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Four Subunits That Are Shared by the Three Classes of RNA Polymerase Are Functionally Interchangeable between *Homo sapiens* and *Saccharomyces cerevisiae*

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Four cDNAs encoding human polypeptides hRPB7.0, hRPB7.6, hRPB17, and hRPB14.4 (referred to as Hs10α, Hs10β, Hs8, and Hs6, respectively), homologous to the ABC10α, ABC10β, ABC14.5, and ABC23 RNA polymerase subunits (referred to as Sc10α, Sc10β, Sc8, and Sc6, respectively) of *Saccharomyces cerevisiae*, were cloned and characterized for their ability to complement defective yeast mutants. Hs10α and the corresponding Sp10α of *Schizosaccharomyces pombe* can complement an *S. cerevisiae* mutant (*rpc10*-Δ::*HIS3*) defective in Sc10α. The peptide sequences are highly conserved in their carboxy-terminal halves, with an invariant motif $CX_2CX_{12}RCX_2CGXR$ corresponding to a canonical zinc-binding domain. Hs10β, Sc10β, and the N subunit of archaeal RNA polymerase are homologous. An invariant CX_2CGX_nCCR motif presumably forms an atypical zinc-binding domain. Hs10β, but not the archaeal subunit, complemented an *S. cerevisiae* mutant (*rpb10*-Δ*1*::*HIS3*) lacking Sc10β. Hs8 complemented a yeast mutant (*rpb8*-Δ*1*::*LYS2*) defective in the corresponding Sc8 subunit, although with a strong thermosensitive phenotype. Interspecific complementation also occurred with Hs6 and with the corresponding Dm6 cDNA of *Drosophila melanogaster*. Hs6 cDNA and the Sp6 cDNA of *S. pombe* are dosage-dependent suppressors of *rpo21-4*, a mutation generating a slowly growing yeast defective in the largest subunit of RNA polymerase II. Finally, a doubly chimeric *S. cerevisiae* strain bearing the Sp6 cDNA and the human Hs10β cDNA was also viable. No interspecific complementation was observed for the human hRPB25 (Hs5) homolog of the yeast ABC27 (Sc5) subunit.

Eukaryotic mRNAs are synthesized by large transcription complexes formed by RNA polymerase II and a number of protein cofactors controlling the selectivity and efficiency of transcriptional initiation, elongation, and termination (14, 36). Purified preparations of RNA polymerase II were obtained for several eukaryotes (references 26, 37, 38, and 54 and references therein) and were found to consist of at least 10 distinct polypeptides ranging from 220 to less than 10 kDa. Their subunit structure is thus much more complex than is that of the three-component bacterial core enzyme $\alpha_2\beta\beta'$. Archaeal RNA polymerases also contain a large number of polypeptides, and most of them are related to eukaryotic subunits (21, 23–25, 38).

The genes encoding the 12 subunits of the yeast enzyme have all been cloned and sequenced (see Table 1). The three largest subunits, Sc1, Sc2, and Sc3, are homologous to the β′, β, and α components of the bacterial core enzyme (28, 38, 54). Sc11 (50) is homologous to AC19, a subunit which is shared by yeast RNA polymerases I and III (15). Sc7 is similar to what is most probably the C25 subunit of RNA polymerase III (39). Sc7 was initially believed to be nonessential for mRNA synthesis in vivo (54), but further studies indicated that deletion of the corresponding gene is lethal (29). Five small subunits, Sc5, Sc6, Sc8, Sc10α, and Sc10β (22, 44, 49, 52), are present in all three nuclear RNA polymerases (11, 12, 37, 46). These 10 specific or common subunits are essential components of the transcription apparatus, as strains carrying the corresponding null alleles are nonviable. In contrast, the deletion of the genes encoding the RNA polymerase II-specific Sc4 and Sc9 subunits leads to slowly growing but viable mutants (48, 51).

The human RNA polymerase II, although less extensively characterized, contains at least 10 distinct subunits (19, 26). The yeast and animal enzymes are closely related antigenically (18), indicating a strong evolutionary conservation. This was directly established by cloning and sequencing seven human cDNAs encoding RNA polymerase II subunits Hs1, Hs2, Hs3, Hs5, Hs6, Hs9, and Hs11 (1–3, 32–34, 47), which all showed significant homology to the corresponding yeast subunits (see Table 1). However, sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of the human enzyme (26) failed to reveal small polypeptides of less than 10 kDa that would correspond to the Sc10α and Sc10β subunits shared by all three yeast RNA polymerases (12).

It was recently demonstrated that the common subunit Sc6 of *Saccharomyces cerevisiae* can be functionally replaced in vivo by homologs from *Schizosaccharomyces pombe* and hamsters (30, 41). Therefore, human genes cloned by sequence homology may be functionally identified by interspecific complementation in *S. cerevisiae*. We describe here three human cDNAs, encoding small polypeptides of 7.0 kDa (Hs10α), 7.6 kDa (Hs10β), and 17 kDa (Hs8), that have strong sequence homologies to Sc10α, Sc10β, and Sc8 and are interchangeable with them in vivo. This also applies to the Sp10α homolog of *S. pombe*. Moreover, Hs6 and Dm6 from *Drosophila melanogaster*

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TABLE 1. Simplified nomenclature for yeast and human RNA polymerases II[a]

Organism and subunit	Subunit	Gene(s)
S. cerevisiae		
Sc1	B220	*RPB1* and *RPO21*
Sc2	B150	*RPB2*
Sc3	B44	*RPB3*
Sc4	B32	*RPB4*
Sc5	ABC27	*RPB5*
Sc6	ABC23	*RPB6* and *RPO26*
Sc7	B16	*RPB7*
Sc8	ABC14.5	*RPB8*
Sc9	B12.6	*RPB9*
Sc10α	ABC10α	*RPC10*
Sc10β	ABC10β	*RPB10*
Sc11	B12.5	*RPB11*
H. sapiens		
Hs1	hRPB220	*POLR2* A
Hs2	hRPB140	*POLR2* B
Hs3	hRPB33	*POLR2* C
Hs5	hRPB25	*POLR2* E
Hs6	hRPB14.4	*POLR2* F
Hs7	hRPB19	*POLR2* G
Hs8	hRPB17	*POLR2* H
Hs9	hRPB14.5	*POLR2* I
Hs10α	hRPB7.0	*POLR2* K
Hs10β	hRPB7.6	*POLR2* L
Hs11	hRPB14	*POLR2* J

[a] For *S. cerevisiae*, homogeneous nomenclature (leftmost column) was adopted in the present work, biochemical nomenclature (middle column) is as defined by Sentenac (37), and genetic nomenclature (rightmost column) is as defined in the original literature (5, 38, 54). For *H. sapiens*, homogeneous nomenclature was adopted in the present work, biochemical nomenclature is as proposed by Pati and Weissman (32), and genetic nomenclature is in agreement with that proposed by the Human Gene Mapping workshops.

complement a yeast mutant lacking Sc6. Hs6 and Sp6 have an extragenic suppression effect on a conditional mutant defective in the largest subunit of RNA polymerase II. This structural and functional conservation of the yeast and human small subunits shared by all three nuclear RNA polymerases underscores their fundamental but still elusive role in transcription.

