Encephalomyocarditis Virus Infection of Mouse Plasmacytoma Cells

II. Effect on Host RNA Synthesis and RNA Polymerases

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The effect of encephalomyocarditis virus infection of MOPC 460 mouse plasmacytoma cells on host RNA synthesis and RNA polymerases was investigated. Consistent with work performed in other virus host systems, rates of RNA synthesis appeared to be inhibited in infected cells, whereas RNA degradation appeared normal. These results were further extended with isolated nuclei, in which distinct RNA polymerase activities could be studied under conditions where problems with RNA turnover and endogenous nucleotide pool sizes were insignificant. Endogenous nuclear RNA polymerase II activity was inhibited early postinfection and at 1 to 2 h prior to endogenous RNA polymerase I plus III activity. However, the solubilized enzymes were fully active with exogenous DNA as template. In fact, the levels of RNA polymerases I, II, and III, isolated from infected cells and nuclei, were indistinguishable from levels in uninfected cells and nuclei at each stage of their partial purification procedure. The chromatographic properties of the enzymes on DEAE-Sephadex were also unaltered. Furthermore, the RNA synthetic activity of these isolated enzymes, or of nuclei isolated from uninfected cells, was resistant to extracts of nuclei or of cytoplasmic fractions from infected cells. These results are discussed in terms of a possible inhibition of RNA synthesis *in vivo* at the level of transcription initiation.

Infection of animal cells with picornavirus provokes an inhibition of the apparent rate of host RNA synthesis (19), the biochemical mechanism for which is not determined. A functional virus genome is required for this inhibition (10, 15, 21), although a productive infection need not occur (5, 19). Because the rates of RNA degradation appear normal in picornavirus-infected cells (4, 9, 10), the abnormal RNA metabolism seems to reflect primarily an inhibition of RNA synthesis. However, analyses of the molecular mechanism behind this phenomenon often have resulted in conflicting observations. A requirement for viral or host protein synthesis has not been consistently demonstrated (5, 16, 19). Some studies have implicated protein (1) or lipid (11) factors as inhibitors of RNA synthesis (which might inactivate RNA polymerase), whereas others (12) have failed to detect any soluble inhibitors. Finally, in mengovirus-infected L cells (7), and in poliovirus-infected HeLa cells (6), an early inhibition of 45S rRNA precursor synthesis and a later inhibition of extranucleolar RNA synthesis (HnRNA) was observed. By contrast, in isolated nuclei from mengovirus-infected L cells, the

situation appears reversed (20). Endogenous RNA polymerase II activity, which accounts for HnRNA synthesis (see below), is inhibited early after infection, and a putative RNA polymerase I activity, which accounts for rRNA synthesis (see below), is not inhibited until late in infection.

It seems obvious that the inhibition of RNA synthesis in virus-infected cells involves the RNA polymerases; but detailed studies of isolated enzymes have not been performed. Three classes of RNA polymerases are ubiquitous to eukaryote cells (26; L. B. Schwartz, V. E. F. Sklar, J. A. Jaehning, R. Weinmann, and R. G. Roeder, *J. Biol. Chem.*, in press) and account for the synthesis of all major classes of RNA. The nucleolar RNA polymerase I (27) synthesizes rRNA (2, 23, 29, 31); the nucleoplasmic RNA polymerase II (27) synthesizes HnRNA (2, 23, 31); and the nucleoplasmic (27; L. B. Schwartz et al., *J. Biol. Chem.*, in press) RNA polymerase III synthesizes 4S and 5S RNA (29). In normal somatic cells, enzymes I and II account for the majority, whereas enzyme III accounts for less than 15% of the total RNA polymerase activity (24; L. B. Schwartz et al.,

J. Biol. Chem., in press). In addition, because class I, II, and III enzymes have distinct alpha-amanitin sensitivities, their relative activities can be distinguished in solubilized extracts with exogenous DNA or in nuclei where the enzymes remain bound to their cellular chromosome template (29; L. B. Schwartz et al. J. Biol. Chem., in press).

