THE INABILITY OF YEAST TRANSFER RNA TO SUPPRESS AN AMBER MUTATION IN AN *E. coli* SYSTEM¹

JOHN A. KIGER, JR. AND CAROL J. BRANTNER

*Department of Biochemistry and Biophysics,
Oregon State University, Corvallis, Oregon 97331*

Manuscript received August 11, 1972

Revised copy received September 11, 1972

ABSTRACT

Transfer RNA from super-suppressor mutants of *Saccharomyces cerevisiae* cannot suppress an amber mutation *in vitro* in an *E. coli* protein synthesizing system. It is tentatively concluded that the yeast amber suppressor does not contain a transfer RNA altered in the anticodon.

IT is well established that mutant transfer RNA is capable of suppressing nonsense codons and polar mutations in *E. coli*. The site of the suppressor mutation usually is thought to be in the anticodon of the transfer RNA molecule (GAREN 1968; GOODMAN *et al.* 1968) but can be elsewhere in the molecule (HIRSH 1971). Amber (UAG) and ochre (UAA) codons produced by mutation have also been identified in the yeast, *Saccharomyces cerevisiae*; these codons act as terminators and some of them exhibit polar effects. These mutations are suppressed by the super-suppressor mutants of yeast which are by analogy thought also to represent altered tRNA species. These super-suppressors are allele specific but not gene specific, just as are the nonsense suppressors of *E. coli* (GILMORE, STEWART and SHERMAN 1971; SHERMAN and STEWART 1971; STEWART and SHERMAN 1972).

Suppression in an *in vitro* *E. coli* protein-synthesizing system has been demonstrated using a messenger RNA from the bacteriophage f2 containing an amber mutation (sus 3) at the position of the sixth amino acid of the coat protein (ENGELHARDT *et al.* 1965). When this RNA is used as messenger in a protein-synthesizing system derived from a su⁻ strain, only the N-terminal fragment (fmet-ala-ser-asn-phe-thr) of the coat protein is synthesized. In the presence of tRNA from a su⁺ strain, however, it is possible to detect synthesis of the whole coat protein resulting from suppression of the amber codon.

We have employed the f2 system to determine if tRNA from super-suppressor strains of yeast can suppress an amber mutation *in vitro* in an *E. coli* system.

MATERIALS AND METHODS

Strains: Yeast strains were obtained from DR. F. SHERMAN: D609-24B *atyr* 7-1 *arg* 4-17

¹ This work was supported in part by a grant from the National Science Foundation, GB-25358, and by a grant from The American Cancer Society, Oregon Division.

his 5-2 *lys* 1-1 (*su*⁻); SL9-2C *αSUP* 7-1 *arg* 4-17 *leu* 1-12 (*trp* 5-48 *his* 5-2 *lys* 1-1?) (*su-UAA*) and SL41-2B *αSUP* 7-2 *tyr* 7-1 *trp* 1-1 *lys* 1-1 *arg* 4-17 *his* 5-2 (*su-UAG*).

E. coli strains D24 (used to make S-30 extract) and K37 (host for *f2sus3*) were obtained from Dr. H. LODISH as was *f2sus3*. *f2*⁺ phage were purchased from Miles Laboratories.

Transfer RNA: Yeast tRNA was purified according to HOLLEY (1967). Glycogen was removed from the crude tRNA preparations by centrifugation at 30,000 rpm for 2 hours in a Spinco 30 rotor. The purification upon DEAE cellulose was omitted.

Transfer RNA preparations were deacylated by incubation in 2 M Tris, pH 9.1, at 37°C for 30 minutes.

Crude phenol extracts of strains CA 265 *su*_{III}⁺ (*UAG*), CA 169 *su*_c⁺ (*UAA*) and CA 244 *su*⁻ were a gift from Dr. J. MENNINGER. The crude extracts were treated with DNAase, re-extracted with phenol and deacylated as described above.

Purified tRNA from *E. coli* B was purchased from Schwartz/Mann.

Acylation of tRNA: Aminoacyl-tRNA synthetases were prepared from *E. coli* D24 by the method described by TWARDZIK, GRELL and JACOBSON (1971). Aminoacyl-tRNA synthetases from yeast strain D609-24B were prepared as described by GILLAM *et al.* (1967), except that the cells were lysed with an Eaton press. ³H-tyrosine tRNA and ³⁵S-methionine tRNA were synthesized essentially as described by TWARDZIK *et al.* (1971) and purified by phenol extraction as described by MAXWELL, WIMMER and TENER (1968). The final pellet was dissolved in 0.01 M Na acetate pH 5.0 and stored at -80°C.

