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Review

# Biogenesis and roles of tRNA queuosine modification and its glycosylated derivatives in human health and diseases

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## SUMMARY

Various types of post-transcriptional modifications contribute to physiological functions by regulating the abundance and function of RNAs. In particular, tRNAs have the widest variety and largest number of modifications, with crucial roles in protein synthesis. Queuosine (Q) is a characteristic tRNA modification with a 7-deazaguanosine core structure bearing a bulky side chain with a cyclopentene group. Q and its derivatives are found in the anticodon of specific tRNAs in both bacteria and eukaryotes. In metazoan tRNAs, Q is further glycosylated with galactose or mannose. The functions of these glycosylated Qs remained unknown for nearly half a century since their discovery. Recently, our group identified the glycosyltransferases responsible for these tRNA modifications and elucidated their biological roles. We, here, review the biochemical and physiological functions of Q and its glycosylated derivatives as well as their associations with human diseases, including cancer and inflammatory and neurological diseases.

## INTRODUCTION

RNAs undergo a wide variety of chemical modifications after transcription. To date, about 150 types of RNA modifications have been found across all domains of life.[1] These modifications are involved in physiological processes through the regulation of gene expression. RNA modifications are universally present in almost all RNAs, including mRNA, long non-coding RNA, rRNA, tRNA, and nuclear small RNA. They include base and ribose methylation, acetylation, hydroxylation, dehydration cyclization, sulfuration, selenylation, reduction, isomerization, and the addition of amino acids and sugars.

While some RNA modifications are highly conserved across three domains of life, many are unique to specific lineages or species. This suggests that diverse RNA modifications were acquired by organisms during evolution, contributing to functional differentiation. In recent years, several groups, including ours, have reported novel RNA modifications, and the chemical space of RNA modifications is expected to expand.[2] Approximately 80% of RNA modifications have been found in tRNAs; these are involved in the accuracy and efficiency of decoding during translation as well as the stabilization of the tertiary structure of tRNA.

Although tRNAs are ubiquitously expressed in all tissues and cells, their expression patterns are tissue- and cell type-specific. Additionally, tRNA-modifying enzymes are dynamically regulated in different tissues in a spatiotemporal manner.[3,4] The proper regulation of the tRNA abundance and modification status optimizes the translation rate of each mRNA in a tissue-specific fashion. This regulation controls mRNA stability[5–7] and ensures correct folding of nascent proteins,[8] thereby maintaining optimal translation and proteostasis in each cell type.[9]

The deficiency or dysregulation of tRNA modification can result in pathological conditions, including cancer, diabetes, developmental disorders, neurological disorders, and mitochondrial diseases. These findings have led to the proposal of a unique category of human diseases, termed RNA modopathies, caused by aberrant RNA modification.[2,3,10–12]

## QUEUOSINE, A UNIQUE tRNA MODIFICATION

Queuosine (Q) is a complex modified nucleoside with a 7-deazapurine core structure bearing a bulky side chain with a cyclopentene ring (Figure 1).[13,14] Q is present at the first position (position 34) of the anticodons in tRNAs for Tyr, His, Asn, and Asp, which decode NAY (Y=U or C) codons. Q is found in tRNAs of many organisms, ranging from bacteria to eukaryotes, and mitochondria.[15] However, it is absent in archaea and various specific species including *Mycoplasma*, budding yeast, and *Arabidopsis*.

## BIOGENESIS OF Q IN BACTERIA

In general, RNA modifications involve the enzymatic addition of functional groups to RNA bases or riboses. However, Q is introduced to tRNAs through a unique mechanism involving a base replacement reaction catalyzed by a transglycosylase.[16–18] In eukaryotes and mitochondria, the Q base, queuine (q), is

