RPC82 Encodes the Highly Conserved, Third-Largest Subunit of RNA Polymerase C (III) from *Saccharomyces cerevisiae*

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RNA polymerase C (III) promotes the transcription of tRNA and 5S RNA genes. In *Saccharomyces cerevisiae*, the enzyme is composed of 15 subunits, ranging from 160 to about 10 kDa. Here we report the cloning of the gene encoding the 82-kDa subunit, *RPC82*. It maps as a single-copy gene on chromosome XVI. The *UCR2* gene was found in the opposite orientation only 340 bp upstream of the *RPC82* start codon, and the end of the *SKI3* coding sequence was found only 117 bp downstream of the *RPC82* stop codon. The *RPC82* gene encodes a protein with a predicted M_r of 73,984, having no strong sequence similarity to other known proteins. Disruption of the *RPC82* gene was lethal. An *rpc82* temperature-sensitive mutant, constructed by in vitro mutagenesis of the gene, showed a deficient rate of tRNA relative to rRNA synthesis. Of eight RNA polymerase C genes tested, only the *RPC31* gene on a multicopy plasmid was capable of suppressing the *rpc82*(Ts) defect, suggesting an interaction between the polymerase C 82-kDa and 31-kDa subunits. A group of RNA polymerase C-specific subunits are proposed to form a substructure of the enzyme.

The eukaryotic nuclear RNA polymerases A (I), B (II), and C (III) are among the most complex enzymes known today. They are each composed of 12 to 15 distinct polypeptides. The aggregate molecular masses of the holoenzymes range between 500 and 650 kDa, with the two largest subunits of each enzyme contributing over 50% to the total mass. RNA polymerase A synthesizes rRNA precursors in the nucleolus, RNA polymerase B synthesizes mRNA precursors, and RNA polymerase C synthesizes small stable RNAs, such as U6 RNA, tRNAs, and 5S RNA (reviewed in reference 38).

RNA polymerase C has been purified from different species of protozoan, fungal, insect, plant, mouse, and human cells (13, 38, and references therein). *Saccharomyces cerevisiae* RNA polymerase C is composed of 15 subunits whose apparent sizes on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels are 160, 128, 82, 53, 40, 37, 34, 31, 27, 23, 19, 14.5, and 10 (two comigrating subunits) kDa. During the past few years, different genes encoding RNA polymerase C subunits have been isolated and used to determine the role of these subunits in transcription (reviewed in references 12 and 30). Studies on eukaryotic RNA polymerases suggest the existence of a minimal enzyme, composed of the two largest subunits having several domains of homology to the β' and β subunits of the *Escherichia coli* enzyme (1, 19). In addition, putative homologs of the prokaryotic α subunit have been identified for the yeast RNA polymerases (9, 21, 27). These include the 44.5-kDa subunit for the B enzyme (20) and the 19- and 40-kDa subunits common to the A and C enzymes (9, 24). Three subunits of 27, 23, and 14.5 kDa and two of about 10 kDa are common to all three yeast RNA polymerases (7, 38). The remaining subunits of 82, 53, 37, 34, 31, and 26 kDa are specific to RNA polymerase C (17, 46).

The 82-kDa subunit has no immunological cross-reactivity with other subunits of the same enzyme or with subunits of

RNA polymerase A or B (17). This subunit is highly conserved in that nearly all preparations of RNA polymerase C from different eukaryotes contain a characteristic subunit of M_r 80,000 to 90,000 (38), with the exception of insect enzyme C, which contains a smaller polypeptide of about 65 kDa (15, 41). Polyclonal antibodies against the RNA polymerase C 82-kDa subunit (C82) inhibit the specific transcription in vitro of a tRNA gene, indicating that C82 is required for RNA polymerase C activity (17).

In this article, we present the nucleotide sequence of the *RPC82* gene and show that a conditional *RPC82* mutant is affected in tRNA synthesis, providing evidence that C82 is a part of the holoenzyme. The search for extragenic suppressors of the mutation among other RNA polymerase C subunit genes provided genetic evidence of an interaction between C82 and another enzyme C-specific subunit.

MATERIALS AND METHODS

Strains and media. The *E. coli* strain XL1-blue (*recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac* [F' *proAB lacI^qZAM15 Tn10* (Tet^r)]) was obtained from Stratagene and used for the cloning experiments. Bacterial media were prepared as described by Maniatis et al. (23). The genotypes of the yeast strains are given in Table 1. The yeast genetic techniques and media used were those described by Guthrie and Fink (16). Transformation of yeast cells was done by the lithium acetate method (18). Electroporation (28) was performed with an excess of DNA (>200 ng) and a 10-ms square-wave pulse of 3.5 kV/cm, giving a transformation efficiency of about 2,000 transformants per μ g of DNA. The 5-fluoroorotic acid medium was described by Boeke et al. (4) and used for the plasmid shuffle test (24).

