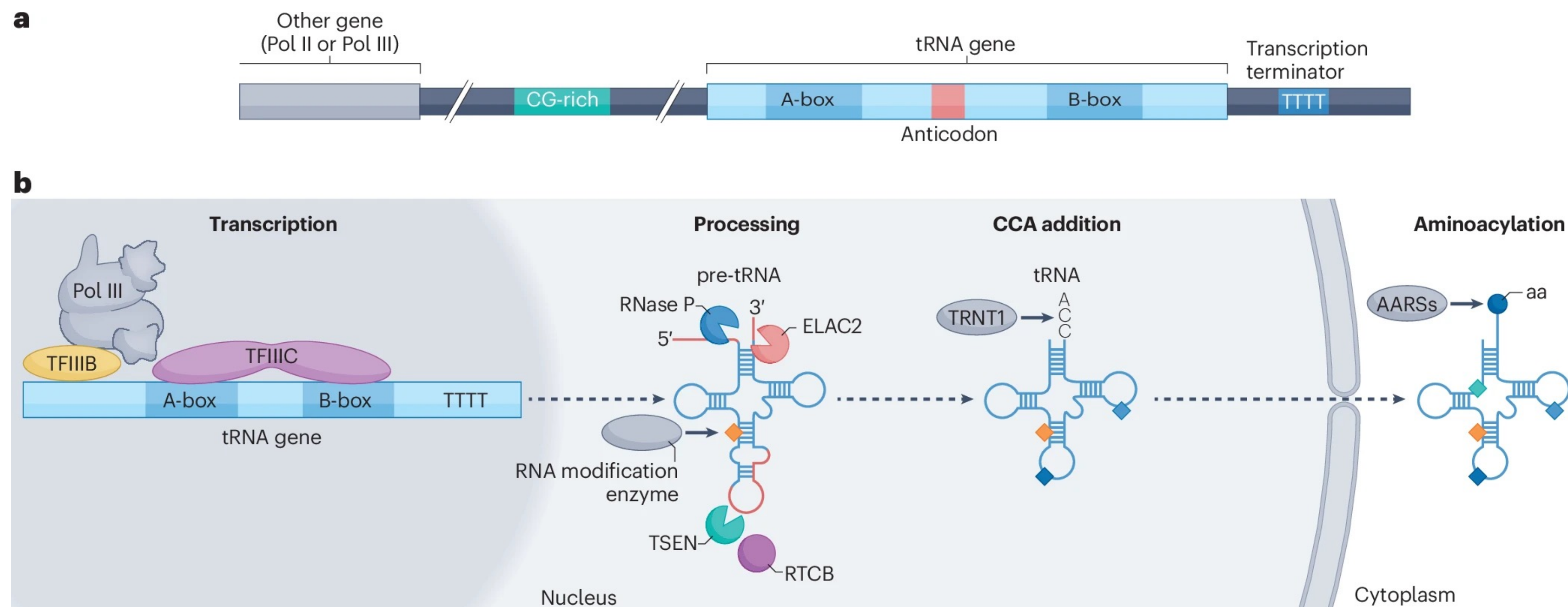
tRNA modifications

tRNA biogenesis and processing

Nuclear-encoded tRNA genes are transcribed by Pol III, supported by the transcription initiation factors TFIIIC, which binds the A-box and B-box and promotes TFIIIB recruitment, and TFIIIB, which binds upstream of the transcription start site and recruits Pol III. A 3′ poly(dT) tract (poly(U) in the nascent tRNA) terminates transcription. tRNA maturation involves removal of a 5′ leader by RNase P and of a 3′ trailer by ELAC2, which in intron-containing tRNAs is followed by tRNA splicing endonuclease (TSEN)–RTCB-mediated splicing. Most tRNA processing takes place in the nucleus, but the location of splicing remains under debate. In human cytoplasmic (and mitochondrial) tRNAs, the CCA tail is added to the 3′ end post-transcriptionally by the nucleotidyltransferase TRNT1. Finally, nucleotide A76 is charged with the cognate amino acid (aa) by the corresponding aminoacyl-tRNA synthetases (aaRSs). RNA modifications of varying chemical complexity and functions are added pre-processing and post-processing in the nucleus and cytoplasm.

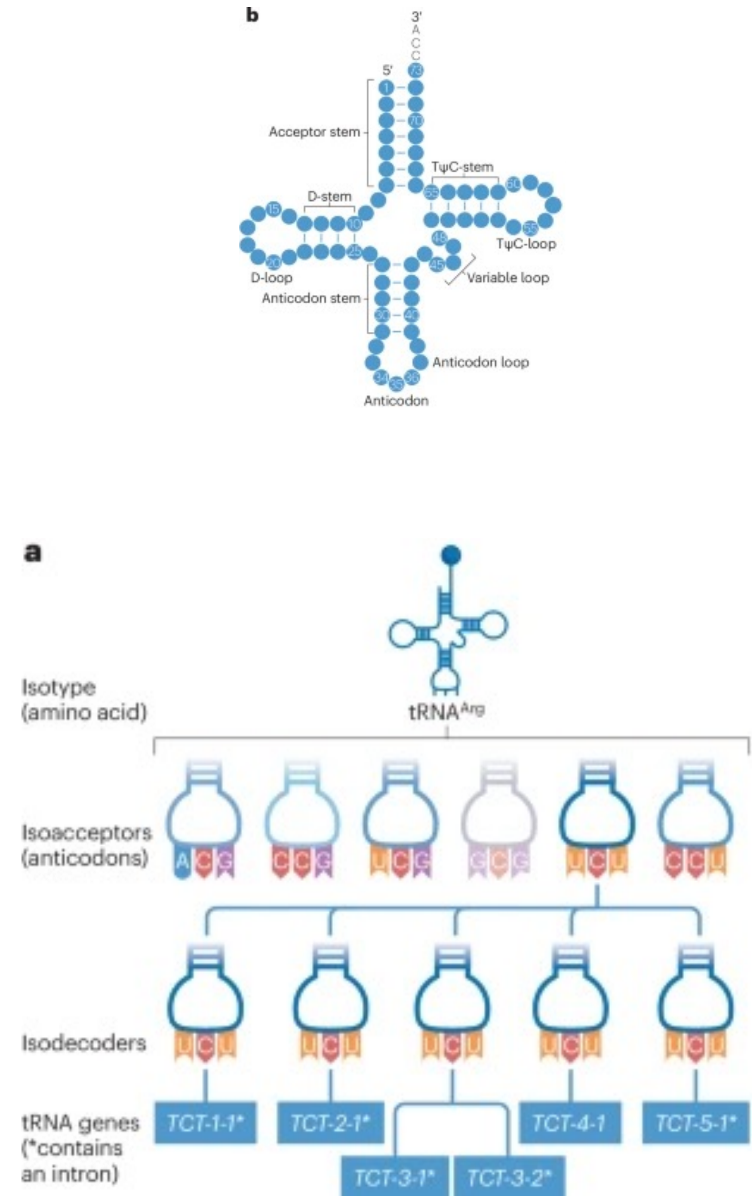


tRNA types and nomenclature

tRNA isotypes: all tRNAs that are charged with the same amino acid, regardless of their anticodon sequences.

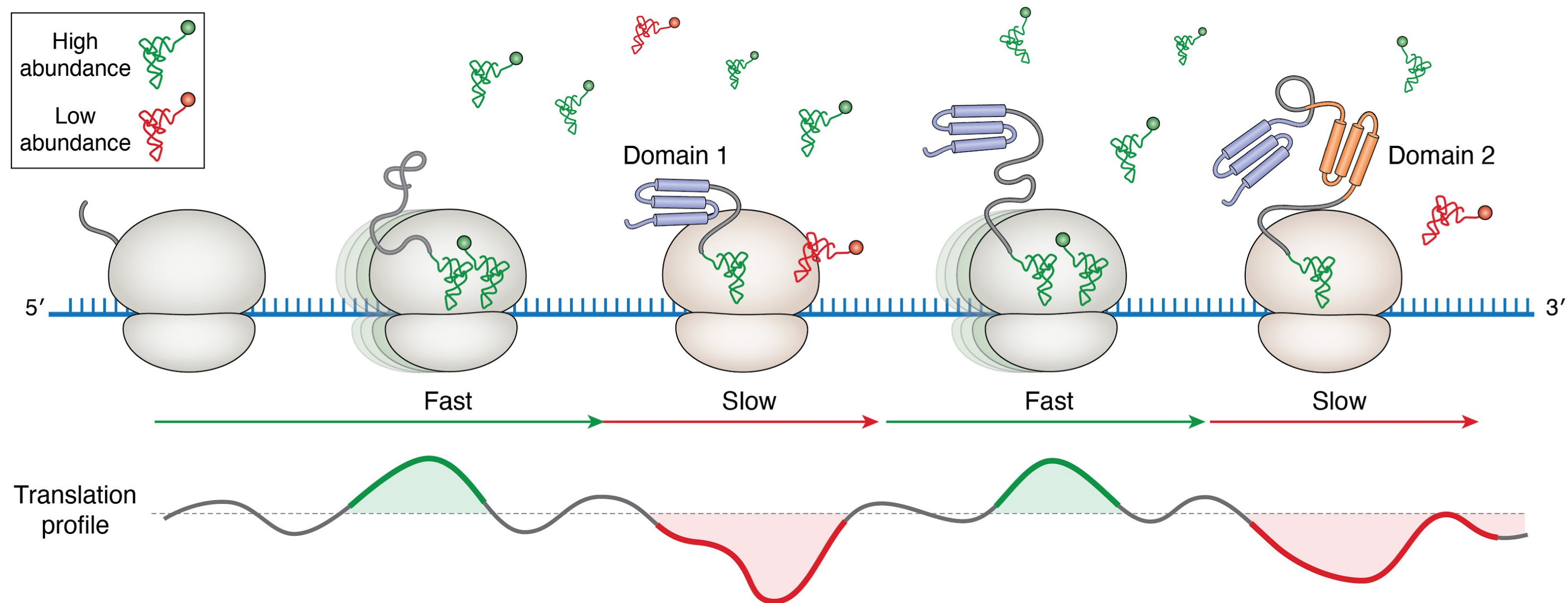
tRNA isoacceptors: carry the same amino acid but have different anticodons (for example, Arg is encoded by six codons, for which there are six possible anticodons; in humans, the Arg isotype includes five isoacceptors, the tRNAArg(GCG) is missing, as CGC is decoded by other isoacceptors through wobbling pairing).

tRNA isodecoders: tRNAs with the same anticodon but different sequences outside the anticodon, which may affect their transcription, processing, stability and/or function in translation (For example, there are five unique tRNA-Arg-UCU isodecoders, which are encoded by six different tRNA genes). Isodecoder pools varies substantially across different human cell types.



