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Cell-Free Transfer of Leucine by Transfer Ribonucleic Acid from Mouse Liver and Plasma Cell Tumors into Rabbit Hemoglobin*

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ABSTRACT: Chromatographic differences in leucyl transfer ribonucleic acids have been previously demonstrated among several mouse plasma cell tumors which synthesize κ-type immunoglobulin light chains. To investigate whether these chromatographically different transfer ribonucleic acids also differed functionally, transfer ribonucleic acid from two such tumors as well as from normal mouse liver was used to transfer leucine into hemoglobin in a cell-free system derived from rabbit reticulocytes. The two tumors chosen, MOPC 46B and MOPC 149, secrete light chains differing in leucine placement in at least one position and have different leucyl transfer ribonucleic acid patterns when chromatographed on a reversed-phase column. The leucyl transfer ribonucleic acid from MOPC 46B was like that from mouse liver except for differences in the relative amounts of each distinguishable isoaccepting transfer ribonucleic acid. MOPC 149, on the other hand, showed the apparent loss of a component peak of leucyl transfer ribonucleic acid compared with MOPC 46B. Despite these qualitative and quantitative differences in chromatographic pattern, all the mouse leucyl transfer ribonucleic acid preparations, normal and neoplastic, transferred labeled leucine into the proper hemoglobin α-chain peptides.

Transacylation under these conditions of protein synthesis was ruled out by demonstrating that two fractions of rabbit liver leucyl transfer ribonucleic acid separated on DEAE-Sephadex transferred leucine into different hemoglobin peptides. Thus, it is concluded that transfer ribonucleic acid from tumors as well as normal tissue can be utilized by protein-synthesizing components from another mammal. Further, these experiments show no evidence for variation in translation of natural hemoglobin α-chain message when transfer ribonucleic acid from either of two different mouse plasma cell tumors is used.

Previous work from this laboratory has demonstrated significant, stable, and reproducible differences in Leu-tRNA from κ-type-light-chain-producing mouse plasma cell tumors analyzed chromatographically on a reversed-phase column (Mushinski and Potter, 1969). For the purpose of investigating the functional significance of these findings, the Leu-tRNAs from two such tumors were studied for their ability to transfer leucine into protein as directed by a natural mammalian messenger.

The possibility of translational variations playing a role in shaping mammalian protein sequences has been postulated for immunoglobulins (Potter *et al.*, 1965; von Ehrenstein, 1966; Mach *et al.*, 1967; Campbell, 1967) and also for rabbit hemoglobin (von Ehrenstein, 1966). One of the possibilities for shaping protein structure during translation would be a regulation of the populations of tRNAs available during polypeptide synthesis. If certain exceptional messengers had regions or codons which could be translated by several tRNAs

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bearing different amino acids, then the final protein structure would be determined by which of these tRNAs were present to translate the ambiguous codons. It has been established that the immunoglobulin secreting cells of mouse plasma cell tumors do contain variable numbers of isoaccepting tRNAs for certain amino acids (Mach *et al.*, 1967; Yang and Novelli, 1968; Mushinski and Potter, 1969) and that certain of these isoaccepting species are missing in certain tumors over long-term transplantation (Mushinski and Potter, 1969). These chromatographic findings could be reflecting the presence in one tumor of one combination of tRNAs responsive to a common ambiguous κ-chain messenger and the absence or replacement of one or more of these tRNAs in another tumor line resulting in a different, though similar light-chain product. In bacterial systems work on nonsense suppressors (Capecchi and Gussin, 1965; Engelhardt *et al.*, 1965) and on missense suppressors (Yanofsky, 1967) has shown that a single cistron can code for more than one polypeptide chain when one codon is recognized by two or more different tRNAs carrying different amino acids. In the present report we examined this possibility in mammalian tissues using tRNA molecules from neoplastic and normal cells of a highly inbred mouse strain and cell-free protein-synthesizing components from another species.

Rabbit reticulocyte polyribosomes have been used successfully for synthesizing hemoglobin using tRNA from *Escherichia coli* (von Ehrenstein and Lipmann, 1961) and from guinea pig liver (Bishop *et al.*, 1961), so they were chosen to supply a natural messenger and synthetic machinery. These ribosomes were incubated with purified tRNA from two mouse sources which had been charged separately with leucine labeled with different radioactive isotopes. The resulting hemoglobin was examined to see if the different mouse tRNAs successfully transferred the labeled amino acid into the complete protein and to see if different mouse tRNAs inserted leucine into different places in the growing polypeptide chain.

