Correlation between a Specific Isoaccepting Lysyl Transfer Ribonucleic Acid and Cell Division in Mammalian Tissues†

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ABSTRACT: Comparisons of the lysyl-tRNA patterns of several mammalian tissues were carried out with reversed-phase chromatography. One species of lysyl-tRNA, Lys-tRNA$_4$, was found to be present in variable amounts depending upon the proliferative rate of the tissue examined. This tRNA was absent in nondividing cells such as brain, muscle, and lens cortex, but was present in variable amounts in other tissues. Highest values were seen in bone marrow and spleen where Lys-tRNA$_4$ represented 8 and 12% of the total Lys-tRNA, respectively. Elevated levels of this tRNA were also seen in two Morris Hepatomas and in HTC cells and mouse leukemic cells grown in tissue culture. Mouse leukemic cells, grown in suspension cultures with a doubling time of 10 hr, had the maximum amount of Lys-tRNA$_4$. In these cells Lys-tRNA$_4$ was the major lysyl-tRNA and accounted for over 40% of the total.

Samples of neonatal rat liver tRNA showed slightly higher levels of Lys-tRNA$_4$ which decreased with age. Increases in Lys-tRNA$_4$ were seen in regenerating rat liver also, however, this was principally seen at 48 and 72 hr of posthepatectomy. Conversely, when mouse leukemic cells were continuously grown in the same culture media, Lys-tRNA$_4$ levels decreased with increasing cell density until at stationary phase, Lys-tRNA$_4$ had decreased threefold. From these results it is suggested that Lys-tRNA$_4$ is present in cells which are dividing, or can be stimulated to divide, but is absent in cells which lack this ability.

Several reports have appeared recently on the separation and coding properties of lysyl-tRNAs from mammalian tissues (Rudloff and Hilse, 1971; Volkers and Taylor, 1971; Liu and Ortwerth, 1972). From these studies it is becoming increasingly apparent that there are more isoaccepting species of lysyl-tRNA than there are code word assignments. The number of these isoaccepting tRNAs depends upon the separation system employed; however, multiple peaks have been seen with both benzoylated DEAE-cellulose (Taylor, 1970) and reversed-phase Freon chromatography (Rudloff and Hilse, 1971). During the past several years we have been studying the distribution of lysyl-tRNAs in many mammalian tissues by both reversed-phase Freon chromatography (RPC-2) and reversed-phase Plaskon chromatography (RPC-5). With these systems we have been able to detect at least two major and three minor species of lysyl-tRNA. One of these tRNAs, Lys-tRNA$_4$, has been shown to be present in various amounts in different mammalian tissues and in different experimental situations. A consideration of these data suggests that the level of this tRNA is proportional to the number of proliferative cells in all tissue examined.

Materials and Methods

Isolation of tRNA. Transfer RNA was isolated from all tissues by phenol extraction and purified by DEAE-cellulose chromatography as described previously (Ortwerth, 1971). Liver, spleen, and pancreas were removed from several rats, pooled, and homogenized in a Teflon-in-glass homogenizer with three volumes of buffer A (0.25 M sucrose, 0.05 M Tris-HCl (pH 7.5), 0.05 M KCl, 0.01 M $MgCl_2$, and 0.001 M Na_2-EDTA). Bentonite was added to a final concentration of 20 mg/ml when homogenizing with pancreas and 40 mg/ml with spleen to bind ribonuclease. No bentonite was added to rat liver since it contains an endogenous ribonuclease inhibitor. Calf livers were obtained from 1- to 2-day old animals shortly after death from the University of Missouri slaughterhouse. Each liver was homogenized with three volumes of buffer A in a high-speed Waring blender. Calf muscle tRNA was prepared as described previously (Ortwerth, 1971). Calf brain, fetal calf liver, and swine bone marrow were obtained fresh frozen from Pel-Freeze Biologicals, Rogers, Ark. Each tissue was homogenized directly with a high-speed Waring blender with buffer A. Bentonite was added to 20 mg/ml with the swine bone marrow and fetal calf liver and 40 mg/ml with the bovine brain. Fresh eyes were obtained from a local slaughterhouse within 1–2 hr after the death of the animal. The lenses were removed and nicked with a knife. This allowed the capsule to be cleanly removed from the rest of the lens. Using this method all the lens epithelial cells are removed with the capsule. The lens cortex was then removed from the lens nuclear region by punching out the hard nuclear region with a no. 9 cork borer. Lens capsules were homogenized with three volumes of buffer A and 20 mg/ml of bentonite using either a glass-in-glass homogenizer or a small Waring blender. Lens cortex was homogenized in a Waring blender with three volumes of a buffer containing 0.15 M KCl, 0.001 M Tris-HCl buffer (pH 7.5), 0.01 M $MgCl_2$, and 0.001 M Na_2EDTA. Morris Hepatomas 7800 and 9618A were grown in the legs of Buffalo rats. These rats were obtained as a gift from Dr. H. D. Brown, Ellis Fischel Cancer Research Center, Columbia, Mo. The tumors were excised, trimmed, and homogenized in a Teflon-in-glass homogenizer with three volumes of buffer A and 20 mg/ml of bentonite. 7288C HTC cells were grown in monolayers in Swim's S-77 medium supplemented with 1.8 mM $CaCl_2$, 2 mM glutamine, 0.5 mM cystine, 10 mM $NaHCO_3$, 50

