Proc. Nat. Acad. Sci. USA
Vol. 73, No. 2, pp. 405–409, February 1976
Biochemistry

Position of aminoacylation of individual *Escherichia coli* and yeast tRNAs

[isomeric tRNAs/CTP(ATP):tRNA nucleotidyltransferase/aminoacyl-tRNA synthetases/protein biosynthesis/enzyme specificity]

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Communicated by George H. Büchi, November 24, 1975

ABSTRACT **Transfer RNAs terminating in 2′- or 3′-deoxyadenosine were prepared from unfractionated *E. coli* and yeast (*Saccharomyces cerevisiae*) tRNAs and purified to remove unmodified tRNAs. The modified tRNA species were assayed for aminoacylation with each of the 20 amino acids to determine the initial position of tRNA aminoacylation. The *E. coli* and yeast aminoacyl-tRNA synthetases specific for arginine, isoleucine, leucine, methionine, phenylalanine, and valine, as well as the *E. coli* glutamyl-tRNA synthetase, aminoacylated only those cognate tRNAs terminating in 3′-deoxyadenosine (i.e., those having a 2′-OH group). On the other hand, those *E. coli* and yeast synthetases specific for alanine, glycine, histidine, lysine, proline, serine, and threonine, as well as the yeast synthetase specific for glutamine, utilized exclusively those tRNAs having an available 3′-OH group on the 3′-terminal nucleoside, while the *E. coli* and yeast synthetases specific for asparagine, cysteine, and tyrosine, and the yeast aspartyl-tRNA synthetase, utilized both of the modified cognate tRNAs. The only observed difference in specificity between the *E. coli* and yeast systems was for $tRNA^{Trp}$, which was aminoacylated on the 2′-position in *E. coli* and the 3′-position in yeast. The results indicate that the initial position of aminoacylation is not uniform for all tRNAs, although for individual tRNAs the specificity has been conserved during the evolution from a prokaryotic to eukaryotic organism.**

Although aminoacyl-tRNA undoubtedly exists in solution as a rapidly equilibrating mixture of the 2′- and 3′-*O*-aminoacyl species, a description of the initial position of aminoacylation of individual tRNAs by their cognate aminoacyl-tRNA synthetases is of interest as part of a complete description of the precise mechanism of peptide bond formation. Several reports have described the preparation of modified tRNAs terminating in 2′- and 3′-deoxyadenosine and 2′- and 3′-*O*-methyladenosine and the utilization of those tRNAs in certain of the partial reactions of protein biosynthesis (1–4). Substrate activity of the modified tRNAs in phenylalanine acceptor assays was limited to those species terminating in 3′-deoxyadenosine and 3′-*O*-methyladenosine; the latter was shown to be aminoacylated poorly relative to unmodified tRNA, also indicating an apparent steric requirement in the aminoacylation reaction. More recently a report has appeared describing the substrate activity of a number of *Escherichia coli* tRNAs terminating in 2′- and 3′-deoxyadenosine (5) and some of the conclusions were supported by a related study utilizing tRNAs terminating in 2′(3′)-amino-2′(3′)-deoxyadenosine (6).

Abbreviations: tRNA-C-C_{OH}, tRNA missing the 3′-terminal adenosine moiety; DBAE-cellulose; *N*-[*N*′-(*m*-dihydroxyborylphenyl)succinamyl]aminoethyl-cellulose; A_λ unit, the amount of material giving an A of 1.0 at wavelength λ when dissolved in 1 ml of solution and measured in a cell with a 1 cm light path; nucleoside Q, 7-(4,5-*cis*-dihydroxy-1-cyclopenten-3-ylaminomethyl)-7-deazaguanosine.

