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Synthesis of large rRNAs by RNA polymerase II in mutants of *Saccharomyces cerevisiae* defective in RNA polymerase I

(*GAL7* promoter/glucose repression/rRNA processing)

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ABSTRACT **The 35S rRNA gene of the yeast *Saccharomyces cerevisiae* was fused to the *GAL7* promoter. This hybrid gene, when present on a multicopy plasmid and induced by galactose, suppressed the growth defects of a temperature-sensitive RNA polymerase I (pol I) mutant and those of a mutant in which the gene for the second largest subunit of pol I was deleted. Analysis of pulse-labeled RNA directly demonstrated that rRNA synthesis in this deletion mutant is from the *GAL7* promoter. These experiments show that the sole essential function of pol I is the transcription of the rRNA genes, that pol I is not absolutely required for the synthesis of rRNA and ribosomes or cell growth if 35S rRNA synthesis is achieved by some other means, and that the tandemly repeated structure of the chromosomal rRNA genes is also not absolutely required for the synthesis of rRNA and ribosomes.**

In eukaryotic organisms, there are three distinct RNA polymerases, RNA polymerase I, II, and III (pol I, II, and III) responsible for transcription of nuclear-encoded genes (for review, see ref. 1); pol I transcribes tandemly repeated large rRNA genes. For example, in the yeast *Saccharomyces cerevisiae* the tandemly repeated rRNA genes are transcribed by pol I to produce an initial transcript, 35S rRNA, which undergoes a series of posttranscriptional processing steps, yielding the 18S, 5.8S, and 25S rRNAs found in ribosomes (for reviews, see refs. 2 and 3). In this article we describe experiments relevant to two questions about pol I. (*i*) The tandemly repeated rRNA gene arrangement is observed in almost all eukaryotic organisms, but its significance has not been clearly elucidated. Although formation of a mininucleolus structure from a single rDNA unit was recently shown in *Drosophila melanogaster* (4), whether the tandemly repeated arrangement of rRNA genes that constitutes the nucleolus organizer is essential for rRNA synthesis, processing, and ribosome assembly has not been completely answered. (*ii*) Is the essential function of pol I solely transcription of rRNA genes, as has been generally assumed without actual experimental evidence? We have now constructed a system in which a single unit of the 35S rRNA gene is fused to the *GAL7* promoter and is transcribed by pol II under control of the *GAL* regulatory system. Yeast strains carrying defective pol I could grow when the 35S rRNA gene was transcribed by pol II in the presence of galactose. These results demonstrate that the sole essential function of pol I is, in fact, transcription of the rRNA genes and that transcription of tandemly repeated chromosomal rRNA genes is not required for cell growth if rRNA synthesis is achieved by some other means.

MATERIALS AND METHODS

Media, Plasmids, and Strains. YEP-glucose medium is 1% yeast extract/2% Bacto peptone (Difco)/2% glucose. YEP-galactose medium is the same, except that 2% galactose is substituted for glucose. Synthetic galactose medium (SGal) is 2% galactose/0.67% Bacto-yeast nitrogen base (Difco), which, when indicated, was supplemented with amino acids or bases, as described by Sherman *et al.* (5). Concentration of Casamino acids used as a supplement was 0.5%. Synthetic glucose medium is the same as SGal, except that 2% glucose is substituted for galactose. For making solid medium, 2% agar was added. Yeast strains and plasmids used are listed in Table 1 and Fig. 1. Transformation of yeast cells was done by using the lithium acetate method (13). Other methods used for plasmid construction were as described by Maniatis *et al.* (14).

