Hartman, F. C. (1970), *Biochemistry 9*, 1776.
Kapoulas, V. M., and Thompson, G. A. (1969), *Biochem. Biophys. Acta 187*, 594.
Patterson, M. S., and Greene, R. C. (1965), *Anal. Chem. 37*, 854.
Rieder, S. V., and Rose, I. A. (1959), *J. Biol. Chem. 234*, 1007.
Rose, I. A., and Rieder, S. V. (1958), *J. Biol. Chem. 231*, 315.
Snyder, F., Blank, M. L., Malone, B., and Wykle, R. L. (1970a), *J. Biol. Chem. 245*, 1800.
Snyder, F., Malone, B., and Blank, M. L. (1970b), *J. Biol. Chem. 245*, 1790.
Snyder, F., Rainey, W. T., Jr., Blank, M. L., and Christie, W. H. (1970c), *J. Biol. Chem. 245*, 5853.
Thompson, G. A., Jr., and Hanahan, D. J. (1963), *Biochemistry 2*, 641.
Wykle, R. L., and Snyder, F. (1970), *J. Biol. Chem. 245*, 3047.

Possible Anticodon Sequences of $tRNA^{His}$, $tRNA^{Asn}$, and $tRNA^{Asp}$ from *Escherichia coli* B. Universal Presence of Nucleoside Q in the First Position of the Anticondons of These Transfer Ribonucleic Acids*

Fumio Harada and Susumu Nishimura

ABSTRACT: An unidentified nucleoside, Q, was isolated from *Escherichia coli* $tRNA_1^{His}$, $tRNA^{Asn}$, and $tRNA_1^{Asp}$ as well as from $tRNA^{Tyr}$. The nucleotide sequences of oligonucleotides containing Q, obtained by RNase T_1 digestion of these three tRNAs, were determined by conventional techniques. The sequences of these oligonucleotides are A-U-U-Q-U-Gp for $tRNA_1^{His}$, A-C-U-Q-U-U-N*-A-ψ-C-C-Gp for $tRNA^{Asn}$, and C-C-U-Q-U-C-m^2A-C-Gp for $tRNA_1^{Asp}$. From these results and other supporting evidence, Q seems to be located in the first position of the anticodons of all these tRNAs. Q was not found in other *E. coli* tRNAs. Thus there is a strict relation between the presence of Q and codon recognition of *E. coli* tRNA. *E. coli* tRNAs which recognize U and C in the third position and A in the second position of code words always contain Q. It was shown that Q has more affinity for U than for C in codon–anticodon base pairing, since among the trinucleotides corresponding to code words, those ending with U always cause most stimulation of the binding of tRNAs containing Q to ribosomes.

Elucidation of the primary sequences of numerous tRNAs has clearly established the cloverleaf structure of tRNA molecule which was first proposed by Holley *et al.* (1965a). One of the most important contributions of this model is the establishment of the anticodon of tRNA which pairs with the codon of mRNA. As proposed first by Crick (1966a) and Nirenberg *et al.* (1966), the first nucleotide in the anticodon has specific wobbling characteristics to recognize multiple codons. The wobble theory (Crick, 1966b), proposed to explain the mechanism of recognition of the third letter of the codon, postulated that G in the first position of the anticodon will pair with U or C in the codon. In fact, elucidation of the primary sequences of tRNAs which recognize XY_C^U-type codons, such as yeast $tRNA^{Tyr}$ (Madison *et al.*, 1966; Madison and Kung, 1967), yeast $tRNA^{Phe}$ (RajBhandary *et al.*, 1967; RajBhandary and Chang, 1968), yeast $tRNA^{Asp}$ (Gangloff *et al.*, 1971), Torulopsis $tRNA^{Tyr}$ (Hashimoto *et al.*, 1969), wheat germ $tRNA^{Phe}$ (Dudock *et al.*, 1969; Dudock and Katz, 1969), *Escherichia coli* $tRNA^{Phe}$ (Barrell and Sanger, 1969), *E. coli* $tRNA_2^{Leu}$ (Blank and Söll, 1971), *E. coli* $tRNA^{Ile}$ (Yarus and Barrell, 1971), *E. coli* $tRNA_3^{Gly}$ (Squires and Carbon, 1971), *E. coli* $tRNA_2^{Val}$ (Yaniv and Barrell, 1971), and *E. coli* $tRNA_3^{Ser}$ (H. Ishikura, Y. Yamada, and S. Nishimura, unpublished results), actually showed that the first letter of the anticodons of these tRNAs were occupied either by G or 2′-*O*-methylguanosine, and proved the wobble theory. However, in the case of *E. coli* $tRNA^{Tyr}$, the first nucleoside of the anticodon is not normal guanosine, but modified guanosine designated as either Q^1 (RajBhandary *et al.*, 1969), G* (Goodman *et al.*, 1968, 1970), or R (Doctor *et al.*, 1969) is located in that position. The structure of Q is not known yet but it is reasonable to imagine that such modification of G to Q must have a specific function in the decoding process by *E. coli* $tRNA^{Tyr}$. As an approach to understand the function of Q, we examined most of the purified *E. coli* tRNAs which were available in our laboratory to see whether Q was present in other *E. coli* tRNAs besides *E. coli* $tRNA^{Tyr}$. As reported in this communication, we could show a relation between the presence of Q and codon recognition of *E. coli* tRNA. Thus in addition to $tRNA^{Tyr}$ other *E. coli* tRNAs which recognize U and C in the third position,

* From the Biology Division, National Cancer Center Research Institute, Chuo-ku, Tokyo, Japan. *Received September 8, 1971.*

[1] The abbreviations used are: Q, unidentified minor nucleoside; Q*, Q modified with periodate; s^4U, 4-thiouridine; Gm, 2′-*O*-methylguanosine; N, 2-methylthio-N^6-(Δ^2-isopentenyl)adenosine; m^7G, 7-methylguanosine; m^2A, 2-methyladenosine; N*, *N*-[9-(β-D-ribofuranosyl)purin-6-ylcarbamoyl]threonine; PDase, snake venom phosphodiesterase; pancRNase, bovine pancreatic RNase; PNPase, *E. coli* polynucleotide phosphorylase; Tyr, *E. coli* $tRNA^{Tyr}$; Tyr 2, *E. coli* $tRNA_2^{Tyr}$; His 1, *E. coli* $tRNA_1^{His}$; Asn, *E. coli* $tRNA^{Asn}$; Asp 1, *E. coli* $tRNA_1^{Asp}$; optical density unit (ODU), an amount of material with an absorbance of 1.0 at 260 mμ when dissolved in 1 ml of water and measured with a 1-cm light path.

and A in the second position of the code word, *i.e.*, $tRNA^{His}$, $tRNA^{Asn}$, and $tRNA^{Asp}$, contain Q. Other *E. coli* tRNAs checked so far did not contain Q. The sequences of oligonucleotides containing Q, derived from RNase T_1 digests of *E. coli* $tRNA^{His}$, $tRNA^{Asn}$, and $tRNA^{Asp}$, were determined. The results showed that Q is possibly located in the first position of the anticodons of all these tRNAs. In addition, experiments on the binding of these tRNAs to ribosomes showed that Q has more affinity for U than C in codon–anticodon base pairing.

