
# RNA PROCESSING

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# RNA PROCESSING

- **RNA processing** includes the addition of a methylated guanine residue to the 5' end (called the cap)
- Removing segments (introns) of the **RNA** internally by a **process** called **RNA splicing**,
- And adding 100–200 adenine nucleotides to the 3' end (a **process** called polyadenylation).

# PRIMARY TRANSCRIPT

- A newly synthesized RNA molecule is called a primary transcript.
- The primary transcript for a eukaryotic mRNA typically Contains sequences encompassing one gene, although the sequences encoding the polypeptide may not be contiguous.
- Non coding tracts that break up the coding region of the transcript are called introns, and the coding segments are called exons .

- Splicing, the introns are removed from the primary transcript and the exons are joined to form a continuous sequence that specifies a functional polypeptide.

# POLYADENILATION

Eukaryotic mRNAs are also modified at each end. A modified residue called a 5' cap is added at the 5' end. The 3' end is cleaved, and 80 to 250 A residues are added to create a poly(A) "tail. Which is called polyadenylation.

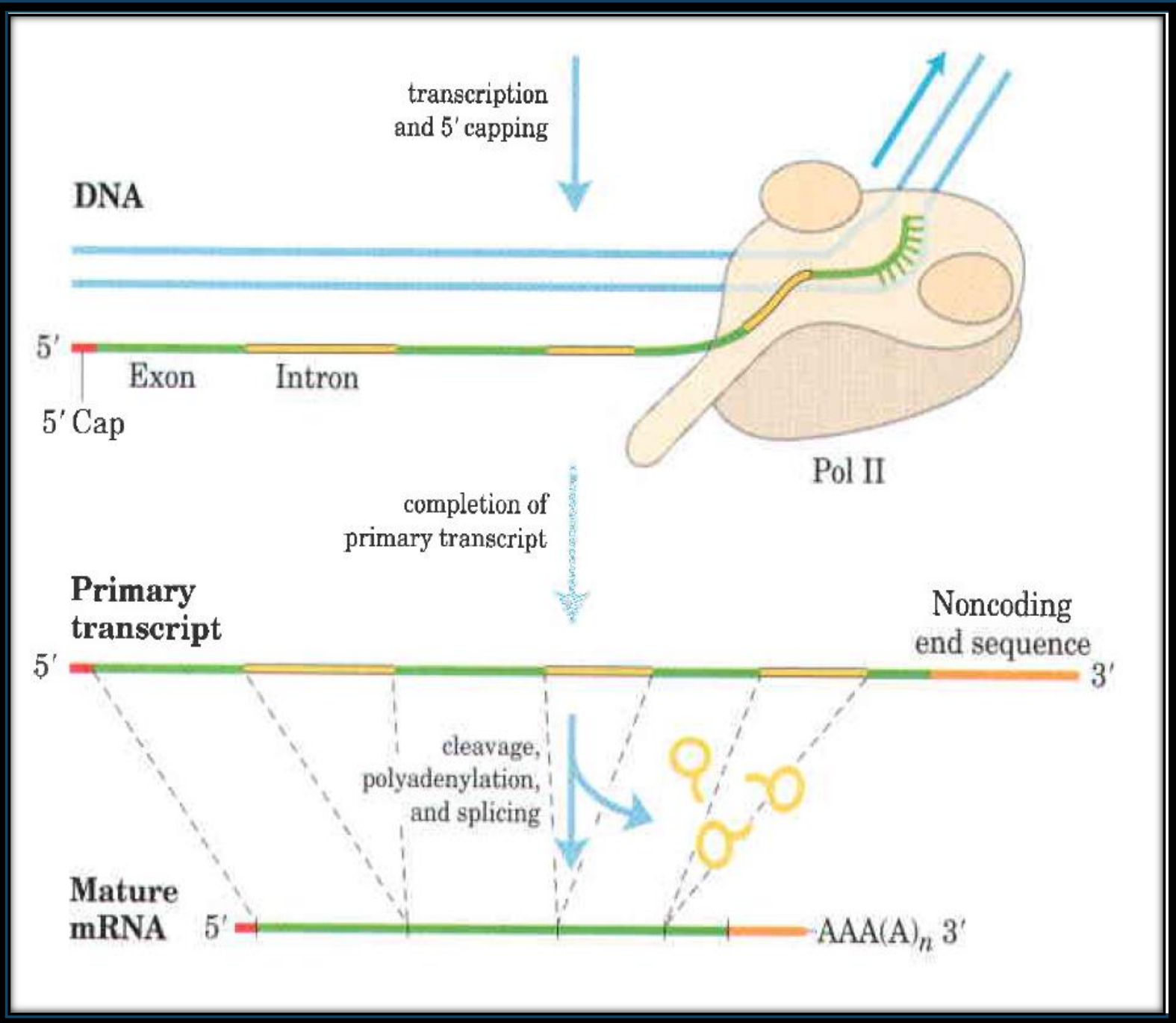


FIGURE Formation of the primary transcript and its processing during maturation of mRNA in a eukaryotic cell. The 5' cap (red) is added before synthesis of the primary transcript is complete. A. noncoding sequence intron following the last exon is shown in orange. Splicing can occur either before or after the cleavage and polyadenylation steps. All the processes shown here take place in the nucleus.

