Subunits shared by eukaryotic nuclear RNA polymerases

Nancy A. Woychik, Sha-Mei Liao, Peter A. Kolodziej, and Richard A. Young

Whitehead Institute for Biomedical Research, Cambridge, Massachusetts 02142 USA; Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139 USA

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*]

Received September 18, 1989; revised version accepted January 3, 1990.

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; Breant 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.

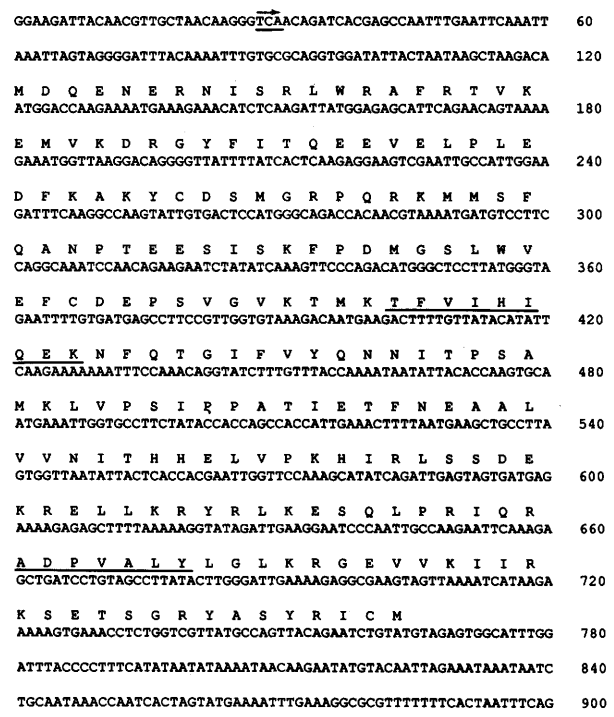

Figure 1. Sequence of *RPB5* DNA and the predicted amino acid sequence of the RPB5 subunit. The tryptic RPB5 peptides TFVIHIQEK and ADPVALY are underlined. The mRNA start site (±1) is underlined and marked with an arrow.

1985; Riva et al. 1986; Mann et al. 1987; Sweetser et al. 1987; Memet et al. 1988b; Kolodziej and Young 1989; Woychik and Young 1989). Here, we describe the isolation and characterization of genes that encode the three common subunits of yeast RNA polymerases I, II, and III.

Results

General strategy for isolation of the common subunit genes

Yeast RNA polymerase II was purified using a standard procedure (Valenzuela et al. 1978). The 27-, 23-, and 14.5-kD proteins (RPB5, RPB6, and RPB8) were isolated from SDS-polyacrylamide gels. Tryptic fragments of each of the three subunits were generated and separated by high-performance liquid chromatography (HPLC), and at least two peptide sequences were obtained from each subunit. The peptide sequences obtained for RPB5, RPB6, and RPB8 were used to design DNA oligonucleotides for use in isolating the RNA polymerase subunit genes (Table 1).

The oligonucleotides were labeled and used to probe Southern blots of *S. cerevisiae* genomic DNA cleaved with various restriction enzymes. The hybridization and wash conditions that produced the best signal-to-noise ratio were determined for each oligonucleotide.

RPB5

RPB5 was cloned from a λEMBL3a library of *S. cerevisiae* S288C genomic DNA. From six plates containing ~2×10^4 plaques, we selected a total of 10 λEMBL3a recombinant clones that produced positive signals with radiolabeled *RPB5* oligonucleotides. Each plate contained ~50 plaques that produced a strong positive signal. All 10 clones selected contained an 8-kb *Sal*I DNA restriction fragment that hybridized to both *RPB5* probes in a Southern blot. The 8-kb *Sal*I DNA fragment was subcloned into plasmid pBluescript KS$^+$ to produce pRP51, and this clone was subjected to sequence analysis using as primers the same oligonucleotides that were used as probes to isolate the λEMBL3a clones. The sequence confirmed that pRP51 contains *RPB5* DNA because the DNA sequence accurately predicts the RPB5 amino acid sequence determined by peptide sequencing. The *RPB5* sequence was then obtained for both DNA strands and is shown in Figure 1.

The *RPB5* transcription initiation start site was deduced by primer extension of *S. cerevisiae* poly(A)$^+$ RNA (Fig. 1). This information and the partial amino acid sequences obtained for RPB5 protein permitted unambiguous assignment of the RNA polymerase subunit-coding sequence. The *RPB5* sequence predicts a protein of 215 amino acid residues with a molecular mass of 25,038 daltons. This is only slightly less than the apparent molecular mass of 27 kD estimated by SDS-PAGE.

A human 23-kD RNA polymerase II subunit gene has recently been isolated and sequenced (Pati and Weissman 1989) and is a homolog of the yeast *RPB5* gene. The two proteins are similar in length. Yeast RPB5 consists of 215 amino acid residues, and its human relative contains 197 residues. Alignment of the amino acid sequences of the human and yeast RNA polymerase subunits revealed that 30% of the amino acids are identical (Fig. 2). An additional 26% of the residues are conservative amino acid replacements, scoring +1 or greater in the mutation data matrix of Dayoff et al. (1983).

