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Two Genes for Ribosomal Protein 51 of *Saccharomyces cerevisiae* Complement and Contribute to the Ribosomes

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We cloned and sequenced the second gene coding for yeast ribosomal protein 51 (*RP51B*). When the DNA sequence of this gene was compared with the DNA sequence of *RP51A* (J. L. Teem and M. Rosbash, Proc. Natl. Acad. Sci. U.S.A. 80:4403–4407, 1983), the following conclusions emerged: both genes code for a protein of 135 amino acids; both open reading frames are interrupted by a single intron which occurs directly after the initiating methionine; the open reading frames are 96% homologous and code for the same protein with the exception of the carboxy-terminal amino acid; DNA sequence homology outside of the coding region is extremely limited. The cloned genes, in combination with the one-step gene disruption techniques of Rothstein (R. J. Rothstein, Methods Enzymol. 101:202–211, 1983), were used to generate haploid strains containing mutations in the *RP51A* or *RP51B* genes or in both. Strains missing a normal *RP51A* gene grew poorly (180-min generation time versus 130 min for the wild type), whereas strains carrying a mutant *RP51B* were relatively normal. Strains carrying mutations in the two genes grew extremely poorly (6 to 9 h), which led us to conclude that *RP51A* and *RP51B* were both expressed. The results of Northern blot and primer extension experiments indicate that strains with a wild-type copy of the *RP51B* gene and a mutant (or deleted) *RP51A* gene grow slowly because of an insufficient amount of RP51 mRNA. The growth defect was completely rescued with additional copies of *RP51B*. The data suggest that *RP51A* contributes more RP51 mRNA (and more RP51 protein) than does *RP51B* and that intergenic dosage compensation, sufficient to rescue the growth defect of strains missing a wild-type *RP51A* gene, does not take place.

Ribosomal protein genes and ribosomal protein mRNAs have been cloned from a number of eucaryotic organisms (1, 3, 5, 6, 15, 29, 31). Among the most surprising results of these initial studies is that many of these coordinately controlled genes could be repeated. Most cloned ribosomal protein genes generate multiple bands when used as probes on Southern blots with genomic DNA (16). Even for yeast cells, only 3 of 23 cloned ribosomal protein genes yield an unambiguous single band of genomic Southern blots, i.e., most of the cloned ribosomal protein genes have one additional genomic region with some nucleic acid homology (6).

There are a number of possible explanations for the presence of two bands when a single cloned probe is utilized on a genomic Southern blot. The most likely of these is either that one band consists of the real ribosomal protein gene and the other is a pseudogene or that both genes code for ribosomal proteins. The two genes could code for the same ribosomal protein, or they could code for different proteins which share nucleic acid sequence homology. The definition of a single protein can be problematic since two similar, but nonidentical, polypeptides can perform the same function and be interchangeable. In this context, one can point to studies on the duplicated *H2B* histone genes of yeasts (10, 23, 30). Were a similar situation to occur for some (but not all) yeast ribosomal protein genes, it would raise the intriguing regulatory problem of how to synthesize equimolar amounts of ribosomal proteins, when some ribosomal protein genes are repeated and others are unique (6, 31).

To approach this problem for ribosomal protein genes, we cloned and sequenced the second yeast gene coding for ribosomal protein 51 (RP51). When the DNA sequence of the second *RP51* gene was compared with that of the first, the data indicated that the two genes code for proteins of virtually identical primary sequence. The availability of both *RP51* genes and the elegant one-step gene disruption techniques of Rothstein (22) allowed us to initiate a genetic analysis of this gene pair.

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MATERIALS AND METHODS

Bacterial strains and plasmids. *Escherichia coli* strains HB101 (*leu pro thi thr lacY1* Str *hsdR hsdM recA*) and JM103 [Δ(*lac-pro*) *supE thi rpsL endA sbcB15 hsdR4* F *traD36 proAB lacI*^{q}ZM13] were used to grow and propagate plasmids and M13 subclones, respectively.

Plasmids CV9 (18), YIP5 (27), and YEP24 (2) were the generous gifts of Mary Ann Osley. mp8 and mp9 phage were obtained from Bethesda Research Laboratories.

Cloning of *RP51B*. DNA (5 μg) extracted from *Saccharomyces cerevisiae* strain A364A (9) was digested to completion with *Eco*RI and ligated to an equal amount of *Eco*RI-cut and phosphatased pBR322. Strain HB101 transformants were selected on tetracycline plates. Approximately 10^4 colonies were screened by using the kinased *Hin*dIII-*Sal*I insert from pHS2 by the procedure of Gergen et al. (7).

pR1-4 was the plasmid isolated by this procedure, and it contains the 6-kilobase (kb) *Eco*RI fragment described in Fig. 1B.

pYI51B is a 3-kb *Hin*dIII-*Eco*RI fragment containing *RP51B* subcloned into plasmid YIP5.

pYE51B was obtained by insertion into pYI51B of the *Eco*RI fragment from plasmid YEP24 carrying the 2-μm-plasmid origin of replication.

Plasmids HA1RF and HA2RF contain the double-stranded forms of the 1.2-kb *Hae*III fragment containing *RP51B*, cloned in opposite orientations into the *Sma*I site of phage mp8 (14). Complete digestion of these plasmids with *Ava*II and *Hin*cII provided subclones for sequencing, as well as Bal 31 deletions generated from flanking sites in the polylinker.

The *Pvu*II-*Hae*III fragment was subcloned into mp8 to determine the 5′ upstream sequences.

pYI51A is a 3-kb *Eco*RI-*Hin*dIII fragment containing *RP51A* subcloned into plasmid YIP5.

Plasmid HFR-4 contains the 235-base pair (bp) *Hin*cII fragment from the 3′ exon of *RP51A* subcloned into the *Sma*I site of phage mp8; the single-stranded insert in the phage is complementary to the coding region of both RP51A and RP51B mRNA.

