Transcription

Piyush Tailor

Transcription

Four Steps

- Intiation
- Elongation
- Termination
- Post-transcription modification.

Mammalian RNA Polymerases

DNA dependant RNA polymerases.

- RNAp type I (A) = Synthesis of r-RNA
- RNAp type II (B) = Synthesis of m-RNA
- RNAp type III (C) = Synthesis of t-RNA

Bacterial RNA Polymerases

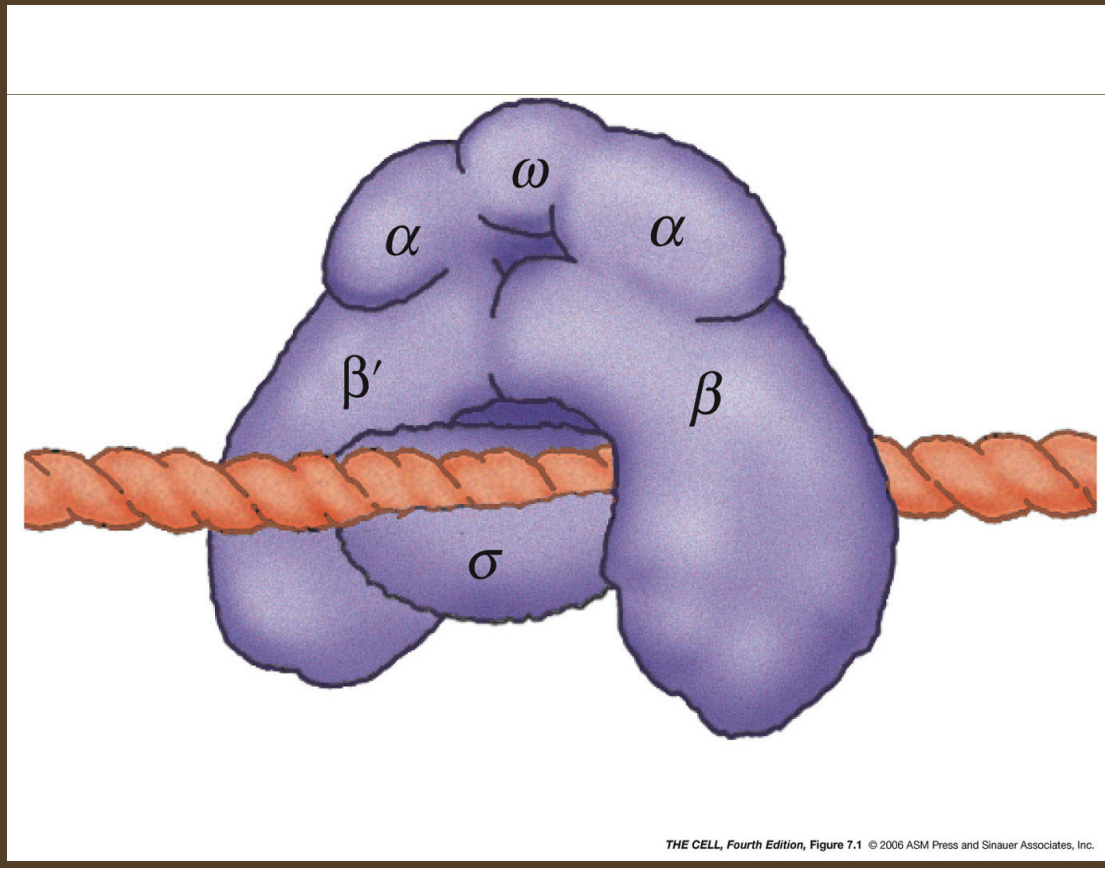
2 alpha subunit (α)

2 Beta subunit (β)

1 Sigma subunit (σ)

1 Omega subunit (ω)

Core Enzyme

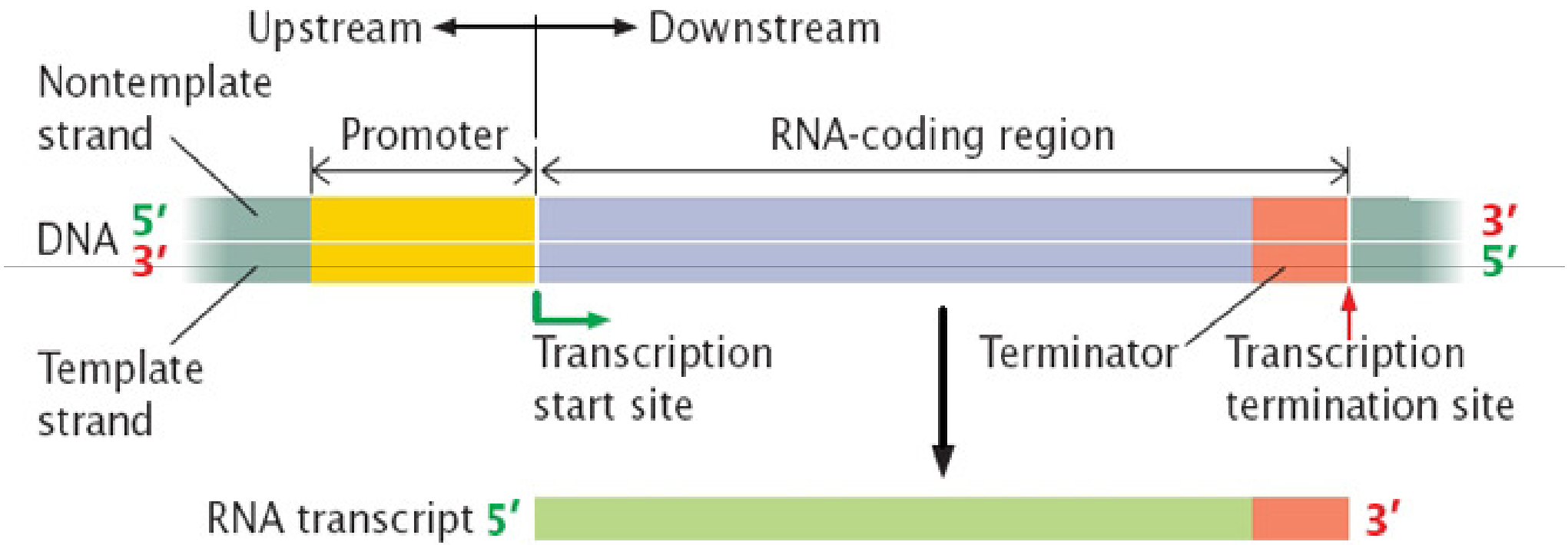


Prokaryotic RNA polymerase structure

Subunit	Role
α	Uncertain (Lack of Specificity)
β ,β'	Bind DNA Form Phosphodiester Bond.
σ	Recognizes Promoter site Facilitates initiation of Transcription (Specific)

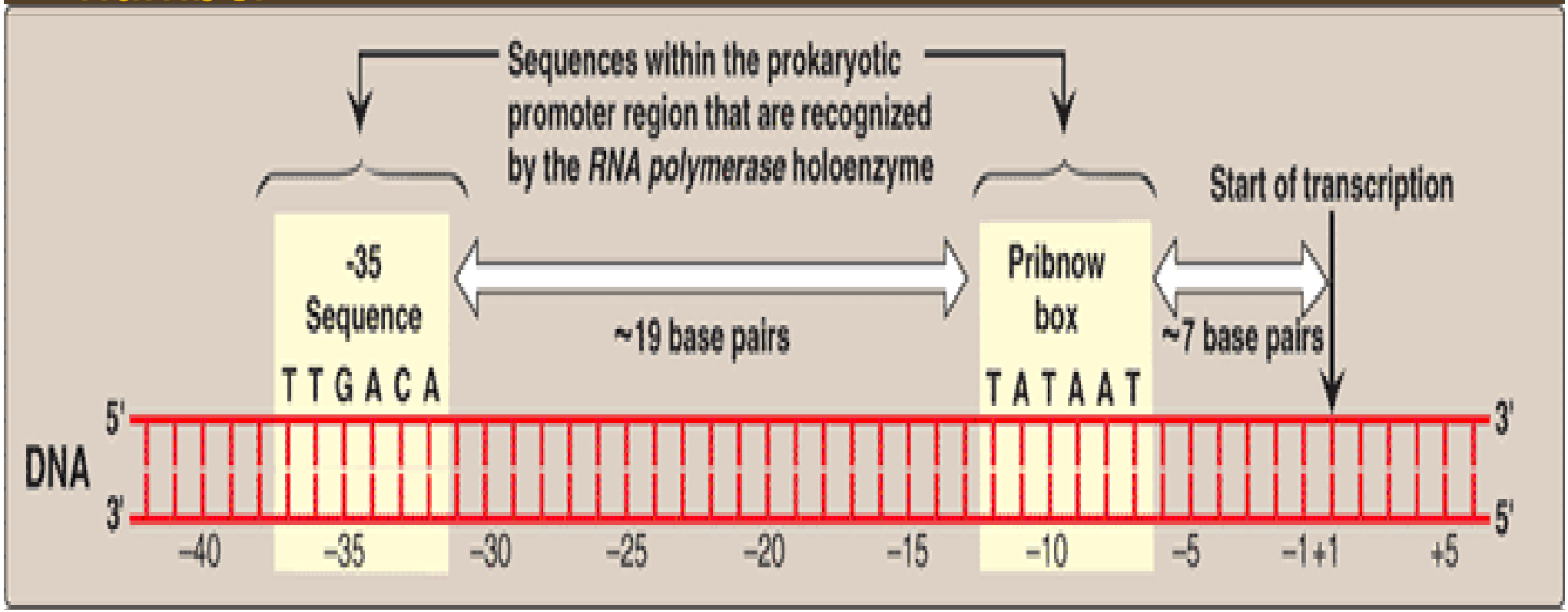
$\alpha_2\beta\beta'\sigma \rightleftharpoons \alpha_2\beta\beta' + \sigma$