This investigation concerns primarily the effect(s) of encephalomyocarditis (EMC) virus infection of mouse plasmacytoma (MOPC 460) cells on host RNA synthesis and RNA polymerases. Because viral effects on host RNA metabolism vary somewhat with the conditions of infection (6) and with different virus and host cell systems (18), the kinetics of RNA synthesis and degradation were defined for the present system. These studies have been performed both with intact infected cells and with infected cell nuclei, because, in the latter case, complicated problems concerning the distribution and equilibration of endogenous and exogenous nucleotide substrates are diminished. However, of primary concern is the mechanism(s) by which the RNA polymerase activities are inhibited during viral infection. Experiments to detect soluble inhibitors of RNA synthesis have been performed, and the levels of activity of solubilized RNA polymerases I, II, and III in infected cells and cell nuclei have been monitored to detect a possible inactivation of RNA polymerase.

MATERIALS AND METHODS

Infection of plasmacytoma cells. The growth of plasmacytoma MOPC 460 ascites cells and the infection of these cells with EMC virus have been described (17). In these experiments, cells were infected at a cell concentration of 2×10^6 to 2.5×10^6 cells per ml and with 5×10^{-2} to 10×10^{-2} HAU/cell of EMC virus. Under these conditions, about 80% of the cells were infected and the remaining population was resistant to infection. After an initial period of 0.5 h at room temperature to allow for virus attachment, the cultures were incubated at 37 C for the duration of the infection. Uninfected controls were treated identically to infected cells except for the addition of virus.

Kinetics of uridine incorporation into RNA and of RNA degradation in intact uninfected and infected cells. At 2 h after infection, [^3H]uridine (35 Ci/mmol, New England Nuclear, Boston, Mass.) was added to 10-ml cultures of uninfected and infected cells at a final concentration of 10 $\mu\text{Ci/ml}$. After 15 min actinomycin D was added to a final concentration of 10 $\mu\text{g/ml}$ which completely inhibits host cell RNA synthesis (unpublished data). At various times, 0.5-ml portions were withdrawn and pipetted directly into ice-cold 10% trichloroacetic acid. The precipitated cells were collected by centrifugation, dissolved in 1.0 ml of 0.5% sodium dodecyl sulfate, and reprecipitated with 1.0 ml of 10% trichloroacetic acid.

The precipitate was collected on glass-fiber filters (Whatman GF/C), dried, and counted in a toluene-based scintillation fluor.

Rate of uridine incorporation into RNA in infected cells. The apparent rate of RNA synthesis in intact cells was estimated by pulse-labeling portions of uninfected and infected cultures with [^3H]uridine. At various times after infection, 1.0-ml portions of each culture were removed, and [^3H]uridine was added to a final concentration of 2 $\mu\text{Ci/ml}$. The cells were incubated for 15 min at 37 C, and the incubation was terminated by the addition of ice-cold phosphate-buffered saline (3 ml; Grand Island Biological Co., Grand Island, N.Y.). The cells were washed two times by centrifugation in phosphate-buffered saline (3 ml), dissolved in 0.5 ml of 0.5% sodium dodecyl sulfate, and precipitated with 0.5 ml of cold 10% trichloroacetic acid. The precipitate was collected on glass-fiber filters, and the radioactivity in acid-precipitable material was determined.

Preparation of cytoplasmic extracts. After 3.5 h of infection, uninfected and infected cultures were centrifuged at 1,200 rpm for 5 min, and the pelleted cells were suspended in an ice-cold solution of 0.035 M Tris-hydrochloride (pH 7.5) and 0.14 M NaCl and recentrifuged. The supernatant fluid was aspirated, and the cells were suspended in 2 volumes of 0.01 M KCl, 0.0015 M magnesium acetate, and 0.02 M Tris-hydrochloride (pH 7.5), and homogenized with 25 strokes in a tight-fitting Dounce homogenizer. The homogenate was adjusted to 0.12 M KCl, 0.005 M magnesium acetate, and 0.03 M Tris-hydrochloride (pH 7.5), and centrifuged at $30,000 \times g$ for 10 min. Low-molecular-weight material was removed by passing the supernatant fraction over a Sephadex G-25 column equilibrated with 0.12 M KCl, 0.05 M magnesium acetate, and 0.03 M Tris-hydrochloride (pH 7.5). The cytoplasmic extract was frozen and stored in liquid nitrogen.

