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Review

A-to-I editing of microRNAs: Regulating the regulators?

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ABSTRACT

An important epigenetic mechanism in mammals is adenosine deamination, which generates transcriptome variety through the conversion of single adenosines into inosines in RNA molecules. Inosine is interpreted as guanosine by the translational machinery, and when A-to-I RNA editing occurs in the coding region of pre-mRNA molecules this substitution can result in non-synonymous codon changes and subsequent altered protein function. Furthermore, editing can also take place in non-coding RNA molecules, including pri-miRNAs. In this review I intend to give an overview on the interplay between miRNA-mediated control of gene expression and RNA editing, and how editing could impact cellular behavior by influencing mature miRNA expression levels.

Contents

1. Introduction

RNA molecules have only recently been recognized as important elements for generating cellular complexity. For a long time the role of RNA molecules was thought to be confined to a limited number of classical processes. However, the elucidation of the human genome, and with that the notion that humans do not have a substantially higher number of protein-coding genes in comparison to lower organisms, alluded to the potential function of RNA molecules as an additional regulatory layer [1]. Today, RNA molecules have indeed been proven to be important players in controlling gene expression on transcriptional and post-transcriptional levels, as well as contributing to other crucial cellular processes [1,2].

The strength of the transcriptome lies within its great plasticity. Both coding and non-coding RNA molecules define protein levels and ultimately cellular behavior. Moreover, enzymatic modifications within these RNA molecules result in a huge transcriptome and subsequent proteome diversity. This diversity and flexibility contribute to the development and evolution of complex cellular processes and organisms, arising from a limited set of genes encoded within the DNA. An important enzymatic process that generates transcriptome diversity is A-to-I RNA editing [3,4], which causes the conversion of single adenosines into inosines in coding and non-coding RNA molecules. This form of editing has recently also been observed in the precursors of micro(mi-)RNAs and small interfering (si-)RNAs, which are essential classes of gene regulatory RNA molecules. Editing of these RNA molecules might interfere with their functionality, resulting in an intriguing interplay between editing activity and small RNA-mediated control of gene expression. In this review I will therefore mainly focus on the consequences of editing in these important classes of small non-coding RNA molecules.

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2. The editing mechanism

Editing was initially observed in the coding regions of gene transcripts. As inosine has similar functional characteristics as guanosine, this A-to-I conversion can have profound consequences on protein function. For example, editing occurs in the pre-mRNA transcripts of the glutamate receptor GluR-2B subunit [5]. At one specific position it causes the alteration of a codon, CAG (Q) into CIG (R), and thus a different amino acid is incorporated into the final protein than would be expected when solely looking at the genomic sequence. This Q/R switch alters the ability of GluR-2 to regulate Ca^{2+} permeability, and mice with a decreased Q/R conversion rate suffer from severe epileptic seizures and die within three weeks after birth [6].

Editing at this position occurs in over 99% of the transcripts. In contrast to this nearly complete conversion, the modified and unmodified variants of most pre-mRNA editing substrates usually exist side-by-side. For instance, editing occurs at five major sites in transcripts of the 5-HT_{2C} serotonin receptor [7]. At each of these positions either an adenosine or an inosine can be present, theoretically generating 32 (2^5) different transcripts, which arise from one genomically encoded sequence. This underscores the importance of A-to-I editing as an epigenetic mechanism to create transcriptome and proteome diversity.

The only true pre-requisite for an editing substrate is the presence of a double-stranded RNA foldback structure. Thus, any RNA molecule that partly folds back onto itself may be targeted by the editing enzymes, and it is therefore suspected that a substantial amount of low-level recoding A-to-I conversions occurs [8,9]. At this moment it is still unclear why some double-stranded substrates are more potent editing targets than others, although certain 5′ and 3′ base preferences with respect to the targeted adenosine exist [10–12].

