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Absence of the Sequence G-T-ψ-C-G(A)- in Several Eukaryotic Cytoplasmic Initiator Transfer RNAs

(tritium fluorography/polynucleotide kinase labeling/fingerprinting)

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ABSTRACT **The nucleotide sequence G-T-ψ-C-G(A)- has previously been found in every tRNA of known sequence that is active in protein biosynthesis. An exception to this generalization is the recently sequenced initiator tRNA from yeast cytoplasm. It is now reported that cytoplasmic initiator tRNAs from wheat germ, rabbit liver, and sheep mammary gland also lack the G-T-ψ-C-G(A)- sequence. Thus: (*i*) nucleoside composition analyses show the absence of T in all these tRNAs; (*ii*) analyses of oligonucleotide fragments produced by T1 ribonuclease show the absence not only of the T-ψ-C-G(A)- sequence, but also of U-ψ-C-G(A)- or U-U-C-G(A)- sequences in such digests. The absence of G-T-ψ-C-G(A)- in the eukaryotic cytoplasmic initator tRNAs is, therefore, not simply due to lack of enzymatic modification of U to T.**

A specific methionine tRNA is used for the initiation of protein biosynthesis in both prokaryotes and eukaryotes. The initiator methionine tRNA is used either as methionyl-tRNA or as *N*-formylmethionyl-tRNA; this choice depends upon the source of the protein-synthesizing system. Bacteria, chloroplasts, and mitochondria use predominantly formylmethionyl-tRNA (fMet-tRNA^{fMet}), whereas the cytoplasmic protein-synthesizing system of eukaryotes uses methionyl-tRNA (Met-tRNA) without formylation (1). Because of this distinction in the form in which the initiator tRNA is used, we have investigated the structural similarities or differences between prokaryotic and eukaryotic initiator tRNAs.

A specific methionine tRNA (tRNA$_f^{Met}$) has been characterized as the cytoplasmic initiator tRNA of baker's yeast (2, 3). The nucleotide sequence of this tRNA was determined recently (4). It was found that this tRNA lacked the sequence G-T-ψ-C-G(A)-, which had previously been found in every tRNA of known sequence that is active in protein synthesis, including the *Escherichia coli* initiator tRNA. We now report that cytoplasmic initiator tRNA from wheat germ (5, 6) also lacks G-T-ψ-C-G(A)-. In addition, rabbit liver and sheep mammary gland cytoplasmic initiator tRNAs (7, 8) have been shown independently by Petrissant (30) to lack the above sequence, and here we confirm his results using totally different techniques. The absence of G-T-ψ-C-G(A)- is thus a feature common to all eukaryotic cytoplasmic initiator tRNAs.

Abbreviations: tRNA^{fMet}, formylmethionine tRNA; tRNA$_f^{Met}$, a species of cytoplasmic methionine tRNA from eukaryotes that can be formylated *in vitro* by bacterial extracts subsequent to aminoacylation; A′, C′, U′, G′, ψ′, T′, nucleoside trialcohol derivatives obtained by oxidation of the corresponding nucleosides with sodium metaperiodate and subsequent reduction with sodium borohydride.

MATERIALS AND METHODS

General. Yeast tRNA$_f^{Met}$ and *E. coli* tRNA$_{II}^{Tyr}$ were purified as described (2, 9). Initiator tRNA from wheat germ was a generous gift of Dr. H. P. Ghosh (6); rabbit liver and sheep mammary gland initiator tRNAs were kindly provided by Dr. G. Petrissant. Most of the enzymes used in this work were from described sources (4, 9). *E. coli* alkaline phosphatase, freed of nucleases by DEAE-cellulose chromatography, and polynucleotide kinase from bacteriophage T4 were provided by Dr. J. H. van de Sande. Yeast hexokinase was purchased from Boehringer Mannheim as a suspension in ammonium sulfate.

[γ-^{32}P]ATP was prepared by the method of Glynn and Chappell (10). [^{3}H]NaBH$_4$ (5 Ci/mmol) was purchased from New England Nuclear Corp. It was diluted with unlabeled NaBH$_4$ and stored as a 0.1 M solution in 0.1 N NaOH at a specific activity of 2 Ci/mmol at −90°.

Nonradioactive markers of U′, C′, A′, G′, T′, and ψ′ were prepared and purified (11); pψ was a gift of Dr. R. W. Chambers. The preparation of pAp, pCp, and pUp has been described (12, 13).

