Specific changes in Q-ribonucleoside containing transfer RNA species during Friend leukemia cell erythroid differentiationVictor K. Lin¹, Walter R. Farkas² and Paul F. Agris¹¹Division of Biological Sciences, University of Missouri, Columbia, MO 65211, and ²Memorial Research Center, University of Tennessee, Knoxville, TN 37920, USA

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ABSTRACT

Changes in specific tRNA isoacceptors during Friend leukemia cell (F.L.C.) erythroid differentiation have been found to be concomitant with differences in the extent of the Q-base modification in certain species of tRNA. Transfer RNA was isolated from F.L.C. cultures after 0, 36, 48, 72, and 96 hr of DMSO induced differentiation. Changes in 17 isoacceptors of tRNA^{asn}, tRNA^{asp}, tRNA^{his} and tRNA^{tyr} were compared by RPC-5 chromatography. Isoacceptors of these tRNA changed in relative amounts, following consistent trends throughout cell differentiation. The amount and distribution of Q-base containing tRNA isoacceptors was assayed by measuring the guanine-tRNA transferase catalyzed incorporation of [³H]-labeled guanine into tRNA species undermodified in Q-base followed by RPC-5 chromatography of the tRNA. The amount of Q-base containing tRNA species decreased in the first 48 hr after the induction, then increased again, indicating the level of Q-modification is correlated to the process of differentiation. Isoacceptors that lacked the Q-base were eluted late from RPC-5.

INTRODUCTION

Prokaryotic and eukaryotic tRNA^{asn}, tRNA^{asp}, tRNA^{his}, and tRNA^{tyr} contain the hypermodified ribonucleoside Q¹ or its glycosylated derivative Q* in the first position of the anticodon (1). The Q ribonucleoside may be important for regulatory functions of these tRNAs. Recent work has shown that mammalian cells synthesize the Q-containing (Q⁺) tRNAs from precursor tRNAs that have guanine in the wobble position by excision of the guanine followed by insertion of queuosine, the base of Q (2). The amount of Q relative to unmodified G in these tRNAs varies at different stages in the life cycle of *Drosophila*, suggesting that the presence of Q in tRNA is related to cell differentiation (3, 4). A significant decrease in the amount of Q in tRNA has been associated with malignancy (5-7).

In this report, we have used reversed-phase plaskon chromatography to re-

¹ Abbreviations and explanation of terminology: Q, queuosine, 7-(4,5-cisdi-hydroxy-1-cyclopenten-3-ylaminomethyl)-7-deazaguanosine; (Q⁺)tRNA, tRNA that contains Q in the first position of the anticodon; (Q⁻)tRNA, the precursor of (Q⁺)tRNA. In this study, Q is used generically to designate Q and its saccharide derivative Q*.

solve and quantitatively analyze the 17 isoaccepting species of tRNA^{asn}, tRNA^{asp}, tRNA^{his}, and tRNA^{tyr} during erythroid differentiation of Friend leukemia cells, F.L.C. (8). This cell has the ability to undergo erythroid-like differentiation when exposed to dimethylsulfoxide (DMSO) and in this process, passes from a proliferative, non-hemoglobin synthesizing state to a non-proliferative, hemoglobin synthesizing state. In the previous adjoining paper, we reported on the changes in 47 isoacceptors for 12 other amino acids (9). During F.L.C. erythroid differentiation, relative amounts of isoaccepting species of tRNA^{asn}, tRNA^{asp}, tRNA^{his}, and tRNA^{tyr} changes significantly, and the amount of Q ribonucleoside modification of these tRNA species was found to be correlated to the process of differentiation.

MATERIALS AND METHODS

Materials: The sources of the cell line, cell culture medium, chromatographic materials and radiochemicals and the specific activities of the radiochemicals are found in the adjoining paper (9). Labelled [³H] guanine for guanylation was purchased from ICN with the specific activity of 1 Ci/mmol.

Cell Culture and Erythroid Induction with Dimethylsulfoxide (DMSO):

Cultures of F.L.C. were grown and the cells were induced to differentiate as described (9).

Isolation and Aminoacylation of Transfer RNA: Transfer RNA was extracted, purified and aminoacylated as described in the adjoining report (9). tRNA^{asn} was aminoacylated with [³H] asparagine in the presence of unlabelled aspartate (1mM) to insure that tRNA^{asp} would not become labelled by aminoacylation with deaminated asparagine. Aminoacyl-tRNA synthetase was prepared as also described in the adjoining report (9).

Reversed-Phase Chromatography: RPC-5 column chromatography was accomplished as described in the adjoining report (9).

