Protein family review

**Adenosine deaminases acting on RNA (ADARs): RNA-editing enzymes**
Liam P Keegan, Anne Leroy, Duncan Sproul and Mary A O’Connell

Address: Medical Research Council Human Genetics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, UK.

Correspondence: Liam P Keegan. E-mail: Liam.Keegan@hgu.mrc.ac.uk

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### Summary

Adenosine deaminases acting on RNA (ADARs) were discovered as a result of their ability extensively to deaminate adenosines in any long double-stranded RNA, converting them to inosines. Subsequently, ADARs were found to deaminate adenosines site-specifically within the coding sequences of transcripts encoding ion-channel subunits, increasing the diversity of these proteins in the central nervous system. ADAR1 is now known to be involved in defending the genome against viruses, and it may affect RNA interference. ADARs are found in animals but are not known in other organisms. It appears that ADARs evolved from a member of another family, adenosine deaminases acting on tRNAs (ADATs), by steps including fusion of two or more double-stranded-RNA binding domains to a common type of zinc-containing adenosine-deaminase domain.

### Gene organization and evolutionary history

**ADAR genes in chordate and invertebrate genomes**

The role of ADARs is to deaminate adenosine to inosine non-specifically in long double-stranded RNA (dsRNA) or site-specifically in transcripts. Non-specific adenosine deamination has a role in defence against viruses that have dsRNA stages in their life-cycle [1], as hypermutation of the viral genome by adenosine-to-inosine conversion disrupts viral open reading frames. The second role of ADARs, site-specific deamination of individual adenosines in pre-mRNAs, can change individual codons in open reading frames, including start or stop codons, or it may affect splicing or untranslated regions [2].

The first ADAR gene to be identified was the vertebrate ADAR1. The protein was purified from *Xenopus*, bovine liver and calf thymus using non-specific deamination of dsRNA as an assay [3-5], and peptide-sequence information was used to design PCR primers to clone the gene [6,7]. The ADAR1 gene was found to encode three double-stranded-RNA binding domains (dsRBDS) and a deaminase domain with zinc-binding motifs (Figure 1), similar to those of zinc-dependent cytosine deaminases and to the cytosine-to-uracil RNA-editing enzyme APOBEC1. The first example of site-specific editing, in a pre-mRNA encoding the vertebrate glutamate-receptor subunit *GluR-B*, was found at the same time; editing converts a glutamine codon to an arginine codon (Q to R in the single-letter amino-acid code) [8]. ADAR1 was unable to catalyze site-specific editing of the Q/R editing site in the *GluR-B* transcript *in vitro*, however. This observation led to the identification of ADAR2, which edits the *GluR-B* Q/R site specifically [9,10]. ADAR2 contains two dsRBDS and an adenosine-deaminase domain (Figure 1). Other ADAR genes in vertebrates and ADAR genes in other organisms were identified either by PCR using primers derived from the deaminase motifs or by sequence homology.

There are two ADAR-like genes in vertebrate genomes, encoding enzymes with unknown function. The ADAR3 gene, also called Red2, is very similar to ADAR2 (Figure 2) [11]. It has all the key conserved residues that are known to be required for catalysis, but it has been shown to lack non-specific and site-specific dsRNA adenosine-deaminase activity [12]. ADAR3 may have arisen from ADAR2 by gene
duplication within the chordate lineage. *ADAR3* is very well conserved in fish genomes, even though no function has been ascribed to it, suggesting that it is active on an unknown substrate. A fourth *ADAR*-like gene in vertebrates, *TENR*, is expressed in the male germline and has only one dsRBD [13]. *TENR* lacks a key catalytic glutamate residue in the deaminase domain and also lacks zinc-chelating residues; the protein has not been characterized. We have been able to identify *TENR* genes only in mammalian and not in fish genomes.

The presence of four *ADAR* genes in vertebrates represents a degree of gene-family expansion in the chordate lineage similar to that found in many other gene families, notably including the cytosine deaminases acting on RNA (CDARs) [14]. The vertebrate *ADAR* genes are unlinked in the genome, but some chordate genomes contain multiple copies of particular *ADAR* genes: for example, the *Xenopus* genome, which is large and not completely sequenced, contains two *ADAR1* genes, and *Takifugu rubripes* has two *ADAR2* genes.

