MOLECULAR AND CELLULAR BIOLOGY, Nov. 1988, p. 4792–4798
0270-7306/88/114792-07$02.00/0

Vol. 8, No. 11

Gene Dosage Alteration of L2 Ribosomal Protein Genes in *Saccharomyces cerevisiae*: Effects on Ribosome Synthesis

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Received 23 May 1988/Accepted 1 August 1988

In *Saccharomyces cerevisiae*, the genes coding for the ribosomal protein L2 are present in two copies per haploid genome. The two copies, which encode proteins differing in only a few amino acids, contribute unequally to the L2 mRNA pool: the L2A copy makes 72% of the mRNA, while the L2B copy makes only 28%. Disruption of the L2B gene (ΔB strain) did not lead to any phenotypic alteration, whereas the inactivation of the L2A copy (ΔA strain) produced a slow-growth phenotype associated with decreased accumulation of 60S subunits and ribosomes. No intergenic compensation occurred at the transcriptional level in the disrupted strains; in fact, ΔA strains contained reduced levels of L2 mRNA, whereas ΔB strains had almost normal levels. The wild-type phenotype was restored in the ΔA strains by transformation with extra copies of the intact L2A or L2B gene. As already shown for other duplicated genes (Kim and Warner, J. Mol. Biol. 165:79–89, 1983; Leeret al., Curr. Genet. 9:273–277, 1985), the difference in expression of the two gene copies could be accounted for via differential transcription activity. Sequence comparison of the rpL2 promoter regions has shown the presence of canonical HOMOL1 boxes which are slightly different in the two genes.

The presence of equimolar amounts of rRNA and ribosomal proteins (rp) in the ribosome is ensured by specific mechanisms which control the coordinated synthesis of all of these components. In eucaryotes, many different mechanisms of regulation have been proposed, including transcriptional and posttranscriptional processes (3, 5, 13, 15–17, 25). In *Saccharomyces cerevisiae* the first level of regulation seems to operate at the transcriptional level: the mRNAs for the various rp are present in roughly equimolar amounts and have very similar half lives (10). Also, changes in the rate of transcription appear to be responsible for the increased or decreased production of ribosomal components observed in response to different environmental conditions (7). Since the rp genes have been shown to be present in single or duplicated form, there should be quantitative differences in the transcription of single- or multiple-copy genes in order to maintain similar levels of mRNA.

In this study, we have analyzed the activity of the genes which code for the rp L2 (rpL2), which is part of the large subunit of the ribosome. We have previously shown that these genes are present in the *S. cerevisiae* genome in two copies (18a), analogous to the situation in *Xenopus laevis*, where the genes coding for the rp L1, which is homologous to the yeast rpL2, are also present in two copies per haploid genome (4).

Genetic manipulation of yeast cells permits the alteration of the gene dosage in haploids and the analysis of the effects of deleting one copy of the gene. By following this approach, it is also possible to understand the functional and evolutionary role of gene duplications. The results of this analysis have shown that both genes are active, although their contributions to the mRNA pool are different. Disruptions of the two gene copies have different phenotypic effects which allow us to examine the regulatory mechanisms controlling the activity of these genes and their role in the complex process of ribosome biosynthesis.

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MATERIALS AND METHODS

Strains and plasmids. The yeast strains used and their characteristics were the following: S150-2B **a** *leu2-3 leu2-112 ura3-52 trp1-289 his3-Δ1*; H3-4 **a**/α *leu2-3 leu2-112/leu2-3 leu2-112 ura3-Δ52/ura3-Δ52*. ΔA and ΔB strains were obtained by transforming S150-2B cells with a *Pvu*II fragment of the L2A gene (from position 70 to 904 with respect to the ATG) containing, in the internal *Stu*I site, a 3-kilobase (kb) *Bgl*II fragment harboring the *LEU2* gene. Diploid strains disrupted in the L2A (H3-4 ΔA) or L2B (H3-4 ΔB) gene were obtained by transforming H3-4 cells with the same linear DNA fragment. Spores from these diploids were obtained by standard genetic techniques. Strains ΔA-L2A and ΔA-L2B have been obtained by transforming the ΔA strain with plasmids yex-L2A and yex-L2B, respectively. These are pEMBLyex4 (6) derivatives containing in the *Bam*HI site the L2A and L2B gene, respectively (18a). As a control, the strain ΔA-yex4 has been derived by transformation of ΔA with plasmid pEMBLyex4 alone.

Yeast media and transformation. Haploid strains were grown at 30°C in YPD medium (21) with the exception of ΔA-L2A, ΔA-L2B, and ΔA-yex4, which were grown in SC medium lacking uracil (21). Diploids were grown in SC medium lacking leucine. Transformations were performed by the method of Sherman et al. (21) with purified plasmid DNA or with linear fragments to generate mutations in the rpL2 genes.

