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Ribosomal Protein L2 in *Saccharomyces cerevisiae* Is Homologous to Ribosomal Protein L1 in *Xenopus laevis*

ISOLATION AND CHARACTERIZATION OF THE GENES*

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By cross-hybridization with a cDNA probe for the *Xenopus laevis* ribosomal protein L1 we have been able to isolate the homologous genes from a *Saccharomyces cerevisiae* genomic library. We have shown that these genes code for a ribosomal protein which was previously named L2. In yeast, like in *X. laevis*, these genes are present in two copies per haploid genome and, unlike the vertebrate counterpart, they do not contain introns.

Amino acid comparison of the *X. laevis* L1 and *S. cerevisiae* L2 proteins has shown the presence of a highly conserved protein domain embedded in very divergent sequences. Although these sequences are very poorly homologous, they confer an overall secondary structure and folding highly conserved in the two species.

We have previously reported the isolation and characterization of cDNA clones for six *Xenopus laevis* r-proteins[1] (1, 2) and of two genomic clones coding for the r-proteins L1 and L14 (3–5). In particular we have focused our attention on the gene for the r-protein L1 which has shown a very interesting post-transcriptional regulation operating at the level of splicing and stability of the precursor RNA (6, 7). Because the L1 seemed to be a highly regulated protein, and in order to know how the regulation of expression had evolved, we decided to isolate and analyze the corresponding gene in the yeast *Saccharomyces cerevisiae*. The comparison of the primary sequence of the same protein from two distantly related species would have offered interesting information on the structural domains of the protein which must be conserved in order to ensure its correct activity.

The gene coding for the protein corresponding to the *X. laevis* L1 is present in yeast, as in *X. laevis*, in two copies. Both are intronless and code for a protein, previously named L2 (8), which is 34 amino acids shorter than the vertebrate counterpart.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) J03195.

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[1] The abbreviations used are: r-proteins, ribosomal proteins; kb, kilobase; SDS, sodium dodecyl sulfate.

MATERIALS AND METHODS

Strains

Yeast—All the experiments were carried out with *S. cerevisiae* strain S150-2B (a,leu2,ura3,trp1,his3) or PT1-7B (a,rna2,ade1,ura1, his7,met).

Bacteria, Phage, and Plasmid—The *S. cerevisiae* genomic library, partial *Sau*3A in EMBL4, and the positive clones were propagated in *Escherichia coli* Q358. Subclones were constructed in plasmid pSP65 (9) and propagated in *E. coli* HB101.

Identification of Recombinant Phages and Subcloning

The library was screened according to the Benton and Davis method (10) utilizing as probe the 1.3-kb *Eco*RI insert of plasmid pSP-103 which corresponds to the full length cDNA coding for the r-protein L1 of *X. laevis* (4). The hybridization was carried out at 65 °C in 0.6 M NaCl, 8 mM EDTA, 120 mM Tris-HCl, pH 8.0, 10 × Denhardt's solution, 20 μg/ml salmon sperm DNA. Washes were performed at 48 °C in 0.1 × SSC. The positive plaques were screened again by hybridization with the same probe. The *Eco*RI fragments of the positive clones which showed hybridization to the L1 probe have been subcloned in pSP65.

DNA Sequence Analysis

DNA sequencing was carried out according to Maxam and Gilbert (11) with the addition of a T-specific reaction (12). Fragments were end-labeled with T4 polynucleotide kinase and strand separated. The chemical reaction products were electrophoresed on urea:acrylamide (30:1) gels, 40 × 20 × 0.03 cm (one 20% and two or three 6%), yielding an average of 200–300 nucleotides of sequence per labeled end.

S1 Mapping and Northern Blot

Appropriate restriction fragments were end-labeled either with T4 polynucleotide kinase (5′ end) or with Klenow polymerase (3′ end). The labeled fragments were coprecipitated with 20 μg of total yeast RNA, annealed in R-loop conditions, and S1-treated as already described (3). For Northern analysis, 20 μg of total yeast RNA was run on formaldehyde/formamide gels and blotted as already described (3).