Materials and Methods

Preparation of Purified E. coli tRNAs. tRNA was prepared from *E. coli* B cells harvested in the late-log phase of growth as described by Zubay (1962) except that treatment with alkali was omitted. The purified *E. coli* tRNAs used in this work were usually obtained by a combination of DEAE-Sephadex A-50 column chromatography (Nishimura *et al.*, 1967; Nishimura, 1971) with other column chromatographic procedures such as reverse-phase partition chromatography (Kelmers *et al.*, 1965), benzoylated DEAE-cellulose (BD-cellulose) chromatography (Gillam *et al.*, 1967), and DEAE-Sephadex A-50 column chromatography at pH 4.0 (Yoshida *et al.*, 1971). The procedures used to obtain *E. coli* $tRNA_2^{Tyr}$ and $tRNA^{Phe}$ were described previously (Nishimura *et al.*, 1967, 1969; Nishimura, 1971). *E. coli* $tRNA_1^{His}$ and $tRNA_1^{Asp}$ were also obtained by a procedure described previously (Nishimura, 1971) and further purified by DEAE-Sephadex A-50 column chromatography at pH 4.0. *E. coli* $tRNA^{Asn}$ was obtained by successive DEAE-Sephadex A-50 column chromatographies at pH 7.5 and 4.0, BD-cellulose column chromatography, and reverse-phase partition chromatography. Details of this purification procedure will be published elsewhere. The purities of the $tRNA_2^{Tyr}$, $tRNA_1^{His}$, $tRNA^{Asn}$, $tRNA_1^{Asp}$, and $tRNA^{Phe}$ thus obtained were estimated to be more than 90% as judged from their amino acid acceptor abilities and the chromatographic profiles of their RNase T_1 and pancRNase digests. Reports on the purification of the following *E. coli* tRNAs have appeared: $tRNA_1^{Ser}$ and $tRNA_3^{Ser}$ (Ishikura *et al.*, 1971); $tRNA^{fMet}$, $tRNA_1^{Met}$, and $tRNA_2^{Met}$ (Seno *et al.*, 1968); $tRNA_1^{Val}$ (Nishimura *et al.*, 1967; Nishimura, 1971); $tRNA_{1\text{-}a\text{-}2}^{Leu}$, $tRNA_{1\text{-}b}^{Leu}$, $tRNA_{1\text{-}a\text{-}1}^{Leu}$, and $tRNA_2^{Leu}$ (K. Ishii *et al.*, manuscript in preparation); and $tRNA^{Cys}$ (Nishimura, 1971).

Trinucleoside Diphosphate. U-U-U, U-U-C, U-A-U, U-A-C, C-A-U, and C-A-C were synthesized chemically and kindly given by Dr. H. G. Khorana. A-A-U and A-A-C were prepared by fractionation of a pancRNase digest of *E. coli* unfractionated tRNA, and dephosphorylation by treatment with *E. coli* alkaline phosphomonoesterase. G-A-U and G-A-C were also prepared from pancRNase digests of *E. coli* $tRNA_1^{Val}$ and $tRNA_3^{Ser}$, respectively.

Enzymes. RNase T_1 and RNase T_2 were obtained from Sankyo Co. Ltd. RNase U_2 was a gift from Dr. H. Okazaki of the Central Research Laboratories, Sankyo Co. Ltd. Panc RNase, *E. coli* alkaline phosphomonoesterase, and snake venom phosphodiesterase were products of Sigma Chemical Co., Miles Laboratories, Inc., and Worthington Biochemicals, respectively. Partially purified polynucleotide phosphorylase (fraction V) was prepared from *E. coli* B by a slight modification of the procedure of Singer (1966).

Other Materials. Thin-layer glass plates coated with Avicel SF cellulose were purchased from Funakoshi Pharmaceutical Co. Filter paper, No. 51A, equivalent to Whatman No. 1 was obtained from Toyo Roshi Co., Ltd. [U-^{14}C]Amino acids were obtained from the New England Nuclear Co. The specific activities of [^{14}C]tyrosine, [^{14}C]histidine, [^{14}C]asparagine, [^{14}C]aspartic acid, and [^{14}C]phenylalanine were 379, 256, 178, 171, and 392 mCi per mmole, respectively.

General Procedures for Isolation and Identification of Oligonucleotides. The general methods used for isolation and identification of oligonucleotides, such as column chromatography, paper chromatography, thin-layer chromatography, elution of nucleotides and oligonucleotides from paper or thin-layer plates, enzymatic degradation of tRNA or oligonucleotide by various RNases and *E. coli* alkaline phosphomonoesterase, and measurement of ultraviolet absorbance, were essentially the same as those used for determination of the primary sequence of *E. coli* $tRNA_1^{Val}$ (Harada *et al.*, 1971; Kimura *et al.*, 1971). The solvent systems used for two-dimensional chromatography were: system 1, first dimension, isobutyric acid–0.5 N NH_4OH (5:3, v/v); second dimension, isopropyl alcohol–concentrated HCl–H_2O (70:15:15, v/v); and system 2, first dimension, isobutyric acid–0.5 N NH_4OH (5:3, v/v); second dimension, *tert*-butyl alcohol–ammonium formate buffer, pH 3.8 (1:1, v/v), as described by Rushizky and Knight (1960).

Assay of Binding of AA-tRNA to Ribosomes. [^{14}C]AA-tRNAs and *E. coli* ribosomes were prepared as described previously (Nishimura *et al.*, 1969). The assay procedure described by Nirenberg and Leder (1964) was used. The reaction mixture (0.05 ml) contained 0.1 M Tris-HCl (pH 7.5)–0.05 M KCl, 1 ODU of ribosomes, 0.01 or 0.02 M magnesium acetate, [^{14}C]AA-tRNA, and trinucleoside diphosphate as specified. Incubation was carried out at 25° for 15 min with 0.02 M magnesium ion, or at 37° for 8 min with 0.01 M magnesium ion.

Results

Presence of Q in RNase T_2 Digests of E. coli $tRNA^{Tyr}$, $tRNA_1^{His}$, $tRNA^{Asn}$, and $tRNA_1^{Asp}$. For detection of a minor component in an individual tRNA, 2–4 ODU of tRNA were extensively hydrolyzed by RNase T_2, and the resulting nucleotide mixture was analyzed by two-dimensional thin-layer chromatography (system 1). As shown in Figure 1, chromatograms of each tRNA digest showed that 1 mole of minor nucleotide, Qp, was found in *E. coli* $tRNA_2^{Tyr}$, $tRNA_1^{His}$, $tRNA^{Asn}$, and $tRNA_1^{Asp}$, together with Tp, ψp, and s^4Up. Although not shown in the figures, minor species of tRNA for these amino acids, *i.e.*, *E. coli* $tRNA_1^{Tyr}$, $tRNA_2^{His}$, and $tRNA_2^{Asp}$, also contained Q. To study the nature of Q further, digests of 20 ODU of each tRNA with RNase T_2 were fractionated by two-dimensional paper chromatography in the same way. The spots containing Qp were eluted with water, and their uv absorption spectra were measured. As shown in Figure 2, the uv absorption spectra of samples of Qp isolated from four different tRNAs were identical in all respects. In addition, it was shown that the 3′-phosphates of 2-methylthio-N^6-(Δ^2-isopentyl)adenosine (N) (Harada *et al.*, 1968) and 2′-*O*-methylguanosine from $tRNA_2^{Tyr}$, *N*-[9-(β-D-ribofuranosyl)purin-6-ylcarbamoyl]threonine (N*) (Schweizer *et al.*, 1969) from $tRNA^{Asn}$, 2-methyladenosine (m^2A) (M. Saneyoshi, *et al.*, 1972) from $tRNA_1^{His}$ and $tRNA_1^{Asp}$, and 7-methylguanosine from $tRNA_1^{His}$, $tRNA^{Asn}$, and $tRNA_1^{Asp}$ were detected as other minor components. It should be mentioned that Q was not detected in other purified amino acid specific tRNAs such as $tRNA^{Phe}$, $tRNA_{1\text{-}a\text{-}2}^{Leu}$ (UU series), $tRNA_1^{Ser}$ (UCU,UCA,UCG), $tRNA^{Cys}$, $tRNA^{Trp}$, $tRNA_{1\text{-}a\text{-}1}^{Leu}$ (CU series), $tRNA_{1\text{-}b}^{Leu}$ (CU series), $tRNA_2^{Leu}$ (CU series), $tRNA^{Arg}$ (CG series), $tRNA^{Ile}$, $tRNA^{fMet}$, $tRNA_1^{Met}$, $tRNA_2^{Met}$, $tRNA^{Thr}$, $tRNA^{Lys}$, $tRNA_3^{Ser}$ (AGU,AGC), $tRNA_1^{Val}$ (GUU,GUA,GUG), $tRNA_1^{Glu}$,

