FEBS Letters

FEBS PRESS science publishing by scientists

REVIEW ARTICLE

The epitranscriptome in translation regulation: mRNA and tRNA modifications as the two sides of the same coin?

Namit Ranjan[1] and Sebastian A. Leidel[2,3]

1 Department of Physical Biochemistry, Max Planck Institute for Biophysical Chemistry, Goettingen, Germany
2 Max Planck Research Group for RNA Biology, Max Planck Institute for Molecular Biomedicine, Münster, Germany
3 Department of Chemistry and Biochemistry, University of Bern, Bern, Switzerland

Correspondence
N. Ranjan, Department of Physical Biochemistry, Max Planck Institute for Biophysical Chemistry, Goettingen, Germany
Tel: +49 5512012958
E-mail: namit.ranjan@mpibpc.mpg.de
S. A. Leidel, Department of Chemistry and Biochemistry, University of Bern, Bern, Switzerland
Tel: +41 316314296
E-mail: sebastian.leidel@dcb.unibe.ch

(Received 2 April 2019, revised 7 June 2019, accepted 11 June 2019, available online 27 June 2019)

doi:10.1002/1873-3468.13491

Edited by Maria Papatriantafyllou

Translation of mRNA is a highly regulated process that is tightly coordinated with cotranslational protein maturation. Recently, mRNA modifications and tRNA modifications – the so called epitranscriptome – have added a new layer of regulation that is still poorly understood. Both types of modifications can affect codon–anticodon interactions, thereby affecting mRNA translation and protein synthesis in similar ways. Here, we describe an updated view on how the different types of modifications can be mapped, how they affect translation, how they trigger phenotypes and discuss how the combined action of mRNA and tRNA modifications coordinate translation in health and disease.

Keywords: disease; epitranscriptome; post-transcriptional modifications; ribosome; RNA modification; RNA sequencing; translation

Protein synthesis is essential to life. Thus, it is regulated by a variety of sophisticated mechanisms and comprises four phases: (a) initiation; (b) elongation; (c) termination; and (d) ribosome recycling. Initiation is the key regulatory step of translation, and different mechanisms have been discovered that lead either to a global or transcript-specific control of translation initiation [1]. However, translation initiation is not the only option for the cell to regulate protein synthesis. Translation elongation comprises mRNA decoding, peptide-bond formation and tRNA–mRNA translocation, resulting in nascent-peptide elongation, and each of these steps bears regulatory potential for translation. Furthermore, mRNA translation occurs in concert with protein maturation. As soon as the nascent-peptide chain emerges from the ribosome during protein synthesis, proteins begin to fold into their final three-dimensional structure and acquire protein modifications [2].

Some of the key players in translation like mRNA, tRNA or ribosomes are RNA molecules or contain RNA molecules, essential to their function. These can be post-transcriptionally modified by a plethora of chemical modifications, which currently amount to more than a hundred seventy [3–5]. Some of these RNA modifications are evolutionary conserved and were linked to a human disease [6–8]. Since RNA modifications change the structural and chemical properties of RNA molecules, some modifications of either mRNA, tRNA or rRNA are thought to optimize translation dynamics in the cell, whereas others may be neutral to translation [5]. In

Abbreviations
ac^4C, N^4-acetylcytidine; eIF, eukaryotic initiation factor; hm^5C, 5-hydroxymethylcytosine; IP, immunoprecipitation; m^5C, 5-methylcytosine; m^6A, N^6-methyladenosine; m^6Am, N^6,2′-*O*-dimethyladenosine; RT, reverse transcription; SAM, *S*-adenosyl-L-methionine; τm^5U, 5-taurinomethyluridine; U34, wobble uridine.

particular internal mRNA modifications and tRNA anticodon modifications likely lead to very similar effects, since the codon and the anticodon interact directly during decoding. Importantly, the reversibility of both types of modifications suggests a new, essentially unexplored layer of the control of gene expression that has been termed the epitranscriptome [9,10]. However, how particular mRNA or tRNA modifications affect translation is largely unknown because the analysis of eukaryotic translation *in vitro* or *in vivo* and the identification of internal mRNA modifications is very challenging.

Here, we discuss our emerging understanding of the role of mRNA modifications [N^6-methyladenosine (m^6A), 5-methylcytosine (m^5C), N^4-acetylcytidine (ac^4C), 2′-*O*-methylation] and tRNA wobble uridine (U34) modifications in translation and how our understanding of the roles of mRNA modifications is influenced by current mapping approaches. Finally, we comment on general implications for disease. This article aims to convey a broad picture of how mRNA and tRNA modifications act in concert to control translation. For a more focused discussion of mRNA and tRNA modifications we refer the readers to several recent reviews [9,11–20].

Internal mRNA modifications and their mapping

Internal modifications of mRNA and long noncoding RNA have already been discovered in the 1970s [21–23]. However, due to their low abundance they generally evaded biochemical analyses and we lacked the ability to map their positions in a transcriptome-wide manner. mRNA modification mapping has become possible only recently, with the advent of deep sequencing and the implementation of specialized sequencing-based protocols. In general, successful mapping approaches are based on three strategies: (a) the induction or detection of specific mutations in RNAseq experiments; (b) termination of reverse-transcription (RT) reactions and (c) antibody-based enrichment of modification sites (extensively reviewed in [18]). Direct RNA sequencing appears as a promising future alternative and has been shown to detect modified bases in synthetic RNA strands or abundant cellular targets [24]. However, these protocols have not been shown to work reliably on a transcriptome-wide level, yet. It is important to realize that the low abundance of mRNA modifications in combination with false-positive rates in their detection has remained a challenge. Due to the lack of quantitative high-quality mRNA modification maps for most organisms we cannot easily correlate translational phenotypes to the occurrence of mRNA modifications. This has prevented us from understanding the *in vivo* roles of mRNA modifications and in particular from understanding their effects during translation.

The most abundant internal mRNA modification is m^6A, which is present on average at 3 positions per mRNA molecule (Box 1). In addition to m^6A, pseudouridine (Ψ), N^1-methyladenosine (m^1A), N^6,2′-*O*-dimethyladenosine (m^6Am), as well as m^5C (Box 2) and its oxidation product 5-hydroxymethylcytosine (hm^5C), ac^4C and 2′-*O*-methylated nucleotides were mapped to the transcriptome. Additional modifications were reported based on measurements by RNA mass spectrometry but their position has not been determined throughout the transcriptome [9,25]. The complex modification pattern of the transcriptome likely differs between species and even between different tissues in one organism [26]. However, a complete map of all modifications in all cell types, still needs to be achieved and will require concerted efforts from many labs comparable to consortia like the ENCODE project [27].

