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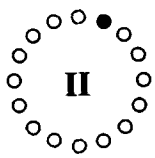

Yeast Sequencing Reports

Nucleotide Sequence and Characterization of the *Saccharomyces cerevisiae RPL19A* Gene Encoding a Homolog of the Mammalian Ribosomal Protein L19

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A gene designated *RPL19A* has been identified in the region downstream from the 3′-end of the *Saccharomyces cerevisiae MIS1* gene encoding the mitochondrial C_1-tetrahydrofolate synthase. The gene codes for the yeast ribosomal protein YL19 which exhibits 57·5% identity with the mammalian ribosomal protein L19. *RPL19A* is one of two functional copies of the YL19 gene located on chromosome II. The disruption of *RPL19A* has no effect on the growth of the yeast. The *RPL19A* gene contains an intron located near the 5′-end. The 5′-flanking region contains one similar and one complete UAS_{rpg} upstream activating sequence. *RPL19A* was also found to be adjacent to the chromosome II *AAC3* gene, encoding the mitochondrial ADP/ATP carrier protein. The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL data bank with the accession number Z36751.

KEY WORDS — *Saccharomyces cerevisiae*; chromosome II; ribosomal protein; intron; yeast

INTRODUCTION

In our attempts to investigate the genes coding for proteins involved in folic acid and one-carbon metabolism, we have found that an amino acid sequence deduced from a nucleotide sequence in the region downstream from the *Saccharomyces cerevisiae MIS1* gene encoding the mitochondrial C_1-tetrahydrofolate synthase (Shannon and Rabinowitz, 1988) shows significant identity with the carboxyl-terminal portion of rat ribosomal protein L19 (Chan *et al.*, 1987). Accordingly, the nucleotide sequence was extended downstream from *MIS1* in order to obtain an open reading frame (ORF) and its regulatory sites. The peptide sequence predicted from this ORF shows significant identity with the ribosomal protein L19 family from human, rat and mouse cells (Kumabe *et al.*, 1992; Chan *et al.*, 1987; Nakamura *et al.*, 1990), *Dictyostelium discoideum* (Singleton *et al.*, 1989) and *Halobacterium marismortui* (Scholzen and Arndt, 1991), and with a predicted L19 peptide from *Methanococcus vannielii* (Auer *et al.*, 1989). Its amino-terminal peptide sequence matches the yeast YL19 (also referred to as L23, YL14, RP33 or RP15L) amino-terminus previously determined by direct amino-terminal sequence analysis (Otaka *et al.*, 1983; Takakura *et al.*, 1992). This gene, designated as *RPL19A*, is one of the two functional copies of the YL19 gene and occurs relative to several other genes, *TEC1* (Laloux *et al.*, 1990), *MIS1* (Shannon and Rabinowitz, 1988) and *AAC3* (Kolarov *et al.*, 1990), on the right arm of chromosome II. The order, proximal to distal, is *TEC1-MIS1-RPL19A-AAC3*.

Ribosomal proteins assemble cooperately with ribosomal RNAs to form a ribosome which then carries out translation. Synthesis of ribosomal protein is mainly regulated at the level of transcription

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Table 1. *S. cerevisiae* strains.

Strain	Genotype
GT48	α *ade3-130 ser1-172 ura3-52*
JS87-7A	**a** *ade3-130 his3*-Δ trp1-Δ ura3-52
JS-88	**a**/α *ade3-130/ade3-130 HIS3/his3*-Δ *TRP1/ trp1*-Δ *SER1/ser1-172 ura3-52/ura3-52*
S-36	**a**/α *ade3-130/ade3-130 HIS3/his3*-Δ *TRP1/ trp1*-Δ *SER1/ser1-172 ura3-52 RPL19A/ rpl19a::URA3*
S36-1A	**a** *rpl19a::URA3 ade3-130 ura3-52*
S37	α *ade3-130 mis1-5 ser1-172 ura 3-52*

of their genes. The genes for many ribosomal proteins of *S. cerevisiae* have been cloned and sequenced (Warner, 1989; Woodford and Warner, 1991). Unlike most other yeast genes, more than half of the cloned ribosomal protein genes are duplicated. In each case tested, both copies are expressed, but usually not at the same level. Many ribosomal protein genes contain an intron, usually near the initiation codon, and have two adjacent copies of an upstream activating sequence designated UAS_{rpg}. A general transcriptional factor Rap1p binds to the UAS_{rpg} and activates the transcription of most of the genes containing UAS_{rpg} (Woodford and Warner, 1991).