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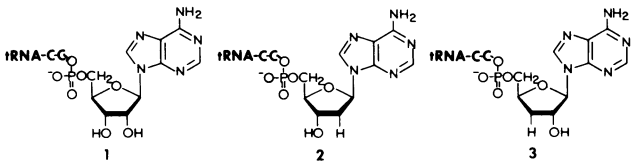
This report is concerned with the conversion of unfractionated tRNA to tRNA-C-C_{OH} in high yield, with the preparation of purified tRNAs terminating in 2′- and 3′-deoxyadenosine, and with the utilization of those purified tRNAs to measure the relative rates of aminoacylation of tRNA species 1–3 from *E. coli* and yeast. The results, which are



not entirely consistent with recent reports for the *E. coli* system (5, 6), indicate which modified tRNA species (2 or 3) can be aminoacylated with each amino acid and presumably reflect the initial site of aminoacylation in unmodified tRNA.

MATERIALS AND METHODS

Tritiated samples of alanine, glycine, leucine, and tyrosine were obtained from ICN Pharmaceuticals, Inc.; [^{3}H]arginine, -asparagine, -isoleucine, and -lysine were from Schwarz/Mann Biochemicals. The remaining amino acids ([^{14}C]cystine and -glutamine, [^{3}H]asparagine, -glutamic acid, -histidine, -methionine, -phenylalanine, -proline, -serine, -threonine, -tryptophan, and -valine) as well as [^{3}H]CTP, [^{14}C]ATP, [^{3}H]cordycepin, [^{3}H]-2′-deoxyATP and ^3H_2O were purchased from New England Nuclear Corp. Unlabeled cordycepin (3′-deoxyadenosine) and 2′-deoxy ATP were purchased from Sigma Chemical Co., as was aminoethyl cellulose. 3′-Deoxyadenosine was phosphorylated (7) and converted to the 5′-triphosphate (8) by known methods. Purified venom phosphodiesterase was obtained from Boehringer Mannheim; DEAE cellulose and GF/A glass fiber discs were from Whatman. Unfractionated tRNA was derived from *E. coli* M72, from an F^-, suppressor$^-$, lactose$^-$ tryptophan revertant (*trp*$^+$) of strain K_{12} resistant to streptomycin and phages T_1 and T_5, or from *Saccharomyces cerevisiae* Y185. The tRNA was obtained by phenol extraction. Large nucleic acids were removed by precipitation with lithium chloride and the tRNA was isolated after several precipitations with ethanol and cetyltrimethylammonium bromide. Partially fractionated aminoacyl-tRNA synthetase solutions were obtained from the same strains of *E. coli* and yeast as described previously (1), except that the final chromatography in each case was carried out on Sephadex G-100. Yeast CTP(ATP):tRNA nucleotidyltransferase was a gift from Dr. Paul Sigler and Mrs. Margaret Rosa.

Preparation of Abbreviated tRNA (tRNA-C-C_{OH}). Samples of abbreviated *E. coli* and yeast tRNA (tRNA-C-C_{OH})

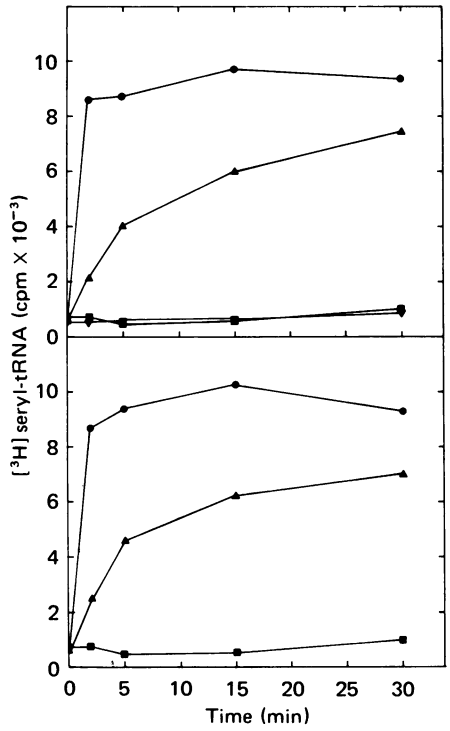


FIG. 1. Aminoacylation of $tRNA^{Ser}$ species 1 (●), 2 (▲), and 3 (▼), relative to a control lacking tRNA (■) in the presence of ATP (upper panel) or 2′-deoxyadenosine 5′-triphosphate (lower panel). Experimental details are given in the legend to Table 1.

