Perspective

The Regulation of RNA Modification Systems: The Next Frontier in Epitranscriptomics?

Matthias R. Schaefer [ID]

Centre for Anatomy & Cell Biology, Division of Cell-and Developmental Biology, Medical University of Vienna, Schwarzspanierstrasse 17, Haus C, 1st Floor, 1090 Vienna, Austria; matthias.schaefer@meduniwien.ac.at; Tel.: +43-1-40160-37702

Abstract: RNA modifications, long considered to be molecular curiosities embellishing just abundant and non-coding RNAs, have now moved into the focus of both academic and applied research. Dedicated research efforts (epitranscriptomics) aim at deciphering the underlying principles by determining RNA modification landscapes and investigating the molecular mechanisms that establish, interpret and modulate the information potential of RNA beyond the combination of four canonical nucleotides. This has resulted in mapping various epitranscriptomes at high resolution and in cataloguing the effects caused by aberrant RNA modification circuitry. While the scope of the obtained insights has been complex and exciting, most of current epitranscriptomics appears to be stuck in the process of producing data, with very few efforts to disentangle cause from consequence when studying a specific RNA modification system. This article discusses various knowledge gaps in this field with the aim to raise one specific question: how are the enzymes regulated that dynamically install and modify RNA modifications? Furthermore, various technologies will be highlighted whose development and use might allow identifying specific and context-dependent regulators of epitranscriptomic mechanisms. Given the complexity of individual epitranscriptomes, determining their regulatory principles will become crucially important, especially when aiming at modifying specific aspects of an epitranscriptome both for experimental and, potentially, therapeutic purposes.

Keywords: epitranscriptomics; regulation; reproducibility

1. Introduction

For over half a century, chemical RNA modifications have been known to exist in RNA [1–4]. For most of this time, these modifications were considered to be chemical additions to the sequence of primarily non-coding RNAs (ncRNAs) which affected their biogenesis, stability and, likely, function. Curiously, various modifications had also been detected in coding RNAs, namely *N7*-methylguanosine (m7G), *N6*-methyladenosine (m6A), inosine (I), ribose methylation (Nm) and poly-A additions, indicating the potential for a regulatory role in messenger RNAs (mRNAs) [5–8].

Research on mRNA modifications lay largely dormant for many years with the exception of RNA editing (C-to-U and later A-to-I) due to the relatively easy detection by DNA and RNA sequencing [9–19]. Then, a thought-provoking commentary in 2010 boldly suggested the existence of molecular mechanisms, called "RNA epigenetics", which, akin to epigenetics affected RNA functionally [20]. This idea was based on the earlier identification of an α-ketoglutarate-dependent dioxygenase activity (AlkB) that was able to demethylate not only m6A-modified DNA but also m6A-modified RNA [21]. This finding together with the identification of another enzymatic activity capable of removing m6A from RNA [22] provided the necessary spark that triggered renewed interest in chemical modifications, especially in their the regulatory potential for mRNAs. Between then and now, feverishly productive years have resulted in an impressive number of studies reporting on links between specific types of RNA modifications and many essential biological processes. Importantly, the discovery of dedicated molecular machineries that

can dynamically change RNA identity led to coining the terms "epitranscriptome" and "epitranscriptomics" [23,24]. Ever since, proteins installing specific RNA modifications are called writers, activities that modify or even remove the same modification are considered to be modifiers/erasers, and proteins that interpret the RNA modification signature are called readers. Accordingly, the resulting transcriptome-wide RNA modification landscape, established by a particular epitranscriptomic machinery, is now called an epitranscriptome. There is still semantic confusion in the field. Some authors are calling only specific and reversible mRNA modifications, containing carbon-nitrogen bonds (i.e., $m^6A(m)$, m^1A) as epitranscriptomic. Others use the term for any RNA modification, be it terminally or internally placed on any RNA including non-coding RNAs. Despite of these semantic issues, determining individual epitranscriptomes (through 'epitranscriptomics') is now a rapidly developing field that is (currently) focused on a limited number of RNA modifications (m^6A, m^5C, m^1A, Ψ, m^7G, and Nm; structural information in [25]). This limitation does exist because reliable and reproducible detection of a given RNA modification is a key requirement when studying the biological impact of RNA modifications. Hence, during the last 10 years, the major share in epitranscriptomics research was invested in the development of mass spectrometry- and sequencing-based methodologies to map specific RNA modifications [25–28]. This technological progress has resulted in extensive and transcriptome-wide studies and the publication of large data sets originating from different cell types, tissues and organisms. Importantly, RNA modifications have also been associated with human health. In particular, deregulation of particular writers, readers and modifiers/erasers, and thereby specific epitranscriptomes, has been observed in multiple human cancers and various diseases (reviewed in [29–33]). Rather than reiterating the extensive amount of accumulated knowledge here, the interested reader is referred to the multitude of excellent reviews written on the available technologies and the consequences of mutations in epitranscriptomic circuitry [25,26,34–39]. Instead, this article aims at highlighting some of the open questions in epitranscriptomics with a focus on the need to better understand the regulation of the enzymes that establish, modulate and erase individual epitranscriptomes.

2. Key Questions in Epitranscriptomics

2.1. Once Is Never: How Reproducible Is Current Modification Mapping?

While the existence of RNA modifications and their effects on RNA functionality is measurable, our understanding as to how specific epitranscriptomes are established, dynamically modulated and partially erased is very much incomplete. To better define epitranscriptomic mechanisms and to be able to decipher their phenotypic consequences, one basic prerequisite is reproducibly determining individual epitranscriptomes. As of now, many of the published efforts to map specific epitranscriptomes (mapping the same RNA modification transcriptome-wide) have produced contradictory primary data sets. This has resulted in an "uneasy" debate about the limitations of current technologies and, importantly, how to draw conclusions from the existing data sets [25,40–47]. Yet constantly, an increasing number of modification mapping experiments is being published (still using debated technology [48]), along with descriptions of a staggering variety of RNA modification-dependent phenotypes. In contrast, few follow-up studies have been published that utilized the available mapping data as basis for addressing the biological impact of a specific RNA modification in a specific RNA at a specific position. Notable exceptions are studies on the functional consequences of RNA editing, which can draw on reproducible mapping data produced by multiple (and independent) laboratories [49–52]. Inaccurate mapping of any RNA modification will greatly affect all experimental conclusions, hypothesis building, and importantly, ongoing bioinformatics efforts to predict RNA modification patterns in silico [53–61]. A persistent question, therefore, is how to reliably map specific epitranscriptomes, not only in reproducible fashion but also sufficiently robust to technical variation.

2.2. Stoichiometry: How Many Molecules Need to Be Modified for Biological Impact?

The biological impact of specific RNA modifications likely depends on the percentage of individual transcripts that are modified. For instance, a modification affecting RNA stability is unlikely to have impact if only a few transcripts are modified. On the other hand, a modification such as A-to-I editing at a specific mRNA position resulting in the translation of a particular protein isoform, might have major impact even when produced at low levels. Quantitative measurements revealed that the relative levels of various eukaryotic mRNA modifications (other than RNA editing) were: 0.2 to 0.6% for m^6A/A, 0.015 to 0.054% for m^1A/A, 0.025 to 0.1% for m^5C/C, 0.001–0.004% for hm^5C/C, 0.2–0.6% for Ψ/U, and only 0.003% for m^6Am/all nucleosides (listed and individually referenced in [27]). These are rather low values. And even though m^6A is repeatedly mentioned as the most abundant mRNA modification, the actual values translate into 1 to 3 m^6A-modifiations per 1000 nucleotides of an mRNA. Do these values represent nucleotides from a fraction of RNAs with the same sequence (sub-stoichiometric modification) or is every RNA molecule modified, yet is only expressed in a few cells (stoichiometric modification)? State-of-the-art liquid chromatography-mass spectrometry (LC-MS) methods allow the absolute quantification of individual RNA modifications [62–65]. However, this technology still requires relatively large (and pure) quantities of the RNA analyte, making it unsuitable for high-throughput analyses [28]. Therefore, sequencing-based methods will need to be developed for determining the stoichiometry of RNA modifications in complex and low-input samples. Methods such as site-specific cleavage and radioactive-labelling followed by ligation-assisted extraction and thin-layer chromatography (SCARLET) and m^6A-level and isoform-characterization sequencing (m^6A-LAIC-seq) can be used to quantify m^6A/A levels at candidate loci and in transcriptome-wide fashion [66,67]. Furthermore, RiboMeth-Seq allows quantitative insights into ribose methylation (Nm) levels at specific sites [68]. Also, RNA bisulfite sequencing, a method allowing the mapping of m^5C at single nucleotide resolution [69], could be used to quantify the ratio of m^5C/C in specific transcripts. However, when using sequencing-based approaches for quantitative RNA modification measurements, it will be crucially important to include unique molecular identifiers (UMIs) during sample preparation [70], a technical detail that was often not included in existing RNA modification mapping data.

2.3. RNA Modification Come and Go: Constitutive or Dynamic Signatures?

Epitranscriptomics has largely been defined by the transcriptome-wide interrogation of one specific epitranscriptome at a given time. With respect to occurrence, stoichiometry and latency in the same RNA sequence, the obtained results allow conceptually separating at least two categories of epitranscriptomic signatures. In one category, RNA modifications can be detected in almost every sequence of a specific RNA class, type or species. For instance, every functional mRNA contains specific terminal RNA modifications such as m^7G at cap structures and non-templated poly-A-tails. Individual tRNAs, rRNAs and snRNAs contain invariant RNA modifications at specific positions with high stoichiometry indicating that these modifications are constitutive (reviewed in [71–74]). In contrast, RNA modifications are also detectable at sub-stoichiometric levels suggesting that their placement is not constitutive but (dynamically) regulated. This notion is supported by observations showing that RNA modifications at specific positions can differ quantitatively, especially in response to particular cellular, developmental or environmental changes [75–83]. Convincing examples exist for A-to-I editing, which, while constitutive in repetitive RNAs [17,84,85], appears to be dynamically modulated in particular mRNAs [80,82,86]. Another intriguing example is the developmental stage-dependent activity of Initiator of Meiosis 4 (IME4), the yeast homolog of mammalian METTL3, mediating m^6A only during meiosis and sporulation in *Saccharomyces cerevisiae* [87,88]. Recent evidence indicated that also microbiome-dependent regulation of eukaryotic RNA modifications. The micronutrient queuine, produced by prokaryota, is not only a precursor for substituting guanosine with queuosine (Q) in specific tRNAs [89], but placement of Q also affects m^5C levels at specific tRNA positions [90,91]. It

follows that future epitranscriptomics would gain from incorporating any context-relevant information (cellular, developmental, environmental, disease) when publishing and depositing RNA modification mapping data into databases.

2.4. The Multi-Substrate/Promiscuity Problem

The chemical universe of RNA modifications is rather complex but most known modification reactions involve methylation groups, followed by isomerizations and deamination reactions [92–95]. Many of the enzymes with the potential to modify RNAs have been only bioinformatically identified and await characterization [96–99]. However, various knockout or knockdown approaches addressing specific epitranscriptomic systems followed by transcriptome-wide determination of respective epitranscriptomes have revealed the substrate specificity for a select number of writers and modulators/erasers. While many enzymes modifying ncRNAs such as tRNAs, rRNAs or small nuclear RNAs (snRNAs) appear to have limited and often evolutionary conserved substrate specificity, others display pronounced substrate promiscuity. Examples for limited substrate specificity are highly conserved enzymes such as particular members of the NOP2/Sun domain (NSUN) or methyltransferase-like (METTL) family of proteins targeting single nucleotides in rRNAs [100–105], tRNAs [106–108] and snRNAs [109]. Another writer displaying minimal substrate specificity is Apolipoprotein B mRNA-editing enzyme 1 (APOBEC-1), which mediates the C-to-U deamination of only one position in apolipoprotein B (apoB) mRNA [110]. Expanded substrate specificity is represented highly promiscuous NSUN family members targeting various cytosolic and mitochondrial tRNAs, other small ncRNAs and hundreds of different mRNAs [111–117]. Similarly, various pseudo-uridine synthetases (PUS) modify miRNAs, tRNAs and mRNAs [118,119], and the METTL3/METTL14 complex addresses hundreds to thousands of different mRNAs [23,40,120], pri-miRNAs [121], long ncRNAs [122] as well as circular RNAs (circRNAs) [123]. The most extended multi-substrate RNA modification enzymes are the adenosine deaminases acting on RNA (ADARs) targeting hundreds to millions of adenosines [124]. Such substrate promiscuity complicates obtaining a more detailed understanding of a given epitranscriptomic system since it will remain unclear whether the modification of a particular position in only one RNA species or the combination of different positions in different RNAs is causative for an observed phenotype. Hence, an important but unresolved question in epitranscriptomics remains: how to experimentally address RNA modifications in specific RNAs without modulating the rest of the respective epitranscriptome?

2.5. Phenotypic Pleiotropy: Boon or Bust for Deciphering the Impact of Epitranscriptomes?

To gain first insights into the impact of specific epitranscriptomes on cellular processes, "early-stage" epitranscriptomics has mostly employed classic reverse genetics. Gene-specific knockout or overexpression constructs have been used to interfere with or enhance the function of various writers, readers or modifiers/erasers). Especially, one RNA modification, the addition of a methyl group onto adenosine resulting in m^6A, has become the "flagship" modification for most of current epitranscriptomics. m^6A writers, readers and modifiers/erasers have been genetically manipulated in different cells, tissues and organisms. The wide range of reported phenotypes indicated that modulating this particular RNA modification system is affecting literally every aspect in cell biology but raises also important conceptual questions. Just to make the point, here is a non-exhaustive list of processes affected when interfering with m^6A RNA modification systems: proliferation [125], mRNA splicing and adipogenesis [126–129], RNA stability [76,130,131], mRNA translation and decay [75,130,132,133], mitotic entry [134], DNA damage response [135], circadian rhythm [136], signaling pathways [137], oocyte maturation [138,139], maternal-zygotic transition [140], sex determination [141,142], spermatogenesis [143,144], reprogramming to pluripotency [145,146], stem cell renewal and differentiation [147–151], tumorigenicity and cancers [137,149,152–155], yet also anti-tumor immunity [156], neural development and differentiation [139,157], axon regeneration [158], neurotransmitter-related circuitry [159],

learning and memory [160], CNS myelination [161], neural development [157,162], miRNA processing [121] and viral infection [163–167]. The combination of these observations has mostly been interpreted as proof for the notion that m^{6}A in RNA is necessary and crucially required for cellular functions. However, the sum of the observed phenotypes points both at experimental and conceptual problems. Specifically, the sheer scope of affected biological processes indicates that classic knockdown or knockout approaches makes it virtually impossible to separate cause from consequence. Phenotypic pleiotropy, albeit more limited, has also been observed when modulating other epitranscriptomic systems as those responsible for A-to-I editing (reviewed in [168,169]), for m^{5}C (reviewed in [170]), for Ψ (reviewed in [171]) and for Nm (reviewed in [172,173]). In summary, phenotypic pleiotropy suggest that interfering with gene products with multi-substrate promiscuity and acting upstream of a complex system of effectors is not necessarily resulting in a better understanding of that particular system. How then should one interrogate the impact of an RNA modification without completely removing its writers, readers or modulators/erasers from a complex system?