³H-tyrosine (6.5 c/mmole) was purchased from Schwartz/Mann. ³⁵S-methionine was made from carrier-free ³⁵S-sulfate as described by SANGER, BRETSCHER and HOCQUARD (1964); its specific activity was not determined. ³⁵S-methionine tRNA was synthesized from *E. coli* B tRNA.

Protein Synthesizing System: The S-30 preparation and its use in protein synthesis was described by SALTER, GESTELAND and BOLLE (1967) except that polyethylene glycol was omitted and the S-30 preparation was not dialysed (YOUNG 1970). The concentration of *f2*⁺ RNA in incubation mixtures was 5.2 A₂₆₀/ml and that of *sus3* RNA was 6.8 A₂₆₀/ml. Incubation mixtures (0.10 ml) always contained 0.6 A₂₆₀ or less of ³⁵S-methionine tRNA as a source of label to determine protein synthesizing activity.

Following incubation at 37°C for 15 minutes, 0.5 ml bovine serum albumen (0.5 mg/ml) and 0.10 ml of 1 N NaOH were added per 0.10 ml incubation mixture, and incubation continued for 10 minutes more to hydrolyze the remaining labeled aminoacyl tRNA. Radioactively labeled protein was precipitated by the addition of 0.5 ml of cold 10%, trichloroacetic acid. The precipitate was collected on glass fiber filters (Whatman GF/A) which were washed with cold 2% trichloroacetic acid. The dried filters were counted in a toluene based fluor in a Packard Liquid Scintillation Counter.

RESULTS

The data in Table 1 demonstrate that exogenous ³H-tyrosine tRNA from either *E. coli* or yeast can act as a donor of ³H-tyrosine in *f2* messenger-directed protein synthesis. Yeast ³H-tyrosine tRNA readily participates in this *E. coli* protein synthesizing system. As much as 70% of all the added yeast ³H-tyrosine tRNA can transfer its amino acid to acid-precipitable protein. In this system exogenous ³H-tyrosine tRNA is competing with endogenous tRNA. Under these conditions it is clear that yeast tRNA competes as well as, if not better than, exogenous ³H-tyrosine tRNA of *E. coli*.

It should be noted in Table 1 that the activity of the system as measured by ³⁵S-methionine incorporation is markedly dependent on the amount of added ³H-tyrosine tRNA, regardless of the source of the exogenous tRNA. No way of eliminating this inhibitory effect has been found and therefore attempts to detect amber suppression by exogenous aminoacyl tRNA have been made at several

TABLE 1

f2⁺ messenger directed protein synthesis

Content of protein synthesizing system	A ³⁵ S-met incorporation (CPM)	B ³ H-tyr tRNA added (picomoles) *	C ³ H-tyr incorporation (picomoles)	Percent incorporation C/B × 100
No messenger	660†			
f2 ⁺ messenger	5,390			
f2 ⁺ messenger and yeast ³ H-tyr tRNA				
D609-24B (su ⁻)	3,000	0.97	0.51	53
	4,190	0.49	0.25	51
	5,410	0.25	0.13	52
SL41-2B (UAG)	3,100	1.13	0.53	47
	4,850	0.56	0.39	70
	6,200	0.28	0.18	64
SL9-2C (UAA)	2,580	1.47	0.57	39
	4,200	0.74	0.43	58
	4,940	0.37	0.18	49
f2 ⁺ messenger and <i>E. coli</i> ³ H-tyr tRNA				
CA 244 (su ⁻)	1,533	1.33	0.45	34
	2,798	0.67	0.19	28
	4,133	0.33	0.15	46
CA 265 (amber)	2,344	1.02	0.42	41
	3,698	0.51	0.22	43
	4,373	0.26	0.10	39

* The specific activity of the ³H-tyrosine was 2,000 CPM/picomole.

† This figure represents incorporation directed by endogenous messenger in the absence of added tRNA. Exogenous tRNA decreases this incorporation. This background was determined for each concentration of exogenous tRNA and is subtracted from the appropriate figures below.

concentrations that allow a reasonable level of protein synthesis to be detected using f2⁺ RNA as messenger.

The N-terminal peptide (fmet-ala-ser-asn-phe-thr) of the coat protein of f2 produced by the sus 3 amber mutation is acid-soluble, and since this mutation has a polar effect on the rest of the f2 messenger, no acid-precipitable protein is produced in its presence. Suppression of the sus 3 mutation allows the incorporation of radio-active label into acid-precipitable protein.

