Transfer RNA Modifications: From Biological Functions to Biomedical Applications

Adrian Gabriel Torres and Lluís Ribas de Pouplana

Contents

Abstract Transfer RNAs (tRNAs) are essential components of the protein translation machinery. In order to become fully active, they need to be heavily modified post-transcriptionally. Such modifications affect the structure, stability and functionality of tRNAs; however, their exact roles at the molecular level remain largely elusive. Here we focus on the biological functions of tRNA modifications associated to human diseases and how such information can be used for biomedical applications. We put an emphasis on mitochondrial-linked dysfunctions, metabolic disorders, neurological defects and cancer. We also present methods and approaches currently used in the clinic to detect and monitor different human

A.G. Torres
Institute for Research in Biomedicine (IRB Barcelona), Barcelona Institute for Science and Technology, C/Baldiri Reixac 10, Barcelona 08028, Catalonia, Spain
e-mail: adriangabriel.torres@irbbarcelona.org

L. Ribas de Pouplana (✉)
Institute for Research in Biomedicine (IRB Barcelona), Barcelona Institute for Science and Technology, C/Baldiri Reixac 10, Barcelona 08028, Catalonia, Spain

Catalan Institution for Research and Advanced Studies (ICREA), P/Lluis Companys 23, Barcelona 08010, Catalonia, Spain
e-mail: lluis.ribas@irbbarcelona.org

S. Jurga et al. (eds.), *Modified Nucleic Acids in Biology and Medicine*, RNA Technologies, DOI 10.1007/978-3-319-34175-0_1

pathologies involving tRNA modifications or tRNA modification enzymes, and, additionally, we propose novel tRNA modification-based strategies that could be used for diagnosis, prognosis or treatment of human diseases.

Keywords tRNA • tRNA modification • Protein translation • Human disease • Biomedicine

1 Transfer RNAs Are Post-Transcriptionally Modified

Transfer RNAs (tRNAs) play a key role in the protein translation machinery. They are transcribed as long primary tRNAs that are processed during their biogenesis to yield 70–100 nucleotides long RNA species, that fold into a cloverleaf-shape (2^{ry} structure) (Fig. 1) and L-shape (3^{ry} structure) arrangement (Piñeyro et al. 2014). Following maturation, they are charged at their 3′-end with their cognate amino acid, which will be incorporated into the growing polypeptide chain during protein synthesis. Residues 34, 35 and 36 of the tRNA form the tRNA 'anticodon' (Fig. 1) that pairs specifically with nucleotide triplets on the messenger RNA (mRNA) called 'codons'. Each mRNA codon codifies for a specific amino acid; hence, tRNAs are adaptor molecules that translate specific mRNA codons into specific amino acids during translation (Piñeyro et al. 2014).

During maturation, tRNAs are required to go through a series of post-transcriptional chemical modifications. In general, for a given tRNA, about 10–15 % of the tRNA residues are found modified (Phizicky and Alfonzo 2010). There are more than 50 different chemical modifications described for eukaryotic tRNAs, that include methylations, thiolations, deaminations, acetylations, isomerizations and hydroxylations, among others (Machnicka et al. 2013). Such modifications affect the structure, processing, stability and overall functionality of tRNAs.

Modifications in particular regions of the tRNA affect different aspects of tRNA functionality. In general, modifications in the main body of the tRNA affect the rigidity/flexibility of the molecule. For example, pseudouridines increase the binding affinity of tRNA residues by inducing a C3′-endo sugar conformation, and dihydrouridines make these interactions more flexible by retaining the sugar pucker into a C2′-endo conformation (El Yacoubi et al. 2012). Modifications at the anticodon region of the tRNA have a direct role on codon recognition and prevent frameshifting during protein translation. On the one hand, modifications at position 34 of the tRNA increase (or restrict) the number of codons a tRNA can recognize, by promoting or inhibiting tRNA 'wobbling' (non-Watson-Crick nucleotide pairing) (Crick 1966). Some examples include A34-to-I34 editing (deamination) that allows the modified tRNAs to decode codons ending not only in uridine (U) but also in adenine (A) and cytosine (C) (Torres et al. 2014b) or U34 modifications, such as the one on $tRNA^{Lys}_{(UUU)}$, which allows the tRNA to decode its cognate codons AAA and AAG but restricts the recognition of the near-cognate asparagine

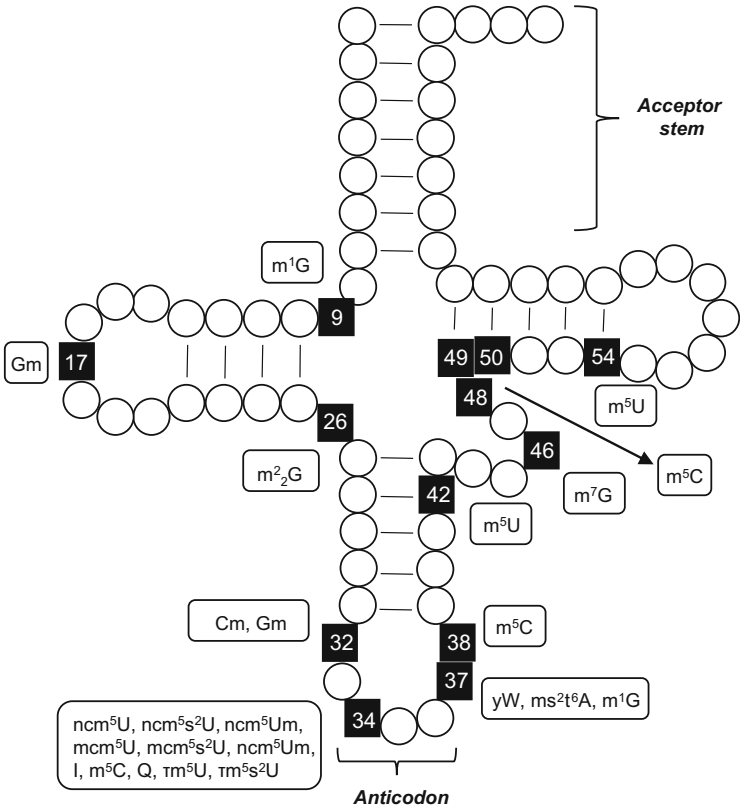

Fig. 1 Representation of the tRNA secondary structure ('cloverleaf'). Post-transcriptionally modified tRNA residues associated to human diseases are shown (*black boxes*). *Abbreviations:* m^1G, 1-methylguanosine; Gm, 2′-*O*-methylguanosine; $m^2{}_2G$, N2,N2-dimethyl guanosine; Cm, 2′-*O*-methylcytidine; ncm^5U, 5-carbamoylmethyluridine; ncm^5s^2U, 5-carbamoylmethyl-2-thiouridine; ncm^5Um, 5-carbamoylmethyl-2′-*O*-methyluridine; mcm^5U, 5-methoxycarbonylmethyluridine; mcm^5s^2U, 5-methoxycarbonylmethyl-2-thiouridine; mcm^5 Um, 5-methoxycarbonylmethyl-2′-*O*-methyluridine; I, inosine; m^5C: 5-methylcytosine; Q, queuosine; τm^5U, 5-taurinomethyluridine; τm^5s^2U, 5-taurinomethyl-2-thiouridine; yW, wybutosine; ms^2t^6A, 2-methylthio-N^6-threonyl carbamoyladenosine; m^5U, 5-methyluridine; m^5 U, 5-methyluridine; m^7G, 7-methylguanosine

codons AAU and AAC (Yarian et al. 2002). On the other hand, modifications at position 37 (adjacent to the anticodon) are usually associated to keeping in-frame translation. These are in general bulk modifications that stabilize codon/anticodon pairing by generating base-stacking interactions. Examples include the wybutosine 37 (yW37) modification that prevents −1 frameshifts (Waas et al. 2007) or the 1-methylguanosine 37 (m^1G37) modification that impedes +1 frameshifting (Urbonavicius et al. 2003). Finally, modifications in the acceptor stem of the tRNA (the stem formed by the 5′- and 3′-ends of the tRNA; Fig. 1) usually serve as identity elements for aminoacyl tRNA synthetases, the enzymes that charge the tRNA with its cognate amino acid. A representative example is the post-

transcriptional addition of a G residue at the 5′-end of $tRNA^{His}$ (G_{-1} modification) that is essential for correct charging by histidine-tRNA synthetase (Rudinger et al. 1994). For detailed information on the biological functions of tRNA modifications, several comprehensive reviews are available (Phizicky and Alfonzo 2010; El Yacoubi et al. 2012; Jackman and Alfonzo 2013; Piñeyro et al. 2014).

Interestingly, while tRNA modifications seem very important for tRNA function, the vast majority of them are not essential for cell viability (Piñeyro et al. 2014). In fact, in many cases, the modulation of tRNA modifications does not affect significantly the tRNA function and usually results in subtle phenotypes. However, it is not possible to generalize on this matter, and known observations need not apply to different cellular systems. Indeed, tRNA modification-based phenotypes can be associated to specific tissues. It is well documented that the tRNA pool and the expression of proteins carrying a particular codon bias may vary in a tissue-dependent manner (Kirchner and Ignatova 2015). Additionally, the 'penetrance' of a phenotype may be linked to the degree of the tRNA modification misregulation. This has been shown for mutations on mitochondrial tRNA genes that prevent the tRNA to be modified. A cell has a variable number of mitochondria, each of which carries their own mitochondrial genome. Only when a significant amount of mitochondria carrying the mutated mitochondrial genome variant accumulate, clear phenotypes can be observed (Abbott et al. 2014). Finally, sometimes tRNA modifications need to be considered not as individual modifications but as a part of a whole set of modifications leading to a significant phenotype. In yeast, overall tRNA modification patterns change in the tRNA pool when cells are subjected to stress conditions (Chan et al. 2012), suggesting a coordinated regulation of tRNA modifications as a mechanism for stress response.

