Subunits shared by eukaryotic nuclear RNA polymerases

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RNA polymerases I, II, and III share three subunits that are immunologically and biochemically indistinguishable. The Saccharomyces cerevisiae genes that encode these subunits (RPB5, RPB6, and RPB8) were isolated and sequenced, and their transcriptional start sites were deduced. RPB5 encodes a 25-kD protein, RPB6, an 18-kD protein, and RPB8, a 16-kD protein. These genes are single copy, reside on different chromosomes, and are essential for viability. The fact that the genes are single copy, corroborates previous evidence suggesting that each of the common subunits is identical in RNA polymerases I, II, and III. Furthermore, immunoprecipitation of RPB6 coprecipitates proteins whose sizes are consistent with RNA polymerase I, II, and III subunits. Sequence similarity between the yeast RPB5 protein and a previously characterized human RNA polymerase subunit demonstrates that the common subunits of the nuclear RNA polymerases are well conserved among eukaryotes. The presence of these conserved and essential subunits in all three nuclear RNA polymerases and the absence of recognizable sequence motifs for DNA and nucleoside triphosphate-binding indicate that the common subunits do not have a catalytic role but are important for a function shared by the RNA polymerases such as transcriptional efficiency, nuclear localization, enzyme stability, or coordinate regulation of rRNA, mRNA, and tRNA synthesis.

[Key Words: RNA polymerase; subunit; transcription; Saccharomyces cerevisiae]

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Eukaryotic RNA polymerases I, II, and III are highly conserved enzymes that are responsible for rRNA, pre-mRNA, and small stable RNA synthesis, respectively (Lewis and Burgess 1982; Sentenac 1985). Although these nuclear RNA polymerases differ in promoter utilization, their shared functions are as noteworthy as their differences. The RNA polymerases are multisubunit enzymes that must properly initiate, elongate, and terminate the synthesis of RNA in a template-dependent fashion.

The common functions of the three RNA polymerases are reflected in features that are shared by these enzymes. The two large subunits of RNA polymerase I are related in size and sequence to the two large subunits of RNA polymerases II and III (Allison et al. 1985; Memet et al. 1988a). Considerable effort has gone into studying these two RNA polymerase subunits because they are thought to have important roles in transcription initiation and because the genes that encode them were among the first subunit genes to be isolated from the yeast Saccharomyces cerevisiae (Young and Davis 1983; Ingles et al. 1984; Allison et al. 1985; Riva et al. 1986; Sweetser et al. 1987; Memet et al. 1988b), Drosophila (Searles et al. 1982; Greenleaf 1983; Biggs et al. 1985; Faust et al. 1986; Falkenburg et al. 1987), and mammals (Cho et al. 1985; Corden et al. 1985; Ahearn et al. 1987). The other major feature shared by the nuclear RNA polymerases is a set of three subunits that appear to be common to the three enzymes in all eukaryotes examined thus far (Sentenac 1985). The structure and function of these three subunits are poorly defined, in part, due to the fact that the genes that encode them have not yet been described.

The S. cerevisiae nuclear RNA polymerases are among the best studied eukaryotic RNA polymerases. These enzymes are each composed of 10–13 polypeptides (Sentenac 1985). The three subunits that appear to be shared by the nuclear RNA polymerases, with apparent molecular masses of 27, 23, and 14.5 kD, are referred to here as RPB5, RPB6, and RPB8, respectively. Whether isolated from RNA polymerase I, II, or III, any one of the common subunits appears to be identical in all three enzymes by SDS-polyacrylamide gel mobility, fingerprint patterns (Buhler et al. 1976b, Valenzuela et al. 1976), isoelectric point (Buhler et al. 1976b), and antigenic recognition (Buhler et al. 1980, Huet et al. 1982, Brent et al. 1983). However, it is not yet clear whether each of the common subunits is actually identical or just very closely related.

