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Alterations in tRNA isoaccepting species during erythroid differentiation of the Friend leukemia cell

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ABSTRACT

The chromatographic profiles of isoaccepting tRNAs were analyzed at five time points during the 96 hr, dimethylsulfoxide induced, erythroid-like differentiation of Friend leukemia cells. Sixty-four isoaccepting species of tRNA for 16 amino acids were resolved by RPC-5 chromatography. The relative amounts of $tRNA^{Phe}$, $tRNA^{Ile}$, and $tRNA^{Val}$ species were maintained by the cells during differentiation; whereas the relative amounts of some of the isoacceptor tRNAs for the other 13 amino acids changed significantly. Fluctuations in amounts of isoacceptors occurred between 36 and 72 hr after addition of dimethylsulfoxide, corresponding to globin mRNA appearance and hemoglobin synthesis, respectively. In most cases, the predominant tRNA isoacceptors of uninduced cells were retained throughout differentiation. Notable exceptions were tRNA species for threonine, proline, and methionine. Some of the isoacceptors occurring in relatively smaller amounts were not expressed at all times. These changes possibly reflect the cell's functional adaptation of tRNA in differentiation for hemoglobin synthesis.

INTRODUCTION

Protein biosynthesis appears to be controlled by regulation of translation as well as transcription. The intracellular levels of specific species of tRNA are believed to be tightly correlated with the needs of the cell for various amounts of particular amino acids and certain anticodons in protein synthesis (1). In order to study the specialization of tRNA content for protein synthesis, a number of different systems which synthesize predominantly one protein have been developed. These include fibroin synthesis by silk worms (1,2), crystallin synthesis by bovine lens tissue (3,4), hemoglobin synthesis in reticulocytes (5-7), and collagen synthesis in chicken embryos (8-10). Evidence has already been presented which demonstrates that changes in tRNA isoaccepting species are associated with differentiation, sporulation, phage and viral infection, hormonal stimulation, malignant degeneration, as well as with different cell culture conditions. (For reviews, see reference #11). However, the significance of a multiplicity of isoaccepting species of eucaryotic tRNA

redundant for the 61 translated coding triplets still remains unclear. It is not clear yet to what extent the cell actually uses variations in specific tRNA levels or specific isoaccepting forms of tRNA to, for instance, control the relative rates of translation of different mRNA.

In order to study various aspects of translational control, our laboratory has investigated the tRNA isoaccepting species of a system consisting of a cloned cell line that is fully inducible for differentiation in culture. The alteration of 64 isoaccepting species of tRNA for 16 amino acids coincident with Friend leukemia cell (F.L.C.) differentiation was studied by reversed-phase plaskon chromatography. Concomitant with alterations of cellular functions in the transition between dividing, undifferentiated cells to non-dividing, predominantly hemoglobin synthesizing cells, dramatic quantitative and qualitative changes in the distribution of tRNA isoaccepting species would be expected. We have characterized these tRNA changes at five time points in the differentiation of F.L.C. Results for 47 isoacceptors of tRNA for 12 amino acids are reported here. Changes in 17 isoaccepting tRNA species for the four aminoacyl-tRNAs containing the hypermodified ribonucleoside Q are described separately in the adjoining paper (12).

MATERIALS AND METHODS

Materials. A cloned line of Friend leukemia cells (F.L.C.) designated as GM86 (clone 745A) was obtained from Human Genetic Mutant Repository (Camden, NJ). The biology of this cell line including its erythroid-like differentiation has been reviewed (13). Polychlorotrifluoroethylene (plaskon CTFE; Allied Chemical Co.), adogen 464, and washed bentonite were kindly provided by Dr. B.J. Ortwerth (University of Missouri, Columbia, MO). All [^{3}H], [^{14}C], and [^{35}S] labelled amino acids were purchased from New England Nuclear. Amino acids labelled with [^{3}H] had the following specific activities in Ci/mmol: aspartic acid, 16,9; histidine, 10.9; isoleucine, 99.2; leucine, 54.6; lysine, 60.0; phenylalanine, 60.0; proline, 111.0; serine, 2.76; threonine, 4.2; tryptophan, 20.0; tyrosine, 42.3; and valine, 11.18. Amino acids labelled with [^{14}C] had the following specific activities in mCi/mmol: alanine, 160.6; asparagine, 179.0; and lysine, 285. Cysteine-[^{35}S] and methionine-[^{35}S] had specific activities of 489.56 Ci/mmol and 323.7 Ci/mmol, respectively, at the time they were used in these studies.