Hybrid Released Translation

10 μg of pSP13 and pSP65 DNA was bound to a nitrocellulose filter (1) and hybridized to 200 μg of total yeast RNA in 1 ml of 50% formamide, 0.75 M NaCl, 100 mM Tris-HCl, pH 7.5, 10 mM EDTA, 0.5% SDS for 20 h at 37 °C. After hybridization, the filter was washed 30 min at 37 °C in the same hybridization buffer, 1 h in 0.1 × SSC, 0.2% SDS at 52 °C and four times in cold 0.1 × SSC to avoid SDS presence on the filters. These were minced in Eppendorf tubes, and the hybridized RNA was eluted by two sequential washes at 100 °C for 1 min in 10 mM KCl and ethanol-precipitated.

The RNA was translated in a rabbit reticulocyte lysate system (Du Pont-New England Nuclear translation kit) containing [^{35}S]methionine (1000 Ci/mmol). After incubation, samples were extracted with 66% acetic acid and analyzed on a two-dimensional acrylamide gel electrophoresis (13). Purified cold *S. cerevisiae* ribosomal protein markers were mixed with the *in vitro* translation products before electrophoresis.

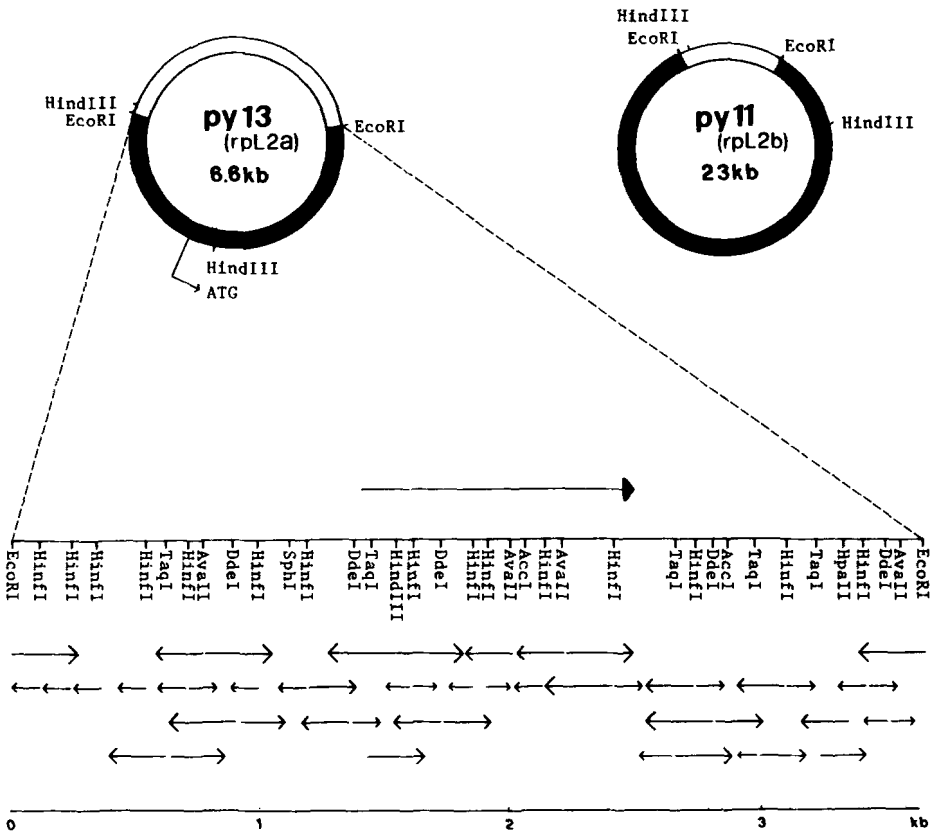

FIG. 1. **Recombinant plasmids containing sequences homologous to the *X. laevis* L1 r-protein gene.** py13 contains a 3.6-kb *Eco*RI fragment (*filled segment*) inserted in the *Eco*RI site of pSP65. py11 contains an *Eco*RI fragment of approximately 20 kb cloned in the same vector. The *arrow* in the circular map of py13 indicates the start and orientation of the coding region. Circular maps are not drawn to scale. The linear map shows a partial restriction map of the 3.6-kb *Eco*RI insert as well as the length and location of the coding region (*arrow*). Restriction sites listed have been used for sequencing or S1 mapping. The sequence strategy is shown *below* the map. *Arrows* show the direction and extent of sequence analysis.

RESULTS

Isolation of the Genes Homologous to the X. laevis r-protein L1—A full length cDNA clone (pSP-103) containing the sequences coding for the L1 r-protein of *X. laevis* (4) has been utilized as a probe for screening a genomic library of the yeast *S. cerevisiae*. Several positive clones have been isolated which fall into two classes by restriction map analysis. The representatives of the two groups, subcloned in pSP65, are diagrammed in Fig. 1.

