Vesicular Stomatitis Virus Infection Reduces the Number of Active DNA-dependent RNA Polymerases in Myeloma Cells*

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Infection of mouse myeloma (MPC-11) cells with vesicular stomatitis virus resulted in rapid loss in activity of cellular RNA polymerases associated with nuclear chromatin. No RNA polymerase inhibitor could be detected in extracts of infected cell nuclei. Reconstitution experiments with solubilized RNA polymerases dissociated from chromatin of infected and uninfected cells demonstrated that vesicular stomatitis viral infection did not affect the ability of the polymerases to function on endogenous or exogenous templates; nor did infection alter the template capability of the chromatin. Measurement of the number of actively growing RNA chains revealed that infected cell nuclei contained fewer active polymerase units; however, the rates of RNA chain elongation were the same in nuclei from infected and uninfected cells. Quantitation of the number of polymerase units active in nuclear chromatin revealed that the α-amantin-sensitive polymerase II was more severely reduced by viral infection than were polymerases I and III.

Inhibition of host cellular RNA synthesis during infection with vesicular stomatitis virus has been examined extensively using infectious B virions (1–3) and virions rendered noninfectious by ultraviolet irradiation (3–6). The mechanism by which VS virus shuts off host RNA synthesis is not well understood. Degradation of cellular mRNA is not enhanced in VS virus-infected cells (7), and there is no evidence for modification of post-transcriptional events such as polyadenylation or transport of RNA (8). In contrast to inhibition of cellular RNA synthesis by poliovirus infection (9), VS virus does not appear to induce a cytoplasmic inhibitor of nuclear polymerases, although in vitro transcription is markedly reduced in nuclei isolated from VS virus-infected cells (8).

A number of investigators, who have studied other virus-cell systems, have reported findings that support the hypothesis that viral infection decreases the initiation of cellular RNA synthesis. Among the best studied is mengovirus, which rapidly causes a shutdown of cellular RNA metabolism by differentially inhibiting the major polymerase activities of the cell nucleus (10–12). Mengovirus infection does not increase the rate of degradation of cellular mRNA (13), but rather, it apparently inhibits initiation of RNA synthesis without grossly modifying either the RNA polymerase or the template capacity of the cellular chromatin (14). Schwartz et al. (15) have reported similar findings in cells infected with encephalomyocarditis virus, which contain the same levels of soluble RNA polymerases as uninfected cells, even though host RNA synthesis was severely retarded.

The negative strand RNA viruses, such as VS virus, are quite different from positive strand picornaviruses and, therefore, might differ in their mode of action in shutting off cellular RNA synthesis. The studies reported here were designed to examine the mechanism by which VS virus inhibits the DNA-dependent RNA polymerases of host cells. Examination of endogenous polymerase activities in cell chromatin, and the use of solubilized polymerases from infected cells revealed that binding of enzyme to template was inhibited in vivo but not in vitro. Direct quantitation of the active polymerases in nuclei isolated from infected cells at various times postinfection showed that the number of polymerases engaged in chain elongation was reduced but that the rates of elongation were unaltered. These data agree closely with information obtained from other virus-cell systems and are compatible with the idea that viral infection reduces initiation by RNA polymerases in vivo.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Virus Production**—Mouse myeloma cells, MPC-11, were obtained by W. M. Kuehl of our department from the American Type Culture Collection, Rockville, Md. The cells were grown in Dulbecco's modified Eagle's medium, 10% heat-inactivated horse serum, and nonessential amino acids, all obtained from Grand Island Biological Co., Grand Island, N. Y. 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 United States Agriculture Research Center, Beltsville, Md. (16). Clones of this virus were selected from plaques picked from L-cell monolayers, and virus stocks were prepared by growth on BHK-21 cells. Infectivity of virus preparations was titrated by assay of plaque-forming units (pfu) on monolayers of L-cells. The infection of myeloma cells was performed as previously described (8) and routinely done at input multiplicities of 10 pfu/cell.

