Evolutionary Conservation of the Synthetase Recognition Site of Alanine Transfer Ribonucleic Acid*

W. French Anderson

ABSTRACT: The species specificity for transfer ribonucleic acid and aminoacyl transfer ribonucleic acid synthetase between *Escherichia coli* and human spleen was tested for eight amino acids. Three classes of reaction were found: (1) complete cross-reactivity: alanine, arginine, lysine: (2) human spleen transfer ribonucleic acid recognized by the *E. coli* enzyme, but the *E. coli* transfer ribonucleic acid only weakly recognized by human enzyme: valine; and (3) partial or no cross-reactivity: leucine, phenylalanine, proline, serine. Alanine acylation was examined further. Human spleen, rabbit liver, and *E. coli* B transfer ribonucleic acid were fractionated by Freon reversed-phase chromatography, and the alanine transfer ribonucleic acid species from each organism were identified. Heterologous acylation assays indicated that each alanine transfer ribonucleic acid species could be acylated by enzyme isolated from any of the three organisms.

As an evolutionary divergent source of material, lobster muscle transfer ribonucleic acid was purified. This transfer ribonucleic acid was also acylated to an equal extent by enzymes from human, rabbit, and *E. coli*. It appears, therefore, that there is a complete cross-reactivity of alanine transfer ribonucleic acid species with alanyl transfer ribonucleic acid synthetases. This conclusion implies that the synthetase recognition site for alanine transfer ribonucleic acid has been conserved throughout the evolutionary development of bacteria to man.

The species specificity of aminoacyl-tRNA synthetases in the acylation of tRNA with amino acids has been examined by several workers (see review by Novelli, 1967). For example, Doctor and Mudd (1963) examined the extent of acylation for 14 amino acids when the unfractionated tRNA and the enzyme were isolated from the same or from different organisms. In most cases, they found a variable degree of cross-reactivity for certain combinations of tRNA and synthetases. However, as Barnett and Jacobson (1964) have shown, there are cases where an apparent cross-reactivity between organisms is due, in fact, to the spurious acylation of an amino acid onto tRNA species which normally do not accept the amino acid. Consequently, it is not possible to draw an accurate conclusion concerning cross-reactivity without examining the identity of the individual species of tRNA which are being acylated (Bennett *et al.*, 1965).

In conjunction with our studies on the effects of tRNA species on the regulation of protein synthesis in *Escherichia coli* (Anderson, 1968, 1969) and in mammalian cells (Anderson and Gilbert, 1969), we have examined the degree of species specificity of tRNA and aminoacyl-tRNA synthetases for several amino acids. The tRNA species from *E. coli*, rabbit, and human tissues which accept alanine were examined individually. A complete cross-reactivity between $tRNA^{Ala}$ species and alanyl-tRNA synthetases appears to exist.

Materials

L-[U-^{14}C]Amino acids, each at a specific activity of 100 mCi/mmole, were purchased from Tracerlabs. The materials for reversed-phase chromatography were obtained as follows: tricaprylylmethylammonium chloride (Aliquat 336) from General Mills, Inc.; tetrachlorotetrafluoropropane (Freon 214) from E. I. duPont de Nemours and Co.; Chromosorb W (acid washed, dimethyldichlorosilane treated, 100–120 mesh size) from Johns-Manville Products Corp.

Methods

Fractionation of tRNA. Human spleen tRNA was obtained from a 1750-g spleen removed from a patient suffering idiopathic hypersplenism. After removing a portion for pathological diagnosis, the tissue was placed directly into liquid nitrogen within 10–12 min after the blood supply to the organ had been clamped. The pathological diagnosis was reported as splenomegaly consistent with leukemic infiltrate. Unfractionated tRNA was prepared using modifications of the procedure of Delihas and Staehelin (1966); 50 g of frozen spleen was homogenized with 150 ml of buffer A (0.01 M Tris-Cl (pH 8.0)–0.001 M $MgCl_2$–0.3 M sucrose) and 150 ml of water-saturated phenol for 1.5 min in a Waring Blendor at room temperature. The aqueous phase was extracted twice more with phenol at room temperature; each phenol phase was reextracted with three-tenths volume of buffer A; all aqueous phases were pooled. The tRNA was precipitated by the addition of one-tenth volume of 20% potassium acetate and two volumes of 95% ethanol followed by standing at −20° for 2 hr or overnight. The precipitate was pelleted by centrifugation at 15,000*g* for 10 min, dissolved in 0.001 M $MgCl_2$, dialyzed 4 hr against three changes of 0.001 M $MgCl_2$, then made to 1.0 M NaCl, and allowed to stand at 4° for 16 hr with slow stirring. The supernatant was collected after pelleting the high molecular weight precipitated material by centrifugation at 15,000*g* for 10 min. The pellet was washed three times with 1.0 M NaCl; all supernatant fractions were pooled and the tRNA was precipitated by addition of two volumes of 95% ethanol, pelleted by cen-

