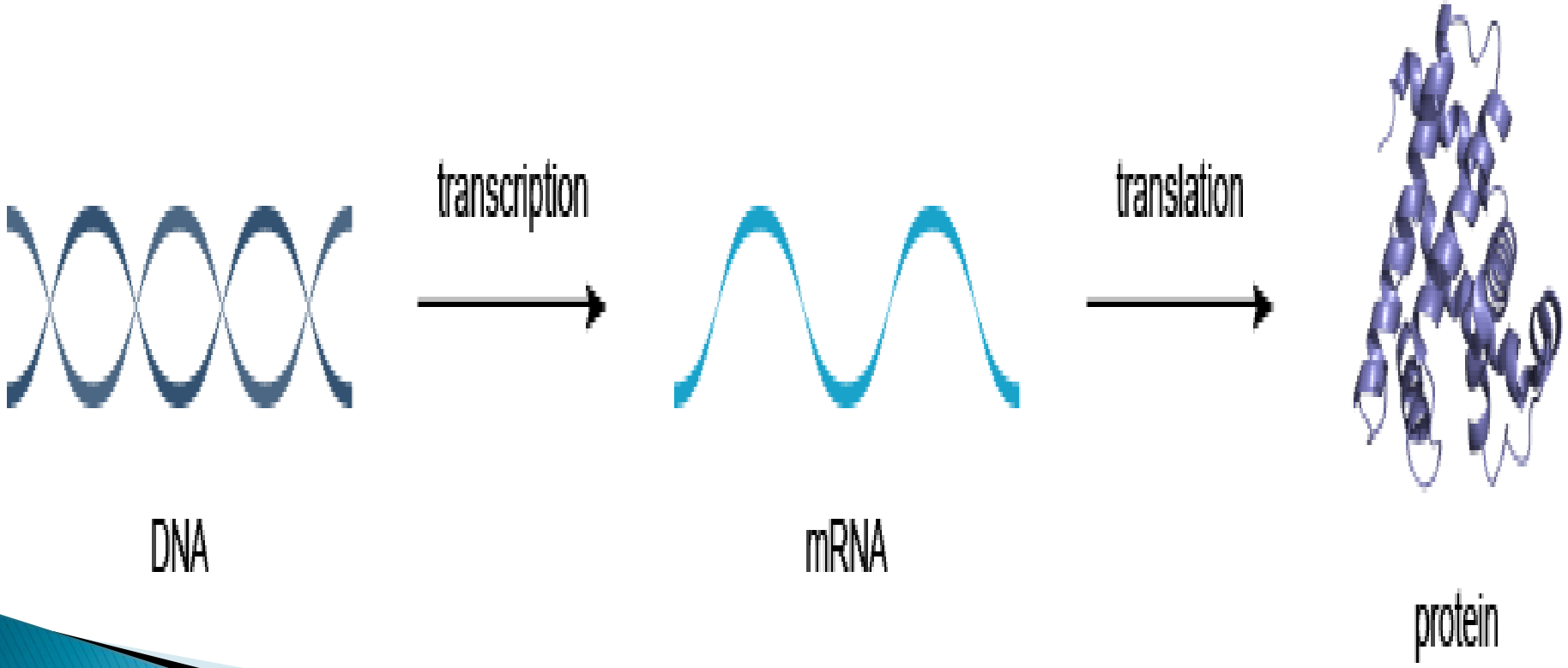
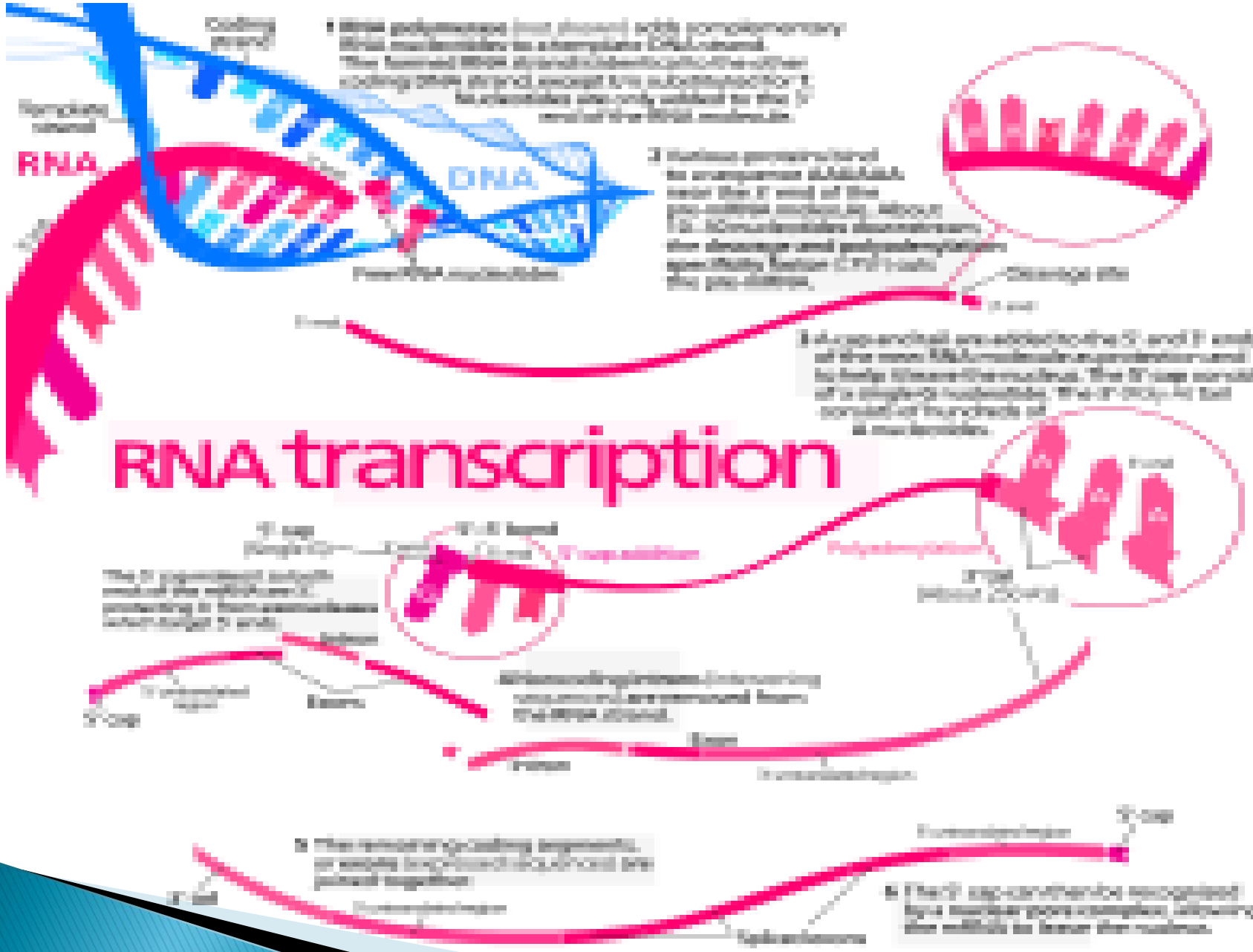
DISCUSS TRANSCRIPTION MECHANISM

