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Natural UAG suppressor glutamine tRNA is elevated in mouse cells infected with Moloney murine leukemia virus

YOSHIYUKI KUCHINO*, HILDBURG BEIER†, NORIKO AKITA*, AND SUSUMU NISHIMURA*

*Biology Division, National Cancer Center Research Institute, Chuo-ku, Tokyo 104, Japan; and †Institut für Biochemie, Bayerische Julius-Maximilian-Universität, Röntgenring 11, D-8700 Würzburg, Federal Republic of Germany

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ABSTRACT **Two species of glutamine tRNA were isolated from mouse liver and their nucleotide sequences were determined. The minor glutamine tRNA(tRNA$^{Gln}_{UmUG}$) that possesses UmUG (where Um stands for 2′-*O*-methyluridine) as the anticodon sequence was found to have suppressor activity for the UAG termination codon of tobacco mosaic virus RNA in a rabbit reticulocyte *in vitro* translation system. The amount of this suppressor glutamine tRNA in mouse liver was 1–2% of the amount of the major glutamine tRNA(tRNA$^{Gln}_{CUG}$) that has the CUG anticodon sequence, but it was markedly increased in NIH 3T3 cells infected with Moloney murine leukemia virus and in Ehrlich ascites cells. These results support the hypothesis that tRNA$^{Gln}_{UmUG}$ actually functions *in vivo* as a suppressor tRNA that recognizes the UAG termination codon located at the *gag-pol* gene junction of Moloney murine leukemia virus and results in the synthesis of the virus-encoded protease.**

We have reported that *Tetrahymena*, a ciliated protozoan, contains two glutamine tRNAs that have CUA and UmUA (where Um stands for 2′-*O*-methyluridine) as their anticodons (1, 2). These two glutamine tRNAs, tRNA$^{Gln}_{CUA}$ and tRNA$^{Gln}_{UmUA}$, were shown to recognize UAG and either UAG or UAA codons, respectively, in a rabbit reticulocyte, cell-free, protein synthesizing system (2). These results, as well as data on the nucleotide sequences of several ciliate structural genes including *Tetrahymena* genes, strongly suggested that ciliates use UAG and UAA as glutamine codons, but not as termination codons (3–6). This raised the question of whether this kind of deviation from the universality of the genetic code also occurs in other organisms.

Pure *et al.* (7) reported that yeast glutamine tRNA produced *in vivo* by transfection of multiple copies of the corresponding tRNA genes with a TTG anticodon sequence weakly suppresses the UAA termination codon.

In addition, Yoshinaka *et al.* (8) showed that a viral protease, coded by Moloney murine leukemia virus (Mo-MuLV), is a read-through product of the UAG termination codon between the *gag* and *pol* genes, with glutamine inserted at the site of the termination codon. These data suggest that glutamine suppressor tRNAs might also be present in other organisms that use the normal genetic code, as a sign of some shared ancestral trait, although, in ciliates, the use of UAG and UAA as glutamine codons may have been a strongly pronounced feature throughout their evolution.

This hypothesis prompted us to attempt to isolate natural glutamine suppressor tRNA from other higher eukaryotes, especially from mammalian tissue such as mouse liver. We report here the isolation and nucleotide sequence analysis of a glutamine suppressor tRNA that can recognize the UAG termination codon of tobacco mosaic virus (TMV) RNA in an *in vitro* protein biosynthesis system. We also report that the amount of this natural glutamine suppressor tRNA, which exists as a minor species of glutamine tRNA in normal mouse cells, is markedly increased in Mo-MuLV-infected mouse cells as well as in Ehrlich ascites cells.

MATERIALS AND METHODS

The mouse cell line NIH 3T3 and Mo-MuLV-infected NIH 3T3 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% (vol/vol) calf serum. The mouse ascites cells used were Ehrlich ascites, mouse sarcoma S180A, and mouse leukemia L1210, which were continuously transplanted i.p. about once a week in BDF-1 female mice, ddY female mice, and BDF-1 female mice, respectively. BDF-1 female mice were used for preparation of regenerating liver.

