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MODIFICATION OF GUANINE TO QUEUINE IN TRANSFER RNAs DURING DEVELOPMENT AND AGING*

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Summary: The degree of modification of guanine to queuine in the four queuine-containing tRNAs (Q-tRNAs) has been studied from rats of various age groups, and bacterial cells in different growth phases by measuring the amount of G-tRNA present in these tRNA preparations by tRNA-guanine transferase. In very young (one-week old) animals, only a small amount of G to Q modification was observed. However, this modification was essentially complete in the tRNAs of nine-month old animals, thereafter, the amount of Q decreased steadily. Studies of tRNAs from leukemic lymphocytes and bacterial cells indicated that the degree of G to Q modification was related to the metabolic state of the cell. The possible role of the Q-deficient isoacceptors in translation control is discussed.

Transfer RNA populations have been suggested to reflect regulatory events, many of which are age-dependent.[1] Defective tRNAs, a result of changes in the tRNA population, could produce errors in translation, possibly leading to an "error catastrophe".[2] For example, under-modified tRNA may have a capacity of readthrough of nonsense codon resulting in formation of new proteins. If tRNAs are incompletely modified, or if the modifications occur differently during the aging process, perhaps as a result of regulatory changes, the resulting undermodified tRNAs can exhibit lower fidelities of amino-acid selection,[3] slower rates of aminoacylation,[4] or less efficient

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The abbreviations used are: Q, queuosine, that is, 7-{[(*cis*-4,5-dihydroxy-2-cyclopenten-1-yl)amino]methyl}-7-deazaguanosine; Q-tRNA, transfer RNA containing queuine in the "wobble" base position; G-tRNA, queuine characteristic transfer RNA containing guanine instead of queuine (absence of G to Q modification).

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ribosome binding.[5] This work examines the degree of modification of G to Q in Q-tRNAs from various age groups and in other tRNAs from different metabolic states. tRNA-guanine transferase catalyzes an exchange of free G with the G (but, not Q) located in the first position of the anticodon of Q-tRNAs in which this G has failed to modify into Q.[6-8] The degree of G to Q modification in various tRNAs was compared by measuring the G-tRNA with appropriate controls by tRNA-guanine transferase.

MATERIALS AND METHODS

Transfer RNA was isolated from the pooled livers of six animals (Fischer white male rats, Charles River Breeding Lab., Wilmington, MA) of different age groups (0.25, 5, 9, 18 and 26 months), as described elsewhere.[9] The rats (strain No. DCF 344) maintained in a germ-free environment, were nourished *ad libitum* with autoclaved Purina rat chow. tRNA-guanine transferase enzyme was purified from *Escherichia coli*.[10] Bacterial tRNAs from different growth phases and tRNAs from leukemic lymphocytes were prepared as described elsewhere.[11,12] Salmon DNA was purchased from Sigma Chem., St. Louis, MO. Mammalian rRNAs and tRNAs were prepared as described elsewhere.[13] From unfractionated liver and *E. coli* tRNAs, Q-tRNAs were removed by chromatography on boronate columns[14] followed by chromatography on affinity matrices containing Q-specific lectins.[13,15] Absence of Q-tRNAs in these preparations was further confirmed by their failure to aminoacylate with each amino acid specific to a Q-tRNA. [8-^{3}H]Guanine of two different specific activities (5.5 and 8.0 Ci/mmol) from Schwarz-Mann Radiochem., Orangeburg, NY was used in this work.

Assay of guanine exchange. A typical reaction mixture contained 70 m*M* tris-HCl, pH 7.5, 20 m*M* $MgCl_2$, 7.5 µCi (1.3 nmol) of [8-^{3}H]guanine, 0.3 A_{260} unit of unfractionated tRNA and 40 µl of the enzyme preparation (specific activity, 6,500 units per mg) in a total volume of 150 µl. The mixture was incubated at 37° for 1 hour and then cooled in ice. The tRNA was precipitated on a glass fiber disc by adding three volumes of 10% trichloroacetic acid and the pellet was washed successively with 5% trichloroacetic acid, 75% ethanol, and 100% ethanol to remove unreacted labelled guanine. Finally, the disc was dried and radioactivity assayed in a liquid scintillation counter.[9]

RESULTS AND DISCUSSION

Base Specificity of Guanine Exchange.