The two plasma cell tumors selected for isolation of this tRNA were MOPC 46B and MOPC 149 whose κ-type light-chain structures have been shown to differ in at least the fourth residue from the amino terminus: leucine in MOPC 46B and methionine in MOPC 149 (Appella and Perham, 1967). The Leu-tRNAs from these tumors and from normal mouse liver were compared chromatographically, and that of MOPC 149 was found to be markedly deficient in a peak prominent in the other two tissues. Despite the chromatographic differences, tRNA preparations from normal liver and from both tumors transferred leucine into rabbit hemoglobin. Further, when transfers were performed in pairs utilizing double-label techniques, both Leu-tRNA preparations in each pair transferred leucine into the appropriate positions in the hemoglobin α chain with a constant ratio. Randomization of the isotope due to transacylation during incubation was ruled out by showing that under the same conditions pools of rabbit liver Leu-tRNA separated on DEAE-Sephadex transferred leucine into different positions in the rabbit hemoglobin α chain.

Materials and Methods

Plasma Cell Tumors. Mineral oil induced plasma cell tumors were induced and maintained in BALB/c mice as described by Potter (1966). Two κ-chain secreting tumors, MOPC 46B and MOPC 149, were chosen for this study. (For available amino acid sequence data for the proteins secreted by these two tumors, see Appella and Perham, 1967.)

tRNA Preparations. Tumors were harvested from mice before extensive necrosis occurred. Subcutaneous tumors were pooled from several mice, and tRNA was prepared by Sephadex G-100 fractionation of phenol-extracted whole tumors as previously described (Mushinski and Potter, 1969).

tRNA from the livers of BALB/c mice was extracted by the same procedure. In the preparation of tRNA from rabbit liver, the passage over a G-100 Sephadex column was omitted.

To remove any endogenously acylated amino acids, concentrated solutions of tRNA were brought to pH 8 by addition of 0.1 volume of 1 M Tris-HCl (pH 8) and incubated at 37° for 30 min.

Preparation of Aminoacyl-tRNA Synthetases. Crude supernatant enzymes were used for aminoacylation reactions. Solid plasma cell tumor or mouse liver (*ca.* 5 g) was minced and then homogenized by hand in a Teflon-glass homogenizer with 1.5 volumes (v/w) of cold medium A.[1] The homogenate was first centrifuged 20 min at 30,000*g* and the supernatant was then centrifuged 2 hr at 100,000*g*. The ribosome-free supernatant was removed with a Pasteur pipet, and free amino acids and nucleotides were removed by passage through a G-25 Sephadex column (1 × 15 cm) equilibrated with medium A,[1] with sucrose omitted. Reduced glutathione was added to a final concentration of 0.01 M and the enzyme preparation was used without further concentration. For rabbit liver synthetase preparation the homogenization was performed in 0.35 M sucrose, 0.03 M Tris-HCl (pH 7.6), 0.01 M $MgCl_2$, 0.05 M KCl, and 0.006 M 2-mercaptoethanol and was used immediately after 100,000*g* centrifugation without further treatment.

Aminoacylation of tRNA. The mouse tRNA aminoacylating reaction mixture contained 0.01 M $MgCl_2$, 0.1 M Tris-HCl (pH 7.4), 0.01 M reduced glutathione, 0.001 M ATP, 0.01 M phosphoenolpyruvate, 0.02 mg/ml of pyruvatekinase, 0.5–10 mg/ml of RNA, 2×10^{-5} M L-[U-^{14}C]leucine (240 mCi/mmole) or L-[4,5-^{3}H]leucine (2 Ci/mmole) (New England Nuclear), 2×10^{-4} M 19 [^{12}C]amino acids omitting leucine, and charging enzyme from liver for liver or MOPC 149 tRNA or enzyme from MOPC 46B for its own tRNA, between 0.01 and 0.1 mg per ml of protein. For rabbit liver tRNAs the same conditions were used except for the following: 0.0014 M 2-mercaptoethanol, 0.002 M ATP, 0.01 mg/ml of pyruvate kinase, 1–2 mg/ml of tRNA, 1.1×10^{-5} N L-[U-^{14}C]-leucine (180 mCi/mmole) or L-[4,5-^{3}H]leucine (7.6 Ci/mmole) (Schwartz BioResearch, Inc.) in a total volume of 5.0 ml including 2.0 ml of liver enzyme preparation. The reaction proceeded at 37° for 30 min, when a plateau of trichloroacetic acid precipitable radioactivity was reached under all the concentrations of tRNA and enzymes, at which time the charged tRNA was shaken with a pinch of Bentonite and an equal volume of water-saturated phenol. After two to three additional phenol extractions, the aqueous phase was mixed with 0.1 volume of 20% K acetate (pH 5.5) and precipitated at −20° with three volumes of absolute ethanol. The precipitate was collected by centrifugation, drained in

