Yeast omnipotent supressor SUP1 (SUP45): nucleotide sequence of the wildtype and a mutant gene

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ABSTRACT

The primary structures of the yeast recessive omnipotent supressor gene $\underline{SUP1}$ ($\underline{SUP45}$) and one of its mutant alleles ($\underline{sup1-ts36}$) was determined. The gene codes for a protein of 49 kD. The mutant protein differs from the wildtype form in one amino acid residue (Ser instead of Leu) in the N-terminal part. The codon usage differs significantly from that of yeast ribosomal protein genes. However, an upstream element resembling a conserved oligonucleotide in the region 5' to ribosomal protein genes in S. cerevisiae has been found. A DNA probe internal to the $\underline{SUP1}$ gene does not exhibit detectable homology to genomic DNA neither from higher eucaryotes nor from eu- or archaebacteria. The hypothetical function of this protein in control of translational fidelity is discussed.

INTRODUCTION

The phenomenon of omnipotent recessive supression in Saccharomyces cerevisiae has been known for long years (1), but was never elucidated on a biochemical basis. Mutations resulting in this phenotype were mapped at two genetic loci, SUP1 (SUP45) and SUP2 (SUP35), on chromosomes IIR and IVR, respectively, and induce pleiotropic effects on various cellular functions (2,3). Similarities with certain bacterial (Escherichia coli) mutants altered in ribosomal proteins S4 (ramA) and S5 (ramC) (4), and with another omnipotent but dominant suppressor mutation in yeast (SUP46), altered in a small ribosomal subunit protein (5), suggested that SUP1 and SUP2 genes may code for ribosomal proteins. Recently the SUP1 structural gene has been cloned from S. cerevisiae (6), and a 1.6 kb transcript and a 54 kd protein have been identified (7). Sequence determination revealed an unsplit gene coding for a protein of 49 kd molecular weight and deviating in codon usage significantly from the one typical for

ribosomal protein genes and other highly expressed genes in yeast (8, 9). The DNA sequence of a mutant gene (<u>sup1-ts36</u>) (6) differed in several nucleotides from that of the wildtype only one of which led to an amino acid exchange in the N-terminal segment of the sup1 protein.

MATERIALS AND METHODS

Organisms, plasmids and transformation

Yeast strain 7B-D244, Escherichia coli HB101 (as a plasmid receptor), the derivation of plasmids pPBM10 (SUP1) and pPBM25 (sup1-ts36), and the conditions for transformation of DNA were described earlier (6). Plasmids pPBM102 and pPBM1022 were constructed by replacing the small SalI/XbaI fragment of pACYC184 (10) by the 2.6 kb SalI/XbaI fragments from pPBM25 and pPBM10, respectively. As a yeast/E. coli shuttel vector YEp13 (11) was used.

Preparation and manipulation of DNA and RNA

Preparations of DNA, total cellular RNA and polyA+ mRNA were performed as given elsewhere (6,12). Restriction endonuclease cleavage, ligation and other enzymatic treatment was carried out as recommended by the suppliers of the enzymes (Boehringer-Mannheim, New England Biolabs). Hybridization of radioactively labelled DNA probes was done according to Southern (13). Samples of genomic DNA were from the following sources: Methanococcus vannielii DSM 1224, human placenta, mouse spleen (line C57Bl/6) and wheat were kindly provided by H. Auer and J. Hauber, Munich. Sequence analysis and S1 mapping

The nucleotide sequences of the cloned <u>SUP1</u> alleles were determined using the chemical modification and cleavage method (14). Nuclease S1 mapping of the 5' end of the <u>SUP1</u> mRNA was determined and interpreted as described by others (12, 15-17).

In vitro translation

Translation of mRNA selected as hybrids towards the 1060 bp BglII/BamHI fragment, internal to the <u>SUP1</u> coding region, was carried out in a rabbit reticulocyte system (Amersham-Buchler) according to published procedures (12).

Computer analysis

The protein sequence data base was that of the PIR, release 4.0,

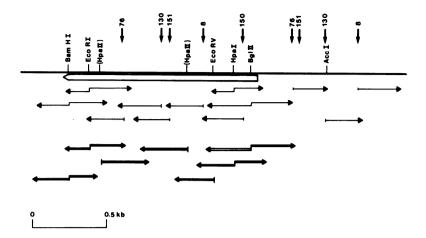


Fig. 1. Strategy for sequencing the gene SUP1 (single-lined arrows) and its mutant allele (sup1-ts36) (double-lined arrows) from S. cerevisiae. The bar represents the SUP1 coding region (see Fig.2). Restriction endonuclease cleavage sites are symbolized as follows: Ac = AccI, B = BamHI, Bg = BglII, E = EcoRI, EV = EcoRV, Hp = HpaI, Mb = MboI, X = XhoI, Xb = XbaI; brackets mean that not all of the sites for the respective enzyme are shown; the numbered vertical arrows mark the location of HindIII sites introduced into the wildtype sequence (see Materials and Methods).

