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Biochemistry

Role of ribothymidine in mammalian tRNA^{Phe}

(mammalian protein synthesis)

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ABSTRACT **We have previously reported that mammalian tRNAs^{Phe} from various tissues contain different amounts of ribothymidine and that a uridine methylase from *Escherichia coli* can quantitatively convert these tRNAs to species that contain their full complement of ribothymidine at position 23 from the 3′ terminus. The role of ribothymidine in mammalian tRNAs has now been investigated by studying the ability of several highly purified mammalian tRNAs^{Phe}, differing only in their ribothymidine content, to support poly(U)-directed poly(Phe) synthesis under various conditions. Our results indicate that the ribothymidine content of mammalian tRNA^{Phe} can be correlated with the ability of these tRNAs to function *in vitro* in a low-magnesium (6 mM), ribosome wash factor-dependent, poly(U)-directed poly(Phe) synthesis system from rat liver. Specifically, the effect of increasing the ribothymidine content in a class C mammalian tRNA becomes manifest in an increased apparent maximum velocity for the overall synthesis of poly(Phe), while the apparent Michaelis constant (K_m) remains essentially unchanged. It is postulated that the modified nucleoside ribothymidine might be involved in the regulation of protein synthesis at the level of translation in mammalian liver.**

In recent years, tRNA has been shown to act not only as a pivotal macromolecule in protein synthesis but also as a regulatory macromolecule at both the transcriptional and translational levels (1–10). These studies include those by Ofengand and Henes (8) and Shimizu *et al.* (9) who demonstrated that the T-Ψ-C-G loop region in prokaryotic tRNA (loop IV) was involved in ribosome binding during protein synthesis. In addition, the more recent work by Sprinzl *et al.* (10) indicated that the tetranucleotide fragment T-Ψ-C-Gp competitively inhibits the binding of aminoacyl tRNA to the prokaryotic ribosomal amino acid acceptor ("A") site.

Because tRNA from prokaryotic cells contains 1 mol of ribothymidine per mol of tRNA, whereas that of eukaryotic cells contains less than molar amounts of ribothymidine (11–18), our laboratory has been interested in elucidating the function of this modified nucleoside in highly purified mammalian tRNAs. We recently reported (17) that, based on which nucleoside was present at position 23 from the 3′ terminus, mammalian tRNA can be grouped into four classes: class A, those tRNAs in which ribothymidine is completely replaced by adenosine; class B, those tRNAs that contain their full complement of ribothymidine, pseudouridine, or 2′-*O*-methylribothymidine; class C, those tRNAs that have only a partial conversion of uridine to ribothymidine; and class D, those tRNAs in which only uridine occupies position 23 from the 3′ terminus. Because tRNAs in classes A and B contain either adenosine, pseudouridine, ribothymidine, or 2′-*O*-methylribothymidine at position 23, these tRNAs are not substrates for the *Escherichia coli* uridine methylase. At that time we also reported that the class C tRNAs, which partially lack ribothymidine, are acceptable substrates for the *E. coli* uridine methylase. Recent evidence indicates that the mammalian tRNAs in class D can be methylated by the *E. coli* uridine methylase (18), but at a much lower rate than the wheat germ tRNA^{Gly} (B. A. Roe and K. Roesch, unpublished data).

It has also been shown that class A tRNA (including the initiator tRNA) binds to a site that is different from the binding site of the noninitiator tRNAs (19). In a recent review, Erdmann (20) hypothesized that, because the 5.8S RNA contained an unusually high number of sequences that were complementary to the T-Ψ-C-G region of noninitiator tRNAs, this RNA would be a good candidate to function in eukaryotic ribosomes in a fashion similar to that of the 5S RNA in prokaryotic ribosomes (10). Therefore, the initiator tRNA might bind at the ribosomal peptidyl ("P") site through interactions involving 5S RNA, whereas the noninitiator tRNAs might initially bind to the ribosomal aminoacyl ("A") site through interactions involving the 5.8S RNA. Marcu and Dudock (21) demonstrated that wheat tRNA^{Gly} functions better in protein synthesis when it totally lacks ribothymidine.

In the present studies we compared the kinetics of poly(U)-directed poly(Phe) synthesis by using mammalian tRNAs^{Phe} differing only in their ribothymidine content. The results imply that the partial ribothymidine content of specific mammalian tRNAs may act to regulate eukaryotic protein synthesis at the level of translation.