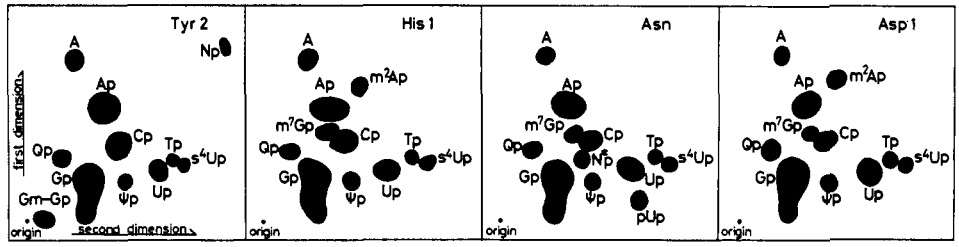

FIGURE 1: Two-dimensional thin-layer chromatograms of RNase T_2 digests of *E. coli* $tRNA_2^{Tyr}$, $tRNA_1^{His}$, $tRNA^{Asn}$, and $tRNA_1^{Asp}$. Guanosine 3′,5′-diphosphate was superimposed in a lower part of spot corresponding to Gp. In fact, pGp was isolated from $tRNA^{Tyr}$, $tRNA^{His}$, and $tRNA^{Asp}$ as 5′-hydroxyl terminal during the course of the further sequential study.

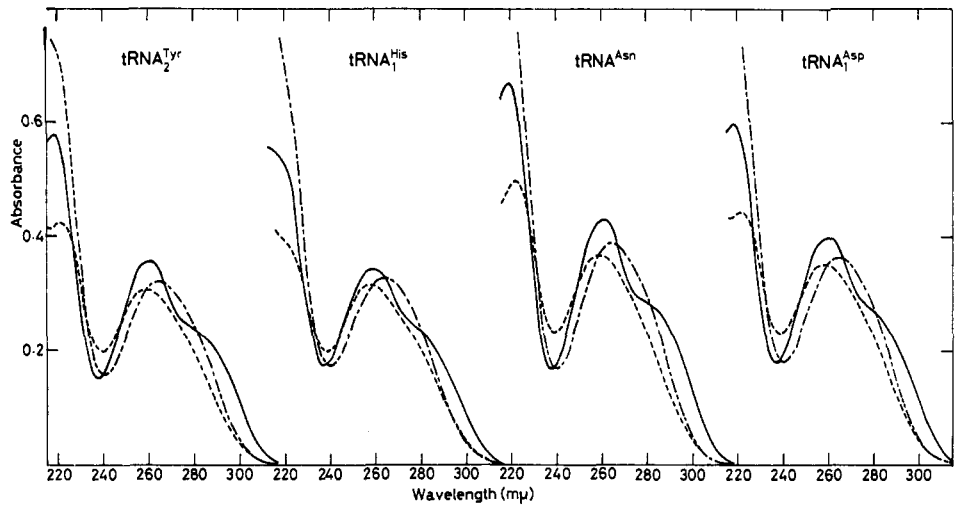

FIGURE 2: Ultraviolet absorption spectra of Qp, isolated from *E. coli* $tRNA_2^{Tyr}$, $tRNA_1^{His}$, $tRNA^{Asn}$, and $tRNA_1^{Asp}$. 1 N HCl, -------; pH 2.0 and 7.0,———; pH 12.0,– – – –.

$tRNA_2^{Glu}$, and $tRNA^{Gly}$, when analyzed by the same method of two-dimensional chromatography.

Determination of the Location of Q in Molecules of $tRNA_1^{His}$, $tRNA^{Asn}$, and $tRNA_1^{Asp}$. Each particular minor component is known to be located in a specific region of the tRNA molecule, as arranged in a cloverleaf structure (for examples, see review by Zachau, 1969). Q is known to be located in the first position of the anticodon of *E. coli* $tRNA^{Tyr}$ (Goodman *et al.*, 1968, 1970; RajBhandary *et al.*, 1969), so it was likely that Q was also located in the same position of *E. coli* $tRNA_1^{His}$, $tRNA^{Asn}$, and $tRNA_1^{Asp}$. To determine the location of Q in each tRNA without obtaining their total primary sequences, each tRNA was hydrolyzed by RNase T_1, and the resulting oligonucleotides were fractionated by DEAE-Sephadex A-25 column chromatography. Oligonucleotides containing Q were isolated, and their nucleotide sequences were determined. Previously m^2A was found in the position next to the 3′-hydroxy end of the anticodon of *E. coli* $tRNA_2^{Glu}$ (Ohashi *et al.*, 1970). Therefore, in the case of $tRNA_1^{His}$, the sequence of the oligonucleotide containing m^2A was also determined to obtain more information on the anticodon loop structure of $tRNA_1^{His}$. In the following sections, determination of these oligonucleotides will be described in detail.

ISOLATION AND IDENTIFICATION OF THE OLIGONUCLEOTIDES CONTAINING Q OR m^2A, FROM AN RNase T_1 DIGEST OF *E. coli* $tRNA_1^{His}$. A sample of *E. coli* $tRNA_1^{His}$ (150 ODU) was dissolved in 0.5 ml of 0.05 M Tris-HCl buffer (pH 7.5) containing 75 μg of RNase T_1. The solution was incubated for 18 hr at 37°. Then solid urea was added to the solution to a concentration of 7 M. The mixture was adsorbed on a column of DEAE-Sephadex A-25, and the column was eluted with a linear gradient of sodium chloride (Rushizky *et al.*, 1964). The elution pattern is shown in Figure 3. Each fraction was desalted, completely hydrolyzed by RNase T_2, and analyzed by two-dimensional thin-layer chromatography (system 1) to detect the

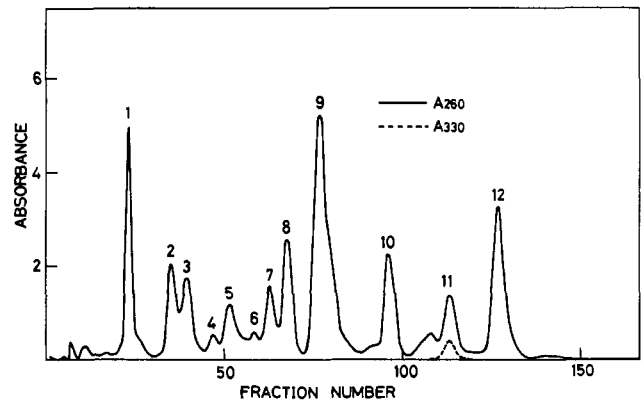

FIGURE 3: Chromatography of an RNase T_1 digest of $tRNA_1^{His}$ on a column (0.3 × 150 cm) of DEAE-Sephadex A-25 in the presence of 7 M urea. Elution was performed with a linear salt gradient obtained by placing 250 ml of 0.14 M NaCl–0.02 M Tris-HCl (pH 7.5)–7 M urea in the mixing chamber and 250 ml of 0.7 M NaCl–0.02 M Tris-HCl (pH 7.5)–7 M urea in the reservoir. Fractions of 1.8 ml of effluent were collected at a flow rate of 2 ml/hr.

