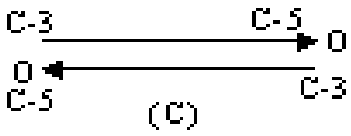
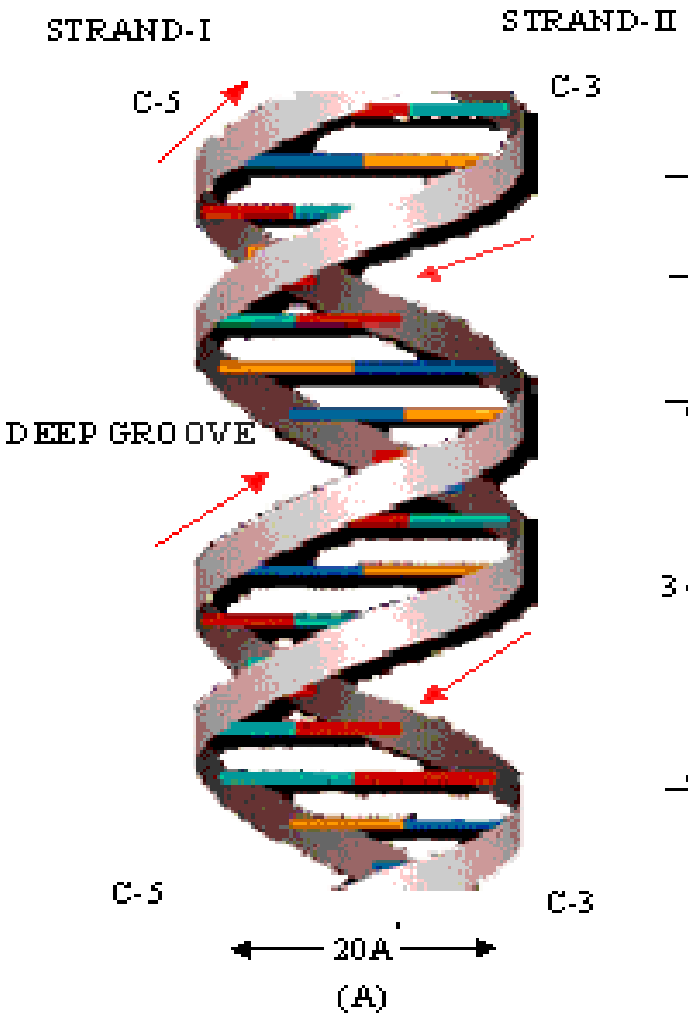
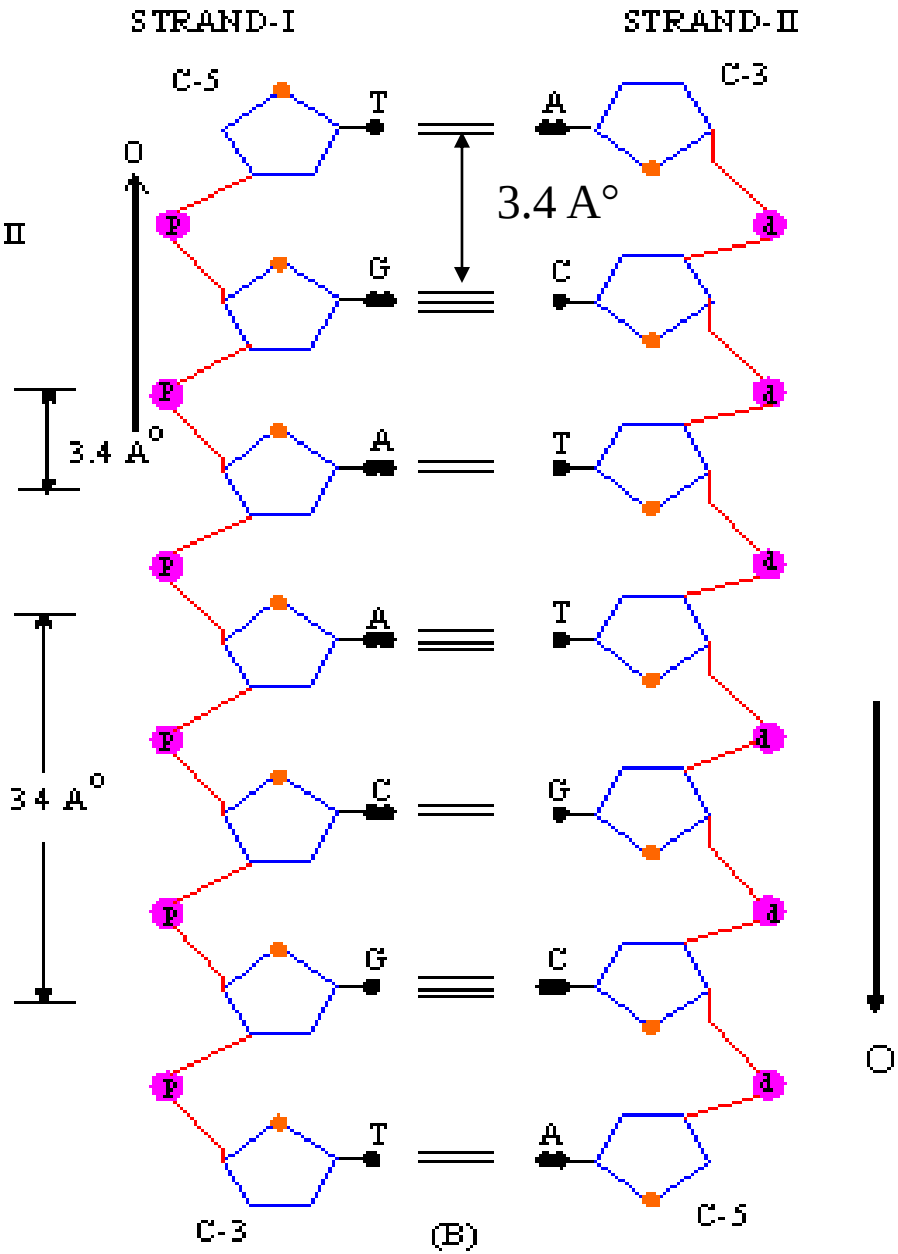
Genetics

