MOLECULAR BIOLOGY: TRANSCRIPTION IN EUKARYOTES AND POST-TRANSCRIPTIONAL MODIFICATION

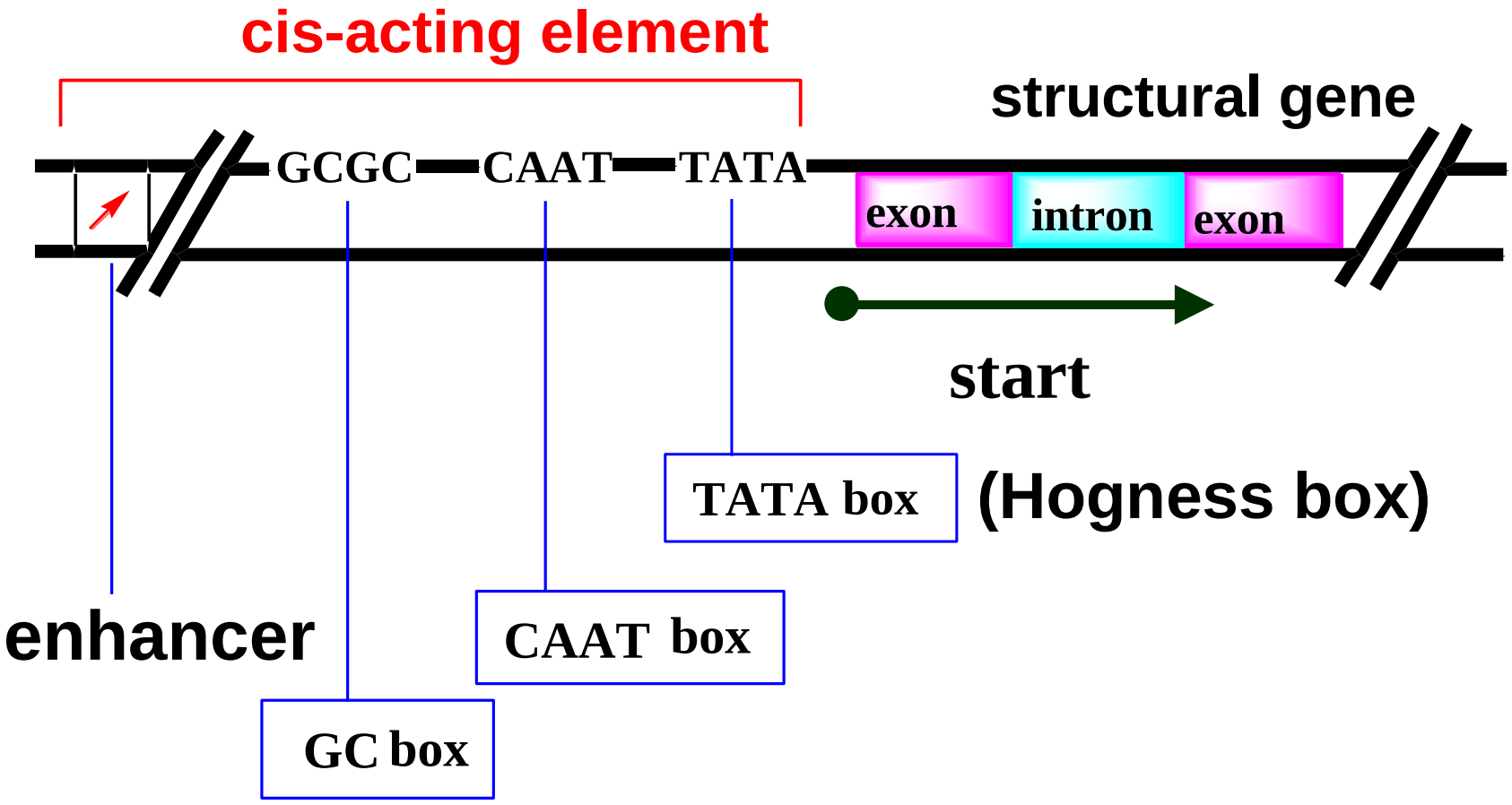
Lecture 3

Transcription in Eukaryotes

Initiation

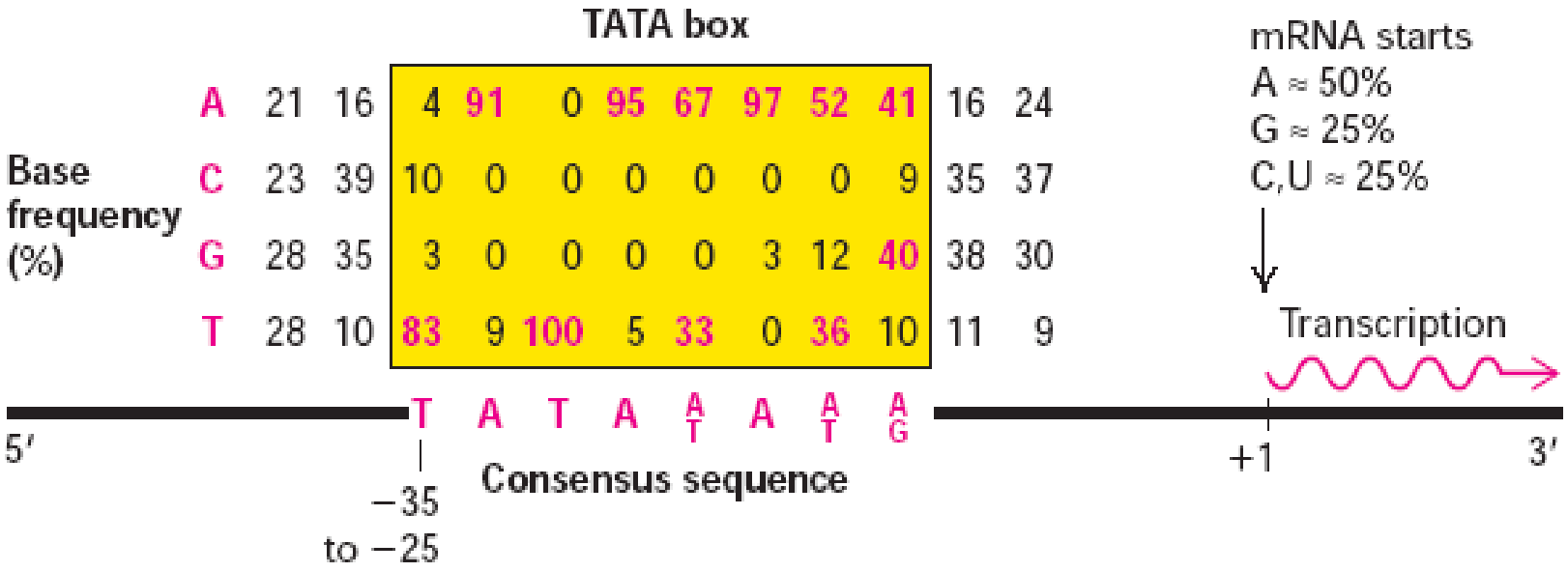
- Transcription initiation needs promoter and upstream regulatory regions.
- The cis-acting elements are the specific sequences on the DNA template that regulate the transcription of one or more genes.
- Promoters are called cis-acting elements because they are always on the same molecule of DNA as the genes being transcribed.
- Cis-acting elements are binding sites for proteins called transcription factors

Cis-acting element



TATA box

•An element with the consensus sequence TATAAAA that begins about 25–30 bp upstream of the start of transcription in most eukaryotic promoters recognized by RNA polymerase II.



