SEPARATION OF SEVEN LYSINE tRNA ISOACCEPTOR SPECIES AND THEIR RELATIONSHIP TO THE GROWTH STATE OF MAMMALIAN CELLS

by

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TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Transfer ribonucleic acid (tRNA) has the cellular function of serving as a link between the genetic word encoded in messenger ribonucleic acid and the corresponding amino acid. Hence, its role in the protein biosynthetic system of the cell is of prime importance. However, it is clear that tRNA is a multifunctional molecule with additional roles of fundamental importance to the cell.

Besides its function in protein biosynthesis, tRNA serves in bacteria (1) as well as in mammals (2), as a mediator in the non-ribosomal enzymatic transfer of certain amino acids to the amino terminal end of specific proteins. Also, in some gram-positive bacteria specific tRNA's work exclusively in the synthesis of cell wall peptidoglycans, such as $tRNA^{Gly}$ in Staphylococcus aureus (3). tRNA may also function as an allosteric effector of specific enzymes in procaryotes (4) and eukaryotes (5).

That tRNA could have a regulatory role in the biosynthesis of proteins has been suggested by several researchers (6,7,8). They have postulated that tRNA, when present in limiting amounts, may modulate the translation of the genetic message. This view is supported by experiments done using an in vitro hemoglobin synthesizing system which indicate that there are isoaccepting tRNAs for a single amino acid with a specificity for certain codons (9,10). It was also shown that the rates of synthesis of the alpha and beta chains are susceptible to variations in the specific concentration of tRNA isoacceptors (11). This effect also is observed with artificial messenger (12).

Biochemical and genetic experiments done in Salmonella typhimurium support the theory (18) that tRNA plays a role in the regulation of transcriptional events. The hisT mutant is deficient in a functional enzyme that forms pseudouridine in the anticodon stem of histidine tRNA (13) and leucine tRNA (14). In these cells this deficiency causes the constitutive production of the histidine (15,16) and isoleucine-valine (17) biosynthetic enzymes. The absence of an adequate repressor mechanism for these operons suggests that the pseudouridine modification is necessary for tRNA to work as a corepressor. Recently, it has been reported that a $tRNA^{Trp}$ system also plays a role in the repression of the tryptophan operon (18) in Escherichia coli. A complex formed by tryptophanyl-tRNA and its aminoacyl-tRNA synthetase or by the aminoacyl-tRNA synthethase and some other unidentified factor binds to a DNA region which apparently corresponds to the second transcriptional control site, the attenuator (19-22), located between the termination of the operator and the beginning of the first structural gene of the tryptophan operon. This site is different from the first transcriptional control site located at the leader region of the operator to which a tryptophan-repressor protein complex binds (20,22).

tRNA may also play a role in the inhibition of translation of exogenously added natural messengers to an in vitro protein synthesizing system from interferon-treated mouse L cells (23). The addition of purified fractions of mammalian tRNA restores the normal rate of translation of these messengers.

tRNA is a dynamic molecule in vivo, undergoing changes with various metabolic states (24). The most direct investigative procedure for studying differences in tRNAs involves chromatographic separation in one of

several systems in common use. Most of the studies described below followed such a procedure. Quantitative and qualitative changes in tRNA isoacceptor populations have been observed in differentiating systems: brine shrimp, insects and wheat seedlings (25); sea urchin (24,26); Drosophila (27); and cotyledons and hypocotyls of soybean seedlings (24). Several workers have analyzed tRNA populations from normal rat liver as compared to liver tumors. Zajdela ascitic hepatoma has several qualitative or quantitative differences from normal liver (28). A new leucine tRNA appears in liver of ethionine-treated rats; however, this alteration disappears in the ethionine-induced hepatomas (29). A fetal phenylalanine tRNA has been found in Morris hepatoma (30); however, it seems that the appearance of this species depends on the synthetase preparation used for aminoacylation (31); a crude preparation shows the presence of the extra species in normal liver as well, whereas a more purified preparation does not. Studies in an immunoglobulin producing plasma cell neoplasm suggested that tRNA may be involved in regulating antibody production (32,33,34). Different tyrosine and aspartic acid tRNAs were observed in a human myeloma cell line when compared to a normal lymphoblast cell line (35). An enzyme-dependent difference in glutamine tRNAs was observed in normal and leukemic human lymphoblasts (36); when tRNA was charged with an aminoacyl-tRNA synthetase-containing fraction from normal cells, an extra species of glutamine tRNA was observed. This difference was not observed when enzymes from leukemic cells were used for aminoacylation.

Viral infections also cause tRNA alterations. Herpes simplex infection of baby hamster kidney cells (37) and adenovirus infection of KB cells (38) altered the profiles of arginine tRNA. Evidence indicates

that the virus genome did not code for arginine tRNA itself and, consequently, was not the direct source of the alterations (38,39). It has been shown that avian myeloblastosis virus contains two kinds of RNA in addition to its 70S RNA: free 4S RNA and "70S associated" 4S RNA. Studies of base composition (40,41) and amino acid acceptor capacity (42,43) have shown that these RNAs are mostly lysine tRNA (44,45). There is also a preference to include lycine aminoacyl-tRNA synthetase in the virion (46). The major species of primer RNA utilized by the avian oncornavirus RNA-directed DNA polymerase for the initiation of DNA synthesis in vitro is a 4S RNA molecule with structural and functional features of tRNA (47-51). This species has been shown to be $tRNA^{Trp}$ as indicated by sequencing and aminoacylation (52) studies. Both, Rous sarcoma (53,54) and avian myeloblastosis (55) reverse transcriptases use host tryptophan tRNA as a primer.

This thesis will report observations on the variations of isoaccepting lysine tRNA patterns in different proliferative states of mammalian cells. Therefore, a survey of work related to lysine tRNA is presented. In general, only two major species of lysine tRNA are observed by different chromatographic systems. Developing brine shrimp (56) and sea urchin (57,58) show a new lysine tRNA. There is also a correlation between the presence of specific lysine tRNAs and the proliferative ability of cells (59); an isoacceptor fraction (in addition to the usually observed major species) was absent in non-dividing cells, such as brain, muscle, and lens cortex; it was present (8-12% of total) in bone marrow, spleen, Morris hepatoma and HTC cells. Larger amounts were observed in rapidly growing mouse leukemic cells.

Quantitative differences in lysine tRNAs, compared with controls, were found in livers of chickens treated with diethylstilbestrol (60) and livers of rats treated with thyroxine (61). Quantitative changes were reported in L-M cells in tissue culture when compared with L-M tumor cells (62). Changes also were observed in Morris hepatoma (30) and Zajdela ascitic hepatoma (28). In other related studies a mouse cell tumor had a larger third peak of lysine tRNA, whereas similar tumors and normal tissues have two major peaks and in some cases a small third peak (63). A comparison of SV-40-infected monkey kidney cells and mouse fibroblasts in tissue culture, using DEAE-sephadex chromatography, show only the two major lysine tRNA peaks in both biological systems. No significant differences were observed (64). Hybridization experiments indicated that the SV-40 genome does not seem to code for any of the observed lysine tRNAs. In another report, lysine tRNA from newborn mice (Balb/c) and SV-40-transformed Balb/3T3 cells in monolayer culture were compared using BD-cellulose (benzoylated DEAE-cellulose) chromatography (65). Only one peak was observed in newborn mice; two major peaks were found in SV-40 transformed cells.

In other studies using cells in monolayer culture, a significant increase in one of the isoaccepting aspartic acid tRNAs was found in polyoma-transformed rat cells (BN line) and polyoma- or SV-40-transformed mouse cells (A1/N line) (66). Changes in aspartic acid tRNA also were observed in SV-40-transformed and SV-40-infected monkey kidney cells (67), in hamster tumor derived by inoculation with SV-40-transformed cells, and in a human mammary tumor (68). Less striking changes have been found for leucine, isoleucine, phenylalanine, threonine, asparagine and histidine tRNAs (67).

Previous work in this laboratory (69) has shown the presence of a major extra species of lysine tRNA in polyoma-transformed 3T3 mouse fibroblasts (Py3T3 cell line). BD-cellulose column chromatography profiles of normal 3T3 cells and mouse embryo primary cultures show the presence of only two major lysine tRNA isoaccepting species as reported by most workers (26,27,36,59,60,63,64,70,71): peak I and peak II. The extra species present in Py3T3 cell cultures was labeled peak III. Further work in this sytem using RPC-5 chromatography (reverse phase chromatography system 5) yielded five well separated peaks of lysine tRNA when Py3T3 material was used. Four peaks were observed with lysine tRNA from 3T3 cells.

Part of the work presented in this thesis will deal with the accomplishment of the correlation between BD-cellulose and RPC-5 chromatographic profiles of lysine tRNA from Py3T3 cells. A combination of these two powerful chromatographic systems revealed the presence of a total of seven lysine tRNA isoaccepting species.

The other part of the work was directed to an analysis of the behavior of these isoacceptors in monolayer cultures of cells in different proliferative states. As mentioned before (59), a specific lysine tRNA isoaccepting species (peak 4) was found to vary with the proliferative conditions of the tissues studied. Since most of this work was done by comparing lysine tRNA populations from different tissues of the whole animal, it was of interest to look for this variation in monolayer cell culture systems which could be manipulated to provide growing and resting conditions in the same cells. Furthermore, the combination of two powerful chromatographic systems (BD-cellulose and RPC-5) allowed us to have a more complete picture of any changes in lysine tRNA isoacceptors.

MATERIALS

All reagents were analytical grade. Glass fiber filters (GF/C) were obtained from Whatman. Eagle's minimum essential medium (MEM), calf serum, penicillin, and streptomycin, were obtained from Grand Island Biological Company. ^{3}H-lysine (7 Ci/mM) and ^{14}C-lysine (312 mCi/mM) were obtained from Schwarz/Mann. Material for RPC-5 and BD-cellulose were prepared according to Kelmers et al. (72) and Gillam et al. (73), respectively. 3T3 and Py3T3 (polyoma virus-transformed 3T3) mouse fibroblasts were obtained from Dr. H. Green. Balb/3T3, KA31 (murine sarcoma virus-transformed Balb/3T3), and SV-T2 (SV-40 virus-transformed Balb/3T3) mouse fibroblasts were obtained from Dr. G. Todaro. A polyoma-free CR1 mouse strain obtained from Charles River Breeders, Brockton, Mass., was the source of livers and embryos for primary tissue cultures. ^{3}H-Lysyl-tRNA from leukemic mouse cells grown in suspension culture was kindly provided by Dr. B. J. Ortwerth (Department of Biochemistry, University of Missouri).

METHODS

Growth of Cells. Cells were grown in 700 cm^2 glass roller bottles at 37° in MEM containing penicillin G (100 U/ml) and streptomycin (100 ug/ml) and supplemented with 10% calf serum. The pH in cultures was held at physiological conditions by bubbling 5% CO_2 into the roller bottles every 48 hr. Sterile conditions were meticulously observed during cell growth.

Preparation of Primary Mouse Embryo Cultures. Pregnant mice in the late stages of gestation were asphyxiated by bottled carbon dioxide fumes and embryos were removed aseptically. The heads and extremities were removed from the embryos, and the remaining tissue was minced and rinsed in PBS (0.01 M sodium phosphate buffer, pH 7.2, 0.15 M sodium chloride) to remove red blood cells. The tissue was treated with trypsin (0.25% trypsin, 0.01 M sodium phosphate buffer, pH 7.2, 0.15 M sodium chloride) for 15 min periods at room temperature. The mixture was centrifuged at 600 x g for 10 min at 4°. Cells were resuspended in MEM containing 10% fetal calf serum to stop the activity of trypsin. This process was repeated 6 to 7 times to disperse individual cells from the remaining tissue. The cells in the final suspension were counted in an AO-Spencer hemacytometer. Approximately 20,000 cells/cm^2 were seeded in each 700 cm^2 roller bottle.