Holo-enzyme — Core polymerase — Sigma factor

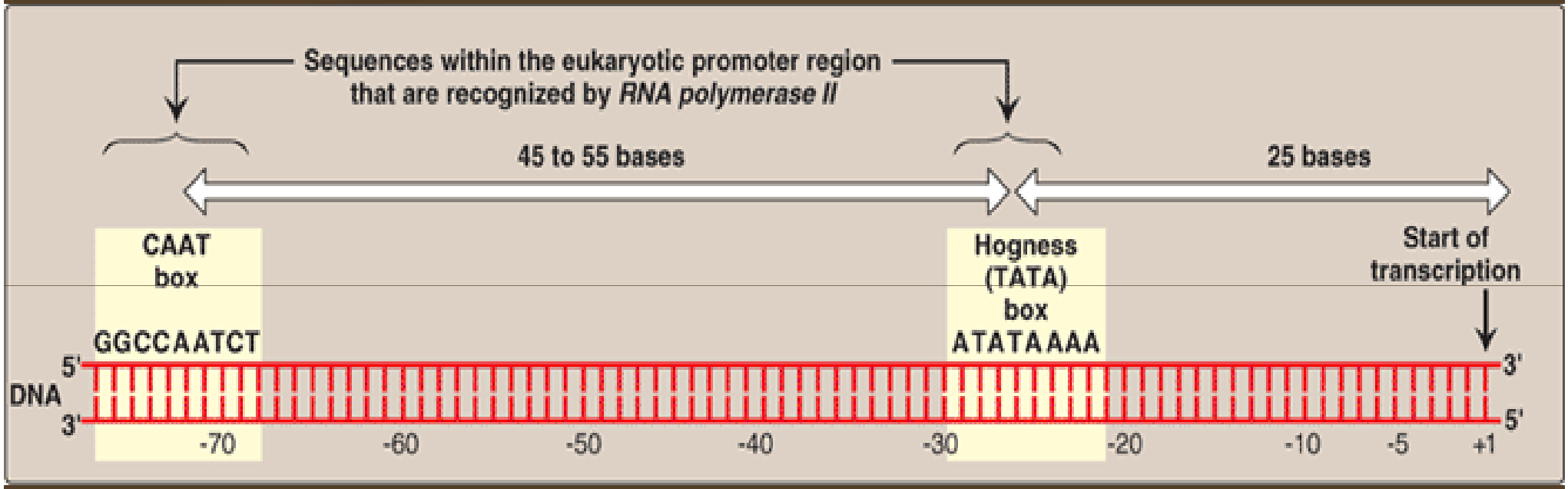

Upstream
Downstream
Nontemplate strand
Promoter
RNA-coding region
DNA 5′
3′
3′
5′
Template strand
Transcription start site
Terminator
Transcription termination site
RNA transcript 5′
3′

Initiation of transcription

- Holoenzyme bind to promoter.
- Promoter site = consensus sequences.
- Consensus = Most frequent .
- Transcription start = +1 position.
- Promoter region (prior to transcription site) = Negative number.



Eukaryotic Promoter consensus sequences



- TATA or Hogness box :
- CAAT box :

Prokaryotic Promoter consensus sequences

Pribnow Box

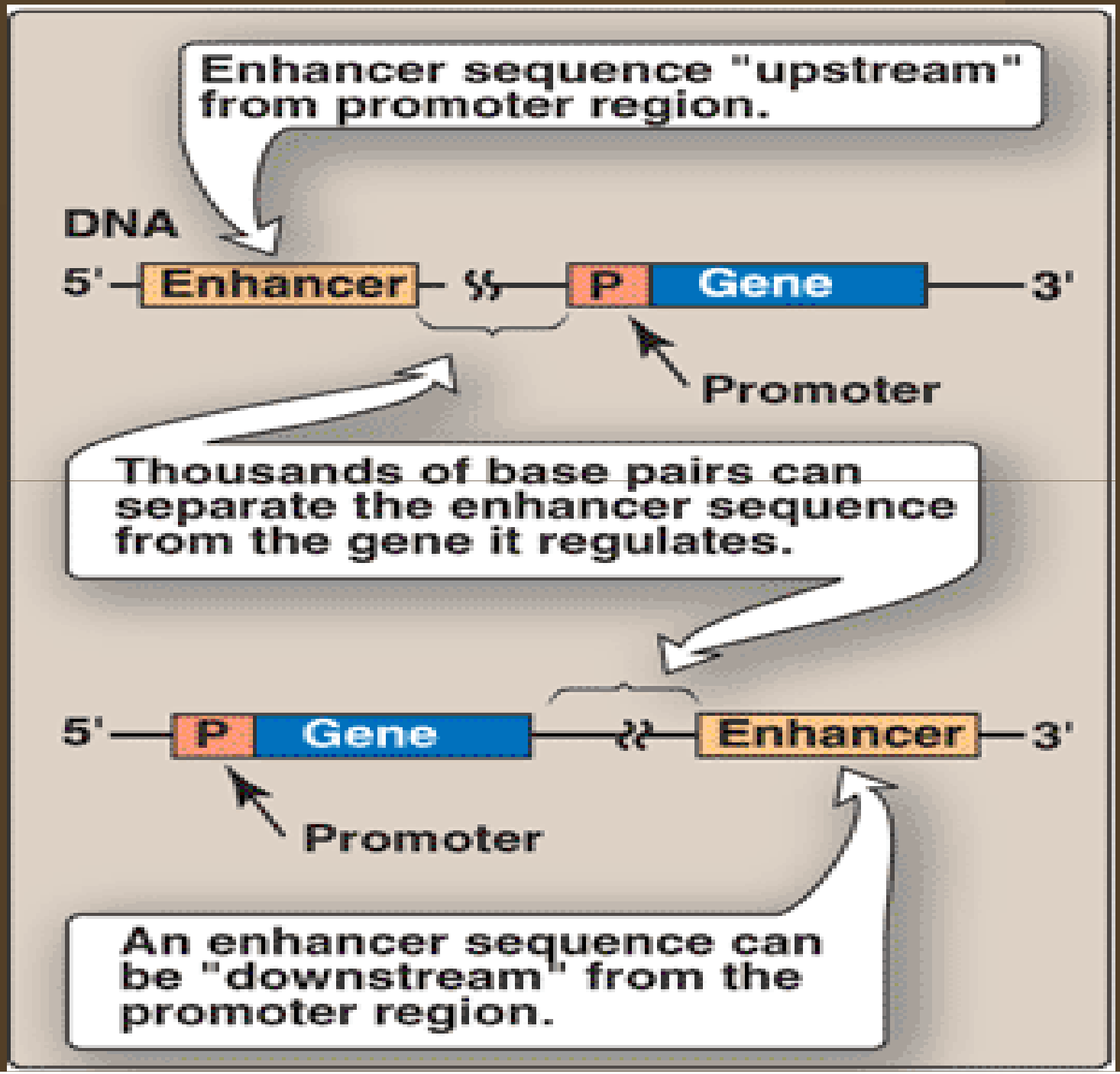
- 10 nucleotides Left to transcription unit
- Six nucleotides (5′-TATAAT-3′).

