RNA Polymerase II Subunit RPBlO Is Essential for Yeast Cell Viability*

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The Saccharomyces cerevisiae gene encoding the smallest RNA polymerase II subunit, RPBlO, was isolated and sequenced. The gene for this subunit is present in single copy and maps to chromosome XV, where two other yeast RNA polymerase II subunits, RPB2 and RPB8, reside. The RPBlO sequence predicts a protein only 46 amino acids in length with a molecular size of 5400 daltons. All but three of these polypeptides appear to be unique to RNA polymerase II. The 27-, 23-, and 14.5-kDa subunits, encoded by RPBl-10, whose apparent molecular masses are 220, 150, 44.5, 32, 27, 23, 16, 14.5, 12.6, and 10 kilodaltons. Sporulation and tetr analysis of diploid cells containing one copy of the RPBlO gene and one copy of HIS3 in place of the RPBlO gene revealed that the RPBlO subunit is essential for viability.

The Saccharomyces cerevisiae nuclear RNA polymerases are among the best studied eukaryotic RNA polymerases. These enzymes are each composed of 10–13 polypeptides (Sentenac, 1985). RNA polymerase II is composed of 10 subunits, encoded by RPBl–10, whose apparent molecular sizes are 220, 150, 44.5, 32, 27, 23, 16, 14.5, 12.6, and 10 kilodaltons. All but three of these polypeptides appear to be unique to RNA polymerase II. The 27-, 23-, and 14.5-kDa polypeptides are shared by all three nuclear RNA polymerases (Buhler et al., 1976; Breant et al., 1983; Woychik et al., 1980).

RNA polymerase II subunit composition and sequence appear to be well conserved among eukaryotes (Sentenac, 1985; Allison et al., 1985; Memet et al., 1988).

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To understand better the structure and function of eukaryotic RNA polymerases, the genes that encode S. cerevisiae RNA polymerase II subunits are being isolated and used to examine the roles of subunits in mRNA transcription. Thus far, the genes for the yeast RNA polymerase II subunit RPBl (Young and Davis, 1983; Ingles et al., 1984; Allison et al., 1985); RPB2 (Sweetser et al., 1987); RPB3 (Kolodziej and Young, 1989); RPB4 (Woychik and Young, 1989); and RPB5, RPB6, and RPB8 (Woychik et al., 1990) have been isolated and sequenced. In this paper we describe the isolation and sequence of the smallest RNA polymerase II subunit, RPBlO, as well as data that demonstrate that it is essential for cell viability.

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Isolation of the RPB10 Gene—The strategy for isolation of the RPB10 gene involved the purification of yeast RNA polymerase II followed by peptide sequence analysis from the amino-terminal end of the RPB10 subunit. Microsequence analysis was performed on RPB10 protein that had been subjected to SDS-PAGE and subsequently Western blotted to synthetic membrane. The peptide sequence obtained from synthetic membrane was compared to the predicted amino acid sequence of the RPB10 subunit. Microsequence analysis confirmed that the purified RPB10 protein contained the amino-terminal end of the RPB10 subunit.

RESULTS

Isolation of the RPB10 Gene—The strategy for isolation of the RPB10 gene involved the purification of yeast RNA polymerase II followed by peptide sequence analysis from the amino-terminal end of the RPB10 subunit. Microsequence analysis was performed on RPB10 protein that had been subjected to SDS-PAGE and subsequently Western blotted to synthetic membrane. The peptide sequence obtained from RPB10 was used to design an 80-mer DNA oligonucleotide probe.

Copy Number Analysis—Hybridization and wash conditions were as described by Davis et al. (1980). Baked nitrocellulose filters containing denatured double-stranded plasmid DNA primers were performed using denatured double-stranded plasmid DNA (Chen and Seeburg, 1985) as suggested by the manual for the Sequetimator version 2.0 DNA sequencing kit (U.S. Biochemical). The plasmid construct used for sequencing was pRP10/3. Sequences were determined for both strands of DNA. Computer analyses of the sequences were carried out using the FASTA program (Pearson and Lipman, 1988) to search the protein databases, and PREDICT89 to determine amino acid content, molecular mass, and isoelectric points (Robert Stroud, University of California, San Francisco).

Copy Number Analysis—Hybridization and wash conditions were as described by Davis et al. (1980). Baked nitrocellulose filters containing denatured genomic DNA restriction fragments were prehybridized for 1 h at 37°C in a solution containing 5 X SSPE + 0.3% SDS (20 X SSPE consists of 3.6 M sodium chloride, 0.2 M sodium phosphate, 0.16 M sodium hydroxide, and 20 mM EDTA), 100 µg/ml denatured salmon sperm DNA, and 50% formamide. Denatured radiolabeled probe was added to the hybridization mix, and the filter was hybridized at 42°C overnight. Filters were washed at 45°C in 2 X SSPE plus 0.2% SDS for 1 h. For copy number analysis of RPB10, the radiolabeled probe was made using the BamHI/StyI fragment shown in Fig. 1.

Chromosomal Localization—S. cerevisiae chromosomes were isolated and separated by pulsed field electrophoresis using the Geneline System (Beckman Instruments). Samples were prepared and electrophoresed following the recommendations of the manufacturer. Southern analysis of the blotted DNA was performed as described above, using the same BamHI/StyI RPB10 DNA fragment as a radioactive probe.

Construction of the RPB10 Deletion—rpb10-1:HIS3 was constructed by replacement of the entire RPB10 coding region of pRP10/3 with a synthetic RPB10 plasmid containing the HIS3 gene. The resulting plasmid, pRP10/3, was used to transform the yeast diploid isolate Z321. Genomic DNA was prepared (Sherman et al., 1986) from the His+ transformant 2431 and subjected to Southern analysis to verify the substitution of the chromosomal copy of RPB10 with HIS3. Z321 cells were sporulated and subjected to tetrad analysis.