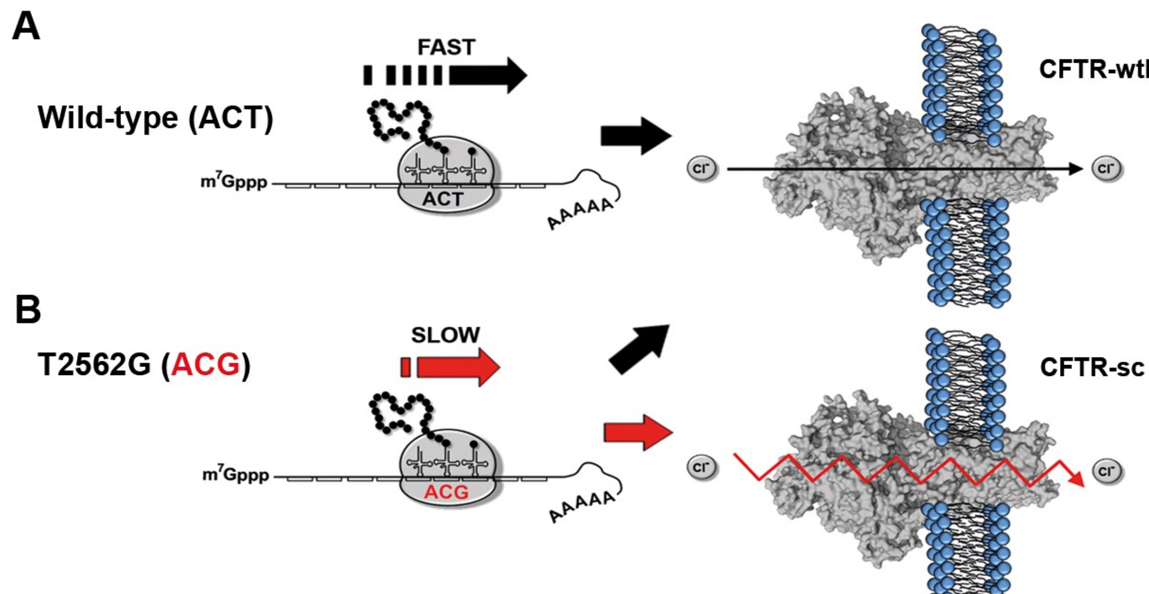
tRNAs as central regulators of mRNA translation dynamics

The codon translation velocity is mainly determined by the concentration of the cognate tRNA; thus, the codon sequence shapes a unique translation profile for each transcript.



The synonymous single nucleotide polymorphism (sSNP) T2562G inverts local translation speed in CFTR mRNA

Change in Thr codon (ACT -> AGT) in the cystic fibrosis transmembrane regulator gene (CFTR) decreases translation velocity by twofold, thereby reducing protein expression by ~30% and altering the CFTR channel's function and conductivity. Notably, the effect is restricted to bronchial tissue, in which the abundance of the corresponding tRNAThr(CGU) decoding the ACG codon is among the lowest. By contrast, in the heart, brain and kidney, in which both tRNAThr(CGU) (decoding ACG) and tRNAThr(AGU) (decoding ACU) are expressed at similar levels, this sSNP has no discernible effect.

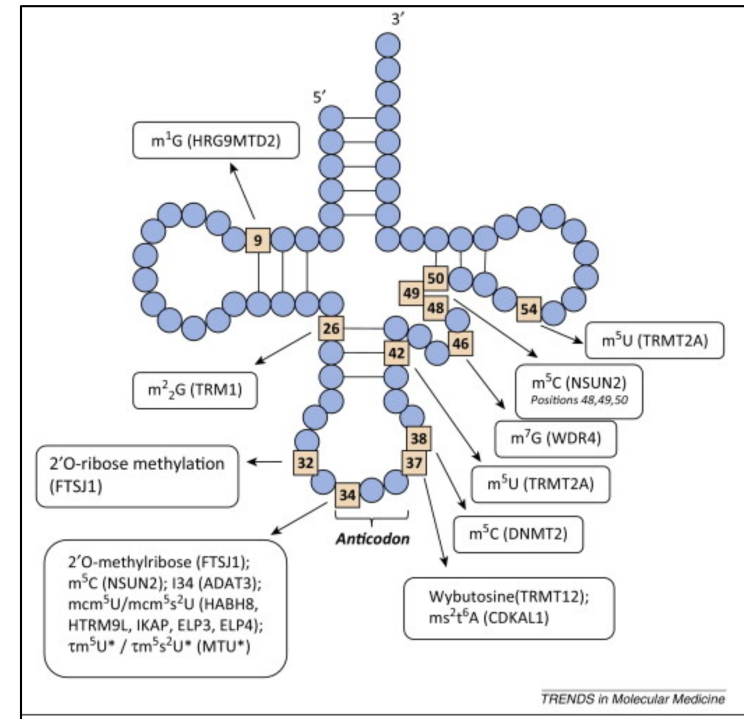


tRNA modifications

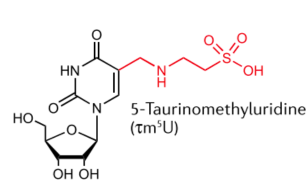
- tRNAs are heavily modified post-transcriptionally during their maturation process. In Eukarya there are more than 50 different chemical modifications described affecting different positions on the tRNA. Some modifications are common to most if not all tRNAs, such as dihydrouridine (in the D-loop) or pseudouridine (in the TψC loop), but others are restricted to a specific tRNA or group of tRNAs. Chemical modifications are crucial for tRNA structure, function, and stability.

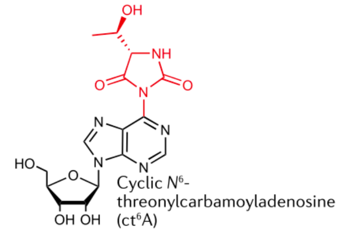
- Because modification is a binary event, a huge population of microspecies can potentially exist. Taking into account only the 13 sites, the number of microspecies for each tRNA can be computed as $2^{13} = 8,192$

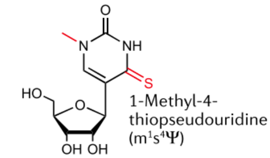
60 million tRNA molecules per mammalian cell !

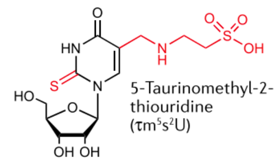


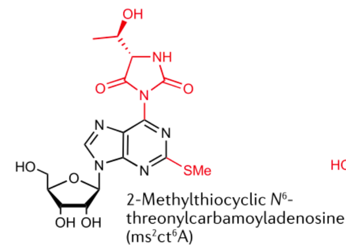
tRNA modifications

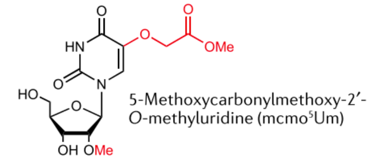


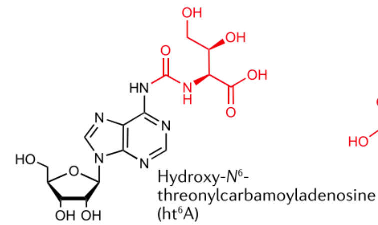


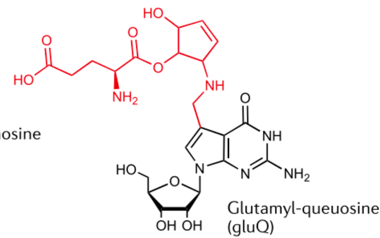


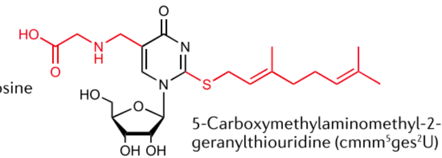


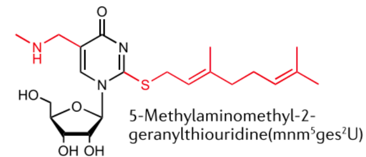


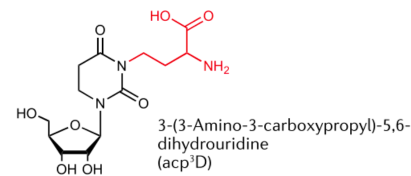


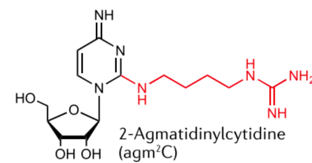


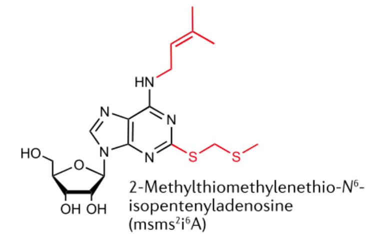


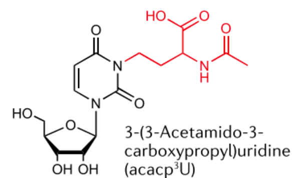


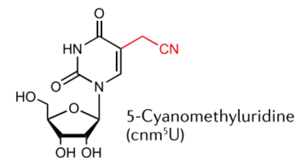


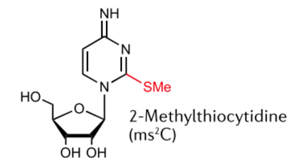












Regulatory role of tRNA base modifications

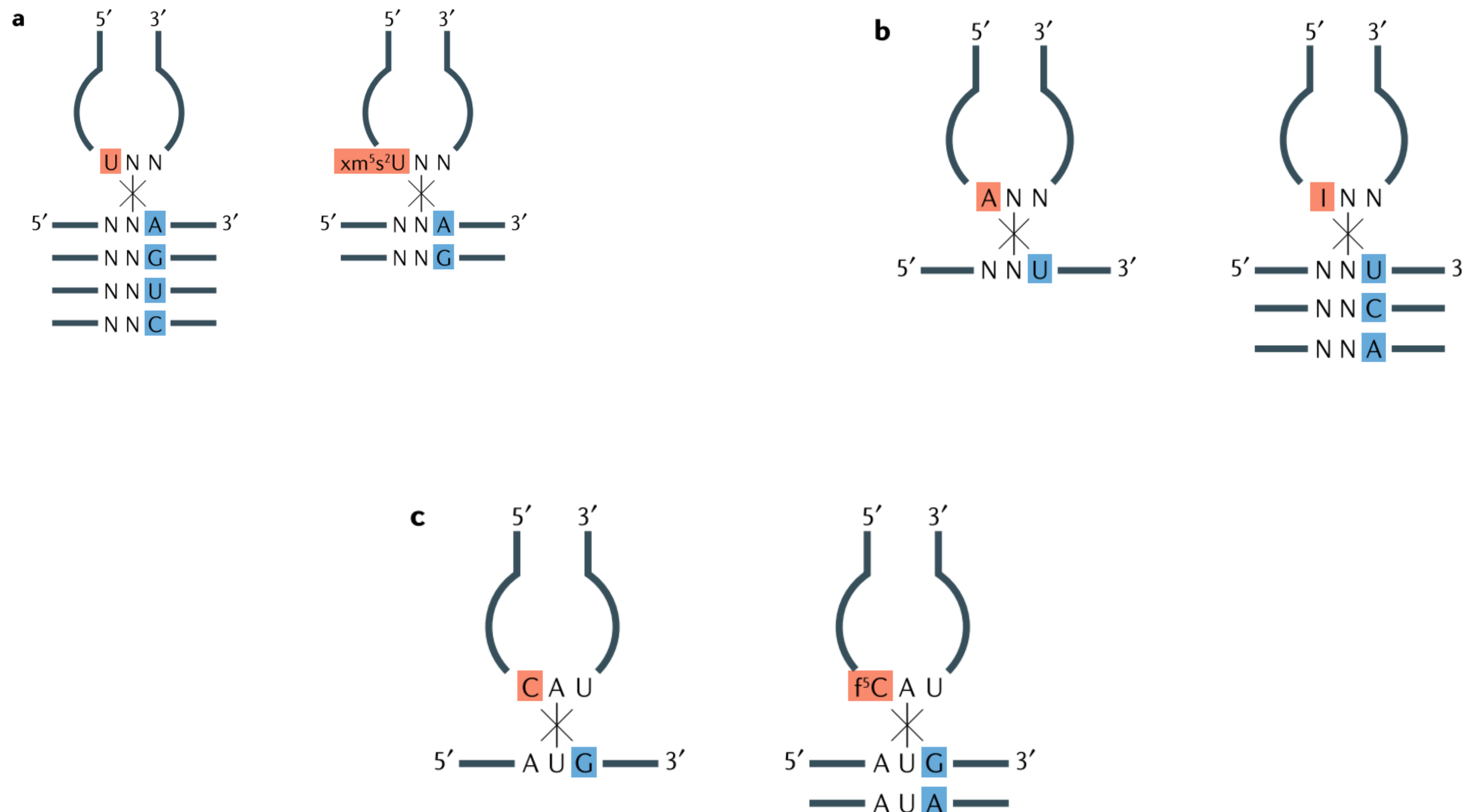
- Codon usage, tRNA abundance and modifications, and codon-anticodon base pairing are crucial for efficient and accurate translation of mRNAs into proteins.