Analysis of Modified Nucleosides in tRNAs. The procedure used was similar to that described by Randerath and Randerath (14), except for the following: (*a*) a 25-fold molar excess of [^{3}H]NaBH$_4$ over sodium metaperiodate was used, (*b*) nonradioactive markers of A′, C′, U′, G′, T′, and ψ′ (0.1 A_{260} unit of each) were added to the reaction mixture before thin layer chromatography, and (*c*) a 5-cm long paper wick (Whatman no. 1) was used during chromatography in the first dimension. Solvent A (acetonitrite–ethyl acetate–*n*-butyl alcohol –isopropanol–6 N NH$_4$OH 7:2:1:1:2.7) was used in the first dimension and solvent B(*t*-amyl alcohol–methyl ethyl ketone–acetonitrile–ethyl acetate–water–formic acid [specific gravity 1.2] 4:2:1.5:2:1.5:0.18) for the second dimension (15).

About 25 nmol of nucleoside mixture obtained from hydrolysis of tRNA was used for the labeling reaction. Blank reactions in the absence of tRNA were also run at all times. The incorporation of ^{3}H into nucleoside derivatives was usually 5- to 10-times the blank value. For the two-dimensional mapping, 1–5 μCi of the [^{3}H]nucleoside trialcohols were used. The x-ray film was exposed for 2–3 days.

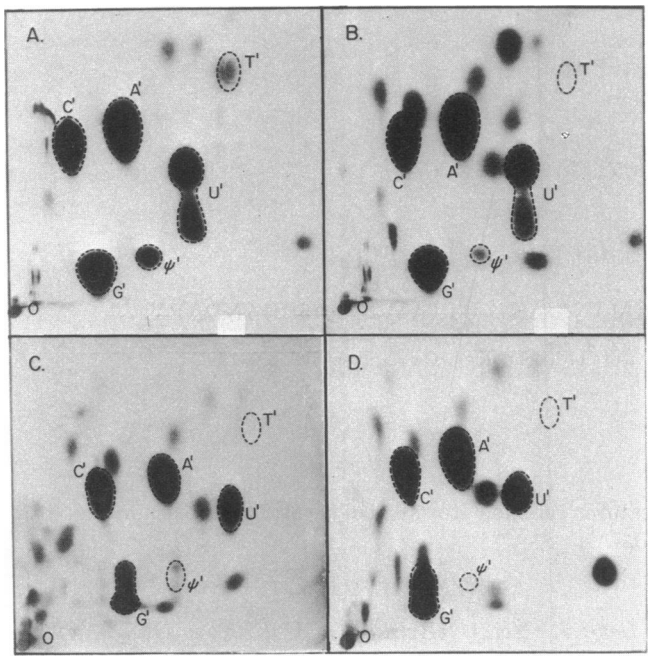

FIG. 1. Fluorograms of ^{3}H-labeled nucleoside trialcohols obtained from *A*, *E. coli* $tRNA_{II}^{Tyr}$; *B*, yeast $tRNA_f^{Met}$; *C*, wheat germ cytoplasmic initiator tRNA; and *D*, rabbit liver cytoplasmic initiator tRNA. *Dotted circles* identify the positions of A′, C′, U′, G′, T′, and ψ′ based on the mobilities of nonradioactive markers. The markers usually gave more compact spots than the fluorograms. Markers of U′ and G′ frequently gave two spots each, due to splitting in the chromatographic solvent used (14). *O*, origin.

5′-End Group Labeling of Fragments Produced by the Action of T1 RNase on tRNAs. The procedure involved is as follows: (*i*) Treatment of tRNA with T1 RNase and removal of 3′-phosphomonoester residues from the fragments with *E. coli* alkaline phosphatase. (*ii*) Inactivation of alkaline phosphatase by treatment with nitrilotriacetic acid. (*iii*) Phosphorylation of 5′-hydroxyl end-groups with [γ-^{32}P]ATP and polynucleotide kinase (16). (*iv*) Destruction of excess ATP by phosphorylation of glucose to glucose-6 phosphate in the presence of yeast hexokinase (17).

