Differential Inhibition of Nuclear RNA Polymerases in L Cells Infected with Mengovirus[1] (36474)

HENRY I. MILLER AND EDWARD E. PENHOET[2]
(Introduced by J. J. Holland)
Department of Biology, University of California, San Diego, La Jolla, California 92037

Soon after picornavirus infection of cells in tissue culture, the rate of cellular RNA synthesis begins to decline (1–4). In Mengovirus (murine poliovirus)-infected L cells, the decline is very marked, cellular RNA synthesis falling to less than 15% of uninfected controls within 2 hr of infection (see below and Ref. 5). It has been demonstrated that this deficiency is paralleled by an impairment in the function of the DNA-dependent RNA synthetic system (6, 7). Holland and Peterson have shown that DNA or deoxyribonucleoprotein extracted from poliovirus-infected cells serves as template for the *Escherichia coli* RNA polymerase as efficiently as the same materials from uninfected control cells. Franklin and Baltimore have shown in the Mengovirus system (2) and Holland and Peterson in the poliovirus system (7) that the RNA polymerase activity associated with a crude DNA–protein complex ("aggregate enzyme") from infected cells is reduced, compared to uninfected controls.

It is reported here that the marked depression of RNA synthesis in the nuclei of L cells infected with Mengovirus is paralleled by a rapid and preferential inhibition of nuclear RNA polymerase II, the enzyme which is localized in the cellular nucleoplasm. This nucleoplasmic enzyme is $Mn^{2+}/(NH_4)_2SO_4$-activated, is inhibited by low concentrations of α-amanitin, and synthesizes primarily "DNA-like" (messenger) RNA (8–10). There is also a postinfection decrease in the activity of RNA polymerase I (the nucleolar enzyme, active primarily in the transcription of the genes coding for ribosomal RNA (10)), but it is much less rapid and not as complete as for polymerase II until, perhaps, very late into infection. Preliminary evidence is cited which suggests that this slower loss of polymerase I activity is a secondary effect of viral inhibition of cellular protein synthesis.

Materials and Methods. Virus and infection of cells. A single stock of Mengovirus grown in monolayer cultures of L cells was employed for all the experiments reported here. Prior to infection, L cells were grown to confluent monolayers at 37° in Eagle's Minimal Essential Medium (MEM) containing 10% fetal calf serum. The medium was removed, the virus added in a small volume at a multiplicity of infection 50–100 pfu/cell and permitted to adsorb for 30 min at 37°. Unadsorbed virus was removed and serum-free MEM was added; this was designated time of infection (t_0). Unless otherwise indicated, cultures were then immediately incubated at 37°.

Fractionation of cells. This was performed essentially as described by Costanzo *et al.* (11), except that after swelling the cells in a hypotonic medium, homogenizing, and pelleting nuclei by centrifugation, the nuclei were resuspended in TGMEDA (50 m*M* Tris-HCl, pH 7.7; 25% (v/v) glycerol; 5 m*M* $MgCl_2$; 0.1 m*M* EDTA; 0.5 m*M* dithiothreitol; 20 m*M* $(NH_4)_2SO_4$) prior to assay for enzymatic activity or radioactivity.

Assay for RNA polymerases. The assay measured the incorporation of $[^3H]$UTP into an acid-insoluble product. The standard as-

---

[1] Supported by National Cancer Institute Grant No. CA10802.

[2] Present Address: Department of Biochemistry, University of California, Berkeley, Berkeley, California 94720.

say mixture, incubated 5 min at 37°, contained the following (in a volume of 0.065 ml): Tris–HCl, pH 7.6 (45 m*M*), 2-mercaptoethanol (2 m*M*), $MnCl_2$ (1.5 m*M*), $(NH_4)_2SO_4$ (70 m*M*), calf thymus DNA (0.25 mg/ml), ATP, GTP, CTP (each 0.5 m*M*), UTP (9 μM), [^{3}H]UTP (2 μCi; sp act 18.6 Ci/mmole), and enzyme. This assay detected incorporation by both polymerases I and II; inclusion in the assay of α-amanitin (10 μg/ml), a potent inhibitor of polymerase II, caused only the activity of polymerase I to be manifest. Thus, the activity of polymerase II was deducible as: (enzymatic activity without α-aminitin) — (enzymatic activity in presence of α-amanitin). Reactions were stopped by spotting 0.05 ml directly onto DE 81 paper circles which were processed for counting according to Blatti *et al.* (10).

