RNA Editing

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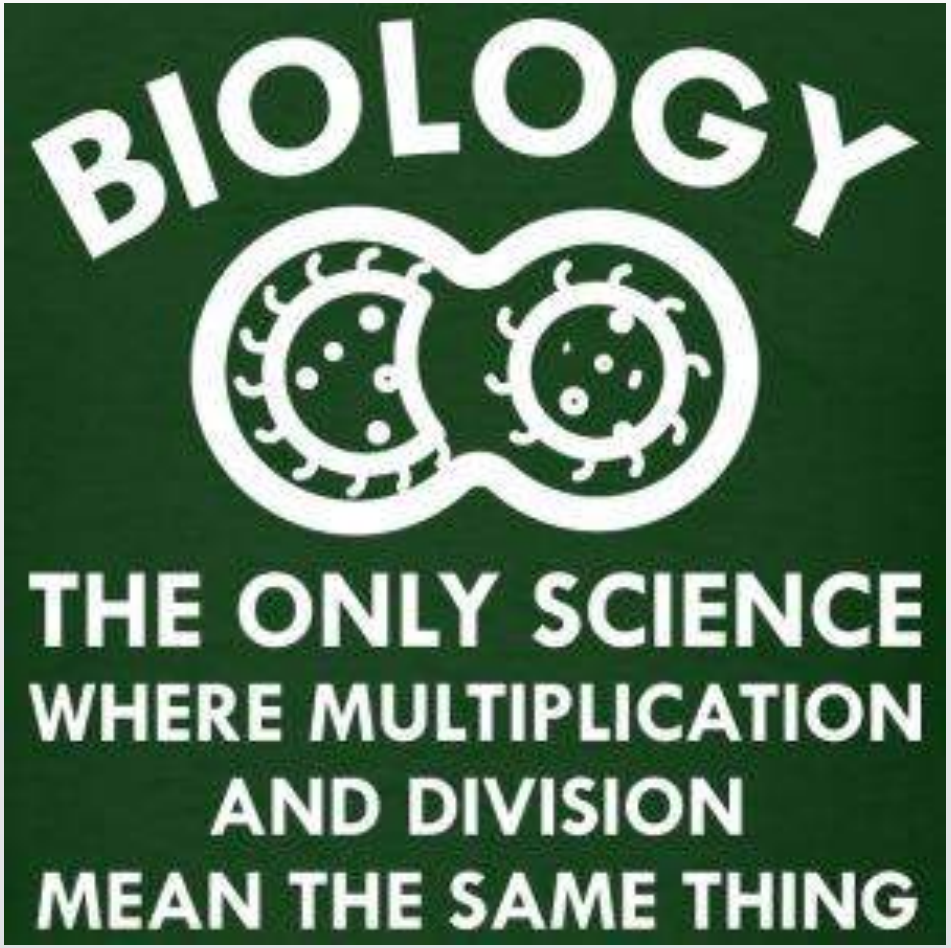
PG Semester –I

Course – Cytology and Genetics


BIOLOGY
THE ONLY SCIENCE
WHERE MULTIPLICATION
AND DIVISION
MEAN THE SAME THING

RNA editing –

the post-transcriptional modification of the base sequence of mRNA.

Thus, the protein produced upon translation is different from that predicted from the gene sequence,