Construction of plasmids for the generation of mutant *RP51* genes. (i) pHS2LEU. The 2.0-kb *Sal*I-*Hpa*I *LEU2* fragment from plasmid CV9 was filled in and blunt end ligated to a partial *Hin*cII digest of pHS2. The resulting plasmid pHS2LEU was cut with *Hin*dIII and *Sal*I, and the insert was excised and gel purified for transformation into yeast cells. Transformants were selected on minimal plates lacking leucine.

(ii) pHS2URA. The 1.2-kb *Hin*dIII fragment from plasmid YEP24 was filled in and ligated to a complete *Hin*cII digest of an mp9 derivative of pHS2 in which the *Sal*I site was obliterated, generating a deletion of the 235-bp internal *Hin*cII fragment when the *URA3* gene was inserted. The resulting replicative form of pHS2URA was cut with *Hin*dIII and *Eco*RI in the polylinker, and the insert was excised for transformation into yeast cells. Transformants were selected on minimal plates lacking uracil.

(iii) pGOBLEU. The *Hin*cII-*Hin*dIII fragment from pHS2 was put into pUC9 cut with *Hin*cII and *Hin*dIII (pUC9-1). The filled-in *Sal*I-*Hpa*I fragment from plasmid CV9 was then ligated into the *Hin*cII site of pUC9-1. The resulting plasmid was cut with *Sma*I and *Bam*HI, and the *Eco*RI-filled-in *Bgl*II fragment from pYI51A was ligated in. The insert was excised from the pUC9 backbone with *Sal*I and *Hin*dIII for yeast transformation; a unique *Sal*I site was present ca. 1 kb from the *Bgl*II site.

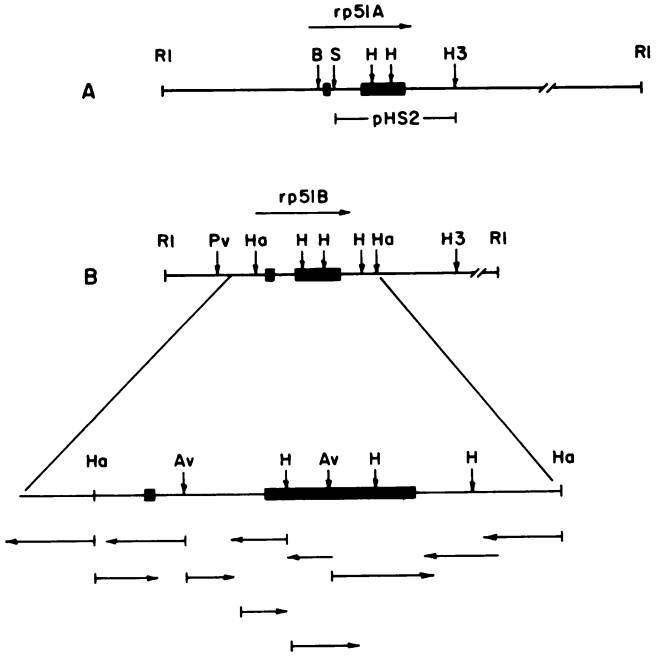

FIG. 1. Yeast genomic *Eco*RI fragments complementary to pHS2. (A) Organization of the *RP51A* gene within the 13.5-kb *Eco*RI fragment (22). Dark boxes, Coding sequences; arrow, direction of transcription. Abbreviations: *Eco*RI (RI), *Bgl*II (B), *Sal*I (S), *Hin*cII (H), *Hin*dIII (H3). (B) Organization of *RP51B* within the 6-kb *Eco*RI fragment and sequencing strategy. Arrows, Strand and extent of sequence determined from each M13 clone (see text). Abbreviations: as above and *Pvu*II (Pv), *Hae*III (Ha), *Ava*II (Av).

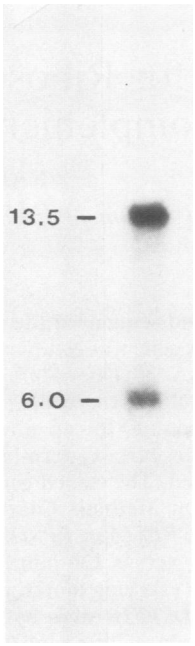

FIG. 2. Southern blot analysis of *Eco*RI digested *S. cerevisiae* genomic DNA. Genomic DNA (5 μg) from strain A364A was cut with *Eco*RI separated on a 0.8% agarose gel and probed with radioactive pHS2, a 1.3-kb *Hin*dIII-*Sal*I subclone of phage 11-138 containing sequences from *RP51A*.

(iv) pHAEURA. The *URA3* gene was inserted as a filled-in *Hin*dIII fragment from plasmid YEP24 into a partial *Hin*cII digest of plasmid HA1RF. The insert was excised with *Hin*dIII and *Eco*RI, enzymes that cut in the polylinker for transformation into yeast cells.

DNA sequence analysis. The mp8 subclones of *RP51B*, generated as described above, were sequenced by using the dideoxy terminator method as described by Sanger et al. (24).

Yeast strains, media, and transformation. Yeast strains as well as the mutants generated in this study are listed in Table 1. All strains were grown at 30°C in YM-1 medium (8) or SD medium containing 0.67% yeast nitrogen base without amino acids and 2% glucose supplemented with amino acids, purines, and pyrimidines, as previously described (25). Transformations were performed as previously described (12), and the transformants were selected on SD plates lacking the nutritional requirements uracil or leucine.

Yeast DNA isolation and Southern blot analysis. Yeast genomic DNA was isolated by the method of Sherman et al. (25). Southern blot analysis was performed as previously described (26, 31) with plasmid HFR-4 single-stranded DNA as a probe labeled by primer extension (11).

RNA extraction and Northern blot analysis. RNA was extracted from 100 ml of culture as described by Rosbash et al. (21). Northern blot analysis was performed as described by Colot and Rosbash (4).

cDNA synthesis. Oligonucleotide-primed cDNA synthesis was performed as described by Teem and Rosbash (28)

except that the reactions contained 25 μM each of dATP, dCTP, and dTTP and 5 μM of ddGTP (see Fig. 7B, lanes 3 and 4).