- tRNA-modifying enzymes have been implicated in translational reprogramming in metabolism, DNA damage response, and cancer.

- tRNA-modifying enzymes are also implicated in the production of functional tRNA fragments.

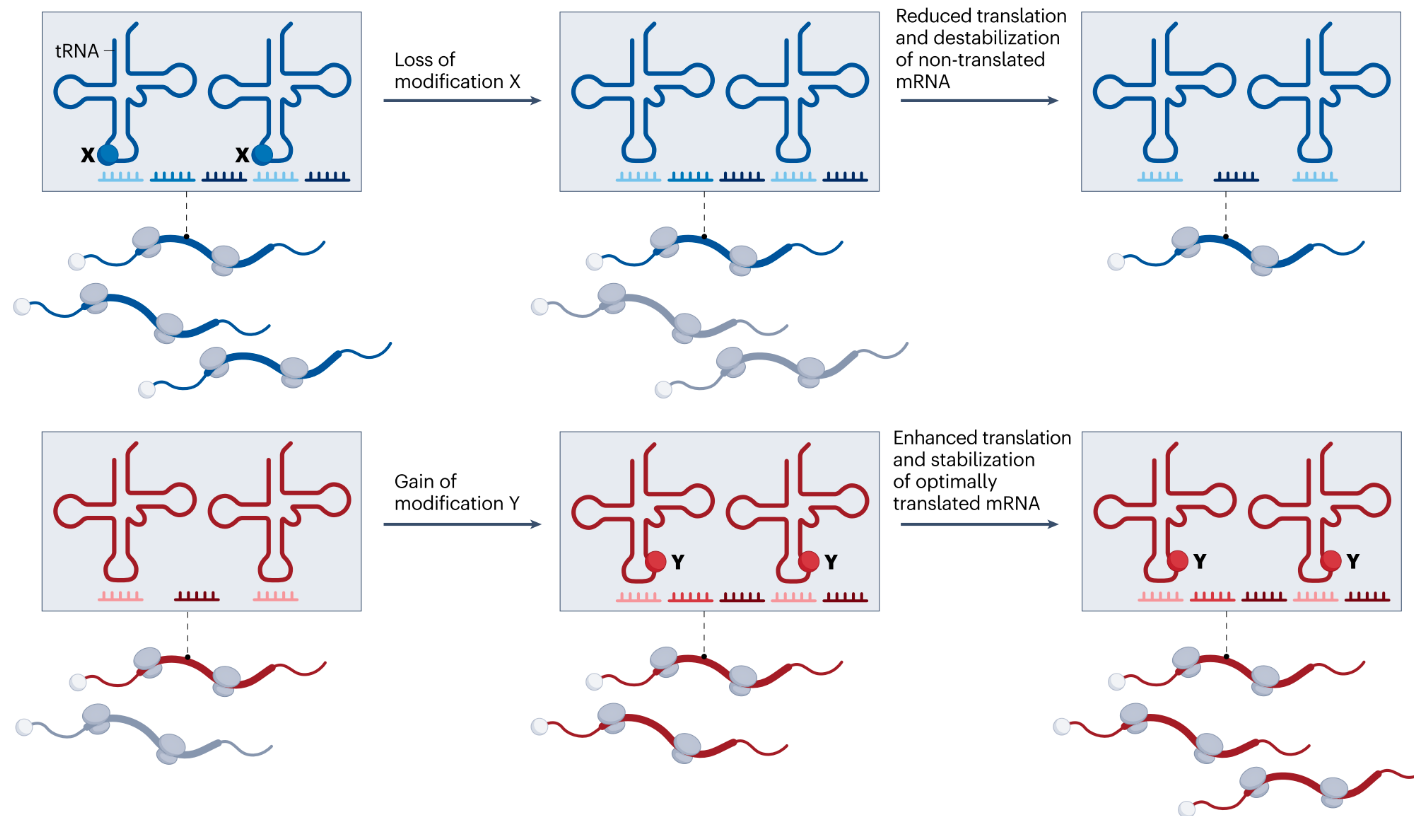
tRNA modifications of the anticodon

A wide variety of modified nuclosides (wobble modifications) are present at the first position (position 34) of the anticodon, which forms **wobble base pairing** with the third position of the codon at the ribosome to ensure accurate decoding of the genetic code. To date, more than 30 types of wobble modifications have been identified, which restrict or expand the decoding properties of the tRNA.



Nucleotide modifications in the anticodon sequence of tRNAs regulate efficient translation and allow optimal codon use

Re-balancing the mRNA translation speed and fidelity towards mRNAs of a different gene set (from blue to red) can rewire the translatome without requiring transcriptional changes in the nucleus. Transcript-specific enhanced translation can thereby determine the outcome of a cellular response.



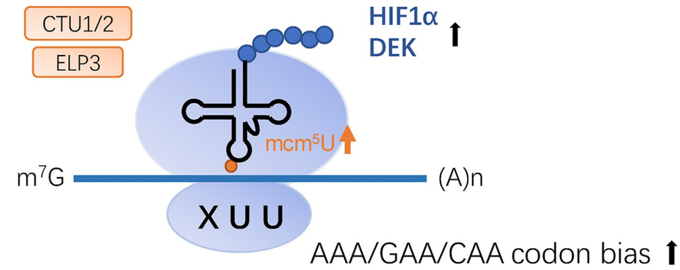
Metabolic control of tRNA modification

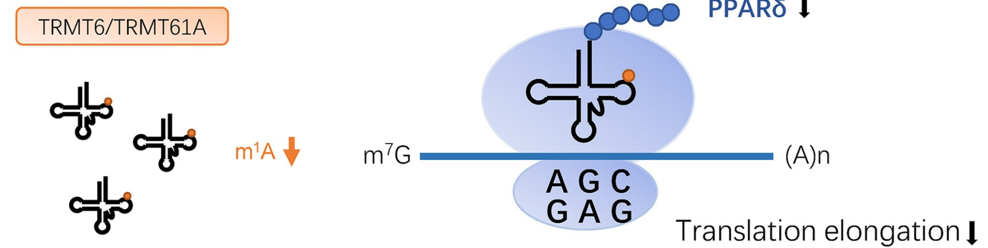
It was once widely believed that tRNA modifications are stable and static, and that their frequencies are rarely regulated, implying that they are structural components that ensure proper functioning of tRNAs in protein synthesis. During tRNA maturation, hypomodified and/or unstructured tRNAs are eliminated by the nuclear surveillance system. However, not all hypomodified tRNAs are eliminated, and many instances of hypomodified tRNAs are detected in healthy as well as pathogenic cells. These observations indicate that tRNA modifications undergo dynamic alteration in certain cellular conditions.

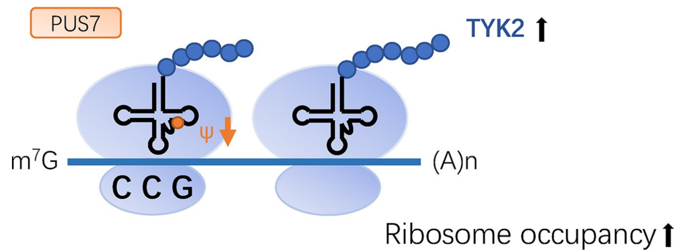
Examples of dynamic regulation of tRNA modification by the cellular metabolic status are:

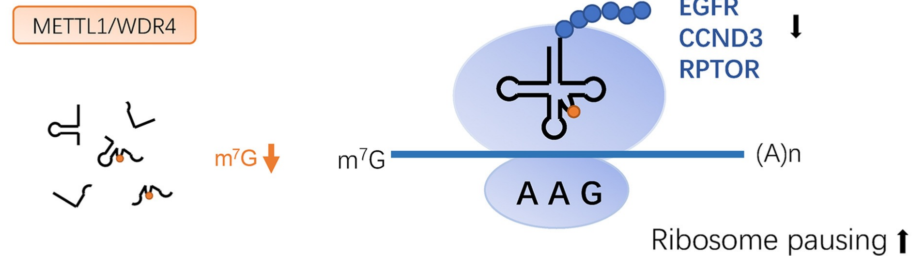
- Chemical switching of tRNA modifications by taurine deficiency
- CO2- sensitive tRNA modification and the Warburg effect
- Nutritional control of translation by queuosine

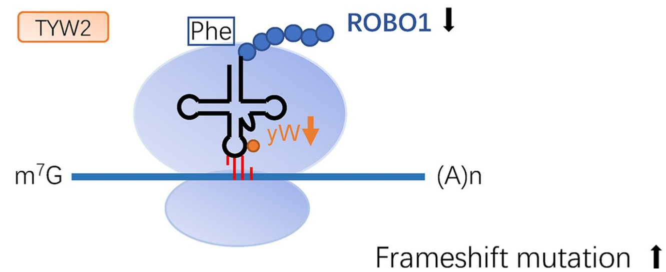
The translational effects associated with tRNA modifications defeats











