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Increased Expression of *Saccharomyces cerevisiae* Translation Elongation Factor 1α Bypasses the Lethality of a *TEF5* Null Allele Encoding Elongation Factor 1β

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ABSTRACT

Translation elongation factor 1β (EF-1β) catalyzes the exchange of bound GDP for GTP on EF-1α. The lethality of a null allele of the *TEF5* gene encoding EF-1β in *Saccharomyces cerevisiae* was suppressed by extra copies of the *TEF2* gene encoding EF-1α. The strains with *tef5::TRP1* suppressed by extra copies of *TEF2* were slow growing, cold sensitive, hypersensitive to inhibitors of translation elongation and showed increased phenotypic suppression of +1 frameshift and UAG nonsense mutations. Nine dominant mutant alleles of *TEF2* that cause increased suppression of frameshift mutations also suppressed the lethality of *tef5::TRP1*. Most of the strains in which *tef5::TRP1* is suppressed by dominant mutant alleles of *TEF2* grew more slowly and were more antibiotic sensitive than strains with *tef5::TRP1* suppressed by wild-type *TEF2*. Two alleles, *TEF2-4* and *TEF2-10*, interact with *tef5::TRP1* to produce strains that showed doubling times similar to *tef5::TRP1* strains containing extra copies of wild-type *TEF2*. These strains were less cold sensitive, drug sensitive and correspondingly less efficient suppressors of +1 frameshift mutations. These phenotypes indicate that translation and cell growth are highly sensitive to changes in EF-1α and EF-1β activity.

THE elongation step of protein synthesis involves binding of aminoacyl-tRNA to the ribosomal A site, formation of a peptide bond, and translocation of the newly formed peptidyl-tRNA to the P site (MOLDAVE 1985). The eukaryotic elongation factors EF-1 and EF-2 have functions analogous to those of the bacterial elongation factors EF-Tu/Ts and EF-G (MOLDAVE 1985; HINNEBUSCH and LIEBMAN 1991). Yeast require another elongation factor, EF-3, as well (SKOGERSON and WAKATAMA 1976; CHAKRABURTTY and KAMATH 1988). The EF-1 complex contains three or four subunits and facilitates binding of aminoacyl-tRNA to the ribosomal A site. The α subunit of EF-1 binds aminoacyl-tRNA in a GTP-dependent manner; this ternary complex then binds to the ribosome (CARVALHO *et al.* 1984). The GTP is hydrolyzed to GDP after the cognate aminoacyl-tRNA is bound at the A site. The GDP remains tightly bound to EF-1α after this reaction. The β subunit of EF-1 stimulates nucleotide exchange to regenerate EF-1α-GTP for the next elongation cycle (SLOBIN and MOLLER 1978). The function of the γ subunit of EF-1 is unknown, although there is evidence that it can stimulate the nucleotide exchange activity of EF-1β (MOLDAVE 1985).

Saccharomyces cerevisiae EF-1α is an abundant protein, the majority of which is isolated as a monomer (DASMAHAPATRA *et al.* 1981). In yeast as well as in other eukaryotes, EF-1α is also found associated with EF-1β and EF-1γ (SAHA and CHAKRABURTTY 1986). EF-1α in *S. cerevisiae* is encoded by two unlinked genes, *TEF1* and *TEF2* (NAGATA *et al.* 1984). EF-1α is essential; a *tef1*Δ *tef2*Δ double null mutation is lethal. Both genes encode identical proteins of 458 amino acids whose sequence is 80% identical to that of EF-1α from *Artemia salina* and humans and 30% identical to *Escherichia coli* EF-Tu (MERRICK *et al.* 1990, 1993). The *TEF5* gene encoding the yeast homologue of EF-1β was isolated by screening a yeast cDNA expression library with antibodies against proteins that associate with calmodulin (HIRAGA *et al.* 1993). *TEF5* is a single copy essential gene. The predicted protein sequence is ~50% identical to *A. salina* and human EF-1β. The yeast *TEF3* and *TEF4* genes encode proteins with sequence homology to EF-1γ (KAMBOURIS *et al.* 1993; KINZY *et al.* 1994). Disruption of both genes is not lethal and results in no observable defects in translation.

Mutations affecting translational fidelity have been found in yeast and bacteria tRNAs (SHERMAN 1982) and ribosomal proteins (ATKINS *et al.* 1972; ROSSET and GORINI 1969). Other yeast genes implicated in fidelity include the omnipotent suppressors *sup35* and *sup45* (HIMMELFARB *et al.* 1985; KUSHNIROV *et al.* 1988) and the recessive antisuppressor *asu9* (LIEBMAN and CAVENAGH 1980). SANDBAKEN and CULBERTSON (1988) isolated nine dominant mutations in the *TEF2* gene encoding EF-1α that suppress +1 frameshift mutations in yeast. Furthermore, increases in the amount of EF-1α protein affects fidelity. SONG *et al.* (1989) found that an extra copy of *TEF1*, encoding EF-1α, increased the suppres-

