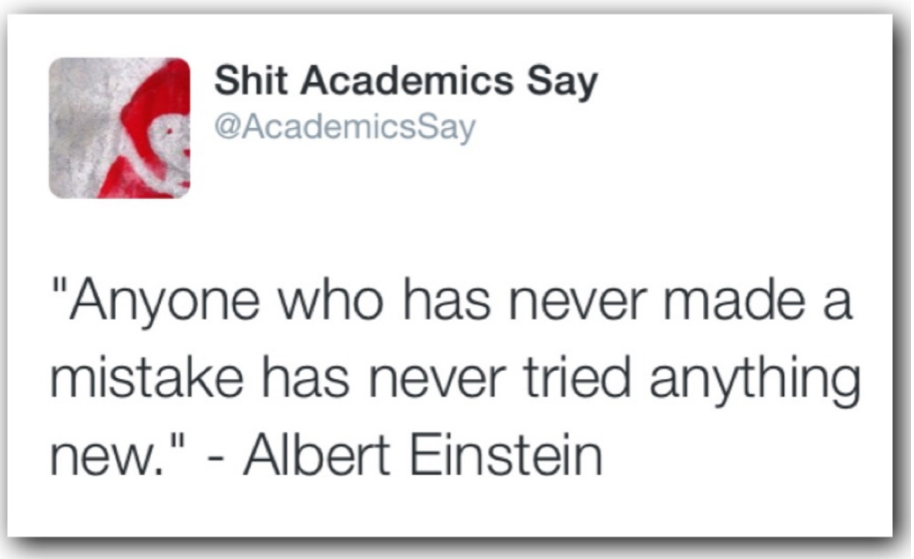
# Lecture 18:

## Transcription in eukaryotes

**Course 371**

# Lessons for life



# AIMS

- Understand the transcription process in eukaryotes.
- Understand the eukaryotic gene structure.
- Understand the sequence of events and the molecular machinery.
- Understand the molecular modifications of eukaryotic transcript and why they take place.
- Compare the transcription process of prokaryotes to that of eukaryotes.

# Eukaryotes RNA polymerase

Unlike prokaryotes, eukaryotes have different kinds of RNA polymerases. Each RNA polymerase is responsible for transcription of specific RNA molecules.

# Eukaryotes RNA polymerase

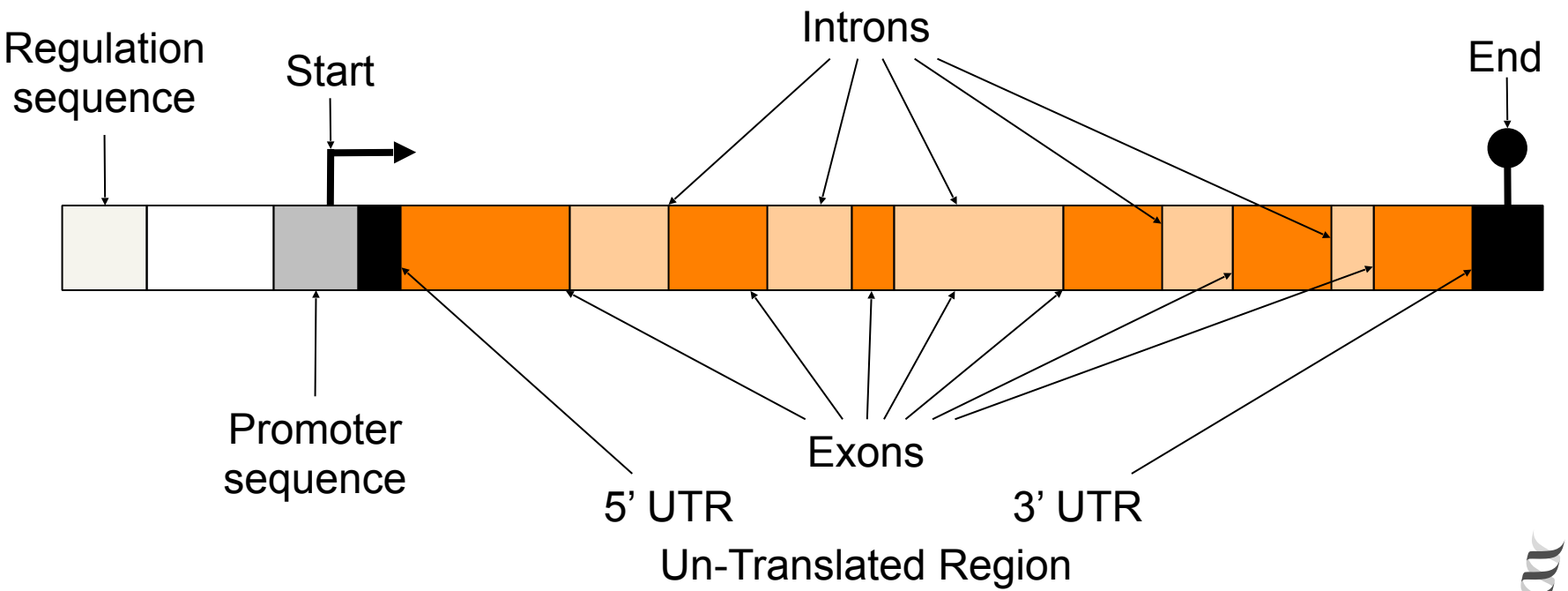
1. **RNA Pol I:** Transcribes RNA found in ribosomes
   - 28s ribosomal RNA
   - 18s rRNA
   - 5.8s rRNA