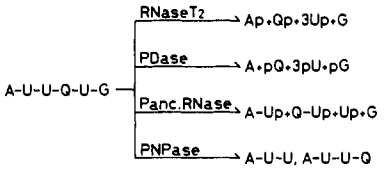

FIGURE 4: Scheme to illustrate the determination of the nucleotide sequence of A-U-U-Q-U-G derived from an RNase T_1 digest of $tRNA_1^{His}$.

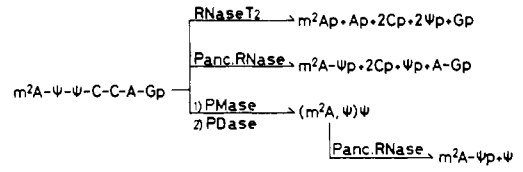

FIGURE 5: Scheme to illustrate the determination of the nucleotide sequence of m²A-ψ-ψ-C-C-A-Gp from an RNase T_2 digest of $tRNA_1^{His}$.

minor component. It was found that peaks 8 and 10 contained Q and m²A, respectively. Therefore, attempts were only made to determine the nucleotide sequences of these two oligonucleotides.

Peak 8 (A-U-U-Q-U-Gp). 3′-Phosphate was removed from this oligonucleotide by treatment with *E. coli* alkaline phosphomonoesterase, and the dephosphorylated oligonucleotide was further purified by DEAE-Sephadex A-25 column chromatography with a linear gradient of triethylammonium bicarbonate buffer. An RNase T_2 digest of this oligonucleotide was separated by two dimensional thin-layer chromatography (system 1). Ap, Qp, Up and guanosine were obtained in a ratio of 0.9[2]:1.0:3.0:1.0. Digestion of the oligonucleotide with snake venom phosphodiesterase showed that adenosine was the 5′-terminal nucleoside. A pancreatic RNase digest of the oligonucleotide was separated by two-dimensional thin-layer chromatography (system 2). A-Up, Q-Up, Up, and guanosine were obtained in a molar ratio of 1.0:1.0:1.0:1.0. The sequence of the oligonucleotide was finally determined by digestion with polynucleotide phosphorylase. Six optical density units of the oligonucleotide were digested as described by Madison *et al.* (1967) and the digest was separated by two-dimensional paper chromatography (system 2). A-U-U and A-U-U-Q were obtained. Therefore, the sequence of the oligonucleotide in peak 8 was determined as A-U-U-Q-U-Gp. A summary of the sequence determination is shown in Figure 4.

Peak 10 (m²A-ψ-ψ-C-C-A-Gp). This oligonucleotide was shown to be homogeneous by rechromatography on DEAE-Sephadex A-25 in 7 M urea at pH 2.7. On two-dimensional thin-layer chromatography (system 1) of an RNase T_2 digest of this peak, m²Ap, Ap, Cp, Gp, and ψp were identified in a molar ratio of 1.0:1.1:2.0:1.0:1.7. Digestion of the oligonucleotide with pancreatic RNase and subsequent separation of the products by two-dimensional paper chromatography (system 2) showed that m²A-ψp, Cp, A-Gp, and ψp were present in a molar ratio of 0.9:2.0:1.0:0.8. The complete sequence was determined by partial digestion with snake venom

[2] Extinction coefficients for the mononucleotides were taken from the literature (Holley *et al.*, 1965b; Dunn and Hall, 1968). The following millimolar extinction coefficients were also used tentatively: N*p, 18.8 (270 mμ at pH 2) and 14.2 (260 mμ at pH 7); Qp, 10.7 (260 mμ at pH 2–7).

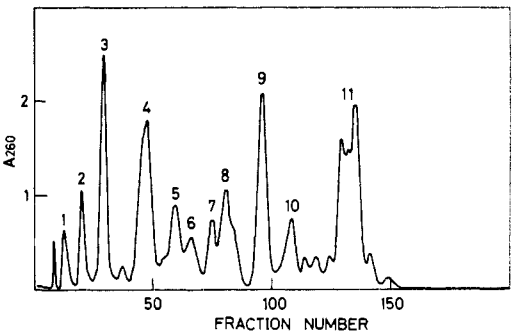

FIGURE 6: Chromatography of an RNase T_1 digest of $tRNA^{Asn}$ on a column (0.6 × 150 cm) of DEAE-Sephadex A-25 in the presence of 7 M urea. Elution was performed with a linear salt gradient as described in Figure 3, except that 750 ml of each elution buffer were used. Fractions of 4.5 ml of effluent were collected at a flow rate of 10 ml/hr.

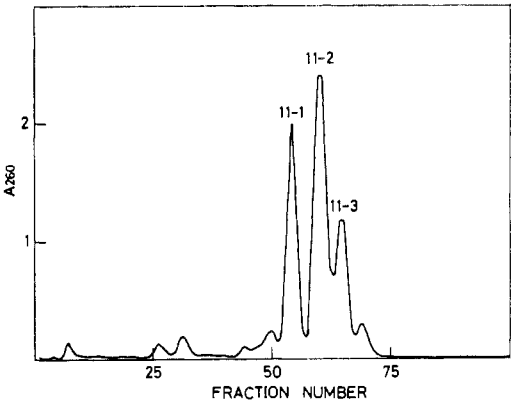

FIGURE 7: Resolution of three oligonucleotides in peak 11 of Figure 6 by DEAE-Sephadex column chromatography at pH 2.7. The mixture of oligonucleotides in peak 11 was dissolved in 1 ml of 7 M urea, and the solution was adjusted to pH 2.7 with HCl. The mixture was chromatographed on a column (0.6 × 100 cm) of DEAE-Sephadex A-25 in the presence of 7 M urea. Elution was performed with a linear salt gradient obtained by placing 200 ml of 0.06 M HCl–7 M urea in the mixing chamber and 200 ml of 0.4 M NaCl–0.06 M HCl–7 M urea in the reservoir. Fractions of 3.5 ml of effluent were collected at a flow rate of 15 ml/hr.

phosphodiesterase as described by Holley *et al.* (1964). The dephosphorylated oligonucleotide (8 ODU) was dissolved in 0.5 ml of 0.05 M NH_4HCO_3 and incubated with 30 μg of snake venom phosphodiesterase at 37° for 20 min. The mixture was then separated by two-dimensional paper chromatography (system 2). The (m²A,ψ)ψ thus obtained was further hydrolyzed by pancRNase and m²A-ψp and pseudouridine were obtained in equimolar amounts. A summary of sequence determination of the material in peak 10 is shown in Figure 5.

