation or RNA polymerase activity (1, 22, 27).

Most research on viral switch-off of cellular RNA synthesis has been done with positive-strand picornaviruses (6, 15, 22, 27), the transcriptional and translational strategy of which is quite different from that of negative-strand RNA viruses (31). The studies reported here are the first in a series designed to examine in detail the effect of VS viral infection on the cellular processes involved in RNA synthesis. Mouse myeloma cells were chosen for these studies because they provided a well-characterized model system for analyzing specific RNAs and

proteins (20, 21) and because of exquisite susceptibility to the effects of VS virus (23); different cell types vary considerably in their response to the effects of VS virus on cellular macromolecular synthesis (3, 32). Since VS virus can undergo complete replication and maturation cycles in enucleated cells (9), it was also essential to measure RNA synthesis in isolated nuclei of infected cells in both the presence and absence of cytoplasmic components.

MATERIALS AND METHODS

Chemicals and radioisotopes. [^{3}H]GTP (13.2 Ci/mol) was purchased from New England Nuclear Corp., Boston, Mass. [^{3}H]uridine (26 Ci/mmol) and Nuclear-Chicago solubilizer were obtained from Amersham/Searle, Arlington Heights, Ill. ATP, CTP, UTP, and dithiothreitol were purchased from Calbiochem, San Diego, Calif. Electrophoresis-grade agarose, acrylamide, *N,N'*-methylenebisacrylamide, and sodium dodecyl sulfate (SDS) were provided by Bio-Rad Laboratories, Richmond, Calif. Nonidet-40 (NP-40) is a product of Shell Oil Co. *N,N,N',N'*-tetramethylethylenediamine was purchased from Eastman Kodak, Rochester, N.Y.

Cells, media, and virus. Mouse myeloma cells, MPC-11, were obtained by W. M. Kuehl of our department from the American Type Culture Collection, Rockville, Md. These myeloma cells secrete immunoglobulin heavy and light chains. The cells were grown in Dulbecco modified Eagle medium, 10% heat-inactivated horse serum, and nonessential amino acids, all obtained from Grand Island Biological Co., Grand Island, N.Y. Comparative studies were done with L cells and BHK-21 cells, as previously described (17). Antibiotics were routinely omitted from all media since these might alter normal cellular RNA synthesis.

The VS virus, strain San Juan (Indiana serotype), used in these experiments was originally obtained from the U.S. Agricultural Research Center, Beltsville, Md. (33). Clones of this virus were selected from plaques picked from L-cell monolayers, and virus stocks were prepared by growth on BHK-21 cells. The resulting virus preparations were titrated by assay of PFU on monolayers of L cells.

Procedure for isotopic labeling of RNA in infected and uninfected cells. MPC-11 cells maintained at 37°C in spinner cultures suspended in Dulbecco modified Eagle medium (without serum) were used in most experiments; several comparative studies with L cells and BHK-21 cells were performed under comparable conditions. Cells were infected with VS virus (or mock infected) by exposing 1-ml volumes of 1×10^7 to 5×10^7 cells to virus at various multiplicities of infection (MOI), but usually at an MOI of 1 PFU/cell. The virus was allowed to adsorb for 30 min at room temperature, after which the cells were washed and maintained at 37°C in 10-ml suspension cultures by agitation with small magnetic stirring bars.

The pulse-labeling of myeloma cells was achieved by removing 0.5-ml portions of cells from spinner cultures and adding these to 0.5 ml of warm medium containing 4 μCi of [^{3}H]uridine (specific activity, 26 Ci/mmol) per ml. Preliminary experiments demonstrated that this final concentration of uridine (2 μCi/ml) was saturating under the conditions used in these experiments. In those experiments requiring continuous labeling, cells were pelleted by centrifugation, resuspended in media containing 2 μCi of [^{3}H]uridine per ml, and returned to suspension cultures. After incorporation of [^{3}H]uridine, cells were washed once in 2 ml of ice-cold medium, resuspended in 0.5 ml of ice-cold reticulocyte standard buffer (RSB), and precipitated by the addition of 0.5 ml of 10% trichloroacetic acid. After at least 10 min on ice, the acid-insoluble material was pelleted by centrifugation at $800 \times g$ for 5 min. When acid-soluble radioactivity was measured, 0.2-ml samples of the supernatant solution were withdrawn for measurement of radioactivity. The resulting precipitate was washed twice with 0.5 ml of ice-cold 5% trichloroacetic acid and solubilized in 0.5 ml of Nuclear-Chicago tissue solubilizer, and 0.2-ml samples were taken for the determination of radioactivity. All samples were counted in a Beckman LS-230 liquid scintillation system with an efficiency of 40% for tritium.

Cell fractionation and RNA extraction. The fractionation of cells into nuclear and cytoplasmic components was achieved by NP-40 lysis (25). After incubation, cells were resuspended in 3 ml of ice-cold RSB, to which were added 0.1 ml of sodium heparin (1,000 U/ml), 0.1 ml of 10 mM spermidine, and 1 ml of 1% NP-40. Cells were lysed by gently shaking the test tubes, and nuclei were pelleted by centrifugation at $800 \times g$ for 5 min at 4°C. The resulting supernatant was carefully decanted and either precipitated by the addition of an equal volume of cold 10% trichloroacetic acid or extracted with phenol and chloroform. The resulting nuclear pellet was resuspended in RSB and precipitated with 10% trichloroacetic acid or subjected to phenol-chloroform extraction after the nuclear pellet was resuspended in 4 ml of extraction buffer containing 10 mM sodium acetate (pH 5.1) and 30 mM NaCl. Ten percent SDS was added to each solution to give a final concentration of 1%, and the RNA was isolated by two extractions with phenol and chloroform at 60°C, followed by two additional extractions at 22°C as described by Penman (25). After extraction, the aqueous phase was removed, NaCl was added to a final concentration of 0.5 M, and the RNA was precipitated in 2 volumes of 95% ethanol in the presence of rRNA as carrier at −20°C overnight. After LiCl treatment, as described by Baltimore and Girard (2), to remove DNA, the RNA was precipitated an additional time in ethanol before its analysis by polyacrylamide gel electrophoresis. Protein concentrations were determined by the method of Lowry et al. (16) and DNA by the colorimetric assay described by Burton (4).

