RNA Editing

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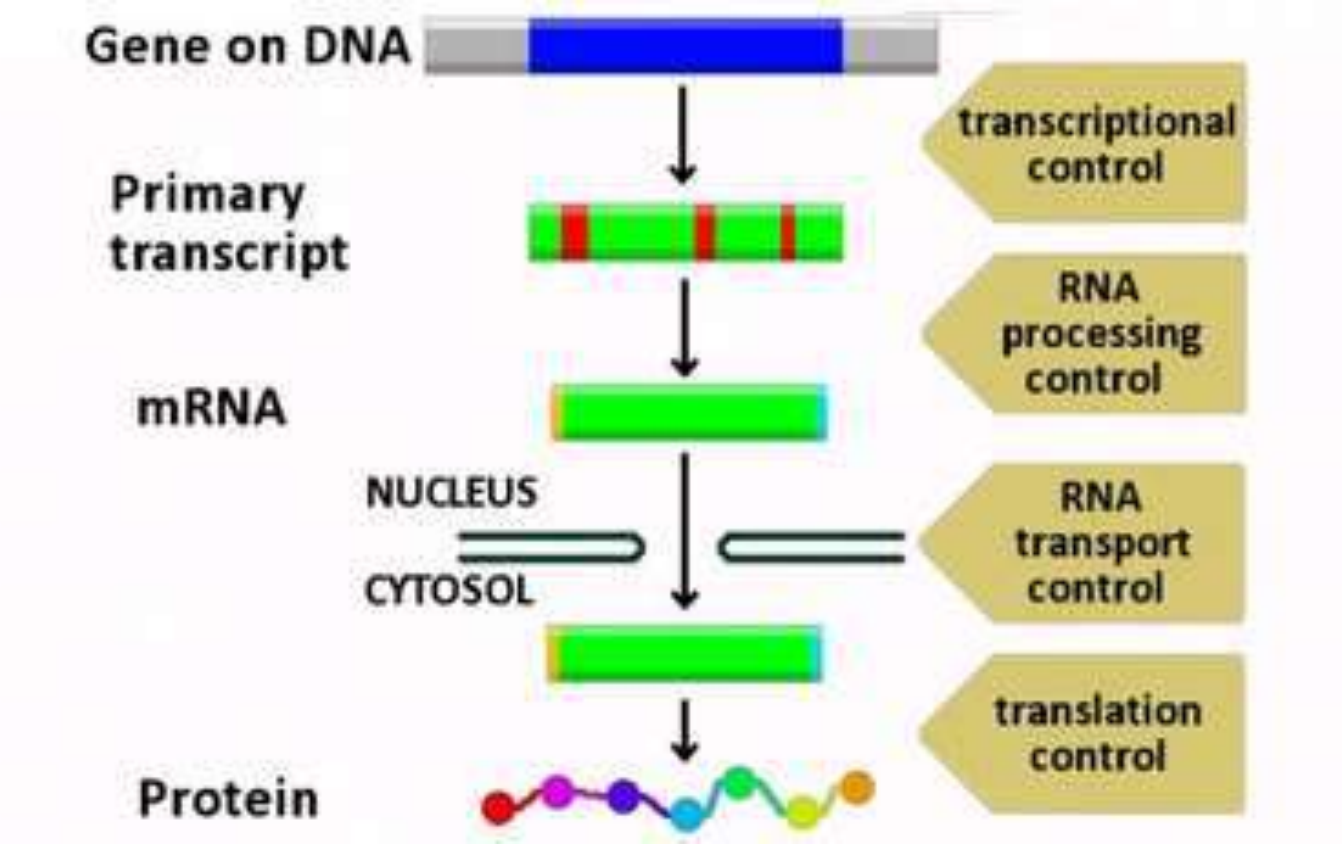
Introduction

- RNA editing is a process of RNA modification in which discrete changes are made in the specific nucleotide sequences within an RNA molecule after transcription without splicing.
- In a simple way, it can be defined as any type of changes in RNA transcript sequence without RNA splicing called RNA editing.
- It was previously thought as rare phenomenon, but it is widespread
- It was firstly discovered in mitochondria of *Trypanosomes*.
- It takes place in cell nucleus and cytosol as well as mitochondria and plastids.
- RNA editing enables a cell to obtain large diversity in proteins by using small number of genes.
- It is mainly achieved by insertion, modification or deletion of one or more bases.
- It happens after transcription, also happens in Poly(A) tail.

RNA Editing

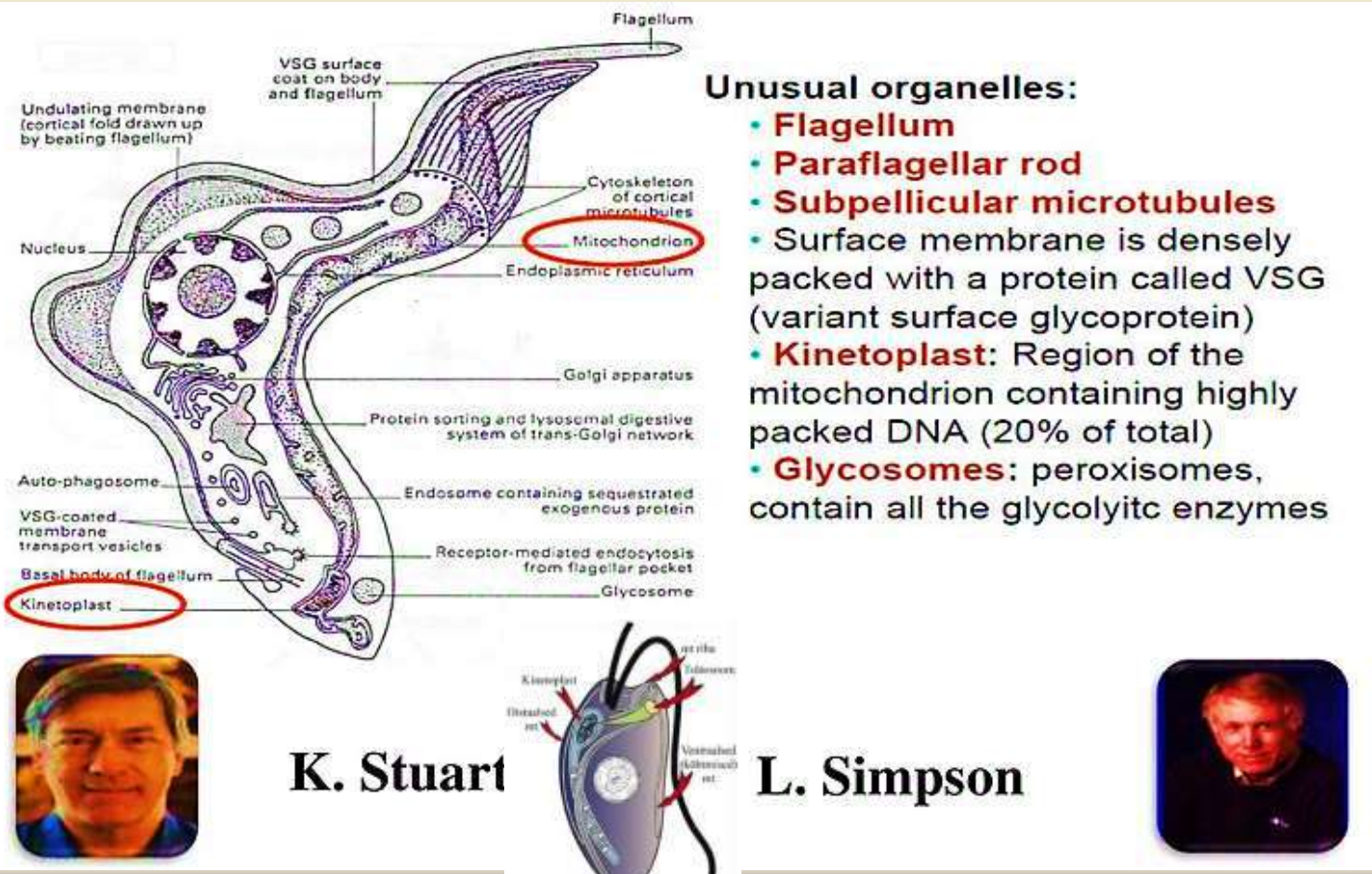


It is reflective of mechanism of translational control in biological system

The mechanism of editing: 3´ to 5´ end; Clue: Partial edit only at but not 5´ end

RNA Editing

- Discovered in *Trypanosome* mitochondria; take place abundantly in kinteoplast.
- Discovered by K. Stuart and L. Simpson.