ISOLATION AND IDENTIFICATION OF THE OLIGONUCLEOTIDE CONTAINING Q FROM AN RNase T_1 DIGEST OF *E. coli* $tRNA^{Asn}$ (A-C-U-Q-U-U-N*-A-ψ-C-C-Gp). A sample of *E. coli* $tRNA^{Asn}$ (300 ODU) was dissolved in 1 ml of 0.05 M Tris-HCl buffer (pH 7.5) containing 150 μg of RNase T_1. The solution was incubated for 18 hr at 37°. The hydrolysate was fractionated by DEAE-Sephadex A-25 column chromatography in the same way as $tRNA_1^{His}$. The elution pattern is shown in Figure 6. Peak 11 contained a mixture of three oligonucleotides. These oligonucleotides were separated by DEAE-Sephadex A-25 column chromatography at pH 2.7 as shown

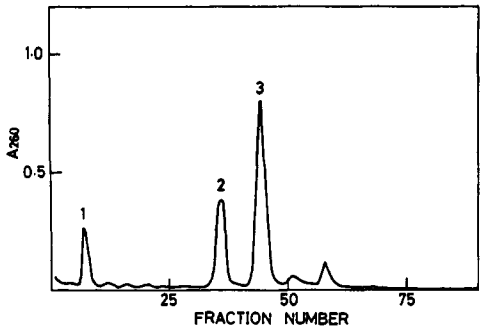

FIGURE 8: Chromatography of an RNase U_2 digest of peak 11-2 of Figure 7 on a column (0.3 × 100 cm) of DEAE-Sephadex A-25 in the presence of 7 M urea. Elution was performed with a linear salt gradient as described in Figure 3, except that 200 ml of each elution buffer were used. Fractions of 2.8 ml of effluent were collected at a flow rate of 6 ml/hr.

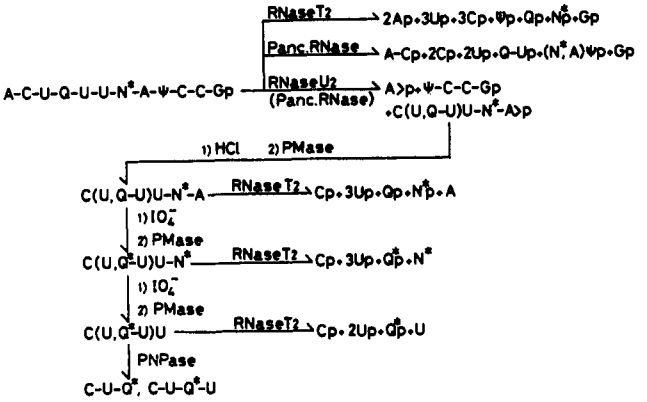

FIGURE 9: Scheme to illustrate the determination of the nucleotide sequence of A-C-U-Q-U-U-N*-A-ψ-C-C-Gp from an RNase T_1 digest of $tRNA^{Asn}$.

in Figure 7. It was found that peak 11-2 contained Q and N*, whereas peak 11-1 and peak 11-3 contained m^7G and pUp, respectively. Therefore, attempts were made to determine the nucleotide sequence of the material in peak 11-2. An RNase T_2 digest of peak 11-2 was separated by two-dimensional thin-layer chromatography (system 1). Ap, Up, Cp, Gp, ψp, Qp, and N*p were obtained in the ratio of 2.0:2.8:3.3:1.1:0.8:1.0:0.9. The products of digestion of peak 11-2 with pancRNase were A-Cp, Cp, Up, Gp, Q-Up, and (N*,A)ψp in the ratio of 0.9:2.2:2.0:1.1:1.0:0.7. An RNase U_2 digest of peak 11-2 (9 ODU) was separated by DEAE-Sephadex A-25 column chromatography. Three peaks were obtained as shown in Figure 8. Peak 1 was identified as A>p. Peak 2 was further digested with pancreatic RNase, and Gp, ψp, and Cp were obtained in a ratio of 1.0:1.1:2.2. Peak 3 was also digested with pancreatic RNase, and Cp, N*-A>p, Up, U-N*-A>p,[3] and Q-Up were obtained in a ratio of 1.0:0.6:1.7:0.4:1.0. From these results, the partial sequence of the dodecanucleotide in peak 11-2 was determined as A-C(U,Q-U)U-N*-A-ψ-C-C-Gp. The order of Up and Q-Up, shown in brackets, was determined as follows. Peak 3, obtained from an RNase U_2 digest of peak 11-2, was treated with 0.1 M HCl for 18 hr at 0° to split terminal cyclic phosphate. It was then dephosphorylated by treatment with *E. coli* alkaline phosphomonesterase. The dephosphorylated oligonucleotide was treated twice with periodate by the method of Khym and Uziel (1968) with the modification that NaCl and CTABr were omitted as described previously (Harada *et al.*, 1971). In each step, the oligonucleotide was desalted and separated from the alkaline phosphomonoesterase by DEAE-cellulose column chromatography. The 3′-terminal nucleoside of the shortened oligonucleotide was determined by analysis of an RNase T_2 digest by two-dimensional thin-layer chromatography. Adenosine, N*, and uridine were identified as the 3′-terminal nucleosides of the original dephosphorylated heptanucleotide, the shortened hexanucleotide and the pentanucleotide, respectively. The pentanucleotide was then digested with polynucleotide phosphorylase, and the digest was separated by two-dimensional paper chromatography (system 2). C-U-Q* and C-U-Q*-U

[3] It should be noted that the phosphodiester bond of uridine becomes resistant to pancRNase if a bulky nucleoside is linked to the phosphate group. The same phenomenon was observed in the sequence determination of the anticodon loop of *E. coli* $tRNA_2^{Glu}$ which contains 5-methylaminomethyl-2-thiouridine (Ohashi *et al.*, 1970). Cory *et al.* (1968) also reported that pancRNase did not attack the U–N* phosphodiester bond.

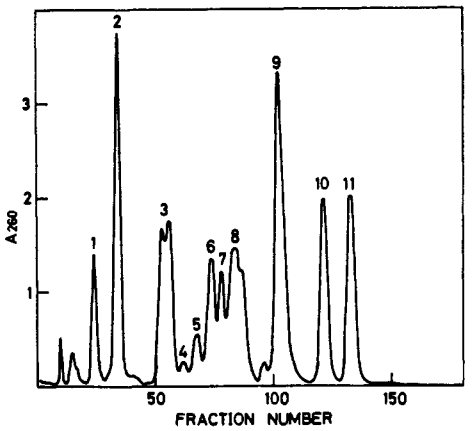

FIGURE 10: Chromatography of an RNase T_1 digest of $tRNA_1^{Asp}$ on a column (0.6 × 150 cm) of DEAE-Sephadex A-25 in the presence of 7 M urea. The chromatographic procedure was as described in Figure 6.

were obtained.[4] A summary of the sequence determination of peak 11-2 is shown in Figure 9.

ISOLATION AND IDENTIFICATION OF OLIGONUCLEOTIDE CONTAINING Q FROM AN RNase T_1 DIGEST OF *E. coli* $tRNA_1^{Asp}$ (C-C-U-Q-U-C-m^2A-C-Gp). A sample of *E. coli* $tRNA_1^{Asp}$ (300 ODU) was dissolved in 1 ml of 0.05 M Tris-HCl buffer (pH 7.5) containing 150 μg of RNase T_1. The solution was incubated for 18 hr at 37°. The hydrolysate was fractionated by DEAE-Sephadex A-25 column chromatography in the same way as $tRNA_1^{His}$ and $tRNA^{Asn}$. The elution pattern is shown in Figure 10. It was found that peak 10 contained Q, and this oligonucleotide was pure as judged by the elution pattern on DEAE-Sephadex A-25 column chromatography at pH 2.7. The RNase T_2 digest of the oligonucleotide contained m^2Ap, Cp, Up, Qp, and Gp in the ratio of 1.0:4.3:1.9:1.0:1.0. A digest of the oligonucleotide with pancreatic RNase contained m^2A-Cp, Q-Up, Cp, Up, and Gp in the ratio of 1.2:1.0:3.1:1.0:0.9. In addition, digestion of dephosphorylated material from peak 10 with polynucleotide phosphorylase gave C-C-U and C-C-U-Q. Thus, the partial sequence of the oligonucleotide was C-C-U-Q-U(C,m^2A-C)Gp. The complete sequence

[4] Q was modified by oxidation with periodate, but the exact mechanism of the reaction is unknown (F. Harada and S. Nishimura, unpublished result).

