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Elongation Factor EF-1α Gene Dosage Alters Translational Fidelity in *Saccharomyces cerevisiae*

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Changes in the dosage of genes encoding elongation factor EF-1α were shown to cause parallel changes in the misreading of nonsense codons. Higher amounts of EF-1α were correlated with increased nonsense suppression, suggesting that the level of EF-1α is critically involved in translational fidelity.

To understand how translational accuracy is controlled, it is important to identify which genes are involved and to determine how they exert their effects. The best-studied mutations that alter translational fidelity in yeast cells are the codon-specific suppressors caused by altered tRNA anticodons (for reviews, see references 32 and 40). Among other genes that affect translational accuracy are the omnipotent suppressors, e.g., *sup35* and *sup45*, which cause non-codon-specific misreading (3, 9, 11, 17, 42), and the recessive antisuppressor, *asu9*, which increases translational fidelity in *sup35* and *sup45* strains (19, 20). While attempting to clone *asu9*, we found that an extra copy of one of two redundant genes coding for the translation elongation factor EF-1α increased suppression efficiency in a *sup45-2 asu9* strain.

EF-1α is an important component of the translational apparatus. It promotes the GTP-dependent binding of aminoacyl-tRNA to the ribosome and participates in the proofreading of the codon-anticodon match (16, 23, 38). Yeast EF-1α is encoded by two unlinked genes, *TEF1* and *TEF2* (5, 25, 26, 31). The two genes are efficiently transcribed at about the same level, and the presence of either gene is sufficient for cell viability and normal growth rates (4). Mutations in the yeast EF-1α and its *Escherichia coli* analog EF-Tu have been found to affect translational fidelity (13, 14, 30, 37, 41). In yeast cells, single mutational changes in *TEF2* were shown to cause dominant suppression of both nonsense and frameshift mutations (30). In this paper, we show that the level of EF-1α also affects translational fidelity.

(Preliminary accounts of some of these results have been reported elsewhere [J. M. Song and S. W. Liebman, Yeast 2:S364, 1986; S. W. Liebman, J. M. Song, J. All-Robyn, E. Griffin, and D. Kelley-Geraghty, NATO ASI Ser. **H14**: 403–414, 1988].)

A DNA clone containing the *TEF1* gene complements *asu9*. *Saccharomyces cerevisiae* strain SL680-4B (**a** *asu9-1 sup45-2 leu2-1 met8-1 trp1-1 ade3-26 his5-2 lys2-1 can1-132 ura3-52*) was chosen as the host for selection of *asu9* complementing clones. In this strain, the *asu9-1* mutation prevented growth on medium containing trichodermin (0.5 μg/ml; kindly provided by Leo Pharmaceutical Products), and the *asu9-1* and *sup45-2* mutations together permitted only a low level of suppression of the UAA marker *leu2-1* and the UAG marker *met8-1* (19; J. M. Song, Ph.D. thesis, University of Illinois, Chicago, 1987). The host was transformed (10, 12) with a library of the yeast genome made in the *URA3*-bearing *CEN* plasmid YCp50 (28). Transformants obtained on synthetic complete medium (SC) (33) lacking uracil were pooled and replated on medium with uracil, leucine, and methionine omitted and containing 0.5 μg of trichodermin per ml to select for *asu9* complementing clones. Plasmid pJS7, isolated from one of the fastest-growing transformants on this medium, caused both a slight reduction in the trichodermin sensitivity and enhanced suppression of *leu2-1* and *met8-1* upon retransformation (15) of SL680-4B. These phenotypes were coretained or colost with the plasmid. The restriction map of pJS7 is identical to the published map (26) of the *TEF1* region (Fig. 1). Furthermore, DNA blot (35) analysis

TABLE 1. Effect of *tef2*-Δ on suppression efficiency

Strain	Partial genotype[a]	No. of *TEF* genes	Growth on medium lacking[b]:				
			Leu	Met	Trp	Ilv	Ura
L-1221	*sup45 tef2*-Δ (YCp50)	1	+	++	±	−	++
L-1222	*sup45 tef2*-Δ (pJS7)	2	++	++	+	−	++
L-1225	*SUP7*-**a** *tef2*-Δ (YCp50)	1	−	++	+	±	++
L-1226	*SUP7*-**a** *tef2*-Δ (pJS7)	2	−	++	++	++	++