Preparation of tRNA. Cells were harvested from exponentially growing cultures unless otherwise indicated. Cells were scraped off the glass surface with a rubber policeman, washed with 20 ml of PBS per roller bottle, recovered in a Kolmer graduated tube by centrifugation for 10 min in a clinical centrifuge at setting 5, and resuspended in a 0.1 M Tris-HCl buffer solution, pH 7.5 (Tris buffer), using 2.5 ml of Tris buffer per

0.5 ml of packed cell volume. Cells then were lysed with 0.5% sodium dodecyl sulfate for 5 min at 0°; tRNA was extracted by vigorous stirring for 3 min at 60° with an equal volume of phenol saturated with Tris buffer. The aqueous phase was recovered by centrifugation of the mixture for 10 min at 16,000 x g. The phenolic phase was re-extracted with 0.5 volume of Tris buffer, the aqueous phase was recovered by centrifugation, and both aqueous phases were pooled. After adding 0.1 volume of 20% potassium acetate, tRNA was precipitated with 2.5 volumes of 95% ethanol. After an additional precipitation, tRNA was purified further on DEAE-cellulose (74), using approximately 0.5 ml of packed DEAE-cellulose per 0.5 ml of packed cell volume. Chromatography was accomplished in disposable plastic syringes: after the last centrifugation, tRNA was dissolved in Water's washing buffer (0.25 M NaCl, 0.01 M $MgCl_2$, 0.001 M EDTA) using 10 ml per 0.5 ml of packed cell volume; under these conditions, tRNA is retained on the column. Then, tRNA was eluted with 4 column volumes of Water's eluting buffer (0.7 M NaCl, 0.01 M $MgCl_2$, 0.001 M EDTA) and precipitated with ethanol after adding potassium acetate as before. After centrifugation, any attached amino acid was discharged by incubating the tRNA for 60 min at 37° in 1.8 M Tris-HCl, pH 7.8 (0.1 ml/0.5 ml of cell pack volume). After precipitation with 3 volumes of 95% ethanol, tRNA was recovered by centrifugation and dissolved in acetate buffer (0.004 M magnesium acetate, 0.04 M potassium acetate, pH 4.5). After two additional reprecipitations, tRNA was dried in vacuo and dissolved in deionized water. The concentration was determined by measuring the absorbance at 260 nm and assuming that 1 mg/ml of tRNA gives 24 A_{260} units when measured in a cell with a 1 cm path length.

Preparation of Aminoacyl-tRNA Synthetases. The cell pellet from 10 roller bottles was washed with PBS, suspended in 2 ml of Tris buffer, and broken in a Ten Broeck glass homogenizer with 15 gentle pestle strokes. The supernatant solution from a 10,000 x g centrifugation, was centrifuged again for 120 min at 105,000 x g. The final supernatant solution was passed through a 20 x 2 cm column of Sephadex G-100 (void volume 20 ml). After sample application, the column was eluted with Tris buffer, and after the first 15 ml of eluate, ten fractions of 3 ml were collected. Each of the ten fractions were assayed for lysyl-tRNA synthetase activity. The pooled active fractions were divided into 100 ul aliquots and stored at -70°.

Aminoacylation of tRNA. tRNA was aminoacylated in a 150 ul reaction containing: Tris-HCl, pH 7.2, 15 umoles; ATP, 0.2 umoles; magnesium acetate, 2 umoles; 2-mercaptoethanol, 5 umoles; 19 non-radioactive L-amino acids (not including lysine), 1 umole each; 25 uCi of ^{3}H-lysine or 2.5 uCi of ^{14}C-lysine; 0.5 A_{260} units of tRNA; and 40 ug of protein from the synthetase preparation. After incubation for 20 min at 37°, aminoacyl-tRNA was recovered by chromatography on a 0.2 ml column of DEAE-cellulose.

Chromatography on BD-Cellulose. Column dimensions were 110 x 0.9 cm. tRNA preparations from test and control systems aminoacylated with ^{3}H- and ^{14}C-lysine, respectively, were mixed with 2.0 mg of Escherichia coli B tRNA as carrier in 0.5 ml of acetate buffer (0.010 sodium acetate, pH 5, 0.010 M $MgCl_2$, 0.65 M NaCl, and 0.4% sodium azide). After sample application, the column was washed with 60 ml of acetate buffer. A 200 ml linear gradient from 0.65 M to 1.2 M NaCl in acetate buffer was used for elution. A final wash consisted of 60 ml of acetate buffer containing 1.5 M NaCl and 15% methoxyethanol. Fractions of 3 ml were collected; each fraction

received 120 ug of yeast RNA and was adjusted to 10% in trichloroacetic acid. The precipitate was collected by filtration on glass fiber filters (2.5 cm diameter), and the amount of radioactivity on the dried filters was determined in 5 ml of a toluene-based scintillation fluid. Absorbance at 254 nm was monitored throughout the column chromatography.

Chromatography on RPC-5. Column dimensions were 110 x 0.9 cm. A high pressure column system was used with pressure varying from 200 to 400 psi. tRNA preparations from test and control systems, aminoacylated with ^{3}H- and ^{14}C-lysine, respectively, were mixed and injected into the column previously equilibrated with acetate buffer (0.010 M sodium acetate, pH 5, 0.010 M $MgCl_2$, 0.5 M NaCl). After sample application, a 500 ml linear gradient from 0.5 M to 0.65 M NaCl in acetate buffer was started. Fractions of 4 ml were collected at a flow rate of 1.3 ml/min. Radioactivity in each fraction was determined in 15 ml of a toluene-based scintillation fluid containing 33% Triton X-100.

Protein Determination. Proteins were determined by Lowry's method (75).

RESULTS

BD-Cellulose Chromatographic Profiles of 3T3 and Py3T3 Lysine tRNAs. As observed in Fig. 1, BD-cellulose chromatography fractionates lysine tRNA from exponentially growing 3T3 cells into three fractions: peak I, IIa, and II. An extra species of lysine tRNA, peak III, is observed in Py3T3 cells. The data agree with the initial finding of an extra species of lysine tRNA in Py3T3 cells as reported by Jacobson et al. (69). An improved resolution in the peak II region shows the additional presence of peak IIa.

RPC-5 Chromatographic Profiles of 3T3 and Py3T3 Lysine tRNAs. The RPC-5 chromatographic profiles of lysine tRNA from exponentially growing cultures of 3T3 and Py3T3 cells are shown in Fig. 2. Lysine tRNA from 3T3 cells is fractionated into four clearly defined peaks: 1, 2, 4 and 5. It is shown below that peak 3 is masked in the peak 4 region. Py3T3 cells show an extra species of lysine tRNA: peak 6.

Correlation of BD-Cellulose and RPC-5 Chromatographic Results. Although the relative chromatographic position of RPC-peak 6 differs from the relative position of BDC-peak III, the possibility that the peaks were identical species of tRNA was tested. The general relationships of the rest of the peaks present in both types of chromatographic systems also was studied. An amount of Py3T3 lysyl-tRNA sufficient for recovery and rechromatography was fractionated on BD-cellulose (Fig. 3). The fractions between the arrows were pooled, concentrated, and rechromatographed in the RPC-5 system with unfractionated lysine tRNA from Py3T3 cells as a chromatographic marker. As shown in Fig. 4, BDC-peak I splits into RPC peaks 1,

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Figure 1.

BD-cellulose chromatography of 3T3 and Py3T3 lysine tRNA. For 3T3, 2,000 cells/cm^2 were seeded in 10 glass roller bottles of 700 cm^2 capacity; cells were harvested at a density of 57,000 cells/cm^2. For Py3T3, 2,000 cells/cm^2 were seeded in 5 glass roller bottles of 700 cm^2 capacity; cells were harvested at a density of 140,000 cells/cm^2. Chromatographic conditions were as described in Methods.

(———) ^{3}H-Lysine tRNA from 3T3 cells.

(-----) ^{14}C-Lysine tRNA from Py3T3 cells.

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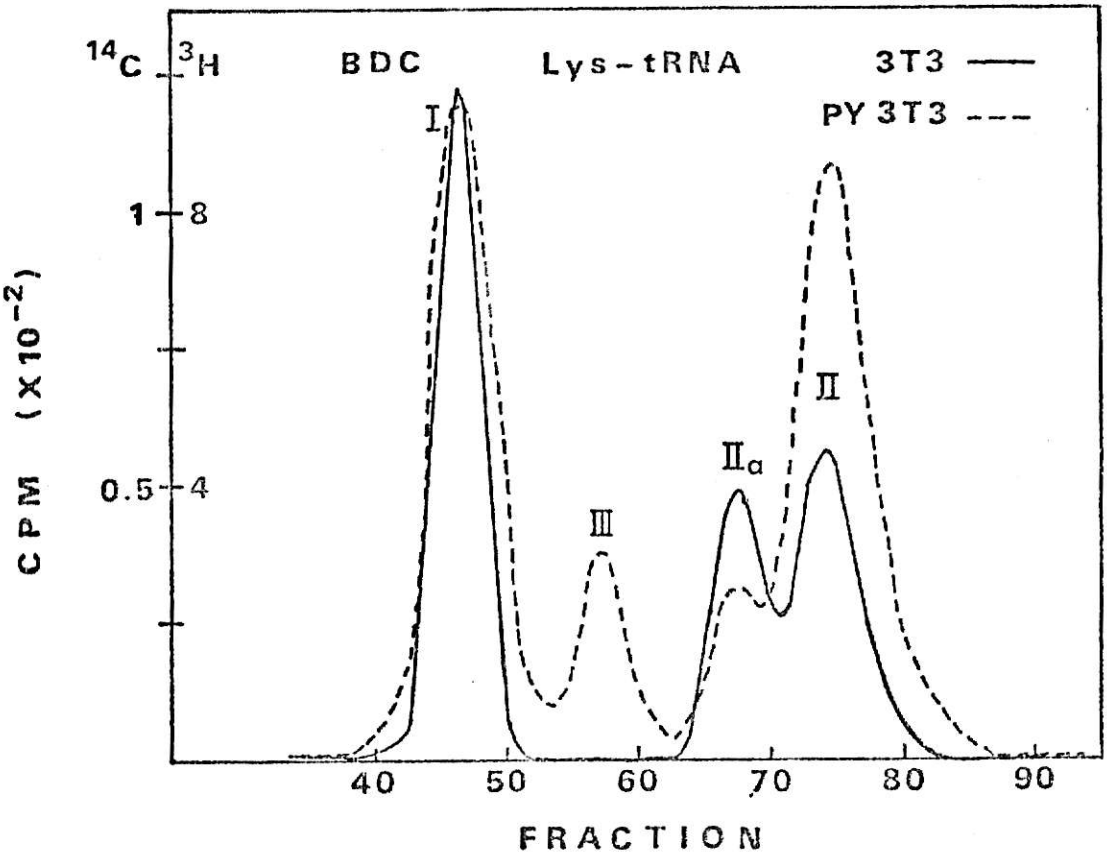

^{14}C ^{3}H
BDC
Lys-tRNA
3T3
PY 3T3
I
II
IIa
III
1
8
0.5
4
CPM (X 10^{-2})
40
50
60
70
80
90
FRACTION

Figure 2.

RPC-5 chromatography of 3T3 and Py3T3 lysine tRNA. Seeding and harvesting conditions were as in Fig. 1. Chromatographic conditions were as specified in Methods.

