Yeast DNA-dependent RNA Polymerase I

A RAPID PROCEDURE FOR THE LARGE SCALE PURIFICATION OF HOMOGENEOUS ENZYME*

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PABLO VALENZUELA,† FANNYELA WEINBERG, GRAEME BELL, AND WILLIAM J. RUTTER

From the Department of Biochemistry and Biophysics, University of California at San Francisco, San Francisco, California 94143

A procedure has been developed for the rapid purification of large amounts of yeast RNA polymerase I (A). The method involves batchwise treatment with phosphocellulose and DEAE-cellulose, ion filtration chromatography on DEAE-Sephadex, sucrose gradient centrifugation, and DNA-cellulose chromatography. The enzyme obtained is apparently homogeneous by sedimentation velocity analysis and has a specific activity of 300 nmol of UMP incorporated into RNA in 10 min per mg of protein. Between 30 and 40 mg of enzyme can be obtained in 5 days from 3.0 kg of yeast cells.

The subunit composition of the enzyme was determined by polyacrylamide gel electrophoresis in the presence of 0.1% sodium dodecyl sulfate. The purified polymerase is composed of 11 putative subunits with molecular weights 185,000 (I. ), 137,000 (Ib), 48,000 (Ic), 44,000 (Id), 41,000 (Ie), 36,000 (If), 28,000 (Ig), 24,000 (Ih), 20,000 (Ii), 14,500 (II), and 12,000 (II).

Yeast polymerase I separates into two forms when subjected to gel electrophoresis under nondenaturing conditions. The main component which migrates faster contains all the subunits except the polypeptides I. and I. The slow migrating component which is present in lower amounts contains all the subunits.

Since the original reports of the existence of multiple forms of RNA polymerases (I, II, and III) in sea urchin, rat liver, and in yeast (1-4), there have been confirmatory findings in virtually all other eukaryotic cells investigated. The purification and subunit structure of some of the enzymes have also been reported (5-8).

Large quantities of homogeneous protein are required for establishing the structure-function relationship of the complex RNA polymerase molecule and for the in vitro study of the mechanism and specificity of transcription in eukaryotic systems.

Yeast is one of the most suitable sources for large scale purification of eukaryotic RNA polymerases since it is readily available at reasonable cost in bulk amounts, growth conditions can easily be manipulated, and yeast genetics is well developed and many well characterized mutants are available (9, 10).

A number of procedures have been reported for the purification of yeast polymerases I and II (11-15), but none is satisfactory, for the methods employed are not easily scaled up, the yields are low, or the specific activity of the product is low. Finally, there may be contamination with polymerase III.

We report here a method for the facile preparation of large quantities of polymerase I in high yield. We have utilized batchwise absorption on phosphocellulose and DEAE-cellulose modified from the procedure of Buhler et al. (15), followed by ion filtration chromatography on DEAE-Sephadex (10), and sucrose gradient centrifugation in 25% glycerol. DNA-cellulose chromatography has been added as a final step to remove contaminating polymerase III and to yield enzyme homogeneous by native gel electrophoresis and sedimentation velocity. Dialysis has been eliminated and dilution of the enzyme is avoided. Smaller quantities of highly purified RNA polymerase III can also be recovered as a by-product. We also report measurements of the size and stoichiometry of the polypeptides present in the RNA polymerase I which reveal a complex quaternary structure.

**EXPERIMENTAL PROCEDURES**

Materials—[3H]UTP (20 Ci/mmol) was obtained from New England Nuclear, Boston, Mass. Dimethylsulfoxide, calf thymus DNA, and phenylmethylsulfonylfluoride were purchased from Sigma Chemical Co., St. Louis, Mo. Dithiothreitol, pyruvate kinase, and phosphoenolpyruvic acid were from Calbiochem, La Jolla, Ca. Nucleotides were from P-L Biochemicals, Milwaukee, Wis. Phosphocellulose (Whatman P10), 7.4 meq/g, and DEAE-cellulose (Whatman DE52), 1.0 meq/g, were purchased from Reeve Angel, New York, N. Y. Sucrose, bidistilled glycerol, and ammonium sulfate were enzyme grade reagents. a-Amanitin was a Boehringer product obtained from Henley Co., New York, N. Y. Saccharomyces cerevisiae yeast cells were the kind gift of Red Star Yeast Co., Oakland, Ca.

