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Alterations in Specific Transfer Ribonucleic Acids in a Spectrum of Hepatomas*

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ABSTRACT: Tyrosyl-, histidyl-, asparaginyl-, and phenylalanyl-tRNAs from a spectrum of Morris hepatomas were compared to the same respective aminoacyl-tRNAs from normal rat liver by means of methylated albumin kieselguhr column chromatography. The tyrosyl-, histidyl-, and asparaginyl-tRNAs from hepatoma 3924A had altered elution profiles similar to those found earlier in Novikoff hepatoma. Hepatomas 9121, 9098, 3683F, and 5123C had altered phenylalanyl-tRNA profiles. Codon recognition properties of tyrosyl-tRNAs from Novikoff hepatoma, hepatomas 3924A and 9098, and normal rat liver were also examined. In the case of Novikoff hepatoma, the tyrosyl-tRNA was further fractionated on a DEAE-Sephadex A-50 column. All of the tyrosyl-tRNAs recognized the normal tyrosine codons UAU and UAC; the response to UAU was always greater than the response to UAC. None of the tyrosyl-tRNAs were bound by the chain terminator codons UAG or UAA. The biological and functional significance of these findings are discussed.

A previous study of the tRNA obtained from the Novikoff hepatoma demonstrated pronounced differences in the MAK[1] column elution profiles of tyrosyl-, histidyl-, and asparaginyl-tRNAs, when compared to the corresponding aminoacyl-tRNAs from control rat liver (Baliga *et al.*, 1969). The appearance of new species of isoaccepting tRNAs and of

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[1] Abbreviation used is: MAK, methylated albumin kieselguhr.

significant changes in the relative amounts of normally existing isoaccepting tRNAs has been observed in association with a variety of biological phenomena (Sueoka and Kano-Sueoka, 1970). Such involvement of tRNAs in phage (Kano-Sueoka and Sueoka, 1966; Weiss *et al.*, 1968) and animal virus infection (Subak-Sharpe *et al.*, 1966), in oncogenesis or oncogenic viral transformation (Mushinski and Potter, 1969; Taylor *et al.*, 1968), in genetic suppression (Carbon *et al.*, 1966, 1969; Capecchi and Gussin, 1965; Gopinathan and Garen, 1970; Goodman *et al.*, 1968), and in differentiation (Kaneko and Doi, 1966; Lee and Ingram, 1967) is suggestive of a regulatory role on their part.

The Novikoff hepatoma is a very rapidly growing tumor which by histologic and biochemical criteria is highly undifferentiated and no longer resembles normal liver. The availability of a well characterized spectrum of hepatomas derived by Morris (Morris and Wagner, 1968; Miyaji *et al.*, 1968; Reuber, 1966), which differ in growth rate and state of differentiation, presents a unique opportunity to gain further insight into the possible role that specific tRNAs might play in cell growth, differentiation, and tumor formation. Extensive data comparing metabolic patterns, enzyme profiles, and karyotypes of these tumors to those of normal liver is available (Weber and Lea, 1967; Nowell *et al.*, 1967; Farina *et al.*, 1968). One of the overall patterns that appears to emerge from these studies is that, in general, with increasing growth rate there is a decrease in cytologic differentiation and a progressive deviation in enzyme profiles from that of normal liver.

In the present study, we have examined the tRNAs for tyrosine, histidine, asparagine, and phenylalanine of five of the Morris hepatomas and compared them to the corresponding tRNAs from livers of normal rats of the same inbred strain, sex, and age. The hepatomas studied in this investigation are described in Table I. The data obtained suggest certain correlations between deviations in tRNA profiles from normal liver and some parameters of these tumors. In addition, the coding properties of tyrosyl-tRNA from some of the hepatomas are compared to those of normal liver. A preliminary report of some of these results has been presented (Srinivasan *et al.*, 1970).

TABLE I: Characterization of Hepatomas.

Hepatoma Line	Generation	Histologic Criteria[a]
3683F	274, 276	Poorly differentiated
5123C	80	Well differentiated, some areas poorly differentiated
3924A	265, 271	Poorly differentiated
9098	34	Predominately intermediate between well-differentiated and poorly differentiated hepatocellular carcinoma
9121	30	Predominately intermediate between well-differentiated and poorly differentiated hepatocellular carcinoma

[a] According to Dr. David R. Meranze.

