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All I's on the RADAR: Role of ADAR in gene regulation

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Abstract

Adenosine to inosine (A-to-I) editing is the most abundant form of RNA modification in mammalian cells, which is catalyzed by adenosine deaminase acting on the double-stranded RNA (ADAR) protein family. A-to-I editing is currently known to be involved in the regulation of the immune system, RNA splicing, protein recoding, microRNA biogenesis, and formation of heterochromatin. Editing occurs within regions of double-stranded RNA, particularly within inverted Alu repeats, and is associated with many diseases including cancer, neurological disorders, and metabolic syndromes. However, the significance of RNA editing in a large portion of the transcriptome remains unknown. Here, we review the current knowledge about the prevalence and function of A-to-I editing by the ADAR protein family, focusing on its role in the regulation of gene expression. Furthermore, RNA editing-independent regulation of cellular processes by ADAR and the putative role(s) of this process in gene regulation will be discussed.

Keywords

ADAR; RNA editing; non-coding RNA; Adenosine to inosine

Introduction

The extent and role of A-to-I RNA editing has only recently come to light with the advent of RNA-sequencing technologies. Given that inosine preferentially base pairs with cytosine, A-to-I editing has the potential to drastically alter the coding and structural properties of RNA. The first observation of RNA editing came from the characterization of an activity in *Xenopus* embryos causing differential migration of RNA:RNA duplexes on a gel [1]. Although this was thought to be a result of duplex unwinding, it was later determined to be caused by the destabilization of RNA duplexes through the C^6 deamination of adenosine to inosine (A-to-I) by ADAR [2]. ADAR was later cloned in mammalian cells [3]. The ADAR family is highly conserved across metazoans, with the presence of ADAR members in the earliest-branching metazoan taxa, including sponges and ctenophores [4]. ADAR was later cloned in mammalian cells [3].

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Humans have three ADAR genes (Figure 1): ADAR1, ADAR2 (also known as ADARB1 or RED1) and ADAR3 (also known as ADARB2 or RED2). ADAR1 has two major isoforms: an interferon inducible ADAR1 p150 that contains both the Zα and Zβ Z-DNA binding domains, and a constitutive ADAR1 p110 that lacks the N-terminal Zα Z-DNA binding domain [5, 6], while ADAR2 lacks any Z-DNA binding domains [7]. The Zβ domain lacks several conserved residues necessary for Z-DNA binding [8, 9]. Consequently, only the Zα domain has been shown to be able to bind Z-DNA [8], although its function is still unclear and debated. Although ADAR3 has not been shown to possess any catalytic activity, it is able to bind to both single (ssRNA) and double-stranded RNA (dsRNA) [7]. The significance of ADAR3 binding to ssRNA through its arginine-rich R-domain is unknown and this activity has only been demonstrated *in vitro*. The R-domain of ADAR3 has been shown to act as a nuclear localization sequence by interacting with importin subunit alpha-1 (KPNA2) [10]. The function of ADAR3 is still being explored, although evidence suggests that it may negatively regulate A-to-I editing in the brain and effect transcriptomic plasticity [7, 11]. In *C. elegans*, the catalytically inactive ADA-1 was found to regulate RNA editing by the catalytically active ADA-2 (Washburn 2014 Cell Reports), although it is unclear if a similar mechanism exists in humans.

Both ADAR1 and ADAR2 are essential for normal growth and development. Loss of ADAR1 is embryonically lethal in mice by embryonic day E12.5 [12] due to impaired hematopoiesis and the development of severe type-I interferonopathy [13]. ADAR1 deficient hematopoietic stem cells (HSCs) and hematopoietic progenitor cells (HPCs) show abolished capacity to repopulate an irradiated recipient, suggesting that ADAR1 has a role in both the differentiation and survival of these cells [14]. Furthermore, ADAR1 is essential for HSC and HPC maintenance in the fetal liver and bone marrow [13] and induced expression of a catalytically dead mutant of ADAR in HSCs results in mice developing severe hematopoietic defects [15]. Embryonic lethality of ADAR1 knockout mice can be rescued by subsequent deletion of MAVS [16], a protein that induces type-I interferon and NF-κB signaling, or the upstream effector MDA5 (also known as IFIH1) [17, 18] that is involved in the viral defense pathway by acting as a sensor for dsRNA [19]. This points to the role of ADAR editing in negatively regulating the MAVS-MDA5 pathway and preventing activation of an immune response by endogenous transcripts by destabilizing host-derived dsRNA structures. This destabilization prevents MDA5 from forming filaments and triggering an immune response as MDA5 is sensitive to bulges and mismatches in dsRNA [20] (see [21] for a more detailed overview). ADAR1 is also able to inhibit RIG-I activation by endogenous dsRNAs by directly competing with its binding for the substrate [22]. Both the editing and binding activities of ADAR are necessary to first of all suppress endogenous interferon responses and further prevent translational shutdown during such a response [23]. Mice harboring a genetic knockout of ADAR2 are born at normal mendelian ratios, suggesting that ADAR2 is dispensable for embryological development [24]. However, postnatal lethality is observed in ADAR2 null mice due to progressively worse brain seizures which result in death within 3 weeks of birth [24].

