# Diet-dependent depletion of queuosine in tRNAs in *Caenorhabditis elegans* does not lead to a developmental block

RAHUL GAUR[1], GLENN R. BJÖRK[2], SIMON TUCK[3,]* and UMESH VARSHNEY[1,]*

[1]*Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore 560012, India*
[2]*Department of Molecular Biology, and* [3]*Umeå Centre for Molecular Pathogenesis, Umeå University, SE-901 87 Umeå, Sweden*

* *Joint corresponding authors (Fax, 91-80-2360 2697; Email, varshney@mcbl.iisc.ernet.in) (Fax, 46 90 77 80 07; Email: Simon.Tuck@ucmp.umu.se)*

Queuosine (Q), a hypermodified nucleoside, occurs at the wobble position of transfer RNAs (tRNAs) with GUN anticodons. In eubacteria, absence of Q affects messenger RNA (mRNA) translation and reduces the virulence of certain pathogenic strains. In animal cells, changes in the abundance of Q have been shown to correlate with diverse phenomena including stress tolerance, cell proliferation and tumour growth but the function of Q in animals is poorly understood. Animals are thought to obtain Q (or its analogues) as a micronutrient from dietary sources such as gut microflora. However, the difficulty of maintaining animals under bacteria-free conditions on Q-deficient diets has severely hampered the study of Q metabolism and function in animals. In this study, we show that as in higher animals, tRNAs in the nematode *Caenorhabditis elegans* are modified by Q and its sugar derivatives. When the worms were fed on Q-deficient *Escherichia coli*, Q modification was absent from the worm tRNAs suggesting that *C. elegans* lacks a *de novo* pathway of Q biosynthesis. The inherent advantages of *C. elegans* as a model organism, and the simplicity of conferring a Q-deficient phenotype on it make it an ideal system to investigate the function of Q modification in tRNA.

[Gaur R, Björk G R, Tuck S and Varshney U 2007 Diet-dependent depletion of queuosine in tRNAs in *Caenorhabditis elegans* does not lead to a developmental block; *J. Biosci*. **32** 747–754]

## 1. Introduction

Queuosine (Q), a complex modification of guanosine, is found at the wobble (34th) position of the GUN anticodons in transfer RNA (tRNA)$^{Tyr, His, Asn, Asp}$ (Harada and Nishimura 1972). Apart from the known exceptions of yeast, *Thermus thermophilus*, mycoplasma and mycobacteria, Q is found in organisms from all major divisions of life (Kasai *et al* 1975; Katze *et al* 1982; Reader *et al* 2004). The tRNA$^{Asp}$ from mitochondria (Randerath *et al* 1984) and chloroplast (Schon *et al* 1992) are also known to possess Q. The biosynthesis of Q in eubacteria involves a series of reactions (Reader *et al* 2004). The starting molecule GTP is transformed by an unknown mechanism to $preQ_{0,}$ which is then converted to $preQ_1$ by QueF (Van Lanen *et al* 2005; Gaur and Varshney 2005). $preQ_1$ is inserted into tRNA by tRNA guanine transglycosylase (Tgt) by replacing the wobble position guanine of the GUN anticodon (Okada *et al* 1979). Subsequently, $preQ_1$ is converted to epoxy-Q(oQ) on the tRNAs by QueA, and the oQ is further modified to Q by an as yet unknown activity (Frey *et al* 1988). In Archaea, the pathway diverges from $preQ_0$ to form a related modified nucleoside, archaeosine (Gregson *et al* 1993). Interestingly, eukaryotes are thought not to carry out *de novo* synthesis of

**Keywords.** *Escherichia coli* B105; mannosylated queuosine (manQ); Q metabolism

Abbreviations used: cAMP, cyclic adenosine monophosphate; mRNA, messenger RNA; Q, queuosine; Tgt, tRNA guanine transglycosylase; oQ, epoxy-Q; tRNA, transfer RNA.

Q (Kirtland *et al* 1988). Instead, they obtain Q or queuine (the base of Q) from the diet or gut microflora (reviewed by Iwata-Reuyl 2003).

Although eukaryotes are thought to lack the ability to synthesize Q *de novo*, the presence of Q in tRNAs has been strongly conserved in evolution. Eukaryotes possess at least two enzymes specific for Q metabolism, one which salvages queuine from Q (Kirtland *et al* 1988) or preQ (Gunduz and Katze 1984), and a second that glycosylates Q in tRNA (Okada and Nishimura 1977; Haumont *et al* 1987). Whereas eubacterial Tgt uses $preQ_0$ or $preQ_1$ as substrate, the eukaryotic TGT has evolved to insert not only $preQ_0$ or $preQ_1$ but also the salvaged queuine into tRNAs (reviewed in Stengl *et al* 2005). Such an elaborate system suggests that the insertion of Q into tRNA serves an important physiological purpose in eukaryotes.