Polyacrylamide gel electrophoresis was used to analyze cellular RNA solubilized in sample buffer containing 35 mM Tris (pH 7.6), 30 mM sodium phosphate, 0.2% SDS, 1 mM EDTA, and 10% sucrose. Bromophenol blue tracking dye and [^{14}C]rRNA markers were added, and the sample was heated for 30 s at 100°C. The RNA was cooled quickly in an ice bath and analyzed on 10-cm cylindrical gels containing 2% acrylamide, 0.1% *N,N'*-methylenebisacrylamide, 1% agarose, 35 mM Tris (pH 7.6), 30 mM sodium

phosphate, 1 mM EDTA, 0.075% ammonium persulfate, and 0.075% *N,N,N',N'*-tetramethylethylenediamine. Electrophoresis was performed at 5 mA/gel for 3 h until the tracking dye was at the bottom of the gel. Gels were sectioned into 1-mm slices on a Mickle gel slicer and solubilized in 0.5 ml of Nuclear-Chicago tissue solubilizer, and radioactivity was measured in 10 ml of toluene-based liquid scintillation fluid.

Measurement of RNA synthesis in isolated nuclei and nucleoli. Nuclei were isolated from infected or uninfected MPC-11 cells according to the technique of Schwartz et al. (27), resulting in a yield of 80 to 90% intact nuclei as measured with a hemocytometer. Nuclei were prepared freshly for each experiment and were used immediately after isolation. Nuclear pellets were gently resuspended in ice-cold 25% glycerol containing 5 mM magnesium acetate, 50 mM Tris (pH 8), 5 mM dithiothreitol, and 0.1 mM EDTA. The conditions used for RNA synthesis were those described by Marzluff et al. (20) for isolated myeloma cell nuclei. RNA synthesis was measured in reaction mixtures (100 μl) containing 12.5% glycerol, 25 mM Tris (pH 8), 0.05 mM EDTA, 5 mM magnesium acetate, 1 mM $MnCl_2$, 0.15 M KCl, 2.5 mM dithiothreitol, 0.4 mM each of ATP, CTP, and UTP, and 1 μCi of [^{3}H]GTP (13.2 Ci/mmol). The reaction mixtures routinely contained 60 to 80 μg of DNA and were incubated in a water bath at 25°C. After incubation, 0.1 ml of ice-cold RSB was added to the reactions along with 1 to 2 mg of bovine serum albumin as carrier, and the entire reaction was precipitated with an equal volume of 10% trichloroacetic acid. The measurement of the amount of radioactive precursor incorporated into acid-insoluble material was determined as described above. When the amount of α-amanitin-sensitive polymerase activity in isolated nuclei was measured, the toxin was added at a concentration of 10 μg per ml of reaction mixture. Preliminary experiments demonstrated that this concentration inhibited 40 to 50% of total nuclear activity, and polymerase II activity was calculated as [(nuclear activity without α-amanitin) − (nuclear activity with α-amanitin)].

Nucleoli were prepared from isolated nuclei according to the procedure of Schwartz et al. (28). After the isolation of nuclei, as described above, the nuclear pellet was resuspended in 1 ml of 0.34 M sucrose and sonically treated for 15-s periods with a Branson Sonicator model W140D with a microtip at a setting of 4. A total sonic treatment time of 30 s resulted in a complete disruption of nuclei. The resulting solution was underlaid with 4 ml of 0.9 M sucrose and centrifuged at 2,000 × *g* for 10 min. The resulting nucleolar pellet was resuspended in ice-cold 25% glycerol containing 5 mM magnesium acetate, 50 mM Tris (pH 8), 5 mM dithiothreitol, and 0.1 mM EDTA. Nucleolar polymerase activity was assayed as described above for isolated nuclei and was found to be completely insensitive to α-amanitin at a concentration of 10 μg/ml.

Preparation of cytosol. Cytosol from MPC-11 cells was prepared as previously described by Weck and Johnson (34). Cells were removed from growth media by centrifugation at 800 × *g*, resuspended in 5 ml of ice-cold RSB, and homogenized in a tight-fitting Dounce homogenizer. The homogenate was centrifuged at 2,000 × *g* for 15 min, and the resulting supernatant was subjected to centrifugation at 100,000 × *g* for 60 min at 4°C. The soluble cytoplasm was dialyzed overnight against a solution containing 10 mM Tris-hydrochloride (pH 7.4), 10 mM KCl, 1.5 mM $MgCl_2$, and 2 mM 2-mercaptoethanol. When cytosol was tested for its effect on isolated nuclei, it was routinely added at a concentration of 100 μg of protein per ml of reaction mixture.