Internal mRNA modifications in translational regulation

The recent identification of proteins that install (writers), recognize (readers) and remove (erasers) m^6A and other modifications has revealed mechanisms how mRNA modifications can affect nearly every aspect of the mRNA life cycle, as well as various cellular, developmental and disease processes. However, for understanding their effects in translation, we currently have to mostly rely on biochemical and biophysical analyses. Using an *in vitro* protein synthesis kit containing the components for *in vitro* transcription and translation, named 'PUR-Express translation system' with m^5C, m^6A, Ψ or 2′-*O*-methylated nucleotides at each position within a codon revealed that protein synthesis is strongly affected by nucleotide modifications in a position-specific manner [28]. 2′-*O*-methylated nucleotides at the 1st codon position affect translation only marginally; however, when placed at the 2nd position the same modification causes an almost complete stop of protein synthesis. Introduction of multiple modified nucleotides within one codon increased the translation inhibition [28]. More specifically, the presence of 2′-*O*-methylation at the 2nd codon position of mRNA strongly delays tRNA accommodation to the modified codon [29]. Furthermore, 2′-*O*-methylation impairs the initial and proofreading selection of aminoacyl-tRNA and the interaction between the codon–anticodon helix and ribosomal-monitoring bases [29]. Such alterations of codon–anticodon interaction can even change the identity of the incorporated amino acid as the presence

FEBS Letters **593** (2019) 1483–1493 © 2019 The Authors. *FEBS Letters* published by John Wiley & Sons Ltd on behalf of Federation of European Biochemical Societies.

Box 1: Mapping of m^6A sites

The mapping of m^6A sites is the classical example for detection based on modification-specific antibodies [80,81]. Early protocols randomly fragment the mRNA and enrich m^6A-containing mRNA fragments by immunoprecipitation (IP) using m^6A-specific antibodies. The purified mRNA pool and a negative control are subsequently converted to cDNA and sequenced. Putative m^6A sites are identified as m^6A peaks that are absent in the control. While this strategy is sufficient to identify the position of m^6A sites at a global level, it does not reach nucleotide resolution. Nucleotide resolution was later achieved by UV cross-linking the antibodies to mRNA prior to the IP called individual-nucleotide-resolution cross-linking and IP (miCLIP) [19,82,83]. The cross-link between RNA and the antibody leads to characteristic nucleotide substitutions that can be detected in the sequencing reaction. Antibody-based purification strategies have also been used for m^1A, m^6Am, hm^5C and ac^4C [18,19]. While m^1A interferes with Watson–Crick basepairing, m^6A is not distinguished from adenosine by standard RT enzymes. However, different strategies can be used to increase the mutation rates in such experiments like varying the concentration of dNTPs, using modified polymerases that are sensitive towards specific modifications or using selenium-modified deoxythymidine triphosphate analogues [84–86]. An orthogonal strategy is to use analogues of *S*-adenosyl-L-methionine (SAM) [87]. Since SAM analogues are unstable under physiological conditions, cells are fed with propargyl-L-selenohomocysteine, which is converted to the respective SAM analogue by the cellular methionine adenosyltransferase [87]. Subsequently, cellular methyltransferases, which are often promiscuous towards their substrate use the SAM analogue and incorporate the methyl derivate into mRNA. The artificial label can then be used to enrich the labelled mRNA by chemical click reactions and to induce mutations and/or strand termination in the RT reaction [87].

Box 2: Mapping of m^5C sites

m^5C was the first modified nucleotide, which was mapped to the entire transcriptome after adapting bisulfite-conversion sequencing to RNA [17]. The method takes advantage of the fact that methylation changes the chemical reactivity of cytidine. While acidic bisulfite deamination converts cytidine to uridine, m^5C is resistant to this conversion reaction. Therefore, the detection of m^5C relies on the identification of cytidines that do not convert in response to bisulfite treatment. In identifying thousands of m^5C sites throughout the transcriptome RNA-bisulphite sequencing has become the first example for identifying internal mRNA modifications in high throughput. However, the high number of m^5C sites has been questioned, showing how much care needs to be taken not to misinterpret modification marks that may be the result of single-nucleotide polymorphisms, inefficient chemical reactions or sequencing errors [20,88].

of m^5C at the 2nd codon position of CCC – a proline codon – leads to an amino acid-substitution by either isoleucine or leucine [28].

A FRET-based assay using a bacterial translation system revealed that m^6A at the 1st codon position strongly inhibits translation elongation dynamics [30]. m^6A acts as a barrier to tRNA accommodation when present at the 2nd codon position. However, when present at the 3rd codon position of the near-cognate codon m^6A essentially does not affect tRNA selection rate during translation elongation [30]. In addition, to the position of the modification, the sequence context also has a significant modulatory effect [28,30]. For instance m^6A in the glutamine (CAG) or proline (CCA) slowed translation elongation and Ψ at the 1st position of the UAA stop codon increases translational read-through [30,31]. However, translation elongation is not the only step that can be affected by nucleotide modifications. m^6A like the cap, when present in the 5′ UTR of mRNAs can modulate translation initiation. Even a single m^6A nucleotide in the 5′ UTR induces direct binding of mRNA to eukaryotic initiation factor 3 (eIF3), and is sufficient to recruit the 43S complex to initiate translation in the absence of the cap-binding initiation factor, eIF4E [32]. Following heat shock, m^6A was found more frequently in Hsp70 mRNA regulating its cap-independent translation [32], an effect that appears to be mediated by ABCF1, a key factor in m^6A-promoted translation under both physiological and stress conditions [33].

Transcriptome-wide mapping of the ac^4C, catalysed by acetyltransferase NAT 10, revealed ac^4C enrichment within coding regions of mRNA [34]. Furthermore, mRNA stability was decreased in NAT10-

deleted HeLa cells indicating that ac^4C actively promotes mRNA stability and enhances translation. ac^4C is strongly enriched in the 5′ end of mRNA, however, no effect of ac^4C on the formation of 48S preinitiation complex was observed by *in vitro* analysis, demonstrating that ac^4C does not affect translation initiation [34]. Finally, ribosome-profiling data show increased ribosome occupancy for acetylated mRNA mediated by NAT10, suggesting that ac^4C intrinsically promotes translation, a finding further supported *in vivo* by the quantification of translation products from parental and NAT10-depleted cells [34].

