Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 6815–6819, October 1987
Genetics

Expression of a set of synthetic suppressor tRNA^{Phe} genes in *Saccharomyces cerevisiae*

JEAN-MICHEL MASSON*†, PIERRE MEURIS*‡, MICHAEL GRUNSTEIN*, JOHN ABELSON§, AND JEFFREY H. MILLER*

*Department of Biology and Molecular Biology Institute, University of California, Los Angeles, CA 90024; and §Division of Biology, California Institute of Technology, Pasadena, CA 91125

Contributed by John Abelson, June 15, 1987

ABSTRACT **Synthetic ochre and amber tRNA suppressor genes derived from the yeast tRNA$^{Phe}_{GAA}$ sequence have been constructed. They were efficiently transcribed *in vitro* and expressed *in vivo* via a synthetic expression cassette. tRNA$^{Phe}_{UUA}$ and tRNA$^{Phe}_{UUA}$ΔIVS (IVS = intervening sequence) are relatively inefficient ochre suppressors. They are toxic to the cell when expressed on a multicopy plasmid, and they do not suppress at all when present as single copies. The intron does not seem to have any effect on suppression. In contrast, the amber suppressor tRNA$^{Phe}_{CUA}$ΔIVS is efficient when expressed from a single-copy plasmid, while its efficiency is reduced on a multicopy vector.**

Suppressor tRNAs are useful genetic tools in investigating the nature of suppressible mutations as well as the translational process. They also provide a very powerful tool for the analysis of structure–function relationships in proteins by allowing defined amino acid substitutions at specified positions. Unfortunately, only a limited set of suppressors can be generated by conventional mutagenesis because (*i*) most tRNA genes would require two or three changes to be converted to suppressor tRNA-encoding genes, and (*ii*) although a number of tRNA genes are known to be redundant in the cell, the replacement of a wild-type tRNA gene by its suppressor counterpart could be deleterious to the cell. This is likely the case in *Saccharomyces cerevisiae*, where only three classes of suppressor tRNAs have been uncovered, leading to insertion of tyrosine, serine, and leucine (1). Other suppressors that theoretically could be obtained by a single base change from the tRNAs leading to insertion of glutamine, glutamic acid, lysine, or tryptophan have not been observed. Moreover, it has been shown that a serine-inserting ochre suppressor is lethal in haploids, while the diploids that bear one intact copy of the corresponding tRNA^{Ser} gene are viable (2, 3).

tRNA genes now can be easily synthesized chemically. Therefore, one straightforward way of generating new suppressors is to synthesize them *de novo*. Such an approach has been shown to be successful in *Escherichia coli*, where a large collection of suppressor tRNAs has been obtained (ref. 4; also J.-M.M., J. Normanly, L. G. Kleina, J.A., and J.H.M., unpublished data); therefore, we decided to extend this strategy to yeast.

The first suppressors we synthesized are derived from tRNA$^{Phe}_{GAA}$ (Fig. 1). The tRNA^{Phe} sequence has been determined at the RNA and DNA levels (6–8), and its structure has been resolved by x-ray crystallography (9, 10). The tRNA^{Phe} gene has an intron of 19 base pairs (bp) (8). We synthesized suppressor tRNA genes with and without the intron. These synthetic genes were cloned into an expression cassette, which we designed based on the sequence of a tRNA^{Tyr} gene,

