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Recognition of Nonsense Codons in Mammalian Cells

(calf liver/aminoacyl-tRNA synthetases/codon UGA)

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ABSTRACT **The tritiated trinucleotide UGA was used in a binding assay to detect transfer RNAs that recognize this nonsense codon from calf-liver cells. Acylation of transfer RNA with labeled amino acids and determination of codon responses of aminoacyl-tRNAs demonstrate that a species of seryl-tRNA and a species of arginyl-tRNA recognize the codon UGA. Slight responses of cysteinyl-tRNA and of trytophanyl-tRNA to the codon UGA were also observed.**

Suppression of nonsense mutations by tRNA in prokaryotic cells has been well established (1, 2). In eukaryotic cells, however, there are only a limited number of studies that suggest that tRNA may suppress nonsense codons. Genetic experiments in yeast (3–6) and in *Neurospora* (7) demonstrated the supression of nonsense mutations. However, direct evidence that tRNA is responsible for this suppression has not been demonstrated. Several tRNA species in higher organisms are capable of recognizing nonsense codons (8–13). As a first step for determining if nonsense-suppressor tRNAs occur in higher organisms, a study of the recognition of nonsense codons by fractionated tRNA from mammalian tissue was undertaken. The technique used was a modification (14) of the binding assay of Nirenberg and Leder (15), in which radioactive oligonucleotides were used to detect tRNA responses. In the present paper, the recognition of the nonsense codon [^{3}H]UGA by fractionated tRNA from calf liver is reported.

METHODS

Transfer RNA was extracted from fresh calf liver with phenol and then with chloroform as described in ref. 13, with the exception that whole tissue was used, applied to a DEAE-cellulose column, eluted with 1 M NaCl, deacylated (13), fractionated on a reverse-phase chromatographic column (RPC I) (16) as described in the legend of Fig. 1, precipitated, collected, and aminoacylated as described in Table 1. Aminoacyl-tRNA synthetases were prepared by the procedure of Muench and Berg (17) as modified by Portugal (18). [^{3}H]-Arginine (4.96 Ci/mmol), [^{14}C]arginine (300 Ci/mol), [^{14}C]-cystine (271 Ci/mol), [^{3}H]glycine (11.1 Ci/mmol), [^{3}H]-leucine (6.0 Ci/mmol), [^{14}C]leucine (327 Ci/mol), [^{3}H]-serine (2.23 Ci/mmol), [^{3}H]tryptophan (4.05 Ci/mmol) and [^{14}C]tryptophan (512 Ci/mol) were purchased from New England Nuclear Corp. and Schwartz BioResearch.

[^{3}H]UGA was prepared from UG and [^{3}H]ADP (2.03 Ci/mmol; New England Nuclear Corp.) in the presence of primer-dependent polynucleotide phosphorylase (EC 2.7.7.8.; P. L. Biochemicals, Inc., Milwaukee, Wis.). The binding of [^{3}H]UGA to *Escherichia coli* ribosomes was determined by the technique of Nirenberg and Leder (15) as modified for labeled oligonucleotides (14) on fractionated tRNA (see Table 1). The wash procedure in binding assays with [^{3}H]UGA was modified to four, 3-ml washes followed by four, 5-ml washes with cold standard buffer, pH 7.3 (15), to reduce nonspecific interactions between [^{3}H]UGA and Millipore filters. *E. coli* MRE600 ribosomes (prepared in collaboration with Dr. F. Portugal) were prepared by the procedure of Pestka (19), with the exception that ribosomes were washed twice in 1 M NH_4Cl.