In this chapter, we will describe the known connections that have recently been established between post-transcriptional tRNA modifications and human diseases. It will become evident that the roles of such modifications on those diseases are complex and that the molecular mechanisms behind the observed phenotypes remain poorly understood. Importantly, we will not address human diseases caused by mutations on tRNA genes or by defects on tRNA processing and maturation, which have been recently reviewed in depth (Abbott et al. 2014; Kirchner and Ignatova 2015), unless a clear direct effect on the tRNA modification pattern has been observed. Finally, we will present different strategies that are being pursued in the clinic for diagnosis, prognosis and treatment of this type of diseases and will propose potentially novel tRNA modification-based therapeutic approaches to be pushed forward in the future.

2 Links Between tRNA Modifications and Human Diseases

While tRNA modifications and the enzymes that catalyse such modifications have not been studied in depth in metazoans, an association between tRNA modifications and human diseases is starting to emerge (Torres et al. 2014a) (Fig. 1 and Table 1). Several human genetic studies have shown links between mutations in genes that

Table 1 Post-transcriptional tRNA modifications discussed in this chapter, including their role in different tissues and associated human diseases

Tissue	Gene	tRNA modification	Affected tRNAs	tRNA residues	Associated human diseases and phenotypes	References
Brain	FTSJ1	Cm, Gm, ncm^5Um	Leu, Phe, Trp	32, 34	Non-syndromic X-linked mental retardation	Hamel et al. (1999), Freude et al. (2004), Ramser et al. (2004), Bonnet et al. (2006), Froyen et al. (2007), Dai et al. (2008), Takano et al. (2008), Giorda et al. (2009)
	TRMT1	$m^2{}_2G$	Several	26	Intellectual disability	Najmabadi et al. (2011)
	ADAT3	I	Ala, Pro, Thr, Val, Ser, Arg, Leu, Ile	34	Intellectual disability and strabismus	Alazami et al. (2013)
	Elongator complex (IKAP, ELP2, ELP3, ELP4)	mcm^5U, ncm^5U, and derivatives	Several	34	Familial dysautonomia	Anderson et al. (2001), Slaugenhaupt et al. (2001), Leyne et al. (2003), Karlsborn et al. (2014)
					Intellectual disability	Najmabadi et al. (2011)
					Amyotrophic lateral sclerosis	Simpson et al. (2009)
					Rolandic epilepsy	Strug et al. (2009)
Lung	Elongator complex (IKAP)	mcm^5U, ncm^5U and derivatives	Several	34	Bronchial asthma in children	Takeoka et al. (2001)
Bladder	HABH8 (ALKBH8)	mcm^5U	Arg, Glu	34	Bladder cancer	Shimada et al. (2009)
Liver	TARBP1 (TRMT3)	Gm	Ser	17	Morris hepatoma	Randerath et al. (1981)
Breast	TRMT12	yW	Phe	37	Breast cancer	Rodriguez et al. (2007)
	TRMT2A (HTF9C)	m^5U	Several	42, 54		Bartlett et al. (2010)

(continued)

Table 1 (continued)

Tissue	Gene	tRNA modification	Affected tRNAs	tRNA residues	Associated human diseases and phenotypes	References
Multiple tissues	NSUN2	m^5C	Several	34, 48, 49, 50	Autosomal-recessive intellectual disability	Abbasi-Moheb et al. (2012), Khan et al. (2012), Martinez et al. (2012), Fahiminiya et al. (2014), Blanco et al. (2014)
					Dubowitz-like syndrome	Martinez et al. (2012)
					Noonan-like syndrome	Fahiminiya et al. (2014)
					Skin, breast and colorectal cancer	Frye and Watt (2006), Pierga et al. (2007), Frye et al. (2010)
	TRMT10A (HRG9MTD2)	m^1G	Several	9	Intellectual disability, microcephaly, short stature	Igoillo-Esteve et al. (2013), Gillis et al. (2014)
					Colorectal cancer	Berg et al. (2010)
					Hyperinsulinaemic hypoglycaemia	Igoillo-Esteve et al. (2013), Gillis et al. (2014)
	HTRM9L	$\underline{m}cm^5U$, $\underline{m}cm^5s^2U$	Several	34	Different types of cancer	Begley et al. (2013)
	*	Q	Asn, Asp, His, Tyr	34	Different types of cancer	Randerath et al. (1984), Vinayak and Pathak (2010)
	CDKAL1	$\underline{ms^2}t^6A$	Lys	37	Type 2 diabetes	Kirchhoff et al. (2008), Stancakova et al. (2008), Quaranta et al. (2009), Wei et al. (2011), Wei and Tomizawa (2011), Xie et al. (2013)
					Cardiovascular diseases	Saade et al. (2011)
					Crohn's disease	Barrett et al. (2008), Quaranta et al. (2009)
					Psoriasis	Quaranta et al. (2009)
	WDR4	m^7G	Several	46	Down's syndrome	Michaud et al. (2000)
					Microcephalic primordial dwarfism	Shaheen et al. (2015)

	DNMT2 (TRDMT1)	m^5C	Asp	38	Increased red blood cell folate levels	Franke et al. (2009)
					Different types of cancer	Schaefer et al. (2009)
Mitochondrial defects	**	τm^5U	mt-Leu	34	MELAS	Hayashi et al. (1993), Kirino et al. (2004), Suzuki and Nagao (2011)
	**	τm^5s^2U	mt-Lys	34	MERRF	Yasukawa et al. (2001), Suzuki and Nagao (2011)
	MTU1 (TRMU)	s^2U ($\tau m^5\underline{s^2}U$)	mt-Lys, mt-Glu, mt-Gln	34	MERRF	Umeda et al. (2005)
					Acute liver failure in infancy accompanied by lactic acidaemia	Zeharia et al. (2009)
					Deafness associated with mutations in mitochondrial 12S ribosomal RNA	Guan et al. (2006)
	TRMT5	m^1G	mt-Leu	37	Lactic acidosis and multiple mitochondrial respiratory complex deficiencies	Powell et al. (2015)

For complex tRNA modifications, the underlined modification refers to the modification step at which the indicated gene is involved
Cm, 2′-*O*-methylcytidine; Gm, 2′-*O*-methylguanosine; ncm^5U, 5-carbamoylmethyluridine; ncm^5Um, 5-carbamoylmethyl-2′-*O*-methyluridine; m^5C, 5-methylcytosine; mcm^5U, 5-methoxycarbonylmethyluridine; yW, wybutosine; m^5U, 5-methyluridine; m^1G, 1-mehtylguanosine; m^7G, 7-methylguanosine; $m^2{}_2G$, N2,N2-dimethylguanosine; Q, queuosine; ms^2t^6A, 2-methylthio-N6-threonyl carbamoyladenosine; I, inosine; τm^5U, 5-taurinomethyluridine; τm^5s^2U, 5-taurinomethyl-2-thiouridine
*No associated gene
**Unknown enzyme

encode (or are expected to encode) for enzymes that catalyse tRNA modifications and a wide range of complex human pathologies, including neurological disorders, cardiac and respiratory defects, cancer, metabolic dysregulations and mitochondrial-linked dysfunctions (see below). In most of these conditions, an in-depth understanding of the molecular mechanisms of pathology is lacking. It will become clear in this section that unravelling the details of such molecular mechanisms will be very challenging given that some of the observed phenotypes associated to tRNA modifications are tissue-specific and are dependent on the degree to which the tRNA is modified or not (Kirchner and Ignatova 2015).

2.1 Neurological Disorders

The brain is perhaps the most sensitive tissue to defects in tRNA modifications. The FtsJ RNA methyltransferase homolog 1 (FTSJ1) gene is likely the human homolog of the yeast tRNA methyltransferase 7 (TRM7) gene that encodes for the enzyme that methylates positions 32 and 34 on $tRNA^{Leu}$, $tRNA^{Trp}$ and $tRNA^{Phe}$ (Towns and Begley 2012). Mutations in FTSJ1 or a complete deletion of this gene has been linked to non-syndromic X-linked mental retardation (Hamel et al. 1999; Freude et al. 2004; Ramser et al. 2004; Bonnet et al. 2006; Froyen et al. 2007; Dai et al. 2008; Takano et al. 2008) and reported to affect cognitive functions in young males of the Han Chinese population (Gong et al. 2008). The tRNA modification state of these patients was not evaluated, but a mutant FTSJ1 transcript was shown to be very unstable and likely degraded by nonsense-mediated mRNA decay (Freude et al. 2004; Takano et al. 2008). Interestingly, the expression of wild-type FTSJ1 in human tissues was reported to be high in foetal brain (Freude et al. 2004) but low in adult brain (Ramser et al. 2004), consistent with a key role for this protein in the developing brain. Notably, patients bearing a chromosomal duplication of regions involving FTSJ1 and other genes presented mild/moderate mental retardation (Bonnet et al. 2006; Giorda et al. 2009), suggesting that overexpression of wild-type FTSJ1 might also be detrimental. However, a patient with mild mental retardation that presented a smaller chromosomal duplication, also involving FTSJ1, did not show increased levels of FTSJ1 mRNA as measured by quantitative PCR in blood; and instead the phenotype was attributed to the overexpression of three other genes (also located within the duplicated chromosomal region): EBP, WDR13 and ZNF81 (El-Hattab et al. 2011).

Mental retardation has also been reported in human patients with mutations in other genes encoding for tRNA modification enzymes. The tRNA methyltransferase 10A (TRMT10A), also known as human RNA (guanine-9) methyltransferase domain containing 2 (HRG9MTD2), is the human ortholog of the yeast enzyme that catalyses the methylation of guanosine at position 9 of several tRNAs (Towns and Begley 2012). Mutations in the human TRMT10A gene have been associated to microcephaly, short stature and intellectual disability (Igoillo-Esteve et al. 2013; Gillis et al. 2014). TRMT10A was shown to be expressed in

human embryonic and foetal brain (Igoillo-Esteve et al. 2013). Furthermore, Gillis and colleagues showed in vitro that the mutant TRMT10A protein could effectively bind to a tRNA substrate but showed a dramatic reduction in methylation activity as compared to the wild-type protein, probably due to impaired ability to bind the methyl donor S-adenosylmethionine (Gillis et al. 2014). As it will be discussed later, defects on this enzyme not only affect brain tissues but also the liver and colon.