Peptide microsequencing. Two milligrams of purified RNA polymerase C (17) was subjected to SDS-PAGE, and the 82-kDa subunit was isolated from the gel. Subsequently, the protein was digested with bovine pancreatic trypsin (Merck) in 0.1 M ammonium acetate (pH 8.2) at 37°C overnight. The resulting peptides were purified by reverse-phase high-pres-

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TABLE 1. Yeast strains used in this study

Strain	Relevant genotype	Source or reference
CMY215	<i>MATα ura3-52 trp1-Δ1 his3-Δ200 ade2-101 lys2-801 can1</i>	Derived from CMY214 (24)
CMY395	<i>MATα ura3-52 trp1-Δ1 his3-Δ200 ade2-101 lys2-801 CAN1 leu2-Δ1</i>	C. Mann
SC55	CMY215 $\times$ CMY395	This study
H71	[<i>rho</i> ⁰] derivative of IL8-8C (<i>MATα trp1 his1</i>)	11
SC63	<i>MATα rpc82::HIS3-1 ura3-52 trp1-Δ1 his3-Δ200 ade2-101 lys2-801 leu2-Δ/pEMBLYP32 (UR43) RPC82</i>	This study
SC108	<i>MATα rpc82::HIS3-1 ura3-52 trp1-Δ1 his3-Δ200 ade2-101 lys2-801 leu2-Δ/pRS314 (TRP1) RPC82</i>	This study
SC116	<i>MATα rpc82::HIS3-1 ura3-52 trp1-Δ1 his3-Δ200 ade2-101 lys2-801 leu2-Δ/pRS314 (TRP1) rpc82-6</i>	This study
SC130	<i>MATα rpc82::HIS3-1 ura3-52 trp1-Δ1 his3-Δ200 ade2-101 lys2-801 leu2-Δ/pRS314 (TRP1) rpc82-6/pFLA4L (UR43) RPC31</i>	This study
SC137	<i>SC55 rpc82::HIS3-2/+</i>	This study

sure liquid chromatography (HPLC) on an Applied Biosystems 130-A microbore HPLC apparatus and microsequenced with an Applied Biosystems model 477-A protein sequencer (44).

Cloning of the *RPC82* gene. Sequence information for peptide 3, LKTEDGFVIPALP (oligonucleotides I and II), and for peptide 4, SSVYEYVIASLT (oligonucleotides III and IV), was used to synthesize the following degenerate oligonucleotides:

AAAACNGAAGATGGNTTTGTNATTCCNGC	pool I
G G C C C	
	A
GCNGGAATNACAAANCCATCTTCTGTTT	pool II
G G G C C	
T	
GTNTATGAATATGTNATTGCNTCNACNTTN	pool III
C G C C AG C	
	A
NAANGTNGANGCAATNACATATTCATANAC	pool IV
G CT G G C G	
	T

These oligonucleotides were subsequently used to amplify a fragment from *S. cerevisiae* ABYS86 genomic DNA by the polymerase chain reaction (PCR). The reaction mixes contained all possible pairwise combinations of the oligonucleotide pools (25 pmol of each pool) along with 100 ng of yeast genomic DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 200 μ M each dATP, dCTP, dGTP, and dTTP, 1 mg of gelatin per ml, and 2.5 U of AmpliTaq polymerase (United States Biochemical) in a total volume of 100 μ l. The DNA was denatured for 4 min at 92°C, and the oligonucleotides were allowed to hybridize for 10 min at 20°C. Subsequently, 1-min cycles consisting of denaturation at 92°C, hybridization at 55°C, and polymerization at 72°C were carried out. After 30 cycles, the reaction was completed for

10 min at 72°C. The products were then phenol-chloroform extracted and purified over a Sepharose 6B-Cl spin column equilibrated in 20 mM Tris-HCl (pH 8.0)–1 mM EDTA. The termini of the DNA fragments were rendered blunt with Klenow polymerase, and the fragments were then ligated into the Bluescript SK plasmid previously linearized with *Sma*I. The PCR-produced fragments were used as specific probes to screen a lambda EMBL3a S288C genomic yeast DNA library (a generous gift of Mike Snyder, Yale University). Positive clones were purified and analyzed by restriction enzyme digestion. A 3-kb *Pst*I fragment was subcloned into the *Pst*I site of Bluescript SK and sequenced.

Sequence analysis. The nucleotide sequences were established for both strands of DNA with the Sequenase kit from United States Biochemical. The DNA sequence was analyzed by the DNA Strider program (26). Homology searches (GenBank data base release 65 and Swissprot release 18 [May 1991]) were performed with the FASTA program (32) with the computer facilities at CITI2 in Paris on a VAX8530 computer. Secondary-structure analysis was performed on the same computer with the University of Wisconsin Genetics Computer Group programs.

Plasmid constructions. The 3-kb *Pst*I fragment containing the entire *RPC82* gene was inserted into the *Pst*I site of either Bluescript SK (obtained from Stratagene), yielding pRST11, or pRS314 (40), yielding Scp120. Cloning of the blunt-ended *Pst*I *RPC82* fragment into the Klenow blunt-ended *Xba*I site of pEMBLYP32 (3, 29) gave rise to Scp80. pRST14 was obtained by cloning the 2-kb *Xmn*I-*Pst*I fragment from pRST11 containing the entire *RPC82* gene into Bluescript SK. The deletion mutants were constructed in the following way: the oligonucleotides V (5'¹⁰⁹¹TTGACTTGG CAAGACATCTCCCCGC₁₁₁₅3') and VI (5'GGGGGTGAC CTA₁₈₃₆TTTAACATCATCTCTATTTCGCC₁₈₁₅3') or V and VII (5'GGGGGTGACCTA₁₇₃₁CATGAAATTATAGGAA TGCG₁₇₁₂3') were hybridized to *RPC82* DNA and amplified by PCR. Subsequently, they were cleaved with *Hind*III and *Sa*I and cloned into pRST11 cleaved with the same enzymes, resulting in pRST21 and pRST22, respectively. During the PCR amplification with oligonucleotides V and VI, one clone contained a point mutation at position 1810, introducing a translational stop codon. The mutated *RPC82* gene was then cloned as a *Sac*I-*Sa*I fragment into the multicopy plasmid pFL45S (5) or the centromeric plasmid pUN20 (10).