[1] Medium A is composed of 0.25 M sucrose, 0.006 M $MgCl_2$, 0.08 M KCl, and 0.03 M, Tris-HCl (pH 7.5).

the cold, dissolved in a convenient volume of 0.001 M $MgCl_2$ and 0.001 M Na_2EDTA, and stored frozen. Alternatively the precipitate was dissolved in water and lyophilized, and, for the ribosomal transfer experiments, the tRNA was dissolved in 0.001 M $MgCl_2$ without EDTA.

Fractionation of Charged tRNA. To examine the subspecies of the Leu-tRNA fraction of each tissue, the reversed-phase chromatographic system of Weiss and Kelmers (1967) was employed. This column was 240 cm high and 1 cm in diameter and packed with Chromosorb W to which an organic phase of 5% Aliquat 336 in Freon 214 had been absorbed. The mobile aqueous phase was a NaCl gradient from 0.35 to 0.55 M in a buffer consisting of 0.01 M Na acetate and 0.01 M Mg acetate (pH 4.5). The column was water jacketed and maintained at 15°. Elution rate was 1.5 ml/min and fractions of 10 ml were collected. When two tissues or tumors were being compared in this system, between 15,000 and 60,000 cpm of [^{3}H]Leu-tRNA from one tissue was mixed with a similar amount of [^{14}C]Leu-tRNA from the other tissue and applied in 1–2 ml of initial buffer to the column.

RNA was precipitated by adding 1 ml of 50% trichloroacetic acid to each fraction and mixing. After at least 30 min at 4°, the precipitates were collected on membrane filters (Millipore HAWP) which were washed with additional ice-cold 5% trichloroacetic acid, placed in glass scintillation vials, and dried in a 100° oven for 20 min. Acid hydrolysis of the precipitate was effected with 0.2 ml of 1 N HCl, in the same vials after capping, by heating in the 100° oven for 20 min. Absolute ethanol (3 ml) and 10 ml of scintillant, 0.6% PPO[2] and 0.006% POPOP in toluene, were added to the vials and samples were counted in a Packard Tri-Carb liquid scintillation spectrometer equipped with automatic external standardization and punched tape output. Three replicate counts were averaged and absolute dpm of [^{3}H] and [^{14}C] were computed in a IBM 1620 computer using a standard quench curve generated with this solvent mixture. Alternatively, the [^{14}C] contribution to the [^{3}H] channel (usually 14%) was subtracted and radioactivity was reported as counts per minute.

Fractionation of Rabbit Liver tRNA. Rabbit liver tRNA was fractionated on DEAE-Sephadex following the procedure of Nishimura *et al.* (1967). A 6 × 55 cm column of DEAE-Sephadex A-50 was preequilibrated with the following starting buffer: 0.02 M Tris-HCl (pH 7.5), 0.008 M $MgCl_2$, and 0.375 M NaCl. About 250 mg of tRNA was dissolved in 25 ml of water, diluted to a final volume of 175 ml with starting buffer, and loaded on the column. Elution was carried out at room temperature by a linear gradient using 3 l. of starting buffer in the mixing chamber and 3 l. of the limit buffer (0.02 M Tris-HCl (pH 7.5), 0.016 M $MgCl_2$, and 0.525 M NaCl) in the reservoir. At the end of the gradient, elution was continued with limit buffer. The flow rate was 120 ml/hr, and the volume of each fraction was 20 ml. The leucine acceptor activity was determined in a reaction mixture containing 0.001 M ATP, 0.007 M phosphoenolpyruvate, 0.01 mg/ml of pyruvate kinase, 1×10^{-5} M L-[U-^{14}C]leucine (50 mCi/mmole) (Schwartz BioResearch, Inc.), and 0.020 ml of rabbit liver charging enzyme; the final volume was 0.5 ml and contained 0.3 ml of the column fraction to be tested. The reaction was carried out at 37° for 20 min at which time it was stopped by the addition of 2 ml of 5% trichloroacetic acid. The samples were filtered onto membrane filters, washed with 5% trichloroacetic acid, and counted in a Nuclear-Chicago gas-flow counter. The absorbance at 260 mμ and the leucine acceptor activity of the column fractions were plotted. For preparative charging with [^{3}H]- or [^{14}C]leucine, appropriate fractions were pooled (see below) and precipitated by the addition of two volumes of absolute ethanol at −20°. The precipitate was collected by centrifugation, dried in a desiccator, and dissolved in water. The charging and reisolation were carried out as described above.