2. **RNA Pol II:** Transcribes
   - m-RNA
   - Some sn-RNA

3. **RNA Pol III:** Transcribes
   - t-RNA
   - 5s rRNA
   - Some sn-RNA

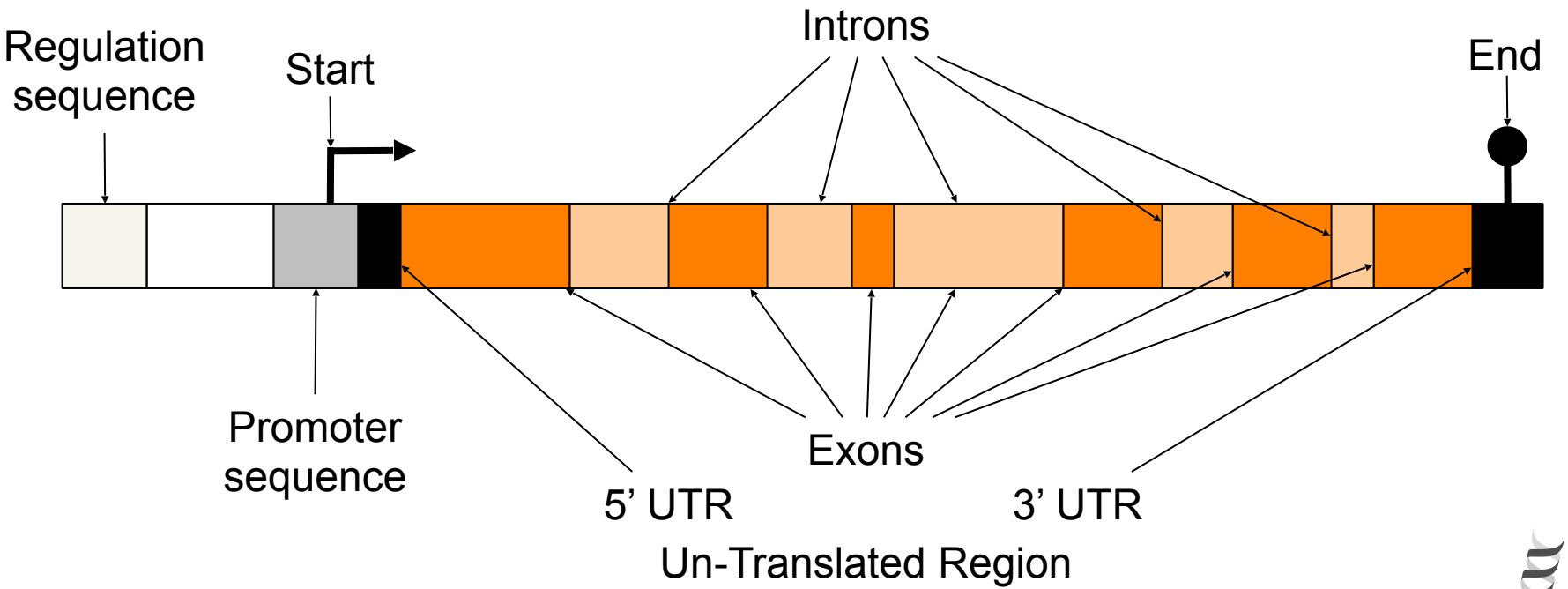
# Eukaryotes Gene structure

- Promoter.
- Promoter proximal elements.
- 5’ Un-translated region (5’ UTR).



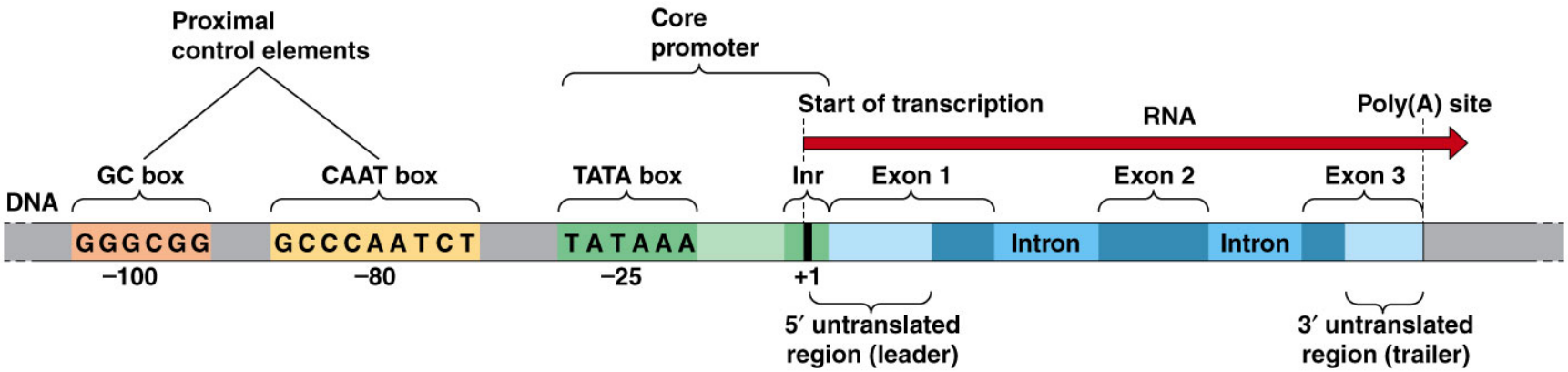
# Eukaryotes Gene structure

- Protein coding sequence.
- 3’ Un-translated region (3’ UTR).
- Exons: amino acid coding sequence.
- Introns: no code for amino acid.



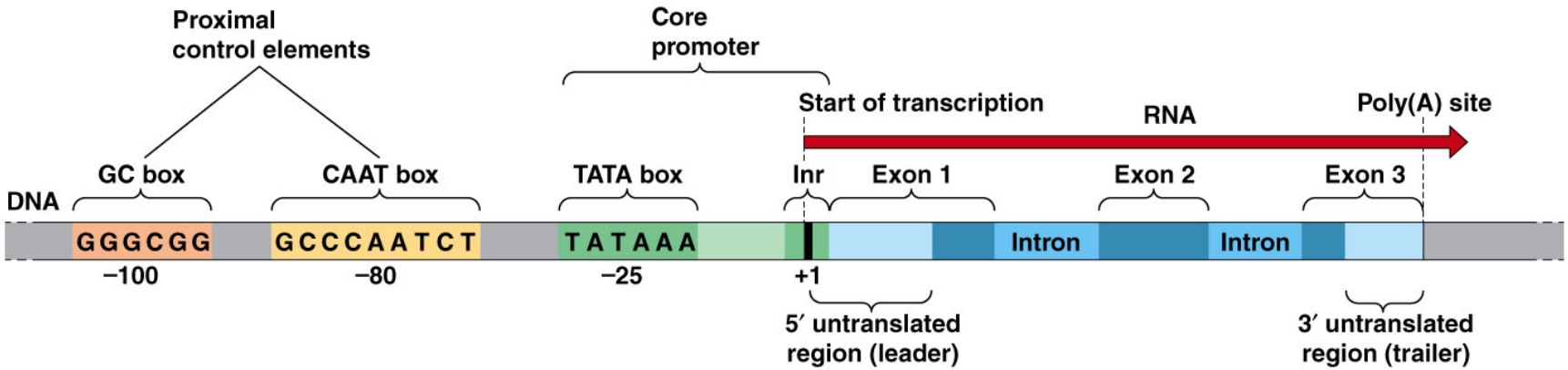
# Eukaryotic promoter

- Short sequence called (**initiator**):
  - **Inr** located at (seq +1).
- **TATA box :**
  - Located at -30 sequence.



# Eukaryotic promoter

- **Promoter proximal element:**
  - located upstream of the TATA box (-50 - -200)
  - Activators.
  - Enhancers.