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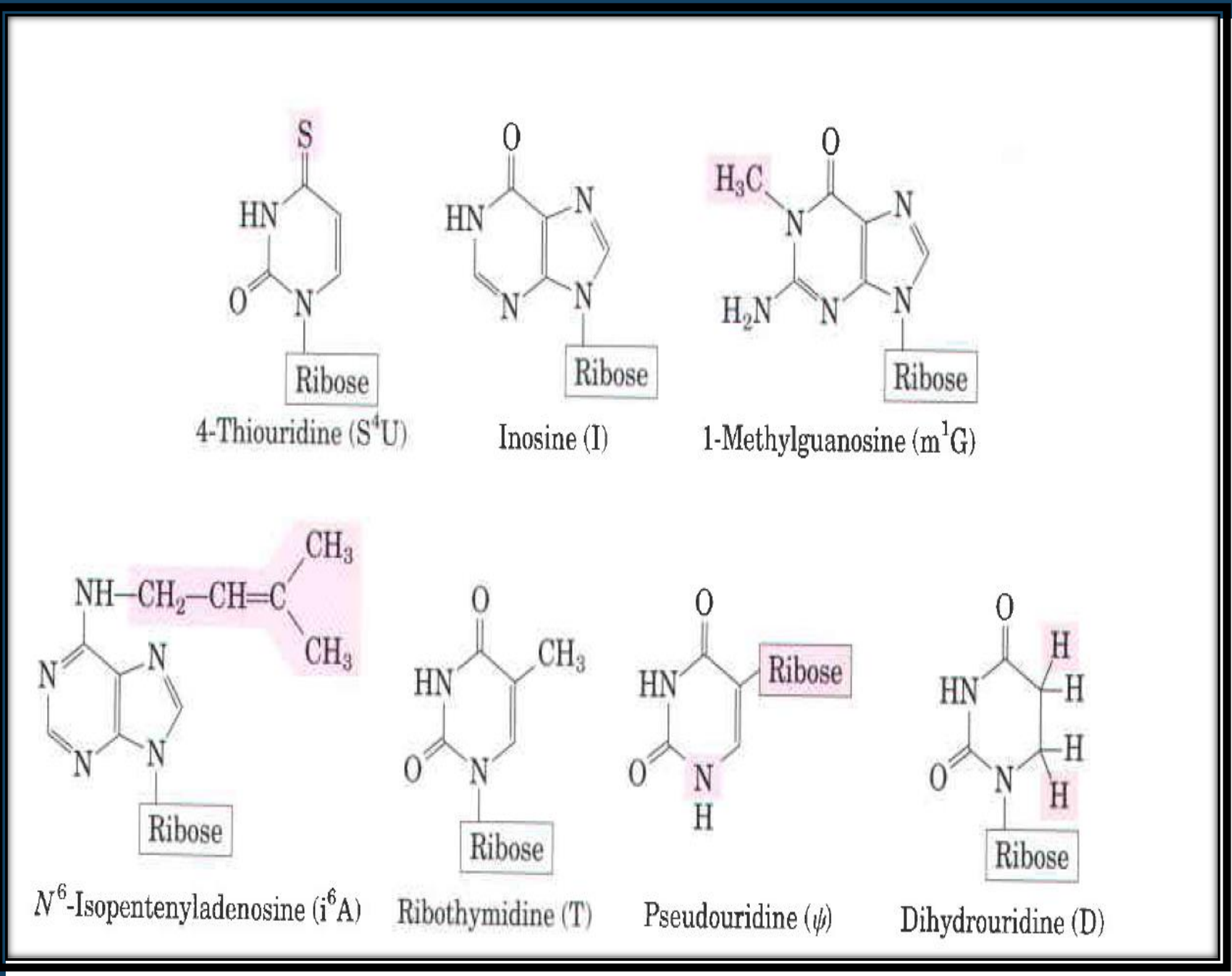