Transfer of Leucine into Rabbit Hemoglobin. Reticulocytes were collected from phenylhydrazine-injected rabbits, and their ribosomes were used to transfer leucine into hemoglobin using the conditions of Weisblum *et al.* (1965) with Leu-tRNA from one source labeled with [^{3}H]leucine and that from another source bearing [^{14}C]leucine. After 30-min incubation, carrier rabbit hemoglobin obtained from the same reticulocytes was added, extracted with phenol, and precipitated as globin in acid–acetone. α and β chains from this globin were separated on CM-cellulose with a pyridine–formic acid gradient, and the α chains were pooled and digested with trypsin (Dintzis, 1961). Soluble tryptic peptides were fractionated on a Dowex 50 × 8 column with a pyridine–acetic acid gradient as described by Jones (1964). When necessary, coeluting peptides were separated by paper electrophoresis in pyridine–acetic acid (pH 6.4). Amino acid composition and radioactivity were determined on a Beckman 120C amino acid analyzer and a Packard Tri-Carb liquid scintillation spectrometer.

Results

Freon Reversed-Phase Fractionation of tRNA Mixtures. Figure 1A,B shows the patterns for mouse Leu-tRNA from the preparations compared below for their ability to transfer leucine into rabbit hemoglobin. In these studies MOPC 46B tRNA was compared with liver (Figure 1A) and MOPC 46B with MOPC 149 (Figure 1B). These and other chromatographic patterns previously described (Mushinski and Potter, 1969) revealed reproducible differences in the Leu-tRNAs from various κ-chain-producing plasma cell tumors. Although Figure 1A shows better resolution of the two major peaks than that achieved in Figure 1B, it is clear from this chromatogram and two repeats (not shown) that MOPC 149 is very deficient in a component (peak 3) that is present in MOPC 46B. The peaks of Leu-tRNA from liver generally resemble the MOPC 46B pattern in this column run and several others (not shown), although relative quantities of the distinguishable peaks differ with relatively less of late eluting components in liver than in MOPC 46B. Thus quantitative differences are seen among all three preparations used in the experiments below, and a major qualitative difference is the lack of a substantial peak 3 in MOPC 149 Leu-tRNA.

Transfer of Leucine into Hemoglobin. Two separate double-label-transfer experiments were performed. In expt 1, the tRNA from MOPC 46B charged with [^{14}C]leucine was compared with [^{3}H]Leu-tRNA prepared from livers of BALB/c mice. In expt 2, tRNA from MOPC 46B charged with [^{3}H]leucine was compared with the tRNA from MOPC

[2] Abbreviations used are: $tRNA^{Leu}$, nonacylated leucine transfer RNA; PPO, 2,5-diphenyloxazole; POPOP, 1,4-bis[2(5-phenyloxazolyl)]-benzene.

TABLE I: Transfer of Leucine from Mouse Liver and Mouse Tumor tRNA into Rabbit Hemoglobin in the Reticulocyte Ribosomal System.[a]

Expt	tRNA	Specific Act. of tRNA (cpm Leucine/mg of tRNA[b] × 10^4)	Total Added (cpm)	Ribosomes (OD_{260} Units)	Transferred to the α Chain (cpm)	Transferred to the β Chain (cpm)
1	[^{3}H]Leucyl-tRNA liver	7.8	15.4 × 10^4	3490	1.8 × 10^4	0.84 × 10^4
	[^{14}C]Leucyl-tRNA MOPC 46B	4.7	10.2 × 10^4		1.1 × 10^4	0.48 × 10^4
	Ratio [^{3}H]:[^{14}]C cpm		1.5		1.6	1.75
2	[^{3}H]Leucyl-tRNA MOPC 46B	5.0	59.5 × 10^4	3153	5.9 × 10^4	5.3 × 10^4
	[^{14}C]Leucyl-tRNA MOPC 149	7.1	34.0 × 10^4		1.3 × 10^4	1.2 × 10^4
	Ratio [^{3}H]:[^{14}C] cpm		1.75		4.5	4.4

