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Isolation of Ribonucleic Acid Polymerases I, II, and III from *Saccharomyces cerevisiae*†

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ABSTRACT: A procedure for the simultaneous purification of RNA polymerases I, II, and III from *Saccharomyces cerevisiae* is described. High yields of each enzyme activity are obtained, allowing the preparation of approximately 10 mg of polymerase I, 25 mg of polymerase II, and 12 mg of polymerase III from 1.2 kg of cells (wet weight). Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate indicates RNA polymerase I contains polypeptides with molecular weights 185 000, 137 000, 41 000, 35 000, 28 000, 24 000, 20 000, 16 000, 14 500, and 12 300; RNA polymerase II contains subunits with molecular weights 170 000, 145 000, 41 000, 33 500, 28 000, 24 000, 18 000, 14 500, and 12 500; and RNA polymerase III contains polypeptides with molecular weights 160 000, 128 000, 82 000, 53 000, 41 000, 37 000, 34 000, 28 000, 24 000, 20 000, 14 500, and 10 700.

The in vitro analysis of transcription from eucaryotc cells is seriously hampered by two characteristics of these systems. First, as a consequence of their genetic complexity, the concentration of a particular gene sequence in the transcripts from isolated DNA or chromatin templates is very low; therefore large amounts of RNA polymerases are required to produce detectable amounts of a specific product. Second, the concentration of the transcriptive enzymes in higher cells is relatively low (Rutter et al., 1974; Schwartz and Roeder, 1974). Thus substantial amounts of the enzymes are very difficult to obtain. These two considerations severely limit the feasibility of studies with homologous templates and polymerases, a condition which may be important for the study of controlling sequences or elements.

To partially diminish these difficulties, we have chosen to develop a system from the lower eucaryote, *Saccharomyces cerevisiae*. The genetic complexity of this cell is several hundred fold lower than higher eucaryotes; the commercial availability of large quantities of cells should permit the isolation of enzymes and other ancillary factors in the required quantity. This should allow the molecular reconstruction of transcription in this system.

Several procedures have been described for the separate preparation of RNA polymerases I (A) and II (B) from yeast to various levels of purity (Ponta et al., 1972; Adman et al., 1972; Dezelee and Sentenac, 1973; Buhler et al., 1974), and the presence of RNA polymerase III has been demonstrated on the basis of chromatographic criteria (Roeder, 1969; Ponta et al., 1971; Adman et al., 1972). We describe here a procedure for the concomitant purification of large amounts of RNA polymerases I, II, and III from yeast, and preliminary studies on the structure of these enzymes. In addition to their application to the study of transcription from yeast templates, the relative simplicity of purification from a single procedure makes these enzymes attractive for transcriptional studies in nonhomologous eucaryotic systems, where the isolation of high levels of RNA polymerase is not practicable.

Materials and Methods

Materials. The following were obtained from Calbiochem: protamine sulfate, A grade; dithiothreitol, A grade; and α-amanitin, A grade. Coomassie brilliant blue R, phenylmethylsulfonyl fluoride, and calf thymus DNA, grade I, were purchased from Sigma Chemical Co. Nucleotide triphosphates were from P-L Biochemicals, Inc., Milwaukee, Wisc., and [^{3}H]UTP (30 Ci/mmol) was from Schwarz/Mann. Poly(dAT·dAT) was obtained from Miles Laboratories Inc. Amicon concentrators (350- and 50-ml capacity) were purchased from the Amicon Corp.

Denatured calf thymus DNA cellulose was prepared as described by Alberts and Herrick (1971), with the following modifications (Litman, 1968). After adsorption of the DNA to the cellulose and lyophilization, the powder was suspended in absolute EtOH, distributed as a 1-cm deep slurry, and ir-

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TABLE I: Purification of RNA Polymerases I, II, and III.[a]

	280/260	Enzyme Composition	Protein (mg)	Act. (units)	Sp Act.	% Yield
Enzyme fraction						
(1) Homogenate	0.62	I, II, III	80 200	32 025	0.4	100
(2) Protamine sulfate supernatant	0.74	I, II, III	66 500			
(3) Agarose A-5m pool	1.20	I, II, III	10 200	23 600	2.3	74
(4) Ion Filtration pool		I, II, III	2 130	32 500	15.2	100
DNA-cellulose pools						
(5)		I, II	350	19 200	55.0	
(6)		III	12	3 000	250	60
DEAE-Sephadex pools						
(7)		I	45	1 580	35.0	33
(8)		II	26	12 900	500	50
Sucrose gradient						
(9)		I	8	960	125	20

[a] Enzyme activities (determined under the standard conditions given in Materials and Methods) and yields are given for a preparation from 1.2 kg of cells (wet weight). The values represent averages of three independent preparations. The total activity present in fraction 1 was determined at a dilution of the crude homogenate sufficiently great to obtain a linear relationship of enzymatic activity with added aliquot of the homogenate.

radiated with a UVL-22 Blak-Ray UV[1] lamp (Ultra-violet Products, Inc., San Gabriel, Calif.) from a distance of 5 in. for 15 min. The slurry was stirred at 2-min intervals to ensure uniform irradiation. The cellulose slurry was air-dried at room temperature, lyophilized, and stored as the dried powder at −20 °C before use.

Yeast Strain and Cell Storage. Baker's yeast, strain F1, was obtained from the Red Star Yeast Corp. in Oakland, Calif. Cells were harvested on ice from the commercial fermenter during the last growth cycle. After one wash with water at 4 °C, cell pellets were suspended in one-tenth (w/v) 85% glycerol, 10% Me_2SO, 0.1 M Tris-HCl, pH 7.9 (4 °C), 0.05 M $MgCl_2$, 1 mM NaEDTA, 5 mM $PhCH_2SO_2F$, 5 mM dithiothreitol. The cell suspensions were frozen by submersion in liquid nitrogen from an apparatus that formed multiple streams of droplets. The resulting pellets were stored under liquid nitrogen for up to 6 months before use.

