TRANSCRIPTION

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Definition

Cellular process in which RNA is synthesized using DNA as a template known as **TRANSCRIPTION**.

DNA

RNA

RNA

- Polymer of ribonucleotide held together by 3'→ 5' phosphodiester bridge & are single stranded.

- Is the only molecule known to function both in the storage & transmission of genetic information & in catalysis.

- All RNAs except the RNA genomes of certain viruses derived from information which is stored permanently in DNA.

RNA

- Three major kinds of RNAs

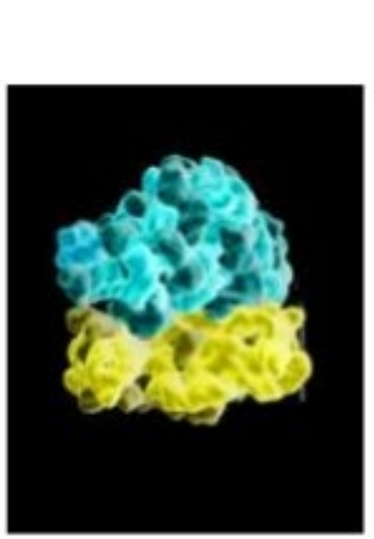
- mRNA (5-10%)→ transfer information of gene to ribosome i.e. encodes the amino acids sequence .

- tRNA (10-20%) → reads codes on mRNA and transfers appropriate AA to mRNA.

- rRNA (60-80%) → constituents of ribosome.

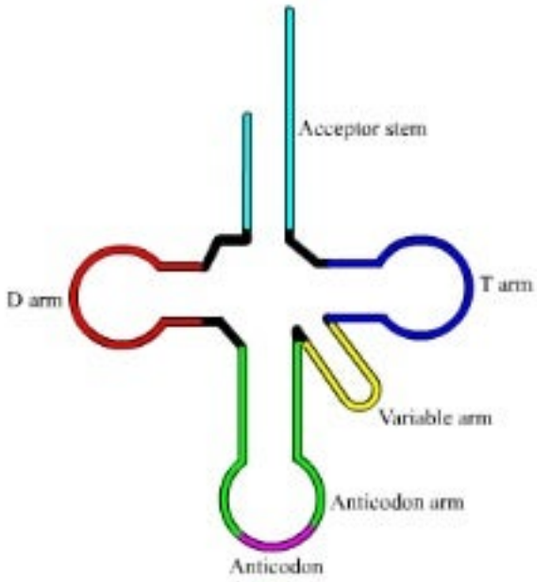
- Many additional specialized RNAs which has catalytic activity or regulatory functions are present in the cell.

DNA

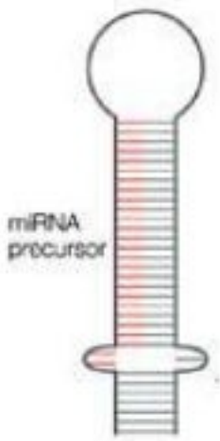


rRNA

mRNA



tRNA



miRNA

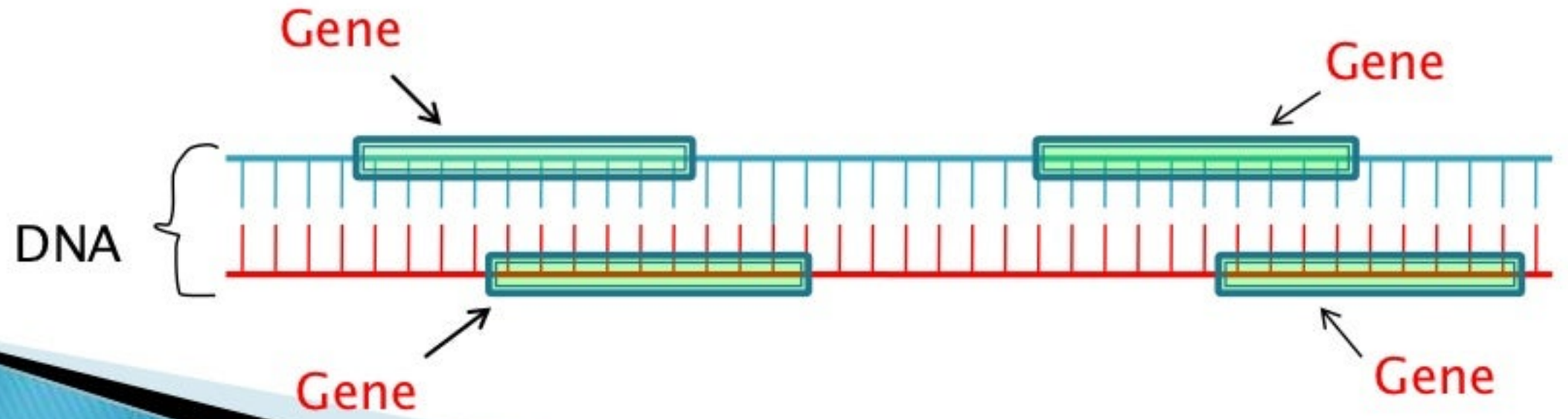
ribozyme

U1RNA

- In replication entire DNA molecule is normally copied.

- In transcription a particular gene or group of genes are copied at any time, & some portions of DNA are never transcribed.

- Gene is a segment of DNA that codes for a type of **Protein** or for **RNA** & may present on any strand of DNA (contain many genes.)



Differences between replication and transcription

	Replication	Transcription
Template	Both strand whole genome	single strand small portion of genome
Primer	yes	no
Enzyme	DNA polymerase	RNA polymerase
Product	dsDNA	ssRNA
Base pair	A-T, G-C	A-U, T-A, G-C
Proof reading	yes	no

Features of transcription

- **1)** It is highly selective.

- This selectivity is due to signals embedded in the nucleotide sequence of DNA.

- Specific sequences mark the beginning and end of the DNA segment which is to be transcribed.

- This signals instruct the enzyme

where to start & stop the transcription
when to start,
how often to start .

features

- **2)** Many of the RNA transcripts are synthesized as precursors that is known as primary transcripts.

- Which on modifications & trimming converted into functional RNA .
- **SITE:**
- **Transcription –** Prokaryotes– cytoplasm(all RNAs).
  Eukaryotes– Nucleus & mitochondria
  a) Nucleolus – rRNA
  b) Nucleoplasm –tRNA & mRNA.

- The basic biochemistry of RNA synthesis is similar in prokaryotes & eukaryotes, but its regulation is more complex in eukaryotes.

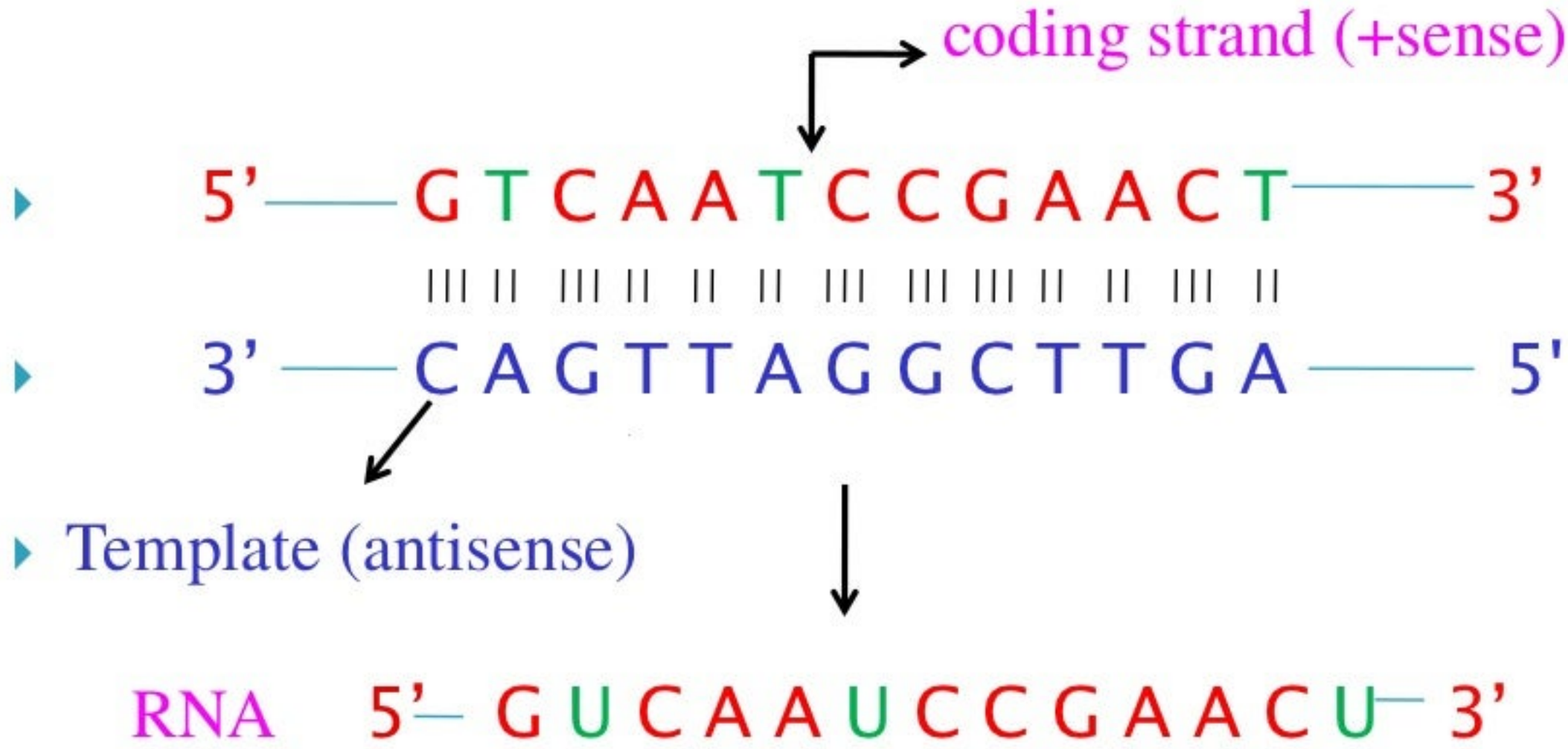
- RNA synthesis in prokaryotes is catalyzed by a large enzyme called as