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mM Tricine, 100 units/ml of penicillin G, 10% bovine serum, and 5% fetal calf serum. Under these conditions, the cells exhibited a doubling time of about 24 hr. The cells were harvested by centrifugation after trypsinization. The cells were broken with 20–30 strokes of a Dounce homogenizer in 10 ml of buffer A containing 20 mg/ml of bentonite and 0.5% sodium dodecyl sulfate. L5178Y mouse leukemic cells were grown in suspension cultures in Fischer's medium without bicarbonate supplemented with 5% horse serum. The cells were harvested by centrifugation and the tRNA was extracted in the manner previously described for HTC cells.

After homogenization, each tissue preparation was centrifuged at 30,000*g* for 15 min to remove nuclei and mitochondria. The supernatants were extracted with an equal volume of water saturated phenol at 0–5°. The aqueous layer was removed and the nucleic acids were precipitated with 2.5 volumes of ethanol. These nucleic acids were separated by DEAE-cellulose chromatography with a stepwise elution procedure. The nucleic acids eluting between 0.3 and 0.7 M NaCl were precipitated and used as the crude tRNA fraction.

The yield of crude tRNAs was extremely variable depending upon the tissue. About 2–3 A_{260} units/g of tissue was usual, but lens and erythrocyte gave 1 A_{260} unit/g of tissue, while rat liver and Morris Hepatoma had about 6–8 A_{260} units/g of tissue. Mouse leukemic cells contained about 1 A_{260} unit/5 $\times$ 10^8 cells. When smaller amounts of tissue culture cells were used, the tRNA was not purified by DEAE-cellulose chromatography, but was aminoacylated as a crude RNA extract.

All the tRNA samples were aminoacylated with either [^{14}C]lysine (312 Ci/mol) or [^{3}H]lysine (3.0 Ci/mmol) under conditions described previously (Ortwerth, 1971). Aminoacylation of all tRNA samples was carried out using a crude preparation of rat liver synthetases which was prepared by passing a 165,000*g* supernatant from rat liver over a Sephadex G-75 column to remove endogenous tRNA. The peak eluting in the void volume was pooled and stored as a 50% glycerol solution. Aminoacylated tRNAs were isolated from the reaction mixture by passage over a 5-ml DEAE-cellulose column according to the method of Yang and Novelli (1968). Each sample was adjusted to 0.3 or 0.5 M NaCl, 0.01 M sodium acetate (pH 4.5), 0.01 M $MgCl_2$, and 0.001 M Na_2EDTA and stored at $-75°$ in an ultrafreezer.

RPC-2 packing was prepared as described by Weiss and Kelmers (1967) and was used in the first part of this work. This was later replaced with RPC-5 packing which proved to be superior. RPC-5 packing was prepared as described by Pearson *et al.* (1971). Each column was run with a 2-l. salt gradient (0.3–0.6 M NaCl with RPC-2 packing and 0.5–1.0 M NaCl with RPC-5 packing). Included in each gradient were 0.01 M sodium acetate (pH 4.5), 0.01 M $MgCl_2$, 0.001 M Na_2EDTA, and 0.003 M β-mercaptoethanol. Fractions of 10 ml were collected. The tRNA was precipitated with Cl_3CCOOH and filtered on Millipore filters (0.45 μ pore size). In almost every separation the tRNA to be tested was run with a lysyl-tRNA preparation giving a known pattern, usually rat liver lysyl-tRNA, to monitor for any chromatographic abnormalities. Column recoveries of 80–100% were routinely obtained.

Results

The separation of lysyl-tRNA from various mammalian tissues has been reported using methylated albumin-kieselguhr (Taylor *et al.*, 1967), benzoylated DEAE-cellulose (Taylor, 1970), and reversed-phase chromatography (Rudloff and Hilse, 1971). Generally, two major species have been seen, however, in some cases greater heterogeneity was suggested by the data presented. Rudloff and Hilse (1971) have reported the presence of four lysyl-tRNAs from reticulocytes in separations using RPC-2. We have seen similar patterns from many mammalian tissues with RPC-2 and particularly with RPC-5. Figure 1 shows a composite separation of lysyl-tRNA as obtained in our laboratory. Lys-tRNA$_1$ is always seen, but is never more than 1–2% of the total lysine acceptance. Lys-tRNA$_2$ and Lys-tRNA$_5$ are the two main species previously reported by many workers. Lys-tRNA$_3$ is a minor species which is present in some rapidly dividing tissues, but is never present at a level greater than 2–3% of the total lysine profile. The novel tRNA which is the subject of this paper is Lys-tRNA$_4$ which always elutes about halfway between Lys-tRNA$_2$ and Lys-tRNA$_5$. The relative amount of this tRNA is variable and appears to correlate with the rate of cell division in the tissue examined.

