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Sequence and Functional Similarity between a Yeast Ribosomal Protein and the *Escherichia coli* S5 *ram* Protein

JAMIE A. ALL-ROBYN,[1]† NINA BROWN,[1] EIKO OTAKA,[2] AND SUSAN W. LIEBMAN[1]*

Laboratory for Molecular Biology, Department of Biological Sciences, University of Illinois at Chicago, Box 4348, Chicago, Illinois 60680,[1] and Department of Biochemistry and Biophysics, Research Institute for Nuclear Medicine and Biology, Hiroshima University, Minami-ku, Hiroshima, 734 Japan[2]

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The accurate and efficient translation of proteins is of fundamental importance to both bacteria and higher organisms. Most of our knowledge about the control of translational fidelity comes from studies of *Escherichia coli*. In particular, *ram* (ribosomal ambiguity) mutations in structural genes of *E. coli* ribosomal proteins S4 and S5 have been shown to increase translational error frequencies. We describe the first sequence of a ribosomal protein gene that affects translational ambiguity in a eucaryote. We show that the yeast omnipotent suppressor *SUP44* encodes the yeast ribosomal protein S4. The gene exists as a single copy without an intron. The SUP44 protein is 26% identical (54% similar) to the well-characterized *E. coli* S5 *ram* protein. SUP44 is also 59% identical (78% similar) to mouse protein LLrep3, whose function was previously unknown (D. L. Heller, K. M. Gianda, and L. Leinwand, Mol. Cell. Biol. 8:2797–2803, 1988). The *SUP44* suppressor mutation occurs near a region of the protein that corresponds to the known positions of alterations in *E. coli* S5 *ram* mutations. This is the first ribosomal protein whose function and sequence have been shown to be conserved between procaryotes and eucaryotes.

Analyses of informational suppressors in *Escherichia coli* have elucidated the components involved in determining the fidelity of translation. In most instances, the factors identified have been either tRNAs or ribosomal proteins (31, 69, 84). Alterations in five different ribosomal proteins have been shown to influence translational accuracy. Mutations in the structural genes for S12 (30), S17 (8), and L6 (53) can cause an increase in accuracy, while mutations in the structural genes for L7/L12 (47), S4 (77), and S5 (13, 71) can decrease fidelity. Such mutations that affect S4 or S5 are known as *ram* (ribosomal ambiguity) mutations because they cause a general ambiguity of translation, suppressing nonsense as well as missense and frameshift mutations (3, 73). More recently, nonsense suppressor phenotypes have also been shown to be associated with mutations in elongation factor EF-Tu (85, 89).

We and others have used a similar genetic approach to identify components of the eucaryotic protein synthesis machinery that control translational fidelity (2, 21, 25, 54, 65, 88, 91, 95). Certain eucaryotic translational fidelity mutations are in many respects analogous to *E. coli ram* mutations in that they have similar phenotypes and seem to affect components of the ribosome. For example, analyses of such mutants in *Podospera anserina* using two-dimensional gel electrophoresis indicate that alterations in ribosomal proteins are often associated with the mutant phenotypes (21). In yeast cells, omnipotent suppressors cause sensitivity to aminoglycoside antibiotics and can suppress all three nonsense codons (2, 65, 88, 91), phenotypes also associated with *E. coli ram* mutations. Two yeast omnipotent suppressors, *sup35* and *SUP46*, show altered ribosomal protein patterns on the basis of two-dimensional gel electrophoresis (25, 40). In addition, 40S ribosomal subunits isolated from *sup35*, *sup45*, *SUP44*, and *SUP46* strains increase in vitro misreading of polyuridylate templates (25, 62).

Despite the suggestive biochemical evidence, the first two yeast omnipotent suppressor genes to be cloned and sequenced ($SUP35^+$ and $SUP45^+$) do not encode ribosomal proteins (10, 11, 17, 18, 38, 54, 57, 95). The $SUP45^+$ gene is predicted to encode a protein of molecular mass 49 kDa (10). Regions of weak similarity to several tRNA synthetases were found, but the function of the gene product is still not known. The $SUP35^+$ gene is predicted to encode a protein of 76.5 kDa that shows a high degree of similarity to EF-1α (45, 54, 95) but is not identical to any of the three biochemically characterized elongation factors from yeast cells. Neither $SUP35^+$ nor $SUP45^+$ appears likely to encode a ribosomal protein, since both predicted gene products are much larger than ribosomal proteins, and their codon usage patterns indicate that, unlike ribosomal protein genes, they are not highly expressed (10, 54, 95).

Here, we describe the sequence of a third yeast omnipotent suppressor gene, $sup44^+$. This suppressor does encode a ribosomal protein. Furthermore, the SUP44 protein shows substantial sequence similarity to the *E. coli* S5 *ram* protein.

MATERIALS AND METHODS

Strains and genetic methods. The following *Saccharomyces cerevisiae* strains were used: SL815-26B [α *met8-1 leu2-1 trp1-1* (*his5-2* and/or *his3-1*) *lys2-1 ura3-52 SUP44*] and SL-982 [**a**/α *ura3-52/ura3-52 met8-1/met8-1 leu2-1/leu2-1 ade3-26/+ his5-2/+ his3-Δ1/+ tyr7-1/+ trp1-1/+*]. Standard yeast genetic procedures of crossing, sporulation, and tetrad analysis were used (83). Strains were propagated on complex glucose medium (YPD). Nutritional markers were scored on omission media (synthetic complete glucose medium lacking a specific amino acid). Sensitivity to the aminoglycoside antibiotic paromomycin was scored on plates containing nutrient medium supplemented with 0.5 or 1 mg of paromomycin sulfate (a kind gift from Warner-Lambert Co.) per ml. *E. coli* DH5α and DH5αF′ were used for growth and

* Corresponding author.
† Present address: Ohio State Medical School, Columbus, OH 43210.

maintenance of plasmid and M13 vectors (61). Phages M13 mp18 and M13mp19 were obtained from Amersham. Procedures for working with M13 phage were adapted from Dale et al. (19).

Bacterial and yeast transformation. Bacterial transformations were done as described previously (60). Yeast cells were transformed by the lithium acetate method (41).

Southern and Northern (RNA) analyses. *E. coli* plasmid DNA and total yeast genomic DNA were isolated, electrophoresed, transferred to Hybond N (Amersham), and hybridized as previously described (94). RNA was isolated from yeast cells by using a modification of a published procedure (24). Aurintricarboxylic acid (100 μM) was used as a nuclease inhibitor. RNA was fractionated on a formaldehyde-agarose gel and transferred to a GeneScreen filter (NEN). Prehybridization and hybridization were done at 42°C without dextran sulfate as described in the GeneScreen manual. All probes were labeled with ^{32}P by either nick translation (76) or random priming (26).

Subcloning. The *sup44*$^+$ gene was previously cloned on plasmid pYsupx, which contains an 8.85-kb fragment of yeast DNA in the *CEN* vector YCp50 (2). Plasmid pJR4 was constructed by partial digestion of pYsupX with *Sau*3A. The resulting DNA was ligated and used to transform *E. coli* DH5α to ampicillin resistance. DNA was extracted from pooled transformants by the alkaline lysis procedure and was then used to transform yeast strain SL815-26B to uracil prototrophy. Resulting colonies were screened for the ability to complement the recessive paromomycin sensitivity associated with the *SUP44* suppressor. One such transformant was obtained and contained plasmid pJR4. Plasmids pJR5 and pJR6 were constructed by *Hin*dIII and *Eco*RI-*Sal*I digestion of pJR4, respectively, and ligation into appropriately cut YCp50. Complementation of *SUP44* was scored on medium containing 0.5 mg of paromomycin sulfate per ml.

Cloning the *SUP44* mutant. The *SUP44* mutant was cloned by the method of integration and excision (96). Strain SL815-26B (*SUP44 ura3-52*) was transformed with YIpJR2 (2), a *URA3*$^+$ integrating vector containing a portion of the *sup44*$^+$ gene on the 6.5-kb *Eco*RI fragment from pYsupX (Fig. 1). Transformants were selected on uracil-deficient medium and were checked for retention of suppressor activity on methionine- and leucine-deficient media and for the presence of a single integrated copy of the vector at the *SUP44* locus by Southern blot analysis. Plasmid containing the mutant *SUP44* allele was obtained by restricting DNA isolated from an appropriate transformant with *Sph*I, followed by ligation and transformation into *E. coli*.