The Q modification in tRNAs has been implicated in cell differentiation and survival of cells during stress. In *E. coli,* Q has been shown to play a role in viability during the stationary phase (Noguchi *et al* 1982). In the pathogen *Shigella flexneri*, absence of Q drastically decreases translation of *virF* mRNA, and nearly abolishes bacterial virulence (Durand *et al* 2000). In *Dictyostelium discoideum*, Q influences aggregate formation (Kersten 1984), apparently by regulating the synthesis of early cyclic adenosine monophosphate (cAMP)-regulated proteins and cAMP surface receptors (Schachner and Kersten 1984). In mammalian cells, queuine is reported to modulate tolerance to hypoxia (Reisser *et al* 1994), their proliferation (Langgut and Kersten 1990; Langgut *et al* 1993) and the expression of lactate dehydrogenase (Pathak and Vinayak 2005). In many tumours the incorporation of Q into tRNAs is reduced (Randerath *et al* 1984; Emmerich *et al* 1985; Baranowski *et al* 1994), and the extent of such a hypomodification correlates with the stage of the malignancy (Emmerich *et al* 1985). Hypomodification can also be induced by transfection of the cells with the *ras* oncogene (Morgan *et al* 1996). Interestingly, administration of queuine rescues hypomodification and prevents tumour growth (Katze and Beck 1980).

Despite these significant observations in various organisms, and the evolutionary conservation of Q in eukaryotic tRNAs, there are only two reports describing the consequence of Q depletion in an animal system. Both *Drosophila melanogaster* and mice have been shown to lack the ability to synthesize Q *de novo* (Reyniers *et al* 1981; Siard *et al* 1991). *Drosophila* embryos raised on a diet lacking Q develop normally but show increased sensitivity to cadmium (Siard *et al* 1991). Mice kept for a year in germ-free conditions on a chemically defined diet lacking Q (Reyniers *et al* 1981) showed no visible defects. Plants may also lack the ability to synthesize Q. tRNA isolated from lupin and parsley cells cultured in suspension in medium lacking Q does not contain Q (Pawelkiewicz *et al* 1986). While these studies are informative, the necessity of raising individuals on chemically defined media under germ-free conditions makes the use of these systems for studying the biology of Q less than optimal.

We report that the nematode worm, *Caenorhabditis elegans* is a convenient organism to study Q metabolism and function. The complete development of this organism has been described at single-cell resolution (Sulston *et al* 1983) and the cultivation of worms in the laboratory is both fast and convenient. We show here that *C. elegans* worms grown under standard conditions contain Q in their tRNA. However, tRNA isolated from worms grown on a diet of bacteria lacking Q does not contain detectable levels of Q suggesting that *C. elegans* lacks the ability to synthesize Q *de novo*.

## 2. Materials and methods

### 2.1 *Growth, culture and brood size counting of Caenorhabditis elegans*

Overnight standing cultures of *E. coli* were grown at 37°C by inoculating isolated colonies into 100 ml 2YT medium (Sambrook *et al* 1989) and stored at 4°C for up to two weeks. Lawns of various *E. coli* strains were made by spreading 0.1–0.2 ml of the culture on nematode growth medium (NGM) agar (Brenner 1974) plates (60 mm) and keeping them at room temperature for 2–3 days. *C. elegans* N2 worms were grown at 20°C on the NGM plates containing the desired bacteria and maintained by consecutive transfers of the L4 progeny worms to fresh plates of the same strain of *E. coli*. For tRNA isolation, the worms were grown as follows. The bacterial cultures (0.6–0.7 ml) were spread on 20 100 mm NGM agar plates, allowed to dry overnight at room temperature and then incubated at 37°C for 24 h. About 10–12 L4 stage larvae from the third generation obtained by continuous culture on one type of bacteria were transferred to each large plate containing the same bacteria and left at 20°C for 5 days. The plates were supplemented with concentrated bacteria when the bacterial lawn was depleted during this period. To determine the brood size, individual worms were grown on plates seeded with either *E. coli* B105 ($Q^+$) or *E. coli* B105 ($Q^-$) and were transferred to a new plate every 24–36 h until they stopped laying eggs. Ten worms were analysed for each bacterial strain. The progeny were counted at the L3/L4 stage.

### 2.2 *tRNA isolation*

The worms were harvested in sterile M9, washed twice with M9 and incubated at room temperature for 20 min to

allow digestion of the bacteria present in the worm intestine. To prepare total RNA, the worm pellet (about 1 ml) was subjected to two cycles of freeze–thaw in liquid $N_2$ and ground in half the volume of TRIzol (Invitrogen) with a prechilled mortar and pestle. The ground slurry was taken up in seven volumes of TRIzol (Invitrogen), vortexed and spun at 20,000 *g* for 15 min. The supernatant was extracted twice with equal volumes of chloroform. The total RNA (present in the aqueous fraction) was precipitated with 0.7 volume of ice cold isopropanol and pelleted by centrifugation. The RNA pellet was washed with 2 ml 70% ethanol, dissolved in 0.5 ml buffer R200 (10 mM Tris-$H_3PO_4$ at pH 6.3, 15% ethanol, 200 mM KCl) and analysed by a Nanodrop® spectrophotometer for concentration and purity.

