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RNA Editing: Molecular Foundations and Relevance to Neurodegenerative Disorders

CONFERENCE

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OVERVIEW

- RNA editing is a major epigenetic regulator of gene expression, involved in the fine-tuning of cellular responses sustaining cellular homeostasis.
- RNA editing systems fall into two general classes: insertion/ deletion and substitution.
- There are several types of RNA editing, among which the deamination of Adenosine (A) to Inosine (I) or Cytidine (C) to Uridine (U) is the most common type in mammals.
- Adenosine deaminases acting on RNA (ADAR), and (apolipoprotein B mRNA editing enzyme catalytic polypeptide-like (APOBEC) enzyme families, catalyzed the major type of RNA editing in mammals (A-to-I and C-to-U, respectively).
- RNA editing may be advantageous for adaptation to changing environments.
- Several studies have associated RNA editing with disease conditions, including a number of neurodegenerative disorders.
- Medical tools have been developed based on RNA editing.

AIM

- To provide an introduction to the molecular basis of RNA editing so that their types, the molecular mechanisms involved, their physiological and adaptive importance, their relevance to neurodegenerative diseases, and some of their medical applications are understood.

OUTLINE

- RNA editing:
  - ✓ Definition
  - ✓ Types
  - ✓ Molecular mechanisms involved

- Functional consequences of RNA editing and its advantages for adaptation

- Relevance of RNA editing for neurodegenerative disorders:
  - ✓ Alzheimer´s disease
  - ✓ Amyotrophic Lateral Sclerosis
  - ✓ Huntington´s disease

RNA EDITING DEFINITION

- The term “RNA editing” was originally coined by Benne *et al.* in 1986 to describe the **process of uridine (U) insertion and deletion** in mitochondrial transcripts of kinetoplastid protozoa.

Benne *et al*. Cell. 1986; 46(6):819-26.

- RNA editing refers to an **epigenetic mechanism that contributes to transcriptome diversification** through the introduction of alterations in RNA species relative to the corresponding genome-encoded information (RNA–DNA differences, RDDs).

Farajollahi and Maas. Trends Genet. 2010; 26(5):221-30.

- RNA editing is a **biological process or tool for repairing or altering RNA.**

Woolf *et al*. Proc. Natl. Acad. Sci. USA 1995; 92: 8298-8.
Booth *et al*. Mol Ther. 2023; S1525-0016(23)00005-9.

TYPES OF RNA EDITING

RNA EDITING

Insertion/Deletion

- The nucleotide sequence of the mature RNA product is **not collinear** with that of its DNA coding sequence: the final RNA product contains extra nucleotides compared to its gene.

A. Insertion/deletion editing

```
... UUG UUU AA    G UUA   UG   G ...
        ↓↓↓   ↓ ↓↓  ↓  ↓ ↓   ↓↓
... UUG --- AAU UUG -UAU UUG UUG ...

... Leu     Asn Leu  Tyr Leu Leu ...
```

Substitution

- Coding sequences of a mature RNA and its gene are **collinear**: they contain the same number of nucleotides but differ at those positions where editing occurs.

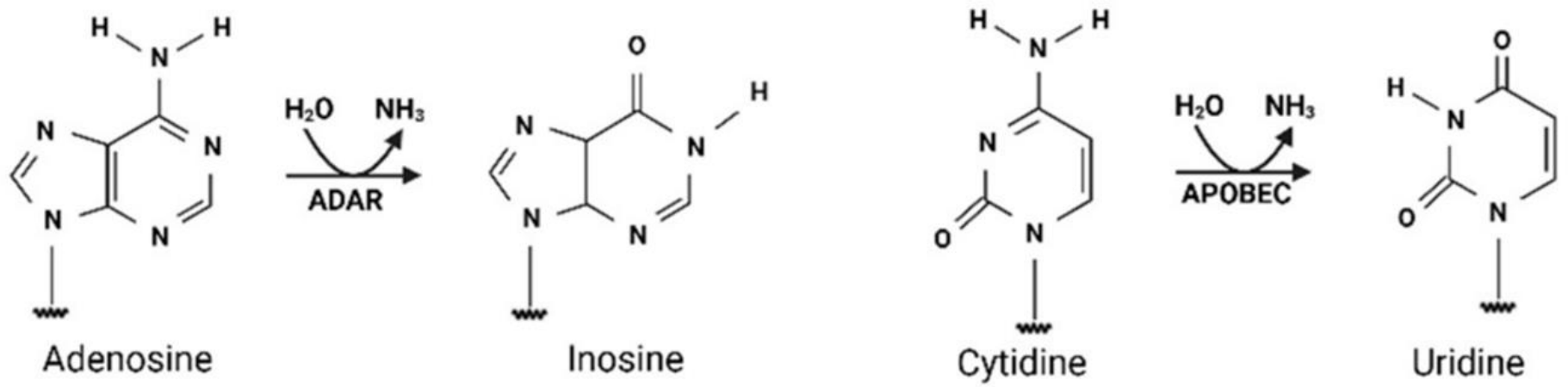
B. Substitution editing

```
... Ile Arg Gly Thr Asn His Ala Phe Thr Pro ...
... AUU CGU GGA ACU AAU CAU GCC UUU ACG CCU ...
        ↓                           ↓
        UGU                         AUG
        Cys                         Met
```

Gray. Biochemistry. 2012; 51(26):5235-42.

TYPES OF RNA EDITING

- A-to-I and C-to-U editing are two types of substitutional RNA editing in mammals:



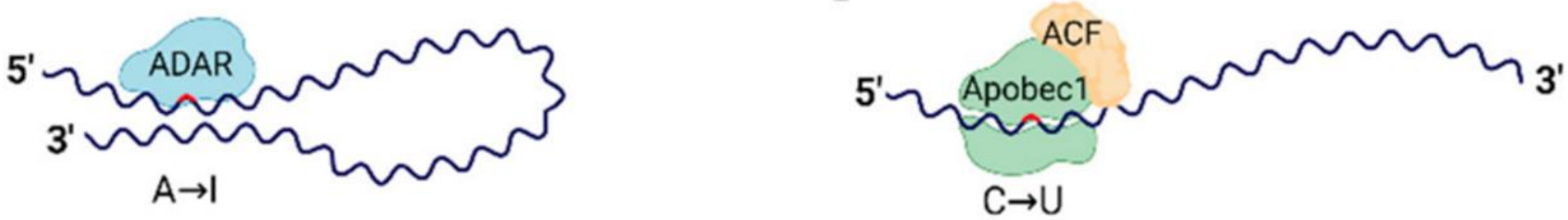
- It is predicted that there are over 100 million human Alu RNA Adenosine to inosine (A-to-I) editing sites, located across the human genome.