Taken together, the emerging data suggest a direct impact of internal mRNA modifications on gene expression, primarily through translation.

tRNA modifications and their mapping

tRNAs contain the largest diversity of modified nucleotides [12,35]. Eukaryotic tRNAs contain on average 13 modifications, including various methylations, pseudouridine, dihydrouridine, thiolation and others. Modifications are found throughout the tRNA molecule, with a hotspot in the anticodon loop. The modifications in the anticodon loop fine-tune decoding, translational fidelity and translational efficiency, whereas tRNA modifications outside the anticodon loop mainly affect tRNA stability and modulate tRNA folding [12]. Furthermore, both classes of modifications act as determinants for aminoacyl-tRNA synthetase binding to tRNA. In recent years, numerous independent studies established an unexpected role of tRNA modifications and the enzymes catalysing such modifications for the aetiology of complex human pathologies including cancer, neurological and respiratory disorders and mitochondrial diseases [6–8,36]. Compared to mRNA, tRNA are very abundant. Hence, their modifications have been characterized using RNA mass spectrometry and biochemical methods, whereas similar methods were challenging for mRNA [37]. Nevertheless, the simultaneous quantification of tRNA and their modifications in high throughput remains challenging, since sequence-specific RNA mass spectrometry is not commonly used and some RNA modifications perturb sequencing-based detection. However, recent tRNA-sequencing protocols like ARM-Seq have improved the situation [38,39].

Wobble uridine in translational processivity

The ability of cells to respond and to adapt to dynamically changing external conditions and stimuli are ensured by the coordinated processes of transcription, translation and maintenance of protein homeostasis. During this process, tRNA modifications appear critical for maintaining this coordination [40–42]. In all known organisms wobble U34 carries a complex modification at its 5′ position and a 2-thio-group (s^2U) in $tRNA^{Lys(UUU)}$, $tRNA^{Gln(UUG)}$ and $tRNA^{Glu(UUC)}$. These tRNA decode A-ending codons of split codon boxes where U- and C-ending codons code for a different amino acid. Furthermore, U34 modifications were shown to be a determinant of efficient aminoacylation of $tRNA^{Glu}$ and $tRNA^{Gln}$ by increasing the binding affinity of the synthetase in *Escherichia coli* but not in yeast [43–45]. On the ribosome, tRNA wobble modifications are implicated in maintaining accurate decoding and translational processivity. In bacteria, cmo^5U34 in $tRNA^{Ala(UGC)}$ facilitates the decoding of codons ending with A, G and U, according to the wobble rules, but also the C-ending codon with reduced efficiency [46]. In $tRNA^{Gln(UUG)}$ s^2U34 affects the hydrolysis of GTP by EF-Tu and subsequently dipeptide formation [47]. Since the ratio between the rates of GTP hydrolysis and peptide bond formation is similar for s^2U modified and unmodified $tRNA^{Gln(UUG)}$, it is likely that the modification preferentially affects GTP hydrolysis and inorganic phosphate (Pi) release with little effect on later steps in decoding [47]. In eukaryotes, $tRNA^{Lys(UUU)}$, $tRNA^{Gln(UUG)}$ and $tRNA^{Glu(UUC)}$ are decorated by 5-methoxycarbonylmethyl-2-thiouridine (mcm^5s^2U34). The absence of either of the two modifications leads to a codon-specific slowdown of translation of the AAA and CAA codons, which are decoded by $tRNA^{Lys(UUU)}$, $tRNA^{Gln(UUG)}$ [40,48]. GAA is not decoded more slowly in the yeast mutant, consistent with the observation that reading-frame maintenance of these codons depends on the mcm^5s^2U modifications only for $tRNA^{Lys(UUU)}$ and $tRNA^{Gln(UUG)}$ [49] and that the overexpression of $tRNA^{Glu(UUC)}$ is not able to rescue growth phenotypes [50,51]. Hence, the absence of the modifications affects the global translation of a subset of mRNAs enriched for codons that are read by these tRNAs [52]. A comparison of the decoding properties of native modified and unmodified tRNAs in an *in vitro* translation system showed that U34 modifications increase the affinity of the tRNA to its cognate codon in the A site of the ribosome [46,52]. Also the rate of peptide-bond formation at saturating concentrations of the ternary complex is slower in the absence of s^2U34 or mcm^5U34 [52]. A real-time kinetic analysis shows that hypomodified $tRNA^{Lys(UUU)}$ that only carries mcm^5U34 but lacks s^2U34 binds to its cognate codon with a lower affinity and is more frequently rejected than the fully modified

$tRNA^{Lys(UUU)}$. Nevertheless, the rate of peptide-bond formation remains unaffected [53]. Altogether, these data demonstrate a role of tRNA wobble uridine modifications at the initial steps of decoding and also during the proofreading step [53].

But how are the observed translational defects linked to phenotypes and to the regulation of gene expression? Several studies have shown that 2-thiolation of $tRNA^{Lys(UUU)}$, $tRNA^{Gln(UUG)}$ and $tRNA^{Glu(UUC)}$ is decreased at elevated temperature in yeast [54–57] and that this effect is reversible when normal growth conditions are restored [56,57]. A study utilizing quantitative mass spectrometry and northern-blot analyses showed a decrease in s^2U34 under permissive growth conditions upon nutrient starvation. However, thiolation pattern remained unaffected when sufficient nutrients were supplied at elevated growth temperature, thereby establishing that the reduction in 2-thiolation under heat stress is independent of nutrient availability [54]. Under most circumstances, the lack of tRNA modifications induces cellular stress and negatively affects cell growth and survival [58]. Nevertheless, the absence of tRNA modifications can also be beneficial in specific cases, for example by conferring resistance to endoplasmic reticulum stress [55] or resistance to long-term nutrient starvation [59]. This indicates that the link between wobble uridine modifications and stress is more complex than it seems.

Hypomodification and protein homeostasis

Modifications of tRNA optimize translation dynamics, which is crucial to maintain cellular homeostasis [40,60]. Several studies have characterized mutants that are deficient in wobble uridine modification using RNAseq, ribosome profiling and quantitative proteomics [40,48,52,54]. While no major translation defect was apparent based on S_{35} incorporation and polysome profiling [40,52], gene ontology analysis of downregulated genes and proteins linked the loss of U34 modifications to processes like rRNA synthesis and processing, ribosome biogenesis, tRNA synthesis and modification, electron transport chain and oxidative phosphorylation, and translation regulation, which are typically downregulated during the response to numerous stresses [40,52,54,61,62]. Analyses of codon translation by ribosome profiling in yeast cells lacking wobble uridine modifications revealed a seemingly mild enrichment of ~ 20% of AAA and CAA codons in the A site of mutant ribosomes relative to wild-type [40,48,63]. This is in agreement with a slight enrichment of AAA, CAA and GAA codons in mRNAs that appear reduced at the proteomic level [52] and an *in vitro* study that showed that the absence of s^2U34 affects the stability of $tRNA^{Lys(UUU)}$ binding to the ribosome during decoding and impedes rotation of ribosomal subunits upon