[a] The reactions took place in a volume of 10 ml of mixture consisting of 0.1 M Tris-HCl (pH 7.6), 0.025 M KCl, 3–4 × 10^{-4} M $MgCl_2$, 0.008 M reduced glutathione, 5 × 10^{-4} GTP, 0.001 M ATP, 0.01 M phosphoenolpyruvate, 0.04 mg/ml of pyruvate kinase, 0.002 M L-[^{12}C]leucine, and 2 × 10^{-4} M each of 19 [^{12}C]amino acids omitting leucine. Reticulocyte ribosome-free supernatant (S-100) (0.5–1.0 ml) was added along with the amounts of reticulocyte ribosomes and radioactive Leu-tRNA as indicated above, and incubation proceeded at 37° for 30 min. [b] Assuming 24.0 OD_{260} units equivalent to 1 mg of tRNA.

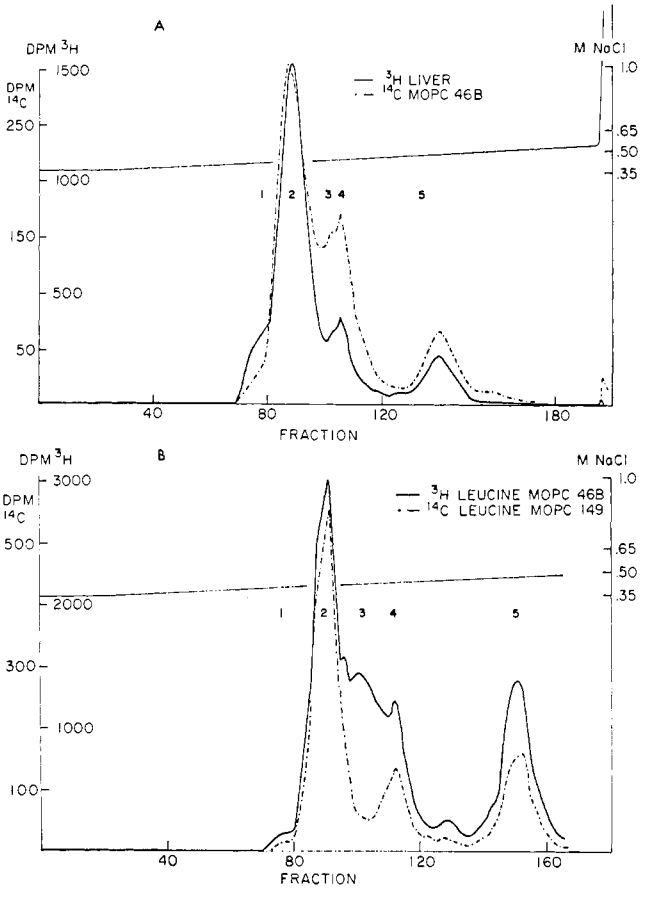

FIGURE 1: Reversed-phase chromatographic comparisons of Leu-tRNA patterns from normal mouse liver and one mouse plasma cell tumor, MOPC 46B (A), and this tumor with a similar tumor, MOPC 149 (B). The NaCl gradients are linear from 0.35 to 0.55 M in 0.01 M Na acetate and 0.01 M Mg acetate (pH 4.5). Peaks are numbered as shown.

149 charged with [^{14}C]leucine. The experimental details for the two transfers are summarized in Table I. The efficiency of transfer for all tRNAs tested was comparable except that MOPC 149 tRNA transferred into hemoglobin only about one-third the fraction of total counts (*ca.* 7%) transferred by the other three preparations (*ca.* 20%). Nonetheless the transfer from MOPC 149 tRNA seems qualitatively like the others with similar amounts going into α and β chains and a constant ratio between [^{3}H] and [^{14}C] counts incorporated. Possibly this preparation suffered more denaturation during isolation, lyophilization, or storage than the other three. The loss of component three of Leu-tRNA may also have played a role in decreasing the efficiency of overall transfer of leucine from MOPC 149 tRNA, but this possibility has not yet been investigated.