To better understand the structure and function of eukaryotic RNA polymerases, the genes that encode S. cerevisiae RNA polymerase subunits are being isolated and used to examine the roles of subunits in transcription (Young and Davis 1983; Ingles et al. 1984; Allison et al.
Table 1. DNA oligonucleotides

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene-specific oligonucleotides (5'→3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPB5</td>
<td>1. TT[T/C]-GTI-ATI-CA[T/C]-ATI-CA[A/G]GA[A/G]-AA</td>
</tr>
<tr>
<td></td>
<td>2. GCI-GA[T/C]-CCI-GTI-GCI-ITI-TA</td>
</tr>
<tr>
<td></td>
<td>2. ATG-AAA[T/C]-CCI-GTI-TTI-TTI-CCI-CGI-ITI-GA[A/G]-GA</td>
</tr>
<tr>
<td>RPB8</td>
<td>1. TGG-IGI-CCI-CA[A/G]-GGI-GGI-GA</td>
</tr>
</tbody>
</table>
RPB6

The RPB6 DNA probes were found to produce signals with λEMBL3a vector DNA; to avoid this problem, RPB6 was cloned from a yeast plasmid sublibrary. A plasmid library enriched in RPB6 DNA was constructed. To deduce which genomic DNA restriction fragments were large enough to likely contain the entire RPB6 gene, Southern blots containing S. cerevisiae genomic DNA digested with a variety of restriction enzymes were probed with the RPB6-specific oligonucleotides. Both RPB6 oligonucleotides hybridized to a 1.5-kb HindIII fragment. S. cerevisiae genomic DNA was digested with HindIII, and DNA fragments in the range of 1–2 kb were gel-purified and ligated to HindIII-cut pBluescript II KS+ DNA. *Escherichia coli* cells were transformed with this DNA, the plasmid library was screened with the RPB6-specific oligonucleotides, and signal-producing colonies were isolated. One positive clone was obtained from ~800 colonies. Plasmid prepared from this clone contained a 1.5-kb HindIII DNA fragment.

The sequence of the entire 1.5-kb HindIII DNA fragment was obtained by sequencing both strands, and a single open reading frame predicted a protein containing the two amino acid sequences obtained from RPB6 tryptic peptides [Fig. 3]. However, this open reading frame did not encode any methionine residues upstream of the coding sequence for the first of the two peptide sequences. This observation led to the suspicion that RPB6 mRNA might be spliced.

Two observations confirm that RPB6 contains an intervening sequence. First, the yeast splice signal sequence 5′-TACTAAC-3′ occurs upstream of the open reading frame obtained from the genomic DNA clone. This sequence is preceded by a potential 5′ splice junction donor sequence 5′-GTATGT-3′ and is followed by a 3′ splice acceptor junction 5′-CAG-3′. 16 nucleotides downstream, suggesting an intron of 76 bp [Fig. 4A]. Second, reverse transcriptase-catalyzed primer extension of both wild-type and ma2-1 poly[A]+ mRNA demonstrated that unspliced RPB6 precursor RNAs accumulate in an ma2-1 strain [Fig. 4B]. *S. cerevisiae* ma2-1 mutants are temperature-sensitive for pre-mRNA splicing at 37°C. Primer extension of RNA from wild-type yeast indicates that RPB6 mRNA has three different 5′ ends [Fig. 4B]. RNA was isolated from ma2-1 cells grown at the permissive temperature (23°C) or shifted to the nonpermissive temperature (37°C) for 1 hr. Primer extension of RPB6 poly[A]+ RNA from ma2-1 cells grown at the nonpermissive temperature revealed the presence of three RNAs ~76 nucleotides longer than each of the wild-type mRNAs [Fig. 4B, ma2-1 37°C]. In contrast, primer extension of wild-type RNA or RNA prepared from ma2-1 cells grown at permissive temperature did not reveal the three RPB6 pre-mRNAs [Fig. 4B, wild-type and ma2-1 23°C]. Additional primer extension experiments using two unique DNA oligonucleotides as primers confirmed the results shown in Figure 4B. The presence of these three pre-mRNAs in mutant ma2-1 cells verifies the presence of a 76-bp intron near the beginning of the RPB6 gene.

The sequence of the RPB6 gene, the positions of the RPB6 transcript start sites, and the predicted amino acid sequence of the RNA polymerase subunit are shown in Figure 3. The spliced mRNA is translated into a 155-residue protein with a molecular mass of 17,857 daltons. This is considerably less than the apparent molecular mass of 23 kD estimated by SDS-PAGE. However, this subunit is phosphorylated [Buhler et al. 1976a; Bell et al. 1977], and phosphorylated proteins often migrate aberrantly on SDS-polyacrylamide gels. The RPB6 protein does not appear similar to any protein sequence accessible through conventional data bases.