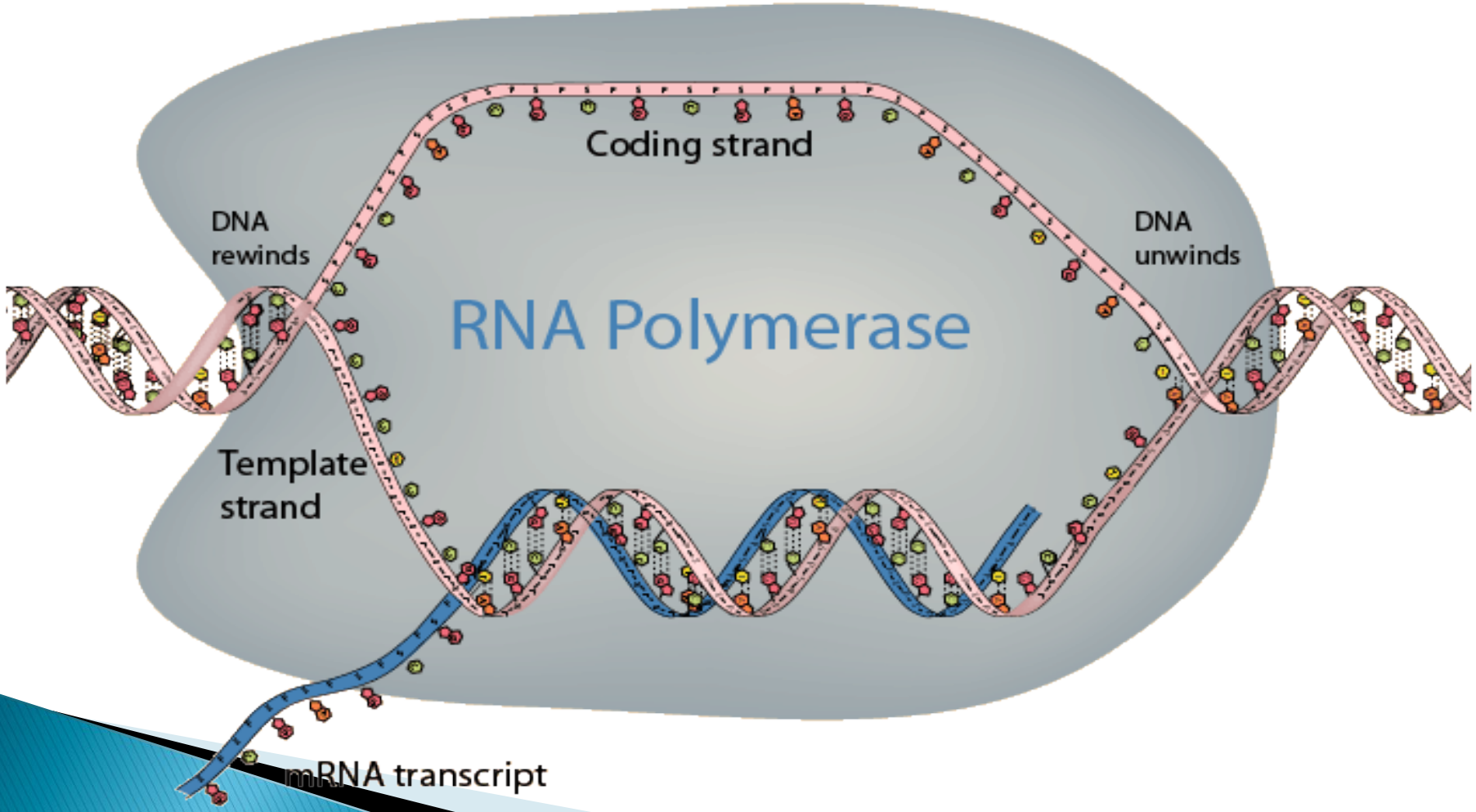
BRIAN JUSTUS MUSUBIRE
16/BSB/BU/R/0001

THE CENTRAL DOGMA

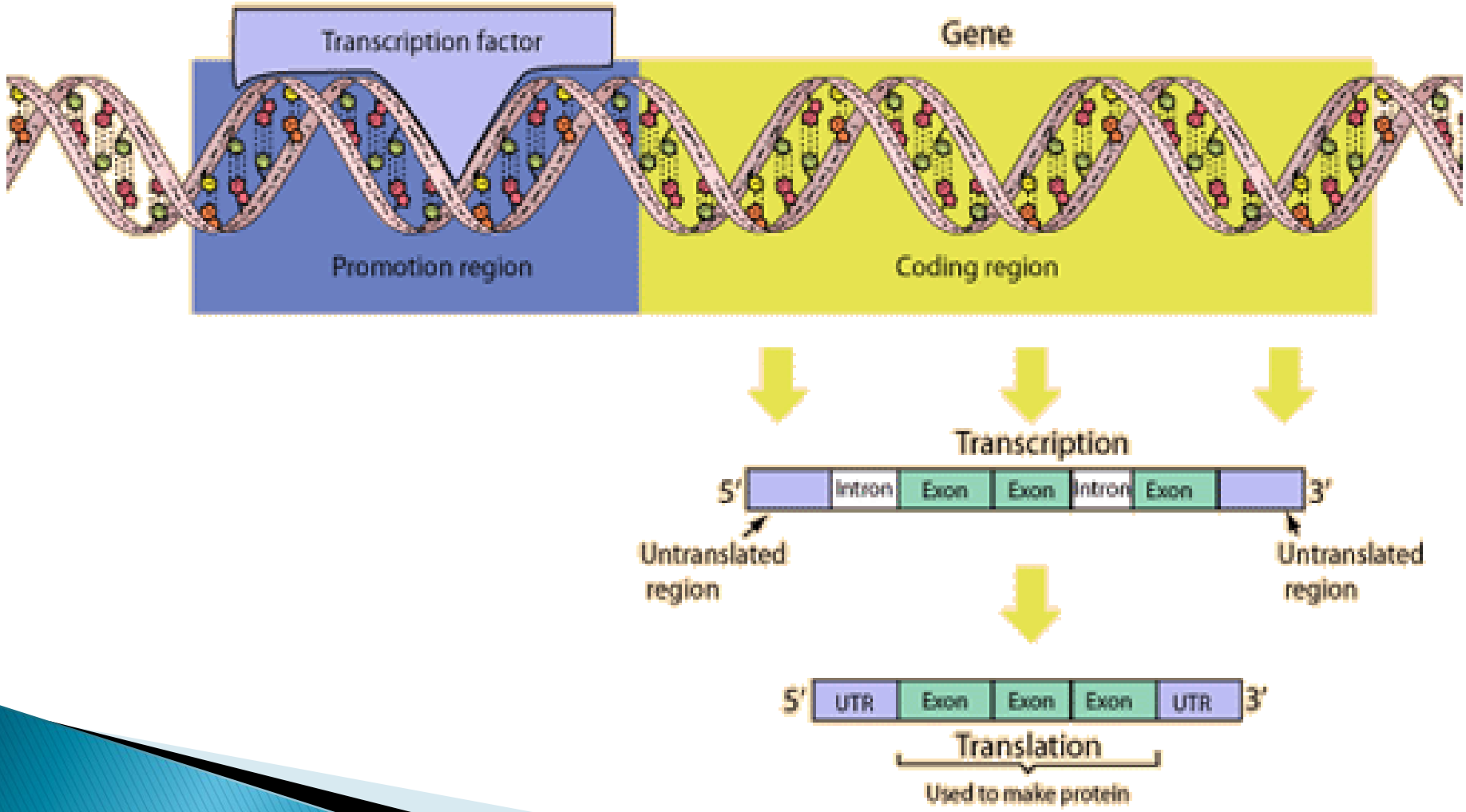



RNA transcription
DNA
RNA

OVERVIEW OF TRANSCRIPTION

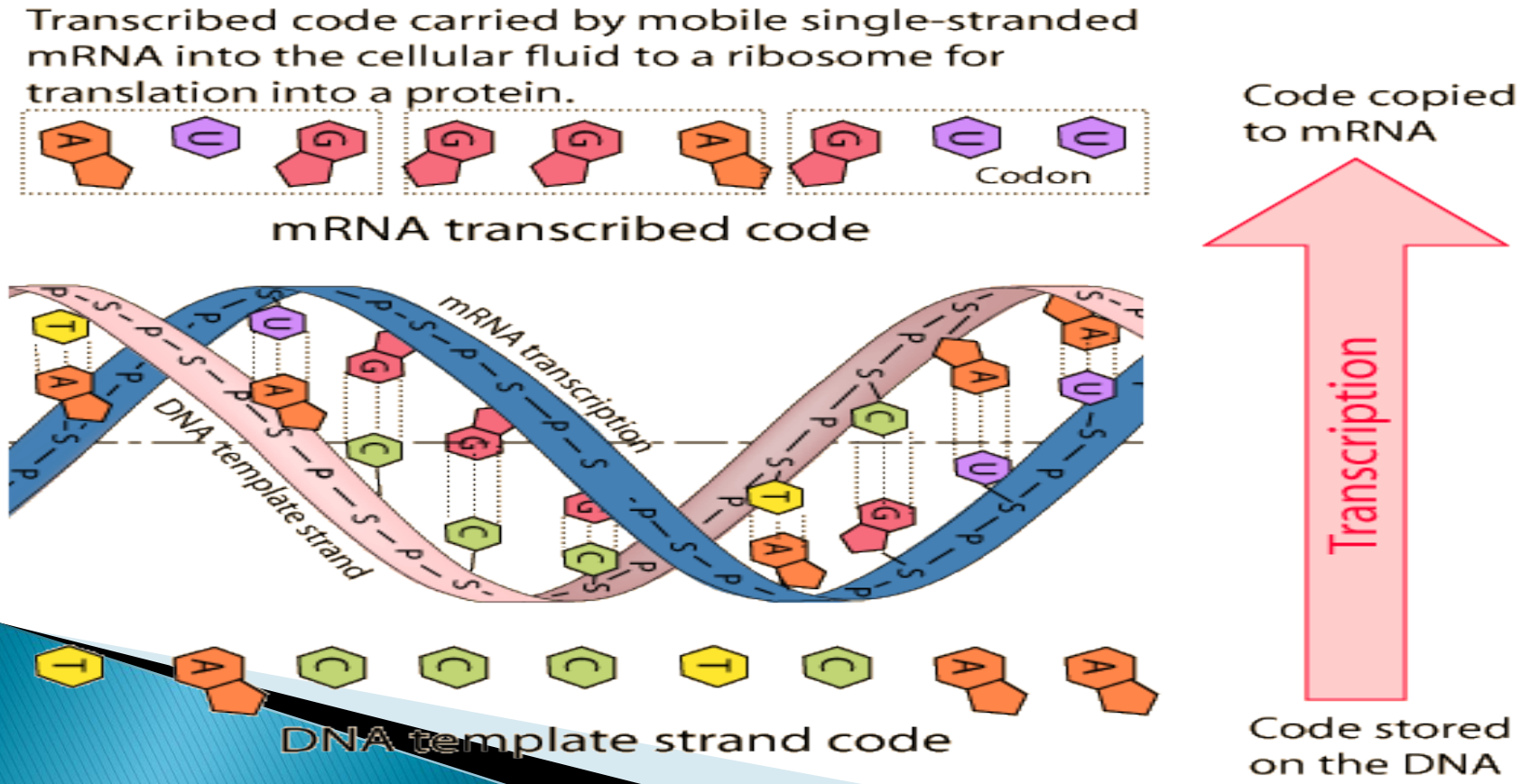


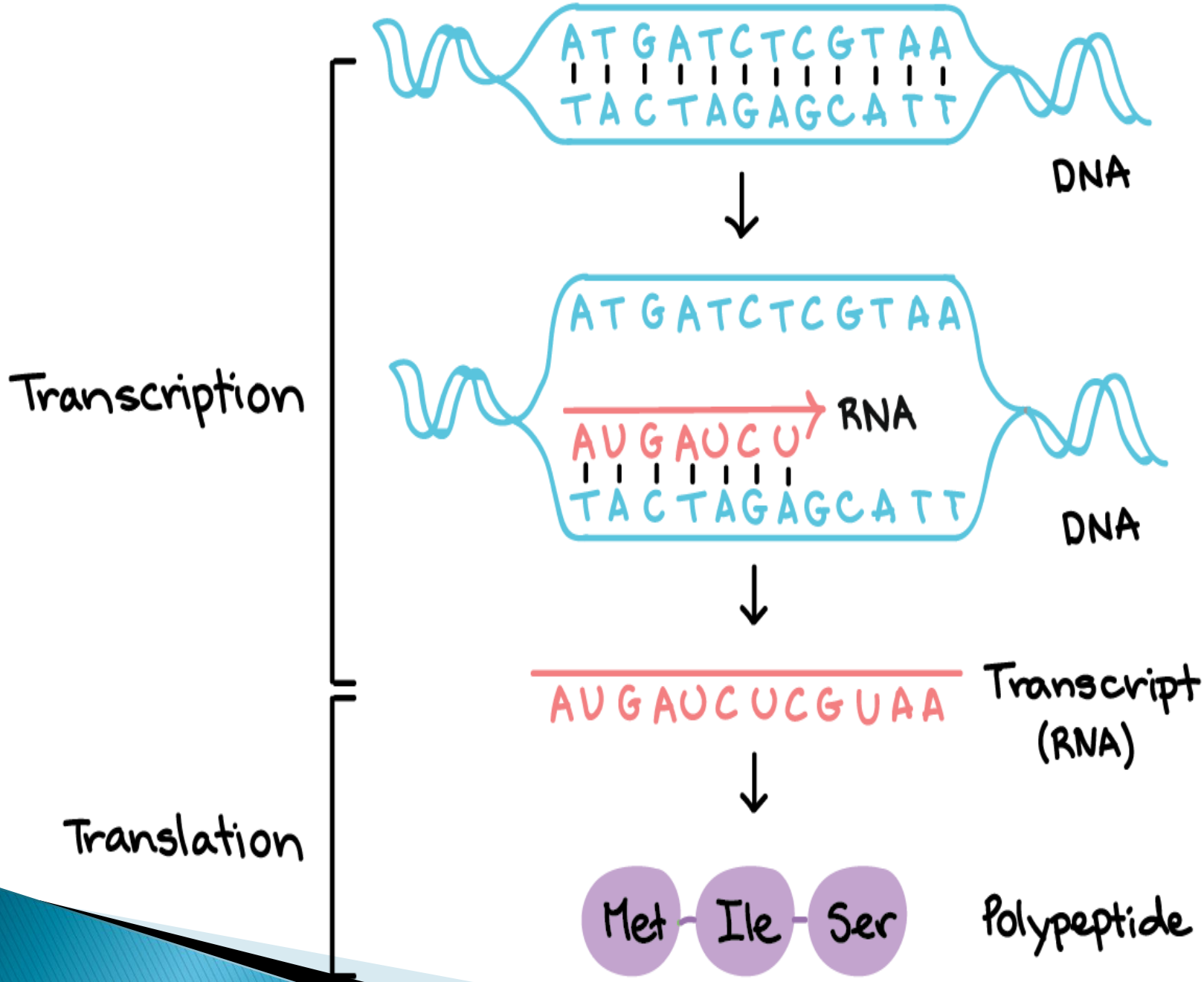
OVERVIEW


Transcription factor
Gene
Promotion region
Coding region
Transcription
5'
Intron
Exon
Exon
Intron
Exon
3'
Untranslated region
Untranslated region
5'
UTR
Exon
Exon
Exon
UTR
3'
Translation
Used to make protein

TRANSCRIPTION

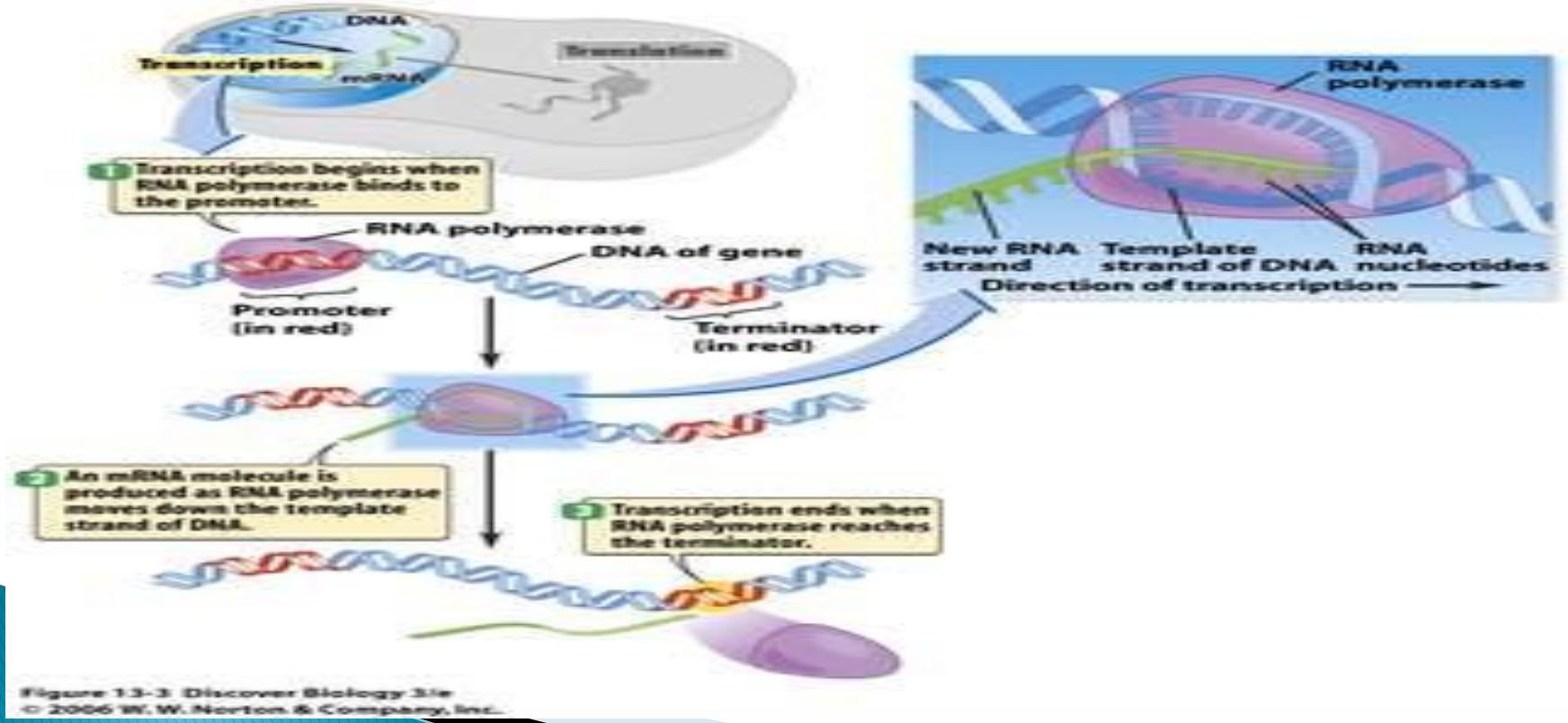
- The transfer of sequence information from a DNA template.




ATGATCTCGTAA
TACTAGAGCATT
DNA
Transcription
ATGATCTCGTAA
AUGAUCU
RNA
TACTAGAGCATT
DNA
AUGAUCUCGUAA
Transcript
(RNA)
Translation
Met
Ile
Ser
Polypeptide

BACKGROUND

- ***ROGER KERNBERG*** got a nobel prize in 2006 for duties on molecular basis of transcription

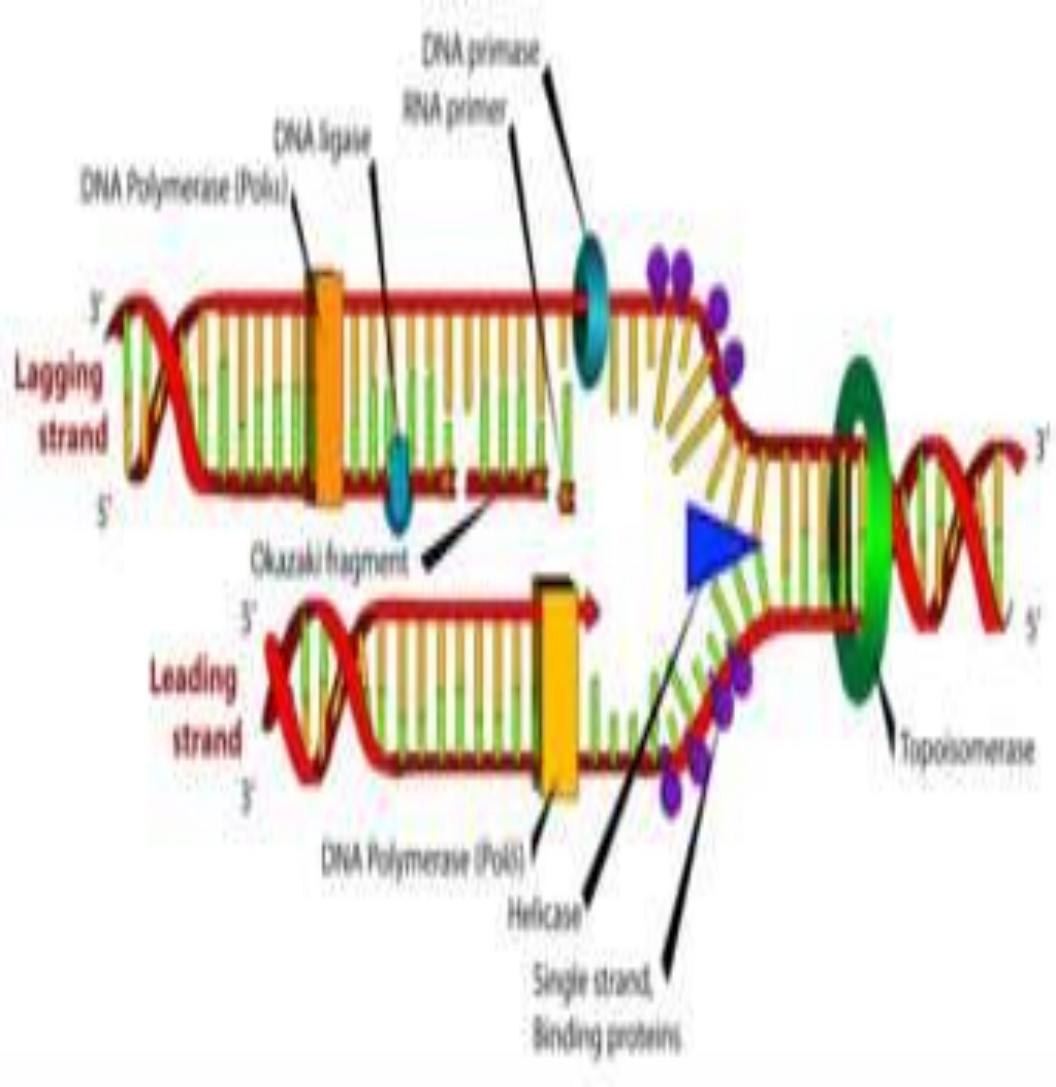


BACKGROUND CONTINUED

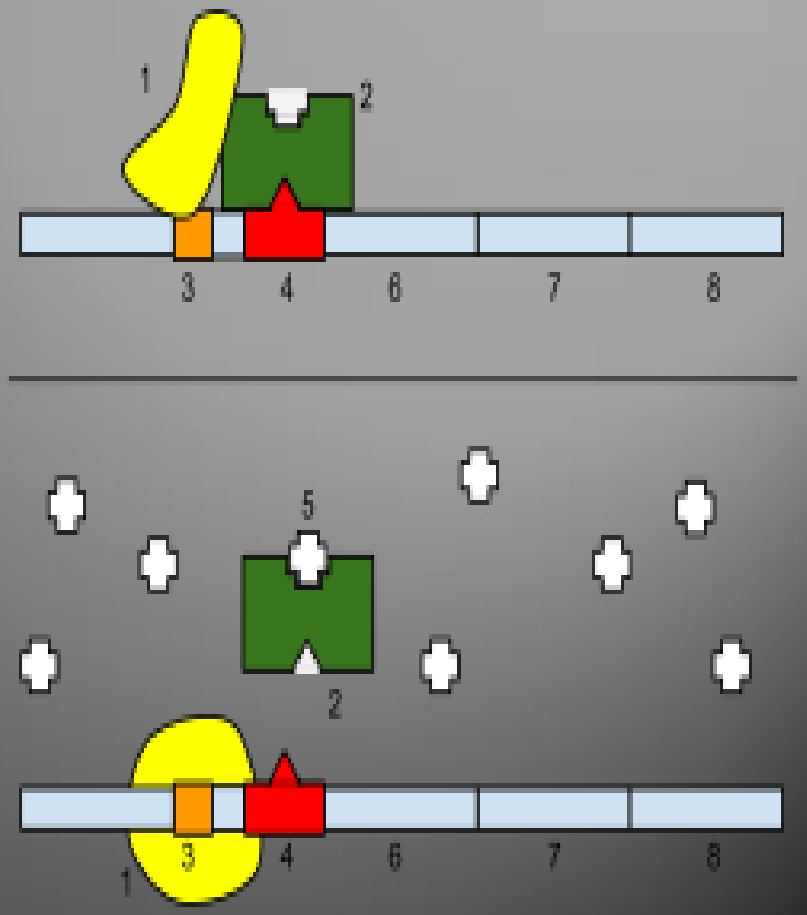
- He is the son of ***ARTHUR KORNBERG***(nobel prize in 1959), and worked on DNA REPLICATION.

