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Dissociation of two polypeptide chains from yeast RNA polymerase A

(RNA polymerase A*/template requirements/α-amanitin)

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ABSTRACT **Yeast RNA polymerase A (RNA nucleotidyltransferase; nucleosidetriphosphate:RNA nucleotidyltransferase; EC 2.7.7.6) can be converted to a new form of enzyme, called RNA polymerase A*, which is lacking two polypeptide chains of 48,000 and 37,000 daltons. Apart from these two missing polypeptides the subunit structures of RNA polymerases A and A* are indistinguishable. RNA polymerase A* differs from the complete enzyme in its electrophoretic and chromatographic behavior, template requirements, and α-amanitin sensitivity. RNA polymerase A* transcribes the alternated copolymer $d(A\text{-}T)_n$ with the same efficiency as RNA polymerase A but its specific activity is greatly reduced with native calf thymus DNA as template. The transcription of a variety of synthetic templates is also altered by removal of the two polypeptide chains. RNA polymerase A* is inhibited by high concentrations of α-amanitin (500 μg/ml), whereas RNA polymerase A is comparatively less sensitive to the toxic peptide. The data are discussed in terms of possible roles of the two dissociable polypeptides.**

There is currently considerable interest in the structural and functional properties of the multiple forms of RNA polymerase (RNA nucleotidyltransferase; nucleosidetriphosphate:RNA nucleotidyltransferase; EC 2.7.7.6) isolated from eukaryotic cells. Several laboratories have purified these enzymes from various organisms (1). Nuclear RNA polymerases are quite complex multimeric proteins which appear to consist of two large subunits in equimolar amount associated with a series of smaller polypeptide chains (2–6). This structural complexity suggests the likelihood that each enzyme form is made of a fundamental enzyme surrounded with regulatory components or specificity determinants having a specialized role in transcription. Although no available evidence yet supports this hypothesis, past experience with other polymerizing enzymes shows that the basic template-directed polymerization reaction can be carried out by relatively simple proteins (7–9). If small polypeptide chains present in the RNA polymerase molecule were indeed endowed with regulatory function one could expect some of them to be loosely and reversibly associated to the basic enzyme.

As our experience with yeast RNA polymerase A accumulated, persistent peculiarities were noted during fractionation which indicated that the enzyme could be resolved into two distinct enzymatic fractions. It is the purpose of this report to detail the experiments which exploited these hints. The data show that two polypeptide chains of 48,000 and 37,000 daltons are reversibly associated to RNA polymerase A. Following gel electrophoresis or phosphocellulose chromatography RNA polymerase A can be converted to RNA polymerase A*, which is lacking these two satellite proteins. RNA polymerase A* differs from the complete enzyme in its template requirements as well as other properties. A preliminary report of this work has been published (10).

MATERIALS AND METHODS

Nucleic Acids and RNA Polymerases. Synthetic polymers were obtained as previously described (11). Calf thymus DNA was purified by nitrocellulose chromatography (12). Homogeneous yeast RNA polymerase B was the phosphocellulose fraction 8 [12]. RNA polymerase A was purified essentially as previously described (6) with the following modifications. After DEAE-cellulose batch adsorption of the enzyme (step 3) the DEAE-cellulose column chromatography was found unnecessary and the glycerol gradient centrifugation was carried out in the presence of 0.3 M instead of 0.05 M ammonium sulfate. ^{35}S-Labeled RNA polymerase A (80,000 cpm/μg of protein) was prepared from 2 g of yeast cells grown in the presence of sodium [^{35}S]sulfate. The radioactive enzyme was purified by a micro-scale adaptation of the previously described purification procedure (6). Assay conditions of yeast RNA polymerases A, B, and *Escherichia coli* RNA polymerase were as previously described (6, 13).