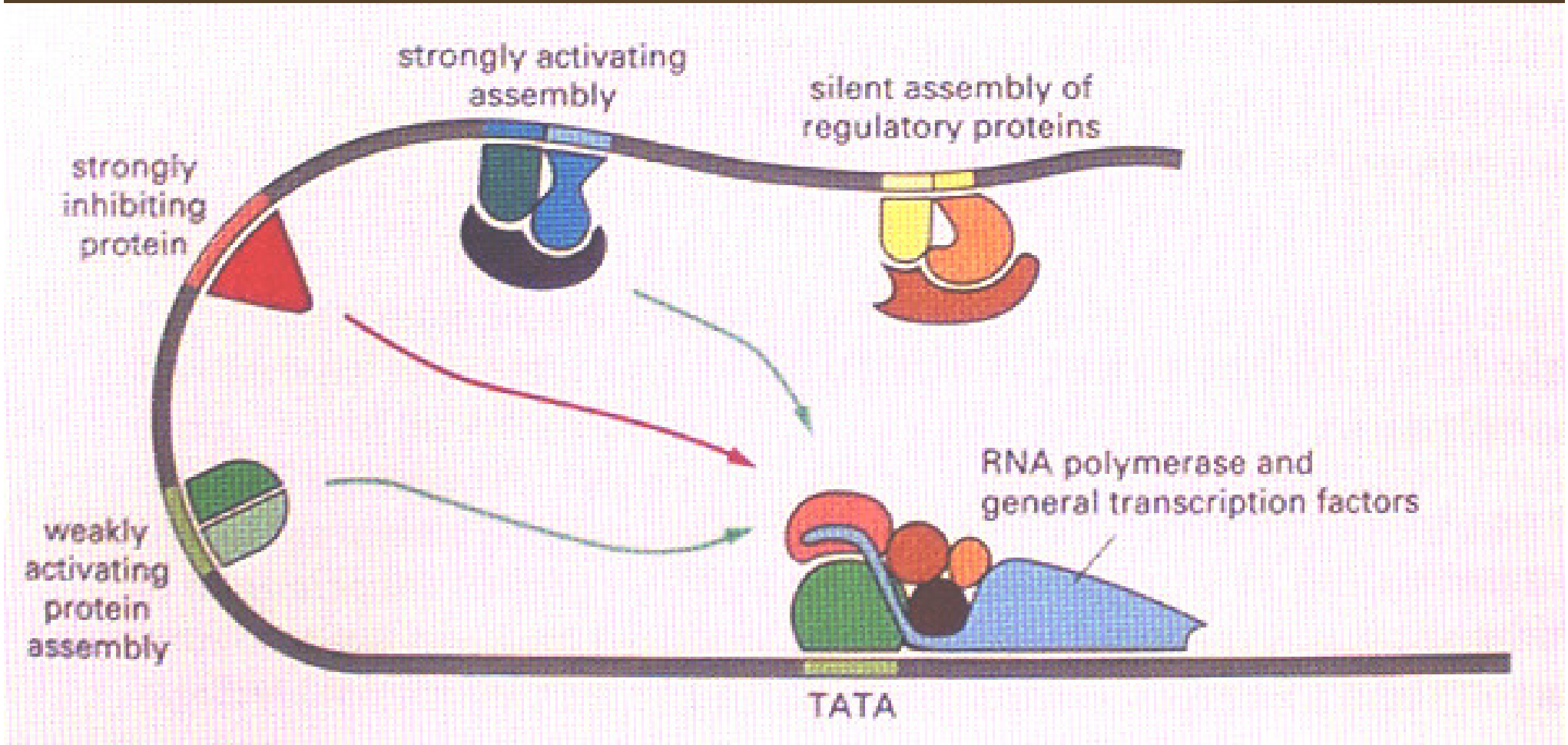
- **35 sequence**

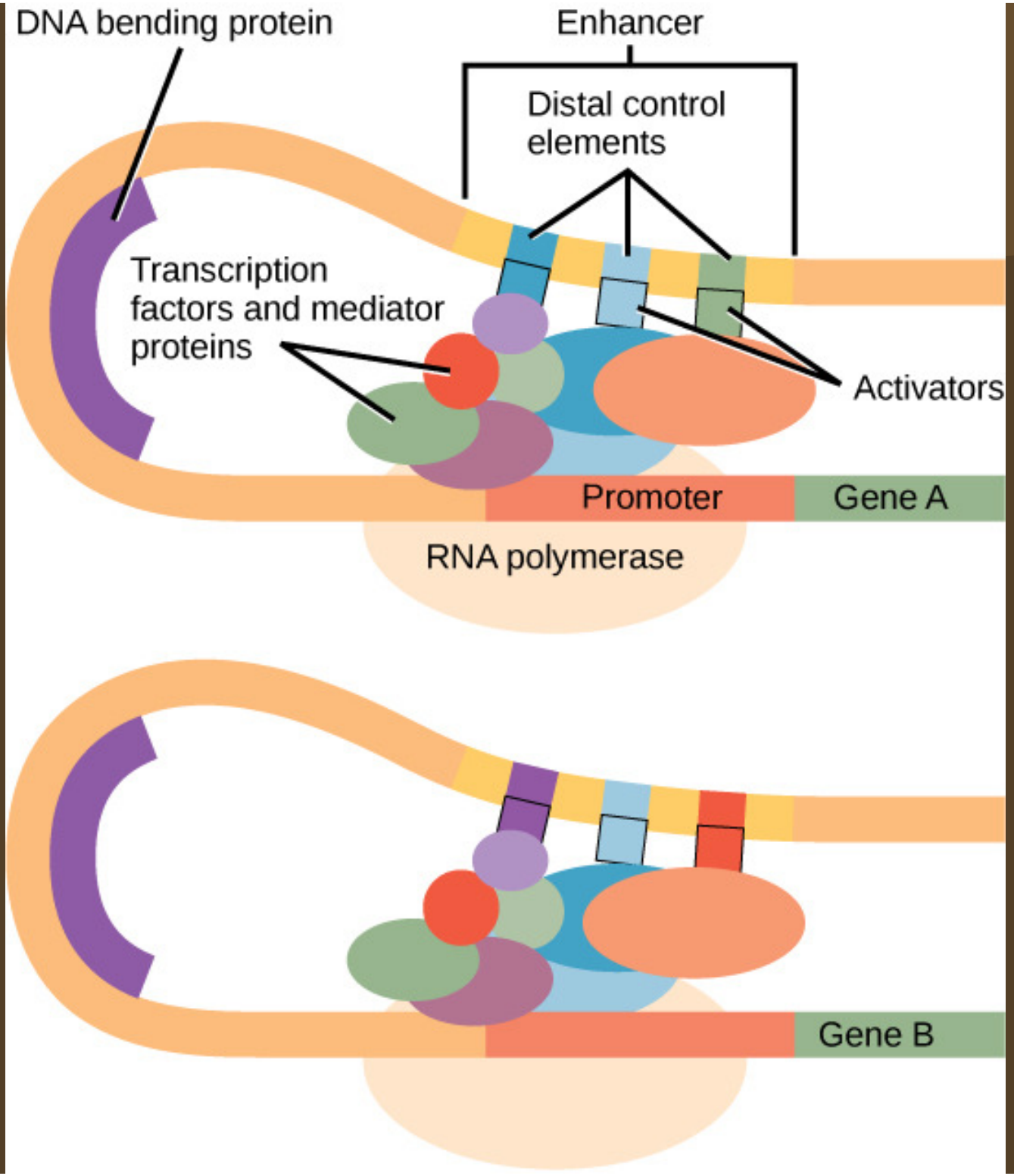
- (5′-TTGACA-3′) is centered about -35 bases.

- **If Mutation is there in Initiation sequence ????**

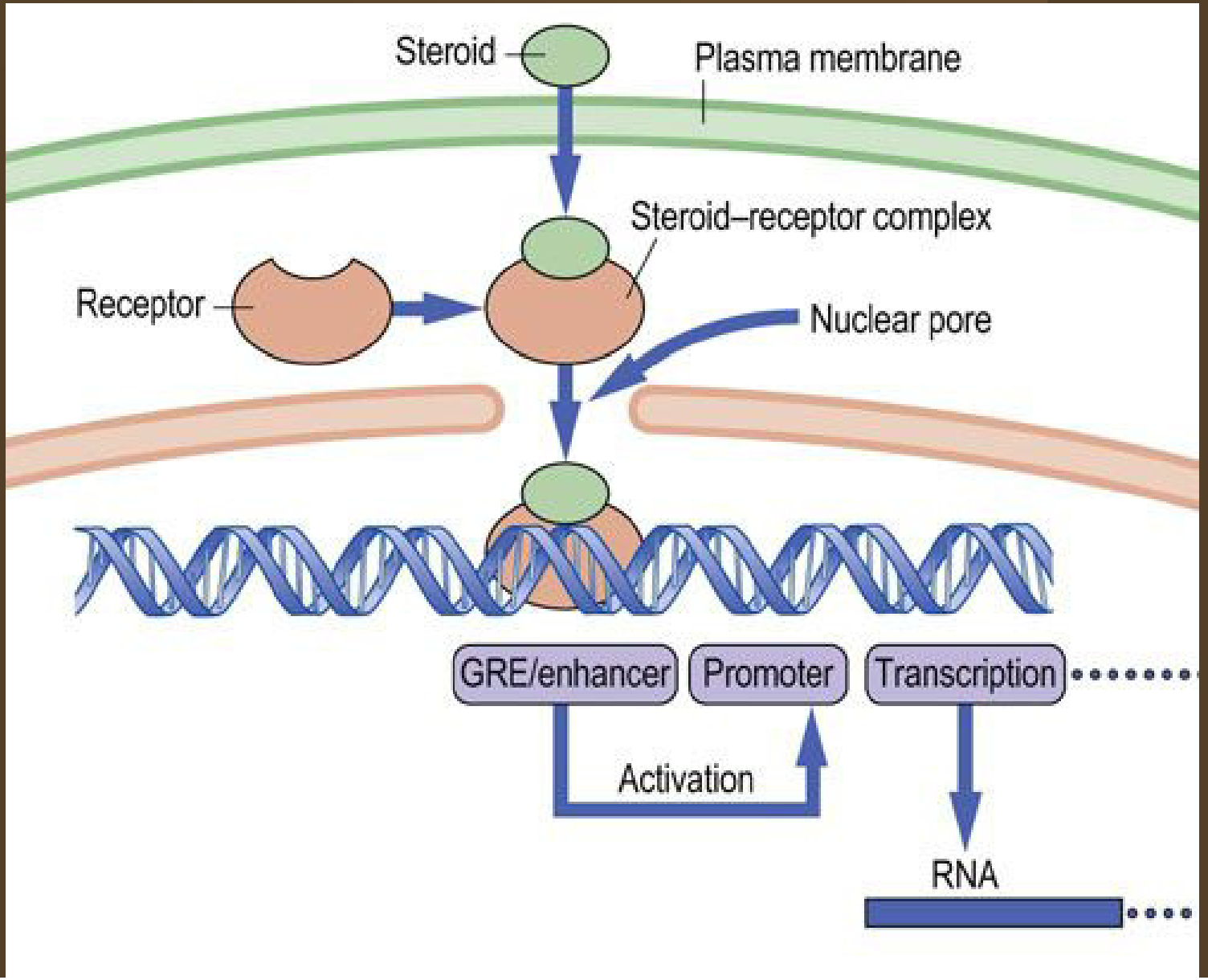
Enhancer sequence

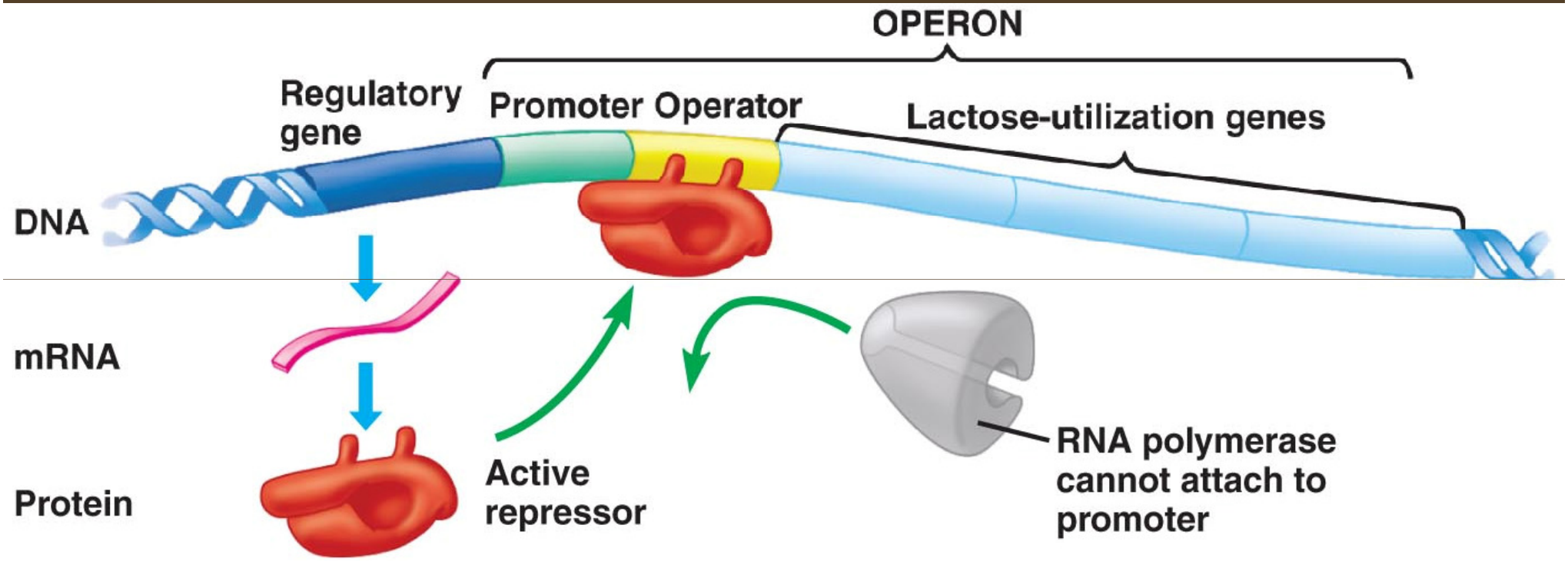



strongly activating assembly
silent assembly of regulatory proteins
strongly inhibiting protein
RNA polymerase and general transcription factors
weakly activating protein assembly
TATA