National Biomedical Research Foundation (Washington, D.C., USA). All comparisons were run on a VAX/VMS.

RESULTS AND DISCUSSION

The cloning, subcloning and identification of the <u>SUP1</u> (<u>SUP45</u>) structural gene of <u>S. cerevisiae</u> and of its mutant allele <u>sup1-ts36</u> was described earlier (6). Because of lacking information about the nature of the gene product, we decided to sequence the gene localized on a 2.6 kb BamHI/XbaI fragment. The strategies for sequencing both the wildtype and mutant (<u>sup1-ts36</u>) alleles are illustrated in Fig.1. The sequence derived contained only a single long open reading frame coding for a hypothetical polypeptide of 438 amino acids and about 49kD molecular weight (Fig.2). This is in agreement with the finding of a single RNA band of 1.5 to 1.6 kb approximate size hybridizing against the coding segment (7; our own observations). In vitro a protein of 50 kD monomer size was translated from

-987 ACTTGCTGTAGCTCTATTCTCTTTCCCAACCACCTTT	TCCTTTATTCCAAAATTTTTAAAACTTTTCTGTTACATTATATAATCTTC							
-900 TGTCTGAAATGTTTGGATATAACGCCTCTTGATCCACTTTGTATATGCGT	GCTATTTATTTTCAGATTTATAAAGAGTATAAGCGTCATTTACATAAATA							
-800 GCTGAAGTTATTCATGGAAAATACGAAGAGCACGYATGTGAGCCAACAGA	ACATTTGACGTAAGACTCTACAATGTGCCAAGAACTGGACAAGTAGAGGA							
-700 CTGAGAACTTTATTTCAATTCATTGCTCCTTTTTGGTTGCGCTACCTTTA	GCGAAGGTCAATGATGAATGTGCACATGCTGTCGAAACCAAAAGCAAATT							
-600 CTAACCAACTTCAAAATGACATAGTCATCTGATATTTCTACTCATTATAG	ATAGTATGGGAGCCTTGAAACGAAAAGTAAAAGCTGGATATGAGC							
-500 Acci ACTATGAGGTAGACCTTAGCTACATCATTTCCCCCAATAGCTGCTGCAAA	TATCTGGTTAAATTTGTGATTCCATGAAGAGGATAACAGACTTGTTAAAA							
-400 AGCATCCTGTCAAAATCTAATTTTTGAAGGGCAGTATTCAATTCATAATT	TACTTTAGCTTAGATCCTTCCAATTTATGGTATTATAACCTGATCATAAA							
-300 CTACAATCTGTCGCTACCGCATGTACGAAGAATACTTAAGTCACTTGCTC	TCTCĀŤĈĀŤŤĠŤĀĈĀŤŦŤTTCAGTAATACCGTTTGATAGCGCCGTCTTT							
-200 *T ATTACCCGGATTATTCCGTTGACCCTGAATGAAAAATTTTTTCAGAAATC	CAGTGCTAAGCGTCAAATCAATGAAATACATCACTGTATTTTTAACTGAT							
-100 ATACTGTTGGTGTGGCCTTAACGACACCTTTATTTCTTAATTCATTTCGG	CTTGTCTCCTTATTAAGACTACAGAAATAGACAAAGGAAATACTTCAATA							
BglII ATG GAT AAC GAG GTT GAA AAA AAT ATT GAG ATC TGG AAG MET Asp Asn Glu Val Glu Lys Asn Ile Glu Ile Trp Lys	78 GTC AAG AAG TTG GTC CAA TCT TTA GAA AAA GCT AGA GGT Val Lys Lys Leu Val Gln Ser Leu Glu Lys Ala Arg Gly							
C AAT GGT ACT TCT ATG ATT TCC TTA GTT ATT CCT CCT AAG Asn Gly Thr Ser Met Ile Ser Leu Val Ile Pro Pro Lys	HpaI 156 GGT CTA ATT CCA CTG TAC CAA AAA ATG TTA ACA GAT GAA Gly Leu Ile Pro Leu Tyr Gln Lys Met Leu Thr Asp Glu							
27 Ser TAT GGT ACT GCC TCG AAT ATT AAA TCT AGG GTT AAT CGT	234 CTT TCC GTT TTA TCT GCT ATC ACT TCC ACC CAA CAA AAG							
Tyr Gly Thr Ala Ser Asn Ile Lys Ser Arg Val Asn Arg	Page V							