Southern blot analysis of total *S. cerevisiae* DNA digested with *Eco*RI or *Bam*HI (enzymes which do not cut inside the gene) always showed two bands of hybridization (not shown). From these results we concluded that the genomic sequences homologous to the *X. laevis* r-protein L1 are present, in the genome of the yeast *S. cerevisiae*, in two copies. This is equivalent to the gene copy number of the L1 r-protein described in *X. laevis* (3).

Sequence Determination—For sequence analysis we choose clone py13 (see Fig. 1) in which the whole gene is contained in a 3.6-kb *Eco*RI fragment. Clone py11 is much larger and does not contain appropriate sites for sorting out a fragment containing the whole gene.

The sequence strategy is shown in Fig. 1. The Maxam and Gilbert (11) chemical degradation technique was employed, and almost the whole sequence was determined on both strands; portions of the fragment for which data were available from only one of the two strands were sequenced several times. The nucleotide sequence of almost 2000 nucleotides is shown in Fig. 2. The sequence shows an open reading frame of 1089 nucleotides. The deduced amino acid sequence is indicated underneath and compared with the one of the *X. laevis* r-protein L1a (4). The yeast sequence proves to be 34 amino acids shorter, 29 are missing in the COOH terminus, 2 in the NH_2 terminus, and 3 in internal regions. The comparison between the two sequences shows that from amino acids 56–107 there is a 98% degree of similarity; such a high amino acid conservation in proteins from two species so distantly related suggests that this protein domain plays an important and conserved function.

On the other hand, the rest of the protein shows a degree of similarity of approximately 54%. Therefore, its contribution to the activity of the protein seems not to be dependent on the conservation of the primary amino acid sequence.

Fig. 2*b* shows the comparison of the hydropathic index (obtained with the PEPPLOT software package; see Ref. 14) of *X. laevis* and the corresponding *S. cerevisiae* protein. As the diagram shows, both the conserved regions and the remaining portions which show divergent primary sequences have corresponding patterns. This shows that the nonconserved regions have undergone substantial diversification during evolution while strictly maintaining the secondary and tertiary folding. It can be postulated that while the region of the protein conserved in primary sequence functions by specific side-chain interactions for rRNA or r-protein recognition, the remaining parts contribute with their structure to the protein function.

The gene copy present in py11 has been almost completely sequenced and shows only few amino acid substitutions with respect to the py13 copy. On the contrary, the flanking regions are considerably different (not shown).

In *X. laevis* we showed that the two gene copies differ mainly because of the insertion in one of them (the "b" copy) of five additional amino acids in the 3′ portion. In yeast, this part of the protein is absent and no deletions or insertions differentiate the coding regions of the two copies, as shown by sequencing and S1 mapping (see below).

We do not know what is the role of this carboxyl-terminal difference in *X. laevis* and the meaning of its absence in yeast.

Internal Structure of the Gene—In *X. laevis*, we have shown

a)

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-660  AGGACCTGATTTTATGAAAATCTTAAAGAAAGAAGAAAATATCAAGAAAAAGTCAAGTGGTGGAGCCAAGTCGGTCATGGCTTGTTTTCAGCATGGTATGCTCAAATGCGCCAAGAAGAT
-540  GAAGAAGACGAAGACGGTCAAGCAAAATCAGACAATCTGTCTGACGACATAGAGAGCGAAGAGGAAGAGCAAGAGGAAGAGGGTGATGATAGCTTGCAATCATTCTATAGTTAACGTAAG
-420  AAAAGCTGCCCTTCTGGCCGAAAAAGACAATCCCCATTCCAAGAAACTGCTTTCCAACTTACTTAAGGAAAAGCAACTACCTTGCATAAAAGGCGACAACTCGTCAAAGATAACAAGTGC
-300  TACTAAAAGCATGCTCCAAAATGCTCCAACACTTGTACAATCGGAGCATAATATCTTAGATAGATGTCTCAAGAGACATATCCTAAATAATATTGAATATGCACTTTTACTATATTAATA
-180  TCACGTCACACGACGCACAGTGAGAAGTGAAAAATTTTTTTTCAATCTGAAAAAAAAAAAAAAAAAAAAAAAAAATTTATATAAACGAATGGTATCTCCTCACATTCTTTTAGCCTCGCA
 - 60  ACTTGTACTTTTCATCACTTTTCTTTGTAATTTAGCAATATCCCAAGAACAATCATCGAA ATG TCC CGT CCA CAA GTT ACT GTT CAC TCT TTG ACT GGT GAA GCT
                                                                    Met Ser Arg Pro Gln Val Thr Val His Ser Leu Thr Gly Glu Ala
                                                          Met Ala Cys Ala Arg Pro Leu Ile Ser Val Tyr Ser Glu Lys Gly Glu Ser