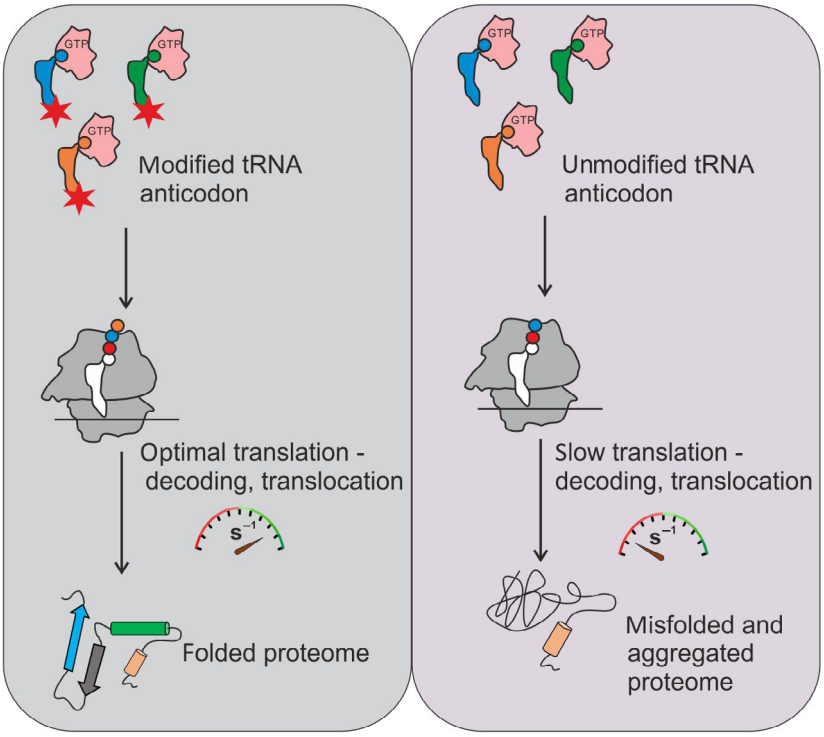

Fig. 1. Cellular outcomes of the presence or absence of tRNA anticodon modifications. Fully modified tRNAs ensure optimal translation through optimal decoding and translocation (left panel) resulting in a properly folded proteome. Slow decoding and translocation occurs due to loss of tRNA anticodon modifications (right panel), resulting in higher ribosome occupancy – indicative of a translation slowdown – at the codons requiring modified tRNAs and protein homeostasis defects.

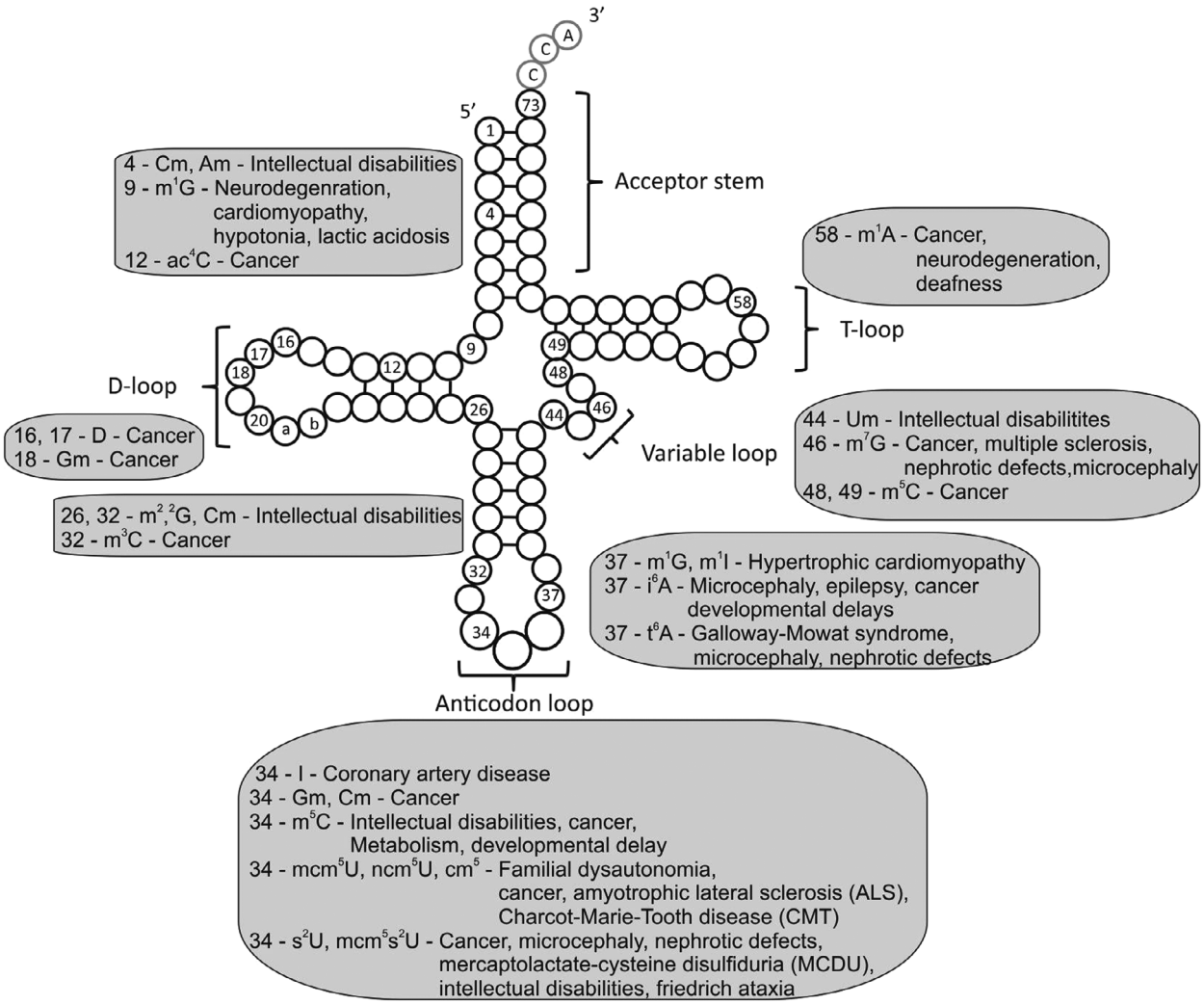

Fig. 2. tRNA modifications associated with human diseases. Schematic representation of a cytoplasmic tRNA with disease-linked modifications and the name of the associated diseases.

