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C25, an Essential RNA Polymerase III Subunit Related to the RNA Polymerase II Subunit RPB7

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We identified a partially sequenced *Saccharomyces cerevisiae* gene which encodes a protein related to the *S. cerevisiae* RNA polymerase II subunit, RPB7. Several lines of evidence suggest that this related gene, *YKL1*, encodes the RNA polymerase III subunit C25. C25, like RPB7, is present in submolar ratios, easily dissociates from the enzyme, is essential for cell growth and viability, but is not required in certain transcription assays in vitro. *YKL1* has ABF-1 and PAC upstream sequences often present in RNA polymerase subunit genes. The sodium dodecyl sulfate-polyacrylamide gel electrophoresis mobility of the YKL1 gene product is equivalent to that of the RNA polymerase III subunit C25. Finally, a C25 conditional mutant grown at the nonpermissive temperature synthesizes tRNA at reduced rates relative to 5.8S rRNA, a hallmark of all characterized RNA polymerase III mutants.

Eukaryotic RNA polymerases I, II, and III are multisubunit enzymes responsible for the synthesis of rRNA, pre-mRNA, and small stable RNAs, including 5S and tRNA, respectively. While each class of RNA polymerase transcribes a nonoverlapping set of nuclear genes, all three enzymes mediate similar functions. In general, each enzyme recognizes transcription factors that facilitate specific promoter recognition, binds template DNA and nucleoside triphosphate substrates, catalyzes the template-dependent synthesis of RNA, and terminates RNA synthesis at or near specific sites.

In the yeast *Saccharomyces cerevisiae*, each nuclear RNA polymerase has 12 to 16 subunits (31). Nearly all of these subunits have been isolated and characterized, including 13 of the 14 RNA polymerase I subunits (8, 18, 22, 25, 31, 32, 35, 38, 40), all 12 RNA polymerase II subunits (2, 4, 16, 20, 30, 32, 34–39, 41), and 13 of the 16 RNA polymerase III subunits (2, 5, 8, 14, 18, 19, 23, 29, 31, 32). The common functions of the three RNA polymerases result from similarities among individual subunits. The two largest subunits of the three enzymes are related to one another and are counterparts of the bacterial β and β′ subunits. In addition, two smaller subunits are related in size and sequence in all three enzymes (RPB3 to the RNA polymerase I and III subunit AC40, and RPB11 to the RNA polymerase I and III subunit AC19). Finally, five subunits are identical in *S. cerevisiae* RNA polymerases I, II, and III.

The 16 RNA polymerase III subunits are designated C160, C128, C82, C53, AC40, C37, C34, C31, ABC27, C25, ABC23, AC19, ABC14.5, C11, ABC10α, and ABC10β according to enzyme class and apparent molecular weight (31). ABC27, ABC23, ABC14.5, ABC10α, and ABC10β are assembled into all three RNA polymerases, AC40 and AC19 are assembled in both RNA polymerase I and III, and the remaining subunits are only represented in RNA polymerase III. We have identified the gene which appears to encode the RNA polymerase III subunit C25. This subunit is similar to the RNA polymerase II RPB7 subunit in sequence and function, providing another example of the parallel roles of subunits in the three classes of eukaryotic nuclear RNA polymerases.

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MATERIALS AND METHODS

Strains. The yeast strains used are described in Table 1.

Plasmids. pRP714 has an approximately 6-kb RPB7-containing *Nsi*I insert in plasmid pGEM9Zf(+). pRP719 has an approximately 10-kb *YKL1*-containing insert in YEp24. pRP729 has a *Bgl*II fragment containing the *YKL1* gene and ~450 bp of 5′ DNA and ~400 bp of 3′ DNA ligated to the *Bam*HI site of the *LEU2* high-copy-number plasmid YEplac181 (11). pRP720 has a PCR-generated fragment containing the *YKL1* open reading frame plus ~215 bp of 5′ DNA with *Bam*HI ends and ~100 bp of 3′ DNA with *Sal*I ends ligated to the *Bam*HI-*Sal*I sites of YEplac181. pRP721 and pRP728 contain the *RPB7* open reading frame plus ~700 bp of 5′ DNA with *Bam*HI ends and ~300 bp of 3′ DNA with *Sal*I ends ligated to the *Bam*HI-*Sal*I sites of YEplac181 and YCplac33 (11), respectively. Deletion of DNA encoding the conserved 20 amino acids of C25 and RPB7 was performed by the method of Ho et al. (13), using linearized pRP719 (C25) and pRP714 (RPB7) as templates. The PCR products for the *RPC25* and *RPB7* genes had *Bam*HI 5′ ends and *Sal*I 3′ ends and were ligated to the *Bam*HI-*Sal*I sites of YEplac181 to yield plasmids pRP724 and pRP725, respectively. The mutant *RPC25* gene in pRP724 has ~215 bp of *RPC25* 5′ DNA and ~100 bp of *RPC25* 3′ DNA. The mutant *RPB7* gene in pRP725 has ~700 bp of *RPB7* 5′ DNA and ~300 bp of *RPB7* 3′ DNA. The other plasmids used in this study are described below.

