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Specific initiation by RNA polymerase I in a whole-cell extract from yeast

(*in vitro* transcription/ribosomal genes/growth control/*Saccharomyces cerevisiae*)

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ABSTRACT **A protocol is described for making a soluble whole-cell extract from yeast (*Saccharomyces cerevisiae*) that supports active and specific transcription initiation by RNA polymerases I, II, and III. Specific initiation by polymerase I decreases in high-density cultures, paralleling the decrease in abundance of the endogenous 35S rRNA precursor. This extract should be useful for studying the molecular mechanisms that regulate rRNA transcription in yeast.**

The yeast *Saccharomyces cerevisiae* has emerged as the preeminent eukaryote for many studies of transcriptional regulation, due to both the ease of its genetic manipulation as well as the ability to inexpensively grow large amounts of the organism for biochemical fractionation. In the case of RNA polymerase I in yeast, definition of cis-acting regulatory elements has been made possible by the development of genetic approaches that circumvent the problems raised by the repetitive nature of the ribosomal genes (1, 2). Study of the trans-acting factors controlling transcription by RNA polymerase I has been hampered, however, by the lack of *in vitro* transcription extracts capable of supporting specific transcription initiation at the site employed *in vivo*.

This situation is now changing. Riggs and Nomura (3) have reported a whole-cell polymerase I extract from yeast and Lue and Kornberg (4) have described a polymerase I extract made from yeast nuclei. And in this paper we wish to report our own, independently developed procedure for accomplishing the same goal. The method that we describe is rapid, produces extracts from whole cells without prior isolation of nuclei or chromatographic fractionation, and can be adapted for large amounts of yeast. We also show that polymerase I initiation activity in this extract varies with the growth state of the cell. This type of extract should therefore be useful in studying the mechanisms coordinating ribosomal gene transcription with cell growth.

MATERIALS AND METHODS

Strains. Transcription extracts were normally prepared from the multiply protease-deficient *S. cerevisiae* strain BJ2168 (*leu2, trp1, ura3-52, prb1-1122, pep4-3, prc1-407, MAT***a**; provided by S. Hahn, Hutchinson Cancer Research Center, Seattle, WA). Extracts were also made using a strain lacking mitochondrial RNA polymerase, rpo 41 [*ade2-101, lys2-801, his3Δ300, tyr1, RPO41*::Tn*10 URA3, MAT***a** (*pet*$^-$); ref. 5], and a similar strain with wild-type mitochondrial polymerase, RPO 41 [*ade2-101, lys2-801, his3Δ300, ura3-52, trp1Δ901, MAT***a** (*PET*$^+$)] (both provided by R. Butow, Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas).

Plasmids. All plasmids were derived from pBD4 (6), which contains the entire *S. cerevisiae* ribosomal DNA (rDNA) repeat.

pSCr20. The 5302-base-pair (bp) *Nar* I–*Sac* I fragment of the rDNA repeat was cloned into pUC18. pSCr20 contains the entire intergenic spacer and therefore includes the 5S rRNA gene.

pYr11-316. An 800-bp *Sma* I–*Hin*dIII fragment of pSCr20, containing the 35S rRNA promoter, was cloned into pUC18 and a 16-bp *Xho* I linker (5′-CGTCAAGACCCTCGAG-3′) was inserted into the *Taq* I site at +25 (*Xho* I site at +36).

pYr12-5. Same as pYr11-316 except that a 26-bp *Xho* I linker (5′-CGATTCTGAGCCTCAAGACCCTCGAG-3′) was inserted into the *Taq* I site at +25 (*Xho* I at +46).

pYr11A. The *Sma* I–*Xho* I rDNA promoter fragment from pYr11-316 was inserted into pGEM-3 (Promega) in several steps which left an *Xba* I site in the polylinker upstream of the insert. pYr11A was then used to make linker-scanner and 5′-deletion mutants of the promoter region (S.Y.C., details to be published elsewhere). In brief, oligonucleotide-directed mutagenesis (7) was used to introduce additional *Xba* I linkers at −109/−102 and at −89/−82 relative to the site of transcription initiation. The 5′-deletion mutants pYr11A5′−103 and −5′−83 were made from these linker-scanner mutants by digesting the plasmid with *Xba* I and recircularizing the large fragment.