RESULTS

Cloning and sequence analysis of *RP51B*. A cloned segment of yeast DNA, pY11-138, had originally been identified by hybrid selection and analysis of the in vitro translation product as coding for RP51. This plasmid was used as a probe in genomic Southern analysis and gave an apparently unique high-molecular-weight band upon digestion of genomic DNA with *Hin*dIII (31). A single high-molecular-weight band was also obtained upon digestion with *Bam*HI (data not shown). On the basis of these preliminary characterizations, RP51 was presumed to be encoded for by a single gene. More recently, we probed *Eco*RI-digested yeast genomic DNA with a small subclone of RP51 DNA. Two bands, of 13.5 and 6 kb, were clearly visible (Fig. 2), indicating the presence of an additional sequence homologous to the subclone pHS2 used as a probe (Fig. 1A).

The higher-molecular-weight (13.5 kb) *Eco*RI fragment had been previously cloned in phage lambda (32) and, on the basis of restriction mapping, corresponds to the *RP51* gene originally cloned in pY11-138 (31). This *RP51* gene (Fig. 1A) has been subcloned and sequenced (28). The results of these analyses indicate that this *RP51* gene codes for a basic protein of 135 amino acids. The gene is interrupted by an intron of 399 bp which occurs after the initiating methionine, i.e., between the first and second codons. To determine the relationship of the 6-kb *Eco*RI fragment to the RP51 DNA found within the 13.5-kb *Eco*RI fragment, an *Eco*RI digest of yeast DNA was cloned in pBR322, and the transformed colonies were screened with the radioactive *Sal*I-*Hin*dIII insert of pHS2 (Fig. 1A). In this way, the 6-kb *Eco*RI fragment was identified and analyzed. The restriction map of this fragment (Fig. 1B) bears little resemblance to the restriction map of the larger fragment (Fig. 1A). By Southern analysis, all of the homology with pHS2 was confined to a 1.2-kb *Hae*III fragment which was subcloned in M13 mp8 (14) in both orientations (data not shown). These subclones, and further subclones thereof, were utilized for sequencing as indicated above and in Fig. 1B.

The sequence of this *Hae*III fragment and 380 nucleotides from the adjacent *Pvu*II-*Hae*III subclone is shown in Fig. 3. A comparison with the previously published sequence of the coding region of the *RP51* gene from the larger *Eco*RI

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                    -510       -500       -490       -480       -470       -460       -450       -440
                    CATCTTTTGC TTAATGTATA TATTTTCAGA CTACGCTGAT ATTTTATCGA AAATGGAGGT GATTAGAGAA AAATTATTTT

-430       -420       -410       -400       -390       -380       -370       -360       -350       -340
TATCATTAAC CTAAACATCG AACTCTGGCA TGTACTTTCC CAATCGTGTT GATTTTCACT TATTTGTTTG GAACGTCGTG AATTTTCTAC ATATATAAGC

-330       -320       -310       -300       -290       -280       -270       -260       -250       -240
TCCCTGTAAC ATCCATACAT TCGGTTAAAA TTACACCCAT ACATTTATAG TATTTTTTCC ATCGTTAGTT TTTCGTATTA TGGGCATTCC GAAGAATTTC

-230       -220       -210       -200       -190       -180       -170       -160       -150       -140
ATCTGGAAGA CGCGCATTGA TTGATATCGT TTTCTCAGAG GTCGTACCGT TTCGAGATGA GGGAGAAGGT TCAGGCATCG AGGTCTACCG GAGCAAGGCC

-130       -120       -110       -100       -90        -80        -70        -60        -50        -40
AGTCCTTCCT CCCGTTAGTA CCTCTGTTAA GGGTAAATTT CAAGTAAACA GATATTTTAC ATATTAATTA TTTATTTTGC GTTGTATAAC CTAGAGAAGA

-30        -20        -10        MET        10         20         30         40         50         60
ATAAATAGAT AAAGAAAAAA GCAGATAAAA ATG GTACGTA CCACGAGATG TTGATGAAGC CGGATGATGA TGGACTGGGC GCTAACACAT

        70         80         90         100        110        120        130        140        150        160
GAAATGAGGG CAAGGTTTGC AGAGAGATTG AAAGCGTTAT GGGAACGAGG GGACCAGCAG GGTATTCTTA TTTATGAGCA GATTAGAAAA CTCCATTACT

       170        180        190        200        210        220        230        240        250        260
GATTAGTTTA GAAGAGCGCT CAATGAAGTA GTAGATATTT AAAAGATCAA CCAAATAACC AATTGCTTTC GAATGGCATA TTCTATCTTA TCCAATGGTC

       270        280        290        300        310                                              10*        *
TTGAAGAGAG GTATTTACTA ACTTAAGTTG TCTCATTTGA TTATTGCTAT TTTTATAG GGT AGA GTT AGA ACC AAG ACC GTC AAA CGT GCC

  *                        20                  *                  30*                                  *   *
TCC AAG GCT TTG ATT GAA CGT TAC TAT CCA AAG TTG ACC TTG GAT TTC CAA ACT AAC AAG AGA CTT TGT GAT GAA ATT GCA

 40                                      50                 *   *                *  60
ACT ATC CAA TCC AAG AGA TTG AGA AAC AAG ATT GCT GGT TAC ACT ACT CAT TTG ATG AAA AGA ATC CAA AAG GGT CCA GTT

          *  70                                        80         *                                  90
AGA GGT ATT TCT TTC AAA TTG CAA GAA GAA GAA AGA GAA AGA AAG GAT CAA TAC GTC CCA GAA GTC TCT GCT TTG GAC TTG

                    100                          *             110                                      120
TCT CGT TCT AAC GGT GTT TTG AAC GTT GAC AAC CAA ACC TCT GAC TTG GTT AAA TCT TTG GGT TTG AAG TTG CCA TTA TCT

  *                 *   *                130                        ASN       730        740        750        760
GTC ATC AAC GTT TCC GCT CAA AGA GAC AGA CGT TAC AGA AAG AGA AAC TAA AAAG CATGAATTGA TATAGTTATT ATCATGTCGT