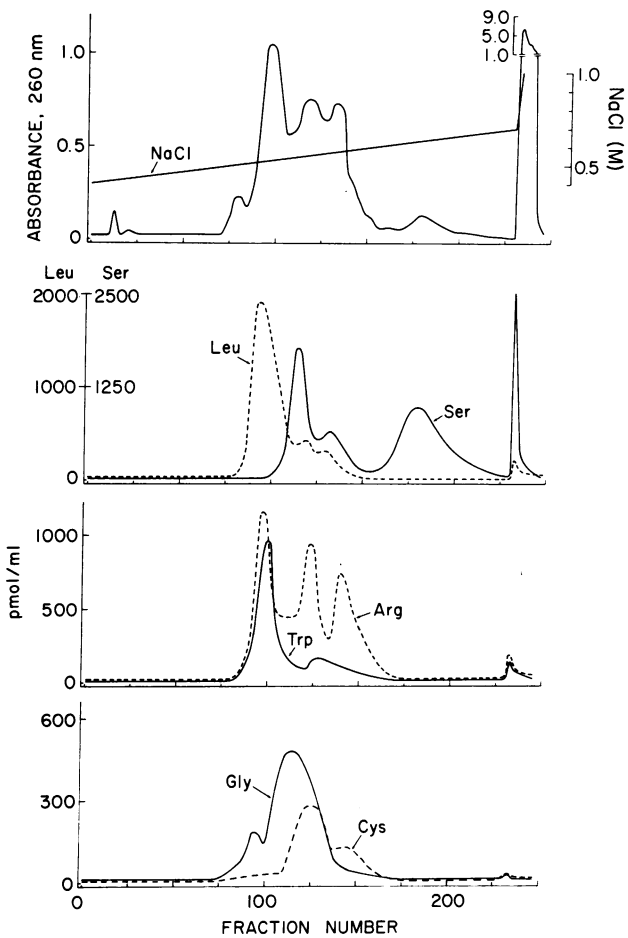

FIG. 1. Fractionation and aminoacylation of calf-liver tRNA. 1013 A_{260} units of calf-liver tRNA were applied to an RPC I column (13, 16). 12.5-ml fractions were collected. Fractions were pooled; the tRNA was precipitated and collected as shown in Table 1. Aminoacylation was as described in Table 1.

TABLE 1. *Fractionation, precipitation, and collection of tRNA for aminoacylation and studies with [^{3}H]UGA*

Fraction nos.*	Fractions pooled†	Aminoacylation‡		[^{3}H]UGA responses§	
		μl/assay	Fraction nos.	μl/assay	Fraction nos.
1– 60	1–30, 31–60	10	1–30, 31–60	—	—
61– 86	61–62, 63–64, etc. through 85–86	5	61–62, 65–66, etc. through 85–86	5	63–64, 67–68, etc. through 83–84
87–146	None	10	88, 90, etc. through 146	10	87, 89, etc. through 145
147–230	147–148, 149–150, etc. through 229–230	5	149–150, 153–154, etc. through 229–230	5	147–148, 151–152, etc. through 227–228
231–240	None	10	231, 232, 233, etc. through 240	10	231, 232, 233, etc. through 240

* Correspond to the fraction numbers in Fig. 1.

† Fractions were pooled on the basis of the absorbance profile shown in Fig. 1 and were then diluted to 0.2 M NaCl with 1 mM EDTA–10 mM magnesium acetate-10 mM sodium acetate (pH 4.5). Three volumes of 95% ethanol were added to each pool. After standing overnight at −20°, the tRNA was collected by a modification of Yang and Novelli's method (22): precipitates were collected on nitrocellulose filters (0.45 μM, Millipore Type HA), then washed with 5 ml of 95% cold ethanol, two 5-ml portions of cold 70% ethanol, and two 5-ml portions of 95% ethanol at room temperature (24°). Residual ethanol was removed by gentle suction. The filters were placed into 13 × 100-mm tubes and tRNA was eluted in 1 ml of H_2O by vortexing for about 1 min at room temperature.

‡ Arg-, Cys-, Gly-, Leu-, Ser-, and Trp-tRNAs were aminoacylated with the fraction listed, and the results are presented in Fig. 1. Conditions of aminoacylation in 50-μl reaction mixtures have been described (13). Reactions contained one [^{14}C], one [^{3}H], and 18 unlabeled amino acids.

§ Responses to [^{3}H]UGA were investigated with the fractions listed, and the results are presented in Fig. 2. Fractions were lyophilized and dissolved in 100 μl of H_2O before assay with the exception of fractions 231 through 240, which were assayed directly.

RESULTS

Aminoacyl-tRNA profiles

The elution profiles of six aminoacyl-tRNAs from calf liver on an RPC I column are shown in Fig. 1. The profiles were obtained after aminoacylation of the fractionated tRNA (Table 1). The six aminoacyl-tRNAs were selected as potential tRNAs that might recognize codon UGA, since substitution of a single base in the anticodon (1, 2) or elsewhere in the tRNA molecule (20), may give rise to a UGA-suppressor tRNA. Suppressors of codon UGA may be Arg-, Cys-, Gly-, Leu-, Ser-, and Trp-tRNAs. Similarly, Leu- and Ser-tRNAs may also contain suppressors of the nonsense codons UAG and UAA, and Trp-tRNA, a suppressor of the nonsense codon UAG.