Assay for (Q⁻)tRNA: The reaction conditions and assay procedures have been described in detail (2), except 7 units of tRNA-guanine transferase (10), and either 0.08 or 0.16 A₂₆₀ units of F.L.C. tRNA were added to the incubation mixture.

RESULTS AND DISCUSSION

Transfer RNA was isolated from uninduced Friend leukemic cells (F.L.C.) and from the same cells 36, 48, 72, and 96 hr after induction of erythroid differentiation by the addition of dimethylsulfoxide (DMSO) to culture medium. The iso-

accepting species of tRNAs which contain the ribonucleoside Q, tRNA^{asn}, tRNA^{asp}, tRNA^{his}, and tRNA^{tyr}, were resolved by reversed-phase plaskon chromatography (RPC-5). Quantitative and qualitative changes of the isoaccepting species for these tRNAs, tabulated in Table 1, reflected significant alterations during erythroid differentiation.

Transfer RNA^{asn} was resolved into 5 isoaccepting species. In Figure 1A, distributions of the tRNA^{asn} isoaccepting species were compared at 5 time points during the course of erythroid differentiation. As shown in the graph and Table 1, tRNA₃^{asn} consistently increased in relative amount during the differentiation, whereas tRNA₂^{asn} and tRNA₅^{asn} decreased to trace amounts. However, tRNA₁^{asn} was rather constant in amount after decreasing in the first 36 hr to approximately 24% of the total tRNA^{asn}, while tRNA₄^{asn} first increased and then decreased to a barely detectable level.

Table 1: Changes in Amino Acid Isoaccepting tRNA Species Which Contain Q-Nucleoside

tRNA	Time During Differentiation ^a	Relative Amounts of Isoaccepting Species ^b				
		1	2	3	4	5
Asn	0	32	26	11	3	28
	36	16	13	42	10	19
	48	24	5	57	13	1
	72	26	0	63	11	0
	96	23	4	71	1	1
Asp	0	3	24	68	5	
	36	6	9	64	21	
	48	4	10	65	21	
	72	2	18	54	26	
	96	0	43	53	4	
His	0	16	16	27	41	
	36	12	13	35	40	
	48	10	10	47	33	
	72	1	13	54	32	
	96	7	13	8	72	
Tyr	0	4	11	27	58	
	36	13	11	12	64	
	48	39	2	9	50	
	72	43	3	0	54	
	96	53	2	0	45	

^aHours after the addition of DMSO

^bpercent of total aminoacyl-tRNA

Figure 1. Alterations in the RPC-5 profile 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 was aminoacylated with [^3H]-labelled amino acid (o--o) and subjected to RPC-5 chromatography along with an internal standard, rat liver [^{14}C]-lysyl-tRNA (●--●). The co-chromatography of [^{14}C]-asparaginyl-tRNA_{FLC} with [^3H]-lysyl-tRNA_{Rat liver} was an exception to this procedure. Details of the chromatography procedures are described in Material and Methods. Panel A shows the separation of isoaccepting asparaginyl-tRNA_{FLC} species; B, aspartyl-; C, histidyl-; and D, tyrosinyl-tRNA_{FLC}.

Transfer RNA^{asp} was resolved into 4 isoacceptors as shown in Figure 1B, tRNA^{asp}₃ was always the predominant species. tRNA^{asp}₁ was present only in trace amounts if present at all. tRNA^{asp}₂ decreased in relative amount at 36 hr and then gradually increased to almost two fold the original level at the completion of differentiation (96 hr). Concomitantly, tRNA^{asp}₄ first increased by 4 fold;

however, at 96 hr, the relative amount of $\text{tRNA}_4^{\text{asp}}$ was comparable to that at 0 hr. Increases in the relative amounts of the late-eluting species of tRNA^{asp} are thought to be favored, though not obligated, by neoplasia, and have been found to be a consistent property of SV40- and Polyoma-transformed cells in culture (11-14). Our results on F.L.C. $\text{tRNA}_3^{\text{asp}}$ and $\text{tRNA}_4^{\text{asp}}$ are consistent with most of these observations.