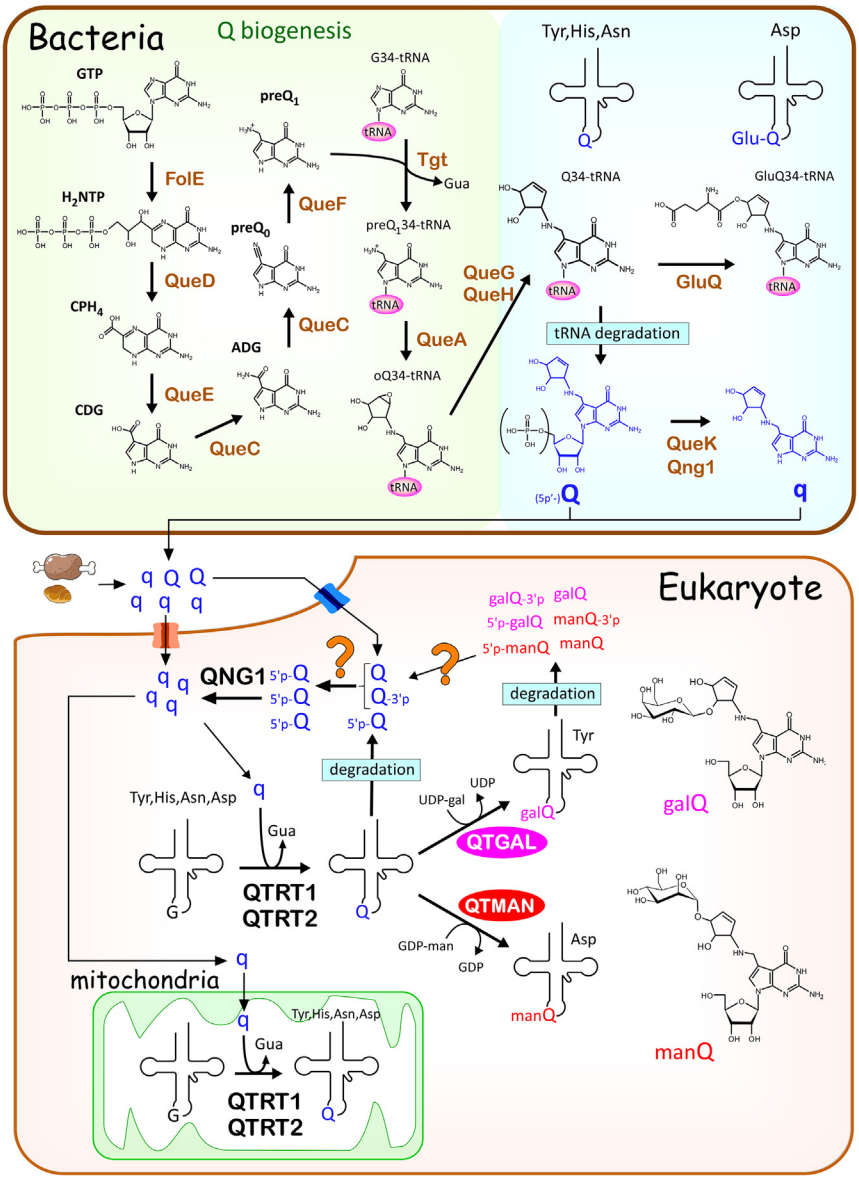


**Figure 1. Biosynthetic pathways of Q and its derivatives in bacteria and eukaryote**

In bacteria, the biosynthesis of queuosine (Q) starts from GTP and progresses through several intermediates: 7,8-dihydroneopterin triphosphate ($H_2NTP$), carboxy-5,6,7,8-tetrahydropterin ($CPH_4$), 7-carboxy-7-deazaguanine (CDG), 7-amido-7-deazaguanine (ADG), 7-cyano-7-deazaguanine ($preQ_0$), and 7-aminomethyl-7-deazaguanine ($preQ_1$). $preQ_1$ is incorporated at position 34 of tRNAs for Tyr, His, Asn, and Asp through a base-replacement reaction catalyzed by bacterial tRNA guanine transglycosylase (bTGT). $preQ_1$ on tRNA is converted to epoxyqueuosine (oQ) by QueA. Finally, oQ on tRNA is converted to Q by QueG or QueH. In some bacteria, Q34 of $tRNA^{Asp}$ is further modified with glutamic acid to form glutamyl-queuosine (gluQ) catalyzed by GluQ (YadB). During the degradation of Q-modified tRNAs, Qng1 releases queuine (q) from 5′-monophospho queuosine (QMP). QueK is another subgroup of Q hydrolase. In eukaryotes, Q and q are obtained from dietary sources and the gut microbiota. Eukaryotic tRNA guanine transglycosylase (eTGT), composed of a heterodimeric complex of QTRT1 and QTRT2, replaces guanine with queuine at the 34th position of tRNAs for Tyr, His, Asn, and Asp. Additionally, in tRNAs for Tyr and Asp, Q is further modified by QTGAL and QTMAN, adding galactose and mannose, respectively, resulting in galactosyl-Q (galQ) and mannosyl-Q (manQ). Fractions of QTRT1 and QTRT2 are imported into the mitochondria and incorporate Q34 modifications to mitochondrial tRNAs for Tyr, His, Asn, and Asp. A Q-to-q salvage pathway in eukaryotic cells is mediated by QNG1; however, the salvage pathways for galQ and manQ remain uncharacterized.

incorporated into tRNA at position 34, the first position of the anticodon, by replacing the guanine base catalyzed by tRNA guanine transglycosylase (TGT) (Figure 1). In bacteria, the precursor of q, $preQ_1$ (7-amino methyl-7-deazaguanine), is introduced into tRNA by TGT, and then converted to Q by further modifications on the tRNA (Figure 1).

In bacteria, $preQ_1$ is biosynthesized from GTP through multi-step enzymatic reactions[19–21] (Figure 1). In *Escherichia coli*, GTP cyclohydrolase I, encoded by *folE*, catalyzes the cleavage of the imidazole ring of GTP and subsequent rearrangement of the ring to produce 7,8-dihydroneopterin triphosphate ($H_2NTP$).[22] $H_2NTP$ is a precursor not only for $preQ_1$ but also for tetrahydrofolate (THF) and tetrahydrobiopterin ($BH_4$).[22,23] Next, 6-pyruvoyltetrahydropterin synthase, QueD, converts $H_2NTP$ into 6-carboxy-5,6,7,8-tetrahydropterin ($CPH_4$).[24,25] The radical SAM enzyme QueE synthesizes 7-carboxy-7-deazaguanine (CDG) from $CPH_4$.[26] In this step, the 7-deazaguanine core structure of Q is formed. QueC converts CDG to 7-amido-7-deazaguanine (ADG) and further synthesizes $preQ_0$ (7-cyano-7-deazaguanine) from ADG. This process consumes two ATP molecules.[27] $preQ_0$ also serves as a substrate for archaeosine, a tRNA modification unique to archaea.[28–30] Finally, the reductase QueF reduces the nitrile group of $preQ_0$ to produce $preQ_1$.[31,32] This is a unique example of an enzymatic reaction to reduce a nitrile group in a natural biosynthetic pathway.

Bacterial (b)TGT introduces $preQ_1$ into tRNA by swapping the guanine base at G34 of the tRNA with $preQ_1$ (Figure 1). bTGT functions as a homodimer and recognizes the Y32U33G34U35 sequence common to the anticodon loops of the four tRNA species bearing Q34.[33,34] Once bTGT binds to the tRNA substrate, an Asp residue crucial for catalytic activity of bTGT forms a covalent bond with the C1 carbon of the G34 ribose, releasing guanine. Then, $preQ_1$ is transferred to the C1′ carbon to form an N-glycosyl bond, releasing the Asp residue of bTGT.[35] $preQ_1$ on the tRNA is then converted to Q via two consecutive reactions. First, QueA transfers the ribosyl group of SAM to the amino group of $preQ_1$, generating epoxyqueuosine (oQ).[36,37] Next, QueG or QueH use an iron-sulfur cluster as a cofactor to reduce oQ to Q.[38,39] Most of these enzymatic reactions for Q synthesis utilize metal ions as cofactors, drawing attention to the

