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Post-Transcriptional Modifications of the Anticodon Loop Region: Alterations in Isoaccepting Species of tRNA's During Development in *Bacillus subtilis*

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Structural similarities of tRNA's were compared using three sets of isoaccepting species that had previously been shown to undergo significant changes in chromatographic elution properties as a function of developmental stage in *Bacillus subtilis*. Comparisons of the structures of the tRNA's were based on the composition of their modified nucleosides, comparisons of oligonucleotide elution profiles from RPC-5 columns, and two-dimensional electrophoretic fingerprint analysis of oligonucleotides. The tRNA's studied were $tRNA_1^{Lys}$ and $tRNA_3^{Lys}$; $tRNA_1^{Tyr}$ and $tRNA_2^{Tyr}$; and $tRNA_1^{Trp}$ and $tRNA_2^{Trp}$. The results suggest that the difference among these pairs of isoaccepting species is a difference in the degree of post-transcriptional modifications of the anticodon loop region. The nucleosides involved were N^6-(Δ^2-isopentenyl)adenosine (i^6A), 2-methylthio-N^6-(Δ^2-isopentenyl)adenosine (ms^2i^6A), and an unknown nucleoside K, which occurred in a position analogous to *N*-[9-(β-D-ribofuranosyl)purin-6-ylcarbamoyl]threonine. The amounts of i^6A and ms^2i^6A, determined using total tRNA from exponential- or stationary-phase cells, suggest that the thiomethylation of i^6A is a pleiotropic phenomenon affecting several tRNA species. As opposed to the situation in *Escherichia coli* tRNA, where ms^2i^6A constitutes about 90% of the total hydrophobic nucleosides at all growth stages, *B. subtilis* tRNA's have i^6A as the predominant hydrophobic nucleoside in exponential growth and ms^2i^6A as the predominant nucleoside in stationary phase. Thus, the enzyme system which forms i^6A and the enzyme system which thiomethylates i^6A are not coordinated during growth in *B. subtilis* as they are in *E. coli*. It is suggested that these changes in anticodon loop modifications in *B. subtilis* may be related to changes in the translational apparatus which occur during sporulation.

Alterations in tRNA's associated with cellular development have been the subject of much interest but have proved to be a complex area of investigation. Changes in isoaccepting species of tRNA have been reported in a variety of differentiating systems as well as cases of abnormal development. As opposed to changes in the levels of groups of tRNA's, characteristic of certain highly differentiated systems that produce a specialized protein in large amounts (i.e., 25), qualitative changes within a set of isoaccepting species seem to be characteristic of other differentiating systems, for example, *Bacillus subtilis* (6, 29) and *Dictyostelium discoideum* (20). These changes were observed as alterations in the chromatographic elution properties of the isoaccepting tRNA's in question. Such a difference in elution properties could be caused by a post-transcriptional modification of tRNA's transcribed from the same gene, a difference in primary structure between two tRNA's, or a change in tertiary conformation. The concept of post-transcriptional alterations is attractive in light of the work of Cortese et al. (7) demonstrating that the post-transcriptional modification of pseudouridine in the tRNA's of a mutant of *Salmonella typhimurium* was part of a "super-control system" for the regulation of the biosynthesis of the enzymes of certain amino acids.

In contrast to the differentiating systems mentioned above, alterations in the chromatographic elution profiles of the tRNA's of *Escherichia coli*, a nondifferentiating procaryote, are not prevalent during normal growth. Skjold et al. (24) investigated the chromatographic profiles for several isoaccepting tRNA's extracted from cells of *E. coli* that had been grown under extremes of growth conditions. They found no alterations in any of the seven tRNA isoaccepting sets studied. One change in the relative levels of tyrosine tRNA's in early and late logarithmic growth stages has been reported by other work-

ers (12). Of course, altered chromatographic profiles for tRNA's in *E. coli* have been produced by various non-physiological conditions; however, we are concerned with those alterations that occur under normal physiological conditions.

In previous studies, we described chromatographic alterations in 10 groups of isoaccepting species of tRNA that occurred during development in *B. subtilis*, and we established the temporal pattern of the alterations for lysyl-, tyrosyl-, tryptophanyl-, and leucyl-tRNA's (28). The differences in their temporal expression implied a difference in the mechanisms that regulated the changes. Subsequently, we studied the state of modification of the tRNA's and identified the types and amounts of modified nucleosides present (22, 27). Although no gross changes in minor nucleosides occurred as a function of development, subtle differences in methyl modifications and in pseudouridine were detected (22). Lysine tRNA's were studied in particular; the two isoaccepting species that showed a change in percentage during growth differed in their codon preference (30) and in their utilization by polyribosomes in vivo (26).

