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Alterations in lysine transfer RNA during erythroid differentiation of the Friend cell

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ABSTRACT

The proportion of lysine tRNA represented by the isoacceptor species lysine $tRNA_4$ has previously been shown to be largest in cells with the greatest ability to proliferate. Using reverse phase chromatography (RPC-5), we have analyzed the changes in the relative quantities of lysine tRNA species which occur in different cellular states of the Friend cell, a transformed murine cell infected with Friend erythroleukemia virus complex. This cell undergoes erythroid differentiation when exposed to various chemicals. Lysine $tRNA_4$ comprises 32% of the total lysine tRNA in rapidly dividing, uninduced Friend cells, but only 16% of the total lysine tRNA in uninduced cells which have reached a density-dependent stationary growth phase. Friend cells undergoing erythroid differentiation divide more slowly than uninduced cells, and finally cease proliferation, but lysine $tRNA_4$ becomes the major lysine tRNA species (greater than 50%). This does not appear to reflect erythroid properties of the cell, since the lysine tRNA of the mouse reticulocyte contains very little lysine $tRNA_4$. The non-dividing erythroid Friend cell, therefore, represents an exception to the finding that non-dividing cells usually have little or no lysine $tRNA_4$ present.

INTRODUCTION

Transfer RNA for many individual amino acids may be resolved by reverse phase chromatography (RPC) into a number of isoacceptor species (Yang et al., 1969). Lysine tRNA from mammalian cells can be resolved on an RPC-5 column into 4-6 species. Ortwerth et al (1973a) found that the amount of total lysine tRNA represented by one of these isoaccepting species, lysine $tRNA_4$, varied in amount according to the proliferative capacity of the tissue. Thus, lysine tRNA from non-dividing brain and lens cells contained very little lysine $tRNA_4$, while the lysine tRNA in a moderately dividing tissue such as liver or bone marrow contained a moderate amount of lysine $tRNA_4$ (7-12% of the total lysine tRNA). Rapidly-dividing embryo or tumor cells were found to have as high as 47% of the total lysine tRNA represented by lysine $tRNA_4$. When rapidly-dividing mouse embryo cells in tissue culture reached a non-dividing, confluent state, the proportion of total lysine tRNA com-

prised of lysine $tRNA_4$ dropped from 47% to 16% (Juarez et al., 1973).

In this report, we have used RPC-5 to analyze the changes in the lysine tRNA profile which occur in different cellular states of the Friend cell, a transformed murine tissue culture cell infected with the Friend erythroleukemia virus complex. This cell has the ability to undergo erythroid differentiation when exposed to a variety of chemical inducers, and in this process, passes from a proliferative to a non-proliferative state. In this paper, we report that during this differentiation, lysine $tRNA_4$ does not decrease as might be expected, but instead becomes the major lysine tRNA isoacceptor species present in the erythroid Friend cell.

MATERIALS AND METHODS

Culture conditions and erythroid induction with tetramethyl urea (TMU). The Friend virus-infected cells used in this study were derived from DBA/2J mice. The cell line used, 745A, was obtained from the Mammalian Genetic Mutant Cell Repository, Institute for Medical Research, Copewood and David Street, Camden, N.J. Cultures were maintained in alpha medium (Stanners, Eliceiri, and Green, 1971) lacking nucleosides and deoxynucleosides, supplemented with 15% fetal calf serum (Microbiological Associates, Walkersville, Maryland), at 37°C under an atmosphere of 95% air, 5% CO_2. Cells from which transfer RNA was isolated were grown in 1-3 liters of culture medium in 3 liter Bellco spinner flasks (Bellco, Vineland, N.J.).

Friend cells were induced by growing them in the presence of 5 mM tetramethyl urea (Preisler et al., 1976; Germinario et al., 1977). Erythroid induction was monitored by staining cells with benzidine, a stain for heme. Benzidine tests were performed on intact cell suspensions as described by Orkin, Harosi, and Leder (1975).

Isolation and aminoacylation of transfer RNA. tRNA was isolated from the mouse reticulocyte and the Friend cell by phenol/chloroform extraction, and purified by 1.0 M NaCl extraction and DEAE-cellulose chromatography, as described by Yang et al. (1971). Reticulocytes were obtained from Swiss mice made anaemic using the phenylhydrazine procedure described by Conkie et al. (1975). Rat liver tRNA was purchased from the Grand Island Biological Company (Grand Island, New York).