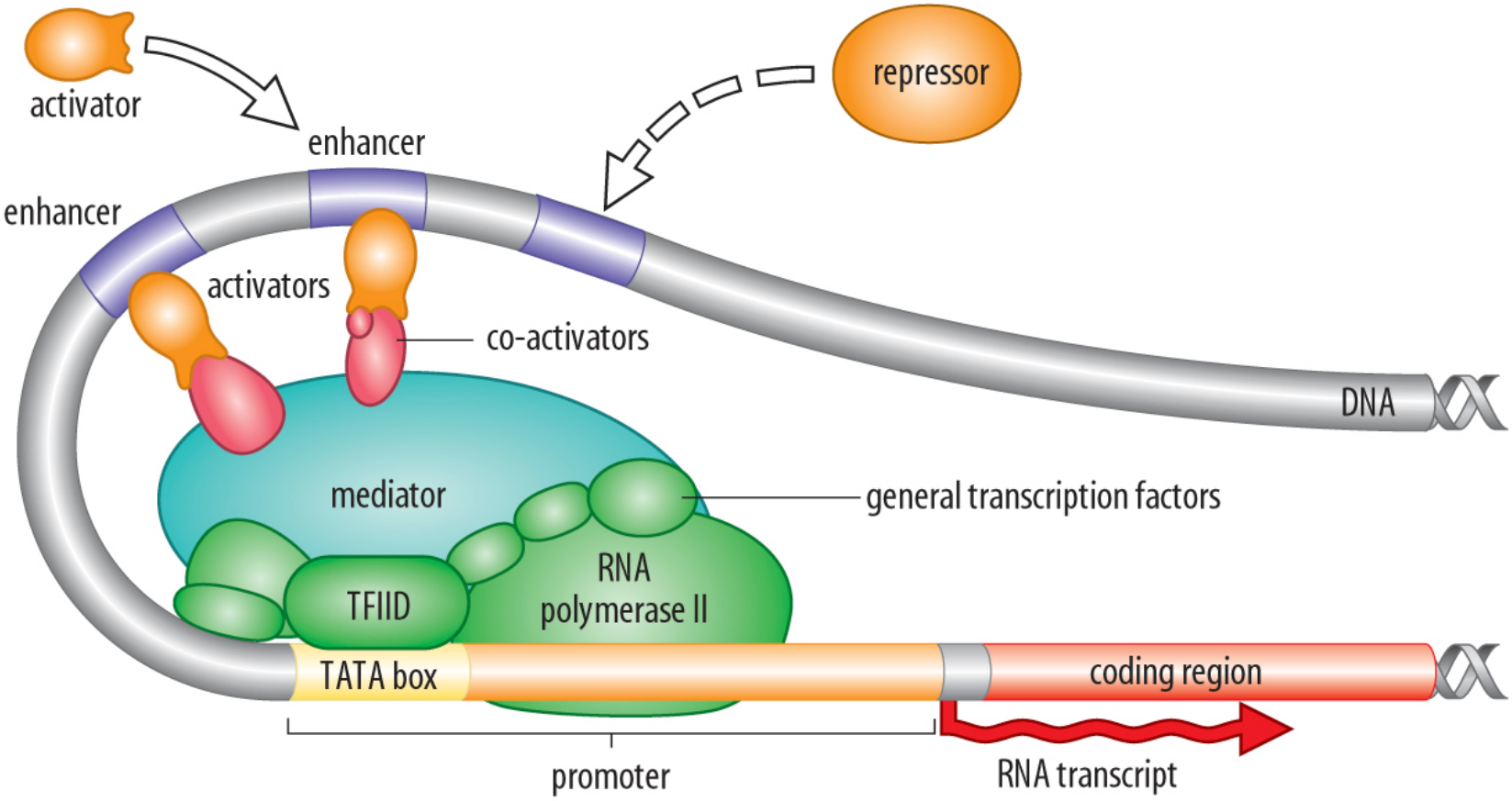


# Eukaryotes RNA polymerase

TATA Box

**Promoter proximal element:**

Activator, Enhancer, or Repressor



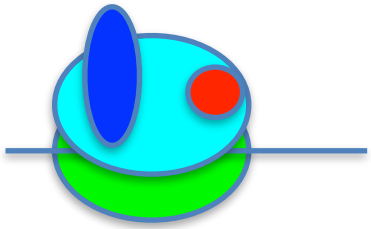
# Transcription process in Eukaryotes

**We will consider the transcription process to make m-RNA by RNA Pol II**

# Transcription process in Eukaryotes

1. **Initiation:** assembly of RNA Pol II with general transcription factors (GTFs) on promoter.

    1. TFIID binds to the TATA box.



    2. **TFIID is composed of two units:**

        - TATA boxes binding protein (TBP)
        - TBP associated factors (TAFs)

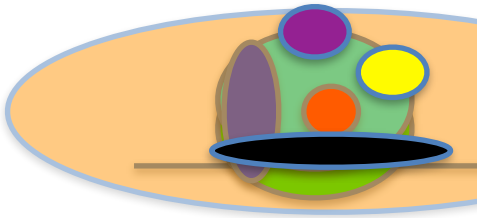
    3. TFIIA and TFIIB bind to TFIID.

# Transcription process in Eukaryotes

1. **Initiation:** assembly of RNA Pol II with general transcription factors (GTFs) on promoter.

    5. RNA Pol II and TFIIF bind to (TFIID, TFIIA, TFIIB)

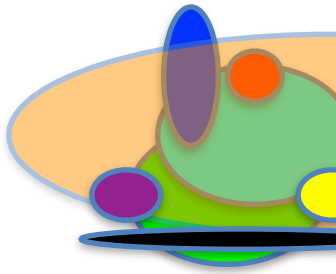
    6. TFIIE binds to the complex.



    7. **TFIIH** (helicase) binds to the complex – will be unwinding the promoter.

    8. All elements make the transcription **Pre-initiation complex (PIC).**

# Transcription process in Eukaryotes



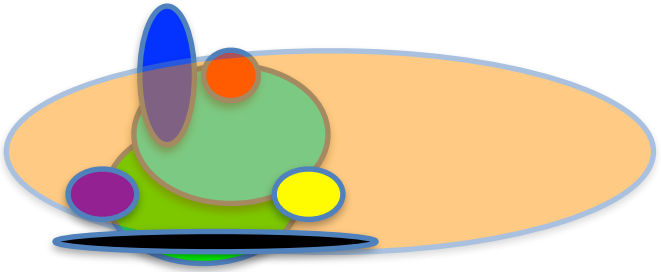
2. **Promoter melting by TFIIH:**

    - The helicase activity of TFIIH unwind the promoter sequence to use a template.

3. **Abortive initiation:**

    - The RNA polymerase synthesizes short sequences of RNA but can not escape the promoter.

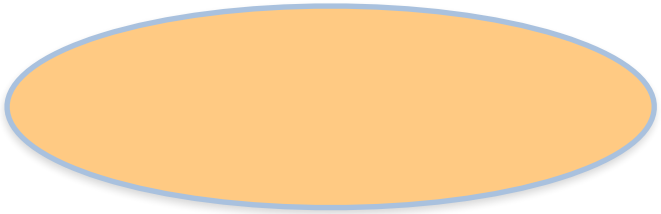
# Transcription process in Eukaryotes



4. **Promoter escape:**

   - RNA Pol breaks the interactions with transcription factors and escape the promoter and start synthesizing RNA.

# Transcription process in Eukaryotes



5. **Elongation:**

- RNA Pol adds the correct complementary NTPs to the template and continue. IF errors made proofreading capabilities fixes the problems.

# Transcription process in Eukaryotes

6. **Termination:**

- When RNA Pol synthesize pass a poly(A) site in the transcript (5’ AAUAAA 3’), the RNA synthesized is cleaved by:
    - CPSF (cleavage and polyadenylation specificity factor) protein.
    - CstF (cleavage stimulating factor) protein.
    - CFI and CFII (cleavage factor proteins).

