

Bioinformatic Approaches for Identification of A-to-I Editing Sites

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Abstract The first discoveries of mammalian A-to-I RNA editing have been serendipitous. In conjunction with the fast advancement in sequencing technology, systematic methods for prediction and detection of editing sites have been developed, leading to the discovery of thousands of A-to-I editing sites. Here we review the state-of-the-art of these methods and discuss future directions.

Keywords A-to-I RNA editing • Alu repeats • Bioinformatics • Deep sequencing

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Adenosine-to-inosine (A-to-I) RNA editing has the potential for a major diversification of the transcriptome beyond its genomic blueprint. This post-transcriptional modification of RNA is catalyzed by enzymes of the ADARs (adenosine deaminases that act on RNA) protein family, which bind double-stranded RNA

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structures and deaminate targeted adenosines (A) within these structures into inosines (I). The inosines seem to be functionally equivalent to guanosines (Gs), and thus A-to-I editing affects downstream RNA processes, such as translation and splicing, resulting in different fates for the edited RNA molecules (Bass 2002; Nishikura 2010).

Twenty years ago, the first mammalian example for A-to-I RNA editing was reported—editing of an adenosine nucleotide within the coding sequence of the glutamate receptor subunit GluRB, resulting in a modified protein with a distinctive biochemical activity (Sommer et al. 1991). Despite much effort, only a handful of additional mammalian editing targets were found till 2003. On the other hand, a number of tantalizing hints suggested that editing is of high importance and wider scope: mice lacking ADARs die in utero or shortly after birth (Hartner et al. 2004; Higuchi et al. 2000; Wang et al. 2000). In addition, a number of neurological pathologies were linked to abnormal editing patterns, including epilepsy, brain tumors, amyotrophic lateral sclerosis (ALS), schizophrenia, depression and neuronal apoptosis following disruption of the blood flow to the brain (Brusa et al. 1995; Gurevich et al. 2002; Kawahara et al. 2004; Maas et al. 2001; Niswender et al. 2001; Paz et al. 2007; Wang et al. 2004). Most recently it was found that editing activity of ADAR1 is essential for hematopoiesis (Hartner et al. 2009; XuFeng et al. 2009). These phenotypes were not all explained by the few editing targets identified. Moreover, pioneering experimental work found that inosine exists in mRNA in large amounts (Paul and Bass 1998), much larger than could be accounted for by the small number of targets known at that time. Accordingly, the search for more targets continued and a variety of experimental methods to detect additional editing events and their levels were developed (Chateigner-Boutin and Small 2007; Chen et al. 2008b; Gallo et al. 2002; Lanfranco et al. 2009; Morse and Bass 1999; Ohlson et al. 2005; Ohlson and Ohman 2007; Sakurai et al. 2010; Suspene et al. 2008; Wong et al. 2009; Zilberman et al. 2009) with various levels of success.

1 Bioinformatic Screens

In principle, detection of editing sites should be straight-forward, analyzing cDNA sequencing data. Resembling the endogenous enzymes, most sequencing reactions also identify an edited adenosine “A” site within cDNA as a guanosine “G”. Therefore, an A-to-G mismatch between a sequenced cDNA and its genomic reference is an indication of an A-to-I editing event. Naively, then, one has to only align the available cDNA data, including millions of publicly available ESTs and full-length RNAs, to the genome, and search for such A-to-G mismatches (Fig. 1). However, a simple application of this idea fails in reality due to the extremely low signal-to-noise ratio. The total fraction of mismatches between the genome and the expressed sequences amounts to 1–2% (Hillier et al. 1996). The main contributors for these discrepancies are then random sequencing errors in the expressed

sequences, which alone account for 1–2 mismatches per 100 bp sequenced. Another important cause of variance between RNA and the genome includes genomic polymorphisms and somatic mutations that result in genomic differences between the different individuals, or the individual cells, contributing to the expressed sequences and the reference genome. In addition, misalignment of the RNA sequences to the genome is a major concern when dealing with repetitive regions of the genome.