MATERIALS AND METHODS

Nomenclature. In view of the rather confusing nomenclatures used to describe RNA polymerase subunits and their genes, we have adopted homogeneous symbols (Table 1), where the two letters stand for the source (Hs, *Homo sapiens*; Dm, *D. melanogaster*; Sp, *S. pombe*; Sc, *S. cerevisiae*). The concordance with biochemical and genetic nomenclatures currently used for *S. cerevisiae* and *H. sapiens* is indicated in Table 1.

Cloning of cDNAs. The Sp10α from *S. pombe* cDNA was cloned in pGEN by PCR amplification of a cDNA library (7) with primers that were derived from a recently described genomic sequence (accession no. X82444) containing two putative exons, one of which has strong homology to Sc10α (see Table 2, plasmid pGVS121). Three independently isolated clones were sequenced and proven to harbor the same 277-nucleotide fragment (accession no. U20867).

The *D. melanogaster* Dm6 cDNA (accession no. Z47726) was cloned by screening a λ-ZapII cDNA library prepared from *D. melanogaster* embryos by using the human Hs6 cDNA as a probe. Several independent clones were obtained, revealing a sequence of 543 nucleotides with a single open reading frame (ORF) of 393 nucleotides encoding a 14.7-kDa protein.

Hs10α (accession no. Z47727) was obtained by PCR amplification of cDNAs prepared from oligo(dT)-primed HeLa cell poly(A)$^+$ RNA. The primers used (GTTCAACCACCAAAGCAGCAGCCAATGATA and AGGCAAATGTAT GAATGAAGATACT) were derived from the region most highly conserved between the yeast Sc10α sequence and the translation product of a murine cDNA (accession no. S63758 [53]) that has homology to the yeast subunit. The amplified fragment of about 190 bp was ^{32}P labelled by nick translation and used to screen λ-Zap cDNA libraries prepared from either random-primed or oligo(dT)-primed HeLa poly(A)$^+$ RNA. These libraries were plated at 40,000 plaques per 13.5-cm-diameter petri dish. Two filters per dish were lifted and hybridized (37°C, 0.9 M NaCl, 35% formamide) with the labelled PCR-derived probe. Eight independent clones were characterized. The inserts were recovered as pBluescriptSK$^-$ recombinants by using a commercial helper-mediated excision system (Stratagene) and characterized by Southern blot analysis of *Eco*RI digests with the PCR-derived probe.

Hs10β (accession no. Z47728 and Z47729) cDNA was cloned by using degenerate oligonucleotide primers derived from two conserved motifs of the yeast Sc10β and archaeal N subunits (10-CGKVVGDKWE-19 and 45-CCRRMI LTHV-54 [Fig. 1]) amplified on HeLa cell cDNAs (see above). An amplified fragment of about 130 bp was ^{32}P labelled by nick translation and used to screen a λ-Zap cDNA library [prepared from random-primed HeLa poly(A)$^+$ RNA]. Five independent clones were characterized and recovered as pBluescriptSK$^-$ recombinants (see above). A λ-EMBL3 genomic library (from partial *Sau*3AI digests of human placental DNA) was screened with the same probe. Positive clones were identified by Southern blotting of *Sac*I, *Not*I, *Sau*3AI, and *Sfi*I digests, and a 4.7-kb *Sac*I fragment was subcloned into pBluescriptSK$^-$.

Hs8 (accession no. Z49199) cDNA was isolated by direct screening of a HeLa cDNA library with an oligonucleotide probe (GACCCCGACGGCAAGAAGT TCGACCGGGT) based on the peptide homology (DPDGKKFDRV) chosen on the basis of the sequence comparison of the putative Sc8 homologs from the genomes of the nematode *Caenorhabditis elegans* (accession no. U12964 and U13875) and the rice species *Oryza sativa* (accession no. D15823) (Fig. 1). Three independent clones encompassing the complete ORF were recovered as pBluescriptSK$^-$ recombinants (see above). PCR of reverse-transcribed poly(A)$^+$ RNA from HeLa cells yielded the same coding sequence, which ruled out the possibility that chimeric artifacts were generated during the preparation of the cDNA library.

Plasmids, strains, and yeast genetic techniques. The plasmids constructed in the present work are listed in Table 2. pYADE4, pYPGE2, pRPO26, pSL103, pASZ11, pFL44L, pLS193, pFL44-RPB10e, pGVS41, and pGVS58 were described previously (9, 10, 22, 41, 43, 44, 49). Yeast strains (Table 2) were grown at 16, 30, or 37°C on YPD, YPGE, FOA, and inositolless media (8, 10, 14a, 40). Minimal SD medium (40) supplemented with 0.1% casein hydrolysate, 0.002% adenine sulfate, and 0.002% uracil or tryptophan was used as tryptophan or uracil omission medium. Sporulation was done on solid potassium acetate medium (40). Strains were constructed by standard genetic techniques based on transformation of lithium-acetate-treated cells, sexual mating, and tetrad analysis (40). Zygotes and spores were isolated by using a de Fonbrunne micromanipulator. Strains JAY212, JAY444, YSL171, and Z431 were provided by Jacques Archambault and Nancy Woychik. Plasmids pRPON, pFL44-RPC10, and pRPB10-5 (Table 2) were gifts of Doris Langer and Dominique Lalo.

Interspecific complementation was tested by examining whether plasmids expressing a given cDNA could bypass the lethal phenotype conferred by the *rpb10*-Δ*1*::*HIS3*, *rpc10*-Δ::*HIS3*, *rpb8*-Δ*1*::*LYS2*, and *rpb6*-Δ::*LEU2* alleles. We used a plasmid-shuffling assay in which the *rpb10*$^-$, *rpc10*$^-$, *rpb8*$^-$, or *rpb6*$^-$ null alleles of haploid tester strains (YGVS017, YGVS020, YGVS043, and JAY444) were complemented by the corresponding wild-type genes borne on the *ADE2*$^+$ or *URA3*$^+$ plasmids pRPB10-5, pFL44-RPC10, pSL103, and pRPO26 (Tables 1 and 2). In a wild-type context, these plasmids are lost at a rate of about 10% per cell division (9). In the tester strains used, this lethal event can be relieved only by heterospecific complementation. Plasmid loss was monitored by the *ade2*$^-$ red sectors or fluoro-orotate-resistant colonies (8) resulting from the loss of the *ADE2* or *URA3* allele of pRPB10-5, pFL44-RPC10, pSL103, or pRPO26. Interspecific complementation for the Sc10α and Sc10β subunits was also tested by using diploid tester strains that are heterozygous for *rpc10*-Δ::*HIS3*/+ (LS137) and *rpb10*-Δ*1*::*HIS3*/+ (YGVS018), respectively. Upon transformation with the appropriate complementing plasmids, these strains were induced to sporulate and subjected to tetrad analysis of their meiotic offspring by microdissection on YPD. The untransformed diploids have a Mendelian segregation of two fully growing colonies bearing the wild-type *RPC10*$^+$ or *RPB10*$^+$ allele and two lethal segregants corresponding to the deleted allele. In contrast, the complementing plasmid yielded additional viable segregants that bear the deleted chromosomal allele (as monitored by histidine prototrophy) together with the complementing plasmid (Fig. 2).

