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# QUEUINE HYPOMODIFICATION OF tRNA INDUCED BY 7-METHYLGUANINE

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SUMMARY: Transfer RNA isolated from Chinese hamster cells transformed by 7-methylguanine is hypomodified for queuine. 7-Methylguanine rapidly induces queuine hypomodification of tRNA in normal Chinese hamster embryo cells under conditions leading to transformation, and the enzyme catalyzing the queuine modification reaction, tRNA: guanine transglycosylase, is inhibited by 7-methylguanine *in vitro*.

Extensive post-transcriptional modification of tRNA generates macromolecules containing a vast array of altered purines and pyrimidines. Queuine (Q-base), a highly modified guanine analog, is one such modification that is found in the first position of the anticodon of tRNA isoacceptors for histidine, tyrosine, aspartic acid, and asparagine (1). Transfer RNA isolated from normal mammalian cells is almost fully modified for Q-base. However, tRNA from tumor tissue or cells transformed *in vitro* exhibits pronounced hypomodification for Q-base (2). This structural change in the anticodon leads to the appearance in transformed cells of unique tRNA isoaccepting species with altered chromatographic characteristics *in vitro* (2-4).

The enzymatic modification generating Q-containing tRNA is unusual, in that it involves a direct replacement of Q-base for guanine (5,6). The enzyme responsible for catalyzing this reaction is tRNA: guanine transglycosylase (5,6), and a generalized reaction scheme for the mammalian enzyme is depicted in Figure 1. The enzyme was discovered in rabbit reticulocytes by its ability

Abbreviations: Q-base, queuine [7-(3,4-*trans*-4,5-*cis*-dihydroxy-1-cyclopenten-3-ylamino- methyl)-7-deazaguanine]; ChH, normal Chinese hamster embryo cells; ChH-1G, Chinese hamster embryo cells transformed with 1-methylguanine. ChH-7G, Chinese hamster embryo cells transformed with 7-methylguanine.

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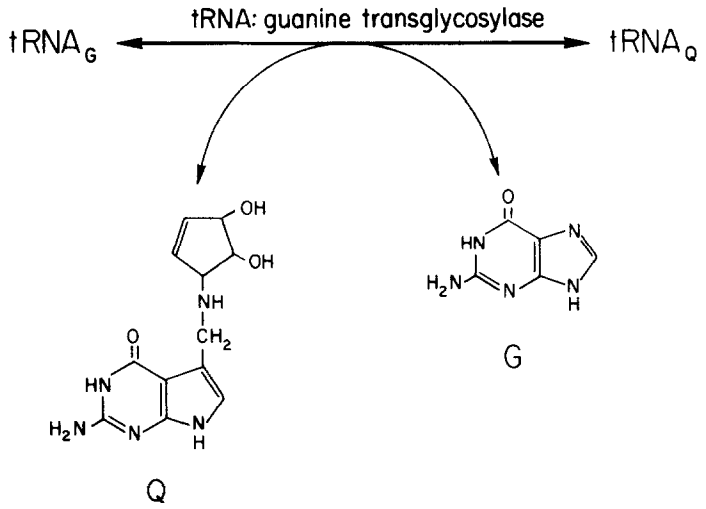


Fig. 1. tRNA: guanine transglycosylase reaction responsible for exchanging Q-base for guanine in the first position of the anticodon of mammalian tRNA for asparagine, aspartic acid, histidine and tyrosine. The abbreviations are: Q, 7-(3,4-trans-4,5-cis-dihydroxy-1-cyclopenten-3-ylaminomethyl)-7-deazaguanine (queuine); and G, guanine.

to insert radiolabeled guanine into homologous tRNA (7), and with appropriate Q-containing tRNA substrates, the reaction is reversible in vitro (8).

Bacterial tRNA also contains Q-base. However, the tRNA: guanine transglycosylase from Escherichia coli utilizes a precursor of Q-base, 7-(aminomethyl)-7-deazaguanine, as the initial substrate, and then the modification is completed at the macromolecular level (9). E. coli tRNA: guanine transglycosylase has been characterized extensively (2,9,10), and it has been shown to be inhibited very effectively by 7-methylguanine (10).

It was demonstrated previously that chronic exposure of normal Chinese hamster embryo cells to 7-methylguanine or 1-methylguanine can lead to altered cellular growth properties, including in vitro neoplastic transformation (11-13). How normal components of cellular nucleic acids (7-methylguanine and 1-methylguanine) can influence phenotypic expression when supplied to the cell's culture environment has not been established. However, the reported inhibition of E. coli tRNA: guanine transglycosylase by 7-methylguanine led us to investigate whether the methylated purines transforming Chinese hamster cells could inhibit Q-base modification of tRNA. It was surmised that a major structural change (guanine vs Q-base) in the anticodon of specific tRNA species

might play some role in altered gene expression in cells exposed to elevated methylated purine concentrations for prolonged periods.