Transfer RNA^{his} was resolved into 4 isoaccepting species as shown in Figure 1C, $\text{tRNA}_3^{\text{his}}$ and $\text{tRNA}_4^{\text{his}}$ were predominant species throughout the first 72 hr of differentiation (Table 1). The minor species, $\text{tRNA}_1^{\text{his}}$, and the major species, $\text{tRNA}_3^{\text{his}}$, particularly attracted our attention because of dramatic changes in their relative amounts during the course of differentiation. Upon completion of differentiation by a majority of the cells at 72 hr after addition of DMSO, $\text{tRNA}_3^{\text{his}}$ had doubled in amount relative to the other isoacceptors, whereas $\text{tRNA}_1^{\text{his}}$ decreased. $\text{tRNA}_3^{\text{his}}$ decreased dramatically in relative amount between 72 and 96 hr, while $\text{tRNA}_4^{\text{his}}$ doubled in the same time period. $\text{tRNA}_2^{\text{his}}$ was maintained by the cells in a constant relative amount throughout the entire course of differentiation. Rabbit reticulocytes were found to contain two isoaccepting species for tRNA^{his} when that tRNA was investigated by various chromatographic methods including RPC-5 (15). The early eluting species contained Q, whereas the late eluting one did not (15, 16). Both isoaccepting tRNA^{his} species were found to incorporate histidine into all of the histidine-containing positions of rabbit hemoglobin (15). Thus, the quantitative changes of tRNA^{his} species 1, 3, and 4 that occurred during erythroid differentiation of F.L.C. may have a more subtle function than recognition of the two histidine codes (CAC and CAU). For instance, the rate of translation may be affected by the increased relative amount of $\text{tRNA}_3^{\text{his}}$.

Transfer RNA^{tyr} was separated into 4 isoacceptors (Figure 1D). $\text{tRNA}_4^{\text{tyr}}$ was the major species and changed relatively little throughout differentiation (Table 1). $\text{tRNA}_1^{\text{tyr}}$ consistently increased in relative amount and finally became a second major species. $\text{tRNA}_2^{\text{tyr}}$ and $\text{tRNA}_3^{\text{tyr}}$, which were not well resolved by the chromatography conditions we employed, decreased gradually during the course of differentiation relative to the other species. Comparative studies of tRNA^{tyr} from normal human placenta and HeLa cells indicated that no significant differences existed in the rate or site of incorporation of tyrosine into tryptic peptides of α -globin, even though there was again a dramatic difference in the RPC-5 chromatographic profiles between the tRNAs of these two tissues (17). The observed changes in F.L.C. tRNA^{tyr} isoacceptors 1, 2, and 3 were coincident with cell differentiation. It has been suggested in two reports that one of the

tRNA^{tyr} isoaccepting species may have a regulatory function concomitant with differentiation, in these cases with regard to pigment cell metabolism (18, 19). The amount of Q ribonucleoside relative to unmodified guanosine in tRNA^{asn}, tRNA^{asp}, tRNA^{his}, and tRNA^{tyr} varies at different stages in the life cycle of *Drosophila* further suggesting that the presence of Q in tRNA is related to differentiation of cells (3).

Differences in amounts of tRNA isoaccepting species for asparagine, aspartate, histidine, and tyrosine consistent with differences in the extent of Q ribonucleoside modification have been observed in a number of mammalian systems (5-7, 13). The presence of Q, which is both positively charged and hydrophilic, in the exposed anticodon of a specific tRNA molecule, suggests that the molecule would be a good receptor of enzymes in reactions associated with regulation of cell function (20, 21), the rate of protein synthesis or the anticodon-codon recognition (22, 23).

The presence of the Q modification and the distribution of tRNA isoacceptors lacking this modification during the course of differentiation were studied. We first determined the amounts of Q ribonucleoside present in F.L.C. tRNA preparations isolated at five time points during differentiation. An enzyme, purified from rabbit erythrocytes, catalyzes the replacement of guanine with queuine at the wobble position of (Q⁻)tRNA (2, 10). In the absence of its true substrate, queuine, this enzyme catalyzes an exchange between free guanine and the guanine in the wobble position (2). This exchange can be used to assay for (Q⁻)tRNA (4, 5, 16, 24). Our results from guanylation of tRNA preparations from F.L.C. at five stages of differentiation are shown in Table 2. There was essentially no guanylation observed for uninduced tRNA; indicating that tRNA from uninduced cells was highly modified with regard to the Q. However, the tRNA

Table 2: Guanine Incorporation for Different F.L.C. tRNA Preparations

tRNA Preparation ^a	0 hr	36 hr	48 hr	72 hr	92 hr
[³ H] Guanine Incorporated into tRNA ^b	1.0	8.0	9.7	5.0	3.7

^atRNA preparations are designated according to the length of time F.L.C. had been induced to undergo erythroid differentiation by exposure to dimethylsulfoxide.