Nucleotide sequence accession numbers. The cDNA sequence data reported here have been assigned the following EMBL Sequence Data Library accession numbers: Hs10α, Z47727; Sp10α, U20867; Hs10β, Z47728 and Z47729; Hs8, Z49199; Dm6, Z47726.

RESULTS

Cloning of the Hs10α and Sp10α cDNAs and in vivo substitution of Sc10α in *S. cerevisiae*. Eight cDNAs encoding Hs10α were obtained and analyzed. Their sequence extended over 237 nucleotides (accession no. Z47727), with a 189-nucleotide ORF. The 48-nucleotide sequence upstream of the ATG contains a stop codon in all three reading frames. The predicted polypeptide has a calculated molecular mass of 6,974 Da. It is identical (except for a single amino acid substitution)

Subunit Hs10α family

```
H. sapiens                1  MDTQKDVQPPKQQPMIYICGECHTENEIKSRDPIRCRECGYRIMYKKRTKRLVVFDAR  58
S. pombe                  1  MNHPTSTGGTAFNPPRPATMIYLCADCGRRNTIQAKEVIRCRECGHRVMYKMRTKRMVQFEAR  63
S. cerevisiae             1  MSREGFQIPTNLDAAAAGTSQARTATLKYICAECSSKLSLSRTDAVRCKDCGHRILLKARTKRLVQFEAR  70
INVARIANTS                                  Y C  C            RC  CG R   K RTKR V F AR
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Subunit Hs10β family

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H. sapiens                1  MIIPVRCFTCGKIVGNKWEAYLGLLQAE---YTEGDALDALGLKRYCCRRMLLAHVDLIEKLLNYAPLEK      67
O. sativa                 1  MIIPVRCFTCGKVIGNKWDLYLDLLQAD---YTEGDALDALGLVRYCCRRMLMTHVDLIEKLLNYNTLEKTE    69
B. napus                  1  MIIPVRCFTCGKVIGNKWDAYLDLLQLD---YTEGDALDALNLVRYCCRRMLMTHVDLIEKLLNYNTLEKSDNS  71
S. cerevisiae             1  MIVPVRCFSCGKVVGDKWESYLNLLQEDE--LDEGTALSRLGLKRYCCRRMILTHVDLIEKFLRYNPLEKRD     70
S. acidocaldarius         1  MIIPIRCFTCGAVVADKWEPFSNRVMGG---EDPEKVLTELGVNRYCCRRMLLSHVNIIREIIHYTRPI        66
H. marismortui            1  MMVPVRCFTCGNVVGEHWEEFKARTREAEEPEDPEKVLDELGVERHCCRRMLVSHKDLVDIVSPYQ           66
Vaccinia virus            1  MVFQLVCSTCGKDISHERYKLIIRKKSL------KDVL--VSVKNECCRLKLSTQIEPQRNLTVQPLLDIN     63
INVARIANTS                   M     C  CG                        L        CCR
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Subunit Hs8 family

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H. sapiens                1  MAGILFEDIFDVKDIDPEGKKFDRVSRLHCESE-SFKMDLILDVNIQIYPVDLGDKFRLVIASTLYEDGTLDDGEYNP------   77
C. elegans                1  MAGIIPDDMPKVKSVDPDGKKFDRVSRYFCDAE-SFKMELIIDINSQIYPLKQNDKVRLVLATTLREDGLADEGEYDP------   77
O. sativa                 1  MAEFLFEDLPTVTRLDPDGKKFDRVSRIEARSD-QFDMYMQLDVATDVYPMHPGDRFTMVLVPTLNLDGTPDSAFFT-------  76
S. cerevisiae             1  MSNTLFDDIFQVSEVDP-G-RYNKVCRIEAASTTQDQCKLTLDINVELFPVAAQDSLTVTIASSLNLEDTPANDSSATRSWRPP  82
INVARIANTS                   M    F D F V   DP G    V R              D      P    D         L

H. sapiens               78  TDDRPSRADQFEYVMYGKVYRIEGDETSTEAAT-RLSAYVSYGGLLMRLQGDANNLHGFEVDSRVYLLMKKLAF          150
C. elegans               78  KAEYP-RIKQYEYVMYGKVYRLEDDDTGTDGG--KLAAYASFGGLLMRLKGEAINLHGFEVDMNLYLLMKKTDF          148
O. sativa                77  QAGRKTLADKYEYVMHGKLYKISEDKDNSDQNAKKVAMYASFGGLLVMLKGLLVMLKGDPPVMR..                  [140]
S. cerevisiae            83  QAGDRSLADDYDYVMYGTAYKPEEVSKD------LIAVYYSFGGLLMRLEGNYRNLNNLKQENA-YLLIPR            146
INVARIANTS                      YVM G  Y                 Y S GGLL  L G    L         YLL
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Subunit Hs6 family

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H. sapiens               40  PSGERPQANQKRITTPYMTKYERARVLGTRALQIAMCAPVMVELEGETD-PLLIAMKELKARKIPIIIRRYLPDGSYEDWGVDELIITD  127
D. melanogaster          43  GAGGGGVPKSKRITTKYMTKYERARVLGTRALQIAMCAPIMVELDGETD-PLQIAMKELKQKKIPIIIRRYLPDHSYEDWSIDELIMVDN  131
S. pombe                 58  AQSGKAVAKEDRTTTPYMTKYERARILGTRALQISMNAPVLVDLEGETD-PLQIAMKELAQKKIPLLVRRYLPDGSYEDWSVAELI      142
S. cerevisiae            68  TLKEKAIPKDQRATTPYMTKYERARILGTRALQISMNAPVFVDLEGETD-PLRIAMKELAEKKIPLVIRRYLPDGSFEDWSVEELIVDL  155
S. acidocaldarius         1  MTIDKINEIFKENWKNKLTKYEIARIISARALQYLWSLTTDTYFYPKSDAVISIA-RGIKRGVLPITIRRIYPNGQVELISVRK        83
H. marismortui            1              MNAQESRYEKARKLGARALQLAHGAPVLIETEHTQ--PILIAAEEYDAGVLPFTVNRSD                                  57
ASFV virus               58  SQTLVIIPDNERITSNVLTTFEATRLVAVRAQQLAINGSTMLKKKYSS--PIDIAKQELFNRKIPLLVMRCIKVTPEGQKIVEI      139
INVARIANTS                                    E  R    RA Q                  IA         P    R
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Subunit Hs5 family

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H. sapiens                1  MDDEEETY--RLWKIRKTIMQLCHDRGYLVTQDELDQTLEEFKAQFGDKPSEGRPRRTDLTVLVAHNDDPTDQ-      71
S. cerevisiae             1  MDQENERNISRLWRAFRTVKEMVKDRGYFITQEEVELPLEDFKAKYCDSM--GRPQRKMMSFQANPTEESISKF      72
INVARIANTS                   MD E E    RLW    T      DRGY  TQ E    LE FKA   D    GRP R

H. sapiens               72  -----MFVFPPEEPKVGIKTIKVYCQRMQEENITRALIVVQQGMTPSAKQSLVDMAPKYILEQFLQQELLINIT      140
S. cerevisiae            73  PDMGSLWVEFCDEPSVGVKTMKTVFIHIQEKNFQTGIFVYQNNITPSAMK-LVPSIPPATIETFNEAALVVNIT      145
S. acidocaldarius         1                                                          MGKIMRSSSKKKIDIS      16
INVARIANTS                       V F  EP VG KT K      QE N      V Q   TPSA   LV   P              I

H. sapiens               141  EHELVPEHVVMTKEEVTELLARYKLRENQLPRIQAGDPVAPYFGIKRGQVVKIIRPSETAGRYITYRLVQ         210
S. cerevisiae            146  HHELVPKHIRLSSDEKRELLKRYRLKESQLPRIQRADPVALYLGLKRGEVVKIIRKSETSGRYASYRICM         215
S. acidocaldarius         17  NHELVPKHEILQLEEAYKLVKELGIKPEQLPWIRASDPVAKSIGAKPGDIIKITRKSPFTGESVTYRIVITG       88
INVARIANTS                    HELVP H      E   L         QLP I   DPVA   G K G   KI R S   G    YR