Unfractionated tRNAs from various mouse cells were prepared by extraction with phenol in 20 mM Tris·HCl buffer (pH 7.5) containing 1 M NaCl, 1 mM Na_2EDTA, 10 mM $MgCl_2$, and 0.3% $NaDodSO_4$. Fractionation of bulk tRNAs by BD-cellulose column chromatography was carried out with a linear gradient of 0.3–1.2 M NaCl in 0.02 M sodium acetate (pH 6.0) unless otherwise specified. The procedures used for RPC-5 column chromatography and polyacrylamide gel electrophoresis for the further purification of tRNA, the nucleotide sequence analysis of purified tRNA with 0.1–1.0 μg by post-labeling techniques were described (9, 10).

Crude aminoacyl tRNA ligase (formerly synthetase) from Ehrlich ascites cells was prepared by a modification of a method described (11). The ascites cells were washed with cold 0.9% NaCl and centrifuged for 10 min at 750 rpm (100 × *g*) to remove erythrocytes. Then they were washed with 0.09% NaCl, suspended in three volumes of 1 mM Hepes buffer (pH 7.5) containing 0.025 M sucrose, 0.5 mM magnesium acetate, and 0.6 mM 2-mercaptoethanol, and incubated in an ice bath for 15 min. The suspension was subsequently homogenized in an Emmanuel–Chaikoff homogenizer. The homogenate was promptly mixed with an equal volume of 0.04 M Hepes buffer, pH 7.5/1 M sucrose/0.02 M magnesium acetate/0.024 M 2-mercaptoethanol and centrifuged successively at 18,000 × *g* for 10 min and at 105,000 × *g* for 1 hr. The ligase in the supernatant was precipitated by addition of ammonium sulfate (0.516 g/ml); the precipitate was collected, dissolved in a minimum volume of 10 mM Hepes buffer (pH 7.5), 5 mM magnesium acetate, 6 mM 2-mercaptoethanol, and 10% (vol/vol) glycerol, and dialyzed overnight against the same buffer. After centrifugation at 18,000 × *g* for 10 min to remove insoluble material, the enzyme solution was mixed with an equal volume of glycerol and stored at −20°C. Glutamine acceptance of mouse tRNA was assayed in reaction mixture (100 μl) containing 50 mM Hepes buffer (pH 7.5), 3 mM magnesium acetate, 2 mM ATP, 10 mM potassium acetate, 1 mM dithiothreitol, 0.1 μCi of [^{14}C]glutamine

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Abbreviations: TMV, tobacco mosaic virus; Mo-MuLV, Moloney murine leukemia virus; Um, 2′-*O*-methyluridine; ψ, pseudouridine.

(specific activity, 250 mCi/mmol; 1 Ci = 37 GBq), and appropriate amounts of aminoacyl tRNA ligase and tRNA. After incubation at 37°C for 15 min, the acid-insoluble radioactivity was measured as described (12).

In vitro translation experiments in a nuclease-treated reticulocyte lysate were performed by a reported method except that lysate not supplemented with calf liver tRNA was used (13, 14). Proteins synthesized in the *in vitro* translation system were analyzed by gel electrophoresis in 8% NaDodSO$_4$/polyacrylamide slab gels as described (15).

RESULTS

For isolation of natural glutamine suppressor tRNA from mouse liver, unfractionated BDF-1 mouse liver tRNA (8000 A_{260} units) was first subjected to column chromatography on BD-cellulose, as shown in Fig. 1. Three peaks of glutamine acceptor activity were obtained, which were designated as fractions I, II, and III in the order of their elution from the column. These fractions were collected separately, and their read-through ability on UAG and UAA termination codons were examined in rabbit reticulocyte, cell-free, protein synthesizing systems using TMV RNA and globin mRNA, respectively (2, 14). As shown in Fig. 2*A*, tRNA from fraction II or fraction III, but not from fraction I produced a substantial amount of a 183-kDa read-through protein, which is the product of TMV RNA-UAG readthrough. However, none of the three tRNA fractions had UAA suppressor ability in the translation of globin mRNA (data not shown).