The conditions used for the isolation of rat liver amino acyl-tRNA synthetases and for the acylation of lysine tRNA were those described by Ortwerth (1971).

Reverse phase chromatography of aminoacylated tRNA. The RPC-5 material was

prepared as described by Pearson et al (1971). 1-2.5 A_{260} units of tRNA charged with radioactive lysine were applied to the column (1.6 cm x 75 cm), and eluted with a one liter salt gradient (0.5 M - 0.7 M sodium chloride). Included in each gradient were 0.01 M sodium acetate, pH 4.5, 0.01 M magnesium chloride, 0.001 M EDTA, and 0.003 M beta-mercaptoethanol. The columns were run at room temperature with a flow rate of approximately 0.8 ml/minute, and 7 ml fractions were collected. The nucleic acid in each fraction was precipitated by adding 2.8 A_{260} units of calf thymus DNA and 1.4 ml of 50% trichloroacetic acid, and left for at least 30 minutes at 0°C. The precipitates were collected on glass fiber filters, washed twice with 70% ethanol, dried under an infrared lamp, placed in scintillation vials containing Omnifluor-toluene, and counted in a liquid scintillation counter.

RESULTS AND DISCUSSION

Using reverse phase chromatography, we have determined the RPC-5 profile of lysine tRNA isolated from rat liver, mouse reticulocytes, and Friend cells present in different cellular states. tRNA was extracted from uninduced Friend cells in both the logarithmic phase of growth (0.7-1.5 x 10^6 cells/ml) and in a stationary growth phase (2.4 x 10^6 cells/ml), and from Friend cells exposed to 5 mM tetramethyl urea (TMU) for various lengths of time. The parameters of growth of these cells cultured in the presence or absence of 5 mM TMU is shown in Figure 1. TMU was first shown to be potent inducer of Friend cell erythroid differentiation by Preisler et al (1976), and Figure 1 shows that when logarithmically growing cells are exposed to 5 mM TMU, the induction of benzidine-positive cells occurs rapidly between 24 and 48 hours after exposure to the inducer, with greater than 90% of the cells being induced. It can be seen that the rate of cell proliferation is slower in the presence of TMU than in its absence, and that growth usually ceases after 4 cell doublings, a phenomenon previously reported using other inducers (Gusella et al., 1976).

Figures 2 and 3 contain the RPC-5 profiles of lysine tRNA isolated from rat liver, mouse reticulocytes, and from the different cellular states of the Friend cell, and Table 1 lists the proportions of the total lysine tRNA represented by each peak. Figure 2A shows the results of a double-label experiment in which rat liver tRNA was charged with ^{3}H-lysine, tRNA isolated from uninduced Friend cells in the logarithmic phase of growth was charged with ^{14}C-lysine, and the samples were co-chromatographed on RPC-5, resulting in the resolution of 5 isoacceptor species for both tRNA samples. By virtue of their identical elution from RPC-5, we have numbered the Friend cell

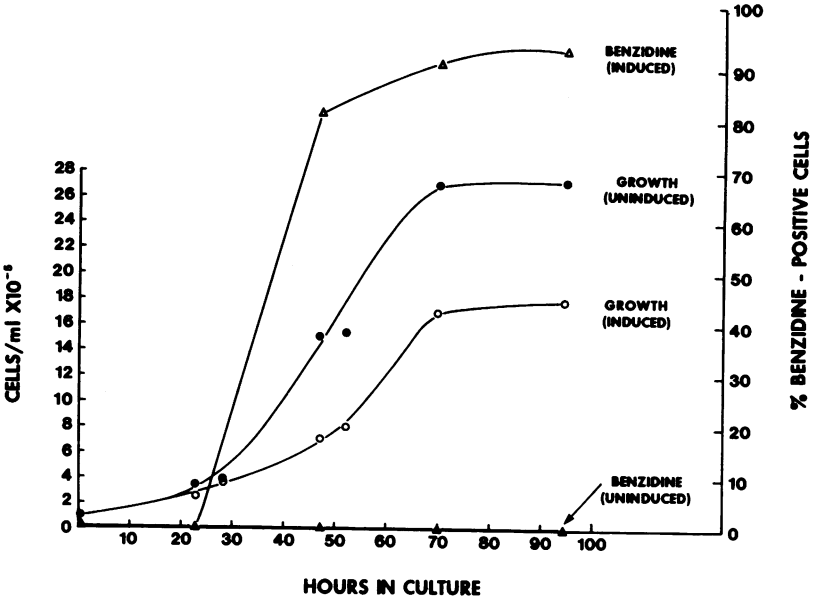

Figure 1. Growth and erythroid differentiation of Friend cells cultured in the presence or absence of 5 mM tetramethyl urea.

species according to the numbering system used by Ortwerth for rat liver lysine tRNA (Ortwerth et al., 1973a).

