Release of RNA Polymerase from Vero Cell Mitochondria after Herpes Simplex Virus Type 1 Infection

TATSUYA TSURUMI† AND I. R. LEHMAN*

Department of Biochemistry, Beckman Center, Stanford University, Stanford, California 94305-5307

Received 22 August 1989/Accepted 2 October 1989

Infection of Vero cells with herpes simplex virus type 1 results in the appearance in soluble extracts of a DNA primase activity. The partially purified enzyme, M_r , ~100,000, is identical in resistance to α -amanitin, pH profile, Mg^{2+} dependence, salt sensitivity, and K_m^{ATP} to the catalytic core of Vero cell mitochondrial RNA polymerase. Moreover, the products synthesized are those expected of an RNA polymerase rather than a DNA primase. Inasmuch as the enzyme is not present in soluble extracts of uninfected Vero cells, we presume that the specific appearance of RNA polymerase in extracts of herpesvirus-infected cells results from infection-induced disruption of the mitochondrial membrane, followed by release of the enzyme into the cytosol.

In a search for a herpesvirus-induced DNA primase, we observed a primase activity in nuclear extracts of herpes simplex virus type 1 (HSV-1)-infected Vero cells as judged by its ability to promote ATP-dependent synthesis of poly(dA) by the large fragment of *Escherichia coli* DNA polymerase I with poly(dT) as the template (2). No such activity was evident in comparable extracts of uninfected or mock-infected Vero cells. Upon partial purification of this activity, we found that it is not a herpesvirus-encoded DNA primase but is very likely the catalytic core of Vero cell mitochondrial RNA polymerase, presumably released from the mitochondria by herpesvirus-induced disruption of the mitochondrial membrane. A herpesvirus-induced primase described by Holmes et al. (5) has properties similar to those of the RNA polymerase described here and may be the same enzyme.

Identification of novel RNA polymerase in soluble extracts of HSV-1-infected Vero cells. When nuclei of HSV-1-infected Vero cells were extracted with a low-salt buffer (20 mM Tris hydrochloride pH 7.6) and chromatographed on a column of single-stranded DNA-agarose, a single peak of RNA poly-

merase activity appeared which eluted at 0.38 M KCl (Fig. 1). This activity was unaffected by concentrations of α -amanitin as high as 1 mg/ml; hence, it was not RNA polymerase II or III (10).

No RNA polymerase activity was detected upon low-salt extraction of nuclei from uninfected Vero cells. However, when the nuclei were extracted with 20 mM Tris hydrochloride buffer containing 1.7 M KCl and 0.2% Nonidet P-40 and chromatographed on a single-stranded DNA-agarose column, RNA polymerase activity appeared; however, it eluted at 0.54 M KCl. Moreover, it constituted less than 3% of the activity observed in the low-salt nuclear extracts of HSV-1-infected cells. This activity also differed from that observed in infected cells in its pH optimum and salt sensitivity (see below). Thus, infection of Vero cells with HSV-1 appeared to induce a novel α -amanitin-insensitive RNA polymerase activity.

The RNA polymerase activity was purified by chromatography on single-stranded DNA-agarose and heparin-agarose and by Superose 12 gel filtration. On the basis of its position of elution from the Superose 12 column relative to a series of marker proteins, we estimated that the molecular weight of the RNA polymerase was approximately 100,000. Chromatography of the heparin-agarose fraction on a Mono Q column gave a single peak of RNA polymerase activity that coincided with a polypeptide with a molecular weight of

FIG. 1. Chromatography of low-salt extract of HSV-1-infected Vero cell nuclei on single-stranded DNA-agarose.

* Corresponding author.

† Present address: Laboratory of Virology, Institute for Disease Mechanism and Control, Nagoya University School of Medicine, Nagoya, Japan.

TABLE 1. Characteristics of α -amanitin-insensitive RNA polymerase activity^a

Omission or addition	dAMP incorporated (% of maximum)
None (complete)	100
Omit enzyme	0
Omit (dT) ₆₀₀₀	0
Omit ATP	1
Omit Mg^{2+}	0
Omit Mg^{2+} plus Mn^{2+} (2 mM)	38
Add α -amanitin (1 mg/ml)	95
Add rifampin (50 μ g/ml)	110
Add <i>N</i> -ethylmaleimide (4 mM)	7
Add NaCl (50 mM)	9

^a The RNA polymerase activity of the Superose 12 fraction was measured by using the coupled assay described by Conaway and Lehman (2). A value of 100% represents 19 pmol of dAMP incorporated per h with (dT)₆₀₀₀ as the template.

FIG. 2. (A) Optimal conditions for RNA polymerase purified from HSV-1-infected Vero cells. The RNA polymerase activity of the Superose 12 fraction was measured with the coupled assay (2), except that the pH and the ATP, NaCl, and MgCl₂ concentrations were varied as indicated. The activity of the DNA polymerase I large fragment with poly(dT)-oligo(dA) as the template primer was not significantly affected by the changes in conditions shown. The 100% value was 52 pmol of dAMP incorporated. (B) Optimal conditions for mitochondrial RNA polymerase. RNA polymerase was partially purified from uninfected Vero cells as described by Walberg and Clayton (13) and assayed as for panel A.