- **DNA dependent RNA polymerase or RNA polymerase**

A single enzyme, RNA polymerase, synthesizes all types cellular RNAs in prokaryotes.

RNA polymerase use one of the DNA strand as template on which complimentary ribonucleotides are incorporate to synthesize RNA.

- The strand of DNA which is transcribed to RNA called as template strand.
- Opposite strand is referred as coding strand.



RNA POLYMERASES

- RNA polymerase synthesize RNA in the direction of 5'-3' that means DNA template is read in 3'-5' direction.
- Ribonucleotides required -- ATP, GTP, CTP & UTP.

- The prokaryotic RNA polymerase is a multimeric enzyme consisting of six subunits, two identical **α**-subunits, similar but not identical **β** and **β'** and **ω** sixth is **σ** factor.

2α,β,β'ω -- core enzyme

2α,β,β'ω + σ --- Holoenzyme

RNA Polymerase of prokaryotes

Subunit	Function
α,α	Determine the DNA to be transcribed
β	Catalyze polymerization
β′	Bind & open DNA template(unwinding)
ω	Function is not known
σ	Recognize the initiation sites called promoter

Functions of RNAP

- A single RNA polymerase performs multiple functions in transcription process.
- 1- search & binds to promoter site
- 2- unwinds a short stretch of double helical DNA.
- 3- selects correct ribonucleotide & catalyze the formation of phosphodiester bond (polymerization according to base pair rule)
- $(RNA)n + NTP \rightleftharpoons (RNA)n+1 + PPi$
- 4- detects termination signals
- 5- interacts with activator & repressor proteins that regulate the rate of transcription.

Transcription in Prokaryotes

Three stages

- Initiation phase: RNA-polymerase recognizes the **promoter** and starts the transcription.
- Elongation phase: the RNA strand is continuously growing.
- Termination phase: the RNA-polymerase stops synthesis and the nascent RNA is separated from the DNA template.

Initiation

- Involves the interaction of RNAP with DNA at a specific site or sequences of DNA.

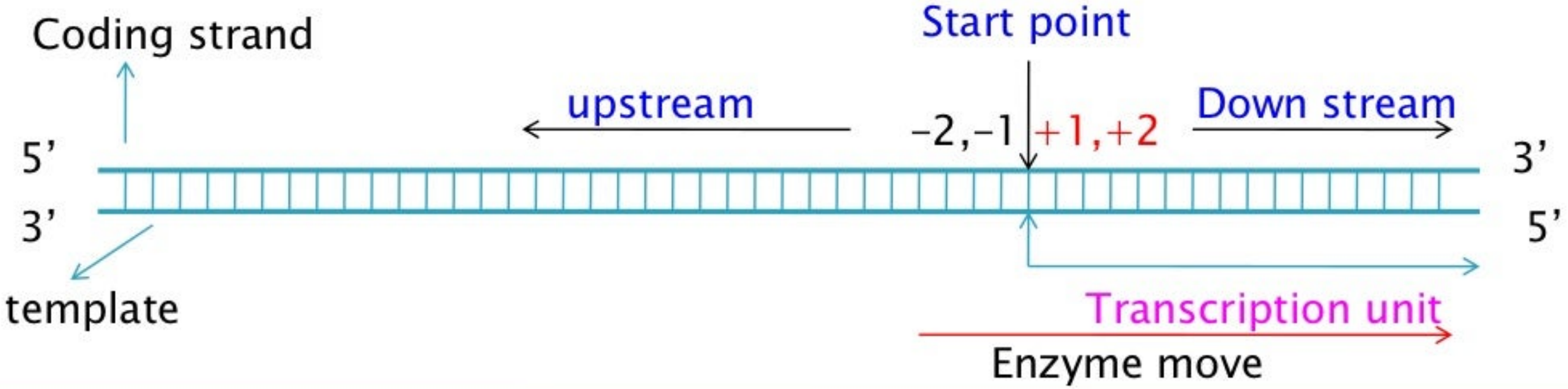
- *The sequence of DNA needed for RNA polymerase to bind to the template and accomplish the initiation reaction defines the promoter.*

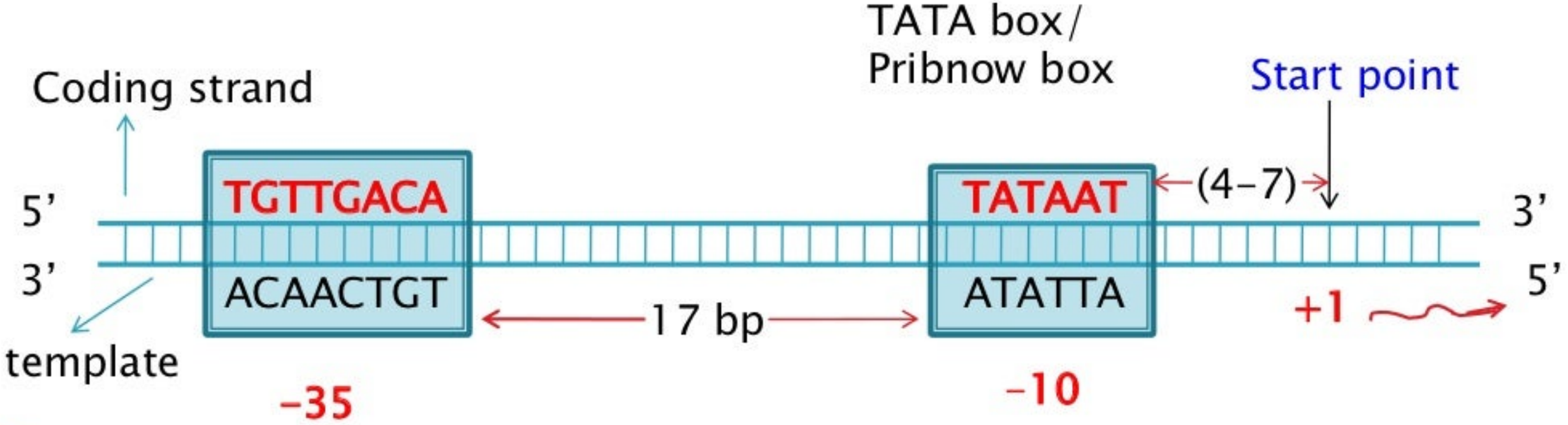
- Promoter are the characteristic sequences of DNA that direct the RNA polymerases to initiate the transcription.

- usually located on coding strand.

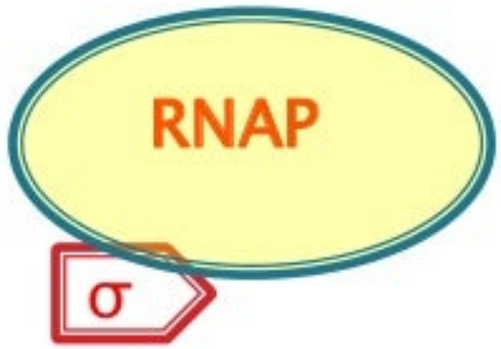
- Simplest type of promoters found in prokaryotes.