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TABLE 1

***S. cerevisiae* strains used in this study**

Strain	Genotype	Source
JM749	*MAT***a***/MATα ura3-52/ura3-52 trp1-Δ101/trp1-Δ101 lys2-801/lys2-801 leu2-Δ1/leu2-Δ1 his3-Δ200/his3-Δ200*	Maddock *et al.* (1994)
MC1160	*MAT***a** *his4-713 met2-1 ura3-52 leu2-3,112 lys2*	Sandbaken and Culbertson (1988)
JWY4134	*MAT***a** *ura3-52 trp1-Δ101 lys2-801 leu2-Δ1 his3-Δ200*	
JWY4175	*MAT***a***/MATα ura3-52/ura3-52 trp1-Δ101/trp1-Δ101 lys2-801/lys2-801 leu2-Δ1/leu2-Δ1 his3-Δ200/his3-Δ200 tef5::TRP1/TEF5*	This study
JWY4200	*MAT***a** *ura3-52 trp1-Δ101 lys2-801 leu2-Δ1 his3-Δ200 tef5::TRP1* [*TEF5 URA3 CEN4*]	This study
JWY4201	*MATα ura3-52 trp1-Δ101 lys2-801 leu2-Δ1 his3-Δ200 tef5::TRP1* [*TEF5 URA3 CEN4*]	This study
JWY4202	*MATα ura3-52 trp1-Δ101 lys2-801 leu2-Δ1 his3-Δ200 tef5::TRP1* [*TEF2 URA3 CEN4*]	This study
JWY4203	*MAT***a** *ura3-52 trp1-Δ101 lys2-801 leu2-Δ1 his3-Δ200 tef5::TRP1* [*TEF2 URA3 CEN4*]	This study
JWY4204	*MAT***a** *ura3-52 trp1-Δ101 lys2-801 leu2-Δ1 his3-Δ200 tef5::TRP1* [*TEF2 URA3 2μ*]	This study
JWY4205	*MAT***a** *ura3-52 trp1-Δ101 lys2-801 leu2-Δ1 his3-Δ200 tef5::TRP1* [*TEF2 URA3 2μ*]	This study
JWY4211	*MATα ura3-52 trp1-Δ101 lys2-801 leu2-Δ1 his3-Δ200 tef5::TRP1* [*TEF2-1 URA3 CEN4*]	This study
JWY4212	*MAT***a** *ura3-52 trp1-Δ101 lys2-801 leu2-Δ1 his3-Δ200 tef5::TRP1* [*TEF2-1 URA3 CEN4*]	This study
JWY4213	*MAT***a** *ura3-52 trp1-Δ101 lys2-801 leu2-Δ1 his3-Δ200 tef5::TRP1* [*TEF2-2 URA3 CEN4*]	This study
JWY4214	*MATα ura3-52 trp1-Δ101 lys2-801 leu2-Δ1 his3-Δ200 tef5::TRP1* [*TEF2-2 URA3 CEN4*]	This study
JWY4215	*MATα ura3-52 trp1-Δ101 lys2-801 leu2-Δ1 his3-Δ200 tef5::TRP1* [*TEF2-3 URA3 CEN4*]	This study
JWY4216	*MAT***a** *ura3-52 trp1-Δ101 lys2-801 leu2-Δ1 his3-Δ200 tef5::TRP1* [*TEF2-3 URA3 CEN4*]	This study
JWY4217	*MATα ura3-52 trp1-Δ101 lys2-801 leu2-Δ1 his3-Δ200 tef5::TRP1* [*TEF2-4 URA3 CEN4*]	This study
JWY4218	*MAT***a** *ura3-52 trp1-Δ101 lys2-801 leu2-Δ1 his3-Δ200 tef5::TRP1* [*TEF2-4 URA3 CEN4*]	This study
JWY4219	*MATα ura3-52 trp1-Δ101 lys2-801 leu2-Δ1 his3-Δ200 tef5::TRP1* [*TEF2-7 URA3 CEN4*]	This study
JWY4220	*MAT***a** *ura3-52 trp1-Δ101 lys2-801 leu2-Δ1 his3-Δ200 tef5::TRP1* [*TEF2-7 URA3 CEN4*]	This study
JWY4221	*MATα ura3-52 trp1-Δ101 lys2-801 leu2-Δ1 his3-Δ200 tef5::TRP1* [*TEF2-9 URA3 CEN4*]	This study
JWY4222	*MAT***a** *ura3-52 trp1-Δ101 lys2-801 leu2-Δ1 his3-Δ200 tef5::TRP1* [*TEF2-9 URA3 CEN4*]	This study
JWY4223	*MAT***a** *ura3-52 trp1-Δ101 lys2-801 leu2-Δ1 his3-Δ200 tef5::TRP1* [*TEF2-10 URA3 CEN4*]	This study
JWY4224	*MATα ura3-52 trp1-Δ101 lys2-801 leu2-Δ1 his3-Δ200 tef5::TRP1* [*TEF2-10 URA3 CEN4*]	This study
JWY4225	*MATα ura3-52 trp1-Δ101 lys2-801 leu2-Δ1 his3-Δ200 tef5::TRP1* [*TEF2-13 URA3 CEN4*]	This study
JWY4226	*MAT***a** *ura3-52 trp1-Δ101 lys2-801 leu2-Δ1 his3-Δ200 tef5::TRP1* [*TEF2-13 URA3 CEN4*]	This study
JWY4227	*MAT***a** *ura3-52 trp1-Δ101 lys2-801 leu2-Δ1 his3-Δ200 tef5::TRP1* [*TEF2-16 URA3 CEN4*]	This study
JWY4228	*MAT***a** *ura3-52 trp1-Δ101 lys2-801 leu2-Δ1 his3-Δ200 tef5::TRP1* [*TEF2-16 URA3 CEN4*]	This study
JWY4231	*MATα ura3-52 leu2 lys2-801 met2-1 his4-713* [*pJWB2937 TEF5 URA3 CEN4*]	This study
JWY4247	*MATα ura3-52 leu2 lys2-801 met2-1 his4-713* [*pJWB3013 TEF5 LEU2 CEN6*]	This study

sion efficiency of an *asu9, sup45-2* double mutant strain. One would further predict that alterations in the specific activity of EF-1α should affect fidelity.

We report that yeast EF-1β, which exchanges bound GDP for GTP on EF-1α, is dispensable for growth when an additional copy of the *TEF2* gene encoding EF-1α is present. Strains lacking EF-1β but containing extra copies of the gene encoding EF-1α showed growth defects and sensitivity to antibiotics that inhibit elongation and affect translational fidelity.

MATERIALS AND METHODS

Strains and media: *E. coli* NM522 was used for plasmid preparation. *S. cerevisiae* strains used in these studies are listed in Table 1. JM749 was the diploid parent of all *tef5::TRP1* strains (Maddock *et al.* 1994). Standard yeast genetic methods were used (Mortimer and Hawthorne 1966; Sherman *et al.* 1986). Yeast cells were grown in either YEPD (1% Bacto yeast extract, 2% peptone, 2% dextrose) or defined synthetic complete media (C or C−) supplemented with 2% dextrose as a carbon source. Yeast were transformed by the lithium acetate method (Ito *et al.* 1983). Strain JWY4231 was obtained by sporulation and dissection of JWY4201 × MC1160 diploids. JWY4247 was obtained by transformation of plasmid pJWB3013 into JWY4231 and selection on 5-fluoro orotic acid for loss of pJWB2937 (Boeke *et al.* 1984).

DNA and RNA manipulations: Recombinant DNA techniques, electrophoresis, blotting and hybridization of DNA and RNA were performed as described (Sambrook *et al.* 1989). Nytran Plus membrane (Schleicher and Schuell, Keene, NH) was used for DNA and RNA blotting. Restriction endonucleases and DNA modifying enzymes were obtained from Boehringer Mannheim Biochemicals (Indianapolis, IN). (α-^{32}P)dATP was obtained from Amersham Corporation (Arlington Heights, IL). Plasmids designed to be used in both *E. coli* and yeast were the *URA3* low copy *CEN* plasmid YCp50 (Johnston and Davis 1984) and the high copy 2μ plasmid YEp24 (Botstein *et al.* 1979). The *TEF5* gene (pJWB2937) was isolated from a yeast genomic library prepared in YCp50 (Rose *et al.* 1987) using an *Eco*RI/*Sal*I DNA fragment probe prepared from plasmid pMOO25 (kindly provided by Dr. Michael Clark, McGill University, Montreal, Canada). A *Pst*I/*Cla*I fragment bearing *TEF5* was subcloned into the pBluescript KS+ vector, producing plasmid pJWB2956. Plasmid pJWB3013 was prepared by ligation of the 2.6-kb *Pst*I/*Xho*I fragment of *TEF5* derived from pJWB2956 between the *Pst*I and *Xho*I sites of pRS315 (Sikorski and Hieter 1989).