- Editing of C-to-U is not as physiologically common as that of A-to-I editing.

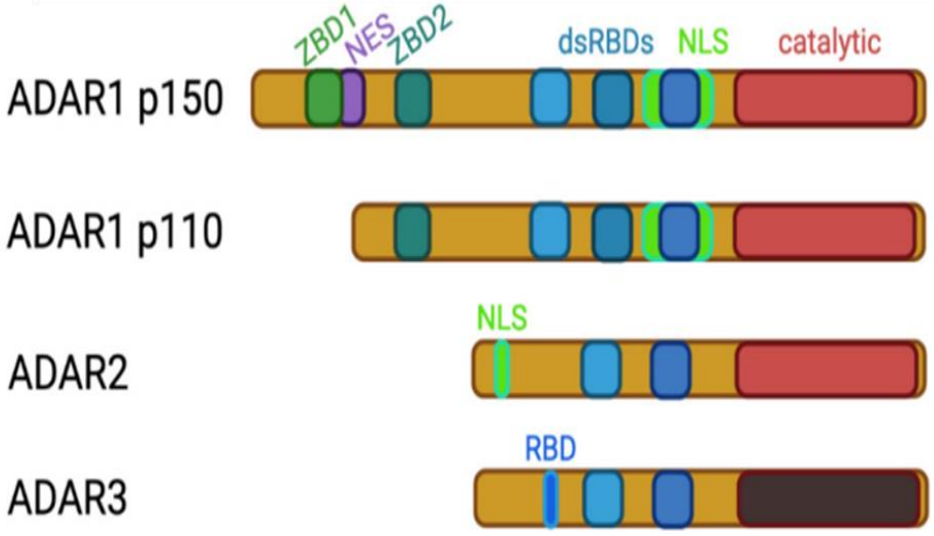
- RNA editing has been observed in diefferent RNA species, including mRNAs, tRNAs, and rRNAs, in mitochondrial and chloroplast encoded RNAs, and in nuclear encoded RNAs.

MOLECULAR MECHANISMS INVOLVED IN RNA EDITING

- The major types of RNA editing in mammals are catalyzed by adenosine deaminases acting on RNA (ADAR) (A-to-I), and apolipoprotein B mRNA editing enzyme catalytic polypeptide-like (APOBEC) (C-to-U) enzyme families.



- There are three members of the ADAR family in mammals: ADAR (ADAR1)(two isoforms: p150 and p110), ADARB1 (ADAR2) and ADARB2 (ADAR3).



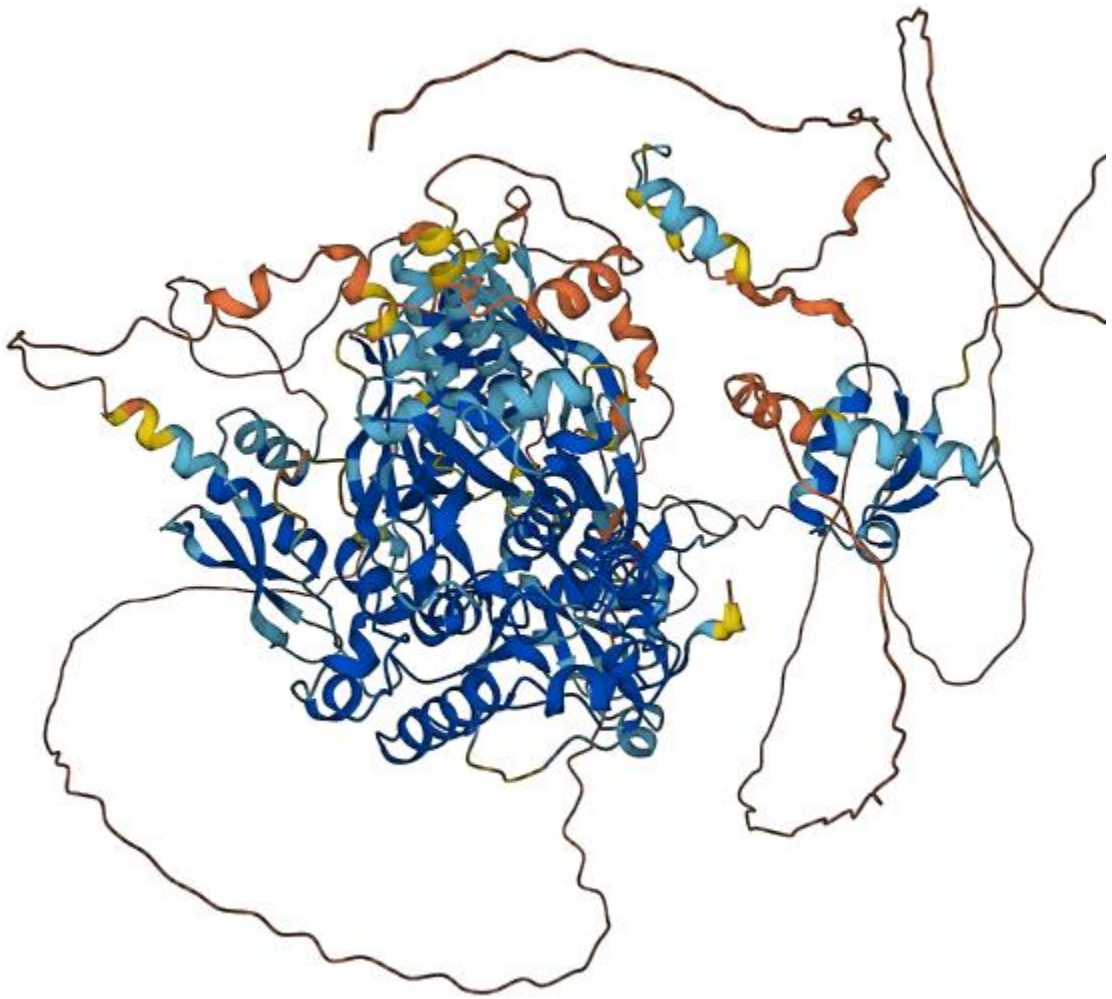
Vesely and Jantsch. Genes (Basel). 2021; 12(7):1026.