In the present study, we report structural studies done on three sets of isoaccepting species of *B. subtilis* tRNA: $tRNA_1^{Lys}$ and $tRNA_3^{Lys}$; $tRNA_1^{Tyr}$ and $tRNA_2^{Tyr}$; and $tRNA_1^{Trp}$ and $tRNA_2^{Trp}$. There are no previously published data on the tryptophan tRNA's of *B. subtilis*. The lysine and tyrosine tRNA's of *B. subtilis* have been studied by others. Chuang et al. (5) reported results of hybridization experiments with the lysine tRNA's of *B. subtilis* W23 that implied that there may be two different primary sequences. In contrast, we found that the lysine tRNA's showing chromatographic alterations during growth have extremely similar sequences and differ in two nucleoside residues. It appeared most likely that there were real differences between the strains used or growth conditions (30). Finding post-transcriptional modification in one system does not exclude the validity of differences in primary structure occurring in another system. On the other hand, the results on the structure of $tRNA_1^{Lys}$ are completely compatible with a sequence analysis for the $tRNA^{Lys}$ of *B. subtilis* 168 that has recently been completed by Yamada and Ishikura (33). Arceneaux and Sueoka (1) presented data to suggest that the 3'-terminal ends of the two isoaccepting species of tyrosine tRNA had the same sequence, although they could compare only a small segment of the molecule by the technique they used. McMillian and Arceneaux (16) observed that adding tyrosine to the medium reduced the extent of the alteration in the amounts of the two tyrosine tRNA species during development and suggested a role for tyrosine in the regulation of the levels of these tRNA's. Others have reported that the change in tyrosine tRNA's that was found in *B. subtilis* as a function of development was the result of a modification involving the N^6-(Δ^2-isopentenyl)adenosine (i^6A) group (13, 17). A comparison of oligonucleotides was not done, however.

MATERIALS AND METHODS

Purification of tRNA's. After the initial extraction and fractionation on Sephadex G-100 (27), tRNA's not labeled with a radioisotope were purified by combinations of standard chromatographic methods using the reverse-phase column, RPC-5 (14); benzoylated diethylaminoethyl-cellulose (11); diethylaminoethyl-Sephadex (19); and/or dihydroxyboryl-cellulose (15) columns. Except for the separations on dihydroxyboryl-cellulose, the isoaccepting species of interest was identified by postcharging of the eluted tRNA's. The ^{32}P-labeled tRNA's for lysine were labeled with sodium [^{32}P]phosphate in dephosphorylated broth (30). After extraction and Sephadex G-100 chromatography, the ^{32}P-labeled tRNA's were precharged with L-[^3H]lysine and chromatographed sequentially over dihydroxyboryl-cellulose, RPC-5, and Aminex A-28 (23) columns. Aminex A-28 columns were eluted with an NaCl gradient of 0.5 to 0.7 M NaCl for $tRNA_1^{Lys}$ and 0.6 to 0.9 M NaCl for $tRNA_3^{Lys}$ in a buffered solution containing 10 mM sodium acetate (pH 4.5), 10 mM $MgCl_2$, 1 mM ethylenediaminetetraacetic acid, 20 mM 2-mercaptoethanol, and 0.1 ml of isoamyl acetate per liter. The purity of the isoaccepting species in all cases was greater than 1,200 pmol of amino acid accepted per amount of tRNA giving an absorbance of 1 unit at 260 nm.

Digestion procedures. For analytical purposes, purified tRNA's were digested to nucleosides by incubation with ribonuclease (RNase) T2 (Sigma Chemical Co., grade V) followed by alkaline phosphatase (Bacterial Alk phosphatase, Worthington) as previously described (27). Large-scale digestion to nucleosides for the experiments with the hydrophobic nucleosides is described in a later section. To obtain nucleotides, tRNA's of the order of 1 absorbancy unit at 260 nm were digested by incubation at 37°C for 6 h with 4 U of RNase T2, 20 U of RNase T1 (Sigma Chemical Co.), and 0.06 mg of pancreatic RNase (Worthington, RNase A) in 20 μl of 0.05 M ammonium acetate (pH 4.5) plus 1 mM ethylenediaminetetraacetic acid. Oligonucleotides were prepared from purified tRNA's by incubation of 1 to 10 absorbancy units at 260 nm of tRNA at 37°C for 5 h with 100 U of RNase T1 in 100 μl of 5 mM tris(hydroxymethyl)aminomethane (pH 7.4). The reaction mixture was brought to pH 4.5 by the addition of acetic acid and then loaded directly onto the RPC-5 column.

