THE JOURNAL OF INVESTIGATIVE DERMATOLOGY
Vol. 60, No. 3

Printed in U.S.A.

PROPERTIES OF RNA POLYMERASES FROM B16 MELANOMA*

I. A. MENON, PH.D., AND H. F. HABERMAN, M.D., F.R.C.P.(C)

ABSTRACT

Some of the properties of RNA polymerases from melanotic and amelanotic B16 melanomas have been studied. Chromatin-bound RNA polymerase is activated by Mg^{++} more than Mn^{++}. Addition of ammonium sulfate was found to activate the enzyme. Preincubation with phospholipase A or phospholipase C as well as extraction with ether or iso-octane decreased the RNA polymerase activity in the presence of Mg^{++} but not that in the presence of Mn^{++}. The polymerase activity in the presence of high salt concentration (ammonium sulfate) was not affected by the phospholipases or by solvent extraction. Preincubation in the presence of trypsin was found to activate enzymatic activity in the presence of Mn^{++} to a greater extent than in the presence of Mg^{++}

RNA polymerase activity of melanoma mitochondria was decreased by treatment with phospholipase A or phospholipase C as well as by extraction with ether or iso-octane. Phospholipase D as well as wheat-germ lipase did not have any effect on the RNA polymerase activities of nuclei or mitochondria. α-amanitin was found to inhibit the nuclear RNA polymerase activity in the presence of Mn^{++} at high salt concentration but not in the presence of Mg^{++}. The RNA polymerase activity of melanoma mitochondria was not inhibited by α-amanitin. The RNA polymerase activity in melanoma nuclei and mitochondria was inhibited by actinomycin D but rifamycin and rifampicin did not have any inhibitory action. Of the various tissues studied, the properties of the RNA polymerase from melanoma closely resembled those of the RNA polymerases from liver.

RNA polymerase activity in mammalian cells is present mostly in the nuclei [1] mainly associated with nucleoli and extranucleolar chromatin [2]. Recent studies have demonstrated that considerable RNA polymerase activity is also present in mitochondria [3, 4]. The RNA polymerase in mammalian cells is present mostly in a membrane-bound form, in contrast to the extensively studied enzymes from microorganisms. The RNA polymerase from several mammalian tissues has been solubilized and partially purified. These studies have revealed the presence of two and in some cases three different forms of the enzyme in certain mammalian cells. Such partially purified preparations have been employed for the study of the regulatory mechanisms involved in the synthesis of RNA in mammalian cells. Some of these studies have pointed out difficulties in correlating the properties of the solubilized RNA polymerase with the activity of the enzyme in vivo [5–8].

Further studies have demonstrated the presence in certain mammalian cells of a factor which specifically enhanced the activity of one form of RNA polymerase [7, 8]. Although studies using solubilized RNA polymerase preparations may be expected to provide valuable information regarding the various forms of the enzyme, it is important to take into consideration the manner in which native enzyme is associated with chromatin. Recent studies from this laboratory have indicated that the activities of RNA polymerase from several mammalian tissues are significantly affected by the degradation of phospholipids associated with chromatin-bound RNA polymerase complex [9–11].

It is well known that tumors in general have an altered pattern of nucleic acid metabolism [13–18]. It has been generally observed that most tumor tissues have an increased rate of RNA synthesis. In general, tumors also have enlarged nucleoli [2, 16] and higher ratio of nucleolar RNA synthesis to RNA synthesis in extranucleolar nucleoplasm [15–17]. Hepatoma cells have been reported to contain a more diverse selection of RNA molecules than are present in normal liver [14]. Nuclear RNA polymerases isolated from spontaneous brain tumors have been found to have different substrate requirements in comparison to normal brain [18]. The available data indicate that there is a wide diversity in the properties of the RNA synthesizing system among different tumors. Melanotic and amelanotic melanomas have been widely used in studies as a tumor and as a melanin synthesizing system. To our knowledge, RNA polymerase from melanomas has not been studied in any great detail. The results described in this communication detail some of the properties of nuclear and mitochon-

Manuscript received April 25, 1972; in revised form November 3, 1972; accepted for publication November 8, 1972.

This work was supported by the Ontario Cancer Treatment and Research Foundation. The electron microscopic studies were supported by a grant from the Medical Research Council of Canada (MA4038). Dr. Haberman is a Fellow of the Ontario Cancer Treatment and Research Foundation.

* From the Clinical Science Division, University of Toronto, Toronto 181, Ontario, Canada.

drial RNA polymerases from transplantable B16 melanotic and amelanotic melanomas.