  45  ACT GCC AAT GCC TTG CCA TTG CCA GCT GTC TTC TCC GCT CCT ATC CGT CCA GAC ATT GTC CAC ACT CTT TTC ACC TCT GTG AAC AAG AAC
      Thr Ala Asn Ala Leu Pro Leu Pro Ala Val Phe Ser Ala Pro Ile Arg Pro Asp Ile Val His Thr Leu Phe Thr Ser Val Asn Lys Asn
      Ser Gly Lys Asn Val Thr Met Pro Ala Val Phe Arg Ala Pro Ile Arg Pro Asp Ile Val Asn Phe Val His Thr Asn Leu Arg Lys Asn

 135  AAG AGA CAA GCT TAC GCT GTT TCT GAA AAG GCT GGT CAC CAA ACC TCC GCT GAA TCT TGG GGT ACC GGT CGT GCC GTC GCT CGT ATT CCA
      Lys Arg Gln Ala Tyr Ala Val Ser Glu Lys Ala Gly His Glu Thr Ser Ala Glu Ser Trp Gly Thr Gly Arg Ala Val Ala Arg Ile Pro
      Asn Arg Gln Pro Tyr Ala Val Ser Lys Leu Ala Gly His Gln Thr Ser Ala Glu Ser Trp Gly Thr Gly Arg Ala Val Ala Arg Ile Pro

 225  AGA GTT GCT GGT GGT GGT ACC GGT AGA TCC GGT CAA CGT GCC TTC GGT AAC ATG TGT CGT GCT GGT CGT ATG TTT GCT CCA ACT AAG ACC
      Arg Val Gly Gly Gly Gly Thr Gly Arg Ser Gly Gln Gly Ala Phe Gly Asn Met Cys Arg Gly Gly Arg Met Phe Ala Pro Thr Lys Thr
      Arg Val Arg Gly Gly Gly Thr His Arg Ser Gly Gln Gly Ala Phe Gly Asn Met Cys Arg Gly Gly Arg Met Phe Ala Pro Thr Lys Thr

 315  TGG AGA AAG TGG AAC GTT AAG GTT AAC CAC AAC GAA AAG CGA TAC GCC ACT GCT TCT GCT ATT GCT GCT ACT GCT GTT GCC TCT TTG GTC
      Trp Arg Lys Trp Asn Val Lys Val Asn His Asn Glu Lys Arg Tyr Ala Thr Ala Ser Ala Ile Ala Ala Thr Ala Val Ala Ser Leu Val
      Trp Arg Arg Trp His Arg Arg Val Asn Thr Thr Gln Lys Arg Tyr Ala Val Cys Ser Ala Leu Ala Ala Ser Ala Leu Pro Ala Leu Ile

 405  TTG GCC AGA GGT CAC AGA GTC GAA ACG ATT CCA GAA ATC CCA TTG GTT GTC TCC ACT GAC TTG GAC TCT ATT CAA AAG ACC AAG GAA GCT
      Leu Ala Arg Gly His Arg Val Glu Thr Ile Pro Glu Ile Pro Leu Val Val Ser Thr Asp Leu Asp Ser Ile Gln Lys Thr Lys Glu Ala
      Met Ser Lys Gly His Arg Ile Glu Glu Ile Pro Glu Val Pro Leu Val Val Glu Asp Lys Val Glu Ser Tyr Lys Lys Thr Lys Glu Ala

 495  GTT GCT GCT TTG AAG GCT GTT GGT GCT CAC TCC GAC TTG TTG AAG GTC TTG AAG TCC AAG AAA TTG AGA GCC GGT AAG GCT AAG TAC AGA
      Val Ala Ala Leu Lys Ala Val Gly Ala His Ser Asp Leu Leu Lys Val Leu Lys Ser Lys Lys Leu Arg Ala Gly Lys Gly Lys Tyr Arg
      Val Leu Leu Leu Lys Lys Leu Lys Ala Trp Asn Asp Ile Lys Lys Val Tyr Ala Ser Gln Arg Met Arg Ala Gly Lys Gly Lys Met Arg