Growth and Freezing of Cells. Cells were inoculated into YEPD (2% yeast extract/1% bactopeptone/2% dextrose) at 1–1.5 × 10^5 per ml and cultured at 30°C with vigorous shaking. Most extracts were prepared from cells harvested at OD_{600} 0.3–2. The cells were cooled by pouring over crushed ice and then spun at 4000 rpm for 4 min in a Sorvall H-6000A rotor. The wet weight of the pellet was determined, and the cells were then washed successively in ice-cold distilled water (2 vol/g of cells) and in 1.3 vol of extraction buffer [100 mM Hepes·KOH, pH 7.9/245 mM KCl/5 mM EGTA/1 mM EDTA/2.5 mM dithiothreitol (added fresh)]. The cell pellet was then resuspended in 1.3 vol of extraction buffer supplemented with protease inhibitors (phenylmethylsulfonyl fluoride, 0.2 mM; benzamidine hydrochloride; 10 mM; L-1-chloro-3-(4-tosylamido)-4-phenyl-2-butanone, 25 μg/ml; pepstatin A, 3.5 μg/ml; leupeptin, 5 μg/ml; aprotinin, 10 μg/ml; and chymostatin, 100 μg/ml) and the cells were pelleted as above. The thick cell paste was loaded into a syringe, extruded into liquid nitrogen, and then stored at −70°C.

Preparation of the Transcription Extract. For most experiments, frozen cells were broken by extensive manual grinding of 2-g lots under liquid nitrogen in a ceramic mortar and pestle. Cell breakage was monitored by assaying soluble protein in the 100,000 × *g* supernatant (8). Under standard conditions, the maximum amount of soluble protein released was about 40 mg/ml; specific transcription was observed with protein concentrations in the range of 13–40 mg/ml.

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Abbreviation: nt, nucleotide(s).

After grinding, all procedures were performed at 4°C. The powder of broken cells was transferred to an ice-cold beaker, and 1.3 vol of extraction buffer with protease inhibitors was added. Thawing of the powder was speeded by slight warming. The final suspension of broken cells was briefly mixed by pipetting and then spun at 100,000 × *g* for 2 hr. (Active extracts were also obtained by spinning at speeds as low as 10,000 × *g*.) The entire supernatant (minus the lipid pellicle) was collected by tube puncture and dialyzed for at least 4 hr against 50 vol of 20 mM Hepes·KOH, pH 7.9/50 mM KCl/5 mM EGTA/0.05 mM EDTA/2.5 mM dithiothreitol (fresh)/20% (vol/vol) glycerol/0.2 mM phenylmethylsulfonyl fluoride with leupeptin at 0.5 μg/ml. This supernatant was used as the transcription extract.

RNA Polymerase I Transcription Reactions. Typical 40-μl reaction mixtures contained 20 μl of extract and 20 mM Hepes·KOH (pH 7.9), 50 mM KCl, 10 mM $MgCl_2$, 5 mM EGTA, 0.05 mM EDTA, 2.5 mM dithiothreotol, 10% glycerol, 100 μM each ribonucleoside triphosphate, α-amanitin at 10 μg/ml, and template DNA at 10–30 μg/ml. After incubation at 25°C for 45 min, reactions were stopped by the addition of 10 vol of 10 mM Tris·HCl, pH 7.5/1% SDS/1 mM EDTA. After phenol/chloroform and chloroform extraction, the products were precipitated with probe and 20 μg of *Escherichia coli* tRNA was added as carrier.