RNA Editing

Types of RNA editing

RNA editing is mainly of two types:

- ***Base modification***
  - Site specific deamination of cytosine and adenine – A to I, C to U, U to C, etc.
  - Enzyme deaminase is involved.

- ***Insertion and /or deletion***
  - ***Guide RNA mediated*** site specific insertion and deletion of uridine base (U insertion/deletion and so on), seen in kinteoplastid protozoa
  - Mono/di nucleotide insertion, seen in *Physarum*
  - Nucleotide replacement, seen in *Acanthamoeba* tRNAs

RNA Editing

Types of RNA editing

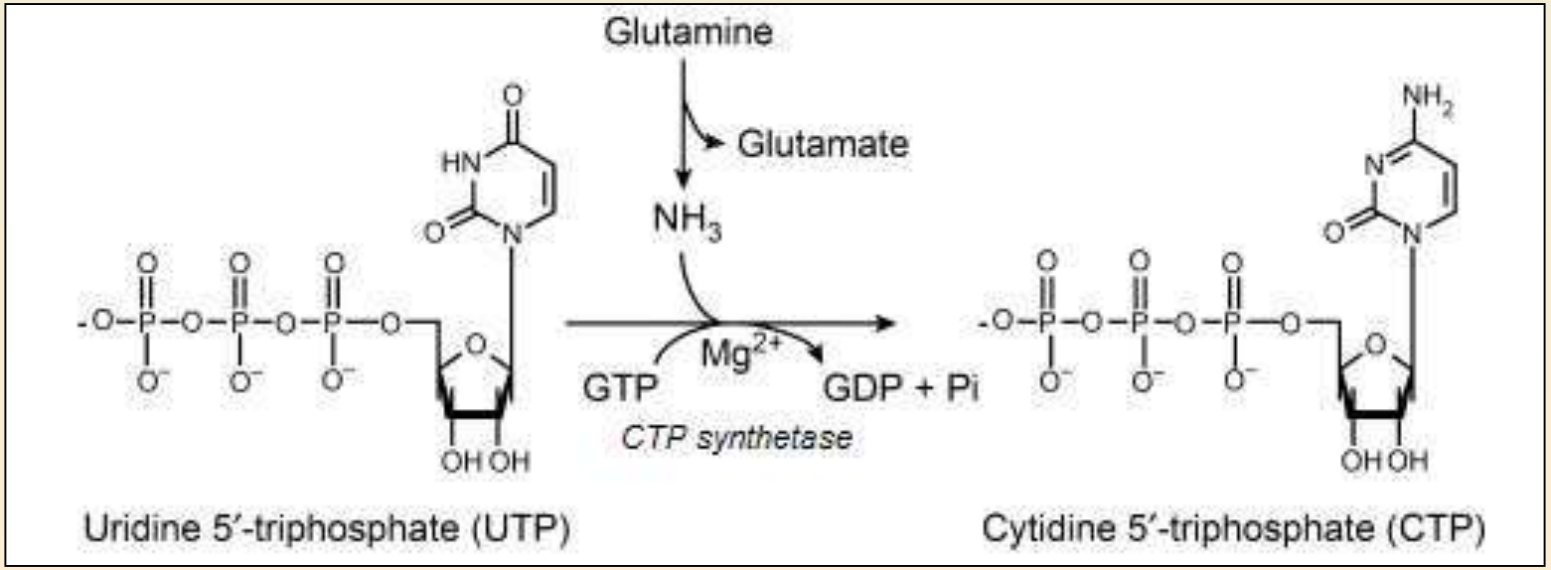
Types	Genomic factors	Resources
A-to-I editing	RNA-specific adenosine deaminases ADARs	Glutamate receptor, hepatitis delta virus, etc.
C-to-U editing U-to-C	Pentatricopeptide repeat (PPR) protein containing the DYW motif	ApoB mRNA(Gln-Stop)#; NF1(Arg-Stop) WT1 mRNA(Leu-Pro)
tRNA editing	HsmtPheRS and HsctPheRS*	Alzheimer's, atherosclerosis, and cataractogenesis,
Sno RNA-mediated nucleotide modification of rRNAs	Serotonin 2C receptor (5HTr2c)	The Prader-Willi syndrome^
G-insertion editing	Total unedited RNA	Paramyxoviruses

RNA Editing

Base modification:

Deamination of cytosine and adenine.

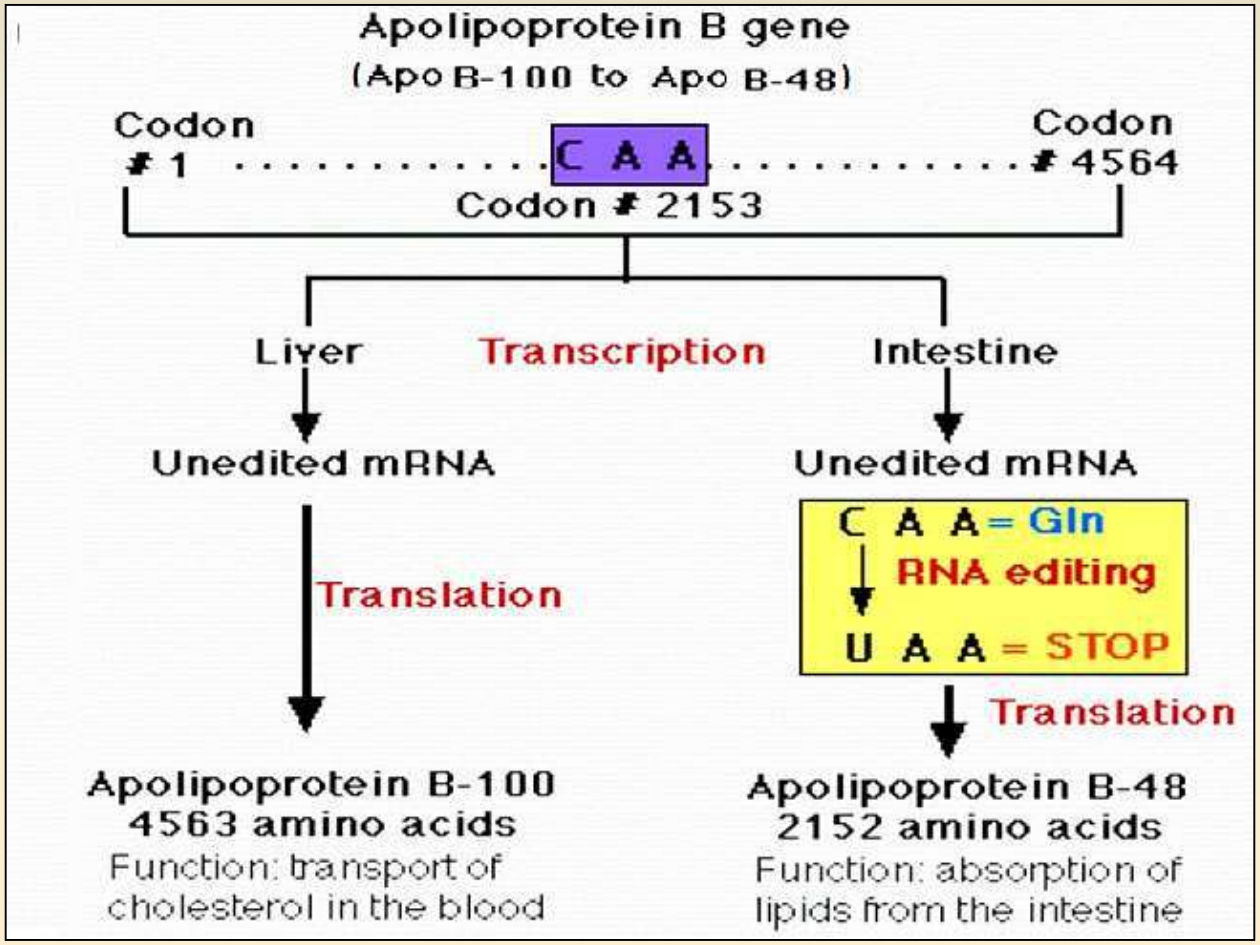
- Deamination of cytidine is tissue or site specific, for example, deamination of cytosine in mRNA of ApoB100 (apolipoprotein B 100) genes to produce ApoB48.



