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Replacement of the Sequence G-T-ψ-C-G(A)- by G-A-U-C-G- in Initiator Transfer RNA of Rabbit-Liver Cytoplasm

(eukaryotic initiator tRNA/fingerprinting/specific chemical cleavage/nucleotide sequence analysis)

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ABSTRACT **Eukaryotic cytoplasmic initiator tRNAs lack the sequence G-T-ψ-C-G(A)-, which is in every tRNA of known sequence that is active in protein biosynthesis. In initiator tRNA of yeast cytoplasm, which is the only eukaryotic initiator tRNA of known sequence, this sequence is replaced by G-A-U-C-G-. We now report the sequence of a 30-nucleotide-long, 3′-terminal fragment of cytoplasmic initiator tRNA of rabbit liver obtained by specific cleavage of the tRNA at the site occupied by the modified nucleoside, 7-methyl guanosine. We show (*i*) that in rabbit-liver initiator tRNA also, the sequence G-T-ψ-C-G(A)- is replaced by G-A-U-C-G- and (*ii*) that the sequences of loop IV of both the yeast and rabbit-liver cytoplasmic initiator tRNAs are identical, A-U-C-G-m¹-A-A-A-.**

Initiation of protein biosynthesis uses a specific methionine tRNA (1). Bacteria, chloroplasts, and mitochondria use formylmethionyl-tRNA, whereas the cytoplasmic protein-synthesizing system of eukaryotes uses methionyl-tRNA without the prerequisite of formylation. In an attempt to explain this variation in the use of initiator tRNAs, our work has aimed at a comparison of the nucleotide sequences of eukaryotic and prokaryotic initiator tRNAs (2, 3).

In initiator tRNA of yeast cytoplasm, the sequence G-T-ψ-C-G(A)-, which is present in all tRNAs that are active in protein biosynthesis, is absent and is replaced by G-A-U-C-G-. Subsequent studies (4–6) have demonstrated that the absence of G-T-ψ-C-G(A)- is a feature common to several cytoplasmic initiator tRNAs of eukaryotes. Since T1 RNase digests of several cytoplasmic initiator tRNAs of eukaryotes yielded a tetranucleotide A-U-C-G or A-U*-C-G-, it was suggested that replacement of G-T-ψ-C-G by G-A-U-C-G- or G-A-U*-C-G- may also be a feature common to all cytoplasmic initiator tRNAs of eukaryotes (5, 6). We now provide evidence for this possibility for cytoplasmic initiator tRNA of rabbit liver.

A method (7) for cleavage of a tRNA at the site occupied by the modified nucleoside m⁷G has been used to isolate a 30-nucleotide-long fragment, which contains the original 3′-end of the cytoplasmic initiator tRNA of rabbit liver. The nucleotide sequence of this fragment has been deduced. The entire sequences of loop IV (commonly called the T-ψ-C-loop) in yeast and rabbit-liver cytoplasmic initiator tRNAs are identical.

MATERIALS AND METHODS

General. Sources of tRNA, enzymes, and radioactive materials, and the techniques used were as described (5, 8). Specific activity of [³H]ATP was 20 Ci/mmol.

3′-End Group Labeling of tRNAs. The procedure involves (*i*) removal of a part of 3′-terminal C-C-A with snake-venom phosphodiesterase, (*ii*) removal of the enzyme, and (*iii*) repair of the 3′-terminal C-C-A in the presence of [¹²C]CTP and either [³H]ATP or [α-³²P]ATP by use of tRNA pyrophosphorylase present in crude preparations of aminoacyl-tRNA synthetases of *Escherichia coli* (9).

The incubation mixture (0.5 ml) contained 50 mM Tris·HCl (pH 8.0), 10 mM $MgCl_2$, cytoplasmic initiator tRNA of rabbit liver (10 A_{260} units), and snake-venom phosphodiesterase (25 μg). Incubation was at 24° for 15 min. The incubation mixture was applied to a column (0.5 × 7 cm) of DEAE-cellulose previously equilibrated with 50 mM Tris·HCl (pH 8.0)–0.3 M NaCl. The column was washed with 0.3 M NaCl in 50 mM Tris·HCl (pH 8.0) until all the snake-venom phosphodiesterase (assayed by hydrolysis of bis-*p*-nitrophenyl phosphate) was completely removed. This usually required 10–15 ml. tRNA was then eluted with 1 M NaCl in 50 mM Tris·HCl (pH 8.0) and 10 mM $MgCl_2$, dialyzed overnight against 8 mM Tris·HCl (pH 8.0)–1 mM $MgCl_2$ (4 liters) at 4°, and stored frozen at −20° until use.

