Isolation, Structure, and General Properties of Yeast Ribonucleic Acid Polymerase A (or I)

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SUMMARY

This paper describes a method for the isolation of RNA polymerase A (or I) from Saccharomyces cerevisiae which is rapid and achieves a 2,000-fold purification. The method involves mainly a batchwise adsorption of enzyme on phosphocellulose and on DEAE-cellulose and a sedimentation on glycerol gradient. The enzyme obtained can be differentiated from RNA polymerase B (or II) by a number of criteria including electrophoretic migration on polyacrylamide gel, sensitivity to alphaamanitin, subunit structure, and optimal conditions for RNA synthesis. Polyacrylamide gel electrophoresis with sodium dodecyl sulfate reveals that yeast RNA polymerase A (or I) is made up of two large subunits in rapid and achieves a 2,000-fold purification. The method involves mainly a batchwise adsorption of enzyme on phosphocellulose and on DEAE-cellulose and a sedimentation on glycerol gradient. The enzyme obtained can be differentiated from RNA polymerase B (or II) by a number of criteria including electrophoretic migration on polyacrylamide gel, sensitivity to alphaamanitin, subunit structure, and optimal conditions for RNA synthesis. Polyacrylamide gel electrophoresis with sodium dodecyl sulfate reveals that yeast RNA polymerase A (or I) is made up of two large subunits in equilibrium with 190,000 and 135,000 daltons and several smaller polypeptide chains, 1 (48,000), 1 (41,000), 2 (29,000), and 2 (15,000). The RNA synthesized on native calf thymus or yeast DNA is constituted of many short chains and of a small percentage (about 10%) of very long chains which represent the bulk of the RNA. Termination factor rho from Escherichia coli inhibits 50% the transcription of native DNA.

Great progress has been made in the isolation and elucidation of the structure of eukaryotic RNA polymerases (1–4). Present knowledge indicates the presence in yeast of two major and possibly three nuclear enzymes (5–9). RNA polymerase A (or I) is generally believed to be involved in ribosomal RNA synthesis (10, 11) for a number of reasons yeast cells are good candidates for the study of ribosomal RNA synthesis. Large quantities of cells are available. About 2% of the yeast genome hybridizes with rRNA which represents some 140 copies of the genes coding for both the 18 S and 25 S RNA (12–16). Purification of high molecular weight yeast DNA can be achieved (17, 18). Moreover, ribosomal RNA synthesis and maturation has been extensively studied (19–23) and one can hope that mutants altered for both the 18 S and 25 S RNA (12–16) will be available. These results prompted us to isolate RNA polymerase A of high specific activity from yeast cells.

Previous communications have described the extensive purification and subunit structure of yeast RNA polymerase B (or II) (0, 24). The present report describes the purification and general properties of RNA polymerase A and demonstrates by several criteria, including subunit structure, that RNA polymerase A and B are distinct and different enzymes. In the accompanying paper the template requirements of the two polymerases are reported (25).

EXPERIMENTAL PROCEDURE

Material—Phosphocellulose P11, 7.4 meq per g, was purchased from Sigma. Unless otherwise stated, "native" DNA corresponds to calf thymus DNA purified on nitrocellulose as previously described (24). d(A-T) was obtained from Biopolymers Inc., unlabeled nucleotides from P-L Biochemicals. [3H]UTP and [14C]UTP from Commissariat a l'Energie Atomique (Saclay). Prior to use, labeled nucleotides were lyophilized to remove the alcohol. Labeled nucleic acids were obtained as previously described (24). Thymymethylthio-
The homogenate was adjusted to pH 8 with 1 cell extract was cooled immediately with an ice and salt mixture. A sample of 2 ml of cold trichloroacetic acid (w/v). Acid-insoluble radioactivity was collected on Millipore filter RAP 025 and washed with about 30 ml of cold trichloroacetic acid. The filter was dried and counted in an Intertechnique counter with 10 ml of a toluene-scintillation fluid. One unit of activity corresponds to 1 m mole of UMP incorporated per hour under the above conditions. Specific activity of enzyme is expressed as units per mg of protein. Protein was measured by the procedure of Lowry et al. (28) with crystalline bovine serum albumin as a standard.