Gene disruption. The one-step gene replacement procedure was used to disrupt the *sup44*$^+$ gene (79). A 1.8-kb *Hin*dIII fragment extending from the leftmost site within the *sup44*$^+$ gene in plasmid pJR4 to the site in the YCp50 vector was inserted into vector pBR322-1. (The *Eco*RI site in pBR322 was filled in with the large fragment of *E. coli* DNA polymerase I to form pBR322-1.) The new plasmid was digested with *Eco*RI and was ligated to a 1.2-kb *Eco*RI fragment containing the *URA3* gene, obtained from plasmid pLS10 (90; kind gift from L. Symington). This ligation yielded plasmid p*sup44*::*URA3*, in which the *sup44*$^+$ coding sequence was disrupted at the internal *Eco*RI site. A 2.4-kb *Hin*dIII-*Sph*I fragment from p*sup44*::*URA3* containing the disruption was used to transform diploid strain SL-982 to uracil prototrophy. Southern analysis confirmed that a single integration had occurred at *sup44*$^+$.

DNA sequencing. Sequencing reactions were performed on DNA fragments cloned into the polylinker region of phages M13mp18 and M13mp19 or on plasmid pJR4. Appropriate oligonucleotides were used to prime chain termination sequencing reactions (80) using [α-^{35}S]dATP (6). Double-stranded sequencing was done as described in the U.S. Biochemical Corp. manual "Step-by-Step Protocols for DNA Sequencing with Sequenase Version 2.0." Reaction products were resolved by electrophoresis through 6 and 8% polyacrylamide gels under denaturing conditions and were detected by autoradiography overnight at room temperature. All of the cloning junction points were sequenced in plasmid pJR4 by using appropriate oligonucleotides. All sequence for the wild-type *sup44*$^+$ allele was confirmed for both DNA strands. Only a single strand of the mutation was sequenced except in the region containing the alteration, which was confirmed on both strands.

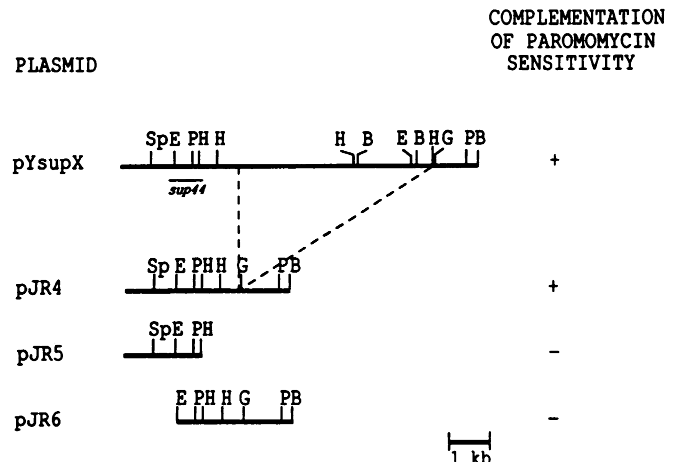

FIG. 1. Localization of the *sup44*$^+$ gene. Plasmids were tested for their ability to complement the recessive paromomycin sensitivity associated with the *SUP44* mutation. The column on the right shows the ability (+) or inability (−) of the various fragments to complement the *SUP44*-associated paromomycin sensitivity when cloned into the YCp50 vector and transformed into yeast strain SL815-26B (α *ura3-52 SUP44*). The exact location of *sup44* as indicated in the first line was established later from the DNA sequence. Restriction sites: B, *Bam*HI; E, *Eco*RI; G, *Bgl*II; H, *Hin*dIII; P, *Pvu*II; Sp, *Sph*I. (An additional two *Hin*dIII sites restrict the 3.4-kb *Hin*dIII region into 1.4-, 1.3-, and 0.7-kb fragments. The exact locations of these sites have not been determined. The single *Hin*dIII site shown near the 3′ end of the *sup44* gene is actually composed of two *Hin*dIII sites within 21 bp of each other, as shown in Fig. 3.)

Peptide sequencing. Purified yeast S4 protein was obtained as described previously (37). Proteolysis and peptide sequencing was performed by the Harvard Microchemistry facility. S-V8 fragments were purified by using a Vydac C18 column, and the sequence was obtained by automated Edman degradation on an Applied Biosystems Inc. 470A protein sequencer equipped with a 120A on-line phenylthiohydantoin-amino acid analyzer.

Nucleotide sequence accession number. The nucleotide sequence data reported have been submitted to GenBank and assigned accession number M38029.

RESULTS

Characterization of the *sup44*$^+$ gene. We have previously described the isolation of the *sup44*$^+$ gene on an 8.85-kb DNA fragment (2). The position of the gene within the larger insert was established by assessing the ability of various

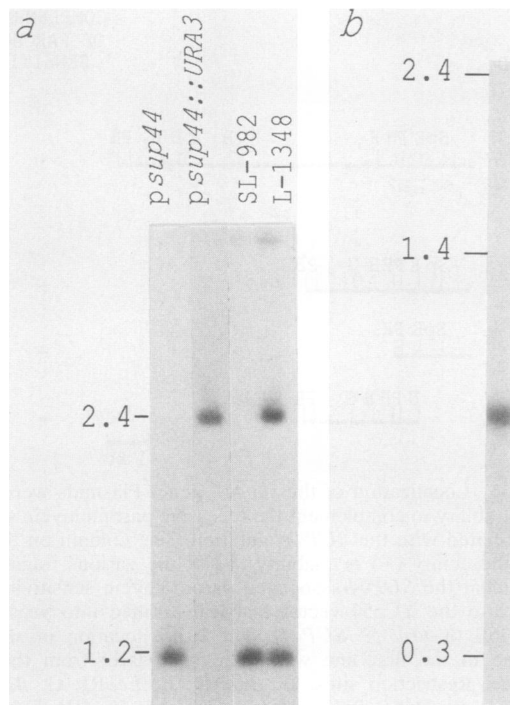

FIG. 2. Southern and Northern blot analysis. (a) Southern blot analysis. *E. coli*-grown plasmid p*sup44* contains the intact *sup44*$^+$ gene; plasmid p*sup44*::*URA3* contains the *sup44*$^+$ gene disrupted at the *Eco*RI site with a 1.2-kb *Eco*RI fragment carrying the *URA3* gene. The 2.4-kb *Hin*dIII-*Sph*I fragment from p*sup44*::*URA3* containing the disrupted *sup44* gene was used to transform the diploid yeast strain SL-982 (**a**/α *ura3-52*/*ura3-52*) having two undisrupted copies of *sup44*$^+$. The diploid transformant, L-1348, is isogenic to SL-982 except that one copy of *sup44*$^+$ is disrupted (*sup44*::*URA3*). The 2.4-kb *Hin*dIII-*Sph*I fragment contains the disrupted *sup44* gene (*sup44*::*URA3*). The 1.2-kb *Hin*dIII-*Sph*I fragment contains the undisrupted *sup44*$^+$ gene. Occasionally, weakly hybridizing fragments were also seen. (b) Northern analysis. Numbers indicate the lengths of RNA ladder markers (Bethesda Research Laboratories) in kilobases. Filters were probed with the ^{32}P-labeled internal 500-bp *Eco*RI-*Hin*dIII fragment of *sup44* cloned into M13mp19.

restriction fragments and deletions to complement the recessive paromomycin sensitivity associated with the *SUP44* suppressor (Fig. 1). These results localized *sup44*$^+$ to a 4-kb *Sau*3A partial-digestion fragment and suggested that the gene spans an *Eco*RI-*Hin*dIII fragment of 500 bp. With this 500-bp fragment used as a probe, Northern blot analysis demonstrated the presence of a single transcript of approximately 800 bp (Fig. 2).

The DNA sequence is shown in Fig. 3. There is a 762-bp open reading frame that, as predicted from the subcloning results, overlaps the *Eco*RI and *Hin*dIII sites. Consensus TATA-like elements are located at positions −36 and −91. The consensus termination signal (TTTTTATA) proposed by Henikoff et al. (35) is present at position +794. The proposed AATAAA polyadenylation signal (75) is not present, but a closely related sequence ATTAAA is located at position +809.

The protein predicted from the *sup44*$^+$ sequence contains many features characteristic of yeast ribosomal proteins. The low molecular mass (27.5 kDa) and basic amino acid composition (pI 11.23) are both characteristic of yeast ribosomal proteins (92). The codon bias indices of ribosomal protein mRNAs are similar to those found for other highly expressed yeast genes, ranging from 0.75 to 0.99 (5, 82). The codon bias index of *sup44*$^+$ is 0.91. In addition, like most transcripts for ribosomal proteins, the *sup44*$^+$ transcript is barely large enough (800 bp) to encode the open reading frame (762 bp) (27).