The requirement of a base-paired RNA structure arises from the presence of double-stranded RNA binding domains in the editing enzymes [13]. Editing is catalyzed by the family of Adenosine Deaminase acting on RNA (ADARs). Three ADAR enzymes exist in mammals (ADAR1–3) [13], of which ADAR1 and ADAR2 are nearly ubiquitously expressed, while the expression of ADAR3 is restricted to the brain [4]. To date there has not been any editing activity observed for ADAR3, while ADAR1 and ADAR2 catalyze the A-to-I conversions of all currently characterized editing substrates. ADAR1 and ADAR2 can often convert the same substrate, although they exhibit different efficiencies as they have an overlapping but distinct substrate specificity [4].

How editing activity is controlled *in vivo* is still largely unknown. However, both ADAR1 and ADAR2 enzymes exist as different isoforms, which can determine their subcellular localization. Especially recoding editing is a nuclear event, and editing might thus in part be regulated through the controlled nuclear localization of the different editing enzymes. In this respect, the long ADAR1-p150 isoform is actively shuttled between the nucleus and cytoplasm, while the shorter ADAR1-p110 isoform is retained inside the nucleus [14]. Similarly, different protein variants of ADAR2 might influence the import of this protein into the nucleus [15].

ADARs bind and edit long double-stranded RNA foldback structures with high efficiency. These structures often arise in the primate transcriptome due to the presence of oppositely orientated Alu repeat sequences within one RNA molecule. Computational analysis has estimated that at least 87% of all pre-mRNA molecules contain inosine at least at one position due to the presence of these Alu sequences [10]. At this moment, the biological significance of editing within Alu repeat sequences is still largely unclear. It can lead to exonization through the alteration of splice sites [10,16], might result in the silencing of retrotransposons [17], or could cause nuclear retention [18].

Editing events can occur in the protein-coding and non-coding regions of pre-mRNA molecules, but also in non-coding RNA transcripts. One such class of edited non-coding RNA molecules is microRNAs, which are important regulators of gene expression levels.

3. microRNAs: a short introduction

microRNAs are a widespread class of small regulatory RNA molecules. In humans, there are currently over a thousand microRNAs annotated in the publicly available database miRBase [19], and they are expected to influence the expression of as much as one-third of the protein-encoding genes [20]. miRNAs are only 20–23 nt in length and bind to partly complementary sequences present most frequently in the 3′-UTRs of mRNA molecules [21]. This miRNA–mRNA interaction results in the inhibition of protein translation or an altered RNA stability [22]. Although the effect of one miRNA on the protein level of one mRNA is often only marginal, it is the regulation of many genes simultaneously by one or several miRNAs [20] that can cause major alterations in cellular behavior.

Within the human genome a substantial fraction of miRNAs clusters together, which can be related to the evolutionary mechanism of how they spread throughout the genome [23]. These clustered microRNAs are often transcribed as one long polycistronic transcript, and subsequently processed into individual mature miRNAs. Alternatively, miRNAs exist as individual genes, each independently transcribed by RNA polymerase II [22]. In general, a microRNA is transcribed as a long primary transcript, called primary (pri-)miRNA, which can be several kilobases long. Through several consecutive processing steps, pri-miRNAs give rise to mature miRNA sequences which influence the expression levels of target genes [22]. A-to-I RNA editing can interfere with the efficiency of these processing steps [4], and thus with the miRNA homeostasis within a cell.

3.1. The impact of RNA editing on miRNA biogenesis

The processing steps that generate mature miRNAs are schematically outlined in Fig. 1A. As mentioned above, most miRNAs are transcribed as long primary miRNA transcripts. During and after transcription the pri-miRNA molecule folds back into an imperfectly double-stranded hairpin structure. This fold-back structure is the substrate for the microprocessor complex, which consists of at least 20 distinct proteins including the RNAse III enzyme Drosha and the RNA binding enzyme DGCR8 [24]. DGCR8 then guides Drosha to cleave the pri-miRNA hairpin structure, giving rise to precursor miRNAs (pre-miRNAs).