TTG AAG CTA TAT AAT ACT TTG CCC AAG AAC GGT TTA GTT Leu Lys Leu Tyr Asn Thr Leu Pro Lys Asn Gly Leu Val 79	390							
AAA AAG GTC ACT TTT GAC ATC GAA CCT TAC AAA CCT ATC Lys Lys Val Thr Phe Asp Ile Glu Pro Tyr Lys Pro Ile 105	AAC ACA TCC TTA TAT TTG TGT GAT AAC AAA TTT CAT ACA Asn Thr Ser Leu Tyr Leu Cys Asp Asn Lys Phe His Thr							
GAA GTT CTT TCG GAA TTG CTT CAA GCT GAC GAC AAG TTC Glu Val Leu Ser Glu Leu Leu Gln Ala Asp Asp Lys Phe 131	GGT TTT ATA GTC ATG GAC GGT CAA GGT ACT TTG TTT GGT Gly Phe Ile Val Met Asp Gly Gln Gly Thr Leu Phe Gly							
TCT GTG TCC GGT AAT ACG AGA ACT GTT TTA CAT AAA TTT Ser Val Ser Gly Asn Thr Arg Thr Val Leu His Lys Phe 157	T 546 ACT GTC GAT CTG CCA AAA AAG CAT GGT AGA GGT GGT CAA Thr Val Asp Leu Pro Lys Lys His Gly Arg Gly Gly Gln							
TCT GCG CTT CGT TTT GCT CGT TTA AGA GAA GAA AAA AGA Ser Ala Leu Arg Phe Ala Arg Leu Arg Glu Glu Lys Arg 183	CAT AAT TAT GTG AGA AAG GTC GCC GAA GTT GCT GTT CAA His Asn Tyr Val Arg Lys Val Ala Glu Val Ala Val Gln							
AAT TTT ATT ACT AAT GAC AAA GTC AAT GTT AAG GGT TTA Asn Phe Ile Thr Asn Asp Lys Val Asn Val Lys Gly Leu 209	$\begin{array}{c} & C & 702 \\ \text{ATT TTA GCT GGT TCT GCT GAC TTT AAG ACC GAT TTG GCT} \\ \text{Ile Leu Ala Gly Ser Ala Asp Phe Lys Thr Asp Leu Ala} \end{array}$							
AAA TCT GAA TTA TTC GAT CCA AGA CTA GCA TGT AAG GTT Lys Ser Glu Leu Phe Asp Pro Arg Leu Ala Cys Lys Val 235	780							
TTC AAC CAG GCT ATC GAA CTT TCT GCC GAA GCG TTG GCC Phe Asn Gln Ala Ile Glu Leu Ser Ala Glu Ala Leu Ala	858 AAT GTC AAG TAT GTT CAA GAA AAG AAA TTA TTG GAG GCA Asn Val Lys Tyr Val Gin Glu Lys Lys Leu Leu Glu Ala							
261 TAT TTT GAC GAA ATT TCC CAG GAC ACT GGT AAA TTC TGT Tyr Phe Asp Glu Ile Ser Gln Asp Thr Gly Lys Phe Cys 287	TAT GGT ATA GAT GAT ACT TTA AAG GCA TTG GAT TTA GGT							
	6 1014							
GCA GTC GAA AAA TTA ATT GTT TTC GAA AAT TTG GAA ACT Ala Val Glu Lys Leu Ile Val Phe Glu Asn Leu Glu Thr 313								
ATA AAA TTC GCT GAA CCA GAA GCC AAG GAC AAG TCG TTT Ile Lys Phe Ala Glu Pro Glu Ala Lys Asp Lys Ser Phe 339								
TCC GAA GAA CCT TTA ATT GAA TGG CTA GCA GCT AAC TAC Ser Glu Glu Pro Leu Ile Glu Trp Leu Ala Ala Asn Tyr 365								
TCT TCA GAA GGT GCC CAA TTT GTC ACA GGT TTT GGT GGT Ser Ser Glu Gly Ala Gln Phe Val Thr Gly Phe Gly Gly 391	Ile Gly Ala Met Leu Arg Tyr Lys Val Asn Phe Glu Gln							
CTA GTT GAT GAA TCT GAG GAT GAA TAT TAT GAC GAA GAT Leu Val Asp Glu Ser Glu Asp Glu Tyr Tyr Asp Glu Asp 417	BamHI 1330 GAA GGA TCC GAC TAT GAT TTC ATT TAAATAAATAAAAGGGGGA Glu Gly Ser Asp Tyr Asp Phe Ile							
A. GAAAAAAATCGAATCAAAAAGAATTTAATCACTAGATGCCAGATTTAAGT	TAAATTCGCTTTTAATTTTTTGTACAATATAATATATATA							
G	AGGACCGTGCATATACGTAGAAAATACAGTGAAAGGAGAGTTTCTCTTCA							
1559 AAAGCCTCGAGATTAATTATTTCTCTTTT								

