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mRNA Transcription in Nuclei Isolated from *Saccharomyces cerevisiae*

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We developed an improved method for the isolation of transcriptionally active nuclei from *Saccharomyces cerevisiae*, which allows analysis of specific transcripts. When incubated with α-^{32}P-labeled ribonucleoside triphosphates in vitro, nuclei isolated from haploid or diploid cells transcribed rRNA, tRNA, and mRNAs in a strand-specific manner, as shown by slot blot hybridization of the in vitro synthesized RNA to cloned genes encoding 5.8S, 18S and 28S rRNAs, tRNA^{Tyr}, and *GAL7*, *URA3*, *TY1* and *HIS3* mRNAs. A yeast strain containing a high-copy-number plasmid which overproduced *GAL7* mRNA was initially used to facilitate detection of a discrete message. We optimized conditions for the transcription of genes expressed by each of the three yeast nuclear RNA polymerases. Under optimal conditions, labeled transcripts could be detected from single-copy genes normally expressed at low levels in the cells (*HIS3* and *URA3*). We determined that the α-amanitin sensitivity of transcript synthesis in the isolated nuclei paralleled the sensitivity of the corresponding purified RNA polymerases; in particular, mRNA synthesis was 50% sensitive to 1 μg of α-amanitin per ml, establishing transcription of mRNA by RNA polymerase II.

Nuclei isolated from a number of cell types have been shown to accurately transcribe RNA in vitro (8, 10, 13, 14, 17, 19). In a subset of these systems there is reinitiation of RNA synthesis (10, 13, 17), and in some cases polyadenylation occurs in the isolated nuclei (8, 10, 14). In one example nuclei have been shown to accurately initiate transcription of exogenous DNA added to the incubation mixture (13). Recently, runoff transcription in isolated nuclei has been used to study the role of enhancer elements (24). The environment of the isolated nuclei can be precisely controlled so it is possible that these nuclei can be used to study the effects of soluble factors on transcription.

The abundance of genetic and molecular biological data available for the yeast *Saccharomyces cerevisiae* make it especially desirable to develop a yeast nuclear isolation procedure capable of accurately reconstructing in vivo events. The first step in achieving this goal is to demonstrate that yeast nuclei contain active RNA polymerases I, II, and III. Yeast RNA polymerases I and II are 50% inhibited at α-amanitin concentrations of 300 to 600 and 1 μg/ml, respectively (11, 18), and RNA polymerase III is insensitive to α-amanitin concentrations of up to 2.4 mg/ml (18, 19). Previous studies have shown that α-amanitin inhibits the synthesis of some size classes of RNA in isolated nuclei (14, 19), but the RNAs were not positively identified. We used these unique drug sensitivities to detect the presence and activity of the three RNA polymerases by measuring the sensitivity of rRNA synthesis, tRNA synthesis, and mRNA synthesis to α-amanitin. The low levels of most mRNA-encoding genes make detection of a unique message difficult. To overcome this problem, a strain containing a high-copy-number plasmid encoding the *GAL7* gene was used for the initial mRNA detection studies (S. M. Baker, M. R. Karl, S. A. Johnston, J. E. Hopper, and J. A. Jaehning, submitted for publication). It has been shown previously that transcription from this plasmid accurately mimics the regulated expression of *GAL7* in its chromosomal location (2). Optimizing transcription of this abundant mRNA [as much as 50% of the total poly(A)$^+$ RNA] allowed us to define conditions under which we could detect transcription of less abundant mRNAs, such as *URA3* and *HIS3* [0.01 to 0.1% of the total poly(A)$^+$ RNA] (1, 23). We found that the synthesis of mRNAs is sensitive to low levels of α-amanitin, which is consistent with synthesis by RNA polymerase II.

MATERIALS AND METHODS

Yeast strains. The strains of *S. cerevisiae* used for this investigation were strain YJJ51 *MATa/α lys1/LYS1 gal2/GAL2*, strain YJJ86 *MATa/α ade5/ade5 leu2-3,112/leu2-3,112 trp1-289/trp1-289 ura3-52/ura3-52 gal7Δ102/gal7Δ102* [cir^0], and strain YJJ106 *MATα, ade5 trp1-289 leu2-3,112 gal7*Δ102 *his3*::ADH1φ*GAL4*φ*URA3 gal80*::*HIS3* [cir^0]. Strains YJJ86 and YJJ106 and transformants containing YEp731 and plasmid vector pJDB219 containing the *GAL7* gene on a *Bam*HI fragment insertion were provided by S. Baker of our laboratory (2; Baker et al., submitted).