Buffers. Buffer A contained 10% (v/v) glycerol, 0.05 M Tris-HCl, pH 7.9 (4 °C), 5 mM $MgCl_2$, 0.1 mM NaEDTA, 0.2 mM dithiothreitol. Buffer B was identical with buffer A, except that the glycerol concentration was 25% (v/v).

Sodium Dodecyl Sulfate Gel Electrophoresis. Electrophoresis was carried out in 1.5 mm × 10 cm × 17.5 cm polyacrylamide slabs in a Hoefer Scientific Instruments slab gel apparatus using the discontinuous buffer system described by Laemmli (1970). Protein samples were heated to 100 °C for 2 min in the presence of 2% sodium dodecyl sulfate and 2-mercaptoethanol prior to electrophoresis. Bromophenol blue (0.004%) was added as a dye marking the position of the discontinuous buffer front. After electrophoresis gels were gently swirled on a reciprocal shaker for 20 min in 10% trichloroacetic acid–50% 2-propanol. The gel was then stained overnight in 10% trichloroacetic acid–25% 2-propanol–0.1% Coomassie brilliant blue and destained in 10% acetic acid. Gels were either photographed directly or scanned at 600 nm with a Gilford linear transport attached to a Gilford spectrophotometer.

RNA Polymerase Assay. The standard reaction mixture contained the following: 0.05 M Tris-HCl, pH 7.4 (30 °C), 6 mM NaF, 0.5 mM dithiothreitol, 2 mM $MnCl_2$, 0.6 mM GTP, CTP, and ATP, 0.01 mM [^{3}H]UTP, 1 mCi/μmol, 50 μg/ml native calf thymus DNA, 50 μg/ml heat-denatured calf thymus DNA, and 0.05 M ammonium sulfate. Reactions were terminated as described by Mead (1964) by spotting 50 μl of the incubation mixture on 2.4-cm Whatman DE 81 filter paper chromatography paper circles (Reeve Angle, Clifton, N.J.). The circles were washed seven times with 5% Na_2HPO_4, once each with water, EtOH, and diethyl ether, dried, and counted in a Packard liquid scintillation counter.

One unit of enzyme activity catalyzes the incorporation of 1 nmol of UMP into DE 81-bound RNA in 10 min at 30 °C under the reaction conditions given above, except the concentration of UTP is adjusted to 0.1 mM.

Results

The procedure described yields each of the three major species of RNA polymerase present in *Saccharomyces cerevisiae* in high yield (Table I). Processing of the enzymes through the initial steps is rapid, and the use of techniques, such as high-speed centrifugation, that would preclude scaling the method up to the multiple-kilogram level are avoided.

To achieve parallel purification without the procedure becoming unwieldy, common properties of the enzymes were exploited to maintain them as a single pool throughout the first four steps of the procedure. Through these steps, the concentration of the enzymes is increased from 0.15% in the homogenate to approximately 6% of the protein present in fraction 4, while recovery of enzymatic activity is essentially 100% and nucleic acid is quantitatively removed.

Cell Breakage. An homogenate of 1.2 kg of cells (wet weight) is prepared by passing the cells (taken directly from liquid-nitrogen storage) in 120-g aliquots through a modified Eaton press (Bhargava and Halvorson, 1971) which has been precooled for 3 min in a CO_2–EtOH bath. If cells are taken directly from liquid-nitrogen storage just before breaking, the heat absorbed by the cells maintains the Eaton cell at a low

[1] Abbreviations used: Me_2SO, dimethyl sulfoxide; EDTA, ethylenediaminetetraacetate; $PhCH_2SO_2F$, phenylmethylsulfonyl fluoride; UV, ultraviolet; Tris, tris(hydroxymethyl)aminomethane; DEAE, diethylaminoethyl.

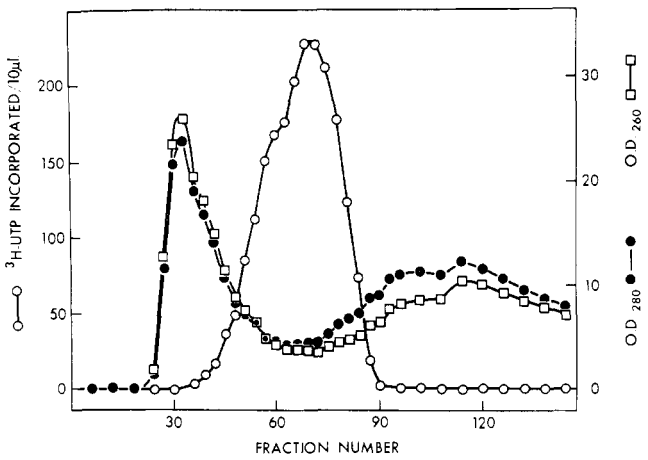

FIGURE 1: Agarose chromatography. The ammonium sulfate precipitate of fraction 2 prepared from 1.3 kg of cells was chromatographed on agarose A-5m as described under Results. Twenty-seven-milliliter fractions were collected, and the optical densities at 260 and 280 nm were determined after appropriate dilution with 1 M ammonium sulfate. RNA polymerase activity was measured with 10-µl aliquots under the standard reaction conditions. Fractions 50–85 were combined to form enzyme fraction 3.