3. Dynamic RNA Modifications Likely Require Context-Specific Regulation

The observed substrate promiscuity of writers and modulators/erasers, the dynamic nature as well as the sub-stoichiometric levels of some RNA modifications suggest that the components of RNA modification systems are subject to regulation. This article will ignore regulatory principles such as gene expression since expression changes cannot explain how different RNA species are modified at varying stoichiometries by the same enzyme within the same cell. Hence, the following paragraphs will focus on the post-transcriptional and post-translational regulation of epitranscriptomic activities through changes in their subcellular localization, interactions with proteins and/or RNAs, protein modifications and the availability of co-factors.

3.1. Subcellular Localization: Regulated or by Chance?

The enzymes establishing and modulating/erasing RNA modifications as well as their RNA substrates need to find each other within cells. While this is rather obvious, many text books still define the interior of a cell, including subcellular compartments, as spaces filled with molecules, which find each other stochastically and thereby by chance. How do epitranscriptomic systems that affect most RNAs sub-stoichiometrically come together? Most RNAs undergo either co- or post-transcriptional modification, close to the source of transcription, as part of elaborate processing and maturation steps (reviewed in [73,174]). Some RNAs, such as snRNAs and tRNAs, are not only modified in the nucleus, but become exported into the cytoplasm for additional modifications before being reimported into the nuclear compartment to undergo final processing (reviewed in [71,175,176]). These observations indicate a network of RNA modification enzymes residing in various cellular compartments. However, proteins do also dynamically change localization. Examples for intracellular trafficking of RNA modification activities are substrate-promiscuous enzymes. For instance, particular ADAR isoforms reside exclusively in the nucleus while others shuttle in and out of the nucleus or can be detected in stress- or infection-induced subcellular structures (reviewed in [177]). Another example is the complex localization pattern of NSUN2 (a promiscuous cytosine-5 RNA methyltransferase), which is mostly nucleolar but is also imported into the mitochondrial matrix [116,117]. In addition, NSUN2 re-localizes to different subcellular regions depending on the cell cycle stage and on environmental stress exposure [111,116,117,178,179]. Furthermore, individual members of the pseudo-uridine synthetase (PUS) protein family display diverse localization patterns (reviewed in [180]). And, last but not least, context-dependent and complex subcellular localization has also been revealed for writers and modulators/erasers of m^{6}A (reviewed in [34]). Currently, defining the subcellular localization of the various components of epitranscriptomic circuitry is largely focused on proteins rather than RNAs. However, there is ample evidence for the regulated and dynamic localization of individual RNAs within cells (reviewed

in [181]). The potential influence of RNA localization on (sub-stoichiometric) RNA modification levels is an exciting but still unexplored possibility. Hence, future attempts that aim at understanding epitranscriptomic systems might consider not only addressing the localization of writers, modulators and erasers, but also determine the localization of specific substrate RNAs. In doing so, one should preferably be implementing single molecule imaging techniques, which can distinguish single nucleotide changes/modifications such as fluorescent in-situ hybridization (FISH) techniques involving hybridization chain reaction (HCR) [182–184], or click-encoded rolling FISH (ClickerFISH, [185]), which could be combined with in vivo RNA localization approaches (reviewed in [186]). Combining in vivo localization of specific RNAs with the systematic analysis of spatially restricted proteomes in a particular cell type and context (reviewed in [187]) will allow determining the subcellular details of "where and when" epitranscriptomic systems act.

3.2. Guiding Epitranscriptomic Activities: Context-Dependent Protein-Protein Interactions

Mutating the most upstream components of various RNA modification systems (writers or modifiers/erasers) has resulted in pleiotropic phenotypes, which are hard to interpret mechanistically. In order to modulate epitranscriptomic signatures in a more targeted fashion, determining the downstream interactions of writers and modifiers/erasers would allow defining points of interference that would not necessarily result in removing an entire epitranscriptome. Besides protein-protein interactions that determine the localization of epitranscriptomic activities (and thereby their substrate choice), specific interactions could also directly inhibit or enhance of their catalytic function. Some efforts have already been made towards determining how the most abundant RNA modifications such as m^6A, Ψ, or A-to-I could be regulated through interacting proteins. While various writers were able to modify RNAs under minimal in vitro assay conditions, not unexpectedly, those proteins acted in multiprotein complexes in vivo. For instance, the core components of the m^6A system for modifying mRNAs (METTL3 and METTL14) form a heterodimeric writer complex, which methylate RNAs in vitro. In this complex, METTL3 is the catalytically active subunit while METTL14, unable to bind the essential co-factor S-adenosyl-methionine (SAM), plays a structural role that is critical for substrate recognition [188]. This suggests that context-dependent interactions of METTL14 could greatly affect the activity of METTL3. Importantly, interactions with the splicing factor WTAP and (so far) five more proteins (VIRMA, RBM15/RBM15B, ZC3H13 and HAKAI) are required for localization to nuclear speckles and m^6A deposition on mRNA in vivo (reviewed in [189]). It was proposed that these accessory proteins are directing methylation specificity towards coding and non-coding RNAs through interaction with particular RNA-binding proteins [122,190]. Similar conclusions can be drawn for the A-to-I editing system, in which the function of a catalytically inactive ADAR family member (ADAR3) [191] is thought to be regulating other ADARs, for instance by binding to and blocking substrate RNAs [83]. Similarly, ADARs (A-to-I editing) and PUS (Ψ) can act as stand-alone enzymatic activities in vitro, but interact with a plethora of proteins in vivo (reviewed in [171,180,192]). While these findings are not surprising, the challenge now lies in how to disentangle this complexity in order to better understand the effects of individual protein interactors on the activity of particular writers and modifiers/erasers in vivo. In order to do so, more systematic approaches are needed, preferably by in vivo mapping using advanced proximity biotinylation and ligation techniques (reviewed in [187,193]), and in combination with cross-linking mass spectrometry, which allows delineating the interaction surfaces of interacting proteins but also reveals structural information (reviewed in [194]).

3.3. Non-Coding RNAs: Guides and Modulators of Epitranscriptomic Activities

RNA-guided processing or degradation of DNAs and RNAs is evolutionary conserved. For instance, bacteria and archaea produce RNAs from genomically encoded "clustered regularly interspaced short palindromic repeats" (CRISPR) [195,196], which served as guide RNAs (gRNAs) for CRISPR-associated (Cas) proteins. Cas proteins act

as endonucleases, which upon being guided to complementary DNA or RNA sequences, degrade such sequences (reviewed in [197–199]). Particular Cas proteins can also target and degrade RNAs (reviewed in [200]). Similar to CRISPR-Cas systems, eukaryotic RNAi pathways require small RNAs (microRNAs, miRNAs; small interfering RNAs, siRNAs; piwi-associated RNAs, piRNAs) to guide particular proteins (Argonautes) towards their RNA targets resulting in sequence-specific processing or degradation of complementary nucleic acids (reviewed in [201]).

Importantly, enzymes writing or modulating/erasing RNA modifications can interact with non-substrate RNAs resulting not only in guiding but potentially also in the modulation of their activities. A prominent example for how RNA modification enzymes are guided by RNAs was discovered in *Trypanosoma brucei* [9]. In trypanosomes, many mitochondrial RNAs undergo post-transcriptional uridine insertions and deletions as a prerequisite for the production of functional messengers. The information for these RNA editing processes is provided by trans-acting small RNAs guiding a multiprotein complex to particular positions in the mitochondrial transcriptome (reviewed in [202]). Later, it was found that gRNA-mediated RNA modifications are not restricted to protozoa nor to the process of RNA editing. A common theme for the underlying mechanisms is the assembly of multiprotein complexes that are guided by RNAs to base-pair with target RNAs and thereby direct modification of specific ribonucleotides. For instance, hundreds of gRNAs have been identified that target activities introducing Nm and Ψ into various RNAs and different species. These small gRNAs have been named small nucleolar RNAs (snoRNAs) and classified into box C/D RNAs, directing Nm and box H/ACA RNAs that target activities to introduce Ψ. A representative example is U6 snRNA, which acquires eight Nm and three Ψ for full functionality [203,204]. The introduction of such gRNA-mediated modifications are highly conserved in respect to sequence context and their existence affect the processing and function of ribosomal RNAs as well as spliceosomal snRNAs (reviewed in [205]). Importantly, small RNAs can not only guide but could also affect the activity of RNA modification enzymes on their substrate RNAs. While there is not much evidence for this scenario yet, one example is snoRNA (SNORD115), which targets Nm to an ADAR2-mediated pre-mRNA editing site, thereby specifically interfering with ADAR2 activity [206]. Furthermore, ADAR1 interacts through one of its three dsRNA binding domain with the nuclear import receptor (Transportin 1), which is mutually exclusive with binding to substrate dsRNAs [207] suggesting that the availability of specific dsRNAs can determine the localization and thereby the substrate choice of this writer. In this respect, it is also noteworthy that not all RNAs that are targeted by various writers and modifiers/erasers need to be substrate RNAs with a biological function. A case in point is the sub-stoichiometric activity of the (cytosine-5) methyltransferases NSUN2 and NSUN6 in mRNAs resembling the sequence contexts and structures of their respective tRNA substrates [114,208]. This observation raises the question as to whether these sites represent consequential mRNA modifications or are (only) off-targets, which could affect the activity of these enzymes on tRNAs. While these selected examples highlight the potential for RNAs to affect the localization, protein interactions and substrate specificity of RNA modification enzymes, the challenge lies now in complement the mapping of direct targets for a particular epitranscriptomic systems with additional RNA interactions that could have regulatory function. Approaches such as artificial intelligence-based predictions trained by chemical context profiling [209], proximity labelling in subcellular compartments combined with protein-RNA crosslinking [210–214] together with monitoring the activity of writers/modifiers/erasers on specific RNAs will likely uncover which RNAs affect the activity of RNA modification circuitry in a specific subcellular compartment and biological context.

3.4. Post-Translational Modifications of Epitranscriptomic Activities

Even though post-translational modifications (PTMs) occur in most proteins including RNA modification enzymes, specific PTMs might be an inroad into experimentally

addressing the specificity and dynamic nature of epitranscriptomic activities. To date, a role of PTMs for the turnover, localization and catalytic activity of selected writers and modifiers/erasers has been reported. For instance, ADAR1 isoforms are subjected to context-dependent PTMs such ubiquitination resulting in proteasomal degradation [215], or phosphorylation facilitating exportin 5-mediated transport into the cytoplasm [216]. In addition, nuclear import of ADAR2 requires phosphorylation, which, if disturbed, results in poly-ubiquitination by E3 ligase activities and proteasomal degradation [217]. Furthermore, fat mass and obesity-associated protein (FTO), an eraser of m^{6}A, can become SUMOylated at a single lysine residue, which promotes FTO degradation thereby affecting the balance between adenosine methylation and demethylation [218]. Also, direct effects on RNA modification activities have been observed. For instance, SUMOylation of ADAR1 can reduce its editing activity without causing degradation or altering the subcellular localization [219]. Furthermore, the catalytic subunit of the m^{6}A methyltransferase complex (METTL3) can be modified by small ubiquitin-like modifier 1 (SUMO1) at various lysine residues both in vitro and in vivo [220]. These PTMs did neither affect METTL3 stability, localization nor the interaction with METTL14/WTAP but inhibited m^{6}A deposition on mRNAs. However, the impact of many existing PTMs remains unclear. For instance, mutating individual phosphorylation sites in METTL3 or METTL14 did not affect heterodimer formation or the catalytic activity of METTL3 on model substrates [221] suggesting context-specific effects that cannot be observed in vitro. Hence, more systematic approaches need to be implemented to obtain a better understanding of the impact of PTMs on individual epitranscriptomes in vivo. This could involve combinatorial studies such as global profiling of PTMs in whole and, importantly, context-specific proteomes (reviewed in [222]). Once, context-specific PTMs on writers and modifiers/erasers are known, the generation of site-specific substitutions and structural mimics (reviewed in [223]), by using genome-editing tools, will facilitate functional in vivo studies.