Cell growth and isolation of nuclei. Strains YJJ86 and YJJ106 containing YEp731 were grown in defined synthetic media (2) lacking leucine but containing 3% glycerol plus 2% DL-lactic acid; mid-log phase corresponded to an optical density at 600 nm of 0.6 under these conditions. Strain YJJ51 was grown in YPG (1% yeast extract, 2% peptone [Difco Laboratories], 2% galactose); mid-log phase corresponded to an optical density at 600 nm of 1.2 under these conditions. Galactose, lactic acid, and amino acids were obtained from Sigma Chemical Co.

Cells were harvested in mid-log phase by centrifugation for 10 min at 20,000 × *g*, and they were converted to spheroplasts by using modifications of the two-step procedure of Cabib (4); the volumes indicated below were per gram of yeast cells. The cells were preincubated for 30 min at 30°C in 3.5 ml of 40 mM EDTA (pH 8)–100 mM β-mercaptoethanol. Following centrifugation at 20,000 × *g* for

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10 min, the resulting pellet was washed with 5 ml of 1 M sorbitol. The cells were converted to spheroplasts at 30°C in 6.7 ml of 1 M sorbitol containing 1 mM EDTA and Zymolyase 5000 (Miles Laboratories, Inc.). The extent of spheroplast formation was determined by microscopically examining a 1:20 dilution of cells in 1% sodium dodecyl sulfate (SDS). The amount of Zymolyase 5000 needed dependend on the strain being converted to spheroplasts. Typically, 5 to 7 mg of Zymolyase 5000 per 3 g of cells resulted in 90% of the cells being converted to spheroplasts in 30 to 40 min. The degree to which the cells were converted to spheroplasts was a major determinant in the final yield of nuclei. If spheroplast formation was stopped before the first cells lysed, the yield was considerably lower than if spheroplast formation was continued until 90% of the cells became spheroplasts, even though some of the cells lysed.

The spheroplasts were collected by centrifugation for 10 min at 2,000 × *g* and were then washed by suspension in 7.5 ml of 1 M sorbitol and centrifugation for 10 min at 2,000 × *g*. The spheroplasts either could be used immediately or could be stored in 1 M sorbitol at 4°C for up to 2 days. If they were to be stored, the washing procedure was repeated twice. The spheroplasts were then incubated for 35 to 40 min with gentle shaking at 30°C in YPG containing 1 M Sorbitol (20 ml/g of original cells). This recovery incubation increased total transcription and induced transcription of the *GAL7* gene (21). The spheroplasts were harvested by centrifugation for 10 min at 2,000 × *g*, washed with 7.5 ml of 1 M sorbitol containing 1.7 mM phenylmethylsulfonyl fluoride, and collected by centrifugation at 4,000 × *g* for 10 min. The spheroplasts were lysed, and the nuclei were isolated essentially as described by Schultz (19) (see below). The nuclear pellet was suspended in at least five volumes of buffer A (20 mM KH_2PO_4, pH 6.5, 18% [wt/vol] Ficoll [Pharmacia], 0.5 mM $CaCl_2$, 1.7 mM phenylmethylsulfonyl fluoride). The suspension was centrifuged at 2,000 × *g* for 5 min to pellet the lysed cells. The nuclei were then collected by centrifugation of the supernatant at 30,000 × *g* for 25 min.

The pelleted nuclei were suspended in 2 volumes of buffer B (20 mM KH_2PO_4, pH 6.5, 1 M sorbitol, 7% [wt/vol] Ficoll, 20% [wt/vol] glycerol, 0.5 mM $CaCl_2$, 1.7 mM phenylmethylsulfonyl fluoride), and additional buffer B was then added to 10 volumes. Suspending the nuclei at this point required fairly vigorous procedures (e.g., vortexing) to break up aggregates, which reduced yields in subsequent steps. Unlysed cells were removed by centrifugation at 3,000 × *g* for 5 min. The supernatant was centrifuged at 20,000 × *g* for 25 min to pellet the nuclei. The nuclei were suspended in 1 ml of buffer C (50 mM Tris hydrochloride, pH 7.5, 0.6 M sucrose, 1 mM $MgCl_2$, 10 mM NaCl, 1 mM dithiothreitol, 35% glycerol) per 3 g of original cells. Stored at −20°C, the nuclei remained transcriptionally active for at least 2 weeks. A drastic reduction in transcriptional activity was observed if the nuclei were frozen.