 585  AAC AGA AGA TGG ACT CAA AGA AGA GGT CCA TTA GTT GTC TAC GCT GAA GAC AAC GGT ATC GTC AAG GCC TTG AGA AAC GTT CCA AGT GTT
      Asn Arg Arg Trp Thr Gln Arg Arg Gly Pro Leu Val Val Tyr Ala Glu Asp Asn Gly Ile Val Lys Ala Leu Arg Asn Val Pro Ser Val
      Asn Arg Arg Arg Ile Gln Arg Arg Gly Pro Cys Val Ile Tyr Asn Glu Asn Asn Gly Leu Val Lys Ala Phe Arg Asn Ile Pro Gly Ile

 675  GAA ACT GCC AAC GTT GCT TCT TTG AAC TTG TTG CAA TTG GCT CCA AGT GCT CAC TTG GGT AGA TTC GTT ATC TGG ACC GAA GCT GCT TTC
      Glu Thr Ala Asn Val Ala Ser Leu Asn Leu Leu Gln Leu Ala Pro Ser Ala His Leu Gly Arg Phe Val Ile Trp Thr Glu Ala Ala Phe
      Thr Leu Leu Asn Val Ser Lys Leu Asn Leu Leu Arg Leu Ala Pro Gly Gly His Val Gly Arg Phe Cys Ile Trp Thr Glu Ser Ala Phe

 765  ACC AAG TTG GAC CAA GTC TGG GGT TCC GAA ACC GTT --- GCC TCC TCC AAG GTC GGC TAC ACT TTG CCA TCC CAT ATC ATC TCC ACT TCT
      Thr Lys Leu Asp Gln Val Trp Gly Ser Glu Thr Val --- Ala Ser Ser Lys Val Gly Tyr Thr Leu Pro Ser His Ile Ile Ser Thr Ser
      Arg Lys Leu Asp Asp Leu Tyr Gly Thr Trp Arg Ser Lys Ala Lys Leu Lys Ala Asp Tyr Asn Leu Pro Met His Lys Met Thr Asn Thr

 852  GAT GTC ACC AGA ATT ATC AAC TCT TCC GAA ATC CAA TCT GCT ATC AGA CCA GCT GGC CAA GCT ACT CAA AAG CGT ACT CAC GTT TTG AAG
      Asp Val Thr Arg Ile Ile Asn Ser Ser Glu Ile Gln Ser Ala Ile Arg Pro Ala Gly Gln Ala Thr Gln Lys Arg Thr Lys Val Leu Lys
      Asp Leu Thr Arg Ile Leu Lys Ser Gln Glu Ile Gln Arg Ala Leu Arg Ala Pro Asn Lys Lys Val Lys Arg Arg Glu Leu Lys --- ---

 942  AAG AAC CCA TTG AAG AAC AAG CAA GTC TTG TTG AGA TTG AAC CCT TAC GCC AAG GTC TTT GCT GCT GAA AAG CTA GGT TCC AAG AAG GCT
      Lys Asn Pro Leu Lys Asn Lys Gln Val Leu Leu Arg Leu Asn Pro Tyr Ala Lys Val Phe Ala Ala Glu Lys Leu Gly Ser Lys Lys Ala
      Lys Asn Pro Leu Lys Asn Leu Arg Ile Met Met Arg Leu Asn Pro Tyr Ala Lys Thr Ala Arg Arg Lys Ala Ile Leu Gln Gln Leu Glu

1032  GAA AAG ACT GGT ACC AAG CCA GCT GCT GTT TTC ACC GAA ACT TTG AAA CAC GAT TAA ACTTATTAGTACATTAAAAATATTCTTTTATTTTTTATATACA
      Glu Lys Thr Gly Thr Lys Pro Ala Ala Val Phe Thr Glu Thr Leu Lys His Asp
      Asn Ile Lys Ala Lys Glu Lys Lys Pro Asp Asp Gly Lys Pro Lys Ala Lys Lys Pro Leu Asp Ala Lys Thr Lys Met Ile Lys Leu Ala