ENZYMES INVOLED IN TRANSCRIPTION

- Topoisomerase
- Helicase
- Primase
- Ligase
- Ssbp
- RNA polymerase



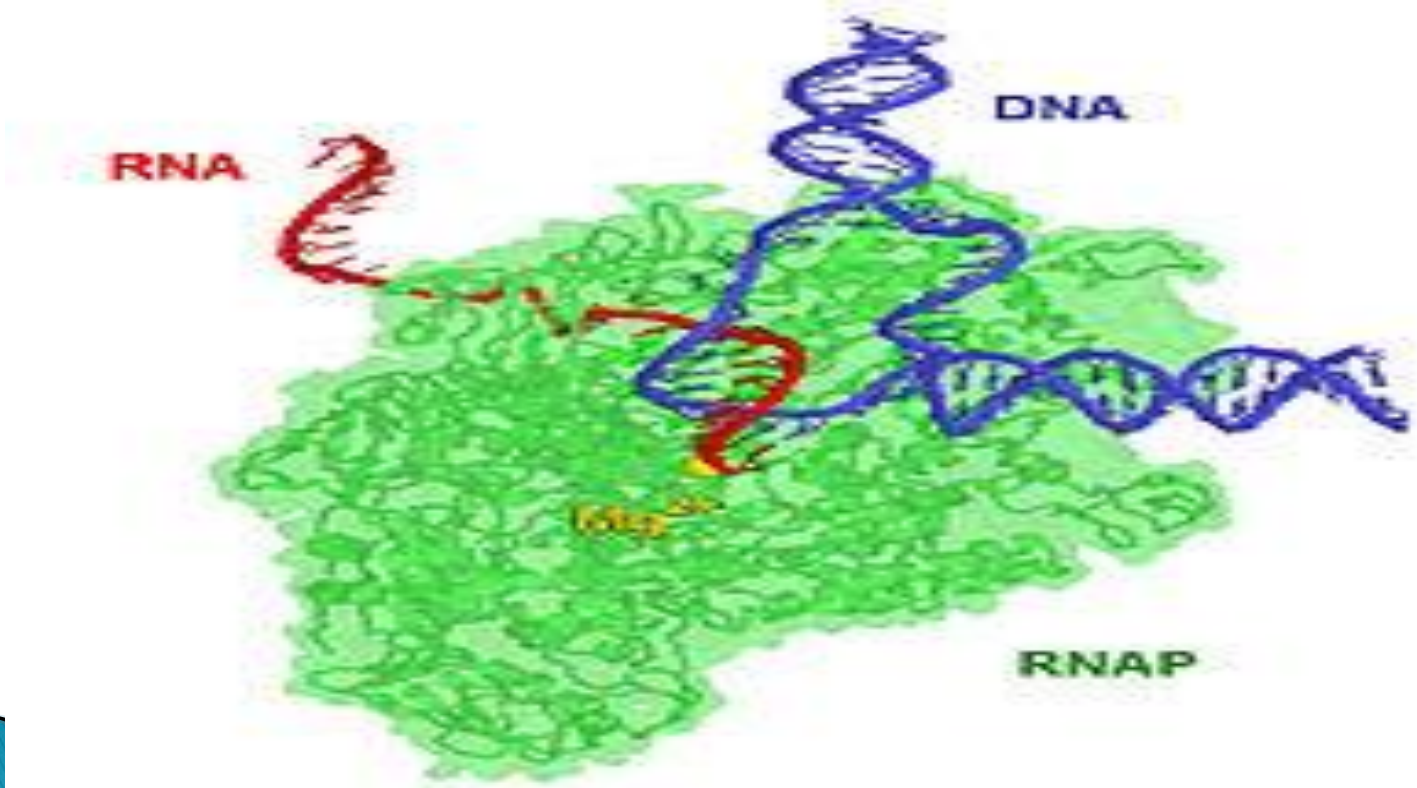
- 1.RNAP
- 2.Repressor
- 3.Promoter
- 4.Operator
- 5.Lactose



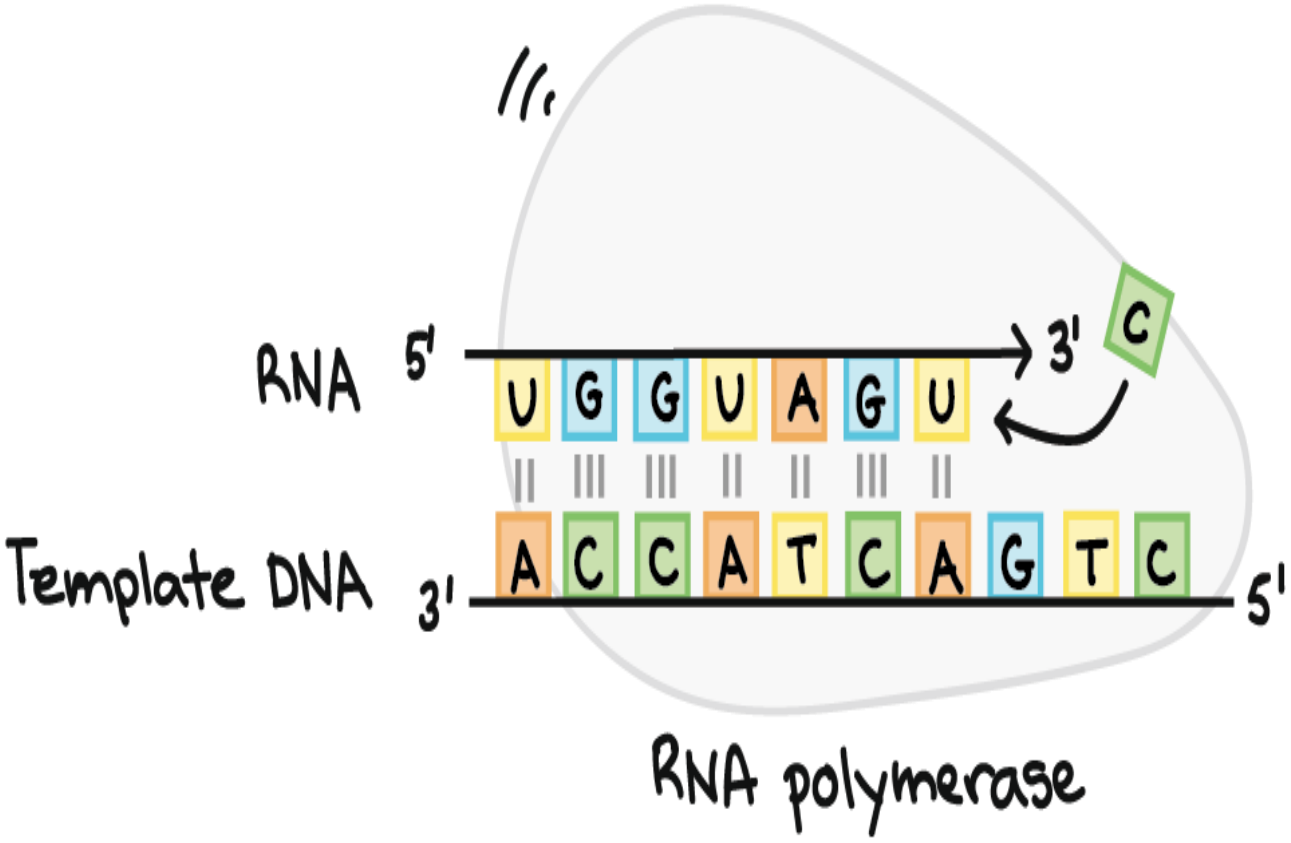
- There is no lactose to inhibit the repressor, so the repressor binds to the operator, which obstructs the RNA polymerase from binding to the promoter .
- Lactose inhibits the repressor, hence RNA polymerase binds with the promoter

STRUCTURE OF RNA POLYMERASE

- Catalytic sites of RNA polymeases include 2 metal ions normally Mg2+.



RNA POLYMERASE



continued

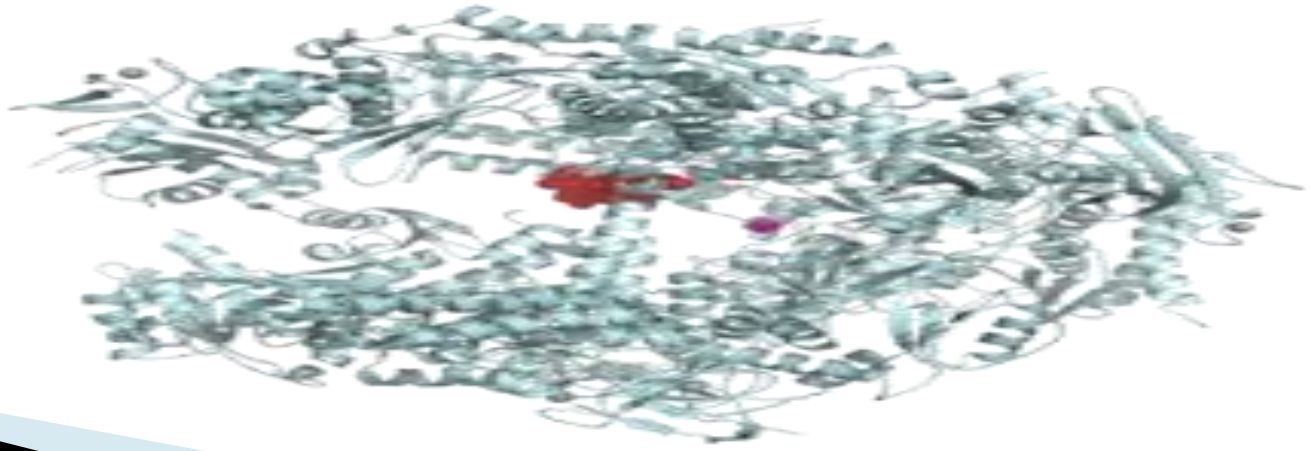
- One ion remains tightly bound to the enzyme while the other ion comes in with the nucleoside triphosphate and leaves with the pyrophosphate.

FUNCTIONS OF RNA POLYMERASES

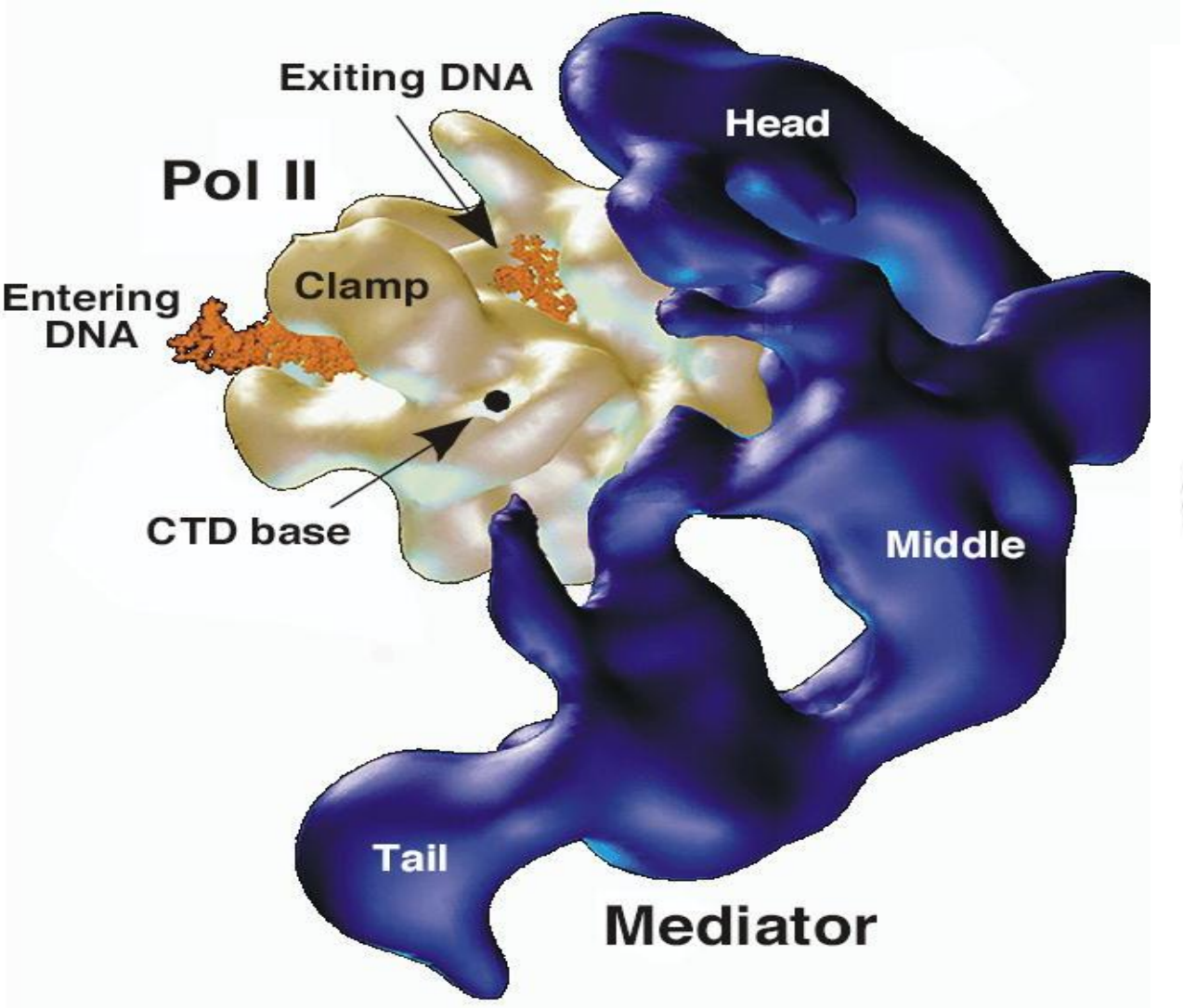
- Search DNA for promoter sites.
- Unwind a short stretch of double helix
- Select the correct ribonucleotide triphosphate
- Catalyze formation of phosphodiester bonds
- Detect termination signals
- Interact with activator and repressor proteins

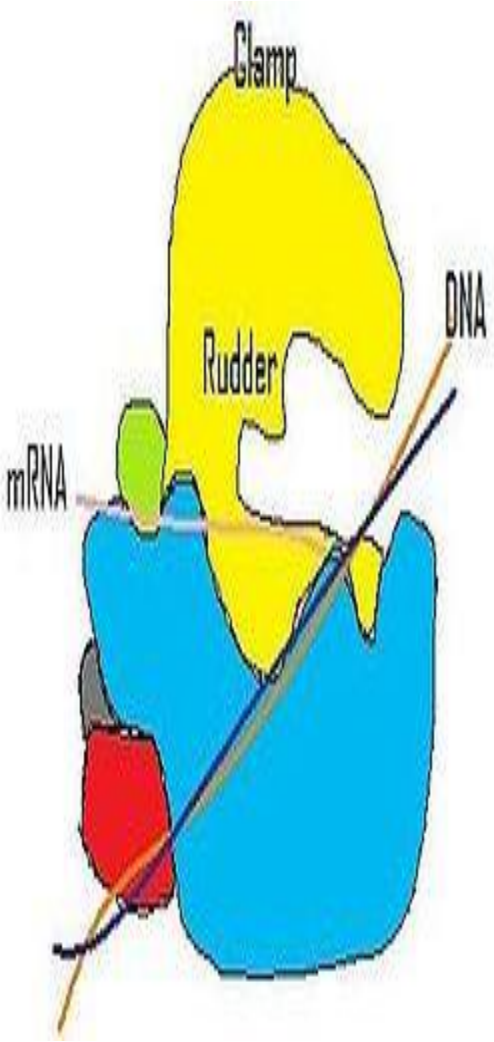
THE 3 DIFFERENT DNA DEPENEDENT RNAP

- RNAP type II or B:Main enzyme for the synthesis of mRNAs.
- Its inhibited by alpha amanitin(a toxin from mushroom amanita phalloides),these block the translocation of RNAP during mRNA synthesis.