but this example is perhaps
even more dramatic than the case of splicing—

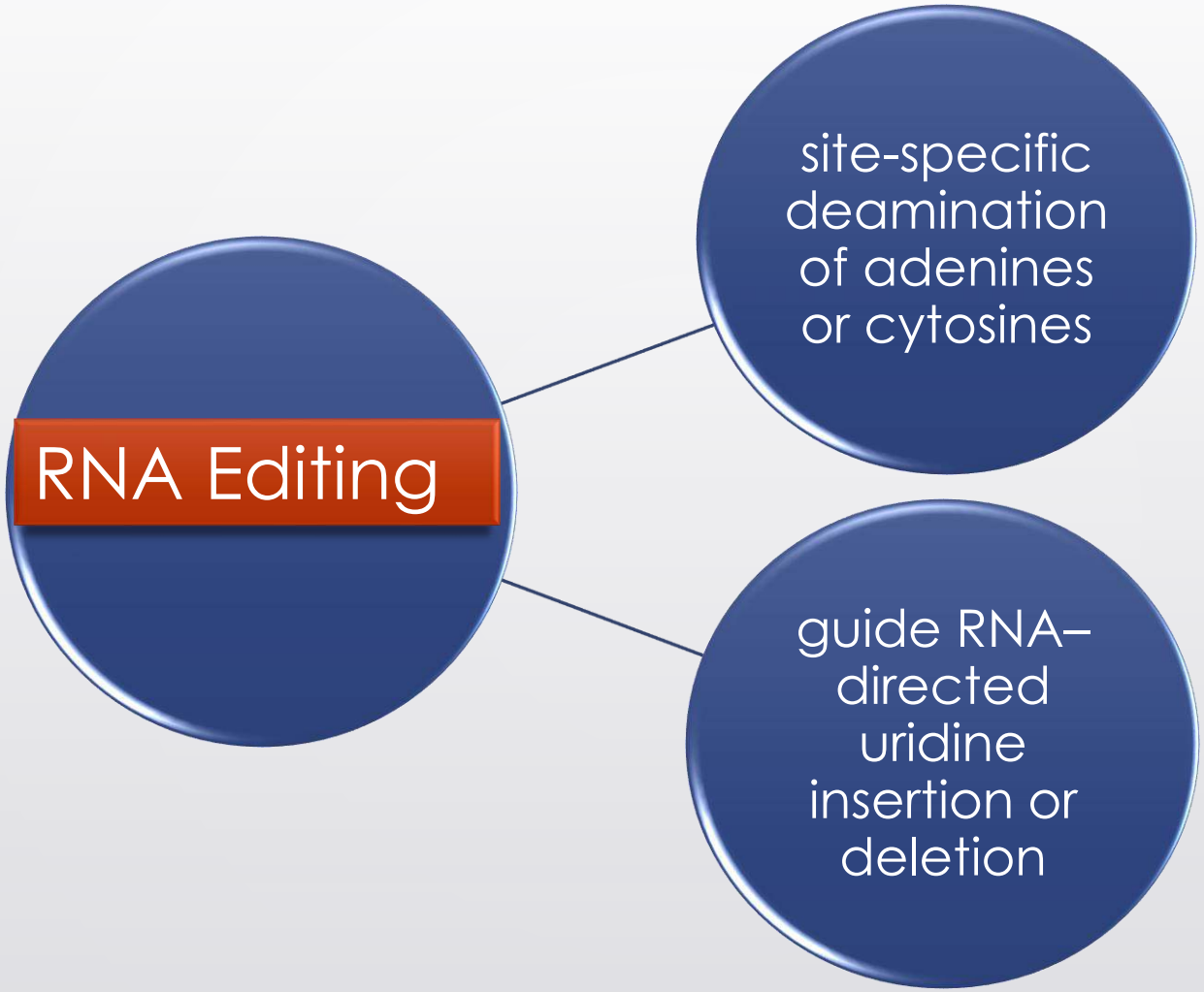
instead of stretches of the mRNA being reassorted, during editing,
individual bases are either inserted,
deleted, or changed.

That is, the coding information in the RNA is altered.

RNA editing was first discovered in trypanosomes, and later found to occur in viruses, plants, slime molds, mammals (including humans and marsupials), squid, and dinoflagellates.

It is now recognized as a widespread mechanism for changing gene-specified codons and thus protein structure and function.