3.5. Co-factor Requirements and Context-Dependent Metabolic Interactions

Most known RNA modifications involve enzymatic reactions attaching specific chemical moieties to nucleic acids [95]. Importantly, these reactions require the availability of co-factors or co-substrates, many of which are provided by micronutrients (i.e., vitamins and minerals) either through dietary intake or specific microbial activities (reviewed in [224]). It follows that the availability of such co-factors and co-substrates will affect many RNA modifications. Since about 70 % of all known RNA modifications contain one or more methyl groups [93,94], methyl donors such as S-adenosylmethionine (SAM), are of utmost importance for efficient RNA methylation (reviewed in [225]). Indeed, it has been shown that limiting micronutrient levels, including SAM depletion, have major impact on cell growth [226–228]. In addition, SAM and its demethylation product, S-adenosyl-homocysteine (SAH), are integral molecules in the folate and methionine cycles. Products of this so-called one-carbon metabolism are crucially important for basic processes such as the biosynthesis of phospholipids, polyamines and nucleotides, amino acid homeostasis, the redox defense system, and, importantly, for nucleic acid and protein methylation (reviewed in [229]). Because of its central role in cellular functions, the one-carbon metabolism is tightly regulated. This includes feed-back and feed-forward mechanisms that respond to changes and imbalances in nutrition, stress exposure or aging [230]. Interestingly, the synthesis of SAM appears to be under the control of epitranscriptomic mechanisms. For instance, SAM depletion resulted in reduced m^{6}A in the 3' UTR of MAT2A, encoding a ubiquitous mammalian SAM synthetase, and its concomitant upregulation through splicing-dependent mRNA stabilization [231,232]. Furthermore, manipulation of the cytosine-5 RNA methyltransferase NSUN2 resulted in changes in the methionine and tricarboxylic acid (TCA) cycles as well as synthetic pathways for amino acids [233]. Specifically, higher levels of methionine and SAM were observed in *NSUN2* mutant cells indicating changes in the output of the methionine cycle [233]. In addition, RNAs can interact with various metabolic enzymes (reviewed in [234]). The reason for

these interactions are not completely understood but it has been proposed that RNAs (and their modification status) could act as sensors for cellular changes that require metabolic adjustments (reviewed in [92,235]). These observations underscore the intriguing complexity involving RNAs, epitranscriptomic activities and metabolic pathways (reviewed in [236]). Some even might explain the phenotypic pleiotropy when manipulating epitranscriptomic systems since metabolite-mediated (secondary) effects including epigenome and gene expression changes have been reported [237]. To start disentangling the (direct or indirect) interplay of RNA modification systems with metabolic pathways, more sophisticated experimentation than gene knockout studies followed by gene expression analyses will be required. This might include context-dependent manipulation of particular writers or modifiers/erasers, for instance by targeted protein degradation (reviewed in [238]), and the concurrent measurement of metabolic and gene expression patterns by combining single cell transcriptomics and metabolomics (reviewed in [239]).

4. The Next Frontier: Getting a Closer Look at the Details

The last 10 years have defined yet another frontier in molecular biology, the existence of epitranscriptomes. Early efforts in epitranscriptomics have been awarded with regular publicity, mostly for developing technologies that allow mapping individual epitranscriptomes at transcriptome-wide fashion. In addition, first insights into the impact of specific epitranscriptomic systems have produced a breath-taking picture of their immense complexity. However, both the accumulation of epitranscriptomic mapping data as well as the multitude of phenotypes resulting from malfunctioning RNA modification systems, have caused another kind of scientific competition. Rather than paving the way for more detailed and in-depth studies of specific (and known) RNA modification signatures, the amount of data and its complexity appears to deter from being used when formulating and testing new hypotheses. This is at odds with the immense amount of compiled mapping data and some of the precision tools now available that would allow "digging into the data". Rather than doing that, a large fraction of the field focuses on only one particular RNA modification system, the m^6A system modulating mRNAs, and reports with regularity on its involvement in anything that resonates with the notion that biology does not work without m^6A-modified mRNAs. Another fraction is in "discovery mode" continuously "hunting" for "novel" RNA modifications by mass spectrometry or sequencing-based approaches. In contrast, a minority fraction in the field is conducting experiments aimed at investigating the impact of particular RNA modifications in specific RNAs. Some examples for the latter are studies on the impact of A-to-I editing at specific sites in specific RNAs in particular human disease models [240–242], the analysis of how specific RNA modifications affect innate immune responses (reviewed in [243]), or the impact of chemical modifications on RNA structures [244–247]. These approaches are facilitated by the recent development of in vivo methodology allowing site-specific introduction of RNA modifications [248–253] or their removal [251,254–256], by the use of nucleoside analogues (reviewed in [257]) and through variations of single molecule imaging techniques allowing to query positional information on modified RNAs [258]. A guiding example as to how continuous investment in deciphering the molecular details of the impact of particular RNA modifications can result in amazing knowledge leaps is the recent breakthrough for RNA-based therapeutics including the development and approval of mRNA vaccines, which could not have happened without focusing on the impact of particular modifications on RNA stability and interactions with the innate immune system [259–266].

Is the use of modified mRNAs in vaccine development all of what RNA modifications can teach us about biology, the ingenuity of human adaptability, technological progress and disease? Likely not. However, in order to move epitranscriptomics from an emerging and experimentally tractable phenomenon to one that can be better understood, the experimental focus needs to divert from counting numbers to addressing the mechanistic details of the modification reactions preferably with atomic scale resolution. To do so, better definitions of the biological, developmental and environmental context in which

particular epitranscriptomic systems modulate RNA identity need to be incorporated into experimental designs. Furthermore, the context-dependent regulation of specific RNA modification activities needs to be systematically addressed, preferably by utilizing the great variety of established vertebrate and non-vertebrate model organisms, which offer many advantages over mammalian (cancer) cells constantly (evolving) in culture. Most importantly, structural knowledge will be required as the very prerequisite for an in-depth understanding of any RNA modification system. While some studies have addressed the structural basis for the activity of particular RNA modification enzymes, largely focusing on m^6A and Ψ circuitry, more efforts will have to be made to determine the structures of other RNA modification enzymes, preferably in combination with their respective RNA substrates. This will result in arriving at a more solid understanding of the structure-function relationships between RNAs and enzymes that determine the complexities of individual epitranscriptomes.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Cohn, W.E. Pseudouridine, a carbon-carbon linked ribonucleoside in ribonucleic acids: Isolation, structure, and chemical characteristics. *J. Biol. Chem.* **1960**, *235*, 1488–1498. [CrossRef]
2. Rottman, F.; Shatkin, A.J.; Perry, R.P. Sequences containing methylated nucleotides at the 5′ termini of messenger RNAs: Possible implications for processing. *Cell* **1974**, *3*, 197–199. [CrossRef]
3. Davis, F.F.; Allen, F.W. Ribonucleic acids from yeast which contain a fifth nucleotide. *J. Biol. Chem.* **1957**, *227*, 907–915. [CrossRef]
4. Ozban, N.; Tandler, J.; Sirlin, J.L. Methylation of nucleolar RNA during development of the amphibian ooecyte. *J. Embryol. Exp. Morphol.* **1964**, *12*, 373–380.
5. Desrosiers, R.; Fridrich, K.; Rottmann, F. Identification of methylated nucleosides in messenger RNA from novikoff hepatoma cells. *Proc. Natl. Acad. Sci. USA* **1974**, *71*, 3971. [CrossRef]
6. Perry, R.P.; Kelley, D.E.; LaTorre, J. Synthesis and turnover of nuclear and cytoplasmic polyadenylic acid in mouse L cells. *J. Mol. Biol.* **1974**, *82*, 315–331. [CrossRef]
7. Perry, R.P.; Kelley, D.E.; Friderici, K.; Rottman, F. The methylated constituents of L cell messenger RNA: Evidence for an unusual cluster at the 5′ terminus. *Cell* **1975**, *4*, 387–394. [CrossRef]
8. Muthukrishnan, S.; Morgan, M.; Banerjee, A.K.; Shatkin, A.J. Influence of 5″-terminal m7G and 2′-O-methylated residues on messenger ribonucleic acid binding to ribosomes. *Biochemistry* **1976**, *15*, 5761–5768. [CrossRef]
9. Benne, R.; Van den Burg, J.; Brakenhoff, J.P.; Sloof, P.; Van Boom, J.H.; Tromp, M.C. Major transcript of the frameshifted coxII gene from trypanosome mitochondria contains four nucleotides that are not encoded in the DNA. *Cell* **1986**, *46*, 819–826. [CrossRef]
10. Driscoll, D.M.; Wynne, J.K.; Wallis, S.C.; Scott, J. An in vitro system for the editing of apolipoprotein B mRNA. *Cell* **1989**, *58*, 519–525. [CrossRef]
11. Köhler, M.; Burnashev, N.; Sakmann, B.; Seeburg, P.H. Determinants of Ca^{2+} permeability in both TM1 and TM2 of high affinity kainate receptor channels: Diversity by RNA editing. *Neuron* **1993**, *10*, 491–500. [CrossRef]
12. Johnson, D.F.; Poksay, K.S.; Innerarity, T.L. The mechanism for apo-B mRNA editing is deamination. *Biochem. Biophys. Res. Commun.* **1993**, *195*, 1204–1210. [CrossRef]
13. Yang, J.-H.; Sklar, P.; Axel, R.; Maniatis, T. Editing of glutamate receptor subunit B pre-mRNA in vitro by site-specific deamination of adenosine. *Nature* **1995**, *374*, 77–81. [CrossRef] [PubMed]
14. Rueter, S.M.; Burns, C.M.; Coode, S.A.; Mookherjee, P.; Emeson, R.B. Glutamate receptor RNA Editing in vitro by enzymatic conversion of adenosine to inosine. *Science* **1995**, *267*, 1491–1494. [CrossRef] [PubMed]
15. Melcher, T.; Maas, S.; Herb, A.; Sprengel, R.; Higuchi, M.; Seeburg, P.H. RED2, a brain-specific member of the RNA-specific adenosine deaminase family. *J. Biol. Chem.* **1996**, *271*, 31795–31798. [CrossRef] [PubMed]
16. Fujino, T.; Navaratnam, N.; Scott, J. Human apolipoprotein B RNA editing deaminase gene (APOBEC1). *Genomics* **1998**, *47*, 266–275. [CrossRef] [PubMed]

17. Levanon, E.Y.; Eisenberg, E.; Yelin, R.; Nemzer, S.; Hallegger, M.; Shemesh, R.; Fligelman, Z.Y.; Shoshan, A.; Pollock, S.R.; Sztybel, D.; et al. Systematic identification of abundant A-to-I editing sites in the human transcriptome. *Nat. Biotechnol.* **2004**, *22*, 1001–1005. [CrossRef]

18. Picardi, E.; Horner, D.S.; Chiara, M.; Schiavon, R.; Valle, G.; Pesole, G. Large-scale detection and analysis of RNA editing in grape mtDNA by RNA deep-sequencing. *Nucleic. Acids Res.* **2010**, *38*, 4755–4767. [CrossRef]

19. Lerner, T.; Papavasiliou, F.; Pecori, R. RNA editors, cofactors, and mRNA targets: An overview of the C-to-U RNA editing machinery and its implication in human disease. *Genes* **2019**, *10*, 13. [CrossRef]

20. He, C. Grand challenge commentary: RNA epigenetics? *Nat. Chem. Biol.* **2010**, *6*, 863–865. [CrossRef] [PubMed]

21. Yi, C.; Yang, C.-G.; He, C. A non-heme iron-mediated chemical demethylation in DNA and RNA. *Acc. Chem. Res.* **2009**, *42*, 519–529. [CrossRef] [PubMed]

22. Jia, G.; Fu, Y.; Zhao, X.; Dai, Q.; Zheng, G.; Yang, Y.; Yi, C.; Lindahl, T.; Pan, T.; Yang, Y.-G.; et al. N6-methyladenosine in nuclear RNA is a major substrate of the obesity-associated FTO. *Nat. Chem. Biol.* **2011**, *7*, 885–887. [CrossRef]

23. Meyer, K.D.; Saletore, Y.; Zumbo, P.; Elemento, O.; Mason, C.E.; Jaffrey, S.R. Comprehensive analysis of mRNA methylation reveals enrichment in 3' UTRs and near stop codons. *Cell* **2012**, *149*, 1635–1646. [CrossRef] [PubMed]

24. Saletore, Y.; Meyer, K.; Korlach, J.; Vilfan, I.D.; Jaffrey, S.; Mason, C.E. The birth of the Epitranscriptome: Deciphering the function of RNA modifications. *Genome Biol.* **2012**, *13*, 175. [CrossRef]

25. Wiener, D.; Schwartz, S. The epitranscriptome beyond m6A. *Nat. Rev. Genet.* **2021**, *22*, 119–131. [CrossRef]

26. Helm, M.; Motorin, Y. Detecting RNA modifications in the epitranscriptome: Predict and validate. *Nat. Rev. Genet.* **2017**, *23*, 234. [CrossRef] [PubMed]

27. Li, X.; Xiong, X.; Yi, C. Epitranscriptome sequencing technologies: Decoding RNA modifications. *Nat. Methods* **2016**, *14*, 23–31. [CrossRef]

28. Limbach, P.A.; Paulines, M.J. Going global: The new era of mapping modifications in RNA. *Wiley Interdiscip Rev. RNA* **2017**, *8*. [CrossRef]

29. Hwang, T.; Park, C.-K.; Leung, A.K.L.; Gao, Y.; Hyde, T.M.; Kleinman, J.E.; Rajpurohit, A.; Tao, R.; Shin, J.H.; Weinberger, D.R. Dynamic regulation of RNA editing in human brain development and disease. *Nat. Neurosci.* **2016**, *19*, 1093–1099. [CrossRef]

30. Maas, S.; Kawahara, Y.; Tamburro, K.M.; Nishikura, K. A-to-I RNA editing and human disease. *RNA Biol.* **2006**, *3*, 1–9. [CrossRef]

31. Dimitrova, D.G.; Teysset, L.; Carré, C. RNA 2′-O-Methylation (Nm) modification in human diseases. *Genes* **2019**, *10*, 117. [CrossRef] [PubMed]

32. Torres, A.G.; Batlle, E.; de Pouplana, L.R. Role of tRNA modifications in human diseases. *Trends Mol. Med.* **2014**, *20*, 306–314. [CrossRef] [PubMed]

33. Barbieri, I.; Kouzarides, T. Role of RNA modifications in cancer. *Nat. Rev. Cancer* **2020**, *169*, 1–20. [CrossRef]

34. Shi, H.; Wei, J.; He, C. Where, when, and how: Context-dependent functions of RNA methylation writers, readers, and erasers. *Mol. Cell* **2019**, *74*, 640–650. [CrossRef]

35. Wetzel, C.; Limbach, P.A. Mass spectrometry of modified RNAs: Recent developments. *Analyst* **2016**, *141*, 16–23. [CrossRef] [PubMed]

36. Linder, B.; Jaffrey, S.R. Discovering and mapping the modified nucleotides that comprise the epitranscriptome of MRNA. *Cold Spring Harb. Perspect. Biol.* **2019**, *11*, a032201. [CrossRef]

37. Schwartz, S. Cracking the epitranscriptome. *RNA* **2016**, *22*, 169–174. [CrossRef]

38. Zhao, B.S.; Roundtree, I.A.; He, C. Post-transcriptional gene regulation by mRNA modifications. *Nat. Rev. Mol. Cell Biol.* **2017**, *18*, 31–42. [CrossRef]