The *Drosophila* genome encodes one *ADAR*, which has two dsRBDs and a deaminase domain and is more similar to vertebrate *ADAR2* than to *ADAR1* [15]. We searched for *ADAR* genes in the complete genome sequences of the primitive chordates *Ciona intestinalis* and *Ciona savignyi* (our unpublished work); predicted deaminase domains were found that seem to correspond to *ADAR1* and *ADAR2* as well as a
Figure 2
A phylogenetic tree of core deaminase sequences. Protein sequences that are predicted from genomic sequences rather than from full cDNA sequences are indicated by asterisks. The vertebrate genes listed include one from the crab-eating monkey *Macacus fascicularis*. The fish genomes examined are from the zebrafish *Danio rerio* and pufferfish, *Takifugu rubripes* and *Tetraodon fluviatilis*. Two tunicate genomes have been examined, from *Ciona intestinalis* and *Ciona savignyi*. One squid (*Loligo pealeii*) is included (J. Rosenthal, personal communication). The insect genomes are of the two *Drosophila* species, *Drosophila melanogaster* and *Drosophila pseudoobscura*, and the malaria mosquito *Anopheles gambiae*. Alignments were made using T-COFFEE, the boundaries of the core deaminase domain were specified and a tree based on the alignment was generated using MEGA. Bootstrap values are given on all branches.
separate ADAT1 gene (see below; Figure 2) [16]. We have not proven that these are full ADAR1 genes, because there is no cDNA sequence available for them and because the full genes with dsRBDs are difficult to predict from the genome sequence. ADAR1-like deaminase domains are clearly present in basal chordates, however. This is a little surprising and argues against the idea that ADAR1 and ADAR2 diverged from a parental gene during the period of widespread gene duplications in early vertebrate evolution; it suggests, instead, that the ADAR duplication occurred earlier (our unpublished work). An ADAR2 gene has been cloned from the squid Loligo pealeii, but it is not clear whether squid also have an ADAR1 gene.

The genomes of the nematodes Caenorhabditis elegans and Caenorhabditis briggsae contain two ADAR genes, adr1 and adr2 [17]. The deaminase domains of ADR1 and ADR2 are not closely related to each other, and their names do not reflect a direct correspondence with vertebrate ADAR1 and ADAR2 proteins; the sequences differ so much from vertebrate ADARs that it is impossible to make such links (Figure 2). Both adr1 and adr2 are required for editing activity in vivo, and the proteins may form heterodimers. ADR1 has two dsRBDs; ADR2 has only one and lacks the active-site glutamate. The C. elegans adr1 gene structure shows some resemblance to the Drosophila Adar gene, however. In addition to encoding an ADAR with two dsRBDs, C. elegans adr1, like Drosophila Adar, produces several isoforms by alternative 3′ splice-site usage affecting the sequence between the dsRBDs.

Human ADAR1 has two transcription start sites and two promoters, leading to production of a shorter isoform that lacks the first 296 residues of the long form (Figure 1). The first 530 amino acids of human ADAR1, up to the middle of the first dsRBD, is encoded by a single exon. Interestingly, each of the regions encoding the three dsRBDs in ADAR1 is interrupted in the middle by an intron in a conserved position, suggesting that they could have arisen by duplication of a pair of exons. The deaminase motifs are encoded on separate exons. Human ADAR2 has both dsRBDs on one exon, as does ADAR3. As in human ADAR1 the deaminase motifs are encoded on separate exons but intron positions in the deaminase domain are not well conserved between human ADAR1 and ADAR2. Human ADAR2 produces two isoforms one of which (ADAR2-L) has an exonized Alu sequence that inserts an additional 40 amino acids between the second and third deaminase motifs (the ADAR2-S form lacking the Alu sequence is shown in Figures 1 and 3). These splice variants should not be confused with the two distinct ADAR2a and ADAR2b genes found in the genome of T. rubripes. Alternative splice forms of ADAR3 or TENR have not been described.

**Evolution of ADAR genes from ADAT genes**

So far, ADARs have been found only in multicellular animals; they are clearly absent from yeast and have not been reported in protozoa. Searches in the yeast and Drosophila genomes for proteins with ADAR-type deaminase motifs led to the identification of an adenosine deaminase acting on tRNA (Tad1/ADAT) that deaminates adenosine to inosine in tRNA\textsuperscript{Ala} at position 37, adjacent to the anticodon [16]. ADAT1 has an adenosine-deaminase domain but lacks dsRBDs. Our present view of ADAR evolution is that the first ADAR evolved in protozoans or lower multicellular animals from an ADAT1-like protein by acquisition of dsRBDs and residue changes in the adenosine-deaminase domain.

