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Transcription in Yeast: Separation and Properties of Multiple RNA Polymerases

(eukaryote/transcription/rifamycin AF/013)

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ABSTRACT **Four peaks of DNA-directed RNA polymerase activity are resolved by salt gradient elution of a sonicated yeast cell extract on DEAE-Sephadex. The enzymes, which are named IA, IB, II, and III in order of elution, all appear to come from cell nuclei. Only enzyme II is sensitive to α-amanitin. All enzymes are more active with Mn^{++} than with Mg^{++} as divalent ion. Enzymes IB and II have salt optima in the range 0.05–0.10 M $(NH_4)_2SO_4$, whereas enzyme III is maximally active at 0.20–0.25 M $(NH_4)_2SO_4$. With optimal salt concentration and saturating DNA, the template preference ratio, activity on native calf-thymus DNA divided by activity on denatured calf-thymus DNA, is 2.2 for IB, 0.4 for II, and 3.5 for III. None of the yeast polymerases was inhibited by rifamycin SV. Rifamycin AF/013 effectively inhibited polymerases IB, II, and III.**

Genetic transcription in bacteria is catalyzed by a single enzyme: DNA-directed RNA polymerase. The complete purification of this enzyme (1) has been closely followed by studies of the catalytic role of each protein subunit (2) and of the various subunit alterations that accompany development (3, 4). Essential to this rapid progress in phage and bacterial transcription has been the availability of phage and bacterial mutants, particularly those affecting properties of RNA polymerase and systems that modify this enzyme (5–8).

In nearly every eukaryotic organism, more than one RNA polymerase is found (9). Substantial progress has been made on the separation, enzymologic characterization, and subunit analysis of the two major RNA polymerases found in animal cell nuclei (10, 11). In order to proceed beyond descriptive enzymology to a study of the biological function of each eukaryotic RNA polymerase and of the role each may play in developmental change, a combination of many experimental approaches will be required. Among these will be the use of RNA polymerase mutations and the other powerful tools of genetic analysis. Of the eukaryotes, *Saccharomyces* is the one that has undergone the most extensive formal genetic study (12). Because of the existence of many conditional macromolecular synthesis-deficient mutants (13) and the possibilities for selecting drug-resistant mutants with inhibitors of RNA polymerase, the prospects for developing RNA polymerase genetics in yeast appear to be excellent.

After the initial discovery that sonication in media containing high salt concentrations released multiple RNA polymerases from sea urchin and rat liver nuclei (9), Roeder did an analogous experiment on nuclear material from *S. cerevisiae* (14). DEAE-Sephadex chromatography of the sonicate of a crude yeast nuclear pellet produced a four-peak pattern (14), differing only slightly from that obtained with sea urchin nuclei. In our experiments, a similar pattern has been seen, with three major components and one minor one, with enzymes from both yeast nuclei and whole cells. The three major enzymes differ from one another in their response to salt and their sensitivity to the inhibitor, α-amanitin. In contrast, a chemically-modified rifamycin derivative (rifamycin AF/013) effectively inhibits each of the enzymes.

MATERIALS AND METHODS

Solutions. Extraction buffer is 0.10 M Tris·HCl (pH 7.9, measured at 4°), 20% v/v glycerol, 0.02 M $MgCl_2$, 0.8 M $(NH_4)_2SO_4$, 1.0 mM EDTA, and 1.0 mM dithiothreitol. Immediately before use, phenylmethylsulfonyl fluoride was added at a concentration of 0.5 mg/ml. TGED is 0.05 M Tris·HCl (pH 7.9, 4°), 25% v/v glycerol, 0.5 mM EDTA, and 0.5 mM dithiothreitol.

Yeast Strains, Media, and Cell Growth. The same pattern of multiple RNA polymerases (Fig. 1) was obtained from vegetatively-growing cells of several different yeast strains. All cells were grown in high glucose with aeration. The diploid strain S41 (15) was grown in YEPD medium (15) at 30° to a density of 9×10^7 cells/ml, corresponding to late-log phase. A diploid made by crossing strains A364A and 79-20-3 (13) and another diploid strain (Z 186) were grown in YEPD at 30° and harvested at a cell density of 2.5×10^7/ml. The haploid strain A364A was grown at 22° in YM-1 medium (13) to a density of 4.0×10^7 cells/ml.