MATERIALS AND METHODS

Chemicals. ATP, CTP, GTP, and UTP (all as sodium salts), phospholipase C, wheat-germ lipase, and phospholipase D were obtained from Sigma Chemical Company. Trypsin was obtained from Calbiochem. [^{3}H]-CTP was purchased from Schwarz/Mann (specific activity 20 Ci/mmole). Purified phospholipase A was prepared as described previously [9].

Transplantation of tumor. Melanotic and amelanotic B16 melanomas were originally obtained from Dr. A. Kopf, New York University Medical Center, New York, N. Y. They were maintained by serial transplantation into C57 BL/6J mice as described previously [16].

Preparation of nuclear RNA polymerase. Samples of melanoma were homogenized in 10 volumes of 0.25 M sucrose containing 0.01 M Tris-HCl buffer, pH 7.5. The homogenates were filtered through four layers of Miracloth. The filtrates were centrifuged at 600 *g* for 10 minutes. The sediments were washed three times by resuspending in 0.25 M sucrose and centrifuging at 600 *g* for 10 minutes. The sediments were finally suspended in 0.05 M Tris-HCl buffer, pH 7.5, and kept in ice for 30 minutes. The suspensions were centrifuged at 10,000 *g* for 15 minutes. The supernatant fractions were saved and used as soluble RNA polymerase. The sediments were suspended with homogenization in 0.05 M Tris-HCl buffer, pH 8.1. These suspensions were employed as a source of chromatin-bound RNA polymerase.

Preparation of mitochondria. Mitochondria from melanoma were prepared by the method of Schneider and Hogeboom [20]. The purity of these preparations was checked by electron microscopic studies and enzyme assays. The washed mitochondria were suspended in 0.05 M PO_4 buffer, pH 8.1, and incubated at 37° C for 15 minutes to increase permeability as recommended by Schmerling [21].

Pretreatment of polymerase preparations with phospholipase A. In experiments where the enzyme was treated with phospholipase, 0.2 ml of enzyme preparation was incubated with 0.05 ml phospholipase solution at the appropriate concentration. Control tubes contained 0.05 ml water instead of the phospholipase solution. The incubation was carried out at 37° C for 1 hour. At the end of this time the tubes were placed in ice and immediately employed for the RNA polymerase assay.

Extraction of RNA polymerase. The RNA polymerase preparations were extracted three times with 5 volumes of ether. Traces of ether in the aqueous phase were removed by bubbling nitrogen. The volume of the aqueous phase was adjusted to the original volume with 0.05 M Tris-HCl buffer, pH 8.1, and mixed thoroughly by homogenization. The extraction with iso-octane was carried out in a similar manner.

Assay of RNA polymerase. RNA polymerase activity was determined by measuring the incorporation of radioactive CMP into RNA. The usual incubation medium contained 100 mM Tris-HCl buffer, pH 8.1; 1 mM of ATP, GTP, and UTP; 1 μM CTP containing 1 μc [^{3}H]-CTP; 3 mM mercaptoethanol; and enzyme as specified. Total volume was 0.5 ml. Mg^{++} or Mn^{++} was added as specified in the legends. Incubation was carried out at 37° C for 10 minutes. The incorporation of [^{3}H]-CMP was determined as described previously [10]. When ammonium sulfate or other salts were added, they were added after the pretreatment; the RNA polymerase reaction was started by the addition of a mixture of nucleoside triphosphates. When soluble RNA polymerase was employed, the assay system also contained 300 μg denatured calf-thymus DNA. In agreement with previous reports, the RNA polymerase activities of particulate preparations were not further increased by addition of native or denatured calf-thymus DNA. Therefore the standard assay medium for these preparations did not contain added DNA.

RESULTS

Properties of soluble and chromatin-bound RNA polymerases from melanoma. Some elementary characteristics of the soluble and chromatin-bound RNA polymerases from melanoma are shown in Table I. When the three nucleotides ATP, GTP, and UTP were omitted, the incorporation of radioactive CMP was decreased to approximately 8 percent of that in the complete system. The radioactivity present in the acid-insoluble precipitate could be completely extracted by perchloric acid at 90° C as well as by KOH at 37° C. When soluble RNA polymerase was employed, the incorporation of radioactivity was decreased to approximately 10 percent when DNA was not added to the system. The polymerase activity was also inhibited by actinomycin D. These results show that the incorporation of

TABLE I

Properties of RNA polymerase from melanoma nuclei

Enzyme source[a]	RNA polymerase system[b]	Extraction of RNA[c]	CMP[d]
Nuclei	Complete system	Procedure I	1.90
Nuclei	ATP, GTP and UTP omitted	Procedure I	0.22
Nuclei	Complete system	Procedure II	1.85
Nuclei	Complete system	Procedure III	1.95
Soluble enzyme	Complete system	Procedure I	1.56
Soluble enzyme	DNA omitted	Procedure I	0.15
Soluble enzyme	20 μg actinomycin D added	Procedure I	0.48

[a] Nuclei containing 0.9 mg protein or soluble enzyme containing 1.7 mg protein were employed.