Phosphocellulose Chromatography. Phosphocellulose P_{11} (Whatman) was washed as described (6) and used to purify RNA polymerase A. 2 mg of RNA polymerase A (glycerol gradient step) were diluted in 10 ml of a buffer containing 0.02 M Tris·HCl, pH 8, 0.5 mM EDTA, 0.01 M 2-mercaptoethanol, 0.05 M ammonium sulfate, and 10% glycerol (v/v). The sample was applied to a phosphocellulose column (0.5 cm^2 × 12 cm) equilibrated with the same buffer. The column was washed at a constant flow rate of 15 ml/hr with 20 ml of the above buffer then with 100 ml of a linear gradient from 0.05 to 0.45 M ammonium sulfate in the same buffer. The fractions containing the activity were pooled and concentrated by dialysis against the same buffer containing 60% glycerol (v/v) for 12 hr at −15° and stored at −80°.

Polyacrylamide Gel Electrophoresis. Two-dimensional electrophoresis was used to analyze the subunit pattern of ^{35}S-labeled RNA polymerase A. Electrophoresis of the native enzyme was performed in the first dimension under nondenaturing conditions on standard gel slab (6). Dodecyl sulfate detergent was used in the second dimension. The corresponding strip of gel was rinsed for 30 min at room temperature with the upper gel solution described by Laemmli (14), layered on top of a polymerized 12.5% acrylamide gel slab containing 0.1% sodium dodecyl sulfate, and embedded in the upper gel. After electrophoresis, the gel was dried over a paper sheet and the radioactive spots of proteins were located by autoradiography.

Antiserum Preparation. The largest subunit of 190,000 daltons from RNA polymerase A was purified by preparative electrophoresis with sodium dodecyl sulfate on 5% polyacrylamide slab gel (5 mm thick). The protein was eluted from the gel by electrophoresis. One and a half milligrams

