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Role of tRNA modifications in human diseases

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Transfer RNAs (tRNAs) are key for efficient and accurate protein translation. To be fully active, tRNAs need to be heavily modified post-transcriptionally. Growing evidence indicates that tRNA modifications and the enzymes catalyzing such modifications may play important roles in complex human pathologies. Here, we have compiled current knowledge that directly link tRNA modifications to human diseases such as cancer, type 2 diabetes (T2D), neurological disorders, and mitochondrial-linked disorders. The molecular mechanisms behind these connections remain, for the most part, unknown. As we progress towards the understanding of the roles played by hypomodified tRNAs in human disease, novel areas of therapeutic intervention may be discovered.

Structure of tRNAs and molecular role of tRNA modifications

tRNAs (see Glossary) are key adaptor molecules in the protein translation machinery. In their mature form, they are approximately 70–100 nucleotides long and fold into a 'clover leaf' secondary structure (Figure 1) and an L-shaped tertiary structure. After maturation, tRNAs are charged with their cognate amino acid at the 3′-end and, through their anticodon loop, pair specifically with codons in messenger RNAs (mRNAs). The nucleobases in the tRNA anticodon that interact with the mRNA triplets are those located at positions 34, 35 and 36 of the tRNA. Position 34 can wobble and pair with different nucleotides at the third position of the mRNA codon via non-Watson–Crick interactions. This flexibility allows the genetic code to be degenerate at the third position of codons (i.e., the third position of the codon does not alter the amino acid decoding); therefore, wobbling at position 34 of tRNAs is important because it allows some tRNAs to decode different sets of codons coding for the same amino acid, and some codons to be recognized by more than one anticodon sequence [1].

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 (The tRNA Modification Database: http://mods.rna.albany.edu/home). Most of these modifications and the enzymes responsible for catalyzing them are well described in the yeast *Saccharomyces cerevisiae* [2]; however, in recent years the human homologs for many of those enzymes and the biological role of the modifications they catalyze have started to be documented [3]. With this novel information, a link between tRNA modifications and human diseases is becoming increasingly clear (Table 1).

Chemical modifications are crucial for tRNA structure, function, and stability. In general, hypomodified tRNAs are targeted for degradation, thus a primary role of tRNA modifications is to prevent tRNAs from entering specific degradation pathways [2]. From a functional point of view, specific modifications in the anticodon loop can directly affect the behavior of tRNAs during gene translation. For example, several modifications at position 37 (adjacent to the anticodon) help to stabilize codon:anticodon interactions by providing base-stacking interactions at this position. Such modifications were shown to prevent translational frameshifting [4]. Modifications at position 34 are generally associated with decoding, because base modifications at this position are usually necessary for codon:anticodon wobbling to occur; however, such modifications can also prevent translational frameshifting. Typical examples of wobble modifications include uridine (U) 34 modifications such as incorporation of hydroxyl, methyl, and thiol groups, and adenosine (A) 34 modifications such as adenosine-to-inosine (A-to-I) editing [2,5].

Modifications in the main body of the tRNA usually have structural and stabilizing roles in tRNAs. Those modifications that drive the sugar conformation of the nucleobase into the C3′-endo increase binding affinity and rigidify the tRNA structure (e.g., pseudouridines); whereas other modifications, such as dihydrouridines, help to maintain the flexibility of the tRNA structure [4]. In some cases, certain modifications serve as identity elements for tRNAs (e.g., aminoacyl tRNA synthetase recognition). Post-transcriptional addition of a guanosine (G) at the 5′-end of $tRNA^{His}$ is critical for charging the tRNA with histidine by histidinyl-tRNA synthetase [6]. Another example is the 2′O-ribosyl phosphate modification at position 64 of $tRNA^{Met}$ from *S. cerevisiae*, which was shown to serve for discrimination between the initiator $tRNA^{Met}$ and the elongator $tRNA^{Met}$ [7]. Chemical structures of the tRNA modifications discussed are depicted in Figure 2.