**Assays for Nuclease Activities—**DNase, RNase, and ribonuclease II activities were estimated by incubating, under the standard assay conditions, RNA polymerase samples (Step 2 or 5) and RNase A with varying amounts of DNase I and RNase A to estimate the maximum activity of each nuclease. The activities were then calculated to detect about 1 ng per ml of RNase A and 10 ng per ml of DNase I.

**Polyacrylamide Gel Electrophoresis—**Standard polyacrylamide gel, pH 8.3, containing 5% acrylamide was prepared as previously described (26). Electrophoresis of native RNA polymerase (7 to 10 μg of protein) was run at 5 mA per gel for 60 min at 4°C. The gels were stained with Coomassie brilliant blue R-250 and destained with Dowex AG 1-X2, chloride form. Assay for enzymatic activity after gel electrophoresis was performed by cutting out unstained gel and incubating the slices 0.5 mm thick under the standard assay conditions with d(A-T)2 as template for 1 hour at 28°C. RNA was recovered with the usual acid precipitation technique. The slices were removed from the filter after the washing procedure and stained with Coomassie blue. Under these conditions, recovery of input activity was 12%. Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate was performed according to the procedure described by Laemmli (30).

**RESULTS**

**Purification of RNA Polymerase A**

In the fractionation procedure to be described, the temperature was maintained at 0-5°C. The results are summarized in Table 1.

<table>
<thead>
<tr>
<th>Fraction or step in purification</th>
<th>volume</th>
<th>protein</th>
<th>total activity</th>
<th>specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. High speed centrifugation</td>
<td>m</td>
<td>mg</td>
<td>units</td>
<td>units/mg</td>
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<tr>
<td>2. Phosphocellulose batch</td>
<td>290</td>
<td>185</td>
<td>38,000</td>
<td>203</td>
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<tr>
<td>3. DEAE-cellulose batch</td>
<td>300</td>
<td>21</td>
<td>25,000</td>
<td>1,200</td>
</tr>
<tr>
<td>4. DEAE-cellulose chromography</td>
<td>30</td>
<td>2.5</td>
<td>3,000</td>
<td>1,200</td>
</tr>
<tr>
<td>5. Glycerol gradient</td>
<td>5</td>
<td>0.5</td>
<td>900</td>
<td>1,800</td>
</tr>
</tbody>
</table>

* RNA polymerase A and B are not separated at this stage.

and clarified by centrifugation for 30 min at 9,000 rpm in the JA10 rotor of the Beckman J21 centrifuge. The supernatant was again centrifuged for 2 hours at 20,000 rpm in polycarbonate tubes with the use of a 21 rotor of the Beckman ultracentrifuge. The clear part of the supernatant was pooled to give Fraction 1.

**Phosphocellulose Batch—**This step is based on previous observations indicating that RNA polymerase A and B are adsorbed on phosphocellulose at pH 8 and eluted, respectively, with 0.275 M and 0.12 M ammonium sulfate. Phosphocellulose (120 g, 7.4 mg per g), previously equilibrated in Buffer I containing 0.15 M ammonium sulfate (Buffer I is 0.02 M Tris-HCl, pH 8, 0.01 M 2-mercaptoethanol, 0.5 mM EDTA, and 10% glycerol), was added to Fraction 1 (530 ml). Packed phosphocellulose (1 g wet weight) was used for every 350 A280 units of supernatant. After thorough mixing, the ammonium sulfate concentration was reduced to 0.15 M by addition of 1 volume of Buffer I (530 ml). The ion strength was lowered in the presence of phosphocellulose in order to prevent the binding of RNA polymerase to nucleic acids. The suspension was stirred slowly for 30 min at 4°C then filtered with gentle suction on a Buchner funnel with a sintered glass No. 3. The phosphocellulose was not allowed to dry. The cake was poured into a beaker well suspended in 1 liter of Buffer I containing 0.15 M ammonium sulfate and again collected by filtration. This washing procedure was repeated four times. After the last filtration, the packed phosphocellulose was suspended in 300 ml of Buffer I containing 0.4 M ammonium sulfate, gently stirred 30 min, and collected on the funnel as above. Suction was applied to draw the maximum of solution from the phosphocellulose cake while avoiding foaming. About 300 ml of filtrate resulted, containing RNA polymerase A (Fraction 2).