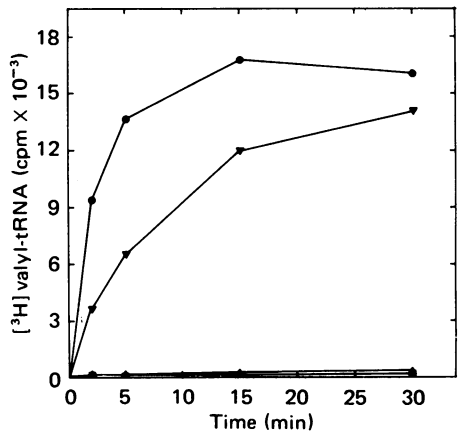


FIG. 2. Aminoacylation of $tRNA^{Val}$ species 1 (●), 2 (▲), and 3 (▼), relative to a control lacking tRNA (■) in the presence of ATP. Experimental details are given in the legend to Table 1.

were prepared by modification of an earlier procedure (1), by incubating 500 A_{260} units of unfractionated tRNA and 50 μg of venom exonuclease in 15 mM Tris–acetate buffer (pH 8.8) for 30 min. This procedure afforded tRNA which was without significant phenylalanine acceptor activity and which accepted 0.8–1.4 equivalents of [^{3}H]CTP per equivalent of tRNA in the presence of CTP(ATP):tRNA nucleotidyl transferase. The venom-treated tRNA was reconstituted by the same enzyme in the presence of unlabeled CTP to afford tRNA-C-C_{OH}. The abbreviated tRNA samples (tRNA-C-C_{OH}) accepted 80–100% of the theoretical amount of [^{3}H]ATP when incubated with [^{3}H]ATP and the CTP(ATP):tRNA nucleotidyltransferase and the fully reconstructed tRNA which resulted could be aminoacylated with phenylalanine essentially as well as untreated tRNA.

Preparation of tRNAs Terminating in 2′- and 3′-Deoxyadenosine. To 5.0 ml (total volume) of 0.01 M Tris·HCl (pH 8.7) containing 10 mM $MgCl_2$, 75 A_{260} units of tRNA-C-C_{OH}, and 75 A_{258} units of 2′- or 3′-deoxyadenosine 5′-triphosphate was added 200 μl of yeast CTP(ATP):tRNA nucleotidyltransferase solution. The combined solution was maintained at room temperature for 4 hr and then treated with 2 volumes of cold ethanol. The precipitated tRNA was redissolved in water and applied to a column of DEAE-cellulose (0.9 × 10 cm); elution was with a linear gradient of sodium chloride (200 ml total volume; 0–0.8 M; 2 ml fractions). Fractions 42–75 were combined, dialyzed against water, concentrated, and precipitated with cold ethanol. The precipitated tRNA was redissolved in 0.05 M morpholine·HCl (pH 8.7) containing 1.0 M NaCl, 0.1 M $MgCl_2$, and 20% dimethylsulfoxide and applied to a column of *N*-[*N*′-(*m*-dihydroxyborylphenyl)succinamyl]aminoethyl-cellulose (DBAE-cellulose) (9) (0.9 × 10 cm) which had been equilibrated with the same buffer at 4°. The column was washed with 60 ml of the same buffer and 1-ml fractions were collected and assayed for A_{260}. The tRNA peak was combined, dialyzed against distilled water, concentrated, and treated with 2 volumes of cold ethanol to precipitate the purified tRNAs terminating in 2′- or 3′-deoxyadenosine. The column was washed with 0.05 M sodium 2-(*N*-morpholino)ethanesulfonate (Mes) (pH 5.5, containing 1 M sodium chloride) to effect elution of tRNA and tRNA-C-C_{OH}.