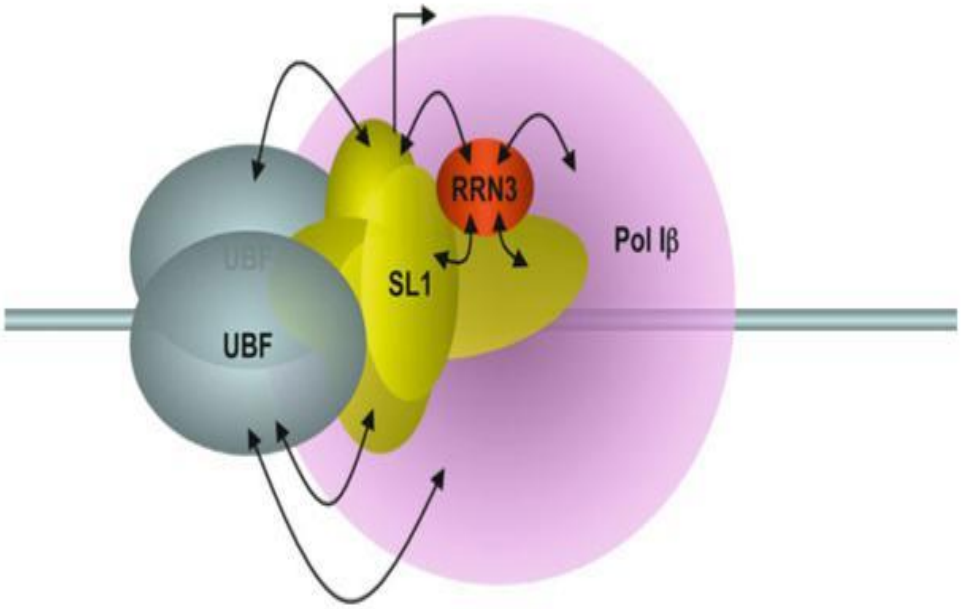
POLYMERASE II STRUCTURE

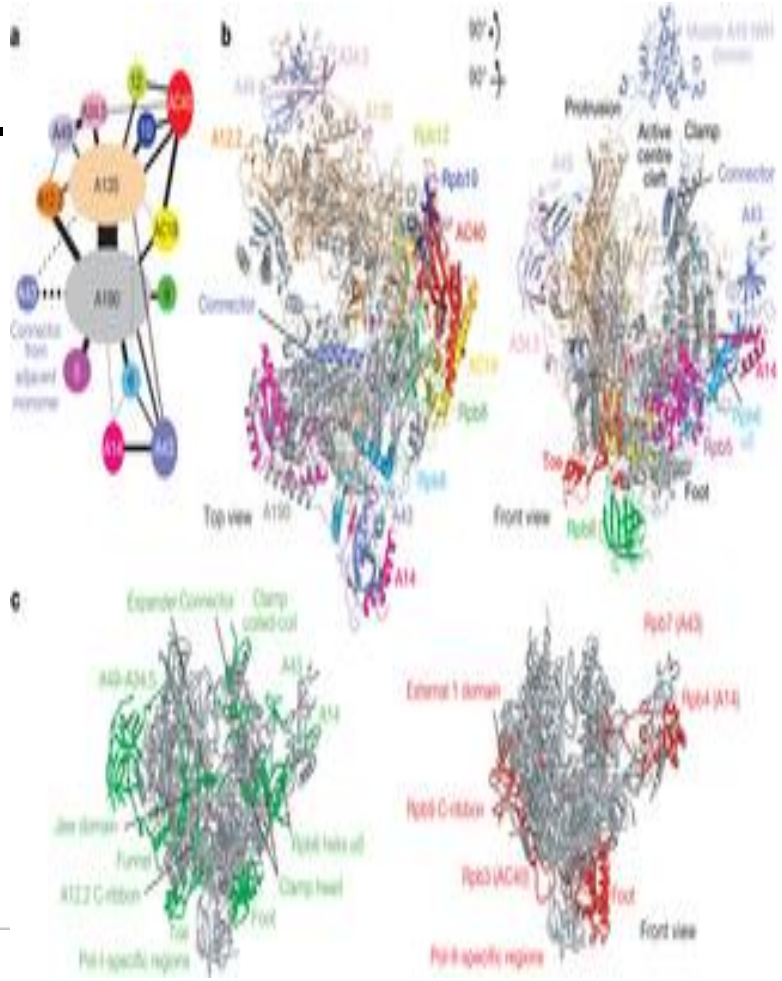




2.RNAP type I or A

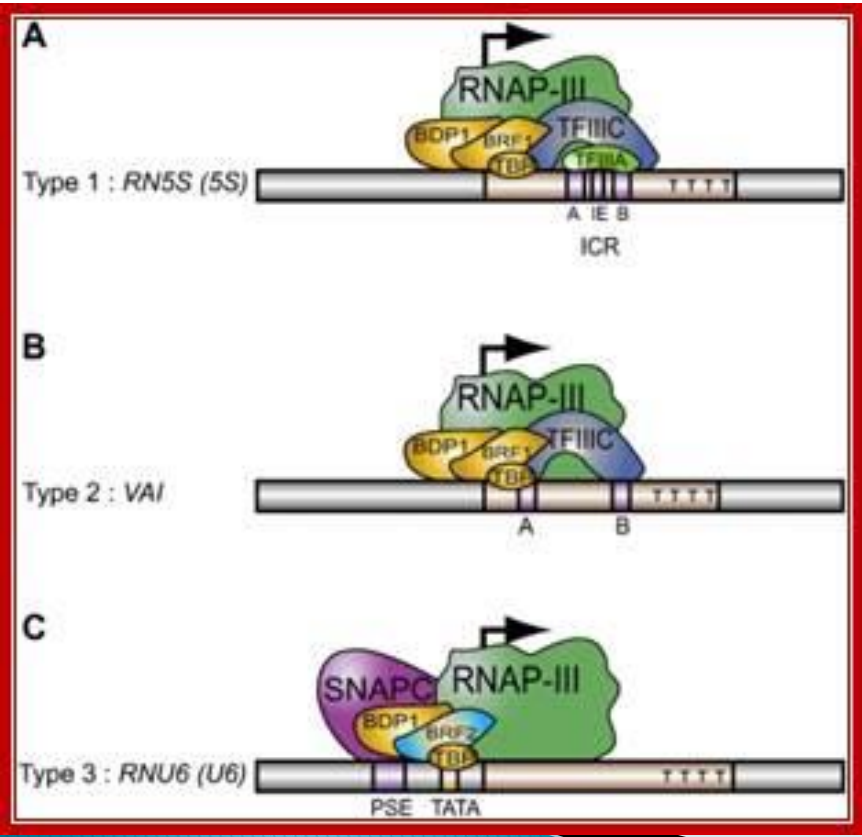
- Responsible for the synthesis of ribosomal (rRNA).
- Not inhibited by amanitin.

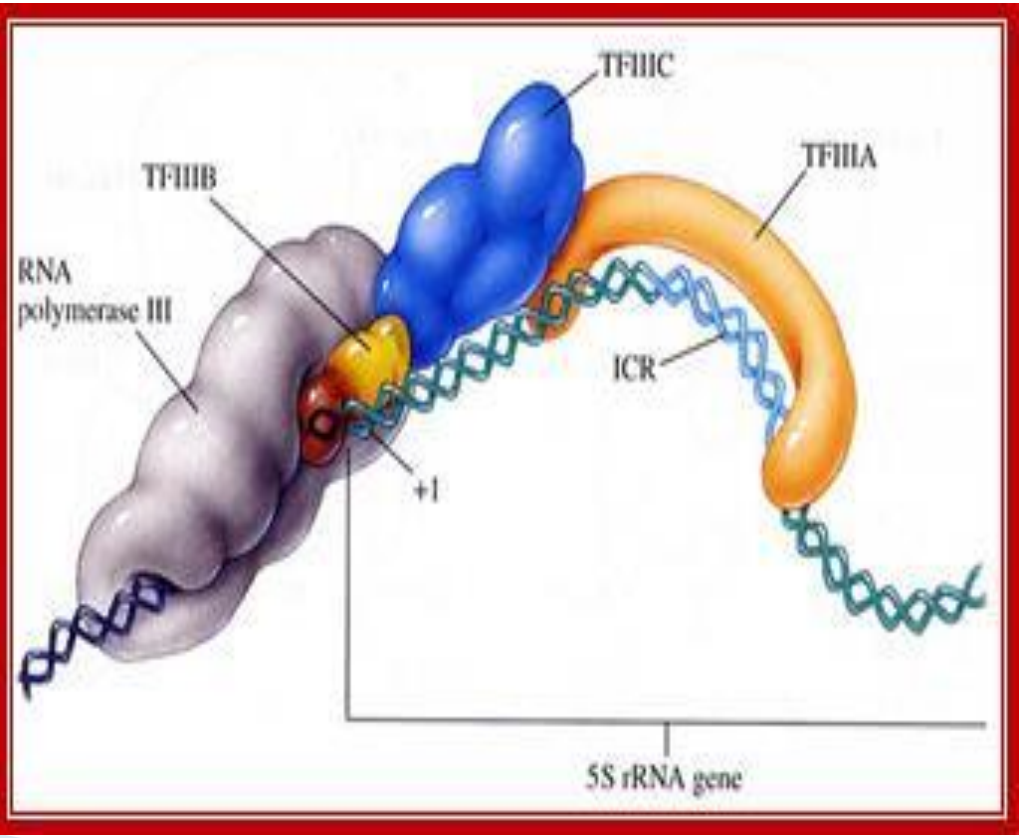




3.RNAP III or C

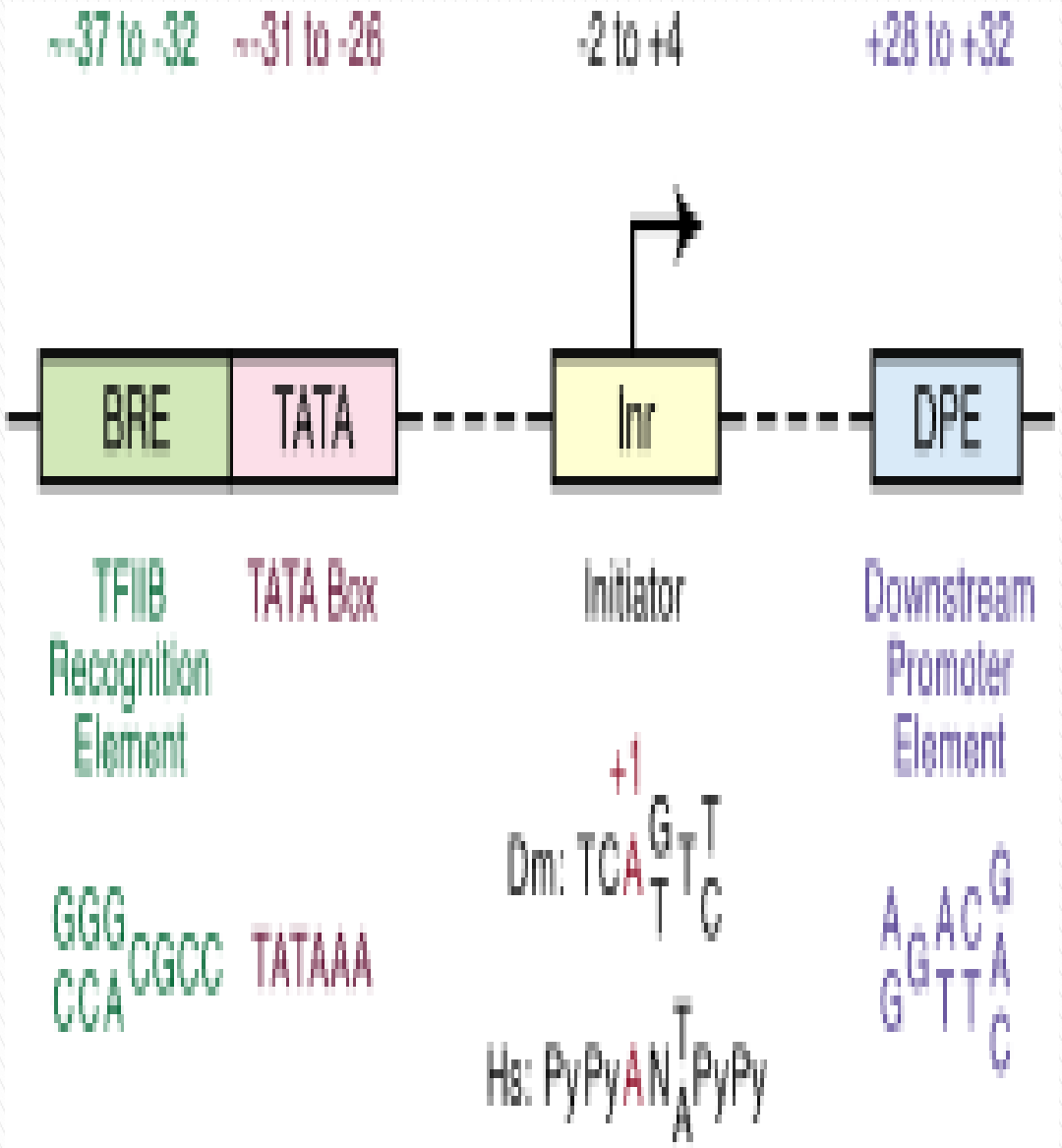
- Responsible for synthesis of tRNA.
- Moderately sensitive to amanitin.





