Kaltschmidt, E., and Wittmann, H. G. (1972), *Biochimie 54*, 167.
Michalski, C. J., Sells, B. H., and Morrison, M. (1973), *Eur. J. Biochem. 33*, 481.
Moore, P. (1971), *J. Mol. Biol. 60*, 169.
Mora, G., Donner, D., Thammana, P., Lutter, L., and Kurland, C. G. (1971), *Mol. Gen. Genet. 112*, 229.
Morrison, M., Bayse, G., and Donner, D. J. (1970), *in* Biochemistry of the Phagocytic Process, Schultz, J., Ed., Amsterdam, North-Holland Publishing Co., p 51.
Morrison, M., Hamilton, H. B., and Stotz, E. (1957), *J. Biol. Chem. 228*, 767.
Morrison, M., and Hultquist, D. E. (1963), *J. Biol. Chem. 238*, 2843.
Nirenberg, M. W. (1963), *Methods Enzymol. 6*, 17.
Pellegrini, M., Oen, H., and Cantor, C. R. (1972), *Proc. Nat. Acad. Sci. U. S. 69*, 837.
Phillips, D. R., and Morrison, M. (1971), *Biochemistry 10*, 1766.
Traub, P., Mizushima, S., Lowry, C. V., and Nomura, M. (1971), *Methods Enzymol. 20*, 391.

Oligonucleotide Composition of a Yeast Lysine Transfer Ribonucleic Acid†

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ABSTRACT: The purification of $tRNA_2^{Lys}$ from baker's yeast and the identification of the products of complete digestion with pancreatic ribonuclease and ribonuclease T1 are described. Seventeen modified nucleoside residues were found, including a 5′-terminal pseudouridine and 2-thio-5-carboxymethyluridine methyl ester in the first position of the anticodon. An alternative form of the latter nucleoside was found after purification of the tRNA. The alternative form of this nucleoside apparently was produced during isolation of the tRNA.

The nucleotide sequence of one of the two lysine transfer ribonucleic acids in baker's yeast has been reported (Madison *et al.*, 1972). The purification of the $tRNA^{Lys}$ and the isolation and determination of the nucleotide sequences of the oligonucleotides produced by complete digestion with RNase I and RNase T1 are reported here. The isolation of large fragments and reconstruction of the nucleotide sequence are described in the following paper (Madison and Boguslawski, 1974).

Materials and Methods

$tRNA_2^{Lys}$ Purification. Bulk yeast tRNA was extracted from commercial baker's yeast (Fleischmann's yeast) as described by Holley (1963). Countercurrent distribution of the crude tRNA was carried out with the ammonium sulfate system (Kirby, 1960) as described by Doctor (1967). Up to 12 g of bulk tRNA was dissolved in 800 ml of the ammonium sulfate system and distributed for 225 transfers at 25 ± 1°. The faster moving $tRNA^{Lys}$ peak was removed from the countercurrent system with $CetMe_3NBr$[1] (Eastman, technical) as previously described (Madison *et al.*, 1967), except that eight tubes from the countercurrent were combined (640 ml), and $CetMe_3NBr$ equal to about ten times the weight of RNA present was added. After the addition of diethyl ether, the gelatinous RNA–$CetMe_3NBr$ complex was collected in a 40-ml tube and centrifuged. As much as possible of the ether and aqueous layers was removed with a Pasteur pipet. The complex was dissociated with 10–20 ml 2 M NaCl and the RNA precipitated with 3 vol of 95% ethanol. The RNA was dissolved in water and reprecipitated with ethanol to remove residual NaCl and $CetMe_3NBr$.

$tNRA_2^{Lys}$ was further purified by reverse-phase chromatography (Weiss and Kelmers, 1967). About 250 mg of RNA from countercurrent distribution was absorbed on a 2.6 × 100 cm column of Chromosorb W (dimethylchlorosilane treated, acid washed, 100–120 mesh, obtained from Applied Science, Inc.[2]), treated with Aliquat 336. The RNA was eluted with a concave gradient made from 2 l. of 0.01 M $MgCl_2$, 0.01 M sodium acetate (pH 5.0), and 0.23 M NaCl in the mixing chamber and 2 l. of 0.4 M NaCl containing 0.01 M $MgCl_2$ and 0.01 M sodium acetate (pH 5). The columns were run at room temperature. The tubes could be assayed directly for lysine acceptor activity (Madison *et al.*, 1967). The peaks of $tRNA^{Lys}$ were combined, enough $CetMe_3NBr$ was added to make the $CetMe_3NBr$:RNA ratio about 150, and the RNA was isolated as above.