Nuclei isolation. To obtain nuclei, MOPC 460 cells (10^6 cells) in tissue culture medium were cooled to 0 to 4 C and pelleted by centrifugation at 1,500 rpm for 5 min in an International PR6 centrifuge. (All subsequent procedures concerning nuclei or solubilized RNA polymerase were performed at 0 to 4 C except when stated otherwise.) The supernatant fraction was removed by aspiration, and the cell pellet was suspended in 2 ml of 0.20 M sucrose, 0.2 mM Tris-hydrochloride (pH 7.9; 23 C), 0.1 mM EDTA, 5 mM MgCl_2 , 2% Nonidet P-40, and 0.5% Triton X-100. This suspension was homogenized with five strokes in a glass-Teflon homogenizer. More than 95% of the cells were judged to be broken upon examination under the phase-contrast microscope. This 2-ml nuclear suspension was underlaid with 10 ml of 1.5 M sucrose, 5 mM MgCl_2 , and 0.2 mM Tris-hydrochloride (pH 7.9; 23 C). Nuclei were then pelleted by centrifugation at 2,000 rpm for 20 min in a swinging bucket rotor of an International PR6 centrifuge. The supernatant fraction was removed; pelleted nuclei were suspended in 1 ml of 0.05 M Tris-hydrochloride (pH 7.9), 25% (vol/vol) glycerol, 0.1 mM EDTA, and 0.5 mM dithioerythritol, and were judged free of contaminating particulate matter by phase-contrast microscopy. The yield of nuclei was always greater than 90% as determined

with a hemocytometer. These nuclei were used immediately for determination of either endogenous nuclear or solubilized nuclear RNA polymerase activity (see below).

Measurement of RNA polymerase activity. Assays for RNA polymerase activity were performed in final volumes of 50 μ liters. Solubilized RNA polymerase activity was determined as described previously (L. B. Schwartz et al., *J. Biol. Chem.*, in press). Assays for endogenous RNA polymerase activity in nuclei contained 15 to 30 μ g of nuclear DNA, 2 mM $MnCl_2$, 50 mM Tris-hydrochloride (pH 7.9), 0.6 mM unlabeled nucleoside triphosphates (GTP, ATP, CTP), 0.05 mM unlabeled UTP, 1 μ Ci of [^3H]UTP (10 to 20 Ci/mmol), and appropriate quantities of ammonium sulfate and α -amanitin (Henley and Co., New York). Incubations were performed at 37 C. Reactions were terminated exactly as described for solubilized RNA polymerase assays, except that with nuclei 5 μ liters of a 2-mg solution of DNase I per ml (Worthington, electrophoretically purified) was added to the incubation mixture 20 s before pipetting the mixture onto DE52 (Whatman) filter paper. One unit of activity represents incorporation of 1 pmol of UMP into RNA at 20 min for solubilized and endogenous RNA polymerase activities. In the present studies, a concentration of 0.5 μ g of α -amanitin per ml was used to distinguish RNA polymerase II activity (sensitive) from that of RNA polymerase I plus III (insensitive). This is possible since the class I, II, and III enzymes have unique toxin sensitivities (29; L. B. Schwartz et al., *J. Biol. Chem.*, in press). At 0.5 μ g of α -amanitin per ml class II enzymes are completely inhibited, whereas class I and class III enzymes are completely resistant. Class III enzymes can be inhibited, but only by much higher toxin concentrations (50% inhibition at 10 to 20 μ g/ml; >95% inhibition at 200 μ g/ml), whereas class I enzymes show no sensitivity even at 1 mg of α -amanitin per ml.

