

## Detection of unique tRNA species in tumor tissues by *Escherichia coli* guanine insertion enzyme

(modified nucleoside Q/tRNA<sup>Asp</sup>/post-transcriptional modification/cancer diagnosis)

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**ABSTRACT** The guanine insertion enzyme from *Escherichia coli* catalyzes exchange of guanine located at the first position of the anticodon of tRNA with radioactive guanine (N. Okada and S. Nishimura, unpublished data). tRNA isolated from various tumors, including slowly growing Morris hepatoma 7794A, incorporated considerable guanine with *E. coli* guanine insertion enzyme, whereas tRNA isolated from all normal tissues so far tested, except regenerating rat liver, incorporated scarcely any. In the rat ascites hepatoma AH7974, the guanine was mostly incorporated into minor isoaccepting species of tRNA<sup>Asp</sup> that contained the guanine residue instead of Q base in the first position of the anticodon. This is a sensitive and easy method for identifying unique tRNA species in tumor tissues.

Many new isoaccepting tRNA species have been found in particular tissues, cells at various stages of differentiation, tumor tissues, transformed cells, and cells grown under different culture conditions (for reviews, see refs. 1-3). Various techniques have been used in attempts to detect unique tRNA species in tumor cells, and it has been found that the amount of methylated nucleosides in tRNA and the activities of tRNA methylases are generally high in tumor tissues (3, 4). However, analyses of modified nucleosides using total unfractionated tRNA have given results dependent upon the tumor tissues examined (5). Recently, Kuchino and Borek (6) demonstrated that the tRNA<sup>Phe</sup> that specifically appeared in Novikoff hepatoma and Ehrlich ascites tumor cells contains 1-methylguanine, unlike tRNA<sup>Phe</sup> in normal tissues. The new tRNA<sup>Phe</sup> species that appears in some tumor tissues is due to lack of modified base Y in the position next to the anticodon (7, 8). A new isoaccepting species of tRNA has often been detected in tumor cells by analyzing changes in the chromatographic profile of amino acid acceptor activity (3). However, new tRNA cannot always be detected in this way because the elution position of tRNA may be influenced by several factors. In addition, most methods used previously require a large quantity of material and pure species of tRNA, which are difficult to obtain from tumor tissues.

In this paper, we report a method for detecting unique tRNA species specifically present in tumor cells. We previously reported that the guanine insertion enzyme from rabbit reticulocytes, discovered by Farkas (9), specifically catalyzes the exchange of modified base Q in *Escherichia coli* tRNA with guanine without breaking the polynucleotide chain (10). A guanine insertion enzyme has also been found in other organisms, such as *E. coli* (N. Okada and S. Nishimura, unpublished data) and Ehrlich ascites tumor cells (11), and an extensively purified preparation from *E. coli* catalyzed exchange of guanine with guanine, but not of Q base in tRNA with guanine (N.

Okada and S. Nishimura, unpublished data). With this enzyme it is easy to detect tRNA species for tyrosine, histidine, asparagine, and aspartic acid, which contain guanine instead of Q base in the first position of the anticodon. In the present work with this enzyme, much guanine was incorporated into tRNA from various tumor tissues, but scarcely any was incorporated into tRNA from normal tissues. The tRNA species that accepts guanine was purified from rat ascites hepatoma AH7974 cells and identified as a minor species of tRNA<sup>Asp</sup> containing guanine instead of Q base. Thus use of this enzyme provides a readily applicable method for clearly distinguishing tumor-specific tRNA.