(———) ^{3}H-Lysine tRNA from 3T3 cells.

(-----) ^{14}C-Lysine tRNA from Py3T3 cells.

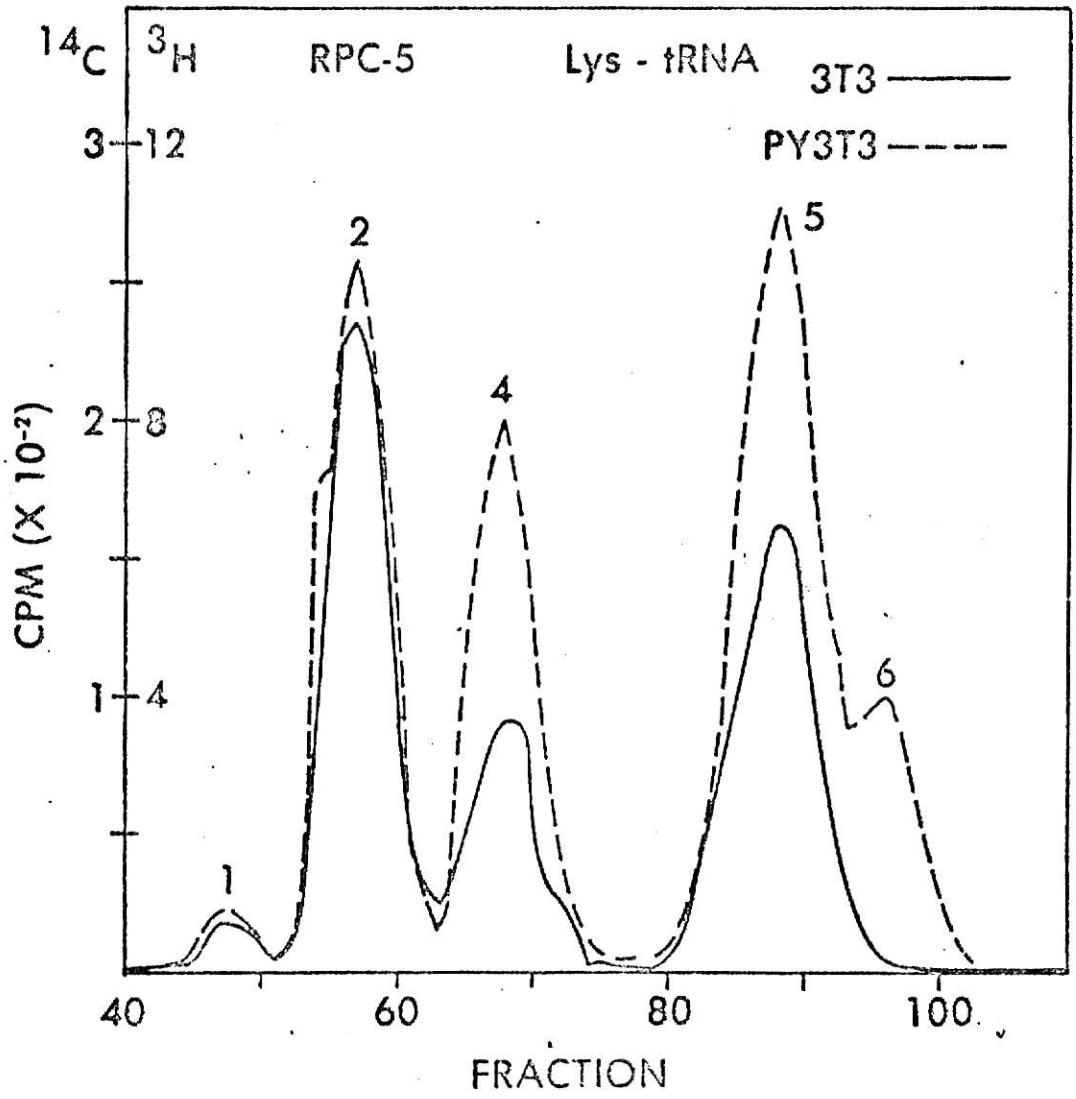

^{14}C ^{3}H
RPC-5
Lys - tRNA
3T3
PY3T3
3—12
2—8
1—4
CPM (X 10^{-2})
1
2
4
5
6
40
60
80
100
FRACTION

Figure 3

Preparative BD-cellulose chromatography of Py3T3 lysine tRNA. tRNA, 2.5 A_{260} units (five times the usual amount), was aminoacylated with ^{3}H-lysine and chromatographed on BD-cellulose. Fractions between the arrows were collected, pooled, and recovered by precipitation for rechromatography in the RPC-5 system.

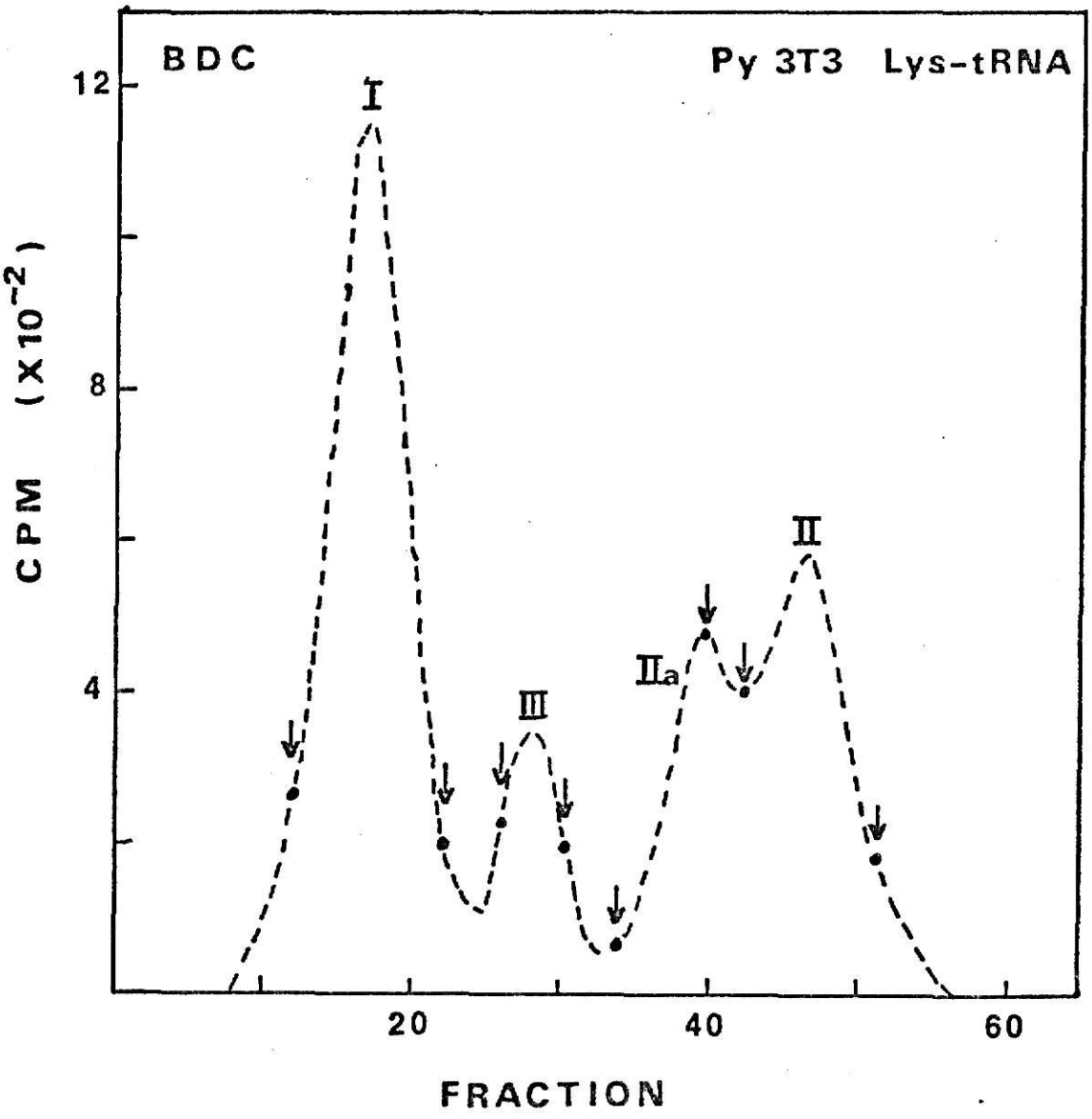
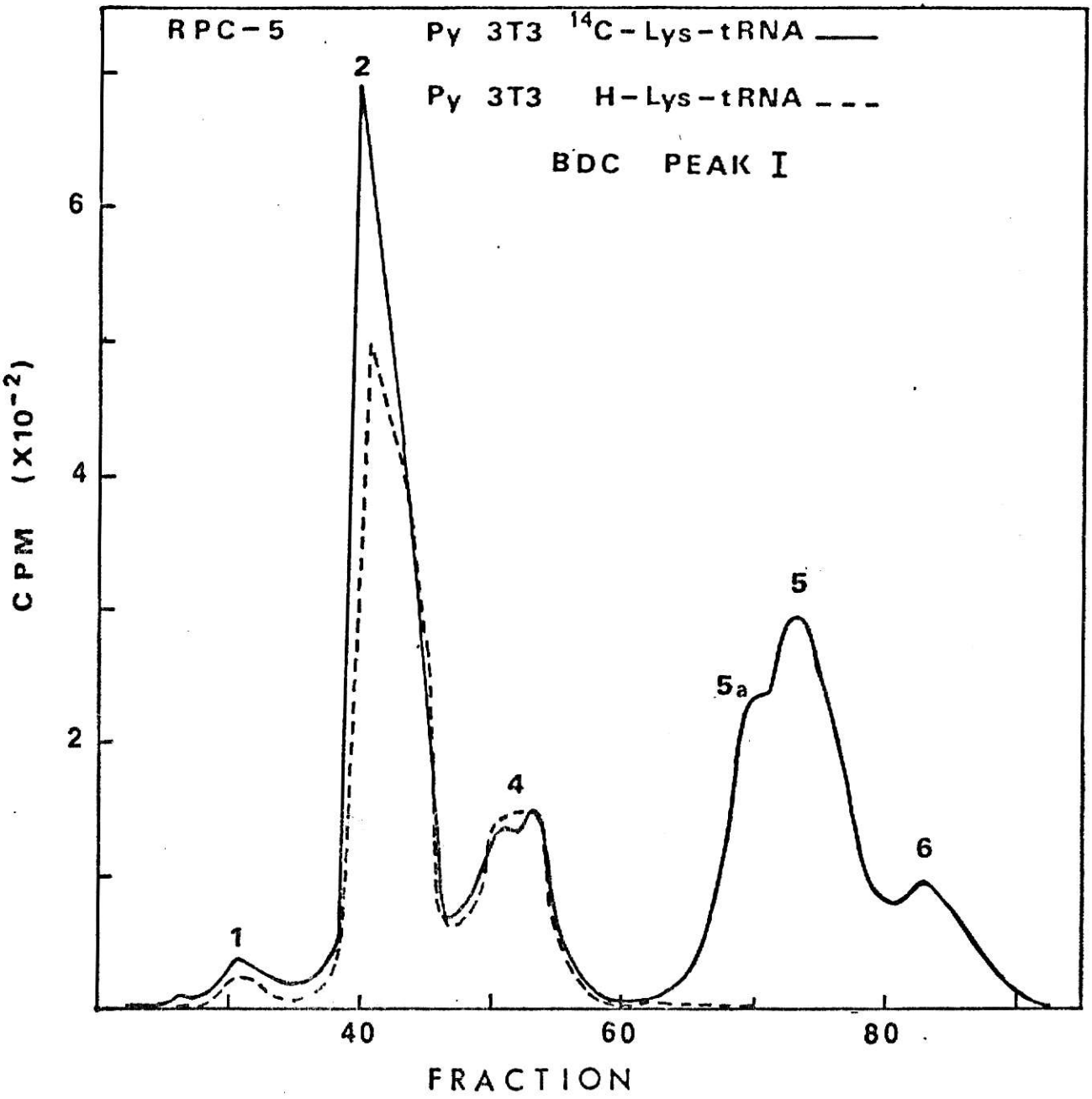

BDC
Py 3T3 Lys-tRNA
I
II
IIa
III
12
8
4
CPM (X10^{-2})
20
40
60
FRACTION

Figure 4

RPC-5 chromatography of BDC-peak I from Fig. 3 with lysine tRNA from Py3T3 cells.

