Yeast ochre suppressor SUQ5-o1 is an altered tRNA^{Ser}_{UCA}

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ABSTRACT

Ochre suppressor tRNA was partially purified from strains of *Saccharomyces cerevisiae* containing the serine-inserting class III suppressor SUQ5-o1. RNA sequence analysis of this tRNA indicates that the suppressor is derived from a UCA-decoding tRNA^{Ser} by a G → U substitution in the middle position of the anticodon. The suppressor further differs from the wild-type UCA-decoding tRNA^{Ser} in that the mutant anticodon lacks the modified uridine found in the wobble position of the wild-type tRNA and contains instead another modification in or near the anticodon.

INTRODUCTION

Nonsense suppressors allow amino acids to be incorporated into growing polypeptide chains at codons that normally signal the termination of translation. The only eukaryotes in which such suppressors have been characterized biochemically are the budding yeast *Saccharomyces cerevisiae* and the fission yeast *Schizosaccharomyces pombe*. In these cases nonsense suppression is mediated by tRNAs (1-3) with anticodons complementary to the termination codons that they suppress (4-9).

To date the structures of eukaryotic ochre (UAA) suppressor tRNAs have been inferred from the DNA sequences of cloned genes (5,9). However, the DNA sequences alone do not provide information on base modifications which may determine some properties of the suppressor tRNAs. To investigate possible roles of base modifications we have characterized the suppressor tRNA encoded by the SUQ5-o1 gene of *S. cerevisiae*.

The SUQ5 gene (alternatively designated SUP16) (10) was first identified in a mutant form capable of suppressing a number of auxotrophic mutations (11). This suppressor mutation, SUQ5-o1, causes insertion of serine in response to ochre codons (12). The efficiency of SUQ5-o1 suppression is influenced by the extra-chromosomal genetic

element [psi] (11): in [psi]⁺ SUQ5-01 strains, the efficiency of ochre read-through is about ten fold higher than in [psi]⁻ SUQ5-01 strains (13). The molecular basis of this difference is not known. SUQ5-01, like other ochre suppressors in yeast, decodes only ochre (UAA) codons. This is in contrast to bacterial ochre suppressors which recognize both ochre and amber (UAG) codons. It has been suggested that the specificity of the yeast ochre suppressors may be determined by modifications of the wobble base in the tRNA which restricts pairing to A residues in the third position of the cognate codon (14,15).

Our studies did not reveal any effect of [psi] genotype on the production or properties of SUQ5-01 tRNA. We did, however, find a novel modified base, in or near the anticodon, which may determine the ochre specificity of this suppressor.

MATERIALS AND METHODS

Strains and growth conditions. We used the following haploid strains of S. cerevisiae: BSC 466/6c (suq5⁺, [psi]⁺), BSC 468/1d (SUQ5-01, [psi]⁻, MT141-4/6d and D898 (SUQ5-01, [psi]⁺). D898 was kindly provided by Fred Sherman (University of Rochester); the other strains were from the collection of the Botany School, Oxford.

Cultures were grown at 30° C with shaking in YEPD (10g yeast extract/1, 10g bactopectone/1, 20g glucose/1) and harvested during exponential growth (5-8 g cells (wet weight)/1). For the preparation of [³²P]-labeled tRNA, cells were grown in the low phosphate medium of Rubin (16) containing 0.3 mCi carrier-free [³²P]-orthophosphate/ml.

Preparation of tRNA. Total tRNA for column chromatography was prepared by phenol extraction of whole cells followed by salt extraction, stripping of amino acids, and passage over DEAE-cellulose (2). Total tRNA for sequencing was prepared in the same way except that the tRNA was not stripped of its amino acids.

tRNA charging assays. Amino acid acceptor activities of tRNA fractions were assayed for the incorporation of [³H]-serine into acid-precipitable material (1). The charging enzyme was supplied as S-100 extract from yeast cells broken by vortexing for five minutes with an equal weight of extraction buffer and three weights of glass beads (0.5mm diameter, Sigma).

In vitro suppression assays. We used the suppressor assay of

Gesteland et al. (2) in which RNA from bacteriophage Q β is translated in cell-free extracts containing ribosomes and the pH5 fraction from mouse ascites cells and initiation factors from rabbit reticulocytes (17). Addition of ochre-suppressing tRNA gives a read-through product that is 2800 daltons longer than the normal synthetase and can be resolved by sodium dodecyl sulphate-polyacrylamide gel electrophoresis.