### MATERIALS AND METHODS

**Isolation of *E. coli* Guanine Insertion Enzyme.** Guanine insertion enzyme was isolated from cells of *E. coli* B harvested in the late logarithmic phase. The cells were ground with glass beads and the enzyme present in the 100,000 × *g* supernatant was precipitated by 50% saturation with ammonium sulfate. The precipitate dissolved in buffer A (10 mM Tris-HCl, pH 7.5/10 mM MgCl<sub>2</sub>/1 mM EDTA/6 mM 2-mercaptoethanol/10% by vol glycerol) was dialyzed against buffer A and loaded on a column of DEAE-cellulose (DE52). Elution was performed with linear gradient of NaCl in buffer A. The enzyme eluted with 0.2 M NaCl was collected and dialyzed against buffer A. The dialyzed preparation was fractionated by DEAE-Sephadex A-50 column chromatography using a gradient of NaCl (0-0.4 M) and pH (pH 7.5-8.8). The enzyme eluted from the column was dialyzed against buffer B (10 mM potassium phosphate buffer, pH 6.6/6 mM 2-mercaptoethanol/10% glycerol) and subjected to phosphocellulose column chromatography. The enzyme eluted with 0.3 M NaCl was used for assay. The preparation catalyzed the incorporation of 50 pmol of guanine per 1 μg of enzyme in 1 hr under the standard assay conditions, and was thus more than 100 times as active as the enzyme preparation isolated previously from Ehrlich ascites tumor (12). The enzyme had no detectable RNase activity. Details of the purification procedure and properties of the enzyme will be published elsewhere.

**Conditions for Assay of Guanine Insertion.** For assay using the *E. coli* guanine insertion enzyme, the reaction mixture (0.4-1 ml) contained 1-4 A<sub>260</sub> units of tRNA, 2.0 μg of the enzyme, and 0.1-4 μCi of [<sup>14</sup>C]guanine (55 Ci/mol) in 70 mM Tris-HCl, pH 7.5/20 mM MgCl<sub>2</sub>. After incubation at 37° for the period specified, an aliquot of the reaction mixture was mixed with 1/3 vol of 30% (wt/vol) trichloroacetic acid. The precipitate formed was trapped on a membrane filter and washed with 5% (wt/vol) trichloroacetic acid, and the radioactivity remaining in the filter was measured in a liquid scintillation counter. When <sup>3</sup>H-labeled guanine with high specific activity was used for assay, the tRNA added was reduced to less

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than 1/20th of its initial amount. For assay using the enzyme from Ehrlich ascites tumor cells, the reaction mixture (0.25 ml) contained 2.3  $\mu$ Ci of [ $^3$ H]guanine (12 Ci/mmol) and tRNA as specified in 80 mM Tris-HCl, pH 8.0/12 mM MgCl<sub>2</sub>/0.4 mM 2-mercaptoethanol. Incubation was at 37° for 1 hr. When tRNA eluted from a column of RPC-5 was assayed, 1 ml of eluate was mixed with 3 vol of ethanol. The mixture was left at -20° C for 3 hr, the precipitated tRNA was collected by centrifugation, and the total precipitate was used for the assay.

**Other Procedures.** The guanine insertion enzyme from Ehrlich ascites tumor cells was prepared as described previously (12). Unfractionated tRNA from various mammalian tissues was isolated by homogenization of the tissues with phenol and buffer, and 2-propanol fractionation (13). Crude rat liver aminoacyl-tRNA synthetase was prepared as described by Nishimura and Weinstein (14). The conditions for assay of amino acid acceptor activity were described in the same paper (14). Postlabeling of an RNase T1 digest of tRNA and fingerprint analysis by Sanger's method were carried out as described by Simsek *et al.* (15). Modified nucleosides were analyzed by postlabeling as described by Silberklang *et al.* (16). Rat ascites hepatoma tRNA was fractionated by reversed phase (RPC-5) column chromatography as described by Pearson *et al.* (17). Purification of tRNA by two-dimensional polyacrylamide gel electrophoresis was carried out as described by Ikemura and Dahlberg (18).

**Materials.** [ $^8$ - $^{14}$ C]Guanine (55 Ci/mol) and [ $^8$ - $^3$ H]GTP (12 Ci/mmol) were obtained from New England Nuclear and the Radiochemical Centre, respectively. For preparation of [ $^8$ - $^3$ H]guanine, [ $^8$ - $^3$ H]GTP was dissolved in 1 M HCl in a capillary tube, and the tube was sealed and heated at 100° C for 1 hr. The hydrolysate was subjected to paper chromatography in isobutyric acid and 0.5 M NH<sub>4</sub>OH (5:3, vol/vol), and the spot of guanine was eluted. [ $\gamma$ - $^{32}$ P]ATP used for the polynucleotide kinase reaction was prepared as described by Glynn and Chappell (19). Unfractionated yeast tRNA was a product of Boehringer. Unfractionated *E. coli* tRNA was prepared from *E. coli* B cells by Zubay's procedure (20). Rat liver tRNA<sup>Asp</sup> was isolated by concanavalin A-Sepharose affinity chromatography (21). Acetylated DBAE-cellulose was obtained from Collaborative Research. T4 polynucleotide kinase and nuclease P1 were gifts from H. G. Khorana of Massachusetts Institute of Technology and A. Kuninaka of the Research Laboratory of Yamasa Shoyu Co., Choshi, Japan, respectively. Plaskon coated with Adogen 464, used for RPC-5 chromatography, was kindly provided by A. D. Kelmers of Oak Ridge National Laboratory.