By convention, the site at which RNA polymerase begins transcription is numbered +1. Downstream denotes the direction in which a template DNA strand is transcribed i.e. toward 3' end relative to the start site while Upstream denotes the opposite direction. Nucleotide positions in the DNA sequence downstream from a start site are indicated by a positive (+) sign; those upstream, by a negative (-) sign.

RNA-polymerase of eukaryotes

RNA-pol	I	II	III
products	45S rRNA	hnRNA	5S rRNA tRNA snRNA
Sensitivity to Amanitin	No	high	moderate

α-amanitin (from *Amanita phalloides* , a highly poisonous species of mushroom) is a specific inhibitor of RNA-polymerase. This toxin can cause catastrophic and fatal liver damage within 48 hrs.

Amanita phalloides is also called the 'death cup' or 'destroying angel'

Transcription factors (TFs)

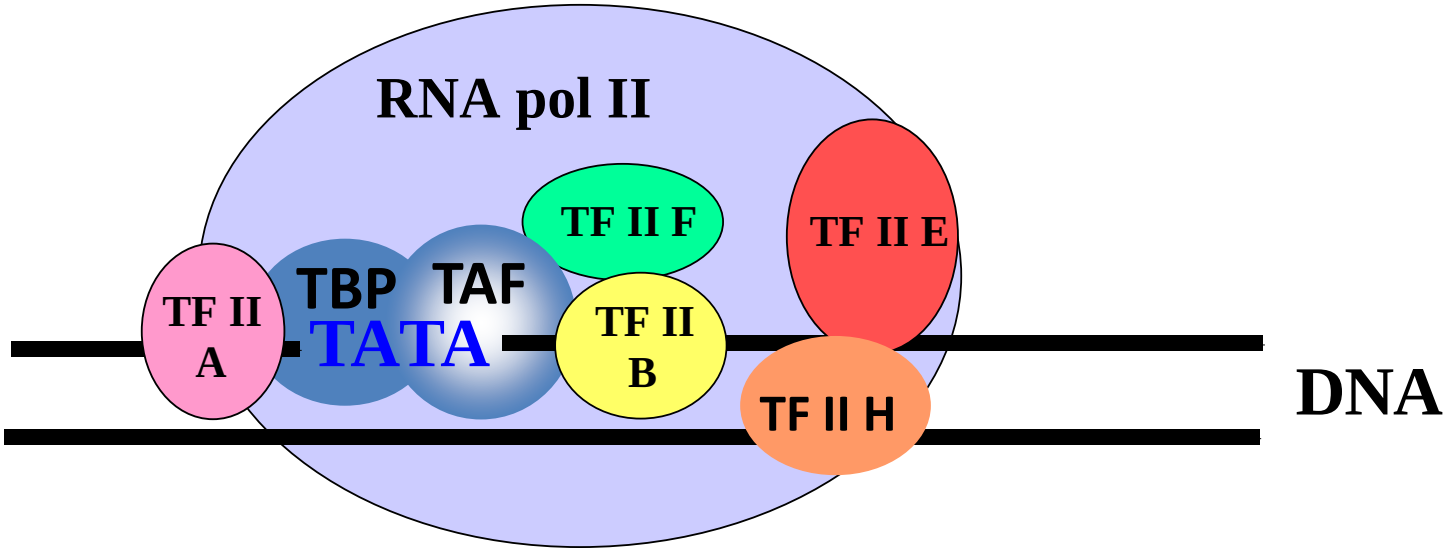
- Unlike in prokaryotes, in eukaryotic systems RNA-polymerase does not bind the promoter directly.
- These RNA polymerases require various protein factors (called transcription factors) to help them locate promoters and initiate transcription.
- RNA-polymerase II associates with six transcription factors, TFII A - TFII H.
- The trans-acting factors are the proteins that recognize and bind directly or indirectly cis-acting elements and regulate its activity.

TF for eukaryotic transcription

Transcription protein	Function (s)
TBP (TATA-Binding protein)	Specifically binds TATA Box in class II promoters
TFIIA	Stabilizes the binding of TFIID to the promoter
TFIIB	Helps TFIIF plus RNA polymerase II bind to the promoter
TFIID	serves as a nucleation site around which the preinitiation complex (PIC) assembles.
TFIIE	Recruits TFIIH; Has ATPase and helicase activities
TFIIF	Prevents binding of pol II to nonspecific DNA sequences
TFIIH	Has protein kinase activity (Phosphorylates pol II within the CTD) and DNA helicase activity (Unwinds DNA at promoter). Recruits nucleotide-excision repair proteins

Formation of the Pre-initiation complex (PIC)

- TBP of TFII D binds TATA
- TFII A and TFII B bind TFII D
- TFII F-RNA-pol complex binds TFII B
- TFII F and TFII E open the dsDNA (helicase and ATPase)
- TFII H: completion of PIC