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The RPB10 DNA probe was found to produce signals with the yeast genome under a variety of hybridization conditions.
with the RPBlO oligonucleotide probe, and signal-producing peptide was found within one of the open reading frames. The transformed with this DNA, the plasmid library was screened for DNA of interest was included within a 1.5-kb PstI/SphI fragment. Approximately 400 colonies containing S. cerevisiae genomic DNA digested with a variety of restriction enzymes were probed with the RPBlO-specific oligonucleotide. The RPBlO oligonucleotide hybridized to a 3.0-kb PstI DNA fragment. The SphI fragment containing the RPBlO coding sequence revealed that the 2.5-kb insert DNA was enriched in restriction digests of S. cerevisiae genomic DNA digested with a variety of restriction enzymes. Southern analysis of restriction digests of this insert DNA using the oligonucleotide probe did not change over a range of hybridization and wash conditions and was the same as that observed with the oligonucleotide probe used for gene isolation and characterization. The RPBlO gene was localized to chromosome XV by probing a Southern blot containing S. cerevisiae genomic DNA with gene-specific DNA fragments (Fig. 5). Two other RNA polymerase II subunit genes, RPB2 and RPB8, have also been mapped to chromosome XV (Scafe et al., 1990; Woychik et al., 1990). Examination of predicted amino acid sequences verified that RPBlO has a potential metal-binding domain, C-X-X-H, where X is any amino acid. Previous studies of the iron-binding protein rubredoxin revealed that two of these sequence motifs are involved in binding one Fe⁺ (Adman et al., 1975). Although this metal-binding sequence motif occurs rarely among proteins in the database, it is found in the amino terminus of eukaryotic and prokaryotic RNA polymerases (Fig. 3).

Copy Number and Chromosomal Location of Common Subunit Genes—Southern blots containing immobilized restriction digestes of S. cerevisiae genomic DNA were probed with RPBlO DNA fragments at moderate stringency as described under "Experimental Procedures" (Fig. 4). The pattern of hybridization in which only a single band producing a strong signal was observed, indicated that RPBlO is a single copy gene in haploid yeast. The pattern of hybridization obtained with the RPBlO DNA probe did not change over a range of hybridization and wash conditions and was the same as that obtained with the oligonucleotide probe used for gene isolation and characterization.

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RPBlO Is Essential for Cell Viability—Most, but not all, of the RNA polymerase II subunits studied thus far are essential for yeast cell viability (Nonet et al., 1987; Sweetser et al., 1987; Kolodziej and Young, 1989; Woychik and Young, 1989; Woychik et al., 1990). To determine whether RPBlO is essential for cell viability, the gene was replaced with the yeast nutritional marker HIS3. The entire protein coding region of RPBlO was first deleted and replaced with two unique restriction sites using oligonucleotide-directed mutagenesis (Kunkel, 1985). A HIS3 DNA fragment was inserted into the newly created restriction sites to produce the rpblOΔ1::HIS3 allele. One chromosomal copy of the gene in diploid yeast cells was replaced using the method of Rothstein (1983). This method relies on homologous recombination of RPBlO flanking DNA with the chromosomal DNA, resulting in the replacement of search of conventional databases did not reveal the existence of any protein sequences significantly similar to RPBlO. However, RPBlO has a potential metal-binding domain, C-X-X-H, where X is any amino acid. Previous studies of the iron-binding protein rubredoxin revealed that two of these sequence motifs are involved in binding one Fe⁺ (Adman et al., 1975). Although this metal-binding sequence motif occurs rarely among proteins in the database, it is found in the amino terminus of eukaryotic and prokaryotic RNA polymerases (Fig. 3).

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one of the chromosomal copies of the subunit gene with a selectable marker. The diploid cells obtained by this approach have 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 the deletion of RPBlO produces nonviable haploid cells (Table II). Therefore, RPBlO is essential for cell viability.

**DISCUSSION**

RPBlO is the smallest of the 10 RNA polymerase II subunits, consisting of 46 amino acids with a molecular mass of 5.4 kDa. It is a single copy gene located on chromosome XV. Despite its size, the RPBlO subunit is required for cell viability.

Eight of the 10 RNA polymerase II subunits have now been identified (Table I). The two largest subunits, RPBl and RPB2, are homologs of the bacterial RNA polymerase subunits, β' and β, respectively. The two large subunits of eukaryotic and prokaryotic RNA polymerases can bind DNA and nucleoside triphosphate substrates and are thought to contain the catalytic site (Chamberlin, 1982; Yura, T., and Ishihama, A. (1979) Annu. Rev. Genet. 13, 59-97). Amino acid sequence similarity between RPB3 and the bacterial α-subunit coupled with the fact that the behavior of RPB3 assembly mutants parallels that of α-subunit assembly mutants suggests that RPB3 is an α homolog. The other RNA polymerase II subunits do not exhibit extensive sequence similarity to other prokaryotic RNA polymerase subunits. All but one of the eight yeast RNA polymerase II subunits isolated to date are essential for cell viability. The exception, RPB4, is not required for viability but is essential for high and low temperature yeast cell growth.

RPBlO is among the RNA polymerase II polypeptides that play a critical role in transcription. The function of RPBlO is unclear, but the constraints of its size would appear to limit its functional potential. It seems likely that RPBlO plays either a structural role or acts as an accessory to the function of other subunits. Precisely defining the function of RPBlO will require further molecular genetic approaches.

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