Kinetics of RNA synthesis and degradation in nuclei. Nuclei were isolated from infected and uninfected cells 2.5 h after infection. RNA synthesis was determined as detailed above. Degradation of RNA was determined after a 30-min period of RNA synthesis, at which time 0.06 μ mol of unlabeled UTP (2 μ liters) was added to each 50- μ liter incubation mixture. Disappearance of [^3H]UMP-labeled RNA was subsequently determined at appropriate time intervals (up to 2 h) after the addition of this excess UTP.

Solubilization of RNA polymerase activity. Either 10⁸ MOPC 460 cells or nuclei were suspended in 1 ml of buffer A (0.05 M Tris-hydrochloride [pH 7.9], 25% (vol/vol) glycerol, 0.1 mM EDTA, and 0.5 mM dithioerythritol), and the enzyme activity was solubilized (L. B. Schwartz et al., *J. Biol. Chem.*, in press) as follows: (i) suspensions were adjusted to 0.3 M ammonium sulfate and subjected to sonic extract; (ii) the sonically treated material was subjected to high-speed centrifugation (150,000 $\times g$ for 50 min); (iii) the supernatant fraction (F1) was diluted with buffer A to 0.1 M ammonium sulfate; and (iv) the resultant chromatin precipitate was pelleted by another centrifugation (150,000 $\times g$ for 50 min). The supernatant fraction (F2) was again diluted with buffer A to 0.05 M ammonium sulfate, and additional

aggregated material was again removed by high-speed centrifugation (150,000 $\times g$ for 50 min). This supernatant fraction (F3) was applied directly to a DEAE-Sephadex column.

DEAE-Sephadex chromatography. DEAE-Sephadex was prepared and equilibrated with buffer A containing 0.05 M ammonium sulfate as described (L. B. Schwartz et al., *J. Biol. Chem.*, in press). Solubilized RNA polymerase fractions (fraction F3, in buffer A containing 0.05 M ammonium sulfate) from 10⁸ cells or nuclei were then loaded onto 6-ml columns and washed with 8 ml of buffer A containing 0.05 M ammonium sulfate. RNA polymerase activity was eluted with a 15-ml linear 0.05 to 0.5 M ammonium sulfate gradient in buffer A. Fraction volumes of 0.45 ml were collected at a flow rate of 0.5 ml/min. Salt concentrations in the sample fraction were measured as described previously.

RESULTS

RNA synthesis in infected cells and isolated nuclei. The apparent rates of RNA synthesis in control and EMC-infected MOPC 460 cells were determined by measuring the incorporation of [^3H]uridine into acid-precipitable radioactivity as described above. During infection, there is a rapid decline in the apparent rate of total RNA synthesis, which commences within an hour postinfection and is about 70% inhibited by 2 h (Fig. 1).

Endogenous RNA synthetic activities in nuclei can be assigned to specific RNA polymerases, because each enzyme has a characteristic α -amanitin sensitivity that is identical, whether the enzyme is assayed in a solubilized form (with exogenous template) or in isolated nuclei (29; L. B. Schwartz et al., *J. Biol. Chem.*, in press). The endogenous RNA polymerase II and the endogenous RNA polymerase I plus III activities in control and infected cells are compared in Fig. 2. For comparative purposes, the data in Fig. 1 have been replotted in Fig. 2 with the apparent rate of RNA synthesis expressed as a percentage of the rate in control cells. In isolated nuclei, the inhibition of endogenous RNA polymerase II activity is about 50% at 2 h postinfection and closely parallels the *in vivo* inhibition of the apparent rate of total RNA synthesis. The combined endogenous RNA polymerase I plus III activity is inhibited only 10 to 15% at 2 h, but at later times is inhibited to a greater extent (30 to 60% by 3 h). The early inhibition of total RNA synthesis observed *in vivo* may be accounted for by the preferential decrease in RNA polymerase II activity in nuclei, which suggests that, primarily, RNA polymerase II activity is measured in a 15-min pulse *in vivo*. This is consistent with the observation that 75 to 85% of the RNA labeled in other cultured

FIG. 1. RNA synthesis in intact cells after infection with EMC virus. Portions of uninfected and infected cells were pulse-labeled with $[^3\text{H}]$ uridine.

cells during a 10- to 20-min pulse represents HnRNA (28).