For identification of the tRNA species responsible for UAG suppressor activity, each tRNA fraction was further fractionated by column chromatography on RPC-5 and then by polyacrylamide gel electrophoresis, to isolate pure species of glutamine tRNA (10). During this purification, the material with glutamine acceptor activity in fraction II was separated into two species. The glutamine tRNA species that constituted the minor fraction of the glutamine acceptor activity was found to recognize the UAG termination codon, whereas the major species from fraction II had no UAG suppressor activity. The nucleotide sequences of all the purified glutamine tRNAs were determined by the post-labeling technique (10). Some of the purified glutamine tRNA species were found to be identical except for the different modifications described below. Thus, the nucleotide sequence data indicated that mouse liver contains two species of glutamine tRNA (major $tRNA^{Gln}_{CUG}$ and minor $tRNA^{Gln}_{UmUG}$, designated according to their anticodon sequences). The amount of

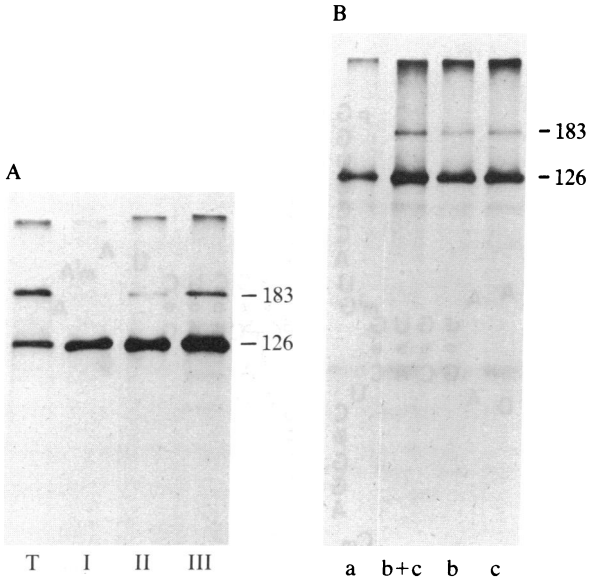

FIG. 2. (*A*) Fluorogram of [S^{35}]methionine-labeled proteins on 8% NaDodSO$_4$/polyacrylamide gel. Translation *in vitro* of TMV RNA using a rabbit reticulocyte lysate was carried out in the presence of tRNA (50 μg/ml) from the fraction specified. Lanes: T, tobacco $tRNA^{Tyr}_{G\psi A}$ (50 μg/ml); I, fraction I (50 μg/ml); II, fraction II (50 μg/ml); III, fraction III (50 μg/ml). (*B*) UAG suppressor activity of purified, mouse liver glutamine tRNAs in the translation system *in vitro*. The translation of TMV RNA was carried out in the presence of each glutamine tRNA at 25 μg/ml. Lanes contain the following tRNAs: a, normal $tRNA^{Gln}_{CUG}$; b, hypomodified $tRNA^{Gln}_{UmUG}$; c, normal $tRNA^{Gln}_{UmUG}$. Molecular sizes in kDa are indicated.

$tRNA^{Gln}_{UmUG}$ was 1–2% of that of the major species $tRNA^{Gln}_{CUG}$. The nucleotide sequences of the two glutamine tRNAs, arranged in the clover-leaf configuration are shown in Fig. 3. The nucleotide sequence of $tRNA^{Gln}_{CUG}$ is the same as that of rat liver $tRNA^{Gln}_{CUG}$ reported by Yang *et al.* (16), except for some differences in modification. The major portion of glutamine tRNA in fraction I and the minor portion of glutamine tRNA in fraction II were hypomodified species of $tRNA^{Gln}_{CUG}$ and $tRNA^{Gln}_{UmUG}$, respectively. Both hypomodified tRNAs contained unmodified guanosine at residue 18, instead of 2′-*O*-methylguanosine. The UAG read-through abilities of the purified glutamine tRNA species are shown in Fig. 2*B*. Both $tRNA^{Gln}_{UmUG}$ species from fractions II and III