MATERIALS AND METHODS

Materials. Poly(U) (molecular weight, 10^5–10^6) was obtained from Biogenics Res. Corp., Chagrin Falls, OH. Creatine phosphokinase and creatine phosphate were purchased from Grand Island Biological Co.; [^{14}C]phenylalanine at 50 and 430 mCi/mmol was from Schwarz/Mann and ICN, respectively; and *S*-adenosyl-L-[^{14}C]methionine at 52 mCi/mmol, from ICN. All other reagents were as described (17, 22, 23).

Preparation of tRNA, Methylated tRNA, and Sham-Methylated tRNA. Rat liver, beef liver, and human placenta tRNA^{Phe} were obtained at a purity of at least 1.2 nmol/A_{260} unit* as described (17, 23). These tRNAs were fully methylated with a partially purified preparation of *E. coli* uridine methylase as described (17, 24, 25). Sham-methylated tRNA^{Phe} was prepared under similar conditions (17) by incubation with *E. coli* uridine methylase in the absence of *S*-adenosyl-L-methionine. The *in vitro* methylated and sham-methylated mammalian tRNAs^{Phe} were further purified by gradient elution from a small (0.5 × 10 cm) RPC-5 column at pH 7.6 under conditions similar to those described (23, 24). This additional purification step removed an apparent inhibitor of the

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Abbreviation: buffer A, 0.25 M sucrose/0.1 mM EDTA/1 mM dithiothreitol.

* An A_{280} unit is that amount of material that gives an absorbance of 1 when dissolved in 1 ml of solvent when the light path is 1 cm.

poly(U)-directed poly(Phe) synthesis, evidenced at high tRNA concentrations. The purity of each methylated or sham-methylated tRNA was subsequently determined by direct measurement of the radioactivity from the *in vitro* methylation reaction (17) or by measurement of the amino acid acceptor activity (22, 23). In all cases, only those fractions that gave a specific activity of at least 1.2 nmol/A_{260} unit were pooled and used in the subsequent studies. In addition, all data reported for the methylated tRNA have been corrected for the radioactivity of the ^{14}C-labeled methyl group.

Preparation of KCl-Washed Rat Liver Ribosomes, Ribosome Wash Factors, and Aminoacyl-tRNA Synthetases. A partially fractionated rat liver protein synthesis system was isolated by modifying the procedures described by Reyes *et al.* (26) and Shafritz and Anderson (27). This procedure entailed homogenization of 80 g of liver in 100 ml of 0.25 M sucrose/0.05 M Tris·HCl, pH 7.6/2 mM $MgCl_2$ containing 25 mM KCl, centrifugation at 20,000 × *g* for 15 min, and then centrifugation at 30,000 × *g* for 30 min and at 105,000 × *g* for 1 hr. The supernatant from the last centrifugation, which contained the aminoacyl-tRNA synthetases, was precipitated with ammonium sulfate (45 g/100 ml), dialyzed against 0.01 M Tris·HCl, pH 7.6/0.01 M $MgCl_2$/0.01 M 2-mercaptoethanol/10% (vol/vol) glycerol, and stored at −20° at a final concentration of 90–100 A_{280} units/ml. The pellet from the 105,000 × *g* centrifugation was homogenized in 5 ml of supernatant, layered on 0.75 M sucrose (containing 0.05 M Tris·HCl, pH 7.6, and 0.01 M $MgCl_2$) and pelleted through 1.5 M sucrose by centrifugation at 105,000 × *g* for 3 hr. The resulting pellet was suspended in buffer A (0.25 M sucrose/0.1 mM EDTA/1 mM dithiothreitol) containing 0.025 M KCl, and 4.0 M KCl was added to give a final concentration of 0.5 M. After incubation at 4° for 1 hr, the suspended pellet was centrifuged at 105,000 × *g* for 1.5 hr, and the ribosomal pellet was dispersed in buffer A to a final concentration of 150–200 A_{280} units/ml and stored at −70° in small portions. The supernatant fraction containing the 0.5 M KCl ribosome wash factors was diluted to 0.01 M KCl by addition of buffer A, adsorbed to DEAE-cellulose, eluted with buffer A containing 0.3 M KCl, and stored at −70° at a final concentration of 2–3 A_{280} units/ml.