The first discoveries of A-to-I editing sites resided all within the coding parts of mRNAs. These editing sites were shown to be functional—their editing results in a modified protein, with biochemical properties different than those of the unedited version. Studying these sites, it has been noticed that the genomic sequence surrounding them is highly conserved among species (Hoopengardner et al. 2003). This can be readily understood in terms of an additional evolutionary constraint: in addition to the sequence conservation against changes in the amino-acid coding information, the double-stranded RNA structure must be left intact in order to preserve the editing event. This constraint leads to higher conservation at the DNA level, and has proven to be a very useful signature of editing sites, to be employed in bioinformatic searches (Clutterbuck et al. 2005; Hoopengardner et al. 2003; Levanon et al. 2005a). The first study identified highly conserved regions and then used extensive sequencing to look for editing sites, resulting in 16 novel sites in *Drosophila melanogaster* and one in human (Hoopengardner et al. 2003). The conservation may be further used to sift through the mismatches in available cDNA data. Unlike editing sites, sequencing errors and genomic polymorphisms are not often shared between species. Note, however, that specific types of sequencing errors are not random but rather follow from a given pattern in the neighboring sequence (Zaraneck et al. 2010), these would seem as ‘conserved’ between species. Focusing on mismatches that reoccur in different species allows one to find the few editing recoding sites among tens of millions of mismatches between ESTs/RNAs and the genome. This strategy was applied by a number of groups: looking for such conserved mismatches located in the exact same position in human and mouse resulted in a few additional A-to-I editing substrates (Levanon et al. 2005a; Ohlson et al. 2007; Sie and Maas 2009). The newly discovered sites are now under investigation in order to determine their biological function and regulation potential (Galeano et al. 2010; Hideyama et al. 2010; Kwak et al. 2008; Nicholas et al. 2010; Nishimoto et al. 2008; Riedmann et al. 2008; Rula et al. 2008). One might have used an additional characterization of the editing sites to further improve this analysis, namely the requirement for having a dsRNA structure at the editing site. However, based on the examples of editing sites known so far, it seems that the typical dsRNA structures are rather weak and hard to predict computationally (Bhalla et al. 2004). Interestingly, many of the novel editing sites appear in the SNP database (dbSNP), due to an erroneous interpretation of the variability among expressed sequences in these sites as a sign for a single-nucleotide polymorphism (SNP) (Eisenberg et al. 2005a). A careful analysis of dbSNP could result in more editing sites hidden as mis-annotated SNPs (Gommans et al. 2008).

2 Editing Within Repetitive Elements

In 1990s, experimental evidence for a significant amount of inosine in total RNA has emerged. In the decade to follow, we have witnessed an impressive growth in the number of known editing sites within the coding sequence (editing of which might modify the encoded protein), especially as deep-sequencing methods have been introduced in the past 2 years (see below). However, these are far from being able to account for the total inosine levels observed: the currently known editing sites within the coding region amount to about 400 sites. Thus, they represent roughly 1:150,000 of all nucleotides in exons. The editing efficiency is spread between 0 and 100%, with average efficiency less than 50%, so one expects not more than 1:300,000 inosine to adenosine ratio in total mRNA. This rough estimate is at odds with an observed ratio of 1:17,000nt in rat's brain (Paul and Bass 1998) and results showing up to one inosine per 2,000 nt in poly-adenylated mRNA from human brain (Blow et al. 2004). In addition, a number of clusters of editing events were found in non-coding regions, providing first hints for the significance of the non-coding RNA for the global A-to-I editing pattern (Morse and Bass 1999).

In 2004, three groups have devised computational methods for identifying such clusters, based on analysis of mismatches in otherwise almost perfect alignments of RNA (Athanasiadis et al. 2004; Eisenberg et al. 2005b; Kim et al. 2004; Levanon et al. 2004). The methods differ by the clustering criteria used and the statistical analysis employed. Remarkably, the three independent procedures resulted in highly similar results: A-to-G substitutions, which could arise from A-to-I editing events, account for more than 80% of the 12 possible types of mismatches in the selected set of transcripts. As this disparity in mismatches distribution is unlikely to occur for genomic polymorphisms and sequencing errors, it provides a clear signature of editing in tens of thousands of sites within the human transcriptome.