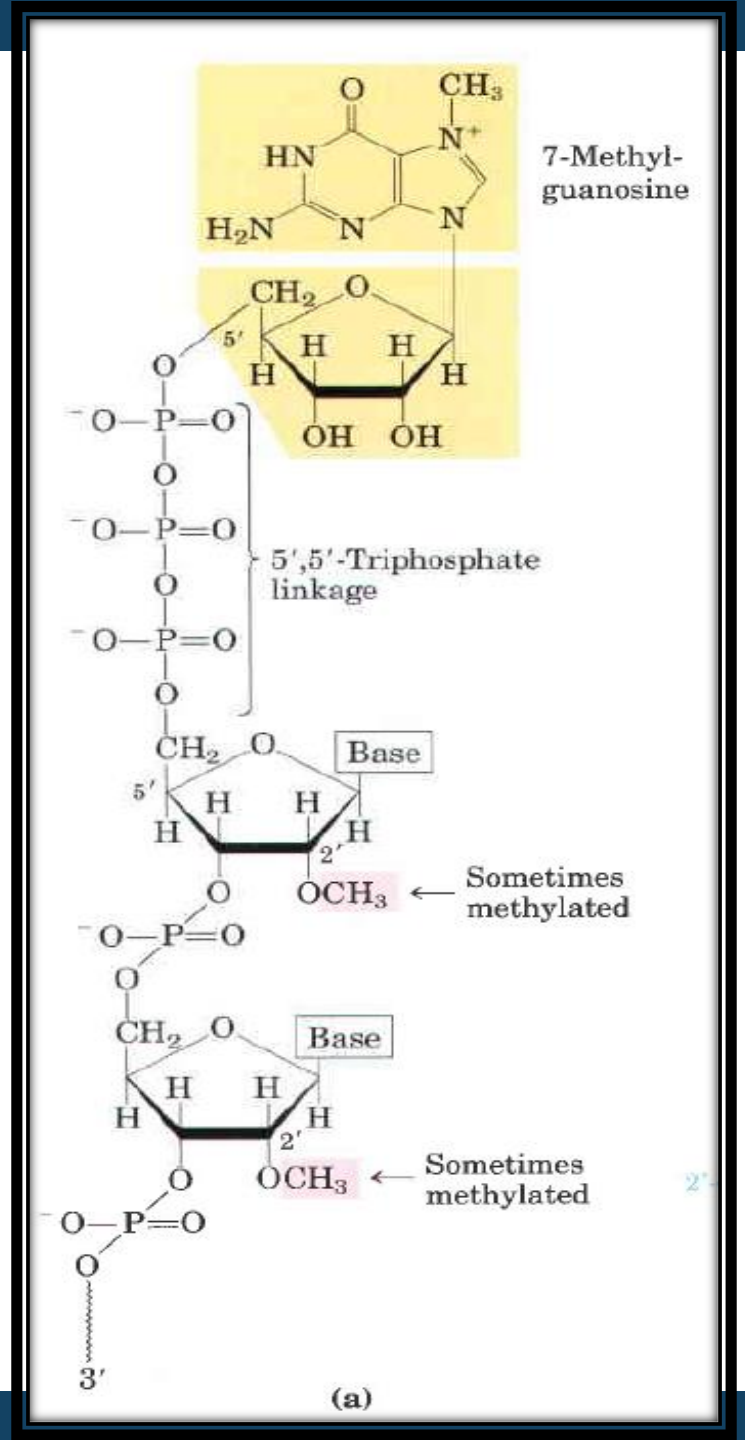

The primary transcripts of bacterial and eukaryotic tRNAs are processed by the removal of sequences from each end (cleavage) and in a few cases by the removal of introns (splicing). Many bases and sugars in tRNAs are also modified; mature tRNAs are replete with unusual bases not found in other nucleic acids fig 3.

Fig3 Some modified bases of rRNAs and tRNAs, produced in posttranscriptional reactions.

# EUKARYOTIC m RNAs are CAPED AT 5/ END

- Most eukaryotic mRNAs have a 5' cap, a residue of 7-Methyl guanosine linked to the 5'-terminal residue of the mRNA through an unusual 5',5'-triphosphate linkage a. The 5' cap helps protect mRNA from ribonuclease It. Also binds to a specific cap-binding complex of proteins and participates in binding of the mRNA to the ribosome to initiate translation .

The 5' cap is formed by condensation of a molecule of GTP with the triphosphate at the 5' end of the transcript. The guanine is subsequently methylated at N-7, and additional methyl groups are often added at the 2' hydroxyls of the first and second nucleotides adjacent to the cap (Fig. a).

