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A-to-I editing of coding and non-coding RNAs by ADARs

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Abstract

Adenosine deaminases acting on RNA (ADARs) convert adenosine to inosine in double-stranded RNA. This A-to-I editing occurs not only in protein-coding regions of mRNAs, but also frequently in non-coding regions that contain inverted Alu repeats. Editing of coding sequences can result in the expression of functionally altered proteins that are not encoded in the genome, whereas the significance of Alu editing remains largely unknown. Certain microRNA (miRNA) precursors are also edited, leading to reduced expression or altered function of mature miRNAs. Conversely, recent studies indicate that ADAR1 forms a complex with Dicer to promote miRNA processing, revealing a new function of ADAR1 in the regulation of RNA interference.

Adenosine to inosine (A-to-I) RNA editing was originally discovered as a mysterious enzymatic activity causing unwinding of double-stranded RNA (dsRNA) in *Xenopus laevis* oocytes and embryos[1]. Soon after, it became clear that this activity is carried out by an adenosine deaminase acting on RNA (ADAR)[2,3]. These discoveries established the field of A-to-I RNA editing[4,5]. Initially, a limited number of editing sites were discovered serendipitously in protein-coding regions of mRNAs, when comparing human genomic DNA versus cDNA sequences. However, the development of deep sequencing and recent advancements in bioinformatics made it possible to screen A-to-I RNA-editing sites globally. Surprisingly, the most frequent and widespread targets of A-to-I RNA editing are dsRNAs made from inverted Alu repetitive elements (Alu dsRNAs), which are located within introns and untranslated regions[6–19].

Precursors of certain microRNAs (miRNAs) also undergo A-to-I RNA editing, which negatively regu lates the expression and function of the mature miRNAs[20–24]. Conversely, recent studies indicate that ADAR1 forms a complex with Dicer to promote miRNA processing and RNA interference (RNAi) efficacy[25,26]. This Review summarizes our current knowledge on A-to-I RNA editing and ADARs in mammals. Its focus, however, is on the

Competing interests statement

The author declares no competing financial interests.

DATABASES

National Cancer Institute Drug Dictionary: http://www.cancer.gov/drugdictionary

Online Mendelian Inheritance in Man (OMIM): http://www.omim.org/ *ADAR1* | *ADAR2* | *ADAR3*

RADAR editing sites database: http://rnaedit.com

DARNED editing sites database: http://beamish.ucc.ie

miRNA editing sites databases: http://www.cs.tau.ac.il/~mirnaed/

miRBase database: http://www.mirbase.org/

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significance of non-coding, repetitive RNA editing and on the interactions between the RNA-editing and RNAi mechanisms. For reviews focusing on the relevance of A-to-I RNA editing to brain functions, viral infection and human diseases, see REFS 4,5,27–35. For reviews on other types of RNA editing, see REFS 36–39.

Mechanism and regulation of RNA editing

A-to-I RNA editing is mediated by ADAR family members, which are conserved in the animal kingdom[40] (FIG. 1a).

ADARs and their domain structures

Vertebrates have three ADAR genes, *ADAR1* (REF. 41), *ADAR2* (REF. 42) and *ADAR3* (REFS 43,44). ADARs have common functional domains (FIG. 1a). The dsRNA-binding domain (dsRBD) (~65 amino acids), which has an α-β-β-β-α configuration, makes direct contact with dsRNA[45]. The carboxy-terminal region contains the deaminase domain that forms the catalytic centre of an ADAR. Certain structural features are unique to particular ADAR members: ADAR1 contains two Z-DNA-binding domains (Zα and Zβ)[46], whereas ADAR3 contains an Arg-rich single-stranded RNA (ssRNA)-binding domain (R domain) at its amino-terminal region[43]. The functional significance of these unique domains is not well understood. The enzymatic activities of ADAR1 (REF. 41) and ADAR2 (REF. 42) have been demonstrated, but the A-to-I RNA-editing activity of ADAR3 is yet to be shown[43,44,47]. ADAR1 is ubiquitously expressed[41], whereas ADAR2 is most highly expressed in the brain, but is also expressed in other tissues[42]. ADAR3 expression is restricted to the brain[43,44]. In addition to these three ADARs, testis nuclear RNA-binding protein (TENR; also known as ADAD1), which is specifically expressed in testes and is required for spermatogenesis[48], and TENR-like (TENRL; also known as ADAD2), which is expressed in the brain[49], have sequence and domain-structure similarity to ADAR but have no deaminase activity, owing to the lack of amino acid residues that are crucial for the catalytic reaction. In *Drosophila melanogaster*, only a single *ADAR2*-like gene, *dAdar*, is present, and in *Caenorhabditis elegans*, two ADAR genes, *adr1* and *adr2*, are known (FIG. 1a). ADARs are absent in all protozoa, yeast and plants[40].

Mechanism of deamination and editing-site selectivity

During the A-to-I RNA editing process, adenosine is converted to inosine by hydrolytic deamination at the C6 position[2,3] (FIG. 1b). The translation machinery reads the inosine as if it were guanosine, base-pairing it with cytosine (FIG. 1b). In this manner, A-to-I RNA editing can result in the incorporation of amino acids that are not directly encoded in the genome. X-ray crystallographic analysis of the catalytic domain of human ADAR2 revealed that His394, Glu396, Cys451 and C516 are involved in the coordination of a zinc ion and formation of the catalytic centre[50]. A base-flipping mechanism probably places the targeted adenosine in the catalytic pocket for the deamination reaction[50]. Structural studies also revealed the presence of inositol hexakisphosphate ($InsP_6$) buried within the enzyme core, surrounded by many Arg and Lys residues and located very close to the catalytic centre. The $InsP_6$ molecule is predicted to have a crucial role during the deamination reaction, although its exact function is currently not known[50].

ADAR acts on both inter- and intramolecular dsRNAs of >20 bp in length[51]. More than half of all adenosines of long (>100 bp), fully base-paired dsRNAs can be edited by ADARs. By contrast, only a few adenosines of short and/or partially base-paired dsRNAs are selectively edited, perhaps indicating that the secondary structure of substrates dictates editing-site selectivity[52]. For example, site selectivity in the glutamate receptor GRIA2 (formerly known as GluR2 and GluRB) precursor mRNA (pre-mRNA) at the Q/R site requires an intramolecular dsRNA structure that is formed between the exonic sequence around the editing site and a downstream intronic complementary sequence termed the ECS (editing site complementary sequence)[53]. Owing to this requirement for the intron, A-to-I editing at this site is believed to occur in the nucleus, either before or simultaneously with splicing. Although no strict sequence specificity is required for A-to-I RNA editing, a preference for editing adenines neighbouring 5′ uridine and 3′ guanosine has been reported[54]. Certain sites are edited by ADAR1 only or ADAR2 only, whereas other sites are edited equally well by both[54–57] (TABLES 1, 2).

Regulation of ADAR expression and localization

Transcription from separate promoters generates two isoforms of ADAR1, a full-length, interferon-inducible ADAR1p150 and a shorter and constitutively expressed ADAR1p110, which lacks the N-terminal portion of the protein, including the Zα domain[58,59] (FIG. 1a). ADAR2 expression is positively regulated by the transcription activator CREB (cyclic adenosine monophosphate response element-binding protein) in the brain[60], and by c-Jun N-terminal kinase 1 (JNK1; also known as MAPK8) in pancreatic β-cells[61]. Interestingly, CREB also suppresses transcription from the ADAR1p110 promoter in metastatic melanomas[62]. Homodimerization is required for the A-to-I RNA-editing activities of ADAR1 and ADAR2 (REFS 63–65). The third dsRBD of ADAR1 and the first dsRBD of ADAR2 are required for their homodimerization[26,64]. ADAR3 is unable to homodimerize, which may underlie its lack of A-to-I RNA editing activity[63].

Both ADAR1p150 and ADAR1p110 shuttle between the nucleus and the cytoplasm[66–68] (FIG. 2a). Binding of the nuclear export factor exportin 1 (XPO1; also known as CRM1) to the nuclear export signal (NES) located within the Zα domain, together with RAN·GTP, regulates nuclear export of ADAR1p150 (REF. 69). The nuclear localization signal (NLS) located in the third dsRBD is responsible for localization of ADAR1 in the nucleus and nucleolus[66,68]. Nuclear export of ADAR1p110 is mediated by XPO5–RAN·GTP and is regulated by dsRNA binding to the dsRBDs, whereas nuclear import of ADAR1p110 is mediated by binding of transportin 1 (TRN1) to the third dsRBD, which is inhibited by binding of dsRNA[68,70]. The predominantly nucleolar localization of ADAR2 is regulated by the binding of karyopherin subunit α1 (KPNA1) and KPNA3 (REF. 71) to an Arg-rich NLS in the N-terminal region[66,71,72] (FIG. 2b). Post-translational modification regulates the nuclear localization and stability of ADAR2. Phosphorylation of Thr32 activates ADAR2 interaction with the prolyl-isomerase PIN1 in a dsRNA-binding dependent manner, which isomerizes Pro33 and posi tively controls the nuclear localization and stability of ADAR2. By contrast, the E3 ubiquitin ligase WWP2 promotes the degradation of ADAR2 in the cytoplasm[73] (FIG. 2b).