DNA bending protein
Enhancer
Distal control elements
Transcription factors and mediator proteins
Activators
Promoter
Gene A
RNA polymerase
Gene B

Steroid Hormone Action = Glucocorticoid Receptor Element



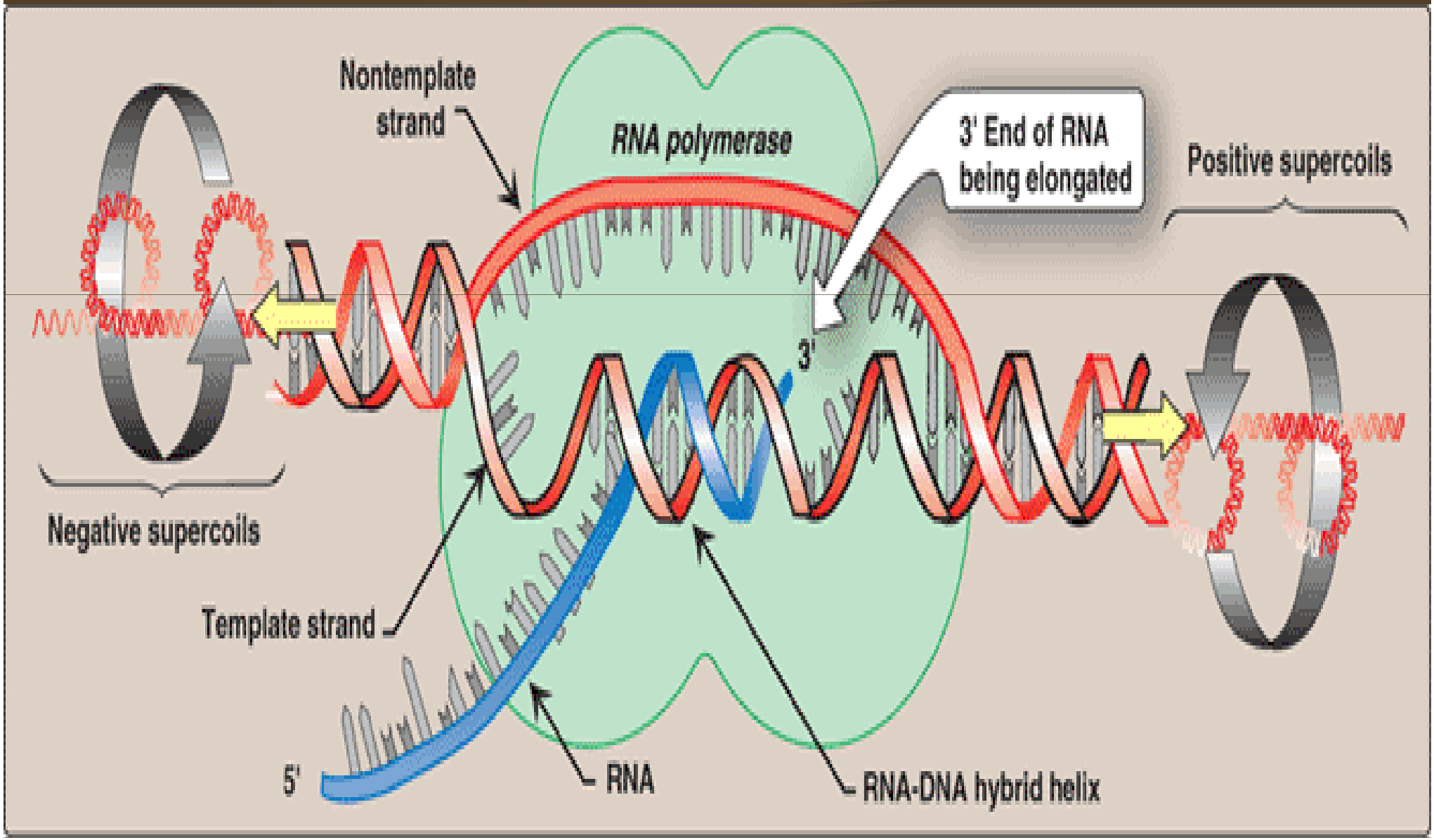


Operon turned off (lactose absent)

Elongation of Transcription

- Holoenzyme (Sigma factor)
  - Recogniz Promoter region.
  - Local Unwinding of the DNA helix.
- Removal of Supercoils = DNA topo-isomerase I & II .
- RNA polymerase = elongation of transcription unit.
- Sigma is then released
- Short DNA-RNA hybrid helix is formed.
- Uses nucleoside triphosphates as substrates
- Releases pyrophosphate each time a nucleoside monophosphate is added to the growing chain.
- Always in the 5′→3′ direction.
- Not require a primer
- No proofreading activity.