To determine the amount of DNA present, the nuclei were suspended in 10 volumes of lysis buffer (1% SDS, 0.5 M NaCl, 10 mM EDTA, pH 8.0). Proteinase K (Beckman Instruments, Inc.) was added to a concentration of 50 mg/ml, and the mixture was incubated at 37°C for 30 min. Following extraction with an equal volume of phenol-chloroform (1:1), the DNA was ethanol precipitated in the presence of 2 M ammonium acetate. The amount of DNA in the pellet was determined by the diphenylamine method (9). We estimated that our yield of nuclei ranged from 20 to 50% in different preparations, based on the level of DNA recovered.

Preparation of cloned DNA for use in hybridization studies. The 5.3-kilobase (kb) Sc4811 *Eco*RI DNA fragment (18) containing DNA corresponding to the entire *GAL7* transcript and most of the *GAL10* transcript was subcloned into the *Eco*RI site of pUC9 (25). A 6.05-kb *Hin*dIII fragment containing the genes for 18S, 25S, and 5.8S rRNAs was isolated from pBD4 (3) and was subcloned into the *Hin*dIII site of pUC9 (pJJ141). A 284-base pair *Eco*RI fragment containing the gene for $tRNA^{Tyr}$ (20) (generously provided by S. Sandmeyer) and a 1.25-kb *Eco*RI fragment containing DNA corresponding to the 5′ end of the *TY1* transcript were subcloned into the *Eco*RI site of pUC9 (pJJ143 and pJJ91, respectively); the *TY1* fragment was an *Eco*RI linker construction of the *Hpa*II-to-*Eco*RI fragment from *TY1* isolate D15 (5), which was provided by M. Woontner, Department of Microbiology, University of Illinois. Plasmids were isolated in cesium chloride-ethidium bromide gradients (6). Following digestion of the plasmids with the appropriate restriction enzyme, the fragments were separated from vector sequences by sucrose gradient centrifugation (25). A 1.7-kb *Bam*HI fragment containing the *HIS3* gene (23), single-stranded M13 DNA containing 1 kb of DNA coding for *URA3* (1), 90 or 500 nucleotides of DNA corresponding to the 5′ end of the *GAL7* transcript in both orientations, and double-stranded M13 DNA was provided by G. T. Marczynski of our laboratory. Single-stranded M13 DNA containing the gene for $tRNA^{Tyr}$ was provided by M. R. Karl of our laboratory.

Transcription reactions and their analysis. Transcription reactions were performed in 50-μl volumes (unless otherwise specified) by using 25 μl of a suspension of nuclei (1.6 to 2.6 μg of nuclear DNA) per reaction. The final concentrations in the reaction mixture were 0.5 mM ATP, 0.5 mM CTP, 0.5 mM UTP (P-L Biochemicals, Inc.), 0.5 mM dithiothreitol, 2.5 mM Tris hydrochloride (pH 7.5), 0.05 M NaCl, 0.3 M sucrose, 17.5% glycerol, and 100 μM [α-^{32}P]GTP (the [α-^{32}P]GTP was obtained from Amersham Corp. and was diluted with unlabeled GTP obtained from P-L Biochemicals to a specific activity of 1×10^4 to 2×10^4 cpm/pmol for reactions to be analyzed by hybridization to specific DNAs and to a specific activity of 4×10^3 cpm/pmol for reactions whose total incorporation was to be determined by counting portions of the reaction mixture in a liquid scintillation counter). The concentrations of KCl, $MgCl_2$, and α-amanitin were varied (see figure legends). The reaction mixtures were incubated at 30°C for 15 min, and 100 μl of a 0.5% SDS–10 mM EDTA solution was then added to stop the reaction. If the amount of [^{32}P]GMP incorporated was to be determined by scintillation counting, 5 μl of the mixture was applied to Whatman DE81 paper and washed as described previously (16). When hybridization was to be performed, the reaction mixtures were extracted three times with 2 volumes of phenol-chloroform (1:1), ammonium acetate was added to a final concentration of 2 M, and the RNA was precipitated with 2 volumes of ethanol at −20°C for 3 to 4 h. The pellet was dissolved in 0.5 ml of diethylpyrocarbonate (Sigma)-treated water, heated for 2 min at 100°C, and quick chilled on ice before being added to the hybridization solution.

DNA for the slot blots was prepared by denaturing 1 μg of the appropriate DNA (at least a six-fold excess over the expected amount of RNA) in 100 μl of 0.2 N NaOH at 22°C for 10 min and then increasing the salt to 2× SSC (16) and incubating the preparation on ice for 10 min. The DNA solution was applied to Gene Screen Plus (New England Nuclear Corp.) by using a slot blot apparatus (Schleicher & Schuell, Inc.). Each well was washed three times with 120 μl of water. Prehybridization was performed for 4 h at 42°C in

4 ml of a solution containing 50% formamide, 5× Denhardt solution (16), 0.1% SDS, 5× SSPE (16), and 0.2 mg of denatured salmon sperm DNA per ml. For hybridization 0.5 ml of ^{32}P-labeled RNA was added to 3.5 ml of a solution containing 50% formamide, 1× Denhardt solution, 0.1% SDS, 5× SSPE, and 0.1 mg of salmon sperm DNA per ml. Hybridization was performed at 46°C for 14 h unless otherwise indicated.