Other examples of genes encoding for tRNA modification enzymes and mental retardation include the gene encoding for tRNA methyltransferase 1 (TRMT1), an enzyme that dimethylates guanosines at position 26 of several tRNAs (Liu and Straby 2000), and the gene encoding for ELP2 (a component of the elongator complex; see below). Both enzymes were reported as novel markers for recessive cognitive disorders (Najmabadi et al. 2011). Notably, in mice, a potential homolog of the human TRMT1 named 'TRM1-like' was shown to have a role in motor coordination and exploratory behaviour (Vauti et al. 2007), suggesting a conserved role for this protein in the brain. Finally, the human WD repeat domain 4 (WDR4) gene was found as a potential candidate marker for phenotypes observed in Down's syndrome (Michaud et al. 2000); and mutations in this gene have recently been associated to a distinct form of microcephalic primordial dwarfism (Shaheen et al. 2015). This gene is the human homolog of the yeast TRM82, one of the subunits of the heterodimeric enzyme responsible for the 7-methylguanosine modification at position 46 in several tRNAs (Towns and Begley 2012). Two transcript variants for this gene were described in humans, where the shorter one (of about 1.5 kb) was highly expressed in foetal tissues and the longer one (of about 2.5 kb) showed faint expression in most tissues (Michaud et al. 2000), suggesting a key function for the shorter transcript in developmental processes. Interestingly, the chromosomal region where WDR4 maps has already been associated to several genetic disorders such as maniac-depressive psychosis, autosomal-recessive deafness, Knobloch syndrome and holoprosencephaly (Michaud et al. 2000).

Recently, the human adenosine deaminase acting on tRNA 3 (ADAT3) was validated as one of the subunits of the heterodimeric enzyme responsible for adenosine-to-inosine editing at position 34 of 8 different tRNAs (Torres et al. 2015). A single missense mutation in the human ADAT3 gene was reported to cause intellectual disability and strabismus (Alazami et al. 2013). Interestingly, this protein and its catalytic partner ADAT2 were reported essential in yeast, *Trypanosoma brucei*, *Arabidopsis thaliana* and likely also in human cell lines (Gerber and Keller 1999; Rubio et al. 2007; Zhou et al. 2014; Torres et al. 2015). This suggests that patients carrying mutations in the ADAT3 gene probably still have some residual ADAT activity. However, a more detailed analysis of the functional importance for this enzyme in mammals remains to be addressed (Torres et al. 2014b).

NOP2/Sun RNA methyltransferase family member 2 (NSUN2) is an enzyme that methylates cytosine at positions 34, 48, 49 and 50 on different tRNAs (Brzezicha et al. 2006; Hussain et al. 2013) . Mutations in the NSUN2 gene have been associated to autosomal-recessive intellectual disability (Abbasi-Moheb

et al. 2012; Khan et al. 2012; Martinez et al. 2012; Fahiminiya et al. 2014). Khan and colleagues investigated the subcellular localization of wild-type and mutant NSUN2 and found that mutant NSUN2 failed to localize to nucleoli (Khan et al. 2012). Abbasi-Moheb and colleagues further showed that deletion of the fly NSUN2 ortholog resulted in a short-term memory phenotype (Abbasi-Moheb et al. 2012), suggesting an evolutionary conserved function for tRNA methylation in the brain. A recent study has addressed some mechanistic aspects on how NSUN2 defects would be contributing to human disease. The authors showed that lack of 5-methylcytosine (m^5C) on tRNAs results in increased tRNA endonucleolytic cleavage mediated by angiogenin, leading to the accumulation of 5′-tRNA halves that reduce protein translation rates and activate stress pathways in human and mouse cells. Moreover, NSUN2-deficient brains were more sensitive to oxidative stress, and this phenotype could be rescued by inhibiting angiogenin during embryogenesis (Blanco et al. 2014).

Two other reports have associated mutations in NSUN2 to different degrees of mental retardation. Patients with these mutations showed overlapping phenotypes to that of the Dubowitz syndrome (Martinez et al. 2012) and to that of the Noonan syndrome (Fahiminiya et al. 2014). In the first case, the authors showed that patients suffering from this Dubowitz-like syndrome were lacking the m^5C modification at positions 47 and 48 on $tRNA^{Asp}_{(GTC)}$, one of the substrates of NSUN2 (Martinez et al. 2012). In the second case, the link to a Noonan-like syndrome suggests a role for NSUN2 beyond the brain, as this is a pathology that affects mainly the cardiac tissue and only about 25 % of affected patients also suffer mental retardation. As it will be discussed later, NSUN2 has also been linked to different forms of cancer (see below) suggesting that this enzyme has key roles not only in the brain and heart but also in different tissue types.

A set of very well studied tRNA modifications associated to neurological disorders are those involving the formation of 5-methoxycarbonylmethyluridine (mcm^5U) and 5-carbamoylmethyluridine (ncm^5U) at position 34 of several tRNAs. These complex tRNA modifications (and derivatives of them) require a methylation step catalysed by the elongator complex. This complex is highly conserved from yeast to humans, where it was shown to be composed of six subunits: IκB kinase complex-associated protein (IKAP/yeast ELP1), Stat3-interacting protein (StIP1/yeast ELP2), elongator protein homolog 3 (ELP3), ELP4 and two unidentified polypeptides (Hawkes et al. 2002).

Mutations in the gene encoding for IKAP (*IKBKAP*) have been linked to familial dysautonomia (FD) (Anderson et al. 2001; Slaugenhaupt et al. 2001; Leyne et al. 2003; Karlsborn et al. 2014). Although some of these mutations are missense mutations, the most prevalent mutation (>99.5 %) was found in homozygosity and involved a point mutation resulting in exon-skipping and aberrant protein truncation (Anderson et al. 2001; Slaugenhaupt et al. 2001). Moreover, levels of mcm^5s^2U34 were shown to be reduced in brain tissue and fibroblast cell lines derived from FD patients (Karlsborn et al. 2014). Strikingly, even though patients were homozygous for the exon-skipping mutation, they presented variable levels of wild-type IKAP in a tissue-specific manner, where brain cells primarily expressed the mutant

IKBKAP mRNA (Slaugenhaupt et al. 2001). In this regard, *IKBKAP* function may also be important for pulmonary function, as a mutation in this gene has been associated to bronchial asthma in children (Takeoka et al. 2001). Altogether, it seems that a tissue-dependent control of exon skipping of the *IKBKAP* transcript might be central to the symptoms caused by *IKBKAP* mutations.

Mutations in other genes encoding for proteins of the elongator complex have also been linked to neurological dysfunctions (Chen et al. 2009; Simpson et al. 2009; Strug et al. 2009; Najmabadi et al. 2011). As mentioned above, ELP2 has been linked to intellectual disability (Najmabadi et al. 2011). In a study that involved 38 US families, variants of ELP4 have been associated to the electroencephalographic abnormality of centrotemporal sharp waves, a typical clinical trait of Rolandic epilepsy (Strug et al. 2009). Lastly, mutations in ELP3 have been associated to amyotrophic lateral sclerosis (Simpson et al. 2009). Interestingly, in flies and zebrafish, impaired function of ELP3 resulted in neurological phenotypes (Simpson et al. 2009), and in *Caenorhabditis elegans*, mutations in ELPC1 and ELPC3 (homologs of IKAP and ELP3, respectively) also resulted in neurological abnormalities (Chen et al. 2009). This suggests a conserved functional role in metazoan neuronal tissues for these components of the elongator complex.

2.2 Cancer

Mounting evidence suggests that different types of cancer could be attributed to a dysregulation on tRNA modification enzymes (Torres et al. 2014a). In some cases, such dysregulation involves an upregulation of the tRNA modification enzymes, while in other cases, the tRNA modification enzymes (or the modifications itself) are found downregulated. Examples of the first case include upregulation of NSUN2, TRMT12 and human AlkB homolog 8 (HABH8 or HALKBH8), among others. In breast cancer patients and in several breast cancer cell lines, the genomic region encompassing the NSUN2 gene is frequently found duplicated (Pierga et al. 2007; Frye et al. 2010). Indeed, NSUN2 expression was reported to be low in normal tissues but high in different tumour types such as squamous cell carcinoma, breast cancer and colorectal cancer (Frye and Watt 2006). NSUN2 was reported to have a role in keratinocyte proliferation mediated by the proto-oncogene Myc. Further, knockdown of NSUN2 reduced tumour formation in a mice xenograft model for squamous cell carcinoma (Frye and Watt 2006).

TRMT12 is one of the enzymes involved in the formation of wybutosine at position 37 of $tRNA^{Phe}$. This gene was also found amplified in breast cancer cell lines and breast cancer tumours (Rodriguez et al. 2007). Finally, knockdown of HABH8 (the protein likely responsible for the formation of mcm^5U34 on $tRNA^{Arg}$ and $tRNA^{Glu}$) was shown to suppress angiogenesis, invasion and growth of bladder cancers in vivo (Shimada et al. 2009). Many more examples exist of upregulated tRNA modification enzymes and hypermodified tRNAs in cancer. This is especially true for tRNA methyltransferases that, already in the early 1970s, were known to be

either upregulated or having higher methyltransferase activity in neoplastic tissues as compared to normal tissues (Kerr and Borek 1973).

Downregulation of tRNA modifications/tRNA modification enzymes has also been found in different types of cancer. Such is the case of another potential human homolog of the yeast TRM9, named tRNA methyltransferase 9-like (HTRM9L) that was reported to be downregulated in bladder, cervix, breast, testicular and colorectal cancer. Moreover, two colorectal carcinoma cell lines showed a dramatic reduction in tumour growth in vivo when HTRM9L expression was recovered (Begley et al. 2013). Similarly, the 2′-*O*-methylguanosine modification at position 17 of $tRNA^{Ser}_{(IGC)}$ has been shown to be absent in Morris hepatoma (Randerath et al. 1981).