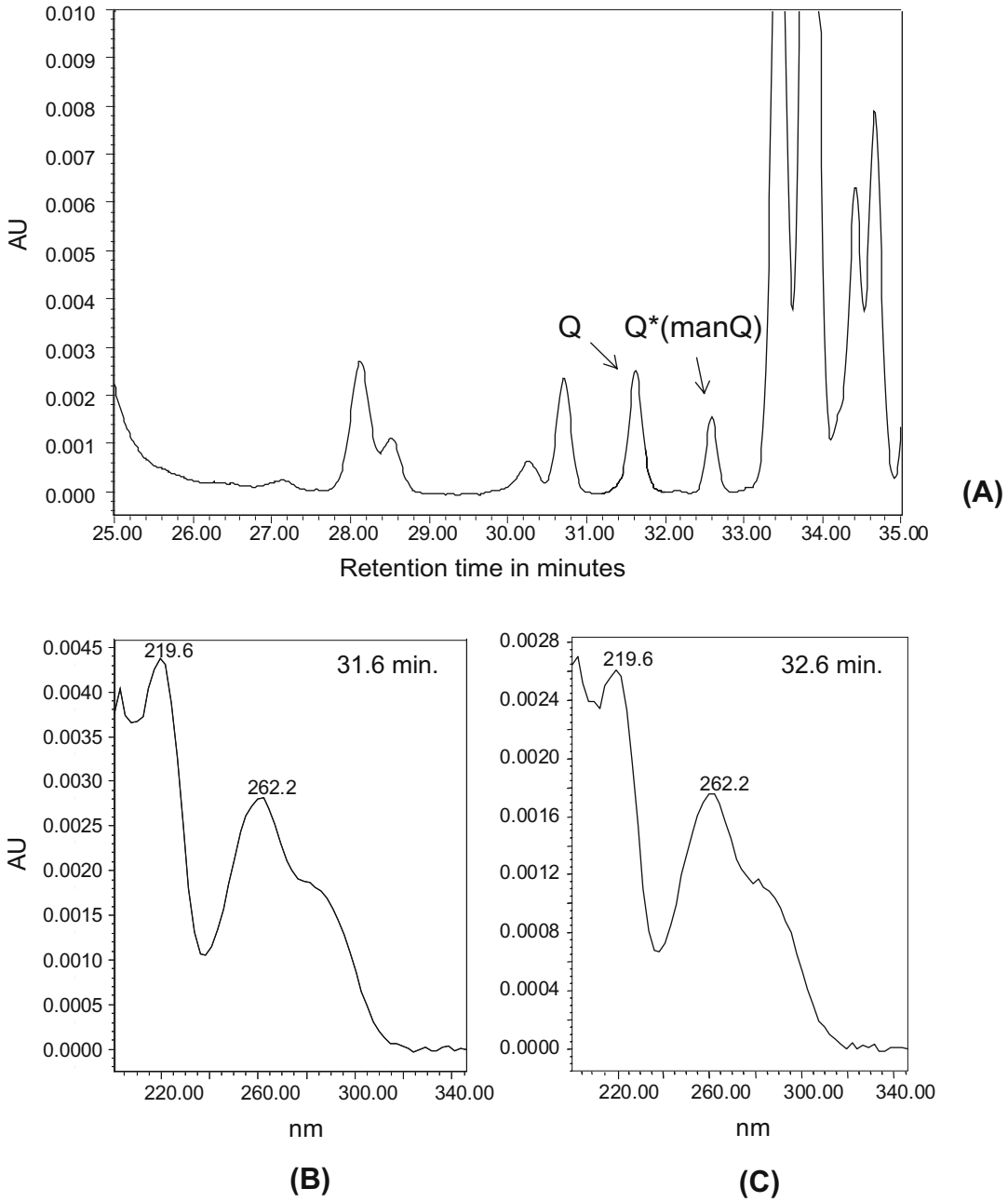


**Figure 1.** HPLC profile of tRNA isolated from a mixed population of *C. elegans* N2 worms fed on *E. coli* OP50. The tRNA fraction was digested overnight with P1 nuclease followed by treatment with alkaline phosphatase. The preparation was then loaded onto a Supelcosil LC-18 column and run according to Gehrke and Kuo (1990). Panel **A** shows the HPLC profile in the 25–35 min region. Panel **B** is the UV spectrum of the Q peak, and panel **C** is the spectrum for the manQ peak.

To isolate tRNA, the volume of total RNA was made up to 2 ml with R200 and loaded onto a Nucleobond AX500 column (Macherey-Nagel Gmbh & Co.), pre-equilibrated with the same buffer, washed with 6 ml R200, followed by 2 ml R650 (same as R200, except that it contains 650 mM KCl) and the tRNA was eluted with 6 ml R650. The tRNA was precipitated with isopropanol, pelleted by centrifugation, washed with 70% ethanol, vacuum dried and dissolved in 100 $\mu$l sterile MilliQ water. Yields of 200–240 $\mu$g total tRNA from 4 mg total RNA were obtained.

### 2.3 *Base analysis by HPLC and mass spectrometry*

tRNA was treated with nuclease P1 followed by bacterial alkaline phosphatase and the hydrolysates were analysed using a Supelcosil LC-18S column (250 x 4.6 mm) on a Waters HPLC system Model 2695 as described by Gehrke and Kuo (1990). The chromatograms presented were obtained at 254 nm. For mass spectrometry, a Waters Micromass ZQ system was used in conjunction with HPLC. The buffers used in HPLC were as described in Pomerantz and McCloskey (1990), except that the ammonium acetate concentration was 5 mM and the flow rate 1.8 ml/min. The ion source was 120 and the desolvation temperature was 300°C.