Fractionation of oligonucleotides on RPC-5. Oligonucleotides were fractionated on RPC-5 columns, 0.6 by 100 cm, jacketed at 37°C. A gradient of 0.5 to 3.0 M ammonium acetate-acetic acid (pH 4.5)

was used to elute the oligonucleotides according to the procedure of Egan (8). The elution of oligonucleotides was monitored by an Altex UV monitor, model 152.

Analysis of nucleosides on Aminex A-6. The composition of major and minor nucleosides was determined as previously described (22) using Aminex A-6 columns and an Altex UV monitor.

TLC of nucleotides. Nucleotides were analyzed on 20- by 20-cm cellulose thin-layer chromatography (TLC) plates, with isobutyric acid-0.5 M ammonium hydroxide (5:3, vol/vol) in the first dimension and isopropanol-concentrated HCl-water (70:15:15, vol/vol/vol) in the second dimension. ^{32}P-labeled nucleotides were located by autoradiography.

Analysis of hydrophobic nucleosides on Sephadex LH-20. To obtain the tRNA required for this experiment, *B. subtilis* 168 *trpC2* was grown in 8 liters of tryptone-yeast extract medium in a fermentor. Cells were harvested by centrifugation, washed once with tris(hydroxymethyl)aminomethane-sucrose, and frozen. The cells were subsequently thawed and subjected to sonic disruption. The tRNA's were extracted with phenol and chloroform-isoamyl alcohol (24:1, vol/vol), precipitated with ethanol, and fractionated on a column of Sephadex G-100. For complete release of i^6A type of nucleosides, a mixture of RNases was used to digest the tRNA. tRNA (300 absorbancy units at 260 nm) was dissolved in 1.0 ml of 0.05 M ammonium acetate (pH 4.5) plus 1 mM ethylenediaminetetraacetic acid and incubated at 37°C overnight with 150 U of RNase T2, 1,000 U of RNase T1, and 2.5 mg of pancreatic RNase. The solution was then adjusted to 5 mM in tris(hydroxymethyl)aminomethane, and the pH was brought to 7.5. After the addition of 75 U of alkaline phosphatase, the incubation was continued for 6 h at 37°C. The resulting nucleosides were separated on Sephadex LH-20 as described by Armstrong et al. (2) except that the ethyl acetate extraction step was eliminated. Nucleosides were loaded directly on the Sephadex LH-20 column (0.9 by 23 cm), which was equilibrated and eluted with 35% ethanol at room temperature.

RESULTS

Nucleoside composition of tyrosine tRNA's and tryptophan tRNA's. Tyrosine and tryptophan tRNA species were purified by standard chromatographic methods, using RPC-5, benzoylated diethylaminoethyl-cellulose, diethylaminoethyl-Sephadex, and dihydroxyboryl-cellulose. Total nucleoside digests of each purified tRNA were then made, and the nucleosides were analyzed on columns of Aminex A6 (27) (Table 1). In addition to our data, values for similar *E. coli* tRNA's are given for comparison. The composition is expressed as residues per tRNA molecule. Since the sequence of none of the *B. subtilis* tRNA's had been determined at the time of these analyses, we assumed a total nucleoside number for *B. subtilis* tRNA's approximating that for the *E. coli* tRNA's. *B. subtilis* tRNA's have some unique characteristics, such as the presence of 1-methyladenosine and low amounts of 4-thiouridine, which are usually thought of as characteristic of eucaryote tRNA's as opposed to procaryote tRNA's. It is noteworthy, therefore, that the tryptophanyl-tRNA's of *B. subtilis* did not contain 4-thiouridine or 7-methylguanosine, as the corresponding *E. coli* tRNA's did. Also, the *B. subtilis* tyrosine tRNA's contained 1-methyladenosine, but the *E. coli* tyrosine tRNA did not.

It is clear that the two tryptophanyl-tRNA isoaccepting species of *B. subtilis* are extremely similar in composition of major and minor nucleosides. By the Aminex technique, we could not detect a significant difference between the isoaccepting species of the tryptophan or of the tyrosine tRNA's of *B. subtilis*, except a possible difference in pseudouridine. However, both of these tRNA groups contain a cytokinin-active nucleoside, presumably i^6A or 2-methylthio-N^6-(Δ^2-isopentenyl)adenosine (ms^2i^6A). Therefore, we explored the difference in the hydrophobic nucleosides as discussed in the following section.