Assay of RNA Polymerase I Transcription. S1 nuclease protection assays were performed essentially according to Labhart and Reeder (9), using single-stranded DNA probes. The S1 nuclease protection probe for transcripts of the pYr11 series of plasmids was a 50-nucleotide (nt) single-stranded oligonucleotide complementary to pYr11A from −15 to +35 (probe A). Correctly initiated transcripts yielded a 35-nt probe fragment. The S1 probe for detecting transcripts from pYr12-5 (probe B) was prepared by labeling the 266-bp *Sma* I–*Xho* I fragment of pYr12-5 and then separating the strands in a nondenaturing 8% acrylamide gel. Correctly initiated transcripts protect a 50-nt-long fragment. For primer extensions, primer A was hybridized to the reaction products in a final volume of 20 μl containing 10 mM Tris·HCl (pH 8), 300 mM KCl, and 0.2 mM EDTA. Reverse transcription was performed with recombinant Moloney murine leukemia virus reverse transcriptase according to the manufacturer's instructions (BRL). Primer A (5′-CGTTTCCAAACTCTTTTCG-3′) was designed to detect both the endogenous rRNA precursor and the transcripts from the pYr11 series or pYr12-5. It hybridized to the rRNA sequence just downstream of the *Xho* I linker in these plasmids and detected the endogenous transcripts as a 44-nt product, and the transcripts from the added template as a 60-nt (pYr11) or 70-nt (pYr12-5) product. The transcripts were resolved in denaturing polyacrylamide gels. Transcript production was quantitated by image analysis of autoradiographs scanned with a Videk 1K × 1K CCD camera coupled to a Sun 3/260 computer (Sun Microsystems, Mountain View, CA) running VISAGE 2000 software (BioImage, Ann Arbor, MI).

Mapping the Start Site of RNA Polymerase I Transcripts. The products of *in vitro* transcription of pYr12-5 were analyzed by primer extension as above. The 19-mer primer was also used for sequencing the template.

Chromatography of the 100,000 × *g* Supernatant. The protein machinery required for specific transcription was concentrated by chromatography on DEAE-Sepharose CL-6B (Pharmacia). Undialyzed 100,000 × *g* supernatant was adjusted to ≈100 mM KCl and 20% glycerol by dilution with 1.3 vol of 20 mM Hepes·KOH, pH 7.9/5 mM EGTA/0.05 mM EDTA/2.5 mM dithiothreitol/36% glycerol/0.2 mM phenylmethylsulfonyl fluoride containing leupeptin at 0.5 μg/ml. The extract was then applied to a DEAE column preequilibrated with CB100 (same as dilution buffer, except that it contained 100 mM KCl and 20% glycerol). The column was washed with CB100 until no more protein was eluted. CB350 (same as CB100, except that KCl was at 350 mM) was then applied and the fractions of highest protein concentration were pooled for dialysis, frozen, and stored as for the 100,000 × *g* supernatant.

Bulk polymerase I activity was separated from polymerases II and III by gradient elution from DEAE-Sepharose as described in the legend to Fig. 5.

RESULTS

Preparation of the Extract. A detailed protocol for producing the extract is given in *Materials and Methods*. In brief, washed cells were frozen in liquid nitrogen (10), placed in a ceramic mortar, and broken under liquid nitrogen by manual grinding with a pestle. Broken cells were then thawed in a minimum volume of extraction buffer containing protease inhibitors and centrifuged at various speeds (ranging from 10,000 × *g* for 10 min to 100,000 × *g* for 120 min). The supernatant was assayed for total protein concentration as well as for the ability to initiate transcription at a yeast rDNA promoter.

Several aspects of this procedure are worthy of mention. (*i*) The calcium chelator EGTA was present in all buffers and magnesium was left out until just before the assay for transcription. (*ii*) Specific initiation activity on an added rDNA template increases as soluble protein in the extract increases (data not shown). Therefore, it is desirable to obtain breaking efficiencies approaching 100% (complete breakage was determined empirically to give a protein concentration of about 40 mg/ml in the 100,000 × *g* supernatant).

