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# Six Human RNA Polymerase Subunits Functionally Substitute for Their Yeast Counterparts

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**To assess functional relatedness of individual components of the eukaryotic transcription apparatus, three human subunits (hsRPB5, hsRPB8, and hsRPB10) were tested for their ability to support yeast cell growth in the absence of their essential yeast homologs. Two of the three subunits, hsRPB8 and hsRPB10, supported normal yeast cell growth at moderate temperatures. A fourth human subunit, hsRPB9, is a homolog of the nonessential yeast subunit RPB9. Yeast cells lacking RPB9 are unable to grow at high and low temperatures and are defective in mRNA start site selection. We tested the ability of hsRPB9 to correct the growth and start site selection defect seen in the absence of RPB9. Expression of hsRPB9 on a high-copy-number plasmid, but not a low-copy-number plasmid, restored growth at high temperatures. Recombinant human hsRPB9 was also able to completely correct the start site selection defect seen at the *CYC1* promoter in vitro as effectively as the yeast RPB9 subunit. Immunoprecipitation of the cell extracts from yeast cells containing either of the human subunits that function in place of their yeast counterparts in vivo suggested that they assemble with the complete set of yeast RNA polymerase II subunits. Overall, a total of six of the seven human subunits tested previously or in this study are able to substitute for their yeast counterparts in vivo, underscoring the remarkable similarities between the transcriptional machineries of lower and higher eukaryotes.**

The eukaryotic mRNA transcription apparatus comprises RNA polymerase II, general transcription factors and their associated factors, and gene-specific factors (reviewed in references 6, 13, and 23). RNA polymerase II (pol II) is a multisubunit enzyme with ~12 to 15 subunits, depending on the organism, that plays a major role in mRNA synthesis since it possesses the catalytic machinery for the formation of the 3′-5′ phosphodiester bonds between ribonucleoside triphosphates and presumably responds to signals from the multiple factors involved in regulating its function during initiation and elongation of mRNA synthesis.

Yeast *Saccharomyces cerevisiae* pol II has been a useful paradigm for enzyme function since its subunit structure and amino acid sequences are strikingly similar to pol II subunits from a variety of eukaryotes and even archaebacteria. *S. cerevisiae* pol II has 12 subunits, designated RPB1-RPB12, ranging in size from ~190 to ~8 kDa (reviewed in references 26 and 27). Five of these subunits (RPB5, RPB6, RPB8, RPB10, and RPB12) are also present in RNA polymerases I and III and are usually referred to as the common subunits.

Since the functions of most pol II subunits are unclear, one approach to understanding their role in transcription has been to isolate the genes encoding human RNA polymerase subunits and test for functional similarities by determining if the human subunit can function in place of its yeast homolog. Only a few of the human subunit genes have been tested for heterocomplementation in *S. cerevisiae*. hsRPB6 has a highly conserved carboxyl half and can fully function in yeast cells (17), hsRPB7 can substitute for its yeast counterpart at moderate temperatures (12), and the human homolog of the smallest subunit (ABC10α, or RPB12) can support growth in the absence of essential subunit RPB10 or RPB12 (21a) (EMBL/GenBank accession number Z477727). In this study, we tested the level of functional conservation of four additional human subunits and demonstrated that three are able to support yeast cell growth in vivo.

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## MATERIALS AND METHODS

**Plasmids, yeast strains, and media.** Plasmids used in this study are listed in Table 1. Yeast complete minimal dropout medium used for nutrient selection was prepared by a standard method (24). When used, 5-fluoroorotic acid (5-FOA) was added to 1 mg/ml. The parent and isogenic yeast strains used for complementation analysis were N222, *MAT*α *ura3-52 his3*Δ*200 leu2-3 leu2-112 lys2*Δ*201 ade2*; WY-32, *MAT*α *ura3-52 his3*Δ*200 leu2-3 leu2-112 lys2*Δ*201 ade2 RPB5*Δ*1*::*HIS3*, pRP510, for RPB5 complementation; WY-67, *MAT***a** *ura3-52 his3*Δ*200 leu2-3 leu2-112 lys2*Δ*201 ade2 RPB8*Δ*1*::*LYS2*, pRP84, for RPB8 complementation; WY-40 (16), *MAT***a** *ura3-52 his3*Δ*200 leu2-3 leu2-112 lys2*Δ*201 ade2 RPB10*Δ*1*::*HIS3*, pRP10/10, for RPB10 complementation; and WY-9, *MAT*α *ura3-52 his3*Δ*200 leu2-3 leu2-112 lys2*Δ*201 ade2 RPB9*Δ*1*::*HIS3* for RPB9 complementation.

**cDNA library screening.** In order to identify human genes encoding the subunits of pol II, a database generated at Human Genome Sciences and the Institute of Genomic Research containing expressed sequence tags generated from multiple human tissues was searched with the BLAST algorithm. Expressed sequence tag clones were discovered by established methods (3, 4). Multiple tags showing homology to yeast RPB8 and RPB10 were identified, and full-length cDNAs for each were rescued from a Jurkat cell library, a lung cell library, and a pituitary cell library, respectively. Each of the three libraries was constructed in lambda Zap, with the cDNAs directionally cloned between the *Eco*RI site and the *Xho*I site. The full-length cDNAs were subjected to multiple rounds of automated sequence analysis to ensure accuracy.