All but two of the sequenced yeast ribosomal protein genes contain one or more *RAP1*/*GRF1* sequences (formerly called HOMOL or RPG) within 100 to 500 bases upstream that are necessary for transcription and that bind the *RAP1* transcription factor (39, 56, 78, 86, 101). The two ribosomal protein genes, L3 and S33, that lack *RAP1*/*GRF1* sequences have been found to contain a *GF1* consensus sequence that binds a different transcription factor, *ABF1* (22, 32). No *RAP1*/*GRF1* sequence has been found within 500 bp of the AUG codon in *sup44*$^+$, but a sequence that fits the *GF1* consensus is located at position −295 to −283 (Fig. 3). In addition, a polypyrimidine stretch characteristic of ribosomal protein genes (78) is found at −164 to −151.

Many, but not all, yeast ribosomal protein genes contain a single intron and are duplicated (92). Consensus signals for splicing were not found in the *sup44*$^+$ open reading frame or in the flanking regions. Southern blot analyses indicated that *sup44*$^+$ is unique because a single band hybridized strongly to the internal 500-bp *Eco*RI-*Hin*dIII *sup44*$^+$ probe when DNA was digested with *Hin*dIII-*Sph*I (Fig. 2), *Bam*HI, *Eco*RI, *Hin*dIII, or *Xho*I (data not shown). Furthermore, disruption of one copy of the *sup44*$^+$ open reading frame in diploid strain SL-982 (Fig. 2) resulted in the segregation of 2 viable:2 inviable meiotic products upon sporulation. Of 24 tetrads dissected, 23 contained two viable spores and 1 contained a single viable spore. Since none of the viable segregants contained the *URA3* disruption of *sup44*$^+$, the 762-bp open reading frame encodes an essential protein. In contrast, the isogenic undisrupted diploid gave rise to three or four viable meiotic products in 24 of 26 tetrads dissected.

Characterization of the mutant *SUP44* gene. The identical DNA region was isolated from a *SUP44* strain on an integrating *URA3* plasmid. The plasmid was verified to contain the suppressor, since Ura$^+$ transformants of a *sup44*$^+$ *ura3* strain contained suppressor activity (R. Liu and S. Liebman, unpublished results). DNA sequence analysis of the complete *SUP44* coding region identifies a single-base change relative to the sequence of the wild-type allele. The serine (TCT) at position +200 in the wild type is changed to tyrosine (TAT) in the mutant (Fig. 3). Thus, the 762-bp open reading frame encodes the *SUP44* gene product.

Identification of the SUP44 protein as the yeast S4 ribosomal protein. Since it appeared likely that *sup44*$^+$ encodes a ribosomal protein, the SUP44 sequence was compared with the 50 yeast ribosomal protein sequences that are completely or partially known (92). No match was found. However, many of the yeast ribosomal proteins that have not been sequenced have been purified, and their molecular weights and amino acid compositions are known (37, 42). Thus, we compared the SUP44 predicted molecular weight and amino acid composition with those of all experimentally characterized yeast ribosomal proteins (37, 42, 66). Using the Compsim comparison program of PC/GENE (IntelliGenetics), we found that the amino acid composition of SUP44 was more similar to that of the small subunit ribosomal protein identified as S4 (also called YS5, YP9, or rp12; 37, 67, 74) than to that of any other experimentally characterized yeast ribosomal protein or any other protein in the Swiss-protein data base (Table 1). The predicted size of the SUP44 protein, 27.5 kDa, is also very close to that measured for the S4 protein, 28 kDa (63).

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-520                                                                                GTTTACACGTATTTTAGTAATTGGCTATTCACTAAGGATT
-480 CTTAAGGTTTTCTTAATAGTTTTCTACGTCGGCATGCGATTGTTTGGTTTAGAAGACTGCTTTCTAAATATGGTTGGGTGTATTTAAGCTAGACCCATACACCCGCTCTATGGGATTATT
-360 TACTTGTTTGAATTTTAAGATTTGTGATAATGGAACTGGACGCAAACATTTGATGGAAAACGCATGTCATCATTAACGAGGTAAGCTAGGTATCTGTCCTGCCTTAGTATTGCACGCAGC
-240 TTCCCAGGAGCCCTAGCTATTTTTTCATCTATTCCCCTCTGTAGTAACGTAAGAGTTTTCAAGTTTTTAATTCAGACTTTCTCTTCCTTTGTTTCCAATTTCCTTCCTTACTGCTTGATA
-120 CCTTTTCAATCCCAAAGAAACCGTGTTCTTTATATATTGTCGATTGAAAGTTACCTACATCAACTTTCCGTGTTCCATTCCGACTATAACAAACAACCAATAAGCTCAACTAATTAAGTA

  +1 ATGTCTGCTCCAGAAGCTCAACAACAAAAGAGAGGTGGTTTCGGTGGCCGTAACAGAGGCCGTCCAAACAGAAGAGGACCAAGAAACACTGAAGAAAAGGGATGGGTTCCAGTTACCAAA
   1 MetSerAlaProGluAlaGlnGlnGlnLysArgGlyGlyPheGlyGlyArgAsnArgGlyArgProAsnArgArgGlyProArgAsnThrGluGluLysGlyTrpValProValThrLys

                                                                                             EcoRI
+121 CTAGGTAGATTAGTCAAGGCTGGTAAGATTACCACCATTGAAGAAATCTTCTTGCACTCTTTGCCAGTCAAGGAATTCCAAATCATTGACACTTTGTTGCCAGGTTTGCAAGACGAAGTC
  41 LeuGlyArgLeuValLysAlaGlyLysIleThrThrIleGluGluIlePheLeuHisSerLeuProValLysGluPheGlnIleIleAspThrLeuLeuProGlyLeuGlnAspGluVal

+241 ATGAACATCAAGCCAGTTCAAAAGCAAACCAGAGCCGGTCAAAGAACCAGATTTAAGGCTGTTGTCGTTGTTGGTGACTCTAACGGTCACGTTGGTTTGGGTATCAAGACCGCCAAGGAA
  81 MetAsnIleLysProValGlnLysGlnThrArgAlaGlyGlnArgThrArgPheLysAlaValValValValGlyAspSerAsnGlyHisValGlyLeuGlyIleLysThrAlaLysGlu

                                                                                             KpnI
+361 GTTGCTGGTGCCATCAGAGCTGGTATCATTATTGCCAAGTTGTCCGTTATCCCAATCAGAAGAGGTTACTGGGGTACCAACTTGGGTCAACCACATTCTTTGGCCACCAAGACCACTGGT
 121 ValAlaGlyAlaIleArgAlaGlyIleIleIleAlaLysLeuSerValIleProIleArgArgGlyTyrTrpGlyThrAsnLeuGlyGlnProHisSerLeuAlaThrLysThrThrGly

                                                                                PvuII                                  A*
+481 AAGTGTGGTTCCGTCACTGTTAGATTGATCCCAGCCCCAAGAGGTTCTGGTATCGTCGCTTCTCCAGCTGTCAAAAAGTTGTTGCAATTGGCTGGTGTTGAAGATGTCTACACCCAATCT
 161 LysCysGlySerValThrValArgLeuIleProAlaProArgGlySerGlyIleValAlaSerProAlaValLysLysLeuLeuGlnLeuAlaGlyValGluAspValTyrThrGlnSer

+601 AACGGTAAGACTAGAACTTTGGAAAACACCTTGAAGGCTGCTTTCGTTGCTATTGGTAACACATACGGTTTCTTGACTCCAAACTTGTGGGCCGAACAACCATTGCCAGTTTCTCCATTG
 201 AsnGlyLysThrArgThrLeuGluAsnThrLeuLysAlaAlaPheValAlaIleGlyAsnThrTyrGlyPheLeuThrProAsnLeuTrpAlaGluGlnProLeuProValSerProLeu

                HindIII                    HindIII
+721 GACATCTACTCCGATGAAGCTTCTGCTCAAAAGAAGAGATTCTAAGCTTGTTGTCTACAAATTATAAAATAGTTTTTTATAATCAACCATTAAATAATTTTGTACACTTATAATAACACT
 241 AspIleTyrSerAspGluAlaSerAlaGlnLysLysArgPheTer

+841 TTATTTCTCGTTCCAATTATGCGATGCATGTGCCTTCCGTTTAGAGTGAGCATTATCAAATATAAAGTCAAATCCTTTTGGCATTTATTTTGATTGAAACCCAGTCTG
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FIG. 3. DNA sequence and predicted amino acid sequence of the *sup44* gene. Locations of relevant restriction sites are shown. Numbers on the left refer to amino acid and nucleotide positions. Candidate TATA sequences at −36 and −89, a consensus termination signal at +794, and the sequence that fits the *GF1* box consensus at −295 to −283 (RTCRYNNNNNACG) (22) are underlined. The peptide sequence (residues 33 to 55), which was obtained by microsequencing of an S-V8 peptide purified from the isolated yeast S4 ribosomal protein, is found in the *sup44*$^{+}$ open reading frame and is underlined. The asterisk indicates the nucleotide altered in the mutant *SUP44*, causing the serine (TCT) at position 200 to become tyrosine (TAT).