**Isolation of MPC-11 Cell Nuclei**—Nuclei were isolated from infected or uninfected MPC-11 cells according to the technique of Schwartz et al. (15), resulting in a yield of 80 to 90% intact nuclei as measured by microscopic examination in a hemocytometer. Nuclei were prepared freshly for each experiment and were used immediately after isolation. Nuclear pellets were gently resuspended in ice-cold 20% glycerol containing 5 mM magnesium acetate, 20 mM Tris (pH 8), 5 mM dithiothreitol, and 0.1 mM EDTA.
Preparation of Chromatins—The preparations of chromatin for the measurement of endogenous RNA polymerase activity and a 0.12 M KCl nuclear extract were performed as described by Marzluff and Huang (17). The chromatin pellet was resuspended in 25% glycerol buffer, sheared by passage through a 16-gauge needle, and tested under conditions identical with those described for isolated nuclei.

Solubilization of Polymerases—RNA polymerases were extracted from isolated nuclei by treating the nuclei in a buffer containing 0.05 M Tris (pH 8), 0.005 M dithiothreitol, 0.025 M KCl, and 0.1 M NaOH in a Dounce homogenizer. The resulting homogenate was incubated for 10 min at 25°C in order to extract the nuclear RNA polymerases which results in solubilization of 70% of the original nuclear activity (18). Glycerol was added to a final concentration of 16% (w/v) and the nuclear lysate was centrifuged for 30 min at 208,000 × g in a Beckman SW 60 Ti Rotor at 4°C. The resulting supernatant fluid was tested for RNA polymerase activity.

The chromatin pellet was resuspended in 10% glycerol containing 0.5 M NaCl and 1 ml of distilled water and maintained at 4°C overnight. When used for in vitro transcription, the chromatin was pelleted, resuspended in 25% glycerol buffer, and sheared by passage through a 16-gauge needle.

RNA Synthesis—The conditions used to measure RNA synthesis in isolated nuclei or chromatins were basically those described by Marzluff et al. (19) for myeloma cell nuclei. Isolated nuclei were employed for determination of the number of active polymerases and reaction mixtures (1 ml) containing 12.5% glycerol, 25 mM Tris (pH 8), 0.05 mM EDTA, 5 mM magnesium acetate, 1 mM UTP, 0.15 M KCl, 2.5 mM dithiothreitol, 0.03 mM ATP, CTP, and GTP, and 10 μCi of [3H]UTP (35.5 Ci/mmol). Following incubation at 25°C, the nuclei were resuspended in 1 ml of buffer containing 10 mM Tris (pH 7.6), 10 mM KCl, 1.5 mM MgCl₂, and precipitated by the addition of 1 ml of 5% perchloric acid.

The measurement of endogenous or added polymerase activity in chromatin preparations was performed under conditions identical with those for isolated nuclei except that 100 μl reactions contained 0.4 μM each ATP, CTP, and UTP, and 1 μCi of [3H]GTP (10.6 Ci/mmol). Following incubation at 25°C, reaction mixtures were precipitated in trichloroacetic acid and prepared for liquid scintillation counting as described previously (8).

Hydrolysis and Analysis of RNA Products—The perchloric acid precipitates from reactions of isolated nuclei, described above, were dissolved in 100 μl of 0.3 N KOH and hydrolyzed for 16 to 18 h at 37°C (20). The hydrolysate was then filtered on FEP-cellulose thin layer plates prewashed in 10% aqueous NaCl, distilled water, and air-dried. The plates were developed in distilled water, the spots corresponding to uridine and UMP were cut out, and the radioactivity was eluted with 10 μl of 0.1 N HCl for 15 min, incubated in 0.5 ml Nuclear Chicago solubilizer for 1 h at 37°C, and measured in 10 ml of toluene-based scintillation fluid.