hybrid selected polyA+ mRNA (not shown), and is probably identical to the protein (54 kD) shown by others to be translated from a transcript read from the same chromosomal segment (7). The stop codon (TAA) is the same as in most other yeast genes. It is concluded that the open reading frame corresponds to the SUP1 structural gene.

Most yeast ribosomal protein genes (15 out of 17 sequenced) contain introns close to their 5' ends (8, 9). Especially a 100% conserved oligonucleotide (TACTAAC) is present in all introns. However, the SUP1 gene is unsplit, and no sequences resembling those characteristic for introns in S. cerevisiae are found in the whole sequence. The amino acid composition (Table 1) reveals a more acidic nature of the SUP1 protein unlike most ribosomal proteins. Also, the codon usage deviates considerably from the one typically used in yeast ribosomal protein genes (Table 2), or other highly expressed yeast genes (10). Together with the observation that the SUP1 (SUP45) gene is expressed at much lower rate as cytoplasmic ribosomal proteins (7) and the fact that the protein translated is larger than all known ribosomal proteins in S. cerevisiae, all this suggests that the earlier hypotheses (18, 19) concerning the nature of the SUP1 gene product have to be modified: If the protein is a translational factor interacting with cytoplasmic ribosomes, it is not an integral and stoichiometric component of the organelle. The presumed SUP1 (SUP45) transcript was roughly mapped by others to start beween the HpaI and the BglII sites (7; cf. Fig. 1). S1 mapping, however, revealed that the transcript started left of the BglII site (Fig.3) and thereby proved that the first ATG in the open reading frame is most likely the gene start.

Fig. 2. Nucleotide and deduced amino acid sequence of the SUP1 gene of S. cerevisiae. The location of relevant restriction sites is indicated. The arrows mark the transcription initiation sites within the purin-rich block (36 nucleotides) 5' to the initiation codon. The oligonucleotide resembling the conserved element HOMOL1, found upstream of several ribosomal protein genes of yeast (8, 9), is marked by a broken line. Nucleotide exchanges found in the $\frac{\sup 1-ts 36}{\sup 1-ts 36}$ sequence are written over the respective position of the wildtype sequence, one of which results in an amino acid exchange from Leu to Ser (pos. 34 of the protein sequence).

Amino acid	Number	Percentage
Ala	29	6.62
Arg	13	2.97
Asn	22	5.02
Asp	32	7.31
Cys	4	0.91
Gln	14	3.20
Glu	39	8.90
Gly	31	7.08
His	4	0.91
Ile	27	6.16
Lys	41	9.36
Leu	42	9.59
Met	6	1.37
Phe	23	5.25
Pro	10	2.28
Ser	25	5.71
Thr	24	5.48
Trp	2	0.46
Tyr	17	3.88
Val	32	7.31

Table 1. Calculated amino acid composition of the yeast $\underline{SUP1}$ protein

There are other features of DNA primary structure in the region upstream of the <u>SUP1</u> gene supporting this conclusion: (i) the transcript starts in a poly-purin stretch, and (ii) its initiation site is preceded by a typical poly-pyrimidin block (pos. -37 to -74; Fig.2). (iii) A TATA-box (around pos. -100) is