The cells remain frozen as they are broken and thaw directly into the presence of protease inhibitors. With these precautions it appears that special protease-deficient strains of yeast are not required in order to obtain active extracts. We initially worked with a protease-deficient strain (BJ2168) but subsequently made equally active extracts from strain rpo 41 (deficient in mitochondrial polymerase) and its wild-type parent, RPO 41 (see Fig. 2). We expect that the procedure will work for most strains of yeast. Accurate, promoter-dependent initiation was observed after centrifugation at all speeds tested (10,000–100,000 × *g*). Since template optima varied, depending upon the speed of centrifugation, it is difficult to make quantitative comparisons among extracts spun at different speeds. We conclude, however, that one could use low-speed centrifugation to rapidly make extracts for preliminary testing of a variety of different cell strains or growth conditions. Spinning at higher speeds, sufficient to remove ribosomes, is probably better suited to extracts intended for further biochemical fractionation.

We have tested various salt concentrations in the extraction buffer. So far the one that works best is about 0.25 M KCl, which is also the optimal salt for making S100 extracts of cultured *Xenopus laevis* cells (11). The method of breaking cells has also been varied. For all the experiments shown in this paper, we manually ground relatively small amounts of cells (up to 2 g) in a mortar and pestle. However, we have also prepared successful extracts from larger amounts of cells by breakage in a Waring blender that was especially modified to allow grinding under liquid nitrogen (10). We expect that the Waring blender will be especially useful for producing extracts for further biochemical fractionation. Extracts of somewhat lower activity were also obtained from thawed cells broken by agitation with glass beads.

For further purification a 100,000 × *g* supernatant was loaded onto a DEAE-Sepharose column in a buffer containing 0.1 M KCl, the column was washed with 0.1 M KCl, and a fraction was step-eluted with 0.35 M KCl (referred to hereafter as the DEAE 0.35 fraction). The DEAE 0.35 fraction contains all of the components required for specific initiation by polymerase I and was the fraction used in the

experiment in Fig. 1. Both the 100,000 × *g* supernatant and DEAE 0.35 fraction are stable at −70°C for 3 months and remain active through multiple rounds of freeze–thawing.

We estimate that the 100,000 × *g* supernatant produces 0.001 transcript per template per hr, whereas the DEAE 0.35 fraction is about 20 times more active on a per milligram of protein basis.

Initiation Is at the Previously Identified *in Vivo* Start Site. Previous *in vivo* studies have shown that yeast ribosomal genes produce a major transcript initiating with an adenine residue (+1 as indicated in Fig. 1*A*). A large fraction of these 5′ termini bear a polyphosphate, suggesting that they are termini of primary transcripts (12, 13). A third group reported additional *in vivo* termini that mapped to about −30 (14). When ribosomal minigenes are introduced into yeast, variable numbers of additional termini are observed, particularly at −10, and in the vicinity of −40 (1, 15, 16).

Fig. 1*A* shows an experiment in which a template containing a yeast ribosomal gene promoter was transcribed using the DEAE 0.35 fraction and primer extension was used to locate 5′ termini produced *in vitro*. A major 5′ terminus was detected that maps precisely to the +1 site previously identified as the major *in vivo* site of initiation.

Fig. 1*B* shows that specific initiation continues for at least 2 hr in the DEAE 0.35 fraction. Again, the major 5′ terminus (in this experiment located by S1 nuclease protection) maps to the same adenine residue at +1. Initiation at the *in vivo* +1 site is consistent with this transcription being due to RNA polymerase I.

In addition to initiation at +1, the S1 analysis in Fig. 1*B* shows an approximately equal amount of full-length probe protection, indicative of additional readthrough transcription originating upstream of the polymerase I promoter. As we will show below, both the initiation at +1 and the upstream readthrough transcription behave as though it is due to polymerase I.

We point out that the promoter fragment used in these experiments was derived from a cloned ribosomal gene repeat that has the ability to support normal growth rates when it is the only source of ribosomes in a yeast cell (E. Morgan, personal communication).