39. Zaccara, S.; Ries, R.J.; Jaffrey, S.R. Reading, writing and erasing mRNA methylation. *Nat. Rev. Mol. Cell Biol.* **2019**, *20*, 608–624. [CrossRef]

40. McIntyre, A.B.R.; Gokhale, N.S.; Cerchietti, L.; Jaffrey, S.R.; Horner, S.M.; Mason, C.E. Limits in the detection of m6A changes using MeRIP/m6A-seq. *Sci. Rep.* **2020**, *10*, 1–15. [CrossRef]

41. Ke, S.; Pandya-Jones, A.; Saito, Y.; Fak, J.J.; Vågbø, C.B.; Geula, S.; Hanna, J.H.; Black, D.L.; Darnell, J.E.; Darnell, R.B. m6A mRNA modifications are deposited in nascent pre-mRNA and are not required for splicing but do specify cytoplasmic turnover. *Genes Dev.* **2017**, *31*, 990–1006. [CrossRef] [PubMed]

42. Darnell, R.B.; Ke, S.; Darnell, J.E. Pre-mRNA processing includes N6methylation of adenosine residues that are retained in mRNA exons and the fallacy of "RNA epigenetics". *RNA* **2018**, *24*, 262–267. [CrossRef]

43. Zhao, B.S.; Nachtergaele, S.; Roundtree, I.A.; He, C. Our views of dynamic N6-methyladenosine RNA methylation. *RNA* **2018**, *24*, 268–272. [CrossRef] [PubMed]

44. Schwartz, S. m1A within cytoplasmic mRNAs at single nucleotide resolution: A reconciled transcriptome-wide map. *RNA* **2018**, *24*, 1427–1436. [CrossRef]

45. Smemo, S.; Tena, J.J.; Kim, K.-H.; Gamazon, E.R.; Sakabe, N.J.; Gómez-Marín, C.; Aneas, I.; Credidio, F.L.; Sobreira, D.R.; Wasserman, N.F.; et al. Obesity-associated variants within FTO form long-range functional connections with IRX3. *Nature* **2014**, *507*, 371–375. [CrossRef]

46. Eisenstein, M. Epitranscriptomics: Mixed messages. *Nat. Methods* **2017**, *14*, 15–17. [CrossRef]

47. Grozhik, A.V.; Olarerin-George, A.O.; Sindelar, M.; Li, X.; Gross, S.S.; Jaffrey, S.R. Antibody cross-reactivity accounts for widespread appearance of m1A in 5′UTRs. *Nat. Commun.* **2019**, *10*, 5126. [CrossRef] [PubMed]

48. Helm, M.; Lyko, F.; Motorin, Y. Limited antibody specificity compromises epitranscriptomic analyses. *Nat. Commun.* **2019**, *10*, 1428. [CrossRef] [PubMed]

49. Picardi, E.; D'Erchia, A.M.; Giudice, C.; Pesole, G. REDIportal: A comprehensive database of A-to-I RNA editing events in humans. *Nucleic Acids Res.* **2017**, *45*, D750–D757. [CrossRef]

50. Giudice, C.; Pesole, G.; Picardi, E. REDIdb 3.0: A comprehensive collection of RNA editing events in plant organellar genomes. *Front. Plant Sci.* **2018**, *9*, 482. [CrossRef]

51. Li, M.; Xia, L.; Zhang, Y.; Niu, G.; Li, M.; Wang, P.; Zhang, Y.; Sang, J.; Zou, D.; Hu, S.; et al. Plant editosome database: A curated database of RNA editosome in plants. *Nucleic Acids Res.* **2019**, *47*, D170–D174. [CrossRef]

52. Kiran, A.M.; O'Mahony, J.J.; Sanjeev, K.; Baranov, P.V. Darned in 2013: Inclusion of model organisms and linking with Wikipedia. *Nucleic Acids Res.* **2013**, *41*, D258–D261. [CrossRef] [PubMed]

53. Sun, P.P.; Chen, Y.B.; Liu, B.; Gao, Y.X.; Han, Y.; He, F.; Ji, J.C. DeepMRMP: A new predictor for multiple types of RNA modification sites using deep learning. *Math. Biosci. Eng.* **2019**, *16*, 6231–6241. [CrossRef]

54. Chen, W.; Tang, H.; Ye, J.; Lin, H.; Chou, K.-C. iRNA-PseU: Identifying RNA pseudouridine sites. *Mol. Ther. Nucleic Acids* **2016**, *5*, e332.

55. Jiang, J.; Song, B.; Tang, Y.; Chen, K.; Wei, Z.; Meng, J. m5UPred: A Web Server for the Prediction of RNA 5-methyluridine sites from sequences. *Mol. Ther. Nucleic Acids* **2020**, *22*, 742–747. [CrossRef] [PubMed]

56. Ahmed, S.; Hossain, Z.; Uddin, M.; Taherzadeh, G.; Sharma, A.; Shatabda, S.; Dehzangi, A. Accurate prediction of RNA 5-hydroxymethylcytosine modification by utilizing novel position-specific gapped k-mer descriptors. *Comput. Struct. Biotechnol. J.* **2020**, *18*, 3528–3538. [CrossRef]

57. Yang, Y.-H.; Ma, C.; Wang, J.-S.; Yang, H.; Ding, H.; Han, S.-G.; Li, Y.-W. Prediction of N7-methylguanosine sites in human RNA based on optimal sequence features. *Genomics* **2020**, *112*, 4342–4347. [CrossRef] [PubMed]

58. Tang, Y.; Chen, K.; Song, B.; Ma, J.; Wu, X.; Xu, Q.; Wei, Z.; Su, J.; Liu, G.; Rong, R.; et al. m6A-Atlas: A comprehensive knowledgebase for unraveling the N6-methyladenosine (m6A) epitranscriptome. *Nucleic Acids Res.* **2021**, *49*, D134–D143. [CrossRef] [PubMed]

59. Li, J.; Huang, Y.; Zhou, Y. A Mini-review of the computational methods used in identifying RNA 5-methylcytosine sites. *Curr. Genom.* **2020**, *21*, 3–10. [CrossRef] [PubMed]

60. Salekin, S.; Mostavi, M.; Chiu, Y.-C.; Chen, Y.; Zhang, J.M.; Huang, Y. Predicting sites of epitranscriptome modifications using unsupervised representation learning based on generative adversarial networks. *Front. Phys.* **2020**, *8*, 196. [CrossRef] [PubMed]

61. Chen, Z.; Zhao, P.; Li, F.; Wang, Y.; Smith, A.I.; Webb, G.I.; Akutsu, T.; Baggag, A.; Bensmail, H.; Song, J. Comprehensive review and assessment of computational methods for predicting RNA post-transcriptional modification sites from RNA sequences. *Brief Bioinform.* **2020**, *21*, 1676–1696. [CrossRef] [PubMed]

62. Paulines, M.J.; Limbach, P.A. Stable isotope labeling for improved comparative analysis of RNA digests by mass spectrometry. *J. Am. Soc. Mass Spectrom.* **2017**, *28*, 551–561. [CrossRef] [PubMed]

63. Kellner, S.; Ochel, A.; Thüring, K.; Spenkuch, F.; Neumann, J.; Sharma, S.; Entian, K.-D.; Schneider, D.; Helm, M. Absolute and relative quantification of RNA modifications via biosynthetic isotopomers. *Nucleic Acids Res.* **2014**, *42*, e142. [CrossRef]

64. Popova, A.M.; Williamson, J.R. Quantitative analysis of rRNA modifications using stable isotope labeling and mass spectrometry. *J. Am. Chem. Soc.* **2014**, *136*, 2058–2069. [CrossRef]

65. Glasner, H.; Riml, C.; Micura, R.; Breuker, K. Label-free, direct localization and relative quantitation of the RNA nucleobase methylations m6A, m5C, m3U, and m5U by top-down mass spectrometry. *Nucleic Acids Res.* **2017**, *45*, 8014–8025. [CrossRef] [PubMed]

66. Liu, N.; Parisien, M.; Dai, Q.; Zheng, G.; He, C.; Pan, T. Probing N6-methyladenosine RNA modification status at single nucleotide resolution in mRNA and long noncoding RNA. *RNA* **2013**, *19*, 1848–1856. [CrossRef]

67. Molinie, B.; Wang, J.; Lim, K.S.; Hillebrand, R.; Lu, Z.-X.; Van Wittenberghe, N.; Howard, B.D.; Daneshvar, K.; Mullen, A.C.; Dedon, P.; et al. m(6)A-LAIC-seq reveals the census and complexity of the m(6)A epitranscriptome. *Nat. Methods* **2016**, *13*, 692–698. [CrossRef] [PubMed]

68. Krogh, N.; Jansson, M.D.; Häfner, S.J.; Tehler, D.; Birkedal, U.; Christensen Dalsgaard, M.; Lund, A.H.; Nielsen, H. Profiling of 2′-O-Me in human rRNA reveals a subset of fractionally modified positions and provides evidence for ribosome heterogeneity. *Nucleic Acids Res.* **2016**, *44*, 7884–7895. [CrossRef]

69. Schaefer, M.; Pollex, T.; Hanna, K.; Lyko, F. RNA cytosine methylation analysis by bisulfite sequencing. *Nucleic Acids Res.* **2009**, *37*, e12. [CrossRef]

70. Kivioja, T.; Vähärautio, A.; Karlsson, K.; Bonke, M.; Enge, M.; Linnarsson, S.; Taipale, J. Counting absolute numbers of molecules using unique molecular identifiers. *Nat. Methods* **2011**, *9*, 72–74. [CrossRef]

71. Bohnsack, M.T.; Sloan, K.E. Modifications in small nuclear RNAs and their roles in spliceosome assembly and function. *Biol. Chem.* **2018**, *399*, 1265–1276. [CrossRef] [PubMed]

72. Maden, B.E.; Hughes, J.M. Eukaryotic ribosomal RNA: The recent excitement in the nucleotide modification problem. *Chromosoma* **1997**, *105*, 391–400. [CrossRef] [PubMed]

73. Sloan, K.E.; Warda, A.S.; Sharma, S.; Entian, K.-D.; Lafontaine, D.L.J.; Bohnsack, M.T. Tuning the ribosome: The influence of rRNA modification on eukaryotic ribosome biogenesis and function. *RNA Biol.* **2017**, *14*, 1138–1152. [CrossRef]

74. Phizicky, E.M.; Hopper, A.K. tRNA biology charges to the front. *Genes Dev.* **2010**, *24*, 1832–1860. [CrossRef] [PubMed]

75. Zhou, J.; Cao, G.; Li, H.-B.; Wan, J.; Gao, X.; Yin, Z.; Zhang, X.; Flavell, R.A.; Jaffrey, S.R.; Qian, S.-B. Dynamic m(6)A mRNA methylation directs translational control of heat shock response. *Open Biol.* **2015**, *526*, 591–594. [CrossRef]

76. Mauer, J.; Luo, X.; Blanjoie, A.; Jiao, X.; Grozhik, A.V.; Patil, D.P.; Linder, B.; Pickering, B.F.; Vasseur, J.-J.; Chen, Q.; et al. Reversible methylation of m6Am in the 5′ cap controls mRNA stability. *Nature* **2016**, *541*, 371–375. [CrossRef]

77. Chan, C.T.Y.; Dyavaiah, M.; DeMott, M.S.; Taghizadeh, K.; Dedon, P.C.; Begley, T.J. A quantitative systems approach reveals dynamic control of tRNA modifications during cellular stress. *PLoS Genet.* **2010**, *6*, e1001247. [CrossRef]

78. Dedon, P.C.; Begley, T.J. A system of RNA modifications and biased codon use controls cellular stress response at the level of translation. *Chem. Res. Toxicol.* **2014**, *27*, 330–337. [CrossRef] [PubMed]

79. Chan, C.T.Y.; Pang, Y.L.J.; Deng, W.; Babu, I.R.; Dyavaiah, M.; Begley, T.J.; Dedon, P.C. Reprogramming of tRNA modifications controls the oxidative stress response by codon-biased translation of proteins. *Nat. Commun.* **2012**, *3*, 937. [CrossRef] [PubMed]

80. Garrett, S.; Rosenthal, J.J.C. RNA editing underlies temperature adaptation in K+ channels from polar octopuses. *Science* **2012**, *335*, 848–851. [CrossRef]

81. Yu, X.; Willmann, M.R.; Vandivier, L.E.; Trefely, S.; Kramer, M.C.; Shapiro, J.; Guo, R.; Lyons, E.; Snyder, N.W.; Gregory, B.D. Messenger RNA 5′ NAD+ capping is a dynamic regulatory epitranscriptome mark that is required for proper response to abscisic acid in arabidopsis. *Dev. Cell* **2020**, *56*, 125–140. [CrossRef] [PubMed]

82. Riemondy, K.A.; Gillen, A.E.; White, E.A.; Bogren, L.K.; Hesselberth, J.R.; Martin, S.L. Dynamic temperature-sensitive A-to-I RNA editing in the brain of a heterothermic mammal during hibernation. *RNA* **2018**, *24*, 1481–1495. [CrossRef] [PubMed]

83. Tan, M.H.; Li, Q.; Shanmugam, R.; Piskol, R.; Kohler, J.; Young, A.N.; Liu, K.I.; Zhang, R.; Ramaswami, G.; Ariyoshi, K.; et al. Dynamic landscape and regulation of RNA editing in mammals. *Nature* **2017**, *550*, 249–254. [CrossRef]

84. Li, J.B.; Levanon, E.Y.; Yoon, J.-K.; Aach, J.; Xie, B.; Leproust, E.; Zhang, K.; Gao, Y.; Church, G.M. Genome-wide identification of human RNA editing sites by parallel DNA capturing and sequencing. *Science* **2009**, *324*, 1210–1213. [CrossRef] [PubMed]

85. Schaffer, A.A.; Levanon, E.Y. ALU A-to-I RNA Editing: Millions of sites and many open questions. *Methods Mol. Biol.* **2021**, *2181*, 149–162.