Blots were washed twice in 100 ml of 2× SSC at 22°C for 5 min, twice in 200 ml of 2× SSC–0.1% SDS at 65°C for 30 min, once in 200 ml of 1× SSC at 22°C for 30 min, and once in 500 ml of 0.3 M NaCl–2 mM EDTA–10 mM Tris hydrochloride (pH 7.5) containing 10 μg of RNase A per ml at 37°C for 30 min. Autoradiography was performed at −70°C with a Cronex Lightning Plus intensifying screen and Kodak XAR-5 film. To accurately determine the amount of ^{32}P-labeled RNA hybridized, several exposures of the blots were obtained, and the relative intensities of the signals were determined by using a densitometer (Helena Laboratories). In some experiments, control slots containing vector DNA (pUC or M13) were included, and no hybridization to vector sequences was detected.

RESULTS AND DISCUSSION

Recovery incubation increases transcriptional activity of isolated yeast nuclei. Previously used procedures for the isolation of yeast nuclei usually included a lysis step directly after removal of the cell wall by lytic enzymes (4, 12, 19). Since yeast cells are metabolically inactive during the spheroplast formation procedure (typically, extended incubation in osmotically stabilizing media with no available carbon or nitrogen source), the nuclei isolated from them might be expected to have low transcriptional activity. Including a recovery incubation step in galactose-containing media allows the cells to reestablish metabolic activity and also induces a high level of transcription from the plasmid-borne *GAL7* gene (2, 21; Baker et al., submitted).

In the transformed strain used in this study, which contained high levels of the positive activator *GAL4* (*ADH1*ϕ*GAL4* fusion gene) and lacked a functional copy of the negative regulator *GAL80* (*gal80*::*HIS3* disruption), as much as 25 to 50% of the total poly(A)$^+$ RNA was *GAL7* mRNA under inducing conditions (Baker et al., submitted). The advantage of using such an abundant mRNA is shown in Fig. 1; the hybridization signal from the *GAL7* DNA was readily apparent after relatively short exposure times. In this experiment, nuclei isolated from galactose-induced spheroplasts were incubated with α-^{32}P-labeled nucleoside triphosphates in the absence or presence of 100 μg of α-amanitin per ml. Total label RNA was isolated and hybridized to different DNA samples which had been immobilized by slot blotting (see Materials and Methods). Only the *GAL7*-hybridizable material showed sensitivity to this moderate level of α-amanitin, supporting the assumption that RNA polymerase II is responsible for mRNA transcription (10, 21) (see below). Labeled RNA isolated from spheroplasts incubated in glycerol-lactate rather than galactose showed very little hybridization to the *GAL7* probe (data not shown).

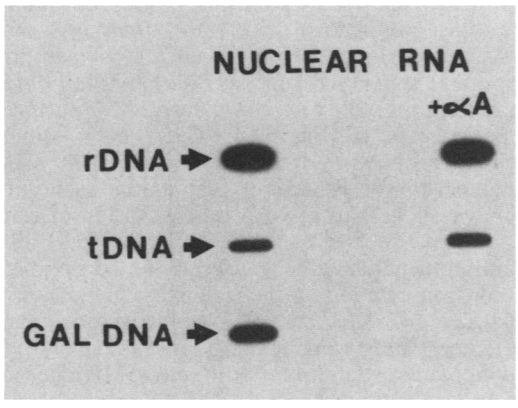

FIG. 1. Analysis of nuclear transcripts. Nuclei isolated from strain YJJ106(YEp731) containing 1.6 μg of nuclear DNA per reaction mixture were incubated in the absence or presence (+αA) of 100 μg of α-amanitin per ml, ^{32}P-labeled RNA was isolated, and hybridization and autoradiography were performed as described in Materials and Methods. The labeled arrows indicate the positions of immobilized double-stranded DNA encoding yeast rRNA, tRNA^{Tyr}, and *GAL7* mRNA. The autoradiogram shown was from a 6-h exposure.