## 3. Results and Discussion

### 3.1 *Analysis of C. elegans tRNA shows the presence of queuosine (Q)*

Queuosine is present in organisms as diverse as bacteria, starfish, coconut and humans (Kasai *et al* 1975; Katze

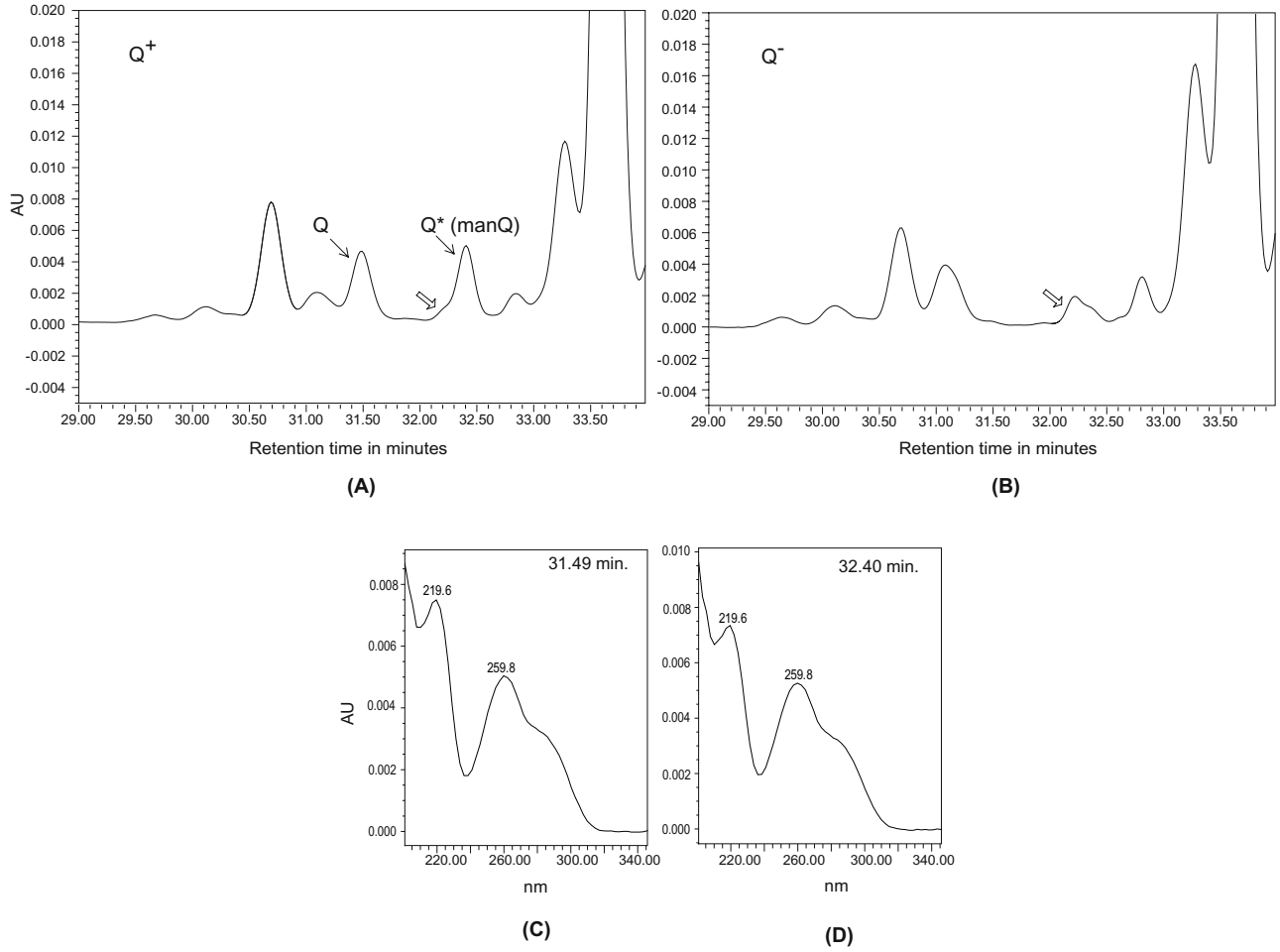


**Figure 2.** HPLC profiles of tRNA isolated from a mixed population of *C. elegans* N2 worms fed with either *E. coli* B105($Q^+$) **(A)** or *E. coli* B105($Q^-$) **(B)**. Panels **C** and **D** show UV absorbance of the Q and manQ peaks, respectively. The Q peaks (black arrows) are missing in the profile from worms grown on *E. coli* B105($Q^-$). A small peak (empty arrow) seen in the 32.5 min region has an absorbance that is different from the characteristic absorbance of Q and gal/manQ. This peak is also present as a shoulder of the gal/manQ in the $Q^+$ sample but is more prominent in the $Q^-$ sample which had about 40% more material than the $Q^+$ sample.