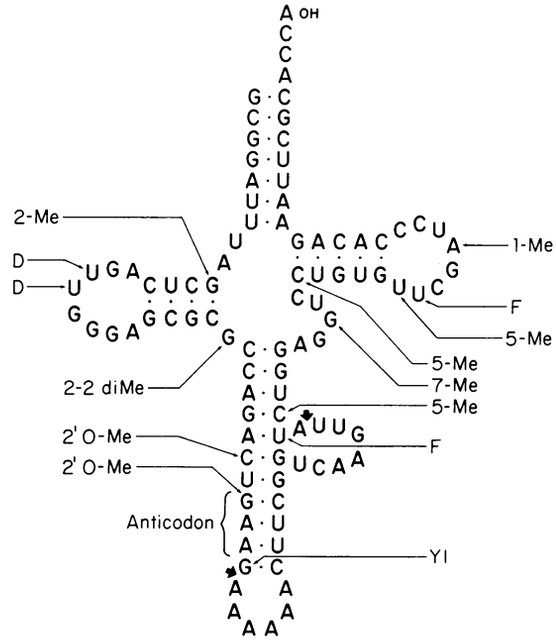

FIG. 1. Structure of the precursor tRNA$^{Phe}_{GAA}$. Numbering is that of Nishimura (5). The 18-base intron is delineated by the thick arrows. Positions of the base modifications are indicated. Abbreviations: Me, methyl; 2′-*O*-Me, 2′-*O*-methyl; D, dihydroxy; and YI, hypermodified.

SUP4. Expression of these genes was studied *in vitro* in a yeast RNA polymerase III system and *in vivo* by transformation of the genes into yeast.

MATERIALS AND METHODS

Strains, Plasmids, and Media. *E. coli* strain MC1061 was used for transformation and large-scale preparation of the various plasmids. *S. cerevisiae* strain AHA75 (*trp1, ade2-o, arg8-o, his4-o, leu2-o, lys2-o, tyr1-o, ura4-o*, where *o* designates an ochre mutation) (11) was provided by Maynard Olson. *S. cerevisiae* strain S2 (*trp1, met8-1-a, tyr7-1-a, ade3-26-a*, where *a* designates an amber mutation) was constructed by crossing strain AHA75 with strain SL183-21C provided by Susan Liebman. *E. coli* strains were grown on LB medium with ampicillin as described (12), and *S. cerevisiae* strains were grown on yeast extract/peptone/dextrose medium or drop-out plates of synthetic complete medium minus one amino acid as described by Sherman *et al.* (13).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: IVS, intervening sequence.

†Present address: Departement de genie biochimique et alimentaire, I.N.S.A., Avenue de Rangueil, 31077 Toulouse Cedex, France.
‡Present address: Centre de Recherche de Biochimie et de Genetique Cellulaires, 31062 Toulouse, France.

Oligonucleotides and Gene Synthesis. The following oligonucleotides were synthesized on an Applied Biosystems (Foster City, CA) 380A DNA synthesizer:

```
1, CGCGGATTTAGCTCAGTTGGGAGAGCGCCAGCCTTTAG;
2, AAAAAACTTCGGTCAAGTT;
3, ATCTGGAGGTCCTGTGTTCGATCC;
3, ATCTGGAGGTCCTGTGTTCGATCC;
4, ACAGAATTCGCATTTTTTGGTAC;
5, CAAAAAATGCGAATTCTGTGGATCGAACACAGGAC;
6, CTCCAGATAACTTGACCGAAGTTTTTTCTAAAGTCT;
7, GGCGCTCTCCCAACTGAGCTAAATCCGCGAGCT;
8, CTCCAGCTCTAAAGTCT;
9, CGCGGATTTAGCTCAGTTGGGAGAGCGCCAGACTCTAG;
10, CTCCAGATCTAGAGTCT;
11, CGTATACTCTTTCTTCAACAATTAGAGCTCCCGGGTACCAT;
12, CGATGGTACCCGGGAGCTCTAATTGTTGAAGAAAGAGTATA.
```

The oligonucleotides were purified, phosphorylated with kinase, and hybridized as described (4). The rationale for the gene synthesis was as follows: oligonucleotides 11 and 12 hybridized together are the expression cassette. The ochre suppressor gene was constructed from oligonucleotides 1 through 7. Modifications to the original $tRNA^{Phe}_{GAA}$ sequence were: (*i*) the anticodon was changed from GAA to TTA and the complementary intron sequence was changed from TTC to TAA to keep the base pairing between anticodon and intervening sequence (IVS); (*ii*) the 3′ transcription termination signal (a stretch of six thymidine residues) was included in the tRNA gene oligonucleotide sequence instead of the expression cassette to avoid the risk of incorrect maturation of the 3′ end of the tRNA, which could have resulted from the introduction of a restriction site between the coding sequence and the termination signal; and (*iii*) *Sac* I and *Kpn* I restriction sites were created at the extremities of the tRNA gene sequence.