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to the C-terminal part of the product of an ORF present on a previously reported mouse cDNA (accession no. S63758). Curiously, this murine cDNA activates transcription from a metal response element of the mouse metallothionein I gene inserted into the yeast genome (53).

As shown in Fig. 1, the amino acid sequences of Hs10α, the *S. pombe* Sp10α (accession no. U20867) cDNA gene product (predicted molecular mass, 7,289 Da), and the *S. cerevisiae* Sc10α subunit are closely related to each other. All three polypeptides are very basic, with predicted pIs of 9.27, 10.21, and 9.84, respectively. Their C-terminal 25 residues are identical or strongly conserved, whereas their N-terminal ends are poorly conserved in size and sequence. In keeping with the zinc-binding properties of Sc10α in vitro (45), there is an invariant canonical $CX_2CX_{13}CX_2C$ zinc-binding motif. The single intron of the *S. pombe* gene falls between the first two cysteines, within the codon corresponding to position D-26 of the amino acid sequence (Fig. 1).

Upon being subcloned in suitable expression vectors, the human and *S. pombe* cDNAs were found to complement the defective *rpc10*-Δ::*HIS3* allele, as determined by tetrad analysis assays as well as a plasmid-shuffling assay based on the spontaneous loss of pFL44-RPC10 (Fig. 2). The doubling time for the complemented strains was 2 h on YPD (30°C), similar to the wild-type level. It has been repeatedly observed (e.g., see reference 51) that yeast mutants with a partially defective RNA polymerase II fail to grow without inositol. We have indeed observed a somewhat less effective complementation on inositolless medium at 30°C and an almost complete inositol auxotrophy at 37°C (data not shown), indicating that the Hs10α and Sp10α subunits do not fully replace their yeast homolog.

Cloning of the Hs10β gene and its cDNA. A PCR-amplified HeLa cell cDNA fragment spanning two domains that are conserved in the homologous *S. cerevisiae* Sc10β and *Sulfolobus acidocaldarius* N subunits was used to screen a HeLa cell cDNA library (see Materials and Methods). Several independent overlapping clones were isolated and sequenced, and they gave a sequence of 381 nucleotides containing an ORF of 201 bp. The corresponding polypeptide (Fig. 1) has a calculated molecular mass of 7,645 Da and a pI of 7.65.

The first methionine codon of the ORF lies within a context compatible with translation initiation (16) but could not be unambiguously identified as a translation initiator because of the lack of an in-frame stop codon up to the 5′ extremity of the cDNA sequence. This raised the possibility that the N terminus of the subunit was missing from the cloned cDNAs. To answer this question, we cloned the corresponding genomic region as a 4.7-kb *Sac*I fragment isolated from a λ-EMBL3 human genomic library (accession no. Z47728 and Z47729). Comparing the genomic sequence with that of the cDNA indicated that the entire Hs10β coding sequence was contained within the genomic fragment and revealed an intervening sequence of about 2.1 kb that falls within the codon for G-32 in the amino acid sequence (Fig. 1).

The 5′ end of the mRNA was determined by reverse-transcribed primer extension mapping of HeLa cell poly(A)$^+$ RNA (2), which resulted in one major band starting 21 nucleotides upstream of the putative initiator ATG (data not shown). The RNA transcribed from this site encodes the same ORF as that predicted from the cDNA analysis, thus confirming the position of the translation initiation site. Moreover, there is no other potential start codon in the upstream region sequenced so far.

Examination of the nucleotide sequence upstream of the transcription start sites did not reveal signatures of typical promoter elements, with the exception of a significantly high GC content (77%) within the 195-bp 5′-flanking sequences, which is a characteristic of promoters of many housekeeping genes (42). The sequence surrounding the 5′ end (GCAGTC) is similar although not identical to the TCATTC consensus found for the initiators (42) and may nevertheless be functional, as only one major mRNA band was detected by reverse-transcribed primer extension.

Structure and sequence conservation of Hs10β. As shown in Fig. 1, there is a very strong similarity between Hs10β and Sc10β, with 49 identical amino acids out of 70 (70%). Remarkably, half of these residues are invariant in the N subunit of the archaeal (*S. acidocaldarius*) RNA polymerase (23) and have a less pronounced but significant homology to a 7-kDa component of the vaccinia virus enzyme (4). In addition to showing homology to these three bona fide subunits, Hs10β shows significant homology to putative ORFs present in the genome of an archaeon, *Haloarcula marismortui*, and in the cDNAs of a rice species (*O. sativa*) and a turnip species (*Brassica napus*), as revealed by DNA data bank screening. After optimal sequence alignment, 70, 45, and 22% of the Hs10β residues were found to be identical at corresponding positions of the yeast, archaeal, and viral sequences, respectively. The corresponding scores were 79, 58, and 38% when the similarities between amino acid residues were taken into account. Six strictly invariant residues form a CX_2CGX_nCCR motif that may define an atypical zinc finger, consistent with the zinc-binding properties of Hs10β in vitro (45). The two pairs of cysteines are encoded by distinct exons in the human gene. They are separated by a domain that is highly conserved among eukaryotes but differs somewhat in size and sequence in the archaeal and viral subunits.