(———) BDC-peak I ^{3}H-lysine tRNA.

(-----) ^{14}C-Lysine tRNA from Py3T3 cells.


RPC-5
Py 3T3 ^{14}C-Lys-tRNA
Py 3T3 H-Lys-tRNA
BDC PEAK I
CPM (X10^{-2})
FRACTION
1
2
4
5
5a
6
2
4
6
40
60
80

2 and 4. From Fig. 5, it is observed that BDC-peak II is RPC-peak 5, which shows a leading edge in the control. This edge, as shown in Fig. 6, corresponds to BDC-peak IIa. Finally, BDC-peak III splits into RPC-peaks 3 and 6 (Fig. 7).

The data are summarized in Table 1, and indicate the existence of at least seven lysine tRNA isoaccepting species: RPC peaks 1, 2, 3, 4, 5a, 5, and 6; peaks 5a and 5 are much better fractionated in the BD-cellulose chromatographic system into peaks IIa and II, respectively.

Influence of the Proliferative State of Cells on the Distribution of Lysine tRNAs. A significant difference in the distribution of peaks is observed when lysine tRNA from growing and resting mouse primary cell cultures are compared on RPC-5. Cells from whole mouse embryo and growing primary cells in culture have a significant amount of peak 4 (Fig. 8). On the other hand, comparison of growing primary cell cultures with resting primary cell cultures or adult mouse liver (Figs. 9 and 10) reveals almost no peak 4 in nongrowing cells. Moreover, peak 4 is the main species in rapidly dividing mouse leukemic cells growing in suspension (Fig. 11).

A summary of the data from Figs. 8, 9, 10, and 11 is provided in Table II. Peak 4 is five-fold larger in tRNA from growing mouse embryo cells when compared with tRNA from mouse liver, and it is almost three-fold larger in tRNA from growing primary mouse cells than in resting cultures. In both instances, a smaller amount of peak 2 accompanies the larger amount of peak 4.

Time Course of Variation of Lysine tRNA Species in Growing Cells. As the preceding data show, the populations of isoaccepting lysine tRNAs from resting and growing cells differ. KA31 cells (murine sarcoma

Figure 5

RPC-5 chromatography of BDC-peak II from Fig. 3 with unfractionated lysine tRNA from Py3T3 cells.

(-----) BDC-peak II ^{3}H-lysine tRNA.

(———) ^{14}C-lysine tRNA from Py3T3 cells.

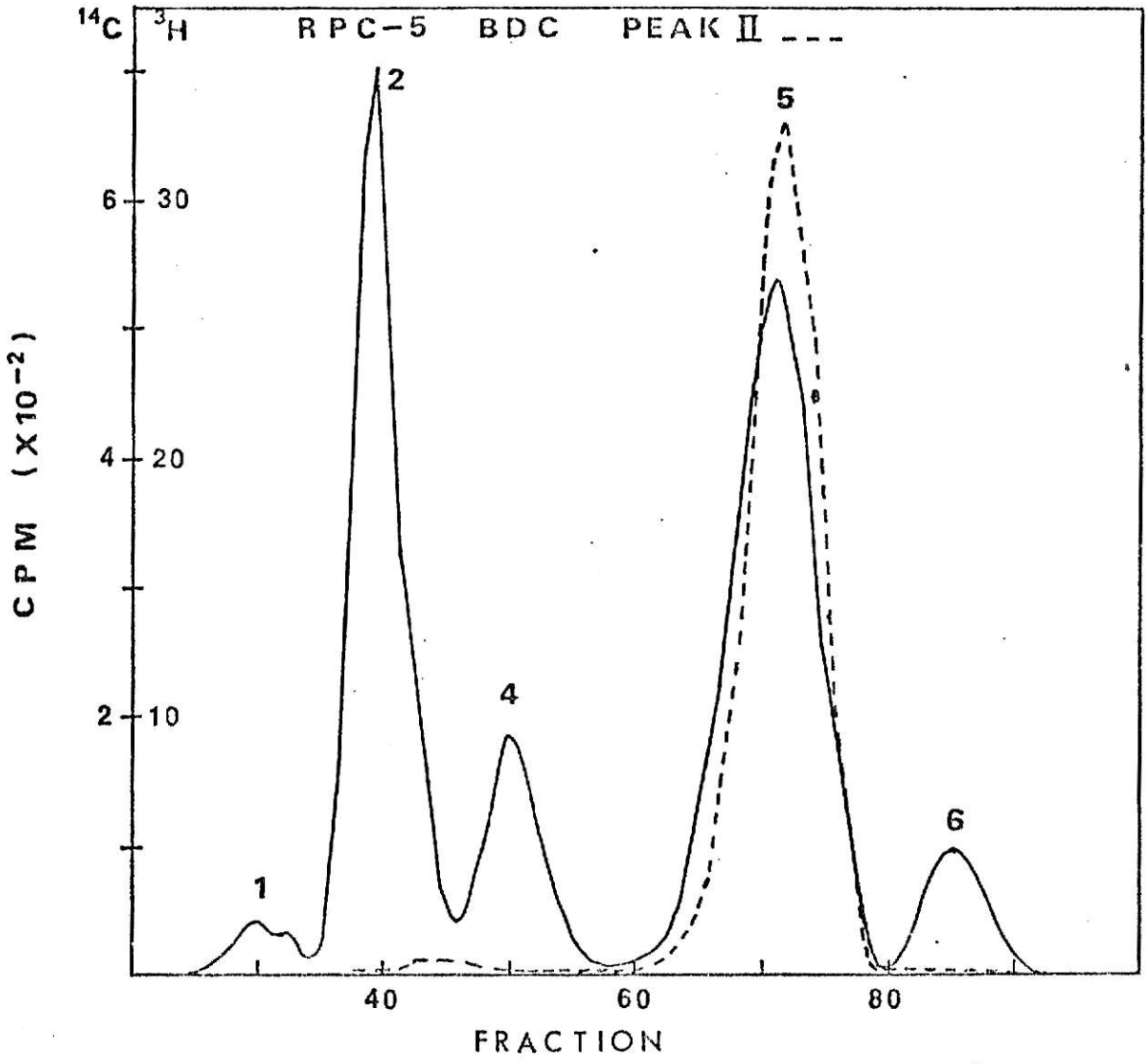

^{14}C ^{3}H RPC-5 BDC PEAK II ---
CPM (X10^{-2})
6
4
2
30
20
10
1
2
4
5
6
40
60
80
FRACTION

Figure 6

RPC-5 chromatography of BDC-peak IIa from Fig. 3 with unfractionated lysine tRNA from Py3T3 cells.

(-----) BDC-peak IIa ^{3}H-lysine tRNA.

(———) ^{14}C-lysine tRNA from Py3T3 cells.

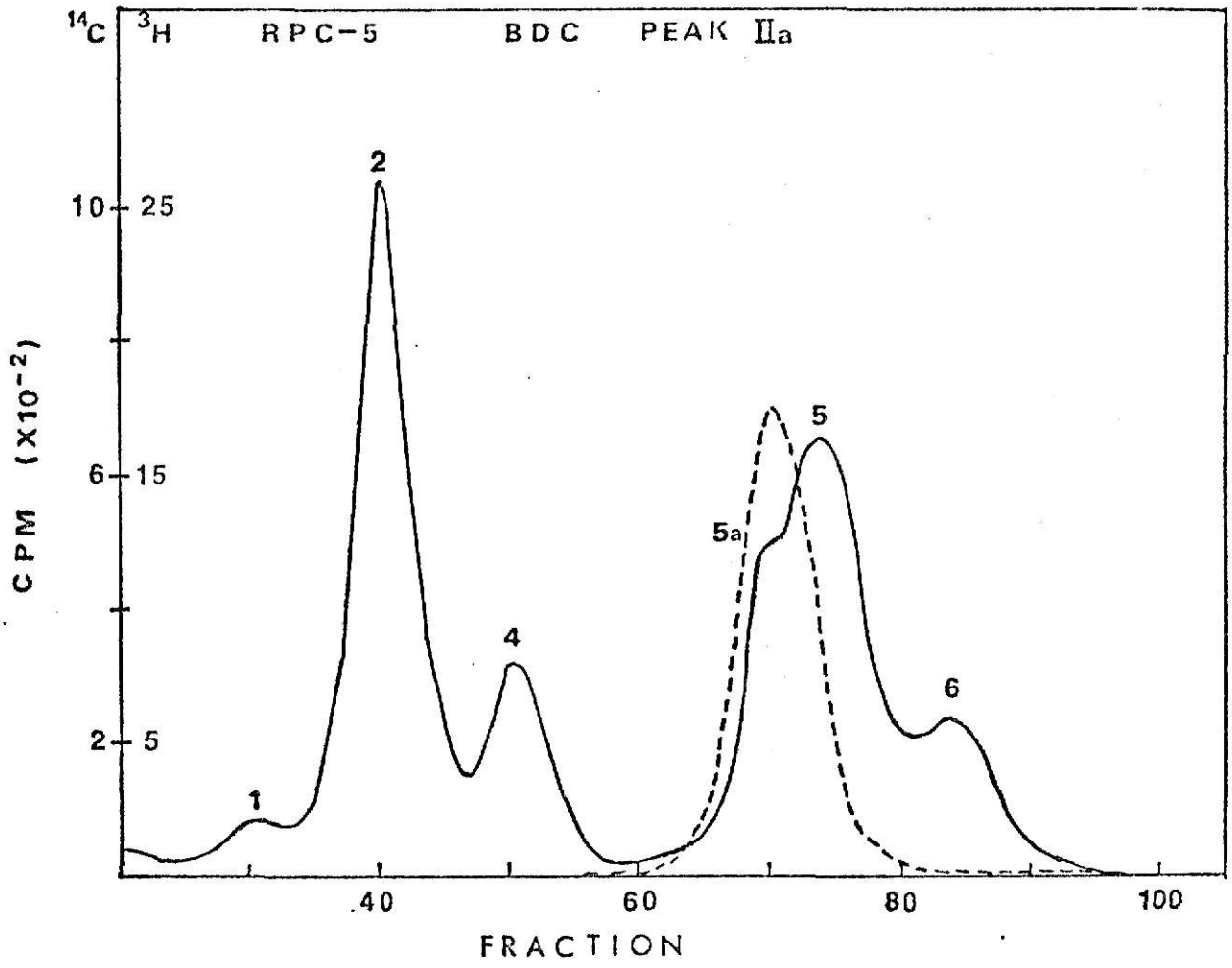

^{14}C ^{3}H RPC-5 BDC PEAK IIa
CPM (X10^{-2})
10
25
6
15
2
5
1
2
4
5
5a
6
40
60
80
100
FRACTION

Figure 7

RPC-5 chromatography of BDC-peak III from Fig. 3 with unfractionated lysine tRNA from Py3T3 cells.