To examine base specificity in the guanine exchange reaction, and to study the stability of the product, two yeast tRNA samples (0.6 A_{260} unit) were treated with labelled guanine and the transferase enzyme. One reaction mixture was precipitated and washed with trichloroacetic acid (see Methods), but the other was mixed with three volumes of ethanol. In each reaction, the precipitated tRNA was hydrolyzed enzymatically to nucleosides.[16] Label-

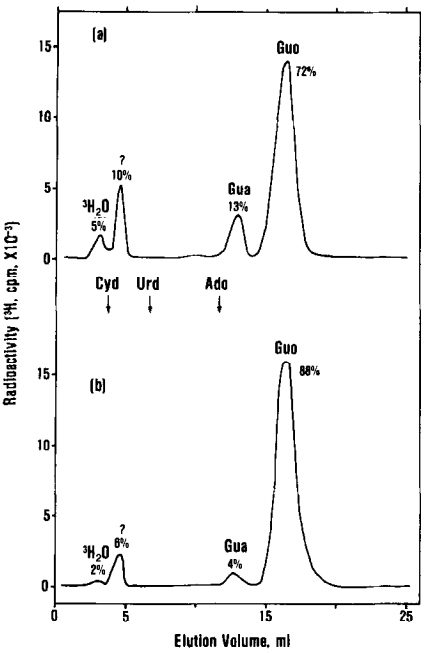

Figure 1. *Separation of nucleosides obtained from the enzymatic hydrolysis of yeast tRNA after reaction with labelled guanine and tRNA-guanine transferase.* The hydrolysis product was separated by anion-exclusion chromatography (BioRad Aminex A-6, 26 x 0.64 cm column) using 20 m*M* $(NH_4)_2CO_3$ brought to pH 9.8 with NH OH and at 50°. (*a*) tRNA-protein precipitate washed with ethanol, (*b*) tRNA-protein precipitate washed with trichloroacetic acid.

The presence of labelled G was perhaps due to incomplete removal of the unreacted G during the washings with ethanol and trichloroacetic acid. Washing of the tRNA-protein precipitate with trichloroacetic acid was more efficient than with ethanol. In each case, a small amount of ^3H_2O was derived by simple exchange with [8-^{3}H]guanosine (or guanine). The component eluting immediately after cytidine could not be characterized. Very little exchange of the tritium from [8-^{3}H]guanosine into ^3H_2O was observed. Treatment of the tRNA-protein precipitate with trichloroacetic acid was more effective than ethanol in removing unreacted labelled G.

led products of the tRNA hydrolysate were separated by anion-exclusion chromatography.[17]

The results in Figure 1 indicate that the radioactivity was mainly present in guanosine and in no other nucleosides. The assay procedure used for this work involved immobilization of the tRNA on a filter disc followed by extensive washing with trichloroacetic acid, etc. (see Methods). This procedure produced very little background radioactivity which was subtracted from each assay (see below).

TABLE I. NON Q-NUCLEIC ACIDS AS SUBSTRATE FOR THE ENZYME tRNA-GUANINE TRANSFERASE

Nucleic Acid	Source	Guanine Incorporated[a]
DNA	Salmon	0.18
rRNA	Bovine liver	0.15
	Rat liver	0.13 ± 0.02
G-tRNA[b]	*E. coli*	0.15 ± 0.01
	Bovine liver	0.15 ± 0.01
Glycine $tRNA_3$[c]	*E. coli*	0.17 ± 0.03
None	(water)	0.11

[a] Picomoles of guanine incorporated into one A_{260} unit of nucleic acid.
[b] Unfractionated tRNAs from which Q-tRNAs have been removed by repeated affinity chromatography.
[c] The anticodon structure of this particular tRNA is very similar to those of Q-tRNAs, however it contains a G in place of Q in position 34 (18).

In the transferase reaction, anything inhibitory for this enzyme would also be interpreted as an indication of Q, therefore, reactions with non-Q-nucleic acids were performed to rule out the presence of any inhibitor in this reaction. In Table I, guanine incorporation into non Q-nucleic acids was about the same as that in a control reaction. Little or no reaction of guanine with $tRNA_3^{Gly}$ (*E. coli*) further indicated the strict specificity of this enzyme. (No radioactivity was found in Q-tRNAs when free guanine was replaced by free adenine.)

Deficiency of Queuine in tRNAs.

The degree of Q deficiency in the Q-tRNAs of five age groups of rats was examined (Table II). These data show a rapid drop-off in the level of G incorporation per unit of tRNA, as the age of the rats increases. In particular, there is a sharp drop off in the amount of G-tRNAs after one week and, after that, the level simply fluctuates around 3.5 ± 0.2 pmol of G-tRNAs per ≈1.8 nmol of the "global" tRNAs. Thus, the major item of interest is the initial drop off in G incorporation. The G incorporation reaches a plateau

TABLE II. CHANGES IN THE AMOUNT OF G-tRNAs WITH AGE[a]

Age (months)	Guanine Incorporated[b]
0.25	42.2 ± 0.6
5	4.1 ± 0.1
9	1.8 ± 0.3
18	2.7 ± 0.1
26	5.6

[a] tRNAs from pools of male rat livers of various age groups were assayed for G incorporation by the guanine-tRNA transferase reaction.

[b] Picomole of guanine incorporated into one A_{260} unit of the unfractionated tRNA. Average mean of three independent assays.

under our reaction conditions (Singhal, *et al.*, unpublished results). Therefore, assuming that the enzyme remains fully active during incubation, this was the maximum amount of G that can be incorporated in these tRNAs (Table II).