Monika LöfflerElke Zameitat, in Encyclopedia of Biological Chemistry, 2004

RNA Editing

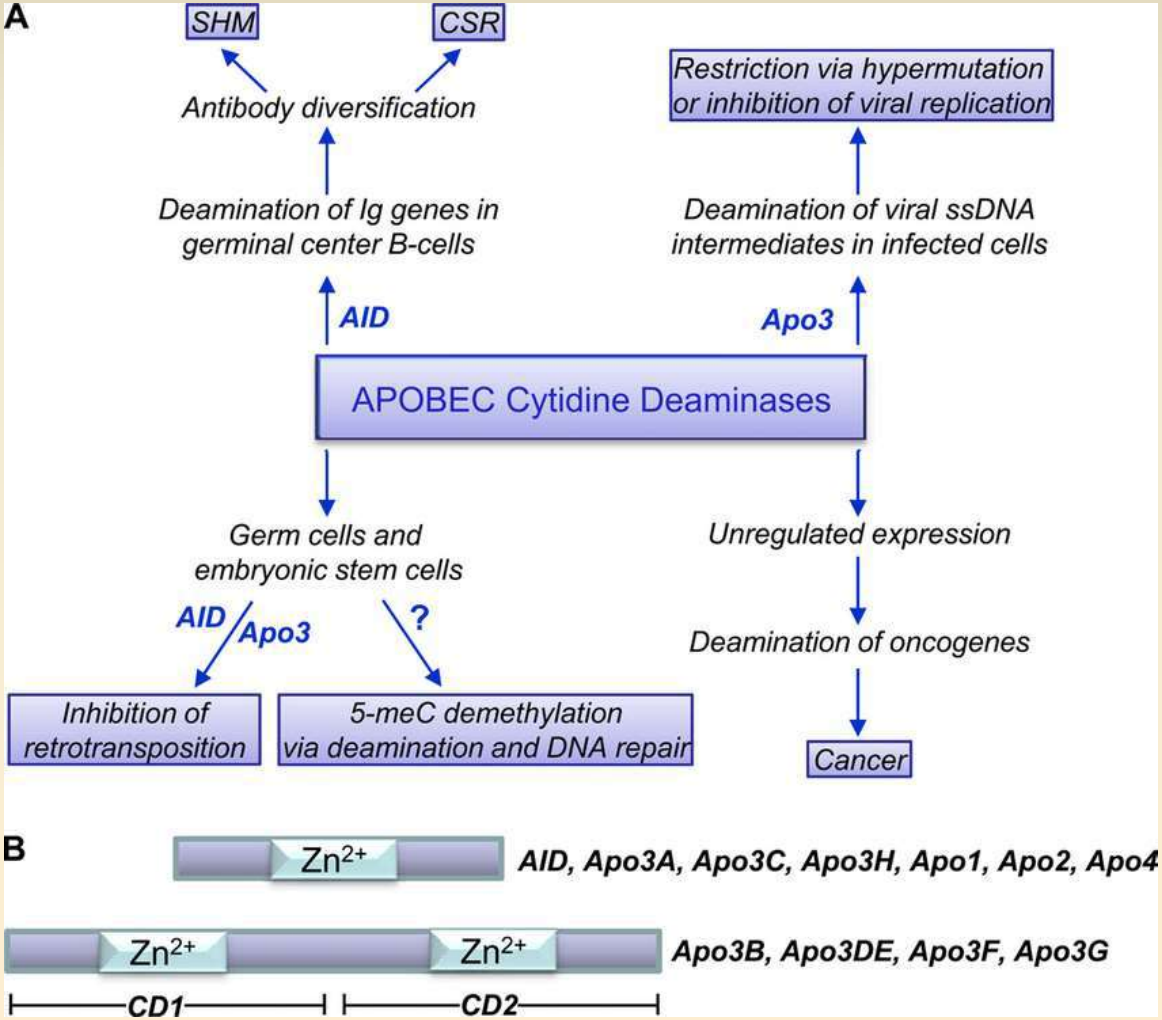
Base modification:



RNA Editing

Base modification:

- A cytidine deaminase activity is involved in Apobec protein.
- Another protein ACF (apobec complimentation factor) is also required.
- Apobec + ACF complex called as editisome.
- Both recognize the sequences flanking the C to edited.

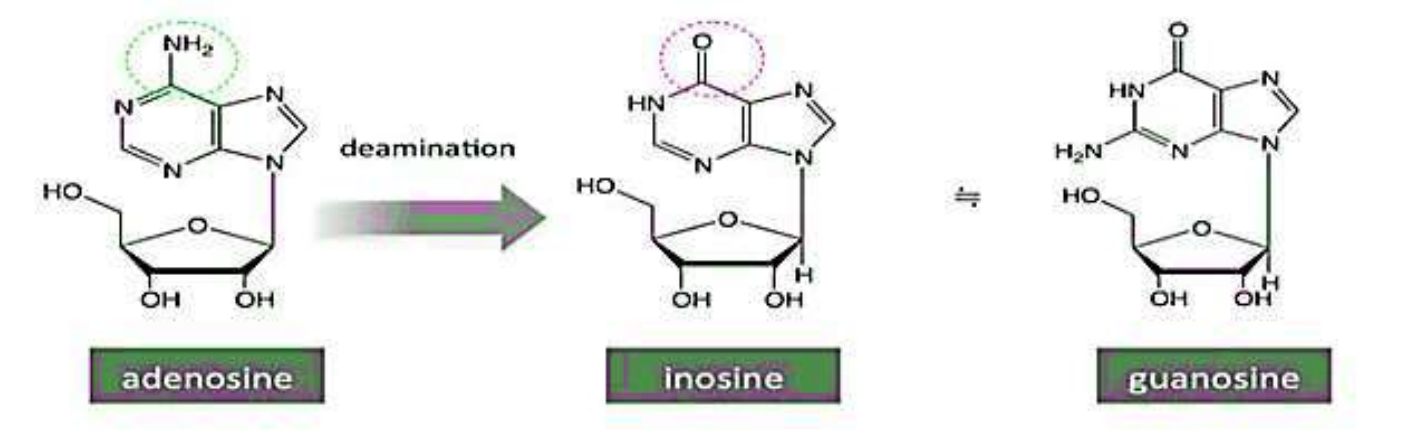


RNA Editing

Base modification:

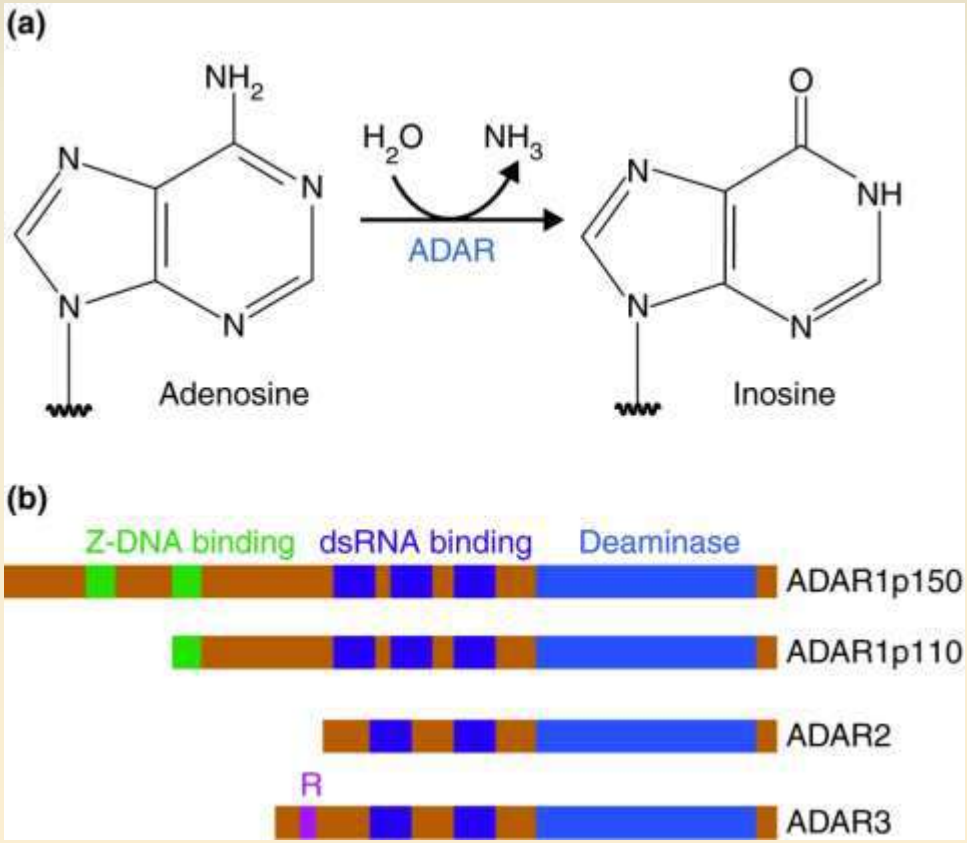
Deamination of adenine and inosine

- Deamination of adenine and inosine (A to I modification) usually occurs in secondary strructure regions of RNA.
- Set of protein required for deamination of adenosine called ADAR or dsRNA dependent
- This inosine can be base paired with wither A, T, G or C.
- Inosine is recognized as guanine during translation.
- Example includes different types of Glut receptors for insulin.



RNA Editing

Base modification: Deamination of adenine and inosine



Slotkin & Nishikura Genome Medicine 2013; 5, 105

- ADAR enzymes catalyze the A-to-I hydrolytic deamination reaction, by which an adenosine loses an amine group.
- There are four main proteins of the ADAR enzyme family: two isoforms of ADAR1 (p110 and p150), ADAR2 and ADAR3.
- All of these enzymes contain a conserved deaminase domain, shown in blue. The double-stranded (ds)RNA-binding domains, shown in purple, determine substrate specificity.
- The two ADAR1 isoforms differ in their Z-DNA-binding domains, shown in green.
- ADAR3 contains an arginine-rich domain, shown in pink, which binds single-stranded RNA.

RNA Editing

Base modification: Deamination of adenine and inosine

Examples of A-to-I editing

Gene
Glutamate receptor GluR-2 (NM_000826)
Potassium channel Kv1.1 (NM_000217)
Serotonin receptor 5HT2C (NM_000868)
Pri-miRNA-99b
Pri-miRNA 133a2
Lin28
Nuclear prelamin A recognition factor (NARF)

RNA Editing

Insertion or deletion

- RNA editing through the addition and deletion of uracil has been found in kinetoplasts from the mitochondria of *Trypanosoma brucei*.
- It is sometimes seen in a large fraction of the sites in a gene, therefore it is also called as 'pan editing' to distinguish it from typical editing of one or a few sites.
- It starts with the base-pairing of the unedited primary transcript with a guide RNA (gRNA), which contains complementary sequences to the regions around the insertion/deletion points.
- The newly formed double-stranded region is then enveloped by an editosome, a large multi-protein complex that catalyzes the editing.
- The *editosome opens the transcript* at the first mismatched nucleotide and starts inserting uridine.
- The *inserted uridine base-pairs with the guide RNA,* and insertion continues as long as A or G is present in the guide RNA and stops when a C or U is encountered.
- The inserted nucleotide then causes a frameshift and result in a translated protein that differs from its gene.
- The mechanism involves *an endonucleolytic cut at the mismatch point* between the guide RNA and the unedited transcript by editosome.