* From the Section on Human Biochemistry, National Heart Institute, National Institutes of Health, Bethesda, Maryland 20014. *Received May 12, 1969.*

trifugation, dissolved in buffer B (0.01 M Tris-Cl (pH 7.5)–0.01 M $MgCl_2$–0.01 M KCl), and stored frozen. Approximately 8 A_{260} units of material were obtained from each gram wet weight of spleen.

Pooled tRNA fractions (5900 A_{260} units) in 27 ml of buffer B were applied to a 5.0 × 89 cm Sephadex G-200 column, equilibrated with buffer B, and eluted at room temperature with the same buffer at a flow rate of 3.0 ml/min (9 ml/cm² per hr). High molecular weight material eluting at the void volume represented 1600 A_{260} units, the tRNA peak 4000 A_{260} units, and the low molecular weight material 300 A_{260} units. The final yield of tRNA represented a recovery of approximately 5 A_{260} units/g of spleen. The tRNA peak was utilized for studies on unfractionated tRNA and a portion of this material was fractionated by Freon reversed-phase chromatography according to the procedure of Weiss and Kelmers (1967). After equilibrating a 2.5 × 31 cm Freon column with buffer C (0.01 M Tris-Cl–0.01 M $MgCl_2$–0.20 M NaCl–0.5 mM NaEDTA, pH 7.0, 37°), 3100 A_{260} units of unfractionated human spleen tRNA in 50 ml of buffer C were pumped onto the column at the rate of 1.9 ml/min (0.4 ml/cm² per min), followed by a wash of 200 ml of buffer C. A 4-l. concave gradient with buffer C and buffer D (0.01 M Tris-Cl–0.01 M $MgCl_2$–0.40 M NaCl–0.5 mM NaEDTA, pH 7.0, 37°), using a nine-chamber gradient maker as described by Weiss and Kelmers (1967), was run at the rate of 1.9 ml/min at 37°. Fractions of 60 ml were collected, and the column was then washed with 60 ml each of 0.5, 1.0, and 2.0 M NaCl. The tRNA from each fraction was precipitated with two volumes of 95% ethanol, pelleted by centrifugation, dialyzed against 0.001 M $MgCl_2$, lyophilized, and stored in liquid nitrogen.

Rabbit liver tRNA was purchased from General Biochemicals, (Lot No. 686061), and further purified by Sephadex G-200 chromatography. Using the Freon column and the procedures described above, 1650 A_{260} units of tRNA were fractionated using a 3-l. concave gradient of buffer C and buffer E (0.01 M Tris-Cl–0.01 M $MgCl_2$–0.35 M NaCl–0.5 mM NaEDTA, pH 7.0, 37°). Fractions of 60 ml were collected, and the column was then washed with 60 ml each of 0.4, 0.5, 1.0, and 2.0 M NaCl (the 2.0 M NaCl wash eluted no additional A_{260}-absorbing material). The tRNA in each fraction was isolated and stored as described above.

E. coli B tRNA was purchased from General Biochemicals. After Sephadex G-200 chromatography, 4000 A_{260} units of tRNA were fractionated on the Freon column with a 3-l. concave gradient of buffer C and buffer E. In this case, 10-ml fractions were collected and every third tube was assayed for ability to accept [^{14}C]alanine. Two well-separated peaks were obtained. Tubes corresponding to these peaks were pooled and the tRNA was isolated and stored as described above.