The two tyrosine tRNA's were also extremely similar in composition of major and minor nucleosides. The *B. subtilis* tyrosine tRNA's, as opposed to tryptophan tRNA's, did contain 4-thiouridine. The nucleoside composition of the tyrosine tRNA's of *B. subtilis* reported by others is also given in the table. Using TLC analysis, Keith et al. (13) concluded that these species were the same except that $tRNA_1^{Tyr}$ contained i^6A whereas $tRNA_2^{Tyr}$ contained ms^2i^6A. Our results on nucleoside composition are in agreement. However, before drawing the conclusion that these species were derived from the same gene, we thought that some further structural evidence should be given, so we compared oligonucleotide elution patterns. Figure 1 shows the elution profiles of oligonucleotides from the two purified tyrosine tRNA's on RPC-5 columns. The similarity of the profiles provides further evidence that these two tRNA species share the same basic primary sequence.

Quantitation of i^6A and ms^2i^6A at two different growth stages. If the difference between the two tyrosine tRNA's and the two tryptophan tRNA's was that the later-eluting species contained an ms^2i^6A as opposed to i^6A, we might expect an elevation in ms^2i^6A relative to i^6A in unfractionated tRNA's to occur as a function of growth. It has been shown with *E. coli* $tRNA^{Tyr}$ that removal of the isopentenyl group or the thiomethyl group shifts the elution position of the tRNA to an earlier position on a reversed-phase column (10). Therefore, the addition of a thiomethyl group would be expected to cause a shift to a later-eluting position. As far as we know, there is only one enzyme system for the thiomethylation of i^6A (9). Therefore, the

TABLE 1. *Nucleoside composition of tryptophan and tyrosine tRNA's as determined by Aminex A-6 analysis*

Nucleoside[a]	Tryptophan-tRNA[b]			Tyrosine-tRNA[b]			
	E. coli[c]	*B. subtilis*		*E. coli*[c]	*B. subtilis*		*B. subtilis*[d]
		1[e]	2[e]		1[e]	2[e]	
U	11	13	11	11	12	10	12-14
G	22	26	26	21	27	25	23-25
C	20	20	20	27	23	25	21-23
A	14	14	16	18	13	14	14-16
m^5U	1	1.0	0.9	1	1.1	1.3	1
ψ	1	1.9	1.4	2	2.2	2.6	2
hU	3	—	—	No	—	—	1
ms^2i^6A	1	—	—				
s^4U[f]	1	No	No	2	Yes	Yes	1
C_m	1	—	—				
m^7G	1	No	No				
ms^2i^6A or i^6A				1	—	—	1
m^1A and m^6A				No	1.7	1.9	1
G_m				1	—	—	—
Q				1	—	—	1
Total	76			85			

[a] Nucleosides: U, uridine; G, guanosine; C, cytosine; A, adenosine; m^5U, 5-methyluridine; ψ, pseudouridine; hU, dihydrouridine; s^4U, 4-thiouridine; C_m, 2'-*O*-methylcytidine; m^7G, 7-methylguanosine; m^1A, 1-methyladenosine; m^6A, N^6-methyladenosine; G_m, 2'-*O*-methylguanosine; Q, 7-(4,5-*cis*-dihydroxy-1-cyclopenten-3-ylaminomethyl)-7-deazaguanosine.

[b] —, The nucleoside wasn't looked for or could not have been detected by the method used; No, indicates the nucleoside could have been detected but wasn't there; Yes, indicates the nucleoside was detected but its amount could not be accurately quantitated.

[c] Nucleoside compositions for *E. coli* tRNA's were taken from the *Handbook of Nucleic Acid Sequences* (3).

[d] Nucleoside composition determined by Keith et al. (13). One residue of i^6A is present in $tRNA_1^{Tyr}$, whereas ms^2i^6A is present in $tRNA_2^{Tyr}$.

[e] Designation of which isoaccepting species. Species were numbered by their order of elution from RPC-5.

[f] The presence of s^4U was determined by following the absorbance in the region of 340 nm of the purified tRNA's before hydrolysis.