Aminoacyl-tRNA Activity, Ribothymidine Content, Enzymatic Conversion of Uridine to Ribothymidine, and Poly(Phe) Synthesis. Measurement of aminoacyl-tRNA activity was performed as described (22), with a saturating amount of aminoacyl-tRNA synthetase. The kinetics of aminoacylation were determined by using a limiting amount of aminoacyl-tRNA synthetase under both the optimal aminoacylation conditions described (22) and the poly(Phe) synthesis conditions described herein. The ribothymidine content of the various mammalian tRNAs used in these studies was determined by the ^{3}H post-labeling technique described by Randerath *et al.* (28, 29). The enzymatic conversion of uridine to ribothymidine at position 23 from the 3′ terminus by a partially purified preparation of *E. coli* uridine methylase has been reported (16).

Measurement of poly(Phe) incorporation was performed in a 0.10-ml incubation mixture containing 7.5 mM Tris·HCl (pH 7.6), 130 mM KCl, 1 mM ATP, 0.2 mM GTP, 5 mM or 10 mM magnesium acetate (6 mM or 11 mM final concentration), 1 mM dithiothreitol, 10 μg of creatine phosphokinase (115 enzyme units/mg), 0.2 mM creatine phosphate, 1.2 nmol of [^{14}C]phenylalanine (430 mCi/mmol), 2.0 nmol of the 19 other nonradioactive amino acids, 40 μg of poly(U), 0.75–1.00 A_{280} unit of 0.5 M KCl-washed ribosomes, 0.001–0.03 A_{260} unit of pure tRNA, 0.4–0.5 A_{280} unit of total aminoacyl-tRNA synthetase, and 0.02–0.03 A_{280} unit of 0.5 M KCl ribosome wash factors. Incubations were at 37° for 6, 12, or 30 min; reactions were terminated by the addition of 3 ml of 10% (wt/vol) trichloroacetic acid. The mixture then was heated at 85° for 30 min, cooled to 0°, filtered through a glass fiber disc (Reeve Angel, grade 934AH), washed with 2% trichloroacetic acid, and dried. Radioactivity was determined as described (22).

Table 1. Reaction requirements for poly(Phe) synthesis

Incubation mixture	Polymerization*	Polymerization†
Complete	100 %‡	100 %§
−ATP	2	3
−GTP	40	60
−Mg	2	3
−tRNA	4	4
−Poly(U)	3	5
−Ribosomes	4	9
−Aminoacyl tRNA synthetase	1	1
−Creatine phosphate and creatine phosphokinase	74	70
−19 other amino acids	70	66
−Ribosomal wash fraction	60	—¶
+Ribosomal wash fraction	—¶	100

* The complete incubation mixture was at a final Mg^{2+} concentration of 6 mM and contained 0.2 A_{280} unit of the 0.5 M KCl ribosome wash fraction. All other components were present as described in *Materials and Methods*, except that 0.02 nmol of $tRNA^{Phe}$ was used.

† The complete incubation mixture was at a final Mg^{2+} concentration of 11 mM and the 0.5 M KCl ribosome wash fraction was omitted. All other components were present as described in *Materials and Methods*, except that 0.02 nmol of $tRNA^{Phe}$ was used.

‡ 100% polymerization represents 24.4 pmol of [^{14}C]phenylalanine retained on a glass fiber filter disc after treatment as described in *Materials and Methods*.

§ 100% polymerization represents 20.3 pmol of [^{14}C]phenylalanine retained on a glass fiber filter disc after treatment as described in *Materials and Methods*.

¶ Same as complete.

All experiments were performed in replicate (duplicate or greater), as indicated in the figure legends, and were reproduced for different preparations of 0.5 M KCl-washed ribosomes, 0.5 M KCl ribosome wash factors, and sham- and *in vitro*-methylated $tRNA^{Phe}$. In some cases the data presented have been corrected for variations (not greater than 20%) in the ribosome activities for different preparations by normalization to either a human placenta or beef liver $tRNA^{Phe}$ control and plotted with the error bars representing the maximum standard deviation for the normalized data.