[b] Complete system as described under *Materials and Methods*. 10 mM Mg^{++} was added. When soluble enzyme was employed, 300 μg of denatured calf-thymus DNA were added per tube.

[c] Procedure I: as described previously [10]. Procedure II: The precipitate was washed with TCA and ethanol as in Procedure I. The precipitate was then extracted twice with 5% perchloric acid at 90° C for 20 min. 0.3 ml of the combined extract was employed for measurement of radioactivity as previously described [19]. Procedure III: The precipitate was washed with TCA and ethanol as in Procedure I. The precipitate was incubated with 1 N KOH at 37° C for 2 hr, and centrifuged. The precipitate was once more extracted with 1 N KOH. The combined extracts were employed for measurement of radioactivity as in [19].

[d] The results are described in terms of p moles of [^{3}H]-CMP incorporated per incubation.

radioactivity by the soluble polymerase is dependent on the presence of all of the four nucleotides, and that the reaction is dependent upon DNA. These data are in agreement with the properties of DNA-dependent RNA polymerases from other sources.

Effects of ions and ammonium sulfate on the activity of chromatin-bound RNA polymerase from melanoma. The effects of various concentrations of Mg^{++} and Mn^{++} and ammonium sulfate on polymerase activity are shown in Figure 1. It was found that in the absence of either Mg^{++} or Mn^{++}, only very low RNA polymerase activity could be detected. The RNA polymerase activity was considerably enhanced by addition of Mg^{++}. Since salts such as ammonium sulfate have been reported to produce considerable changes in the RNA polymerase activity from several normal tissues [10], the effects of ammonium sulfate on melanoma RNA polymerase were investigated. The RNA polymerase activity in the presence of Mg^{++} was slightly decreased by 40 mM ammonium sulfate; addition of 400 mM ammonium sulfate decreased the polymerase activity to a still greater extent. Mn^{++} was much less effective than Mg^{++} in supporting the RNA polymerase activity of this preparation. Addition of 40 mM ammonium sulfate did not have any effect on the RNA polymerase activity. However, addition of 400 mM ammonium sulfate enhanced the RNA polymerase activity by approximately 100 percent. NaCl and KCl were found to have similar effects to ammonium sulfate in this system.

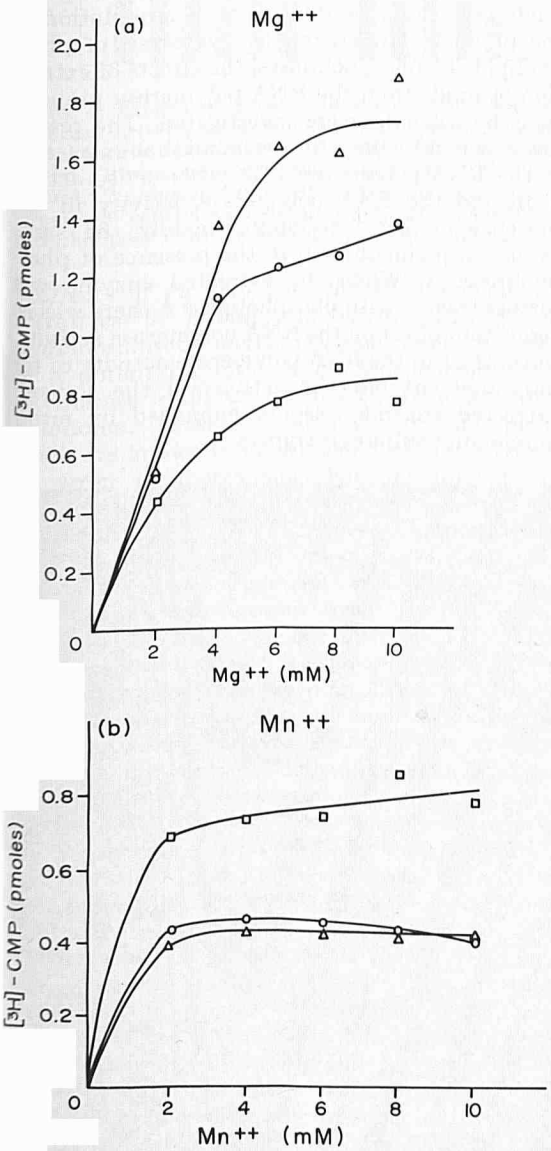

FIG. 1. Effects of ions and ammonium sulfate on the activity of chromatin-bound RNA polymerase from melanoma. 0.2 ml enzyme containing 0.7 to 1.0 mg protein was used. RNA polymerase activity was determined as described under *Materials and Methods*. The results are expressed in terms of [^{3}H]-CMP incorporated per tube. (a) Mg^{++}; (b) Mn^{++}. Δ——Δ: no ammonium sulfate; O——O: 40 mM ammonium sulfate; □——□: 400 mM ammonium sulfate.