TABLE 1. Comparison of the amino acid analysis of yeast S4 ribosomal protein and the predicted amino acid composition of the SUP44 protein

Amino acid	Composition (mol%)	
	S4[a]	SUP44[b]
Ala	8.5	8.2
Arg	6.8	7.8
Asx	7.7	6.2
Glx	10.6	9.8
Gly	11.6	11.0
His	1.4	1.1
Ile	6.3	6.6
Leu	8.9	8.2
Lys	7.9	7.0
Met	0.5	0.7
Phe	2.8	2.7
Pro	6.0	6.2
Ser	4.1	4.7
Thr	6.7	7.4
Tyr	1.8	1.5
Val	8.5	8.2

[a] From Higo and Otaka (37). No corrections for incomplete acid hydrolysis or for decomposition were made. Tryptophan and cysteine were not measured.
[b] Predicted from the *sup44*$^+$ sequence.

To test the hypothesis that *sup44* encodes the yeast S4 ribosomal protein, the S4 protein was isolated, and the sequence of 23 residues of a purified S-V8 proteolytic fragment was determined. All assignments were clearly observed chromatographically, yielding high confidence sequence with no ambiguities. The sequence reported to us blind from the Harvard Microchemistry facility (Table 2) exactly matched residues 33 to 55 of the predicted amino acid sequence of the SUP44 protein (Fig. 3). Thus, it is clear that *sup44* encodes the yeast S4 ribosomal protein.

TABLE 2. Sequence data for an S-V8 proteolytic fragment isolated from yeast S4 ribosomal protein

Cycle	PTH-AA[a]	Yield (pmol)[b]
1	Lys	128.6
2	Gly	83.5
3	Trp	37.5
4	Val	24.1
5	Pro	25.9
6	Val	9.2
7	Thr	11.9
8	Lys	8.7
9	Leu	8.8
10	Gly	7.4
11	Arg	8.7
12	Leu	4.7
13	Val	2.6
14	Lys	3.1
15	Ala	3.0
16	Gly	2.0
17	Lys	1.5
18	Ile	2.1
19	Thr	1.5
20	Thr	1.5
21	Ile	2.1
22	Glu	0.7
23	Glu	1.1

[a] PTH-AA, Phenylthiohydantoin-amino acid.
[b] Corrected for previous cycle background of assigned amino acid. Average repetitive yield calculated for residues 1 through 21 = 81.4%.

Comparison of the SUP44 protein with other ribosomal proteins. The predicted amino acid sequence of the *sup44*$^+$ product was compared with protein sequences in the NBRF data bank (29), using the algorithm of Lipman and Pearson (58). The results indicated that SUP44 has significant similarity to ribosomal protein S5 from both *E. coli* (99) and *Bacillus stearothermophilus* (46). A search of the DNA GenBank data bank (7) revealed extensive similarity (60% identity in a 728-bp overlap) to a mouse cDNA clone, *LLrep3*, that was known to contain an open reading frame capable of encoding a 25-kDa protein (33, 34). (The putative LLrep3 protein is not in the Swiss-protein and NBRF data bases.) Since there are nine AUGs in the *LLrep3* 5′ leader, each followed by small open reading frames, we suspect that the cDNA clone is not a faithful copy of the functional gene's mRNA. Possibly the cDNA is related to one of the 200 pseudogene copies of *LLrep3*. We propose that *sup44*$^+$ in yeast cells and *LLrep3* in mouse cells encode ribosomal proteins equivalent to the bacterial S5. The four sequences are aligned in Fig. 4 (residues identical to SUP44 are boxed). The overall identities between the SUP44 protein and the S5 proteins of *E. coli* and *B. stearothermophilus* are 26 and 30%, respectively; when conservative substitutions are considered, similarities are 54 and 55%, respectively. The LLrep3 predicted protein is 59% identical to SUP44, 78% when conservative substitutions are considered, over the entire overlapping sequence.

The similarity between SUP44 and the bacterial proteins is highest in regions that correspond to the most similar regions between the bacterial sequences. When the bacterial sequences are compared, positions 17 to 57 and 98 to 144 show 73 and 68% identity, respectively. In contrast, the residues from positions 75 to 97 show only 50% identity (46). A comparison of residues 17 to 57 and 98 to 144 between the bacterial S5 and SUP44 sequences also shows a high level of conservation: 51 and 34% identity to the respective *B. stearothermophilus* S5 regions, and 41 and 34% identity to the respective *E. coli* S5 regions. By contrast, the region at positions 75 to 97 that was less conserved between the bacterial sequences actually contains an insertion in the two eucaryotic sequences (Fig. 4). The highly conserved and less conserved regions are clearly depicted on the dot matrix homology plots in Fig. 5. A correlation method that quantitatively evaluates the structural similarity predicted from the sequences (51, 52, 68) gave similar results when used to compare the four sequences (data not shown). The three most conserved regions indicated by this analysis correspond to *B. stearothermophilus* residues 13 to 20, 36 to 60, and 99 to 123.

DISCUSSION

The sequences of all 52 *E. coli* ribosomal proteins and their approximate positions in the ribosome relative to each other and the rRNA have been determined (12, 14, 64, 97). Eucaryotic ribosomes, which contain about 75 ribosomal proteins, are not as well characterized. The sequences of about 37 rat and 28 yeast ribosomal proteins are now known. Since 20 of the 37 sequenced rat ribosomal proteins have a homolog among the 28 known yeast sequences, it appears that it will be possible to correlate the ribosomal proteins among eucaryotes. Eliminating homologs from the yeast and rat data sets, approximately 45 distinct eucaryotic ribosomal protein sequences are known. Of these, only 12 show significant similarity to any *E. coli* ribosomal proteins (reviewed in references 92 and 100). Since the sequences of all