Omitting oligonucleotides 2 and 6 and replacing them by single-junction oligonucleotide 8 in the hybridizing reaction yielded the $tRNA^{Phe}_{UUA}\Delta IVS$ gene. The corresponding amber suppressor $tRNA^{Phe}_{CUA}\Delta IVS$ gene was constructed by hybridizing oligonucleotides 3, 4, 5, 7, 9, and 10.

Cloning, Transformation, and Selection. The 41-bp expression cassette was introduced at the *Cla* I site of the multicopy plasmid YRp7 (14). This site was chosen because it lies between the −10 and the −35 region of the tetracycline promoter. Inserts at that site frequently result in a tetracycline-sensitive phenotype. The screening for ampicillin-resistant tetracycline-sensitive clones yielded plasmids with the insert in either orientation (YJM2, clockwise).

The synthetic suppressor tRNA genes were constructed and cloned into the two vectors YJM1 and YJM2 cut with *Sac* I and *Kpn* I. Competent *E. coli* MC1061 cells were transformed by the calcium chloride method (15). While all YJM2:Phe (with intron) or YJM2:PheΔIVS (without intron) plasmids conferred both ampicillin and tetracycline resistance, the clones with YJM1:Phe or YJM1:PheΔIVS remained tetracycline sensitive.

The suppressor genes were also cloned in the single-copy plasmid YJM3, which was constructed by introducing at the *Bam*HI site of YJM2 a 627-bp *SAU3AI–Bam*HI fragment from plasmid pJW3 (34) containing the *CEN3* sequence. The sequences of these cloned tRNA genes were determined by dideoxy sequencing.

The plasmids extracted from the *E. coli* strain were purified by using cesium chloride and used to transform *S. cerevisiae* as described by Sherman *et al.* (13).

***In Vitro* Transcription and Base Modification.** *In vitro* reactions were performed with a yeast nuclear extract (16) essentially as described (17, 18). The *in vitro* synthesized RNAs were digested with different RNases (19), and the modified nucleosides were separated by the method of Saneyoshi *et al.* (20) as described (19).

***In Vivo* Suppressor Assays.** The efficiency of the suppressors *in vivo* was assayed by the method of Shaw and Olson (11). In brief, serial dilutions of cells were plated as 5-μl aliquots (10^7 to 10^1 cells on selective plates), and the plates were scored for confluent growth after 5 days at 30°C in the case of the ochre suppressors or 2 days at 30°C in the case of the amber suppressors.

RESULTS

Transcription of the $tRNA^{Phe}_{UUA}$ Genes Is Efficient *in Vitro*. Transcription of tRNA genes in eukaryotes is accomplished by RNA polymerase III (21). It is primarily controlled by two sequences internal to the coding region that are highly conserved among eukaryotic tRNA genes (22–25). Nonetheless, some additional features external to the coding region have been shown to play an important role in transcription. These include a series of thymidine residues at the 3′ boundary of the transcription unit and a 5′ leader sequence (26, 27).

To efficiently express the synthetic suppressor tRNA genes in yeast and ensure correct processing of the transcripts, we added a stretch of six thymidine residues at the 3′ end of the tRNA gene sequences and cloned them in vectors YJM1 and YJM2 with a short synthetic expression cassette (Fig. 2). The role of this cassette is to provide a 5′ leader sequence as well as the *Sac* I and *Kpn* I cloning sites. Shaw and Olson (11) have shown that 27–36 bases of the 5′ flanking sequence of the *SUP4-o* gene are required for optimal expression of the gene. Therefore, we decided to include in our expression cassette a sequence of 27 bp derived from the leader sequence of the *SUP4-o* gene. A cytosine residue was added at the 5′ end to create a CG cohesive end, and the 3′ end was modified to a *Sac* I restriction site. Additional restriction sites were included at the 3′ end, allowing this cassette to be used in other shuttle vectors.