Editing of protein-coding sequences

Transcripts of a relatively small number of genes are edited within their coding regions; this is termed recoding-type editing[28,29]. These include physiologically important mammalian genes, such as those encoding the glutamate receptor subunit GluR2 (REF. 53), the G protein-coupled serotonin receptor 5-$HT_{2C}R$[74], the potassium channel Kv1.1 (REF. 75) and the α3 subunit of $GABA_A$ (γ-aminobutyric acid type A) receptor (GABRA3)[76]. Recoding-type editing often dramatically alters protein functions[28,29] (TABLE 1). For instance, editing of the Q/R site (recoding of Gln to Arg) located in the channel-pore-loop domain of GluR2 results in a channel that is impermeable to Ca^{2+} (REF. 53), whereas editing of five adenosines located in the second intracellular loop domain of 5-$HT_{2C}R$ changes the G protein-coupling functions of the receptor[74]. Editing of the I/V site of Kv1.1 substantially reduces the inactivation rate of this voltage-gated channel[75], and the trafficking and proper localization of GABRA3 are reduced by editing of its I/M site[76]. Recent transcriptome deep sequencing and global screening for editing sites have revealed that the recoding type of A-to-I editing also occurs in genes other than those encoding neurotransmitter receptors and ion channels. However, recoding-type editing to any significant degree (>20% editing) is rare; the functions of ~80 mammalian genes in total might be regulated by A-to-I editing[7,13,15,17–19].

Deficiency in A-to-I RNA editing

Adar2-null mutant mice die several weeks after birth following frequent epileptic seizures, which are caused by neuronal death owing to excess influx of Ca^{2+}. This is a result of severe deficiency in the editing of an almost exclusive ADAR2 target, *GRIA2* pre-mRNA, at its Q/R site[56]. Deficient editing of the Q/R site seems to underlie the loss of motor neurons in patients with sporadic amyotrophic lateral sclerosis (ALS)[77], and death of motor neurons and other symptoms of ALS are indeed detected in motor neuron-specific knockouts of *Adar2* in mice[78]. Furthermore, deficiency in GluR2 Q/R-site editing and the consequent excess Ca^{2+} influx have been proposed to activate the kinase AKT and lead to glioblastoma proliferation[79], as well as neuronal death in forebrain ischemia[60]. The inactivation of *A*DAR1 in mice results in an embryonic-lethal phenotype that is characterized by defective erythropoiesis, aberrant activation of interferon signalling and widespread apoptosis[55,57,80–82]. ADAR1 seems to protect organisms from the deleterious effects of interferon activation, which is relevant to many human pathological processes, such as chronic inflammation and autoimmune disorders[80]. The embryonic lethal phenotype of *Adar1*-null mice can be rescued by the simultaneous inactivation of mitochondrial antiviral signalling adaptor protein (MAVS)[83] or melanoma differentiation-associated protein 5 (MDA5; also known as IFIH1)[81], the upstream genes involved in the interferon activation pathway. This indicates the relevance of ADAR1 function in the regulation of the interferon pathway to the embryonic-lethal phenotype of *Adar1*-null mice[81,83]. Endogenous long dsRNAs produced from inverted retrotransposon repeats of the LINE (long interspersed nuclear elements) and SINE (short interspersed nuclear elements) families and located in the 3′ untranslated regions (UTRs) of several genes, such as Krüppel-like factor 1 (*Klf1*), optineurin (*Optn*), and OPA-interacting protein 5 homologue (*Oip5*), have been proposed as crucial ADAR1-substrate RNAs. Failure to edit these dsRNAs may lead to the activation of

cytosolic dsRNA sensing by MDA5 and of MAVS-mediated interferon signalling in *Adar1*-null mouse embryos[81].

Dysfunction of A-to-I editing causes human diseases

Nine *ADAR1* mutations were found in a subset of patients with Aicardi–Goutières syndrome (AGS)[84]. AGS is an autosomal-recessive inflammatory disorder that affects the brain and skin and is characterized by an aberrant immune response and increased interferon-α expression[84]. Failed editing of certain endogenous dsRNAs such as Alu by the mutant ADAR1, and the consequent activation of interferon signalling, seem to underlie the pathogenesis of AGS[83,85]. Indeed, the overproduction of interferons is detected in the brains and spinal cords of conditional *Adar1*-null mice[85]. A large number of *ADAR1* mutations (>130) are also associated with dyschromatosis symmetrica hereditaria (DSH), which is an autosomal-dominant disorder that is mainly found in Asian individuals and is characterized by hypo- and hyper-pigmentation of the skin[86]. *ADAR1* haploinsufficiency, as well as dominant-negative effects of the mutant ADAR1, are likely to underlie the pathogenesis of DSH[65,86].

The 5-HT_{2C}R pre-mRNA is edited at five sites by ADAR1 and ADAR2 (REFS 55–57), and the combinatorial editing of these sites results in the expression of 24 protein isoforms. Mutant mouse lines expressing either the unedited or the fully edited forms of 5-HT_{2C}R have been established[87,88]. Mutant mice expressing fully edited 5-HT_{2C}R had significantly decreased fat mass and increased energy expenditure, resulting from hyperactivation of the sympathetic nervous system, suggesting that 5-HT_{2C}R mRNA editing has a regulatory role in lipolysis and metabolism[87]. The same 5-HT_{2C}R mRNA-editing mutant mice exhibit a phenotype similar to Prader–Willi syndrome (PWS), which is characterized by obesity and a range of developmental abnormalities[88]. However, the mutant mice lacked certain PWS-associated symptoms such as obesity, and thus the relevance of over-editing of 5-HT_{2C}R mRNA to PWS needs further investigation. The 5-HT_{2C}R mRNA-editing mutant mice (both over- and under-edited) exhibit anti-depressive and exaggerated anxiety- like behaviours, indicating a relevance of 5-HT_{2C}R mRNA editing to psychiatric disorders[89]. An association of altered editing patterns of 5-HT_{2C}R with several psychiatric disorders, including anxiety, depression, bipolar disorder, schizophrenia and suicide, as well as with autism, has been reported, although the findings have often been inconsistent owing to the difficulty of analysing post-mortem human brain samples[28,29,90].

Alu dsRNA editing and its implications

Recent advances in sequencing technology, as well as in bioinformatics analyses of sequence databases, have made it possible to globally screen for previously unknown A-to-I editing sites in normal tissues and in various cancers. This has resulted in the identification of many millions of new editing sites in human transcripts[6–19]. Surprisingly, almost all of these new sites reside in introns and 3′ UTRs that harbour Alu dsRNAs.What is the fate of RNAs carrying highly edited Alu repeats? Are there any proteins that specifically recognize and interact with these highly edited RNAs? Are there any functions for highly edited Alu dsRNAs?

Exonization of intronic Alu sequences

An inosine is recognized by the splicing machinery as a guanosine. Thus, A-to-I editing of Alu sequences can generate splice donor and acceptor sites. For instance, AU-to-IU editing will generate a sequence that could be recognized as the canonical 5′ splice donor site GU (IU as GU), and AA-to-AI editing will generate a sequence that could be recognized as the 3′ splice acceptor site AG (AI as AG). Self-editing by ADAR2 of an intronic sequence of its own pre-mRNA indeed results in the creation of an alternative 3′ splice acceptor site and the suppression of ADAR2 expression, thereby functioning as a negative autoregulatory mechanism[91,92]. A-to-I editing-mediated exonization of Alu sequences (FIG. 3a) has been reported for several genes, including G protein-coupled receptor 107 (REF. 6), nuclear prelamin A[93] and seryl-tRNA synthetase[19].

Retention of edited Alu dsRNAs in paraspeckles

The inosine-specific RNA-binding protein p54^{nrb} was proposed to mediate the specific retention in nuclear paraspeckles of mRNAs containing extensively edited Alu dsRNA[94] (FIG. 3b). At least 333 human genes contain Alu dsRNA sequences in their 3′ UTRs[95]. A-to-I editing and p54^{nrb}-dependent nuclear retention of transcripts of one such human gene, nicolin 1, has been demonstrated[95]. Another example is the mouse *Ctn* gene[96]. Ctn transcripts contain a long dsRNA formed of inverted repeats of mouse SINEs in the 3′ UTR[96]. Under conditions of stress, Ctn RNA is post-transcriptionally cleaved and polyadenylated at an alternative site, resulting in the loss of the edited SINE sequences and the release of the protein-coding *mCat2* (also known as *Slc7a2*) mRNA to the cytoplasm, where it is translated into cationic amino acid transporter 2 proteins[96] (FIG. 3b). More recent studies have revealed that the nuclear paraspeckle assembly transcript 1 (NEAT1), a long non-coding RNA, is also required for the formation of nuclear paraspeckles and for the retention mechanism. Human embryonic stem cells (ES cells) lack NEAT1 and do not form paraspeckles. Accordingly, LIN28 mRNAs containing extensively edited Alu dsRNA in their 3′ UTRs can be detected in the cytoplasm despite the presence of p54^{nrb}, indicating that both p54^{nrb} and NEAT1 are required for the retention mechanism[97].