tRNA–mRNA translocation [53]. The lack of s^2U34 modification increases the length of the decoding steps and slows down translocation such that the residence time of the ribosomes on Lys-codon AAA increased by 20–40%, clearly accounting for the modest increase in the ribosome occupancy revealed by ribosome profiling [40,48,53]. Strikingly, however, the subtle translation defects are accompanied by a perturbation of in cellular protein homeostasis leading to the upregulation of cytoplasmic chaperones and the proteasome and lead to the formation of protein aggregates in the mutants [40] (Fig. 1). Similar phenotypes were observed in different modification mutants and may point to a common mechanism how defects in tRNA modifications induce phenotypes [41,64]. Interestingly, protein aggregates isolated from such cells are similar to those induced by the loss of ribosome-associated chaperones, responsible for cotranslational folding of nascent polypeptides and preventing them from incorrect folding [40,65]. Finally, the protein aggregates in wobble uridine modification mutants are not enriched for AAA, CAA and GAA codons, but contain mainly proteins that are known to be metastable [40,65]. Taken together, the absence of tRNA modifications induces seemingly mild effects on translation dynamics. However, those perturbations can significantly impact on cellular viability through its profound negative effect on protein homeostasis.

The epitranscriptome: implication in disease

Mutations in nearly half of the RNA modification enzymes have been linked to human diseases, including

cancer, neurological disorders, genetic birth defects, cardiovascular diseases, mitochondrial-linked defects and metabolic disorders [8] (Fig. 2). Interestingly, amongst the diseases that have been associated with mutations in RNA modifying enzymes, neurological disorders are the most prevalent. This is in agreement with the observed enrichment of various RNA modifications in neuronal tissues [66,67]. The m^5C methyltransferase NSUN2 has been associated with defects in memory and learning in Drosophila [68]. Furthermore, tRNA fragments were shown in NSUN2-deficient mice, which may activate apoptosis in the brain [69]. RNA demethylation has also been linked to neurological defects. The deletion of the RNA demethylase FTO in mice results in an impairment of dopamine receptor control of neuronal activity and behaviorial responses [70]. ALKBH5, a second m^6A demethylase has been associated to major depressive disorders [71]. In addition, mutations that affect 5-taurinomethyluridine (τm^5U) biosynthesis in mitochondrial tRNAs are associated with mitochondrial diseases and affect the translation of lysine and leucine codons. MTO1 and GTPBP3 catalyse the formation τm^5U and GTPBP3-knockout exhibit respiratory defects and reduced mitochondrial translation, however, little is known about the mechanism [72–74].

Defects in tRNA modifications and mRNA modifications have also been directly tied to cell proliferation and malignancy in a number of lymphomas, leukaemias and carcinomas, including breast, bladder and colorectal cancers [6,75–77]. Elp3 and Ctu1/2, enzymes responsible for mcm^5s^2 modification are upregulated in breast cancer and were shown to be required to sustain metastasis [78]. NSUN2 is a direct target gene of c-Myc, a well-known proto-oncogene and has been found to be upregulated in primary tumours and metastases of breast carcinomas [79]. The reduction in m^6A levels through knockdown of *Mettl3* results in tumour progression [77]. Taken together, these point to a crucial role of RNA modifications in human diseases.

Concluding remarks

This review aims to summarize the emerging view of internal mRNA and tRNA modifications – the so called epitranscriptome – as regulators of translation and protein homeostasis. In the last decade we have learned how RNA modifications – in particular in the tRNA anticodon and the newly discovered internal mRNA modifications – affect RNA metabolism in unexpected ways, thus adding an unchartered layer to translation regulation. It will be exciting to follow how new modifications will be mapped to the transcriptome under different conditions. However, most importantly we will discover how those marks in trigger phenotypes and facilitate physiological effects in different species. An important step will be the further improvement of modification mapping. Deep-sequencing-based methods have already made invaluable contributions to our understanding of modified nucleosides and the integration of additional enzymes and further improvements of antibody-based strategies, will provide us with novel insights.

One of the major recent advances was the discovery how internal mRNA modifications affect gene expression and translation. While most of the studies were performed using single mRNA modifications, it will be very interesting to study the cross-talk between various of this modification marks. Furthermore, it will be important to understand whether tRNA modifications play a role in decoding modified mRNA nucleotides and in particular how modified mRNA nucleotides interact with tRNA anticodon modifications during translation. In the light of data highlighting the impact of tRNA wobble modifications for translation quality through optimizing decoding, translocation and ribosome density, it appears worthwhile to explore the roles of other tRNA modifications. It is now well established that gene expression regulation and human diseases are affected by tRNA modifications. It will be similarly important to understand which human pathologies are linked to mRNA modifications. The importance of RNA modifications for translation dynamics as well as their potential to perturb translation elongation will eventually tell us whether the two types of modifications are indeed equal sides of the coin or whether translation is a coin with a flipside.

Acknowledgements

Decades of work by many laboratories has uncovered the roles of RNA modifications. In particular, wobble uridine modifications are amongst the most studied modifications. We are therefore, grateful to the scientists in the field who have provided many important insights even though we have not been able to adequately reference their work due limited space. However, our review is also the result of discussions with numerous colleagues who have shared their insights and opinions. NR and SAL thank for the support by the Deutsche Forschungsgemeinschaft (DFG) Priority Programme SPP1784 Chemical Biology of native Nucleic Acid Modifications to NR (RA 3194/1-1) and SAL (LE3260/2) and the Max Planck Society. SAL further acknowledges the support by DFG grant (LE 3260/3-1).

References

1 Hinnebusch AG, Ivanov IP and Sonenberg N (2016) Translational control by 5′-untranslated regions of eukaryotic mRNAs. *Science* **352**, 1413–1416.

2 Rodnina MV and Wintermeyer W (2016) Protein elongation, co-translational folding and targeting. *J Mol Biol* **428**, 2165–2185.

3 Machnicka MA, Milanowska K, Osman Oglou O, Purta E, Kurkowska M, Olchowik A, Januszewski W, Kalinowski S, Dunin-Horkawicz S, Rother KM *et al.* (2013) MODOMICS: a database of RNA modification pathways-2013 update. *Nucleic Acids Res* **41**, D262–D267.

4 Milanowska K, Mikolajczak K, Lukasik A, Skorupski M, Balcer Z, Machnicka MA, Nowacka M, Rother KM and Bujnicki JM (2013) RNApathwaysDB-a database of RNA maturation and decay pathways. *Nucleic Acids Res* **41**, D268–D272.

5 Grosjean H (2009) Nucleic acids are not boring long polymers of only four types of nucleotides: a guided tour. In DNA and RNA Modification Enzymes: Structure, Mechanism, Function and Evolution (Grosjean H, ed), pp. 1–18. Landes Bioscience, Austin, TX.