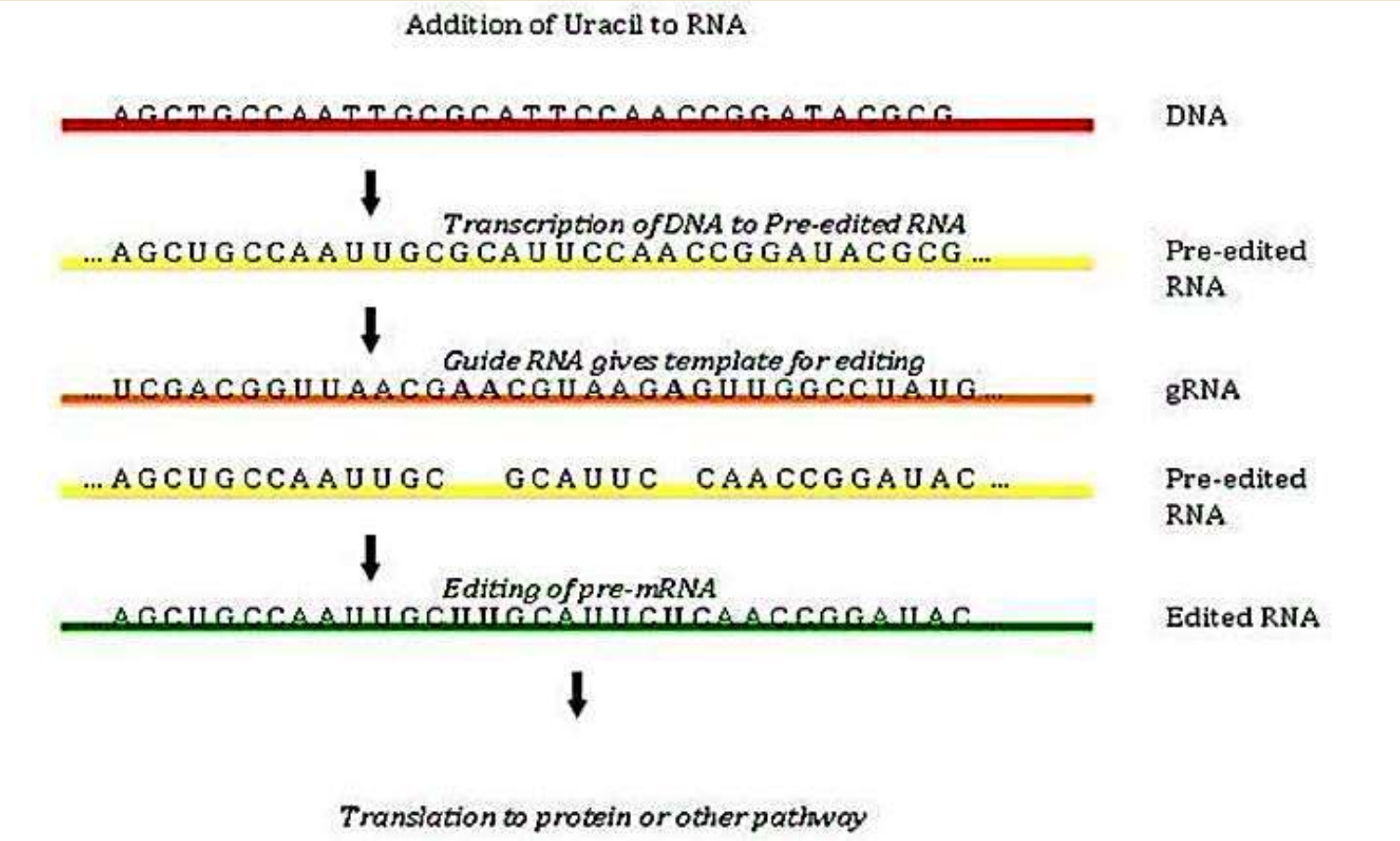
RNA Editing

Insertion or deletion

- Then Us (uridine) are added from UTP at the 3´ end of the mRNA by one of the enzyme of the complex called terminal U-transferase.
- Unpaired Us are removed ny another set of enzymes known as U-specific exoribonuclease.
- Editing leads to the formation of mRNA complementary to gRNA, which then rejoined at the ends of the edited mRNA transcripts by RNA ligase.
- As a consequence, the editosome can edit ***only in a 3´ to 5´ direction*** along with the primary RNA transcripts.
- The complex can act on only a single guide RNA at a time. Therefore, a RNA transcript requiring extensive editing needs more than one guide RNA and editosome complex.

RNA Editing

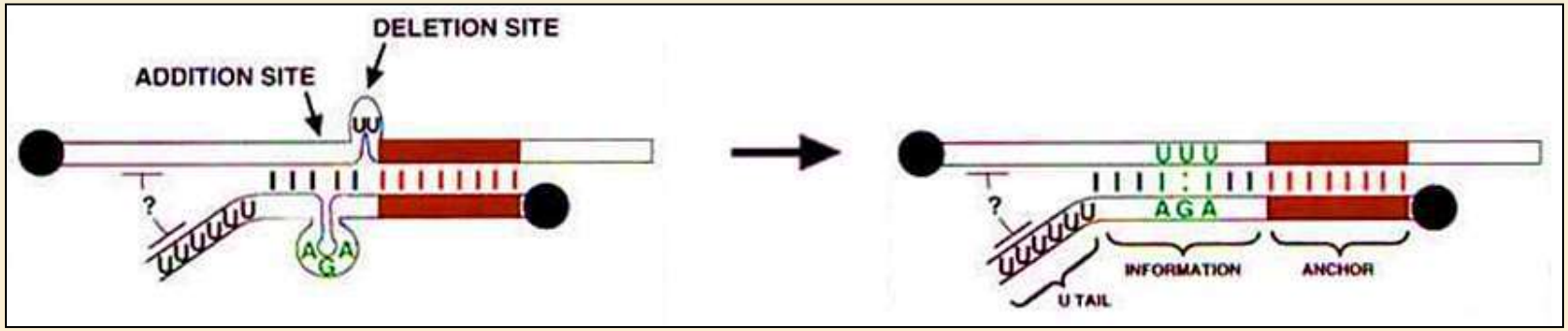
Insertion or deletion



RNA Editing

Guide RNA

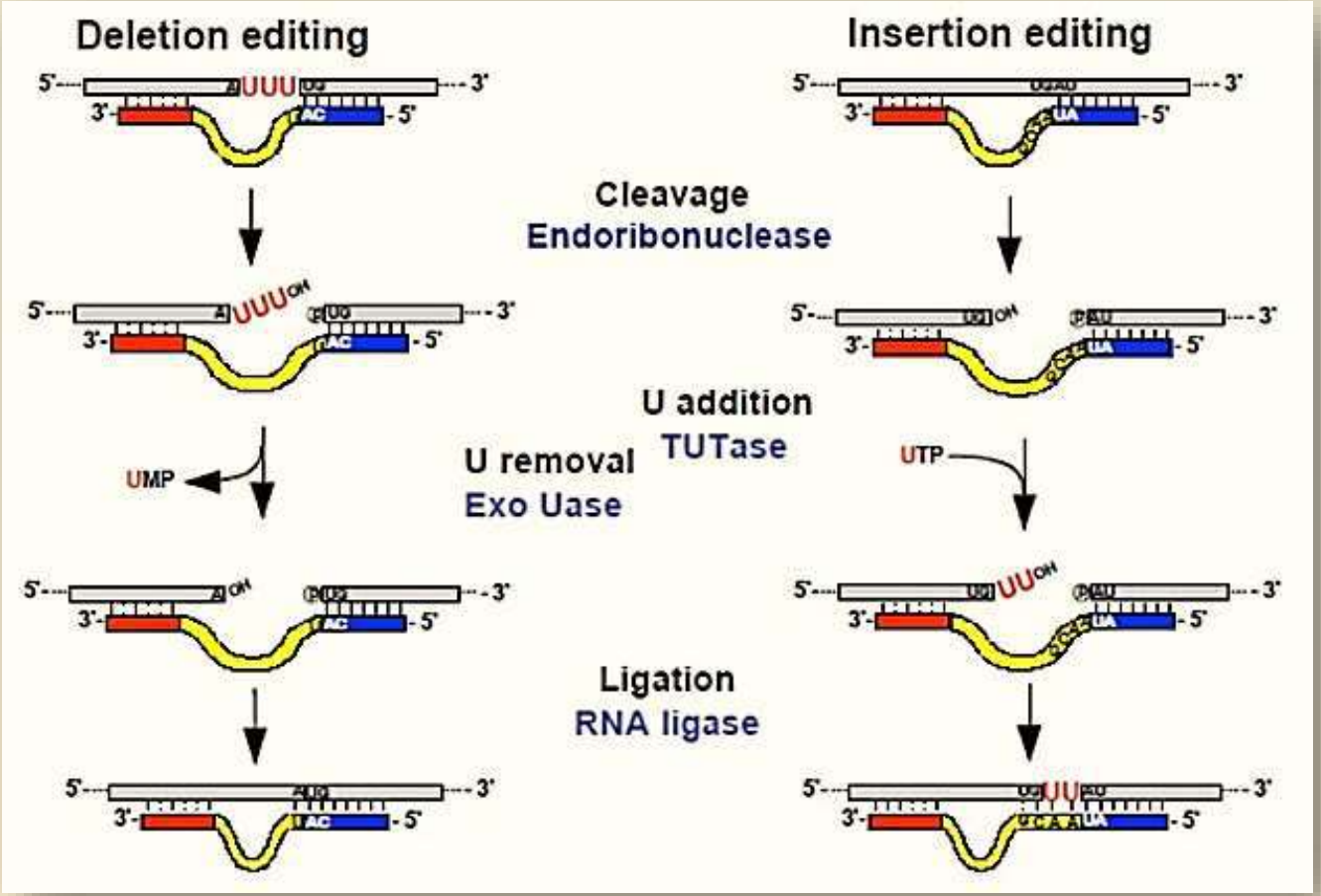
- **Guide RNAs** (gRNAs) direct editing
  - gRNAs are small (40-70 nt) and complementary to portions of the mRNA
  - Structural elements: anchor, informational part and Oligo(U)tail
  - Base-pairing of gRNA with unedited RNA gives mismatched regions, which are recognized by the editing machinery
  - Machinery includes an **Endonuclease**, a **Terminal Uridylyl Transferase (TUTase),**and a **RNA ligase**
- Editing is **directional**, from 3' to 5'