Lobster tRNA was prepared from 50 g of tail muscle from a 3-lb Maine lobster. The same procedure as that described for human spleen tRNA was utilized except that 0.002 M sodium thiosulfate was present at all times and a DEAE-cellulose step was added prior to the 1.0 M NaCl precipitation in order to remove opaque, non-A_{260}-absorbing material from the preparation. The nucleic acid pellet from 50 g of tissue which was obtained after the (post-phenol)ethanol precipitation was dissolved in 200 ml of buffer B which had been made 0.1 M NaCl and 0.002 M $Na_2S_2O_3$. This opaque solution was passed at the rate of 3 ml/min at 4° through a 2.5 × 21 cm DEAE-cellulose column which had been equilibrated with the same buffer. Nucleic acid was bound to the DEAE-cellulose while the opaque material emerged in the effluent. The column was washed with the same buffer until the effluent was clear. Nucleic acid was then eluted with buffer B made to 1.0 M NaCl and 0.002 M $Na_2S_2O_3$. The 1.0 M NaCl precipitation and Sephadex G-200 chromatography steps were then performed, and the tRNA preparation was dialyzed, lyophilized, and stored as described above.

Preparation of Aminoacyl-tRNA Synthetases. Human spleen synthetase (from the same organ used for the tRNA preparation) was obtained by homogenizing 15 g of frozen tissue in 45 ml of buffer composed of 0.40 M potassium phosphate (pH 6.5), 0.001 M $MgCl_2$, and 10% glycerol in an Omni-Mixer at 4° for 40 sec at top speed. All procedures were carried out at 4°. After centrifugation at 30,000*g* for 15 min and at 205,000*g* for 30 min, the upper three-fourths of the supernatant fractions (42-ml total) was passed at the rate of 3 ml/min through a 2.5 × 28 cm DEAE-cellulose column equilibrated with 0.30 M potassium phosphate (pH 6.5), 0.001 M $MgCl_2$, and 10% glycerol. The leading 27 ml of the eluate peak were pooled and dialyzed for 4 hr against three changes of 0.01 M potassium phosphate (pH 6.6), 0.001 M $MgCl_2$, and 10% glycerol. The A_{280}/A_{260} ratio of the final solution was 1.30; it was stored at a concentration of 9.4 mg/ml at −20° in the presence of 50% glycerol. Incubation of the preparation with [^{14}C]alanine in the absence of added tRNA produced no trichloroacetic acid precipitable counts above background.

Rabbit liver aminoacyl-tRNA synthetase was prepared in a manner identical with that described for human spleen. Rabbit reticulocyte enzyme also was prepared similarly after the lysate fraction was obtained by adding four volumes of 0.001 M $MgCl_2$ at 0° to washed red blood cells prepared from phenylhydrazine-treated rabbits. The preparation of *E. coli* synthetase was described previously (Anderson, 1969).

Assay of Amino Acid Acceptance of tRNA. Acylation assays were carried out in 25-μl reactions containing (unless otherwise stated) 0.1 M potassium phosphate (pH 6.6), 0.005 M $MgCl_2$, 0.002 M ATP, 1×10^{-4} M L-[^{14}C]amino acid (100 mCi/mmole), 1×10^{-4} M each of the other 19 cold L-amino acids, 0.05–0.20 A_{260} unit of tRNA, and synthetase protein. In all cases, [^{14}C]amino acid acceptance was limited by tRNA concentration. Reactions were incubated for 15 min at 37° and were terminated by addition of 1 ml of 10% trichloroacetic acid at 4°. Precipitates were washed on nitrocellulose filters (Millipore, type HA, 0.45 μ pore size, 25 mm diameter) with 5% trichloroacetic acid at 4°. The filters were dried, immersed in 10 ml of scintillation fluid (Liquifluor in toluene), and assayed for radioactivity in a liquid scintillation counter (Packard, Model 3380) at an efficiency of 82% for ^{14}C.

Results

Acylation of Unfractionated tRNA. Unfractionated tRNA and crude aminoacyl-tRNA synthetase preparations from *E. coli* and human spleen were tested for extent of acylation for eight amino acids when the source of the enzyme and of the tRNA in the reaction mixture were the same or different. The data are shown in Table I. The amino acids fall into three classes: (1) complete cross-reactivity: alanine, arginine, lysine; (2) human spleen tRNA recognized by *E. coli* synthetase but the *E. coli* tRNA only weakly acylated by the human enzyme: valine; and (3) partial or no cross-reactivity: leucine, phenylala-

TABLE I: Species Specificity for Acylation of Unfractionated tRNA with [^{14}C]Amino Acids.[a]