RESULTS

The reaction mixture components required for the polymerization of phenylalanine in the *in vitro* poly(Phe) synthesis system, with a highly purified rat liver $tRNA^{Phe}$ (1.2 nmol/A_{260} unit), are shown in Table 1. Reaction component requirements were similar for both the low magnesium (6 mM final concentration) 0.5 M KCl ribosome wash factor-dependent system and the high magnesium (11 mM final concentration) factor-independent system, although the total phenylalanine polymerized under the low magnesium conditions was greater than that obtained under the high magnesium conditions (18,000 and 15,000 cpm, respectively). In a separate experiment, these two magnesium concentrations for the *in vitro* polymerization re-

action, with or without added 0.5 M KCl ribosome wash factors, were determined to be optimal (data not shown) and to be in agreement with those reported with total tRNA rather than highly purified mammalian $tRNA^{Phe}$ (31). The experiment shown in Table 1 demonstrated that the polymerization reaction was completely dependent upon added poly(U), ATP, ribosomes, tRNA, and aminoacyl-tRNA synthetases and less dependent upon the addition of an ATP regenerating system, the 19 other amino acids, and GTP.

The rates of poly(Phe) synthesis with either the human placenta or the rat liver $tRNA^{Phe}$ (50 and 78% ribothymidine, respectively) were compared under the conditions shown for the complete reaction mixture components (Table 1). This rate study indicated that both the rate and the extent of the poly(U)-directed poly(Phe) synthesis were directly proportional to the ribothymidine content of the tRNA (data not shown but similar to Fig. 1). Because the complete nucleotide sequence for rat liver $tRNA^{Phe}$ has yet to be determined, although from nucleoside composition data (17) its sequence is probably identical to that of the human $tRNA^{Phe}$ (50% ribothymidine), we repeated this rate study with beef liver $tRNA^{Phe}$ (72% ribothymidine) which has a nucleotide sequence identical (except for ribothymidine content) to that of human placenta $tRNA^{Phe}$ (16, 30). The purity of this preparation of beef liver $tRNA^{Phe}$ was 1.2 nmol/A_{260} unit, and its nucleoside composition has been reported earlier (17). Based on the above observations, the beef liver $tRNA^{Phe}$ was used in the subsequent studies instead of the rat liver $tRNA^{Phe}$.

Both the rate and extent of poly(Phe) formation, at 6 mM magnesium with added 0.5 M KCl ribosome wash fraction, were proportional to the ribothymidine content of mammalian $tRNA^{Phe}$, when the tRNAs were at the same rate-limiting concentrations (Fig. 1 *left*). Sham-methylated and untreated beef liver $tRNA^{Phe}$ had identical rates and extents, indicating that pretreatment of the tRNA with the *E. coli* uridine methylase, followed by purification through RPC-5, did not alter the ability of these tRNAs to function in the poly(Phe) synthesizing system. When a similar experiment was performed under identical conditions except in the absence of the 0.5 M KCl ribosome wash factors, no difference was observed in either the rate or the extent of poly(Phe) synthesis, regardless of which $tRNA^{Phe}$ was used (Fig. 1 *right*).

Because this *in vitro* poly(U)-directed poly(Phe) system entails the use of nonaminoacylated tRNA, which is subsequently aminoacylated during incubation under the above-described conditions, we attempted to determine if the ribothymidine content of these highly purified mammalian tRNAs affected either the rate or the extent of the aminoacylation reaction. These studies (data not shown), which were performed under the same reaction conditions and aminoacyl-tRNA concentration as used in the rate and kinetic studies shown in Figs. 1 and 2, demonstrated that there was no significant difference in either the rate or extent of the aminoacylation reaction for the various tRNAs. In addition, under the *in vitro* poly(Phe) synthesis conditions, essentially all of the tRNA present was aminoacylated within at most the first 1 min of incubation.

These results prompted us to perform kinetic experiments in an attempt to determine which kinetic parameter was affected by the ribothymidine content of the mammalian $tRNAs^{Phe}$ in the poly(U)-directed poly(Phe) synthesis system. The rates of polymerization of phenylalanine in the *in vitro* rat liver poly(U)-directed system at varying tRNA concentrations with several mammalian $tRNAs^{Phe}$ are shown in Fig. 2. As shown in Fig. 2 *A–C*, at high magnesium concentration in either