Both ADAR1 and ADAR2 have been reported to form homodimers and heterodimers with each other [25] presenting the possibility of multiple regulatory mechanisms controlling editing Recently, the editing and stability of CTN-RNA was found to be dependent upon the

interaction between ADAR1 and ADAR2 in cells [26], suggesting that editing of select RNA substrates is dependent upon the cooperativity between these two ADAR members. It is however, unclear if this editing occurs through the heterodimerization of ADAR1 and ADAR2, or rather whether binding and editing of CTN-RNA by one ADAR isoform promotes the binding and editing activity of the other. Discrepancy between *in vitro* and *in vivo* heterodimerization data may suggest that post-translational modifications or other protein binding partners may be required to facilitate this interaction. The expression of ADAR3 however is restricted to certain brain regions and post-mitotic neurons [7, 27].

Role of A-to-I editing

Protein recoding

Given that A-to-I editing changes the base pairing properties of RNA, it is easy to envision its ability to alter the amino acid sequence of a protein. Although this is not the major role of ADAR editing, there are many notable examples where RNA editing changes protein function. ADAR2 editing of the AMPA type glutamate receptor 2 subunit (GRIA2) occurs at near 100% efficiency and results in an amino acid change of a glutamine to an arginine (Q/R) [28]. Editing of the Q/R site reduces Ca^{2+} permeability through the receptor by up to 30 times [24, 28]. This is essential for survival and genetic mutation of Q/R site rescues postnatal lethality of ADAR2 knockout mice [24]. Our understanding of why editing of the Q/R site occurs and is conserved [29] is incomplete, as a single point mutation could abrogate the need for any editing to occur. One postulated idea is that editing of GRIA2 may play a role in neural cell development and differentiation [30]. While earlier studies did not observe any obvious architectural differences in brain slices of $GRIA2^{R/R}$ mice [31], this idea cannot yet be discredited as changes may also occur at the microscopic or biochemical level. Recently, ADAR3 was found to be an important negative regulator of the editing of GRIA2 [11]. Notably, hypo-editing of GRIA2 in the setting of glioblastoma [32] is accompanied by an increase in ADAR3 protein expression [11]. The dsRNA binding domains of ADAR3 were found to be necessary for this activity, suggesting that ADAR3 is directly competing for editing site binding with other ADAR family members.

Many known examples of protein recoding by RNA editing require the formation of specific dsRNA structures (see [33] for a more comprehensive overview) and occur at regions where the underlying DNA sequence is evolutionarily conserved, although overall conservation between all identified ADAR targets is very low [34]. Recently, cephalopods have been found to be able to undergo extensive A-to-I editing in order to diversity their proteome, especially in neural cells in which recoding leads to alterations in neural excitability and morphology [35]. However, this appears to be an event specific to cephalopods and occurs at the expense of genomic diversity, as all recoding sites are highly conserved within the taxon [35].

RNA export, splicing and stability

ADAR1 can shuttle in and out of the nucleus through interactions with the nuclear import receptor transportin-1 [36] and the nuclear export receptor exportin-5 [36, 37]. While nuclear import of ADAR is inhibited by its binding of dsRNA substrates, nuclear export is

not [36]. As such, it is believed that ADAR1 may mediate the export of specific RNAs out of the nucleus (Figure 2). A-to-I editing itself mediates nuclear retention of hyper-edited dsRNAs through selective binding of inosine-rich RNAs to NONO (Figure 2) [38]. One function of nuclear retention is to permit those RNAs that are not required in the cytoplasm under normal conditions to be quickly exported immediately after some form of cellular stress. The classical example of nuclear retention involves modulation of the levels of mCAT2, a protein encoding for an arginine transporter and involved in the nitric oxide pathway [39]. CTN-RNA is transcribed from the mCAT2 gene using an alternate upstream promoter and contains an extended 3'UTR sequence [40]. The 3'UTR of CTN-RNA forms a dsRNA structure that is extensively bound and edited by ADAR2, promoting its stabilization and half-life [26, 40, 41]. Upon cellular stress, the 3'UTR of CTN-RNA is cleaved which allows for its subsequent nuclear export into the cytoplasm where it results in increased production of the mCAT2 protein [40]. A similar mechanism has been found for the Nicolin 1 gene [42]. It is feasible that A-to-I hyper-edited sites exist in a subset of RNAs that are retained in the nucleus to be released under specific stimuli. As a large proportion of A-to-I editing occurs within intronic sequences [43], an obvious function of A-to-I editing mediated nuclear retention is to retain incorrectly spliced or un-spliced transcripts in the nucleus. Interestingly, as paraskeckles are not found in human embryonic stem cells (ESCs) due to the lack of the non-coding RNA NEAT1 and only appear upon ESC differentiation, there is no nuclear retention of edited RNAs [44] despite the high level of ADAR editing in ESCs [45]. Although there is ongoing debate as to whether paraspeckles are essential for cellular function, as mice lacking NEAT1 are viable and display no obvious phenotypic changes under laboratory condition [46], many argued that they may only be necessary during times of stress [47]. This idea is in line with the functional retention in edited RNAs for quick export to the cytoplasm in the event of a stimulus. For instance, NEAT1 is dramatically upregulated following viral infection and exerts antiviral effects [48] and NEAT1 knockout mice fail to become pregnant [49], showing clear phenotypic effects. It would be interesting to see the effects of challenging NEAT1 knockout mice with infection or a tumor on mouse survival.

