REVIEW

The Epitranscriptome and Innate Immunity

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Abstract

Our knowledge of the variety and abundances of RNA base modifications is rapidly increasing. Modified bases have critical roles in tRNAs, rRNAs, translation, splicing, RNA interference, and other RNA processes, and are now increasingly detected in all types of transcripts. Can new biological principles associated with this diversity of RNA modifications, particularly in mRNAs and long non-coding RNAs, be identified? This review will explore this question by focusing primarily on adenosine to inosine (A-to-I) RNA editing by the adenine deaminase acting on RNA (ADAR) enzymes that have been intensively studied for the past 20 years and have a wide range of effects. Over 100 million adenosine to inosine editing sites have been identified in the human transcriptome, mostly in embedded Alu sequences that form potentially innate immune-stimulating dsRNA hairpins in transcripts. Recent research has demonstrated that inosine in the epitranscriptome and ADAR1 protein establish innate immune tolerance for host dsRNA formed by endogenous sequences. Innate immune sensors that detect viral nucleic acids are among the readers of epitranscriptome RNA modifications, though this does preclude a wide range of other modification effects.

Introduction

Conventional RNA-Seq is unable to address how much RNA modification occurs in mRNA and noncoding RNAs. Reverse transcriptases used in cDNA synthesis have evolved tolerances for diverse types of base modification in the RNA template; amazingly, they can even make cDNA copies of highly modified sections of tRNAs and rRNAs [1]. This evolutionary feature of reverse transcriptases is likely to reflect the presence of a range of modified bases in RNAs but it also means that standard protocols for cDNA synthesis and sequence analysis do not reveal most modified bases. Until now, identifications of modified bases in mRNAs have relied mainly on mass spectrometry or antibodies specific for the modified base, or on the detection of different responses of the modified base versus the normal base to some chemical modification ([2] and references therein). However, there have been some recent developments to improve the detection of certain modifications [3].

RNA base modifications—in particular, base methylations in mRNAs and noncoding RNAs—have been described as the “epitranscriptome” [4–6], suggesting that effects of modified RNA bases also involve reader, writer, and eraser proteins. Base modification enzymes have switched between DNA and RNA substrates in evolution. Studies on adenosine to inosine...
(A-to-I) editing by adenine deaminases acting on RNA (ADARs) now show that innate immune nucleic acid sensors are one set of readers of modified bases in RNA [7]. It is now clear that modified bases in either DNA or RNA aid innate immune sensors in discriminating between host and viral RNAs.

**N^6^-methyladenosine (m^6^A) Writers, Readers, and Erasers in mRNAs and Noncoding RNAs**

The emerging roles of N^6^-methyladenosine (m^6^A) have received a lot of recent attention. m^6^A does not change base-pairing preferences of RNA and cannot recode open reading frames. It has been found in approximately 7,000 mRNAs with an enrichment around the stop codon and in the 3’UTR regions of transcripts [8]. This is the RNA base modification that has been characterised in the terms of the epitranscriptome model, as the addition and removal of a methyl group is reminiscent of DNA methylation and epigenetics. The modification is introduced by IME4 in *Drosophila* and by the METTL3 and METTL14 proteins in vertebrates; YTH and hnRNP C proteins bind to RNAs containing the m^6^A base as readers, and the fat mass and obesity-associated gene (FTO) and ALKBH5 enzymes are erasers able to demethylate the m^6^A (for review, see [9]).

*Drosophila Ime4* mutants fail to induce meiosis and mouse *Mettl3* mutant embryonic stem (ES) cells fail to differentiate. Pluripotency transcripts that normally have m^6^A accumulate to higher levels when they are unmodified in the *Mettl3* mutant ES cells and do not decrease to allow differentiation [10]. A possible explanation for this is that the *Mettl3* phenotype is due to an altered balance between transcript production and turnover; the reader proteins YTH and hnRNP C may facilitate the turnover of subsets of m^6^A-containing transcripts.

Although m^5^C modification at CpG islands in DNA is the canonical example of an epigenetic modification, studies on m^5^C in RNA are still at an early stage. Several different methods have been used to identify m^5^C positions in RNAs, with little overlap between these sites found within either mRNA or ncRNA (for review, [11]). The biological role of m^5^C in mRNA and ncRNA is largely unknown, and proteins binding m^5^C in RNA have not yet been identified.

**Diverse Roles of A-to-I RNA Editing by ADARs**

Studies on ADAR RNA editing can also be interpreted in relation to the epitranscriptome model. The earliest work on ADARs focused on their recoding of codons in open reading frames but recent findings on ADAR1 have uncovered effects normally associated with other types of base modification.