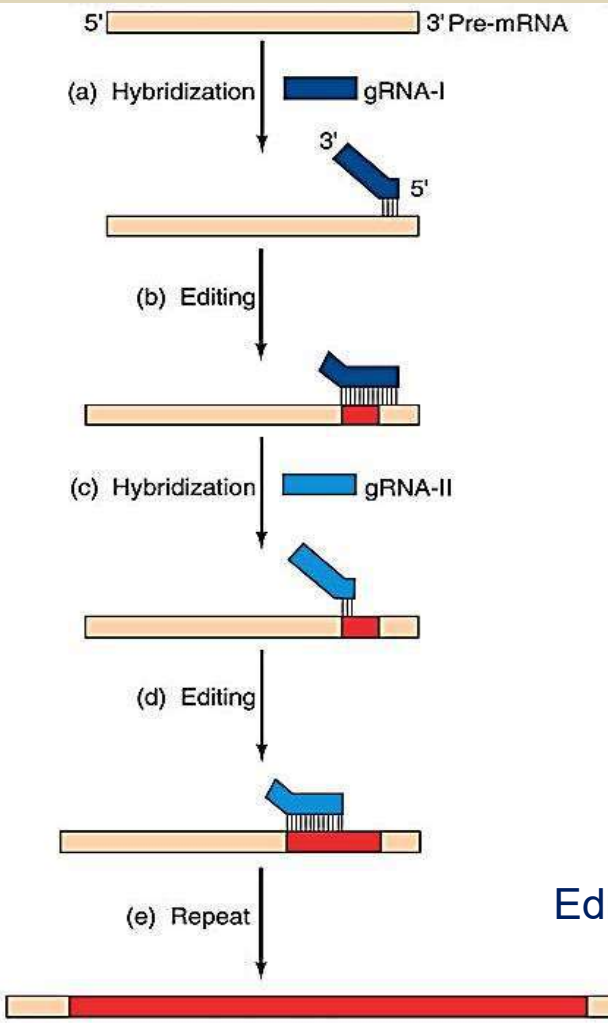
Source: Abhishek DAs

RNA Editing

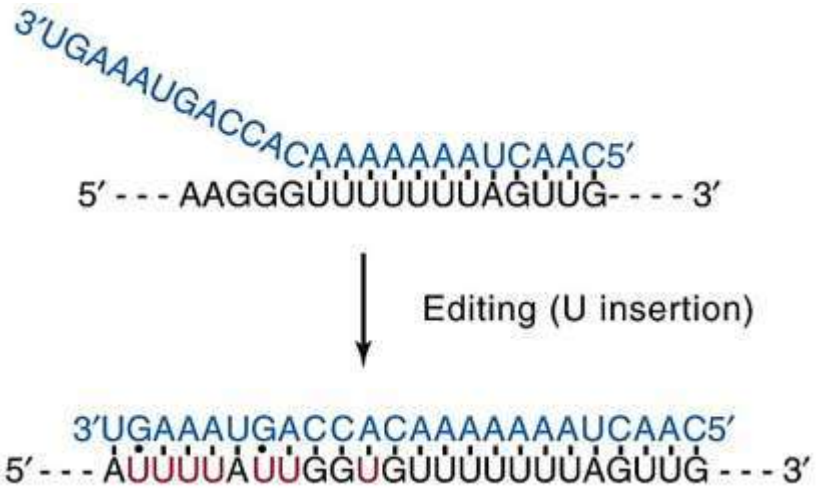
RNA editing mechanism



RNA Editing



Guide RNAs Direct Editing in Trypanosomes.



Editing is from 3’ to 5’ along an unedited RNA.

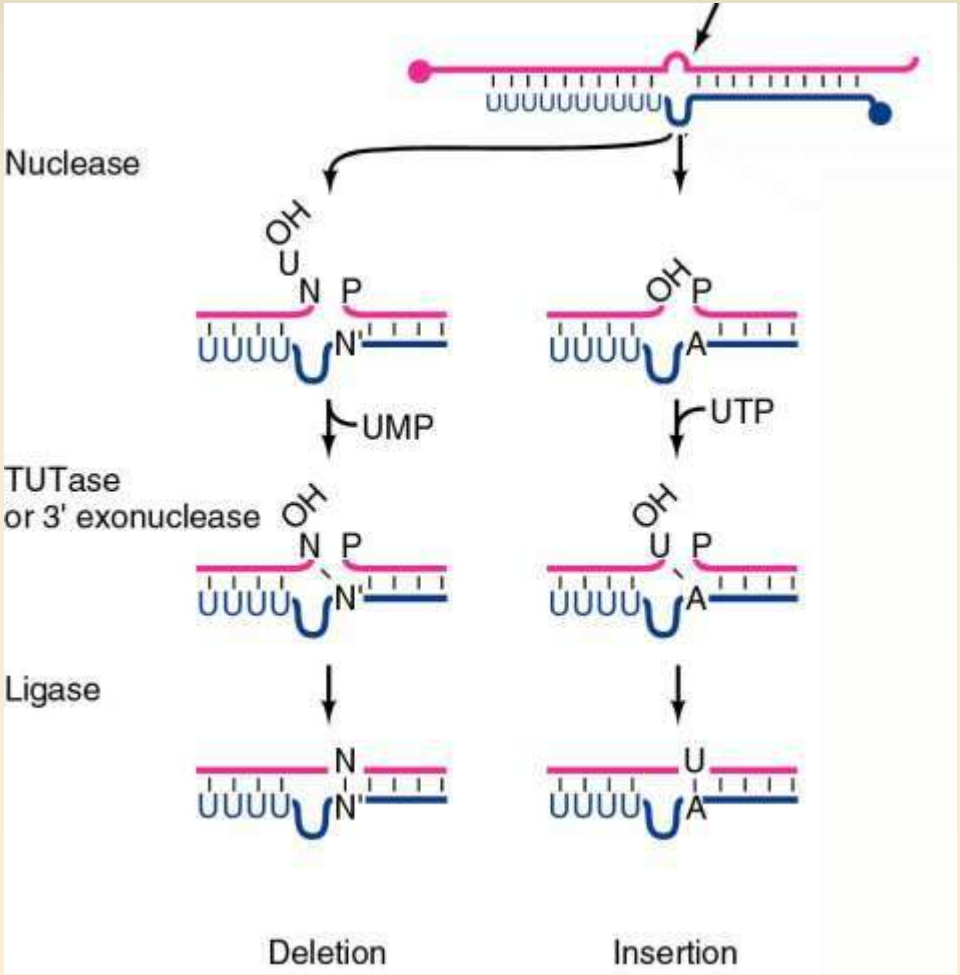
RNA Editing

RNA editing mechanism

- Editing is catalyzed by a multiprotein complexes that have not been fully defined yet.
- It contains following four key enzymes as characterized from glycreol gradients of 20S editosome.
  - ***Endonuclease:*** cleavage in vitro occurs at an unpaired nucleotide immediately upstream of the gRNA-mRNA anchor duplex.
  - ***Exonuclease:*** It removes non-base paired U nucleotides after cleavage of deletion editing sites.
  - ***Terminal uridyl transferase (TUTase):*** It adds Us to the 3´ end of the 5´ pre-mRNA fragment as specified by the gRNA.
  - ***RNA II ligase:*** The natural editing ligase substrates are nicked dsRNAs that are completely base-paired after the correct addition or removal of U nucleotides.
  - ***Helicase:*** It provides ATP-dependent unwinding activity in RNA editing, thus it is involved in gRNA displacement directly from gRNA/edited mRNA duplex or indirectly, to allow 5´ gRNA to form an anchor duplex with the edited mRNA to initiate another block of editing. Examples include REH1 (RNA editing helicase 1) in Trypanosoma.
  - ***Other 20S editosome proteins***