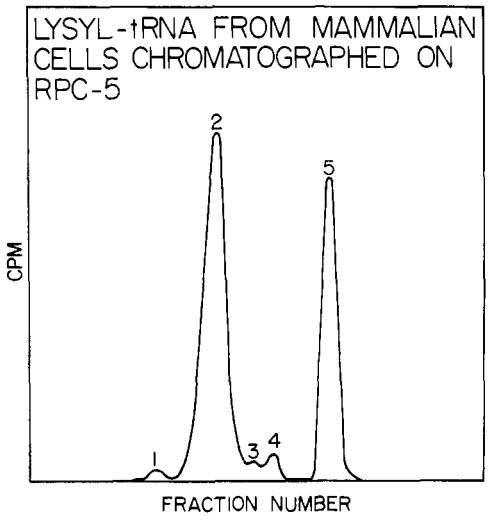

FIGURE 1: Composite profile of Lys-tRNA from mammalian tissues on RPC-5.

This correlation of Lys-tRNA$_4$ with cell division is presented in Figure 2. This figure shows the levels of Lys-tRNA$_4$ seen in 12 different tissues which we have examined. Panel A shows that there is essentially no Lys-tRNA$_4$ in lens cortex cells. These cells do not carry out any cell division in the lens and most of the cells have lost their nuclei during development. The profiles obtained from muscle and brain (panels B and C) show little or no Lys-tRNA$_4$. This is consistent with the almost complete lack of cell division in these tissues. Lens epithelial cells are found exclusively on the outside of the lens. In this cell layer, only the cells at one location, the germinative zone, are able to divide. In adult animals the rate of division of these cells is very slow, but cell division does continue. The tRNA from this tissue does show the definite presence of Lys-tRNA$_5$, but it only amounts to 3.3% of the total (panel D). The same thing is true for rat liver where cell division is very slow in adult animals. Likewise Lys-tRNA$_4$ is only 3.2% of the total (panel E). A slightly higher value of 5.9% was seen for rat pancreas (panel F); however, when we looked specifically at tissues which are responsible for cell proliferation, even higher values were obtained. The tissues which could be obtained in sufficient quantity were bone marrow and spleen. Correspondingly, both of these tissues had increased levels of Lys-tRNA$_4$. The