A large deficiency of Q in the tRNAs from one-week old animals can be regarded *a priori* as being caused by a reduced level of the G to Q modifying enzymes or by a high degree of turnover of the tRNAs during development, or both. To test this hypothesis, several bacterial tRNAs derived from actively dividing log phase cells were examined. A high degree of modification of G to Q was observed in the four bacteria examined (Table III). The cells growing in the stationary phase lacked Q more significantly than in the log phase. The high turnover of tRNAs in one-week old animals can be compared with similar turnovers in leukemic lymphocytes (*cf.* Tables II and III).

The changes observed in tRNAs may, in several cases, represent incomplete modification of G to Q in position 34 of the anticodon. Q-deficient isoacceptors (G-tRNAs) in a given system may serve the postulated role of tRNA in translation control.[19] There are indications that the relative amount of hexosylqueuosine is higher in ascites hepatomas than in normal rat liver.[20] During the differentiation of *Drosophila* pupae, the level of Q

TABLE III. CHANGES IN THE AMOUNT OF G-tRNAs FROM DIFFERENT ORGANISMS

tRNAs (source)	Guanine Incorporated[a]	
	Cells harvested in log phase	Cells harvested in stationary phase
Bacteria		
E. coli	0.73	3.6
B. megaterium	4.5	14.7
B. subtilis	3.4	--
B. lichieniformis	6.3	--
Non Bacteria		
S. cerevisiae	45.6	
Leukemic lymphocytes	10.6	

[a] Picomoles of guanine incorporated into one A_{260} unit of the unfractionated tRNA. The values are average of three independent reactions, and are in excess of the guanine exchanged with rRNA, used as the control.

fluctuates.[21] Our results indicate that the level of Q in young, mature and old rats appears to change in an ordered manner. Thus, the synthesis of Q in tRNAs is perhaps related to the metabolic state of the cell. While this modification is very low (one week old animals) or significantly absent in actively-dividing mammalian cells (leukemic lymphocytes), it is relatively complete in bacterial tRNAs harvested from the cells in exponentially growing log phase. This difference could be the result of differences in G to Q modification which may exist in these two types of organisms.

REFERENCES

1. Adelman, R. (1975) Federation Proc. 34, 179-182.
2. Orgel, L. E. (1963) Proc. Natl. Acad. Sci., U.S., 49, 517-521.
3. Peterkofsky. A., Jesensky, C., and Capra, J. (1966) Cold Spring Harbor Symp. Quant. Biol. 31, 515-524.
4. Kitchingman, G., Webb, E. and Fournier, M. J. (1976) Biochemistry 15, 1848-1857.
5. Gefter, M. and Russell, R. L. (1969) J. Mol. Biol. 39, 145-157.
6. Farkas, W. R. and Chernoff, D. (1976) Nucleic Acids Res. 3, 2521-2529.
7. Okada, N., Shindo-Okada, N., Sato, S., Itoh, Y. H., Oda, K. and Nishimura, S. (1978) Proc. Natl. Acad. Sci., U.S.A. 75, 4247-4251.
8. McKinnon, R. D., Wosnick, M. A., and White, B. N. (1978) Nucleic Acids Res. 5, 4865-4876.

9. Kopper, R. A. and Singhal, R. P. (1979) Intl. J. Biol. Macromol. 1, 65-72.
10. Okada, N. and Nishimura, S. (1979) J. Biol. Chem. 254, 3061-3066.
11. Singhal, R. P. and Vold, B. (1976) Nucleic Acids Res. 3, 1249-1261.
12. Singhal, R. P., Delmez, B. L., Street, T. L., Hiesterman, G. W. and Yeary, T. J., in Proceedings of Interntl. Symp. Biomol. Structure, Conformation, Function and Evolution, Pergamon Press, Oxford, 1980, Vol. 1, pp. 285-292.
13. Vakharia, V. N. and Singhal, R. P. (1979) J. Appl. Biochem. 1, 210-220.
14. Singhal, R. P., Bajaj, R. K., Buess, C. M., Smoll, D. G. and Vakharia, V. N. (1980) Anal. Biochem. 109, 1-11.
15. Garcia, C. M. and Singhal, R. P. (1979) Biochim. Biophys. Res. Commun. 86, 697-703.
16. Singhal, R. P. (1974) Biochemistry 13, 2924-2932.
17. Singhal, R. P. (1974) Separation Purification Mds 3, 339-398.
18. Singhal, R. P. and Fallis, P. A. M. (1979) Progr. Nucleic Acid Res. Mol. Biol. 23, 227-292.
19. Simpson, D. R., Arfin, S. M., and Hatfield, G. W. (1975) Fed. Proc. 34, 586.
20. Kasai, H., Kuchino, Y., Nihei, K. and Nishimura, S. (1975) Nucleic Acids Res. 2, 1931-1939.
21. White, B. N., Tener, G. H., Holden, J. and Suzuki, D. T. (1973) J. Mol. Biol. 74, 635-651.