RPB8

A plasmid library enriched for RPB8 DNA was constructed to facilitate the isolation of the gene. Southern analysis of restriction enzyme-digested *S. cerevisiae* genomic DNA showed that the RPB8-specific oligonucleotide probes [Table 1] hybridized to a 7-kb EcoRI fragment. Yeast genomic DNA was digested with EcoRI, 6- to 8-kb DNA fragments were purified and ligated to pBluescript II KS+ cleaved with EcoRI, and ~800 *E. coli* transformants were screened with the RPB8 probe. Restriction mapping of plasmid DNA from the three positive clones selected revealed that each contained a 7-kb EcoRI insert with identical internal restriction sites. The DNA sequence of one of these clones was determined, and the predicted RPB8 amino acid sequence was found to contain the three peptide sequences obtained from proteolytic fragments of RPB8 [Fig. 5]. The RPB8 mRNA start site was deduced from primer extension analysis of poly[A]+ RNA. The predicted RPB8 protein is 146 amino acids long and has a molecular mass of 16,468 daltons,
similar to the molecular mass of 14.5 kD estimated by SDS-PAGE. Computer search of conventional data bases did not reveal the existence of any protein sequences significantly similar to RPB8.

Copy number and chromosomal location of common subunit genes

Southern blots containing immobilized restriction digests of *S. cerevisiae* genomic DNA were probed with RPB5, RPB6, and RPB8 DNA fragments at moderate stringency, as described in Methods (Fig. 6). The pattern of hybridization with each probe, in which only a single band producing a strong signal was observed, indicated that RPB5, RPB6, and RPB8 are single-copy genes in haploid yeast. The pattern of hybridization obtained with RPB5, RPB6, and RPB8 DNA fragment probes did not change over a range of hybridization and wash conditions and was the same as that obtained with the oligonucleotide probes used for gene isolation and characterization.

The RPB5 gene was localized to chromosome II, RPB6 to chromosome XVI, and RPB8 to chromosome XV by probing a Southern blot containing *S. cerevisiae* chromosome separated by pulsed-field electrophoresis with gene-specific DNA fragment (not shown).

Figure 3. Sequence of RPB6 DNA and the predicted amino acid sequence of the RPB6 subunit. The tryptic peptides IVTGGNGPEDFQQ and LQISMNAPVFVDLEGET are underlined. The approximate mRNA start sites (±1) are underlined and marked with an arrow. The 76-bp intron is boxed.

Figure 4. Identification of a 76-bp intron near the amino terminus of the RPB6 gene. [A] Position and features of the 76-nucleotide RPB6 intervening sequence. The 5' splice donor sequence, splice signal sequence, and 3' splice junction are boxed. The start sites (±1) of the three RPB6 transcripts are numbered. [B] Primer extension analysis of wild-type and ma2-1 poly[A]+ RNA. The end-labeled DNA oligonucleotide 5'-CTCTCTATCGGAAATATGCTCATCTACATC-3' was annealed to wild-type poly[A]+ RNA (wild-type) or ma2-1 poly(A)+ RNA prepared from cells grown at the permissive temperature (ma2-1, 23°C), or shifted to the nonpermissive temperature (37°C) for 1 hr (ma2-1, 37°C). After reverse transcriptase-catalyzed DNA synthesis, equivalent amounts of each reaction were loaded adjacent to sequencing reactions primed with the same oligonucleotide used for primer extension. The three transcript start sites correspond to those represented in A. Each lane represents the primer extension products obtained using 0.7 pmole of labeled oligonucleotide annealed to 5 μg of poly(A)+ RNA. The sequencing reactions were run adjacent to the primer extension products to accurately determine transcript lengths.
Figure 5. Sequence of RPB8 DNA and the predicted amino acid sequence of the RPB8 subunit. The trypic peptides SWRPPQAGDR, LADDYDYVM, and NLNNLKQENA are underlined. The approximate mRNA start sites (±1), determined by primer extension, are underlined and marked with an arrow.