Quantitation of Active Polymerase Molecules—The methodology of Cox (22) was employed to quantify the number of elongating polymerase molecules in isolated nuclei. The amounts of [14C]TP incorporated into RNA and released as uridine, after hydrolysis, was determined as the counts per min per μg of DNA. The following formula was used to determine the number of active enzyme molecules per cell:

Number of active polymerases = (U) × (specific activity)/(base correction) × (efficiency) × (cells/DNA)

where U = uridine counts per min per μg of DNA; specific activity of UTP = 2.38 × 10⁸ molecules/dpm; base correction = 25%; counting efficiency = 45%; and cells/DNA = 1.8 × 10⁷ cells/μg of DNA. Polymerase II activity was calculated as that amount of total nuclear activity which was sensitive to α-amanitin at a concentration of 10 μg/ml of reaction mixture.

RESULTS

Endogenous RNA Polymerase Activity in Chromatin from Infected and Uninfected MPC-11 Cells—Intact nuclei from VS virus-infected myeloma cells had previously exhibited a marked reduction in the activity of nuclear DNA-dependent RNA polymerases compared with that of uninfected cells (8). Although no cytoplasmic inhibitor of RNA synthesis could be detected in earlier experiments, it still seemed possible that some viral product or altered cellular component within the isolated nuclei might be responsible for the inhibited nuclear activity. In an effort to examine this possibility, a 0.12 M KCl extract was prepared from nuclei isolated from infected and uninfected myeloma cells. When this extract was tested in transcribing chromatin preparations, it was apparent that inhibition of nuclear polymerase activities was not being mediated by any type of soluble protein (Fig. 1). In fact, the presence of the nuclear extract from either infected or uninfected cell nuclei caused a slight increase in transcription in chromatin from both sources (Fig. 1). However, there was no RNA polymerase activity detectable in the protein extract when calf thymus DNA was used as template, which is in agreement with the results of Marzluff and Huang (17). Thus, the chromatin prepared from nuclei isolated from infected cells was inhibited, approximately 40% (Fig. 1), and this inhibition could not be enhanced by the addition of an extract, which has been shown to contain 40 to 50% of the nuclear protein and almost 90% of the nuclear RNA (17).

Comparative Activities of Solubilized Polymerases and Templates of Infected and Uninfected Cells—Although it was apparent that cellular chromatin activity was significantly reduced by viral infection, the site of the alteration could involve either the DNA template or the RNA polymerase itself. In order to test these two possibilities, nuclear polymerases were solubilized and their RNA-synthesizing activities were tested by reconstitution with calf thymus DNA or uninfected and infected cell chromatin (Table I). When polymerases were examined in this manner, essentially no difference could be detected in their abilities to transcribe the various template preparations (Table I). These results indicate that...
the enzymatic activities of infected and uninfected cellular poly-

merases are comparable, and that viral infection does not cause irreversible damage to the polymerase molecules them-

selves. In addition, chromatin prepared from infected cells was found to serve as well as uninfected control cell chromatin as a DNA template for RNA synthesis. Although indirect, this observation suggests that viral infection does not affect the number of open sites available for transcription within the nucleus.

It is interesting to note that polymerase activity was signifi-
cantly higher on a template of calf thymus DNA than on myeloma cell chromatin. This is probably due to the presence of proteins on the DNA in the chromatin preparations, and possibly the fact that calf thymus DNA contains nicks which can serve as nonspecific initiation sites (29). In any event, these significant differences in transcription of exogenous DNA and chromatin are not simply the result of variation in background incorporation since the prepared chromatin contained essentially no activity in the absence of added poly-

merases (data not shown).

