Review

Molecular diversity through RNA editing: a balancing act

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RNA editing by adenosine deamination fuels the generation of RNA and protein diversity in eukaryotes, particularly in higher organisms. This includes the recoding of translated exons, widespread editing of retrotransposon-derived repeat elements and sequence modification of microRNA (miRNA) transcripts. Such changes can bring about specific amino acid substitutions, alternative splicing and changes in gene expression levels. Although the overall prevalence of adenosine-to-inosine (A-to-I) editing and its specific functional impact on many of the affected genes is not yet known, the importance of balancing RNA modification levels across time and space is becoming increasingly evident. In particular, transcriptome instabilities in the form of too much or too little RNA editing activity, or misguided editing, manifest in several human disease phenotypes and can disrupt that balance.

Transcript and protein diversity through RNA editing

RNA editing is broadly defined as the post-transcriptional alteration of RNA sequences through the insertion, deletion or modification of nucleotides but not including RNA processing events such as splicing, polyadenylation or the degradation of RNA molecules [1]. Of the various types of RNA editing (Box 1), A-to-I base modification is the most widespread in higher eukaryotes (for a comprehensive review see [2]). Furthermore, both the complexity of the molecular machinery that mediates A-to-I editing and the number of editing targets seem to increase from lower to higher organisms [2–4].

The diversity generated by A-to-I editing affects gene expression at several levels and targets different types of transcripts. Here, we review the emerging insights on molecular diversity generated through RNA editing and the implications of tipping the complex balance of editing patterns in experimental models and human disease.

The three major sequence classes undergoing A-to-I editing are protein-coding exons in pre-mRNAs, repetitive sequence elements in untranslated exons as well as introns as well as miRNA precursor transcripts (Figure 1). A key distinguishing feature among the three kinds of targets is that the type of RNA secondary structure formed influences how the RNA editing machinery interacts and modifies them. Whereas RNA folds involving repetitive sequence elements are characterized by extended, almost perfectly base-paired duplex structures that undergo heavy and multiple site editing, the secondary structures that lead to miRNA editing consist of short RNA hairpins with small bulges and loops – a hallmark feature of miRNA precursors. RNA editing events in pre-mRNAs that do not involve repetitive elements are mediated by composite secondary structures with multiple small base-pairing segments separated by bulges and loops. These types of structures often give rise to highly site-selective and high efficiency base modification by adenosine deaminases acting on RNA (ADARs).

Target substrates, functions and fates

Editing within pre-mRNAs can generate or destroy splice sites, regulate alternative splicing events and influence the dynamics of constitutive splice sites [2]. Of particular interest are instances in which A-to-I editing within protein-coding exons results in a non-synonymous codon change (reviewed in [5]). Usually, the protein sequence of a gene product can be faithfully deduced from the nucleotide sequence of the translated exons. However, this is not

Glossary

Alu repeat elements: large family of retrotransposon-derived sequence elements, each about 300 nucleotides long, that entered the primate genomes more than 60 Mya and have since expanded in number (reviewed in [94]). The human genome harbors about 1.4 million Alu sequences, constituting about 10% of the total genome content and leading to an average frequency of about one dozen Alus per gene. Any two Alu sequences are at least 70–80% identical in sequence, which leads to high base complementarity between pairs of Alus that are oppositely oriented within the same RNA molecule. Some Alus are still active in retrotransposition today causing about one reinsertion event in humans every 100–200 births [94].

Editing site identification: to determine if an RNA is subject to A-to-I editing *in vivo*, the gDNA and cDNA from the gene in question is analyzed from the same specimen to exclude any genomic variations from the epigenetic modification. Through the gene-specific amplification and sequencing of gDNA and cDNA covering the same region, a mixed signal for A and G is obtained only in the cDNA read, and the editing level can be estimated directly from the relative signals for A and G in the sequence electropherogram.

Inosine: the product of adenosine deamination. The properties of inosine closely resemble those of guanosine both during RNA folding and the translation of inosine-containing codons. Therefore, any A-to-I change in a protein-coding sequence is equivalent to making an A-to-G mutation. A-to-I editing is the only mechanism known to generate inosine within RNA molecules.

Recoding editing: the alteration through A-to-I editing of non-synonymous codon positions in protein-coding genes, which results in protein variants harboring a single amino acid substitution.

RNA editing frequency: the fraction of edited RNA molecules ranges from a few to almost 100% of the gene's transcripts. Thus, edited and unedited variants are usually coexpressed within the same cell providing for transcriptome variation without the all-or-nothing effect of DNA mutations in the genome.

Specificity of editing: intrinsically, the A-to-I RNA editing machinery is promiscuous in that it will modify without site-selectivity many of the adenosines that are located within an extended, perfect dsRNA. The high site-specificity of physiological recoding targets (glutamate receptors, 5-HT_{2C}, Gabra-3) lies within the intricate 3D RNA fold, which includes base-paired regions as well as bulges and loops. Although the exact mode of interaction of the editing enzymes with their targets is unknown, these partially base-paired RNA structures are believed to guide the machinery to edit a single nucleotide with high efficiency.

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Box 1. Types of RNA editing

The term ''RNA editing'' was initially introduced over 20 years ago after the discovery of mitochondrial mRNA modification in kinetoplastid protozoa. The different types of editing distinguished today differ substantially in their molecular mechanisms, machineries and species distributions (for review see [1]).

Insertion and deletion: Affects most mitochondrial transcripts in kinetoplastids and involves the addition and deletion of non-genomically encoded uridine residues in pre-mRNA transcripts. The required information for site selection and editing extent is provided by gRNAs, which are complementary to the fully edited mRNA, and is further mediated by multiprotein complexes. Another type of insertional editing is observed in mitochondria of the slime mold *Physarum polycephalum*. The majority of the editing events observed in this organelle involve the cotranscriptional insertion of cytosines [1].