6 Sarin LP and Leidel SA (2014) Modify or die?–RNA modification defects in metazoans. *RNA Biol* **11**, 1555–1567.

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

8 de Crecy-Lagard V, Boccaletto P, Mangleburg CG, Sharma P, Lowe TM, Leidel SA and Bujnicki JM (2019) Matching tRNA modifications in humans to their known and predicted enzymes. *Nucleic Acids Res* **47**, 2143–2159.

9 Roundtree IA, Evans ME, Pan T and He C (2017) Dynamic RNA modifications in gene expression regulation. *Cell* **169**, 1187–1200.

10 Guy MP, Young DL, Payea MJ, Zhang X, Kon Y, Dean KM, Grayhack EJ, Mathews DH, Fields S and Phizicky EM (2014) Identification of the determinants of tRNA function and susceptibility to rapid tRNA decay by high-throughput *in vivo* analysis. *Genes Dev* **28**, 1721–1732.

11 Advani VM and Ivanov P (2019) Translational control under stress: reshaping the translatome. *BioEssays* **41**, e1900009.

12 Phizicky EM and Hopper AK (2010) tRNA biology charges to the front. *Genes Dev* **24**, 1832–1860.

13 Shepherd J and Ibba M (2015) Bacterial transfer RNAs. *FEMS Microbiol Rev* **39**, 280–300.

14 Ontiveros RJ, Stoute J and Liu KF (2019) The chemical diversity of RNA modifications. *Biochem J* **476**, 1227–1245.

15 Zhao BS, Roundtree IA and He C (2017) Post-transcriptional gene regulation by mRNA modifications. *Nat Rev Mol Cell Biol* **18**, 31–42.

16 Nachtergaele S and He C (2018) Chemical modifications in the life of an mRNA transcript. *Annu Rev Genet* **52**, 349–372.

17 Squires JE, Patel HR, Nousch M, Sibbritt T, Humphreys DT, Parker BJ, Suter CM and Preiss T (2012) Widespread occurrence of 5-methylcytosine in human coding and non-coding RNA. *Nucleic Acids Res* **40**, 5023–5033.

18 Motorin Y and Helm M (2019) Methods for RNA modification mapping using deep sequencing: established and new emerging technologies. *Genes (Basel)* **10**, E35.

19 Linder B, Grozhik AV, Olarerin-George AO, Meydan C, Mason CE and Jaffrey SR (2015) Single-nucleotide-resolution mapping of m6A and m6Am throughout the transcriptome. *Nat Methods* **12**, 767–772.

20 Helm M and Motorin Y (2017) Detecting RNA modifications in the epitranscriptome: predict and validate. *Nat Rev Genet* **18**, 275–291.

21 Adams JM and Cory S (1975) Modified nucleosides and bizarre 5′-termini in mouse myeloma mRNA. *Nature* **255**, 28–33.

22 Desrosiers R, Friderici K and Rottman F (1974) Identification of methylated nucleosides in messenger RNA from Novikoff hepatoma cells. *Proc Natl Acad Sci USA* **71**, 3971–3975.

23 Dubin DT and Taylor RH (1975) The methylation state of poly A-containing messenger RNA from cultured hamster cells. *Nucleic Acids Res* **2**, 1653–1668.

24 Garalde DR, Snell EA, Jachimowicz D, Sipos B, Lloyd JH, Bruce M, Pantic N, Admassu T, James P, Warland A *et al.* (2018) Highly parallel direct RNA sequencing on an array of nanopores. *Nat Methods* **15**, 201–206.

25 Haag S, Sloan KE, Ranjan N, Warda AS, Kretschmer J, Blessing C, Hubner B, Seikowski J, Dennerlein S, Rehling P *et al.* (2016) NSUN3 and ABH1 modify the wobble position of mt-tRNAMet to expand codon recognition in mitochondrial translation. *EMBO J* **35**, 2014–2119.

26 Delatte B, Wang F, Ngoc LV, Collignon E, Bonvin E, Deplus R, Calonne E, Hassabi B, Putmans P, Awe S *et al.* (2016) RNA biochemistry. Transcriptome-wide distribution and function of RNA hydroxymethylcytosine. *Science* **351**, 282–285.

27 ENCODE Project Consortium (2012) An integrated encyclopedia of DNA elements in the human genome. *Nature* **489**, 57–74.

28 Hoernes TP, Clementi N, Faserl K, Glasner H, Breuker K, Lindner H, Huttenhofer A and Erlacher MD (2016) Nucleotide modifications within bacterial messenger RNAs regulate their translation and are

