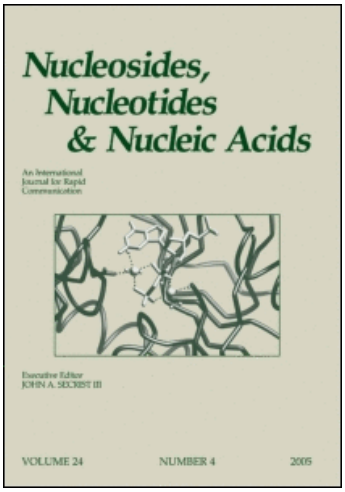

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Queuine, The Q-Containing tRNAs and the Enzymes Responsible for Their Formation

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QUEUINE, THE Q-CONTAINING tRNAs AND THE ENZYMES RESPONSIBLE FOR THEIR FORMATION

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Discovery of Q and elucidation of its structure

Queuine was first observed by Goodman *et al.* during studies on suppression of nonsense mutations in *E. coli* (1). This report was soon confirmed by Doctor *et al.* (2). In that study the molecule responsible for suppression of nonsense was found to be a mutated $tRNA^{Tyr}$. While the suppressor $tRNA^{Tyr}$ contained cytidine in the first position of the anticodon, wild-type $tRNA^{Tyr}$ contained a modified derivative of guanine in the first position of the anticodon. The modified nucleoside was called G^* and had an ultraviolet absorption spectrum very similar to guanosine. They reported that G^* had an additional basic group with a pKa between 5 and 6. T_1 ribonuclease, which cleaves RNA to form oligonucleotides ending in G at their 3' end, did not cleave the phosphodiester bond on the 3' side of G^*. The $tRNA^{Tyr}$ containing G^* had the same codon recognition properties as if unmodified guanine was present, leading the authors to speculate that the modification was on the imidazole or non-base pairing part of the purine ring (1). A similar compound was found in rat liver tRNA by Rogg and Staehelin (3). When they cleaved the tRNA with pancreatic RNase, G^* was found exclusively in the dinucleotide $G^*_pU_p$. The spectrum of the mammalian compound was identical to the compound isolated from bacteria (4).

In 1971 Saneyoshi and Nishimura for the first time called the newly discovered nucleoside Q, and they showed that Q reacted with BrCN resulting in loss of a positive charge (5). The BrCN-treated tRNA could

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still be aminoacylated, and it became possible to determine which tRNAs contained Q by charging BrCN-treated tRNA with a labeled amino acid and comparing the chromatographic mobility with unreacted tRNA. A shift in the elution profile of the tRNA after reaction with BrCN indicated that the tRNA contained Q (5,6,7). Using this technique, they demonstrated that $tRNA^{Asp}$, $tRNA^{Asn}$, and $tRNA^{His}$ as well as $tRNA^{Tyr}$ contained Q (8). An examination of the genetic code revealed that these tRNAs formed a set in that they all responded to the generalized codons NAPy where N was one of the canonical RNA bases and Py was either U or C. Q was located in the first or wobble position which explained why it was always found exclusively as the dinucleotide Q_pU_p in pancreatic RNase digests of tRNA. The nucleoside on the 5' side of the anticodon is a universal U. The composition of the bases adjacent to Q in the anticodon would have to be $U_pQ_pU_pN_p$ and the specificity of pancreatic RNase for pyrimidines dictated that Q would be present in the dinucleotide Q_pU_p. Interestingly, there is one exception to this generalization. In rat liver $tRNA^{Asp}$, there is a cytidine instead of the universal uridine next to the 5' end of the anticodon (9). The only other case where cytidine is found in this position is in the eukaryote initiator tRNAs.

The structure of Q shown in Fig. 1 was deduced by Kasai *et al.* (10), and the absolute configuration determined as 2-amino-5-(3S,4R,5S-4,5-dihydroxycyclopent-1-en-3-ylaminomethyl)-7-(β-*D*-ribofuranosyl)pyrrolo-[2,3-d]pyrimidin-4-one by Ohgi *et al.* (11). The structure was derived from UV absorbance, mass spectrometry, proton magnetic reasonance spectroscopy and studies of chemical reactivity. Whereas the phosphodiester bond Q_pU was not split by T_1 or pancreatic RNase, it was cleaved by T_2 RNase (10). The UV absorption spectrum was similar to G but λ max was shifted to longer wavelength (262 nm) and there was a second peak at 220 nm. There were three ionizing groups with pKa values of 1.1, 7.7 and 10.4. Unlike the purine nucleosides, the N glycoside bond of Q was not hydrolized under acid conditions which was not unexpected since Q is not a true purine. The N glycoside bond of Q is stable in 1 N HCl at 100° for 1 hr. Also, the hydrogen at C-8 does not undergo exchange with tritiated water as occurs with adenosine and guanosine. This latter fact has hindered queuine research since [^{3}H] labeled Q has not been available. The presence of the *cis* diol group was confirmed by experiments using isopropylidine formation and oxidation with periodate. The