RESULTS

Parameters of VS viral inhibition of cellular RNA synthesis. Initial experiments were performed to determine the optimal conditions under which VS virus shuts off cellular RNA synthesis. MPC-11 cells were found to be most susceptible to viral infection and were used as the model system for most experiments. To characterize the rate and extent of RNA synthesis inhibition, spinner cultures of MPC-11 cells infected with VS virus (MOI ≃ 1) were compared with uninfected MPC-11 cells by labeling with [^{3}H]uridine for 15 min at hourly intervals. Similar experiments were performed with suspension cultures of L cells and BHK-21 cells. Total amounts of [^{3}H]uridine incorporated into trichloroacetic acid-precipitable RNA were measured as described in Materials and Methods.

Infection of myeloma cells with VS virus resulted in progressive diminution in capacity to synthesize RNA; by 4 h after infection, the RNA-synthesizing level of infected cells was <10% that of uninfected cells (Fig. 1A). The exact amount of viral RNA synthesized at this time was not determined, but represents only a limited portion of the total intracellular RNA; much of the newly synthesized viral RNA was released from infected cells in the form of virions. This rapid reduction in host cell RNA synthesis correlated well with production of infectious VS virions, which also reached a maximal level in the MPC-11 cell system at 4 h after infection (data not shown).

Figure 1B compares the effects of VS virus on inhibition of RNA synthesis in MPC-11, L, and BHK-21 cells. As noted, infection of L cells and BHK cells resulted in shut-off of RNA synthesis that was much less rapid and less extensive than the effect on MPC-11 cells; maximal reductions in RNA synthesis were 75% for BHK cells and 63% for L cells, compared with >90% for MPC-11 cells. Differential susceptibility among cell types to the effect of VS virus on cellular macromolecular synthesis has been reported by Baxt and Bablanian (3). Subsequent experiments in this series were carried out with the more susceptible MPC-11 cells.

MOI is another factor that has been shown to influence the degree to which cell macromo-

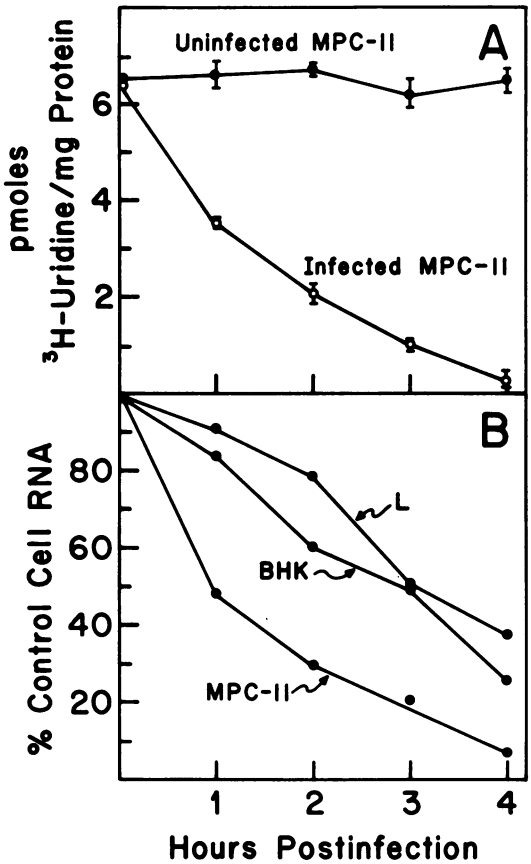

FIG. 1. *Effect of VS viral infection on RNA synthesis in MPC-11 cells, mouse L cells, and BHK-21 cells. After adsorption of virus (MOI ≃ 1) or mock infection, samples of uninfected and infected cells were pulse-labeled for 15 min with [³H]uridine (2 μCi/ml) at hourly intervals after incubation at 37°C. Cells were harvested after labeling, and trichloroacetic acid-precipitable radioactivity was determined as described in Materials and Methods. (A) Average RNA synthesis in duplicate cultures of uninfected and infected MPC-11 cells (vertical bars represent range of values). (B) Comparative effect of VS viral infection on [³H]uridine incorporation by MPC-11, L, and BHK-21 cells plotted as the percentage of incorporation of [³H]uridine by control uninfected cultures of each cell type.*

lecular synthesis is shut off (13, 37). To examine the relationship between input multiplicity and inhibition of cellular RNA synthesis, MPC-11 cells were infected with different amounts of virus and pulse-labeled with [³H]uridine at hourly intervals after infection. Figure 2A shows no significant difference in inhibition of RNA synthesis in cells infected at multiplicities of 1 or 50. On the other hand, an MOI of 0.1 resulted in no significant reduction in cellular RNA synthesis; this result is not surprising, because not more than 10% of host cells could be infected at this multiplicity. However, it is necessary to assume that VS virus particles unable to form plaques can also shut off cellular RNA synthesis in order to account for the similar effects at multiplicities of 1 and 50. If only plaque-forming VS virus is effective, the Poisson distribution would dictate that RNA synthesis can be inhibited in only 63% of cells exposed at MOI ≃ 1.