Phosphorylation of RNA-polymerase

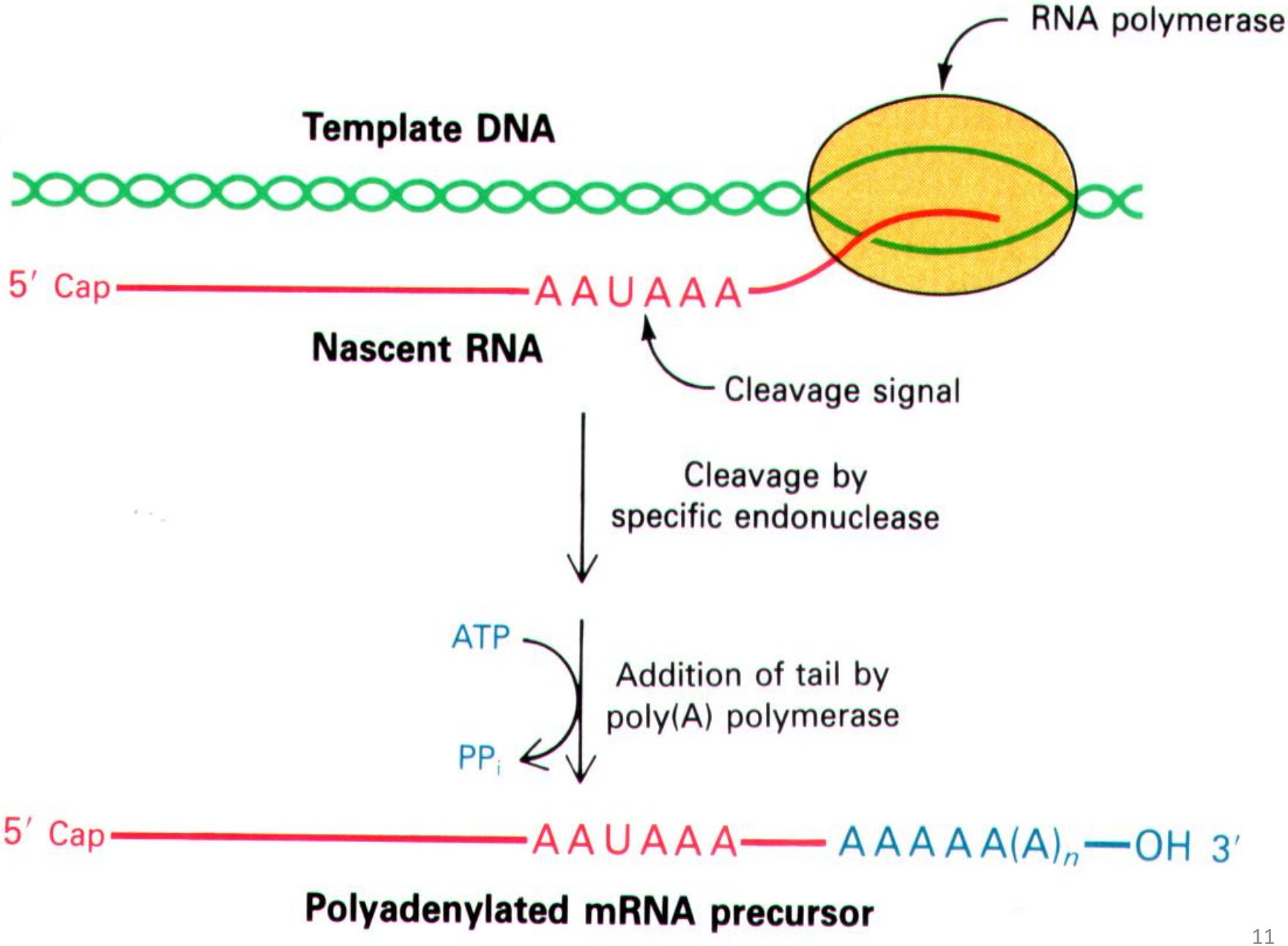
- TF II H has protein kinase activity to phosphorylate CTD of RNA-polymerase(CTD is the C-terminal domain of RNA-pol)
- Only the phosphorylated RNA-pol can move toward the downstream, starting the elongation phase.
- Most of the TFs fall off from PIC during the elongation phase.

Elongation

- The elongation is similar to that of prokaryotes.
- Unlike in prokaryotes, for eukaryotic systems transcription and translation do not take place simultaneously since they are separated by nuclear membrane.

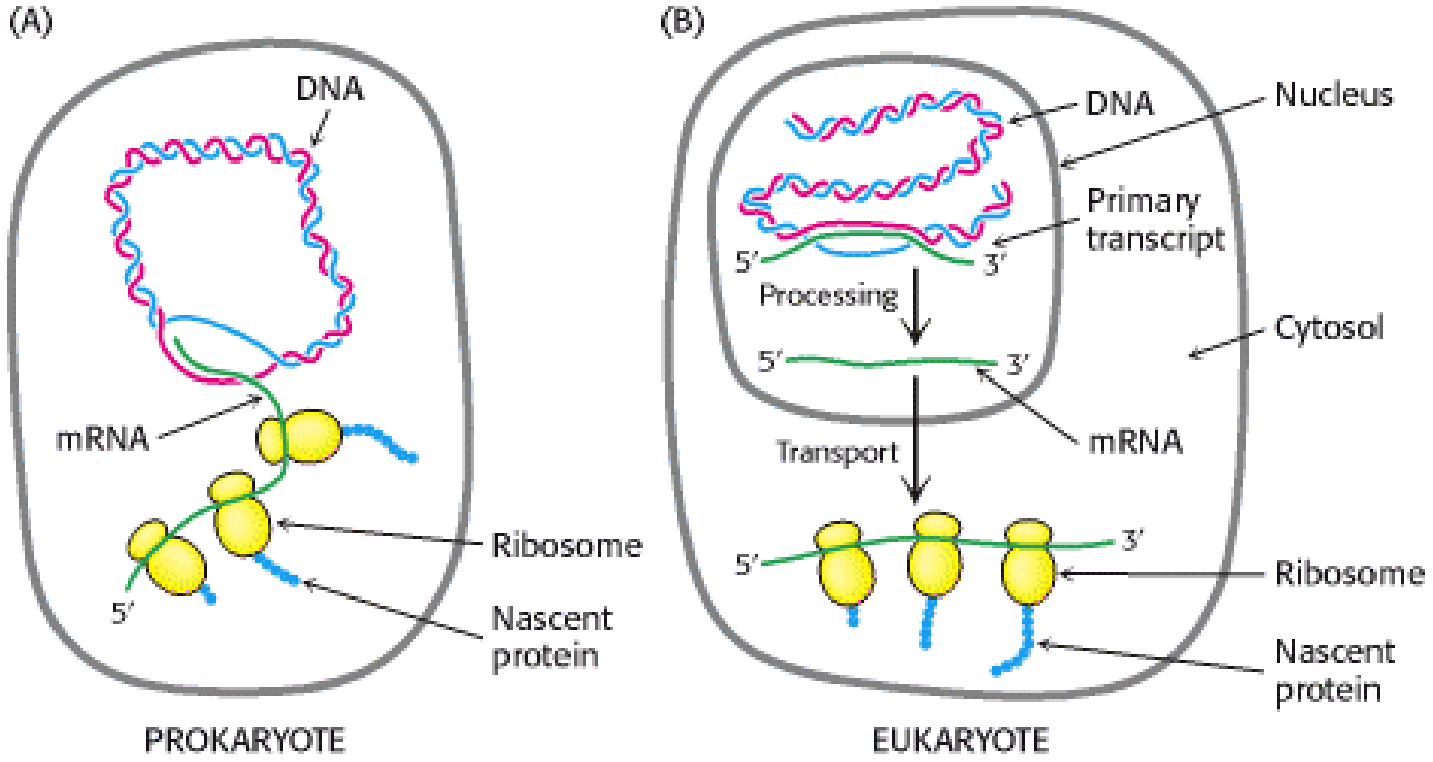
Termination

- The termination sequence is AATAAA followed by GT repeats.
- The termination is closely related to the post - transcriptional modification.