86. Buchumenski, I.; Bartok, O.; Ashwal-Fluss, R.; Pandey, V.; Porath, H.T.; Levanon, E.Y.; Kadener, S. Dynamic hyper-editing underlies temperature adaptation in Drosophila. *PLoS Genet.* **2017**, *13*, e1006931. [CrossRef]

87. Shah, J.C.; Clancy, M.J. IME4, a gene that mediates MAT and nutritional control of meiosis in Saccharomyces cerevisiae. *Mol. Cell Biol.* **1992**, *12*, 1078–1086. [CrossRef]

88. Clancy, M.J.; Shambaugh, M.E.; Timpte, C.S.; Bokar, J.A. Induction of sporulation in Saccharomyces cerevisiae leads to the formation of N6-methyladenosine in mRNA: A potential mechanism for the activity of the IME4 gene. *Nucleic Acids Res.* **2002**, *30*, 4509–4518. [CrossRef]

89. Fergus, C.; Barnes, D.; Alqasem, M.A.; Kelly, V.P. The queuine micronutrient: Charting a course from microbe to man. *Nutrients* **2015**, *7*, 2897–2929. [CrossRef]

90. Becker, M.; Müller, S.; Nellen, W.; Jurkowski, T.P.; Jeltsch, A.; Ehrenhofer-Murray, A.E. Pmt1, a Dnmt2 homolog in Schizosaccharomyces pombe, mediates tRNA methylation in response to nutrient signaling. *Nucleic Acids Res.* **2012**, *40*, 11648–11658. [CrossRef]

91. Tuorto, F.; Legrand, C.; Cirzi, C.; Federico, G.; Liebers, R.; Müller, M.; Ehrenhofer-Murray, A.E.; Dittmar, G.; Gröne, H.-J.; Lyko, F. Queuosine-modified tRNAs confer nutritional control of protein translation. *EMBO J.* **2018**, *37*, e99777. [CrossRef]

92. Grosjean, H. *DNA and RNA Modification Enzymes: Structure, Mechanism, Function and Evolution*; CRC Press: Boca Raton, FL, USA, 2009. [CrossRef]

93. Helm, M.; Alfonzo, J.D. Posttranscriptional RNA Modifications: Playing metabolic games in a cell's chemical Legoland. *Chem. Biol.* **2014**, *21*, 174–185. [CrossRef]

94. Motorin, Y.; Helm, M. RNA nucleotide methylation. *Wiley Interdiscip. Rev. RNA* **2011**, *2*, 611–631. [CrossRef]

95. Boccaletto, P.; Machnicka, M.A.; Purta, E.; Piatkowski, P.; Baginski, B.; Wirecki, T.K.; de Crécy-Lagard, V.; Ross, R.; Limbach, P.A.; Kotter, A.; et al. MODOMICS: A database of RNA modification pathways. 2017 update. *Nucleic Acids Res.* **2018**, *46*, D303–D307. [CrossRef] [PubMed]

96. Koonin, E.V.; Rudd, K.E. SpoU protein of Escherichia coli belongs to a new family of putative rRNA methylases. *Nucleic Acids Res.* **1993**, *21*, 5519. [CrossRef] [PubMed]

97. Gustafsson, C.; Reid, R.; Greene, P.J.; Santi, D.V. Identification of new RNA modifying enzymes by iterative genome search using known modifying enzymes as probes. *Nucleic Acids Res.* **1996**, *24*, 3756–3762. [CrossRef] [PubMed]

98. Anantharaman, V.; Koonin, E.V.; Aravind, L. Comparative genomics and evolution of proteins involved in RNA metabolism. *Nucleic Acids Res.* **2002**, *30*, 1427–1464. [CrossRef] [PubMed]

99. Reid, R.; Greene, P.J.; Santi, D.V. Exposition of a family of RNA m(5)C methyltransferases from searching genomic and proteomic sequences. *Nucleic Acids Res.* **1999**, *27*, 3138–3145. [CrossRef]

100. Heissenberger, C.; Rollins, J.A.; Krammer, T.L.; Nagelreiter, F.; Stocker, I.; Wacheul, L.; Shpylovyi, A.; Tav, K.; Snow, S.; Grillari, J.; et al. The ribosomal RNA m5C methyltransferase NSUN-1 modulates healthspan and oogenesis in Caenorhabditis elegans. *eLife Sci.* **2020**, *9*, 970.

101. Bourgeois, G.; Ney, M.; Gaspar, I.; Aigueperse, C.; Schaefer, M.; Kellner, S.; Helm, M.; Motorin, Y. Eukaryotic rRNA modification by yeast 5-methylcytosine-methyltransferases and human proliferation-associated antigen p120. *PLoS ONE* **2014**, *10*, e0133321. [CrossRef]

102. Metodiev, M.D.; Spåhr, H.; Polosa, P.L.; Meharg, C.; Becker, C.; Altmueller, J.; Habermann, B.; Larsson, N.-G.; Ruzzenente, B. NSUN4 is a dual function mitochondrial protein required for both methylation of 12S rRNA and coordination of mitoribosomal assembly. *PLoS Genet.* **2014**, *10*, e1004110. [CrossRef]

103. Sharma, S.; Yang, J.; Watzinger, P.; Kötter, P.; Entian, K.-D. Yeast Nop2 and Rcm1 methylate C2870 and C2278 of the 25S rRNA, respectively. *Nucleic Acids Res.* **2013**, *41*, 9062–9076. [CrossRef]

104. Heissenberger, C.; Liendl, L.; Nagelreiter, F.; Gonskikh, Y.; Yang, G.; Stelzer, E.M.; Krammer, T.L.; Micutkova, L.; Vogt, S.; Kreil, D.P.; et al. Loss of the ribosomal RNA methyltransferase NSUN5 impairs global protein synthesis and normal growth. *Nucleic Acids Res.* **2019**, *47*, 11807–11825. [CrossRef]

105. Janin, M.; Ortiz-Barahona, V.; de Moura, M.C.; Martínez-Cardús, A.; Llinàs-Arias, P.; Soler, M.; Nachmani, D.; Pelletier, J.; Schumann, U.; Calleja-Cervantes, M.E.; et al. Epigenetic loss of RNA-methyltransferase NSUN5 in glioma targets ribosomes to drive a stress adaptive translational program. *Acta Neuropathol.* **2019**, *138*, 1053–1074. [CrossRef]

106. Haag, S.; Sloan, K.E.; Ranjan, N.; Warda, A.S.; Kretschmer, J.; Blessing, C.; Hübner, B.; Seikowski, J.; Dennerlein, S.; Rehling, P.; et al. NSUN3 and ABH1 modify the wobble position of mt-tRNAMet to expand codon recognition in mitochondrial translation. *EMBO J.* **2016**, *35*, 2104–2119. [CrossRef] [PubMed]

107. Van Haute, L.; Dietmann, S.; Kremer, L.; Hussain, S.; Pearce, S.F.; Powell, C.A.; Rorbach, J.; Lantaff, R.; Blanco, S.; Sauer, S.; et al. Deficient methylation and formylation of mt-tRNA(Met) wobble cytosine in a patient carrying mutations in NSUN3. *Nat. Commun.* **2016**, *7*, 12039. [CrossRef] [PubMed]

108. Ignatova, V.V.; Kaiser, S.; Ho, J.S.Y.; Bing, X.; Stolz, P.; Tan, Y.X.; Lee, C.L.; Gay, F.P.H.; Lastres, P.R.; Gerlini, R.; et al. METTL6 is a tRNA m^{3}C methyltransferase that regulates pluripotency and tumor cell growth. *Sci. Adv.* **2020**, *6*, eaaz4551. [CrossRef] [PubMed]

109. Chen, H.; Gu, L.; Orellana, E.A.; Wang, Y.; Guo, J.; Liu, Q.; Wang, L.; Shen, Z.; Wu, H.; Gregory, R.I.; et al. METTL4 is an snRNA m6Am methyltransferase that regulates RNA splicing. *Cell Res.* **2020**, *30*, 544–547. [CrossRef] [PubMed]

110. Teng, B.; Burant, C.F.; Davidson, N.O. Molecular cloning of an apolipoprotein B messenger RNA editing protein. *Science* **1993**, *260*, 1816–1819. [CrossRef]

111. Blanco, S.; Dietmann, S.; Flores, J.V.; Hussain, S.; Kutter, C.; Humphreys, P.; Lukk, M.; Lombard, P.; Treps, L.; Popis, M.; et al. Aberrant methylation of tRNAs links cellular stress to neuro-developmental disorders. *EMBO J.* **2014**, *33*, 2020–2039. [CrossRef]

112. Hussain, S.; Sajini, A.A.; Blanco, S.; Dietmann, S.; Lombard, P.; Sugimoto, Y.; Paramor, M.; Gleeson, J.G.; Odom, D.T.; Ule, J.; et al. NSun2-mediated cytosine-5 methylation of vault noncoding RNA determines its processing into regulatory small RNAs. *Cell Rep.* **2013**, *4*, 255–261. [CrossRef]

113. Yang, X.; Yang, Y.; Sun, B.-F.; Chen, Y.-S.; Xu, J.-W.; Lai, W.-Y.; Li, A.; Wang, X.; Bhattarai, D.P.; Xiao, W.; et al. 5-methylcytosine promotes mRNA export-NSUN2 as the methyltransferase and ALYREF as an m(5)C reader. *Cell Res.* **2017**, *27*, 606–625. [CrossRef]

114. Selmi, T.; Hussain, S.; Dietmann, S.; Heiss, M.; Borland, K.; Flad, S.; Carter, J.-M.; Dennison, R.; Huang, Y.-L.; Kellner, S.; et al. Sequence- and structure-specific cytosine-5 mRNA methylation by NSUN6. *Nucleic Acids Res.* **2020**, *49*, 1006–1022. [CrossRef]

115. Haag, S.; Warda, A.S.; Kretschmer, J.; Günnigmann, M.A.; Höbartner, C.; Bohnsack, M.T. NSUN6 is a human RNA methyltransferase that catalyzes formation of m5C72 in specific tRNAs. *RNA* **2015**, *21*, 1532–1543. [CrossRef]

116. Van Haute, L.; Lee, S.-Y.; McCann, B.J.; Powell, C.A.; Bansal, D.; Vasiliauskaite, L.; Garone, C.; Shin, S.; Kim, J.-S.; Frye, M.; et al. NSUN2 introduces 5-methylcytosines in mammalian mitochondrial tRNAs. *Nucleic Acids Res.* **2019**, *47*, 8720–8733. [CrossRef]

117. Shinoda, S.; Kitagawa, S.; Nakagawa, S.; Wei, F.-Y.; Tomizawa, K.; Araki, K.; Araki, M.; Suzuki, T.; Suzuki, T. Mammalian NSUN2 introduces 5-methylcytidines into mitochondrial tRNAs. *Nucleic Acids Res.* **2019**, *47*, 8734–8745. [CrossRef] [PubMed]

118. Schwartz, S.; Bernstein, D.A.; Mumbach, M.R.; Jovanovic, M.; Herbst, R.H.; León-Ricardo, B.X.; Engreitz, J.M.; Guttman, M.; Satija, R.; Lander, E.S.; et al. Transcriptome-wide mapping reveals widespread dynamic-regulated pseudouridylation of ncRNA and mRNA. *Cell* **2014**, *159*, 148–162. [CrossRef]

119. Song, J.; Zhuang, Y.; Zhu, C.; Meng, H.; Lu, B.; Xie, B.; Peng, J.; Li, M.; Yi, C. Differential roles of human PUS10 in miRNA processing and tRNA pseudouridylation. *Nat. Chem. Biol.* **2020**, *16*, 160–169. [CrossRef]

120. 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. Topology of the human and mouse m6A RNA methylomes revealed by m6A-seq. *Nature* **2012**, *485*, 201–206. [CrossRef] [PubMed]

121. Alarcón, C.R.; Lee, H.; Goodarzi, H.; Halberg, N.; Tavazoie, S.F. N6-methyladenosine marks primary microRNAs for processing. *Nature* **2015**, *519*, 482–485. [CrossRef] [PubMed]

122. Patil, D.P.; Chen, C.-K.; Pickering, B.F.; Chow, A.; Jackson, C.; Guttman, M.; Jaffrey, S.R. m6A RNA methylation promotes XIST-mediated transcriptional repression. *Nature* **2016**, *537*, 369–373. [CrossRef]

123. Zhou, C.; Molinie, B.; Daneshvar, K.; Pondick, J.V.; Wang, J.; Van Wittenberghe, N.; Xing, Y.; Giallourakis, C.C.; Mullen, A.C. Genome-Wide Maps of m6A circRNAs identify widespread and cell-type-specific methylation patterns that are distinct from mRNAs. *Cell Rep.* **2017**, *20*, 2262–2276. [CrossRef] [PubMed]

124. Bazak, L.; Haviv, A.; Barak, M.; Jacob-Hirsch, J.; Deng, P.; Zhang, R.; Isaacs, F.J.; Rechavi, G.; Li, J.B.; Eisenberg, E.; et al. A-to-I RNA editing occurs at over a hundred million genomic sites, located in a majority of human genes. *Genome Res.* **2014**, *24*, 365–376. [CrossRef] [PubMed]

125. Boissel, S.; Reish, O.; Proulx, K.; Kawagoe-Takaki, H.; Sedgwick, B.; Yeo, G.S.H.; Meyre, D.; Golzio, C.; Molinari, F.; Kadhom, N.; et al. Loss-of-function mutation in the dioxygenase-encoding FTO gene causes severe growth retardation and multiple malformations. *Am. J. Hum. Genet.* **2009**, *85*, 106–111. [CrossRef] [PubMed]

126. Xiao, W.; Adhikari, S.; Dahal, U.; Chen, Y.-S.; Hao, Y.-J.; Sun, B.-F.; Sun, H.-Y.; Li, A.; Ping, X.-L.; Lai, W.-Y.; et al. Nuclear m(6)A Reader YTHDC1 Regulates mRNA Splicing. *Mol. Cell* **2016**, *61*, 507–519. [CrossRef]

127. Zhao, X.; Yang, Y.; Sun, B.-F.; Shi, Y.; Yang, X.; Xiao, W.; Hao, Y.-J.; Ping, X.-L.; Chen, Y.-S.; Wang, W.-J.; et al. FTO-dependent demethylation of N6-methyladenosine regulates mRNA splicing and is required for adipogenesis. *Cell Res.* **2014**, *24*, 1403–1419. [CrossRef] [PubMed]