Analyses. Nuclease digestions, isolation of oligonucleotides, and analysis of nucleotide composition have all been described (Madison *et al.*, 1967) except that the chromatography on DEAE-cellulose in 7 M urea was done in the presence of 0.02 M morpholinopropanesulfonic acid (pH 7.0). The columns were run at room temperature with a flow rate of about 15 ml/hr. Two-dimensional thin-layer chromatography on Avicel plates (Analtech, Wilmington, Del.) using iso-

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[1] Abbreviations used are: $CetMe_3NBr$, hexadecyltrimethylammonium bromide; mcm^5S, 2-thio-5-carboxymethyluridine methyl ester (can also be named 2-thiouridine-5-acetic acid methyl ester); mcm^5S^*, modified form of mcm^5S; t^6A, *N*-[9-(β-D-ribofuranosyl)purin-6-ylcarbamoyl]-L-threonine (can also be named threonylcarbamoyladenosine); N*, unknown compound whose spectra is shown in Figure 8; RNase I, bovine pancreatic ribonuclease.

[2] Mention of a trademark or company name does not constitute a guarantee or warranty of the product by the U. S. Department of Agriculture.

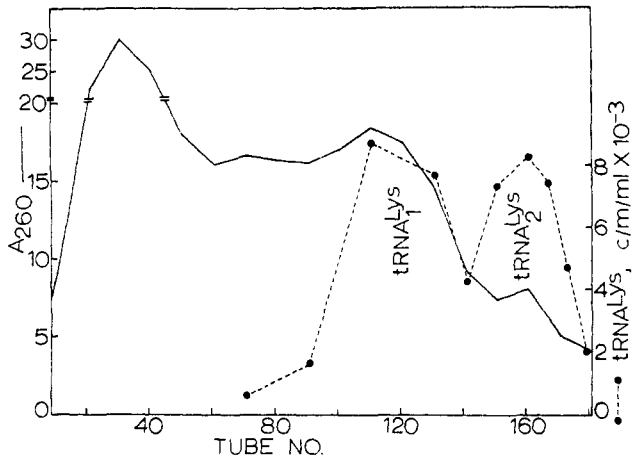

FIGURE 1: Countercurrent distribution of 12 g of yeast tRNA in the ammonium sulfate system showing the two peaks of lysine acceptor activity. Details are given under Materials and Methods.

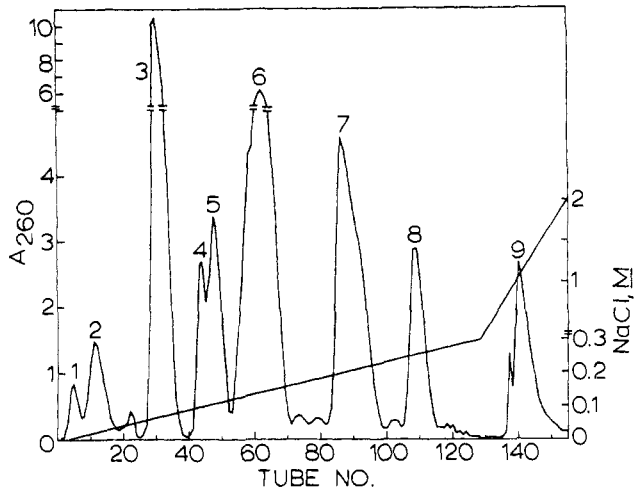

FIGURE 3: Chromatography of RNase I digestion products of purified tRNA$_2^{Lys}$ on a 0.35 × 25 cm column of DEAE-cellulose in 7 M urea. Each tube contained 1.8 ml.

butyric acid–concentrated NH_4OH–H_2O (66:1:33) followed by isopropyl alcohol–concentrated HCl–H_2O (68:16.4:15.6) was used instead of paper chromatography.

Iodine Oxidation. mcm^{5}S was treated with iodine as described by Carbon *et al.* (1965) except that the pH had to be raised to 7.5–8.0. The excess I_2 was extracted with chloroform and KI separated by gel filtration on a 1 × 62 cm column of Sephadex G-10 in 0.1 M ammonium bicarbonate.

Results

A typical countercurrent distribution pattern is shown in Figure 1. tRNA$_2^{Lys}$ was purified about fivefold, while tRNA$_1^{Lys}$ was about fourfold enriched. Attempts to further purify tRNA$_1^{Lys}$ (the slower moving peak) by reverse-phase chromatography were not successful. An example of the results of a reverse-phase column are shown in Figure 2. tRNA$_2^{Lys}$ was purified an additional sixfold to an estimated purity of about 90%. Only traces of phenylalanine, asparagine, threonine, and histidine acceptor activity and no activity for isoleucine, tyrosine, aspartic acid, glutamine, and arginine were found in the purified tRNA$_2^{Lys}$ fractions.