Determination of RNA synthesis in infected cells. The rate of RNA synthesis was determined by a method similar to that of Costanzo *et al.* (11). Infected cell monolayers (and uninfected control cultures) were pulse-labeled with [^{3}H]uridine at various times after infection. The medium was removed and replaced with serum-free MEM containing 20 μCi/ml [^{3}H]uridine (sp act .026 mCi/mmole) and a 15–20-fold excess of unlabeled thymidine. After 15 min at 37°, incorporation was stopped by washing the cultures with ice-cold 0.15 *M* NaCl. Cell monolayers were then immediately processed as described above to obtain a nuclear and a cytoplasmic fraction. [^{3}H]RNA in these fractions was quantified by spotting aliquots on DE81 paper and processing according to Blatti *et al.* (10).

Protein and DNA determinations. Protein was quantitated by the method of Lowry *et al.* using BSA as a standard (12). Amounts of DNA were determined by means of the fluorimetric technique of LePecq and Paoletti (13).

Materials. [^{3}H]UTP and [^{3}H]uridine were obtained from Schwarz BioResearch. DNA (calf thymus) was purchased from Calbiochem. Whatman DE81 paper was obtained from H. Reeve Angel and Co.

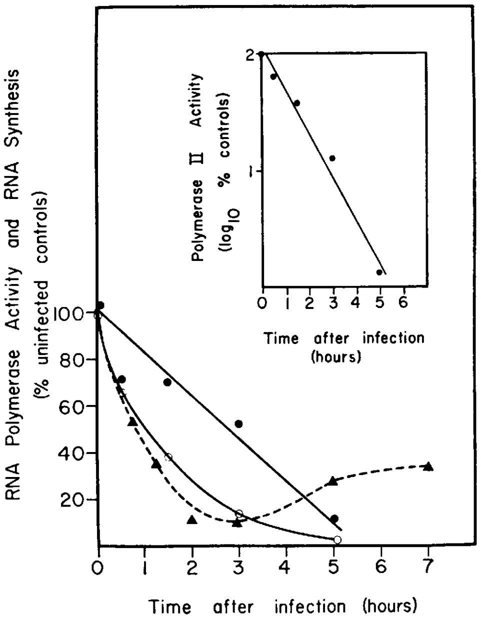

FIG. 1. Kinetics of RNA polymerases I and II and rate of RNA synthesis in the nuclei of L cells infected with Mengovirus. Nuclear fractions were prepared and assays performed as described in Methods. Counts per minute representing RNA polymerase activities and RNA synthesis were normalized to weight of DNA contained in each nuclei preparation prior to comparison of extracts from infected cells to those from uninfected controls. (In nuclear extracts from control cultures, RNA polymerase activities were in the range 100–200 pmoles [^{3}H]UMP/mg DNA.) Each time point represents the average value from two separate experiments. ●—●, Polymerase I activity; O—O, Polymerase II activity; ▲- - -▲, RNA synthesis. Insert: Polymerase II activity plotted as log$_{10}$ (% activity in uninfected controls) *vs.* time after infection.

Results. The kinetics of RNA synthesis and the activities of RNA polymerases I and II were measured in nuclei isolated from L cells after their infection by Mengovirus. It was found that while there is a loss of activity by both of these enzymes beginning very early during the course of infection, the kinetics of these inhibitions are quite different.

Figure 1 indicates that the precipitous decline in nuclear RNA synthesis during the 2 hr postinfection is closely followed by a de-

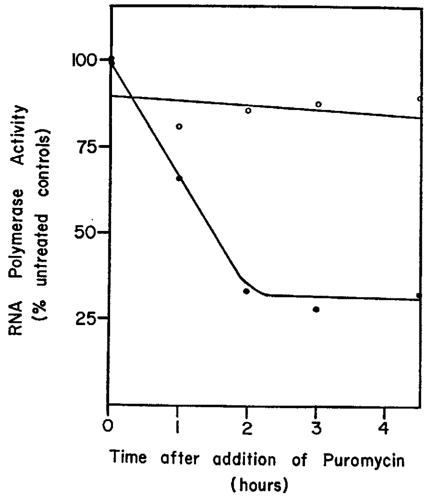

FIG. 2. Kinetics of RNA polymerases I and II in the nuclei of L cells after addition of puromycin. Medium was removed from all cultures at time = 0, and serum-free MEM was added to the control cultures and serum-free MEM containing 25 μg/ml puromycin to the experimental cultures. Details are as described in the legend to Fig. 1. ●—●, Polymerase I activity; ○—○, Polymerase II activity.

crease of similar magnitude in the activity of polymerase II. Plotting $\log_{10}$ (inhibition of polymerase II) *vs.* time after infection illustrates that activity decreases as a true exponential function (Fig. 1, insert).

