Nuclear Deoxyribonucleic Acid-Dependent Ribonucleic Acid Polymerases from *Saccharomyces cerevisiae*

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Two deoxyribonucleic acid (DNA)-dependent ribonucleic acid (RNA) polymerases (I, II) have been solubilized from isolated *Saccharomyces cerevisiae* nuclei. The enzymes can be separated by chromatography on *O*-diethylaminoethyl Sephadex. Both enzymes are active with high-molecular-weight nuclear yeast DNA, although RNA polymerase I has a higher affinity for polydeoxyadenylic-thymidylic acid and RNA polymerase II for denatured DNA. RNA polymerase I is active only with manganese. α-Amanitin inhibits only the activity of RNA polymerase II.

The isolation of ribonucleic acid (RNA) polymerase in a variety of organisms has provided new approaches to the study of gene regulation. In prokaryotic organisms, two interesting examples of the role of the RNA polymerases in the control of gene expression are the development of the bacteriophages T4 (19, 24) and T7 (5, 22) and the sporulation of *Bacillus subtilis* (12, 13). However, very little is known about the role of the RNA polymerase in the control of the gene expression in euakaryotic organisms, where the presence of multiple RNA polymerase has been reported (3, 6, 9, 16, 17, 21).

Since yeast exhibits a programmed synthesis of macromolecules during the cell cycle (23) and undergoes a typical developmental process of sporulation, it is an attractive system for the study of the role of the RNA polymerase in the regulation of transcription. The availability of methods to isolate nuclei (1) and high-molecular-weight deoxyribonucleic acid (DNA) (M. M. Bhargava, J. H. Cramer, and H. O. Halvorson, Anal. Biochem, *in press*) from *Saccharomyces cerevisiae* has facilitated the study of the RNA polymerase present in yeast nuclei.

In this paper we report the solubilization of two DNA-dependent RNA polymerases from *S. cerevisiae* nuclei which differ in their chromatographic properties, template specificity, metal requirements, and drug sensitivity.

**MATERIALS AND METHODS**

**Yeast strains.** Two strains of *S. cerevisiae*, Y55 and IL46, were used. The cells were grown in medium made of 1% yeast extract, 2% peptone, and 2% glucose.

**Solutions.** Buffer A consisted of 50 mM tris(hydroxymethyl)aminomethane (Tris)-hydrochloride, 2 mM MgCl₂, 1 mM dithioerythritol, 0.2 mM ethylenediaminetetraacetic acid, and 20% glycerol, pH 7.5.

**Nuclei and DNA isolation.** Nuclei from *S. cerevisiae* were isolated by the method of Bhargava and Halvorson (1), and high-molecular-weight nuclear DNA was isolated from *S. cerevisiae* Y55 by the method of Bhargava et al. (M. M. Bhargava, J. H. Cramer, and H. O. Halvorson, Anal. Biochem., *in press*). The absorbance ratio 260 nm:280 nm of the DNA was 1.9, and the average molecular weight exceeded 40 x 10⁶ as determined by zonal sedimentation in cesium chloride gradients in the model E centrifuge.

**RNA polymerase assay.** The standard assay mixture has in a final volume of 0.3 ml: 50 mM Tris-hydrochloride, pH 7.5; 1 mM dithioerythritol; 10 mM of MgCl₂; 2 mM MnCl₂; 0.15 mM each of adenosine triphosphate (ATP), guanosine triphosphate (GTP), and cytidine triphosphate (CTP), 0.01 mM uridine triphosphate (UTP), 0.5 μCi of ³H-UTP (specific activity 13 Ci/mmol), 15 μg of native nuclear yeast DNA and enzyme preparation. When polydeoxyadenylic-thymidylic acid (dAT) was used as a template, 0.025 A₅₅₀ units were included in the assay mixture. Denatured DNA was obtained by heating the DNA solution in boiling water for 10 min followed by rapid cooling in an ice-salt bath. The assay mixture was incubated at 35 C, and the reaction was stopped by the addition of 2 ml of cold 5% trichloroacetic acid containing 1% tetrasodium pyrophosphate. After standing 15 min in an ice water bath, the trichloroacetic acid-insoluble material was collected on membrane filters (Millipore type HA, 0.45 μm pore size), presoaked in water, and washed.
with 50 ml of the trichloroacetic acid-Na$_2$P$_2$O$_7$ mixture. The filters were dried and suspended in 10 ml of the scintillation liquid containing 2,5-diphenyloxazole (4 g/liter) and 1,4-bis-2-(5-phenyloxazolyl)benzene (0.5 g/liter) and counted in a Beckman LS-225 liquid scintillation counter.