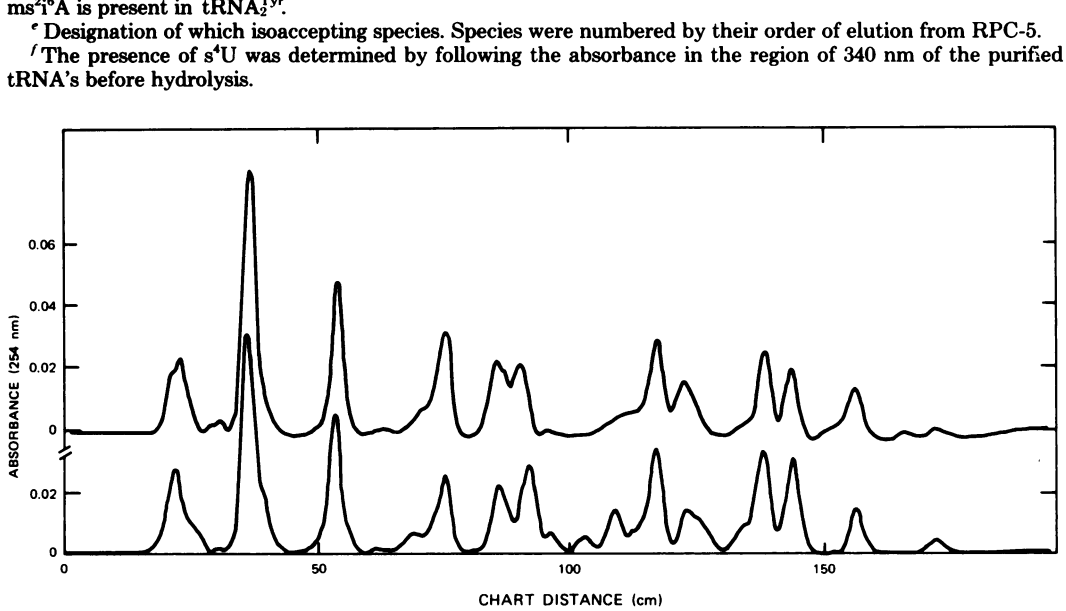

FIG. 1. *Fractionation of RNase T1 oligonucleotides of tyrosine tRNA's on RPC-5. Top section: oligonucleotides of $tRNA_1^{Tyr}$. Bottom section: oligonucleotides of $tRNA_2^{Tyr}$.*

thiomethylation of i^6A during growth might be a pleiotropic phenomenon. This possibility was also suggested by Menichi and Heyman (17). To investigate this possibility, the amounts of i^6A and ms^2i^6A were quantitated after the nucleosides were separated on a column of Sephadex

LH-20. Cells were harvested in mid-exponential growth or in stationary phase, 3 h after the end of exponential growth. After the tRNA's were isolated and digested to nucleosides, the nucleosides were fractionated on a column of Sephadex LH-20 and eluted with 35% ethanol, according to the procedure described by Armstrong et al. (2). The absorbance of the eluate was followed by means of an Altex UV monitor at 254 nm. The elution profile for tRNA's from exponentially growing cells is shown in Fig. 2. Before integrating the areas representing i^6A, the material was collected and rechromatographed on Sephadex LH-20 to remove nonhydrophobic nucleosides. Unlike Armstrong et al. (2), we did not use an extraction with ethyl acetate to enrich for the hydrophobic nucleosides before chromatography. We found that rechromatography of the i^6A was more direct. The contents of i^6A and ms^2i^6A in tRNA from two growth stages are given in Table 2. The identity of the i^6A peak was confirmed by co-chromatography with an i^6A standard and by its absorbance profile. The identity of the ms^2i^6A was inferred by its later elution position off Sephadex LH-20 and from the fact that its absorbance profile gave a maximum at a longer wavelength than that for i^6A.

Evidence from *E. coli* suggests that there are two different enzyme systems for the formation of i^6A and for the thiomethylation of i^6A. The significant point to notice from Table 2 is that these two enzyme systems are not coordinated in *B. subtilis*. This is not the case for *E. coli*