Editing events couple with splicing, thus they may occur in introns as well. However, computational approaches based on expressed sequences are obviously limited in their ability to detect editing within introns. Therefore, it is anticipated that the actual number of editing sites in the human genome is even much higher than the tens of thousands sites reported in the above works. Indeed, direct sequencing of human brain total RNA has revealed that up to 1 in 1,000 bp of the expressed regions are being edited, compared to only 1:2000 bp in poly-adenylated mRNA (Blow et al. 2004).

Virtually all clusters of editing sites are harbored within Alu repetitive elements (Levanon et al. 2004). Alu elements are short interspersed elements (SINEs), roughly 300 bp long each. Humans have about a million copies of Alu, accounting for ~10% of its genome (Lander et al. 2001). Since these repeats are so common, especially in gene-rich regions, pairing of two oppositely-oriented Alus located in the same pre-mRNA structure is likely. Such pairing produces a long and stable dsRNA structure, an ideal target for the ADARs. Alu repeats are primate specific

(Batzer and Deininger 2002), but other mammals have a similar number of different SINEs. For example, the number of rodent-specific SINEs in the mouse genome is larger than the number of Alu SINEs in humans, and they occupy a similar portion of the genome (7.6% in mouse, 10.7% in human) (Waterston et al. 2002). However, genome-wide analysis of the properties of these SINE repeats explains the order-of-magnitude difference in the global editing levels observed in measurements of total inosine abundance as well as in bioinformatic screens editing sites (Eisenberg et al. 2005b; Kim et al. 2004). It turns out that the shorter length and higher diversity of the mouse SINE repeats are responsible for this disparity in editing levels (Neeman et al. 2006). This global difference between human and other mammals such as mouse is intriguing, as it is generally believed that cellular mechanisms are generally conserved between human and mouse. However, the significance of this difference is not clear yet, as the role of editing in non-coding repeats is yet elusive.

3 Deep-Sequencing Approaches

Recent advancements in massively parallel sequencing technologies open a new era in analysis of genome to transcriptome discrepancies. The first bioinformatic works studied the publicly available transcription data in GenBank, a result of group-effort of hundreds of labs around the world. In comparison, it is possible today to produce a similar amount of data in a single 1000\$ experiment. Thus, one could start sequencing whole transcriptomes in order to determine the full scope of RNA editing. However, observing a consistent discrepancy between the RNA sequence and the reference genome is not sufficient to prove the site to be an editing site. One must exclude the possibility of genomic diversity between the reference genome and the genome of the RNA source tissue. It then follows that identifying RNA editing sites requires sequencing of both genomic DNA and cDNA from the same source, or two RNA samples of a wild-type and editing-deficient mutant. Second, editing levels vary among tissues, and therefore one would need to repeat the experiment for a wide variety of tissues in order to obtain the full organism-wide repertoire of editing. Current technology still renders this kind of experiment quite expensive. Accordingly, current usage of deep-sequencing to look at transcriptome-wide editing is usually limited to a single tissue, or to a limited part of the transcriptome (e.g. micro-RNAs, or a specific gene(s) of interest) throughout a number of tissues or developmental stages. Such studies are currently conducted by several groups, and are expected to significantly increase the scope of known editing levels, and may even detect consistent RNA–DNA mismatches other than A-to-G, reflecting RNA modifications beyond the dominant (at least when one includes Alu repeats in the analysis) A-to-I editing.

In a first and pioneering work in this direction, Rosenberg et al. (2011) have implemented a deep-sequencing approach followed by DNA–RNA mismatches analysis to discover 32 novel targets of APOBEC1 C-to-U RNA editing, edited in

epithelial cells from the small intestines of a mouse. As it was the case for A-to-I editing, while the first known example of APOBEC1 editing (apoB mRNA) resides in the coding region (Chen et al. 1987; Powell et al. 1987), transcriptome-wide analysis have revealed editing in the non-coding regime. The functional role of this extensive catalytic activity in non-coding parts of the transcripts is an open challenge.