Sequence of the L2B promoter. From plasmid py11, which harbors the L2B gene copy (18a), a 3-kb *Hin*dIII-*Bam*HI fragment was derived which contained the 5′ upstream region of the gene. This fragment was subcloned, and a region of almost 500 nucleotides upstream to the ATG codon was sequenced by the method of Maxam and Gilbert (14).

Southern blot analysis. DNA was extracted from 5-ml cultures (21), and 5 μg was digested with restriction enzymes, run on agarose gels, blotted, and hybridized either to an *X. laevis* probe (pSP-103 [12]), which is homologous to

the 5′ half of the rpL2 coding region (18a), or to a *Leu2* probe.

S1 mapping and Northern analysis. Total RNA was extracted from cultures grown to an OD_{600} of 0.6, and 15 μg was analyzed by S1 mapping (5) or by Northern (RNA) blot (16).

Sucrose gradient analysis of ribosomal components. Cultures (250 ml) were grown to an A_{600} of 0.6, treated with 100 μg of cycloheximide per ml for 10 min, chilled, and harvested. Polysomes were prepared essentially as described by Abovich et al. (1) and fractionated on a 15 to 30% sucrose gradient for 4.5 h at 26,000 rpm in an SW28 rotor.

RESULTS

Disruptions of the rpL2 genes. We have previously shown the presence of two copies of the genes coding for rpL2 in the yeast genome (18a). The easy genetic manipulation of yeast cells offers the possibility of understanding whether both copies are functional and whether they play a different physiological role in the cell. In order to answer these questions, we created artificial mutant strains, disrupting one copy of the rpL2 genes at a time (20). Transformation of yeast cells with linear DNA gives a high frequency of homologous recombination driven by the free ends of the DNA molecule. Figure 1 shows the scheme followed in order to obtain these mutants. A 3-kb fragment carrying the yeast *LEU2* gene was inserted in the unique *Stu*I site of the L2A coding region of plasmid pScrpL2A (18a). The DNA region between the two *Pvu*II sites at positions 70 and 904 from the ATG was isolated and used to transform haploid Leu^- S150-2B *S. cerevisiae* cells. The recombinogenic ends of this fragment have sequence homology with both genes which show very few nucleotide substitutions in the coding region (unpublished data). Leu^+ transformants were selected, and successful disruption of the L2A or L2B gene was determined by Southern blot with enzymes appropriate to analyze the two different disruptions; examples are shown in Fig. 2. Bands A and B (Fig. 2a) correspond to the wild-type copies of the L2A and L2B genes, respectively (lane 1). The DNA was digested with *Eco*RI and *Bam*HI and hybridized with a probe (pSP-103) which recognizes only the 5′ half of the gene up to the *Stu*I site. The insertion of the *LEU* fragment, which contains an *Eco*RI site, inside the L2B copy (ΔB strain), caused the disappearance of band B and the appearance of a faster-migrating band (band C′, lane 2).

The disruption of the L2A copy (ΔA strain) was checked by digestion of the DNA with *Bam*HI. In Fig. 2b, bands A and B correspond to the wild-type gene copies L2A and L2B, respectively, and band C″ corresponds to the disrupted L2A copy. Band C″, which was present only in the ΔA DNA (lane 2), showed an increased size of 3 kb with respect to band A: this was determined by the insertion of the *LEU* fragment, which does not contain *Bam*HI sites. The lower intensity of the B bands with respect to the others was due to the size of this DNA fragment (22 kb). Its migration in lane 2b was altered because of the overloading of the slot. To be sure of the integrity of the L2B copy in this strain, we used other enzymes and confirmed that no exogenous DNA was inserted inside this gene. Both kinds of disruptions were checked by hybridizing duplicated filters with a *LEU* probe. As shown in panels a′ and b′, only the C bands showed specific hybridization to the *LEU* probe other than the endogenous *LEU2* gene (Leu bands).

Interestingly, the frequency of transformants disrupted in the L2B copy was much higher than those disrupted in the L2A copy. Out of 16 transformants analyzed by Southern blot, only 2 appeared to be disrupted in the L2A copy; furthermore, these 2 transformants showed a reduced size of the colonies.

Disruptions performed on diploid strains showed no difference in the frequency of the transformants disrupted in the L2A (H3-4 ΔA) or the L2B (H3-4 ΔB) gene. In Fig. 2c, an example of disruption of one L2A copy in diploid strains (H3-4 ΔA) is shown; besides the A and B bands, the C″ band is also present (lane 2).