- Two general types of sequence elements are found.
- One sequence element is believed to promote initial binding of the enzyme RNAP.
- Other element usually has high content of adenine & thymine.
- These sequences are 6 to 8 nt in length and located about -35 & -10 bp upstream of the start point of transcription.
- These are on coding strand indicates duplex DNA required for transcription.
- Change in only one base pair in promoter region decrease the rate of transcription


Coding strand
Start point
upstream
Down stream
-2,-1
+1,+2
5'
3'
3'
5'
template
Transcription unit
Enzyme move


TATA box/
Pribnow box
Coding strand
Start point
TGTTGACA
TATAAT
(4-7)
5'
3'
3'
5'
ACAATCGT
ATATTA
17 bp
+1
template
-35
-10

Initiation process



Coding strand

Start point

Closed complex

5’

3’

3’

5’

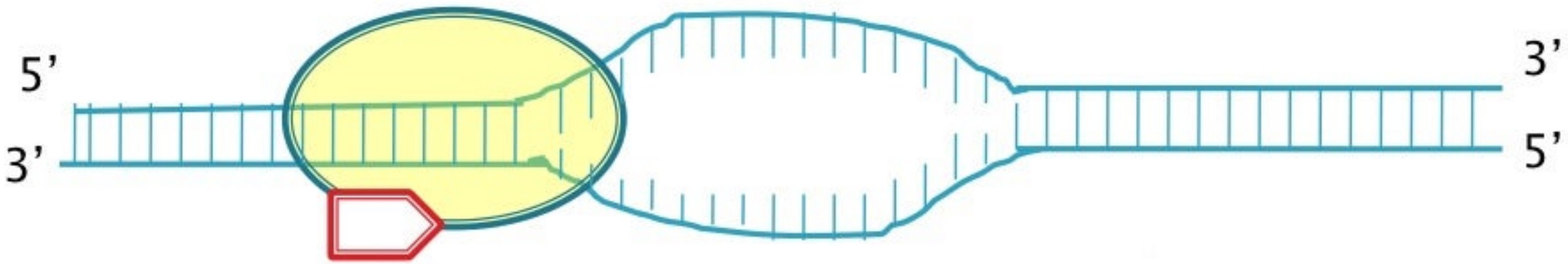
−35

−10

+1

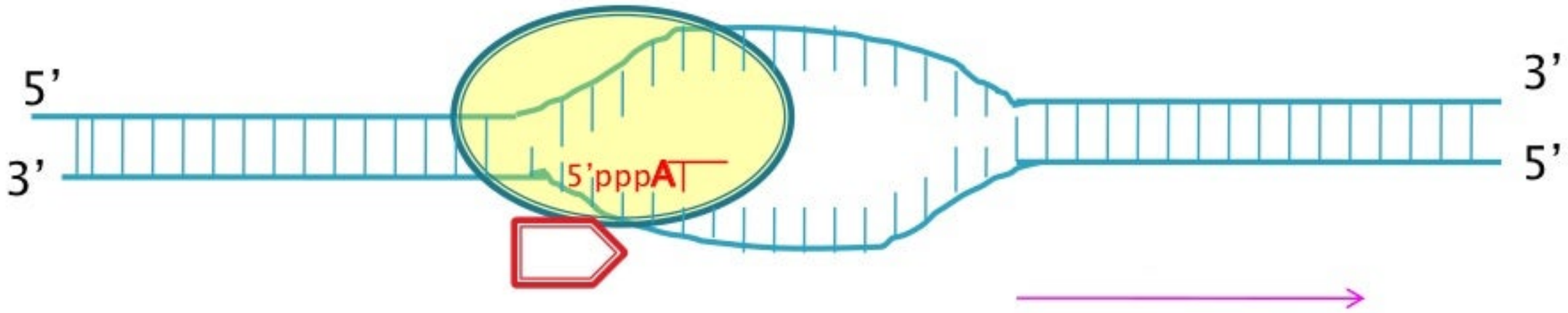
promoter

template



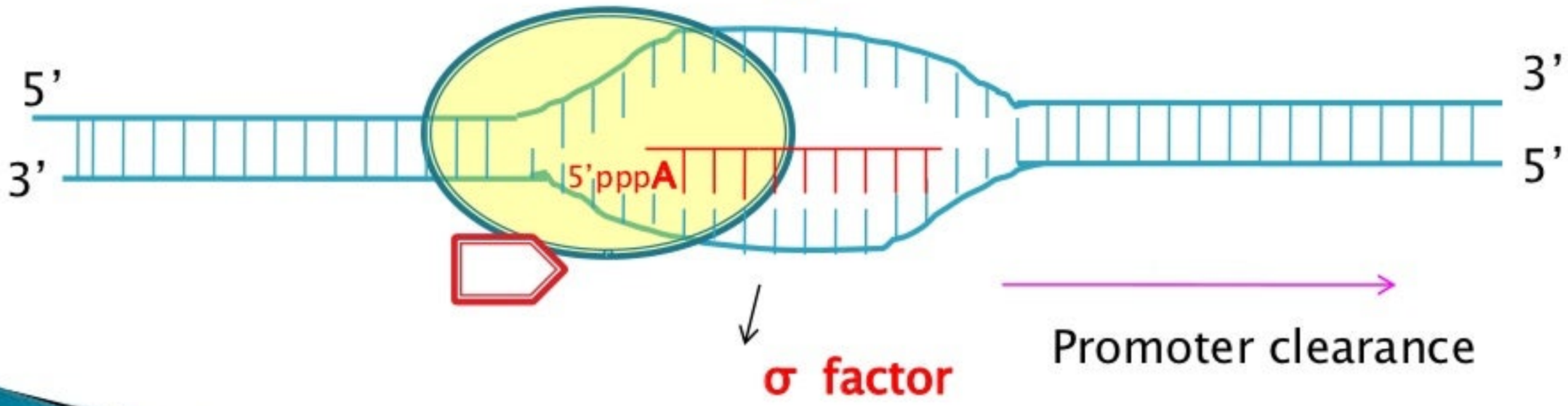
open complex

Initiation



pppA + pppN ⇌ pppApN +ppi

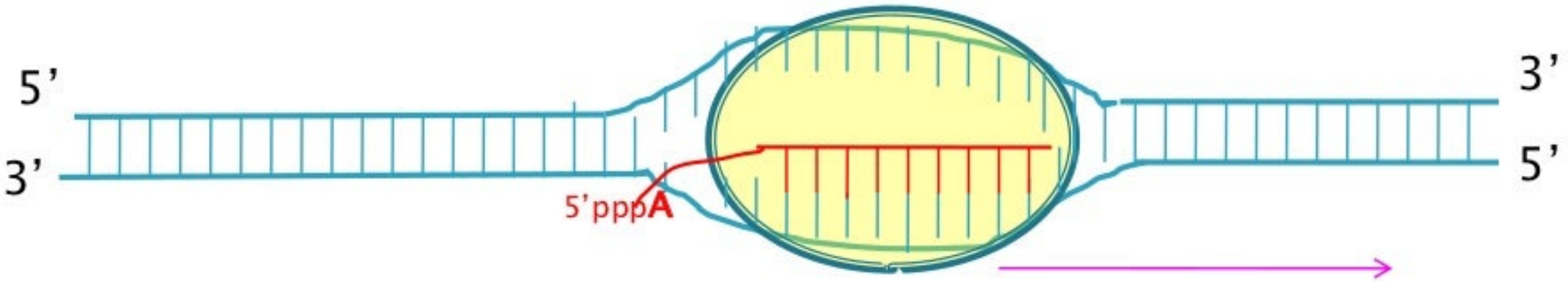