The incubation mixture (200 μl) for repair of the C-C-A end contained 40 mM Tris·HCl (pH 8.0), 8 mM $MgCl_2$, 2 mM 2-mercaptoethanol, tRNA treated with snake-venom

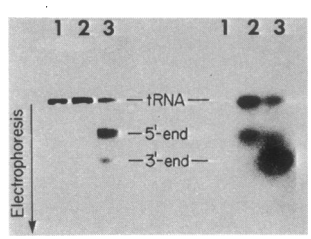

FIG. 1. Polyacrylamide-gel electrophoresis of [3′-³²P] tRNA of rabbit liver after cleavage at m⁷G. Electrophoresis in an E.C. apparatus was on 12% polyacrylamide-gel slabs (20 cm long) containing 5% bis acrylamide–6 M urea and was for 3 hr at 300 V. Running buffer contained 90 mM Tris·HCl–90 mM boric acid–4 mM EDTA at pH 8.5 (20). (*Left*) nucleotidic material detected by staining with methylene blue; (*right*) ³²P detected by autoradiography. *Slot 1*, nonradioactive tRNA; *slot 2*, [3′-³²P]tRNA after treatment with 0.05 N NaOH for 15 min; *slot 3*, as in *2* followed by cleavage in presence of aniline.

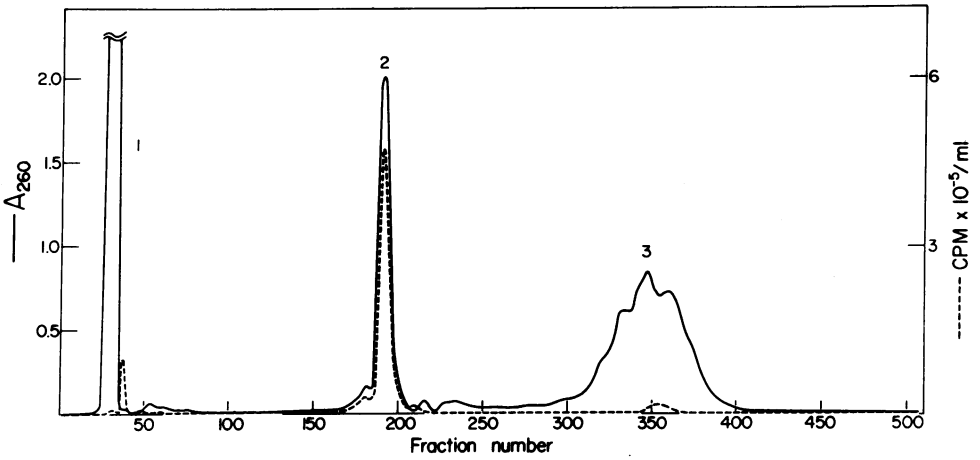

FIG. 2. Chromatography of the fragments obtained by cleavage of 3′-^{3}H-labeled initiator tRNA of rabbit-liver cytoplasm (150 A_{260} units) on a column (0.7 × 200 cm) of DEAE-cellulose (chloride form) in 7 M urea at pH 3.0. Elution was with a linear gradient (0.1–0.5 M, total volume 1600 ml) of NaCl in 7 M urea at pH 3.0. Fractions of 3 ml were collected every 15 min. Peak *1*, aniline; peak *2*, the 3′-terminal fragment; peak *3*, probably a mixture of related fragments from the 5′-end of the tRNA.

phosphodiesterase (2.4 A_{260} units), 135 μM [^{3}H]ATP or [α-^{32}P]ATP, 90 μM [^{12}C]CTP, and 320 μg of crude *E. coli* aminoacyl-tRNA synthetases. Incubation was at 37° for 30 min. Isolation of the tRNA labeled at the 3′- end that was freed of proteins by phenol extraction, and subsequent work-up, was as described (8). The labeled tRNA was separated from excess of radioactive ATP by chromatography on a column (1 × 10 cm) of superfine Sephadex G-50. Fractions containing tRNA were pooled, dialyzed against 0.1 mM EDTA (2 liters) at 4° for 4 hr, and stored frozen at −20° until use.