The pre-miRNA molecules are subsequently transported out of the nucleus by the nuclear-pore complex exportin-5 and RanGTP [25]. In the cytoplasm the pre-miRNAs are subsequently processed into mature miRNA molecules. This occurs through cleavage of the pre-miRNA molecules into 20–23 nt mature miRNA sequences by the RNAse III enzyme Dicer. Dicer alone is sufficient for cleaving the pre-miRNA but does interact with a series of other proteins, including TRBP [26]. The protein TRBP can bridge miRNA processing and miRNA function, as it facilitates complex formation between Dicer and Argonaute (Ago) [26], an essential member of the miRNA-induced silencing complex (miRISC). In general, the miRNA strand with the thermodynamically less stable 5′-end is incorporated into miRISC, while the other strand is usually rapidly degraded [22]. The miRISC loaded with a miRNA subsequently scans the 3′-UTRs of mRNA molecules for sequences partly complementary to the miRNA, and causes inhibition of protein expression.

The double-stranded nature of primary miRNA transcripts makes them suitable substrates for the RNA editing machinery

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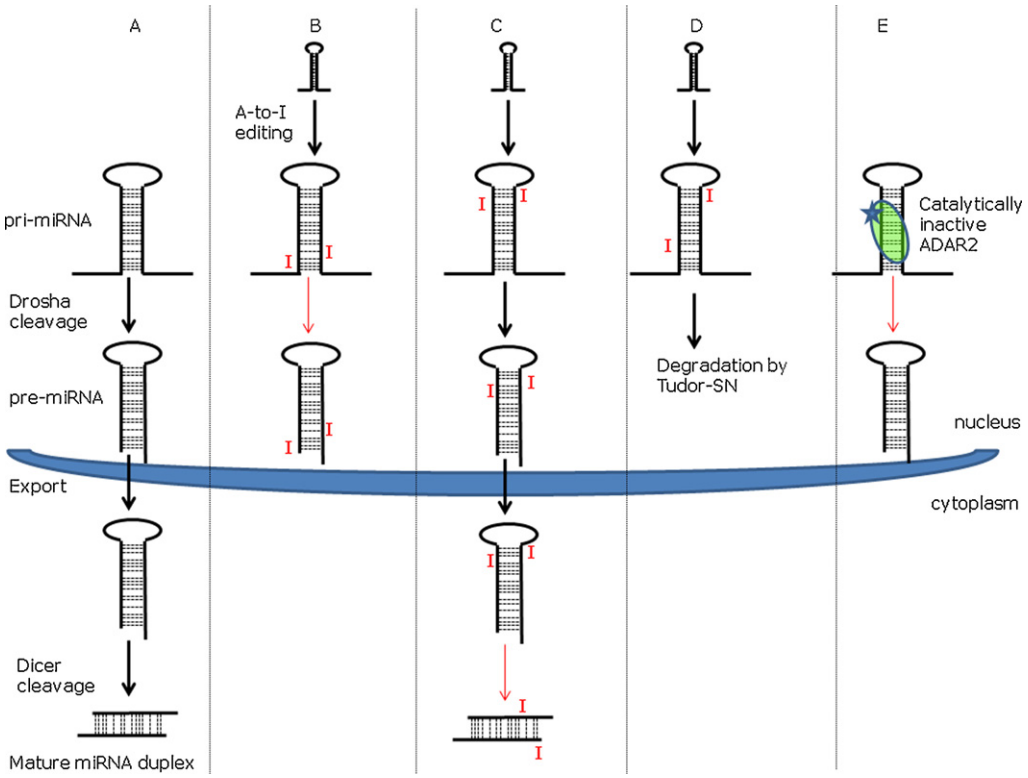

Fig. 1. Schematic representation of the influence of A-to-I editing on miRNA biogenesis. (A) Canonical miRNA processing pathway. After transcription, the pri-miRNA molecules are cleaved by Drosha into pre-miRNAs. The pre-miRNAs are subsequently transported out of the nucleus, followed by Dicer-mediated cleavage into mature miRNA duplexes. The strand with the less thermodynamically stable 5′-end is incorporated into miRISC to repress protein expression levels. (B–E) Editing can influence the miRNA processing at the Drosha (B) and/or Dicer (C) mediated cleavage step, or through degradation of the inosine-containing transcripts by Tudor-SN (D). Binding of ADAR2 without editing can also block Drosha-mediated cleavage (E). miRNA processing steps are shown until the step affected by editing, as indicated by a red arrow. For (B), editing has been shown to either enhance or reduce the efficiency of this processing step. For (B and C), inhibition of the processing could either result in a reduced or complete absence of the mature edited miRNAs. Inosine (I) and inhibited processing steps are indicated in red. For details, see text.