RNA polymerase
Template DNA
5′ Cap
A A U A A A
Nascent RNA
Cleavage signal
Cleavage by
specific endonuclease
ATP
PPi
Addition of tail by
poly(A) polymerase
5′ Cap
A A U A A A
A A A A A(A)n
OH 3′
Polyadenylated mRNA precursor

Post-Transcriptional Modification

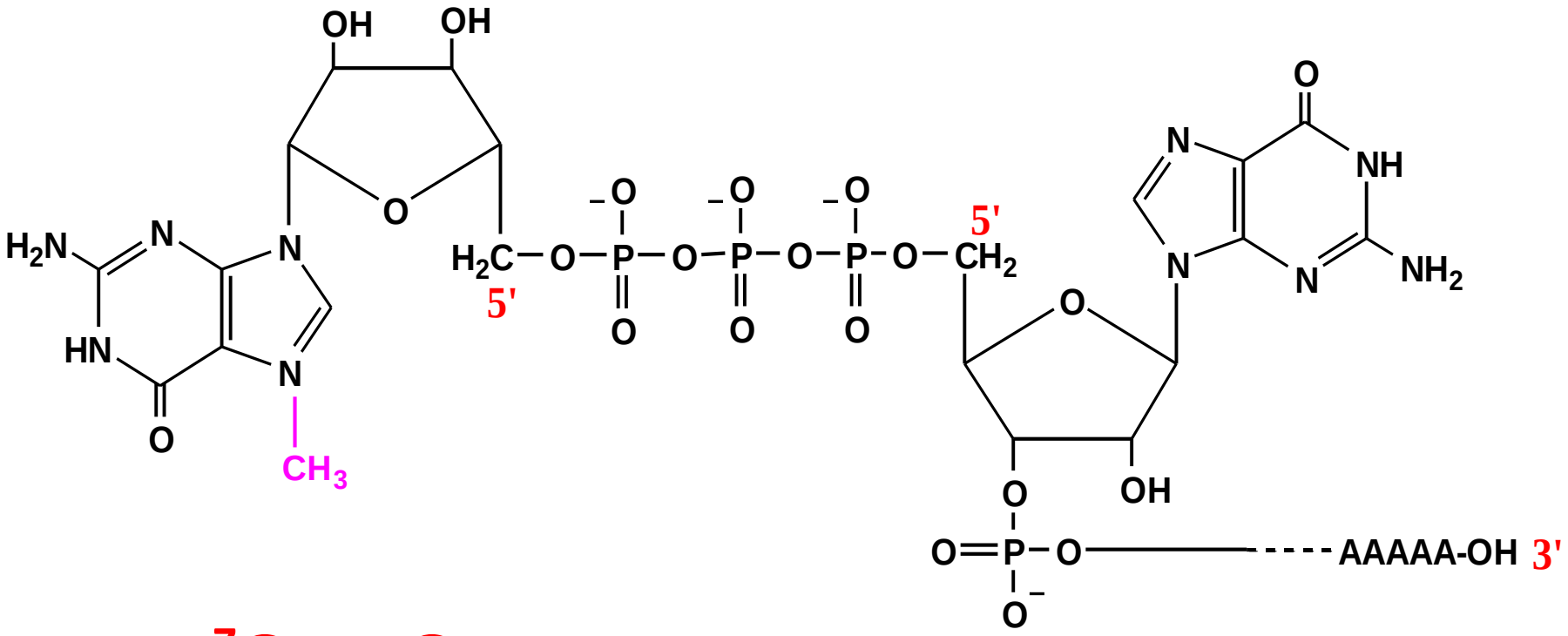
- The nascent RNA, also known as primary transcript, needs to be modified to become functional tRNAs, rRNAs, and mRNAs.
- The modification is critical to eukaryotic systems.



Modification of hnRNA

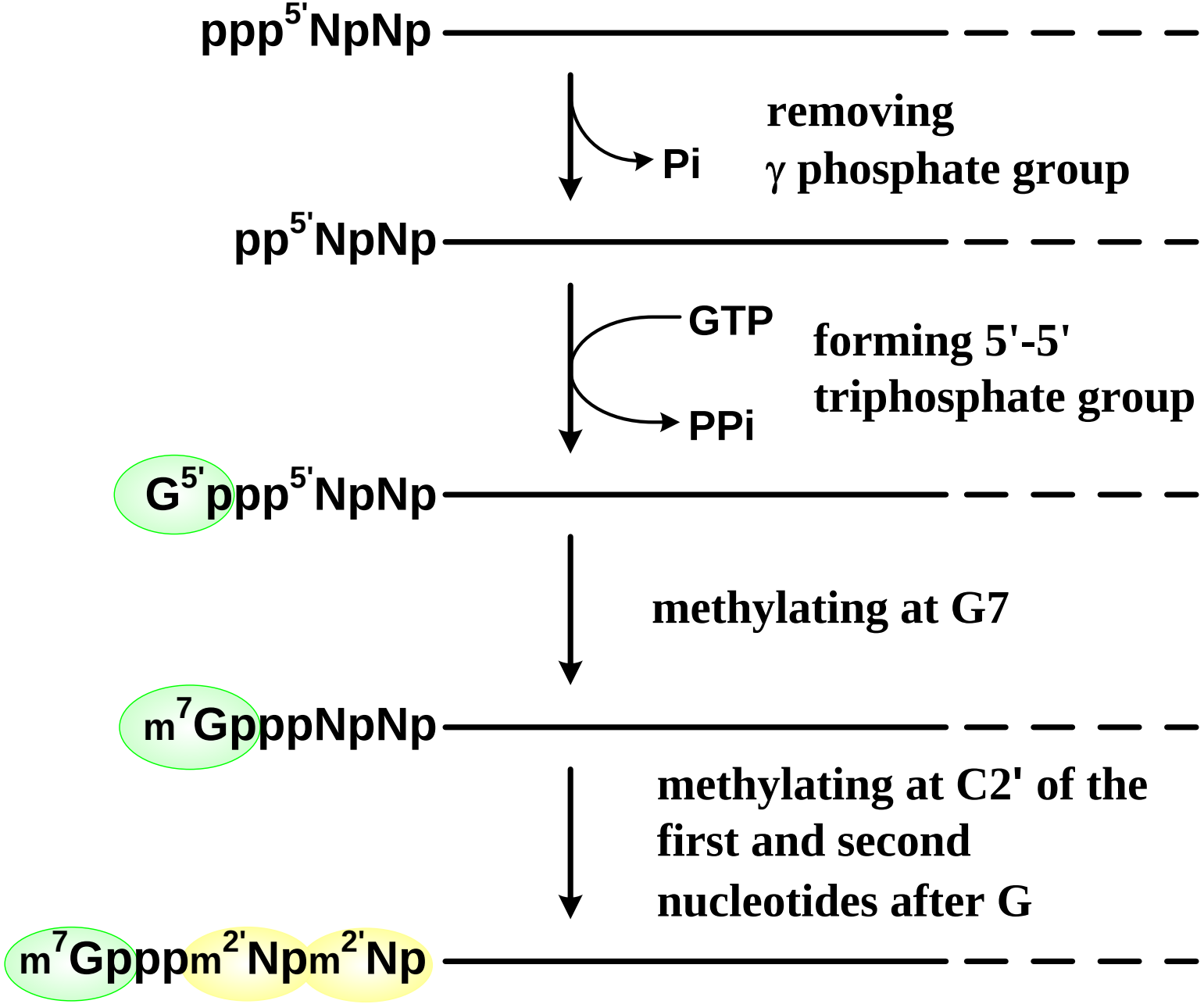
- Primary transcripts of mRNA are called as heteronuclear RNA (hnRNA).
- hnRNA are larger than matured mRNA by many folds.
- Modification includes
  - Capping at the 5′- end
  - Tailing at the 3′- end
  - mRNA splicing
  - RNA editing