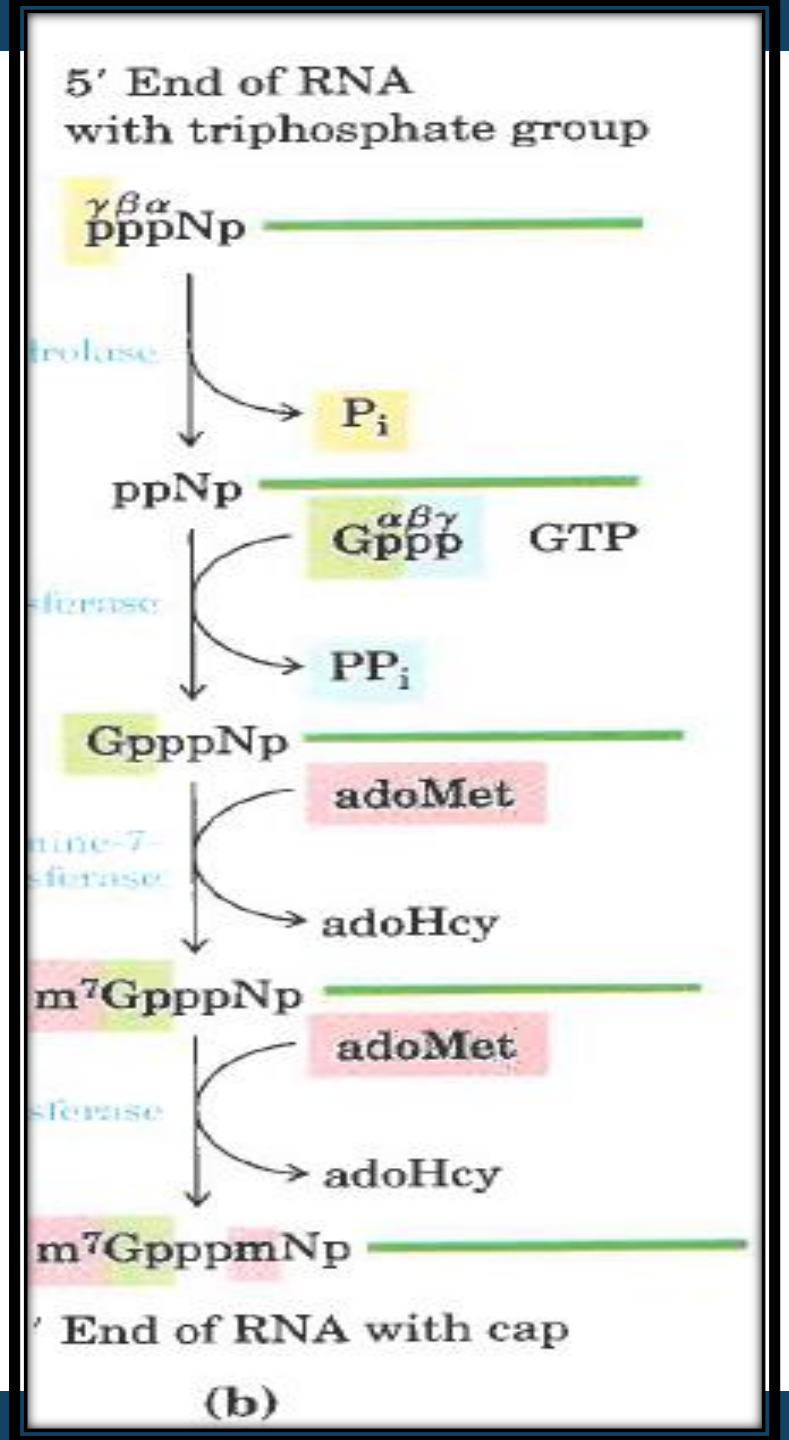



(a) 7-Methylguanosi (name' G) is Joined to the 5'end of almost all eukaryotic mRNA is an unusual 5',5'triphosphate linkage Methyl groups CH3 are often found a t the 2' position of the of first and second nucleotides RNAs in yeast cells lack the2 '-methyl groups. The2 'methyl group on the second nucleotides generally, found only in RNAs from vertebrate cells.

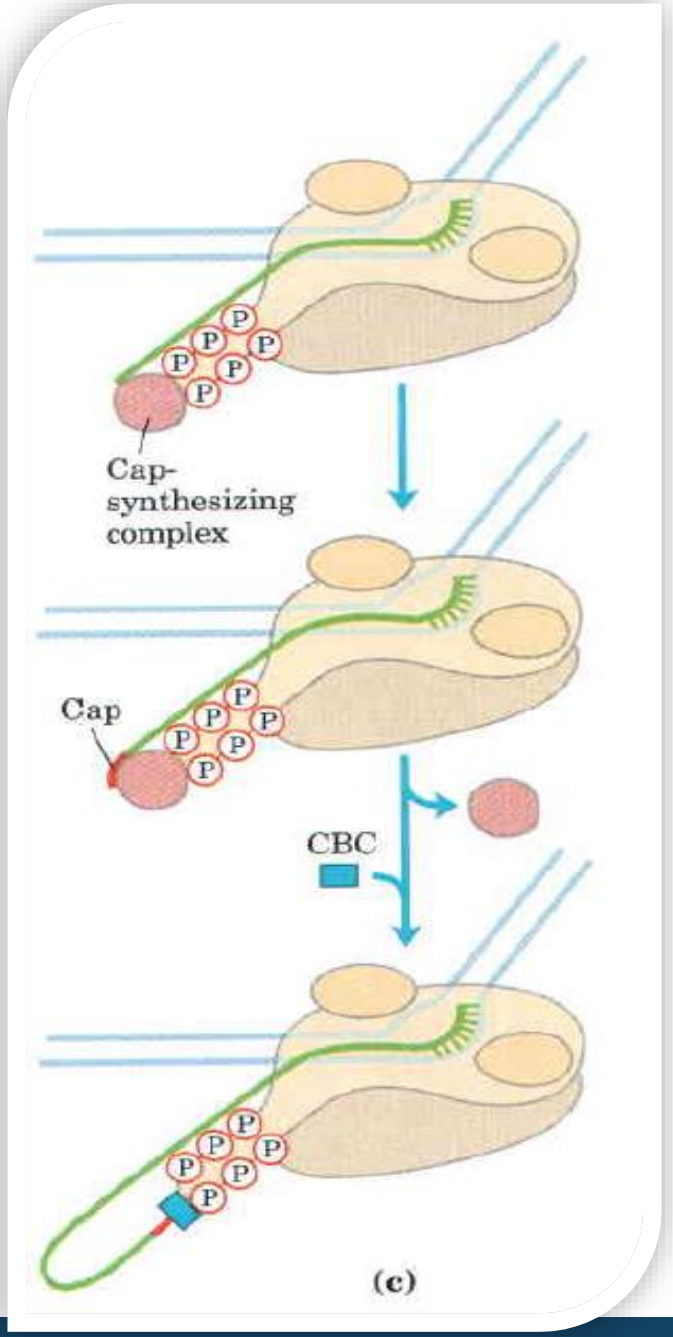
The methyl groups are derived from, S-adenosylmethionin . All these reactions occur very early in transcription, after the first 20 to 30 nucleotides of the transcript have been added.

All three of the capping enzymes, and through them the 5' end of the transcript itself, are associated with the RNA polymerase II CTD.

(The C-terminal domain (CTD) of the RNA polymerase II largest subunit consists of multiple heptad repeats (consensus Tyr1–Ser2–Pro3–Thr4–Ser5–Pro6–Ser7), varying in number from 26 in yeast to 52 in vertebrates. The CTD functions to help couple transcription and processing of the nascent RNA and also plays roles in transcription elongation and termination). until the cap is synthesized .The capped5 ' end is then released from the capping enzymes and bound by the cap-binding complex




(b) Generation of the 5' cap involves four to five separate steps (adoHcy is S adenosyl homocysteine)




C, Synthesis of the cap is carried out by enzymes tethered to the CTD of Pol Il. The cap remains tethered to the CTD through an association with the cap-binding complex(CBC).

# RNA catalyse the Splicing of Introns

There are four classes of introns. The first two, the group I and group II introns, differ in the details of their splicing mechanisms but share one surprising characteristic: they are self -splicing-no protein enzymes are involved.

Group I introns are found in some nuclear, mitochondrial, and chloroplast genes that code for rRNAs, mRNAs, and tRNAs.