Degradation of edited Alu dsRNAs by endonuclease V

A ribonuclease activity that specifically cleaves both RNA strands of a dsRNA that contains multiple I·U base pairs (that is, extensively edited Alu dsRNA) has been reported[98]. Endonuclease V (EndoV) was recently identified as this ribonuclease[99]. Tudor staphylococcal nuclease (Tudor-SN) seems to promote the activity of EndoV as a cofactor[100]. Thus, A-to-I editing of Alu dsRNAs may lead to degradation by EndoV together with Tudor-SN, which in turn might control the expression levels of genes harbouring Alu dsRNA (FIG. 3c). A-to-I hyperedited RNAs are easily detected in steady-state mRNA pools, and thus their degradation is not constitutive and must be regulated, perhaps by compartmentalization of EndoV and Tudor-SN: cytoplasmic as well as nucleolar localization of EndoV has been reported[99], whereas Tudor-SN localizes to stress granules in stress conditions[101,102]. Interestingly, ADAR1p150 seems to bind to extensively edited dsRNA via its Zα domain and localizes together with Tudor-SN to stress granules in stress conditions, although the roles of ADAR1p150 and Tudor-SN, as well as the fate of the extensively

edited dsRNA in stress granules, remain to be established[102,103]. Thus, EndoV–Tudor-SN could regulate the expression of genes containing extensively edited Alu dsRNA in special circumstances, for instance, during stress or viral infection.

Suppression of the interferon response

The interferon signalling pathway is activated in response to infection by pathogens such as viruses and bacteria, as well as by long dsRNAs such as synthetic poly(I:C). A biological function for extensively edited dsRNAs in the suppression of the interferon signalling pathway has been proposed, using a synthetic dsRNA that contains multiple and consecutive IU-mismatched wobble base pairs (IU-dsRNA)[104]. IU-dsRNA, which resembles extensively edited Alu dsRNA, forms a unique configuration[104] and inhibits the activation of interferon signalling and of interferon-stimulated genes (ISGs) induced by poly(I:C) dsRNAs in cultured cell lines. The IU-dsRNA was proposed to competitively inhibit binding to poly(I:C) dsRNA by retinoic acid-inducible gene 1 (RIG1; also known as DDX58) or MDA5, both of which are cytosolic sensors for dsRNA and upstream regulators of the MAVS-mediated interferon activation pathway[104] (FIG. 3d). The same IU-dsRNA was shown to suppress the interferon pathway that is aberrantly activated in *Adar1*-null mouse embryonic fibroblasts[83]. Recent studies identified LINE and SINE dsRNAs present in 3′ UTRs of *Klf1, Optn,* and *Oip5* as candidate sources of endogenous IU-dsRNAs[81]. Failure in hyper-editing these dsRNAs by ADAR1 may lead to their sensing by RIG1 and MDA5, and consequent activation of MAVS-mediated interferon signalling, which is perhaps relevant to *Adar1*-null mouse phenotypes, as well as to the pathology of AGS[81].

Heterochromatin formation and gene silencing

The involvement of RNAi and its components (such as endogenous short interfering RNAs (endo-siRNAs) and PIWI-interacting RNAs) in the establishment of heterochromatin and in silencing the expression of repetitive sequences and transposons is well known in plants, fission yeast and various other eukaryotes. However, it has been debated whether similar mechanisms operate in mammalian cells[105]. Interestingly, vigilin, which is a multi-KH-domain protein, binds to inosine-containing RNAs such as extensively edited Alu dsRNA and forms a complex with ADAR1, the KU86 (86 kDa subunit of Ku antigen; also known as XRCC5)–KU70 heterodimer (which is involved in the repair of DNA double-strand breaks), ATP-dependent RNA helicase A (RHA) and heterochromatin protein 1 (HP1)[106]. The *D. melanogaster* homologue of vigilin, Ddp1, localizes to heterochromatin and is essential for gene silencing in flies. Vigilin also interacts with SUV39H1, which methylates histone H3 on Lys9 (H3K9me). The H3K9me epigenetic mark provides a binding site for HP1, which mediates the formation of heterochromatin and gene silencing[107] (FIG. 3e). Association of Alu elements with H3K9me and their involvement in heterochromatin formation have been reported[108]. These findings suggest a possible contribution of extensively edited Alu dsRNAs to heterochromatin formation and gene silencing of a region enriched in Alu sequences.

By contrast, antagonistic effects of *dAdar* (which is the only known *D. melanogaster* ADAR gene) on hetero chromatic gene silencing of *Hoppel* transposable elements were reported. It was proposed that dAdar edits a long dsRNA generated from *Hoppel* loci, thereby

preventing Dicer from processing it into endo-siRNAs, which are required for RNAi-mediated hetero chromatin formation and gene silencing[109]. However, the involvement of Dicer in this transposon-silencing mechanism and the generation of endo-siRNAs from *Hoppel* elements remain to be shown.

Editing of miRNAs and its consequences

Primary miRNA (pri-miRNA) transcripts fold to form dsRNA (hairpin) structures, which are processed in the nucleus into precursor-miRNAs (pre-miRNAs) of ~70 nt in length by the RNase III protein Drosha, in complex with the pri-miRNA recognition factor DGCR8 (FIG. 4a). Pre-mRNAs are then exported to the cytoplasm, where they are processed further by another RNase III protein, Dicer, in complex with TAR RNA-binding protein (TRBP; also known as TARBP2) to generate double-stranded, mature miRNAs of ~22 nt in length. Mature miRNAs are loaded onto Argonaute (AGO) proteins and together form the core of the RNA-induced silencing complex (RISC). The miRNA guide strand (the functional strand retained by AGO) directs the RISC to the target mRNAs, causing translation repression or mRNA decay. Nucleotides 2–8 of the guide strand, known as the seed sequence, are particu larly important in directing the selection of mRNA targets[110]. miRNA-mediated gene silencing has crucial roles in many biological processes, such as tissue differentiation, cell proliferation, embryonic development and apoptosis, and its misregulation can result in human diseases[111,112]. Certain pri-miRNAs undergo A-to-I editing (TABLE 2), which affects their biogenesis and function.

Approximately 20% of pri-miRNAs are edited in the adult human brain[21]. In addition, editing of several pri-miRNAs encoded by DNA viruses (Epstein–Barr virus (EBV) and Kaposi sarcoma-associated herpes virus HHV-8) has been reported[20,113,114]. It was anticipated that next-generation sequencing of small RNAs would reveal many new A-to-I editing sites in miRNAs. However, only a small number of new sites that are edited at significant frequency (>5% editing) were identified in mature miRNAs, indicating that the expression of edited mature miRNAs is relatively rare[115,116]. This may be because editing of pri-miRNAs results mostly in inhibition of miRNA biogenesis.

Suppression of miRNA biogenesis

ADARs can suppress miRNA maturation at different processing stages by editing-dependent and editing-independent mechanisms. The recognition of pri-miRNA hairpin structures by the Drosha–DGCR8 complex can be affected by A-to-I editing, as was first demonstrated for pri-miR-142. Editing of pri-miR-142 at the +4 and +5 positions (counting from the 5′ end of the mature miRNA sequence) by ADAR1 and ADAR2 inhibits its cleavage by Drosha–DGCR8 (REF. 24) (FIG. 4a). As expected, the expression of miR-142-5p is substantially higher in the spleen of *Adar1*- and of *Adar2*-null mice compared with wild-type mice[24]. Although editing of pri-miR-142 prevents its processing to pre-miR-142, no accumulation of edited pri-miR-142 was detected in HEK293 cells ectopically overexpressing ADAR1 and ADAR2, owing to their degradation by EndoV–Tudor-SN[99]. Degradation of edited fly pri-miR-125, presumably by EndoV–Tudor-SN, was also reported[117]. Thus, A-to-I editing-dependent degradation of pri-miRNAs could be considered to be a control mechanism of miRNA biogenesis and activity. Inhibition of cleavage by Drosha was also reported for pri-

miR-33, pri-miR-133a2 and pri-miR-379 (REF. 21). By contrast, editing increases Drosha cleavage — although very slightly — for pri-miR-197, and substantially for pri-miR-203 (REF. 21), as well as for *D. melanogaster* pri-miR-100 (REF. 117). Editing of pri-miR-455 at the +2 and +17 positions by ADAR1 and suppression of the Drosha cleavage step were also reported in human melanocytes[62]. Suppression of ADAR1 expression, and thus reduced editing of these sites, results in increased expression of miR-455-5p and suppression of its target, the tumour suppressor cytoplasmic polyadenylation element-binding protein 1 (CPEB1), which could be relevant to metastasis of melanomas[62].