Purification of tRNAs Containing Nucleoside Q. Four aminoacyl-tRNAs from *E. coli* contain nucleoside Q, 7-(4,5-*cis*-dihydroxy-1-cyclopenten-3-ylaminomethyl)-7-deazaguanosine (10). The modified tRNAs derived from these species were prepared as above, except that the final chromatography was carried out on a column of DBAE-cellulose (0.9 × 10 cm; 40 A_{260} units of tRNA) which had been equilibrated at room temperature with 0.05 M morpholine·HCl, pH 8.7, containing 1 M NaCl and 0.1 M $MgCl_2$. The column was washed with 50 ml of this buffer at room temperature and then with 30 ml of 0.05 M sodium 2-(*N*-morpholino)ethane sulfonate, pH 5.5, containing 1 M NaCl (11). One-milliliter fractions were collected. Fractions 7–30 were pooled, desalted by dialysis against water, concentrated, and treated with ethanol. This sample consisted of nucleoside-Q-containing tRNAs terminating in 2′- or 3′-deoxyadenosine (as well as all possible species derived from tRNAs not containing nucleoside Q). Fractions 61–72 consisted of tRNA and tRNA-C-C_{OH} corresponding to those tRNAs containing nucleoside Q ($tRNA^{Asp}$, $tRNA^{Asn}$, $tRNA^{His}$, and $tRNA^{Tyr}$).

RESULTS

The abbreviated tRNA (tRNA-C-C_{OH}) was prepared by treatment of samples of unfractionated *E. coli* and yeast tRNA with purified venom exonuclease until no significant phenylalanine acceptor activity remained. The venom-treated tRNAs were then incubated with CTP in the presence of CTP(ATP):tRNA nucleotidyltransferase to afford tRNA-C-C_{OH}. The abbreviated tRNA samples incorporated 80–100% of the theoretical amount of ATP when tRNA-C-C_{OH} was further incubated with the tritiated nucleotide in the presence of the CTP(ATP):tRNA nucleotidyltransferase. After ATP incorporation, the reconstituted tRNAs incorporated phenylalanine >95% as well as an untreated sample.

Incubation of tRNA-C-C_{OH} with 2′- and 3′-deoxyadenosine 5′-triphosphate in the presence of yeast CTP(ATP):tRNA nucleotidyltransferase resulted in incorporation of the deox-

Table 1. Percent aminoacylation relative to unmodified tRNA (species 1) of modified tRNA species 2 and 3

	Transfer RNA terminating in			
	2′-Deoxyadenosine (2)		3′-Deoxyadenosine (3)	
Amino acid	Yeast	*E. coli*	Yeast	*E. coli*
Alanine	74	51	2	0
Arginine	3	0	25	100
Asparagine	86	39	81	15
Aspartic acid	66	—[a]	31	—[a]
Cysteine	99	47	84	100
Glutamine	45	—[a]	0	—[a]
Glutamic acid	—[a]	0	—[a]	91
Glycine	34[b] (28)	100	9[b] (6)	5
Histidine	24	37	1	4
Isoleucine	5	11	100	100
Leucine	0	1	100	100
Lysine	47	46	4	0
Methionine	0	12	21	76
Phenylalanine	0	2	71	100
Proline	100	27	5	2
Serine	58	77	9	0
Threonine	49[b] (27)	51	9[b] (4)	6
Tryptophan	39	5	7	100
Tyrosine	100	27	100	24
Valine	1	3	88	100

The aminoacylation assays were carried out in 0.09 M NH_4^+-piperazine-*N*,*N*′-bis(2-ethanesulfonate)(Pipes) buffer (pH 7.0; total volume 110 μl) containing 0.09 M KCl, 13.5 mM $MgCl_2$, 0.45 mM EDTA, 9 μM of the labeled amino acid being tested, 9 μM of each of 18 other unlabeled amino acids (except cysteine), 0.9 mM nucleoside triphosphate (ATP, 2′-deoxyadenosine 5′-triphosphate or 3′-deoxyadenosine 5′-triphosphate) and 0.2–0.8 A_{260} units of tRNA species **1**, **2**, or **3**. The reactions were initiated by the addition of 5 or 10 μl of aminoacyl-tRNA synthetase solution and maintained at room temperature. Twenty-five microliter aliquots were withdrawn after 2, 5, 15, and 30 min and applied to glass fiber discs which had been presoaked with 100 μl of 0.05 M cetyltrimethylammonium bromide solution in 1% acetic acid. (In some cases, particularly Tyr, Trp, His, Lys, and Arg, 20 μl of 0.01–0.05 M solutions of the unlabeled amino acids were also added to the discs to reduce the background.) The discs were washed thoroughly with 1% acetic acid solution and then used to determine radioactivity. Percent aminoacylation is recorded here for those aliquots removed after 30 min of incubation.