A-to-I editing and N6-methyladenosine (m6A) RNA modifications are generally mutually exclusive [50]. A-to-I edited RNA residues can no longer be methylated by the m6A complex and 6mA modified RNA is a poor substrate for A-to-I editing [51]. Indeed, there is a negative correlation between m6A and levels of A-to-I editing in cells [50] suggesting that these two types of RNA modifications can directly antagonize the functions of one another.

A-to-I editing has been heavily implicated in the regulation of RNA splicing by introducing a splice donor or acceptor site, or abolishing a splice branch site. ADAR2 self-regulates its expression and catalytic activity by modulating its own alternative splicing [52]. A-to-I editing by ADAR2 can convert an intronic AA dinucleotide into an AI dinucleotide that can function like the canonical AG 3' splice site acceptor [52]. The ability of editing events to alter splicing has been documented for several other genes [53–56]. As many such editing events occur in the context of inverted Alu repeats, it has been argued that Alu expansion in the human genome helped to promote transcript diversity through alternative splicing and exonization (reviewed in [57]). The binding of ADAR to RNA has also been proposed to

interfere with spliceosomal assembly [58], which could in part explain the role of ADAR in controlling aberrant exonization of Alu elements in the human genome [59].

ADAR is directly involved in regulating the stability of certain RNA species inside the cell. The binding of ADAR2 was found to antagonize the binding of the RNA decay-promoting protein PARN, an RNA deadenylase, to CTN-RNA in an editing independent manner [41]. Likewise, ADAR1 binding to inverted Alu repeats in the 3'UTR of multiple transcripts is believed to directly inhibit the binding of the RNA-decay protein and stress-response gene Stuaufen-1 [60]. This function of ADAR1 acts to protect cells from stress-induced apoptosis in an editing independent manner [60]. Alternatively, ADAR1 interaction with HuR increases transcript stability [61]. HuR belongs to the embryonic lethal abnormal vision (ELAV), an RNA-binding protein family that preferentially binds to single stranded AU-rich RNA sequences [62, 63]. AU-rich elements (AREs) are found in the 3'UTR of up to 8% of all mRNAs, including cytokines and cell cycle genes [64, 65], and are one of the best characterized elements involved in RNA instability. The binding of ADAR1 and HuR to such RNAs is cooperative to increase transcript stability as knockdown of ADAR1 abolishes HuR binding [61].

RNAi

Given that miRNA biogenesis requires the formation of dsRNA intermediates, it is easy to envision the role of ADAR in this process. ADAR can antagonize miRNA biogenesis through editing dependent or independent means. Editing of pri-miRNA or pre-miRNAs can result in many outcomes that depend upon the location and extend of A-to-I editing. RNA editing has large and global effects on RNA secondary structure [66]. Editing of a particular pri-miRNA can suppress its subsequent processing by Drosha [67] or DICER [68]. Alternatively, if editing occurs in the "seed" region of the miRNA it has the potential to alter its target binding sites and change the function of the miRNA as it will now silence a completely different set of genes, as has been demonstrated for miR-376 [69] and a miRNA encoded by human herpesvirus [70]. ADAR protein binding can also suppress miRNA biogenesis and the efficacy of siRNA by directly competing with RNAi processing machinery for the binding of the pre-miRNA in an editing-independent fashion [71, 72]. More recently, ADAR1 has been shown to heterodimerize with DICER to stimulate miRNA biogenesis [73–75], although what determines the formation of this interaction between DICER and ADAR to facilitate either a stimulatory or inhibitory effect remains to be determined. Furthermore, ADAR binding to RNA can antagonize miRNA by reducing the accessibility go the AGO2-miRNA complex to its target site [76].