Substitution: Occurs in both pre-mRNAs and tRNAs. Apart from A-to-I modifications, cytosine deamination is a form of RNA editing also found in mammalian nuclear genes, although only a few physiological targets of the C-to-U RNA editing machinery are known. A well-characterized C-to-U editing target is human apolipoprotein B (*APOB100*), which is essential for the removal of low-density lipoproteins. Tissue-specific *APOB100* deamination introduces an in-frame stop codon, generating a truncated protein (ApoB48) with altered physiological functions[1]. Intriguingly, the enzymatic component of the C-to-U editing activity is the cytidine deaminase APOBEC 1 (APOB mRNA-editing enzyme catalytic polypeptide 1), which is related to the APOBEC 2/3 family of DNA-specific modification enzymes active in retroviral restriction [84]. C-to-U base conversion in RNA is more common in plant mitochondria [1].

tRNA editing: Adenosine deamination in tRNAs is found across organisms from prokaryotes to mammals and includes the generation of the wobble base in the tRNA anticodon. A-to-I modification is accomplished by ADATs (adenosine deaminase acting on tRNAs), a family of deaminases that share sequence similarity with the catalytic deaminase domain of ADARs but lack dsRBDs (for review see [95]).

Other types of substitution: G-to-A, U-to-C or other conversions are occasionally reported [2]. Those types of changes would often require the cleavage and religation of the RNA molecules and to date neither the molecular mechanism(s) nor the involved enzymes are known.

the case if the gene is subject to A-to-I RNA editing, because a fraction of the primary transcripts undergo a recoding event. Because inosine (Box 2) is interpreted as a guanosine by the translational machinery, RNA editing can change the meaning of codons. As a result, a fraction of the protein output will carry a single amino acid substitution compared with the non-edited version. Until recently, only a small number of proteins with amino acid substitutions caused by editing were known, most of them identified by chance. Recent studies, facilitated by bioinformatics and deep sequencing approaches, support the notion that hundreds of genes undergo recoding editing resulting in amino acid substitutions [6,7]. However, it seems that many of the recoding events identified more recently display low level modification rates and that despite considerable high-throughput sequencing relatively few novel sites become validated [6,7]. Thus, because RNA editing is fractional and might be restricted in time and/or space, the comprehensive mapping of all recoding editing sites within the human transcriptome will require the combination of bioinformatics-based editing site prediction with deep sequencing and/or targeted specimen analysis.

For most cases of recoding editing characterized in mammals, subtle to dramatic changes in protein function result from single amino acid differences. Intriguingly, many of the modified codons specify highly conserved residues that might otherwise be excluded from variation through genomic mutations by purifying selection [4,8]. A-to-I RNA editing might, therefore, play an evolutionary role allowing for the exploration of sequence space at a small, tolerable rate [4,8].

The highest level of transcript diversity caused by editing is generated within transposon-derived repeat sequences. Especially in primate genomes, the prevalence and genetic properties of Alu-type repeat elements [94] make pairs of Alu elements within primary transcripts the most prominent editing targets. Tens of thousands of individual editing sites in thousands of mRNAs with Alu elements have been mapped within the human transcriptome [9–12], and deep sequencing analysis further indicates that many more editing events exist in Alu elements already known to harbor editing sites [13]. The properties of the intrinsically promiscuous RNA editing machinery paired with the characteristic Alu pair RNA secondary structure induces highly efficient multiple site editing in Alu elements. In fact, predictions based on the existing data posit that more than 98% of all pre-mRNAs are subject to Alu-mediated RNA editing [9].

What could be the functional impact of the abundant editing of repetitive sequences, especially primate-specific Alu elements? Most Alu repeats (as well as other types of repeat elements) are located in introns and non-translated exons; in these cases, editing will not directly influence protein function. Still, editing changes within those sequences have the potential to indirectly alter protein expression or function. For example, sometimes editing can induce alternative pre-mRNA splicing through the creation of a cryptic splice donor (AT to IT) or acceptor (AA to AI) site [9,14], modulate alternative splicing efficiency through the modification of splicing enhancer or inhibitor sequences, or eliminate a consensus splice acceptor site (AG to IG). Alternatively, editing can modulate other types of functional RNA elements, such as miRNA-binding sites in mRNAs [15]. However, most of the time, the outcome of Alu editing is simply an RNA with multiple inosines present within one or more regions of the pre-mRNA or within the untranslated regions of a spliced mRNA. It seems that there is not a single mechanism, but rather several outcomes, for the fate of such an Alu-edited RNA. On one hand, experimental evidence shows that Alu-edited RNAs often become sequestered in the nucleus by a protein complex with a specific affinity for inosine in RNA molecules [16,17]. On the other hand, these and other Alu-edited transcripts sometimes get exported and associate with polysomes despite being edited [18,19]. So far, it is unknown what might regulate such distinct behaviors. In one specific example of a heavily edited 3'-UTR (untranslated region) of the mouse cationic amino acid transporter (*Cat2*) gene, nuclear- retained transcripts become mobilized for export and translation following cellular stress through the cleavage of the inosine-containing 3'-UTR from the rest of the mRNA [20]. Furthermore, a more general switch activating the