Two mechanisms that mediate editing:



Site-specific deamination of cytosines

Cytidine to uridine (C → U) editing

In contrast to widespread A → I editing, C → U editing has only been identified in the apolipoprotein B (ApoB) (see below) and neurofibromatosis type 1 (NF1) transcripts. Neurofibromatosis type 1 is a dominantly inherited disease that predisposes affected individuals to various forms of cancer. An editing event that introduces a C → U modification results in a premature stop codon. The truncated form of the NF1 (neurofibromin) protein lacks tumor suppressor function.

mammalian apolipoprotein B gene.

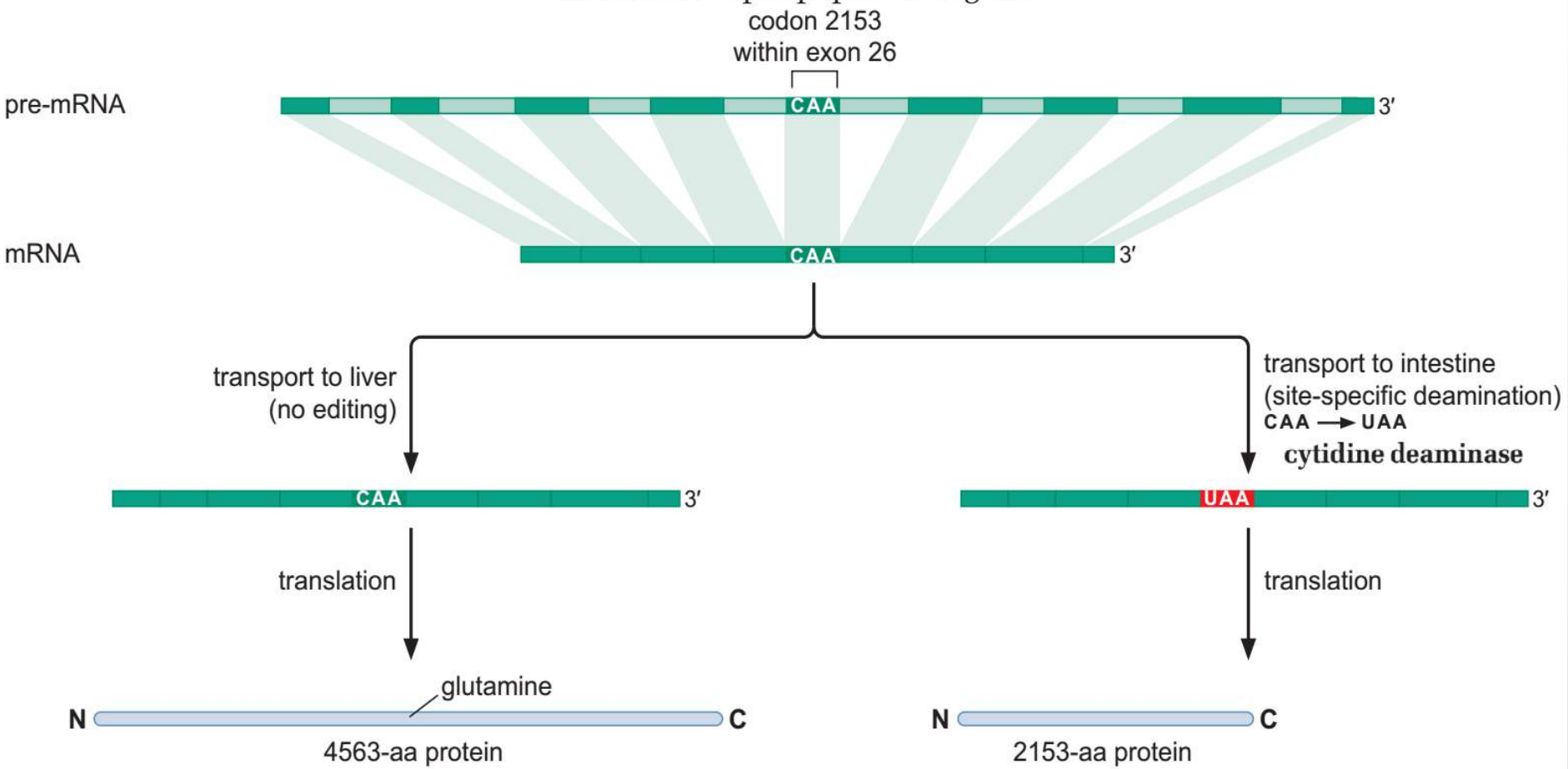


FIGURE 14-30 **RNA editing by deamination.** The RNA made from the human apolipoprotein gene is edited in a tissue-specific manner by deamination of a specific cytidine to generate a uridine. This event occurs in RNAs in the intestine, but not in those found in the liver. The result, as described in the text, is that a stop codon introduced into the intestinal mRNA generates a shorter protein than that produced in the liver. The figure is not drawn to scale: Thus the edited exon is exon 26, and the codon marked as filling it is in reality only a very short part of that exon.

The two forms of apolipoprotein B are both involved in lipid metabolism. The longer form, found in the liver, is involved in the transport of endogenously synthesized cholesterol and triglycerides. The smaller version, found in the intestines, is involved in the transport of dietary lipids to various tissues.

Site-specific deamination of adenines

Adenosine to inosine (A → I) editing

A → I editing occurs frequently, affecting > 1600 genes. It is catalyzed by members of the double-stranded RNA-specific ADAR (*a*denosine *d*eaminase *a*cting on *R*NA) family (Fig. 13.24). The recent crystal structure of ADAR revealed an unusual feature. The enzyme requires inositol hexakisphosphate as a cofactor. Inositol hexakisphosphate is abundant in mammalian cells, but only recently has been implicated as a versatile molecule with important roles in controlling diverse cellular activities.

- These editing events could affect splicing, RNA localization, RNA stability, and translation.

- A $\rightarrow$ I RNA editing is particularly abundant in brain tissues, occurring primarily in receptors and ion channels.

- Not surprisingly, editing defects occur in a number of neurological disorders. Altered editing patterns are associated with inflammation, epilepsy, depression, malignant gliomas (brain tumors), and amyotrophic lateral sclerosis (ALS).