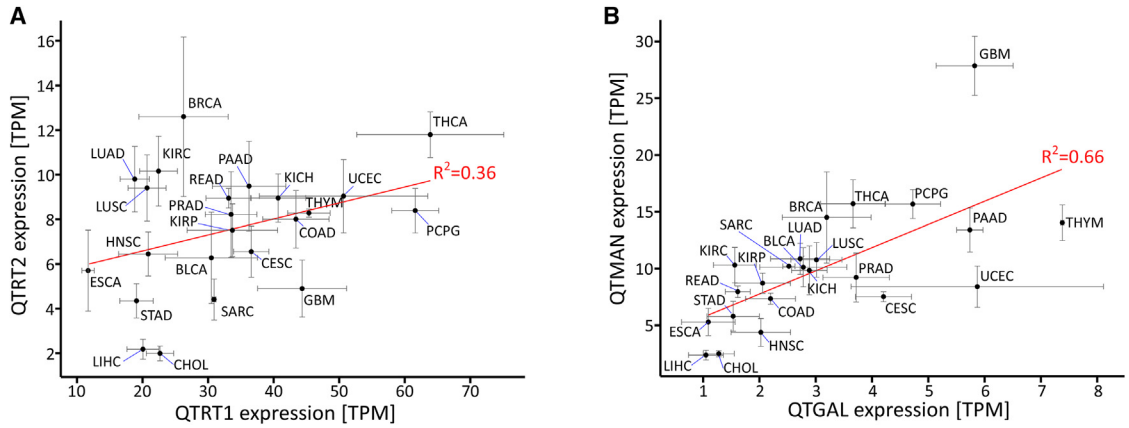


**Figure 2. Comparison of expression levels for Q and glycosylated Q modifications across human tissues**

The mRNA levels (TPM) of each gene in various normal tissues were obtained from The Cancer Genome Atlas Program (TCGA) using the UALCAN database, and plotted across various human tissues for *QTRT1* versus *QTRT2* (A), and *QTGAL* versus *QTMAN* (B).

The normal tissues corresponding to each tumor are indicated by the abbreviations of the respective tumor tissues as follows; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; THCA, thyroid carcinoma; ESCA, esophageal carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; THYM, thymoma; SARC, sarcoma; BRCA, breast invasive carcinoma; LIHC, liver hepatocellular carcinoma; PAAD, pancreatic adenocarcinoma; CHOL, cholangiocarcinoma; STAD, stomach adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; COAD, colon adenocarcinoma; UCEC, uterine corpus endometrial carcinoma; READ, rectum adenocarcinoma; CESC, cervical squamous cell carcinoma; PRAD, prostate adenocarcinoma; BLCA, bladder urothelial carcinoma.

relationship between protein synthesis modulated by Q biogenesis and intracellular metal ion concentrations.[40,41]

## BIOGENESIS OF Q IN EUKARYOTES

Unlike bacteria, eukaryotes, including humans, do not synthesize q or its precursors *de novo*. Instead, they acquire q from the gut microbiota or dietary sources as a micronutrient and use it for tRNA modification[15] (Figure 1). In the cell, tRNAs are degraded and converted into nucleotides (or nucleosides) including modified nucleotides. Eukaryotes and some bacteria possess a salvage pathway in which q is released by a queuosine hydrolase (Qng1) that hydrolyzes an N-glycosyl bond of the Q nucleotide[42–45] (Figure 1). The salvage pathway plays a crucial role in maintaining intracellular levels of q.[42] In experiments, cultured cells take up q from the serum in the media to synthesize Q. Of note, horse serum has low q concentration and hence is often used as a q-deficient condition.[46,47]

In the cytoplasm of eukaryotic cells, a QTRT1 and QTRT2 heterodimer, a eukaryotic TGT, incorporates q and synthesizes Q34 in tRNAs for Tyr, His, Asn, and Asp.[48–51] QTRT1 and QTRT2 are paralogous. Only QTRT1 possesses catalytic activity, while QTRT2 plays a crucial role in tRNA binding.[52] The high conservation of these enzymes indicates the broad distribution of Q modification in eukaryotes. A portion of the cytoplasmic pools of QTRT1 and QTRT2 proteins are imported into the mitochondria[53,54] and contribute to Q34 formation in mitochondrial tRNAs.[55,56] However, the mechanism by which the QTRT1/QTRT2 complex is translocated to mitochondria remains unclear, as neither protein has apparent mitochondria-targeting signals.

## VARIATION IN Q MODIFICATION

The Q frequency varies among tissue types. For example, in mice, the Q frequency is high in brain, heart, and skeletal muscle and low in spleen and skin.[57] This variation in the Q frequency may be correlated with the expression levels of *Qtrt1* and *Qtrt2* and with the turnover rate of each cell type.[57,58]

Additionally, the frequencies of Q modification and derivatives vary depending on the developmental stage and differentiation state.[59–61] In fetal mice and young rats, the Q frequency is low and increases with age.[62,63] In flies, the Q frequency decreases gradually from the larval stage and is nearly depleted in adults but eventually recovers.[64] In zebrafish, tRNA-modifying enzymes for Q and its derivatives are maternally deposited in fertilized eggs, and the steady-state levels of these mRNAs gradually decrease after fertilization.[65] These changes suggest that Q modification is closely associated with developmental and morphological processes. However, as mentioned previously, because q is largely obtained from dietary sources and the internal salvage pathway, the effects of diet and environmental conditions on the Q frequency should also be considered.[66–68]

## Q DERIVATIVES

In some bacteria, the *allyl*-OH (12-OH) group of Q34 in $tRNA^{Asp}$ is further acylated with glutamate, forming glutamyl-queuosine (gluQ) (Figure 1).[69–73] gluQ is formed by glutamyl-Q-tRNA(Asp) synthetase (GluQ or YadB). GluQ shares partial homology with glutamyl-tRNA synthetase and catalyzes a reaction similar to aminoacylation. GluQ first adenylates Glu to form Glu-AMP and then transfers the Glu to the *allyl*-OH of Q34 in $tRNA^{Asp}$, forming gluQ. The functions of gluQ are not well characterized.