Fractionation of tRNA. The total tRNA was applied to an RPC-5 (18) column in 0.3M NaCl, 50 mM Tris-HCl pH 7.6. The bulk of the tRNA was eluted with 0.6 M NaCl, 50 mM Tris-HCl pH 7.6. The column was then developed with a linear gradient of 0.6M to 0.8M NaCl in 50 mM Tris-HCl pH 7.6. Remaining tRNA was eluted from the column with 2 M NaCl in 50 mM Tris-HCl pH 7.6. Suppressor-containing fractions from the RPC-5 column were precipitated with ethanol and applied to a Sepharose-4B (19) column in buffer A (10 mM sodium acetate pH 4.5, 10 mM MgCl₂, 1 mM EDTA, 6 mM β -mercaptoethanol) containing 1.3 M (NH₄)₂SO₄. The column was developed with a concave gradient from 1.3 M to zero (NH₄)₂SO₄ in buffer A. Pooled fractions were dialyzed for 16 hours against 20 mM Tris-HCl pH 7.6, 6 mM β -mercaptoethanol and then for 6 hours against distilled water to remove (NH₄)₂SO₄.

tRNAs were also fractionated by two-dimensional gel electrophoresis, first through a 40 cm gel of 10% polyacrylamide, then at right angles through a 20 cm gel of 20% polyacrylamide (20).

RNA sequence analysis. Purified tRNA was digested with RNase T₁ and the products separated by electrophoresis at pH 3.5 on cellulose acetate followed by DEAE homochromatography with homomix c (21). The RNase T₁ products were redigested with RNase A and with RNase U₂ and the products characterized by their electrophoretic mobilities on DEAE-paper at pH 3.5 (RNase A) or pH 1.9 (RNase U₂). The base compositions of RNase T₁ fragments and redigestion products were determined by complete digestion with RNase T₂ followed by two dimensional thin layer chromatography (22).

RESULTS AND DISCUSSION

In vitro suppressor activities of total tRNAs. Total tRNA from SUQ5-01 strains was shown to have ochre suppressor activity by addition of the tRNA to a mammalian cell-free protein-synthesizing system programmed with Q β -RNA and measurement of read-through of the synthetase UAA terminator (Figure 1). tRNA from wild-type (suq5⁺)

strains does not cause this read-through (Figure 1).

Using this *in vitro* assay for ochre suppression we examined the influence of the extra-chromosomal genetic element *[psi]*. Although *in vivo* *SUQ5-ol* efficiently suppresses ochre mutations in *[psi]⁺* strains only, we failed to observe any effect of *[psi]* on the amount of ochre suppressor activity in tRNA preparations extracted from *SUQ5-ol* cells. Furthermore, the *SUQ5-ol* tRNAs from *[psi]⁺* and *[psi]⁻* strains were indistinguishable by their chromatographic and electrophoretic properties (see below).

Partial purification of *SUQ5-ol* suppressor tRNA. Total tRNA from *SUQ5-ol* strains was fractionated by reverse phase chromatography; the fractions which caused ochre suppression in the *in vitro* translation assay were those which contained the first serine-accepting activity eluted from the column (Figure 2). Further fractionation of the suppressor-containing tRNA by chromatography through Sepharose 4B yielded only one serine-accepting activity which co-purified with the only ochre-suppressing activity (Figure 3). Two-dimensional polyacrylamide gel electrophoresis of the suppressor tRNA purified as described here gave only one spot which stained intensely with methylene blue in the position to which tRNA^{Ser}_{UCA/G} migrates (6,23) (Figure 4).

SUQ5-ol suppressor tRNA is a mutant form of tRNA^{Ser}_{UCA/G}. Wild-type tRNA^{Ser}_{UCA/G} consists of two closely related species of serine-accepting

Figure 2. RPC-5 fractionation of total tRNA from an SUQ5-o1 strain. Pooled fractions were monitored for absorbance at 260 nm (●—●), [³H]-serine accepting activity (o---o) and in vitro ochre-suppressor activity (see insert).

tRNAs present in a ratio of ~3:1. The anticodon of the major species is UGA while that of the minor is CGA; they are presumed to decode UCA and UCG respectively (23). The UCA-decoding species could become a

Figure 3. Sepharose 4B chromatography of suppressor-containing fractions from RPC-5 column. Pooled fractions were monitored for absorbance at 260 nm (●—●), [³H]-serine accepting activity (o---o), and in vitro ochre-suppressor activity (see insert).