Queuosine (Q) is incorporated at position 34 of $tRNA_{GUN}$ (i.e. tRNA Asn, Asp, His and Tyr), replacing G34, in a reaction catalysed by eukaryotic TGTase (Vinayak and Pathak 2010). Eukaryotes are unable to synthesize queuine, the base form of nucleoside Q, and need to obtain it from food intake, the intestinal flora, or by a queuine salvage pathway from tRNA turnover. It is well documented that neoplastic tissues and transformed cells contain hypomodified Q-tRNAs. For example, Morris hepatoma is characterized by a lack of Q on mitochondrial $tRNA^{Asp}$ (Randerath et al. 1984). Moreover, the degree of Q hypomodification can correlate closely with the degree of malignancy for several types of cancer, making it a promising marker for the prognosis of the disease (Vinayak and Pathak 2010) (see Sect. 3). However, despite strong efforts to understand the biology of Q and Q-tRNAs, a clear picture of the molecular mechanisms linking this tRNA modification to human diseases remains elusive.

2.3 *Metabolic Dysregulations*

Some human diseases associated to tRNA modifications involve complex metabolic dysregulations. DNA (cytosine-5)-methyltransferase-like protein 2 (DNMT2), also known as tRNA aspartic acid methyltransferase 1 (TRDMT1), is responsible for the methylation of cytosine (m^5C) at position 38 of $tRNA^{Asp}$ (Goll et al. 2006). A polymorphism in TRDMT1 was linked to increased red blood cell folate levels that seemed to reduce the risk of developing spina bifida aperta in individuals of Dutch ethnicity (Franke et al. 2009). Interestingly, loss of m^5C at position 34 of $tRNA^{Leu}_{(CAA)}$ by knocking out NSUN2 (see above) resulted in an approximately 30 % weight loss in mice. Moreover, mice also presented alopecia, suggesting that NSUN2 would be playing a role in maintaining skin homeostasis (Blanco et al. 2011). Notably, while single knockout mice for TRDMT1 (DNMT2) or NSUN2 were viable, DNMT2/NSUN2 double knockouts were synthetic lethal and presented an underdeveloped phenotype (Tuorto et al. 2012). These reports highlight a role for the loss of m^5C at different tRNA positions and in different tRNA species with a range of complex phenotypes that could be attributed to metabolic dysfunctions.

Links between tRNA modifications and perturbed glucose metabolism have also been reported. Patients with mutations in TRMT10A (see above) also suffered from hyperinsulinaemic hypoglycaemia (Gillis et al. 2014). It was postulated that the abnormalities in glucose homeostasis triggered by the TRMT10A deficiency may be due to accelerated β-cell apoptosis (Gillis et al. 2014). In line with these results, Igoillo-Esteve and colleagues showed that TRMT10A was enriched in pancreatic islets and that a nonsense mutation on the TRMT10A gene was linked to young-onset diabetes. Moreover, knockdown of TRMT10A in primary rat β-cells and in dispersed human islets induced apoptosis and increased sensitivity to free fatty acids and endoplasmic reticulum stress (Igoillo-Esteve et al. 2013).

Several reports have shown a correlation between mutations in the CDK5 regulatory subunit-associated protein 1-like 1 (CDKAL1) gene and type 2 diabetes (T2D) (Kirchhoff et al. 2008; Stancakova et al. 2008; Wei et al. 2011; Wei and Tomizawa 2011; Xie et al. 2013). CDKAL1 is a methylthiotransferase responsible for the 2-methylthio-N6-threonyl carbamoyladenosine (ms^2t^6A) modification at position 37 of $tRNA^{Lys}_{(UUU)}$ (Wei and Tomizawa 2011). Patients carrying mutations in CDKAL1 show reduced levels of ms^2t^6A37 and impaired proinsulin-to-insulin conversion and insulin secretion (Kirchhoff et al. 2008; Stancakova et al. 2008; Xie et al. 2013). Moreover, misreading of Lys codons in proinsulin was detected in CDKAL1 knockout β-cells from mice (Wei et al. 2011). Notably, polymorphisms on CDKAL1 have also been associated to myocardial infarction and coronary artery disease, probably as a consequence of its influence on T2D (Saade et al. 2011). Mutations in CDKAL1 have also been linked to Crohn's disease and psoriasis (Barrett et al. 2008; Quaranta et al. 2009). These associations were shown to be independent of T2D (Quaranta et al. 2009). Therefore, it is likely that CDKAL1 plays a role in disease in a variety of tissues; and probably, the observed phenotypes are associated to mistranslation of Lys codons on particular tissue-specific Lys-enriched transcripts.

2.4 Mitochondrial-Linked Dysfunctions

The mitochondria are responsible for supplying energy to the cell. As such, defects in mitochondrial metabolism can have severe and complex phenotypes especially in tissues that require high amount of energy, such as the brain and muscle. More than 200 different mutations in mitochondrial tRNA (mt-tRNA) genes have been associated to human diseases (Ruiz-Pesini et al. 2007). Here we will focus on two well-characterized mitochondrial-linked human diseases caused by mutations in mt-tRNA genes that result in tRNA hypomodification: mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS) and myoclonic epilepsy with ragged-red fibres (MERRF) (Suzuki and Nagao 2011). Mostly, these diseases arise from the lack of taurine modifications at position 34 of $mt\text{-}tRNA^{Leu}_{(UUR)}$ and $mt\text{-}tRNA^{Lys}$, respectively (Suzuki and Nagao 2011).

MELAS patients were found containing mutations at different residues of the mt-tRNA$^{Leu}_{(UUR)}$ gene that resulted in a complete lack of 5-taurinomethyluridine (τm^5U34) on this tRNA. However, patients having other mutations in this same gene that resulted in retention of the τm^5U34 modification were diagnosed with mitochondrial diseases other than MELAS such as mitochondrial myopathy (MM), chronic progressive external ophthalmoplegia (CPEO) and maternally inherited mitochondrial myopathy and cardiomyopathy (MMC) (reviewed in Suzuki and Nagao 2011). This suggests that the lack of τm^5U34 on mt-tRNA$^{Leu}_{(UUR)}$ is the causative of MELAS disease. The τm^5U34 modification allows for mt-tRNA$^{Leu}_{(UUR)}$ to read both UUR codons: UUA and UUG. However, in the absence of this modification, mt-tRNA$^{Leu}_{(UUR)}$ is only capable of reading its cognate UUA codon (Kirino et al. 2004). Given that in these patients only the decoding of UUG Leu codons is affected, only those transcripts enriched in this codon show translation defects. One such case is that of the mitochondrially encoded NADH dehydrogenase 6 (*ND6*) gene. *ND6* encodes for a component of the respiratory chain complex I. Cybrid cell lines derived from MELAS patients lacking τm^5U34 showed a marked reduction in complex I activity and a reduced rate of translational activity of ND6, while general mitochondrial protein synthesis seemed unaffected (Hayashi et al. 1993). Therefore, the specific translational defects of UUG-enriched mitochondrial transcripts caused by the lack of τm^5U34 on mt-tRNA$^{Leu}_{(UUR)}$ largely explains the molecular mechanism behind MELAS disease.

MERRF patients lack 5-taurinomethyl-2-thiouridine (τm^5s^2U) at position 34 of mt-tRNA^{Lys}. As a result, the unmodified mt-tRNA^{Lys} is unable to efficiently decode any of its Lys codons (AAA and AAG), leading to overall mitochondrial translation defects and a more severe pathological phenotype than that observed in MELAS patients (Yasukawa et al. 2001). In addition to the importance of the taurine modification, this effect may indicate a key role of the s^2U34 modification in efficient tRNA decoding. Knockdown of the mitochondrial tRNA-specific 2-thiouridylase 1 (MTU1, also known as TRMU) in HeLa cells resulted in a reduction of s^2U34 modification on mt-tRNA^{Lys}, reduction on oxygen consumption and defective mitochondrial membrane potentials, all of which are characteristics of MERRF (Umeda et al. 2005). Notably, mutations in MTU1 have been associated to other mitochondrial disorders such as acute liver failure in infancy accompanied by lactic acidaemia (Zeharia et al. 2009) and deafness associated with mutations in mitochondrial 12S ribosomal RNA (Guan et al. 2006).

In this section we have described only two mitochondrial-linked diseases associated to the lack of tRNA modifications. These two pathologies are the ones that have been studied more in depth thus far (see Suzuki and Nagao 2011). However, more reports are emerging with further associations between tRNA modification enzymes and mitochondrial disorders. One such example is the recently published article by Powell and colleagues showing that individuals carrying mutations on the tRNA methyltransferase 5 (*TRMT5*) gene presented lactic acidosis and multiple mitochondrial respiratory complex deficiencies, particularly in the skeletal muscle. The authors also validated that TRMT5 is responsible for m^1G37 modification on mitochondrial tRNA^{Leu} (Powell et al. 2015). We refer the reader to a recent review on mitochondrial tRNA mutations and disease for more information (Abbott et al. 2014).

3 Biomedical Strategies Based on tRNA Modifications

The role of tRNA modifications and human diseases is still an emerging field (Torres et al. 2014a). As such, the full potential of tRNA modification-based approaches to be used in the clinic has not been exploited thus far. In this section we will review the cases where studying and modulating the levels of tRNA modifications or tRNA modification enzymes have proved useful for therapeutic applications; and we will also hypothesize on other tentative approaches that could be worth pursuing in the future to tackle tRNA modification-linked diseases.

3.1 Diagnosis and Prognosis

Several studies have focused on finding typical 'gene signatures' associated to different human diseases. These gene signatures can be in the form of gene expression patterns using 'omics' (e.g. microarrays, RNAseq, proteome profiling, etc.) or as a set of mutations in particular genes obtained by performing population genetic studies in human patients. Interestingly, in many of such type of studies, genes encoding (or expected to encode) for tRNA modification enzymes emerge.