Levels of expression of the L2 genes. To compare the expression of the two gene copies, S1 mapping on rpL2 mRNA isolated from wild-type strains was performed. A 2,049-nucleotide-long *Hin*dIII-*Eco*RI fragment, which was derived from the cloned L2A copy of plasmid pScrpL2A (18a) and covers 935 nucleotides of the 3′ coding region, was used as a probe. Two protected bands were detected by using wild-type RNA (Fig. 3a, lane 1). They identified the transcripts originating from the two gene copies, which differ in the 3′ untranslated region (18a). The slower-migrating band (band A) maps 87 nucleotides downstream of the termination codon and identifies the L2A mRNA, whereas the faster-migrating band (band B) maps at the termination codon and identifies the L2B mRNA. By densitometric analysis from multiple experiments, it was calculated that the ratio of intensity of the two bands was 2.5:1, signifying that the L2A gene contributed 72% of the L2 mRNA whereas the L2B copy contributed only 28%. The same probe was used in order to ensure that no complete mRNA for the A or B copy of the L2 gene was produced in those strains in which the corresponding gene was disrupted. Figure 3a shows the S1 mapping on the transcripts derived from these two strains. The band corresponding to the L2A mRNA was absent in the ΔA strain (lane 2) and the L2B band was absent in the ΔB strain (lane 3). The RNA from ΔA and ΔB strains gave an additional band 510 nucleotides long (band C, lanes 2 and 3). This band corresponds to transcripts extending from the 5′ region down to the *Stu*I site, the site used for the gene disruption (see diagram of Fig. 3). This demonstrates that the promoters of the disrupted genes were still active and that their transcripts became fused to *LEU2* sequences.

In order to compare the L2 mRNA levels of the disrupted strains with those of the wild type, Northern analysis was performed. A 15-μg amount of total RNA extracted from wild-type, ΔA, and ΔB strains was run on formaldehyde gels, blotted, and hybridized to the *Hin*dIII-*Stu*I fragment, which covers the 5′ half of the transcripts (see diagram of Fig. 3). In the ΔA strain (Fig. 3b, lane 2), there was a drastic decrease in the overall amount of L2 mRNA (only 25% remaining with respect to wild type), whereas in the ΔB strain (lane 3) there was only a slight reduction. The faster-migrating bands (ΔL2) present in ΔA and ΔB RNAs represent the transcripts originating from the disrupted genes. It is interesting that the abundance of these RNAs was higher in ΔA strains and lower in ΔB strains. This result confirms that the two genes have different activities which correspond to those described by S1 analysis in the wild type.

Phenotypic characterization of the L2 mutants. In order to analyze the effects of the disruptions of the two rpL2 genes, we measured the colony size and the growth rate of the different strains in liquid medium (Table 1). The disruption of the L2B copy (strain ΔB) had no effect on growth rate. In contrast, an unfunctional L2A gene (strain ΔA) resulted in a strong reduction of the growth rate. In fact, while the generation time in wild-type and ΔB strains was 120 to 130