Kinetics of RNA synthesis and degradation. To demonstrate that the apparent inhibition of RNA synthesis, observed in the above experiments, is not caused by an increase in the rate of turnover of newly synthesized RNA, the kinetics of precursor incorporation into RNA and its subsequent degradation in the absence of RNA synthesis were studied in vivo in infected cells and in vitro in nuclei from infected cells.

The kinetics of $[^3\text{H}]$ uridine incorporation into acid-insoluble material in cells analyzed at 2 h after infection is shown in Fig. 3. At this time, viral RNA synthesis is not yet detectable, and radioactivity is incorporated only into cellular RNA (unpublished observations). Incorporation of $[^3\text{H}]$ uridine in infected cells is inhibited by about 70% when compared to that in uninfected control cells. After 15 min, actinomycin D was added (10 $\mu\text{g}/\text{ml}$) to completely inhibit cellular RNA synthesis. In the presence of actinomycin D, there was no suggestion of an increased rate of degradation of newly synthesized RNA in infected cells (Fig. 3). Thus, it is evident that the viral infection caused an inhibition of RNA synthesis.

Similar results were obtained when the kinetics of $[^3\text{H}]$ UMP incorporation into RNA was

FIG. 2. RNA synthesis in intact cells and in isolated nuclei after infection with EMC virus. The apparent rates of RNA synthesis in vivo were determined by monitoring $[^3\text{H}]$ uridine incorporation into RNA during 15-min pulses, and the endogenous RNA polymerase activities in isolated nuclei were monitored in the presence of 0.2 M ammonium sulfate. Symbols: ●, percentage of $[^3\text{H}]$ uridine incorporation in uninfected cells (6×10^3 to 11×10^3 counts per min per 2×10^6 cells) remaining in infected cells; Δ, percentage of uninfected cell nuclear RNA polymerase II activity (1,500 pmol of UMP incorporated per 30 min per mg of DNA) remaining in infected cell nuclei (0.5 μg of α -amanitin per ml sensitive); ○, percentage of uninfected cell nuclear RNA polymerase I plus III activity (600 pmol of UMP incorporated per 30 min per mg of DNA) remaining in infected cell nuclei (0.5 μg of α -amanitin per ml insensitive).

FIG. 3. Kinetics of RNA synthesis and degradation in vivo. Rates of $[^3\text{H}]$ uridine incorporation into RNA and of RNA degradation in infected (○) and uninfected (●) cells (2.5 h postinfection).

studied in nuclei from infected cells as shown in Fig. 4. When initial rates of incorporation (0 to 5 min) were considered, endogenous RNA polymerase II activity was inhibited 47% and RNA polymerase I plus III activity 36%, relative to uninfected controls. After a 30-min incubation with [^{3} H]UTP, the amount of radioactivity incorporated into RNA had plateaued, suggesting either steady-state kinetics or termination of RNA synthesis with no product degradation. To distinguish between these possibilities, an excess of unlabeled UTP was added at 20 min to prevent further incorporation of label, and the fate of the labeled material was then followed. (Control experiments showed that when this concentration of UTP was added initially, less than 5% of the normal amount of label was incorporated into RNA after 30 min. Previously labeled RNA is stable under these conditions, and the kinetics of labeling, therefore, reflect the rate of RNA synthesis (Fig. 4).

The decreased rates of RNA synthesis observed both *in vivo* and *in vitro* thus appear to reflect an inhibition of RNA polymerase activities.

Levels of solubilized RNA polymerases.