A possible explanation for rapid shut-off of cellular RNA synthesis is early death of cells after exposure to VS virus. The cell-killing potential of VS virus and its relationship to viral functions and compromised cell functions have

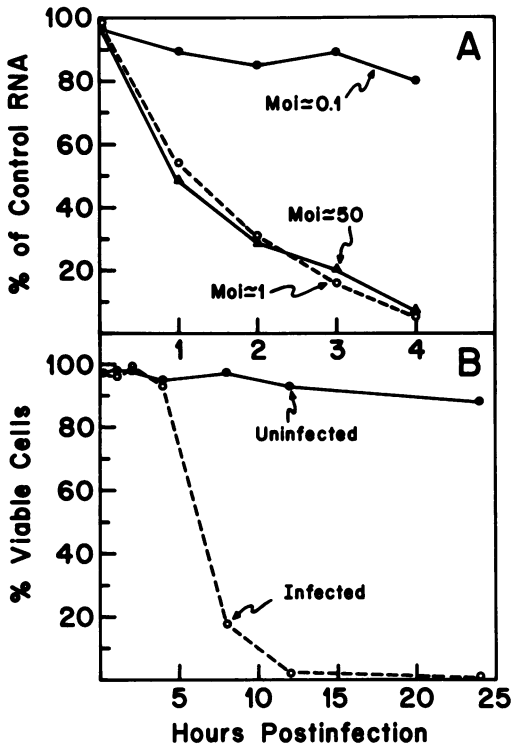

FIG. 2. *Comparative effect of VS virus on (A) RNA synthesis in MPC-11 cells infected at different MOI and (B) viability of uninfected MPC-11 cells and infected MPC-11 cells (MOI ≃ 1) as determined by trypan blue dye exclusion at intervals after incubation at 37°C. RNA synthesis was measured by pulse-labeling cells for 15 min at hourly intervals and was plotted as the percentage of incorporation of [³H]-uridine into trichloroacetic acid-precipitable material in cells infected at MOI of ~0.1, ~1.0, and ~50 as compared with comparable uninfected cells. Cell viability was determined by exclusion of trypan blue by cells examined in a hemocytometer and scored as percentage of viable cells; the data represent the average of two independent experiments in which each determination was made in triplicate.*

been extensively investigated by Marcus (18, 19). To rule out the possibility that inhibition of RNA synthesis was only secondary to cell killing, the viability of cells infected with VS virus (MOI ≃ 1) was tested by their capacity to exclude the vital dye trypan blue at various times after infection. Figure 2B demonstrates that ~95% of infected cells retained their ability to exclude trypan blue up to 4 h postinfection, a time at which >90% of cell RNA synthesis was already shut off. At later times after infection the number of viable cells was drastically reduced, and almost total cell destruction was evident by 24 h. In contrast, most (90%) uninfected control MPC-11 cells survived. These data suggest that RNA synthesis is not shut off simply as a secondary consequence of cell killing by VS virus.

Effect of VS viral infection on cellular uptake of [^{3}H]uridine. The question arose of whether the decreased rates of cellular RNA synthesis after VS viral infection could be due to altered permeability of cells to [^{3}H]uridine, as was reported by Genty (10). To determine whether VS viral infection interfered with [^{3}H]-uridine transport across the cell membrane, acid-soluble and acid-insoluble radioactivity was measured in MPC-11 cells at various times after infection, as well as in uninfected cells. The amounts of acid-soluble radioactivity found in infected cells was actually slightly higher than that in uninfected cells, despite a two- to three-fold reduction in acid-insoluble radioactivity in the infected cells (Table 1). More significantly, when calculation of the amount of [^{3}H]uridine in acid-insoluble RNA products was corrected for total cellular radioactivity (soluble plus insoluble), the activity present in uninfected cells remained at least twofold greater than that in infected cells at 2 h postinfection (Table 1). These results provide assurance that reduced incorporation of [^{3}H]uridine in infected MPC-11 cells was due to a decrease in the actual rate of RNA synthesis and was not the result of decrease in membrane transport of uridine. Comparable results were also obtained when endogenous UTP pools were first depleted by incubating cells in glucosamine, as described below, before being labeled with [^{3}H]uridine (data not shown).

TABLE 1. *Comparative uptake and incorporation into RNA of [^{3}H]uridine in uninfected MPC-11 cells and MPC-11 cells 2 h after infection with VS virus*[a]

MPC-11 cells	Time after [^{3}H]uridine pulse (min)	Acid-soluble radioactivity (pmol/mg of protein)	Acid-insoluble radioactivity (pmol/mg of protein)	Acid-insoluble/total radioactivity[b]
Uninfected	10	8.1	3.9	0.32
	20	11.7	7.6	0.39
	40	13.6	12.4	0.48
Infected	10	9.2	1.3	0.12
	20	14.9	2.8	0.16
	40	19.2	4.8	0.20

[a] After mock infection or infection with VS virus (MOI ≃ 1) for 2 h at 37°C, cells were labeled continuously for 40 min with [^{3}H]uridine. Suspended cell samples removed at intervals after the [^{3}H]uridine pulse were assayed for their content of trichloroacetic acid-soluble and TCA-insoluble radioactivity as described in Materials and Methods.

[b] Corrected values to demonstrate the proportions of [^{3}H]uridine incorporated into RNA based on the total level of radioactivity present in the cells at each time.

RNA stability in MPC-11 cells infected with VS virus. Measurements of RNA synthesis really represent the net rates of RNA transcription and degradation. If VS viral infection enhances degradation of rapidly synthesized cellular RNA, normal rates of [^{3}H]uridine incorporation would appear to be reduced. Rates of [^{3}H]RNA degradation in prelabeled infected and uninfected MPC-11 cells were measured by depleting the endogenous uridine pool to halt further incorporation of [^{3}H]uridine (36). Such a technique circumvents any adverse effects that an inhibitor of RNA synthesis, such as actinomycin D, might have on RNA processing (11, 24). Under the conditions used, measured rates of RNA degradation should not be influenced by promoters or inhibitors of RNA synthesis.