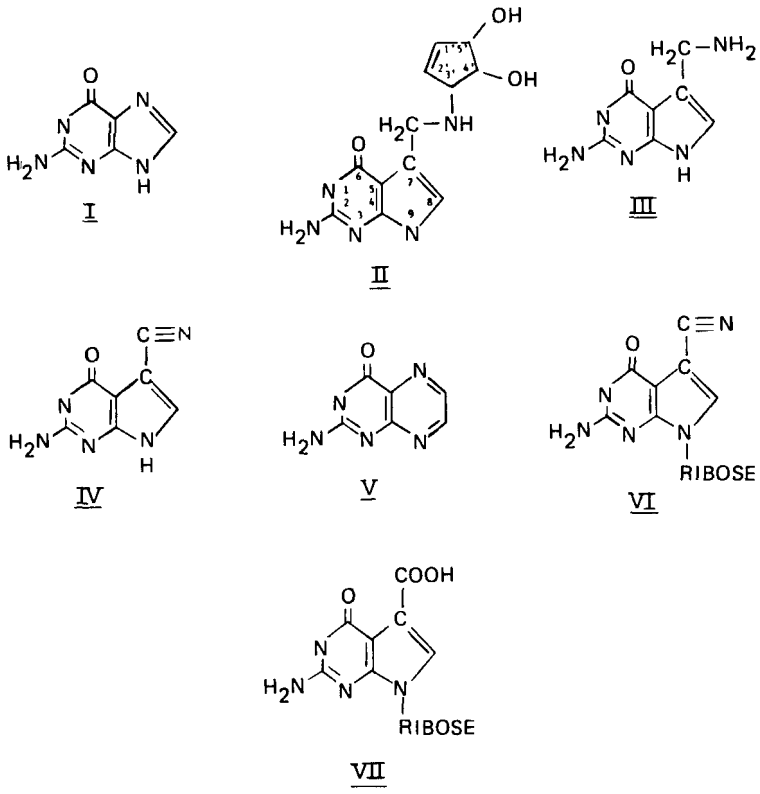

FIG. 1

Structure of queuine and related compounds. I, guanine; II, queuine, queuosine when ribose is present at N9. Two derivatives of Q in which either mannose or galactose are attached at the 4' position are referred to in the literature as Q*. III, 7-(aminomethyl)-7-deazaguanine; IV, 7-(cyano)-7-deazaguaine; V, pterine; VI, toyocamycin; VII, cadeguomycin.

reaction with periodate, like the reaction with BrCN, is useful for detecting the (Q+)tRNAs during chromatography (6,12).

Kasai *et al.* examined the species distribution of Q and found that it was present in the tRNAs of plants, invertebrates, *E. coli* and mammals. It was not found in the tRNAs of yeast and the bacterium *Thermus thermophilus* (13). Q has been observed in human tRNA (14), and unpublished work from our laboratory shows that Q is also present in fish and brine shrimp. Even though it was absent in yeast, it was present in another lower eukaryote, the slime mold (15). During the studies on the species distribution of Q, Kasai *et al.* found two compounds that had most of the chemical and spectral properties of Q, but they had different

chromatographic properties. In the literature, these derivatives of Q were designated as Q*. They were later identified as glycosylated derivatives of Q (16). The carbohydrate moieties have been identified as galactose in $tRNA^{Tyr}$, mannose in $tRNA^{Asp}$ and they have been found attached to the hydroxyl at the 4'-position of the cyclopentene diol moiety of the Q molecule.