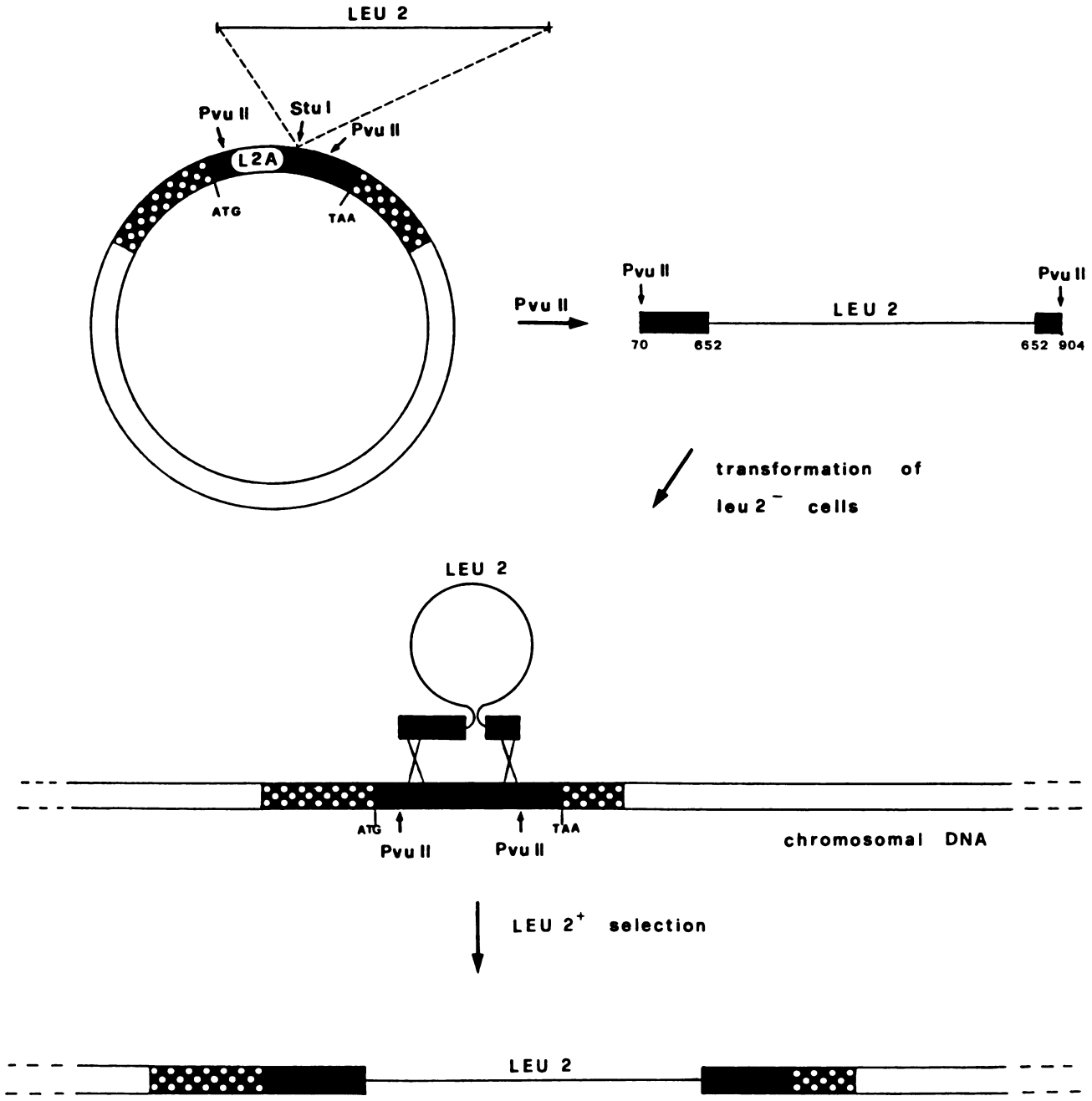

FIG. 1. Schematic representation of the strategy used for disrupting the L2 genes in haploid and diploid cells. Solid line, L2 sequences; stippled areas, L2 flanking sequences; circular open areas, pEMBLEX4 sequences; linear open areas, yeast chromosomal DNA. Numbers indicate the distance from the ATG of the L2 gene.

min, ΔA strain generation time was 230 min. Transformation of the ΔA strain with multicopy plasmids containing the L2A gene (pyex-L2A) or the L2B gene (pyex-L2B) gave rise to cells which had a normal growth rate of 135 min (Table 1).

The L2B gene was able to rescue the mutant phenotype of ΔA, most likely because it compensates for its lower transcriptional activity with the gene dosage. This result supports the idea that the products of the two gene copies are interchangeable and that the defect of the ΔA strain is the limiting amount of L2 mRNA.

Mutational analysis on diploids showed that the disruption of a single L2B copy (H3-4 ΔB) had no effect on the growth phenotype of the cells, whereas the disruption of one of the two L2A copies (H3-4 ΔA) produced a small reduction of the growth rate (175 min, Table 1). One possible explanation is that the diploid cell is able to adapt the rate of transcription to the different gene dosage: in the case of the rpL2 genes, each L2A copy could produce 36% of the mRNA instead of 72% as in haploid cells. It is important to note that the promoter of the ΔA copy was not affected by the disruption and was fully active, although the transcripts were not functional. The disruption of one L2A gene copy could then result in a reduction of rpL2 just below the limits of normal physiological growth.

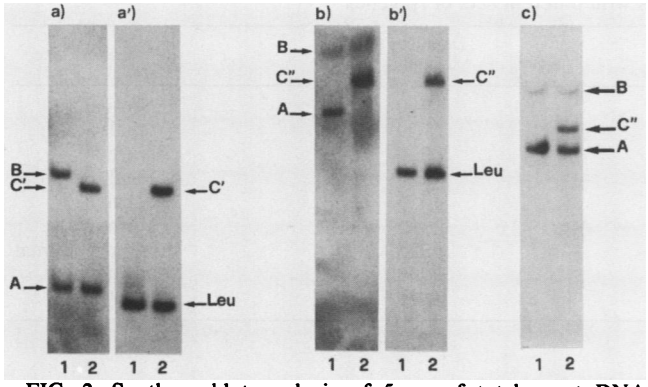

FIG. 2. Southern blot analysis of 5 μg of total yeast DNA hybridized to (a, b, c) the rpL2 probe (see Materials and Methods) or (a' and b') the *LEU2* probe. Panels a and a', S150-2B (lanes 1) and ΔB (lanes 2) DNA digested with *Bam*HI and *Eco*RI. Panels b and b', S150-2B (lanes 1) and ΔA (lanes 2) DNA digested with *Bam*HI. Panel c, H3-4 (lane 1) and H3-4 ΔA (lane 2) DNA digested with *Bam*HI. Band designations are explained in the text.