**DEAE-cellulose Batch—**Fraction 2 was thoroughly mixed in a beaker with 100 g of DEAE-cellulose (1 meq per g) well equilibrated in Buffer II containing 0.1 M ammonium sulfate. (Buffer II is 0.02 M Tris-HCl, pH 8.4, 0.01 M 2-mercaptoethanol, and 0.5 mM EDTA.) Packed DEAE-cellulose (1 g wet weight) was used for every 3 A280 units. The ammonium sulfate concentration was lowered to 0.1 M by addition of 3 volumes of Buffer II (900 ml). The suspension was slowly stirred for 60 min, filtered over a Buchner funnel, and washed four times with 500 ml of Buffer II containing 0.1 M ammonium sulfate as described above. To elute RNA polymerase, the cellulose cake was suspended with stirring for 30 min in 300 ml of Buffer II containing 0.3 M ammonium sulfate. The maximum of solution was drawn by gentle suction to yield about 300 ml of Fraction 3.
DEAE-cellulose and eluted as described in the text. Fractions of 3 ml were collected and assayed for RNA polymerase activity on 10-μl aliquots for 15 min under the standard assay conditions.

Proteins were determined according to the method of Lowry et al. (28). RNA polymerase activity was measured on 15-μl aliquots for 10 min under standard conditions. The peak fractions were pooled and stored at -70°C (Fraction 5).

At this stage the enzyme reached a specific activity of 1,800 units per mg of protein. The final recovery of protein and enzymatic activity was low. The yield was much increased when the glycerol gradient step was performed directly after the DEAE-cellulose batch, but then the enzyme preparation was still contaminated with nucleic acids even if sedimentation was carried out in 0.4 M ammonium sulfate.

Contaminating Nucleic Acids and Nuclease Activities—At Stage 3 (DEAE-cellulose batch), the RNA polymerase preparation was slightly contaminated with DNase and RNase activities (about 5 ng of RNase A equivalents per mg of protein), whereas no ribonuclease H could be detected. After the glycerol gradient step, the preparation did not contain detectable RNase and ribonuclease H activities, and the maximum level of DNase activity corresponded to 30 ng of DNase I equivalents per mg of protein. The ratio of absorbance at 280 nm to that at 260 nm was 1.6, suggesting that less than 0.3% (by weight) nucleic acid was present in Fraction 5.

Features Distinguishing RNA Polymerases A and B

Chromatographic Separation of Enzymes—The former criterion for the presence of multiple RNA polymerase in eukaryotic cells was their different behavior during DEAE-Sephadex chromatography (33). The phosphocellulose step described above was devised to adsorb selectively RNA polymerase A from the cell extract while leaving RNA polymerase B and possibly other polymerase activities in solution. The material not retained on phosphocellulose at 0.15 M ammonium sulfate (flow-through) was analyzed by chromatography on DEAE-cellulose in order to check the selective removal of enzyme A. As a control, an untreated cell extract was chromatographed under identical conditions. Both elution profiles are shown in Fig. 3. Two peaks of activity, A and B, were seen in the control. After phosphocellulose treatment, the peak corresponding to RNA polymerase A disappeared, whereas RNA polymerase B was fully recovered. A small peak of activity, not well resolved when the two main activities were present, was revealed in the phosphocellulose-treated sample. This activity eluted definitely between RNA polymerase A and B.

Differential Susceptibility of RNA Polymerases A and B to α Amanitin—To further show that the enzyme isolated on phosphocellulose was free from RNA polymerase B, its sensitivit
Selective removal of RNA polymerase A by phosphocellulose as evidenced by DEAE-cellulose chromatography of a treated and untreated cell extract. For phosphocellulose treatment, 2 ml of Fraction 1 (high speed centrifugation step), with an absorbance of 80 at 280 nm, were mixed with packed phosphocellulose (0.5 g wet weight). The suspension was diluted successively with 2 ml of Buffer I and 6 ml of Buffer I containing 0.15 M ammonium sulfate. After 30 min with gentle stirring, phosphocellulose was removed by low speed centrifugation in the cold and 6 ml of supernatant fluid were diluted with 12 ml of Buffer II without ammonium sulfate. A second sample of 2 ml of Fraction 1 was treated in the same way except that phosphocellulose was omitted. Simultaneous chromatography of the two samples was carried out on two identical DEAE-cellulose columns (2 cm² x 5 cm) packed from the same batch of ion exchanger. The columns were washed with 30 ml of Buffer II plus 0.05 M ammonium sulfate. Elution was achieved with 100 ml of a linear gradient from 0.05 to 0.5 M ammonium sulfate in Buffer II. In order to achieve identical conditions, gradients were delivered at identical elution rates (15 ml per hour) from one pump and from the same gradient mixer. Fractions of 2 ml were collected and assayed for polymerase activity on 25-μl aliquots. Absorbance at 280 nm was recorded with an ISCO dual beam absorbance monitor. The gradient was started at tube 25 (arrow).