Figure 4. Two dimensional polyacrylamide gel electrophoresis of total yeast tRNA. The gel was stained with ethidium bromide. The arrow indicates the position to which suppressor-containing tRNA from the Sepharose 4B column would migrate.

UAA-decoding ochre suppressor by a single mutation substituting U for G in the middle position of the anticodon. Such a change would eliminate a site sensitive to RNase T₁ and generate a novel T₁ oligonucleotide containing the complete anticodon sequence:

Furthermore, if SUQ5-o1 were derived from one of the genes coding for $\text{tRNA}^{\text{Ser}}_{\text{UCA}}$, we would expect to observe not only the appearance of this new oligonucleotide but also a reduction in the molar yield of the wild-type UCA-decoding anticodon relative to the UCG-decoding species.

To test this hypothesis, ³²P-labeled RNA from an SUQ5-o1-containing strain fractionated as in Figure 4 was digested with RNase T₁ and the products separated by electrophoresis on cellulose acetate followed by homochromatography on DEAE cellulose plates (21) (Figure 5a,b). The pattern of oligonucleotides observed is nearly identical to that

Figure 5. RNase T₁ Digestion Products of tRNA^{Ser} from an SUQ5-ol-containing strain. (a) Autoradiograph of separated fragments. (b) is a representation of the autoradiograph shown in (a). The arrow indicates the oligonucleotide (1) unique to tRNA^{Ser} of SUQ5-ol strains. Sequences and molar yields of the numbered products are given in Table 1.

of tRNA^{Ser}_{UCA/G} from wild-type (suq5⁺) cells (23; D.C. and C.W., unpublished) but includes one additional large oligonucleotide (spot 1). Sequence analysis of the RNase T₁ oligonucleotides was performed by characterizing products of re-digestion with RNases T₂, A and U₂. These data (reported in Table I) are consistent with the above prediction that the sequence of the SUQ5-ol-specific oligonucleotide is ACUUUA(i⁶A,A)Ψ(C,U)G.

The relative amounts of the suppressor tRNA, tRNA^{Ser}_{UCG} and tRNA^{Ser}_{UCA} present in the SUQ5-ol strain can be estimated by comparing the molar yields of the anticodon oligonucleotides unique to each (spots 1,2

Table I

Sequences and Molar Yields of Anticodon-containing
RNase T₁ Oligonucleotides of tRNA^{Ser}

Sequence	Molar ^b Yield	Products of redigestion with		
		RNase T ₂	RNase A	RNase U ₂ ^c
(1) ^a ACUUUAi ⁶ AAΨCUGp	0.71	Ap,Cp,Gp, Up,Ψp,i ⁶ Ap, *p	ACp,Ai ⁶ AAΨp, Gp,Cp,Up,	(C,U ₃)Ap, (Ψ,C,U)Gp
(2) Ai ⁶ AAΨCUCUUmGp	1.00	Ap,Cp,Up UmGp,i ⁶ Ap, Ψp	Ai ⁶ AAΨp, Cp,Up,UmGp	N.D. ^d
(3) Ai ⁶ AAΨCUGp	2.09	Ap,i ⁶ Ap,Ψp, Cp,Up,Gp	Ai ⁶ AAΨp, Cp,Up,Gp	(Ψ,C,U)Gp, Ap,i ⁶ Ap
(4) ACUUGp/CUCUGp	N.D.	Ap,Cp,Up Up,Gp	ACp,Up,Gp,Cp	(C,U,U*)Gp, Ap,(C,U)Gp

- The oligonucleotides listed are the digestion products shown in Figure 5.
- The molar yields were calculated by normalizing the radioactivity in each product to oligonucleotide 2, the 3' portion of the tRNA^{Ser}_{UCG} anticodon.
- The order of nucleotides within the RNase U₂ products has not been determined.
- N.D.; not determined.

and 3 respectively). In wild-type (suq5^+) strains the ratio of $\text{tRNA}_{\text{UGC}}^{\text{Ser}}$ to $\text{tRNA}_{\text{UCA}}^{\text{Ser}}$ is 1:3 (23). As shown in Table I the SUQ5-01 strain contains the $\text{tRNA}_{\text{UGC}}^{\text{Ser}}$, $\text{tRNA}_{\text{UCA}}^{\text{Ser}}$, and suppressor tRNA in relative yields of 1:2.1:0.7. This finding suggests that one of several $\text{tRNA}_{\text{UCA}}^{\text{Ser}}$ genes has mutated to the ochre suppressor. The same conclusion has been deduced from the DNA sequence of the cloned SUQ5-01 gene (9; J. Broach, personal communication).