To study degradation of RNA, the technique described by Wertz (36) was used to deplete MPC-11 cells of endogenous unlabeled UTP before pulse-labeling with [^{3}H]uridine. After infection, cells were incubated in complete medium containing 20 mM glucosamine for 1 h before pulse-labeling. The cells were pelleted by centrifugation and resuspended in medium containing [^{3}H]uridine (2 μCi/ml) and incubated at 37°C. After pulse-labeling, the cells were washed and resuspended in medium containing 20 mM glucosamine and 100 μg of unlabeled uridine per ml. At various times, portions of cells were removed and the amount of radioactivity in acid-precipitable material was determined.

As expected, MPC-11 cells previously infected with VS virus incorporated less [^{3}H]uridine than did uninfected cells (Fig. 3). After the chase period and [^{3}H]uridine depletion with glucosamine (time zero), the levels of acid-precipitable RNA plateaued and then declined in both uninfected and infected cells. Degradation of RNA was somewhat greater in infected cells; 74% of the RNA remained at 60 min after chase, com-

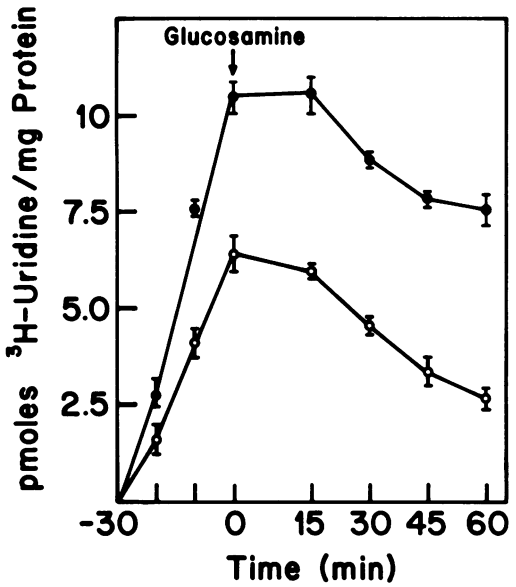

FIG. 3. *Degradation of RNA in uninfected (●) and VS virus-infected (○) MPC-11 cells. At 1 h after infection (MOI ≃ 1), cells were pelleted by centrifugation at 800 × g, resuspended in medium containing 20 mM glucosamine, and reincubated for an additional hour. After this incubation period, the cells were labeled for 30 min with [³H]uridine (2 μCi/ml), washed, and resuspended in fresh medium containing 20 mM glucosamine and unlabeled uridine (arrow, time zero); incubation was continued for an additional hour. The amount of acid-insoluble radioactivity was determined at intervals after labeling and before the chase (−30 to 0 min) and after the chase (0 to 60 min).*

pared with 52% of uninfected cells. However, this slight difference in degree of degradation is insufficient to account for the markedly reduced rate of RNA synthesis in infected cells.

Effect of VS virus on transport of nuclear RNA to cytoplasm. Although transcription is the primary event in the production of RNA, it has become evident that post-transcriptional events such as processing, polyadenylation, and transport of RNA across the nuclear membrane also play an important role (7). To determine whether infection with VS virus alters such events, uninfected and infected cells (2 h post-infection) were incubated in medium containing [³H]uridine; at various intervals thereafter, samples of cells were removed and fractionated, and the amount of acid-insoluble radioactivity in the cytoplasm was determined. If viral infection inhibits RNA transport, then one would expect to find lesser amounts of [³H]RNA appearing in the cytoplasm of infected cells than the differences detected in the total incorporation of the radioactive precursor (see Table 1).

The data recorded in Table 2 reveal rapid accumulation of RNA in the cytoplasm of uninfected cells and a two- to threefold decrease in the amounts of RNA in the cytoplasm of infected cells. This decrease was quite similar to that measured for the total incorporation of [³H]uridine into unfractionated cells infected with VS virus. This experiment indicates that the rate of transport of RNA from the nucleus to the cytoplasm in infected cells is not altered by viral infection. Therefore, the observed reduction in the amounts of [³H]RNA accumulating in the cytoplasm must be due to the inhibition of cellular RNA synthesis. No significant difference could be detected in the proportions of cytoplasmic mRNA that was polyadenylated in infected compared with uninfected cells (Table 2). This observation agrees with the data obtained in other virus-cell systems in which viral infection did not enhance the degradation or size of host polyadenylic acid-containing mRNA (6, 14).

Characterization of RNA products synthesized in infected and uninfected cells. Since VS virus shuts off RNA synthesis so rapidly, it was of interest to compare the nature and size distribution of the RNA products made in the two cell populations. Electrophoresis on polyacrylamide-agarose gels was used to examine the rapidly synthesized nuclear RNA of VS virus-infected and uninfected cells at 2 h post-infection. [³H]RNA was extracted from nuclei isolated after a 15-min [³H]uridine pulse of intact

TABLE 2. *Cytoplasmic accumulation of total and polyadenylated RNA in uninfected MPC-11 cells and MPC-11 cells 2 h after infection with VS virus*[a]

Time after [³H]uridine addition (min)	Total cytoplasmic [³H]RNA (pmol/mg of protein		Poly(A)[b] RNA in cytoplasm (% of total RNA)	
	Uninfected	Infected	Unifected	Infected
15	0.6	0.2	6.2	6.0
30	4.5	2.2	11.7	12.0
60	10.1	3.4	10.4	13.0
120	23.8	12.0	9.7	8.8

[a] After mock infection or infection with VS virus (MOI ≃ 1) for 2 h at 37°C, MPC-11 cells were incubated for 120 min in medium containing 2 μCi of [³H]uridine per ml. At times indicated, samples of cells were removed and separated into cytoplasmic and nuclear fractions. The resulting cytoplasmic fractions were divided and either precipitated with 10% trichloroacetic acid to measure total radioactivity or extracted with phenol and chromatographed on an oligodeoxythymidine column to determine the proportion of RNA that was polyadenylated (see Materials and Methods and reference 17).