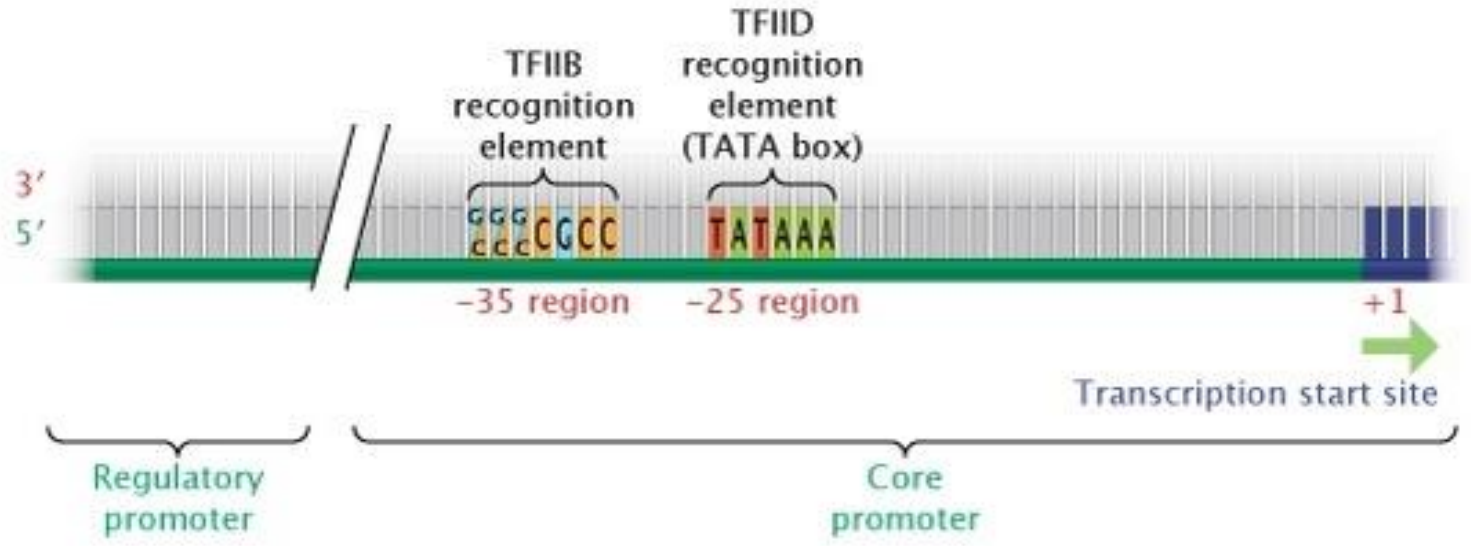
TATA BOX,

The ***TATA box*** is grouped with the TFIIB and the transcription initiator site and the downstream promoter element are located several base pairs away



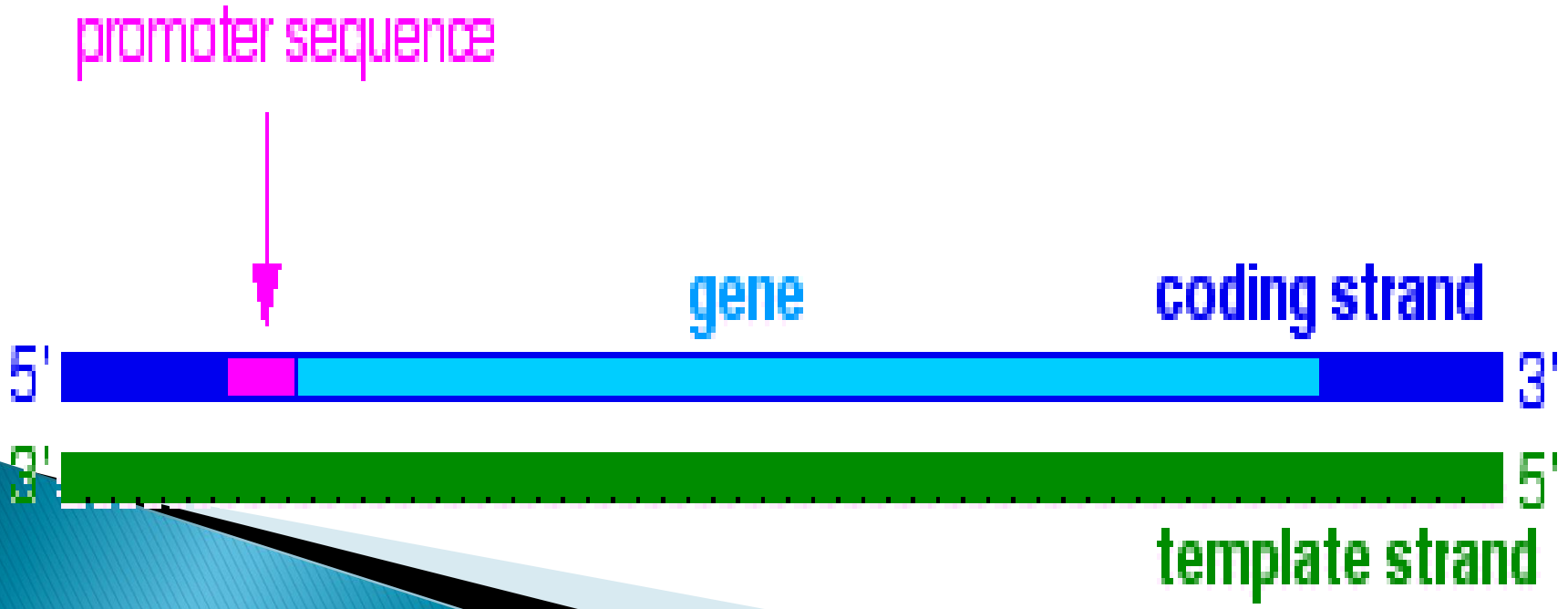
INITIATION SIGNALS

- 1.PROMOTERS:Specific areas on DNA act as starting signals for initiation.
- RNAP attaches at these sites on the DNA template.



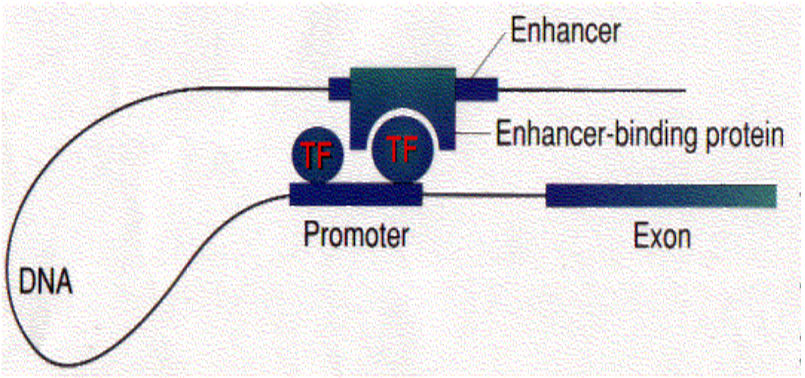
2.GOLBERGHOGNESS BOX

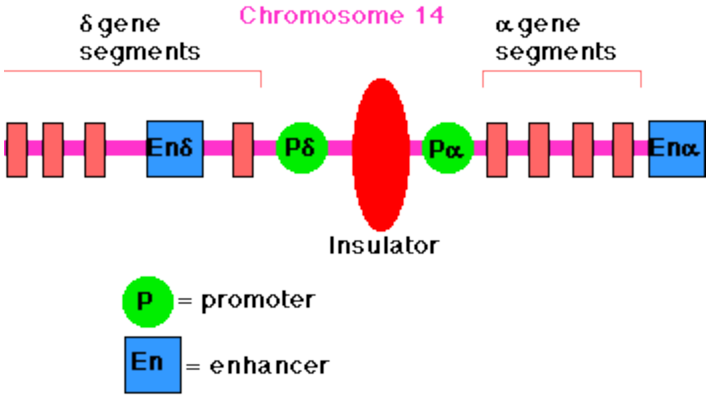
- In humans,the exact sequence in the TATA box is slightly different(TATAA).
- This acts as a region for the start region



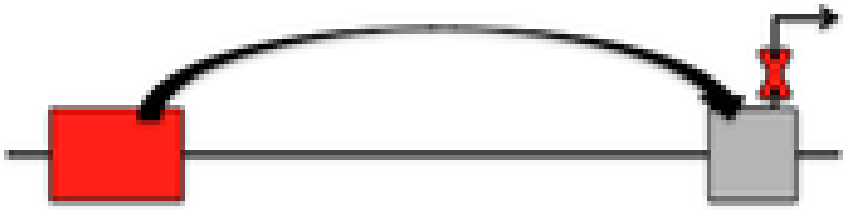
ENHANCERS AND SILENCERS

- Enhancers increase the rate of transcription and the silencers decrease the fate on transcription.



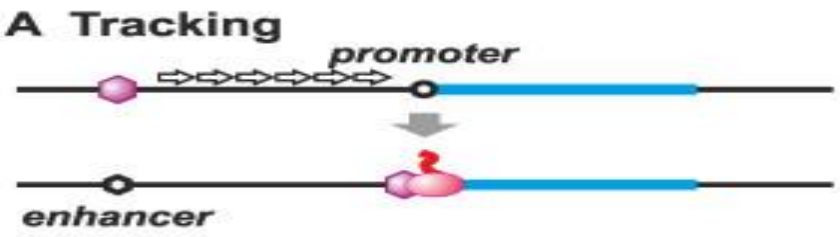


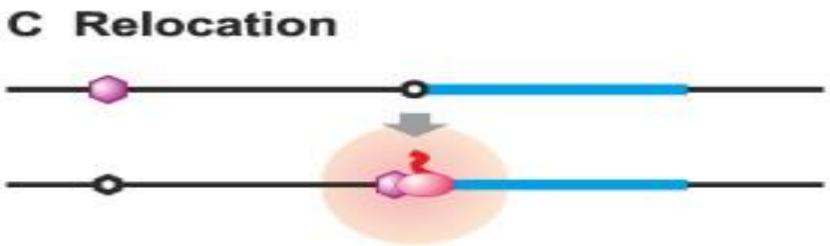
(A)

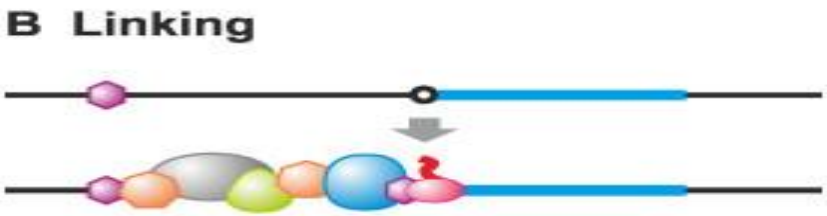


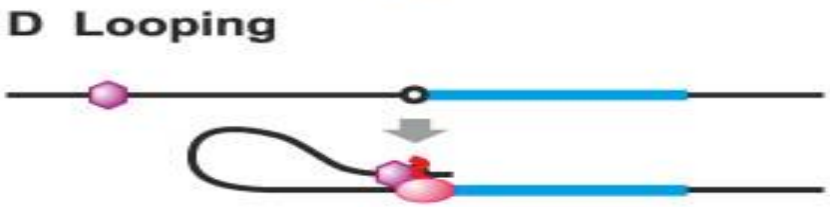
(B)