438

99.77

Table	2.	Codon	usage	of	the	yeast	SUP1	gene	and	yeast
		riboso	omal bi	cote	ein d	renes		-		_

total

				F		5	-								
		SUP1	YRPa)			SUP1	YRP			SUP1	YRP			SUP1	YRP
Phe	UUU	14	4	Ser	UCU	12	53	Tyr	UAU	14	6	Cys	UGU	4	8
Phe	UUC	9	55	Ser	UCC	9	35	Tyr	UAC	2	47	Cys	UGC	-	1
Leu	UUA	17	16	Ser	UCA	1	5	-		1	11	•		_	-
Leu	UUG	12	107	Ser	UCG	3	-			-	-	Trp	UGG	2	13
Leu	CUU	5	1	Pro	CCU	5	6	His	CAU	5	12		CGU		26
Leu	CUC	-	-	Pro	CCC	1	1	His	CAC	-	31	Arg	CGC	-	_
Leu	CUA	5	11	Pro	CCA	4	64	Gln	CAA	12	55	Arg	CGA	-	-
Leu	CUG	3	-	Pro	CCG	-	-	Gln	CAG	2	1	Arg	CGG	-	-
Ile	AUU	14	46	Thr	ACU	13	53	Asn	AAU	14	6	Ser	AGU	-	2
Ile	AUC	10	50	Thr	ACC	4	43	Asn	AAC	8	58	Ser	AGC	_	4
Ile	AUA		-	Thr	ACA	6	2	Lys	AAA	22	31	Arg	AGA	8	151
Met	AUG	6	37	Thr	ACG	1	-	Lys	AAG	19	168	Arg	AGG	1	1
Val	GUU	18	87	Ala	GCU	15	113	Asp	GAU	18	37	Gly	GGU	29	138
Val	GUC	11	77	Ala	GCC	8	38	Asp	GAC	14	46	Gly	GGC	1	5
Val	GUA	-	-	Ala	GCA	5	5	Glu	GAA	33	97	Gly	GGA	1	2
Val	GUG	3	3	Ala	GCG	2	2	Glu	GAG	6	2	Gly	GGG	-	1

a) from 11 yeast ribosomal protein genes, compiled from Ref. 8.

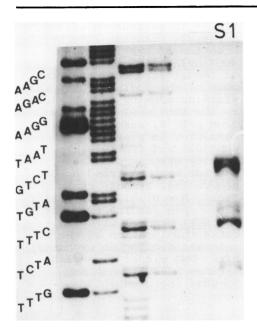


Fig. 3. S1 nuclease mapping of the 5' end of the SUP1 mRNA. For duplex formation the 254 bp HaeIII/TaqI fragment from position -84 through +170 (see Fig.2) was used. The sequencing reaction was started at the TaqI site; only the relevant section is shown.

present in a distance where this element is also found ahead of many other yeast genes, which is thought to have its significance in the initiation of transcription by RNA polymerase II, and which gave rise to controversial discussions (20, 21). (iv) In a distance (around pos. -240), where upstream of ribosomal protein genes a conserved oligo-nucleotide, called HOMOL1, was observed (3, 9), a very similar sequence was found (Fig.2). The last finding also could mean that the gene may be recognized by factors modulating transcription of components of the translational apparatus, and, therefore, is somehow co-regulated together with ribosomal protein genes.

The mutant allele ($\underline{\sup 1-ts36}$) from strain 78-D244 when sequenced (cf. Fig.1, 2) showed several nucleotide exchanges in and outside the coding segment, which probably reflects that the allele was selected in a strain of \underline{S} . cerevisiae differing in derivation from the one ($\underline{S283C}$) form which the wildtype allele was cloned (6; M.D. Ter-Avanesyan, personal communication). Only one nucleotide exchange (T to C; pos. +101 in Fig.2), however, was non-conservative and led to an amino acid exchange (Leu to Ser) in the N-terminal part of the $\underline{SUP1}$ protein. Additional prove, that the mutation was located in this segment of the gene,

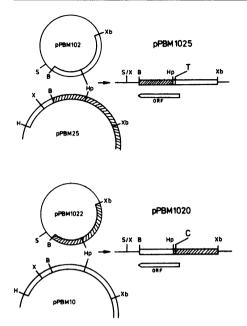


Fig. 4. Localization of the sup1-ts36 mutation site by creation of hybrid SUP1/ sup1-ts36 alleles. The
SalI=HpaI fragments covering most of the coding region in plasmids pPBM102 and pPBM1022 by the XhoI/HpaI fragments from pPBM10, respectively, resulting in plasmids pPBM1025 and pPBM1020. The primary structure of both hybrids was confirmed by DNA sequencing. Symbols as in Fig.1, H = HindIII, S = SalI; ORF = open reading frame of the SUP1 gene; T or C indicates the location of the relevant nucleotide exchange; bars: DNA cloned from yeast strains S288C (open) and 7B-D244 (hatched).