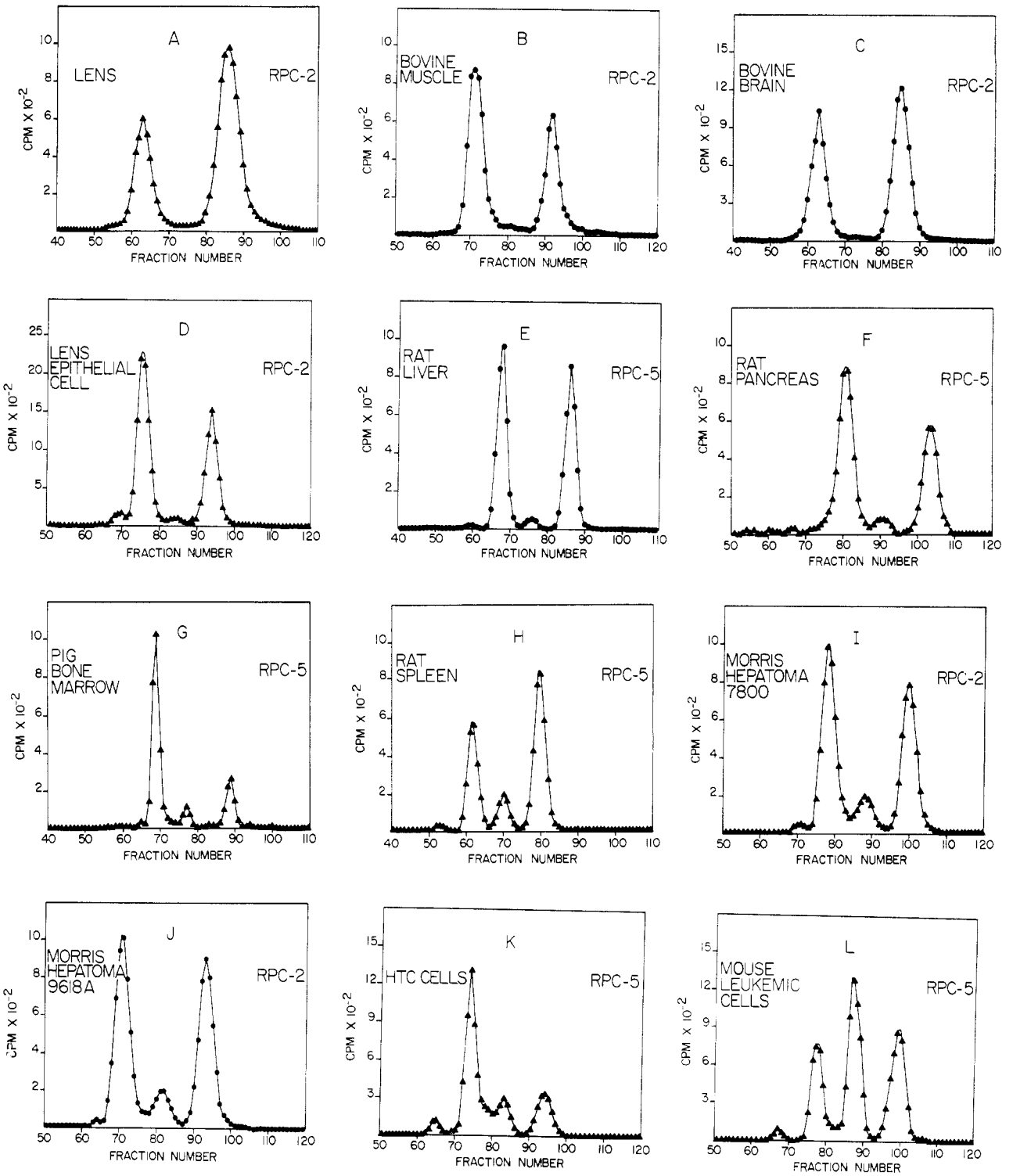

FIGURE 2: Profiles of Lys-tRNA from various mammalian tissues, hepatomas, and tissue culture cells. Each sample of either ^{3}H (▲) or ^{14}C (●) Lys-tRNA was run on either RPC-2 or RPC-5 as indicated. Two liter gradients from 0.3 to 0.6 M or 0.5 to 1.0 M NaCl were run with RPC-2 (1 × 240 cm column) and RPC-5 (2.5 × 100 cm column), respectively. Fractions of 10 ml were collected in every case.

pattern for bone marrow is shown in panel G and Lys-tRNA$_4$ accounts for 7.2% of the total Lys-tRNA. Spleen Lys-tRNA is shown in panel H and Lys-tRNA$_4$ accounts for 12.2% in this tissue. In a similar way, we examined the Lys-tRNA patterns in several tumors. As shown in panels I and J, Lys-tRNA$_4$ is elevated in two Morris Hepatomas accounting for 9.8% of the total in Hepatoma 7800 and 10.7% in Hepatoma 9618A. Both of these are slow-growing, minimal deviation Hepatomas requiring roughly 2- and 4-months generation time for 7800 and 9618A, respectively. As an example of more rapidly dividing cells, we looked at tumor cells grown in monolayer (HTC cells) and tumor cells grown in suspension cultures (mouse leukemic cells). HTC cells (panel K) had still higher levels of Lys-tRNA$_4$ reaching 15–16% of the total; however, the most striking example is shown in panel L. In this panel it can be seen that Lys-tRNA$_4$ is the major

TABLE I: Variation in Lys-tRNA$_4$ in Developing Rat Livers.

Age of Rats (Days)	Lys-tRNA$_4$ (% of Total)	
	Expt	Adult[a]
10	4.6	3.4
15	4.4	2.7
20	3.8	3.0
30	3.5	3.0
40	3.9	3.3

[a] Each sample of Lys-tRNA from developing rat livers was chromatographed with a sample of adult rat liver Lys-tRNA as control.

species of Lys-tRNA in mouse leukemic cells. These cells have a doubling time of only 10 hr and are dedifferentiated cells. In this case Lys-tRNA$_4$ makes up 40–45% of the total Lys-tRNA.

The profiles presented in Figure 2 appear to be an accurate reflection of the tRNA^{Lys} population in each tissue. Rat liver tRNA isolated both with and without the use of phenol extraction gave identical profiles. Similarly, crude rat liver tRNA and rat liver tRNA purified by DEAE-cellulose chromatography gave identical results. Degradation due to the presence of nucleases in the crude rat liver synthetase preparation seems unlikely due to the presence of high concentrations of a ribonuclease inhibitor protein in rat liver tissue. Also, assays of the rat liver synthetase preparation for ribonuclease activity were negative.