As explained above, there is a strong link between tRNA modifications (and the enzymes that catalyse them) and different forms of cancer. Therefore, tRNA modifications could serve as means of diagnosis and/or prognosis of cancer. One example is Mammostrat, a tool used to stratify patients with early-stage breast cancer into groups having risks of a relapse following treatments with tamoxifen, in order to inform treatment decisions (e.g. if chemotherapy would be additionally recommended or not) (Bartlett et al. 2010). This prognostic tool measures the levels of five genes (i.e. gene signature), one of which is the tRNA methyltransferase homolog 2A (TRMT2A, also known as HTF9C), predicted to be responsible for the formation of 5-methyluridine at positions 42 and 54 of several tRNAs (Towns and Begley 2012).

Another study compared high-resolution genome copy number variation in microsatellite-stable colorectal tumours to identify susceptibility loci (Berg et al. 2010). Ten genomic loci were identified, comprising about 500 protein-coding genes, as markers for susceptibility to early-onset colorectal cancer. Moreover, combined genomics and transcriptomics allowed for the identification of a gene signature of 7 genes that correlates with an increased risk for colorectal cancer. One of these signature genes was TRMT10A (also known as HRG9MTD2; see above) (Berg et al. 2010).

The monitoring of gene signatures involving tRNA modification enzymes for diagnosis or prognosis of disease is not restricted to cancer. Najmabadi and colleagues have performed homozygosity mapping, exon enrichment and next-generation sequencing in 136 consanguineous families and have identified 50 novel genes with potentially pathogenic variants for intellectual disability. Two

of these genes encode for tRNA modification enzymes: TRMT1 and ELP2 (discussed above) (Najmabadi et al. 2011). Similarly, a study identified genes related to folate metabolism that are involved in the aetiology of spina bifida. One of the 4 novel genes described in that work was TRDMT1 (DNMT2) (Franke et al. 2009).

Just like genes encoding for tRNA modification enzymes can be monitored to predict or evaluate the development of human diseases, measuring the levels of tRNA modifications themselves can prove useful. The measurement of the levels of Q34 in human lung cancer and normal lung tissue from different patients correlated well with the grade of tumour malignancy and was suggested to be a viable approach to predict patient survival (Huang et al. 1992). Given that other types of cancer also show Q34 hypomodification (see above) (Vinayak and Pathak 2010), it would not be surprising if a similar approach for quantification of Q34 could be applied for prognosis of other cancer types. Likewise, measurement of ms^2 modification on $tRNA^{Lys}_{(UUU)}$ by a quantitative PCR technique proved useful to monitor the activity levels of CDKAL1 and assess the risk of developing type 2 diabetes (Xie et al. 2013). Moreover, analysis of total RNA extracted from human peripheral blood samples showed lower levels of ms^2 modification in individuals carrying the CDKAL1 genotype associated to T2D that correlated with lower levels of insulin secretion (Xie et al. 2013).

Even though to date there are few cases reported of successful analyses of tRNA modifications and tRNA modification genes to diagnose and monitor different human diseases, the mounting evidence is strong enough to establish a proof of principle. It is therefore not difficult to imagine that other strategies will emerge soon. These can involve using already available data (e.g. if TRMT1 and ELP2 regulation is associated to intellectual disability, maybe measuring the levels of $m^2{}_2G$ and mcm^5U/ncm^5U could serve as well as molecular markers for this disease) or by performing further 'omics'-based screenings for novel candidate genes in different pathological scenarios. Moreover, as technology progresses, these findings will result in further high-throughput, cost-effective and minimally invasive methods for monitoring disease, as already described for breast cancer (i.e. Mammostrat) or T2D (i.e. quantitative PCR method from peripheral blood samples).

3.2 Potential Therapeutic Treatments

Developing drugs for therapeutic applications is often harder than developing tools for diagnosis and prognosis of a disease. However, there is some evidence in the literature of potential approaches that could be helpful for the treatment of complex human diseases based on the biology of tRNA modifications.

For some pathologies, the desired drug effect would be to reduce the levels of tRNA modifications. As discussed above, different types of cancer show high levels of tRNA methylation (Kerr and Borek 1973). Azacytidine, a cytosine analogue, has been developed as a drug for epigenetic cancer therapy through the inhibition of DNA methylation. However, azacytidine, which is commonly used for treatment of

myeloid leukaemia, was also shown to specifically inhibit m^5C38 formation on $tRNA^{Asp}$ by DNMT2 (Schaefer et al. 2009). Demethylation of m^5C38 on $tRNA^{Asp}$ in myeloid leukaemia cell lines correlated well with azacytidine, and it has therefore been proposed as a suitable novel molecular marker for azacytidine response (Schaefer et al. 2009). Inhibition of tRNA methyltransferase activity can also be achieved by using different types of adenine analogues, some of which are cytokinins (Wainfan and Borek 1967; Wainfan and Landsberg 1971). In fact, adenine analogues have been proposed as effective compounds for treatment of acute myeloid leukaemia (Honma 2003), while cytokinin ribosides were reported to impair cell viability (Casati et al. 2011).

Another strategy to reduce the levels of tRNA modifications is to directly knockdown tRNA modification enzymes. An example of this approach is the downregulation of NSUN2, shown to inhibit Myc-induced keratinocyte proliferation and to reduce the growth of human squamous cell carcinoma in mice in a dose-dependent manner (Frye and Watt 2006). Likewise, downregulation of HABH8 (ALKBH8) was suggested as a new therapeutic strategy to target urothelial carcinomas (discussed in Sect. 2.2) (Shimada et al. 2009). The same knockdown approaches could be used also for other tRNA modification enzymes such as TRMT12, which is amplified in breast cancer tumours (Rodriguez et al. 2007).

In other cases the desired effect of drugs would be to restore the levels of tRNA modifications. The method to achieve this will be dependent on the type of tRNA modification and the type of tRNA modification enzyme. Enhancing tRNA methyltransferase activity could be useful in patients of neurological disorders caused by mutations in FTSJ1, TRMT10A, TRMT1, WDR4, etc. This could be accomplished by using hormone treatments. As an example, the levels of N2 and N2-dimethylguanine in uteri could be recovered upon addition of estradiol in ovariectomized rats (Sharma et al. 1971).

A completely different mechanism would be the one involving the Q modification. As discussed above, queuine is not readily synthesized by eukaryotes and need to be obtained via different mechanisms in order to produce Q (see above). Queuine has been proposed as an anticarcinogenic agent (Vinayak and Pathak 2010). Externally administered Q reduces cell proliferation in vitro and in vivo probably due to restoration of Q-tRNAs (Pathak et al. 2007). In this scenario, the absence of Q-tRNAs could be due to deficiencies in conversion of queuine into Q; however, the lack of Q-tRNAs could also be due to poor activity of the TGTase, reduced uptake of queuine or dysfunctions in the queuine salvage system (reviewed in Vinayak and Pathak 2010). Therefore, different therapeutic strategies can be devised depending on the affected molecular mechanism for the generation of Q-tRNAs.

An interesting approach to restore tRNA modification is the one currently being pursued for the treatment of FD. It is possible to correct aberrant splicing of *IKBKAP* transcripts by using different types of compounds. Kinetin, a plant cytokinin, is one of the most promising candidates for treating this disease. Patients homozygous for the FD splice mutation were treated with 23.5 mg/kg/day kinetin for 28 days and presented an increase in wild-type *IKBKAP* mRNA expression in

leukocytes (Axelrod et al. 2011). Other small molecules are also being investigated as splice-correction agents for treating FD including epigallocatechin gallate (found in green tea), genistein and daidzein (present in soy) and tocotrienols (members of the vitamin E family) (Anderson et al. 2003a, b, 2012; Anderson and Rubin 2005). Importantly, combined treatment using these compounds was shown to have synergistic effects on *IKBKAP* mRNA splicing correction (Anderson et al. 2003a, 2012). The exact molecular mechanism for the efficacy of these small molecules to correct splicing is unclear. A kinetin responsive sequence element has been mapped at the 5′-splice site of *IKBKAP* exon 20; and the actual FD mutation on *IKBKAP* was not required for kinetin activity (Hims et al. 2007). In the case of epigallocatechin gallate, the compound was shown to downregulate the protein HNRNP A2/B1 that promotes the preferential use of the intron distal 5′-splice site that is selected when generating the *IKBKAP* mRNA mutant splice variant (Anderson et al. 2003a). It is conceivable that in the future other small molecules with the ability to correct the FD splicing defect will be described, as well as other splice-correction strategies such as the use of oligonucleotide analogues conjugated to cell-penetrating peptides to mask splicing sites (Betts et al. 2012).

A more dramatic approach to restore tRNA modification levels would be to re-express/overexpress the wild-type versions of tRNA modification enzymes following gene replacement therapies in those cases where patients contain mutations on genes encoding for such tRNA modification enzymes. As mentioned before, re-expression of HTRM9L showed promising potential for controlling tumour growth in a colorectal carcinoma model (see Sect. 2.2) (Begley et al. 2013). Given that gene expression usually needs to be carefully controlled, this kind of approach will probably be more effective when replacing enzymes that are believed to be constitutively active physiologically. This could be applied to the overexpression of wild-type ADAT3 to correct the intellectual disability and strabismus phenotypes (Alazami et al. 2013). It is believed that hetADAT activity is saturated in cells given that apparently $tRNA_{(ANN)}$ seems to be fully modified to I34 (Torres et al. 2014b).

Restoring tRNA modifications associated to mitochondrial-linked diseases opens a whole new set of different therapeutic approaches. MELAS patients are unable to modify mt-$tRNA^{Leu}_{(UUR)}$ with τm^5U34 (see Sect. 2.4). Recovery of MELAS phenotypes could be observed in a lung carcinoma cybrid cell line bearing a mutation in the anticodon sequence of the mt-$tRNA^{Leu}_{(CUN)}$ gene (the other isoacceptor of mt-$tRNA^{Leu}_{(UUR)}$). This mutant resulted in a mt-$tRNA^{Leu}_{(CUN)}$ having the anticodon UAA (the same anticodon as mt-$tRNA^{Leu}_{(UUR)}$) instead of its wild-type anticodon UAG. Notably, this mutant tRNA was found modified with τm^5U34 and was therefore capable of decoding UUG codons (Kirino et al. 2006). This shows that a potential therapeutic strategy against MELAS is to overexpress a related mt-$tRNA^{Leu}$ isoacceptor bearing the anticodon of mt-$tRNA^{Leu}_{(UUR)}$, which can be modified to τm^5U34.