## MATERIALS AND METHODS

Establishment and propagation of primary cultures of Chinese hamster embryo (ChH) cells were as previously described except that the culture medium was supplemented with 5% fetal bovine serum (11,12). Establishment of transformed ChH cell lines by chronic exposure to 10 μM 1-methylguanine (ChH-1G) and 10 μM 7-methylguanine (ChH-7G) was reported previously (11). Fetal bovine serum deficient in Q-base was prepared by treatment with dextran-coated charcoal as described by Katze (3). Proliferating cells were homogenized in hypotonic buffer, and tRNA was isolated as described by Wilkinson and Kerr (14).

Transfer RNA: guanine transglycosylase was isolated from E. coli MRE 600 cells (Grain Processing Corp.) as described by Okada and Nishimura (10). However, purification of the enzyme was carried only through the DEAE-cellulose chromatography step, since the preparation was free of RNase activity when the RNase-deficient strain MRE 600 was utilized. The E. coli tRNA: guanine transglycosylase was used to assay for Q-hypomodified tRNA as reported by Okada et al. (2). The assay procedure was that of Howes and Farkas (15) with a modified reaction mixture containing: 10 μmoles Tris-HCl (pH 7.4), 53 μmoles KCl, 5 μmoles 2-mercaptoethanol, 1 nmole [8-$^3$H]guanine (1 Ci/mmole), 0.05 to 0.25 $A_{260}$ units tRNA, and 6 units E. coli tRNA: guanine transglycosylase in a total volume of 0.6 ml. Transfer RNA was precipitated and collected on glass fiber filters for scintillation counting (15).

Rabbit erythrocyte lysates were prepared as the source of a mammalian tRNA: guanine transglycosylase as described by Howes and Farkas (15). The lysate was centrifuged at 20,000 x g for 20 minutes and 105,000 x g for 90 minutes and desalted on a Sephadex G-25 column. This enzyme preparation was used to assay 1-methylguanine and 7-methylguanine as enzyme inhibitors. The reaction mixture was as described above for Q-hypomodified tRNA using the E. coli enzyme except 2.0 $A_{260}$ units of Q-deficient yeast tRNA was included as a substrate and the guanine concentration was 1 μM. In this case, the reaction was terminated and extracted with phenol as described by Farkas and Singh (7) before precipitating and collecting the tRNA on glass fiber filters.

## RESULTS AND DISCUSSION

Transfer RNA isolated from transformed ChH-1G and ChH-7G cells was assayed for deficiency of Q-base using the E. coli enzyme, and the results are presented in Table 1. The tRNAs from cells transformed by the methylated purines were approximately 5-fold better tRNA: guanine transglycosylase substrates than the tRNA from normal ChH cells. The tRNAs from the transformed cells were also better substrates in the assay than the positive control, yeast tRNA.

To determine whether 1-methylguanine and/or 7-methylguanine can inhibit Q-base modification of cellular tRNA directly, normal ChH cells were exposed to the individual methylated purines for six population doublings. E. coli tRNA:

TABLE I. Queuine-hypomodified tRNA from Chinese hamster cells transformed by methylated purines.

| tRNA Source | Guanine Incorporation (pmoles/hr/$A_{260}$ unit)[a] |
|---|---|
| ChH | 3.9 ± 0.4 |
| ChH-1G | 17.6 ± 1.5[b] |
| ChH-7G | 19.9 ± 6.8[b] |
| Yeast | 6.6 ± 1.8 |
| E. coli | <0.2 |

[a] Mean ± standard deviation from 4 experiments.

[b] Significantly different from normal ChH cells; $P \leq 0.05$.

guanine transglycosylase was again used to assay for deficiency of Q-base in the tRNA isolated from the cells, and these results are presented in Figure 2. As can be seen, the short-term exposure of the normal diploid cells to 10 μM 7-methylguanine led to the induction of Q-deficient tRNA. However exposure to

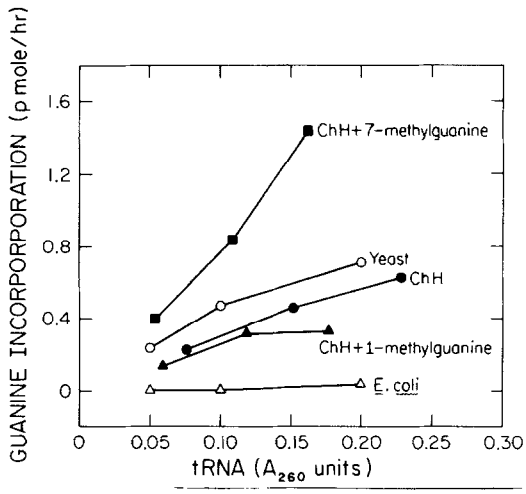


Fig. 2. Hypomodification of ChH tRNA for Q-base induced by 7-methylguanine. Transfer RNA was isolated from ChH cells treated for 6 population doublings with 10 μM 7-methylguanine (■), from ChH cells treated for 6 population doublings with 10 μM 1-methylguanine (▲), and from matched untreated ChH cells (●). Yeast tRNA (○) and E. coli tRNA (△) were used as Q-deficient and Q-sufficient controls respectively. Triplicate reactions were run at each tRNA concentration. See Materials and Methods for additional details.