While this work was in progress, Suzuki and Wool (1993) reported that the existence of an amino acid sequence deduced from the nucleotide sequence downstream from *MIS1* has significant identity with the carboxyl-terminal portion of the rat ribosomal protein L19. In this paper, we present the entire sequence of the *RPL19A* gene that encodes ribosomal protein YL19 and its many features that are characteristic of yeast ribosomal protein genes.

The function of the ribosomal protein L19 is not known, although there is a suggestion that rat L19 is associated in the 60S subunit with the small rRNAs to participate in the binding of aminoacyl-tRNA to ribosomes (Chan *et al.*, 1987). The availability of *RPL19A* may provide more opportunity to investigate the functional role of the ribosomal protein L19.

MATERIALS AND METHODS

Strains, media and genetic procedures

The *S. cerevisiae* strains used are listed in Table 1. Standard yeast media and growth conditions were used in this study (Sherman *et al.*, 1986). Standard procedures for mating, sporulation and tetrad analysis were used to construct appropriate strains and to analyze gene segregations (Sherman and Lawrence, 1974). *Escherichia coli* DH5α was used to propagate plasmids.

Plasmids

Plasmids YEpKS6 and YEpKS17 (Shannon and Rabinowitz, 1988) are derivatives of episomal plasmid YEp24 and both carry the *RPL19A* gene. Plasmid pKS31 (Shannon and Rabinowitz, 1988) is a derivative of pUC4K and carries the *RPL19A* gene. Plasmid pJS68 was constructed by replacing the 2.38-kb *Xba*I-*Kpn*I DNA fragment of pKS31 with the 1.11-kb *Hin*dIII-*Sma*I fragment containing the *URA3* gene to create the *rpl19a::URA3* disruption gene (Figure 1).

Preparation of nucleic acids and transformation of cells

Yeast DNA was isolated as previously described (Sherman *et al.*, 1986). Total yeast RNAs were isolated as described (Collart and Oliviero, 1993). Plasmid DNA from *E. coli* was isolated as described (Crouse *et al.*, 1983). Yeast transformation was performed by treatment with lithium acetate as described (Ito *et al.*, 1983). *E. coli* transformation was performed as recommended by the supplier of frozen competent DH5α cells purchased from GIBCO BRL.

Southern and Northern blot analysis

DNA digested with restriction enzymes or yeast total RNA was fractionated on agarose gels as described previously (Collart and Oliviero, 1993), transferred to Nytran and hybridized with a DNA probe (Schleicher & Schull Manual). The DNA probe was prepared with a DNA labeling kit (Pharmacia Biotech Inc.) with ^{32}P-labeled dCTP and purified on a Sephadex G-25 spin column (Boehringer Mannheim Biochemicals). The chromosomal location was determined by hybridization to a chromosome blot (kindly provided by R. Hyman and K. Song).

DNA sequencing

The DNA from plasmids YEpKS6 and YEpKS17 was used for the *RPL19A* sequencing. Double-stranded DNA sequencing utilizing chain termination was performed by using the Sequenase

