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An *Escherichia coli* tyrosine transfer RNA is a leucine-specific transfer RNA in the yeast *Saccharomyces cerevisiae*

(amber suppression in yeast/aminoacyl-tRNA synthetase/cross-species tRNA recognition/*Escherichia coli* suppressor tRNA/relationships between tRNAs)

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ABSTRACT **While the *Escherichia coli Su-3* (*tyrT*) tyrosine tRNA suppressor inserts only tyrosine at amber codons in *E. coli*, we show here that in *Saccharomyces cerevisiae* this tRNA inserts leucine and no significant amounts of any other amino acid. Thus, the *E. coli* tyrosine tRNA is functionally a leucine tRNA in yeast cytoplasm. This functional identity may correlate with a structural relationship between the *E. coli* tyrosine and yeast leucine tRNAs, which are both members of the uncommon type II class of tRNA structures. The results raise the possibility that in evolution a tRNA may be more closely related to a tRNA of different acceptor specificity, but of the same type class, than to one with the same amino acid specificity, but of a different type class.**

In view of the universal language of the genetic code, the identification of determinants for recognition of some tRNAs by their cognate aminoacyl-tRNA synthetases (1, 2) has raised interest in the question of the conservation of determinants in evolution. Recent experiments established that a single G3·U70 base pair in the acceptor helix is a major determinant of the identity of *Escherichia coli* and of the cytoplasmic *Bombyx mori* and human alanine tRNAs (3). The alanine tRNA from *E. coli* and its eukaryotic counterparts are all members of the common type I class of tRNA structures, which typically have four or, less often, three base pairs in the dihydrouridine stem and four to six nucleotides in the variable loop. In contrast, the less common type II tRNAs have large variable loops of 13–22 nucleotides and three base pairs in the dihydrouridine stem (4). In *E. coli*, only the leucine, serine, and tyrosine tRNAs are type II tRNAs. Like its *E. coli* counterpart, the *Saccharomyces cerevisiae* mitochondrial tyrosine tRNA is a type II structure, and there is evidence that the determinants for identity between the *E. coli* and yeast mitochondrial tyrosine tRNAs are also conserved (5). In contrast, the cytoplasmic tyrosine tRNA in *S. cerevisiae* is a type I structure, and the only cytoplasmic type II tRNAs in *S. cerevisiae* are those specific for leucine and serine.

To test for conservation of determinants for identity between the *E. coli* and yeast cytoplasmic tyrosine tRNA, the *E. coli Su-3* (also called *tyrT*) amber suppressor tyrosine tRNA was expressed in yeast (6). At a level of expression 40% of that of an average yeast tyrosine tRNA gene (5% of total yeast tyrosine tRNA), the *E. coli* suppressor was unable to suppress amber mutations in a tester strain. However, the *E. coli* tRNA can function with the yeast translational apparatus, because coexpression of the *E. coli* suppressor with the *E. coli* tyrosyl-tRNA synthetase in yeast led to efficient suppression of three amber alleles. As the *E. coli* tRNA is a heterologous substrate and must compete with endogenous tRNAs, it is possible that much higher levels of the heterologous tRNA are required for suppression in yeast.

In this study, we used a new vector to raise the level of expression of the *E. coli* suppressor tRNA in yeast to levels up to 300% of total yeast tyrosine tRNA. The increased expression of the *E. coli* tRNA in yeast resulted in suppression of two different amber alleles, in the absence of any *E. coli* aminoacyl-tRNA synthetase. To determine the specificity of aminoacylation of the *E. coli* tRNA in yeast, we used a test protein (chitinase) that was translated from an mRNA that had an amber codon near its amino terminus, analogous to the way in which amino acid insertions into suppressed proteins have been studied in *E. coli* (7). We were able to obtain amber suppression in yeast at a sufficiently high efficiency to isolate enough of the suppressed test protein to determine which amino acid(s) was inserted by the *E. coli* tRNA.