**In vivo complementation.** Plasmid shuffle experiments (5) were performed with the three essential yeast genes as follows. For RPB5, WY-32 was transformed with plasmid pRP519 (human) or pRP520 (yeast control) to create WY-100 and WY-102, respectively. After 5-FOA treatment only the control strain, WY-102, remained viable since hsRPB5 could not support growth. For RPB8, WY-67 was transformed with pRP86 (human) to create WY-92 and, subsequently, WY-93 after 5-FOA treatment. For RPB10, WY-40 was transformed with pRP10/17 (human) or pRP10/15 (yeast control) and treated with 5-FOA to create WY-99 and WY-42, respectively. Human subunit-containing strains remaining viable after 5-FOA treatment were spotted onto yeast extract-peptone-dextrose plates and tested for growth at 12, 30, and 37°C relative to their respective isogenic control strain.

For complementation testing of hsRPB9, WY-9 cells were transformed with pRP924 (low-copy-number hsRPB9), pRP957 (high-copy-number RPB9), or

TABLE 1. Plasmids used in this study

| Plasmid | Description |
|---|---|
| *hsRPB5/RPB5* | |
| pRP56 | 1.1-kb *RPB5*-containing *Eco*RV-*Spe*I fragment in pGEM5Zf(+) (Promega) |
| pRP510 | 1.5-kb *Xba*I-*Spe*I *RPB5*-containing fragment ligated to the *Xba*I site of pRS416 (*URA3 CEN6 ARS4*) |
| pRP518 | ~780-bp *Apa*I (5′)-*Sac*I (3′) fragment containing an *Xba*I site replacing the RPB5 coding region flanked by the ~750-bp RPB5 5′ DNA and ~30-bp 3′ DNA ligated to the *Apa*I and *Sac*I sites of pRS415 (*LEU2 CEN6 ARS4*) |
| pRP519 | hsRPB5 coding region with *Xba*I ends ligated (in the correct orientation relative to promoter DNA) to the *Xba*I site of pRP518 |
| pRP520 | RPB5 coding region with *Xba*I ends ligated (in the correct orientation relative to promoter DNA) to the *Xba*I site of pRP518 |
| pRP521 | 1.2-kb *hsRPB8*-containing *Eco*RI fragment ligated to the 3.5-kb fragment from *Eco*RI-cut pRP56 |
| *hsRPB8/RPB8* | |
| pRP82 | 2.5-kb *RPB8*-containing *Pst*I-*Xba*I fragment in pBluescript II KS(+) |
| pRP83 | ~800-bp *Eco*RI-*Xho*I *hsRPB8*-containing fragment in pBluescript II SK(−) |
| pRP84 | pRP82 insert in YCplac33 (*URA3 CEN4 ARS1*) |
| pRP85 | ~600-bp *Eco*RI (5′)-*Pst*I (3′) fragment containing *Bam*HI and *Xba*I sites replacing the RPB8 coding region flanked by the ~500-bp RPB5 5′ DNA and ~100-bp 3′ DNA ligated to the *Eco*RI and *Pst*I sites of YCplac111 (*LEU2 CEN4 ARS1*) |
| pRP86 | hsRPB8 coding region with *Bam*HI and *Xba*I ends ligated to the *Bam*HI and *Xba*I sites of pRP85 |
| pRP87 | RPB8 coding region with *Bam*HI and *Xba*I ends ligated to the *Bam*HI and *Xba*I sites of pRP85 |
| *hsRPB9/RPB9* and related plasmids | |
| pRP916 | 3.0-kb *RPB9*-containing *Hin*dIII fragment in pBluescript KS(+) (Stratagene) |
| pRP922 | ~500-bp *Eco*RI-*Xho*I *hsRPB9*-containing fragment in pBluescript II SK(−) |
| pRP923 | hsRPB9 coding region with *Bam*HI ends ligated (in the correct orientation relative to promoter DNA) to the *Bam*HI site of pRP956 |
| pRP924 | *hsRPB9*-containing *Eco*RI-*Hin*dIII fragment from pRP923 ligated to the *Eco*RI and *Hin*dIII sites of YCplac111 (*LEU2 CEN4 ARS1*) |
| pRP925 | *RPB9*-containing *Eco*RI-*Hin*dIII fragment from pRP951 ligated to the *Eco*RI and *Hin*dIII sites of YCplac111 (*LEU2 CEN4 ARS1*) |
| pRP951 | RPB9 coding region with *Bam*HI ends ligated (in the correct orientation relative to promoter DNA) to the *Bam*HI site of pRP956 |
| pRP953 | dmRPB9 coding region with *Bam*HI ends ligated (in the correct orientation relative to promoter DNA) to the *Bam*HI site of pRP956 |
| pRP956 | ~320-bp *Eco*RI (5′)-*Hin*dIII (3′) fragment containing a *Bam*HI site replacing the RPB9 coding region flanked by the ~260-bp RPB9 5′ DNA and ~60-bp 3′ DNA ligated to the *Eco*RI and *Hin*dIII sites of YEplac195 (*URA3* 2μm *ori*) |
| pRP957 | *hsRPB9*-containing *Eco*RI-*Hin*dIII fragment from pRP923 ligated to the *Eco*RI and *Hin*dIII sites of YEplac181 (*LEU2* 2μm *ori*) |
| pNW84 | A12.2 coding region with *Eco*RI ends ligated to the *Eco*RI site of pGEX-2T (Pharmacia) |
| pNW85 | A12.2 coding region with *Bam*HI ends ligated (in the correct orientation relative to promoter DNA) to the *Bam*HI site of pRP956 |
| *hsRPB10/RPB10* | |
| pRP10/2 | 1.5-kb *RPB10*-containing *Sph*I-*Pst*I fragment in the *Sph*I and *Pst*I sites of pGEM5Zf(+) (Promega) |
| pRP10/11 | 1.5-kb *RPB10*-containing *Sph*I-*Pst*I fragment in the *Sph*I and *Pst*I sites of YCplac33 (*URA3 CEN4 ARS1*) |
| pRP10/12 | ~1.3-bp *Sph*I (5′)-*Sac*I (3′) fragment containing *Xho*I and *Sma*I sites replacing the RPB10 coding region flanked by the ~650-bp RPB10 5′ DNA and ~630-bp 3′ DNA ligated to the *Sph*I and *Sac*I sites of pRS415 (*LEU2 CEN6 ARS4*) |
| pRP10/15 | RPB10 coding region with *Xho*I and *Sma*I ends inserted into the *Xho*I and *Sma*I sites of pRP10/12 |
| pRP10/16 | ~300-bp *Eco*RI-*Xho*I *hsRPB10*-containing fragment in pBluescript II SK(−) |
| pRP10/17 | hsRPB10 coding region with *Xho*I and *Sma*I ends inserted into the *Xho*I and *Sma*I sites of pRP10/12 |