*et al* 1982). The observation that the genome of *C. elegans* harbours the TGT gene(s) (ZK829.6 and Y39B6A.35) indicated that Q should be present in the worm tRNA. However, the worm tRNA has never been analysed for the presence of Q. We analysed the base composition of the tRNA of worms grown on *E. coli* OP50, the bacterial strain most commonly used to grow *C. elegans*. As shown in figure 1, the HPLC profile showed a peak at the elution time expected for Q. Further, the ultraviolet (UV) absorbance spectrum of this peak is characteristic of Q, indicating that the worm tRNAs contain Q. Interestingly, another peak (referred to as Q*) eluting near Q and with a similar UV spectrum was also observed. Two sugar derivatives of Q, galactosylated Q (galQ; Kasai *et al* 1976) and mannosylated Q (manQ; Kuchino *et al* 1981; Haumont *et al* 1984) are known to exist in animal tRNAs. Gehrke and Kuo (1990) reported the mean retention times for Q (28.31 min ± 0.311), manQ (29.15 min ± 0.275) and galQ (29.70 min ± 0.314) from 16 experiments. Using data from 5 experiments, we obtained a mean retention time of 31.13 min ± 0.378 for Q and 32.15 min ± 0.286 for the Q*. The ratio of retention times Q*/Q (1.03, rounded off to two decimal places) calculated from our data is similar to the manQ/Q (1.03) ratio but not to the galQ/Q (1.05) ratio (Gehrke and Kuo 1990), suggesting that Q* is manQ.

### 3.2 *Q and its derivative are depleted from C. elegans tRNA isolated from worms fed on E. coli defective in queuosine biosynthesis*

To investigate whether Q is present in tRNA isolated from worms raised on a diet lacking Q, *C. elegans* N2 worms were cultured for four consecutive generations on either *E. coli* B105($Q^-$), a mutant strain lacking Q modification (Dineshkumar *et al* 2002), or *E. coli* B105($Q^+$), an isogenic strain harbouring Q (Gaur and Varshney 2005). The

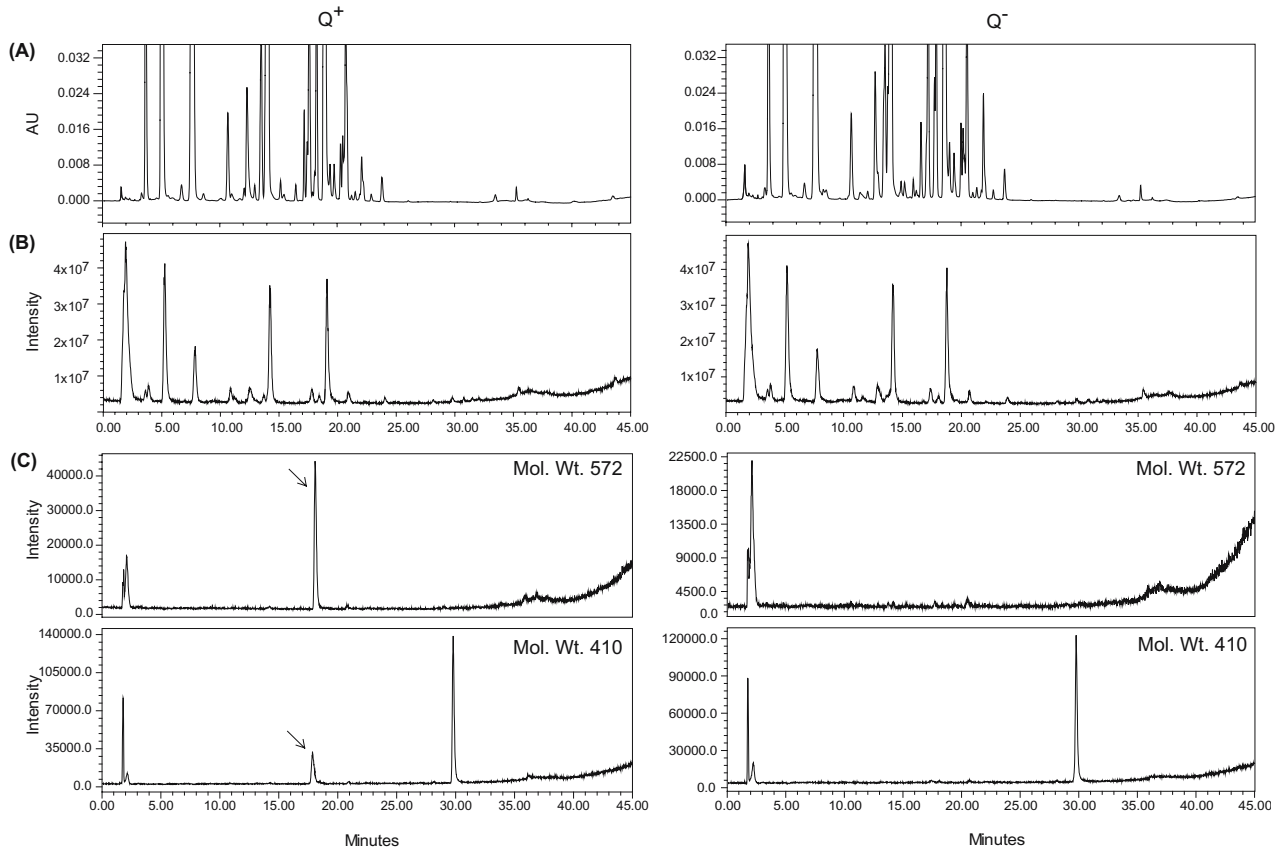


**Figure 3.** Mass spectrometry analysis of tRNA from $Q^+$ and $Q^-$ worms. **(A)** UV absorbance of tRNA hydrolysates in the buffer system after Pomerantz and McCloskey (1990). The Q and its other forms elute at around 18 min in this buffer system. **(B)** Total ionic counts in the tRNA hydrolysates. **(C)** Intensity plotted against elution time for the molecular weights 572 and 410, respectively. 572 is the molecular weight of man/galQ; 410 is the molecular weight of Q itself. Note that peaks (indicated by arrows) are present at 18 min for both molecular weights in the spectra obtained with tRNA from worms grown on $Q^+$ bacteria. The corresponding peaks are absent from the spectra obtained from tRNA isolated from worms grown on the $Q^-$ strain.