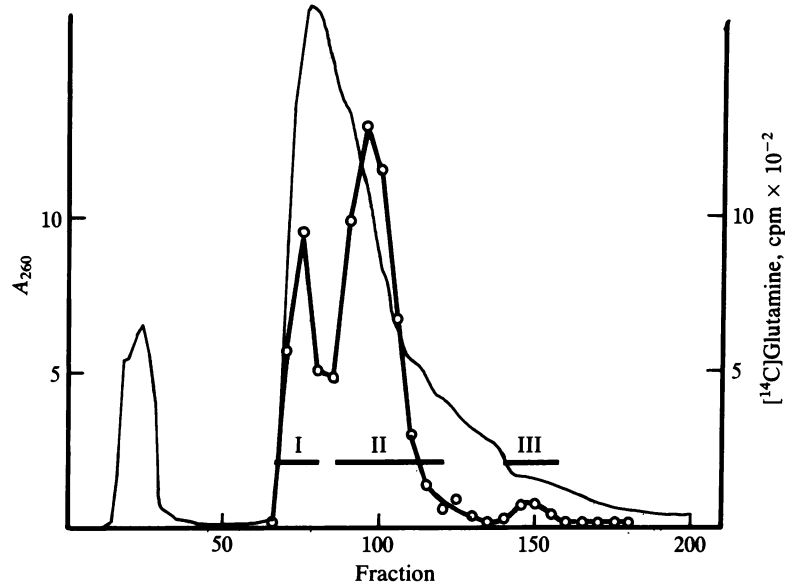

FIG. 1. Fractionation of mouse liver tRNA by column chromatography on BD-cellulose. Unfractionated mouse liver tRNA (8000 A_{260} units) was loaded on a BD-cellulose column (100 × 2 cm). Elution was carried out with a linear gradient produced by 1 liter of 0.02 M sodium acetate buffer, pH 6.0/1.2 M NaCl in the reservoir, and 1 liter of 0.02 M sodium acetate buffer, pH 6.0/0.3 M NaCl in the mixing chamber. Fractions (10 ml) were collected, and the A_{260} was measured (thin line). Samples (100 μl) of each fraction were used to assay glutamine acceptor activity (open circles, thick line) with a crude preparation of aminoacyl tRNA ligases from Ehrlich ascites cells. The fractions indicated by horizontal bars were used for assay of suppressor activity and for further purification of tRNAs.