The incubation mixture (100 μl) contained 25 μg of tRNA, 50 mM Tris·HCl (pH 7.5), and 2.5 units of T1 RNase. After 3 hr at 37°, 5 units of purified *E. coli* alkaline phosphatase was added and the reaction mixture was further incubated for 2 hr at 37°. Nitrilotriacetic acid (40 mM at pH 7.0–7.5) was added, to a final concentration of 5 mM. After incubation for 20 min at room temperature, the mixture was heated at 100° for 2 min, then cooled. For the labeling of 5′-end groups, the reaction mixture (15 μl) contained 5 μl of the above digest, 2.5 nmol of [γ-^{32}P]ATP (10–15 Ci/mmol), 4 units of T4 polynucleotide kinase, and 10 mM $MgCl_2$–15 mM Tris·HCl (pH 8.0)–15 mM 2-mercaptoethanol. Incubation was at 37° for 30 min. To this mixture was added 30 nmol of glucose, 10 nmol of nonradioactive ATP, and 0.1 μg of yeast hexokinase. After 10 min at 37°, 5 nmol of ATP was added and incubation was continued for an additional 10 min. The reaction was terminated by heating at 100° for 2 min. 5-μl aliquots were used for two-dimensional electrophoresis (18).

5′-End Group Analyses of ^{32}P-Labeled Fragments. The radioactive spots were eluted with 2 M triethylammonium bicarbonate adjusted to pH 10 with triethylamine. The eluate was evaporated to dryness, then used for degradation with T2 RNase. The incubation mixture (50 μl) contained 1–2 pmol of ^{32}P-labeled fragment, 2 units of T2 RNase, and 20 mM ammonium acetate (pH 4.5). After 2 hr at 37°, the incubation mixtures were evaporated to dryness on parafilm and dissolved in 2–5 μl of a solution containing 0.1 A_{260} unit each of pAp, pCp, and pUp. The solutions were chromatographed on thin-layer cellulose plates in isobutyric acid–concentrated ammonia–water 66:1:33 for 5–6 hr. The nonradioactive markers, which were clearly separated, were located under a Mineralight UV lamp, while ^{32}P was detected by autoradiography.

RESULTS

Eukaryotic Cytoplasmic Initiator tRNAs Lack T; Except for Wheat Germ tRNA, They Also Lack ψ. A direct approach for demonstrating the absence of the sequence G-T-ψ-C-G(A)- in a tRNA would be to show the absence of T and ψ. In any analysis for these nucleosides, the following points are noteworthy: (*i*) The absence of T and ψ is evidence that the sequence G-T-ψ-C-G(A)- is absent as such in the tRNA. However, in contrast to T, which is present in a tRNA in a specific location (19), ψ may also be present elsewhere in the tRNA. Consequently, the presence of ψ in a tRNA does not necessarily signify the presence of the G-T-ψ-C-G(A)- or of G-U-ψ-C-G(A)-. (*ii*) The sequence G-T-ψ-C-G(A)- has been