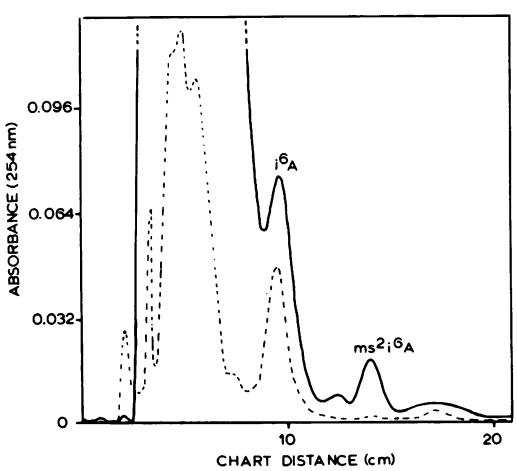

FIG. 2. *Separation of i^6A and ms^2i^6A on Sephadex LH-20. The absorbance in the eluate was followed by an Altex UV monitor. The solid line shows the elution pattern obtained from the initial chromatography. The first area of material absorbing in the UV that eluted from the column represents all nonhydrophobic nucleosides. The dashed line shows the results of re-chromatography of the area containing i^6A.*

TABLE 2. *Contents of i^6A and ms^2i^6A in tRNA's from two different growth stages*[a]

Growth stage	i^6A	ms^2i^6A (% of total)	Total
Mid-log	0.095	0.038 (29)	0.133
Stationary	0.032	0.066 (67)	0.098

[a] Values are given as mol per 100 mol of total nucleosides from a digest of tRNA, assuming an $\epsilon \times 10^{-3}$ (254 nm and neutral pH) of 14 for i^6A, 20 for ms^2i^6A, and an average value of 11 for the nonhydrophobic nucleosides.

tRNA. Bartz et al. (4) have quantitated the levels of i^6A and ms^2i^6A in *E. coli* tRNA and found that 90 to 93% of the i^6A moieties were thiomethylated at whatever growth stage was examined. In addition, they observed no difference in the chromatographic elution patterns of the isoaccepting species of tRNA for tyrosine, serine, phenylalanine, cysteine, tryptophan, and leucine when these species were compared from early-, mid-, or late-logarithmic phases.

Nucleoside (nucleotide) composition of lysine tRNA's. The nucleosides obtained from unlabeled lysine tRNA's analyzed by the Aminex A-6 technique are given in Table 3. In addition, the nucleotides obtained from ^{32}P-labeled lysine tRNA's separated by TLC are given in Table 3. A comparison of the data obtained by the two different techniques should substantiate significant differences. Thus, although the composition of major nucleosides, of 5-methyluridine, and of dihydrouridine are the same in the two tRNA's, $tRNA_3^{Lys}$ seems to contain one more pseudouridine than $tRNA_1^{Lys}$. By both types of analysis, two unknown nucleosides were detected. Although one of the unknowns chromatographed in the same position as the *N*-[9-(β-D-ribofuranosyl)purin-6-ylcarbamoyl]threonine (t^6A) standard on Aminex A-6, no nucleotide was detected on the TLC plates in a position characteristic of the 3'-phosphate of t^6A. An autoradiogram of ^{32}P-labeled nucleotides from purified lysine tRNA's on the two-dimensional TLC plates is shown in Fig. 3. On the TLC plates, the two unknowns are designated as spot 1 and spot 2. Both of the unknowns migrated to a position similar to a nucleotide called K recently discovered by Yamada and Ishikura from TLC analyses of the nucleotides of lysine $tRNA_1$ from *B. subtilis* (33). Further work will be required to characterize these unknowns, but the most likely interpretation is that the two unknown spots represent K and a modified form of K.

Oligonucleotide comparisons. [^{32}P]-$tRNA_1^{Lys}$ and [^{32}P]$tRNA_3^{Lys}$ were purified, and the RNase T1 digests were separated by two-dimensional electrophoresis using the standard

TABLE 3. *Nucleoside composition of lysine tRNA's determined by Aminex A-6 analysis of nucleosides or TLC of ^{32}P-labeled nucleotides*[a]

Aminex A-6 analysis			Two-dimensional TLC analysis		
Nucleoside	$tRNA_1^{Lys}$	$tRNA_3^{Lys}$	Nucleotide	$tRNA_1^{Lys}$	$tRNA_3^{Lys}$
U	16	16	Up	15	17
G	22	23	Gp	21	21
C	20	17	Cp	17	15
A	14	16	Ap	18	18
m^5U	1.0	1.0	m^5Up	0.4	0.4
ψ	1.2	1.7	ψ	1.2	1.6
hU	—	—	hUp	1.5	1.5
t^6A (?)	Yes	Yes	pGp	1.3	0.8
			Free ^{32}P	0.1	0.2
Unknown	Yes	Yes	1[b] K*p (?)	0.1	0.5
			2 Kp (?)	0.2	ND

[a] The lysine tRNA's purified for Aminex analysis were isolated from cells grown in the absence of isotopic label. The lysine tRNA's purified for TLC analysis were grown in the presence of sodium [^{32}P]phosphate. The values are given as residues per 76 to 77 residues. Technical procedures are described in the text. Symbols: U, uridine; G, guanosine; C, cytosine; A, adenosine; m^5U,5-methyluridine; ψ, pseudouridine; hU, dihydrouridine; pGp, 3'5'-guanosine diphosphate; p (suffix) indicates the 3'-phosphate form.