Cell Culture and Erythroid Induction with Dimethylsulfoxide (DMSO): Cultures were maintained in Eagle's minimum essential medium supplemented with 10% fetal calf serum (GIBCO) at 37^{o}C under an atmosphere of 95% air, 5% CO_2.

Friend cells were induced to differentiate by growing them in the presence of 270 mM dimethylsulfoxide (14). Erythroid induction was monitored by benzidine staining of intact cell suspensions as described (15).

Isolation of Transfer RNA: Transfer RNA was phenol extracted from F.L.C. and purified by DEAE-cellulose chromatography as described previously (16-18). Transfer RNA from rat liver served as internal standard for the RPC-5 chromatography and was extracted as has been described (19). The yield of unfractionated tRNA was about 1 A_{260}unit per 5 x 10^8 cells in culture and 5 A_{260} units per gram of rat liver tissue. The contamination of 5S RNA in tRNA preparations was assessed by polyacrylamide slab gel electrophoresis (20) and was found to be less than 15% for these preparations.

Aminoacyl-tRNA Synthetase Preparation: The aminoacyl-tRNA synthetase used throughout this work was obtained from Sephadex G 100 filtration of a 160,000xg supernatant extract of rat liver as described (19).

Aminoacylation: Aminoacylation of tRNA samples was carried out in a 0.5 ml reaction mixture containing: 0.2 ml of buffer (0.4 M Tris-HCl, pH 7.5, 8 mM $MgCl_2$, 20 mM KCl, 8 mM ATP, 1.6 mM CTP, 4 mM dithiothreitol); 0.1 ml of rat liver synthetase; 2 µCi of [^{14}C] or 10 µCi of [^{3}H] amino acid; and 15 µg of tRNA. After a 15 min incubation at 37°C, 0.5 ml of 1 M sodium acetate, pH 4, was added to the reaction mixture. Aminoacylated tRNAs were isolated from the reaction mixture by either phenol extraction at pH 4.5 or by DEAE-cellulose chromatography as described by Yang and Novelli (21).

Reversed-phase Chromatography: Plaskon CTFE was coated with adogen 464 by method C as described by Pearson et al. (22). The RPC-5 column resin was packed into a 0.9 x 40 cm high pressure Altex analytical column as described previously (23). The column was equilibrated with 0.5 M NaCl buffer (0.01 M sodium acetate, pH 4, 0.01 M $MgCl_2$, 0.001 M Na_2EDTA, 3.0 mM 2-mercaptoethanol and 0.5 M NaCl). Rat liver [^{14}C] or [^{3}H] lysyl-tRNA (6,000 CPM) was added to each sample as an internal standard directly before sample injection. After injection of the sample the tRNA was eluted with a 100 ml linear gradient of 0.5 M to 1.0 M NaCl in the above buffer. The operation pressure was 200 PSI at a flow rate of 1 ml/min. Two hundred fractions of 0.5 ml were then collected directly into scintillation vials. A volume of 4.5 ml of Triton X/toluene scintillation counting solution (3.2 g PPO and 0.08 g POPOP dissolved in 800 ml toluene and 400 ml Triton X-100) was then added to each vial and the sample radioactivity counted in a Packard Tricarb liquid scintillation counter. Throughout the work, the rat liver lysyl-tRNA internal standard produced a consistent chromatographic profile. Isoaccepting tRNA separations were repro-

ducible as determined from fifteen separate tRNA preparations, three from each of the five time points, being analyzed for each of the 16 amino acids.

