Levin, D. H., Kyner, D., & Acs, G. (1973) *J. Biol. Chem. 248*, 6416.
McMaster, G. K., & Carmichael, G. G. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 4835.
Mills, D. R., & Kramer, F. R. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 2232.
Mills, D. R., Dobkin, C., & Kramer, F. R. (1978) *Cell 15*, 541.
Shapiro, R., & Pohl, S. H. (1968) *Biochemistry 7*, 448.
Shatkin, A. J. (1976) *Cell 9*, 645.
Shatkin, A. J., & LaFiandra, A. J. (1972) *J. Virol. 10*, 698.
Smith, R. M., & Furuichi, Y. (1980) *Virology 103*, 279.
Sonenberg, N., Rupprecht, K. M., Hecht, S. M., & Shatkin, A. J. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 4345.
Sonenberg, N., Trachsel, H., Hecht, S., & Shatkin, A. J. (1980) *Nature (London) 285*, 331.
Testa, D., & Banerjee, A. K. (1978) *Biochem. Biophys. Res. Commun. 82*, 655.
Venkatesan, S., & Moss, B. (1980) *J. Biol. Chem. 255*, 2835.
Warrington, R., Hayward, D., & Kapuler, A. M. (1973) *Biochim. Biophys. Acta 331*, 231.

Purification, Subunit Structure, and Immunological Properties of Chromatin-Bound Ribonucleic Acid Polymerase I from Cauliflower Inflorescence†

Tom J. Guilfoyle

ABSTRACT: The large-scale purification of deoxyribonucleic acid (DNA) dependent ribonucleic acid (RNA) polymerase I from cauliflower inflorescence has been achieved following solubilization of the enzyme from the chromatin template. The initial step of purification of the enzyme by heparin–Sepharose chromatography at high ionic strength prevents reaggregation of chromatin proteins which can occur if salt concentrations are reduced. The enzyme was further purified by phosphocellulose, diethylaminoethyl (DEAE)-Sephadex, and DEAE-cellulose chromatography and glycerol density gradient centrifugation. The purified RNA polymerase I has a specific activity of 400–450 nmol of UMP incorporated into RNA in 20 min per mg of protein. From 1 kg of tissue, ~300 μg of purified enzyme is obtained with an overall yield of >30%. The purification of the enzyme to homogeneity takes about 4–5 days. The overall purification is ~10000-fold from total tissue and ~250-fold from isolated chromatin. Cauliflower RNA polymerase I purified by this procedure consists of seven polypeptides of 190000, 170000, 125000, 38000, 25000, 22000, and 17500 daltons as determined by one- (dodecyl sulfate) and two- (8 M urea, pH 8.7, followed by dodecyl sulfate) dimensional polyacrylamide gel electrophoresis. Polypeptides of 125000, 38000, and 22000 have stoichiometries of ~1, the 25000 and 17500 subunits have stoichiometries of 1.5–2, and the 190000 plus 170000 polypeptides sum to a stoichiometry of 1. The 170000 polypeptide probably arises from the 190000 polypeptide by proteolysis during purification of the enzyme. Comparison of the subunit structure of cauliflower RNA polymerase I with cauliflower RNA polymerases II and III by one- and two-dimensional polyacrylamide gel electrophoresis indicates that three polypeptides of 25000, 22000, and 17500 daltons associated with each class of enzyme have identical mobilities, and this suggests that there is a common pool of low molecular weight subunits in RNA polymerases I, II, and III. By use of immunological methods [Renart, J., Reiser, J., & Stark, G. R. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 3116–3120], it is shown that the three putative common subunits as well as the 38000 subunit of RNA polymerase I cross-react with antibodies raised against cauliflower RNA polymerase II.

Eukaryotic cells contain three distinct classes of nuclear DNA[1]-dependent RNA polymerases which are generally referred to as RNA polymerases I, II, and III. Each class of RNA polymerase has characteristic chromatographic and catalytic properties, transcriptional functions, intracellular localizations, and subunit structures [reviewed by Roeder (1979)]. RNA polymerase I is localized in the nucleolus and transcribes ribosomal DNA. RNA polymerase II is found in the nucleoplasm and catalyzes the synthesis of precursors to messenger RNA. RNA polymerase III is also localized in the nucleoplasm and transcribes sequences coding for 5S ribosomal RNA, precursors to transfer RNA, and some other small RNAs. Each class of enzyme has a characteristic sensitivity to inhibition by the fungal toxin, α-amanitin. While RNA polymerase I in higher eukaryotes is refractory to α-amanitin, RNA polymerases II and III are inhibited by 50% at about 0.01–0.05 μg/mL and 10–20 μg/mL α-amanitin, respectively. In some cases, however, RNA polymerase III is inhibited only at concentrations in the range of 1–2 mg/mL α-amanitin (Guilfoyle, 1976; Renart & Sebastian, 1976) or is refractory to the toxin (Sklar et al., 1976), and in yeast, RNA polymerase I is inhibited by α-amanitin (Schultz & Hall, 1976; Valenzuela et al., 1976b). Although each class of RNA polymerase has a distinct subunit structure, certain of the subunits appear to be common to RNA polymerases I, II, and III (Buhler et al., 1976; Valenzuela et al., 1976a; Roeder, 1976; D'Alessio et al., 1979) as judged by one- and two-dimensional polyacrylamide gel electrophoresis. In yeast, genetic data (Thonart et al.,

† From the Department of Botany, University of Minnesota, St. Paul, Minnesota 55108. *Received June 2, 1980.* This research was supported by U.S. Public Health Service Grant GM 24096 and USDA/SEA Competitive Research Grant 5901-0410-9-0312-0.

[1] Abbreviations used: ABM, aminobenzyloxymethyl; DBM, diazobenzyloxymethyl; Tris, tris(hydroxymethyl)aminomethane; $NaDodSO_4$, sodium dodecyl sulfate; DNA, deoxyribonucleic acid; RNA, ribonucleic acid; UMP, uridine 5′-phosphate.