# pre-mRNA

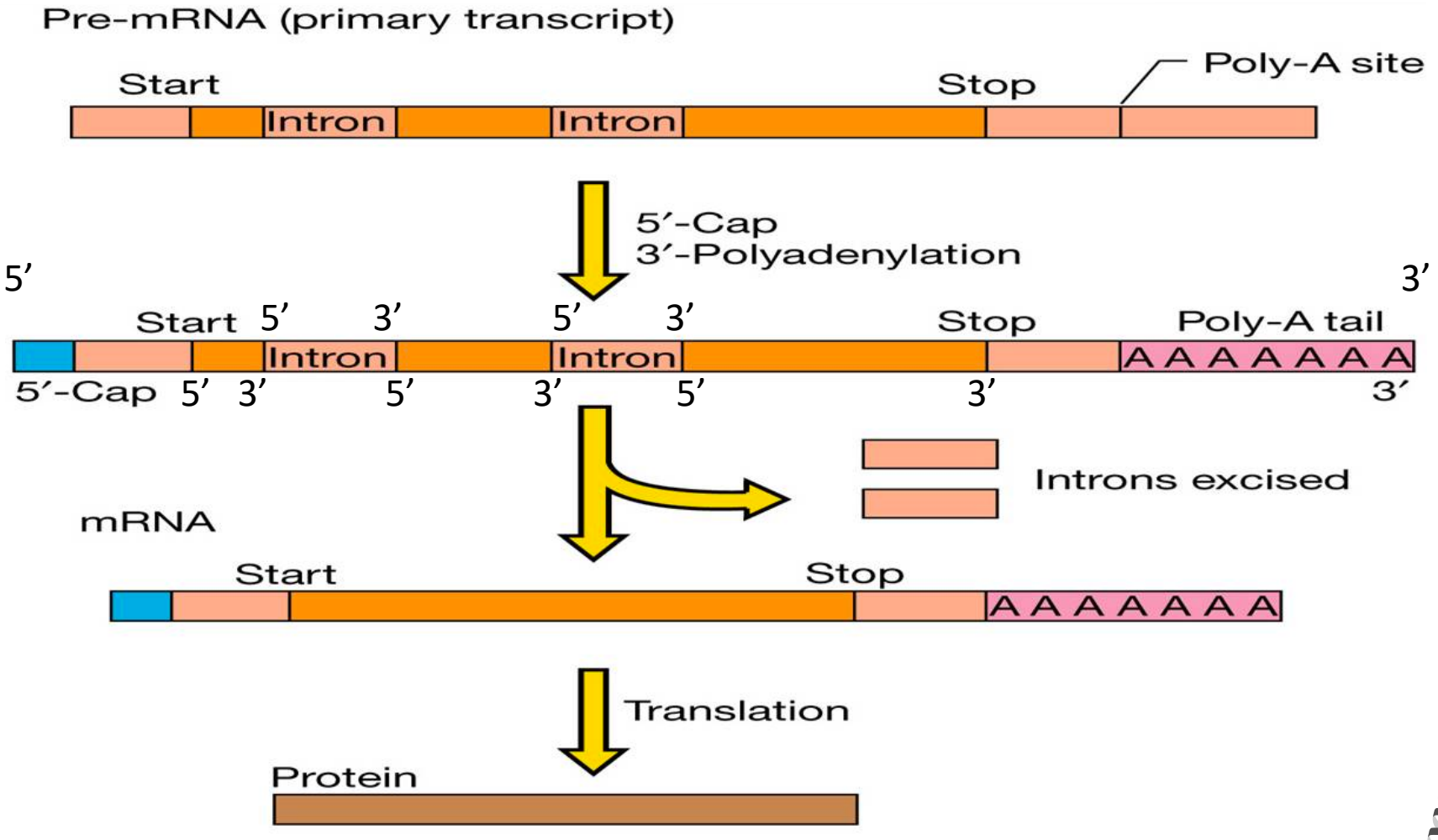
- The first product of transcription in eukaryotes is called pre-mRNA.
- Unlike the transcript of a prokaryote that get translated while being transcribed (**coupled transcription and translation**), the eukaryotic transcript needs some modification before leaving the nucleus to be translated.
- The pre-mRNA of eukaryotes gets processed to a mature mRNA.

# pre-mRNA

**What are the processes to produce mature mRNA?**

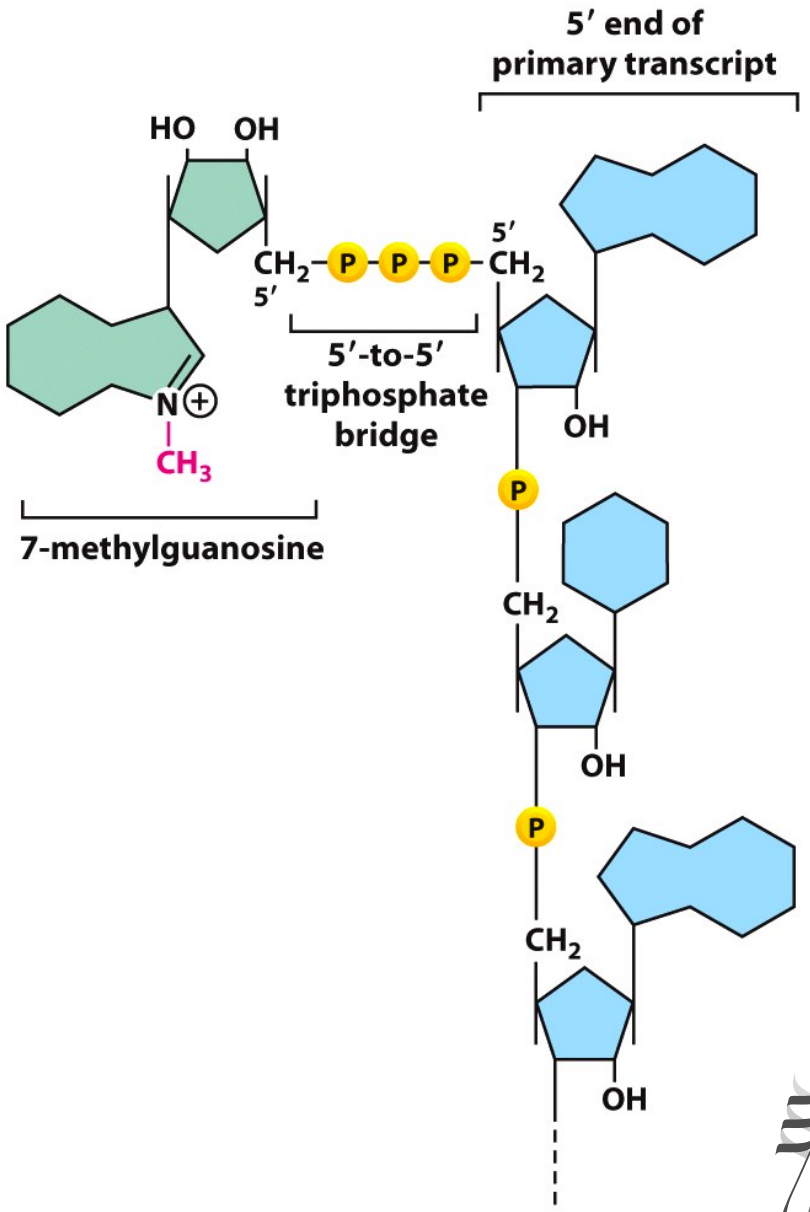
(1) 5’ modification
(2) 3’ modification
(3) Intron splicing