RESULTS

Transfer RNA was isolated from Friend leukemia cells (F. L. C.) in culture before the addition of the erythropoietic inducer, dimethylsufoxide, and 36 hr, 48 hr, 72 hr, and 96 hr afterward. Cultures of induced F.L.C. exhibited a growth lag during the initial 36-48 hr. Resumption of cell division at an enhanced rate enabled the induced cultures to obtain a cell density comparable to that of uninduced cultures after 96 hr. An increase in the number of hemoglobin producing cells, detected by positive benzidine staining, was seen as early as 48-60 hr after induction. Each of the five isolated unfractionated tRNA preparations (0, 36, 48, 72 and 96 hr after addition of DMSO) was then separately aminoacylated with each of 16 radio-labelled amino acids: ala, asn, asp, cys, his, ile, leu, lys, met, phe, pro, ser, thr, trp, tyr and val. Isoaccepting species of these 80 aminoacyl-tRNA preparations were then resolved by reversed-phase plaskon chromatography. The internal standard of rat liver tRNA amino-acylated with [^{3}H] or [^{14}C]-lysine allowed direct comparison of chromatograms by recognition of rat liver tRNA^{lys} isoacceptors 2, 4 and 5.

The tRNAs for the 16 different amino acids were resolved into 64 isoaccep-ting species. However, the numbers of isoacceptors and the relative amounts of each for particular aminoacyl-tRNAs were either constant, variable or extremely variable during differentiation of F.L.C. The Q-base containing tRNA, asp, asn, his, and tyr (24), were among the most variable species. Changes in the iso-acceptors for these four aminoacyl-tRNAs and their extent of Q-modification over the period of erythroid differentiation are considered separately in the adjoining paper (12).

The relative amounts of each isoaccepting species of valyl-, isoleucyl-, and phenylalanyl-tRNAs isolated from cells at the five time points of erythroid differentiation mentioned above are summerized in Table I. The chromatographic profiles of these three tRNAs (profiles not shown)+ reflect similar distribu-tions of isoaccepting species, and are not considered significantly altered during differentiation. Valyl-tRNA was resolved into two isoaccepting species with an almost identical distribution over the five time points. The resolu-tion of the minor species of phenylalanyl-tRNA was rather poor. However, at

+Chromatographic profiles not shown are available from the authors upon request.

Table 1: Amino Acid Isoaccepting tRNA Species that Change Slightly During Differentiation of Friend Leukemic Cell

tRNA	Time During Differentiation[a]	Relative Amounts of Isoaccepting Species[b]					
		1	2	3	4	5	6
Val	0	79	21				
	36	82	18				
	48	78	22				
	72	78	22				
	96	71	29				
Phe	0	4	1	0	95		
	36	6	3	2	89		
	48	10	11	0	79		
	72	14	9	11	66		
	96	2	8	0	90		
Ile	0	29		30[c]		41	
	36	21		54		25	
	48	33		30		37	
	72	24		44		32	
	96	36		6		58	

[a] Hours after the addition of DMSO
[b] Percent of total aminoacyl-tRNA
[c] Percent of total $tRNA^{ile}$ for species 2, 3 and 4 combined

least four isoaccepting species could be defined. The one major peak designated species 4 consistently eluted very late. Species 2 increased in relative amount and species 3 was detected only at 72 hr. Isoleucyl-tRNA has been resolved into two major isoaccepting species designated 1 and 5. Species 1 and 5 were maintained by the cells at constant distributions over the time course of differentiation (Table 1). The remaining minor isoaccepting species which were not resolved, but appeared as shoulders to the major peaks, had larger changes in relative amounts increasing at 36 hr and then decreasing. These minor species of $tRNA^{ile}$ disappeared when the cells had completely differentiated.