128. Louloupi, A.; Ntini, E.; Conrad, T.; Ørom, U.A.V. Transient N-6-methyladenosine transcriptome sequencing reveals a regulatory role of m6A in splicing efficiency. *Cell Rep.* **2018**, *23*, 3429–3437. [CrossRef]

129. Zhou, K.I.; Shi, H.; Lyu, R.; Wylder, A.C.; Matuszek, Z.; Pan, J.N.; He, C.; Parisien, M.; Pan, T. Regulation of Co-transcriptional Pre-mRNA Splicing by m6A through the Low-Complexity Protein hnRNPG. *Mol. Cell* **2019**, *76*, 70–81. [CrossRef]

130. Du, H.; Zhao, Y.; He, J.; Zhang, Y.; Xi, H.; Liu, M.; Ma, J.; Wu, L. YTHDF2 destabilizes m(6)A-containing RNA through direct recruitment of the CCR4-NOT deadenylase complex. *Nat. Commun.* **2016**, *7*, 12626. [CrossRef]

131. Wang, X.; Lu, Z.; Gomez, A.; Hon, G.C.; Yue, Y.; Han, D.; Fu, Y.; Parisien, M.; Dai, Q.; Jia, G.; et al. N6-methyladenosine-dependent regulation of messenger RNA stability. *Nature* **2014**, *505*, 117–120. [CrossRef]

132. Choi, J.; Indrisiunaite, G.; DeMirci, H.; Ieong, K.-W.; Wang, J.; Petrov, A.; Prabhakar, A.; Rechavi, G.; Dominissini, D.; He, C.; et al. 2′-O-methylation in mRNA disrupts tRNA decoding during translation elongation. *Nat. Struct. Mol. Biol.* **2018**, *25*, 208–216. [CrossRef]

133. Wang, X.; Zhao, B.S.; Roundtree, I.A.; Lu, Z.; Han, D.; Ma, H.; Weng, X.; Chen, K.; Shi, H.; He, C. N(6)-methyladenosine modulates messenger RNA translation efficiency. *Cell* **2015**, *161*, 1388–1399. [CrossRef]

134. Fei, Q.; Zou, Z.; Roundtree, I.A.; Sun, H.-L.; He, C. YTHDF2 promotes mitotic entry and is regulated by cell cycle mediators. *PLoS Biol.* **2020**, *18*, e3000664. [CrossRef]

135. Xiang, Y.; Laurent, B.; Hsu, C.-H.; Nachtergaele, S.; Lu, Z.; Sheng, W.; Xu, C.; Chen, H.; Ouyang, J.; Wang, S.; et al. RNA m6A methylation regulates the ultraviolet-induced DNA damage response. *Nature* **2017**, *543*, 573–576. [CrossRef]

136. Fustin, J.-M.; Doi, M.; Yamaguchi, Y.; Hida, H.; Nishimura, S.; Yoshida, M.; Isagawa, T.; Morioka, M.S.; Kakeya, H.; Manabe, I.; et al. RNA-methylation-dependent RNA processing controls the speed of the circadian clock. *Cell* **2013**, *155*, 793–806. [CrossRef]

137. Liu, J.; Eckert, M.A.; Harada, B.T.; Liu, S.-M.; Lu, Z.; Yu, K.; Tienda, S.M.; Chryplewicz, A.; Zhu, A.C.; Yang, Y.; et al. m6A mRNA methylation regulates AKT activity to promote the proliferation and tumorigenicity of endometrial cancer. *Nat. Cell Biol.* **2018**, *20*, 1074–1083. [CrossRef]

138. Qi, S.T.; Ma, J.Y.; Wang, Z.B.; Guo, L.; Hou, Y.; Sun, Q.Y. N6-methyladenosine sequencing highlights the involvement of mRNA methylation in oocyte meiotic maturation and embryo development by regulating translation in *Xenopus laevis*. *J. Biol. Chem.* **2016**, *291*, 23020–23026. [CrossRef] [PubMed]

139. Ivanova, I.; Much, C.; Di Giacomo, M.; Azzi, C.; Morgan, M.; Moreira, P.N.; Monahan, J.; Carrieri, C.; Enright, A.J.; O'Carroll, D. The RNA m6A Reader YTHDF2 Is Essential for the Post-transcriptional Regulation of the Maternal Transcriptome and Oocyte Competence. *Mol. Cell* **2017**, *67*, 1059–1067. [CrossRef] [PubMed]

140. Zhao, B.S.; Wang, X.; Beadell, A.V.; Lu, Z.; Shi, H.; Kuuspalu, A.; Ho, R.K.; He, C. m(6)A-dependent maternal mRNA clearance facilitates zebrafish maternal-to-zygotic transition. *Nature* **2017**, *542*, 475–478. [CrossRef]

141. Lence, T.; Akhtar, J.; Bayer, M.; Schmid, K.; Spindler, L.; Ho, C.H.; Kreim, N.; Andrade-Navarro, M.A.; Poeck, B.; Helm, M.; et al. m(6)A modulates neuronal functions and sex determination in Drosophila. *Nature* **2016**, *540*, 242–247. [CrossRef]

142. Haussmann, I.U.; Bodi, Z.; Sanchez-Moran, E.; Mongan, N.P.; Archer, N.; Fray, R.G.; Soller, M. m6A potentiates Sxl alternative pre-mRNA splicing for robust Drosophila sex determination. *Nature* **2016**, *540*, 301–304. [CrossRef]

143. Hsu, P.J.; Zhu, Y.; Ma, H.; Guo, Y.; Shi, X.; Liu, Y.; Qi, M.; Lu, Z.; Shi, H.; Wang, J.; et al. Ythdc2 is an N6-methyladenosine binding protein that regulates mammalian spermatogenesis. *Cell Res.* **2017**, *27*, 1115–1127. [CrossRef]

144. Lin, Z.; Hsu, P.J.; Xing, X.; Fang, J.; Lu, Z.; Zou, Q.; Zhang, K.-J.; Zhang, X.; Zhou, Y.; Zhang, T.; et al. Mettl3-/Mettl14-mediated mRNA N6-methyladenosine modulates murine spermatogenesis. *Cell Res.* **2017**, *27*, 1216–1230. [CrossRef] [PubMed]

145. Chen, T.; Hao, Y.-J.; Zhang, Y.; Li, M.-M.; Wang, M.; Han, W.; Wu, Y.; Lv, Y.; Hao, J.; Wang, L.; et al. m(6)A RNA methylation is regulated by microRNAs and promotes reprogramming to pluripotency. *Cell Stem Cell* **2015**, *16*, 289–301. [CrossRef]

146. Aguilo, F.; Zhang, F.; Sancho, A.; Fidalgo, M.; Di Cecilia, S.; Vashisht, A.; Lee, D.-F.; Chen, C.-H.; Rengasamy, M.; Andino, B.; et al. Coordination of m(6)A mRNA methylation and gene transcription by ZFP217 regulates pluripotency and reprogramming. *Cell Stem Cell* **2015**, *17*, 689–704. [CrossRef] [PubMed]

147. Wen, J.; Lv, R.; Ma, H.; Shen, H.; He, C.; Wang, J.; Jiao, F.; Liu, H.; Yang, P.; Tan, L.; et al. Zc3h13 Regulates Nuclear RNA m6A Methylation and Mouse Embryonic Stem Cell Self-Renewal. *Mol. Cell* **2018**, *69*, 1028–1038.e6. [CrossRef]

148. Wang, Y.; Li, Y.; Toth, J.I.; Petroski, M.D.; Zhang, Z.; Zhao, J.C. N(6)-methyladenosine modification destabilizes developmental regulators in embryonic stem cells. *Nat. Cell Biol.* **2014**, *16*, 191–198. [CrossRef]

149. Weng, H.; Huang, H.; Wu, H.; Qin, X.; Zhao, B.S.; Dong, L.; Shi, H.; Skibbe, J.; Shen, C.; Hu, C.; et al. METTL14 Inhibits Hematopoietic Stem/Progenitor Differentiation and Promotes Leukemogenesis via mRNA m6A Modification. *Cell Stem Cell* **2018**, *22*, 191–205. [CrossRef] [PubMed]

150. Batista, P.J.; Molinie, B.; Wang, J.; Qu, K.; Zhang, J.; Li, L.; Bouley, D.M.; Lujan, E.; Haddad, B.; Daneshvar, K.; et al. m(6)A RNA modification controls cell fate transition in mammalian embryonic stem cells. *Cell Stem Cell* **2014**, *15*, 707–719. [CrossRef] [PubMed]

151. Geula, S.; Moshitch-Moshkovitz, S.; Dominissini, D.; Mansour, A.A.; Kol, N.; Salmon-Divon, M.; Hershkovitz, V.; Peer, E.; Mor, N.; Manor, Y.S.; et al. Stem cells. m6A mRNA methylation facilitates resolution of naïve pluripotency toward differentiation. *Science* **2015**, *347*, 1002–1006. [CrossRef]

152. Li, Z.; Weng, H.; Su, R.; Weng, X.; Zuo, Z.; Li, C.; Huang, H.; Nachtergaele, S.; Dong, L.; Hu, C.; et al. FTO plays an oncogenic role in acute myeloid leukemia as a N 6 -Methyladenosine RNA Demethylase. *Cancer Cell* **2017**, *31*, 127–141. [CrossRef]

153. Yang, S.; Wei, J.; Cui, Y.-H.; Park, G.; Shah, P.; Deng, Y.; Aplin, A.E.; Lu, Z.; Hwang, S.; He, C.; et al. m6A mRNA demethylase FTO regulates melanoma tumorigenicity and response to anti-PD-1 blockade. *Nat. Commun.* **2019**, *10*, 2782. [CrossRef]

154. Zhang, S.; Zhao, B.S.; Zhou, A.; Lin, K.; Zheng, S.; Lu, Z.; Chen, Y.; Sulman, E.P.; Xie, K.; Bögler, O.; et al. m 6 A Demethylase ALKBH5 maintains tumorigenicity of glioblastoma stem-like cells by sustaining FOXM1 expression and cell proliferation program. *Cancer Cell* **2017**, *31*, 591–606.e6. [CrossRef] [PubMed]

155. Lin, X.; Chai, G.; Wu, Y.; Li, J.; Chen, F.; Liu, J.; Luo, G.; Tauler, J.; Du, J.; Lin, S.; et al. RNA m6A methylation regulates the epithelial mesenchymal transition of cancer cells and translation of Snail. *Nat. Commun.* **2019**, *10*, 2065. [CrossRef] [PubMed]

156. Han, D.; Liu, J.; Chen, C.; Dong, L.; Liu, Y.; Chang, R.; Huang, X.; Liu, Y.; Wang, J.; Dougherty, U.; et al. Anti-tumour immunity controlled through mRNA m6A methylation and YTHDF1 in dendritic cells. *Nature* **2019**, *566*, 270–274. [CrossRef] [PubMed]

157. Edens, B.M.; Vissers, C.; Su, J.; Arumugam, S.; Xu, Z.; Shi, H.; Miller, N.; Rojas Ringeling, F.; Ming, G.-L.; He, C.; et al. FMRP modulates neural differentiation through m6a-dependent mRNA nuclear export. *Cell Rep.* **2019**, *28*, 845–854. [CrossRef]

158. Weng, Y.L.; Wang, X.; An, R.; Cassin, J.; Vissers, C.; Liu, Y.; Liu, Y.; Xu, T.; Wang, X.; Wong, S.Z.H.; et al. Epitranscriptomic m6A regulation of axon regeneration in the adult mammalian nervous system. *Neuron* **2018**, *97*, 313–325.e6. [CrossRef] [PubMed]

159. Hess, M.E.; Hess, S.; Meyer, K.D.; Verhagen, L.A.W.; Koch, L.; Brönneke, H.S.; Dietrich, M.O.; Jordan, S.D.; Saletore, Y.; Elemento, O.; et al. The fat mass and obesity associated gene (FTO) regulates activity of the dopaminergic midbrain circuitry. *Nat. Neurosci.* **2013**, *16*, 1042–1048. [CrossRef] [PubMed]

160. Shi, H.; Zhang, X.; Weng, Y.L.; Lu, Z.; Liu, Y.; Lu, Z.; Li, J.; Hao, P.; Zhang, Y.; Zhang, F.; et al. m6A facilitates hippocampus-dependent learning and memory through YTHDF1. *Nature* **2018**, *563*, 249–253. [CrossRef]

161. Xu, H.; Dzhashiashvili, Y.; Shah, A.; Kunjamma, R.B.; Weng, Y.L.; Elbaz, B.; Fei, Q.; Jones, J.S.; Li, Y.I.; Zhuang, X.; et al. m6A mRNA Methylation Is Essential for Oligodendrocyte Maturation and CNS Myelination. *Neuron* **2020**, *105*, 293–309.e5. [CrossRef]

162. Li, M.; Zhao, X.; Wang, W.; Shi, H.; Pan, Q.; Lu, Z.; Perez, S.P.; Suganthan, R.; He, C.; Bjørås, M.; et al. Ythdf2-mediated m6A mRNA clearance modulates neural development in mice. *Genome Biol.* **2018**, *19*, 1187. [CrossRef]

163. Tirumuru, N.; Zhao, B.S.; Lu, W.; Lu, Z.; He, C.; Wu, L. N6-methyladenosine of HIV-1 RNA regulates viral infection and HIV-1 Gag protein expression. *eLife Sci.* **2016**, *5*, 165.