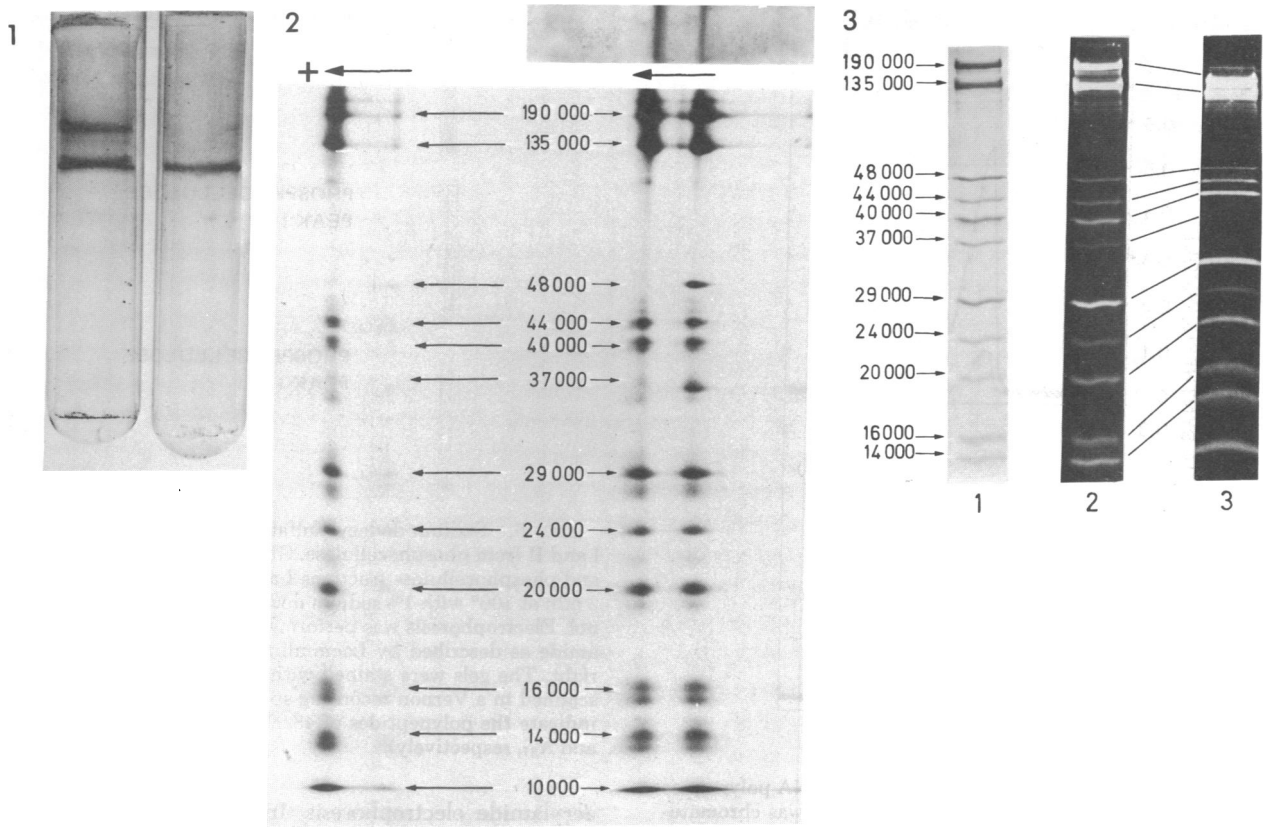

FIG. 1. Polyacrylamide gel electrophoresis of yeast RNA polymerase A. Electrophoresis of native RNA polymerase A on 5% acrylamide gel was performed under nondenaturing conditions (6) using two different amounts of enzyme, 12 μg (left) or 4 μg (right).

FIG. 2. Analysis of ^{35}S-labeled RNA polymerase A by two-dimensional electrophoresis. Radioactive RNA polymerase A (1.5 μg; 125,000 cpm) was subjected to electrophoresis on 5% acrylamide gel, with or without addition of 8 μg of carrier unlabeled enzyme. Subsequently the gel was subjected to a second-dimension electrophoresis with sodium dodecyl sulfate as described under *Materials and Methods* and the radioactive subunits were located by autoradiography. *Left*, analysis of 1.5 μg of enzyme; *right*, 9.5 μg of enzyme. The molecular weights of the polypeptide chains are indicated in the figure. On top of the figure (right) is shown the Coomassie blue staining of the two protein bands in the presence of carrier enzyme, after the first dimension electrophoresis.

FIG. 3. Analysis of antibody precipitate of ^{35}S-labeled RNA polymerase A. A 20 μl sample of radioactive RNA polymerase A (1.2 μg; 100,000 cpm) was mixed with 20 μl of an antiserum preparation. After 15 min incubation at 30°, 8 μg of carrier unlabeled enzyme was added and the mixture was further incubated for 30 min at 30°. The precipitate was collected by low-speed centrifugation and washed twice with a buffer containing 0.02 M Tris·HCl, pH 8.4, 1 mM 2-mercaptoethanol, 0.5 mM EDTA, and 0.25 M ammonium sulfate. About 90% of the input radioactivity was recovered. The washed precipitate was solubilized by boiling 2 min in 50 μl of sample buffer described by Laemmli (14) and subjected to polyacrylamide gel electrophoresis with sodium dodecyl sulfate. The polypeptide pattern obtained from ^{35}S-labeled RNA polymerase A (glycerol gradient step) is also shown for comparison: (*1*) Coomassie brilliant blue staining of radioactive glycerol gradient RNA polymerase A; (*2*) autoradiography of the above gel; (*3*) polypeptide pattern of the antibody precipitate. The molecular weights of the various proteins bands are given in the figure (6).

of purified subunit, obtained from 12 mg of RNA polymerase A, were used to prepare rabbit antibodies. The serum was used directly or purified by precipitation with ammonium sulfate followed by chromatography on DEAE-cellulose. The gammaglobulin fraction inhibited selectively RNA polymerase A but not RNA polymerase B (10).

RESULTS

Behavior of RNA Polymerase A During Gel Electrophoresis. We have already noted (6) that RNA polymerase A activity often exhibited peculiarities during purification which suggested the existence of two fractions. Polyacrylamide gel electrophoresis of the native enzyme often gave rise to two prominent bands of protein both coincident with enzymatic activity (10). Interestingly, the relative amount of these two bands varied, depending on the amount of enzyme applied on the gel (Fig. 1). The slower band of protein, which was barely visible when less than 5 μg of protein were analyzed, progressively increased and eventually predominated when higher amounts of enzyme were used, whereas the relative intensity of the faster band was decreased proportionally. This concentration dependence was suggestive of a reversible dissociation process.