Chemical and biochemical synthesis of Q

The chemical synthesis of Q nucleoside, Q base, and some of their derivatives, have been achieved (17-19) although the biosynthetic pathway leading to Q remains obscure. That at least part of the purine skeleton of the guanine was utilized in the biosynthesis of Q, was indicated by the fact that, when 2-[^{14}C]guanine was added to the growth medium of a guanine auxotroph of *Salmonella typhimurium* and the Q isolated from the bacterial tRNA, the radioactivity was retained. When 8-[^{14}C]guanine was added, no radioactivity was found in Q (20). Further information about the biosynthesis of Q has accrued from the observation of Katze and Mosteller that the amount of (Q+)tRNA was greatly reduced in a relaxed methionine auxotroph (21). Okada *et al.* showed that a precursor of Q, which they identified as 7-(aminomethyl)-7-deazaguanosine (Fig. 1, compound III) rather than Q, was present in the methyl-deficient tRNA (22). When radioactive methionine was added to the medium, no part of its carbon skeleton was incorporated into queuine. Evidently methionine is required for a post-transcriptional modification of tRNA, the absence of which does not prevent the insertion of the amino compound into tRNA but which is a required prerequisite to the addition of the cyclopentenediol residue to the amino compound. These workers also isolated other mutants without nutritional requirements that were deficient in the biosynthesis of (Q+)tRNA and found free amino compound in the cytoplasm as well as in tRNA of these cells. These workers also found a second compound, 7-(cyano)-7-deazaguanosine (Fig. 1, IV) in these mutants. Although knowledge about the biosynthesis of Q is incomplete, a partial putative biosynthetic pathway can be postulated by drawing analogies with the biosynthesis of another pyrrolopyrimidine, toyocamycin. Toyocamycin is an antibiotic excreted by *Streptomyces rimosus*. It is 7-cyano-7-deazaadenosine (see Fig. 1, VI) (23). Elstner and Suhadolnik showed that GTP was a precursor of toyocamycin (24). The first step being the open-

ing of the imidazole ring with concomitant production of formic acid from the C-8. This reaction is also the first step in the biosynthesis of the pteridines and riboflavin. The nitrogen at position 7 must then be removed, but the enzymatic reaction(s) that do this have not been elucidated. Carbon 7, 8 and the cyano group of toyocamycin are derived from the 3', 2', and 1' positions of the ribose moiety of GTP. It was also established that the pyrrole ring was not synthesized until a second ribose was attached to N-9 (23). Presumably a similar mechanism results in the biosynthesis of the cyano intermediate in Q synthesis. The cyano compound IV is likely a precursor of the amino compound III. Recently, another pyrrolopyrimidine antibiotic named cadeguomycin (compound VII), which will likely turn out to be an intermediate or side product of queuine biosynthesis, has been isolated from a strain of *Streptomyces hygroscopicus* (25,26). None of the enzymes of Q or toyocamycin biosynthesis, with the exception of the one catalyzing the first step, GTP cyclohydrolase, have been described.

As will be described below, in bacteria the amino compound rather than queuine is inserted into tRNA. The cyclopentendiol moiety is added later at the polynucleotide level. The biosynthesis of the cyclopentendiol residue and how it is attached to the amino group is unknown. The enzyme that inserts mannose into the cyclopentendiol of $tRNA^{Asp}$ has been found in rat liver (72), but the enzyme that transfers galactose to the Q residue in $tRNA^{Tyr}$ has not been described.

Conformational studies have been carried out on 5' QMP by x-ray crystallography, and this conformation was used to extrapolate what the conformation would be if the Q were in the anticodon of a tRNA for which the 3-dimensional structure has been determined. The results indicated that the bulky group attached to C-7 would not interfere with codon-anticodon interaction (27) which is consistent with reports that there are only minor differences between the binding of (Q+) and (Q-)tRNA to ribosomes in response to triplet codons (8,28).

Isolation and identification of queuine-containing tRNAs

The most widely used procedure for separating isoacceptor tRNAs is known as reversed phase chromatography-5 or RPC-5 (29). RPC-5 is an anion exchange system in which a trialkyl (C_8-C_{10}) methyl ammonium chloride is adsorbed onto polychlorotrifluoroethylene. Separation occurs due to par-

tition of the tRNA between the immobilized quaternary amine and a mobile aqueous phase. This technique separates the (Q+)tRNAs from the (Q-) isoacceptors. The tRNAs are usually charged with a radioactively labeled amino acid and the tRNA is then adsorbed to the resin. The column is developed with a linear NaCl gradient at pH 4.5. The (Q+) isoacceptors are eluted before their (Q-) counterparts (30). The presence of Q can be confirmed by reacting the tRNA with BrCN or periodate prior to RPC-5 chromatography. Reaction with either of these reagents causes the (Q+)tRNA to be eluted at higher ionic strength. The chromatographic mobility of (Q-)tRNA isn't affected by either of these reagents (5-7,12). A typical RPC-5 chromatogram to identify (Q+)tRNA is shown in Fig. 2. The (Q+) peak had disappeared and a new peak that eluted at fraction number 72 appeared. The elution position of the (Q-)$tRNA^{Asn}$ was not changed by BrCN. Polystyrene anion exchange columns can also be used to separate (Q+) and (Q-)tRNAs (31). Affinity chromatography based on complex formation between the *cis* hydroxyl groups of Q with boronic acids has also proven to be useful for isolating the (Q+)tRNAs. McCutchan *et al.* prepared acetylated dihydroxyboryl-cellulose (DBAE-cellulose) and found that (Q+)tRNAs form stable complexes with this resin via the *cis*-diol group at slightly