In higher eukaryotes, such as vertebrates, Q34 in cytoplasmic $tRNA^{Tyr}$ is further modified with galactose to form galactosyl-queuosine (galQ) (Figure 1), and $tRNA^{Asp}$ is modified with mannose to form mannosyl-queuosine (manQ) (Figure 1).[74,75] Initial structural analyses showed that galQ and manQ have galactose and mannose attached in a β-configuration at

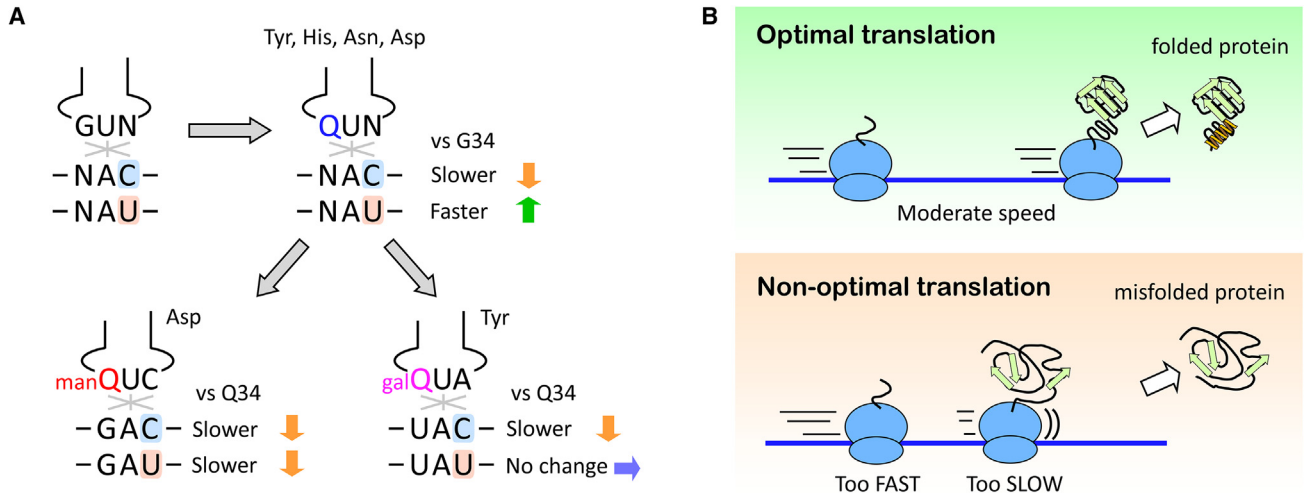


**Figure 3. Decoding speed tuned by Q and glycosylated Qs and proteostasis**

(A) Q modifications of four tRNA species speed up the decoding of U-ending codons but slow down that of C-ending codons. For $tRNA^{Asp}$, manQ slows the translation of both GAU and GAC codons. For $tRNA^{Tyr}$, galQ does not significantly affect UAU decoding but slows down UAC decoding.

(B) Optimal translation regulated by tRNA abundance or modification facilitates proper folding of nascent proteins to maintain cellular proteostasis. Non-optimal translation due to lack of tRNA modification or dysregulation of tRNA abundance impairs protein folding and proteostasis.

the *homoallyl*-OH (11-OH) group of the cyclopentene ring.[75] Later comparisons with chemically synthesized compounds confirmed the stereochemical configuration of galQ[61] (Figure 1) but revised the stereochemical configuration of manQ, revealing that mannose is attached in an α-configuration to the *allyl*-OH (12-OH) group of Q34 in $tRNA^{Asp}$[76] (Figure 1).

## BIOGENESIS AND REGULATION OF galQ AND manQ

Last year, our group identified two glycosyltransferases, QTGAL (originally B3GNTL1) and QTMAN (originally GTDC1), which transfer galactose and mannose to Q34 of tRNAs for Tyr and Asp, respectively[65] (Figure 1). QTGAL and QTMAN use UDP-galactose and GDP-mannose as sugar donors for Q-glycosylation, respectively. QTGAL belongs to the GT2 glycosyltransferase family with a GT-A fold and catalyzes an $S_N2$ reaction to form galQ by inverting the α-configuration of the anomeric carbon of UDP-galactose to the β-configuration of galactose attached to *homoallyl*-OH (11-OH) group of the cyclopentene ring of galQ (Figure 1). QTMAN belongs to the GT4 glycosyltransferase family with a GT-B fold and catalyzes an $S_Ni$-like retaining type sugar transfer reaction to form manQ in the α-configuration of the anomeric carbon of mannose attached to the *allyl*-OH (12-OH) group of the cyclopentene ring of manQ (Figure 1).

Phylogenetic analyses revealed that QTRT1 and QTRT2 are highly conserved across most eukaryotes, whereas QTGAL and QTMAN are broadly conserved in metazoans, particularly in vertebrates, chordates, echinoderms, and mollusks, and are present in arthropods and nematodes.[65] Interestingly, *Caenorhabditis elegans* possesses only QTGAL, while *Drosophila* has only QTMAN.

QTGAL and QTMAN are ubiquitously expressed; however, the frequencies of galQ and manQ vary among different organs in mice.[61] QTRT1, QTRT2, QTMAN, and QTGAL exhibit distinct expression patterns across various tissues (Figures 2A and 2B).[77,78] The expression of QTRT1 and QTRT2 shows a weak correlation ($R^2$ = 0.36) (Figure 2A). Both proteins are expressed at high levels in the thyroid but at low levels in the bile ducts, stomach, and liver (Figure 2A). Interestingly, we observed a stronger correlation in the expression profiles of QTGAL and QTMAN across tissues ($R^2$ = 0.66) (Figure 2B), suggesting that glycosylated Q modifications may function cooperatively. QTMAN is highly expressed in the brain, whereas QTGAL is highly expressed in the thymus (Figure 2B). Notably, the expression profiles of both QTGAL and QTMAN show a higher correlation with QTRT1 ($R^2$ = 0.65 and 0.54, respectively) (Figures S1A and S1B) and a weaker correlation with QTRT2 ($R^2$ = 0.33 and 0.38, respectively) (Figures S1C and S1D).