^bGuanylation was assayed as described in Materials and Methods and is shown as pmole [³H] guanine incorporated per A₂₆₀ unit of tRNA.

from F.L.C. after 36 hr of differentiation accepted 8.0 pmole of guanine per A_{260} of tRNA. tRNA from cells after 48 hr of induction exhibited the maximum guanine incorporation which then decreased significantly at 72 and again at 96 hr. These results indicate that concomitant with differentiation, F.L.C. continually decreased the synthesis of Q in newly synthesized tRNA until 48 hr after the addition of DMSO, the time at which the tRNA had the lowest level of Q. The level of Q in tRNA increased consistently as the greater portion of the F.L.C. differentiated during the next 48 hours. Tumor cell tRNA contains mostly guanosine in the wobble position instead of Q (5,7); however, these results were obtained from tRNA extracted from tumor cells propagated *in vivo* or from tumor cell cultures that were not monitored for cell differentiation. Our results from guanylation of tRNA of F.L.C. undergoing well-defined differentiation adds another dimension to these findings. The Q content of F.L.C. tRNA was not constant during differentiation.

Theoretical acceptance of guanine in *in vitro* guanylation should be approximately 400 pmoles/ A_{260} ; yet we obtained a maximum incorporation of 10 pmoles/ A_{260} . The same result has been observed by other investigators using F.L.C. tRNA preparations and *E. coli* guanine tRNA transferase (Randall Kopper and Sam Singhal, personal communication). For yeast tRNA, which contains no Q we observed an uptake of 45.6 pmoles 3H guanine per A_{260} of tRNA. Using the guanine tRNA transferase from *E. coli* and yeast tRNA, Okada et al. obtained a guanine incorporation of 110.7 pmoles per A_{260} (5) indicating that regardless of the source of enzyme the amount of guanine that can be accepted by a sample of tRNA is indicative of the amount of (Q-) tRNA (5) but considerably less than theoretical uptake. The reason for this is not clear; however, at present, there are four possibilities to be considered: 1) that not all isoaccepting species are substrate for guanine tRNA transferase; 2) that some isoacceptor might already have a "pre-Q" in place, and therefore, can not be a substrate for guanine tRNA transferase; 3) that the sample tRNA lacks other modifications necessary for recognition by the guanylation enzyme; and 4) that the *in vitro* assay might not be the absolute analogue of the *in vivo* system.

In order to examine more closely the tRNA species which may serve as substrates for guanylation, F.L.C. tRNAs isolated from different time points during the induced differentiation were guanylated and then fractionated by RPC-5 chromatography (Figure 2). The results showed that a minor group of guanylated tRNA eluted early and a major group of guanylated tRNA eluted late, approximately corresponding to the elution position of the internal

Figure 2. RPC-5 chromatographic comparison of guanylated tRNA from F.L.C. undergoing erythroid differentiation. tRNA isolated from F.L.C. 36, 48 and 96 hr after addition of DMSO to cultures, was guanylated with [^3H]-labelled guanine and subjected to RPC-5 chromatography. This chromatogram compares the elution profiles of these three guanylated tRNA (36 hr, $\bullet\bullet\bullet$; 48 hr, $\blacksquare\blacksquare\blacksquare$; 96 hr, $\square\square\square$). Details of the guanylation and the chromatography procedures are described in Materials and Methods.

standard tRNA $^{\text{lys}}_5$. Thus, it appears that these isoaccepting species of tRNA $^{\text{asn}}$, tRNA $^{\text{asp}}$, tRNA $^{\text{his}}$, and tRNA $^{\text{tyr}}$ capable of being modified for the synthesis of Q have similar retention times on RPC-5 chromatography under the condition used. The isoaccepting species of tRNAs that are fully modified with respect to Q are not detected by this method of guanylation and fractionation. Therefore, from the elution positions of the 17 tRNA species for the four amino acids, we suggest that tRNA $^{\text{asn}}_4$, tRNA $^{\text{asn}}_5$, tRNA $^{\text{asp}}_4$, tRNA $^{\text{his}}_3$, tRNA $^{\text{his}}_4$, and tRNA $^{\text{tyr}}_4$ may contain guanosine in the wobble position. The relative amounts of tRNA $^{\text{asn}}_4$, tRNA $^{\text{asp}}_4$, and tRNA $^{\text{his}}_3$ were altered corresponding to the level of guanylation in total tRNA at different time points during the differentiation of F.L.C.

It has been suggested that the degree of tRNA modification in tissue culture cells may be affected by cell density (13, 25). However, a recent report (10) has shown that cell density and/or proliferative state are not major variables in controlling the expression of Q and has therefore indicated that the previous results may be an artifact due to drastic changes of pH or other conditions in culture. Thus, we consider that changes of tRNA $^{\text{asn}}$, tRNA $^{\text{asp}}$, tRNA $^{\text{his}}$, and tRNA $^{\text{tyr}}$ isoaccepting species at various stages of F.L.C. differentiation were not due to changes in cell density, but due to cell differentiation.

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