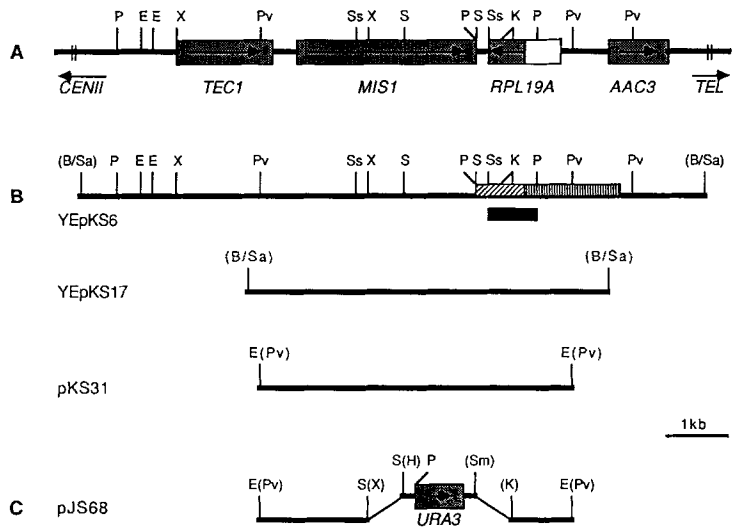

Figure 1. Organization of the *RPL19A* locus. (A) *S. cerevisiae RPL19A* locus. Some restriction sites are shown. Stippled boxes with arrowheads represent the ORFs with arrows showing the direction of transcription. The *RPL19A* gene is interrupted by an intron (open box). Arrowheads indicate localization of the *RPL19A* gene on the right arm of chromosome II. (B) Restriction maps of plasmids carrying the *RPL19A* gene. The nucleotide sequence determined in this study is indicated as the vertically hatched bar and the redetermined sequence is indicated as the hatched bar. The black bar below the line indicates the region used as a probe for Southern and Northern blot analysis. (C) Representation of the disruption of *RPL19A* by replacing the 2·38-kb *Xba*I-*Kpn*I fragment containing the *RPL19A* gene with the 1·11-kb *Hin*dIII-*Sma*I fragment containing the *URA3* gene. Restriction sites: B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; K, *Kpn*I; P, *Pst*I; Pv, *Pvu*II; S, *Sal*I; Sa, *Sau*3AI; Sm, *Sma*I; Ss, *Sst*I; X, *Xba*I. The eliminated sites are shown in parentheses.

Version 2·0 DNA sequencing kit (U. S. Biochemical Corp.) with synthetic oligonucleotides as primers.

RESULTS AND DISCUSSION

Identification of RPL19A

A 460-nucleotide sequence downstream of *MIS1* has been reported (Shannon and Rabinowitz, 1988) and is stored in the GenBank/EMBL data bank under accession number J03724. Computer analysis of an amino acid sequence deduced from this sequence showed 77 identities in 144 residues of the carboxyl-terminal portion of rat ribosomal protein L19. In order to identify an ORF encoding for the predicted ribosomal protein L19, the nucleotide sequence was extended 1597 nucleotides downstream from the reported sequences of *MIS1* in the insert of plasmids YEpKS6 and YEpKS17 (Figure 1). The search for an ORF found a shift in an ORF encoding a predicted peptide which shows 57.5% identity with the mammalian ribosomal protein L19. Accordingly, the 702-nucleotide sequence downstream from the *Sal*I site at the 3′-end of *MIS1* was redetermined on both strands. The omission of one nucleotide on the 43rd codon was confirmed to be the cause of the frameshift. In addition, two nucleotides on the 53rd codon was corrected from AGC to GCC, but had no effect on the homology with rat L19.

The nucleotide sequence of *RPL19A* (Figure 2) has many features characteristic of yeast ribosomal protein genes. A 567-bp ORF is interrupted with a predicted 506-bp intron near its 5′-end. The intron contains a 5′ splice site (GTATGT) and a similar lariat site (AACTAAC). The 5′-flanking sequence contains one similar and one complete UAS_{rpg} upstream activating sequence at positions −404 and −420, respectively. There is a TATA-like sequence at position −91 and a T-rich region from −366 to −386. The 3′-non-translated region contains a polyadenylation signal AATAAA