The relative distributions over the course of erythroid differentiation of prolyl-, lysyl-, methionyl-, leucyl-, seryl-, cysteinyl-, tryptophanyl-, threonyl-, and alanyl-tRNA species are shown in Table 2. These nine aminoacyl-tRNAs exhibited more than 38 isoaccepting species of which 27 were well resolved by the RPC-5 chromatography. Some of these species remained almost

Table 2: Amino Acid Isoaccepting tRNA Species that Change Dramatically During DMSO Induced Differentiation of Friend Leukemic Cell

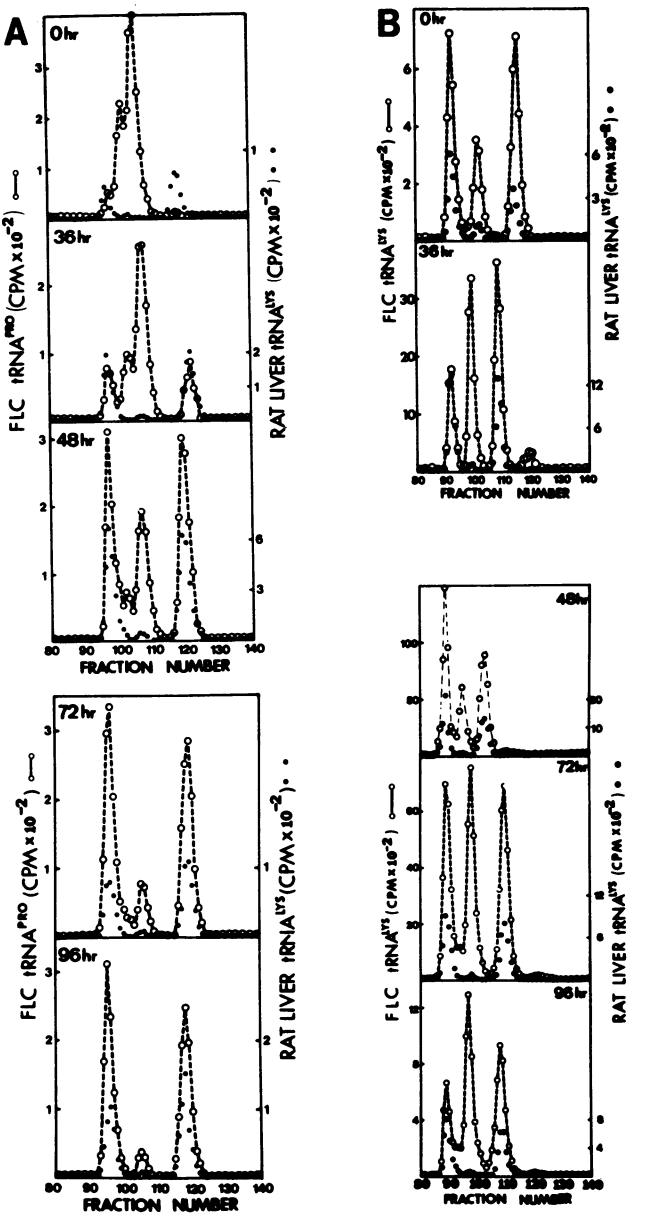
tRNA	Time During Differentiation[a]	Relative Amounts of Isoaccepting Species[b]					
		1	2	3	4	5	6
Pro	0	5	27	67	1		
	36	13	20	52	15		
	48	30	8	25	37		
	72	47	0	11	42		
	96	49	0	6	45		
Lys	0	0	36	0	22	41	1
	36	0	20	0	35	40	5
	48	0	43	0	21	34	2
	72	0	33	0	32	33	2
	96	0	21	0	42	35	2
Met	0	64	17	19			
	36	47	13	40			
	48	12	18	70			
	72	66	12	22			
	96	60	18	22			
Leu	0	17	7	36	40	0	
	36	6	31	0	49	15	
	48	27	17	0	36	10	
	72	5	49	0	24	22	
	96	30	23	0	39	8	
Ser	0	20	0	40	24	16	
	36	30	8	51	0	11	
	48	20	28	40	0	12	
	72	24	0	39	19	18	
	96	49	0	51	0	0	
Cys	0	79	9	12			
	36	81	5	14			
	48	68	20	12			
	72	50	21	29			
	96	51	31	18			
Trp	0	24	42		34[c]		
	36	21	24		55		
	48	30	32		38		
	72	29	32		39		
	96	45	55		0		
Thr	0	14	30	32	24		
	36	16	20	32	32		
	48	23	12	18	47		
	72	38	5	7	50		
	96	39	0	4	57		
Ala	0	15[d]		54	29[d]		
	36	30		51	19		
	48	31		51	18		
	72	18		59	23		
	96	13		73	14		