Cleavage of tRNA at the Site Occupied by m^{7}G. The procedure used is a modification of that described by Wintermeyer and Zachau (7) and results in almost quantitative cleavage of the phosphodiester bond at this site. The 3′-labeled tRNA (see above) was diluted 5-fold with nonradioactive cytoplasmic initiator tRNA of rabbit liver and dialyzed overnight against 0.1 mM EDTA (1 liter) at 4°. The tRNA (3 A_{260} units) was evaporated to dryness and dissolved in 50 mM NaOH (100 μl). After 15 min at room temperature (25°), the pH of the solution was adjusted to 4.5–5.0 by addition of 5 N acetic acid (about 2 μl). To the solution was added 0.3 N aniline hydrochloride (pH 4.5, 100 μl), and the mixture was then incubated at 37° or 50° for 4 hr.

For analysis of the cleavage reaction by gel electrophoresis, the reaction mixtures were diluted with water (2 ml) and applied to a column (0.7 × 5 cm) of DEAE–cellulose equilibrated with 50 mM triethylammonium bicarbonate buffer at pH 8.0. The column was thoroughly washed with the same buffer to remove aniline and chloride ions. Nucleotidic material was eluted with 2 M triethylammonium bicarbonate (pH 8.0). Fractions were pooled and evaporated to dryness; the evaporation was repeated a few times with addition of water until all the triethylammonium bicarbonate was completely removed. The residue was dissolved in water (10 μl) and used for gel electrophoresis (Fig. 1).

No difference in the pattern or extent of cleavage was observed whether incubation with aniline-hydrochloride was done at 37° or 50°. For large-scale reactions involving [3′-^{3}H]tRNA, incubation was, therefore, at 37°. The fragments produced were separated by direct application of the reaction mixture on columns of DEAE–cellulose (Fig. 2).

5′-End Group Labeling of Fragments Produced by the Action of Pancreatic RNase. This procedure was similar to that described (5) except for the use of pancreatic RNase instead of T1 RNase. Excess [γ-^{32}P]ATP was completely destroyed by avoiding the addition of nonradioactive ATP during the first step of incubation with yeast hexokinase (unpublished observation of J. Ziegenmeyer). The 5′-^{32}P-labeled fragments were then separated by two-dimensional electrophoresis (10).

Partial Digestion of 3′-Terminal Fragment of Cytoplasmic Initiator tRNA of Rabbit Liver with Pancreatic RNase. The incubation mixture (50 μl) contained 100 mM Tris·HCl (pH 7.5), 10 mM $MgCl_2$, the 3′-terminal fragment (peak *2* of Fig. 2; 1 A_{260} unit), and pancreatic RNase (0.25 μg). After 30 min at 0°, the incubation mixture was diluted with water (50 μl) and extracted with phenol (0.1 ml) equilibrated with 50 mM

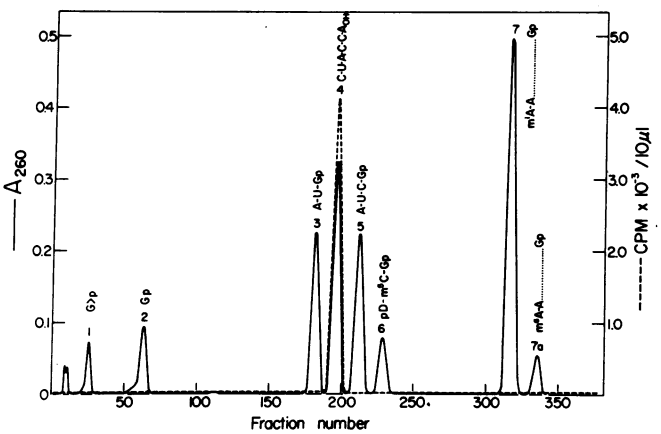

FIG. 3. Chromatography of a complete T1 RNase digest of material from peak *2* of Fig. 2. on a column (0.7 × 50 cm) of DEAE–cellulose in 7 M urea–20 mM Tris·HCl (pH 7.5). The 3′-terminal fragment (21 A_{260} units) was incubated with T1 RNase (24 units) in 50 mM Tris·HCl (pH 7.5) (0.64 ml) at 37° for 5 hr. The incubation mixture was made 7 M by addition of solid urea and then applied to the column. Elution was with a linear gradient (0–0.3 M, total volume 600 ml) of NaCl in 7 M urea–20 mM Tris·HCl (pH 7.5). Fractions of 2 ml were collected every 15 min.