Instructor: Dr. Jihad Abdallah

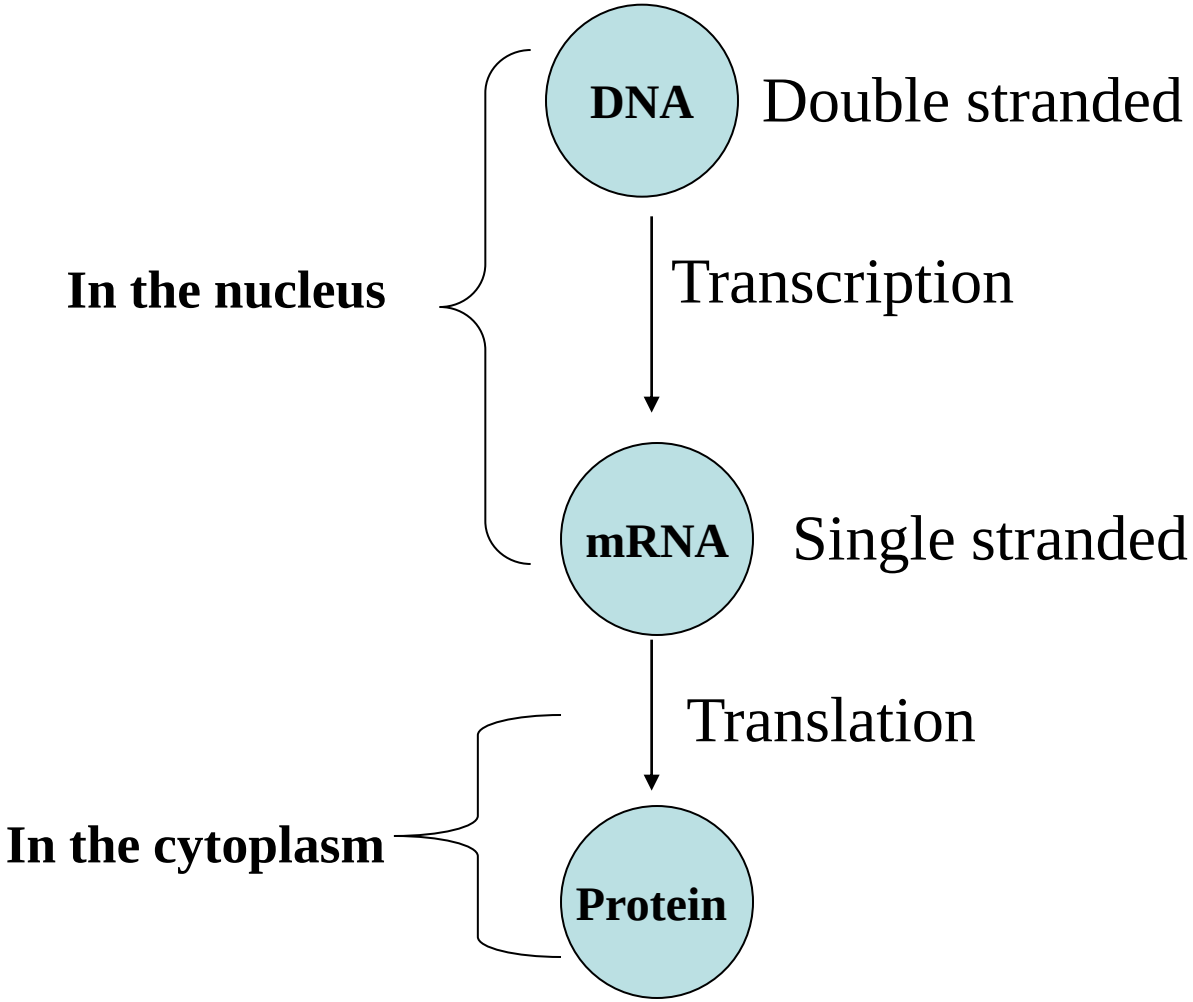
Transcription of DNA

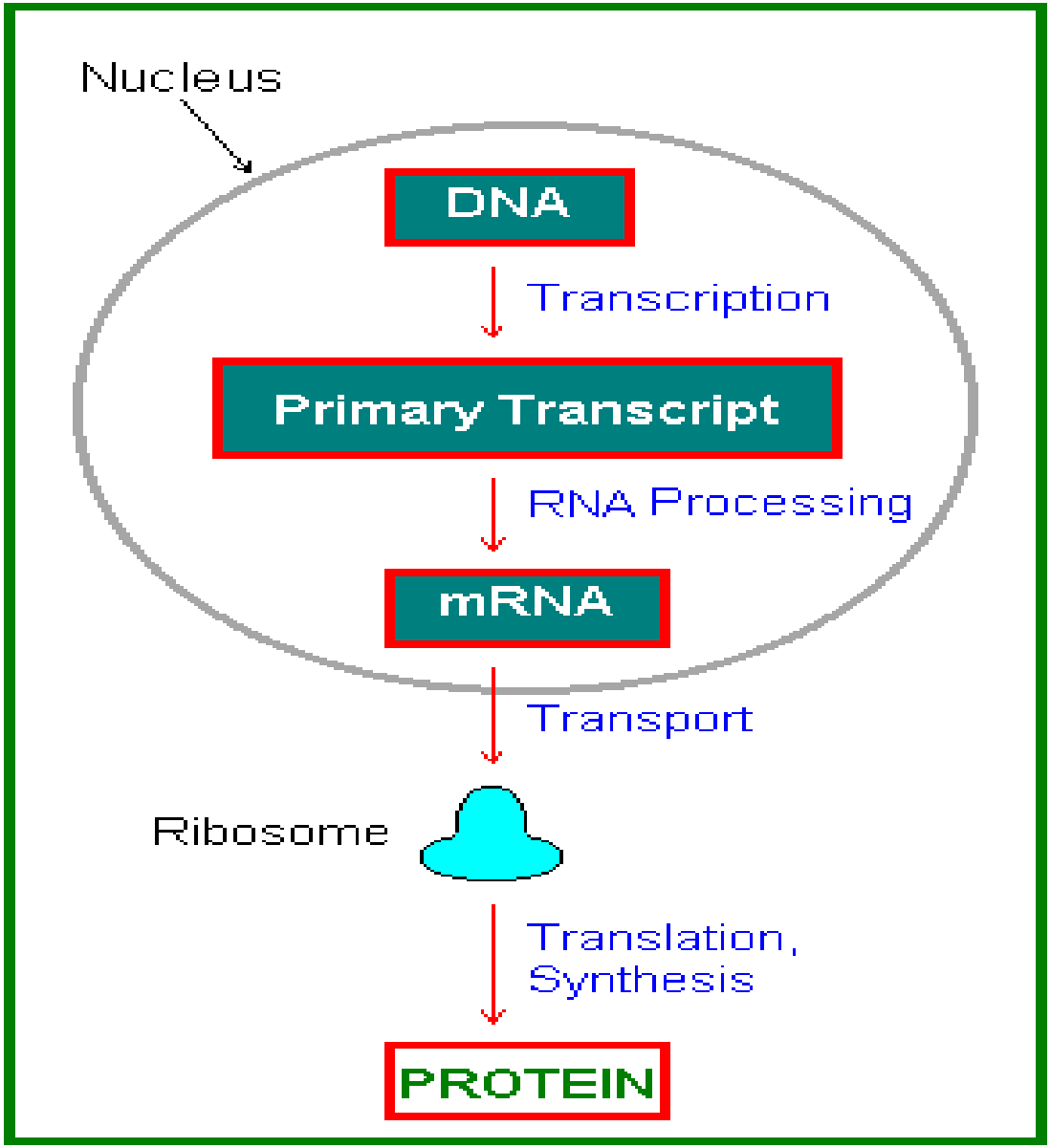