Isolation of the complete *YKL1* gene. Using primers within the published *YKL1* open reading frame, we screened an *S. cerevisiae* YEp24 genomic plasmid library (prepared in the D. Botstein laboratory and kindly provided by the G. Fink laboratory) by PCR to isolate plasmid pRP719, containing the complete *YKL1* promoter sequences and open reading frame in an ~10-kb insert.

Construction of the *RPC25* deletion. *RPC25/RPC25Δ1::HIS3* yeast cells were selected on His⁻ plates following the transformation of the yeast diploid WY-22 with a PCR-generated fragment containing the *HIS3* gene (1.3 kb) in place of most of the *RPC25* open reading frame (48 bp encoding the amino-terminal end of *YKL1* and 30 bp encoding the carboxyl end of

TABLE 1. Yeast strains used in this study

Strain	Genotype
WY-22	*MATa/MATα ura3-52/ura3-52 his3Δ200/his3Δ200 leu2-3/leu2-3 leu2-112/leu2-112 lys2Δ201/lys2Δ201 ade2/ade2*
WY-57	*MATa/MATα ura3-52/ura3-52 his3Δ200/his3Δ200 leu2-3/leu2-3 leu2-112/leu2-112 lys2Δ201/lys2Δ201 ade2/ade2 RPC25/RPC25Δ1::HIS3*
WY-58	*MATa ura3-52 his3Δ200 leu2-3 leu2-112 lys2Δ201 ade2 RPC25Δ1::HIS3* [pRP719]
WY-59	*MATa ura3-52 his3Δ200 leu2-3 leu2-112 lys2Δ201 ade2 RPC25Δ1::HIS3* [pRP719, pRP724]
WY-60	*MATa ura3-52 his3Δ200 leu2-3 leu2-112 lys2Δ201 ade2 RPB7Δ1::LEU2* [pRP728, pRP725]
WY-61	*MATa ura3-52 his3Δ200 leu2-3 leu2-112 lys2Δ201 ade2 RPC25Δ1::HIS3* [pRP719, pRP722]
WY-62	*MATa ura3-52 his3Δ200 leu2-3 leu2-112 lys2Δ201 ade2 RPC25Δ1::HIS3* [pRP719, pRP723]
WY-63	*MATa ura3-52 his3Δ200 leu2-3 leu2-112 lys2Δ201 ade2 RPC25Δ1::HIS3* [pRP722]
WY-64	*MATa ura3-52 his3Δ200 leu2-3 leu2-112 lys2Δ201 ade2 RPC25Δ1::HIS3* [pRP723]
WY-65	*MATa ura3-52 his3Δ200 leu2-3 leu2-112 lys2Δ201 ade2 RPC25Δ1::HIS3* [pRP719, pRP721]

YKL1 remained) flanked by ~300 bp of *RPC25* 5′ DNA and ~150 bp of *RPC25* 3′ DNA. Genomic DNA was prepared from the His^+ transformant WY-57 and subjected to PCR analysis to verify the substitution of one chromosomal copy of *RPC25* with *HIS3*. WY-57 cells were then sporulated and subjected to tetrad analysis.

Mutagenesis of C25. The two temperature-sensitive *rpc25-1* and *rpc25-2* mutants were isolated from a plasmid shuffle screen (3) of PCR-mutagenized DNA. WY-58 cells were transformed by electroporation with a population of randomly mutagenized *RPC25*-containing PCR fragments plus the gapped pRP720 plasmid (cut with *Nde*I-*Nru*I to eliminate most of the *RPC25* open reading frame) as described by Muhlrad et al. (24). His^+ Leu^+ Ura^+ transformants were transferred to plates containing 1 mg of 5-fluoroorotic acid per ml to select against the wild-type *RPC25 URA3* plasmid. Several hundred individual transformants were then tested for growth at the nonpermissive temperature (38°C).