able to rewire the genetic code. *Nucleic Acids Res* **44**, 852–862.
29 Choi J, Indrisiunaite G, DeMirci H, Ieong KW, Wang J, Petrov A, Prabhakar A, Rechavi G, Dominissini D, He C *et al*. (2018) 2′-O-methylation in mRNA disrupts tRNA decoding during translation elongation. *Nat Struct Mol Biol* **25**, 208–216.
30 Choi J, Ieong KW, Demirci H, Chen J, Petrov A, Prabhakar A, O'Leary SE, Dominissini D, Rechavi G, Soltis SM *et al*. (2016) N(6)-methyladenosine in mRNA disrupts tRNA selection and translation-elongation dynamics. *Nat Struct Mol Biol* **23**, 110–115.
31 Karijolich J and Yu YT (2011) Converting nonsense codons into sense codons by targeted pseudouridylation. *Nature* **474**, 395–398.
32 Meyer KD, Patil DP, Zhou J, Zinoviev A, Skabkin MA, Elemento O, Pestova TV, Qian SB and Jaffrey SR (2015) 5′ UTR m(6)A promotes cap-independent translation. *Cell* **163**, 999–1010.
33 Coots RA, Liu XM, Mao Y, Dong L, Zhou J, Wan J, Zhang X and Qian SB (2017) m(6)A facilitates eIF4F-independent mRNA translation. *Mol Cell* **68**, 504–514.e7.
34 Arango D, Sturgill D, Alhusaini N, Dillman AA, Sweet TJ, Hanson G, Hosogane M, Sinclair WR, Nanan KK, Mandler MD *et al*. (2018) Acetylation of cytidine in mRNA promotes translation efficiency. *Cell* **175**, 1872–1886.e24.
35 Ranjan N and Rodnina MV (2016) tRNA wobble modifications and protein homeostasis. *Translation* **4**, e1143076.
36 Jonkhout N, Tran J, Smith MA, Schonrock N, Mattick JS and Novoa EM (2017) The RNA modification landscape in human disease. *RNA* **23**, 1754–1769.
37 Gaston KW and Limbach PA (2014) The identification and characterization of non-coding and coding RNAs and their modified nucleosides by mass spectrometry. *RNA Biol* **11**, 1568–1585.
38 Cozen AE, Quartley E, Holmes AD, Hrabeta-Robinson E, Phizicky EM and Lowe TM (2015) ARM-seq: AlkB-facilitated RNA methylation sequencing reveals a complex landscape of modified tRNA fragments. *Nat Methods* **12**, 879–884.
39 Zheng G, Qin Y, Clark WC, Dai Q, Yi C, He C, Lambowitz AM and Pan T (2015) Efficient and quantitative high-throughput tRNA sequencing. *Nat Methods* **12**, 835–837.
40 Nedialkova DD and Leidel SA (2015) Optimization of codon translation rates via tRNA modifications maintains proteome integrity. *Cell* **161**, 1606–1618.
41 Thiaville PC, Legendre R, Rojas-Benitez D, Baudin-Baillieu A, Hatin I, Chalancon G, Glavic A, Namy O and de Crecy-Lagard V (2016) Global translational impacts of the loss of the tRNA modification t(6)A in yeast. *Microb Cell* **3**, 29–45.
42 Klassen R, Grunewald P, Thuring KL, Eichler C, Helm M and Schaffrath R (2015) Loss of anticodon wobble uridine modifications affects tRNA(Lys) function and protein levels in *Saccharomyces cerevisiae*. *PLoS ONE* **10**, e0119261.
43 Rogers MJ, Weygand-Durasevic I, Schwob E, Sherman JM, Rogers KC, Thomann HU, Sylvers LA, Ohtsuka E, Inokuchi H and Soll D (1993) The recognition of *E. coli* glutamine tRNA by glutaminyl-tRNA synthetase. *Nucleic Acids Symp Ser*, **29**, 211–213.
44 Sylvers LA, Rogers KC, Shimizu M, Ohtsuka E and Soll D (1993) A 2-thiouridine derivative in tRNAGlu is a positive determinant for aminoacylation by *Escherichia coli* glutamyl-tRNA synthetase. *Biochemistry* **32**, 3836–3841.
45 Weygand-Durasevic I, Rogers MJ and Soll D (1994) Connecting anticodon recognition with the active site of *Escherichia coli* glutaminyl-tRNA synthetase. *J Mol Biol* **240**, 111–118.
46 Kothe U and Rodnina MV (2007) Codon reading by tRNAAla with modified uridine in the wobble position. *Mol Cell* **25**, 167–174.
47 Rodriguez-Hernandez A, Spears JL, Gaston KW, Limbach PA, Gamper H, Hou YM, Kaiser R, Agris PF and Perona JJ (2013) Structural and mechanistic basis for enhanced translational efficiency by 2-thiouridine at the tRNA anticodon wobble position. *J Mol Biol* **425**, 3888–3906.
48 Zinshteyn B and Gilbert WV (2013) Loss of a conserved tRNA anticodon modification perturbs cellular signaling. *PLoS Genet* **9**, e1003675.
49 Manickam N, Joshi K, Bhatt MJ and Farabaugh PJ (2016) Effects of tRNA modification on translational accuracy depend on intrinsic codon-anticodon strength. *Nucleic Acids Res* **44**, 1871–1881.
50 Esberg A, Huang B, Johansson MJ and Bystrom AS (2006) Elevated levels of two tRNA species bypass the requirement for elongator complex in transcription and exocytosis. *Mol Cell* **24**, 139–148.
51 Leidel S, Pedrioli PG, Bucher T, Brost R, Costanzo M, Schmidt A, Aebersold R, Boone C, Hofmann K and Peter M (2009) Ubiquitin-related modifier Urm1 acts as a sulphur carrier in thiolation of eukaryotic transfer RNA. *Nature* **458**, 228–232.
52 Rezgui VA, Tyagi K, Ranjan N, Konevega AL, Mittelstaet J, Rodnina MV, Peter M and Pedrioli PG (2013) tRNA tK(UUU), tQ(UUG), and tE(UUC) wobble position modifications fine-tune protein translation by promoting ribosome A-site binding. *Proc Natl Acad Sci USA* **110**, 12289–12294.
53 Ranjan N and Rodnina MV (2017) Thio-modification of tRNA at the wobble position as regulator of the