The pattern observed after chromatography on DEAE-cellulose in 7 M urea of RNase I digestion of tRNA$_2^{Lys}$ is shown in Figure 3. The last peak could not be eluted with NaCl, but was eluted with a second gradient made from 0.3 M NaCl–7 M urea–0.02 M morpholinopropanesulfonic acid (pH 7.0) in the mixing chamber and 2.0 M NaCl–0.1 N HCl–7 M urea in the reservoir.

The sequences assigned to the RNase I oligonucleotides are shown in Table I along with the evidence by which the sequence was determined. Most of the peaks from Figure 3 could be separated into pure oligonucleotides by chromatography on DEAE-cellulose in 7 M urea containing 0.1 M formic acid (Rushizky and Sober, 1964). The only fragments that were not separated by chromatography in urea–formic acid were the dinucleotides m_2^2G-ψp and mcm^{5}S*-Up and the trinucleotides G-G-Cp, t^{6}A-A-Cp, and pψp. Thin-layer chromatography was used to separate these components.

The sequence of the hexanucleotide, A-G-G-G-G-Tp, was very difficult to define. This hexanucleotide was not eluted from the column with NaCl but was eluted when HCl was added to the reservoir. This fragment was also very resistant to nuclease digestion. The results shown in Table I were obtained after an overnight digestion with snake venom phosphodiesterase. Even after this long period of digestion, more than half of the starting material had not been hydrolyzed. The fact that all the adenine recovered was in the form of adenosine, no pA was detected, while 70% of the guanine was in the form of pG is evidence that the one Ap in the molecule is at the 5′ terminus. Tp must be at the 3′ terminus since it is the only pyrimidine present.

The oligonucleotides produced by RNase T1 digestion of tRNA$_2^{Lys}$ are shown in Figure 4. The oligonucleotides from Figure 4 were chromatographed on DEAE-cellulose in urea–

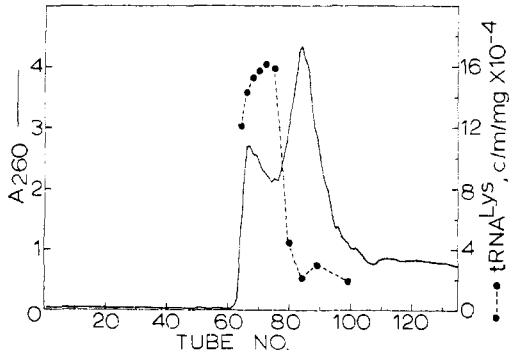

FIGURE 2: Reverse-phase chromatography of the tRNA$_2^{Lys}$ peak from Figure 1. Details are given under Materials and Methods.

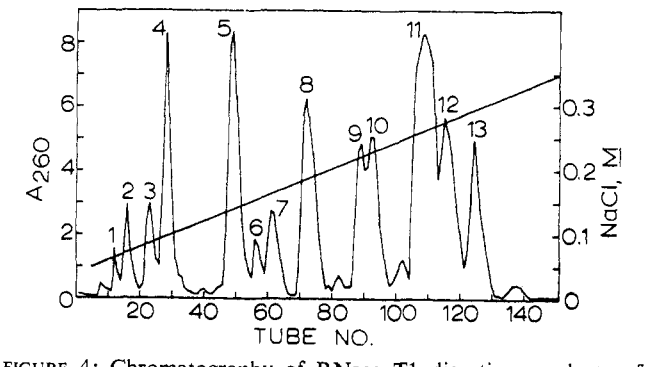

FIGURE 4: Chromatography of RNase T1 digestion products of tRNA$_2^{Lys}$ on a 0.35 × 120 cm column of DEAE-cellulose in 7 M urea. Each tube contained 1.8 ml.

TABLE I: Products of Ribonuclease I Digestion of $tRNA_2^{Lys}$.