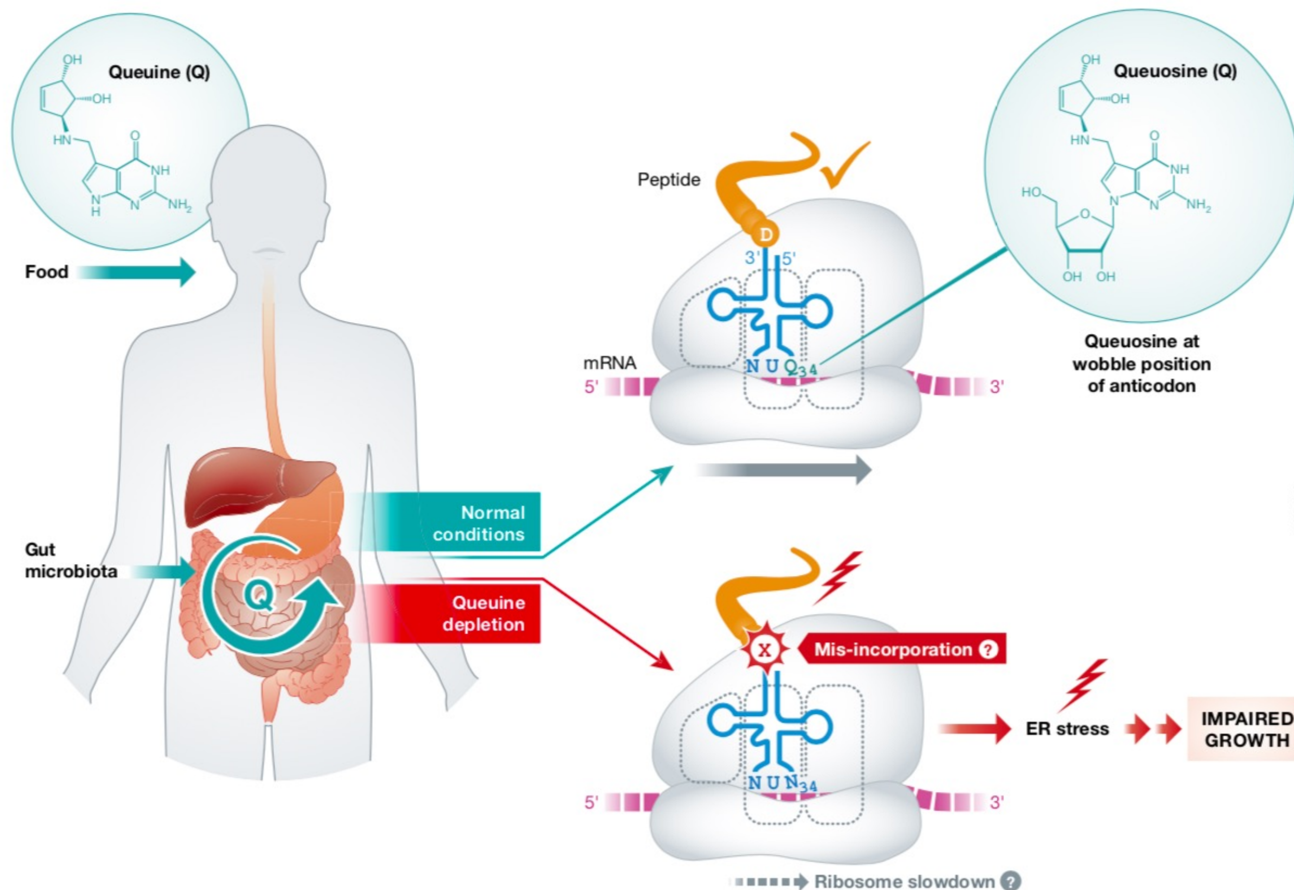
Aberrant tRNA modifications in human diseases

Category	Human genes	tRNA modifications	Effect	Diseases
Cancer	*METTL1/WDR4*	m^7G46	Upregulated	Intrahepatic cholangiocarcinoma, lung cancer, hepatocarcinoma, head and neck squamous cell carcinoma, esophageal squamous cell carcinoma, bladder cancer
	PUS7	$\Psi 8$	Upregulated	Glioblastoma
	ELP3/CTU1/2	mcm^5U34, mcm^5s^2U34	Upregulated	Breast cancer, melanoma
	ELP5	mcm^5U34, mcm^5s^2U34	Downregulated	Gallbladder cancer
	TRMT6/TRMT61A	m^1A58	Upregulated	Hepatocellular carcinoma, bladder cancer
	TYW2	G37 yW	Downregulated	Colon cancer
Neurological disorder	*ELP1*	mcm^5U34, mcm^5s^2U34	Downregulated	Familial dysautonomia
	ELP2	ncm^5U, mcm^5U34, mcm^5s^2U34	Downregulated	Intellectual disability and autism spectrum disorder
	ELP3	mcm^5s^2U34	Downregulated	Amyotrophic lateral sclerosis
	FTSJ1	2′-O-methylation	Downregulated	Nonsyndromic X-linked mental retardation, intellectual disability
	NSUN2	m^5C34	Downregulated	Neurodevelopmental disorders
	PUS7	$\Psi 13$	Downregulated	Intellectual disability and microcephaly
	DALRD3	m^3C32	Downregulated	Epileptic encephalopathy
	TRDC/OSGEP	t^6A37	Downregulated	Galloway-Mowat syndrome
Mitochondrial disease	*mt-tRNA$^{Leu(UUR)}$*	τm^5U34	Downregulated	MELAS
	mt-tRNA$^{Lys(UUU)}$	τm^5S^2U34, m^1A58, t^6A37	Downregulated	MERRF
	GTPBP3/MTO1	τm^5U34	Downregulated	Hypertrophic cardiomyopathy, lactic acidosis, and encephalopathy
	CDK5RAP1	2′-O-methylation	Downregulated	Myopathy
	mtDNA (A4295G/A4435G)	m^1G37	Upregulated	Deafness and maternally inherited hypertension

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

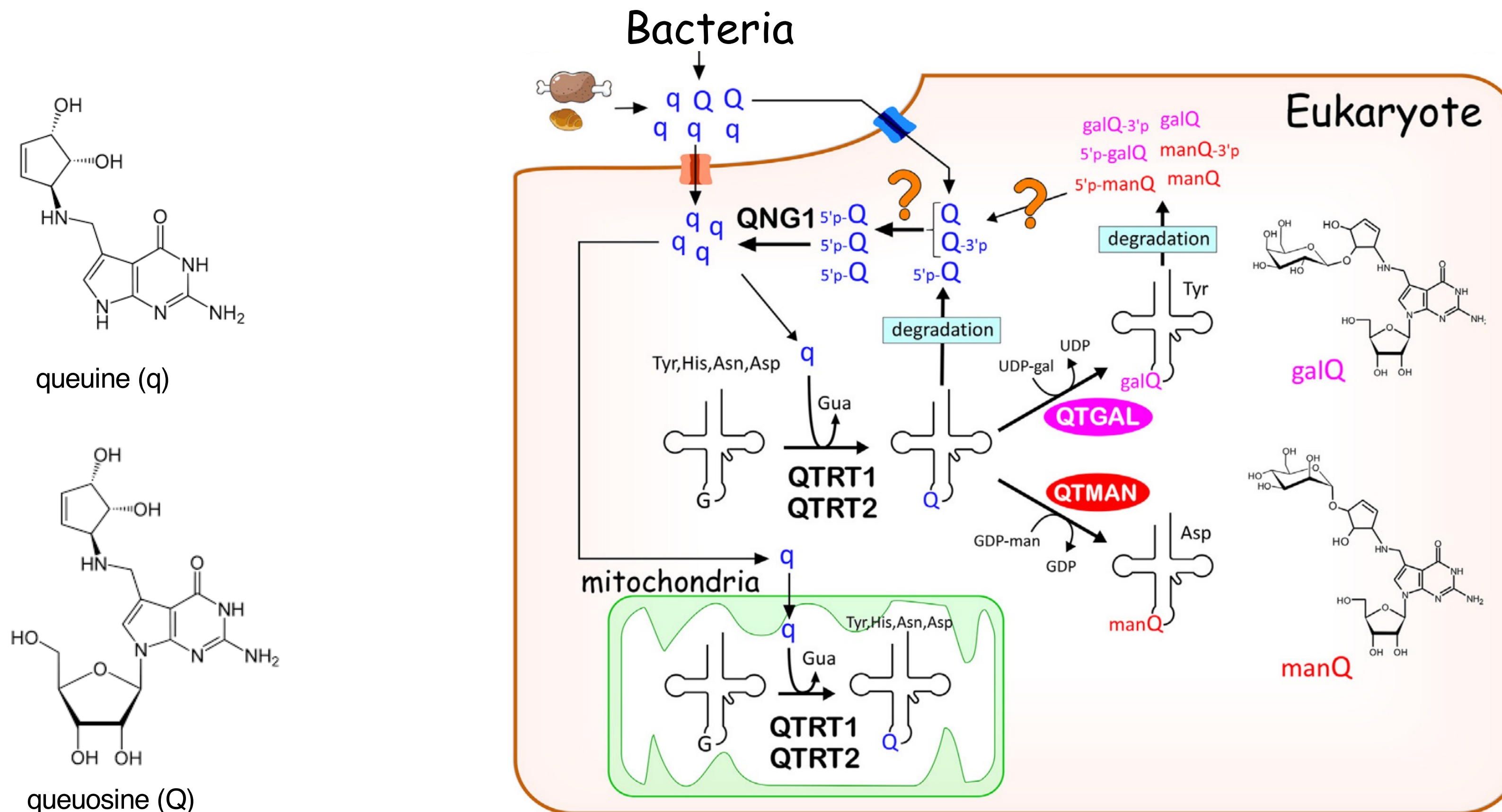
Queuosine-modified tRNAs confer nutritional control of protein translation

Queuine is a micronutrient obtained from food and the gut microbiota. Under normal conditions, queuine is used to modify the wobble position of certain tRNAs and this modification is required for proper translation. Queuine depletion results in deregulation of translation that leads to ER stress and impaired growth.



Biosynthetic pathways of queuosine in eukaryote

In eukaryotes, **queuine (q)** is 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 to obtain **queuosine (Q)** for Tyr-, His-, Asn-, and Asp-tRNAs. 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.



Physiological importance of Q and glycosylated Qs

Germfree mice subjected to dietary Tyr depletion develop a range of developmental issues, including neurological abnormalities and die within two weeks after birth. Lethality of germfree mice was entirely rescued by a diet supplemented with 100 nM queuine.