Editing also occurs at position 34 within the anticodons of several vertebrate tRNAs. Searches for adenosine-deaminase domain sequences in the yeast genome led to the identification of Tad2/ADAT2, which, as a heterodimer with Tad3/ADAT3, deaminates position 34 of tRNAs. This is the wobble base-pairing position in the anticodons of seven tRNAs in yeast [18]. Unlike ADAT1, the ADAT2 and ADAT3 proteins are essential in yeast. The loop between deaminase motifs II and III of the ADARs and ADAT1 is absent from ADAT2 and ADAT3 (Figure 1). A deaminase domain with motifs II and III closer together is characteristic of the free-nucleotide cytosine deaminases and CDARs such as APOBEC1 and its relatives. The presence of such a domain in CDARs and ADATs further supports the idea that ADARs, ADATs and CDARs had a common ancestor [19]. Only ADAT2, and not ADAT3, has the conserved glutamate residue associated with adenosine deaminase motif I, so the heterodimer has only one active site.

ADAT1, ADAT2 and ADAT3 are present in all eukaryotes examined, although we have not found any sequence encoding an ADAT1 in the nematode genomes. ADAT2 and ADAT3 are homologous to each other over a stretch of 120 amino acids containing the deaminase motifs, but the remaining parts of the proteins are not clearly homologous to one another or to the larger deaminase domains of ADAT1s or ADARs. The carboxy-terminal region in vertebrate ADAT2 proteins is even shorter than in the yeast ADAT2 proteins. We have taken the region of homology between ADAT2 and ADAT3 to define a core deaminase domain and used protein sequence alignments of this region to construct the evolutionary tree shown in Figure 2.

An Escherichia coli homolog of ADAT2, tadA, forms homodimers and is able to edit position 34 in tRNA\textsuperscript{Arg2}, which is the only known target in E. coli [20]. The evolution of a heterodimeric ADAT2/ADAT3 in eukaryotes, presumably from a tadA-like precursor, coincided with an increase in the number of tRNAs that have inosine at the wobble position. In higher eukaryotes, eight tRNA classes are edited, and editing also occurs at position 34 within the anticodons of several vertebrate tRNAs. Searches for adenosine-deaminase domain sequences in the yeast genome led to the identification of Tad2/ADAT2, which, as a heterodimer with Tad3/ADAT3, deaminates position 34 of tRNAs. This is the wobble base-pairing position in the anticodons of seven tRNAs in yeast [18]. Unlike ADAT1, the ADAT2 and ADAT3 proteins are essential in yeast. The loop between deaminase motifs II and III of the ADARs and ADAT1 is absent from ADAT2 and ADAT3 (Figure 1). A deaminase domain with motifs II and III closer together is characteristic of the free-nucleotide cytosine deaminases and CDARs such as APOBEC1 and its relatives. The presence of such a domain in CDARs and ADATs further supports the idea that ADARs, ADATs and CDARs had a common ancestor [19]. Only ADAT2, and not ADAT3, has the conserved glutamate residue associated with adenosine deaminase motif I, so the heterodimer has only one active site.

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The tadA protein edits a minisubstrate consisting of the anticodon stem-loop of E. coli tRNA\textsuperscript{Arg2} [20]. ADAT1 from yeast,
Figure 3
An alignment of core deaminase domains from vertebrate ADAR1 and ADAR2 proteins, showing residues characteristic for each. Asterisks indicate cysteines and histidines that chelate zinc, as in Figure 1, and the three active-site motifs are bracketed.

however, clearly requires other parts of the tRNA in addition to the anticodon stem and loop, and it evidently recognizes a longer portion of the tRNA substrate [16]. The definition of ADARs and ADATs on the basis of whether their substrate is tRNA or mRNA does not coincide with classification based on the structures of their deaminase domains and their interactions with RNA. ADAT2 and ADAT3 are different in size, structure and mode of interaction with tRNA from ADAT1, which has a clearly ADAR-like deaminase domain. The ADAT1-type deaminase could have evolved from an ADAT2-like protein in one of two ways: either a loop was inserted between the second and third zinc-chelating residues to give ADAR deaminase motif III, or ADARs have a inserted between the second and third zinc-chelating ADAT2-like protein in one of two ways: either a loop was
what is now the loop, with ADAR motif III and the carboxy-terminal part of the deaminase domain deriving from another protein.