In vivo substitution of Sc10β by the human Hs10β but not by the archaeal N subunit. The Hs10β cDNA was cloned into yeast vectors allowing its constitutive (pGEN) expression and tested for its ability to complement the defective *rpb10*-Δ*1*::*HIS3* mutation by using the plasmid-shuffling and tetrad analysis assays described in Materials and Methods. Viable haploid segregants bearing the *rpb10*-Δ*1*::*HIS3* chromosomal allele and

FIG. 1. Sequence alignments of the five human subunits with eukaryotic, archaeal, and viral homologs. Amino acids that are invariant in pairwise alignments are denoted by asterisks. Invariant amino acids shared by all sequences are indicated below each set of sequences. For the subunit Hs10α family, shown is alignment of the human Hs10α (this work, accession no. Z47727) with the *S. pombe* Sp10α (this work, accession no. U20867) and *S. cerevisiae* subunit Sc10α (44). For the subunit Hs10β family, shown is alignment of the human Hs10β cDNA product (this work, accession no. Z47728 and Z47729) with the translated products of cDNAs from rice (*O. sativa*) and turnip (*B. napus*) species (accession no. D23218 and U12133, respectively), the *S. cerevisiae* Sc10β (22), the N subunit of *S. acidocaldarius* (23), the translated product of an *H. marismortui* ORF (accession no. M76567), and the 7-kDa subunit of vaccinia virus RNA polymerase (4). For the subunit Hs8 family, shown is alignment of the human Hs8 cDNA (this work, accession no. Z49199) with the translated products deduced from a genomic DNA from *C. elegans* (accession no. U12964 and U13875, allowing for putative introns) and from an incompletely sequenced rice (*O. sativa*) cDNA (accession no. D15823, allowing for a frameshift sequencing error) and with the *S. cerevisiae* subunit Sc8 (49). For the subunit Hs6 family, shown is alignment of the most highly conserved portion of the human Hs6 (3) with the corresponding sequences of *D. melanogaster* (this work, accession no. Z47726), *S. pombe* (Sp6 [41]), *S. cerevisiae* (Sc6 [49]), the K subunit of *S. acidocaldarius* RNA polymerase (25), the translated product of an *H. marismortui* ORF, and the PMVK-CL ORF of African swine fever virus (ASFV) (27). For the subunit Hs5 family, shown is alignment of Hs5, corrected for a frameshift error in the initial sequence (32; see also references 13 and 38), with the *S. cerevisiae* subunit (Sc5 [49]) and the H subunit of *S. acidocaldarius* RNA polymerase (21, 38).

TABLE 2. Yeast plasmids and strains used

Plasmid or strain	Nonbacterial genes and cDNAs or genotype[a]	Construction, origin, or reference
Plasmids		
pGEN	2μm *TRP1* pPGK	Modified pYPGE2 (10) created by inserting AATTCGCTAGCACTAGTCCTAGG TCTAGA into *Eco*RI site to generate unique *Eco*RI, *Nhe*I, and *Avr*II sites
pGVS102	2μm *URA3 RPB10*	Deletion of 1.3-kb *Pvu*II fragment from pFL44-RPB10e (22), removing most of *MGM1* gene downstream of *RPB10*
pGVS108	2μm *TRP1* pADH2 *RPB10*	Directional cloning of *S. cerevisiae RPB10* ORF (Sc10β) between *Sma*I and *Eco*RI sites of pYADE4 (10) by PCR with pGVS102, by using CGTAAACCCGGGTAA GCAAAATAATAATAC and GAAAAGAAGAATTCAAAAACAGCCATATTG primers in front of ATG initiator and behind stop codon
pRPB10-5	*CEN6 ARSx ADE2 RPB10*	Cloning of *Bam*HI-*Kpn*I fragment (1.6 kb) from pFL44-RPB10e to pASZ11 vector (43)
pGEN-Sc10β	2μm *TRP1* pPGK *RPB10*	Cloning of *RPB10* ORF (Sc10β) into *Nhe*I site of pGEN by PCR amplification of a pGVS102 probe with TGCTAGCATGATTGTCCCAGTCAGA and GGCTAGC TTAATCTCTTTTTTCTAA primers to create an in-frame *Nhe*I site in front of ATG and behind stop codon
pGEN-Hs10β	2μm *TRP1* pPGK Hs10β	PCR cloning of Hs10β cDNA between *Nhe*I and *Spe*I sites of pGEN, by using GGGCTAGCATGATCATCCCTGTACGCTGC and GGACTAGTTCACTTCTC CAGGGGTGCATA primers to create an in-frame *Nhe*I site in front of ATG and an in-frame *Spe*I site behind stop codon
pRPON	*RpoN* (*S. acidocaldarius*)	Cloning of 1.2-kb *Eco*RI-*Hin*dIII fragment bearing *S. acidocaldarius* gene *RpoN* into pBluescript KS$^+$ (22a)
pGVS106	2μm *TRP1* pADH2 *RpoN*	PCR cloning of *RpoN* ORF between *Sma*I and *Eco*RI sites of pYADE4 (10) by using GCGCCCGGGTGAAACTTCATGATTATTCCGA and CTAACTGAATTC TGTTCCTCTCTCCTTC primers in front of ATG (replacing TTG in *RpoN*, underlined) and behind stop codon
pGVS107	2μm *TRP1* pADH2 *RpoN*	Blunt-end cloning of *RpoN* ORF into *Sma*I site of pYADE4 (10) by PCR amplification of pRPON with GCGCCCGGGTGAAACTTCATGATTATTCCGA and CTAACTGAATTCTGTTCCTCTCTCCTTC primers in front of ATG initiator and behind stop codon
pGEN-Hs8	2μm *TRP1* pPGK Hs8	PCR mutagenesis of Hs8 cDNA with primers CACTAGTATGGCGGGCATCCTG TTTGAGGA and GTCTAGATCAGGCGAGGTTCAGAAGGCTAG, introducing a *Spe*I restriction site in front of ATG codon and an *Xba*I site behind stop codon. The *Spe*I-*Xba*I fragment was inserted in the *Nhe*I site of pGEN
pGEN-Hs6	2μm *TRP1* pPGK Hs6	PCR mutagenesis of Hs6 cDNA, introducing a *Nhe*I restriction site in front of ATG codon and a *Spe*I site behind stop codon, as indicated above for the Hs10β ORF. The *Nhe*I-*Spe*I fragment was then inserted in the *Nhe*I site of pGEN
pGEN-Dm6	2μm *TRP1* pPGK Dm6	PCR mutagenesis of Dm6 cDNA, introducing a *Nhe*I restriction site in front of ATG codon and a *Spe*I site behind stop codon. The *Nhe*I-*Spe*I fragment was then inserted in the *Nhe*I site of pGEN
pGEN-Hs10α	2μm *TRP1* pPGK Hs10α	PCR mutagenesis of Hs10α cDNA, introducing a *Nhe*I restriction site in front of ATG and a *Spe*I site behind stop codon, as indicated above for the Hs10β ORF. The *Nhe*I-*Spe*I fragment was then inserted in the *Nhe*I site of pGEN
pGVS121	2μm *TRP1* pPGK *rpc10*$^+$ *Szp*	Cloning of *rpc10* ORF of *S. pombe* (Sp10α) between *Bam*HI and *Eco*RI sites of pGEN by PCR amplification of an *S. pombe* cDNA library (7) by using TTTAAC TGGATCCAAATACACTAAAAAAGTT and AAGTTAGAATTCCGTTTTTCT CTTTTCATAG primers in front of ATG and behind stop codon
