MOLECULAR AND CELLULAR BIOLOGY, Apr. 1985, p. 816–822
0270-7306/85/040816-07$02.00/0

Vol. 5, No. 4

Isolation of the *SUP45* Omnipotent Suppressor Gene of *Saccharomyces cerevisiae* and Characterization of Its Gene Product

H. J. HIMMELFARB, E. MAICAS, AND J. D. FRIESEN*

Department of Medical Genetics, University of Toronto, Toronto, Ontario, Canada M5S 1A8

Received 27 September 1984/Accepted 8 January 1985

The *Saccharomyces cerevisiae SUP45*$^+$ gene has been isolated from a genomic clone library by genetic complementation of paromomycin sensitivity, which is a property of a mutant strain carrying the *sup45-2* allele. This plasmid complements all phenotypes associated with the *sup45-2* mutation, including nonsense suppression, temperature sensitivity, osmotic sensitivity, and paromomycin sensitivity. Genetic mapping with a *URA3*$^+$-marked derivative of the complementing plasmid that was integrated into the chromosome by homologous recombination demonstrated that the complementing fragment contained the *SUP45*$^+$ gene and not an unlinked suppressor. The *SUP45*$^+$ gene is present as a single copy in the haploid genome and is essential for viability. In vitro translation of the hybrid-selected *SUP45*$^+$ transcript yielded a protein of M_r = 54,000, which is larger than any known ribosomal protein. RNA blot hybridization analysis showed that the steady-state level of the *SUP45*$^+$ transcript is less than 10% of that for ribosomal protein L3 or rp59 transcripts. When yeast cells are subjected to a mild heat shock, the synthesis rate of the *SUP45*$^+$ transcript was transiently reduced, approximately in parallel with ribosomal protein transcripts. Our data suggest that the *SUP45*$^+$ gene does not encode a ribosomal protein. We speculate that it codes for a translation-related function whose precise nature is not yet known.

Omnipotent suppressor mutants of the yeast *Saccharomyces cerevisiae* are named for their ability to suppress simultaneously UAG, UGA, and UAA nonsense mutations. These suppressors were first identified by Inge-Vechtomov and Andrianova (19) and were mapped to two loci that have been designated *sup35* and *sup45* (15), *sup2* and *sup1* (19), or *supP* and *supQ* (10). *sup45* and a more recently isolated omnipotent suppressor, *sup46* (31), map near *lys2* on chromosome 2R but are presumably distinct (31), and *sup35* is on chromosome 4R (15). Unlike tRNA suppressors, omnipotent suppressors are usually recessive; they display a variety of allele-specific pleiotropic effects in vivo, including osmotic sensitivity, high or low temperature sensitivity, respiratory deficiency, and sensitivity to aminoglycoside antibiotics such as paromomycin (15, 19, 46, 47). Ribosomes isolated from omnipotent suppressor strains have been reported to show increased misreading in vitro (43, 44) and, at least for *sup46* (26), this misreading is enhanced by paromomycin. Paromomycin induces phenotypic suppression of nonsense and presumed missense mutations (4, 42) and has been shown to decrease the fidelity of translation in vitro, both in *S. cerevisiae* (33, 42) and in *Escherichia coli* (5) by binding to ribosomes (33). In vivo, *sup45* and paromomycin have been shown to act synergistically in their suppression of nonsense mutations of nutritional markers (46). Ribosomes purified from *sup1* (*sup45*) mutant strains show an increased dissociability into subunits in vivo (44). One low-temperature-sensitive allele of *sup1* (reference 45) is defective in 60S subunit assembly. All in all, the characteristics of omnipotent suppressors suggest that their phenotype is due to a ribosomal alteration that leads to decreased translation fidelity or perhaps aberrant translation termination.

Analogous suppression based on streptomycin-induced misreading has been well-characterized in *E. coli*. Translation fidelity both in vivo and in vitro is affected by mutations that alter specific ribosomal proteins. Mutations in *rpsL* (ribosomal protein S12; *strA*) increase translational fidelity (38), as do mutations which alter the structure of S17 (reference 2) and L6 (reference 21). In contrast, *ram* mutations in *rpsP* or *rpsE* (ribosomal protein S4 or S5) increase translational error frequencies (3, 35, 38) above the estimated normal level of 6×10^{-4} per codon (7).

The characteristics of omnipotent suppressors of yeast, especially when viewed in the light of analogous mutants in bacteria, make them good candidates for the study of the eucaryotic translation machinery. To begin this, we have isolated the *SUP45*$^+$ gene by genetic complementation. Analysis of its RNA and protein products suggests that *SUP45*$^+$ is unlikely to be the structural gene for any of the currently recognized ribosomal proteins.

MATERIALS AND METHODS

Strains, plasmids, and growth conditions. *S. cerevisiae* strains SL183-21C [*MAT*α *met8-1*(Am) *tyr7-1*(Am) *trp1-1*(Am) *ade3-26*(Am) *ilv1-1*(Am) *his5-2*(Oc) *lys2-1*(Oc) *leu2-1*(Oc)] and its derivative SL428-1C (same as SL183-21C but *sup45-2*) were kindly provided by S. Liebman. Strains YHS11 [*MAT***a** *met8-1*(Am) *leu2-1*(Oc) *ilv1-1*(Am) *lys2-1*(Oc) *trp1-1*(Am) *ura3-52 sup45-2*], YEM35 [*MAT*α *met8-1*(Am) *leu2-1*(Oc) *ura3-52*] and YHU101 (*MAT***a**/*MAT*α *ADE1*/*ade1 LEU2*/*leu2-3,-112 ura3-52*/*ura3-52 HIS4*/*his4-912*) were constructed for this study. Strain LL20 (*MAT*α *leu2-3,-112 his3-11,-15*) was constructed by L. Lau. Strains A364A (*RNA2*$^+$) and the *rna2* mutant ts368 (13) were obtained from the Yeast Genetic Stock Center, University of California, Berkeley. *E. coli* K-12 strain JF1754 (*hsdR* Lac$^-$ Gal$^-$ *metB leuB hisB*) was used as the bacterial host. The cloning vehicle was pJM94, which is a yeast shuttle vector expressing *HIS3*$^+$, the herpes simplex thymidine kinase (TK$^+$) gene, and ampicillin resistance (27). pJH18 (from J. Haber) consists of pBR322 with the URA3$^+$ gene carried on a 1.1-kilobase (kb) *Hin*dIII DNA fragment.