Core enzyme

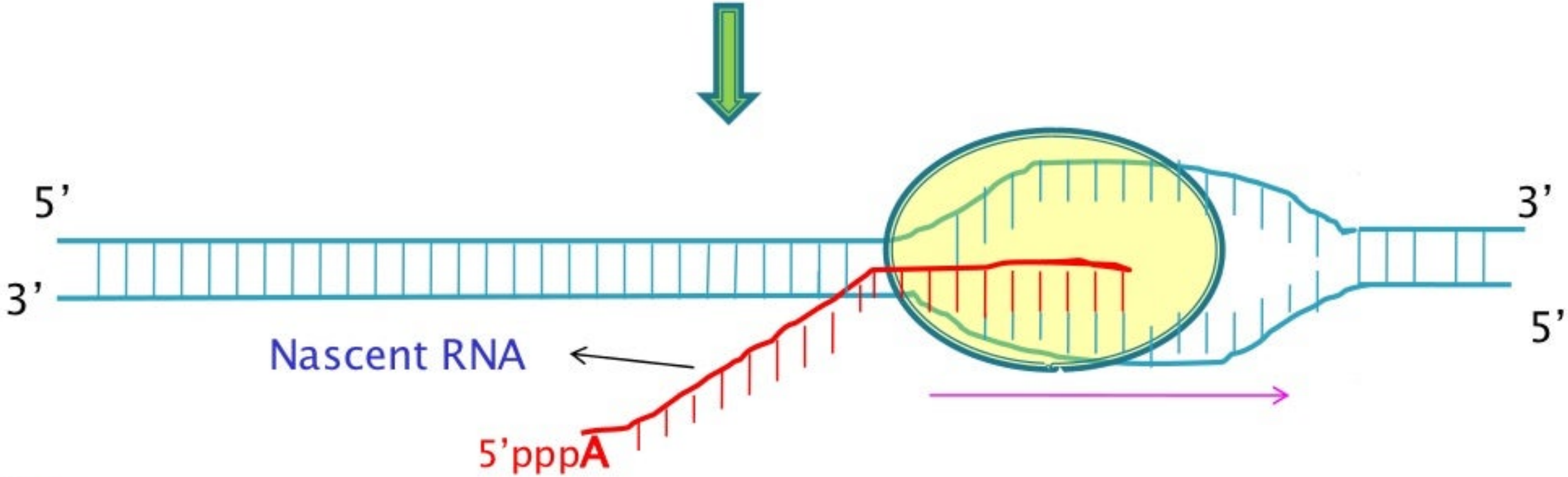


σ factor

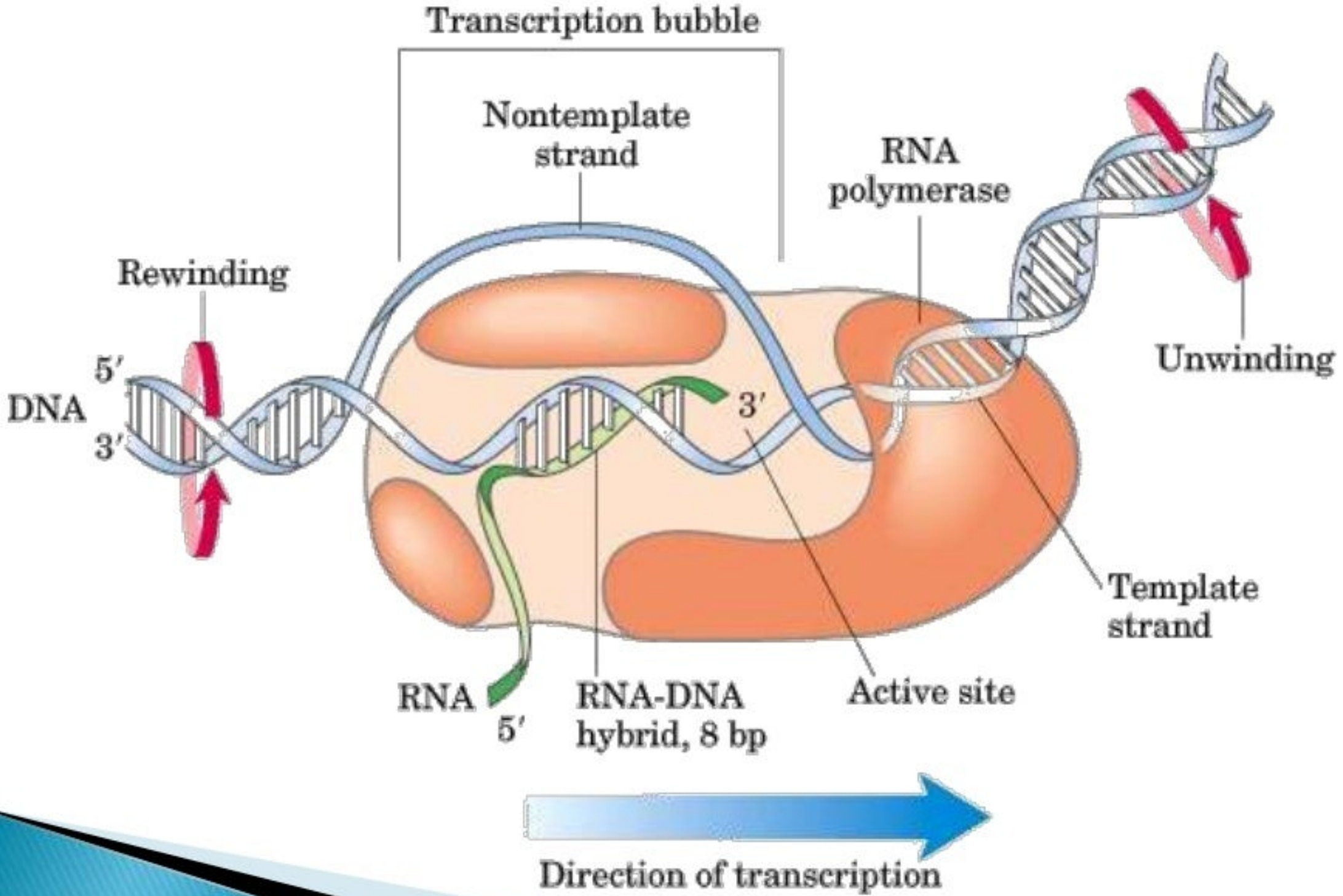
Promoter clearance

Elongation





Transcription bubble



Termination

- The transcribed region of DNA template contain stop signals.
- Prokaryotes have two classes of termination signals.
- 1. relies on protein factor called rho(ρ) rho-dependent termination.
- 2 other is rho-independent termination.

Rho- dependent termination

- Signalled by a sequence in the template strand of the DNA molecule
- Which are 40bp in length & are inverted repeat or hyphenated. These signals recognized by a termination protein, the rho (ρ) factor.

- Rho is an ATP-dependent RNA-stimulated helicase which binds to the signals. Thus RNAP cannot move further, so it dissociates from DNA that disrupts the nascent RNA-DNA complex., release nascent RNA.

TERMINATION

Nascent RNA

Rho factor identifies stop signal

Rho

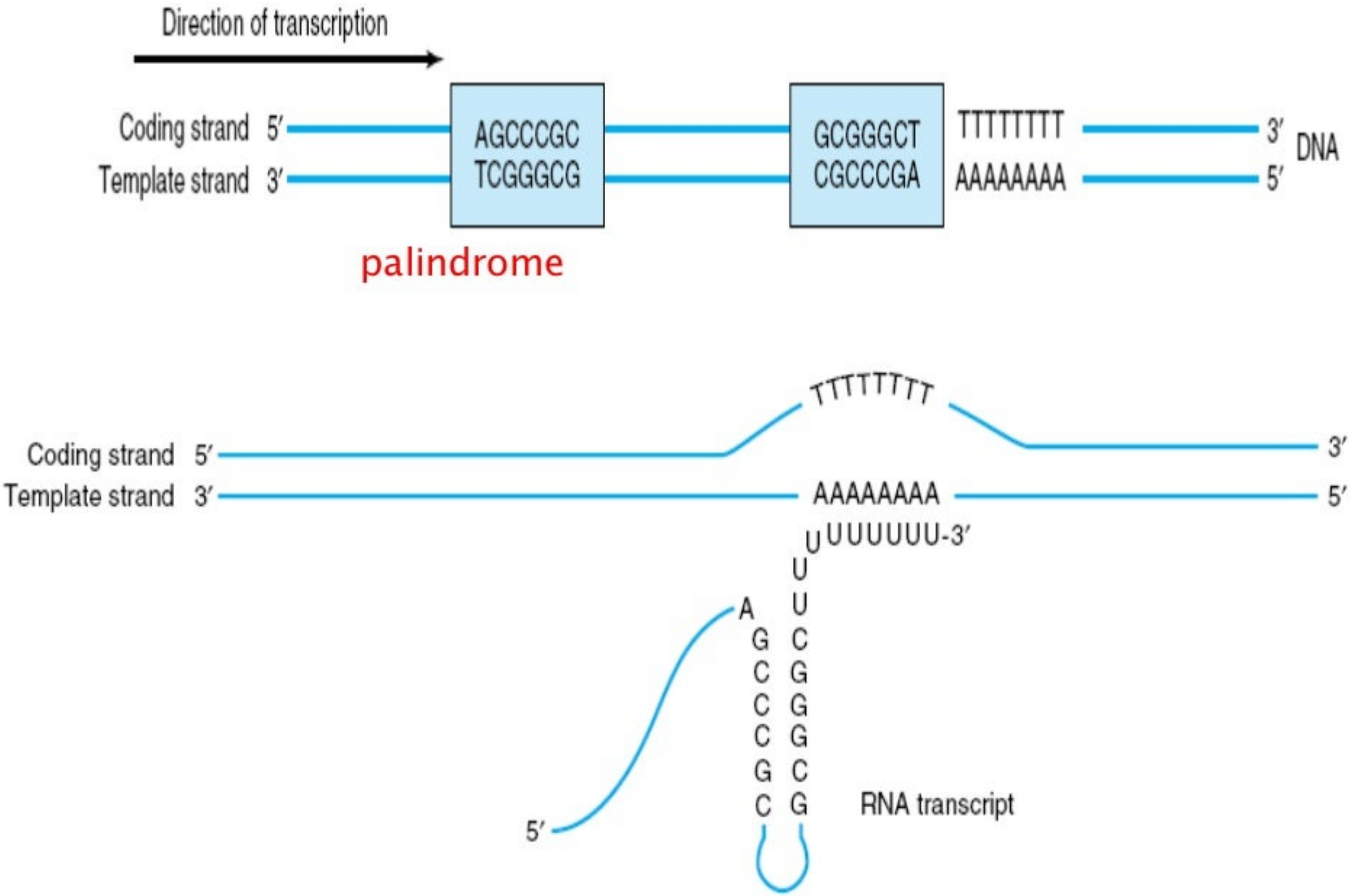
Rho independent

Most -independent terminators have two distinguishing features.