Group II introns are generally found in the primary transcripts of mitochondrial or chloroplast mRNAs in fungi, algae, and plants. Group I and group II introns are also found among the rare examples of introns in bacteria. Neither class requires a high energy cofactor (such as ATP) for splicing. The splicing mechanisms in both groups involve two transesterification reaction steps

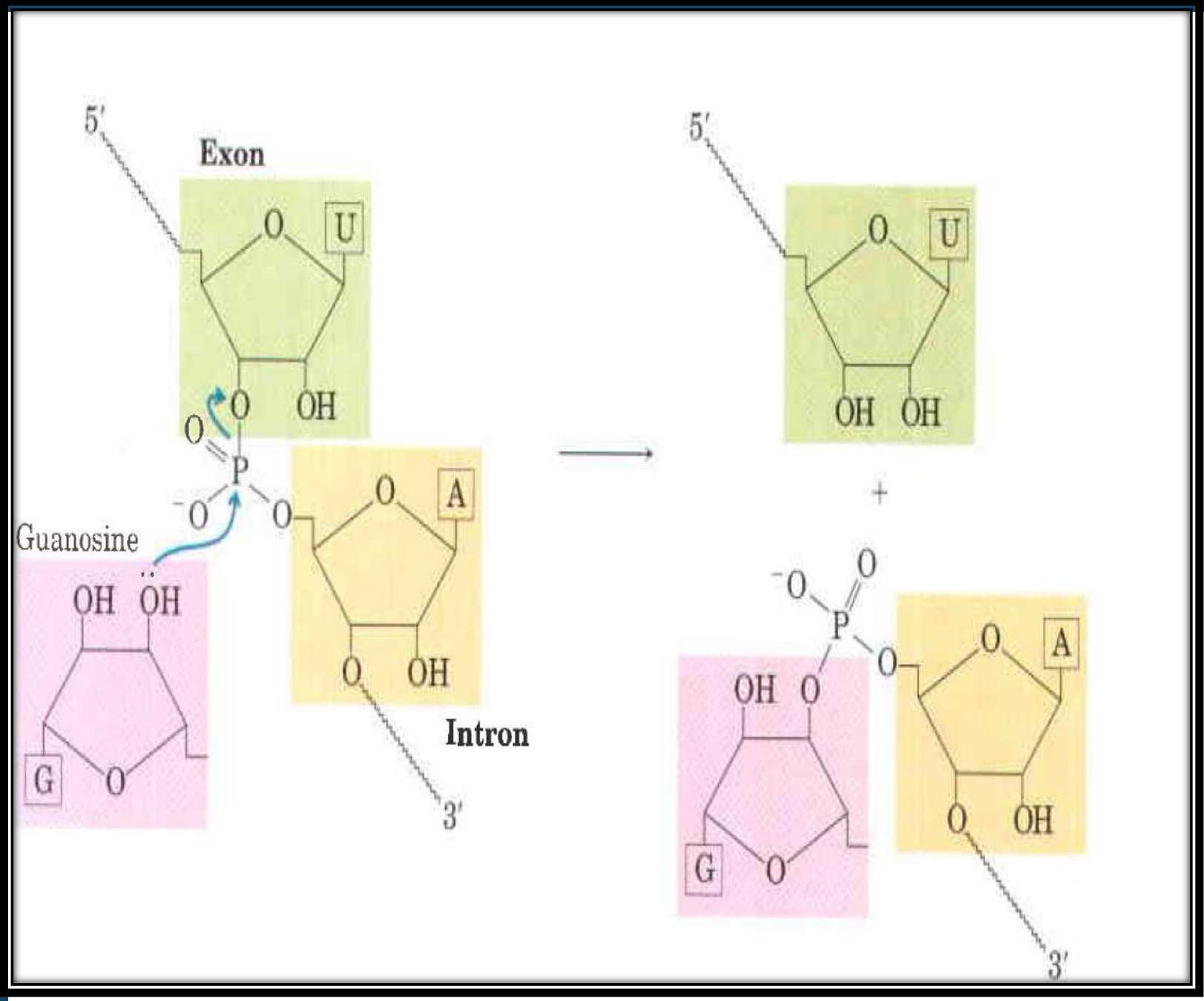



FIGURE : Transesterification - action. Shown here is the first step in the two-step splicing of group I introns. In these example the 3' OH of a guanosine molecule acts as nucleophile, attacking the phosphodiester linkage between U and A residue as an exon-intron junction of an mRNA molecule (F ig.b). (Fig. a), in which a ribose 2, - or 3'-hydroxyl group makes a nucleophilic attack

(The **nucleophile** can **attack** from the top or the bottom and therefore create a racemic product. It is important to use aprotic

(which has no O-H or N-H bonds,the A means without and protic refers to proton or hydrogen atoms..the molecules have no H atoms on O or N.)

on a phosphorus and a new phosphodiester bond is formed at the expense of the old, maintaining the balance of energy. These reactions are very similar to the DNA breaking and Re-joining reactions promoted by topoisomerase (f ig.)

Topoisomerases catalyze the interconversion of DNA topological isomers via coupling of DNA phosphodiester bond cleavage and relegation with the passage of DNA through the break. By maintaining global DNA supercoiling at optimal level and removing local topological barriers, DNA topoisomerases play vital roles in DNA replication, transcription, repair and recombination