Preparation of Resins—Phosphocellulose and DEAE-cellulose were precycled and equilibrated as described by Buhler et al. (15). Denatured calf thymus DNA-cellulose was prepared by the method of...
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Alberts and Herrick (17). After extensive washing the resin contained 0.3 mg of DNA/ml of resin.

Salt, DNA, and Protein Measurements.—Conductivity was measured with a Potentiometer CDM2 conductivity meter following the vol-

U-100 fold dilution in water, and salt concentration determined by reference to a standard curve. DNA was measured by the method of Burton (18). Protein was determined after precipitation with 10% trichloroacetic acid by the method of Lowry et al. (19) using crystalline bovine serum albumin (Pentex type I) as standard.

Polymerase Assay.—The standard incubation mixture contained in 0.060 ml: 60 mM Tris-HCl, pH 7.9, 1.6 mM MgCl2, or 10 mM MgCl2, 0.6 mM ATP, CTP, and GTP, 0.01 mM UTP, 0.5 pCi of [3H]-

UTP, 10 mM NaF, 10 mM 2-mercaptoethanol, 120 µg of bovine serum albumin, 1.1 mM EDTA, 10% glycerol, and 0.5 µg of native E. coli RNA. For assay of RNA polymerase crude extracts the incubation mixture included 0.2 µg of crystalline pyruvate kinase and 70 mM phospho

enolpyruvnic acid. This reaction mixture containing nonsaturating levels of UTP was used in routine assays. However, when the specific activities of the different fractions were measured, the UTP concentra-

tion was raised to 0.6 mM. After incubation for 10 min at 37°, a 50-µl aliquot was withdrawn from each tube and applied directly onto Whatman DE81 filter disks. The filters were washed seven times with 5% NaHCO3, twice with water, with ethanol, and dried. Radioactivity was measured by immersing the discs in 3 ml of a solu-

tion containing 10% acetic acid, 20% ethanol, and 10% NCS. One unit of activity corresponds to the incorporation of 1 nmol of RNA polymerase A and B by passage through a column of Sephadex G-75.

Table I. A detailed description of each step in the procedure follows.

Preparation of Cell Extract.—Yeast cells in late log phase were harvested by low speed centrifugation, washed by resus-

pending first in cold distilled water, and then in 2 liters of extraction buffer (0.02 M Tris-HCl, pH 8.0, 10% glycerol, 0.01 M MgCl2, 0.01 M 2-mercaptoethanol, 0.5 mM EDTA, 0.3 M ammonium sulfate, 1 mM phenylmethylsulfonyl fluoride, and 1% dimethylsulfoxide) per 1000 g, wet weight, of cells. The washed cells are suspended in a small amount of extraction buffer and the heavy paste is added dropwise to liquid N2 and the pellets stored at -80°. Cells prepared in this way can be stored for several months with no noticeable change in RNA polymerase activity. The yeast extract was prepared by the method of Bhargava and Halvorson (24) as modified by Hager and Holland of this laboratory.1 The cells are disrupted by crushing the frozen pellets through a 200-ml Eaton pressure cell (25) at a pressure of 10,000 psi and cooled to -30°. The broken cells are stirred with 9,900 ml of extraction buffer and the pH is adjusted to 8.0 with solid Tris. Unless otherwise stated all RNA polymerase purification steps were performed at 0-4°. The homogenate is centrifuged 60 min at 27,000 × g (Sorvall rotor GSA). The supernatant is removed and cen-

trifuged for 2 hours at 60,000 × g in Beckman ultracentrifuge rotor (21). The clear part of the supernatant is pooled to give about 2.5 liters of Fraction 1. On the average this fraction contains 30 mg/ml of protein. At this stage, due to the high amounts of RNA and presence of hydrolytic enzymes, the RNA polymerase assay is not reliable and gives about 20,000 total