Materials and Methods

Hepatomas 9121, 9098, and 3924A were grown intramuscularly in the thigh muscle of male rats of the inbred ACI/T[2] strain, and hepatoma 5123C in the thigh muscle of male rats of the Buffalo strain. Hepatoma 3683F was grown intraperitoneally in males of the ACI/T strain. The hepatomas from 7 to 10 rats were harvested before the onset of significant necrosis, dissected free of any necrotic or normal tissue, frozen in liquid nitrogen, and stored at −70°. Livers from normal male rats of the same strains and age were similarly rapidly excised, frozen, and stored.

tRNA was prepared by phenol extraction, essentially as described by Wevers *et al.* (1966). The tRNA was stripped of endogenous amino acids by incubation in 0.05 M sodium carbonate buffer at pH 10 for 20 min at 37°. One-tenth volume of 4 M NaCl was then added and the tRNA was precipitated by the addition of two volumes of 95% ethanol and placed at −20° overnight. The precipitate was collected by centrifugation, dissolved in 0.005 M sodium acetate buffer (pH 5.0), and stored at −20°.

[2] T = Texas Inbred Mouse Co.

Aminoacyl-tRNA synthetases were prepared by homogenizing the minced tissue in one volume of 0.25 M sucrose containing 0.005 M Tris-HCl, 0.005 M magnesium acetate, 0.06 M KCl, and 0.007 M β-mercaptoethanol (pH 7.6), by means of a motor-driven Ten Broeck homogenizer with a Teflon pestle. After centrifugation at 105,000*g* for 90 min, the clear supernatant fraction was passed through a Sephadex G-100 column as described by Taylor *et al.* (1968). All operations were carried out at 0–5°.

Aminoacylation of tRNA. The tRNAs were aminoacylated in a 1-ml reaction mixture containing: 0.1 M Tris-HCl (pH 7.4), 0.005 M $MgCl_2$, 0.005 M ATP (neutralized), 0.0025 M CTP, 0.04 mM ^{14}C-labeled amino acid (10 μCi), or 0.04 mM ^{3}H-labeled amino acid (40 μCi), a mixture of 19 other nonradioactive amino acids (0.5 mM each, neutralized with Tris), 1 mg of tRNA, and sufficient enzyme to achieve maximum charging in 15 min at 37° as determined by prior assay. When tRNA was charged with tyrosine, 0.0025 M reduced glutathione was added to decrease nonspecific incorporation. This did not affect the net charging. When tRNA was charged with asparagine, 0.4 mM nonradioactive aspartic acid was added to prevent significant charging by radioactive aspartic acid which might arise from the hydrolysis of radioactive asparagine. After incubation at 37° for 15 min, one-fourth volume of 0.05 M sodium acetate (pH 5.0) was added, and the mixture deproteinized with phenol previously equilibrated with 0.05 M sodium acetate (pH 5.0). One-tenth volume of 2 M sodium acetate (pH 5.0) and three volumes of 95% ethanol were added to the aqueous layer. After standing at −20° for 2 hr, the precipitate was collected by centrifugation, washed with 80% ethanol, dried in a vacuum desiccator, and resuspended in 0.005 M sodium acetate (pH 5.0).

Column Chromatography. MAK was prepared as described by Mandell and Hershey (1960). The suspension (8.5 ml; prepared within 24 hr prior to use) was added to a column (9 mm diameter). A mixture of the [^{3}H]- and [^{14}C]aminoacyl-tRNAs (0.4–0.8 mg, 20,000–40,000 cpm of each) in 0.1 M sodium chloride–0.05 M sodium phosphate buffer (pH 6.7) was applied to the column. The column was then washed with 30 ml of the same buffer, followed by a linear gradient of NaCl. The salt concentrations of the gradients are given in the legends to the figures. The chromatography was carried out at 4°. Frac-