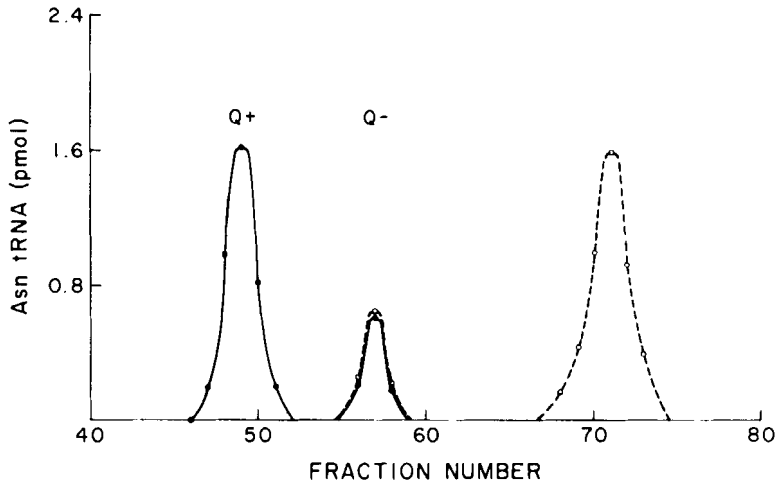

FIG. 2

Effect of BrCN on the RPC-5 profiles of $tRNA^{Asn}$. A sample of tRNA was reacted with BrCN and then charged with [^{3}H]Asn. A control sample of tRNA that was not reacted with BrCN was charged with [^{14}C]Asn and the samples were cochromatographed. (---) Asn tRNA that was reacted with BrCN; (——) control Asn tRNA.

alkaline pH. After tRNAs that do not contain Q are eluted, the (Q+)tRNAs are eluted with dilute acid (32). This technique has also been used to isolate (Q+)tRNA precursors that have not yet been trimmed by precursor tRNA-processing nucleases (33). Some plants contain proteins called lectins that bind to certain carbohydrates with high specificity, and these lectins can be bound to inert insoluble matrices such as Sepharose. Since $tRNA^{Asp}$ and $tRNA^{Tyr}$ have a mannose or galactose residue respectively bound to Q, these lectin-Sepharoses can be used to isolate these two tRNAs. When unfractionated liver tRNA was passed through a concanavalin A Sepharose column, only $tRNA^{Asp}$ was bound. (Concanavalin A has a high affinity for mannose.) When the bulk tRNA was passed through a *Ricinus communis* lectin-Sepharose column, only $tRNA^{Tyr}$ was retained on the column since this lectin has an affinity for galactose (16). Other modified nucleosides found in tRNAs, such as 7-methylguanine, have been shown to be antigenic and antibody specificity has been used to purify tRNAs (34). The antibody technique has not yet been applied to the (Q+)tRNAs.

The enzymes that insert queuine or its precursors into tRNA

The enzymatic modification that leads to the insertion of Q into tRNA is unique. The synthesis of the other modified nucleosides involves chemical alterations of one of the canonical RNA nucleosides by enzymes that operate directly on the nucleoside at the polynucleotide level. Queuine is synthesized separately from tRNA and it or one of its precursors is then inserted into the polynucleotide chain without cleavage or synthesis of a phosphodiester bond. Another unique character of queuine in tRNA is that it is the only case where the heterocyclic skeleton of a purine or pyrimidine is altered.

The first indication of the existence of this unusual enzyme was the observation that, when rabbit reticulocytes were incubated in the presence of [^{14}C]guanine, the guanine was incorporated into the tRNA (35). Since mammalian reticulocytes have no nucleus, contain no DNA and cannot synthesize RNA, the guanine had to be incorporated post-transcriptionally into the tRNA. When the tRNA was chromatographed by reversed phase chromatography, it was found that there were only two peaks that contained [^{14}C]-guanine. The tRNAs comprising these peaks were identified as $tRNA^{His}$ (36) and $tRNA^{Asn}$ (12). The insertion of guanine was shown to be enzymatic (36), and the enzyme was purified to homogeneity from rabbit erythrocytes

(7,37,38). Since $tRNA^{Asn}$ and $tRNA^{His}$ were part of the Q family of tRNA, it was suggested that the enzyme that "guanylated" tRNA was somehow involved in the metabolism of Q (7,10,39). However, since queuine was not available, it was not possible to test this hypothesis at that time. Proof that the enzymes was involved in Q metabolism came from a seemingly unrelated series of experiments. A factor (thought to be a hormone-like substance) was identified in fetal calf serum that induced tissue culture cells to produce (Q+)tRNA (40). The factor was found in greater amounts in bovine amniotic fluid and was found to be a potent inhibitor of the guanine insertion reaction catalyzed by the reticulocyte enzyme (41). The factor was purified and was shown to be identical to queuine. The K_m of the enzyme for queuine was 4.5×10^{-8}M which was one-third lower than the K_m for guanine (41). Queuine insertion was also shown to be the true role of this enzyme by the work of Okada *et al.* (42).