pFL44-RPC10	2μm *URA3 RPC10*	Directional cloning of *RPC10* (Sc10α) containing 2.2-kb *Sal*I-*Bam*HI fragment of pLS193 (44) into pFL44L (9, 21a)
Strains		
YPH499	*MAT***a** *ade2-1 lys2-801 ura3-52 trp1-Δ63 his3-Δ200 leu2-Δ1*	Yeast Genetic Stock Center, Berkeley, Calif.
YPH500	*MAT*α *ade2-1 lys2-801 ura3-52 trp1-Δ63 his3-Δ200 leu2-Δ1*	Yeast Genetic Stock Center, Berkeley, Calif.
JAY212	*MAT*α *CAN1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 ade2-1 rpo21-4*	5
JAY444	*MAT*α *CAN1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 ade2-1 rpb6-Δ::LEU2*(pRPO26: *CEN ARS URA3 RPB6* [Sc6])	5
YGVS003	*MAT*α *CAN1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 ade2-1 rpb6-Δ::LEU2*(pGEN-Hs6: 2μm *TRP1* pPGK Hs6)	Plasmid shuffling in JAY444
YGVS030	*MAT*α *CAN1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 ade2-1 rpb6-Δ::LEU2*(pGVS41: 2μm *URA3 rpb6 Szp* [Sp6])	Plasmid shuffling in YGVS31
YGVS031	*MAT*α *CAN1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 ade2-1 rpb6-Δ::LEU2*(pGVS58: *CEN4 ARSH4 HIS3* pADC1 *rpb6* Szp [Sp6])	Plasmid shuffling in JAY444

Continued on following page

TABLE 2—*Continued*

Plasmid or strain	Nonbacterial genes and cDNAs or genotype[a]	Construction, origin, or reference
YGVS041	*MAT*α *CAN1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 ade2-1 rpb6-*Δ*::LEU2*(pGEN-Dm6: 2μm *TRP1* pPGK Dm6)	Plasmid shuffling in JAY444
Z431	*MAT***a**/*MAT*α *his3-*Δ*200 leu2-3,112 lys2-*Δ*201 ade2-1 ura3-52 RPB10*/*rpb10-*Δ*1::HIS3*	52
YGVS013	*MAT*α *his3-*Δ*200 leu2-3,112 lys2-*Δ*201 ade2-1 ura3-52 rpb10-*Δ*1::HIS3* (pRPB10-5: *CEN6 ARSx ADE2 RPB10* [Sc10β])	Segregant of Z431(pRPB10-5)
YGVS015	*MAT*α *his3-*Δ*200 leu2-3,112 lys2-*Δ*201 ade2-1 ura3-52 rpb10-*Δ*1::HIS3* (pGVS102: 2μm *URA3 RPB10* [Sc10β])	Shuffling in YGVS013
YGVS017	*MAT*α *ura3-52 his3-*Δ*200 leu2* lys2* ade2-1 trp1-*Δ*63 rpb10-*Δ*1::HIS3* (pRPB10-5: *CEN6 ARSx ADE2 RPB10* [Sc10β])	Segregant of YGVS013 × YPH499
YGVS018	*MAT***a**/*MAT*α *ura3-52 his3-*Δ*200 leu2* lys2* ade2-1 trp1-*Δ*63 RPB10*/*rpb10-*Δ*1::HIS3*	YGVS017 × YPH499, cured of pRPB10-5
YGVS021	*MAT*α *ura3-52 his3-*Δ*200 leu2* lys2* ade2-1 trp1-*Δ*63 rpb10-*Δ*1::HIS3* (pGEN-Hs10β: 2μm *TRP1* pPGK Hs10β)	Segregant of YGVS018(pGEN-Hs10β)
YGVS022	*MAT***a** *ura3-52 his3-*Δ*200 leu2* lys2* ade2-1 trp1-*Δ*63 rpb10-*Δ*1::HIS3* (pGEN-Hs10β: 2μm *TRP1* pPGK Hs10β)	Segregant of YGVS018(pGEN-Hs10β)
YGVS026	*MAT*α *ura3-52 his3-*Δ*200 leu2* lys2* ade2-1 trp1-*Δ*63 rpb10-*Δ*1::HIS3* (pGVS108: 2μm *TRP1* pADH2 *RPB10* [Sc10β])	Plasmid shuffling in YGVS017
YGVS032	*MAT***a** *CAN1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 ade2-1 rpb6-*Δ*::LEU2 rpb10-*Δ*::HIS3* (pGEN-Hs10β: 2μm *TRP1* pPGK Hs10β + pGVS41: 2μm *URA3 rpb6*$^+$ *Szp* [Sp6])	Segregant of YGVS030 × YGVS022
LS137	*MAT***a**/*MAT*α *CAN1-100*/+ *his3-*Δ*200 lys2-801 trp1-*Δ*1 ura3-52 ade2-1 RPC10*/*rpc10-*Δ*::HIS3*	44
YGVS020	*MAT***a** *his3-*Δ*200 lys2-801 trp1-*Δ*1 ura3-52 ade2-1 rpc10-*Δ*::HIS3* (pFL44-RPC10: 2μm *URA3 RPC10* [Sc10α])	Segregant of LS137(pFL44-RPC10)
YGVS039	*MAT***a** *his3-*Δ*200 lys2-801 trp1-*Δ*1 ura3-52 ade2-1 rpc10-*Δ*::HIS3* (pGEN-Hs10α: 2μm *TRP1* Hs10α)	Plasmid shuffling in YGVS020
YGVS040	*MAT***a** *his3-*Δ*200 lys2-801 trp1-*Δ*1 ura3-52 ade2-1 rpc10-*Δ*::HIS3* (pGVS121: 2μm *TRP1 rpc10 Szp* [Sp10α])	Plasmid shuffling in YGVS020
YSL171	*MAT***a** *his3-*Δ*200 lys2-*Δ*201 leu2-3,112 ura3-52 ade2-1 rpb8-*Δ*1::LYS2* (pSL103: *CEN URA3 RPB8* [Sc8])	49
YGVS043	*MAT***a** *his3-*Δ*200 lys2* leu2* ura3-52 ade2-1 trp1-*Δ*63 rpb8-*Δ*1::LYS2* (pSL103: *CEN URA3 RPB8* [Sc8])	Segregant of YSL171 × YPH500
YGVS045	*MAT***a** *his3-*Δ*200 lys2* leu2* ura3-52 ade2-1 trp1-*Δ*63 rpb8-*Δ*1::LYS2* (pGEN Hs8: 2μm *TRP1* pPGK Hs8)	Plasmid shuffling in YGVS043

[a] *leu2**, *leu2-3,112* or *leu2-*Δ*1*; *lys2**, *lys2-801* or *lys2-*Δ*201*.

the pGEN-Hs10β plasmid were readily recovered (Fig. 2). As with Hs10α, the doubling time on YPD was indistinguishable from that of a wild-type control, but complementation was less effective on inositolless medium, especially at 37°C (data not shown). In keeping with a previous report (31), heterologous expression of the archaeal N subunit (plasmids pGVS106 and pGVS107 [Table 2]) failed to complement *rpb10-*Δ*1::HIS3*.

Cloning of the Hs8 cDNAs and in vivo substitution of Sc8 in *S. cerevisiae*. Three independent cDNAs encoding Hs8 were obtained and analyzed. Their sequence extended over 813 nucleotides up to the poly(A) site (accession no. Z49199), with a 450-nucleotide ORF frame that was confirmed by reverse-transcribed PCR. No other ATG codon was found in the 5′ untranslated 78 nucleotides. The predicted 150-amino-acid polypeptide has a calculated molecular mass of 17,143 Da, and, like Sc8 (pI of 4.6), is rather acidic, with a pI of 4.34. When the sequence was aligned with that of Sc8, only 49 identical residues (33%) were observed (Fig. 1); when similarities are taken into account, the score is raised to 51%. The yeast and human sequences were also aligned with the putative amino acid sequences from *C. elegans* (accession no. U12964 and U13875) and *O. sativa* (partial sequence, accession no. D15823) as deduced from entries in current DNA data banks.

The Hs8 cDNA was cloned into the pGEN expression vector and tested for its ability to complement the defective *rpb8-*Δ*1*::*LYS2* mutation (49). As shown in Fig. 3, complementation was observed at 30°C but not at 37°C. Again, growth on inositolless medium (tested at 30°C; data not shown) was less effective.

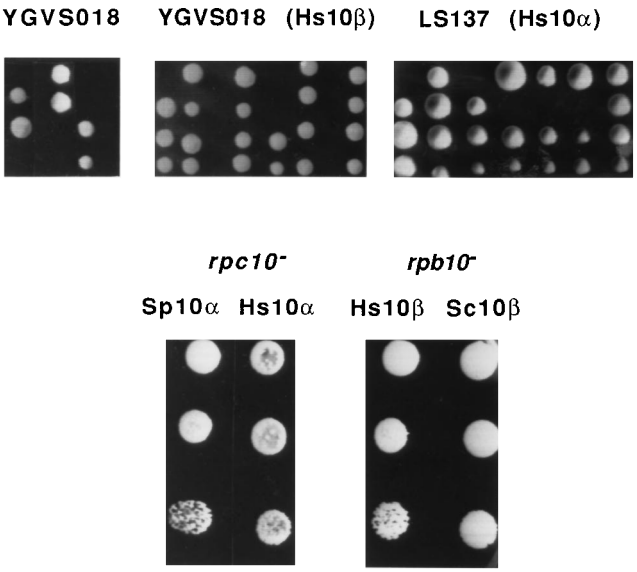