The mutagenized *RPC25* fragment was synthesized by using pRP719 as a template and the same oligonucleotides used to create the pRP720 *RPC25* fragment. The PCR mixture was as recommended by the manufacturer (Perkin-Elmer Cetus) except that $MnCl_2$ was at 0.5 mM, $MgCl_2$ was added to either 4, 4.5, 5, 5.5, or 6 mM, dimethyl sulfoxide was added to 10%, and 2-mercaptoethanol was added to 0.1 M. The cycle parameters used were 94°C for 1 min, 55°C for 1 min, and 72°C for 1.5 min for 24 cycles followed by a final cycle at 72°C for 10 min. Plasmid pRP722 contained the *rpc25-1* mutant gene; pRP723 contained the *rpc25-2* mutant gene.

In vivo labeling and RNA isolation. WY-61 and WY-62 were labeled in synthetic complete medium plus 5 μg of uracil per ml with [^{3}H]uracil (53 mCi/mmol; Amersham) and handled as described by Mann et al. (19). The permissive temperature was 30°C, and the nonpermissive temperature was 38°C. The cultures were harvested, and total RNA was isolated by using hot acidic phenol (6). Twenty micrograms of total RNA was loaded per lane and run on a 6% acrylamide–7 M urea denaturing gel. The relative amounts of tRNA, 5S rRNA, and 5.8S rRNA were measured by densitometry of the autoradiograph and by direct measurement of radioactivity in the regions of the gel containing each of the three RNAs.

Bacterial expression of C25. A PCR-generated fragment consisting of the *YKL1* open reading frame with a 5′ *Bam*HI site and a 3′ *Eco*RI site was cloned into the *Bam*HI-*Eco*RI sites of pGEX-2T (Pharmacia) to create pRP726. The control glutathione *S*-transferase (GST) protein was prepared after induction of bacterial cells containing pGEX-2T instead of pRP726.

The majority of GST-C25 fusion protein produced in *Escherichia coli* was in inclusion bodies and insoluble after standard lysis procedures. We scaled up the standard induction procedure suggested by the manufacturer to isolate the small percentage of soluble GST-C25 fusion protein. One liter of bacteria containing pRP726 was grown at 37°C to an optical density at 550 nm of 0.5 to 0.8, isopropyl-β-D-thiogalactopyranoside (IPTG) was added to 1 mM, and the culture grown for an additional 2 h at 37°C. The cells were pelleted, washed with TSE (50 mM Tris [pH 8.0], 1 mM EDTA, 100 mM NaCl) containing 2 mM phenylmethylsulfonyl fluoride, pelleted, and resuspended in TSE containing 2 mM phenylmethylsulfonyl fluoride and 200 μg of lysozyme per ml. The mixture was incubated on ice for 10 to 15 min and sonicated at 75% of the microtip limit with four 20-s pulses, and Triton X-100 was added to a final concentration of 1%. The resulting cell extract was centrifuged for 20 min at 10,000 rpm; the supernatant was removed and incubated with 200 μl of washed glutathione-Sepharose 4B resin at 4°C for 1 h under gentle agitation. To obtain the GST-C25 fusion protein, the GST-C25 bound to glutathione-Sepharose 4B was washed first with 2 ml of TSE containing 1% Triton X-100 and then with two 0.5-ml washes in 50 mM Tris (pH 8.0). The fusion protein was then eluted from the resin upon incubation in 200 μl of 50 mM Tris (pH 8.0) plus 5 mM reduced glutathione (freshly prepared) with gentle agitation at room temperature for 10 to 15 min.

Preparation of the GST-C25 protein to be cleaved with thrombin was the same as described above except after the first wash of the fusion protein bound to resin. Following the wash with 2 ml of TSE plus 1% Triton X-100, the resin with bound protein was washed with a buffer containing 50 mM Tris (pH 8.0), 50 mM NaCl, and 2.5 mM $CaCl_2$ and resuspended in 200 μl of the same buffer. To cleave the C25 from the GST-C25 bound to glutathione-Sepharose 4B, thrombin (Sigma) was added to a final concentration of 0.025 U/μl and incubated at 4°C overnight.

Nucleotide sequence accession number. The GenBank/EMBL accession number for the reported sequence is U11048.

RESULTS

The *YKL1* open reading frame encodes a protein related to the RNA polymerase II subunit RPB7. Database searches revealed that the amino acid sequence encoded by the *S. cerevisiae* partial open reading frame named *YKL1* (1) was significantly similar to the seventh-largest subunit, RPB7, of *S. cerevisiae* RNA polymerase II. We isolated a plasmid containing the complete *YKL1* gene from an *S. cerevisiae* plasmid library. The *YKL1* gene encodes a 212-amino-acid protein with a predicted molecular mass of 24,300 Da. Alignment of RPB7 and YKL1 revealed that 25% of the residues are identical and 50% are conserved overall (Fig. 1). The alignment score of the two proteins is 11 standard deviations from the mean.