Construction of a *tef5::TRP1* null allele: An *Nde*I site at the initiator AUG of *TEF5* was created in plasmid pJWB2956 using site-directed mutagenesis with oligonucleotide TEF5-NDE1 (5′-CGAATATATACACACATATGGCATCCACC-3′) (KUNKEL *et al.* 1987). Approximately 75% of the *TEF5* gene was removed by digesting the resulting plasmid (pJWB2964) with *Nde*I, filling in the 5′ overhang with Klenow fragment, and subsequently digesting with *Nco*I. The entire *TRP1* gene on a 2.5-kb *Nco*I/*Sma*I restriction fragment was then ligated into *Nco*I/*Nde*I digested pJWB2964 to produce plasmid pJWB2968 (Figure 1A). A *Sma*I/*Cla*I fragment containing this *tef5::TRP1* disruption was transformed into diploid yeast JM749, homozygous for *trp1-Δ101*, and Trp$^+$ colonies were selected (ROTHSTEIN 1983). The expected transplacement to produce yeast strain JWY4175 was confirmed by genomic Southern blotting (data not shown).

Isolation of strains with *tef5::TRP1* suppressed by *TEF2*: To test for suppression of the lethality caused by *tef5::TRP1*, low or high copy plasmids bearing wild-type alleles of the genes encoding EF-1 subunits were transformed into JWY4175. Centromere (low copy) plasmids bearing the selectable yeast marker *URA3* and *TEF5* (plasmid pJWB2937), *TEF3* (plasmid pJWB2853), *TEF4* (plasmid pJWB2814), or *TEF2* (plasmid YCpMS29), as well as a control plasmid bearing only *URA3* (YCp50) and a high copy (2μ) plasmid bearing *TEF2* (plasmid YCpMS42), were transformed into diploid strain JWY4175 heterozygous for the *tef5::TRP1* disruption. Ura$^+$ transformants were selected and sporulated. YCp50 plasmids bearing the nine dominant mutant alleles of *TEF2* (*TEF2-1, TEF2-2, TEF2-3, TEF2-4, TEF2-7, TEF2-9, TEF2-10, TEF2-13* and *TEF2-16*) (SANDBAKEN and CULBERTSON 1988) were also transformed into JWY4175, and Ura$^+$ transformants were selected and sporulated. Spores were incubated at 30° for 6–7 days on YEPD to allow slow germinating and slow growing colonies to appear. Spore clones obtained are listed in Table 1 (strains JWY4200–JWY4228). Strains with *tef5::TRP1* suppressed by the *TEF2* alleles and containing chromosomal UAG nonsense (*lys2-801*) and +1 frameshift (*his4-713, met2-1*) mutations were obtained by transformation of YCp50 plasmids bearing wild-type *TEF2* or mutant alleles into JWY4247 and loss of the *TEF5 LEU2* helper plasmid (strains JWY4248–JWY4257).

Temperature sensitivity, translational fidelity and growth of *tef5::TRP1* strains: Temperature sensitivity was assayed by spotting 10 μl of a suspension of *tef5::TRP1* strains containing each plasmid tested onto YEPD plates, followed by incubation at 13, 23, 30 and 37° for 3–7 days. Phenotypic suppression of +1 frameshift and nonsense mutations was determined by spotting 10 μl of each strain containing *tef5::TRP1* and a *TEF2* plasmid, derived from JWY4247, onto complete medium (C) or complete medium lacking methionine (C-met), histidine (C-his) or lysine (C-lys) and incubating for 5–8 days at 30°. Doubling times were determined by measuring the growth in liquid culture of at least two independent strains. Cultures grown for 2 days in YEPD at 30° were diluted to an A_{600} of 0.1 in fresh YEPD and grown at 30° with vigorous shaking. Optical density (A_{600}) was assayed approximately every 2 hr. Cultures were diluted into fresh YEPD when the A_{600} reached mid-log phase (0.4 units) to allow continued monitoring. The doubling time was determined for each independent growth curve; the standard deviation was calculated with a minimum $n = 4$.

Drug sensitivity: Two-milliliter cultures of each strain were grown for 2 days at 30° in YEPD to mid-log phase. At least two independent *tef5::TRP1* spore clones were assayed with each *TEF2* plasmid tested. For each culture, 0.2 ml was spread plated onto YEPD plates. Twenty microliters of each drug was pipetted onto sterile BBL 1/4-inch diameter paper discs and allowed to dry. The concentrations of drugs used were as follows: 0.65 mM cryptopleurine, 2.5 mM cycloheximide, 5 mM neomycin, 5 mg/ml tetracycline, 5 mM hygromycin B, 5 mM kanamycin, 5 mM streptomycin and 48 mM paromomycin. A maximum of four filters were placed on each plate, and the plates were incubated for 3 days at 30°. Sensitivity to each drug was measured by the radius of inhibition around each disc.

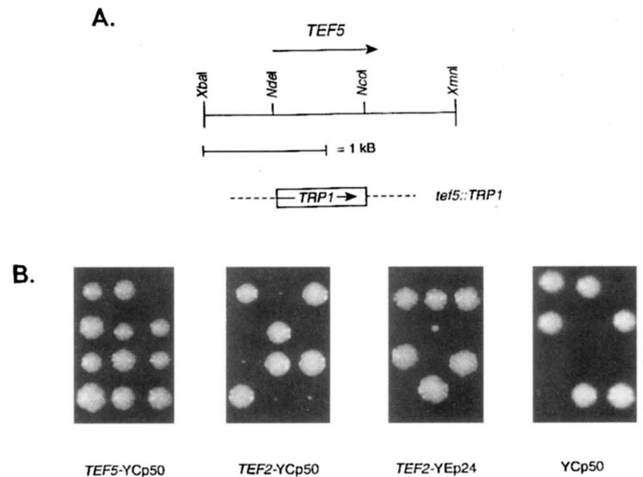

FIGURE 1.—Suppression of a *tef5::TRP1* null allele by extra copies of *TEF2*. (A) Partial restriction map of the *TEF5* genomic subclone. The 0.8-kb *TEF5 Nde*I-*Nco*I fragment was replaced with the yeast *TRP1* gene. A 2.5-kb *Cla*I-*Sma*I fragment was used for one step gene replacement in diploid yeast JM749. (B) Tetrads from diploid JWY4175 transformed with a *TEF5 URA3 CEN4* plasmid (pJWB2937), a *TEF2 URA3 CEN4* plasmid (YCpMS29), a *TEF2 URA3* 2μ plasmid (YCpMS42) and YCp50. Spores were allowed to germinate for 6–7 days at 30° on YEPD plates. Each vertical line of colonies represents a tetrad.