MATERIALS AND METHODS

Construction of Expression Vectors. Plasmids pHET78-1Aam, pHET78-1Bam, pHET78-3Bam, and pHET78-6Bam were constructed by inserting a *Sal* I fragment, which carries the gene for the *E. coli* tyrosine amber suppressor tRNA, into the *Sal* I site of pMP78-1. Four different isolates were recovered, which differ in the number of gene inserts and orientation of the inserts. Plasmids with inserts were detected by phenotypic suppression of the *lacZ* and *argE* amber alleles in *E. coli* strain XAC-I as described (4). The presence of the *Sal* I insert, the number of inserts, and the orientation (indicated A or B in the name of each plasmid) of the insert closest to the 3′ end of the ampicillin resistance gene, *Ap*, were determined by restriction analysis. Plasmids pHET78-1Aam and pHET78-1Bam each contain a single tRNA gene insert in opposite orientations. Plasmids pHET78-3Bam and pHET78-6Bam contain three and six tRNA gene inserts, respectively (Fig. 1). (It is likely that the multiple insertions in pHET78-3Bam and pHET78-6Bam are in head-to-tail arrangements because they are structurally stable.) Only the orientation of the first insert (located on the 3′ side of *Ap*) was determined directly.

Preparation and Amino Acid Sequence Analysis of Chitinase. Suspensions of chitinase were run on 0.1% SDS/7% polyacrylamide gels and transferred electrophoretically to 0.45-μm Immobilon transfer membranes (Millipore) as described (8). Protein sequence was obtained from filters on an Applied Biosystems gas-phase sequenator.

RESULTS

Expression Vectors for *E. coli* Amber Suppressor in *S. cerevisiae*. We inserted the *Sal* I fragment that encodes the *E.*

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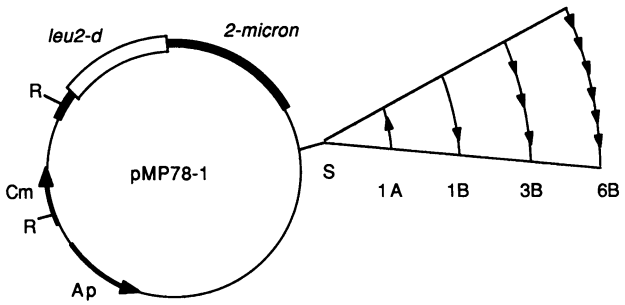

FIG. 1. Plasmids pHET78-1Aam, pHET78-1Bam, pHET78-3Bam, and pHET78-6Bam. The four different constructions differ in the number of gene inserts (arrowheads) and orientation of the inserts (arrowheads point in the 5'-to-3' direction) in the parent, pMP78-1. Ap, ampicillin resistance gene; Cm, chloramphenicol resistance gene; *leu2-d*, defective allele of *LEU2*. Restriction sites: R = *Eco*RI and S = *Sal* I. The thick line indicates DNA of the 2-μm yeast plasmid.

coli amber suppressor tRNA from plasmid pHET120am (6) into the *Sal* I site of pMP78-1, a 2-μ vector which has a copy number about 4.5 times higher than typical 2-μ plasmids in yeast (9). This vector carries the selectable marker *leu2-d*, a defective allele of *LEU2* that must be present in high copy to give a leucine-independent (Leu^+) phenotype.

Several derivatives of pMP78-1 that contain one or more copies of the *E. coli* tRNA gene insert were isolated (Fig. 1). Strain HEY301-129 (6) was transformed with vector pMP78-1 or one of the four derivatives that encode the *E. coli* amber suppressor tRNA. Total RNA was isolated from Leu^+ transformants of each plasmid and was analyzed by Northern blotting to determine the level of tRNA. The pMP78-derived plasmids generate much more tRNA than does pHET120am. A quantitative analysis indicated that the pMP78-derived vectors produce the *E. coli* tyrosine suppressor tRNA at levels up to 300% of the level of total endogenous tyrosine tRNA (data not shown).