Immunoprecipitation demonstration that the RPB6 gene product is a component of RNA polymerases I, II, and III

The fact that RPB5, RPB6, and RPB8 are single-copy genes and that closely related sequences were not detected by hybridization to whole genome Southern blots indicates that each of the common subunits is identical in the three nuclear RNA polymerases. To confirm that the product of one of these genes, RPB6, is actually incorporated into all three nuclear RNA polymerases, we used immunoprecipitation of specific RNA polymerase subunits to investigate their association with the three nuclear RNA polymerases. An influenza hemagglutinin epitope-sequencing code (Field et al. 1988) was added to the amino-terminal sequence of RPB3, which encodes a subunit unique to RNA polymerase II (Kolodziej and Young 1989), and to the amino-terminal-sequencing code of RPB6. The modified RPB3 and RPB6 gene products complement their respective deletion mutations, and cells containing either epitope-tagged protein grow at wild-type rates throughout the normal temperature range for wild-type cells.

Immunoprecipitation of epitope-tagged RPB3 from [35S]methionine-labeled extracts results in the coprecipitation of the 10 subunits expected for RNA polymerase II (Fig. 7; Sentenac 1985). In contrast, immunoprecipitation of epitope-tagged RPB6 from [35S]methionine-labeled extracts not only results in the precipitation of the 10 RNA polymerase II subunits but also in polypeptides whose sizes are consistent with those described for the larger subunits of yeast RNA polymerases I and III (Sentenac 1985). These large RNA polymerase polypeptides have mobilities consistent with RPA1 (190 kD), RPA2 (135 kD), RPA4 (43 kD), and RPAC5 (40 kD) and with RPC1 (160 kD), RPC2 (128 kD), and RPC3 (82 kD). A shorter exposure allows the resolution of RPC1 and RPB2. With the exception of the common subunits RPB5, RPB6, and RPB8, the smaller subunits described for RNA polymerases I and III are not clearly observed in this experiment. This is due to the appearance of lower levels of RNA polymerases I and III relative to RNA polymerase II and to the small number of methionine residues in the smaller proteins in Figure 7. These results indicate that the RPB6 gene product is assembled with the RPA1, RPA2, RPA4, and RPAC5 subunits.

All three RNA polymerase common subunits are essential for cell viability

Most, but not all, of the RNA polymerase subunit genes 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). To determine whether the common subunits are essential for cell viability, each of the common subunit genes was replaced with a yeast nutritional marker. One chromosomal copy
Discussion

We have isolated and characterized the genes that encode the three subunits common to nuclear RNA polymerases in *S. cerevisiae*. We find that RPB5, RPB6, and RPB8 are single-copy genes that reside on chromosomes II, XVI, and XV, respectively. Although the RPB5, RPB6, and RPB8 subunits of RNA polymerase II appear, by a variety of criteria, to be identical to their counterparts in RNA polymerases I and III (Buhler et al. 1976b, 1980; Valenzuela et al. 1976; Huet et al. 1982; Breant et al. 1982), the mobility of the RPB6 subunit is slightly shifted. This shift is due to the addition of the influenza hemagglutinin epitope.

of each gene in diploid yeast cells was replaced using the method of Rothstein (1983). This method relies on homologous recombination of *RPB5*, *RPB6*, or *RPB8*-flanking DNA with the chromosomal DNA, resulting in the replacement of the chromosomal copy of the subunit gene with a selectable marker. Approximately two-thirds of the *RPB5* gene was removed and replaced with the *HIS3* gene to produce the allele *rpb5A1 :: HIS3* (Fig. 8A). The entire protein-coding regions of *RPB6* and *RPB8* were replaced by *URA3* [rpβ6Δ1 :: *URA3*] (Fig. 8B) and *LYS2* [rpβ8Δ1 :: *LYS2*] (Fig. 8C), respectively. 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 either *RPB5*, *RPB6*, or *RPB8* produces nonviable haploid cells (Table 2). Therefore, all three of the common subunit genes are essential for cell viability.