The observation that, in diploids, the disruption of one L2A copy is not compensated for by the presence of the other intact copy supports the conclusion that the rate of transcription per gene does not change once it has been established, even if the amount of the final products becomes limiting for the correct growth of the cell. Furthermore, no strong posttranscriptional compensation seems to occur.

Spores obtained from these diploids behaved exactly like the corresponding mutants obtained by transformation of the haploid strain, with the growth rate being reduced for disruption of the L2A copy and remaining normal for all the other genotypes.

Comparative analysis of the L2A and L2B gene promoters. The S1 analysis showed that the two rpL2 gene copies contributed differently to the amount of L2 mRNA accumulated in the cell. This feature could be explained by different transcriptional activity of the two genes. In order to analyze this possibility, we sequenced 500 nucleotides of the upstream region of the L2B gene and compared them with the corresponding region of L2A which was determined previously (18a). The presence of two conserved elements, originally designated HOMOL1 and RPG box, together with an A- or T-rich stretch was described in the upstream regions of 21 cloned yeast rp genes (11, 22). Deletion analysis of a

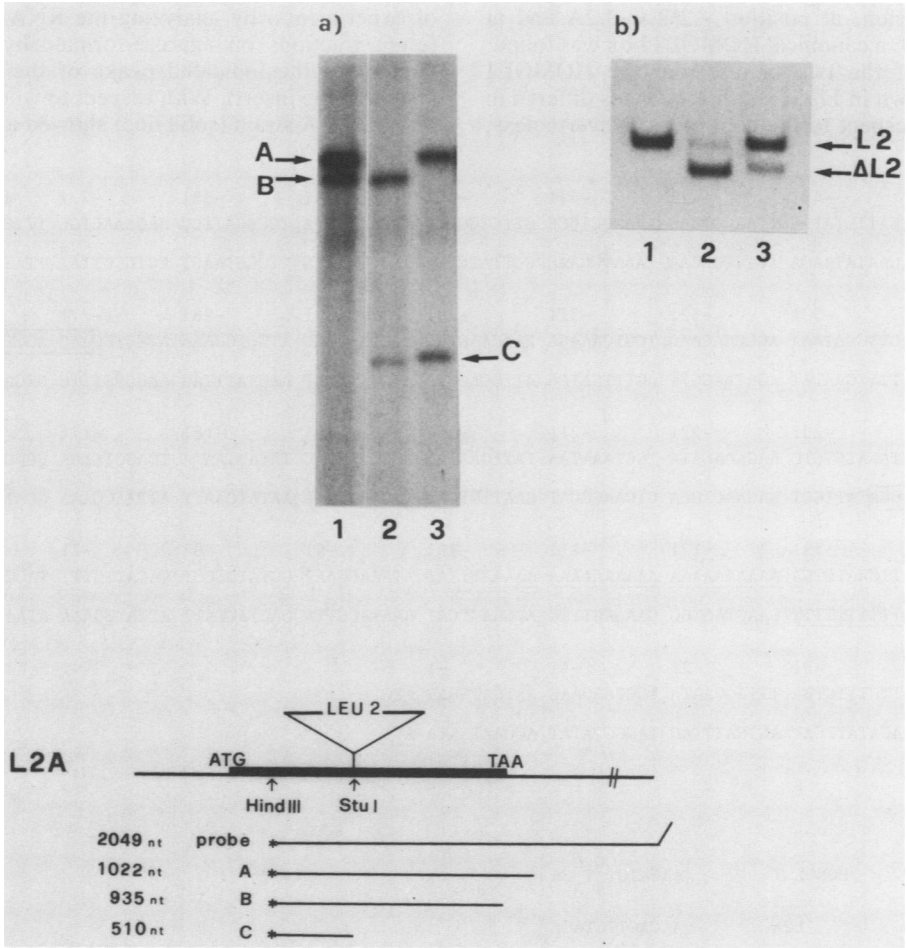

FIG. 3. S1 mapping (a) and Northern analysis (b) of transcripts derived from the L2A and L2B genes. Lanes 1, RNA from S150-2B cells; lanes 2, RNA from strain ΔA; lanes 3, RNA from strain ΔB. In panel a, the arrows indicate the S1-protected fragments corresponding to L2A mRNA (A), L2B mRNA (B), and mRNA deriving from the disrupted genes (C). The lower panel shows a diagrammatic representation of the probe used and the protected fragments obtained. (Asterisks indicate the labeled DNA ends, and arrows indicate the restriction sites used for preparing the probe [*Hin*dIII] and for gene disruption [*Stu*I].) In panel b, the arrows indicate the L2 mRNA (L2) and the mRNA originating from the disrupted gene (ΔL2). nt, Nucleotides.