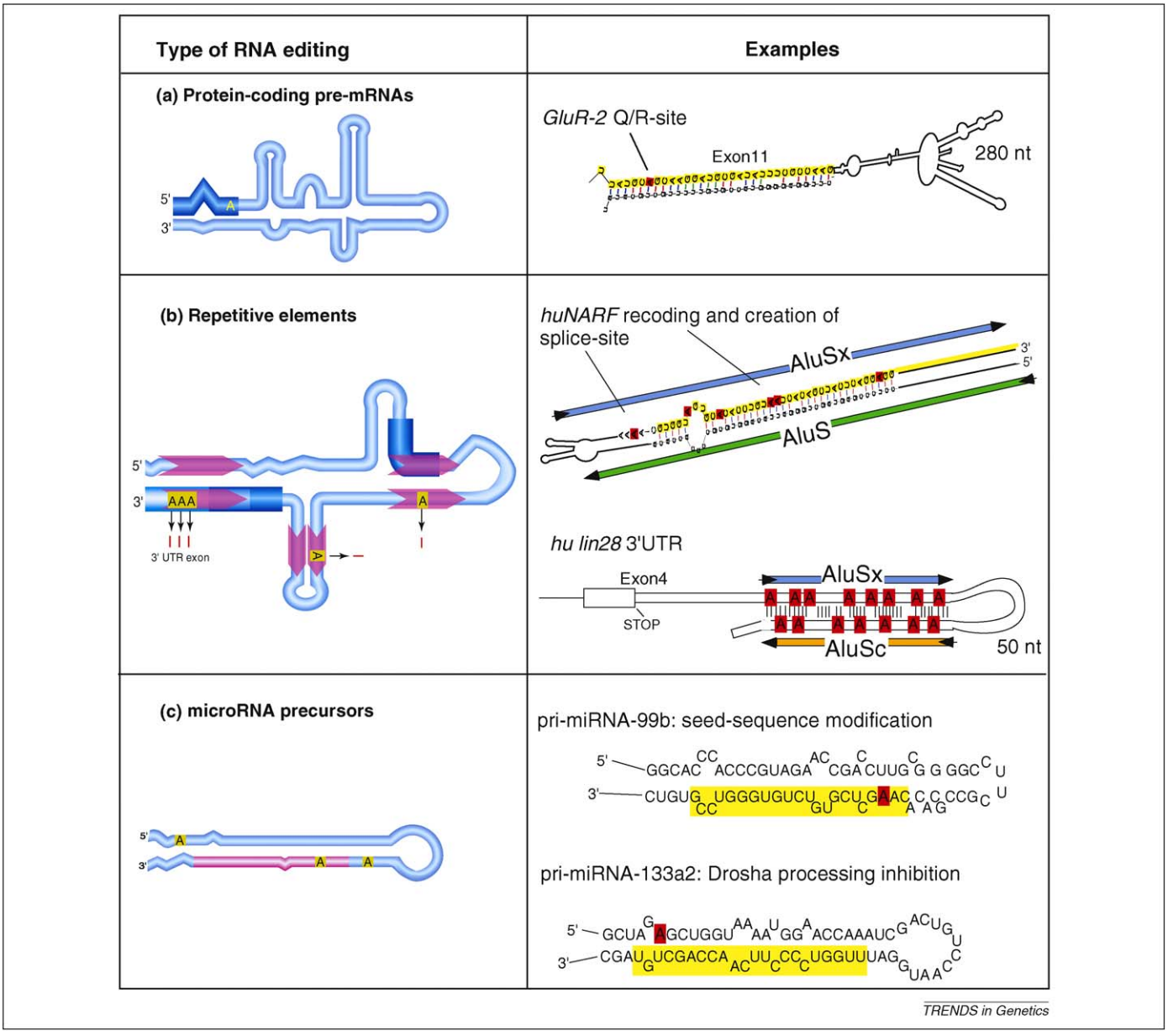

Figure 1. Illustration of the three major types of A-to-I RNA editing targets and their fates. Panels on the left show a schematic of RNA secondary structures highlighting a translated exon sequence (dark blue box), untranslated exon sequence (light blue boxes), location of repetitive sequence elements (red arrows), non-coding and intronic RNA sequence (light blue lines) and location of mature miRNA sequence (light red line). **(a)** The pre-mRNA editing of protein-coding genes with a composite RNA secondary structure leads to highly site-selective recoding if it affects a non-synonymous codon site. For example, the glutamate receptor subunit *GRIA2* exon 11 Q/R site [2] forms an experimentally validated secondary RNA structure between exon 11 (marked in yellow) and intron 11. **(b)** Pairs of repetitive elements, such as primate Alus located in coding or non-coding exons or introns, can generate RNA secondary structures targeted by the RNA editing machinery. For example, the editing of the intramolecular RNA fold between two Alu elements in human nuclear prelamin A recognition factor (*NARF*) causes recoding within the Alu exon (marked in yellow) and leads to the creation of the 3′-splice consensus site upstream of the Alu exon, thereby regulating alternative splicing of this exon. In the case of human *lin28*, extensive RNA editing within its non-coding, 3′-UTR mediated by a pair of Alu elements leads to the nuclear retention of the mRNA. **(c)** The characteristic secondary structure of pre-miRNAs is a frequent target of ADARs. For example, pri-miRNA-99b editing alters a nucleotide within the seed of the mature miRNA (marked in yellow and edited position highlighted in red) and, therefore, has the potential to alter the target interaction profile of this miRNA [26], whereas the modification of an adenosine outside of the mature miRNA region in pri-miRNA-133a2 causes a change in the processing rate by the RNase Drosha [26].

retention of inosine-containing RNAs in the nucleus of human cells is provided by the induction of the non-coding RNA nuclear paraspeckle assembly transcript 1 *(NEAT1)* [19]. Human embryonic stem cells do not express *NEAT1* and export heavily edited RNAs. By contrast, differentiation induces *NEAT1* expression, leading to the formation of nuclear structures called paraspeckles, which not only colocalize with the proteins known to bind inosine-containing RNAs, but also prevent the export of heavily edited Alu-containing transcripts [19]. Despite these intriguing examples of the regulation of gene expression involving edited Alu repeats, it will be necessary to elucidate the molecular mechanisms that lead to the nuclear binding, storage, degradation or release of these RNAs to understand the bigger picture of why and when a particular transcript that undergoes Alu-mediated editing enters a specific pathway.