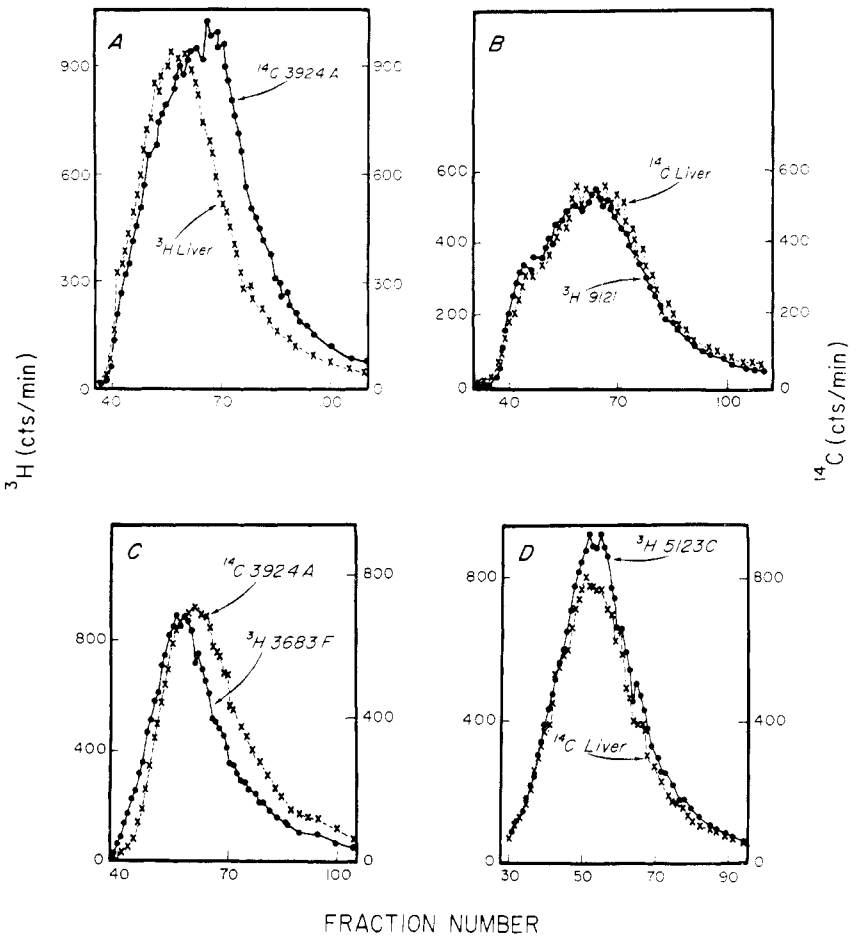

FIGURE 1: MAK column elution profiles of tyrosyl-tRNA. The normal liver tRNA in A and B is from the ACI/T strain; in D it is from the Buffalo strain. A linear gradient of 0.2–0.5 M NaCl in 0.05 M sodium phosphate buffer (pH 6.7) was used.

tions of 1.25 ml were collected and precipitated by the addition of one drop of carrier DNA (0.5 mg/ml) and 2 ml of 10% trichloroacetic acid. The precipitates were collected on Whatman GF/C filters, dried, and counted in a liquid scintillation spectrometer with toluene–phosphor. The recovery of radioactivity from the MAK columns ranged from 75 to 100%.

DEAE-Sephadex A-50 chromatography was performed as described by Nishimura and Weinstein (1969); specific details are given in the legend to Figure 5.

Ribosomes were prepared from *Escherichia coli* B as described by Nirenberg (1963), except that the ribosomal pellet was washed twice with 0.01 M Tris-acetate buffer (pH 7.8) containing 2 M KCl and 0.03 M magnesium acetate and finally suspended in 0.01 M Tris-acetate buffer (pH 7.2) containing 0.01 M magnesium acetate and 0.05 M KCl and stored frozen under liquid nitrogen.

Triribonucleoside diphosphates UpApU and UpApC were prepared by enzymatic syntheses catalyzed with ribonuclease A by a modification of Bernfield's method (1966). Uridine 2′,3′-cyclic phosphate (ammonium salt, 400 μmoles) and ApU or ApC (100 μmoles) were incubated for 5 hr at 0° in 0.125 M Tris-HCl (pH 8.06) with 150 μg of ribonuclease A (Calbiochem, five-times recrystallized). The whole reaction mixture was applied immediately onto two sheets of Whatman No. 3MM paper and dried with a stream of air and descending chromatography performed in the solvent system butanol–acetic acid–water, 5:2:3, v/v. The slowest moving band, containing the triplets, was eluted and further purified by electrophoresis on Whatman No. 3MM paper in 0.05 M triethylammonium bicarbonate buffer (pH 7.6) for 1 hr at 50 V/cm. The electrophoretic mobility of the triplets (referred to UMP mobility as 1.0) was 0.65. The analytical results obtained after T2-ribonuclease hydrolysis were in good agreement with the calculated values. UpApA and UpApG were products of Miles Laboratories.

Assay of Aminoacyl-tRNA Binding to Ribosomes. The general procedure of Nirenberg and Leder (1964) was used. The incubation mixture (0.05 ml) contained: 0.10 M Tris-acetate (pH 7.2), 0.05 M KCl, 0.03 M magnesium acetate, 1.5–2.5 A_{260} units of ribosomes, and the amounts of trinucleotides and labeled aminoacyl-tRNAs indicated in the table and figures. After incubation (24°, 20 min) the reaction mixtures were diluted with 3 ml of the same buffer, filtered on cellulose nitrate filters (Millipore, 25 mm diameter, 0.45 μ pore size), and washed with four 5-ml portions of cold buffer, and the radioactivity was determined in a liquid scintillation counter.

Radioactive Amino Acids. The sources and specific activities (in milliCuries per millimole) of labeled amino acids were as follows: Schwarz BioResearch: L-[^{14}C]tyrosine (454), L-[^{3}H]-tyrosine (8000), L-[^{14}C]histidine (312), L-[^{3}H]histidine (5150), L-[^{3}H]phenylalanine (2740); New England Nuclear: L-[^{3}H]-asparagine (1100); Amersham & Searle: L-[^{14}C]asparagine (207); Nuclear-Chicago: L-[^{14}C]phenylalanine (504).