Peak No. (Fig. 3)	mol/mol of tRNA	Product	Basis for Structure Assignment[a]
1		C_{OH}	CpC_{OH} found in RNase T1 digest
2	1–3	C>p, U>p	A, D, B: Cp, Up
3	10.6	Cp	D, B
3	7.1	Up	D, B
3	0.9[b]	ψp	D, B
3	1.4[c]	m^5Cp	D, B
6	1	pψp	J, D, B: ψ_{OH}
3	1.06	m^7G-Dp + m^7G-Up	G, D, B, C: m^7Gp, 1; Up, 0–0.3; D, 0.7–1.0
4	1	mcm^5S^*-Up	G, D, B: mcm^5S^*p, 1; Up, 1 (assuming that the extinction coeff of mcm^5S^* is the same as that of mcm^5S)
4	1	m_2^2G-ψp	A, D, B: m_2^2Gp, 1.2; ψp, 1
5	1	A-ψp	A, D, B: Ap, 1; ψp, 1
5	1	G-Up	A, D, B: Gp, 0.8; Up, 1
6	1	A-m^2G-Cp	A, D, B: Ap, 1.1; m^2Gp, 1; Cp, 1 F, L: A-m^2Gp, 0.6; Cp, 1
6	1	G-G-Cp	A, D, B: Gp, 1.9; Cp, 1
6	1	t^6A-A-Cp	G, D, B: t^6Ap, 1.1; Ap, 1.2; Cp, 1 H, D: 5′-terminal t^6A_{OH} Acid hydrolysis of t^6Ap produced Thr and traces of several other ninhydrin positive spots after paper chromatography
6	1	A-G-Dp	C, A, D, B: Ap, 1.1; Gp, 1.1; Dp, 0.8 F, L: A-Gp
6	1	G-m^1A-G-Cp	A, D, B: Gp, 2.1; m^6Ap, 0.9; Cp, 1 H, D: 5′-terminal G_{OH} F, L: m^1A-Gp, Gp, Cp Presence of m^1Ap inferred since tetranucleotide chromatographed with trinucleotides and dinucleotide, m^1A-Gp, with mononucleotides
7	1	A-G-A-G-Cp	A, D, B: Ap, 2; Gp, 2.2; Cp, 1 F, L: A-Gp, 1.6; Cp, 1
7	1	G-A-A-A-Up	A, D, B: Gp, 1.2; Ap, 2.9; Up, 1 F, L: A-A-A-Up, Gp
9	1	A-G-G-G-G-Tp	A, D, B: Ap, 1; Gp, 4.3; Tp, 1.1 H, D: A_{OH}, 0.8; G_{OH}, 1.2; pG, 2.8
8	1	G-A-G-G-A-G-Cp	A, D, B: Gp, 4; Ap, 2; Cp, 1 H, D: 5′-terminal G_{OH} F, L: A-Gp, 2; Gp, 2; Cp, 1 I, L: G-A-G_{OH}, G-A-G-G-$_{OH}$

[a] Methods used for the sequence assignment: A, alkaline hydrolysis; B, ultraviolet spectra at pH 2, 7, and 13. For a summary of ultraviolet spectra of nucleotides see Hall (1971). The numbers following the nucleotide symbols are molar ratios; C, decrease in A_{230} in 0.1 N KOH (Batt *et al.*, 1954); D, two-dimensional thin-layer or paper chromatography; F, RNase T1 digestion; G, RNase T2 digestion, 100 units of enzyme (Calbiochem) in 0.2 ml of 0.05 M ammonium acetate (pH 5.5), 37°, 1–2 hr; H, complete snake venom phosphodiesterase digestion of oligonucleotide with 3′-phosphate, 0.02 mg of enzyme (Boehringer Mannheim) in 0.2 ml of 0.05 M Tris (pH 7.5)–0.025 M $MgCl_2$, 37°, 3–4 hr; I, alkaline phosphatase followed by partial snake venom phosphodiesterase digestion; J, bacterial alkaline phosphatase digestion; L, chromatography on DEAE-cellulose in 7 M urea (pH 7.0). Pyrimidine must be the 3′ terminus because of the specificity of RNase I. [b] Expected 2.0 mol/mol of tRNA. [c] Expected 1.0 mol/mol of tRNA.

formic acid (Rushizky and Sober, 1964) to check for homogeneity. Only peaks 11 and 12 were separated into more than one component by this procedure. The two oligonucleotides in peak 8 were separated by thin-layer chromatography with the isobutyric acid–ammonium hydroxide system. Peak 3 was separated into two components by chromatography on a 0.35 × 30 cm column of DEAE-cellulose and eluting with a 0–0.1 M gradient of ammonium bicarbonate. Sephadex G-10 was used to separate the components in peak 1. The evidence on which the nucleotide sequences of the oligonucleotides were assigned is given in Table II. The only RNase T1 oligonucleotide for which the sequence assignment was not completely straightforward was C-C-C-C-C-U-A-ψ-Gp. Partial digestion with snake venom or polynucleotide phosphorylase did not give all the possible intermediate sized fragments. In addition, polynucleotide phosphorylase produced the frag-

TABLE II: Products of Ribonuclease T1 Digestion of $tRNA_2^{Lys}$.