[b] Polyadenylic acid.

myeloma cells, a pulse time during which primarily heterogeneous nuclear RNA is labeled (30). Analysis of the RNA from the two different cell populations revealed that there was no difference in the polydisperse nature of the nuclear RNA (Fig. 4).

Linear sucrose-SDS gradient analysis and the examination of RNA synthesized by cells at later times postinfection yielded similar results (data not shown). Thus, the observed shut-off of RNA synthesis by VS virus is not accompanied by a shift in the size distribution or heterogeneous nature of the nuclear RNA products.

RNA synthesis in isolated nuclei and the effect of cytoplasm from infected and uninfected MPC-11 cells. A more direct measurement of nuclear polymerase activity can be achieved by assaying the activity of isolated nuclei (21, 29). When nuclei were isolated from MPC-11 cells at 2 h postinfection and tested in vitro for incorporation of [^{3}H]GTP, there was an approximate 50% decline in the rate of RNA synthesis by nuclei from infected cells (Fig. 5B) compared with that of uninfected cells (Fig. 5A). This measured decrease in the RNA polymerase activity of nuclei was quite similar to that detected for total incorporation of [^{3}H]uridine by intact cells (see Table 1). These results demonstrate that isolated nuclei reflect what is occurring in intact myeloma cells during viral infection.

Factors in the cytoplasm of the eukaryotic cell may cause significant alterations in the activity of isolated nuclei (34), and a cellular inhibitor of host RNA synthesis has been isolated from cells after poliovirus infection (12). To determine whether such a factor exists in the cytoplasm of MPC-11 cells infected with VS virus,

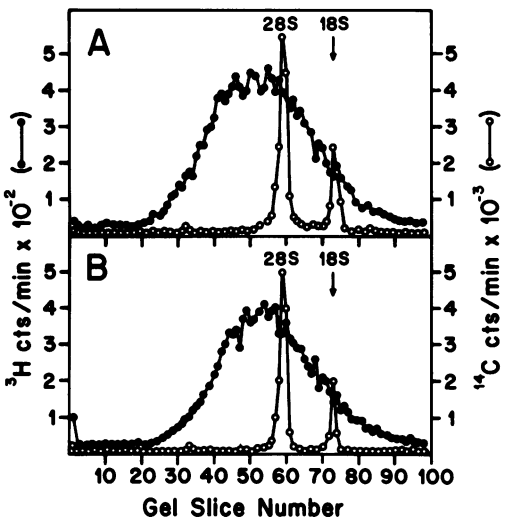

FIG. 4. *Electropherograms of nuclear RNA synthesized by (A) uninfected MPC-11 cells and (B) cells infected with VS virus at MOI ≃ 1. At 2 h postinfection, cells were labeled for 15 min with [^{3}H]uridine and fractionated, and the nuclear RNA was phenol-chloroform extracted as described in Materials and Methods. After ethanol and LiCl precipitation, the RNA samples were heated for 30 s at 100°C, cooled in an ice bath, and subjected to polyacrylamide gel electrophoresis. [^{14}C]rRNA markers were included in the samples; bromophenol blue was used as a dye marker. The samples contained 16,650 and 18,950 cpm in uninfected- and infected-cell nuclear RNA, respectively.*

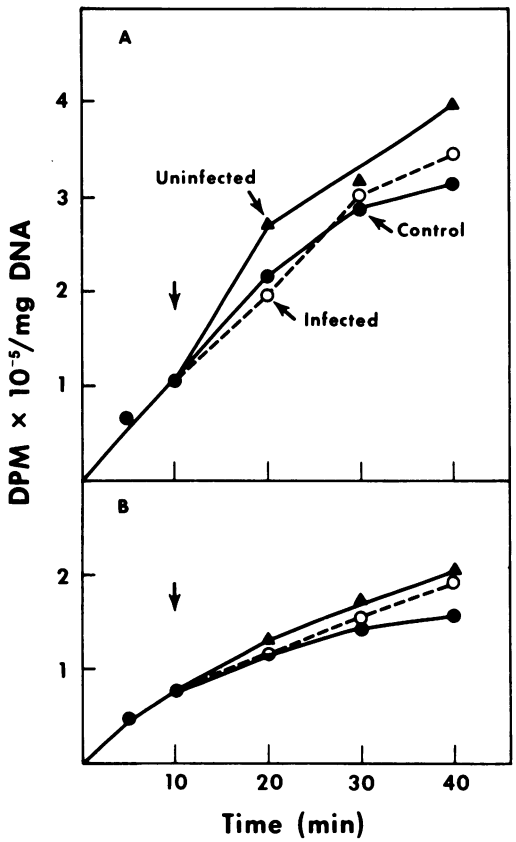

FIG. 5. *Effect of cytosol from infected or uninfected MPC-11 cells on synthesis of RNA by isolated nuclei of (A) uninfected MPC-11 cells and (B) MPC-11 cells infected with VS virus (MOI ≃ 10). Two hours postinfection, nuclei were isolated, and RNA synthesis was measured by incorporation of [^{3}H]GTP as described in Materials and Methods. Cytosol from either infected (○) or uninfected (▲) MPC-11 cells was prepared at 2 h after infection and was added to a final concentration of 100 μg of protein per ml of reaction mixture at 10 min (arrow). Control reactions (●) received a comparable volume of the buffer in which the cytosol was prepared. The amounts of [^{3}H]GTP incorporated into acid-insoluble material were determined at the times indicated.*