This data verifies their previous reports that mammalian lysine tRNA can be resolved on an RPC-5 column into 4-6 peaks. Under somewhat altered chromatographic conditions, an additional peak, lysine $tRNA_3$, can sometimes be seen eluting between lysine $tRNA_2$ and lysine $tRNA_4$; this material never amounts to more than 2-3% of the total lysine tRNA. Juarez et al (1975) reported that lysine $tRNA_5$ could further be resolved into two species on benzoylated DEAE cellulose. There may therefore be at least seven lysine tRNA species present in mammalian cells.

It can be seen that lysine $tRNA_4$ is a prominant species in the lysine tRNA of rapidly-dividing, uninduced Friend cells (over 30%), while the proportion of total lysine tRNA represented by this species in the liver, a moderately-dividing tissue, is only 10% of that found in the Friend cell.

Figure 2B shows a similar experiment in which the tRNA from mouse reticulocytes was charged with ^{14}C-lysine, and analyzed together with rat liver tRNA (^{3}H-lysine) on RPC-5. Although lysine $tRNA_4$ is a minor component in reticulocyte lysine tRNA, especially when compared to the amount found in rapidly-dividing Friend cells, it still represents a higher fraction of the

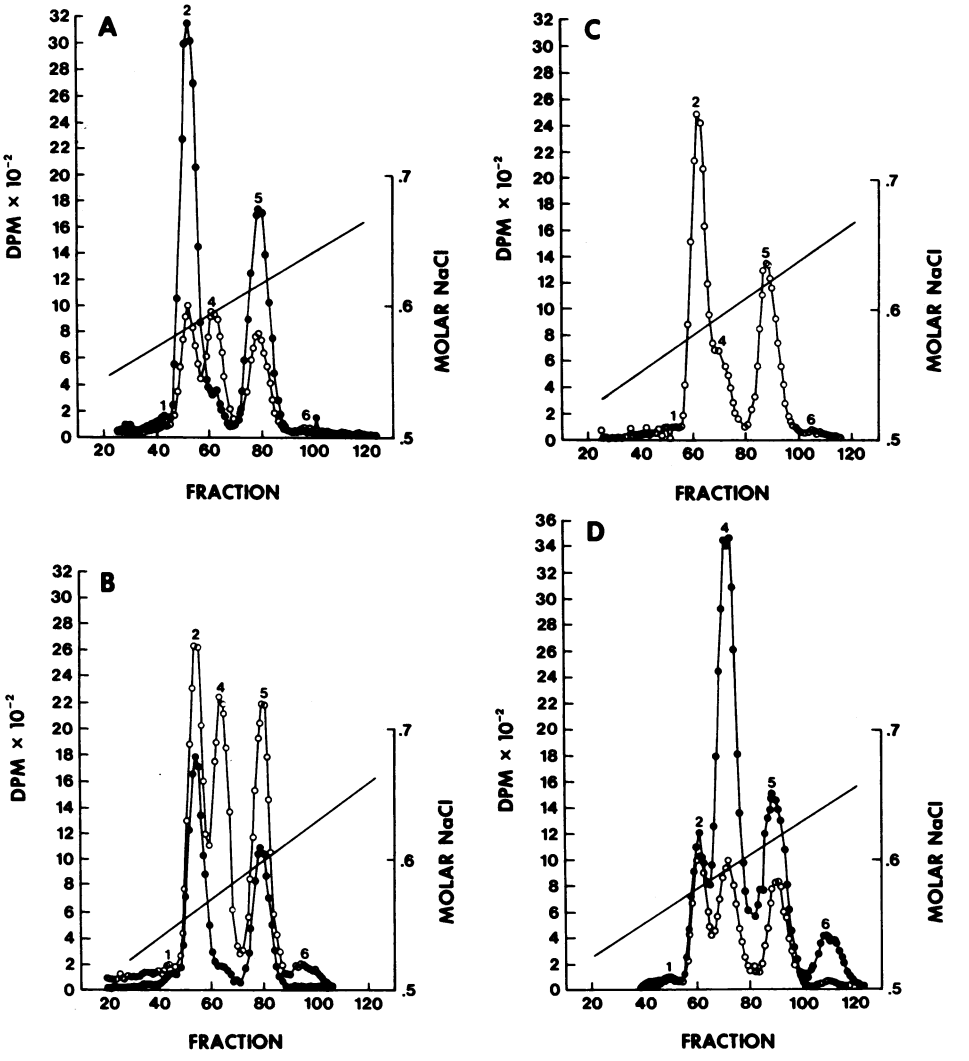

Figure 2. RPC-5 profiles of tRNA isolated from rat liver, mouse reticulocyte, and the Friend cell in different cellular states. The tRNA was charged with either ^{3}H-lysine or ^{14}C-lysine as described in the text. (A) —●—●— , rat liver (^{3}H); —○—○—, uninduced Friend cells in the logarithmic growth phase (^{14}C). (B) —●—●—, mouse reticulocyte (^{14}C); —○—○—, uninduced Friend cells in the logarithmic growth phase (^{3}H). (C) —○—○—, uninduced Friend cells in the stationary growth phase (^{3}H). (D) —●—●—, induced Friend cell exposed for four days to 5 mM tetramethyl urea (^{3}H); —○—○—, uninduced Friend cell in the logarithmic growth phase.

total lysine tRNA (5.4%) than the values reported by Ortwerth et al (1973a) for lysine tRNA isolated from the non-dividing cells of lens and brain (in which lysine $tRNA_4$ is essentially non-detectable). This might be due to a