The possibility that tRNA$_4^{Lys}$ might be formed or affected by the presence of tRNA modifying enzymes in the crude rat liver synthetase also appears doubtful. When tRNA preparations from rat liver, calf liver, Morris Hepatoma, and mouse leukemia cells were chromatographed on RPC-5 and then assayed to determine the tRNA^{Lys} acceptance profile, the amounts of tRNA$_4^{Lys}$ were identical with those obtained by aminoacylation and then chromatography. Further tRNA$_4^{Lys}$ does not represent an inactive configuration of a major tRNA^{Lys} since it can be isolated, discharged, and recharged again. This material also elutes in the original position upon rechromatography.

The results in Figure 2, therefore, appear to be an accurate reflection of tRNA^{Lys} distribution in the various tissues and strongly suggest that tRNA$_4^{Lys}$ may be associated with cell division in some manner. It is possible, however, that some other correlations exist which we have not considered. To strengthen our argument we felt it was necessary to alter the rate of cell division within a given tissue and then see what changes occurred in Lys-tRNA$_4$. The first situation we considered was the postnatal development of rat liver. It is known that there is an increase in rat liver cell number after birth (Greengard *et al.*, 1972). We, therefore, looked at the Lys-tRNA profiles of the livers from 10-, 15-, 20-, 30-, and 40-day old rats. In each case adult rat liver Lys-tRNA was also run on the same column. The amount of Lys-tRNA$_4$ was measured and these data are presented in Table I. These results show that young rats have slightly increased amounts of Lys-tRNA$_4$ which decreases with age approaching adult levels. An average of 15 determinations of the percentage of Lys-tRNA$_4$ in adult rat liver gives a value of 3.2 ± 0.2% as a standard deviation. These values were obtained using several different perparations of tRNA and multiple preparations of

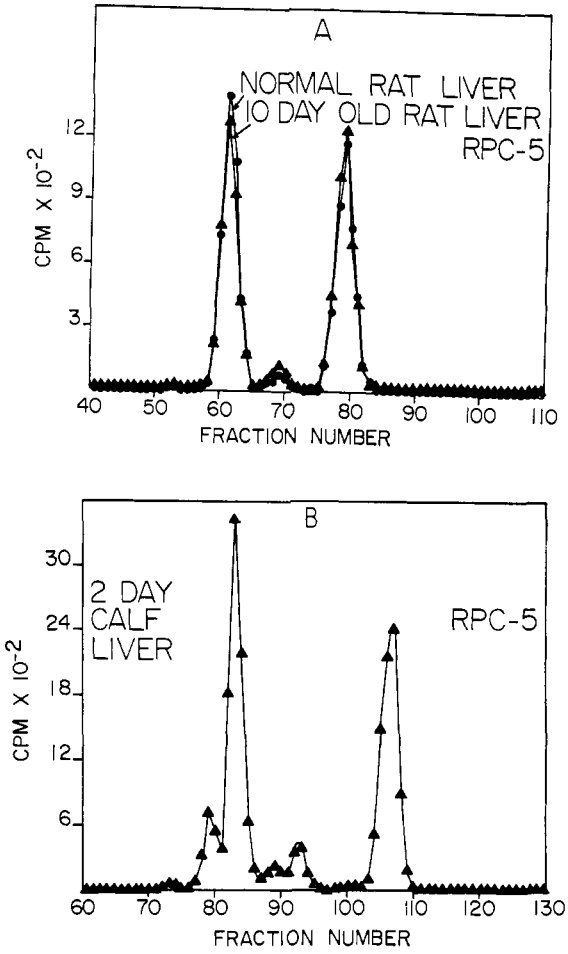

FIGURE 3: RPC profiles of Lys-tRNA from (A) 10-day old (▲) and adult (●) rat liver and (B) 2-day old calf liver.

lysyl-tRNA. While the increase in Lys-tRNA$_4$ may be mathematically significant, the comparison of 10-day old and adult rat liver Lys-tRNA in panel A of Figure 3 shows that the increase is indeed small. A slightly larger increase was seen in 2-day old calf liver. In this tissue Lys-tRNA$_4$ represented 5.4% of the total. The profile is shown in panel B of Figure 3.

One of the classic systems used to study the process of cell division in mammalian cells is that of regenerating rat liver. We therefore, employed this system to stimulate cell division and looked at the Lys-tRNA profiles. Partial hepatectomy was carried out on several rats by removing the left lateral and median lobes of the liver. The rats were then sacrificed at 24, 48, and 72 hr after the operation and the tRNA was isolated from each liver. As shown in Figure 4, progressive changes were seen in the lysyl-tRNA pattern with each day of regeneration. No difference was seen, however, in a sham operated control. A close inspection of the profile for 24-hr regenerating rat liver showed that there was little or no difference in Lys-tRNA$_4$; however, an increase was seen at a position between Lys-tRNA$_2$ and Lys-tRNA$_4$. This is the tRNA we have numbered as Lys-tRNA$_3$. A consideration of the RPC-5 profiles shown in Figure 2 and other profiles not reported showed that Lys-tRNA$_3$ was present in most of the rapidly dividing tissues studied; however, it never amounted to more than 2–3% of the total. The profiles for 48- and 72-hr regenerating rat liver show that Lys-tRNA$_3$ stays relatively constant while Lys-tRNA$_4$ then increases. These data may possibly mean that Lys-tRNA$_3$ is a precursor of Lys-tRNA$_4$; however, additional evidence will be required.