0006-2960/80/0419-5966$01.00/0

1976) and peptide mapping of subunits with identical mobilities (Buhler et al., 1976) provide additional evidence that RNA polymerases I and II possess common subunits.

Cauliflower inflorescence or curd is a dense mass of actively dividing cells that provides a rich source of transcriptionally active plant nuclei and RNA polymerases (Guilfoyle, 1976). In addition, the cauliflower inflorescence offers a source of plant material that is relatively free of chloroplasts and is commercially available. In contrast to wheat germ (Jendrisak & Burgess, 1975) and other ungerminated plant embryonic axes (Jendrisak & Guilfoyle, 1978; Guilfoyle & Malcolm, 1980) which are good sources of soluble RNA polymerases (e.g., RNA polymerases which are not engaged on the chromatin template), cauliflower inflorescence contains a large proportion of its RNA polymerases (especially RNA polymerases I and III) as template-engaged or chromatin-bound enzymes. I have previously reported on the partial purification and characterization of RNA polymerases I, II, and III from isolated nuclei of cauliflower inflorescence (Guilfoyle, 1976). In this paper, I report the purification of chromatin-bound RNA polymerase I from cauliflower inflorescence to homogeneity. I have compared the subunit structure and α-amanitin sensitivity of purified RNA polymerase I to that of cauliflower RNA polymerases II and III. I have shown that RNA polymerases I, II, and III have three low molecular weight subunits with identical mobilities on polyacrylamide gels and that four subunits in RNA polymerase I share antigenic determinants with RNA polymerase II from cauliflower.

Experimental Procedures

Source of RNA Polymerases. Cauliflower inflorescence (*Brassica oleracea* botrytis) was purchased from H. Brooks and Co., Minneapolis, MN. Terminal branches of the curd were excised, collected on ice, and washed thoroughly with ice-cold distilled water.

Materials. The source of materials used in the purification and assay of RNA polymerase is described by Jendrisak & Guilfoyle (1978) and Guilfoyle & Malcolm (1980). Heparin–Sepharose was prepared with Sepharose 4B (Pharmacia) by the method of Davison et al. (1979). ABM- and DBM-paper were prepared by the method of Alwine et al. (1977). *Staphylococcus aureus* [^{125}I]protein A was purchased from New England Nuclear.

RNA Polymerase Assay and Protein Determination. RNA polymerase activity was assayed as described by Guilfoyle et al. (1976). Protein concentrations were determined by the method of Bradford (1976).

Polyacrylamide Gel Electrophoresis. Procedures used for one- and two-dimensional polyacrylamide gel electrophoresis are described by Jendrisak & Burgess (1977) and Jendrisak & Guilfoyle (1978). When RNA polymerase subunits were blotted from polyacrylamide gels to DBM-paper, composite polyacrylamide–agarose gels with ethylene diacrylate cross-linker were constructed as described by Renart et al. (1979).

Buffers. Homogenization buffer was 50 mM Tris-HCl (pH 7.9 at 4 °C), 2 mM $MgCl_2$, 0.1 mM ethylenediaminetetraacetic acid, and 15 mM 2-mercaptoethanol. Chromatography buffer was 50 mM Tris-HCl (pH 7.9 at 4 °C), 1 mM ethylenediaminetetraacetic acid, 15 mM 2-mercaptoethanol, and 25% (v/v) ethylene glycol. Storage buffer was 50 mM Tris-HCl (pH 7.9 at 4 °C), 1 mM ethylenediaminetetraacetic acid, 1 mM dithiothreitol, and 50% (v/v) glycerol.

Immunological Methods. Antibodies to cauliflower RNA polymerases II and III were raised in New Zealand white rabbits, and the γ-globulin or IgG fraction was prepared from sera by the methods described by Linn et al. (1973). Double immunodiffusion was performed as described by Jendrisak & Guilfoyle (1978). Common antigenic determinants in subunits of cauliflower RNA polymerases I and II were detected by the methods outlined by Renart et al. (1979). Briefly, 2 μg each of RNA polymerases I and II from cauliflower were separated into subunits by gel electrophoresis in the presence of dodecyl sulfate using composite polyacrylamide–agarose gels with ethylene diacrylate cross-linker. Following electrophoresis, gels were incubated by gentle rocking in 250 mL of 0.25 M ammonium hydroxide for 30 min. This procedure was repeated in a second volume of ammonium hydroxide, and the gels were transferred to 250 mL of 0.5 M sodium phosphate (pH 7.2) for 30 min. The gels were then incubated twice for 15 min each in 50 mM sodium phosphate buffer (pH 7.2). RNA polymerase subunits were then transferred from the gels to freshly prepared DBM-paper by blotting for 24 h at 22 °C using 50 mM sodium phosphate buffer (pH 7.2) and the blotting procedure described by Southern (1975). After blotting, gel adhering to the DBM-paper was scraped off with a razor blade, and the paper was incubated at 37 °C in a Sears "Seal-N-Save" bag containing 500 mL of 0.1 M Tris-HCl (pH 9.0 at 25 °C), 10% (v/v) ethanolamine, and 0.25% (w/v) gelatin. Incubation was for 2 h to inactivate remaining diazonium groups.

This solution was removed and replaced with the anti-RNA polymerase II γ-globulin fraction (5 mg/mL) diluted 1:25 with 50 mM Tris-HCl (pH 7.4 at 25 °C), 150 mM NaCl, 5 mM ethylenediaminetetraacetic acid, 0.25% (w/v) gelatin, and 0.05% (v/v) Nonidet P-40 in a final volume of 50 mL. After incubation with gentle rocking for 12 h at 37 °C, the paper was washed in 500 mL of the same buffer minus the γ-globulin fraction for an additional 12 h at 37 °C. This solution was removed and replaced with 25 mL of the same buffer containing 2 μCi of [^{125}I]protein A. After incubation for 2 h at 37 °C, the label was removed and the paper was rinsed thoroughly with distilled water. The paper was then incubated twice for 2 h in 50 mM Tris-HCl (pH 7.4 at 25 °C), 1 M NaCl, 5 mM ethylenediaminetetraacetic acid, and 0.4% (w/v) Sarkosyl at 37 °C in a volume of 500 mL. After incubation, the paper was removed from the bag, rinsed briefly with distilled water, blotted dry, and autoradiographed with Kodak X-Omat R X-ray film and a Du Pont Cronex Lightning-plus intensifying screen (Laskey & Mills, 1977).