Models for enhancer function



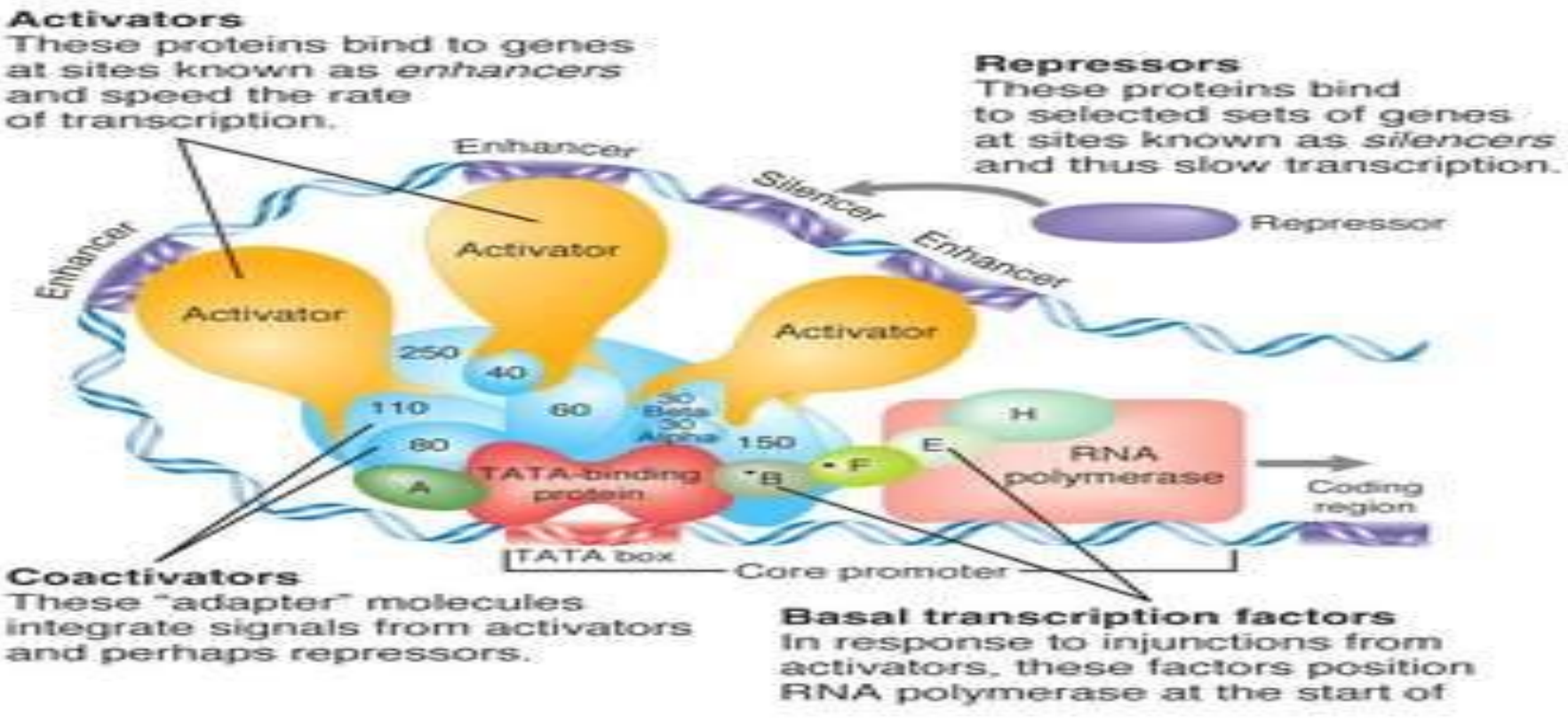


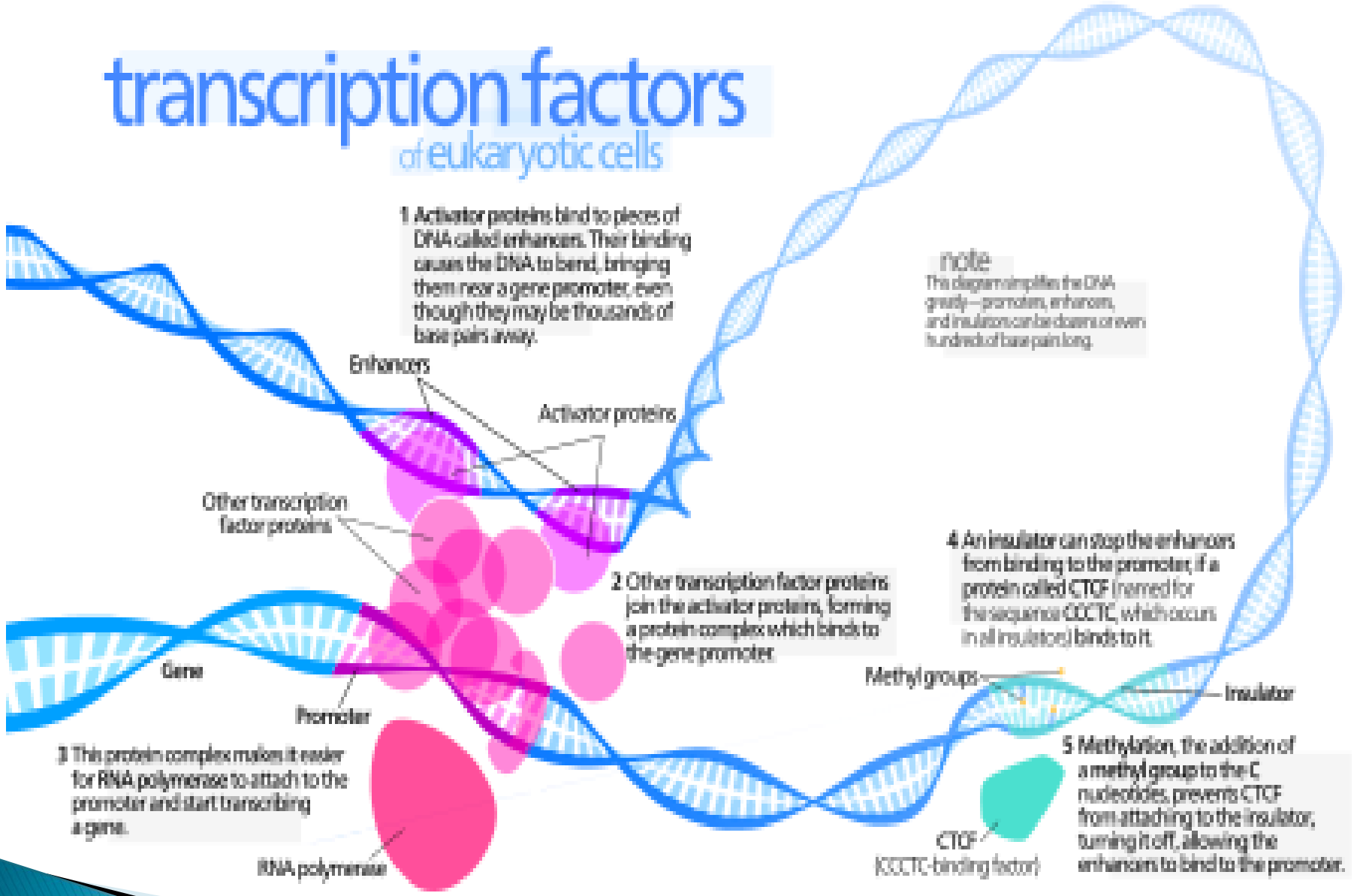




OTHER REGULATORY HORMONES INCLUDE

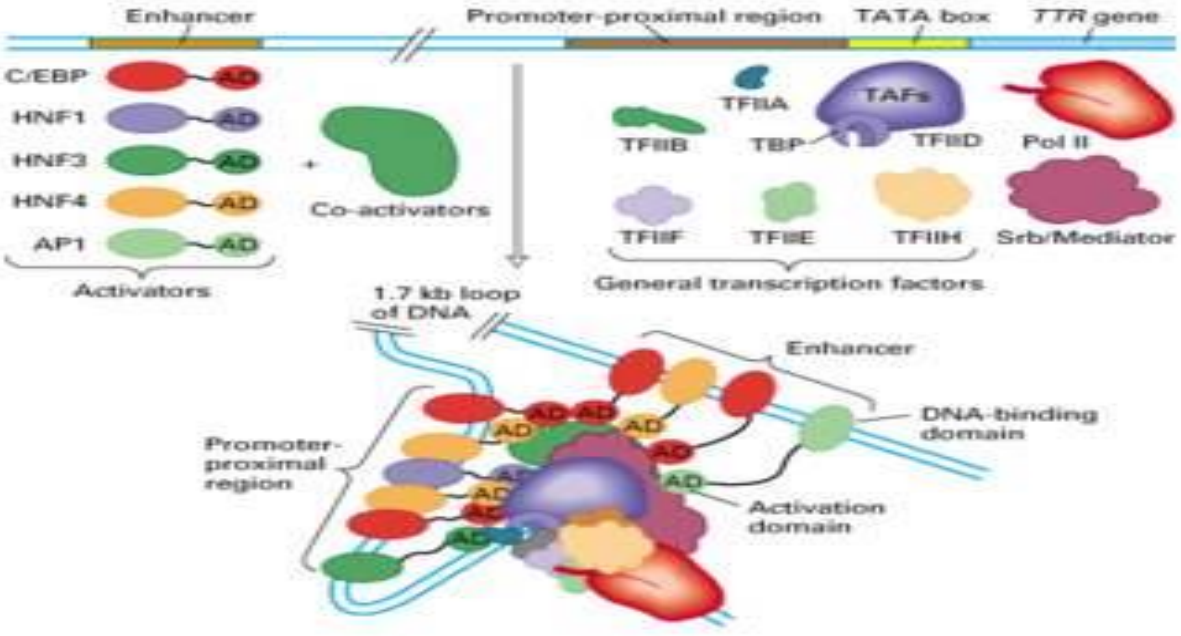
- Hormone Response Elements(HRE).
- Repressors
- Inducers and depressors.

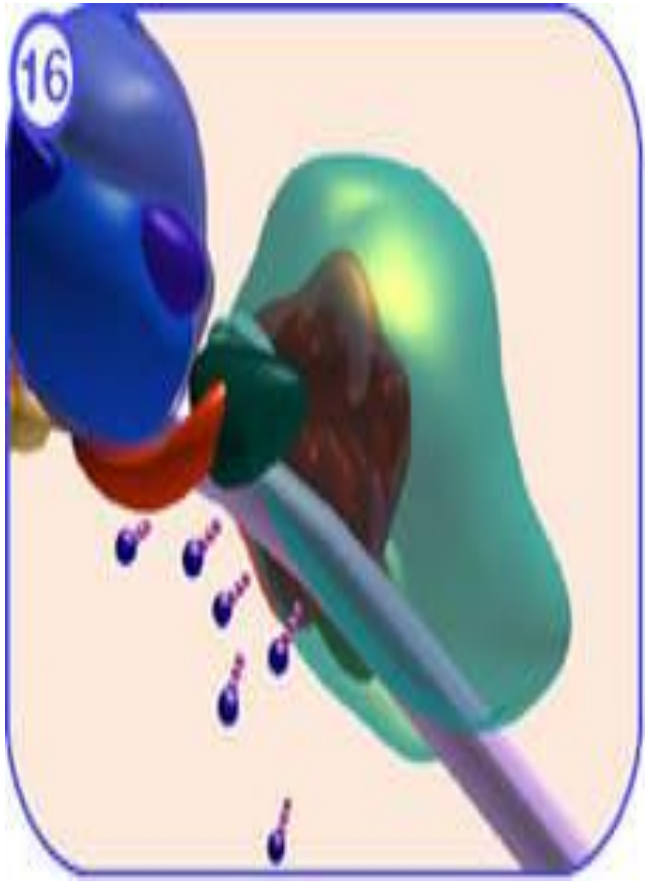



transcription factors
of eukaryotic cells
1 Activator proteins bind to pieces of DNA called enhancers. Their binding causes the DNA to bend, bringing them near a gene promoter, even though they may be thousands of base pairs away.
Enhancers
Activator proteins
Other transcription factor proteins
2 Other transcription factor proteins join the activator proteins, forming a protein complex which binds to the gene promoter.
Gene
Promoter
3 This protein complex makes it easier for RNA polymerase to attach to the promoter and start transcribing a gene.
RNA polymerase
note
This diagram simplifies the DNA greatly — promoters, enhancers, and insulators can be dozens or even hundreds of base pairs long.
4 An insulator can stop the enhancers from binding to the promoter, if a protein called CTCF (named for the sequence CCCTC, which occurs in all insulators) binds to it.
Methyl groups
Insulator
5 Methylation, the addition of a methyl group to the C nucleotides, prevents CTCF from attaching to the insulator, turning it off, allowing the enhancers to bind to the promoter.
CTCF
(CCCTC-binding factor)

THE 3 CLASSES OF TRANSCRIPTION FACTORS

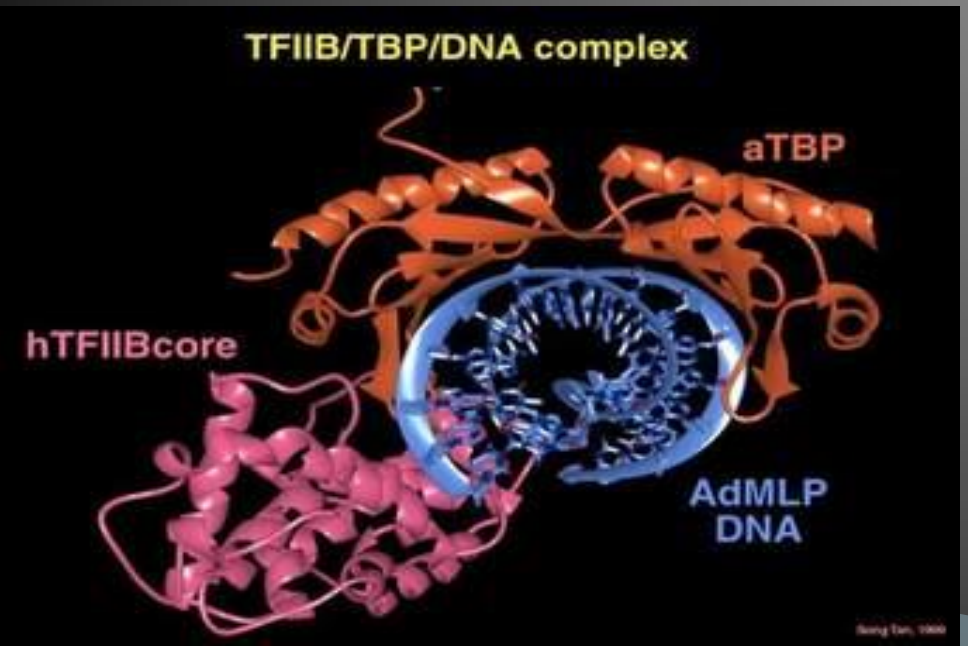
- Basal components e.g. TBP,TFIIA,B,EF and H
- Co-regulators e.g. TAFs, TFIID, chromatin modifiers.
- Activation such as;SP1,ATF,API

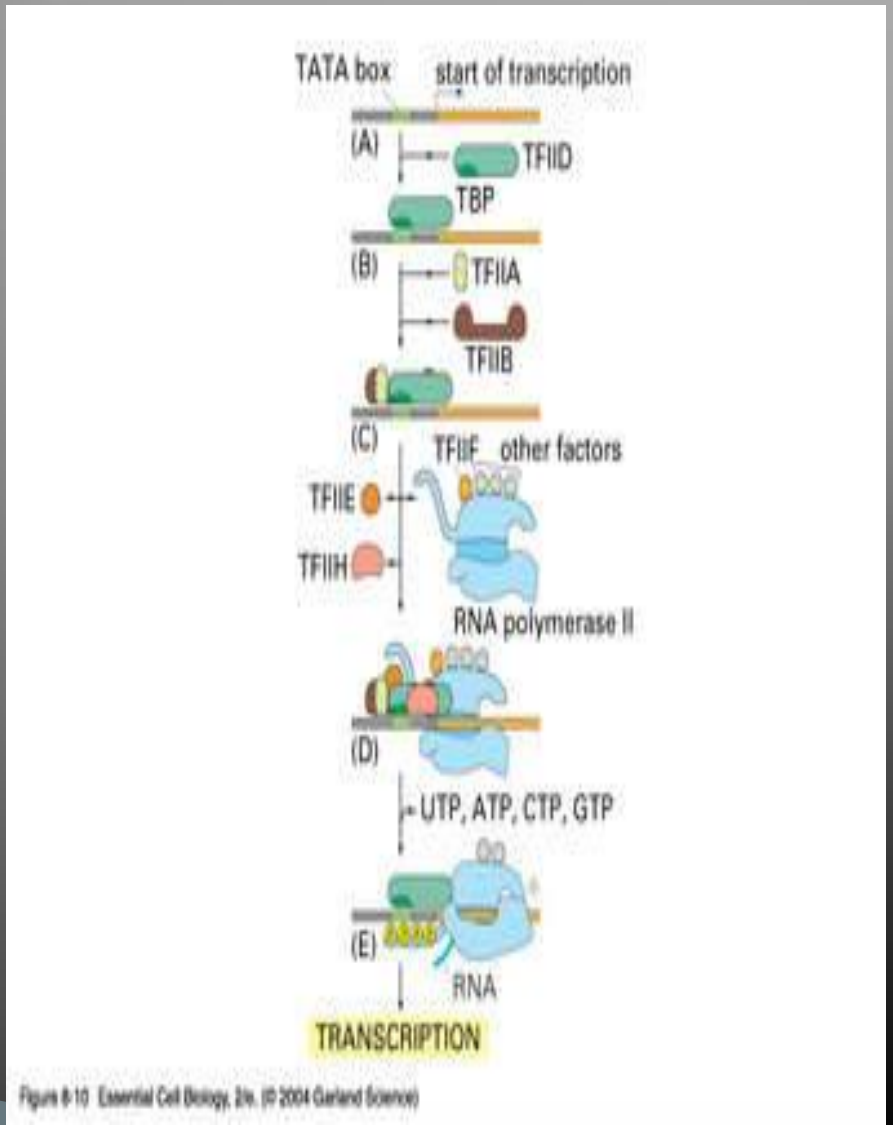




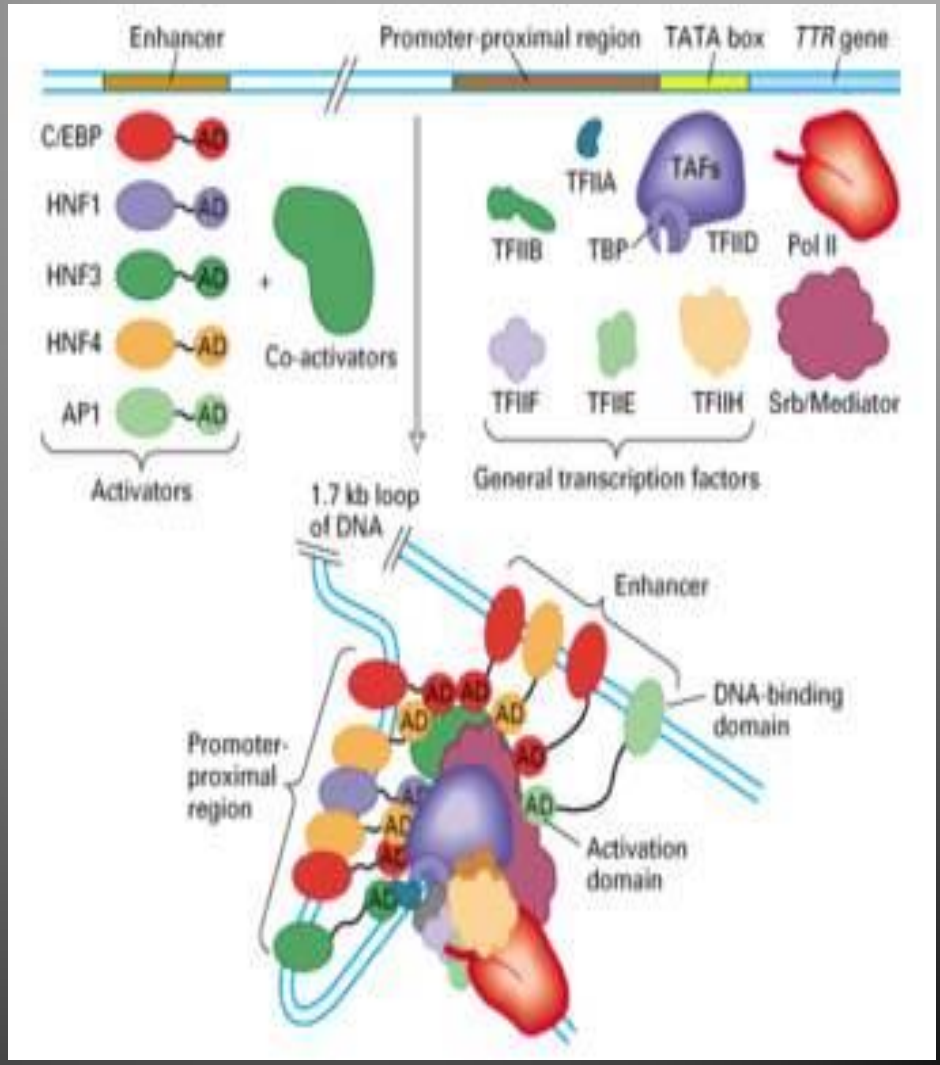
THE 7 TRANSCRIPTION FACTORS COLLECTIVELY CALLED TF-II

- 1st,the TATA box is recognized by TBP(TATA Binding Protein).
- Instead of the sigma factor, SLL factor ensures that RNAP could locate the start point.