pRP925 (yeast control) to create WY-105, WY-118, and WY-104, respectively, and tested for growth at 12, 30, and 37°C.

All final strains were tested by PCR to confirm the presence or absence of the expected plasmids and selectable markers, e.g., DNA from the hsRPB8-containing yeast strain (WY-93) yielded PCR products verifying that the *hsRPB8* and *LEU2*, but not *RPB8* and *URA3*, genes were present.

**Cell labeling and immunoprecipitation.** Cells were labeled with [$^{35}$S]methionine for 1 h and immunoprecipitated by the method of Kolodziej and Young (14) using the anti-RPB1 carboxyl-terminal domain antibody 8WG16 (22). Following sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), the gel was treated as suggested by the manufacturer with Entensify (Dupont) and dried prior to exposure to XAR-5 film.

**In vitro transcription assays.** Nuclear extracts were prepared from isogenic wild-type (N222) and *RPB9Δ1*::*HIS3* (WY-9) strains by the method of Lue et al. (15). In general, transcription reactions were performed at 23°C with 60 to 80 mg of extract and 200 to 300 ng of a pGAL4CG$^-$ template. Transcription reactions were performed in the absence of recombinant GAL4-VP16.

To test the effects of glutathione *S*-transferase (GST), GST-hsRPB9, GST-dmRPB9, and GST-A12.2 fusion proteins on in vitro transcription, a range of concentrations of each recombinant protein was added to reaction mixtures containing 60 mg of extract and 300 ng of the pGAL4CG$^-$ template before incubation.

**Preparation of GST fusion proteins.** Induction of GST fusion protein expression in *Escherichia coli* DH5α cells was initiated by the addition of 100 mM IPTG (isopropyl-β-D-thiogalactopyranoside). Cells were grown for 2 h following induction, harvested, and lysed according to Pharmacia guidelines. GST fusion pro-

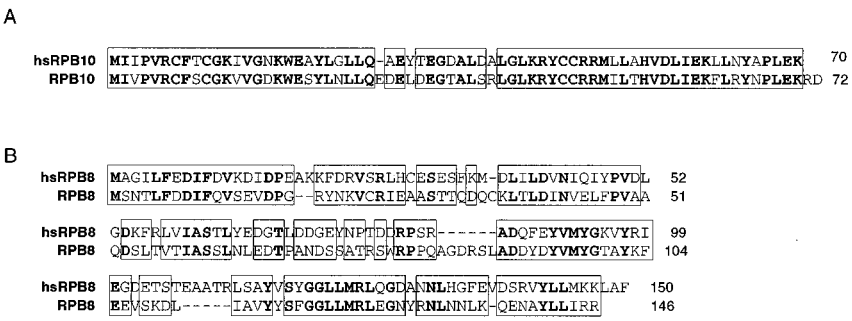


FIG. 1. Alignment of hsRPB8 and hsRPB10 with their human counterparts. Conserved residues are boxed; identical residues are boldfaced.

teins were purified from cell lysates by using glutathione Sepharose 4B (Pharmacia) as suggested by the manufacturer.

**Nucleotide sequence accession numbers.** The nucleotide sequences of *hsRPB8* and *hsRPB10* have been deposited in GenBank under accession numbers U37689 and U37690, respectively.