164. Lu, W.; Tirumuru, N.; St Gelais, C.; Koneru, P.C.; Liu, C.; Kvaratskhelia, M.; He, C.; Wu, L. N6-Methyladenosine–binding proteins suppress HIV-1 infectivity and viral production. *J. Biol. Chem.* **2018**, *293*, 12992–13005. [CrossRef] [PubMed]

165. Gokhale, N.S.; McIntyre, A.B.R.; McFadden, M.J.; Roder, A.E.; Kennedy, E.M.; Gandara, J.A.; Hopcraft, S.E.; Quicke, K.M.; Vazquez, C.; Willer, J.; et al. N6 -methyladenosine in flaviviridae viral RNA genomes regulates infection. *Cell Host Microbe* **2016**, *20*, 654–665. [CrossRef]

166. Hesser, C.R.; Karijolich, J.; Dominissini, D.; He, C.; Glaunsinger, B.A. N6-methyladenosine modification and the YTHDF2 reader protein play cell type specific roles in lytic viral gene expression during Kaposi's sarcoma-associated herpesvirus infection. *PLoS Pathog.* **2018**, *14*, e1006995. [CrossRef]

167. Lu, M.; Zhang, Z.; Xue, M.; Zhao, B.S.; Harder, O.; Li, A.; Liang, X.; Gao, T.Z.; Xu, Y.; Zhou, J.; et al. N6-methyladenosine modification enables viral RNA to escape recognition by RNA sensor RIG-I. *Nat. Microbiol.* **2020**, *5*, 584–598. [CrossRef] [PubMed]

168. Jain, M.; Jantsch, M.F.; Licht, K. The editor's i on disease development. *Trends Genet.* **2019**, *35*, 903–913. [CrossRef] [PubMed]

169. Eisenberg, E.; Levanon, E.Y. A-to-I RNA editing-immune protector and transcriptome diversifier. *Nat. Rev. Genet.* **2018**, *19*, 473–490. [CrossRef] [PubMed]

170. Bohnsack, K.E.; Höbartner, C.; Bohnsack, M.T. Eukaryotic 5-methylcytosine (m5C) RNA Methyltransferases: Mechanisms, cellular functions, and links to disease. *Genes* **2019**, *10*, 102. [CrossRef]

171. Borchardt, E.K.; Martinez, N.M.; Gilbert, W.V. Regulation and function of RNA pseudouridylation in human cells. *Annu. Rev. Genet.* **2020**, *54*, 309–336. [CrossRef] [PubMed]

172. Ayadi, L.; Galvanin, A.; Pichot, F.; Marchand, V.; Motorin, Y. RNA ribose methylation (2′-O-methylation): Occurrence, biosynthesis and biological functions. *Biochim. Biophys. Acta Gene Regul. Mech.* **2019**, *1862*, 253–269. [CrossRef] [PubMed]

173. Höfler, S.; Carlomagno, T. Structural and functional roles of 2′-O-ribose methylations and their enzymatic machinery across multiple classes of RNAs. *Curr. Opin. Struct. Biol.* **2020**, *65*, 42–50. [CrossRef] [PubMed]

174. Mattaj, I.W.; Tollervey, D.; Séraphin, B. Small nuclear RNAs in messenger RNA and ribosomal RNA processing. *FASEB J.* **1993**, *7*, 47–53. [CrossRef] [PubMed]

175. Will, C.L.; Lührmann, R. Spliceosomal UsnRNP biogenesis, structure and function. *Curr. Opin. Cell Biol.* **2001**, *13*, 290–301. [CrossRef]

176. Hopper, A.K.; Pai, D.A.; Engelke, D.R. Cellular dynamics of tRNAs and their genes. *FEBS Lett.* **2010**, *584*, 310–317. [CrossRef]

177. Sinigaglia, K.; Wiatrek, D.; Khan, A.; Michalik, D.; Sambrani, N.; Sedmík, J.; Vukić, D.; O'Connell, M.A.; Keegan, L.P. ADAR RNA editing in innate immune response phasing, in circadian clocks and in sleep. *Biochim. Biophys. Acta Gene Regul. Mech.* **2019**, *1862*, 356–369. [CrossRef] [PubMed]

178. Hussain, S.; Benavente, S.B.; Nascimento, E.; Dragoni, I.; Kurowski, A.; Gillich, A.; Humphreys, P.; Frye, M. The nucleolar RNA methyltransferase Misu (NSun2) is required for mitotic spindle stability. *J. Cell Biol.* **2009**, *186*, 27–40. [CrossRef] [PubMed]

179. Sakita-Suto, S.; Kanda, A.; Suzuki, F.; Sato, S.; Takata, T.; Tatsuka, M. Aurora-B regulates RNA methyltransferase NSUN2. *Mol. Biol. Cell* **2007**, *18*, 1107–1117. [CrossRef]

180. Rintala-Dempsey, A.C.; Kothe, U. Eukaryotic stand-alone pseudouridine synthases-RNA modifying enzymes and emerging regulators of gene expression? *RNA Biol.* **2017**, *14*, 1185–1196. [CrossRef]

181. Sato, H.; Das, S.; Singer, R.H.; Vera, M. Imaging of DNA and RNA in living eukaryotic cells to reveal spatiotemporal dynamics of gene expression. *Annu. Rev. Biochem.* **2020**, *89*, 159–187. [CrossRef]

182. Marras, S.A.E.; Bushkin, Y.; Tyagi, S. High-fidelity amplified FISH for the detection and allelic discrimination of single mRNA molecules. *Proc. Nat. Acad. Sci. USA* **2019**, *116*, 13921–13926. [CrossRef] [PubMed]

183. Bi, S.; Yue, S.; Zhang, S. Hybridization chain reaction: A versatile molecular tool for biosensing, bioimaging, and biomedicine. *Chem. Soc. Rev.* **2017**, *46*, 4281–4298. [CrossRef]

184. Choi, H.M.T.; Chang, J.Y.; Trinh, L.A.; Padilla, J.E.; Fraser, S.E.; Pierce, N.A. Programmable in situ amplification for multiplexed imaging of mRNA expression. *Nat. Biotechnol.* **2010**, *28*, 1208–1212. [CrossRef] [PubMed]

185. Chen, F.; Bai, M.; Cao, X.; Zhao, Y.; Xue, J.; Zhao, Y. Click-encoded rolling FISH for visualizing single-cell RNA polyadenylation and structures. *Nucleic Acids Res.* **2019**, *47*, e145. [CrossRef]

186. Chao, J.A.; Lionnet, T. Imaging the life and death of mRNAs in single cells. *Cold Spring Harb. Perspect. Biol.* **2018**, *10*, a032086. [CrossRef] [PubMed]

187. Bosch, J.A.; Chen, C.L.; Perrimon, N. Proximity-dependent labeling methods for proteomic profiling in living cells: An update. *Wiley Interdiscip. Rev. Dev. Biol.* **2021**, *10*, e392. [CrossRef] [PubMed]

188. Wang, P.; Doxtader, K.A.; Nam, Y. Structural basis for cooperative function of Mettl3 and Mettl14 methyltransferases. *Mol. Cell* **2016**, *63*, 306–317. [CrossRef] [PubMed]

189. Lence, T.; Paolantoni, C.; Worpenberg, L.; Roignant, J.-Y. Mechanistic insights into m6A RNA enzymes. *Biochim. Biophys. Acta (BBA) Gene Regul. Mech.* **2019**, *1862*, 222–229. [CrossRef] [PubMed]

190. Yue, Y.; Liu, J.; Cui, X.; Cao, J.; Luo, G.; Zhang, Z.; Cheng, T.; Gao, M.; Shu, X.; Ma, H.; et al. VIRMA mediates preferential m6A mRNA methylation in 3′UTR and near stop codon and associates with alternative polyadenylation. *Cell Discov.* **2018**, *4*, 10. [CrossRef] [PubMed]

191. Chen, C.X.; Cho, D.S.; Wang, Q.; Lai, F.; Carter, K.C.; Nishikura, K. A third member of the RNA-specific adenosine deaminase gene family, ADAR3, contains both single- and double-stranded RNA binding domains. *RNA* **2000**, *6*, 755–767. [CrossRef]

192. Deffit, S.N.; Hundley, H.A. To edit or not to edit: Regulation of ADAR editing specificity and efficiency. *Wiley Interdiscip. Rev. RNA* **2016**, *7*, 113–127. [CrossRef] [PubMed]

193. Samavarchi-Tehrani, P.; Samson, R.; Gingras, A.-C. Proximity dependent biotinylation: Key enzymes and adaptation to proteomics approaches. *Mol. Cell Proteom.* **2020**, *19*, 757–773. [CrossRef] [PubMed]

194. Matzinger, M.; Mechtler, K. Cleavable cross-linkers and mass spectrometry for the ultimate task of profiling protein–protein interaction networks in vivo. *J. Proteome Res.* **2020**, *20*, 78–93. [CrossRef]

195. Mojica, F.J.M.; Díez-Villaseñor, C.; García-Martínez, J.; Soria, E. Intervening sequences of regularly spaced prokaryotic repeats derive from foreign genetic elements. *J. Mol. Evol.* **2005**, *60*, 174–182. [CrossRef]

196. Jansen, R.; Embden, J.D.A.V.; Gaastra, W.; Schouls, L.M. Identification of genes that are associated with DNA repeats in prokaryotes. *Mol. Microbiol.* **2002**, *43*, 1565–1575. [CrossRef]

197. Hille, F.; Charpentier, E. CRISPR-Cas: Biology, mechanisms and relevance. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **2016**, *371*, 20150496. [CrossRef]

198. Makarova, K.S.; Koonin, E.V. Annotation and classification of CRISPR-cas systems. *Methods Mol. Biol.* **2015**, *1311*, 47–75.

199. Jiang, F.; Doudna, J.A. The structural biology of CRISPR-Cas systems. *Curr. Opin. Struct. Biol.* **2015**, *30*, 100–111. [CrossRef]

200. Garcia-Doval, C.; Jinek, M. Molecular architectures and mechanisms of Class 2 CRISPR-associated nucleases. *Curr. Opin. Struct. Biol.* **2017**, *47*, 157–166. [CrossRef]

201. Meister, G. Argonaute proteins: Functional insights and emerging roles. *Nat. Rev. Genet.* **2013**, *14*, 447–459. [CrossRef]

202. Read, L.K.; Lukeš, J.; Hashimi, H. Trypanosome RNA editing: The complexity of getting U in and taking U out. *Wiley Interdiscip. Rev. RNA* **2016**, *7*, 33–51. [CrossRef]

203. Tycowski, K.T.; You, Z.H.; Graham, P.J.; Steitz, J.A. Modification of U6 spliceosomal RNA is guided by other small RNAs. *Mol. Cell* **1998**, *2*, 629–638. [CrossRef]

204. Ganot, P.; Jady, B.E.; Bortolin, M.L.; Darzacq, X.; Kiss, T. Nucleolar factors direct the 2′-O-ribose methylation and pseudouridylation of U6 spliceosomal RNA. *Mol. Cell Biol.* **1999**, *19*, 6906–6917. [CrossRef] [PubMed]

205. Terns, M.P.; Terns, R.M. Small nucleolar RNAs: Versatile trans-acting molecules of ancient evolutionary origin. *Gene Expr.* **2002**, *10*, 17–39.

206. Vitali, P.; Basyuk, E.; Le Meur, E.; Bertrand, E.; Muscatelli, F.; Cavaillé, J.; Huttenhofer, A. ADAR2-mediated editing of RNA substrates in the nucleolus is inhibited by C/D small nucleolar RNAs. *J. Cell Biol.* **2005**, *169*, 745–753. [CrossRef] [PubMed]

207. Fritz, J.; Strehblow, A.; Taschner, A.; Schopoff, S.; Pasierbek, P.; Jantsch, M.F. RNA-regulated interaction of transportin-1 and exportin-5 with the double-stranded RNA-binding domain regulates nucleocytoplasmic shuttling of ADAR1. *Mol. Cell Biol.* **2009**, *29*, 1487–1497. [CrossRef] [PubMed]

208. Huang, T.; Chen, W.; Liu, J.; Gu, N.; Zhang, R. Genome-wide identification of mRNA 5-methylcytosine in mammals. *Nat. Struct. Mol. Biol.* **2019**, *26*, 1–15. [CrossRef]

209. Parisien, M.; Wang, X.; Perdrizet, G.; Lamphear, C.; Fierke, C.A.; Maheshwari, K.C.; Wilde, M.J.; Sosnick, T.R.; Pan, T. Discovering RNA-protein interactome by using chemical context profiling of the RNA-protein interface. *Cell Rep.* **2013**, *3*, 1703–1713. [CrossRef]

210. Kaewsapsak, P.; Shechner, D.M.; Mallard, W.; Rinn, J.L.; Ting, A.Y. Live-cell mapping of organelle-associated RNAs via proximity biotinylation combined with protein-RNA crosslinking. *eLife Sci.* **2017**, *6*, e29224.

211. Benhalevy, D.; Anastasakis, D.G.; Hafner, M. Proximity-CLIP provides a snapshot of protein-occupied RNA elements in subcellular compartments. *Nat. Methods* **2018**, *15*, 1074–1082. [CrossRef]

212. Fazal, F.M.; Han, S.; Parker, K.R.; Kaewsapsak, P.; Xu, J.; Boettiger, A.N.; Chang, H.Y.; Ting, A.Y. Atlas of Subcellular RNA Localization Revealed by APEX-Seq. *Cell* **2019**, *178*, 473–490. [CrossRef]

213. Youn, J.-Y.; Dunham, W.H.; Hong, S.J.; Knight, J.D.R.; Bashkurov, M.; Chen, G.I.; Bagci, H.; Rathod, B.; MacLeod, G.; Eng, S.W.M.; et al. High-density proximity mapping reveals the subcellular organization of mrna-associated granules and bodies. *Mol. Cell* **2018**, *69*, 517–532. [CrossRef]

214. Padrón, A.; Iwasaki, S.; Ingolia, N.T. Proximity RNA labeling by APEX-Seq reveals the organization of translation initiation complexes and repressive RNA granules. *Mol. Cell* **2019**, *75*, 875–887. [CrossRef] [PubMed]

215. Li, L.; Qian, G.; Zuo, Y.; Yuan, Y.; Cheng, Q.; Guo, T.; Liu, J.; Liu, C.; Zhang, L.; Zheng, H. Ubiquitin-dependent turnover of adenosine deaminase acting on RNA 1 (ADAR1) is required for efficient antiviral activity of type I interferon. *J. Biol. Chem.* **2016**, *291*, 24974–24985. [CrossRef]

216. Sakurai, M.; Shiromoto, Y.; Ota, H.; Song, C.; Kossenkov, A.V.; Wickramasinghe, J.; Showe, L.C.; Skordalakes, E.; Tang, H.-Y.; Speicher, D.W.; et al. ADAR1 controls apoptosis of stressed cells by inhibiting Staufen1-mediated mRNA decay. *Nat. Struct. Mol. Biol.* **2017**, *24*, 534–543. [CrossRef]