Table 1. *DNA oligonucleotides*

Gene	Gene-specific oligonucleotides (5′ → 3′)
RPB5	1. TT(T/C)-GTI-ATI-CA(T/C)-ATI-CA(A/G)-GA(A/G)-AA 2. GCI-GA(T/C)-CCI-GTI-GCI-ITI-TA
RPB6	1. ATI-GTI-ACI-GGI-GGI-AA(T/C)-GGI-CCI-GA(A/G)-GA(T/C)-TT(T/C)-CA(A/G)-CA 2. ATG-AA(T/C)-GCI-CCI-GTI-TT(T/C)-GTI-GA(T/C)-ITI-GA(A/G)-CA(A/G)-GA
RPB8	1. TGG-IGI-CCI-CCI-CA(A/G)-GCI-GGI-GA

Figure 2. Sequence similarities between RPB5 and the 23-kD human RNA polymerase II HP23 subunit. Identical amino acid residues are boxed and shaded.

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 be likely to contain the entire *RPB6* gene, Southern blots containing *S. cerevisiae* genomic DNA digested with a variety of restriction enzymes were probed with the *RBP6*-specific oligonucleotides. Both *RBP6* oligonucleotides hybridized to a 1.5-kb *Hin*dIII fragment. *S. cerevisiae* genomic DNA was digested with *Hin*dIII, and DNA fragments in the range of 1–2 kb were gel-purified and ligated to *Hin*dIII-cut pBluescript II KS$^+$ DNA. *Escherichia coli* cells were transformed with this DNA, the plasmid library was screened with the RPB6 oligonucleotide probes, and signal-producing colonies were isolated. One positive clone was obtained from ~800 colonies. Plasmid prepared from this clone contained a 1.5-kb *Hin*dIII DNA fragment.

The sequence of the entire 1.5-kb *Hin*dIII 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 *RBP6* 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 *rna2-1* poly(A)$^+$ mRNA demonstrated that unspliced *RPB6* precursor RNAs accumulate in an *rna2-1* strain (Fig. 4B). *S. cerevisiae rna2-1* 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 *rna2-1* 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 *rna2-1* cells grown at the nonpermissive temperature revealed the presence of three RNAs ~76 nucleotides longer than each of the wild-type mRNAs (Fig. 4B, *rna2-1* 37°C). In contrast, primer extension of wild-type RNA or RNA prepared from *rna2-1* cells grown at permissive temperature did not reveal the three *RPB6* pre-mRNAs (Fig. 4B, wild-type and *rna2-1* 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 *rna2-1* 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 *Eco*RI fragment. Yeast genomic DNA was digested with *Eco*RI, 6- to 8-kb DNA fragments were purified and ligated to pBluescript II KS$^+$ cleaved with *Eco*RI, 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 *Eco*RI 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,

```
TTCGTATAGTATGATATTTAATTGAAAAAAAAATTTTTTTTTTTTAAGAATATCATTCA   60

AAGGCATCAATCACAACCTTGAAGAAAGGCTAAAAAGACACATTTTGCAGGTAACAGTGT  120

                    M  S  D  Y  E  E  A
AAAGATTAAGGCTACAAGTGATCATGTCAGACTACGAGGAGGGTATGTGCATTCATATT   180

GGAGAAATTGCGTGCTTTAGTTTTAATTATATTTACTAACTTCTATCATTGTGATACAGG  240

F  N  D  G  N  E  N  F  E  D  F  D  V  E  H  F  S  D  E  E
TTTAACGACGGTAATGAGAACTTCGAAGATTTTGATGTAGAGCATTTTTCTGATGAGGAG  300

T  Y  E  E  K  P  Q  F  K  D  G  E  T  T  D  A  N  G  K  T
ACTTATGAGGAAAAACCTCAATTCAAGGATGGTGAAACAACCGATGCCAACGGTAAGACC  360

I  V  T  G  G  N  G  P  E  D  F  Q  Q  H  E  Q  I  R  R  K
ATCGTTACTGGTGGTAATGGCCCAGAAGATTTTCAACAGCATGAGCAAATAAGAAGAAAG  420

T  L  K  E  K  A  I  P  K  D  Q  R  A  T  T  P  Y  M  T  K
ACACTTAAGGAAAAGGCCATCCCAAAAGACCAAAGAGCAACTACTCCATACATGACCAAA  480

Y  E  R  A  R  I  L  G  T  R  A  L  Q  I  S  M  N  A  P  V
TATGAAAGGGCAAGAATTTTAGGTACAAGAGCCCTACAAATTTCCATGAATGCGCCAGTT  540

F  V  D  L  E  G  E  T  D  P  L  R  I  A  M  K  E  L  A  E
TTTGTAGATTTAGAAGGTGAAACTGATCCATTGCGTATTGCCATGAAGGAGTTGGCTGAA  600

K  K  I  P  L  V  I  R  R  Y  L  P  D  G  S  F  E  D  W  S
AAGAAAATTCCTTTGGTTATTAGAAGATATTTACCAGATGGTTCCTTTGAGGACTGGAGT  660

V  E  E  L  I  V  D  L
GTGGAGGAACTCATTGTGGATTTGTGATTACACCTGCATTTTCCTTTATGTGTATTTGCT  720

TTGTTTGTATATTATTATAACTATAAAGAACGATTTATTTTGTAATGCAAATAACTGGAC  780
```

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.

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).