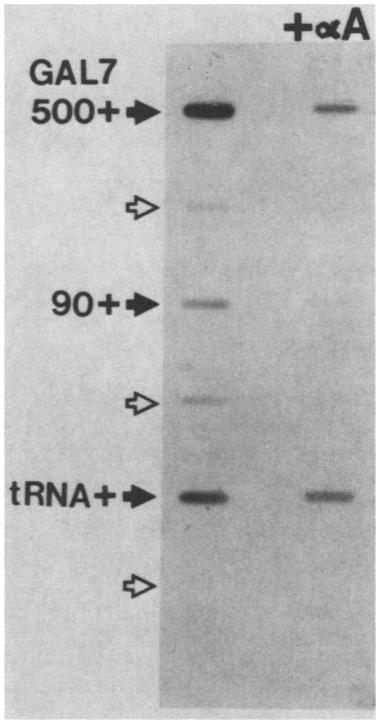

FIG. 2. Strand specificity of nuclear transcripts. DNA was isolated from single-stranded M13 phage containing both orientations of 90 or 500 nucleotides of the 5′ coding region of the *GAL7* gene or of the entire tRNA^{Tyr} gene. The arrows indicate the positions of immobilized DNA (1 μg) corresponding to the sense (solid arrows) or antisense (open arrows) strand of each of the DNAs. The slot blots were hybridized with ^{32}P-labeled RNA transcribed in the absence or presence (+αA) of 100 μg of α-amanitin per ml, as described in Materials and Methods.

The *GAL7* RNA synthesized in the isolated nuclei was also transcribed strand specifically (Fig. 2). Labeled nuclear RNA was hybridized to single-stranded DNAs corresponding to the sense or antisense strand of the first 500 or first 90 nucleotides of *GAL7* mRNA. Based on the hybridization signal from the 500-nucleotide probe, we concluded that the newly synthesized RNA hybridized to the sense DNA, indicating transcription from the correct strand in the isolated nuclei. Although hybridization was observed to the

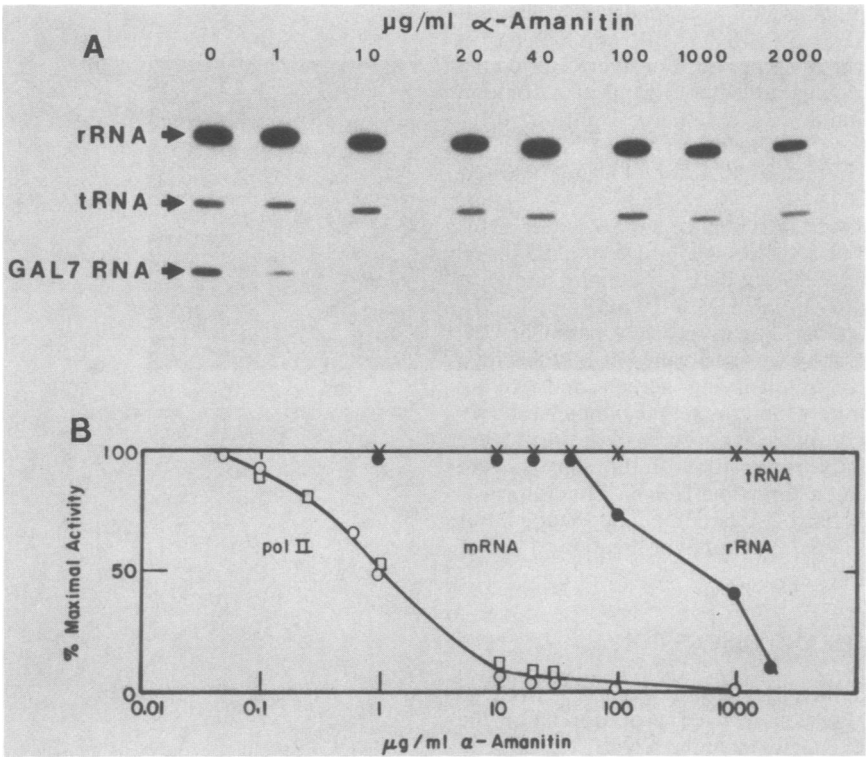

FIG. 3. α-Amanitin sensitivity of RNA polymerases in isolated nuclei. (A) Hybridization of labeled transcripts to immobilized DNA. Transcription reactions were performed at different α-amanitin concentrations by using nuclei isolated from strain YJJ106(YEp731). RNA was isolated and hybridized to immobilized double-stranded DNAs as described in Materials and Methods. (B) Percentage inhibition of transcription of nuclear RNA polymerases and of partially purified RNA polymerase II. We obtained several exposures of the slot blot shown in panel A and of a similar experiment in which lower α-amanitin concentrations were used. Densitometer tracings of the autoradiograms were used to determine the intensity of each signal. The tRNA signal, which had previously been shown to be unaffected by α-amanitin at a concentration of 2.4 mg/ml (21), was used as a control for normalizing to correct for slight variations in the total amount of RNA isolated from each experimental point. Symbols: ×, $tRNA^{Tyr}$; ●, rRNA, ○, *GAL7* mRNA. Transcription reactions in which we used partially purified RNA polymerase II (pol II) (□) were performed as described previously (26).