- All ADARs contain a deaminase domain at their C-terminus. However, the catalytic domain of ADAR3 is enzymatically inactive.
- Substrate engagement of the ADARs is mediated via a variable number of double-stranded RNA-binding domains (dsRBDs) that interact with structured and double-stranded RNAs.
- The cellular transcriptional and translational machinery recognizes Inosine (I) as Guanosine (G); thus, processed ADAR edited transcripts display a G at the edited site (A-I-G editing).

MOLECULAR MECHANISMS INVOLVED IN RNA EDITING

ADAR (ADAR1)

(Adenosine Deaminase RNA Specific)



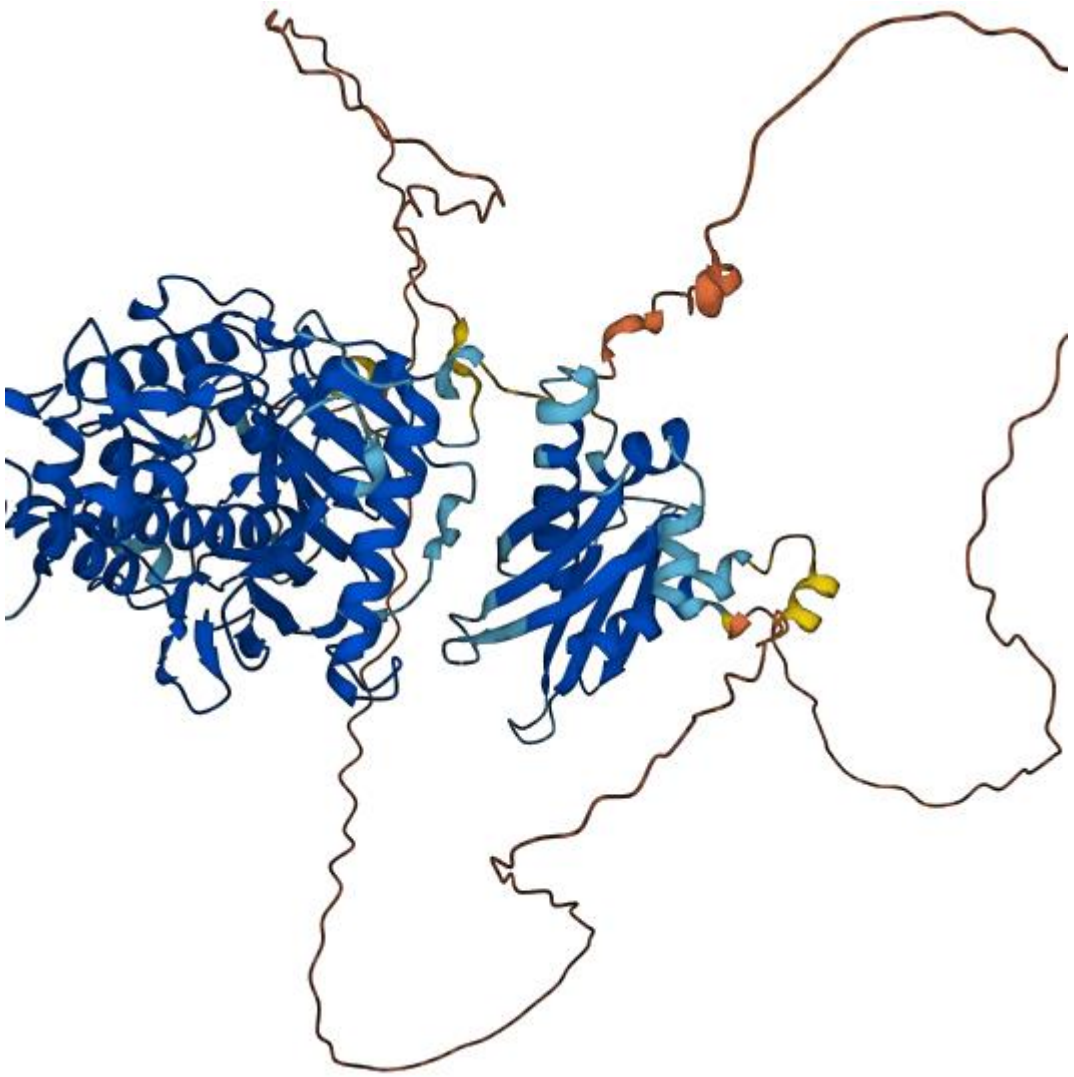
AlphaFold Protein Structure Database (ebi.ac.uk)

- Catalyzes the hydrolytic deamination of adenosine to inosine in double-stranded RNA (dsRNA) referred to as A-to-I RNA editing.
- Can edit both viral and cellular RNAs.
- Can edit RNAs at multiple sites (hyper-editing) or at specific sites (site-specific editing).
- Its cellular RNA substrates include: bladder cancer-associated protein (BLCAP), neurotransmitter receptors for glutamate (GRIA2) and serotonin (HTR2C) and GABA receptor (GABRA3).

MOLECULAR MECHANISMS INVOLVED IN RNA EDITING

ADARB1 (ADAR2)

(Adenosine Deaminase RNA Specific)