ADAR1 edits the −1 and +3 positions of pri-miR-151 in certain tissues, such as amygdala, cerebral cortex and lung, which results in a complete block of pre-miR-151 cleavage by Dicer–TRBP and inhibition of miR-151-3p expression[22] (FIG. 4a). Binding of the Dicer–TRBP complex to unedited and to edited pre-miR-151 are comparable, indicating that Dicer cleavage, not binding, is inhibited by editing. Partial prevention of pre-let-7g cleavage by Dicer due to editing of the +4 position by ADAR2 was also reported[21].

Loading of miRNA onto AGO2-containing RISC can also be inhibited by editing. ADAR1 edits the +20 position of pri-miR-BART6, a miRNA encoded by the EBV, which results in the inhibition of miR-BART6-5p loading onto RISC and thus of its function; this, in turn, affects the latency state of EBV[20] (FIG. 4a). When not edited, miR-BART6-5p specifically targets the human Dicer mRNA, which is evidence that EBV has developed a unique strategy to suppress host RNAi[20]. Thus, editing of pri-miR-BART6 by ADAR1 could have evolved as a human counteractive strategy against the suppression of RNAi by EBV.

Owing to its dsRNA-binding capacity, ADAR2 seems to sequester and thus inhibit the processing of pri-miR-376a1 and pri-miR-376a2, most probably at the Drosha cleavage step[118]. Editing-independent suppression by ADAR2 of miRNA processing and its tumour-promoting role in glioblastoma were also proposed[119]. Expression of miRNAs is indeed altered in *Adar2*-null mouse embryos, most probably through an editing-independent mechanism[120]. ADAR1 also suppresses the expression of many miRNAs, including the stem cell self-renewal-promoting miR-302 family of miRNAs, in an RNA editing-independent manner, which is essential for neural differentiation of human ES cells[121]. Editing-independent suppression of miR-222 expression by ADAR1 and the consequent upregulation of ICAM1, and the relevance of this to melanoma immune resistance, have also been reported[122]. Finally, global screening for ADAR1-binding sites suggested that ADAR1 might compete with DGCR8 for binding to many pri-miRNAs[123]. Thus, a larger subset of miRNAs than those edited might be affected by both ADAR1 and ADAR2, independently of their catalytic functions.

Alteration of miRNA target specificity

In some cases pri-miRNA editing does not inhibit miRNA maturation, leading to the expression of edited mature miRNAs that can be loaded onto AGO2–RISC. However, as editing — even at a single site — can alter the base pairing properties of the miRNA, it can also affect recognition of its target mRNA, especially if editing takes place within the seed sequence of the miRNA.

Members of the miR-376 cluster are transcribed as one transcript and processed to individual pre-miRNAs[23]. In miR-376a, at least two main sites are edited, corresponding to +4 (located in the 5p seed sequence) and +44 (or alternatively numbered as the +6 site; located in the 3p seed sequence). ADAR2 edits the +4 site, whereas ADAR1 edits the +44 site. Interestingly, edited miR-376a targets an almost completely different set of genes than unedited miR-376a. One of the targets specific to the miR-376a-5p edited at the +4 site is phosphoribosyl pyrophosphate synthetase 1 (PRPS1), which is an essential enzyme involved in purine metabolism and in the uric acid synthesis pathway (FIG. 4b). A human disorder characterized by gout and neuro-developmental impairment with hyperuricaemia is caused by substantially increased PRPS1 expression, indicating the importance of tightly regulated expression of this enzyme. *Adar2*-null mice have both PRPS1 and uric acid levels upregulated approximately twofold in the cortex. No increase in PRPS1 or uric acid levels was detected in the livers of *Adar2*-null mice, consistent with the fact that the +4 site is barely edited in wild-type mouse liver[23]. This proves that editing of the +4 site of pri-miR-376a by ADAR2 tightly regulates uric acid levels in a tissue-specific manner by redirecting miRNA target specificity[23] (FIG. 4b). Editing of miR-376 cluster miRNAs increases from embryonic day 19 (E19) in mouse embryos, suggesting that it may be important for embryo development[124]. Furthermore, the differential silencing of RAS-related protein RAP2A and of the E3 ubiquitin ligase AMFR by unedited and edited miR-376a-5p, respectively, were reported to affect glioblastoma metastasis[125]. Seed sequence editing of several other mature miRNAs has also been reported[21,114,124] and is likely to alter target gene specificity (FIG. 4a). Silencing of the tumour suppressor *Dice1* (deleted in cancer 1; also known as *Ints6*) by the EBV-encoded miR-BART3-5p is antagonized owing to editing of pri-miR-BART3 at the +5 site (seed sequence) by ADAR1 (REF. 113).

Regulation of RNAi by ADARs

Both A-to-I RNA-editing and RNAi pathways act on dsRNA. It has been speculated that the A-to-I editing and RNAi may interact with each other by competing for shared dsRNA substrates[126]. Indeed, the two processes antagonistically interact (FIG. 5a) (see the discussion on miRNA editing in the previous section and the discussion below). Conversely, a stimulative interaction between RNA editing and RNAi machinery proteins exists, as ADAR1 forms a complex with Dicer and promotes its activity (FIG. 5b).

Suppression of RNAi

Analyses of ADAR-null *C. elegans* strains have revealed the presence of an antagonistic interaction between A-to-I RNA-editing and RNAi pathways[127,128]. *C. elegans* strains that contain homozygous deletions of both *adr1* and *adr2* genes have a chemotaxis-defective phenotype[128], which is rescued by crossing these worms with RNAi-defective worms, indicating that enhanced RNAi leading to the suppression of a chemotaxis gene underlies the chemotaxis-defective phenotype of ADAR-null worms[127,128]. A-to-I RNA editing of a dsRNA made of a chemotaxis gene transcript could perhaps inhibit its silencing by RNAi[127,128] (FIG. 5a). However, details of the putative interaction between RNA editing and RNAi, as well as the identity of the chemotaxis gene, remain unknown.

It has recently been reported that endo-siRNAs derived from loci enriched with inverted repeats and transposons are dramatically upregulated in ADAR-null mutant worms. A-to-I RNA editing of dsRNA regions of transcripts derived from these loci seems to inhibit their entry into the RNAi silencing pathway and to consequently suppress synthesis of endo-siRNAs from these transcripts[129]. A separate study suggested that biogenesis of not only endo-siRNAs, but also of miRNAs, is significantly affected in ADAR-null mutant worms[130] (FIG. 4a). In the fruit fly *white+* eye reporter system, antagonistic effects on RNAi were observed by the introduction of human ADAR1p150, but not of ADAR1p110 or ADAR2. Interestingly, it is not the A-to-I editing but the dsRNA-binding activity of ADAR1p150 that seems to be responsible for its RNAi-antagonistic function[118].

ADAR1 interacts with Dicer and promotes its activity

Analysis of proteins that interact with epitope-tagged Dicer or ADAR1 has revealed a robust and direct interaction between Dicer and ADAR1 (REF. 26). Although both ADAR1p150 and ADAR1p110 can form the complex *in vitro*, ADAR1p110 seems to be the true partner of Dicer *in vivo*[25]. ADAR1 distinguishes between its functions in RNA editing and in RNAi by the formation of two different complexes: ADAR1–ADAR1 homodimers in the nucleus for RNA editing, and Dicer–ADAR1 heterodimers in the cytoplasm. ADAR1 in complex with Dicer has no A-to-I RNA-editing activity, perhaps reflecting the fact that homodimerization is required for its A-to-I RNA-editing activity[63]. In addition to Dicer, ADAR1 interacts indirectly with AGO2 through its interaction with Dicer, resulting in the formation of Dicer–ADAR1–AGO2 complexes of ~450 kDa in size[26]. Dicer contains a DEAD-box RNA helicase domain in its N-terminal region, followed by DUF283 and PAZ domains, two catalytic RNase III domains, and a dsRNA-binding domain at the C-terminus. The second dsRBD (dsRBD2) of ADAR1, and the DEAD-box RNA helicase and DUF283 domains of Dicer, are required for the formation of the Dicer–ADAR1 complex[26].