Capping at the 5′- end



$m^7GpppGp$----

•A 5’cap is a residue of 7-methylguanosine linked to the 5’-terminal residue of the mRNA through an unusual 5’,5’-triphosphate linkage. The 5’cap helps protect mRNA from ribonucleases and assists in its export to the cytoplasm. It also facilitates initiation of translation.


ppp5'NpNp
Pi
removing
γ phosphate group
pp5'NpNp
GTP
PPi
forming 5'-5'
triphosphate group
G5'ppp5'NpNp
methylating at G7
m7GpppNpNp
methylating at C2' of the
first and second
nucleotides after G
m7Gpppm2'Npm2'Np

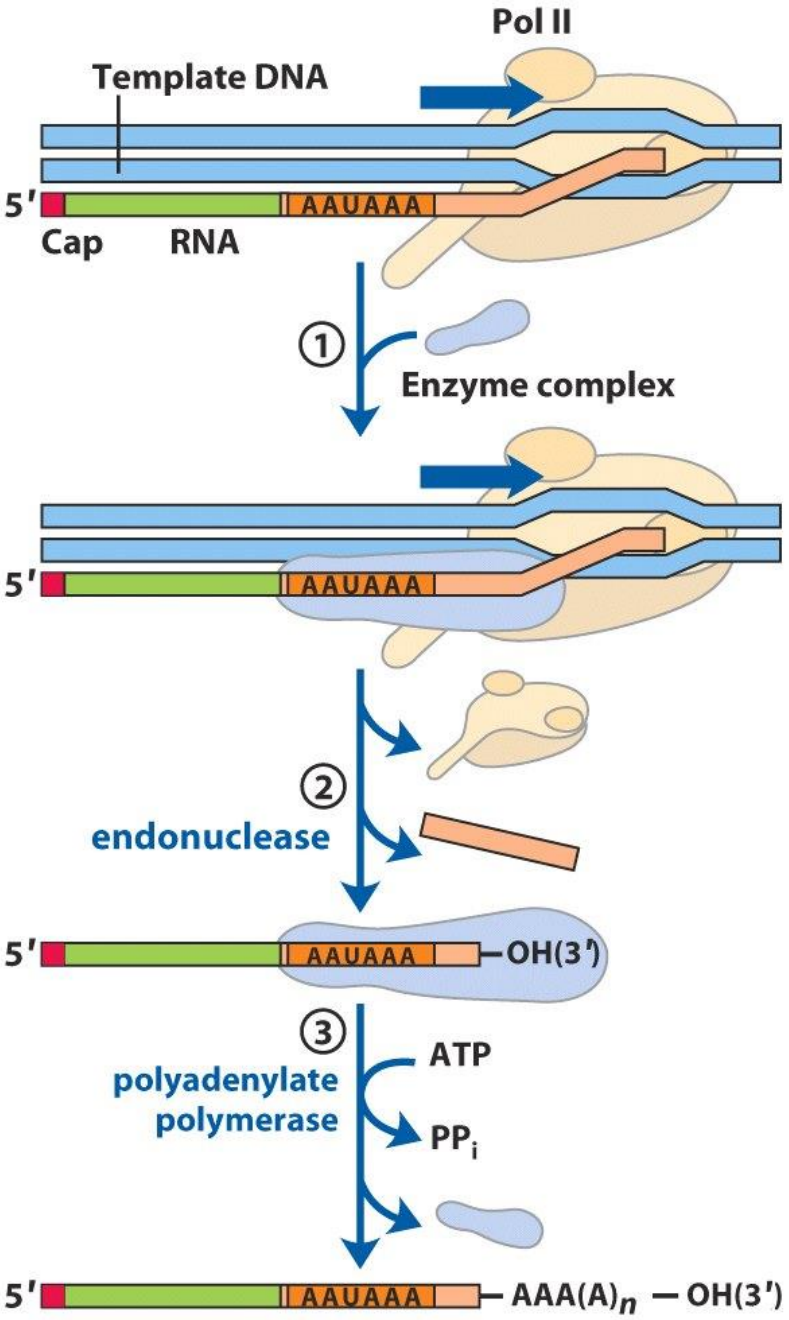
Poly-A tailing at 3′ - end

- There is no poly(dT) sequence on the DNA template. $\Rightarrow$ The tailing process does not depend on the template.
- The tailing process occurs prior to the splicing.
- The tailing process takes place in the nucleus

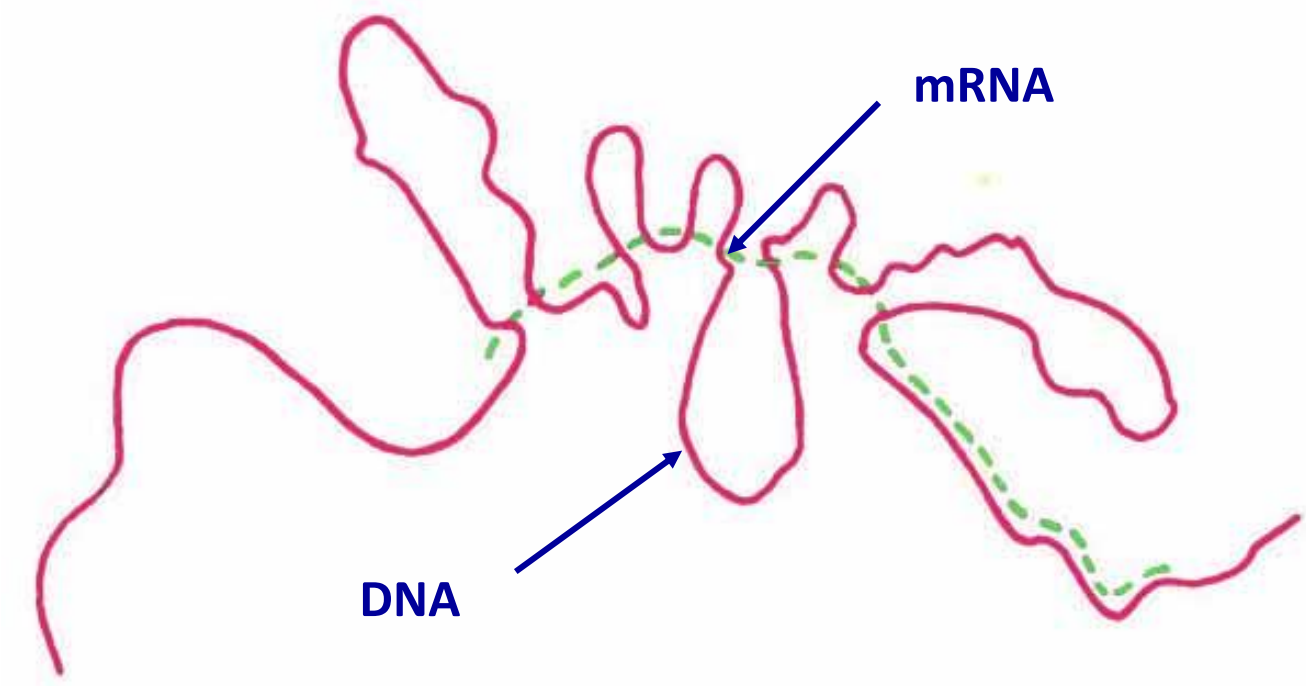
•At their 3’ end, most eukaryotic mRNAs have a string of 80 to 250 A residues, making up the poly(A) tail.