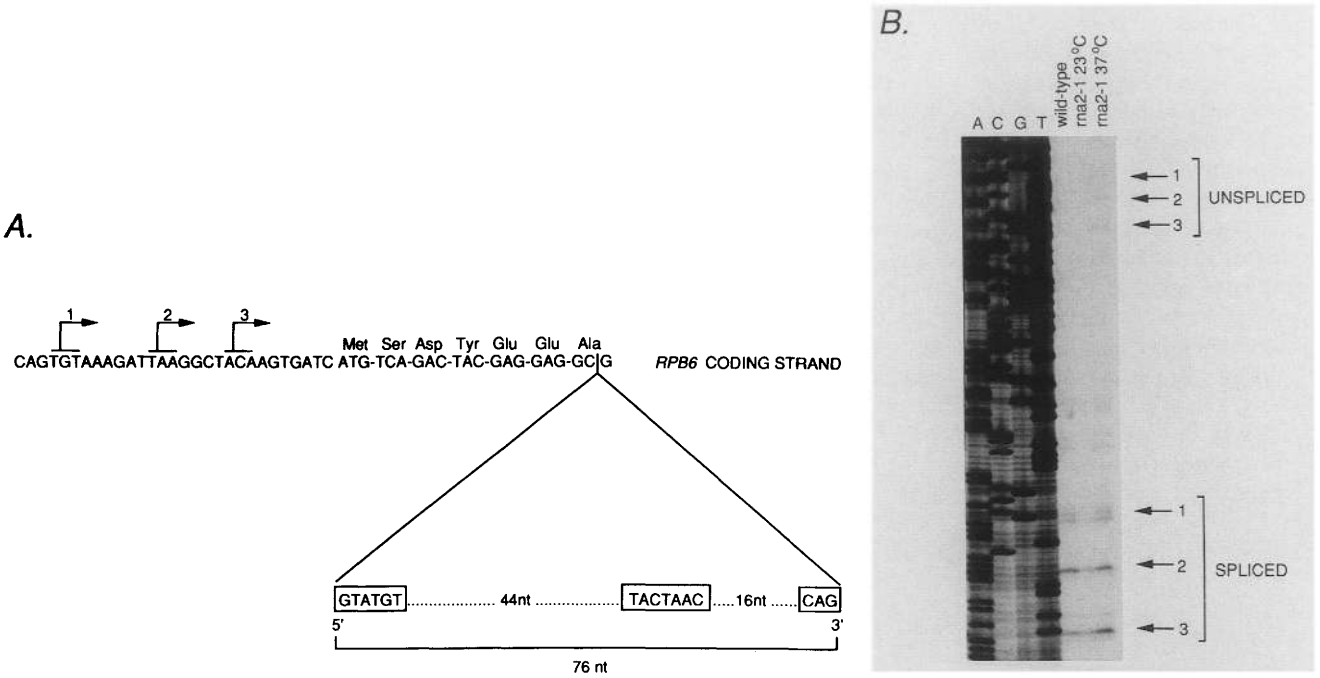

Figure 4. Identification of a 76-bp intron near the amino terminus of the *RPB6* gene. (*A*) Position and features of the 76-nucleotide *RPB6* intervening sequence. The 5′ splice donor sequence, splice signal sequence, and 3′ splice junction are boxed. The start sites (±1) of the three *RPB6* transcripts are numbered. (*B*) Primer extension analysis of wild-type and *rna2-1* poly(A)$^+$ RNA. The end-labeled DNA oligonucleotide 5′-CTCCTCATCAGAAAAATGCTCTACATC-3′ was annealed to wild-type poly(A)$^+$ RNA (wild-type) or *rna2-1* poly(A)$^+$ RNA prepared from cells grown at the permissive temperature (rna2-1, 23°C), or shifted to the nonpermissive temperature (37°C) for 1 hr (rna2-1, 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.

```
TTTTCACATTTTTTTTTTTCTAAAGCGAAAACTAAGATGAAAAATTTCAGAATATTTAAA   60

CTGTGATGGCAGCTATAAAAGCCCAGTTGCCAGCTCATTGCTTCCATAACAACCGCAGCA  120

M  S  N  T  L  F  D  D  I  F  Q  V  S  E  V  D  P  G  R  Y
ATGTCTAACACTCTATTTGATGATATATTCCAAGTCTCGGAAGTTGATCCCGGTCGTTAT  180

N  K  V  C  R  I  E  A  A  S  T  T  Q  D  Q  C  K  L  T  L
AACAAAGTCTGCCGTATCGAAGCCGCATCTACCACTCAGGACCAATGCAAACTAACCTTG  240

D  I  N  V  E  L  F  P  V  A  A  Q  D  S  L  T  V  T  I  A
GATATAAATGTTGAATTGTTTCCCGTCGCCGCACAAGATTCTTTGACAGTGACTATTGCA  300

S  S  L  N  L  E  D  T  P  A  N  D  S  S  A  T  R  S  W  R
TCCTCTTTAAACCTCGAAGACACCCCAGCTAACGACTCTTCTGCGACAAGAAGCTGGAGA  360

P  P  Q  A  G  D  R  S  L  A  D  D  Y  D  Y  V  M  Y  G  T
CCTCCACAGGCTGGTGACAGATCCCTTGCAGATGATTATGATTACGTCATGTATGGTACC  420

A  Y  K  F  E  E  V  S  K  D  L  I  A  V  Y  Y  S  F  G  G
GCTTACAAGTTTGAGGAAGTAAGCAAGGATCTAATTGCCGTTTACTACTCATTCGGTGGC  480

L  L  M  R  L  E  G  N  Y  R  N  L  N  N  L  K  Q  E  N  A
CTCTTAATGAGATTAGAAGGTAACTATAGAAATTTGAATAACTTGAAGCAAGAGAACGCT  540

Y  L  L  I  R  R
TATCTTTTGATTCGTCGTTAGCAGCATTGGACCGAGTGCTAAAAAAAAGGGCGATCACTT  600
```