       770        780        790        800        810        820        830        840        850
CTGTTTTACT CCATTTCATT GAGGACTCTC CTTAATGTAT AGTTAAATAA TTTCAATATC ATCTAAAATA TGAAGATATG ATTCATTATT

       860        870        880        890        900        910        920        930        940        950
AGGGATGTAT TCTCTTAATT ATAAGCATTT TGTTACTGAA TATAGCACCT TTGATCAACT TTATGAAGCG TTTATATAAT ATTGAGAATT TCATTAGCC
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FIG. 3. DNA sequence of *RP51B*. The protein coding sequence has been underlined, and within it the codons are numbered. Outside this region the nucleotides are numbered starting from the first nucleotide of the initiation codon. The amino acid that is different from that of *RP51A* has been indicated. *, Nucleotide difference within the protein-coding regions of both genes.

fragment (28) indicates that the 6.0-kb *Eco*RI fragment codes for a second copy of an almost identical protein. Thus, the first copy of this gene (Fig. 1A) has been denoted *RP51A*, and the second copy of this gene (Fig. 1B) has been denoted *RP51B*. On the basis of the two DNA sequences, the relationship between the proteins RP51A and RP51B can be summarized as follows. Both genes code for a protein of 135 amino acids (Fig. 3) (28). The amino acid sequences of the two proteins are identical, except for the last amino acid which is valine in RP51A (28) and asparagine in RP51B (Fig. 3). There are 16 third-base changes in the coding regions of the 3′ exons; thus, the two coding regions are 96% homologous. Analogy to an extensive analysis of RP51A transcripts (28) indicates that the *RP51B* coding region is also interrupted by a single intron which occurs between codons 1 and 2, i.e., the intron occurs after the initiating methionine in both genes. There is relatively little homology within the introns (19) or the flanking DNA (Teem et al., manuscript in preparation).

The DNA sequence of the two genes, including the conserved open reading frames, suggests that both genes are functional. Also, gene-specific subclones from both genes were constructed which hybridized to poly(A)$^+$ RNA (data not shown). (These subclones contained mostly intron sequences and the very short 5′ exon sequences. They hybridized well to intron-containing precursor RNAs but very poorly to the mature mRNAs.) A genetic analysis provided a definitive means of demonstrating that both genes were functional and of assessing the relationship between them.

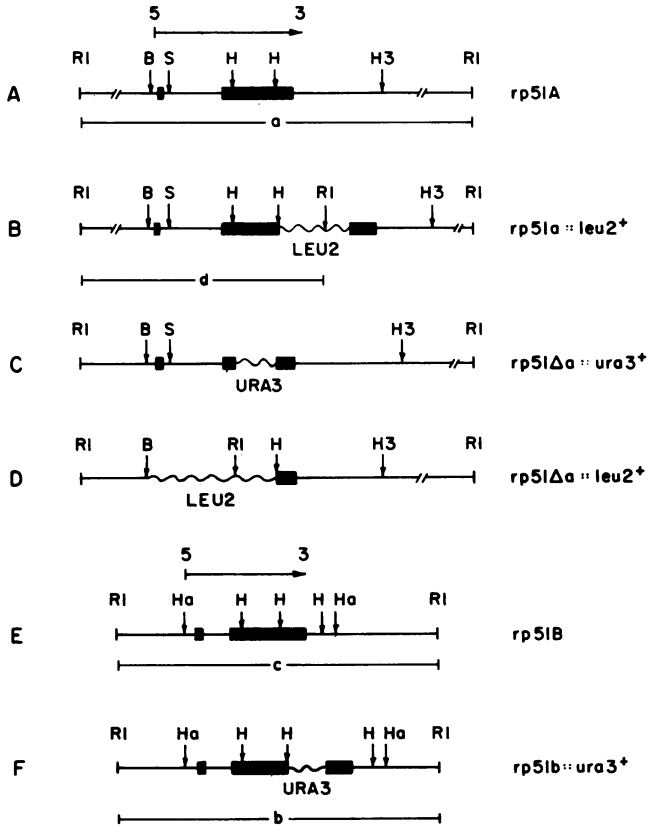

FIG. 4. Structure of the mutations generated in the *RP51* genes. (A and E) Structures of the wild-type *RP51A* and *RP51B* genes, respectively. (B, C, and D) Structures of three different mutations generated in *RP51A* by transformation, as described in the text, with fragments derived from pHS2LEU, pHS2URA, and pGOBLEU, respectively. (F) Structure of the mutation generated in *RP51B* by transformation with *pHAEURA*. Fragments a, b, c, and d correspond to the bands in Fig. 5. Restriction site abbreviations are as in Fig. 1.

Generation of mutations in the *RP51* genes. Recent methods of Rothstein allow one to utilize recombinant DNA as homologous, site-specific mutagenic agents (22). Mutations in both *RP51A* and *RP51B* were generated as shown in Fig. 4. To compare the phenotypes of different kinds of mutations, three *rp51a* mutations were generated. The first (Fig. 4B) consisted of a 2-kb *Sal*I-*Hpa*I fragment containing the *LEU2* gene inserted into the second *Hin*cII site in the 3′ exon of *RP51A*. This disrupted the *RP51A* open reading frame such that the wild-type RP51A protein was no longer synthesized. This *rp51a*::*LEU2* mutant should synthesize only the first 101 amino acids (instead of 135 amino acids) of RP51 followed by eight amino acids from the *LEU2* fragment. The *rp51Δa*::*URA3* mutant (Fig. 4C) was generated in a similar fashion, except that a 235-bp internal *Hin*cII fragment (coding for 78 of the 135 amino acids of *RP51A*) was deleted and replaced with a 1.2-kb *Hin*dIII fragment containing the *URA3* gene. The *rp51Δa*::*LEU2* (Fig. 4D) was missing *RP51A* DNA, from the *Bgl*II site (where transcription begins) to the second *Hin*cII site (at amino acid 101) within the coding region. The *Sal*I-*Hpa*I fragment carrying the *LEU2* gene was inserted in its place. One mutant *rp51b* gene, *rp51b*::*URA3*, was generated in a fashion similar to that in which the first *rp51a* mutant gene was generated, as described above; the *URA3* gene was inserted at the second *Hin*cII site within the coding region. This mutant *rp51b* gene should synthesize the first 101 amino acids of RP51B followed by 12 amino acids from the *URA3* fragment.