Solubilization of RNA Polymerase Activity. Cells were disrupted by agitation for 2 min with glass beads (0.12 mm diameter, Minnesota Mining and Manufacturing Co.) in a Braun homogenizer (model MSK 2876, Bronwill Scientific, Inc.), in a mixture of 1 part cells, 1 part glass beads, and 1 part extraction buffer. The resulting supernatant was sonicated for 1 min at setting 45 in a Branson J17-V sonicator (Branson Instruments, Inc.) and then centrifuged at 48,000 rpm for 90 min in a Spinco type 60 Ti rotor. The resulting crude extract was diluted with 7 volumes of TGED buffer and immediately subjected to DEAE-Sephadex chromatography (see Fig. 1). Since these experiments were done, we have found that the use of 0.45-mm glass beads for disruption gives a higher yield of polymerase without affecting the relative amount of each enzyme.

Assay for RNA Polymerase Activity. The standard incubation mixture (0.2 ml) was 0.05 M Tris·HCl (pH 7.9), 1.6 mM $MnCl_2$, 0.5 mM each in ATP, CTP, and GTP, and 0.1 mM UTP containing 5.0 μCi of [^{3}H]UTP/ml, and it contained 100 μg/ml DNA. Ammonium sulfate concentrations are given

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in the figure and table legends. The reactions were initiated by addition of a mixture of DNA and other reaction components to a solution of the enzyme in TGED buffer. The resulting mixture was incubated at 30° for 30 min after which the reaction was stopped by rapid cooling to 0°, followed by addition of 100 μg bovine serum albumin in 0.1 ml and 1.0 ml of cold 10% Cl_3CCOOH containing 0.12 M sodium pyrophosphate. The precipitates were collected on Whatman GF/C filters and washed 5 times with 5 ml of 5% Cl_3CCOOH containing 0.06 M sodium pyrophosphate. The filters were dried, placed in vials containing Liquifluor (New England Nuclear Corp.) in toluene, and counted in a liquid scintillation counter.

DEAE-Sephadex Chromatography. The chromatography procedure is described in the legend to Fig. 1. For preparative purposes the enzyme activities observed in the column profile were individually concentrated by pooling those fractions from each peak with an activity at least one-half that of the peak fraction. The pooled fractions were diluted with two volumes of TGED buffer and applied to a 0.9 × 7 cm DEAE-Sephadex (A-25) column equilibrated with 0.05 M $(NH_4)_2SO_4$ in TGED buffer. The enzymes were then eluted with 5 ml of 0.35 M $(NH_4)_2SO_4$ in TGED buffer. 1-ml fractions were collected, and the enzyme activity was primarily found in a single fraction.

Substrates. Unlabeled ribonucleoside triphosphates were obtained from Sigma Chemical Co. [5-^{3}H]UTP (13 Ci/mmol) was obtained from Schwartz/Mann.

DNA Templates. Yeast DNA was prepared by lysing protoplasts (16) with sodium dodecyl sarcosinate, then subjecting the lysate to two CsCl equilibrium centrifugation steps. The yeast strain used, (RD-1B), was a petite lacking mitochondrial DNA. Salmon-sperm DNA was prepared by Pronase treatment of salmon testis nuclei, followed by two $CHCl_3$–octanol extractions, one phenol extraction, and dialysis against 0.01 M Tris·HCl (pH 7.9)–0.05 M KCl–0.1 mM EDTA. Calf-thymus DNA (Type V) was purchased from Sigma Chemical Co. Poly d(A-T) was purchased from Miles Laboratories.

RESULTS

DEAE-Sephadex Column Chromatography. A crude extract was first analyzed by DEAE-Sephadex chromatography. Elution of the column by a salt gradient resolved four peaks of enzyme activity, emerging at 0.12–0.13, 0.16–0.18, 0.225–0.245, and 0.29–0.31 M $(NH_4)_2SO_4$ (Fig. 1). We designate† these, in order of elution from the column, enzymes IA, IB, II, and III. RNA synthesis catalyzed by each of these peaks is completely dependent upon added DNA. Synthesis continues for longer than 30 min at 30°.