In vitro transcription-translation of the *RPC82* gene. The transcription-translation reaction was performed with the reticulocyte in vitro system purchased from Promega Corporation. pRST14 linearized at the single *Sma*I site served as the template for the mRNA synthesis. Protein synthesis was carried out in the presence of [³⁵S]methionine, and the products were separated on a 10% polyacrylamide–SDS gel prior to autoradiography overnight.

Disruption of the *RPC82* gene. The *RPC82* gene was disrupted by substituting the 1.7-kb *Bam*HI fragment containing the *HIS3* gene (43) for the two adjacent *Bgl*II fragments of *RPC82*, thereby deleting 1.0 kb of the 1.96 kb of *RPC82* coding sequence (see Fig. 2A). The resulting 3.8-kb fragment was used to transform the diploid strain SC55. Integration of the transforming DNA at the *RPC82* locus was confirmed by Southern analysis with a 1.5-kb *Pvu*II fragment of *RPC82* as the probe. *rpc82::HIS3-1* transformants were sporulated for tetrad analysis. The heterozygous diploid was further transformed by Scp80 (*RPC82 UR43*) and allowed to sporulate. A haploid *rpc82::HIS3-1*/Scp80 strain (SC63) was

obtained after tetrad dissection and used in the plasmid shuffle tests.

A complete and precise deletion of the *RPC82* gene (the *rpc82::HIS3-2* mutation) was also constructed. The 3-kb *RPC82 PstI* fragment was cloned into M13mp19. *BamHI* sites were created at either side of the *RPC82* start and stop codons by site-specific mutagenesis. The plasmid was mutated simultaneously with three oligonucleotides that changed the A at position -8 to G, T at 1338 to C, T at 1980 to G, A at 1984 to C, and A at 1985 to C (the numbering scheme is as indicated in Fig. 1). The effect of these mutations was to create *BamHI* sites at positions -8 and 1980 and to remove the *BamHI* site located at 1355. The mutagenized plasmid was called M13mp19-*RPC82*-1001. The *BamHI* restriction site from the M13mp19 polylinker was removed by cutting M13mp19-*RPC82*-1001 with *EcoRI* and *SalI*, filling in the sticky ends, and religating the plasmid. The *BamHI* fragment containing the *RPC82* coding sequence was then replaced by the 1,764-bp *BamHI HIS3* fragment. Digestion of this plasmid with *EcoRV* and *SphI* yielded a 2,734-bp *rpc82::HIS3-2* fragment that was used to transform the diploid strain SC55.

Random mutagenesis with hydroxylamine. *Scp120 (TRP1 RPC82)* DNA was mutagenized with hydroxylamine. For each sample, 100 μ l of hydroxylamine solution (0.09 g of NaOH, 0.35 g of hydroxylamine-HCl in 5 ml of ice-cold water, made up fresh before use) was added to an equal volume of 10 μ g of DNA in 10 mM Tris-HCl-1 mM EDTA. DNA was incubated at 75°C. Samples were taken every 15 min from 0 to 120 min, and the reactions were terminated by the addition of 4 μ l of 5 M NaCl, 10 μ l of 1-mg/ml bovine serum albumin, and 235 μ l of ethanol. After precipitation, the DNA was resuspended in 0.3 M sodium acetate and reprecipitated. The redissolved DNA was used directly for transformation of the yeast strain SC63 [*trp1-Δ1 rpc82::HIS3-1/Scp80 (RPC82 URA3)*] by electroporation. *Trp*⁺ transformants decreased as incubation time increased. Two hundred eighty transformants obtained with the plasmid mutagenized for 45 to 120 min were analyzed. Among them, eight transformants (2.8%) were unable to grow on plates containing 5-fluoroorotic acid (4). These transformants presumably received plasmids carrying *rpc82* null mutations. The other 272 transformants were tested for the ability to grow at 16 or 37°C after curing of the plasmid containing the wild-type *RPC82* gene. One temperature-sensitive candidate was retained. The corresponding mutagenized plasmid contained in these cells was recovered in *E. coli* and then retested after transformation back into yeast cells. This plasmid again conferred temperature-sensitive growth to the *rpc82*-disrupted strain, indicating that the mutation was indeed carried by the plasmid.

In vivo labeling of RNAs. Strains SC108 and SC116 were complemented for uracil auxotrophy by transforming cells with the *URA3* gene carried on the multicopy plasmid pFL44D. Cells of both strains were then grown exponentially at 30°C in glucose synthetic medium without uracil. The cells were labeled for 30 min by adding 5.5×10^5 Bq of [5,6-³H]uracil per 10^7 cells ($A_{600} \approx 0.3$) either at 30°C or after having transferred the cells to 37°C for 3 or 15 h. Cells were pelleted, washed in cold water, and stored at -20°C. RNAs were phenol-extracted at 65°C (36), with a yield of 50 to 100 μ g of RNA. Small RNAs of interest were recovered in a reproducible manner (42). RNA samples were separated on a 7 M urea-6% polyacrylamide gel and autoradiographed after the gel was treated with Amplify (Amersham).