The frequency of glycosylated Q is influenced not only by different expression levels of QTGAL and QTMAN but also by changes in intracellular concentrations of nucleotide diphosphate sugars, UDP-galactose and GDP-mannose, in various tissues. Kinetic analyses of Q-glycosylation suggest that QTGAL and QTMAN have relatively high *Km* values for their sugar donors, particularly indicating that QTMAN-mediated manQ formation might be susceptible to variation in intracellular GDP-mannose concentrations.[65]

## TRANSLATION SPEED OPTIMIZED BY Q AND GLYCOSYLATED QS

Early studies have reported that Q deficiencies affect the accuracy of translation, leading to increased frameshifting,[79,80] miscoding,[81–83] and readthrough of stop codons.[84]

Ribosome profiling of human cells lacking Q modification revealed that Q accelerates the translation elongation speed of U-ending codons but slows down C-ending codons in the cytoplasmic translation (Figure 3A).[65,85] In mitochondrial translation, Q deficiency results in slower UAU decoding by mitochondrial $tRNA^{Tyr}$.[55] Regarding Q-glycosylation, Q-galactosylation of $tRNA^{Tyr}$ does not affect the UAU decoding significantly but slows down UAC decoding (Figure 3A). Q-mannosylation of $tRNA^{Asp}$ slows down the decoding of both GAU and GAC codons (Figure 3A).

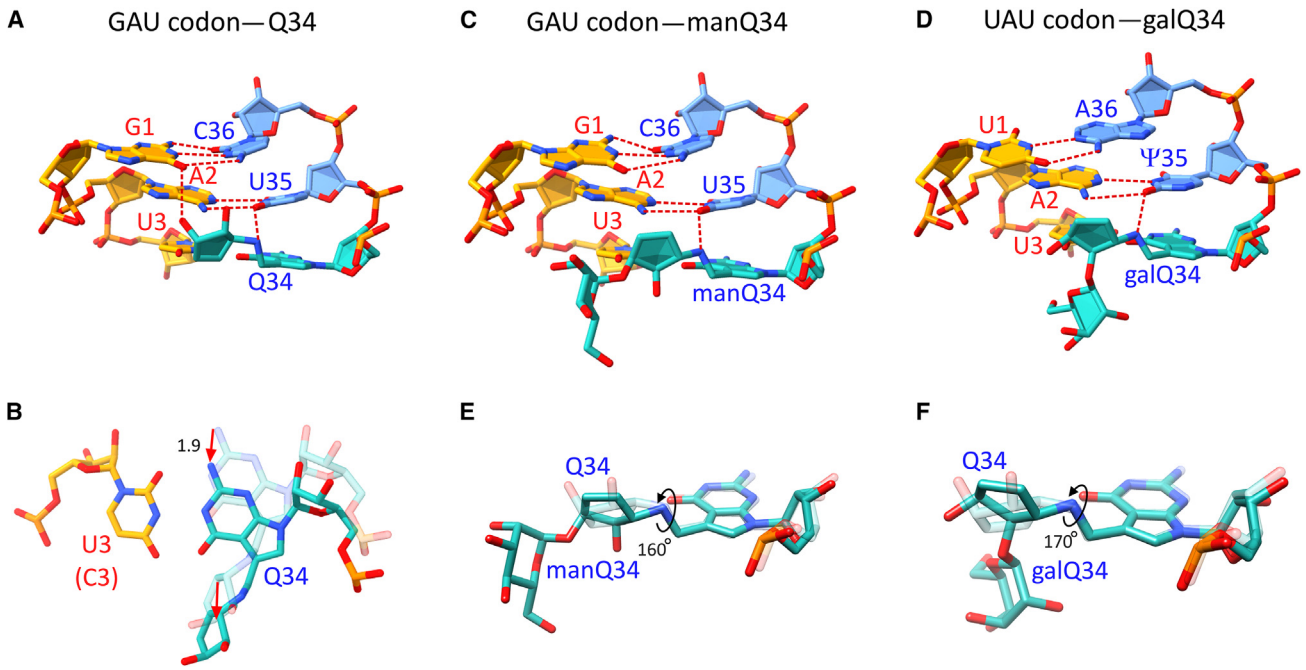


**Figure 4. Codon recognition by Q and glycosylated Qs**

(A, C, and D) Cryo-EM structures of the codon–anticodon interaction at the A-site of human 80S ribosome with human $tRNA^{Asp}$ bearing Q34 (cyan) and GAU codon (orange) (PDB: 8JDJ) (A), human $tRNA^{Asp}$ bearing manQ34 (cyan) and GAU codon (orange) (PDB: 8JDK) (C), and human $tRNA^{Tyr}$ bearing galQ34 (cyan) and UAU codon (orange) (PDB: 8JDM) (D), viewed from the major groove side. H-bonds are shown as dotted lines.

(B) Structural difference between Q34-C3 (opaque) and Q34-U3 (translucent) pairs. The Q34-C3 pair is superimposed on Q34-U3 by aligning C3 and U3. N2 and N10 of Q34 are shifted by 1.9 Å as indicated by arrows. The cyclopentene moiety of Q34 moves away from the major groove of the codon-anticodon helix.

(E and F) Rotation of the cyclopentene moiety caused by Q34 glycosylation. Q34 (opaque) (PDB: 8JDJ) is aligned with manQ34 (translucent) (PDB: 8JDK) (E) or galQ34 (translucent) (PDB: 8JDM) (F). Compared with Q34, the cyclopentene moieties of manQ34 and galQ34 are rotated by 160° and 170° in the clockwise direction, respectively.