Initiation Is Not Due to Polymerase II or III. The extract contains an active transcription system for RNA polymerase II and will support specific initiation at the *CYC1* promoter (M.C.S., M. Woontner, and J. A. Jaehning, unpublished data; template and reaction conditions are described in ref. 17). However, both the specific initiation and the readthrough seen in Fig. 1 are resistant to concentrations of α-amanitin up to 100 μg/ml (data not shown). We can be certain, therefore, that neither type of transcript is due to RNA polymerase II.

We have also observed that neither the signal at +1 nor the readthrough is inhibited by the bacterial inhibitor Tagetin (18) at levels that nearly abolish transcription by RNA polymerase III (data not shown). Thus we further conclude that initiation at the ribosomal gene promoter is not due to RNA polymerase III.

Initiation Is Not Due to Mitochondrial Polymerase. Whole cell extracts from yeast are known to contain mitochondrial RNA polymerase activity (19). It was essential, therefore, to determine whether or not the initiation at +1 on the ribosomal gene promoter was due to contamination by this polymerase. Parallel extracts were made from a yeast strain in which the gene coding for the catalytic subunit of mitochondrial RNA polymerase is disrupted (rpo 41; ref. 5) as well as from its wild-type parent (RPO 41). As shown in Fig. 2, 35,000 × *g* supernatants from both strains showed comparable levels of specific initiation on the ribosomal gene promoter at two different template concentrations (5 and 10 μg/ml) as well as comparable levels of readthrough transcription. The differential activity seen at the higher template concentration is probably due to different template optima in the two extracts. We conclude that neither type of transcription is due to mitochondrial polymerase.

Initiation *in Vitro* Requires Promoter Sequences Similar to Those Previously Identified *in Vivo*. The approximate location of the yeast ribosomal gene promoter has been defined *in vivo* (15, 20). In more detailed analysis, assay of 5′-deletion mutants *in vivo* has indicated that a major 5′ boundary of the ribosomal gene promoter is located between −154 and −134 since deletion to the 5′ side of −134 essentially abolishes transcription (1). Fig. 3 shows that a similar result is obtained *in vitro*. Deletion of the 5′ side of −103 reduces specific

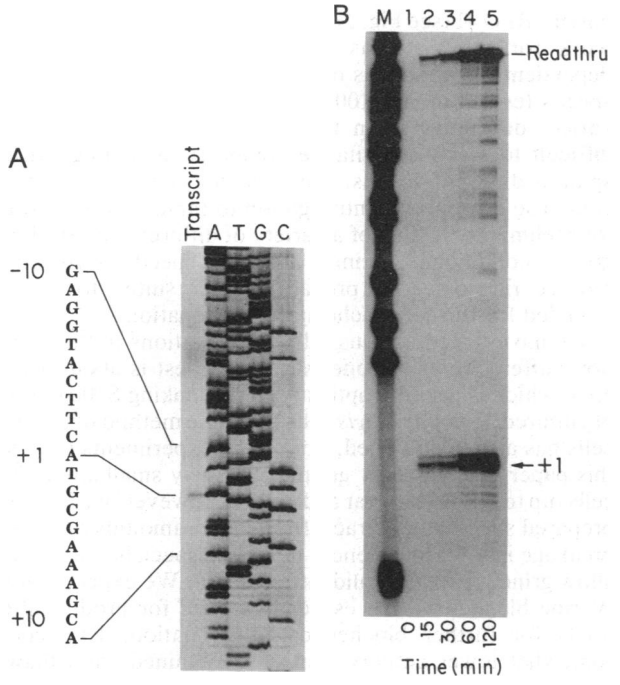

FIG. 1. The extract supports specific transcription initiation at +1 on the yeast 35S rRNA gene. (*A*) Template pYr12-5 was transcribed in the DEAE 0.35 fraction, and specific initiation was assayed by primer extension. To determine the precise site of 5′ end formation, the same primer was used for dideoxy sequencing of the +1 region of the template. (*B*) Specific initiation continues for at least 120 min in the DEAE 0.35 fraction. Specific initiation on template pYr12-5 was assayed by S1 nuclease protection using probe B.