first 90 nucleotides of the sense strand, the signal was weaker, and significant hybridization to the antisense probe was apparent. This may reflect the absence of *GAL7* transcript reinitiation in the isolated nuclei. tRNA transcription, which was monitored as a control, was clearly highly strand specific (Fig. 2).

In addition to being highly active (2- to 40-fold more active than previous reports under optimal conditions [see below], the nuclei isolated by this procedure were very stable when they were stored as described in Materials and Methods (in portions at −20°C, without freezing). The spheroplasts could also be stored for later nuclear isolation, thus facilitating multiple experiments. The activity and yield of nuclei were critically dependent on the spheroplast formation step, as described in Materials and Methods.

α-Amanitin sensitivity of mRNA transcription confirms synthesis by RNA polymerase II. As shown above, synthesis of *GAL7* mRNA was decreased significantly by the addition of 100 μg of α-amanitin per ml (Fig. 1). To titrate the effect of α-amanitin on transcription in isolated nuclei, transcription reactions were performed in the presence of increasing concentrations of α-amanitin. RNA was isolated and was hybridized to DNA encoding rRNA, $tRNA^{Tyr}$, and *GAL7* mRNA (Fig. 3A). As previously described by Shultz (19), synthesis of tRNA was highly resistant to even the highest concentrations of α-amanitin tested (2 mg/ml). The tRNA signal was used to normalize the hybridization to the rRNA and mRNA probes (Fig. 3B). We found that rRNA synthesis was sensitive to high levels of α-amanitin; the signal was decreased to 50% at 500 μg of α-amanitin per ml, in agreement with previously published values for inhibition of purified yeast polymerase I (11, 18). Synthesis of *GAL7* mRNA was sensitive to low levels of α-amanitin (50% inhibition at 1 μg/per ml); in fact, its inhibition curve was exactly superimposable on a curve derived for purified yeast RNA polymerase II (Fig. 3B). These data confirm the synthesis of mRNAs by RNA polymerase II in yeast cells and establish the usefulness of α-amanitin sensitivity as a criterion for successful in vitro reconstitution of transcription.

Optimizing conditions for transcription. To optimize conditions for transcription by the isolated yeast nuclei, ranges of $MgCl_2$ (Fig. 4), KCl, and GTP concentrations and incubation temperatures were tested (data not shown). Under each set of reaction conditions both total GMP incorporation (in the absence and presence of 100 μg of α-amanitin per ml) and incorporation into individual transcripts (rRNA, $tRNA^{Tyr}$, and *GAL7* mRNA) were analyzed as described in Materials and Methods. An example of such an analysis is shown in Fig. 4; in this experiment the KCl concentration was 100 mM, and the $MgCl_2$ concentration was varied from 0.5 to 40 mM. Total incorporation was maximal between 10 and 20 mM $MgCl_2$; higher levels of $MgCl_2$ result in decreased total incorporation, but the percentage of α-amanitin-