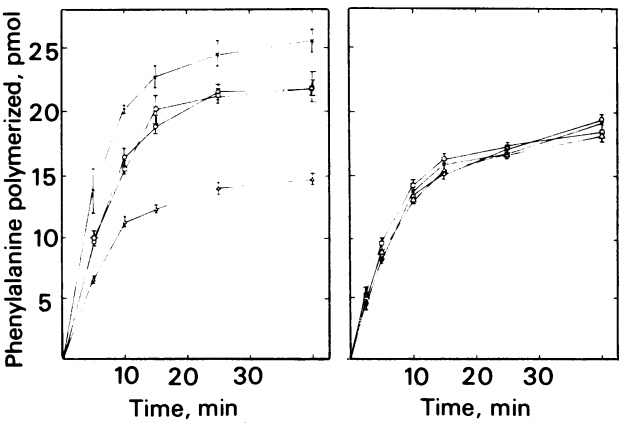

FIG. 1. Rate of poly(U)-directed poly(Phe) synthesis. (*Left*) Under the conditions of low magnesium concentration in the presence of 0.025 A_{280} unit of 0.5 M KCl ribosome wash factors, 0.5 A_{280} unit of aminoacyl-tRNA synthetase, and 0.00314 nmol of the specific mammalian tRNAs as described in *Materials and Methods*. ×, Methylated beef liver $tRNA^{Phe}$; ○, beef liver $tRNA^{Phe}$; □, sham-methylated beef liver $tRNA^{Phe}$; △, human placenta $tRNA^{Phe}$. (*Right*) As *Left*, except that the 0.5 M KCl ribosome wash factors were replaced by 0.3 M KCl.

the absence or presence of the 0.5 M KCl ribosome wash factors or at low magnesium concentration in the absence of 0.5 M KCl ribosome wash factors, the rates of poly(Phe) synthesis as a function of tRNA concentration were identical for all preparations of $tRNA^{Phe}$. However, as shown in Fig. 2*D*, at low magnesium concentration in the presence of 0.5 M KCl ribosome wash factors, the rate of poly(Phe) synthesis as a function of tRNA concentration was directly proportional to the ribothymidine content for all four mammalian $tRNAs^{Phe}$. Also, at all concentrations, the naturally occurring beef liver $tRNA^{Phe}$ (72% ribothymidine) gave higher values than those obtained with the naturally occurring human placenta $tRNA^{Phe}$ (50% ribothymidine). Similar results were obtained when naturally occurring rat liver $tRNA^{Phe}$ (78% ribothymidine) was compared to the human placenta $tRNA^{Phe}$ (data not shown). A similar correlation between the rate of phenylalanine polymerization and ribothymidine content was also obtained for the *in vitro* treated and subsequently RPC-5 purified sham- and completely methylated beef liver $tRNAs^{Phe}$.

The data presented in Fig. 2 *D* were analyzed by using the weighted least squares method for: $v = (V_{max} \cdot [S])/(K_m + [S])$ as described by Wilkinson (32), in which the velocity v was obtained for each tRNA concentration $[S]$. The apparent K_m, expressed in tRNA concentration ($\times 10^8$), and apparent V_{max}, expressed in pmol of phenylalanine polymerized per A_{280} unit of 0.5 M KCl washed rat liver ribosomes per min, were: 12.3 ± 3.1 and 8.26 ± 1.23 for the *in vitro* methylated beef liver $tRNA^{Phe}$ (100% ribothymidine); 9.2 ± 1.8 and 4.54 ± 0.39 for the *in vitro* sham-methylated beef liver $tRNA^{Phe}$ (72% ribothymidine); 10.0 ± 1.6 and 4.99 ± 0.37 for the untreated beef liver $tRNA^{Phe}$ (72% ribothymidine); and 14.0 ± 4.2 and 3.77 ± 0.61 for the untreated human placenta $tRNA^{Phe}$ (50% ribothymidine). These results indicate that, whereas the ribothymidine content of the various $tRNAs^{Phe}$ had no effect on the apparent K_m, the effect on the apparent V_{max} was significant. In addition, they strongly suggest that, only under the conditions of low magnesium in the presence of the 0.5 M KCl ribosome wash factors, the ribothymidine content of mammalian $tRNA^{Phe}$ is critical for the optimal polymerization reaction and that the methylation of uridine to ribothymidine at position 23 from the 3′ terminus results in an increased apparent V_{max}.