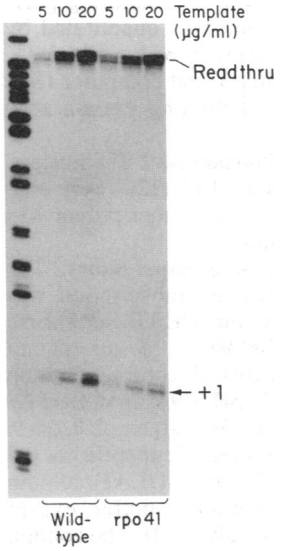

FIG. 2. Specific initiation at the 35S rRNA gene promoter is not due to mitochondrial RNA polymerase. Supernatants (35,000 × *g*) were prepared from strain rpo 41 (lacking the catalytic subunit of mitochondrial RNA polymerase) or from its wild-type parent, RPO 41. Template pYr12-5 was transcribed in either extract at various concentrations and assayed by S1 nuclease protection using probe B. The extracts yield approximately equal amounts of specific initiation at +1.

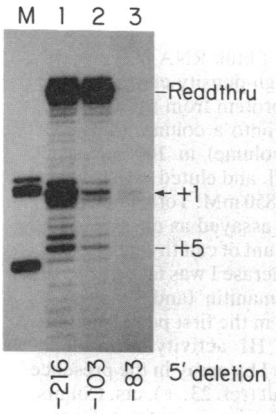

FIG. 3. Specific initiation at the 35S rRNA gene promoter requires approximately the same sequences as are required *in vivo*. Various 5'-deletion mutants of pYr11A were transcribed in the 100,000 × *g* supernatant and were assayed for specific initiation by S1 nuclease protection using probe A. Initiation at +1 is strongly inhibited by deletion to the 5' side of −103, while further deletion to −83 nearly abolishes the signal. Readthrough transcription is also abolished by deletion to −83.

initiation about 10-fold, while further deletion to the 5' side of −83 nearly eliminates transcription. These preliminary results indicate that initiation both *in vivo* and *in vitro* requires approximately the same promoter sequences.

The readthrough transcription observed in Figs. 1–3 behaves as though it is also due to polymerase I, particularly since it is eliminated by destroying the ribosomal gene promoter (Fig. 3, deletion to the 5' side of −83). We do not understand why partially damaging the promoter (deletion to the 5' side of −103) still allows abundant readthrough. However, it is known from other systems that the interaction between readthrough and specific initiation can be rather complex (21).

From the data in Figs. 1–3 we conclude that the observed *in vitro* transcription initiation at the ribosomal gene promoter was due to specific initiation by RNA polymerase I.

Activity of the Extract Is Subject to Growth Regulation. Yeast cells vary the rate of rRNA transcription according to the physiological state of the cell (2). Our initial experiments indicate that this *in vivo* regulation can be reflected in the activity of the extract *in vitro* (Fig. 4). Extracts were made from yeast grown to various cell densities (more grinding is required to completely break cells from high-density cultures) and each extract was assayed for specific polymerase I initiation ability. Initiation was monitored by primer extension using a primer that can detect both the endogenous 35S rRNA precursor as well as transcripts initiated on the exogenous template.

Fig. 4 summarizes two experiments. In both experiments specific initiation activity in the extract decreases as cell density increases, and this decrease roughly parallels a similar decrease in the steady-state concentration of the 5' end of the 35S rRNA precursor. This suggests that the activity seen in the extracts reflects endogenous transcription activity. We have mixed active extracts from early-phase cells with inactive extracts from late-phase cells and consistently found that the activity of the mixture was only slightly different from that of the active extract alone (data not shown). This indicates that the inactive extracts do not contain any grossly inhibitory substance.