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Glossary

Anticodon: a sequence formed by residues 34, 35, and 36 on tRNAs that recognize specific codons in mRNAs.
Base stacking: noncovalent interactions between the aromatic rings of the nucleotide bases.
Codon: a sequence of three nucleotides present in mRNAs that encode for a specific amino acid (or stop signal) during protein synthesis.
Dubowitz syndrome (DS): rare genetic disorder characterized by microcephaly, growth and mental retardation, eczema and peculiar facial features (small and round face, broad nose, wide set eyes with drooping eyelids).
Exon skipping: the omission of an exon by the splicing machinery during mRNA maturation. In some circumstances, therapies can induce the splicing machinery to leave out a mutated or misaligned exon, resulting in a transcript encoding for a truncated but functional protein.
Familial dysautonomia (FD): a hereditary sensory and autonomic neuropathy characterized by complex clinical traits such as decreased sensitivity to pain and temperature; cardiovascular, respiratory, and gastrointestinal dysfunction; lack of overflow tears; excessive sweating; and hypertension.
Gene therapy: the use of DNA as a therapeutic agent to treat a disease (e.g., supplement the patient with a DNA encoding for the functional version of a gene to replace the endogenous mutated one).
Homolog: protein or DNA sequences from different organisms that are similar due to a shared phylogenetic ancestry.
Insulin: a peptide hormone produced in the pancreas to regulate the carbohydrate and fat metabolism. It induces cells in skeletal muscles and liver and fat tissue to absorb glucose from the blood.
Intellectual disability: refers to patients with limitations in cognitive function and deficits in two or more adaptive behaviors such as reduced communication or social skills.
Isoacceptors: different tRNA species that code for the same amino acid. They carry a different anticodon sequence and hence recognize a different mRNA codon for a particular amino acid.
Messenger RNA (mRNA): a family of RNA molecules that are transcribed from the genomic DNA sequence and carry the information for protein translation. The coding region of mRNAs is defined by codons which are recognized by tRNAs to translate its nucleotide sequence into a peptidic sequence.
Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS): a syndrome linked to mutations in the mitochondrial genome. As such, it is inherited from the female parent. It primarily affects the nervous system and muscles.
Myoclonus epilepsy associated with ragged-red fibers (MERRF): a syndrome linked to mutations in the mitochondrial genome. As such, it is inherited from the female parent. It is characterized by progressive myoclonus epilepsy, short stature, hearing loss, lactic acidosis, and exercise intolerance.
Noonan syndrome: relatively common congenital disorder characterized by heart problems (pulmonary valve stenosis, atrial septal defects, hypertrophic cardiomyopathy, etc.) and multiple malformations (widely set eyes, low set ears, webbed neck, chest deformity, etc.). Patients also develop mental retardation in approximately 25% of cases.
Non-syndromic intellectual disability: a form of mental retardation that is milder than the syndromic intellectual disability with no other phenotypic abnormalities. It can be linked to the X chromosome in which case males are affected whereas females are carriers.
Oncogene: a gene that has the potential to produce cancer by inducing cell survival and proliferation.
Ortholog: a homolog derived from a speciation event.
Post-transcriptional modification: modifications that occur on RNA molecules after being transcribed from DNA and are therefore not encoded in the genome.
Protein translation accuracy: refers to the accurate incorporation of the correct amino acid into the growing polypeptide chain.
Protein translation efficiency: refers to the speed at which a protein is being translated and directly reflects on the protein abundance.
Rolandic epilepsy: a benign form of childhood epilepsy. It can start at age 3 years but usually stops at around age 14–18 years.
Strabismus: a condition where the eyes are not properly aligned with each other (also known as heterotropia).
Squamous cell carcinoma: a type of non-melanoma skin cancer.
Transfer RNA (tRNA): a family of non-coding RNA of approximately 70–100 nucleotides in length that serve as an adaptor molecule between mRNAs and the growing polypeptide chain during protein translation. tRNAs carry a specific amino acid at its 3′-end and specifically recognizes codons in the mRNA through the tRNA anticodon.
Translational frameshift: refers to a shift in the ribosome reading frame where the second or the third position of the mRNA codon is read as the first position of the codon.
Type 2 diabetes (T2D): metabolic disorder resulting in elevated levels of glucose in the blood either due to resistance to insulin or due to a relative deficiency in insulin production.
Urothelial carcinoma: a type of cancer that occurs in the urinary system (kidneys, urinary bladder, and accessory organs).
Watson–Crick pairing: nucleotide pairing between a purine and a pyrimidine (guanine:cytosine and adenine:thymine/adenine:uracil).
Wobble pairing: non-Watson–Crick pairing between residue 34 of tRNAs and the third residue of the mRNA codon.
Xenograft: a tissue graft or organ transplant from a donor of a different species than the recipient.

Because tRNA modifications can affect translation accuracy and efficiency as well as general tRNA stability, it would be expected that lack of such modifications could have profound and generalized effects on protein synthesis. By contrast, certain tRNA modifications might affect the translation of only a defined subset of transcripts enriched in certain types of codons, which could all be linked to a common cellular pathway [8]. In either case, we can hypothesize that the regulation of the tRNA modification levels could be used as a method to modulate protein expression and regulate complex cellular processes. In agreement with this hypothesis, many reports point at a clear link between defects in tRNA modifications and human diseases such as cancer, T2D, neurological disorders, and mitochondrial-linked disorders (Table 1). We therefore propose that tRNA modifications play crucial roles in human diseases, and that novel therapeutics based on modulation of such modifications could lead the way for tackling complex human pathologies for which, to date, there are no effective treatments.

tRNA modifications and neurological disorders

Intellectual disability is a major health problem worldwide with an estimated prevalence of up to 3% in the total population. In approximately 25% of cases, the disease is due to chromosomal rearrangements that are either cytogenetically visible (e.g., Down's syndrome trisomy 21) or submicroscopic (smaller deletions or duplications in the DNA). However, the remaining 75% of cases are believed to be caused by different single gene mutations that may or may not be linked to the X chromosome [9]. Several reports associate human intellectual disability and mutations in genes that encode for tRNA modification enzymes [10–17].

The FtsJ RNA methyltransferase homolog 1 (*FTSJ1*) gene has been described as the closest human homolog to the yeast tRNA methyltransferase 7 (*TRM7*) gene, which encodes for a methyltransferase acting at positions 32 and 34 on $tRNA^{Leu}$, $tRNA^{Phe}$, and $tRNA^{Trp}$ [3,18] (Figure 1). In humans, *FTSJ1* maps to the X chromosome, and genetic analyses revealed mutations in this gene to be associated with non-syndromic X-linked mental retardation [11–14]. Additionally, a strong correlation between the genetic variations in *FTSJ1* and cognitive functions in young males of the Chinese Han population has also been observed [19]. Mutant *FTSJ1* transcripts were reported to be very unstable and, in at least one case, the aberrant transcript was shown to be degraded via nonsense-mediated mRNA decay [11,14]. Northern blot analysis on a panel of normal human tissues revealed that wild type *FTSJ1* was expressed in several fetal tissues, most prevalently in the fetal brain [11]. In a different study, the expression of the gene in a panel of adult tissues was found to be highest in the heart and liver, and very low in the brain [12]. Thus, current data point towards a critical role