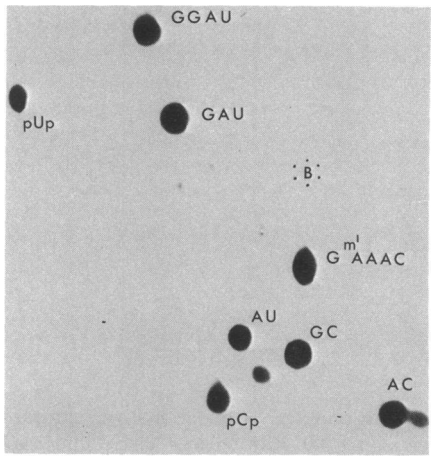

FIG. 4. Autoradiogram of [5′-^{32}P]oligonucleotides obtained from pancreatic RNase digestion of the 3′-terminal fragment (peak *2* of Fig. 2). Characterization of the oligonucleotides included (*i*) 5′-end group analyses and (*ii*) partial digestion with snake-venom phosphodiesterase and comparison of the pattern of digestion with the same oligonucleotides obtained from cytoplasmic initiator tRNA of yeast. *B*, blue dye marker.

Tris·HCl (pH 7.5). After separation of the layers by centrifugation, the aqueous layer was again extracted with phenol (0.1 ml). The combined phenol layers were then back-extracted with 0.1 ml of 50 mM Tris·HCl (pH 7.5). The aqueous layers were mixed, extracted several times with ether to remove phenol, and evaporated to dryness.

The residue was dissolved in water (50 μl), and small oligonucleotide fragments were removed by electrophoresis on DEAE–cellulose paper. Electrophoresis was in 7% formic acid and at 12 V/cm for 15 hr. Material remaining at the origin was eluted with 2 M triethylammonium bicarbonate (pH 9.8), evaporated to dryness, and used for labeling of 5′-end groups.

5′-End Group Labeling of Fragment Present in Partial Pancreatic RNase Digests. This procedure was as described (5)

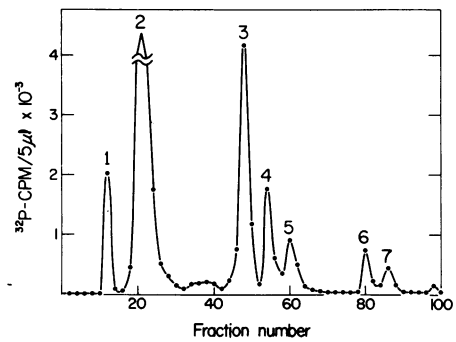

FIG. 5. Chromatography of fragments obtained by partial digestion of peak *2* of Fig. 2 with pancreatic RNase and subsequent labeling of 5′-end groups with ^{32}P on a column (0.7 × 23 cm) of DEAE–cellulose (chloride form) in 7 M urea (pH 3.0). Elution was with a linear gradient (0–0.4 M, total volume 300 ml) of NaCl in 7 M urea (pH 3.0). Fractions of 2.1 ml were collected every 10 min. Aliquots (5 μl) were counted for radioactivity. Peak *2*, glucose-6-phosphate.

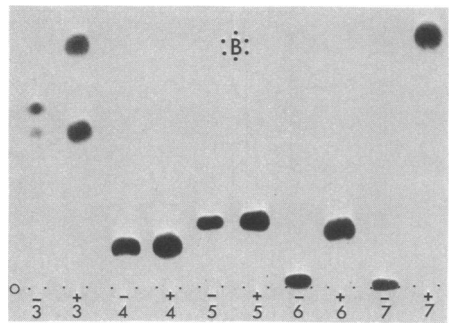

FIG. 6. Autoradiogram of DEAE–cellulose paper electrophoresis on peaks *3–7* of Fig. 6 before (−) and after (+) complete digestion with pancreatic RNase. The incubation mixture (10 μl) contained [5′-^{32}P]oligonucleotide (15,000 cpm), 50 mM Tris·HCl (pH 8.0), and pancreatic RNase (2.5 μg), when present. Incubation was in sealed capillary tubes at 37° for 5 hr. Electrophoresis was in 7% formic acid and at 9 V/cm for 15 hr. *B*, blue dye marker.

except that the incubation mixture contained only *E. coli* alkaline phosphatase and no T1 or pancreatic RNase. After destruction of excess [γ-^{32}P]ATP, the incubation mixture was made 7 M with respect to urea and then applied directly onto DEAE–cellulose.