100,000 (8). Several other polypeptides were present in smaller amounts; hence, it is not certain that enzymatic activity is associated with the 100,000-molecular-weight polypeptide.

The Superose 12 fraction of RNA polymerase was essentially free of DNase, DNA-dependent ATPase, and DNA polymerase activities.

Characterization of RNA polymerase activity. The properties of the RNA polymerase, i.e., pH profile and effects of mono- and divalent cations and ATP concentration, are shown in Fig. 2A and Table 1. The enzyme exhibited a high pH optimum, between pHs 8.7 and 9.3. It was very salt sensitive; 50% inhibition occurred at 20 mM NaCl. Activity was completely suppressed by addition of 4 mM *N*-ethylmaleimide but was insensitive to 1 mg of α -amanitin per ml. These characteristics are very similar to those observed for the catalytic core of the mitochondrial RNA polymerase partially purified from cultured Vero cells (Fig. 2B).

Analysis of products. The products synthesized by the RNA polymerase with (dT)₆₀₀₀ as the template in the presence of [³²P]ATP and unlabeled dATP were analyzed by

FIG. 3. Products of RNA polymerase synthesis. Products were synthesized with the Superose 12 fraction with M13 single-stranded (ss) DNA (lanes 1 to 3), (dT)₆₀₀₀ (lanes 4 and 5), poly(dA-dT) (lane 6), and native calf thymus (CT) DNA (lane 7) as the templates; urea-10% polyacrylamide gel electrophoresis was performed as previously described (8). Lanes: 1, 400 μ M [³²P]ATP, 100 μ M CTP, 100 μ M GTP, and 100 μ M UTP; 2, 400 μ M [³²P]ATP; 3, 400 μ M ATP, 100 μ M [³²P]dCTP, 100 μ M [³²P]dGTP, and 100 μ M [³²P]dTTP; 4, 400 μ M [³²P]ATP and 100 μ M dATP; 5, 400 μ M ATP and 100 μ M [³²P]dATP; 6 and 7, 400 μ M ATP, 100 μ M CTP, 100 μ M GTP, and 10 μ M [³²P]UTP.

urea-10% polyacrylamide gel electrophoresis. An alkali-sensitive poly(rA) ladder was generated, and each oligonucleotide differed in length by one nucleotide, extending from 15 to greater than 100 residues (Fig. 3, lane 4). There was no detectable incorporation of [³²P]dATP (lane 5). The same distribution of products appeared without dATP.

The products of the reaction with M13 single-stranded DNA as the template were also analyzed. In the presence of all four ribonucleoside triphosphates and [³²P]ATP, a series of oligonucleotides ranging in size from less than 15 to greater than 80 residues was synthesized (Fig. 3, lane 1); however, the predominant products were in the range of 30 to 60 nucleotides. In the presence of ATP alone, the products (lane 2) formed a series of oligonucleotides extending

from less than 10 to 30 residues. Thus, the enzyme can synthesize poly(rA) with M13 single-stranded DNA as the template, presumably by the reiterative mechanism that has been described for *E. coli* RNA polymerase and DNA polymerase I (7). Deoxynucleotides could not replace ribonucleotides as substrates (Fig. 3, lane 3). Finally, the enzyme used the alternating duplex copolymer poly(dA-dT) as a template (lane 6) but was unable to use native calf thymus DNA (lane 7). These properties are all characteristic of an RNA polymerase rather than a DNA primase (1, 2, 6, 11, 12, 14, 15).

Thus, our attempts to detect a primase specifically induced upon infection of Vero cells with HSV-1 led to the identification and partial purification of an RNA polymerase activity that can catalyze the synthesis of a ribo-oligonucleotide capable of priming poly(dA) synthesis by the large fragment of *E. coli* DNA polymerase I on a poly(dT) template. Although this assay has served to identify the eucaryotic DNA primases associated with DNA polymerase α (2), it can also be used to assay RNA polymerase activity (1, 6).

The RNA polymerase activity that we identified was present only in nuclear extracts of HSV-1-infected cells, and therefore it appears to be induced by herpesvirus infection. However, it is clearly not encoded by the HSV-1 genome. Thus, Western blotting (immunoblotting) analysis of enzyme fractions obtained after chromatography on single-stranded DNA-agarose and heparin-agarose and Superose-12 gel filtration with antisera against the UL5, UL8, and UL52 gene products showed that they were immunologically unrelated to these polypeptides which comprise the herpesvirus-encoded helicase-primase (3; T. Tsurumi and I. R. Lehman, unpublished data). The four remaining HSV-1-encoded enzymes required for HSV-1 DNA replication, i.e., the origin-binding protein (UL9) (4), the DNA polymerase-UL42 complex (J. J. Crute and I. R. Lehman, *J. Biol. Chem.*, in press), and ICP8 (13), do not have primase or RNA polymerase activity (J. J. Crute and I. R. Lehman, unpublished data).