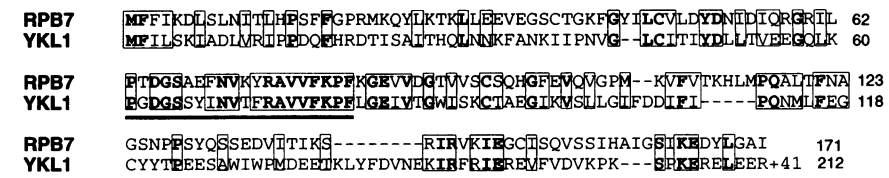

FIG. 1. *YKL1* encodes a protein related to RPB7. Conserved residues are boxed, identical amino acid residues are boxed and in boldface, and the essential conserved 20-amino-acid region is underlined.

Deletion of *YKL1* is lethal and cannot be rescued by *RPB7*. Most RNA polymerase subunits are essential for cell growth and viability. RPB7 is one of the 10 essential subunits of the 12-subunit RNA polymerase II enzyme. To test if the single-copy *YKL1* gene (1) is also essential, we inserted the *HIS3* selectable marker in place of most of the open reading frame. WY-57 diploid cells obtained by this approach had one chromosome with a wild-type RNA polymerase subunit gene and one chromosome with a deletion allele. Tetrad analysis of the sporulation products of these diploid cells revealed that *YKL1* is an essential gene since there were two viable and two inviable spores per tetrad. None of the spores containing the deletion allele produced viable cells (i.e., all viable spores were His^-). Deletion of *YKL1* did not result in defective germination since microscopic observation revealed that His^+ spores germinated but failed to divide after a few generations.

In addition to tetrad analysis, we used complementation to demonstrate that expression of a plasmid containing a wild-type copy of *YKL1* can rescue the lethality caused by the deletion of *YKL1*. To do this, we transformed WY-57 diploid cells with a wild-type *YKL1* plasmid, sporulated the transformants, and isolated a viable haploid spore containing the *YKL1* chromosomal deletion plus the complementing *YKL1* plasmid. This yeast strain, designated WY-58, was used throughout this study in plasmid shuffle experiments.

Using the plasmid shuffle method (3), we also tested if expression of *RPB7* could substitute for YKL1 function. A high-copy-number yeast plasmid containing the *RPB7* gene and marked with *LEU2* was transformed into WY-58 yeast cells. WY-58 cells have a deleted chromosomal copy of *YKL1* replaced with *HIS3* plus a complementing plasmid containing a wild-type copy of *YKL1* on a yeast multicopy plasmid marked with *URA3*. Independent His^+ Leu^+ Ura^+ transformants were subsequently placed on medium containing 5-fluoroorotic acid to prevent growth of $URA3^+$ cells. Selection for the loss of the wild-type *YKL1* Ura^+ plasmid revealed that overexpression of *RPB7* did not support growth and therefore cannot compensate for lack of *YKL1*. This result demonstrates that YKL1 is not a functionally redundant RPB7 homolog.

The *YKL1* promoter contains a PAC box and ABF1-binding site. *YKL1* is located on chromosome XI in one of the most highly transcribed regions in eukaryotic chromosomes (1). It is divergently transcribed with the gene encoding the mitochondrial protein *YKL2* (1) and directly downstream of *YTA3*, the gene encoding yeast Tat-binding protein. The 300-bp region between *YKL1* and *YKL2* contains the ABF1-binding site RTCRYBN$_4$ACG (7). The transcription factor ABF1 binds to this sequence in promoter regions of many housekeeping genes (7, 8), especially RNA polymerase genes (8). The *YKL1-YKL2* divergent promoter region also contains a PAC box (8), TG(C/A)GATGAG, followed by a purine-rich sequence, found in the promoters of many yeast polymerase I and III subunit genes. The presence of these two motifs in the *YKL1* promoter coupled with the similarity of the YKL1 protein to RPB7 suggested that *YKL1* encoded a subunit of RNA polymerase I or III.