[^{3}H]UGA responses

The response of [^{3}H]UGA to fractionated calf-liver tRNA at 30 mM Mg^{++} is shown in Fig. 2. Three major peaks of [^{3}H]-UGA were observed. These studies were performed, as described in Table 1, with the fractionated tRNA used for aminoacylation. Therefore, a correlation between [^{3}H]UGA responses (Fig. 2) and aminoacyl-tRNA profiles (Fig. 1) can be made. The two [^{3}H]UGA peaks occurring between fractions 100 and 170 (Fig. 2) correspond closely to the Cys-tRNA profile shown in Fig. 1. The [^{3}H]UGA peak, which is present in the fractions that eluted from the column in 1 M NaCl, corresponds to that of a Ser-tRNA (Fig. 1). A response of Cys-tRNA to codon UGA has been reported with both fractionated (9) and unfractionated (8) guinea-pig liver tRNA. A specific response of a mammalian seryl-tRNA, which elutes from the RPC I column in 1 M NaCl, to codon UGA has been reported (13).

To determine which tRNAs are responsible for the observed responses to [^{3}H]UGA, aliquots were taken from the fractions shown in Fig. 2 (designated I–V) and pooled (Table 2). $tRNA^{Arg}$, $tRNA^{Gly}$, $tRNA^{Leu}$, and $tRNA^{Trp}$ in Fraction I; $tRNA^{Arg}$, $tRNA^{Cys}$, $tRNA^{Gly}$, $tRNA^{Leu}$, and $tRNA^{Trp}$ in Fraction II; $tRNA^{Arg}$, $tRNA^{Cys}$, $tRNA^{Gly}$, and $tRNA^{Trp}$ in Fraction III; $tRNA^{Arg}$, $tRNA^{Gly}$, and $tRNA^{Trp}$ in Fraction IV; and $tRNA^{Ser}$ in Fraction V were acylated under optimal conditions with labeled amino acid, and responses of each to codons were examined (see below). The data shown in Fig. 1 demonstrate that portions of the Gly-, Leu-, Trp-, and Arg-tRNA profiles elute before the responses to [^{3}H]UGA shown in Fig. 2. Since the [^{3}H]UGA studies (Fig. 2) were performed with deacylated tRNA, and since acylated and deacylated tRNA are known to respond differently in the ribosome-binding assay with labeled trinucleoside diphosphate (14), it was necessary to examine

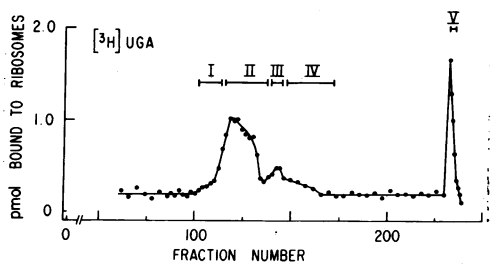

FIG. 2. [^{3}H]UGA Responses to fractionated calf-liver tRNA. Fractions were pooled, and the tRNA was precipitated and collected as described in Table 1. Assays were as described in refs. 14 and 15, with the exception that 50 mM Tris·acetate buffer (pH 7.3) was used. Reactions contained 2.1 A_{260} units of ribosomes, 0.148 A_{260} units of [^{3}H]UGA (1348 cpm/pmol at a counting efficiency of 30%), and 5 or 10 μl of the fractions given in Table 1. Most of the fractions used in [^{3}H]-UGA assays were lyophilized and dissolved in 100 μl of H_2O (see Table 1).