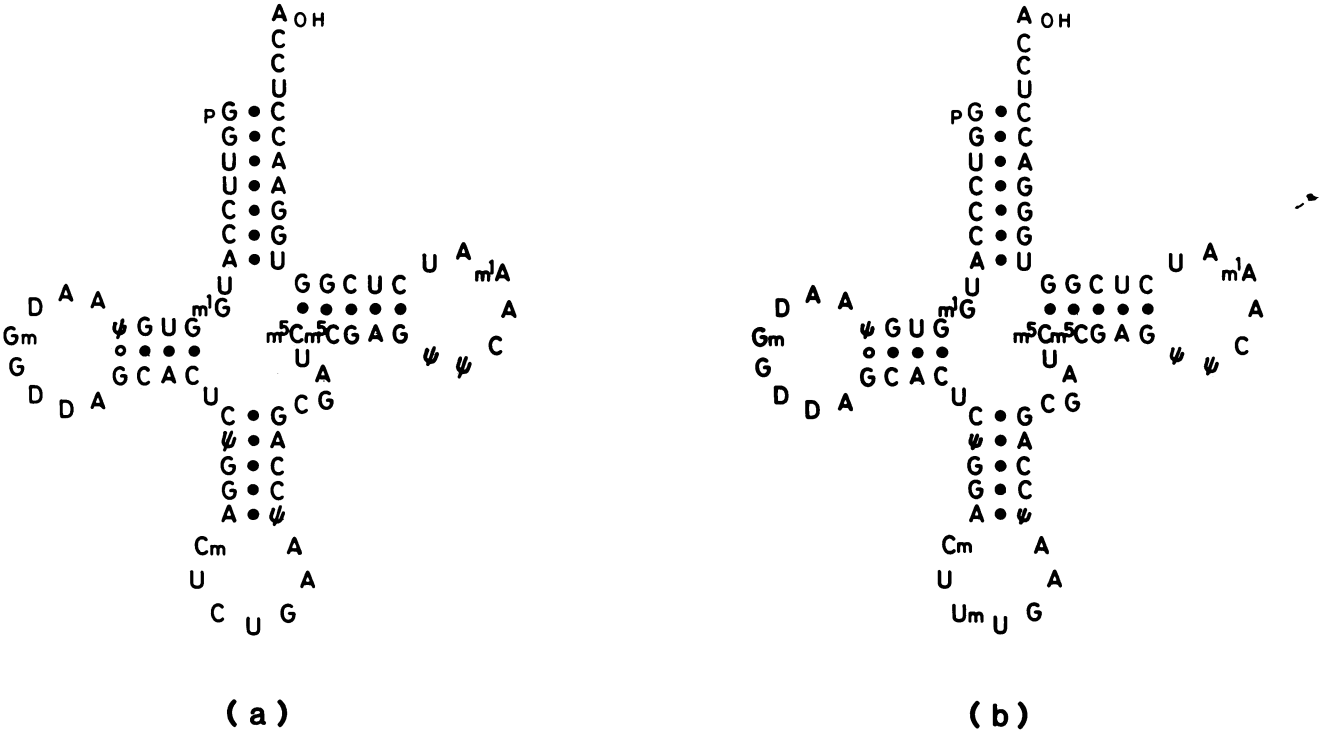

FIG. 3. Cloverleaf structures of two glutamine tRNAs from mouse liver. (*a*) $tRNA^{Gln}_{CUG}$. (*b*) $tRNA^{Gln}_{UmUG}$.

recognized the UAG termination codon of TMV RNA, whereas the two $tRNA^{Gln}_{CUG}$ species from fractions I and II did not. The suppressor ability of $tRNA^{Gln}_{UmUG}$ was weaker than that of tobacco $tRNA^{Tyr}_{G\psi A}$, which has the anticodon sequence GψA (where ψ stands for pseudouridine), and is known to be a natural UAG suppressor tRNA in plants (14) (Fig. 2).

These findings raised the question of how such a small amount of $tRNA^{Gln}_{UmUG}$ with weak suppressor activity is efficiently used to generate the read-through product that is subsequently cleaved into *gag* proteins and polymerase or the protease in Mo-MuLV-infected cells. One possibility is that the amount of $tRNA^{Gln}_{UmUG}$ is increased in Mo-MuLV-infected cells to allow synthesis of a sufficient amount of the protease, which in fact has been shown to be the case. The BD-cellulose chromatographic profiles of the glutamine acceptor activity of tRNA, isolated from Mo-MuLV-infected cells and from non-infected NIH 3T3 cells, were compared. As shown in Fig. 4, the glutamine acceptor activity in the region of fraction III was greatly increased in the bulk of tRNA from virus-infected cells. The increased level of glutamine acceptor activity in fraction III was also found in the tRNA from Ehrlich ascites cells (Fig. 5), but not in S180A mouse sarcoma, in L1210 mouse leukemia cells, or in regenerating mouse liver (data not shown). To prove that the increase of glutamine acceptor activity in Mo-MuLV-infected NIH 3T3 cells and Ehrlich ascites cells was caused by an actual increase in the amount of suppressor $tRNA^{Gln}_{UmUG}$, we isolated glutamine tRNAs from fraction III of both cells and determined their nucleotide sequences by post-labeling, including the use of the wanderling technique (17), and a method of chemical degradation (10). Normal mouse cells contain two species of $tRNA^{Gln}_{UmUG}$ with UAG suppressor activity. In the tRNA of normal NIH 3T3 cells, as shown in Fig. 4*A*, the hypomodified $tRNA^{Gln}_{UmUG}$ was eluted in the earlier peak of fraction III, forming a shoulder of fractions I and II. The normal $tRNA^{Gln}_{UmUG}$ of NIH 3T3 cells constituted the later minor peak of glutamine acceptor activity in fraction III of Fig. 4*A*. On the other hand, fraction III of the Mo-MuLV-infected NIH 3T3 cells and of the Ehrlich ascites cells, with increased glutamine acceptor activity, consisted of a single peak, from which two species of glutamine tRNA, both with a UmUG anticodon sequence, were isolated and found to have nucleotide sequences exactly the same as those of their normal counterparts (data not shown). Furthermore, it was found that the $tRNA^{Gln}_{UmUG}$ isolated from fraction III of the virus-infected or ascites cells had UAG suppressor activity (Fig. 6). These results clearly indicate the increased level of glutamine tRNA in fraction III to be due to the presence of suppressor $tRNA^{Gln}_{UmUG}$.