Figure 7. Immunoprecipitation of epitope-tagged RPB6 and RPB3. (Lane RPB6) Immunoprecipitation of RNA polymerases I, II, and III using epitope-tagged RPB6 [RPB6*]. RPA1, RPA2, and RPA4 represent bands with mobilities of the large RNA polymerase I subunits; RPC1, RPC2, and RPC3 indicate bands with mobilities of RNA polymerase III subunits. RPAC5 is a subunit common to both RNA polymerase I and III. (Lane RPB3) Immunoprecipitation of RNA polymerase II subunits with epitope-tagged RPB3 [RPB3*]. Band ACTD is a proteolytic product of the RPB1 subunit that lacks the 26-heptapeptide repeat units at the carboxyl terminus of the protein. The mobility shifts seen for the RPB3* and RPB6* subunits are due to the addition of the 9-amino-acid influenza hemagglutinin epitope. The apparent molecular masses of the RNA polymerase II subunits are 220 kD [RPB1], 150 kD [RPB2], 44.5 kD [RPB3], 32 kD [RPB4], 27 kD [RPB5], 23 kD [RPB6], 16 kD [RPB7], 14.5 kD [RPB8], 12.6 kD [RPB9], and 10 kD [RPB10].

Figure 8. Construction of *RPB5*, *RPB6*, and *RPB8* gene disruptions. The boxed regions indicate coding sequence. The numbers below each starting DNA fragment represent approximate length (in kb). The resulting gene replacements are not drawn to scale. The EcoRI restriction fragment containing *RPB5* DNA includes 65 bp of DNA 5' to the *RPB5*-coding sequence; this 65-bp region is within the 5'-untranslated leader of the *RPB5* mRNA.
Eukaryotic RNA polymerase common subunits

1983), it is not clear whether each of the common subunit proteins is encoded by a single gene or multiple related genes. The fact that RPB5, RPB6, and RPB8 are single-copy genes indicates that each of the common subunits is truly identical in the three nuclear RNA polymerases. In addition, the observation that epitope-tagged RPB6 protein coprecipitates with polypeptides described for RNA polymerases I, II, and III provides supporting evidence that the RPB6 gene product is assembled into all three nuclear RNA polymerases. Finally, the common subunit genes specify essential components of the eukaryotic RNA polymerases, as deletion of any one of these genes is lethal to haploid yeast cells.

The proteins encoded by RPB5, RPB6, and RPB8 have predicted molecular masses of 25,038, 17,857, and 16,468 daltons, respectively. RPB5 is a very basic protein with a pI of 10.15, whereas both RPB6 and RPB8 are quite acidic, with pI values of 5.15 and 4.28, respectively. The predicted molecular mass of RPB6 is somewhat less than that its apparent molecular mass of 23 kD, as estimated by SDS-PAGE (Sentenac 1985). RPB6 is phosphorylated (Buhler et al. 1976a; Bell et al. 1977), accounting for its reduced SDS-polyacrylamide gel mobility.

RNA polymerases I, II, and III share a variety of features and functions. They are large multisubunit enzymes composed of two very large subunits and 8-11 smaller proteins that include the three common subunits. Each of the RNA polymerases are assembled and transported into the nucleus. All three enzymes must recognize transcription factors that facilitate specific promoter recognition. The polymerases also bind template DNA and nucleoside triphosphate substrates and catalyze the template-dependent synthesis of RNA. Finally, these enzymes terminate RNA synthesis at or near specific sites, probably in concert with termination factors.

Which of the functions shared by the three nuclear RNA polymerases are carried out by the highly conserved two large subunits, and which are performed by the three common subunits? The two large subunits of prokaryotic RNA polymerases have the ability to interact with transcription factors, to bind DNA, to bind nucleoside triphosphate substrates, and to catalyze RNA synthesis (Yura and Ishihama 1979; Chamberlin 1982). The sequence similarity of the two large RNA polymerase subunits in eukaryotes and prokaryotes has led investigators to suggest that the two large subunits perform similar functions in eukaryotes and prokaryotes. Indeed, the two large eukaryotic RNA polymerase II subunits are capable of binding DNA and nucleoside triphosphates, and the second largest subunit is thought to contain the catalytic site (Cho and Kimball 1982; Carroll and Stollar 1983; Riva et al. 1987).

The sequences of the common subunits provide clues to their functions by helping to eliminate some possible functions. The common subunits lack known DNA-binding domains such as zinc fingers, leucine zippers, or helix-turn-helix motifs. They also lack nucleoside-binding motifs. Thus, these subunits are probably not directly involved in DNA or nucleoside triphosphate binding.