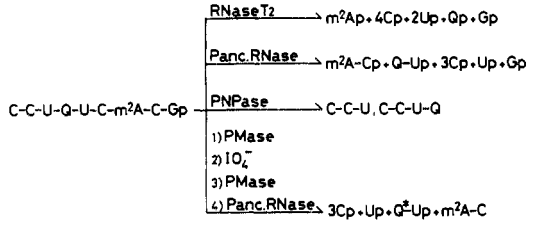

FIGURE 11: Scheme to illustrate the determination of the nucleotide sequence of C-C-U-Q-U-C-m²A-C-Gp from an RNase T_1 digest of $tRNA_1^{Asp}$.

was determined using periodate oxidation, followed by dephosphorylation and digestion with pancRNase. The digest was separated by two-dimensional thin-layer chromatography, and m²A-C was obtained as the 3′-terminal sequence. A summary of the sequence determination is shown in Figure 11.

Stimulation of the Binding of [¹⁴C]*Tyrosyl-tRNA*$_2^{Tyr}$, [¹⁴C]*-Histidyl-tRNA*$_1^{His}$, [¹⁴C]*Asparaginyl-tRNA*^{Asn}, [¹⁴C]*Aspartyl-tRNA*$_1^{Asp}$ *and* [¹⁴C]*Phenylalanyl-tRNA*^{Phe} *to Ribosomes by the Corresponding Codon Triplets.* As discussed later, analysis of the nucleotide sequence of oligonucleotides containing Q from $tRNA_1^{His}$, $tRNA^{Asn}$, and $tRNA_1^{Asp}$ strongly suggested that Q is located in the first position of the anticodon of these tRNAs. Therefore, it was of interest to investigate the codon recognizing properties of these tRNAs to see whether Q possesses particular wobbling characteristics compared with normal guanosine. For this purpose, the binding of purified [¹⁴C]-aminoacyl-tRNAs to ribosomes in the presence of the corresponding codon triplets was measured as described by Nirenberg and Leder (1964). As a control, the binding of *E. coli* [¹⁴C]phenylalanyl-tRNA^{Phe} to ribosomes in the presence of U-U-U or U-U-C was also measured, since this tRNA contains normal guanosine in the first position of the anticodon (Barrell and Sanger, 1969). As seen in Figure 12, binding of tRNAs containing Q to ribosomes is always stimulated more by the trinucleotide ending with U, whereas binding of tRNA^{Phe} to ribosomes was stimulated more by U-U-C than U-U-U. This tendency was much more pronounced when the assay was carried out at 37° in the presence of 0.01 M magnesium ion, as shown in Table I.

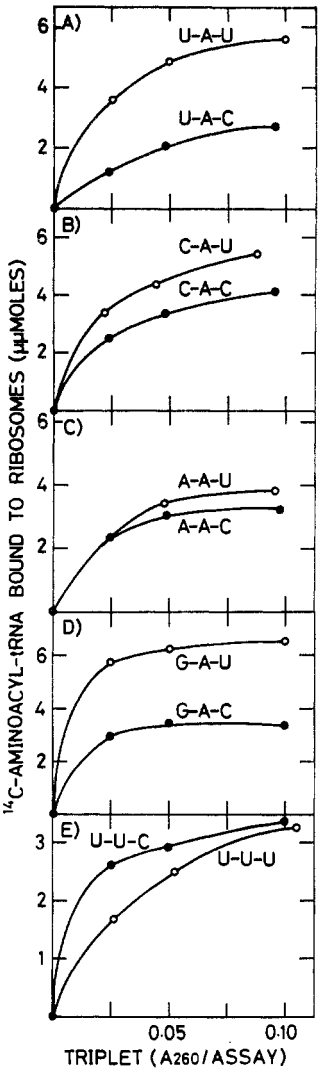

FIGURE 12: Stimulation of binding of *E. coli* [¹⁴C]tyrosyl-tRNA$_2^{Tyr}$, [¹⁴C]histidyl-tRNA$_1^{His}$, [¹⁴C]asparaginyl-tRNA^{Asn}, [¹⁴C]aspartyl-tRNA$_1^{Asp}$, and [¹⁴C]phenylalanyl-tRNA^{Phe} to ribosomes by the corresponding codon triplets in the presence of 0.02 M magnesium ion. The reaction mixture (0.05 ml) contained: (A) [¹⁴C]tyrosyl-tRNA$_2^{Tyr}$ (12.5 pmoles), (B) [¹⁴C]histidyl-tRNA$_1^{His}$ (15.6 pmoles), (C) [¹⁴C]-asparaginyl-tRNA^{Asn} (13.3 pmoles), (D) [¹⁴C]aspartyl-tRNA$_1^{Asp}$ (12.2 pmoles), and (E) [¹⁴C]phenylalanyl-tRNA^{Phe} (13.7 pmoles).

TABLE I: Stimulation of the Binding of [¹⁴C]Aminoacyl-tRNAs Containing Q to Ribosomes by the Corresponding Triplets.

Aminoacyl-tRNA[b]	Aminoacyl-tRNA Bound to Ribosomes (pmoles)[a]		
	Triplet[c]	−Triplet	+Triplet
[¹⁴C]Tyrosyl-tRNA$_2^{Tyr}$	U-A-U	0.11	0.42
	U-A-C	0.11	0.24
[¹⁴C]Histidyl-tRNA$_1^{His}$	C-A-U	0.07	1.22
	C-A-C	0.07	0.78
[¹⁴C]Asparaginyl-tRNA^{Asn}	A-A-U	0.08	0.98
	A-A-C	0.08	0.64
[¹⁴C]Aspartyl-tRNA$_1^{Asp}$	G-A-U	0.06	2.86
	G-A-C	0.06	0.85
[¹⁴C]Phenylalanyl-tRNA^{Phe}	U-U-U	0.47	1.61
	U-U-C	0.47	1.99

[a] The reaction was carried out in 0.1 M Tris-HCl (pH 7.5) containing 0.01 M magnesium acetate for 8 min at 37°. [b] The amounts of [¹⁴C]aminoacyl-tRNA in the reaction mixture (0.05 ml) were the same as Figure 12. [c] The reaction mixture contained 0.1 ODU of trinucleoside diphosphate.