Heterochromatin formation and gene regulation

Vigilin is an RNA binding protein that contains 14 K-homology (KH) domains and has been implicated in both heterochromatin formation and chromosomal segregation in *Drosophila* and humans [77, 78]. The C-terminal domain of Vigilin has been shown to interact with SUV39H1 [78], a histone methyltransferase involved in depositing the heterochromatic H3K9me3 mark [79]. Vigilin has been shown to bind to hyper-edited RNAs (predominantly inverted Alu repeats) (Figure 3) and interact with both ADAR and the Ku70/86 complex that is involved in non-homologous end joining (NHEJ) [79], suggesting that ADAR editing may

play a role in both the formation of heterochromatin and the DNA repair pathway. However, a direct role of ADAR in either of these processes has never been shown. Of note, the DNA damage response (DDR) results in an increase in type I interferon signaling [80]. In certain cell types including macrophages, this interferon response may promote the DDR pathway [81]. Given that ADAR edited RNAs have been implicated in NHEJ and type I interferon signaling promotes expression of ADAR1p150, it would be interesting to see if this protein has any role in the DDR.

Several recent mass spectrometry screens have identified ADAR1 enrichment in heterochromatin [82] with the H3K9me3 mark [83, 84], as well as in association with the Polycomb Repressive Complex (PRC) components EED and PRC1 [85], suggesting that ADAR may directly play a role in the formation of heterochromatin (Figure 3) in an editing-dependent or editing-independent way. The Zα domain has been shown to be involved in protein localization to cytoplasmic stress granules [86, 87] and viral defense [88]. Z-DNA is a left-helical form of DNA [89] that may be transiently formed in alternating purine-pyrimidine DNA stretches in response to torsional stress [90, 91] such as negative supercoiling that arises in the region behind a moving polymerase [92]. Expression of the Zα domain of human ADAR1 fused to the Gal4 activation domain in yeast enhanced transcription from a reporter containing a poly[d(CG)] Z-DNA forming region in its promoter [93], suggesting that ADAR1 p150 may also have an editing independent role in transcriptional regulation (Figure 3), though this has yet to be experimentally determined. As ADAR1 p150 is an interferon inducible transcript that may have the capability of binding to Z-DNA it is tempting to speculate that ADAR1 p150 may regulate gene expression during an immune response.

Regulation of A-to-I editing

Editing-site selectivity

Early studies suggested that inosine was present at levels up to 1 in every 17,000 nucleotides in rat tissue [94], making it one of the most abundant mammalian RNA modifications. Moreover, RNA editing is at least 100 times more prevalent in primates compared to rodents [95], likely due to the high abundance of Alu elements in our genomes which may contribute to transcriptomic complexity [96, 97]. However, due to the difficulties of detecting A-to-I editing events, we have only recently characterized a substantial proportion of the editing sites in human tissues. ADAR editing requires the formation of double-stranded RNA [2]. Interestingly, both dsRNA and DNA:RNA duplexes have structural similarities as they both form an A-form helix [98, 99]. While, *in vitro* studies showed the ability of both ADAR1 and ADAR2 to modify the DNA strand of a DNA-RNA duplex [100], no significant DNA editing was observed above background levels when human ADAR was fused to Cas9 which would put ADAR in the context of such a duplex [101]. Interestingly, deamination of the RNA strand of DNA:RNA duplexes was shown *in vitro* [100], presenting the possibility of RNA editing in the absence of double-stranded RNA formation, although this is yet to be shown *in vivo*. However, EMSA data has also shown that binding of the catalytic domain of ADAR2 to a target substrate is sensitive to 2’ deoxy substitutions opposite the editing site [102], although this study was never carried out with the full-length enzymes.

The mechanism guiding editing site selectivity are poorly understood. While ADAR1 and ADAR2 can edit some of the same substrates, most substrates can only be edited by one of the two enzymes. Prediction of ADAR editing sites remains poor [103]. ADAR editing involves a base-flipping mechanism in which the reactive base is flipped out of the RNA duplex to be accessed by the active site of the enzyme [104]. Due to the structural constraints of such a feat, there is a nearest neighboring preference for A or U on the 5' side and G on the 3' side of the A to be edited [104, 105]. Catalytic activity is further stimulated if there is a C mismatch opposite the base to be edited [106]. Selectivity of editing sites is contributed to by both the catalytic domain and the dsRBDs [103, 107].

To date, over 400,000 individual editing events have been reported in the human transcriptome [108]. However, earlier studies estimated that up to 100 million editing sites may exist in the human genome, with many editing events occurring at levels of under 1% [109]. This discrepancy is due to the computational difficulty of identifying *bona fide* editing sites within sequencing data, given that editing sites must be discriminated from single nucleotide polymorphisms (SNPs) and the difficulty of identifying editing sites in repetitive regions of the genome where they are most abundant and hyper editing tends to occur. Moreover, it is unclear if such low editing frequencies are functionally relevant. The majority (97.7%) of editing sites occur within repetitive sites, particularly in the context of inverted Alu repeats [108].