[a] Strains L-1221 and L-1222 resulted from transformation of strain SL895-2B (**a** *sup45-2 leu2-1 met8-1 trp1-1 ilv1-1 ade3-26 his5-2 tef2*-Δ *lys2-201 ura3-52*) with the *URA3*-containing plasmids YCp50 and pJS7, respectively. Likewise, strains L-1225 and L-1226 were transformants of SL899-1C (α *SUP7*-**a** *leu2-1 met8-1 trp1-1 aro7-1 ilv1-1 ade3-26 his5-2 tef2*-Δ *lys2-201 ura3-52*). The *met8-1*, *trp1-1*, *aro7-1*, *ilv1-1*, *ade3-26*, and *can1-132* alleles are UAG (amber) mutations; the *leu2-1*, *his5-2*, *lys1-1*, and *lys2-1* alleles are UAA (ochre) mutations (21). The *ura3-52* allele is not suppressible and does not revert (27).

[b] There was no difference in growth rate in SC or SC lacking uracil between isogenic strains with one or two copies of *TEF*. The efficiency of suppression was determined by the level of growth that the suppressor permitted on appropriate omission media. Reliable growth rates could not be obtained in liquid medium in which suppression was required for growth, because faster-growing revertants tended to take over the population. Thus, growth was measured by comparing the intensities of spots on plates made by inoculations with suspensions of cells. Here, the occasional spotty growth of a revertant could be distinguished from that of the nonrevertant background. ++, Good growth by 1 or 2 days; +, good growth by 3 days; ±, some growth by 4 days; −, no sign of growth by 7 days. The efficiency of suppression and the effect of *tef2*-Δ varied with the markers scored. Markers that were already suppressed efficiently with one *TEF* gene (e.g., *met8-1*) were not suppressed any more efficiently by two copies of *TEF*.