Besides the two high-molecular-weight subunits present in equimolar amount, 10 lower molecular weight components repeatedly copurified with the enzyme (6). The existence of this considerable number of putative subunits prompted us to investigate whether all these components remained associated with the two enzyme bands during gel electrophoresis. To facilitate the analysis, a small amount of native ^{35}S-labeled RNA polymerase A was analyzed with or without addition of carrier unlabeled enzyme. Subsequently the gel was subjected to a second dimension electrophoresis with sodium dodecyl sulfate in order to resolve the subunit pattern of each protein band (Fig. 2). Autoradiography of the gel shows that radioactive enzyme migrated as a single band of protein (the rapid band) which displayed all the components normally found in the glycerol gradient enzyme, except for two polypeptide chains of 48,000 and 37,000 daltons. On the other hand, after addition of 8 μg of carrier enzyme, as ex-

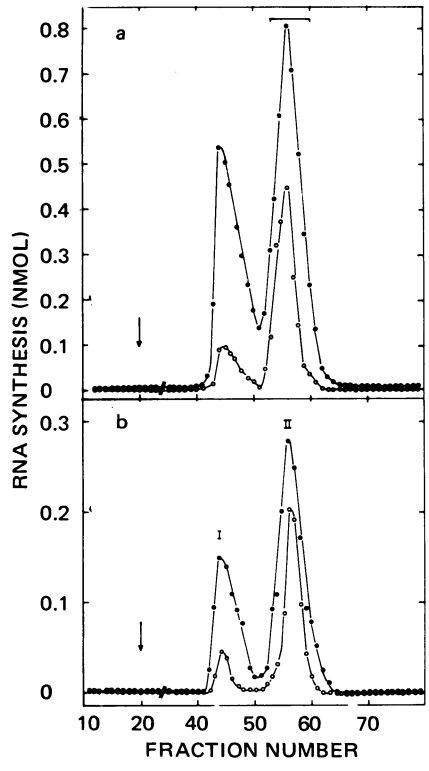

FIG. 4. Phosphocellulose chromatography of RNA polymerase A. (a) Glycerol gradient enzyme (2 mg of protein) was chromatographed as described under *Material and Methods*. Fractions (1.2 ml) were collected and assayed for RNA polymerase activity on 10 μl aliquots using native calf thymus DNA (○) or d(A-T)$_n$ (●) as template. Assay conditions were as described in Table 1, using 5 mM $MgCl_2$. (b) Fractions 53–60 (0.7 mg of protein) were pooled, and rechromatographed under similar conditions. RNA synthesis, with native DNA as template (○), or d(A-T)$_n$ (●), is given as nmol of total polymerized nucleotides calculated from base composition of template.

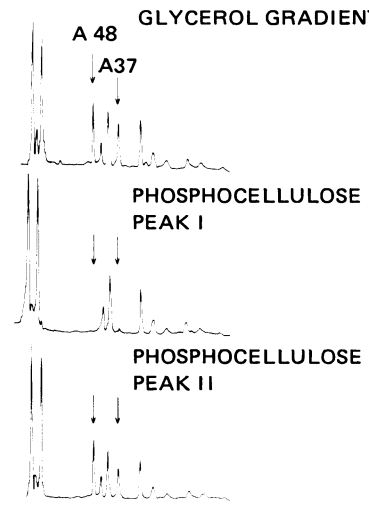

FIG. 5. Sodium dodecyl sulfate gel electrophoresis of fractions I and II from phosphocellulose. Glycerol gradient RNA polymerase and phosphocellulose fractions I and II (15–20 μg) were heated for 2 min at 100° with 1% sodium dodecyl sulfate–1% 2-mercaptoethanol. Electrophoresis was performed in gels containing 12.5% acrylamide as described by Laemmli (14). Migration was from left to right. The gels were stained with Coomassie blue, destained, and scanned in a Vernon recording spectrophotometer (6). The arrows indicate the polypeptides of 48,000 and 37,000 daltons (called A_{48} and A_{37}, respectively).

pected, two radioactive bands of protein were observed. As above, the rapid band was lacking the two components, but the slower band showed the complete subunit pattern of the native enzyme. A very similar observation was independently made by Schwartz and Roeder with RNA polymerase I isolated from mouse myeloma cells except that a single polypeptide component was missing in one form of enzyme (15).