DISCUSSION

This work showed that mouse liver contains a minor species of glutamine tRNA ($tRNA^{Gln}_{UmUG}$) that possesses UmUG as the anticodon sequence and that recognizes the UAG termination codon (Figs. 2 and 3). A more striking observation was that the amount of this natural, suppressor glutamine tRNA was greatly increased in cells infected with Mo-MuLV (Fig. 4). This increase in levels of a glutamine tRNA that can read an amber codon explains why the protease coded by Mo-MuLV RNA is a read-through product of the UAG termination codon between *gag* and *pol* genes, with glutamine inserted at the site of the termination codon (8). It is, therefore, quite likely that the $tRNA^{Gln}_{UmUG}$ actually functions as an UAG suppressor tRNA *in vivo*.

There have been many reports describing alteration of the chromatographic profiles of tRNA from tumor cells (transformed by viruses, chemically induced, and established cell lines) (18). However, these altered tRNA species in tumor cells have almost always subsequently been found to be the result of a change in post-transcriptional modification (19–21). Therefore, our results on retrovirus-infected cells and Ehrlich ascites cells are, to our knowledge, the first demonstration of increase in the quantity of a natural suppressor glutamine tRNA in mammals.

Recognition of the UAG codon by $tRNA^{Gln}_{UmUG}$ is facilitated by two wobble base pairings in the anticodon—i.e., at the first and third positions of the anticodon. Wobbling of the guanine residue at the third position of an anticodon with uracil in the first position of the codon in mRNA in suppres-