kinetics of decoding and translocation on the ribosome. *J Am Chem Soc* **139**, 5857–5864.
54 Tyagi K and Pedrioli PG (2015) Protein degradation and dynamic tRNA thiolation fine-tune translation at elevated temperatures. *Nucleic Acids Res* **43**, 4701–4712.
55 Damon JR, Pincus D and Ploegh HL (2015) tRNA thiolation links translation to stress responses in *Saccharomyces cerevisiae*. *Mol Biol Cell* **26**, 270–282.
56 Lu J, Huang B, Esberg A, Johansson MJ and Bystrom AS (2005) The *Kluyveromyces lactis* gamma-toxin targets tRNA anticodons. *RNA* **11**, 1648–1654.
57 Alings F, Sarin LP, Fufezan C, Drexler HC and Leidel SA (2015) An evolutionary approach uncovers a diverse response of tRNA 2-thiolation to elevated temperatures in yeast. *RNA* **21**, 202–212.
58 Schaffrath R and Leidel SA (2017) Wobble uridine modifications-a reason to live, a reason to die?!. *RNA Biol* **14**, 1209–1222.
59 Laxman S, Sutter BM, Wu X, Kumar S, Guo X, Trudgian DC, Mirzaei H and Tu BP (2013) Sulfur amino acids regulate translational capacity and metabolic homeostasis through modulation of tRNA thiolation. *Cell* **154**, 416–429.
60 Laguesse S, Creppe C, Nedialkova DD, Prevot PP, Borgs L, Huysseune S, Franco B, Duysens G, Krusy N, Lee G *et al*. (2015) A dynamic unfolded protein response contributes to the control of cortical neurogenesis. *Dev Cell* **35**, 553–567.
61 Chan CT, Pang YL, Deng W, Babu IR, Dyavaiah M, Begley TJ and Dedon PC (2012) Reprogramming of tRNA modifications controls the oxidative stress response by codon-biased translation of proteins. *Nat Commun* **3**, 937.
62 Gasch AP, Spellman PT, Kao CM, Carmel-Harel O, Eisen MB, Storz G, Botstein D and Brown PO (2000) Genomic expression programs in the response of yeast cells to environmental changes. *Mol Biol Cell* **11**, 4241–4257.
63 Ingolia NT, Ghaemmaghami S, Newman JR and Weissman JS (2009) Genome-wide analysis *in vivo* of translation with nucleotide resolution using ribosome profiling. *Science* **324**, 218–223.
64 Schaffrath R and Klassen R (2017) Combined tRNA modification defects impair protein homeostasis and synthesis of the yeast prion protein Rnq1. *Prion* **11**, 48–53.
65 Willmund F, del Alamo M, Pechmann S, Chen T, Albanese V, Dammer EB, Peng J and Frydman J (2013) The cotranslational function of ribosome-associated Hsp70 in eukaryotic protein homeostasis. *Cell* **152**, 196–209.
66 Chi L and Delgado-Olguin P (2013) Expression of NOL1/NOP2/sun domain (Nsun) RNA methyltransferase family genes in early mouse embryogenesis. *Gene Expr Patterns* **13**, 319–327.
67 Paul MS and Bass BL (1998) Inosine exists in mRNA at tissue-specific levels and is most abundant in brain mRNA. *EMBO J* **17**, 1120–1127.
68 Abbasi-Moheb L, Mertel S, Gonsior M, Nouri-Vahid L, Kahrizi K, Cirak S, Wieczorek D, Motazacker MM, Esmaeeli-Nieh S, Cremer K *et al*. (2012) Mutations in NSUN2 cause autosomal-recessive intellectual disability. *Am J Hum Genet* **90**, 847–855.
69 Blanco S, Dietmann S, Flores JV, Hussain S, Kutter C, Humphreys P, Lukk M, Lombard P, Treps L, Popis M *et al*. (2014) Aberrant methylation of tRNAs links cellular stress to neuro-developmental disorders. *EMBO J* **33**, 2020–2039.
70 Hess ME, Hess S, Meyer KD, Verhagen LA, Koch L, Bronneke HS, Dietrich MO, Jordan SD, Saletore Y, Elemento O *et al*. (2013) The fat mass and obesity associated gene (Fto) regulates activity of the dopaminergic midbrain circuitry. *Nat Neurosci* **16**, 1042–1048.
71 Du T, Rao S, Wu L, Ye N, Liu Z, Hu H, Xiu J, Shen Y and Xu Q (2015) An association study of the m6A genes with major depressive disorder in Chinese Han population. *J Affect Disord* **183**, 279–286.
72 Asano K, Suzuki T, Saito A, Wei FY, Ikeuchi Y, Numata T, Tanaka R, Yamane Y, Yamamoto T, Goto T *et al*. (2018) Metabolic and chemical regulation of tRNA modification associated with taurine deficiency and human disease. *Nucleic Acids Res* **46**, 1565–1583.
73 Suzuki T, Suzuki T, Wada T, Saigo K and Watanabe K (2002) Taurine as a constituent of mitochondrial tRNAs: new insights into the functions of taurine and human mitochondrial diseases. *EMBO J* **21**, 6581–6589.
74 Kirino Y, Yasukawa T, Ohta S, Akira S, Ishihara K, Watanabe K and Suzuki T (2004) Codon-specific translational defect caused by a wobble modification deficiency in mutant tRNA from a human mitochondrial disease. *Proc Natl Acad Sci USA* **101**, 15070–15075.
75 Emmerich B, Zubrod E, Weber H, Maubach PA, Kersten H and Kersten W (1985) Relationship of queuine-lacking transfer RNA to the grade of malignancy in human leukemias and lymphomas. *Cancer Res* **45**, 4308–4314.
76 Rodriguez V, Chen Y, Elkahloun A, Dutra A, Pak E and Chandrasekharappa S (2007) Chromosome 8 BAC array comparative genomic hybridization and expression analysis identify amplification and overexpression of TRMT12 in breast cancer. *Genes Chromosom Cancer* **46**, 694–707.
77 Batista PJ (2017) The RNA modification N(6)-methyladenosine and its implications in human disease. *Genomics Proteomics Bioinformatics* **15**, 154–163.

78 Delaunay S, Rapino F, Tharun L, Zhou Z, Heukamp L, Termathe M, Shostak K, Klevernic I, Florin A, Desmecht H *et al*. (2016) Elp3 links tRNA modification to IRES-dependent translation of LEF1 to sustain metastasis in breast cancer. *J Exp Med* **213**, 2503–2523.
79 Frye M and Watt FM (2006) The RNA methyltransferase Misu (NSun2) mediates Myc-induced proliferation and is upregulated in tumors. *Curr Biol* **16**, 971–981.
80 Meyer KD, Saletore Y, Zumbo P, Elemento O, Mason CE and Jaffrey SR (2012) Comprehensive analysis of mRNA methylation reveals enrichment in 3′ UTRs and near stop codons. *Cell* **149**, 1635–1646.
81 Dominissini D, Moshitch-Moshkovitz S, Schwartz S, Salmon-Divon M, Ungar L, Osenberg S, Cesarkas K, Jacob-Hirsch J, Amariglio N, Kupiec M *et al*. (2012) Topology of the human and mouse m6A RNA methylomes revealed by m6A-seq. *Nature* **485**, 201–206.
82 Ke S, Alemu EA, Mertens C, Gantman EC, Fak JJ, Mele A, Haripal B, Zucker-Scharff I, Moore MJ, Park CY *et al*. (2015) A majority of m6A residues are in the last exons, allowing the potential for 3′ UTR regulation. *Genes Dev* **29**, 2037–2053.
83 Hawley BR and Jaffrey SR (2019) Transcriptome-wide mapping of m(6) A and m(6) Am at single-nucleotide resolution using miCLIP. *Curr Protoc Mol Biol* **126**, e88.
84 Maden BE (2001) Mapping 2′-O-methyl groups in ribosomal RNA. *Methods* **25**, 374–382.
85 Aschenbrenner J, Werner S, Marchand V, Adam M, Motorin Y, Helm M and Marx A (2018) Engineering of a DNA polymerase for direct m(6) A sequencing. *Angew Chem Int Ed Engl* **57**, 417–421.
86 Hong T, Yuan Y, Chen Z, Xi K, Wang T, Xie Y, He Z, Su H, Zhou Y, Tan ZJ *et al*. (2018) Precise antibody-independent m6A identification via 4SedTTP-involved and FTO-assisted strategy at single-nucleotide resolution. *J Am Chem Soc* **140**, 5886–5889.
87 Hartstock K, Nilges BS, Ovcharenko A, Cornelissen NV, Pullen N, Lawrence-Dorner AM, Leidel SA and Rentmeister A (2018) Enzymatic or *in vivo* installation of propargyl groups in combination with click chemistry for the enrichment and detection of methyltransferase target sites in RNA. *Angew Chem Int Ed Engl* **57**, 6342–6346.
88 Khoddami V, Yerra A, Mosbruger TL, Fleming AM, Burrows CJ and Cairns BR (2019) Transcriptome-wide profiling of multiple RNA modifications simultaneously at single-base resolution. *Proc Natl Acad Sci USA* **116**, 6784–6789.