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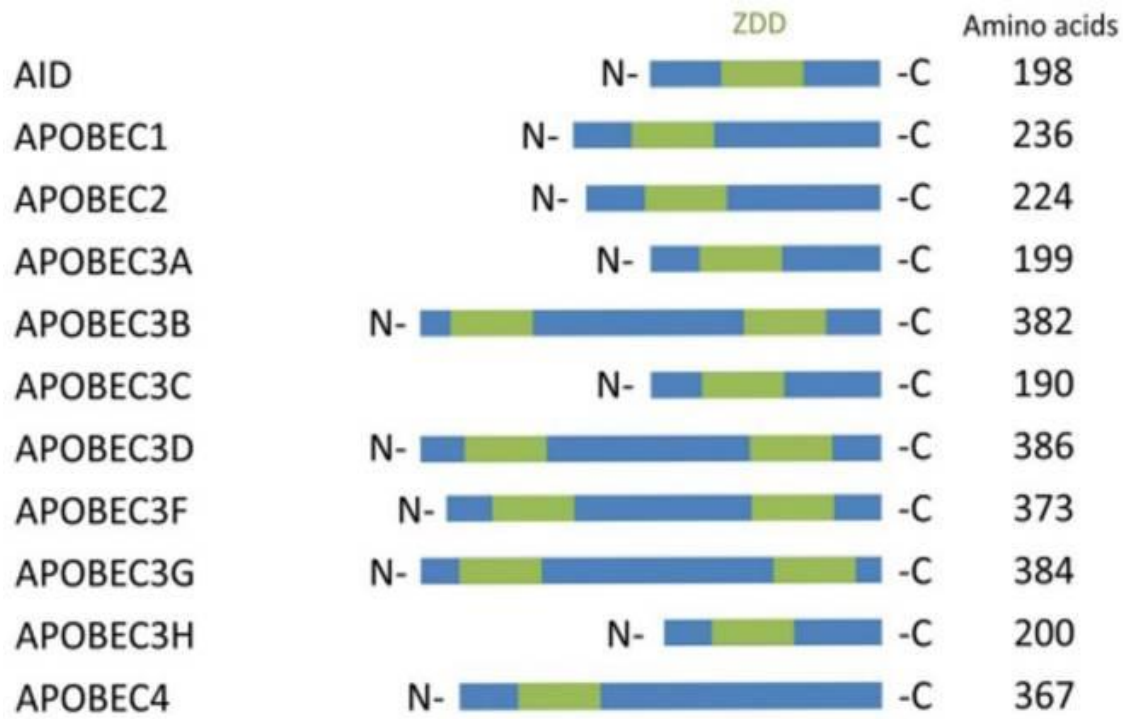
- Its cellular RNA substrates include: bladder cancer-associated protein (BLCAP), neurotransmitter receptors for glutamate (GRIA2 and GRIK2) and serotonin (HTR2C), GABA receptor (GABRA3) and potassium voltage-gated channel (KCNA1).

MOLECULAR MECHANISMS INVOLVED IN RNA EDITING

- One of the major types of RNA editing in mammals is catalyzed by the apolipoprotein B mRNA editing enzyme catalytic polypeptide-like (APOBEC) (C-to-U) enzyme family.

- There are eleven members of the APOBEC family in mammals: APOBEC1, APOBEC2, APOBEC3A, APOBEC3B, APOBEC3C, APOBEC3D, APOBEC3F, APOBEC3G, APOBEC3H, APOBEC4, and AICDA/AID.



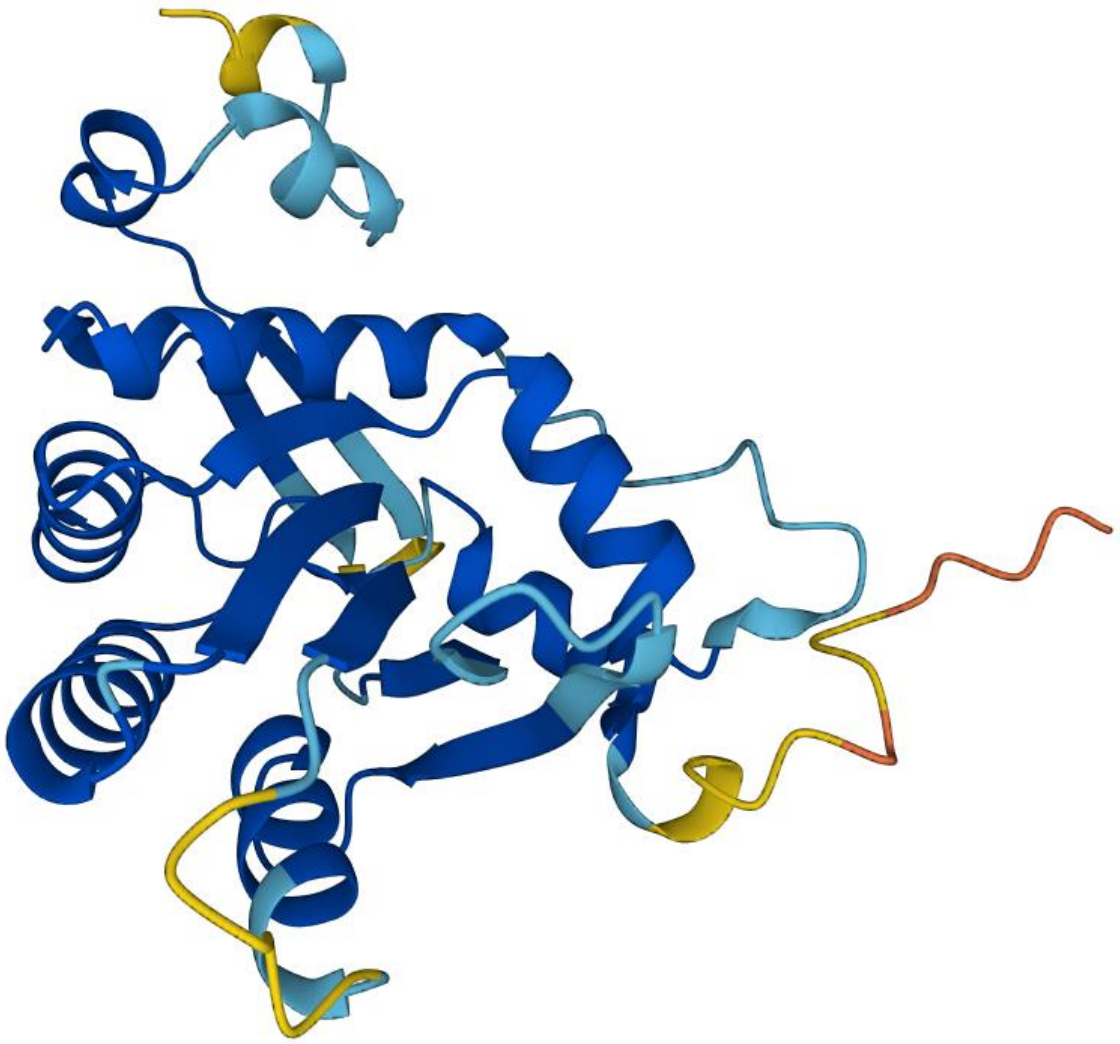
- All members of the APOBEC family in mammals have a zinc-dependent deaminase domain (ZDD).