Two general types of mechanisms could account for the observed inhibition of RNA synthesis: a direct inactivation of the RNA polymerase molecule(s), or an inhibition at some biochemical step involved in transcription, such as initiation or elongation. The former possibility was investigated by solubilizing RNA polymer-

ase activity from uninfected and infected intact cells or nuclei to render the activity totally dependent on exogenous template (26). RNA polymerase activities, at various stages in the solubilization procedure, were distinguished by their sensitivities to 0.5 μ g of α -amanitin per ml and are summarized in Table 1. As with the enzymes from other cell types, RNA polymerases I, II, and III from plasmacytoma cells can be resolved by chromatography on DEAE-Sephadex (24; L. B. Schwartz et al., *J. Biol. Chem.*, in press). The elution profiles of the solubilized RNA polymerases from uninfected and infected intact cells and nuclei were analyzed by this method, and results for intact cells are shown in Fig. 5, where peaks of enzyme I, II, and III activities are readily apparent. The minor peak of activity seen with calf thymus as template in the presence of low concentrations of α -amanitin (to specifically inhibit RNA polymerase II) was identified as RNA polymerase III by its increased activity with poly(d[A-T]) relative to calf thymus DNA as template (Fig. 5), and by its unique and complete sensitivity to high concentrations of α -amanitin (29; L. B. Schwartz et al., *J. Biol. Chem.*, in press) (data not shown). These experiments were performed 2 h after infection, at which time total RNA synthesis *in vivo* had been inhibited by 70% and RNA polymerase II activity in nuclei by 50%. No significant differences in the chromatographic elution profiles of the three RNA polymerase species were detected. The data are summarized quantitatively in Table 1, and show that

FIG. 4. Kinetics of RNA synthesis and degradation in nuclei. Rates of RNA synthesis and degradation in isolated nuclei (2.5 h postinfection) from infected (---) and uninfected (—) cells were determined in the presence (○) or absence (●) of α -amanitin. All assays were performed in the presence of 0.2 M ammonium sulfate. The time scale was shortened after the first 30-min period of RNA synthesis by a factor of 7.5.

TABLE 1. Solubilized RNA polymerase activities from isolated nuclei and intact cells^a

Polymerase	Activity (U/10 ⁶ cells or nuclei) ^b			
	Nuclei		Intact cells	
	Uninfected	Infected	Uninfected	Infected
F1 RNA polymerases I plus III	4,519	4,030	5,125	5,220
RNA polymerase II	530	686	1,825	2,940
F2 RNA polymerases I plus III	5,657	5,866	9,200	8,825
RNA polymerase II	2,551	1,826	3,850	4,150
F3 RNA polymerases I plus III	4,195	4,809	6,120	7,720
RNA polymerase II	4,400	3,650	4,010	3,980
DEAE-Sephadex ^c				
RNA polymerases I plus III	4,800	4,850	9,480	8,600
RNA polymerase II	3,050	3,200	3,720	3,610

^a RNA polymerase was solubilized from nuclei and whole cell preparations. Final supernatant fraction (F3) was applied directly to DEAE-Sephadex and developed.

^b Values from average of two experiments.

^c Calculated from chromatography experiments.

FIG. 5. DEAE-Sephadex chromatography of RNA polymerases I, II, and III from infected and uninfected cells. RNA polymerase was solubilized from uninfected and infected (2 h postinfection) cells. No losses of activity were apparent during the prechromatographic fractionations. Symbols: ●, calf thymus DNA; ○, α -amanitin (0.5 μ g/ml) and calf DNA; △, α -amanitin (0.5 μ g/ml) and poly(d[A-T]) as template.

the total solubilized RNA polymerase levels (DEAE-Sephadex step) obtained from uninfected and infected cells or from nuclei derived from such cells are the same. Similar results were also obtained with activity solubilized from whole cells at 4 h after infection (data not shown). The RNA polymerase molecules themselves, therefore, do not appear to be affected by virus infection, suggesting that shut-off of host RNA synthesis occurs at some specific biochemical event in transcription.

Absence of detectable inhibitor of RNA synthesis. It has been suggested that an inhibitor of RNA polymerase is responsible for the inhibition of RNA synthesis during infection (1, 11). A number of experiments have been performed to detect such an inhibitor in the EMC-MOPC 460 system.

The absence of any difference in RNA polymerase activities in the crude fractions solubilized from uninfected and infected nuclei (Table 1) indicate the absence of an inhibitor, which

would be detected under conditions used to measure RNA polymerase activity *in vitro* with exogenous templates.