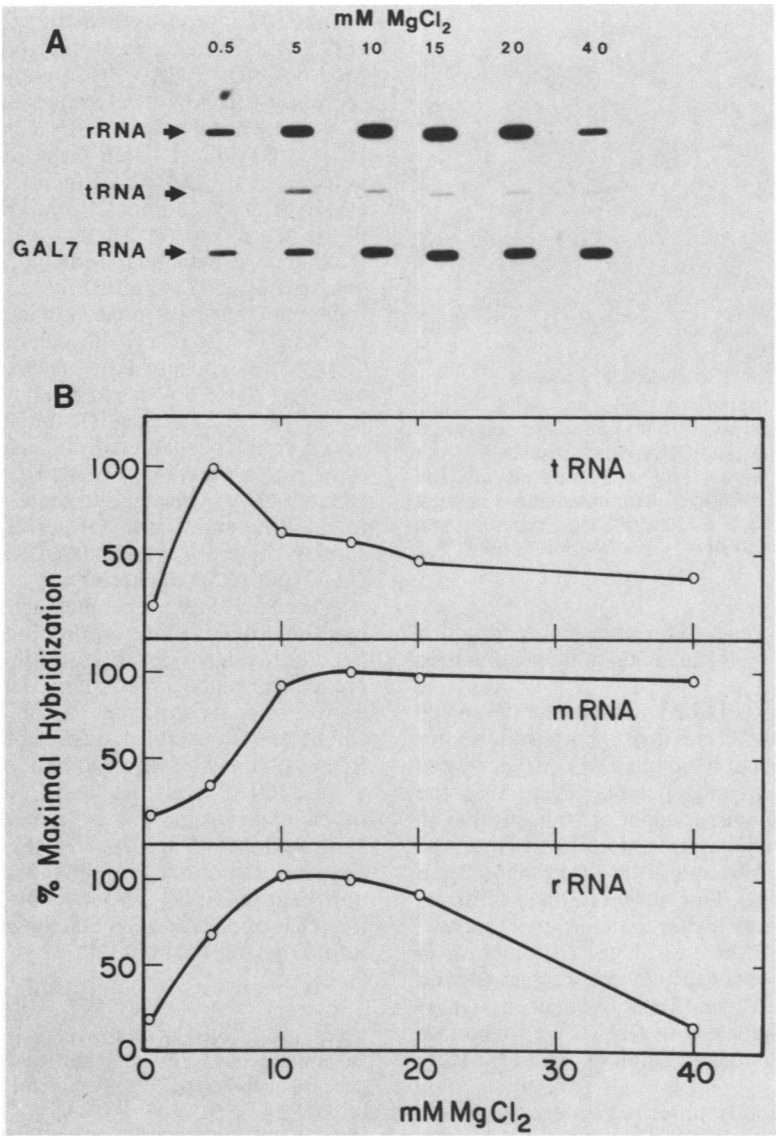

FIG. 4. $MgCl_2$ optima of RNA polymerases I, II, and III in isolated yeast nuclei. (A) Hybridization of labeled transcripts to immobilized DNA. Nuclei were incubated at different $MgCl_2$ concentrations, and the ^{32}P-labeled RNA was hybridized to immobilized DNAs as described in Materials and Methods. (B) Densitometer tracings of several exposures of the slot blots shown in panel A were obtained to determine the relative intensity of each signal. The most intense signal from each DNA was given a value of 100%.

sensitive activity increased (data not shown). When individual transcripts were analyzed by hybridization (Fig. 4A), rRNA synthesis and tRNA synthesis showed relatively sharp optima at 10 and 5 mM $MgCl_2$, respectively (quantitation of the autoradiogram is shown in Fig. 4B). In contrast, *GAL7* mRNA synthesis increased at $MgCl_2$ concentrations between 0.5 and 15 mM and actually remained at its maximal level as the concentration was further increased to 40 mM, the highest level tested.

The effect of KCl was measured by using a relatively high level of $MgCl_2$ (20 mM), which enhanced the mRNA signal. Under these conditions *GAL7* mRNA synthesis displayed a broad optimum at KCl concentrations ranging from 200 to 400 mM. rRNA synthesis peaked at 200 mM KCl, and tRNA synthesis was maximal at KCl concentrations of over 100 mM under these conditions (data not shown). We also tested the effect of varying the concentration of GTP from 12.5 to 400 μM with an $MgCl_2$ concentration of 20 mM and a KCl concentration of 200 mM. Incorporation showed a strong dependence on GTP concentration in this range, with approximately twofold higher incorporation at 400 μM than at 100 μM when the nuclei were incubated at 30°C (see below).

To determine the temperature and length of time needed to obtain maximal transcription, nuclei were incubated at 20, 25, and 30°C in reaction mixtures containing 100 μM [α-^{32}P]GTP, 10 or 20 mM $MgCl_2$, and 100 or 150 mM KCl. At both 25 and 30°C incorporation increased linearly for the first 15 min, with maximum incorporation achieved between 15 and 20 min. The amount of label incorporated remained constant for 45 to 60 min. After 60 min of incubation at both 25 and 30°C, the amount of GMP incorporated consistently decreased; the decrease may reflect degradation of the labeled RNA (see below). When the nuclei were incubated at 20°C, accumulation of ^{32}P-labeled RNA was much slower; after 15 min only 57% as much had accumulated as accumulated after the same length of time at 30°C. At 20°C incor-

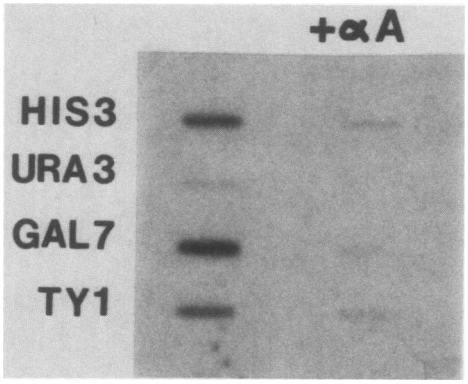

FIG. 5. Detection of transcripts from single- and multiple-copy chromosomal genes. Nuclei isolated from untransformed strain YJJ51 containing 6.0 μg of nuclear DNA were incubated in the absence or presence (+αA) of 100 μg of α-amanitin per ml. The isolated ^{32}P-labeled RNA was hybridized with immobilized double-stranded DNA samples for 40 h as described in Materials and Methods. The autoradiogram shown was exposed for 72 h.

poration continued at a slower rate for at least 1 h, at which time it had reached about 90% of the maximal level obtained at 30°C.