Nucleotide sequence accession number. The nucleotide

sequence data reported in this article will appear in the EMBL, GenBank and DDBJ nucleotide sequence data bases under accession number X63500.

RESULTS

Isolation of the *RPC82* gene. Microsequence data were obtained for several tryptic peptides of the electrophoretically purified C82 subunit (Materials and Methods). The following sequences were obtained: LVTPEVDMTISSLEQ (peptide 1), YNSVEIQEVP (peptide 2), LKTEDGFVIPAL PAAVSK (peptide 3), SSVYEYVIASTLGPSAMR (peptide 4), LLSL?EVFQMA (peptide 5), and IINKPNELSQL TVDPK (peptide 6). The underlined sequences in peptides 3 and 4 were used to synthesize a degenerate set of oligonucleotides. Oligonucleotide pools I through IV (Materials and Methods) were used to amplify an *RPC82*-specific DNA fragment from yeast genomic DNA by the PCR method. All different combinations of the four oligonucleotides were tested, but a fragment of 304 bp containing a single *BamHI* site was obtained only with oligonucleotides I and IV. This fragment was then subcloned into the Bluescript SK plasmid, and sequence analysis confirmed that it encoded the entire peptide 3 and the amino-terminal part of peptide 4. Subsequently, the PCR fragment was used as a probe to screen a lambda EMBL3a yeast genomic DNA library. Five positive clones were analyzed, and from one of them, a 3-kb *PstI* fragment was subcloned into the vector Bluescript SK, giving rise to plasmid pRST11.

Nucleotide sequence of *RPC82*. The sequence of the 3-kb *PstI* insert in pRST11 showed one large open reading frame (ORF) encoding 655 amino acids which encoded all six C82 peptides, as determined by protein microsequencing (Fig. 1). Since the predicted mass of the *RPC82* protein (74 kDa) was substantially less than that estimated by migration on SDS-polyacrylamide gels (82 kDa), we determined whether the cloned *RPC82* gene contained all the information necessary for encoding the C82 protein by in vitro translation. The *XmnI-PstI* fragment containing the entire ORF (Fig. 2A) was placed under the control of a T7 RNA polymerase promoter and assayed in an in vitro transcription-translation system. The in vitro-synthesized protein comigrated with the authentic C82 subunit of the RNA polymerase C enzyme on an SDS-polyacrylamide gel (data not shown). This result established that the isolated 3-kb DNA fragment contained the entire *RPC82* gene and that the size discrepancy was not due to extensive modification of the translation product. A discrepancy between the apparent molecular mass on SDS-PAGE and that calculated from the nucleotide sequence was also observed for several other RNA polymerase subunits (45).

The end of the *SKI3* gene (34) was found just 117 bp downstream of the *RPC82* stop codon. Upstream of the *RPC82* gene, one incomplete reading frame was found in the opposite direction. By sequence comparison, it was identified as the *UCR2* gene (31). To our surprise, the identity of the region upstream of the published *UCR2* gene with our clone did not extend beyond the *Sau3A* site at position -4 of the *RPC82* gene. This observation raised the possibility that during the construction of the genomic library by *Sau3A* partial digestion, a scrambling of DNA fragments had occurred. In order to test this hypothesis, we performed a PCR amplification with genomic DNA and pRST11 using different sets of oligonucleotides hybridizing to *UCR2* and *RPC82* sequences on either side of the *Sau3A* site in question (Fig. 2A). In addition, probes specific for *UCR2* and for *RPC82*