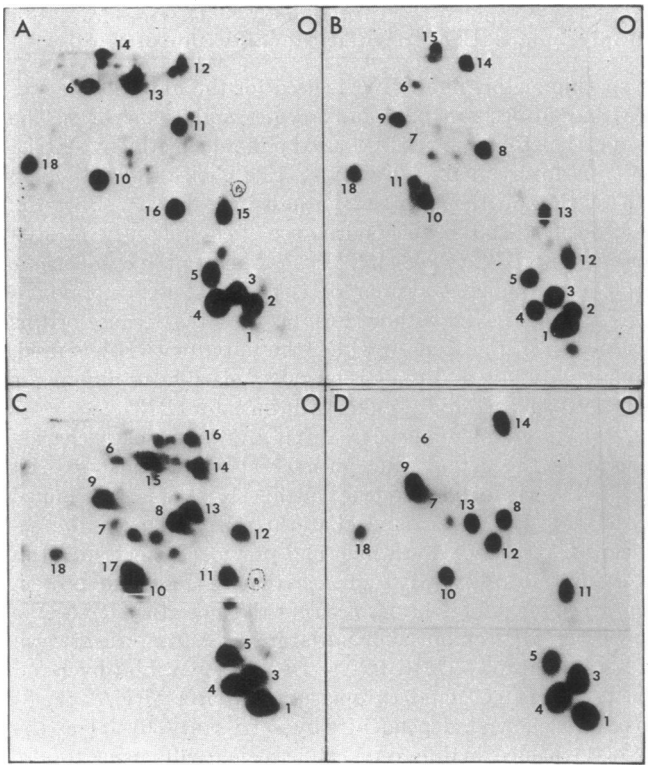

FIG. 2. Autoradiograms of 5′-^{32}P-labeled oligonucleotides obtained upon T1-RNase digestion of tRNAs. *A*, *E. coli* $tRNA_{II}^{Tyr}$; *B*, yeast $tRNA_f^{Met}$; *C*, wheat germ cytoplasmic initiator tRNA; and *D*, rabbit liver cytoplasmic initiator tRNA. Electrophoresis in the first dimension was on cellulose acetate strips for 90 min at 100 V/cm (*right* to *left*), and in the second dimension was on DEAE-cellulose paper for 15 hr at 7 V/cm (*top* to *bottom*). (B), position of blue dye marker; *O*, origin. Autoradiography was usually for 3–9 hr.

TABLE 1. *5'-End-group analysis and quantitation of the major radioactive spots in Fig. 2*

Spot no.	Identity	*E. coli* $tRNA_{II}^{Tyr}$ 5'-end	*E. coli* $tRNA_{II}^{Tyr}$ mol/mol tRNA	Yeast $tRNA_f^{Met}$ 5'-end	Yeast $tRNA_f^{Met}$ mol/mol tRNA	Wheat germ tRNA 5'-end	Wheat germ tRNA mol/mol tRNA	Rabbit liver tRNA 5'-end	Rabbit liver tRNA mol/mol tRNA
1	pC-G	C	0.12	C	5.3	C	3.3	C	3.5
2	pC-C-G	C	0.9	C	0.92	—	—	—	—
3	pC-A-G	C	0.85	C	1.44	C	1.26	C	2.3
4	pA-G	A	2.1	A	1.0	A	1.58	A	3.18
5	pA-A-G	A	0.9	A	0.9	A	1.32	A	1.16
6	pT-ψ-C-G	T	0.94	T	0.15	T+C	0.11	T	0.05
7	ATP		0.03		0.05	—	0.07		0.6
8	pA-U(U*)-C-G	—	—	A	1.12	A	1.00	A	0.93
9	pU-m^1G-m^2G	—	—	U	0.80	U	1.10	U	1.0
10	pU-G or pH_2U-G	U	1.0	H_2U	1.0	H_2U or U	1.0	H_2U or U	0.98
11	†	U	0.8	C	0.47	A	0.76	C	0.97
12	†	A	0.62	m^1A	0.73	m^1A	0.45	C	0.85
13	†	U+A	1.6	C	0.80	A	1.06	A	1.12
14	†	A	0.44	A	1.0	m^5C	0.73	C+m^1A	1.54
15	†	C	0.72	C	0.57	C	1.22	—	—
16	†	C	0.85	—	—	C	0.50	—	—
17	pψ-G	—	—	—	—	ψ	1.0	—	—
18	pG cyclic p	G	0.35	G	0.6	G	0.17	G	0.45

Quantitation of radioactive spots: The spots were excised and counted in a toluene-based scintillation solvent.

† In the numbering system used, several radioactive spots from the different autoradiograms have been assigned the same numbers (spots 11–16). This does not imply that the corresponding spots 11–16 in the different autoradiograms are identical. The identification of these spots is not complete. Their probably identity can, however, be inferred in case of *E. coli* $tRNA_{II}^{Tyr}$ and yeast $tRNA_f^{Met}$, whose total nucleotide sequences are known.

found in virtually every tRNA (19). Thus, unless the preparation of cytoplasmic initiator tRNAs used are completely homogeneous, contamination by any other tRNA will yield small amounts of T and ψ. A background level of 5–10% of T and slightly more of ψ if the contaminating tRNA contains several ψ residues (19) might, therefore, be expected in these analyses.

Fig. 1 shows the fluorograms of ^{3}H-labeled nucleoside trialcohols obtained from *E. coli* $tRNA_{II}^{Tyr}$ (used as a control), and yeast, wheat germ, and rabbit liver cytoplasmic initiator tRNAs. *E. coli* $tRNA_{II}^{Tyr}$, which contains the sequence G-T-ψ-C-G-, yielded—as expected—radioactive spots corresponding to added markers of T′ and ψ′ in addition to the four major nucleoside trialcohols (Fig. 1*A*). None of the eukaryotic cytoplasmic initiator tRNAs studied (Fig. 1*B*, *C*, and *D*)—including sheep mammary gland (not shown)—showed any radioactive spot corresponding to T′. Also, except for wheat germ initiator tRNA (Fig. 1*C*), no radioactive spot corresponding to ψ′ was seen above the expected background level of 5–20% due to contaminating tRNAs. We conclude that T is absent from all the eukaryotic cytoplasmic initiator tRNAs and that ψ is absent from all except the wheat germ tRNA. (Preliminary analysis indicates the presence of 1 mol of ψ per mol of wheat germ tRNA. It is shown below that this ψ is present in the sequence G-ψ-G-.)