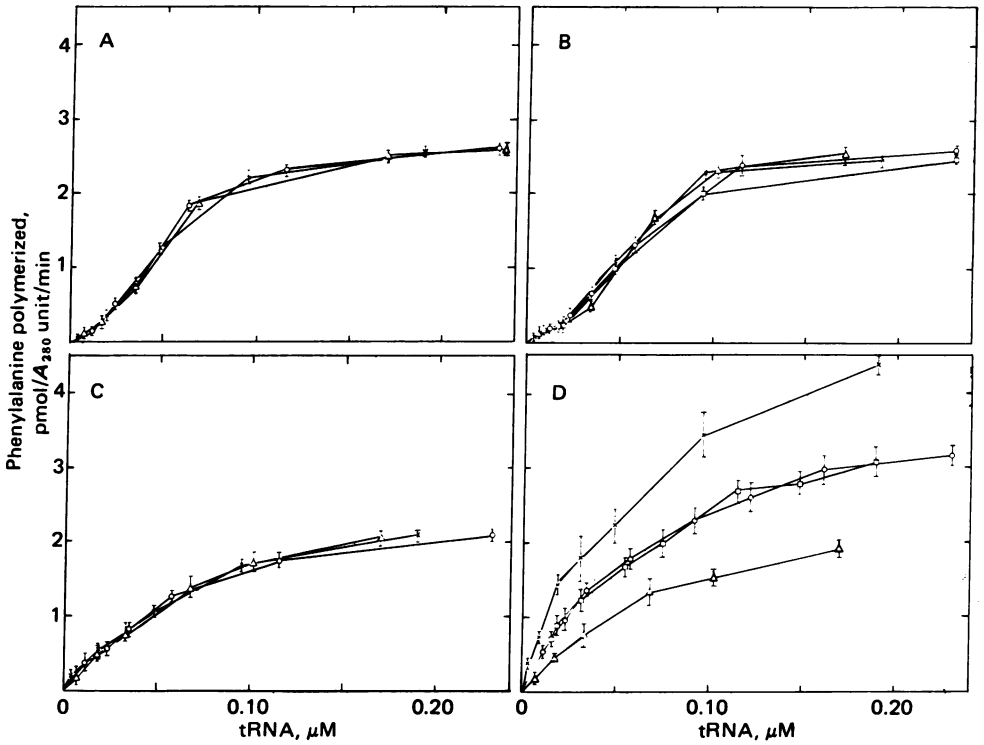

FIG. 2. tRNA concentration-dependent formation of poly(Phe). (*A*) Under conditions as described in *Materials and Methods* at 11 mM final Mg^{2+} concentration in the presence of 0.5 A_{280} unit of aminoacyl-tRNA synthetase but with the 0.5 M KCl ribosome wash replaced by 0.3 M KCl. ×, Methylated beef liver $tRNA^{Phe}$; ○, beef liver $tRNA^{Phe}$; △, human placenta $tRNA^{Phe}$. (*B*) Same as *A* except in the presence of 0.025 A_{280} unit of 0.5 M KCl ribosome wash factor. ×, Methylated beef liver $tRNA^{Phe}$; ○, beef liver $tRNA^{Phe}$; □, sham-methylated beef liver $tRNA^{Phe}$; △, human placenta $tRNA^{Phe}$. (*C*) Same as *A* except at 6 mM final Mg^{2+} concentration with the 0.5 M KCl ribosome wash factor replaced by 0.3 M KCl. ×, Methylated beef liver $tRNA^{Phe}$; ○, beef liver $tRNA^{Phe}$; △, human placenta $tRNA^{Phe}$. (*D*) Same as *A* except at 6 mM final Mg^{2+} concentration in the presence of 0.025 A_{280} unit of 0.5 M KCl ribosome wash factors. ×, Methylated beef liver $tRNA^{Phe}$; ○, beef liver $tRNA^{Phe}$; □, sham-methylated beef liver $tRNA^{Phe}$; △, human placenta $tRNA^{Phe}$.

DISCUSSION

In the experiments reported here, we attempted to ascertain the functional significance of the methylated uridine (ribothymidine) in class C mammalian tRNAs that partially lack this modification. These experiments were performed by comparing the ability of the $tRNA^{Phe}$ from rat liver (78% ribothymidine), beef liver (72% ribothymidine), human placenta (50% ribothymidine), and fully methylated mammalian $tRNA^{Phe}$ (100% ribothymidine based on modification of uridine to ribothymidine at position 23 from the 3′ terminus) to support poly(Phe) synthesis under various of conditions.

Our results indicate that, although the ribothymidine content of mammalian $tRNAs^{Phe}$ has no effect on their ability to function in the aminoacylation reaction, there is a significant effect of ribothymidine content on their ability to support poly(Phe) synthesis. Specifically, the effect of greater ribothymidine content for a class C mammalian $tRNA^{Phe}$ becomes manifest in an increased V_{max} rather than an altered apparent K_m for the poly(Phe) synthesis reaction. This effect was only observed under the more native conditions of low (6 mM) magnesium concentration in the presence of the 0.5 M KCl ribosome wash factors. Because under these conditions both the rate and extent of aminoacylation are identical for several mammalian $tRNAs^{Phe}$ differing only in their ribothymidine contents, and the ribosome wash factor fraction contains several initiation and elongation factors required for protein synthesis (27), it is tempting to speculate that the *in vivo* effect of ribothymidine in mammalian tRNA is to regulate protein synthesis, in concert with one or more of these factors.