In principle, ADAR1 as part of the Dicer–ADAR1 complex could be inhibitory with respect to Dicer function. In fact, however, it increases the V_{max} of Dicer-mediated cleavage of pre-miRNAs fourfold in comparison with the reaction with Dicer alone[26]. Similar analyses on Dicer cleavage of a long dsRNA indicated that ADAR1 also promotes processing of endo-siRNAs, showing that ADAR1 upregulates the turnover rate of Dicer and substantially increases the overall rate of miRNA and endo-siRNA production[26] (FIG. 5b). miRNAs generated by the Dicer–ADAR1 complex were found to be fully functional when tested in various miRNA silencing assays[26]. In addition, ADAR1 substantially pro- motes RISC assembly and loading of miRNAs[26] (FIG. 5b). Interestingly, neither the dsRNA-binding nor the deamin ase activities of ADAR1 is required for promoting the miRNA-processing and RISC-loading activities of Dicer. The catalytic activity of Dicer is auto-inhibited by its DEAD-box RNA helicase domain[131]. Thus, the enhancement of Dicer activity by ADAR1 may be due to ADAR1 binding to the Dicer DEAD-box RNA helicase domain, thereby blocking its auto-inhibitory effect[26].

ADAR1 upregulates miRNA expression in mouse embryos

Analysis of miRNA expression levels in mouse embryos indicates that a rapid and dramatic increase of miRNA production occurs globally at around E11–E12 (REFS 25,26), which is

likely to be essential for embryo development. This developmental stage-specific increase in miRNA production seems to be caused by concomitant upregulation of Dicer and ADAR1p110 (REF. 26). Global suppression of miRNA production is detected in *Adar1*-null mouse embryos, which die at around E12 (REFS 25,26). In contrast to the rapid increase in ADAR1p110 expression, TRBP expression remains very low around this period, perhaps indicating that the contribution of ADAR1 is more important than that of TRBP in the miRNA-mediated RNAi mechanism, at least during embryonic development of the E11–E12 stage[26].

The considerable upregulation of miRNA production at the E11–E12 stage cannot occur in *Adar1*-null mouse embryos, owing to the lack of the Dicer–ADAR1p110 complex[25,26]. This seems to result in dysregulated expression of many genes, which would otherwise be repressed by these miRNAs during normal development, as dramatic changes of global gene expression patterns are detected in the *Adar1*-null embryos. The target genes have different functions, but cell death control and activation of interferon signalling are two of the most significant functions represented[26]. Thus, deficiency in the RNAi function of ADAR1 may underlie, at least partly, the embryonic lethality of *Adar1*-null mice around E12 (REFS 25,26).

Concluding remarks and outlook

It is now almost 30 years since the discovery of A-to-I RNA editing mediated by ADAR[4,5]. Since then, considerable progress has been made in understanding the editing mechanism, characterizing invertebrate and vertebrate ADAR genes, identifying numerous A-to-I editing sites in a wide range of coding and non-coding RNAs, and unravelling the relevance of A-to-I RNA editing to human diseases and revealing its interactions with RNAi pathways.

Nonetheless, we realize that many important questions in this field remain to be answered. For example, what is the selective advantage that initially drove the evolution of A-to-I RNA editing in the animal kingdom? ADAR genes are absent in plant, fungi and yeast genomes. In these organisms, very powerful RNAi pathways utilize dsRNA and play a major part in many important processes, such as silencing of transposons and heterochromatin formation. Did A-to-I RNA editing evolve as a mechanism to assist or replace RNAi? ADAR expression levels are not necessarily correlated well with A-to-I RNA-editing levels of target RNAs within a given tissue or developmental stage, indicating the presence of a currently unidentified mechanism (or mechanisms) that determines editing levels. Certain ADAR gene family members seem to have functions in addition to A-to-I RNA editing, for example, the function of ADAR1 in RNAi. However, it is not known how the balance between the A-to-I RNA-editing and the RNAi functions of ADAR1 is regulated. Exciting findings are likely to be made in the field of A-to-I RNA editing, through future investigations addressing these questions.

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Alu

A type of retrotransposon of the short interspersed nuclear elements (SINE) family found in primate genomes. There are about 1.4 million copies of Alu in the human genome.

Z-DNA

A left-handed form of DNA that is different from the common A and B structural isoforms of DNA. Its biological functions are largely unknown.

Deamination

The chemical process that replaces a primary amino group by a hydroxyl group, resulting in conversion of one nucleoside to another.

Inositol hexakisphosphate

($InsP_6$). An intracellular organic compound that is found throughout the animal kingdom and is affiliated with a wide range of important physiological activities such as modulation of haemoglobin structure and function.

Retrotransposon

A class of genetic elements that includes endogenous retroviruses and transposable elements, which propagate in the genome through an intermediate RNA stage.

Nuclear paraspeckles

Discrete, irregularly shaped nuclear compartments. Usually, approximately 10–30 paraspeckles are present in the interphase mammalian nucleus. Their function is not known, but they may trap certain proteins in the nucleus.

Wobble base pairs

Pairs of nucleotides other than G:C and A:U, such as thermodynamically less stable I:U and G:U pairs. Wobble base pairs, like Watson–Crick base pairs, participate in RNA folding and the formation of secondary structures.

Endogenous short interfering RNAs

(endo-siRNAs). siRNAs derived from endogenous double-stranded transcripts and repetitive elements such as Alu or other retrotransposons.

RNase III protein

A double-stranded RNA (dsRNA)-specific endonuclease that cleaves dsRNA into short fragments with a 3′ overhang and a recessed 5′ phosphate. The RNA interference (RNAi) factors Drosha and Dicer are such proteins.

RNA-induced silencing complex

(RISC). A complex containing short interfering RNAs (siRNAs) or microRNAs (miRNAs) and an Argonaute protein, which mediates the degradation or translation inhibition of target mRNAs that have high sequence complementarity to the small RNAs.

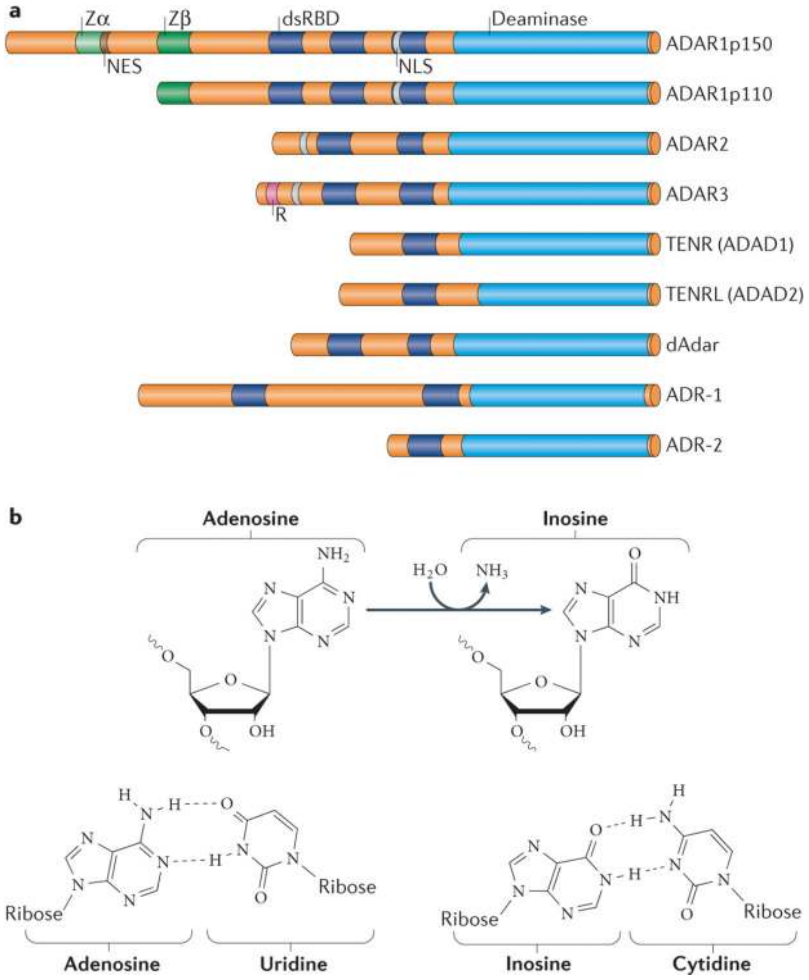

Figure 1. Deamination of adenosine to inosine by adenosine deaminases acting on RNA (ADAR) proteins

a | Three human ADAR family members (ADAR1, ADAR2 and ADAR3), two human ADAD (adenosine deaminase domain-containing) family members (TENR and TENRL), *Drosophila melanogaster* dAdar, and two *Caenorhabditis elegans* ADAR proteins (ADR-1 and ADR-2), share common functional domains. These include two or three repeats of the double-stranded RNA (dsRNA)-binding domain (dsRBD) and a catalytic deaminase domain. Certain structural features, such as Z-DNA-binding domains (Zα and Zβ) and the Arg-rich, single-stranded RNA (ssRNA)-binding R domain, are unique to particular ADAR members. **b** | ADARs catalyse a hydrolytic deamination reaction that converts adenosine to inosine (top). Whereas adenosine base-pairs with uridine, inosine behaves like a guanosine, as it base-pairs with cytidine in a Watson–Crick-bonding configuration (bottom). NES, nuclear export signal; NLS, nuclear localization signal. Part **b** reprinted with permission from REF. 5, Annual Reviews.

