PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 170, 328–332 (1982)

Yeast Cells Cannot Incorporate Queuine into Their tRNA (41438)

TOM WALDEN, JON P. REYNIERS, VIVIAN HIATT, AND W. R. FARKAS[1]

Department of Medical Biology, University of Tennessee Center for the Health Sciences, 1924 Alcoa Highway, Knoxville, Tennessee 37920

Abstract. The compound known as queuine has been found in the tRNAs of virtually all animals, plants, and bacteria. It is absent in yeast tRNA. Mice have a dietary requirement for queuine and are dependent either on their diet or intestinal flora for this hypermodified purine. We tested the possibility that yeast were like higher mammals in that they could not synthesize queuine but would incorporate it into their tRNA if it was made available to them. This report shows that this is not the case and that yeast cells not only cannot synthesize queuine but they cannot incorporate exogenous queuine into their tRNA.

Queuine is a hypermodified derivative of guanine found in the first or wobble position of the anticodon of the tRNAs for aspartic acid, asparagine, tyrosine, and histidine (1). For each of these tRNAs, there is also an isoacceptor that contains guanine instead of queuine in the wobble position. The guanine-containing isoacceptor, known as (Q−)tRNA, is a precursor of the queuine-containing isoacceptor known as (Q+)-tRNA. The guanine in the wobble position of (Q−)tRNA is enzymatically excised and queuine is inserted in its place (2). Although the function of queuine is still unknown, it has been found in every species examined with the exception of yeast and mycoplasma (3). Enzymes which insert queuine into tRNA are known as guanine−queuine tRNA transglycosylases and are characterized by their ability to exchange free guanine with the guanine in the first position of the anticodon in the absence of their true substrate, queuine (2, 4). The mouse, and probably other mammals, must obtain their queuine from their diet (5). Kasai *et al.* (3) reported that the tRNA of yeast cells grown under laboratory conditions on a defined medium to which no queuine has been added contained no queuine. However, the natural environment in which yeast cells are ordinarily found is likely to contain queuine. Therefore, we decided to grow yeast cells on their natural substrate, fresh fruits and also on growth media supplemented with queuine in order to determine if, like mice, yeast cells will survive in the absence of exogenous queuine but will utilize it to make (Q+)tRNA if the queuine is available. The possibility that yeast cells might contain queuine was also suggested by the reports of Wosnick and White (6) and Owenby *et al.* (7) who found that the tRNAs extracted from *Drosophila* grown on media supplemented with yeast cells contained much more queuine than did the tRNAs of fruit flies grown on media without yeast supplementation.

Materials and Methods. Yeast (*Saccharomyces cerevisiae*) strain (139) was grown in media consisting of 10 g yeast extract, 20 g bactopeptone, and 20 g of dextrose per liter of water at 37°, and harvested during log phase. Queuine was isolated from bovine amniotic fluid using a modification of the method of Katze and Farkas (2). Yeast aminoacyl tRNA synthetases were prepared and charging of the tRNA was carried out by the procedures described by Bhanot *et al.* (8). Rabbit liver aminoacyl tRNA synthetase was prepared according to Katze and Farkas (2). [^{3}H]-Guanine (1 Ci/mmole) and [^{3}H]aspartic acid (10 Ci/mmole) were from Amersham; [^{14}C]-aspartic acid (50 mCi/mmole) was from Schwarz/Mann and [^{3}H]tyrosine (20 Ci/mmole) was from ICN. ^{3}H-reduced queuine was prepared by catalytically tritiating queuine (Amersham) and was generously supplied by Dr. Jon Katze. It had a specific

[1] To whom reprint requests should be addressed.

0037-9727/82/070328-05$01.00/0

activity of 152 mCi/mmole. Yeast tRNA was prepared according to Holley (9). Assays for guanine–queuine tRNA transglycosylase activity was performed according to Howes and Farkas (4). Reverse phase column 5 chromatography (RPC-5) was performed according to Pearson *et al.* (10), using a 100-ml 0.45 to 0.85 *M* NaCl gradient. The NaCl was dissolved in 0.01 *M* sodium acetate, pH 4.5, 0.005 *M* β-mercaptoethanol, 0.01 *M* $MgCl_2$, and 0.001 *M* EDTA. BrCN treatment of the tRNA was carried out as described by DuBrul and Farkas (11); periodate oxidation of the tRNA was according to Farkas and Chernoff (12).