It is not surprising that RPB7 is related to a subunit in another RNA polymerase class since three other yeast RNA polymerase II subunits are related to subunits in RNA polymerase I or both polymerases I and III. The same level of sequence similarity seen between RPB7 and YKL1, where 50% of the residues are identical or conserved, exists between RPB3 and AC40 as well as RPB11 and AC19 (36). AC19 and AC40 are subunits present in both RNA polymerases I and III. In the third example, RPB9 is related in size and sequence to the RNA polymerase I subunit A12.2 (25). While the sequences of the Zn-binding domains are conserved between RPB9 and A12.2, the level of overall sequence similarity is somewhat lower (37% of the residues are identical or conserved) than for other examples.

A *YKL1* conditional mutant is defective in RNA polymerase III transcription. Since we suspected that YKL1 is an RNA polymerase I or III subunit, we compared its size with sizes of the subunits whose genes have not yet been identified and sequenced. Only one yeast RNA polymerase I subunit gene, encoding the 14-kDa protein A14, has not been characterized. The predicted molecular mass of YKL1 (24 kDa) is nearly double that for A14, eliminating the possibility that YKL1 is an RNA polymerase I subunit and suggesting it is instead an RNA polymerase III subunit. To investigate whether YKL1 is indeed an RNA polymerase III subunit, we obtained conditional *YKL1* mutants and determined if they had defects in RNA polymerase III transcription under nonpermissive conditions. Two temperature-sensitive mutants, *rpc25-1* and *rpc25-2*, were obtained from a plasmid shuffle screen of a mutagenized *YKL1* plasmid library (Fig. 2). The mutant phenotype of *rpc25-1* was marginal, while *rpc25-2* did not support growth at the nonpermissive temperature.

Mutants in RNA polymerase III subunit genes have characteristic defects in tRNA synthesis. In all RNA polymerase III subunit mutants studied to date, there is a decrease in the synthesis of tRNA relative to 5.8S rRNA (5, 8, 12, 19, 23, 29). However, there is no substantial decrease in the synthesis of the other major RNA polymerase III transcript, 5S rRNA, relative to 5.8S rRNA. To test the effects of *rpc25-1* and *rpc25-2* on tRNA synthesis, the two mutant and isogenic

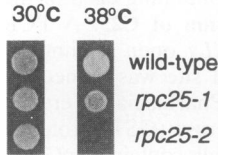

FIG. 2. Temperature-sensitive mutants in *RPC25*. Equivalent numbers of cells were spotted onto YPD plates and incubated at the temperature shown above each panel.

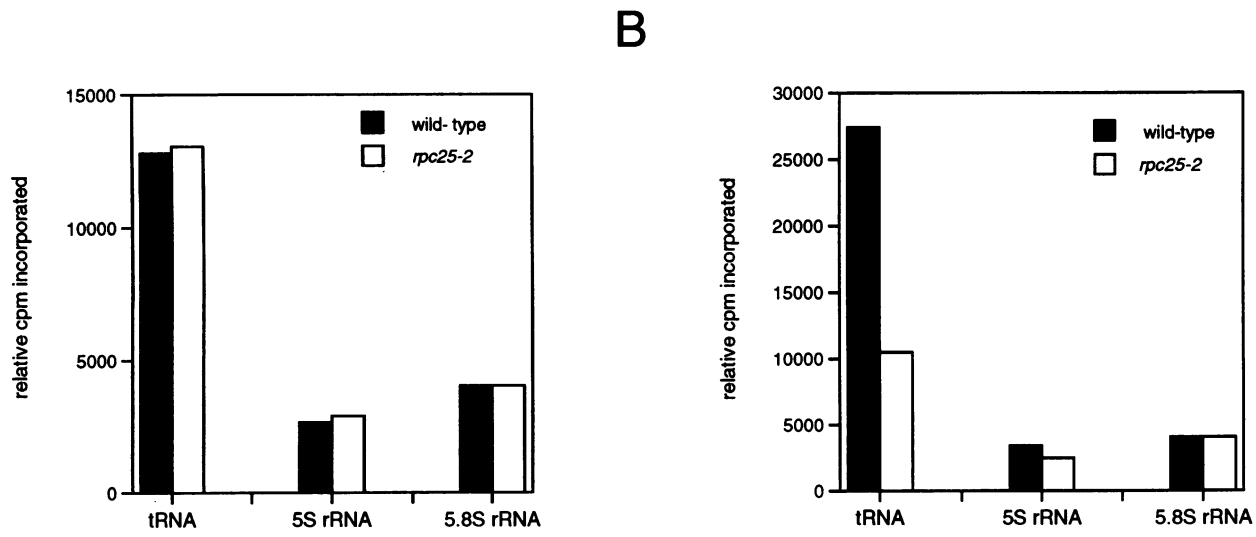

FIG. 3. *rpc25-2* is defective in RNA polymerase III transcription. Mutant and wild-type cells were radioactively labeled with [^{3}H]uracil at the permissive (30°C; A) or nonpermissive (38°C; B) temperature to assess the level of synthesis of 5S rRNA, tRNA, and 5.8S rRNA. Total RNA purified from each strain was subjected to denaturing PAGE, and the levels of the three RNAs were quantified and normalized to the 5.8S rRNA values.