Suppression of Amber Alleles in *S. cerevisiae* by an *E. coli* tRNA. To test for function of the amber suppressor, tester strain HEY301-129 was transformed with each of the plasmids. In addition to markers for plasmid selection (*leu2-3,112, ura3-1*), this strain contains three amber alleles, *met8-1*, *trp1-1*, and *his4-580*, which render it auxotrophic for the amino acids methionine, tryptophan, and histidine, respectively. Leu^+ transformants were isolated, put in patches on yeast extract/peptone/dextrose (YPD) plates, and replica plated to minimal medium supplemented with various amino acids. In contrast to transformants which carried pHET-100am (YEp21 with one tRNA gene insert) or pHET120am (YEp21 with two tRNA gene inserts), Leu^+ transformants which carried plasmids pHET78-1Aam, pHET78-1Bam, pHET78-3Bam, or pHET78-6Bam were found to be Met^+, indicating suppression of the *met8-1* amber allele (Fig. 2). These transformants were Trp^- His^-, however, which indicates that the *E. coli* suppressor is unable to suppress the *trp1-1* and *his4-580* alleles. Leu^+ Ura^+ transformants which carried pHET120am plus pHEF200Δ1, which encodes sequences of the *E. coli* tyrosyl-tRNA synthetase on a *URA3* selectable plasmid (5), were found to be Met^+ Trp^+ His^+, confirming earlier findings (6), while Leu^+ transformants which contained the vectors alone (YEp21 or pMP78-1) showed no suppression (Fig. 2).

The plasmid linkage of the suppression phenotype was verified by segregation analysis. One transformant carrying each tRNA-encoding plasmid was grown in rich medium to allow plasmid loss, plated onto rich plates, and then screened for nutritional markers by replica plating. Segregation of the tRNA-encoding plasmids occurred at frequencies comparable to loss of the parent vector from the same strain. In each case (11/11 pHET78-1Aam, 14/14 pHET78-1Bam, 6/6 pHET78-3Bam, and 8/8 pHET78-6Bam segregants), loss of the plasmid, indicated by a Leu^- phenotype, was accompanied by loss of the suppression phenotype.

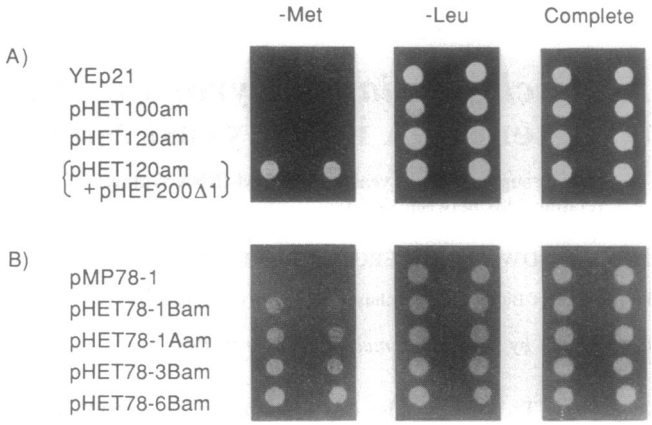

FIG. 2. Suppression of the *met8-1* amber allele in yeast by *E. coli* suppressor tRNA. Leu^+ transformants of HEY301-129 (relevant genotype *leu2-3,112 met8-1* amber) were selected and streaked on selective minimal medium. Single colonies were grown in minimal medium supplemented with required amino acids minus leucine to select for plasmid. Saturated cultures were diluted 1:4.5 (left columns) or 1:6 (right columns) in sterile distilled water in microtiter dishes and were plated onto minimal medium with an inoculating manifold to score nutritional markers. The plasmids carried by each transformant are indicated at the left. The tRNA-encoding vectors in *A* are derived from vector YEp21 while the tRNA-encoding vectors in *B* are derived from vector pMP78-1. Suppression of the *met8-1* allele is indicated by growth in the absence of methionine (-Met). The presence of the plasmids is indicated by growth in the absence of leucine (-Leu). Growth on a minimal plate supplemented with all required amino acids is shown at right (complete).

To extend these results, the pMP78-derived plasmids were recovered from yeast by transformation of *E. coli*. DNA from two yeast transformants of each plasmid (or from one yeast transformant of pHET78-1Bam) was used to transform *E. coli*. For each yeast transformant, DNA from three *E. coli* transformants was analyzed. Restriction digestions of the plasmids recovered indicated that no gross structural alterations had occurred. Sequence analysis of an isolate of pHET78-1Aam and of pHET78-1Bam showed no changes in sequence had occurred in these suppressor tRNA genes. A representative of each type of plasmid recovered from yeast was used to back-transform HEY301-129. The results from three transformations and the back-transformation are summarized in Table 1. This experiment confirmed the observation of suppression and plasmid linkage of the suppression phenotype and, coupled with the structural analysis, suggested that no alteration in the tRNA was required for suppression.