In some of those cases in which aminoacylation of the modified tRNAs is recorded as "100%," these species were actually aminoacylated to a greater extent than unmodified tRNA. This may be due to partial or complete removal of some tRNA species during the modification procedures (such as the tRNAs containing nucleoside Q), resulting in effective enrichment of the other species.

In several cases (e.g., the *E. coli* glycyl-, isoleucyl-, methionyl-, and threonyl-tRNA synthetases and the prolyl, isoleucyl, methionyl, and lysyl enzymes from yeast, initial aminoacylation experiments were unsuccessful and the experiments were repeated with a more concentrated enzyme solution which was not purified on Sephadex G-100.

[a] Uncertain.

[b] This value was obtained after 60 min. The 30 min value is in parentheses.

ynucleotides into tRNA to the extent of 45–65%. The modified tRNAs were separated from unreacted tRNA-C-C_{OH} and from intact tRNA by chromatography on DBAE-cellulose, which retained the latter species when washed with 0.05 M morpholine·HCl (pH 8.7, containing 1.0 M NaCl, 0.1 M $MgCl_2$, and 20% dimethylsulfoxide) at 4° by virtue of interaction with the vicinal glycol functionality of the 3′-terminal cytidine moiety. However, four *E. coli* tRNAs ($tRNA^{Asn}$, $tRNA^{Asp}$, $tRNA^{His}$, and $tRNA^{Tyr}$) contain nucleoside Q, which also has a *cis*-diol group. Modification of these species thus afforded tRNA-C-C_{OH} with two vicinal glycol moieties. Reconstruction of these abbreviated tRNAs with 2′- or 3′-deoxyadenosine gave samples of tRNA species **2** and **3** still having one vicinal glycol group. Therefore, the initial separation of unfractionated tRNA species **2** and **3** from tRNA-C-C_{OH} on DBAE-cellulose was ineffective in separating the abbreviated nucleoside Q-containing tRNAs from their respective modified species (**2** and **3**). The abbreviated (tRNA-C-C_{OH}) and modified (**2** and **3**) species derived from these four tRNAs were separated by carrying out the final chromatography on DBAE-cellulose under conditions (0.05 M morpholine·HCl, pH 8.7, containing 1 M NaCl and 0.1 M $MgCl_2$; room temperature) (11) which retained selectively those species having two *cis*-diol groups (intact tRNA and tRNA-C-C_{OH} having the nucleoside Q). Elution with this buffer thus afforded tRNA species **2** or **3** derived from $tRNA^{Asn}$, $tRNA^{Asp}$, $tRNA^{His}$, and $tRNA^{Tyr}$, and tRNA-C-C_{OH} derived from those tRNAs not containing nucleoside Q.

E. coli and yeast tRNA species **2** and **3** were aminoacylated using *E. coli* and yeast aminoacyl-tRNA synthetase solutions, respectively. These were purified by ammonium sulfate precipitation of the crude cell extract, followed by chromatography of the preparation on DEAE-Sephadex A-25 and then on Sephadex G-100. Aminoacylation was carried out for each ^3H- or ^{14}C-labeled amino acid in the presence of the same concentration of 18 other unlabeled amino acids and the extent of aminoacylation was measured at four time points over a period of 30 min. Each of the aminoacylation reactions was carried out initially using ATP as an energy source. To assure that the ATP was not also utilized by CTP(ATP):tRNA nucleotidyltransferase activity in the synthetase preparation to convert tRNA species **2** or **3** to species **1**, each of the apparently successful *E. coli* aminoacylation reactions (and several from the yeast system) was then repeated utilizing 2′- or 3′-deoxyadenosine 5′-triphosphate as the energy source.