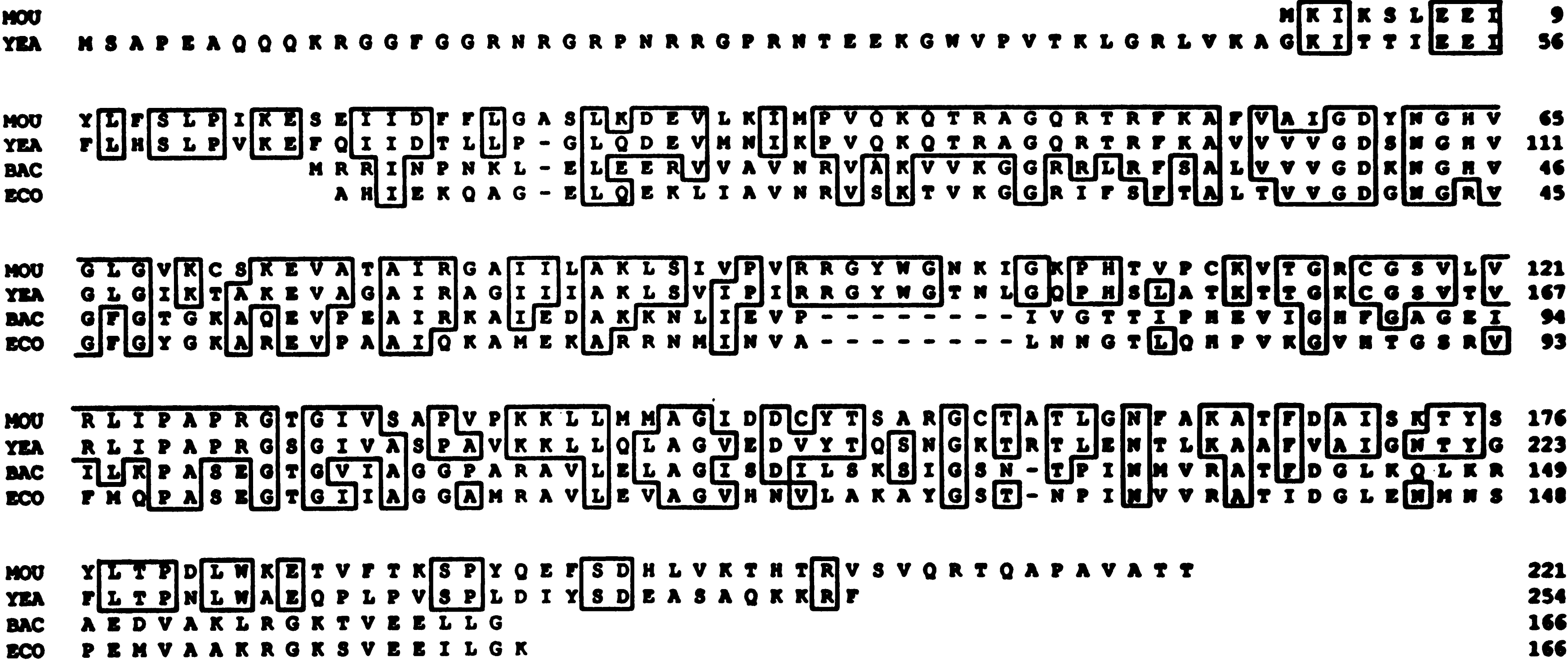

FIG. 4. Correlation of four amino acid sequences (single-letter code): yeast SUP44 protein (YEA), S5 ribosomal proteins of *E. coli* (99) (ECO) and *B. stearothermophilus* (46) (BAC), and mouse LLrep3 (33, 34) (MOU). The sequences were aligned by using a correlation method (51, 52, 68) that quantitatively evaluates the structural similarity predicted by the sequences. Residues identical to those in the yeast SUP44 sequence are boxed. The start of the mouse protein is unknown (see text). Numbers on the right refer to the amino acid positions in the respective gene products. Conservative substitutions referred to in the text are from Dayhoff (20): a = S,A,P,T,G; d = D,E,Q,N; h = K,R,H; i = V,L,I,M; f = Y,W,F; c = C.

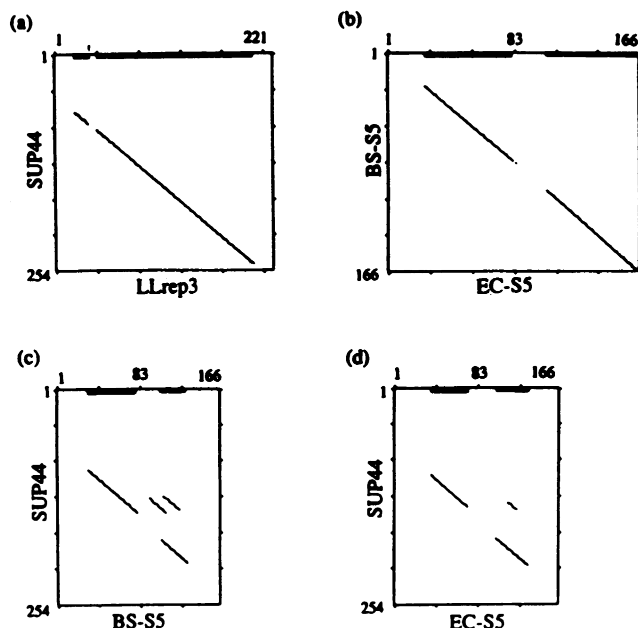

FIG. 5. Dot matrix comparisons between members of the S5 family of ribosomal proteins. Each dot for SUP44/LLrep3 (a) and *B. stearothermophilus* S5 (BS-S5)/*E. coli* S5 (EC-S5) (b) represents 12 exact matches in a window of 27 amino acids. Each dot for SUP44/*B. stearothermophilus* S5 (BS-S5) (c) and SUP44/*E. coli* S5 (EC-S5) (d) represents 8 exact matches in a window of 27 amino acids. Plots were generated by the homology plot program of HIBIO PROSIS (Hitachi America, Ltd). The horizontal black bars compare the relative positions of the homology among the proteins.

of the *E. coli* ribosomal proteins are known, it appears that most procaryotic and eucaryotic ribosomal proteins have diverged to the point that we can no longer trace their relationships. It is reasonable to hypothesize that those eucaryotic ribosomal proteins that can be correlated with *E. coli* ribosomal proteins are likely to have the same function and relative position within the ribosome as do their procaryotic homologs. Our results support this hypothesis. We show that *sup44*$^+$ encodes ribosomal protein S4 in yeast cells. This protein is conserved in sequence, as well as function, compared with the bacterial S5 *ram* protein (77, 99).

Mutations in the *E. coli* gene *rspE*, which encodes S5, cause one of three different phenotypic effects: (i) resistance to spectinomycin (28, 72); (ii) reversion from streptomycin dependence to independence, which also causes a *ram* phenotype (43); and (iii) reversion of a temperature-sensitive alanyl-tRNA synthetase mutation (98). The amino acid changes in the S5 protein that are associated with each of these phenotypes are clustered in what appear to be independent functional domains (73). In the spectinomycin-resistant mutants, alterations occurred in the N-terminal region at position 19, 20, or 21; the S5 *ram* mutants had changes in positions 103 and 111; the single characterized tRNA synthetase revertant contained a deletion of residues 161 to 164 and 166 at the C terminus. Only a single *SUP44* allele has been studied so far, and it falls into the second class of mutations since it causes a *ram* phenotype. The *SUP44* allele is associated with an amino acid substitution of tyrosine for the serine residue at position 200 in the yeast protein, corresponding to an alteration at position 126 in the *E. coli* protein, not far from the known fidelity alterations at positions 103 and 111.

Extensive similarity between *sup44* and the mouse sequence *LLrep3* (33) was found at both the DNA and protein levels. *LLrep3* is a member of a 200-copy repeated sequence family that appears to consist almost entirely of pseudogenes. It is a highly conserved gene that is also present in 200 copies in the human genome (34, 83a) but in a single copy in chicken, *Xenopus laevis*, fish, and nematode genomes (33). The transcript of *LLrep3* is abundant [0.5% of the poly(A)$^+$ RNA in CHO and hepatoma cells] and is strongly growth regulated (33). From these data, it was hypothesized that *LLrep3* had an important housekeeping function. We find that the protein predicted from *LLrep3* is 59% identical to SUP44 (78% with conservative substitutions) over the entire overlapping sequence (Fig. 4 and 5). In addition, the DNA sequences of the two genes are about 60% identical. We propose that both *SUP44* and *LLrep3* are eucaryotic ribosomal protein genes related to the *E. coli* gene that encodes ribosomal protein S5 and that is associated with *ram* mutations. While mammalian ribosomal protein genes are generally encoded by one functional gene, they are usually associated with only 10 to 20 pseudogene copies (23, 48, 70, 93).

The 5′ region of the LLrep3 protein is not precisely defined. The sequenced cDNA clone contains a 1-kb 5′ leader with nine AUGs before the AUG that starts the large open reading frame. The first AUG is in a good context for efficient initiation of translation, while the 10th AUG is in a poor context (50). Since the first AUG almost always is the initiator codon in eucaryotes (49), and since the 5′ leader of eucaryotic ribosomal protein messages is generally short, 50 to 100 bp, it appears that this cDNA does not correspond to the bona fide message. Furthermore, the open reading frame directly 5′ to the initiator AUG proposed by Heller et al. (33) shows a strong similarity to the yeast sequence, suggesting that the 10th AUG is not the initiator codon. Indeed, a sequence equivalent to 10 amino acids preceding the 10th AUG is identical to the corresponding region of SUP44. The further 5′ sequence, which is strongly glycine and arginine rich, also shows good similarity.

The SUP44 protein begins to correspond to its S5 bacterial counterparts at position 66 or 67 (Fig. 4). Within the noncorresponding N-terminal region, the SUP44 protein contains a sequence (RGGFGGRNRG) that shows striking similarity to glycine-arginine-rich sequence repeats found in a variety of other nucleolar proteins. These repeats are found in nucleolin, the major nucleolar protein (three copies of RGGF GGR) (9); fibrillarin, located in the fibrillar compartments of nucleoli (three copies of RGGFGGR) (36, 81); yeast single-stranded RNA-binding protein SSB1, located predominantly in the nucleolus (three copies of RGGFRGR) (44); a helix-destabilizing, single-stranded binding heterogeneous nuclear ribonucleoprotein A1 (one copy of RGGFGGS and other similar sequences) (16); and a suspected nuclear antigen from Epstein-Barr virus (three copies of RGGSGGR) (4). The arginine residues in some of these sequences have been shown to be dimethylated posttranslationally (15, 55, 59). While the function of this sequence repeat is not known, it has been hypothesized to be involved in RNA binding, cooperative protein-protein associations (15, 36), or integration into the nucleolus (81). Possibly, this sequence in SUP44 functions similarly to those in the other nucleolar proteins. A similar sequence (RGGFGSGLRGRGRGRGR GRGRGRG) is found within the glycine-arginine-rich region in LLrep3 mentioned above. However, we note that the sequence is not found in other eucaryotic ribosomal proteins presently known. Thus, if the sequence is important to

SUP44 and LLrep3, its function has been replaced by other sequences or has disappeared in other ribosomal proteins.