(-----) BDC-peak III ^{3}H-lysine tRNA.

(———) ^{14}C-lysine tRNA from Py3T3 cells.

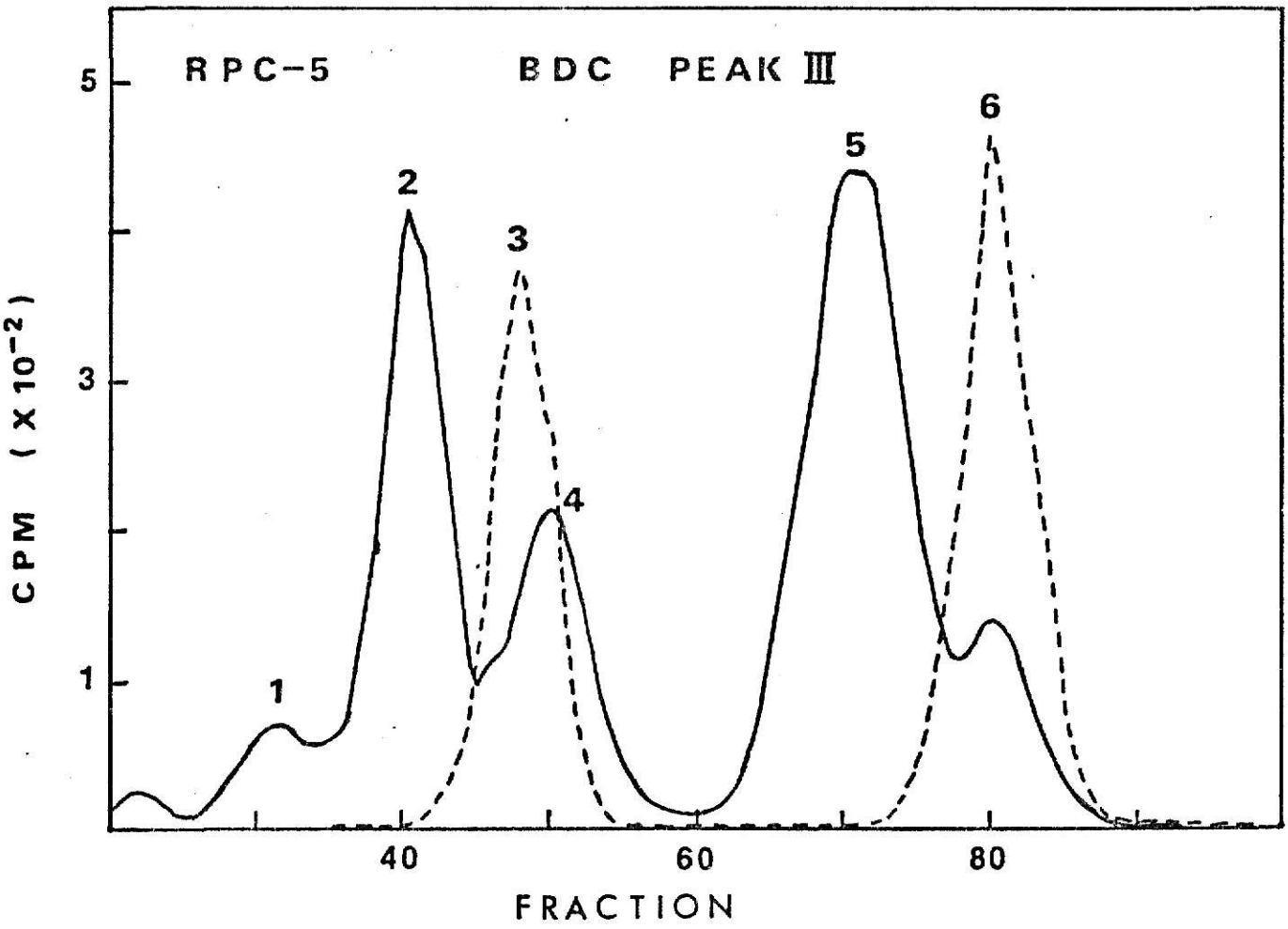

RPC-5
BDC PEAK III
1
2
3
4
5
6
5
3
1
CPM (X 10⁻²)
40
60
80
FRACTION

TABLE I

Comparison of Isoaccepting Lysine tRNA's by BD-Cellulose and RPC-5 Chromatography

BD-Cellulose peak	Corresponding peaks on RPC-5
I	1, 2, 4
II	5
IIa	5a
III	3, 6

Figure 8

RPC-5 chromatography of lysine tRNA from mouse embryo and growing primary cells. Cells were collected at a density of 55,000/cm^2.

(-----) ^{3}H-lysine tRNA from growing primary cells.

(——) ^{14}C-lysine tRNA from mouse embryo.

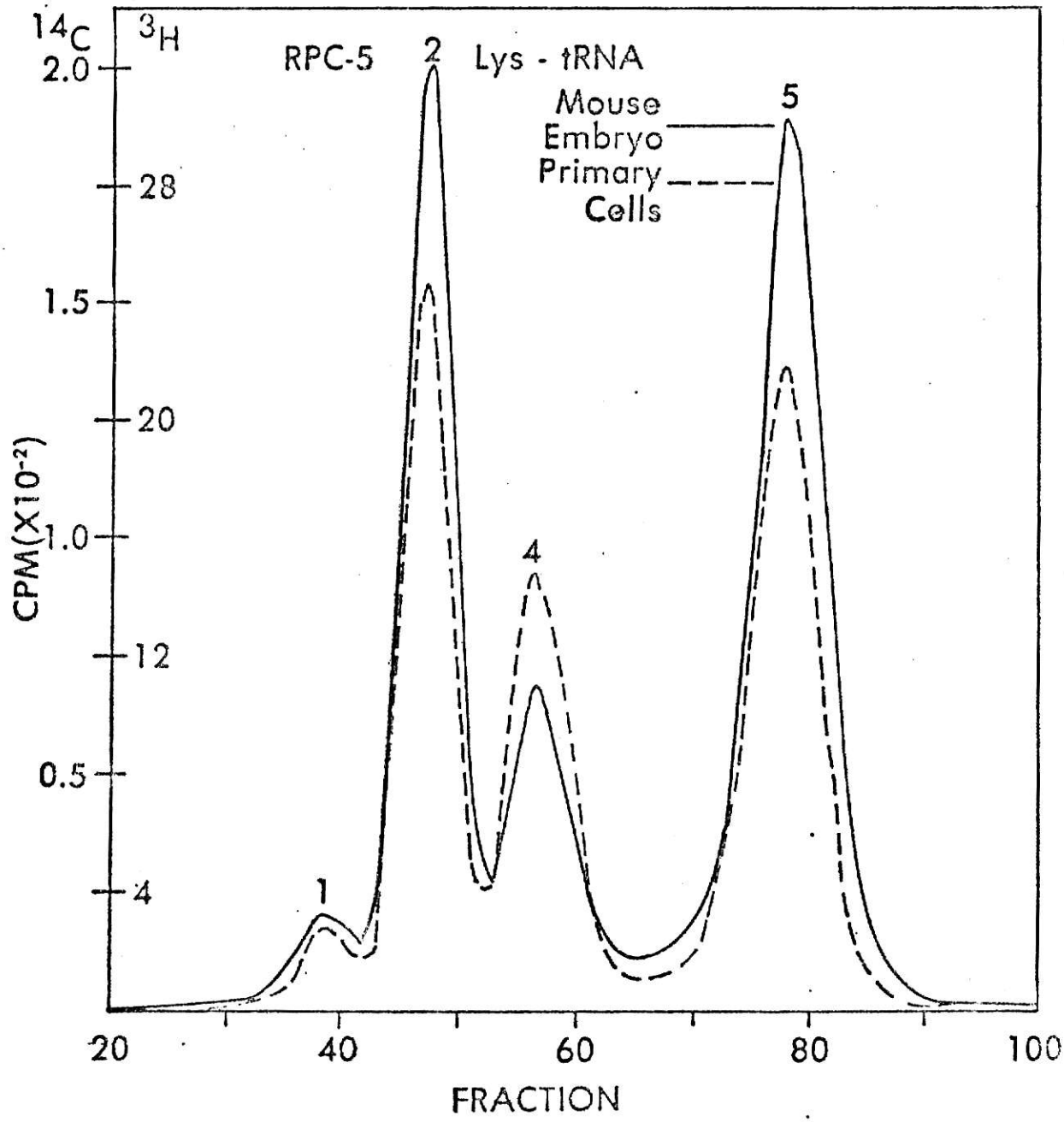

^{14}C
^{3}H
RPC-5
2
Lys - tRNA
Mouse Embryo Primary Cells
5
4
1
2.0
28
1.5
20
1.0
12
0.5
4
CPM(X10^{-2})
20
40
60
80
100
FRACTION

Figure 9

RPC-5 chromatography of lysine tRNA from growing and resting mouse primary cell cultures. Resting primary cell cultures were harvested at a density of 94,000 cell/cm^2.

(-----) ^{3}H-lysine tRNA from growing primary cells.

(———) ^{14}C-lysine tRNA from resting primary cells.

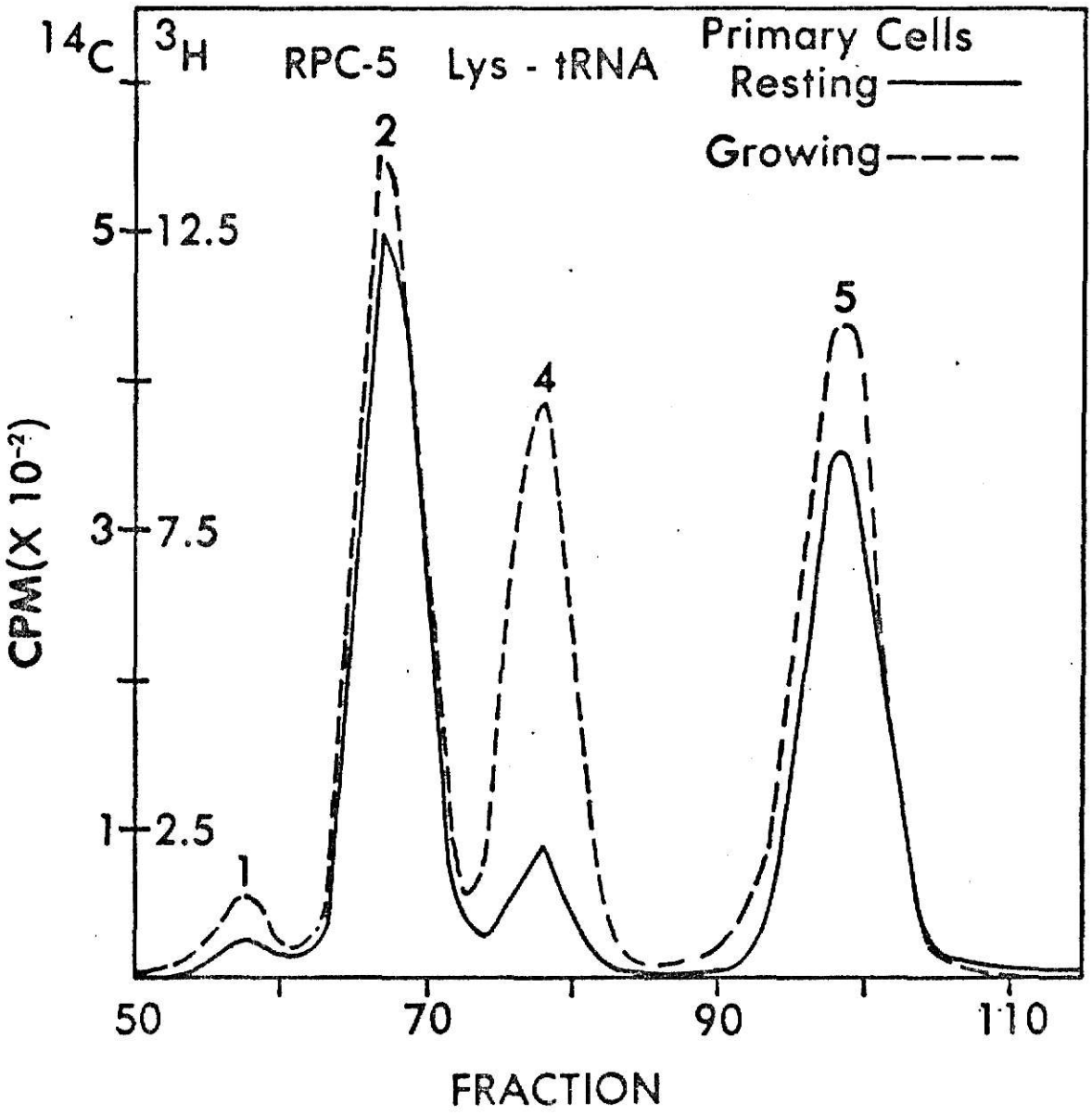

^{14}C ^{3}H
RPC-5 Lys - tRNA
Primary Cells
Resting
Growing
5
12.5
3
7.5
1
2.5
CPM(X 10^{-2})
1
2
4
5
50
70
90
110
FRACTION