Discussion

As shown in this communication, the unidentified nucleoside Q, known to be present in the first position of the anticodon of *E. coli* tRNA^{Tyr}, was also found in *E. coli* tRNA^{His}, tRNA^{Asn}, and tRNA^{Asp}. Nucleoside Q isolated from these three tRNAs was identical with that from *E. coli* tRNA^{Tyr} with respect to its ultraviolet absorption spectra, chromatographic mobilities, and possession of one additional positive charge. The nucleotide sequences of oligonucleotides containing Q and other minor components which were obtained from RNase T_1 digests of the tRNAs were identified. It was found

that Q is located in the first position of the anticodons of all three of these tRNAs, as shown in Figure 13. This was concluded without obtaining the total primary sequences of the tRNAs for the following reasons. (1) The cloverleaf structure of tRNA that have emerged from known tRNA sequences (*cf.* review by Jukes, 1969) established the general nature of the anticodon loop structure. Namely, the looped-out region consists of seven nucleotide residues. The anticodon placed in the middle of the looped out sequence is preceded by pyrimidine-U- at the 5′-hydroxy end, and generally followed by a modified purine nucleoside on the 3′-hydroxy side. The anticodon loop structures deduced for the three tRNAs completely satisfies these general characteristics. (2) The coding properties of tRNAs for histidine, aspargine, and aspartic acid strongly suggested that Q-U-G, G-U-U and Q-U-C are placed as the anticodon sequences for the respective tRNAs. (3) m^2A was placed in a position to the anticodons of *E. coli* $tRNA_1^{His}$ and $tRNA_1^{Asp}$ and has already been found in the same position in *E. coli* $tRNA_2^{Glu}$ (Ohashi *et al.*, 1970, 1972). Similarly, N* placed adjacent to the anticodon of *E. coli* $tRNA^{Asn}$ was also found in the same position in *E. coli* $tRNA^{Ile}$, $tRNA_1^{Met}$, $tRNA^{Thr}$, $tRNA^{Lys}$ and $tRNA_3^{Ser}$ (Ishikura *et al.*, 1969; Kimura-Harada *et al.*, 1972) and Torulopsis $tRNA^{Ile}$ (Takemura *et al.*, 1969a,b). The consistent presence of N* adjacent to anticodons of tRNAs which recognize codons starting from A has previously been shown (Ishikura *et al.*, 1969; Kimura-Harada *et al.*, 1972). These indirect pieces of evidence support the conclusion that the anticodon loop structures proposed for the three tRNAs must almost certainly be correct.

Q was not found in any other *E. coli* tRNAs examined so far. Thus there is a strict relation between the presence of Q and the codon recognition of *E. coli* tRNA. *E. coli* tRNAs which recognize U and C in the third position and A in the second position of code words always contain Q. Therefore, it is very likely that the function of Q is more or less related with codon recognition of tRNA. The experiments on ribosome–tRNA binding reported in this paper showed that, without exceptions, Q has more affinity for U than for C in codon–anticodon base pairing since among the trinucleotides corresponding to code words those ending with U always stimulated the binding of tRNAs containing Q to ribosomes most (Figure 12 and Table I). Doctor *et al.* (1966) also reported preferential binding of *E. coli* $tRNA^{Tyr}$ to ribosomes by U-A-U rather than U-A-C. They showed that with low magnesium concentration, binding of *E. coli* $tRNA^{Tyr}$ to ribosomes by UAU was three to five times higher than by UAC, whereas in the case of yeast $tRNA^{Tyr}$ which contained G instead of Q there was essentially no difference. The discrepancy between the data on tRNA binding observed in earlier work on codon assignment (Söll *et al.*, 1965; Nirenberg *et al.*, 1965) and the present data might be explained by the fact that unfractionated tRNA was used in the earlier work, while purified individual tRNAs were used in this experiment. This preferential recognition of code words ending with U by $tRNA_2^{Tyr}$, $tRNA_1^{His}$, $tRNA^{Asn}$, and $tRNA_1^{Asp}$ must be due to the presence of Q, since the binding of $tRNA^{Phe}$, which contains normal G instead of Q in the first position of the anticodon, was stimulated much more by U-U-C than by U-U-U (Figure 12 and Table I). In this connection, it should be noted that Q is derived from a guanosine residue (Goodman *et al.*, 1968, 1970), although its exact structure is not known yet. The structure of Q must be investigated to understand the molecular mechanism of wobbling of Q.

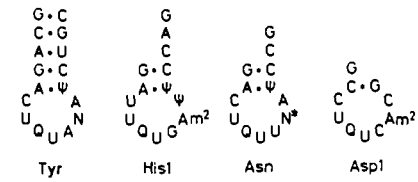

FIGURE 13: Structures of the anticodon regions of *E. coli* $tRNA^{Tyr}$, $tRNA_1^{His}$, $tRNA^{Asn}$, and $tRNA_1^{Asp}$.

Although it was proved that modification of G to Q resulted in change in the efficiency of codon recognition *in vitro*, it is not clear whether such modification is absolutely necessary for protein synthesis in cells *in vivo*. If the function of Q is simply to change the efficiency of recognition of code words, it is unlikely that replacement of G by Q in the first position of the anticodons has any drastic affects on *in vivo* protein synthesis, since the extent of preferential recognition of code words ending with U is not so great. Another possible explanation of the function of Q is that it prevents any miscoding which might occur if G is present in the first position, and U in the second position of the anticodon of *E. coli* tRNAs. We previously showed that binding of *E. coli* [^{14}C]aspartyl-tRNA to ribosomes was greatly stimulated by G-A-A, when the magnesium concentration was increased (Nishimura *et al.*, 1965). This indicates that misrecognition of the third letter of a code word actually occurs at a high magnesium concentration. Similarly, poly(U-G), containing a repeating dinucleotide sequence, directed the synthesis of valine–tyrosine copolypeptide in the presence of 0.02 M magnesium ion and/or in the presence of purified *E. coli* $tRNA^{Tyr}$ (Nishimura *et al.*, 1969). It was concluded that U-G-U is ambiguously read as tyrosine, indicating that misrecognition of the second letter of the code word occurred. Therefore, it is possible that this type of miscoding might occur even under normal conditions, if G were present in the first position of the anticodon rather than Q.

At present, it is not known how modification of G to Q occurs in certain types of *E. coli* tRNA. As discussed earlier, *E. coli* $tRNA^{Phe}$, $tRNA_2^{Leu}$, $tRNA^{Ile}$, $tRNA_3^{Gly}$, $tRNA_2^{Val}$, and $tRNA_3^{Ser}$ contain a normal guanosine residue in the first position of the anticodons. The consistent presence of Q might be accounted for by the recognition specificity of the modification enzyme(s) involved in the conversion of G to Q. If the enzyme can modify G to Q by recognizing the U-G-U sequence in the anticodon loop, only *E. coli* $tRNA^{Tyr}$, $tRNA^{His}$, $tRNA^{Asn}$, and $tRNA^{Asp}$ can be modified. The presence of Q seems to be specific for *E. coli* tRNA, since yeast $tRNA^{Tyr}$, yeast $tRNA^{Asp}$, and Torulopsis $tRNA^{Tyr}$ contain normal guanosine in the first position of the anticodon.[5] The absence of Q in yeast tRNAs might simply be due to lack of the enzyme(s) involved. This possibility should be tested by isolating the modification enzyme, and using it to modify yeast tRNAs.

Acknowledgment

We are grateful to the Laboratories of Kaken Chemicals Ltd. for large-scale isolation of crude *E. coli* tRNA. The au-

[5] Rogg and Staehelin (1969) found a minor component similar to Q in rat liver tRNA. However, its precise characteristics were not reported. Therefore at present it is uncertain whether the two minor components are identical.

thors are indebted to Dr. H. G. Khorana for a gift of trinucleoside diphosphates, and to Dr. H. Okazaki for a gift of RNase U_2. This work was supported in part by grants from the Princess Takamatsu Cancer Research Fund and the Japanese Ministry of Education.

References

Barrell, B. G., and Sanger, F. (1969), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 3*, 275.