Comparative Quantitation of Active Polymerase Units on Chromatin of Infected and Uninfected Cells—In recent years, a method has been developed for determining the number of polymerase units involved in elongating RNA chains in isolated nuclei (22). Since this technique is based on analysis of the 3'-terminus of growing RNA chains, it is essential to determine the incorporation of the radioactively labeled precursor in the form of either nucleosides or nucleotides in the alkaline hydrolysis products of nuclear RNA (24). When the incorporation of [3H]UTP by isolated nuclei was measured in this manner, there was a significant reduction in the incorporation of both uridine and UMP by nuclei isolated from myeloma cells at about 3-h postinfection (Fig. 2, A and B), suggesting that there is a decrease in the number of active RNA polymerase(s) in the cells. These data also indicate that there is no generation of false 3'-terminal during short incu-
bations of the nuclei since the measurable levels of free uridine in the hydrolysis products do not increase with time (Fig. 2 A).

When the values in Fig. 2, A and B, are compared as a ratio of UMP to uridine, there is no difference between infected and uninfected cell nuclei (Fig. 2C), a result which demon-

strates that rates of elongation of RNA chains are the same for infected and uninfected cells. Thus, viral infection reduces the number of actively growing RNA chains without altering the rates at which they are being elongated.

The measurement of the number of growing DNA chains allows a calculation to be made of the actual number of active RNA polymerase units (22). By measuring the amount of DNA per nucleus (1 μg of DNA = 1.8 x 10⁶ cells), by knowing the specific activity of the radioactively labeled precursor (2.38 x 10⁶ molecules/dpm), and by assuming that 25% of the 3'-termini contain radioactive uridine, the number of active RNA polymerases per cell can be determined. The measure-

ment of the 3'-termini of RNA molecules in five independent experiments demonstrated that there was an approximate 50% reduction in the number of active RNA polymerases in infected cell nuclei as compared to uninfected control cell nuclei (Table II). It should be noted that the number of polymerases does not increase significantly with time of incubation as would be expected for a system measuring RNA chain elon-


gation.

Measurements of the Number of Active Units of Polymerase I, II, and III During Viral Infection—Previous work with isolated myeloma cell nuclei had indicated that the different classes of nuclear polymerases showed preferential sensitivity to viral infection (8). The quantitation of these polymerases, depicted in Fig. 3, supports these earlier findings. In order to measure these polymerases, nuclei were isolated at 2, 4, or 6 h postinfection and incubated with [3H]UTP in the presence or absence of α-amanitin. The resulting [3H]-labeled RNA was subjected to alkali hydrolysis and analysis on PEI-cellulose,

and the number of active polymerases calculated as described above.

As depicted in Fig. 3A, the active α-amanitin-resistant pol-

ymerases I and III were not reduced in number until 3 to 4 h postinfection. In contrast, the number of active units of poly-

merase II declined sharply during the first 2 h of infection, and by 6 h the remaining nuclear polymerase activity was totally insensitive to α-amanitin (Fig. 3B). Although the kinetic data

Table I

<table>
<thead>
<tr>
<th>RNA synthesis by solubilized polymerases dissociated from VS virus-infected and uninfected myeloma (MPC-11) cells</th>
</tr>
</thead>
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| At 3 to 4 h after infection, nuclei were isolated from infected and uninfected cells; chromatin or RNA polymerases were prepared from the nuclei as described under "Experimental Procedures." The solub-

ilized polymerases (600 to 750 μg of protein) were added to reaction mixtures containing chromatin from infected cells or uninfected cells, as well as calf thymus DNA (50 μg), and assayed for [3H]UTP incorporation at 25°C for 10 min. All results are corrected for minor incorporation measured in the absence of added polymerases. |

<table>
<thead>
<tr>
<th>Template chromatin from</th>
<th>Nuclear polymerase activity from</th>
<th>Uninfected cells</th>
<th>Infected cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calf thymus DNA</td>
<td>3.18</td>
<td>3.14</td>
<td></td>
</tr>
<tr>
<td>Uninfected cells</td>
<td>1.80</td>
<td>1.77</td>
<td></td>
</tr>
<tr>
<td>Infected cells</td>
<td>1.77</td>
<td>1.95</td>
<td></td>
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</tbody>
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Table II