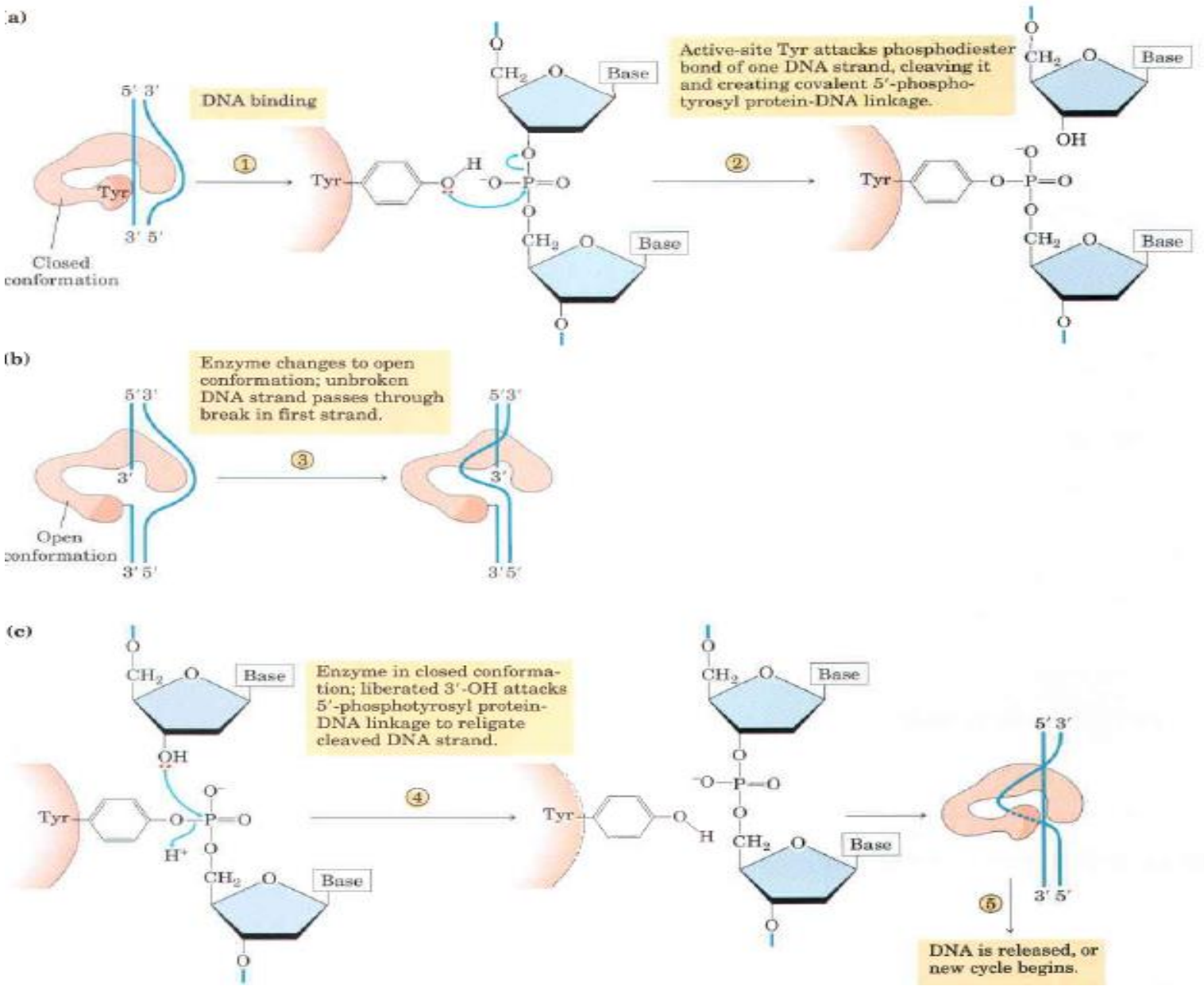



Fig : Bacterial type I topoisomerase result after linking number. A proposed reaction sequence for the bacterial topoisomerase is lustrate. The. Enzyme has closed and open conformations. (a)A DNA molecule binds to the closed conformation and one DNA strand is cleaved. (b) The enzyme changes to its open conformation, and the other DNA strand moves through the break in the first strand. (c) In the closed conformation the DNA strand is relighted. (regenerated)

# GROUP I SPLICING

The group I splicing reaction requires a guanine nucleoside or nucleotide cofactor, but the cofactor is not used as a source of energy; instead, the 3'-hydroxylgroup of guanosine is used as a nucleophile in the first step of the splicing pathway. The guanosine 3'-hydroxyl group forms a normal 3',5'-phosphodiester bond with the 5' end of the intron (Fig.).