The specific activity of enzymes IB, II, and III is about 10 pmol of UMP incorporated per μg protein per 10 min at 30° (assayed with excess UTP). In preparations extracted and chromatographed by our standard procedure (see *Methods*), all active RNA polymerase is retained by DEAE-Sephadex, while 90% of total protein is washed through in the loading buffer. After concentration on a second, small DEAE-Sephadex column, and storage at −10° in 25% glycerol,

† The basis for assigning these particular numbers to the peaks is explained in the *Discussion*.

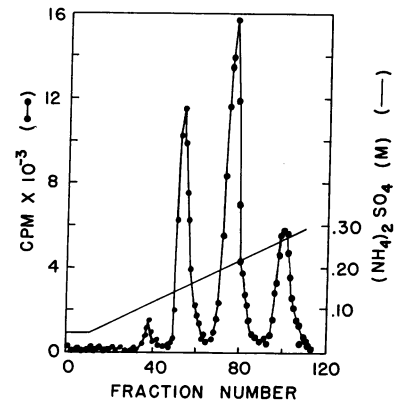

FIG. 1. Resolution of yeast RNA polymerase activities by DEAE-Sephadex chromatography. 7.0 ml of crude extract, from 10 g of packed cells, was diluted with 49 ml of TGED buffer and loaded on a 2.5 × 12.5 cm DEAE-Sephadex (A-25) column equilibrated with 0.05 M $(NH_4)_2SO_4$ in TGED buffer. After loading, the column was washed with one column volume of 0.05 M $(NH_4)_2SO_4$ in TGED buffer and then eluted with 12 column volumes of a linear gradient of 0.05–0.425 M $(NH_4)_2SO_4$ in TGED buffer. A flow rate of 35 ml/hr was used. 6-ml Fractions were collected, and 50-μl aliquots were assayed with carrier-free [^{3}H]UTP in a final incubation volume of 100 μl containing, in final concentrations: 0.05 M Tris·HCl (pH 7.9), 1.6 mM $MnCl_2$, 0.5 mM each of ATP, CTP, and GTP, 5.0 μCi/ml [^{3}H]UTP (13 Ci/mmol), and 50 μg/ml each of native and denatured calf-thymus DNA. Incubation was at 30° for 30 min, after which [^{3}H]UTP incorporation was measured (see *Methods*). (●——●), activity in cpm for each fraction; (——), $(NH_4)_2SO_4$ molarity.

enzymes IB and III retain 50% of the initial activity for 2 weeks. Under these conditions, the half-life of enzyme II is 4 weeks or more.

DEAE-Sephadex chromatography has been done more than 60 times with extracts made from many different batches of *S. cerevisiae*. A pattern of enzyme activity like that in Fig. 1 is reproducibly obtained, provided the standard conditions of cell growth, breakage, extraction, and chromatography are maintained. The apparent activity is always high for enzymes IB, II, and III, with II being generally highest in total activity. The greatest variability is seen in enzyme IA, which ranges from an undetectable amount to 25% of the total activity found for enzyme IB. Among different enzyme preparations extracted from the same batch of yeast cells, the relative amount of enzyme IA varies only slightly. Neither the number of peaks of RNA polymerase on DEAE-Sephadex nor the degree of resolution was affected by variation of the $(NH_4)_2SO_4$ concentration from 0.0 to 0.8 M during sonication or by variation of the time of sonication from 0 to 2 min.