A-to-I editing events occur in either a site specific or promiscuous manner. Site-specific editing occurs at sites that are more conserved in the genome and form imperfect dsRNA structures and includes editing of coding regions and miRNA sites [34, 95, 110]. Promiscuous editing however occurs in the context of long dsRNA structures that have few bulges and mismatches [111], making inverted Alu repeats perfect candidates for this type of editing. Alu elements are ~300 nucleotide primate-specific short interspersed nuclear elements (SINEs) that comprise 10% of the human genome. Given that Alu elements frequently occur in clusters and are over-represented in gene-rich regions of the genome [112], this presents the intriguing notion that Alu elements may have been co-opted by the ADAR machinery to diversify the human transcriptome. Indeed, editing of inverted Alu repeats has been shown to be involved in many processes including circular RNA biogenesis, induction of editing in *cis*, transcriptional elongation and splicing (Figure 4).

Circular RNAs (circRNAs) are a class of non-coding RNAs (ncRNAs) that have a variety of cellular functions including acting as micro RNA (miRNA) sponges [113], regulating RNA polymerase II associated transcription [114], and interacting with RNA-binding proteins to perform diverse functions such as controlling cell cycle progression [115]. One of the major methods of circRNA biogenesis involves the presence of reverse complementary sequences such as Alu repeats flanking the RNA and promoting back-splicing and circularization [116]. ADAR editing negatively regulates circRNA biogenesis by editing and destabilizing the flanking Alu repeat sequences, making circRNA production less favorable [117]. Alu editing has also been shown to act as *cis*-acting editing inducer elements (EIEs) for distant sites on the same RNA molecule and allow for the editing of short double-stranded sites that could not otherwise be edited efficiently by ADAR [118]. This has been shown for a number of transcripts including the I/M editing site in the Gabra transcript [118] and the Q/R editing

site of the GluA2 transcript [119]. Transcriptome wide analyses have shown that adjacent Alu sequences inserted in the same orientation are overrepresented in the human genome, compared to those inserted in an inverted orientation [120–122]. The presence of inverted Alu elements within a gene appeared to interfere with RNA Pol II activity as polymerase occupancy was decreased downstream of a transcribed inverted Alu region, indicating that the double-stranded structure formed by the RNA influences polymerase activity and gene expression [122]. This secondary structure formation by inverted repeat Alus appears to influence the kinetics of RNA polymerase, enabling for a global mechanism to control for gene expression rate. Indeed, transcripts containing inverted Alu elements are more lowly expressed compared to those that do not [57, 122]. Others have found that the presence of inverted Alu sequences in the 3'UTR has the potential to alter translation efficiency [123]. Whether ADAR editing of these inverted repeats has implications for both transcription and translation by possibly destabilizing and alternating the double-stranded structures or recruiting a different repertoire of RNA binding proteins is an unanswered question in the field.

The expression of ADAR1 and ADAR2 has been reported to be differentially regulated by the DNA-methyltransferase (DNMT) inhibitor 5'-azacytidine and the global histone deacetylase (HDAC) inhibitor trichostatin A [124], suggesting an interplay between global gene expression and ADAR activity. DNMT inhibitors are known to induce interferon signaling through the upregulation of endogenous retroviruses (ERVs) [125]. Consequently, differential regulation of ADAR activity in the setting of DNMT and HDAC inhibitors could be part of an endogenous protective mechanism against ERVs.

A-to-I editing in disease

Mutations in ADAR1 are known to contribute to or cause several inflammatory disorders including Aicardi-Goutières syndrome (AGS) [126] and systemic lupus erythematosus (SLE) [127]. While homozygous mutations in ADAR that abolish its catalytic activity are embryonically lethal, heterozygous mutations cause dyschomatosis symmetrica hereditaria (DSH) [128]. DSH often manifests simply as a skin disease causing hyper- or hypo-pigmentation of the hands and feet and rarely results in neurological deterioration [129] although the pathogenesis of this disorder is unknown. Altered levels of A-to-I editing has also been reported for many cognitive and psychiatric disorders including Alzheimers [130, 131], depression [132] and schizophrenia [133, 134]. Interestingly, editing of the serotonin 2C receptor (5-HT2C) is altered in depressed suicide patients [135, 136]. Treatment of mice with fluoxetine (Prozac), a drug commonly used to treat depression, resulted in editing changes to 5-HT2C that were exactly opposite to those seen in depressed suicide patients [135], suggesting that one mechanism through which this drug works is by targeting and altering ADAR editing in neural tissue. ADAR2 may also play a role in the pathogenesis of type 2 diabetes (T2D), as knockdown of ADAR2 in rat insulinoma cells and primary pancreatic islets reduced glucose-stimulated insulin secretion [137]. Furthermore, ADAR2 is metabolically regulated in islet cells [138].