A novel modified base in SUQ5-01 suppressor tRNA. Our RNA sequence analysis suggests that the SUQ5-01 suppressor tRNA differs from the wild-type $\text{tRNA}_{\text{UCA}}^{\text{Ser}}$ only in the anticodon oligonucleotide. One change is the substitution of U for G in the middle position of the anticodon (Figure 6), a conclusion that is confirmed by the DNA sequence data of Olson *et al.* (9). Wild-type $\text{tRNA}_{\text{UCA}}^{\text{Ser}}$ and the SUQ5-01 suppressor tRNA also differ in the modified base composition of their anticodon oligonucleotides. Wild-type $\text{tRNA}_{\text{UCA}}^{\text{Ser}}$ contains a modified uridyl residue ($\ddot{\text{U}}$) in the wobble position of the anticodon (23) which is absent in the suppressor. However, modified nucleotide analysis of the oligonucleotide containing the SUQ5-01 anticodon reveals the

Figure 6. Sequence of $\text{tRNA}_{\text{UCA}}^{\text{Ser}}$ and the base change found in the SUQ5-01 suppressor tRNA. The sequence of $\text{tRNA}_{\text{UCA}}^{\text{Ser}}$ is based on the results of Piper (6), Etcheverry *et al.* (23) and Olson *et al.* (9). The RNase T_1 oligonucleotide which contains the ochre suppressor anticodon includes the nucleotide $\ddot{\text{U}}$. Because its precise location has not been determined (see text), $\ddot{\text{U}}$ is not included in the structure.

presence of a distinct modification, here designated $\dot{T}$ (see Figure 7 and Table I); this residue is not present in the wild-type tRNA. We do not know the structures of $\dot{U}$ or $\dot{T}$ but preliminary experiments suggest that $\dot{U}$ is 5-carbamoylmethyluridine (ncm^5U ; 24) and $\dot{T}$ is 5-(methoxycarbonylmethyl)-2-thiouridine ($\text{mcm}^5\text{s}^2\text{U}$; H. Laten, personal communication). The modified base mcm^5U or $\text{mcm}^5\text{s}^2\text{U}$ occurs in the wobble position of UGA-decoding suppressor tRNAs of *Schizosaccharomyces pombe* (25). We have not been able to determine the exact position of $\dot{T}$ in the SUQ5-01 sequence due to lability of the $\dot{T}$ modification. However, since $\dot{U}$ is absent from the suppressor anticodon and both $\dot{U}$ and $\dot{T}$ are uridine derivatives, we suggest that $\dot{U}$ is replaced by $\dot{T}$ as a secondary consequence of the $G \rightarrow U$ change which generated a UAA-decoding tRNA.

It has been suggested that yeast ochre-specific suppression is determined by modification of the wobble base in the anticodon (14,15). The $\dot{T}$ modification observed in the SUQ5-01 tRNA is thus a candidate for this modification. This hypothesis is strengthened by the recent

Figure 7. Minor nucleotide analysis of the anticodon oligonucleotides of tRNA^{Ser} and SUQ5-01 tRNA. (a) the tRNA^{Ser} RNase U, oligonucleotide CUUGp and (b) the SUQ5-01 T_1 oligonucleotide ACUUUAi⁶AApCUG were digested to mononucleotides using RNases A, T_1 and T_2 and the products separated by thin layer chromatography (22). B indicates the position of the indicator dye xylene cyanol. The novel minor nucleotide observed in the anticodon oligonucleotide of the suppressor tRNA has been assigned the trivial name $\dot{T}_p$ solely on the basis of the similarity of its behavior to that of T_p on thin layer chromatography ($\dot{T}_p$ migrates slightly faster than T_p in both dimensions).

demonstration that the same modification can be identified in oligonucleotides containing the anticodons of the distinct serine-inserting ochre suppressor SUP61 (derived from tRNA^{Ser}_{UCG}) and the tyrosine-inserting ochre suppressor SUP4 (Colby, Etcheverry and Guthrie, manuscript in preparation).