To determine if there is an inhibitor in infected cell nuclei that would inhibit endogenous RNA polymerase activity, nuclei from uninfected and infected cells were mixed in the same reaction, and RNA polymerase activities were determined. Nuclei were isolated at 3 h after infection, at which time RNA polymerases I plus III as well as RNA polymerase II were markedly inhibited. Nuclei isolated from uninfected and infected cells were assayed either separately or together in the presence of 0.2 M ammonium sulfate. At this salt concentration nuclei lyse, facilitating thorough mixing of their components, whereas preinitiated RNA polymerase template complexes remain intact. The RNA polymerase activities of mixtures of such nuclei reflect the average activity of the homologous nuclear suspensions (Table 2). Similar results were obtained when assays were performed at 0.05 M ammonium sulfate (data not shown). Furthermore, cytoplasmic fractions from uninfected or infected cells had no effect on nuclear RNA polymerase activities, as detailed in Table 3. The results provide no evidence for the existence of a specific inhibitor of RNA polymerase in infected cells.

DISCUSSION

A number of mechanisms have been proposed to explain the apparent picornavirus-effected shut-off of host RNA synthesis. One possibility is that lysosomes release degradative enzymes which could affect net RNA synthesis, either via template or RNA polymerase inactivation or via changes in RNA stability. However, the nuclear cytopathology observed in picornavirus-infected cells has been shown to precede the release of detectable levels of lysosomal en-

TABLE 2. Effects of mixtures of uninfected and infected cell nuclei on endogenous RNA polymerase activities^a

Nuclei source	Activity (pmol of UMP incorporated per 30 min per mg of DNA)	
	RNA polymerases I and III	RNA polymerase II
Uninfected	510	1,560
Infected	214	423
Uninfected and infected	370 (362) ^b	1,030 (992) ^b

^a Nuclei were isolated at 3 h postinfection and assayed in the presence of 0.2 M ammonium sulfate.

^b Theoretical average endogenous activities.

TABLE 3. *Effects of cytoplasmic fractions on the endogenous RNA polymerase activities of isolated nuclei^a*

Nuclei source	Activity (pmol of UMP incorporated per 30 min per mg of DNA)			
	Nuclei (infected)		Nuclei (uninfected)	
	RNA polymerases I and III	RNA polymerase II	RNA polymerases I and III	RNA polymerase II
Cytoplasm (uninfected)	383	562	374	834
Cytoplasm (infected)	405	541	400	913
Buffer A	410	638	408	1017

^a Ten μ liters of cytoplasm or buffer A and 20 μ liters of nuclei (2 h postinfection) from infected and uninfected cells were mixed and assayed in the presence of 0.2 M ammonium sulfate.

zymes (16). Furthermore, in infected cells, the DNA is not preferentially fragmented (10) and, as discussed below, the RNA polymerases are not preferentially inactivated. Previous studies have also shown that no abnormal degradation of total cellular RNA (9, 10) or of polyadenylic acid-containing cytoplasmic RNA (4) occurs upon picornavirus infection. The present studies on intact infected cells confirm the data on total RNA stability. In addition, studies on endogenous levels of RNA synthesis in isolated nuclei provide more convincing evidence for decreased rates of RNA synthesis following virus infection, because complications related to RNA turnover or to unknown nucleotide pool sizes and specific activities are avoided. These data clearly suggest a defect in the RNA synthetic processes in infected cells.

Other studies have suggested that RNA polymerases are inactivated directly by components in virus-infected cells (1, 11). This is compatible with the decreased endogenous RNA polymerase activities in infected cell nuclei. However, the present data demonstrate, for the first time, that the cellular activity levels and the relative proportions of RNA polymerases I, II, and III (when solubilized and assayed in the presence of exogenous template) are identical in virus-infected and uninfected cells, not only at 2 h but also at 4 h after infection. Similarly, the levels and relative proportions of the solubilized RNA polymerase activities are also identical in nuclei from these cells, demonstrating that the enzymes are not preferentially or selectively lost from infected cell nuclei during the isolation procedure. The three RNA polymerase classes from infected and uninfected plasmacytoma cells also chromatographed identically on DEAE-Sephadex. Similar results have been found for adenovirus-infected KB cells, where solubilized RNA polymerase I levels in nuclei are normal even though endogenous activities are completely repressed (R. Weinmann, H. Raskas, and R. G.