## RESULTS

The guanine insertion enzyme from *E. coli* catalyzes the exchange of free guanine with guanine located in the first position of the anticodon of tRNA<sup>Tyr</sup>, tRNA<sup>His</sup>, tRNA<sup>Asn</sup>, and tRNA<sup>Asp</sup> but not the exchange of guanine with Q base in these tRNAs (N. Okada and S. Nishimura, unpublished data). Therefore, when *E. coli* tRNA, which is usually fully modified and contains Q nucleoside (22), was used as acceptor, no guanine incorporation was observed, whereas yeast tRNA, which does not contain Q nucleoside (23), was found to be a very good acceptor (Table 1). With purified yeast tRNA<sup>Tyr</sup> as an acceptor, the [ $^{14}$ C]guanine incorporated into yeast tRNA was found to be located in the first position of the anticodon (N. Okada and S. Nishimura, unpublished data). Rat ascites hepatoma AH7974 tRNA was also a good acceptor of guanine (approximately 1/10th as good as yeast tRNA), whereas no [ $^{14}$ C]guanine was incorporated into rat liver tRNA (Fig. 1). These results suggest that there is a

Table 1. Incorporation by *E. coli* enzyme of guanine into various tumor tRNAs

Source of tRNA	Guanine incorp., pmol/A <sub>260</sub> unit per hr
AH7974 rat ascites hepatoma	18.1
Morris hepatoma 7794A	25.3
Ehrlich mouse ascites tumor	13.2
SN36 mouse ascites tumor	18.4
Sarcoma 180 mouse ascites tumor	6.9
MM46 mouse ascites tumor	8.8
Clone T22 (simian virus 40-transformed AGMK cells)	40.7
Clone C14 (established cell line of AGMK)	14.0
Regenerating rat liver (24 hr after hepatectomy) (Donryu)	28.3
Regenerating rat liver (48 hr after hepatectomy) (Donryu)	32.1
Rat fetal liver (Donryu)	1.5
Rabbit reticulocytes	1.0
Rabbit liver	<0.3
Rat liver (Donryu)	<0.3
Rat liver (Buffalo)	<0.3
Mouse liver (ddY)	<0.3
Rat kidney (Donryu)	<0.3
Rat spleen (Donryu)	<0.3
Rat testis (Donryu)	<0.3
Yeast	110.7
<i>E. coli</i>	<0.3

AH7974 rat ascites hepatoma was transplanted into Donryu rats. Morris hepatoma 7794A was transplanted intramuscularly into the hind legs of 9- to 10-week-old Buffalo strain rats. Two months after transplantation, rats were killed by decapitation and the tumors were removed. Rabbit reticulocytes were prepared as described by Farkas and Singh (9). Ehrlich ascites tumor and sarcoma 180 ascites tumor were transplanted into ddY and ICR mice, respectively. An established cell line of AGMK, clone C14, and simian virus 40-transformed AGMK cells, clone T22 (24), were cultivated in Eagle's medium with 10% calf serum and 5% tryptose phosphate at 37° C and harvested at confluency. SN36 is a transplantable ascites tumor derived from mouse leukemia cells, and passaged in ICR mice (25). MM46 is a transplantable ascites tumor derived from a spontaneous mammary carcinoma in a C3H/He mouse (26). For each of these ascites tumors 10<sup>6</sup>-10<sup>7</sup> cells were injected, and tumor cells were collected 6-8 days later.

unique tRNA species containing guanine instead of Q base in ascites hepatoma tRNA, but not in normal rat liver tRNA.