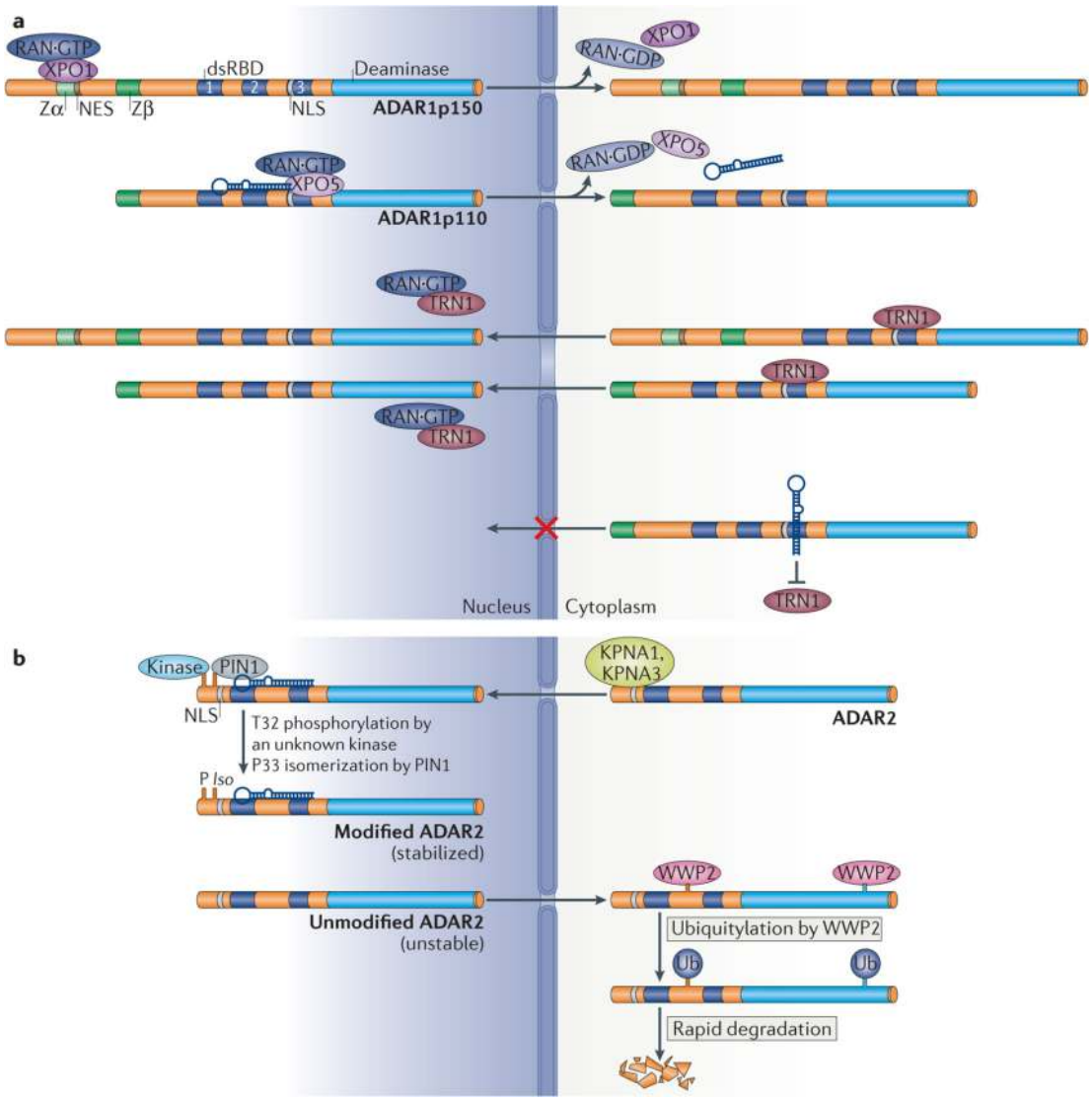

Figure 2. Cellular localization of adenosine deaminases acting on RNA 1 (ADAR1) and ADAR2

a | Exportin 1 (XPO1) binds to the nuclear export signal (NES) located within the Zα domain of ADAR1p150 and regulates its nuclear export together with RAN·GTP. Nuclear export of ADAR1p110 is mediated by XPO5–RAN·GTP and regulated by double-stranded RNA (dsRNA) binding to its dsRNA-binding domains (dsRBDs). The nuclear localization signal (NLS) located in dsRBD3 is responsible for localization of both ADAR1p150 and p110 in the nucleus and nucleolus. Nuclear import of ADAR1p110 is mediated by binding of transportin 1 (TRN1) to dsRBD3, which is inhibited by binding of dsRNA. **b** | The nuclear and nucleolar localization of ADAR2 is regulated by binding of karyopherin subunit α1 (KPNA1) and KPNA3 to an NLS located in the amino-terminal region. Phosphorylation of Thr32 by a currently unknown kinase enables interaction of ADAR2 with the prolyl-isomerase PIN1 in a dsRNA-binding-dependent manner, which isomerizes Pro33 and positively controls the nuclear localization and stability of ADAR2. The E3 ubiquitin ligase WWP2 promotes rapid degradation of ADAR2 in the cytoplasm, which is why ADAR2 is usually not detected in the cytoplasm. Ub, ubiquitin.

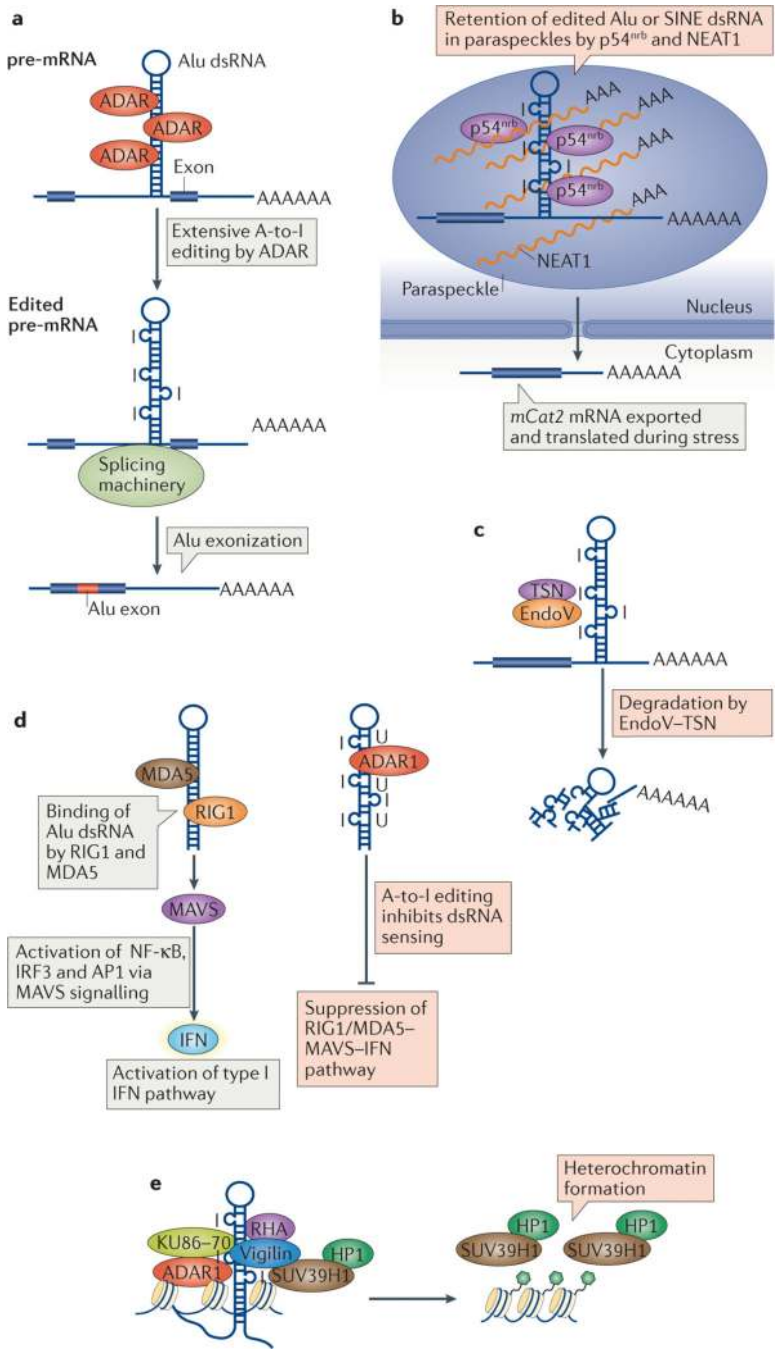

Figure 3. Editing of Alu double-stranded RNAs (dsRNAs) and its consequences

a | Genome-wide inverted Alu repeats in introns and in 3′ untranslated regions (UTRs) form intramolecular RNA duplexes (Alu dsRNAs), which are edited by adenosine deaminases acting on RNA (ADARs). Inosines at intronic Alu dsRNAs are recognized by the splicing machinery as guanosines, thus effectively creating new splice sites, which results in the inclusion of intronic Alu sequences in the mature mRNAs (Alu exonization). **b** | Extensively edited Alu dsRNAs are retained in nuclear paraspeckles by a mechanism involving p54^{nrb} and the long non-coding RNA nuclear paraspeckle assembly transcript 1(NEAT1).The