The absence of T from all, and ψ from most, of these initiator tRNAs is not due to a general lack of modified nucleosides in these tRNAs. The eukaryotic initiator tRNAs contain several additional spots due to modified nucleosides as compared to those present in *E. coli* $tRNA_{II}^{Tyr}$ (Fig. 1). Most of these modified nucleosides have been identified by their position on the fingerprint (14) and by their presence in the nucleotide sequence of the yeast initiator tRNA (4).

G-T-ψ-C-G(A)- Is Not Replaced by G-U-U-C-G(A)- in Eukaryotic Cytoplasmic Initiator tRNAs. The above results do not exclude the possibility that the sequence G-T-ψ-C-G(A)- is replaced by G-U-U-C-G(A)-, and that the absence of T and ψ is a specific consequence of the lack of enzymatic modification of the U residues. If so, T1-RNase digestion of these tRNAs should produce either U-U-C-G- or a longer fragment U-U-C-A-. . . . .G-, which contains the sequence U-U-C at the 5′ end. As shown below, neither of these fragments is present in T1-RNase digests of these tRNAs.

tRNAs were hydrolyzed with T1 RNase, the 3′-terminal phosphomonoester groups were removed by incubation with *E. coli* alkaline phosphatase, and the 5′-hydroxyl group of each fragment was phosphorylated with ^{32}P by the use of [γ-^{32}P]ATP and T4 polynucleotide kinase. Fig. 2 shows the autoradiograms obtained from *E. coli* $tRNA_{II}^{Tyr}$ (used as a control), and yeast, wheat germ, and rabbit liver cytoplasmic initiator tRNAs. Table 1 lists the total radioactivity and the 5′-end group of each of the major radioactive spots. The results can be summarized as follows: (*i*) The autoradiogram of *E. coli* $tRNA_{II}^{Tyr}$ (Fig. 2*A*), spot 6, and the data in Table 1 show that T-ψ-C-G- when present in a tRNA is phosphorylated and recovered quantitatively. (*ii*) As expected from the known sequence of the yeast cytoplasmic initiator tRNA (4), the spot corresponding to ^{32}pT-ψ-C-G is almost totally absent in this tRNA (Fig. 2*B* and Table 1). (*iii*) None of the other eukaryotic cytoplasmic initiator tRNAs examined, those from wheat germ (Fig. 2*C*), rabbit liver (Fig. 2*D*), and

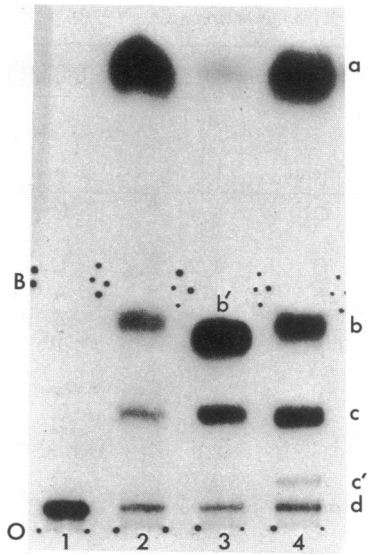

FIG. 3. Autoradiograms of partial digests with snake venom phosphodiesterase of spot 8 of Fig. 2 (*B–D*). The incubation mixture (50 μl) contained 40 mM Tris·HCl (pH 8.0), 4 pmol of ^{32}P-labeled oligonucleotide, and 0.5 μg of snake venom phosphodiesterase. Incubation was at room temperature for 4 min. A 25-μl aliquot was directly applied on DEAE-cellulose paper and subjected to electrophoresis at pH 3.5 for 15 hr at 7 V/cm. *1*, spot 8 of Fig. 2*B*, no enzyme added; *2*, spot 8 of Fig. 2*B*; *3*, spot 8 of Fig. 2*C*; and *4*, spot 8 of Fig. 2*D*. Band *c'* is a minor contaminant that is also present in unincubated samples and is resistant to snake venom phosphodiesterase. *B* and *O* indicate the positions of blue dye marker and origin, respectively.