The site-selective modification of miRNA precursor molecules represents another frequent event of A-to-I RNA editing. miRNAs are small, regulatory RNA molecules

Box 2. A-to-I editing: chemical mechanism and machinery

A-to-I RNA editing by ADARs proceeds via a hydrolytic deamination mechanism without the requirement for RNA backbone breaks (proposed mechanism in Figure I). Only adenosines within the context of RNA molecules are targeted by ADARs. Inosine largely behaves like a guanosine in RNA folding and is also interpreted as G by the translation machinery. Editing occurs within sections of RNA that are completely or partially double- stranded and does not require any essential cofactors [2,3].

ADAR proteins have been characterized from different organisms, including worms, insects and vertebrates [2,3]. They share a common domain structure with two or three dsRBDs and a C terminal catalytic deaminase domain. The C terminal deaminase domain, which is highly conserved between ADARs, coordinates a Zn^{2+}-ion in its catalytic center and the functional deaminase fold requires the incorporation of an inositol hexaphosphate IP6 molecule [96].

Three ADARs (ADAR1, ADAR2 and ADAR3) have been identified in humans (Figure II). ADAR1 protein is the largest of the three family members and is expressed in two major splice variants ADAR1 p150 and ADAR1 p110. ADAR1 p150 contains an extended N terminus including two Z-DNA/RNA- binding motifs (Z_α and Z_β) [2,3]. ADAR2 and ADAR3 share high sequence similarity (50% protein sequence identity), but to date no catalytic activity has been documented for ADAR3. The ADAR3 R-domain, a 13–15 amino acid, arginine-rich sequence motif, mediates ssRNA binding [2,3] and serves as a nuclear localization signal [31]. ADAR2 can also be expressed to include a N terminal R-domain [97], which can exhibit a different cellular localization from the major ADAR2 splice form. ADARs undergo both homo- and heterodimerization and are likely to be catalytically active as dimers [37].

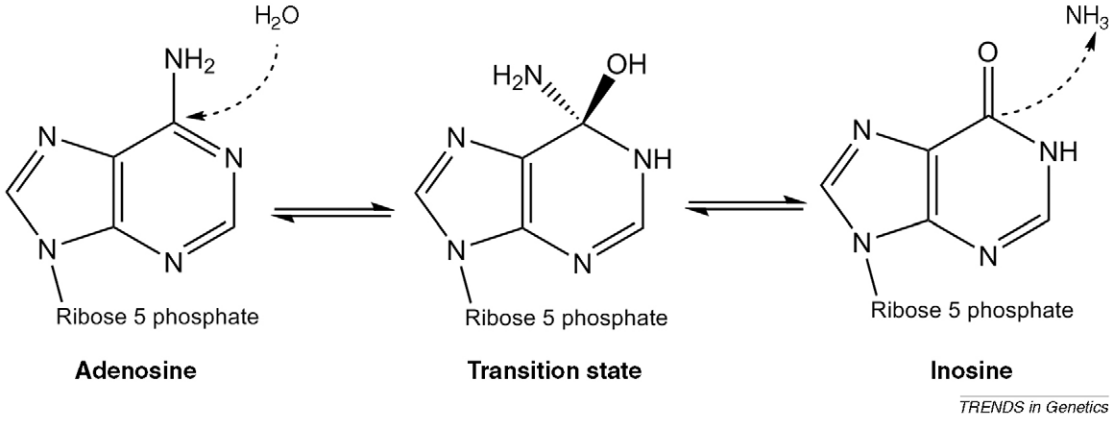

Figure I. Mechanism of adenosine deamination.

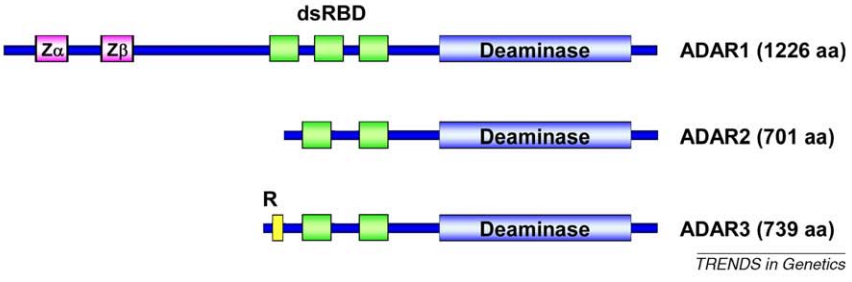

Figure II. ADAR domain structure.

with diverse roles in development, differentiation and cell cycle regulation [21]. Each of the small RNAs is excised from longer, hairpin-structured precursors through the sequential action of the RNases Drosha and Dicer. Following the initial reports of miRNA sequence editing [22,23], additional miRNA precursors have subsequently been shown to undergo editing, and current estimates posit that ~16% of all human miRNA genes are subject to A-to-I modification. The editing of nucleotides in the vicinity of Dicer or Drosha processing sites can prevent the further maturation and expression of the miRNA [2,24,25]. Intriguingly, if A-to-I editing modifies a nucleotide within the miRNA seed sequence that is critical for target recognition, then the edited mature miRNA can exhibit a distinct target profile from the non-edited variant. This is the case for human miR-376 and possibly for four other miRNAs [26,27]. Yet, the predominant outcome of pre-miRNA editing is the modulation of miRNA biogenesis through the inhibition of Drosha- or Dicer-mediated cleavage [26]. miRNA function can also be influenced through the editing of miRNA- binding sites on their target sequences [28]. Although this aspect has not been fully explored, human miR-513 and miR-769-3p/-450b-3p provide examples of when A-to-I editing in the target mRNA generates a consensus target sequence [15].