Effects of phospholipases on the activity of chromatin-bound RNA polymerase from melanoma. Some of the properties of chromatin-bound RNA polymerase from B16 melanoma are shown in Figure 2. Previous reports have shown that treatment with phospholipases produces drastic changes in the RNA polymerase activities of several tissues [9–11]. Preincubation of the melanoma RNA polymerase in the presence of phospholipase A or phospholipase C was found to decrease the RNA polymerase activity in the presence of Mg^{++} by approximately 65 and 53 percent respectively. However, phospholipase D did not have any effect on this system. When the RNA polymerase activity was determined in the presence of Mg^{++} and 400 mM ammonium sulfate, no further inhibition by phospholipases

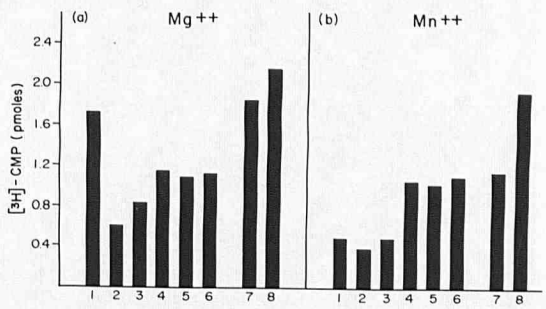

FIG. 2. Properties of chromatin-bound RNA polymerase from melanoma. 0.2 ml enzyme was mixed with 0.05 ml of a solution of phospholipase A (0.05 units), phospholipase C (50 μg) or trypsin (5 μg) as stated below. Control tubes contained 0.05 ml water. The samples were preincubated at 37° C for 1 hr. The RNA polymerase activity was determined immediately after preincubation. The results are expressed in terms of [^{3}H]-CMP incorporated per tube. (a) 10 mM Mg^{++}; (b) 10 mM Mn^{++}. 1: control; 2: phospholipase A; 3: phospholipase C; 4: control with 400 mM ammonium sulfate; 5: phospholipase A with 400 mM ammonium sulfate; 6: phospholipase C with 400 mM ammonium sulfate; 7: control; 8: trypsin.

could be observed. The RNA polymerase activity in the presence of Mn^{++} was not significantly affected by preincubation in the presence of phospholipase A or phospholipase C. Previous results have shown that RNA polymerase activity in liver and brain were increased by treatment with trypsin. The results in Figure 2 show that the melanoma RNA polymerase activity in the presence of Mg^{++} or Mn^{++} was enhanced by treatment with trypsin, although the activity in the latter system was increased to a considerably greater extent.

Effects of phospholipases on the RNA polymerase activity in melanoma mitochondria. Freshly prepared mitochondria were employed to study the purity of the mitochondrial preparations. Electron microscopic studies showed that the above preparations from melanotic melanoma contained mostly mitochondria and melanosomes, while those from amelanotic melanoma contained mostly mitochondria. Some lysosome-like structures were present, but there was no significant contamination by nuclei (see Fig. 3). These preparations also had high cytochrome oxidase, succinic oxidase, pyruvic oxidase, and glutamic oxidase activities. Figure 4 shows the effects of preincubation in the presence of phospholipases on the RNA polymerase activity of melanoma mitochondria. It was found that incubation in the presence of phospholipase A or phospholipase C decreased the RNA polymerase activity of mitochondria in the presence of either Mg^{++} or Mn^{++}. Addition of 400 mM ammonium sulfate did not affect these inhibitory effects. Preincubation in the presence of trypsin under similar conditions to those used for the nuclei (Fig. 2) did not enhance the RNA polymerase activity of mitochondria.

Effects of extraction with solvents upon the activities of nuclear and mitochondrial RNA polymerase from melanoma. In order to investigate the possibility that the above effects of the phospholipases could be due to the accumulation of the products formed by the hydrolysis of phospholipids by phospholipases, the effects of extraction of lipids from the RNA polymerase preparations by solvents, were investigated. The results are shown in Figure 5. It was found that extraction of the RNA polymerase with ether or iso-octane decreased the RNA polymerase activity in the presence of Mg^{++} to approximately the same extent as preincubation in the presence of phospholipase A. When the extracted enzyme was further treated with phospholipase A there was no significant effect on the RNA polymerase activity. In contrast to the RNA polymerase activity of the untreated enzyme, the activity of the solvent-extracted enzyme was not enhanced by either ammonium sulfate or trypsin.