•This tail serves as a binding site for one or more specific proteins. The poly(A) tail and its associated proteins probably help protect mRNA from enzymatic destruction.

•The polyadenylate polymerase synthesizes a poly(A) tail 80 to 250 nucleotides long, beginning at the cleavage site.



mRNA splicing



The matured mRNAs are much shorter than the DNA templates.
Transcripts from genes containing introns undergo splicing, the removal of the introns and joining of the exons
Distinct isoforms of multidomain proteins often are expressed in specific cell types as the result of alternative splicing of exons.

Exon and intron

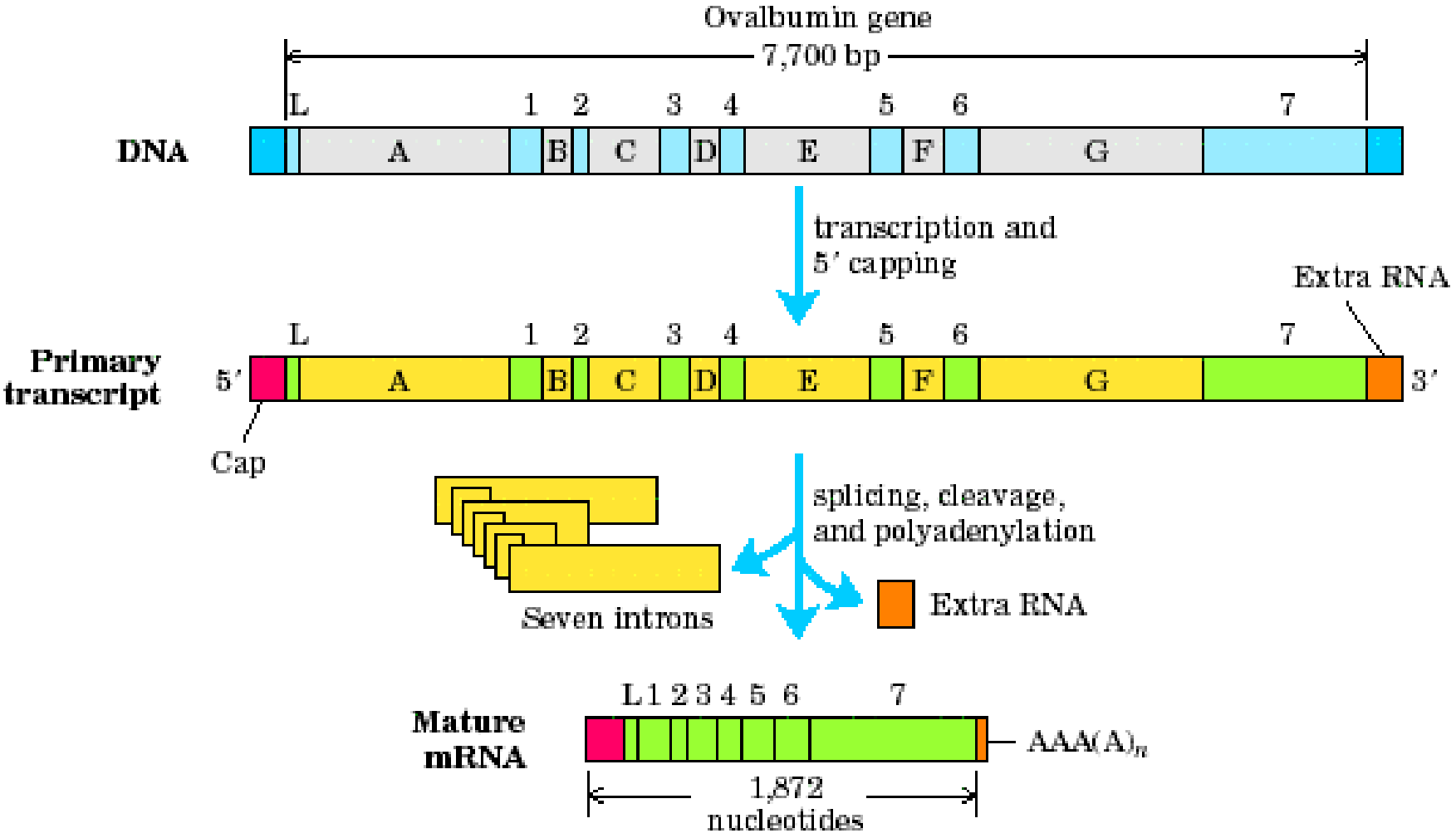
Exons are the coding sequences that appear on split genes and primary transcripts, and will be expressed to matured mRNA.

Introns are the non-coding sequences that are transcribed into primary mRNAs, and will be cleaved out in the later splicing process.

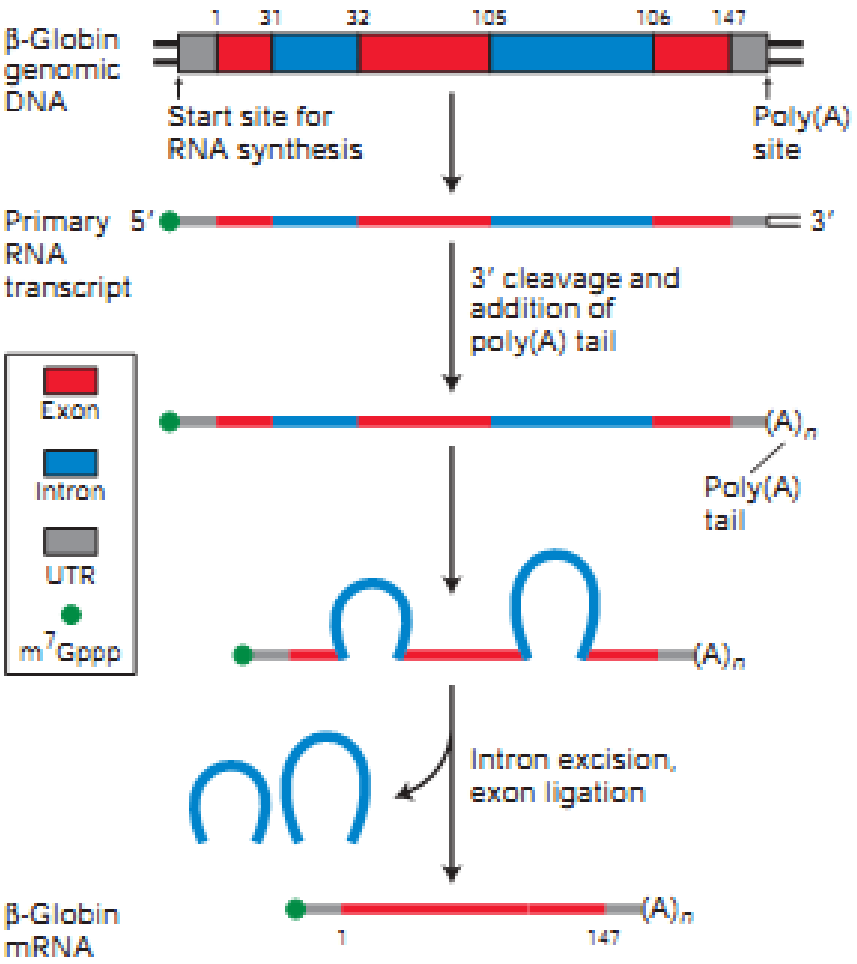
Messenger RNA splicing is an intricate process dependent on many molecular events.