The molecular mode of action of the *E. coli* ribosomal proteins that affect translational accuracy is now under investigation. Since the S4-S5-S12 cluster is found on the opposite side of the 30S subunit from the site of the codon-anticodon interaction, it has been suggested that its effect on the codon-anticodon interaction is probably indirect (1). In a recent study using structure-sensitive chemical probes, mutations in S4 and S12 that induced or restricted ambiguity were shown to alter the higher-order structure of 16S rRNA (1). For example, reactivity of the 16S rRNA nucleotide A-908 to the structure-sensitive chemical probe dimethyl sulfate generally paralleled the translational error frequency of the ribosome. The correlations of rRNA structural alterations with changes in translational accuracy suggest that the rRNA conformation directly affects fidelity. Although it is possible that the correlations are simply coincidental, the hypothesis of a causative relationship is further supported by the fact that molecular modeling studies on 16S rRNA place A-908 between the decoding site and the S4-S5-S12 cluster. The authors suggest that the reactivity of A908 is a measure of an equilibrium between two 16S rRNA conformations that are held in balance in wild-type ribosomes by the opposing effects of a ribosomal protein such as S4 or S5 versus S12. They suggest that alterations in these proteins may affect translational accuracy via a change in the 16S rRNA structure (1). A further test of this model requires the examination of additional mutations in ribosomal protein genes affecting translational accuracy (e.g., mutations in the genes encoding S17 and S5).

Other investigators (87) have used in vitro experiments to show that alterations in S4 or S12 can change the rate of EF-Tu–GDP dissociation. Since this rate is thought to be an internal kinetic standard by which EF-1α distinguishes between cognate and noncognate amino acylated tRNAs, small rate changes may have a dramatic effect on translational fidelity (87). Possibly, alterations in S4, S5, or S12 affect the 16S rRNA conformation, which in turn affects translational accuracy by changing the rates of EF-Tu–GDP dissociation. As mutations in yeast ribosomal protein genes analogous to the *E. coli* fidelity mutations are isolated and identified, they can be used to test these proposed mechanisms in a eucaryotic ribosome.

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LITERATURE CITED

1. **Allen, P. N., and H. F. Noller.** 1989. Mutations in ribosomal proteins S4 and S12 influence the higher order structure of 16S ribosomal RNA. J. Mol. Biol. **208**:457–468.
2. **All-Robyn, J. A., D. K. Kelley-Geraghty, E. Griffin, N. Brown, and S. W. Liebman.** 1990. Isolation of omnipotent suppressors in an [*eta*+] yeast strain. Genetics **124**:505–514.
3. **Atkins, J. F., D. Elseviers, and L. Gorini.** 1972. Low activity of β-galactosidase in frameshift mutants of Escherichia coli. Proc. Natl. Acad. Sci. USA **69**:1192–1195.
4. **Baer, R., A. T. Bankier, M. D. Biggin, P. L. Deininger, P. J. Farrell, T. J. Gibson, G. Hatfull, G. S. Hudson, S. C. Satchwell, C. Seguin, P. S. Tuffnell, and B. G. Barrell.** 1984. DNA sequence and expression of the B95-8 Epstein-Barr virus genome. Nature (London) **310**:207–211.
5. **Bennetzen, J. L., and B. D. Hall.** 1982. Codon selection in yeast. J. Biol. Chem. **257**:3026–3031.
6. **Biggin, M. O., T. J. Gibson, and G. F. Hong.** 1984. Buffer gradient gels and ^{35}S label as an aid to rapid DNA sequence determination. Proc. Natl. Acad. Sci. USA **80**:3963–3965.
7. **Bilofsky, H. S., C. Burks, J. W. Fickett, W. B. Goad, F. I. Lewitter, W. P. Rindone, C. D. Swindell, and C. S. Tung.** 1986. The GenBank data bank. Nucleic Acids Res. **14**:1–6.
8. **Bollen, T., T. Cabezon, D. DeWilde, R. Villaroel, and A. Herzog.** 1975. Alteration of ribosomal protein S17 by mutation linked to neamine resistance in *E. coli*. I. General properties of neaA mutants. J. Mol. Biol. **99**:795–806.
9. **Bourbon, H. M., B. Lapeyre, and F. Amalric.** 1988. Structure of the mouse nucleolin gene. J. Mol. Biol. **200**:627–638.
10. **Breining, P., and P. Piepersberg.** 1986. Yeast omnipotent suppressor *SUP1* (*sup45*): nucleotide sequence of the wild type and a mutant gene. Nucleic Acids Res. **14**:5187–5197.
11. **Breining, P., A. P. Surguchov, and W. Piepersberg.** 1984. Cloning and identification of a DNA fragment coding for the *sup1* gene of *Saccharomyces cerevisiae*. Curr. Genet. **8**:467–470.
12. **Brimacombe, R., J. Atmadja, W. Stiege, and D. Schuler.** 1988. A detailed model of the three-dimensional structure of *Escherichia coli* 16S ribosomal RNA *in situ* in the 30S subunit. J. Mol. Biol. **199**:115–136.
13. **Cabezon, T., A. Herzog, M. Dewilde, R. Villaroel, and A. Bollen.** 1976. Cooperative control of translational fidelity by ribosomal proteins in *E. coli*. Mol. Gen. Genet. **144**:59–62.
14. **Capel, M. S., D. M. Engelman, B. R. Freeborn, M. Kjeldgaard, J. A. Langer, V. Ramakrishnan, D. G. Schindler, K. K. Schneider, B. P. Schoenborn, I. Y. Sillers, S. Yabuki, and P. B. Moore.** 1987. A complete mapping of the proteins in the small ribosomal subunit of *Escherichia coli*. Science **238**:1403–1406.
15. **Christensen, M. E., and K. P. Fuxa.** 1988. The nucleolar protein, B-36, contains a glycine and dimethylarginine-rich sequence conserved in several other nuclear RNA-binding proteins. Biochem. Biophys. Res. Commun. **155**:1278–1283.
16. **Cobianchi, F., D. N. SenGupta, B. Z. Zmudzka, and S. H. Wilson.** 1986. Structure of rodent helix-destabilizing protein revealed by cDNA cloning. J. Biol. Chem. **261**:3536–3543.
17. **Crouzet, M., C. M. Izgu, C. M. Grant, and M. F. Tuite.** 1988. The allosuppressor gene *sal4* encodes a protein important for maintaining translational fidelity in *Saccharomyces cerevisiae*. Curr. Genet. **14**:537–543.
18. **Crouzet, M., and M. F. Tuite.** 1987. Genetic control of translational fidelity in yeast: molecular cloning and analysis of the allosuppressor gene *sal3*. Mol. Gen. Genet. **210**:581–583.
19. **Dale, R. M. K., B. A. McClure, and J. P. Houchins.** 1985. A rapid single-stranded cloning strategy for producing a sequential series of overlapping clones for use in DNA sequencing: application to sequencing the corn mitochondrial 18S rDNA. Plasmid **13**:31–40.
20. **Dayhoff, M. O.** 1978. Atlas of protein sequence and structure, vol. 5, suppl. 1 to 3. National Biomedical Research Foundation, Washington, D.C.
21. **Dequard-Chablat, M., E. Coppin-Raynal, M. Picard-Bennoun, and J. J. Madjar.** 1986. At least seven ribosomal proteins are involved in the control of translational accuracy in a eukaryotic organism. J. Mol. Biol. **190**:167–175.
22. **Dorsman, J. C., M. M. Doorenbosch, C. T. C. Maurer, J. H. DeWinde, W. H. Mager, R. J. Planta, and C. A. Grivell.** 1989. An ARS/silencer binding factor also activates two ribosomal protein genes in yeast. Nucleic Acids Res. **17**:4917–4923.
23. **Dudov, K. P., and R. P. Perry.** 1984. The gene family encoding the mouse ribosomal protein L32 contains a uniquely expressed intron-containing gene and an unmutated processed pseudogene. Cell **37**:457–468.
24. **Elder, R. T., E. Y. Loh, and R. W. Davis.** 1983. RNA from the yeast transposable element Ty1 has both ends in the direct repeats, a structure similar to retrovirus RNA. Proc. Natl. Acad. Sci. USA **80**:2432–2436.