came from a fragment exchange experiment (Fig.4): Complementation in strain 7B-D244 of the sup1-ts36 mutation, resulting in a temperature-resistant and adenine-requiring phenotype (6), could only be achieved, when the BamHI/XbaI fragment from pPBM1025 was introduced into vector YEp13 (BamHI/XbaI) and transformed. The same fragment derived from pPBM1020, however, was not able to relieve the suppressor phenotype of the mutant allele. Therefore, the conclusion seems justified, that this segment within the SUP1 protein is of major importance for its function, and, when slightly altered, exerts a severe effect on various other cellular functions. Alterations immediately at the C-terminus, on the other hand, for instance fusions at the single BamHI site (replacing the 5 terminal amino acids) as in the sup1-ts36 complementing clone pPBM8 (6) do not seem to interfere with the proteins function.

The function of the <u>SUP1</u> protein remains a mystery. Some elucidation could come from comparison of the nucleotide and amino acid sequences with the library of sequence data available. Interesting, though weak, homologies were found with some amino-acyl-tRNA synthetase sequences: (i) Methionyl-tRNA synthetase

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A

Metrs S.c. Thr asp Glu Tyr Gly Thr Ala Thr Glu Thr Lys Ala Leu Act Gat GaA Tat GGt ACT GCC ACG GAA ACT AAA GCT TTG G **

SUP1 S.c. ACG AT GAA TAT GGT ACT GCC TCG AAT ATT AAA TCT AGG G Thr Asp Glu Tyr Gly Thr Ala Ser Asn Ile Lys Ser Arg

B

30 40 50

Tyrrs B.s. L N E E R V T L Y C G F D P T A D S L H I G H L A Tyrrs E.c. L A Q G P I A L Y C G F D P T A D S L H I G H L V

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SUP1 S.c. L P K N G L V L Y C G D I I T E D G K E K K V T F
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Fig. 5. Comparison of the SUP1 sequence with sequences of aminoacyl-tRNA synthetases. (A) Area of homology to the MetRS gene from S. cerevisiae (22). Asterisks mark homologous base pairs; amino acid homologies are boxed; the numbers refer to the amino acid residues within the protein. (B) Protein homology between TyrRS from Bacillus stearothermophilus (23) and E. coli (24) with SUP1. Homologous amino acids are indicated by asterisks; the overlined peptide indicates an area highly conserved in several aminoacyl-tRNA synthetases (23).

(MetRS) from yeast (22) contains a stretch of accordance in both nucleotide and polypeptide sequence, where the respective rading frames are in phase with each other (Fig.5A).(ii) Homology on the protein level exists between SUP1 sequence and a segment from tyrosyl-tRNA synthetases (TyrRS) from B. stearothermophilus (23) and E. coli (24), which is highly conserved between those two enzymes in primary and probably tertiary structure (25), and close to a sequence conserved in several tRNA synthetases (23) (Fig.5B). Also the predicted secondary structure of the SUP1 protein in this small segment is consistent with the one described for the TyrRS polypeptides, where this section could be part of the ATP binding site (26). (iii) Another weak homology exists between the N-terminal portions (when the first 100 amino acids were compared) of both the ras2 gene product (27) and the SUP1 protein. Again the N-terminal half is highly conserved between ras-like factors (27). All this could indicate that SUP1 protein is a nucleotide or tRNA binding factor controlling, directly or indirectly, the codon-anticodon fitting during cytoplasmic translation. Though present probably in sub-stoichiometric amounts relative to the 80S ribosomes (7), it would at least part of the time interact with nascent or mature ribosomes, as can be judged from the pleiotropic effects on ribosome structure and function it exerts (3, 18, 19).

Since factor <u>SUP1</u> is an essential protein in yeast it could be conserved also in other organisms. However, when a DNA fragment internal to the coding region is used as a probe for hybridization against total genomic DNA, from human, mouse, plant, eubacterial (<u>E. coli</u>) or archaebacterial (<u>Methanococcus vanniellii</u>) origin, digested by various restriction enzymes, no homology was found (not shown). Therefore the factor is either unique to lower eucaryotes, or it was not highly conserved during evolution.

The gene isolation and the derived structure of the coded protein of yeast <u>SUP1</u> factor will make it possible in future to express the gene product in bacteria, to purify and test it for its effects on in vitro translation in a highly purified yeast system. Also it will be possible now to test for its relationship to the gene product(s) encoded by <u>SUP2</u> (<u>SUP35</u>) since this gene was cloned recently (A.P. Surguchov, personal communication).

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