Strain HEY303-66 (a *met8-1 aro7-1 leu2-3,112 ura3-1 ade1 lys1-1 can1-100*) was constructed (6) to test for suppression of a second amber allele. This strain contains the *leu2-3,112* allele for selection of the pMP78-derived plasmids, in addition to the amber alleles, *met8-1* and *aro7-1*. The *aro7-1* allele results in a requirement for the aromatic amino acids phenylalanine and tyrosine (Aro^-). HEY303-66 was transformed with the pMP78-derived plasmids, and Leu^+ transformants were isolated. Transformants were patched to YPD and replica plated to minimal media supplemented with various amino acids to score for suppression. Transformants carrying pHET78-1Aam and pHET78-1Bam showed weak suppression of the *met8-1* allele in this background, but were all Aro^-. In contrast, transformants carrying plasmids pHET78-3Bam and pHET78-6Bam were Met^+ and Aro^+. The parent vector pMP78-1 gave

Table 1. Summary of suppression data

Vector 1 (tRNA)	Vector 2 (synthetase)	Met	Trp	His	No. screened
pMP78-1	None	−	−	−	36
pHET78-1Aam	None	+	−	−	37
pHET78-1Bam	None	+	−	−	29
pHET78-3Bam	None	+	−	−	40
pHET78-6Bam	None	+	−	−	47
pHET120am	pHEF200Δ1	+	+	+	12

Suppression phenotypes of HEY301-129 carrying the gene for the *E. coli* suppressor tRNA (on pMP78-1). Strain HEY301-129 (relevant genotype *leu2-3,112 ura3-1 met8-1am trp1-1am his4-580am*) was transformed with plasmid that encodes the *E. coli* tyrosine amber suppressor tRNA (vector 1, selectable marker *leu2-d*). Leu^+ transformants were isolated, patched to YPD, and replica plated to minimal medium supplemented with various amino acids to score for suppression of the three amber alleles. As a control, HEY301-129 was transformed with pHET120am plus pHEF200Δ1 (which encodes the *E. coli* tyrosyl-tRNA synthetase). The Met, Trp, and His phenotypes of the transformants are indicated. A + indicates growth in the absence of the amino acid (suppression); − indicates no growth in the absence of the amino acid (no suppression). One pHET78-6Bam transformant showed a Met^+ Trp^+ Ade^+ phenotype (not recorded in table); however, Leu^- segregants of this transformant retained Met^+ Ade^+ phenotypes, indicating the presence of a chromosomal (omnipotent) suppressor.

rise to Met^- Aro^- transformants only, and all transformants were Ade^- Lys^- (data not shown). Lack of suppression of the *ade1* (UGA) or *lys1-1* (UAA) alleles of HEY303-66 confirms the specificity of the *E. coli* suppressor for amber alleles in yeast observed with other constructions.

Leucine Is Inserted at an Amber Codon by *E. coli* Tyrosine tRNA Amber Suppressor. The amino acid sequence of a suppressed protein can be determined directly. A system (to be published) for direct determination of the amino acid inserted was developed in this laboratory, with the chitinase gene from yeast. The strain W303∇*cts1* was constructed; it secretes no detectable chitinase protein, because the chromosomal copy of the chitinase gene is partly deleted and disrupted by the insertion of the *HIS3* gene (M. J. Kuranda and P. W. Robbins, personal communication) (Fig. 3, lane a). Transformants of W303∇*cts1* which carry a cloned copy of the chitinase gene on pCT28 secrete large amounts of plasmid-encoded chitinase (Fig. 3, lane b). In contrast, a derivative of pCT28 called pCTam, which has an amber mutation near the N terminus (at Asn-8 of the mature protein), produces no detectable chitinase.