In order to determine if [^{3}H]queuine was taken up by yeast cells, the cells were suspended in either phosphate-buffered saline or yeast growth medium and ^{3}H-labeled queuine obtained by reducing queuine with tritium gas (performed by Amersham). The cell suspension was incubated at 37° for 10 min. The percentage of the total volume occupied by the cells was determined with a hematocrit centrifuge. The cells were separated from the medium by centrifugation at 15,000*g*. An aliquot of the medium was added to a vial to determine the amount of labeled compound that had not been taken up by the cells. The tubes were rotated 180° so that the cell pellet was now on top and centrifugation was repeated. The small amount of liquid that had adhered to the cells was removed with a Pasteur pipet. This process was repeated until no more medium was removed from the pellet after centrifugation. The cells were now suspended in 2 ml of water and added to a vial containing 10 ml Scintiverse (Fisher). The cells were uniformly suspended in the Scintiverse with vigorous shaking before the counting mixture solidified.

Results and Discussion. Transfer RNA was extracted from yeast cells which had been grown on queuine-supplemented media (69 μg queuine per liter of media). The queuine added to the media was five times the amount that would be necessary to convert all of the (Q−)tRNA to (Q+)-tRNA. This tRNA was charged with [^{3}H]-aspartic acid and cochromatographed on RPC-5 with [^{14}C]aspartyl tRNA prepared from cells grown in the absence of queuine. Queuine-containing tRNAs elute from RPC-5 columns earlier than (Q−)tRNA because queuine has one more positive charge than guanine (11–13). As shown in Fig. 1, the two tRNAs chromatograph identically; thus, queuine was not incorporated into yeast $tRNA^{Asp}$. If queuine had been incorporated, the Asp tRNA would have eluted with a peak at fraction 20 instead of at fraction 25. The absence of queuine in $tRNA^{Asp}$ is especially significant because the queuine insertion enzyme has a greater affinity for $tRNA^{Asp}$ than for the other tRNAs of the queuosine family (14).

Further tests were conducted to confirm that queuine was not incorporated into the yeast tRNA. The tRNA from the cells cultured in queuine-containing media was treated with BrCN and in a separate experiment, with periodate. The reaction of BrCN with the secondary amino group of queuine causes the tRNA to elute at higher ionic strength during RPC-5 chromatography (11). Periodate reacts with the cyclopentenediol residue of queuine causing the

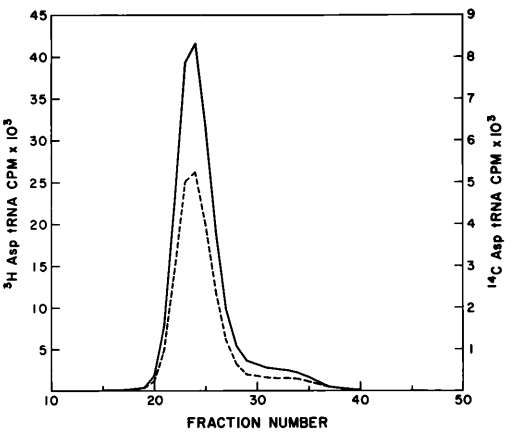

FIG. 1. RPC-5 cochromatography of [^{3}H]aspartyl-"Q supplement" yeast tRNA (——) and [^{14}C]aspartyl-normal yeast tRNA (– – –). The Q supplement tRNA was isolated from cells grown in the presence of queuine. The "normal" tRNA was extracted from yeast cells grown in identical medium but in the absence of queuine. The tRNAs were eluted with a linear gradient of 100 ml of 0.45–0.85 *M* NaCl dissolved in RPC-5 buffer and collected in 1-ml fractions.

tRNA to bind so tightly to RPC-5 columns that it is not eluted by the eluate routinely used for RPC-5 chromatography (12). Neither of these reagents altered the elution profiles of yeast tRNAs extracted from cells grown in the presence or absence of queuine, indicating that those tRNAs charged by the yeast aminoacyl tRNA synthetases did not contain queuine.

E. coli does not utilize queuine for the synthesis of (Q+)tRNA. The substrate for the bacterial enzyme analogous to guanine–queuine tRNA transglycosylase is 7-(aminomethyl)-7-(deazaguanine) rather than queuine (15). This compound is a precursor of queuine. The cyclopentenediol group is added at the polynucleotide level. A possible reason why no (Q+)tRNA was synthesized in yeast cells grown in queuine-containing media was that a derivative of queuine found in the natural substrates rather than queuine itself, was the substrate for the yeast tRNA transglycosylase. The presence of queuine in a large number of animal and vegetable sources has recently been reported (16).