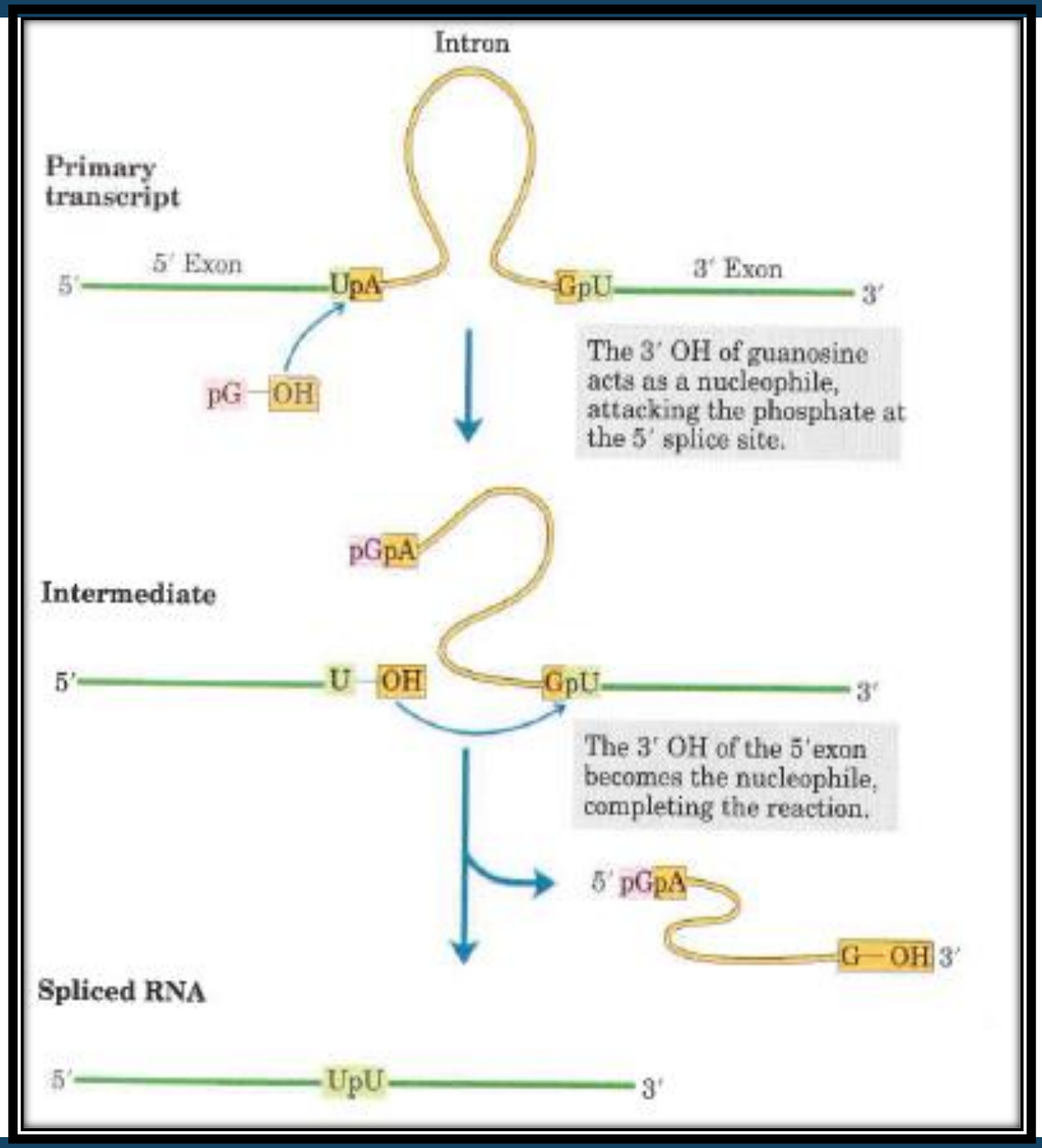
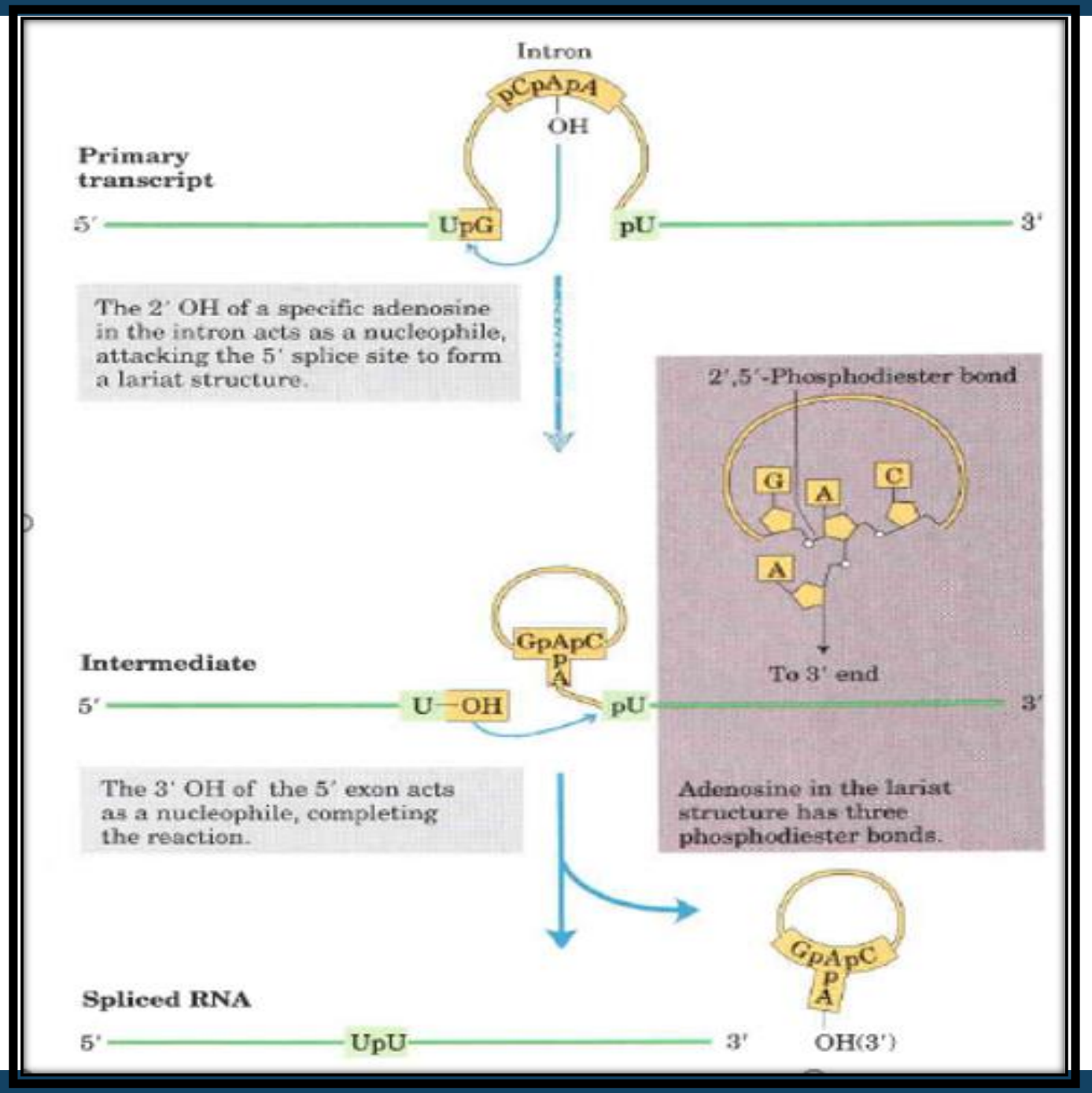



FIGURE:a: Splicing mechanism of group I introns. The nucleophile in the first step maybe guanosine GMP, GDP, GTP.The spliced intron Is eventually degraded. The B, hydroxyl of the exon that is displace in this step then act nucleophile in a similar reaction at the 3' end of the intron. The result is precise excision of the intron and ligation of the exons.