Additionally, the MELAS mutation on $tRNA^{Leu}_{(UUR)}$ was also shown to reduce the levels of aminoacylation for this tRNA, and overexpression of the human mitochondrial leucyl-tRNA synthetase (LeuRS) in cells carrying such MELAS

mutation recovered respiratory function in a dose-dependent manner (Park et al. 2008; Li and Guan 2010). Park and colleagues showed that, under these conditions, cells showed increased steady-state levels of $tRNA^{Leu}_{(UUR)}$, but the fraction of aminoacylated $tRNA^{Leu}_{(UUR)}$ remained unchanged. Likewise, rates of mitochondrial translation where not increased either in MELAS cells overexpressing LeuRS. The phenotypic recovery observed in these cells was instead attributed to an increase in protein stability (Park et al. 2008). On the contrary, Li and Guang found that overexpression of LeuRS improved aminoacylation efficiency, mt-tRNA stability and mitochondrial translation (Li and Guan 2010). These observations propose yet another therapeutic strategy to treat MELAS, although the molecular mechanism behind the phenotypic recovery is controversial.

Finally, we should also mention mitochondrial gene replacement therapy as means to overcome human diseases associated to mutations in the mitochondrial genome. While this is a sensitive approach due to potential ethical reasons ('three-parent in vitro fertilization'), the technique has already been successfully applied, using the spindle transfer method, in nonhuman primates resulting in healthy offspring (Tachibana et al. 2009). Furthermore, initial studies, using the same mitochondrial gene transfer method, have been reported in human oocytes with promising results (Tachibana et al. 2013). However, even if this approach is taken further into the clinic, it will serve to prevent the transfer of mitochondrial-linked diseases to a child but will not serve to cure existing patients with mitochondrial pathologies.

Clearly, new therapeutic strategies will emerge as our understanding of the molecular and cellular mechanisms triggered by tRNA modification misregulation improves. As exemplified above (see Sect. 2.1), inhibiting angiogenin during embryogenesis could recover some of the phenotypes observed in brain from NSUN2-deficient mice by preventing the accumulation of 5′-tRNA halves derived from tRNAs lacking m^5C (Blanco et al. 2014). It is likely that unexpected or non-canonical mechanisms (e.g. mechanisms beyond aberrant protein translation) play important roles in these different pathologies, and while it will take some time to fully unravel them, they will be key to developing further therapeutic approaches to treat tRNA modification-linked diseases.

4 Conclusions and Perspectives

The knowledge on the roles that tRNA modifications play in human diseases is increasing at a very fast pace. This is opening a whole new and exciting field for research and for development of novel therapeutics. However, in order to exploit its full potential, several key aspects still need to be addressed.

We need to improve our insights into the biological functions of tRNA modifications. For example, a direct role for different types of tRNA modifications on protein translation is often assumed. Not only those potential roles need to be

experimentally verified, but also it is important to understand the extent to which protein fidelity and/or efficiency is affected when tRNAs are hypo- or hypermodified. Further, a key question is whether in pathological scenarios general protein synthesis, or only a subset of genes, is affected (Novoa and Ribas de Pouplana 2012). Moreover, tRNA modifications may be important for non-canonical functions of tRNA, such as those performed by tRNA fragments (Durdevic and Schaefer 2013; Anderson and Ivanov 2014). Therefore, we also need to keep an open mind and take into account that some of the observed cellular phenotypes could be due to non-canonical functions of tRNAs driven by the modulation of tRNA modifications.

In addition, there is a need for novel model systems to study tRNA modifications. The vast majority of the published research on tRNA modifications has been carried out in vitro, in bacteria, in lower eukaryotes and, to a less extent, in mammalian cell lines. In many cases, the data obtained using these models cannot be extrapolated to in vivo systems; and in fact our knowledge of tRNA modifications in metazoans is scarce. Therefore, it is critical to develop in vivo models to study tRNA modifications, especially mammalian models. This will not only expand our understanding of the biological functions of tRNA modifications but also will serve as models for the design of tRNA modification-based therapeutics. In this regard, mouse models have been developed to study the function of IKAP (familial dysautonomia) (Dietrich et al. 2012) and NSUN2 (neurological disorders) (Blanco et al. 2014). Other animal models (*Caenorhabditis elegans*, *Drosophila melanogaster* and *Danio rerio*) have also been used to study, for example, other members of the elongator complex (Chen et al. 2009; Simpson et al. 2009).

Lastly, it will also be essential to design new technologies that would allow for proper detection and quantification of tRNA modifications. Fortunately, for some modifications, this is being achieved successfully. Detection of m^5C at transcriptomics scale can be performed using methylation individual-nucleotide resolution cross-linking and immunoprecipitation (miCLIP) or 5-azacytidine-mediated RNA immunoprecipitations (Aza-IP) (Hussain et al. 2013; Khoddami and Cairns 2013). Direct sequencing of tRNAs (tRNAseq) is still very challenging, but novel strategies are being developed that allow for more efficient quantification of tRNA species (Pang et al. 2014) and detection of tRNA modifications (Iida et al. 2009; Torres et al. 2015). For example, the levels of I34 on human tRNAs were successfully monitored, using tRNAseq, in human cell lines upon downregulation of ADAT2 (Torres et al. 2015). Finally, techniques based on HPLC coupled to mass spectrometry are also being developed and have been already proven useful to study the dynamics of a diverse set of tRNA modifications in yeast tRNAs upon cellular stress (Chan et al. 2010). Altogether, it is likely that new technologies will soon allow for strong detection and quantification of tRNA modifications in a cost-efficient and high-throughput manner.

From a strictly therapeutic point of view, the challenges are even higher. On the one hand, the value of tRNA modification-based tools for diagnosis and prognosis will largely depend on the technologies available to detect and quantify tRNA modifications (see above). Moreover, the models that could be used to develop

these tools will have to closely mimic pathological scenarios. In this regard, using patient-specific induced pluripotent stem cells (iPSCs) could prove valuable, an approach that has already been tried to model familial dysautonomia (Lee et al. 2009). Likewise, cancer models could be recapitulated using iPSCs and differentiated cells derived from them. On the other hand, the therapeutic strategies based on modulating tRNA modifications will have to be carefully controlled. Many tRNA modification enzymes seem to play different roles in different tissues (Table 1). For example, NSUN2 deficiency in the brain leads to neurological disorders (Abbasi-Moheb et al. 2012; Khan et al. 2012; Martinez et al. 2012; Fahiminiya et al. 2014), while its overexpression in the skin or breast has oncogenic potential (Frye and Watt 2006; Pierga et al. 2007; Frye et al. 2010). Therefore, developing tissue-specific targeting drugs will be key for success.

We have previously predicted that in the future 'epi-tRNAomes' (quantification of the tRNA pool and the evaluation of the modification levels) will be used in personalized medicine (Torres et al. 2014a). We believe that this will become a reality with the advent of cost-effective technologies that combines mass spectrometry, transcriptomics and proteomics and the parallel development of strong bioinformatics that will allow for the rapid and efficient analysis of large datasets.

References

Abbasi-Moheb L, Mertel S, Gonsior M et al (2012) Mutations in NSUN2 cause autosomal-recessive intellectual disability. Am J Hum Genet 90:847–855

Abbott JA, Francklyn CS, Robey-Bond SM (2014) Transfer RNA and human disease. Front Genet 5:158

Alazami AM, Hijazi H, Al-Dosari MS et al (2013) Mutation in ADAT3, encoding adenosine deaminase acting on transfer RNA, causes intellectual disability and strabismus. J Med Genet 50:425–430

Anderson P, Ivanov P (2014) tRNA fragments in human health and disease. FEBS Lett 588:4297–4304

Anderson SL, Rubin BY (2005) Tocotrienols reverse IKAP and monoamine oxidase deficiencies in familial dysautonomia. Biochem Biophys Res Commun 336:150–156

Anderson SL, Coli R, Daly IW et al (2001) Familial dysautonomia is caused by mutations of the IKAP gene. Am J Hum Genet 68:753–758

Anderson SL, Qiu J, Rubin BY (2003a) EGCG corrects aberrant splicing of IKAP mRNA in cells from patients with familial dysautonomia. Biochem Biophys Res Commun 310:627–633

Anderson SL, Qiu J, Rubin BY (2003b) Tocotrienols induce IKBKAP expression: a possible therapy for familial dysautonomia. Biochem Biophys Res Commun 306:303–309

Anderson SL, Liu B, Qiu J et al (2012) Nutraceutical-mediated restoration of wild-type levels of IKBKAP-encoded IKAP protein in familial dysautonomia-derived cells. Mol Nutr Food Res 56:570–579

Axelrod FB, Liebes L, Gold-Von Simson G et al (2011) Kinetin improves IKBKAP mRNA splicing in patients with familial dysautonomia. Pediatr Res 70:480–483

Barrett JC, Hansoul S, Nicolae DL et al (2008) Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. Nat Genet 40:955–962

Bartlett JM, Thomas J, Ross DT et al (2010) Mammostrat as a tool to stratify breast cancer patients at risk of recurrence during endocrine therapy. Breast Cancer Res 12:R47

Begley U, Sosa MS, Avivar-Valderas A et al (2013) A human tRNA methyltransferase 9-like protein prevents tumour growth by regulating LIN9 and HIF1-alpha. EMBO Mol Med 5:366–383

Berg M, Agesen TH, Thiis-Evensen E et al (2010) Distinct high resolution genome profiles of early onset and late onset colorectal cancer integrated with gene expression data identify candidate susceptibility loci. Mol Cancer 9:100