Haploid strains carrying either one or two mutant *rp51* genes were obtained as indicated in Table 1. Strains carrying a mutant *rp51b* gene, such as PB-8 and PB-9, as well as strain PB-12 carrying a mutant *rp51a* gene, were obtained by direct transformation of the haploid strains HR125-2D and DB745. Strains PB-10 and PB-11 carrying mutant *rp51a* genes and strains PB-13 and PB-14 carrying mutations in both *rp51a* and *rp51b* were obtained by sporulation and tetrad dissection of the indicated transformed diploid strains (Table 1).

Successful disruption of the *RP51* genes was determined by Southern blot analysis of the DNA extracted from the various haploid strains (Fig. 5). Bands a and c of Fig. 5 correspond to the wild-type copies of *RP51A* and *RP51B* genes, respectively (Fig. 5, lane 1). The replacement of *RP51B* with *rp51b*::*URA3* as in strain PB-8 caused the disappearance of band c (Fig. 5) and the appearance of a new band, band b (Fig. 5), which was larger than band c by the size of the *URA3* gene insertion (Fig. 5, lane 2). As expected, this *Eco*RI band also hybridized with a *URA3* probe (data not shown). The replacement of *RP51A* with *rp51a*::*LEU2* caused the disappearance of band a (Fig. 5) and the appearance of a smaller band, band d (Fig. 5), because there was an *Eco*RI site within the inserted *LEU2* gene. Lanes 4 and 5 (Fig. 5) show only the *RP51B* band c, since in the construction of strains PB-11 (lane 4) and PB-12 (lane 5), the *Hin*cII fragment used as a probe was deleted from the *rp51a* gene (Fig. 4). The pattern of the two haploid strains PB-13 and PB-14, both of which carry two mutant genes, is shown in lanes 6 and 7 of Fig. 5; as expected, both wild-type *Eco*RI bands are absent.

Phenotypic characterization of *rp51* mutants. The effect of the mutations upon the growth properties of the strains, as scored by colony size and growth rate in liquid media, is indicated in Table 1. The absence of the *RP51B* gene, as in

TABLE 1. Characterization of strains carrying mutant *rp51* genes

Strain	Relevant genotype	Source	Colony size[a]	Doubling time[b] (min)
HR125-2D	**a** *RP51A RP51B*	HR125-2D	Normal	133
PB-8	**a** *RP51A rp51b::URA3*	Transformation of HR125-2D	Normal	142
PB-9	α *RP51A rp51b::URA3*	Transformation of DB745	Normal	146
PB-10	α *rp51a::LEU2 RP51B*	Sporulation of PB-2[c]	Small	200
PB-11	*rp51Δa::URA3 RP51B*	Sporulation of PB-7[d]	Small	183
PB-12	α*rp51Δa::LEU2 RP51B*	Transformation of DB745[e]	Small	200
PB-13	α*rp51a::LEU2 rp51b::URA3*	Sporulation of PB-6[f]	Tiny	366
PB-14	**a** *rp51Δa::LEU2 rp51b::URA3*	Sporulation of PB-15[g]	Tiny	525

[a] Determined for cells plated on YM-1 plates.
[b] Determined at 30°C in completed SD medium as described in the text.
[c] Obtained by transforming a diploid made by mating strains HR125-2D (**a** *leu2(2-3,112) ura3-52 trp1 his3 his4*; source, I. Herskowitz) and HR125-RD (α *leu2(2-3,112) ura3-52 trp1 his3 his4*; source, I. Herskowitz).
[d] Obtained by transforming strain DBY1091 (**a**/α *ade2/+ his4/+ can1-101ura3-53/ can¹ura3-52*; source, D. Botstein) with pHS2URA.
[e] α*ura3-52 ade1-100 leu2(2-3,112)*; source, D. Botstein.
[f] Obtained by transforming a diploid made from strains PB-8 and PB-9 with pHS2LEU.
[g] Obtained by transforming a diploid made from strains PB-8 and PB-9 with pGOBLEU.

strain PB-8, had relatively little effect on growth rate. In contrast, the absence of the *RP51A* gene resulted in a markedly slow growth rate (180 to 200 min of generation time versus 130 min for the wild type). Strains PB-10, PB-11, and PB-12 carry different mutations of *RP51A*, yet all three strains manifested a similar phenotype. The two double-mutant strains carrying mutations in both the *RP51A* and *RP51B* genes have a very pronounced phenotype in that they grow extremely poorly (6 to 9 h of generation time). Thus, although a mutant *rp51b* gene had relatively little phenotype in a wild-type background (strains PB-8 and PB-9), there was a striking effect of mutating the *RP51B* gene in a strain which already carries a mutant *rp51a* gene. From these results we conclude that both genes were expressed and that both can contribute to the RP51 protein pool.

To compare the expression of the two genes, Northern blot analysis of RP51 RNA, isolated from wild-type and mutant haploid strains, was performed (Fig. 6A and B). RNA from strain PB-8, a strain containing the *rp51b::URA3* mutant, is shown in lane 2 (Fig. 6). The size and intensity of the major RP51 transcripts were similar to these characteristic of the wild type (Fig. 6, lane 1), consistent with the finding that this strain grew normally. A strain carrying a major deletion in the *RP51A* gene, *rp51Δa::LEU2* (Fig. 6,