Under optimal conditions (10 to 20 mM $MgCl_2$, 100 to 200 mM KCl, 400 μM GTP, 30°C), maximal incorporation after 15 min of incubation was equal to 80 pmol of GMP per μg of nuclear DNA. This level was significantly higher than the levels obtained in two previous studies of transcription in isolated yeast nuclei, approximately 40-fold higher than the level reported by Schultz (19), and 2- to 4-fold higher than the level reported by Ide (12). Our observed level of incorporation was also considerably higher than the level reported for hen oviduct nuclei by Ernest et al. (8) (20 pmol/μg of DNA in 3 h) and was equivalent to the levels achieved with highly active nuclei isolated from *Drosophila* sp. by Gross and Ringler (10) (75 to 100 pmol/μg of DNA). We found that nuclei isolated from both haploid and diploid yeast cells were transcriptionally active.

The fact that we consistently observed a decrease in the amount of total RNA transcribed when the nuclei were incubated for long periods may indicate that reinitiation did not occur in our system. However, a low level of initiation would have been obscured if degradation was also occurring. Studies with *Drosophila* (10) and hen oviduct (8) nuclei have indicated that bovine serum albumin apparently stabilizes either the transcription machinery or the RNA itself, since increased total RNA accumulation was observed when bovine serum albumin was included in the incubation mixture. In both of these systems bovine serum albumin had no significant effect on the early stages of RNA synthesis, but rather enhanced the long-term stability of the RNA (9) or resulted in increased total synthesis (7). We found that the addition of bovine serum albumin to levels comparable to those used in the studies described above (8, 10) had no affect on apparent degradation (data not shown).

Detection of transcripts from chromosomal genes. After optimal mRNA transcription conditions had been established as described above, we isolated nuclei from an untransformed yeast strain (strain YJJ51) with wild-type, single copy genes for *GAL7* (21), *HIS3* (20), and *URA3* (1) and multiple chromosomal copies of the yeast-transposable element *TY1* (5, 7). The mRNAs from these genes accumulated to levels significantly below the level for the plasmid-borne *GAL7* gene used in the earlier parts of this study. *GAL7* and *TY1* represent approximately 0.5 and 5% of the total poly(A)$^+$ RNA, respectively (7, 21). *HIS3* and *URA3* represent very low-level transcripts [approximately 0.01 and 0.1% of the total poly(A)$^+$ RNA, respectively] (1, 23). To try to detect synthesis from these lower-expression genes, labeled RNA was isolated from nuclear transcription reactions and hybridized to immobilized DNA probes encoding each of the genes (Fig. 5). The DNA fragment used to detect the *HIS3* gene also included sequences homologous to two other yeast genes, *PET56* and *DED1* (22, 23). Hybridization to this fragment reflected predominantly the *DED1* and *HIS3* mRNAs (G. Marczynski, personal communication). Although the exposure times necessary for detecting the signals for these lower-expression genes were necessarily longer (see figure legends), transcripts from all four of the genes could be detected. In each case transcription was sensitive to α-amanitin (Fig. 5). It is interesting that more labeled RNA hybridized to the *GAL7* and *HIS3* DNAs than to the *TY1* and *URA3* DNAs. This may reflect a greater stability of the *TY1* and *URA3* RNAs compared with the *GAL7* and *HIS3* transcripts.

Although it has not been established yet whether accurate reinitiation of mRNA synthesis occurs in yeast nuclei isolated as we have described in this report, we feel that the procedure will be an important addition to the study of yeast transcriptional regulation. Runoff synthesis in isolated nuclei can be used to establish transcription by a particular class of RNA polymerase; in combination with measurements of in vivo mRNA levels, such as Northern analysis, nuclear runoff experiments can be used to determine the relative contributions of initiation rates and stability to mRNA abundance and to determine the mechanism of activation by upstream promoter elements (24). They may also be useful for other applications, including studies of chromatin structure, DNA replication, and RNA processing and transport.

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