Results

The MAK column elution profiles of tyrosyl-tRNAs are presented in Figure 1. The tRNA extracted from hepatoma 3924A and charged with tyrosine had a component which eluted at a higher salt concentration than the control rat liver tyrosyl-tRNA. The tyrosyl-tRNAs from hepatomas 9121, 3683F, and 5123C and from 9098 (not shown here)

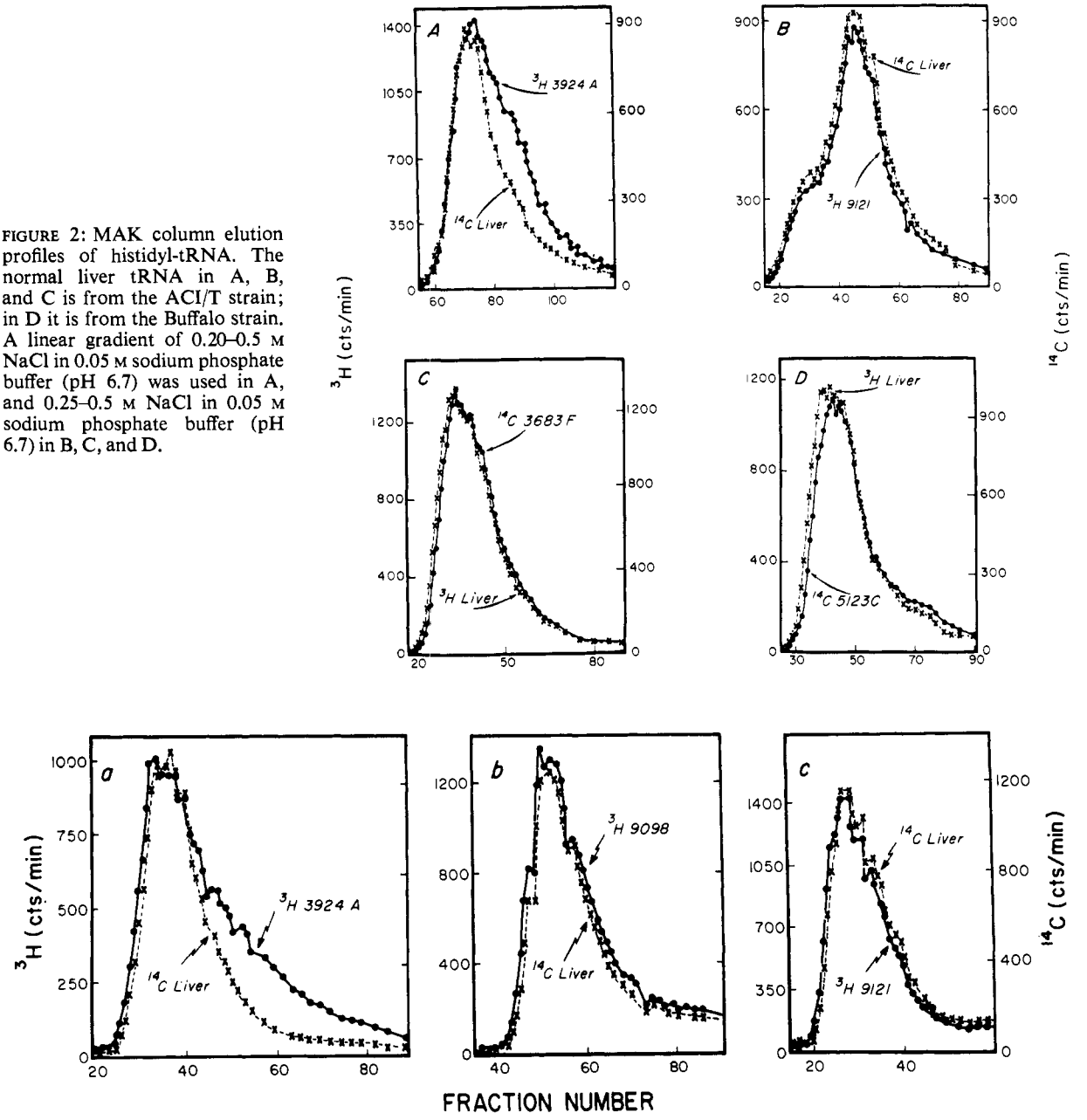

FIGURE 2: MAK column elution profiles of histidyl-tRNA. The normal liver tRNA in A, B, and C is from the ACI/T strain; in D it is from the Buffalo strain. A linear gradient of 0.20–0.5 M NaCl in 0.05 M sodium phosphate buffer (pH 6.7) was used in A, and 0.25–0.5 M NaCl in 0.05 M sodium phosphate buffer (pH 6.7) in B, C, and D.

