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Review

RNA editing in regulating gene expression in the brain

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Abstract

Adenosine to inosine RNA editing, catalyzed by *A*denosine *D*eaminases *A*cting on *R*NA (ADARs), represents an evolutionary conserved post-transcriptional mechanism which harnesses RNA structures to produce proteins that are not literally encoded in the genome. The species-specific alteration of functionally important residues in a multitude of neuronal ion channels and pre-synaptic proteins through RNA editing has been shown to have profound importance for normal nervous system function in a wide range of invertebrate and vertebrate model organisms. ADARs have also been shown to regulate neuronal gene expression through a remarkable variety of disparate processes, including modulation of the RNAi pathway, the creation of alternative splice sites, and the abolition of stop codons. In addition, ADARs have recently been revealed to have a novel role in the primate lineage: the widespread editing of *Alu* elements, which comprise approximately 10% of the human genome. Thus, as well as enabling the cell-specific regulation of RNAi and selfish genetic elements, the unshackling of the proteome from the constraints of the genome through RNA editing may have been fundamental to the evolution of complex behavior.

Keywords: RNA editing; ADAR; RNA interference; *Alu* element; Ion channel; Pre-synaptic protein

1. Introduction

The brains of higher metazoans are amongst the most intricate systems known to science. In recent years, it has become increasingly clear that the protein repertoires encoded by literal translation of metazoan genomes are insufficient to generate the complex behaviors required for successful survival and reproduction. Rather, a series of post-transcriptional and -translational mechanisms are also deployed to increase the complement of neuronal transcripts and protein isoforms [1–4].

The diversification of neuronal mRNAs is accomplished through two processes: alternative splicing and RNA editing. The pinnacle of alternative splicing may perhaps be reached in transcripts of the *Drosophila* axon guidance receptor *dscam* (Down syndrome cell adhesion molecule), where up to 38,000 different isoforms may be generated through the differential inclusion of alternative exons [5]. RNA editing encompasses a broad range of 'subtle' RNA modifications, including the insertion, deletion, and enzymatic conversion of single or multiple ribonucleotides in an mRNA molecule [6]. RNA editing can act in concert with alternative splicing to further enhance transcript diversity. For example, in the *para* locus encoding a *Drosophila* voltage-gated Na^+ channel, two dozen processing sites encompassing alternative splicing and RNA editing may potentially combine to generate more than two million Na^+ channel isoforms [7–10]. Here we focus on the most common form of RNA editing in the nervous system, adenosine to inosine (A–I) RNA editing, and its potential role in regulating the expression and sequence of neuronal transcripts.

2. Adenosine to inosine RNA editing

2.1. The structure and evolution of ADARs

A–I RNA editing is the hydrolytic deamination of adenosine to inosine in an mRNA template, catalyzed by ADARs (*A*denosine *D*eaminases *A*cting on *R*NA) [11,12]. Inosine is

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recognized by the ribosome (and reverse transcriptases) as a guanosine [13]. Therefore, an A–I transition in the coding region of an mRNA has the potential to alter the resulting protein sequence (Fig. 1A).

ADARs are conserved throughout the animal kingdom, and are present in the genomes of organisms as divergent as nematodes, insects and mammals [14], but appear to be absent in the plant and fungi kingdoms (Fig. 1B). The genomes of both nematodes and mammals contain multiple *adar* loci. Two genes encoding ADARs (*adr-1* and *adr-2*) are present in the *C. elegans* genome [15], and three in mammalian genomes (*ADAR1*, *ADAR2* and *ADAR3/RED2*) [16–19], though deaminase activity of ADAR3 has yet to be demonstrated [19]. Insect genomes, in contrast, appear to have only a single *adar* locus [20]. However, the relative lack of genomic diversity in insects such as *Drosophila* may be compensated by the fact that transcripts arising from the single *adar* locus undergo both alternative splicing and auto-editing, thus generating multiple ADAR isoforms [20].

All ADARs contain a catalytic deaminase domain and generally between one and three double-strand RNA binding domains (dsRBDs) (Fig. 1B) [14]. The catalytic domain of ADARs exhibits weak homology to the cytosine–uradine deaminase APOBEC1 [21] and those of ADATs (*A*denosine *D*eaminases *A*cting on *t*RNA), which have been hypothesized to be the evolutionary ancestors of ADARs [22,23].