To assess the transcriptional efficiency of the expression cassette, we analyzed the transcription of our constructs in a yeast nuclear extract. The level of transcription of the $tRNA^{Phe}_{UUA}$ genes on YJM2 did not differ significantly from that of a *Sup53* gene cloned on a 2-μm plasmid (18) (Fig. 3). The primary transcript was not only produced at the same level as that of the control gene but also was processed equally well into pre-tRNA in the case of YJM2:Phe or into mature tRNA in the case of $YJM2:Phe_o\Delta IVS$. In this extract there was no tRNA splicing activity, so pre-$tRNA^{Phe}_{UUA}$ containing the intron remained intact. Contrary to what was observed for the YJM2 constructs, there was no transcription from the tRNA genes on the YJM1 plasmids. Thus, in one orientation (YJM2), there was efficient transcription of the tRNA gene, whereas in the reverse orientation (YJM1), transcription was apparently inhibited by the surrounding sequences.

The Ochre Suppressor Is Relatively Inefficient *in Vivo*. Competent *S. cerevisiae* AHA75 cells were transformed and selected for their ability to grow without tryptophan. The transformants were then replicated on the various selective media and tested for suppression of the six different ochre mutations of AHA75 (Table 1). Ability to suppress a given nonsense mutation to the point where confluent growth could be observed after 4–5 days at 30°C was scored as a "+" (Table 1).

The pattern of suppression that can be deduced from this table is very similar but not identical to the one obtained with the ochre tyrosine suppressor tRNAs (11): *his4-o* was the best suppressed mutation; *arg8-o*, *leu2-o*, and *lys2-o* were equally suppressed to a lesser extent; and *tyr1-1* was poorly suppressed, as was the case with *SUP4-o*. The *ade2-o* mutation,