Transformants of W303∇*cts1* containing pCTam and various suppressor constructs were obtained. Chitinase from these strains was purified, loaded onto an SDS/polyacrylamide gel, and then transferred to an Immobilon filter for amino acid analysis (8). (All sequences were obtained twice in two disruption strains.) Consistent with its inability to suppress amber alleles in yeast, the amber suppressor encoded by pHET120am produced no detectable chitinase from pCTam (Fig. 3, lane c). However, in the presence of coding sequences of the gene for *E. coli* tyrosyl-tRNA synthetase (cloned in the *Sal* I site of pCTam), significant amounts of protein were obtained (Fig. 3, lane d). Amino acid analysis of the protein produced under these conditions revealed that the suppressor tRNA inserts tyrosine only at the amber codon (Fig. 4*A*). Thus, the chitinase protein is secreted and is stable when tyrosine is inserted.

We found that cotransformants of pCTam and pMP78-1 produced no detectable chitinase (Fig. 3, lane e), but that cotransformants of pCTam and pHET78-6Bam (with six tyrosine tRNA gene inserts) generated enough chitinase to produce a sequence (Fig. 3, lane f). Surprisingly, the *E. coli* tyrosine tRNA suppressor inserts 100% leucine at the amber codon (Fig. 4*B*). This result implies that, in yeast, the *E. coli* tyrosine tRNA is specifically aminoacylated by leucyl-tRNA synthetase.

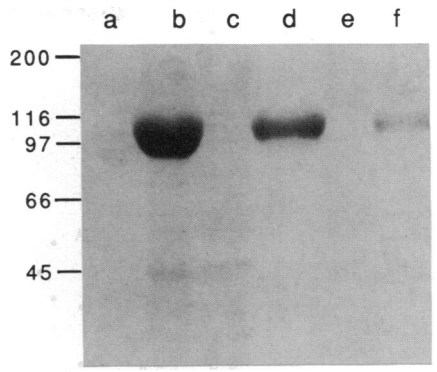

FIG. 3. Suppression of the *cts1-82,84* amber allele by *E. coli Su-3* tyrosine tRNA. Chitinase was isolated from W303∇*cts1* transformants that carried no plasmid (lane a), pCT28 (lane b), pCT28am + pHET120am (lane c), pCTam/*tyrS* + pHET120am (lane d), pCT28am + pMP78-1 (lane e), or pCT28am + pHET78-6Bam (lane f). The chitinase fractions (100 μl in lanes a and c–f, 50 μl in lane b) were electrophoresed on a 0.1% SDS/7% polyacrylamide gel. The gel was stained with Coomassie brilliant blue R-250. Chitinase has an apparent molecular weight of 110,000 when glycosylated. The locations and sizes ($\times 10^{-3}$) of the molecular weight markers are indicated on the left (rabbit skeletal muscle myosin, *E. coli* β-galactosidase, rabbit muscle phosphorylase *B*, bovine serum albumin, and hen ovalbumin; Bio-Rad).

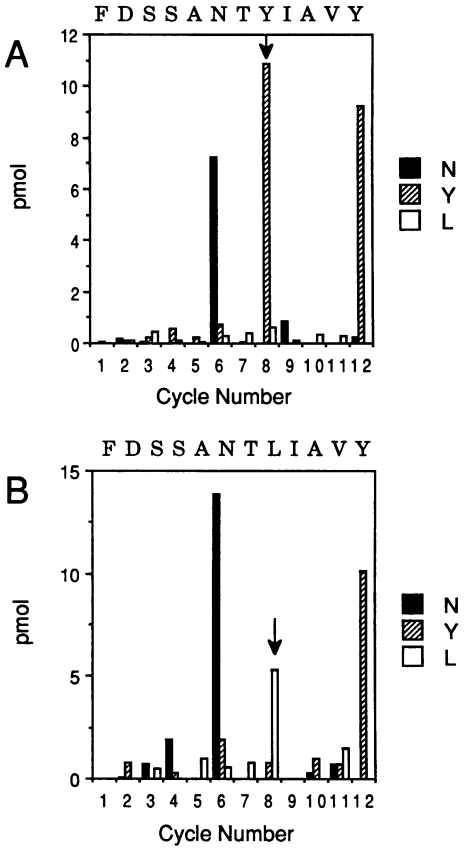

FIG. 4. Amino acid sequences of suppressed proteins. The sequence of the first 12 amino acids of chitinase isolated from W303∇*cts1* transformants which carry plasmids pCTam/*tyrS* + pHET120am (*A*) or pCTam + pHET78-6Bam (*B*) is shown. The sequence obtained in each case is given at the top of each panel. The yield of amino acid (pmol) is plotted against the residue number.