[a] Hours after the addition of DMSO
[b] Percent of total aminoacyl-tRNA
[c] Percent of total $tRNA^{trp}$ for species 3, 4 and 5 combined
[d] Percents of total $tRNA^{ala}$ for species 1 and 2, and 4 and 5, combined

constant in relative amount during differentiation of F.L.C., others varied in a discernable pattern, while still others changed without pattern. Prolyl-tRNA (Figure 1A) has been resolved by chromatography into four isoaccepting species. Species 1 and 4 constituted very little of the prolyl-tRNA population, if they were present at all, in the tRNA preparations from uninduced cells (0 hr, Table 2). However, both species 1 and 4 continually increased in relative amounts while $tRNA^{pro}$ species 2 and 3 decreased during differentiation. Lysyl-tRNA has been resolved into as many as six species. We have numbered the four lysine species found in F.L.C. (Figure 1B) according to a standardized designation for simplicity and continuity (19). It is worth noting that the relative amount of $tRNA^{lys}_4$ was reduced at the 48 hr time point and then increased again to greater than the original level.

Methionyl-tRNA was separated into three isoaccepting species (Figure 1C). The relative amount of species 2 fluctuated throughout the course of differentiation. However, the relative amount of the major species, designated 1, decreased at 48 hr to approximately one-fifth that at 0 hr, then increased to the original level. The chromatograms for leucyl-tRNA (profiles not shown) showed an alteration in the amounts of the five isoacceptors without any consistent pattern over the time course. Species 3 completely disappeared, whereas 5 appeared after the addition of DMSO. The chromatograms of seryl-tRNA showed two major peaks (1 and 3) and three minor peaks. The two major species 1 and 3 were present without alteration throughout the time course and represent the only serine isoacceptors after 96 hr of differentiation (Table 2). Species 2 was only present at the 36 and 48 hr time points, whereas species 4 was absent at these times. All three minor species disappeared at 96 hr after the addition of DMSO.

Cysteinyl-tRNA has been resolved into three species. Species 1 continually decreased in relative amount throughout the differentiation while 2 and 3 increased. Tryptophanyl-tRNA has been separated into five isoacceptors. However, the late eluting minor $tRNA^{trp}$ species (peaks 3, 4 and 5) were poorly resolved under the conditions used and disappeared at 96 hr after the addition of DMSO. The isoaccepting species of $tRNA^{thr}$ exhibited continual changes in relative amounts during the course of F.L.C. differentiation (Figure 1D). Four isoaccepting species were resolved. Species 1 and 4 increased in relative amount consistently, while 2 and 3 decreased throughout the time course. Alanyl-tRNA exhibited five isoaccepting species with only one major species (peak 3) which was maintained in constant relative quantity during the first 72 hr of differentiation and then increased by 50% at 96 hr. Species 4 and 5