(Fig. 1B–E). It was first demonstrated by Luciano et al. that pri-miRNAs can indeed be edited, by showing that low-level editing of the ubiquitously expressed pre-miRNA-22 occurs in both human brain and testis [27]. Several years later, a more thorough investigation of 99 pri-miRNAs in ten different human tissues resulted in the conservative estimation that approximately 6% of all human pri-miRNAs are edited at least at one position [28]. The extent of editing within these pri-miRNAs differed between tissues and editing sites, which may reflect both editing substrate preferences and the spatiotemporal regulation of the editing machinery.

The authors of these initial studies already speculated that editing of miRNAs has consequences on miRNA function, such as miRNA biogenesis or selective strand loading into the miRISC complex. These important questions were partly answered in a study by Yang et al. [29]. In this study, an *in vitro* editing assay indicated that pri-miRNA-142 harbors several editing sites, which replace A-U or U-A Watson-Crick pairs within the pri-miRNA stem-loop structure with I·U or U·I wobble pairs. As the integrity of the stem-loop structure is essential for pri-miRNA cleavage [30], editing at two of these positions drastically reduced the capacity of Drosha-DGCR8 to process pri-miRNA-142 into pre-miRNA molecules. Importantly, this pri-miRNA was also shown to be edited endogenously by ADAR1 and ADAR2, and in the spleens of mouse strains lacking either ADAR1 or ADAR2 expression, increased levels of mature miRNA-142-5p and -3p were observed [29]. The inosine-containing pri-miRNA could subsequently be targeted for degradation by Tudor-SN, which is a component of the RISC and can either mediate or itself execute inosine-dsRNA-specific ribonuclease activity [29,31]. Altogether, editing can thus influence the miRNA homeostasis within a cell at the level of pri-miRNA processing, through inhibition of the Drosha cleavage step (Fig. 1B) as well as the subsequent rapid degradation of the highly edited pri-miRNA by Tudor-SN (Fig. 1D).

The generated pre-miRNA is transported into the cytoplasm by the nuclear-pore complex exportin-5 and RanGTP [25]. Recently it was shown that ADAR1-p110 shuttles between the cytoplasm and the nucleus in some cell lines, and the nuclear export of ADAR1 is mediated through its interaction with exportin-5 and is dependent on ds-RNA binding [32]. Thus, ADAR1-p110 seems to mediate the export of certain ds-RNA molecules into the cytoplasm and it will be interesting to investigate if these ds-RNAs molecules comprise (edited) pre-miRNAs as well.

In the cytoplasm, pre-miRNAs are further processed into mature miRNA molecules by the Dicer-containing protein complex. Editing can influence this processing step as well (Fig. 1C), as was first demonstrated for pri-miRNA-151. This miRNA has been shown to be edited endogenously at the minor −1 site and major +3 site with respect to the 5′-end of the mature sequence of miRNA-151-3p [28,33], which replaces an A–U Watson Crick pair with an I·U wobble pair within the stem-loop structure. These two editing sites did not affect the pri-miRNA processing by Drosha-DGCR8 but severely reduced the efficiency of the DICER-mediated cleavage step, resulting in only unedited mature miRNA molecules. Interestingly, editing of pri-miRNA-151 is mediated primarily by ADAR1, and although this enzyme as well as miRNA-151 are expressed in many different tissues, no editing of pri-miRNA-151 was observed in tissues outside the central nervous system [33]. The underlying reason for this is unknown, but might be due to the complex and as of yet unrevealed regulatory mechanisms of editing activity [4,34].