The ADAR RNA-editing enzymes convert adenosine (A) to inosine (I) by hydrolytic deamination of adenosine bases within double-stranded (ds)RNA [12]. Individual adenosine bases are edited in pre-mRNAs when exons form short RNA hairpin structures, usually with nearby introns. Editing within exons can result in recoding of open reading frames because inosine is read as guanosine by the translational machinery [13]. Inosine prefers to form base pairs with cytosine, so ADAR RNA editing sites are easily identified since A in the genomic sequence appears as G in cDNA sequences [14]. ADARs primarily recognize duplex RNA that is in the A-form; they prefer certain bases beside the edited A but do not recognize a strong consensus sequence [15]. Studies on site-specific RNA editing have focused on ADAR2 which is enriched in the brain and is responsible for editing the Glutamine to Arginine Q/R site in the *Gria2* transcript and many of the other specific sites in CNS transcripts [16]. Editing the *Gria2* Q/R site appears to be the main function of ADAR2 as *Adar2* mutant mice, which die from seizures three weeks after birth, can be rescued by knocking-in the edited isoform *Gria2* R [17].

The ADAR proteins are comprised of two or three dsRNA binding domains (dsRBDS) at the amino terminus and a catalytic adenosine deaminase domain at the carboxyl terminus.
Mammals have five related ADAR proteins (Fig 1). ADAR1 and ADAR2 are enzymatically active (for review, [18]) but enzymatic activity has never been demonstrated for the brain-specific ADAR3 protein, even though it closely resembles ADAR2 [19]. The more divergent testis-specific adenine deaminase domain-contain proteins (ADAD1 and ADAD2) lack key catalytic residues but are still evolutionarily conserved, presumably acting as dsRNA-binding proteins [20,21]. Conservation of deaminase domain fold structure and key active site residues show that the ADARs are members of the cytidine deaminase protein family (CDAs); different cytosine deaminases edit cytosine to uracil in RNA or DNA or both [22].

Whereas ADAR2 has a major role in site-specific editing, ADAR1 performs “promiscuous” editing of transcripts encoding repetitive elements (for review, [23]). This is reflected in their different biological roles. ADAR1 is more stable than ADAR2 and thus was the first to be identified and purified [24,25]. Unlike recombinant ADAR2, purified endogenous ADAR2 is not stable [26]. It is post-translationally regulated by phosphorylation, proline isomerization, and ubiquitination [27]. The first distinctive features of ADAR1 were its wider tissue distribution and the higher levels of expression in most tissues than ADAR2. Another distinctive feature of ADAR1 is that it expresses two different isoforms—a constitutive and predominantly nuclear

![Fig 1. A schematic representation of the ADAR and related ADAD proteins present in humans. All proteins have dsRNA binding domains (grey box) and a C-terminal deaminase domain (purple box). A nuclear localization sequence (NLS) is marked in red, whereas the nuclear export signal (NES) present in ADAR1 is marked in green. Z-DNA binding domains are indicated by an orange box in ADAR1. A region enriched in arginine/lysine residues, R domain is present in ADAR3 (blue box). The number of amino acids is indicated on the right.](doi:10.1371/journal.pgen.1005687.g001)
110 kDa isoform and a larger interferon-induced and mainly cytoplasmic 150 kDa isoform [28–30]. Both ADAR1 isoforms shuttle between cytoplasm and nucleus [31]. One area of controversy is whether ADAR1 is pro- or antiviral (for review, [32] and references therein). There is evidence for both points of view; however, viruses sometimes hijack the cell’s defence system so that antiviral proteins appear to have a pro-viral role [33].

Transgenic Adar1−/− mice die at day E12.5 with defects in haematopoiesis and generalized induction of interferon and the interferon-stimulated gene (ISG) transcripts [34–36]. It was presumed that the Adar1 mutant phenotype would be due to loss of a site-specific editing event that alters a protein or microRNA required for stem cell maintenance or blood system development [36]. An intense search ensued to identify ADAR1-edited transcript(s) or noncoding RNAs. This approach proved unsuccessful and for over ten years the molecular cause of the mortality in the Adar1−/− mice remained elusive.

### Innate Immune Sensor Proteins As Readers of Inosine in dsRNA

Eventually, a different genetic approach was undertaken to rescue the Adar1 mutant mice [7], based on the idea that the mutant defect is caused by aberrant activation of the innate immune system by cellular dsRNA and not due to a defect in site-specific editing. ADAR1 is primarily responsible for promiscuous editing of transcripts encoding repetitive elements such as short interspersed nuclear elements (SINEs) which, in humans, are Alu elements [37,38]. On average, human pre-mRNAs contain about ten embedded Alu elements. Alu elements can readily form RNA duplexes that are promiscuously edited at low levels when they lie within one or two kilobases of each other in inverted orientations; this accounts for the majority of all identified RNA editing events in human transcripts [38]. Some transcripts have Alu duplexes in their 3’ UTRs that can reach the cytoplasm [39], where dsRNA can activate the innate immune responses.