C-3
C-5
O
O
C-5
C-3
(C)

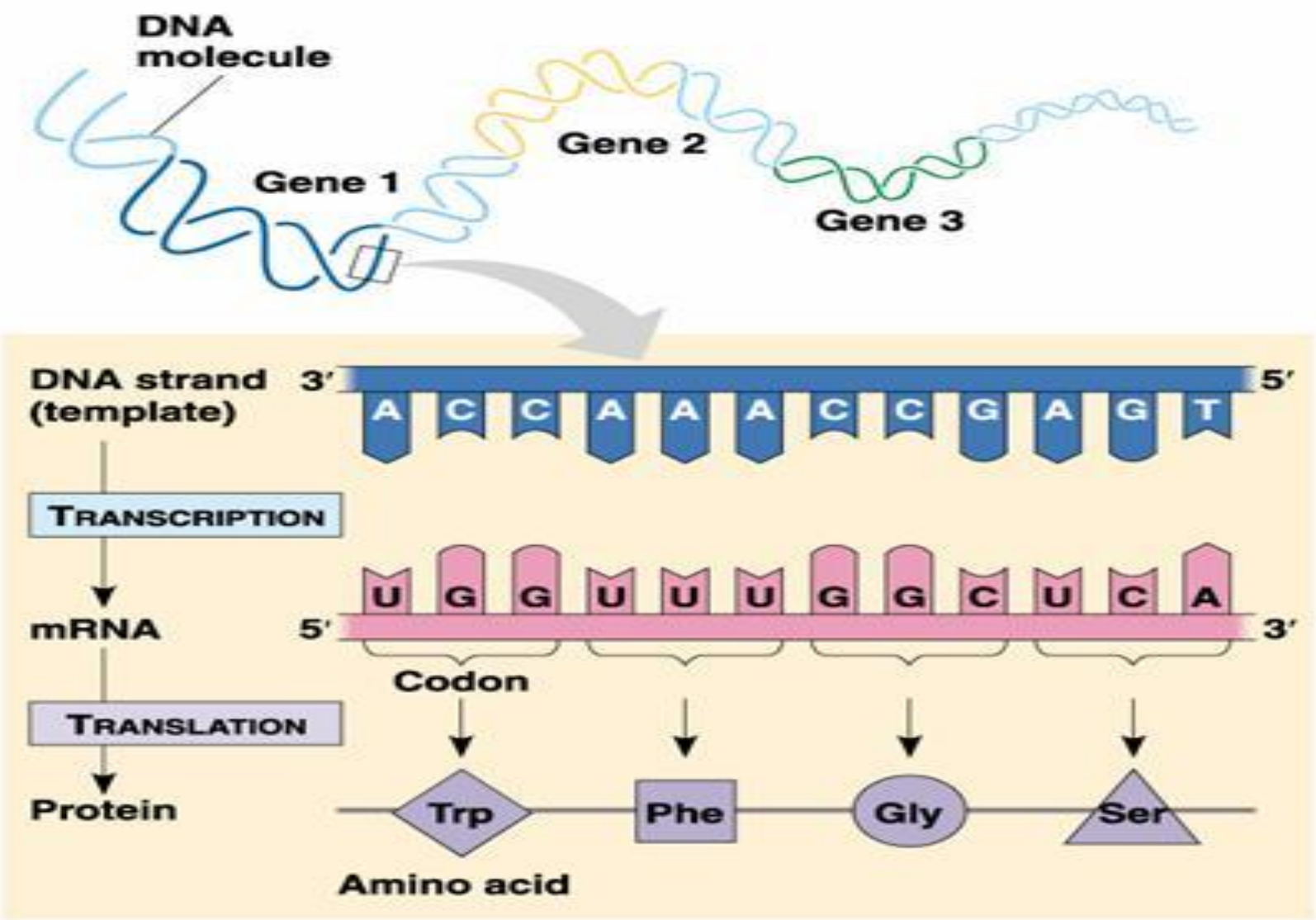

STRAND-I
STRAND-II
C-5
C-3
DEEP GROOVE
C-5
C-3
20A°
(A)