A
0hr
36hr
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FLC tRNA^PRO (CPM x10^-2)
RAT LIVER tRNA^LYS (CPM x 10^-2)
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B
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FLC tRNA^LYS (CPM x10^-2)
RAT LIVER tRNA^LYS (CPM x10^-2)
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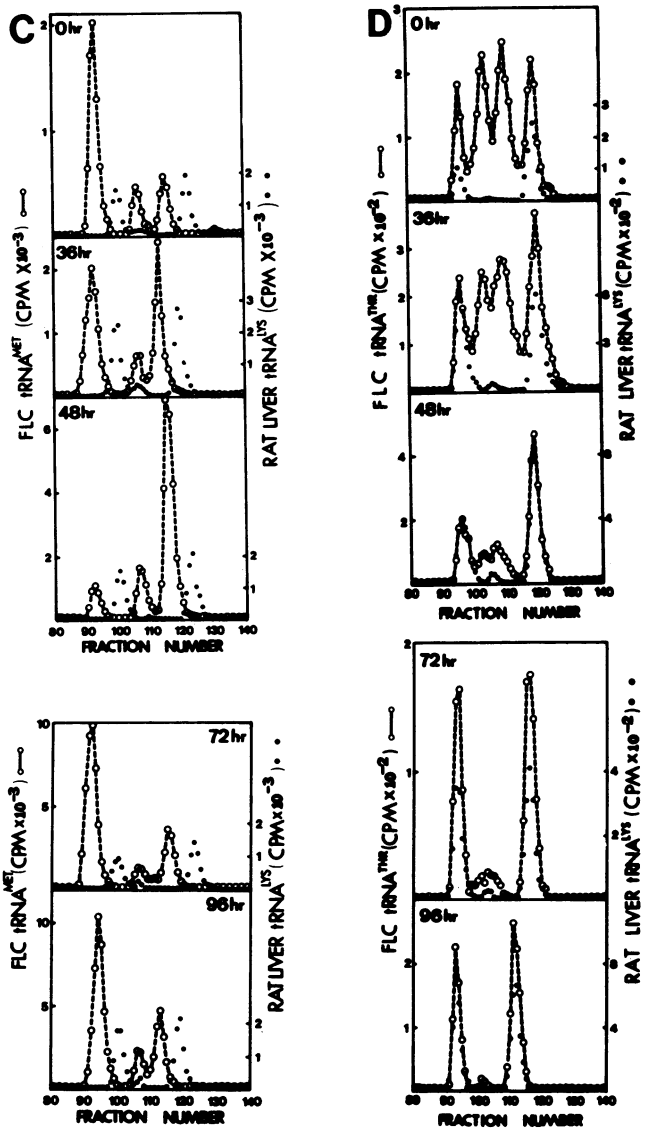

Figure 1. Alterations in the RPC-5 profiles of tRNA species extracted from Friend leukemia cells undergoing erythroid differentiation. tRNA was isolated from the cells after 0, 36, 48, 72 and 96 hr of induction. Each of the five tRNA preparations were aminoacylated with one [^{3}H]-labeled amino acid (o--o) and subjected to RPC-5 chromatography along with an internal standard, rat liver [^{14}C]-lysyl-tRNA (●--●). Exceptions to this procedure included the co-chromatography of [^{35}S]-methionyl-tRNA$_{FLC}$, and [^{3}H]-lysyl-tRNA rat liver. Details of the chromatography procedures are described in Materials and Methods. Panel A shows the separation of the isoaccepting prolyl-tRNA species; B, lysyl-; C, methionyl-; D, threonyl-tRNA.

essentially decreased in relative amount throughout the differentiation.