1. One is palindromic G-C rich region which is followed by an A-T rich region.

Thus RNA transcript of this palindrome is self complementary sequences, permitting the formation of a hairpin structure.

2. The second feature is a highly conserved string of **A** residues in the template strand that are transcribed into **U** residues. The RNA transcript ends within or just after them.


Direction of transcription
Coding strand 5′
Template strand 3′
AGCCCGC
TCGGGCG
GCGGGCT
CGCCCGA
TTTTTTTT
AAAAAAAA
3′
5′
DNA
palindrome
TTTTTTTT
Coding strand 5′
Template strand 3′
3′
5′
DNA
AAAAAAAA
UUUUUUU-3′
U
U
A U
G C
C G
C G
C G
G C
C G
5′
RNA transcript

Transcription in Eukaryotes

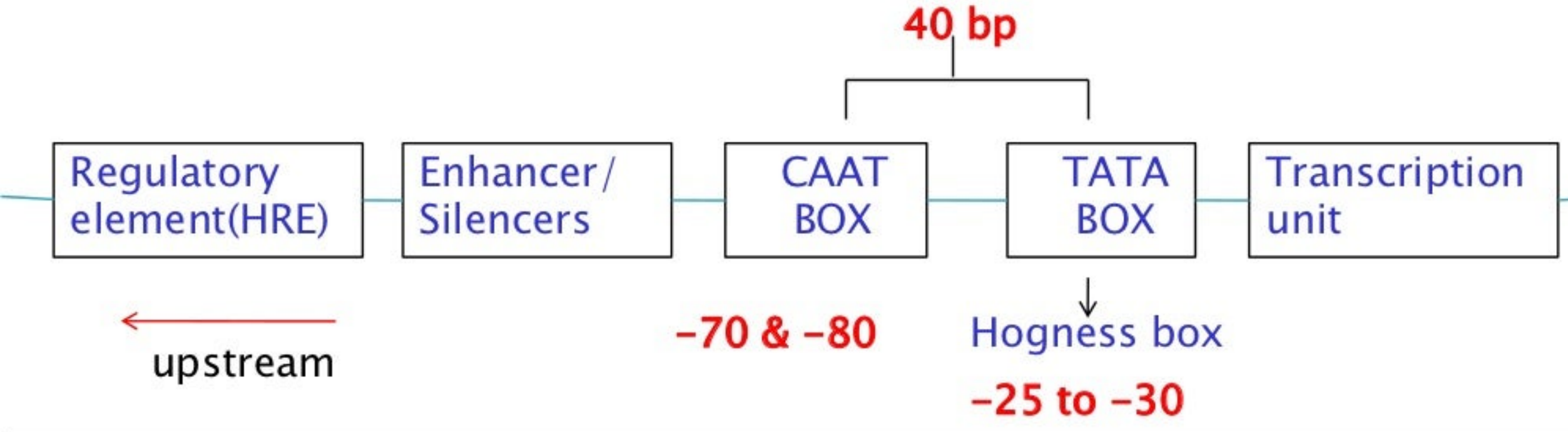
- 1. Much more complicated.
- 2. Three different RNA polymerases.
- 3. Required many transcription factor protein.
- 4. Transcription initiation needs promoter and upstream regulatory regions.
- 5. Enhancers /silencers are DNA sequences that regulate the rate of initiation of transcription by RNA polymerase II

RNA polymerases of eukaryotes

RNA-polymerases	I	II	III
products	rRNA (28S,18S, 5.8S)	mRNA (hn RNA) snRNA	5S rRNA tRNA
Sensitivity to Amanitin	No	high	moderate

Amanitin is a specific inhibitor of RNA polymerases .

Eukaryote Promoters



Post transcriptional modifications

- The nascent RNA, also known as primary transcript, needs to be modified to become functional tRNAs, rRNAs, and mRNA.

- _Primary transcripts of mRNA are called as heterogenous nuclear RNA (hnRNA).

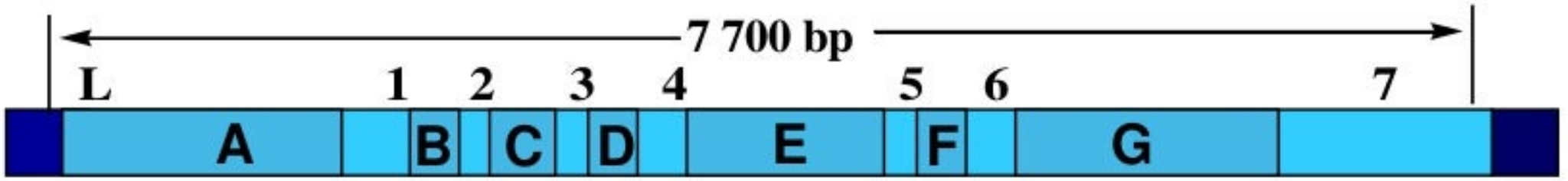
- hnRNA are larger than matured mRNA .