Figure 10

RPC-5 chromatography of lysine tRNA from growing mouse primary cells with adult mouse liver.

(-----) ^{3}H-lysine tRNA from growing primary cells.

(———) ^{14}C-lysine tRNA from adult mouse liver.

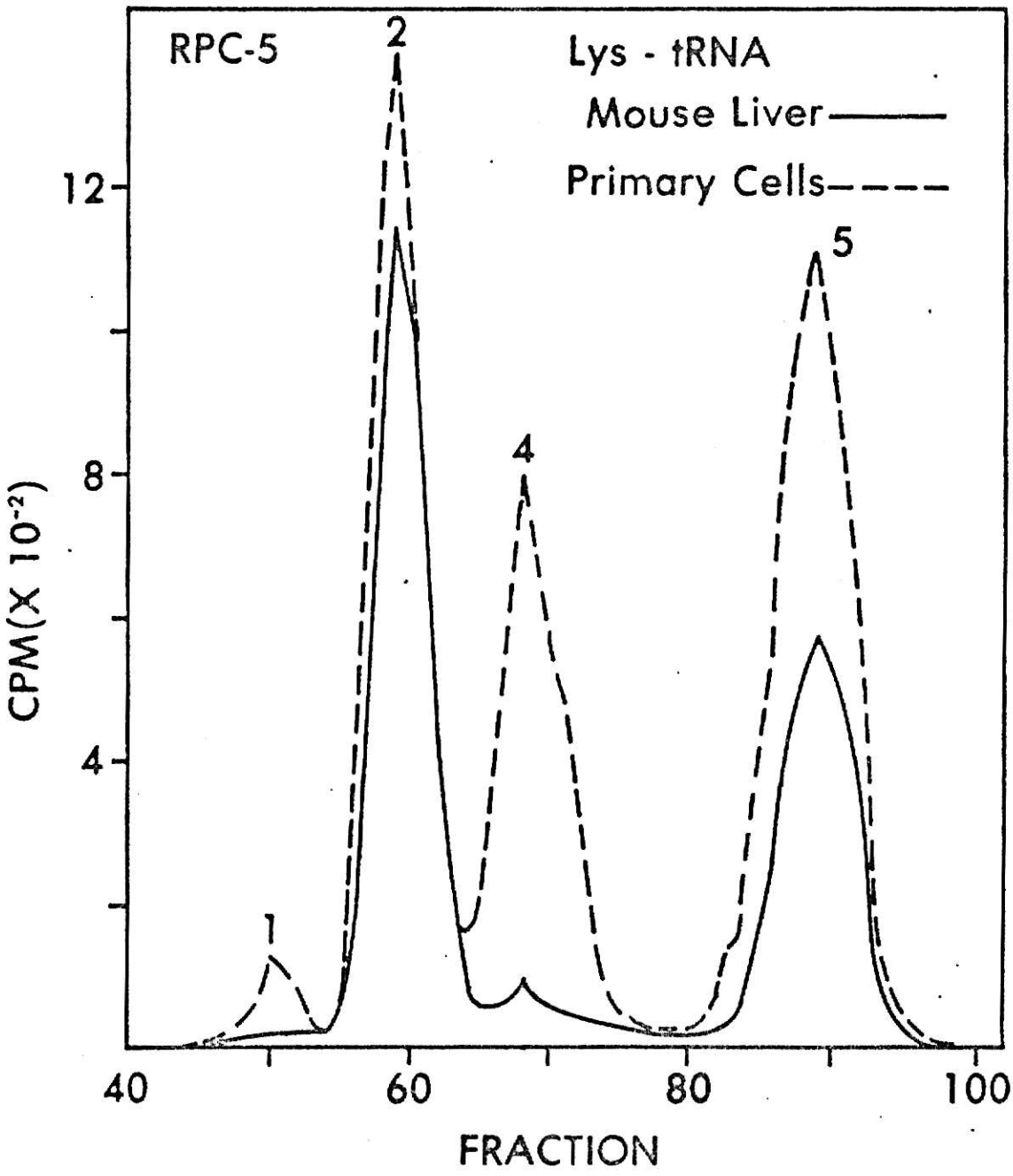

RPC-5
Lys - tRNA
Mouse Liver
Primary Cells
1
2
4
5
12
8
4
CPM(X 10⁻²)
40
60
80
100
FRACTION

Figure 11

RPC-5 chromatography of lysine tRNA from leukemic mouse cells grown in suspension and Py3T3 cells.

(-----) ^{3}H-lysine tRNA from leukemic mouse cells.

(———) ^{14}C-lysine tRNA from Py3T3 cells.

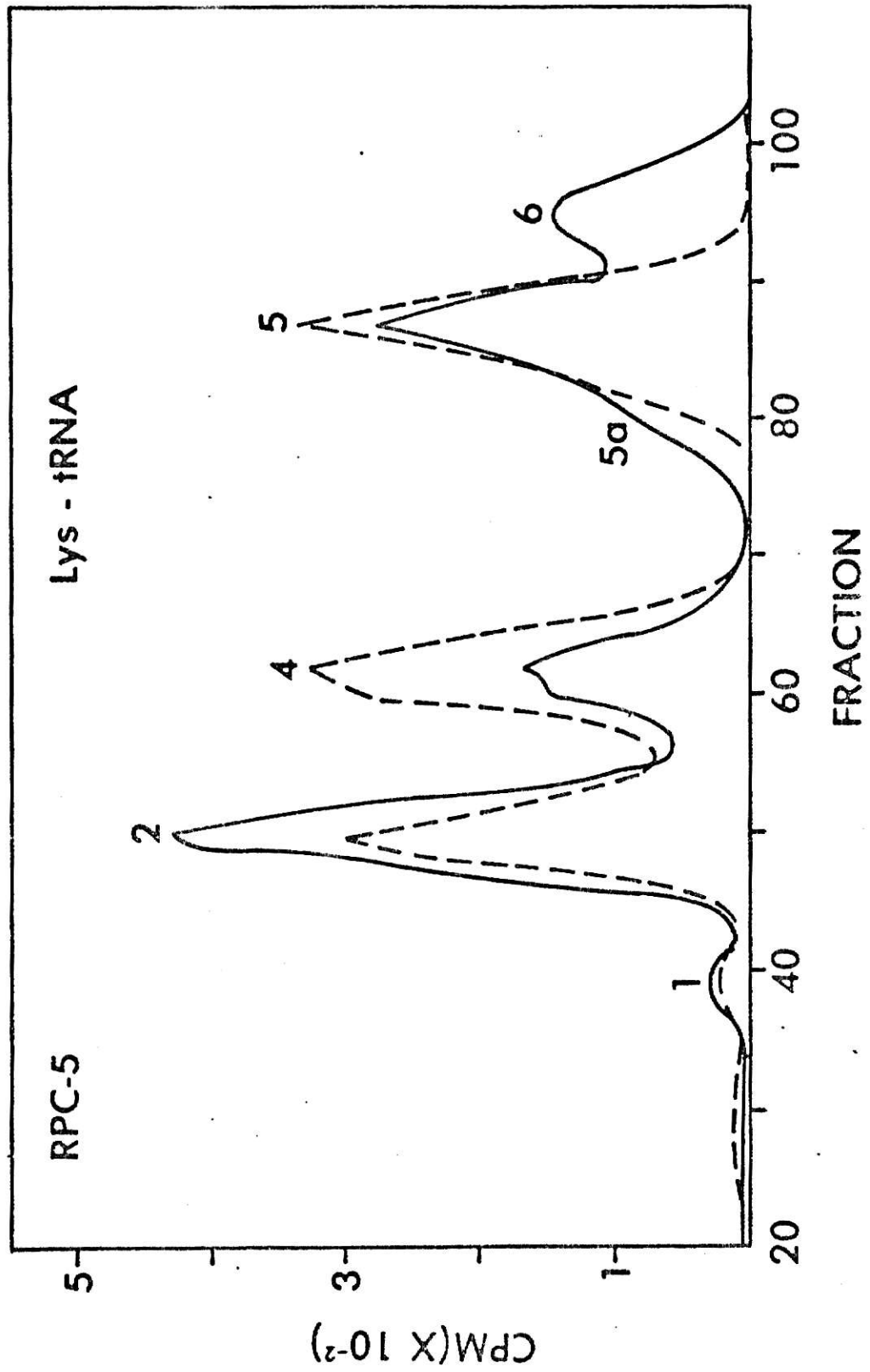

RPC-5
Lys - tRNA
1
2
4
5
5a
6
20
40
60
80
100
FRACTION
1
3
5
CPM(X 10-²)

TABLE II

Effect of State of Growth of Cells on Distribution of Isoacceptors of Lysine tRNA in the RPC-5 System

Cells	Growth State	Distribution of Lysine tRNA Isoacceptors % of total				
		1	2	4	5	6
Mouse liver	Resting	-	58	3	38	-
Mouse embryo	Growing	4	37	15	44	-
Primary embryo	Resting	2	50	9	40	-
Primary embryo	Growing	2	35	24	37	-
Mouse leukemia	Growing	1	28	37	35	-

virus-transformed Balb/3T3 cells) were used to look at the time course of these changes as cells grew to confluency. The distributions of lysine tRNA species from cells harvested at 24, 72, 96, and 120 hr after seeding as a subconfluent monolayer are shown in Table III. As expected, there is a significant decrease in the amount of peak 4 (from 20 to 5 per cent) as the culture reaches higher density; peak 2 increases from 25 to 38 per cent. In both cases there is about a 15 per cent variation; following the same pattern, peak 5 increases by the same amount that peak 6 decreases.

The distribution on BD-cellulose of lysine tRNA species from the same cells described above, is shown in Table IV. While peak I remains constant, peak IIa decreases from 13 to 0 per cent, and peak III decreases from 21 to 16 per cent, whereas peak II increases from 20 to 38 per cent. Again, the variations in peak IIa and III seem to complement the increase in peak II.