Peak No. (Fig. 4)	mol/mol of tRNA	Product	Basis for Structure Assignment[a]
1	0.3	CpC_{OH}	E, D, B: Cp, 1; C_{OH}, 0.9
1	?	N*	Structure unknown, see text
2	1–2[b]	G>p	A, D, B: Gp
3	<0.5[c]	C-m_2^2G>p	A, D, B: m_2^2Gp, 1; Cp, 1 Cyclic phosphate assumed since dinucleotide eluted before Gp
3	1	m^1A-Gp	A, D, B: m^6Ap, 0.9; Gp, 1 Presence of m^1A inferred since dinucleotide eluted before Gp
4	6[b]	Gp	D, B
5	3	A-Gp	A, D, B: Ap, 1; Gp, 1
6	1	D-D-Gp	C, A, D, B: Dp, 1.4; Gp, 1
7	1	D-A-Gp	C, A, D, B: Dp, 0.7; Ap, 0.9; Gp, 1 E, L: A-Gp
8	1	T-ψ-C-Gp	A, D, B: Tp, 1.2; ψp, 1; Cp, 1.2; Gp, 1 K, L: T-ψ-C_{OH}; Tp must be 5′-terminal nucleotide since it is found in A-G-G-G-G-Tp in RNase A digest
8	1	U-U-A-m^2Gp	A, D, B: Up, 2.1; Ap, 1; m^2Gp, 1 E, L: Up, 2.2; A-m^2Gp, 1
8	<0.5[c]	ψ-ψ-C-Gp	Could not be separated from T-ψ-C-Gp; sequence determined as part of peak 10
9	1	C-U-C-A-Gp	A, D, B: Cp, 2; Up, 1.2; Ap, 1; Gp, 1 E, L: Cp, 2.1; Up, 1; A-Gp, 1 H, D: 5′-terminal C_{OH} I, L: C-U-C_{OH}
10	1	C-m_2^2G-ψ-ψ-C-Gp	A, D, B: Cp, 2.1; m_2^2Gp, 1.2; ψp, 1.9; Gp, 1 H, D: 5′-terminal C_{OH} I, L: C-m_2^2G-ψ_{OH} K, L: C-m_2^2G-ψ-ψ_{OH} and C-m_2^2G-ψ-ψ-C_{OH}
11	1	pψ-C-C-U-U-Gp (U)	A, D, B: pψp, 0.9; Cp, 2.2; Up, 2.3; Gp, 1 I, L: ψ-C-C-U_{OH}, ψ-C-C-U-U_{OH} J, A, D, B: ψp, Cp, Up, G_{OH}
11	1	A-A-A-U-m^7G-D-m^5C-A-Gp	A, D, B: Ap, 4; Up, 1.6; m^5Cp, 1; Gp, 1 C: Dp, 0.4–1 (measured on m^7G-Dp formed by RNase I) E, L: A-A-A-Up, m^7G-Dp, m^5Cp, A-Gp H, D: 5′-terminal A_{OH} K, L, G, D: A-A-A-U-m^7G_{OH}, A-A-A-U-m^7G-D_{OH} (D_{OH} assumed, no nucleoside detected), A-A-A-U-m^7G-D-m^5C_{OH}, A-A-A-U-m^7G-D-m^5C-A_{OH}
12	1	C-C-C-C-C-U-A-ψ-Gp	A, D, B: Cp, 5; Up, 1.3; ψp, 0.8; Ap, 1.3; Gp, 1 E, L: A-ψp, Cp, Up, Gp K, L: A-ψ-G_{OH}[d] plus C-C-C_{OH}, C-C-C-C_{OH}, C-C-C-C-C_{OH} H, L: pUp formed from C-C-C-C-C-Up[d]
13	1	C-U-mcm^{5}S*-U-U-t^{6}A-A-C-C-Gp	A, D, B: Cp, 3.2; Up, 3.1; Ap, 1.9; Gp, 1 H, D: 5′-terminal C_{OH} E, L: mcm^{5}S*-Up, t^{6}A-A-Cp, Up, Cp, Gp K, L, G: C-U-mcm$^5S^*_{OH}$, C-U-mcm^{5}S*-U_{OH}, C-U-mcm^{5}S*-U-U_{OH}, C-U-mcm^{5}S*-U-U-t^6A_{OH} I, L, G: C-U-mcm^{5}S*-U-U-t^{6}A-A-C_{OH}, C-U-mcm^{5}S*-U-U-t^{6}A-A-C-C_{OH}

[a] Methods used for the sequence assignment, see footnote *a* to Table I: E, RNase I digestion; K, alkaline phosphatase digestion followed by polynucleotide phorphorylase digestion. Gp or a modified G must be present at the 3′ terminus because of specificity of RNase T1. [b] G>p plus Gp should be 6 mol/mol of tRNA. [c] Also found as part of C-m_2^2G-ψ-ψ-C-Gp. [d] Anomalous cleavage product of polynucleotide phosphorylase.

ments A-ψ-G_{OH} and C-C-C-C-C-Up, which could have resulted from an anomalous cleavage by polynucleotide phosphorylase or from the action of a contaminating nuclease. The sequence given is the only one that fits all the data.