TABLE 1. Characterization of strains with different sets of rp genes

Strain	Relevant genotype	Doubling time (min)	Colony size
S150-2B	a *RPL2A RPL2B*	120	Normal
ΔA	a *rpL2a::LEU2 RPL2B*	230	Small
ΔB	a *RPL2A rpL2b::LEU2*	130	Normal
ΔA-yex4	a *rpL2a::LEU2 RPL2B* (pyex4)$_n$[a]	220	Small
ΔA-L2A	a *rpL2a::LEU2 RPL2B* (pyex-L2A)$_n$	135	Normal
ΔA-L2B	a *rpL2a::LEU2 RPL2B* (pyex-L2B)$_n$	135	Normal
H3-4 ΔA	a/α *RPL2A/rpL2a::LEU2 RPL2B/RPL2B*	175[b]	Almost normal
H3-4 ΔB	a/α *RPL2A/RPL2A RPL2B/rpL2b::LEU2*	130[b]	Normal
ΔA spore[c]	α *rpL2a::LEU2 RPL2B*	235	Small

[a] *n*, Plasmid copy number (varies from 10 to 15).
[b] Measured in both leucineless and YPD media.
[c] Strain H3-4 ΔA was sporulated, and a spore containing the disrupted L2A gene was analyzed.

number of different yeast rp genes has shown that these elements are implicated in the activation of transcription of the rp genes (19, 26). Footprinting experiments demonstrated that a protein factor called TUF binds to both HOMOL1 and RPG boxes, though with different affinity (9, 23). Figure 4 shows the comparison of the 450 nucleotides upstream of the ATG codon of the two rpL2 genes. A stretch of A's at position −105 was present in the L2A promoter, and a stretch of T's at position −131 was found in the L2B copy. Further upstream, at position −262 in L2A and at position −238 in L2B, a canonical HOMOL1 box was found.

The divergence of the two boxes from the HOMOL1 sequence is also shown in Fig. 4: both sequences differed in two positions with respect to the consensus. Nevertheless, the C in position 5 in L2A is sometimes present, whereas an A or in general a purine has never been found (11, 13).

Accumulation of 40S subunits in L2 mutants. In order to evaluate whether the reduced growth rate of the ΔA strains had any biochemical correlation with the protein synthesis machinery, we examined the polysomal profiles of wild-type and mutant strains. Figure 5 shows the A_{260} profiles of cell extracts fractionated on sucrose gradients; the positions of the 40S and 60S subunits were determined (from double sets of experiments) by analyzing the RNA content of the different fractions on agarose-formaldehyde gels (the RNA content of the indicated peaks of the wild-type strain is shown in the insert). With respect to wild-type cells (broken line), the ΔA strain (solid line) showed a relative increase in

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          -441       -431       -421       -411       -401       -391       -381       -371       -361       -351
L2A  AGCTTGGAAT CATTCTATAG TTAACGTAAG AAAAGCTGCC CTTCTGGCCG AAAAAGACAA TCCCCATTCC AAGAAACTGC TTTCCAACTT ACTTAAGGAA

L2B  ATTAGGGTTG CAATATAAGA ACATTCGCAA TAAAGGCAAC CCTTATCCGG ACCCTACATG TTCCGTAAGT CCTGCCTTAT TTATCGTAAC GGTATTCGTC

          -341       -331       -321       -311       -301       -291       -281       -271       -261       -251
     AAGCAACTAC CTTGCATAAA AGGCGACAAC TCGTCAAAGA TAACAAGTGC TACTAAAAGC ATGCTCCAAA ATGCTCGAAC ACTTGTACAA TCGGAGCATA

     TTACAGGAAT TTTTGAATAT AGATAACGTT AGTTTGATAG ATTGGAACAA ATGGGAAAAT AACAATTTAG AAGCGCTTTC CTCAGCGTAT CTGAATTATA

          -241       -231       -221       -211       -201       -191       -181       -171       -161       -151
     ATATCTTAGA TAGATGTCTC AAGAGACATA TCCTAAATAA TATTGAATAT GCACTTTTAC TATATTAATA TCACGTCACA CGACGCACAG TGAGAAGTGA

     AAACAACTGC GCATATTGCT AAGAACTGCA CTGAAGGCAT GAATTGTCTA TTCCGTTTGA AATATCAATT ATTTACGAAA GCATCGCGCA GACGCAATTT

          -141       -131       -121       -111       -101        -91        -81        -71        -61        -51
     AAAATTTTTT TTCAATCTGA AAAAAAAAAA AAAAAAAAAA AAAAATTTAT ATAAACGAAT GGTATCTCCT CACATTCTTT TAGCCTCGCA ACTTGTACTT