tRNA from the two worm populations was analysed for the presence of Q by HPLC. As shown in figure 2A, the tRNA from worms fed on B105($Q^+$) contains Q and Q* (manQ), whereas the tRNA from worms fed on B105($Q^-$) is deficient Q (figure 2B). To further confirm the observation, the tRNA preparation was analysed by mass spectrometry. As shown in figure 3C, the peaks for the mass of Q and man/galQ are seen in the tRNA from the worms fed on *E. coli* B105($Q^+$) but absent from those fed on *E. coli* B105($Q^-$). An extra peak of mass 410 is also seen in both the profiles (figure 3C). Although this mass corresponds to the mass of Q, the retention time of this peak (~30 min) is different from that of Q (~18 min). As a thorough analysis of the modified nucleosides in worm tRNAs is unavailable (*http://medlib.med.utah.edu/RNAmods/*), the identity of this novel peak remains unknown at present.

Using the data from HPLC (figures 1 and 2) and mass spectrometric analysis (figure 3), we conclude that the worms possess Q and a sugar derivative of Q, both of which are depleted when the worms are fed on an *E. coli* mutant lacking the ability to synthesize Q. Interestingly, this observation suggests that *C. elegans* lacks the ability to synthesize Q *de novo*.

The time taken to produce worms depleted of Q (two weeks) compares favourably with that required to produce mice lacking Q (one year or more). Furthermore, whereas the generation of mice or flies lacking Q requires careful husbandry of animals on a Q-free diet under germ-free conditions, growing worms on $Q^-$ bacteria is not technically demanding. The large brood sizes (approximately 300 for each hermaphrodite) coupled with the short generation time means that it is possible to produce a population of worms in which Q is undetectable within two weeks.

### 3.3 *Analysis of C. elegans fed on $Q^+$ and $Q^-$ E. coli*

The gross morphology of the young $Q^-$ worms was similar to that of $Q^+$ worms. All of the L1 worms ($n$ = 100) of both $Q^+$ and $Q^-$ type survived a 2 h heat shock at 33°C. When a 5 h heat shock was given to L4 worms at 33°C, the ratio of survivors to dead worms was 56% for $Q^+$ and 52% for $Q^-$ worms ($n$ = 125 for both populations). Comparison was also made of metal stress tolerance between $Q^+$ ($n$ = 62) and $Q^-$ populations ($n$ = 120). All of the L1 worms of both types reached the L4 stage when 20 mM $CdCl_2$ was included in the plates. When 200 mM $CuSO_4$ was included in the plates, 45% of $Q^+$ L1 worms reached the L4 stage compared with 50% of $Q^-$ worms. Also, the $Q^-$ worms had a similar brood size (312 ± 29.4) as the $Q^+$ worms (321 ± 41.5) indicating that the $Q^-$ worms were generally healthy. Thus in the phenotypes analysed by us, the responses of the $Q^+$ and $Q^-$ worm populations were found to be similar.

## 4. Conclusion

We have carried out the first analysis of Q in *C. elegans* and shown that like other metazoans, *C. elegans* tRNAs possess the Q modification and a sugar derivative of Q. Importantly, both the forms of Q disappear from *C. elegans* tRNA when *C. elegans* is grown on a diet of $Q^-$ *E. coli*, suggesting that nematodes, like other animals, cannot synthesize Q *de novo* and obtain the modified nucleoside from their diet. The method employed by us to deplete Q in *C. elegans* has the distinct advantage of precluding the use of a chemically defined medium. This method is fast, straightforward and maintains the organism under growth conditions similar to those routinely used in the laboratory.

The worms lacking Q obtained using this system were subjected to heat and metal stress. However, the $Q^-$ and the $Q^+$ worms behaved in a similar fashion in these experiments suggesting that Q does not play a detectable role in the physiological response of *C. elegans* to these stresses. In *Drosophila* larvae, Q has been shown to be involved in cadmium resistance (Siard *et al* 1991) but a similar phenomenon was not observed in the $Q^-$ worms tested by us. On the other hand, the fact that $Q^-$ mice did not show any abnormalities (Reyniers *et al* 1981) is in agreement with our observations in $Q^-$ *C. elegans*. However, our experiments do not rule out a role of Q modification in tRNAs in subtle mechanisms relevant to the *in vivo* function and to the fitness of the animal in its natural habitat. A TGT orthologue containing the consensus catalytic and tRNA-binding residues has been identified in *C. elegans* (Romier *et al* 1997). Intuitively, one would consider that a high degree of conservation of the molecular machinery involved in salvage and incorporation of Q in eukaryotic tRNAs would imply an important role for Q in metazoan biology. Further investigations under varied conditions may reveal the physiological role of Q in metazoan biology. With the development of a facile method of Q depletion in *C. elegans,* many such experiments now become feasible. Moreover, this system also serves as an excellent platform to address important issues of Q metabolism such as the Q salvage pathway and the identities of enzymes that glycosylate Q.