[b] 1 and 2 refer to the areas of the TLC plate represented in Fig. 3. * Designates further unknown modification of K.

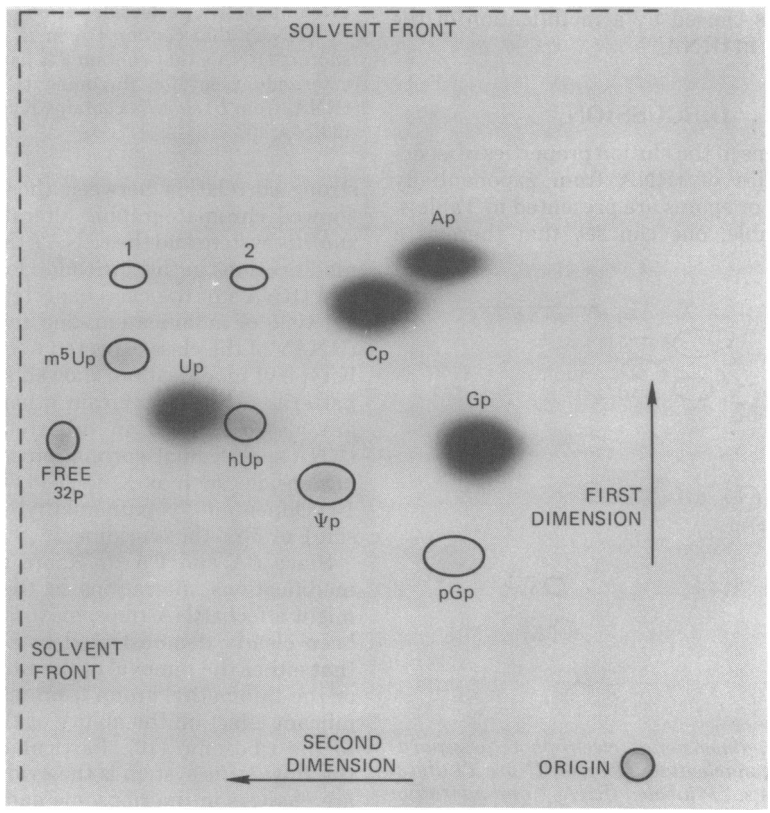

FIG. 3. *Autoradiogram of ^{32}P-labeled nucleotides from purified lysine tRNA's on two-dimensional TLC. Solvent systems given in the text. The numbered areas represent nucleotides of uncertain assignment.*

Sanger technique (21). Preliminary observations in collaboration with Abelson and Otsuka at the University of California-San Diego indicated that one and maybe two large oligonucleotides differed between the two tRNA's. Final analyses were done in collaboration with Ishikura and

Yamada at the Jichi Medical School in Japan. Our [^{32}P]tRNA$_1^{Lys}$ and [^{32}P]tRNA$_3^{Lys}$ were each digested along with their lysine tRNA (in an unlabeled form) in amounts high enough that the UV absorbance of the oligonucleotides could be detected. Oligonucleotides from the [^{32}P]-tRNA$_1^{Lys}$ and the unlabeled lysine tRNA cochromatographed after digestion by either RNase T1 or pancreatic RNase. A representation of the two-dimensional electrophoretic separation of the oligonucleotides from [^{32}P]-tRNA$_3^{Lys}$ and nonradioactive tRNA$_1^{Lys}$ is shown in Fig. 4. It is clear that one large oligonucleotide was found in tRNA$_3^{Lys}$ that was not found in tRNA$_1^{Lys}$. The sequence data given in the figure come from the data of Yamada and Ishikura (33). The large oligonucleotide may be related to the oligonucleotide that includes the anticodon loop, the largest oligonucleotide present. Since the pseudouridine modification is not likely to significantly change the mobility of that oligonucleotide, we postulate that the alteration in mobility is caused by a modification of the nucleoside K in tRNA$_3^{Lys}$

DISCUSSION

Comparisons of the elution properties of isoaccepting species of tRNA from exponentially growing cells or spores are presented in Table 4. From this table, one can see that there is a strong correlation between those tRNA's that showed chromatographic alterations in the *B. subtilis* system and the tRNA's that have certain modifications in the anticodon loop. Four out of five tRNA's of the class expected to contain an i^{6}A type of modification, and five out of seven tRNA's of the class expected to contain a t^{6}A or K type of modification, showed shifts in elution patterns. Although certain other modifications are also restricted in occurrence to specific tRNA's, no similar correlation can be made for nucleosides such as X, Q [7-(4,5-*cis*-dihydroxy-1-cyclopenten-3-ylaminomethyl)-7-deazaguanosine], or 5-methoxyuridine.