Amino Acid	Source of Enzyme	Source of tRNA: Human Spleen[c]	Source of tRNA: *E. coli*[c]
Alanine	Human spleen	17	75
	E. coli	17	82
Arginine	Human spleen	22	71
	E. coli	21	80
Lysine	Human spleen	6	32
	E. coli	7.5	32
Valine	Human spleen	19	9
	E. coli	17	75
Leucine	Human spleen	32	13
	E. coli	0	122
Phenylalanine	Human spleen	7.5	4
	E. coli	4	41
Phenylalanine[b]	Human spleen	43	
	E. coli	0	
Proline	Human spleen	7.5	0
	E. coli	0	34
Serine	Human spleen	15	9
	E. coli	0	26

[a] Three tRNA concentrations (usually 0.05, 0.10, and 0.20 A_{260} units per 25 μl of reaction mixture) were tested and averaged for each value listed. [b] Because of the low level of acylation for unfractionated tRNA^{Phe} from human spleen, tRNA from fraction 39 of the Freon reversed-phase chromatographic separation shown in Figure 2 was used as a source of fractionated tRNA^{Phe}. [c] In $\mu\mu$moles/A_{260} unit.

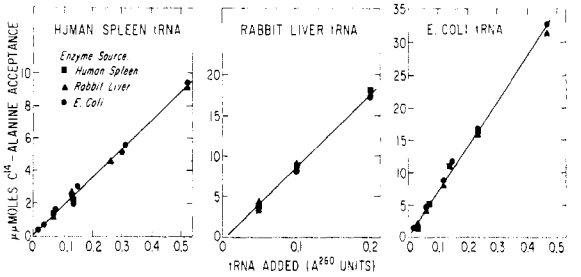

FIGURE 1: [^{14}C]Alanine acceptance by unfractionated tRNA from human spleen, rabbit liver, and *E. coli* B using aminoacyl-tRNA synthetases isolated from each of the three organisms. Enzyme preparations and acylation conditions are described in Methods. The data for human spleen and *E. coli* tRNA represent a composite of values from several different experiments. The micromicromoles of [^{14}C]alanine accepted per A_{260} unit of tRNA as calculated from the slope of each plot are: human spleen tRNA, 17; rabbit liver tRNA, 88; *E. coli* B tRNA, 70.

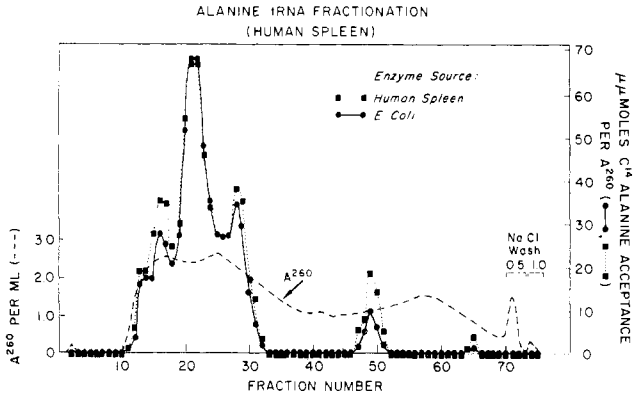

FIGURE 2: Fractionation of human spleen tRNA by Freon reversed-phase chromatography. The lyophilized tRNA from each fraction (see Methods) was brought to a concentration of 50 A_{260}/ml by addition of distilled water. Each point represents the [^{14}C]alanine acceptance per A_{260} unit of tRNA. The tRNA from six fractions was examined further and the data are shown in Table II: I, fraction 13; II, fraction 16; III, fraction 21; IV, fraction 29; V, fraction 49; VI, fraction 65.

nine, proline, serine.

We wished to determine if a case of true cross-reactivity between *E. coli* and human tissue does exist and, therefore, we examined one of the amino acids, alanine, in detail. The study of arginine was also initiated but was complicated by the presence of an arginine transferase in human spleen similar to that described by Soffer in sheep thyroid (Soffer, 1968).

Acylation of Unfractionated tRNA^{Ala}. The acceptance of [^{14}C]alanine by unfractionated tRNA isolated from *E. coli*, rabbit liver, or human spleen, when catalyzed by aminoacyl-tRNA synthetase prepared from each of the three organisms, is shown as a function of tRNA concentration in Figure 1. As can be seen, the extent of acylation of each unfractionated tRNA is only dependent upon the amount of tRNA^{Ala} in the preparation, not on the enzyme utilized to catalyze the reaction.