To find out whether this tRNA was also present in other tumors, we isolated tRNAs from various tumors and normal tissues and tested them as acceptors in the guanine insertion reaction. As shown in Table 1, the tRNAs from all tumors tested, originating from various organs and having different growth rates, were found to be acceptors in the guanine insertion reaction, while none of those from normal tissues, such as liver, kidney, spleen and testis were acceptors. Slight incorporation of guanine was observed with tRNA isolated from rat fetal liver and rabbit reticulocytes, but the guanine incorporated into them was less than 1/20th of that incorporated into tRNA from most tumor tissues.

Incorporation of guanine into tRNA from regenerating rat liver (24 and 48 hrs after hepatectomy) and from an established cell line of African green monkey kidney (AGMK), clone C14, was as high as that into tRNA from tumor cells. However, the incorporation into tRNA from simian virus 40-transformed AGMK cells, clone T22, was almost 3 times as much as that into tRNA from untransformed cells.

**Characterization of Guanine-Accepting tRNA Present in Rat Hepatoma Cells.** A large amount of AH7974 tRNA was

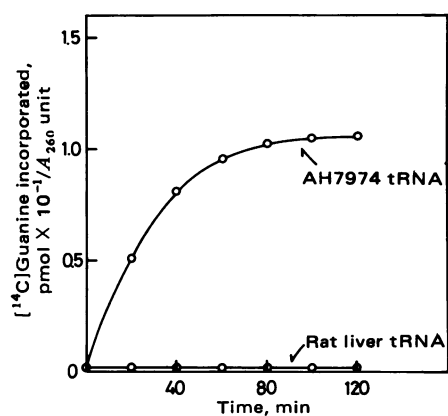


FIG. 1. Incorporation of guanine into rat ascites hepatoma tRNA, catalyzed by *E. coli* guanine insertion enzyme.

fractionated by RPC-5 column chromatography at pH 7.5 for characterization of the guanine-accepting tRNA species present in tumor tissues. As shown in Fig. 2A, the guanine-accepting activity was separated into several discrete fractions on the column. The main fraction (fractions 34–37), containing more than 85% of the guanine acceptor activity, was eluted in the same fractions as a minor peak of aspartic acid accepting activity. Upon rechromatography of this peak on RPC-5 at pH 4.5 (Fig. 2B), most of the guanine-accepting activity emerged from the column as a sharp peak, coinciding with aspartic acid acceptor activity but completely separated from tyrosine and histidine acceptor activities. The purity of the tRNA<sup>ASP</sup> in this fraction (fractions 43–47 of Fig. 2B) was estimated to be more than 20% in terms of guanine and aspartic acid acceptor activities, assuming that 1  $A_{260}$  unit of tRNA is equal to 1.6 nmol.