The common subunits are probably important for a function shared by the nuclear RNA polymerases such as transcriptional efficiency, nuclear localization, enzyme stability, or coordinate regulation of rRNA, mRNA, and tRNA synthesis. It is attractive to consider the possibility that the cell could coordinate a general increase or reduction in RNA synthesis via the common subunits. Modification of one or more of the common subunits might permit a rapid and general response to changes in cell cycle or growth conditions.

Seven yeast RNA polymerase II subunit genes have been isolated and characterized, including those for RNA polymerase common subunits. Table 3 summar-

### Table 2. Tetrad analysis of diploid cells with RPB5, RPB6, or RPB8 gene replacements

<table>
<thead>
<tr>
<th>Subunit</th>
<th>Genotype at target locus</th>
<th>Viable spores per tetrad&lt;br&gt;^a</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>RPB5&lt;br&gt;RPB5/Δ::HIS3</td>
<td>13</td>
<td>34</td>
<td>0</td>
</tr>
<tr>
<td>RPB6&lt;br&gt;RPB6/Δ::URA3</td>
<td>15</td>
<td>28</td>
<td>0</td>
</tr>
<tr>
<td>RPB8&lt;br&gt;RPB8/Δ::LYS2</td>
<td>12</td>
<td>22</td>
<td>0</td>
</tr>
</tbody>
</table>

^a All viable spores were His" for RPB5, Ura" for RPB6, or Lys" for RPB8.

### Table 3. Yeast RNA polymerase II subunit genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>SDS-PAGE mobility [kD]</th>
<th>Protein mass [kD]</th>
<th>Gene copy number</th>
<th>Chromosomal location</th>
<th>Deletion viability</th>
<th>Sequence similarity</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPB1&lt;br&gt;220</td>
<td>190</td>
<td>1</td>
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<td>inviable</td>
<td>β^a</td>
<td></td>
</tr>
<tr>
<td>RPB2&lt;br&gt;150</td>
<td>140</td>
<td>1</td>
<td>XV</td>
<td>inviable</td>
<td>β^b</td>
<td></td>
</tr>
<tr>
<td>RPB3&lt;br&gt;45</td>
<td>35</td>
<td>1</td>
<td>IX</td>
<td>inviable</td>
<td>RPC40^c</td>
<td></td>
</tr>
<tr>
<td>RPB4&lt;br&gt;32</td>
<td>25</td>
<td>1</td>
<td>X</td>
<td>conditional</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RPB5&lt;br&gt;27</td>
<td>25</td>
<td>1</td>
<td>II</td>
<td>inviable</td>
<td>23-kD human^d</td>
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</tr>
<tr>
<td>RPB6&lt;br&gt;23</td>
<td>18</td>
<td>1</td>
<td>XVI</td>
<td>inviable</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RPB8&lt;br&gt;14</td>
<td>16</td>
<td>1</td>
<td>XV</td>
<td>inviable</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

^a Allison et al. (1985).
^b Sweetser et al. (1987).
^c Mann et al. (1987).
izes the principal features of these genes and the proteins that they encode. Each of the subunits is encoded by a single-copy gene. All of the RNA polymerase II subunit genes are essential for viability except RPB4, whose deletion produces an enzyme that is thermally unstable. Although the two largest RNA polymerase II subunits have homologs in the prokaryotic RNA polymerase, the remaining eukaryotic subunits are not similar in sequence to the bacterial RNA polymerase subunits α or β. Further investigation will be required to define precisely the functions of each of the eukaryotic RNA polymerase subunits, and the application of molecular genetic approaches using cloned subunit genes should facilitate these studies.

**Methods**

**Yeast media**

Strains were grown on YPD medium [2% yeast extract, 1% Bacto-Peptone (Difco Laboratories, Detroit, Michigan), 2% glucose], YPD plates contain 2% agar. Dropout medium minus histidine, uracil, or lysine and low sulfate synthetic medium (LSM) has been described elsewhere (Julius et al. 1984; Nonet et al. 1987; Sherman et al. 1986). The ammonium sulfate concentration in LSM is 26 mg/liter. No sulfate medium (NSM) is LSM without ammonium sulfate.