RESULTS

Extra copies of *TEF2* suppress the lethality of a *tef5::TRP1* disruption: Plasmids bearing wild-type genes encoding the α, β or γ subunits of EF-1 and a vector bearing only *URA3* were transformed into the diploid yeast strain JWY4175 heterozygous for a *tef5::TRP1* disruption, and Ura$^+$ transformants were selected. The diploids were sporulated and tetrads dissected. Two viable Trp$^-$ spores were recovered from each of 20 tetrads bearing the YCp50 control plasmid lacking any *TEF* gene (Figure 1B), confirming that *TEF5* is essential for mitotic growth (HIRAGA *et al.* 1993). Diploids containing plasmids bearing either *TEF3* or *TEF4* also produced no viable Trp$^+$ spore clones in the 22 or 20 tetrads dissected, respectively (Table 2). Many diploids bearing a *URA3 TEF5* plasmid produced four viable spore clones (Figure 1B), two of which were Trp$^-$ and two of which were Trp$^+$ and Ura$^+$, indicating that the plasmid-borne *TEF5* on a low copy plasmid allowed growth of *tef5::TRP1* haploid spore clones. The size of the four spore clones was approximately equal. Diploids bearing *TEF2* on either a low copy or high copy plasmid produced two large Trp$^-$ spore clones and several small

TABLE 2

Spore clones obtained from dissection of JWY4175 (*tef5::TRP1/TEF5*) bearing the wild-type *TEF2, TEF3, TEF4* and *TEF5* alleles or the *TEF2*-dominant mutant alleles

Plasmid	Four-spore tetrads	Three-spore tetrads	Two-spore tetrads	One-spore tetrads	Total tetrads
YCp50	0	0	20	0	20
TEF2 CEN	7	5	11	0	23
TEF2 2μ	1	2	45	0	48
TEF3	0	0	22	0	22
TEF4	0	0	20	0	20
TEF5	4	1	6	0	11
TEF2-1	2	1	11	4	18
TEF2-2	3	2	12	0	17
TEF2-3	2	2	4	1	9
TEF2-4	8	1	17	1	27
TEF2-7	4	3	9	1	17
TEF2-9	1	3	5	0	9
TEF2-10	2	2	6	2	12
TEF2-13	3	5	13	2	23
TEF2-16	1	1	7	2	11

slowly growing spores (Figure 1B, Table 2). These small spore clones were Trp^+ and Ura^+, indicating that extra copies of *TEF2* can bypass the lethality of a *tef5::TRP1* disruption. *tef5::TRP1* haploid spore clones with either the low or high copy *TEF2* plasmid contained increased levels of *TEF2* mRNA. No significant difference in *TEF2* mRNA levels was detectable between strains bearing the low and high copy *TEF2* plasmids (data not shown).

To quantitate the growth defect in the suppressed strains and to confirm that the reduced size of suppressed spore clones was not solely due to delayed germination, growth rates of the strains containing the *tef5::TRP1* null allele and plasmid-borne *TEF5* or *TEF2* were assayed in liquid cultures. Strains with *tef5::TRP1* containing a *TEF5* plasmid showed essentially wild-type growth rates. However, the *tef5::TRP1* strains containing extra copies of *TEF2* exhibited increased doubling times, although no significant difference was seen between the strains bearing low and high copy *TEF2* plasmids (Table 3). Therefore, the small size of spore clones in which *tef5::TRP1* is suppressed by extra copies of *TEF2* is also due to a reduced growth rate and not simply a germination defect. Strains with *tef5::TRP1* and either a high or low copy *TEF2* plasmid grew more slowly than strains bearing a *TEF5* plasmid at 13, 23, 30 and 37° and were particularly sensitive to cold temperatures (Cs^-, 13°, Figure 2). Wild-type strain JWY4134 containing extra copies of *TEF2* on either low or high copy plasmids showed no growth defects at 13 or 37°.

***TEF2*-mediated suppression of *tef5::TRP1* results in altered sensitivity to translation inhibitors:** We tested the sensitivity of the wild-type and suppressed strains to several compounds: paromomycin and hygromycin B; aminoglycosides, which affect translational fidelity; cycloheximide and cryptopleurine, which affect eukaryotic translation elongation; and the antibiotics tetracycline, streptomycin, neomycin, and kanamycin, which inhibit prokaryotic translation. No strains were sensitive to tetracycline, streptomycin, neomycin and kanamycin (data not shown). Strains with *tef5::TRP1* suppressed by extra copies of *TEF2* exhibited increased sensitivity to cycloheximide, cryptopleurine, paromomycin and hygromycin B, as measured by the zone of inhibition of growth around a drug-saturated filter paper disc. The strains in which *tef5::TRP1* is suppressed by extra copies of *TEF2* were more sensitive to cycloheximide (8 *vs.* 9.5 mm, *TEF5 vs. TEF2*), cryptopleurine (4 *vs.* 6 mm), and hygromycin B (0.5 *vs.* 3 mm) than *tef5::TRP1* strains bearing a *TEF5* plasmid (Table 4). Additionally, these strains were sensitive to paromomycin (0 *vs.* 3.5 mm). No significant difference in drug sensitivity was seen between *tef5::TRP1* strains bearing *TEF2* on low and high copy plasmids. Drug sensitivity was assayed in a

TABLE 3

Doubling times of strains with *tef5::TRP1* bearing *TEF2* plasmids

TEF2 Allele	Doubling time (hr)
TEF5	1.86 ± 0.31
TEF2 CEN	3.05 ± 0.41
TEF2 2μ	2.83 ± 0.18
TEF2-1	5.06 ± 0.24
TEF2-2	4.28 ± 0.16
TEF2-3	4.51 ± 0.61
TEF2-4	3.65 ± 0.59
TEF2-7	4.65 ± 0.69
TEF2-9	5.26 ± 0.80
TEF2-10	3.27 ± 0.61
TEF2-13	4.87 ± 0.91
TEF2-16	6.64 ± 0.49

Values are means ± SD.

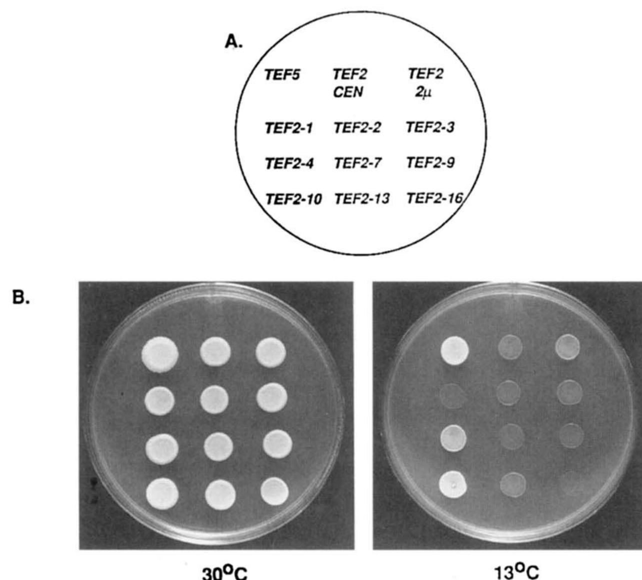

FIGURE 2.—Strains with *tef5::TRP1* containing extra copies of *TEF2* are cold sensitive. (A) Strains tested, from upper left: JWY4200 (*TEF5*), JWY4202 (*TEF2 CEN*), JWY4204 (*TEF2 2μ*), JWY4211 (*TEF2-1*), JWY4213 (*TEF2-2*), JWY4215 (*TEF2-3*), JWY4217 (*TEF2-4*), JWY4219 (*TEF2-7*), JWY4221 (*TEF2-9*), JWY4223 (*TEF2-10*), JWY4225 (*TEF2-13*), and JWY4227 (*TEF2-16*). (B) All strains were spotted onto YEPD plates and incubated for 3 days at 30° or 7 days at 13°.

wild-type strain (JWY4134) containing an extra copy of *TEF2* on either a low or a high copy plasmid. No increase in sensitivity to any translation inhibitors was observed at the concentrations tested (data not shown).