Inosine has been found in archael tRNA [21,22], so the core adenosine-deaminase domain may be very ancient. Inosine could have been produced easily from adenosine even earlier, by RNA-mediated catalysis, and inosine may have been excluded rather than unavailable when the canonical base set for Watson-Crick base-pairing evolved to give unambiguous rules for RNA replication. The wobble base-pairing of tRNAs now appears to be a means to economize on tRNAs rather than a relic of some ancient type of translation apparatus, as the inosine in archael tRNA is not in the anticodon. In eukaryotes, there have been two rounds of evolutionary expansion in targets of the inosine-producing RNA-editing apparatus, first to increase the number of tRNAs edited at the anticodon wobble position by Tad2/ADAT2 and Tad3/ADAT3 and second (in animals) to include pre-mRNAs and dsRNA as targets of ADARs.

**Characteristic structural features**

**The adenosine deaminase domain**

Experimental exchanges of the deaminase domains of ADAR1 and ADAR2 have shown that the deaminase domain determines the difference in specificity between the two proteins [23]. With the availability of more genomic sequences, we have aligned 14 different sequences of ADAR1 and ADAR2 deaminase domains and found a high degree of conservation in a region of 179 amino acids (Figure 3). The amino acids that are downstream of the second deaminase motif appear to specify the enzyme that is encoded. The sequence differences between ADAR1 and ADAR2 that are highlighted (Figure 3) make it possible to assign newly sequenced vertebrate ADAR genes as ADAR1 or ADAR2 orthologs, even in cases in which there is no full-length cDNA sequence and the number of dsRNA-binding domains is not known. With this obvious difference close to the catalytic site, it is not surprising that ADAR1 and ADAR2 show very little overlap in the specific sites they edit in transcripts, despite the fact that they both perform the same enzymatic activity. The three active site sequence motifs are particularly characteristic of ADARs. Each motif contains a cysteine or histidine that is thought to chelate zinc at the active site (asterisks in Figure 3); the first deaminase motif (CHAE) includes a glutamate (E) that is required for catalysis.

Structural information is available for one *E. coli* free-nucleotide cytosine deaminase [24]. This protein has deaminase-domain motifs similar to ADAT2 and tAD and dimerizes so that each monomer contributes to form the active site; it has very little homology to ADARs apart from the zinc-binding motifs, and it is not clear whether the ADARs dimerize in an equivalent way around the active site. The additional sequence between motif II and motif III in ADARs may be incompatible with this mechanism of dimerization. Structural information from this cytosine deaminase cannot be used reliably to model ADAR deaminase domains but may be more useful for modeling the deaminase domains of ADAT2 and ADAT3.

We do not know how the deaminase domain interacts with RNA. The ADAR2 deaminase domain without dsRBDs is capable of site-specific editing in vitro [25], and the same is true of the *Drosophila* ADAR deaminase domain (G. Ring and M.O'C., unpublished observations). The ADAR deaminase domain was therefore a dsRNA-binding domain even before it acquired dsRBDs. It clearly evolved from ADAT1 [19], but the residues responsible for binding to tRNA in ADAT1 in the heterodimer ADAT2/ADAT3 are as yet unknown. Considering the sequence homology between the ADARs and ADAT1, the intriguing possibility arises that recognition of pre-mRNA and tRNA substrates by these proteins follows a common structural model. Both ADARs and ADAT1 probably flip the target adenosine out of the DNA and into the enzyme’s active site; studies with a fluorescent adenine analog at a second GluR-B DNA-editing site (the R/G site) are consistent with base-flipping by ADAR2 [26,27]. (The R/G site is separated from the Q/R site and is more experimentally accessible.) Consistent with this idea, some similarity to DNA methyltransferases that flip out the target base has been found in the deaminase domain of ADARs [13].