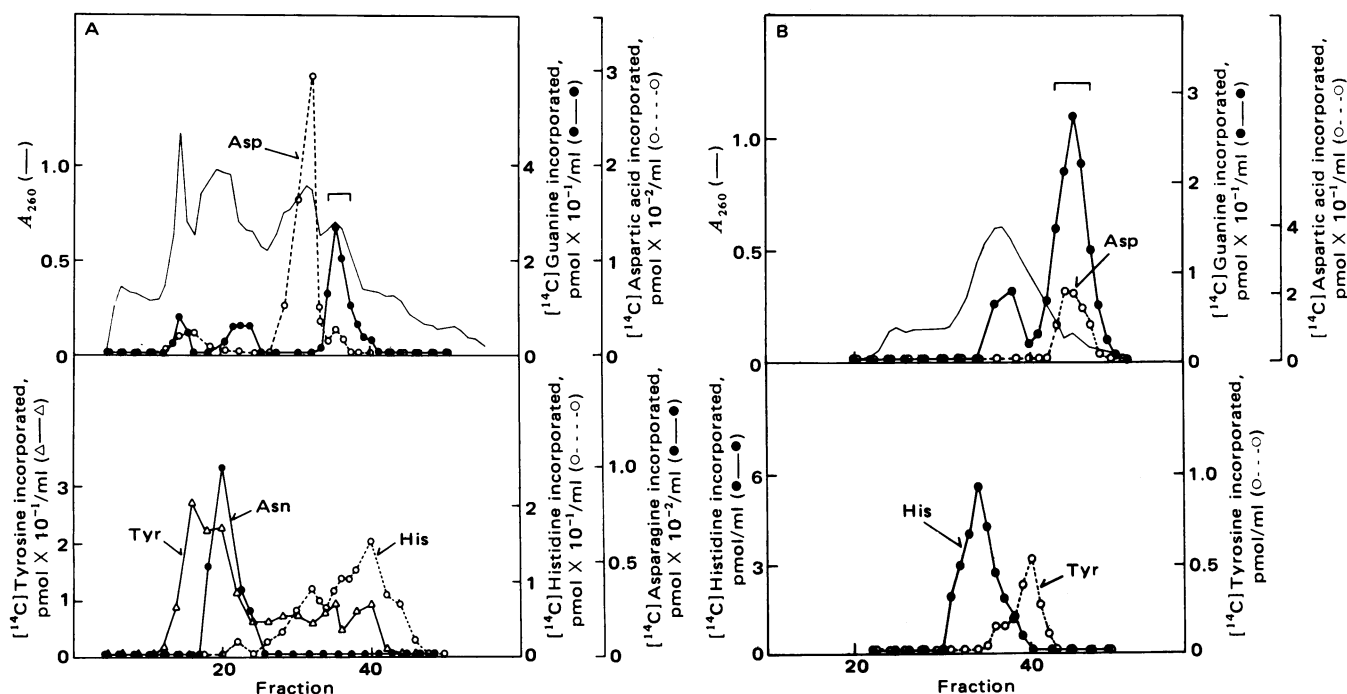


FIG. 2. Fractionation of guanine-accepting tRNA in rat ascites hepatoma AH7974 by RPC-5 column chromatography. (A) First fractionation by RPC-5 column chromatography at pH 7.5. tRNA (1000  $A_{260}$  units) was fractionated by RPC-5 column chromatography (1.5 × 85 cm). Linear gradient elution was carried out using 1 liter of 10 mM Tris-HCl, pH 7.5/10 mM  $MgCl_2$ /1 mM 2-mercaptoethanol/500 mM NaCl in the mixing chamber, and 1 liter of 10 mM Tris-HCl, pH 7.5/10 mM  $MgCl_2$ /1 mM 2-mercaptoethanol/900 mM NaCl in the reservoir. The flow rate was 50 ml/hr. Effluent fractions collected were 20 ml. (B) Second fractionation by RPC-5 column chromatography at pH 4.5. Guanine-accepting tRNA from the first column of RPC-5 (80  $A_{260}$  units, fractions 34–37 of A) was loaded on a second column (0.8 × 60 cm). Linear gradient elution was performed with 250 ml of 10 mM sodium acetate buffer, pH 4.5/10 mM  $MgCl_2$ /2 mM 2-mercaptoethanol/500 mM NaCl in the mixing chamber, and 250 ml of 10 mM sodium acetate buffer, pH 4.5/10 mM  $MgCl_2$ /2 mM 2-mercaptoethanol/1 M NaCl in the reservoir. The flow rate was 20 ml/hr, and 5-ml fractions were collected.

We conclude from these findings that the guanine-accepting tRNA species present in AH7974 cells are mostly minor isoaccepting species of tRNA<sup>ASP</sup>, and that from the properties of the *E. coli* guanine insertion enzyme, the minor isoaccepting tRNA<sup>ASP</sup> should contain guanine instead of Q\* (manQ, Q containing mannose) (21, 27) in the first position of anticodon.