DISCUSSION

Several different hypotheses have been advanced which propose that tRNA molecules are regulatory factors during cell differentiation. A number of studies have shown that the tRNA population of a tissue specialized for the production of one protein reflects the amino acid composition of that protein (2, 25). Garel (1, 2) has discussed in some detail this functional adaptation of tRNA populations in a number of systems. The primary purpose of this investigation was to determine if qualitative or quantitative differences in tRNA isoaccepting species existed between the undifferentiated Friend leukemia cells (F.L.C.) and the same cells induced to undergo differentiation for hemoglobin synthesis. Such differences would suggest that levels of particular tRNA species may regulate hemoglobin synthesis because synthesis of this protein amounts to at least 24% of total protein synthesis in differentiated F.L.C. (13). Studies reported here and in the adjoining paper (12) demonstrated that thirteen of sixteen tested aminoacyl-tRNAs had quantitatively and qualitatively altered distributions of isoaccepting species during the course of DMSO induced erythroid differentiation.

The change in lysine isoacceptor patterns during various cellular events has been extensively studied (9, 26) and it is of interest to compare the changes of $tRNA^{lys}$ in F.L.C. with some of these studies. $tRNA^{lys}_4$ is of greatest interest since its presence has been correlated with the ability of mammlian cells to divide (18). In mouse leukemic cells (L5178Y) in suspension, $tRNA^{lys}_4$ was a major species, accounting for as much as 40% of total $tRNA^{lys}$. The level of this species decreased progressively at higher cell densities until it was less than 20% of the total $tRNA^{lys}$ (18). It has also been reported that confluent cultures of F.L.C. contained a decreased level of $tRNA^{lys}_4$; however, when these cells were induced to differentiate by the addition of tetramethyl urea, a sharp increase in $tRNA^{lys}_4$ was seen (27).

Our experimental results showed that the relative amounts of $tRNA^{lys}_4$ (as well as other isoacceptors) were similar to those previously reported (27). However, unlike the earlier report, we have analyzed $tRNA^{lys}$ isoacceptors at 36 hr as well as 0, 48, 72, and 96 hr after addition of DMSO. We found that a marked decrease in the relative amount of $tRNA^{lys}_4$ repeatedly occurred between 36 and 48 hours after the addition of DMSO to the culture. The decreased amount of $tRNA^{lys}_4$, 48 hours after the induction, corresponds in time to an accumulation of globin mRNA, the beginning of globin polypeptide synthe-

sis, and to the reduced rate of F.L.C. division that occurs in the initial 48 hr of differentiation.

Our studies have resolved three $tRNA^{met}$ isoaccepting species from F.L.C. corresponding to $tRNA^{met}_1$, $tRNA^{met}_3$ and $tRNA^{met}_4$ according to Elder and Smith (28). It is worth noting that the relative amount of $tRNA^{met}_1$, initiator $tRNA^{met}$, has a rather constant value during the time course of differentiation except for a dramatic decrease relative to other isoacceptors at 48 hours. DMSO treated F.L.C. cultures show a 24-48 hour lag before logarithmic growth commences (14, 29). It has also been shown that total protein and RNA synthesis in F.L.C. decreased in the first 48 hours after the addition of DMSO (29), and that globin polypeptide synthesis did not increase until 48 hours (13). Thus, relatively low levels of initiator $tRNA^{met}$ may reflect this transition state of low protein synthesis between the undifferentiated and differentiated stages of the cells.

F.L.C. expressed one major and three minor species of $tRNA^{phe}$. The changes in these tRNA were small; however, even small changes could reflect important events in differentiation. Ortwerth, et al. (4) have shown that a $tRNA^{phe}$ species eluting earlier on RPC-5 chromatography than the major $tRNA^{phe}$ was unique to the lens and not present in other normal mammalian tissue. This novel $tRNA^{phe}$ was further shown to increase as a result of lens cell differentiation. Furthermore, in the same report, it was shown that the extra lens $tRNA^{phe}$ was almost absent in the epithelial cells but present in the fiber cells, suggesting that induction of a new lens $tRNA^{phe}$ occurred upon differentiation. The profiles of $tRNA^{phe}$ that we obtained showed a unique pattern in which $tRNA^{phe}_1$ was always present in very small amounts but that $tRNA^{phe}_2$ was not present until 36 hours after the addition of inducer and reached its maximal relative amount of 11% at 48 hours. A third minor species, designated 3, appeared at 72 hr after induction of F.L.C.