FIGURE 3: MAK column elution profiles of asparaginyl-tRNA. The normal liver tRNA is from the ACI/T strain. A linear gradient of 0.18–0.43 M NaCl in 0.05 M sodium phosphate (pH 6.7) was used.

eluted exactly as the control liver tyrosyl-tRNA. Figure 1C clearly demonstrates the difference between the tyrosyl-tRNAs from hepatoma 3924A and 3683F. MAK column elution profiles of tRNAs charged with histidine are presented in Figure 2. The histidyl-tRNA from 3924A also had a component which eluted at a higher salt concentration than the histidyl-tRNA from control rat liver. The histidyl-tRNAs from hepatomas 9121, 3683F, and 5123C cochromatographed with the histidyl-tRNA from control rat liver. The histidyl-tRNA from tumor 9098 also had a normal elution profile (not shown here). Asparaginyl-tRNA from 3924A also had a component which eluted at a higher salt concentration than the asparaginyl-tRNA of normal liver (Figure 3). Asparaginyl-tRNAs from hepatomas 9098 or 9121 were similar to that of normal liver.

In contrast to the results obtained with tyrosyl-, histidyl-, and asparaginyl-tRNAs, the elution profile of phenylalanyl-tRNA from hepatoma 3924A was identical with that of normal liver (Figure 4). This was also true with tRNA from the Novikoff hepatoma (Baliga *et al.*, 1968). On the other hand, hepatomas 9121 and 3683F had phenylalanyl-tRNAs which eluted somewhat earlier than the control or than the 3924A tRNAs. Similar changes were noted with phenylalanyl-tRNA from hepatomas 9098 and 5123C (not shown here), but they were less pronounced. The small, early peak observed in each of the phenylalanyl-tRNA elution profiles is most probably an artifact, occurring only when the tRNA is charged with [^{3}H]-phenylalanine.

The alteration noted in the MAK column elution profile of the tyrosyl-tRNA of hepatoma 3924A was also seen on reversed-phase Freon columns. The preparation of the reversed-phase Freon columns and the chromatographic procedure were the same as that described in the studies of the Novikoff hepatoma (Baliga *et al.*, 1969). The hepatoma 3924A tyrosyl-

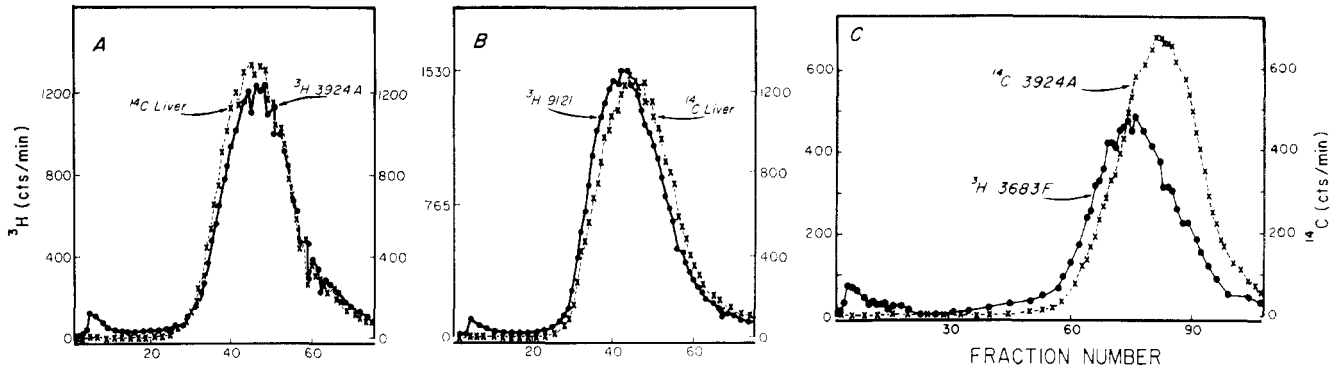

FIGURE 4: MAK column elution profiles of phenylalanyl-tRNA. The normal liver tRNA in A and B is from the ACI/T strain. A linear gradient of 0.25–0.50 M NaCl in 0.05 M sodium phosphate buffer (pH 6.7) was used.

tRNA had a component which eluted later than the normal tyrosyl-tRNA similar to that observed in the case of Novikoff hepatoma (Baliga *et al.*, 1969).

The above-noted differences between hepatomas and normal liver in profiles of tyrosyl-, histidyl-, asparaginyl-, and phenylalanyl-tRNAs were confirmed when the ^{14}C and ^{3}H isotope labeling of the tRNAs was reversed. Furthermore, the same changes in elution profiles were noted whether the tRNAs were charged with normal rat liver enzymes or with hepatoma 3924A enzymes. There was no marked difference in the molar acceptance of a given amino acid by any of the tRNAs in the presence of either the control liver enzyme or tumor enzyme. Therefore, the changes in aminoacyl-tRNA noted with these tumors, as with the Novikoff hepatoma (Baliga *et al.*, 1969), are properties of the tRNAs and not of the aminoacyl-tRNA synthetases. The differences in elution profiles between certain hepatoma tRNAs and control liver tRNAs could be due to differences in primary structures or to differences in their conformations. The 3924A and 9121 tRNAs were, therefore, denatured and renatured in 0.02 M $MgCl_2$ at 60° for 5 min (Lindahl *et al.*, 1967) prior to charging and column chromatography. The above described differences in elution profiles of aminoacyl-tRNAs were still apparent. It seems likely, therefore, that the changes in chromatographic behavior of tyrosyl-, histidyl-, and asparaginyl-tRNAs of the Novikoff and 3924A hepatomas and of the phenylalanyl-tRNA from hepatomas 9121, 3683F, 9098, and 5123C are due to differences in their primary structures.