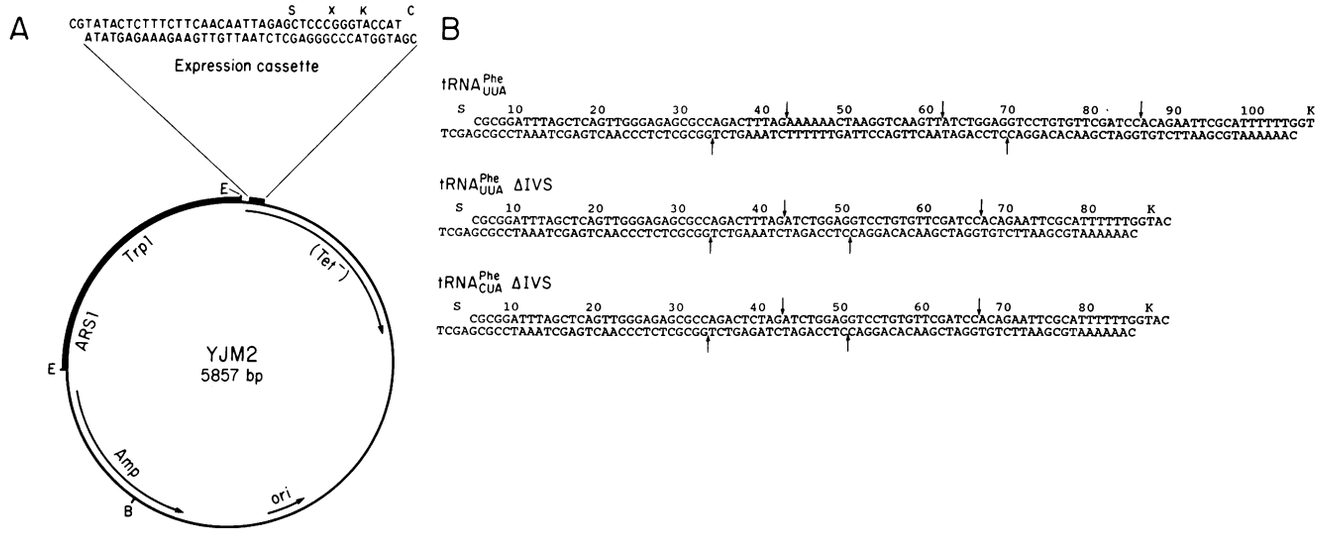

FIG. 2. The cloning vector YJM2 and the synthetic suppressor genes. (*A*) The plasmid YJM2 was constructed by inserting the 41-bp expression cassette in YRp7 at the *Cla* I site. C, *Cla* I; E, *Eco*RI; K, *Kpn* I; S, *Sac* I; X, *Xho* I; Amp, ampicillin; Tet, tetracycline. (*B*) The synthetic suppressor genes were assembled from oligonucleotides 6–8 and cloned between the *Sac* I and *Kpn* I sites of YJM2 or YJM3.

which was well suppressed by *SUP4-o*, was not suppressed at all with $tRNA^{Phe}_{UUA}$. Although the ochre suppressor was expressed, resulting in the growth of colonies on selective media, there was clearly a deleterious effect of the suppressor because even cells displaying no ochre suppression grew poorly on SD medium. On rich medium the YJM2 plasmid carrying the ochre suppressor was rapidly lost. We conclude that the ochre suppressor gene is toxic when present on a multicopy vector.

If the toxicity were indeed due to overproduction of the suppressor tRNA on a multicopy plasmid, this effect might be reduced by lowering the number of copies of the $tRNA^{Phe}$ gene in the cell. This was done by two independent procedures: (*i*) by selecting for single-copy integration of the plasmid YJM2:Phe_o linearized with *Bgl* II (28), and (*ii*) by introducing into the plasmid a yeast centromeric sequence (*CEN3*) that ensures a copy number of YJM3:Phe_o of one to two per cell (29). In both cases the transformed cell grew on medium without tryptophan with a growth rate similar to that of cells transformed with YRp7. However, we found that the $tRNA^{Phe}_{UUA}$ gene did not suppress at all in single copy, either integrated in the yeast genome or on a *CEN3* vector (Table 1).

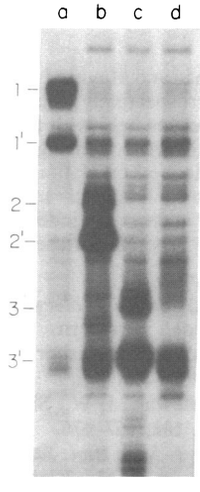

FIG. 3. *In vitro* transcription of the YJM1 and YJM2 constructs. Cesium chloride-purified plasmid (100 ng) was transcribed with a yeast nuclear extract (16) in the presence of [α-^{32}P]UTP. After extraction with phenol/chloroform and precipitation with ethanol, the RNA products were separated on an 8% acrylamide/7 M urea gel. Lanes: a, control, YIpLEU2SUP53 (band 1, primary transcript; band 1', end-processed pre-tRNA); b, YJM2:Phe_o (band 2, primary transcript; band 2', end-processed pre-$tRNA^{Phe}_{UUA}$); c, YJM2:Phe_oΔIVS (band 3, primary transcript; band 3', end-processed mature $tRNA^{Phe}_{UUA}$); d, YJM1:Phe_o, no transcript.

The Intron Has no Obvious Effect on Suppression. Two studies, on *SUP6* and *SUP53*, have shown that deletion of the intron from a tRNA gene results in under modification of the tRNA with marked effects on the suppressor efficiency (18, 19). As described above, we assembled $tRNA^{Phe}_{UUA}$ suppressors with and without the intron. Fig. 2 shows that both of these genes were equally well transcribed. We then compared the *in vitro* base modifications of the tRNA products by transcribing both genes *in vitro* in the presence of the methyl donor *S*-adenosylmethionine (30) and [α-^{32}P]UTP, [α-^{32}P]ATP, or [α-^{32}P]CTP. The labeled, end-processed RNAs were digested with RNases T_1, T_2, and A, producing nucleoside 3' monophosphates labeled by nearest-neighbor transfer. Fig. 4 compares the digests obtained with the precursor and the mature tRNAs in the case of [α-^{32}P]UTP labeling.