TCCATGGACCTTAACAGCCAATGTAGATATTTAGTAGGTGCGTCTCTAGCGGAAACGGTCAACCTTCTAACTGACCCCTGGGCAAATTG -361
 CAATCTAGCTGCTGACAACATCAACGTTCTTCTTTTTCTTTTAAATAATTTAACTCTCCCGCTTAATCGTTCGAGCACACTGCTGTT -271
 AAAAACCTTATTAACCTTCTAAACCTTCGTAGCAAAACGCTCTGAGGAAAGGCCCTTGTCTTTATATATATATTTGGTAGGCTAACGGAT -181
 ATAAGACGGGGCGGCCCTCTATTGGTTTGGTGGGAATGATCAGAAATTTTCAAAAGCTCATCGCAATTGAAGGAAAAATAATTTTGG -91
 ATCAAAATAATAATAGTGAAGAAATATTGAGAACAATAGAGAATTGCTGACACAGGAATAGGGAAAAATTTTGTGAGCAAGATCCAA -1
 ATGGACGAGTTACTGGGAGAAGCGCTTAGTGAGAGAACCAACAGGTGAGAGCACTGTGAACTCTGAGAAGTTGGTCACCCCGAAGAT 90
 M D E L L G E A L S A E N Q T G E S T V E S E K L V T P E D
 GTTATGACCATATCATCTTGGAACAGAGAACCTTGAATCCCGATTGTTTCTTTACAAAGAGTTGGTGAAGGCACATCTGGGTGAAAGA 180
 V M T I S S L E Q R T L N P D L F L Y K E L V K A H L G E R
 GCAGCTTCTGTAATGGGATGTTGGTTGCGCTGGGAGATTGAGTGTGCGGAGTTAGTGGAGAAAAATAGATGGTATGGATGTAGACAGC 270
 A A S V I G M L V A L G R L S V R E L V E K I D G M D V D S
 GTCAAGACGACACTAGTCTCATTAACTCAATTAAGGTGTGTTAAGTACCTGCAAGAAACAGCAATATCCGGGAAAAAACGACTTATTAT 360
 V K T T L V S L T Q L R C V K Y L Q E T A I S G K K T T Y Y
 TACTATAACGAGGAAGGGATACACATTTTACTCTACTCAGGGTGATTATTGACGAAATTTATACGCAATGCGTGTAACGACGAAGAG 450
 Y Y N E E G I H I L L Y S G L I I D E I I T Q M R V N D E E
 GAGCATAACAGCTGGTGGCTGAAATTTACAAAACGTTATATCATTAGGGTCCTTAACAGTGAAGATTATCTAAGTAGTGTACAGTCA 540
 E H K Q L V A E I V Q N V I S L G S L T V E D Y L S S V T S
 GATTCCATGAAGTACACCATTTTCATCACTATTCGTGCAACTGTGTGAAATGGGATATCTTATCAAAATATCAAAATTCATTATACCCCA 630
 D S M K Y T I S S L F V Q L C E M G Y L I Q I S K L H Y T P
 ATCGAAGATCTCTGGCAATTTTATATGAAAAACATTACAAAATATCCCGAGAACTCCCGTTGTCCGATTGAAGAAAAGATCCCAA 720
 I E D L W Q F L Y E K H Y K N I P R N S P L S D L K K R S Q
 GCCAAGATGAATGCGAAAACCTGATTTTGCCTAAATCATAAATAAACCAATGAGCTTTTACAAATCTTAACGGTTGATCCAAAGACCTCA 810
 A K M N A K T D F A K I I N K P N E L S Q I L T V D P K T S
 TTGAGGATTGTTAAACCTACAGTCTCTCTGACGATTAATTTGGATAGATTATGAAAGGTAGAAGATCTAAACAATTGATTAACTTGCC 900
 L R I V K P T V S L T I N L D R F M K G R R S K Q L I N L A
 AAGACAAGAGTCGGCTCCGTCACAGCTCAAGTTTACAAATGCATTAAGACTCACAGAGCAAAAGTCTCTAAATTAGAGATCCACTA 990
 K T R V G S V T A Q V Y K I A L R L T E Q K S P K I R D P L
 ACTCAAACCTGGGCTTTTACAAAGATTGGAGGAAGCAAAATCGTTTCAAGACGAAGCCGAACCTTGTGAGGAGAAGACACAGGGCTTACC 1080
 T Q T G L L Q D L E E A K S F Q D E A E L V E E K T P G L T
 TTTAATGCTATTGACTTGGCAAGACATCTCCCGCAGAATTGGACTTGCCTGGAAGCTTATTATCTAGAAAGCCCTCTGACAAATAAAAG 1170
 F N A I D L A R H L P A E L D L R G S L L S R K P S D N K K
 CGTTCTGGCTCAACGCTGCTGCATCATTACCCAGTAAAAAATAAAAACCGAAGATGGATTGTGATCCCGCGCTACCTGCTGCTGTT 1260
 R S G S N A A A S L P S K K L K T E D G F V I P A L P A A V
 TCCAAATCTCTTCAAGAAAGTGGAGATACACAAGAAGACGAAGAGGAGGAGATTGGATGCAGACACTGAGGATCTCTACTAGCT 1350
 S K S L Q E S G D T Q E E D E E E E D L D A D T E D P H S A
 TCTTTGATAAATAGTCATTATAAAATCTTAGCATCATCAAAATTTCCCTTTTGAACGAAACCAACAGGTGTTTACTATGTTCCATAT 1440
 S L I N S H L K I L A S S N F P F L N E T K P G V Y Y V P Y
 AGCAAACTAATGCCAGTATTAAAGTCATCTGTTTATGAGTATGTTATCGCGTCTACGTTGGGACCTCAGCAATGCGTTTAAGCCGTTGT 1530
 S K L M P V L K S S V Y E Y V I A S T L G P S A M R L S R C
 ATTCGTGATAACAAGCTGGTGTCTGAGAAAAATTCAACTCAACTGCGTTAATGAAGGAAAGGATATAAGATCTACATTAGCATCTTTA 1620
 I R D N K L V S E K I I N S T A L M K E K D I R S T L A S L
 ATTAGATACAATTCTGTGGAATCCAGGAAGTTCCAAGAAGACGCGATAGATCAGCATCAAGGGCAGTTTTTTGTTTAGATGCAAGAA 1710
 I R Y N S V E I Q E V P R T A D R S A S R A V F L F R C K E
 ACGCATTCCTATAATTTTCATGAGACAAAACCTGGAATGGAATATGGCAATTTACTTTTTAAAAAGGAGAAATTAAGCAAGAAAACCT 1800
 T H S Y N F M R Q N L E W N M A N L L F K K E K L K Q E N S
 ACATTATTAAGAAAGCAAAATAGAGATGATGTTAAAGGAAGAGAGAACTACTATTACCAAGCGAATTGAATCAATTAAGATGGTC 1890
 T L L K K A N R D D V K G R E N E L L L P S E L N Q L K M V
 AATGAAGAGAGCTAAATCTTTTCGCTCGTTTATCGAGTTGCTATCGCTTGGGAAGTTTCCAAATGGCATGATTACTCTATACAGCT 1980
 N E R E L N L F A R L S R L L S L W E V F Q M A *
 GATAAACTCTGCTAATAACCTTTTATACCTCTATAGAAAAGATAAAATTTTACTAATGTTACATTAAGGTTTGATTGACTATCTCGAA 2070
 TCCAAATTTTGAAGAACTTCGTTTAGCGCCTTCACTGCAG 2111
 SKI3