# pre-mRNA maturation



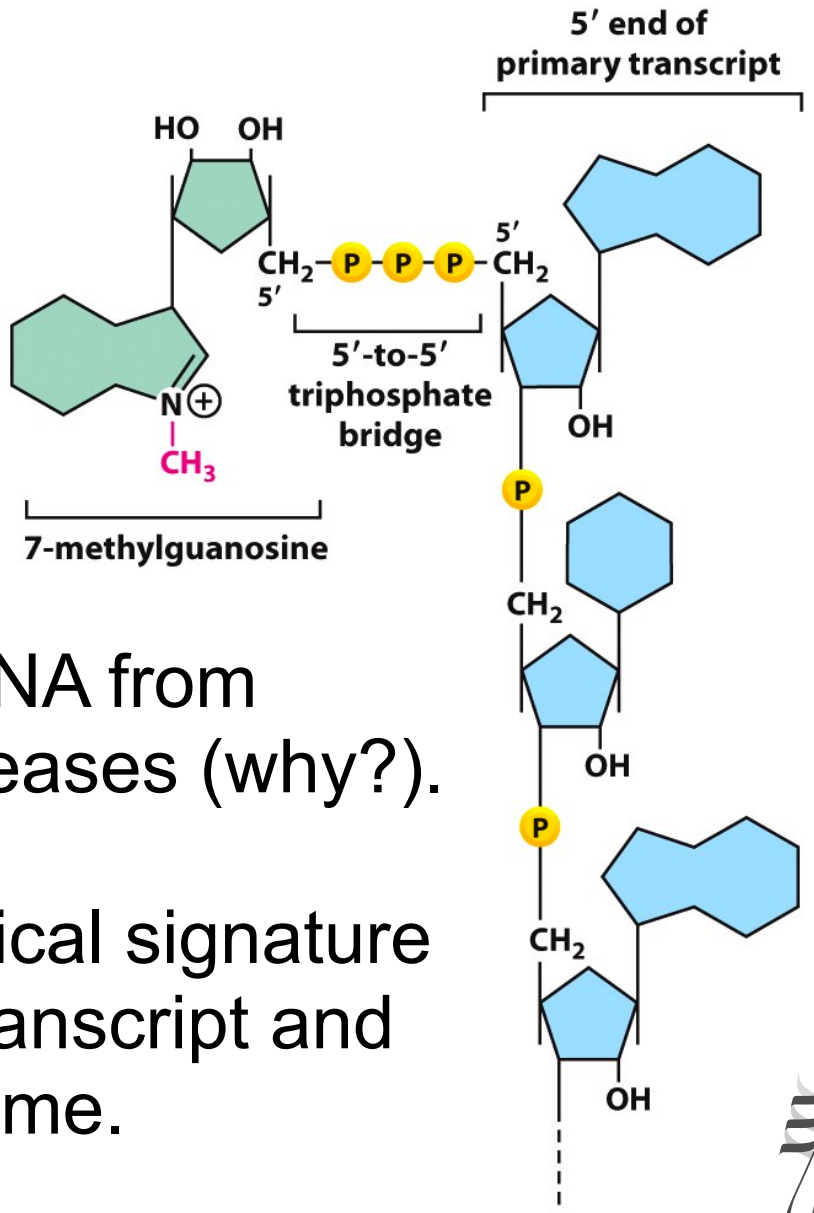
# 5’ modification of eukaryotic mRNA

- It is referred to as the **Capping of the 5’ end.**
- Adding a 7-methyl Guanine ($m^7G$) to the 5’ end using a (5’ – 5’) linkage.



# 5’ modification of eukaryotic mRNA

**This modification is important for:**




- Protecting the mature mRNA from degradation from exonucleases (why?).
- This is an important chemical signature for the translation of the transcript and initial binding to the ribosome.

# 3’ modification of eukaryotic mRNA

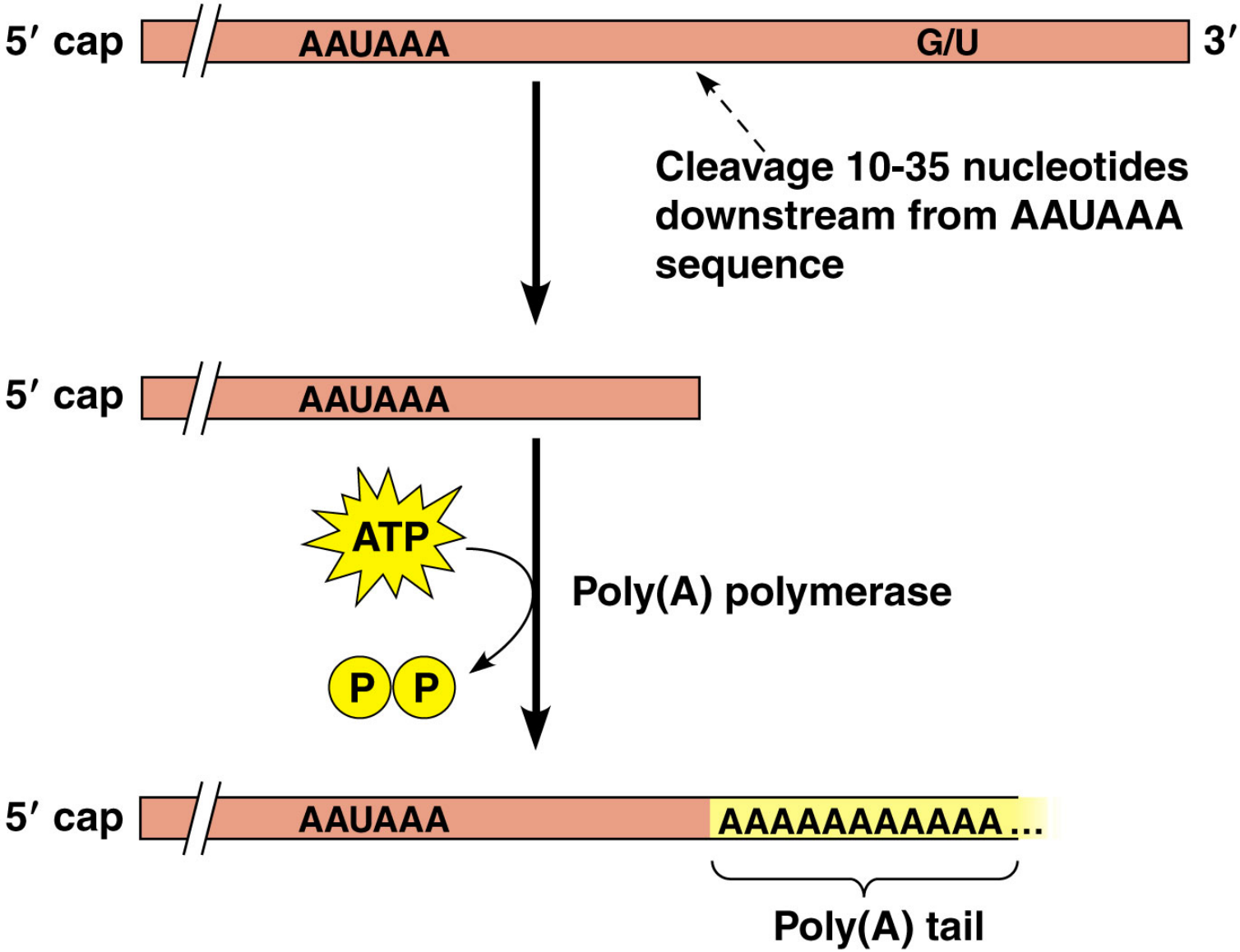
- This modification involve the addition of 50-250 adenine nucleotides at the 3’ end.
- This is called **poly(A) tails.**
- The poly(A) tails is synthesized without a template by an enzyme called **poly(A) polymerase (PAP).**

# 3’ modification of eukaryotic mRNA

## Why poly(A) tails?

- Help to export the mRNA from the nucleus.
- Prevent the mRNA from degradation by exonucleases.
- Helps in the initiation of translation.
- Helps in the regulation of translation.