As shown in Fig. 1, for example, tRNA species **2** derived from *E. coli* $tRNA^{Ser}$ was aminoacylated to the extent of 77% after 30 min, relative to unmodified *E. coli* $tRNA^{Ser}$, while the corresponding tRNA species **3** was not a substrate for *E. coli* seryl-tRNA synthetase. The experiment was repeated using 2′-deoxyadenosine 5′-triphosphate as the energy source for tRNA species **1** and **2**. Virtually identical results were obtained. Fig. 2 shows the results of aminoacylation of yeast tRNA species **1–3** with [^3H]valine. After 30 min in the presence of ATP, the $tRNA^{Val}$ terminating in 3′-deoxyadenosine (**3**) was aminoacylated to the extent of 88%, relative to normal $tRNA^{Val}$, while the corresponding species **2** was not a substrate for the synthetase. *E. coli* and yeast transfer RNA species **1–3** were aminoacylated with each of the remaining 18 amino acids and the initial results, which were obtained in each case using ATP as the energy source, were verified for each of the *E. coli* enzymes and several of the yeast enzymes in separate experiments using the appropriate deoxyadenosine 5′-triphosphate. The results of these experiments are summarized in Tables 1–3.

DISCUSSION

Transfer RNAs terminating in 2′- and 3′-deoxyadenosine (2, 4) and 2′- and 3′-*O*-methyladenosine (1) are of potential utility in studies of the formation and functioning of individual positional isomers of aminoacyl- and peptidyl-tRNAs,

Table 2. Initial site of aminoacylation of *E. coli* tRNA

Hydroxyl group at 3'-terminus of tRNA which is aminoacylated			
2'-OH	3'-OH	2'- and 3'-OH	Uncertain
Arg	Ala	Asn	Asp
Glu	Gly	Cys	Gln
Ile	His	Tyr	
Leu	Lys		
Met	Pro		
Phe	Ser		
Trp	Thr		
Val			

since the absence of a vicinal diol moiety precludes the isomerization of one species into another. Aminoacylation of these species (1, 2, 4), and of related species (3), was carried out initially using [^{3}H]phenylalanine. The formation of phenylalanyl-tRNA^{Phe} was limited to modified tRNA species of type 3, i.e., those having a free 2'-hydroxyl group, and was interpreted as evidence that tRNA^{Phe} is normally aminoacylated on the 2'-position. The generality of this conclusion for other aminoacyl-tRNAs was tested by carrying out the aminoacylation of unfractionated samples of the modified *E. coli* tRNAs in the presence of a partially fractionated aminoacyl-tRNA synthetase solution and a commercial mixture of 15 ^{3}H-labeled amino acids (2). Although this experiment also indicated a strong preference for aminoacylation of tRNA species 3, additional experiments carried out with single ^{3}H- or ^{14}C-labeled amino acids and their cognate aminoacyl-tRNA synthetases revealed several which utilized the corresponding tRNA species 2 exclusively, e.g., alanyl-, prolyl-, and seryl-tRNA synthetases. Similar experiments have also been carried out in other laboratories and two recent reports (5, 6) have described aminoacylation results obtained with partially modified *E. coli* tRNAs in the presence of larger amounts of unmodified tRNAs that were ostensibly destroyed chemically either before (5) or after (6) the aminoacylation procedure. It should be noted that in a number of assays the resulting aminoacylation attributed to the modified tRNAs was only several percent, relative to that obtained with unmodified tRNA (5, 6).

In the present case, we have converted unfractionated yeast and *E. coli* tRNAs to tRNA-C-C$_{OH}$ under carefully controlled conditions to minimize the loss of tRNA isoacceptors during the overall modification process. Samples of abbreviated tRNAs (tRNA-C-C$_{OH}$) were recovered in high yield and could be reconstituted in yields of 80–100%, as judged by incorporation of [^{3}H]ATP and aminoacylation of the resulting tRNAs with phenylalanine. Use of the enzymatic degradation procedure, as opposed to successive treatments of the tRNA with periodate and an amine (5), also circumvented destruction of nucleoside Q in *E. coli* tRNA^{Asn}, tRNA^{Asp}, tRNA^{His}, and tRNA^{Tyr}, and the more general degradation of the tRNAs by this procedure (5, 12). The modified tRNAs (2 and 3) were separated from intact tRNA and unreacted tRNA-C-C$_{OH}$ by chromatography on DBAE-cellulose.