In group II introns the reaction pattern is similar except for the nucleophile in the first step which in this case is the 2 '-hydroxyl group of an A residue within the intron A branched lariat(loop) structure is formed as an intermediate not designated with a group number.

Most introns are not self-splicing and these types are not designated with a group number.

FIGURE:bSplicing mechanism of group ll introns. The chemistry is similar to that of group I intron splicing, except for the identity of the nucleophile in the first step and formation of a lariat like intermediate in which one branch is a 2',5'-phosphodiesbteorn bond



The third and largest class of introns includes those found in nuclear mRNA primary transcripts. These are called spliceosomal introns, because their removal occurs within and is catalysed by a large protein complex called a spliceosome. Within the spliceosome the introns undergo splicing by the same lariat-forming mechanism as the group I introns.

The spliceosome is made up of specialized RNA-protein complexes small nuclear RNA (snRNPs, often pronounced "snurps")‘Each snRNP contains one of a class of eukaryotic RNAs, 100 to 200 nucleotides long, known as smell nuclear RNAs (snRNAs). Five snRNAs (U1, U2, U4, U5, and U6) involved in splicing reactions are generally found in abundance in eukaryotic nuclei. The RNAs and proteins in snRNPs are highly conserved in eukaryotes from yeasts to humans. mRNA Splicing, Spliceosomal introns generally have the dinucleotide sequences GU at 5/ end and AG at 3/ end, and these sequences mark the sites where splicing occurs.

# 3' processing :Cleavage and polyadenylation

The pre-mRNA processing at the 3' end of the RNA molecule involves cleavage of its 3' end and then the addition of about 250 adenine residues to form a poly(A) tail.

The cleavage and adenylation reactions occur primarily if a polyadenylation signal sequence (5'- AAUAAA-3') is located near the 3' end of the pre-mRNA molecule, which is followed by another sequence, which is usually **(5'-CA-3')** and is the site of cleavage.

A **GU-rich sequence** is also usually present further downstream on the pre-mRNA molecule. More recently, it has been demonstrated that alternate signal sequences such as UGUA upstream off the cleavage site can also direct cleavage and polyadenylation in the absence of the AAUAAA signal. It is important to understand that these two signals are not mutually independent and often coexist.

After the synthesis of the sequence elements, several multi-subunit proteins are transferred to the RNA molecule.( by CPSF For UGUA dependent processing sites), binding of the multi protein complex is done by Cleavage Factor I (CF I). The resultant protein complex formed contains additional cleavage factors and the enzyme Polyadenylate Polymerase (PAP).

This complex cleaves the RNA between the polyadenylation sequence and the GU-rich sequence at the cleavage site marked by the (5'-CA-3') sequences. Poly(A) polymerase then adds about 200 adenine units to the new 3' end of the RNA molecule using ATP as a precursor.

As the poly(A) tail is synthesized, it The transfer of these sequence specific binding proteins cleavage and polyadenylation specificity factor (CPSF), Cleavage Factor I (CF I) and cleavage stimulation factor (CStF) occurs from RNA Polymerase II. The three factors bind to the sequence elements. The AAUAAA signal is directly bound binds multiple copies of poly(A)-binding protein, which protects the 3'end from ribonuclease digestion by enzymes.

# SUMMARY OF RNA PROCESSING

1. Eukaryotic mRNAs are modified by addition of a7-methylguanosine residue at the 5' end and by cleavage and polyadenylation at the 3' end to form a long poly(A) tail.

2. Many primary mRNA transcripts contain introns (noncoding regions), which are removed by splicing. Excision of the group I introns found in Some rRNAs requires a guanosine cofactor.

3, Some group I and group II introns are capable of self-splicing; no protein enzymes are required. Nuclear mRNA precursors have a third (the largest) class of introns, which are spliced with the aid of RNA protein 4. 4.complexes called snRNPs  assembled in to spliceosomes A.  fourth class of introns, found in some tRNAs, consists of the only introns known to be spliced by Protein enzymes.

5, The function of many eukaryotic mRNAs is regulated by complementary microRNAs (miRNAs). The miRNAs are themselves derived from larger precursors through a series of processing reaction.

6. Ribosomal RNAs and transfer RNAs are derived from longer precursor RNAs, trimmed bynucleases Some bases are modified enzymatically during the maturation processes. Some nucleoside modifications are guided by snoRNAs, within protein complexes called noRNps

7. The self-splicing introns and the RNA component of RNase P (which cleanest at 5, end of tRNA precursors) are two examples of ribozymes. These biological catalysts have the properties of true enzymes. They generally promote hydrolytic cleavage and transesterification using RNA as substrate. Combinations of these reactions can be promoted by the excised group I intron of Tetrahymena rRNAs, resulting in a type of RNA polymerization reaction. Polynucleotide phosphorylase reversibly forms

8.RNA-like polymers from ribonucleoside 5' –diphosphates adding or removing ribonucleotides at the 3'-hydroxyl end of the polymer. The enzyme degrades RNA in vivo