**Yeast strains**

Yeast strains are listed in Table 3 (X2180-2) or S288C. Strain Z374, expressing an epitope-tagged RPB6 subunit, was constructed as follows. The plasmid pY2442 was introduced into Z320, a diploid heterozygous for the RPB6+ allele. 1-2 kb deletions were observed for sporulation of the Z320 derivatives and scored for complementation of the rpβ6Δ1 lethal phenotype. Leu+ cells with wild-type growth phenotypes were recovered, indicating that the amino-terminal modification did not adversely affect RPB6. Strain Z374, which carries pY2442, was derived from this dissection. Plasmids and yeast strains used for epitope-tagging experiments involving RPB3 are described in Kolodziej and Young (1989).

**Protein sequence analysis**

Amino acid sequence was obtained for purified X2180-2 S. cerevisiae RNA polymerase II subunits using the method of Aebi et al. (1987). Briefly, subunits were separated by SDS-PAGE, the polypeptides were electrophoretically transferred onto nitrocellulose as described by Matsudaira (1987), and the proteins were stained with Ponceau S to visualize individual subunit bands. Denaturation of nucleic acids was achieved by placing the filters in the autoclave for 2 min on the dry cycle. After baking in a 80°C vacuum oven for 1–2 hr, the filters were washed for 30 min in 2 x SSC (Duby et al. 1989) containing 0.1% SDS. All plasmids were probed with DNA oligonucleotides in sodium chloride/sodium citrate, as described by Duby et al. (1989).

**Plasmids**

RPB5 An 8-kb SalI fragment of DNA was the smallest fragment contained in aEMBL3a recombinant clones that hybridized to the RPB5 probes. An 8-kb SalI fragment containing RPB5 DNA was ligated into the SalI site in pBluescript II K5+ (Stratagene, San Diego, California), and the resulting plasmid was designated pRP51. The pRP56 plasmid contains the RPB5 gene in a 1.1-kb EcoRV–SpeI DNA fragment that has been ligated into the EcoRV–SpeI sites of pGem5.

**RPB6** A pBluescript II K5+ plasmid sublibrary was constructed with 1- to 2-kb HindIII yeast genomic DNA fragments. A clone isolated from this library, pRP61, was found to contain two HindIII fragments of 1.5 and 1.3 kb. The 1.5-kb HindIII fragment containing the RPB6 gene was isolated and ligated into pGem7, and plasmids containing both orientations of the insert were recovered, called pRP66 and pRP67. Plasmid pRP61 containing the epitope-tagged RPB6 gene was constructed by oligonucleotide-directed mutagenesis (Kunkel 1985) using the 61-mer oligonucleotide 5'-GGCTACAAGTGATCATGTACCCATACGACGTCCCA-

**RPB8** The positive clones pSL104 and pSL105 were isolated from a pBluescript II K5+ plasmid sublibrary. Both contained a 7.2-kb EcoRI insert. Plasmid pSL106 is pBluescript II K5+ with a 2.5-kb PstI–XbaI RPB8 DNA fragment derived from pSL104 inserted into the PstI and XbaI sites.

**DNA sequence and primer extension analysis**

All sequencing reactions with nondegenerate DNA primers were performed using denatured double-stranded plasmid DNA (Chen and Seeburg 1985), as suggested by the manual for the Sequenase Version 2.0 DNA Sequencing Kit (U.S. Biochemicals, Cleveland, Ohio). Sequencing reactions with the degenerate DNA oligonucleotides were carried out following the suggestions of Nichols and Dixon (1988), using the 5′S Sequencing Pack [New England Biolabs, Beverly, Massachusetts]. The plasmid constructs used for sequencing were pRP51, pRP61, and pSL104. Sequences were determined for both strands of DNA. Computer analysis of the sequences was carried out using the FASTA program (Pearson and Lipman 1988) to search the NBRF protein data base, ALIGN to align RPB5 and its human homolog, and PREDICT89 to determine amino acid content and isoelectric points (Robert Stroud, University of California at San Francisco).
poly(A) + RNA, followed by extension with reverse transcriptase (Williams and Mason 1985). Poly(A) + RNA was prepared of an end-labeled DNA oligonucleotide to
Hybridization and wash conditions were as described by Davis
denatured salmon sperm DNA, and 50% formamide. Den-
This probe included the entire
tide 385 to the
tides 50-698 (Fig. 1). The probe used for
was made from a 650-bp
BP3-coding region of pRP66 with a
 restriction site
Baker nitrocellulose filters containing blotted gene-
troduced plasmid was added, 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 hr.
Sources: This study.