The two above described examples are just a snap-shot of the impact of A-to-I editing on the biogenesis of miRNAs. To get a firmer

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grasp on the frequency of pri-miRNA editing, a large-scale survey with 209 human pri-miRNAs was performed [35]. From this analysis, it was estimated that around 16% of human pri-miRNAs are edited in the brain with an efficiency of more than 10%. In addition, there seems to be a preference for editing in the 5′-strand, as well as a favored UAG triplet for A-to-I editing, which is consistent with the local sequence preferences of the RNA editing machinery as determined previously [10–12]. As expected, it was shown that some pri-miRNAs are a preferential substrate for ADAR1 while others are only edited by ADAR2. Most importantly, when analyzing six randomly chosen edited pri-miRNA sequences it appeared that editing in each of these pri-miRNAs alters the processing of either Drosha or Dicer, by enhancing or inhibiting cleavage efficiency. This indicates that A-to-I editing of pri-miRNAs is a widespread phenomenon and a major consequence of miRNA editing is its influence on miRNA biogenesis.

In addition to editing-dependent effects on miRNA processing, ADAR2 also functions as an editing-independent modulator of miRNA maturation. miRNA processing can be influenced by many other proteins which bind to either the pri- or pre-miRNA structure [36]. Binding of (a catalytically inactive) ADAR2 to the pri-miRNA structure of miRNA-376a2 substantially inhibits Drosha processing [37], indicating that not only editing but mere binding of the pri-miRNA structure can be sufficient to affect miRNA biogenesis (Fig. 1E).

A miRNA can push protein levels below detection even though there is gene transcription [38]. If subsequently transcription levels increase or miRNA levels decrease, a threshold can be passed which results in a detectable protein and a subsequent cellular response. RNA editing can contribute to pushing protein levels below or above this threshold by influencing miRNA biogenesis. A recent study indeed showed overexpression of 80 gene transcripts in the brain of ADAR2 knockout mice compared to control mice [39], potentially through the modulation of miRNA levels. In addition, in cancerous tissues editing levels are altered [40] which might contribute to the observed global reductions in mature miRNA expression levels [41]. Several animal models have indicated that a reduction in editing activity is not likely to be a causative effect for tumor development [34] but possibly it could augment the progression through editing-dependent miRNA expression alterations. Thus through influencing miRNA processing efficiency, editing could influence gene expression levels and ultimately impact cellular behavior.

3.2. Impact of A-to-I conversions in the mature miRNA sequence

For recognition of the target mRNA, complementarity between nucleotides 2–7 of the miRNA (the 'seed' sequence) and the mRNA are often crucial [21]. Conversion of an adenosine into an insosine within the miRNA seed region could thus drastically alter the mRNA target profile. Proof of this concept has indeed been demonstrated for the miRNA-376 cluster [42]. The miRNAs within this cluster are transcribed as one long primary transcript, and all six members are edited at specific positions in select mouse and human tissues. Editing of these pri-miRNAs does not substantially affect the processing of the primary transcripts into mature miRNAs, but it does skew the targeting profile as two major editing sites are located within the seed sequence. Indeed, the unedited version of miRNA-376a-5p represses the expression of different genes than the edited variant, as was demonstrated *in silico*, *in vitro*, as well as *in vivo* [42]. This means that editing can influence the functionality of miRNAs through editing of specific positions within the miRNA seed sequence.

Besides altering the miRNA target recognition profile, editing within mature miRNAs can also affect miRNA loading efficiency into the miRISC. A survey for edited miRNAs derived from the Epstein–Barr Virus (EBV) resulted in the detection of edited sites in 4 out of 23 pri-miRNAs [43]. Aside from EBV, miRNA editing has thus far only been reported in one other virus (KSHV), but the significance of editing in KSHV-miR-K12-10 is currently unknown [44]. In contrast, editing of ebv-miR-BART6 at a site close to the 3′-end of the mature miRNA is the first case where editing was shown to substantially inhibit the loading of a miRNA into the RISC, likely due to the altered thermodynamic stability of the miRNA duplex [43]. Hence, A-to-I conversions within the processed mature miRNA can have several consequences, such as a shift in the target gene set or modulation of miRISC loading efficiency.

3.3. Editing of mature miRNA molecules: large-scale surveys

To obtain a thorough answer to the question to what extent edited mature miRNA sequences actually exist, massive parallel sequencing of small RNAs may be the way to go. Inosine appears as a guanosine within the sequencing trace, and hence, the alignment of the genomic sequence to the cDNA trace will show an A/G discrepancy.