1 G. L. Hager and M. Holland, unpublished results.
RNA polymerase with a specific activity of 32 units/mg of ammonium sulfate, stirred slowly for 30 min, and filtered as Fraction 2 (1.3 liters). After thorough mixing the ammonium cake is suspended in 1.2 liters of Buffer A containing 0.4 M ethanol, and 0.5 mM EDTA), 400 g (wet weight) are added to denatured DNA-cellulose previously equilibrated in Buffer A containing 0.15 M ammonium sulfate. The suspension is slowly stirred for 30 min and then filtered. Treatment with Phosphocellulose—Fraction 1 (2.5 liters) was added to 500 g (wet weight) of phosphocellulose previously equilibrated in Buffer A containing 0.15 M ammonium sulfate (Buffer A is 0.02 M Tris-HCl, pH 8.0, 0.01 M 2-mercaptoethanol, 0.5 mM EDTA, and 10% glycerol). After thorough mixing the ammonium sulfate concentration is reduced to 0.15 M by addition of 1 volume of Buffer A (2.5 liters). The suspension is slowly stirred for 30 min and then filtered through filter paper in a Buchner funnel without allowing the phosphocellulose to dry. The cake is suspended in 4 liters of Buffer A containing 0.15 M ammonium sulfate, stirred for 10 min, and collected by filtration. This procedure is repeated three more times. After the last filtration the phosphocellulose cake is suspended in 1.2 liters of Buffer A containing 0.4 M ammonium sulfate, stirred slowly for 30 min, and filtered as above. About 1.3 liters of filtrate are obtained, which contain RNA polymerase with a specific activity of 32 units/mg of protein (Fraction 2). Treatment with DEAE-cellulose—Of DEAE-cellulose previously equilibrated in Buffer B containing 0.1 mM ammonium sulfate (Buffer B is 0.02 M Tris-HCl, pH 8.4, 0.01 M 2-mercaptoethanol, and 0.5 mM EDTA), 400 g (wet weight) are added to Fraction 2 (1.3 liters). After thorough mixing the ammonium sulfate concentration is lowered to 0.1 M by addition of 3 volumes of Buffer B. The suspension is gently stirred for 30 min, filtered as above, and washed four times with 2 liters of Buffer B containing 0.1 mM ammonium sulfate. RNA polymerase is eluted by suspending and stirring the cellulose cake in 800 ml of Buffer B containing 0.3 mM ammonium sulfate for 30 min. After filtration 900 ml of enzyme solution with a specific activity of 106 units/mg of protein is obtained (Fraction 3). At this stage the enzyme can be conveniently stored by precipitating it with ammonium sulfate (see below) and dissolving in Buffer B containing 50% glycerol.

### Ion Filtration Chromatography

RNA polymerase (Fraction 3) is precipitated by addition of 35 g of solid ammonium sulfate/100 ml of solution. After dissolution of the salt the precipitate is left overnight at 4°C and collected by centrifugation (27,000 rpm for 45 min in rotor SW-27 of Beckman ultracentrifuge). The pellet is dissolved in 12 ml of Buffer B containing 30% glycerol and 0.35 mM ammonium sulfate and loaded onto a column (4 x 30 cm) packed with DEAE-Sephadex A-25. If the preparation is started with 2,000 g of cells, the pellet is dissolved in 20 ml of buffer and a column (4 x 50 cm) is used. The column was previously equilibrated with 0.02 M Tris-HCl, pH 8.0, 25% glycerol, 0.5 mM EDTA, 0.02 M 2-mercaptoethanol, and 0.1 mM ammonium sulfate. The column is developed with the same buffer containing 0.35 mM ammonium sulfate (Fig. 1). The fractions containing activity are pooled (53 ml). The enzyme obtained has a specific activity of 195 units/mg of protein (Fraction 4). The experimental conditions for purifying RNA polymerases by ion filtration chromatography were developed by Michael Goldberg in this laboratory.