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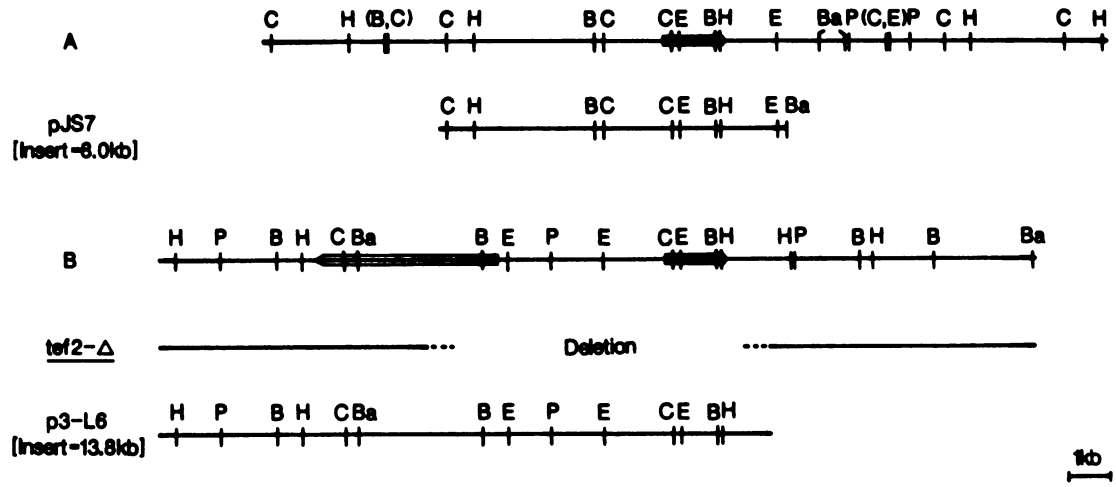
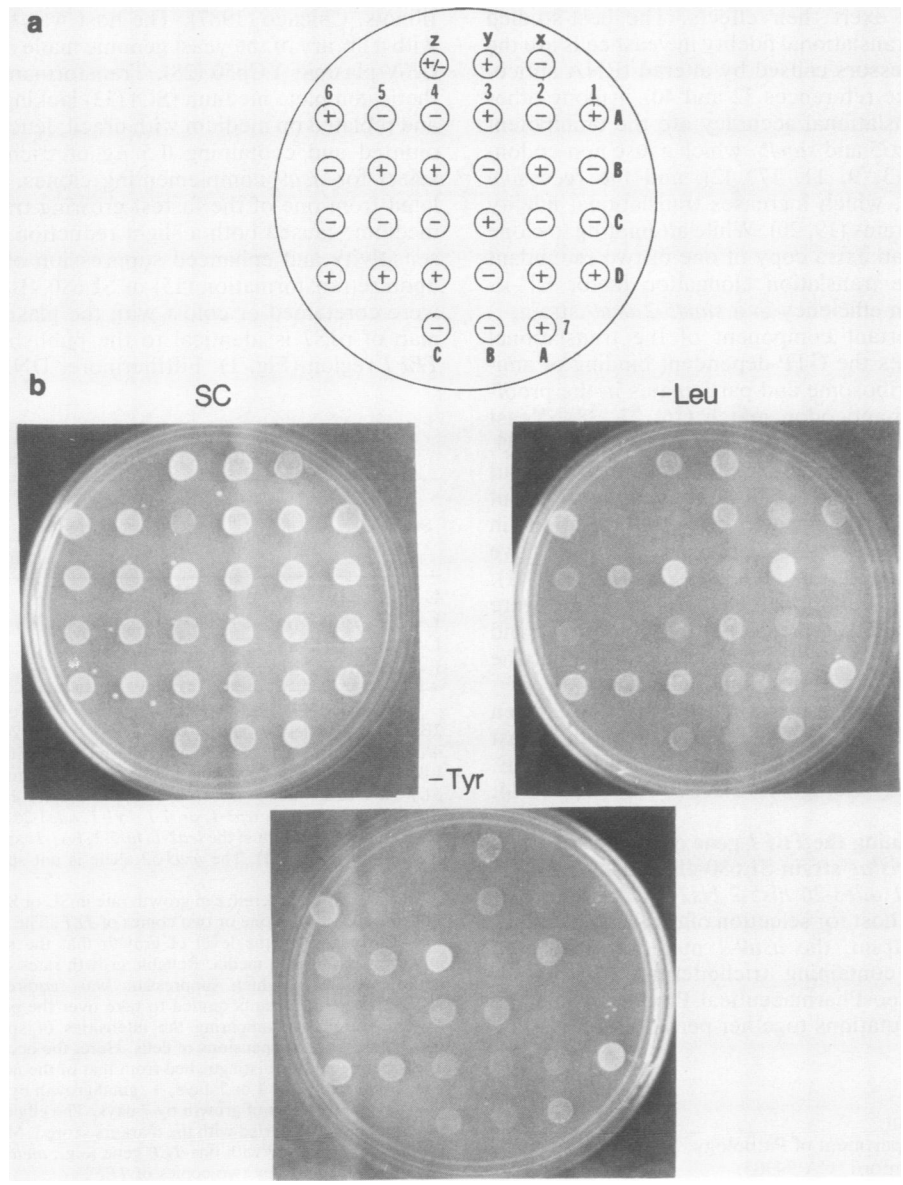


FIG. 1. Restriction maps of plasmids carrying the EF-1α genes. Symbols: ■, EF-1α genes; □, *LYS2* gene. Arrowheads indicate the direction of transcription. Restriction enzyme abbreviations: B, *Bgl*II; Ba, *Bam*HI; C, *Cla*I; E, *Eco*RI; H, *Hin*dIII; P, *Pst*I. (A) Map of the *TEF1* region (26); (B) map of the *TEF2-LYS2* region (31). The extent of the deletion in the *tef2*-Δ (or *lys2-201*) allele is also shown (31, 34). It includes the *LYS2* 5′ region and most of *TEF2*.