Table 1. Suppressor phenotypes of the strains containing the ochre and amber suppressors

	Ochre suppressors						Amber suppressors		
Strains	*tyr1*	*arg8*	*ade2*	*lys2*	*leu2*	*his4*	*met8-1*	*tyr7-1*	*ade3-26*
YJM2:Phe_o	+	++	−	++	++	+++			
YJM2:Phe_o-ΔIVS	+	++	−	++	++	+++			
YJM3:Phe_o	−	−	−	−	−	−			
YJM2:Phe_a-ΔIVS							++++	+++	+++
YJM3:Phe_a-ΔIVS							++	+	+

Suppression phenotypes as determined by the test of Shaw and Olson (11). Serial dilutions of cells in 5-μl aliquots were assayed for confluent growth on selective media at 30°C after 5 days for the ochre suppressors (signified by subscript o) because of their low efficiency or after 2 days for the amber suppressors (signified by subscript a). In the table the number of cells required to give confluent growth on a given selective media is depicted as "+" to "++++" (10^7 cells, + to 10^3 cells, ++++) or − (no growth at whatever concentration).

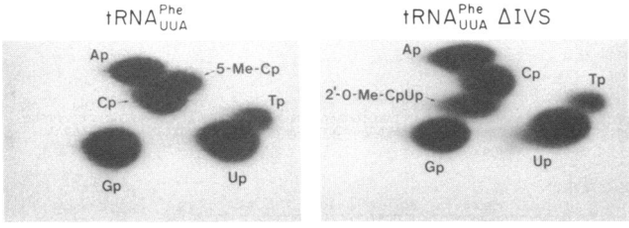

FIG. 4. Modified nucleotide analysis of tRNA$^{Phe}_{UUA}$ and tRNA$^{Phe}_{UUA}$ΔIVS. The end-processed transcript made *in vitro* in the presence of a [α-^{32}P]UTP and the methyl donor *S*-adenosylmethionine were digested and chromatographed as described (19). The nucleotide 3'-monophosphates are identified with abbreviations consistent with those in Fig. 1. 2'-*O*-Me-CpUp, 2'-*O*-methylcytosine-uridine dinucleotide 3' monophosphate (this dinucleotide is not hydrolyzed by the RNase cocktail).

Among the modified bases that could be resolved clearly with this system were some common to both patterns (pseudouridine-39 and -55 and ribothymidine-54), denoting modification enzymes that work on either intron-containing precursor or intact tRNA. Other modifications were present only on the mature tRNA (2'-*O*-methylcytosine-32), while the only modification that was specific to the pre-tRNA and therefore required the presence of the intron was the 5-methylation of cytosine-40 and probably cytosine-48.

In the case of *SUP6* (19) and *SUP53* (18), it has been shown that the presence of the intron was required for the modification of bases within the anticodon, these modifications being important for the efficiency of the suppressors. In tRNA^{Phe}, there was no such modification of the bases of the anticodon, and this can probably account for the fact that when the two constructs were assayed *in vivo*, no difference could be seen in the level of their suppression (Table 1). There was no clear difference in suppressor efficiency between the gene with an intron and the gene without an intron.

The Synthetic Amber Suppressor Gene Is Efficient *in Vivo*. In yeast, the amber suppressors, especially those inserting tyrosine, are very efficient (1). Replacing oligonucleotides 1 and 8 by oligonucleotides 9 and 10 yielded the intronless amber suppressor gene tRNA$^{Phe}_{CUA}$ΔIVS. This gene was cloned into both the YJM2 vector and the YJM3 vector and was used to transform cells of *S. cerevisiae* strain S2. The transformants were selected on SD medium without tryptophan and then assayed for the suppression of the three amber markers, *met8-1-a, tyr7-1-a*, and *ade3-26-a*.