Phenotypic suppression of +1 frameshift and nonsense mutations is increased in strains with *tef5::TRP1* suppressed by *TEF2*: A *tef5::TRP1* strain containing *TEF5* on a *LEU2 CEN* plasmid (JWY4247) was constructed bearing two +1 frameshift alleles (*met2-1* and *his4-713*) and a UAG nonsense allele (*lys2-801*). Low and high copy plasmids bearing *TEF2* were transformed into JWY4247, and a variant was identified in which the plasmid bearing wild-type *TEF5* was spontaneously lost. The ability to grow on complete media (C) or complete media lacking methionine (C-met), histidine (C-his) or lysine (C-lys) was then assayed for strains containing the wild-type *TEF2* plasmids. The *tef5::TRP1* strains bearing *TEF2* on either a low copy or a high copy plasmid grew better on C-met and C-his medium than the same strain bearing a *TEF5* gene, indicating increased phenotypic suppression of the +1 frameshift mutation (Table 5, Figure 3). The suppressed strains also grew better than control strains on C-lys medium, indicating increased phenotypic suppression of the UAG nonsense mutation *lys2-801* (Table 5, Figure 3).

Dominant *TEF2* frameshift suppressors bypass the lethality of *tef5::TRP1*: Dominant mutant alleles of *TEF2* were isolated as suppressors of frameshift mutations (SANDBAKEN and CULBERTSON 1988). We tested the ability of these *TEF2* alleles to suppress the lethality of *tef5::TRP1* haploid spore clones by transforming each YCp50 plasmid into the diploid JWY4175 heterozygous for the *tef5::TRP1* disruption. Diploids were sporulated and tetrads dissected. All nine *TEF2* mutant alleles allowed growth of Trp^+ Ura^+ spore clones (Table 2). As observed for *tef5::TRP1* strains containing extra copies of wild-type *TEF2*, most of these spore clones produced colonies that were smaller in size than *TEF5* spore clones (data not shown). There were, however, obvious differences in the sizes of the *tef5::TRP1* spore clones containing the *TEF2* alleles, with *TEF2-4* and *TEF2-10* producing the largest *tef5::TRP1* spore clones.

Growth rates were measured for multiple independent isolates of strains with *tef5::TRP1* and one of the nine *TEF2* mutant alleles (Table 3). Strains with *tef5::TRP1* and *TEF2-2*, *TEF2-3*, *TEF2-7*, or *TEF2-13* had doubling times within experimental error of each other and were ~50% slower growing than a strain with *tef5::TRP1* and extra copies of wild-type *TEF2* (Table 3).

TABLE 4

Radius of inhibition of growth in *tef5::TRP1* strains suppressed by *TEF2*

Complementing gene	1.3 mM Cryptopleurine	5 mM Hygromycin B	2.5 mM Cycloheximide	48 mM Paromomycin
TEF5	4[a]	0.5	8	0
TEF2 CEN	6	3	9.5	3.5
TEF2 2μ	6	3	8.5	3.5
TEF2-1	8	3	14	6.6
TEF2-2	6.5	2.5	10	4.5
TEF2-3	6.5	2.25	10.5	3
TEF2-4	5.5	2.5	10	2.75
TEF2-7	6	1.5	11.25	3.5
TEF2-9	8	3.5	11	4.25
TEF2-10	4.75	1.25	8	2.5
TEF2-13	8	4	11	3.75
TEF2-16	9.5	4	15.5	4.5

[a] Radius of inhibition is the region with no cell growth around the corresponding drug-saturated filter disc. Measurements are given in mm and represent an average of at least four experiments.

TABLE 5

Frameshift and nonsense suppression in *tef5::TRP1* strains suppressed by extra copies of *TEF2*[a]

Plasmid-borne *TEF* allele	Complete	C−met (+1)	C−his (+1)	C−lys (UAG)
TEF5	3	0	0	0
TEF2 CEN	3	2	1	1
TEF2 2μ	3	2	1	1
TEF2-1	3	2	2	2
TEF2-2	3	2	1.5	1
TEF2-3	3	2	1	1.5
TEF2-4	3	0	0.5	0
TEF2-7	3	1	0.5	2
TEF2-9	3	2	1	0.5
TEF2-10	3	1	0.5	0.5
TEF2-13	3	1	0	0

[a] Scale is from 0 (no growth) to 3 (best growth).

Strains with *tef5::TRP1* and *TEF2-1* or *TEF2-9* showed a more pronounced growth defect. The *tef5::TRP1* strain containing *TEF2-16* grew significantly slower than all others, with a doubling time of 6.64 ± 0.49 hr. The *TEF2-4* and *TEF2-10* plasmids suppressed the growth defect of a strain with *tef5::TRP1* approximately as well as increased copies of the wild-type *TEF2* gene. These two mutant alleles contain different replacements of the same amino acid, glutamic acid 122, in EF-1α. Only *tef5::TRP1* strains bearing an extra copy of *TEF2-4* and *TEF2-10* showed reduced cold sensitivity compared with strain JWY4202 containing *tef5::TRP1* and an extra copy of wild-type *TEF2* (Figure 2). Wild-type yeast JWY4134 containing extra copies of these *TEF2* alleles were neither Cs^- or Ts^- (data not shown).

Strains with *tef5::TRP1* suppressed by *TEF2* mutant alleles exhibit different sensitivities to translation inhibitors: The sensitivity to eight different translation inhibitors was determined for *tef5::TRP1* strains suppressed by each of the nine dominant *TEF2* mutant alleles. None of the strains with *tef5::TRP1* and a dominant *TEF2* mutant allele were sensitive to tetracycline, streptomycin, neomycin or kanamycin (data not shown). However, the *tef5::TRP1* strains containing *TEF2* mutant alleles showed significant differences in their sensitivity to the other drugs (Table 4). Strains with *tef5::TRP1* and the *TEF2-9* and *TEF2-16* mutant alleles exhibited increased sensitivity to all four drugs. Of the other strains with *tef5::TRP1* that contain *TEF2* mutant alleles, several showed a significant increase in sensitivity to cryptopleurine (*TEF2-1* and *TEF2-13*), hygromycin B (*TEF2-13*), cycloheximide (*TEF2-1, TEF2-7* and *TEF2-13*), and paromomycin (*TEF2-1* and *TEF2-2*). Most interestingly, several strains with *tef5::TRP1* and *TEF2* dominant alleles are less sensitive to hygromycin B (*TEF2-2* and *TEF2-7*); hygromycin B and paromomycin (*TEF2-3*); hygromycin B, paromomycin and cryptopleurine (*TEF2-4*); and all four drugs (*TEF2-10*) compared with a *tef5::TRP1* strain with *TEF2*. The cycloheximide and cryptopleurine sensitivity of the *TEF2-10 tef5::TRP1* strain was reduced to levels equal to, or very near to, strains bearing wild-type *TEF5*. Wild-type yeast JWY4134 containing extra copies of the nine *TEF2* alleles were not sensitive to tetracycline, streptomycin, neomycin, kanamycin, or hygromycin B, and most showed little to no change in sensitivity to cycloheximide and cryptopleurine (data not shown). Some dominant mutant alleles of *TEF2* showed increased paromomycin sensitivity