Figure 5. Sequence of *RPB8* DNA and the predicted amino acid sequence of the RPB8 subunit. The tryptic 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-coding sequence (Field et al. 1988) was added to the amino-terminal coding sequence of *RPB3*, which encodes a subunit unique to RNA polymerase II (Kolodziej and Young 1989), and to the amino-terminal-coding sequence 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 [^{35}S]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 [^{35}S]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

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

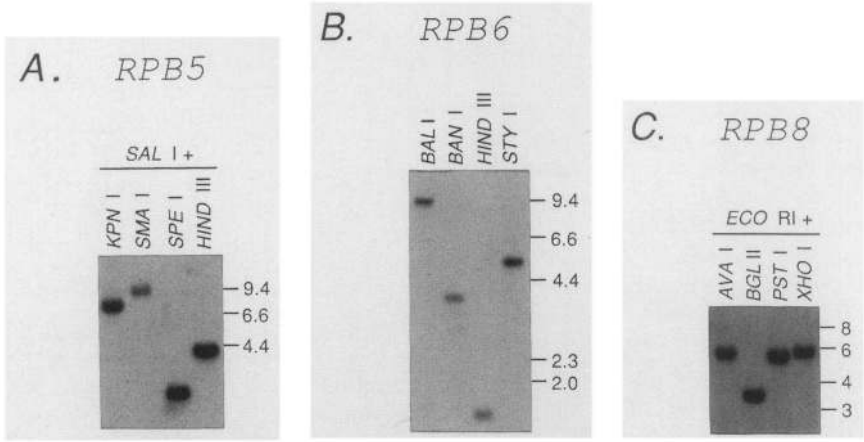

Figure 6. Copy number of the *RPB5* (*A*), *RPB6*, (*B*), and *RPB8* (*C*) genes. Genomic DNA prepared from haploid yeast cells was digested with the enzyme(s) indicated. DNA restriction fragments used to radiolabel probes are described in Methods. DNA size markers (in kb) are shown at *right*.

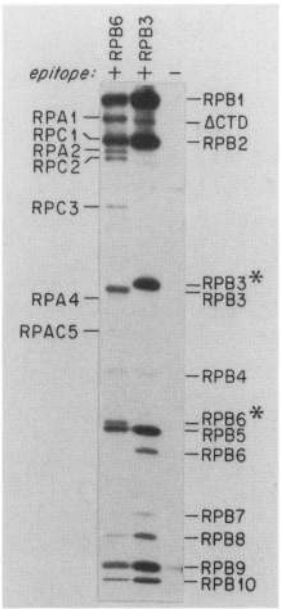

Figure 7. Immunoprecipitation of epitope-tagged RPB6 and RPB3. (Lane *RPB6*) Immunoprecipitation of RNA polymerases I, II, and III using epitope-tagged RPB6 (RPB6*). RPA1, RPA2, and RPA4 represent bands with mobilities of the large RNA polymerase I subunits; RPC1, RPC2, and RPC3 indicate bands with mobilities 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 ΔCTD 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).

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 *rpb5Δ1 :: HIS3* (Fig. 8A). The entire protein-coding regions of *RPB6* and *RPB8* were replaced by *URA3* (*rpb6Δ1 :: URA3*) (Fig. 8B) and *LYS2* (*rpb8Δ1 :: 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.

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.