FIG. 1. Nucleotide sequence of the *RPC82* gene. The first nucleotide of the start codon is designated +1. The translation of the nucleotide sequence is given in the one-letter amino acid code. The underlined amino acids indicate the peptides determined by microsequencing. The four circled leucine residues in the carboxy-terminal part of the C82 protein indicate a putative leucine zipper motif. The asterisk at the end of the peptide sequence refers to the translational stop codon. A consensus binding site for the ABFI protein (in reverse orientation,

FIG. 2. (A) Restriction map of the *RPC82* locus and structure of the *rpc82::HIS3-1* disruption mutation. The *UCR2* (open box) and *RPC82* (hatched box) ORFs are transcribed in opposite directions, as indicated by the arrows. The black bar shows the DNA fragment synthesized by PCR with degenerate oligonucleotides. Dotted lines represent DNA fragments synthesized by PCR with seven different oligonucleotides, positioned relative to the *Sau*3A site, proving that the genomic arrangement of the *UCR2* and *RPC82* genes is as shown. The 1.8-kb *Bam*HI *HIS3* fragment was inserted into the *RPC82* *Bgl*II sites for gene disruption. (B) Southern blot analysis. Total genomic DNA from a wild-type ABYS86 yeast strain was digested with eight different restriction enzymes and some combinations of them, separated by electrophoresis on a 1% agarose gel, and transferred to a nylon membrane. The probe used was the 304-bp PCR-synthesized fragment (black bar in panel A). Restriction sites: P, *Pst*I; Xm, *Xmn*I; S3, *Sau*3A; V, *Pvu*II; Bg, *Bgl*II; B, *Bam*HI; Xb, *Xba*I; C, *Cl*aI; E, *Eco*RI; S, *S*alI; H, *H*indIII. DNA sizes are indicated in kilobases.

were hybridized to yeast genomic DNA and pRST11 (results not shown). Both methods showed identical DNA fragment lengths for both pRST11 and genomic DNA, confirming that the genomic arrangement of *UCR2* and *RPC82* is as shown in this article. Thus, the DNA sequence beyond the *Sau*3A site published by Oudshoorn et al. (31) was not colinear with the *UCR2* gene. We suggest that the plasmid analyzed by Oudshoorn et al. resulted from the ligation of two nonadjacent *Sau*3A genomic DNA fragments.

A consensus ABFI-binding site (8) is found at positions -146 to -139 of the *RPC82* promoter region (Fig. 1).

Downstream of this site at positions -122 to -114, a PAC site was identified. The PAC sequence and ABFI- or TUF/GRF/RAP-binding sites are found in the promoter regions of other yeast RNA polymerase A and C genes (9). The significance of these two sites for *RPC82* transcriptional regulation remains to be shown. The sequence of *RPC82* was compared with those of the other subunits of RNA polymerases A, B, and C. In addition, the *RPC82* protein sequence was compared to the GenBank DNA sequence data base (release 65) translated into all six reading frames and to the Swissprot data base (release 18, May 1991). No significant similarity was found either at the DNA or at the amino acid level with other sequences in the data base. However, a heptad repeat of four leucine residues was found close to the carboxy-terminal end of C82 (Fig. 1) in a region predicted to contain an α -helical structure.

***RPC82* is a single-copy gene on chromosome XVI.** The copy number of *RPC82* was determined by Southern hybridization of a 304-bp *RPC82* coding sequence to genomic DNA (Fig. 2B). One major band hybridized with the probe in each case, except for *Bam*HI-cut DNA, which gave rise to two bands because of the *Bam*HI site present in the hybridization probe. Faint higher-molecular-weight bands were seen in some of the lanes. These bands may be due to partial restriction enzyme digestion or to the presence of weakly cross-hybridizing sequences in the yeast genome. The absolute requirement for the *RPC82* gene for viability (see below) indicates that it is functionally unique. In order to determine the chromosomal location of *RPC82*, the 3-kb *Pst*I fragment from pRST11 was used as a probe to hybridize to yeast chromosomes that had been separated by pulsed-field gel electrophoresis. A signal was detected at the position of the comigrating chromosomes XIII and XVI. Therefore, the experiment was repeated with chromosomes from strain H71, in which chromosomes XIII and XVI are clearly separated by gel electrophoresis. In this case, the *RPC82* probe hybridized only to chromosome XVI (data not shown).

***RPC82* is essential for viability.** A heterozygous (*rpc82::HIS3-1/+*) diploid strain was constructed by one-step gene disruption (35) of SC55. Upon sporulation, all 10 asci analyzed gave rise to two viable spores that were all *His*⁻. Haploid segregants containing the *rpc82::HIS3-1* allele were recovered by transforming the heterozygous diploid with the centromeric plasmid Scp80, containing the *RPC82* gene. All of the resulting *His*⁺ meiotic segregants had retained the complementing plasmid, showing that the 3-kb DNA fragment on plasmid Scp80 is necessary and sufficient to complement the disrupted gene.