As mentioned above, studying a wide variety of tissues using a straight forward deep-sequencing approach is still impractical. An alternative approach was demonstrated recently by Li et al. (2009b). Combining a computational approach together with a novel targeted sequencing technique, they aimed to get a transcriptome-wide editing profile in a multi-tissues experiment. A bioinformatic search used alignments of eight million human ESTs against the human reference genome, in the spirit of the older bioinformatic approaches. After the repetitive portion of the human genome and known genomic polymorphisms were removed, there remained $\sim 60,000$ mismatches, which potentially could signal edited sites. A targeted capture and sequencing approach was employed to specifically deep-sequence the predicted sites. For each of the predicted sites, a padlock probe (also known as molecular inversion probe) was designed for specific anchoring and amplification (Li et al. 2009a). All sites were simultaneously captured, amplified and sequenced using genomic DNA and cDNA from several different tissues (mainly brain), all derived from a single donor in order to rule out polymorphisms among populations. The pool of probes was hybridized to the DNA and cDNA in separate amplification reactions. The amplicons were sequenced, and the resulting sequences were scanned in order to identify A-to-G mismatches between the genomic DNA and the RNA-derived cDNA. This method allows for parallel sequencing of tens of thousands of suspected sites in a single reaction. It resulted in detection of hundreds of novel A-to-I editing sites residing out of repetitive elements. This technology can now be applied to study the hundreds of confirmed editing sites (instead of the 60,000 candidates) in a large panel of tissues. In particular, it provides a promising cost-effective approach to study in large scale possible associations between the editing profile and various pathologies.

Similarly, Enstero et al. (2010) have first identified $\sim 2,500$ conserved regions which form putative double-stranded RNA structures, and then used deep sequencing of only 45 regions that were considered particularly promising based on sequence conservation and the existence of A-to-G mismatches in the public databases. This study has resulted in ten new editing sites, eight of which recode codons. However, the editing efficiency of these sites was minute-0.6–2.4%.

In addition to the improvement in detection of RNA editing, the deep-sequencing technology allows for much better quantification of the editing level. Counting the number of edited and non-edited reads is easy enough. However, an important (often neglected) concern is the possibility of an alignment bias. Current deep-sequencing technology often results in short reads, with a non-negligible amount of sequencing errors (1–2%). Due to the large amount of reads, fast-alignment protocols must be used. These often allow only a small number of mismatches within the read, e.g., retaining only reads with up to two mismatches.

An edited read will appear as if it has a mismatch in the edited site. Therefore, only a single additional mismatch is allowed if the read is to be aligned at all, while unedited reads will be aligned even if exhibiting two mismatches. This creates a bias against edited reads, resulting in an apparently lower level of editing. Situation is even worse considering the fact that many editing sites appear in clusters, where editing of the different sites within the cluster is often positively correlated. A satisfactory algorithmic solution for this problem has not been reported yet. As such solution will become easier as technology is shifting toward producing longer reads.

Finally, let us mention that the same methods described here for identification of RNA editing are applicable for the study of DNA editing and somatic mutations. Bioinformatic approaches for these phenomena are only beginning to emerge (Zaranek et al. 2010), but are expected to increase as large deep-sequencing data is accumulating.

4 Structural and Sequence Determinants of A-to-I Editing

A-to-I editing is characterized by a puzzling specificity and selectivity. In some targets, such as the AMPA receptor gluR-B subunit in mice (Seeburg et al. 1998) and the E1 site within the Alu-based alternative exon in the NARF gene (Lev-Maor et al. 2007), 100% of the transcripts are being edited at a specific adenosine. In contrast, most sites in the coding region show only a partial editing. Looking at sites in Alu repeats, a seemingly random editing pattern is observed: virtually all adenosines are targeted with varying editing efficiency, but only a few are edited in any given clone of the transcript. However, it was recently shown that editing in Alu repeats is also highly reproducible: the variability among healthy individuals in editing level at a given site within a specific Alu repeat is much lower than the site-to-site differences (Greenberger et al. 2010). The wide range of efficiencies and the significant consistency between individuals call for a sequence and structural motifs that determine the editing efficiency of each site relative to others. The sequence and the resulting dsRNA structure formed by Alu vary significantly from site-to-site, but are shared by all samples. Sequence analysis of editing sites revealed a number of weak motifs: C and T are over-represented at the nucleotide 5' to the editing site, while G is under-represented. At the nucleotide 3' to the site, G is significantly over-represented (Kleinberger and Eisenberg 2010; Lehmann and Bass 2000; Melcher et al. 1996; Polson and Bass 1994; Riedmann et al. 2008). However, these alone cannot account for the observed tightly regulated editing profiles. Therefore, the question still stands: what controls the editing level at each given site?