To test this possibility, yeast cells were grown in one liter of media supplemented with 400 g of homogenized fruit (either white grapes or nectarines) to determine if the natural substrate might induce yeast to synthesize queuine-containing tRNA. The isolated tRNAs from "nectarine" or "grape-supplemented" media was charged with [^{3}H]aspartic acid and chromatographed on RPC-5 with [^{14}C]aspartyl tRNA isolated from cells grown on typical laboratory media. The results were identical to the experiment summarized in Fig. 1 indicating that no (Q+)aspartyl tRNA has been synthesized.

Whatever the function of queuine may be in other organisms, this compound is not necessary for yeast cells, and we feel that these experiments demonstrate that yeast does not incorporate queuine into its tRNA. Furthermore, we were unable to detect the enzyme guanine–queuine tRNA transglycosylase in extracts of yeast cells. In our attempts to detect the enzyme in yeast we first assayed for the enzyme in the crude extract of yeast cells that we used as our source of aminoacyl tRNA synthetase. When we found no activity, we passed the extract through a Sephadex G-25 column and again found no activity in the excluded fraction. We also fractionated the yeast extract with ammonium sulfate taking fractions of 0–40, 40–50, and 50–70% of saturation. These fractions were assayed after ammonium sulfate had been removed by passage through Sephadex G-25 columns and were found to be inactive. The mammalian enzyme has a requirement for a monovalent cation (4) and the enzyme from *E. coli* has a requirement for Mg^{2+} (17). We also repeated the assay for the yeast enzyme in the presence of Mg^{2+} at 5, 10, 15, and 20 m*M* and again found no activity.

Since queuine was not incorporated into yeast tRNA, it was of interest to determine if queuine could be taken up by yeast cells. (In order to do this, yeast cells were suspended to the cell densities indicated in Table I; tritiated queuine was added and the samples were incubated for 10 min.) The data summarized in Table I show that the distribution of counts between the cells and the medium was identical to the percentage of cells in the medium indicating that the tritiated queuine was neither excluded from nor concentrated by the yeast cells. The

TABLE I. YEAST CELLS TAKE UP ^{3}H-REDUCED QUEUINE

Type of medium	Percentage yeast cells in medium	Percentage tritiated queuine in yeast cells
Phosphate-buffered saline	14	16
Phosphate-buffered saline	14	12
Yeast growth medium	17	16
Yeast growth medium	17	17

tritiated compound used in this experiment is identical to queuine except that ^3H has been added across the double bond of the cyclopentene ring. This compound is a substrate for the eukaryote guanine–queuine-tRNA transglycosylase (to be published elsewhere) and is readily inserted into yeast tRNA at a rate that is only slightly less than for queuine itself.

Even though the possibility seemed an unlikely one, we decided to determine if queuine might have been incorporated into the tRNAs but that the queuine-containing tRNAs were not aminoacylated by the yeast enzymes. Therefore, we charged the yeast tRNA extracted from cells grown in the presence of queuine with rabbit aminoacyl tRNA synthetases.

As shown in Fig. 2, for aspartic acid the species recognized by the rabbit enzyme was entirely different from that recognized by the yeast enzyme. The yeast tRNA mischarged with Asp by the mammalian enzyme did not contain queuine, as was shown by its failure to react with BrCN (results not shown). The identity of this mischarged peak is unknown but it did not chromatograph with AsptRNA, AsntRNA, HistRNA, or TyrtRNA. The rabbit enzyme also mischarged yeast tRNA with tyrosine. In this case, the mischarged peak was histidyl-tRNA. The complete mischarging of yeast tRNA by the rabbit enzyme with aspartic acid and tyrosine emphasizes the problems that may arise in using an interspecies system.

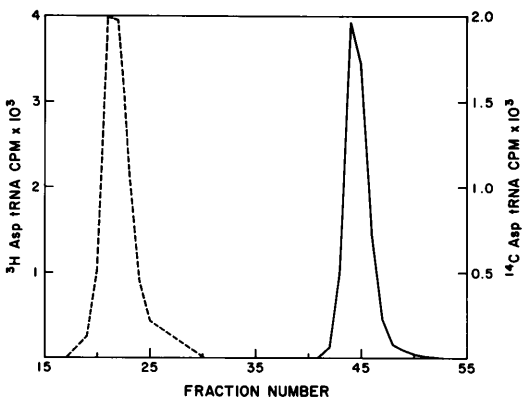

FIG. 2. Mischarging of yeast tRNA with aspartic acid by rabbit aspartyl tRNA synthetase. Yeast tRNA was charged with [^{14}C]aspartic acid using a yeast aminoacyl-tRNA synthetase (– – –), while a similar sample was charged with [^3H]aspartic acid using a rabbit liver aminoacyl-tRNA synthetase (——). Both samples were then cochromatographed on an RPC-5 column and eluted as described in Fig. 1.