RESULTS

Cleavage of Cytoplasmic Initiator tRNA of Rabbit Liver at m^{7}G and Isolation of Fragment Containing the 3′-Terminal End. The modified nucleoside m^{7}G, when present in a tRNA, is always located in a specific position as nucleoside number 31 from the 3′-end (11). Cleavage of a tRNA at the site occupied by m^{7}G is therefore useful for isolation of a 30-nucleotide-long fragment, which includes loop IV (normally containing the G-T-ψ-C- sequence) and extends onto the 3′-end of the tRNA (7). In the present work, the use of a tRNA that carried a radioactive label at the 3′-end allowed us to follow the progress of the cleavage reaction (Fig. 1, *right*) and further simplified the location of the fragment during purification by column chromatography.

The 3′-terminal fragment can be isolated pure in a single step of column chromatography. Fig. 2 shows the chromatographic profile obtained when the cleavage reaction was done on 3′-^{3}H-labeled cytoplasmic initiator tRNA and of rabbit-liver. The reaction went essentially to completion, since more than 80% of the total ^{3}H recovered from the column was present in a sharp peak (peak *2*), and in a position different from that of intact tRNA. The close correspondence of ultraviolet absorbance and radioactivity in this peak combined with the results of further analyses (see below) suggests that the material in peak *2* is the desired 3′-terminal fragment.

Complete Digestion of 3′-Terminal Fragment with T1 RNase. Fig. 3 shows the pattern obtained upon chromatography of a T1 RNase digest of the 3′-terminal fragment. The products are 1 mol of G-cyclic-p and Gp combined and 1 mol each of A-U-Gp, C-U-A-C-C-A$_{OH}$, A-U-C-Gp, pD-m^{5}C-Gp, and m^{1}A-A-A-C-C-A-U-C-C-U-C-U-Gp. (*i*) Since the sequence pD-m^{5}C-Gp contains a 5′-terminal phosphomonoester group, pD-m^{5}C-Gp (peak *6*) must represent the 5′-end of the fragment. (*ii*) Similarly, since C-U-A-C-C-A$_{OH}$ is the only fragment that does not contain a 3′-terminal Gp and since it contains all the ^{3}H present originally at the 3′-end of the tRNA,

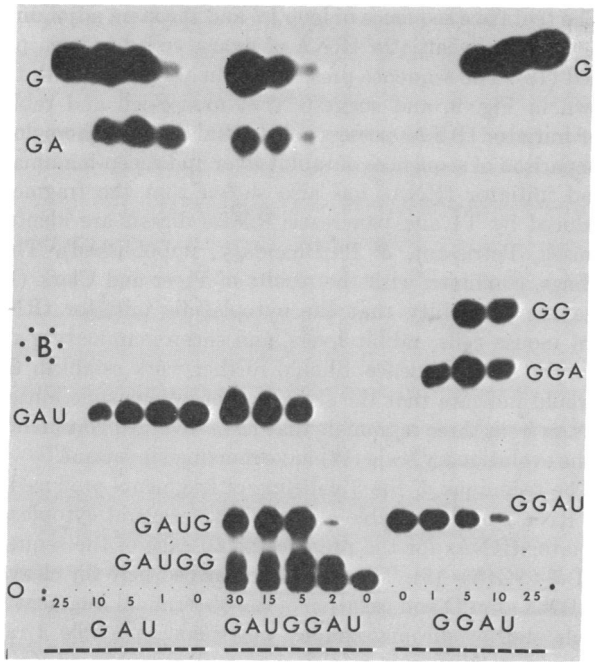

FIG. 7. Autoradiograms of partial digests by snake-venom phosphodiesterase on peak *6* of Fig. 6 (5). Also included are similar digests on [*5'*-^{32}P]G-G-A-U and [*5'*-^{32}P]G-A-U, which provide the necessary markers. Electrophoresis was on DEAE-cellulose paper (57 cm long) in 7% formic acid and was at 9 V/cm for 15 hr. *B*, blue dye marker. *Numbers* represent minutes of incubation with snake-venom phosphodiesterase.