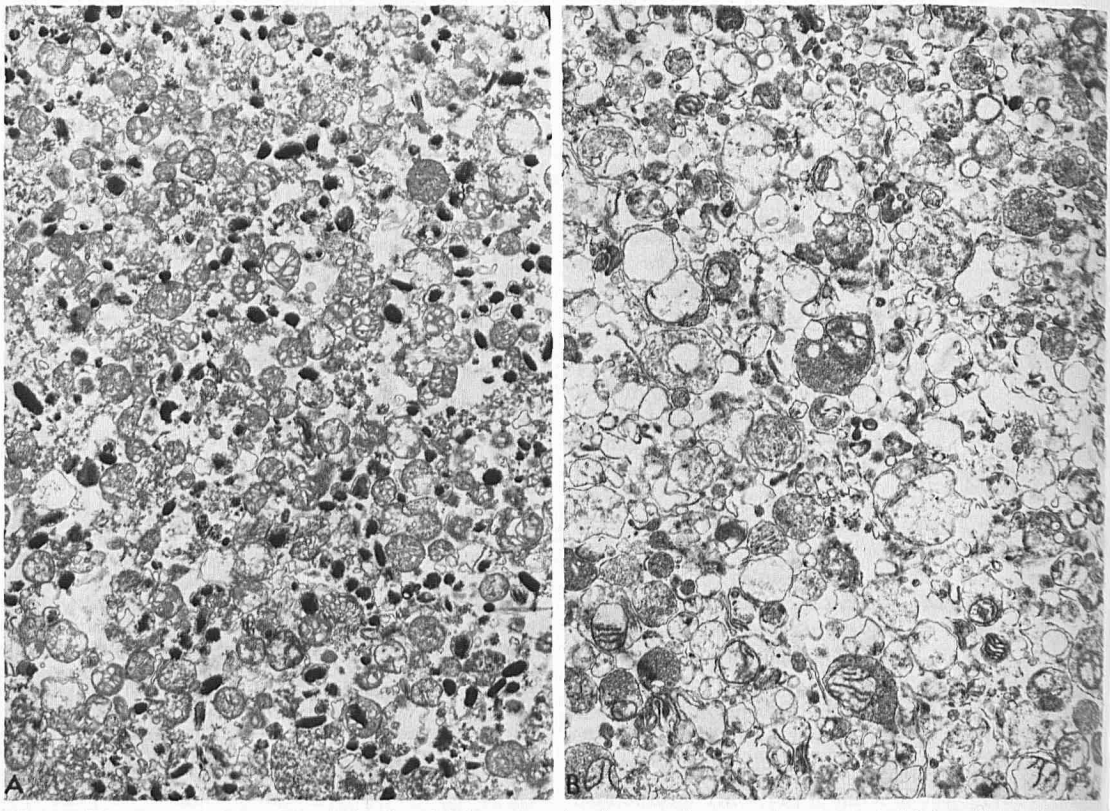

FIG. 3. Electron micrographs of mitochondrial preparation from melanoma. × 17,000. A. Melanotic melanoma; B. Amelanotic melanoma.

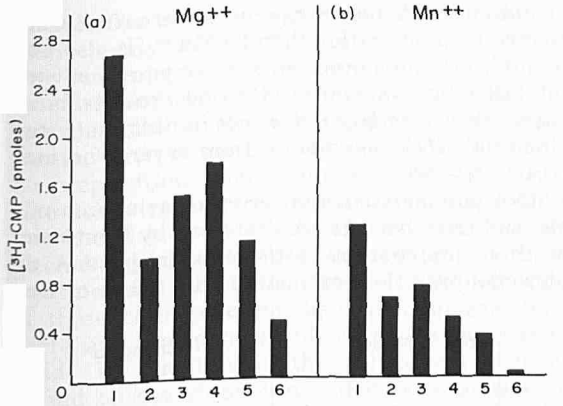

FIG. 4. Effects of phospholipases on the RNA polymerase activity in melanoma mitochondria. 0.2 ml mitochondria containing 1.2–1.5 mg protein was employed. Other conditions were the same as in Fig. 2. 1: control; 2: phospholipase A; 3: phospholipase C; 4: trypsin; 5: control with 400 mM ammonium sulfate; 6: phospholipase A with 400 mM ammonium sulfate.