Several large-scale miRNA deep-sequencing analyses explored the number and levels of miRNA A-to-I editing events [45–48]. Investigation of six different rat tissues resulted in the detection of six miRNAs that showed editing levels of a certain nucleotide position above 10%[48]. Five of those miRNAs arose from the miRNA-376 cluster, which had previously been shown to be edited in human and mouse [42]. In addition, brain-specific miRNA-377 was confirmed as a novel editing target [48]. When taking into account that this analysis was based upon the investigation of 689 rno-miRNAs, the observed editing in only 6 mature miRNAs is surprisingly low.

Similar results were obtained after investigation of fly [45], mouse [46], and human [47] mature miRNA profiling sets. For *D. melanogaster*, small RNA sequencing libraries obtained from S2 cells were mined for potential editing events [45]. For miRNAs, A to G alterations did not occur more frequently than other types of nucleotide changes, suggesting very low levels of mature miRNA editing. After designing a specific computational pipeline that could predict editing events, only 18 RNA editing candidates were detected with different levels of confidence [45]. Analysis of small RNA sequencing reads in mouse brain tissues gave a similar overall picture [46]. Only 0.61% of the sequences contained an A to G alteration, which resembled other single nucleotide mismatches. A more thorough computational analysis resulted in sixteen potential editing events, of which nine were located within the seed sequence and could thus alter the target recognition profile of that miRNA. Interestingly, 7 of the 16 edited miRNAs also showed a significant shift with respect to the 5′-nucleotide [46], indicating that the specificity of Drosha or Dicer cleavage can be affected through editing. This alteration in the Drosha or Dicer cleavage site can change the overall target profile of the miRNA as well, as the seed sequence is located at a fixed position with respect to the 5′-end of the miRNA [21]. Yet another study investigated the editing levels in a human monocytic leukemia cell line after deep-sequencing. They stringently corrected for potential cross-mapping effects, and found only one edited miRNA in this cell line [47].

There are several explanations for observing such low amounts of edited mature miRNAs. First, single A to G modifications could lead to incorrect mapping to the reference genome. The mature miRNA sequence is only around 20–23 nt in length, and there are currently over a thousand different human miRNAs annotated in the miRNA storage database miRBase [19]. There is thus a reasonable possibility that an A-to-I editing event can generate another miRNA species that is similar in sequence, which results in underestimating the frequency of editing events. Second, editing activity could be present but only on a low level. Sequencing platforms generate a certain number of sequencing errors. If editing levels fall

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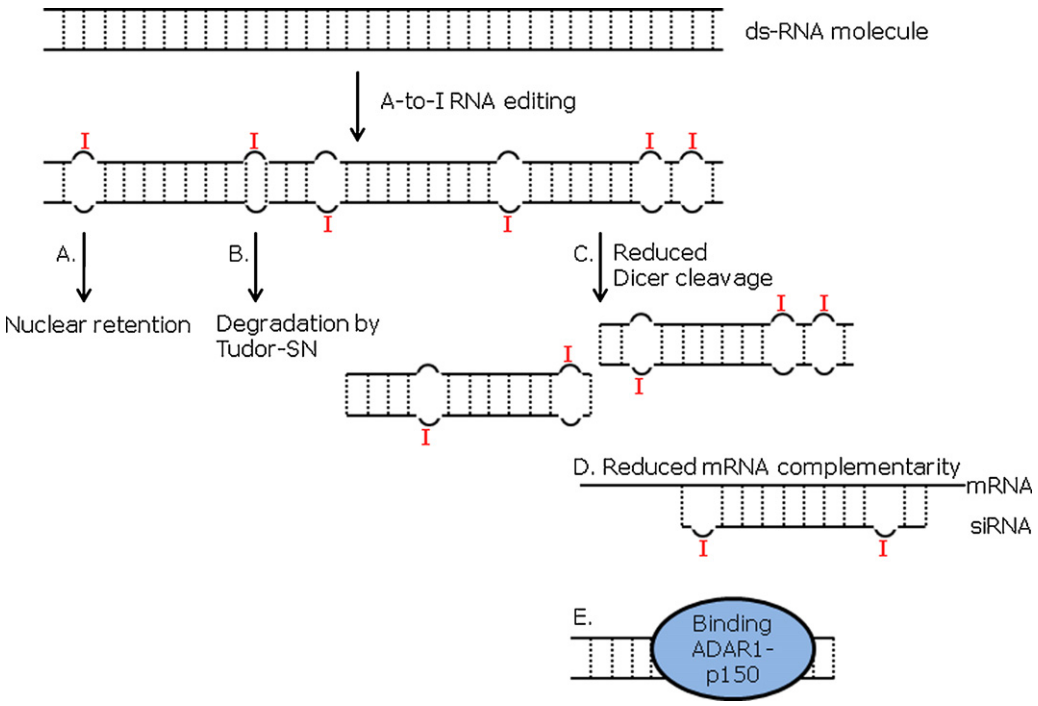