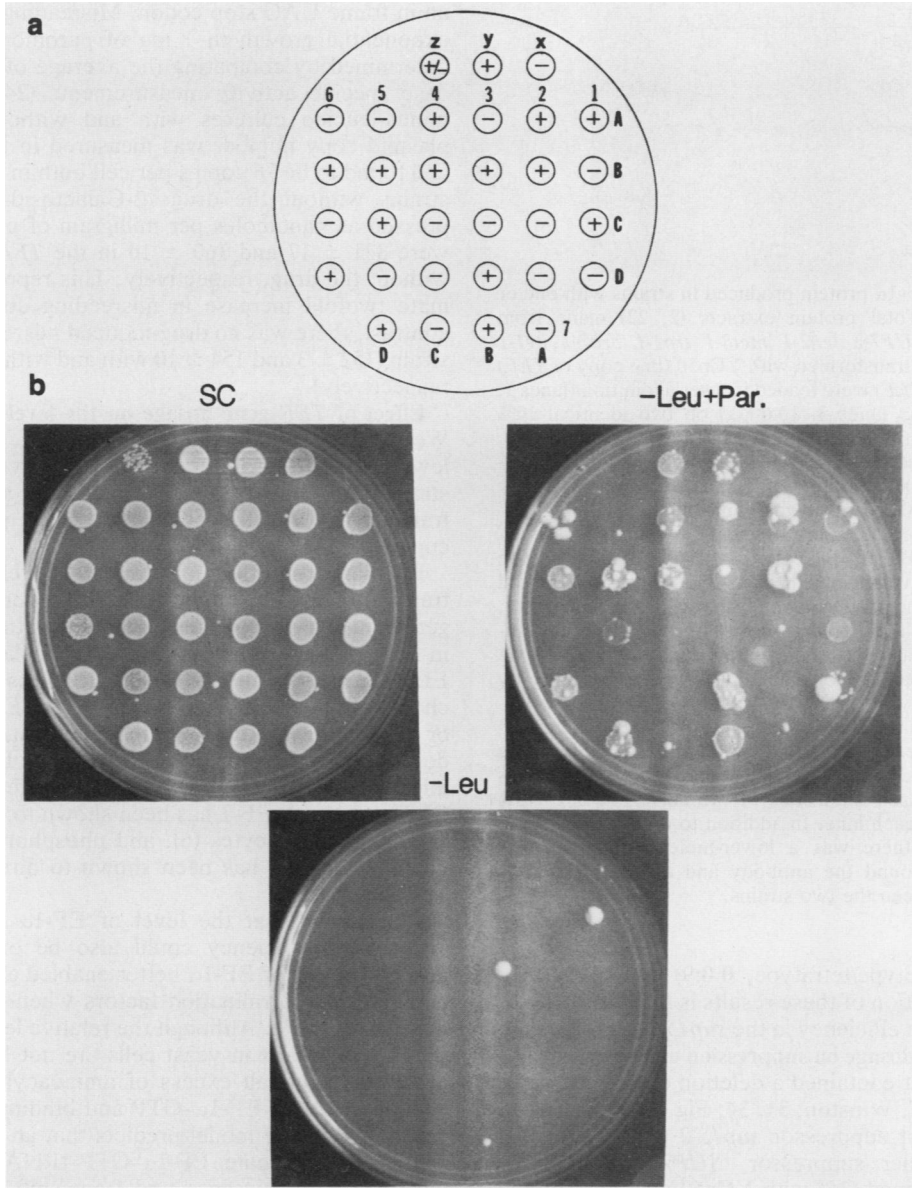


FIG. 3. Cosegregation of reduced paromomycin-induced misreading with *tef2*-Δ. (a) Strains represented: x, *tef2*-Δ (SL895-6A, **a** *leu2-1 met8-1 ade3-26 his5-2 tef2*-Δ *lys2-201 ura3-52*); y, *TEF2* (SL787-6B, α *leu2-1 met8-1 trp1-1 aro7-1 ilv1-1 ade3-26 his5-2 lys1-1 can1-132 cyc1-76*); z, diploid made from crossing SL895-6A and SL787-6B. Seven complete tetrads were obtained. All strains carry the UAA marker *leu2-1*. + and −, Genotypes, *TEF2* and *tef2*-Δ, respectively. (b) Growth of cells. Patches of strains indicated in panel a were spotted on SC, SC minus leucine (−Leu), and SC minus leucine with 0.1 mg of paromomycin per ml (−Leu + Par) by inoculations with suspensions of cells. Growth of spots on these plates were recorded in photographs after 14 days of incubation. The occasional spotty growth of revertants that arose during the 14 days of incubation can be distinguished from the nonrevertant background.