Purification of Cauliflower RNA Polymerase I from Isolated Chromatin. (a) Preparation of Cell Extract. All purification procedures were performed at 0–4 °C. Ten kilograms of terminal branches from cauliflower inflorescence was homogenized in homogenization buffer (2 volumes/weight tissue) at top speed in a Waring Blender and filtered through eight layers of cheesecloth and then through one layer of Miracloth (Calbiochem). Triton X-100 was added to a final concentration of 0.5% (v/v) with rapid stirring for 5 min. Chromatin was pelleted by centrifugation of the extract at 10000*g* for 30 min. The supernatant was decanted, and the chromatin was suspended in 1 L of homogenization buffer and repelleted at 10000*g* for 30 min. Greater than 90% of the RNA polymerase I in the tissue extract is recovered in the chromatin fraction with little or no detectable activity in the supernatant fraction which contains the bulk of RNA polymerases II and III.

(b) Solubilization of Chromatin-Bound RNA Polymerases. Chromatin was suspended in 500 mL of chromatography buffer and adjusted to 0.5 M ammonium sulfate by adding solid ammonium sulfate with rapid stirring. The viscous solution was homogenized at top speed with a Brinkmann Po-

lytron PT20ST to shear the chromatin DNA. Homogenization was continued until the chromatin solution dripped freely from the end of the polytron probe which indicated that the viscosity of the solution had been reduced. The sheared chromatin was centrifuged at 50 000 rpm for 60 min in a Beckman 60 Ti rotor, and the supernatant containing the solubilized RNA polymerases was recovered. A protein fraction containing the solubilized RNA polymerases was precipitated by addition of 0.35 g/mL solid ammonium sulfate. The solution was stirred for 1 h at 4 °C, and the precipitated protein was collected by centrifugation at 50 000 rpm for 60 min in a Beckman 60 Ti rotor.

(*c*) *Heparin–Sepharose Chromatography.* The ammonium sulfate precipitate was suspended in chromatography buffer, adjusted to 0.25 M ammonium sulfate, and applied to a 2.5 × 30 cm column of heparin–Sepharose equilibrated with chromatography buffer and 0.25 M ammonium sulfate. The column was washed with the same buffer until no protein could be detected in the eluate. RNA polymerases were then eluted with a step gradient of 0.75 M ammonium sulfate in chromatography buffer. RNA polymerases elute as a sharp peak in ~50 mL at a specific activity of ~68 units/mg of protein (Table I). The RNA polymerase activity eluted from heparin–Sepharose is ~70% resistant to α-amanitin inhibition in the presence of 1 μg/mL toxin.

(*d*) *Phosphocellulose Chromatography.* Fractions containing RNA polymerase from heparin–Sepharose chromatography were adjusted to 0.3 g/mL with solid ammonium sulfate and stirred for 60 min at 4 °C. The ammonium sulfate precipitate was collected by centrifugation at 50 000 rpm for 60 min with a Beckman 60 Ti rotor. The pellet was suspended in chromatography buffer and adjusted to 0.05 M ammonium sulfate. This solution was applied to a 1.5 × 30 cm column of phosphocellulose equilibrated with chromatography buffer containing 0.05 M ammonium sulfate. The phosphocellulose column was washed with 10 column volumes of the chromatography buffer containing 0.075 M ammonium sulfate, and RNA polymerases were eluted with a 100- plus 100-mL gradient of 0.075–0.5 M ammonium sulfate in chromatography buffer. RNA polymerase activity eluted as three overlapping peaks at 0.11, 0.15, and 0.23 M ammonium sulfate. The latter two peaks were resistant to inhibition by 1 μg/mL of α-amanitin while the first peak disappeared when assayed in the presence of the toxin. The latter two peaks were pooled and had a specific activity of 237 units/mg of protein (Table I).

(*e*) *DEAE-Sephadex Chromatography.* The pooled fractions from phosphocellulose chromatography were dialyzed against 1 L of chromatography buffer containing 0.03 M ammonium sulfate. The dialyzate was loaded onto a 1.5 × 30 cm column of DEAE-Sephadex equilibrated at 0.05 M ammonium sulfate in chromatography buffer. After application of the sample, the column was washed with 5 column volumes of chromatography buffer containing 0.05 M ammonium sulfate, and RNA polymerase were eluted with a 0.05–0.5 M ammonium sulfate gradient in chromatography buffer (75 plus 75 mL). Two peaks of RNA polymerase activity were eluted at 0.15 and 0.28 M ammonium sulfate. The first peak of activity is RNA polymerase I and is refractory to α-amanitin. About 90% of the RNA polymerase activity in the second peak is resistant to inhibition by 100 μg/mL toxin. Further purification of the second peak of RNA polymerase by DEAE-cellulose chromatography and glycerol density gradient centrifugation indicates that this activity is almost entirely composed of RNA polymerase III (T. J. Guilfoyle, unpublished experiments; see Figure 1). Fractions which contained the bulk of RNA polymerase I were pooled and had a specific activity of 354 units/mg of protein (Table I).

Table I: Summary of Purification of Chromatin-Bound RNA Polymerase I from 10 kg of Cauliflower Inflorescence

fraction	total units[a]	protein (mg)	sp act. (units/mg of protein)	yield (%)[c]
$(NH_4)_2SO_4$ precipitate[b]	1760	570	3.1	
heparin–Sepharose	3930	58	67.8	100
phosphocellulose	2750	11.6	237	70
DEAE–Sephadex	1950	5.5	354	50
DEAE–cellulose	1470	3.6	405	37
glycerol density gradient	1250	2.8	435	32

[a] Total units are units of α-amanitin resistant activity in nanomoles of UMP incorporated into RNA in 20 min. [b] This is the ammonium sulfate precipitate of the solubilized chromatin proteins (see text for details). [c] Yield (%) is based on the heparin–Sepharose fraction since an ~2.5-fold increase over the activity present in the ammonium sulfate precipitate is achieved by this chromatographic procedure.

(*f*) *DEAE-cellulose Chromatography.* The pooled fractions from DEAE-Sephadex chromatography were dialyzed against 1 L of 0.03 M ammonium sulfate in chromatography buffer. The dialyzate was loaded onto a 0.75 × 10 cm column of DEAE-cellulose equilibrated with 0.05 M ammonium sulfate in chromatography buffer. After the column was washed with 5 column volumes of the same buffer, RNA polymerase I was eluted with a 0.05–0.5 M ammonium sulfate gradient in chromatography buffer (20 plus 20 mL). RNA polymerase activity eluted as a single peak at 0.125 M ammonium sulfate. Peak fractions were pooled and had a specific activity of 405 units/mg of protein (Table I).

(*g*) *Glycerol Density Gradient Centrifugation.* The pooled fractions from DEAE-cellulose chromatography were dialyzed against 1 L of 0.075 M ammonium sulfate in chromatography buffer for 5 h and applied to a 0.75 × 5 cm column of phosphocellulose equilibrated with 0.075 M ammonium sulfate in chromatography buffer. RNA polymerase I was concentrated by step elution of the enzyme from phosphocellulose at 0.35 M ammonium sulfate in chromatography buffer. Concentrated fractions of 0.25 mL at a protein concentration of about 1–2 mg/mL were loaded onto glycerol gradients constructed as described by Spindler et al. (1978) and centrifuged at 50 000 rpm for 16 h in a Beckman SW 50.1 rotor at 4 °C. Fractions of 0.25 mL were collected, and a specific activity of ~435 units/mg of protein was obtained for each fraction containing RNA polymerase activity (Table I).

Results

Purification Summary. Table I summarizes the purification of cauliflower RNA polymerase I to homogeneity. Overall purification is ~250-fold from isolated chromatin and ~150-fold from solubilized chromatin protein but ~10 000-fold from total tissue. The overall yield is ~32%, and ~0.3 mg of RNA polymerase I is obtained/kg of cauliflower curd. The final specific activity is ~435 nmol of UMP incorporated into RNA at 30 °C in 20 min which is similar to that of other highly purified eukaryotic RNA polymerases assayed under similar conditions (Guilfoyle & Jendrisak, 1978; Guilfoyle et al., 1980). The final purification step, glycerol density gradient centrifugation, results in a homogeneous preparation of cauliflower RNA polymerase I as evidenced by the symmetrical activity peak on glycerol density gradient centrifugation, by

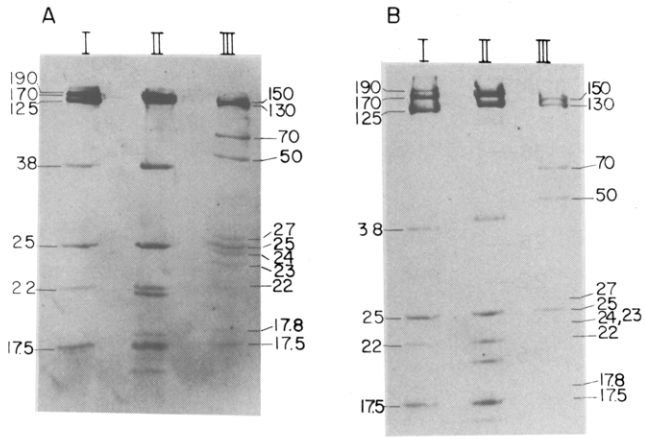

FIGURE 1: $NaDodSO_4$–polyacrylamide gel electrophoresis of cauliflower RNA polymerases I, II, and III on (A) 15% and (B) 10–16% gradient polyacrylamide gels. RNA polymerase I was purified through the glycerol gradient step (Table I). RNA polymerase II was purified as described by Jendrisak & Guilfoyle (1978). RNA polymerase III was purified through the DEAE-Sephadex step as described in the text for RNA polymerase I and was further purified by DEAE-cellulose chromatography and glycerol density gradient centrifugation (T. J. Guilfoyle, unpublished experiments). Approximately 2 μg each of RNA polymerases I and II and 0.5 μg of RNA polymerase III were applied to the gels. After electrophoresis, gels were stained with Coomassie brilliant blue and destained as described by Burgess & Jendrisak (1975). Numbers adjacent to RNA polymerases I and III indicate the molecular masses of the subunits in each enzyme in kilodaltons. Molecular masses of RNA polymerase II subunits are given in Jendrisak & Guilfoyle (1978) and in Figure 3.

the constancy of specific activity across the glycerol density gradient peak, by the high specific activity of the enzyme, by the migration of the purified enzyme as a single polypeptide in polyacrylamide gels subjected to electrophoresis under nondenaturing conditions (data not shown), and by the subunit composition of the enzyme resolved on one- and two-dimensional polyacrylamide gels subjected to electrophoresis in the presence of dodecyl sulfate (Figures 1 and 3).

RNA Polymerase I Subunit Structure. The subunit structure of cauliflower RNA polymerase I is compared to that of cauliflower RNA polymerase II and III on 15% and 10–16% gradient $NaDodSO_4$–polyacrylamide gels in Figure 1. The molecular weights of the putative subunits were estimated by comparing the mobilities of RNA polymerase I polypeptides with those of wheat germ RNA polymerase II, cauliflower RNA polymerase II, and *Escherichia coli* RNA polymerase. Polypeptides associated with cauliflower RNA polymerase I are 190 000 (a), 170 000 (a′), 125 000 (b), 38 000 (c), 25 000 (d), 22 000 (e), and 17 500 (f). The amount of 190 000 polypeptide compared to that of the 170 000 polypeptide is somewhat variable, and preliminary evidence suggests that the 170 000 polypeptide has a similar peptide map to the 190 000 polypeptide as determined by the peptide mapping procedure of Cleveland et al. (1977) utilizing *S. aureus* V8 protease. The proteolysis of an RNA polymerase I a subunit to an a′ polypeptide during purification procedures has been previously demonstrated in rat liver (Coupar et al., 1977).

Figure 2 shows densitometer tracings of cauliflower RNA polymerase I polypeptides which were separated on $NaDodSO_4$–polyacrylamide gels identical with those displayed in Figure 1. The molar ratio or stoichiometry of each putative subunit was determined by calculating the area under each polypeptide peak from the densitometer scans (Burgess & Jendrisak, 1975). Molar ratios for the subunits are (a) 0.41, (a′) 0.63, (b) 1, (c) 0.85, (d) 1.7, (e) 0.8, and (f) 1.9. Although the ratio of a subunit to a′ polypeptide was variable, both

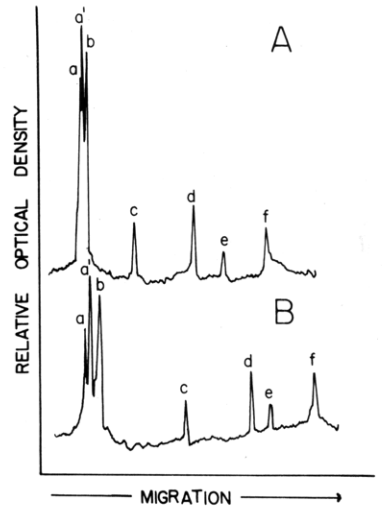

FIGURE 2: Densitometer tracings of 2-μg amounts of cauliflower RNA polymerase I subunits separated on (A) 15% and (B) 10–16% gradient $NaDodSO_4$–polyacrylamide gels. Subunits are labeled a–f (see text). The gels are identical with those shown in Figure 1.

polypeptides together consistently summed to a molar ratio of ~1.

On both 15% and 10–16% gradient $NaDodSO_4$–polyacrylamide gels, three polypeptides of 25 000, 22 000, and 17 500 have identical mobilities in RNA polymerases I, II, and III. These polypeptides also have identical mobilities on two-dimensional polyacrylamide gels as demonstrated with RNA polymerases I and II (Figure 3). These results suggest that the three classes of RNA polymerase possess three common subunits. This is also supported by the observation that the molar ratios of the 25 000, 22 000, and 17 500 subunits in each class of enzyme are 1.7–2.0, 0.8–1.0, and 1.5–2.0, respectively. Thus, each putative common subunit has an identical molecular weight, an identical charge, and a similar molar ratio in each class of RNA polymerase.

Antigenic Relatedness of RNA Polymerases I and II. Figure 4 shows that RNA polymerase I from cauliflower forms precipitin lines with antibody raised against cauliflower RNA polymerases II and III in double immunodiffusion analysis. This result indicates that the three classes of enzyme possess common antigenic determinants. To elucidate which subunits cross-react in RNA polymerases I and II, I used a procedure similar to that described by Renart et al. (1979) for studying SV-40 virion polypeptides (see Experimental Procedures). Equivalent amounts of purified cauliflower RNA polymerases I and II were denatured in dodecyl sulfate sample buffer (Laemmli, 1970) and subjected to electrophoresis on 16% polyacrylamide–agarose composite gels (Renart el al., 1979). After electrophoresis, RNA polymerase polypeptides were transferred to DBM-paper and sequentially incubated with anti-RNA polymerases II γ-globulin and then with [^{125}I]-protein A from *S. aureus* (see Experimental Procedures for details). The paper was subjected to autoradiography, and Figure 5 shows that the 38 000, 25 000, 22 000, and 17 500 polypeptides of RNA polymerase I and all of the polypeptides associated with RNA polymerase II react with the [^{125}I]protein A. The 190 000, 170 000, and 125 000 polypeptides of RNA polymerase I do not react. This result further supports the concept that RNA polymerases I and II possess three common subunits but, in addition, indicates that the 38 000 subunit of RNA polymerase I is antigenically related to some RNA polymerase II polypeptide.

Sensitivity of RNA Polymerases I, II, and III to Inhibition by α-Amanitin. Figure 6 shows the sensitivity of purified

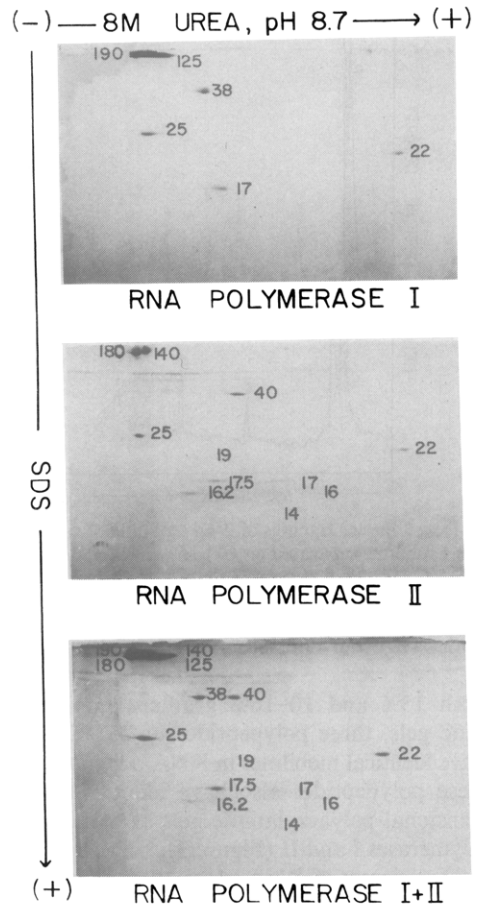

FIGURE 3: Polypeptide subunits of cauliflower RNA polymerases I and II separated on two-dimensional polyacrylamide gels as described by Jendrisak & Burgess (1977). The first dimension (horizontal direction) consisted of electrophoresis of RNA polymerases I, II, or I and II on 7.5% polyacrylamide gels containing 8 M urea (pH 8.7). The second dimension (vertical direction) consisted of electrophoresis of polypeptides from the urea into a 1.5 mm thick 15% polyacrylamide slab gel containing dodecyl sulfate. Approximately 25 μg of RNA polymerases I, II, or I and II were subjected to analysis. After electrophoresis, gels were stained and destained as described by Burgess & Jendrisak (1975). Directions of migration are indicated in the figure. Subunit molecular masses in kilodaltons are adjacent to subunit polypeptides.

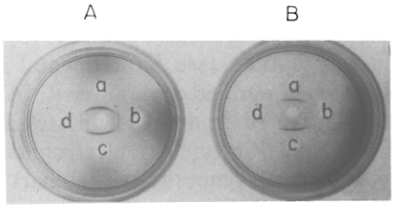

FIGURE 4: Double immunodiffusion analysis (Ouchterlony) of cauliflower RNA polymerases I, II, and III with antibody raised against cauliflower RNA polymerases II and III. Approximately 5 μL of a 1 mg/mL solution of each enzyme was applied to outer wells, and 5 μL of a 5 mg/mL solution of the γ-globulin fraction was applied to the center well. (A) Center well contains antibody raised against cauliflower RNA polymerase II. Outer wells contain (a) cauliflower RNA polymerase I, (b) wheat germ RNA polymerase II, (c) cauliflower RNA polymerase II, and (d) yeast RNA polymerase II. (B) Center well is antibody raised against cauliflower RNA polymerase III. Outer wells contain (a) cauliflower RNA polymerase I, (b) wheat germ RNA polymerase II, (c) cauliflower RNA polymerase III, and (d) yeast RNA polymerase II.

cauliflower RNA polymerases I, II, and III to inhibition by the fungal toxin, α-amanitin. RNA polymerase II is inhibited by 50% at ~0.05 μg/mL α-amanitin which is similar to that reported for other plant class II enzymes (Jendrisak & Guilfoyle, 1978; Guilfoyle & Jendrisak, 1978). Purified RNA polymerase III is inhibited 50% at about 1–2 mg/mL α-amanitin which is similar to the inhibition reported earlier for a less pure preparation of the enzyme (Guilfoyle, 1976). RNA polymerase I is refractory to the toxin even at concentrations in the range of 1–2 mg/mL α-amanitin.

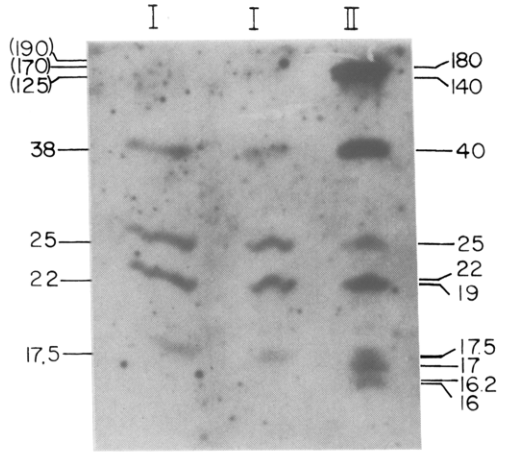

FIGURE 5: Specificity of anti-cauliflower RNA polymerase II antibody reacted with subunits of cauliflower RNA polymerases I and II. Procedures for separating the RNA polymerase subunits by NaDodSO$_4$–polyacrylamide gel electrophoresis, transfer of polypeptides to DBM-paper, incubation with anti-RNA polymerase II, and incubation with [^{125}I]protein A are described in the text. The autoradiogram displayed in this figure is for cauliflower RNA polymerase I purified through DEAE-cellulose (see Table I) in the left lane, glycerol gradient purified cauliflower RNA polymerase I in the center lane, and cauliflower RNA polymerase II purified to homogeneity in the right lane. Subunit molecular masses for RNA polymerase I (on the left) and RNA polymerase II (on the right) are in kilodaltons. RNA polymerase I subunits that do not cross-react with anti-RNA polymerase II are indicated in parentheses on the left.

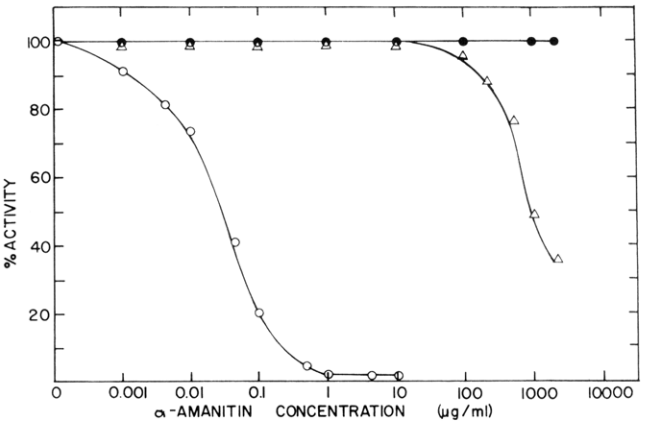

FIGURE 6: Effect of α-amanitin on the activities of cauliflower RNA polymerases I (●), II (○), and III (△). Assays were conducted with enzymes of the purity shown in Figure 1 at a concentration of 1.25 μg/mL RNA polymerase protein.

Discussion

RNA polymerases which are actively engaged in transcription are associated with the chromatin template and are released as soluble enzymes only after high-salt extraction and shearing of the chromatin DNA [reviewed by Jacob (1973; Chambon (1974); Roeder, (1976)]. In this paper, I have described a procedure for the large-scale purification of chromatin-bound RNA polymerase I from cauliflower to homogeneity with an overall yield of >30%. About a 400-fold purification of the enzyme is achieved by simply pelleting the chromatin from the total tissue extract. Subsequent purification of the enzyme by heparin–Sepharose chromatography

results in RNA polymerase I that is about 15–20% pure after a single chromatographic step. Chromatography on heparin–Sepharose is advantageous as an initial purification procedure with RNA polymerases that have been solubilized from chromatin since the chromatographic column can be loaded at 0.25 M ammonium sulfate. The use of this high salt concentration not only leads to a selective binding of RNA polymerases to the resin, but also prevents aggregation of chromatin proteins which occurs if the solubilized chromatin proteins are diluted into low ionic strength buffers prior to chromatography. Purification of the enzyme to homogeneity from 1–30 kg of tissue can be achieved in 4–5 days, and milligram quantities of pure RNA polymerase I are easily obtained.

One- (dodecyl sulfate) and two- (8 M urea, pH 8.7, followed by dodecyl sulfate) dimensional polyacrylamide gel electrophoresis conducted under denaturing conditions indicates that cauliflower RNA polymerase I consists of seven polypeptides of 190 000, 170 000, 125 000, 38 000, 25 000, 22 000, and 17 500. On two-dimensional polyacrylamide gels, subunits are separated primarily on the basis of charge in the first dimension and on the basis of molecular weight in the second dimension. Molar ratios are near unity for the 190 000 plus 170 000, 125 000, 38 000, and 22 000, and a stoichiometry of ~2 is obtained for the 25 000 and 17 500 subunits. Peptide mapping, stoichiometries, and inconsistency in the relative amounts of the 190 000 subunit compared to the 170 000 polypeptide suggest that the 170 000 polypeptide may arise artificially at the expense of the 190 000 subunit during purification of the enzyme. We have previously reported that the largest subunit of soybean RNA polymerase I shows some heterogeneity (Guilfoyle et al., 1976) as does the largest subunit of class II RNA polymerases (Jendrisak & Guilfoyle, 1978; Guilfoyle & Jendrisak, 1978; Guilfoyle & Malcolm, 1980). Coupar et al. (1977) have demonstrated that the method employed for extraction and purification of rat liver RNA polymerase I could influence the relative amount of 195 000 to 175 000 polypeptide, and they suggested that the 175 000 polypeptide arises as an artifact during purification procedures. Although it is likely that the 170 000 polypeptide associated with purified cauliflower RNA polymerase I also arises artificially during enzyme purification, I have not been successful in purifying enzyme that is devoid of the 170 000 polypeptide even though a number of purification procedures have been employed and a number of protease inhibitors have been utilized (Guilfoyle, 1976; T. J. Guilfoyle, unpublished experiments).

Comparison of the subunit structure of cauliflower RNA polymerase I with those of cauliflower RNA polymerases II and III on one-dimensional $NaDodSO_4$–polyacrylamide gels (as well as two-dimensional polyacrylamide gels in the case of RNA polymerase II) indicates that each class of RNA polymerase has a distinct subunit structure but that each enzyme also possesses three subunits of 25 000, 22 000, and 17 500 which have identical molecular weights, identical charges, and similar stoichiometries. This suggests that three subunits may be common to cauliflower RNA polymerase I, II, and III. Common subunits of 27 000, 23 000, and 14 500 have been reported in RNA polymerases I and II from yeast (Buhler et al., 1976; Valenzuela et al., 1976a), and common subunits of 22 500, 15 500, and 13 300 have been reported in RNA polymerases I, II, and III in *Acanthamoeba castellanii* (D'Alessio et al., 1979). In both of the above studies, putative common subunits have identical mobilities on one- and two-dimensional polyacrylamide gels. In two other examples limited to resolution of polypeptides on one-dimensional $NaDodSO_4$–polyacrylamide gels, wheat germ RNA polymerases I, II, and III were reported to contain putative common subunits of 20 000, 17 800, and 17 000 (Jendrisak, 1980), and mouse plasmacytoma RNA polymerases I, II, and III were shown to possess subunits 29 000 and 19 000 (Roeder, 1976). In general, data on the subunit structures of the three classes of eukaryotic RNA polymerase support there being at least three common low molecular weight subunits: (a) a polypeptide of ~25 000 that is basic in charge (p*I* of ~9) with a stoichiometry of ~2; (b) a polypeptide of ~20 000 that is acidic in charge (p*I* of ~4.5) with a stoichiometry of 1; (c) a polypeptide of ~13 000–17 500 of intermediate charge (p*I* of ~4.7) with a stoichiometry of 1–2.

In a further effort to determine the relatedness of polypeptides in cauliflower RNA polymerases I, II, and III, I analyzed the RNA polymerase subunits for immunological cross-reactivity by using antibody raised against cauliflower RNA polymerase II. By using the procedures described by Renart et al. (1979), I first determined that all of the cauliflower RNA polymerase II subunits are antigenic (Figure 5; T. J. Guilfoyle, unpublished experiments). Since all of the RNA polymerase II subunits showed some antigenicity, this indicated that any common subunits or common antigenic determinants should be detectable in RNA polymerase I. Using the method of Renart et al. (1979), I have demonstrated that RNA polymerase I subunits of 38 000, 25 000, 22 000, and 17 500 react with antibody raised against cauliflower RNA polymerase II. Although this would be expected for the three putative common subunits in the enzymes, the cross-reactivity of the 38 000 subunit is surprising in that a polypeptide of this molecular weight or charge is not detected in RNA polymerase II. This result indicates that the relatedness of polypeptides in the three classes of RNA polymerase can not be dismissed simply on the basis of nonidentical mobilities on one- or two-dimensional polyacrylamide gels. It is possible that the 38 000 subunit of RNA polymerase I and the 40 000 subunit of RNA polymerase II share some common peptide sequences and antigenic determinants even though they have slightly different molecular weights and charge densities; however, it is possible also that the 38 000 subunit is unrelated to the 40 000 subunit but shares antigenic determinants with some other RNA polymerase II polypeptide. The results with cauliflower RNA polymerase II antibody also indicate that the 190 000, 170 000, and 125 000 polypeptides of RNA polymerase I do not react with anti-RNA polymerase II. It is obvious from the above results that if specific antibody is to be made to a class of RNA polymerase that does not cross-react with other classes of RNA polymerase, it is probably necessary to make this antibody to one of the two largest subunits of the enzyme rather than the native enzyme.

In addition to the three putative common subunits in cauliflower RNA polymerases I and II, there may be other common subunits in RNA polymerases I, II, and III. The subunit structure presented here for cauliflower RNA polymerases may be over simplified due to the following reasons: (1) subunits of less than stoichiometric amounts or subunits which stain poorly with Coomassie brilliant blue might not be detected in this study, (2) certain subunits may dissociate from the RNA polymerases during purification procedures, and (3) certain subunits may be proteolytically degraded or modified during purification procedures. In yeast (Valenzuela et al., 1976a) and *A. castellanii* (D'Alessio et al., 1979), as many as six subunits may be common to RNA polymerases I and III.

The presence of common polypeptides and common antigenic determinants in the three classes of eukaryotic RNA polymerase suggests that these subunits may be required for a common role such as in the polymerization of RNA or the interaction of the enzymes with the chromatin template. The presence of common subunits also presents interesting problems for the regulation of the biosynthesis of RNA polymerases I, II, and III, especially in cases where the relative quantities of RNA polymerases I, II, and III vary with the cell cycle (Sebastian et al., 1974) or are altered by hormone or drug administration (Jaehning et al., 1975; Guilfoyle et al., 1980).

Acknowledgments

I acknowledge the skillfull technical assistance of Sandra Malcolm, especially for her effort in raising antibodies to the cauliflower RNA polymerases, and the critical comments on the manuscript by Gretchen Hagen.

References

Alwine, J. C., Kemp, D. J., & Stark, G. R. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 5350–5354.

Bradford, M. M. (1976) *Anal. Biochem. 72*, 248–254.

Buhler, J. M., Iborra, F., Sentenac, A., & Fromageot, P. (1976) *J. Biol. Chem. 251*, 1712–1717.

Burgess, R. R., & Jendrisak, J. J. (1975) *Biochemistry 14*, 4634–4638.

Chambon, P. (1974) *Enzymes, 3rd Ed. 10*, 261–331.

Cleveland, D. W., Fischer, S. G., Kirschner, M. W., & Laemmli, U. K. (1977) *J. Biol. Chem. 252*, 1102–1106.

Coupar, B. E. H., Chesterton, C. J., & Butterworth, P. H. W. (1977) *FEBS Lett. 77*, 273–276.

D'Alessio, J. M., Perna, P. J., & Paule, M. R. (1979) *J. Biol. Chem. 254*, 11282–11287.

Davison, B. L., Leighton, T., & Rabinowitz, J. C. (1979) *J. Biol. Chem. 254*, 9220–9226.

Guilfoyle, T. J. (1976) *Plant Physiol. 58*, 453–458.

Guilfoyle, T. J., & Jendrisak, J. J. (1978) *Biochemistry 17*, 1860–1866.

Guilfoyle, T. J., & Malcolm, S. (1980) *Dev. Biol. 78*, 113–125.

Guilfoyle, T. J., Lin, C. Y., Chen, Y. M., & Key, J. L. (1976) *Biochim. Biophys. Acta*, 344–357.

Guilfoyle, T. J., Olszewski, N., & Zurfluh, L. (1980) in *Genome Organization and Expression in Plants* (Leaver, C. J., Ed.) pp 93–104, Plenum Press, New York.

Jacob, S. T. (1973) *Prog. Nucleic Acid Res. Mol. Biol. 13*, 93–126.

Jaehning, J. A., Stewart, C. C., & Roeder, R. G. (1975) *Cell (Cambridge, Mass.) 4*, 51–57.

Jendrisak, J. J. (1980) in *Genome Organization and Expression in Plants* (Leaver, C. J., Ed.) pp 77–92, Plenum Press, New York.

Jendrisak, J. J., & Burgess, R. R. (1975) *Biochemistry 14*, 4639–4645.

Jendrisak, J. J., & Burgess, R. R. (1977) *Biochemistry 16*, 1959–1964.

Jendrisak, J. J., & Guilfoyle, T. J. (1978) *Biochemistry 17*, 1322–1327.

Laemmli, U. K. (1970) *Nature (London) 227*, 680–685.

Laskey, R. A., & Mills, A. D. (1977) *FEBS Lett. 82*, 314–316.

Linn, T. G., Greenleaf, A. L., Shorenstein, R. G., & Losick, R. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70*, 1865–1869.

Renart, J., & Sebastian, J. (1976) *Cell Differ. 5*, 97–107.

Renert, J., Reiser, J., & Stark, G. R. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 3116–3120.

Roeder, R. G. (1976) in *RNA Polymerase* (Losick, R., & Chamberlin, M., Eds.) pp 285–329, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Schultz, L. D., & Hall, B. D. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 1029–1033.

Sebastian, J., Takano, I., & Halvorson, H. O. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 769–773.

Sklar, V. E. F., Jaehning, J. A., Gage, L. P., & Roeder, R. G. (1976) *J. Biol. Chem. 251*, 3794–3800.

Southern, E. M. (1975) *J. Mol. Biol. 98*, 503–517.

Spindler, S. R., Duester, G. L., D'Alessio, J. M., & Paule, M. R. (1978) *J. Biol. Chem. 253*, 4669–4675.

Thonart, P., Bechet, J., Hilger, F., & Burny, A. (1976) *J. Bacteriol. 125*, 25–32.

Valenzuela, P., Bell, G. I., Weinberg, F., & Rutter, W. J. (1976a) *Biochem. Biophys. Res. Commun. 71*, 1319–1325.

Valenzuela, P., Hager, G. L., Weinberger, F., & Rutter, W. J. (1976b) *Proc. Natl. Acad. Sci. U.S.A. 73*, 1024–1028.