## RESULTS AND DISCUSSION

**Human counterparts of RPB8 and RPB10.** We isolated the cDNAs of two genes encoding human counterparts of the yeast *S. cerevisiae* nuclear RNA polymerase subunits. Alignment of the human subunits, designated hsRPB8 and hsRPB10, with their yeast counterparts (Fig. 1) revealed extensive amino acid sequence conservation. The most striking similarity exists between the common subunits hsRPB10 and RPB10, whose sequences are 70% identical and 91% conserved overall. hsRPB8 is somewhat less conserved, with 78% conservation (47% identity) upon alignment to its yeast homolog.

**In vivo complementation.** We used the plasmid shuffle method (5) to see if expression of *hsRPB8* and *hsRPB10* or two additional human genes, *hsRPB5* and *hsRPB9*, complemented the growth defect in cells lacking their respective yeast counterpart. Three of the four yeast homologs, RPB5, RPB8, and RPB10, are essential for cell growth and viability. The exception, RPB9, is required only for growth at high and low temperatures.

For complementation analysis of the three essential genes, each human gene was placed under control of its respective yeast promoter in a plasmid marked with the *LEU2* gene. This low-copy-number (*CEN4*) plasmid marked with *LEU2* was transformed into yeast cells that have the chromosomal copy of the appropriate yeast subunit gene deleted plus a complementing plasmid containing a wild-type copy of the corresponding essential yeast gene on a *URA3* plasmid. Independent transformants were subsequently placed on medium containing 5-FOA to prevent growth of *URA3*$^+$ cells. Selection for the loss of the wild-type Ura$^+$ plasmids enabled us to determine if the remaining plasmid containing the human gene supports yeast cell growth. All yeast strains were tested for the presence or absence of appropriate markers and subunit genes by PCR of their DNA to verify that growth is due to expression of only the human subunit gene and not a yeast gene. The *hsRPB8* and *hsRPB10* genes (Fig. 2), but not *hsRPB5*, could substitute for their yeast counterparts at normal (30°C) growth temperatures. The inviability of the hsRPB5 yeast strain was not altered by expression of *hsRPB5* on a high-copy-number plasmid (data not shown). Growth profiles of the hsRPB8- and hsRPB10-containing yeast strains (Fig. 3) revealed that both human subunits support adequate, but not necessarily optimal, cell growth at temperature extremes.

To test for complementation of the nonessential gene, RPB9, a low- or high-copy-number hsRPB9 plasmid was transformed into the yeast strain lacking RPB9 and the resulting strain was tested on plates for growth at the nonpermissive temperature (Fig. 4). Low-copy-number expression of the *hsRPB9* gene did not support cell growth on plates at 37°C. However, growth on plates at 37°C was restored by high-copy-number expression of the *hsRPB9* plasmid. In contrast, high-copy-number expression of *RPA12* (the gene encoding the A12.2 RNA pol I subunit that is related to RPB9 [18]) did not support high-temperature growth (Fig. 4).

**hsRPB8 and hsRPB10 appear to efficiently assemble with yeast RNA pol II.** To test if hsRPB8 and hsRPB10 assemble with the complete set of pol II subunits, we immunoprecipitated radioactively labeled cell extracts with an antibody to the RPB1 carboxyl-terminal domain (Fig. 5). In each case, antibody binding to the RPB1 carboxyl-terminal domain appeared to result in the coimmunoprecipitation of the 11 other pol II subunits, suggesting that hsRPB8 and hsRPB10 can assemble with intact *S. cerevisiae* pol II. Assembly of hsRPB10 with the yeast subunits was detectable since it has a lower mobility than RPB10 (which normally migrates with RPB12). Assembly of hsRPB8 with the yeast pol II subunits was also distinguishable upon SDS-PAGE. In this case, hsRPB8 has a mobility similar to that of yeast RPB8 (consistent with molecular mass predications), but the band appears more intense. The increase in intensity is predicted upon labeling since hsRPB8 has five methionine residues while RPB8 has only three. These experiments suggest that hsRPB8 and hsRPB10 are stable and assembled efficiently in yeast cells. Since RPB9 and RPB11 comigrate during SDS-PAGE and hsRPB9 has a molecular mass nearly identical to that of RPB9, we were not able to assess assembly of hsRPB9 by immunoprecipitation. The subunit profile obtained after immunoprecipitation of hsRPB9-containing cells was indistinguishable from the wild-type profile (data not shown).

**hsRPB9 can complement the transcription initiation defect in RPB9-deficient cell extracts.** In vitro transcription at the *CYC1* promoter using nuclear extracts from RPB9 deletion or null mutants, coupled with in vivo primer extension analysis,

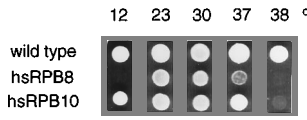


FIG. 2. hsRPB8 and hsRPB10 support yeast cell growth. hsRPB8 (WY-93) or hsRPB10 (WY-99) yeast cells containing plasmids expressing *hsRPB8* or *hsRPB10*, respectively, in place of their corresponding yeast gene were spotted on yeast extract-peptone-dextrose plates next to the wild-type isogenic background strain (N222) and incubated at the temperatures indicated.