Given the dependence of ADAR activity upon the formation of a double-stranded RNA structure, it is plausible that structural motifs also play a role. Indeed, also some evidence has been accumulated supporting this idea. The editing level in a given Alu repeat can be shown to correlate with the existence of a nearby

and reversely-oriented repeat, in support of the paired-Alu model. Analysis of thousands of examples has shown that effective editing requires a distance of roughly 2000 bp or less between the two Alus. Furthermore, the level of editing increases with the number of reverse complement Alus present within this distance (Athanasiadis et al. 2004; Blow et al. 2004; Kim et al. 2004; Levanon et al. 2004). These characteristics of the editing pre-requisites are instrumental in devising future searches for editing targets in human and other organisms. Interestingly, edited adenosines within the dsRNA structure are paired with a “U” or a “C” in the reverse strand, meaning that editing either strengthens or weakens the dsRNA structure, but virtually never has a neutral effect on the dsRNA-pairing energy (Levanon et al. 2004). However, a detailed analysis comparing editing levels of specific inosines within an Alu repeat is still not available. A first step in this direction has been done recently (Kleinberger and Eisenberg 2010), but results are still far from being able to explain in full the variability in editing levels. Ideally, one would like to have a predictive model which, given the genomic sequence, will provide the relative efficiencies of editing for all adenosines in the given sequence.

5 Correlations Between Editing Sites

Many edited targets include a number of editing sites. Analysis of correlations between editing of neighboring sites might reveal details regarding ADAR binding and catalytic activity. A recent study (Enstero et al. 2009) has identified positive correlations between different editing sites, as far as 25 bp apart. These positive correlations might support a model in which ADAR is attracted to a specific ‘strong’ editing site, and then edits weaker sites in its vicinity. Indeed such weak ‘satellite’ editing sites have been observed in the vicinity of several editing sites. A more complex pattern is observed when one looks at site–site correlations after correcting for the whole-transcript editing affinity. Then, a rich pattern of positive and negative correlations is seen, including pair and triple correlations for editing sites as far as 150 bp apart (Paz-Yaacov et al. 2010). These intriguing results may suggest that as editing of one site changes locally the double-stranded binding energy, it might induce changes in the global structure, which in turn may enhance or diminish editing efficiency in remote sites.

6 RNA Editing and Micro-RNAs

The role of Alu editing is yet to be explored. Recent observations suggest that editing is involved in molecular mechanisms based on dsRNA structure, such as RNAi (Tonkin and Bass 2003) and miRNA (de Hoon et al. 2010; Kawahara et al.

2008; Kawahara et al. 2007b; Luciano et al. 2004). miRNAs are short non-coding RNAs, endogenously expressed in the living cell, that bind to mRNAs and induce suppression of translation, by either leading to degradation of the RNA or inhibiting translation. The primary sequence of the miRNAs processes in the nucleus by Drosha and then further processes in the cytoplasm by Dicer, resulting in a mature sequence ~ 21 nucleotides long. These short RNAs are binded by the RISC complex (Bartel 2004). RNA editing is potentially coupled to miRNAs throughout their life cycle. The biogenesis of miRNAs through Drosha and Dicer processing hinges upon their double-stranded RNA structure. As these stages, editing of these pre-miRNA (or pri-miRNA) and double-stranded RNAs might interfere with the proper production of mature miRNAs, or even result in modified mature miRNA sequences, exhibiting a different set of targets. Furthermore, miRNA targets are often present in the 3' UTRs, regions heavily targeted by RNA editing. Thus, editing might influence miRNA targets, increasing or decreasing their affinity toward miRNA binding.