Table 1. Yeast strains and plasmids used

Designation	Genotypes and comments
Strain	
NOY401*	*MATa rpa190-3 ura3 leu2 trp1 can1*
NOY398	*MATa/MATα ade2-1/ade2-1 ura3-1/ura3-1 his3-11/his3-11 trp1-1/trp1-1 leu2-3,112/ leu2-3,112 can1-100/can1-100 rpa135::LEU2/ RPA135* (ref. 6)
NOY408	NOY398 pNOY102
NOY408-1a†	*MATα rpa135::LEU2 ade2-1 ura3-1 his3-11*
(also -1d)	*trp1-1 leu2-3,112 can1-100* pNOY102
NOY408-1b†	*MATa ade2-1 ura3-1 his3-11 trp1-1*
(also -1c)	*leu2-3,112 can1-100* pNOY102
W303-1a	*MATa ade2-1 ura3-1 his3-11 trp1-1 leu2-3,112 can1-100* (ref. 7)
NOY409	W303-1a pNOY102
Plasmid	
pAA7	Galactose-inducible expression vector, 2μ, *URA3* (ref. 8; see Fig. 1)
pC1/1	Ultra-high copy vector, 2μ, *leu2-d* (ref. 9)
pNOY100	pC1/1 carrying *GAL7*–35SrDNA (see Fig. 1)
pNOY102	pAA7 carrying *GAL7*–35SrDNA (see Fig. 1)

*NOY401 was constructed by crossing NOY265 (10) and W303-1a (7) and then screening meitotic segregants for a temperature-sensitive (*rpa190-3*) and strongly galactose-positive phenotype.
†NOY408-1a, -1b, -1c, and -1d are four haploid segregants derived from a single ascospore produced by diploid strain NOY408 on galactose plates.

Both pNOY100 and pNOY102 carry 35S rDNA from +1 to +6922 (transcription start site being +1), which includes all rRNA-coding regions and the enhancer element (+6734 to +6922; ref. 15) located distal to the 25S rRNA-coding region (see Fig. 1). The fusion of the *GAL7* promoter to the rRNA-encoding DNA is at one of the *GAL7* transcription start sites (cytidine at +2) followed by CAGATCC derived from linker sequences, and thus the sequence at the fusion site is 5′ - - - -(−10)GATAAAAAAAA$\overset{-1}{A}\overset{*}{A}\overset{*}{C}$*CAGATCC*$\overset{+1}{A}$TGC-GAAAGC(+10)- - - 3′ (the *GAL7* start site being either the

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Abbreviations: SGal, synthetic galactose medium; pol I, II, and III, RNA polymerase I, II, and III, respectively.

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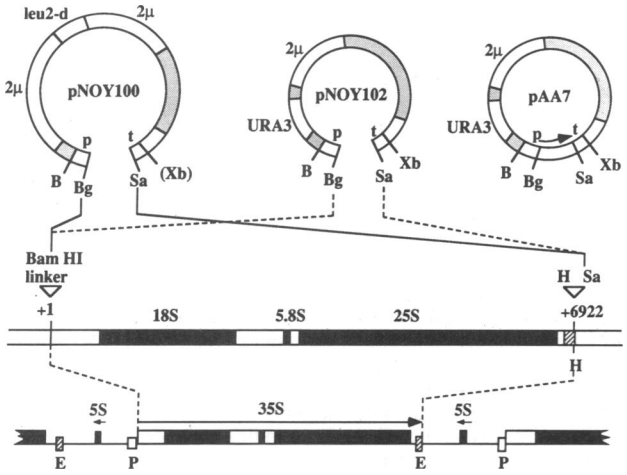