### Sucrose Gradient Centrifugation

Fraction 4 is precipitated by overnight dialysis against Buffer B saturated with ammonium sulfate. The precipitate is collected by centrifugation (Beckman SW-27 rotor, 27,000 rpm, 45 min) and dissolved in a final volume of 3.6 ml with Buffer B containing 20% glycerol and 0.2 M KCl. Aliquots of 0.6 to 1.0 ml are layered on 11 ml of a 5 to 20% (w/v) sucrose gradient in 0.05 M Tris-HCl, pH 8.0, 25% glycerol, 0.5 mM EDTA, 0.2 M KCl, and 0.02 M 2-mercaptoethanol. The gradients are centrifuged at 40,000 rpm for 28 hours at 4°C (in a Spinco SW-41 rotor). A profile of the sucrose density is shown in Fig. 2. The enzyme pooled from the active fractions has a specific activity of 300 units/mg and a protein concentration of 0.75 to 1.5 mg/ml. It is stored at −80°C (Fraction 5).

The conditions of the sucrose gradient centrifugation step were obtained from a study in which the protein load, nature and concentration of salt, and the glycerol concentration were systematically varied. The best yield and reproducibility was obtained with gradients containing protein loads of at least 6 mg/tube, 0.2 to 0.3 M KCl, and no Mg2+2. At salt concentrations lower than 0.2 M KCl partial aggregation of the enzyme occurs resulting in broadening of the protein peak.

### DNA-cellulose Chromatography

Fraction 5 is diluted three times with Buffer C (0.02 M Tris-HCl, pH 8.0, 25% glycerol, 0.1 mM EDTA, and 0.02 M 2-mercaptoethanol) to bring the KCl concentration to 0.066 M and applied to a 25-ml column of demineralized DNA-cellulose previously equilibrated with Buffer C containing 0.066 M KCl. The column was washed with 40 ml of Buffer C containing 0.1 M KCl and eluted with 150 ml of a linear gradient from 0.1 M to 0.8 M KCl in Buffer C. Fractions of 2.4 ml are collected. A profile is shown in Fig. 3. RNA polymerase I activity elutes in a sharp peak at 0.43 M KCl. Contaminant RNA polymerase III activity elutes at 0.7 M KCl. Active fractions are pooled and stored in liquid N2. For long term storage the enzyme is concentrated by precipitation with ammonium sulfate and dissolved in Buffer C containing 50% glycerol.

### Enzyme Purity

The enzyme obtained after sucrose gradient centrifugation is completely dependent on added DNA for activity and has a A260/A280 ratio of 1.78. Direct analysis of DNA by the method of Burton (18) shows no detectable DNA (less than 15 μg of...
of the enzyme when centrifuged at 44,000 rpm at a concentration of 0.4 mg/ml. An $s_{20, w}$ value of 16.2 was obtained, which in combination with the approximate molecular weight of 650,000 $\pm$ 50,000 calculated from the molecular weights and molar ratios of the individual subunits (see below).