The S2 cells bearing the multicopy version of the amber suppressor gene (YJM2:Phe$_a$ΔIVS) grew at a significantly slower rate than the ones bearing the single copy of the suppressor gene (YJM3:Phe$_a$ΔIVS) and lost the plasmid at a high frequency (95% loss in 72 hr). Nonetheless, the toxicity of the multicopy amber suppressor gene was much lower than that of its ochre counterpart, and its suppression efficiency was substantially higher (Table 1). On the other hand, the presence of YJM3:Phe$_a$ΔIVS did not seem to impede cell growth because there was no measurable loss of the plasmid on SD-minus-tryptophan medium, and a strong suppression effect for all three amber markers was observed (Table 1).

DISCUSSION

We have demonstrated that new suppressors can be generated in yeast via gene synthesis. The first such suppressors, derived from the tRNA$^{Phe}_{GAA}$ gene sequence, have been expressed *in vivo* through a short synthetic expression cassette. We find that tRNA$^{Phe}_{CUA}$ΔIVS is an efficient amber suppressor when expressed on a single-copy plasmid, while its efficiency is reduced when it is expressed on a multicopy plasmid. In contrast, the ochre suppressor tRNA$^{Phe}_{UUA}$ΔIVS (or tRNA$^{Phe}_{UUA}$) gene codes for a rather inefficient suppressor and is functional only on a multicopy plasmid, where it is toxic to the cells. This contrast can be accounted for by a combination of factors: (*i*) the high instability of the multicopy plasmids combined with the toxicity of the suppressors, the toxicity being enhanced by the high number of copies; (*ii*) an obvious difference in intrinsic toxicity; and (*iii*) a difference in the intrinsic efficiency between the ochre and the amber suppressors. Such a difference in efficiency between two isogenic suppressors has not been encountered before in yeast. More detailed biochemical studies are needed to determine whether the cause of this discrepancy lies at the level of the interaction with the tRNA synthetase or with the ribosome.

A number of yeast nuclear tRNA genes contain an intron, while others do not. This difference can be found even between isoacceptors (31). The only important role that has been assigned to these introns is to ensure correct modification of some of the bases of the anticodon loop. These modified bases are critical to the translational efficiency (32) or the suppression efficiency (18, 19). The tRNA^{Phe} gene has an intron of 18 bp that may be required for the methylation of the cytosine-40 or -48. The only difference in the modification pattern between tRNA$^{Phe}_{UUA}$ and tRNA$^{Phe}_{UUA}$ΔIVS that could be detected in the region of the anticodon loop is the 2'-*O*-methylation of cytosine-32 that is only present on the mature tRNA. We saw no evidence of modification on the anticodon bases of the tRNA^{Phe} gene, and this can be related to the fact that the intron seems to play no role in the efficiency of this suppressor.

Other synthetic suppressor genes can be expressed *in vivo* by using the same multicopy or single-copy vectors, and this system should ultimately allow one to obtain a larger set of suppressors in yeast, as has been achieved in *E. coli* by a similar strategy (ref. 4; J.-M.M., J. Normanly, L. G. Kleina, J.A., and J.H.M., unpublished data). Developing such a tool also requires a more detailed understanding of the rules governing suppression in yeast. The gene synthesis approach chosen here provides a convenient system to investigate the function of yeast suppressors as well as the means of improving their efficiency by specific base changes. Since yeast suppressors have been shown to work in other eukaryotic cells (33), this approach should have widespread significance.

We thank Susan Liebman and Maynard Olson for providing strains SL183-21c and AHA75, respectively; Sandra Ebeling for technical assistance; Marge Strobel for help with the *in vitro* reactions; and Susan Whoriskey for the DNA sequencing. This work was supported in part by a National Science Foundation Grant (DMB 8417353) to J.A. and J.H.M. and by a National Institutes of Health Grant (GM23674) to M.G. The authors are indebted to the Biotechnology Research and Education Program for use of equipment.