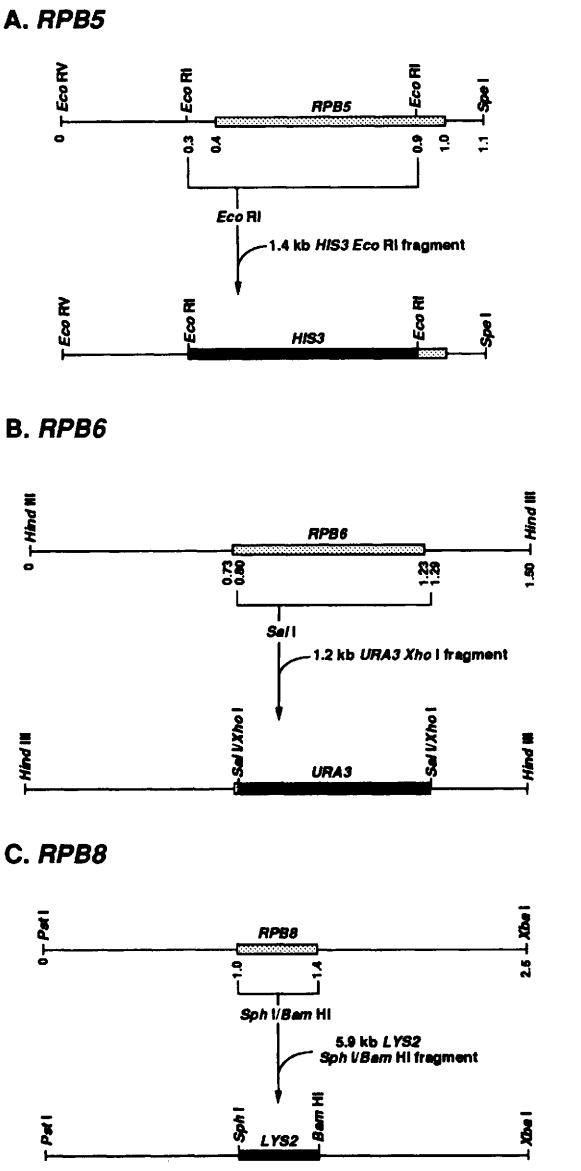

Figure 8. Construction of *RPB5*, *RPB6*, and *RPB8* gene disruptions. The boxed regions indicate coding sequence. The numbers below each starting DNA fragment represent approximate length (in kb). The resulting gene replacements are not drawn to scale. The *Eco*RI restriction fragment containing *RPB5* 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.

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

Subunit	Genotype at target locus	Viable spores per tetrad[a] 1	2	3	4
RPB5	*RPB5/rpb5Δ1 :: HIS3*	13	34	0	0
RPB6	*RPB6/rpb6Δ1 :: URA3*	15	28	0	0
RPB8	*RPB8/rpb8Δ1 :: LYS2*	12	22	0	0

[a]All viable spores were His$^-$ for *RPB5*, Ura$^-$ for *RPB6*, or Lys$^-$ for *RPB8*.

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 summa-

Table 3. *Yeast RNA polymerase II subunit genes*

Gene	SDS-PAGE mobility (kD)	Protein mass (kD)	Gene copy number	Chromosomal location	Deletion viability	Sequence similarity
RPB1	220	190	1	IV	inviable	β′[a]
RPB2	150	140	1	XV	inviable	β[b]
RPB3	45	35	1	IX	inviable	*RPC40*[c]
RPB4	32	25	1	X	conditional	
RPB5	27	25	1	II	inviable	23-kD human[d]
RPB6	23	18	1	XVI	inviable	
RPB8	14	16	1	XV	inviable	

[a]Allison et al. (1985).
[b]Sweetser et al. (1987).
[c]Mann et al. (1987).
[d]Pati and Weismann (1989).

rizes 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 σ. 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 contained 2% agar. Dropout medium minus histidine, uridine, 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 4. X2180 is the diploid derivate of 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 *rpb6Δ1* deletion mutant, in which *RPB6* is replaced by *URA3*. Tetrads were dissected after sporulation of the Z320 derivatives and scored for complementation of the *rpb6Δ1* lethal phenotype. Leu$^+$ Ura$^+$ 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 Aebersold et al. (1987). Briefly, subunits were separated by SDS-PAGE, the polypeptides were electroblotted onto nitrocellulose as described by Matsudaira (1987), and the proteins were stained with Ponceau S to visualize individual subunit bands. Nitrocellulose containing the blotted subunit protein was destained and digested with trypsin, and tryptic peptides were purified by HPLC. Amino acid sequence was obtained from chromatography column fractions containing individual peptides from trypsin-digested 27- (RPB5), 23- (RPB6), or 14.5-kD (RPB8) polypeptides.

Recombinant DNA libraries

The λEMBL3a yeast genomic library was a gift of M. Snyder (Stanford University School of Medicine, Stanford, California) and was constructed with DNA from *S. cerevisiae* S288C. Plasmid sublibraries were constructed with DNA from *S. cerevisiae* N306, which is a S288C derivative. Plating and filter lifts of bacteriophage libraries were performed according to Davis et al. (1980). Transfer of bacterial colonies to filter membranes was done by placing a dry nitrocellulose circle over the colonies on the surface of a chilled plate (4°C) for 1 min. The filter was removed and left to air-dry for 10 min. Lysis of the cells and denaturation of nucleic acids were 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× SSC (Duby et al. 1989) containing 0.1% SDS. All libraries were probed with DNA oligonucleotides in sodium chloride/sodium citrate, as described by Duby et al. (1989).

Plasmids

RPB5 An 8-kb *Sal*I DNA fragment was the smallest fragment contained in λEMBL3a recombinant clones that hybridized to the RPB5 probes. The 8-kb *Sal*I fragment containing *RPB5* DNA was ligated into the *Sal*I site in pBluescript II KS$^+$ (Stratagene, San Diego, California), and the resulting plasmid was designated pRP51. The pRP56 plasmid contains the *RPB5* gene in a 1.1-kb *Eco*RV–*Spe*I DNA fragment that has been ligated into the *Eco*RV–*Spe*I sites of pGem5.

RPB6 A pBluescript II KS$^+$ plasmid sublibrary was constructed with 1- to 2-kb *Hin*dIII yeast genomic DNA fragments. A clone isolated from this library, pRP61, was found to contain two *Hin*dIII DNA fragments of 1.5 and 1.3 kb. The 1.5-kb *Hin*dIII 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 pRP611 containing the epitope-tagged *RPB6* gene was constructed by oligonucleotide-directed mutagenesis (Kunkel 1985) of pRP66 using the 61-mer oligonucleotide 5′-GGCTACAAGTGATCATGTACCCATACGACGTCCCA-GACTACGCTTCAGACTACGAGGAGGC-3′, which includes the 9-amino-acid influenza hemagglutinin epitope-coding sequences (underlined) flanked by *RPB6* sequences. The 1.5-kb *Hin*dIII fragment was excised from pRP611 as a *Sac*I–*Xho*I fragment and inserted into the *Sac*I–*Xho*I-cut yeast centromere plasmid pRP315 (Sikorski and Hieter 1989) containing the selectable marker *LEU2* to yield plasmid pY2442.