Finally, ADAR activity has been heavily implicated in the pathogenesis and progression of many cancer types including lung, breast and blood cancers [139–143]. For example, editing

of focal adhesion kinase (FAK) RNA by ADAR promotes transcript stability and results in increased FAK protein expression that enhances cancer cell invasion and metastasis [139]. As another example, JAK/STAT signaling was shown to increase the expression of ADAR1 in leukemic stem cells, allowing for the editing and impairment of let-7 miRNA biogenesis [142]. Let-7 has known tumor suppressor functions [144] and its expression must be suppressed in stem cells to allow for self-renewal [145]. Genome-wide studies have identified many editing sites that are hypo- or hyper-edited in the setting of cancer [146]. Many of these sites are found in non-coding regions, however a subset of these editing sites were identified to result in non-synonymous RNA mutations. For example, editing of antizyme inhibitor 1 (AZIN1) by ADAR1 in the context of liver cancer confers increased cell proliferation by promoting AZIN1 protein stability and increasing polyamine production [147]. For a more detailed review on the role of ADAR enzymes in cancer, see [148, 149].

Conclusions

In conclusion, we show the pervasive role of ADAR in the regulation of many aspects of RNA function, including regulating the biogenesis of circRNA, miRNA, RNA export and splicing. The advent of sequencing technologies has allowed us to better understand the breadth of RNA editing and its contribution to transcriptomic diversity in a variety of cell, tissue, and disease settings. However, many outstanding question remain in the field including: (1) how ADAR activity is regulated in cells by nucleo-cytoplasmic shuttling, or by post-translational modifications and other protein-protein interactions, (2) the role of ADAR in gene silencing by heterochromatin, (3) whether ADAR activity influenced the evolution and exaptation of Alu elements in the human genome, (4) the prevalence and function of nuclear retained hyper-edited RNAs, (5) the interplay between A-to-I editing and 6mA base modification and how these modifications influence target gene expression, as well as (6) the role of the RNA editing enzymes in cancer progression and autoimmune disorders.

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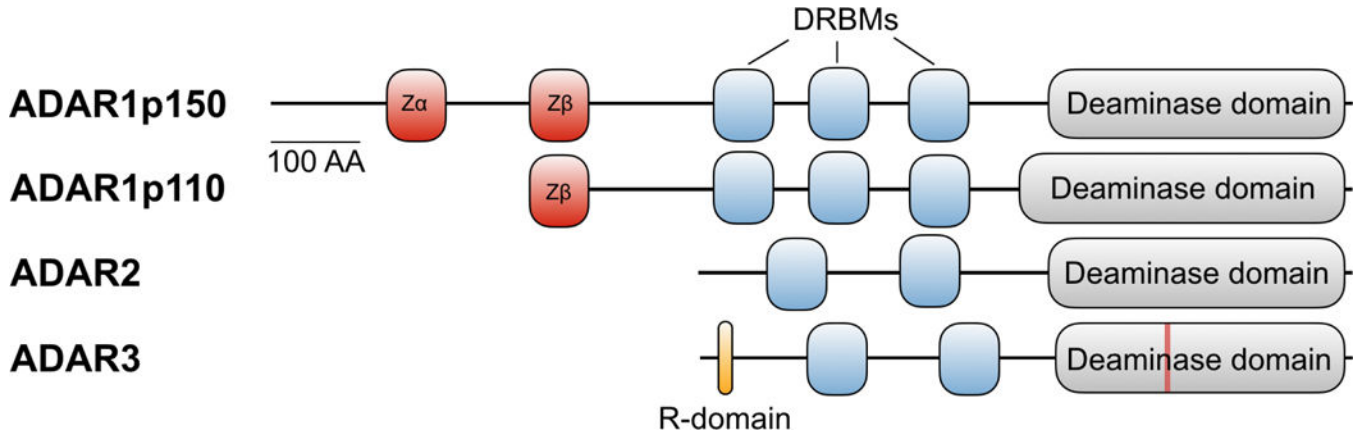

Figure 1.

Human ADAR protein family. All ADAR family members contain double stranded RNA binding motifs (DRBMs) and a deaminase domain, which is mutated at a critical residue in ADAR 3 (red line). ADAR1 is expressed as a constitutive ADAR1p110 isoform, or as an interferon inducible ADAR1p110. AA, amino acids; Zα, Zα DNA binding motif; Zβ, Zβ DNA binding motif; R-domain, arginine-rich domain.