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

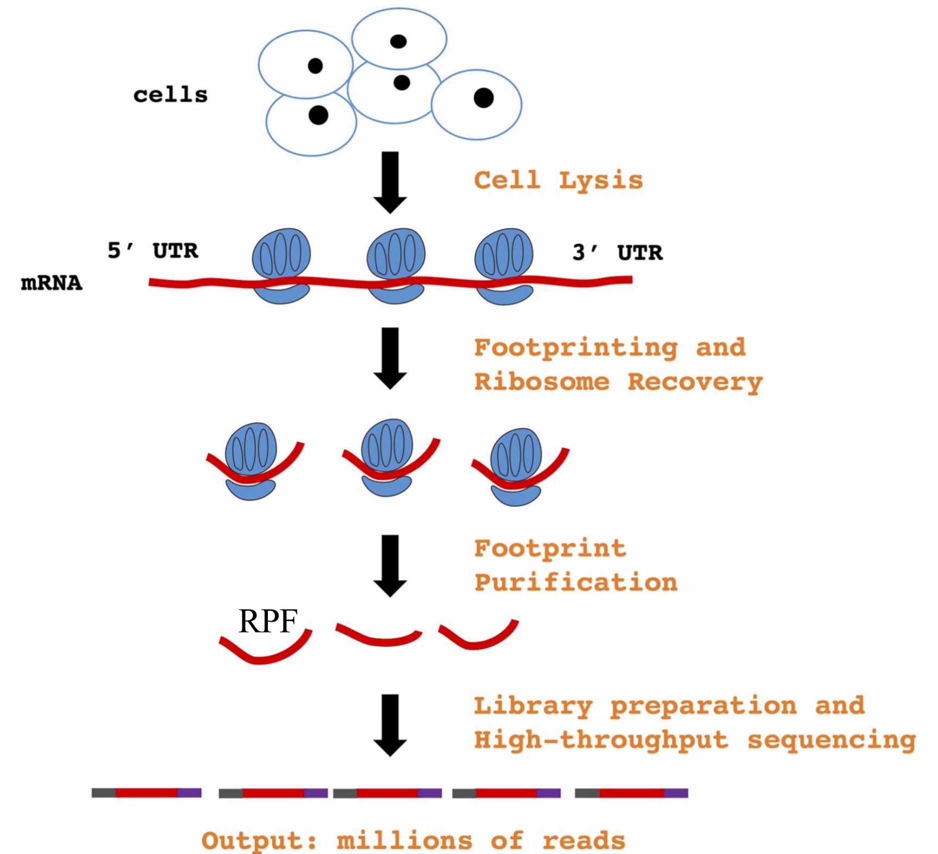
Maternal-zygotic mutants of qtrt1, qtgal, and qtman in zebrafish resulted in viable fish that displayed a shorter body length, suggesting that Q-glycosylation is required for efficient post-embryonic growth in zebrafish.

Both qtrt1 KO and qtgal KO increased eIF2a phosphorylation, suggesting that there is a connection between translational perturbation and the integrated stress response.

Ribo-seq analysis in tRNA modification studies

A variety of algorithms, tools and online resources have been developed to meet the ever-increasing demand for Ribo-seq data analysis. Essentially, the tools include the following analysis steps:

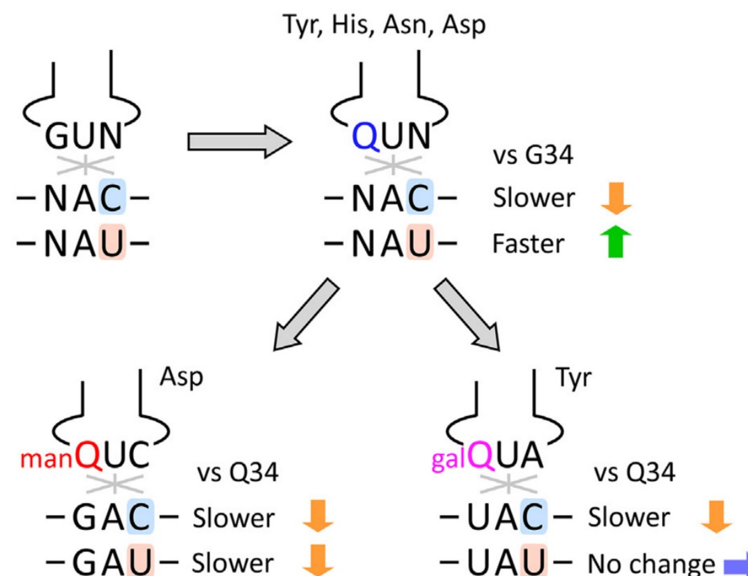
- **read mapping**, which maps RPF to coding sequence regions (CDS);
- **differential expression analysis**, which compares gene expression levels between different experimental groups using statistical methods;
- **translated open reading frame (ORF) detection**, which identifies regions within a genome that are being translated into proteins;
- **calculation of translation efficiency**, which normalizes ribosome counts to transcript abundance (the integration of RNA-seq data and Ribo-seq data is needed);
- **A-site detection**, **P-site detection** and **codon occupancy analysis**.



Decoding speed tuned by Q and glycosylated Qs

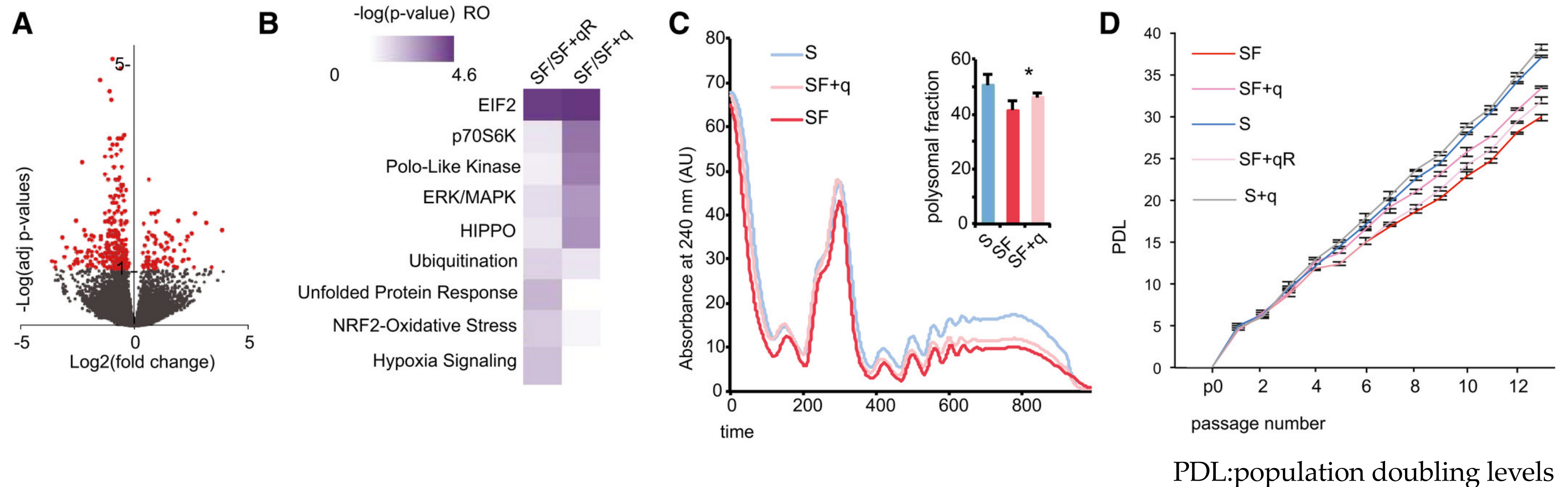
Ribosome profiling of human cells revealed that Q deficiencies affect the accuracy of translation, leading to increased frameshifting and miscoding.

- **Q** accelerates the translation elongation speed of U-ending codons but slows down C-ending codons in the cytoplasmic translation.
- **Q-galactosylation** of tRNA-Tyr does not affect the UAU decoding significantly but slows down UAC decoding .
- **Q-mannosylation** of tRNA-Asp slows down the decoding of both GAU and GAC codons.



Q-dependent phenotypes in cultured cell lines

They performed gene ontology (GO) enrichment analysis on mRNAs that were differentially translated in Q-depleted cells (A). This revealed a strong enrichment for genes involved in eIF2 signaling (B). The affected signaling pathways are strictly linked to the observed cellular phenotypes in the absence of Q: reduced global protein translation capacities (C) and reduced cell proliferation rates (D). these results indicated that q-dependent protein translation affects cell growth signaling and activated stress signaling.

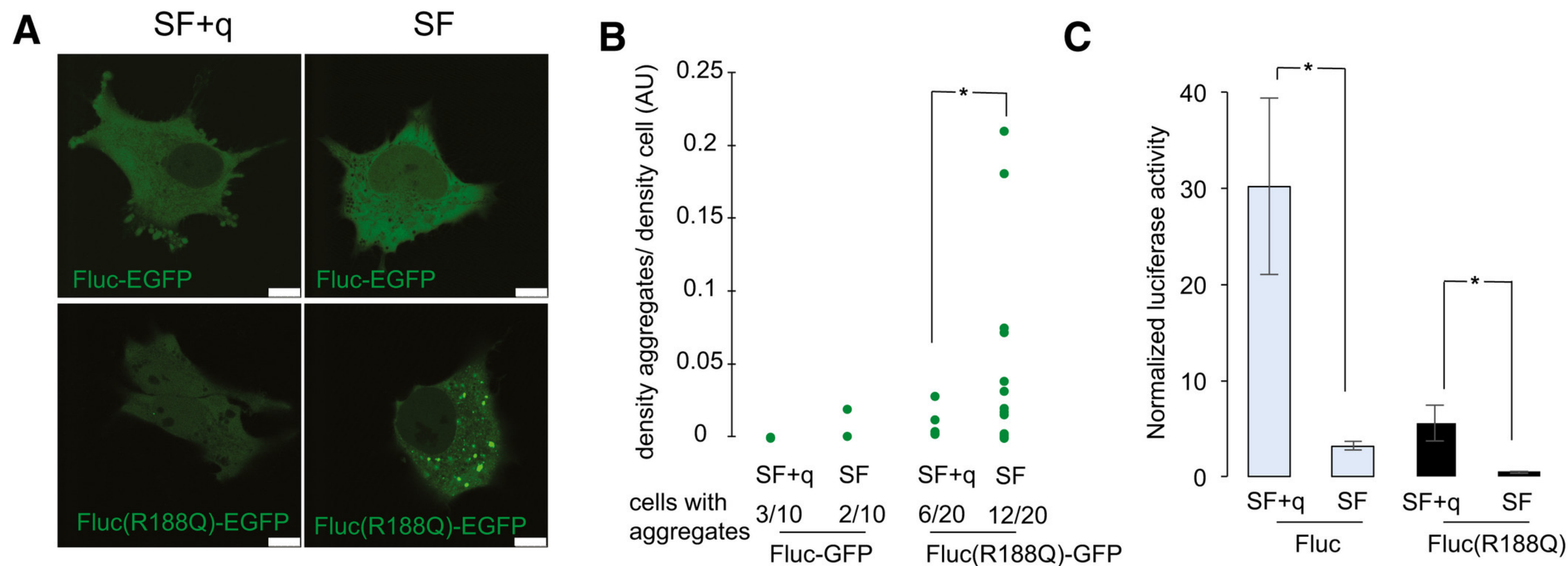


PDL:population doubling levels

Tuorto et al., 2018. EMBO J.