RPB8 The positive clones pSL104 and pSL105 were isolated from a pBluescript II KS$^+$ plasmid sublibrary. Both contained a 7.2-kb *Eco*RI insert. Plasmid pSL106 is pBluescript II KS$^+$ with a 2.5-kb *Pst*I–*Xba*I *RPB8* DNA fragment derived from pSL104 inserted into the *Pst*I and *Xba*I 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 ^{35}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, IALIGN 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).

Table 4. *Yeast strains*

Name	Genotype	Alias
Z303	*MATa/MATα ura3-52/ura3-52 his3Δ200/his3Δ200 leu2-112/leu2-112 lys2Δ201/lys2Δ201 ade2/ade2 rpb8/rpb8Δ1 :: LYS2*	YSL167
Z319	*MATa/MATα ura3-52/ura3-52 his3Δ200/his3Δ200 leu2-112/leu2-112 lys2Δ201/lys2Δ201 ade2/ade2 rpb5/rpb5Δ1 :: HIS3*	WY-5
Z320	*MATa/MATα ura3-52/ura3-52 his3Δ200/his3Δ200 leu2-112/leu2-112 lys2Δ201/lys2Δ201 ade2/ade2 rpb6/rpb6Δ1 :: URA3*	WY-6
Z374	*MATa/MATα ura3-52/ura3-52 his3Δ200/his3Δ200 leu2-112/leu2-112 lys2Δ201/lys2Δ201 ade2/ade2 rpb6/rpb6Δ1 :: URA3* (pY2442)	—
Z321	*MATa/MATα ura3-52/ura3-52 his3Δ200/his3Δ200 leu2-112/leu2-112 lys2Δ201/lys2Δ201 ade2/ade2*	N222 × N225
N38	*MATα rna2-1 ura3-1 ura3-2 ade1 ade2 tyr1 GAL⁺*	RY15
N306	*MATα ura3-52 his3Δ200 ade2-101 leu2-77 lys2-801 trp1-901 tyr1-501 gal4-542 gal80-538*	—
X2180-2	*MATa/MATα mal⁻/mal⁻ gal2/gal2*	Y4

Source: 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× SSPE plus 0.3% SDS (20× 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× SSPE plus 0.2% SDS for 1 hr.

For copy number analysis of *RPB5*, the radiolabeled probe was made from a 650-bp *Eco*RI fragment that includes nucleotides 50–698 (Fig. 1). The probe used for *RPB6* consisted of a 500-bp *Xmn*I–*Hin*dIII fragment containing DNA from nucleotide 385 to the *Hin*dIII site 200 bp 3′ of the *RPB6*-coding region (Fig. 3). A 700-bp *Spe*I 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 (5×10^7) grown in LSM were harvested by centrifugation at 2000 rpm for 5 min, resuspended in 1 mCi of [^{35}S]methionine (New England Nuclear, 686 Ci/mmole) and 12 μl of 5× 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 *Eco*RI 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 *Eco*RV and *Spe*I, and the yeast diploid Z321 was transformed 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 *Sal*I restriction site using oligonucleotide-directed mutagenesis (Kunkel 1985). *URA3* was inserted into the *Sal*I site as a *Xho*I DNA fragment created by addition of *Xho*I linkers to a 1.14-kb *Hin*dIII *URA3* fragment. The resulting plasmid, pRP68, was cut with *Hin*dIII, 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 *Sph*I and *Bam*HI sites using oligonucleotide-directed mutagenesis to produce pSL111. *LYS2* was inserted into the *Sph*I–*Bam*HI site of pSL111 as a 5.9-kb *Sph*I–*Bam*HI fragment. The resulting plasmid, pSL119, was cleaved with *Pst*I and *Xba*I, 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.

Acknowledgments

We thank William Lane of the Harvard Microchemistry Facility for HPLC and protein sequence analysis of RPB5, RPB6, and RPB8 peptides and Mike Nonet for *S. cerevisiae* poly(A)$^+$ RNA. This work was supported by U.S. Public Health Service Grant GM-34365, by U.S. Public Health Service postdoctoral fellowship GM-11605 to N.A.W. from the National Institutes of Health, and by fellowship DRG969 to S.-M.L. from the Damon Runyon–Walter Winchell Cancer Fund. P.A.K. is a National Institutes of Health predoctoral trainee and R.A.Y. is a Burroughs Wellcome Scholar.

References

Aebersold, R.H., J. Leavitt, R.A. Saavedra, and L.E. Hood. 1987. Internal amino acid sequence analysis of proteins separated by one- or two-dimensional gel electrophoresis after in situ protease digestion on nitrocellulose. *Proc. Natl. Acad. Sci.* **84:** 6970–6974.

Ahearn, J.M., Jr., M.S. Bartolomei, M.L. West, L.J. Cisek, and J.L. Corden. 1987. Cloning and sequence analysis of the mouse genomic locus encoding the largest subunit of RNA polymerase II. *J. Biol. Chem.* **262:** 10695–10705.

Allison, L.A., M. Moyle, M. Shales, and C.J. Ingles. 1985. Extensive homology among the largest subunits of eukaryotic and prokaryotic RNA polymerases. *Cell* **42:** 599–610.