TABLE 2. *Responses of codons to fractionated calf-liver aminoacyl-tRNAs*

Aminoacyl-tRNA	Fraction*	10 mM Mg^{++} (Δ pmol)†						pmol added	A_{260} units added
		UGA	AGA	CGU	CGA	CGG	None‡		
[^{14}C]Arg-tRNA	A	0	0.02	0.02	0.06	0.87	(0.13)	4.97	0.097
[^{14}C]Arg-tRNA	I	−0.04	0.01	0.43	0.59	0.65	(0.19)	4.02	0.076
[^{14}C]Arg-tRNA	II	0	0.03	0.87	0.83	0.13	(0.21)	3.88	0.066
[^{14}C]Arg-tRNA	III	0.03	0.05	0.05	0.92	0.26	(0.19)	6.85	0.057
[^{14}C]Arg-tRNA	IV	0.05	0.07	0.04	0.21	0.54	(0.17)	3.91	0.054
		UGA	UGU	UGC	UGG	None‡			
[^{14}C]Cys-tRNA	II	0.01	0.10	0.19	0	(0)		6.68	0.395
[^{14}C]Cys-tRNA	III	0.02	0.09	0.12	—	(0.03)		6.22	0.496
		UGA	UCU	None‡					
[^{3}H]Ser-tRNA	V	0.078	0.046	(0.024)				2.480	0.044
		UGA	UAG	UGG	None‡				
[^{3}H]Trp-tRNA	A	0.004	0	0.027	(0.011)			0.905	0.094
[^{3}H]Trp-tRNA	I	0.005	0.003	0.041	(0.001)			2.770	0.082
[^{3}H]Trp-tRNA	II	0.004	0.003	0.036	(0.001)			0.850	0.123
[^{3}H]Trp-tRNA	III	0.001	0.001	0.014	(0.015)			1.320	0.247
[^{3}H]Trp-tRNA	IV	−0.007	−0.003	0.015	(0.006)			1.740	0.315
		20 mM Mg^{++} (Δ pmol)†							
		UGA	AGA	CGU	CGA	CGG	None‡		
[^{14}C]Arg-tRNA	A	−0.03	0.08	0.17	0.11	0.73	(0.47)	4.97	0.097
[^{14}C]Arg-tRNA	I	−0.08	0.05	0.68	1.27	0.86	(0.63)	4.02	0.076
[^{14}C]Arg-tRNA	II	0.01	0.06	1.21	1.50	0.35	(0.75)	3.88	0.066
[^{14}C]Arg-tRNA	III	0.34	0.46	0.29	1.79	1.03	(0.58)	6.85	0.057
[^{14}C]Arg-tRNA	IV	0.29	0.50	0.07	0.30	0.93	(0.68)	3.91	0.054
		UGA	UGU	UGC	UGG	None‡			
[^{14}C]Cys-tRNA	II	0.07	0.16	0.25	0.02	(0.04)		6.68	0.395
[^{14}C]Cys-tRNA	III	0.08	0.19	0.26	—	(0.12)		6.22	0.496
		UGA	UCU	None‡					
[^{3}H]Ser-tRNA	V	0.949	0.562	(0.308)				2.480	0.044
		UGA	UAG	UGG	None‡				
[^{3}H]Trp-tRNA	A	0.009	0.002	0.147	(0.015)			0.905	0.094
[^{3}H]Trp-tRNA	I	0.014	0	0.113	(0.005)			2.770	0.082
[^{3}H]Trp-tRNA	II	0.001	0.001	0.105	(0.009)			0.850	0.123
[^{3}H]Trp-tRNA	III	−0.005	−0.008	0.025	(0.031)			1.320	0.247
[^{3}H]Trp-tRNA	IV	−0.009	−0.006	0.020	(0.021)			1.740	0.315

the possibility that these earlier eluting species of $tRNA^{Arg}$, $tRNA^{Gly}$, $tRNA^{Leu}$, and $tRNA^{Trp}$ may recognize codon UGA only in the aminoacylated form. Therefore, $tRNA^{Arg}$, $tRNA^{Gly}$, $tRNA^{Leu}$, and $tRNA^{Trp}$ in Fraction A (see Table 2) were acylated with labeled amino acid and their responses to codons were studied (see below).

Responses of bovine-liver Ser-tRNA to codon UGA have been reported (13). Therefore, only the peak of Ser-tRNA that corresponded to the [^{3}H]UGA response (Fraction V, Fig. 2) was studied. $tRNA^{Cys}$ in Fractions I and IV was not aminoacylated for codon studies. A minor peak of $tRNA^{Cys}$, which elutes before the major peaks of $tRNA^{Cys}$, has been reported (ref. 8 and unpublished data). A very slight front-eluting shoulder of Cys-tRNA was observed (see Fig. 1) that may correspond to the reported minor peak. This shoulder was not present in sufficient quantities for codon assay.

Aminoacyl-tRNA responses to codons

The responses at concentrations of 10, 20, and 30 mM Mg^{++} of the labeled aminoacyl-tRNAs prepared from Fractions I–V (see Fig. 2) and from Fraction A to codons are shown in Table 2. [^{14}C]Arg-tRNA responded to codon UGA in fractions III and IV at concentrations of 10–30 mM Mg^{++}. This response corresponds to that of Arg-tRNA to the arginine codon AGA (21) in these fractions. Slight responses of Arg-tRNA to codon AGA were observed in Fractions I and II. Clearly, the response to codon UGA in Fractions III and IV does not correspond to that observed with codon CGA, which is very pronounced in Fraction III, but reduced in Fraction IV. Responses of Arg-tRNA to codon UGA were observed in Fractions I and II and in Fraction A, but only at a concentration of 30 mM Mg^{++} (see Table 2 and below). Responses to the arginine codons CGU and CGG (21) were also observed.