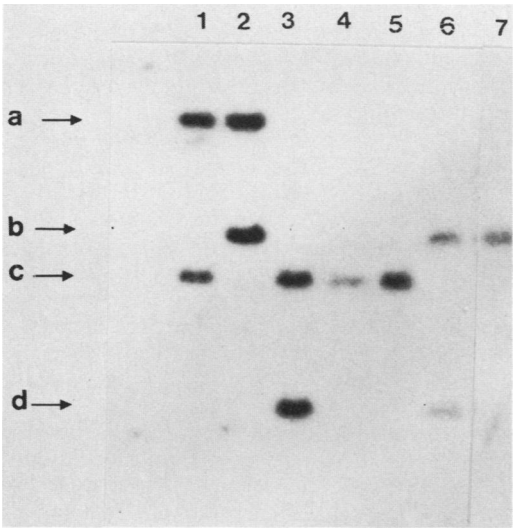

FIG. 5. Southern blot analysis of the mutations generated in the *RP51* genes. Genomic DNA (2.5 μg) isolated from the corresponding strains was digested with *Eco*RI, electrophoresed in a 0.7% agarose gel for 16 h at 125 V, blotted to nitrocellulose, and hybridized to 10 × 10^6 cpm of single-stranded HFR-4, the 235-bp *Hin*cII subclone of pHS2 labeled by primer extension. Lanes: 1, DB745; 2, PB-8; 3, PB-10; 4, PB-11; 5, PB-12; 6, PB-13; 7, PB-14. For strain designation and relevant genotypes, see Table 1. Fragments a, b, c, and d correspond to the *Eco*RI fragments labeled in Figure 4.

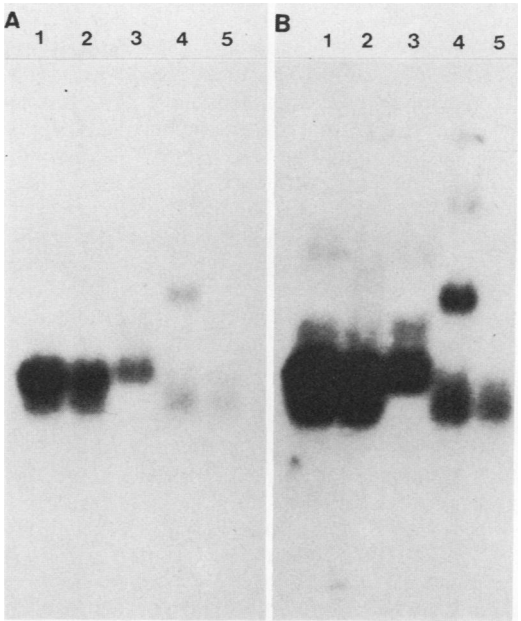

FIG. 6. Northern blot analysis. (A) 90-min exposure; (B) 5-h exposure of the same filter. Total RNA (5 μg) from the indicated haploid strains was separated in a 1.5% agarose-formaldehyde gel run at 175 V for 4 h. After transfer to nitrocellulose, the filters were hybridized to 5 × 10^6 cpm of HFR-4 labeled by primer extension. Lanes: 1, HRD125-2D; 2, PB-8; 3, PB-12; 4, PB-13; 5, PB-14.

lane 3), had considerably less RP51 RNA than did the wild type (compare lanes 3 and 1), consistent with the finding that this strain grew poorly, probably due to an insufficient amount of RP51 mRNA (see below). The residual RP51 transcripts in this strain, ca. 30 to 40% of the level found in the wild-type strain by densitometric scanning of this and similar autoradiograms, were products of the *RP51B* gene. Lane 4 (Fig. 6) contains RNA from strain PB-13 and consists of a mixture of aberrant transcripts that fuse *RP51A* sequences to the *LEU2* fragment and *RP51B* sequence to the *URA3* fragment (see Table 1). Presumably, the sizes of these transcripts are determined by the positions and strengths of polyadenylation signals within these fragments. The origins of these fusion transcripts were assigned by comparing lanes 4 and 5 of Fig. 6 in a longer exposure of this filter shown in Fig. 6B. Lane 5 contains RNA from strain PB-14. Since this strain contains an *rp51a* deletion and an *rp51b*::*URA3* gene, the transcript in lane 5 can be assigned to the mutant *rp51b* gene. This same transcript is also visible in lane 4. The high-molecular-weight *rp51b*::*URA3* transcript present in lane 2 (RNA from strain PB-8, containing a wild-type *RP51A* gene and a mutant *rp51b*::*URA3* gene) is also present in lane 4, and by subtraction, the other transcripts in lane 4 can be assigned to the mutant *rp51a*::*LEU2* gene also present in this strain. Presumably the transcript visible in lane 5 is also present in lane 2 but not detectable due to the large amount of comigrating wild-type RP51A transcripts. In summary, the structures of the RP51 RNA species observed were consistent both with the structure of the mutant genes shown in Fig. 4 and with the observed phenotypes of the strains.

The molecular phenotypes of the mutants suggest that, in the wild-type strain, the *RP51A* gene contributes ca. 60 to 70% of the RP51 mRNA, and the *RP51B* gene contributes ca. 30 to 40%. However, this conclusion requires that the level of RP51B mRNA is unaffected by the deletion of the *RP51A* gene and, therefore, is identical to the level of RP51B mRNA in a wild-type strain, i.e., the amount of RP51B mRNA visible in Fig. 6, lane 3, is identical to the amount of RP51B mRNA in Fig. 6, lane 1. To test this possibility requires the ability to measure separately the levels of RP51A and RP51B transcripts. Because our existing gene-specific subclones have very limited homology with mature RP51 transcripts, we decided to exploit primer extension as a means of measuring the levels of individual RP51 transcripts.

A previously described synthetic oligonucleotide (28), the sequence of which is indicated in Fig. 7A, was used to prime cDNA synthesis complementary to RP51 mRNA. The primer was perfectly complementary to RP51A mRNA and had a one-base mismatch with RP51B mRNA. The primer-extended products obtained with wild-type RNA as the template are shown in Fig. 7B, lane 1. Only the RP51A products are easily visible (28). The RP51B products are very faint, almost certainly a reflection of the lower abundance of these transcripts, the mismatch with the primer, and an even more heterogeneous set of 5′ ends. The RP51B-specific products are shown in Fig. 7, lane 2, where the RNA was prepared from the strain that carries a deletion mutation of *rp51a* and extrachromosomal copies of *RP51B*.