A difference in primary structure, due to a change in major base composition or a secondary modification of one of the major bases, could directly or indirectly affect the function of the anticodon region of that tRNA. It was of interest, therefore, to examine the codon recognition properties of tyrosyl-tRNAs from some of these hepatomas and compare them to those of normal liver. In the case of the Novikoff hepatoma, partially purified fractions of tyrosyl-tRNAs were first prepared by column chromatography. The Novikoff tRNA (150 mg) was fractionated on a DEAE-Sephadex A-50 column (Figure 5), as described previously for normal rat liver tRNA (Nishimura and Weinstein, 1969). When the fractions were assayed for tyrosine acceptance activity a sharp peak was apparent at the beginning of the major A_{260} peak, which corresponded to that previously described in normal rat liver tRNA. This was immediately followed by a second component which was not seen with the rat liver tRNA. The tRNAs present in fractions corresponding to these two components, fractions 39 and 49, respectively, were recovered by ethanol precipitation, charged with [^{14}C]tyrosine, and tested for codon recognition in the ribosomal binding assay. Figure 6 shows that unfractionated [^{14}C]tyrosyl-tRNA from liver of the Holtzman strain of rats recognized the tyrosine codons

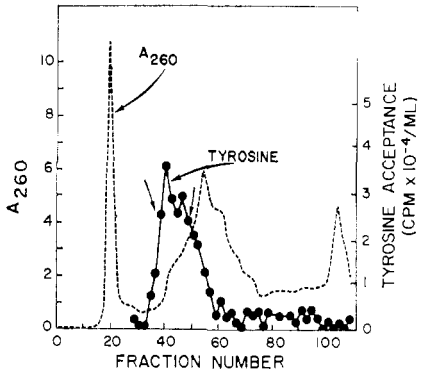

FIGURE 5: DEAE-Sephadex column chromatography of tRNA from the Novikoff hepatoma. The column (1 × 150 cm) was equilibrated with 0.02 M Tris-HCl (pH 7.5), 0.0075 M $MgCl_2$, and 0.375 M NaCl and the tRNA, 150 mg in 20 ml of starting buffer, was applied. A linear gradient elution was carried out using 1 l. of 0.02 M Tris-HCl (pH 7.5), 0.016 M $MgCl_2$, and 0.525 M NaCl in the reservoir, and 1 l. of the starting buffer in the mixing chamber. The flow rate was 10 ml/hr. Each fraction contained 7 ml of effluent. Fractions were assayed for A_{260} and acceptance capacity for [^{14}C]tyrosine. For subsequent codon recognition studies, the tRNA present in fractions 39 and 49, respectively, indicated by arrows, was recovered by ethanol precipitation and charged with [^{14}C]tyrosine.

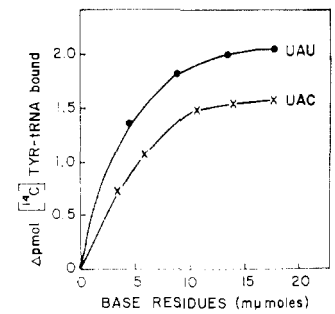

FIGURE 6: Effect of UpApU and UpApC on the binding of [^{14}C]-tyrosyl-tRNA from rat liver to ribosomes. Experimental conditions are described in Methods. 1.9 A_{260} units of *E. coli* ribosomes and 5.1 pmoles of [^{14}C]Tyr-tRNA (51.5 pmoles of [^{14}C]Tyr/1 A_{260} of tRNA) were added.

TABLE II: Effect of Trinucleotides on Binding of [^{14}C]Tyr-tRNA from Rat Liver and Hepatomas to Ribosomes.[a]

Codon (0.1 A_{260} Unit)	[^{14}C]Tyr-tRNA Bound					
	ACI/T Liver (3 pmoles/0.2 A_{260})		Hepatoma 9098 (3.9 pmoles/0.2 A_{260})		Hepatoma 3924A (3.5 pmoles/0.2 A_{260})	
	pmol	Δpmol	pmol	Δpmol	pmol	Δpmol
	0.19		0.15		0.24	
UAU	0.68	0.49	0.67	0.52	0.81	0.57
UAC	0.53	0.34	0.51	0.36	0.68	0.44
UAG	0.08	−0.11	0.07	−0.08	0.08	−0.16
UAA	0.08	−0.11	0.10	−0.05	0.09	−0.15

[a] Incubation conditions and processing of the samples are described in Methods. The amount of [^{14}C]Tyr-tRNA and trinucleotides are specified.