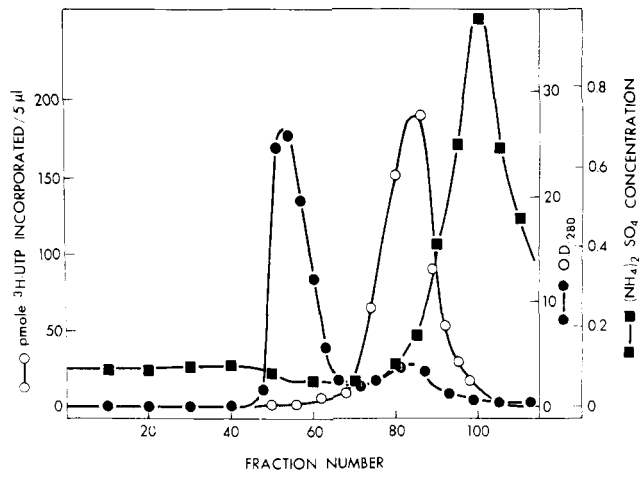

FIGURE 2: Ion-filtration chromatography. The ammonium sulfate precipitate of fraction 3 was subjected to ion-filtration chromatography as described under Results. Twenty-eight-milliliter fractions were collected and 5-µl aliquots assayed for RNA polymerase activity under the standard reaction conditions.

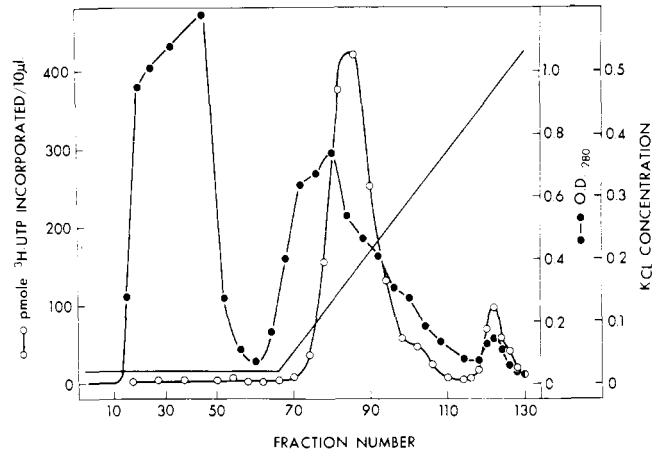

FIGURE 3: DNA-cellulose chromatography. Fraction 4 was applied to a 360-ml DNA-cellulose column as described under Results. After washing with 200 ml of 0.02 M KCl in buffer B, the column was eluted with a 2000-ml linear salt gradient–0.02–0.60 M KCl in buffer B. Fifteen-milliliter fractions were collected and 10-µl aliquots assayed for enzymatic activity under the standard reaction conditions.

enough temperature to permit continuous processing of aliquots. A cell disruption efficiency of greater than 80% is consistently achieved using this technique. The frozen extrudate is thawed (in 200-g aliquots) by the addition of a one-to-one ratio (w/v) of buffer A containing 0.7 M $(NH_4)_2SO_4$, 1% Me_2SO, 0.5 mM dithiothreitol and freshly dissolved $PhCH_2SO_2F$ to 0.5 mM. Aliquots (600-ml) of this viscous fraction were subjected to 30-s sonication at 100 W, using a Heat-Systems Ultrasonics Inc. sonicator fitted with a 1.3-cm probe. The sonicate is then centrifuged for 20 min at 10 000*g* to remove unbroken cells and debris. The supernatant from this centrifugation is diluted with 1.3 volumes of buffer A, bringing the $(NH_4)_2SO_4$ concentration to 0.15 M (fraction 1). The protein concentration of this fraction is approximately 17 mg/ml, and the optical absorbance at 260 nm is approximately 85 units/ml.

Protamine Sulfate Precipitation. A 2.5% solution of protamine sulfate in buffer A containing 0.15 M $(NH_4)_2SO_4$ is prepared by stirring overnight at 30 °C. Two-hundred and eighteen milliliters of this solution is rapidly added with vigorous stirring per liter of fraction 1 at 4 °C, resulting in a final temperature not greater than 8 °C. This suspension is stirred at 4 °C for 30 min and centrifuged at 8000*g* for 20 min. The supernatant from this centrifugation (fraction 2) is adjusted to 82% ammonium sulfate saturation by the addition of 560 g of ammonium sulfate per l. of solution. The pH (measured with a glass electrode on an undiluted aliquot) is maintained above 7 during this step by the dropwise addition of 6 N NH_4OH.

Agarose A-5m Chromatography. After stirring at 4 °C for 30 min, the fraction 2 precipitate is centrifuged at 8000*g* for 1 h. The precipitate is resuspended in buffer A to a volume of 600 ml (100 mg/ml protein) utilizing a Dounce homogenizer. This suspension is applied to a 57 cm × 9.5 cm agarose A-5m column previously equilibrated with buffer A containing 0.10 M ammonium sulfate. The column is eluted with the same buffer (Figure 1). This step removes a large peak of residual contaminating nucleic acid, increasing the average 280 nm/260 nm optical density ratio of enzyme-containing fractions to 1.2, indicating the presence of less than 2% nucleic acid. Fractions containing significant enzyme activity are pooled (fraction 3).

Ion-Filtration Chromatography. Fraction 3 is adjusted to 82% ammonium sulfate saturation by the addition of 560 g/l. ammonium sulfate. The suspension is centrifuged at 8000*g* for 1 h in a Sorvall GS3 rotor, and the precipitate is dissolved in buffer A to a final volume of 250 ml. This solution (40 mg/ml protein) is applied to a 42 × 9.5 cm DEAE-Sephadex A-25 column previously equilibrated with buffer A containing 0.1 M ammonium sulfate. The column is eluted with buffer A containing 0.3 M ammonium sulfate (Figure 2). Fractions containing 95% of the total recovered enzyme activity are pooled (fraction 4). This rapid step removes the final traces of nucleic acid and achieves a further fivefold purification with respect to protein.