Q-deficiencies trigger protein aggregation

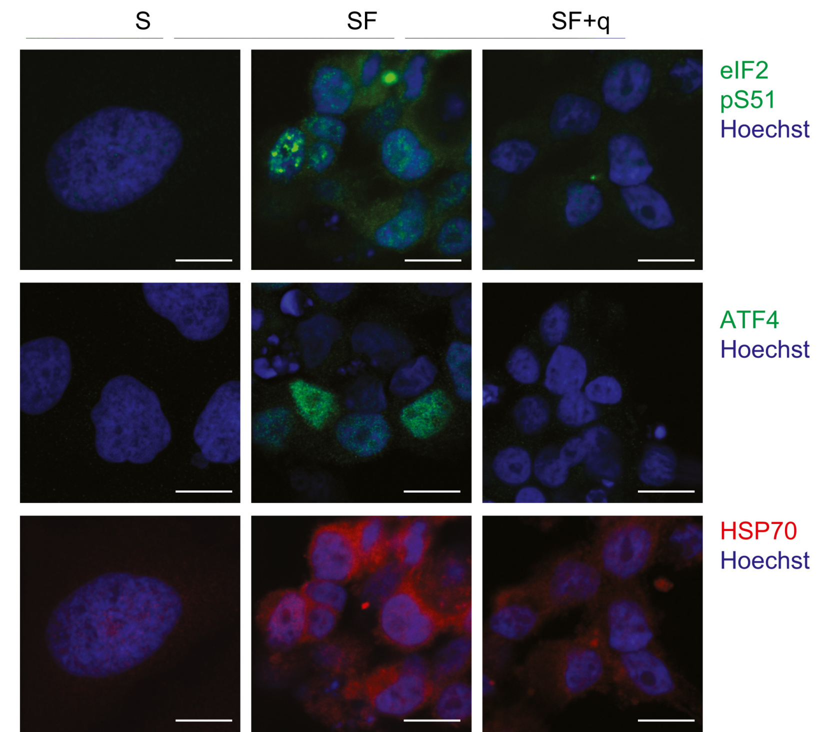
They used a destabilized variant of firefly luciferase (FlucR188Q) fused to GFP. The luminescence activity of Fluc mutation reflects imbalances in cellular protein homeostasis, and it requires chaperone surveillance to maintain soluble and enzymatically active state. The GFP-tagged Fluc variant could efficiently be used to measure the formation of protein aggregates in the absence of Q (A,B). Furthermore, and consistent with a decreased folding capacity, the destabilized luciferase had a decreased luminescence activity in the absence of Q (C).



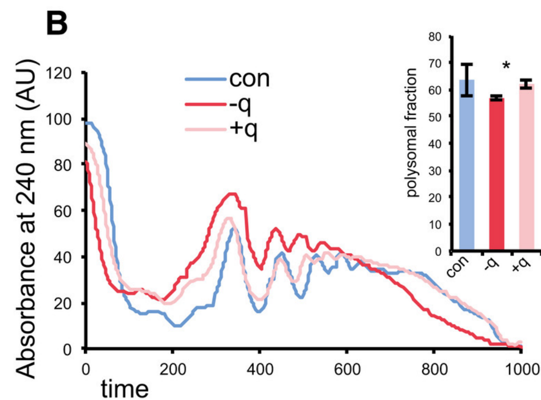
Tuorto et al., 2018. EMBO J.

Q depletion induces ER stress and UPR response

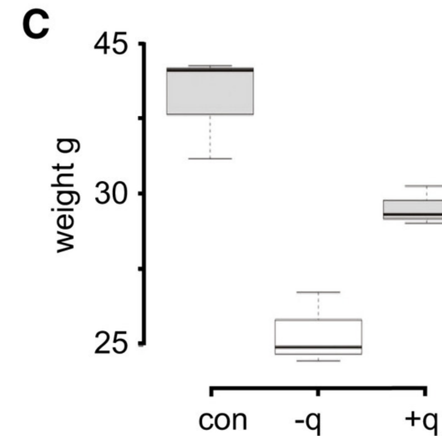
They observed an increase in phosphorylated eIF2α in Q-depleted cells, which was in agreement with the observed enrichment of eIF2 signaling genes among the mRNA that is differentially translated upon Q depletion. In addition, they also detected increased expression for the ATF4 transcription factor and the HSP70 chaperone, two key components of the UPR, that are known to increase the protein folding capacity of the ER under stress conditions



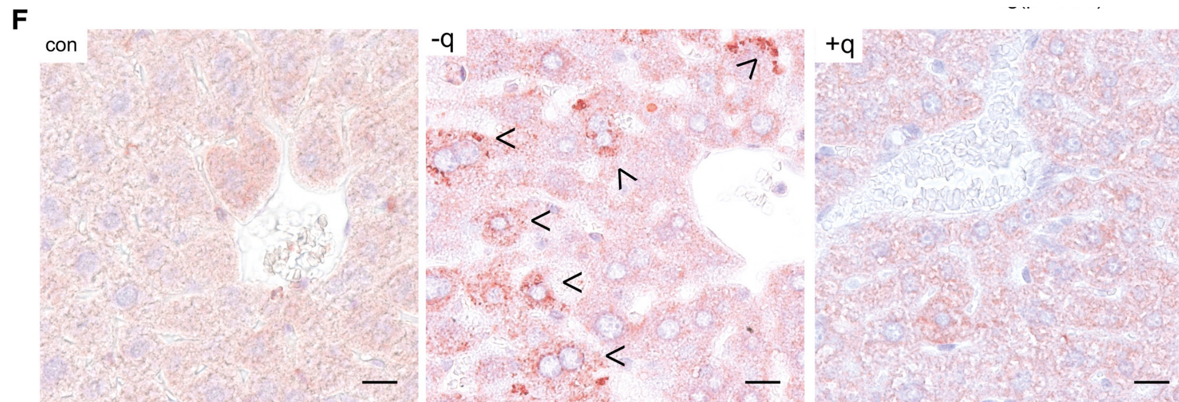
Q-dependent phenotypes in mice



B. Polysome profiles showing a reduction in the protein translation rate in the liver of mice fed with a q-free synthetic diet. Addition of q to the q-free diet significantly rescued the translation rate.

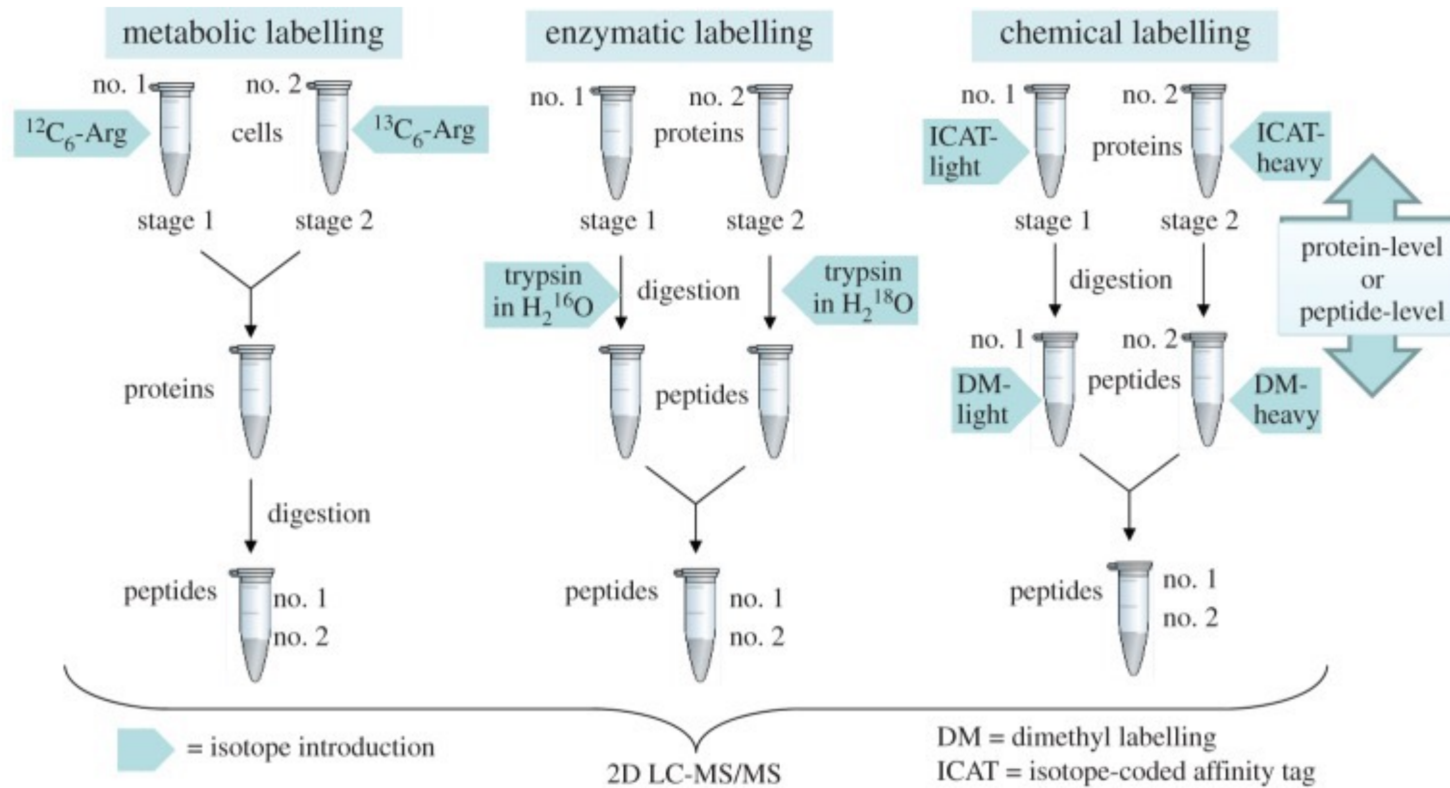


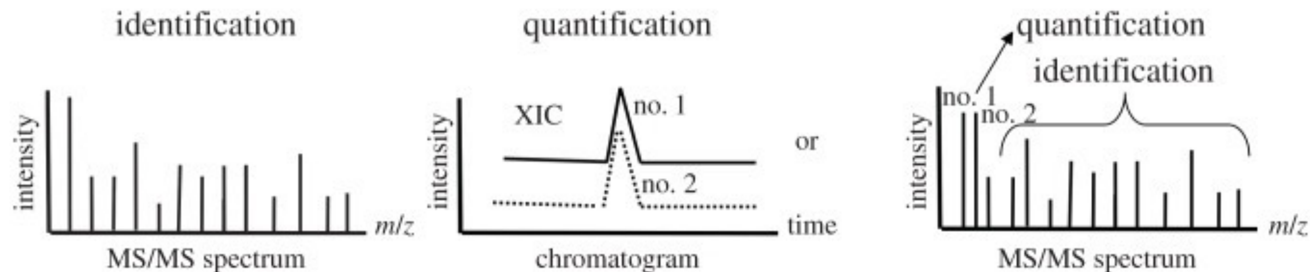
C. Box plot showing the body weight of mice fed a q-free synthetic diet for 60 days.