* Corresponding author.

Growth and transformation of *S. cerevisiae* and *E. coli* were essentially as described previously (27). The complex rich medium used for the growth of yeast cells was YPD (1% yeast extract [Difco Laboratories], 2% Bacto-peptone, 2% glucose). The defined yeast medium consisted of 0.67% yeast nitrogen base (Difco) and 2% glucose, buffered to pH 5.8 with sodium succinate (10 g of succinic acid and 6 g of sodium hydroxide per liter). Required amino acids and nitrogenous bases were added as described previously (40). Selection for yeast transformants expressing TK^+ activity was accomplished by pouring 7 ml of transformed spheroplasts in regeneration agar (containing YPD plus 200 μg of thymidine per ml) on a petri plate, incubating this for 12 to 18 h at 34°C, and transferring the thin agar slab to the surface of YPD agar containing 5 mg of sulfanilamide per ml (Sigma Chemical Co.), 100 μg of amethopterin per ml (Lederle Laboratories), and 200 μg of thymidine per ml. Paromomycin sulfate was a gift of the Warner-Lambert Company.

Genetic mapping by integrative transformation. The entire DNA insert of pJH109 (see Fig. 1) carried on the vector pJH18 was linearized at the unique *Hpa*I or *Xho*I site and introduced into strain YHS11 by transformation, selecting for Ura^+. Complementation of the *sup45-2* phenotype (suppression of nonsense mutations; sensitivity to paromomycin) was verified. Several independent clones were mated to strain YEM35, and the diploids thus obtained were sporulated. The tetrads were dissected and grown on rich medium, and the phenotype of the haploids was determined by replica plating on defined medium.

DNA and RNA manipulations. Enzymes and reagents were purchased from Boehringer-Mannheim Canada, Bethesda Research Laboratories, New England Nuclear Corp., and New England Biolabs. Enzyme reactions were carried out according to procedures supplied by the manufacturers. DNA manipulations were by standard methods (25). Yeast DNA for blot-hybridization analysis was purified as described earlier (6). Total yeast RNA from cells growing exponentially in rich medium was prepared as described previously (17). DNA blotting, RNA blotting, nick translation, and nucleic acid hybridization have been described previously (25).

Quantitation of the *SUP45*$^+$ transcript. For measurement of the steady-state level of the *SUP45*$^+$ transcript relative to ribosomal protein (L3 and rp59) and nonribosomal protein (actin) transcripts, strain LL20 was grown at 30°C in 25 ml of defined medium supplemented with yeast extract (10 g/liter), peptone (20 g/liter), and leucine and histidine (50 μg/ml) (14). [2-^{3}H]adenine (New England Nuclear; 15.5 Ci/mmol) was added to a final concentration of 200 μCi/ml plus 10 μg of nonradioactive adenine per ml, and the cells were grown exponentially for at least three generations to an optical density at 600 nm (OD_{600}) of 1.6 (41). Samples were taken from this culture at various times during growth to ensure that when the sample (5 ml) was taken for the evaluation of steady-state mRNA levels (OD_{600} of 0.70; see Table 1), the specific radioactivity of cellular RNA (disintegrations per minute of [2-^{3}H]adenine per microgram of RNA) was constant (data not shown). RNA was extracted as described previously (17), and the integrity of the final RNA preparation was checked by RNA blot-hybridization analysis, probing with the cloned *SUP45*$^+$ gene (data not shown). DNA-RNA hybridization was performed as described below, except that the extracted RNA was precipitated once with an equal volume of 5 M LiCl before being added to the hybridization reaction.

For the pulse-labeling experiments, a 50-ml culture of strain LL20 was grown at 23°C in defined medium supplemented only with leucine and histidine (50 μg/ml) to OD_{600} = 0.70. The culture was then shifted to 36°C, and 10, 30, and 55 min thereafter, 4-ml aliquots were labeled for 7 min with [5-^{3}H]uracil (New England Nuclear; 27.2 Ci/mmol) at a final concentration of 200 μCi/ml. A 4-ml sample was similarly labeled immediately before the temperature shift. Each radioactive sample was then poured on crushed ice, and the cells were pelleted and washed once with 10 ml of ice-cold water. RNA was extracted as described previously (17).

The radioactive RNA preparations were hybridized to filter-bound plasmid DNA under conditions of DNA excess as described previously (20). Each 0.5-ml hybridization reaction contained 18 μg of radioactive RNA and five 10-mm nitrocellulose filter disks to each of which 30 μg of a different denatured plasmid DNA had been bound. The plasmids used were pBR322, pEM14 (the *SUP45*$^+$ gene fragment of pEM8 carried in pBR322; see below), pAV7 (the *tcm1* gene carried in pBR325; reference 39), pAV11 (the *cry1* gene carried in pBR325; reference 17), and pEM15 (the *S. cerevisiae* actin gene carried in pBR322; reference 28). In each case, the cloned yeast DNA fragment encodes only a single transcript. The mixtures were incubated for 70 h at 40°C with gentle shaking. The filters were then washed as described previously (20) and dried, and the radioactivity bound to each filter was determined in a toluene-based scintillation mix. The "dpm hybridized" shown in Table 1 were corrected for nonspecific ^{3}H binding to a filter blank.