Viral dsRNA in the cytoplasm is detected by the innate immune sensor proteins Retinoic acid-Inducible Gene 1 (RIG-I) and Melanoma Differentiation-Associated protein 5 (MDA5) (review in [40]). These RIG-I-like (RLR), innate immune sensors bind dsRNA with RNA helicase domains; they do not unwind dsRNA, as they lack the required structure elements, but instead they scan dsRNA by using ATPase activity to translocate smoothly along it or to disassociate and re-associate [41–43]. The key adapter protein mediating antiviral responses and interferon induction by dsRNA through the RLR pathway is the mitochondrial antiviral-signalling protein (MAVS), also known as VISA, IPS-1, and CARDIF (for review, [44]). Double homozygous Adar1; Mavs mice survive to birth, and ISG transcripts that were aberrantly activated in the Adar1 mutant embryos returned to normal in the double mutant embryos (Fig 2) [7]. Therefore, the Adar1 mutant defect in these mice is due to an aberrant innate immune response, and ADAR1 is not required primarily for development of the embryonic haematopoietic system [34–36] nor is it essential for the function of DICER in early development [45] as, otherwise, the Adar1; Mavs mice would not survive to birth [46]. However, ADAR1 may affect these processes indirectly.

Restoring expression of either ADAR1 or an ADAR2 mutant that localizes to the cytoplasm in stably transfected Adar1; P53 MEF cells reduces the level of transcription of ISGs, whereas catalytically inactive ADAR1 does this to an observable but considerably lower extent [7]. Thus, the Adar1 mutant phenotype appears to be mainly due to loss of inosine in RNA, with Mavs rescuing as it blocks the aberrantly activated signalling from both of the RLR dsRNA sensors. The effect of inosine-containing dsRNA is dominant; dsRNA oligonucleotide-containing inosine can bind to RIGI and MDA5, thus inhibiting the innate immune response [47].
Part of the ADAR1 effect is due to an editing-independent function. A recent study demonstrated that knocking-in the \textit{Adar1E861A} catalytically inactive mutation in mice gives an embryonic phenotype that is similar to, but not as severe as, the complete null [48]. The \textit{Adar1E861A} catalytically inactive mutant embryos die two days later (E14.5 versus E12.5) [48], and a double mutant that eliminates just one of the RLR sensors, \textit{MDA5 (Ifih1-/-)} (43], rescues the \textit{Adar1E861A} mutant but not \textit{Adar1} null mutant embryos [7]. This implies that the presence of the inactive \textit{Adar1E861A} protein partially rescues the \textit{Adar1} null mutant phenotype in the whole embryo, presumably because it still binds the most critical immune-inducing RNAs. Enzymes that introduce epigenetic modifications in chromatin also show partial rescues by catalytically-inactive mutants [49,50]. Maintaining the structure of a particular protein complex on the correct nucleic acid partially substitutes for lack of the epigenetic modification. ADARs bind to more dsRNAs than they edit, and the inosine base in a substrate does not necessarily change the binding affinity [51,52]. For epitranscriptome RNA base modifications in general, persistent binding of writer enzymes and interactions with other proteins could contribute in parallel with the base modification itself.

While it is tempting to associate the ADAR1 editing-independent activity with Alu or SINE RNAs, this remains an entirely open question. ADAR proteins are not abundant, and many adenosines in Alu RNA hairpins are edited at efficiencies below 1%. The editing-independent ADAR1 effect may involve particular immune-inducing ADAR target RNAs present in both mice and humans. ADAR1 tightly binds many of the conserved pre-mRNA structures that are site-specifically edited by ADAR2; some such RNA structures are entirely in exons or UTR regions and might reach the cytoplasm to affect innate immune sensors. The inactive ADARs, the brain specific ADAR3, and the two testis-specific ADAR-related ADAD proteins present in the genome may also bind particular RNAs that are tissue-specific (for review [23]).
The importance of epitranscriptome base modification for innate immune regulation is well illustrated by Aicardi-Goutières syndrome (AGS), which is caused by mutations in ADAR1 [53]. AGS is a fatal childhood congenital encephalopathy with interferon overexpression, and children with ADAR1 mutations also present with childhood dystonia due to bilateral striatal neurodegeneration, again with interferon expression. One could envisage that when an AGS child with ADAR1 mutations catches a transient viral infection, reduced ADAR1 dosage or activity leads to unedited cellular RNAs in the cytoplasm binding to RLRs, stimulating an innate immune response (Fig 2). After 12 hours, ADAR1p150, which is mainly cytoplasmic, is induced by interferon. It should edit all RNAs in the cytoplasm, thus turning off the innate immune signal to limit self-damage caused by interferon responses [7]. Resolution of the interferon response fails in the AGS patient; the inflammation is chronic and fails to correctly resolve.

Innate Immune Sensors Read Other Epitranscriptome Modifications

Data from the Weissman group has demonstrated that in vitro transcribed RNA containing various modified nucleotides such as m5C, m6A, m5U, pseudouridine, or 2'-O-methylated nucleotides dampen the innate immune response when transfected into mammalian dendritic cells, whereas RNA that is unmodified will stimulate it [54–56]. The responses observed are likely to involve RLR as well as TLR sensors.

In the case of inosine effects on RLRs, the inosine–uracil (I-U) wobble base pair weakens dsRNA base pairing and several I-U base pairs together cause dsRNA melting [57]. It is likely that it is this perturbation of the helical structure by the I-U wobble base pair that is detected by RLRs [7]. However, many other types of modified bases could also perturb dsRNA structure sufficiently for innate immune sensors to distinguish self dsRNA from the perfect dsRNA that is formed directly from virus replication. For instance, m6A also weakens base pairing in dsRNA even though it does not alter base pairing preferences [58]. 2’-O-methylated groups in the minor groove would also be easy for RLRs to detect, and these are known to prevent innate immune induction by dsRNA.