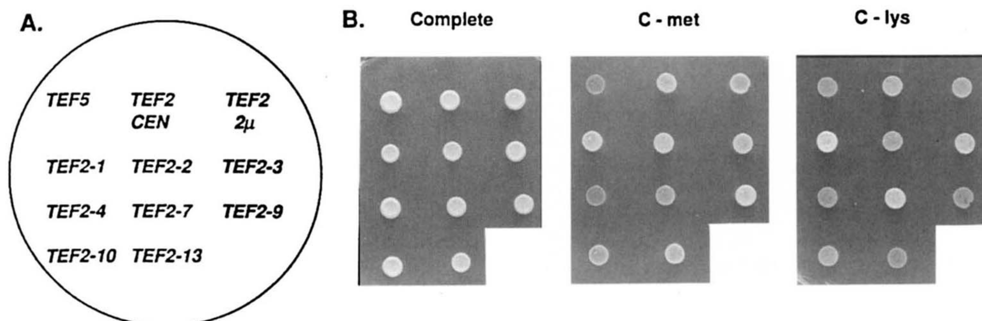

FIGURE 3.—Phenotypic suppression is increased in many strains with *tef5::TRP1* and extra copies of *TEF2*. (A) Strains tested, from upper left: JWY4247 (*TEF5*), JWY4248 (*TEF2 CEN*), JWY4249 (*TEF2 2μ*), JWY4250 (*TEF2-1*), JWY4251 (*TEF2-2*), JWY4252 (*TEF2-3*), JWY4253 (*TEF2-4*), JWY4254 (*TEF2-7*), JWY4255 (*TEF2-9*), JWY4256 (*TEF2-10*), and JWY4257 (*TEF2-13*). (B) Cell suspensions of these strains were spotted onto complete, C-met and C-lys solid medium and incubated for 5 days at 30°.

in a wild-type strain, as described previously (SANDBAKEN and CULBERTSON 1988).

Increased phenotypic suppression of +1 frameshift and nonsense mutations correlates with paromomycin sensitivity of strains with *tef5::TRP1* and extra copies of *TEF2* dominant alleles: Phenotypic suppression of +1 frameshift and nonsense mutations was assayed for strains derived from JWY4231 bearing *tef5::TRP1* and each of eight dominant *TEF2* mutant alleles. Strains with *tef5::TRP1* bearing *TEF2-16* grew very poorly and were not assayed. Strains with *tef5::TRP1* and *TEF2-4* or *TEF2-10* were the least sensitive to drugs, less sensitive than a strain with *tef5::TRP1* suppressed by the wild-type *TEF2* allele. Both +1 frameshift mutations (*met2-1* and *his4-713*) and a UAG nonsense allele (*lys2-801*) were suppressed significantly less efficiently in a *tef5::TRP1* strain containing extra copies of *TEF2-4* than in strains containing extra copies of the other *TEF2* mutant alleles or wild-type *TEF2* (Table 5, Figure 3). Frameshift or nonsense suppression in a *tef5::TRP1 TEF2-10* strain was approximately half as efficient compared with a *tef5::TRP1* strain containing extra copies of wild-type *TEF2*. Strains with *tef5::TRP1* and either *TEF2-7* or *TEF2-13* showed slightly increased phenotypic suppression of the *met2-1* allele compared with wild-type strains (Figure 3, Table 5). Frameshift mutations are suppressed most efficiently in the *tef5::TRP1* strain containing extra copies of *TEF2-1* (Table 5). Strains with *tef5::TRP1* and *TEF2-1, TEF2-3* or *TEF2-7* exhibited increased phenotypic suppression of a nonsense mutation relative to a *tef5::TRP1* strain with either *TEF5* or *TEF2* (Figure 3, Table 5). *TEF2-3, TEF2-9* and *TEF2-13* were unable to suppress UAG codons when overexpressed in a wild-type strain (SANDBAKEN and CULBERTSON 1988). In the background of a *tef5::TRP1* disruption, *TEF2-3* suppressed a UAG nonsense allele, whereas *TEF2-9* showed a slight increase in phenotypic suppression (Figure 3, Table 5).

DISCUSSION

We determined that overexpression of *TEF2*, encoding the α subunit of EF-1, can suppress the lethality of a *tef5::TRP1* disruption. A low copy plasmid containing either the *TEF3* or *TEF4* gene encoding a protein similar in sequence to EF-1γ could not overcome the requirement for EF-1β. Thus, suppression was limited to the substrate for the exchange activity of EF-1β, EF-1α, but not other subunits of the EF-1 complex. These results suggest that the spontaneous release rate for GDP by EF-1α is sufficient for viability, provided excess EF-1α is available to the cell. Further overexpression of EF-1α might bypass the requirement for EF-1β more efficiently. We found no significant difference in the level of suppression by *TEF2* when it is present on either low copy or high copy plasmid. This might be a result of the levels of *TEF2* mRNA, which were approximately the same in strains with either plasmid.

The suppression of *tef5::TRP1* by extra copies of *TEF2* yielded slow-growing and cold-sensitive strains, indicating that cellular functions are not proceeding at wild-type rates or levels. This effect was specific to EF-1β-deficient strains, because an extra copy of the *TEF2* gene in a wild-type *TEF5* strain did not show the cold sensitivity and slow growth phenotype. Strains with *tef5::TRP1* bearing an extra copy of *TEF2* were sensitive to two drugs that affect translation elongation, cryptopleurine and cycloheximide, as well as two aminoglycosides, paromomycin and hygromycin B, that are known to affect translational fidelity in yeast (PALMER *et al.* 1979; SINGH *et al.* 1979). Previous studies with *TEF2* mutant alleles, as well as increased levels of EF-1α expression, showed a correlation between EF-1α function and fidelity (SANDBAKEN and CULBERTSON 1988; SONG *et al.* 1989). Thus, fidelity may require a small window of optimum EF-1 activity, because excess activity or altered EF-1α proteins lead to changes in translational fidelity.

Although the amount of EF-1α expression is increased, the lack of the exchange activity by EF-1$\beta\gamma$ results in a net loss of EF-1 activity. The EF-1$\alpha\beta\gamma$ complex is two- to threefold more active in polyphenylalanine synthesis *in vitro* than EF-1α alone. Free EF-1α also has a 10-fold tighter binding constant for GDP compared with EF-1$\alpha\beta\gamma$ (SAHA and CHAKRABURTTY 1986). It has been estimated based on the elongation and nucleotide exchange rates that recycling of active EF-1α may be the rate-limiting step in translation (JANSSEN and MOLLER 1988b). These calculations do not take into account any negative effects on translation due to the loss of other functions of EF-1β or EF-1γ, such as their proposed roles in directing the location of translation within the cell (JANSSEN and MOLLER 1988a). The need to optimize the rate of translation yet control the level of EF-1α protein may be another important factor in the requirement for EF-1β in yeast. Overexpression of EF-1α may produce unexpected and potentially deleterious effects. For example, overexpression of EF-1α causes transformation of some cell lines (TATSUKA *et al.* 1992).