10 μM 1-methylguanine did not lead to Q-hypomodification of tRNA after six population doublings (Figure 2).

It has been reported that mouse cells either cannot synthesize Q-base or cannot synthesize enough for their needs (16), and therefore, cells in culture obtain most of this modified purine from the serum utilized to supplement the growth medium (5,17). To establish whether ChH cells also require exogenous Q-base for proper tRNA maturation, cells were grown for two population doublings in Q-deficient serum after which the tRNA was assayed for Q-hypomodification. The results are presented in Figure 3, and the requirement for exogenous Q-base is clearly evident.

Transfer RNA: guanine transglycosylase from rabbit erythrocytes was used to assess the direct inhibition of tRNA modification in vitro by 1-methylguanine and 7-methylguanine. These results are presented in Figure 4. As can be seen, 10 μM 7-methylguanine effectively inhibited the mammalian enzyme, whereas 1-methylguanine was much less effective. These results for the isolated rabbit erythrocyte enzyme are consistent with those for the cultured ChH cells presented in Figure 2. However, it remains to be established whether the induction of Q-hypomodification of ChH tRNA by 7-methylguanine occurs by a direct inhibition of the ChH transglycosylase or by the inhibition of transport of exogenous Q-base. These studies are underway, as are examinations of the kinetics of enzyme inhibition.

The possibility that tRNA: guanine transglycosylase inhibitors are responsible for the Q-hypomodification of tRNA in malignant cells in vivo was suggested by other investigators based on comparable enzyme activities being observed in normal Q-sufficient and malignant Q-deficient tissues (6,18). The nature of these putative inhibitors was not established. However, it is known that malignant tissues contain aberrant tRNA methyltransferases (19), and that cancer patients excrete highly elevated levels of tRNA catabolites; especially methylated derivatives (19,20). Therefore, it is possible that increased endogenous methylated purines (e.g. 7-methylguanine) may be involved in inducing the Q-hypomodification of tRNA associated with neoplasia. This could

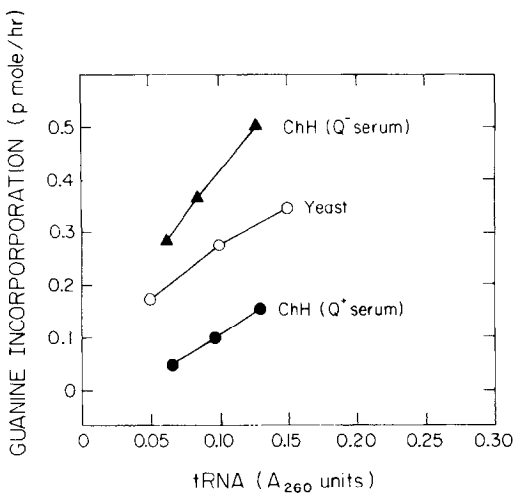


Fig. 3. Hypomodification of ChH tRNA for Q-base induced by culturing cells in Q-deficient serum. Transfer RNA was isolated from ChH cells grown for 2 population doubling in Q-deficient ($Q^-$) serum (▲) or matched Q-sufficient ($Q^+$) serum (●). See legend to Figure 2 and Materials and Methods for additional details.

explain how Q-deficient tRNA is generated in normal tissues far from the tumor origin in animals (21). In addition, it offers an explanation for the source of Q-deficient tRNA in the ChH-1G cells, since it was demonstrated previously that the tRNA methyltransferase activity in these cells is elevated significantly (11).

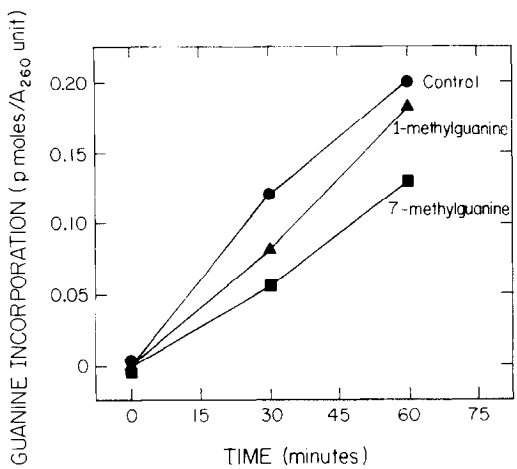


Fig. 4. Inhibition of tRNA: guanine transglycosylase from rabbit erythrocytes by methylated guanines. The assays were carried out as described in the Materials and Methods with the guanine substrate concentration at 1 μM. The methylguanines were added at time zero at a final concentration of 10 μM. The reactions are: Control (●), plus 1-methylguanine (▲), and plus 7-methylguanine (■).

Studies are underway to determine if inhibition of tRNA modification by 7-methylguanine or other methylated purines excreted at high levels by cancer patients has some fundamental role in the expression of carcinogenesis. A model invoking such a role was proposed previously (22).

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