Levels of base modifications often change in response to stress or virus infection. Pseudouridine has been found in mRNA in both yeast and mammalian cells in the past year by three groups [59–61], all using a similar method of treating the RNA with CMC (N-cyclohexyl-N0-(2-morpholinoethyl)-carbodiimide metho-p-toluenesulphonate) a chemical that forms a stable interaction with pseudouridine [62]. When this RNA is subsequently used for cDNA synthesis, reverse transcriptase will terminate when it encounters the artificial base derivative. When a stress is applied to cells, such as heat shock of the yeast cells or serum starvation of the mammalian cells, this generally increases the level of pseudouridine; however, the biological function of this stress-induced increase in pseudouridine in mRNA is unclear [63].

Base modification is important in the conflict between virus and host because viruses can benefit from evading host innate immune responses if they can capture some modifications. Thus, m6A occurs in some viral RNA at a higher level than would be predicted from a random occurrence; for example, Rous sarcoma viral mRNA has seven m6A sites, whereas SV40 has more than ten [64]: it has been shown that TLR 3 is not activated when m6A is present in RNA [56].

Helm and co-workers have demonstrated that stimulation of the innate immune receptor TLR7, by tRNA purified from Escherichia coli but not by tRNA from human cells in PBMCs [65], is due to at least three different modifications on the human tRNAs—two of which are methylations of ribose 2’hydroxyls on tRNA guanosines 18 and 34. These naturally occurring
modifications in native tRNA not only prevent stimulation of TLR7 by the modified tRNA but also appear to antagonize TLR7 signalling to suppress immunostimulation by unmodified test tRNAs, similar to the inhibitory effect of inosine in dsRNA on the activation of RLRs.

Conflicts between self and non-self nucleic acids are much older than the animal innate immune systems [66], and tRNA modifications may also affect these conflicts. For instance, the γ-toxin from Kluyveromyces lactis cleaves tRNAsGlu/Gln/Lys from Saccharomyces cerevisiae on the 3’ side of an anticodon base that is modified. The γ-toxin recognizes the cleavage sites due to the 5-methoxycarbonylmethyl-2-thiouridine wobble base modification present on these tRNAs in S. cerevisiae but not in K. lactis [67–69].

Recent Advances

Some modified bases lead to particular patterns of misincorporation at the modified base position in standard RNA-Seq and indications of RNA modification events can be obtained from existing RNA sequence databases [70]. New RNA-Seq protocols identify modified sites by cloning cDNAs synthesised using lower nucleotide concentrations and more discriminating reverse transcriptases [71] that give increased misincorporation and termination. Also, more detailed examinations of modified base effects on RLRs are beginning to be investigated [72]. However, considering the abundance and variety of modification in RNA, it will take the development of new technology before one can be confident that the encoded RNA is truly being sequenced.

Conclusion

As Albert Einstein once said, “Fundamental ideas of science are essentially simple.” The use of host RNA modification to distinguish between host and parasite nucleic acids is reminiscent of the restriction and modification that occurs in bacterial DNA. With the plethora of modifications in RNA, it’s easy to envisage these would generate a unique bar code that would be species-specific, allowing intricate “self”-“non-self” discrimination.

References

1. Watanabe Y, Kawarabayasi Y. Experimental confirmation of a whole set of tRNA molecules in two archaeal species. Int J Mol Sci. 2015; 16(1):2187–203. doi: 10.3390/ijms16012187 PMID: 25608653; PubMed Central PMCID: PMC4307357.
2. Machnicka MA, Milanowska K, Osman Oglou O, Purta E, Kurkowska M, Olchowik A, et al. MODO-MICS: a database of RNA modification pathways—2013 update. Nucleic Acids Res. 2013; 41(Database issue):D262–7. Epub 2012/11/03. doi: 10.1093/nar/gks1007 PMID: 23118484; PubMed Central PMCID: PMC3531130.
3. Hauenschild R, Tserovski L, Schmid K, Thuring K, Winz ML, Sharma S, et al. The reverse transcription signature of N-1-methyladenosine in RNA-Seq is sequence dependent. Nucleic Acids Res. Epub 2015 Sep 13. doi: 10.1093/nar/gkv895 PMID: 26365242.
4. Meyer KD, Jaffrey SR. The dynamic epitranscriptome: N6-methyladenosine and gene expression control. Nat Rev Mol Cell Biol. 2014; 15(5):313–26. doi: 10.1038/nrm3785 PMID: 24713629; PubMed Central PMCID: PMC4393108.
5. Saletore Y, Chen-Kiang S, Mason CE. Novel RNA regulatory mechanisms revealed in the epitranscriptome. RNA Biol. 2013; 10(3):342–6. doi: 10.4161/rna.23812 PMID: 23434792; PubMed Central PMCID: PMC3672275.
6. Saletore Y, Meyer K, Korlach J, Vilfan ID, Jaffrey S, Mason CE. The birth of the Epitranscriptome: deciphering the function of RNA modifications. Genome Biol. 2012; 13(10):175. doi: 10.1186/gb-2012-13-10-175 PMID: 23119964; PubMed Central PMCID: PMC3491402.
7. Mannion NM, Greenwood SM, Young R, Cox S, Brindle J, Read D, et al. The RNA-editing enzyme ADAR1 controls innate immune responses to RNA. Cell Rep. 2014; 9(4):1482–94. doi: 10.1016/j.celrep.2014.10.041 PMID: 25456137.
8. Dominissini D, Moshitch-Moshkovitz S, Schwartz S, Salmon-Divon M, Ungar L, Osenberg S, et al. Topology of the human and mouse m6A RNA methylomes revealed by m6A-seq. Nature. 2012; 485(7397):201–6. PMID:22575960. doi:10.1038/nature11112