STRAND-I
STRAND-II
C-5
C-3
O
3.4 A°
3.4 A°
3 4 A°
T
A
G
C
A
T
A
T
C
G
G
C
T
A
C-3
C-5
(B)
O

Expression of Genetic information

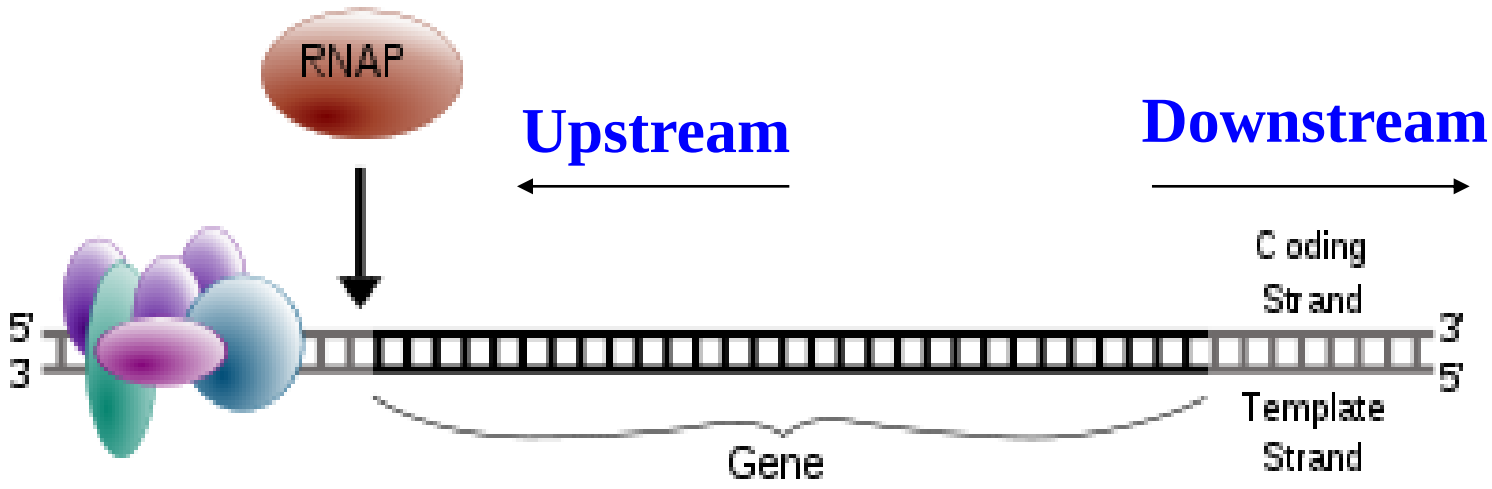



Nucleus
DNA
Transcription
Primary Transcript
RNA Processing
mRNA
Transport
Ribosome
Translation,
Synthesis
PROTEIN



Transcription

- Is the synthesis of mRNA from a DNA template.
- DNA has two strands but only one strand is transcribed.
- One strand is called the **coding strand** **(**also called **sense strand** or **non-template strand)**. This strand has the same sequence as the mRNA except that the T (Thymine) is replaced by U (Uracil) in the mRNA.
- The other (complementary) strand, is the one that is read by RNA Polymerase and called the **template strand** (also called **non-coding strand** or **non-sense strand)**. It serves as a template to synthesize a complimentary copy of mRNA.



- The **coding strand** is oriented 5`→ 3` and the **template strand** is oriented 3`→ 5`
- **RNA polymerase** starts synthesizing mRNA in the 5`→ 3`, therefore the resulting mRNA is also oriented 5`→ 3`.

- **Promoter:** a short sequence of DNA provides a site to begin transcription. Transcription is initiated when RNA Polymerase binds to the promoter.
- **Terminator:** is a sequence which signals the end of transcription. The piece of DNA that is transcribed into mRNA is the one which extends from the end of promoter to the terminator.
- **Transcription factors:** proteins which play an important role in transcription. They bind to the DNA sequence of the promoter. They enable RNA polymerase to bind to the promoter region and initiate transcription.

- Transcription involves three steps:
  - Initiation
  - Elongation
  - Termination

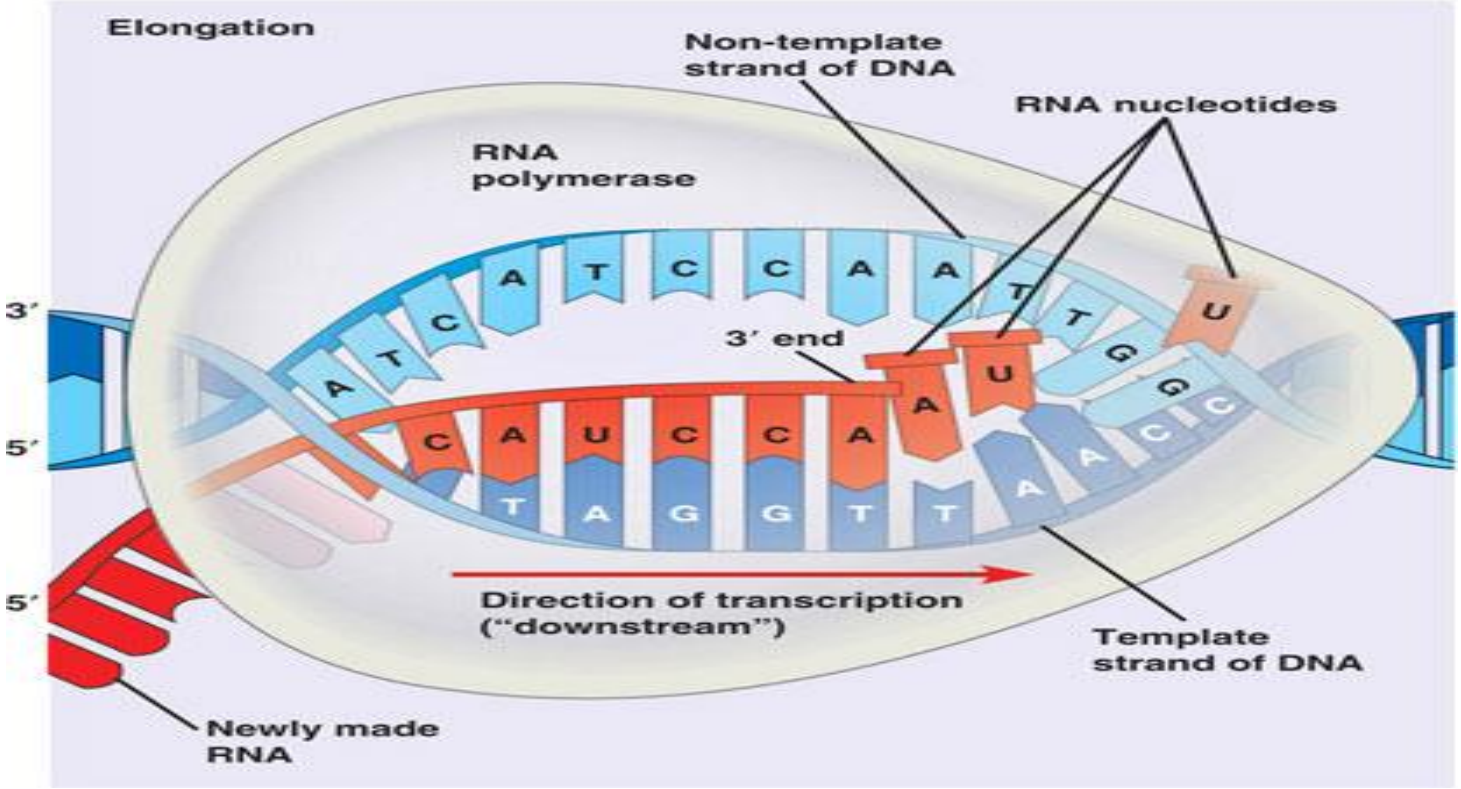
1. Initiation:

- RNA Polymerase binds to the promoter helped by transcription factors

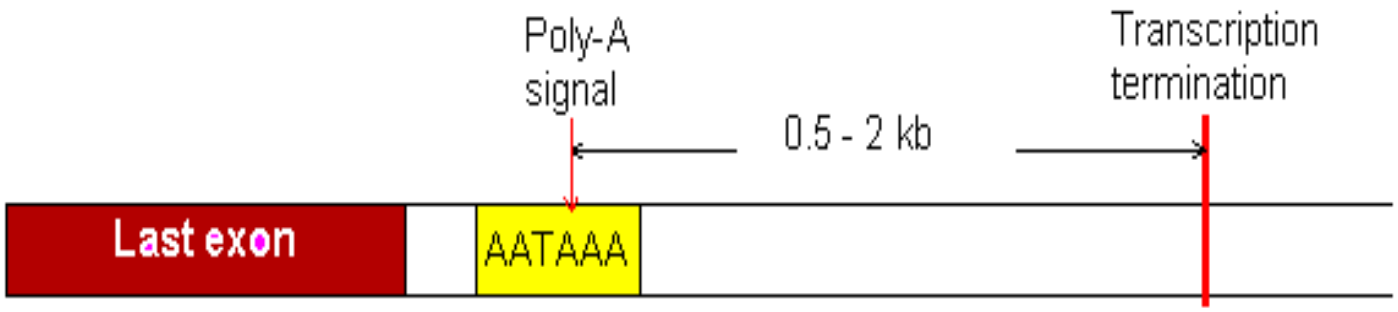
- Following binding to the promoter, the DNA double helix unwinds forming a **transcription bubble** (or **open complex**) and RNA polymerase initiates RNA synthesis

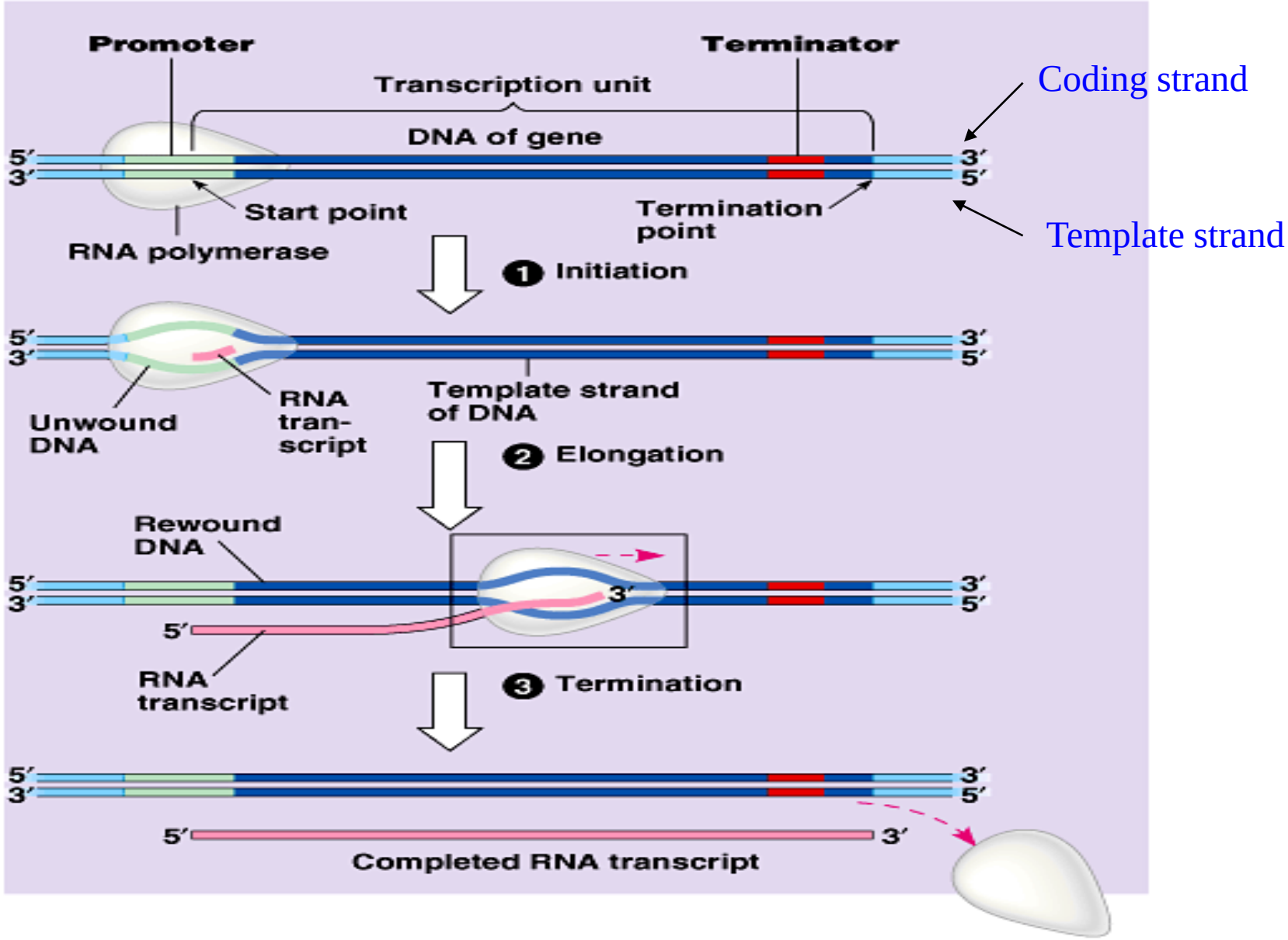
2. Elongation (synthesis of mRNA):

- The polymerase moves along the template strand to synthesize mRNA.
- As it moves, the DNA unwinds, the RNA transcript grows in length and the DNA rewinds itself behind.