The enzyme has been purified to homogeneity from the three major domains of life, animals (rabbit erythrocytes), plants (wheat germ) and a prokaryote (*E. coli*) (38,43,44). In addition, the enzyme has been partially purified from rat liver (45). The physical chemical properties of these enzymes are remarkably different (Table 1). The molecular weight of the bacterial enzyme is 46K and consist of only one subunit. The erythrocyte enzyme has a molecular weight of 104K and consists of 60K and 43K subunits; and the wheat-germ enzyme has a molecular weight of 140K and consists of two 68K subunits. The rat-liver enzyme weighs in at 80K. The *E. coli* enzyme and the wheat-germ enzyme have requirements for Mg^{2+}, a property that is not observed in the mammalian enzymes. The enzyme in brine shrimp (*Artemia salina*) also has a requirement for Mg^{2+} (V. Hiatt and W.R. Farkas, unpublished). The pH optima for all of the enzymes are similar. The mammalian and plant enzymes insert queuine into (Q-)tRNA whereas the bacterial enzymes uses 7-(aminomethyl)-7-deazaguanine (Fig. 1, compound III) rather than queuine as substrate. All of the enzymes catalyze the exchange of free guanine with the guanine in the wobble position in the absence of their true substrates and all have strong affinities for guanine. For the wheat-germ enzyme, the affinity for guanine is actually slightly greater than for queuine, raising the possiblity that the guanine exchange may have some biochemical function rather than being just another futile cycle. Nevertheless, the guanine exchange is very valuable to investigators in queuine research because it presents a very facile method for assaying for the enzyme.

TABLE 1

COMPARISON OF GUANINE,QUEUINE-tRNA TRANSGLYCOSYLASE FROM DIFFERENT SOURCES

PROPERTY	*E. COLI*	RABBIT ERYTHROCYTE	RAT LIVER	WHEAT GERM
Substrate	7-(aminomethyl)-7-deazaguanine	queuine	queuine	queuine
Molecular Wt.	46,000	104,000	80,000	140,000
No. of subunits	1	2	-	2
Molecular Wt. of subunit	46,000	60,000+43,000	-	68,000+68,000
pH optimum	7.0	7.8	7.3	7.6
K_m guanine	5.3×10^{-8}M	1.5×10^{-7}M	8.3×10^{-7}M	6.1×10^{-8}M
K_m queuine	not a substrate	4.5×10^{-8}	2.9×10^{-7}M	9.5×10^{-8}M
K_m amino compound	1.4×10^{-8}M	-	2.1×10^{-6}M	-
Specific activity of pure enzyme	50,000	153	55	2,029
Requirement for Mg^{2+}	yes	no	no	yes

The mammalian enzyme will replace guanine in the wobble position of mammalian tRNA with queuine but will not reverse this process and excise the queuine and replace it with guanine (41,45). However, the enzyme will excise queuine from the wobble position of *E. coli* tRNA and replace it with guanine (45). The enzyme from *E. coli* does not utilize queuine as a substrate, and it will not replace queuine in mammalian tRNA with guanine (44).

When one compares the specific activities of the enzymes from the different sources from which it has been purified, a great disparity is apparent. The *E. coli* enzyme is 327X more active than the erythrocyte enzymes and 25X more active than the enzyme from wheat germ.