Acylation of Fractionated Human Spleen tRNA^{Ala}. A Freon reversed-phase chromatographic fractionation of human spleen tRNA is shown in Figure 2. Using either human spleen or *E. coli* alanyl-tRNA synthetase, six peaks for tRNA^{Ala} can be seen. *E. coli* (and, in separate experiments, rabbit liver, and rabbit reticulocyte) enzyme does not appear to catalyze the [^{14}C]alanine acylation of any tRNA other than the species acylated by the homologous enzyme.

In order to more closely examine the recognition of these peaks by the three enzyme preparations, tRNA^{Ala} fractions as described in the legend to Figure 2 were studied individually. The results are shown in Table II. The data indicate that fractions I–V are recognized by all three enzymes, with the efficiency of the reaction for fractions I, II, IV, and V, under the conditions tested, decreasing in order of synthetase source as follows: human spleen > rabbit liver > *E. coli*. Fraction III, however, was acylated to a greater extent by *E. coli* synthetase. Acylation of fraction VI was variable (see Discussion).

Acylation of Fractionated Rabbit Liver tRNA^{Ala}. A Freon reversed-phase chromatographic fractionation of rabbit liver tRNA is shown in Figure 3. Four tRNA^{Ala} peaks are obtained. Since fewer fractions were taken, the resolution in this experiment was not as great as that seen in Figures 2 and 4. As in the case of human spleen tRNA, the pattern of [^{14}C]alanine acceptance is similar with each synthetase preparation. The efficiency of the enzyme preparation decreases, under the condi-

TABLE II: [^{14}C]Alanine Acylation of Human Spleen tRNA^{Ala} Fractions by Human Spleen, Rabbit Liver, and *E. coli* Enzymes.[a]

Fraction of tRNA^{Ala} from Human Spleen	Source of Enzyme		
	Human Spleen[b]	Rabbit Liver[b]	*E. coli*[b]
I	17	13	8
II	28	26	21
III	64	62	71
IV	32	28	28
V	22	15	6

[a] The tRNA^{Ala} fractions are described in the legend to Figure 2. Each value listed is the average of data obtained from three or more tRNA concentrations. Acylation of fraction VI was low and not reproducible. [b] In $\mu\mu$moles/A_{260} unit.

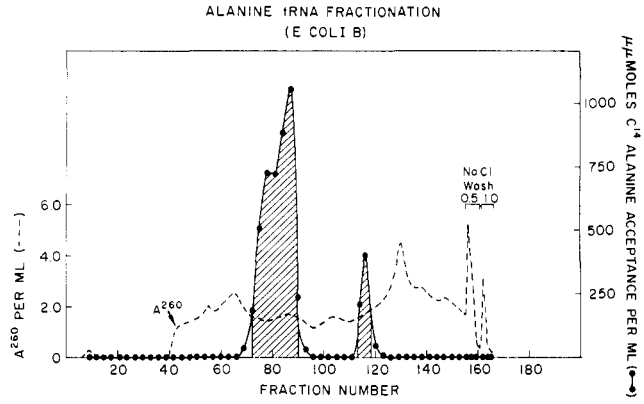

FIGURE 4: Fractionation of *E. coli* B tRNA by Freon reversed-phase chromatography. [^{14}C]Alanine acceptance by 50 μl of each column fraction was measured (see Methods) and plotted as micromicromoles per milliliter Those fractions represented by the shaded areas were pooled. Fraction I is the tRNA from tubes 72 to 90; fraction II is the tRNA from tubes 113 to 119. Fraction I possibly contains two tRNA^{Ala} species.

tions tested, in the order: rabbit liver > human spleen > *E. coli*.

Acylation of Fractionated E. coli B tRNA^{Ala}. When *E. coli* B was fractionated by Freon reversed-phase chromatography, two peaks of alanine acceptance activity were obtained as shown in Figure 4. The first peak may contain two tRNA^{Ala} species. Acceptance of [^{14}C]alanine by each peak was assayed using synthetase preparations from human spleen, rabbit liver, and *E. coli* under two sets of acylation conditions: phosphate buffer at pH 6.6 and cacodylate buffer at pH 6.9. The data are shown in Table III. Using phosphate buffer, each tRNA^{Ala} peak is acylated to a similar extent by all three enzymes. However, with cacodylate buffer containing 7 mM β-mercaptoethanol and 10 mM $MgCl_2$, the heterologous acylations proceeded to only a limited degree.