The recessive lethality of the *rpc82::HIS3-1* disruption suggested to us that the C82 subunit was essential for yeast cell viability. However, a referee of our manuscript was concerned that C82 might not be an essential protein, but rather that a portion of the C82 protein might be expressed from the *rpc82::HIS3-1* disruption and that this mutant protein might be able to prevent yeast cell growth in the absence of the wild-type subunit. We thus created, by site-specific mutagenesis, a total deletion of the *RPC82* gene (the *rpc82::HIS3-2* mutation; see Materials and Methods). Tetrad

RTCRYYN₄ACG) and the PAC consensus sequence in the upstream region are indicated. The arrow at position -340 marks the start codon for the *UCR2* gene, transcribed in the opposite direction from *RPC82*. Deletions of 72 (CA72), 55 (CA55), and 42 (CA42) carboxy-terminal amino acids of the coding sequence are indicated. Also shown are the positions of the *UCR2* start codon and the *SKI3* stop codon (TAA), as well as the orientation of the two genes relative to *RPC82*.

FIG. 3. Growth of the SC108 (wild type), SC130 (*rpc82-6/pFL44L-RPC31*), and SC116 (*rpc82-6*) strains in rich medium at 37°C for 30 h. Cell growth was measured by optical density at 600 nm.

dissection of the heterozygous diploid strain SC137 *rpc82::HIS3-2/+* never gave rise to more than two viable spores, and these spores were always auxotrophic for histidine. Identical results were thus obtained for the partial *rpc82::HIS3-1* deletion and the total *rpc82::HIS3-2* deletion, from which we conclude that *RPC82* is indeed an essential gene.

We tried to investigate the functional significance of the carboxy-terminal leucine repeats of C82 by constructing *rpc82* mutants on *TRP1*-containing plasmids in which 77, 51, or 42 carboxy-terminal amino acids of the subunit were deleted (Fig. 1). The *rpc82* mutants were introduced into strain SC63 [*rpc82::HIS3-1/Scp80 (URA3 RPC82)*]. None of these deletion mutants could complement the chromosomal disruption of *RPC82* either on a multicopy or on a centromeric plasmid, as shown by the inability of these cells to lose the *Scp80 (URA3 RPC82)* plasmid. We were thus unable to determine the importance of the leucine repeats by this approach, because deletion of only the last 42 amino acids of C82 (carboxy-terminal to the leucine repeats) was already sufficient to inactivate C82 function.

Temperature-sensitive *RPC82* mutation affects tRNA synthesis. The centromeric plasmid *Scp120* was randomly mutagenized with hydroxylamine. *RPC82* function was assayed by genetic complementation of the *rpc82::HIS3-1* disruption in a plasmid shuffle test. Eight of 280 transformants were unable to grow without a wild-type *RPC82* gene, indicating a frequency of *rpc82* null mutants of 2.8% after mutagenesis. One transformant (of 272 analyzed), containing the *rpc82-6* mutant allele, showed normal growth at 30°C (doubling time of 1.7 h) but exhibited a temperature-sensitive phenotype. When transferred from 30 to 37°C on rich medium, the temperature-sensitive mutant grew for the first 10 h with a doubling time of 2.5 h, as did the wild-type control strain. From 10 h onwards, the growth rate declined, and after 30 h at 37°C, no further growth was observed (Fig. 3). This behavior suggests that the mutant RNA polymerase may have a temperature-sensitive assembly defect.

In vivo synthesis of small RNAs was assayed 3 and 15 h after transfer of *rpc82-6* mutant or wild-type cells to 37°C to avoid transitory heat shock inhibition of maturation (48) or of initiation of rRNA gene transcription (47). The growth rate was unchanged in the first 3 h at 37°C, during which time one cell division took place. After pulse-labeling with [5,6-³H]uracil for 30 min, RNA samples were analyzed by gel electrophoresis at constant radioactivity. Synthesis of 5S RNA and tRNA (by RNA polymerase C) was compared with

FIG. 4. Autoradiogram of the in vivo pulse-labeling experiments, showing 5.8S and 5S RNAs and tRNAs. [5,6-³H]uracil incorporation into strains SC108 (wild type) and SC116 (*rpc82-6*) was done for 30 min at 30°C or after 3 h (37.3) or 15 h (37.15) of growth at 37°C. RNA samples containing equal amounts of radioactivity were analyzed to reflect an equal quantity of newly synthesized total RNA. RNAs were separated on a 7 M urea-6% polyacrylamide gel and autoradiographed.

the synthesis of 5.8S RNA (by RNA polymerase A), which served as an internal standard. Three hours after the shift to 37°C, the quantity of newly synthesized tRNA in the mutant strain had already diminished compared with that in the wild-type strain at the same temperature (Fig. 4). After 15 h at 37°C, tRNA synthesis declined more considerably in the mutant strain. In contrast, 5S RNA synthesis remained similar in both the temperature-sensitive and wild-type strains under the same growth conditions. Greater inhibition of tRNA than of 5S RNA synthesis has also been observed for mutants carrying mutations that affect the C160 (14), C53 (25), C34 (42), and C31 (29) subunits.