Primer extension of the RPB5 transcripts involved annealing of an end-labeled DNA oligonucleotide to S. cerevisiae poly[A]^+ RNA, followed by extension with reverse transcriptase (Williams and Mason 1985). Poly[A]^+ RNA was prepared from yeast cells according to Elder et al. (1983).

Gene copy number: Southern analysis

Hybridization and wash conditions were as described by Davis et al. (1980). Baked nitrocellulose filters containing blotted genomic DNA restriction fragments were prehybridized for 1 hr at 37°C in a solution containing 5 x SSPE plus 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 radioabeled 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 hr.

For copy number analysis of RPB5, the radiolabeled probe was made from a 650-bp EcoRI fragment that includes nucleotides 50–698 [Fig. 1]. The probe used for RPB6 consisted of a 500-bp XmnI–HindIII fragment containing DNA from nucleotide 385 to the HindIII site 200 bp 3' of the RPB6-coding region [Fig. 3]. A 700-bp SphI fragment was used to probe RPB8 DNA. This probe included the entire RPB8-coding sequence plus 209 bp 5' and 48 bp 3' of the coding sequence.

Cell labeling
Cells [5 x 10^7] grown in LSM were harvested by centrifugation at 2000 rpm for 5 min, resuspended in 1 mCi of [35S]methionine [New England Nuclear, 686 Ci/mmole] and 12 µl of 5 x NSM, and incubated for 5 min at the appropriate temperature. One milliliter of LSM was added, and the culture was transferred to a disposable 125-ml Erlenmeyer flask. After incubation with shaking for 20 min, 10 ml of LSM was added, and growth continued for 95 min.

Immunoprecipitation of RNA polymerases
Immunoprecipitation with the 12CA5 antibody was performed as described [Kolodziej and Young 1989], except that immunoprecipitates were washed twice with buffer B [20 mM HEPES–NaOH [pH 7.9], 5% glycerol, 10 mM EDTA] containing 0.4 M ammonium sulfate and once with buffer B containing 0.05 M ammonium sulfate, prior to resuspension in loading buffer [Laemmli 1970]. Immunoprecipitates were examined by SDS-PAGE and fluorography as described [Kolodziej and Young 1989].

Construction of RPB5, RPB6, and RPB8 deletions
The rpb5Δ1 :: HIS3 allele was constructed by removing a 650-bp EcoRI fragment [which encodes approximately two-thirds of the RPB5 protein] from pRP56 and inserting a 1.8-kb HIS3 fragment. This plasmid is called pRP57. DNA containing the rpb5Δ1 :: HIS3 allele was removed from pRP57 by digestion with EcoRV and SpeI, and the yeast diploid Z321 was transferred with this DNA. Genomic DNA was prepared (Sherman et al. 1986) from the His^+ transformant Z319 and subjected to Southern analysis to verify the substitution of the chromosomal copy of RPB5 with RPB5Δ1 :: HIS3. Z319 cells were sporulated, and tetrad analysis was performed [Sherman et al. 1986].

rpb6Δ1 :: URA3 was constructed by replacement of the entire RPB6-coding region of pRP66 with a SalI restriction site using oligonucleotide-directed mutagenesis [Kunkel 1985]. URA3 was inserted into the SalI site as a Xhol DNA fragment created by addition of Xhol linkers to a 1.14-kb HindIII URA3 fragment. The resulting plasmid, pRP68, was cut with HindIII, and the URA3-containing fragment was used to transform Z321. The Ura^+ transformant Z320 was checked for appropriate replacement of RPB6 with rpb6Δ1 :: URA3, sporulated, and subjected to tetrad analysis.

rpb8Δ1 :: LYS2 was constructed by replacing the entire RPB8-coding region of pSL106 with adjacent Spbl and BamHI sites using oligonucleotide-directed mutagenesis to produce pSL111. LYS2 was inserted into the Spbl–BamHI site of pSL111 as a 5.9-kb Spbl–BamHI fragment. The resulting plasmid, pSL119, was cleaved with PstI and Xbol, and the DNA fragments were used to transform Z321. The Lys^+ transformant Z303 was checked for appropriate replacement of RPB8 with rpb8Δ1 :: LYS2, sporulated, and subjected to tetrad analysis.
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