Table 3. Initial site of aminoacylation of yeast tRNA

Hydroxyl group which is aminoacylated			
2'-OH	3'-OH	2'- and 3'-OH	Uncertain
Arg	Ala	Asn	Glu
Ile	Gln	Asp	
Leu	Gly	Cys	
Met	His	Tyr	
Phe	Lys		
Val	Pro		
	Ser		
	Thr		
	Trp		

The aminoacylation of modified yeast and *E. coli* tRNA species 2 and 3 was measured as a function of time, utilizing ATP as the energy source. As shown in Table 1, the arginyl-, isoleucyl-, leucyl-, methionyl-, phenylalanyl-, and valyl-tRNA synthetases from both yeast and *E. coli* aminoacylated only those cognate tRNAs of type 3 (Tables 2 and 3). The same was also true of the *E. coli* glutamyl-tRNA synthetase. On the other hand, the alanyl-, glycyl-, histidyl-, lysyl-, prolyl-, seryl-, and threonyl-tRNA synthetases from yeast and *E. coli*, as well as the glutaminyl-tRNA synthetase from yeast, aminoacylated only tRNA species 2. The asparaginyl-, cysteinyl-, and tyrosyl-tRNA synthetases from yeast and *E. coli* and the aspartyl-tRNA synthetase from yeast may be regarded as a third class of enzyme, in that they utilize both cognate tRNA species 2 and 3 as substrates. Thus the position of aminoacylation of the modified tRNAs, and presumably of unmodified tRNA as well, is not uniform for all aminoacyl-tRNA synthetases and indeed shows a surprising degree of diversity. The possibility that the apparent utilization of cognate tRNA species 2 and 3 by the last class of enzymes might be due to the presence of tRNA isoacceptors with different positional specificity can be excluded for yeast tRNA^{Asn}, tRNA^{Cys}, and tRNA^{Tyr}, since both isomers were utilized to virtually the same extent as unmodified tRNA.

Perhaps the most interesting of the amino acids studied was tryptophan, whose *E. coli* aminoacyl-tRNA synthetase aminoacylated tRNA^{Trp} species 3 (but not species 2) as well as unmodified tRNA^{Trp}, while the corresponding *E. coli* enzyme gave 39% aminoacylation of yeast tRNA^{Trp} species 2 after 30 min, relative to unmodified tRNA (but did not utilize species 3). At present this represents the only confirmed example of change in specificity during the evolution from a prokaryotic to eukaryotic organism. All of the other aminoacyl-tRNA synthetases whose activities could be measured in both the *E. coli* and yeast systems utilized the same modified tRNA species in their respective systems.

In terms of comparison between the data contained in Table 1 and those published previously (5, 6), one may note that both the yeast and *E. coli* asparaginyl-tRNA synthetases aminoacylated homologous tRNA species 2 and 3 to the same extent after 30 min, while previous reports for *E. coli*, the apparent conclusions of which are in conflict with each other, have indicated exclusive aminoacylation of the 2'- (5) and 3'-OH (6) groups. Also, for both *E. coli* and yeast, we found that only tRNA^{Thr} species 2 was a substrate for the threonyl-tRNA synthetases while Sprinzl and Cramer (5) reported that aminoacylation of *E. coli* tRNA^{Thr} occurred only with the isomeric tRNA species 3.

The relative rates of aminoacylation of 2 and 3 were also instructive. It was found, for example, that in those cases in which tRNA species 2 was the only modified tRNA that acted as a substrate for aminoacylation, aminoacylation generally proceeded much more slowly than with the unmodified tRNA, while most of the modified tRNAs which were

aminoacylated exclusively on the 2'-OH group (tRNA species 3) were utilized at a rate and to an extent more nearly comparable to that obtained with the unmodified tRNAs. This effect was especially pronounced for the tRNAs from *E. coli* and may well have contributed to the observation of predominant aminoacylation of the unfractionated tRNA species 3 (as compared with 2) in the presence of a mixture of ^{3}H-labeled amino acids and aminoacyl-tRNA synthetases (2). Although any possible physiological significance of this observation is unclear, it could reflect a difference in chemical reactivity of the vicinal hydroxyl groups.