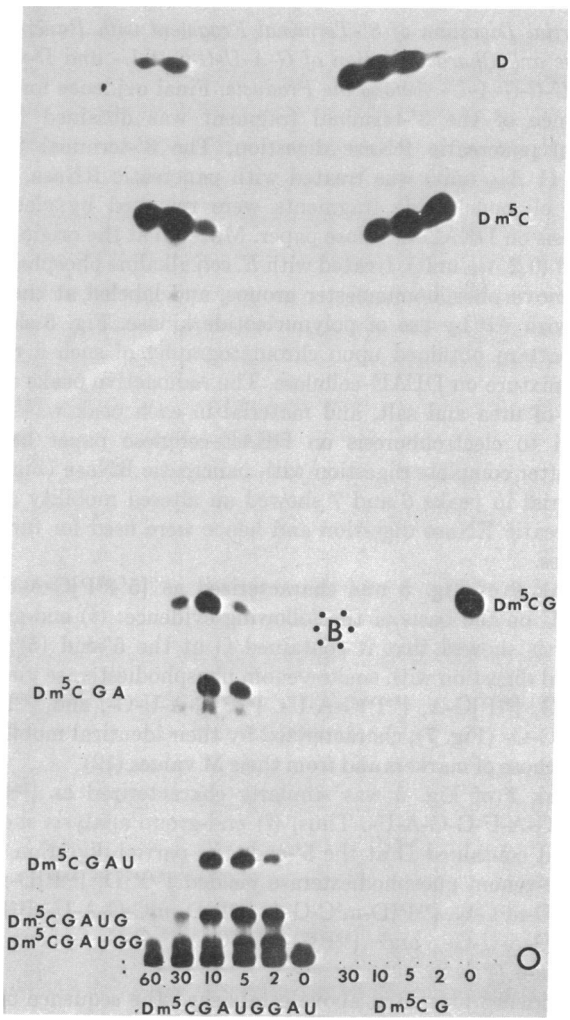

FIG. 8. Autoradiograms of partial digests by snake-venom phosphodiesterase on peak *7* of Fig. 6 (5). Also included is a similar digest on [*5'*-^{32}P]D-m^{5}C-G, which provides the necessary markers. Electrophoresis was on DEAE-cellulose paper (85 cm long) in 7% formic acid and was at 13 V/cm for 15 hr. *B*, blue dye marker. *Numbers* represent minutes of incubation with snake-venom phosphodiesterase.

C-U-A-C-C-A_{OH} (peak *4*) must comprise the 3'-end of the fragment. (*iii*) The modified nucleoside m^{1}A, when present in a tRNA, is usually located as the nineteenth nucleoside from the 3'-end (11). This finding suggests that the sequence m^{1}A-A-A-C-C-A-U-C-C-U-C-U-Gp (peak *7*) precedes C-U-A-C-C-A_{OH}, and this result is confirmed by analysis of products of partial digestion with T1 RNase (not shown). The partial sequence of the 3'-terminal fragment can, therefore, be represented as pD-m^{5}C-Gp(A-U-Gp, Gp, A-U-C-Gp)m^{1}A-A-A-C-C-A-U-C-C-U-C-U-G-C-U-A-C-C-A_{OH}. These possible sequences are shown in Table 1.

Complete Digestion of 3'-Terminal Fragment with Pancreatic RNase and Fingerprinting of the Products. Further support for the above partial sequence is obtained by complete digestion with pancreatic RNase. The oligonucleotides produced were labeled with ^{32}P at the 5'-end and then separated by two-dimensional electrophoresis. Fig. 4 shows the autoradiogram obtained and the oligonucleotides present therein. The characterization of [*5'*-^{32}P]G-m^{1}A-A-A-C in these digests suggests that this oligonucleotide is preceded by a pyrimidine and hence rules out sequences (*e*) and (*f*) of Table 1.