Roeder, Proc. Nat. Acad. Sci. U.S.A., in press). Hence, solubilized levels of RNA polymerase activity do not necessarily reflect *in vivo* rates of RNA synthesis in intact cells or in isolated nuclei where the activity of endogenous enzyme template complexes is measured. In the present system, attempts were also made to inhibit RNA synthesis in uninfected cell nuclei by incubation in the presence of either cytoplasmic or nuclear fractions from infected cells, but no inhibition could be detected.

These observations, however, do not exclude the presence in EMC virus-infected cells of factors that inhibit the RNA polymerases. The failure to detect inhibition of RNA polymerase activity in solubilized extracts (with exogenous templates) could be due to the loss or inactivation of such factors during the solubilization procedures. Alternatively, the mechanism of initiation by RNA polymerase on natural templates *in vivo* (at specific sites) and on heterologous templates *in vitro* may be somewhat different. Even with homologous enzymes and templates, most initiations *in vitro* by solubilized and partially purified enzymes appear to be at nonspecific sites (14, 25), presumably because other undefined components are required for specific initiations. Thus, an inhibitor affecting only natural (specific) initiation would probably not be detected in these studies. Furthermore, because the RNA synthesized by isolated nuclei represents, primarily, elongation of preinitiated RNA molecules (23, 29), inhibitors of initiation (which may be present *in vivo*) would not be detected by addition of infected cell extracts (nuclei or cytoplasm) to active nuclei from uninfected cells. In fact, the present data suggest that inhibition is at the level of initiation. RNA synthesis in nuclei isolated from infected and uninfected cells plateaus at approximately the same time. Assuming similar rates of elongation for RNA polymerases in infected and uninfected cell nuclei, this observation suggests that fewer enzyme molecules

are active in transcription in infected nuclei, and is consistent with the hypothesis that initiation of RNA synthesis is inhibited in infected cells in vivo.

Because the onset of inhibition of endogenous RNA polymerase II activity in nuclei from infected cells occurs 1 to 2 h prior to inhibition of endogenous RNA polymerase I plus III activity (20; Fig. 2), different inhibition mechanisms may be operative for different RNA polymerases. For example, inhibition of an RNA polymerase activity in vivo could result indirectly via the reduced synthesis of a short-lived host protein factor required for natural initiation. Because such a factor has been implicated for enzyme I (30), and because the inactivation of enzyme II apparently precedes that of enzyme I in virus-infected cells, RNA polymerase I may be inactivated as a consequence of the inhibition of HnRNA (mRNA) synthesis and of host protein synthesis, whereas RNA polymerase II may be inactivated as a more direct consequence of virus infection.

Another possibility, however, is that the template (by virtue of structural alterations) in virus-infected cells is refractile to transcription by RNA polymerase. The cessation of RNA synthesis during mitosis in normal cells (8) may result from such modifications, since isolated chromatin does not support transcription by exogenous RNA polymerase (3). However, *Escherichia coli* RNA polymerase has been found to transcribe chromatin from picornavirus-infected and uninfected cells equally well (13). Similar results were also obtained with purified preparations of both myeloma RNA polymerases I and II and limiting amounts of chromatin templates from uninfected and EMC-infected MOPC 460 cells (L. B. Schwartz, unpublished observations). However, because the regions transcribed in chromatin may not be entirely the same as those transcribed in nuclei or in intact cells (14, 22), template restrictions affecting proper in vivo transcription units may not be detected in vitro.

Whereas the detailed biochemical mechanism of inhibition of RNA polymerase activity during viral infection has not been determined in this investigation, the experiments presented are most consistent with inhibition in vivo occurring at the level of initiation of RNA synthesis. Further elucidation of the mechanism involved may require an in vitro system in which RNA synthesis is dependent on natural initiation.

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