F. Liver sections from mice were stained with anti-ER stress antibody

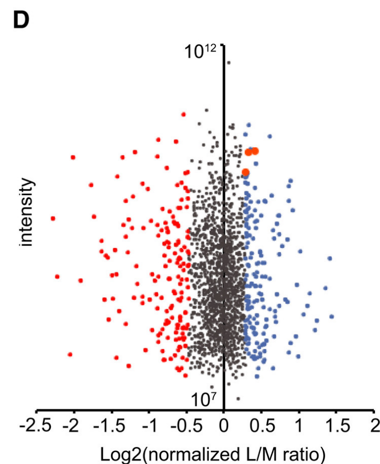
Stable-isotope labelling methods for MS-based quantitative proteomics



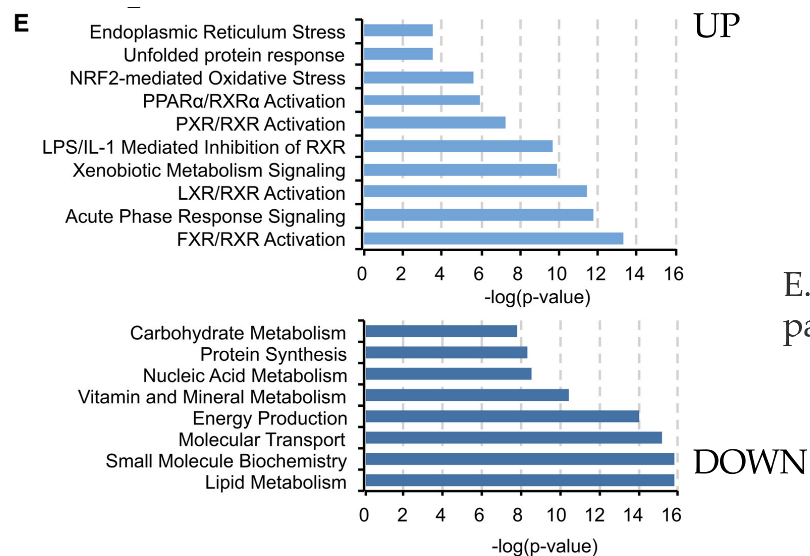


Q-dependent phenotypes in mice

They performed proteome-wide profiling of Q-depleted liver samples using dimethyl-labeling analysis (1690 proteins).

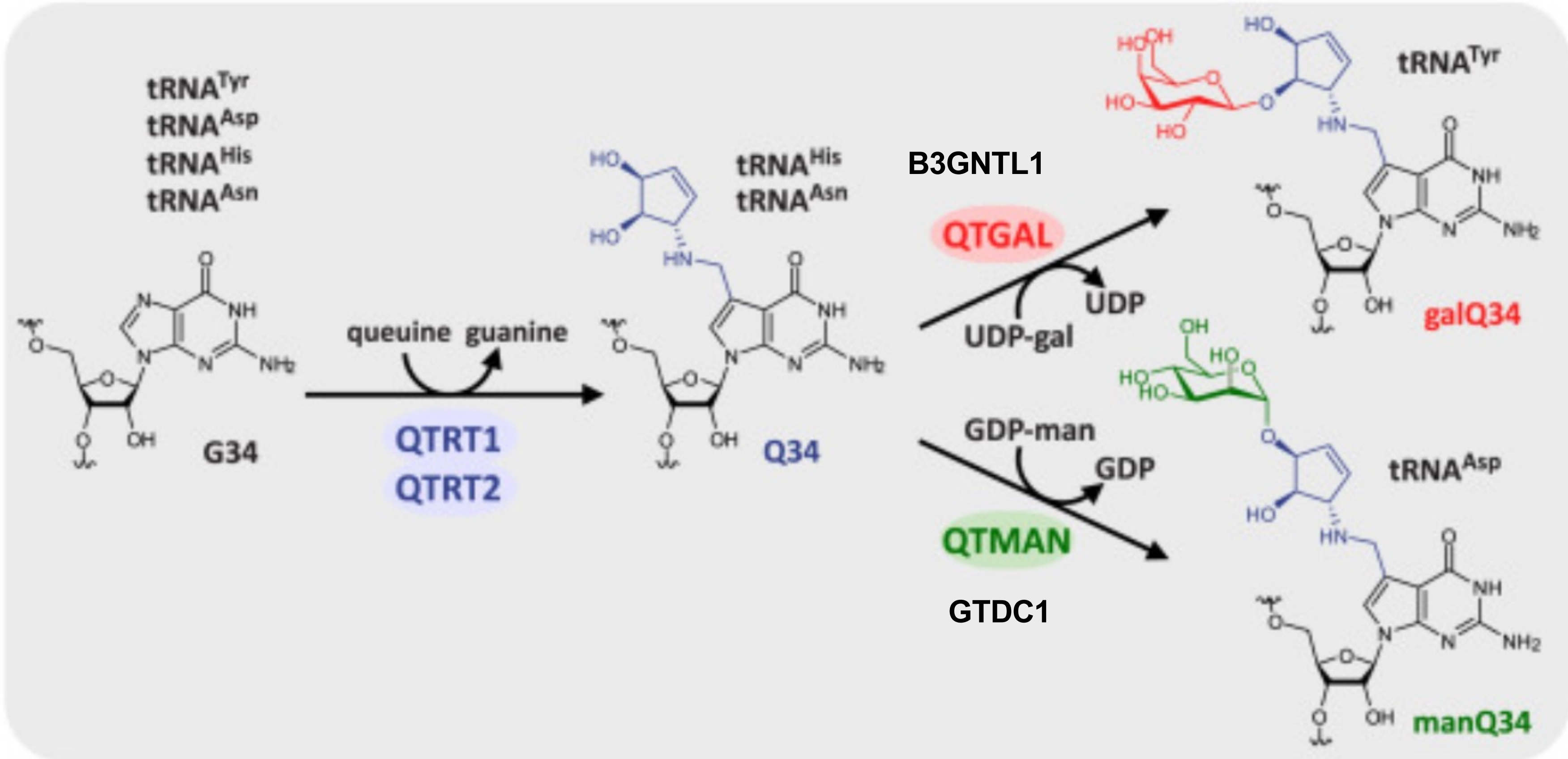


D. Dimethyl-labeling proteome analysis of −q versus +q liver tissue. Top 10% of deregulated proteins are indicated in red and blue. UPR effectors: HSPA5/BiP, HSP90b1, and CALR are in orange.



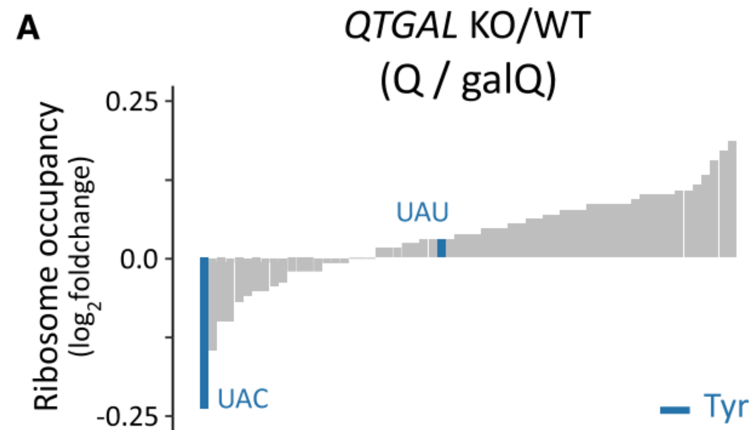
E. Gene ontology analysis of deregulated proteins. Signaling pathways and biological functions are presented

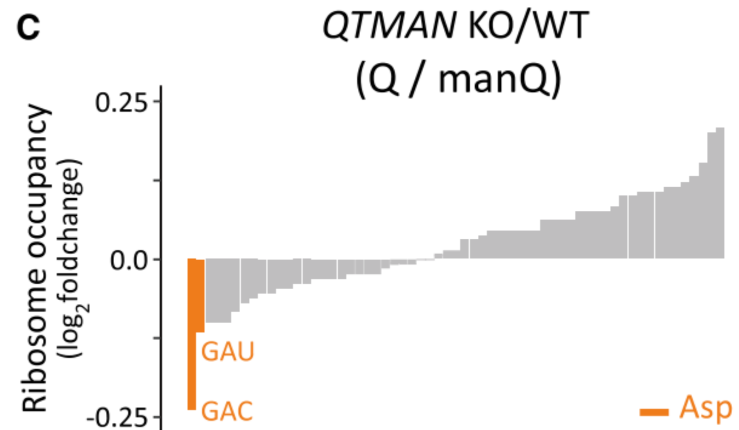
Q-glycosylations regulate codon-specific translation

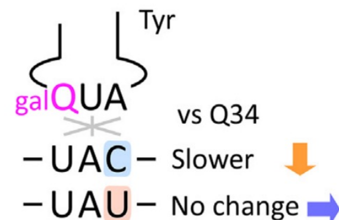


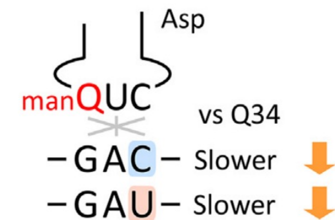
Q-glycosylations regulate codon-specific translation

Q-glycosylation was studied in HEK293 cells KO for QTGAL and QTMAN. Ribo-seq was utilized to analyse the fold-changes in ribosome occupancy at the A-site codon. QTGAL KO#1 (A) and QTMAN KO#1 (C) resulted in a significant decrease in ribosome density at specific codons, which included GalQ and ManQ-decoded codons: **tRNA-Asp and tRNA-Tyr**, suggesting that translation of these codons was faster in the absence of the Q-modification.