Some of the mutations leading to **β-thalassemia** interfere with the splicing of β-globin mRNA precursors. This causes genetic defect called sickle cell anemia (HbS).

mRNA splicing



Splicing of the β-globin mRNA



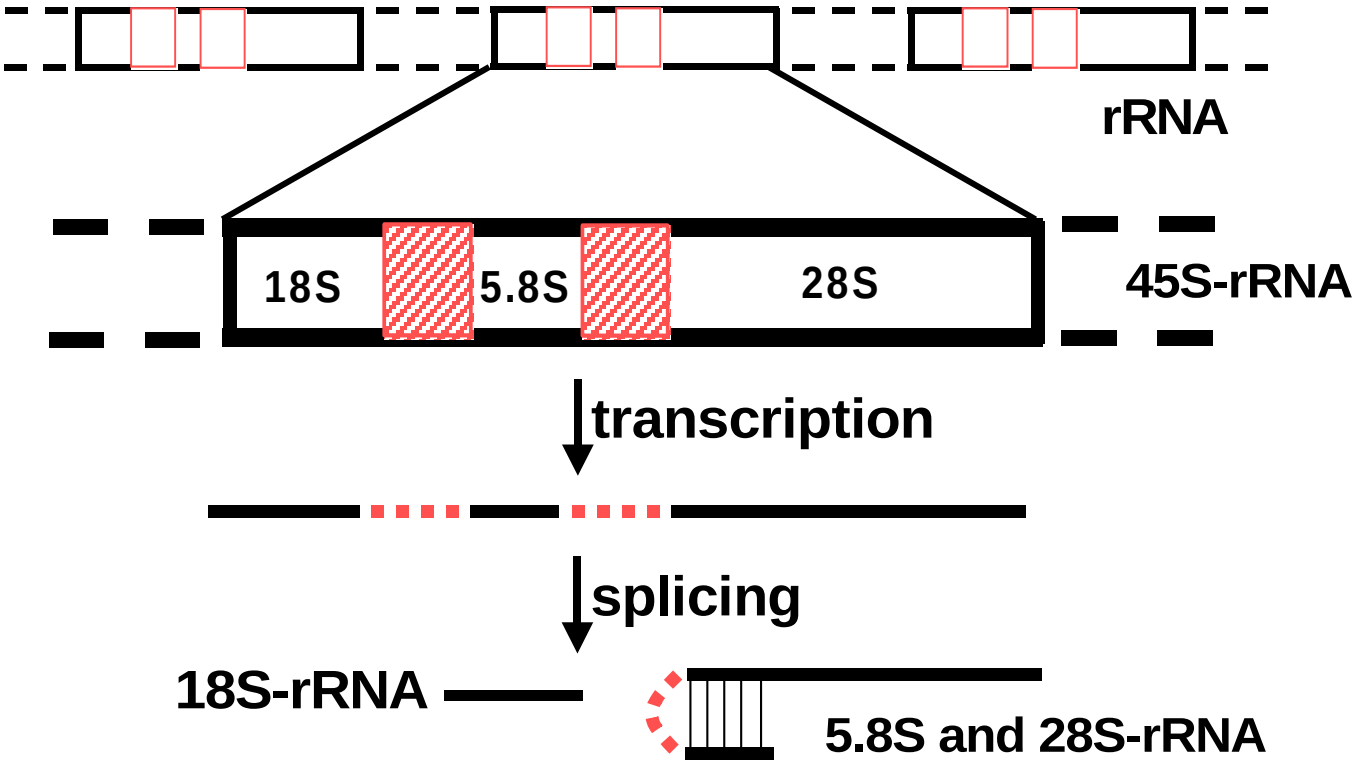
Splicing removes the introns and joins the exons.

The small numbers refer to positions in the 147* amino acid sequence of β-globin.

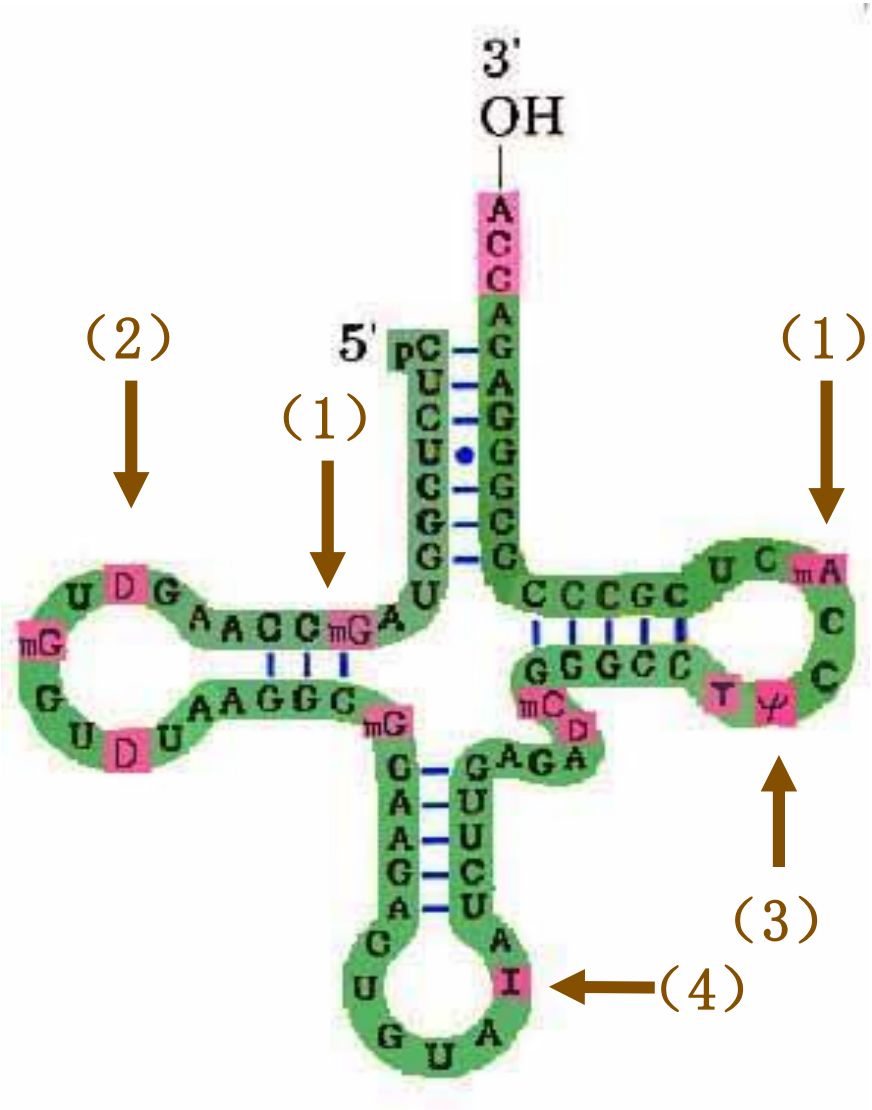
*Size of human β-globin is 146 amino acids

Modification of rRNA

- 45S transcript in nucleus is the precursor of 3 kinds of rRNAs.
- The matured rRNA will be assembled with ribosomal proteins to form ribosomes that are exported to cytosolic space.