Post transcriptional modifications

- Splicing
- Addition of 5’ cap
- Creation of poly A tail
- RNA editing

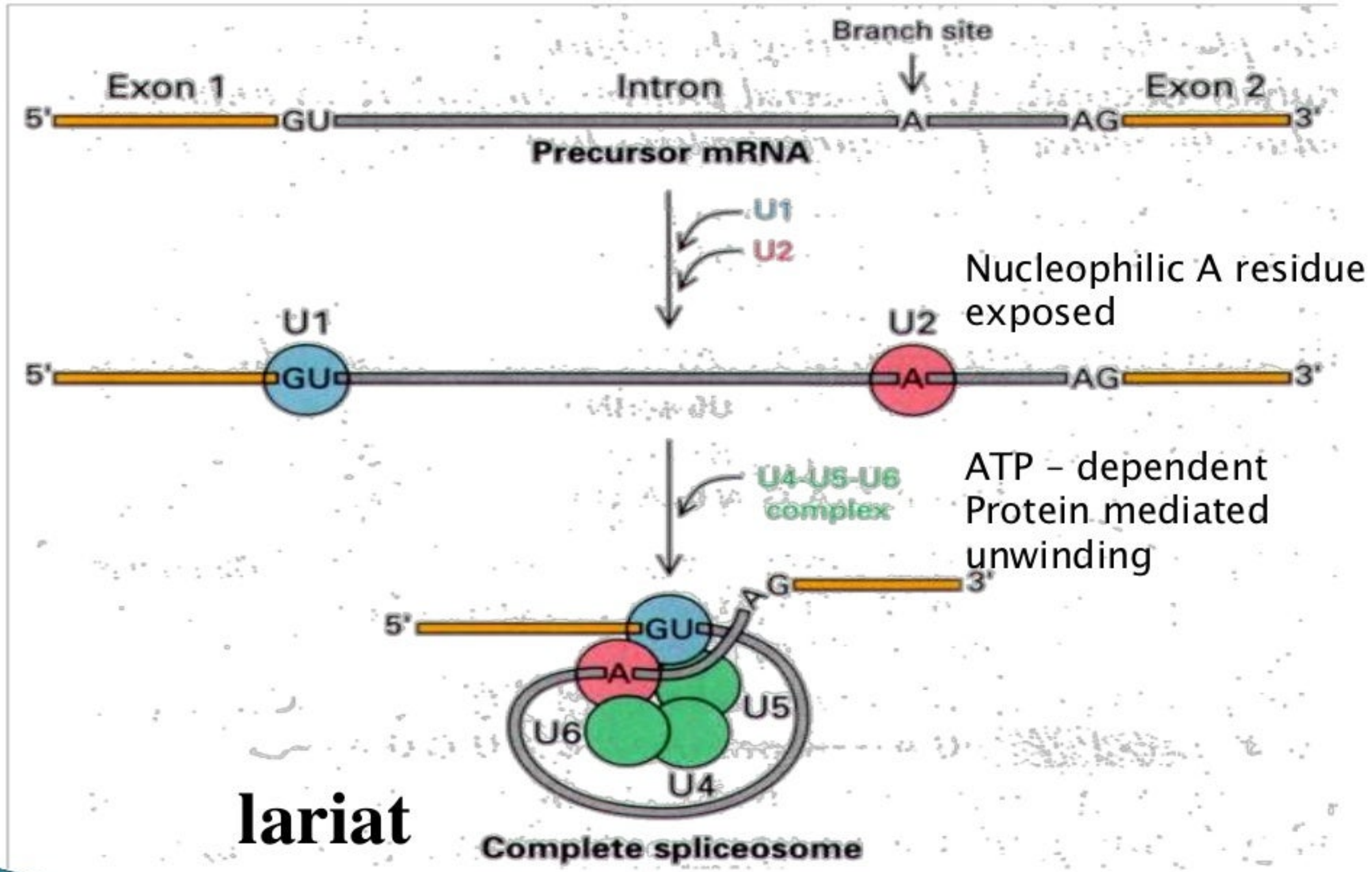
Splicing

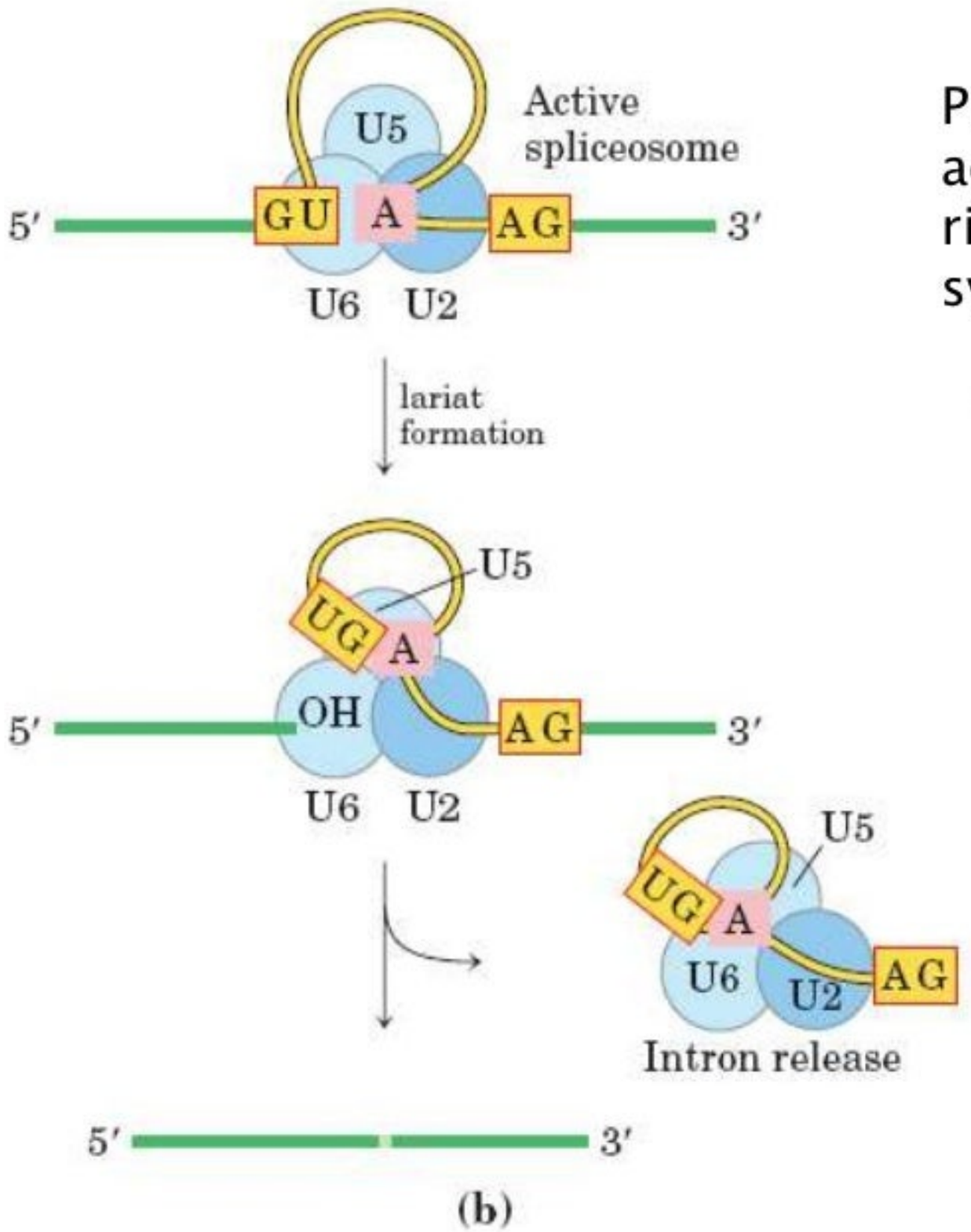
The structural genes are composed of coding and non-coding regions that are alternatively separated.



A-G non-coding region 1-7 coding region

Noncoding sequences called intervening sequences or Introns & coding sequences called Exons


Branch site
Exon 1
Intron
Exon 2
5'
GU
A
AG
3'
Precursor mRNA
U1
U2
Nucleophilic A residue exposed
U1
U2
5'
GU
A
AG
3'
U4-U5-U6 complex
ATP – dependent Protein mediated unwinding
3'
A G
5'
GU
A
U5
U6
U4
lariat
Complete spliceosome



(b)

Production of auto antibodies against small nuclear rionucleoprotein (SnRNPs) cause systemic lupus erytromatosis.

5’capping

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.

Formation of cap require three steps.

1 5’ terminal phosphate group removes by phosphatase enzyme leaving diphosphate.

2 GTP is added by releasing pyrophosphate.

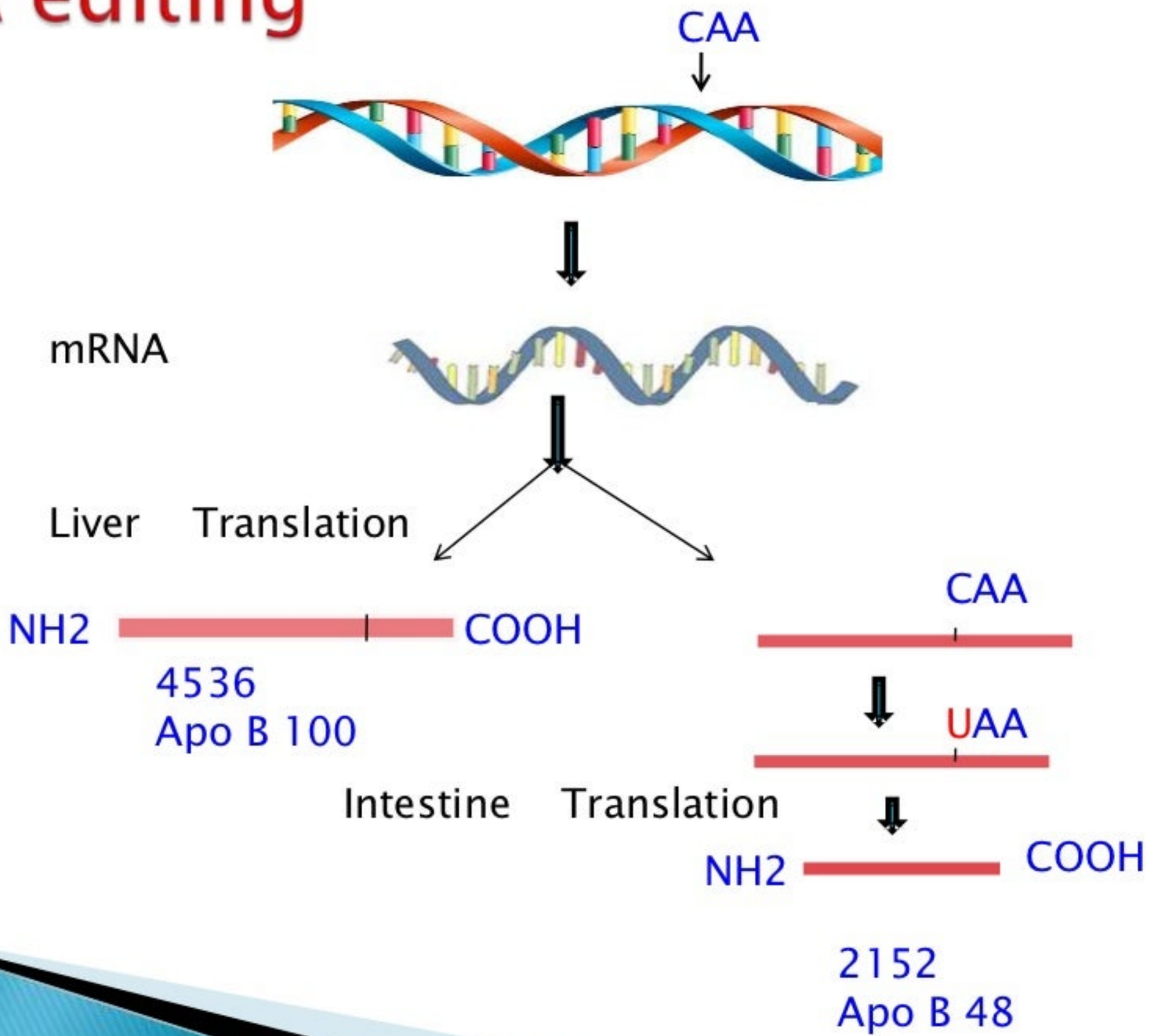
3 7th N of guanine is methylated by methyl transferase enzyme. Methyl group donor is S-adenosyl methionine.