1132  TACATAAGCCTTTAATACCTTTAAAGCGGTAACGACTGCCTCTTGAACTTAAAATTATTCTTTTTTTTGGATATTTGTTCGTTATGTTTAATGTATATATGTTGATGTTCGATCAGTTT
                                                  ▲
      Lys Ala Lys Lys Arg Gln Ala Arg Glu Ala Ala Lys Ala Ala Glu Thr Lys

1252  TACGATATGCTCTTATAGGAACATTTACAGTTTCCCCGCATTGAGTCCAGACCGAAGGTTACATACAAGATTTCTTCAGCGTTTGTTACTTCAGGAAGGCAATGGTAATCATACGTAT

1372  CACATTGAGCAACCTACTGAGGGAGAAAGGAGATTTGTGCTTGGAACTACTTCGTGTAGACAAAGGAATATACCCCTTTGATACTTCTCAACCAATTGGTAGTATGCAGGTGCTGCTCC

1492  CCCACGCTAGTAATTACCCCGGTTCCGCTCAATTTTATCTTTTTGACGCGGCTAGAAACTCGGTTTCGAGTAGTCCGTTAACTTAAACTACAGTGAAAATCATTGGTTAAACAACACTC
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b)

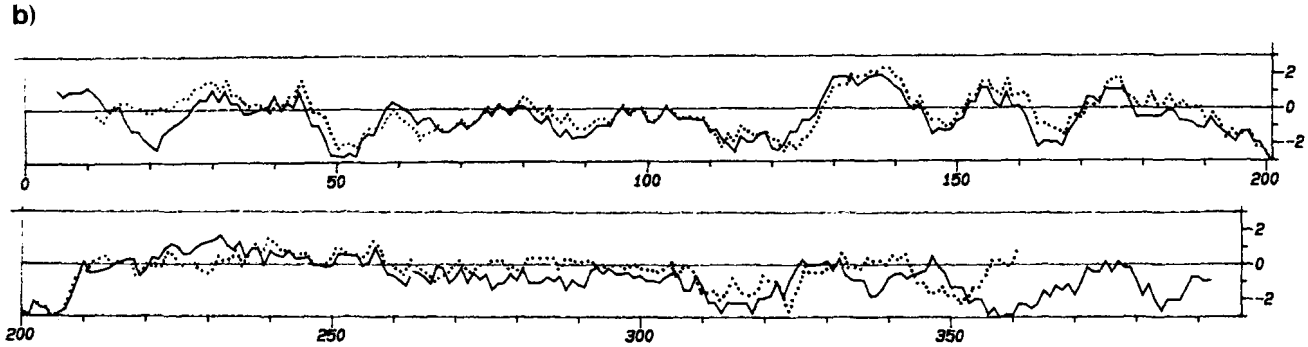

FIG. 2. **Nucleotide and amino acid sequence analysis.** *Panel a*, the sequence of 2232 nucleotides of py13 extending from 660 base pairs upstream to the ATG to 363 nucleotides downstream to the termination codon are

that the gene coding for the L1 r-protein contains nine introns (4). From the sequence of the corresponding gene in *S. cerevisiae*, no introns seem to interrupt the coding region. It is known that many r-protein genes in *S. cerevisiae* contain one intron very often located in the 5′ region right after the ATG (15) and that in other eukaryotes it can also occur in the 5′-nontranslated region (16).

To rule out the possibility of an intron in the 5′-nontranslated regions of the two gene copies we utilized two different approaches: S1 mapping and analysis of rna2 mutants. For S1 mapping, a 430-nucleotide-long *Hin*dIII-*Sph*I fragment extending from position −287 to position +143 with respect to the ATG has been utilized (see Fig. 3). This fragment was 5′ end-labeled and annealed to total yeast RNA. Fig. 3*a*, *lane 2*, shows that after S1 treatment several bands of S1 protection are obtained: three major sites are localized at −31, −25, and −23 from the ATG and two minor sites at −16 and −15 (see Fig. 2). No GT or TACTAAC sequences, diagnostic for the presence of an intron, have been localized in proximity of this site; whereas the canonical box Homo11 described in the promoters of yeast r-protein genes (17, 18) has been found at position −273 with respect to the ATG as well as the adenosine stretch which is present at position −132. These results suggest that the signals found by S1 mapping represent multiple transcription initiation sites, which is quite a common feature of many eukaryotic "housekeeping" genes (19).