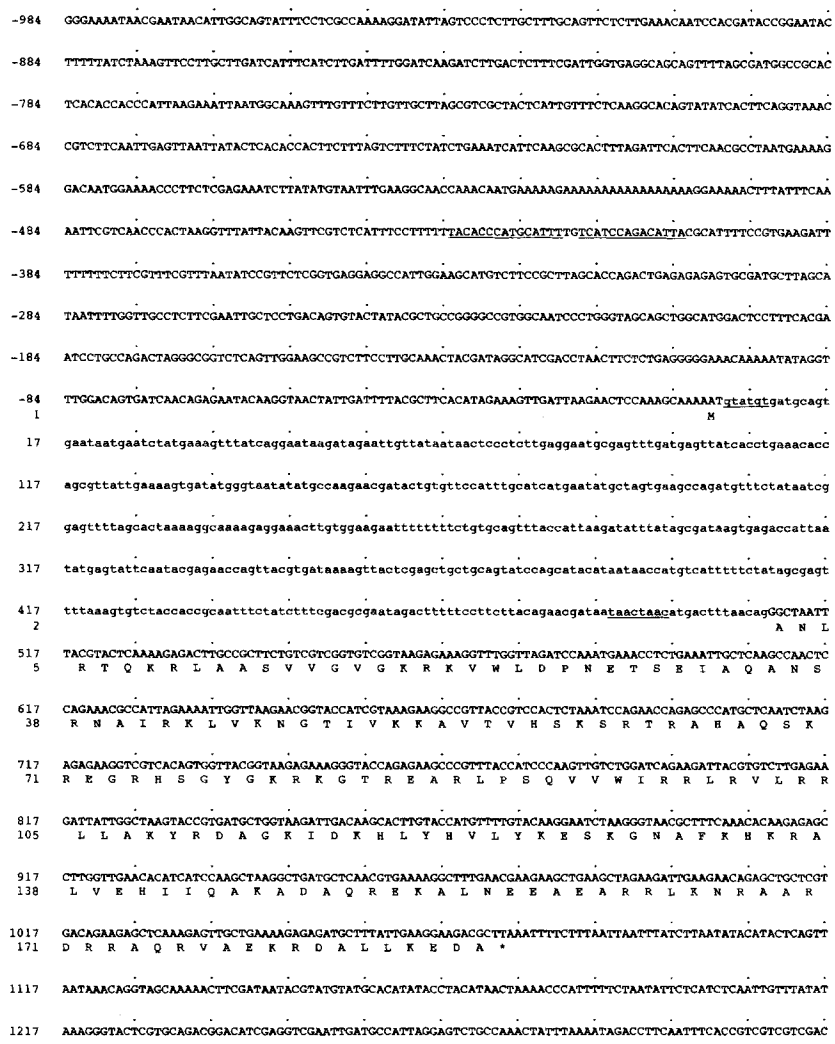

Figure 2. Nucleotide sequence and predicted amino acid sequence of the *RPL19A* gene. Numbers on the left refer to nucleotide and amino acid positions. The intron is indicated in lower case. The consensus sequence of the intron as well as the UAS_{rpg} upstream activating sequences at positions −404 and −420 are underlined. The start codon of the *AAC3* sequence and the stop codon of the *MIS1* sequences are located at positions −721 and +1285 respectively.

at +1117 and sequences TTTTTCTA and TTTATATA at +1184 and +1210, respectively, which are closely related to the consensus termination signal (TTTTTATA). The amino-terminal amino acid sequence predicted from the nucleotide sequence of *RPL19A* matches the sequence determined previously by amino-terminal sequencing of yeast ribosomal protein YL19 (Otaka *et al.*, 1983; Takakura *et al.*, 1992), confirming that *RPL19A* encodes the ribosomal protein YL19.