- Among all of these APOBEC subfamily proteins only APOBEC-1, 3A, 3 B, and 3G have been proven to mediate the C-to-U RNA editing; however, APOBEC1 is the main C-U editing enzyme in mammals.

MOLECULAR MECHANISMS INVOLVED IN RNA EDITING

APOBEC1

(Apolipoprotein B mRNA Editing Enzyme Catalytic Subunit 1)

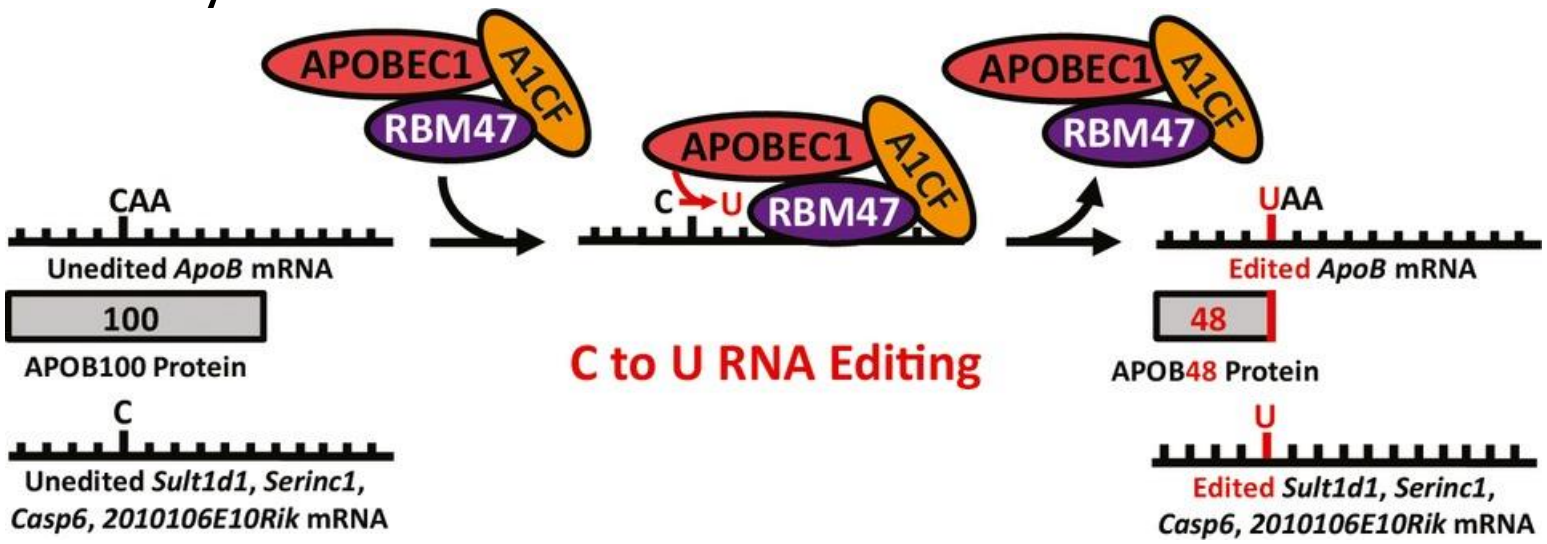


AlphaFold Protein Structure Database (ebi.ac.uk)

- Catalyzes the cytidine to uridine postranscriptional editing of a variety of mRNAs.
- Form complexes with cofactors that confer differential editing activity and selectivity.
- Responsible for the postranscriptional editing of a CAA codon for Gln to a UAA codon for stop in the apolipoprotein B mRNA.
- Also involved in CGA (Arg) to UGA (Stop) editing in the NF1 mRNA.

MOLECULAR MECHANISMS INVOLVED IN RNA EDITING

- APOBEC1-mediated editing is highly specific and requires the formation of the editosome, a protein complex that comprises an enzyme homodimer, an essential co-factor (A1CF or RBM47) and auxiliary proteins that regulate enzymatic activity.



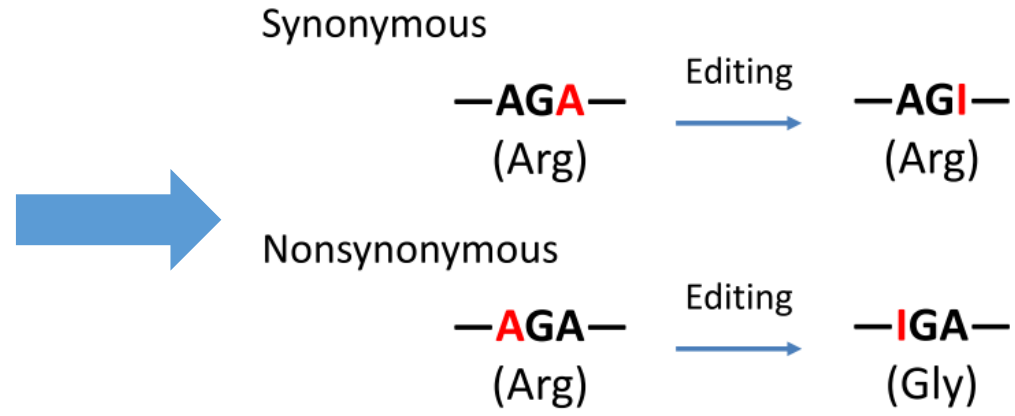
Fossat et al. EMBO Rep. 2014; 15(8):903-10.

- APOBEC1 targets are ssRNAs and display specific sequence elements, corresponding to the mooring sequence (an 11 nt consensus sequence located downstream the C undergoing deamination, required for A1CF binding) and an AU-rich ‘efficiency region’, located upstream of the edited residue.

- The cellular transcriptional and translational machinery recognizes Uridine (U) as Thymine (T); thus, processed APOBEC edited transcripts display a T at the edited site (C-U-T editing).