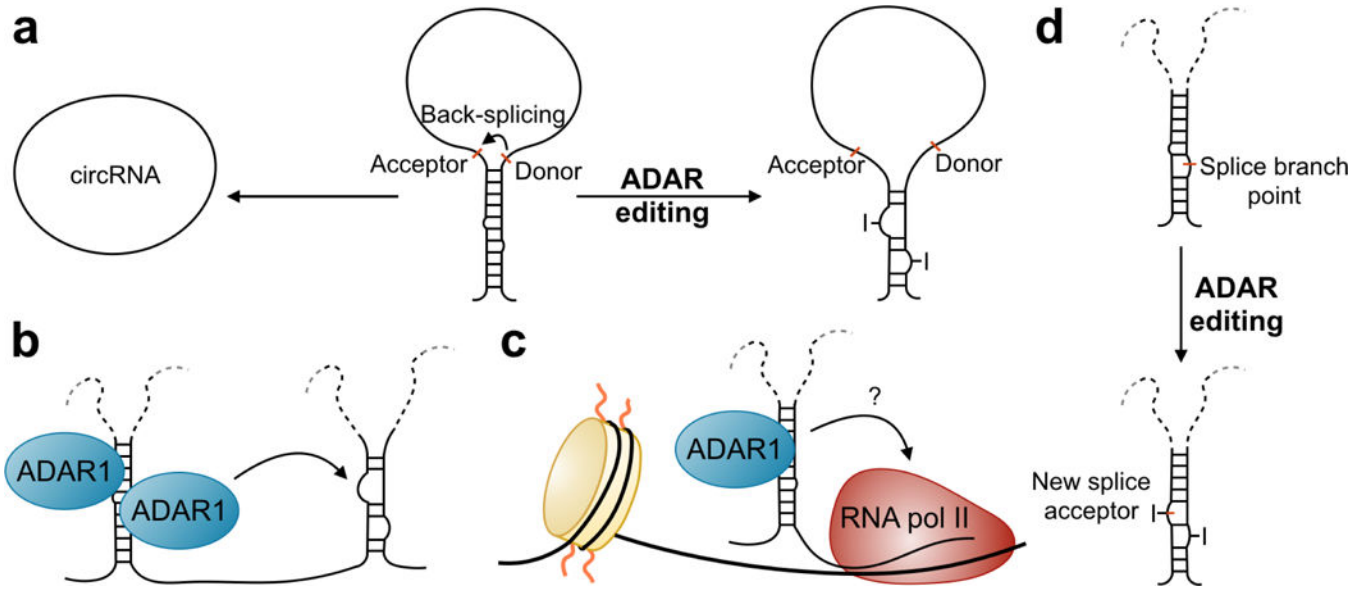

Figure 2.

ADAR editing at inverted Alu repeat regions. Editing of Alu repeats can antagonize circRNA formation (a), induce editing of regions in-*cis* by bringing ADAR in proximity to a less-favorable binding site (b), effect transcription (c), and create or destroy splice sites (d).

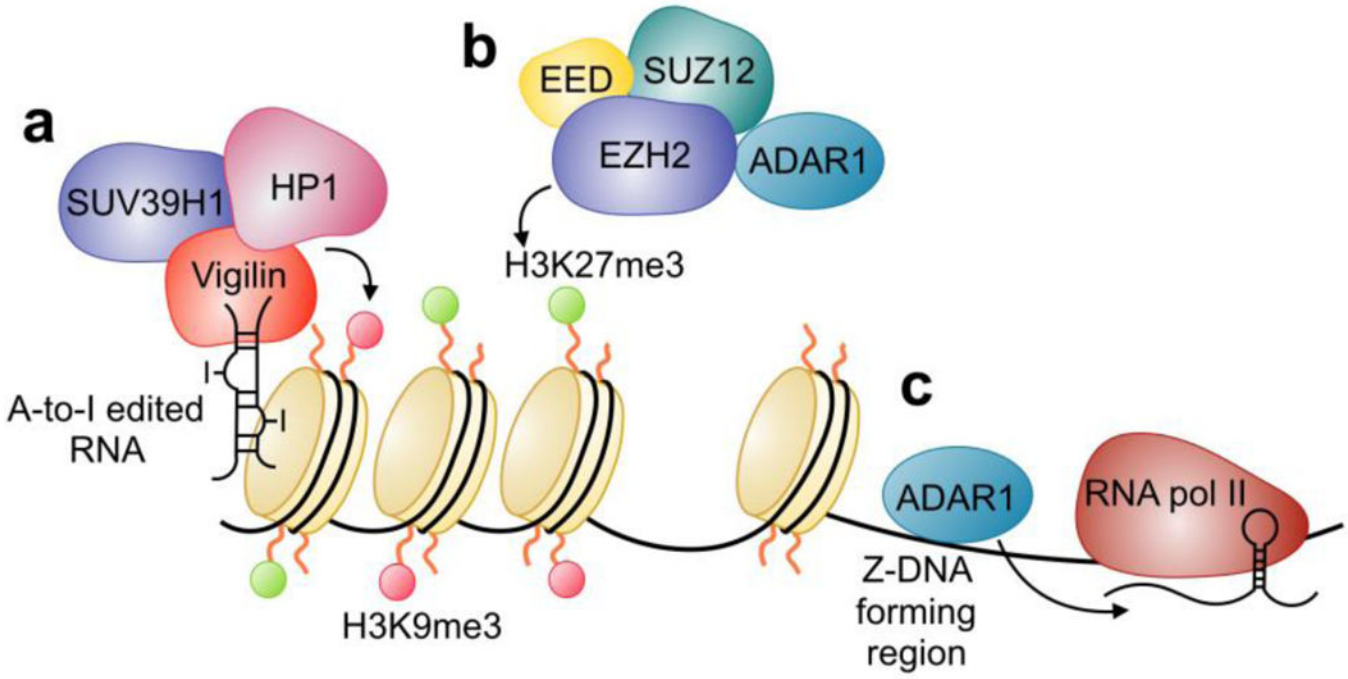

Figure 3.