soluble cytoplasmic preparations were added to isolated nuclei from either infected or uninfected MPC-11 cells (Fig. 5). Cytosol from these two cell populations had no significant effect on incorporation of [^{3}H]GTP when added to isolated nuclei. Although uninfected cell cytosol may have slightly enhanced incorporation, there was definitely no inhibition of RNA synthesis in nuclei incubated in the presence of infected-cell cytosol (Fig. 5). Varying the amount of cytoplasmic protein added to the reaction mixtures also had no effect on nuclear activity. It appears that although VS virus replicates in the cytoplasm of the cell, no cytoplasmic inhibitor of nuclear transcription can be detected.

Loss of nuclear and nucleolar polymerase activities after VS viral infection. The toxin α-amanitin, which specifically inhibits RNA polymerase II (35), has been used extensively to demonstrate the existence of various RNA polymerases in the nuclei of eukaryotic cells. As reported by Weinmann and Roeder (35), approximately 40 to 50% of the endogenous polymerase activity in myeloma cell nuclei is sensitive to α-amanitin at a concentration of 10 μg/ml (see legend to Fig. 6). This concentration of α-amanitin inhibits only polymerase II and therefore allows the determination of the activity of different polymerases at various times after infection.

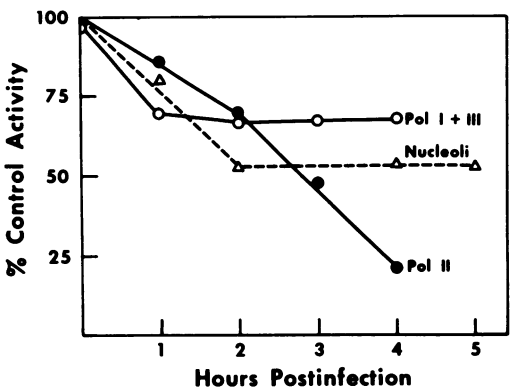

FIG. 6. *α-Amanitin-sensitive polymerase activities in isolated nuclei and nucleoli from MPC-11 cells infected with VS virus (MOI ≃ 1). At hourly intervals postinfection, nuclei or nucleoli were isolated from uninfected and infected cells, and RNA synthesis was measured for 15 min in standard reaction mixtures with or without α-amanitin (10 μg/ml), as described in Materials and Methods. The amounts of [^{3}H]GTP incorporated into acid-insoluble material were determined, and the results are depicted as the percentage of uninfected-cell nuclear polymerase activities that are sensitive (●, polymerase II) or insensitive (○, polymerase I and III) to the toxin. Nucleolar activity (△) is expressed as the percentage of activity of nucleoli isolated from uninfected cells at the various times indicated. Total nuclear activity before infection (time zero) was 4.4 × 10^{-5} dpm per mg of DNA in the absence of α-amanitin and 2.4 × 10^{-5} dpm per mg of DNA in its presence. [^{3}H]GTP incorporation by isolated nucleoli at time zero was 4.16 × 10^{-5} dpm per mg of DNA and was totally insensitive to α-amanitin.*

Figure 6 compares the effect of VS viral infection on the individual polymerase activities of MPC-11 cells as determined by relative sensitivity to α-amanitin. As noted, there was a rapid loss of about 30% of combined activities of polymerases I and III by 1 h postinfection. However, inhibition of enzymatic activity by viral infection did not progress further after this initial loss. In contrast, the activity of polymerase II declined steadily over a period of 4 h to a level approximately 20% that of the uninfected control cells (Fig. 6). This finding clearly indicates that VS viral infection exerts a more drastic reduction in the synthesis of RNA catalyzed by polymerase II than by polymerases I and III.

Another means of assaying polymerase I activity is by isolating nucleoli from cells at various times after infection and testing their activity in vitro. Since isolated nucleoli contain only polymerase I (35), this procedure provides a means of measuring a specific enzyme activity in the absence of nucleoplasmic influences. Similar to the results obtained with α-amanitin and intact nuclei, the polymerase I activity dropped sharply at first and then plateaued for the remainder of the infection (Fig. 6). Even when examined at 5 h postinfection, <50% of nucleolar polymerase I activity was inhibited.

This differential inhibition of the nuclear and nucleolar polymerases still supports the evidence that VS virus shuts off cellular RNA synthesis primarily at the level of transcription.

DISCUSSION

Attempts to elucidate the mechanism(s) responsible for viral shut-off of host nucleic acid synthesis have resulted in numerous reports on the subject, but there still remains little agreement on how such a shut-off might occur. Since cellular RNA metabolism involves a number of complex events (7), it has been difficult to determine whether one particular step or all steps involved in the production of RNA are inhibited by viral infection. The question has also been complicated by the fact that the outcome of virus-cell interactions is dependent on the type of virus-host system studied (3). Similar differences in susceptibility to viral infection are shown for MPC-11, mouse L, and BHK-21 cells described in this report.