One unit of enzyme activity represents the incorporation of 1 pmol of uridine monophosphate (UMP) in the trichloroacetic acid-insoluble material in 10 min.

**RNase assay.** The exonuclease and endoribonuclease activities were measured by the method described by Burgess (4) using 18S yeast ribosomal RNA label with $^{32}$P as a substrate. T1 ribonuclease (RNase) was used as a control of the activity of the system.

**Protein determination.** Proteins were determined by the method of Lowry et al. (14) using bovine serum albumin as standard.

**Column chromatography.** O-Diethylaminoethyl (DEAE)-Sephadex A25 from Pharmacia was washed and equilibrated with buffer A.

### RESULTS

**Solubilization of the RNA polymerase activity from S. cerevisiae nuclei.** Homogenates of nuclei lysed with buffer A show an active synthesis of RNA when they are incubated with either native or denatured nuclear yeast DNA, nucleoside triphosphates, and manganese. Chromatography on DEAE-Sephadex columns of crude extracts prepared from these homogenates shows the presence of one peak of RNA polymerase activity which is eluted with 0.3 M ammonium chloride.

Treatment of the nuclei with buffer A plus 0.5 M potassium chloride leads to a substantial increase of the total activity solubilized and to the appearance of two peaks of enzyme activity which are eluted from the DEAE-Sephadex column with 0.25 M and 0.3 M ammonium chloride.

A further increase in the amount of RNA polymerase solubilized can be obtained by treating the nuclei with the following mixture: 0.5 M KCl, 0.2% Nonidet NP-40, and 0.1% sodium deoxycholate in buffer A. The isolated nuclei are homogenized and incubated in this mixture for 30 min at room temperature and then centrifuged at 50,000 rpm for 1 h. Ninety-nine percent of the activity of the homogenate is solubilized in the supernatant crude extract. The remaining 1% can be removed from the remaining membranes by further treatment with the detergent mixture or by sonic treatment.

Sonic treatment of the whole nuclei also leads to the solubilization of two RNA polymerase activities which can be separated on DEAE-Sephadex columns, but the activity solubilized is less than 50% of that obtained following treatment with the detergent mixture and high ionic strength.

Table 1 summarizes the efficiency of the different treatments in the solubilization of the RNA polymerase activity from the nuclei. It can be seen that the treatment with KCl and the mixture of the nonionic detergents produces the highest yield of activity solubilized.

The activity of the RNA polymerase solubilized by this method is dependent on the presence of DNA and nucleoside triphosphates. Figure 1 shows the kinetics of the reaction and its partial inhibition with $\alpha$-amanitin. The specific activity of the RNA polymerase solubilized from the nucleus is 200 pmol in 10 min per mg of protein using native nuclear yeast DNA as template.

Table 2 summarizes the inhibition by $\alpha$-amanitin of nuclear RNA polymerase activity with different DNA templates. Although transcription of native nuclear yeast DNA and poly dAT is inhibited to approximately the same extent by $\alpha$-amanitin (43 and 35%), transcription of denatured calf thymus DNA was reduced by 81%. Since inhibition by $\alpha$-amanitin has been shown to be independent of the template used in the reaction (10), these results suggest the presence of more than one RNA polymerase in nuclear extracts.