confirmed that pJS7 contains one of the two genes encoding EF-1α, since the 0.87-kilobase *Eco*RI-*Hin*dIII fragment from the pJS7 insert hybridized with the p3L6 *TEF2* probe (kindly provided by C. Falco; data not shown). However, neither *TEF1* nor *TEF2* is the *ASU9* gene, since *TEF1* maps approximately 16 centimorgans from *aro7* on chromosome XVI (30) and *TEF2* is tightly linked to *lys2* on chromosome II (Fig. 1; 31, 34), whereas *asu9* is unlinked to either *aro7* (parental

FIG. 2. Cosegregation of inefficient *sup35-2* suppression with *tef2*-Δ. (a) Strains represented: x, *sup35-2 tef2*-Δ (SL898-1A, **a** *sup35-2 leu2-1 met8-1 trp1-1 aro7-1 ade3-26 his5-2 tef2*-Δ *lys2-201 ura3-52*); y, *sup35-2 TEF2* (SL429-7D, α *sup35-2 leu2-1 met8-1 trp1-1 aro7-1 ilv1-1 ade3-26 his5-2 lys1-1 can1-132 cyc1-76*); z, diploid made from crossing SL898-1A and SL429-7D. Six complete tetrads and one with only three viable spores were obtained from the diploid. All strains carry the UAA marker *leu2-1* and the UAG marker *aro7-1*. + and −, Genotypes *TEF2* and *tef2*-Δ, respectively. (b) Growth of cells. Patches of strains indicated in panel a were spotted on SC, SC-minus-leucine (−Leu), and SC-minus-tyrosine (−Tyr) plates by inoculations with suspensions of cells. Growth of spots on the plates was recorded in photographs after 2 days of incubation. The suppression efficiency varied somewhat in different strains having the same pertinent genotypes, probably because of numerous modifying genes.

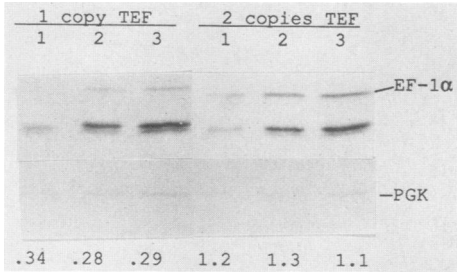


FIG. 4. Levels of EF-1α protein produced in strains with one or two copies of *TEF*. Total protein extracts (2, 22) made from SL899-1C (α *tef2-Δ SUP7-a leu2-1 met8-1 trp1-1 aro7-1 ilv1-1 ade3-26 his5-2 ura3-52*) transformed with YCp50 (one copy of *TEF*) or pJS7 (two copies of *TEF*) were loaded in a serial dilution (lanes 1, 2.5 μg; lanes 2, 5.0 μg; lanes 3, 10.0 μg) on two identical 10% polyacrylamide gels. After electrophoresis (18) and blotting (39), filters were incubated sequentially with blocking solution (5% instant nonfat dry milk, 200 mM NaCl, 50 mM Tris [pH 7.4]), primary antibody (diluted 1:1,000), and a goat anti-rabbit alkaline phosphatase conjugate (Bio-Rad Laboratories). They were then developed by using Bio-Rad BCIP/NBT color development solution. The blot of one gel was developed with a polyclonal antibody raised against yeast EF-1α (the kind gift of E. J. Mellor, Biochemistry, University of Oxford). The parallel blot was probed with an antibody raised against yeast phosphoglycerate kinase (PGK; Sigma Chemical Co.) as a loading control. Levels were quantified by determining the intensity of the EF-1α bands relative to the corresponding PGK band by using a scanning densitometer. The differences in intensity between the twofold dilutions of the PGK bands varied between 0.25 and 0.36. The intensities of the EF-1α bands divided by the normalized intensities of the corresponding PGK bands are listed below each lane. In addition to the EF-1α band at about 50 kilodaltons, there was a lower-molecular-mass cross-reacting protein that bound the antibody and did not show any difference in level between the two strains.

ditype/nonparental ditype/tetratype, 0:0:9) or *lys2* (0:2:7). The simplest explanation of these results is that extra EF-1α increases suppression efficiency in the *sup45-2 asu9-1* strain.