25. **Eustice, D. C., L. P. Wakem, J. Wilhelm, and F. Sherman.** 1986. Altered 40S ribosomal subunits in omnipotent suppressors of yeast. J. Mol. Biol. **188**:207–214.
26. **Feinberg, A. P., and B. Vogelsteon.** 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132**:6–13.
27. **Fried, H. M., N. J. Pearson, C. H. Kim, and J. Warner.** 1981. The genes for fifteen ribosomal proteins of *Saccharomyces cerevisiae*. J. Biol. Chem. **256**:10176–10183.
28. **Funatsu, G., K. Nierhaus, and B. Wittmann.** 1972. Ribosomal proteins: studies on the altered protein S5 from a spectinomycin resistant mutant of *Escherichia coli*. J. Mol. Biol. **64**:201–209.
29. **George, D. G., W. C. Barker, and L. T. Hunt.** 1986. The protein identification resource (PIR). Nucleic Acids Res. **14**: 11–15.
30. **Gorini, L.** 1970. Informational suppression. Annu. Rev. Genet. **4**:107–134.
31. **Gorini, L.** 1974. Streptomycin and misreading of the genetic code, p. 791–803. *In* M. Nomura, A. Tissières, and P. Lengyel (ed.), Ribosomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
32. **Hamil, K. G., H. G. Nam, and H. M. Fried.** 1988. Constitutive transcription of yeast ribosomal protein gene *TCM1* is promoted by uncommon *cis*- and *trans*-acting proteins. Mol. Cell. Biol. **8**:4328–4341.
33. **Heller, D. L., K. M. Gianda, and L. Leinwand.** 1988. A highly conserved mouse gene with a propensity to form pseudogenes in mammals. Mol. Cell. Biol. **8**:2797–2803.
34. **Heller, D., M. Jackson, and L. Leinwand.** 1984. Organization and expression of non-Alu family interspersed repetitive DNA sequence in the mouse genome. J. Mol. Biol. **173**:419–436.
35. **Henikoff, S., J. D. Kelly, and C. H. Cohen.** 1983. Transcription terminates in yeast distal to control sequences. Cell **33**:607–614.
36. **Henriquez, R., G. Blobel, and J. P. Aris.** 1990. Isolation and sequencing of NOP1. J. Biol. Chem. **265**:2209–2215.
37. **Higo, K., and E. Otaka.** 1979. Isolation and characterization of fourteen ribosomal proteins from the small subunits of yeast. Biochemistry **18**:4191–4196.
38. **Himmelfarb, H. J., E. Maicas, and J. D. Friesen.** 1985. Isolation of the *sup45* omnipotent suppressor gene of *Saccharomyces cerevisiae* and characterization of its gene product. Mol. Cell. Biol. **5**:816–822.
39. **Huet, J., and A. Sentenac.** 1987. *TUF*, the yeast DNA-binding factor specific for UAS-RPG upstream activating sequences: identification of the protein and its DNA binding domain. Proc. Natl. Acad. Sci. USA **84**:3648–3652.
40. **Ishiguro, J., B. Ono, M. Masurekar, C. S. McClaughlin, and F. Sherman.** 1981. Altered ribosomal protein S11 from the *SUP46* suppressor in yeast. J. Mol. Biol. **147**:391–397.
41. **Ito, H., H. Fukuda, K. Murata, and A. Kimura.** 1983. Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153**:163–168.
42. **Itoh, T., K. Higo, and E. Otaka.** 1979. Isolation and characterization of twenty-three ribosomal proteins from large subunits of yeast. Biochemistry **18**:5787–5793.
43. **Itoh, T., and H. G. Wittmann.** 1973. Amino acid replacements in proteins S5 and S12 of two *Escherichia coli* revertants from streptomycin dependence to independence. Mol. Gen. Genet. **127**:19–32.
44. **Jong, A. Y., M. W. Clark, M. Gilbert, A. Oehm, and J. L. Campbell.** 1987. *Saccharomyces cerevisiae* SSB1 protein and its relationship to nucleolar RNA-binding proteins. Mol. Cell. Biol. **7**:2947–2955.
45. **Kikuchi, Y., H. Shimatake, and A. Kikuchi.** 1988. A yeast gene required for the G_1-to-S transition encodes a protein containing an A-kinase target site and GTPase domain. EMBO J. **7**:1175–1182.
46. **Kimura, M.** 1984. Proteins of the *Bacillus stearothermophilus* ribosome: the amino acid sequences of proteins S5 and L30. J. Biol. Chem. **259**:1051–1055.
47. **Kirsebom, L. A., and L. A. Isaksson.** 1985. Involvement of ribosomal protein L7/L12 in control of translational accuracy. Proc. Natl. Acad. Sci. USA **82**:717–721.
48. **Klein, A., and O. Meyerhas.** 1984. A multigene family of intron-lacking and containing genes coding for mouse ribosomal protein L7. Nucleic Acids Res. **12**:3763–3776.
49. **Kozak, M.** 1980. Evaluation of the "scanning model" for initiation of protein synthesis in eucaryotes. Cell **22**:7–8.
50. **Kozak, M.** 1981. Possible role of flanking nucleotides in recognition of the AUG initiator codon by eukaryotic ribosomes. Nucleic Acids Res. **9**:5233–5252.
51. **Kubota, Y., K. Nishikawa, S. Takahashi, and T. Ooi.** 1982. Correspondence of homologies in amino acid sequence and tertiary structure of protein molecules. Biochim. Biophys. Acta **701**:242–252.
52. **Kubota, Y., S. Takahashi, K. Nishikawa, and T. Ooi.** 1981. Homology in protein expressed by correlation coefficients. J. Theor. Biol. **91**:347–361.
53. **Kuhlberger, R., W. Piepersberg, A. Pretzet, B. Buckel, and A. Bock.** 1979. Alteration of ribosomal protein L6 in gentamycin-resistant strain of *E. coli*: effects on fidelity of protein synthesis. Biochemistry **18**:187–193.
54. **Kushnirov, V. V., M. Ter-Avanesyan, M. V. Telckov, A. P. Surguchov, V. N. Smirnov, and S. G. Inge-Vechtomov.** 1988. Nucleotide sequence of the *sup2* (*sup35*) gene of *Saccharomyces cerevisiae*. Gene **66**:45–54.
55. **Lapeyre, B., F. Amalric, S. H. Ghaffari, S. V. V. Rao, T. S. Dumbar, and M. O. J. Olson.** 1986. Protein and cDNA sequencing of a glycine-rich, dimethylarginine-containing region located near the carboxyl-terminal end of nucleolin (C23 and 100 kDa). J. Biol. Chem. **261**:9167–9173.
56. **Leer, R. J., M. M. C. Vanraamsdonk-Duin, W. H. Mager, and R. J. Planta.** 1985. Conserved sequences upstream of yeast ribosomal protein genes. Curr. Genet. **9**:273–277.
57. **Liebman, S. W., J. M. Song, J. All-Robyn, E. Griffin, and D. Kelley-Geraghty.** 1988. Omnipotent suppressors, allosuppressors and antisuppressors of yeast, p. 403–414. *In* M. F. Tuite, M. Picard, and M. Bolotin-Fukuhara (ed.), Genetics of translation. Springer-Verlag KG, Berlin.
58. **Lipman, D. J., and W. R. Pearson.** 1985. Rapid and sensitive protein similarity searches. Science **227**:1435–1441.
59. **Lischwe, M. A., R. G. Cook, Y. S. Ahn, L. C. Yeoman, and H. Busch.** 1985. Cluster of glycine and N^G,N^G-dimethylarginine in nucleolar protein C23. Biochemistry **24**:6025–6028.
60. **Mandel, J., and R. Higa.** 1970. *E. coli* transformation. J. Mol. Biol. **53**:159–162.
61. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
62. **Masurekar, M., E. Palmer, B. Ono, J. M. Wilhelm, and F. Sherman.** 1981. Misreading of the ribosomal suppressor *SUP46* due to an altered 40S subunit in yeast. J. Mol. Biol. **147**:381–390.
63. **Michel, S., R. R. Traut, and J. C. Lee.** 1983. Yeast ribosomal proteins: electrophoretic analysis in four two-dimensional gel systems—correlation of nomencclature. Mol. Gen. Genet. **191**:251–256.
64. **Moore, P. B.** 1988. The ribosome returns. Nature (London) **331**:223–227.
65. **Ono, B., M. Tanaka, I. Awano, F. Okamoto, R. Satok, N. Yamagishi, and Y. Ishinoarao.** 1989. Two new loci that give rise to dominant omnipotent suppressors in *Saccharomyces cerevisiae*. Curr. Genet. **16**:323–330.
66. **Otaka, E., K. Higo, and S. Osawa.** 1982. Isolation of seventeen proteins and amino-terminal amino acid sequences of eight proteins from cytoplasmic ribosomes of yeast. Biochemistry **21**:4545–4550.
67. **Otaka, E., and S. Osawa.** 1981. Yeast ribosomal proteins. V. Correlation of several nomenclatures and proposal of a standard nomenclature. Mol. Gen. Genet. **181**:176–182.
68. **Otaka, E., K. Suzuki, and T. Hashimoto.** 1990. Examination of protein sequence homologies. VII. The complementary molecular coevolution of ribosomal proteins equivalent to *Escherichia coli* L7/L12 and L10. Protein Seq. Data Anal. **3**:11–19.