FIG. 1. Structures of plasmids pNOY100 and pNOY102, which carry the *GAL7*–35SrDNA hybrid gene. The structure of pAA7 (8), from which the *GAL7* promoter (p) and the *GAL7* terminator (t) were derived, is also shown. At bottom, the structure of tandemly repeating rRNA genes is shown. The 18S, 5.8S, and 25S rRNA-coding regions are shown as black boxes, as is the 5S RNA-coding region transcribed by pol III. Boxes designated as P and E are the pol I promoter and enhancer elements, respectively. Regions derived from the bacterial plasmid (pBR322) are stippled. Nucleotide sequence numbers +1 and +6922 are given to indicate the transcription start site and the *Hin*dIII site (H) that defines the distal end of the enhancer element, respectively. B, *Bam*HI; Bg, *Bgl* II; Sa, *Sal* I; and Xb, *Xba* I. The 35S rDNA portion (without the promoter) was derived from plasmid prib105 (11). The *Bam*HI-sensitive sequence (GGATCC) was inserted between −1 and +1 of the rDNA sequence by site-directed mutagenesis using the oligonucleotide (36-mer) 5′-CAACTGCTTTCGCAT*GGATC*-*C*GAAGTACCTCCCAAC-3′ and was used to fuse the 35S rDNA to the *GAL7* promoter (the linker sequence is indicated by a triangle). The structure of pNOY102 is the same as pAA7, except that the small *Bgl* II–*Sal* I fragment containing the *GAL7* protein-coding region was replaced with the 35S rDNA [from +1 to +6922 (derived from prib105) together with 15 nucleotides derived from the multiple cloning site of pRS306 (12) used during plasmid construction; the presence of the extra nucleotides is indicated by a triangle at the end of rDNA in the figure]. pNOY100 carries the same *GAL7*–35SrDNA fusion, except that the vector portion was derived from the "ultra-high-copy-number" vector plasmid pC1/1 (9), which carries the *leu2-d* allele as a selectable marker to increase copy number. In addition, the *Xba* I site present in pAA7 was abolished by the insertion of four nucleotides during construction [indicated as (Xb)].

adenosine or cytidine indicated with asterisks), the rDNA segment start at adenosine is indicated as +1, and the linker sequence is indicated by italics (see refs. 16 and 17).

Immunoblot Analysis. Cell extracts were prepared and subjected to SDS/PAGE and then transferred to a nitrocellulose membrane. The membrane was then treated with a mixture of anti-A135 antibodies and anti-A190 antibodies, and bound antibody was visualized by using an alkaline phosphatase-linked detection system (Bio-Rad immunoassay kit), as described in our previous studies (6, 18).

Analysis of RNA Labeled *in Vivo*. [^{3}H]Uridine (174 mCi/mg, 100 μCi/ml; 1 Ci = 37 GBq) was added to 8 ml of yeast cultures at cell density (OD_{600}) of 0.2–0.3 (see legend to Fig. 5). After 30 min at 30°C, the labeling was stopped by immersing the culture flasks into a dry-ice bath. Frozen cells were subsequently thawed, collected by centrifugation, and washed with cold water; RNA was then isolated as described by Elder *et al.* (19). Aliquots of radioactive RNA preparations were analyzed by electrophoresis on a 2% polyacrylamide/0.5% agarose gel (20).

RESULTS

The Temperature-Sensitive *rpa190-3* Mutant Can Grow at Nonpermissive Temperatures When the 35S rRNA Gene Is Expressed from the *GAL7* Promoter. We first used a strain of *S. cerevisiae* carrying a temperature-sensitive mutation, *rpa190-3* (10) in the gene (*RPA190*) coding for the largest subunit (A190) of pol I to examine whether functional expression of the 35S rRNA gene can be achieved from a pol II promoter, specifically from the *GAL7* promoter. Fig. 1 shows that the promoter and upstream DNA elements for the 35S rRNA gene were deleted and replaced by the *GAL7* promoter. The rRNA-encoding DNA fused to the *GAL7* promoter (called *GAL7*–35SrDNA) includes an enhancer element (15), one or more probable transcription-termination sites (21), and a processing site for an endonuclease, which in conjunction with an exonucleolytic process generates the mature 3′ terminus of 25S rRNA (21, 22). To the distal end of the 35S rRNA gene we placed a distal segment of the *GAL7* gene carrying the terminator and/or a processing site for poly(A) addition (23, 24). Of the two plasmids constructed that carry *GAL7*–35SrDNA, pNOY100 carried *leu2-d* as a selectable marker to increase plasmid copy number (9). Because transcription of rRNA genes makes up a substantial fraction of total transcription (see ref. 2), we initially intended to increase plasmid copy numbers by using the *leu2-d* allele as a selective marker in medium lacking leucine to achieve enough 35S rRNA for cell growth.