sheep mammary gland (not shown), yielded any significant amounts of ^{32}pT-ψ-C-G (Table 1). (*iv*) The electrophoretic mobility of ^{32}pU-U-C-G is expected to be similar to that of ^{32}pT-ψ-C-G (M. Gefter, personal communication). The absence of any appreciable radioactive spot in the immediate vicinity of ^{32}pT-ψ-C-G in these autoradiograms (Fig. 2*B–D*) also shows that these tRNAs do not contain the sequence G-U-U-C-G-. (*v*) Under the electrophoretic conditions used, the migration on DEAE-cellulose paper of a fragment that contains the sequence ^{32}pU-U-C-A- . . . . .G would be negligible (18). Accordingly, radioactive spots lying at or near the origin of the DEAE-cellulose paper were analyzed for their 5'-end group. The data in Table 1 show that none of these possess a 5'-terminal U residue.

The Sequence G-A-U-C-G- or G-A-U-C-G- Is Present in All the Eukaryotic Cytoplasmic Initiator tRNAs Studied.* In the yeast cytoplasmic initiator tRNA, the sequence G-T-ψ-C-G(A)- is replaced by G-A-U-C-G- (4). From a comparison of the autoradiograms in Fig. 2*B–D* and those obtained from sheep mammary gland cytoplasmic initiator tRNA (not shown), it is noted that in addition to the common di- and trinucleotides (Table 1), another product, designated spot **8**, is present in an identical position in T1-RNase digests of all these tRNAs. This spot has been identified as ^{32}pA-U-C-G for the yeast, rabbit liver, and sheep mammary gland tRNAs, and as ^{32}pA-U*-C-G for the wheat germ tRNA:

(*i*) Upon thin layer chromatography along with appropriate markers of di-, tri-, tetra-, penta-, and hexanucleotides in *n*-propyl alcohol–NH_4OH–H_2O 55:10:35, the radioactive material migrated as a tetranucleotide.

(*ii*) End-group analysis showed that it contained ^{32}pA at the 5'-end (Table 1).

(*iii*) Electrophoresis of partial digests of spot **8** (Fig. 2) with snake venom phosphodiesterase yielded a series of radioactive spots that contained the 5'-end (20). The electrophoretic mobilities of the mono- (pA), di- (pA-U), tri- (pA-U-C), and tetra- (pA-U-C-G) nucleotides (Fig. 3, bands *a–d*, respectively) were identical for yeast, for rabbit liver, and for sheep mammary gland tRNAs. The pattern obtained from spot **8** of the wheat germ initiator tRNA differs from the other initiator tRNAs in that the dinucleotide (Fig. 3, band *b'*) has a lower mobility than that of ^{32}pA-U (band *b*); further studies have shown that it is also chromatographically distinct from ^{32}pA-U (not shown). Because this dinucleotide (Fig. 3, band *b'*) is also relatively resistant to further degradation with snake venom phosphodiesterase, we conclude that it has the sequence ^{32}pA-U* (U* indicates a derivative of U). Although the possibility that U* is ψ is not ruled out, we consider the possibility unlikely, since the wheat germ tRNA contains only 1 mol of ψ and this ψ residue is present in the sequence G-ψ-G- (see below). Further studies are necessary to clarify the nature of U*.

ψ Present in Wheat Germ Cytoplasmic Initiator tRNA Is in the Sequence G-ψ-G-. The fingerprints of T1-RNase digests of wheat germ initiator tRNA yielded a radioactive spot (spot **17**) absent from the other fingerprints (Fig. 2). This spot has been characterized as ^{32}pψ-G. (*i*) Its electrophoretic migration, immediately behind ^{32}pH_2U-G is consistent with that of ^{32}pψ-G. (*ii*) Digestion with T2 RNase (Table 1) yielded a radioactive nucleoside 3',5'-diphosphate that had a chromatographic mobility slower than pUp and identical to that expected for pψp. (*iii*) Digestion with snake venom phosphodiesterase produced ^{32}pψ. The ^{32}pψ thus obtained cochromatographed with an internal marker of pψ in the following three solvents: (*a*) isobutyric acid–NH_4OH–H_2O 66:1:33; (*b*) isopropanol–HCl–H_2O 680:176:144; and (*c*) isopropanol–5% ammonium formate (pH 3.7) 60:25.