     TTTTAACATT TTTTTTTTTT CAAGTACTCG GAAAAGTTTC ATGAAGTCAT GAATACGTTA CACTACTATT ATTATTGAAA ATAATATTTC TTTCCTATCA

           -41        -31        -21        -11         -1
     TTCATCACTT TTCTTTGTAA TTTAGCAATA TCCCAAGAAC AATCATCGAA ATG

     GCAATCGCTT ACATATTCAT AGTCATTTAC TAGGGTATAT ACCAATAATA ATG
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HOMOL I    AACATC[CG/TA]T[G/A]CA
L2A        AACACTTGTACA
L2B        AACAACTGCGCA
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FIG. 4. Nucleotide sequence of 450 bases upstream from the L2A and L2B genes. The sequences are aligned from the ATG, which has been underlined. Also underlined is the stretch of A's in the L2B gene and the stretch of T's in the L2A. The conserved UASrpg regions have been boxed, and the direct comparison with the HOMOL1 consensus sequence is shown underneath. Dots indicate the nonconserved bases.

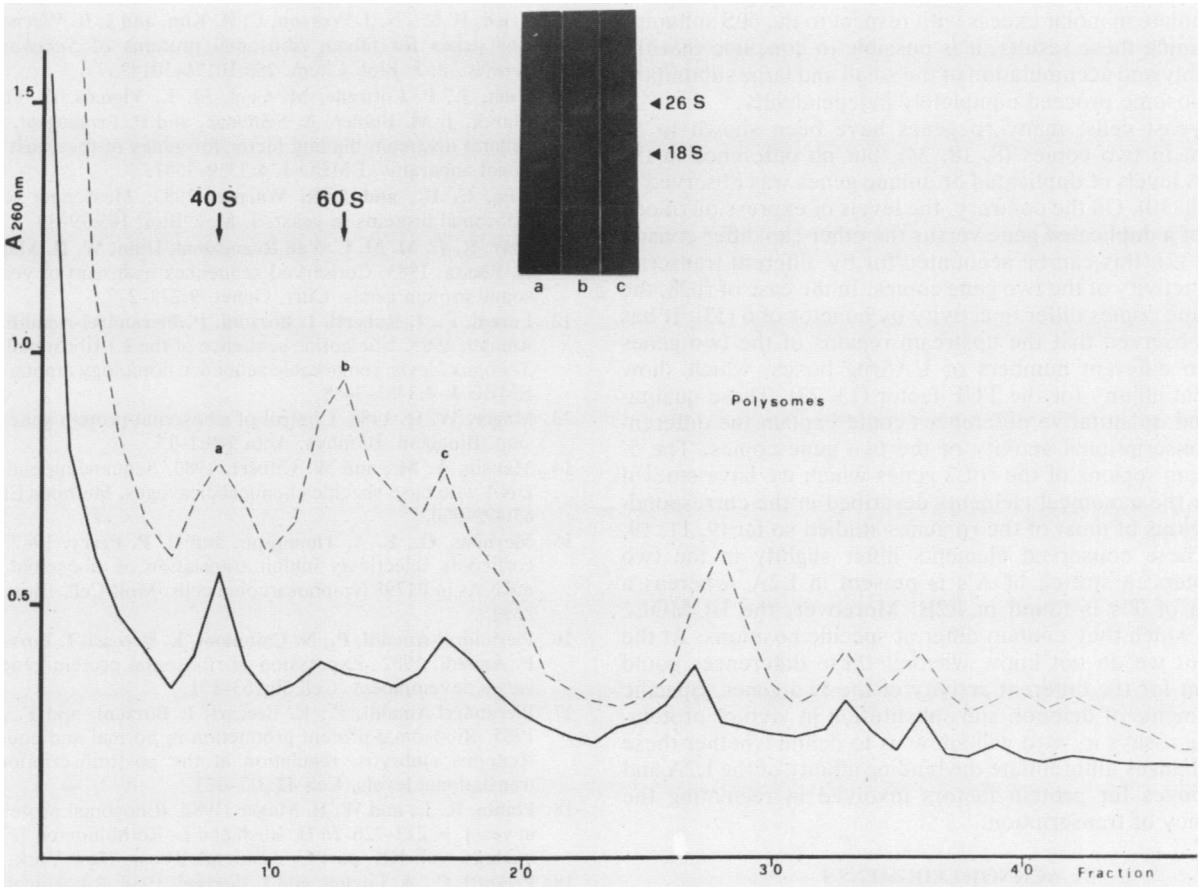

FIG. 5. Polysomal profiles of wild-type S150-2B (– – –) and ΔA (——) strains. The arrows indicate the positions of the 40S and 60S subunits, which were identified indirectly by the presence of either 18S or 26S rRNA. Inset: Gel of RNA extracted from the indicated fractions.

absorbance at the position of the 40S subunits, which paralleled a drastic reduction of the 60S subunits and ribosomes. Furthermore, no visible amount of 26S RNA was found in the 40S peak of ΔA strains, showing that no strong degradation of the 60S into the 40S peak had occurred. From these data, we conclude that the reduced growth rate of ΔA strains is due to insufficient synthesis of the 60S subunits, most likely determined by reduced production of rpL2.