TABLE III

Time Course of Variation of Lysine tRNA Species in Growing Cells: RPC-5 Chromatography

Time After Seeding, hours	Distribution of Lysine tNRA Isoacceptors % of total				
	1	2	4	5	6
24	5	25	20	33	17
72	6	30	12	38	14
96	6	32	8	41	13
120	5	38	5	40	11

*KA31 cells were seeded at 20,000 cells/cm^2 in glass roller bottles (700 cm^2) at 0 time. Five roller bottles were used for each experimental point. The culture density at 120 hr was 72,000 cells/cm^2.

TABLE IV

Time Course of Variation of Lysine tRNA Species in Growing Cells: BD-Cellulose Chromatography*

Time after Seeding, hours	Distribution of Lysine tRNA Isoacceptors % of total			
	I	IIa	II	III
24	46	13	20	21
72	46	10	28	16
120	45	0	38	16

*The experiment was described in Table III.

DISCUSSION

The results presented in this thesis show a relationship between changes in the isoacceptor pattern of lysine tRNAs and the mitotic activity of cells. Previous work in Dr. B. J. Ortwerth's laboratory (59) have shown a correlation between the variation of one of the lysine tRNA isoaccepting species and the proliferative state of several tissues of the whole animal. Therefore, it was of interest to analyze this phenomenon in different growth stages of a single biological system. The use of monolayer tissue cultures of established cell lines and primary cells, instead of whole animal tissues, permitted carefully controlled cell growth conditions and a study of different aspects of the relationship of lysine tRNA isoacceptor patterns to cell density and contact inhibition of growth. Also, since mouse leukemic cells, analyzed in Dr. Ortwerth's laboratory (59), show a dramatically increased level of lysyl-tRNA peak 4, it was of interest to determine what levels of peak 4 were present in virus-transformed cell lines and whether, as in mouse leukemic cells, large amounts of peak 4 were peculiar to the transformed state. Furthermore, to maximally demonstrate variations in the lysine tRNA isoacceptor pattern, it was necessary to use a combination of two chromatographic systems. An increase in the resolving ability of BD-cellulose provided a good separation of peaks II and IIa. Peak IIa previously was visualized only as a shoulder on peak II (69). This was an important improvement, as peak IIa proved to be one of the peaks responding to changes in culture density. Although RPC-5 chromatography fractionates lysine tRNA into more peaks than does BD-cellulose, BDC-peak IIa is masked in the RPC-peak 5 region, rendering this system useless for

detecting changes in peak IIa. Such heterogeneity in this region of RPC-5 column chromatograms could explain the ambiguity of codon recognition experiments observed in different systems including mammalian tissues (76,77) and baker's yeast (78). In these systems, the late eluting major species of lysine tRNA (peak 5 in mammalian tissues and peak 2 in most other systems) responds preferentially to ApApA codon in a binding system with E. coli ribosomes. This result is consistent with the presence of a thiouridine residue at the "wobble" position of the anticodon (79). However, there is always a residual activity which binds to ApApG as well. Recently, Ortwerth and Carlson (80) have shown that, at least for rat liver lysine tRNA, peak 5 contains two isoacceptor species, 5 and 5_B. Peak 5 responds to ApApA, while 5_B binds to ApApG. Both seem to have a modified thiouridine residue as they both are sensitive to iodine (81) or CNBr (82) treatment. It is possible that peak 5_B could correspond to the RPC-5 peak 5a (BDC-peak IIa) reported here. I have shown, in preliminary work, that BDC-peaks II and IIa are sensitive to iodine oxidation, which suggests the presence of a thiouridine residue in both species. Several other reports involving RPC-5 chromatography of lysine tRNA are in the literature (83-88); although it is not obvious that these particular lysine tRNAs contained BDC-peak IIa, it would be of interest to explore this possibility.

As reported in Results, RPC-peak 4 also responds to the growth stage of cells. This peak is well separated from other isoacceptors in RPC-5 chromatography, but chromatographs with peak 2 on BD-cellulose. The results agree with other published reports showing the sensitivity of this peak to the mitotic activity of cells (76,86); peak 4 decreases several fold as the cell density of cultures increases. It is almost absent in nondividing

tissues like mouse liver (3%) and is present in considerable quantity in rapidly dividing tissues such as mouse embryo (15%). In addition to the systems reported here, the same phenomenon has been observed when comparing light and heavy cultures of Balb/3T3 and Py3T3 cell lines. As in the case of RPC-5 peaks 1 and 2, peak 4 is resistant to iodine oxidation and binds exclusively to ApApG triplet (76).

A striking result was the splitting of BDC-peak III into RPC-5 peaks 3 and 6. Peak III was previously reported as being present in Py3T3 and absent in "normal" 3T3 cells (69). This peak is present in KA31 and PyBalb/3T3 cell lines and absent in Balb/3T3 and SVT2 (SV-40 virus-transformed Balb/3T3) cell lines. The same is true for RPC-peak 6, which is present in Py3T3, KA31, PyBalb/3T3, and mouse leukemic cells and absent in 3T3, Balb/3T3 and SVT2 cells. RPC-5 peak 3 is present in small amounts in normal and transformed cell lines. RPC-peak 6 or BDC-peak III does respond to the growth state of the cell, although to a lesser degree than BDC-peak IIa or RPC-peak 4 (see Tables III and IV). The coding properties of RPC peaks 3 and 6 are unknown. The isoacceptor distribution is influenced in different ways by a number of variables such as serum age and source, pH, CO_2 or O_2 tension and perhaps the number of passages of the cells. More work is necessary to assess these effects.

The time course of changes in isoacceptors of KA31 cells (Tables III and IV) suggests that a precursor relationship exists between RPC peaks 4 and 2 as well as between BDC peaks III, IIa, and II. RPC peaks 2 and 5 are most probably the mature, fully modified species. The relationship between RPC peaks 4 and 2 seems rational because they both recognize the same triplet (ApApG), and peak 2 increases by about the same amount that

peak 4 decreases. Triplet binding assignments are not known for BDC peaks III and IIa, although, as discussed previously, there is the possibility that BDC-peak IIa is the peak 5_B described by Ortwerth and Carlson (80) which binds to ApApG. Complicating this simple view is the observation that peak II increases by about the same amount that the combination of peaks IIa and III decrease.

Obviously, definitive experiments are required to demonstrate precursor relationships, and fingerprinting, if not sequencing, of the isoacceptors would be preferred to biological experiments in mammalian cells, which lack the versatility of manipulation that is available with bacterial cells (89).

The results presented do not include experiments designed to provide insight into the purpose of variation in isoaccepting tRNA populations. Nevertheless, it is tempting to speculate that a reasonable function will include a mechanism related to regulation of cell growth. Data is accumulating in the literature showing that the tRNA population of cells is adapted for translation of specific messengers. tRNA-dependent cell-free systems from Ehrlich ascites cells translate efficiently globin mRNA, oviduct mRNA, and encephalomyocarditis (EMC) viral RNA when the system contained ascites cell tRNA. In contrast, reticulocyte tRNA could only promote the translation of globin mRNA and was inefficient for the translation of oviduct mRNA and EMC viral RNA (90). The data agree with early suggestions of functional specialization of reticulocyte tRNA for hemoglobin synthesis (91). The same correlation between function and tRNA content has been observed in reticulocytes of sheep (92), silk gland of silkworm during fibroin synthesis (93,94), the lactating mammary gland (95), rat fibroblasts

during collagen synthesis (96), bovine lens tissue synthesizing crystallin (97), and in rooster livers during estrogen-induced phosvitin synthesis (98). Thus, if the tRNA population of cells is adapted for a specific physiological state, the finding of changes in systems undergoing shifts in regulatory or physiological processes would be expected. As established in the introduction, such changes have been observed during embryonic development, differentiation, neoplasia (24,99), systems under the influence of hormones (100), virus infected cells (37) and bacteriophage-infected E. coli (101,102,103).

The data may be interpreted as an adaptation of the tRNA population of cells to the translation of new proteins, as has been suggested earlier in different forms of the modulation theory (6,7,8). Experiments in a cell-free protein synthesizing system dependent on exogenous lysine tRNA from various cell growth stages are necessary to test a modulation function or a direct effect of lysine tRNA isoacceptors.

It is necessary to emphasize that the data present an average of changes in a heterogeneous cell population in which cells are at different stages of the cell cycle. Experiments with synchronized cell cultures would yield a better understanding of the changes observed.

Another possibility for consideration is that lysine tRNA species sensitive to growth conditions may be acting as transcriptional regulators as is the case with histidine tRNA in Salmonella typhimurium (13-16). An experimental approach to explore this possibility currently is not feasible.

In conclusion, the lysine tRNA isoacceptor family is, apparently, highly responsive to changes in cell physiological conditions. On one hand, we have an extra species present in specific virus-transformed cell lines;

on the other, we have two more isoacceptor species responding to changes in cell density in normal and virus-transformed cell lines. The exploration of the interrelations and significance of these phenomena holds, without doubt, exciting possibilities for future work in this area.