9. Fu Y, Dominissini D, Rechavi G, He C. Gene expression regulation mediated through reversible m(6)A RNA methylation. Nat Rev Genet. 2014; 15(5):293–306. doi:10.1038/nrg3724 PMID:24662220.

10. Geula S, Moshitch-Moshkovitz S, Dominissini D, Mansour AA, Kol N, Salmon-Divon M, et al. Stem cells. m6A mRNA methylation facilitates resolution of naive pluripotency toward differentiation. Science. 2015; 347(6225):1002–6. doi:10.1126/science.1261417 PMID:25569111.

11. Hussain S, Alekseev J, Blanco S, Dietmann S, Frye M. Characterizing 5-methylcytosine in the mammalian epitranscriptome. Genome Biol. 2013; 14(11):215. doi:10.1186/gb4143 PMID: 24286375; PubMed Central PMCID: PMC4053770.

12. Nishikura K, Yoo C, Kim U, Murray JM, Estes PA, Cash FE, et al. Substrate specificity of the dsRNA unwinding/modifying activity. EMBO J. 1991; 10:3523–32. PMID:1915306

13. Basilio C, Wahba AJ, Lengyel P, Speyer JF, Ochoa S. Synthetic polynucleotides and the amino acid code. Proc Natl Acad Sci USA. 1962; 48:613–6. PMID:13865603

14. Bass BL, Weintraub H, Cattaneo R, Billeter MA. Biased hypermutation of viral RNA genomes could be due to unwinding/modification of double-stranded RNA. Cell. 1989; 56:331. PMID:2914324

15. Polson AG, Bass BL. Preferential selection of adenosines for modification by double-stranded RNA adenosine deaminase. EMBO J. 1994; 13:5701–11. PMID:7527340

16. Higuchi M, Single FN, Kohler M, Sommer B, Sprengel R, Seeburg PH. RNA editing of AMPA receptor subunit GluR-B: a base-paired intron-exon structure determines position and efficiency. Cell. 1993; 75(7):1361–70. PMID:8269514.

17. Higuchi M, Maas S, Single FN, Hartner J, Rozov A, Burnashev N, et al. Point mutation in an AMPA receptor gene rescues lethality in mice deficient in the RNA-editing enzyme ADAR2. Nature. 2000; 406(6791):78–81. PMID:10894545.

18. Hogg M, Paro S, Keegan LP, O’Connell MA. RNA editing by mammalian ADARs. Adv Genet. 2011; 73:87–120. PMID:21310295. doi:10.1016/B978-0-12-380860-8.00003-3

19. Melcher T, Maas S, Herb A, Sprengel R, Higuchi M, Seeburg PH. RED2, a brain specific member of the RNA-specific adenosine deaminase family. Proc Natl Acad Sci USA. 1994; 91:10596–60. PMID:7937998

20. O’Connell MA, Gerber AP, Keller W. Purification of human double-stranded RNA-specific editase 1 (hRED1) involved in editing of brain glutamate receptor B pre-mRNA. J Biol Chem. 1997; 272(1):473–8. PMID:8995285.

21. Keegan LP, Leroy A, Sproul D, O’Connell MA. Adenosine deaminases acting on RNA (ADARs): RNA-editing enzymes. Genome Biol. 2004; 5(2):209. PMID:14759252.

22. Gerber AP, Keller W. RNA editing by base deamination: more enzymes, more targets, new mysteries. Trends Biochem Sci. 2001; 26(6):376–84. PMID:11406411

23. Mannion N, Arieti F, Gallo A, Keegan LP, O’Connell MA. New Insights into the Biological Role of Mammalian ADARs; the RNA Editing Proteins. Biomolecules. 2015; 5(4):2338–62. doi:10.3390/biom5042338 PMID:26437436.

24. Kim U, Garner TL, Sanford T, Speicher D, Murray JM, Nishikura K. Purification and characterization of double-stranded RNA adenosine deaminase from bovine nuclear extracts. J Biol Chem. 1994; 269:13480–9. PMID:8175781

25. O’Connell MA, Keller W. Purification and properties of double-stranded RNA-specific adenosine deaminase from calf thymus. Proc Natl Acad Sci USA. 1994; 91:10596–600. PMID:7937998

26. O’Connell MA, Gerber A, Keller W. Purification of human double-stranded RNA-specific editase 1 (hRED1) involved in editing of brain glutamate receptor B pre-mRNA. J Biol Chem. 1997; 272(1):473–8. PMID:8995285.