Differential sensitivity of RNA polymerase A and B to α-amanitin. Incubation mixtures (0.25 ml) as described under “Experimental Procedure” contained 0.3 unit of RNA polymerase A (Fraction 2 phosphocellulose step) (Ο) or 0.7 unit of RNA polymerase A (Fraction 5) (△). Incubation was for 30 min at 30°. Control incorporation of [α-32P]UTP was 0.1 and 0.2 n mole, respectively. Standard incubation mixtures for RNA polymerase B (24) (0.25 ml) contained 4 μg of denatured calf thymus DNA and 5 units of RNA polymerase B (Fraction 8 phosphocellulose) (Ο). Control incorporation of [α-32P]UTP in 30 min at 30° was 2 n moles.

Electrophoretic Migration and Subunit Composition of RNA Polymerase A—Native RNA polymerase A migrated as a main band of protein on polyacrylamide gel electrophoresis under nondissociating conditions (Fig. 5). By cutting an unstained gel and incubating the slices it was verified that the enzymatic activity migrated exactly at the level of the stained band. These results suggest that the enzyme was at least 90% pure. RNA polymerases A and B clearly migrated differently on polyacrylamide gel electrophoresis (Fig. 5). The slower migration of RNA polymerase A as compared to RNA polymerase B reflected a difference in either charge or molecular weight of the two enzymes, or both. Sedimentation of yeast RNA polymerase A in 0.05 M ammonium sulfate with E. coli RNA polymerase as the internal marker indicated a sedimentation coefficient of 18 S, somewhat higher than that of RNA polymerase B (17 S) under similar conditions. This sedimentation value agrees well with those reported by Brogt and Planta (6) and Ponta et al. (5). This result suggested that RNA polymerase A had only a slightly higher molecular weight than enzyme B, in the range of 550,000. Therefore, the difference in electrophoretic migration of the two proteins mainly arose from a difference in net charge as evidenced by their respective behavior on ion exchangers.

The molecular subunit structure of RNA polymerase A was revealed by polyacrylamide gel electrophoresis with sodium...
varying amounts of calf thymus DNA. Alkali denaturation of enzyme obtained from repeated experiments was 1 (mol wt 41,000), 2 (mol wt 29,000), and 2 (mol wt 16,000). Other polypeptide chains were also present. Their weight ratio was estimated by scanning the stained bands and their weight ratio was estimated by scanning the stained bands.

Addition of Mn$^{2+}$ at various concentrations together with 5 mM Mg$^{2+}$ did not lead to a very significant increase in the rate of RNA synthesis (about 15%). Therefore, for the sake of simplicity, only Mg$^{2+}$ was added to the assay mixture. Transcription was not stimulated by ammonium sulfate concentration between 0.1 and 10 mM. Above 10 mM this salt became inhibitory. The optimal temperature was 30°C.

**Effect of DNA Template on RNA Synthesis**—In the absence of DNA there was no detectable synthesis of RNA by the purified RNA polymerase A (Fractions 3 to 5). Native DNA, including yeast DNA, was less efficient than a denatured template with Fractions 3 or 5. The effect of concentration of native and denatured DNA on RNA synthesis is shown in Fig. 7. At low DNA concentration, denatured DNA was a much better template than native DNA. A higher DNA enzyme ratio (10:1, w/w) was required for optimal synthesis on native DNA. With DNA excess, the rate of synthesis with native DNA reached 65% of the maximum rate obtained with the denatured template. These results may vary slightly with the enzyme preparation and the procedure used for DNA denaturation. A more efficient synthesis was obtained with alkali-denatured DNA than with heat-denatured template.