Dietary requirement for queuine by mammals

Since bacteria synthesize Q, experiments to determine whether or not higher mammals can synthesize queuine had to be carried out with germfree mice. A group of mice were fed a chemically defined diet (CD diet) that did not contain queuine, whereas a second group of germfree mice were fed commercial mouse chow. After four weeks (9.3 tRNA half lives), there was a marked decrease in the proportion of (Q+)tRNA in the mice fed the CD diet. The loss of (Q+)tRNA was limited to $tRNA^{Asn}$ and $tRNA^{His}$; $tRNA^{Asp}$ and $tRNA^{Tyr}$ were entirely in the (Q+)state (46). The queuine-deficient diet was also fed to mice maintained in a conventional rather than a germfree facility, and there was no depletion of (Q+)tRNA from the livers of these mice, indicating that the depletion of (Q+)tRNA was not due to a toxic effect of the CD diet. This experiment also indicated that the intestinal flora was capable of providing queuine (46). When the length of time that the mice were maintained on the queuine-deficient diet was extended to one year, the livers were completely depleted of (Q+)tRNA. These results are summarized in Table 2. The (Q+)tRNA could be restored to normal levels, either by injecting the queuine i.p. or by adding it as a dietary supplement; (Q+)tRNA was also a good source of dietary queuine (47). Since the mammalian gua,que-tRNA transglycosylase uses queuine and not queuosine as its substrate, the latter observation presages that there is a mammalian enzyme that cleaves the N glycoside bond of queuosine. When the queuine-depleted mice were titrated with queuine, the $tRNA^{Asp}$ was converted to the (Q+) form before $tRNA^{His}$, showing that the affinity of the queuine insertion enzyme was greater for $tRNA^{Asp}$ than for $tRNA^{His}$.

TABLE 2

PERCENTAGE OF THE (Q+) ISOACCEPTOR IN $tRNA^{Asn}$, $tRNA^{His}$, $tRNA^{Asp}$ and $tRNA^{Tyr}$ IN GERMFREE AND CONVENTIONAL MICE FED A CHEMICALLY DEFINED DIET AND A NORMAL DIET

	GERMFREE MICE			CONVENTIONAL MICE	
	Normal diet	CD diet		Normal diet	CD diet
		4 wk	1 yr		4 wk
$tRNA^{Asn}$	85	15	0	85	85
$tRNA^{His}$	85	15	0	85	88
$tRNA^{Asp}$	100	100	0	100	100
$tRNA^{Tyr}$	100	100	0	100	100

Queuine is in the category of compounds such as biotin and vitamin B_{12} for which a dietary requirement is difficult to demonstrate due to synthesis of these compounds by the gut flora. Deficiency in the transport of vitamin B_{12} leads to the highly fatal disease known as pernicious anemia, and a genetic defect in the processing and possibly the transport of biotin has been described (48). Whether or not similar metabolic dyscrasias involving the transport or processing of queuine also exist, remains to be determined. The queuine-deficient mice have a normal appearance and appear to be as active as control mice. Some of the mice were subjected to extensive post-mortem examination by light and electron microscopy and no obvious pathology was found. Experiments on the effect of queuine deficiency on reproduction and aging are in progress.

As mentioned above, yeast do not contain queuine in their tRNA. The possibility that yeast, like higher mammals, could not synthesize queuine but would make (Q+)tRNA if a source of queuine were available, has been explored. Since the medium on which yeast cells are grown under laboratory conditions does not contain queuine, queuine was added to the yeast growth medium and the yeast cells were also grown on natural substrates (fresh fruits) and the yeast tRNA still did not contain queuine, indicating that yeast cells not only do not contain Q but will not incorporate it into their tRNA even if it is present (49).

Function of queuine in tRNA

Since Q is in the wobble position and should be able to base-pair with either C or U, one of the first studies that was carried out was to determine the binding efficiency of the *E. coli* (Q+)tRNAs to NAC and NAU codons. These studies indicated that there was a slightly greater tendency for Q to wobble with U than with C (8). The codon-directed binding of mammalian (Q+) and (Q-)$tRNA^{Asn}$ and $tRNA^{His}$ was studied by Smith *et al.* who showed that both (Q+) and (Q-)His tRNA were bound to ribosomes in response to both of the histidine codons CAC and CAU. For both (Q+) and (Q-)His tRNA, the binding was somewhat stronger in response to CAU than to CAC (28). These workers also found that histidine bound to either (Q+) or (Q-)$tRNA^{His}$ was distributed equally among all of the histidine residues of hemoglobin (50). These findings indicate no significant differences in reading NAC or NAU codons by (Q+) and (Q-)tRNA. This is not surprising since the chemical alteration of guanine to form queuine should not interfere with the Watson Crick rules for base pairing. Hatfield *et al.* have found that human reticulocytes have more (Q-)$tRNA^{Asp}$, $tRNA^{Asn}$ and $tRNA^{His}$ relative to the corresponding (Q+)tRNA than do rabbit reticulocytes (51). The major differences in frequency of codon usage between human and rabbit globin mRNAs occur in the codons of these three amino acids (51 and references therein). The significance of these facts remains to be established. Landin *et al.* have reported that there is more (Q-)$tRNA^{His}$ in fetal sheep liver than in adult sheep liver. This (Q-)tRNA cochromatographs on RPC-5 with the minor (Q-)$tRNA^{His}$ of rabbit reticulocytes shown by DuBrul and Farkas (7) to be the reactive tRNA in guanine exchange. Since fetal liver performs an erythropoietic function and adult liver does not, Landin *et al.* suggested that (Q-)$tRNA^{His}$ plays a role in hematopoiesis (52). However, it is also a fact that the amount of (Q-)-tRNA is elevated in rapidly growing cells (see below) and, this observation might reflect the fact, that fetal liver cells are proliferating at a greater rate than adult liver, rather than that (Q-)$tRNA^{His}$ has a hematopoietic function. Regenerating adult liver also has more (Q-)tRNA than does normal adult liver (53,54).