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REFERENCES

- 1 Capecchi, M.R., Hughes, S. H. and Wahl, G. M. (1975) *Cell* 6, 269-277
- 2 Gesteland, R.F., Wolfner, M., Grisafi, P., Fink, G., Botstein, D. and Roth, J.R. (1976) *Cell* 7, 381-390
- 3 Kohli, J., Kwong, T., Altruda, F., Söll, D., and Wahl, G. (1979) *J. Biol Chem* 254, 1546-1551
- 4 Piper, P.W., Wasserstein, M., Engbaek, F., Kaltoft, K., Celis, J.E., Zeuthen, J., Leibman, S. W. and Sherman, F. (1976) *Nature* 262, 757-761
- 5 Goodman, H.M., Olson, M.V. and Hall, B.D. (1977) *Proc. Natn. Acad. Sci. USA* 74, 5453-5457
- 6 Piper, P. W. (1978) *J. Molec. Biol.* 122, 217-235
- 7 Rafalski, A., Kohli, J., Agris, P. and Söll, D. (1979) *Nucl. Acid Res.* 6, 2683-2695
- 8 Wetzel, R., Kohli, J., Altruda, F. and Söll, D. (1979) *Molec. Gen. Genet.* 172, 221-228
- 9 Olson, M.V., Page, G. F., Sentenac, A., Piper, P., Worthington, M., Weiss, R. and Hall, B.D. *Nature* (in press)
- 10 Ono, B., Stewart, J.W. and Sherman, F. (1979) *J. Molec. Biol.* 128, 81-100
- 11 Cox, B.S. (1965) *Heredity* 20, 505-521
- 12 Liebman, S. W., Stewart, J. W. and Sherman, F. (1975) *J. Molec. Biol.* 94, 595-610
- 13 Ono, B., Wills, N., Stewart, J. W., Gesteland, R.F., and Sherman, F. (1981) *J. Molec. Biol.* (in press)

- 14 Sherman, F., Ono, B. and Stewart, J. R. (1979) in Nonsense mutations and tRNA suppressors. Celis, J. E. and Smith, J. D., Eds., pp 133-153, Academic Press, London
- 15 McCloskey, J. A. and Nishimura, S. (1977) *Acc't. Chem. Res.* 10, 403-410
- 16 Rubin, G. M. (1974) *J. Biol. Chem.* 248, 3860-3875
- 17 Atkins, J.F., Lewis, J.B., Anderson, C.W. and Gesteland, R.F. (1975) *J. Biol. Chem.* 250, 5688-5695
- 18 Pearson, R. L., Weiss, J.F. and Kelmers, D. (1971) *Biochim. Biophys. Acta* 228, 770-774
- 19 Holmes, W.M., Hurd, R.E., Reid, B.R., Rimerman, R.A. and Hatfield, G.W. (1975) *Proc. Natn. Acad. Sci. USA* 72, 1068-1071
- 20 Fradin, A., Gruhl, H. and Feldmann, H. (1975) *FEBS Letts.* 50, 185-189
- 21 Barrell, B.G. (1971) in *Procedures in nucleic acids research*, Cantoni, G.L. and Davies, D.R., Eds., pp751-779, Harper & Row, New York
- 22 Saneyoshi, M., Ohashi, Z., Harada, F. and Nishimura, S. (1972) *Biochim. Biophys. Acta* 262, 1-10
- 23 Etcheverry, T., Colby, D. and Guthrie, C. (1979) *Cell* 18, 11-26
- 24 Piper, P.W. (1980) in *tRNA: Biological Aspects*, ed. Soll, D., Abelson, J.N. and Schimmel, P.R. pp.379-394. Cold Spring Harbor Lab., Cold Spring Harbor, New York.
- 25 Kohli, J., Altruda, F., Kwong, T., Rafalski, A., Wetzel, R., Soll, D., Wahl, G. and Leupold, U.(1980) in *tRNA: Biological Aspects*, ed. Soll, D., Abelson, J.M. and Schimmel, P.R. pp.407-419. Cold Spring Harbor Lab., Cold Spring Harbor, New York.