Betts C, Saleh AF, Arzumanov AA et al (2012) Pip6-PMO, a new generation of peptide-oligonucleotide conjugates with improved cardiac exon skipping activity for DMD treatment. Mol Ther Nucleic Acids 1, e38

Blanco S, Kurowski A, Nichols J et al (2011) The RNA-methyltransferase Misu (NSun2) poises epidermal stem cells to differentiate. PLoS Genet 7, e1002403

Blanco S, Dietmann S, Flores JV et al (2014) Aberrant methylation of tRNAs links cellular stress to neuro-developmental disorders. EMBO J 33:2020–2039

Bonnet C, Gregoire MJ, Brochet K et al (2006) Pure de-novo 5 Mb duplication at Xp11.22-p11.23 in a male: phenotypic and molecular characterization. J Hum Genet 51:815–821

Brzezicha B, Schmidt M, Makalowska I et al (2006) Identification of human tRNA:m5C methyltransferase catalysing intron-dependent m5C formation in the first position of the anticodon of the pre-tRNA Leu (CAA). Nucleic Acids Res 34:6034–6043

Casati S, Ottria R, Baldoli E et al (2011) Effects of cytokinins, cytokinin ribosides and their analogs on the viability of normal and neoplastic human cells. Anticancer Res 31:3401–3406

Chan CT, Dyavaiah M, DeMott MS et al (2010) A quantitative systems approach reveals dynamic control of tRNA modifications during cellular stress. PLoS Genet 6, e1001247

Chan CT, Pang YL, Deng W et al (2012) Reprogramming of tRNA modifications controls the oxidative stress response by codon-biased translation of proteins. Nat Commun 3:937

Chen C, Tuck S, Bystrom AS (2009) Defects in tRNA modification associated with neurological and developmental dysfunctions in *Caenorhabditis elegans* elongator mutants. PLoS Genet 5, e1000561

Crick FH (1966) Codon–anticodon pairing: the wobble hypothesis. J Mol Biol 19:548–555

Dai L, Xing L, Gong P et al (2008) Positive association of the FTSJ1 gene polymorphisms with nonsyndromic X-linked mental retardation in young Chinese male subjects. J Hum Genet 53:592–597

Dietrich P, Alli S, Shanmugasundaram R et al (2012) IKAP expression levels modulate disease severity in a mouse model of familial dysautonomia. Hum Mol Genet 21:5078–5090

Durdevic Z, Schaefer M (2013) tRNA modifications: necessary for correct tRNA-derived fragments during the recovery from stress? Bioessays 35:323–327

El Yacoubi B, Bailly M, de Crecy-Lagard V (2012) Biosynthesis and function of posttranscriptional modifications of transfer RNAs. Annu Rev Genet 46:69–95

El-Hattab AW, Bournat J, Eng PA et al (2011) Microduplication of Xp11.23p11.3 with effects on cognition, behavior, and craniofacial development. Clin Genet 79:531–538

Fahiminiya S, Almuriekhi M, Nawaz Z et al (2014) Whole exome sequencing unravels disease-causing genes in consanguineous families in Qatar. Clin Genet 86(2):134–41

Franke B, Vermeulen SH, Steegers-Theunissen RP et al (2009) An association study of 45 folate-related genes in spina bifida: involvement of cubilin (CUBN) and tRNA aspartic acid methyltransferase 1 (TRDMT1). Birth Defects Res A Clin Mol Teratol 85:216–226

Freude K, Hoffmann K, Jensen LR et al (2004) Mutations in the FTSJ1 gene coding for a novel S-adenosylmethionine-binding protein cause nonsyndromic X-linked mental retardation. Am J Hum Genet 75:305–309

Froyen G, Bauters M, Boyle J et al (2007) Loss of SLC38A5 and FTSJ1 at Xp11.23 in three brothers with non-syndromic mental retardation due to a microdeletion in an unstable genomic region. Hum Genet 121:539–547

Frye M, Watt FM (2006) The RNA methyltransferase Misu (NSun2) mediates Myc-induced proliferation and is upregulated in tumors. Curr Biol 16:971–981

Frye M, Dragoni I, Chin SF et al (2010) Genomic gain of 5p15 leads to over-expression of Misu (NSUN2) in breast cancer. Cancer Lett 289:71–80

Gerber AP, Keller W (1999) An adenosine deaminase that generates inosine at the wobble position of tRNAs. Science 286:1146–1149

Gillis D, Krishnamohan A, Yaacov B et al (2014) TRMT10A dysfunction is associated with abnormalities in glucose homeostasis, short stature and microcephaly. J Med Genet 51:581–586

Giorda R, Bonaglia MC, Beri S et al (2009) Complex segmental duplications mediate a recurrent dup(X)(p11.22-p11.23) associated with mental retardation, speech delay, and EEG anomalies in males and females. Am J Hum Genet 85:394–400

Goll MG, Kirpekar F, Maggert KA et al (2006) Methylation of tRNAAsp by the DNA methyltransferase homolog Dnmt2. Science 311:395–398

Gong P, Li J, Dai L et al (2008) Genetic variations in FTSJ1 influence cognitive ability in young males in the Chinese Han population. J Neurogenet 22:277–287

Guan MX, Yan Q, Li X et al (2006) Mutation in TRMU related to transfer RNA modification modulates the phenotypic expression of the deafness-associated mitochondrial 12S ribosomal RNA mutations. Am J Hum Genet 79:291–302

Hamel BC, Smits AP, van den Helm B et al (1999) Four families (MRX43, MRX44, MRX45, MRX52) with nonspecific X-linked mental retardation: clinical and psychometric data and results of linkage analysis. Am J Med Genet 85:290–304

Hawkes NA, Otero G, Winkler GS et al (2002) Purification and characterization of the human elongator complex. J Biol Chem 277:3047–3052

Hayashi J, Ohta S, Takai D et al (1993) Accumulation of mtDNA with a mutation at position 3271 in tRNA(Leu)(UUR) gene introduced from a MELAS patient to HeLa cells lacking mtDNA results in progressive inhibition of mitochondrial respiratory function. Biochem Biophys Res Commun 197:1049–1055

Hims MM, Ibrahim EC, Leyne M et al (2007) Therapeutic potential and mechanism of kinetin as a treatment for the human splicing disease familial dysautonomia. J Mol Med (Berl) 85:149–161

Honma Y (2003) Adenine analogs as potential differentiation therapy agents for acute myeloid leukemia. Drug Dev Res 59:14–22

Huang BS, Wu RT, Chien KY (1992) Relationship of the queuine content of transfer ribonucleic acids to histopathological grading and survival in human lung cancer. Cancer Res 52:4696–4700

Hussain S, Sajini AA, Blanco S et al (2013) NSun2-mediated cytosine-5 methylation of vault noncoding RNA determines its processing into regulatory small RNAs. Cell Rep 4:255–261

Igoillo-Esteve M, Genin A, Lambert N et al (2013) tRNA methyltransferase homolog gene TRMT10A mutation in young onset diabetes and primary microcephaly in humans. PLoS Genet 9, e1003888

Iida K, Jin H, Zhu JK (2009) Bioinformatics analysis suggests base modifications of tRNAs and miRNAs in Arabidopsis thaliana. BMC Genomics 10:155

Jackman JE, Alfonzo JD (2013) Transfer RNA modifications: nature's combinatorial chemistry playground. Wiley Interdiscip Rev RNA 4:35–48

Karlsborn T, Tukenmez H, Chen C, Bystrom AS (2014) Familial dysautonomia (FD) patients have reduced levels of the modified wobble nucleoside mcmsU in tRNA. Biochem Biophys Res Commun 454:441–445

Kerr SJ, Borek E (1973) Regulation of the tRNA methyltransferases in normal and neoplastic tissues. Adv Enzyme Regul 11:63–77

Khan MA, Rafiq MA, Noor A et al (2012) Mutation in NSUN2, which encodes an RNA methyltransferase, causes autosomal-recessive intellectual disability. Am J Hum Genet 90:856–863

Khoddami V, Cairns BR (2013) Identification of direct targets and modified bases of RNA cytosine methyltransferases. Nat Biotechnol 31:458–464