Figure 1 demonstrates that the enzymatic activity of polymerase I decreases linearly to about 10% of the control values at 5 hr postinfection. It is possible that this decline in activity of the nucleolar enzyme is caused by degeneration of nucleoli, reflecting the pathogenic condition of the cell. However, several observations argue against this possibility. First, no morphological alteration is observed in nucleoli until late in infection and, even then, they become only denser and smaller with no apparent signs of disintegration (2). Second, if the disappearance of polymerase I is due simply to damage to its normal site of localization, one might expect that the activity could be recovered elsewhere, perhaps in the cytoplasm. However, in no case was significant polymerase I activity detected in cytoplasmic fractions; cytoplasmic polymerase I activity never exceeded 5% of that present in the nuclei (the same level of activity found in the cytoplasm of uninfected cells).

Figure 2 describes the kinetics of decay of RNA polymerase activities in L cells (uninfected) whose protein synthesis has been arrested by treatment with puromycin. While polymerase II activity is only slightly affected by puromycin treatment for up to 4.5 hr, polymerase I activity decreases linearly for several hours, then plateaus at approximately 30% of control values.

Discussion. The concomitance of loss of cellular RNA synthesis and polymerase II activity suggests that inhibition of this enzyme may be a primary mechanism mediating the impairment of the cell's RNA-synthesizing ability. The loss of polymerase II activity with first-order kinetics is apparently due to selective inactivation or degradation of this enzyme and not a secondary result of viral inhibition of protein synthesis since it occurs very rapidly after infection and in light of the experiments described in Fig. 2; these indicate that inhibition of protein synthesis results in the selective decay of polymerase I activity, while that of polymerase II is relatively unaffected. These findings are consistent with earlier work from this laboratory showing that puromycin preferentially inhibits the transcription of ribosomal RNA (14). Furthermore, the recent work of Franze-Fernandez and Pogo (15) corroborates the above observation and these authors conclude that puromycin does affect preferentially the function or synthesis of RNA polymerase I.

The results (Fig. 1) also indicate a slow, linear decrease in the activity of polymerase I, the nucleolar enzyme, after infection. The absence of obvious trauma to the nucleoli and the inability to recover polymerase I activity in the cytoplasm argue against nucleolar damage causing the loss of activity. Alternatively, the observed decrease in polymerase I activity may result from early viral inhibition of cellular mRNA and protein synthesis, if the enzyme itself or a related protein factor required for activity turns over at a high rate. The experiments cited above with cells whose protein synthesis has been arrested by puromycin support this latter hypothesis.

Summary. The rate of nuclear RNA synthesis in L cells infected with Mengovirus, as measured by [^{3}H]uridine incorporation, falls precipitously within several hours after infection. This decline in RNA synthesis is paralleled by the preferential, exponential inhibition of one of the two major species of nuclear RNA polymerase, polymerase II. While there is some decline after infection of the other major polymerase, the decay of its activity is less marked and less rapid and evidence is presented which suggests that it is a secondary result of viral inhibition of cellular protein synthesis.

---

1. Holland, J. J., Proc. Nat. Acad. Sci. U.S.A. **49**, 23 (1963).

2. Franklin, R. M., and Baltimore, D., Cold Spring Harbor Symp. Quant. Biol. **27**, 175 (1962).

3. Crocker, T. T., and Pfendt, E., Science **145**, 401 (1964).

4. Fenwick, M. L., Virology **19**, 241 (1963).

5. McCormick, W., and Penman, S., Virology **31**, 135 (1967).

6. Baltimore, D., and Franklin, R. M., Proc. Nat. Acad. Sci. U.S.A. **48**, 1383 (1962).

7. Holland, J. J., and Peterson, J. A., J. Mol. Biol. **8**, 556 (1964).

8. Roeder, R. G., and Rutter, W. J., Proc. Nat. Acad. Sci. U.S.A. **65**, 675 (1970).

9. Lindell, T. J., Weinberg, F., Morris, P. W., Roeder, R. G., and Rutter, W. J., Science **170**, 447 (1970).

10. Blatti, S. P., Ingles, C. J., Lindell, T. J., Morris, P. W., Weaver, R. F., Weinberg, F., and Rutter, W. J., Cold Spring Harbor Symp. Quant. Biol. **35**, 649 (1970).

11. Costanzo, F., LaPlaca, M., Palenzona, A. M., Novello, F., and Stirpe, F., Nature (London) **227**, 294 (1970).

12. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. **193**, 265 (1951).

13. LePecq, J.-B., and Paoletti, C., Anal. Biochem. **17**, 100 (1966).

14. Holland, J. J., Proc. Nat. Acad. Sci. U.S.A. **50**, 436 (1963).

15. Franze-Fernandez, M. T., and Pogo, A. O., Proc. Nat. Acad. Sci. U.S.A. **68**, 3040 (1971).

Received Sept. 20, 1971. P.S.E.B.M., 1972, Vol. 140.