Garel (2) has found that the population of tRNAs for glycine, alanine, serine and tyrosine, which comprise 93% of amino residues in silkworm Bombyx mori fibroin, increase as much as 3 fold over that of other tRNAs with increasing fibroin production and thereby postulated a "functional adaptation of tRNA." Similar phenomena have been found in the calf lens for the biosynthesis of crystallins (4) as well as in the rabbit reticulocyte for the biosynthesis of hemoglobin (6, 25). The numbers and amounts of isoacceptors for differentiated F.L.C. (72 and 96 hr after addition of DMSO) approximate those of rabbit reticulocyte tRNAs (25).

Since alanine comprises approximately 15% of the amino acids residues in

both mouse α and β globin, while serine makes up approximately 10% of amino acids residues in α globin and 6% in β globin, we might expect that certain isoaccepting species of these two tRNAs would increase in relative amounts during the erythro-differentiation. Indeed, the minor $tRNA^{ala}$ isoaccepting species 1 and 2 have a 2-fold increase at 48 hours compared with $tRNA^{ala}$ from uninduced cells, while $tRNA^{ser}_2$, suspected as a precursor to $tRNA^{ser}_4$ has an almost 2.5-fold increase from 36 to 38 hr. The nucleotide sequence of mouse β globin mRNA has recently been reported (30). Five of the six leucine codons are present in the β globin mRNA sequence and would most likely require four different isoaccepting species. We have found that F.L.C. cultures producing hemoglobin express four isoacceptors, of which two, $tRNA^{leu}_2$ and $tRNA^{leu}_5$ have greatly increased in relative amount during differentiation. $tRNAP^{ro}_2$ and $tRNAP^{ro}_3$ were major species while $tRNAP^{ro}_1$ and $tRNAP^{ro}_4$ were represented only in trace amounts in uninduced cells. However, during differentiation, $tRNAP^{ro}_2$ and $tRNAP^{ro}_3$ consistently decreased in their relative amounts to barely detectable levels, while $tRNAP^{ro}_1$ and $tRNAP^{ro}_4$ consistently increased to become the major species. Only two of the four possible proline codons are present in β globin mRNA (2, CCU and 1, CCC). $tRNA^{thr}$ consisted of four species in relatively equal amounts in uninduced cultures. $tRNA^{thr}_2$ and $tRNA^{thr}_3$ consistently decreased in relative amount throughout differentiation. At 96 hours they were almost non-existent. Of the four possible threonine codons, only those ending in a pyrimidine are present in β globin mRNA (3, ACU and 3, ACC). These observations suggest F.L.C. switches expression of $tRNA^{pro}$ and $tRNA^{thr}$ isoacceptors in order to adapt to the specific protein synthesis needed in differentiation for hemoglobin production. This change of expression could be by way of post-transcriptional modification of $tRNA^{pro}$ and $tRNA^{thr}$ isoacceptors or by differential gene expression.

Minor isoaccepting tRNA species appear transiently during differentiation of F.L.C. in nearly all the aminoacyl-tRNA chromatographic profiles. Since these minor species are only present during the differentiation process, they may be involved in regulating the transition between non-hemoglobin synthesizing and hemoglobin synthesizing stages of the cells. We have resolved 64 isoaccepting species of tRNA for 16 amino acids as described here and the adjoining paper (12). Therefore, we presume that more than 80 tRNA species are produced by F.L.C. for the 20 amino acids used in protein synthesis. The present study comparing isoaccepting tRNA species during the <u>in vitro</u> induced differentiation of a cloned cell line, provides additional information concerning possible roles for tRNA species in differentiation. Identi-

fication of the tRNA isoacceptors that change during F.L.C. differentiation is an important first step in using this easily controlled, homogeneous population of cells for studying the role of tRNA in cell differentiation.

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