wild-type strains were grown at the permissive temperature, shifted to the nonpermissive temperature for 6 h, and then labeled for 1 h with [^{3}H]uracil. Quantification of the band densities of the labeled RNAs revealed that only the *rpc25-2* mutant had decreased levels of tRNA synthesis relative to 5.8S rRNA (Fig. 3). Since the *rpc25-1* conditional phenotype was very marginal, a significant transcriptional defect was not expected. *rpc25-2* mutants displayed a 2.5-fold decrease in tRNA synthesis, comparable to that seen for conditional mutations in all yeast RNA polymerase III mutants tested (including those from six individual subunits, C160, C82, C53, C34, C31, and AC19). Therefore, the *YKL1* gene encodes an RNA polymerase III subunit.

***YKL1* encodes the RNA polymerase III subunit C25.** Only 3 of the 16 yeast RNA polymerase III subunit genes have not been isolated, C37, C25, and C11. The C25 subunit has a predicted molecular weight most consistent with the molecular mass of the 24-kDa YKL1 protein. To test if the gel mobility of the YKL1 protein was consistent with its predicted molecular mass, we expressed the *YKL1* gene in *E. coli* as a GST fusion protein (Fig. 4A). The fusion protein migrated at approximately 50 kDa. Since GST is 26 kDa, the contribution of the YKL1 protein to the overall size of the fusion protein is predicted to be near 25 kDa. Cleavage of the YKL1 protein from GST attached to an affinity resin generated a 25-kDa band which comigrates with the C25 subunit of *S. cerevisiae* RNA polymerase III (Fig. 4B). The mobility of the C25 protein isolated from yeast cells is not altered by the presence of phosphate groups since this subunit is not one of the three RNA polymerase III subunits, ABC23, AC19, and C53, known to be phosphorylated (31). Therefore, the *YKL1* gene encodes the RNA polymerase III subunit C25.

C25 and RPB7 share an essential conserved domain. Alignment of C25, RPB7, and the plant homolog of RPB7 (33) revealed that there is an ~20-amino-acid region which is well conserved between these three related proteins (Fig. 1). We deleted 20 amino acids within this region of RPB7 and C25 and used plasmid shuffle to determine if the mutant subunits were able to support yeast cell growth. Selective loss of the wild-type plasmid in yeast strains WY-59 and WY-60 revealed that the mutant subunits cannot support cell growth; therefore, this conserved region is essential for function of both RPB7 and YKL1. Although the function of this region is unknown, the evolutionary conservation relative to the rest of the subunit emphasizes its importance.

C25 has a counterpart in archaebacteria. Archaebacteria have a single DNA-dependent RNA polymerase with a subunit architecture comparable to that of eukaryotic nuclear RNA polymerases. Recent database searches revealed that RPB7 and C25 are related to an RNA polymerase subunit from archaebacteria (Fig. 5). The amino-terminal 112 amino acids are 29% identical and 71% conserved between C25 and subunit E of *Sulfolobus acidocaldarius* RNA polymerase. A similar level of conservation is found between RPB7 and *S. acidocaldarius* subunit E. Archaebacterial RNA polymerase subunits with similarities to RPB1, RPB2, and RPB3 and to RPB5, RPB6, and RPB10 have also been identified (15, 17, 21, 26, 28). The archaebacterial homolog of the related subunits RPB3 and AC40 is equally similar (57% conserved overall) to its RNA polymerase II counterpart (RPB3) as it is to its relative in RNA polymerase I and III (AC40). The carboxy-terminal portion of *S. acidocaldarius* subunit E also has a cysteine metal-binding motif, as do several eukaryotic nuclear RNA polymerase subunits. These observations suggest that archaebacterial RNA polymerase subunits have functions which are a hybrid of those possessed by RNA polymerases I, II, and III, consistent with the evolutionary placement of archaebacteria closer to eukaryotes than to eubacteria.