RNA Editing

RNA editing mechanism



Involvement of Enzymes in RNA Editing

TUTase, or terminal uridylyl transferase, adds U(s) to the 3' end created by cleavage of the pre-mRNA

RNA Editing

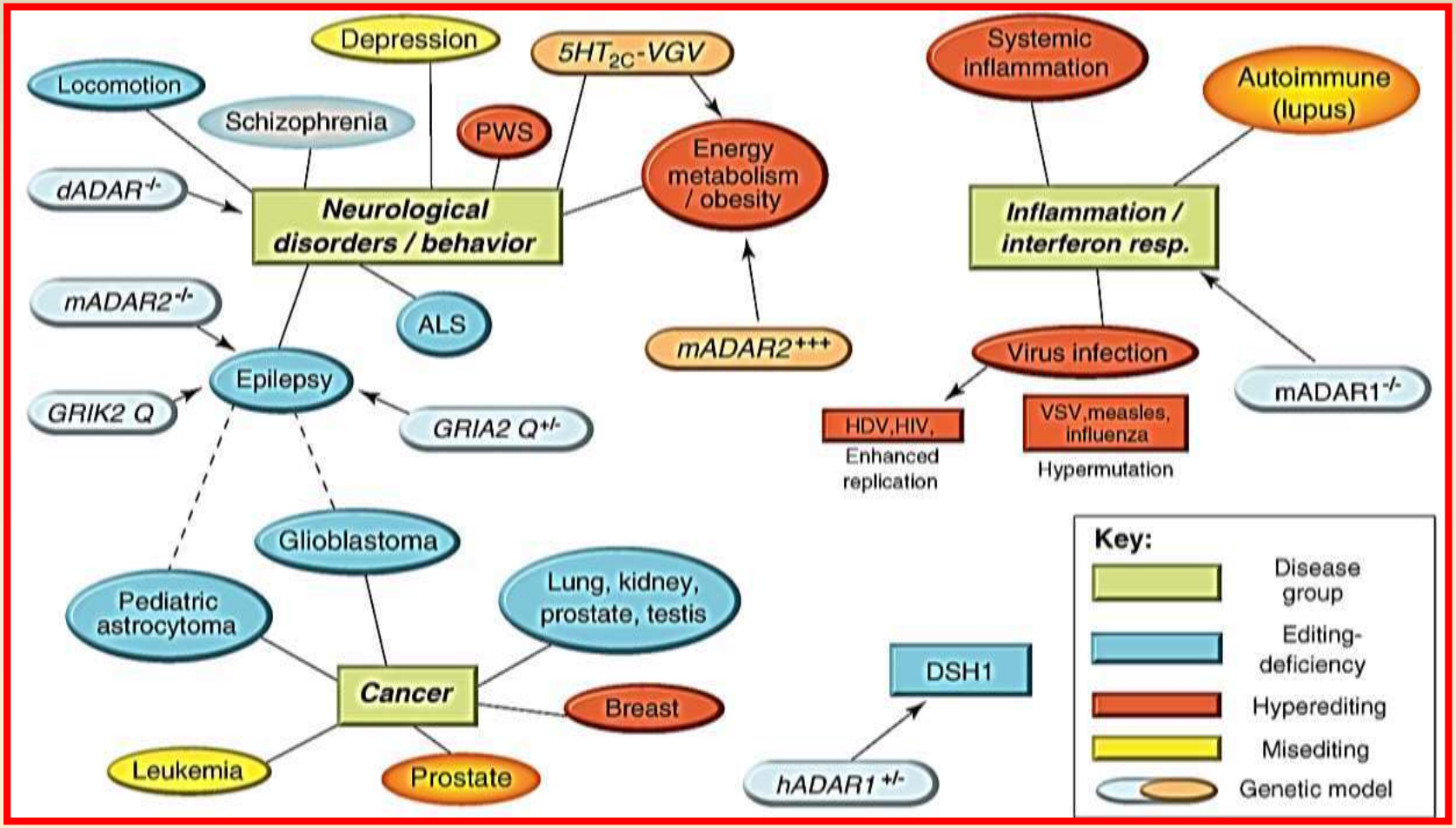
RNA editing mechanism

TABLE 1. Proteins identified in editosomes by mass spectrometry

Proteins	Function	Tag-N1	Tag-B2	Tag-N2	A2-IP	SP-Q-S6
KREPA1 (MP81)	Interaction	√	√	√	√	√
KREPA2 (MP63)	Interaction	√	√	√	√	√
KREPA3 (MP42)	Interaction	√	√	√	√	√
KREPA4 (MP24)	Interaction	√	√	√	√	√
KREPA5 (MP19)	Interaction[a]	√	N.D.	√	√	√
KREPA6 (MP18)	Interaction[a]	√	√	√	√	√
KREN1 (MP90)	Endonuclease	√	N.D.	N.D.	√	√
KREPB2 (MP67)	Endonuclease[a]	N.D.	√	N.D.	√	√
KREN2 (MP61)	Endonuclease	N.D.	N.D.	√	√	√
KREPB4 (MP46)	Interaction[a]	√	√	√	√	√
KREPB5 (MP44)	Interaction	√	√	√	√	√
KREPB6 (MP49)	Interaction[a]	N.D.	N.D.	N.D.	N.D.	N.D.
KREPB7 (MP47)	Interaction[a]	N.D.	N.D.	√	N.D.	√
KREPB8 (MP41)	Interaction[a]	√	N.D.	N.D.	N.D.	√
KREX1 (MP100)	Exonuclease	√	N.D.	N.D.	√	√
KREX2 (MP99)	Exonuclease	√	√	√	√	√
KREL1 (REL1)	RNA ligase	√	√	√	√	√
KREL2 (REL2)	RNA ligase	√	√	√	√	√
KRET2 (RET2)	TUTase	√	√	√	√	√
KREH1 (mHel61p)	Helicase	N.D.	N.D.	N.D.	√	√

TAP-tagged KREN1 (Tag-N1), KREPB2 (Tag-B2), and KREN2 (Tag-N2) complexes were isolated by sequential IgG and Calmodulin affinity chromatography. The other complexes were purified by immunoaffinity chromatography using a monoclonal antibody specific for KREPA2 (A2-IP) or by biochemical methods using sequential SP Sepharose, Q Sepharose, and Superose 6 column chromatography (SP–Q–S6) (Panigrahi et al. 2003). √ indicates protein identified; N.D. indicates not detected by mass spectrometry.
[a]Putative function.