FIG. 2. Interspecific complementation of *rpc10*-Δ::*HIS3* and *rpb10*-Δ*1*::*HIS3* by cDNAs of *H. sapiens* and *S. pombe*. (Upper panels) Lethal 2+:2− segregation in tetrad analysis of the *rpb10*-Δ*1*::*HIS3* allele in the control heterozygous diploid strain (YGVS018) and recovery of meiotic asci with four or three viable spores in the presence of the pGEN-Hs10β plasmid bearing Hs10β expressed under control of the yeast pPGK promoter [YGVS018 (Hs10β)]. Viable segregants bearing the deleted *rpb10*-Δ*1*::*HIS3* allele invariably harbored the complementing plasmid. Similar data are presented for the heterozygous *RPC10*/*rpc10*-Δ::*HIS3* strain LS137, which exhibits a lethal 2+:2− segregation of the *rpc10*-Δ::*HIS3* allele (data not shown and reference 44) with recovery of meiotic asci with four or three viable spores in the presence of the pGEN-Hs10α plasmid bearing Hs10α expressed under control of the yeast PGK promoter [LS137 (Hs10α)]. (Lower panels) Haploid strains harboring the *rpc10*-Δ::*HIS3* allele (left) were complemented by pGVS121 (constitutively expressing the Sp10α subunit of *S. pombe*) or pGEN-Hs10α (constitutively expressing the human Hs10α subunit). Haploid strains bearing the *rpb10*-Δ*1*::*HIS3* allele (right) were complemented by pGEN-Hs10β (constitutively expressing the human Hs10β cDNA) or by multiple copies of the *RPB10* gene (subunit Sc10β) of *S. cerevisiae*. These constructions were obtained by plasmid shuffling and are genetically equivalent to the segregants obtained by tetrad analysis as described above. Growth was tested by dropping 10 μl of a fresh liquid culture (undiluted and after 10- and 100-fold dilutions) on YPD plates and incubating at 30°C for 3 days (similar results were obtained at 16 and 37°C).

An *S. cerevisiae* mutant defective in the largest subunit of RNA polymerase II is suppressed by overexpression of Hs6 and Sp6 subunits. Figure 1 presents an alignment of Sc6 with the predicted products of the Sp6 cDNA from *S. pombe* (41), Hs6 from *H. sapiens* (3), and Dm6 from *D. melanogaster* (this work; accession no. Z47726). These eukaryotic sequences are very well conserved with only a few conservative substitutions, except for their N-terminal domain, which is quite variable in size, albeit very acidic in all four cases (data not shown). Figure 1 also depicts a gene product of the African swine fever virus with low-level but significant homology to the Sc6 family, including an acidic N-terminal domain (27), and archaeal sequences corresponding to the K subunit of RNA polymerase, which lacks the acidic domain (23, 25).

In keeping with previous results obtained for the *S. pombe* and hamster cDNA products (30, 41), we were able to use both Hs6 and Dm6 to complement the *S. cerevisiae* strain, JAY444, bearing the *rpb6*-Δ::*LEU2* allele defective in the Sc6 subunit. Unlike what was observed with all other subunits tested (see above), heterospecific complementation was fully effective on both YPD and inositolless medium. A double mutant lacking both Sc6 and Sc10β subunits was shown to be complemented by the human Hs10β and *S. pombe* Sp6 subunits (strain YGVS032 [Table 2]).

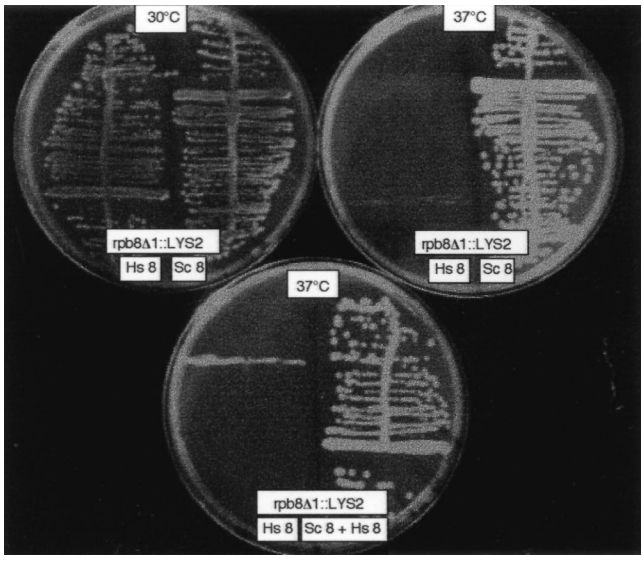

FIG. 3. Interspecific complementation of *rpb8*-Δ*1*::*LYS2* by the Hs8-encoding cDNA of *H. sapiens*. (Upper plates) Cultures grown on YPD plates for 3 days at 30 and 37°C. Plates contain *rpb8*-Δ*1*::*LYS2* segregants with plasmid pGEN-Hs8 (Hs8, left half of plate) or pSL103 (Sc8, right half of plate). (Lower plate) Same *rpb8*-Δ*1*::*LYS2* segregant bearing either the plasmid pGEN-Hs8 (Hs8, left half of plate) or the two plasmids pGEN-Hs8 and pSL103 (Sc8 + Hs8, right half of plate) after 3 days at 37°C.

Archambault et al. (5) also observed that the *RPB6*$^+$ gene of *S. cerevisiae* is a dosage-dependent suppressor of *rpo21-4*, a mutation generating a slow-growth phenotype due to a defect in the largest subunit of RNA polymerase II. As shown in Fig. 4, this phenotype was also partially corrected by a high dosage of the human Hs6 and *S. pombe* Sp6 cDNAs homologous to Sc6, indicating that the corresponding polypeptides can mimic their *S. cerevisiae* counterpart not only in terms of interspecific complementation but also by their extragenic suppression properties.

Human Hs5 does not substitute in vivo for Sc5. The Hs5 human subunit is homologous to Sc5 (32) (Table 1). The Hs5

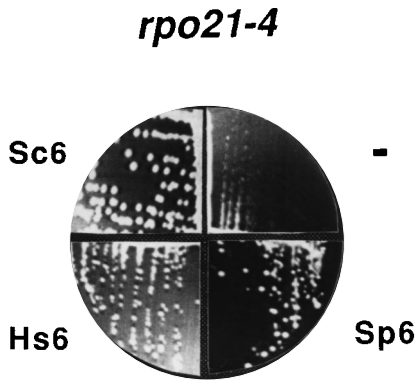

FIG. 4. Extragenic suppression of *rpo21-4*. Strain JAY212 bearing mutation *rpo21-4* was transformed by multicopy plasmids harboring the human Hs6 (pGEN-Hs6) or *S. pombe* Sp6 (pGVS41) cDNAs encoding polypeptides homologous to *S. cerevisiae* subunit Sc6. A control strain harbored pRPO26 encoding the *S. cerevisiae* subunit Sc6. Transformants were streaked on YPD plates and scored after 3 days of incubation at 23°C. The *rpo21-4* mutant was initially described as being temperature sensitive with little or no growth at 37°C (5). Under our experimental conditions, however, JAY212 behaved as a slowly growing mutant at all temperatures tested, including 37°C.

cDNA was reisolated by PCR amplification. After correction for a sequencing error in the initial report (32), the Hs5 cDNA was found to encode a polypeptide of 210 amino acids (24.5 kDa) with 44% identity and 80% similarity to the yeast Sc5 subunit (see also reference 13). Upon being subcloned into pGEN, Hs5 was transferred to strains bearing the corresponding *rpb5*-Δ::*URA3* null allele of *S. cerevisiae* (6) but failed to complement that allele as determined by plasmid-shuffling and tetrad analysis assays (data not shown).

DISCUSSION