Effect of *TEF* gene dosage on suppression efficiency. Strains were constructed that contained a deletion of *TEF2* (*tef2-Δ*) (kindly supplied by F. Winston; 31, 34; Fig. 1) coupled with either the omnipotent suppressor *sup45-2* or the tyrosine-inserting tRNA amber suppressor, *SUP7-a* (21). These strains were transformed (15) with YCp50 as a control and with plasmid pJS7, which contains the *TEF1* insert. Since pJS7 is a *CEN* plasmid, it should be present in about one copy per cell (1). Suppression efficiency was lower in the YCp50 transformants with one *TEF* gene than in the corresponding pJS7 transformants with two copies of *TEF* (Table 1).

Similar results were obtained from crosses that were heterozygous for *tef2-Δ* but homozygous for *SUP7-a* or *sup35*. Here, a decreased level of suppression segregated with *tef2-Δ* (e.g., see Fig. 2). In addition, in a cross without suppressors, a decreased level of misreading induced by the aminoglycoside antibiotic paromomycin (kindly provided by Warner-Lambert) segregated with *tef2-Δ* (Fig. 3).

A gene fusion assay that requires misreading of a UAG codon for β-galactosidase activity (8; M. Firoozan, C. M. Grant, and M. F. Tuite, submitted for publication) was also used to compare the levels of UAG misreading induced by paromomycin in *TEF2* and *tef2-Δ* strains. Both strains were transformed with the 2μm *LEU2-d* (7) plasmid pUKC352, which contains the yeast *PGK1* promoter and amino-terminal segment fused in frame with the *E. coli lacZ* gene via an in-frame UAG stop codon. Misreading induced by 5 h of exponential growth in 1 mg of paromomycin per ml was determined by comparing the average of three β-galactosidase specific activity measurements (24) in parallels SC-minus-leucine cultures with and without the drug. The plasmid copy number was measured in these cultures (36) and found to be 76 copies per cell both in strains with and in strains without the drug. β-Galactosidase activities, expressed as nanomoles per milligram of protein per minute, were 331 ± 17 and 160 ± 10 in the *TEF2* strain with and without the drug, respectively. This represents an approximate twofold increase in misreading due to the drug. In contrast, there was no drug-induced misreading in the *tef2-Δ* strain (152 ± 3 and 154 ± 10 with and without paromomycin, respectively).

Effect of *TEF* gene dosage on the level of EF-1α protein. Western blot (immunoblot) analysis (Fig. 4) showed that the level of EF-1α protein was significantly lower in a *tef2-Δ* strain (one copy of *TEF*) than in the same *tef2-Δ* strain transformed with the single-copy *TEF1* plasmid pJS7 (two copies of *TEF*).

In this paper, we show that the level of EF-1α affects translational fidelity. Similar findings were reported by Vijgenboom et al. (41), who showed that natural UGA leakiness in *E. coli* is dependent on the intracellular concentration of EF-Tu. One way to explain these results is to suppose that changes in the relative concentration of EF-1α and enzymes or substrates used in EF-1α modification may alter the degree of modification. This alteration in EF-1α modification might in turn be responsible for the effect on translational fidelity. Indeed, EF-1 has been shown to be phosphorylated in rabbit reticulocytes (6), and phosphorylation of EF-2 in mammalian cells has been shown to directly control EF-2 activity (29).

Our finding that the level of EF-1α is proportional to suppression efficiency could also be explained if higher concentrations of EF-1α better enabled aminoacyl-tRNA to compete with termination factors when a nonsense codon was in the A site. Although the relative levels of aminoacyl-tRNA and EF-1α in yeast cells are not known, this model predicts an overall excess of aminoacyl-tRNA capable of complexing with EF-1α–GTP and binding to the ribosome. Furthermore, the model predicts that an excess of noncognate versus cognate EF-1α–GTP–tRNA ternary complex would lead to missense and frameshift suppression. This would be expected to occur in cases where there are limiting amounts of cognate but not noncognate aminoacyl-tRNA species.

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