REFERENCES

1. M. J. Leibowitz, and R. L. Soffer
Biol. Chem. 246 5207 (1971).

2. R. L. Soffer
Biol. Chem. 246 1602 (1971).

3. T. S. Stewart, R. J. Roberts, and J. L. Strominger
Nature 230 36 (1971).

4. G. W. Hatfield and R. O. Burns
Proc. Natl. Acad. Sci. U.S. 66 1027 (1970).

5. K. B. Jacobson
Nat. New Biol. 231 17 (1971).

6. H. Itano, in Abnormal Hemoglobins in Africa (J.H.P. Jonxis, ed.).
Oxford Univ. Press, London, p. 3 (1965).

7. B. N. Ames and P. Hartman,
Cold Spring Harbor Symp. Quant. Biol. 28 349 (1963).

8. G. S. Stent
Science, 144 816 (1964).

9. F. Gonano
Biochem. 6 977 (1967).

10. B. Weisblum, F. Gonano, G. von Ehrenstein, and S. Benzer
Proc. Natl. Acad. Sci. USA, 53 328 (1965).

11. W. F. Anderson and J. M. Gilbert
Biochem. Biophys. Res. Commun. 36 456 (1969).

12. W. F. Anderson
Proc. Natl. Acad. Sci. USA, 62 566 (1969).

13. C. E. Singer, F. R. Smith, R. Cortese, and B. N. Ames
Nature New Biol. 238 72 (1972).

14. H. S. Allaudeen, S. K. Yang, and D. Soll
FEBS Letters, 28 205 (1972).

15. S. Schlesinger and B. Magasanik
J. Mol. Biol. 9 670 (1964).

16. D. F. Silber, G. R. Fink, and B. N. Ames
J. Mol. Biol. 22 335 (1966).

17. M. Freundlich
Science, 157 823 (1967).

18. D. E. Morse and A. N. C. Morse
J. Mol. Biol. 103 209 (1976).

19. E. N. Jackson and C. Yanofsky
J. Mol. Biol. 76 89 (1973).

20. K. Bertrand and C. Yanofsky
J. Mol. Biol. 103 339 (1976).

21. K. Bertrand, L. Korn, F. Lee, T. Platt, C. L. Squires, and C. Yanofsky
Science, 189 22 (1975).

22. K. Bertrand, C. Squires, and C. Yanofsky
J. Mol. Biol. 103 319 (1976).

23. J. Content, B. Lebleu, A. Zilberstein, H. Berissi, and M. Revel
FEBS Letters 41 125 (1974).

24. N. Sueoka and T. Kano-Sueoka
Progr. Nucleic Acid Res. Mo. Biol. 10 23 (1970).

25. J. C. Bagshaw, F. J. Finamore, and G. D. Novelli
Developmental Biology 23 23 (1970).

26. C. Spadafora, T. Igo-Kemenes, and H. G. Zachau
Biochim. Biophys. Acta, 312 674 (1973).

27. B. N. White, G. M. Tener, J. Holden, and D. T. Suzuki
Dev. Biol. 33 185 (1973).

28. J. J. Befort, J. Mercier, N. Befort, G. Beck and J. P. Ebel
Biochimie, 54 1327 (1972).

29. R. Axel, I. B. Weinstein, and E. Farber
Proc. Natl. Acad. Sci. USA, 58 1255 (1967).

30. F. Gonano, F. Pirro, and S. Silvetti
Nature New Biol. 242 236 (1973).

31. J. F. Mushinski
Nature, 248 332 (1974).

32. B. Mach, H. Koblet, and D. Gros
Cold Spring Harbor Symp. Quant. Biol. 31 269 (1967).

33. W. K. Yang and G. D. Novelli
Proc. Natl. Acad. Sci. USA, 59 208 (1968).

34. J. F. Mushinski and M. Potter
Biochem. 8 1684 (1969).

35. S. Fujioka and R. C. Gallo
Blood, 38 246 (1971).

36. R. C. Gallo and S. Pestka
J. Mol. Biol. 52 195 (1970).

37. H. Subak-Sharpe, W. M. Shepherd, and J. Hay
Cold Spring Harbor Symp. Quant. Biol. 31 583 (1966).

38. K. Raska, Jr., D. H. Frohwirth, and R. W. Schlesinger
J. Virol. 5 464 (1970).

39. V. L. Morris, K. E. Wagner, and B. Roizman
J. Mol. Biol. 52 247 (1970).

40. K. Randerath, L. J. Rosenthal, and P. C. Zamecnik
Proc. Natl. Acad. Sci. USA, 68 3233 (1971).

41. M. Travnicek and J. Riman
Biochim. Biophys. Acta, 199 283 (1970).

42. G. S. Beaudreau, L. Sverak, R. Zischka, and J. W. Beard
Natl. Cancer Inst. Monograph, 17 791 (1964).

43. J. W. Garnegie, A. O'C. Deeney, K. C. Olson, and G. S. Beaudreau
Biochim. Biophys. Acta, 190 274 (1969).

44. L. J. Rosenthal and P. C. Zamecnik
Proc. Natl. Acad. Sci. USA, 70 1184 (1973).

45. L. J. Rosenthal and P. C. Zemecnik
Proc. Natl. Acad. Sci. USA, 70 865 (1973).

46. M. Travnicek and J. Riman
Nature New Biol. 241 60 (1973).

47. J.E.R. Dahlberg, R. C. Sawyer, J. M. Taylor, A. J. Faras, W. E. Levinson, H. M. Goodman, and J. M. Bishop
J. Virol. 13 1126 (1974).

48. A. J. Faras, J. E. Dahlberg, R. C. Sawyer, F. Harada, J. M. Taylor, W. E. Levinson, J. M. Bishop, and H. M. Goodman
J. Virol. 13 1134 (1974).

49. A. J. Faras and N. A. Dibble
Proc. Natl. Acad. Sci. USA, 72 859 (1975)

50. A. J. Faras, J. M. Taylor, W. E. Levinson, H. M. Goodman, and J. M. Bishop
J. Mol. Biol. 79 163 (1973).

51. R. C. Sawyer, F. Harada, and J. E. Dahlberg
J. Virol. 13 1302 (1974)

52. F. Harada, R. C. Sawyer, and J. E. Dahlberg
J. Biol. Chem. 250 3487 (1975).

53. W. R. Folk, and A. J. Faras
J. Virol. 17 1049 (1976).

54. J. J. Eiden, K. Quade, and J. L. Nichols
Nature 259 245 (1976)

55. L. C. Waters, B. C. Mullin, T. Ho, and W. K. Yang
Proc. Natl. Acad. Sci. USA, 72 2155 (1975).

56. J. C. Bagshaw, F. J. Finamore, and G. D. Novelli
Dev. Biol. 23 23 (1970).

57. S. S. Yang and D. G. Comb
J. Mol. Biol. 31 139 (1968).

58. J. G. Zeikus, M. W. Taylor, and C. A. Buck
Exptl. Cell Res. 57 74 (1969).

59. B. J. Ortwerth and L. P. Liu
Biochem. 12 3978 (1973).

60. W. F. Busby and P. Hele
Biochim. Biophys. Acta, 224 413 (1970).

61. S. S. Yang and D. R. Sanadi
J. Biol. Chem. 244 5081 (1969).

62. W. K. Yang, A. Hellman, D. H. Martin, K. B. Hellman, and G. D. Novelli
Proc. Natl. Acad. Sci. USA, 64 1411 (1969).

63. W. K. Yang
Cancer Res. 31 639 (1971).

64. T. Sekiya and K. I. Oda
Virol. 47 168 (1972).

65. F. H. Portugal, J. S. Simonds, D. Twardzik, and M. Oskarsson
J. Virol. 12 1616 (1973).

66. R. E. Gallagher, R. C. Ting, and R. C. Gallo
Biochim. Biophys. Acta, 272 568 (1972).

67. C. Vennegoor and H. Bloemendal
Europ. J. Biochem. 26 462 (1972).

68. W. T. Briscoe, W. Taylor, A. C. Griffin, R. Duff, and F. Rapp
Cancer Res. 31 1753 (1972).

69. E. L. Jacobson, H. Juarez, C. Hedgcoth, and R. A. Consigli
Arch. Biochem. Biophys. 163 666 (1974).

70. R. Tidwell, B. J. Bruce, and A. C. Griffin
Cancer Res. 32 1002 (1972).

71. M. W. Taylor, G. A. Granger, C. A. Buck, and J. J. Holland
Proc. Natl. Acad. Sci. USA 57 1712 (1967).

72. R. L. Pearson, J. F. Weiss, and A. D. Kelmers
Biochim. Biophys. Acta, 228 770 (1971).

73. I. Gillam, S. Millward, D. Blew, M. von Tigerstrom, E. Wimmer, and G. M. Tener
Biochemistry 6 3043 (1967).

74. W. K. Yang, and G. D. Novelli
Biochem. Biophys. Res. Commun. 31 534 (1968).

75. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall
J. Biol. Chem. 193 265 (1951).

76. B. J. Ortwerth, G. R. Yonuschot, and J. V. Carlson
Biochemistry, 12 3985 (1973).

77. E. Rudloff and K. Hilse
Eur. J. Biochem. 24 313 (1971).

78. G. C. Sen and H. P. Ghosh
Biochim. Biophys. Acta, 308 106 (1973).

79. G. C. Sen and H. P. Ghosh
Mucleic Ac. Res. e 523 (1976).

80. B. J. Ortwerth and J. V. Carlson
Arch. Biochem. Biophys. 178 278 (1977)

81. J. A. Carbon, L. Hung, and D. S. Jones
Proc. Natl. Acad. of Sci. USA, 53 979 (1965).

82. M. Saneyoshi and S. Nishimura
Biochim. Biophys. Acta, 204 389 (1970).

83. E. Rudloff and K. Hilse
Hoppe-Zeyler's Z. Physiol. Chem. 356 1359 (1975).

84. P. J. Christener and J. Rosenbloom
Arch. Biochem. Biophys. 172 399 (1976).

85. W. R. Woodward and E. Herbert
Science, 177 1197 (1972).

86. J. R. Katze
Biochim. Biophys. Acta, 407 392 (1975).

87. C. M. Palatnik, E. R. Katz, and M. Brenner
J. Biol. Chem. 252 694 (1977).

88. D. Hentzen
Cancer Res. 36 3082 (1976).

89. H. Juarez, A. C. Skjold, and C. Hedgcoth
J. Bact. 121 44 (1975).

90. O. K. Sharma, D. N. Beezley, and W. K. Roberts
Biochemistry, 15 4313 (1976).

91. D. W. E. Smith and A. L. McNamara
Science, 171 577 (1971).

92. M. Litt and D. Kabat
J. Biol. Chem. 247 6659 (1972).

93. J. P. Garel, P. Mandel, G. Chavaney, and J. Daillie
Biochimie, 53 1195 (1971).

94. P. Delaney and M. A. O. Siddiqui
Dev. Biol. 44 54 (1975).

95. A. Elska, G. Matsuka, U. Matiash, I. Nasarenko, and N. Semenova
Biochim. Biophys. Acta, 247 430 (1971).

96. K. W. Lanks and I. B. Weinstein
Biochem. Biophys. Res. Commun. 40 708 (1970).

97. J. P. Garel, N. Virmaux, and J. P. Mandel
Bull. Soc. Chim. Biol. 52 987 (1970).

98. P. H. Maenpaa and M. R. Bernfield
Biochemistry, 8 4926 (1969).

99. E. Borek and S. J. Kerr
Adv. Cancer Res. 15 163 (1972).

100. O. K. Sharma and E. Borek
Adv. Enzyme Regul. 12 85 (1974).

101. N. Sueoka and T. Kano-Sueoka
Proc. Natl. Acad. Sci. USA, 52 1535 (1964).

102. L. C. Waters and G. D. Novelli
Proc. Natl. Acad. Sci. USA, 57 979 (1967).

103. L. C. Waters and G. D. Novelli
Biochem. Biophys. Res. Commun. 32 971 (1968).

SEPARATION OF SEVEN LYSINE tRNA ISOACCEPTOR SPECIES AND THEIR RELATIONSHIP TO THE GROWTH STATE OF MAMMALIAN CELLS

by

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MASTER OF SCIENCE

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Previous work from this laboratory using benzoylated DEAE-cellulose (BD-cellulose) column chromatography has shown the presence of an extra species of lysine tRNA (peak *III*) *in polyoma virus-transformed mouse* fibroblasts (Py3T3 cell line). Others have reported that the amount of one of the isoacceptor species of lysine tRNA (peak 4) decreased as the proliferative rate of tissues decreased. Because this observation was *made using a reverse phase chromatographic system* (RPC-5), a correlation of both sets of results was not possible. Work presented in this thesis shows a correlation of BD-cellulose and RPC-5 column chromatographic systems, demonstrating the presence of a total of seven lysine tRNA isoacceptor species; peaks 1, 2, 3, 4, 5, 5a, and 6. To investigate the variation of these species with the proliferative state of the cell, monolayer tissue culture systems were used instead of whole animal tissues as in previous work. The tissue culture system made it possible to observe a single cell population at different proliferative stages. Also, through the use of virus-transformed cell lines, it was possible to investigate whether large amounts of peak 4 was a characteristic of tumor tissue as previously reported in leukemic mouse cells. It was found that peak 4 is present in considerable amounts (15%) in cell systems with a high-proliferative rate (frowing mouse embryo primary cultures and whole mouse embryo); this amount decreases drastically (3%) as mitotic activity decreases (resting mouse embryo primary cultures and adult mouse liver). Another lysine tRNA isoacceptor species was found to be as sensitive as peak 4 to cell *growth conditions*: peak 5a.

Peak 4 was present in about the same amount in different proliferating cell systems (mouse embryo primary cultures, whole mouse embryo, 3T3, Py3T3,

and KA31 cell lines), regardless of their normal or transformed states.

Peak 6, which corresponds to part of BD-cellulose peak III, was found to be present in specific virus-transformed cells (Py3T3, KA31 cell lines), and absent in 3T3, Balb/3T3, mouse embryo primary cultures, whole mouse embryo, adult mouse liver and kidney, and SV-40 virus-transformed mouse fibroblasts (SVT2 cell line).