formation of paraspeckles is absolutely dependent on NEAT1.p54^{nrb}, and perhaps also NEAT1, bind specifically to inosine-containing RNAs such as extensively edited Alu dsRNA. Under certain conditions, such as stress, the paraspeckle-trapped RNA may be released into the cytoplasm for translation, as seen with the mouse Ctn RNA. Editing of short interspersed nuclear element (SINE) dsRNA within the 3′ UTR of the Ctn RNA leads to its nuclear retention. When cells are placed under stress, Ctn RNA is cleaved and polyadenylated at an alternative site, resulting in the loss of the edited SINE sequences and release of the mRNA from the nucleus as the protein-coding *mCat2* (cationic amino acid transporter 2) mRNA. **c** | Extensively edited Alu dsRNAs may be degraded by endonuclease V (EndoV) together with Tudor-SN (TSN), thereby controlling the expression levels of genes harbouring Alu repeats. **d** | Extensively edited Alu dsRNA containing multiple and consecutive I·U mismatched wobble pairs (I·U-dsRNA) may suppress the interferon (IFN) signalling pathway, which would otherwise be activated by unedited Alu dsRNAs. Unedited long dsRNAs (viral and cellular) are potent inducers of IFN signalling. I·U-dsRNA may competitively inhibit the binding of dsRNA to retinoic acid-inducible gene 1(RIG1)or melanoma differentiation-associated protein 5 (MDA5), both of which are cytosolic sensors for dsRNA and upstream regulators of the mitochondrial antiviral signalling adaptor protein (MAVS)-mediated IFN activation pathway. **e** | Extensively edited Alu dsRNAs containing multiple inosines may contribute to heterochromatin formation and gene silencing. Vigilin binds to RNA containing multiple inosines, such as extensively edited Alu dsRNAs. Vigilin has been shown to form a complex with ADAR1, RNA helicase A (RHA), and 86 kDa subunit of Ku antigen (KU86)–KU70, which recruits the histone methyltransferase SUV39H1. SUV39H1 catalyses the methylation of H3 Lys9 (H3K9me), an epigenetic mark that is recognized by heterochromatin protein 1 (HP1), leading to the formation of heterochromatin and to gene silencing. AP1, activator protein-1; IRF3, interferon regulatory factor 3; NF-κB, nuclear factor-κB; pre-mRNA, precursor mRNA. Part **a** adapted from REF. 146, Nature Publishing Group.

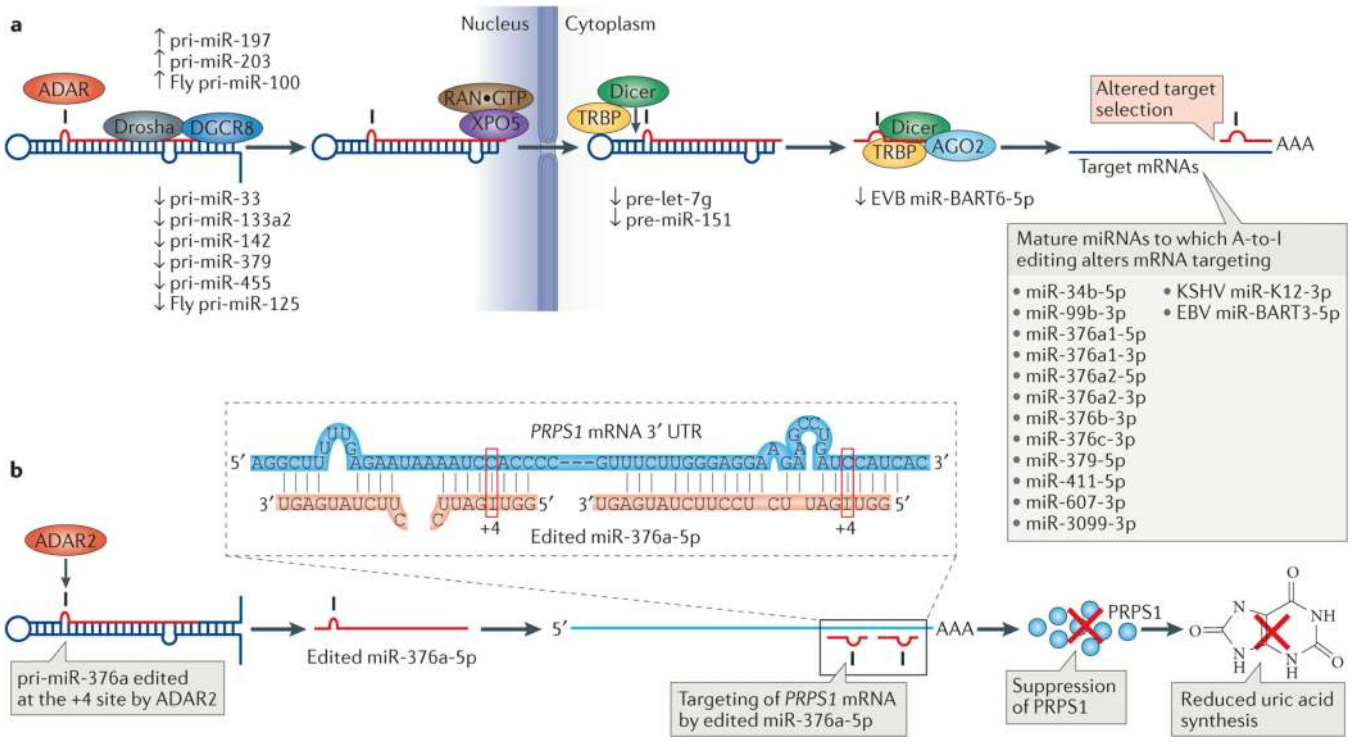

Figure 4. Regulation of microRNA (miRNA) processing, expression and selectivity by RNA editing

a | Primary miRNAs (pre-miRNAs) are processed by the Drosha–DGCR8 complex into precursor miRNAs (pri-miRNAs) in the nucleus, exported to the cytoplasm by exportin 5 (XPO5)–RAN·GTP and processed by the Dicer–TAR RNA-binding protein (TRBP) complex into mature miRNA duplexes. One strand of this duplex is then loaded onto the RNA-induced silencing complex (RISC), which results in the degradation or the inhibition of translation of target mRNAs. Editing can affect any of the miRNA biogenesis steps, including Drosha cleavage, Dicer cleavage and RISC loading, as well as miRNA target selection. Known examples of miRNA editing and their consequences are shown. **b** | Silencing of phosphoribosyl pyrophosphate synthetase 1 (PRPS1) by miR-376a-5p edited at the +4 site by ADAR2 (adenosine deaminases acting on RNA2) and the consequent suppression of uric acid synthesis. A single A-to-I nucleotide change in the seed sequence of miR-376a-5p results in redirection of target gene selection. One of those genes, specifically targeted by the edited miR-376a-5p, is *PRPS1*, which encodes an essential enzyme involved in purine metabolism and the uric acid synthesis pathway. Repression of *PRPS1* by the edited miR-376a-5p results in reduced expression of uric acid in certain tissues, such as brain, in which uric acid levels need to be tightly regulated. The 3′ untranslated region (UTR) of PRPS1 mRNA has two target sites for the edited miR-376a-5p (inset). AGO2, Argonaute 2; EBV, Epstein–Barr virus; KSHV, Kaposi sarcoma-associated herpes virus.