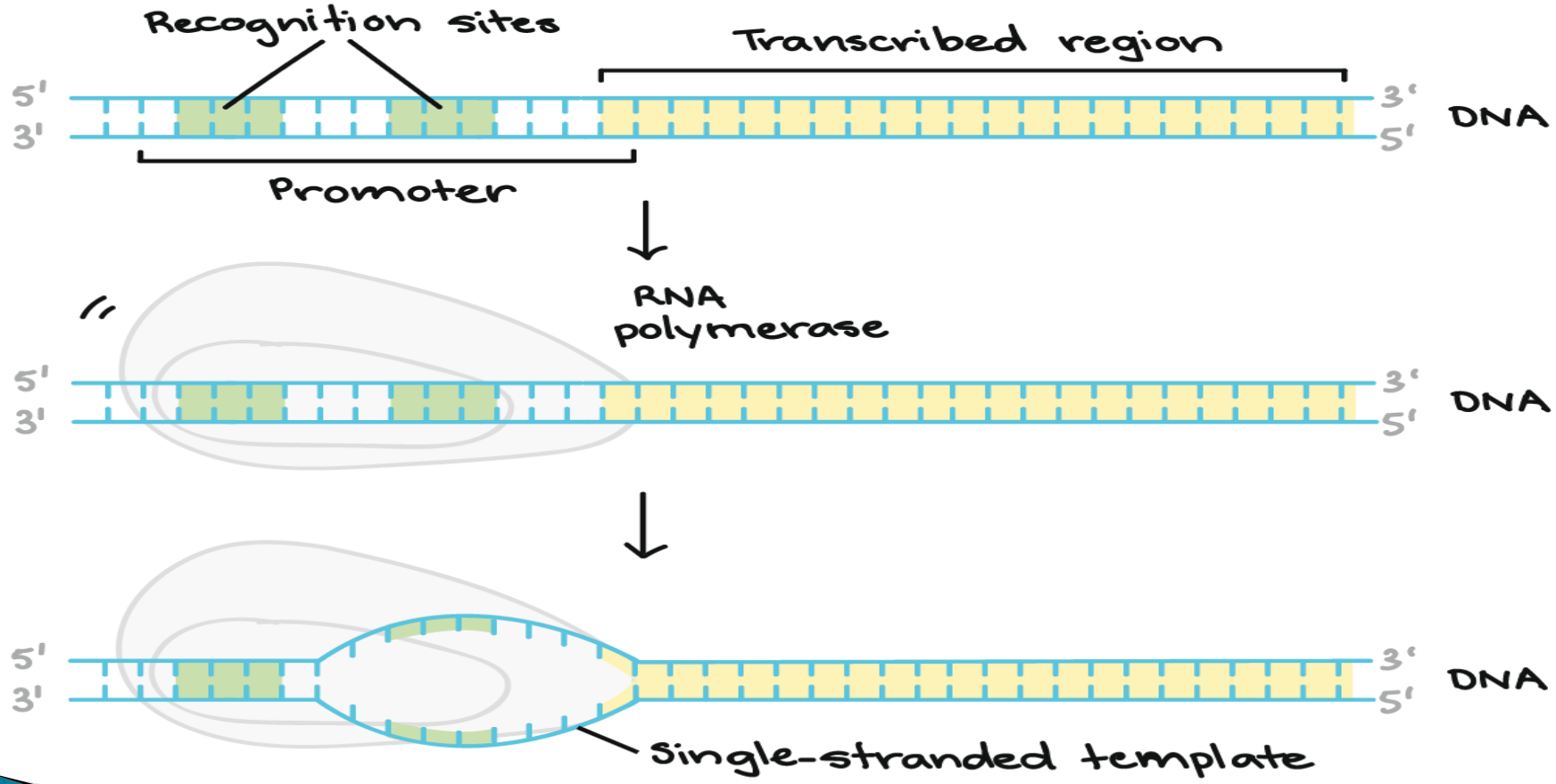




- TBP Associated Factors(TAF) bind with TBP to form TFIID.
- Binding of TFIID complex to the TATA box is the first step of transcription process.

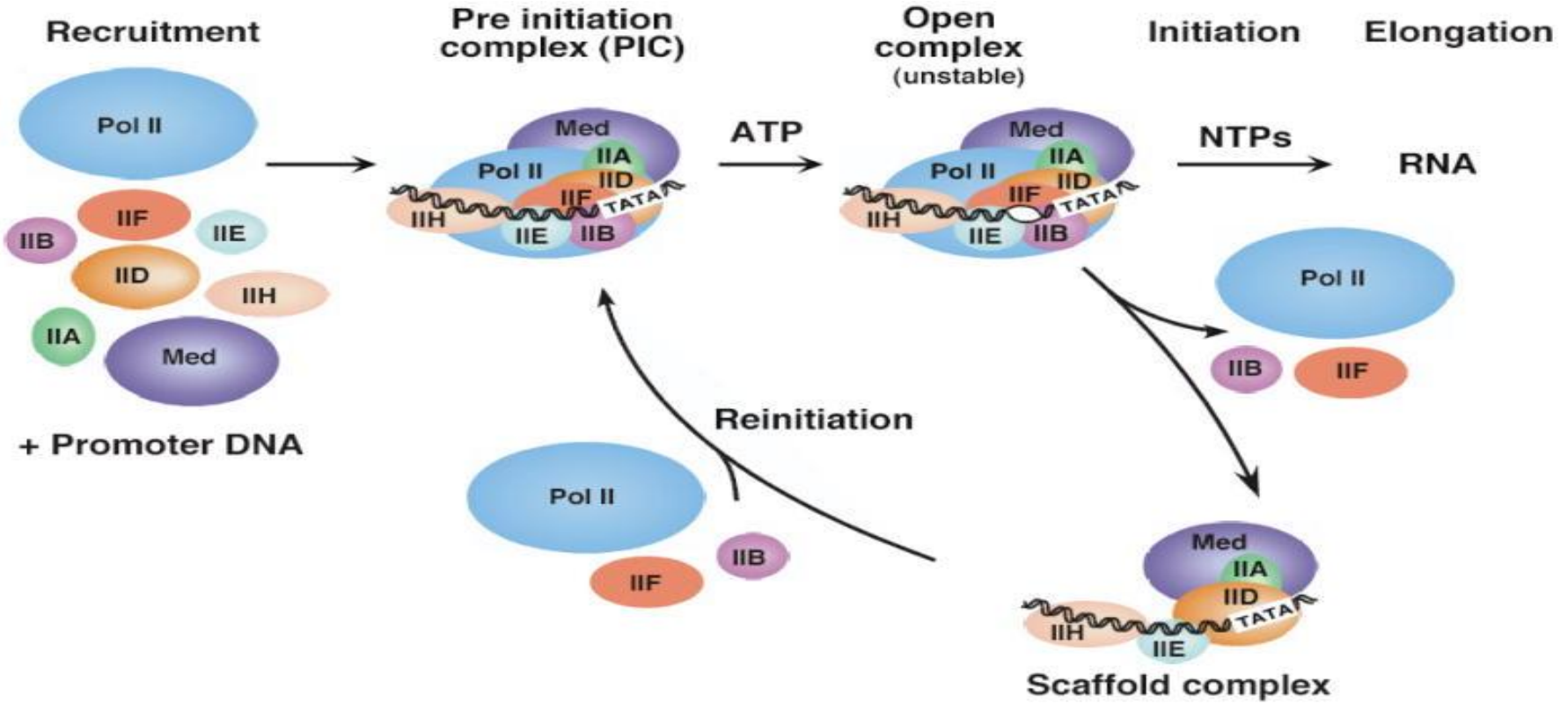


INITIATION


Recognition sites
Transcribed region
5'
3'
3'
5'
DNA
Promoter
RNA polymerase
5'
3'
3'
5'
DNA
5'
3'
3'
5'
DNA
Single-stranded template

RNA POLYMERASES ACTIONS

- The fundamental reason of RNA synthesis is formation of the phosphodiester bonds.

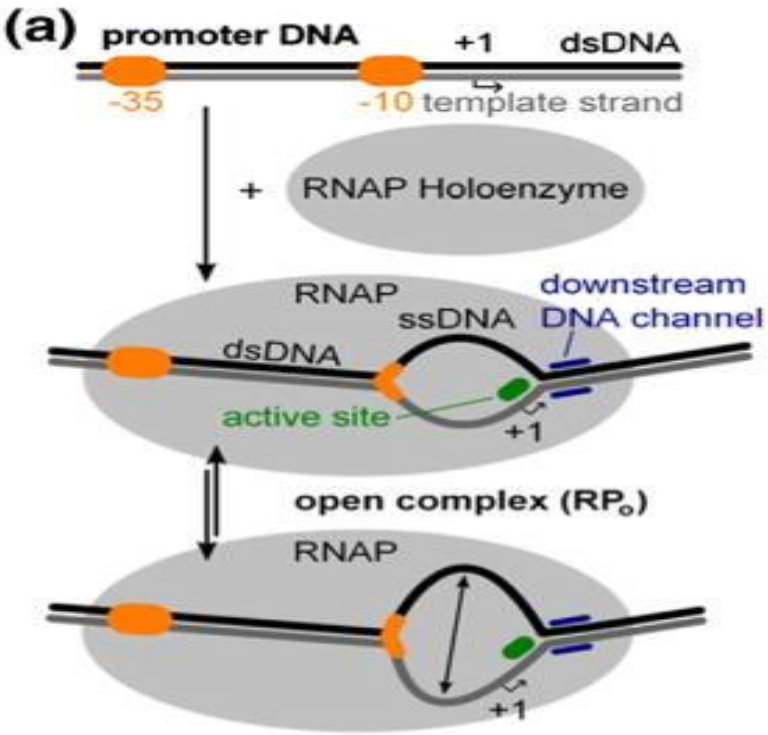


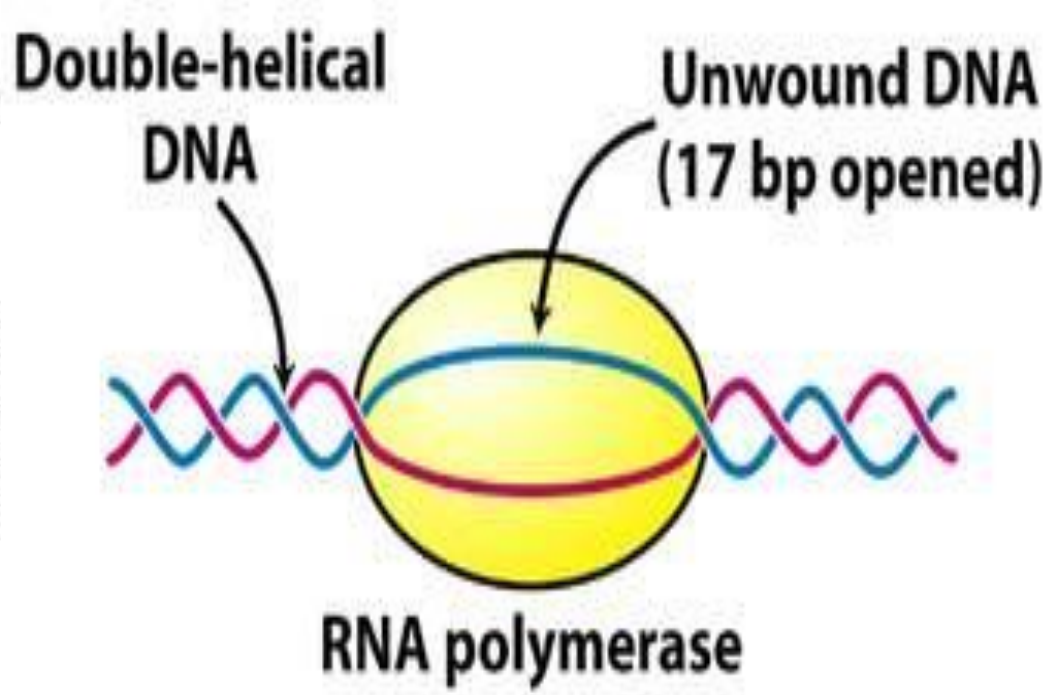
continued

- The 3”-hydroxyl group of the last nucleotide in the chain nucleophillically attacks the alpha phosphoryl group of the incoming nucleoside triphosphate with release of a pyrophosphate.

TRANSCRIPTION BUBBLE

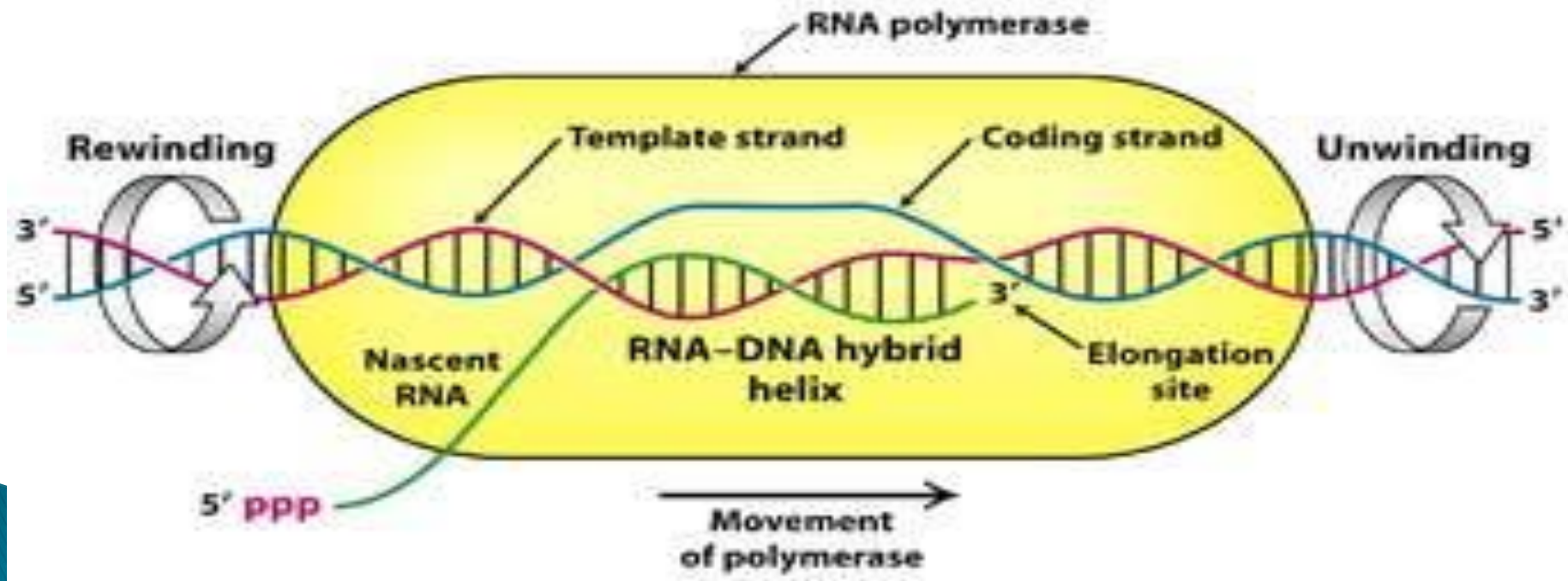
- RNA separates a region of the double helix forming a structure called a transcription bubble.