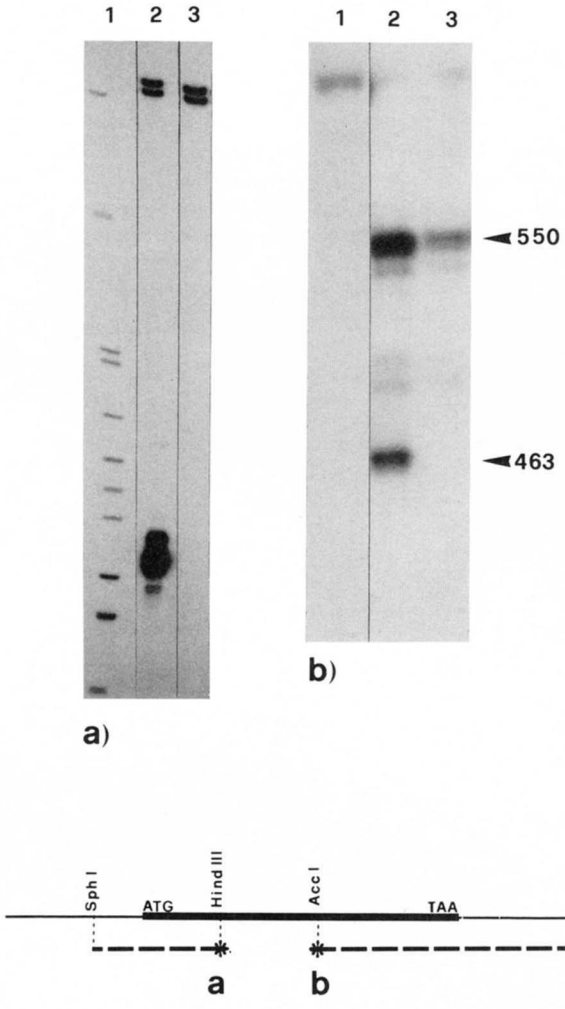

FIG. 3. **S1 nuclease mapping of the 5′ and 3′ ends of transcripts of the gene.** *Panel a*, *lane 1*, *Msp*I-pBR322 DNA; *lane 2*, probe (*a*) annealed with 20 μg of total yeast S150-2B RNA; *lane 3*, nonannealed probe (*a*). *Panel b*, *lane 1*, nonannealed probe (*b*); *lane 2*, probe (*b*) annealed with 20 μg of total yeast S150-2B RNA; *lane 3*, probe (*b*) annealed with 20 μg of total yeast Δ11 RNA. The *arrowheads* indicate the size in nucleotides of the corresponding bands. *Below* is a schematic diagram of the gene regions recognized by probes *a* and *b*.

FIG. 4. **Northern blot analysis of RNA from wild type and rna2 mutants.** PT1-7B cells were grown at 23 °C and RNA prepared either directly (*lane 1*) or after the cells had been incubated at 36 °C for 45 min (*lane 2*). RNA was run on formaldehyde-agarose gels blotted and hybridized to the *X. laevis* cDNA probe for protein L1 (pSP-103).

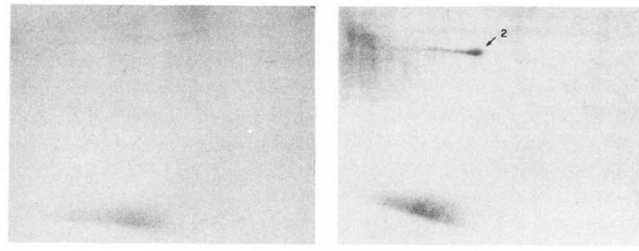

FIG. 5. **Hybrid-released translation.** Autoradiogram of two-dimensional gel electrophoresis of translation products of mRNA hybridized to pSP65 DNA (*a*) or to py13 DNA (*b*). The large spot present in *a* and *b* is globin from residual endogenous synthesis of the reticulocyte system. The other product present in *b* and indicated by *2* co-migrates with the cold r-protein L2.

shown. The ATG and TAA are *boxed*. The 5′ and 3′ termini of the transcribed region are shown by *arrowheads*. The HomoII and the adenosine stretch are *underlined*. The deduced amino acid sequence is shown *below*, together with the corresponding amino acid sequence of the *X. laevis* r-protein L1. The conserved amino acids are indicated. *Panel b*, comparison of the hydropathic indexes for the two amino acid sequences, *X. laevis* (*continuous line*), *S. cerevisiae*, (*dotted line*).