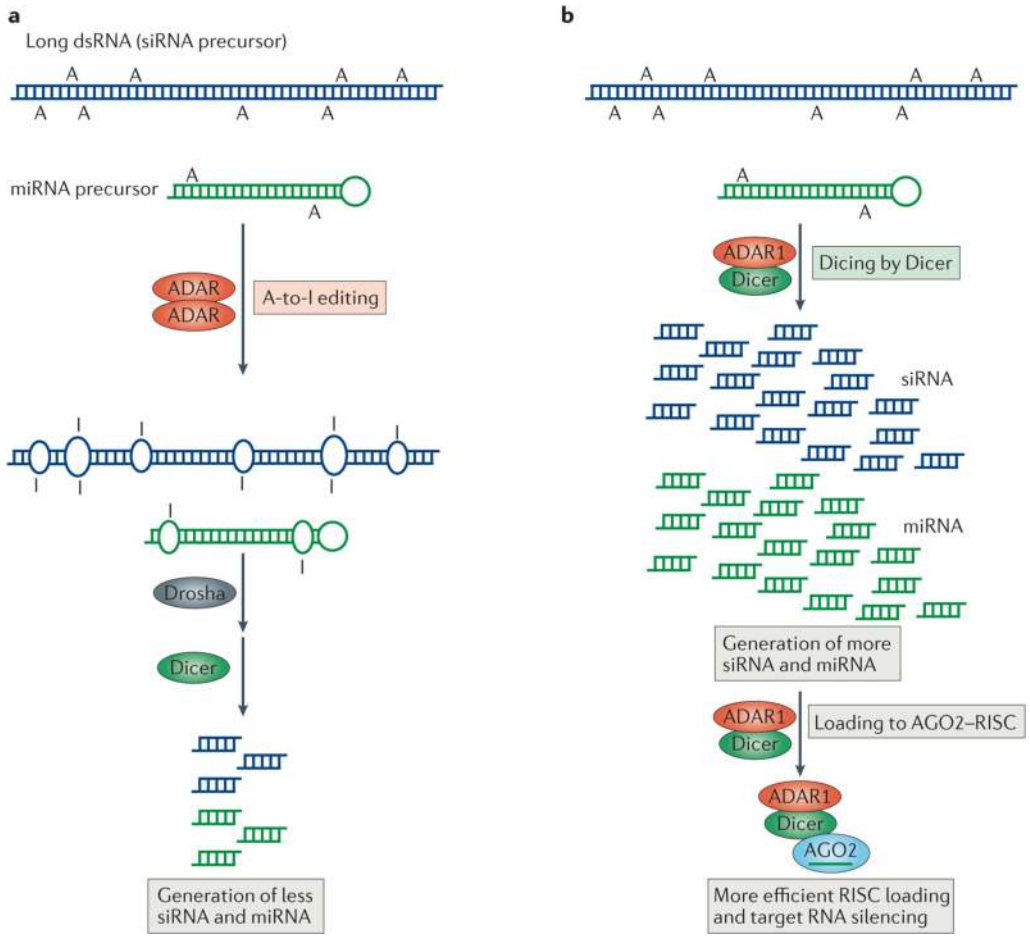

Figure 5. Regulation of RNA interference (RNAi) by adenosine deaminases acting on RNA (ADARs)

Two different types of interaction between RNA-editing and RNAi pathways are known, one antagonistic and the other stimulative. **a** | In antagonistic interactions, ADAR–ADAR homodimers edit long double-stranded RNA (dsRNA) and certain microRNA (miRNA) precursors. Editing changes the dsRNA structure and makes it less accessible to Drosha and/or Dicer, which consequently decreases the efficacy of RNAi by reducing the production of short interfering RNAs (siRNAs) and miRNAs. **b** | In the case of stimulative interactions, ADAR1, as part of a Dicer–ADAR1 heterodimer, promotes RNAi by increasing the Dicer cleavage reaction rate, thereby generating more siRNAs and miRNAs and enhancing RISC (RNA-induced silencing complex) loading and target mRNA silencing. AGO2, Argonaute 2. Figure adapted with permission from REF. 26, Elsevier.

Table 1

A-to-I editing in selected mammalian protein-coding sequences and its functional consequences

Gene	Protein	Recoding	ADAR responsible	Function	Refs
GRIA2	GluR2 subunit of AMPA glutamate receptor	Q→R	ADAR2	Change in Ca^{2+} permeability	132
		R→G	ADAR1, ADAR2	Change in receptor desensitization	133
GRIA3	GluR3 subunit of AMPA glutamate receptor	R→G	ADAR1, ADAR2	Change in receptor desensitization	133
GRIA4	GluR4 subunit of AMPA glutamate receptor	R→G	ADAR1, ADAR2	Change in receptor desensitization	133
GRIK1	GluR5 subunit of kainate glutamate receptor	Q→R	ADAR1, ADAR2	Change in Ca^{2+} permeability	134
GRIK2	GluR6 subunit of kainate glutamate receptor	Q→R	ADAR1, ADAR2	Change in Ca^{2+} permeability	135, 136
		I→V	ADAR1, ADAR2		
		Y→C	ADAR2		
HTR2C	Serotonin receptor 2C	I→V, M	ADAR1	Change in G protein-coupling functions	74
		N→S, G, D	ADAR1, ADAR2		
		I→V	ADAR2		
KCNA1	Voltage-gated K^+ channel (Kv1.1)	I→V	ADAR2	Change in channel inactivation	75
GABRA3	$GABA_A$ receptor, subunit α3	I→M	ADAR1, ADAR2	Kinetics of activation and inactivation, receptor trafficking	76
BLCAP	Bladder cancer-associated protein	Y→C	ADAR1, ADAR2	Not determined	137, 138
		Q→R	ADAR1, ADAR2		
		K→R	ADAR1, ADAR2		
CYFIP2	Cytoplasmic FMR1-interacting protein 2	K→E	ADAR2	Not determined	137
FLNA	Filamin-α	Q→R	ADAR1, ADAR2	Not determined	137
FLNB	Filamin-β	M→V	ADAR1, ADAR2	Liver cancer progression	139
COPA	Coatomer protein complex subunit-α	I→V	ADAR2	Suppression of liver cancer	139
IGFBP7	Insulin-like growth factor-binding protein 7	K→R	–	Proteolytic cleavage sensitivity	137
		R→G			
AR	Androgen receptor	T→A	ADAR1, ADAR2	Prostate cancer progression	140
				Inhibition of interaction with androgen ligands	
AZIN1	Antizyme inhibitor 1	S→G	ADAR1	Liver cancer progression	141
				Change in affinity for antizyme	
NEIL1	DNA repair enzyme NEI-like protein 1	K→R	ADAR1	Change in efficiency or specificity of damaged base removal	142
GLI1	Glioma-associated oncogene 1	R→G	ADAR1, ADAR2	Increased transcription enhancement	143
RHOQ	RAS homology family member Q	N→S	–	Colorectal cancer metastasis	144
				Disruption of interaction with Rap–RapGAP	

ADAR, adenosine deaminases acting on RNA; $GABA_A$, γ-aminobutyric acid type A.

Table 2

A-to-I editing of mammalian microRNAs (miRNAs)

miRNA*	Position‡	Editing levels (%)§		ADAR responsible	Refs
		Human	Mouse		
let-7g	+7	10	0	–	21
	+ 10	30	20	ADAR2	
let-7-2-5P	+10	10	0	–	21
miR-27a-5P	−6	30	0	–	21
	+1	50	20	ADAR2	
	+7	10	0	–	
miR-33a-5P	+10	30	0	–	21
miR-34b-5P	+11	–	40	–	124
miR-99a-5P	+1	20	20	ADAR2	21
miR-99b-3P	−1	10	10	ADAR1	21
	+3	50	10	ADAR1	
miR-122-5p	−7	30	–	ADAR2	145
miR-142-5P	+4	–	5	ADAR1	24
	+5	–	5	ADAR1	
miR-142-3P	+4 (+40)//	–	10	ADAR1, ADAR2	24
miR-151-3P	−1	–	10	ADAR1	22
	+3	40	30	ADAR1	
miR-153-1-3P	+7	10	0	–	21
miR-153-2-3P	+7	30	0	–	21
miR-197-3P	−34	30	–	–	21
miR-203-3P	+21	60	–	ADAR2	21
miR-214-3p	+6	10	–	ADAR2	145
miR-376a-1-5P	+3 (+4)//	50	–	ADAR2	23
miR-376a-1-3P	+6 (+44)//	40	0	ADAR1	23
miR-376a-2-5P	+4	90	50	ADAR2	23
miR-376a-2-3P	+6 (+44)//	100	0	ADAR1	23
miR-376b-3p	+6 (+44)//	95	50	ADAR1	23
miR-379-5P	+5	60	20	ADAR2	21
miR-381-3P	+4	6	13	–	115, 116
miR-411-5P	+5	80	60	ADAR1	21
miR-423 + 5P	−4	40	20	ADAR1	21
miR-497-5P	+2	6	10	–	115, 116
miR-532-5P	+15	10	0	–	21
miR-589-3P	+6	70	–	–	115

miRNA*	Position‡	Editing levels (%)§		ADAR responsible	Refs
		Human	Mouse		
miR-607-3P	+6	70	–	–	21
	+17	80	–	–	
	+20	80	–	–	
miR-652-5P	−10	40	0	–	21
miR-3099-3P	+7	–	80	–	124

ADAR, adenosine deaminase acting on RNA.

*Only editing sites verified by sequencing of specific primary mRNAs (pri-miRNAs) or detected independently more than once by deep sequencing of mature miRNAs are listed.

‡The 5′ end of the human mature miRNA sequence registered at the miRBase database is counted as +1.

§Editing levels indicate fractions of edited miRNAs over edited and unedited miRNAs. The highest editing level reported either in total brain tissue, in sub-regions of the brain or in cultured cells is presented.

//Alternative numbering used in certain references is indicated in parentheses.