### Table 1. RNA polymerase activity solubilized from nuclei of Saccharomyces cerevisiae

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Activity units/g of nuclei (wt/wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer A</td>
<td>2,000</td>
</tr>
<tr>
<td>Buffer A + 0.5 M KCl</td>
<td>6,100</td>
</tr>
<tr>
<td>Buffer A + 0.5 M KCl + 0.2% Nonidet NP-40 + 0.1% sodium deoxycholate</td>
<td>8,500</td>
</tr>
<tr>
<td>Buffer A + sonic treatment$^b$</td>
<td>4,000</td>
</tr>
<tr>
<td>Buffer A + 0.5 M KCl + sonic treatment$^b$</td>
<td>6,400</td>
</tr>
</tbody>
</table>

$^a$Two grams (wt/wt) of isolated nuclei from *S. cerevisiae* were divided into five portions, and each was homogenized in 5 ml of buffer A with the additions indicated in the table. The nuclei homogenates were centrifuged at 50,000 rpm in an SW65 rotor for 1 h, and the RNA polymerase activity was measured in the supernatants using nuclear yeast DNA as template.

$^b$Sonic treatment was carried out at the intensity of 3 A for 20 s in a sonifier cell disruptor.
ammonium chloride. This suggests there is no interconversion between the two enzymes.

The recovery of the activity after the DEAE-Sephadex chromatography is almost 100%, and a purification of nearly 100-fold is obtained for each of the enzymes. The fractions which contain the RNA polymerase I and RNA polymerase II are devoid of detectable endoribonuclease and exoribonuclease activities.

**Template specificity of RNA polymerase I and II.** RNA polymerase I and II differ in their template specificity (Table 3). dAT is the best template for RNA polymerase I; native or denatured yeast or calf thymus DNA are less effective templates. RNA polymerase II has a strong preference for denatured DNA, either yeast or calf thymus, and is less active with dAT and native DNA. These findings indicate that RNA polymerase II corresponds to the enzyme purified and described previously by Frederick et al. (7).

**Metal specificity of RNA polymerase I and II.** Both enzymes are dependent on the presence of a divalent cation in the incubation mixture, although they have different optima. Figure 4 shows the effect of different concentrations of manganese and magnesium on the enzymatic activity of the RNA polymerases. RNA polymerase I has a maximal activity at 2 mM manganese and is inhibited at concentrations lower than 1 mM or higher than 2 mM. The enzyme is also active with magnesium, but the activity is only 50% of the activity with manganese. The activity of RNA polymerase II is almost completely dependent on the presence of manganese, with an optimal concentra-

**Table 2. Template specificity of α-amanitin inhibition of RNA polymerase activity in nuclear extracts from Saccharomyces cerevisiae**

<table>
<thead>
<tr>
<th>DNA template</th>
<th>Activity (units/mg of protein)</th>
<th>Percent inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>−α-amanitin</td>
<td>+α-amanitin</td>
</tr>
<tr>
<td>Native nuclear yeast</td>
<td>220</td>
<td>125</td>
</tr>
<tr>
<td>Polydeoxyadenylic-thymidylic acid</td>
<td>810</td>
<td>520</td>
</tr>
<tr>
<td>Denatured calf thymus</td>
<td>700</td>
<td>130</td>
</tr>
</tbody>
</table>

*Isolated nuclei from *S. cerevisiae* were homogenized in buffer A plus 0.5 M KCl plus 0.2% Nonidet NP-40 plus 0.1% sodium deoxycholate. After 30 min, the homogenate was centrifuged at 50,000 rpm for 1 h in an SW65 rotor. The RNA polymerase solubilized in the supernatant crude extract was assayed with the three DNA templates indicated in the table in the presence and absence of 50 μg of α-amanitin/ml.
the nuclei with 0.5 M KCl-detergents leads to a higher amount of activity solubilized, although the specific activity is lower than using 0.5 M KCl alone or in combination with sonic treatment due to increased solubilization of proteins from the nuclear membranes. Treatment of the nuclei with a lower salt concentration (e.g., 0.1 M KCl) selectively solubilizes RNA polymerase II. Treatment of the nuclei with a higher ionic strength leads to the solubilization of RNA polymerase I which can be separated from RNA polymerase II by DEAE-Sephadex chromatography. These results indicate that RNA polymerase I and II have, in vivo, a different degree of association with the chromatin or a different location inside the nucleus (e.g., nucleolus). It has been suggested that the dark crescents observed under phase micro-

**DISCUSSION**

The use of purified nuclei to study the enzymes involved in RNA synthesis in yeast has the advantage of a lower level of ribonuclease activity than is present in a lower level of cytoplasm or in the mitochondria. This low RNase activity in the nucleus makes it possible to study the RNA synthesized in vitro by nuclei homogenates under different conditions. The use of intact nuclei instead the homogenates is unfortunately limited by their osmotic fragility (1).