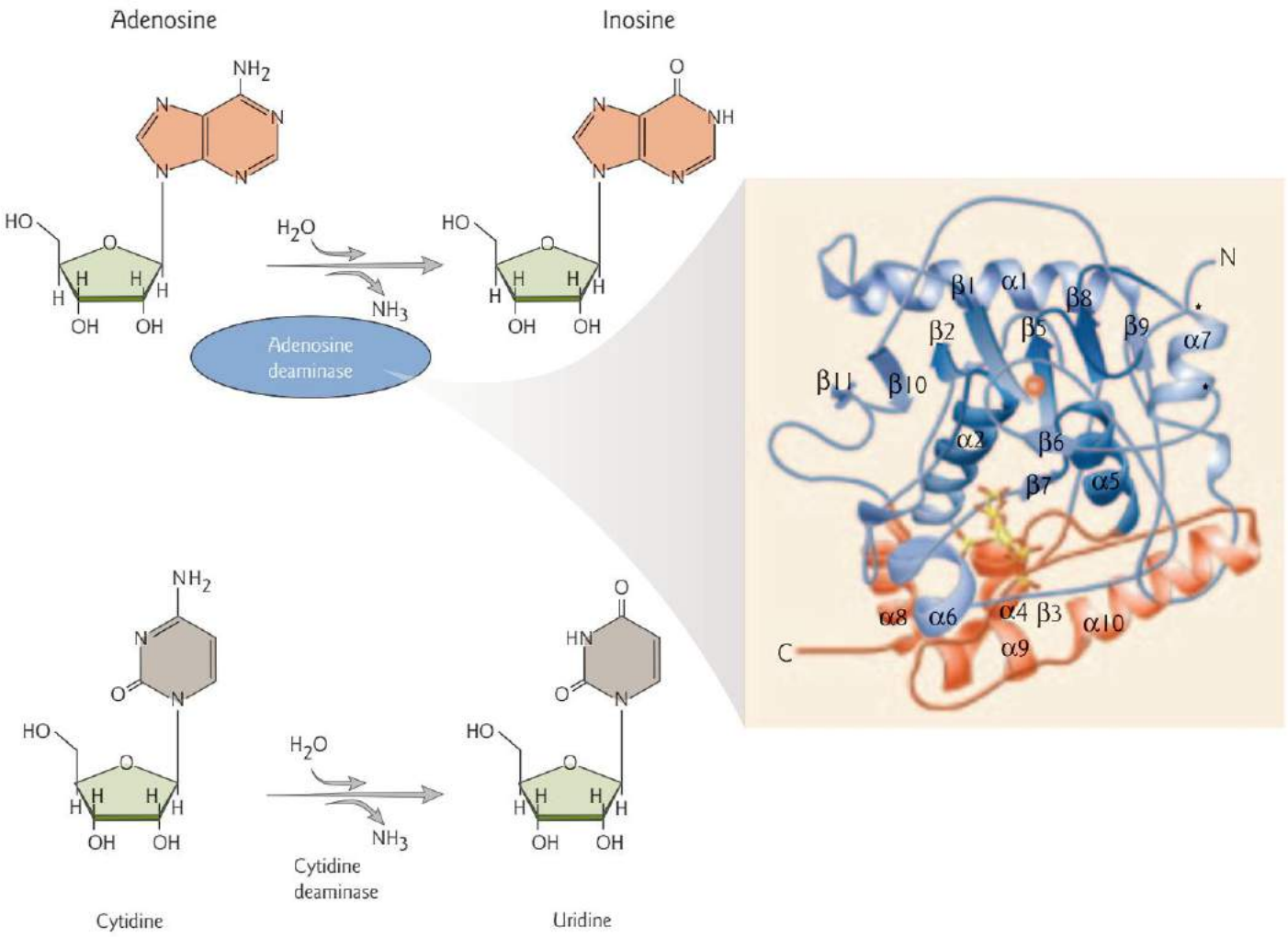


Figure 13.24 The two main classes of RNA editing enzymes in mammals. Adenosine deaminase (e.g. ADAR) generates inosine from adenosine, and cytidine deaminase generates uridine from cytidine (e.g. APOBEC1). (Inset) Ribbon model of the catalytic domain of human ADAR2. The active-site zinc atom is represented by a magenta sphere. The N-terminal domain is colored cyan; the deamination motif region is dark blue; and the C-terminal helical domain which, with contributions from the deamination motif, makes the major contacts to inositol hexakisphosphate (IP_6, ball and stick) is colored red. (Protein Data Bank, PDB:1ZY7. Reprinted with permission from Macbeth, M.R., Schubert, H.L., VanDemark, A.P., Lingam, A.T., Hill, C.P., Bass, B.R. 2005. Inositol hexakisphosphate is bound in the ADAR2 core and required for RNA editing. *Science* 309:1534–1539. Copyright © 2005 AAAS.)

Guide RNA–directed uridine insertion or deletion

- A very different form of RNA editing is found in the RNA transcripts that encode proteins in the mitochondria of trypanosomes.

- In this case, **multiple Us** are inserted into specific regions of mRNAs after transcription (or, in other cases, Us may be deleted).

- These insertions can be so extensive that, in an extreme case, they amount to as many as **half the nucleotides of the mature mRNA**. The addition of Us to the message changes codons and reading frames, completely altering the "meaning" of the message.

What are guide RNAs (gRNAs)?