# 3’ modification of eukaryotic mRNA



# Intron splicing

- Introns are the non-coding sequence of the gene that get transcribed but has to be removed before translation.
- Introns usually start with **5’-GU**.
- Introns usually end with **3’-AG**.
- The removal of introns from the pre-mRNA is called **intron splicing.**

# Intron splicing

- The molecular machinery that carries out splicing of the premature transcript is called **spliceosome.**

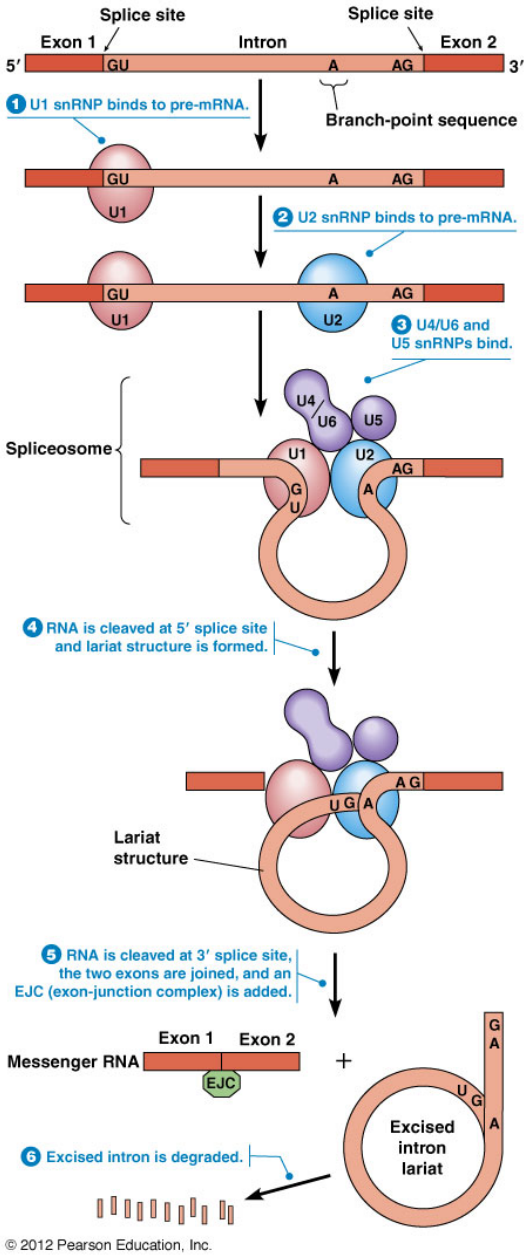
- The pre-mRNA binds to **small nuclear ribonucleoprotein particles (snRNPs).**

- **snRNPs** (U1, U2, U4, U5, U6) **are composed of:**
    - Small nuclear RNA (snRNA).
    - Associated proteins.

# Intron splicing process

1. U1 snRNP binds to the introns 5’ bind site.

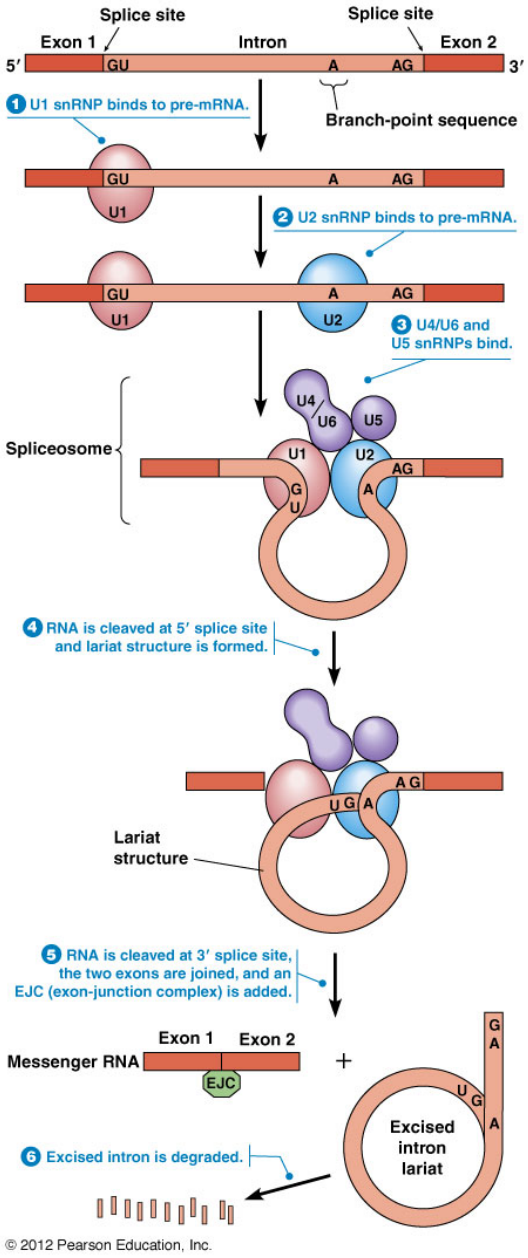
2. U2 snRNP binds to the **branch point sequence** in the middle of the intron (usually Adenine (A)).



# Intron splicing process

3. U4/U6 snRNP and U5 snRNP interact forming a unit.

4. The U4,U5,U6 bind to U1 and U2 making the intron loop and bend.



# Intron splicing process

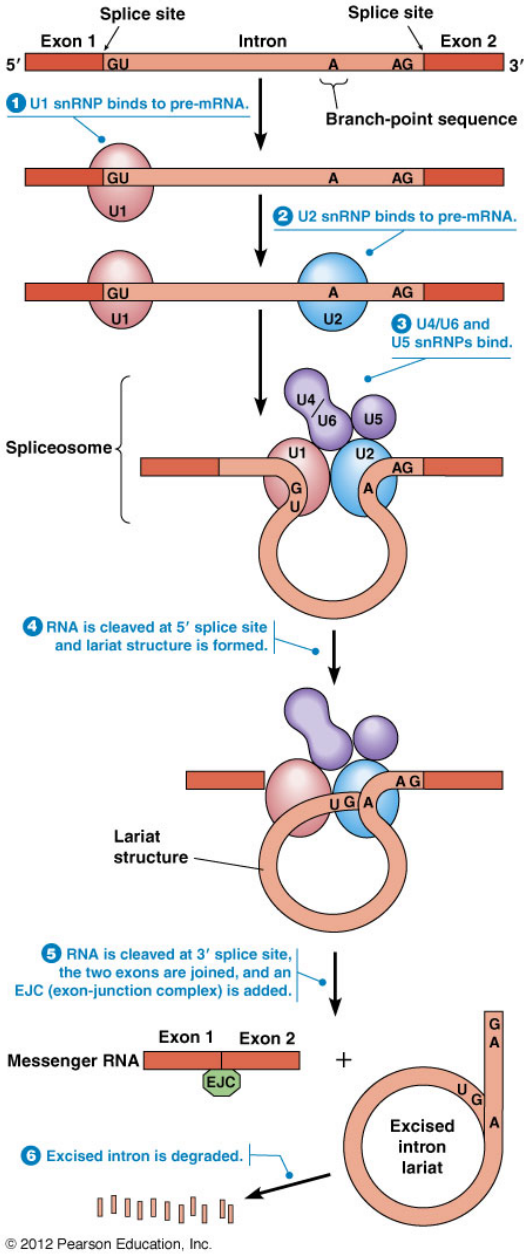
5. This brings the 5’ splice site close to the 3’ splice site.