Fig. 2. The impact of editing on siRNA-mediated gene repression. A-to-I editing of long double-stranded RNA molecules can interfere with RNAi in several ways. Inosine-containing transcripts can be retained in the nucleus (A), degraded by Tudor-SN (B), and/or inhibit the Dicer-cleavage step (C). In addition, the generated siRNA molecules that contain inosines have a reduced complementarity to the target mRNA molecules (D) and cytoplasmic ADAR1-p150 can bind siRNA molecules (E), thereby preventing the siRNA to bind to the target mRNA instead.

below this background cut-off, editing events may be wrongly discarded as sequencing errors. Third, editing activity is regulated in a spatiotemporal manner [4,34,49], and if other time points or tissues are investigated novel miRNA editing events could be picked up. There are also differences in miRNA editing levels between species, as A-to-G transitions seem to occur at 4-fold lower frequency in mouse miRNAs compared to human miRNAs [50]. However, in general editing events in mature miRNAs seem to be either limited, or potentially tissue-specific. This observation is in agreement with the notion that the editing of pri-miRNAs can interfere with both Drosha and Dicer cleavage steps [29,33,35], resulting in a reduced number of edited mature miRNAs.

3.4. The other way around: editing of miRNA target sites

A-to-I RNA editing can cause codon alterations within a limited set of genes, often resulting in an altered functionality of the protein. However, editing most frequently occurs within introns and 5′- and 3′-untranslated regions [4,9]. Editing events within the 3′-untranslated region of a protein-encoding gene could alter a miRNA target site. There are several commonly used criteria for detecting miRNA target sites *in silico*; for example, the target site should contain a stretch of nucleotides complementary to the seed sequence of the miRNA, have an open secondary structure to be accessible for binding of the miRNA, and/or the sequence should be conserved across several species [21]. However, these criteria still result in large numbers of false-positive as well as false-negative sites, causing the correct prediction of miRNA targets to be challenging [21].

The conservation criterion makes it difficult to look for target sites that are generated or lost upon editing, as human editing sites in the 3′-UTRs tend to be located in primate-specific Alu elements as described above. Nonetheless, Liang et al. [51] set out to detect miRNA binding sites that were either generated or lost upon editing, by using a miRNA target prediction model that does not take conservation into account. With the consideration that these miRNA target sites might thus be predicted with a low level of confidence, overlapping the A-to-I editing sites with the miRNA target prediction sites indicated that editing seems to avoid miRNA binding sites. In addition, they found approximately 200 positions where editing could actually create a miRNA binding site [51], adding an interesting layer of control to miRNA-mediated repression. A more extended study including all miRNAs known at the time indicated that over 3000 editing sites located in Alu sequences could generate miRNA-binding sites upon A-to-I deamination [52]. Alu sequences are notable for harboring human miRNA seed matches [53], and these sequences are potent substrates for the editing machinery [10,12,54]. A general function for Alu editing in primate mRNA 3′-UTRs could thus be the modulation of miRNA-mediated repression.