- gRNAs range from 40 to 80 nucleotides in length and are encoded by genes distinct from those that encode the mRNAs on which they act.

- Each gRNA is divided into three regions:

  - The first, at the 5’ end, is called the anchor and directs the gRNA to the region of the mRNA it will edit;

  - the second determines exactly where the Us will be inserted within the edited sequence;

  - and the third, at the 3’ end, is a poly-U stretch.

Mechanism of editing

The overall directionality of editing is from 3′ to 5′ along the mRNA. The process of U insertion and deletion occurs by a series of enzymatic reactions through the following steps (Fig. 13.23):

1. Anchoring: formation of an "anchor" duplex between the pre-mRNA and the gRNA by complementary base pairing near the editing site. The 5′ regions of the gRNAs recognize the pre-mRNA by a complementary region of 4–14 nt adjacent to the region to be edited.
2. Cleavage: at the first point of mismatch between the pre-mRNA and gRNA upstream of the anchor duplex, an endoribonuclease cleaves the pre-mRNA. gRNAs also have a 3′ oligo (U) tail of unknown function that is added post-transcriptionally. The U tail is dispensible *in vitro* but other reports imply that it is essential *in vivo*. The tail is proposed to stabilize the interaction of the 3′ region of the gRNA with the 5′ cleavage product of mRNA.
3. Uridine insertion or deletion: at the site of cleavage, a U is inserted by a 3′ terminal uridylyl transferase (TUTase), or removed by a U-specific 3′ → 5′ exoribonuclease (ExoUase).
4. Ligation: the RNA ends at the site of U addition or deletion are ligated by an RNA ligase.
5. Repeat of editing cycle: the process is repeated at the next mismatch until the RNA is fully edited. The central information regions of the gRNAs specify the editing sites and the numbers of Us to be added or removed. Complete editing results in continuous base pairing between the gRNA and the pre-mRNA.