DISCUSSION

The most important finding from our present work and that of Petrissant (30) is that every eukaryotic cytoplasmic initiator tRNA examined lacks the sequence of G-T-ψ-C-G(A)-. The previous observation (4) that yeast cytoplasmic initiator tRNA lacks the G-T-ψ-C-G(A)- sequence is thus extended to include several other eukaryotic initiator tRNAs. The cytoplasmic initiator tRNAs used in these studies have covered a wide spectrum of eukaryotic organisms and include fungal, plant, and mammalian tRNAs. It would thus appear that absence of the sequence G-T-ψ-C-G(A)- is a feature common to all eukaryotic cytoplasmic initiator tRNAs.

It is important to note that G-T-ψ-C-G(A)- is not simply replaced by G-U-ψ-C-G(A)- in the eukaryotic cytoplasmic initiator tRNAs. Consequently, the absence of G-T-ψ-C-G(A)- is not due to lack of enzymatic modification of U to T, as is the case with tRNAs from mycoplasma (21)‡. Preliminary results indicate that G-A-U-C-G(A)- or G-A-U*-C-G(A)- might replace G-T-ψ-C-G(A)- in these tRNAs. Thus, (*i*)

‡ Some purified tRNA species of rabbit liver also lack T (ref. 22, and H. J. Gross and M. Saneyoski, personal communication). Whether this absence of T is due to lack of modification of U to T in these tRNAs is not known.

In the only eukaryotic cytoplasmic initiator tRNA of known sequence (4), G-T-ψ-C-G- is replaced by G-A-U-C-G-. (*ii*) T1-RNase digestion of every eukaryotic cytoplasmic initiator tRNA yielded a tetranucleotide A-U-C-G- or A-U*-C-G-. Conclusive proof of the exact location of G-A-U-C-G- or G-A-U*-C-G- in these eukaryotic initiator tRNAs will require further sequence work. Such studies are in progress in collaboration with Drs. G. Petrissant and H. P. Ghosh.

Previously, the sequence G-T-ψ-C-G(A)- was found in every tRNA of known sequence, including the *E. coli* initiator tRNA (19). The single exception was the $tRNA^{Gly}$ of *Staph. epidermidis*, which is used for cell-wall biosynthesis and is inactive in protein synthesis (23). The finding that all the eukaryotic cytoplasmic initiator tRNAs lack G-T-ψ-C-G(A)- suggests that this feature may be related to the function of these tRNAs. The most likely possibility is that the absence of G-T-ψ-C-G-(A)- (or its replacement by another sequence) enables these tRNAs to initiate protein biosynthesis in eukaryotic cytoplasmic systems as methionyl-tRNA, but without the prerequisite of formylation. The additional possibility that eukaryotic cytoplasmic ribosomes and/or initiation factors also play an important role in this selection of nonformylated initiator tRNA cannot, however, be excluded.

An alternative possibility is that the absence of G-T-ψ-C-G(A)- in a tRNA prevents this tRNA from binding to the acceptor site on the ribosome. Such tRNAs would be unable to participate in polypeptide chain elongation and would not be able to transfer aminoacid into internal peptidic linkages. Although the yeast initiator tRNA is inactive in inserting methionine internally (3, 24), recent reports indicate, however, that both bacterial and other eukaryotic cytoplasmic initiator tRNAs, including the wheat germ initiator tRNA used in these experiments (25–28), are capable of inserting methionine *in vitro* into internal positions of a polypeptide chain.

Finally, the technique for the 5′-end-group labeling of fragments produced by T1 RNase has allowed a rapid screening of several tRNAs for the presence or absence of G-T-ψ-C-G(A)- in a process that requires no more than a few micrograms of the tRNA. Fingerprints of the radioactive fragments from *E. coli* $tRNA_{II}^{Tyr}$ (Fig. 2*A*) are consistent with those obtained from $tRNA_{II}^{Tyr}$ labeled *in vivo* with ^{32}P (29). Particularly encouraging was the observation that labeling was quantitative for most of the fragments, except for the very long ones (Table 1). The few additional minor spots observed in the fingerprint (Fig. 2*A*) probably arose from small amounts of other contaminating tRNAs. We hope that the technique will be of general use for comparing the fingerprints of different tRNAs and for establishing the extent of homology of nucleotide sequences between various related tRNAs. A potential further application of this technique would be for sequencing tRNAs or similar-sized fragments obtained from other RNAs, e.g., messenger RNAs, without requiring *in vivo* labeling of RNA with unstable isotopes.

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