Plasmids pNOY100 and pC1/1, a control plasmid carrying *leu2-d* but not *GAL7*–35SrDNA, were individually introduced into NOY401, a strain carrying a temperature-sensitive mutation (*rpa190-3*) in the gene for the A190 subunit of pol I. We found that both resultant strains grew better on glucose than on galactose at 23°C, as expected for normal yeast strains. At 37°C, only the strain carrying pNOY100 formed colonies on galactose plates, but this strain failed to form colonies on glucose plates. The strain carrying pC1/1 did not grow on glucose or galactose plates at 37°C. The galactose-dependent growth of the strain carrying pNOY100 at 37°C was confirmed in liquid cultures (data not shown). The results demonstrate that the 35S rRNA gene is transcribed from the *GAL7* promoter by pol II and that the strain carrying the *GAL7*–35SrDNA hybrid gene can grow without functional pol I.

Suppression of *rpa135* Deletion by *GAL7*–35SrDNA Hybrid Gene. We next examined whether yeast strains carrying *GAL7*–35SrDNA can grow in the complete absence of intact pol I. For this purpose, we used a deletion of the gene (*RPA135*; ref. 6) for the second largest subunit (A135) of *S. cerevisiae* pol I. In a diploid strain, NOY398, one of the two copies of *RPA135* was disrupted by replacing ≈85% of the *RPA135*-coding region with the *LEU2* gene (Table 1 and Fig. 2*B*; see ref. 6). Plasmid pNOY102 was introduced into this diploid, and after sporulation, tetrads were dissected, and haploid segregants were analyzed. The pNOY102 plasmid carried the same *GAL7*–35SrDNA hybrid gene as pNOY100, but instead of the *leu2-d* allele, pNOY102 had *URA3* as a selectable marker. On galactose plates, most tetrads yielded two large colonies and two (or one) small colonies. Upon restreaking these colonies on glucose and galactose plates, we found that the large colonies (segregants b and c in Fig. 3*A*) grew normally both on galactose and glucose plates but that the small colonies (segregants a and d in Fig. 3*A*) grew only on galactose plates and failed to form colonies on glucose plates (Fig. 3*A*). Analysis of leucine requirements as well as direct Southern blot analysis of DNA (Fig. 2*C*) demonstrated that the former segregants (NOY408-1b and -1c) had the intact *RPA135* gene but the latter (NOY408-1a and -1d) had only the *rpa135*::*LEU2* deletion allele and no intact *RPA135*. Dependency of growth of the latter strains on galactose was also confirmed in liquid cultures. The strains grew in SGal supplemented with adenine, tryptophan, and Casamino acids with a doubling time of 5–6 hr at 30°C, but after shift to medium containing glucose instead of galactose,