The data in Table 2 demonstrate the efficacy of ³H-tyrosine tRNA from suppressor and non-suppressor strains of *E. coli* and yeast in suppressing the sus 3 amber mutation. Incorporation of ³⁵S-methionine into protein is quite apparent in the presence of tRNA from the *E. coli* amber suppressing strain and, to a lesser degree, is apparent for tRNA from the *E. coli* ochre suppressor. Ochre suppressors are generally less efficient than amber suppressors (GAREN 1968). Suppression of the UAG codon by ochre suppressors is expected according to the wobble hypothesis (CRICK 1966).

It is clear that despite the ability of yeast tyrosine tRNA to participate in f2⁺-directed protein synthesis, neither suppressor strain of yeast can suppress the

TABLE 2
f2sus3 messenger directed protein synthesis

Content of protein synthesizing system	A ³⁵ S-met incorporation (CPM)	B ³ H-tyr tRNA added (picomoles) *	C ³ H-tyr incorporation (picomoles)	Percent incorporation C/B × 100
No messenger	680†			
<i>f2sus3</i> messenger	50			
<i>f2sus3</i> messenger and yeast ³ H-tyr tRNA				
D609-24B (su ⁻)	0‡	0.97	0.07	7
	0	0.49	0.07	14
	0	0.25	0.05	20
SL41-2B (UAG)	22	1.13	0.09	8
	7	0.56	0.03	6
	-1	0.28	0.04	14
SL9-2C (UAA)	-35	1.47	0.08	5
	-27	0.74	0.06	8
	-43	0.37	0.04	11
<i>f2sus3</i> messenger and <i>E. coli</i> ³ H-tyr tRNA				
CA 244 (su ⁻)	0‡	1.33	0.01	1
	0	0.67	0.02	3
	0	0.33	0.00	0
CA 265 (amber)	292	1.02	0.15	15
	578	0.51	0.11	22
	478	0.26	0.06	23
CA 169 (ochre)	122	1.11	0.01	1
	215	0.55	0.00	0
	199	0.28	0.02	7

* See footnote to Table 1.

† See footnote to Table 1.

‡ *f2sus3* messenger added to the protein-synthesizing system containing exogenous tRNA is observed to slightly decrease the incorporation of ³⁵S-methionine. To take this factor into account the data for tRNA from suppressor strains have been normalized with respect to the pertinent nonsuppressor strain. This has resulted in negative values for some points.

sus3 amber mutation as determined by incorporation of ³⁵S-methionine into the coat protein.

It will be noted that some incorporation of ³H-tyrosine does occur in the systems with yeast tRNA, but it is not correlated with the presence of a suppressor. This may be due to some degradation of the *sus3* messenger, yielding fragments which escape the polar effect of the amber mutation, leading to some protein synthesis with improper initiation. Again, the yeast ³H-tyrosine tRNA seems to participate more readily than the *E. coli* ³H-tyrosine tRNA as noted in the data of Table 1.

DISCUSSION

There are at least three known genes for tyrosine tRNA in the *E. coli* sup-

pressor strain employed here. GOODMAN *et al.* (1968) have estimated that only 15% of the tyrosine tRNA of CA265 is capable of recognizing the amber codon. As su_c^+ is probably allelic with su_{III}^+ this is probably also true for the ochre-suppressing tyrosine tRNA. Thus the level of suppression observed in Table 2 is the result of the activity of only 15% of the indicated amount of added *E. coli* 3H -tyrosine tRNA.

Yeast contains eight genes capable of mutation to super-suppressors which insert tyrosine at known ochre or amber codons (GILMORE *et al.* 1971). Thus there are at least eight genes coding for tyrosine tRNA in yeast. Thus, if each of the eight genes for tyrosine tRNA in yeast were equally expressed, 12% of the yeast tyrosine tRNA would be expected to be capable of recognizing the amber codon.

In *E. coli* the efficiency of *in vivo* suppression of amber codons by amber suppressors is approximately 50% while the efficiency of suppression by ochre suppressors is 5–15% (GAREN 1968). In yeast the efficiencies of nonsense suppression by SUP 7-1 (UAA) and SUP 7-2 (UAG) are 9% and 50% respectively (GILMORE *et al.*, 1971; and F. SHERMAN, personal communication).

Studies have shown that in yeast, unlike in *E. coli*, ochre (UAA) suppressors do not suppress amber (UAG) codons (HAWTHORNE 1969). One possible explanation of this fact is that in yeast ochre codons are suppressed by topaz or sepia anticodons (GILMORE *et al.* 1971). Thus in the test employed here we might not expect to see suppression of *sus3* by the yeast ochre suppressor as we do with the *E. coli* ochre suppressor.

If the yeast amber suppressor is the result of an altered anticodon and, if, as outlined above, each yeast tyrosine tRNA gene is equally expressed, we would expect to see suppression of *sus3* by yeast tRNA at a level comparable to that seen for the *E. coli* amber suppressor.