possible contamination of the mouse reticulocyte preparation with peripheral blood lymphocytes, but might also be a result of the fact that the reticulocytes are the recent products of massive erythroid cell division in the bone marrow and spleen which enables the mouse to replenish the blood cells lost by the phenylhydrazine-induced hemolytic anemia. The lysine $tRNA_4$ of non-dividing reticulocytes may be a remnant of the reticulocytes' dividing precursor cells; lens and brain cells may be much further removed in time from their dividing precursor cells, and so contain little or no remnant of lysine $tRNA_4$.

In Figure 2C, the RPC-5 profile of lysine tRNA isolated from uninduced Friend cells in the stationary growth phase is shown, and it can be seen that the proportion of lysine tRNA represented by lysine $tRNA_4$ decreases in these non-proliferating cells. The data shown in panels A, B, and C of Figure 2 confirm the previous findings that lysine $tRNA_4$ varies in amount according to the proliferative capacity of the cell. The proportion of lysine $tRNA_4$ in total lysine tRNA increases when we examined cells with increasing proliferative capacity (reticulocytes and liver versus growing Friend cells), and decreases 50% when uninduced, rapidly-dividing Friend cells are brought to a stationary phase of growth.

However, the Friend cell undergoing erythroid differentiation appears to be an exception to this rule. tRNA isolated from Friend cells exposed for four days to an inducer of erythroid differentiation, 5 mM TMU, was charged with ^{14}C-lysine, and in Figure 2D, the RPC-5 profile of this tRNA is shown, accompanied by ^{3}H-lysine tRNA from uninduced, logarithmically growing Friend cells. Lysine $tRNA_4$ appears to be the major lysine tRNA isoaccepting species in the induced erythroid Friend cell, even though this cell no longer divides. Figure 3 shows a more detailed analysis of the alterations in the RPC-5 profile of lysine tRNA which occur during a five day period in which Friend cells are exposed to 5 mM TMU. During this time, there is a significant increase in lysine $tRNA_4$, so that this species becomes the major lysine tRNA isoacceptor (greater than 50%) in the erythroid Friend cell. During this time there is also a small increase in lysine $tRNA_6$, and a decrease in lysine $tRNA_2$ similar in amount to the increase in lysine $tRNA_4$. Lysine $tRNA_5$ appears to remain relatively constant.

The fact that the fraction of lysine tRNA represented by the sum of lysine $tRNA_2$ and lysine $tRNA_4$ remains constant (last column, Table I) implies a possible precursor-product relationship between these two species. This hypothesis is further strengthened by the findings of Ortwerth *et al* (1973b)