Substrate Saturation. For determination of optimal conditions for assay, the dependence of RNA synthesis upon UTP concentration was measured for enzymes IB, II, and III in the presence of excess ATP, CTP, and GTP. Increasing UTP beyond the concentration used for DEAE-Sephadex column assays (0.38 μM) produced a substantial increase in RNA synthesis, which reached a plateau for each enzyme between 0.05 and 0.1 mM UTP. With UTP held at 0.1 mM, the other three triphosphate substrates were simultaneously varied in concentration. For enzyme IB, maximal [^{3}H]UTP incorporation was achieved with 0.25 mM each of ATP, GTP, and

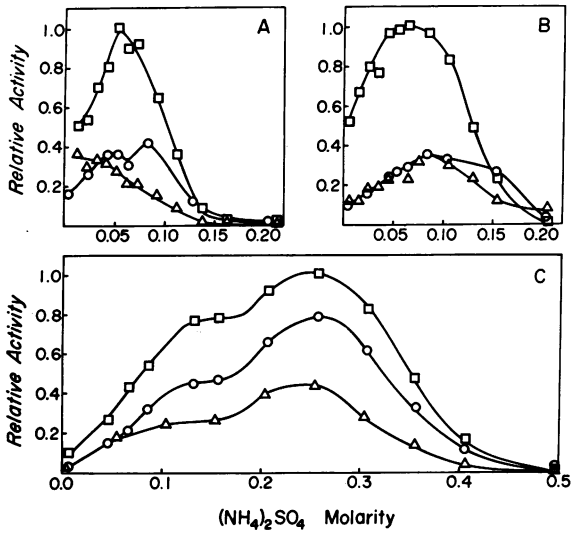

FIG. 2. Relative activities of yeast RNA polymerases with various native DNA templates in the presence of increasing ammonium sulfate concentration. The enzymes used are (*A*), enzyme IB; (*B*), enzyme II; and (*C*), enzyme III. Assays were as described in *Methods*, except that the final concentration of unlabeled UTP was 0.05 mM for enzymes IB and II. For a given enzyme, salt against activity curves for the various DNA templates were not all determined on the same day. The curves shown have been normalized to correct for differences in the quantity of active enzyme used with different templates. The scale of each salt dependency curve has been adjusted so as to make the relative activities on salmon-sperm DNA and native calf-thymus DNA equal to that obtained in Table 1 at the particular $(NH_4)_2SO_4$ concentrations used in the experiments of Table 1. The templates are (□—□), native calf-thymus DNA, 100 μg/ml; (○——○), native salmon-sperm DNA, 100 μg/ml; (△——△), native yeast DNA, 50 μg/ml.

CTP. For enzymes II and III, a concentration of 0.5 mM of each of these three triphosphates was needed to achieve the maximum rate of [^{3}H]UTP incorporation.

Divalent Ion Requirement. Each of the four forms of yeast RNA polymerase eluted from DEAE-Sephadex was more active in the presence of Mn^{++} than in Mg^{++}. For Mg^{++} the curves of enzyme activity against ion concentration all displayed a broad optimum at 8 mM, whereas Mn^{++} gave maximum activity at 1.6 mM. For each of the enzymes, about 3-times higher activity was obtained with optimal Mn^{++} than with the optimal Mg^{++} concentration.

Salt Dependence of Enzyme Activity. Enzymes IB and II have similar curves of activity against $(NH_4)_2SO_4$ concentration on native calf-thymus and salmon-sperm DNA (Fig. 2*A* and *B*). For native calf-thymus DNA, the optimum salt concentration is 0.05 M $(NH_4)_2SO_4$ for both enzymes IB and II; for native salmon-sperm DNA, 0.08 M. With native yeast DNA as template, the salt optimum for enzyme IB is still lower (0.02 M or below), whereas it is near 0.08 M for enzyme II.

Enzyme III (Fig. 2*C*) is most active on all native DNA templates at 0.25 M $(NH_4)_2SO_4$, a salt concentration at which IB and II have no activity (Fig. 2*A* and *B*). The salt curves for enzyme III are also distinctive in having a pronounced shoulder on the low-salt side (Fig. 2*C*). The unusual salt dependence of enzyme III provides a useful means of identification, independent of its position of elution from DEAE-Sephadex.

With denatured calf-thymus DNA as template, enzymes IB, II, and III displayed different $(NH_4)_2SO_4$ dependencies (data not shown). The salt optima were: 0.02 M for enzyme IB, 0.08 M for II, and 0.2 M for III.