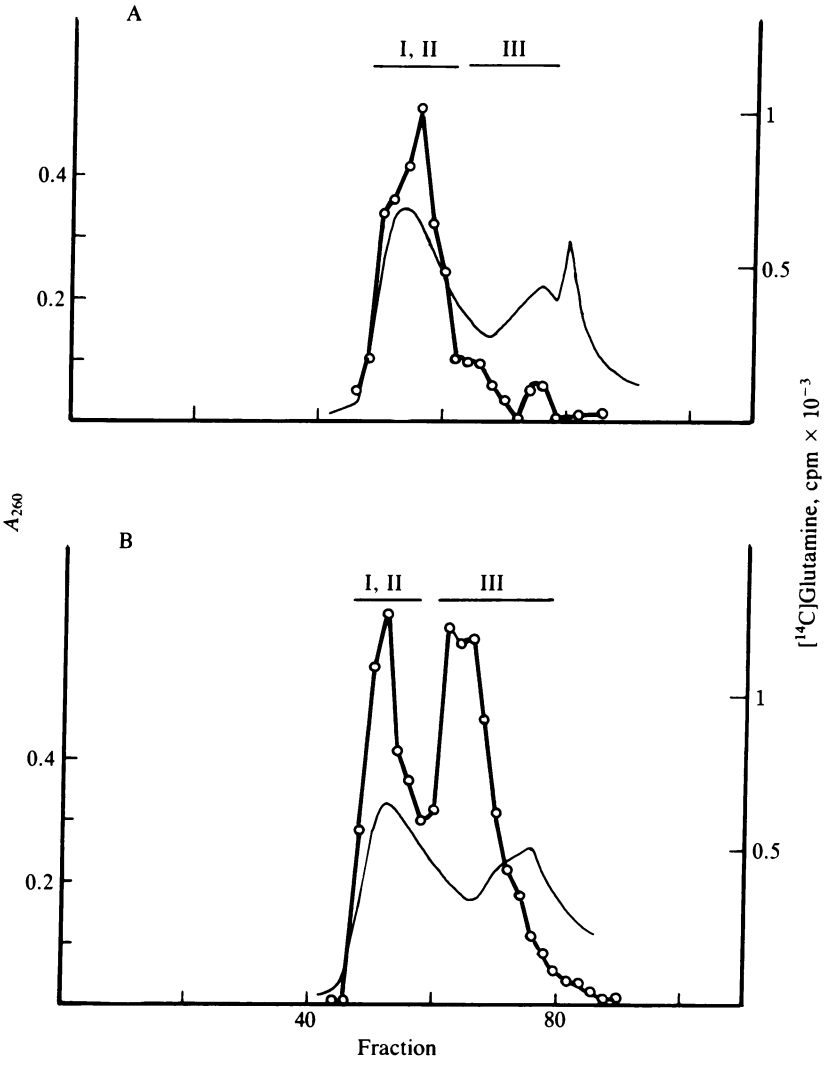

FIG. 4. (*A*) Chromatography of glutamine tRNA from NIH 3T3 cells on a BD-cellulose column (3 × 0.5 cm). Unfractionated tRNA (40 A_{260} units), prepared from NIH 3T3 cells, was loaded on a column and eluted with an 80-ml linear gradient of 0.3–1.2 M NaCl in 0.02 M sodium acetate buffer (pH 6.0). Fractions (1 ml) were collected, and the A_{260} was measured (thin lines). The glutamine acceptor activity of each fraction was assayed with a crude preparation of Ehrlich ascites aminoacyl tRNA ligase (open circles, thick lines). (*B*) Chromatography of glutamine tRNA from NIH 3T3 cells infected with Mo-MuLV on a BD-cellulose column (3 × 0.5 cm). Unfractionated tRNA (40 A_{260} units), prepared from Mo-MuLV-infected NIH 3T3 cells, was loaded on a column. The conditions for elution of tRNA and assay of glutamine acceptor activity were as described in *A*. The horizontal bars indicate the fractions pooled for assays of suppressor activity and for further purification of glutamine tRNAs.

sion of the UAA codon in yeast has been demonstrated *in vivo* by introduction of multiple copies of yeast glutamine tRNA genes (7). Yeast glutamine tRNA, which normally decodes CAA, can weakly suppress the UAA termination codon. The major mouse glutamine tRNA ($tRNA^{Gln}_{CUG}$), with CUG as its anticodon, has no UAG read-through ability, although its nucleotide sequence is identical to that of suppressor glutamine tRNA ($tRNA^{Gln}_{UmUG}$), except for two altered bases in the CCA-stem and the presence of 2′-*O*-methyluridine-34 in the first position of the anticodon. The major species of glutamine tRNA from *Tetrahymena*, with UmUG as its anticodon sequence, also showed no UAG read-through activity (2). Perhaps the conformation of tRNA is another important factor that governs UAG suppressor activity. In this connection it should be noted that UAG-