It appears, therefore, that the RNA polymerase that we identified in extracts of herpesvirus-infected Vero cells results from induction of a preexisting host enzyme. Of the six candidates, RNA polymerases I, II, and III, the host DNA primase (normally associated with host DNA polymerase α), the mitochondrial DNA primase, and the mitochondrial RNA polymerase, only the catalytic core of mitochondrial RNA polymerase corresponds to the RNA polymerase activity that we identified.

Cytological examination of HSV-1-infected Vero cells has revealed profound changes in the structure of subcellular organelles (9). In particular, mitochondria appear to be distended and abnormal in shape. Thus, it is possible that the mitochondrial membrane is altered such that the soluble mitochondrial RNA polymerase that we observed in low-salt nuclear extracts of HSV-1-infected cells may result from association of a portion of the enzyme released from the mitochondria with the nuclear fraction. In uninfected cells, the mitochondrial membrane remains intact and the enzyme would not be expected to appear in nuclear or cytosolic extracts. A less likely possibility is that herpesvirus infection of Vero cells results in enhanced transcription of the mitochondrial RNA polymerase gene.

Holmes et al. have partially purified a DNA primase activity from extracts of HSV-1-infected Vero cells that is

similar to the mitochondrial RNA polymerase that we have described (5). Although it differs in molecular weight (40,000 versus 100,000), pH optimum (pH 8.0 to 8.7 versus 8.7 to 9.3), and salt sensitivity, these differences may be more apparent than real, reflecting the relative states of purification of the two enzymes.

We are grateful to David A. Clayton, Caroline M. Kane, and Edward S. Mocarski for helpful discussions and Melissa Parisi for excellent technical assistance.

This research was supported by Public Health Service grant AI26538 from the National Institutes of Health.

LITERATURE CITED

1. Chambon, P. 1974. Eucaryotic RNA polymerases, p. 261-332. In P. D. Boyer (ed.), *The enzymes*, vol. X. Academic Press, Inc., New York.
2. Conaway, R. C., and I. R. Lehman. 1982. A DNA primase activity associated with DNA polymerase α from *Drosophila melanogaster* embryos. *Proc. Natl. Acad. Sci. USA* **79**:2523-2527.
3. Crute, J. J., T. Tsurumi, L. Zhu, S. K. Weller, P. D. Olivo, M. D. Challberg, E. S. Mocarski, and I. R. Lehman. 1989. Herpes simplex virus 1 helicase-primase: a complex of three herpes-encoded gene products. *Proc. Natl. Acad. Sci. USA* **86**:2186-2189.
4. Elias, P., M. E. O'Donnell, E. S. Mocarski, and I. R. Lehman. 1986. A DNA binding protein specific for an origin of replication of herpes simplex virus type 1. *Proc. Natl. Acad. Sci. USA* **83**:6322-6326.
5. Holmes, A. M., S. M. Wietstock, and W. T. Ruyechan. 1988. Identification and characterization of a DNA primase activity present in herpes simplex virus type 1-infected HeLa cells. *J. Virol.* **62**:1038-1045.
6. Kelly, J. L., and I. R. Lehman. 1986. Yeast mitochondrial RNA polymerase: purification and properties of the catalytic subunit. *J. Biol. Chem.* **261**:10340-10347.
7. Kornberg, A. 1980. DNA replication. W. H. Freeman & Co., San Francisco.
8. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* **227**:680-685.
9. Roizman, B., and D. Furlong. 1974. The replication of herpesviruses, vol. 3, p. 229-403. In H. Fraenkel-Conrat and R. R. Wagner (ed.), *Comprehensive virology*. Plenum Publishing Corp., New York.
10. Schwartz, L. B., V. E. F. Sklar, J. A. Jaehning, R. Weinmann, and R. G. Roeder. 1974. Isolation and partial characterization of the multiple forms of deoxyribonucleic acid-dependent ribonucleic acid polymerase in the mouse myeloma, M09C 315. *J. Biol. Chem.* **249**:5889-5897.
11. Walberg, M. W., and D. A. Clayton. 1983. In vitro transcription of human mitochondrial DNA: identification of specific light strand transcripts from the displacement loop region. *J. Biol. Chem.* **258**:1268-1275.
12. Wang, T. S.-F., S.-Z. Hu, and D. Korn. 1984. DNA primase from KB cells: characterization of a primase activity tightly associated with immunoaffinity purified DNA polymerase α . *J. Biol. Chem.* **259**:1854-1865.
13. Weller, S. K., K. J. Lee, D. J. Sabaurin, and P. A. Schaffer. 1983. Genetic analysis of temperature-sensitive mutants which define the gene for the major herpes simplex type 1 DNA-binding protein. *J. Virol.* **45**:354-366.
14. Wong, T. W., and D. A. Clayton. 1985. Isolation and characterization of a DNA primase from human mitochondria. *J. Biol. Chem.* **260**:11530-11535.
15. Wong, T. W., and D. A. Clayton. 1986. DNA primase of human mitochondria is associated with structural RNA that is essential for enzymatic activity. *Cell* **45**:817-825.