Hybrid selection of the *SUP45*$^+$ transcript. Polyadenylated RNA from strain LL20 that was growing exponentially in rich medium was purified by oligodeoxythymidylate-cellulose (Collaborative Research, Inc.) affinity binding. Polyadenylated RNA (50 μg) was hybridized to 10 μg of plasmid DNA that had been heat denatured and bound to nitrocellulose as described previously (25). The selected RNA was translated in a wheat germ extract (Bethesda Research Laboratories) in the presence of [^{35}S]methionine (New England Nuclear). The products were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (22) and autoradiography. Ribosomal proteins for markers were prepared as described previously (11).

RESULTS

Cloning the *SUP45*$^+$ gene. *S. cerevisiae* SL428-1C carries the (recessive) *sup45-2* omnipotent suppressor mutation and the suppressible *met8-1*, *trp1-1*, and *tyr1-1* amber and *leu2-1* ochre alleles. In addition, strain SL428-1C is temperature sensitive for growth at 37°C, osmotically sensitive, and sensitive to a low concentration of paromomycin (1 mg/ml in YPD); wild-type *S. cerevisiae* is resistant to this concentration of the drug.

The wild-type *SUP45*$^+$ allele of the omnipotent suppressor gene was isolated from a clone library of partially digested *Sau*3A DNA fragments prepared from the DNA of *S. cerevisiae* YF138 (*SUP45*$^+$ *cry1*; reference 17). The vector pJM94 (27) carries the herpes simplex virus TK^+ gene and thus transformants may be selected in rich medium with appropriate additions (27) (see above). This was used to advantage in the present study because the *sup45-2* mutation suppresses nutritional markers that are often used for selection of yeast plasmids. Strain SL428-1C was transformed with the clone library as described above; transformants were selected on rich medium agar containing sulfanilamide, amethopterin, and thymidine to select for the vector-borne TK^+ marker, plus paromomycin at 1 mg/ml to select for *SUP45*$^+$ complementation of paromomycin sensitivity,

which is due to the *sup45-2* mutation. One TK^+, paromomycin-resistant clone was obtained. Plasmid DNA was prepared and was used to transform *E. coli* JF1754. A single plasmid type (pJH109) was recovered from *E. coli*, a restriction map of which is shown in Fig. 1. This plasmid was used in a second transformation of *S. cerevisiae* SL428-1C, selecting again for TK^+ activity and resistance to paromomycin. Fifty such transformants were picked and tested for complementation of the various phenotypes exhibited by *sup45-2*. pJH109 complemented all of the characteristics of the *sup45-2* mutant phenotype in each of the 50 transformants that were tested, including nonsense suppression, osmotic sensitivity, temperature sensitivity, and paromomycin sensitivity.

Genetic mapping of the cloned fragment by integrative transformation. For verification that the DNA fragment carried in pJH109 originated from the *SUP45*$^+$ locus on chromosome 2R, the entire insert DNA fragment was cloned in the integrating plasmid pJH18. Strain YHS11 (*MATa sup45-2 met8-1 leu2-1 ura3-52 lys2-1*) was transformed with this plasmid after directing integration into the chromosome by a restriction cut within the *SUP45*$^+$ insert (32). As expected, all Ura^+ transformants no longer supressed the *met8-1* and *leu2-1* markers. Several of these transformants (Ura^+, Lys^-, and nonsuppressing) were each mated to strain YEM35 (*MATα SUP45*$^+$ *met8-1 leu2-1 ura3-52 LYS2*$^+$). (It was necessary to mate to a *SUP45*$^+$ strain because the *sup45-2* mutation does not allow normal spore germination.) Diploid cells were then sporulated, and a total of 148 tetrads were dissected and scored for Ura and Lys phenotypes. In this experiment, the *URA3*$^+$ gene substitutes as a genetic marker for the *sup45-2* gene and one scores for linkage between *URA3*$^+$ and the test marker, in this case *LYS2*$^+$. Ninety-seven percent of dissected asci contained four spores. Of the 143 four-spored tetrads, 59 tetratype, 84 parental ditype, and zero nonparental ditype asci were scored. This corresponds to a map distance (reference 24) of 20.5 centimorgans, a value that agrees well with the published map distance between *sup45* and *LYS2*$^+$ of 26 centimorgans (reference 16).

***SUP45*$^+$ *RNA*.** To define the location of the $SUP45^+$ gene within the DNA fragment carried in pJH109, parts of this insert were subcloned into pJH18. pEM4 carries the 1.1-kb portion of DNA that lies to the left of the *Xho*I site (Fig. 1), and pEM1 carries the 2.4-kb portion to the right of that *Xho*I site. Total cellular RNA isolated from exponentially growing

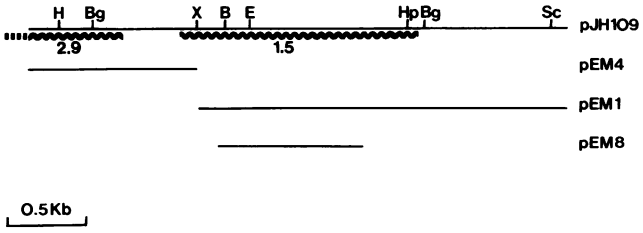

FIG. 1. Restriction map of the *S. cerevisiae* DNA insert that complements *sup45-2*, and several derivatives of the plasmid. Abbreviations: E, *Eco*RI; B, *Bam*HI; H, *Hin*dIII; Bg, *Bgl*II; X, *Xho*I; Hp, *Hpa*I; Sc, *Sac*II. pJH18 was used as vector for the fragments carried in pEM1, pEM4, and pEM8. Plasmids pEM1 and pEM4 are subclones of portions of the pJH109 DNA insert that were transferred to pJH18. The insert carried in pEM8 was obtained by BAL31 digestion of the *Xho-Hpa*I 1.4-kb DNA fragment. The wavy lines represent transcripts. Note that the 2.9-kb transcript is only partly coded by pJH109, as indicated by the dashed line.