**Subunit Structure**

The subunit structure of the purified enzyme was examined by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. Fig. 6 shows the pattern of individual polypeptides in 9% acrylamide gels. Nine protein bands are observed in all cases. The ratio of the amount of these polypeptides to enzyme activity is roughly constant. Scanning of each fraction shows no significant changes in the pattern except for subunit d which is present in slightly higher amounts in the first fractions of the DNA-cellulose peak. A more detailed analysis of the subunit structure is presented in Fig. 7 which shows the stained gel and scanning pattern of enzyme from pooled fractions of DNA-cellulose chromatography. Approximate molecular weights were calculated from the observed mobilities using protein standards of known molecular weights (Fig. 8). Protein bands appeared at 185,090 (a), 137,000 (b), 48,000 (c), 44,000 (d), 41,000 (e), 36,000 (f), 28,000 (g), 24,000 (h), 20,000 (i), 14,500 (j), and 12,000 (k). The same protein bands were obtained when purified enzyme was subjected to electrophoresis on a 5% acrylamide slab gel as described under "Experimental Procedures." 

The purified RNA polymerase I is completely resistant to 40 $\mu$g/ml of $\alpha$-amanitin. Thus it is free from enzyme II. This is confirmed by the subunit composition of the enzyme examined by polyacrylamide gel electrophoresis in sodium dodecyl sulfate, which shows the absence of the large molecular weight polypeptides of yeast polymerase II (26).

RNA polymerase III, as identified by its subunit structure (27) and elution behavior in DEAE-Sephadex column chromatography (Fig. 1) under nondenaturing conditions (Fig. 3) in 9% acrylamide gels. Nine protein bands are observed in all cases. The ratio of the amount of these polypeptides to enzyme activity is roughly constant. Scanning of each fraction shows no significant changes in the pattern except for subunit d which is present in slightly higher amounts in the first fractions of the DNA-cellulose peak. A more detailed analysis of the subunit structure is presented in Fig. 7 which shows the stained gel and scanning pattern of enzyme from pooled fractions of DNA-cellulose chromatography. Approximate molecular weights were calculated from the observed mobilities using protein standards of known molecular weights (Fig. 8). Protein bands appeared at 185,090 (a), 137,000 (b), 48,000 (c), 44,000 (d), 41,000 (e), 36,000 (f), 28,000 (g), 24,000 (h), 20,000 (i), 14,500 (j), and 12,000 (k). The same protein bands were obtained when purified enzyme was subjected to electrophoresis on a 5% acrylamide slab gel as described under "Experimental Procedures." 

**DNA/mg of protein**. Thus the endogenous DNA present is less than 0.2% of the DNA added in the standard assay.
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Figure 5. Sedimentation velocity of purified yeast RNA polymerase I. Enzyme was sedimented in 0.05 M Tris-HCl, pH 7.9, 0.01 M MgCl₂, 0.2 M KCl, and 0.007 M 2-mercaptoethanol at a protein concentration of 0.4 mg/ml. r is the distance from the boundary to the center of rotation. b shows the boundary at 20.4 x 10⁻⁴ g.

Figure 6. Polyacrylamide sodium dodecyl sulfate gel electrophoresis patterns of yeast RNA polymerase I from peak tubes of the sucrose gradient of Fig. 2 and peak tubes of DNA-cellulose chromatography of Fig. 3. The 0.1% sodium dodecyl sulfate/9% acrylamide gels from sucrose gradient were loaded with 4 to 6 µg of protein/gel, gels from DNA-cellulose with 6 to 10 µg of protein/gel.

Possible electrophoretic heterogeneity of yeast RNA polymerase I, suggested by the presence of two protein bands in native gel electrophoresis was further investigated by two-dimensional slab gel electrophoresis. A 5% acrylamide gel run under nondenaturing conditions in the first dimension, resolves the enzyme into two forms with slightly different electrophoretic mobility (Fig. 4). Electrophoresis under denaturing conditions in a second dimension was used to establish the subunit composition of each form. The results are shown in Fig. 9. The main component which migrates faster contains all the subunits except the polypeptides I₈ and I₉. These two polypeptides are not found in the gels. They are more basic than the enzyme complex and presumably migrate toward the cathode in the conditions of the electrophoresis. The slower migrating component, which comprises 10 to 15% of the total protein, contains all the subunits. Although Fig. 9 does not show subunits I₈ and I₉, their presence of these peptides in both forms of polymerase I was established by using a 12% acrylamide gel in the second dimension (results not shown). Compared to enzyme from DNA-cellulose, the slow migrating component has slightly lower amounts of polypeptides I₈ and I₉.