TABLE 2. (*Continued*)

Aminoacyl-tRNA	Fraction*	10 mM Mg^{++} (Δ pmol)†						pmol added	A_{260} units added
		30 mM Mg^{++} (Δ pmol)†							
		UGA	AGA	CGU	CGA	CGG	None‡		
[^{14}C]Arg-tRNA	A	0.04	0.11	0.32	0.25	0.59	(0.72)	4.97	0.097
[^{14}C]Arg-tRNA	I	0.04	0.10	0.83	1.08	0.84	(1.00)	4.02	0.076
[^{14}C]Arg-tRNA	II	0.13	0.10	1.08	1.35	0.33	(1.08)	3.88	0.066
[^{14}C]Arg-tRNA	III	0.63	0.70	0.18	1.62	1.32	(1.08)	6.85	0.057
[^{14}C]Arg-tRNA	IV	0.39	0.57	0.09	0.35	0.73	(1.14)	3.91	0.fi54
		UGA	UGU	UGC	UGG	None‡			
[^{14}C]Cys-tRNA	II	0.08	0.19	0.35	0.01	(0.13)		6.68	0.395
[^{14}C]Cys-tRNA	III	0.07	0.32	0.52	—	(0.27)		6.22	0.496
		UGA	UCU	None‡					
[^{3}H]Ser-tRNA	V	1.047	0.460	(0.925)				2.480	0.044
		UGA	UAG	UGG	None‡				
[^{3}H]Trp-tRNA	A	0.019	0.005	0.223	(0.018)			0.905	0.094
[^{3}H]Trp-tRNA	I	0.019	0.008	0.185	(0.020)			2.770	0.082
[^{3}H]Trp-tRNA	II	0.003	−0.002	0.130	(0.016)			0.850	0.123
[^{3}H]Trp-tRNA	III	0.004	0.005	0.033	(0.030)			1.320	0.247
[^{3}H]Trp-tRNA	IV	0.005	0	0.030	(0.018)			1.740	0.315

Responses of codons to fractionated calf-liver aminoacyl-tRNAs. Labeled aminoacyl-tRNAs were prepared under optimal conditions (13). After aminoacylation, protein was removed with phenol, and the reaction mixture was passed through a Sephadex G-25 column (13). Aminoacyl-tRNAs were then precipitated, collected, and washed as described in Table 1. Codon-assay conditions were the same as those of Nirenberg and Leder (15) except that 50 mM Tris·acetate buffer (pH 7.3) was used. Reaction mixtures contained 2.1 A_{260} units of *E. coli* MRE600 ribosomes and between 0.12 and 0.18 A_{260} units of trinucleoside diphosphate. Nonspecific interactions of labeled aminoacyl-tRNAs with Millipore filters were established in all codon assays with reactions that contained all components, except trinucleoside diphosphate and ribosomes. Therefore, all values given have subtracted from them that which bound nonspecifically to Millipore filters. Values obtained with amino acids of specific activities greater than 1.0 Ci/mmol were carried to three places, and less than 1.0 Ci/mmol to two places.

* Fractions A and I–V correspond to pooled aliquots of the fractions shown in Figs. 1 and 2 as follows: A = 79–99, I = 101–113, II = 115–137, III = 139–145, IV = 147–171, and V = 231–233.

† Amount of aminoacyl-tRNA bound to ribosomes in presence of codon minus the amount bound in absence of codon.

‡ Amount of aminoacyl-tRNA bound to ribosomes in absence of codon.

Responses of fractionated Arg-tRNA of guinea-pig liver to the arginine codons have been reported by Caskey *et al.* (9).

[^{14}C]Cys-tRNA in Fractions II and III responded to the cysteine codons UGU and UGC (21) and slightly to codon UGA (Table 2). Responses of fractionated Cys-tRNA of guinea-pig liver to the cysteine codons and to UGA have been reported by Caskey *et al.* (9).