The function of queuine in tRNA remains obscure. However, a recent report shows that in the presence of (Q–)tRNA^{Tyr} there is a tendency of the protein synthesizing apparatus of xenopus oocytes to read through the amber and ochre termination codons when the oocytes are programmed with TMV RNA (18). It is possible that at least in the case of tRNA^{Tyr}, the role of Q is to prevent read through of termination codons. In yeast cells the factors that control termination of translation may be more efficient than in other cells thereby obviating the necessity for having Q in the anticodon of tRNA^{Tyr}

This work was supported by United States Public Health Service Grants HD14062, ES01323, and GM20546.

1. Harada F, Nishimura S. 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 anticodons of these transfer ribonucleic acids. Biochemistry 11:301–308, 1972.
2. Katze JR, Farkas WR. A factor in serum and amniotic fluid is a substrate for the tRNA-modifying enzyme tRNA-guanine transferase. Proc Nat Acad Sci USA 76:3271–3275, 1979.
3. Kasai H, Kuchino Y, Nihei K, Nishimura S. Distribution of the modified nucleoside Q and its derivatives in animal and plant transfer RNA's. Nucleic Acids Res 2:1931–1939, 1975.
4. Howes NK, Farkas WR. Studies with a homogeneous enzyme from rabbit erythrocytes catalyzing the insertion of guanine into tRNA. J Biol Chem 253:9082–9087, 1978.
5. Farkas WR. Effect of diet on the queuosine family of tRNAs of germfree mice. J Biol Chem 255:6832–6835, 1980.
6. Wosnick MA, White BN. A doubtful relationship between tyrosine tRNA and suppression of the vermilion mutant in *Drosophila*. Nucleic Acids Res 4:3919–3929, 1977.
7. Owenby RK, Stulberg MP, Jacobson KB. Alteration of the transfer RNA's in adult *Drosophila*

melanogaster as a function of age, nutrition, and genotype. Mech Aging Devel **11**:91–103, 1979.

8. Bhanot OS, Kucan Z, Aoyagi S, Lee FC, Chambers RW. Purification of tyrosine:tRNA ligase, valine:tRNA ligase, alanine:tRNA ligase, and isoleucine:tRNA ligase from *Saccharomyces cerevisiae* αS288C. In: Grossman L, Moldave K, eds. Methods in Enzymology. New York, Academic Press, Vol29:p547, 1974.
9. Holley RW. Large-scale preparation of yeast "soluble" ribonucleic acid. Biochem Biophys Res Commun **10**:186–188, 1963.
10. Pearson RL, Weiss JF, Kelmers AD. Improved separation of transfer RNA's on polychlorotrifluoroethylene-supported reversed phase chromatography columns. Biochim Biophys Acta **228**: 770–774, 1971.
11. DuBrul EF, Farkas WR. Partial purification and properties of the reticulocyte guanylating enzyme. Biochim Biophys Acta **442**:379–390, 1976.
12. Farkas WR, Chernoff D. Identification of the minor guanylated tRNA of rabbit reticulocytes. Nucleic Acids Res **3**:2521–2529, 1976.
13. Katze JR. Alterations in SVT2 cell transfer RNAs in response to cell density and serum type. Biochim Biophys Acta **383**:131–139, 1975.
14. Reyniers JP, Pleasants JR, Wostmann BS, Katze J, Farkas WR. Administration of exogenous queuine is essential for the biosynthesis of the queuosine-containing transfer RNAs in the mouse. J Biol Chem **256**:11591–11594, 1981.
15. Okada N, Noguchi S, Kasai H, Shino-Okada N, Ohgi T, Goto T, Nishimura S. Novel mechanism of post-transcriptional modification of tRNA. Insertion of bases of Q precursors into tRNA by a specific tRNA transglycosylase. J Biol Chem **254**:3067–3073, 1979.
16. Katze JR, Basile B, McCloskey JA. Queuine, a modified base incorporated post-transcriptionally into eukaryote tRNA, is widely distributed in nature. Science, in press.
17. Okada N, Nishimura S. Isolation and characterization of a guanine insertion enzyme, a specific tRNA transglycosylase, from *Escherichia coli*. J Biol Chem **254**:3061–3066, 1979.
18. Bienz M, Kubli E. Wild-type $tRNA_G^{Tyr}$ reads the TMV RNA stop codon, but Q base-modified $tRNA_Q^{Tyr}$ does not. Nature (London) **294**:188–190, 1981.

Received December 7, 1981. P.S.E.B.M. 1982, Vol. 170.