It seems quite likely from the data in Table 2 that we would have detected suppression by a level of activity of 10% or less of that exhibited by the *E. coli* amber suppressor. Thus, were the SUP 7-2 gene expressed only with 10% of the level of the other tyrosine tRNA genes, we would expect to detect it, but we do not.

HIRSH (1971) has shown that suppression of UGA by tryptophan tRNA in *E. coli* is due to an alteration in the tRNA molecule outside of the anticodon. BRUENN and JACOBSON (personal communication) have shown that other amber (SUP5-1) and ochre (SUP5-2 and SUP3-1) suppressor strains of yeast, genetically distinct from those employed here, which also insert tyrosine, possess new chromatographically distinct species of tyrosine tRNA.

The most reasonable interpretation of the results presented here is that the species of tyrosine tRNA responsible for suppression of UAG in yeast is altered outside of the anticodon and that an *E. coli* system is not capable of recognizing this species of tRNA as an amber suppressor. We would suggest that this is also true for the yeast UAA suppressor (as suggested by GILMORE *et al.* 1971), although the experiments do not rule out the possibility of topaz or sepia anti-

codons in the tRNA. The possibility remains, however, that in yeast a base change in the anticodon results in a conformational change in the rest of the molecule such that it cannot participate with *E. coli* ribosomes.

We wish to thank Dr. H. LODISH for gifts of messenger RNA with which this work was initiated, and for advice on analyzing the protein synthesized from them *in vitro*.

LITERATURE CITED

- CRICK, F., 1966 Codon-Anticodon Pairing: The wobble hypothesis. *J. Mol. Biol.* **19**: 548-555.
- ENGELHARDT, D., R. WEBSTER, R. WILHELM and N. ZINDER, 1965 *In vitro* studies on the mechanism of suppression of a nonsense mutation. *Proc. Nat. Acad. Sci., USA* **54**: 1791-1797.
- GAREN, A., 1968 Sense and nonsense in the genetic code. *Science* **160**: 149-159.
- GILLAM, I., S. MILLWARD, D. BLEW, M. VON TIGERSTROM, E. WIMMER and G. TENER, 1967 The separation of soluble ribonucleic acids on benzoylated diethylaminoethylcellulose. *Biochemistry* **6**: 3043-3056.
- GILMORE, R., J. STEWART and F. SHERMAN, 1971 Amino acid replacements resulting from super-suppression of nonsense mutants of Iso-1-cytochrome *c* from yeast. *J. Mol. Biol.* **61**: 157-173.
- GOODMAN, H., J. ABELSON, A. LANDY, S. BRENNER and J. SMITH, 1968 Amber Suppression: a nucleotide change in the anticodon of a tyrosine transfer RNA. *Nature* **217**: 1019-1024.
- HAWTHORNE, D. C., 1969 Identification of nonsense codons in yeast. *J. Mol. Biol.* **43**: 71-75.
- HIRSH, D., 1971 Tryptophan transfer RNA as the UGA suppressor. *J. Mol. Biol.* **58**: 439-458.
- HOLLEY, R., 1967 Isolation of sRNA from intact yeast cells. *Methods in Enzymology XII* part A, 596-598. Edited by GROSSMAN and MOLDAVE.
- MAXWELL, I., E. WIMMER and G. TENER, 1968 The isolation of yeast tyrosine and tryptophan transfer ribonucleic acids. *Biochemistry* **7**: 2629-2634.
- SALSER, W., R. GESTELAND and A. BOLLE, 1967 *In vitro* synthesis of bacteriophage lysozyme. *Nature* **215**: 588-591.
- SANGER, F., M. BRETSCHER and E. HOCQUARD, 1964 A study of the products from a polynucleotide-directed cell-free protein synthesizing system. *J. Mol. Biol.* **8**: 38-45.
- SHERMAN, F. and J. STEWART, 1971 Genetics and biosynthesis of cytochrome *c*. *Annual Review of Genetics* **5**: 257-296.
- STEWART, J. and F. SHERMAN, 1972 Demonstration of UAG as a nonsense codon in bakers' yeast by amino-acid replacements in iso-1-cytochrome *c*. *J. Mol. Biol.* **68**: 429-443.
- TWARDZIK, D., E. GRELL and K. JACOBSON, 1971 Mechanism of suppression in *Drosophila*: a change in tyrosine transfer RNA. *J. Mol. Biol.* **57**: 231-245.
- YOUNG, E., 1970 Cell-free synthesis of bacteriophage T4 glucosyl transferase. *J. Mol. Biol.* **51**: 591-604.