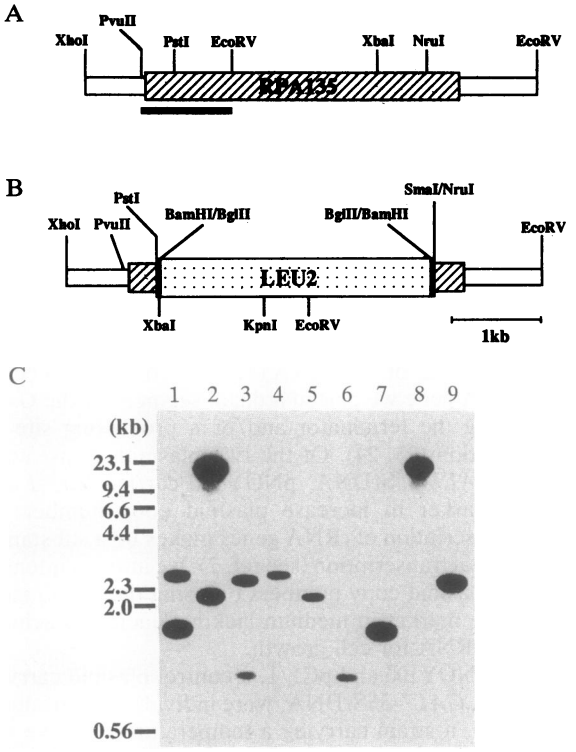

FIG. 2. Southern blot analysis of genomic DNAs prepared from NOY398, NOY408-1a, and NOY408-1b with a *RPA135* probe. Restriction enzyme maps of a cloned DNA fragment containing the intact *RPA135* gene (*A*) and the fragment carrying *rpa135*::*LEU2* used for disruption of the chromosomal *RPA135* (*B*) are indicated (cf. ref. 6). For the autoradiogram of *C*, chromosomal DNAs prepared from NOY398 (lanes 1–3), NOY408-1a (lanes 4–6), and NOY408-1b (lanes 7–9) were digested with *Xho* I and *Eco*RV (lanes 1, 4, and 7), with *Xho* I and *Kpn* I (lanes 2, 5, and 8), or with *Xho* I and *Xba* I (lanes 3, 6, and 9), and the digests were electrophoresed in a 1% agarose gel and probed with the ^{32}P-labeled 1-kilobase (kb) *Pvu* II–*Eco*RV fragment (indicated as a block bar in *A*). Sizes and positions of the DNA markers are shown at left.

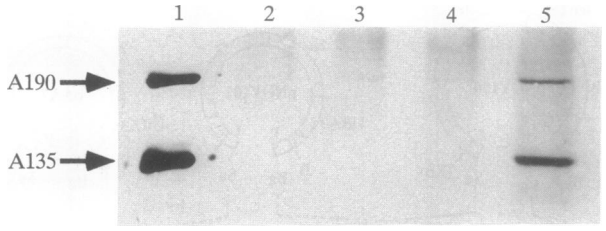

FIG. 4. Immunoblot analysis of A135 and A190 subunits. Samples were prepared from NOY408-1a (lanes 2–4) and NOY408-1b (lane 5) growing exponentially at 30°C in SGal medium supplemented with adenine, tryptophan, and Casamino acids. Samples containing 60 μg (lanes 4 and 5), 120 μg (lane 3), and 180 μg (lane 2) of protein were analyzed. A mixture of anti-A135 and anti-A190 antibodies was used to detect A135 and A190 subunits. Purified RNA pol I was also analyzed as a reference (lane 1) to indicate positions of A135 and A190 subunits. The alkaline phosphatase-stained filter was then photographed.

the growth rate gradually declined, and the cells eventually ceased growth (Fig. 3*B*).

Immunoblot analysis confirmed the absence of intact pol I in NOY408-1a growing in galactose medium. As expected, no evidence for the presence of A135 subunit was obtained by using anti-A135 antibodies (Fig. 4). In addition, analysis by using anti-A190 antibodies showed that the amount of the largest subunit, A190, in NOY408-1a was greatly reduced relative to that in the control strain (Fig. 4; in other experiments not shown, the amount of A190 subunit in NOY408-1a was estimated to be ≈10% or less of control). We presume that in the absence of A135 subunit synthesis the A190 polypeptide is unstable and largely degraded in these strains carrying the *rpa135* deletion, although the possibility cannot be excluded that *RPA190* and *RPA135* are coregulated post-transcriptionally. We conclude that these yeast strains (such as NOY408-1a) can grow in the absence of physically intact pol I, provided that the expression of the 35S rRNA gene from the *GAL7* promoter is induced by galactose.