To confirm the identity of the acceptor, we purified the minor tRNA<sup>ASP</sup> further by two-dimensional gel electrophoresis (18) and analyzed its nucleotide sequence by Sanger's fingerprinting technique using a post-labeled RNase T1 digestion product. Fig. 3 shows a comparison of fingerprints of the RNase T1 digest of the minor and major tRNA<sup>ASP</sup> species. The two fingerprint patterns were similar, indicating that the primary structures of two tRNA<sup>ASPs</sup> are identical or that structural alterations, if any, are very small. The main differences in the patterns were that spot 1 of the major tRNA<sup>ASP</sup> was missing from the minor tRNA<sup>ASP</sup>, and that the latter gave two new spots (spots 2 and 3). Probably the oligonucleotide of the major tRNA<sup>ASP</sup> giving spot 1 contains manQ, while the corresponding oligonucleotide of the minor tRNA<sup>ASP</sup> is hydrolyzed by RNase T1 to produce two oligonucleotides (spots 2 and 3), because replacement of manQ by G in the minor tRNA<sup>ASP</sup> results in the cleavage of the oligonucleotide into two portions. For confirmation of the presence of manQ in the oligonucleotide of spot 1, it was eluted and completely hydrolyzed by RNase T2; the resulting 3'-nucleotides were converted to nucleoside 5',3'-diphosphates with T4 polynucleotide kinase and  $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ ; the 5'- $^{32}\text{P}$ -labeled nucleoside diphosphates were converted to 5'-mononucleotides by treatment with nuclease P1; and the  $^{32}\text{P}$ -labeled 5'-nucleotides were analyzed by two-dimensional thin-layer chromatography (16, 23). Fig. 4 shows that 5'-phosphate of modified nucleoside manQ was clearly detected in the oligonucleotide of spot 1.

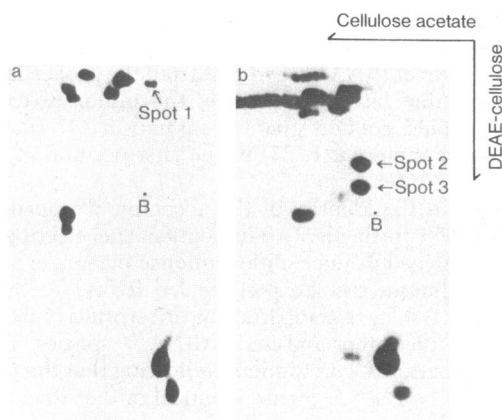


FIG. 3. Comparison of RNase T1 digestion products of major tRNA<sup>Asp</sup> with that of minor tRNA<sup>Asp</sup> by postlabeling technique. Each tRNA (0.005 A<sub>260</sub> unit) was digested with RNase T1. After treatment with bacterial alkaline phosphatase, the digests were labeled with <sup>32</sup>P by T4 polynucleotide kinase as described by Simsek *et al.* (15). Electrophoresis on cellulose acetate was in pyridine/acetate buffer (pH 3.5) for 50 min at 4000 V. Electrophoresis with DEAE-cellulose in the second dimension was performed in 7% (wt/vol) formic acid for 5 hr at 750 V. (a) manQ-containing major tRNA<sup>Asp</sup>; (b) G-containing minor tRNA<sup>Asp</sup>. B is the blue dye marker.

**Guanine Insertion Reaction with Ehrlich Ascites Tumor Enzyme.** Itoh *et al.* (11, 12) reported that the guanine insertion enzyme isolated from Ehrlich ascites tumor cells specifically catalyzed the incorporation of guanine into homologous tRNA but not into normal mouse liver tRNA. Because the Ehrlich ascites tumor enzyme catalyzes exchange of Q base in *E. coli* tRNA with guanine, unlike the *E. coli* guanine insertion enzyme, the question arises of whether the only species of Ehrlich ascites tumor tRNA that act as acceptors in the guanine insertion reaction are those with guanine instead of Q. To answer this question, we fractionated Ehrlich ascites tumor tRNA by RPC-5 column chromatography and measured the guanine accepting activity of each fraction with the enzymes from *E. coli* and Ehrlich ascites tumor. As shown in Fig. 5, guanine-accepting activity was separated into multiple peaks, and the pattern of acceptor activity obtained with the enzyme from Ehrlich ascites tumor cells was almost identical with that obtained with the *E. coli* enzyme, although the extent of incorporation was much less with the Ehrlich ascites tumor enzyme. These results