The tryptic peptides of the purified α chain were separated on a Dowex 50 column. Figure 2A,B shows the elution pattern of expt 1 and 2, respectively, plotting ninhydrin assay, [^{3}H] and [^{14}C] radioactivity. The tracings of the two radioactivities overlap perfectly except for the peak (12) of the Leu-Arg dipeptide αT_{11} which had a [^{14}C] maximum one tube after the [^{3}H] maximum in expt 1. The chromatographic behavior of this small peptide appeared to differ depending on the presence of [4,5-^{3}H]leucine (mol wt 135) or of [U-^{14}C]-leucine (mol wt 143) since a small difference in molecular weight of isotopic leucine alters a significant percentage of the total molecular weight of the peptide. The peptides under each peak were purified by paper electrophoresis in pyridine–acetate (pH 6.4), as described in detail elsewhere (Galizzi, 1969). The radioactivity of the pure peptides and the [^{3}H]:[^{14}C] ratio are shown in Table II for expt 1 and 2. The ratio of [^{3}H]:[^{14}C] of the peptides is constant (±10%) within each experiment except for peptide αT_4 in expt 1. The total radioactivity of this peptide, however, is too low to make this difference significant.

Note that peptide αT_{6a} (von Ehrenstein, 1966) is not

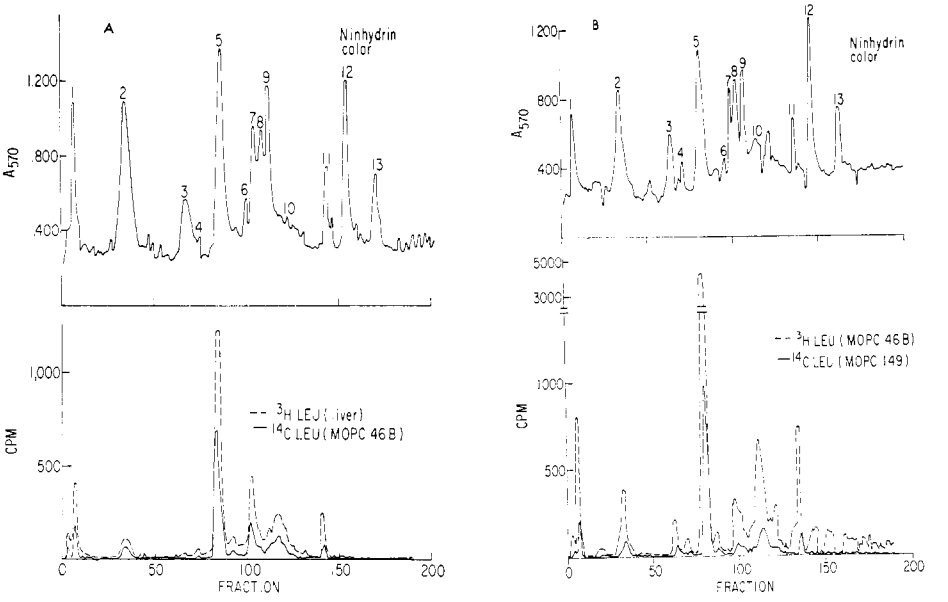

FIGURE 2: Dowex 50 × 8 chromatographic comparisons of the labeling of the tryptic peptides from hemoglobin α chains synthesized by rabbit cell-free system supplemented by isotopically labeled Leu-tRNA normal mouse liver and one mouse plasma cell tumor, MOPC 46B, and this tumor with a similar tumor, MOPC 149 [expt 1 (A), expt 2 (B)]. Peptides found in each ninhydrin-positive peak are as follows (using peptide nomenclature from Baglioni (1961) and von Ehrenstein (1966) and leucine-containing peptides in bold type): 1, **αT_{14}**; 2, **αT_1**, **αT_5**, **αT_9**, αT_{12}; 3, **αT_{1+2}**; 4, COOH-terminal of **αT_{13}** (Ala-Ser-Leu-Asp-Lys); 5, αT_2, **αT_4**, **αT_{10}**; 6, αT_8; 7, **αT_{6a}**, **αT_{8+9}**; 8, αT_{6b}, **αT_{11+12}**; 9, αT_3; 10, **αT_{13}**; 11, **αT_{11}**; 12, αT_7; 13, αT_{15}.

TABLE II: Labeling of Peptides of the α Chain of Rabbit Hemoglobin in the Reticulocyte Ribosomal System with Mouse Liver and Mouse Myeloma Tumor tRNA.