[^{3}H]Ser-tRNA in Fraction V responded to codon UGA and to the serine codon UCU (21) (see Table 2). The pronounced response to codon UCU most certainly is due to the elution in 1 M NaCl of a portion of the Ser-tRNA peak, which elutes third from the column (see Fig. 1) and is known to respond to codon UCU (13). The Ser-tRNA, which recognizes codon UGA, responds specifically to this codon (13) and has recently been resolved into two species, which recognize codon UGA (unpublished data).

Slight responses of [^{3}H]Trp-tRNA to codon UGA were observed in fractions A, I, and II. Responses of [^{3}H]Trp-tRNA to the tryptophan codon UGG (21) in Fractions A and I–IV were observed (Table 2).

Although responses of Gly-tRNA to the glycine codons, GGU, GGC, and GGA, (21) and of Leu-tRNA to the leucine codons, UUG and UUA, (21) were observed, no responses of these aminoacyl-tRNAs were observed to codon UGA (data not shown).

[^{3}H]UGA responses to tRNA and aminoacyl-tRNA in Fractions A and I–IV

Responses of labeled aminoacyl-tRNAs to codon UGA were more pronounced in Fractions III and IV than in Fractions I and II (Table 2). Responses of [^{3}H]UGA, however, were more pronounced with the tRNA (see Fig. 2) that was pooled to give Fractions I and II than with that pooled to give Fractions III and IV. This discrepancy was investigated with [^{12}C]aminoacyl-tRNA and tRNA from Fractions I–IV, and with [^{3}H]UGA at a concentration of 30 mM Mg^{++}. The results demonstrated that at limiting tRNA concentrations, much more pronounced responses with tRNA and aminoacyl-tRNA from Fractions III and IV were observed than with those from Fractions I and II. However, [^{3}H]UGA responses were inhibited with increasing concentrations of tRNA, and

the inhibition was greater with tRNA and aminoacyl-tRNA from Fractions III and IV than with those from Fractions I and II.

The basis of the inhibition of [^{3}H]UGA recognition with increasing tRNA concentrations is not clear. However, one interpretation is that the tRNA used in [^{3}H]UGA-binding experiments shown in Fig. 2 is at such high concentrations that it may saturate ribosome-binding sites preventing codon attachment. The fact that greater [^{3}H]UGA inhibition was observed in fractions containing two species of tRNA that recognize this codon support this proposal.

The binding of [^{3}H]UGA to ribosomes was stimulated with limiting concentrations of [^{12}C]aminoacyl-tRNA, but not with tRNA, in Fraction A. These data are consistent with the data shown in Fig. 2 (where no response of [^{3}H]UGA to fractionated tRNA was observed) and in Table 2 (where a slight response of [^{14}C]Arg-tRNA and of [^{3}H]Trp-tRNA to codon UGA at a concentration of 30 mM Mg^{++} was observed).

DISCUSSION

Codon [^{3}H]UGA responded to fractionated calf-liver tRNA. Three peaks of [^{3}H]UGA recognition were observed. Acylation of the tRNA in each peak with labeled amino acids and determination of their codon responses demonstrated that a species of Arg-tRNA and a species of Ser-tRNA recognize codon UGA. Slight responses of Cys-tRNA and of Trp-RNA to this codon were also observed. Correlation of this data to that of the [^{3}H]UGA responses showed that one peak recognized $tRNA^{Cys}$ and possibly $tRNA^{Trp}$; a second peak, $tRNA^{Cys}$ and $tRNA^{Arg}$; and the third peak, $tRNA^{Ser}$. Therefore, of the six aminoacyl-tRNAs that are most likely to recognize codon UGA, the binding of four to *E. coli* ribosomes was stimulated by codon UGA. The Arg-tRNA, which recognizes codon UGA, apparently is the species that also recognizes codon AGA. It is interesting to note that Portugal (12) has reported that Lys-tRNA of chicken liver and chick embryo recognizes the nonsense codons UAA and UAG in addition to its own codons, AAA and AAG. Similarly, calf-liver Lys-tRNA recognizes UAA and UAG (unpublished data). Thus, it appears that, at least in calf liver, nonsense codons recognize aminoacyl-tRNAs whose codewords contain an adenosine in the 5′ position and contain the same two terminal bases as nonsense codons.

Fractionated Cys-tRNA of guinea-pig liver has been reported by Caskey *et al.* (9) to recognize codon UGA. The Ser-tRNA, which responds to codon UGA, has been reported to specifically recognize this codon (13) and has recently been resolved into two species of Ser-tRNA that recognize codon UGA (unpublished results).

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