27. Marucci R, Brindle J, Paro S, Casadio A, Hempel S, Morrice N, et al. Pin1 and WWP2 regulate GluR2 Q/R site RNA editing by ADAR2 with opposing effects. Embo J. 2011; 30(20):4211–22. PMID:21847096. doi:10.1038/emboj.2011.303

28. George CX, Samuel CE. Characterization of the 5-flanking region of the human RNA-specific adenosine deaminase ADAR1 gene and identification of an interferon-inducible ADAR1 promoter. Gene. 1999; 229(1–2):203–13. PMID:10095120

29. Hartwig D, Schoeneich L, Greeve J, Schutte C, Dom I, Kirchner H, et al. Interferon-alpha stimulation of liver cells enhances hepatitis delta virus RNA editing in early infection. J Hepatol. 2004; 41(4):667–72. PMID:15464249.
30. Patterson JB, Thomis DC, Hans SL, Samuel CE. Mechanism of interferon action: double-stranded RNA-specific adenosine deaminase from human cells is inducible by alpha and gamma interferons. Virology. 1995; 210(2):508–11. PMID: 7618288.
31. Strehblow A, Hallegger M, Jantsch MF. Nucleocytoplasmic distribution of human RNA-editing enzyme ADAR1 is modulated by double-stranded RNA-binding domains, a leucine-rich export signal, and a putative dimerization domain. Mol Biol Cell. 2002; 13(11):3822–35. PMID: 12429827.
32. Samuel CE. Adenosine deaminases acting on RNA (ADARs) are both antiviral and proviral. Virology. 2011; 411(2):180–93. PMID: 21211811. doi: 10.1016/j.virol.2010.12.004
33. Charoenthongtrakul S, Zhou Q, Shembade N, Harhaj NS, Harhaj EW. Human T cell leukemia virus type 1 Tax inhibits innate antiviral signaling via NF-kappaB-dependent induction of SOCS1. J Virol. 2011; 85(14):6955–62. doi: 10.1128/JVI.00007-11 PMID: 21593151; PubMed Central PMCID: PMC3126571.
34. Hartner JC, Schmittwolf C, Kispert A, Muller AM, Higuchi M, Seeburg PH. Liver Disintegration in the Mouse Embryo Caused by Deficiency in the RNA-editing Enzyme ADAR1. J Biol Chem. 2004; 279(6):4894–902. PMID: 14615479.
35. Wang Q, Miyakoda M, Yang W, Khillan J, Stachura DL, Weiss MJ, et al. Stress-induced apoptosis associated with null mutation of ADAR1 RNA editing deaminase gene. J Biol Chem. 2004; 279(6):4952–61. Epub 2003/11/14. doi: 10.1074/jbc.M310162200 [pii]. PMID: 14619393.
36. Hartner JC, Walkley CR, Lu J, Orkin SH. ADAR1 is essential for the maintenance of hematopoiesis and suppression of interferon signaling. Nat Immunol. 2009; 10(1):109–15. PMID: 19060901. doi: 10.1038/ni.1680
37. Osenberg S, Paz Yaacov N, Safran M, Moshkovitz S, Shtrichman R, Sherf O, et al. Alu sequences in undifferentiated human embryonic stem cells display high levels of A-to-I RNA editing. PLoS ONE. 2010; 5(8):e11173. PMID: 20574523. doi: 10.1371/journal.pone.0011173
38. BAZAK L, HAVIV A, BARAK M, JACOB-HIRSCH J, DENG P, ZHANG R, et al. A-to-I RNA editing occurs at over a hundred million genomic sites, located in a majority of human genes Genome Res. Epub 2013 Dec 17. PubMed Central PMCID: PMC24347612.
39. Hundley HA, Krauchuk AA, Bass BL. C. elegans and H. sapiens mRNAs with edited 3' UTRs are present on polysomes. RNA. 2008; 14(10):2050–60. doi: 10.1261/rna.116508 PMID: 18719245; PubMed Central PMCID: PMC2553745.
40. Barral PM, Sarkar D, Su ZZ, Barber GN, DeSalle R, Racaniello VR, et al. Functions of the cytoplasmic RNA sensors RIG-I and MDA-5: key regulators of innate immunity. Pharmacol Ther. 2009; 124(2):219–34. PMID: 19615405. doi: 10.1016/j.pharmthera.2009.06.012
41. Leung DW, Amarasinghe GK. Structural insights into RNA recognition and activation of RIG-I-like receptors. Curr Opin Struct Biol. 2012; 22(3):297–303. doi: 10.1016/j.sbi.2012.03.011 PMID: 22560447; PubMed Central PMCID: PMC3383332.
42. Kowalinski E, Lunardi T, McCarthy AA, Louber J, Brunel J, Grigorov B, et al. Structural basis for the activation of innate immune pattern-recognition receptor RIG-I by viral RNA. Cell. 2011; 147(2):423–35. Epub 2011/10/18. doi: 10.1016/j.cell.2011.09.039 S0092-8674(11)0150-0 [pii]. PMID: 22000019.
43. Luo D, Ding SC, Vela A, Kohlway A, Lindenbach BD, Pyle AM. Structural insights into RNA recognition by RIG-I. Cell. 2011; 147(2):409–22. Epub 2011/10/18. doi: 10.1016/j.cell.2011.09.023 S0092-8674(11)01884-1 [pii]. PMID: 22000018; PubMed Central PMCID: PMC3222294.
44. Wu B, Hur S. How RIG-I like receptors activate MAVS. Curr Opin Virol. 2015; 12:91–8. doi: 10.1016/j.coovi.2015.04.004 PMID: 25942693; PubMed Central PMCID: PMC4470786.
45. Ota H, Sakurai M, Gupta R, Valente L, Wulfef BE, Ariyoshi K, et al. ADAR1 forms a complex with Dicer to promote microRNA processing and RNA-induced gene silencing. Cell. 2013; 153(3):575–89. Epub 2013/04/30. doi: 10.1016/j.cell.2013.03.024 S0092-8674(13)00348-2 [pii]. PMID: 23622242; PubMed Central PMCID: PMC3651894.
46. Bernstein E, Kim SY, Carmell MA, Murchison EP, Alcorn H, Li MZ, et al. Dicer is essential for mouse development. Nat Genet. 2003; 35(3):215–7. doi: 10.1038/ng1253 PMID: 14528307.
47. Vitali P, Scadden AD. Double-stranded RNAs containing multiple IU pairs are sufficient to suppress interferon induction and apoptosis. Nat Struct Mol Biol. 2010; 17(9):1043–50. PMID: 20694008. doi: 10.1038/nsmb.1864
48. Liddicoat BJ, Piskol R, Chalk AM, Ramaswami G, Higuchi M, Hartner JC, et al. RNA editing by ADAR1 prevents MDA5 sensing of endogenous dsRNA as nonself. Science 2015; 349(6252):1115–20. doi: 10.1126/science.aac7049 PMID: 26275108
49. Eskeland R, Leeb M, Grimes GR, Kress C, Boyle S, Sproul D, et al. Ring1B compacts chromatin structure and represses gene expression independent of histone ubiquitination. Mol Cell. 2010; 38(3):452–64. doi: 10.1016/j.molcel.2010.02.032 PMID: 20471950; PubMed Central PMCID: PMC3132514.
50. Dunican DS, Ruzov A, Hackett JA, Meehan RR. xDnmt1 regulates transcriptional silencing in pre-MBT Xenopus embryos independently of its catalytic function. Development. 2008; 135(7):1295–302. doi: 10.1242/dev.016402 PMID: 18305009.