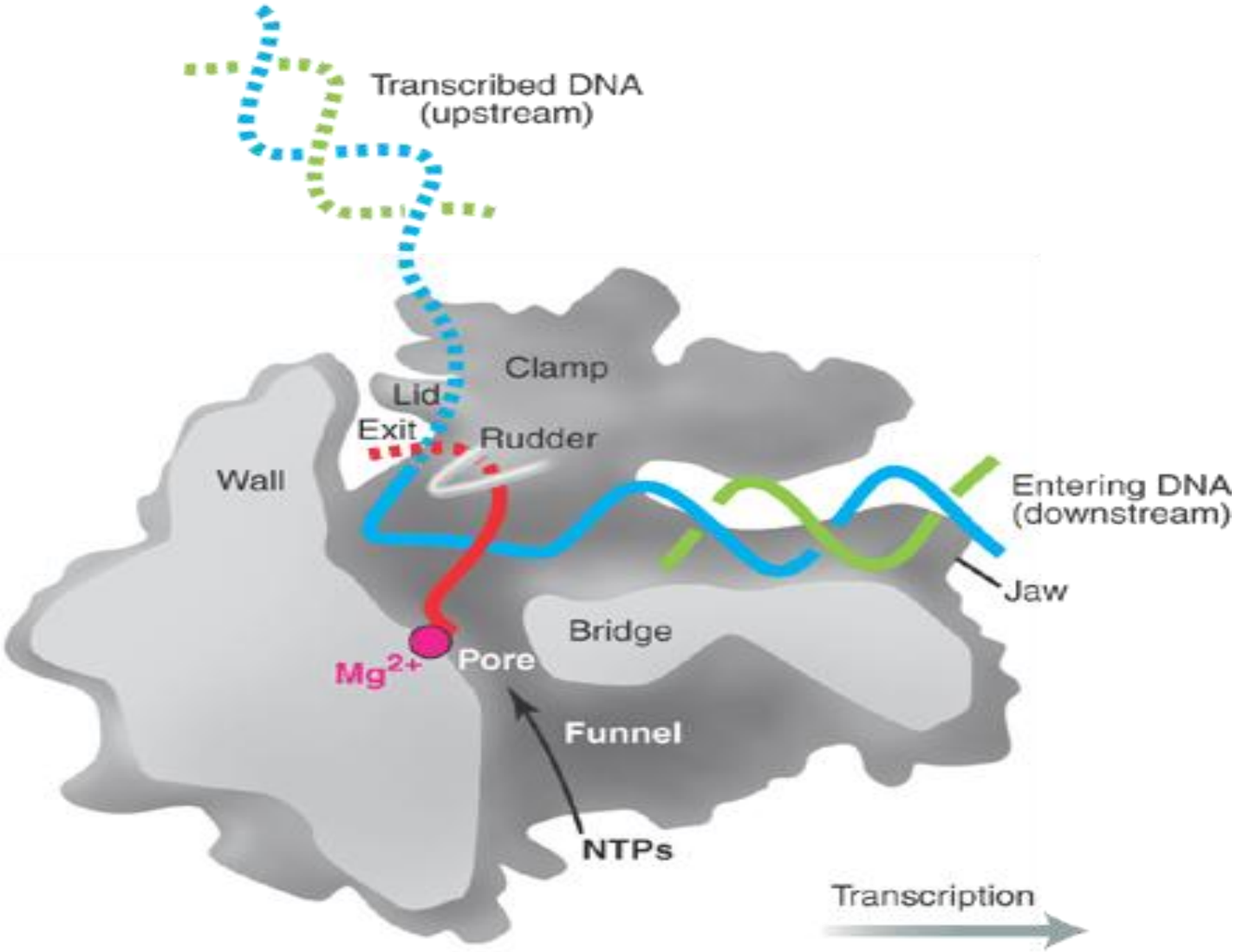
3. **Termination:** a termination signal is reached (a **poly-A signal)** that causes cessation of transcription and causes the RNA polymerase and the RNA transcript to dissociate from the strand of DNA.





RNA polymerases

- DNA in the nucleus of Eukaryotes are transcribed by three different **RNA polymerase** enzymes:
- **RNA polymerase I:** transcribes all of the genes that encode ribosomal RNA (rRNA) except the 5S rRNA.
- **RNA polymerase II:** transcribes all structural genes and certain snRNA genes needed for pre-mRNA splicing. It is responsible for the synthesis of all mRNA
- **RNA polymerase III:** transcribes all of the tRNA genes and the 5S rRNA gene


Transcribed DNA
(upstream)
Clamp
Lid
Exit
Rudder
Wall
Entering DNA
(downstream)
Jaw
Bridge
Mg2+
Pore
Funnel
NTPs
Transcription