Elongation of Transcription



Termination

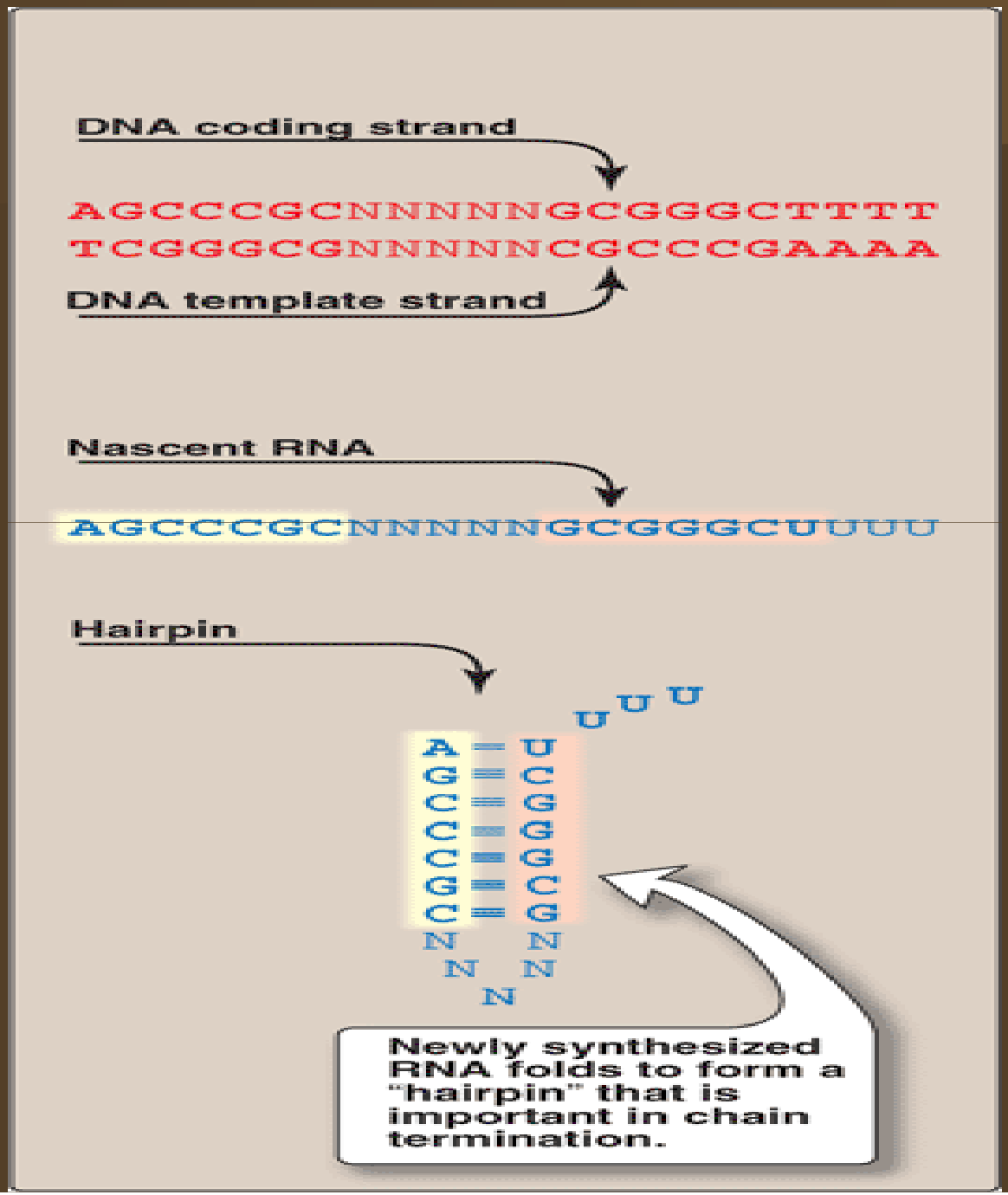
ρ (rho) independent termination

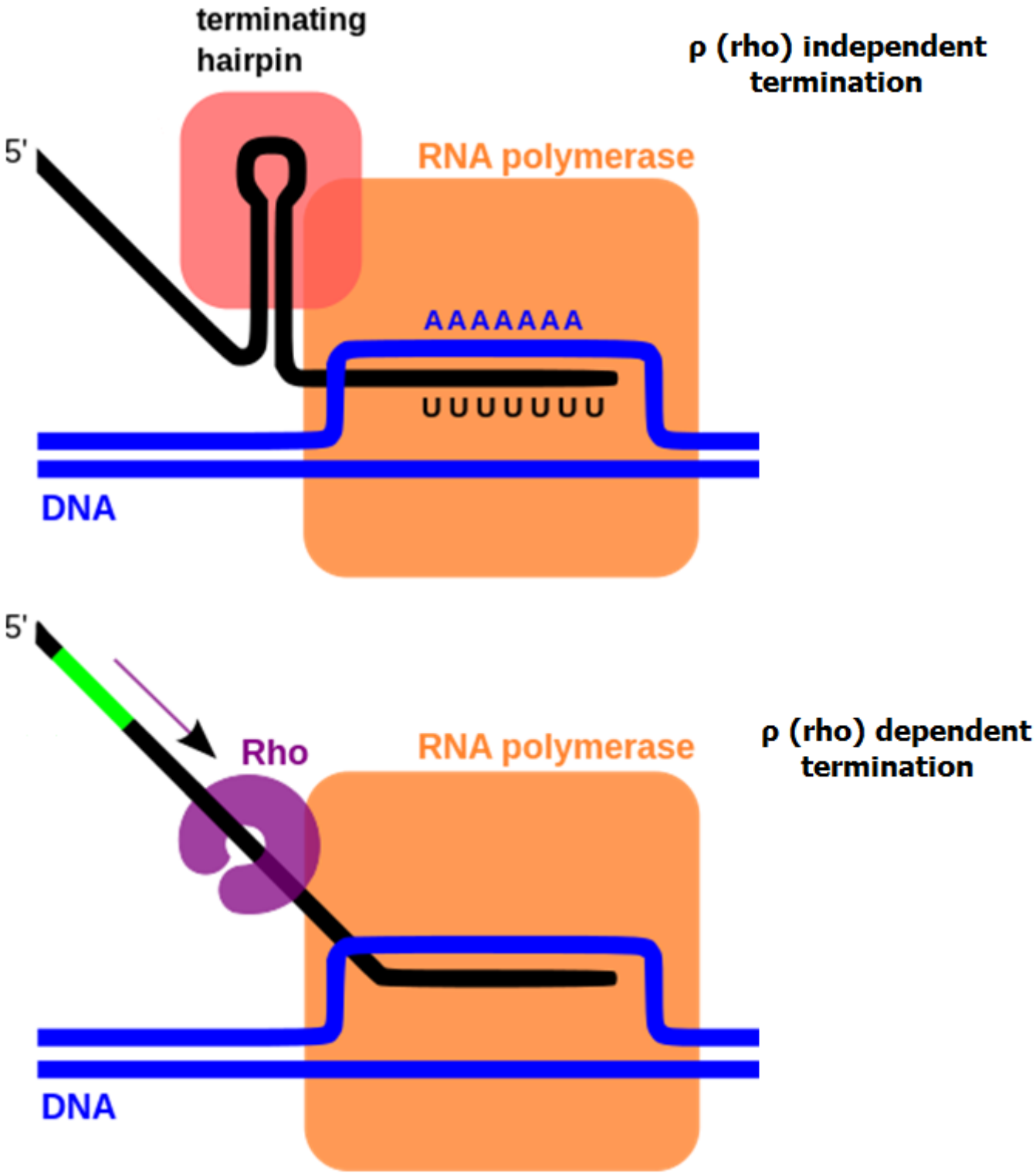
- In prokaryotic genes.
- DNA template generate a sequence self-complementary in newly made RNA.
- Allows the RNA to fold back on itself, forming a loop.
- Known as a hairpin.
- This facilitates the separation of the newly synthesized RNA from its DNA template

ρ (rho) dependent termination

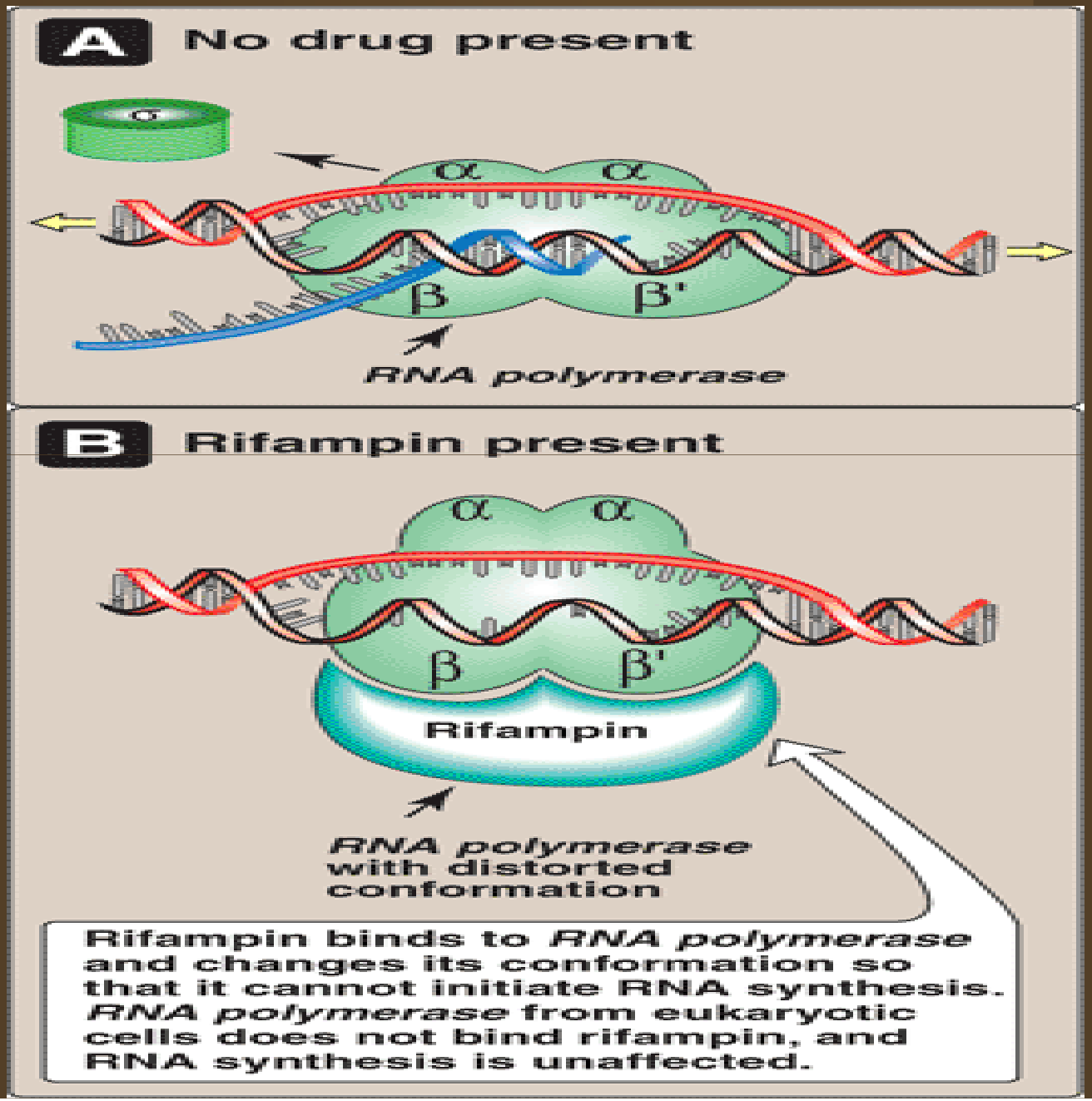
- An additional protein, rho = ρ factor.
- which is a hexameric with ATPase activity.
- It binds a C-rich "rho recognition site" near the 3′-end of the nascent RNA.
- The activity of rho separates the RNA-DNA hybrid helix, causing the release of the RNA.

ρ independent termination

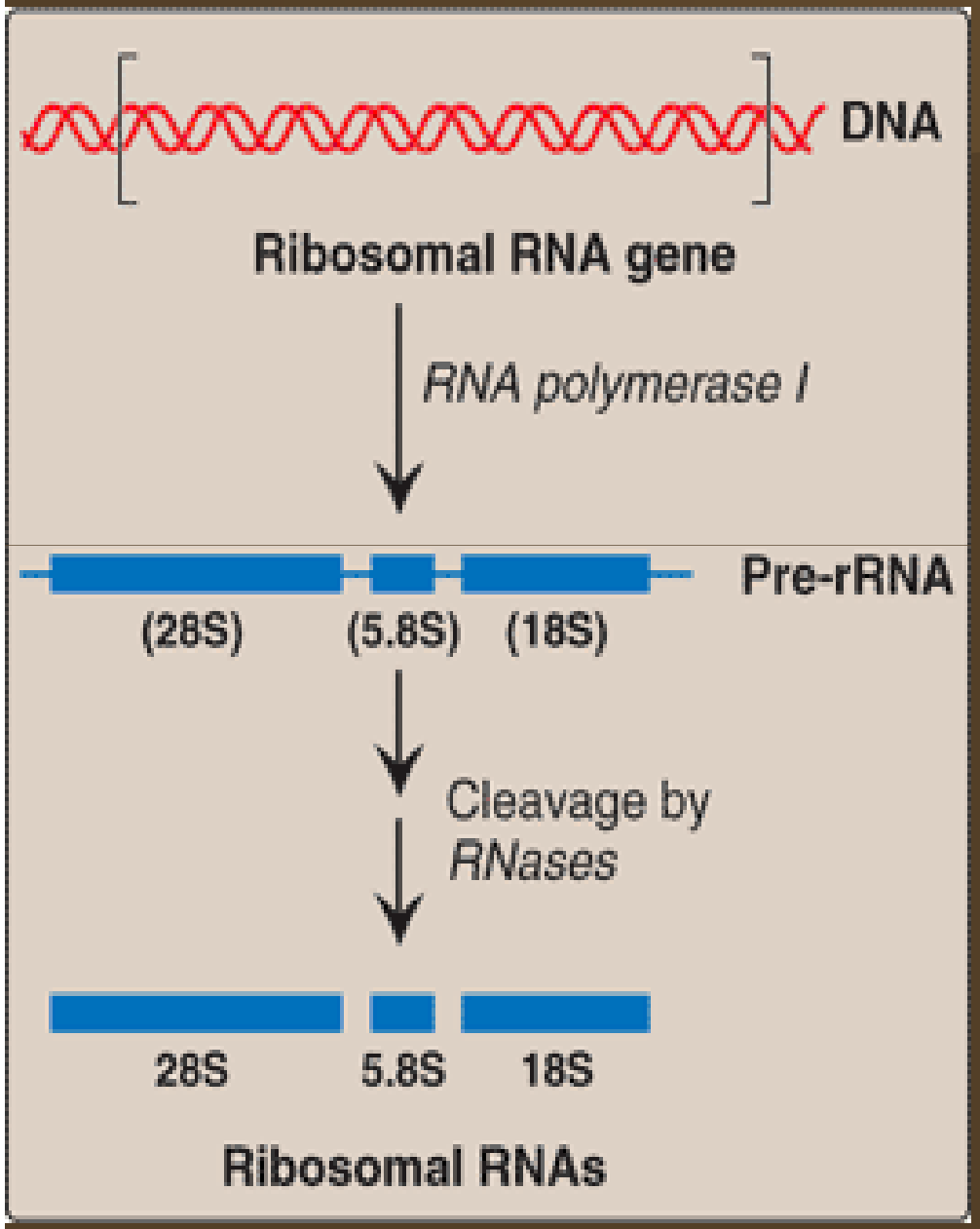



terminating
hairpin
ρ (rho) independent
termination
5'
RNA polymerase
AAAAAAA
UUUUUUU
DNA
5'
Rho
RNA polymerase
ρ (rho) dependent
termination
DNA

Action of Antibiotic = Rifampicin

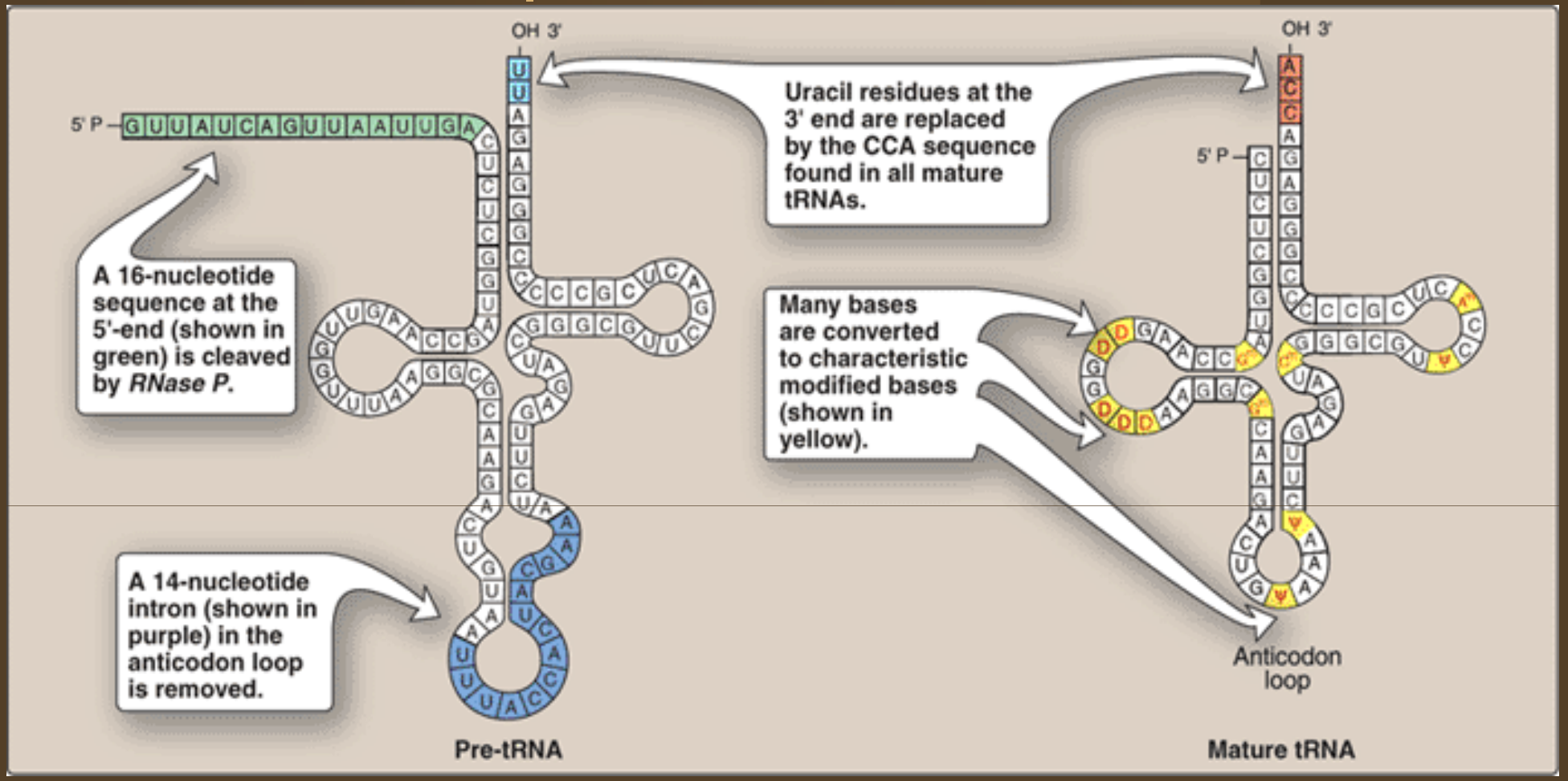


Post-transcription modification of r-RNA



- Synthesized from long precursor molecules called Preribosomal RNAs.
- Further "trimmed" to produce the required RNA species.

Post-transcription modification of t-RNA



- An intron removed from the anticodon loop.
- – CCA added by Nucleotidyltransferase to the 3′- end.
- "Unusual Bases" added at specific positions.

Post-transcription modification of eukaryotic m-RNA

- The collection of all the precursor molecules for mRNA is known as heterogeneous nuclear RNA (hnRNA).
- Modifications usually include:

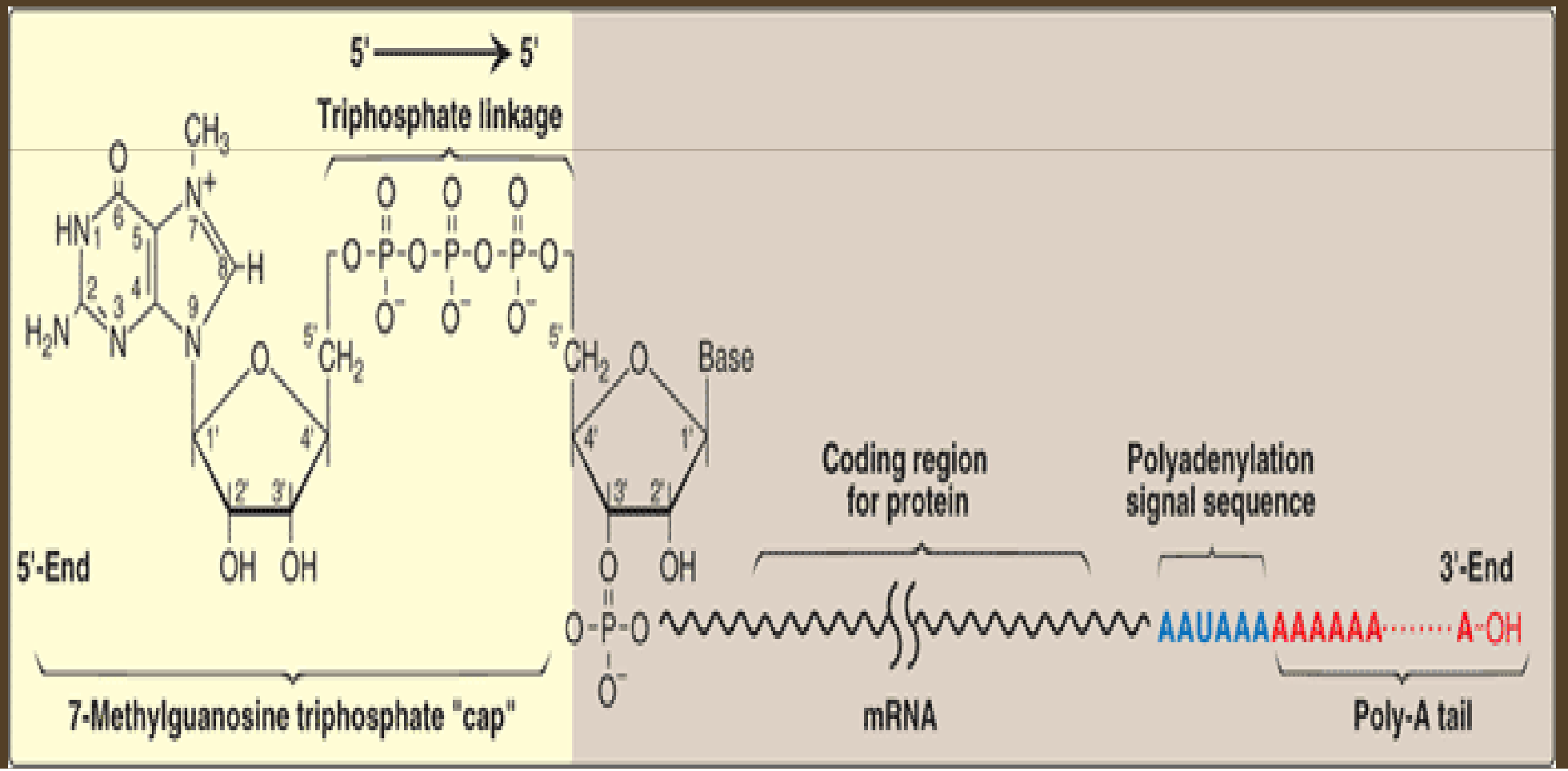
1. 7-methylguanosine Capping at 5’ end
2. Poly A tailing at 3’ end
3. Removal of introns
4. Splicing of m-RNA

1. 7-methylguanosine Capping at 5′ end

- Attached "backward" to the 5′-terminal end.
- Unusual 5′→5′ triphosphate linkage.
- Requires the nuclear enzyme Guanyl transferase.
- S-adenosylmethionine is the source of the methyl group.
- Permits the initiation of translation
- helps stabilize the mRNA.
- **Eukaryotic mRNA = No cap**
- **= No efficient translation.**

7-methylguanosine Capping at 5′ end

5′→5′ triphosphate linkage



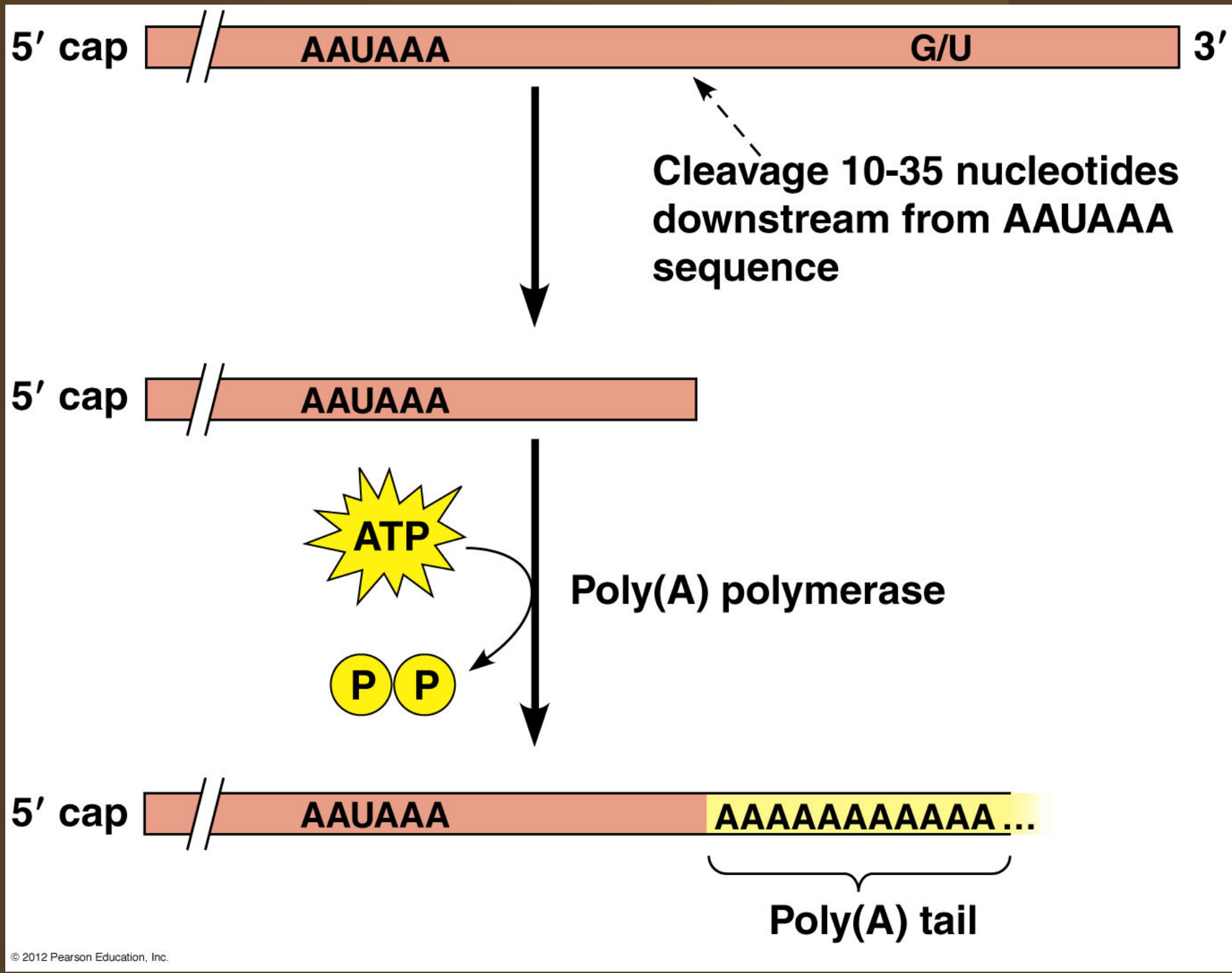
2. Poly A tailing at 3′ end

- 40–200 adenine nucleotides attached 3′-end.
- 3′ end of mRNA is cleaved downstream from Polyadenylation consensus sequence (AAUAAA)
- Then poly-A tail is added to the new 3′-end.

❖ **Significant**

- ✓ Protect from 3′ exonuclease activity.
- ✓ Useful for isolate m-RNA in laboratory.
- ✓ Help stabilize the mRNA
- ✓ Facilitate their exit from the nucleus.

- Gradually shortened,After entery in cytosol
- m-RNA for histone does not contain poly A tail.



3. Removal of Introns

- Removal of RNA sequences, which do not code for protein (introns) from the primary transcript.
- Remaining sequences, Exons, are joined
- Splicing = The process of removing introns and joining exons .
- Spliceosome = Does it.

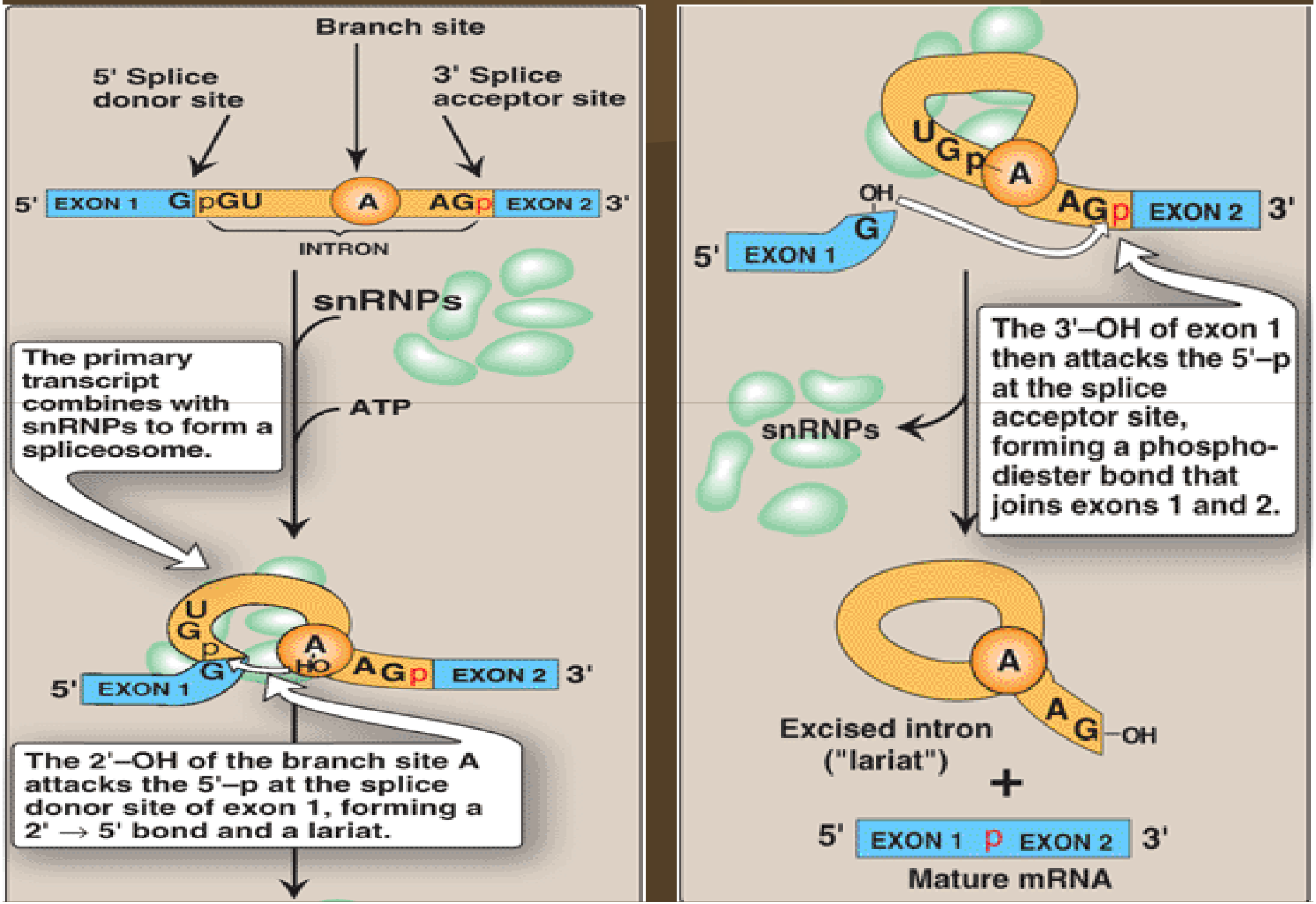
3. Removal of Introns

- snRNP (small nuclear Ribonucleoprotein Particle)
- snRNP = “snurps”
- Mediate splicing.
- They facilitate the removal of intron segments.

Mechanism of Splicing

- snRNP brings he neighboring exons into the correct alignment.
- Introns have been removed and exons joined
- Mature mRNA molecules leave the nucleus and pass into the cytosol through pores in the nuclear membrane.
- Mutations at splice sites can lead to improper splicing.
- 50 % of all genetic diseases are a result of mutations that affect RNA splicing.
- For example, incorrect splicing of β-globin mRNA are responsible for some cases of β-thalassemia.