Comparison of Various DNA Templates. As a first step toward comparing the efficacy of various DNA templates, DNA saturation experiments were done with each DNA–enzyme combination. For the quantities of enzyme used, DNA saturation was achieved at or before 120 μg of DNA/ml with all templates except poly d(A-T). For each enzyme–template combination, the relative activity with saturating DNA is listed in Table 1. The $(NH_4)_2SO_4$ concentration used for each enzyme was that optimal for transcription of native calf-thymus DNA (Fig. 2).

Several major effects are evident from these data (Table 1):

(*1*) Whereas enzymes IB and III are 2- to 3-times more active on native than on denatured calf-thymus DNA, en-

TABLE 1. *Activities* of yeast RNA polymerases with saturating levels of various DNA templates*

DNA template	Enzyme IB		Enzyme II		Enzyme III	
	DNA sat'n level (μg/ml)	Rel.† act.	DNA sat'n level (μg/ml)	Rel act.	DNA sat'n level (μg/ml)	Rel act.
Poly d(A-T)	120	0.62	200‡	0.12	200	0.17
					<200	2.09§
Native yeast	40	0.40	40	0.35	60	0.44
Native calf-thymus	100	1.00	80	1.00	120	1.00
Native salmon-sperm	30	0.44	30	0.33	120	0.77
Denatured calf-thymus	60	0.45	60	2.61	30	0.28

Assays for polymerase activity were performed as described in *Methods* except that the concentration of DNA template was varied from 0 to 200 μg/ml. Final ammonium sulfate concentrations for enzymes IB, II, and III were 0.04 M, 0.075 M, and 0.25 M, respectively.

* Activities reported are an average value for the points on the plateau regions of activity against DNA-concentration profiles.

† Activity of the indicated RNA polymerase on a given DNA template relative to the activity of the same RNA polymerase on native calf-thymus DNA. The amount of UMP incorporated with native calf-thymus DNA template was 218, 204, and 29 pmol for enzymes IB, II, and III, respectively.

‡ Saturation not obtained at this level.

§ This assay was performed in 0.10 M $(NH_4)_2SO_4$.

zyme II greatly prefers denatured DNA as template. In this respect it resembles RNA polymerase II from animal cell nuclei (9).

(2) The ratio of activity on native calf-thymus DNA to activity on poly d(A-T) is 1.6 for enzyme IB, 8 for enzyme II, 0.5 for enzyme III in 0.10 M $(NH_4)_2SO_4$, and 6 for enzyme III in 0.25 M $(NH_4)_2SO_4$. With respect to this property, enzymes IB and II clearly differ. Enzyme III changes from a preference for poly d(A-T) in low salt concentration to a preference for calf-thymus DNA in high salt concentration.

(3) For both enzymes IB and II, native calf-thymus DNA has a 2- to 3-fold greater effectiveness as template than native yeast or salmon-sperm DNA.

This last observation suggested that the native calf-thymus DNA might contain more single-strand interruptions than either the salmon-sperm or yeast DNA. However, alkaline sedimentation velocity analysis gave a frequency of single-strand interruptions of 1 for each 4400 nucleotides for calf-thymus DNA and one in 1100 for salmon-sperm DNA. The native molecular weights were about 10×10^6 for calf-thymus DNA and 5×10^6 for salmon-sperm DNA. The yeast DNA preparation used exhibited a bimodal distribution of both single- and double-strand molecular weights. A substantial fraction (25%) had a double-strand molecular weight $> 10^8$ and single-strand molecular weight $> 2 \times 10^7$; however, for the major fraction of yeast DNA (50%) these values were 2.3×10^6 and 1.8×10^5, respectively. These results suggest that some feature of calf-thymus DNA other than a high frequency of single-strand nicks is responsible for its unusually high template activity for enzymes IB and II.

Effects of Inhibitors of RNA Polymerase. The mushroom toxin α-amanitin inhibited enzyme II almost entirely (Fig. 3) and inhibited the RNA polymerase activity of a crude extract by about 50%. Enzymes IA, IB, and III were unaffected by α-amanitin concentrations as high as 20 μg/ml. The pattern of α-amanitin inhibition of yeast RNA polymerases resembles that observed in *Blastocladiella* (17), sea urchins, and mammalian cells (18). For these organisms, α-amanitin inhibits the second RNA polymerase eluted from DEAE-Sephadex by an $(NH_4)_2SO_4$ gradient. This enzyme, believed to be of extranucleolar origin, has been designated RNA polymerase II (9).