The loss of specific initiation activity documented in Fig. 4 could be due to a general decrease in the amount of RNA polymerase I in high-density cultures. Alternatively, it could be due to a loss of polymerase's ability to recognize the promoter (with the bulk amount of polymerase remaining unchanged). To distinguish between these alternatives we measured the amount of bulk polymerase I activity in a low-density versus a high-density culture by using nicked calf thymus DNA as a template for nonspecific initiation (Fig. 5). Equal amounts of protein from a low-density (OD_{600} 3) culture and from a high-density (OD_{600} 13) culture were loaded onto columns of DEAE-Sepharose and were eluted with a salt gradient. Under these conditions mitochondrial polymerase

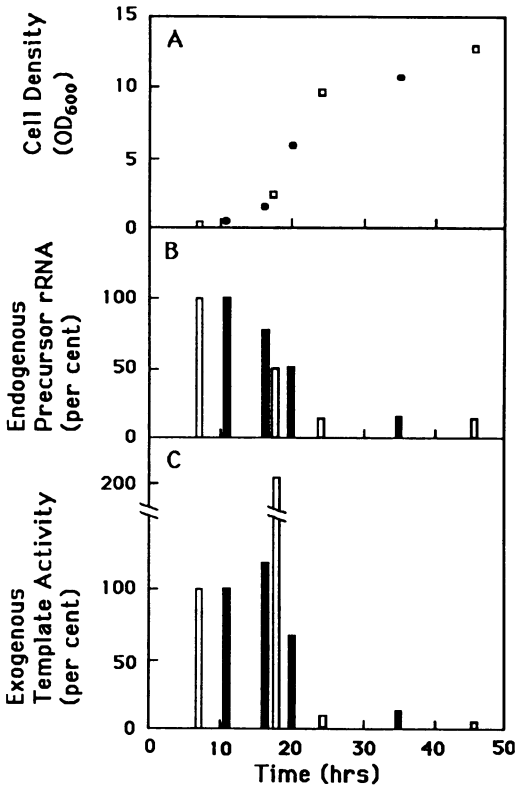

FIG. 4. Specific initiation activity in the extract parallels 35S rRNA precursor concentration at various stages of the growth curve. Yeast strain BJ2168 was grown in liquid culture (YEPD) to various cell densities and aliquots were taken to prepare 100,000 × *g* supernatants. Each extract was used to transcribe template pYr11-316, and primer extension was used to assay both initiation at +1 and the relative concentration of the 5' end of the endogenous 35S rRNA precursor. Open symbols, experiment 1; filled symbols, experiment 2. (*A*) Growth curve of strain BJ2168 in YEPD medium. (*B*) Relative concentration of endogenous 35S rRNA precursor 5' termini. Autoradiographic signals produced by primer extension were measured with a densitometer. The concentration of 5' termini at the earliest point in the growth curve in each experiment was taken as 100%. (*C*) Relative specific initiation activity on exogenously added pYr11-316. Quantitation was as in *B*. Data were normalized to protein concentration by adding the same amount of protein to each transcription reaction. To check that this normalization was valid, additional aliquots of cells from the lowest and highest densities of experiment 2 were separately ground to yield extracts of identical protein concentrations. Upon assay, these extracts showed the same difference between low- and high-density cells as is shown in the figure.

does not bind to the column and is eluted in the wash (3). Under optimal conditions the nuclear polymerases then are fractionated from each other and are eluted in the order I, II, III (24, 25). In this particular experiment polymerase I was eluted first (as judged by the α-amanitin resistance and salt sensitivity of the first peak; see refs. 23 and 26), while polymerases II and III were eluted later but were not resolved from each other. As a measure of bulk polymerase I activity we summed the α-amanitin-resistant activity in fractions 15–20. The high-density culture had essentially the same amount (93%) of bulk polymerase I as did the low-density culture.