***RPC31* suppresses the *rpc82-6* mutation.** Copies of either *RPC160*, *RET1*, *RPC53*, *RPC40*, *RPC34*, *RPC31*, *RPC19*, or *RPB10* (encoding, respectively, the C160, C128, C53, AC40, C34, C31, AC19, and ABC10 β subunits) contained on multicopy plasmids were tested for their ability to suppress the temperature-sensitive phenotype of the *rpc82-6* strain (SC116). Only the plasmid containing *RPC31* suppressed the mutation (Fig. 5). No inhibition of *rpc82-6* mutant cell

FIG. 5. Suppression of the *rpc82-6* mutation by overexpression of RNA polymerase C subunits. SC116 yeast strains transformed by multicopy plasmids containing either the C160, C128 (*RET1*), C82, C53, AC40, C34, C31, AC19, or ABC10 β subunit gene were plated on rich medium at 37°C. Only *RPC82* transformants, and to a lesser extent *RPC31* transformants, formed colonies on the plate.

growth was observed for any of the plasmids. At the restrictive temperature, suppression by *RPC31* was visible after 10 h compared with the control strain transformed with the vector alone (Fig. 3). The growth rate was measured for more than 50 h, showing a doubling time of 6.5 h at 37°C. Suppression of the *rpc82-6* mutation by multiple copies of the *RPC31* gene required *RPC31* overexpression, since such suppression was not seen when the *RPC31* gene was carried on a centromeric plasmid.

DISCUSSION

In this article, we present the nucleotide sequence of the *RPC82* gene, encoding the 82-kDa subunit of the *S. cerevisiae* RNA polymerase C. The theoretical molecular weight of C82 is 73,984. However, in vitro transcription-translation of the gene gave rise to a protein with the same mobility on SDS-PAGE gels as the 82-kDa subunit in the holoenzyme, indicating that the aberrant gel migration is not due to posttranslational modification of the subunit in yeast cells. The single-copy gene was localized on chromosome XVI, adjacent to *UCR2* and *SKI3*.

Four leucine heptad repeats were found in a predicted α -helical region at the carboxy-terminal end of the C82 subunit. Such repeats are characteristic of the leucine zipper motif found in several transcriptional activators, e.g., GCN4, yAP-1, and others (reviewed in reference 6). However, no basic domain or helix-loop-helix motif is associated with the C82 leucine repeats, in contrast to what is found for DNA-binding proteins containing a leucine zipper. Leucine repeat motifs have been shown to mediate homo- and heterodimer formation between proteins (22). Since C82 has been estimated to have a stoichiometry of 1 in RNA polymerase C (46), we suggest that heterodimers may be formed via the leucine repeats of C82 and other polymerase C subunits. Recently, Werner et al. have shown that a mutation in the zinc finger domain of the largest subunit, C160, caused simultaneous loss of C82, C34, and C31 after heat inactivation of the mutant enzyme (49). Moreover, it was reported that C82, C34, and C31 are loosely bound to RNA polymerase C (slightly different molecular weights were indicated by Valenzuela et al. [46]). Heptad repeats of three and four leucine residues are also found in the N-terminal region of C34 (42) and in the carboxy-terminal region of ABC23 (2). In the latter case, point mutations that changed the leucine residues were shown to be lethal in an *rpb1* mutant strain, in which the largest subunit of RNA polymerase B was mutated. Attempts to investigate the role of the leucine repeats by analysis of deletion mutants underscored the need for the carboxy-terminal part of C82. The simple deletion of 42 residues (that preserved the leucine repeats) was lethal to the cells.

The possible interaction of C82 with different subunits of RNA polymerase C was further investigated by multicopy plasmid suppression. Eight of 13 RNA polymerase C subunit genes were tested for their effect on an *rpc82* temperature-sensitive mutant, obtained by in vitro mutagenesis of the cloned gene. Only in the presence of the *RPC31* gene was a suppression of the mutant phenotype observed. This correlates with the biochemical data and supports the hypothesis that C82 and C31 interact directly. Taken together, these results support a model in which the RNA polymerase C-specific subunits C31, C34, and C82 form a subassembly associated with the RNA polymerase C core enzyme, composed of C160 (β' homolog), C128 (β homolog), and AC40 and AC19 (α homologs) (12).

The role of C82 is still conjectural. This subunit is essential, as shown by gene disruption experiments. Sequence analysis did not disclose any similarity with proteins of known function, such as class B transcription factors and other class A or class B polymerase subunits. We have shown that tRNA synthesis is specifically inhibited relative to synthesis of 5S and 5.8S rRNA in an *rpc82* temperature-sensitive mutant at the restrictive temperature. This result confirms a previous in vitro study indicating a role for C82 in tRNA gene transcription by antibody inhibition (17). The preferential inhibition of tRNA synthesis relative to 5S RNA synthesis at the nonpermissive temperature could indicate either that C82 is uniquely involved in tRNA gene transcription or that the mutation preferentially affects RNA polymerase C transcription of tRNA genes versus 5S RNA genes. We favor the latter hypothesis, as different mutations in several subunits, such as the C160 (14), C53 (25), C34 (42), and C31 (29) subunits, all inhibit tRNA synthesis to a greater degree than 5S RNA synthesis. The general effect of all these different mutations may be to decrease the RNA polymerase C level in the cell by affecting enzyme assembly or stability. tRNA gene transcription could be inherently more sensitive to partial inactivation of RNA polymerase C than is 5S RNA gene transcription. The explanation for this difference is not known, but we can suggest at least two possibilities. First, although the tRNA and 5S RNA genes both require transcription factors IIIB and IIIC for their expression, only the 5S RNA genes also require TFIIIA (37, 39). Perhaps RNA polymerase C in association with TFIIIA, -B, and -C is more stable than that in association only with TFIIIB and -C on tRNA genes. A second possibility is suggested by the unorthodox localization of 5S RNA genes in the nucleolus in *S. cerevisiae* (34). RNA polymerase C may be more concentrated or more stable within the yeast nucleolus than in the nucleoplasm.

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The first two authors contributed equally to this work.

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