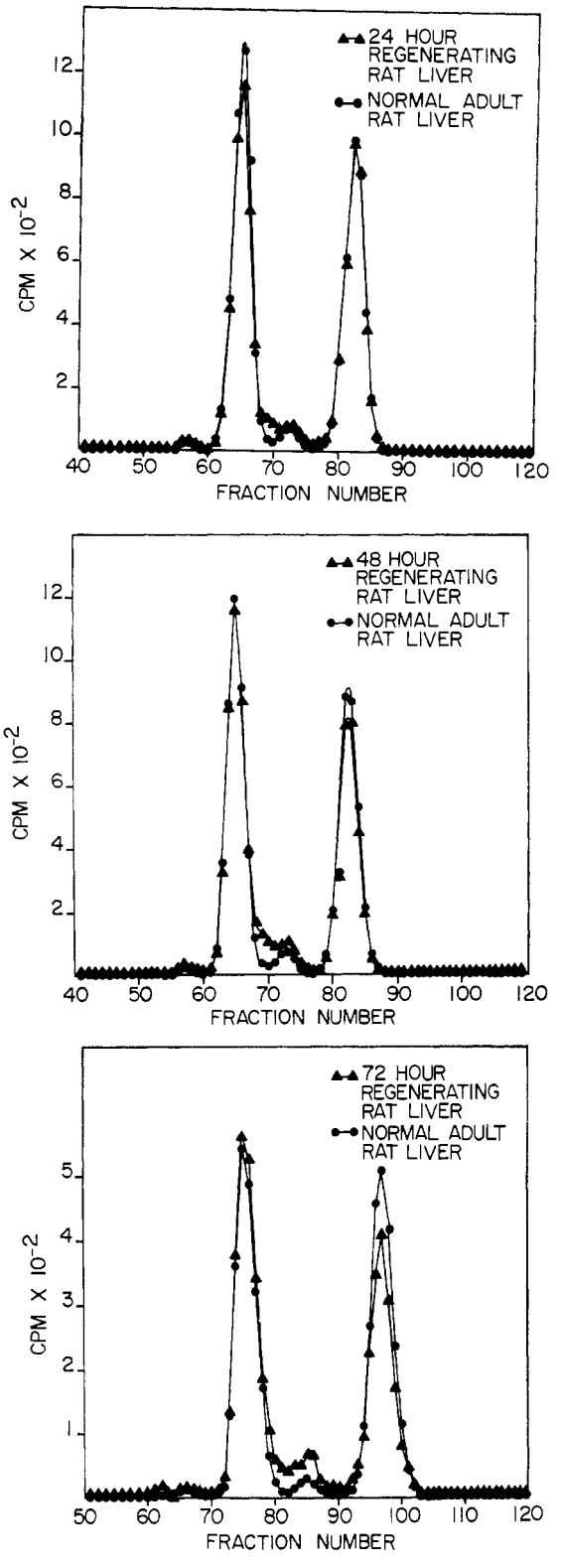

FIGURE 4: Cochromatography of [^{3}H]lysyl-tRNA from 24-, 48-, and 72-hr regenerating rat liver with [^{14}C]lysyl-tRNA from normal rat liver.

Since we have demonstrated an increase in Lys-tRNA$_4$ accompanying increases in cell division, we next wanted to demonstrate a loss of Lys-tRNA$_4$ when cell division is inhibited. For these experiments we used the mouse leukemic cells grown in suspension cultures. We attempted to inhibit cell division by removing the serum from the medium and also by replacing the serum with an equivalent amount of lens cortex extract which is known to contain a mitotic inhibitor. In both of these cases the cells must have been damaged, for no tRNA could be isolated from the packed cells. Finally, we attempted to grow the cells to stationary phase by allowing the cells to exhaust the medium. In this case stationary cells were defined as cells harvested when the cell number begins decreasing and some cell debris can be seen. A comparison of the Lys-tRNA from these cells with the Lys-tRNA from log phase cells is shown in Figure 5. These profiles show that a dramatic decrease in Lys-tRNA$_4$ with a corresponding increase in Lys-tRNA$_2$ occurs when the cells reach stationary phase. Actually this change is not instantaneous but occurs throughout the entire growth of the cells. This is shown in Table II. Mouse leukemic cells were grown to different densities and harvested. The tRNA was isolated, aminoacylated with lysine, and chromatographed with normal log phase cells. The percentage of each peak was determined and the ratio of Lys-tRNA$_2$ to Lys-tRNA$_4$ was determined. As can be seen, the ratio increases slightly at 1×10^6 cells/ml and even more at 2×10^6 cells/ml even though the cells are still growing at a logarithmic rate. After 2×10^6 cells/ml, however, the rate of growth slows considerably and at stationary phase the amount of Lys-tRNA$_4$ is only 15% of the total compared with 45% for Lys-tRNA$_4$ from cells at 5×10^5 cells/ml. This shows that Lys-tRNA$_4$ is markedly decreased when cells stop dividing and in fact this decrease may actually precede the inhibition of cell division.