Singhal has recently found that the (Q+) isoacceptor of $tRNA^{Asp}$ of mammalian liver is aminoacylated with a 16-fold higher V_{max} than the (Q-) isoacceptor, although the K_ms of both isoacceptor tRNAs for AsptRNA synthetase are identical (55). Thus, failure of a cell to modify (Q-)$tRNA^{Asp}$

to (Q+)tRNA^{Asp} could serve to slow down the rate of protein synthesis. If this putative mechanism operated in controlling protein biosynthesis, one would expect to find a large number of nascent polypeptide chains on polysomes with C terminal amino acid just before aspartic acid in cells that contained elevated levels of (Q-)tRNA such as reticulocytes and tumor cells. Since the major protein synthesized by reticulocytes is hemoglobin and the amino acid sequence of hemoglobin is well known, it should be possible to test this interesting hypothesis.

There is another biological system that lends credence to this theory. The slime mold, *Dictyostelium discoideum*, is an amoeboid organism when grown on nutritionally enriched media. However, when *D. discoideum* is shifted to nutrient-starved media, it undergoes a sequence of morphological changes resulting in fungus-like characteristics. These morphological changes are accompanied by biochemical alterations such as a decrease in polyribosomes and a change in the types of proteins being synthesized (56,57). Dingermann *et al.* have recently found that two minutes after the onset of the developmental changes described above, the aminoacylation of tRNA^{Asn} was reduced by 30%; whereas 10 other tRNA species were found to be charged to normal levels with their cognate amino acids. The decrease in charged tRNA^{Asn} could be accounted for by a single unaminoacylated tRNA^{Asn} isoacceptor which was (Q-) (58).

The genetic code letters for tyrosine are UAU and UAC which differ by only a single nucleoside from the amber and ochre termination codons UAG and UAA. Lagerkvist has pointed out that in many cases, only the first two nucleosides of the codon are required for interaction between the mRNA and the anticodon of the tRNAs (59). The termination codons are supposed to be an exception to this two-letter code but as will be described below, (Q-)tRNA^{Tyr} does, to a limited extent, recognize UAG.

Tobacco mosaic virus contains two proteins of molecular weights 160K and 110K with identical amino terminal sequences. A possible explanation of this is schematically described in Fig. 3. The two proteins are translated from the same mRNA which contains a termination triplet after the 110K sequence. If a tRNA would occasionally read through this stop signal, the 160K protein would be synthesized. To test whether the (Q-) and (Q+)tRNA^{Tyr} isoacceptor might be involved in this phenomenon, Bienz and Kubli coinjected either (Q+) or (Q-)tRNA^{Tyr} along with TMV mRNA into Xenopus oocytes and incubated the oocytes in medium containing [^{35}S]-

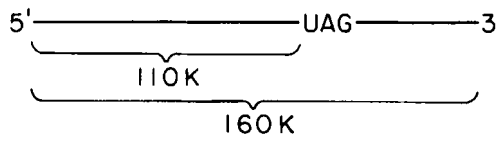

FIG. 3

Schematic diagram of a TMV mRNA containing information for 110K and 160K polypeptides.

methionine. They analyzed the proteins synthesized by SDS gel electrophoresis. The results showed that no 160K protein was made when (Q+)-$tRNA^{Tyr}$ was injected and that there was a marked enhancement of the amount of 160K synthesized in the presence of (Q-)$tRNA^{Tyr}$, indicating that the $tRNA^{Tyr}$ with Q in the anticodon was less likely to recognize and read-through a termination codon. Thus at least in $tRNA^{Tyr}$, the function of Q may be to prevent the misreading of the termination codons UAG and UAA (60).