(Trupin *et al.*, 1965) UpApU and UpApC. Both fractions 39 and 49 from the Novikoff hepatoma recognized the normal tyrosine codons UAU and UAC (Figure 7). With the normal tyrosyl-tRNAs, and with fractions 39 and 49 from the Novikoff hepatoma, the response to UAU was always greater than the response to UAC, even when tested across a range of trinucleotide concentrations. The greater response to UAU has been noted in a previous study of liver tyrosyl-tRNA (Nishimura and Weinstein, 1969) and is also apparent with *E. coli* tyrosyl-tRNA (Trupin *et al.*, 1965). Possible subtle differences between fractions 39 and 49 of the Novikoff tumor were sought by studying the response of each to UAC across a range of Mg^{2+} concentrations. Both materials, however, revealed similar optima of 0.03 M Mg^{2+}. In addition, neither fraction responded to the chain-terminator codon UAG (also see study below).

Studies comparing the codon recognition properties of unfractionated tyrosyl-tRNAs from hepatomas 3924A and 9098 to those of normal liver tyrosyl-tRNA obtained from the same ACI/T strain of rats are given in Table II. As in the above study with rat liver tRNA from the Holtzmann strain, liver [^{14}C]tyrosyl-tRNA from the ACI/T strain recognized the tyrosine codons UAU and UAC, and the response to UAU was greater than that to UAC. Similar results were obtained with tyrosyl-tRNA from hepatomas 9098 and 3924A. Neither normal nor hepatoma tRNAs recognized the chain-terminator codons UAG and UAA.

Discussion

The results presented here provide strong evidence that the tRNA population of certain tumors differs qualitatively from that of the normal tissue from which the tumor was derived. Other tumor cells which display such differences include the Ehrlich ascites tumor, hamster cells transformed by either adenovirus 7, SV 40, or the Rous sarcoma virus (Taylor *et al.*, 1968), certain plasma cell tumors (Yang and Novelli, 1968; Mushinski and Potter, 1969), Hepatoma 5123 (Gonano and Chiarugi, 1969), and a human lymphocytic leukemia cell line (Gallo, 1969). It is of interest that these examples include tumors induced by either chemical carcinogens or oncogenic viruses. The changes in tRNA are not simply a function of the growth environment of the tumor since they are apparent whether tumor cells grow intraperitoneally, as in the case of hepatoma 3683F and of the Novikoff hepatoma (Baliga *et al.*, 1969); in muscle, as in the case of hepatoma 3924A; or in tissue culture (Taylor *et al.*, 1968). Nor can these alterations simply be attributed to the rapid growth of the tumor cells since the altered tyrosyl-tRNA seen in the Novikoff hepatoma and hepatoma 3924A is not seen in the rapidly growing hepatoma 3683F or in regenerating liver (Agarwal *et al.*, 1970). It is equally unlikely that the alteration in tRNAs seen in certain hepatomas can be ascribed to a pattern which mimics the tRNA profile of certain adult tissues other than liver since Taylor *et al.* (1968) have shown that several aminoacyl-tRNAs from different organs in the same animal or across widely divergent mammalian species are similar in their column elution profiles.

It is noteworthy that the hepatomas which are generally least deviated from liver with respect to their karyotype, histology, growth rate, and enzyme patterns, namely hepatomas 9121 and 9098, exhibit changes only in their phenylalanyl-tRNA profiles (Table III), whereas new species of tyrosyl-, histidyl-, and asparaginyl-tRNAs are seen only in those hepatomas which are generally representative of advanced states of malignancy, namely hepatoma 3924A and Novikoff hepatoma. Since hepatomas 3683F and 5123C do not fit exactly into either category, the possibility that specific changes in tRNA reflect the biochemical individuality of each

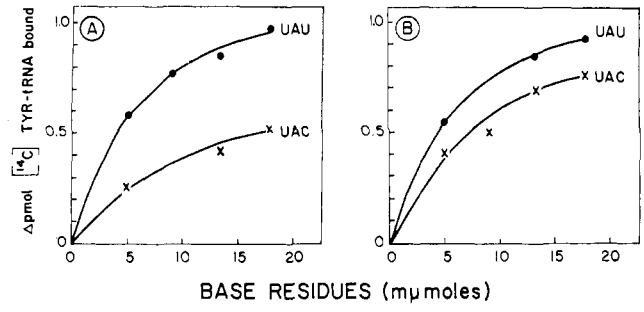

FIGURE 7: Effect of UpApU and UpApC on the binding of [^{14}C]-tyrosyl-tRNA from Novikoff hepatoma to ribosomes. (A) Assays contained 8.9 pmoles of [^{14}C]Tyr-tRNA (89 pmoles of [^{14}C]Tyr/1 A_{260} of tRNA) prepared from fraction 39 of the DEAE-Sephadex column (see Figure 5). Experimental conditions are described in Methods. (B) Assays contained 3.7 pmoles of [^{14}C]Tyr-tRNA (34 pmoles of [^{14}C]Tyr/1 A_{260} of tRNA) prepared from fraction 49 of the DEAE-Sephadex column (see Figure 5). Experimental conditions are described in Methods.