Expt		tRNA	Peptide[a] αT_1	αT_4	αT_5	$\alpha T_6 S$	αT_9	αT_{10}	$\alpha T_{11}+_{12}$	αT_{13}	αT_{14}
1	Total cpm	[^{3}H]Leucyl-tRNA liver	8	36	94	466	105	2050	74	1196	24
		[^{14}C]Leucyl-tRNA MOPC 46B	6	10	44	194	56	956	30	598	12
	Ratio [^{3}H]:[^{14}C]			(3.58)	2.20	2.39	1.85	2.16	1.75	2.01	2.01
	2 Total cpm	[^{3}H]Leucyl-tRNA MOPC 46B	<1	<1	330	<1	315	3419	115	Na[b]	Na
		[^{14}C]Leucyl-tRNA MOPC 149	<1	<1	80	<1	75	865	30	Na	Na
	Ratio [^{3}H]:[^{14}C]				4.1		4.2	3.9	3.8		

[a] Nomenclature adapted from Baglioni (1961) by von Ehrenstein (1966). [b] Not analyzed.

labeled with either isotope in expt 2 although leucine residues on both sides of this residue are labeled. Since tRNA from MOPC 46B is capable of transferring leucine into this peptide as shown in expt 1, some factor other than the tRNA must be responsible for this difference between the two transfers. Analysis of the peptides from the two experiments (not shown here) revealed a very small amount of αT_{6a} (Leu-Ser at positions 48 and 49) in the carrier isolated from expt 2 compared with the peptides of expt 1. In expt 2, the polyribosomal messenger (from a small number of rabbits) may have had a low content of leucine codons at position 48 of the α chain and, instead, consisted primarily of phenylalanine codons yielding a peptide with Phe-Thr at positions 48 and 49 (αT_{6b}). This result cannot be interpreted as an ambiguous translation since the ratios of incorporation of the two isotopes was always identical for the two paired comparisons studied here. Instead, it is consistent with recent findings (Hunter and Munro, 1969) that rabbit hemoglobin α-chain variants act as alleles.

Absence of Transaminoacylation between Leu-tRNAs from

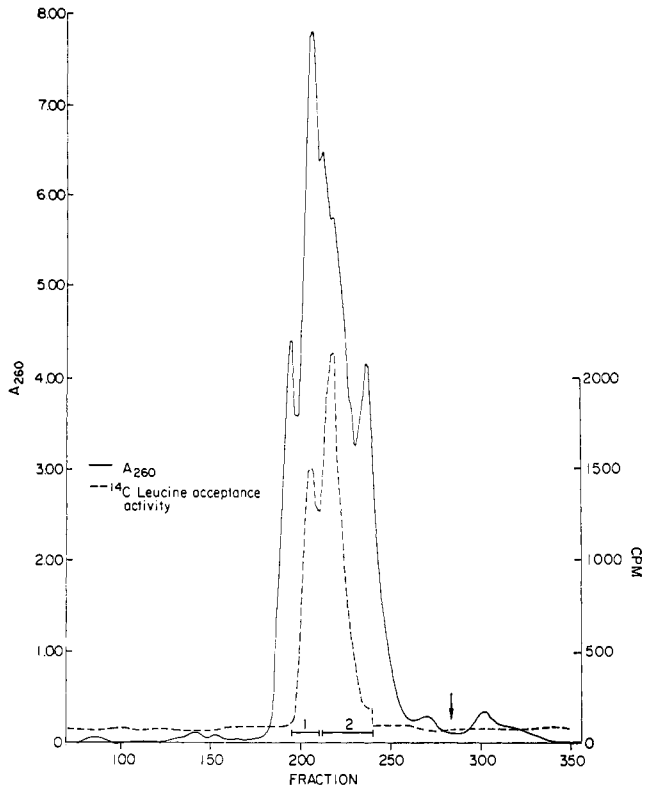

FIGURE 3: DEAE-Sephadex chromatography of rabbit liver tRNA. Solid line indicates absorbance at 260 mμ and dotted line indicates [^{14}C]leucine acceptance activity. Pools I and II, as indicated, were charged separately with [4,5-^{3}H]- and L-[U-^{14}C]leucine and used for *in vitro* transfer into rabbit hemoglobin (see Table IV). Arrow indicates end of gradient and start of elution with limit buffer.