Antibody Precipitation of RNA Polymerase A. Greenleaf *et al.* (16) and Goff and Weber (17) have used antibody precipitation of bacterial RNA polymerase to analyze the polypeptide chains associated to the enzyme. We have taken advantage of this method to further investigate whether all the components of RNA polymerase A remain firmly bound to each other. Native ^{35}S-labeled RNA polymerase A was precipitated using antibodies prepared against the largest subunit of the enzyme. The washed precipitate was solubilized and analyzed by dodecyl sulfate–polyacrylamide gel electrophoresis. Fig. 3 shows the autoradiography of the gel in comparison with ^{35}S-labeled glycerol gradient enzyme. It should be noted that all the bands are labeled with ^{35}S although the relative specific radioactivities of the polypeptide chains varied to a large extent. Practically all the components usually found in purified preparations of RNA polymerase A were precipitated with the antibodies, except the polypeptide of 37,000 daltons which was poorly represented.

In a somewhat different experiment ^{35}S-labeled RNA polymerase A was adsorbed to a column of Sepharose-bound antibodies. After washing the column the enzyme subunits were eluted with 8 M urea and subjected to dodecyl sulfate–acrylamide electrophoresis. In this case, the band pattern was completely lacking the polypeptides of 48,000 and 37,000 daltons (10).

Isolation of RNA Polymerase A* by Phosphocellulose Chromatography. The reduction of RNA polymerase A to a more basic form of enzyme was also achieved by phosphocellulose chromatography. When chromatographed on phosphocellulose RNA polymerase A was separated in two enzymatic fractions (Fig. 4). The two fractions were analyzed by dodecyl sulfate–acrylamide gel electrophoresis. As shown in Fig. 5 the subunit pattern of the two enzymatic fractions was clearly different. Fraction II presented the band pattern of complete RNA polymerase A (corresponding to that of the glycerol gradient enzyme), whereas fraction I was lacking the two polypeptides of 48,000 and 37,000 daltons. Except for the two missing polypeptide chains, no significant difference was found in the subunit pattern of the two enzymes. Fraction I, which is now referred to as RNA polymerase A*, is therefore derived from RNA polymerase A by dissociation of two polypeptide chains. Further evidence in favor of such a dissociation process was obtained by rechromatography of fraction II on phosphocellulose. As shown in Fig. 4, this step again generated a mixture of RNA polymerase A* (fraction I) and RNA polymerase A (fraction II).

Template Requirements of RNA Polymerase A*. The template specificity of RNA polymerase A* was compared with that of the complete enzyme using a variety of synthetic or natural templates. Three groups of synthetic templates were used: single-stranded homopolymers, double-stranded homopolymer pairs, and alternating copolymers. Table 1 reports a comparative study of the various polymers with respect to the relative template efficiency of each. The first observation, already evident in Fig. 4, was that RNA polymerase A* is much less active with native calf thymus DNA as template than RNA polymerase A. In repeated experiments, using enzymes from separate preparations, a 4- to 6-fold difference in specific activity was observed. The difference is even much greater when the assay is performed at