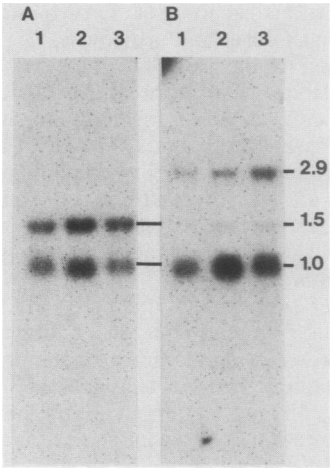

FIG. 2. Blot analysis of the *SUP45*$^+$ mRNA. Lanes 1, total RNA from strain ts368 (*rna2*) grown at the permissive temperature (23°C); lanes 2, total RNA from strain ts368 incubated at the nonpermissive temperature (37°C); lanes 3, total RNA from strain A364A grown at 37°C. (A) The blot was probed with pEM1 plasmid DNA and radiolabeled by nick translation. (B) The blot was probed with pEM4 and radiolabeled by nick translation. The sizes are in kb. The 1.0-kb band is the *URA3*$^+$ transcript detected by the cloning vehicle, pJH18, from which pEM1 and pEM4 were constructed. Control experiments with the actin gene (28) as probe indicated that this transcript was not processed at 37°C in the *rna2* mutant strain (unpublished data).

strain ts368 (*rna2*) was used in an RNA blot-hybridization experiment. In addition to the 1.0-kb *URA3*$^+$ transcript, pEM1 hybridized to a 1.5-kb transcript (Fig. 2A, lanes 1 and 2); pEM4 hybridized only weakly to a 1.5-kb transcript and more strongly to a 2.9-kb RNA (Fig. 2B, lanes 1 and 2). This result is best explained by assuming that the transcripts are positioned on the DNA as shown in Fig. 1. Thus, the weak hybridization of the 1.5-kb transcript to pEM4 suggests only a very small overlap with the DNA fragment carried on that plasmid. Note that the 2.9-kb transcript hybridizes only to pEM4 and thus lies on the left portion of pJH109; since the DNA fragment carried on pEM4 is only 1.1 kb in length, it cannot encode the entire 2.9-kb transcript. Therefore, only the 1.5-kb transcript is entirely encoded on pJH109, and this must correspond to the *SUP45*$^+$ RNA. This transcript was shown to be polyadenylated by specific binding to an oligodeoxythymidylate-cellulose column (unpublished observation).

It has been shown that RNA isolated from an *rna2* mutant strain grown at the nonpermissive temperature contains unprocessed (unspliced) mRNA precursors which appear as higher-molecular-weight species in an RNA blot hybridization (1, 37); coupled with this is a depletion of mature transcripts. Comparison in Fig. 2A of the 1.5-kb transcripts in lane 1 (*rna2* at 23°C), lane 2 (*rna2* at 37°C), and lane 3 (*RNA2*$^+$ at 37°C) reveals no such larger precursor appearing at the nonpermissive temperature. Therefore, within the level of resolution of this analysis, the *SUP45*$^+$ gene does not contain an intron. This is in contrast to most currently known ribosomal protein genes (1, 37).

The relative abundance of the *SUP45*$^+$ transcript was estimated with respect to those of ribosomal protein genes (*tcm1*, ribosomal protein L3 [9, 39]; *cry1*, ribosomal protein rp59 [17, 23]) as well as to actin (28). As described above, we measured by hybridization both the relative steady-state amounts of four yeast transcripts during exponential growth

TABLE 1. Relative abundance of the transcripts of *SUP45*$^+$, two ribosomal proteins, and actin[a]

Protein	Dpm hybridized from 130 × 10^6 input cpm			Transcript size (kb)	Dpm/kb ratio
	Filter 1	Filter 2	Net avg[b]		
pAV7 (*tcm1*)	21,867	23,942	20,391	1.4	14,565
pAV11 (*cry1*)	14,910	15,418	12,651	0.66	19,168
pEM14 (*SUP45*$^+$)	4,367	4,288	1,814	1.5	1,209
pEM15 (actin)	11,192	13,676	9,922	1.3	7,632
pBR322	2,659	2,368	0		

[a] ^{3}H-labeled RNA (50 μg) was hybridized to excess filter-bound plasmid DNA as described in the text. Two filters (1 and 2) were hybridized to separate 50-μg portions of RNA.
[b] The net average was calculated by averaging the disintegrations per minute bound to filters 1 and 2 and then subtracting the average of the pBR322 blank.

and the relative transcription rates upon mild heat shock; a transient decrease in the latter is characteristic of ribosomal proteins (20). The abundance of *SUP45*$^+$ mRNA is less than 10% relative to two ribosomal protein transcripts (Table 1). *SUP45*$^+$ mRNA synthesis is subject to approximately the same degree of transient inhibition upon mild heat shock as are ribosomal protein transcripts (Table 2); thus, the *SUP45*$^+$ gene has at least one characteristic in common with ribosomal protein genes.

A blot analysis of RNA isolated from strain YHS11 transformed with pJH109 showed that the presence of the *SUP45*$^+$ gene on this 2-μm based plasmid increases the steady-state level of the 1.5-kb *SUP45*$^+$ transcript at least fivefold (unpublished data). This increase parallels the observed copy number of such plasmids (8) and is presumably due to a ca. fivefold increase in gene dosage.