Bell, G.I., P. Valenzuela, and W.J. Rutter. 1977. Phosphorylation of yeast DNA-dependent RNA polymerases in vivo and in vitro. *J. Biol. Chem.* **252:** 3082–3091.

Biggs, J., L.L. Searles, and A. L. Greenleaf. 1985. Structure of the eukaryotic transcription apparatus: Features of the gene for the largest subunit of *Drosophila* RNA polymerase II. *Cell* **42:** 611–621.

Breant, B., J. Huet, A. Sentenac, and P. Fromageot. 1983. Analysis of yeast RNA polymerases with subunit-specific antibodies. *J. Biol. Chem.* **258:** 11968–11973.

Buhler, J.-M., F. Iborra, A. Sentenac, and P. Fromageot. 1976a. The presence of phosphorylated subunits in yeast RNA polymerases A and B. *FEBS Lett.* **71:** 37–41.

———. 1976b. Structural studies on yeast RNA polymerases. Existence of common subunits in RNA polymerases A(I) and B (II). *J. Biol. Chem.* **251:** 1712–1717.

Buhler, J.-M., J. Huet, K.E. Davies, A. Sentenac, and P. Fromageot. 1980. Immunological studies of yeast nuclear RNA polymerase at the subunit level. *J. Biol. Chem.* **255:** 9949–9954.

Carroll, S.B. and B.D. Stollar. 1983. Conservation of a DNA-binding site in the largest subunit of eukaryotic RNA polymerase II. *J. Mol. Biol.* **170:** 777–790.

Chamberlin, M.J. 1982. Bacterial DNA-dependent RNA polymerases. In *The enzymes* (ed. P. Boyer), pp. 61–86. Academic Press, New York.

Chen, E.Y. and P.H. Seeburg. 1985. Supercoil sequencing: A fast and simple method for sequencing plasmid DNA. *DNA* **4:** 165–170.

Cho, J.M. and A.P. Kimball. 1982. Probes of eukaryotic DNA-dependent RNA polymerase II-I. Binding of 9-β-D-arabinofuranosyl-6-mercaptopurine to the elongation subsite. *Biochem. Pharmacol.* **31:** 2575–2581.

Cho, K.W.Y., K. Khalili, R. Zandomeni, and R. Weinmann. 1985. The gene encoding the large subunit of human RNA polymerase II. *J. Biol. Chem.* **260:** 15204–15210.

Corden, J.L., D.L. Cadena, J.M. Ahearn, and M.E. Dahmus. 1985. A unique structure at the carboxyl terminus of the largest subunit of eukaryotic RNA polymerase II. *Proc. Natl. Acad. Sci.* **82:** 7934–7938.

Davis, R.W., D. Botstein, and J.R. Roth. 1980. *Advanced bacterial genetics: A manual for genetic engineering*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Dayoff, M.O., W.C. Barker, and L.T. Hunt. 1983. Establishing homologies in protein sequences. *Methods Enzymol.* **91:** 524–545.

Duby, A., K.A. Jacobs, and A. Celeste. 1989. Screening of recombinant DNA libraries. Using synthetic oligonucleotides as probes. In *Current protocols in molecular biology* (eds. F.M. Ausubel, R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith, and K. Struhl), pp. 6.4.1–6.4.10. Greene/John Wiley, New York.

Elder, R.T., E.Y. Loh, and R.W. Davis. 1983. RNA from the yeast transposable element *Ty1* has both ends in direct repeats, a structure similar to retrovirus RNA. *Proc. Natl. Acad. Sci.* **80:** 2432–2436.

Falkenburg, D., B. Dworniczak, D. Faust, and E.K.F. Bautz. 1987. RNA polymerase II in *Drosophila*. Relation of its 140,000 M_r subunit to the β subunit of *Escherichia coli* RNA polymerase. *J. Mol. Biol.* **195:** 929–937.

Faust, D.M., R. Renkawitz-Pohl, D. Falkenburg, A. Gasch, S. Bailojan, R.A. Young, and E.K.F. Bautz. 1986. Cloning and identification of the gene coding for the 140-kd subunit of *Drosophila* RNA polymerase II. *EMBO J.* **5:** 741–746.

Field, J., J.-I, Nikawa, D. Broek, B. MacDonald, L. Rodgers, I.A. Wilson, R.A. Lerner, and M. Wigler. 1988. Purification of a *RAS*-responsive adenylyl cyclase complex from *Saccharomyces cerevisiae* by use of an epitope addition method. *Mol. Cell. Biol.* **8:** 2159–2165.

Greenleaf, A.L. 1983. Amanitin-resistant RNA polymerase II mutations are in the enzymes's largest subunit. *J. Biol. Chem.* **258:** 13403–13406.

Huet, J., L. Phalente, G. Buttin, A. Sentenac, and P. Fromageot. 1982. Probing yeast RNA polymerase A subunits with monospecific antibodies. *EMBO J.* **1:** 1193–1198.

Ingles, J.C., H.J. Himmelfarb, M. Shales, A.L. Greenleaf, and J.D. Friesen. 1984. Identification, molecular cloning, and mutagenesis of *Saccharomyces cerevisiae* RNA polymerase genes. *Proc. Natl. Acad. Sci.* **81:** 2157–2161.