Table 1. Template specificities of RNA polymerase A* and RNA polymerase A

Template	Substrates	RNA synthesis (pmol)			
		RNA polymerase A*		RNA polymerase A	
		Mg	Mg + Mn	Mg	Mg + Mn
Native DNA	*UTP, GTP, CTP, ATP	470	810	3100	4000
Denatured DNA	*UTP, GTP, CTP, ATP	1150	2150	2950	4600
$(dC)_n$	*GTP	790	1850	1240	1080
$(dT)_n$	*ATP	25	670	325	930
$(dA)_n$	*UTP	46	410	125	310
$(dG)_n$	*CTP	5	10	5	10
$d(A-T)_n$ (3 μg)	*ATP, UTP	1200	1060	960	1170
$d(A-T)_n$ (10 μg)	*ATP, UTP	2400	2540	2300	2340
$d(I-T)_n$	*ATP, CTP	35	25	25	25
$d(I-C)_n$	*GTP, CTP	1300	2200	3700	2500
$d(G-C)_n$	*GTP, CTP	190	1040	760	1120
$(dA)_n \cdot (dT)_n$	*ATP	25	675	50	430
$(dA)_n \cdot (dT)_n$	*UTP	50	40	40	30
$(dG)_n \cdot (dC)_n$	*GTP	125	100	10	20
$(dG)_n \cdot (dC)_n$	*CTP	0	5	0	5
$(dI)_n \cdot (dC)_n$	*GTP	10	5	10	5
$(dI)_n \cdot (dC)_n$	*CTP	5	5	15	5
$(rC)_n$	*GTP	0	50	10	130
$(rU)_n$	*ATP	20	20	20	30
$(rA)_n$	*UTP	35	100	50	140
$(rG)_n$	*CTP	0	0	0	0
$(rI)_n$	*CTP	0	5	5	5

The basic incubation mixture (0.1 ml) contained 0.07 mM Tris-HCl (pH 8), 1 mM dithiothreitol, 5 mM $MgCl_2$ or 5 mM $MgCl_2$ plus 3 mM $MnCl_2$, 1 mM of the unlabeled ribonucleoside triphosphates and 0.05 mM of one ^{14}C-labeled nucleoside triphosphate (the asterisk indicates the labeled nucleotide). The template used was either native or alkali-denatured calf thymus DNA (10 μg) or a synthetic template (1.5–2 μg) as indicated in the table. The reaction was started by addition of 2 μg of RNA polymerase A* or 2.5 μg of RNA polymerase A. Following incubation for 60 min at 30° acid-insoluble radioactivity was collected on membrane filters and counted (13). RNA synthesis is given as pmol of total polymerized nucleotides per μg of protein and per hr.

higher ionic strength (J. Huet, to be published). In contrast, both enzymatic fractions showed the same specific activity when provided with $d(A-T)_n$ as template. Hence this alternating copolymer was routinely used to assay the enzymes during fractionation. Other synthetic templates were also transcribed by RNA polymerase A and A*. However, the two forms of enzyme displayed different template specificity, especially when Mg^{2+} ions were used to stimulate transcription. Thus, in the case of homopolymers, $(dC)_n$ supported an extensive synthesis of $(rG)_n$ by RNA polymerase A. Although less efficiently, $(dT)_n$ and $(dA)_n$ stimulated the incorporation of the complementary nucleoside triphosphate. On the other hand, RNA polymerase A* was relatively less active with $(dC)_n$ and practically did not transcribe $(dT)_n$ or $(dA)_n$ homopolymers. Only in the presence of Mn^{2+} ions were the homopolymers $(dC)_n$, $(dT)_n$, and $(dA)_n$ transcribed with similar efficiency by RNA polymerases A and A*. This was due to the fact that manganese ions drastically stimulated transcription mainly by RNA polymerase A*. A similar observation was made with the alternating copolymers $d(I-C)_n$ and $d(G-C)_n$. With Mg^{2+}, these polymers were more actively transcribed by the complete enzyme, whereas no significant difference in the activity of the two enzymes was observed in the presence of Mn^{2+}. Interestingly the reverse situation occurred with $(dG)_n \cdot (dC)_n$, which was active only with RNA polymerase A*. This preliminary survey strongly suggested that removal of the two polypeptide components altered the template specificity of the enzyme.