It should be noted that the numbers in Table 1 were recorded after 30 min and do not represent the ultimate extent of aminoacylation of many of those species whose aminoacylation was not comparable to that obtained with unmodified tRNA. Thus, for a number of the tRNAs in Table 1, values less than those obtained with the corresponding unmodified tRNAs may reflect an inherent property of the modified tRNAs or the fact that the experimental conditions used for aminoacylation were not optimized for each amino acid. On the other hand, we cannot exclude the possibility that there was a disproportionate loss of certain amino-acid acceptor activities during the modification procedure or that reconstruction of tRNA-C-C$_{OH}$ to afford species 2 and 3 proceeded to a different extent for individual tRNAs. Moreover, especially for those modified tRNAs whose rate of aminoacylation was slow compared with unmodified tRNA, the preferential aminoacylation of a single isomeric tRNA (i.e., 2 or 3) does not necessarily imply that the aminoacylation of unmodified tRNA (1) occurs at the same position.

CTP(ATP):tRNA nucleotidyltransferase activity contained in the aminoacyl-tRNA synthetase solutions utilized in these experiments could in principle effect the following type of transformation:

$$\text{tRNA-C-C-dA} \xrightarrow[\text{Enz}]{\text{ATP}} \text{tRNA-C-C-A}$$

While control experiments (and the exclusive aminoacylation of single isomers of the modified tRNAs) suggested that this was not a serious problem under the conditions utilized for aminoacylation of the modified species, the experiments were, nonetheless, verified by repeating many of the aminoacylations in the presence of the deoxynucleoside 5'-triphosphate corresponding to the deoxynucleotide at the terminus of the tRNA being assayed. No qualitative differences in tRNA aminoacylation were noted using this procedure and both the 2'- and 3'-deoxynucleotides were accepted as substrates by the enzymes in all cases tested, with the exception that 3'-deoxyadenosine 5'-triphosphate appeared not to effect the aminoacylation of *E. coli* tRNA^{Gln}. However, the rates of aminoacylation obtained with the deoxynucleotides, relative to those obtained with ATP, varied greatly from one aminoacyl-tRNA synthetase to another.

Thus the results of aminoacylation of the modified tRNAs from *E. coli* and yeast indicate an apparent conservation of the initial position of tRNA aminoacylation during the evolution from a prokaryotic to eukaryotic organism. The only change in specificity was that observed for tRNA^{Trp}, which was aminoacylated on the 2'-position in *E. coli* and the 3'-position in yeast. There seem to be no obvious relationships between amino acid or tRNA structure and the modified tRNA species (2 or 3) which was aminoacylated, although such correlations may exist. The maintenance of positional specificity for aminoacylation is of interest in that there would seem to be no selective advantage in protein biosynthesis to the maintenance of such specificity, since the isomeric tRNAs can equilibrate quickly relative to the time scale on which the partial reactions of protein biosynthesis occur. Alternatively, positional specificity may be part of the process by which tRNA misacylation is prevented (13) or may simply reflect the development of certain discrete types of synthetase active sites which have been maintained during evolution. The extent to which maintenance of positional specificity has occurred and the possible significance of this common feature of tRNA aminoacylation should be easier to assess once the positional specificity is known in other types of organisms.

We thank Prof. Paul Sigler and Mrs. Margaret Rosa for a sample of yeast CTP(ATP):tRNA nucleotidyltransferase and Mr. Steven Hassur for a sample of yeast aminoacyl-tRNA synthetase. This work was supported by a grant from the National Science Foundation. S.M.H. is a Research Career Development Awardee of the National Cancer Institute (1975–1980) and an Alfred P. Sloan Research Fellow (1975–1977). A.C.C. was supported by a National Science Foundation Predoctoral Fellowship (1971–1974) and by a Sloan Research Traineeship (1974–1975).

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