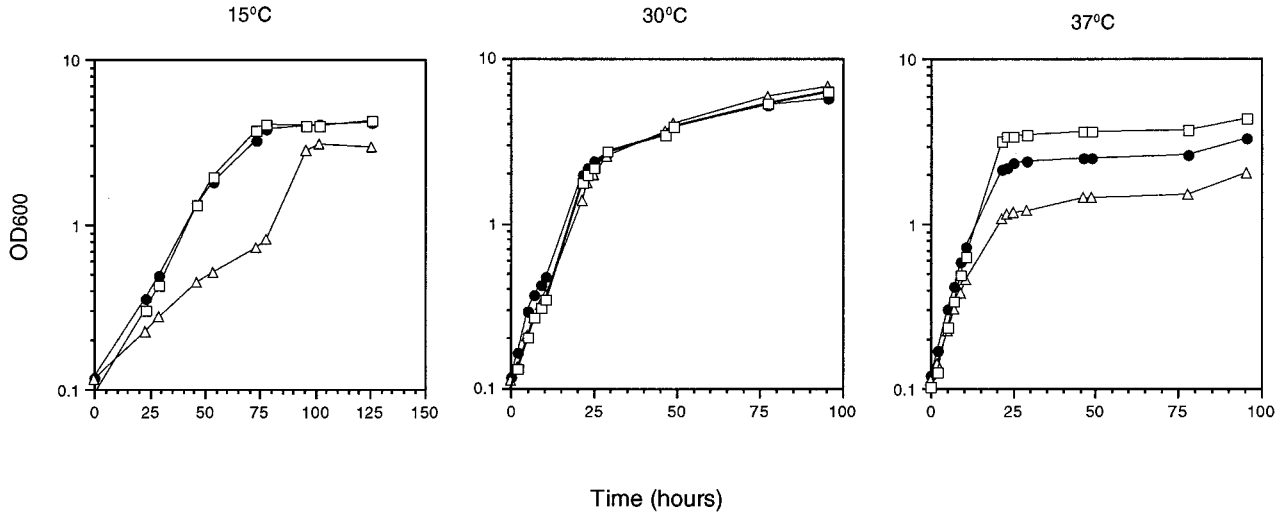


FIG. 3. Growth curves of yeast strains containing hsRPB8 or hsRPB10. Yeast cells containing plasmids expressing *hsRPB8* (WY-93) (△) or *hsRPB10* (WY-99) (●) in place of their corresponding yeast gene were grown in selective (complete minimal dropout) liquid medium and compared with growth of the wild-type isogenic background strain (N222) (□) at the temperatures indicated. The results shown were reproducible in three independent experiments. Estimated doubling times at each temperature are as follows: *hsRPB8* at 15°C, 25 h; *hsRPB10* and the wild type at 15°C, 10 h; *hsRPB8*, *hsRPB10*, and the wild type at 30°C, 6 h; *hsRPB8*, *hsRPB10*, and the wild type at 37°C, 9.5, 5, and 4.5 h, respectively.

has revealed that RPB9 is essential for accurate selection of mRNA initiation sites for multiple genes in vivo (11). These results were consistent with earlier findings linking RPB9 to start site selection after it was identified in a genetic screen for genes that influence TATA element-to-transcription start site spacing (9, 10). RPB9 mutants have a defect in mRNA start site selection that results in an overall upstream shift of the transcription profiles for several classes of genes (9–11).

Since addition of recombinant RPB9 to the mutant extracts completely restores normal start site selection in vitro (11), we used this assay to test if hsRPB9 could also correct the transcriptional defect (Fig. 6). Identical ranges of concentrations of purified GST-hsRPB9 and GST-RPB9 were added to the transcription reaction mixtures. The hsRPB9 completely corrected the start site defect and functioned as effectively as the yeast subunit at each concentration tested. The concentrations of recombinant protein added to the extracts are undoubtedly much higher than the physiological concentrations of the subunit resulting from normal expression from a single-copy gene. Thus, these in vitro results are consistent with the in vivo growth experiments which demonstrated that hsRPB9 expression at high, but not low, levels enabled yeast cells to grow well at elevated temperatures. Since hsRPB9 can completely and efficiently complement the RPB9 start site defect in vitro, its function in humans likely parallels that of yeast RPB9.

**Drosophila RPB9, but not A12.2, corrects the transcription initiation defect in RPB9-deficient cell extracts.** We also tested

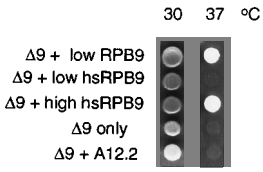


FIG. 4. Expression of *hsRPB9* on a high-copy-number plasmid supports high-temperature growth. Equivalent amounts of cells lacking the *RPB9* gene (Δ9) but containing high (high hsRPB9 or A12.2)- or low (low hsRPB9)-copy-number plasmids expressing the *RPB9*, *hsRPB9*, or *RPA12* gene were spotted onto yeast extract-peptone-dextrose plates and incubated at the temperatures indicated.