TABLE III: Rate of Growth, Karyotype, and the Occurrence of Alterations in Elution Profiles of Aminoacyl-tRNAs in the Hepatomas.

Hepatoma	Rate of Growth	Karyotype		New Species of tRNA			
		Ploidy	Structural Changes	Tyr	His	Asn	Phe
9121	Slow	Diploid	Min	−	−	−	+
9098	Slow	Diploid	Min	−	−	−	+
3683F	Rapid	Hypodiploid	28 Abn	−	−		+
5123C	Slow	Hypertetraploid	Min	−	−		+
3924A	Rapid	Hypotetraploid	10 Abn	+	+	+	−
Novikoff	Rapid	Hypotetraploid		+	+	+	−

tumor cannot be ruled out. In this respect our findings are reminiscent of the evidence for antigenic individuality of chemically induced tumors (Prehn, 1968) and also of the limited correlation between enzyme profiles and other parameters of different tumors. It is not yet apparent whether these diversities are due to (1) the clonal origin of tumors from a diverse population of normal liver cells, (2) the possibility that tumors represent cells arrested at various stages of normal differentiation, (3) a generalized aberration in the overall control of gene expression which manifests itself differently in each tumor, (4) other unknown mechanisms (for a discussion of these ideas, see Potter, 1968).

The molecular basis for the chromatographic alterations in certain tRNAs obtained from hepatomas might be due to (1) a change in primary sequence not related to secondary modifications; (2) a change in secondary modification, such as overmethylation, of tRNA species normally present in liver; or (3) a change in the relative abundance of isoaccepting species of tRNAs normally present in liver. The clearest example of the appearance of unique tRNAs, which differ in primary structure from those usually present, are the bacterial suppressor tRNAs (Garen, 1968). These arise as mutations in host cistrons for tRNA, or they may be coded for by the genome of an infecting virus. Our results indicate that the tumor tyrosyl-tRNAs have normal codon recognition (also see discussion below) and therefore it is unlikely that they have an altered sequence in their anticodons. The new species of tRNAs present in the tumors are probably altered in another region.

The possibility that the altered chromatographic profiles are due to secondary modifications is quite an attractive one, since the total tRNA of certain tumors appears to contain higher levels of methylated bases than the tRNA from corresponding control tissues (Bergquist and Matthews, 1962; Viale *et al.*, 1967). In addition, a variety of tumors have increased levels of tRNA-methylating enzymes (Tsutsui *et al.*, 1966; D. Srinivasan and P. R. Srinivasan, unpublished observations). Overmethylation of the tRNA population would also explain why in certain hepatomas there is a simultaneous and qualitatively similar change in the tRNAs for at least three amino acids. On the other hand, the third possibility cannot be excluded since there is evidence that the broad peak of liver tyrosyl-tRNA seen in the present studies may actually represent at least three components which are not well resolved (Nishimura and Weinstein, 1969; Yang *et al.*, 1969). An increase in the relative abundance of a late component (and possibly an associated decrease in an early component) would explain the apparent shift in elution profile of certain hepatoma tRNAs. A final decision between these alternatives will require complete purification and structural analysis of one of the hepatoma tRNAs and a comparison to related tRNAs present in liver.

The functional significance of the alterations in tRNAs noted in certain hepatomas is not apparent. Since the tyrosyl-tRNAs of hepatoma 3924A and the Novikoff hepatoma recognized the normal tyrosine codons UAU and UAC, and did not recognize the chain terminator codons UAG and UAA, they are not the same as the tyrosyl-tRNA nonsense suppressors of bacteria. It is also unlikely that these tRNAs function as missense suppressors, but studies employing more purified preparations of the "abnormal" tyrosyl-tRNA component in protein-synthesizing systems are required to be certain that it functions normally with respect to codon recognition and protein synthesis. If this proves to be the case, then it is possible that these altered tRNAs are truly "redundant" species. Even in normal rat liver there is considerable redundancy in isoaccepting species of tRNA, suggesting that they may play an as yet unknown role perhaps related to regulatory mechanisms. There is evidence that in bacterial systems tRNAs are involved in enzyme repression (Silbert *et al.*, 1966; McLaughlin *et al.*, 1969) and feedback inhibition (Duda *et al.*, 1968). If this proves to be the case in mammalian cells, then alterations in the tRNA population of certain tumors may contribute to the disturbance in gene expression and control of growth manifested by tumor cells.

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