Defects in RNA Editing



Disruption of the RNA editing balance

Tools of RNA Editing – CRISPR

Clustered

Regularly

Interspersed

Short

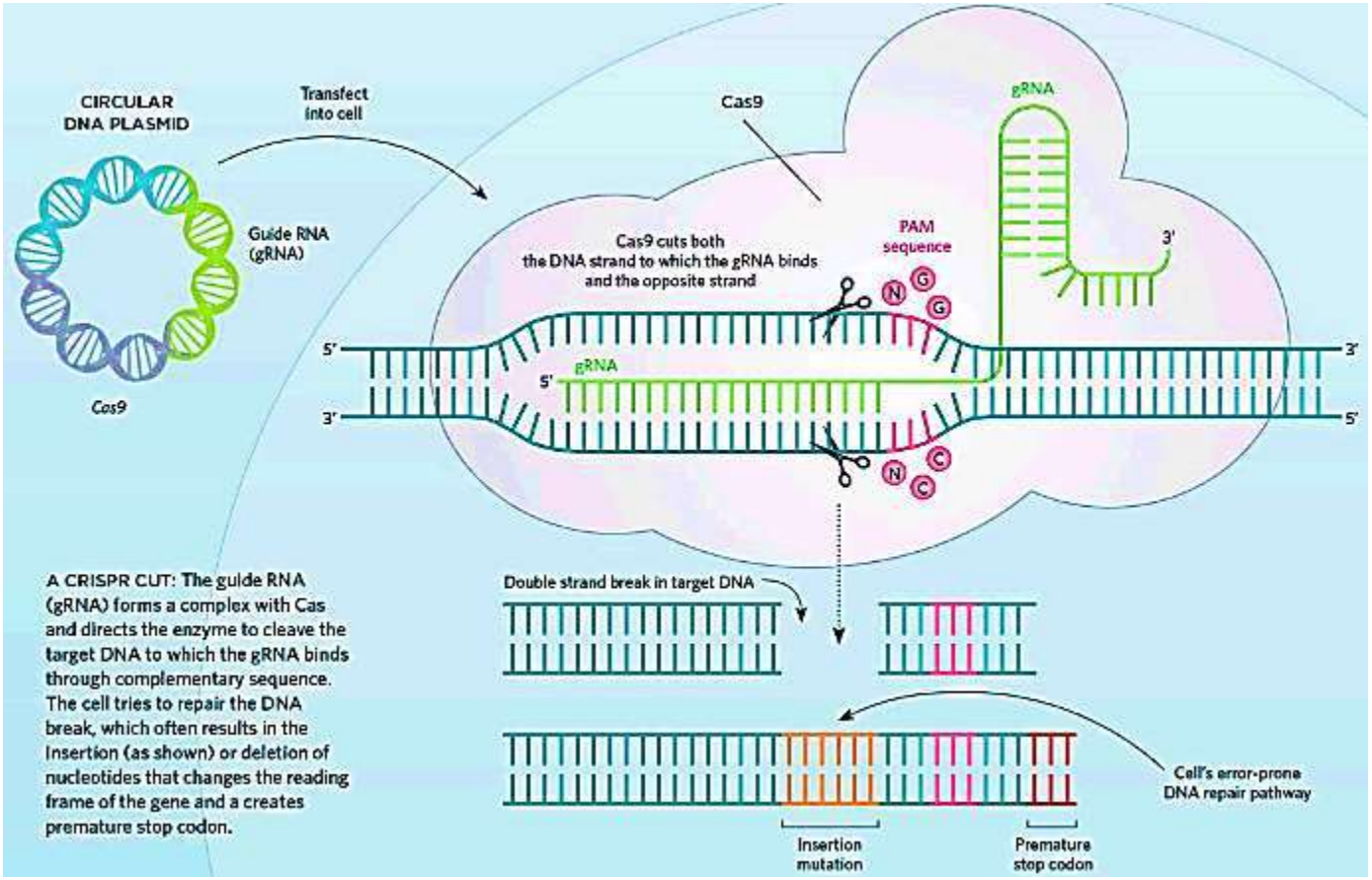
Palindromic

Repeats

- CRISPR is a powerful tool for editing genomes – it allows researchers to easily alter DNA sequences and modify gene function.
- It has many potential applications, including correcting genetic defects, treating and preventing the spread of diseases, and improving the growth and resilience of growth. However, it has some ethical concern.
- It utilizes CRISPR-associated 9 (Cas9), which is an enzyme that acts like a pair of molecular scissors, capable of cutting strands of DNA. Thus it is a RNA guided gene editing mechanism called as CRISPR-Cas9.
- It was adapted from the natural defense mechanism of bacteria and archaea. These organisms use CRISPR-derived RNA, a molecular cousin to DNA and various Cas proteins to foil attacks by viruses.

Tools of RNA Editing – CRISPR

C R I S P R

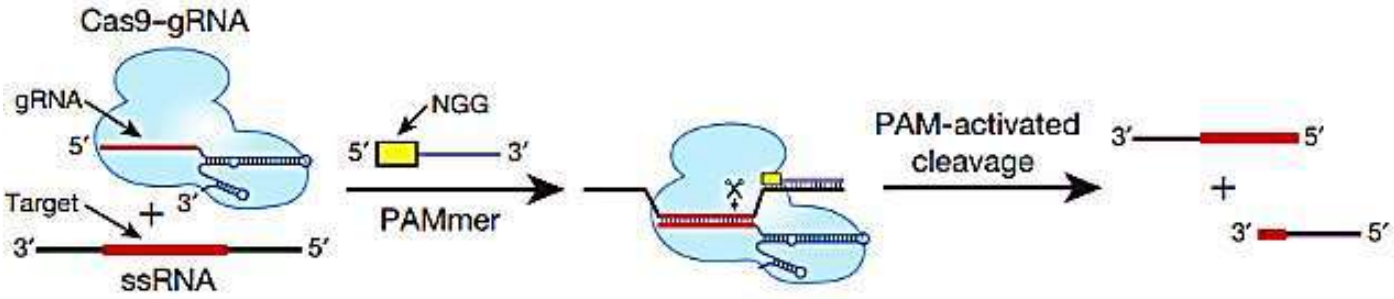


CRISPR/Cas9 process includes a targeted double strand break

https://www.livescience.com/58790-crispr-explained.html

Tools of RNA Editing – CRISPR

C R I S P R



RNA-guided Cas9 cleaves single stranded RNA (ssRNA) targets in the presence of a short PAMpresenting DNA oligonucleotide (PAMmer)

Requires only synthesis of a matching gRNA and complementary PAMmer and does not require affinity tags

Figure and text: Connell & Doudna et al: Programmable RNA recognition and cleavage by CRISPR/Cas9; Nature (2014) 516, 263–266
http://www.nature.com/nature/journal/v516/n7530/full/nature13769.html

https://www.livescience.com/58790-crispr-explained.html

Significance of RNA Editing

- It is essential in regulating gene expression of organisms.
- RNA editing mutant was reported with strong defects in organelle development.
- Defects in RNA editing cause diseases.
- It is a mechanism to increase the number of different proteins available without the need to increase the number of genes in the genome.
- It may help protect the genome against some viruses.

RNA Editing

RNA editing may be involved in RNA degradation

A study looked at the involvement of RNA editing in RNA degradation. The researchers specifically looked at the interaction between ADAR and UPF1, an enzyme involved in the nonsense-mediated mRNA decay pathway (NMD). They found that ADAR and UPF1 are found within the suprasliceosome and they form a complex that leads to the down-regulation of specific genes. The exact mechanism or the exact pathways that these two are involved in are unknown at this time. The only fact that this research has shown is that they form a complex and down-regulate specific genes.

Further reading

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