To circumvent these difficulties and to visualize specifically *RP51B* products, we carried out the primer extensions in the absence of dGTP and in the presence of an excess of ddGTP. The strategy, dictated by the DNA sequences shown in Fig. 7A, was designed to stack the heterogeneous cDNA products into two distinct bands, one from RP51A mRNA and the other from RP51B mRNA. The approach takes advantage of the fact that the first G incorporated into cDNA is in a different position in RP51A than in RP51B.

Lanes 3 and 4 of Fig. 7B show the cDNA products synthesized under these conditions with RNA from a wild-type strain and from a mutant strain deleted of *RP51A*, respectively. The two bands are of exactly the sizes predicted by the DNA sequences of the two mRNAs. As expected, the more intense RP51A band was absent in the *rp51a* deletion mutant. Since the intensity of the RP51B band was unchanged in the *rp51a* deletion, we conclude that RP51B mRNA levels do not change in the absence of RP51A mRNA. This in turn allows the conclusion, based on the Northern data, that in wild-type cells, RP51 mRNA is ca. 60 to 70% RP51A mRNA and 30 to 40% RP51B mRNA.

The RNA analyses and the phenotypes of the double-mutant strains as compared with the single-mutant strains indicate that both wild-type *RP51* genes are expressed. If the two proteins are interchangeable and if the different growth rates observed in the various single-mutant strains are only because *RP51B* is expressed poorly relative to *RP51A*, the slow growth of strain PB-12 (missing the *RP51A* gene with a single copy of the *RP51B* gene) should be rescued by providing extra copies of the *RP51B* gene. To this end, we transformed strain PB-12 with a derivative of plasmid YEP24 (2) which contains the *RP51B* gene (pYE51B). As controls, strain PB-12 and the wild-type parent DB745 were transformed in parallel with plasmid YEP24. The PB-12 (pYE51B) transformants appeared 1 day earlier than the PB-12 (YEP24) transformants. The data in Table 2 show that the growth defect of the *RP51A* deletion was overcome by the extra copies of *RP51B*. The results support the view that the proteins encoded by these two genes are largely, if not entirely, interchangeable and that differences in the phenotypes of the mutants are due to differences in the expression of the *RP51A* and *RP51B* genes.

DISCUSSION

The data presented above indicate that both *RP51* genes are functional and code for essentially interchangeable proteins. The dramatic phenotype of the double mutants (6 to 8.5-h generation time) as compared with the single mutants or to wild-type strains indicates convincingly that mutations in *RP51A* and *RP51B* complement. This interpretation is consistent with the DNA sequences of the two genes and with the phenotypes of a number of *RP51* mutants and mutant-plasmid combinations.

The phenotypes of the single mutants (cells missing either a wild-type *RP51A* gene or a wild-type *RP51B* gene) provide additional insight into the possible modes of regulation of ribosomal protein gene expression. The relatively minor effect of the *RP51B* mutant is consistent with the observation that the contribution of the *RP51B* gene to the RP51 mRNA pool is relatively minor (30 to 40%). The phenotype of the three *RP51A* mutants is consistent with the fact that *RP51A* gene expression contributes 60 to 70% of the RP51 mRNA. Although self-consistent, the data do not explain why there are two *RP51* genes. Perhaps *RP51B* makes a small but important contribution to RP51 synthesis. Alternatively, perhaps the two genes are differentially regulated such that the *RP51B* gene makes a more substantial or necessary contribution under certain physiological conditions.

The data indicate further that the presence of a single *RP51B* gene is not sufficient to compensate fully for the absence of *RP51A* expression. Since extra copies of the *RP51B* gene rescue completely (Table 2), neither the gene nor its product is intrinsically inadequate; rather, the normal