One possible effect of viral infection is an alteration in the permeability of the cell membrane (5). In chicken embryo cells, VS virus can

inhibit the permeability of the cells to uridine, resulting in a decreased uptake of the RNA precursor and, consequently, significant reduction in the apparent rate of synthesis of RNA (10). This loss of the ability to transport uridine was reported to increase exponentially after infection, and therefore it was deemed to be the primary cause for the reduced incorporation of uridine into RNA in these infected cells. In contrast to these findings, infection of myeloma cells with VS virus did not result in a decline in the ability of cells to take up [^{3}H]uridine, but rather caused a slight increase in the amounts of intracellular soluble radioactivity (see Table 1). Although it is possible that viral infection might alter the specific activity of the soluble precursor pool, this was most likely not the case in myeloma cells, since experiments in which glucosamine was used to deplete the endogenous pools of UTP yielded results essentially identical to those shown in Table 1. Even though uridine transport was not altered in myeloma cells, it still remains possible that VS viral infection can distort the concentration of monovalent ions inside the cell as suggested by Carrasco (5).

Recent evidence from quite different virus-cell systems has demonstrated that viral infection does not alter either the stability of nuclear RNA (27) or the rate of degradation of cellular polyadenylated mRNA (6). The present data agree with these observations. When RNA stability was measured in VS virus-infected and uninfected myeloma cells, there was only a small difference in the degree of degradation of previously synthesized RNA in the two cell populations. Thus, the liberation of lysosomal enzymes causing increased degradation of nucleic acids (15) does not appear to be the principal explanation for the decreased synthesis of RNA in myeloma cells infected with VS virus. In addition to these small differences in the rates of degradation (Fig. 3), polyacrylamide gel electrophoresis of nuclear RNA demonstrated that the size distribution of the RNA products remained unaltered (Fig. 4). These results indicate that viral infection does not cause a shift in size to lower-molecular-weight RNA products, which would be expected if RNA degradation were enhanced. These observations with VS virus are in close agreement with those of Colby et al. (6), who showed that the integrity of host mRNA was maintained after infection of L cells with mengovirus.

A number of important post-transcriptional events take place before the exit of RNA molecules from the nucleus to the cytoplasm of the cell (7, 30). If shut-off by virus of cellular RNA synthesis also resulted in a change in the processing of RNA, this alteration should be reflected by a change in the rate of accumulation of RNA in the cytoplasm of the cell. However, when such measurements were made in VS virus-infected and uninfected mouse myeloma cells, there was no significant decrease in the relative proportions of RNA transported from the nucleus to the cytoplasm (Table 2). In addition, oligodeoxythymidine-cellulose separation of cytoplasmic RNA extracted from infected and uninfected cells revealed that the proportion of polyadenylated RNA was the same in both cell populations (Table 2). This observation reveals that the post-transcriptional polyadenylation of RNA is not disturbed for at least 3 h after infection, a result which is similar to that obtained in other virus-cell systems (6, 14). The fact that transport and polyadenylation of nuclear RNA products are unaffected does not rule out the possibility that other key events, such as methylation and capping of the 5′ end of the RNA molecule (7, 8), may be disturbed after viral infection.

The use of nuclei isolated from cells after infection with VS virus allowed a direct measurement of nuclear RNA polymerase activities in either the presence or absence of cytoplasmic factors. Isolated nuclei have been shown to transcribe r- and tRNA genes and have provided a means for studying the major polymerases of the eukaryotic cell (26, 35). The use of such a system circumvents the problems associated with intracellular soluble precursor pools and allows the addition or deletion of cytoplasmic components that can alter nuclear activity. Under conditions that allow faithful transcription in myeloma cell nuclei (20, 29), there was a two- to threefold inhibition of nuclear RNA synthesis (Fig. 5). This measured decrease was similar to the amount of inhibition detected in intact cells and indicates that the primary effect of VS viral infection on myeloma cell RNA synthesis is at the level of transcription. The restoration of cytoplasmic influences had little if any effect on in vitro nuclear activity, and no inhibition of RNA synthesis by isolated nuclei could be attributed to cytosol (Fig. 5). Although it is apparent that no soluble cytoplasmic constituent is responsible for inhibition of cellular RNA synthesis, it remains to be determined which viral component or function is required to induce this switch-off of nuclear transcription.

Infection of MPC-11 cells with VS virus resulted in considerable inhibition of all three DNA-dependent RNA polymerases; however, the activity of polymerase II was compromised to a greater extent and progressively declined throughout infection. The interesting aspect of these results is the fact that the α-amanitin-resistant polymerase I and III activities did not continue to decline throughout the infection, but rather dropped to a level of about 65% of

control activity fairly early in the infection (Fig. 6). The 10% greater effect of viral infection on loss of polymerase I activity, as measured in isolated nucleoli compared with the α-amanitin-resistant activity in intact nuclei, is probably due to the loss of polymerase III during the isolation of nucleoli. Thus, polymerase I appears to be somewhat more sensitive to viral infection than polymerase III. Nevertheless, isolated nucleolar activity also declined rapidly at first, but did not fall below a level of about 55% of uninfected-cell nucleoli as late as 5 h postinfection (Fig. 6). This loss of nuclear polymerase activities in MPC-11 cells infected with VS virus is quite different from that reported for L cells after infection with mengovirus, where activity of both of these major polymerases declined steadily after infection, similar to the very rapid decline in activity of polymerase II (1, 22).

The data presented here indicate that VS virus exerts its effect on host RNA synthesis primarily through a differential inhibition of the various RNA polymerases and that this inhibition cannot be attributed to a cytoplasmic component. In addition, a number of the other multiple steps involved in cellular RNA metabolism evidently remain unaltered.

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