We draw several conclusions from the experiments shown in Figs. 4 and 5. First, highly active extracts capable of specific initiation can be made from yeast grown to an OD_{600} of at least 5.0, which makes it relatively easy to obtain large amounts of material for biochemical fractionation. Second, the reduction of initiation activity in late-growth-phase extracts is due not to a general loss of RNA polymerase I but to a loss of the ability to recognize a ribosomal gene pro-

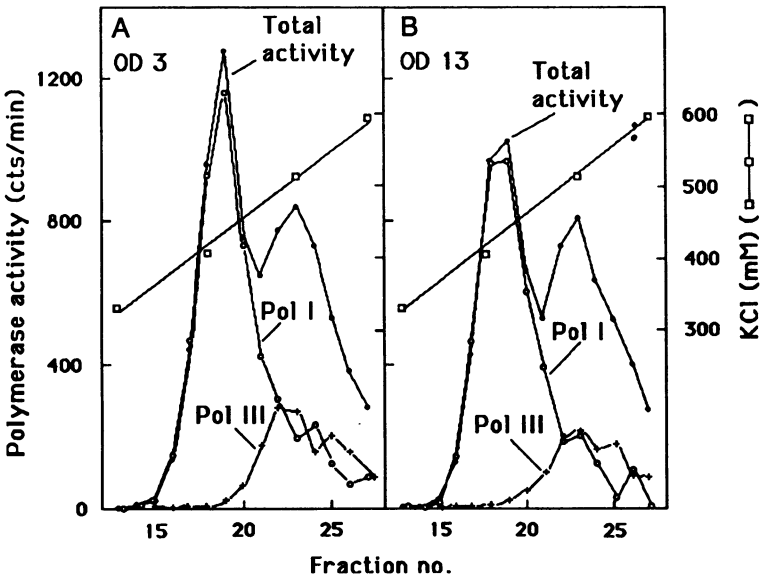

FIG. 5. Comparison of bulk RNA polymerase I activity in low- versus high-density cultures. Fourteen milligrams of total protein from a 100,000 × *g* supernatant was loaded onto a column of DEAE-Sepharose (10-ml bed volume) in 100 mM KCl, washed with 100 mM KCl, and eluted with a 120-ml KCl gradient from 100 to 850 mM. Total nonspecific polymerase activity was assayed as described (22) by using a saturating amount of calf thymus DNA as template (●). RNA polymerase I was taken to be the activity resistant to α-amanitin (and sensitive to high salt) that was eluted in the first peak (fractions 15–20; ○). Polymerase III activity was distinguished from polymerase I by assay in the presence of α-amanitin plus high salt (ref. 23; +). cts, Counts. (A) Polymerase activities in an extract from a low-density culture (OD_{600} 3). (B) Polymerase activities in an extract from a high-density culture (OD_{600} 13). Fractions 15–20 of the high-density culture contained 93% the amount of bulk polymerase I activity as was present in the same fractions from the low-density culture.

moter. It will require further fractionation to determine whether this loss is due to an alteration in the RNA polymerase I or in one of the trans-acting factors associated with polymerase I. Previous studies on *Acanthamoeba* and mammalian transcription systems indicate that the lesion will probably be tightly associated with the polymerase itself (reviewed in ref. 27).

DISCUSSION

Protocols are now available for making yeast extracts that are capable of supporting specific initiation by all three nuclear RNA polymerases. Extracts for polymerase III transcription have been available for some time (for example, see refs. 28 and 29). More recently, both nuclear (30) and whole cell (17) extracts for polymerase II have been reported. In addition, two methods have been reported for making active polymerase I extracts, one involving nuclear isolation (4) and one using whole cells (3).

The main advantages of the method we report in this paper are its ease and the fact that specific initiation activity can be reliably measured early in the procedure. Thus the method should be adaptable to rapid screening of multiple samples. Preliminary experiments indicate that the cells can be broken in a Waring blender as well as in a manual mortar and pestle. This, coupled with the fact that nuclear isolation is not required, suggests that the method could be readily adapted for large-scale biochemical fractionation. In this regard, it is encouraging that initiation-competent polymerase I activity per cell is nearly constant with culture density until an OD_{600} of at least 5.0, thus making it relatively easy to accumulate large amounts of active cells. The protocol has worked well on three different strains of yeast, suggesting that it will not be necessary to use specialized, protease-deficient strains. Although we have focused on polymerase I, preliminary tests indicate that the extract has good activity for polymerases II and III as well.

We hope, therefore, that the method reported here will be of general use for researchers studying the enzymology of transcription in *S. cerevisiae*.

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