<table>
<thead>
<tr>
<th>Effect of VS virus infection on the number of active RNA polymerase units in nuclei isolated from MPC-11 cells</th>
</tr>
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| Nuclei were isolated at 3 to 4 h postinfection from infected and uninfected myeloma cells and incubated with [3H]UTP at 25°C for the times indicated. The resulting RNA products were hydrolyzed, the hydrolysate separated on PEI-cellulose, and the number of active polymerases calculated as described under "Experimental Proce-

dures." |

<table>
<thead>
<tr>
<th>Incubation time</th>
<th>Cell source of nuclei</th>
<th>Per cent uninfected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninfected</td>
<td>Infected</td>
<td></td>
</tr>
<tr>
<td>5 min</td>
<td>30,486</td>
<td>20,673</td>
</tr>
<tr>
<td>10 min</td>
<td>32,181</td>
<td>15,749</td>
</tr>
</tbody>
</table>
Following this 15-min pulse, the RNA was precipitated, hydrolyzed, and observed (8), the observation that they are less sensitive to viral infection is quite evident. However, the chromatin prepared under such conditions that are known to remove a large portion of nucleus-associated proteins and RNA. Howe, the chromatin extracted from infected cells under conditions which might alter the final results (22). In the system employed for the experiments reported here, there appeared to be very little if any generation of false 3'-termini as evidenced by the fact that free uridine residues, representing the number of growing chains, did not increase with incubation time while the number of UMP (internal residues) did (Fig. 2). More importantly, there was a significant reduction in the number of growing RNA chains in nuclei isolated from infected cells, but the rates of chain elongation remained unaltered. When these data were expressed in terms of the number of active polymerase units, an approximate 50% reduction in total nuclear polymerase activity was calculated (Table II). A measurement of the decline in the number of active RNA polymerases clearly demonstrated that during VS virus infection there was a drastic and rapid loss in the quantity of polymerase II units engaged in chain elongation (Fig. 3). In sharp contrast, polymerases I and III were inhibited later in the infection cycle. Whether this represents changes in rates of initiation or premature termination of RNA molecules remains to be determined. This type of differential loss of nuclear polymerases has also been measured in mengovirus-infected cells (10, 11) and in cells infected with encephalomyocarditis virus (19).

The dissociation of RNA polymerases from the chromatin of the cell and its subsequent reconstitution with various DNA templates revealed that there were no drastic alterations in the ability of polymerases to function on exogenous templates (Table I). This fact, coupled with the initial observation that chromatin-bound polymerases are inhibited (Fig. 1), indicates that the loss of cellular polymerase activity occurs at the level of binding of the enzyme to its template while still within the cell. These observations made with mengovirus cell chromatin agree closely with those of Aprilett and Penhoet (14) who could detect no changes in the binding of Escherichia coli RNA polymerase to chromatin prepared from uninfected and mengovirus-infected cells. Therefore, as suggested by these authors and by Schwartz et al. (15), viral infection of mammalian cells results in an inhibition of the initiation of RNA synthesis. Based on the results presented here, VS virus apparently exerts its effect by the same mechanism.

**REFERENCES**


**FIG. 3.** Loss of active cellular RNA polymerases during VS virus infection. At 2-h intervals, nuclei were isolated from infected (○) and uninfected (●) myeloma cells and incubated with [3H]UTP at 25°C for 15 min in the absence or presence of a-aminonucleoside (10 μg/ml). Following this 15-min pulse, the RNA was precipitated, hydrolyzed overnight, and the amounts of radioactive uridine and UMP were determined by thin layer chromatography. The number of elongating RNA polymerase I and II units (A) and RNA polymerase II units (B) was determined as described under "Experimental Procedures."
VS Virus Inhibition of Cellular RNA Polymerases

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