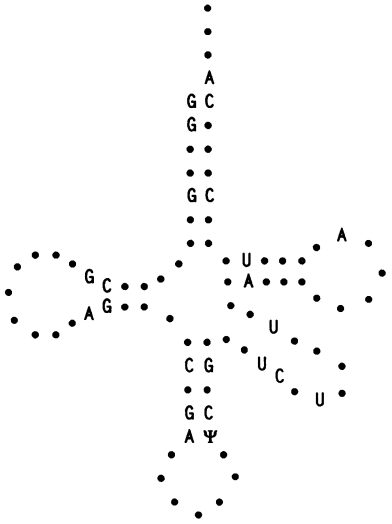

FIG. 5. Comparison of tyrosine tRNAs. A composite of the sequences of the *E. coli Su-3* tyrosine tRNA suppressor and the two known *S. cerevisiae* leucine tRNAs is shown in schematic cloverleaf form. Nucleotides that are conserved among the three tRNAs are indicated by the single-letter code, while those that are conserved among all tRNAs or that are not identical in all three tRNAs are represented by a dot.

DISCUSSION

The results reported here are consistent with several observations of little or no aminoacylation of pure *E. coli* tyrosine tRNA with tyrosine by yeast enzymes (data not shown and see refs. 10–12). Using a crude preparation of yeast enzymes, we observed limited but reproducible *in vitro* charging of pure *E. coli* tyrosine tRNA with leucine, but we cannot rule out the possibility that it is due to minor contamination of the tyrosine tRNA with *E. coli* leucine tRNA. With another system, *in vitro* aminoacylation of a tRNA that was charged *in vivo* was observed only with a pure enzyme preparation, using a stoichiometric excess of enzyme (13).

Misacylation *in vivo* of certain *E. coli* amber suppressors (CUA anticodon) with glutamine or lysine has been reported (2), and in these cases, specific recognition of one or more bases of the artificial CUA anticodon is responsible for mischarging. In contrast, the CUA anticodon substitution in the *E. coli Su-3* tyrosine tRNA, and in 10 other *E. coli* tRNAs that have been tested, does not alter the charging specificity of these tRNAs when they are expressed in *E. coli* (2).

Previously some cross-species *in vitro* misacylation of tRNAs has been observed (14). Among other possibilities, these cross-acylations may be due to the avoidance by the heterologous enzyme of cryptic blocking determinants on tRNAs that prevent misacylations in the homologous host. However, the experiments reported here establish that, when confronted with all 20 enzymes *in vivo*, the charging of a foreign tRNA can be resolved into a single amino acid specificity that operationally may reflect heretofore unanticipated relationships between tRNAs from different organisms.

The observations suggest that the determinants for tyrosine tRNA identity are not conserved between yeast cytoplasm and *E. coli*, contrasting with other studies that have found that the major determinants for tRNA identity are conserved or partially conserved between species (1, 3, 5) when the tRNAs are in the same type class. While it is a formal possibility that the mitochondrial leucyl-tRNA synthetase is responsible for charging of the *E. coli* tyrosine tRNA suppressor in the yeast cytoplasm (cf. ref. 15), the mitochondrial leucine tRNA is also a type II tRNA (16), so that the *E. coli* tyrosine tRNA would still be charged by a leucyl-tRNA synthetase specific for type II tRNAs in this case. The unexpected finding that the *E. coli* tyrosine tRNA is a leucine-specific tRNA in yeast may mean that this tRNA is more closely related to the yeast leucine tRNA than to the yeast tyrosine tRNA.

A comparison of the sequence of the *E. coli* tyrosine tRNA with the two known leucine tRNA sequences from yeast cytoplasm (16) reveals that these tRNAs have the exact same number of bases in each region of the type II tRNA structure (ref. 4; Fig. 5). It is possible that, in the absence of the appropriate type of tRNA of the same specificity in yeast, the *E. coli* tRNA assumes the identity of another, and presumably closely related, tRNA of the same structural class, such as the leucine tRNA. The other bases shared between the *E. coli* tyrosine tRNA and the leucine tRNAs may result in leucine identity rather than serine identity.

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