4. RNA editing of (endo-)siRNA molecules

Besides miRNAs, many other classes of small regulatory RNA molecules exist, such as transcription initiation (ti)RNAs, promoter-associated short RNAs (PASRs), piwi-interacting (pi)RNAs, small nucleolar (sno)RNAs, and endogenous (endo)siRNAs [2], generating an immensely complex network of regulatory signals. Endo-siRNAs have now been discovered in a number of organisms [55], and arise from long double-stranded RNA molecules that are cleaved by Dicer to enter the RNAi pathway. These extended ds-RNA structures can originate from transposon-derived transcripts, but also from pseudogenes and their cognate protein coding genes [55]. Interestingly, two research groups recently demonstrated editing within this novel class of small RNA molecules [45,56].

The main difference between the editing of miRNA or (endo-)siRNA precursors is most likely the number and location of edited positions. Pre-miRNAs have a rather short stemloop structure which can contain bulges and loops. Editing of these molecules is

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therefore expected to take place at highly specific locations. On the contrary, precursors of (endo-)siRNAs are long ds-RNA molecules, giving the ADAR enzymes the opportunity to randomly edit the adenosines located within this long substrate.

The intriguing interplay between RNA editing and RNAi was first suggested by Bass et al. [57] and became strikingly clear in mutant *C. elegans* strains. Studies with *ADAR null* worms indicated that RNA editing can prevent the silencing of transgenes by antagonizing the RNAi machinery [58]. Moreover, editing-deficient *C. elegans* strains display impaired chemotaxis, but this phenotype can be reverted when crossing the strain with RNAi defective worms. This indicates that a currently unknown gene important in the *C. elegans* chemotaxis pathway is protected for RNAi by A-to-I editing [59].

Editing of the long ds-RNA molecules, that give rise to either endogenous or exogenous siRNAs, can influence the RNAi machinery through several potential mechanisms (Fig. 2). The highly edited ds-structures could be retained in the nucleus [18], and thus be prevented from entering the cytoplasm and subsequent cleavage by Dicer. In addition, A-to-I conversions could reduce the efficacy of RNAi through the substitution of an A–U base-pair with an I·U wobble pair between the generated edited siRNA and the target mRNA. Third, *in vitro* assays indicated that editing of these long ds-RNA molecules can alter their double-stranded nature, such as to prevent the production of siRNAs [60]. This is especially true for hyper-edited RNA molecules containing >50% A-to-I substitutions, where the production of siRNAs is completely blocked [60]. Moreover, these long hyper-edited ds-RNA molecules can also be targeted for degradation by Tudor-SN which is a subunit of RISC, thus generating a complex interplay between RNA editing and RNAi [31]. Finally, editing can also interfere with the RNAi machinery in an editing-independent fashion. After cleavage of the long ds-RNA structure into 21-nt (endo-)siRNAs, ADARs are still capable of binding these small double-stranded RNA molecules. Especially the cytoplasmic full-length isoform of ADAR1 (ADAR1-p150) binds with high affinity to siRNAs without editing the substrate [61]. In this manner, ADAR1-p150 binding can titrate the siRNA molecules away from the RNAi machinery, thereby limiting the RNAi effects.

5. Concluding remarks

As outlined above, there is a strong interplay between A-to-I RNA editing and miRNA/siRNA mediated gene silencing. Through several mechanisms, the editing enzymes can either increase or reduce the number of effective small RNA molecules within a cell.

As one miRNA regulates the expression levels of many different genes, induced or decreased editing levels of a specific miRNA precursor could have significant consequences on the transcriptome and final proteome composition. In this respect, editing levels are sensitive to external stimuli [62] and it is therefore tempting to hypothesize that fast cellular responses can occur at least in part through alterations in the editing efficiency of specific miRNA precursors.

RNA editing can also alter the functionality of specific miRNAs. However, there are just a few examples describing editing consequences in mature miRNAs. The main role of editing of pri-miRNAs therefore appears to be to affect miRNA biogenesis and to a lesser extent to influence mature miRNA functions. In addition, the editing of siRNA precursors results in interference with the RNAi machinery. Overall, an unexpected role for RNA editing may be the modulation of gene expression levels through miRNA/siRNA editing, and places A-to-I editing in the middle of these thoroughly investigated mechanisms and widely used research tools.

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