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Genetics

Interactions between three common subunits of yeast RNA polymerases I and III

(RNA polymerases II/*Saccharomyces cerevisiae*/α subunit/nicotinate phosphorybosyltransferase/zinc metalloprotein)

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ABSTRACT **The AC40 and AC19 subunits (encoded by *RPC40* and *RPC19*) are shared by yeast RNA polymerases I and III and have a local sequence similarity to prokaryotic α subunits. Mutational analysis of the corresponding "α motif" indicated that its integrity is essential on AC40 subunit but is not essential on AC19 subunit. By applying the two-hybrid method, these two polypeptides were shown to associate *in vivo*. Extragenic suppression of *rpc19* and *rpc40* mutations confirmed that AC19 and AC40 subunits interact with each other *in vivo* and revealed an interaction with ABC10β subunit [encoded by *RPB10*; Woychick, N. A. & Young, R. A. (1990) *J. Biol. Chem.* 265, 17816–17819], one of the five polypeptides common to all three nuclear RNA polymerases. A correction of the *RPB10* sequence showed that ABC10β subunit is a 70-amino acid polypeptide, as confirmed by peptide microsequencing. These results suggest that the assembly of RNA polymerase I and III requires the association of ABC10β subunit with an AC19/AC40 heterodimer.**

RNA polymerases I, II, and III are required for transcription of the eukaryotic genome. In *Saccharomyces cerevisiae*, these enzymes contain 12–15 distinct proteins (1–3). Their two large subunits are akin to the bacterial β′ and β subunits (4–7). Five subunits (ABC27, ABC23, ABC14.5, ABC10α, and ABC10β) are shared by all three enzymes (2, 8, 9), and several specific subunits are unique to their cognate enzyme (1, 10–15). Moreover, yeast RNA polymerase I and III share subunits AC19 and AC40 (16, 18). As far as examined, the RNA polymerases of other eukaryotes have a similar organization (19). These subunits are strictly essential for growth, except for some of the enzyme-specific ones (3). Biochemical and genetic data have shown that the active site is mainly borne on the two large subunits of the *Escherichia coli* and yeast enzymes (2, 20–22).

The bacterial core enzyme also contains the dimeric α subunit (23) that initiates enzyme assembly (24) and has a C-terminal domain involved in selective interactions with transcriptional regulators (25, 26). The B44 dimer of RNA polymerase II (homologous to the AC40 subunit shared by enzymes I and III) has some sequence similarity to α (17, 27). AC19 subunit also shows some local sequence similarity to α, at the level of a putative "α motif" (18). We show here that these two common subunits associate *in vivo* and interact with the small zinc-binding subunit ABC10β,* which is shared by all three RNA polymerases. Mutational analysis showed that the α motives of AC19 and AC40 subunits are not equivalent, the integrity of the motif being essential in growth for AC40 subunit but not essential in growth for AC19 subunit.

MATERIALS AND METHODS

Strains, Plasmids, and Growth Conditions. Yeast strains and plasmids are listed in Tables 1 and 2. Plasmid pGAD2F and strain Y526 (a derivate of strain YM954 bearing a *GAL1–lacZ* reported fusion integrated at the *URA3* locus) were given by S. Fields and P. Bartel (State University of New York). Multicopy plasmids bearing the *RPC19*, *RPC40*, or *RPB10* genes were isolated from a yeast genomic library on the *URA3* multicopy plasmid pFL44L (34). The two-hybrid system of protein–protein association *in vivo* has been described (35). β-Galactosidase was assayed in appropriate drop-out medium containing ethanol, glycerol, and galactose [each at 2% (wt/vol)] as carbon source. Plate assays were done on colonies grown on drop-out medium with raffinose [2% (wt/vol)], overlaid with 10 ml of a 0.5 M potassium phosphate, pH 7.0 solution containing 0.5% (wt/vol) agarose, 600 μl of *N,N*-dimethylformamide, 100 μl of 10% SDS, and 100 μl of a 4% (wt/vol) 5-bromo-4-chloro-3-indolyl β-D-galactoside solution in formamide.

Mutagenesis. Plasmids p7040 (*URA3 RPC40*) and p3519 (*TRP1 RPC19*) were mutagenized (50) on their α motif and checked by determining their DNA sequence over ≈250 bp around the mutated site. Individual plasmids were introduced in strains DLY11 and DLY200 (see Table 1), respectively, and analyzed by plasmid shuffling (16). DLY11 subclones lacking DLp01 (*TRP1 RPC40 SUP11-1*) were isolated as red sectors on yeast extract/dextrose/peptone (YPD) medium, reflecting the lack of suppression of the ochre allele *ade2-1* in the absence of *SUP11-1* (37). DLY200 subclones lacking p7519 (*URA3 RPC19*) were selected as uracil auxotrophs in the presence of 5-fluoroorotic acid (38). Failure to yield these subclones indicated that the mutant *rpc40* or *rpc19* allele (borne on p7040 or p3519) was unable to complement the chromosomal null alleles *rpc40*-Δ::*HIS3* or *rpc19*-Δ::*HIS3*. Viable subclones isolated at 30°C were also tested at 20°C and 37°C. DLY7C was selected by 5-fluoroorotic acid as a spontaneous uracil auxotrophic subclone of strain DLY7, showing additional auxotrophy for histidine. This double auxotrophy reflected the replacement of the chromosomal deletion *rpc40*-Δ::*HIS3* by the *rpc40-V78R* allele of p7040-V78R and loss of the latter plasmid.

DNA and Protein Sequence Analysis. ABC10β subunit was purified from RNA polymerase I by reverse-phase HPLC, and internal tryptic peptides were microsequenced, as described (9). Plasmids for DNA sequencing were derived from pFL44-RPB10e and -f (Fig. 3*B*) by nested deletions with exonuclease III, with the Pharmacia kit. DNA sequences were obtained by the dideoxynucleotide chain-termination method, using a modified T7 DNA polymerase (Sequenase II from United States Biochemical) with universal primers or ad hoc oligonucleotides prepared in the laboratory. Sequences were analyzed with appropriate software programs (39, 40).

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Abbreviations: ts, temperature sensitive; ORF, open reading frame.
*The sequences reported in this paper have been deposited in the GenBank data base (accession nos. L11274 and L11275).

Table 1. *S. cerevisiae* strains

Strain	Genotype	Origin
FL100	*MATa*	Ref. 28
YM954	*MATa ade2-1* ura3-52 lys2-801 his3-Δ200 trp1-Δ1*† *leu2-3, 112 can1 gal4-542 gal80-538*	Ref. 29‡
YNN281	*MATa ade2-1 ura3-52 lys2-801 his3-Δ200 trp1-Δ1*	Ref. 30
CMY217	*MATa ade2-1 ura3-52 lys2-801 his3-Δ200 trp1-Δ1 rpc40-Δ::HIS3* (YCp50-AC40: *URA3 RPC40*)	Ref. 16
DLY11	*MATa ade2-1 ura3-52 lys2-801 his3-Δ200 trp1-Δ1 rpc40-Δ::HIS3* (DLp01: *TRP1 RPC40 SUP11-1*)	This work
DLY7	*MATa ade2-1 ura3-52 lys2-801 his3-Δ200 trp1-Δ1 rpc40-Δ::HIS3* (p7040/V78R: *URA3 rpc40-V78R*)	This work
DLY7C	*MATa ade2-1 ura3-52 lys2-801 his3-Δ200 trp1-Δ1 rpc40-V78R*	This work
DLY200	*MATα ade2-1 ura3-52 lys2-801 his3-Δ200 trp1-Δ1 rpc19-Δ::HIS3* (p7519:*URA3 RPC19*)	This work
DLY202	*MATα ade2-1 ura3-52 lys2-801 his3-Δ200 trp1-Δ1 rpc19-Δ::HIS3* (p3519/G73D:*TRP1 rpc19-G73D*)	This work

*Ochre allele, also known as *ade2-101*.
†Also known as *trp1-Δ901*.
‡The *gal4* and *gal80* allele numbers of ref. 29 are erroneous (M. Johnston, personal communication).

RESULTS

Sequence Similarity and Site-Directed Mutagenesis of AC40 and AC19 Subunits. The similarity between the α motif of the eubacterial α and eukaryotic AC40, B44 (27, 41), or AC19 (18) subunits is illustrated by Fig. 1*A*. AC40 and B44 subunits are homologous (16, 17) and have a limited, but suggestive, similarity to prokaryotic α subunits (27, 41). Subunit AC19 shows no significant similarity to AC40, B44, or α beyond its putative α motif, but we observed a significant homology between AC19 and the small (67 amino acids) L subunit of the archeal RNA polymerase from *Sulfolobus acidocaldarius* recently sequenced by D. Langer and W. Zillig (personal communication).

Mutations of the putative α motif of AC40 and AC19 subunits were tested for complementation of the chromosomal null-alleles *rpc40-Δ::HIS3* or *rpc19-Δ::HIS3*. Of 22 substitutions at seven conserved positions of AC40 subunit, 17 substitutions were fully lethal (nine alleles) or defective at one of the temperatures tested (Fig. 1*B* and Table 3). The remaining mutations were conservative substitutions (Ala-64 → Gly, Ala-64 → Val, Pro-76 → Thr, and Val-83 → Lys). Thus, the α motif appears crucial for the activity of AC40 subunit. The conditional allele *rpc40-R69C* corresponded to the *rpoA-112 (R45C)* temperature-sensitive (ts) mutation of the α subunit of *E. coli* which has an assembly defect in bacterial RNA polymerase (42). *Rpc40-A64D* corresponded to one of the two substitutions of the ts mutation *rpb3-1* (Ala-30 → Asp and Gly-175 → Ala) in the B44 subunit (41). The ts phenotypes associated with equivalent substitutions on AC40 subunit, B44 subunit, and the bacterial α subunit are consistent with a similar function of the α motif on these polypeptides. Of 12 substitutions at six positions of the putative α motif of AC19 subunit (Fig. 1*B* and Table 3), 9 had no detectable phenotype, including the Asn-74 → Gln, Asn-74 → Asp, Arg-77 → Lys, and Arg-77 → Glu substitutions which, in AC40 subunit, distinctly reduced the growth rate or were lethal. The remaining three mutations are ts: *rpc19-Y78R* brought the AC19 sequence closer to the consensus α motif by restoring a highly conserved pair of arginines (see Fig. 1*A*), whereas *rpc19-G73D* and *rpc19-V86R* corresponded to the ts mutations *rpc40-A64D* and *rpc40-V78R*. Thus, in contrast to AC40 subunit, the amino acid sequence of the AC19 α motif can be appreciably altered without detectable growth defects.

Extragenic Suppression of *rpc40-V78R* and *rpc19-G73D*. Extragenic suppressors of *rpc40-V78R* were selected by transforming strain DLY7C with a genomic library constructed on the *URA3* multicopy vector pFL44L. The corresponding plasmids, when extracted and reintroduced in strain DLY7C, restored growth at 37°C, whereas chasing the plasmid by 5-fluoroacetic acid resulted in a lack of growth at 37°C. On the basis of these two tests, 13 suppressor plasmids were isolated, corresponding to $\approx 5 \times 10^{-5}$ of the plasmid library. Eight plasmids harbored an *RPC40* insert. The remaining five plasmids partially restored growth at 37°C and were allocated to three classes of suppressors by restriction mapping. One of them, pFL44-SRP40, bore an hitherto undescribed gene (*SRP40*) coding for a protein of 406 amino acids unusually rich in serine (48% of serine residues) (accession no. L11275). It had a very weak suppression effect and was not further investigated. Another suppressor plasmid (pFL44-AC19) was shown by restriction mapping to bear *RPC19*. Suppression was also seen with the pYEp-AC19 (*LYS2 RPC19*) multicopy plasmid and extended to several *rpc40* mutations (*rpc40-ts4*, *rpc40-ts74*, *rpc40-ts84*, and *rpc40-ts154-1*, ref. 16) known to be conditionally defective in

Table 2. Plasmids

Name	Yeast genetic markers	Origin
YEp-LYS2	*ORI*(2μ) *LYS2*	Cloning of 1.8-kb *Hin*dIII–*Pst* I 2μ origin of replication (form B) and of 4.8-kb *Eco*RI *LYS2* cassette (31) in pUC19 polylinker
YEp-AC19	*ORI*(2μ) *LYS2 RPC19*	Blunt cloning of 1.8-kb *Sma* I–*Sac* I *RPC19* fragment of pBX1 (18) into *Sma* I site of YEp-LYS2
DLp01	*CEN4 ARS1 TRP1 RPC40 SUP11-1*	Cloning of 1.2-kb *Eco*RI *SUP11-1* fragment (37) in *CMP235* (16)
p7040	*CEN4 ARS1 URA3 RPC40*	Cloning of 4.4-kb *Eco*RI *RPC40* fragment of YCp40-URA3 (16) in pUN70 (46)
p3519	*CEN4 ARS1 TRP1 RPC19*	Same as p7519 (18) but in pUN35 (46)
pRB10-5	*CEN6 ARSx RPB10*	Cloning of 1.5-kb *Bam*HI–*Kpn* I RPB10 fragment (see Fig. 3) in pASZ11 (32)
GAL4(1,147)–AC19	*ORI*(2μ) *HIS3 GAL4*(1–147)::*RPC19*	Cloning of PCR-amplified coding sequence of *RPC19* (with *Bam*HI tail upstream of initiator ATG) at *Bam*HI site of pMA424 (33), creating an in-frame fusion to the GAL4 DNA-binding site
GAL4(1,147)–AC40	*ORI*(2μ) *HIS3 GAL4*(1–147)::*RPC40*	Same as above, but with *RPC40* coding sequence
AC19–GAL4(768,881)	*ORI*(2μ) *LEU2 GAL4*(768–881)::*RPC19*	Same as GAL4(1,147)–AC19, but at the *Bam*HI site of pGAD2F (51), creating an in-frame fusion to *GAL4* DNA activation site
AC40–GAL4(768,881)	*ORI*(2μ) *LEU2 GAL4*(768–881)::*RPC40*	Same as above, but with *RPC40* coding sequence

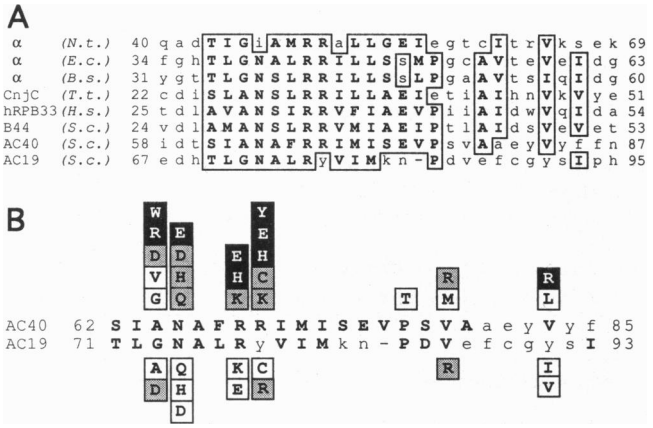

FIG. 1. Sequence alignments and site-directed mutagenesis of the α motif of AC19 and AC40 subunits. (*A*) Local alignments. The aligned sequences include the α subunits of *Nicotiana tabacum* (*N.t.*) (GenBank accession no. Z00044), *E. coli* (*E.c.*) (accession no. X00766), and *Bacillus subtilis* (*B.s.*) (accession no. M13957); the *CnjC* gene product of *Tetrahymena thermophila* (*T.t.*) (accession no. X62317), the human (*Homo sapiens*, *H.s.*) RNA polymerase II subunit hRPB33 (accession no. J05448); the yeast (*Sa. cerevisiae*, *S.c.*) subunits AC40 (accession no. M15499), B44 (accession no. M27496), and AC19 (accession no. M64991). Invariant or conserved residues are boxed and indicated in uppercase letters. Nonconserved residues are given in lowercase letters. The statistical significance of sequence alignment was evaluated by a Monte Carlo analysis (rdf2 test of ref. 39): similarities were highly significant among prokaryotic α subunits or between CnjC, hRPB33, AC40, and B44 subunits but were not significantly similar between these two groups of sequences. (*B*) Effect of amino acid substitutions in the α motif of *RPC40* and *RPC19*. □, No detectable adverse phenotype on yeast extract/peptone/dextrose medium at 20°C, 30°C, and 37°C; ◻, leaky, cold-sensitive or ts phenotypes; ■, fully defective growth phenotype. Phenotypes of conditional mutants are detailed under Table 3.

enzyme assembly. However, the chromosomal *rpc40-Δ::HIS3* deletion was not suppressed by high gene dosage of *RPC19*.

The three remaining plasmids had a strong suppressor effect (Fig. 2), which extended to *rpc40-ts4*, *rpc40-ts74*, *rpc40-ts84*, or *rpc40-ts154-1*. Their inserts overlapped over a 4.9-kb region with two open reading frames (ORFs) (Fig. 3). One of them (*NPT1*) had significant homology to the bacterial nicotinate phosphorybosyltransferase (EC 2.4.2.11) (ref. 43, see Fig. 3). Further subcloning (Fig. 3*B*) showed that suppression required the second ORF (*RPB10*), which, as discussed below, encoded the ABC10β subunit shared by all three nuclear RNA polymerases (9, 36). Suppression was not observed with *RPB10* and *RPC19* borne on a centromeric vector (pRPB10-5 and p7519), indicating that high gene dosage was essential. In a converse experiment, we transformed a conditional *rpc19* mutant (the *rpc19-G73D* strain DLY202) by the pFLL44-RPB10 and pFL44-AC40 plasmids isolated in the previous experiment as harboring *RPB10* or *RPC40* inserts and observed suppression in both cases. Fig. 2 shows that the effect of ABC10β subunit was less strong than on *rpc40-V78R*, whereas suppression by *RPC40* was more efficient. *RPC10*, encoding ABC10α, the other 70-amino acid subunit shared by all three RNA polymerases (9, 45), did not suppress *rpc40-V78R* or *rpc19-A64D* (data not shown).

Association Between AC19 and AC40 Subunits in the Two-Hybrid System. The two-hybrid system (35) was used to examine whether AC19, AC40, and ABC10β subunits can associate in the yeast nucleus. In this system, one protein is fused to the DNA-binding domain of the transcriptional activator GAL4, and the other is fused to the GAL4-activating domain. Heterodimeric association results in a

Table 3. Growth phenotype of *rpc40* and *rpc19* mutants

Mutant	Triplet substitution	Growth at 20°C	30°C	37°C	Phenotype*
		RPC40			
DLY10 (A64D)	GCG/GAC	+	++	−	ts
DLY62 (N65D)	AAT/GAT	+	+	+	l
DLY59 (N65H)	AAT/CAT	+	++	++	l
DLY28 (N65Q)	AAT/CAA	−	++	+++	cs
DLY18 (R68K)	CGT/AAG	+	++	++	l
DLY48 (R69K)	CGT/AAG	+	+	+	l
DLY78 (R69C)	CGT/TGT	−	+	−	cs,ts
DLY7 (V78R)	GTG/AGG	++	+++	−	ts
DLY7C† (V78R)	GTG/AGG	++	+++	−	ts
		RPC19			
DLY202 (G73D)	GGA/GAT	++	+++	−	ts
DLY208 (Y78R)	TAC/CGC	++	+++	++	ts
DLY210 (V86R)	GTA/CGA	++	+++	+	ts

Except for strain DLY7C, the mutations were borne on the centromeric plasmid p7040 (*URA3 RPC40*) or p3519 (*TRP1 RPC19*) in a chromosomal *rpc40-Δ::HIS3* or *rpc19-Δ::HIS3* context. +++, ++, and + denote decreased growth rates, as determined by visual inspection of yeast extract/peptone/dextrose plates incubated between 2 and 7 days at the temperature indicated; +++ indicates the growth level of control strain YNN281 at 30°C (doubling time in liquid medium is 1.7 hr).

*cs, ts, and l stand for cold sensitivity (20°C), heat sensitivity (37°C), and "leaky", respectively, growth at all three temperatures.

†Chromosomal *rpc40-V78R* mutation (Table 1).

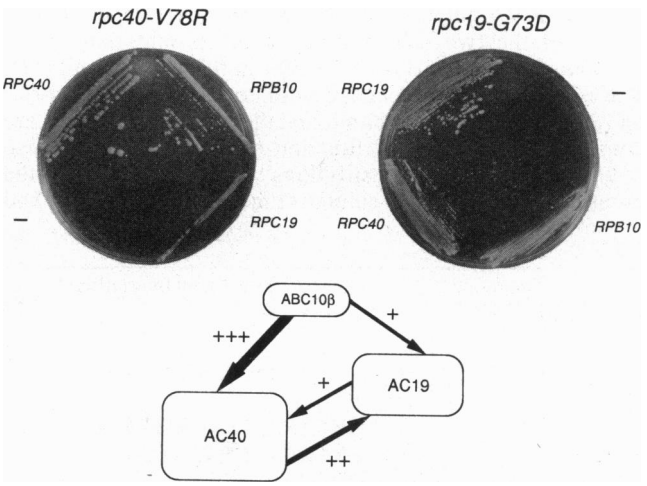

FIG. 2. Extragenic suppression of *rpc40-V78R* and *rpc19-G73D* by multicopy suppressors. Freshly grown cells were streaked on yeast extract/peptone/dextrose medium and incubated at 37°C for 3 days. *RPB10*, *RPC40*, and *RPC19* denote transformants isolated from DLY7C (*Left*) or DLY202 (*Right*) strain after transformation with the multicopy plasmids pFL44-RPB10c (see Fig. 3*A*), pFL44-AC40, and pFL44-AC19. − indicates a control bearing the pFL44L vector without insert. At bottom the suppression pattern between the AC40, AC19, and ABC10β subunits is summarized, as deduced from the corresponding growth phenotype at 37°C. +, ++, and +++ denote increased strengths of suppression.

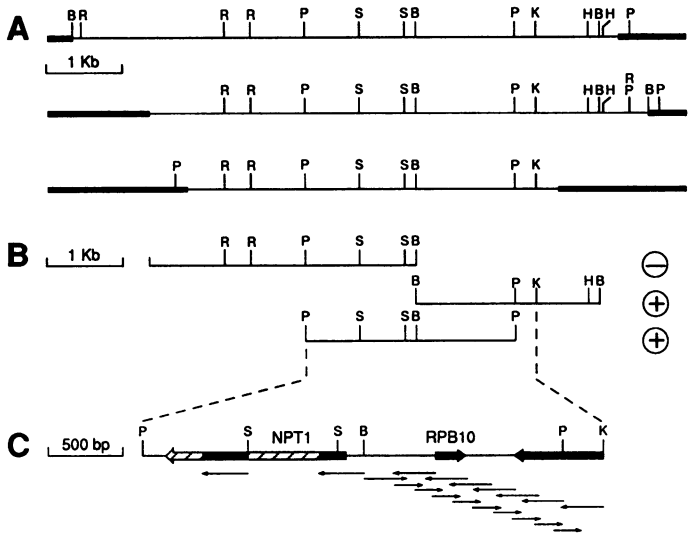

FIG. 3. Organization of *RPB10* locus. (*A*) Structure of pFL44-RPB10 plasmids. Thin lines correspond to yeast genomic inserts; thick lines correspond to the pFL44L vector. B, *Bam*HI; R, *Eco*RI; P, *Pvu* II; S, *Sph* I; K, *Kpn* I; H, *Hin*dIII. (*B*) Subcloning of *RPB10* region from pFL44-RPB10b. ⊖, No suppression of *rpc40-V78R*; ⊕, suppressor-active insert. pFL44-RPB10d is a *Bam*HI deletion of pFL44-RPB10b. The two other plasmids were obtained by subcloning *RPB10* fragments on pFL44L (a 2.5-kb *Bam*HI insert for pFL44-RPB10e and a 2.8-kb *Pvu* II insert for pFL44-RPB10f). (*C*) Sequencing strategy. Thin arrows indicate direction and length of DNA readings. Thick arrows give transcriptional orientation of ORFs. Downstream of *RPB10*, there is the C-terminal end of a putative ORF with no homology to current protein data banks. Upstream of *RPB10*, the *NPT1* ORF (encoding a putative nicotinate phosphoribosyltransferase) was identified by partial sequencing data. The N-terminal fragment of 42 residues and an internal fragment of 111 residues showed 45% (Lfasta optimal score 106) and 35% identity (Lfasta optimal score 194) with the *E. coli* nicotinate phosphoribosyltransferase gene (43). The hatched compartment was not sequenced.

functional chimeric activator, as monitored by the *lacZ* gene under control of the *GAL1* promoter. We tested all pairwise combinations of these three subunits fused to the GAL4 activation or DNA-binding domains. No interaction was seen when combining ABC10β subunit to AC19 or AC40 subunit or any of these three subunits with itself, but a strong positive signal was seen in the two reciprocal constructions combining AC19 and AC40 subunits, as confirmed by a quantitative β-galactosidase assay in liquid cultures (Table 4).

Amino Acid Sequence of ABC10β Subunit. The *RPB10* sequence differed by 1 bp (see Fig. 4) from a previous report (36). *RPB10* encoded a 70-amino acid polypeptide sharing only its first 32 amino acids with the reported sequence. The calculated molecular mass was 8.3 kDa instead of 5.4 kDa, and the pI was of 8.1 rather than 9.6. We sequenced two internal peptides after tryptic digestion of ABC10β subunit (including a peptide corresponding to the C-terminal region) and found a complete identity with the predicted ORF, confirming the coding sequence determined here. The ABC10β amino acid sequence had no significant homology to current data bank releases. ABC10β subunit binds zinc *in vitro* (9). Its sequence contains a half zinc-chelating motive Cys-Xaa$_2$-Cys in the N-terminal region but contains no standard tetra-coordinating motif of the Cys-Xaa$_2$-Cys . . . Cys-Xaa$_2$-Cys (or His-Xaa$_2$-His) type. However, several residues with potential zinc-binding capacities (Cys-45, Cys-46, His-53, and possibly also Asp-28 and Asp-30) may contribute to a noncanonical zinc-binding motif (for review on the chemistry of protein zinc binding, see ref. 47).

Table 4. Quantitative measurement of GAL1–lacZ transactivation in hybrid GAL4 proteins

Plasmid	β-Galactosidase activity
GAL4(1,881)	1925
GAL4(1,147)–AC40	3
GAL4(1,147)–AC19	<1
AC40-GAL4(768,881)	<1
AC19-GAL4(768,881)	<1
GAL4(1,147)–AC40/AC19–GAL4(768,881)	240
GAL4(1,147)–AC19/AC40–GAL4(768,881)	160

β-Galactosidase was assayed in triplicate in strain Y526 transformed by the relevant plasmid(s). GAL4(1,881) corresponds to plasmid pCL1 (35). Enzymatic activities are given in nmol of *o*-nitrophenyl β-D-galactoside hydrolyzed per min per mg of protein.

DISCUSSION

Yeast RNA polymerases I and III share two essential subunits, AC40 and AC19, encoded by the genes *RPC40* (16) and *RPC19* (18). By applying the two-hybrid method (35), we observed that AC19 and AC40 subunits strongly associated in the yeast nucleus. Moreover, conditional *rpc40* mutations were suppressed by high dosage of *RPC19*, and, conversely, an *rpc19* mutation was suppressed by high dosage of *RPC40*. Finally, both classes of mutations responded to high gene dosage of *RPB10*. Because suppression was from high gene dosage and acted on alleles known to prevent enzyme assembly (16), it presumably compensated an assembly defect by increasing the concentration of the interacting subunits. We conclude that AC19 and AC40 subunits form a heterodimer that, in turn, interacts with the *RPB10*-encoded subunit ABC10β, common to all three RNA polymerases.

The B44 subunit specific to RNA polymerase II has a stoichiometry of two and is strongly homologous to AC40 subunit (17). In contrast, AC40 subunit has a stoichiometry

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-295 GTTCATATATGCAAAAGCAGACCTGATAAATCTTTTCATATTCTTGAGGTTTTTAAGCC -237
-226 CAAAATAAGCTACTATCAGCACTCCGATGGCTACTACACTGGAAGTGTCAGCAATTGAG -178
-177 AACACAAAGATCACGTTGAACGAAAAGTAATTTCTCACTCGTTGTATTTGGTAAAAAAA -119
-118 ATTTCATAAGAAAAAAAAAAAGAAACATTGAATAAAATCACAATGGGATGATAGTTAAA  -60
 -59 GTGTATTCAAACTGTAGAGTGTAGAACGTAAACCTCAATAAGCAAAATAATAATACAAA   -1

   1 ATG ATT GTC CCA GTC AGA TGT TTC TCA TGC GGT AAA GTT GTT GGT    45
   1  M   I   V   P   V   R   C   F   S   C   G   K   V   V   G    15

  46 GAC AAG TGG GAA AGC TAC TTA AAC TTG TTG CAA GAA GAT GAG TTG    90
  16  D   K   W   E   S   Y   L   N   L   L   Q   E   D   E   L    30
           *
  91 GAT GAA GGT ACT GCA TTG TCA AGA TTA GGT CTA AAA AGA TAC TGC   135
  31  D   E   G   T   A   L   S   R   L   G   L   K   R   Y   C    45

 136 TGT AGA AGA ATG ATT CTA ACC CAC GTC GAT CTT ATT GAA AAG TTT   180
  46  C   R   R   M   I   L   T   H   V   D   L   I   E   K   F    60

 181 TTA AGA TAC AAC CCA TTA GAA AAA AGA GAT TAA GTGTTCCTCAATATG   228
  61  L   R   Y   N   P   L   E   K   R   D                      70

 229 GCTGTTTTTGAACTTTTCTTTTCCTTGATGGATGAAAAAAAAAAGATGAGAGCGGTCAT  287
 288 GAATATAGAAACGATAACCATTTATTCTCACACACTAAAAGAACTACACGACAACCAGA  346
 347 TGTGTCTCTGCATACTGTAGGAATACCATTCTCTTCCAAAATAATAGAAAATAATATAT  405
 406 ATATATCTTTTGTTTACTCTTCAAGACGATAAAGGAAGTATGTAGATGTTACATCGTGC  464
 465 GATATAAGAGACTTGTATACTATAAGAAAGAGCTTAGATGAAGGGTATGCCTTTATTTT  523
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FIG. 4. Sequence of RPB10 DNA and predicted amino acid sequence of ABC10β subunit. Upstream DNA region: A putative ABF1 box (consensus site RTCRYBNNNNACG, ref. 44) is underlined. Coding sequence: The tryptic peptides sequenced are underlined. Two cysteinyl residues forming a "half-finger", possibly contributing to the zinc-binding properties of ABC10β subunit (9, 36), are indicated in boldfaced type. A star denotes the position where another reported sequence (36) contains an additional adenine.

close to one (48) and fails to homodimerize under the conditions of the two-hybrid assay. Others have described an intermediary complex between B44 subunit and the β-like subunit B150, which further combined with the β'-like subunit B220 (41) in an assembly pathway similar to that of the bacterial enzyme (24). These complexes did not appear to contain ABC10β subunit or any of the other five common subunits. Our data suggest, however, that RNA polymerase assembly may involve an early association of ABC10β subunit (alone or possibly combined with other common subunits) with AC19/AC40 (enzyme I and III), or with a B44 dimer (enzyme II). These structures would interact with the β-like and then with the β'-like subunits of enzymes I–III, allowing segregation of a distinct precursor form for each enzyme. The addition of the smaller enzyme-specific subunits presumably occurs at a later step because several of them can be readily dissociated from wild-type or some mutant forms of RNA polymerase I, II, or III (1–3, 49).

The similarity between the α motif of bacterial α subunits and of AC40, B44, and AC19 subunits (18, 27, 41), with the same HTLGNALR octapeptide present in AC19 subunit and the *E. coli* α subunit, suggested that this domain may be crucial in the synthesis or activity of the cognate RNA polymerases, perhaps by promoting association of the corresponding subunits. We therefore mutagenized the AC40 and AC19 motives, assuming that a functionally crucial domain should be highly sensitive to amino acid substitutions; this sensitivity occurred for AC40 subunit but was absent for AC19 subunit, where most mutations were phenotypically silent, precluding a strict functional equivalence between the AC19 and AC40 motifs. The functional relevance (if any) of the local sequence identity between AC19 subunit and the *E. coli* α remains an open question. In this context, we noted a striking homology between AC19 subunit (but not AC40 subunit) and the L subunit of *Su. acidocaldarius* recently sequenced by D. Langer and W. Zillig (personal communication). AC19 subunit may thus have evolved from an ancestor gene of archaeal origin, unlike B44 and AC40 subunits, where the similarity to bacterial or chloroplastic α subunits tentatively suggests a prokaryotic lineage.

During this work, we revised the amino acid sequence initially proposed for ABC10β subunit (36). Our prediction of a 70-residue polypeptide was fully confirmed by peptide sequencing. ABC10α, another subunit common to all three RNA polymerases, also has a predicted sequence of 70 amino acids and has the same migration rate in SDS/PAGE (9). Both are essential proteins (36, 45). Despite their common size and zinc-binding properties, they have no detectable sequence homology. ABC10α subunit, which has a canonical tetra-coordinating zinc motif, bound zinc *in vitro* much less efficiently than did ABC10β subunit (9), which only has a half-domain of the Cys-Xaa$_2$-Cys type. Thus, ABC10β subunit may bind zinc (as a monomeric subunit) by some atypical motif or participate in subunit–subunit interactions through zinc-mediated hemi-coordination.

Note Added in Proof. A ts *rpb3-1* mutant (kindly supplied by R. A. Young, see ref. 17) was weakly suppressed by pFL44-RPB10e. This result suggests an interaction between subunits ABC10β and B44 of RNA polymerase II, consistent with the sequence homology of B44 and AC40 subunits (17).

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