Poly A tail

- At their 3’ end, most eukaryotic mRNAs have a string of 40 to 200 adenine residues, making up the **poly(A) tail.**

- Adenine nucleotides are added by enzyme adenylate transferase.

RNA editing


CAA
mRNA
Liver Translation
NH2
COOH
4536
Apo B 100
CAA
UAA
Intestine Translation
NH2
COOH
2152
Apo B 48

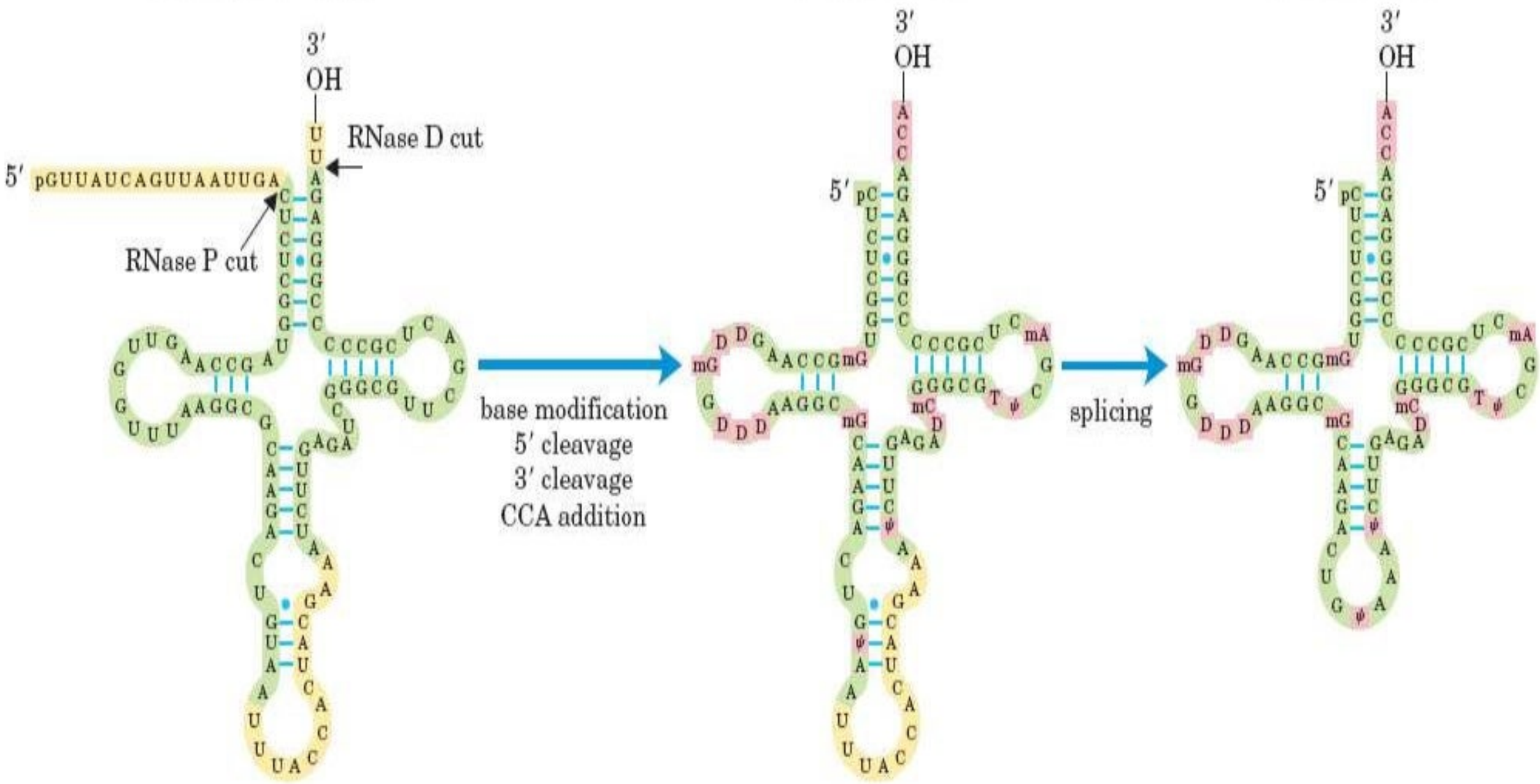
Modification of tRNA

The primary transcripts of prokaryotic 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).