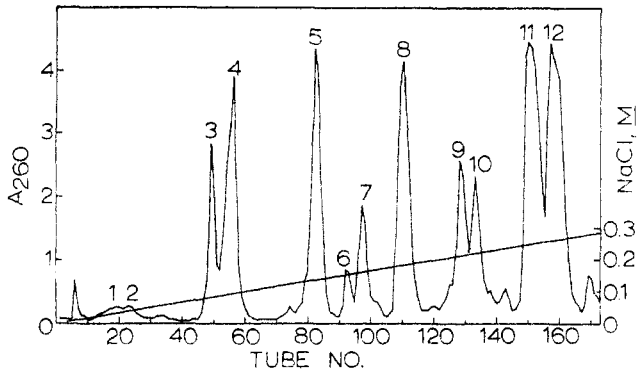

FIGURE 5: Alternative chromatographic pattern of RNase T1 digestion products of $tRNA_2^{Lys}$ on DEAE-cellulose in 7 M urea.

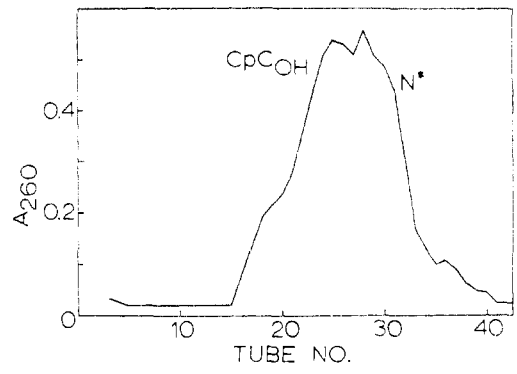

FIGURE 7: Partial separation of CpC_{OH} and an unknown component (N*) by gel filtration. Peak 1 of Figure 4 chromatographed on a 1 × 62 cm column of Sephadex G-10 in water.

In Figure 4, the decanucleotide C-U-mcm^{5}S*U-U-t^{6}A-A-C-C-Gp was found in both peaks 11 and 13. The decanucleotide from both peaks 11 and 13 was digested with RNase I and the trinucleotide t^6-A-A-Cp isolated. The trinucleotide was digested with snake venom phosphodiesterase and the nucleoside t^{6}A isolated by thin-layer chromatography. Paper electrophoresis at pH 7.0 showed that the t^{6}A isolated from peak 11 had no charge and the t^{6}A isolated from peak 13 had a negative charge.

In contrast to the pattern shown in Figure 4, the pattern in Figure 5 was often obtained. In Figure 5, the decanucleotide found in peak 13 of Figure 4 was present with the nonanucleotide C-C-C-C-C-U-A-ψ-Gp in peak 12. Since t^{6}A from the decanucleotide found in peak 12 or peak 13 had a negative charge, a difference in the charge on t^{6}A could not account for the decanucleotide being found in peak 12 or peak 13. It seems more likely that the variation in the chromatographic behavior of the decanucleotide was the result of differences in mcm^{5}S* (or mcm^{5}S). This residue was particularly unstable and subtle changes in its structure may have been responsible for some of the variability in the chromatographic pattern. The spectra of mcm^{5}S* are shown in Figure 6. Both mcm^{5}S* and mcm^{5}S have been detected in both peaks 12 and 13, so some further modification (other than that which is responsible for the change in spectrum) would have to be invoked to account for the chromatographic variations. Cleavage of the ester groups of mcm^{5}S*, which would form a free carboxy group, could explain the retardation of the decanucleotide. mcm^{5}S* (or mcm^{5}S) from peak 13 did not have a free carboxyl. The free carboxy group, if present, could have been detected when the nucleotides were separated by thin-layer chromatography. Surprisingly, no comparable variation in the elution properties of mcm^{5}S*-Up in the RNase I digest was seen.