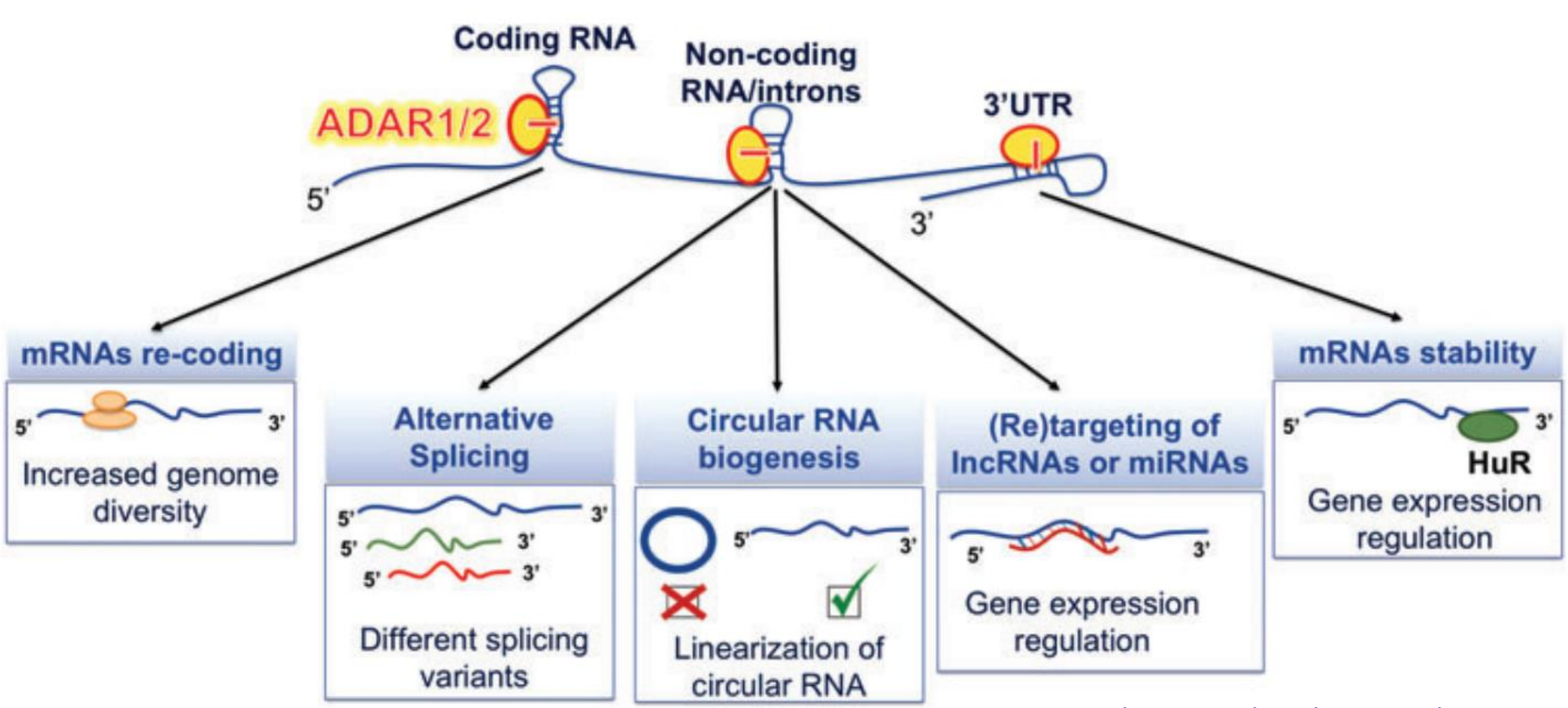
FUNCTIONAL CONSEQUENCES OF RNA EDITING AND ITS ADVANTAGES FOR ADAPTATION

- A-to-I editing could lead to synonymous (not changing the amino acid) or recoding (nonsynonymous, changing the amino acid) events.



Duan *et al*. Wiley Interdiscip Rev RNA. 2022; 13(1):e1666.

Main molecular consequences of ADAR-induced RNA editing



Gatsiou *et al.* Antioxid Redox Signal. 2018; 29(9):846-863.

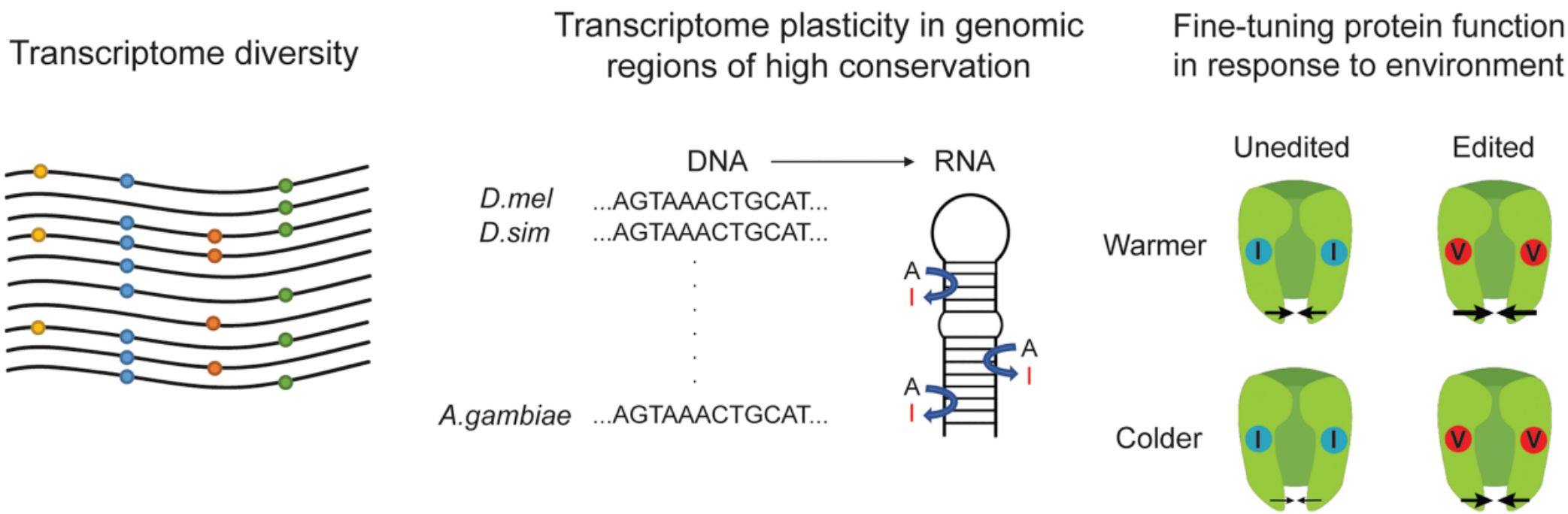
FUNCTIONAL CONSEQUENCES OF RNA EDITING AND ITS ADVANTAGES FOR ADAPTATION

- RNA editing can be advantageous by:

  ✓ Restoring the deleterious effect of a G-to-A DNA mutation.