Occurrence of two functional copies of RPL19 *gene and effect of disruption of* RPL19A

It was previously reported that only one hybridized band appeared when the 4·92-kb*Pvu*II fragment containing the *MIS1* gene was used as a probe for Southern blot analysis of genomic DNA (Shannon and Rabinowitz, 1988). Since *RPL19A* is included in the 4·92-kb *Pvu*II fragment, we naturally assumed that *RPL19A* is a single copy

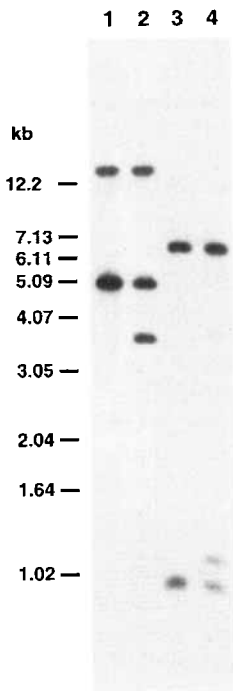

Figure 3. Southern blot analysis of genomic DNA for the *RPL19A* disruption and copy number. DNA isolated from the parent diploid strain JS-88 (Lanes 1 and 3), and its Ura^+ transformant S-36 (lanes 2 and 4), was digested with *Pvu*II (lanes 1 and 2) or *Pst*I (lanes 3 and 4), fractionated on agarose gel, and transferred onto Nytran. The blot was probed with the ^{32}P-labelled 0·66-kb *Sst*I-*Pst*I fragment from the *RPL19A*-coding region. 1-kb DNA ladder (GIBCO BRL) was used as a size marker.

gene. To determine whether *RPL19A* is required for cell growth, we disrupted the *RPL19A* gene *in vitro* and introduced the disrupted gene into the yeast chromosome. The 2·38-kb *Xba*I-*Kpn*I fragment containing the *RPL19A*-coding sequence was replaced with the 1·11-kb *Hin*dIII-*Sma*I fragment carrying the *URA3* gene (Figure 1). The disrupted gene was then excised from the plasmid pJS68 with *Eco*RI and transplaced into an *ura3* homozygous diploid JS-88 constructed with haploid strains GT48 and JS87-7A. Southern blot analysis of genomic DNA isolated from the diploid strains confirmed that the disrupted gene had replaced one of the *RPL19A* genes (Fig. 3). When the 0·66-kb *Sst*I-*Pst*I fragment from the *RPL19A*-coding region was used as a probe (Figure 1), the decreased strength of the bands at 4·92 kb for *Pvu* II and 1·0 kb for *Pst*I and the appearance of new bands at 3·65 kb for *Pvu*II and 1·19 kb for *Pst*I suggest that the diploid strain carries one wild-type copy and one disrupted copy of *RPL19A*. In addition, upper bands at 13·5 kb for *Pvu*II and 6·8 kb for *Pst*I exhibiting the same strength were found, indicating the existence of a second copy of the gene that was not detected previously. This is surprising, but it may be the result of the use of a more defined DNA fragment as a probe in this study. The resulting *RPL19A/rpl19a::URA3* diploid S-36 was sporulated. All the segregants of nine tetrads dissected grew well on YPD medium. Two spores per tetrad were Ura^+, and two were Ura^-. The *rpl19a::URA3* segregants all grew at rates comparable to that of the *RPL19A* segregants and their parent strains.

To search for the chromosomal locations of the *RPL19* gene, a Southern blot analysis of yeast chromosomes resolved by pulsed field electrophoresis was performed. Only one band was found to hybridize with the probe used above (data not shown). This result suggests that both the *RPL19A* and the other gene, designated as *RPL19B*, are located on chromosome II. Indeed, the location of *MIS1* on chromosome II was previously observed by tetrad analysis (K. Shannon and J. C. Rabinowitz, unpublished result). The *mis1::URA3* is 16·7 cM from *lys2* on chromosome II (PD:NPD:T=8:0:4). The 3′-end of *RPL19A* is 212 bp from the 3′-end of *MIS1*.

To determine whether the two copies of *RPL19* gene are functional, a Northern blot analysis was performed. As shown in lane 2 of Figure 4, a broad band was found to hybridize with the probe used above in wild-type yeast cells with respect to *RPL19A*. The size of this band is 0·7 kb and is about 0·13 kb longer than that of the coding region of *RPL19A*. Much greater amounts of the transcript were shown in yeast cells carrying *RPL19A* on multiple copy plasmid YEpKS17 (lane 3 in Figure 4). Yeast cells with a disruption in the *RPL19A* contained a hybridizing band nearly the same size as the *RPL19A* transcript (lane 1 in Figure 4). These results suggest that both the *RPL19* genes are expressed.