Base modification in tRNA



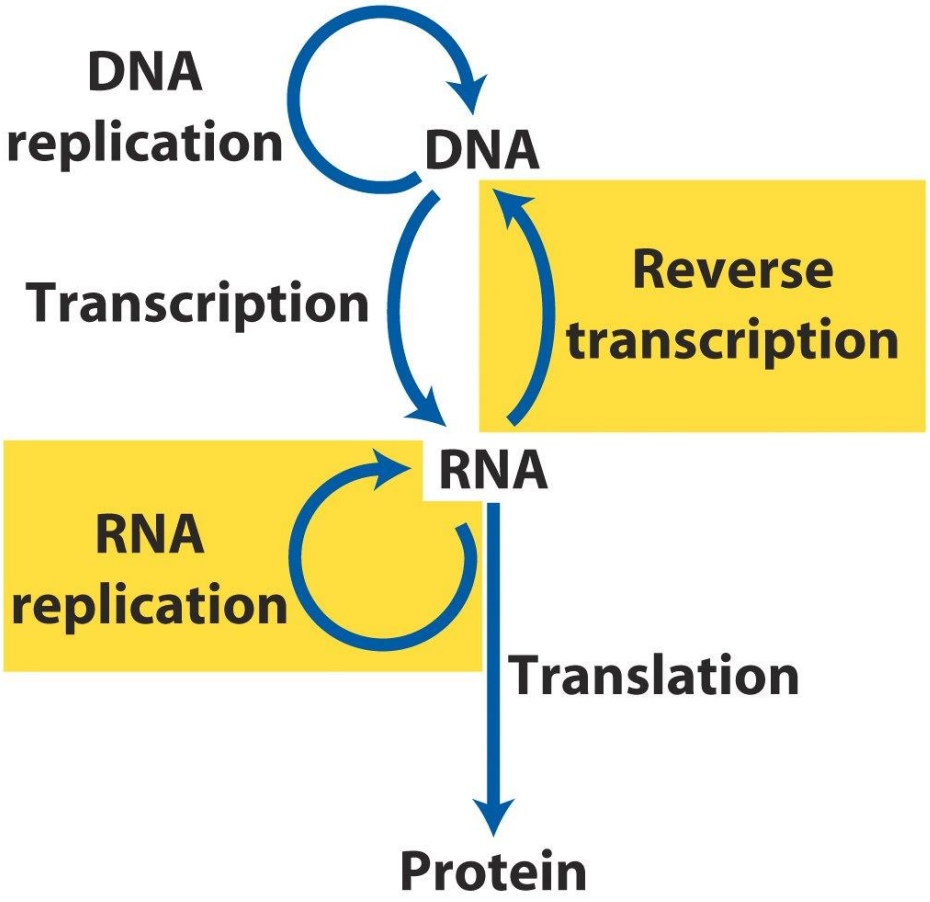
1. **Methylation A→mA, G→mG**
2. **Reduction U→DHU (dihydrouridine)**
3. **Transversion U→ψ (pseudouridine)**
4. **Deamination A→I**

RNA-Dependent Synthesis of RNA and DNA

- In previous slides, the role of template strand has been reserved for DNA.

- However, some enzymes use an RNA template for nucleic acid synthesis.

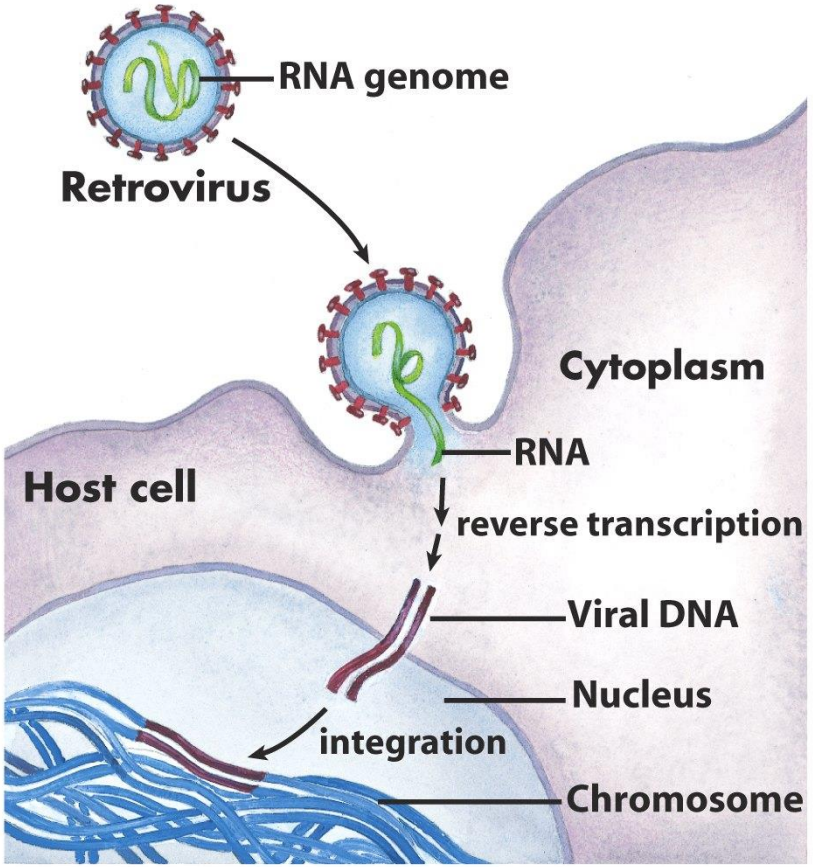
- RNA viruses, are the major source of most RNA-dependent polymerase so far.



RNA-Dependent Synthesis of RNA and DNA

Retroviral infection of a mammalian cell and integration of the retrovirus into the host chromosome.

1. Viral particles entering the host cell carry viral **reverse transcriptase**.
2. The viral RNA is converted to double-stranded DNA by the action of reverse transcriptase.
3. Once converted to double-stranded DNA, the DNA enters the nucleus and is integrated into the host genome. The integration is catalyzed by a virally encoded **integrase.**



Reference Books

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4. David L. Nelson and Michael M. Cox (2008). Lehninger Principles of Biochemistry 5th Edition, New York: W. H. Freeman and Company