51. Ohman M, Kallman AM, Bass BL. In vitro analysis of the binding of ADAR2 to the pre-mRNA encoding the Glu-R-B R/G site. Rna. 2000; 6(5):687–97. PMID: 10836790

52. Klaue Y, Källman AM, Bonin M, Nellen W, Öhman M. Biochemical analysis and scanning force microscopy reveal productive and nonproductive ADAR2 binding to RNA substrates. RNA. 2003; 9(7):839–46. doi: 10.1261/mi.2167603 PMID: 12810917

53. Rice GI, Bond J, Asipu A, Brunette RL, Manfield IW, Carr IM, et al. Mutations involved in Aicardi-Goutières syndrome implicate SAMHD1 as regulator of the innate immune response. Nat Genet. 2009; 41(7):829–32. PMID: 19525956. doi: 10.1038/ng.373

54. Anderson BR, Muramatsu H, Jha BK, Silverman RH, Weissman D, Kariko K. Nucleoside modifications in RNA limit activation of 2′-S-oligoadenylate synthetase and increase resistance to cleavage by RNase L. Nucleic Acids Res. 2011; 39(21):9329–38. doi: 10.1093/nar/gkr586 PMID: 21813458; PubMed Central PMCID: PMC3241635.

55. Anderson BR, Muramatsu H, Nallagatla SR, Bevilacqua PC, Sansing LH, Weissman D, et al. Incorporation of pseudouridine into mRNA enhances translation by diminishing PKR activation. Nucleic Acids Res. 2010; 38(17):5884–92. doi: 10.1093/nar/gkq347 PMID: 20457754; PubMed Central PMCID: PMC2943593.

56. Kariko K, Buckstein M, Ni H, Weissman D. Suppression of RNA recognition by Toll-like receptors: the impact of nucleoside modification and the evolutionary origin of RNA. Immunity. 2005; 23(2):165–75. PMID: 16111635.

57. Serra MJ, Smolter PE, Westhof E. Pronounced instability of tandem IU base pairs in RNA. Nucleic Acids Res. 2004; 32(5):1824–8. PMID: 15037659.

58. Zhou KI, Parisien M, Dai Q, Liu N, Diatchenko L, Sachleben JR, et al. N-Methyladenosine Modification in a Long Noncoding RNA Hairpin Predisposes Its Conformation to Protein Binding. J Mol Biol. Epub 2015 Sep 4. pii: S0022-2836(15)(00486-6) doi: 10.1016/j.jmb.2015.08.021 PMID: 26343757.