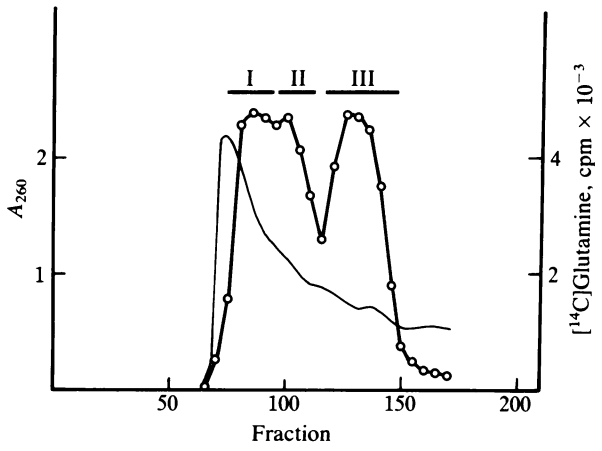

FIG. 5. Chromatography of Ehrlich ascites tRNA on a BD-cellulose column (100 × 1 cm). Unfractionated tRNA (2500 A_{260} units), prepared from Ehrlich ascites cells, was loaded on a column and eluted with a 1.6-liter gradient of 0.3–1.2 M NaCl in 0.02 M sodium acetate buffer (pH 6.0). Fractions (5 ml) were collected, and the A_{260} was measured (thin line). Glutamine acceptor activity was assayed as described in the legend to Fig. 1 (open circles, thick lines). The horizontal bars indicate the fractions pooled for assays of suppressor activity and for further purification of glutamine tRNAs.

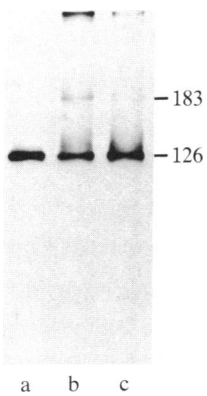

FIG. 6. *In vitro* translation of TMV RNA in the presence of $tRNA^{Gln}_{UmUG}$ from Ehrlich ascites mouse cells. TMV RNA was translated in a mRNA-dependent reticulocyte lysate in the absence of any added tRNA (lane a) or in the presence of purified $tRNA^{Gln}_{UmUG}$ at 25 μg/ml (lane b) and at 10 μg/ml (lane c). Translation products were separated on an 8% $NaDodSO_4$/polyacrylamide gel and identified by fluorography. Molecular sizes of the products are indicated in kDa.

suppressor glutamine tRNAs are always eluted in later fractions from BD-cellulose columns, although these tRNAs do not contain hydrophobic, hypermodified nucleosides.

The high level of $tRNA^{Gln}_{UmUG}$ in NIH 3T3 cells infected with Mo-MuLV is probably due to specific activation of transcription of the tRNA gene for $tRNA^{Gln}_{UmUG}$, although the possibility cannot be excluded that the degradation of $tRNA^{Gln}_{UmUG}$ is inhibited. There may be a specific transcription factor required for the activation of the suppressor tRNA gene, as in the case of the transcription factors IIIB and IIIC; the latter factor is induced by adenovirus infection and nonspecifically stimulates transcription of many tRNA genes (22, 23). The transcription factor for $tRNA^{Gln}_{UmUG}$, if it exists, is different from the factors IIIB and IIIC, since increase in the level of $tRNA^{Gln}_{UmUG}$ in Mo-MuLV infection is very specific and the infection has no effect on the level of the major $tRNA^{Gln}_{CUG}$. The UAG read-through activity in translation *in vivo* may be regulated by the cellular content of suppressor $tRNA^{Gln}_{UmUG}$. Thus, control of transcription of the suppressor tRNA gene seems to be important for replication of the virus. If such a specific transcription factor exists, it would be interesting to characterize it and to elucidate the mechanism by which it is induced by infection with Mo-MuLV.

In Ehrlich ascites cells, the selective increase of suppressor glutamine tRNA might also be regulated by a transcription factor similar to that of Mo-MuLV-infected cells. Cloning of the gene for the UAG suppressor tRNA will certainly facilitate studies on these possibilities. It will also be of interest to investigate to what extent translation of cellular mRNA is affected by the presence of an increased amount of UAG suppressor glutamine tRNA. Read-through protein(s) derived from cellular mRNA, if it is produced, may have an important role in Ehrlich ascites cells and in the phenotype of cells infected by Mo-MuLV.

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