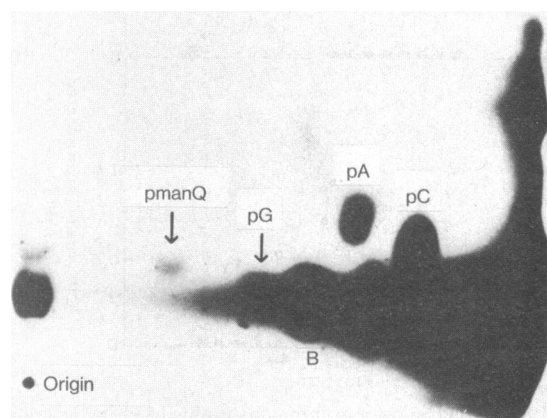


FIG. 4. Detection of manQ in the oligonucleotides of spot 1 by two-dimensional thin-layer chromatography. The dense radioactive spot in the right side of the plate was glucose 6-phosphate produced from [ $\gamma$ -<sup>32</sup>P]ATP by yeast hexokinase (15). B is a contaminant in [ $\gamma$ -<sup>32</sup>P]ATP. The solvent systems used were: first dimension, isobutyric acid/0.5 M NH<sub>4</sub>OH (5:3, vol/vol); second dimension, 2-propanol/concentrated HCl/water (70:15:15, vol/vol/vol).

strongly suggest that the guanine incorporation observed in the homologous system is also due to the incorporation of guanine into undermodified tRNA having guanine instead of Q base. Ehrlich ascites tumor tRNA was fractionated with acetylated DBAE-cellulose (28). All the guanine-accepting activity detected with the Ehrlich ascites tumor enzyme was present in the fraction eluted with buffer I (1 M NaCl/0.1 M MgCl<sub>2</sub>/0.05 M morpholine, pH 8.7) and none was found in the tRNA fraction containing Q, eluted with buffer G (0.2 M NaCl/0.05 M sodium acetate buffer, pH 5.0) (data not shown). This result also supports the above conclusion. The guanine-accepting tRNA species seem to differ in different tumor tissues. Several tRNA species act as acceptors in the guanine insertion reaction in Ehrlich ascites tumor cells, while tRNA<sup>Asp</sup> is the main acceptor in rat ascites hepatoma AH7974 cells.

## DISCUSSION

Abnormal chromatographic profiles of tRNA<sup>Tyr</sup>, tRNA<sup>His</sup>, tRNA<sup>Asn</sup>, and tRNA<sup>Asp</sup> from many tumors and transformed

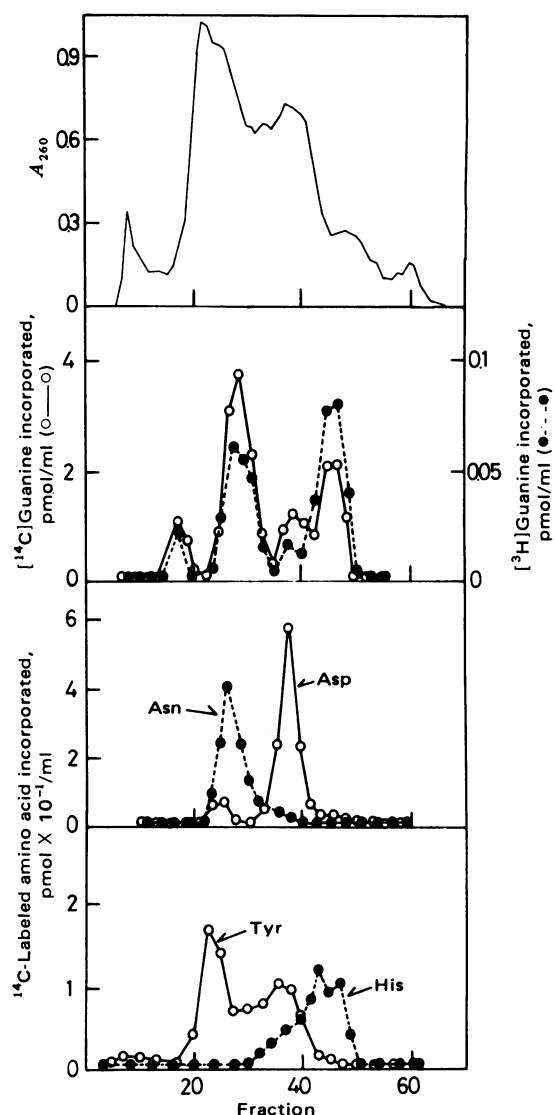


FIG. 5. Fractionation of guanine-accepting activity of Ehrlich ascites tumor tRNA by RPC-5 column chromatography. Conditions for fractionation of tRNA were as described for Fig. 2A. One hundred A<sub>260</sub> units of unfractionated Ehrlich tumor tRNA was loaded on the column. For the second panel down, note that the *E. coli* guanine insertion enzyme was used with [<sup>14</sup>C]guanine and Ehrlich ascites tumor enzyme with [<sup>3</sup>H]guanine.