DISCUSSION

The procedure outlined above has eliminated all major constraints on the large scale preparation of yeast polymerase I without sacrificing yield or specific activity of the final product. The crucial features of this method are: (a) dilution of the extract or enzyme fractions is avoided, so the polymerase remains concentrated throughout the procedure; (b) no high speed centrifugation is necessary; (c) no cumbersome high resolution column chromatographic steps are employed; (d) no dialysis steps are necessary. Routinely 3.0 kg of cells can be processed in 5 days to obtain 30 to 45 mg of pure enzyme.
ion filtration chromatography, a procedure devised by Kirkegaard et al. (16, 29), combining ion exchange and gel filtration. The next step is Mangel (28) for Escherichia coli, because it allows the processing of large amounts of starting material. We have followed this method in the purification of RNA polymerases (8). We have used cellulose and DEAE-cellulose, steps which have been extensively used in the purification of RNA polymerases (8). We have adopted a batchwise procedure similar to that employed by Ponta et al. (11) and Buhler et al. (15) for yeast and Mauggel (28) for Escherichia coli, because it allows the processing of large amounts of starting material. The next step is ion filtration chromatography, a procedure devised by Kirkegaard et al. (16, 29), combining ion exchange and gel filtration in the same column. By adjusting the pH and ionic strength of the column it is possible to elute the purified enzyme after the excluded peak of noninteracting macromolecules and before the liquid column volume so that the enzyme is eluted in the sieving range. For yeast RNA polymerase I these conditions are met by equilibrating a DEAE-Sephadex column with 0.02 M Tris-HCl, pH 8.0, containing 0.1 M ammonium sulfate. The enzyme sample is introduced in the same buffer containing 0.35 M ammonium sulfate. Under these conditions nucleic acids interact stronger with the ion exchanger and are easily resolved from the enzyme. By this procedure a highly concentrated solution of enzyme can be processed in a short period of time (2 hours). This is an important advantage in the purification of RNA polymerases or other enzymes that are sensitive to dilution or dialysis for prolonged periods at 4°C. The enzyme elutes in the sieving range with little dilution, and is easily concentrated for storage by ammonium sulfate precipitation. The salt concentration of the sample load can be as high as 0.35 M ammonium sulfate so that previous dialysis is avoided. The relatively high specific activity (195 units/mg) and the absence of significant endogenous template or RNase and DNase activities make the enzyme at this stage suitable for in vitro transcription studies.

The main contaminant removed in the sucrose gradient centrifugation step is a protein of molecular weight 160,000. This polypeptide, which co-purifies with the enzyme through the ion filtration step in roughly equimolar amounts with the large subunits, has a strong DNA binding ability and stimulates the activity of the purified enzyme especially at high ionic strength.4

The last step of the method is a chromatography on denatured calf thymus DNA-cellulose. This step does not significantly increase the specific activity of the enzyme, but is necessary to resolve RNA polymerase III and to remove other minor protein contaminants which co-purify with enzyme I through the first four steps. Enzyme III interacts very strongly with the DNA-cellulose and is eluted at 0.7 M KCl in a highly purified form.

Examination of the polypeptide composition of purified yeast RNA polymerase I reveals 10 to 11 putative subunits with molecular weights ranging from 185,000 to 12,000. The two large polypeptides (185,000 and 137,000) and some of the smaller polypeptides (48,000, 44,000, 28,000, 24,000, 20,000, and 14,500) are present in stoichiometric amounts. In addition there are polypeptides present in nonstoichiometric amounts (41,000 and 36,000). The molar ratios reported here cannot be taken as definitive. They are estimated from the amount of dye adsorbed, a method which may not be accurate (5). These polypeptides are termed subunits but we recognize there is no evidence that all of them are functionally involved in the RNA polymerase molecule. The very complex structure of the isolated enzyme suggests that some of the polypeptides may be regulatory components accompanying a basic polymerizing unit.