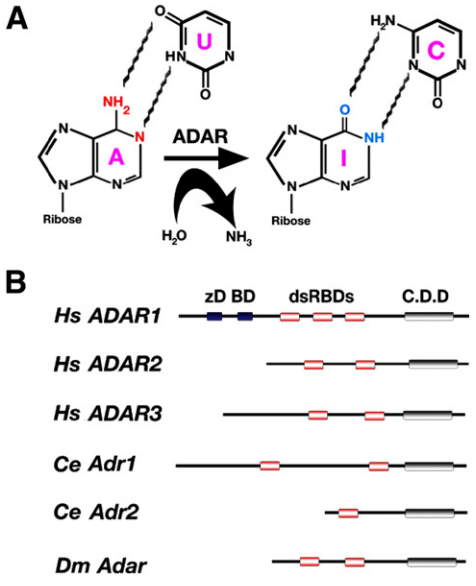

Fig. 1. Mechanism of A–I RNA editing by the ADAR family. (A) Target adenosines within locally paired double-strand RNA duplexes are catalytically deaminated to inosine by ADARs bound to the dsRNA structure. Inosine has similar base-pairing qualities to guanosine, and thus forms Watson–Crick base-pairs with cytosine during translation, reverse-transcription and within dsRNA structures. (B) Selected members of the ADAR family. The human (Hs) genome contains three ADAR loci. ADAR1 encodes a relatively longer polypeptide than ADAR2 or ADAR3 which, in addition to the conserved dsRNA-binding domains (dsRBDs) and catalytic deaminase domain (C.D.D), has two zDNA binding domains (zDBDs) at the N-terminal. ADARs cloned from *C. elegans* (Ce) and *Drosophila* (Dm) contain between one and two dsRBDs and a C-terminal catalytic domain.

2.2. Substrate requirements for ADAR activity

Any dsRNA template greater than ~20 bp may potentially be a substrate for ADARs [24]. However, the enzymatic activity of ADARs on dsRNAs can be broadly classified into two subtypes: non-specific and specific. Long regions of perfectly paired dsRNA may be edited non-specifically at up to 40% of adenosines within a transcript. Such structures may be formed *in vivo* by the base-pairing of inverted *Alu* elements (see below), transposons and viral RNAs [25]. In contrast, shorter regions of imperfectly paired dsRNA may be edited selectively at particular adenosine residues. This form of editing is most often observed in the coding regions of neuronal transcripts, such as pre-mRNAs encoding the *para* sodium channel α-subunit in *Drosophila* [9,10], and the glutamate receptor 2 (*Glur2*) subunit and serotonin receptor 2C receptor (5HT-$_{2C}$R) pre-mRNAs in mammals [26,27]. In these cases, a dsRNA region is formed by imperfect base-pairing between the region surrounding the edited site and an RNA template known as the ECS (*E*diting site *C*omplementary *S*equence). This complementary strand is generally found in neighboring intronic regions [28,29], although in certain cases a single exon alone may fold into a dsRNA structure capable of being recognized by ADAR [9,30,31].

2.3. ADAR activity is required for normal neuronal function

2.3.1. ADARs are required for normal development in vertebrates

The loss of A–I RNA editing has been shown to have profound effects on behavior and survival in several model organisms. The most severe loss of function phenotype occurs in *ADAR1* null mice, which die at the embryonic stage due to defects in erythropoiesis, stress-induced apoptosis and degeneration of the liver [32–34]. ADAR1 exhibits a more widespread expression pattern than ADAR2 and ADAR3, which appear to be expressed predominantly in the brain [17–19,35]. Although ADAR1 clearly edits neuronal RNA templates [27], the phenotype of mice lacking ADAR1 specifically in the nervous system has yet to be examined, precluding studies on its neurobiological function. ADAR2 null mice exhibit profound epileptic seizures and die shortly after birth [36]. Interestingly, this phenotype can be rescued by expressing a constitutively edited form of the GluR2 subunit, suggesting that this mRNA is the major target of ADAR2 *in vivo* [36]. However, several other ion channel subunits are known to be edited in the mouse nervous system, including the Kv1.1 potassium channel, the 5HT-$_{2C}$R, and the α3 GABA$_A$ receptor subunit [27,30,37,38]. Therefore, although editing of the GluR2 subunit may be required for survival, it may be possible that subtle, as yet uncharacterized, neurological phenotypes linked to the absence of editing in these channels and receptors are exhibited in ADAR2$^{-/-}$ mice rescued by expression of edited GluR2. It should also be noted that editing of numerous neuronal targets is still present in ADAR2$^{-/-}$ mice [36], suggesting that ADAR1 and ADAR2 may act redundantly on certain editing sites.