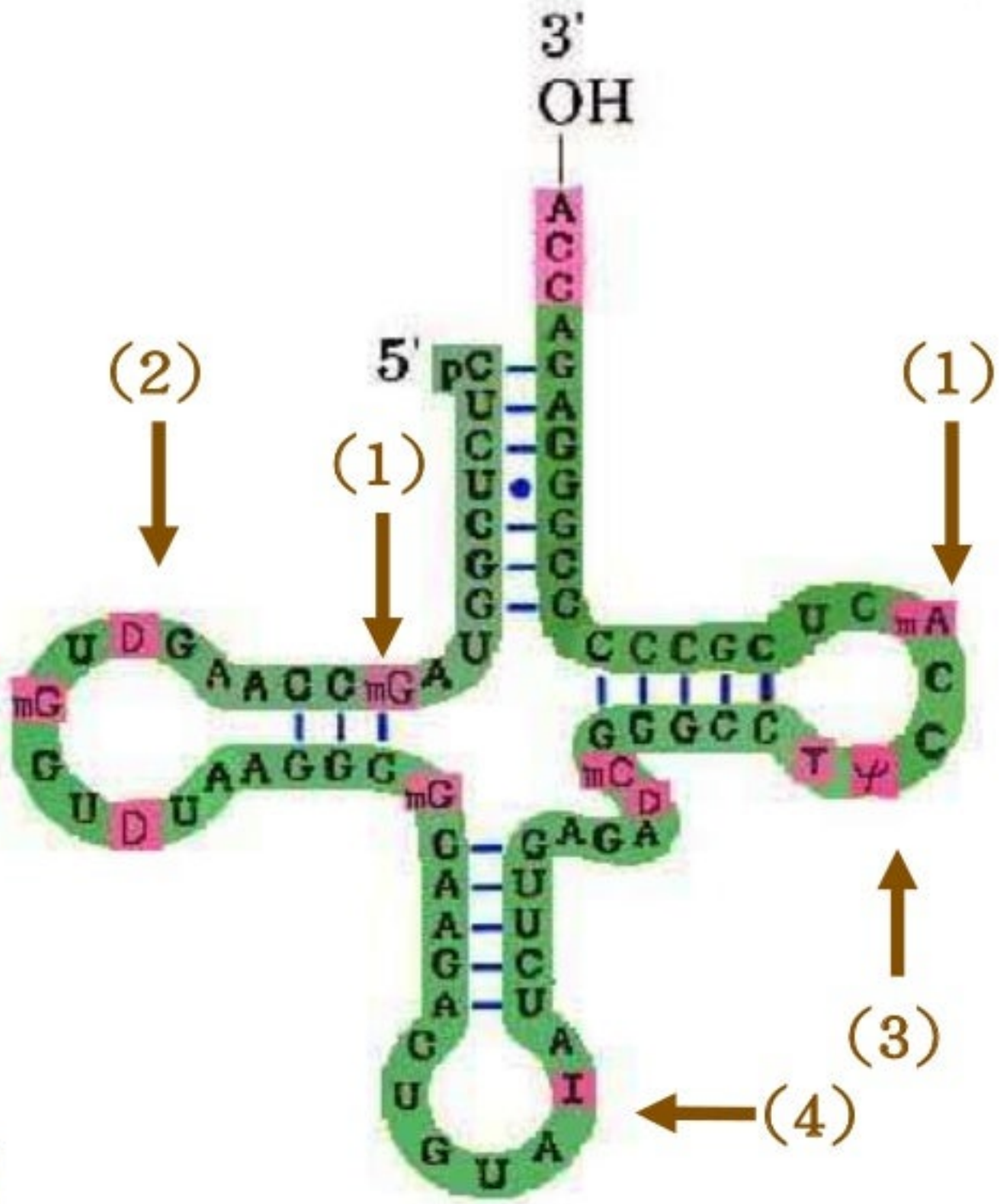
Primary transcript

Intermediate

Mature tRNA^{Tyr}

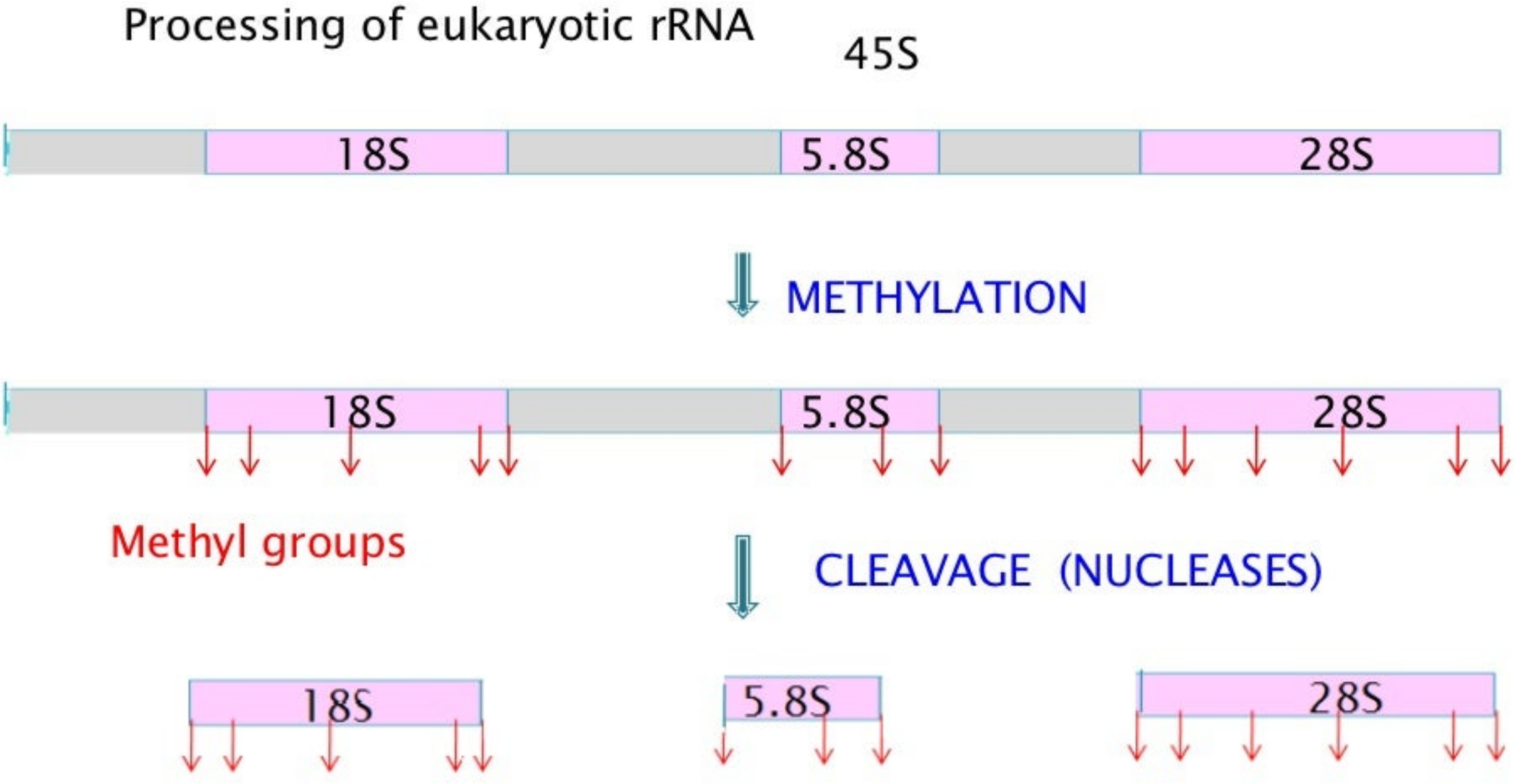


Base modification



1. **Methylation**
   A→mA, G→mG
2. **Reduction**
   U→DHU
3. **Transversion**
   U→ψ
4. **Deamination**
   A→I

Processing of eukaryotic rRNA

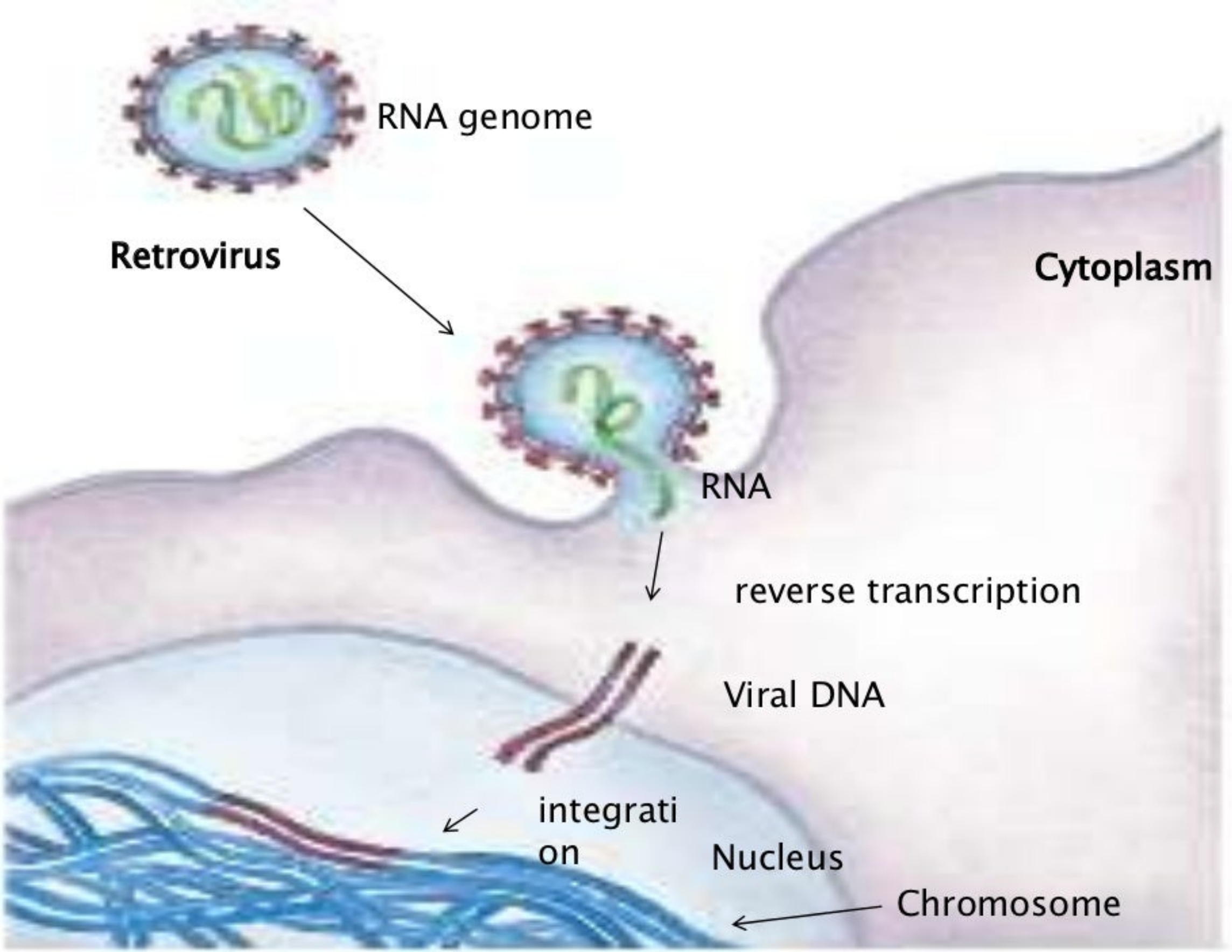


Inhibitors

- Actinomycin D- it binds with DNA template & blocks the movement of RNAP.
- Rifampicin- antibiotic used to treat tuberculosis. It binds with β subunit of prokaryotic RNAP.
- A-Amanitin- Toxin produced by Amanita phalloides, mushroom. It tightly binds with eukaryotic RNA polymerase-II. Thus it inhibits mRNA synthesis.

Reverse transcription

- Retrovirus possess RNA as genetic material
- Enzyme – RNA dependent DNA polymerase
- DNA complementary to viral RNA
- cDNA can be used as a probe to identify the sequence of DNA in genes


RNA genome
Retrovirus
Cytoplasm
RNA
reverse transcription
Viral DNA
integrati
on
Nucleus
Chromosome

- The first nucleotide (the start site) of a transcribed DNA sequence is denoted as +1 and the second one as +2; the
- nucleotide preceding the start site is denoted as -1. These designations refer to the coding strand of DNA. Recall that the
- sequence of the *template strand of DNA is the complement of that of the RNA transcript (see Figure 5.26). In contrast,*
- the *coding strand of DNA has the same sequence as that of the RNA transcript except for thymine (T) in place of uracil*
- (U). The coding strand is also known as the *sense (+) strand, and the template strand as the antisense (-) strand.*