The rna2 mutation of yeast affects the processing of precursor RNAs (20, 21). After shift to the nonpermissive temperature, the splicing of intron containing transcripts is drastically affected: very little mature RNA is detected, whereas the accumulation of unprocessed precursor increases. This has been used as a system in yeast for screening intron containing genes (22). Fig. 4 shows such an analysis performed for our gene: total RNA from rna2 yeast cells grown at 23 °C (*lane 1*) and shifted for 1 h at 36 °C (*lane 2*) has been analyzed by Northern blot. It is clear that after the temperature shift there is a substantial decrease in the amount of mature RNA, but no accumulation of any larger RNA molecule is detected. This result together with the S1 mapping enable us to state that in yeast the genes coding for a protein corresponding to the *X. laevis* L1 do not contain introns.

S1 mapping was also used to map precisely the 3′ end of the transcripts derived from these genes. The probe, 3′ end-labeled, is a 802-nucleotide-long *Acc*I fragment which covers 463 nucleotides upstream to the termination codon and 339 nucleotides downstream to it (see Fig. 3).

Fig. 3*b*, *lane 2*, shows two main bands of protection: the longer band maps 78 bases downstream to the termination codon and localizes the 3′ end of the transcripts derived from the gene copy present in py13, whereas the shorter band maps precisely at the stop codon. This band is due to transcripts derived from the gene copy present in py11. In fact it is absent in S1 experiments performed with RNA derived from a yeast strain (Δ11) in which this gene has been disrupted (Fig. 3*b*, *lane 3*). Therefore the two gene copies differ in their 3′-untranslated regions.

Identification of the Protein Encoded by Clone py13—We performed experiments of "hybrid-released translation" to verify whether the genomic sequences of yeast which are homologous to the *X. laevis* L1 gene actually code for a ribosomal protein and, if so, for which one. RNA specifically hybridized to clone py13 was translated *in vitro* using a reticulocyte cell-free system in the presence of [^{35}S]methionine. To be able to identify the synthesized products, cold yeast r-protein markers were added and the sample analyzed on two-dimensional gel electrophoresis according to Gorenstein and Warner (13). Fig. 5*b* shows that, besides the reticulocyte endogenous globin synthesis, a specific product is obtained which co-migrates with the r-protein marker L2 according to the nomenclature of Kruiswijk and Planta (23) or 2 according to Gorenstein and Warner (13) and YL2 according to Otaka and Osawa (24). It seems that this protein, in the different gel systems used, always results as the r-protein number 2 of the large subunit. Hybrid-released translation on pSP65 DNA shows that no translation products are synthesized beside globin (Fig. 5*a*).

The plasmids containing the two different gene copies have been named pScrpL2a (py13) and pScrpL2b (py11).

DISCUSSION

In *X. laevis*, the expression of the gene coding for the r-protein L1 is under tight control. Increasing the gene dosage, in the oocyte system, no increased production of the corresponding protein is observed, while a specific control operating at the level of splicing and stability of the precursor RNA regulates the amount of mature RNA (6, 7).

The evolutionary study of a highly regulated protein such as *X. laevis* L1 is particularly interesting since an analysis of its homologues in distantly related species provides us with the opportunity of finding out how protein structure and function have evolved and of detecting the way gene expression control mechanisms change in organisms with different gene structure, growth rate, and cell cycle regulation. For these purposes, an attractive system is the simple eukaryote *S. cerevisiae*.

We isolated and characterized the genes which in yeast code for the r-protein homologous to the *X. laevis* L1. This protein, according to the previous nomenclature, is called L2.

The comparison between the *X. laevis* L1 and *S. cerevisiae* L2 r-proteins has revealed the presence of a protein domain, highly conserved in the two species, which could play a very important and evolutionary conserved role. The other regions of the proteins which show a much lower degree of conservation maintain structural similarities suggesting that they contribute to the function of the protein with their overall secondary structure and folding. The easy genetic manipulation of yeast will allow us to approach the problem of whether these domains from yeast and frog could complement each other.

The absence of introns in the *S. cerevisiae* L2 genes is quite peculiar in consideration of the important role that these sequences play in the expression of the corresponding *X. laevis* genes. It will be interesting to relate the structure of the *S. cerevisiae* L2 genes with the regulation of their expression.

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