## Acknowledgements

We thank our laboratory members for their helpful suggestions and discussions. Technical assistance provided by Kerstin Jacobsson for HPLC and mass spectrometry is greatly appreciated. R G was supported by a senior research fellowship of the Council of Scientific and Industrial Research, New Delhi. Research in our laboratories is funded by grants from the Department of Science and Technology, Department of Biotechnology, and Indian Council of Medical Research, New Delhi to U V, the Swedish Cancer

Foundation (Cancerfonden) to S T, and the Swedish Science Research Council (V R) and Cancerfonden to G R B.

## References

Baranowski W, Dirheimer G, Jakowicki J A and Keith G 1994 Deficiency of queuine, highly modified purine base, in transfer RNAs from primary and metastatic ovarian malignant tumors in women; *Cancer Res.* **54** 4468–4471

Brenner S 1974 The genetics of *Caenorhabditis elegans*; *Genetics* **77** 71–94

Dineshkumar T K, Thanedar S, Subbulakshmi C, Varshney U 2002 An unexpected absence of queuosine modification in the tRNAs of an *Escherichia coli* B strain; *Microbiology* **148** 3779–3787

Durand J M, Dagberg B, Uhlin B E, Björk G R 2000 Transfer RNA modification, temperature and DNA superhelicity have a common target in the regulatory network of the virulence of *Shigella flexneri*: the expression of the *virF* gene; *Mol. Microbiol.* **35** 924–935

Emmerich B, Zubrod E, Weber H, Maubach P A, Kersten H and Kersten W 1985 Relationship of queuine-lacking transfer RNA to the grade of malignancy in human leukemias and lymphomas; *Cancer Res.* **45** 4308–4314

Frey B, McCloskey J, Kersten W and Kersten H 1988 New function of vitamin B12: cobamide-dependent reduction of epoxyqueuosine to queuosine in tRNAs of *Escherichia coli* and *Salmonella typhimurium*; *J. Bacteriol.* **170** 2078–2082

Gaur R and Varshney U 2005 Genetic analysis identifies a function for the *queC* (*ybaX*) gene product at an initial step in the queuosine biosynthetic pathway in *Escherichia coli*; *J. Bacteriol.* **187** 6893–6901

Gehrke C W and Kuo K C 1990 Ribonucleoside analysis by reversed-phase high performance liquid chromatography; in *Chromatography and modification of nucleosides. Part A. Analytical methods for major and modified nucleosides* (eds) C W Gehrke *et al* (Amsterdam: Elsevier) **45A** pp A3–A71

Gregson J M, Crain P F, Edmonds C G, Gupta R, Hashizume T, Phillipson D W and McCloskey J A 1993 Structure of the archaeal transfer RNA nucleoside G*-15 (2-amino-4,7-dihydro-4-oxo-7-beta-D-ribofuranosyl-1H-pyrrolo[2,3-d]pyrimidine-5-carboximidamide (archaeosine)); *J. Biol. Chem.* **268** 10076–10086

Gunduz U and Katze J R 1984 Queuine salvage in mammalian cells. Evidence that queuine is generated from queuosine 5′-phosphate; *J. Biol. Chem.* **259** 1110–1113

Harada F and Nishimura S 1972 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; *Biochemistry* **11** 301–308

Haumont E, Nicoghosian K, Grosjean H and Cedergren RJ 1984 The nucleotide sequence of mannosyl-Q-containing $tRNA^{Asp}$ from *Xenopus laevis* oocytes; *Biochimie* **66** 579–582

Haumont E, Droogmans L and Grosjean H 1987 Enzymatic formation of queuosine and of glycosyl queuosine in yeast tRNAs microinjected into *Xenopus laevis* oocytes. The effect of the anticodon loop sequence; *Eur. J. Biochem.* **168** 219–225

Iwata-Reuyl D 2003 Biosynthesis of the 7-deazaguanosine hypermodified nucleosides of transfer RNA; *Bioorg. Chem.* **31** 24–43

Kasai H, Kuchino Y, Nihei K and Nishimura S 1975 Distribution of the modified nucleoside Q and its derivatives in animal and plant transfer RNAs; *Nucleic Acids Res*. **2** 1931–1939

Kasai H, Nakanishi K, Macfarlane R D, Torgerson D F, Ohashi Z, McCloskey J A, Gross H J and Nishimura S 1976 Letter: The structure of Q* nucleoside isolated from rabbit liver transfer ribonucleic acid; *J. Am. Chem. Soc.* **98** 5044–5046

Katze J R, Basile B, McCloskey J A 1982 Queuine, a modified base incorporated posttranscriptionally into eukaryotic transfer RNA: wide distribution in nature; *Science* **216** 55–56

Katze J R and Beck W T 1980 Administration of queuine to mice relieves modified nucleoside queuosine deficiency in Ehrlich ascites tumor tRNA; *Biochem. Biophys. Res. Commun.* **96** 313–319

Kersten H 1984 On the biological significance of modified nucleosides in tRNA. *Prog. Nucleic Acid Res. Mol. Biol.* **31** 59–114

Kirtland G M, Morris T D, Moore P H, O'Brian J J, Edmonds C G, McCloskey J A and Katze J R 1988 Novel salvage of queuine from queuosine and absence of queuine synthesis in *Chlorella pyrenoidosa* and *Chlamydomonas reinhardtii; J. Bacteriol.* **170** 5633–5641