The kinetics of RNA synthesis with various template is presented in Fig. 8. Native calf thymus DNA was used before and after purification on nitrocellulose (24). The removal of DNA molecules containing extensive single-strand regions apparently did not affect the rate and extent of RNA synthesis, on the contrary to what was observed with yeast RNA polymerase B. RNA synthesis was linear for 30 min and continued for more than 2 hours. Very active synthesis was also observed with d(A-T)₆ as template. Further studies on template requirements are reported in the accompanying paper (25).

The rate of RNA synthesis increased linearly with the amount of enzyme added but some cooperativity was often found at very low enzyme concentration (Fig. 9). Linearity could not be recovered by addition of serum albumin (50 µg per ml) to the incubation mixture but several basic proteins restored the activity.

**Analysis of RNA Product**—As a candidate for rRNA synthesis, RNA polymerase A was expected to synthesize particularly long RNA chains. The length of the RNA product with the use of different templates has been analyzed by chromatography on a MAK column as previously described (36).

Before analysis, the transcription complex was dissociated by sodium dodecyl sulfate or by a short exposure to alkaline pH. The latter treatment was intended to denature possible RNA-DNA hybrid structures. The same elution pattern of the RNA was obtained in both cases and, unexpectedly, the same results were also obtained when no special treatment was used to dissociate the transcription complex. One characteristic elution profile is shown in Fig. 10. The bulk of RNA was eluted after the E. coli ribosomal RNA markers, at higher salt concentrations, indicating the presence of very long RNA chains (36). This conclusion was supported by a double-labeling experiment with [$\gamma$-³²P]GTP and [$\gamma$-³²P]ATP as initiator nucleotides and [³H]UTP as an internal marker. The average chain length of the double-labeled RNA eluted after the ribosomal markers ranged from 4000 to 6000 nucleotides. Most of the³²P label, however, eluted at the level of tRNA and the calculated average chain length of the total RNA was strikingly low, about 200 nucleotides. This

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1 J. M. Buhler, unpublished result.
No inhibition was observed with d(A-T)n or polymerase A (Fig. 11). At saturation of p factor, inhibition was indicated that the majority of the RNA chains initiated by RNA polymerase A were extremely small and did not contribute much to the over-all synthesis of RNA. Only a small percentage of chains (around 10 to 20%) elongated normally and represented the bulk of the RNA observed on the MAK column. Identical results were obtained with native calf thymus DNA or yeast DNA.

Inhibition of RNA Synthesis by Rho Factor—E. coli termination factor p (37) inhibited total RNA synthesis by yeast RNA polymerase A (Fig. 11). At saturation of p factor, inhibition was about 50 to 60%. No inhibition was observed with d(A-T)n or denatured DNA as template. p factor was previously shown to interact with E. coli RNA polymerase. The complex was visualized by polyacrylamide gel electrophoresis (27). With the same method, no such complex could be found between p factor and yeast RNA polymerases A or B. More details concerning the mode of action of p factor will be presented elsewhere.

DISCUSSION

Purification Procedure—RNA polymerase A was formerly obtained as a by-product of RNA polymerase B preparation (9). After DEAE-cellulose chromatography, two additional steps, sedimentation in a glycerol gradient followed by phosphocellulose chromatography, were required to purify the enzyme. However, the yield of enzyme was low as well as its final specific activity. Moreover, RNA polymerase A could not be recovered from stationary cells (9). Based on these results, a rapid purification procedure has been devised which achieved a considerable purification of RNA polymerase A from log phase or stationary cells in a few easy steps. The initial step involves the selective adsorption of RNA polymerase A on phosphocellulose. The following DEAE-cellulose batch yields an enzyme which was about 50% pure as estimated from its specific activity (900 to 1200 units per mg of protein) and gel electrophoresis analysis. Column chromatography did not bring about a better purification than the convenient batch procedure which could be easily adapted to large scale purification. At this stage the yield of enzyme was quite good. However, to obtain a pure enzyme free of nucleic acids, DEAE-cellulose chromatography and glycerol gradient centrifugation were required, and a marked loss of activity resulted. This was due to a low recovery of protein rather than to enzyme "inactivation" since the specific activity of the glycerol gradient enzyme (1500 to 1800 units per mg of protein) was similar to the specific activity of pure calf thymus RNA polymerase A1 (2). Other authors have described the purification of yeast RNA polymerase A (or 1) (6, 8, 34). However, the specific activity reported for homogeneous enzyme was much lower than the specific activity of the present enzyme preparation, in the range of 4 units (34), 9 units (6), and 90 units (8) per mg of protein. The discrepancy might possibly arise in a difference in subunit composition since small molecular weight polypeptide chains could not be observed with certainty in the deficient enzymes (8, 34).
The poor recovery observed could be due to enzyme dissociation or to the presence of nucleic acids in the preparation, or both, which in any case would lead to the loss of protein during the sedimentation step. High losses of total activity also occur during purification of the mammalian enzyme (2). Nucleic acids might also be responsible for separation of enzyme A activity into different peaks which were sometimes observed during phosphocellulose or DEAE-cellulose chromatography or even on gel electrophoresis. In fact, we found no convincing evidence for the presence in yeast of two forms of RNA polymerase A as reported for rat liver cells (38).