Acylation of Unfractionated Lobster Muscle tRNA^{Ala}. In order to examine tRNA from an organism whose evolution represents a divergent pathway from that followed by mammalian cells, tRNA from lobster muscle was tested in heterologous acylation assays with aminoacyl-tRNA synthetase preparations from human spleen, rabbit liver, rabbit reticulocytes, and *E. coli*. The data are shown in Table IV. Each enzyme was able to acylate the lobster tRNA to an equal extent. We have been unable to obtain as yet an active preparation of lobster muscle alanyl-tRNA synthetase.

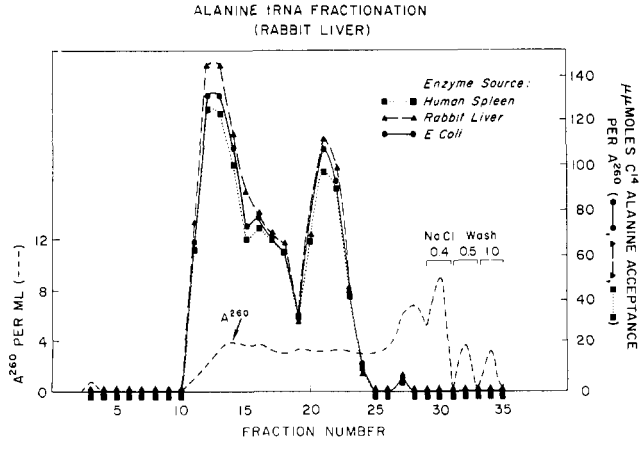

FIGURE 3: Fractionation of rabbit liver tRNA by Freon reversed-phase chromatography. The tRNA from each fraction was assayed as described in Methods and the legend to Figure 2.

TABLE III: [^{14}C]Alanine Acylation of *E. coli* tRNA^{Ala} Fractions by Human Spleen, Rabbit Liver, and *E. coli* Enzymes under Two Acylating Conditions.[a]

Fraction of tRNA^{Ala} from *E. coli*	Buffer	Source of Enzyme		
		Human Spleen[b]	Rabbit Liver[b]	*E. coli*[b]
I	Phosphate	432	465	472
	Cacodylate	99	256	470
II	Phosphate	125	133	144
	Cacodylate	26	71	148

[a] Conditions for incubation in phosphate buffer are described in Methods. Acylation assay mixtures in cacodylate buffer contained the following: 0.1 M potassium cacodylate (pH 6.9), 0.010 M $MgCl_2$, 0.002 M ATP, 0.010 M KCl_2, 0.007 M β-mercaptoethanol, 1×10^{-4} M L-[^{14}C]alanine (100 mCi/mmole), 1×10^{-4} M each of the other 19 cold L-amino acids, 0.05, 0.10, or 0.20 A_{260} units of tRNA, and a saturating level of synthetase protein. The tRNA^{Ala} fractions are described in the legend to Figure 4. Each value listed is the average of the data obtained from the three tRNA concentrations. [b] In $\mu\mu$moles/A_{260} units.

TABLE IV: [^{14}C]Alanine Acylation of Lobster Muscle tRNA by Enzymes Prepared from Different Sources.

Source of Enzyme	[^{14}C]Alanine Acceptance of Lobster Muscle tRNA ($\mu\mu$moles/A_{260} unit)[a]
Human spleen	33
Rabbit liver	33
Rabbit reticulocytes	35
E. coli	36

[a] Each value listed is the average of data obtained from three tRNA concentrations.