59. Schwartz S, Bernstein DA, Mumbach MR, Jovanovic M, Herbst RH, Leon-Ricardo BX, et al. Transcriptome-wide mapping reveals widespread dynamic-regulated pseudouridylation of ncRNA and mRNA. Cell. 2014; 159(1):148–62. doi: 10.1016/j.cell.2014.08.028 PMID: 25219674; PubMed Central PMCID: PMC4180118.

60. Lovejoy AJF, Riordan DP, Brown PO. Transcriptome-wide mapping of pseudouridines: pseudouridinylation actions modify specific mRNAs in S. cerevisiae. PLoS ONE. 2014; 9(10):e110799. doi: 10.1371/journal.pone.0110799 PMID: 25353621; PubMed Central PMCID: PMC4212993.

61. Carlile TM, Rojas-Duran MF, Zinshetyn B, Shin H, Bartoli KM, Gilbert WV. Pseudouridine profiling reveals regulated mRNA pseudouridylation in yeast and human cells. Nature. 2014; 515(7525):143–6. doi: 10.1038/nature13802 PMID: 25192136; PubMed Central PMCID: PMC4224642.

62. Bakin A, Ofengand J. Four newly located pseudouridylate residues in Escherichia coli 23S ribosomal RNA are all at the peptidyltransferase center: analysis by a new sequencing technique. Biochemistry. 1993; 32(37):9754–62. PMID: 8373778.

63. Kariko K, Muramatsu H, Welsh FA, Ludwig J, Kato H, Akira S, et al. Incorporation of pseudouridine into mRNA yields superior nonimmunogenic vector with increased translational capacity and biological stability. Mol Ther. 2008; 16(1):1833–40. doi: 10.1038/mt.2008.200 PMID: 18797453; PubMed Central PMCID: PMC2775451.

64. Niu Y, Zhao X, Wu YS, Li MM, Wang XJ, Yang YG. N6-methyl-adenosine (m6A) in RNA: an old modification with a novel epigenetic function. Genomics Proteomics Bioinformatics. 2013; 11(1):8–17. doi: 10.1016/j.gpb.2012.12.002 PMID: 23453015; PubMed Central PMCID: PMC4357660.

65. Gehrig S, Eberle ME, Botschen F, Rimbach K, Eberle F, Eigenbrod T, et al. Identification of modifications in microbial, native tRNA that suppress immunostimulatory activity. J Exp Med. 2012; 209(2):225–33. Epub 2012/02/09. doi: 10.1084/jem.20111044.10.1083/jem.20111044 [pii]. PMID: 22312113; PubMed Central PMCID: PMC3280868.

66. Vasu K, Nagaraja V. Diverse functions of restriction-modification systems in addition to cellular defense. Microbiol Mol Biol Rev. 2013; 77(1):53–72. doi: 10.1128/MMBR.00044-12 PMID: 23471617; PubMed Central PMCID: PMC3591985.

67. Jablonowski D, Zink S, Mehlkamp C, Daum G, Schaffrath R. tRNA wobble uridine methylation by Trm9 identifies Elongator’s key role for zymocin-induced cell death in yeast. Mol Microbiol. 2006; 59(2):677–88. doi: 10.1111/j.1365-2958.2005.04972.x PMID: 16390450.

68. Jain R, Poulos MG, Gros J, Chakravarty AK, Shuman S. Substrate specificity and mutational analysis of Kluyveromyces lactis lectin gamma-toxin, a eukaryal tRNA anticodon nucleoside. RNA. 2011; 17(7):1336–43. doi: 10.1261/rna.2722711 PMID: 21610213; PubMed Central PMCID: PMC3138569.
69. Lu J, Huang B, Esberg A, Johansson MJ, Bystrom AS. The Kluyveromyces lactis gamma-toxin targets tRNA anticodons. RNA. 2005; 11(11):1648–54. doi: 10.1261/rna.2172105 PMID: 16244131; PubMed Central PMCID: PMC1370851.

70. Ryvkin P, Leung YY, Silverman IM, Childress M, Valladares O, Dragomir I, et al. HAMR: high-throughput annotation of modified ribonucleotides. RNA. 2013; 19(12):1684–92. doi: 10.1261/rna.036806.112 PMID: 24149843; PubMed Central PMCID: PMC3884653.

71. Harcourt EM, Ehrenschwender T, Batista PJ, Chang HY, Kool ET. Identification of a selective polymerase enables detection of N(6)-methyladenosine in RNA. J Am Chem Soc. 2013; 135(51):19079–82. doi: 10.1021/ja4105792 PMID: 24328136; PubMed Central PMCID: PMC3905807.

72. Schuberth-Wagner C, Ludwig J, Bruder AK, Herzner AM, Zillinger T, Goldeck M, et al. A Conserved Histidine in the RNA Sensor RIG-I Controls Immune Tolerance to N1-2’O-Methylated Self RNA. Immunity. 2015; 43(1):41–51. doi: 10.1016/j.immuni.2015.06.015 PMID: 26187414.