Since i^{6}A and t^{6}A or K are anticodon loop modifications, alterations in these nucleosides might affect tRNA-ribosome interactions. It has been clearly demonstrated in *E. coli* tRNA^{Tyr} that either the removal of the isopentenyl group or the thiomethyl group from ms^{2}i^{6}A had a significant effect on the ability of tRNA^{Tyr} to bind to the ribosome (10). Particularly germane to the *B. subtilis* system is the evidence that there are changes in the ribosome and in the translational apparatus during development. The function of the alterations in anticodon loop modifications in tRNA's might be to control the selectivity of codon-anticodon interaction during development.

In vitro, tRNA$_1^{Lys}$ and tRNA$_3^{Lys}$ wobble be-

TABLE 4. *Elution properties of tRNA's from exponentially growing cells or spores on RPC-5*

No change in chromatographic profile	Change in relative amounts of isoaccepting species	Appearance of a unique species
Phenylalanyl-tRNA's[a]	Tyrosyl-tRNA's[a]	Tryptophanyl-tRNA[a]
Isoleucyl-tRNA's[b]	Leucyl-tRNA's[a]	
Methionyl-tRNA[b]	Seryl-tRNA's[a, b]	
Formylmethionyl-tRNA	Lysyl-tRNA's[b]	
Valyl-tRNA's	Threonyl-tRNA's[b]	
Alanyl-tRNA	Asparaginyl-tRNA's[b]	
Aspartyl-tRNA	Glutamyl-tRNA's[b]	
Histidinyl-tRNA's	Glycyl-tRNA's	
Prolyl-tRNA's	Arginyl-tRNA's	

[a] *B. subtilis* tRNA's that contain i^{6}A or a similar cytokinin-active nucleoside (31).

[b] tRNA's that contain t^{6}A in *E. coli* (18). The *B. subtilis* tRNA's that contain t^{6}A have not been characterized except for threonine tRNA (32). Lysine tRNA$_1$ from *B. subtilis* contains K in the place of t^{6}A (33).

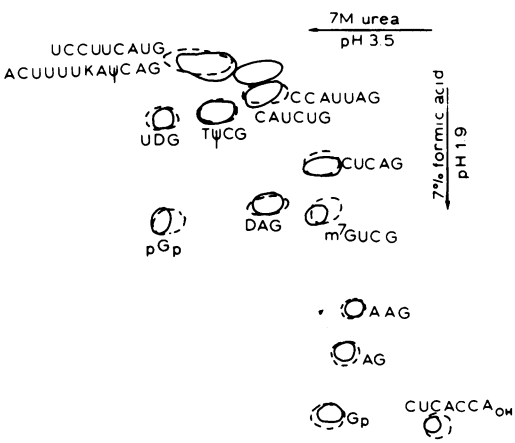

FIG. 4. *Two-dimensional electrophoretic separation of the oligonucleotides from an RNase T1 digest of lysine tRNA's. ^{32}P-labeled tRNA$_3^{Lys}$ and nonradioactive tRNA$_1^{Lys}$ were codigested and chromatographed together. Solid lines represent areas containing radioactivity; dashed lines represent areas absorbing in the UV. The chromatogram was made in the laboratory of H. Ishikura. The composition of nucleotides was determined by Yamada and Ishikura (33).*

tween AAA and AAG but differ in codon preference (30). The amount of the variation in in vitro studies did not suggest that there was much difference between the two lysine species; however, in vivo studies implied that there was a significant difference in cellular physiology. The in vivo studies suggested that the $tRNA_1^{Lys}$ is preferentially bound to polyribosomes beyond what would be expected by simple mass action, and the utilization of $tRNA_3^{Lys}$ was diminished in stationary phase (26). Clearly, further studies on the system in vitro, preferably with natural mRNA and ribosomes from different growth stages, will be worth pursuing.

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