The *SUP45*$^+$ protein. Although the low abundance of the *SUP45*$^+$ transcript relative to two ribosomal protein messages suggests that *SUP45*$^+$ does not encode a ribosomal protein, confirmation requires analysis of the gene product. To this end, the *SUP45*$^+$ mRNA was hybrid selected from polyadenylated RNA with pEM1 DNA; the RNA was translated in vitro with a wheat germ extract (see Fig. 3). One-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of the hybrid-selected in vitro translation product showed that the *SUP45*$^+$ mRNA encodes a major protein of M_r = 54,000 (Fig. 3A, lane 3; Fig. 3B, lane 1), which is larger than the largest known ribosomal protein, L3 (M_r = 43,000; references 9 and 39) Fig. 3A, lane 1; Fig. 3B, lane 2). An additional smaller translation product was usually obtained, which we believe was due to premature termination of translation (34). An M_r of 54,000 agrees with

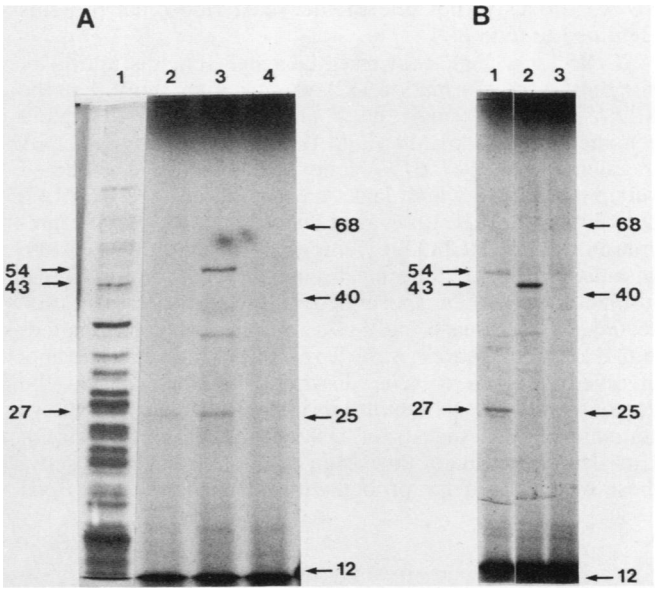

FIG. 3. In vitro translation of the hybrid-selected *SUP45*$^+$ mRNA. The protein products were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. (A) Lane 1, total ribosomal proteins purified from strain LL20; the 43-kd band corresponds to ribosomal protein L3. Lane 2, in vitro translation products of mRNA hybrid-selected with the vector pJH18 only; the 27-kd band corresponds to the *URA3*$^+$ gene product. Lane 3, in vitro translation products of mRNA hybrid-selected with pEM1; the 54-kd band is the presumed *SUP45*$^+$ gene product and the middle band is thought to be due to premature termination of translation. Lane 4 had no RNA. The faint upper bands in lane 1 are due to small amounts of major soluble cell proteins that contaminated the ribosomal protein preparation. (B) Lane 1, in vitro translation products of mRNA hybrid-selected with pEM1; the 54-kd band is the presumed *SUP45*$^+$ gene product. Lane 2, in vitro translation product of mRNA hybrid-selected with pAV7; the 43-kd band is the *tcm1* gene product. Lane 3 had no RNA. Sizes are given in kd calculated with reference to standards that were co-electrophoresed with the translation products.

the expected size of a protein that would be coded in an RNA of 1.5 kb (Fig. 2). Repeated attempts at isoelectric focusing of the *SUP45*$^+$ translation product on two-dimensional polyacrylamide gel electrophoresis systems, both equilibrium (29) and nonequilibrium (30), designed for the resolution of either ribosomal proteins (11) or neutral and basic cellular proteins (29, 30) were unsuccessful. Overall, these characteristics strongly suggest that the product of the

TABLE 2. Effect of a mild temperature shock on relative transcription rates of the *SUP45*$^+$ gene, the genes for two ribosomal proteins, and the actin gene[a]

Protein	Dpm hybridized at time (min) after temp shift (input cpm [10^6])															
	Preshift (4.42)				10 (2.58)				30 (1.76)				55 (3.88)			
	Filter 1	Filter 2	Net avg[b]	% Pre-shift	Filter 1	Filter 2	Net avg	% Pre-shift	Filter 1	Filter 2	Net avg	% Pre-shift	Filter 1	Filter 2	Net avg	% Pre-shift
pAV7 (*tcm1*)	1,258	914	974	100	124	126	47	5	248	244	182	19	1,152	1,183	1,056	108
pAV11 (*cry1*)	1,018	912	852	100	190	195	115	13	283	322	239	28	831	818	712	84
pEM14 (*SUP45*$^+$)	392	220	193	100	115	118	38	20	76	148	48	25	249	319	172	89
pEM15 (actin)	747	750	636	100	800	685	665	104	520	240	316	50	973	1,229	989	155
pBR322	117	108	0		75	81	0		67	61	0		117	106	0	

[a] ^{3}H-labeled RNA (18 μg) from pulse-labeled cells, collected and prepared as described in the text, was hybridized to excess filter-bound plasmid DNA carrying a single yeast gene (see text). Two filters (1 and 2) were hybridized to separate 18-μg portions of RNA.
[b] The net average was calculated by averaging the disintegrations per minute bound to filters 1 and 2, and then subtracting the average of the pBR322 blank.

SUP45$^+$ locus is not one of the yeast ribosomal proteins identified to date (49).