DISCUSSION

The data presented in this study show that the two copies of the rpL2 gene of the yeast *S. cerevisiae* are both functional but contribute unequally to the pool of L2 mRNA. S1 mapping of steady-state RNA has shown that the L2A copy contributes 72% of the total L2 mRNA, and the L2B copy produces the remaining 28%. Gene disruption experiments have shown that strains with an inactivated L2B gene have almost normal levels of L2 mRNA and a normal generation time; in contrast, inactivation of the L2A copy results in a drastic decrease in the L2 mRNA levels of the cell and doubling of the generation time. This feature is determined by the absence of normal amounts of the rpL2 gene products; the wild-type phenotype can be restored by transforming the ΔA strains with multicopy plasmids containing a functional L2A or L2B gene. It can be concluded that in haploids the amount of mRNA produced by a single L2A gene is sufficient to synthesize enough rpL2 to ensure normal growth, whereas the mRNA transcribed from a single active L2B gene produces limiting amounts of rpL2, thus affecting the growth properties of the cell. Furthermore, in consideration of the fact that the L2B gene is also able to restore correct growth properties in ΔA strains, it can be concluded that the proteins encoded by the two gene copies are interchangeable. Sequence analysis has previously shown that the L2B protein has only few amino acid substitutions with respect to the L2A copy (not shown).

An intermediate phenotype was observed in H3-4 ΔA diploids, in which the presence of only one functional L2A gene slightly affected the growth rate of the cell. One possible explanation is that in diploids, in which the rpL2 gene dosage is twice that of haploids, each L2A copy contributes half the amount of the L2A mRNA. In this context, the presence of only one L2A gene can become limiting for normal growth. In conclusion, gene disruption experiments in haploid and diploid cells have shown that no strong intergenic compensation occurs among the rpL2 genes.

The correlation between the levels of rpL2 and the growth conditions of the cell was evidenced by the reduced levels of 60S subunits observed in ΔA strains. Similar results have been obtained previously for other duplicated rp genes: the rp51 gene dosage has an effect both on the accumulation of 40S subunits and on the growth conditions of the cell (2). In that instance, it was shown that in the absence of 40S subunits, the accumulation of 60S particles increased (1). In our experiments, we have observed that the small subunits

accumulate in molar excess with respect to the 60S subunits. Combining these results, it is possible to conclude that the assembly and accumulation of the small and large subunits of the ribosome proceed completely independently.

In yeast cells, many rp genes have been shown to be present in two copies (8, 18, 24), but no difference in the mRNA levels of duplicated or unique genes was observed in the cell (10). On the contrary, the levels of expression of one copy of a duplicated gene versus the other can differ considerably (2); this can be accounted for by different transcriptional activity of the two gene copies. In the case of rp28, the two gene copies differ in activity by a factor of 6 (13). It has been observed that the upstream regions of the two genes contain different numbers of UASrpg boxes, which show different affinity for the TUF factor (13, 23). These qualitative and quantitative differences could explain the differential transcriptional activity of the two gene copies. The 5′ upstream regions of the rpL2 genes which we have studied harbor the canonical elements described in the corresponding regions of most of the rp genes studied so far (9, 11, 19, 22). These conserved elements differ slightly in the two promoters: a stretch of A's is present in L2A, whereas a stretch of T's is found in L2B. Moreover, the HOMOL1 boxes which they contain differ at specific positions. At the moment we do not know whether these differences could account for the different activity of the two genes. Specific experiments of deletion and substitution in vivo or protein-binding assays in vitro will allow us to define whether these base changes differentiate the binding affinity of the L2A and L2B boxes for protein factors involved in regulating the efficiency of transcription.

ACKNOWLEDGMENTS

We thank D. Thiele for helpful suggestions and G. Lucchini for tetrad dissection. We also thank R. M. Menard for revising the manuscript and M. Arceci, G. Pisaneschi, and A. Di Francesco for technical assistance.

This work was supported by grants from Progetto Finalizzato "Ingegneria Genetica e Basi Molecolari delle Malattie Ereditarie" of the Consiglio Nazionale delle Ricerche and from the "Istituto Pasteur Fondazione Cenci-Bolognetti."

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