Cytoplasmic $tRNA^{Tyr}$ acts as a potential suppressor tRNA with stop codon readthrough ability.[84,86] *QTGAL* knockout (KO) cells show a higher readthrough efficiency of UAA and UAG codons than wild-type (WT) cells,[65] indicating that the galactosylation of galQ plays a role in preventing stop codon readthrough. These results provide important insights for the development of therapies using suppressor tRNA aimed at recoding nonsense mutations associated with human diseases.[87]

The optimal translation speed is crucial for proper protein folding and maintaining proteostasis (Figure 3B).[8] Protein aggregates are increased in cells cultured in q-deficient medium[85] as well as in *QTRT2* KO cells.[55,65] Furthermore, reporter protein aggregates are significantly upregulated in both *QTGAL* KO and *QTMAN* KO cells.[65] Thus, Q and Q-glycosylation contribute to the maintenance of proteostasis by optimizing the translation elongation speed.

## MOLECULAR BASIS OF CODON RECOGNITION BY Q AND GLYCOSYLATED QS

The molecular basis of codon recognition mediated by Q and glycosylated Qs has been elucidated by structural analyses.[65] High-resolution cryogenic electron microscopy of human 80S ribosomes complexed with tRNAs with different modification status revealed that the cyclopentene ring of Q recognizes the major groove of the codon-anticodon helix, functioning as a major groove binder to increase the codon recognition efficiency at the A-site of the ribosome (Figure 4A). Specifically, the *cis*-diols of the cyclopentene ring form H-bonds with the first and second base pairs of the codon-anticodon pairing. The Q-C pair of the QUC anticodon paired with the GAC codon adopts a usual Watson-Crick geometry, whereas the Q-U pair with the GAU codon forms a wobble geometry. The cyclopentene ring binds the major groove of the Q-U pairing with greater stability than that for the Q-C-pairing because the Q base moves toward the major groove side by 1.9 Å from Q-U to Q-C pairing (Figures 4B and Data S1); the cyclopentene moiety detaches from the major groove to weaken its binding. This is the molecular basis by which Q facilitates the decoding of U-ending codons but slows down the C-ending codon (Figure 3A). The cyclopentene moieties of manQ and galQ also work as major groove binders, although they lose H-bond networks with the codon-anticodon helix (Figures 4C and 4D). The most striking finding in these structures is a rotation of the cyclopentene moiety upon glycosylation. Mannosylation (Figure 4E) and galactosylation (Figure 4F) rotate the cyclopentene moiety by 160° and 170°, respectively (Datas S2 and S3). This rotation modulates the major groove binding of the cyclopentene moiety. Thus, Q-glycosylation slows down the decoding of cognate codons (Figure 3A) by weakening the interaction between the cyclopentene moiety and codon-anticodon helix. However, the lack of change in the recognition of the UAU codon between galQ34 and Q34 in $tRNA^{Tyr}$ suggests that the rotated cyclopentene ring retains its major groove binding ability through van der Waals interactions (Figure 4D).

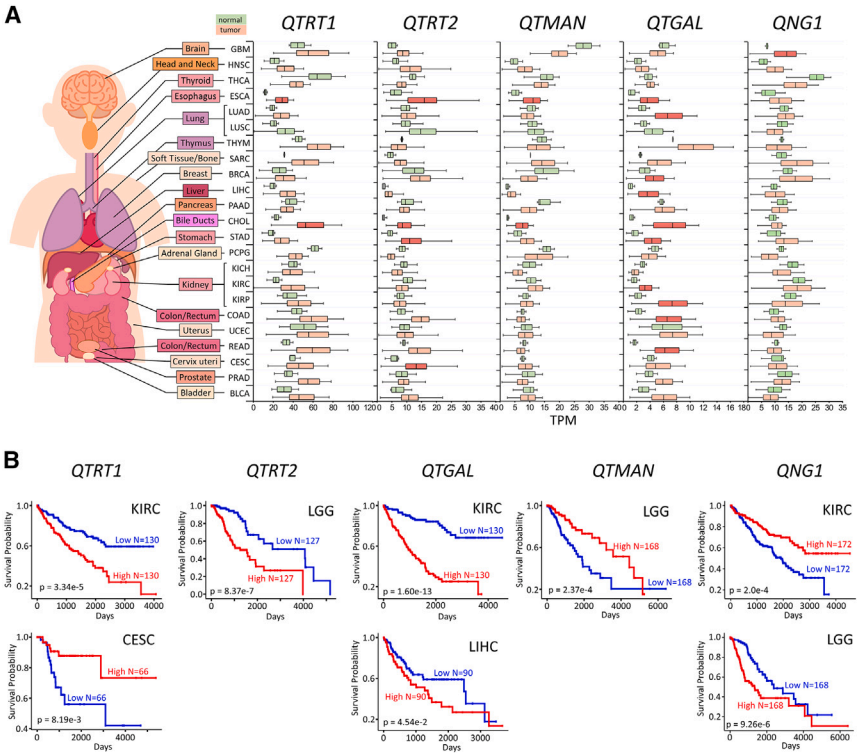


**Figure 5. Expression profiles and prognostic value of *QTRT1*, *QTRT2*, *QTGAL*, *QTMAN*, and *QNG1* in various tissues and cancers**

(A) Data represent the fold change in mRNA levels of each enzyme between normal (light green) and tumor tissues (light red) for different cancer types. mRNAs with significantly elevated levels in tumor tissues are highlighted in thick red color. mRNA levels in normal and tumor tissues along with the *p* values for the comparisons were obtained from The Cancer Genome Atlas Program (TCGA) using the UALCAN database. Abbreviations of cancers are the same as in Figure 2.

(B) Kaplan-Meier survival curves comparing tRNA-modifying enzyme expression using TCGA data. Survival curves were downloaded from the OncoLnc database. *p* values from the long-rank test.