Kuchino Y, Shindo-Okada N, Ando N, Watanabe S and Nishimura S 1981 Nucleotide sequences of two aspartic acid tRNAs from rat liver and rat ascites hepatoma; *J. Biol. Chem.* **256** 9059–9062

Langgut W and Kersten H 1990 The deazaguanine-derivative, queuine, affects cell proliferation, protein phosphorylation and the expression of the proto oncogenes *c-fos* and *c-myc* in HeLa cells; *FEBS Lett.* **265** 33–36

Langgut W, Reisser T, Nishimura S and Kersten H 1993 Modulation of mammalian cell proliferation by a modified tRNA base of bacterial origin; *FEBS Lett.* **336** 137–142

Morgan C J, Merrill F L and Trewyn R W 1996 Defective transfer RNA-queuine modification in C3H10T1/2 murine fibroblasts transfected with oncogenic *ras*; *Cancer Res.* **56** 594–598

Noguchi S, Nishimura Y, Hirota Y and Nishimura S 1982 Isolation and characterization of an *Escherichia coli* mutant lacking tRNA-guanine transglycosylase. Function and biosynthesis of queuosine in tRNA; *J. Biol. Chem.* **257** 6544–6550

Okada N and Nishimura S 1977 Enzymatic synthesis of Q nucleoside containing mannose in the anticodon of tRNA: isolation of a novel mannosyltransferase from a cell-free extract of rat liver; *Nucleic Acids Res.* **4** 2931–2938

Okada N, Noguchi S, Kasai H, Shindo-Okada N, Ohgi T, Goto T and Nishimura S 1979 Novel mechanism of post-transcriptional modification of tRNA. Insertion of bases of Q precursors into tRNA by a specific tRNA transglycosylase reaction; *J. Biol. Chem.* **254** 3067–3073

Pathak C and Vinayak M 2005 Modulation of lactate dehydrogenase isozymes by modified base queuine; *Mol. Biol. Rep.* **32** 191–196

Pawelkiewicz J, Sroga G E, Starzynska E and Zawielak J 1986 Formation of queuine containing $tRNA^{Tyr}$ in higher plants; *Plant Sci.* **47** 83–89

Pomerantz S C and McCloskey J A 1990 Analysis of tRNA hydrolysates by liquid chromatography-mass spectrometry; *Methods Enzymol.* **193** 796–824

Randerath E, Agrawal H P and Randerath K 1984 Specific lack of the hypermodified nucleoside, queuosine, in hepatoma mitochondrial aspartate transfer RNA and its possible biological significance; *Cancer Res.* **44** 1167–1171

Reader J S, Metzgar D, Schimmel P and de Crecy-Lagard V 2004 Identification of four genes necessary for biosynthesis of the modified nucleoside queuosine; *J. Biol. Chem.* **279** 6280–6285

Reisser T, Langgut W and Kersten H 1994 The nutrient factor queuine protects HeLa cells from hypoxic stress and improves metabolic adaptation to oxygen availability; *Eur. J. Biochem* **221** 979–986

Reyniers J P, Pleasants J R, Wostmann B S, Katze J R and Farkas W R 1981 Administration of exogenous queuine is essential for the biosynthesis of the queuosine-containing transfer RNAs in the mouse; *J. Biol. Chem.* **256** 11591–11594

Romier C, Meyer J E and Suck D 1997 Slight sequence variations of a common fold explain the substrate specificities of tRNA-guanine transglycosylases from the three kingdoms; *FEBS Lett.* **416** 93–98

Sambrook J, Fritsch E F and Maniatis T 1989 *Molecular cloning: A laboratory manual* (New York: Cold Spring Harbor Laboratory)

Schachner E and Kersten H 1984 Queuosine deficiency and restoration in *Dictyostelium discoideum* and related early developmental changes; *J. Gen. Microbiol.* **130** 135–144

Schon A, Gough S and Soll D 1992 Chloroplast tRNA(Asp): nucleotide sequence and variation of *in vivo* levels during plastid maturation; *Plant Mol. Biol.* **20** 601–607

Siard T J, Jacobson K B and Farkas W R 1991 Queuine metabolism and cadmium toxicity in *Drosophila melanogaster*; *BioFactors (Oxford, England)* **3** 41–47

Stengl B, Reuter K and Klebe G 2005 Mechanism and substrate specificity of tRNA-guanine transglycosylases (TGTs): tRNA-modifying enzymes from the three different kingdoms of life share a common catalytic mechanism; *Chembiochem* **6** 1926–1939

Sulston J E, Schierenberg E, White J G and Thomson J N 1983 The embryonic cell lineage of the nematode *Caenorhabditis elegans*; *Dev. Biol.* **100** 64–119

Van Lanen S G, Reader J S, Swairjo M A, de Crecy-Lagard V, Lee B and Iwata-Reuyl D 2005 From cyclohydrolase to oxidoreductase: discovery of nitrile reductase activity in a common fold; *Proc. Natl. Acad. Sci. USA* **102** 4264–4269

*MS received 13 December 2006; accepted 3 March 2007*

*e*Publication: 4 April 2007

Corresponding editor: Vidyanand Nanjundiah