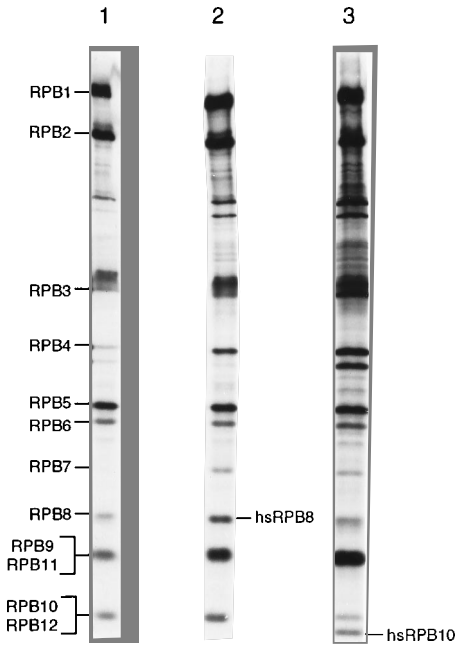


FIG. 5. hsRPB8 and hsRPB10 assemble with yeast RNA pol II subunits. Cells were labeled with [$^{35}$S]methionine, and the cell extracts were immunoprecipitated with an RPB1 anti-carboxyl-terminal domain antibody. Lane 1, wild-type isogenic cells (N222); lane 2, hsRPB8 cells (WY-93); lane 3, hsRPB10 cells (WY-99). The positions of the 12 pol II subunits (RPB1 to RPB12) are indicated. The apparent molecular masses of the yeast pol II subunits in kilodaltons are 220 (RPB1), 150 (RPB2), 45 (RPB3), 32 (RPB4), 28 (RPB5), 24 (RPB6), 16 (RPB7), 14.5 (RPB8), 13 (RPB9 and RPB11), and 8 (RPB10 and RPB12). hsRPB10 has a lower molecular mass and mobility than yeast RPB10. The mobility of hsRPB8 is equivalent to that of its yeast homolog with a similar molecular mass. However, the hsRPB8 band is darker since it has five methionine residues and likely incorporates more radioactive label relative to the yeast subunit with three methionines.

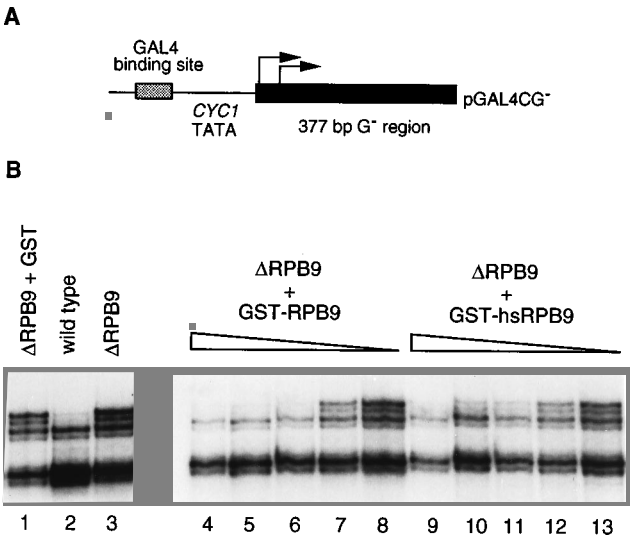


FIG. 6. hsRPB9 corrects the start site selection defect in RPB9-deficient nuclear extracts. The template plasmid used for the transcription reactions, pGAL4CG⁻, contains a single GAL4 binding site and a *CYC1* TATA element controlling the expression of G-less transcripts (A). The two predominant transcripts are approximately 350 and 370 nucleotides. In vitro transcription products obtained from WY-9 extracts were incubated with increased amounts of GST-hsRPB9 or GST-RPB9 (B). Lanes 4 and 9, 125 ng; lanes 5 and 10, 25 ng, lanes 6 and 11, 5 ng; lanes 7 and 12, 1 ng. No recombinant protein was added to lanes 8 and 13. Sixty milligrams of extract and 200 ng of template were used in each reaction.

if the *Drosophila melanogaster* RPB9 homolog (dmRPB9) or the zinc finger-containing RNA pol I subunit A12.2 (18) related to RPB9, could correct the initiation defect in vitro (Fig. 7). Although neither A12.2 or dmRPB9 expressed from high-copy-number plasmids complemented the high-temperature growth defect in cells lacking RPB9 (Fig. 4) (11), recombinant dmRPB9 was able to correct the start site selection defect in vitro. Unlike hsRPB9, dmRPB9 appeared to require addition of relatively more recombinant protein in order to correct the start site deficiency. dmRPB9 also appeared to function less efficiently in vivo since expression of the hsRPB9 high-copy-number plasmid, but not dmRPB9, was able to rescue the temperature sensitivity of RPB9 mutants (11). However, the discrepancy between drosophila and human subunit functions in yeast cells may result from differences in expression efficiency, transcript stability, or protein stability.