We note that control strain NOY408-1b grew in SGal medium (supplemented with adenine, tryptophan, and Casamino acids) with a doubling time of 2–3 hr compared with 5–6 hr for NOY408-1a. Obviously, transcription of ≈150 tandemly repeated 35S rRNA genes on the chromosome by intact pol I must be more efficient for making ribosomes than transcription of the *GAL7*–35SrDNA hybrid gene on the pNOY102 plasmid by pol II. Because this plasmid was a multicopy plasmid with the 2-μm replication origin but did not have the *leu2-d* allele, we were curious about its copy number in the strains lacking intact pol I. We prepared DNA from these strains and measured approximate copy numbers by Southern analysis using a probe containing *URA3*. In the

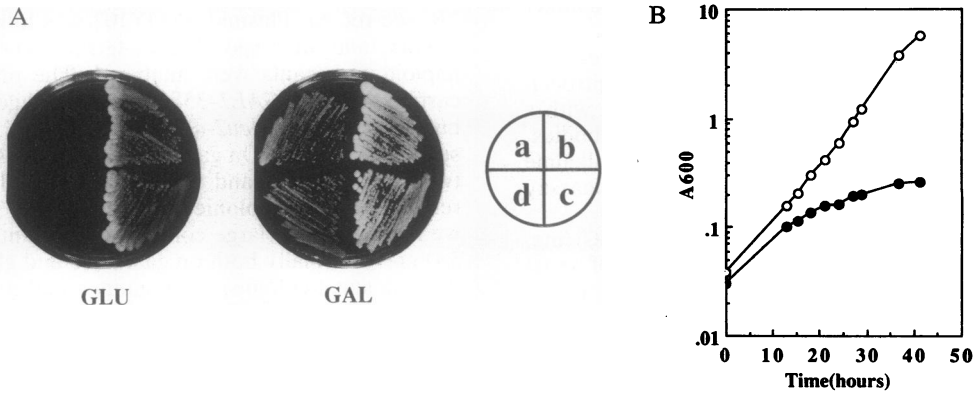

FIG. 3. (*A*) Growth of four haploid segregants derived from an ascospore produced by NOY408 on YEP-galactose and YEP-glucose plates. The four haploid segregants, NOY408-1a, -1b, -1c, and -1d (see Table 1) recovered originally from a YEP-galactose plate were restreaked on YEP-galactose and YEP-glucose plates and incubated for 10 days at 23°C. Positions of the strains are indicated at right. (*B*) Growth of NOY408-1a in galactose and glucose liquid media. Strain NOY408-1a was first grown to a mid-logarithmic phase in SGal medium supplemented with adenine, tryptophan, and Casamino acids at 30°C. Portions of the culture were taken and diluted by the same galactose medium (○) or synthetic glucose medium (●) with the same supplements to give a cell density (OD_{600}) of ≈0.04 (time 0). Incubation was continued at 30°C, and cell growth was measured by optical density changes at 600 nm.

strains with intact pol I (NOY409 and NOY408-1b) pNOY102 was found to be present in 5–10 copies. However, in the strain without intact pol I (NOY408-1a) the plasmid was present in ≈20–50 copies (data not shown). Apparently, although we did not make any specific efforts to select fast-growing colonies, growth on galactose without intact pol I must have selected a population of cells with increased plasmid copy numbers.