DISCUSSION

With 27 of the 30 *S. cerevisiae* nuclear RNA polymerase subunit genes characterized, it is apparent that these three enzymes have significant functional similarities (Fig. 6). Eleven of the twelve RNA polymerase II subunits are related or identical to subunits in RNA polymerase I or III. RPB4 is the only RNA polymerase II subunit which has no known counterpart in other eukaryotic RNA polymerase classes or in any other eukaryotic RNA polymerase II. In fact, eukaryotic homologs have been identified for all yeast RNA polymerase II

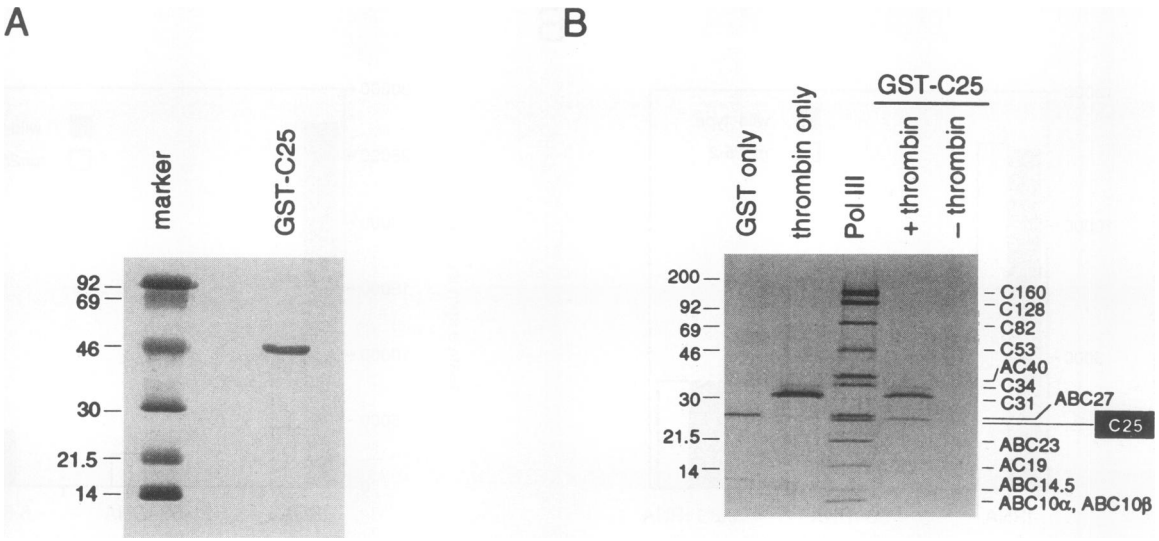

FIG. 4. The gel mobility of the *RPC25* gene product is consistent with the predicted molecular weight of C25. Molecular weight markers (sizes indicated in kilodaltons) are shown at the left of both panels. (A) SDS-PAGE (12% polyacrylamide gel) and Coomassie brilliant blue stain of the GST-C25 fusion protein prepared as described in Materials and Methods. (B) SDS-PAGE (10 to 20% polyacrylamide gradient gel) and silver stain of C25 and *S. cerevisiae* RNA polymerase III. The pure RNA polymerase III is transcriptionally active and was kindly provided by George Kassavetis, in the Peter Geiduschek laboratory. The positions of the RNA polymerase III subunits are shown at the right. The C37 subunit is present at substoichiometric levels and is not visible on this gel. The GST-C25 protein was bound to the glutathione-Sepharose 4B (two right-hand lanes) as described in Materials and Methods. The +thrombin lane represents supernatant loaded after cleavage of glutathione-Sepharose 4B-bound GST-C25 with thrombin to generate free C25 (the thrombin used for cleavage is also present in the supernatant). No protein is visible in the −thrombin lane, demonstrating that the C25 does not come off the glutathione-Sepharose 4B by nonspecific degradation or cleavage.

subunits except for RPB4. RPB4 and RPB7 can dissociate from the enzyme (9, 10, 27), and the stable interaction of RPB7 with RNA polymerase II appears to be dependent on the presence of RPB4 (20).

Since RNA polymerase III has a subunit related to RPB7, it will be interesting to determine whether the sequences of the two unidentified subunit genes encoding C37 and C11 are related to the RPB4 sequence. When the gene encoding C37 is cloned, we suspect that its amino acid sequence will have similarity to RPB4. Consistent with their sequence similarities, C25 and RPB7 have several biochemical properties in common. Both subunits are present in submolar ratios, easily dissociate from the enzyme, are essential for cell growth and viability, but are not required for certain in vitro transcription assays (39, 41). In fact, like RPB4 and RPB7, C37 and C25 are present at substoichiometric levels and can be absent in some transcriptionally active preparations of RNA polymerase III (31).