Rifamycin inhibits the RNA polymerases of bacteria (19) and chloroplasts (20). We have found no rifamycin inhibition of the four yeast RNA polymerases.

Several chemically modified rifamycins inhibit the RNA polymerases of calf-thymus nuclei (21). One of these, rifamycin AF/013, we find to be an effective inhibitor of yeast RNA polymerases IB, II, and III (Fig. 4). At a drug concentration of 20 μg/ml, all three enzymes are more than 90% inhibited. We also observed inhibition of yeast RNA polymerases II and III by rifamycin AF/05, but found that amounts in excess of 100 μg/ml were needed for effective inhibition.

Rifamycin inhibits bacterial RNA polymerase by blocking initiation. Recent results (22) suggest that rifamycin AF/013 likewise inhibits transcription by eukaryotic polymerases at the level of initiation.

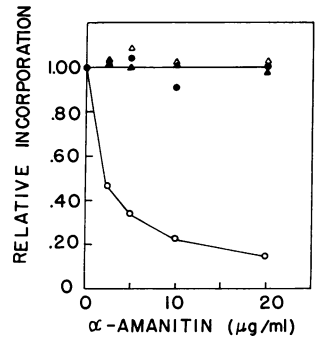

FIG. 3. α-Amanitin sensitivity of yeast RNA polymerases. Assay conditions: 50 μl of DEAE-Sephadex peak fraction was diluted to 100 μl final volume. Tris buffer, Mn^{++}, ATP, GTP, and CTP concentrations were as described in Fig. 1; [^{3}H]UTP, 5 μCi/ml at a specific activity of 20 Ci/mmol. Denatured calf-thymus DNA was used as template at a final concentration of 100 μg/ml. The indicated concentration of α-amanitin was added to each reaction before a 30-min incubation at 30°. In the absence of α-amanitin, the incorporation of UMP was 0.5–1 pmol for each enzyme. (△-△), enzyme IA; (▲-▲), enzyme IB; (○—○), enzyme II; and (●—●), enzyme III.

DISCUSSION

We have begun an exploration of the transcription components of yeast by separating and characterizing the RNA polymerases of vegetatively growing yeast cells. Four RNA polymerase activities are separable by DEAE-Sephadex chromatography. The amounts of RNA polymerase IA isolated were not sufficient for us to study. The three abundant enzymes can readily be distinguished from one another by their catalytic properties. Enzyme IB prefers native DNA as template, is insensitive to α-amanitin, and has a salt optimum below 0.05 M $(NH_4)_2SO_4$. Enzyme II greatly prefers denatured DNA, is sensitive to α-amanitin, and has a salt optimum of 0.06–0.08 M $(NH_4)_2SO_4$. Enzyme III, which prefers native DNA and is insensitive to α-amanitin, differs from both the other enzymes in being extraordinarily salt-resistant. A comparison between these properties of the yeast enzymes and the properties of polymerases I, II, and III isolated from animal cell nuclei (9) has led us to adopt the system of designation used throughout this paper. In salt optima, column elution position, α-amanitin sensitivity, and denatured/native template preference, yeast enzymes IB, II, and III are homologous to sea urchin enzymes I, II, and III (9). Yeast RNA polymerase II has been extensively purified by Dezelee *et al.* (23), who have found a pattern of high molecular weight subunits resembling those of mammalian RNA polymerase II (10, 11).

We have limited information about the intracellular localization of the yeast enzymes. The four enzyme activities we have separated from an extract of whole yeast cells have also been extracted by Roeder (14) and by us from a DNA-rich nuclear fraction of yeast. Thus it appears likely that the RNA polymerases described here came from nuclei. Lacking a reproducible procedure for the isolation of pure yeast nuclei, we cannot show this directly. We can, however, examine the most likely alternative possibility: that one or more of the enzymes comes from mitochondria.