6. U4 snRNP dissociates which activate the **spliceosome.**

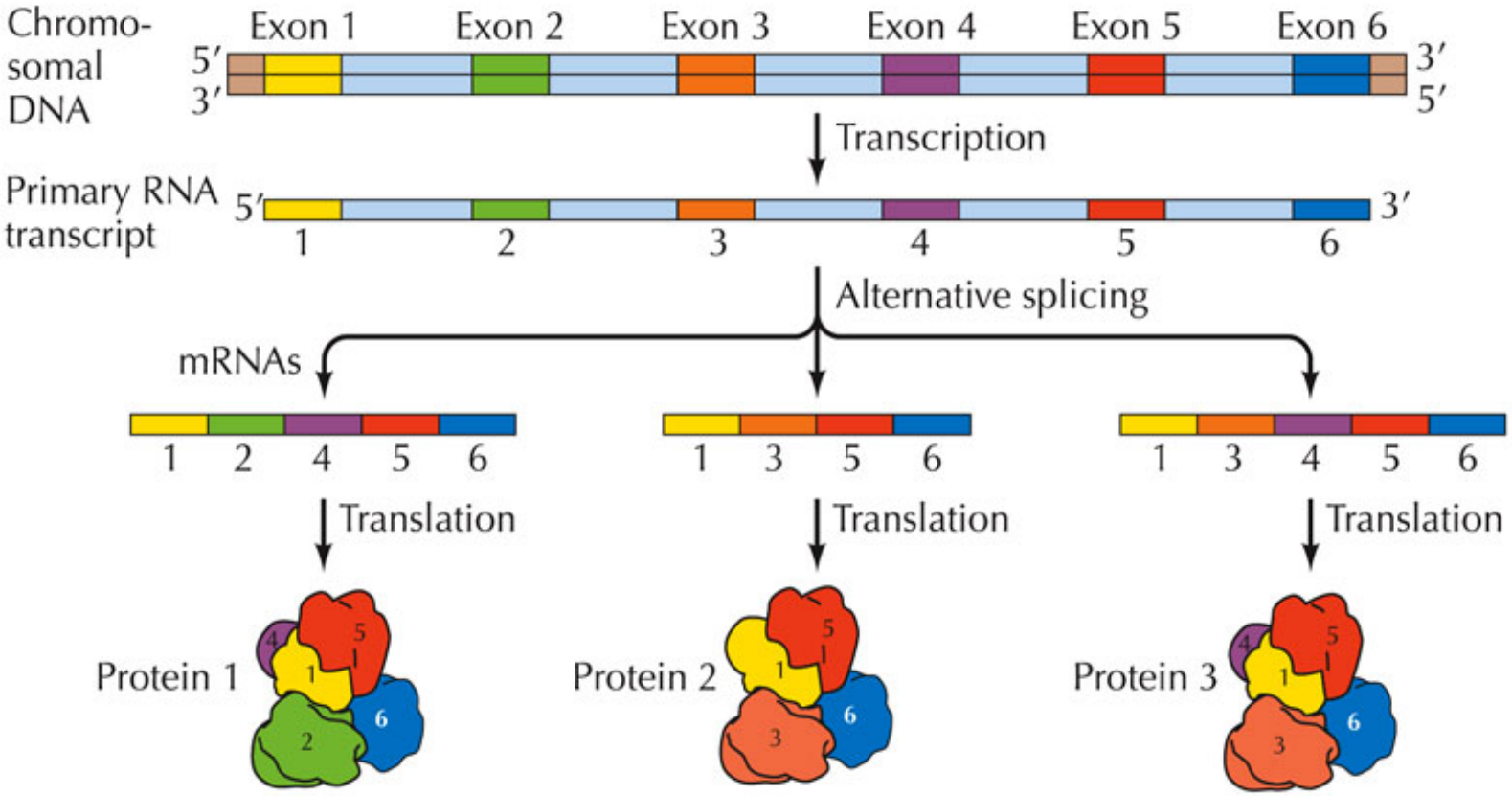
7. The splice sites are excised and the intron is removed.