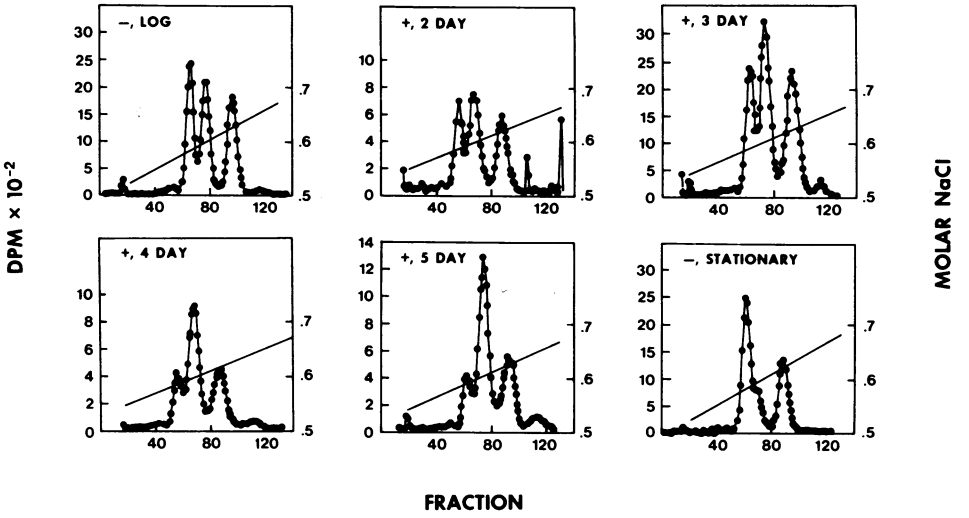

Figure 3. Alterations in the RPC-5 profile of lysine tRNA extracted from Friend cells exposed to 5 mM tetramethyl urea over a five day period. (-), uninduced Friend cells; (+), induced Friend cells. All tRNA samples were charged with ^{3}H-lysine except for +, 4 and 5 day, which were charged with ^{14}C-lysine. The relatively low ^{3}H-DPM seen in +, 2 day relates to the smaller amount of sample put on the column.

TABLE I - PERCENTAGE OF LYSINE TRANSFER RNA REPRESENTED BY THE DIFFERENT ISOACCEPTOR SPECIES

ISOACCEPTOR SPECIES	1	2	4	5	6	2 and 4
Rat Liver	2.8	57.4	3.1	35.9	0.69	60.5
Mouse Reticulocyte	3.4	55.0	5.4	36.0	0.0	60.4
FRIEND CELLS, UNINDUCED:						
Stationary Growth Phase	1.2	48.9	16.0	33.1	0.6	64.9
Logarithmic Growth Phase	1.9	33.7	32.6	29.3	2.4	66.3
TMU-INDUCED FRIEND CELLS:						
2-Day	2.3	28.3	40.0	27.8	1.4	68.3
3-Day	1.2	25.4	39.8	32.0	1.5	65.2
4-Day	1.3	19.1	47.3	26.4	5.7	66.4
5-Day	1.6	12.0	50.2	29.2	6.8	62.2

that lysine $tRNA_2$ and lysine $tRNA_4$ recognize the same codon (AAG) and are both resistant to iodine oxidation, while lysine $tRNA_5$ binds most strongly to the codon AAA, and is susceptible to iodine oxidation.

Why should the slow-dividing, or non-dividing induced Friend cell contain such a high proportion of lysine $tRNA_4$? An answer to this might point out the biological significance of lysine $tRNA_4$. The high proportion of lysine tRNA represented by this species in the differentiated Friend cell is probably not the result of the erythroid properties developed by the cell since the lysine tRNA of mouse reticulocytes contains very little lysine $tRNA_4$. In looking for some common factor in both the dividing uninduced cell and the non-dividing erythroid cell whose appearance is correlated with the appearance of lysine $tRNA_4$, a possible candidate might involve viral gene expression. It has been shown that when uninduced, logarithmically growing Friend cells enter into a density-dependent, stationary-growth phase, there is an inhibition of virus release into the medium (Sheraton et al., 1976). On the other hand, when Friend cells are exposed to the inducer dimethyl sulfoxide, there is an initial large increase in virus released into the medium (Dube et al., 1975), and the amount of viral mRNA in Friend cell polysomes either remains constant or may even increase several fold throughout the differentiation process (Pragnell et al., 1977). The possibility of a role played by lysine $tRNA_4$ in viral gene expression is very interesting because of reports which indicate that lysine and arginine tRNA are the predominant tRNAs found associated with the 70s RNA genome in avian and murine RNA tumor virus, other than the primer tRNA for reverse transcriptase (Waters et al., 1977), and in the avian system, cellular lysine tRNA can hybridize to the viral RNA genome (Waters et al., 1975). Evidence relating lysine $tRNA_4$ to both cellular replication and viral function would strengthen the hypothesis that the function of endogenous viral sequences found in the DNA of all normal mouse cells might be related to the control of cell proliferation.

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