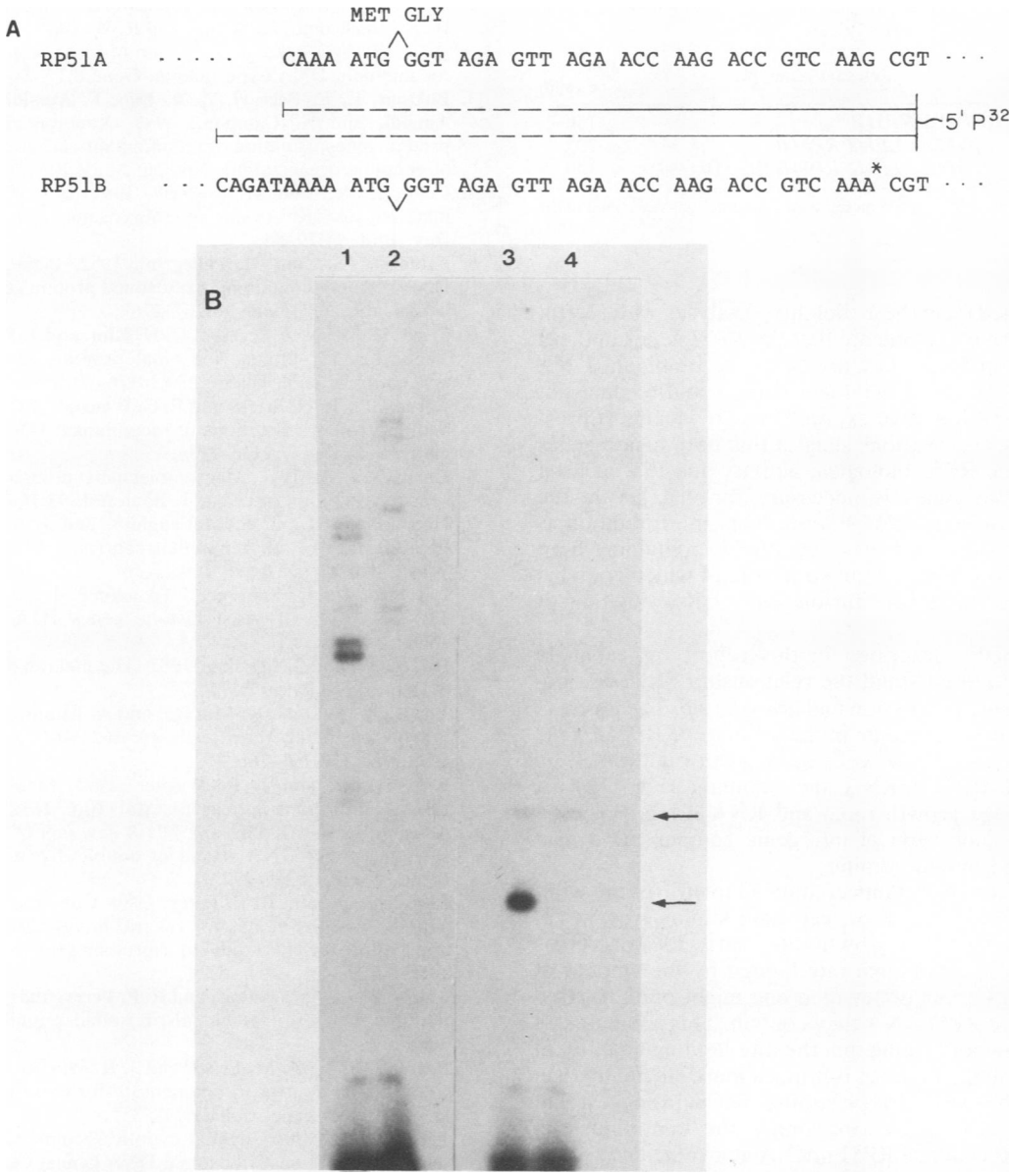

FIG. 7. Analysis of *RP51A*- and *RP51B*-specific transcripts: cDNA synthesis from oligonucleotide-primed mRNA. (A) DNA sequences of *RP51A* and *RP51B* represented as the coding strand. Symbols: ∧, position of the intron; solid line, sequence complementary to the synthetic 19-mer; *, position of a mismatched base in the *RP51B* sequence; dashed line, predicted extension products in −dGTP/+ ddGTP reactions. (B) RP51A and RP51B primer extension products. Total RNA (10 μg) was hybridized to the oligonucleotide primer complementary to the 3′ exon of the *RP51* genes. cDNA extensions of the primer were synthesized with all four nucleotides (lanes 1 and 2) and −dGTP/+ ddGTP (lanes 3 and 4) and was electrophoresed on 6% sequencing gels. Lanes 1 and 3, RNA obtained from wild-type strain HR125-2D; lane 2, RNA obtained from strain PB-12 transformed with PYE51B (see text for description); lane 4, RNA from a strain carrying a deletion of *RP51A*, as in PB-12, but in HR125-2D background.

haploid *RP51B* gene dosage (one) is insufficient to compensate fully for the loss of *RP51A* gene expression. This interpretation is entirely consistent with direct measurements which indicate that intergenic dosage compensation, manifest at the level of mRNA abundance, does not take place (Fig. 7B).

It is likely that the difference in the expression of the two genes is reflected in DNA sequence differences in the noncoding DNA of *RP51A* and *RP51B*. A comparison of the noncoding regions with the aid of computer programs (20) indicates that there is very little overall homology between the noncoding regions of *RP51A* and *RP51B*; nevertheless, short homologous regions do exist. These homologies, and their relationship to other ribosomal protein genes, are discussed in detail elsewhere (Teem et al., manuscript in preparation).

The phenotypes of the double mutants, although indicating that *RP51A* and *RP51B* complement, are somewhat surprising. We expected to be unable to construct these double-mutant strains, i.e., we expected to obtain no viable LEU^+ URA^+ spores from the diploids PB-6 and PB-15. Viable haploid strains of this genotype were, however,

TABLE 2. Additional copies of *RP51B* restore PB-12 to wild-type growth rate[a]

Strain(plasmid)	Relevant genotype	Doubling time (min)
DB745(YEP24)	*RP51A RP51B*	150
PB-12(YEP24)	*rp51Δa::LEU2 RP51B*	205
PB-12(pYE51B)	*rp51Δa::LEU2 RP51B* $(RP51B)_n$	140

[a] Cells were grown at 30°C in SD medium lacking uracil, as described in the text.

routinely obtained from these diploids. Analyses with Northern and Southern blots indicate that the *RP51* genes in these strains are disrupted as they are in the recombinant DNA molecules used to construct them (Fig. 5 and 6; data not shown). Although definitive experiments are lacking at present, preliminary observations suggest that both fusion genes in PB-13 provide RP51 biological activity and that at least one of these two genes is necessary for viability in the absence of a wild-type *RP51* gene. This interpretation is consistent with the fact that strain PB-13, containing both fusion genes, grows better than strain PB-14 which contains only a single *rp51b::URA3* fusion gene and a deletion of *rp51a* (Table 1).

The experiments described in this report represent an initial attempt to understand the relationship between ribosomal protein gene expression and growth rate. The levels of RP51B mRNA do not change in the absence of *RP51A* gene expression, even when the cells are growth rate limited by the amount of RP51 mRNA they contain (Fig. 7B). A comparison of the growth rates and RNA levels suggests, however, that some form of intergenic compensation may take place in the mutant strains.

The *rp51a* deletion mutants contain 30 to 40% of the wild-type RP51 RNA level (Fig. 6), yet these strains grow at ca. 65 to 75% of the wild-type growth rate (180 to 200 min versus 130 min [Table 1]). Although rate limited by the amount of RP51 RNA, they grow better than one might predict based on the amount of RP51 RNA they contain. This anomaly can be reconciled by postulating that the rate-limiting quantity of RP51B mRNA in the mutants is utilized more efficiently than total RP51 mRNA in wild-type strains. The data suggest that the wild-type strains (growing under the conditions described in Table 1) utilize RP51 mRNA at an efficiency of ca. 50% as compared with the *rp51a* deletion strains. Underutilization of RP mRNA in wild-type strains is consistent with results obtained on yeast ribosomal protein gene expression from another laboratory (13, 17). A further examination of the phenotypes of these and other ribosomal protein gene mutants should provide substantial insight into these regulatory problems.

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