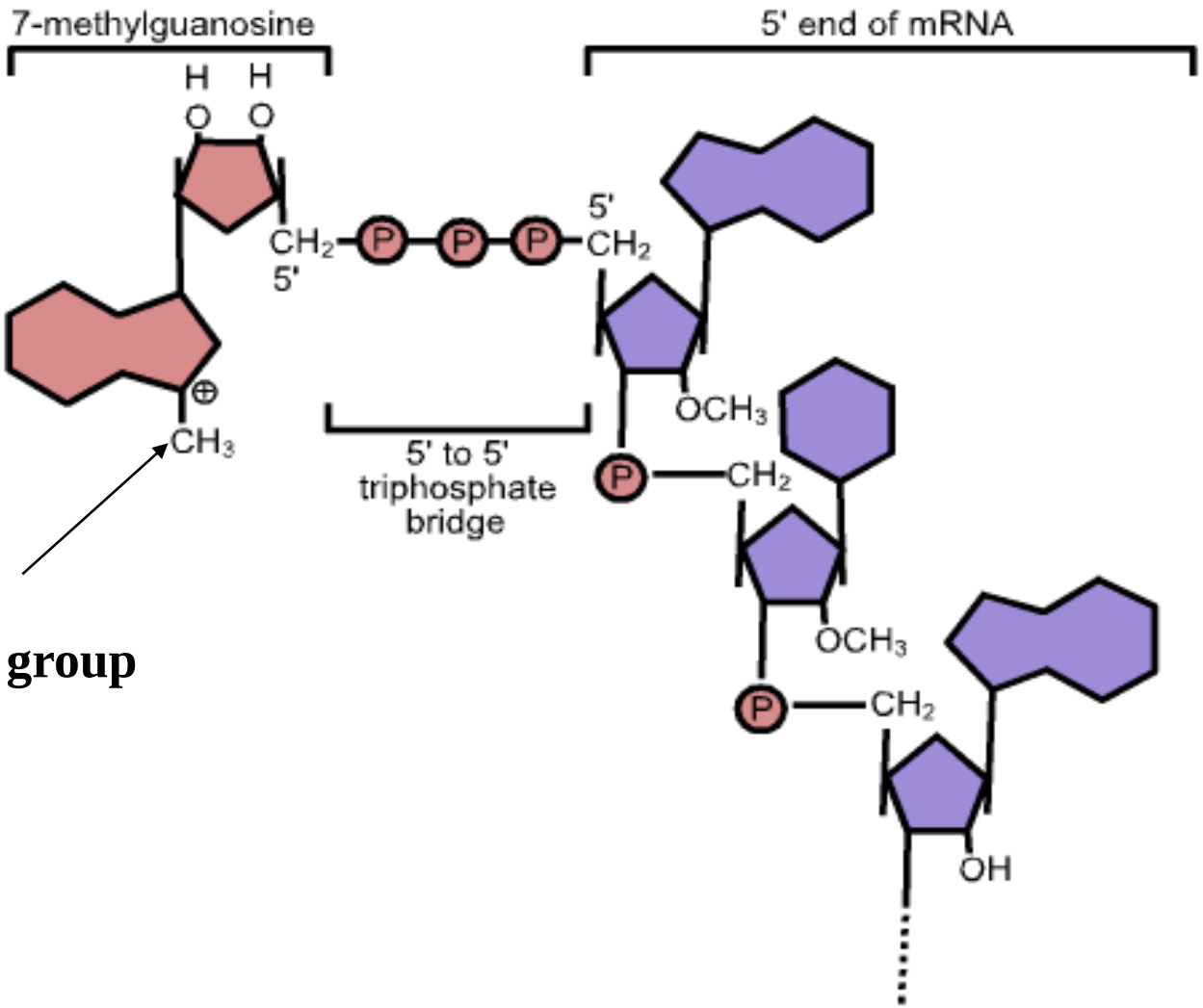
Post-transcriptional modifications

- In eukaryotes, the transcription of structural genes produces a long transcript known as **pre-mRNA**. These long transcripts are also known as **heterogeneous nuclear RNA** (**hnRNA**).
- This pre-mRNA is usually altered by splicing and other modifications before it exits the nucleus. The main modifications include:
  - Capping
  - Poly-A tailing
  - Splicing
  - RNA editing

After these modifications, the resulting RNA transcript (mature mRNA) exits the nucleus to the cytoplasm.

Capping: the attachment of **7-methylguanosine** to the 5`end of the mRNA (called 5`capping). Capping occurs while the pre-mRNA is being synthesized , usually when the transcript is 20 to 25 nucleotides in length. It occurs in **three steps**:

- The nucleotide at the 5`end of the transcript has three phosphate groups, an enzyme called RNA 5`-triphosphatase removes one of the phosphate groups
- A second enzyme, guanylyltransferase, attaches a guanosine monophospahte (GMP) to the 5`end.
- Finally, a methyltransferase attaches a methyl group to the guanine base at the N-7 position .