The possibility that mcm^{5}S* was an oxidation product of mcm^{5}S was considered. The similarities in the spectra of mcm^{5}S* (Figure 6A) and iodine-oxidized mcm^{5}S (Figure 6B) are consistent with this possibility. Treatment of mcm^{5}S with iodine, however, produced about six different components upon thin-layer chromatography. Efforts to reduce mcm^{5}S* to mcm^{5}S were completely unsuccessful. Normally, alkaline hydrolysis of mcm^{5}S*, or hydrolysis of oligonucleotides containing mcm^{5}S*, completely destroyed mcm^{5}S*, since ultraviolet absorbing spots were not detected after thin-layer chromatography. On two occasions small quantities (less than 50%) of 2-thio-5-carboxymethyluridine (mcm^{5}S minus methanol) were detected after alkaline hydrolysis of an oligonucleotide containing mcm^{5}S*. Modifications of t^{6}A and mcm^{5}S (or mcm^{5}S*) apparently were responsible for the observation that the decanucleotide C-U-mcm^{5}S*-U-U-t^{6}A-A-C-C-Gp was found to chromatograph in peaks 11, 12, and 13.

Degradation of mcm^{5}S may also have been responsible for the presence of another unknown component (N*) found in peak 1 of Figure 4. N* could be partially separated from CpC_{OH} by gel filtration on Sephadex G-10 as shown in Figure 7. The first peak contained primarily CpC_{OH} and the second peak primarily the material whose spectrum is shown in Figure 8. Oxidation of mcm^{5}S with I_2 produced a compound with a spectrum identical with that shown in Figure 8 as well as several other compounds.

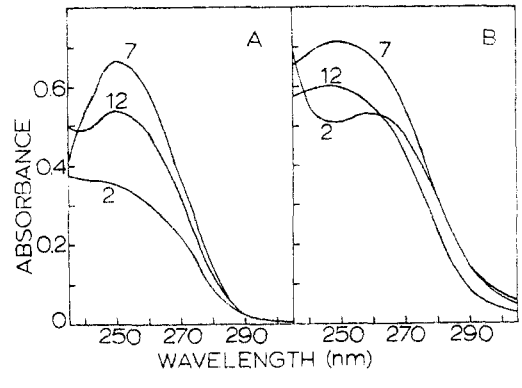

FIGURE 6: Ultraviolet absorption spectra of (A) mcm^{5}S* and (B) mcm^{5}S oxidized with I_2.

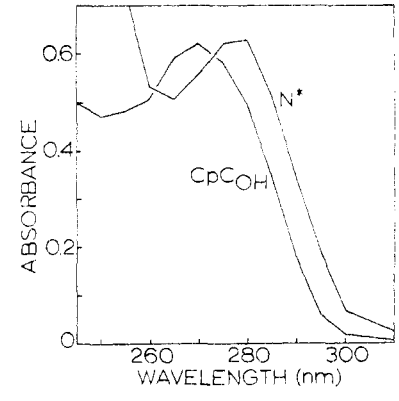

FIGURE 8: Ultraviolet absorption spectra of tubes 25 (CpC_{OH}) and 28 (N*) from Figure 7. Spectra of N* was the same at pH 2, 7, and 12.

Discussion

The nucleotide sequence assignment of A-G-G-G-G-Tp was made very difficult since this oligonucleotide was resistant to enzymic digestion. Apparently four Gp residues in a row are sufficient to cause severe aggregation of oligonucleotides as small as hexanucleotides. Katz and Dudock (1969) found similar aggregation of the hexanucleotide G-G-G-G-A-Up.

All the RNase I oligonucleotides were present in amounts equal to 1 mol/mol of RNA. The amount of ψp found in the mononucleotide region was consistently lower than expected. Since two residues of ψp were found in RNase I digests of the oligonucleotides produced by RNase T1, two is undoubtedly the correct figure.

The only part of the sequence determination of the RNase T1 oligonucleotides that was difficult was the determination of the number of Cp residues present in C-C-C-C-C-U-A-ψ-Gp. It was hard to be sure that there were exactly five residues of Cp present. From the data in Table II it can be seen that if the base composition were calculated using Ap or Up as equal to 1.0, there would be 3.84 residues of Cp calculated to be present, while the Cp:ψp ratio is 6.25:1.00. We were unable to find conditions in which partial digestion with either snake venom phosphodiesterase or polynucleotide phosphorylase produced all the intermediate-sized fragments.

There was some variation in the amount of m^7G-Dp and m^7G-Up found. The amount of Up varied from zero to about 0.3 residue. This most likely represents incomplete modification of the Up at this position. Yeast $tRNA^{Ala}$ also has some Up and some Dp at a comparable location (Holley *et al.*, 1965).