It does not appear that RNA polymerase I has a subunit related to RPB4 since the only subunit whose sequence is not known, A14, has a sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) mobility less than half that of RPB4. RNA polymerase I also lacks a subunit similar to that of RPB7, suggesting that the RPB7 and C25 subunits are unique to RNA polymerases II and III. The RPB7 and C25 subunits may serve roles not required by RNA polymerase I but essential for the other two classes of enzyme such as recognition of TATA-containing promoters or interaction with general factors which do not participate in the RNA polymerase I-mediated transcription.

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S. cerevisiae C25   MFILSKIADLVRIPPDQFHRDTISAITHQLNNKFANKIIPNVGLC  45
Su. acidocaldarius E MFKLVRAKGIVRIPPEYFGQSVDEIAIKILRQEYQEKLIKDIGVV  45

S. cerevisiae C25   ITIYDLLTVEEGQLKPGDGSSYINVTFRAVVFKPFLGEIVTGWIS  90
Su. acidocaldarius E LGIVNAKASEEGFIIFGDGATYHEVEFDMLVYTPIIHEVIEGEVS  90

S. cerevisiae C25   KCTAEGIKVSLLGIFDDIFIPQ----NMLFEGCYYTP--EESA--  127
Su. acidocaldarius E QVDNYGVYVNMGPVDGLVHISQITDDNLKFDSNRGILIGEKSKKS  135

S. cerevisiae C25   -----WIWPMDEETKLYFDVNEKIRFRIERFVFVDVKPKSPKERE  167
Su. acidocaldarius E ITKGDRVRAMIISASMSSGRLPRIALIMKQPYLGKNRMDKSRNSK  180

S. cerevisiae C25   LEERAQLENEIEGKNEETPQNEKPPAYALLGSCQT-------DGM  205
Su. acidocaldarius E GEKVMSEKKGTSFKACKNCRALVPPETSICPLCHSSSFSDEWNGM  225

S. cerevisiae C25   GLVSWWE----------------                      212
Su. acidocaldarius E VIILNENQKLDRCLGLQLLGDTQ                     248
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FIG. 5. C25 has a counterpart in the archaebacteria. Conserved residues are boxed, and identical amino acid residues are boxed and in boldface. The accession number for *S. acidocaldarius* subunit E is X75411.

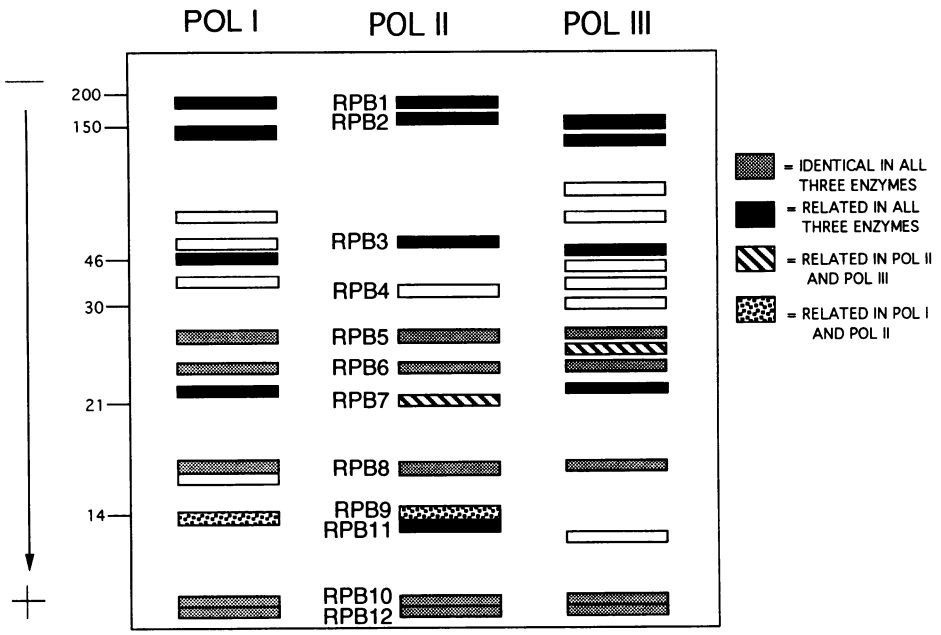

FIG. 6. Subunit functions are conserved in yeast RNA polymerases I, II, and III. Boxes represent relative mobilities of subunit bands in each of the three classes of RNA polymerase in *S. cerevisiae* after SDS-PAGE and staining. The open boxes represent subunits whose sequences are either not similar to any other known yeast subunit or whose genes have not yet been cloned. The molecular weight markers (sizes indicated in kilodaltons) are shown on the left.

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