The full picture of the relationship between miRNAs and A-to-I RNA editing is still missing. However, a number of interesting results have emerged in recent years (Blow et al. 2006; Kawahara et al. 2008; Luciano et al. 2004). It has been demonstrated that both ADAR enzymes edit specific adenosines within pri-miRNAs in human and in viruses (Iizasa et al. 2010; Kawahara et al. 2007a; Kawahara et al. 2007b; Yang et al. 2006). In these cases, editing was reported to suppress the processing by Drosha or Dicer, or prevent loading onto the RISC complex, resulting in a depleted amount of mature miRNA. In some cases, mature miRNAs with an altered sequence have been reported.

Systematic searches for editing sites in miRNAs have not yielded a large number of sites (Chiang et al. 2010; de Hoon et al. 2010; Linsen et al. 2010). However, these studies have focused on rodents, and it is possible that the results for humans might be different. Nevertheless, the low number of editing sites in miRNAs is surprising. It seems to suggest that edited pre-miRNAs are degraded or otherwise prevented from maturation. Alternatively, some other mechanism might be responsible for protection of the miRNA sequences from editing. These questions are yet to be explored.

Finally, modulation of miRNA targets has been also considered. Targets of the miRNA contain a seven nucleotide sequence which complements the miRNA seed. Thus, editing of the miRNA in the seed region should modify its set of targets. Similarly, editing of a target recognition site could alter its binding to the miRNA. Two bioinformatic studies have assessed the scope of this phenomenon (Borchert et al. 2009; Liang and Landweber 2007), concluding that hundreds to thousands of target sites might be affected. In particular, two human miRNAs, miRNA-513 and miRNA-769-3p, target a common motif present in the abundant Alu sequence only when it is edited. Here too, further studies are required to elucidate the importance of target editing for the miRNA regulation process.

7 More functional Roles of RNA Editing of Inverted Repeats

The role of Alu editing is yet to be explored. Recent observations suggest that editing is involved in molecular mechanisms based on dsRNA structure, such as RNAi (Tonkin and Bass 2003) and miRNAs (see above). RNA editing was also shown to be involved in splicing regulation in several cases (Lev-Maor et al. 2007; Moller-Krull et al. 2008), notably the self-editing of ADAR2 (Rueter et al. 1999). It has been suggested that hyper-editing of repetitive elements might result in gene silencing (Wang et al. 2005) or in an anti-retro element defense mechanism (Levanon et al. 2005b). A possibility gathering support in recent years is the suggestion that heavily edited transcripts are retained in the nucleus throughout complexes containing p54nrb (non-POU domain containing, octamer-binding, NONO) (Zhang and Carmichael 2001), later identified as paraspeckles (Chen and Carmichael 2009). Indeed, later studies have shown that a single pair of reversely-oriented Alu repeats in the 3' UTR of a reporting gene strongly represses its expression, in conjunction with a significant nuclear retention of the mRNAs. Nuclear retention was demonstrated in detail for the endogenous Nicotin 1 (NICN11) mRNA harboring inverted Alus in its 3' UTR (Chen et al. 2008a) and for mouse Slc7a2 edited transcripts (Prasanth et al. 2005). However, another group (Hundley et al. 2008) has recently reported no effect of editing within the 3' UTR on mRNA localization and translation of several *Caenorhabditis elegans* and human transcripts, suggesting that the retention phenomenon might be different in different cells types, or conditions.