8. The two exons are ligated.

9. This takes place for every two exons in the gene.



# Alternative splicing

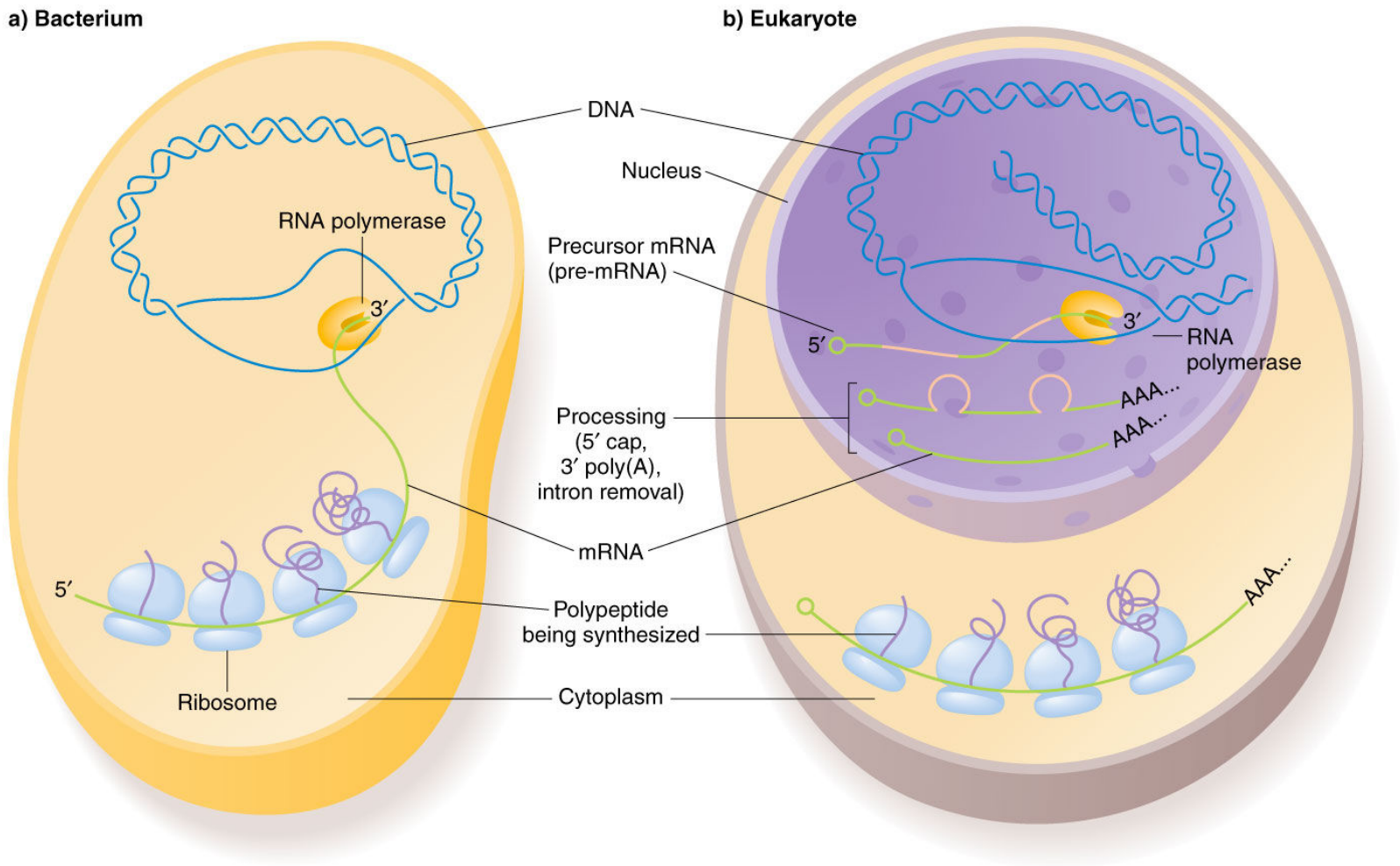



The splicing of introns allows for generating many different combination of exons and thus many different proteins.

# Summary and review

| Prokaryotes | Eukaryotes |
|---|---|
| **No nucleus** | **Nucleus** |
| DNA in cytoplasm | DNA in nucleus |
| **Transcription in cytoplasm** | **Transcription in nucleus** |
| Translation in cytoplasm | Translation in cytoplasm |
| **Polcistronic transcripts (one transcript many genes)** | **Monocistronic transcript (one transcript one gene)** |
| Coupled transcription and translation | Transcription and translation **NOT** coupled |
| **mRNA not processed** | **RNA processed** |
| One RNA polymerase | Many RNA polymerases |

# Summary and review



# To know

polycistronic

Alternative splicing

5’ capping

TFIIB

snRNPs

3’UTR

5’UTR

TFIIA

PIC

RNA Pol I

U4

Spliceosome

AG

promoter

7-methy guanine

Pre-mRNA

Promoter proximal element

exon

RNA Pol III

intron

TFIID

Branch point sequence

Poly(A) tail

U1

U2

PAP

initiator

RNA Pol II

activator

U5

Pre-initiation complex

enhancer

TATA box

U6

monocistronic

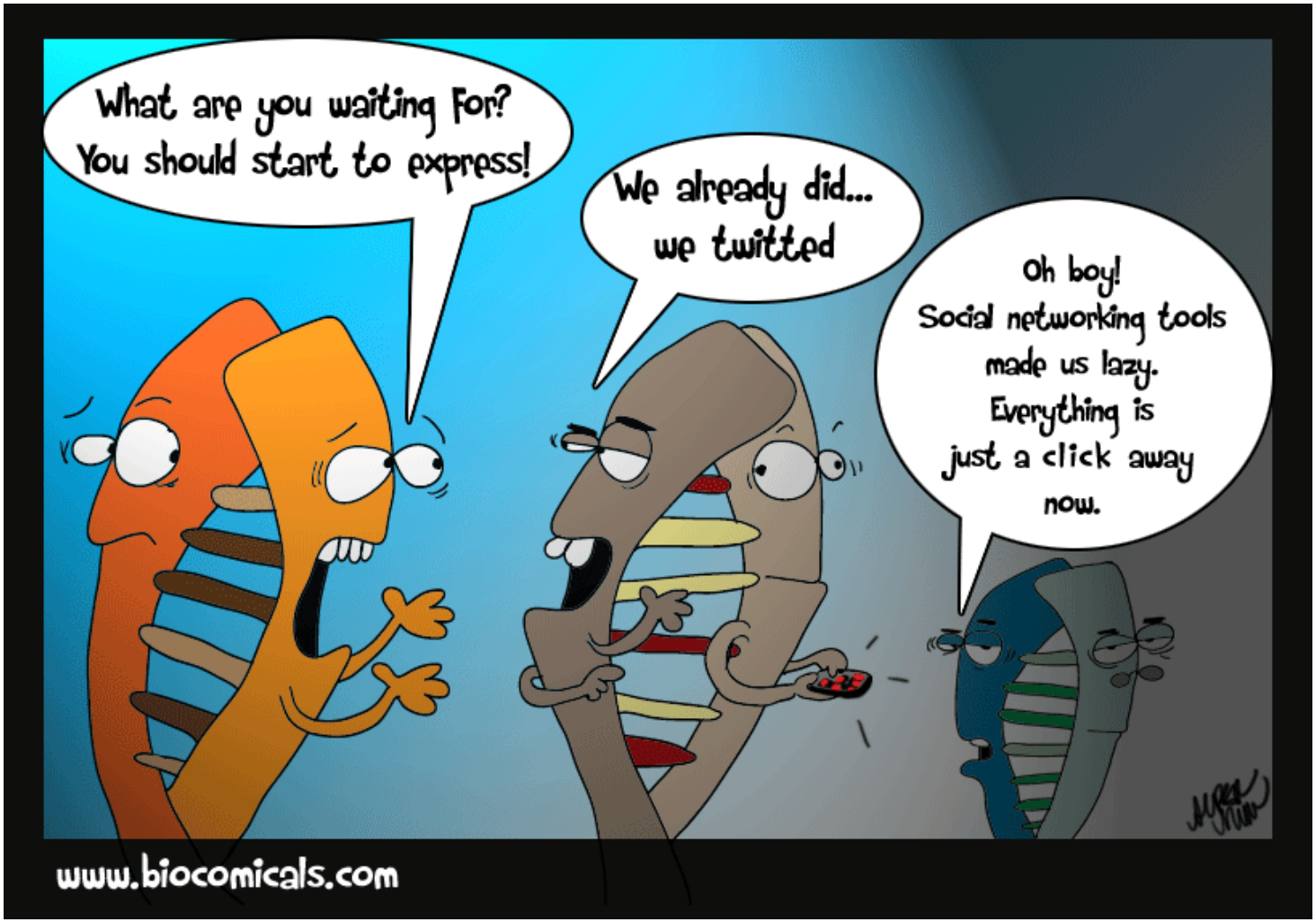
5’-5’ bond

GU

# Expectations

- You know the transcription process in eukaryotes.
- You know what is the eukaryotic gene is composed of.
- You know and memorize the names of the molecular machinery involved in eukaryotic transcription.
- You know the modifications that take place to the mRNA in eukaryotes.
- You know the similarities and differences between the transcription process in prokaryotes to that of eukaryotes.

# For a smile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We already did...
we twitted
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