Comparison of YL19 with other ribosomal proteins

The amino acid sequence of YL19 protein predicted from *RPL19A* was compared to those of proteins in the PIR and Swiss-Prot protein data banks using the computer program FastA (Pearson and Lipman, 1988). YL19 is likely related to the ribosomal protein L19 family: human L19 (Kumabe *et al.*, 1992), rat L19 (Chan *et al.*, 1987), mouse L19 (Nakamura *et al.*, 1990), *D. discoideum*

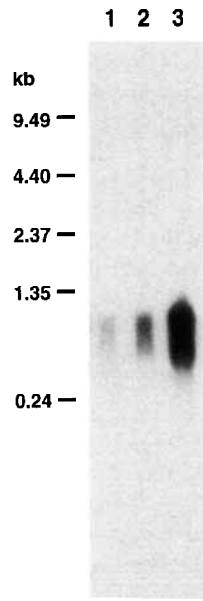

Figure 4. Northern blot hybridization of *RPL19* transcripts. Total RNAs (20 μg) from the indicated strains were transferred to Nytran and hybridized with the ^{32}P-labeled 0·66-kb *Sst*I-*Pst*I fragment from the *RPL19A*-coding region. Lane 1, S36-1A strain with the *rpl19a::URA3* disruption; lane 2, S37 strain, wild-type with respect to *RPL19A*; lane 3, S37 strain carrying *RPL19A* on multiple copy plasmid YEpKS17. RNA ladder (GIBCO BRL) was used as a size marker.

L19e (Singleton *et al.*, 1989), *H. marismortui* HL24 (Scholzen and Arndt, 1991) and *M. vannielii* ORFe (Auer *et al.*, 1989). There is 57·5% identity between YL19 and mammalian L19 over a 186-amino acid overlap, 56·1% identity between YL19 and *D. discoideum* L19e over a 180-amino acid overlap, 35·8% identity between YL19 and *H. marismortui* HL24 over a 148-amino acid overlap, and 35·3% identity between YL19 and *M. vannielii* ORFe over a 136-amino acid overlap (Figure 5). YL19 is seven amino acid residues shorter than the mammalian L19. However, it is three amino acid residues longer than *D. discoideum* L19e, and 40 amino acids longer than both *H. marismortui* HL24 and *M. vannielii* ORFe.

The amino acid sequence deduced from the nucleotide sequence upstream from the 5′-end of the *RPL19A* matches the sequence in a data bank of the amino-terminal portion of the *AAC3* product, mitochondrial ADP/ATP carrier. There was 100% identity in the nucleotide sequence of the coding region and 134 nucleotides from the 5′-end of the *AAC3* when compared with the reported *AAC3* sequence (Kolarov *et al.*, 1990). However, the 127-nucleotide region from −135 to −261 from the 5′-end of the *AAC3* [equal to the first 100-nucleotide sequence of the reported sequence of *AAC3* (Kolarov *et al.*, 1990)] bears a total of

Figure 5. Amino acid sequence alignments of the predicted *S. cerevisiae* YL19 and the ribosomal protein L19 family from other organisms. Human L19 is identical to rat L19. The following symbols are used to indicate the relationship relative to the amino acids of yeast YL19: identical or similar (white on black); different from (black on white). The following abbreviations are used for the organisms: Ddis, *D. discoideum*; Hmar, *H. marismortuei*; Mvan, *M. vannielii*.

28-bp insertions at 16 positions and a 1-bp deletion at position −249. This region is also equivalent to the region from −585 to −459 from the 5′-end of the *RPL19A* (Figure 2), suggesting that the insertions and deletion occurred in the region separating the *RPL19A* and *AAC3* genes. As shown in Figure 1, the location of *AAC3* can now be placed relative to *TEC1, MIS1* and *RPL19A* on the right arm of chromosome II. The order is *CENII-TEC1-MIS1-RPL19A-AAC3-LYS2-*.

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