69. **Ozeki, H., H. Iuokuchi, F. Yamao, M. Kodaira, H. Sakano, T. Ikemura, and Y. Shimura.** 1980. Genetics of nonsense suppressor tRNAs in *Escherichia coli*, p. 341–362. *In* D. Soll, J. N. Abelson, and P. R. Schimmel (ed.), Transfer RNA: biological aspects. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
70. **Pelid-Yalif, E. H., D. Cohen-Binder, and O. Meyuhas.** 1984. Isolation and characterization of four mouse ribosomal protein L18 genes that appear to be processed pseudogenes. Gene **29:**157–166.
71. **Piepersberg, W., A. Bock, and H. G. Wittmann.** 1975. Effects of different mutations in the ribosomal protein S5 of *E. coli* on translational fidelity. Mol. Gen. Genet. **140:**91–100.
72. **Piepersberg, W., A. Bock, M. Yaguchi, and H. G. Wittmann.** 1975. Genetic position and amino acid replacements of several mutations in ribosomal protein S5 from *Escherichia coli*. Mol. Gen. Genet. **143:**43–52.
73. **Piepersberg, W., D. Geyl, H. Hummel, and A. Bock.** 1980. Physiology and biochemistry of bacterial ribosomal mutants, p. 359–377. *In* S. Osawa, H. Ozeki, H. Uchida, and T. Yura (ed.), Genetics and evolution of RNA polymerases, tRNA and ribosomes. Tokyo University Press, Tokyo.
74. **Planta, R. J., W. H. Mager, R. J. Leer, L. P. Woudt, H. A. Raue, and T. El-Baradi.** 1986. Structure and expression of ribosomal protein genes in yeast, p. 699–718. *In* B. Hardesty and G. Kramer (ed.), Structure, function and genetics of ribosomes. Springer-Verlag, New York.
75. **Proudfoot, N. J., and G. G. Brownlee.** 1985. 3′ noncoding sequences in eukaryotic messenger RNA. Nature (London) **263:**211–214.
76. **Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labeling DNA to high specific activity *in vitro* by nick translation with DNA polymerase I. J. Mol. Biol. **113:**237–251.
77. **Rosset, R., and L. Gorini.** 1969. A ribosomal ambiguity mutation. J. Mol. Biol. **39:**95–107.
78. **Rotenberg, M. O., and J. L. Woolford.** 1986. Tripartite upstream promoter element essential for expression of *Saccharomyces cerevisiae* ribosomal protein genes. Mol. Cell. Biol. **6:**674–687.
79. **Rothstein, R.** 1983. One-step gene disruption in yeast. Methods Enzymol. **101:**202–209.
80. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
81. **Schimmang, T., D. Tollervey, H. Kern, R. Frank, and E. C. Hurt.** 1989. A yeast nucleolar protein related to mammalian fibrillarin is associated with small nucleolar RNA and is essential for viability. EMBO J. **8:**4015–4024.
82. **Sharp, P. M., T. M. F. Tuohy, and K. R. Mosurski.** 1986. Codon usage in yeast: cluster analysis clearly differentiates highly and lowly expressed genes. Nucleic Acids Res. **14:**5125–5143.
83. **Sherman, F., and C. W. Lawrence.** 1974. Saccharomyces, p. 359–393. *In* R. C. King (ed.), Handbook of genetics. Plenum Publishing Corp., New York.
83a. **Slynn, G., D. Jenner, W. Potts, P. Elvin, J. E. N. Morten, and A. F. Markham.** 1990. Human cDNA sequence homologous to the mouse LLRep3 gene family. Nucleic Acids Res. **18:**681.
84. **Steege, D. A., and D. G. Soll.** 1979. Suppression, p. 433–485. *In* R. F. Goldberger (ed.), Biological regulation and development, vol. 1. Plenum Publishing Corp., New York.
85. **Tapio, S., and C. S. Kurland.** 1986. Mutant *Ef-tu* increases missense error *in vitro*. Mol. Gen. Genet. **205:**186–188.
86. **Teem, J. L., N. Abovich, N. F. Kaufer, W. F. Schwindinger, J. R. Warner, A. Levy, J. Woolford, J. R. Leer, M. M. C. vanRaamsdonk-Duin, W. H. Mager, R. J. Planta, L. Schultz, J. D. Friesen, H. Fried, and M. Roshash.** 1984. A comparison of yeast ribosomal protein gene DNA sequences. Nucleic Acids Res. **12:**8295–8312.
87. **Thompson, R. C.** 1988. EF-Tu provides an internal kinetic standard for translational accuracy. Trends Biochem. Sci. **13:**91–93.
88. **Tuite, M. F.** 1989. Protein synthesis, p. 161–204. *In* A. H. Rose and J. S. Harrison (ed.), Yeasts, vol. 3, 2nd ed. Academic Press, Inc., New York.
89. **Vijgenboom, E., T. Vink, B. Kraal, and L. Bosch.** 1985. Mutants of the elongation factor EF-Tu, a new class of nonsense suppressors. EMBO J. **4:**1049–1052.
90. **Vincent, A., and T. D. Petes.** 1989. Mitotic and meiotic gene conversion of Ty elements and other insertions in *Saccharomyces cerevisiae*. Genetics **122:**759–772.
91. **Wakem, L. P., and F. Sherman.** 1990. Isolation of omnipotent suppressors in the yeast *Saccharomyces cerevisiae*. Genetics **124:**515–522.
92. **Warner, J. R.** 1989. Synthesis of ribosomes in *Saccharomyces cerevisiae*. Microbiol. Rev. **53:**256–271.
93. **Wiedemann, L. M., and R. P. Perry.** 1984. Characterization of the expressed gene and several processed pseudogenes for the mouse ribosomal protein L30 gene family. Mol. Cell. Biol. **4:**2518–2528.
94. **Wilke, C. M., and S. W. Liebman.** 1989. Integration of an aberrant retrotransposon in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **9:**4096–4098.
95. **Wilson, P. G., and M. R. Culbertson.** 1988. *SUF12* suppressor protein of yeast: a fusion protein related to the EF-1 family of elongation factors. J. Mol. Biol. **199:**559–573.
96. **Winston, F., F. Chumley, B. Valent, and G. R. Fink.** 1984. Eviction and transplacement of mutant genes in yeast. Methods Enzymol. **101:**211–228.
97. **Wittmann, H. G.** 1986. Structure of ribosomes, p. 1–27. *In* B. Hardesty and G. Kramer (ed.), Structure, function and genetics of ribosomes. Springer-Verlag, New York.
98. **Wittmann, H. G., and G. Stoffler.** 1974. Altered S5 and S20 ribosomal protein in revertants of an alanyl-tRNA synthetase mutant of *Escherichia coli*. Mol. Gen. Genet. **134:**225–236.
99. **Wittmann-Liebold, B., and B. Greuer.** 1978. The primary structure of the protein S5 from the small subunit of the *Escherichia coli* ribosome. FEBS Lett. **95:**91–98.
100. **Wool, I. G., Y. Endo, Y.-L. Chan, and A. Glück.** 1990. Structure, function, and evolution of mammalian ribosomes, p. 203–214. *In* W. E. Hill, A. Dahlberg, R. A. Garrett, P. B. Moore, D. Schlessinger, and J. R. Warner (ed.), The ribosome: structure, function, and evolution. American Society for Microbiology, Washington, D.C.
101. **Woudt, L. P., A. B. Smit, W. H. Mager, and R. J. Planta.** 1986. Conserved sequence elements upstream of the gene encoding yeast ribosomal protein L25 are involved in transcription activation. EMBO J. **5:**1037–1040.