Splicing of hn-RNA



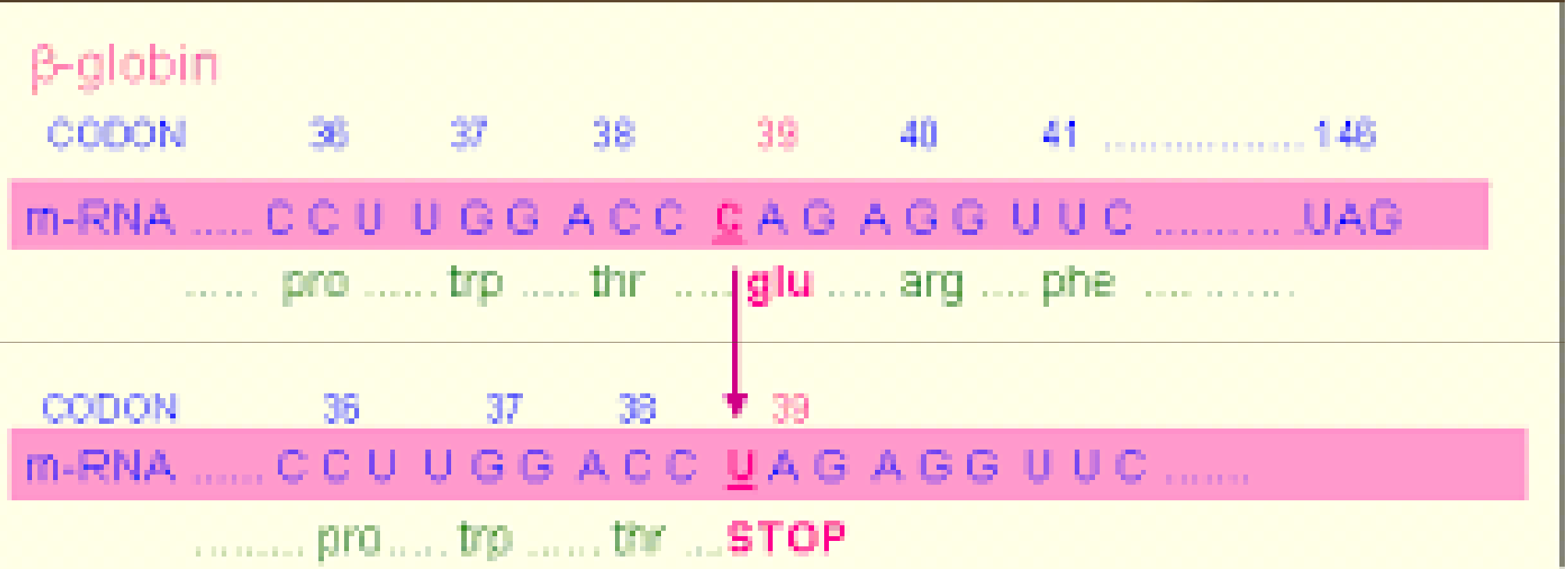
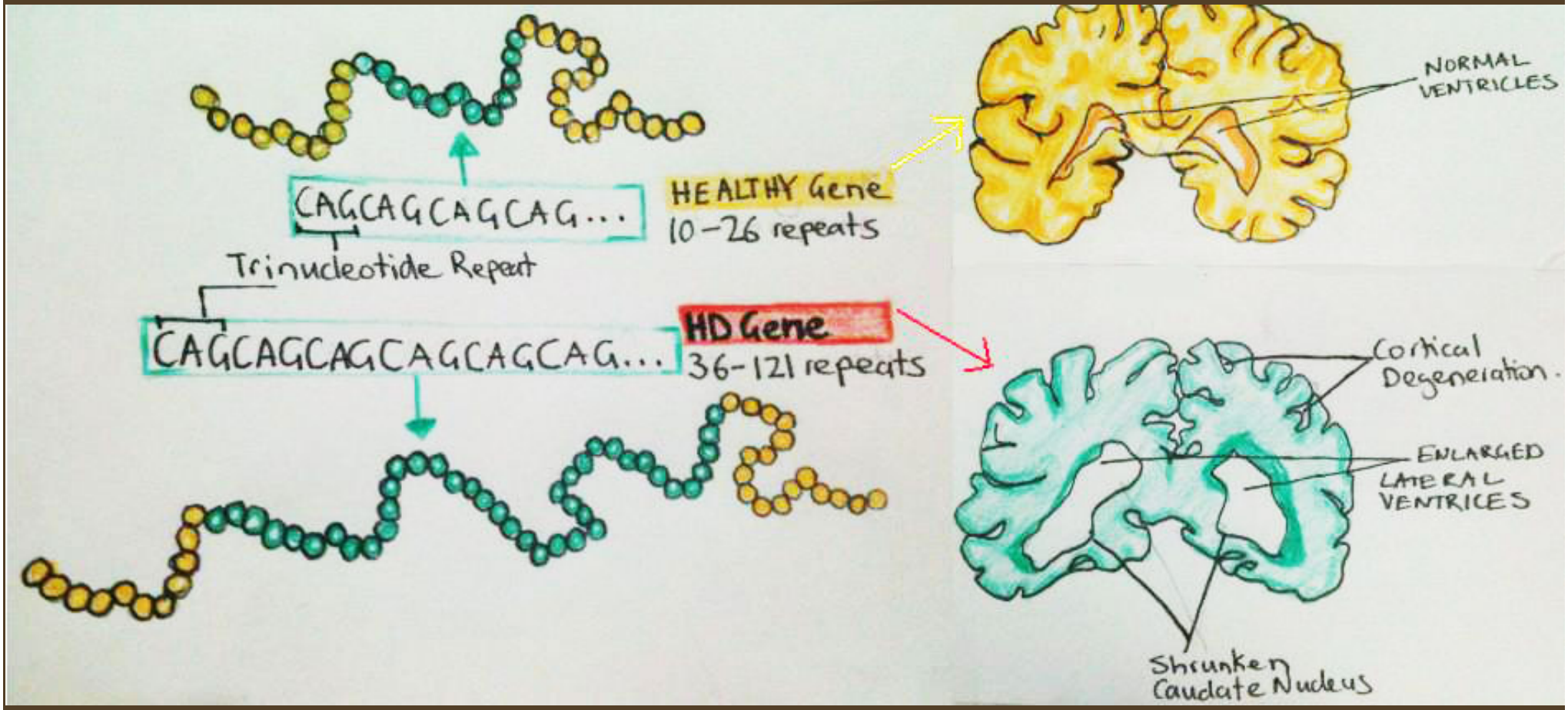
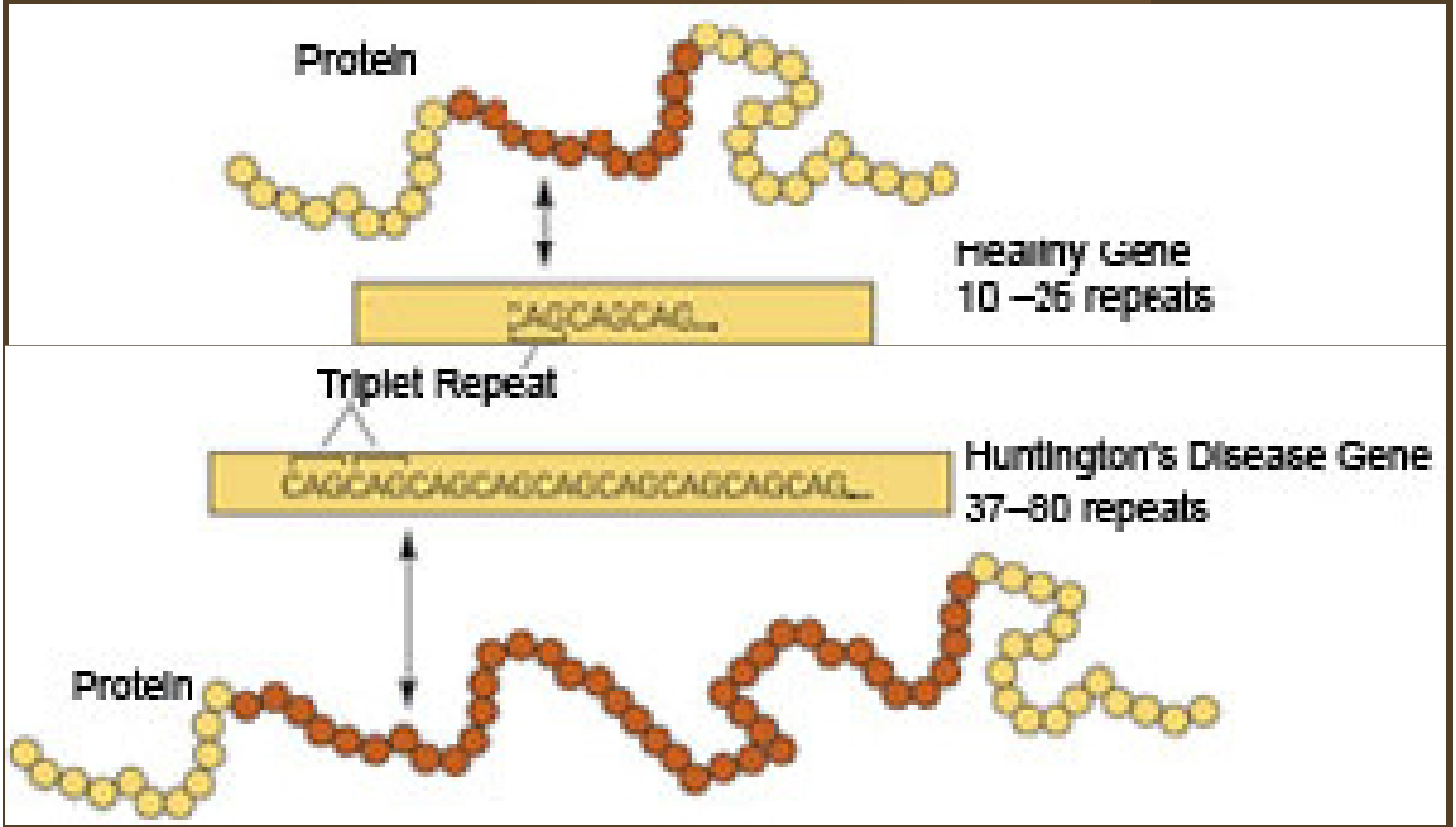


Fig 5.2. The mutation of C to U in codon 39 of the β-globin gene results in the substitution of the amino acid glutamine by a stop signal, resulting in a severely shortened β-globin protein chain.


CAGCAGCAGCAG...
Trinucleotide Repeat
HEALTHY Gene
10-26 repeats
NORMAL VENTRICLES
CAGCAGCAGCAGCAGCAG...
HD Gene
36-121 repeats
Cortical Degeneration
ENLARGED LATERAL VENTRICES
Shrunken Caudate Nucleus


Protein
Healthy Gene
10 –26 repeats
CAGCAGCAG...
Triplet Repeat
CAGCAGCAGCAGCAGCAGCAGCAGCAG...
Huntington's Disease Gene
37–80 repeats
Protein