Effects of inhibitors on the RNA polymerase activities in nuclei and mitochondria from melanoma. Actinomycin D which is well known to be a potent inhibitor of DNA-dependent RNA polymerase inhibited the RNA polymerase from melanoma. However, rifamycin or rifampicin, which are known to inhibit soluble RNA polymerase from microorganisms but not from mammalian cells [26], did not have any effect on the polymerase activity (Table II). α-amanitin has been reported to inhibit one of the nuclear RNA polymerases but not to affect the RNA polymerase activity of mitochondria from several tissues [24–26]. The effects of α-amanitin upon the RNA polymerase activities in nuclei and mitochondria from melanoma are shown in Figure 6. Minute quantities of α-amanitin were found to inhibit the nuclear RNA polymerase activity in the presence of Mn^{++} and ammonium sulfate. Much higher concentrations of this compound did not have any effect on the RNA polymerase activity of nuclei in the *presence* of Mg^{++} or Mn^{++} at low ionic concentration. The RNA polymerase activity of melanoma mitochondria under any of the above conditions was not inhibited by α-amanitin.

Effects of phospholipases on soluble RNA polymerases from melanoma nuclei. Soluble RNA polymerase from melanoma nuclei was prepared by lysis of the nuclei in hypotonic medium as described under *Materials and Methods*. When these preparations were preincubated in the presence of phospholipase or extracted with solvents, the RNA polymerase activity was not altered.

Properties of RNA polymerases from melanotic and amelanotic melanomas. The experiments described above were carried out using RNA polymerase preparations from melanotic and amelanotic B16 melanomas. It was found that in all these experiments similar results were obtained

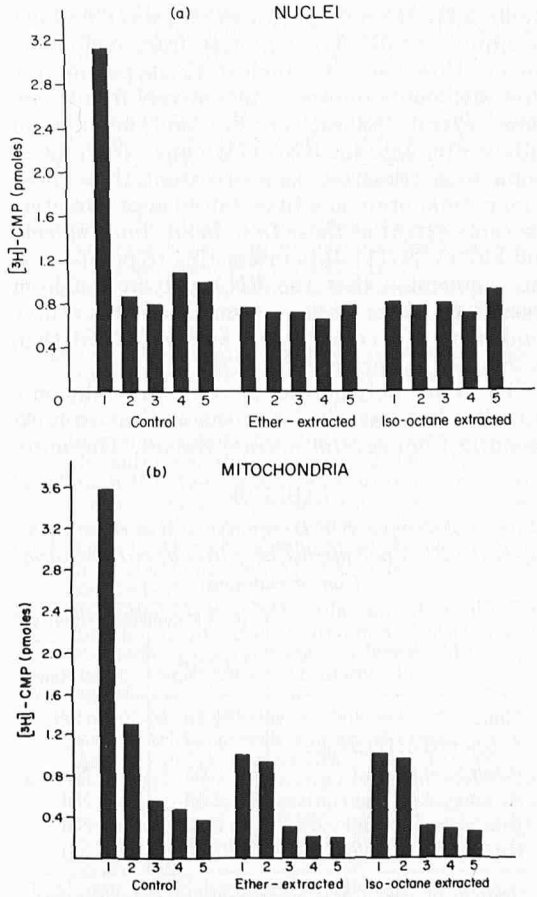

FIG. 5. Effects of extraction with solvents upon the activities of nuclear (a) and mitochondrial (b) RNA polymerases from melanoma. The nuclear and mitochondrial enzymes were extracted with ether or isooctane as described under *Materials and Methods*. The conditions for preincubation and RNA polymerase assay were the same as in Fig. 2. 1: control with Mg^{++}; 2: phospholipase A with Mg^{++}; 3: control with Mn^{++}; 4: trypsin with Mn^{++}; 5: control with Mn^{++} and 400 mM ammonium sulfate.

with the RNA polymerases from these two types of melanomas. In view of these findings, only the results from experiments with amelanotic tumors have been presented.

DISCUSSION

A comparison of the properties of RNA polymerases from melanoma with those of the RNA polymerases from several other tumors is shown in Table III. In several respects the RNA polymerases of melanoma nuclei resemble the nuclear RNA polymerases from normal tissues. For example, the RNA polymerase activity of nuclei from melanoma is activated both by Mg^{++} and Mn^{++} and is enhanced by ammonium sulfate and trypsin. Actinomycin D, which is known to be a potent inhibitor of DNA-dependent RNA polymerase, was found to inhibit the melanoma RNA polymerase. Similarly, rifamycin which does not inhibit

mammalian RNA polymerases [23] was found not to inhibit the RNA polymerase from melanoma nuclei. However, the nuclear RNA polymerase from melanoma differs quantitatively from those from several tissues. Trypsin and ammonium sulfate stimulate the RNA polymerase from melanoma to a relatively smaller extent than those from normal brain and liver, but to approximately the same extent as those from heart, lung, spleen, and kidney [9–11]. It is interesting to point out in this connection that the RNA polymerase from regenerating liver nuclei is stimulated under these conditions to a considerably smaller extent than in the case of normal liver [22].