Discussion

Human spleen tRNA can be fractionated into six $tRNA^{Ala}$ peaks, rabbit liver tRNA into four, and *E. coli* B tRNA into two (possibly three), by Freon reversed-phase chromatography. Since acylation of the sixth peak of human spleen tRNA was weak and variable, it may not represent a distinct $tRNA^{Ala}$ species. Minor tRNA fractions can exist, however, since the $tRNA^{Arg}$ species in *E. coli* which recognize the code words AGA and AGG have been shown to represent only 2% of the total $tRNA^{Arg}$ in the organism (Anderson, 1969). Although the $tRNA^{Ala}$ species themselves vary between organisms, every species can be acylated by enzyme isolated from any of the three organisms tested. It is apparent from Figures 2 and 3, and Tables II and III that the same extent of acylation for a $tRNA^{Ala}$ species is not always reached with enzyme preparations from different organisms. Furthermore, the conditions of acylation can markedly influence the efficiency of acylation as is seen in Table III by the inhibition of acylation of *E. coli* tRNA by mammalian enzyme using cacodylate buffer. Consequently, an accurate assessment of total acylation capacity would require an individual study to determine optimal reaction mixture conditions for every cross. In addition, the present study examines only the final level of acylation, not the rate of the reaction. Nonetheless, although variations in efficiencies occur, it is clear that each enzyme preparation is capable of recognizing all $tRNA^{Ala}$ species tested.

These data indicate, therefore, that the synthetase recognition site(s) for $tRNA^{Ala}$ species began to be conserved in evolution at a very early stage. Although the number of $tRNA^{Ala}$ species appears to have increased during the evolution of bacteria to man, the synthetase recognition site apparently has not been altered substantially. This conclusion is supported further by the finding that synthetase from *E. coli* and three mammalian sources were able to acylate lobster muscle $tRNA^{Ala}$ to an equal extent.

Two studies from other laboratories are related to the finding that the synthetase recognition site in $tRNA^{Ala}$ species is similar in most organisms. First, the work of Nirenberg and others has shown the universality of the genetic code at least for the amino acids tested in *E. coli*, yeast, *Xenopus laevis* liver, guinea pig liver, rat liver, and human placenta (Nirenberg *et al.*, 1966; Marshall *et al.*, 1967; Caskey *et al.*, 1968; Söll *et al.*, 1967; Nishimura and Weinstein, 1969; Matthaei and Schoech, 1967). Second, Krisko and Gordon (1969) have shown that bacterial T factor is interchangeable with the mammalian binding factor in the binding of aminoacyl-tRNA to mammalian ribosomes. All these studies support the conclusion that at least some parts of the genetic machinery of the cell were established in the early stages of biological evolution and that they have remained essentially unchanged despite phylogenetic diversification.

Acknowledgments

I thank Mrs. Anne Baur and Mr. Moses B. Middleton for excellent technical assistance, and Dr. Herbert Weissbach for helpful advice and discussions.

References

Anderson, W. F. (1968), *Fed. Proc. 27*, 341.
Anderson, W. F. (1969), *Proc. Natl. Acad. Sci. U. S. 62*, 566.
Anderson, W. F., and Gilbert, J. M. (1969), *Biochem. Biophys. Res. Commun. 36*, 456.
Barnett, W. E., and Jacobson, K. B. (1964), *Proc. Natl. Acad. Sci. U. S. 51*, 642.
Bennett, T. P., Goldstein, J., and Lipmann, F. (1965), *Proc. Natl. Acad. Sci. U. S. 53*, 385.
Caskey, C. T., Beaudet, A., and Nirenberg, M. (1968), *J. Mol. Biol. 37*, 99.
Delihas, N., and Staehelin, M. (1966), *Biochim. Biophys. Acta 119*, 385.
Doctor, B. P., and Mudd, J. A. (1963), *J. Biol. Chem. 238*, 3677.
Krisko, I., and Gordon, J. (1969), *Fed. Proc. 28*, 726.
Marshall, R. E., Caskey, C. T., and Nirenberg, M. (1967), *Science 155*, 820.
Matthaei, J. H., and Schoech, G. K. (1967), *Biochem. Biophys. Res. Commun. 27*, 638.
Nirenberg, M., Caskey, T., Marshall, R., Brimacombe, R., Kellogg, D., Doctor, B., Hatfield, D., Levin, J., Rottman, F., Pestka, S., Wilcox, M., and Anderson, F. (1966), *Cold Spring Harbor Symp. Quant. Biol. 31*, 11.
Nishimura, S., and Weinstein, I. B. (1969), *Biochemistry 8*, 832.
Novelli, G. D. (1967), *Ann. Rev. Biochem. 36*, 449.
Soffer, R. L. (1968), *Biochim. Biophys. Acta 155*, 228.
Söll, D., Cherayil, J. D., and Bock, R. M. (1967), *J. Mol. Biol. 29*, 97.
Weiss, J. F., and Kelmers, A. D. (1967), *Biochemistry 6*, 2507