Julius, D., R. Schekman, and J. Thorner. 1984. Glycosylation and processing of prepro-α-factor through the yeast secretory pathway. *Cell* **36:** 309–318.

Kolodziej, P.A. and R.A. Young. 1989. RNA polymerase II subunit RPB3 is an essential component of the mRNA transcription apparatus. *Mol. Cell Biol.* **9:** 5387–5394.

Kunkel, T.A. 1985. Rapid and efficient site-specific mutagenesis without phenotypic selection. *Proc. Natl. Acad. Sci.* **82:** 488–492.

Laemmli, U.K. 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature* **227:** 680–685.

Lewis, M.K. and R.R. Burgess. 1982. Eukaryotic RNA polymerases. In *The enzymes* (ed. P. Boyer), pp. 109–153. Academic Press, New York.

Mann, C., J.-M. Buhler, I. Treich, and A. Sentenac. 1987. *RPB40*, a unique gene for a subunit shared between yeast RNA polymerase A and C. *Cell* **48:** 627–637.

Matsudaira, P. 1987. Sequence from picomole quantities of proteins electroblotted onto polyvinylidene difluoride membranes. *J. Biol. Chem.* **262:** 10035–10038.

Memet, S., W. Saurin, and A. Sentenac. 1988a. RNA polymerases B and C are more closely related to each other than to RNA polymerase A. *J. Biol. Chem.* **263:** 10048–10051.

Memet, S., M. Gouy, C. Marck, A. Sentenac, and J.-M. Buhler.

1988b. *RPA190*, the gene coding the largest subunit of yeast RNA polymerase A. *J. Biol. Chem.* **263:** 2830–2839.

Nichols, R. and J.E. Dixon. 1988. Rapid identification of clones using the same degenerate oligonucleotide mixture for both screening and sequencing. *Anal. Biochem.* **170:** 110–115.

Nonet, M., D. Sweetser, and R.A. Young. 1987. Functional redundancy and structural polymorphism in the large subunit of RNA polymerase II. *Cell* **50:** 909–915.

Pati, U.K. and S.M. Weissman. 1989. Isolation and molecular cloning of a cDNA encoding the 23 kDa subunit of human RNA polymerase II. *J. Biol. Chem.* **264:** 13114–13121.

Pearson, W.R. and D.J. Lipman. 1988. Improved tools for biological sequence comparison. *Proc. Natl. Acad. Sci.* **85:** 2444–2448.

Riva, M., S. Memet, J.-Y. Micouin, J. Huet, I. Treich, J. Dassa, R. Young, J.-M. Buhler, A. Sentenac, and P. Fromageot. 1986. Isolation of structural genes for yeast RNA polymerases by immunological screening. *Proc. Natl. Acad. Sci.* **83:** 1554–1558.

Riva, M., A.R. Schaffner, A. Sentenac, G.R. Hartmann, A.A. Mustaev, E.F. Zaychikov, and M.A. Grachev. 1987. Active site labeling of the RNA polymerases A, B and C from yeast. *J. Biol. Chem.* **262:** 14377–14380.

Rothstein, R.J. 1983. One-step gene disruption in yeast. *Methods Enzymol.* **101:** 202–211.

Searles, L.L., R.S. Jokerst, P.M. Bingham, R.A. Voelker, and A.L. Greenleaf. 1982. Molecular cloning of sequences from a *Drosophila* RNA polymerase II locus by P element transposon tagging. *Cell* **31:** 585–592.

Sentenac, A. 1985. Eukaryotic RNA polymerases. *Crit. Rev. Biochem.* **18:** 31–91.

Sherman, F., G.R. Fink, and J.B. Hicks. 1986. *Laboratory course manual for methods in yeast genetics.* Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Sikorski, R.S. and P. Hieter. 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics* **122:** 19–27.

Sweetser, D., M. Nonet, and R.A. Young. 1987. Prokaryotic and eukaryotic RNA polymerases have homologous core subunits. *Proc. Natl. Acad. Sci.* **84:** 1192–1196.

Valenzuela, P., G.I. Bell, F. Weinburg, and W.J. Rutter. 1976. Yeast DNA-dependent RNA polymerases I, II, and III. The existence of subunits common to the three enzymes. *Biochem. Biophys. Res. Commun.* **71:** 1319–1325.

———. 1978. Isolation and assay of eukaryotic DNA dependent RNA polymerases. *Methods Cell Biol.* **19:** 1–26.

Williams, P.G., and J.G. Mason. 1985. Hybridization in the analysis of recombinant DNA. In *Nucleic acid hybridization: A practical approach* (ed. B.D. Hames and S.J. Higgens), pp. 139–160. IRL Press, Washington, D.C.

Woychik, N.A. and R.A. Young. 1989. RNA polymerase II subunit RPB4 is essential for high- and low-temperature yeast cell growth. *Mol. Cell. Biol.* **9:** 2854–2859.

Young, R.A. and R.W. Davis. 1983. Yeast RNA polymerase II genes: Isolation with antibody probes. *Science* **222:** 778–782.

Yura, T. and A. Ishihama. 1979. Genetics of bacterial RNA polymerases. *Annu. Rev. Genet.* **13:** 59–97.

Subunits shared by eukaryotic nuclear RNA polymerases.

N A Woychik, S M Liao, P A Kolodziej, et al.

Genes Dev. 1990, **4:**
Access the most recent version at doi:10.1101/gad.4.3.313