The existence of three distinct transcription enzymes has been documented for all eukaryotes investigated so far; thus, it is a very ancient feature of the transcription machinery in the nucleus. Nevertheless, studies of *S. cerevisiae* have revealed the existence of five small polypeptides (referred to in this paper as Sc5, Sc6, Sc8, Sc10α, and Sc10β [Table 1]) that are strictly common to all three RNA polymerases (11, 12, 37, 46) and are essential for cell growth (44, 49, 52). Previous work indicated that Sc5, Sc6, and Sc8 are antigenically conserved in *D. melanogaster* and, to a lesser extent, in mammals (18), and two human cDNAs (Hs5 and Hs6) have sequence similarity to those encoding Sc5 and Sc6, respectively (3, 13, 32). The present work genetically identifies three additional human polypeptides (Hs8, Hs10α, and Hs10β) that are structurally conserved with their yeast counterparts (Sc8, Sc10α, and Sc10β). Moreover, four of the human polypeptides (Hs6, Hs8, Hs10α, and Hs10β) are functionally interchangeable with their yeast homologs in vivo. Thus, there is a remarkable structural and functional conservation of these subunits from *S. cerevisiae* to *H. sapiens.*

The strong efficiency of heterospecific complementation observed for Hs6, Hs10α, and Hs10β correlates fairly well with their structural conservation. Sequence conservation is particularly striking between Sc10β and its human homolog (the degree of conservation being the highest observed so far among yeast and human RNA polymerase subunits), and almost half of the amino acids are also invariant in the N subunit of the archaeal RNA polymerase (23). Except for a hyperacidic but otherwise variable N-terminal region, Sc6 is highly conserved with its *S. pombe*, *D. melanogaster*, and mammalian homologs, which all efficiently replace Sc6 in vivo (references 30 and 41 and the present work). Sc10α and its human and *S. pombe* homologs have a poorly conserved N-terminal domain but an invariant zinc-binding motif (see below) and a highly conserved C-terminal half. There is a less pronounced (33%) sequence identity between Sc8 and Hs8, which fail to complement a yeast defective for Sc8 at 37°C. Curiously, identity is somewhat higher (44%) between Sc5 and Hs5, with which there was no complementation at all. Recent results (13) suggest that Hs5 interacts with transcriptional regulators such as the X protein of human hepatitis virus and thus might endow RNA polymerase II with transcriptional properties that are incompatible with the faithful expression of the yeast genome. Alternatively, some structural feature of Hs5 may prevent its correct assembly into the yeast enzyme.

The structural and functional conservation of the common subunits emphasizes their central but still elusive role in transcription, also documented by the lethal phenotype conferred by the corresponding null alleles in *S. cerevisiae* (44, 49, 52). The lack of homologous subunits in the bacterial enzyme argues against a direct catalytic role. Hs8 and Hs10α have no known homolog in the archaeal RNA polymerase and thus might be typically eukaryotic, but the sequences of two of the archaeal subunits are still to be determined (23). In contrast, Hs5, Hs6, and Hs10β are related to bona fide subunits of the archaeal RNA polymerase (23), and there is a homolog of Hs10β in the vaccinia virus RNA polymerase (4). The TATA binding protein TBP, which is shared among the three eukaryotic transcription systems (17), also has an archaeal equivalent (35), suggesting the intriguing possibility that Hs5, Hs6, and/or Hs10β functionally interact with this basal transcription factor. Alternatively, the main role of the common subunits may be to trigger the assembly of the three transcription complexes by forming a common heteromultimeric precursor, which would then segregate into the three distinct enzymes by recruiting enzyme-specific subunits.

One outcome of the present work is the identification of several highly conserved motifs, which are obvious candidates for targeted mutagenesis studies. These include the YXS(F/Y)GGLL peptide on Hs8, the 24 C-terminal amino acids and the canonical zinc-chelating domain ($CX_2CX_{12}RCX_2CGXR$) of Hs10α, and the atypical but strictly invariant CX_2CGX_n CCR zinc-chelating motif shared by all members of the Hs10β family. The two putative zinc-chelating domains are of particular interest since the yeast Sc10α and Sc10β subunits have zinc-binding properties in vitro (45). Their invariant glycines could provide the rotational freedom required for proper folding, whereas the invariant arginines may favor zinc coordination by altering the pK of the neighboring cysteines. Given the lack of equivalent subunits in bacteria, these zinc atoms may be required primarily to assemble and maintain the heteromultimeric structure of the eukaryotic enzymes.

The cloning of Hs8, Hs10α, and Hs10β brings to 11 the number of human cDNAs known to encode RNA polymerase II subunits, since a human homolog of Sc7 was recently identified (20). Biochemical studies have failed to reveal polypeptides that have the size of Hs10α and Hs10β in the human enzyme (19, 26), but their identification as genuine subunits (belonging, furthermore, to all three nuclear RNA polymerases) is strongly suggested by our interspecific complementation data. Now that human cDNAs encoding almost all of the RNA polymerase II subunits have been identified, determining which subunit combinations lead to viable chimeras between the human and yeast enzymes may clarify the still elusive pattern of RNA polymerase assembly and will provide an important tool in functionally characterizing the human transcription machinery. Furthermore, it might ultimately be possible to engineer viable yeast strains operating with an RNA polymerase II entirely of human origin.

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ADDENDUM IN PROOF

The *S. pombe* gene encoding a homolog of Sc10β has now been cloned and complements the *rpb10*-Δ*1*::*HIS3* deletion in *S. cerevisiae* in vivo (G. V. Shpakovski, E. N. Lebedenko, and P. Thuriaux, unpublished data).

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