  ✓ Rapidly responding to environmental stresses and adjusting the relative amount of different protein isoforms.

  ✓ Diversifying the transcriptomes and proteomes temporally or spatially.



Yablonovitch *et al*. PLoS Genet. 2017; 13(11):e1007064.

RELEVANCE OF RNA EDITING FOR NEURODEGENERATIVE DISORDERS

- RNA editing events have been associated with several neurodegenerative disorders.

Disorder	Species/Brain Region	Study Type/Target(s)	Methodology/ Validation Method	DE Targets/Trend Relative to Controls	Remarks	Ref.
AD	Human/HPC, temporal and frontal lobe	Focused/recoding in synaptic transcripts (72 targets, 118 sites)	Targeted NGS (mmPCR_seq)	↓ 5-HT2C receptor isoforms, *HPC:* ↓ *Cacna1d, Ddx58, Fbxl6, Fis1, Flj43663, Gria3, Gria4, Igfbp7, Kcna1, Meg3, Narf, Nova1, Ptpn14, Unc80* ↑ *Copa Temporal lobe:* ↓ *Ccni, Fbxl6, Flj43663, Gria2, Gria4, Grik1, Grik2, Meg3, Mfn1, Tme63b, Unc80* ↑ *Narf/Frontal lobe:* ↓ *Mfn1, Grik2, Meg3, Gria2, Unc80, Ddx58*	↓ Recoding	[53]
	Human/HPC	Transcriptome-wide	NGS	11 DE targets, ↓ *Gria2, Gria3, Gria4, Grik1, Grik2* ↑ *Blcap, Copa, Vn1r1, Znf235, Znf397, Znf582*	↓ Recoding	[54]
	Human/ACC/DLPFC/ PCC/aPFC/pSTG/ IFGo/FFG/CB/TC	Transcriptome-wide/focus on database listed A-I editing sites	NGS	↓ Editing in *SYT11, MCUR1, SOD2, ORAI2, HSDL2, PFKP,* and *GPRC5B*	DLPFC Samples: ↓ ADAR1 ↑ *ADAR3 expression in AD cases*	[55]

Karagianni *et al*. Biomolecules. 2022; 12(3):465.

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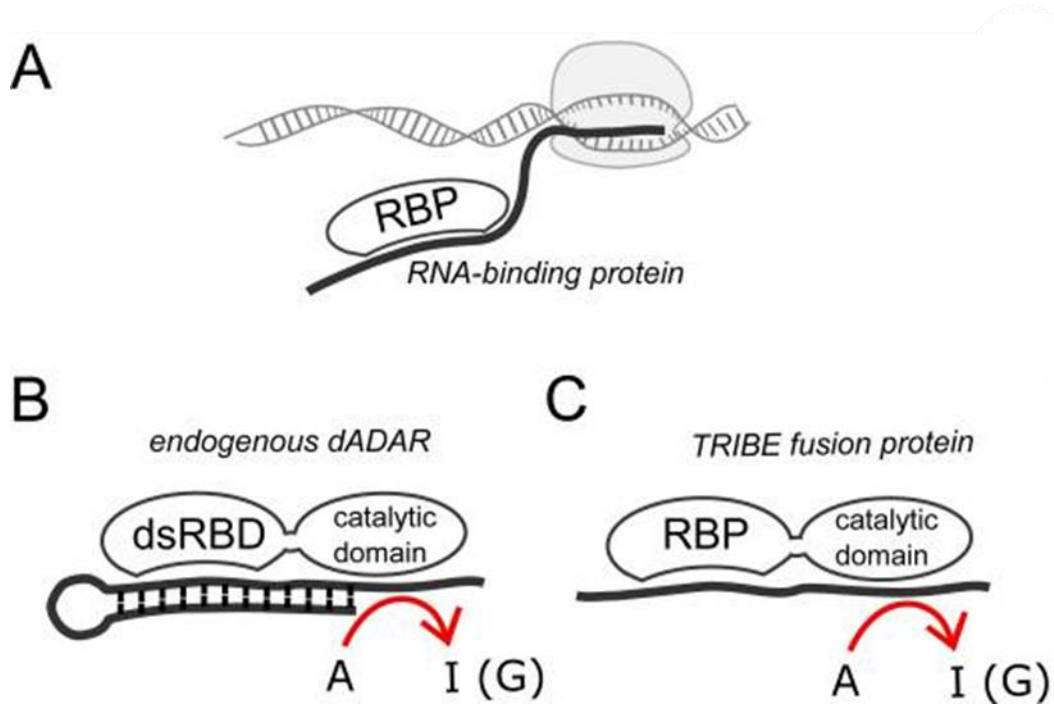
Disorder	Species/Brain Region	Study Type/Target(s)	Methodology/ Validation Method	DE Targets/Trend Relative to Controls	Remarks	
Prion diseases	sCJD and vCJD Rhesus monkeys/CB	Focused/Alu	Cloning and Sanger sequencing	↓ Alu editing	Strain specific differences	[56]
	sCJD Mouse/Cortex	Transcriptome-wide	NGS and Sanger sequencing	3 DE targets experimentally validated, *Mouse pre-clinical: ↓ Sidt2, ↑ Fkrp/Mouse clinical: ↑ Rragd*	↓ Global editing, *Human cross-validation: ↓ Paqr8, ↑ Ctss, Rrgad*	[57]
ALS (C9orf72)	Human/SC, motor cortex, FC, CB	Transcriptome-wide/whole transcriptome	NGS/ADAR1 and/or ADAR2 deficient hiPSC-MNs cells and cells with aberrant ADAR2 localization	1526 DE transcripts	No changes in global editing, region-specific hypo- and hyper-edited patterns	
HD	Human/striatum	Focused/Gria2	RFLPs	Gria2: ↓ Q/R site		
	Human/PFC	Focused/Gria2	RFLPs	Gria2: ↓ Q/R site		
	Human/HPC	Focused/Gria2	Sanger sequencing/ primer extension	Gria2: ↓ Q/R site		

Karagianni *et al*. Biomolecules. 2022; 12(3):465.