**RNA Polymerase A and B as Distinct Enzymes**—The conclusion that the RNA polymerase purified according to this technique is distinctly different from RNA polymerase B is based on five criteria: (a) chromatographic behavior on phosphocellulose and DEAE-cellulose, (b) migration on gel electrophoresis, (c) sensitivity to α-amanitin, (d) subunit structure, and (e) optimal conditions of synthesis. Although the differences in the two RNA polymerases with respect to any one criterion may not be a decisive argument in themselves, the difference in behavior with regard to all of them is more convincing. Behavior on ion exchangers and gel electrophoresis imply that RNA polymerase A is a less acidic protein than the B enzyme. Distinctive differences in protein structure are revealed by electrophoresis of the dissociated enzyme components, 1 (mol wt 48,000), 1 (mol wt 41,000), 2 (mol wt 29,000) and 2 (mol wt 16,000). The unsatisfactory molar ratio of other additional polypeptide chains suggests that they do not belong to the enzyme. Except for minor differences in molecular weight, the above structure is very much like that of the mammalian enzyme (2, 4). The structural difference between enzyme A and B resides in the molecular weight of the large subunits (9) and in the presence of a polypeptide of molecular weight 48,000, which has no equivalent in RNA polymerase B. It cannot be excluded, however, that the enzymes share common subunits since three polypeptide chains appeared to have the same molecular weight (41,000, 29,000 and 10,000). This hypothesis is supported by the finding that the two RNA polymerases are immunologically related (39) (although a contradictory result has been reported by Ponta et al. (5)). It is interesting to note in this respect that two small subunits of RNA polymerase A and B have also identical molecular weights (2, 3). Anyhow, in view of the complexity of these enzymatic structures, the exact knowledge of their subunit composition should await extensive work from different laboratories.

The basis for the dissimilarity in the requirements for RNA synthesis also suggests variations in the structure of RNA polymerases A and B. For instance, Mn²⁺, which inhibits RNA polymerase B (24), stimulates synthesis by enzyme A. More generally, RNA polymerase A activity depends less critically on the ionic conditions than RNA polymerase B (24). If one compares the specific activities of the two enzymes on native and denatured DNA, it appears that RNA polymerase A is much less efficient on denatured templates. On the other hand, it transcribes with a somewhat higher efficiency "native" DNA. In fact, as shown in the accompanying paper (25), the basic requirements of the two enzymes for transcription of double-stranded DNA are very similar. They both require a single-strand gap to perform chain initiation.

RNA polymerase A produces a limited number of very long RNA chains and a large proportion of short chains. This result could account for the lower specific activity of the enzyme as compared with the bacterial RNA polymerase. A similarity between the bacterial and the eukaryotic transcription machineries is suggested by the finding that E. coli ρ factor inhibits RNA synthesis by the yeast enzyme. This result also points to the possible existence of a similar termination factor in eukaryotic cells.

**Acknowledgements**—We wish to thank S. DeZele for providing yeast RNA polymerase B, G. Hervé for the gift of aspartate transcarbamylase, and Mrs. E. Ehrlich for her kind technical advice concerning gel electrophoresis.

**REFERENCES**

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