Maintaining the balance: regulation of RNA editing

The editing of recoding targets is under tight control, and the deregulation of RNA editing in space and/or time is correlated with various human disease phenotypes. The specific molecular mechanisms that govern intracellular RNA editing levels are largely unknown. For example,

although ADAR1 and ADAR2 expression is, in principle, ubiquitous, the presence of ADAR mRNA (or even proteins) rarely correlates with the observed intracellular RNA editing activity (reviewed in [2,3,29]). However, recent insights regarding the developmental and cell-type specific modulation of RNA editing in conjunction with ADAR expression and localization studies have revealed multiple and complex patterns of regulation on the transcriptional, post-transcriptional, translational and post-translational levels. For example, the ADAR proteins are expressed in several alternative splice forms that differ with respect to their intracellular localization, enzymatic activity and/or target specificity [2,3,29].

The editing of pre-mRNAs is often restricted to the nucleus, in particular for editing events that affect intronic sequences or that are mediated through RNA folds involving intronic regions (such as many of the known recoding cases of editing). Most ADAR proteins localize to the nucleus, with the exception of the ADAR1 p150 variant, which is shuttled between the nucleus and cytoplasm, and might perform specific editing or other functions in the cytosol. The p150 isoform of ADAR1 is expressed from an interferon-induced promoter and carries a unique N terminal DNA-binding domain [30]. By contrast, the nuclear ADAR1 p110 variant and the editing enzyme ADAR2 are expressed constitutively. Nuclear RNA editing activity can be regulated through the controlled nuclear import of ADAR proteins. This notion is supported by the observed differential interaction of the nuclear import machinery with individual ADARs [31]. Furthermore, dsRNA binding coregulates the transportin-1- mediated nuclear import of ADAR1 through the competition of double-stranded (ds)RNA and transportin-1 for interaction with the ADAR1 dsRNA- binding domains (dsRBDs) [32]. Within the nucleus, ADARs are shuttled between the nucleoli and nucleoplasma – another potential mechanism for regulating nuclear editing activity [33,34]. Intriguingly, ADAR2 editing activity is further balanced through a feedback mechanism wherein increased functional ADAR2 expression leads to the self-editing of *ADAR2* pre-mRNA, which results in the production of inactive, truncated ADAR2 protein [35]. Similarly, the single ADAR gene in *Drosophila melanogaster* is subject to self-editing; however, in this case the modification results in an amino acid substitution that substantially represses RNA editing activity [36].

Although ADAR1 and ADAR2 seem to be fully functional without the requirement of essential cofactors, homodimer (and potentially heterodimer) formation can modulate target specificity and activity [37]. On that level even ADAR3 might modulate RNA editing activity through heterodimerization with ADAR1 or ADAR2 [37]. The ADAR3 protein shares high sequence similarity with ADAR2, but exhibits no detectable deamination activity [2,3]. Furthermore, the recent identification of additional ADAR interaction partners presents further opportunities for the cell-type specific regulation of editing activity [31,38,39]. Although post-translational modification of ADARs has been suggested as a regulatory mechanism, only ADAR1 sumoylation, which represses editing activity, has been documented to date [40].

The regulation of RNA editing extent and specificity also occurs on the level of individual target transcripts and involves competition between and coregulation of pre-mRNA splicing and editing. In particular, if editing sites are positioned in close proximity to splice consensus sites, the strength of the splicing signal influences RNA editing extent nearby. Similarly, a strong RNA fold mediating editing might promote efficient splicing of only the edited transcript molecules [41–43]. It is often difficult to predict the level of interdependence between editing and splicing because other interactions of RNA-binding proteins (for example, splicing enhancers or silencers) with the RNA target can impact its ability to be edited or spliced. ADARs have also been found to physically associate with the RNA polymerase II C terminus. This colocalization further argues for a close coupling between transcription and editing [44].

For at least one specific case of recoding A-to-I RNA editing, the coregulation of the target through small nucleolar (sno) RNA binding and modification modulates RNA editing activity. The snoRNA *h/mbii-52*, a component of the Prader–Willi syndrome (PWS) imprinting cluster, not only regulates the alternative splicing of the serotonin receptor 2C through specific interaction with its pre-mRNA [45], but also inhibits the site-selective editing of this RNA and leads to the methylation of the adenosine that is also targeted for editing by ADAR2 [46,47].

Tipping the balance: insights from genetics

In recent years, various animal models with hyper-, hypo- or misediting have substantiated the general importance of editing for normal physiology and also revealed some intriguing connections to human disease phenotypes. In flies, which carry a single *ADAR* gene (*dADAR*), the genetic inactivation of A-to-I editing activity yields a strong neurological phenotype with locomotor deficiencies, seizures, premature neurodegeneration and altered reproductive behavior [48]. The ability to both reproduce the phenotype through the neuron-specific knockdown of *dADAR* in adult flies and partially rescue the knockout phenotype in adults using *ADAR* transgenes suggests that the recoding of mostly neuronal targets in fully developed individuals is the primary function of *dADAR* [49]. In rodents, the genetic inactivation of *Adar1* or *Adar2* also leads to severe phenotypes. Indeed, the mouse *Adar1* knockout is embryonically lethal around developmental day E12.5 [50,51]. Although the molecular mechanism for this outcome is unknown, a failure of the hematopoietic system and widespread apoptosis is observed in $Adar^{-/-}$ embryos. Intriguingly, adult-specific *Adar1* inactivation demonstrates that ADAR1 is essential for the maintenance, but not for the establishment, of hematopoietic stem cells (HSCs) and that the increased rate of cell death upon *Adar1* loss is due to a runaway interferon response within these stem cells [52,53]. Of note, ADAR1 p150 is highly expressed in wild type HSCs and might constitute a negative regulator for interferon induction. Currently, it is unknown if this dependency involves a specific RNA modification event or stems from an editing-independent function of ADAR1 p150.