Essentially all of the RNase T1 oligonucleotides were also present in 1:1 ratios except for A-Gp, where 3 mol were found per mol of RNA. Less than equimolar amounts of Cp_{OH} and C-m_2^2G>p were found. The small and variable amount of C-m_2^2G>p found probably arose because C-m_2^2G-ψ-ψ-C-Gp was only partially hydrolyzed. The very poor recovery of Cp_{OH} is hard to explain. It is possible that part of the penultimate pC, as well as the terminal pA residue, is missing from the tRNA as it is isolated, since the addition of CTP to assay mixtures occasionally increased the lysine acceptor activity of the purified tRNA by as much as 30%. No cytidine was detected after RNase T1 digests, however. Nor was a nucleoside found in any other fragment except Cp_{OH}. When $tRNA_2^{Lys}$ was incubated with a yeast tRNA nucleotidyl transferase preparation and [^{14}C]ATP, all the radioactivity that was incorporated was found in peak 3 (Figure 4) after digestion with RNase T1. This peaks contains components with two negative charges, so it would contain C-C-A_{OH}. There is no evidence for any other terminus than G-C-C-A_{OH}.

The nature of mcm^5S^* is unknown and seems likely to be an oxidation product of mcm^5S. mcm^5S^* cannot be a disulfide because if it was, mcm^5S^*-Up formed by RNase I digestion would have six negative charges at pH 7.0 and would chromatograph with the pentanucleotides. mcm^5S^*-Up is found in the dinucleotide region, so mcm^5S^* cannot be a disulfide. mcm^5S^* could also be the result of a photochemical reaction similar to the product formed from s^4U (Favre *et al.*, 1971), but the group attached to mcm^5S cannot be another nucleotide.

All of the evidence suggests that mcm^5S^* is formed from mcm^5S during purification of $tRNA^{Lys}$. mcm^5S was found in bulk yeast tRNA, while the $tRNA_2^{Lys}$ peak after countercurrent distribution contained both mcm^5S and mcm^5S^*. Two peaks of lysine acceptor activity always occurred after reverse-phase chromatography. We studied the faster eluting peak because it was always purified to a much greater extent than the second peak. The relative amounts of the two peaks varied greatly; sometimes the majority of the lysine acceptor activity was found in the second peak. The second peak, even though not pure, was found to contain equimolar amounts of mcm^5S-Up and pψp. mcm^5S apparently was converted to mcm^5S^* during purification probably during countercurrent distribution. Mercaptoethanol (3 mM) added to the solvents during reverse-phase chromatography and during the separation of the fragments in 7 M urea did not prevent the appearance of mcm^5S^*. Yoshida *et al.* (1971) could prevent the destruction of mcm^5S in $tRNA^{Glu}$ by adding 3 mM mercaptoethanol when nuclease digests were chromatographed in 7 M urea.

t^6A was present in two forms. The form that does not move during electrophoresis at pH 7.0 most likely has the carboxy group of threonine esterified, while the form that moves toward the anode has a free carboxy group. At least 75% of the t^6A was found to have a negative charge, so that either the majority of these residues are unmodified or most of the ester bonds are hydrolyzed during purification of tRNA.

References

Batt, R. D., Martin, J. K., Ploeser, J. M., and Murray, J. (1954), *J. Amer. Chem. Soc. 76*, 3663.

Carbon, J. A., Hung, L., and Jones, D. S. (1965), *Proc. Nat. Acad. Sci. U. S. 53*, 979.

Doctor, B. P. (1967), *Methods Enzymol. 12A*, 648.

Favre, A., Michelson, A. M., and Yaniv, M. (1971), *J. Mol. Biol. 58*, 367.

Hall, R. H. (1971), The Modified Nucleosides in Nucleic Acids, New York and London, Columbia University Press.

Holley, R. W. (1963), *Biochem. Biophys. Res. Commun. 10*, 186.

Holley, R. W., Everett, G. A., Madison, J. T., and Zamir, A. (1965), *J. Biol. Chem. 240*, 2122.

Katz, G., and Dudock, B. S. (1969), *J. Biol. Chem. 244*, 3062.

Kirby, K. S. (1960), *Biochim. Biophys. Acta 41*, 338.

Madison, J. T., and Boguslawski, S. J. (1974), *Biochemistry 13*, 527.

Madison, J. T., Boguslawski, S. J., and Teetor, G. H. (1972), *Science 176*, 687.

Madison, J. T., Everett, G. A., and Kung, H. K. (1967), *J. Biol. Chem. 242*, 1318.

Rushizky, G. W., and Sober, H. A. (1964), *Biochem. Biophys. Res. Commun. 14*, 276.

Weiss, J. F., and Kelmers, A. D. (1967), *Biochemistry 6*, 2507.

Yoshida, M., Takeishi, K., and Ukita, T. (1971), *Biochim. Biophys. Acta 228*, 153.