**Conclusion.** Upon biochemical purification, human pol II appears have at least nine subunits (8), two large (~200- and 150-kDa) and seven small (<50-kDa) subunits. However, human pol II is likely have at least as many subunits as the 12-subunit yeast *S. cerevisiae* enzyme since all 12 human genes related to the yeast subunits have now been identified. The genes encoding subunits related to yeast RPB1 (25), RPB2 (1), RPB3 (21), RPB4 (11a), RPB5 (20), RPB6 (17), RPB7 (12), RPB9 (2), RPB11 (19), and RPB12 (21a) have been isolated. In this study, two additional genes, *hsRPB8* and *hsRPB10*, were identified by sequence homology. It is still unclear whether these 12 human pol II subunits represent the complete set of subunits. Human pol II is relatively poorly defined at the biochemical level (8). Therefore, the precise number of human RNA polymerase subunits that copurify with in vitro transcription activity is not known.

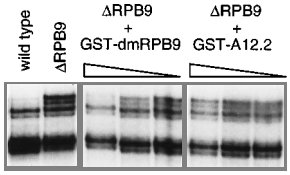


FIG. 7. dmRPB9 but not A12.2 can complement the RPB9 start site defect in vitro. In vitro transcription products obtained from WY9 extracts were incubated with increased amounts (0, 25, or 125 ng) of GST-dmRPB9 or GST-A12.2. Sixty milligrams of extract and 200 ng of template were used in each reaction.

TABLE 2. Human and *S. cerevisiae* RNA pol II subunits

| Yeast subunit | Alternate name(s) | $M_r$ (1,000) | Human homolog | | | In vivo complementation |
|---|---|---|---|---|---|---|
| | | | $M_r$ (1,000) | No. of amino acids | pI | |
| RPB1 | B220, RPO21 | 191 | 217 | 1,970 | 7.3 | ND[a] |
| RPB2 | B150 | 140 | 134 | 1,174 | 6.9 | ND |
| RPB3 | B44 | 35 | 31.4 | 275 | 4.6 | ND |
| RPB4 | B32 | 25.4 | | | | |
| RPB5 | ABC27 | 25.0 | 24.6 | 210 | 5.5 | No |
| RPB6 | ABC23 | 17.9 | 14.4 | 127 | 3.9 | Yes |
| RPB7 | B16 | 19.0 | 19.3 | 172 | 5.3 | Yes |
| RPB8 | ABC 14.5 | 16.5 | 17.1 | 150 | 4.3 | Yes |
| RPB9 | B12.6 | 14.2 | 14.5 | 125 | 4.9 | Yes |
| RPB10 | ABC10β | 8.3 | 7.6 | 67 | 7.8 | ND |
| RPB11 | B12.5 | 13.6 | 13.3 | 117 | 5.7 | Yes |
| RPB12 | ABC10α | 7.7 | 7.0 | 58 | 9.5 | Yes |

[a] ND, not determined.

The conservation in subunit sequence from *S. cerevisiae* to humans also reflects preservation of function since all but one of the subunits tested were able to function in *S. cerevisiae* (Table 2). The inability of hsRPB5 to substitute for RPB5 function may be a consequence of hsRPB5 instability, inadequate communication with putative transcription factors, or defective association with subunits from RNA pol I, II, or III. hsRPB5 has recently been found to associate with the hepatitis B virus activator protein HBx in vivo and in vitro (7). Therefore, this subunit directly communicates with at least one transcriptional activator and perhaps several critical mammalian transcription factors. Yeast cells may not require an accessory factor for hsRPB5 function, or, if they do, this yeast factor may not recognize the particular hsRPB5 domains required for effective signal transmission. Comprehensive studies of intermolecular protein interactions between pol II subunits and other components influencing their functions are necessary for a more thorough understanding of subunit function. It is encouraging that many of the functions of the human subunits can be dissected in more detail because of their apparent ability to function so effectively in *S. cerevisiae*. Once clues to the functions of the *S. cerevisiae* subunits are discovered, several in vivo and in vitro assays should help illuminate the intricate molecular details which underlie their critical but yet elusive functions.