TABLE II: Effect of Increased Cell Density on the Lysyl-tRNA Profile of Mouse Lymphoma Cells in Culture.

	% of Total		
Cell Density (Cells/ml)	Lys-tRNA$_2$	Lys-tRNA$_4$	Lys-tRNA$_5$
0.5×10^6	17	47	36
1.0×10^6	21	42	37
2.0×10^6	25	38	37
2.7×10^6	35	26	39
Stationary cells	41	16	43

Discussion

The recent literature contains numerous examples of changes in tRNA profiles in tumor tissues. In most cases these differences have not been pursued further or have been shown to be artifactual. We feel our results have much greater significance since a new species of tRNA has been shown to be present in tumor tissues which is also present in normal cells which are rapidly dividing. Also since this tRNA is absent in those tissues which are not dividing, we suggest that Lys-tRNA$_4$ is somehow concerned with the basic processes of cell division. In addition to the evidence presented here, several reports in the literature support this idea. These include the work of Taylor (1971) who first showed a difference in the lysyl-tRNA patterns of Morris Hepatoma 9618A and normal rat liver and the work of Yang *et al.* (1969). These workers studied L-M cells grown both in mice and in tissue culture. The Lys-tRNA patterns of the cells grown *in vivo* had an elevated level of Lys-tRNA$_4$ as would be predicted, but when these cells were transferred to tissue culture, the amount of

this tRNA increased about twofold. We interpret this increase to be the result of the increased rate of cell division by the cells in tissue culture. We have examined many other papers for the presence of Lys-tRNA$_4$ but in most cases the separation systems employed were not able to resolve more than two broad peaks of Lys-tRNA.

We have examined all of the data presented here looking for evidence which would suggest how Lys-tRNA$_4$ is formed. This could be a new gene product or a hypo- or hypermodified species of one of the two main Lys-tRNAs. Our data support the latter hypothesis, but are somewhat confusing. The data in Table II show that as Lys-tRNA$_4$ decreases in mouse leukemic cells, there is a corresponding increase in Lys-tRNA$_2$ with little change in Lys-tRNA$_5$. This strongly suggests that Lys-tRNA$_4$ is a modified form of Lys-tRNA$_2$. By the same analogy, however, the data obtained with regenerating rat liver and the profile of HTC cell tRNA suggest that the appearance of Lys-tRNA$_4$ correlates with a loss in Lys-RNA$_5$. Since we have reported previously that Lys-tRNA$_2$ codes specifically for AAG, while Lys-tRNA$_5$ codes for AAA (Liu and Ortwerth, 1972), it seems unlikely that these molecules are similar. This idea is supported by the structural studies carried out with yeast tRNA^{Lys}. In these studies the primary sequences of two tRNA^{Lys} species of yeast were shown to differ at 23 different positions, including the anticodon region (Madison *et al.*, 1972). If the tRNA$_4^{Lys}$ peak seen in various tissues is indeed the same molecule, then the best explanation of our data is that the tRNA$_5^{Lys}$ peak actually contains two tRNAs. One of these being the AAA specific Lys-tRNA, while the other is a modified form of tRNA$_2^{Lys}$. It is the latter tRNA then which is modified to produce tRNA$_4^{Lys}$ in regenerating rat liver and HTC cells. The fact that the tRNA$_5^{Lys}$ peak is heterogeneous has been suggested previously (Liu and Ortwerth, 1972) and has been recently supported by preliminary evidence in our laboratory.

While there is no direct evidence on the mechanism by which tRNA$_4^{Lys}$ is produced, there appears to be a correlation between the levels of tRNA$_4^{Lys}$ and the levels of polyamines in various tissues. This is particularly true for developing and regenerating rat liver (Russell and McVicker, 1972; Raina *et al.*, 1966). Also, Pett and Ginsberg (1968) have shown that KB cells cultured in spent medium have reduced levels of polyamines. They propose that as the medium is depleted the cells can no longer continue to synthesize protein at a rapid rate and since the mRNA for ornithine decarboxylase has a very short half-life, the levels of this enzyme decrease. This causes the decrease in polyamine levels. This correlates with levels of tRNA$_4^{Lys}$ seen in mouse leukemic cells since this tRNA decreased as the cells were continuously grown in the same medium. While it is certainly possible that there is no direct relationship between polyamines and tRNA$_4^{Lys}$, this is a tempting hypothesis in view of the recent work by Leboy (1970, 1971). She has shown that polyamines are effective in stimulating rat liver methylases *in vitro*. The polyamines are active at physiological levels and stimulate not only the activity but also the extent of tRNA methylation. Therefore, the formation of tRNA$_4^{Lys}$ may result from the action of a polyamine-stimulated tRNA methylase.