The RNA polymerase activity in melanoma mitochondria was similar to the enzyme in mitochondria from several normal tissues. The mitochondrial RNA polymerase is preferentially activated by Mg^{++} rather than by Mn^{++}. It also was found that the mitochondrial enzyme was not inhibited by α-amanitin. Previous results have shown that α-amanitin does not inhibit the mitochondrial RNA polymerase from several normal tissues [24–26].

RNA polymerase activities from melanoma nuclei and mitochondria are decreased by treatment of these preparations with phospholipase A or phospholipase C or extraction with ether or iso-

TABLE II

Effects of actinomycin D, rifamycin, and rifampicin upon the RNA polymerase activities in mitochondria from melanoma

Compound	RNA Polymerase Activity	
	CMP[a]	% Inhibition
None	2.10	—
Actinomycin D (20 μg)	0.52	75
Rifamycin (10 μg)	2.05	Nil
Rifamycin (100 μg)	2.19	Nil
Rifampicin (10 μg)	2.04	Nil
Rifampicin (100 μg)	1.96	Nil

[a] pmoles of [^{3}H]-CMP incorporated per incubation.

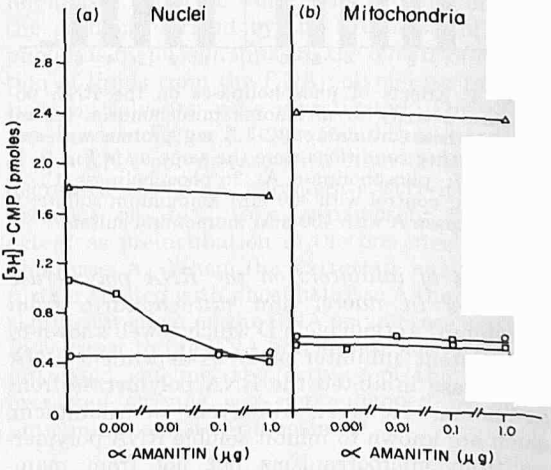

FIG. 6. Effects of α-amanitin upon the RNA polymerase activities in nuclei (a) and mitochondria (b) from melanoma. The experimental conditions were the same as in Fig. 2. Δ——Δ: Mg^{++}; O——O: Mn^{++}; □——□: Mn^{++} with 400 mM ammonium sulfate.

TABLE III

Comparison of the properties of RNA polymerases from melanoma and various normal tissues

Compound added	Tissue														
	Nuclei								Mitochondria						
	Melanoma	Other tissues							Melanoma	Other tissues					
		Liver	Brain	Heart	Lung	Spleen	Kidney	Ependymoblastoma		Liver	Brain	Heart	Lung	Spleen	Kidney	Ependymoblastoma
Mg^{++}	Activation	Activation							Activation	Activation						
Mn^{++}	Activation	Activation							Activation	Activation						
Trypsin	↑	↑↑	↑↑	↑	↑	↑	↑	↑								
$(NH_4)_2SO_4$	↑	↑↑↑	↑↑↑	↑	↑	↑	↑	↑								
Actinomycin D	Inhibition	Inhibition							Inhibition	Inhibition						
α-Amanitin	Inhibition	Inhibition							No effect	No effect						
Phospholipases	↓↓	↓↓	↑↑↑	↓↓	↑	→	↑	↓	↓↓	↓↓	↑↑↑	↓↓	↑	→	↑	↓
Solvent extraction	↓↓	↓↓	↑↑↑	↓↓	↑	→	↑	↓	↓↓	↓↓	↑↑↑	↓↓	↑	→	↑	↓

Activation: ↑↑↑ = high; ↑↑ = medium; ↑ = low
Inhibition: ↓↓↓ = high; ↓↓ = medium; ↓ = low
No effect = →

octane. The RNA polymerase activity in Zimmerman ependymoblastoma was similarly affected. Previous reports have shown that the RNA polymerase activity of liver and heart nuclei and mitochondria is also decreased by these treatments, whereas the polymerase activities of similar preparations from brain are increased [9–11]. The RNA polymerase activities of nuclei and mitochondria from spleen, kidney, and lung are altered only to a relatively small extent by these treatments [11]. Although the biologic importance of these changes is not known, it appears that there are considerable differences in the role of lipids in maintaining the polymerase enzyme bound to the chromatin and it is possible that these differences may have influences on the expression of the RNA polymerases under various physiologic or pathologic conditions. The properties of RNA polymerase from several other tumors are under investigation.

One of the difficulties in these studies of melanoma is the nonavailability of the corresponding normal tissue in sufficiently large quantities for comparative studies. Therefore in the present studies we have attempted to compare the properties of melanoma RNA polymerase with similar properties of RNA polymerases from several normal tissues [9–11]. In these experiments the RNA polymerases from B16 melanoma most closely resemble the polymerases from normal liver.