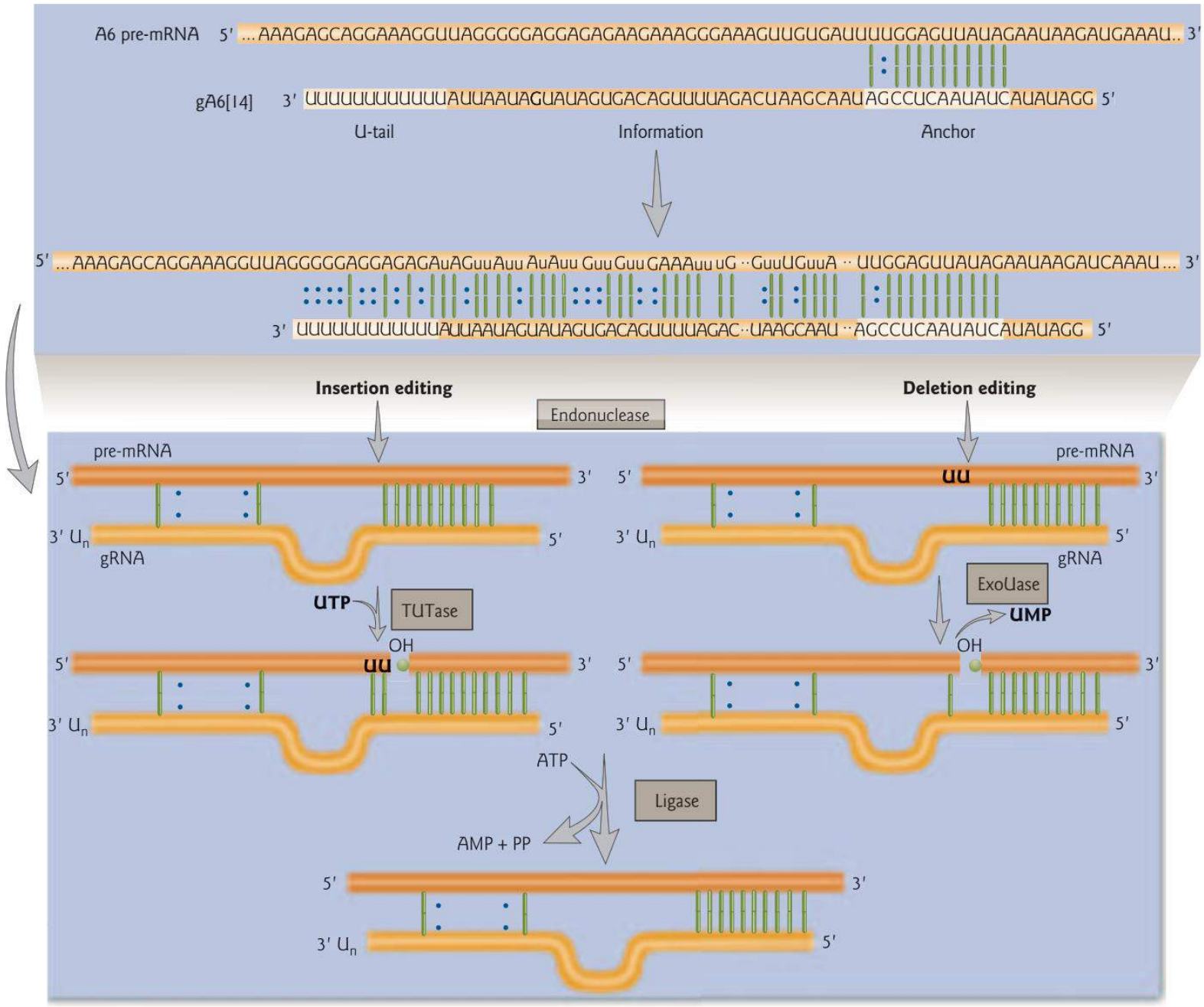


Figure 13.23 General mechanisms of insertion and deletion RNA editing. Pre-mRNAs (dark orange

Figure 13.23 General mechanisms of insertion and deletion RNA editing. Pre-mRNAs (dark orange strands) are edited progressively from 3′ to 5′ with each gRNA (light orange strands) specifying the editing of several sites. Interaction between the RNAs by Watson-Crick base pairs (unbroken green lines) and GU base pairs (blue dots) determines the sites of cleavage and the number of U nucleotides that are added or removed. The gRNAs have a 3′ oligo(U) tail that is added post-transcriptionally. Editing occurs by a series of catalytic steps. Cleavage of the pre-mRNA by an endoribonuclease occurs upstream of the anchor duplex between the pre-mRNA and its gRNA (arrow). Us are either added to the 5′ cleavage fragment by a 3′ terminal uridylyl transferase (TUTase) or removed by a U-specific exoribonuclease (ExoUase), as specified by the sequence of the gRNA. The 5′ and 3′ mRNA fragments are then ligated by an RNA ligase. The process is repeated until all of the sites specified by a gRNA are edited, resulting in complementarity (GU, AU, and GC base pairing) between the edited mRNA and the gRNA, except at the gRNA terminals. Editing by each gRNA creates a sequence that is complementary to the anchor region of the next gRNA to be used. This allows for the sequential use of the multiple gRNAs that are required to edit the mRNAs in full. (Inset) Editing of the first block of *Trypanosoma brucei* ATPase 6 pre-mRNA. The 3′ part of the pre-mRNA is shown with its cognate guide RNA (gA6[14]). The gRNA specifies the insertion of 19 Us and the deletion of four Us. Inserted Us are shown in lowercase letters and the positions of Us that have been deleted from the precursor RNA are indicated by black dots. (Inset redrawn from Kable, M.L., Seiwert, S.D., Heidmann, S., Stuart K. 1996. RNA editing: a mechanism for gRNA-specified uridylate insertion into precursor mRNA. *Science* 273:1189–1195.)