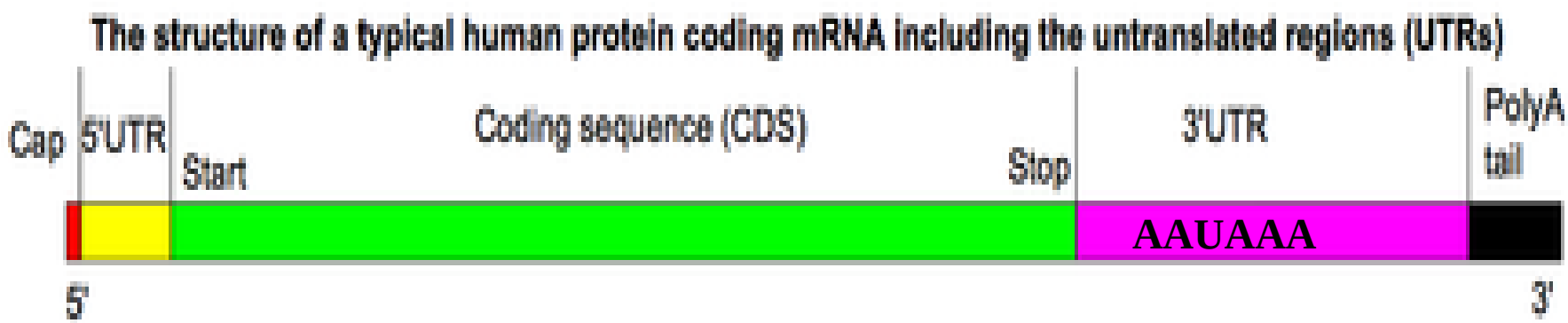
Methyl group

- **Importance of capping:**

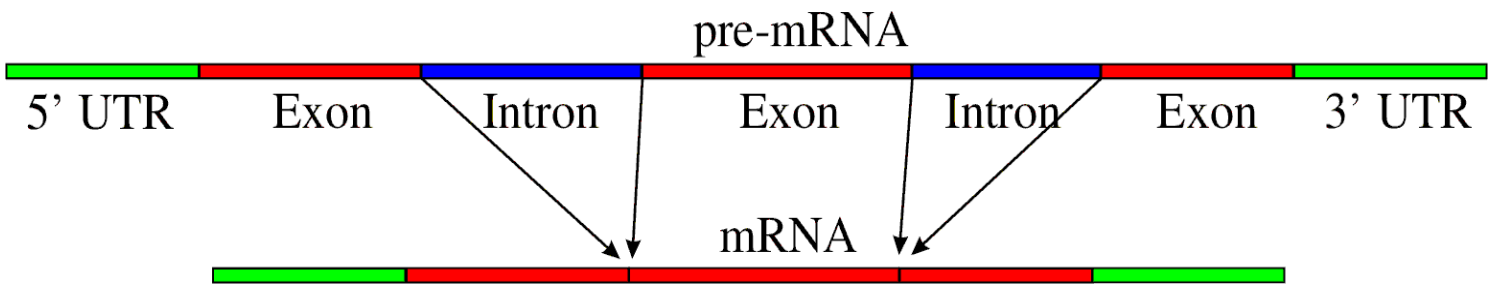
- Protection of mRNA from **nuclease activity**
- Recognition of the ribosome (plays a role in initiation of translation)
- Influences the removal of introns.
- **C**ap-binding proteins are needed for the proper exit of certain RNAs from the nucleus.

2. **Poly-A tailing (Polyadenylation):** a **poly-A** tail of 20 to 200 adenine-containing nucleotides is added at the 3`end of mRNA. This is important for nuclear export, mRNA stability and translation.

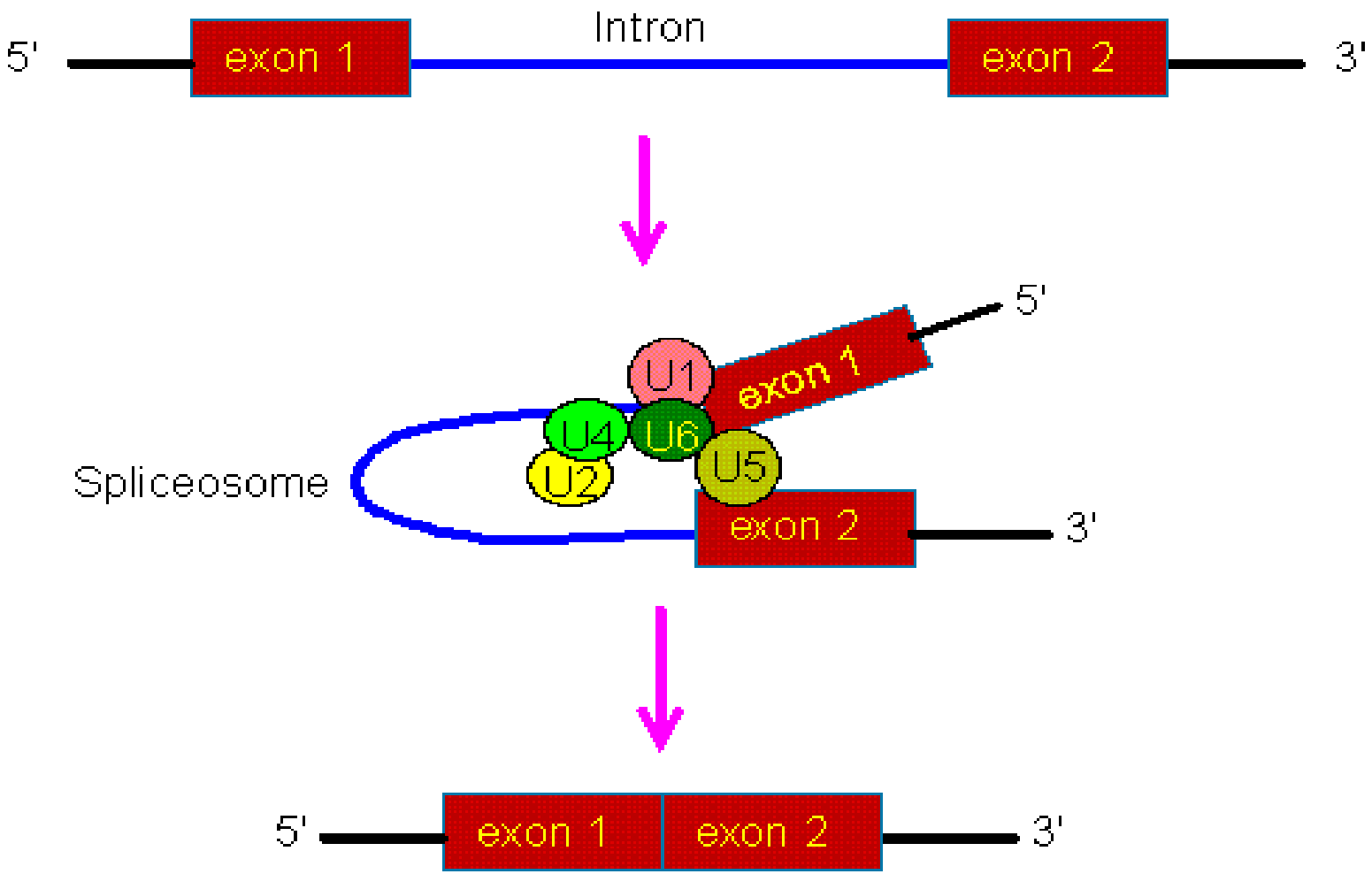
- The process of polyadenylation begins once transcription of a gene has finished. First, endonuclease cuts the polyadenylation sequence present at the 3` end of the newly-made RNA, then PolyA-polymerase adds many adenine containing nucleotides.



3. **Splicing:** removal of **introns** (the intervening sequences; do not code for protein) and rejoining of **exons** (sequences which code for protein) together. Splicing is done by the **spliceosome**. This is a large complex composed of several subunits known as snRNPs (pronounced "snurps"). Each snRNA contains **small nuclear** RNA and a set of proteins.



Simple illustration of exons and introns in pre-mRNA and the formation of mature mRNA by splicing. The UTRs are non-coding parts of exons at the ends of the mRNA.



Schematic drawing for the formation of the spliceosome during RNA splicing. U1, U2, U4, U5 and U6 denote snRNAs and their associated proteins. The U3 snRNA is not involved in the RNA splicing, but is involved in the processing of pre-mRNA.

4. RNA editing: : insertion or deletion of nucleotides.

Example: