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Ribosomal Protein L30 Is Dispensable in the Yeast *Saccharomyces cerevisiae*

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In the yeast *Saccharomyces cerevisiae*, L30 is one of many ribosomal proteins that is encoded by two functional genes. We have cloned and sequenced *RPL30B*, which shows strong homology to *RPL30A*. Use of mRNA as a template for a polymerase chain reaction demonstrated that *RPL30B* contains an intron in its 5′ untranslated region. This intron has an unusual 5′ splice site, C/GUAUGU. The genomic copies of *RPL30A* and *RPL30B* were disrupted by homologous recombination. Growth rates, primer extension, and two-dimensional ribosomal protein analyses of these disruption mutants suggested that *RPL30A* is responsible for the majority of L30 production. Surprisingly, meiosis of a diploid strain carrying one disrupted *RPL30A* and one disrupted *RPL30B* yielded four viable spores. Ribosomes from haploid cells carrying both disrupted genes had no detectable L30, yet such cells grew with a doubling time only 30% longer than that of wild-type cells. Furthermore, depletion of L30 did not alter the ratio of 60S to 40S ribosomal subunits, suggesting that there is no serious effect on the assembly of 60S subunits. Polysome profiles, however, suggest that the absence of L30 leads to the formation of stalled translation initiation complexes.

The ribosome is a complex macromolecular assembly of 4 RNA molecules and approximately 75 proteins. Whereas studies of the structures and functions of the components of the ribosomes of *Escherichia coli* have made substantial progress, similar analyses of eucaryotic ribosomes have lagged, in part because of the lack of genetic approaches to the problem. Consequently, a number of laboratories have undertaken the study of the structures and functions of the genes for the ribosomal proteins of *Saccharomyces cerevisiae*. In this paper, we report on such studies of L30, a protein of the 60S ribosomal subunit.

Whereas the evolutionary conservation of the ribosome would suggest little tolerance of structural aberration, Dabbs has shown, surprisingly, that in *E. coli* almost one-third of the ribosomal protein genes are not essential (3). His laboratory has isolated mutants in every ribosomal protein analyzed on two-dimensional gels. These mutations resulted in a variety of phenotypes; e.g., when either ribosomal protein L29 or L30 was missing, growth was normal, but when S17, L19, L24, or L28 was missing, growth was severely affected. (Regrettably, the numbering systems of the ribosomal proteins of *E. coli* and of *S. cerevisiae* are unrelated.) These investigators were able to construct strains missing up to four proteins, demonstrating the redundancy built into the *E. coli* ribosome. Mutants lacking ribosomal proteins have been used in studies of protein localization on the ribosome (19, 34–36), of ribosomal protein function (15, 37), and of the expression of ribosomal protein operons (4).

In yeast cells, it is relatively simple to determine whether a cloned gene is essential for growth. One of the nine yeast ribosomal proteins that have been examined has been found to be dispensable, but its absence leads to severe defects in cell growth (8). This ribosomal protein gene is fused to one of the genes encoding ubiquitin (*UBI3*); its depletion is also characterized by very inefficient processing of 20S to 18S rRNA, resulting in substantial deficiency in 40S ribosomal subunits. The rat homolog to this ubiquitin fusion tail has been identified as ribosomal protein S27a (26). Sachs and Davis (32) have found that yet another yeast ribosomal protein, L46, can be depleted, resulting in a cold-sensitive phenotype. The other yeast ribosomal proteins thus far tested all play essential roles in cell growth (1, 6, 8, 9, 17, 29).

We have now investigated the functions of the two genes encoding the yeast ribosomal protein L30 (formerly known as RP29). *RPL30A*, which is linked head-to-head with another ribosomal protein gene, *RPL32*, was previously cloned (10) and sequenced (21). *RPL30B* has been cloned and sequenced; the open reading reading frame (ORF) of this gene differs only slightly from its counterpart, whereas the sequences immediately 5′ and 3′ to the ORFs diverge completely. The intron of *RPL30B* is located in its 5′ untranslated region and possesses an unusual 5′ splice site: C/GUAUGU. Each gene is functional, though they are not utilized to the same extent. On mating cells containing disrupted *RPL30A* with cells containing disrupted *RPL30B* and subsequent sporulation, we were surprised to find that cells without any intact gene for L30 are viable. Although their ribosomes entirely lack L30, their doubling times are increased by only 30%. Their ratio of 60S to 40S ribosomal subunits appear normal. These mutants offer the opportunity to study ribosomal protein function, ribosome assembly, and translation.

(The data in this paper are from a thesis submitted in partial fulfillment of the requirements for the Ph.D. degree in the Sue Golding Graduate Division of Medical Sciences, Albert Einstein College of Medicine, Yeshiva University.)

MATERIALS AND METHODS

Strains and media. The strains of *S. cerevisiae* used were W303, W303-1A, and W303-1B, *MAT***a**/*MAT*α, *MAT***a**, and *MAT*α, respectively, and isogenic for *leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15 phi*$^+$ (obtained from R. Rothstein, Columbia University) (39). All yeast strains were grown at 30°C in synthetic complete minimal medium (40) with the necessary supplements. Growth rates were measured in YPD (1% yeast extract, 2% peptone, 2% dextrose).

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Cloning and sequencing of *RPL30B*. An *S. cerevisiae* genomic library based in the *TRP1 CEN3* vector M111 was obtained from V. Mackay (Zymo Genetics, Seattle, Wash.). *E. coli* RR1, transformed with this library, was screened by transferring colonies to nitrocellulose filters (11) and probed with a nick-translated (28) 200-base-pair (bp) *Bgl*II-*Cla*I fragment from the *RPL30A* gene. From those colonies giving a positive signal, DNA was isolated essentially as described by Maniatis et al. (20) and restriction digested to distinguish clones containing *RPL30B* from those containing *RPL30A*. A 4.25-kilobase-pair (kb) *Eco*RV fragment containing *RPL30B* was subcloned into pBLUESCRIPT KS$^+$ (Stratagene), thus generating plasmid DB30B1. DB30B1 was then digested with *Hin*dIII, and the 1.6-kb *Hin*dIII fragment was subcloned into pBLUESCRIPT KS$^+$, producing plasmid DB30B3. DB30B3 was digested at the appropriate restriction enzyme sites flanking the *RPL30B* insert and treated with exonuclease III, using the Erase-a-Base system (Promega Biotec). The resulting DNAs were isolated (16) and sequenced by using Sequenase (U.S. Biochemical Corp.) (16) (see Fig. 2).

Intron splice site localization. RNA was extracted as previously described (7) from W303-1A; the poly(A)$^+$ RNA was then isolated (42) and transcribed with reverse transcriptase (Life Sciences, Inc.), using as a primer a synthetic oligonucleotide complementary to the *RPL30B* transcript. The cDNA generated from this reaction was used as a template for polymerase chain reaction (PCR), utilizing the GeneAmp DNA amplification reagent kit (Perkin-Elmer Cetus). Figure 3A highlights the sequences of the synthetic oligonucleotides used in the PCR reactions. The resulting 92-bp product (Fig. 4B) was ligated into pBLUESCRIPT SK$^-$ and used to transform *E. coli* DH5α. Colonies were screened by transfer to nitrocellulose filters (11) and probed with one of the synthetic oligonucleotides originally used in the PCR reaction, end labeled with polynucleotide kinase (New England BioLabs, Inc.) (20). Plasmid DNA was isolated from four independent colonies giving positive signals and sequenced by using Sequenase (16).

Construction of gene disruption plasmids. DB30A, formerly known was pA13(1.5) (21), was digested with *Cla*I and *Bgl*II, filled in with the Klenow fragment of DNA polymerase I, treated with phosphatase, and ligated to a filled-in 1.1-kb *Hin*dIII fragment containing the intact *URA3* gene in order to construct plasmid DB30A-URA3CB. This plasmid is missing the 270-bp *Cla*I-*Bgl*II fragment of *RPL30A* and was used in the disruption of *RPL30A* (see Fig. 5).

Plasmid DB30B3 DNA (see above) was digested with *Bgl*II, treated with phosphatase, and ligated to a 1.7-kb *Bam*HI fragment containing the intact *HIS3* gene to generate plasmid DB30B3-HIS3. This construct is missing the 250-bp *Bgl*II fragment of *RPL30B* and was used to disrupt *RPL30B* (see Fig. 5).

Yeast transformations and gene disruptions. All yeast transformations were performed by a lithium acetate method similar to that of Ito et al. (13).

Plasmid DB30A-URA3CB was digested with *Eco*RI and *Hin*dIII and used to transform W303a/α, resulting in Δ*A* strains.

DB30B3-HIS3 was digested with *Nci*I and transformed into W303a/α to generate Δ*B* strains.

Southern blot analysis. Genomic yeast DNA was isolated by the method of Carlson and Botstein (2), digested with the appropriate restriction enzymes, separated by electrophoresis on 0.8% agarose gels, and transferred to nitrocellulose (33). These blots were then probed with either nick-translated (28) fragments of *RPL30A*, *URA3*, or *HIS3* or with riboprobes generated from subclones of *RPL30B*, *RPL30A*, or *RPL32* in pBLUESCRIPT (Stratagene) or in pGEM-3Z (Promega Biotec).

Northern (RNA) blot analysis. RNA was extracted as previously described (7) and separated on formaldehyde gels (25). Ethidium bromide was added to the RNA samples before electrophoresis to detect rRNA levels. Gels were blotted to Nytran (Schleicher & Schuell, Inc.) and hybridized with synthetic oligonucleotide probes at the oligonucleotide-specific T_d (T_d represents twice the number of adenine and thymine residues plus four times the number of guanine and cytosine residues) minus 15°C in 5× SSPE (SSPE is 0.18 M NaCl, 0.01 M $NaPO_4$, and 0.01 M EDTA [pH 7.4])–5× Denhardt solution–0.5% sodium dodecyl sulfate–0.1 mg of calf thymus DNA per ml. These blots were washed at the oligonucleotide-specific T_d minus 10°C, using 5× SSC (SSC is 0.15 M NaCl plus 0.015 M sodium citrate [pH 7.0])–0.1% sodium dodecyl sulfate.

Primer extension analysis. Utilizing an oligonucleotide complementary to sequences deleted in both gene disruptions and total RNA isolated as previously described (7) from yeast cells containing these gene disruptions, primer extensions were performed as described by Ju et al. (14). An oligonucleotide complementary to another ribosomal protein gene, *RPL32*, was used as an internal control to measure the RNA levels between the various reactions.

Two-dimensional gel electrophoresis. Ribosomal proteins were isolated from yeast cells and subjected to two-dimensional electrophoresis as described by Warner and Gorenstein (43), using 8 M urea at pH 5 in the first dimension and sodium dodecyl sulfate in the second dimension. Gels were stained with Coomassie blue to visualize the proteins.

Sucrose gradient analysis. Sucrose gradient analysis was performed as described by Warner et al. (44), using an ISCO density gradient fractionator (model 640) and measured by UV absorption at 260 nm.

Nucleotide sequence accession number. The GenBank accession number of the *RPL30B* sequence is M34387.

RESULTS

Cloning *RPL30B*. Many of the ribosomal proteins of *S. cerevisiae* are encoded by two genes. Analysis of genomic DNA suggested that this is also true of ribosomal protein L30 (10; Fig. 1). To isolate the second gene encoding L30, a partially *Sau*3A1-digested genomic library was screened by using an *RPL30*-specific probe. Among 4,500 colonies, representing 36,000 kb of genomic sequences, three colonies giving a positive hybridization signal were identified. Their plasmid DNA was isolated and restriction digested. Two of the plasmids contained the second copy of the gene, *RPL30B*. One of these clones was subcloned and sequenced. Figure 2 shows a partial restriction map of this clone and the scheme used to subclone it for sequencing.

Sequencing *RPL30B*. The sequence of the 1.6-kb fragment of *RPL30B* (Fig. 3A) contains an open reading frame (ORF) of 155 codons. Comparison with that of *RPL30A* revealed only 35 differences among the 468 nucleotides (Fig. 3A), introducing five amino acid changes (Fig. 3B). Four of the five amino acid changes are conservative. Three changes are clustered near the C terminus of the protein. The two genes contain virtually no sequence homology in their 5′ and 3′ noncoding regions. A search through the available ribosomal protein sequences revealed that no homologue to L30 has been found yet in other organisms.

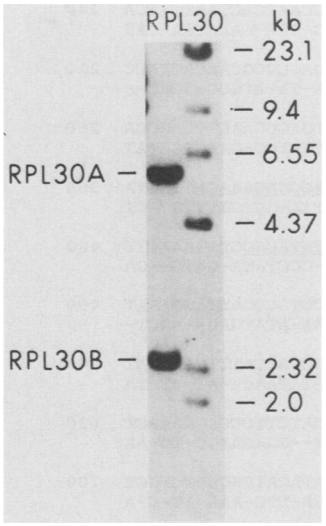

FIG. 1. Southern blot analysis of genomic yeast DNA indicating the presence of two genes encoding L30. DNA was isolated, cut with *Hin*dIII, electrophoresed, transferred to nitrocellulose, and hybridized to a nick-translated fragment containing 270 bp of *RPL30A* coding sequence as described in Materials and Methods. Counterprobing blots such as this one with a probe containing another ribosomal protein gene (*RPL32*) that is tightly linked to *RPL30A* differentiated between the two genes encoding L30.

Identifying splice sites in the 5′ leader of *RPL30B*. *RPL30A* is unusual among yeast genes since it contains an intron in the 5′ untranslated leader (21). In *RPL30B*, the presence at −78 (relative to the ATG) of a TACTAAC box (Fig. 3A), the characteristic site of lariat formation during the splicing of yeast introns (18), suggested that *RPL30B* possesses such an intron as well. However, the canonical 5′ splice sequence, G/GTATGT, was missing. To identify the splice sites definitively, the PCR strategy shown in Fig. 4 was used. Poly(A)$^+$ RNA was used as a template for reverse transcription, using an antisense primer within the coding region but specific for *RPL30B* (oligonucleotide 4 in Fig. 3A). The resulting cDNA was used as a template in a series of PCR reactions. Since neither the 5′ transcription initiation site nor the 5′ splice site was known, several sense primers (oligonucleotides 1, 2, and 3 in Fig. 3A) were used with the hope that one would lie within the first exon of the transcript. Only the reaction using oligonucleotide 3 generated a PCR product of the size expected from a spliced mRNA. No product from the unspliced mRNA is evident, but it would be expected at a level of only 1 to 5% of that generated from the spliced mRNA. The 92-bp product was subcloned, and its sequence was determined in four independent clones (Fig. 4B and C). It is clear that the 5′ splice site is C/GTATGT and that the 3′ splice site lies immediately downstream of the third AG following the TACTAAC box (or 63 nucleotides 3′ of the TACTAAC box) (Fig. 3A and 4C). Both characteristics are unusual: the only other yeast genes that have C/GTATGT at their 5′ splice sites encode RP28 and L44′ (22, 27); the 3′ splice site of yeast genes usually occurs within 20 to 40 nucleotides downstream of the TACTAAC box (reviewed by Woolford [45]). Nevertheless, splicing of this intron is efficient; no unspliced transcript was detected either by Northern analysis or by PCR.

Since neither oligonucleotide 1 nor 2 generated a product (Fig. 4B), the 5′ transcription initiation site must lie between positions −460 and −420 relative to the ATG (or between positions 404 and 443 in Fig. 3A). Primer extension analysis demonstrated a single initiation site at position −420 relative to the ATG (see Fig. 8, described below). Transcription of many ribosomal protein genes is controlled by an upstream activating sequence that includes an RPG box, whose consensus is ACACCCATACATTT (24). In *RPL30B*, the sequence AAACCCAATCACTA (located at position 120 in Fig. 3A) could qualify as a putative RPG box; it is 64%

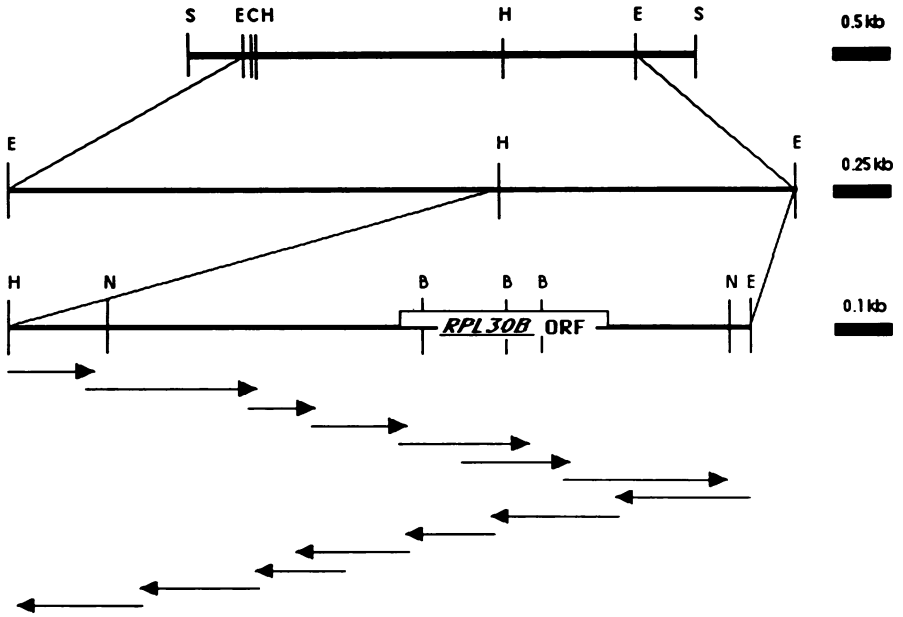

FIG. 2. Partial restriction map of the genomic clone containing *RPL30B* and subcloning scheme (described in Materials and Methods). Arrows indicate the direction and extent of sequence analysis. Restriction sites: S, *Sau*3A1; E, *Eco*RV; C, *Cla*I; H, *Hin*dIII; N, *Nci*I; B, *Bgl*II.

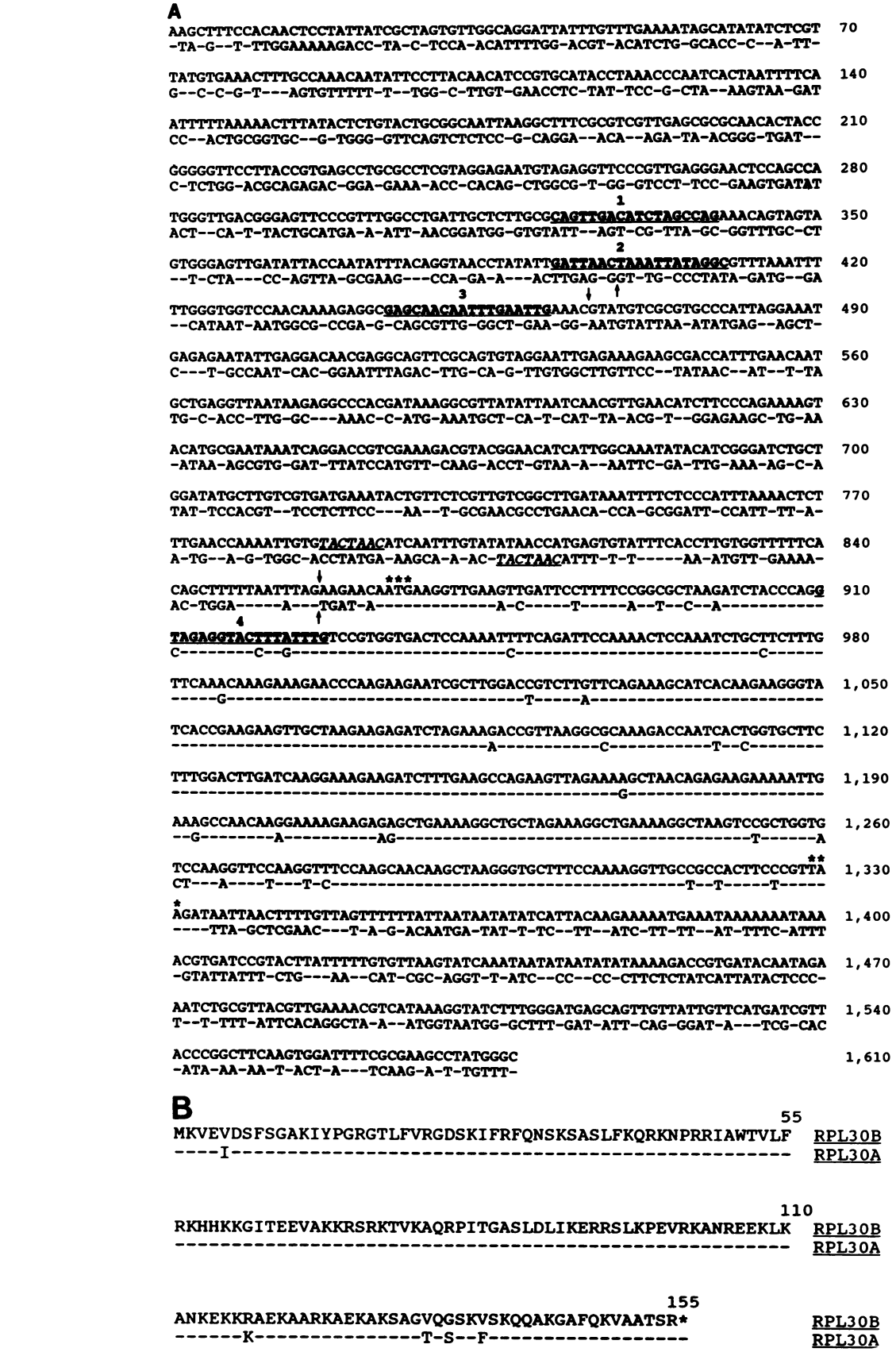

FIG. 3. Sequence comparison of *RPL30B* and *RPL30A*. (A) DNA sequence homology, with *RPL30B* listed first and *RPL30A* compared underneath. Dashed lines represent identical nucleotides. The ATG and the stop codons of the ORFs are starred. The 5′ and 3′ intron splice sites are designated by arrows. The TACTAAC boxes are underlined and italicized. The oligonucleotide sequences used in the intron splice site determination (Fig. 4) are underlined and numbered. (B) Predicted amino acid sequence from both respective ORFs. Dashed lines indicate identical amino acids.

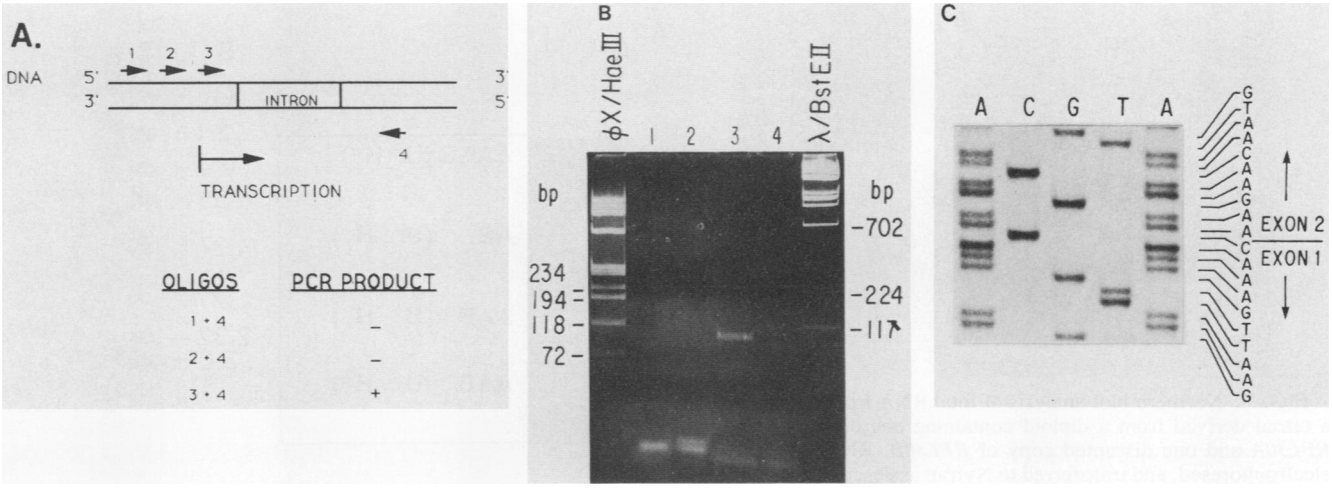

FIG. 4. Determination of *RPL30B* intron splice sites. (A) Schematic representation of the method. A PCR product will be obtained only when the mRNA from which the cDNA is synthesized contains the sequence complementary to the upstream oligonucleotide. If transcription starts downstream of this (e.g., oligonucleotides 1 and 2) or if the sequences are removed by splicing, no product will be obtained. (B) Polyacrylamide gel electrophoresis of the PCR products obtained when using oligonucleotides 1 and 4 (lane 1), 2 and 4 (lane 2), 3 and 4 (lane 3), and 3 and 4 with no RNA in the initial reverse transcription reaction (lane 4). (C) Sequencing gel showing the exon 1-exon 2 junction.

homologous to that of the consensus RPG box and is present 324 nucleotides upstream of the initiation of transcription, a position characteristic of RPG boxes (38).

Disruption of *RPL30A* and *RPL30B*. Taking advantage of the high frequency of homologous recombination in yeast cells, each of the genes encoding L30 was disrupted by using the gene replacement method of Rothstein (30) (Fig. 5). In the disruption of *RPL30A*, 270 bp of the coding sequence were replaced with *URA3*, leaving four N-terminal amino acids intact. In the disruption of *RPL30B*, 250 bp of the coding sequence was replaced by *HIS3*, leaving 11 N-terminal amino acids intact. Each of the two disrupted genes was introduced individually into W303a/α by gene replacement. Sporulation of the diploids gave rise to four viable spores showing that an intact *A* gene or an intact *B* gene is sufficient to support reasonable growth. However, haploid cells of the genotype Δ*A B* grew more slowly than those with the genotype *A* Δ*B* (Table 1), suggesting that *RPL30A* plays a more important role in the production of L30 than does *RPL30B*.

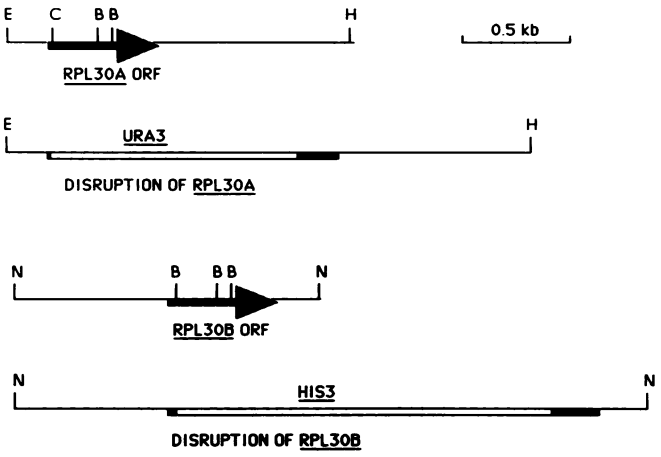

FIG. 5. Schematic diagram of the intact and disrupted *RPL30A* and *RPL30B* genes. Restriction sites: E, *Eco*RI; C, *Cla*I; B, *Bgl*II; H, *Hin*dIII; N, *Nci*I.

RPL30A-specific transcripts could not be detected in cells carrying a disruption of *RPL30A* (Fig. 6A). On the other hand, the probe used in Fig. 6B, which is complementary to the *RPL30B* exon 1-exon 2 junction determined by PCR (Fig. 4C), revealed a relatively rare transcript of approximately 150 to 200 nucleotides in cells carrying the disruption of *RPL30B*, presumably due to the introduction of a termination site within the *HIS3* insert. This truncated transcript would code for only 11 N-terminal amino acids of the *RPL30B* ORF plus a predicted 47 unrelated amino acids from continued translation into the *HIS3* insert.

TABLE 1. Growth rates in YPD medium at 30°C

Strain	Relevant *RPL30* genotype	Doubling time (min)[a]	
Diploids			
W303a/α	A^+/A^+ B^+/B^+	96 (3)	
1	$A^+/\Delta A$ B^+/B^+	110 (2)	
2	A^+/A^+ $B^+/\Delta B$	97 (2)	
3	$A^+/\Delta A$ $B^+/\Delta B$	137 (2)	
Haploids			
W303a	A^+ B^+	105 (5)	
4	A^+ B^+	105 (1)	
5	A^+ B^+	116 (1)	
6	A^+ B^+	100 (1)	
7	A^+ B^+	93 (1)	105 (12)
8	A^+ B^+	120 (1)	
9	A^+ B^+	102 (1)	
10	A^+ B^+	103 (1)	
11	Δ*A* B^+	126 (3)	
12	Δ*A* B^+	132 (3)	130 (9)
13	Δ*A* B^+	131 (3)	
14	A^+ Δ*B*	125 (2)	
15	A^+ Δ*B*	122 (2)	121 (6)
16	A^+ Δ*B*	117 (2)	
17	Δ*A* Δ*B*	137 (3)	
18	Δ*A* Δ*B*	133 (1)	137 (5)
19	Δ*A* Δ*B*	140 (1)	

[a] Values are averages of the number of measurements shown in parentheses.

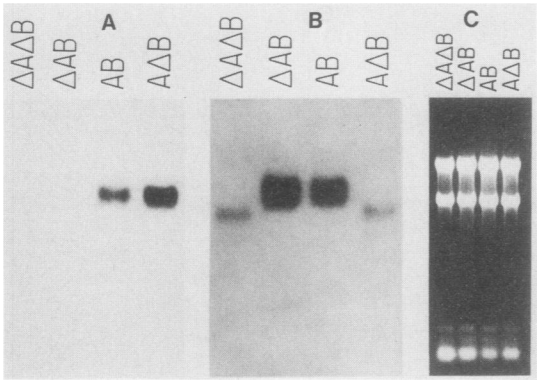

FIG. 6. Northern blot analysis of total RNA from four spores of a tetrad derived from a diploid containing one disrupted copy of *RPL30A* and one disrupted copy of *RPL30B*. RNA was isolated, electrophoresed, and transferred to Nytran as described in Materials and Methods. (A) Northern blot probed with an *RPL30A*-specific oligonucleotide. (B) Northern blot probed with an oligonucleotide complementary to the *RPL30B* exon 1-exon 2 junction. (C) Ethidium bromide-stained formaldehyde gel.

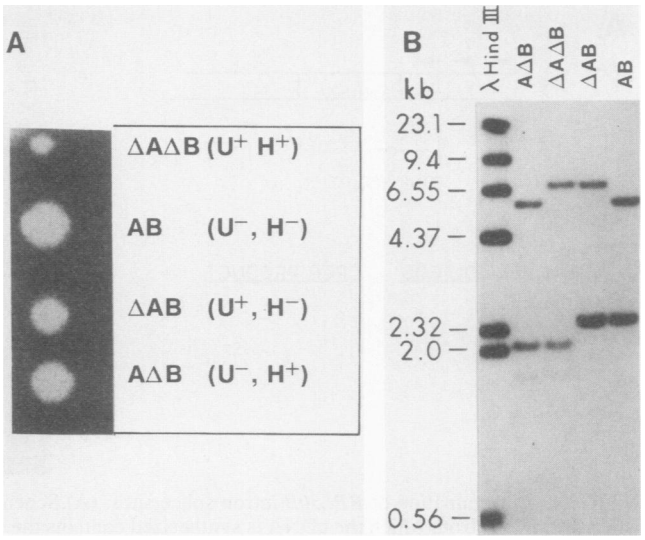

FIG. 7. Illustration of four-spore viability obtained from mating an *RPL30A*-disrupted haploid with an *RPL30B*-disrupted haploid. (A) Photograph of one tetrad with respective phenotypes. U^+ and H^+ indicate autotrophy for uracil and histidine, respectively. (B) Southern blot analysis of genomic yeast DNA from four spores of such a tetrad. DNA was isolated, cut with *Hin*dIII, electrophoresed, and transferred to nitrocellulose as described in Materials and Methods. Hybridization was to two probes simultaneously, *RPL30A* specific and *RPL30B* specific. Bands at 6.0 kb and at 7.1 kb represent the wild-type and disrupted copy of *RPL30A*, respectively. Bands at 2.4 kb and at 2.0 and 1.8 kb represent the wild-type and disrupted copy of *RPL30B*, respectively. Note: The 1.8-kb band displays a faint hybridization signal because of its limited homology with the probe.

Although Fig. 6 suggests that transcription of the intact gene might be compensatory for the disrupted gene, this was not a consistent finding. Indeed, the primer extension experiment of Fig. 8 does not show such compensation.

Haploid strains of genotype *ΔA B* and *A ΔB* were mated, and the resulting diploids were sporulated. Most of the tetrads yielded four viable spores (Fig. 7A). Analysis of the genotypes by using the *URA3* and *HIS3* disruption markers suggested that *RPL30A* and *RPL30B* segregate as unlinked genes. DNA isolated from these spores was characterized by the restriction fragments consistent with the observed Ura and His phenotypes (Fig. 7B). Furthermore, the Ura^+ His^+ spores were viable, suggesting that the cell could grow without any L30.

To determine the relative transcription from each of the L30 genes, primer extension analysis was performed, using a primer complementary to sequences that had been deleted in each gene disruption (Fig. 8). The wild type (lane AB) showed four bands of different intensity. The products of the individual *A* and *B* genes are apparent from the analysis of *A ΔB* and *ΔA B* cells. Similar analysis on RNA from *ΔA ΔB* cells revealed none of the above-mentioned products.

Absence of L30. To demonstrate directly the absence of L30, ribosomes were isolated from cultures grown from four spores of a tetrad derived from a diploid containing one disrupted copy of *RPL30A* and one disrupted copy of *RPL30B*. Ribosomal proteins were purified and subjected to two-dimensional gel electrophoresis (Fig. 9). Ribosomes from cells of genotype *ΔA B* and *A ΔB* appeared to have less than wild-type levels of L30, with the *ΔA B* L30 levels more severely effected. Ribosomes from cells of genotype *ΔA ΔB* had no detectable L30. Such cells grew with a doubling time about 30% longer than that of wild-type cells (Table 1).

Since L30 is a ribosomal protein of the 60S subunit, its absence might be expected to cause a deficiency of 25S rRNA relative to 18S rRNA. Nevertheless, RNA isolated from cells derived from each of the four spores of a tetrad obtained from mating the two disruptions appeared to have the same ratio of 25S to 18S rRNA (Fig. 6C), suggesting that the absence of L30 does not significantly affect the assembly and maturation of the 60S ribosomal subunits. This result was confirmed by sucrose gradient analysis of extracts from the same tetrad, in which the ratios of 60S to 40S subunits were identical (Fig. 10). The polysome profiles were aberrant, however, in cells containing disruptions of either *RPL30A* or *RPL30B* (data not shown) or both genes (Fig. 10), as they revealed stalled translation initiation complexes, also known as halfmers (12). Such stalled translation initiation complexes are thought to be a 43S complex consisting of the 40S ribosomal subunit with attached initiation factors awaiting the addition of the 60S ribosomal subunit. Thus, halfmers represent either a decrease in the quantity of available 60S subunits or defects in the available 60S subunits. The amount of 60S subunits was normal (Fig. 10), suggesting that the 60S subunits are defective in joining to the translation initiation machinery. Polysome profiles also indicated a drastic decrease in the amount of 80S monosomes when both genes encoding L30 were disrupted. Since the monosome peak presumably represents primarily runoff monosomes, or ribosomes that fall off the transcript upon completion of translation, this would imply that L30 may be involved in 60S to 40S ribosomal subunit interactions, either directly or via other factors.

DISCUSSION

A number of criteria confirm that L30 is an authentic ribosomal protein. It is present in roughly stoichiometric amounts within ribosomes (Fig. 9, AB). The transcription of *RPL30A* and *RPL30B* is regulated in parallel with that of other ribosomal protein genes; e.g., transcription is temporarily shut off after a mild heat shock and is responsive to a change in carbon source (reviewed in reference 41) (data not shown). The presence of duplicate genes and of an intron

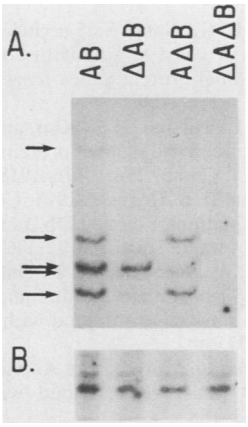

FIG. 8. Primer extension analysis of RNA isolated from four spores of the tetrad described in the legend to Fig. 6. (A) Primer extension performed with a primer specific for both L30-encoding genes. The products are indicated by arrows. (B) In the same reaction was included a primer specific for *RPL32* to control for efficiency of the reaction and concentration of the RNA. Major and minor products are seen as described previously (6).

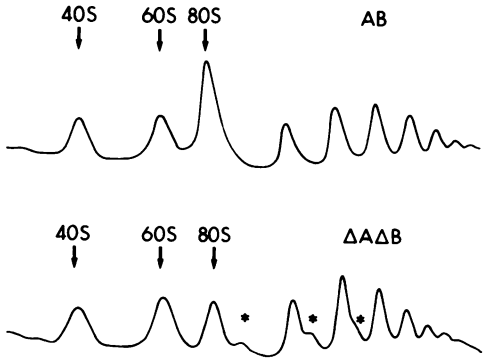

FIG. 10. Polysome profiles of wild-type and L30-deficient strains measured by UV absorption at 260 nm. Sucrose gradient analyses were performed as described in Materials and Methods. Halfmer peaks are starred.

within each is characteristic of genes for ribosomal proteins, yet ribosomal protein L30 is clearly dispensable to the cell.

Nevertheless, L30 is not necessarily innocuous. Cells carrying a different disruption of *RPL30A*, leaving the N-terminal 70 amino acids intact, grow at a severely reduced rate (data not shown). The presence of the truncated L30 perturbs the cell far more than the total absence of L30. It remains to be determined whether the effect of the truncated protein is due to reduced ribosome synthesis or altered ribosome function.

Each of the two genes encoding L30 has been localized to chromosome VII (data not shown). These genes have retained a high degree of homology within their coding regions yet have diverged almost completely on either side. On the other hand, the location of their introns and a short sequence near the end of the introns are highly conserved. Why are the genes for a nonessential protein duplicated? Why is there such a strong selection on the coding region homology when the protein is dispensable? Why have the 3′ splice sites of the two introns been conserved, whereas the noncoding sequences have diverged so rapidly?

The mutants deficient in L30 can be used to study assembly, structure, and function of the eucaryotic ribosome. Such work, pioneered by Dabbs and colleagues for *E. coli* (5), has provided insight into the structure and function of bacterial ribosomes. They have isolated mutants in nearly all of the ribosomal protein genes and have demonstrated that at least 13 of 52 ribosomal proteins of *E. coli* are not essential. A strain missing as many as four ribosomal proteins is viable, albeit barely. Furthermore, several of these dispensable proteins are thought to play important roles in ribosome assembly or function. For instance, L15 and L24 are essential for the in vitro assembly of the *E. coli* large subunit. In

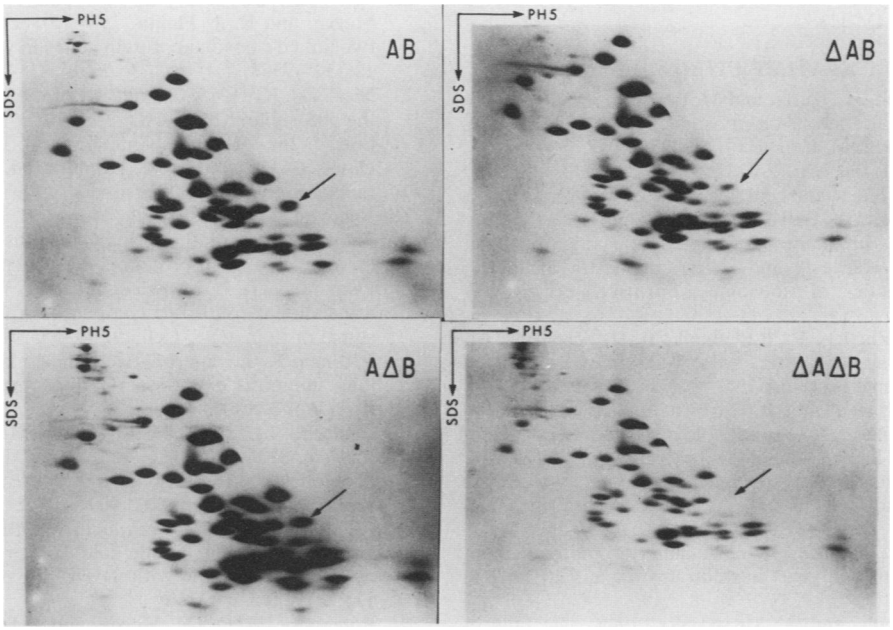

FIG. 9. Two-dimensional gel electrophoresis of ribosomal proteins isolated from four spores of the tetrad described in the legend to Fig. 6. Arrows depict the location of L30 on the gels. See Materials and Methods for experimental procedures.

vivo, mutants lacking L24 are greatly impaired in growth rate and 50S subunit assembly, but mutants lacking L15 are not. *E. coli* mutants deficient in L27, which is located in close proximity to the peptidyltransferase center, grow very poorly. *E. coli* mutants lacking ribosomal proteins have demonstrated that integration of proteins into the ribosome in vivo proceeds differently than during in vitro reconstitution (34); i.e., mutants lacking L11 showed a full complement of each of the other ribosomal proteins despite in vitro studies suggesting that the assembly of L6, L10, L7/L12, and L16 into the 50S subunit requires the presence of L11 (23).

The genetics of *S. cerevisiae* provides a valuable method with which to study the eucaryotic ribosome in the same detail as the studies of *E. coli*. Sachs and Davis (31) have concluded from their studies that a mutation in the gene encoding yeast ribosomal protein L46 suppresses the lethal phenotype caused by disruption of the gene for the poly(A)-binding protein, providing evidence for a link between the 60S subunit and this protein during translation initiation. Our studies indicate that yeast mutants lacking L30 exhibit phenotypes of slow growth, stalling in translation initiation complexes, and delay in rRNA processing associated with the 60S subunit (data not shown). Preliminary results of John Lee (University of Texas, San Antonio) suggest that the absence of L30 alters the protein-protein cross-linking patterns in the yeast 60S ribosomal subunit. It will be interesting to determine whether this structural alteration is involved in 60S to 40S ribosomal subunit interactions or in the binding of 60S-specific translation initiation factors.

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