The results we have obtained with the mammalian $tRNAs^{Phe}$ complement the data reported by Marcu and Dudock (21) for the wheat germ $tRNA^{Gly}$. Because the wheat germ $tRNA^{Gly}$ totally lacks ribothymidine and instead contains a uridine at position 23 from the 3′ terminus (13), it would be a class D tRNA (17). These authors demonstrated that conversion of this uridine to ribothymidine in the wheat germ $tRNA^{Gly}$ resulted in a decreased ability of the tRNA to function in a wheat germ *in vitro* protein synthesis system, compared to sham-methylated $tRNA^{Gly}$. In addition, they observed that the reduced rate of protein synthesis in the presence of methylated wheat germ tRNA could be overcome by the presence of spermine in the reaction mixture. These results are consistent with the hypothesis that eukaryotic tRNAs which totally lack ribothymidine (class D) do not require this methylated uridine for optimal function (21).

Our observation that the methylation of uridine to ribothymidine in mammalian $tRNA^{Phe}$ causes an increase in the apparent V_{max} for the *in vitro* poly(U)-directed synthesis of poly(Phe) is similar to our earlier results (5) on the role of 2-methylguanosine in the aminoacylation of tRNA by yeast phenylalanyl-tRNA synthetase. In that study we demonstrated that the maximum velocity for the aminoacylation of *E. coli* $tRNA^{Phe}$ could be increased by an order of magnitude and coincided with that obtained for the aminoacylation of yeast $tRNA^{Phe}$ when the guanosine at position 10 from the 5′ terminus was fully converted to 2-methylguanosine by a purified preparation of rabbit liver 2-methylguanosine methylase. The results of these two studies as well as the report (21) that spermine eliminates the effect of converting uridine to ribothymidine in wheat germ tRNA suggest that one general function of the

methylation of tRNA might be to alter the tRNA conformation in such a manner as to affect the rate (as manifested in apparent V_{max}) of one or more reactions involving tRNA.

Our observation that the role of ribothymidine in a mammalian tRNA affects the kinetics of protein synthesis by altering V_{max} suggests that it would be quite difficult to observe these effects in an *in vivo* system. These results are consistent with those obtained with *E. coli* mutants that produce ribothymidine-deficient tRNAs, indicating that the *in vivo* approach could not pinpoint the function of ribothymidine in prokaryotic cells (33, 34). However, Bjork and Neidhardt's report (33) that their mutant could not effectively compete for survival when mixed with wild-type *E. coli* indicates a subtle role for ribothymidine in prokaryotic tRNAs. In addition, because we have presented evidence that the discrimination between uridine- or ribothymidine-containing eukaryotic tRNAs is associated with a rat liver ribosomal wash fraction, and *E. coli* has been shown to contain different ribosomal wash factors (35), comparison of our results with those obtained in a prokaryotic system might not be valid.

The present results are consistent with the hypothesis that mammalian class C tRNAs, such as the various mammalian $tRNAs^{Phe}$ which differ only in their *in vivo* content of uridine or ribothymidine, function more efficiently in protein synthesis if they contain this methylated uridine in loop IV. If this observation is confirmed for other incompletely methylated mammalian tRNAs (with regard to their ribothymidine content), these results would imply that this class of tRNA might serve as regulatory macromolecules for mammalian *in vivo* protein synthesis.