***SUP45*$^+$ is a single-copy essential gene.** Gene disruption by integrative transformation (32) was used to show that the *SUP45*$^+$ gene is single copy and essential in a haploid genome. Plasmid pEM8 (Fig. 1) carries an internal DNA fragment of the *SUP45*$^+$ gene subcloned in a modified version of pJH18 which lacks an *Eco*RI site (see Fig. 4A). Diploid strain YHU101 was transformed with pEM8; integration was directed (32) by cutting at the unique *Eco*RI site within the *SUP45*$^+$ internal fragment. Ura$^+$ diploid transformants were then sporulated and 20 tetrads were dissected. In every case, only two of four spores germinated, and all viable spores were Ura$^-$. The molecular events involved in such a gene disruption experiment can be demonstrated by DNA blot hybridization. Figure 4B shows genomic *Hin*dIII digests of DNA from the diploid strain, Ura$^+$ transformants of this strain, and viable spore progeny; these were blotted and probed with the isolated 2.1-kb *Bgl*II *SUP45*$^+$ DNA fragment (Fig. 1). The untransformated diploid strain, YHU101, shows only a single 6.6-kb *Hin*dIII band. Ura$^+$ transformants show this 6.6-kb band plus two additional *Hin*dIII bands of 7.7 and 4.9 kb. These new bands are the result of pEM8 integrative disruption of one of the two *SUP45*$^+$ genes present in the diploid genome (Fig. 4A). The 5.8-kb band that is visible in the transformant DNA corresponds to the size of pEM8 and is due to tandem integration of at least two copies of this plasmid. Finally, viable Ura$^-$ spores show only the single 6.6-kb *Hin*dIII band, which is indicative of an intact *SUP45*$^+$ gene. The tetrad dissections and DNA blot analysis show that integration of pEM8 at the *SUP45*$^+$ locus disrupts the gene, and that such disruption destroys an essential cellular function. These results also indicate that *SUP45*$^+$ is present as only a single copy in the haploid genome.

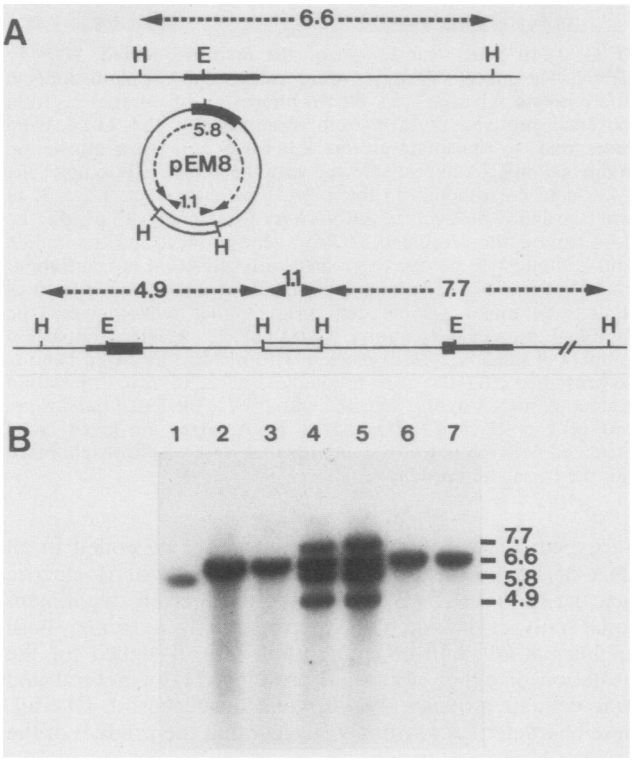

FIG. 4. Disruption of the chromosomal *SUP45*$^+$ gene. (A) Diagram of the recombinant event. For clarity, only the *Hin*dIII (H) and *Eco*RI (E) sites are indicated. The sizes of the *Hin*dIII fragments are in kb. The thin line represents yeast chromosomal or pBR322 sequences, the medium-thick line represents the intact chromosomal *SUP45*$^+$ gene, the black box represents the internal fragment of that *SUP45*$^+$ gene carried by pEM8, and the white box represents the *URA3*$^+$ gene carried by pEM8. The recombination event was directed by linearization of pEM8 at the *Eco*RI site that lies within the deleted *SUP45*$^+$ gene. (B) DNA blot-hybridization analysis of the *SUP45*$^+$ disruption. All genomic DNA samples were digested with *Hin*dIII, and the blot was probed with the 2.1-kb *Bgl*II DNA fragment (see Fig. 1), radiolabeled by nick translation. Lanes 1, pEM8 DNA; 2, LL20 DNA; 3, diploid strain YHU101 DNA; 4 and 5, DNA from two independent Ura$^+$ colonies of diploid strain YHU101 transformed with linear pEM8; 6 and 7, DNA from two viable Ura$^-$ haploid spores derived by sporulation of transformed diploid strain YHU101.

DISCUSSION

The available genetic and biochemical data suggest that *SUP45*$^+$ encodes a ribosome-associated protein, perhaps a ribosomal protein, which is involved in translation fidelity or termination or both (see above). Our data indicate that the *SUP45*$^+$ gene does not encode any of the currently recognized ribosomal proteins for the following reasons. (i) The major in vitro translation product is 54 kilodaltons (kd), a protein some 10 kd larger than the largest known ribosomal protein L3 (45 kd) (9, 39). Moreover, it should be borne in mind that the wheat germ extract appears to be capable of synthesizing mature yeast ribosomal proteins (50). (ii) The steady-state level of the *SUP45*$^+$ mRNA is less than one-tenth that of two known ribosomal protein transcripts, *tcm1* (L3) and *cry1* (rp59). From observations with *E. coli* (12), one might extrapolate that most yeast ribosomal proteins (and thus their transcripts) are present in equimolar amounts. This line of reasoning argues that the *SUP45*$^+$ mRNA, being present at only one-tenth the abundance of ribosomal proteins, does not belong to that class.

If *SUP45*$^+$ is not a ribosomal protein structural gene, yet clearly is related to the translation function (see above), what could it be? We can suggest three possibilities. (i) The *SUP45*$^+$ gene product could code for a function that modifies a ribosomal protein by methylation, acetylation, or phosphorylation (49). (ii) It could be a tightly ribosome-bound translation factor that forms part of the site involved in translational proof reading (18, 48); no tightly bound protein of the appropriate size has yet been described. (iii) *SUP45*$^+$ could encode a translation termination factor, although this is difficult to reconcile with the *sup45*-induced mistranslation in vitro (43, 44) and suppression of presumed missense mutations in vivo (4).