DNA-Cellulose Chromatography. Fraction 4 is rapid-dialyzed in two Bio-Rad hollow-fiber dialyzers connected in series until the conductivity of the solution corresponds to an ammonium sulfate concentration of 0.02 M. The effluent solution from this dialysis is loaded directly on a 6.3 × 11.4 cm column of Whatman CF-11 cellulose containing 2 mg/ml denatured calf thymus DNA, which has been equilibrated with buffer B. The column is developed as described in Figure 3.

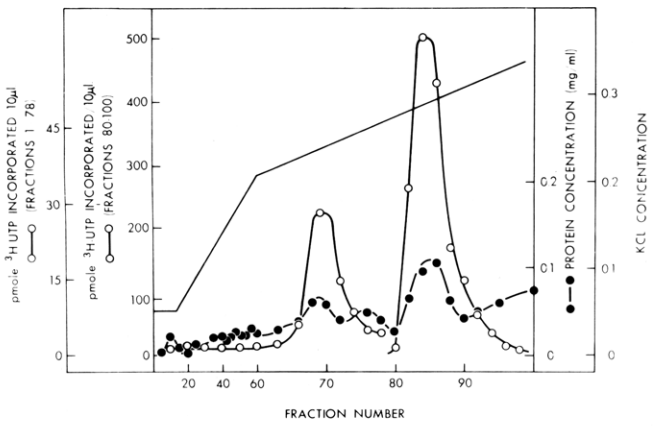

FIGURE 4: DEAE-Sephadex chromatography. Fraction 5 was applied to an 800-ml DEAE-Sephadex A-25 column, previously equilibrated with 0.05 M KCl in buffer B, as described under Results. After washing with 200 ml of 0.05 M KCl in buffer B, the column was eluted with a 5000-ml linear salt gradient–0.05–0.50 M KCl in buffer B. Forty-milliliter fractions were collected, and the protein concentration was determined after the method of Lowry et al. (1951). Ten-microliter aliquots were assayed for RNA polymerase activity under the standard reaction conditions.

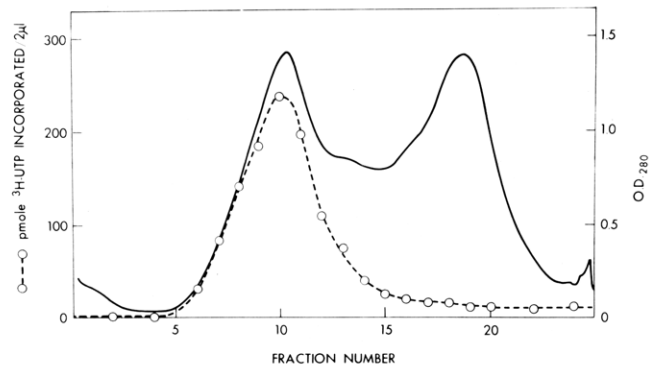

FIGURE 5: Preparative sucrose gradient. The ammonium sulfate precipitate of fraction 7 (see Results) was dissolved in 2 ml of buffer A and layered on three 12-ml 5–20% sucrose gradients prepared in buffer B containing 0.1 M ammonium sulfate. The gradients were centrifuged for 55 h in a Spinco SW 41 rotor at 40 000 rpm and 0.48-ml fractions collected by siphoning from the bottom of the tube. Two-microliter aliquots were assayed for enzymatic activity as under the standard reaction conditions.

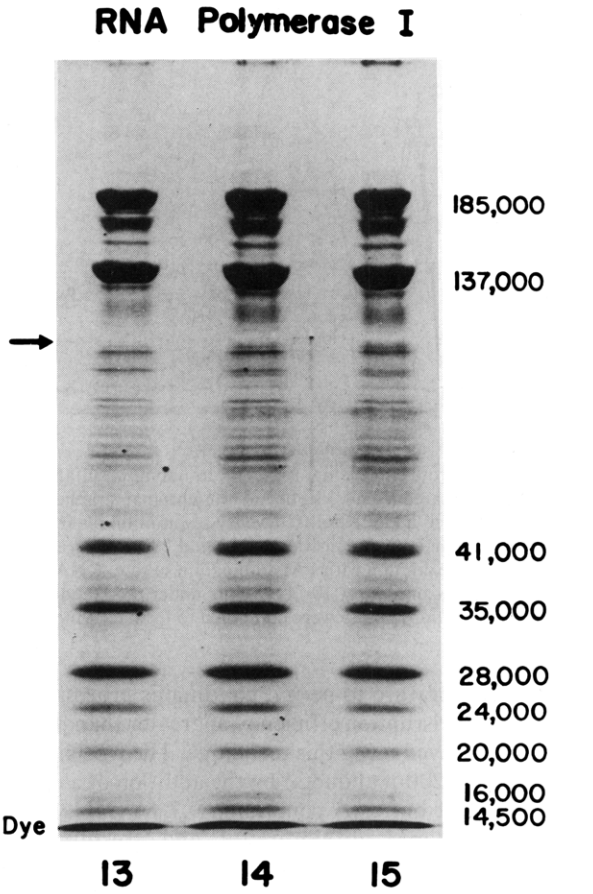

FIGURE 6: Subunit profile of RNA polymerase I. An ammonium sulfate precipitate of 1.5 mg of RNA polymerase I (fraction 9) was dissolved in 0.2 ml of buffer A and layered on a 5.2-ml 5–20% linear sucrose gradient containing 0.1 M ammonium sulfate, 25% (v/v) glycerol, 0.05 M Tris-HCl, pH 7.9, 5 mM $MgCl_2$, 0.1 mM NaEDTA, and 0.2 mM dithiothreitol. After centrifugation in a Spinco SW 65 rotor for 20 h at 60 000 rpm, fractions (0.2 ml) were collected and 2-μl aliquots assayed for RNA polymerase activity under the standard reaction conditions. Fifteen-microliter aliquots from the three fractions containing the highest level of enzyme activity were subjected to gel electrophoresis in the presence of sodium dodecyl sulfate as described in Materials and Methods. Electrophoresis was carried out in discontinuous gels to facilitate simultaneous comparison of the large and small molecular weight subunits. The acrylamide concentration in the upper part of the gel was 8.5 and 13% below the discontinuity indicated by the arrow.