The subunit structure revealed through this work follows the general pattern reported by others for yeast RNA polymerase I (15, 30). However, there are some noticeable differences. Van Keulen et al. (30) reported, for the Saccharomyces carlbergensis enzyme, the presence of a subunit having a molecular weight of 54,000 and found no subunits in the regions 48,000, 41,000, 28,000, and 20,000. Buhler et al. (15) found no polypeptides with molecular weights 24,000 and 20,000 in their preparation. It seems likely that these disparities may arise from the different purification procedures and suggests the

### Table II

*Subunit composition of yeast RNA polymerase I*

Composition was examined by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate with enzyme obtained from DNA-cellulose chromatography. The 9% acrylamide gels were used except for the data of polypeptides j and k which were taken from 12% DNA-cellulose chromatography. The 9% acrylamide gels were used in different preparations and the values shown correspond to those obtained from the data of Fig. 7.

<table>
<thead>
<tr>
<th>Polypeptide</th>
<th>Molecular weight</th>
<th>Molar ratio</th>
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<tbody>
<tr>
<td>a</td>
<td>185,000 ± 10,000</td>
<td>1.00</td>
</tr>
<tr>
<td>b</td>
<td>137,000 ± 5,000</td>
<td>1.12</td>
</tr>
<tr>
<td>c</td>
<td>48,000 ± 2,000</td>
<td>2.00</td>
</tr>
<tr>
<td>d</td>
<td>44,000 ± 2,000</td>
<td>0.98</td>
</tr>
<tr>
<td>e</td>
<td>41,000 ± 2,000</td>
<td>1.50</td>
</tr>
<tr>
<td>f</td>
<td>36,000 ± 2,000</td>
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<td>g</td>
<td>28,000 ± 1,000</td>
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</tr>
<tr>
<td>k</td>
<td>12,000 ± 1,000</td>
<td>1.00</td>
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Fig. 9. Second dimension polyacrylamide sodium dodecyl sulfate gel electrophoresis of yeast RNA polymerase I. Gels contain 9% acrylamide and 0.1% sodium dodecyl sulfate. The first dimension 5% acrylamide gels were used to resolve RNA polymerase I (15, 30). However, there are some noticeable differences. Van Keulen et al. (30) reported, for the Saccharomyces carlbergensis enzyme, the presence of a subunit having a molecular weight of 54,000 and found no subunits in the regions 48,000, 41,000, 28,000, and 20,000. Buhler et al. (15) found no polypeptides with molecular weights 24,000 and 20,000 in their preparation. It seems likely that these disparities may arise from the different purification procedures and suggests the...
existence of dissociable polypeptides which are not necessary for the polymerization reaction but may be required for another function.

Heterogeneity has been found in several eukaryotic RNA polymerases evidenced by chromatography or electrophoresis (26). Our results indicate that RNA polymerase I can be resolved by electrophoresis into two forms. The main component having all the subunits except the polypeptides I₁ and I₂. This suggests that these subunits are more loosely bound than the others. Similar results were reported by Buhler et al. who reported that both forms are active (26, 31).

We have found that yeast RNA polymerases II₁ and/or III₁ also contain polypeptides with molecular weights 41,000, 28,000, 24,000, 20,000, and 14,500 which suggests that there is a common pool of small molecular weight subunits (27). Reconstitution of the enzyme from isolated subunits as well as peptide mapping studies on the small molecular weight polypeptides will be necessary to further elucidate the function, structure, and relationship between the various subunits of yeast RNA polymerases I, II, and III.

The general concepts and methods employed in this procedure may be useful in the preparation of RNA polymerases from other eukaryotic and prokaryotic sources.

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