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1. Rich, A. & RajBhandary, U. L. (1976) *Annu. Rev. Biochem.* **45**, 806–860.
2. Sharma, O. K. & Borek, E. (1976) *Cancer Res.* **36**, 4320–4329.
3. Smith, D. W. E. (1975) *Science* **190**, 529–535.
4. Singer, C. E., Smith, G. R., Cortese, R. & Ames, B. N. (1972) *Nature New Biol.* **238**, 72–74.
5. Roe, B., Michael, M. & Dudock, B. (1973) *Nature New Biol.* **246**, 135–138.
6. Nishimura, S. (1974) in *MTP International Review of Science, Biochemistry of Nucleic Acids*, ed. Burton, K. (Butterworths Univ. Park Press, London), Vol. 6, pp. 289–322.
7. Littauer, U. Z. & Inouye, H. (1973) *Annu. Rev. Biochem.* **42**, 439–470.
8. Ofengand, J. & Henes, C. J. (1969) *J. Biol. Chem.* **244**, 6241–6253.
9. Shimizu, N., Hayashi, H. & Miura, K. (1970) *J. Biochem. (Tokyo)* **67**, 373–387.
10. Sprinzl, M., Wagner, T., Lorenz, S. & Erdmann, V. A. (1976) *Biochemistry* **15**, 3031–3039.
11. Randerath, E., Chia, L. S. Y., Morris, H. P. & Randerath, K. (1974) *Cancer Res.* **34**, 643–653.
12. Faras, A. J., Dahlberg, J. E., Sawyer, R. C., Harada, F., Taylor, J. M., Levinson, W. E., Bishop, J. M. & Goodman, H. M. (1974) *J. Virol.* **13**, 1134–1142.
13. Marcu, K., Mignery, R., Reszelbach, R., Roe, B., Sirover, M. & Dudock, B. (1973) *Biochem. Biophys. Res. Commun.* **55**, 477–483.
14. Piper, R. W. & Clark, B. F. C. (1974) *FEBS Lett.* **47**, 56–59.
15. Gillum, A., Roe, B., Anandaraj, M. P. J. S. & RajBhandary, U. L. (1975) *Cell* **6**, 407–413.
16. Keith, G., Ebel, J-P. & Dirheimer, G. (1974) *FEBS Lett.* **48**, 50–52.
17. Roe, B. A., Chen, E. Y. & Tsen, H. Y. (1976) *Biochem. Biophys. Res. Commun.* **68**, 1339–1347.
18. Reszelbach, R., Greenberg, R., Pirtle, R., Prasad, R., Marcu, K. & Dudock, B. (1977) *Biochim. Biophys. Acta* **475**, 383–392.
19. Grummt, F., Grummt, I., Gross, H. J., Sprinzl, M., Richter, D. & Erdmann, V. A. (1974) *FEBS Lett.* **42**, 15–17.
20. Erdmann, V. A. (1976) in *Progress in Nucleic Acids Research and Molecular Biology*, ed. Cohn, W. E. (Academic Press, New York), Vol. 18, pp. 45–90.
21. Marcu, K. B. & Dudock, B. S. (1976) *Nature* **261**, 159–162.
22. Roe, B. (1975) *Nucleic Acids Res.* **2**, 20–42.
23. Anandaraj, M. P. J. S. & Roe, B. A. (1975) *Biochemistry* **14**, 5068–5073.
24. Roe, B. A., Marcu, K. & Dudock, B. (1973) *Biochim. Biophys. Acta* **319**, 25–36.
25. Roe, B., Sirover, M. & Dudock, B. (1973) *Biochemistry* **12**, 4146–4154.
26. Reyes, R., Vazquez, D. & Ballesta, J. P. G. (1976) *Biochim. Biophys. Acta* **435**, 317–332.
27. Shafritz, D. A. & Anderson, W. F. (1970) *J. Biol. Chem.* **245**, 5553–5559.
28. Randerath, K. & Randerath, E. (1973) in *Methods in Cancer Research*, ed. Busch, H. (Academic Press, New York), Vol. 9, pp. 3–68.
29. Randerath, K., Randerath, E., Chia, L. S. Y/ & Nowak, B. J. (1974) *Anal. Biochem.* **59**, 263–271.
30. Roe, B. A., Anandaraj, M. P. J. S., Chia, L. S. Y., Randerath, E., Gupta, R. C. & Randerath, K. (1975) *Biochem. Biophys. Res. Commun.* **66**, 1097–1105.
31. Shafritz, D. A. & Isselbacher, K. J. (1972) *Biochem. Biophys. Res. Commun.* **46**, 1721–1727.
32. Wilkinson, G. N. (1961) *Biochem. J.* **80**, 324–332.
33. Bjork, G. R. & Neidhardt, F. C. (1975) *J. Bacteriol.* **124**, 99–111.
34. Yang, S., Reinitz, E. R. & Gefter, M. L. (1973) *Arch. Biochem. Biophys.* **157**, 55–62.
35. Weissbach, H. & Ochoa, S. (1976) *Annu. Rev. Biochem.* **45**, 191–216.