We further hypothesized that mutant alleles of *TEF2* that are already compromised for fidelity might exhibit a synthetically lethal effect with a *tef5::TRP1* disruption. Strains with *tef5::TRP1* containing wild-type *TEF2* increased phenotypic suppression of +1 frameshift and nonsense mutations. We tested the nine dominant frameshift suppressor alleles of *TEF2* isolated by SANDBAKEN and CULBERTSON (1988) for the ability to suppress the *tef5::TRP1* lethality. In a wild-type strain these *TEF2* mutant alleles showed varying abilities to suppress three different +1 frameshift mutations: *TEF2-1* suppressed all three well; *TEF2-2, TEF2-3*, and *TEF2-10* suppressed all three but less well; *TEF2-4* and *TEF2-7* suppressed two of three frameshift mutations; *TEF2-9, TEF2-13*, and *TEF2-16* are the least efficient and sup-

pressed only one frameshift (SANDBAKEN and CULBERTSON 1988). Suppression of UAG nonsense mutations was only observed for *TEF2-1, TEF2-2, TEF2-4, TEF2-7,* and *TEF2-10* (SANDBAKEN and CULBERTSON 1988). Five of these mutant alleles also cause paromomycin sensitivity: *TEF2-1, TEF2-2, TEF2-4, TEF2-9* and *TEF2-10* (SANDBAKEN and CULBERTSON 1988). All nine dominant alleles were able to suppress the lethality of *tef5::TRP1.* As predicted, those *tef5::TRP1* strains containing an extra copy of seven of the nine *TEF2* alleles also had decreased growth rates and increased sensitivity to antibiotics compared with the same strain containing an extra copy of the wild-type *TEF2* gene. Wild-type *TEF5* strains bearing the seven mutant alleles of *TEF2* exhibit neither the increased doubling times nor the increased sensitivity to translation inhibitors. However, *tef5::TRP1* strains containing extra copies of two *TEF2* alleles with mutations in glutamic acid 122 (*TEF2-4* E122K and *TEF2-10* E122Q) were not slower growing and were less sensitive to antibiotics than a strain with *tef5::TRP1* and extra copies of wild-type *TEF2.* This surprising result indicates that additional factors are involved in the fidelity effects in addition to the frameshift-suppressing phenotype of the dominant *TEF2* mutant alleles. The growth rates of *tef5::TRP1* strains containing *TEF2-4* or *TEF2-10* were still slow compared with wild-type yeast but had little or no increase in phenotypic suppression of +1 frameshift and UAG nonsense mutations. This indicates that the slow growth of a *tef5::TRP1* strain containing extra copies of *TEF2* is not solely due to effects on translational fidelity. On the other hand, the decreased translational fidelity caused by the *TEF2* mutant alleles in the wild-type background is reflected in the efficiency of frameshift suppression in a *tef5::TRP1* strain. For example, the most efficient frameshift suppressor in a wild-type background, *TEF2-1,* was the most effective frameshift suppressor in a *tef5::TRP1* strain. Likewise, one of the least efficient *TEF2* frameshift suppressors, *TEF2-13,* was an inefficient frameshift suppressor in a *tef5::TRP1* strain. There were, however, significant differences in the translational status of the *tef5::TRP1* strains, because alleles such as *TEF2-3* and *TEF2-9,* which are unable to suppress nonsense mutations in a wild-type strain, exhibited translational readthrough of a UAG stop codon in a *tef5::TRP1* strain. The observation that most of these *tef5::TRP1 TEF2*-suppressed strains are cold sensitive is perhaps due to the fact that other cellular processes have also slowed or that translation elongation is particularly sensitive to cold temperatures. These phenotypes (Cs^-, drug sensitivity, and reduced growth rate) were specific to the *tef5::TRP1* strains and were not seen in a wild-type strain containing the dominant *TEF2* mutant alleles. The *tef5::TRP1* strains containing extra copies of *TEF2* will be an excellent system to compare the rate of elongation and the fidelity of translation.

These results raise an interesting hypothesis for the mechanism by which the mutant alleles of *TEF2* suppress the lethality of the *tef5::TRP1* disruption. The two *TEF2* mutations that suppressed the *tef5::TRP1* null allele most efficiently change the glutamic acid residue at position 122 of EF-1α (*TEF2-4* E122K and *TEF2-10* E122Q). The corresponding residues in the prokaryotic homologue EF-Tu lie within the GTP binding domain (MORIKAWA *et al.* 1978; LA COUR *et al.* 1985). Thus, these two mutations may affect the GDP or GTP binding constants or the rate of GTP hydrolysis, resulting in an effective increase in the concentration of active (GTP-bound) EF-1α. These and previous results linking EF-1α to fidelity may indicate that the fidelity of translation may be affected by the nucleotide recycling activity of EF-1β. Multiple components of the translation machinery, including ribosomal proteins (ROSSET and GORINI 1969; ATKINS *et al.* 1972), EF-1α (SANDBAKEN and CULBERTSON 1988; SONG *et al.* 1989) and perhaps other translation factors (SILAR 1994) such as EF-1β, affect translation fidelity. Further analysis of the effect of the deficiency of EF-1β in yeast, as well as isolation of mutant alleles of *TEF5,* help to define the role of EF-1β in maintaining the balance of fidelity and translation rates.

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LITERATURE CITED

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.

BOEKE, J. D., R. LACROUTE and G. R. FINK, 1984 A positive selection for mutants lacking orotidine-5'-phosphate decarboxylase activity in yeast: 5-fluoro orotic acid resistance. Mol. Gen. Genet. **197:** 345–347.

BOTSTEIN, D., S. C. FALCO, S. E. STEWART, M. BRENNAN, S. SCHERER *et al.,* 1979 Sterile host yeast (SHY): a eukaryotic system of biological containment for recombinant DNA experiments. Gene **8:** 17–24.

CARVALHO, M. G., J. F. CARVALHO and W. C. MERRICK, 1984 Biological characterization of various forms of elongation factor 1 from rabbit reticulocytes. Arch. Biochem. Biophys. **234:** 603–611.

CHAKRABURTTY, K., and A. KAMATH, 1988 Protein synthesis in yeast. Int. J. Biochem. **20:** 581–590.

DASMAHAPATRA, B., L. SKOGERSON and K. CHAKRABURTTY, 1981 Protein synthesis in yeast. II. Purification and properties of elongation factor 1 from *Saccharomyces cerevisiae.* J. Biol. Chem. **256:** 10005–10011.

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.

HINNEBUSCH, A., and S. W. LIEBMAN, 1991 Protein synthesis and translational control in *Saccharomyces cerevisiae,* pp. 627–735 in *The Molecular and Cellular Biology of the Yeast Saccharomyces: Genome Dynamics, Protein Synthesis and Energetics,* edited by J. R. BROACH, E. W. JONES and J. R. PRINGLE. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

HIRAGA, K., K. SUZUKI, E. TSUCHIYA and T. MIYAKAWA, 1993 Cloning

and characterization of the elongation factor EF-1β homologue of *Saccharomyces cerevisiae*. EF-1β is essential for growth. FEBS Lett. **316:** 165-169.