217. Marcucci, R.; Brindle, J.; Paro, S.; Casadio, A.; Hempel, S.; Morrice, N.; Bisso, A.; Keegan, L.P.; Del Sal, G.; O'Connell, M.A. Pin1 and WWP2 regulate GluR2 Q/R site RNA editing by ADAR2 with opposing effects. *EMBO J.* **2011**, *30*, 4211–4222. [CrossRef]

218. Liu, X.; Liu, J.; Xiao, W.; Zeng, Q.; Bo, H.; Zhu, Y.; Gong, L.; He, D.; Xing, X.; Li, R.; et al. SIRT1 Regulates N6-methyladenosine RNA modification in hepatocarcinogenesis by inducing RANBP2-dependent FTO SUMOylation. *Hepatology* **2020**. [CrossRef]

219. Desterro, J.M.P.; Keegan, L.P.; Jaffray, E.; Hay, R.T.; O'Connell, M.A.; Carmo-Fonseca, M. SUMO-1 modification alters ADAR1 editing activity. *Mol. Biol. Cell* **2005**, *16*, 5115–5126. [CrossRef] [PubMed]

220. Du, Y.; Hou, G.; Zhang, H.; Dou, J.; He, J.; Guo, Y.; Li, L.; Chen, R.; Wang, Y.; Deng, R.; et al. SUMOylation of the m6A-RNA methyltransferase METTL3 modulates its function. *Nucleic Acids Res.* **2018**, *46*, 5195–5208. [CrossRef]

221. Schöller, E.; Weichmann, F.; Treiber, T.; Ringle, S.; Treiber, N.; Flatley, A.; Feederle, R.; Bruckmann, A.; Meister, G. Interactions, localization, and phosphorylation of the m6A generating METTL3-METTL14-WTAP complex. *RNA* **2018**, *24*, 499–512. [CrossRef]

222. Yang, F.; Wang, C. Profiling of post-translational modifications by chemical and computational proteomics. *Chem. Commun.* **2020**, *56*, 13506–13519. [CrossRef]

223. Yang, A.; Cho, K.; Park, H.S. Chemical biology approaches for studying posttranslational modifications. *RNA Biol.* **2018**, *15*, 427–440. [CrossRef]

224. Biesalski, H.K. Nutrition meets the microbiome: Micronutrients and the microbiota. *Ann. N. Y. Acad. Sci.* **2016**, *1372*, 53–64. [CrossRef] [PubMed]

225. Mosca, P.; Leheup, B.; Dreumont, N. Nutrigenomics and RNA methylation: Role of micronutrients. *Biochimie* **2019**, *164*, 53–59. [CrossRef] [PubMed]

226. Holley, R.W.; Kiernan, J.A. Control of the initiation of DNA synthesis in 3T3 cells: Low-molecular weight nutrients. *Proc. Natl. Acad. Sci. USA* **1974**, *71*, 2942–2945. [CrossRef]

227. Pardee, A.B. A restriction point for control of normal animal cell proliferation. *Proc. Natl. Acad. Sci. USA* **1974**, *71*, 1286–1290. [CrossRef] [PubMed]

228. Lin, D.-W.; Chung, B.P.; Kaiser, P. S-adenosylmethionine limitation induces p38 mitogen-activated protein kinase and triggers cell cycle arrest in G1. *J. Cell. Sci.* **2014**, *127*, 50–59. [CrossRef] [PubMed]

229. Ducker, G.S.; Rabinowitz, J.D. One-carbon metabolism in health and disease. *Cell Metab.* **2017**, *25*, 27–42. [CrossRef]

230. Clare, C.E.; Brassington, A.H.; Kwong, W.Y.; Sinclair, K.D. One-carbon metabolism: Linking nutritional biochemistry to epigenetic programming of long-term development. *Annu. Rev. Anim. Biosci.* **2019**, *7*, 263–287. [CrossRef]

231. Shima, H.; Matsumoto, M.; Ishigami, Y.; Ebina, M.; Muto, A.; Sato, Y.; Kumagai, S.; Ochiai, K.; Suzuki, T.; Igarashi, K. S-Adenosylmethionine synthesis is regulated by selective N^6-adenosine methylation and mRNA degradation involving METTL16 and YTHDC1. *Cell Rep.* **2017**, *21*, 3354–3363. [CrossRef]

232. Pendleton, K.E.; Chen, B.; Liu, K.; Hunter, O.V.; Xie, Y.; Tu, B.P.; Conrad, N.K. The U6 snRNA m^{6}A Methyltransferase METTL16 regulates SAM synthetase intron retention. *Cell* **2017**, *169*, 824–835. [CrossRef] [PubMed]

233. Gkatza, N.A.; Castro, C.; Harvey, R.F.; Heiss, M.; Popis, M.C.; Blanco, S.; Bornelöv, S.; Sajini, A.A.; Gleeson, J.G.; Griffin, J.L.; et al. Cytosine-5 RNA methylation links protein synthesis to cell metabolism. *PLoS Biol.* **2019**, *17*, e3000297. [CrossRef] [PubMed]

234. Hentze, M.W. Enzymes as Rna-binding proteins-a role for (Di)nucleotide-binding domains. *Trends Biochem. Sci.* **1994**, *19*, 101–103. [CrossRef]

235. Castello, A.; Hentze, M.W.; Preiss, T. Metabolic enzymes enjoying new partnerships as RNA-binding proteins. *Trends Endocrinol. Metab.* **2015**, *26*, 746–757. [CrossRef] [PubMed]

236. Liu, J.Y.; Wellen, K.E. Advances into understanding metabolites as signaling molecules in cancer progression. *Curr. Opin. Cell Biol.* **2020**, *63*, 144–153. [CrossRef]

237. Mentch, S.J.; Mehrmohamadi, M.; Huang, L.; Liu, X.; Gupta, D.; Mattocks, D.; Gómez Padilla, P.; Ables, G.; Bamman, M.M.; Thalacker-Mercer, A.E.; et al. Histone methylation dynamics and gene regulation occur through the sensing of one-carbon metabolism. *Cell Metab.* **2015**, *22*, 861–873. [CrossRef]

238. Wu, T.; Yoon, H.; Xiong, Y.; Dixon-Clarke, S.E.; Nowak, R.P.; Fischer, E.S. Targeted protein degradation as a powerful research tool in basic biology and drug target discovery. *Nat. Struct. Mol. Biol.* **2020**, *27*, 605–614. [CrossRef]

239. Liu, R.; Yang, Z. Single cell metabolomics using mass spectrometry: Techniques and data analysis. *Anal. Chim. Acta* **2021**, *1143*, 124–134. [CrossRef] [PubMed]

240. Stellos, K.; Gatsiou, A.; Stamatelopoulos, K.; Perisic Matic, L.; John, D.; Lunella, F.F.; Jaé, N.; Rossbach, O.; Amrhein, C.; Sigala, F.; et al. Adenosine-to-inosine RNA editing controls cathepsin S expression in atherosclerosis by enabling HuR-mediated post-transcriptional regulation. *Nat. Med.* **2016**, *22*, 1140–1150. [CrossRef]

241. Jain, M.; Mann, T.D.; Stulić, M.; Rao, S.P.; Kirsch, A.; Pullirsch, D.; Strobl, X.; Rath, C.; Reissig, L.; Moreth, K.; et al. RNA editing of Filamin A pre-mRNA regulates vascular contraction and diastolic blood pressure. *EMBO J.* **2018**, *37*, 23. [CrossRef]

242. Van der Kwast, R.V.C.T.; van Ingen, E.; Parma, L.; Peters, H.A.B.; Quax, P.H.A.; Nossent, A.Y. Adenosine-to-inosine editing of microRNA-487b alters target gene selection after ischemia and promotes neovascularization. *Circ. Res.* **2018**, *122*, 444–456. [CrossRef] [PubMed]

243. Freund, I.; Eigenbrod, T.; Helm, M.; Dalpke, A.H. RNA modifications modulate activation of innate toll-like receptors. *Genes* **2019**, *10*, 92. [CrossRef] [PubMed]

244. Liu, N.; Zhou, K.I.; Parisien, M.; Dai, Q.; Diatchenko, L.; Pan, T. N6-methyladenosine alters RNA structure to regulate binding of a low-complexity protein. *Nucleic Acids Res.* **2017**, *45*, 6051–6063. [CrossRef]

245. Barraud, P.; Gato, A.; Heiss, M.; Catala, M.; Kellner, S.; Tisné, C. Time-resolved NMR monitoring of tRNA maturation. *Nat. Commun.* **2019**, *10*, 3373. [CrossRef] [PubMed]

246. Liu, N.; Dai, Q.; Zheng, G.; He, C.; Parisien, M.; Pan, T. N6-methyladenosine-dependent RNA structural switches regulate RNA-protein interactions. *Nature* **2015**, *518*, 560–564. [CrossRef]

247. Tanzer, A.; Hofacker, I.L.; Lorenz, R. RNA modifications in structure prediction-Status quo and future challenges. *Methods* **2019**, *156*, 32–39. [CrossRef]

248. Wettengel, J.; Reautschnig, P.; Geisler, S.; Kahle, P.J.; Stafforst, T. Harnessing human ADAR2 for RNA repair-Recoding a PINK1 mutation rescues mitophagy. *Nucleic Acids Res.* **2017**, *45*, 2797–2808. [CrossRef] [PubMed]

249. Fukuda, M.; Umeno, H.; Nose, K.; Nishitarumizu, A.; Noguchi, R.; Nakagawa, H. Construction of a guide-RNA for site-directed RNA mutagenesis utilising intracellular A-to-I RNA editing. *Sci. Rep.* **2017**, *7*, 1–13. [CrossRef]

250. Marina, R.J.; Brannan, K.W.; Dong, K.D.; Yee, B.A.; Yeo, G.W. Evaluation of engineered CRISPR-cas-mediated systems for site-specific RNA editing. *Cell Rep.* **2020**, *33*, 108350. [CrossRef]

251. Shinoda, K.; Suda, A.; Otonari, K.; Futaki, S.; Imanishi, M. Programmable RNA methylation and demethylation using PUF RNA binding proteins. *Chem. Commun.* **2020**, *56*, 1365–1368. [CrossRef]

252. Wilson, C.; Chen, P.J.; Miao, Z.; Liu, D.R. Programmable m6A modification of cellular RNAs with a Cas13-directed methyltransferase. *Nat. Biotechnol.* **2020**, *38*, 1431–1440. [CrossRef] [PubMed]

253. Liu, X.-M.; Zhou, J.; Mao, Y.; Ji, Q.; Qian, S.B. Programmable RNA N6-methyladenosine editing by CRISPR-Cas9 conjugates. *Nat. Chem. Biol.* **2019**, *15*, 865–871. [CrossRef]

254. Mo, J.; Chen, Z.; Qin, S.; Li, S.; Liu, C.; Zhang, L.; Ran, R.; Kong, Y.; Wang, F.; Liu, S.; et al. TRADES: Targeted RNA demethylation by suntag system. *Adv. Sci.* **2020**, *7*, 2001402. [CrossRef]

255. Li, J.; Chen, Z.; Chen, F.; Xie, G.; Ling, Y.; Peng, Y.; Lin, Y.; Luo, N.; Chiang, C.M.; Wang, H. Targeted mRNA demethylation using an engineered dCas13b-ALKBH5 fusion protein. *Nucleic Acids Res.* **2020**, *48*, 5684–5694. [CrossRef]

256. Rau, K.; Rösner, L.; Rentmeister, A. Sequence-specific m6A demethylation in RNA by FTO fused to RCas9. *RNA* **2019**, *25*, 1311–1323. [CrossRef]

257. Palumbo, C.M.; Beal, P.A. Nucleoside analogs in the study of the epitranscriptome. *Methods* **2019**, *156*, 46–52. [CrossRef]

258. Mellis, I.A.; Gupte, R.; Raj, A.; Rouhanifard, S.H. Visualizing adenosine-to-inosine RNA editing in single mammalian cells. *Nat. Methods* **2017**, *14*, 801–804. [CrossRef] [PubMed]

259. Kormann, M.S.D.; Hasenpusch, G.; Aneja, M.K.; Nica, G.; Flemmer, A.W.; Herber-Jonat, S.; Huppmann, M.; Mays, L.E.; Illenyi, M.; Schams, A.; et al. Expression of therapeutic proteins after delivery of chemically modified mRNA in mice. *Nat. Biotechnol.* **2011**, *29*, 154–157. [CrossRef]

260. Karikó, K.; Muramatsu, H.; Ludwig, J.; Weissman, D. Generating the optimal mRNA for therapy: HPLC purification eliminates immune activation and improves translation of nucleoside-modified, protein-encoding mRNA. *Nucleic Acids Res.* **2011**, *39*, e142. [CrossRef] [PubMed]

261. Terrazas, M.; Kool, E.T. RNA major groove modifications improve siRNA stability and biological activity. *Nucleic Acids Res.* **2009**, *37*, 346–353. [CrossRef]

262. Gehrig, S.; Eberle, M.-E.; Botschen, F.; Rimbach, K.; Eberle, F.; Eigenbrod, T.; Kaiser, S.; Holmes, W.M.; Erdmann, V.A.; Sprinzl, M.; et al. Identification of modifications in microbial, native tRNA that suppress immunostimulatory activity. *J. Exp. Med.* **2012**, *209*, 225–233. [CrossRef] [PubMed]

263. Kreiter, S.; Diken, M.; Pascolo, S.; Nair, S.K.; Thielemans, K.M.; Geall, A. RNA vaccination therapy: Advances in an emerging field. *J. Immunol. Res.* **2016**, *2016*, 9703914. [CrossRef] [PubMed]

264. Singh, M.S.; Peer, D. RNA nanomedicines: The next generation drugs? *Curr. Opin. Biotechnol.* **2016**, *39*, 28–34. [CrossRef] [PubMed]

265. Reautschnig, P.; Vogel, P.; Stafforst, T. The notorious R.N.A. in the spotlight-drug or target for the treatment of disease. *RNA Biol.* **2016**, *14*, 1–18. [CrossRef]

266. Grudzien-Nogalska, E.; Kowalska, J.; Su, W.; Kuhn, A.N.; Slepenkov, S.V.; Darzynkiewicz, E.; Sahin, U.; Jemielity, J.; Rhoads, R.E. Synthetic mRNAs with superior translation and stability properties. *Methods Mol. Biol.* **2013**, *969*, 55–72.