α-Amanitin Sensitivity of RNA Polymerase A*. α-Amanitin sensitivities of RNA polymerase A and A* were compared with native calf thymus DNA as template (Fig. 6). RNA polymerase A was shown to be virtually uninhibited at concentrations of α-amanitin (40–50 μg/ml) which inhibit RNA polymerase B more than 90% (6). However, at higher concentrations of the toxic peptide, RNA polymerase A displayed a low but significant sensitivity to the drug as compared with *E. coli* RNA polymerase. (The inhibitory action

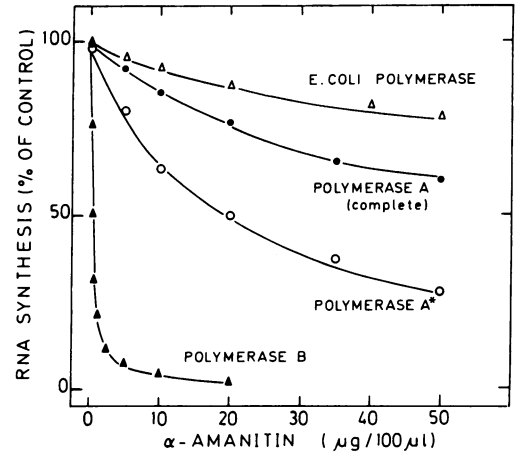

FIG. 6. Sensitivity of RNA polymerases A and A* to α-amanitin. Appropriate amounts of α-amanitin were mixed with the different RNA polymerases and the mixtures were incubated for 90 sec at 30° prior to initiation of the reaction with DNA and nucleoside triphosphates. Control [^3H]UMP incorporations in absence of α-amanitin were, respectively, 0.21 nmol for RNA polymerase A (○), 0.13 nmol for RNA polymerase A* (●), 0.12 nmol for *E. coli* RNA polymerase holoenzyme (△), and 0.15 nmol for yeast RNA polymerase B (▲).

of the drug on the bacterial enzyme could represent the background of unspecific inhibition at high toxin concentration.) Interestingly, the removal of the two polypeptides from RNA polymerase A increased the inhibitory effect of α-amanitin. A similar differential sensitivity of RNA polymerase A* and A was also observed with synthetic templates: d(A-T), $d(I\text{-}C)_n$, $(dC)_n$, and $(dT)_n$. RNA polymerase A was inhibited 50% at about 200 μg/ml. This concentration is still 100-fold higher than that required to inhibit RNA polymerase B to the same extent. One should note in this respect that, in mammalian cells, class C (or III) RNA polymerases differ from class B (or II) RNA polymerases by about a 1000-fold lower sensitivity to α-amanitin (18–20).

DISCUSSION

Earlier studies have been shown that yeast RNA polymerase A is a highly complex multiprotein assembly (6). In the present report we describe a new form of this enzyme called RNA polymerase A* which is derived from RNA polymerase A by dissociation of two polypeptide components of 48,000 and 37,000 daltons. Evidence that these two polypeptides are normally associated to the RNA polymerase molcule stem from the following observations: they copurify with the enzyme through glycerol gradient centrifugation, gel electrophoresis, and ion exchange chromatography. Also they are partly precipitated by antibodies against the largest subunit of the enzyme. Finally, RNA polymerase A* which is lacking these two polypeptides behaves differently on gel electrophoresis and phosphocellulose chromatography than the complete enzyme.

The two forms of RNA polymerase are thus physically but also functionally distinct. The removal of the two components obviously alters the template specificity of the enzyme. Particularly, its ability to transcribe native calf thymus DNA is greatly reduced. The differences observed using synthetic templates in the specificities of the two forms of enzyme strongly suggest that the two polypeptides, or at least one of them, influence some important step in transcription. On the other hand, the fact that both RNA polymerase A and A* transcribe $d(A\text{-}T)_n$ with the same efficiency indicates that these polypeptides are not required for the basic process of polymerization. Schwartz and Roeder (15) recently separated by gel electrophoresis two forms of RNA polymerase I, one of which was lacking a polypeptide chain. However, they did not recover any significant activity in the deficient enzyme.

Multiple forms of RNA polymerase have been classified on the basis of their chromatographic and catalytic properties and α-amanitin sensitivity (1). The results presented here show that two polypeptide components representing about 15% by weight of total polymerase protein can markedly alter the chromatographic behavior, template requirements, and α-amanitin sensitivity of the enzyme. In addition to the properties described here, RNA polymerase A and A* can be further distinguished on the basis of their ionic strength and divalent cation requirements, and also by their thermal sensitivity (J. Huet, to be published). In the light of these results one should consider the possibility that some of the multiple forms of RNA polymerase could mainly differ by the presence or absence of satellite proteins endowed with regulatory functions.