LGG abbreviates for brain lower grade glioma.

## PHYSIOLOGICAL IMPORTANCE OF Q AND GLYCOSYLATED QS

The physiological importance of Q was first reported in nutritional studies on mice.[88,89] Germfree mice subjected to dietary Tyr depletion develop a range of developmental issues, including neurological abnormalities, and die within two weeks after birth. This outcome suggests that certain nutrients obtained from the gut microflora are essential for the survival of germfree mice upon Tyr depletion. Remarkably, lethality of germfree mice was entirely rescued by a diet supplemented with 100 nM queuine. Subsequent analyses revealed that *Qtrt1* KO mice and human cells cultured with queuine-starved conditions exhibited low phenylalanine hydroxylase (PAH) activity, which converts Phe to Tyr, due to reduced levels of tetrahydrobiopterin, a crucial cofactor for PAH.[90] Nevertheless, the molecular mechanisms underlying the characteristic phenotype resulting from a Q deficiency and queuine depletion remain unclear.

*Qtrt1* KO mice show learning and memory deficits due to a global imbalance in translation elongation caused by a loss of Q modification.[57] Curiously, sex differences in cognitive performance were observed; effects were more severe in female mice than in males.

To study the physiological roles of Q-glycosylation in zebrafish, our group constructed maternal-zygotic mutants of *qtrt1*, *qtgal*, and *qtman*.[65] All mutants were viable but displayed a shorter body length than that of WT fish, suggesting that Q-glycosylation is required for efficient post-embryonic growth in zebrafish. Both *qtrt1* KO and *qtgal* KO increased eIF2$\alpha$ phosphorylation, suggesting that there is a connection between translational perturbation and the integrated stress response.

## Q MODIFICATION AND CANCER

Protein synthesis is highly activated in many cancer cells because active translation is required for rapid proliferation.[91,92] Cancer cells often exhibit altered patterns of translation to overexpress specific proteins involved in survival and proliferation. The relationship between tRNA Q modification and cancer cells is well-characterized.[15] Hypomodification of Q has been reported in various malignant tumors, including colon,[93] ovarian,[94] brain,[95] and lung cancers[96] and in leukemia and lymphomas[97] (Table S1). A strong correlation has been reported between Q hypomodification and cancer stage.[93–97] However, not all cancer cells show reduced Q modification,[93,96,98] and mice with q starvation are not prone to cancer,[89,90] suggesting that Q hypomodification is not a direct cause of cancer. The reduction in Q in many cancer cells might be due to the accelerated tRNA turnover and metabolism in cells in which protein synthesis is highly activated and the inability of Q modification to keep up with cell proliferation[99]; alternatively, it may be related to a shortage of q.[100] However, in circulatory cancers, leukemia, and lymphomas, the serum q concentration is not reduced, indicating that Q hypomodification in those cells is not due to a lack of q supply.[99] Hypomodification of Q-tRNA has been frequently observed in many tumors including colon adenocarcinoma.[93,100] Additionally, increased metabolites (biopterin, neopterin, pterin, or $m^7G$) that inhibit tRNA Q modification could also contribute to the reduction.[101–105]

*QTRT1* deficiency inhibits cancer cell proliferation. In a xenograft nude mouse model, the growth of *QTRT1* KO MCF7, a breast cancer cell line, was slower than that of the WT tumor.[106] Although claudin-5 is overexpressed in many breast cancer tumors with a high risk of metastasis,[107,108] it is down-regulated upon *QTRT1* KO, while E-cadherin and β-catenin, crucial factors for cell adhesion, are upregulated.[106] These findings highlight *QTRT1* as a promising target for therapeutic interventions in breast cancer.

According to The Cancer Genome Atlas (TCGA),[78,109,110] *QTRT1*, *QTRT2*, *QTMAN*, and *QTGAL* are upregulated in various types of cancer cells (Figure 5A and Table S1). *QTRT1*, *QTRT2*,

*QTMAN*, and *QTGAL* show elevated expression in cholangiocarcinoma and esophageal cancer. In particular, *QTGAL* shows a marked increase in expression in many tumors. The upregulation of these genes might result from newly synthesized tRNAs associated with increased protein synthesis in tumors. On the other hand, *QNG1* does not show a significant increase in expression in tumor cells compared to the other four genes (Figure 5A). When the expression levels of *QTRT1*, *QTRT2*, *QTMAN*, and *QTGAL* are compared across tissues and tumors, we observe a positive correlation between these genes in normal cells (Figures 2A, 2B, and S1A–S1D). However, little correlation was observed among these genes in tumors (Figures S2A, S2B, S2D, and S2F), with the exception of *QTRT1* versus *QTGAL* ($R^2$ = 0.64) (Figure S2C). These observations suggest that *QTRT1*, *QTRT2*, *QTMAN*, and *QTGAL* are co-expressed in normal cells, whereas this coordination is disrupted in cancer cells. Notably, the positive correlation between *QTRT1* and *QTGAL* appears to be a distinctive feature of cancer cells.