ADAR2 is essential for normal murine brain function because homozygous knockout mice develop epileptic seizures shortly after birth and die within a few weeks of age [41]. This phenotype can be attributed entirely to the consequences of the editing deficiency within a single neuron-specific gene, the glutamate receptor subunit *GRIA2*, which in normal neurons is edited specifically to nearly 100% by ADAR2. The genetic pre-editing of *GRIA2* transcripts through genomic mutation completely rescues the phenotype of *Adar2*$^{-/-}$ mice. This is remarkable because many other RNAs are edited to lower levels in *Adar2*-deficient mice; however, the ensuing functional changes do not seem to interfere with lifespan or normal physiology. In both *Adar1* and *Adar2* knockout mice, the loss of editing activity of one ADAR is partially compensated by the overlapping activity of the other. Moreover, the linkage of editing to other RNA processing events can lead to a partial rescue of editing deficiency being present on the pre-mRNA level. For example, whereas *GRIA2* pre-mRNA is edited to only 10% in *Adar2*$^{-/-}$ knockout mice, the processed mRNA shows editing of 40% because edited primary transcripts are preferentially spliced [41].

Fewer insights are available regarding the consequences of overproducing ADARs *in vivo*. In *Drosophila*, the expression of a *dADAR* mutant that escapes down-regulation through self-editing is lethal and displays a hyperediting phenotype [54]. Mammalian *ADAR2* is also subject to self-editing that leads to a decrease in functional ADAR2 protein [35]; however, mutant mice that lack the ability to edit *Adar2* pre-mRNA show hyperediting, but do not display a discernable behavioral or neurological phenotype [55]. By contrast, the widespread overexpression of a rat *Adar2* transgene in mice results in an obese phenotype [56]. The molecular mechanism for this outcome is unknown; however, this phenotype might not only be due to the increased production of ADAR2, but also a result of the constitutive misexpression of the editing enzyme in cells that do not produce ADAR2 in wild type mice [56].

Dyschromatosis symmetrica hereditaria (DSH1) is an autosomal dominant trait that has been linked to mutations in human *ADAR1* within several Chinese and Japanese families [57]. Characterized by the hyperpigmentation of the hands and feet, many of the mapped mutations suggest a monoallelic inactivation of the functional deaminase. The dominant phenotype could, therefore, be related to a gain-of-function of the truncated or otherwise mutant protein, for example because of its altered RNA- binding properties [57,58]. In addition, recent studies in several centenarian populations have linked polymorphisms in either *ADAR1* or *ADAR2* to human longevity [59].

Connections to cancer

Owing to the diverse impact of RNA editing on gene expression and function, it is possible that its misregulation might play a role in tumorigenesis by either inactivating a tumor suppressor or activating genes that promote tumor development or progression. This notion is supported by observations that link RNA editing alterations with cancer phenotypes (reviewed in [60]). In addition to the general decrease in RNA editing activity detected in several cancer types [61], a specific deficiency in A-to-I editing of glutamate receptor channels is evident in human brain cancers [62,63]. In particular, *GRIA2* Q/R site editing, the molecular determinant for the Ca^{2+}-permeability of AMPA-type glutamate receptors, shows a reduction in modification rates that seems to correlate with tumor stage and has been linked directly to malignant cell behavior, such as migration and invasion [64]. To date, it is unknown if the deregulation in *GRIA2* editing is a causal event for tumor development or represents a marker for tumor classification and progression.

The identification of several cancer-specific editing events within known or potential oncogenes [60,65,66] supports the idea that this epigenetic mechanism could contribute, directly or indirectly, to cancer growth. However, a direct link between these editing events and cancerous growth remains to be shown. Recently, a high-throughput analysis of genome and transcriptome evolution of a lobular breast cancer specimen interestingly identified a few novel cases of human A-to-I recoding editing [7]. However, even though ADAR1 expression was upregulated within the tumor tissue [7], the detected editing events were not restricted to the cancerous cells. The possibility remains that ADAR1 hyperactivity or the deregulation of ADAR2 editing due to ADAR1–ADAR2 heterodimerization might cause aberrant editing.

The frequent A-to-I editing of miRNA transcripts also might contribute to tumorigenesis and cancer progression because RNA editing alters expression levels or the target spectrum of miRNAs that in turn regulate signal transduction pathways involved in cell cycle and growth regulation [67]. For example, both miR-376 and miR-142 undergo editing, and their deregulation is implicated in the molecular signatures of pancreatic cancer and leukemia, respectively [60,68,69].

Although several cancer phenotypes are associated with hypoediting [61–63] (Figure 2), there is no apparent causal relationship between decreased RNA editing levels and the initiation of cancerous growth as judged by currently available animal models of RNA editing deficiency [41,48,52,70,71].