**Double-stranded RNA-binding domains**

DsRBDs are not diagnostic of ADARs, as they occur in a very wide variety of proteins [28]. The number of dsRBDs and the sequence conservation within them and in the linkers connecting them help to distinguish ADAR1 and ADAR2 homologs in vertebrate genomes, however. X-ray crystal-structure determination and nuclear magnetic resonance (NMR) analysis of dsRBD-RNA complexes have revealed that the dsRBDs of other proteins bind non-sequence-specifically to the sugar phosphate backbone of DNA [29,30]. It was therefore surprising when it was demonstrated in *vitro* that the two dsRBDs of ADAR2 recognize the R/G site in GluR-B with some specificity, even in the absence of the deaminase domain [27]. Binding of the dsRBDs alters the conformation of the substrate around the edited position; this is thought to aid base-flipping.

One feature of dsRBDs is that they are multi-functional and are not equivalent to each other. Some have evolved to become protein-protein-interaction domains [28]. The amino terminus and the first dsRBD of *Drosophila* ADAR are required for dimerization in a similar way to the dimerization of dsRNA-dependent protein kinase (PKR) via its dsRBDs [31,32]. The third dsRBD of ADAR1 contains a novel nuclear localization sequence [33].
Localization and function

ADARs are primarily nuclear proteins, but ADAR1 is expressed in two forms and the longer isoform (which is inducible by interferon) is found predominantly in the cytoplasm [34]. The shorter form is a shuttling protein that cycles in and out of the nucleus; blocking its export causes accumulation in the nucleus [35,36]. ADAR1 (both forms) and ADAR2 can accumulate in the nucleolus [36,37]; this accumulation is dependent on binding to RNA, probably nonspecifically in the nucleolus. Transfection of HeLa cells with transcripts that are known to be edited causes both ADAR proteins to relocate from the nucleolus to the nucleoplasm, suggesting that the nucleolus could be a site of accumulation or storage of the proteins when substrate is limiting [36].

The known function of the vertebrate ADARs is to edit transcripts expressed in the central nervous system. The paradigmatic example of site-specific RNA editing is the editing of the GluR-B Q/R site by ADAR2 [8]. Glutamate is the major excitatory neurotransmitter in the vertebrate brain, and GluR-B encodes a subunit of the α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) class of glutamate receptors, which are heterotetramers formed from subunits encoded by several genes. The Q/R editing event changes a key residue within the ion pore of the channel; the unedited Q form of the GluR-B subunit renders AMPA receptors more permeable to calcium ions. The Q/R editing site in the GluR-B transcript in vertebrates is the only known site that is edited more than 99.9% of the time. Studies on knockout mice show that the GluR-B transcript must be edited when it is present [38], but mice with deletions of the GluR-B gene are viable because of redundancy with other subunit genes [39].

Gene-targeting studies in mice show that ADAR2 is required primarily for site-specific editing at the GluR-B Q/R site [38]. Mice with mutations in ADAR2 die as pups, suffering from epilepsy-like seizures and localized neurodegeneration in the hippocampus [38]. These phenotypes are consistent with calcium influx through AMPA receptors. The defects in ADAR2 mutant mice are rescued by expression GluR-B(R). More recently, studies in which mutated forms of GluR-B were expressed in hippocampal-cell cultures have shown that the unedited GluR-B(Q) isoform completes the tetramer formation required to move from the endoplasmic reticulum to the synapse much more quickly than edited GluR-B(R) isoform. RNA editing thereby restricts trafficking of AMPA receptors to the cell membrane [40,41], affecting an important aspect of plasticity at glutamatergic synapses.

Very recent gene-targeting experiments on mouse ADAR1 show that ADAR1 does contribute to site-specific editing and that the gene is essential [44,45]. Mutant embryos die at embryonic day 12.5 with hematopoiesis defects and liver degeneration. Cultured cells from a variety of different tissues in ADAR1 mutant mice undergo stress-induced apoptosis in response to serum starvation. It is not known whether this phenotype arises from failure to edit some unknown site-specific anti-apoptotic RNA-editing target or from failure of a more generalized non-specific RNA-editing activity. The reasons to think ADAR1 might have a wider role than ADAR2 include, in addition to the more severe knockout phenotype [44,45], more widespread expression, an additional promoter for interferon-inducible transcripts and additional Z-DNA binding domains in ADAR1 [46].