MEDICAL APPLICATIONS OF RNA EDITING

- The A-I editing activity of ADAR can be used for identifying targets of RNA-binding proteins (TRIBE and HYPER-TRIBE).
- TRIBE (targets of RNA-binding proteins identified by editing) expresses a fusion protein consisting of a queried RBP and the catalytic domain of the RNA-editing enzyme ADAR (ADARcd), which marks target RNA transcripts by converting adenosine to inosine near the RBP binding sites. These marks can be subsequently identified via high-throughput sequencing.



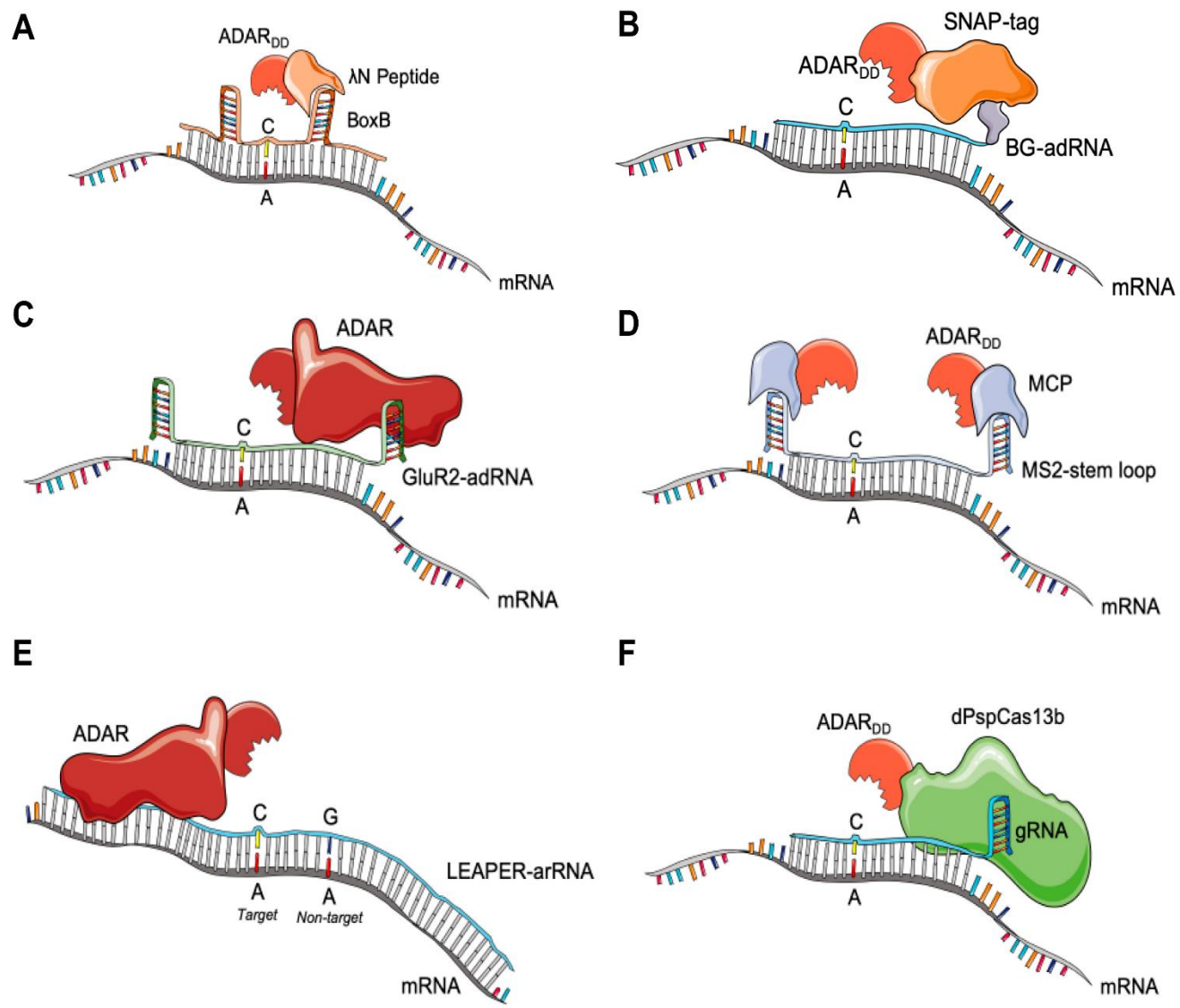
- HyperTRIBE incorporates a previously characterized hyperactive mutation, E488Q, into the ADARcd, then increasing the editing efficiency and reducing sequence bias.

McMahon *et al*. Cell. 2016; 165(3):742-53.
Rahman *et al*. Nat Protoc. 2018; 13(8):1829-1849.

MEDICAL APPLICATIONS OF RNA EDITING

- The A-I editing activity of ADARs can be used for site-directed RNA editing, for therapeutic purposes.
- Several RNA editing approaches have been developed:

(A) BoxB-λN-ADAR with a dual BoxB design to recruit the λN peptide.
(B) SNAP-tag-ADAR fusion with a O6-benzyl-guanine (BG) conjugated adRNA.
(C) Glu-adRNA approach where the GluR2 R/G hairpin recruits exogenous or endogenous full length ADAR.
(D) MS2-MCP-ADAR stem-loop approach with dual MS2 stem-loop hairpins recognized by the MS2 bacteriophage coat binding protein (MCP).
(E) An example of endogenous ADAR recruitment using long LEAPER-arRNAs and G-A mismatches within the guide region to reduce off-target editing.
(F) The REPAIR system with dPspCas13b-ADAR fusions recruited by a guide RNA with a direct repeat.



Fry *et al*. Int J Mol Sci. 2020; 21(3):777.

CONCLUDING REMARKS

- RNA editing is an epigenetic mechanism that contributes to diversifying the transcriptomes and proteomes, and that have been linked to physiological processes and disease.

- RNA editing is very promising as a tool for identifying disease biomarkers and as a therapeutic strategy for genetic disorders.

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