1. Sherman, F. (1982) in *The Molecular Biology of the Yeast Saccharomyces*, eds. Strathern, J. N., Jones, E. W. & Broach, J. R. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 463–486.
2. Brandriss, M. C., Stewart, J. W., Sherman, F. & Botstein, D. (1976) *J. Mol. Biol.* **102**, 467–476.
3. Piper, P. W. (1978) *J. Mol. Biol.* **122**, 217–235.
4. Normanly, J., Masson, J.-M., Kleina, L. G., Abelson, J. & Miller, J. H. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 6548–6552.
5. Nishimura, S. (1979) in *Transfer RNA: Structure, Properties and Recognition*, eds. Soll, D., Abelson, J. N. & Schimmel, P. R. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 518–519.
6. RajBhandary, U. L. & Chang, S. H. (1968) *J. Biol. Chem.* **243**, 598–608.
7. Nielsen, P. E. & Leick, V. (1982) *Biochem. Biophys. Res. Commun.* **106**, 179–185.
8. Valenzuela, P., Venegas, A., Weinberg, F., Bishop, R. &

Rutter, W. J. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 190–194.
9. Robertus, J. D., Ladner, J. E., Finch, J. T., Rhodes, D., Brown, S. R., Clark, B. F. C. & Klug, A. (1973) *Nature (London)* **250,** 546–551.
10. Kim, S.-H. (1979) in *Transfer RNA: Structure, Properties and Recognition*, eds. Soll, D., Abelson, J. N. & Schimmel, P. R. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 83–100.
11. Shaw, K. J. & Olson, M. V. (1984) *Mol. Cell. Biol.* **4,** 657–665.
12. Miller, J. H. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
13. Sherman, F., Fink, G. R. & Lawrence, C. W. (1979) *Methods in Yeast Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
14. Stinchcomb, D. T., Struhl, K. & Davis, R. W. (1979) *Nature (London)* **282,** 39–43.
15. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
16. Engelke, D. R., Gegenheimer, P. & Abelson, J. (1985) *J. Biol. Chem.* **260,** 1271–1279.
17. Newman, A. J., Ogden, R. C. & Abelson, J. (1983) *Cell* **35,** 117–125.
18. Strobel, M. C. & Abelson, J. (1986) *Mol. Cell. Biol.* **6,** 2663–2673.
19. Johnson, P. F. & Abelson, J. (1983) *Nature (London)* **302,** 681–687.
20. Saneyoshi, M., Ohashi, Z., Harada, F. & Nishimura, S. (1972) *Biochim. Biophys. Acta* **262,** 1–10.
21. Sentenac, A. & Hall, B. (1982) in *The Molecular Biology of the Yeast Saccharomyces*, eds. Strathern, J. N., Jones, E. W. & Broach, J. R. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 561–606.
22. Galli, G., Hofstetter, H. & Birnstiel, M. L. (1981) *Nature (London)* **294,** 626–631.
23. De Franco, D., Schmidt, O. & Soll, D. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 3365–3368.
24. Hall, B. D., Clarkson, S. G. & Tocchini-Valentini, G. (1982) *Cell* **29,** 3–5.
25. Stillman, D. J. & Geiduschek, E. P. (1984) *EMBO J.* **3,** 847–853.
26. Ciliberto, G., Castagnoli, L. & Cortese, R. (1983) *Curr. Top. Dev. Biol.* **18,** 59–87.
27. Bogenhagen, D. F. & Brown, D. D. (1981) *Cell* **19,** 261–270.
28. Rothstein, R. J. (1983) *Methods Enzymol.* **101,** 202–212.
29. Clarke, L. & Carbon, J. (1980) *Nature (London)* **287,** 504–509.
30. Brown, A., Pan, C.-J. & Marzluff, W. F. (1982) *Biochemistry* **21,** 4303–4310.
31. Guthrie, C. & Abelson, J. (1982) in *The Molecular Biology of the Yeast Saccharomyces*, eds. Strathern, J. N., Jones, E. W. & Broach, J. R. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 487–528.
32. Weissenbach, J. & Dirheimer, G. (1978) *Biochem. Biophys. Acta* **518,** 530–534.
33. De Robertis, E. M. & Olson, M. V. (1979) *Nature (London)* **278,** 137–143.
34. Wallis, J. W., Rykowski, M. & Grunstein, M. (1983) *Cell* **35,** 711–719.