From the search for homologs of ADARs in other species (Figure 2), it becomes apparent that ADAR3 and TENR are probably functional. Both are thought not to have any enzymatic activity on dsRNA, but ADAR3 is conserved between fish and mammals, and TENR is found in mammals and is testis-specific. Considering the importance of APOPEC-3G (47), which was regarded for many years as a non-functional editing enzyme but now appears to be a DNA-editing enzyme active against human immunodeficiency virus (HIV), these ADAR orphans may yet surprise us.

Mechanism

The ADAR enzymes probably bind to many transcripts nonproductively, as binding and catalysis are independent events [48,49]. Until recently, it was thought that ADARs bind to editing sites as monomers, because ADARs are purified as monomers and because the pre-mRNAs that are edited do not contain any obviously symmetrical RNA sequence that would suggest binding by a dimer. Recent work from many groups has demonstrated, however, that the catalytic ADAR is a dimer [50-52]. Yeast two-hybrid studies with Drosophila ADAR show that ADAR dimerizes using the first dsRBD and sequences amino-terminal to it [52]. Studies in vitro indicate that dimerization requires RNA binding and that ADAR must be a dimer on RNA to be enzymatically active. It is not known whether the minimal dimerization region of one monomer contacts the equivalent part of another monomer or a different part of the protein, such as the deaminase domain.

The dimerization of ADARs is probably what controls the specificity of editing, as two monomers must bind for catalysis to occur. This dimerization model predicts that if another adenosine was placed approximately 17 base-pairs away from a known edited site, this adenosine would also be edited. Evidence consistent with this model comes from an experiment by Herbert and Rich [25], in which multiple base changes in the GluR-B R/G substrate 16–19 base-pairs away from the R/G site created new editing sites at these positions.
and also affected editing at the R/G site itself so that an adjacent adenosine was now also edited.

Frontiers
There are important questions that remain to be answered regarding the structures, substrates and regulation of ADARs and on the links between RNA editing and RNA interference. The structure of an adenosine-deaminase domain from an ADAR or ADAT1 protein remains to be elucidated. The details of how the multiple domains in ADARs interact with substrate RNA must be resolved either with ADAR-RNA cocrysats or structure-function studies guided by the structures of the individual domains. Structural information on a CDAR will also be necessary to resolve finally the relationship between ADARs and the other members of the cytosine deaminase superfamily.

There are likely to be many more transcripts in humans that are edited than have been found so far. Comparison of the human genome with another suitably distant chordate genome sequence is likely to identify them, as indicated by the recent success in using comparison of Drosophila genome sequences to identify 24 novel transcripts with editing at 53 individual sites [53]. Exons encoding editing sites have reduced levels of synonymous codon substitution and are more highly conserved than exons in general, because of the requirement for an RNA duplex. When genomic sequences of D. melanogaster and D. pseudoobscura genes encoding ion-channel subunits and synaptic-vesicle fusion proteins were compared [53], edited exons were identified by their higher conservation. Using this method on the human genome requires identifying a vertebrate genome with sufficient sequence divergence from ours.

Discovering regulatory mechanisms for RNA-editing activity is a key issue in explaining why RNA editing occurs. Editing is certainly tissue-specific - as shown by the expression patterns of ADARs and their substrates - and the expression of the interferon-inducible form of ADAR1 is increased by interferon, viral infection, or dsRNA, but there may be further levels of regulation, particularly in neurons. Understanding regulation may also relate A-to-I RNA editing to other types of editing in mitochondria and chloroplasts that are mechanistically very different but might have some parallel evolutionary rationale.

Work on the C. elegans ADARs implicates RNA editing in the antagonizing of RNA interference [54,55]. ADAR RNA editing is part of a wider biology of dsRNA that has gained renewed attention since the discovery of RNA interference. Double-stranded RNA genomes are now found only in some viruses, and higher eukaryotic cells recognize dsRNA as a hallmark of infection, but dsRNA genomes may have been more widespread in the very distant past. Proteins that recognize the A-form structure of dsRNA are very different from, and some of them may be much more ancient than, the large number of proteins that recognize the B-form helix of DNA. The core deaminase domain of ADARs and ADATs seems to be an ancient protein domain that, like RNA-dependent RNA polymerase and other dsRNA-binding proteins of the RNA interference system, has acquired new roles in recognizing dsRNA genomes as foreign. The conjunction of RNA editing and RNA interference is a lively area for further research. Thus, the study of RNA editing continues to raise fascinating questions about the evolution of genomes, and is likely to give insights into a range of other areas in the near future.

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