Although our data do not allow a choice to be made among these alternatives, they do provide one encouraging hint. The observation that in a mild temperature shock the *SUP45*$^+$ transcript is regulated as if it were a ribosomal protein is remindful of the biosynthetic regulation of some translation elongation factors when bacteria are placed under the stress of amino acid starvation (36), and lends some credence to the suggestion that *SUP45*$^+$ codes for a translation factor. Furthermore, whatever its function, genetic experiments have shown clearly that *SUP45*$^+$ is essential for cell viability.

Though we were able to visualize the *SUP45*$^+$ gene product on a one-dimensional sodium dodecyl sulfate gel, all efforts at characterization in two-dimensional systems failed. It is possible that the isoelectric point of the protein is extremely acidic and thus it ran off of the first dimension of

the gels. Possibly the protein requires a strong detergent for solubility and thus could not enter gels that involve milder denaturation conditions. We are proceeding on the supposition that the best way to obtain an indication of the cellular location and function of the *SUP45*$^+$ gene product is to obtain an antibody to it.

ACKNOWLEDGMENTS

We thank Linda Mamelak for assistance and Lynette Fouser, Fred Pluthero, and Ati Vassarotti for discussion and suggestions.

This work was supported by the National Cancer Institute of Canada and the Natural Sciences and Engineering Council of Canada (grant no. A8060). H.J.H. holds a Medical Research Council of Canada Studentship. E.M. holds an Ontario Graduate Scholarship.

LITERATURE CITED

1. **Bollen, G. H. P. M., C. M. T. Molenaar, L. H. Cohen, M. M. C. van Raansdonk-Duin, W. H. Mager, and R. J. Planta.** 1982. Ribosomal protein genes of yeast contain intervening sequences. Gene **18**:29–38.
2. **Bollen, T., T. Cabezon, M. De Wilde, 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.
3. **Cabezon, T., A Herzog, M. De Wilde, R. Villaroel, and A. Bollen.** 1976. Cooperative control of translation fidelity by ribosomal proteins in *E. coli*. Mol. Gen. Genet. **144**:59–62.
4. **Chattoo, B. B., E. Palmer, B.-I. Ono, and F. Sherman.** 1979. Patterns of genetic and phenotypic suppression of *lys2* mutations in the yeast *Saccharomyces cerevisiae*. Genetics **93**:67–79.
5. **Davies, J., L. Gorini, and B. D. Davis.** 1965. Misreading of RNA codewords induced by aminoglycoside antibiotics. Mol. Pharmacol. **1**:93–106.
6. **Davis, R. W., M. Thomas, J. Cameron, T. P. St. John, S. Scherer, and R. A. Padgett.** 1980. Rapid DNA isolations for enzymatic and hybridization analysis. Methods Enzymol. **65**:404–411.
7. **Edelmann, P., and J. Gallant.** 1977. Mistranslation in *E. coli*. Cell **10**:131–137.
8. **Erhart, E., and C. P. Hollenberg.** 1983. The presence of a defective *LEU2* gene on 2μ DNA recombinant plasmids of *Saccharomyces cerevisiae* is responsible for curing and high copy number. J. Bacteriol. **156**:625–635.
9. **Fried, H. M., and J. R. Warner.** 1981. Cloning of yeast gene for tricodermin resistance and ribosomal protein L13. Proc. Natl. Acad. Sci. U.S.A. **78**:238–242.
10. **Gerlach, W. L.** 1975. Genetic properties of some amber-ochre super-suppressors in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **138**:53–63.
11. **Gorenstein, C., and J. R. Warner.** 1976. Coordinate regulation of the synthesis of eukaryotic ribosomal proteins. Proc. Natl. Acad. Sci. U.S.A. **73**:1547–1551.
12. **Hardy, S. J. S.** 1975. The stoichiometry of the ribosomal proteins of *Escherichia coli*. Mol. Gen. Genet. **140**:253–274.
13. **Hartwell, L. C., C. McLaughlin, and J. R. Warner.** 1970. Identification of ten genes that control ribosome formation in yeast. Mol. Gen. Genet. **109**:42–56.
14. **Hartwell, L. H.** 1967. Macromolecule synthesis in temperature-sensitive mutants of yeast. J. Bacteriol. **93**:1662–1670.
15. **Hawthorne, D. C., and U. Leupold.** 1974. Suppressors in yeast. Curr. Top. Microbiol. Immunol. **64**:1–47.
16. **Hawthorne, D. C., and R. K. Mortimer.** 1968. Genetic mapping of nonsense suppressors in yeast. Genetics **60**:735–742.
17. **Himmelfarb, H. J., A. Vassarotti, and J. D. Friesen.** 1984. Molecular cloning and biosynthetic regulation of the *cryl* gene of *Saccharomyces cerevisiae*. Mol. Gen. Genet. **195**:500–506.
18. **Hopfield, J. J.** 1974. Kinetic proofreading: a new mechanism for reducing errors in biosynthetic processes requiring high specificity. Proc. Natl. Acad. Sci. U.S.A. **71**:4135–4139.
19. **Inge-Vechtomov, S. G., and V. M. Andrianova.** 1970. Recessive super-suppressors in yeast. Genetika **6**:103–115.
20. **Kim, C. H., and J. R. Warner.** 1983. Mild temperature shock alters the transcription of a discrete class of *Saccharomyces cerevisiae* genes. Mol. Cell. Biol. **3**:457–465.
21. **Kuhlberger, R., W. Piepersberg, A. Pretzet, P. Buckel, and A. Bock.** 1979. Alteration of ribosomal protein L6 in gentamicin-resistant strains of *E. coli*. Effects of fidelity of protein synthesis. Biochemistry **18**:187–193.
22. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **277**:680–685.
23. **Larkin, J. C., and J. L. Woolford, Jr.** 1983. Molecular cloning and analysis of the *CRY1* gene: a yeast ribosomal protein gene. Nucleic Acids Res. **11**:403–420.
24. **Ma, C., and R. K. Mortimer.** 1983. Empirical equation that can be used to determine genetic map distances from tetrad data. Mol. Cell. Biol. **3**:1886–1887.
25. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning. A laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
26. **Masurekar, M., E. Palmer, B.-I. Ono, J. M. Wilhelm, and F. Sherman.** 1981. Misreading of the ribosomal suppressor *SUP46* due to an altered 40 S subunit in yeast. J. Mol. Biol. **147**:381–390.
27. **McNeil, J. B., and J. D. Friesen.** 1981. Expression of the *Herpes simplex* virus thymidine kinase gene in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **184**:386–393.
28. **Ng, R., and J. Abelson.** 1980. Isolation and sequence of the gene for actin in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. U.S.A. **77**:3912–3916.
29. **O'Farrell, P. H.** 1975. High resolution two-dimensional electrophoresis of proteins. J. Biol. Chem. **250**:4007–4021.
30. **O'Farrell, P. Z., H. M. Goodman, and P. H. O'Farrell.** 1977. High resolution two-dimensional electrophoresis of basic as well as acidic proteins. Cell **12**:1133–1142.
31. **Ono, B.-I., J. W. Stewart, and F. Sherman.** 1981. Serine insertion caused by the ribosomal suppressor *SUP46* in yeast. J. Mol. Biol. **147**:373–379.
32. **Orr-Weaver, T. L., J. W. Szostak, and R. J. Rothstein.** 1981. Yeast transformation: a model system for the study of recombination. Proc. Natl. Acad. Sci. U.S.A. **78**:6354–6358.
33. **Palmer, E., J. M. Wilhelm, and F. Sherman.** 1979. Phenotypic suppression of nonsense mutations in yeast by aminoglycoside antibiotics. Nature (London) **277**:148–149.
34. **Paterson, B. M., and J. O. Bishop.** 1977. Changes in the mRNA population of chick myoblasts during myogenesis *in vitro*. Cell **12**:751–765.
35. **Piepersberg, W., A. Bock, and H. G. Wittman.** 1975. Effects of different mutations found in ribosomal protein S5 of *E. coli* on translation fidelity. Mol. Gen. Genet. **140**:91–100.
36. **Reeh, S., S. Pedersen, and J. D. Friesen.** 1976. Biosynthetic regulation of individual proteins in *relA*$^+$ and *relA*$^-$ strains of *Escherichia coli* during amino acid starvation. Mol. Gen. Genet. **149**:279–289.
37. **Rosbash, M., P. K. W. Harris, J. L. Woolford, Jr., and J. L. Teem.** 1981. The effect of temperature-sensitive RNA mutants on the transcription products from cloned ribosomal protein genes of yeast. Cell **24**:679–686.
38. **Rosset, R., and L. Gorini.** 1969. A ribosomal ambiguity mutation. J. Mol. Biol. **39**:95–112.
39. **Schultz, L. D., and J. D. Friesen.** 1983. Nucleotide sequence of the *tcm1* gene (ribosomal protein L3) of *Saccharomyces cerevisiae*. J. Bacteriol. **155**:8–14.
40. **Sherman, F., G. Fink, and C. Lawrence.** 1977. Methods in yeast genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
41. **Shulman, R. W., C. E. Sripati, and J. R. Warner.** 1977. Noncoordinated transcription in the absence of protein synthesis in yeast. J. Biol. Chem. **252**:1344–1349.
42. **Singh, A., D. Ursic, and J. Davies.** 1979. Phenotypic suppression and misreading in *Saccharomyces cerevisiae*. Nature (London) **277**:146–148.
43. **Smirnov, V. N., A. P. Surguchov, E. S. Fominykch, L. V. Lizlova, T. V. Saprygina, and S. G. Inge-Vechtomov.** 1976. Recessive nonsense suppression in yeast: further characteriza-