Several methods can be used to solubilize the RNA polymerase activity from the nuclei. These methods involve treatment of the nuclei with nonionic detergents or sonic treatment in a high-ionic-strength medium. Treatment of the nuclei with 0.5 M KCl-detergents leads to a higher amount of activity solubilized, although the specific activity is lower than using 0.5 M KCl alone or in combination with sonic treatment due to increased solubilization of proteins from the nuclear membranes. Treatment of the nuclei with a lower salt concentration (e.g., 0.1 M KCl) selectively solubilizes RNA polymerase II. Treatment of the nuclei with a higher ionic strength leads to the solubilization of RNA polymerase I which can be separated from RNA polymerase II by DEAE-Sephadex chromatography. These results indicate that RNA polymerase I and II have, in vivo, a different degree of association with the chromatin or a different location inside the nucleus (e.g., nucleolus). It has been suggested that the dark crescents observed under phase micro-

**FIG. 2. Separation of RNA polymerase I and II from a nuclear extract of S. cerevisiae by DEAE-Sephadex chromatography.** Isolated nuclei (0.5 g wet wt) were suspended and homogenized in 5 ml of buffer A, 0.5 M KCl, 0.2% NP-40, and 0.1% sodium deoxycholate. After 30 min at room temperature, the nuclei homogenate was centrifuged at 50,000 rpm for 60 min in an SW65 rotor, the supernatant was removed avoiding the floating lipids, and 3.5 ml of this supernatant (4 mg of protein/ml) was loaded on a column (32 by 2 cm) of DEAE-Sephadex A25 equilibrated with buffer A. Stepwise elution was done with 30 ml of buffer A containing the following molarities of ammonium chloride: 0, 0.1, 0.2, 0.25, 0.3, 0.35, 0.4, 0.5, and finally 60 ml of buffer A plus 0.7 M ammonium chloride. Fractions of 3 to 3.5 ml were collected, and 0.1 ml of the fractions was assayed for RNA polymerase activity using native yeast DNA as template. The incubation time was 10 min.

**Inhibition of RNA polymerase II by α-amanitin.** Figure 5 shows the effect of different concentrations of α-amanitin on the activity of RNA polymerase I and II. Whereas RNA polymerase I is insensitive to concentrations up to 50 μg/ml, RNA polymerase II is completely inhibited. At 2 μg/ml it is inhibited by 50%.

Rifampin, a typical inhibitor of the RNA polymerase of bacteria (25), does not have any effect on RNA polymerase I and II at concentrations up to 50 μg/ml.

**FIG. 3. Chromatography on DEAE-Sephadex of RNA polymerase I and RNA polymerase II.** Two columns (10 by 1 cm) of DEAE-Sephadex A25, equilibrated with buffer A, were loaded with 2 ml of a fraction containing RNA polymerase I (660 units/ml with dAT as template) and 2 ml of a fraction containing RNA polymerase II (450 units/ml with dAT as template). Stepwise elution was done with 5 ml of buffer A and 5 ml of buffer A with 0.1, 0.2, 0.25, 0.3, 0.35, and 0.4 M ammonium chloride and 10 ml of buffer A with 0.5 M ammonium chloride. Fractions of 1 ml were collected, and RNA polymerase activity was assayed in the fractions with dAT as template.
TABLE 3. Template specificity of RNA polymerase I and II

<table>
<thead>
<tr>
<th>DNA template</th>
<th>Relative activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RNA polymerase I</td>
</tr>
<tr>
<td>Native nuclear yeast</td>
<td>100</td>
</tr>
<tr>
<td>Denatured nuclear yeast</td>
<td>80</td>
</tr>
<tr>
<td>Native calf thymus</td>
<td>80</td>
</tr>
<tr>
<td>Denatured calf thymus</td>
<td>70</td>
</tr>
<tr>
<td>Polydeoxyadenylic-thymidylic acid</td>
<td>350</td>
</tr>
<tr>
<td>No DNA</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

* RNA polymerase I and II were purified from S. cerevisiae nuclei by chromatography on DEAE-Sephadex. See Materials and Methods for DNA concentration and incubation conditions.