Blank, H. U., and Söll, D. (1971), *Biochem. Biophys. Res. Commun. 43*, 1192.

Cory, S., Marcker, K. A., Dube, S. K., and Clark, B. F. C. (1968), *Nature (London) 220*, 1039.

Crick, F. H. C. (1966a), *Cold Spring Harbor Symp. Quant. Biol. 31*, 3.

Crick, F. H. C. (1966b), *J. Mol. Biol. 19*, 548.

Doctor, B. P., Loebel, J. E., and Kellogg, D. A. (1966), *Cold Spring Harbor Symp. Quant. Biol. 31*, 543.

Doctor, B. P., Loebel, J. E., Sodd, M. A., and Winter, D. B. (1969), *Science 163*, 693.

Dudock, B. S., and Katz, G. (1969), *J. Biol. Chem. 244*, 3069.

Dudock, B. S., Katz, G., Taylor, E. K., and Holley, R. W. (1969), *Proc. Nat. Acad. Sci. U. S. 62*, 941.

Dunn, D. B., and Hall, R. H. (1968), *in* Handbook of Biochemistry, Sober, H. A., Ed., Cleveland, Ohio, Chemical Rubber Co., p G-3.

Gangloff, J., Keith, G., Ebel, J. P., and Dirheimer, G. (1971), *Nature New Biol. 230*, 125.

Gillam, I., Millward, S., Blew, D., von Tigerstrom, M., Wimmer, E., and Tener, G. M. (1967), *Biochemistry 6*, 3043.

Goodman, H. M., Abelson, J., Landy, A., Brenner, S., and Smith, J. D. (1968), *Nature (London) 217*, 1019.

Goodman, H. M., Abelson, J. N., Landy, A., Zadrazil, S., and Smith, J. D. (1970), *Eur. J. Biochem. 13*, 461.

Harada, F., Gross, H. J., Kimura, F., Chang, S. H., Nishimura, S. and RajBhandary, U. L. (1968), *Biochem. Biophys. Res. Commun. 33*, 299.

Harada, F., Kimura, F., and Nishimura, S. (1971), *Biochemistry 10*, 3269.

Hashimoto, S., Miyazaki, M., and Takemura, S. (1969), *J. Biochem. (Tokyo) 65*, 659.

Holley, R. W., Apgar, J., Everett, G. A., Madison, J. T., Marquisee, M., Merrill, S. H., Penswick, J. R., and Zamir, A. (1965a), *Science 147*, 1462.

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

Holley, R. W., Madison, J. T., and Zamir, A. (1964), *Biochem. Biophys. Res. Commun. 17*, 389.

Ishikura, H., Yamada, Y., Murao, K., Saneyoshi, M., and Nishimura, S. (1969), *Biochem. Biophys. Res. Commun. 37*, 990.

Ishikura, H., Yamada, Y., and Nishimura, S. (1971), *Biochim. Biophys. Acta 228*, 471.

Jukes, T. H. (1969), *Curr. Top. Microbiol. Immunol. 49*, 177.

Kelmers, A. D., Novelli, G. D., and Stulberg, M. P. (1965), *J. Biol. Chem. 240*, 3979.

Khym, J. X., and Uziel, M. (1968), *Biochemistry 7*, 422.

Kimura, F., Harada, F., and Nishimura, S. (1971), *Biochemistry 10*, 3277.

Kimura-Harada, F., Harada, F., and Nishimura, S. (1972), *FEBS (Fed. Eur. Biochem. Soc.) Lett.* (in press).

Madison, J. T., Everett, G. A., and Kung, H. (1966), *Science 153*, 531.

Madison, J. T., Holley, R. W., Poucher, J. S., and Connett, P. H. (1967), *Biochim. Biophys. Acta 145*, 825.

Madison, J. T., and Kung, H. (1967), *J. Biol. Chem. 242*, 1324.

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 Sym. Quant. Biol. 31*, 11.

Nirenberg, M. W., and Leder, P. (1964), *Science 145*, 1399.

Nirenberg, M., Leder, P., Bernfield, M., Brimacombe, R., Trupin, J., Rottman, F., and O'Neal, C. (1965), *Proc. Nat. Acad. Sci. U. S. 53*, 1161.

Nishimura, S. (1971), *in* Procedures in Nucleic Acid Research, Cantoni, G. L., and Davies, D. R., Ed., New York, N. Y., Harper & Row (in press).

Nishimura, S., Harada, F., and Hirabayashi, M. (1969), *J. Mol. Biol. 40*, 173.

Nishimura, S., Harada, F., Narushima, U., and Seno, T. (1967), *Biochim. Biophys. Acta 142*, 133.

Nishimura, S., Jones, D. S., Ohtsuka, E., Hayatsu, H., Jacob, T. M., and Khorana, H. G. (1965), *J. Mol. Biol. 13*, 283.

Ohashi, Z., Harada, F., and Nishimura, S. (1972), *FEBS (Fed. Eur. Biochem. Soc.) Lett.* (in press).

Ohashi, Z., Saneyoshi, M., Harada, F., Hara, H., and Nishimura, S. (1970), *Biochem. Biophys. Res. Commun. 40*, 866.

RajBhandary, U. L., and Chang, S. H. (1968), *J. Biol. Chem. 243*, 598.

RajBhandary, U. L., Chang, S. H., Gross, H. J., Harada, F., Kimura, F., and Nishimura, S. (1969), *Fed. Proc., Fed. Amer. Soc. Exp. Biol. 28*, 409.

RajBhandary, U. L., Chang, S. H., Stuart, A., Faulkner, R. D., Hoskinson, R. M., and Khorana, H. G. (1967), *Proc. Nat. Acad. Sci., U. S. 57*, 751.

Rogg, H., and Staehelin, M. (1969), *Biochim. Biophys. Acta 195*, 16.

Rushizky, G. W., Bartos, E. M., and Sober, H. A. (1964), *Biochemistry 3*, 626.

Rushizky, G. W., and Knight, C. A. (1960), *Virology 11*, 236.

Saneyoshi, M., Ohashi, Z., Harada, F., and Nishimura, S. (1972), *Biochim. Biophys. Acta* (in press).

Schweizer, M. P., Chheda, G. B., Baczynskyj, L., and Hall, R. H. (1969), *Biochemistry 8*, 3283.

Seno, T., Kobayashi, M., and Nishimura, S. (1968), *Biochim. Biophys. Acta 169*, 80.

Singer, M. F. (1966), *in* Procedures in Nucleic Acid Research, Cantoni, G. L., and Davies, D. R., Ed., New York, N. Y., Harper & Row, p 245.

Söll, D., Ohtsuka, E., Jones, D. S., Lohrmann, R., Hayatsu, H., Nishimura, S., and Khorana, H. G. (1965), *Proc. Nat. Acad. Sci. U. S. 54*, 1378.

Squires, C., and Carbon, J. (1971), *Nature New Biol. 233*, 274.

Takemura, S., Murakami, M., and Miyazaki, M. (1969a), *J. Biochem. (Tokyo) 65*, 489.

Takemura, S., Murakami, M., and Miyazaki, M. (1969b), *J. Biochem. (Tokyo) 65*, 553.

Yaniv, M., and Barrell, B. G. (1971), *Nature New Biol. 233*, 113.

Yarus, M., and Barrell, B. G. (1971), *Biochem. Biophys. Res. Commun. 43*, 729.

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

Zachau, H. G. (1969), *Angew. Chem. Int. Ed. Engl. 8*, 711.

Zubay, G. (1962), *J. Mol. Biol. 4*, 347.