Kaplan-Meier survival curves[111] show that high *QTRT1* expression is associated with poor prognosis in patients with kidney cancer but high survival rates in patients with cervical cancer (Figure 5B). High *QTRT2* expression is correlated with low survival rates in malignant brain tumors (low-grade gliomas) (Figure 5B and Table S1). High *QTGAL* expression is associated with poor prognosis with kidney and liver cancer (Figure 5B and Table S1). Low *QTMAN* expression is associated with low survival rates in low-grade gliomas (Figure 5B and Table S1). Low expression of *QNG1* is associated with poor prognosis in kidney cancer, whereas high expression of *QNG1* is linked to lower survival rates in low-grade gliomas (Figure 5B and Table S1). A recent multivariate analysis combined with deep learning and artificial intelligence highlighted the importance of *QTRT1* expression in the prognosis of chronic lymphocytic leukemia,[112] suggesting that a multifaceted approach could improve our understanding of the relationship between tRNA Q-modification and cancers. Furthermore, hypomethylation of CpG sites in *QTGAL* is observed in many lung cancers and is a potential biomarker for early lung cancer detection, before symptoms appear.[113–115] *QTMAN* is a senescence-associated gene related to tumor formation and immune infiltration in hepatocellular carcinoma.[116] In addition, it is a key factor in predicting prognosis and responses to immunotherapy, and in the identification of potential drugs for liver hepatocellular carcinoma. These findings provide directions for future research aimed at improving the diagnosis and treatment of cancers related to Q and glycosylated Q modifications. However, the results do not offer a cohesive conclusion, as the connection between Q modifications and cancer is highly complex and often ambiguous. Specifically, the disease relevance of the newly identified QTGAL and QTMAN genes remains largely unknown. Consequently, this review focuses solely on findings with statistical significance. It is also important to acknowledge that alterations in Q modification may not always have therapeutic implications.

## Q MODIFICATION AND OTHER DISEASES

*QTRT1* is also associated with human diseases other than cancer (Table S1). In patients with inflammatory bowel diseases, such as ulcerative colitis and Crohn's disease, *QTRT1* expression is significantly decreased.[117] In the epithelial cells of patients, β-catenin and claudin-5 levels are reduced, claudin-2 is upregulated, and cell junctions and intestinal barrier function are weakened, thereby exacerbating inflammation. Conversely, restoring Q modification improves cell proliferation and junctional activity significantly, with reduced inflammation in epithelial cells. This study indicates that q supplementation from the diet and gut microbiota, along with *QTRT1*-mediated Q modification, might play crucial roles in the pathogenesis of inflammatory bowel disease.

Additionally, q is an abundant metabolite in the brain,[118] implying that Q modification contributes to neural function. A q deficiency in human cells and animals reduces the levels of $BH_4$,[90] a cofactor essential for the conversion from Arg to nitric oxide,[119] Phe to Tyr,[120,121] Tyr to DOPA,[122] and Trp to serotonin.[123] Serotonin, in particular, is associated with numerous neurological disorders, including autism, attention-deficit/hyperactivity disorder, bipolar disorder, and schizophrenia, supporting the physiological importance of Q modification and $BH_4$ formation in brain function.[123]

Behavioral phenotypic analyses of *Qtrt1* KO mice have shown that the loss of Q modification impairs learning and memory functions significantly, especially in female mice[57] (Table S1). The hippocampus of *Qtrt1* KO mice exhibits abnormalities in synapse formation and neuron morphology. Proteomic analyses revealed that proteins related to translation, ribosome metabolism, and nonsense-mediated decay are particularly affected in female mice, suggesting that the abnormal protein synthesis due to the loss of Q modification leads to decreased learning and memory capabilities at the organismal level.[57] Furthermore, *QTRT1* is associated with Alzheimer's disease in patients with Down syndrome[124] (Table S1). Taken together, Q modification has a potential role in the pathology of neurodevelopmental and neurodegenerative disorders.

Introducing Q modification might also be effective in treating multiple sclerosis (MS).[125] Rapidly proliferating cells typically exhibit low levels of Q modification.[59] In a mouse model of MS, the administration of a synthetic q analog successfully controlled the activity of rapidly proliferating immune cells, demonstrating its efficacy in achieving complete remission. Q modification could be a promising therapeutic approach for managing autoimmune diseases, such as MS.

*QTMAN* and *QTGAL* are also associated with various diseases other than cancer (Table S1). Genome-wide association studies (GWASs) related to age at menarche and type 2 diabetes mellitus revealed a single nucleotide polymorphism (SNP) in *B3GNTL1* (*QTGAL*).[126] Additionally, SNPs in *B3GNTL1* are associated with chronic kidney disease in patients who do not have diabetes.[127] *GTDC1* (*QTMAN*) dysfunction may cause neurodevelopmental disorders by the overactivation of NMDA receptors through abnormal glycine metabolism.[128] Additionally, *QTMAN* deficiencies in neural progenitor cells and neurons result in phenotypes similar to those observed in induced pluripotent stem (iPS) cells derived from patients with neurological disorders.[129] Furthermore, studies in zebrafish models have indicated that *QTMAN* is necessary for central nervous system development.[129] *QTMAN* is located upstream of the transcription factor *ZEB2*, which plays a crucial role in the development and organogenesis of multicellular organisms. Genomic deletions in the

region containing both *ZEB2* and *QTMAN* are associated with Mowat-Wilson syndrome,[130,131] severe intellectual disability,[132] and polled and multisystemic syndrome.[133]

There is also growing interest in the relationship between glycemic disorders and Q-glycosylation. Kinetic analyses of Q-glycosylation have revealed that QTMAN has a high Km value of 69 μM for GDP-mannose, suggesting that manQ formation is influenced by the intracellular GDP-mannose concentration. Cellular levels of GDP-mannose are significantly decreased in patients with congenital disorders of glycosylation caused by pathogenic mutations in PMM2 (PMM2-CDG)[134,135] (Table S1). In addition to deficiencies of N-glycans and protein mannosylation, manQ hypomodification might be involved in the pathogenesis of these diseases. The cellular concentration of UDP-galactose altered in patients with galactosemia, an inborn disorder of galactose metabolism.[136] Specifically, the level of galQ might be altered in patients with galactosemia bearing loss-of-function mutations in galactose-1-phosphate uridylyltransferase (GALT) (Table S1).

Additional studies of the roles of *QTGAL* and *QTMAN* in diseases are needed. Physiological studies using knockout mice and other model organisms will enhance our understanding of the distinct functions of these genes in brain and other organs and tissues, in addition to their roles in diseases.

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## AUTHOR CONTRIBUTIONS

All authors discussed the latest findings to write and edit the manuscript.

## DECLARATION OF INTERESTS

The authors declare no competing interests.

## SUPPLEMENTAL INFORMATION

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