Two peaks of activity are observed. The leading fractions of the first peak (fraction 5) are enriched in RNA polymerase II and the later fractions enriched in RNA polymerase I, but, at the high ratios of protein to bound DNA used in this procedure, the resolution is incomplete and irreproducible. The total pool of activity is combined, therefore, and the component enzymes are resolved with DEAE-Sephadex chromatography. The second peak (fraction 6) elutes at 0.45 M KCl and represents a pure fraction of RNA polymerase III. All fractions containing polymerase III are pooled and concentrated by binding to a 10-ml DEAE-Sephadex A-25 column at 0.1 M KCl followed by elution with 1.0 M KCl.

DEAE-Sephadex Chromatography. Fractions from the first DNA-cellulose column peak containing greater than 90% of the enzymatic activity are combined, concentrated in an Amicon concentrator with an XM-50 membrane until the optical density at 280 nm is greater than 1.0, and further concentrated by dialysis overnight against 4 volumes of buffer A saturated with ammonium sulfate. The suspension is centrifuged at 10 000g_{av} for 30 min and the resulting precipitate dissolved in 20 ml of buffer B. After dialysis in a hollow-fiber dialyzer against buffer B until the ammonium sulfate concentration equals 0.05 M, this fraction is applied to a 26 × 6.3 cm DEAE-Sephadex A-25 column, and the column developed as described in Figure 4. Fractions from the second peak (fraction 8, RNA polymerase II) having a constant specific activity are pooled and concentrated as described for fraction 5.

Sucrose Gradient. RNA polymerase I represents 30–50% of the protein in the first DEAE-Sephadex peak (fraction 7) and requires a final sucrose gradient purification step. Fractions containing greater than 90% of the activity are combined and concentrated as described for fraction 5. The final precipitate is dissolved in 2 ml of buffer A and sedimented on three sucrose gradients as described in Figure 5. Fractions having a constant specific activity (fraction 9) are combined and concentrated as described for fraction 5.

Subunit Composition. An aliquot of the final fraction for each RNA polymerase species (fractions 6, 8, and 9) was displayed on a sucrose gradient, and the fractions were ana-

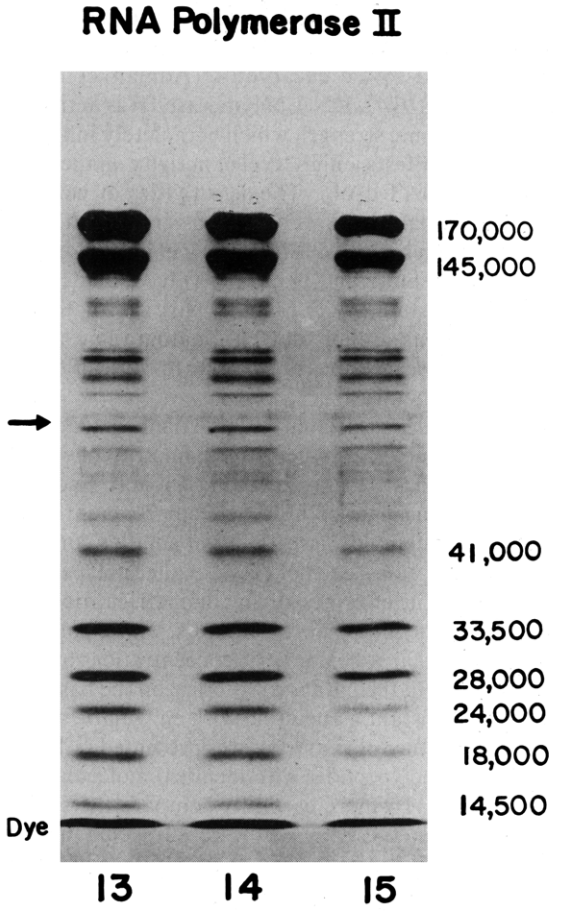

FIGURE 7: Subunit profile of RNA polymerase II. An ammonium sulfate precipitate of 2.1 mg of RNA polymerase II (fraction 8) was subjected to zone sedimentation and analyzed as described in the legend to Figure 6. Ten-microliter aliquots of the three peak activity fractions were electrophoresed also as described in the legend to Figure 6.

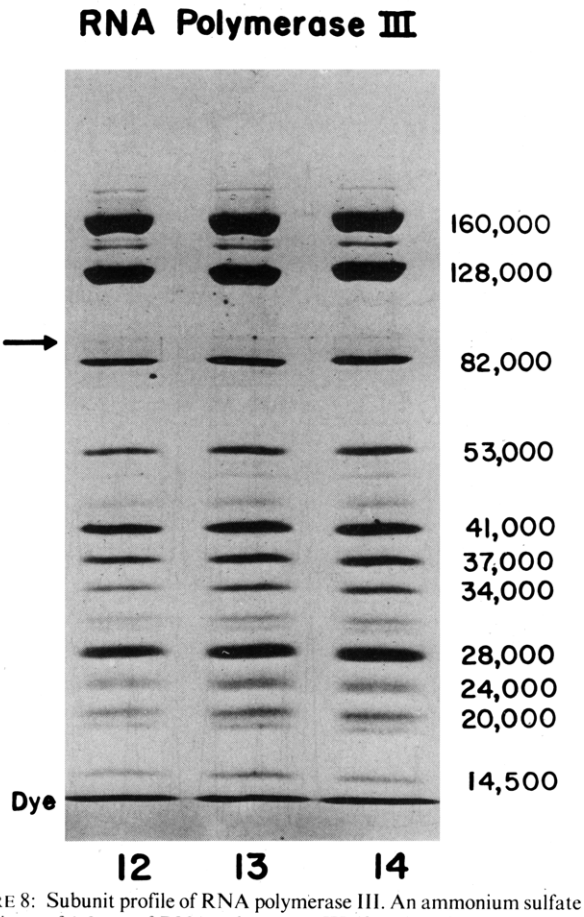

FIGURE 8: Subunit profile of RNA polymerase III. An ammonium sulfate precipitate of 1.3 mg of RNA polymerase III (fraction 6) was analyzed by zone sedimentation and gel electrophoresis (25-μl aliquots) as described in the legend to Figure 6.

lyzed for polypeptides which cosedimented with enzyme activity. The subunit profile after gel electrophoresis in the presence of sodium dodecyl sulfate for the three highest activity fractions of each enzyme is presented in Figures 6–8. Molecular weights for each of the polypeptides were determined as described in the figure legends.

A group of polypeptides with the following molecular weights appears to be specifically associated with RNA polymerase I (Figure 6): 185 000, 137 000, 41 000, 35 000, 28 000, 24 000, 20 000, and 16 000. Additional peptides with molecular weights 14 500 and 12 300 are not resolved from the dye front in this gel but are shown in the uniform concentration gel in Figure 9. RNA polymerase II contains a similar set of putative subunits with the following molecular weights (Figures 7 and 9): 170 000, 145 000, 41 000, 33 500, 28 000, 24 000, 18 000, 14 500, and 12 500. A series of much less prominent bands is presented in both RNA polymerase I and II in the region between 40 000 and 12000. None of these polypeptides is present in stoichiometries greater than 0.1 relative to the high-molecular-weight subunits, and they are presumed to be contaminant proteins. Integration of the total area under these contaminant bands indicates that polymerase I is approximately 95% pure and polymerase II approximately 95% pure.

The profile for RNA polymerase III contains a slightly larger number of polypeptides with the following molecular weights (Figure 8): 160 000, 128 000, 82 000, 53 000, 41 000, 37 000, 34 000, 28 000, 24 000, and 20 000. Again, peptides at 14 500 and 10 700 are unresolved in this gel but are demonstrated in Figure 9.

The concentration of each putative subunit of polymerases I, II, and III was determined as described in Table II, and the stoichiometry relative to the two large subunits was calculated. The experimental values given in the table represent the average of several independent determinations on enzymes from at least three different preparations.

Discussion

We have reported here an effective means of isolating the three major polymerase enzymes in essentially pure form by a single procedure. The key step in this preparation is the removal of the majority of the nucleic acid by precipitation with protamine sulfate. This technique has been frequently employed in other enzyme fractionation procedures, including the purification of both procaryotic (Chamberlin and Berg, 1961) and eucaryotic (Ponta et al., 1972) RNA polymerases. We find that the ionic conditions under which the precipitation is carried out are critical for efficient recovery of enzymatic activity. Below 0.15 M ammonium sulfate there is a severe loss of both α-amanitin sensitive and insensitive activity (data not shown), presumably resulting from the reassociation of polymerase molecules with the chromatin–protamine sulfate complex at these lower salt concentrations. RNA polymerase III is particularly sensitive to these conditions. Chromatography of supernatants from a protamine sulfate precipitation carried out at 0.1 M ammonium sulfate reveals the complete loss of polymerase III activity. Addition of protamine sulfate at ammonium sulfate concentrations greater than 0.16 M also leads

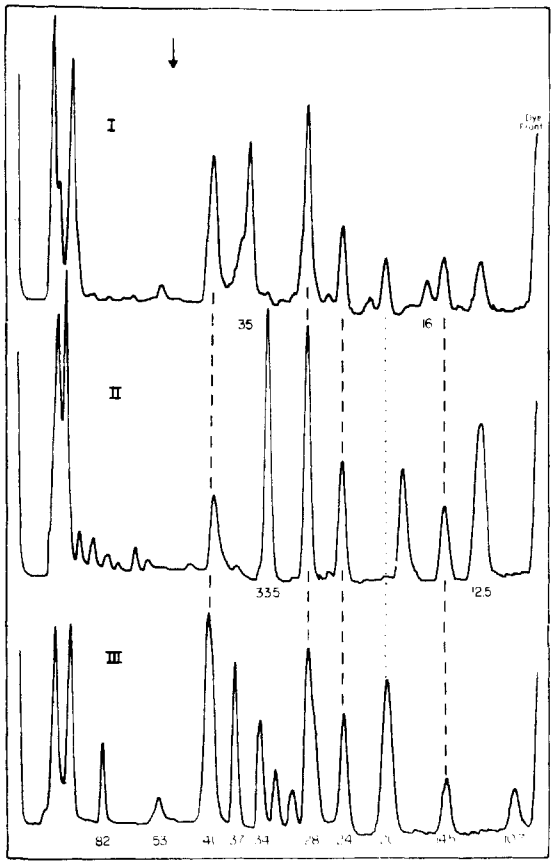

FIGURE 9: Coelectrophoresis of RNA polymerase I, II, and III subunits. Ten micrograms of RNA polymerases I (fraction 9), II (fraction 8), and III (fraction 6) were each electrophoresed in the presence of sodium dodecyl sulfate in a gel containing 13% acrylamide and scanned as described in Materials and Methods. Because of the dense staining of the two high-molecular-weight subunits, the region of the profiles containing polypeptides of lower than 50 000 molecular weight were scanned with a recorder sensitivity four times that used for the high-molecular-weight region. The point of sensitivity change is indicated by the arrow. Subunits of sensitivity change is indicated by the arrow. Subunits of apparently identical molecular weight common to all three enzymes are indicated by a dashed line; those common only to two of the enzymes are indicated by a dotted line.

to a loss of polymerase activity recoverable from the supernatant. Protamine sulfate is increasingly insoluble at these higher ammonium sulfate concentrations; the loss of activity may be due to entrapment of enzyme molecules in the protamine sulfate precipitate.

From a determination of enzyme activity in the presence of 50 μM α-amanitin (specific inhibition of polymerase II), and activity at 0.2 M ammonium sulfate (complete inhibition of polymerases I and II), we estimate that a crude homogenate from 1200 g of yeast contains approximately 30 mg of Rna polymerase I, 55 mg of polymerase II, and 20 mg of polymerase III. On this basis, the overall yield in the final fractions of RNA polymerase protein is approximtely 25% for polymerase I, 50% for polymerase II, and 60% for polymerase III. The yields of enzyme protein reported here represent minimal levels recoverable since the commercial cells used in these experiments were energy-source limited to less than 10% of their maximal growth rate; rapidly growing cells would be expected to contain significantly higher levels of RNA polymerase activity.

The properties of the final enzyme fractions with respect to their activity parameters are presented in Table III. RNA polymerase II demonstrated the classical sensitivity to low levels of α-amanitin and low activity on denatured DNA templates. Also as reported previously for the enzyme from both higher and lower eucaryotes (Adman et al., 1972; Schwartz et al., 1974), RNA polymerase III is active at conditions of high ionic strength which completely inhibit forms I and II and manifests a high level of activity on the synthetic template, poly(dAT·dAT). The sensitivities of polymerases I and III to α-amanitin, however, are reversed with respect to the enzymes from higher cells (Schwartz et al., 1974; Weil and Blatti, 1975). Schultz and Hall (1976) have made a similar conclusion from studies with partially purified fractions of the yeast enzymes, assuming that chromatographic and other properties of these enzymes are similar to those of the higher eucaryotic polymerases.

The polypeptides associated with the final enzyme fractions are shown in Figure 9, and their stoichiometric concentrations in the pure fractions are summarized in Table II. Each enzyme contains a unique pair of high-molecular-weight subunits, together with a complex assortment of smaller components. With a few exceptions, each of these smaller polypeptides are associated with the enzymes at stoichiometric ratios of 1.0 or 2.0, relative to the large pair of subunits. Those polypeptides which are present at stoichiometric concentrations of less than 1.0 are included in the putative structure on the basis of their reproducibly constant association with enzymatic activity.

A striking feature of the structures presented in Table II is the presence of polypeptides with identical molecular weights in each enzyme. To more carefully compare the size of the smaller subunits, the enzymes were subjected to parallel electrophoresis in the presence of sodium dodecyl sulfate in 13% polyacrylamide gels (Figure 9). The following subunits appear to be of identical molecular weight: the 41 000 polypeptide of I and II; the 28 000 polypeptide of enzymes I, II, and III; the 24 000 subunit of enzymes I, II, and III; the 20 000 subunit of enzymes I and III; and the 14 500 subunit of enzymes I, II, and III. These observations suggest that some components may be shared between the three enzymes, probably participating in the basic catalytic structure of the molecules, while other subunits, notably the pair of large subunits, are unique and may be responsible for the specific transcriptional properties of the enzymes.

Sklar et al. (1975) have also reported the presence of identical size subunits in polymerases I, II, and III from *Xenopus laevis* oocytes and mouse myeloma, and Buhler et al. (1976) have made similar observations for polymerases A (I) and B (II) from yeast. The latter authors have extended their studies to fingerprint analysis of the tryptic fragments from the similar subunits (Buhler et al., 1976). They report that the 28 000, 24 000, and 14 500 dalton subunits from enzymes I and II gave identical fingerprints. Our results corroborate the findings of Buhler et al. (1976) regarding the 28 000, 24 000, and 14 500 dalton subunits of polymerases I and II and also indicate that these subunits are probably present in polymerase III. Furthermore, the possibility that polymerase I and III share a common subunit with a molecular weight of 20 000 is suggested by our results (Figure 9).

The specific activities of RNA polymerases I and II obtained by this procedure are each equal to approximately 50% of the activities previously reported for homogeneous preparations of yeast polymerase I (Buhler et al., 1974; Valenzuela et al., 1976) and II (Dezelee and Sentenac, 1973). The RNA polymerase I isolated by this procedure does not contain the polypeptides of molecular weight 48 000 and 44 000 found in the preparations of Buhler et al. (1974) and Valenzuela et al. (1976). Huet et al. (1975) have described an electrophoretic

TABLE II: Polypeptide Composition of RNA polymerases I, II, and III.[a]

RNA Polymerase I		RNA Polymerase II		RNA Polymerase III	
Mol Wt	Stoich.	Mol Wt	Stoich.	Mol Wt	Stoich.
185 000	(1) 1.0	170 000	(1) 1.0	160 000	(1) 1.0
137 000	(1) 1.0	145 000	(1) 1.0	128 000	(1) 1.0
				82 000	(<1) 0.6
				53 000	(<1) 0.7
41 000	(1) 1.0	41 000	(<1) 0.4	41 000	(1) 1.3
				37 000	(1) 0.8
35 000	(1) 1.1			34 000	(<1) 0.6
		33 500	(1) 1.0		
28 000	(2) 1.8	28 000	(1) 1.2	28 000	(2) 1.8
24 000	(1) 1.0	24 000	(1) 0.9	24 000	(1) 0.9
20 000	(1) 0.8			20 000	(1) 1.1
		18 000	(1) 1.0		
16 000	(<1) 0.5				
14 500	(1) 1.0	14 500	(1) 1.0	14 500	(1) 1.0
12 300	(1) 0.9	12 500	(2) 1.9		
				10 700	(1) 1.2

[a] Subunit molecular weights were determined from the observed mobilities in sodium dodecyl sulfate gel electrophoresis (Laemmli, 1970) relative to the following standards: myosin, 200 000; *E. coli* RNA polymerase β', 165 000, β, 155 000; β-galactosidase, 130 000; phosphorylase A, 94 000; bovine serum albumin, 67 000; catalase, 57 000; ovalbumin, 43 000; lactic dehydrogenase, 35 000; carbonic anhydrase, 29 000; and lysozyme, 14 000. Stoichiometric ratios relative to the two large molecular weight subunits were determined from the amount of dye (see Materials and Methods) absorbed to each polypeptide. This procedure assumes equal dye binding independent of amino acid composition for each subunit; the molar ratios obtained must therefore be considered approximate.

TABLE III: Activity Parameters of RNA Polymerases I, II, and III.[a]

Template $(NH_4)_2SO_4$ Concn	Native Calf Thymus		Denatured Calf Thymus		Poly-(dAT·dAT), 0.05	Concn. of α-Amanitin 50% Inhibition, 0.05
	0.05	0.20	0.05	0.20		
I	1.0	0.01	1.0	0.01	1.2	300 μM
II	1.0		3.2	0.05	1.0	10 μM
III	1.0	0.8	1.0	0.7	3.9	$\geq$2 mM

[a] Relative enzyme activities were determined in 10-min reactions under the standard conditions described in Materials and Methods. The template concentration in each case was 50 μg/ml.

and chromatographic variant of polymerase I(A*) that does not contain the 48 000 and 37 000 subunits (corresponding to the 35 000 subunit of this preparation). They report that the activity ratio of the variant on poly(dAT·dAT) (at 30 μg/ml) relative to native calf thymus DNA is 1.3, compared with a ratio of 4.9 for the complete enzyme. The same activity ratio for polymerase I described above is 1.2 (Table III), similar to the ratio for the A* variant of Huet et al. (1975). These observations suggest that the 48 000 subunit, either alone or in combination with the 48 000 and 35 000 subunits, may be required for efficient copying of natural DNA templates. With the availability of procedures for the large-scale isolation of the RNA polymerases from yeast, and assays for the quantitative measurement of their specific products (Holland et al., 1976), the identification of enzyme components or alterations responsible for transcriptive properties should now be possible.

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Characterization of Purified Poly(adenylic acid)-Containing Messenger Ribonucleic Acid from *Saccharomyces cerevisiae*†

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ABSTRACT: Yeast poly(adenylic acid)-containing messenger RNA was isolated from total cellular RNA by affinity chromatography on poly(uridylic acid)-cellulose. The relative complexity of the isolated yeast mRNA was assessed by hybridization analysis with complementary DNA synthesized from the isolated messenger RNA (mRNA) with viral reverse transcriptase. Approximately 25% of the mRNA hybridized at an apparent $C_rt_{1/2}$ of 5×10^{-3} mol s $l.^{-1}$, while the remainder hybridized at an average $C_rt_{1/2}$ of 10^{-1} mol s $l.^{-1}$. Poly(adenylic acid)-containing yeast mRNA was translated in vitro in a wheat germ cell-free extract, and the major polypeptides synthesized have the same molecular weight as the major proteins present in the cell. Four of these proteins were identified by coelectrophoresis and immune precipitation to be pyruvate kinase, enolase, aldolase, and glyceraldehyde-3-phosphate dehydrogenase. These data demonstrate in agreement with the hybridization results that yeast contains major mRNA species and that some of the glycolytic enzyme mRNAs make up part of the major fraction. A procedure is outlined for the preparation of yeast mRNA which is essentially free of ribosomal RNA contamination and is further enriched in the major mRNAs present in the cell.

Yeast is an appealing eucaryotic organism for the study of transcriptive mechanisms, because it shares many of the molecular components found in animal cells. Yeasts contain multiple forms of RNA polymerase which are similar in structure and properties to those of higher eucaryotic cells (Roeder, 1969; Ponta et al., 1971; Adman et al., 1972; Dezelee and Sentenac, 1973; Buhler et al., 1974; Hager et al., 1976; Chambon, 1975 (review)). Yeast mRNA contains poly(A)[1] residues at its 3′ terminus (McLaughlin et al., 1973; Groner et al., 1974) and has been shown to direct the in vitro synthesis of polypeptides which coelectrophorese with those synthesized in the cell (Gallis et al., 1975). Biochemical studies of transcriptional mechanisms are facilitated in yeast because the genetic complexity of the nonreiterated portion of yeast DNA is only a few times that of *Escherichia coli* DNA (Ogur et al., 1952). Yeast is amenable to rigorous genetic analysis (Mortimer and Hawthorne, 1969) and yeast cells are available in large quantity.

It is well known that yeasts are highly specialized metabolically. When grown aerobically or anaerobically on a fermentable carbon source, yeasts maintain a very high intracellular level of the glycolytic enzymes. When yeasts are grown on glucose, for example, the glycolytic enzymes comprise 25–65% of the total cellular protein (Hess et al., 1968). The kinetics of induction of the glycolytic enzymes have been studied in cells shifted from growth on acetate to glucose (Maitra and Zobo, 1974) and the levels of the enzymes have been measured in cells grown on increasing concentrations of glucose (Hommes, 1966). In each case dramatic induction (ranging from 3- to 200-fold) of the glycolytic enzymes was observed. These observations demonstrate that the synthesis of glycolytic enzymes in yeast cells results from regulated differential gene expression rather than constitutive production of these proteins. The study of transcription of these genes both in vivo and in vitro seems feasible and attractive.

In this paper we describe the isolation and characterization of yeast poly(A) containing mRNAs with respect to the number and relative abundance in vegetative cells. We have

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[1] Abbreviations used: poly(U), poly(uridylic acid); poly(A), poly(adenylic acid); cDNA, complementary deoxyribonucleic acid; tRNA, mRNA, and rRNA, transfer, messenger, and ribosomal ribonucleic acids, respectively; YNB, yeast nitrogen base; Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; CM, carboxymethyl; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.