ITO, H., K. Y. FUKUDA, K. MURATA and A. KIMURA, 1983 Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153:** 163-168.

JANSSEN, G. M. C., and W. MOLLER, 1988a Elongation factor 1βγ from *Artemia*: purification and properties of its subunits. Eur. J. Biochem. **171:** 119-129.

JANSSEN, G. M. C., and W. MOLLER, 1988b Kinetic studies on the role of elongation factors 1β and 1γ in protein synthesis. J. Biol. Chem. **263:** 1773-1778.

JOHNSTON, M., and R. DAVIS, 1984 Sequences that regulate the divergent *GAL1-GAL10* promoter in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **4:** 1440-1448.

KAMBOURIS, N. G., D. J. BURKE and C. E. CREUTZ, 1993 Cloning and genetic characterization of a calcium and phospholipid binding protein from *Saccharomyces cerevisiae* that is homologous to translation elongation factor 1-γ. Yeast **9:** 151-163.

KINZY, T. G., T. R. RIPMASTER and J. L. WOOLFORD JR., 1994 Multiple genes encode the translation elongation factor 1-γ in *Saccharomyces cerevisiae*. Nucleic Acids Res. **22:** 2703-2707.

KUNKEL, T. A., J. D. ROBERTS and R. A. ZAKOUR, 1987 Rapid and efficient site-specific mutagenesis without phenotypic selection. Methods Enzymol. **154:** 367-382.

KUSHNIROV, V. V., M. D. TER-AVANESYAN, M. V. TELCKOV, A. P. SURGUCHOV, V. N. SMIRNOV *et al.*, 1988 Nucleotide sequence of the *sup2* (*sup35*) gene of *Saccharomyces cerevisiae*. Gene **66:** 45-54.

LA COUR, T. F. M., J. NYBORG, S. THIRUP and B. F. C. CLARK, 1985 Structural details of the binding of guanosine diphosphate to elongation factor Tu from *E. coli* as studied by X-ray crystallography. EMBO J. **4:** 2385-2388.

LIEBMAN, S. W., and M. CAVENAGH, 1980 An antisuppressor that acts on omnipotent suppressors in yeast. Genetics **95:** 49-61.

MADDOCK, J. R., E. M. WEIDENHAMMER, C. C. ADAMS, R. L. LUNZ and J. J. L. WOOLFORD, 1994 Extragenic suppressors of *Saccharomyces cerevisiae prp4* mutations identify a negative regulator of *PRP* genes. Genetics **136:** 833-847.

MERRICK, W. C., T. E. DEVER, T. G. KINZY, S. C. CONROY, J. CAVALLIUS *et al.*, 1990 Characterization of protein synthesis factors from rabbit reticulocytes. Biochim. Biophys. Acta **1050:** 235-240.

MERRICK, W. C., J. CAVALLIUS, T. G. KINZY and W. L. ZOLL, 1993 Evolution of the EF-Tu family, pp. 669-678 in *The Translational Apparatus*, edited by K. NIERHAUS. Plenum Publishing Corp., New York.

MOLDAVE, K., 1985 Eukaryotic protein synthesis. Annu. Rev. Biochem. **54:** 1109-1149.

MORIKAWA, K., T. F. M. LA COUR, J. NYBORG, K. M. RASMUSSEN, D. L. MILLER *et al.*, 1978 High resolution X-ray crystallographic analysis of a modified form of the elongation factor Tu: guanosine triphosphate complex. J. Mol. Biol. **125:** 325-338.

MORTIMER, R. K., and D. C. HAWTHORNE, 1966 Genetic mapping in Saccharomyces. Genetics **53:** 165-173.

NAGATA, S., K. SAGASHIMA, Y. TSUNETSUGU-YOKOTA, K. FUJIMURA, M. MIYAZAKI *et al.*, 1984 Polypeptide chain elongation factor 1α (EF-1α) from yeast: nucleotide sequence of one of the two genes for EF-1α from *Saccharomyces cerevisiae*. EMBO J. **3:** 1825-1830.

PALMER, E., J. M. WILHELM and F. SHERMAN, 1979 Phenotypic suppression of nonsense mutants in yeast by aminoglycoside antibiotics. Nature **277:** 148-150.

ROSE, M. D., P. NOVICK, J. H. THOMAS, D. BOTSTEIN and G. R. FINK, 1987 A *Saccharomyces cerevisiae* genomic plasmid based on a centromere containing shuttle vector. Gene **60:** 237-243.

ROSSET, R., and L. GORINI, 1969 A ribosomal ambiguity mutation. J. Mol. Biol. **39:** 95-112.

ROTHSTEIN, R. J., 1983 One-step gene disruption in yeast. Methods Enzymol. **101:** 202-211.

SAHA, S. K., and K. CHAKRABURTTY, 1986 Protein synthesis in yeast. Isolation of variant forms of elongation factor 1 from the yeast *Saccharomyces cerevisiae*. J. Biol. Chem. **261:** 12599-12603.

SAMBROOK, J., E. F. FRITSCH and T. MANIATIS, 1989 *Molecular Cloning. A Laboratory Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

SANDBAKEN, M. G., and M. R. CULBERTSON, 1988 Mutations in elongation factor EF-1α affect the frequency of frameshifting and amino acid misincorporation in *Saccharomyces cerevisiae*. Genetics **120:** 923-934.

SHERMAN, F., 1982 Suppression in the yeast *Saccharomyces cerevisiae*, pp. 463-486 in *The Molecular Biology of the Yeast Saccharomyces: Metabolism and Gene Expression*, edited by J. N. STRATHERN, E. W. JONES and J. R. BROACH. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

SHERMAN, F., G. R. FINK and J. B. HICKS, 1986 *Methods in Yeast Genetics: A Laboratory Course Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

SIKORSKI, R. S., and P. HIETER, 1989 A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *S. cerevisiae*. Genetics **122:** 19-27.

SILAR, P., 1994 Is translational accuracy an out-dated topic? Trends Genet. **10:** 71-72.

SINGH, A., D. URSIC and J. DAVIES, 1979 Phenotypic suppression and misreading in *Saccharomyces cerevisiae*. Nature **277:** 146-148.

SKOGERSON, L., and E. WAKATAMA, 1976 A ribosome-dependent GTPase from yeast distinct from elongation factor 2. Proc. Natl. Acad. Sci. USA **73:** 73-76.

SLOBIN, L. I., and W. MOLLER, 1978 Purification and properties of an elongation factor functionally analogous to bacterial elongation factor Ts from embryos of *Artemia salina*. Eur. J. Biochem. **84:** 69-77.

SONG, J. M., S. PICOLOGLOU, C. M. GRANT, M. FIROOZAN, M. F. TUITE *et al.*, 1989 Elongation factor EF-1α gene dosage alters translational fidelity in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **9:** 4571-4575.

TATSUKA, M., H. MITSUI, M. WADA, A. NAGATA, H. NOJIMA *et al.*, 1992 Elongation factor-1α gene determines susceptibility to transformation. Nature **359:** 333-336.

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