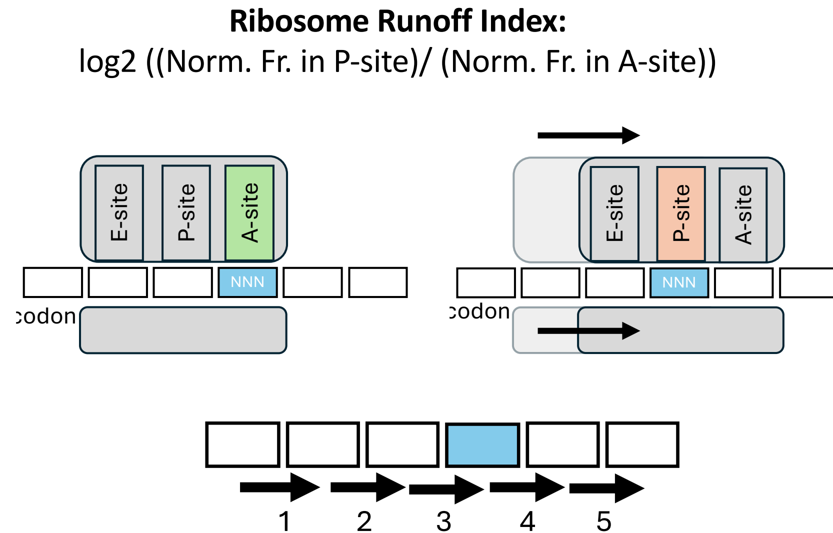






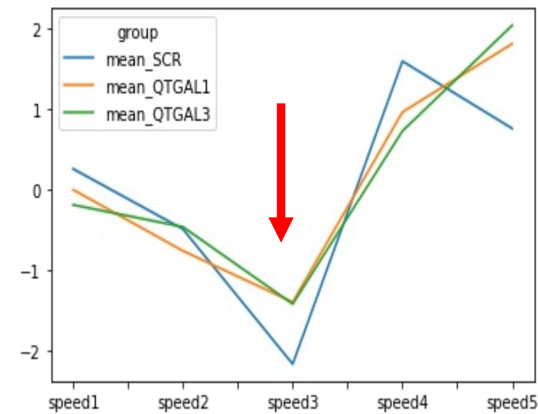


Q-galactosylation of tRNA-Tyr decreases UAC decoding speed

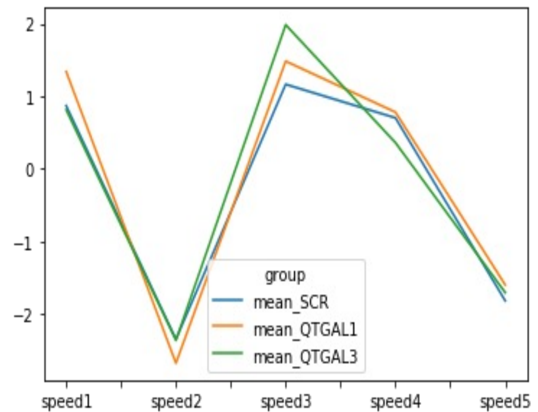


Ribosome Runoff Index

UAC



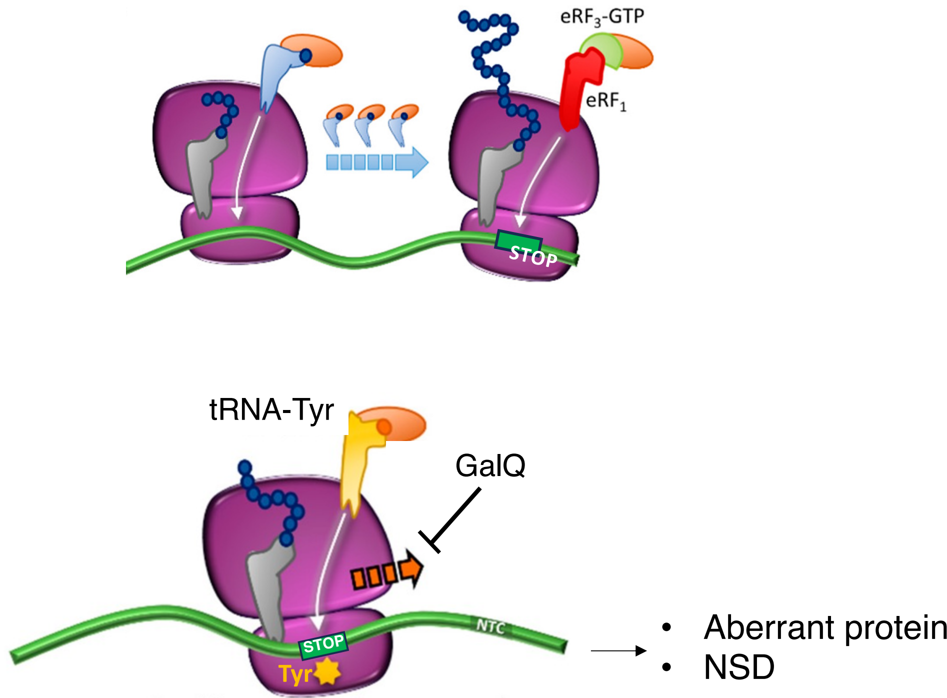
UAU

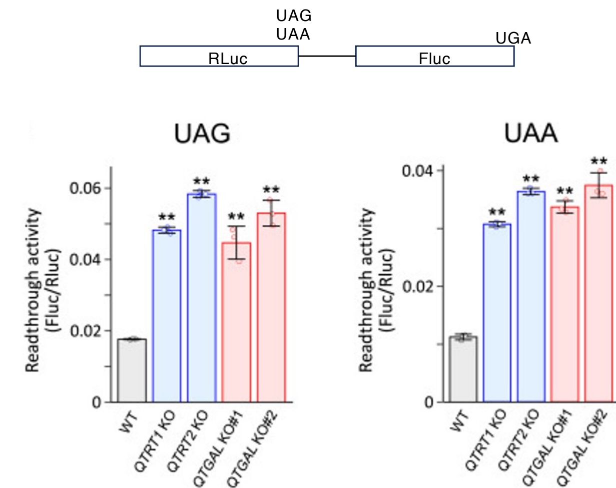


Q-galactosylation of tRNA-Tyr regulates readthrough efficiency

Cytoplasmic tRNA-Tyr acts as a potential suppressor tRNA with stop codon readthrough ability. Tyrosine is the amino acid most frequently incorporated at UAA and UAG codons. QTGAL knockout (KO) cells show a higher readthrough efficiency of UAA and UAG codons than wild-type (WT) cells, indicating that the galactosylation of galQ plays a role in preventing stop codon readthrough.

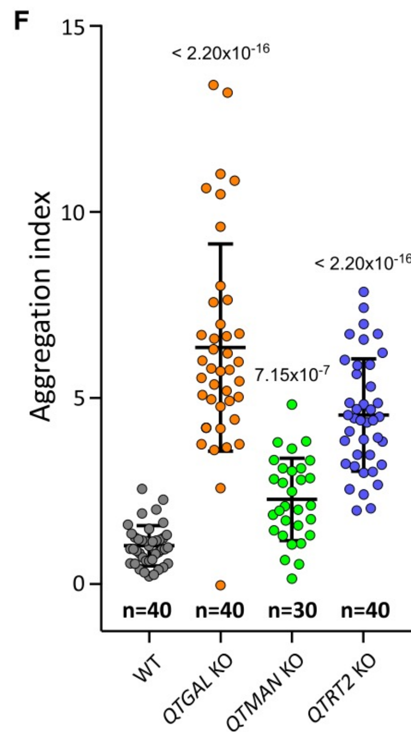
UAU, UAC	Tyr
UAA	**Stop**
UAG	**Stop**

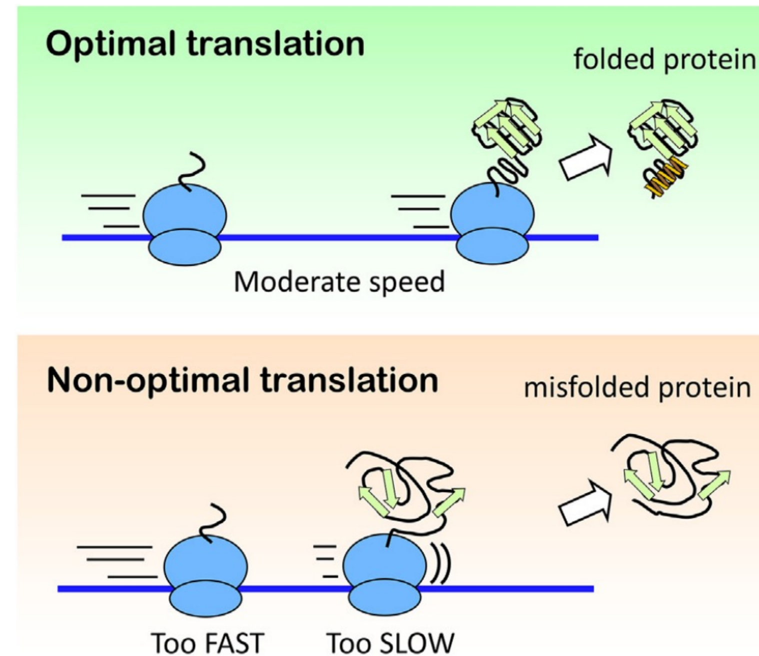




Q-deficiencies trigger protein aggregation

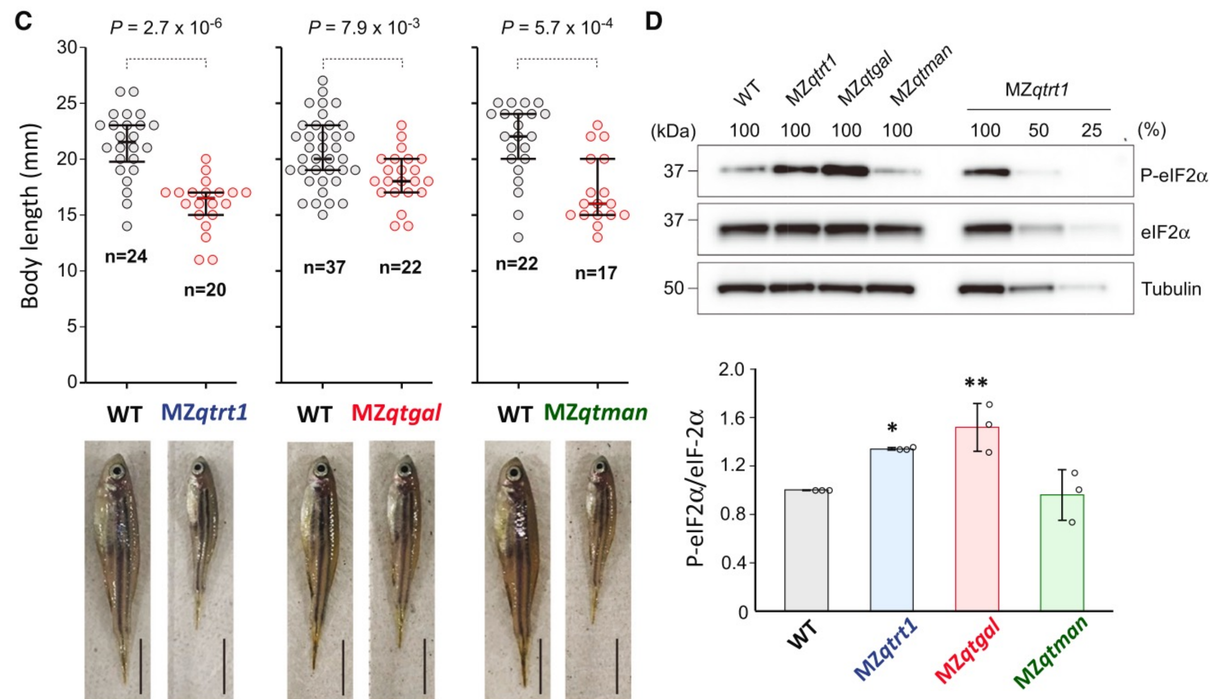
Similarly to Q-depleted cells, by sing an aggregation-prone reporter protein, it was shown that loss QTGAL and QTMAN induced protein aggregation.





Physiological impact of Q-glycosylation in zebrafish

Qtrt1 (-Q), QtGAL (- GalQ) and QtMAn (- ManQ) zebrafish mutants (MZ) were morphologically normal in embryonic and hatching stages. By contrast, body length measurement revealed that MZqtrt1,Mzqtgal, and Mzqtman were significantly shorter than the WT, suggesting growth retardation in the absence of respective tRNA modifications. They also observed upregulation of phosphorylated eIF2 in MZqtrt1 and MZqtgal, but not in Mzqtman, confirming the connection between translation perturbation and induction of integrated stress response. In contrast, the level of phosphorylated eIF2 did not explain the growth phenotype sufficiently.



Q modification and cancer

QTRT1, QTRT2, QTMAN, and QTGAL are upregulated in various types of cancer cells. In particular, QTGAL shows a marked increase in expression in many tumors. However, the potential role Q and glycosylated Q modifications in cancer still need to be addressed.