Nuclear retention of hyperedited transcripts was first interpreted as a means of protection against abnormal transcripts (Zhang and Carmichael 2001). This is supported by the abundance of hyperediting clusters in splicing-defective transcripts (Kim et al. 2004). This idea is in line with a similar proposed mechanism, suggesting that an I-specific cleavage of RNAs can lead to the selective destruction of edited RNAs (Scadden and Smith 2001). However, a recent study (Prasanth et al. 2005) opened a new perspective on the way transcript localization and inosine-specific cleavage might contribute to cell function. It was shown that inverted repeats within the 3' UTR of the mouse Slc7a2 gene form a hairpin dsRNA structure and are highly A-to-I edited. The mRNA is then retained in the nucleus, as a reservoir of mRNAs that can be rapidly exported to the cytoplasm upon cellular stress. It has been demonstrated that under stress conditions, the edited part is post-transcriptionally cleaved, removing the edited SINEs from the 3' UTR. Consequently, the mRNA is exported to the cytoplasm, where it translates into a protein. It thus turns out that A-to-I hyperediting may serve as a powerful means of retaining in the nucleus mRNA molecules that are not immediately needed to produce proteins but whose cytoplasmic presence is rapidly required upon a physiologic stress. This model might provide an elegant functional role to the global editing phenomenon. Naturally, one wonders what the scope of this model is, and whether it is relevant to the thousands of hyper-edited human genes. Some support to this idea has been provided bioinformatically, showing that there

are hundreds of transcripts in public databases exhibiting cleavage of an inverted repeat structure, as if they have been retained in the nucleus and then cleaved and released prior to sequencing (Osenberg et al. 2009). However, it should be pointed out that it is not clear yet whether the nuclear retention is editing-mediated or rather stems from the double-stranded RNA structure.

While editing was shown to be coupled with several regulation mechanisms, it is yet too early to call whether any of these regulation mechanisms is as widespread as Alu editing itself. Thus, Alu editing is a mystery still waiting to be solved.

8 Future Directions

Advances in editing detection methods have opened the door for studies comparing editing levels globally between different conditions, pathologies and developmental stages. First results reporting such difference between normal and tumor brain tissues (Paz et al. 2007), mouse brain developmental stages (Wahlstedt et al. 2009) and along stem-cell differentiation process (Osenberg et al. 2010) have been published recently. We expect to see many more such studies in the near future, which will help to clarify the scope of processes affected by A-to-I RNA editing.

Alu repetitive elements are unique to the primates, but the occurrence of repetitive elements in general is common to all metazoa. Applying the same methods for editing detection to other organisms has shown that editing in human is about 40 times higher as compared with mouse (Eisenberg et al. 2005b; Kim et al. 2004). A similar picture was observed when comparing with rat, chicken and fly (Eisenberg et al. 2005b). The high-editing level in humans is likely due to the fact that humans have only one dominant SINE, which is relatively well-conserved (only ~12% divergence between an average Alu and the consensus). In comparison, mouse has four different SINEs, which are shorter and more divergent (~20% average divergence) (Neeman et al. 2006). This has lead naturally to the question of the relative abundance of editing in humans as compared to other primates. In a recent study (Paz-Yaacov et al. 2010), a two-fold higher level was observed in human compared to chimpanzee and rhesus monkeys, for a set of six genes in which no significant genomic differences occur among the three species. In addition, human-specific Alu repeats have been shown to be associated with neurological pathways and disorders.

The exceptional level of editing in the primate brain makes it tempting to suggest a role in primate evolution. The over-representation of editing in brain tissues and the association of aberrant editing with neurological diseases are consistent with a possible connection between editing and brain capabilities. One thus may speculate that the massive editing of brain tissues is responsible in part for the brain complexity. As this large-scale editing is a direct result of Alu abundance, it follows that if the above idea has any merit then the massive

invasion of Alus into the primate genome had a major impact on primates' evolution (Barak et al. 2009; Britten 2010; Eisenberg et al. 2005b; Gommans et al. 2009; Mattick and Mehler 2008; Paz-Yaacov et al. 2010).

The recent identification of hundreds of non-repetitive human RNA editing sites may be followed by many more very soon. The volume of RNA-sequence data collected in a couple of years already surpasses that the total amount deposited in EST database in two decades. This increasingly growing amount of data will allow for more predicted RNA editing sites. The dbSNP has also grown as a result of recent genomic sequencing efforts, in particular the 1,000 genomes project, improving one's ability to filter rare SNPs. As sequencing cost continues to drop, a comprehensive approach to identifying all RNA editing sites will become possible by sequencing the entire transcriptomes as well as the exomes or genomes. In addition to A-to-I sites, the full scope of other types of RNA editing and modifications are surely going to be revealed by these efforts.

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