Neurological disorders and behavior

Neuronal tissues show high RNA editing activity and many recoding A-to-I editing events affect brain- specific genes. Thus, highly complex systems and their complex physiology and behavior might strongly rely on epigenetic sources of variation, such as A-to-I editing [4,8,72]. In fact, these types of mechanisms could enable and/or accelerate the evolution of highly complex organisms [4,73]. Thus, defects or deregulation in RNA editing might cause or accompany disturbances in higher order function more frequently than they disturb any basic physiological processes. In that respect it is noteworthy that behavioral differences between mouse strains correlate with distinct RNA editing profiles and that several animal models of editing deregulation display behavioral abnormalities [48,74–76] (Figure 2). Editing of the 5-HT_{2C} serotonin receptor, which has established roles in emotion, locomotion, appetite, metabolic rate control, depression, schizophrenia and drug-addiction, provides an illustrative

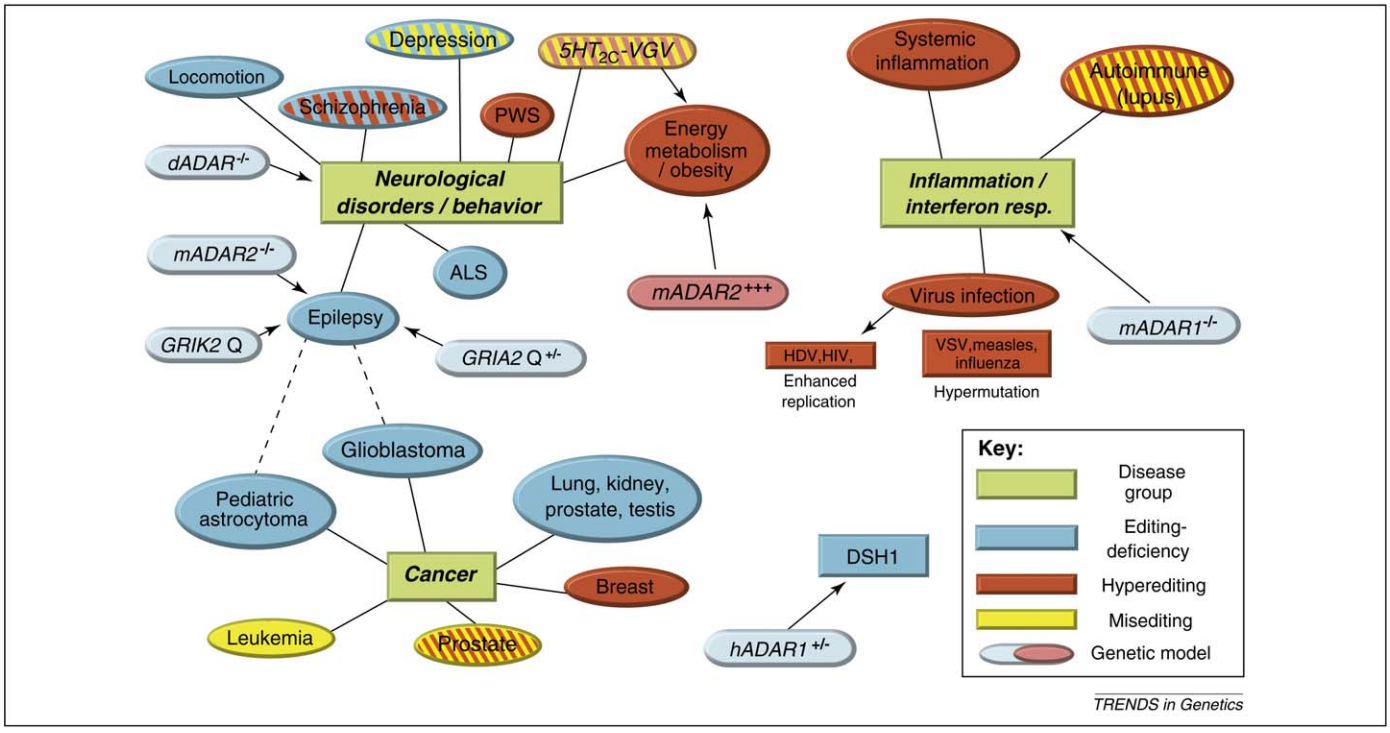

Figure 2. Disruption of the RNA editing balance
Overview of RNA editing phenotypes in various genetic animal models and correlated observations regarding editing in human diseases. Direct causal relationships are indicated by arrows, correlations are shown as lines and possible cross-connections as dotted lines. The partial or complete inactivation of editing has been linked to several neurological and neuropsychiatric disorders. Green shaded areas: main disease groups. Blue shaded areas: disease phenotypes with general or gene-specific editing deficiency (genetic models with hypoediting are in light blue boxes). Red shaded areas states with: increased editing activity (genetic models with hyperediting are in light blue). Yellow shaded areas: changes in editing pattern or misediting without general increase or decrease in editing activity. In PWS, the loss of imprinted sno RNA *mbii-52* leads to the increased editing of *5-HT$_{2C}$* receptor transcripts [46]. DSH1 is linked to haploinsufficiency of ADAR1 [57]. Related references: schizophrenia [80]; locomotion [48,98]; depression [77,79,81]; ALS [99]; epilepsy [41,70,71]; glioblastoma [63]; pediatric astrocytoma [60,62]; leukemia [100]; prostate cancer [66]; breast cancer [7]; lung, kidney, prostate and testicular cancer [61]; systemic inflammation [92]; autoimmune (lupus) [90,91]; virus infection [85–88]; *dADAR$^{-/-}$* [48]; *mADAR2$^{-/-}$* [41]; *GRIK2* Q [71]; *GRIA2* Q$^{+/-}$ [70]; *5HT$_{2C}$-VGV* [74]; *mADAR2^{+++}* [75]; *hADAR1$^{+/-}$* [57]; *mADAR1$^{-/-}$* [52,53].