tion of a defect in translation. FEBS Lett. **66**:12–15.

44. **Surguchov, A. P., Y. V. Berestetskaya, E. S. Fominykch, E. M. Pospelova, V. N. Smirnov, M. D. Ter-Avanesyan, and S. G. Inge-Vechtomov.** 1980. Recessive suppression in yeast *Saccharomyces cerevisiae* is mediated by a ribosomal mutation. FEBS Lett. **111**:175–178.
45. **Surguchov, A. P., E. S. Fominykch, V. N. Smirnov, M. D. Ter-Avanesyan, L. N. Mironova, and S. G. Inge-Vechtomov.** 1981. Further characterization of recessive suppression in yeast. Isolation of the low temperature-sensitive mutant of *Saccharomyces cerevisae* defective in the assembly of 50 S ribosomal subunit. Biochim. Biophys. Acta **654**:149–155.
46. **Surguchov, A. P., E. M. Pospelova, and V. N. Smirnov.** 1981. Synergistic action of genetic and phenotypic suppression of nonsense mutations in yeast *Saccharomyces cerevisiae*. Mol. Gen. Genet. **183**:197–198.
47. **Ter-Avanesyan, M. D., J. Zimmerman, S. G. Inge-Vechtomov, A. B. Sudarikov, V. N. Smirnov, and A. P. Surguchov.** 1982. Ribosomal recessive suppressors cause a respiratory deficiency in yeast *Saccharomyces cerevisiae*. Mol. Gen. Genet. **185**:319–323.
48. **Thompson, R. C., and A. M. Karim.** 1982. The accuracy of protein biosynthesis is limited by its speed: high fidelity selection by ribosomes of aminoacyl-tRNA ternary complexes containing GTP(γS). Proc. Natl. Acad. Sci. U.S.A. **79**:4922–4926.
49. **Warner, J.** 1982. The yeast ribosome: structure, function and synthesis, p. 529–560. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast *Saccharomyces*. Metabolism and gene expression. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
50. **Warner, J. R., and C. Gorenstein.** 1977. The synthesis of eucaryotic ribosomal proteins *in vitro*. Cell **11**:201–212.