A relative activity of 100 represents 15,000 units/mg of protein (RNA polymerase I) and 10,000 units/mg of protein (RNA polymerase II).

Fig. 4. Effect of the concentration of manganese and magnesium on the activity of RNA polymerase I and II. The enzymes were solubilized from S. cerevisiae nuclei and separated by DEAE-Sephadex chromatography. The enzyme preparations were dialyzed against buffer A minus magnesium, and the RNA polymerase activity was measured using native yeast DNA in the presence of different concentrations of manganese (O) and magnesium (C). The incubation time was 10 min.

Fig. 5. Effect of the α-amanitin on the activity of RNA polymerase I and II. The activity of the RNA polymerase I (O) and II (C) purified from nuclei by chromatography on DEAE-Sephadex was assayed in the presence of different concentrations of α-amanitin. Nuclear yeast DNA was used as template, and samples were taken at various intervals during the reaction. The activities shown correspond to a 10-min incubation period under the described assay conditions.

scope in yeast nuclei are nucleoli (15). Sillevis et al. have indicated that the ribosomal RNA precursors are synthesized in this region of the nucleus (20). Our observations indicate that, after treatment of the nuclei with 0.1 M KCl, the dark crescents can still be seen under the phase microscope and they disappear after treatment with a higher salt concentration. These observations suggest a correlation between the solubilization of RNA polymerase I and the disappearance of these dark crescents, suggesting RNA polymerase I may be localized in these structures.

A comparison of the two enzymes separated from the yeast nuclei with the RNA polymerases solubilized from other eukaryotic organisms (2) shows that they share some common properties. RNA polymerase I is active with manganese or magnesium, can use native and denatured DNA as templates, and at least in rat liver has been located in the nucleolus (18). RNA polymerase II is mainly active with manganese, prefers denatured DNA as a template, and is inhibited by α-amanitin. However, the inhibitor concentration of the drug is higher for the enzyme RNA polymerase II of yeast than for the corresponding enzyme of calf thymus (11). In contrast to the RNA polymerases solubilized by Ponta et al. (16) from cellular extracts of S. cerevisiae, the preparations of RNA polymerase I and II described in this paper are much more active with nuclear yeast DNA. Furthermore, the activity of RNA polymerase I is higher with native yeast DNA...
than with denatured calf thymus DNA (Table 3), whereas the corresponding RNA polymerase A of Ponta et al. (16) is 10 times more active with denatured DNA than with native yeast DNA. RNA polymerase II is also more active with yeast DNA than the corresponding RNA polymerase B. We believe that these differences are due to variations in the DNA preparations used as templates.

A third RNA polymerase (III or C) has been reported in various eukaryotic organisms, (2, 16) but its instability has made difficult its characterization. The fact that this enzyme is not present in the nuclei extract prepared with our KCl-detergents treatment can be because it is not solubilized with this treatment or, as in Blastocladia emersonii, is located in the mitochondria (8).

The similarities of the properties of the RNA polymerases from fungi, amphibia, and mammals suggest a common pattern in the transcription of their genomes. To account for a role of these enzymes in the control of the gene expression, a series of specific factors influencing their template specificity would seem likely. Unfortunately none of such factors has been found so far in eukaryotic organisms.

At the present moment the physiological role of the RNA polymerase I and II from nuclei of S. cerevisiae is not known. Our observations suggest that RNA polymerase I can be implicated in the transcription of the ribosomal genes, but an analysis of the RNA made by the two enzymes, when using native nuclear yeast DNA as a template, is necessary to test this possibility. Such product analysis is under current investigation in our laboratory.

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LITERATURE CITED