example [77,78]. The multiple site editing of this receptor subunit regulates the responsiveness of the receptor to serotonin: upon serotonin binding, more strongly edited molecules display decreased coupling efficiency to the downstream G-protein [77]. In human patients with depression, changes in the 5-HT$_{2C}$ editing patterns are apparent and, intriguingly, the treatment of mice with a serotonin uptake inhibitor is accompanied by converse alterations in editing [78,79]. From the analysis of patient specimens, misediting is also observed in some cases of schizophrenia [80]. Mice that misexpress solely the fully edited version of the serotonin receptor (5-HT$_{2C}$-VSV) display increased metabolism, hyperphagia and growth retardation [74]. Although the fully edited serotonin receptor dampens its G-protein coupling efficiency, in this mouse model 5-HT$_{2C}$ neurotransmission is oversensitive to serotonin due to the strongly increased functional expression of the receptors [74]. Straightforward genetic mutations are clearly not sufficient to fully elucidate the physiological role(s) of serotonin receptor editing. The possibility that RNA editing patterns might display dynamic changes in response to external signals such as stress or medication [81] makes the analysis and interpretation of *in vivo* models even more complicated. However, at the same time, if this exciting aspect proved to apply to RNA editing in general, new layers in the cell-type and time-selective regulation of gene expression through RNA editing could emerge.

ADARs on the radar

Several recent reports have suggested that some aspects of ADAR function might be independent of their adenosine deaminase activity. For example, catalytically inactive ADAR2 can suppress the processing of human pri-mir-376a2 without causing editing changes [58], probably based on its selective RNA- binding properties that interfere with the association of miRNA processing factors. Similarly, ADAR1 p150 counteracts siRNA function in mouse *Adar1$^{-/-}$* MEF cells [82] and in a *Drosophila* system [58], also in an editing independent fashion. In summary, the range of ADAR RNA targets could be much larger than the number of edited messages; moreover, the catalytically inactive ADAR3 might exert independent functions that arise from its RNA- binding properties.

The functional roles of the ADAR1 p150 isoform are not well understood. It shares properties with antiviral factors: both are interferon induced [83] and largely localized in the cytosol; moreover, ADAR1 p150 editing activity could target viral RNAs thereby inhibiting their replication in a similar manner to how C-to-U DNA-modifying proteins restrict retroviruses [84]. However, recent studies have documented that ADAR1 can act as a proviral factor during HIV [85,86], vesicular stomatitis virus [87] and measles [88] infections through both editing-dependent and -independent mechanisms. In several cellular systems, including during measles virus infection, ADAR1 overexpression counteracts PKR kinase activity and inhibits apoptosis [88]. In light of these

findings, the escalating interferon response and cellular death observed in *Adar1*-ablated HSCs [52] further supports a role for ADAR1 in downregulating inflammatory response pathways. In that sense, interferon-induced ADAR1 expression does not occur to battle an infection, but instead serves to keep the antiviral response in check. As a result, virus replication is enhanced in cells which express ADAR1. In addition, some viruses might use cytoplasmic ADAR1 p150 to further stimulate viral infection or replication through direct editing of their transcripts [85,88,89].

Another connection between the interferon-mediated induction of ADAR1 p150 and inhibition of apoptosis might lie in the observation of high ADAR1 levels in T-cells and B-cells of lupus erythematosus patients, a severe, systemic autoimmune disease with signs of aberrant RNA editing [90,91]. This hyperediting phenotype is also observed in other inflammatory processes, such as endotoxin-induced systemic inflammation [92] and upon cellular treatment with tumor necrosis factor- α or interferon- γ [92]. As such, the re-equilibration of ADAR1 activity within immune cells could be an effective strategy for the treatment of autoimmune disorders.

Concluding remarks and perspectives

Clearly, A-to-I RNA editing can directly or indirectly affect the expression or function of many genes. The alteration of amino acid codons, splice patterns, stability or localization of protein-coding transcripts, modulation of regulatory RNA biogenesis and function and crosstalk of RNA editing with RNA processing and silencing pathways provide a rich resource for the generation of molecular diversity and gene regulation. These findings also illustrate that we are only beginning to understand how RNA editing is integrated into the biological networks of gene expression, regulatory pathways and genome evolution.

Recent efforts to identify RNA editing events in the human transcriptome using deep sequencing approaches indicate that many editing sites remain to be discovered. However, most recoding sites might be modified only to levels of less than a few percent [6], suggesting that many recoding events might not be of immediate biological relevance, but could represent a form of noise or be part of a broader evolutionary role of editing [4]. Ultimately, the generation of *in vivo* models of gene-specific editing deficiency or hyperediting should shed light on the physiological significance of particular editing events within the organismal context as exemplified by the neuronal glutamate and serotonin receptor targets [70,71,74,93]. However, such a reductionist approach will probably not be appropriate to unravel other aspects of RNA editing biology. For some editing targets, such as repetitive sequence elements, a direct genetic strategy will neither be technically feasible nor expected to yield insights that apply to the whole group of targets. Keeping in mind the complex environment in which RNA editing occurs (highly dynamic RNA folding equilibrium of substrates, divers expression of machinery and targets) and its role in providing additional levels of molecular complexity, it is possible to think of RNA editing as an indicator of complexity states; for example, reflecting higher order brain functions. In diseases where the normal complex states of activity become perturbed, we can therefore also expect to observe a disturbance in RNA editing activity or patterns. We speculate that monitoring the global activity of RNA editing *in vivo* represents a useful early biomarker to detect disturbances in complex systems (such as the brain) even before clinical symptoms become apparent. In that way, learning about editing patterns and dynamics could enhance the understanding of complex biological systems even before all the molecular targets and consequences of RNA editing are elucidated.

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