The authors wish to thank Mrs. H. Li for excellent technical assistance, and Mrs. M. Betlem for the electron microscopic work. We also wish to thank Professor T. Wieland, Max Plank Institute, Heidelberg, Germany, for a gift of α-amanitin, and Dr. A. W. Kopf, New York University School of Medicine, New York, for supplying the melanoma tumor.

REFERENCES

1. Prescott DM: Cellular sites of RNA synthesis, Progress in Nucleic Acid Research and Molecular Biology, vol 3. Edited by NJ Davidson, WE Cohn. New York, Academic Press, 1964, pp 33–57
2. Busch H, Smetana K: The Nucleolus. New York, Academic Press, 1970, p 211
3. Flavell RB: Mitochondrion as a multifunctional organelle. Nature (Lond) 230:504–506, 1971
4. Raven PH: A multiple origin for plastids and mitochondria. Science 169:641–646, 1970
5. Keller W, Goor R: Mammalian RNA polymerase: structural and functional properties. Cold Spring Harbor Symp Quant Biol 35:671–680, 1970
6. Jacob ST, Sajdel EM, Muecke W, et al: Soluble RNA polymerases of rat liver nuclei: properties, template specificity and amanitin responses in vitro and in vivo. Cold Spring Harbor Symp Quant Biol 35:681–691, 1970
7. Seifart KH: The factor stimulating the transcription on double stranded DNA by purified RNA polymerase from rat liver nuclei. Cold Spring Harbor Symp Quant Biol 35:719–725, 1970
8. Stein H, Hausen P: Factors influencing the activity of mammalian RNA polymerase. Cold Spring Harbor Symp Quant Biol 35:709–717, 1970
9. Menon IA: Effects of phospholipases on RNA polymerase activities from various tissues, Abs Pap Am Chem Soc, 1971, p 67
10. Menon IA: Effects of phospholipases on RNA polymerase activity in preparation from rat brain. Can J Biochem 49:1318–1325, 1971
11. Menon IA: A possible role of lipids in RNA polymerase from mammalian cells. Canad J Biochem 50:807–812, 1972
12. Menon IA: Properties of RNA polymerase from a transplantable brain tumor. Brain Res 42:529–533, 1972
13. Bresnick E: Ribonucleic acid polymerase activity in liver nuclei from rat pretreated with 3-methylcholanthrene. Mol Pharmacol 2:406–410, 1966
14. Church RB, Luther SW, McCarthy BJ: RNA synthesis in taper hepatoma and mouse-liver cells. Biochim Biophys Acta 190:30–37, 1969
15. Fukuda P, Akino P, Amano M, et al: RNA synthesis in ascites hepatoma AH-130 cells of rats. Cancer Res 30:1–10, 1970
16. Muramatsu M, Busch H: Isolation, composition and function of nucleoli of tumors and other tissues, Methods in Cancer Research, vol 2. Edited by H Busch. New York, Academic Press, 1967, pp 303–359
17. Potmesil M, Goldfeder A: Nucleolar morphology, nucleic acid synthesis and growth rates of experimental tumors. Cancer Res, 31:789–797, 1971
18. Viale GL: Nucleic acid composition and metabolism in brain tumors, Experimental Biology of Brain Tumors. Edited by WM Kirsch, E Grossi-Paoletti, P Paoletti. Springfield, Charles C Thomas, 1972, pp 357–403
19. Menon IA, Haberman HF: Tyrosinase activity in serum from normal and melanoma bearing mice. Cancer Res 28:1237–1241, 1968
20. Schneider WC, Hogeboom GH: Intracellular distribution of enzymes: further studies on the distribution of cytochrome C. J Biol Chem 183:123–128, 1950
21. Schmerling ZH: The effect of rifamycin on RNA synthesis in the rat liver mitochondria. Biochem Biophys Res Commun 37:965–969, 1969
22. Pogo AO, Allfrey NG, Mirsky AE: Evidence for increased DNA template activity in regenerating liver nuclei. Proc Natl Acad Sci USA 56:550–557, 1966
23. Wehrli U, Nuesch J, Knusel F, et al: Action of rifamycins on RNA polymerase. Biochim Biophys Acta 157:215–217, 1967
24. Menon IA: Differential effects of α-amanitin on RNA polymerase activity in nuclei and mitochondria. Can J Biochem 49:1395–1398, 1971
25. Reid BD, Parsons P: Partial purification of mitochondrial RNA polymerase from rat liver. Proc. Natl Acad Sci USA 68:2830–2834, 1971
26. Saccone C, Gallerani R, Gadaleta MN, et al: The effect of α-amanitin on RNA synthesis in rat liver mitochondria. FEBS Letters 18:339–341, 1971