cells have been reported (29–32). Briscoe *et al.* (32) reported that a new isoaccepting species of tRNA<sup>ASP</sup> was often detected in various tumor tissues by RPC-5 column chromatography. White *et al.* (33) showed that the appearance in *Drosophila* of two isoaccepting tRNA species for each of these four amino acids was due to modification of the first position of the anticodon of these tRNAs. Our results reported here clearly demonstrate that the new tRNA species of tRNA<sup>ASP</sup> appearing in tumor cells is undermodified tRNA<sup>ASP</sup> that contains normal guanine in the anticodon instead of Q base. The guanine-accepting tRNA species in tumor tissues detected by the guanine insertion reaction must be tRNAs corresponding to tyrosine, histidine, asparagine, and aspartic acid that contain guanine instead of Q base or its derivatives. The proportions of these tRNAs differ in different cell types: for example, in rat ascites hepatoma AH7974 cells, tRNA<sup>ASP</sup> was the main component, whereas Ehrlich ascites tumor tRNA contained several tRNA species.

Our method to identify unique tRNA species by using the *E. coli* guanine insertion enzyme has several advantages: (i) Incorporation of guanine was observed in all tumor tissues tested, whereas a change is not always detected by chromatography. (ii) The method is very simple. (iii) Because the *E. coli* enzyme is very active, only a small quantity of cells is needed for the assay: less than 0.05 A<sub>260</sub> unit of unfractionated tRNA is enough.

Because tRNA from regenerating liver also accepts considerable guanine, the presence of undermodified tRNA containing guanine instead of Q base is not directly linked with neoplasia. However, the presence of undermodified tRNA in tumor tissues is not merely linked to the growth rate, because slowly growing Morris hepatoma 7794A, a minimum deviation hepatoma that takes about 2 months to kill the host animal, had higher guanine accepting activity than the rapidly growing ascites hepatoma AH7974, and normal tissues, such as spleen and testis, with rapid cell proliferation have no guanine-accepting activity. Because acceptor activity was found in regenerating rat liver, some properties of malignant cells may transiently be expressed during liver regeneration. This idea is consistent with the finding that in regenerating liver the isozyme of pyruvate kinase is shifted from type L to type M<sub>2</sub>, which is a typical indicator of hepatoma cells (34). The presence of some guanine-accepting tRNA in an established cell line of AGMK suggests that most, if not all, established lines of tissue culture cells may differ in some properties from the cells in normal tissues and organs. However, the level of guanine-accepting tRNA is much higher in simian virus 40-transformed AGMK cells than in untransformed cells. This finding coincides with a previous observation that the amount of a species of isoaccepting tRNA for aspartic acid and histidine is increased in simian virus 40-transformed and -infected AGMK cells (30). Farkas and Chernoff (35) reported that rabbit reticulocyte tRNA accepted guanine with homologous enzyme, and suggested that the tRNA species that accepted guanine might be minor tRNA<sup>ASP</sup> and tRNA<sup>HIS</sup> having guanine instead of Q. Their results agreed with ours with respect to guanine-accepting tRNA species, but we could detect little incorporation of guanine into rabbit reticulocyte tRNA. It is possible that the incorporation into reticulocytes that they observed was less than 1/20th of the amount we observed in tumor tissues.

It is unknown how undermodified tRNA is induced in tumor tissues. The primary structure of the AH7974 minor tRNA<sup>ASP</sup> that accepts guanine seems to be very similar to that of major tRNA<sup>ASP</sup>, but it is still unknown whether the two tRNA<sup>ASPs</sup> have exactly the same primary structure, or whether there are minor differences in their sequences as well as in the extent of their post-transcriptional modification. It is also unknown whether

the induction of such under-modified tRNA in tumor tissues is caused by incomplete modification of G during biosynthesis of Q or by activation of an exchange reaction that replaces Q base by guanine in the tRNA of tumor tissues, as proposed previously (10).

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