The *in vivo* significance of the observed dissociation of RNA polymerase A is still conjectural. By analogy with the reversible association of sigma factor with the bacterial core enzyme one could imagine that the dissociable polypeptides are involved in RNA chain initiation. However, the situation seems to be somewhat more complex, since the complete enzyme itself appears to be unable to initiate an RNA chain on an intact double-stranded template (13). Reversible dissociation of essential cofactor(s) from the enzyme could also be a way to modulate its activity if these polypeptide chains are metabolically unstable and under regulatory control. Recent results actually suggest the existence of a rapidly turning over polypeptide, essential for RNA polymerase A activity (21–23). Another possibility could be that such polypeptides and similar factors direct the synthesis of different classes of RNA. A better understanding of the role of these polypeptide chains in template binding, RNA chain initiation, or elongation awaits further studies on the properties of the two forms of enzyme as well as reconstitution experiments.

1. Chambon, (1974) in *The Enzymes*, ed., Boyer, P. D. (Academic Press, New York), 3rd ed., Vol. 10, pp. 261–331.
2. Weaver, R. F., Blatti, S. P. & Rutter, W. J. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 2994–2999.
3. Kedinger, C., Gissinger, F. & Chambon, P. (1974) *Eur. J. Biochem.* **44**, 421–436.
4. Sklar, V. E. F., Schwartz, L. B. & Roeder, R. G. (1975) *Proc. Nat. Acad. Sci. USA* **72**, 348–352.
5. Dezélée, S., Sentenac, A. & Fromageot, P. (1972) *FEBS Lett.* **21**, 1–6.
6. Buhler, J. M., Sentenac, A. & Fromageot, P. (1974) *J. Biol. Chem.* **249**, 5963–5970.
7. Chamberlin, M., McGrath, J. & Waskell, L. (1970) *Nature* **228**, 227–231.
8. Kornberg, A. (1969) *Science* **163**, 1410–1417.
9. Chang, L. M. S. (1973) *J. Biol. Chem.* **248**, 3789–3795.
10. Buhler, J. M., Dezélée, S., Huet, J., Iborra, F. & Sentenac, A. (1975) in *Control of Transcription* (North Holland Publishing Co.), in press.
11. Dezélée, S., Sentenac, A. & Fromageot, P. (1974) *J. Biol. Chem.* **249**, 5978–5983.
12. Dezélée, S. & Sentenac, A. (1973) *Eur. J. Biochem.* **34**, 41–52.
13. Dezélée, S., Sentenac, A. & Fromageot, P. (1974) *J. Biol. Chem.* **249**, 5971–5977.
14. Laemmli, U. K. (1970) *Nature* **227**, 680–685.
15. Schwartz, L. B. & Roeder, R. (1974) *J. Biol. Chem.* **249**, 5898–5906.
16. Greenleaf, A. L., Linn, T. G. & Losick, R. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 490–494.
17. Goff, C. G. & Weber, K. (1970) *Cold Spring Harbor Symp. Quant. Biol.* **35**, 101–108.
18. Seifart, K. H., Benecke, B. J. & Juhasz, P. P. (1972) *Arch. Biochem. Biophys.* **151**, 519–532.
19. Amalric, F., Nicoloso, M. & Zalta, J. P. (1972) *FEBS Lett.* **22**, 67–72.
20. Schwartz, L. B., Sklar, V. E. F., Jachning, J. A., Weinmann, R. & Roeder, R. G. (1974) *J. Biol. Chem.* **249**, 5889–5897.
21. Yu, F. L. & Feigelson, P. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 2833–2837.
22. Lampert, A. & Feigelson, P. (1974) *Biochem. Biophys. Res. Commun.* **58**, 1030–1038.
23. Gross, K. J. & Pogo, A. O. (1974) *J. Biol. Chem.* **249**, 568–576.