TABLE III: Transfer of Leucine from Rabbit Liver tRNA Fractions into Rabbit Hemoglobin in the Reticulocyte Ribosomal System.[a]

tRNA Fractions	Specific Act. of tRNA (cpm of Leucine/ mg of tRNA × 10^4)	Total Added (cpm × 10^4)	Ribosomes OD_{260} Units	Transferred to the α Chain (cpm × 10^4)
[^{3}H]Leucyl-tRNA$_1^{Leu}$	3.1	20.3	1900	0.7
[^{14}C]Leucyl-tRNA$_2^{Leu}$	1.0	6.4		0.4

[a] Experimental reactions conditions are the same as in Table I footnotes *a* and *b*.

TABLE IV: Differential Labeling of Peptides of the α Chain of Rabbit Hemoglobin in the Reticulocyte Ribosomal System with [^{3}H]- or [^{14}C]Leucine Charged to Two tRNA^{Leu} Fractions from Rabbit Livers.

	Peptide	αT_{6a}	αT_{10}
Total cpm	[^{3}H]Leucyl-tRNA$_1^{Leu}$	4	1190
	[^{14}C]Leucyl-tRNA$_2^{Leu}$	42	307
Ratio [^{3}H]:[^{14}C]		0.09	3.88

Liver. The above experiments show no difference between two tumors tRNAs and between a tumor tRNA and a liver tRNA in the labeling of hemoglobin peptides. This result could have been obtained if there had been an exchange of leucine between the two tRNAs before transfer into polypeptide. This has been demonstrated in an *E. coli* system where different Leu-tRNA species are recognized by the same Leu-tRNA synthetase (Yamane and Sueoka, 1964), and such might well be the case for the combination of mouse tRNA^{Leu} and rabbit Leu-tRNA synthetase. Since a rabbit tRNA–rabbit enzyme combination would be even more likely to show transaminoacylation if the experimental conditions permitted such a process, we tested for this possibility in the completely homologous system.

Rabbit liver tRNA was preparatively fractionated on a DEAE-Sephadex column as described under Materials and Methods. As can be seen from Figure 3, the tRNA^{Leu} can be resolved into two peaks under these conditions. To test for differential labeling of hemoglobin peptides, [^{3}H]leucine was charged to material from peak I and [^{14}C]leucine was charged to material from peak II (Table III). After transfer, differential labeling is observed. As can be seen from Table IV peptide αT_{6a}, with one leucine residue, is labeled exclusively with [^{14}C]leucine from peak II tRNA. On the other hand, peptide αT_{10}, with five leucine residues, is labeled preferentially with [^{3}H]leucine from peak I tRNA although peak II does contribute some leucine here as well. We conclude that there is no exchange of leucine between tRNA species under our transfer conditions.

Discussion

Mouse tRNA, from normal or neoplastic tissue, serves to synthesize hemoglobin in a complete cell-free polyribosomal system from rabbit reticulocytes. The absence of transacylation under the conditions used establishes this system as a feasible means for testing the functional consequences of variations in tRNA revealed chromatographically.

In this report two transfer experiments were successfully performed with tRNA preparations which had been shown to differ quantitatively and qualitatively on reversed-phase chromatography. In both transfers, radioactive leucine was introduced into all the leucine-containing tryptic peptides except for those closest to the amino terminus of the hemoglobin α chain. The ratio of leucine isotopes, which reflects the relative contribution from each member of the pairs compared in a mixed-transfer experiment, remained constant for all peptides analyzed no matter whether the chromatographically similar pair of MOPC 46B and liver were used or the pairing was MOPC 46B with the peak-3-deficient MOPC 149. Therefore, the deficiency of peak 3 in MOPC 149 Leu-tRNA did not influence the labeling of the hemoglobin peptides.

This paper and a previous one (Mushinski and Potter, 1969) establish that different mouse plasma cell tumors have different stabilized chromatographic patterns for Leu-tRNA components. The pattern seems to be characteristic for each tumor, and some tumors (*e.g.*, MOPC 149) have marked deficiencies of Leu-tRNA components present in most other tumors and in normal liver. From the heterologous transfer experiments reported here, these variations do not appear to be meaningful for regulating or modifying hemoglobin α-chain messenger translation. Nonetheless, this does not rule out the possibility that these chromatographic differences represent meaningful, functional differences in the immunoglobulin-synthesizing system.

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