In considering a possible role for tRNA$_4^{Lys}$ in cell division, some conclusions may be drawn. The events occurring during liver regeneneration have been well characterized (Mayfield and Bonner, 1972). These events culminate in a sharp increase in mitotic index at about 24–28 hr after partial hepatectomy. Our results showed little or no difference in the level of Lys-tRNA$_4$ at 24 hr posthepatectomy. Similar results have also been reported by Tidwell *et al.* (1972). They reported no change in the Lys-tRNA profiles at either 12, 18, or 24 hr of posthepatectomy. These data suggest that tRNA$_4^{Lys}$ is not associated with any particular phase of the cell cycle. We feel rather that tRNA$_4^{Lys}$ is associated with the ability of a cell to divide.

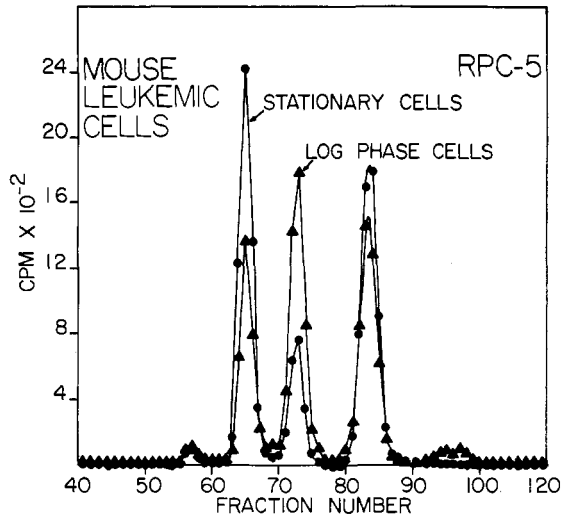

FIGURE 5: Cochromatography of mouse leukemic cells grown in suspension cultures and harvested at either log phase (5×10^5 cells/ml) or stationary phase.

Within a given cell population, three types of cells exist: (A) those cells which are dividing, (B) those cells which are blocked in G_1 or G_2, but which can be stimulated to divide, and (C) those cells which are not capable of division. We feel that cell types A and B contain tRNA$_4^{Lys}$ whereas cell type C does not. The following evidence is consistent with this hypothesis. (A) Cell populations containing only type C cells, such as lens cortex, muscle, and brain do not contain tRNA$_4^{Lys}$. (B) During the first 24 hr of rat liver regeneration there is a change from type B to type A cells. This is not accompanied by an increase in tRNA$_4^{Lys}$; however, as the total cell number increases, type A and B cells represent an increasing proportion of the total liver. This is accompanied by an increase in tRNA$_4^{Lys}$ as seen at 48 and 72 hr of posthepatectomy. (C) Rat liver and salivary glands have an almost negligible mitotic index, but the number of cells which can be stimulated to divide represent a significant number of

TABLE III: Correlation between the Amount of Lys-tRNA$_4$ and the Number of Potentially Dividing Cells.

Tissue	Lys-tRNA$_4$ (% of Total)	No. of Cells Able to Divide (% of Total)
Lens cortex	0	0[a]
Rat liver	3.2	30–40[b]
Rat salivary gland	7.0	60–80[c]
Morris hepatoma	9.8	100[d]
	10.7	

[a] Hanna (1964). [b] Grisham (1962). [c] Baserga (1970). [d] Assumed.

cells. Rat liver cells can be stimulated by partial hepatectomy, while salivary gland cells can be stimulated by a single injection of isoproterenol. The percentage of stimulated cells can be measured by the number of labeled nuclei following [^{3}H]thymidine injection. Table III shows the correlation between the amount of $tRNA_4^{Lys}$ and the number of potentially dividing cells.

This correlation suggests that $tRNA_4^{Lys}$ makes up 10% of the total $tRNA^{Lys}$ in cells which are dividing or can be stimulated to divide, whereas, it is completely absent in cells which cannot divide. If this correlation does prove to be correct then it would suggest that $tRNA_4^{Lys}$ may be a necessary requirement for cell division. The higher levels of $tRNA_4^{Lys}$ seen in HTC cells and especially in mouse leukemic cells may be one of the reasons why these cells have lost control of cell division and can be easily grown in tissue culture.

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