Kirchhoff K, Machicao F, Haupt A et al (2008) Polymorphisms in the TCF7L2, CDKAL1 and SLC30A8 genes are associated with impaired proinsulin conversion. Diabetologia 51:597–601
Kirchner S, Ignatova Z (2015) Emerging roles of tRNA in adaptive translation, signalling dynamics and disease. Nat Rev Genet 16:98–112
Kirino Y, Yasukawa T, Ohta S et al (2004) Codon-specific translational defect caused by a wobble modification deficiency in mutant tRNA from a human mitochondrial disease. Proc Natl Acad Sci U S A 101:15070–15075
Kirino Y, Yasukawa T, Marjavaara SK et al (2006) Acquisition of the wobble modification in mitochondrial tRNALeu(CUN) bearing the G12300A mutation suppresses the MELAS molecular defect. Hum Mol Genet 15:897–904
Lee G, Papapetrou EP, Kim H et al (2009) Modelling pathogenesis and treatment of familial dysautonomia using patient-specific iPSCs. Nature 461:402–406
Leyne M, Mull J, Gill SP et al (2003) Identification of the first non-Jewish mutation in familial Dysautonomia. Am J Med Genet A 118A:305–308
Li R, Guan MX (2010) Human mitochondrial leucyl-tRNA synthetase corrects mitochondrial dysfunctions due to the tRNALeu(UUR) A3243G mutation, associated with mitochondrial encephalomyopathy, lactic acidosis, and stroke-like symptoms and diabetes. Mol Cell Biol 30:2147–2154
Liu J, Straby KB (2000) The human tRNA(m(2)(2)G(26))dimethyltransferase: functional expression and characterization of a cloned hTRM1 gene. Nucleic Acids Res 28:3445–3451
Machnicka MA, Milanowska K, Osman Oglou O et al (2013) MODOMICS: a database of RNA modification pathways–2013 update. Nucleic Acids Res 41:D262–267
Martinez FJ, Lee JH, Lee JE et al (2012) Whole exome sequencing identifies a splicing mutation in NSUN2 as a cause of a Dubowitz-like syndrome. J Med Genet 49:380–385
Michaud J, Kudoh J, Berry A et al (2000) Isolation and characterization of a human chromosome 21q22.3 gene (WDR4) and its mouse homologue that code for a WD-repeat protein. Genomics 68:71–79
Najmabadi H, Hu H, Garshasbi M et al (2011) Deep sequencing reveals 50 novel genes for recessive cognitive disorders. Nature 478:57–63
Novoa EM, Ribas de Pouplana L (2012) Speeding with control: codon usage, tRNAs, and ribosomes. Trends Genet 28:574–581
Pang YL, Abo R, Levine SS, Dedon PC (2014) Diverse cell stresses induce unique patterns of tRNA up- and down-regulation: tRNA-seq for quantifying changes in tRNA copy number. Nucleic Acids Res 42(22), e170
Park H, Davidson E, King MP (2008) Overexpressed mitochondrial leucyl-tRNA synthetase suppresses the A3243G mutation in the mitochondrial tRNA(Leu(UUR)) gene. RNA 14:2407–2416
Pathak C, Jaiswal YK, Vinayak M (2007) Possible involvement of queuine in regulation of cell proliferation. Biofactors 29:159–173
Phizicky EM, Alfonzo JD (2010) Do all modifications benefit all tRNAs? FEBS Lett 584:265–271
Pierga JY, Reis-Filho JS, Cleator SJ et al (2007) Microarray-based comparative genomic hybridisation of breast cancer patients receiving neoadjuvant chemotherapy. Br J Cancer 96:341–351
Piñeyro D, Torres AG, Ribas de Pouplana L (2014) Biogenesis and evolution of functional tRNAs. In: Sesma A, Von der Haar T (eds) Fungal RNA biology. Springer, Switzerland, pp 233–267
Powell CA, Kopajtich R, D'Souza AR et al (2015) TRMT5 mutations cause a defect in post-transcriptional modification of mitochondrial tRNA associated with multiple respiratory-chain deficiencies. Am J Hum Genet 97:319–328
Quaranta M, Burden AD, Griffiths CE et al (2009) Differential contribution of CDKAL1 variants to psoriasis, Crohn's disease and type II diabetes. Genes Immun 10:654–658
Ramser J, Winnepenninckx B, Lenski C et al (2004) A splice site mutation in the methyltransferase gene FTSJ1 in Xp11.23 is associated with non-syndromic mental retardation in a large Belgian family (MRX9). J Med Genet 41:679–683

Randerath E, Gopalakrishnan AS, Gupta RC et al (1981) Lack of a specific ribose methylation at guanosine 17 in Morris hepatoma 5123D tRNASer1IGA. Cancer Res 41:2863–2867

Randerath E, Agrawal HP, 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

Rodriguez V, Chen Y, Elkahloun A et al (2007) Chromosome 8 BAC array comparative genomic hybridization and expression analysis identify amplification and overexpression of TRMT12 in breast cancer. Genes Chromosomes Cancer 46:694–707

Rubio MA, Pastar I, Gaston KW et al (2007) An adenosine-to-inosine tRNA-editing enzyme that can perform C-to-U deamination of DNA. Proc Natl Acad Sci U S A 104:7821–7826

Rudinger J, Florentz C, Giege R (1994) Histidylation by yeast HisRS of tRNA or tRNA-like structure relies on residues -1 and 73 but is dependent on the RNA context. Nucleic Acids Res 22:5031–5037

Ruiz-Pesini E, Lott MT, Procaccio V et al (2007) An enhanced MITOMAP with a global mtDNA mutational phylogeny. Nucleic Acids Res 35:D823–828

Saade S, Cazier JB, Ghassibe-Sabbagh M et al (2011) Large scale association analysis identifies three susceptibility loci for coronary artery disease. PLoS One 6, e29427

Schaefer M, Hagemann S, Hanna K, Lyko F (2009) Azacytidine inhibits RNA methylation at DNMT2 target sites in human cancer cell lines. Cancer Res 69:8127–8132

Shaheen R, Abdel-Salam GM, Guy MP et al (2015) Mutation in WDR4 impairs tRNA m(7)G46 methylation and causes a distinct form of microcephalic primordial dwarfism. Genome Biol 16:210

Sharma OK, Kerr SJ, Lipshitz-Wiesner R et al (1971) Regulation of the tRNA methylases. Fed Proc 30:167–176

Shimada K, Nakamura M, Anai S et al (2009) A novel human AlkB homologue, ALKBH8, contributes to human bladder cancer progression. Cancer Res 69:3157–3164

Simpson CL, Lemmens R, Miskiewicz K et al (2009) Variants of the elongator protein 3 (ELP3) gene are associated with motor neuron degeneration. Hum Mol Genet 18:472–481

Slaugenhaupt SA, Blumenfeld A, Gill SP et al (2001) Tissue-specific expression of a splicing mutation in the IKBKAP gene causes familial dysautonomia. Am J Hum Genet 68:598–605

Stancakova A, Pihlajamaki J, Kuusisto J et al (2008) Single-nucleotide polymorphism rs7754840 of CDKAL1 is associated with impaired insulin secretion in nondiabetic offspring of type 2 diabetic subjects and in a large sample of men with normal glucose tolerance. J Clin Endocrinol Metab 93:1924–1930

Strug LJ, Clarke T, Chiang T et al (2009) Centrotemporal sharp wave EEG trait in rolandic epilepsy maps to Elongator Protein Complex 4 (ELP4). Eur J Hum Genet 17:1171–1181

Suzuki T, Nagao A (2011) Human mitochondrial diseases caused by lack of taurine modification in mitochondrial tRNAs. Wiley Interdiscip Rev RNA 2:376–386

Tachibana M, Sparman M, Sritanaudomchai H et al (2009) Mitochondrial gene replacement in primate offspring and embryonic stem cells. Nature 461:367–372

Tachibana M, Amato P, Sparman M et al (2013) Towards germline gene therapy of inherited mitochondrial diseases. Nature 493:627–631

Takano K, Nakagawa E, Inoue K et al (2008) A loss-of-function mutation in the FTSJ1 gene causes nonsyndromic X-linked mental retardation in a Japanese family. Am J Med Genet B Neuropsychiatr Genet 147B:479–484

Takeoka S, Unoki M, Onouchi Y et al (2001) Amino-acid substitutions in the IKAP gene product significantly increase risk for bronchial asthma in children. J Hum Genet 46:57–63

Torres AG, Batlle E, Ribas de Pouplana L (2014a) Role of tRNA modifications in human diseases. Trends Mol Med 20:306–314

Torres AG, Pineyro D, Filonava L et al (2014b) A-to-I editing on tRNAs: Biochemical, biological and evolutionary implications. FEBS Lett 588:4279–4286

Torres AG, Pineyro D, Rodriguez-Escriba M et al (2015) Inosine modifications in human tRNAs are incorporated at the precursor tRNA level. Nucleic Acids Res 43:5145–5157

Towns WL, Begley TJ (2012) Transfer RNA methytransferases and their corresponding modifications in budding yeast and humans: activities, predications, and potential roles in human health. DNA Cell Biol 31:434–454

Tuorto F, Liebers R, Musch T et al (2012) RNA cytosine methylation by Dnmt2 and NSun2 promotes tRNA stability and protein synthesis. Nat Struct Mol Biol 19:900–905

Umeda N, Suzuki T, Yukawa M et al (2005) Mitochondria-specific RNA-modifying enzymes responsible for the biosynthesis of the wobble base in mitochondrial tRNAs. Implications for the molecular pathogenesis of human mitochondrial diseases. J Biol Chem 280:1613–1624

Urbonavicius J, Stahl G, Durand JM et al (2003) Transfer RNA modifications that alter +1 frameshifting in general fail to affect -1 frameshifting. RNA 9:760–768

Vauti F, Goller T, Beine R et al (2007) The mouse Trm1-like gene is expressed in neural tissues and plays a role in motor coordination and exploratory behaviour. Gene 389:174–185

Vinayak M, Pathak C (2010) Queuosine modification of tRNA: its divergent role in cellular machinery. Biosci Rep 30:135–148

Waas WF, Druzina Z, Hanan M et al (2007) Role of a tRNA base modification and its precursors in frameshifting in eukaryotes. J Biol Chem 282:26026–26034

Wainfan E, Borek E (1967) Differential inhibitors of tRNA methylases. Mol Pharmacol 3:595–598

Wainfan E, Landsberg B (1971) Cytokinins that inhibit transfer RNA methylating enzymes. FEBS Lett 19:144–148

Wei FY, Tomizawa K (2011) Functional loss of Cdkal1, a novel tRNA modification enzyme, causes the development of type 2 diabetes. Endocr J 58:819–825

Wei FY, Suzuki T, Watanabe S et al (2011) Deficit of tRNA(Lys) modification by Cdkal1 causes the development of type 2 diabetes in mice. J Clin Invest 121:3598–3608

Xie P, Wei FY, Hirata S et al (2013) Quantitative PCR measurement of tRNA 2-methylthio modification for assessing type 2 diabetes risk. Clin Chem 59:1604–1612

Yarian C, Townsend H, Czestkowski W et al (2002) Accurate translation of the genetic code depends on tRNA modified nucleosides. J Biol Chem 277:16391–16395

Yasukawa T, Suzuki T, Ishii N et al (2001) Wobble modification defect in tRNA disturbs codon-anticodon interaction in a mitochondrial disease. EMBO J 20:4794–4802

Zeharia A, Shaag A, Pappo O et al (2009) Acute infantile liver failure due to mutations in the TRMU gene. Am J Hum Genet 85:401–407

Zhou W, Karcher D, Bock R (2014) Identification of enzymes for adenosine-to-inosine editing and discovery of cytidine-to-uridine editing in nucleus-encoded transfer RNAs of arabidopsis. Plant Physiol 166:1985–1997