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## REFERENCES


1. **Acker, J., M. Wintzerith, M. Vigneron, and C. Kedinger.** 1992. Primary structure of the second largest subunit of human RNA polymerase II (or B). J. Mol. Biol. **226:**1295–1299.
2. **Acker, J., M. Wintzerith, M. Vigneron, and C. Kedinger.** 1993. Structure of the gene encoding the 14.5 kDa subunit of human RNA polymerase II. Nucleic Acids Res. **21:**5345–5350.
3. **Adams, M. D., M. Dubnick, A. R. Kerlavage, R. Moreno, J. M. Kelley, T. R. Utterback, J. W. Nagle, C. Fields, and J. C. Venter.** 1992. Sequence identification of 2,375 human brain genes. Nature (London) **355:**632–634.
4. **Adams, M. D., J. M. Kelley, J. D. Gocayne, M. Dubnick, M. H. Polymeropoulos, H. Xiao, C. R. Merril, A. Wu, B. Olde, R. F. Moreno, et al.** 1991. Complementary DNA sequencing: expressed sequence tags and human genome project. Science **252:**1651–1656.
5. **Boeke, J. D., J. Trueheart, G. Natsoulis, and G. R. Fink.** 1987. 5-Fluoroorotic acid as a selective agent in yeast molecular genetics. Methods Enzymol. **154:**164–175.
6. **Buratowski, S.** 1994. The basics of basal transcription by RNA polymerase II. Cell **77:**1–3.
7. **Cheong, J., M. Yi, Y. Lin, and S. Murakami.** 1995. Human RPB5, a subunit shared by eukaryotic nuclear RNA polymerases, binds human hepatitis B virus X protein and may play a role in X transactivation. EMBO J. **14:**143–150.
8. **Freund, E., and P. M. McGuire.** 1986. Characterization of RNA polymerase type II from human term placenta. J. Cell. Physiol. **127:**432–438.
9. **Furter-Graves, E. M., R. Furter, and B. D. Hall.** 1991. *SHI*, a new yeast gene affecting the spacing between TATA and transcription initiation sites. Mol. Cell. Biol. **11:**4121–4127.
10. **Furter-Graves, E. M., B. D. Hall, and R. Furter.** 1994. Role of a small RNA pol II subunit in TATA to transcription start site spacing. Nucleic Acids Res. **22:**4932–4936.
11. **Hull, M. W., K. McKune, and N. A. Woychik.** 1995. RNA polymerase II subunit RPB9 is required for accurate start site selection. Genes Dev. **9:**481–490.

11a. **Khazak, V., and E. A. Golemis.** Personal communication.

12. **Khazak, V., P. P. Sadhale, N. A. Woychik, R. Brent, and E. A. Golemis.** 1995. Human RNA polymerase II subunit hsRPB7 functions in yeast and influences stress survival and cell morphology. Mol. Biol. Cell **6:**759–775.
13. **Koleske, A. J., and R. A. Young.** 1995. The RNA polymerase II holoenzyme and its implications for gene regulation. Trends Biochem. Sci. **20:**113–116.
14. **Kolodziej, P. A., and R. A. Young.** 1991. Epitope tagging and protein surveillance. Methods Enzymol. **194:**508–519.
15. **Lue, N. F., P. M. Flanagan, R. J. I. Kelleher, A. M. Edwards, and R. D. Kornberg.** 1991. RNA polymerase II transcription in vitro. Methods Enzymol. **194:**545–550.
16. **McKune, K., and N. A. Woychik.** 1994. Halobacterial S9 operon contains two genes encoding proteins homologous to subunits shared by eukaryotic RNA polymerases I, II, and III. J. Bacteriol. **176:**4754–4756.
17. **McKune, K., and N. A. Woychik.** 1994. Functional substitution of an essential yeast RNA polymerase subunit by a highly conserved mammalian counterpart. Mol. Cell. Biol. **14:**4155–4159.
18. **Nogi, Y., R. Yano, J. Dodd, C. Carles, and M. Nomura.** 1993. Gene RRN4 in *Saccharomyces cerevisiae* encodes the A12.2 subunit of RNA polymerase I and is essential only at high temperatures. Mol. Cell. Biol. **13:**114–122.
19. **Pati, U.** 1994. Human RNA polymerase II subunit hRPB14 is homologous to yeast RNA polymerase I, II, and III subunits (AC19 and RPB11) and is similar to a portion of the bacterial RNA polymerase alpha subunit. Gene **145:**289–292.
20. **Pati, U. K., and S. M. Weissman.** 1989. Isolation and molecular characterization of a cDNA encoding the 23-kDa subunit of human RNA polymerase II. J. Biol. Chem. **264:**13114–13121. (Erratum, **266:**13468, 1991).
21. **Pati, U. K., and S. M. Weissman.** 1990. The amino acid sequence of the human RNA polymerase II 33-kDa subunit hRPB 33 is highly conserved among eukaryotes. J. Biol. Chem. **265:**8400–8403.

21a. **Shpakovski, Y., M. Vigneron, and P. Thuriaux.** Personal communication.

22. **Thompson, N. E., D. B. Aronson, and R. R. Burgess.** 1990. Purification of eukaryotic RNA polymerase II by immunoaffinity chromatography. Elution of active enzyme with protein stabilizing agents from a polyol-responsive monoclonal antibody. J. Biol. Chem. **265:**7069–7077.
23. **Tjian, R., and T. Maniatis.** 1994. Transcriptional activation: a complex puzzle with few easy pieces. Cell **77:**5–8.
24. **Treco, D. A., and V. Lundblad.** 1993. Preparation of yeast media, p. 13.1.1–13.1.7. *In* K. Janssen (ed.), Current protocols in molecular biology, vol. 2. Greene Publishing Associates, Inc., New York.
25. **Wintzerith, M., J. Acker, S. Vicaire, M. Vigneron, and C. Kedinger.** 1992. Complete sequence of the human RNA polymerase II largest subunit. Nucleic Acids Res. **20:**910.
26. **Woychik, N. A., and R. A. Young.** 1994. Exploration of RNA polymerase II structure and function, p. 227–242. *In* R. C. Conaway and J. W. Conaway (ed.), Transcription: mechanisms and regulation, vol. 3. Raven Press, New York.
27. **Young, R. A.** 1991. RNA polymerase II. Annu. Rev. Biochem. **60:**689–715.