Direct Demonstration of rRNA Transcription from the *GAL7* Promoter in the Absence of Intact pol I. Expression of *GAL7* is known to be repressed by glucose, as is expression of *GAL1* or *GAL10* (16, 25). Thus, synthesis of rRNA in NOY408-1a is expected to be subject to glucose repression, in marked contrast to that in normal yeast strains. The synthesis of rRNA in normal yeast cells is known to be stimulated by glucose as a response to nutritional upshift (2). Results of [^{3}H]uridine pulse-labeling experiments (Fig. 5) confirm this expectation; the synthesis of large rRNA (18S, 25S, and 5.8S rRNAs and other precursor rRNAs) was largely repressed by glucose, but the synthesis of 5S RNA and tRNAs was not repressed. The degree of repression of rRNA synthesis relative to 5S RNA synthesis was ≈12-fold by using RNA–DNA filter hybridization (data not shown). These results confirm the conclusion that transcription of the 35S rRNA gene is by pol II from the *GAL7* promoter in this system. In addition, comparison of the radioactive rRNA species synthesized in this NOY408-1a strain (without glucose; lanes 4–6 in Fig. 5) with a control strain (NOY408-1b) without the *rpa135* deletion (lane 7 in Fig. 5) indicate that normal rRNA precursor molecules, such as 27S rRNA and 20S rRNA, were present in the samples from NOY408-1a, and, therefore, primary transcripts synthesized from the *GAL7* promoter are processed in a manner similar, if not identical, to that used for the normal precursors transcribed by pol I. This conclusion was also supported by preliminary experiments in which Northern (RNA) blot analysis with a "precursor-specific" probe detected precursor rRNAs, which exhibited a very similar pattern (data not shown).

It should be noted that in the experiment of Fig. 5, the sample which was pulse-labeled at 30 min after glucose addition showed nearly maximal repression; in contrast, the growth rate declined only very gradually (see Fig. 3*B*). Thus, glucose represses rRNA synthesis in this system quickly, but cells continue to grow for some time by using ribosomes that were synthesized before repression by glucose.

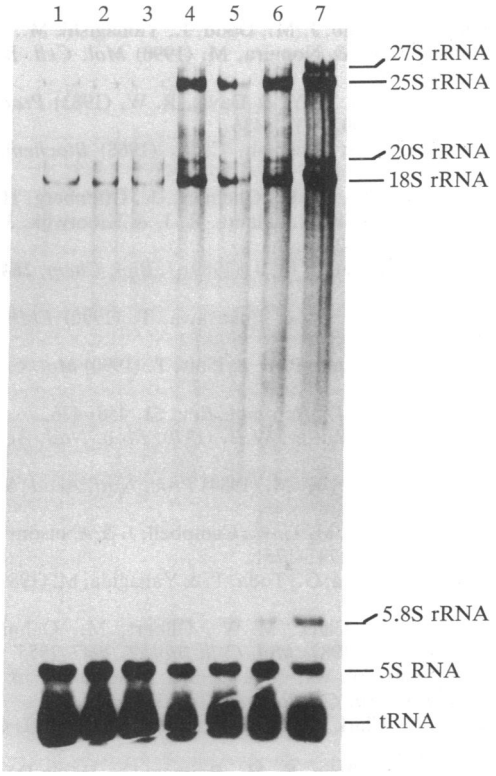

FIG. 5. Polyacrylamide-agarose gel electrophoresis of RNA synthesized in strain NOY408-1a growing in galactose medium with (lanes 1–3) and without (lanes 4–6) further glucose addition. Strain NOY408-1a was grown at 30°C in SGal medium supplemented with adenine, tryptophan, and Casamino acids. At a cell density (OD_{600}) of 0.21, the culture was divided into two parts: glucose (final concentration, 2%) was added to one part (lanes 1–3), and the other served as a control (lanes 4–6). At time 0.5 hr (lanes 1 and 4), 1 hr (lanes 2 and 5), and 2 hr (lanes 3 and 6) after glucose addition, portions of the cultures were withdrawn, and cells were pulse-labeled with [^{3}H]uridine for 30 min. Strain NOY408-1b, which carries intact pol I, was also grown in the same supplemented SGal medium, and pulse-labeling occurred at a cell density (OD_{600}) of 0.22 (lane 7). RNA was isolated from each culture, and samples containing 7×10^4 cpm were analyzed by electrophoresis on a 2% polyacrylamide-0.5% agarose composite gel. An autoradiogram of the dried gel is shown. Autoradiograms obtained after longer gel exposures clearly indicated radioactive RNA bands in lanes 1–6 that corresponded to the 27S and 20S precursor rRNA bands seen in the control cell (lane 7); similarly, 5.8S bands were also detected in all lanes after longer exposures.