Recently, Tsai *et al.* (24) purified yeast mitochondrial RNA polymerases by salt gradient elution from DEAE-Sephadex. Of the three peaks observed, one, minor in amount, was sensitive to α-amanitin and therefore attributed to slight

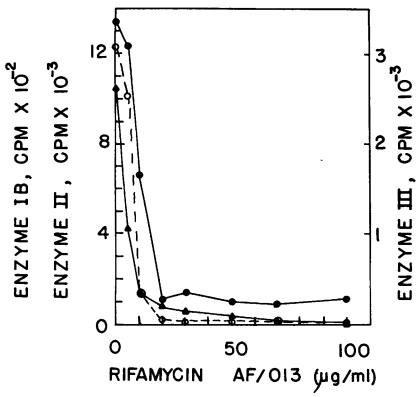

FIG. 4. Effect of rifamycin AF/013 on the activities of yeast RNA polymerases IB, II, and III. The components of the assay were mixed in the following order: to 10 μl of enzyme previously concentrated after DEAE-Sephadex chromatography was added 80 μl TGED buffer, containing sufficient ammonium sulfate to give the optimal initial salt concentration for each enzyme, followed by 10 μl rifamycin AF/103 (a solution in 100% ethanol at 20-times the desired final concentration); 100 μl reaction mix was then added. The composition of reaction mix was such that final incubation conditions were 5% ethanol, 0.05 M Tris·HCl (pH 7.9), 1.6 mM $MnCl_2$, 0.5 mM each of ATP, CTP, and GTP, 0.05 mM UTP, 5 μCi/ml [^{3}H]UTP (13 Ci/mmol), and 100 μg/ml denatured calf-thymus DNA. The final ammonium sulfate concentrations in the assay were 12 mM for enzyme IB, 77 mM for enzyme II, and 110 mM for enzyme III. Incubation was at 30° for 30 min. Acid-precipitable radioactivity was then determined (see *Methods*). (●——●), enzyme IB; (○——○), enzyme II; and (▲——▲), enzyme III.

contamination by nuclear material. The question of possible mitochondrial origin of our enzymes may thus be stated: Are the enzymes IB and III reported here the same as, or different from, mitochondrial enzymes I and III of Tsai *et al.*?

Yeast mitochondrial enzyme I prefers Mg^{++} to Mn^{++} as divalent ion in the range 0–4 mM, where maximum stimulation is obtained (R. Criddle, personal communication). All four of the enzymes we have studied are stimulated 3-fold more by Mn^{++} than by Mg^{++}. Mitochondrial polymerase III elutes from DEAE-Sephadex at higher $(NH_4)_2SO_4$ concentration than does the α-amanitin sensitive peak (24) and thus might be presumed to correspond to our polymerase III. However, the salt optima for the two enzymes are dissimilar, being < 0.05 M $(NH_4)_2SO_4$ for mitochondrial polymerase III (R. Criddle, personal communication) and 0.25 M $(NH_4)_2SO_4$ for our peak III.

A consideration of the known mitochondrial control mechanisms in yeast also suggests that our preparations would not contain mitochondrial RNA polymerase. For glucose-grown cells such as we have used, synthesis of many mitochondrial proteins is repressed (25). Tsai *et al.* (24) have found a very low content of mitochondrial RNA polymerase in glucose-repressed mid log-phase yeast. Thus, it appears unlikely that the enzymes we have described are localized in mitochondria.

The DEAE-Sephadex profile of RNA polymerase activity from wild-type vegetative cells provides a base-line for the analysis of both mutational and developmental variation in RNA polymerases. Our characterization of the activities of enzymes IB, II, and III will allow a comparison to be made between wild-type vegetative yeast, and mutant yeast strains affected in transcription (26) and cells in other stages of the life cycle.

In bacteria, the relationship between structure and function of RNA polymerase has, in several instances, been governed and modified by the action of loosely bound protein subunits (26) and protein factors (27). Despite the impure state of the yeast RNA polymerases we have studied thus far, there are already several strong indications of modifying or regulating factors. Polymerase III, in particular, seems to exist in more than one active condition. A small protein with strong stimulating activity for transcription by enzymes IB and III can be isolated from a high molecular weight fraction of yeast lysates‡.

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