ADAR control of heterochromatin and gene transcription. A-to-I edited RNA binds to Vigilin to influence heterochromatin deposition (a). ADAR interaction with the PRC2 complex may regulate the deposition of H3K27me3 and gene silencing (b). ADAR binding to Z-DNA in transcribed regions could aid in recruiting the enzyme to newly formed transcripts.

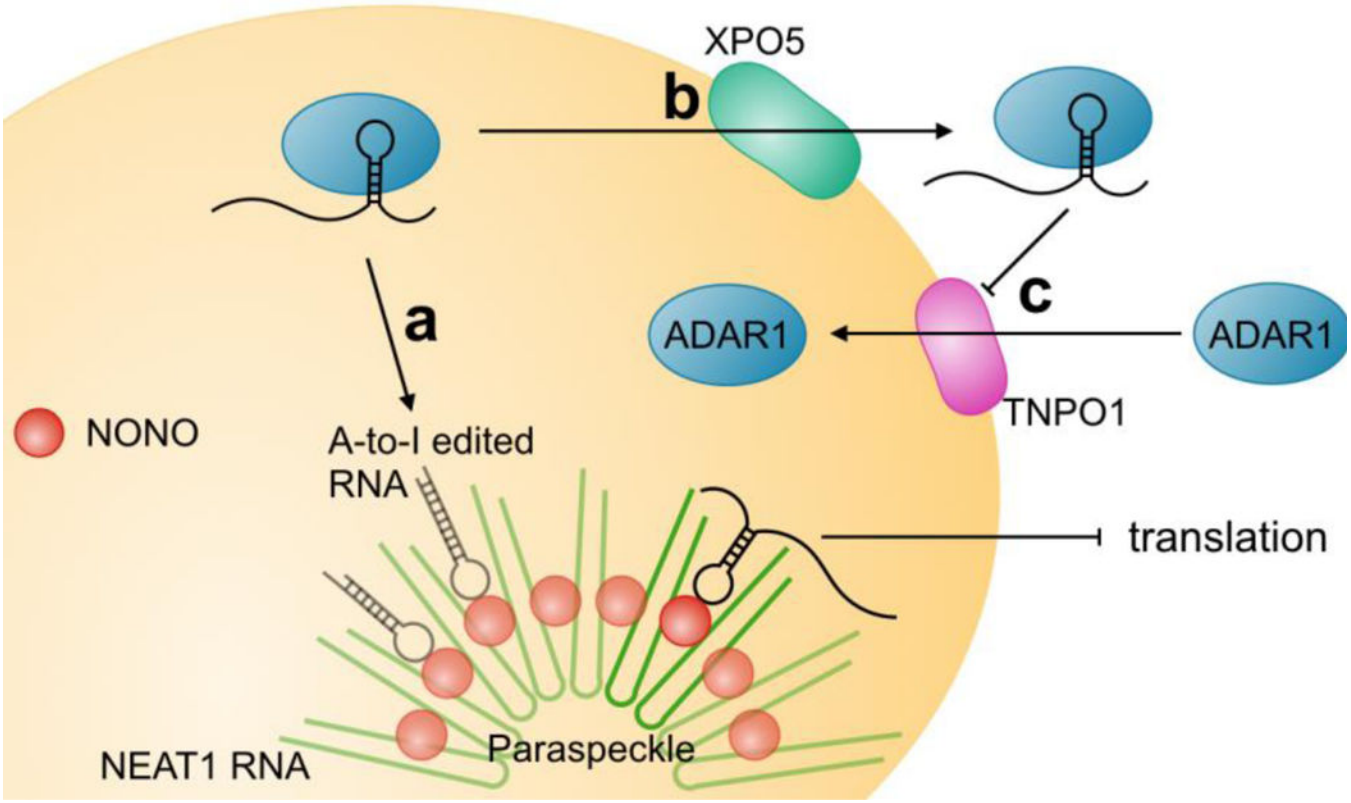

Figure 4.

ADAR control of RNA export. Hyper-editing of RNA by ADAR causes RNA retention through binding of inosine-rich RNA to the RNA-binding protein NONO in paraspeckles (a). While RNA-bound ADAR can be effectively exported from the nucleus (b), nuclear import is inhibited. Only free ADAR is able to shuttle back into the nucleus (c).