DISCUSSION

It has been assumed, but not yet proven, that pol I transcribes only the 35S rRNA genes. Our results show that pol I is dispensable for *S. cerevisiae* as long as rRNA genes are fused to a suitable pol II promoter. We conclude that there are no essential genes transcribed by pol I other than 35S rRNA genes. However, these results do not exclude the possibility that there are some other unidentified genes transcribed by pol I, but such genes, if they do exist, are not essential for mitotic growth.

A corollary of our demonstration that *S. cerevisiae* without intact pol I can grow by using rRNA synthesized from the *GAL7*–35SrDNA hybrid gene is that the tandemly repeated structure of chromosomal rRNA genes is not required for synthesis of rRNA and ribosomes. This conclusion supports and extends the conclusion made by Karpen and coworkers (4) that a single rRNA-encoding DNA unit is sufficient to form a mininucleolus in polytene nuclei in *D. melanogaster* and is functional as judged by partial rescue of bobbed phenotypes caused by deficiencies of endogenous rRNA-encoding DNA. Note, though, that transcriptional activity was monitored in polytene nuclei in their work and, as discussed by these workers, high copy number due to polytenization may have facilitated expression (as well as formation of micronucleolus) by increasing the local concentration of nucleolar components, as does tandem repetition of rRNA genes in the native nucleolus organizer. In addition, transcription of the native tandemly repeated rRNA genes was still allowed in these experiments. In the experiments described here, transcription of the tandemly repeated chromosomal genes should be completely absent, and the morphologically defined yeast nucleolus is also absent as described below.

pol I is known to be localized in the nucleolus (26, 27). This localization has also been demonstrated for the yeasts *S. cerevisiae* (28) and *Schizosaccharomyces pombe* (29). From analysis of a temperature-sensitive mutant (*nuc1*) of *S. pombe* with a mutational alteration in the largest subunit of pol I, Yanagida and coworkers (29) suggested that intact pol I is required for maintenance of proper structure of the nucleolus, which is, in turn, apparently required for maintenance of normal morphology of the nucleus. We have examined the structures of the nucleolus as well as the nucleus in strains growing in the absence of physically intact pol I

(NOY408-1a in galactose media) by immunofluorescence microscopy with suitable nucleolus-specific probes, such as antibodies against SSB1 protein (a yeast nucleolar RNA-binding protein; see ref. 30). We found that the crescent structure revealed in control strains, defined as the nucleolus in this organism (28, 31, 32), does not exist in these mutant strains; instead, several small granules stained by these probes were seen (M. Oakes, Y.N., R.Y., and M.N., unpublished observations). Thus, pol I, in conjunction with the tandemly repeated rRNA genes, appears to play an important structural role in maintaining the normal structure of the nucleolus in *S. cerevisiae*, as was proposed for other organisms, such as *S. pombe* (29) and cultured mammalian cells (33). Because rRNA transcription, processing, and ribosome assembly all occur in the nucleolus, such a structural role of pol I suggested from these observations might be important for the efficient execution of these processes. This explanation is possibly reflected in the reduced rRNA synthesis rates as well as the reduced growth rates of the yeast strains lacking intact pol I but carrying the *GAL7*–35SrDNA hybrid gene, as observed in the present study. Nevertheless, the yeast strains without pol I transcribe and process rRNAs, assemble ribosomes, and do grow. Thus, pol I and the intact crescent structure defined as the *S. cerevisiae* nucleolus are not absolutely required for rRNA processing, ribosome assembly, or cellular growth.

Finally, we note that the present system will be useful for approaching a variety of problems in ribosome research. For example, mutational alterations can be easily introduced into rRNA genes on the *GAL7*–35SrDNA hybrid plasmids and their effects on rRNA processing, ribosome assembly, and/or ribosome function can be directly studied in the absence of transcription of chromosomal rRNA genes by pol I. The present system may also be useful for screening conditionally lethal mutations that affect specifically the transcription of 35S rRNA by pol I.

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