The readthrough phenomenon may be a mechanism for controlling gene expression (61). There is evidence that the genome of RNA retroviruses consist of 4 genetic regions (see Fig. 4). The gag region codes for the internal structural proteins of the virion; pol codes for the RNA-dependent DNA polymerase, also known as reverse transcriptase. Env codes for the glycoprotein of the outer surface, and the src region which is responsible for neoplastic transformation codes for a protein kinase. The env and src regions are coded for by individual mRNAs. One mRNA codes for both the gag and pol products. The gag and pol regions are encoded in the same 35S mRNA, but in infected cells, the ratio of the molar concentration of polymerase protein to the gag structural proteins is between 0.01-0.1, indicating that there must be translational control leading to synthesis of more gag than pol proteins. Evidence for suppression of a termination codon between the gag and pol regions by a suppressor tRNA has been presented. In this scheme a suppressor tRNA would compete with the translational termination factor for the termination codon between gag and pol but the efficiency of the termination factor is greater than that of the nonsense suppressor tRNA for recognizing the termination codon, leading to 10 to 100 fold more gag than pol protein. Presumably a peptidase cleaves the mixed gag-pol polypeptide after translation (62).

The relative amount of (Q+)tRNA and (Q-)tRNA changes very dramatically during *Drosophila* metamorphosis. The life cycle of *Drosophila*

FIG. 4

Genome of a transforming RNA retrovirus.

starts with an egg. After the egg hatches, there are three larval stages called instar 1, 2 and 3. At the end of the third instar stage, the larvae pupate and adult flies emerge from the pupae. White *et al.* showed that for each member of the Q family, there is a steady decline in (Q+)isoacceptors during each of the instar stages. At the third instar stage, there is virtually no (Q+)tRNA. Towards the end of pupation, the (Q+) isoacceptor begins to increase. In the adult flies the increase in the (Q+) form continues for about two weeks until the (Q+) form is about 50% (30). These dramatic changes in the Q family which correlate with defined morphological events in the life cycle were thought to play a role in cell differentiation. However, it has recently been shown that environmental factors, such as diet and temperature, can alter the levels of (Q+)tRNA (63,64).

Another puzzling observation is that when Cd^{2+} is present in the environment during *Drosophila* development, the relative amount of (Q+) tRNAs is increased over that of control cultures (65), suggesting that (Q+)tRNA may have a role in the metabolism of cells growing in the presence of toxic heavy metals.

Relationship between Q and tumorogenesis

With the advent of the reversed phase chromatographic systems for separating tRNAs, investigators began comparing the tRNA of normal cells with those of tumor cells (for reviews see 66,67). Most of these studies were carried out before the discovery of queuine; nevertheless, many differences were observed between the chromatograms of normal and tumor-cell tRNAs involving the Q family of tRNAs. In all cases involving differences in the elution patterns of the Q family, it was observed that the tumor tRNAs had elevated amounts of the (Q-) isoacceptors. Okada *et al.* showed that there was more tRNA in tumor cells that underwent the guanine exchange reaction than in normal cells (53). Their data also showed that the effect was not limited to tumor tRNA but occurred with tRNAs from other rapidly proliferating tissues such as regenerating rat liver (53). Katze showed that the (Q-)tRNA of Ehrlich ascites cells growing in mice

was converted to the (Q+) form when mice were injected with exogenous queuine (68). In Katze's study, there also was a decrease in tumor mass.

Recent studies by Elliot and Trewyn show that 7-methylguanine and 1-methylguanine transform Chinese hamster embryo cells to a neoplastic state (69). The neoplastic transformation is accompanied by the appearance of extensive amounts of (Q-)tRNA. One of these compounds, 7-methylguanine, is a potent inhibitor of gua/que-tRNA transglycosylase, and it is conceivable that inhibition of the enzyme and concomitant production of (Q-)tRNA may be an initiating factor in oncogenic transformation. Mitigating against this mechanism is the fact that 1-methylguanine also transforms Chinese hamster embryo cells with elevation of (Q-)tRNA; however this compound is a poor inhibitor of the transglycosylase (69). Furthermore, the tRNA of mice that have been starved of queuine are extremely deficient in (Q+)tRNA (47), and these Q-deficient mice have no pathological lesions indicating neoplastic transformation (70). The excretion of elevated levels of methylated purines and pyrimidines, including 7-methylguanine, is observed in many forms of cancer (66). It is possible that compounds such as 7-methylguanine might play a role in explaining the appearance of Q-deficient tRNA in normal tissues that are distant from the site of the tumor (71) as suggested in the work of Elliott and Trewyn (69).

FOOTNOTE

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Nomenclature: The nucleoside of queuine is queuosine which can be abbreviated as Q. Transfer RNAs that contain queuine in the anticodon are designated (Q+)tRNA. The precursors of the (Q+)tRNAs that contain guanine rather than queuine in the first position of the anticodon are designated (Q-)tRNA.

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