TABLE 1. *Partial sequence of 3'-terminal fragment of cytoplasmic initiator tRNA of rabbit liver*

(*a*)	pD-m^{5}C-G-A-U-G-G-A-U-C-G-m^{1}A-A-A-C-C-A-U-C-C-U-C-U-G-C-U-A-C-C-A_{OH}
(*b*)	-A-U-C-G-G-A-U-G-
(*c*)	-G-A-U-G-A-U-C-G-
(*d*)	-G-A-U-C-G-A-U-G-
(*e*)	-A-U-G-A-U-C-G-G-
(*f*)	-A-U-C-G-A-U-G-G-

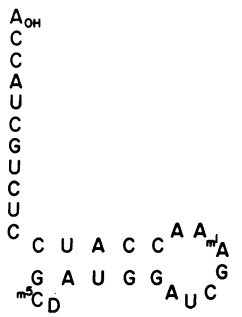

FIG. 9. Nucleotide sequence of 3'-terminal fragment obtained by cleavage of cytoplasmic initiator tRNA of rabbit liver at m^{7}G.

Partial Digestion of 3'-Terminal Fragment with Pancreatic RNase and Characterization of G-A-U-G-G-A-U- and D-m⁵C-G-A-U-G-G-A-U- Among the Products. Final evidence for the sequence of the 3'-terminal fragment was obtained from partial pancreatic RNase digestion. The 3'-terminal fragment (1 A_{260} unit) was treated with pancreatic RNase, and small oligonucleotide fragments were removed by electrophoresis on DEAE–cellulose paper. Material at the origin was eluted (0.2 A_{260} unit), treated with *E. coli* alkaline phosphatase to remove phosphomonoester groups, and labeled at the 5'-end with ^{32}P by use of polynucleotide kinase. Fig. 5 shows the pattern obtained upon chromatography of such a reaction mixture on DEAE-cellulose. The radioactive peaks were freed of urea and salt, and material in each peak was subjected to electrophoresis on DEAE–cellulose paper before and after complete digestion with pancreatic RNase (Fig. 6). Material in peaks *6* and *7* showed an altered mobility after pancreatic RNase digestion and hence were used for further studies.

Peak *6* of Fig. 5 was characterized as [5'-^{32}P]G-A-U-G-G-A-U on the basis of the following evidence: (*i*) end-group analysis showed that it contained G at the 5'-end (5); (*ii*) partial digestion with snake-venom phosphodiesterase yielded [^{32}P]G, [^{32}P]G-A, [^{32}P]G-A-U-, [^{32}P]G-A-U-G-, and [^{32}P]G-A-U-G-G- (Fig. 7), characterized by their identical mobilities with those of markers and from their M values (12).

Peak *7* of Fig. 5 was similarly characterized as [^{32}P]D-m⁵C-G-A-U-G-G-A-U-. Thus, (*i*) end-group analysis showed that it contained D at the 5'-end; (*ii*) partial digestion with snake-venom phosphodiesterase yielded [^{32}P]D, [^{32}P]D-m⁵C, [^{32}P]D-m⁵C-G, [^{32}P]D-m⁵C-G-A, [^{32}P]D-m⁵C-G-A-U-, [^{32}P]D-m⁵C-G-A-U-G-, and [^{32}P]D-m⁵C-G-A-U-G-G- among the products (Fig. 8).

Evidence presented above established the sequence of the 3'-terminal fragment as shown in Table 1 (*a*). Fig. 9 shows the same sequence as part of a cloverleaf structure.

DISCUSSION

The most significant result from this work is that the sequence A-U-C-G-m¹A-A-A- in loop IV of rabbit-liver and yeast cytoplasmic initiator tRNA is identical. Thus, the two unique structural features of yeast cytoplasmic initiator tRNA (3) are also present in the rabbit-liver tRNA (Fig. 9). These are (*i*) replacement of T-ψ-C-G(A)- present in all tRNAs by A-U-C-G- and (*ii*) presence of A as the last nucleoside of this loop instead of a pyrimidine nucleoside as in all other tRNAs (11). Work on two other eukaryotic cytoplasmic initiator tRNAs, from wheat germ and sheep-mammary gland (unpublished), further suggests that these structural features may be common to all eukaryotic cytoplasmic initiator tRNAs.

The above findings support our previous suggestion (3, 5, 6) that these unique structural features may be related to the function of these tRNAs. Although the possibility that these features prevent eukaryotic initiator tRNAs from inserting methionine into internal peptide linkages is not ruled out, a more likely possibility is that they are required for the role of these tRNAs as initiators in protein-synthesizing systems of eukaryotic cytoplasm. Whether these unique features enable these tRNAs to (*i*) bind to eukaryotic initiation factors (13–15), or (*ii*) bind to the 40S ribosomal subunit in the absence of mRNA (16), or (*iii*) initiate protein synthesis as methionyl-tRNA without formylation (17), however, remains to be established.

The tentative sequence of loop IV and the stem adjoining it for cytoplasmic initiator tRNA of mouse cells has been published (18). The sequence presented is in agreement with that shown in Fig. 9, and suggests that mouse-cell and rabbit-liver initiator tRNAs possess substantial sequence homology. Comparison of sequences of rabbit-liver and sheep-mammary-gland initiator tRNAs has also shown that the fragments produced by T1 and pancreatic RNase digests are identical (Simsek, Petrissant, & RajBhandary, unpublished). These findings, combined with the results of Piper and Clark (18), raise the possibility that the cytoplasmic initiator tRNAs from mouse cells, rabbit livers, and sheep mammary glands have the same sequence. Should further work establish this, it would indicate that the sequences of cytoplasmic initiator tRNAs from three mammals that have diverged considerably in the evolutionary scale (19) have remained invariant.

The technique of the 5'-labeling of fragments produced by T1 RNase made possible a screening of several cytoplasmic initiator tRNAs for the presence or absence of the sequence G-T-ψ-C-G(A)- (5). The method described here for cleavage of tRNA at m⁷G and isolation of the 3'-terminal fragment in a single step of chromatography now makes possible a rapid analysis of the sequence that has replaced G-T-ψ-C-G(A)-.

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1. Lucas-Lenard, J. & Lipmann, F. (1971) *Annu. Rev. Biochem.* **40,** 409–448.
2. Dube, S. K., Marcker, K. A., Clark, B. F. C. & Cory, S. (1968) *Nature* **218,** 232–233.
3. Simsek, M. & RajBhandary, U. L. (1972) *Biochem. Biophys. Res. Commun.* **49,** 508–515.
4. RajBhandary, U. L., Simsek, M., Ziegenmeyer, J., Heckman, J., Petrissant, G. & Ghosh, H. P. (1973) *Fed. Proc. Abstr.* **32,** No. 3, 585.
5. Simsek, M., Ziegenmeyer, J., Heckman, J. & RajBhandary, U. L. (1973) *Proc. Nat. Acad. Sci. USA,* **70,** 1041–1045.
6. Petrissant, G. (1973) *Proc. Nat. Acad. Sci. USA* **70,** 1046–1049.
7. Wintermeyer, W. & Zachau, H. G. (1970) *FEBS. Lett.* **11,** 160–164.
8. Walker, R. T. & RajBhandary, U. L. (1972) *J. Biol. Chem.* **247,** 4879–4892.
9. Muench, K. & Berg, P. (1966) *Proc. Nucleic Acid Res.* **1,** 375–383.
10. Sanger, F., Brownlee, G. G. & Barrell, B. G. (1965) *J. Mol. Biol.* **13,** 373–398.
11. Dirheimer, G., Ebel, J. P., Bonnet, J., Gangloff, J., Keith, G., Krebs, B., Kuntzel, B., Roy, A., Weissenbach, J. & Werner, C. (1972) *Biochimie* **54,** 127–144.
12. Barrell, B. G. (1971) *Proc. Nucleic Acid Res.* **2,** 751–779.
13. Chen, Y. C., Woodley, C. L., Bose, K. K. & Gupta, N. K. (1972) *Biochem. Biophys. Res. Commun.* **48,** 1–9.
14. Levin, D. H., Kyner, D. & Acs, G. (1973) *Proc. Nat. Acad. Sci. USA* **70,** 41–45.
15. Zasloff, M. & Ochoa, S. (1972) *Proc. Nat. Acad. Sci. USA* **69,** 1796–1799.
16. Darnbrough, C., Hunt, T. & Jackson, R. J. (1972) *Biochem. Biophys. Res. Commun.* **48,** 1556–1562.
17. Housman, D., Jacobs-Lorena, M., RajBhandary, U. L. & Lodish, H. F. (1970) *Nature* **227,** 913–918.
18. Piper, P. W. & Clark, B. F. C. (1973) *FEBS. Lett.* **30,** 265–267.
19. Dayhoff, M. O. & Eck, R. V. (1968) *Atlas of Protein Sequence and Structure,* edition 1, 21.
20. Philipps, G. R. (1971) *Anal. Biochem.* **44,** 345–357.