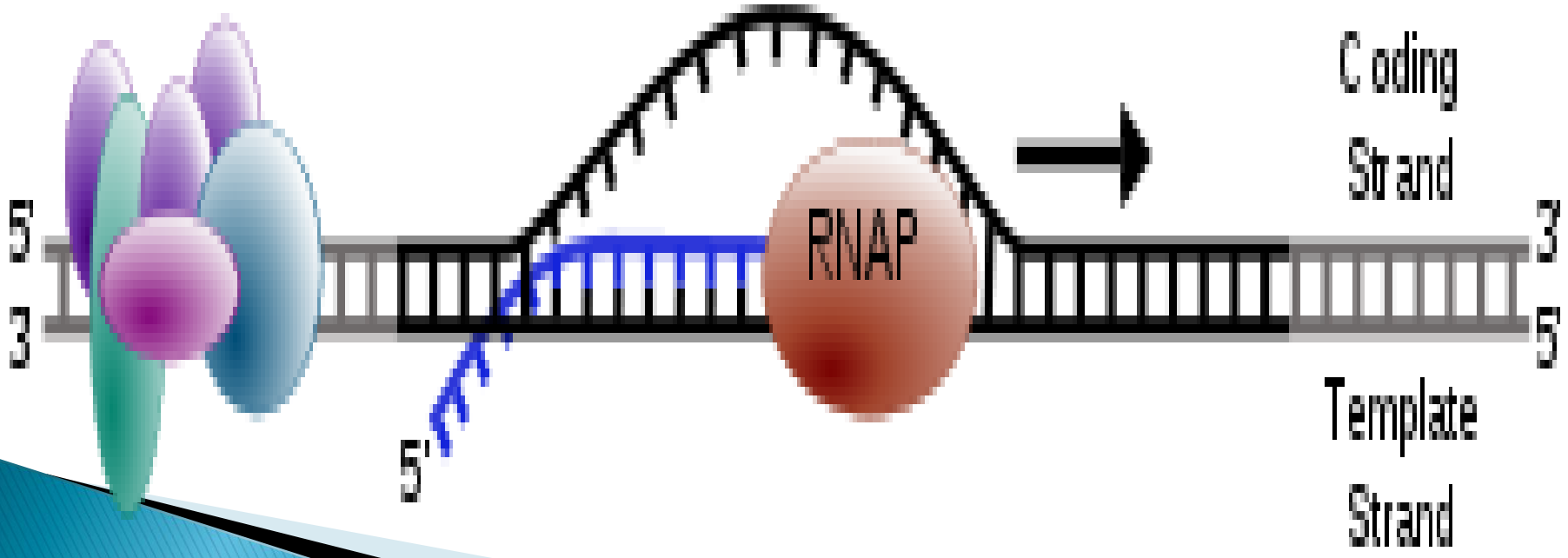
DIRECTION OF GROWTH

- RNAs grow in the 5 to 3 direction
- And the template is read in the 3 to 5 direction.



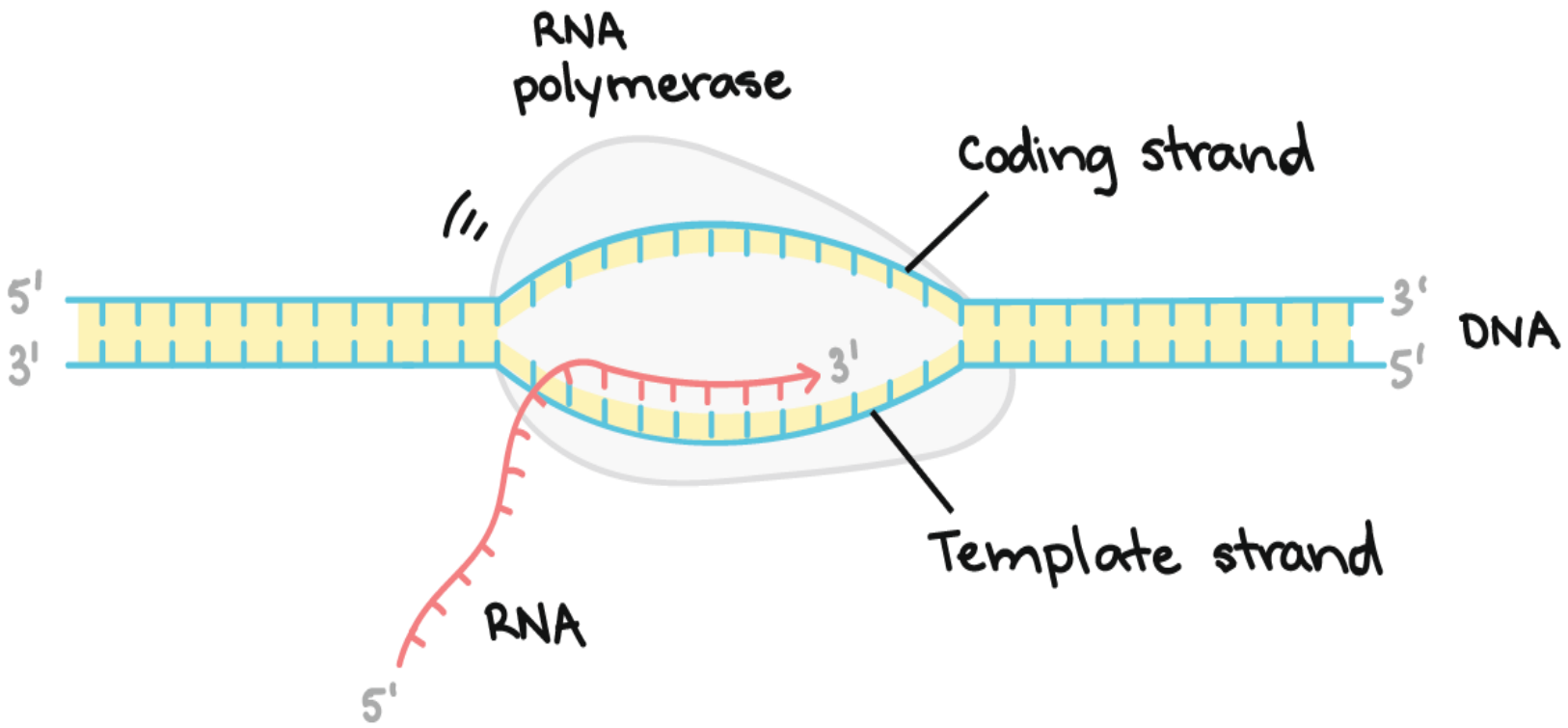
ELONGATION

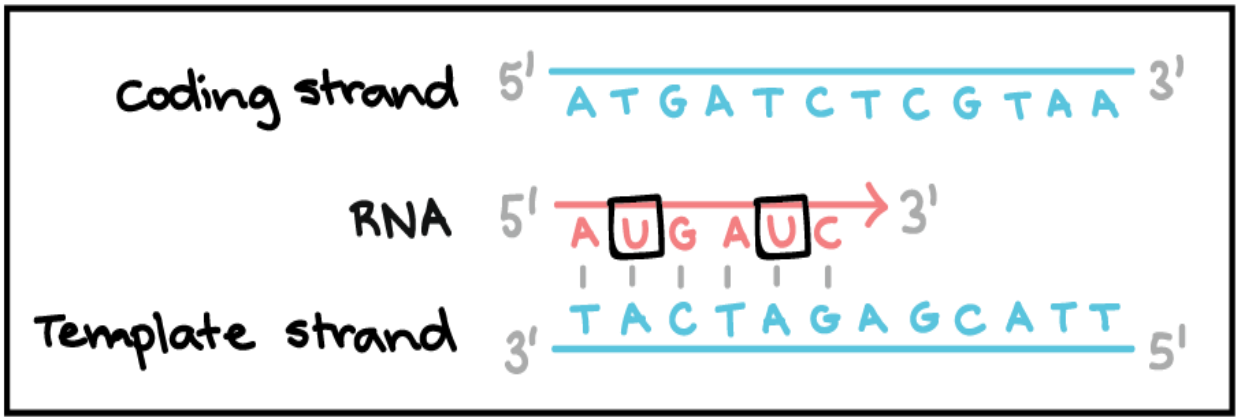
- A ribonucleoside triphosphate binds in the active site of the RNA polymerase directly adjacent the growing RNA chain.



- Incoming nucleoside triphosphate forms a base pair with the template strand.

- The 3 hydroxyl group of the growing RNA chain, oriented and activated by the tightly bound metal attacks the alpha phosphoryl group to form a new phosphodiester bond, displacing pyrophosphate.


RNA polymerase
Coding strand
5'
3'
3'
5'
DNA
3'
Template strand
RNA
5'


Coding strand
5'
ATGATCTCGTAA
3'
RNA
5'
AUGAUC
3'
Template strand
3'
TACTAGAGCATT
5'

continued

- The 3 hydroxyl group at the end of the RNA chain attacks the newly bound nucleotide and forms a new phosphodiester bond releasing pyrophosphate.

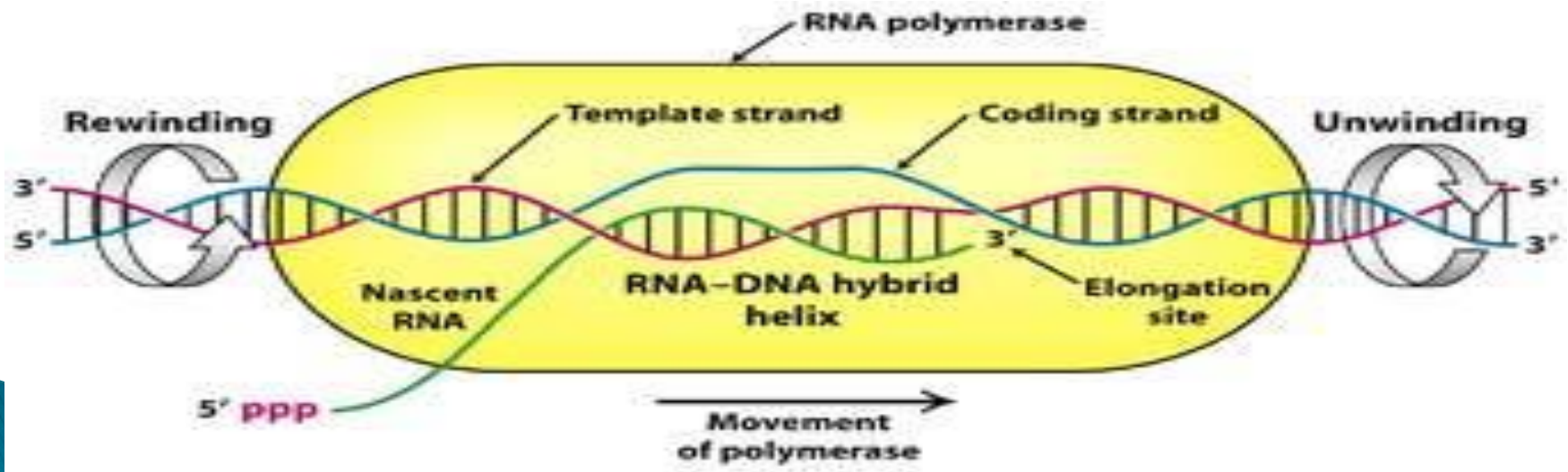
- The RNA-DNA hybrid must move relative to the polymerase to bring the 3 end of the newly added nucleotide into proper position 4 the next nucleotide to be released.

- This translocation step doesn’t need to break any bonds between base pairs and is reversible but, once it has occurred,

- The addition of the next nucleotide favored by triphosphate cleavage and pyrophosphate release and cleavage drives the polymerization reaction forward.

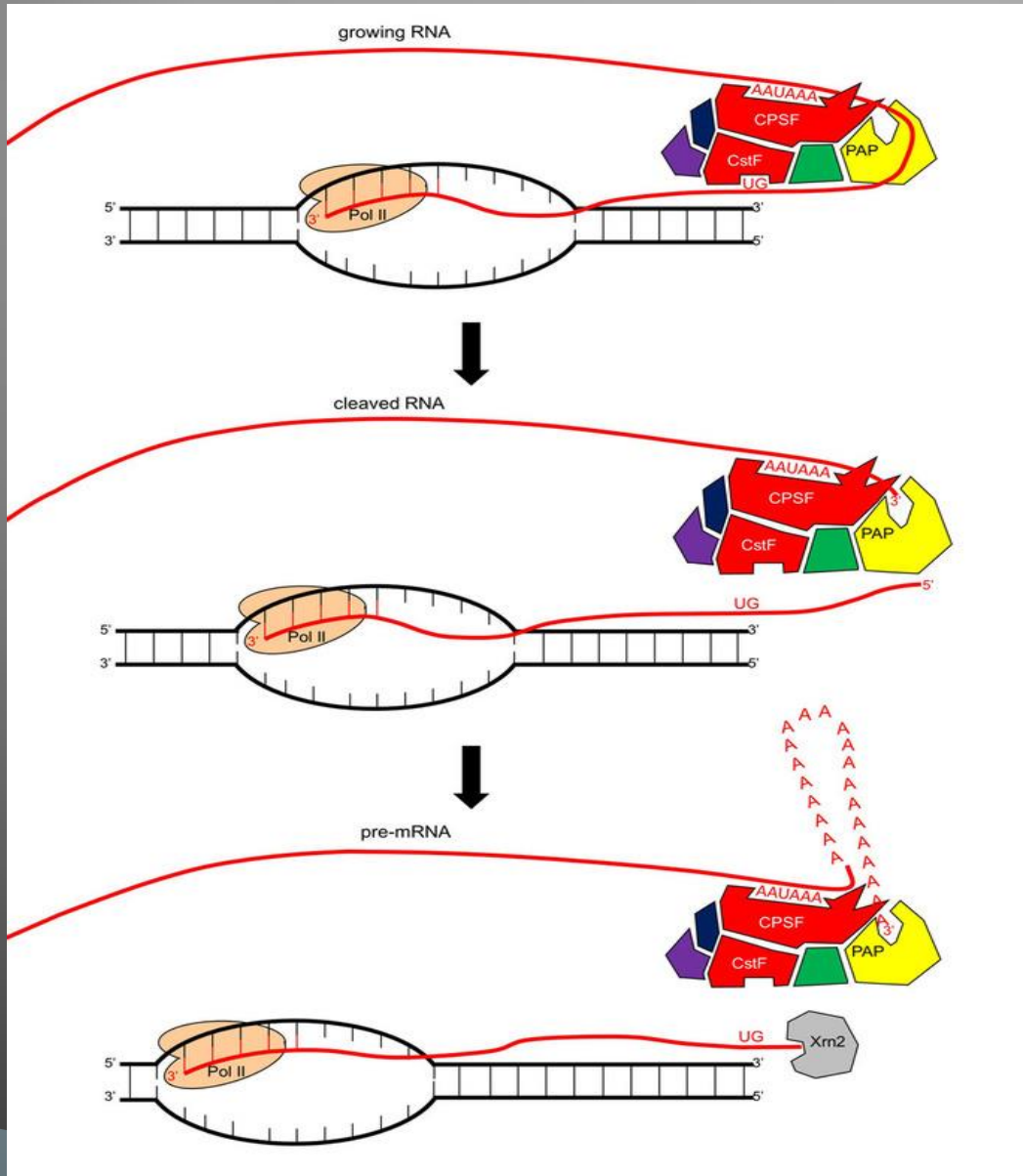
TRANSLOCATION

- After nucleotide addition, the RNA-DNA hybrid can translocate thru the RNA pol brings a new DNA bases into position to base pair with an incoming nucleoside triphosphate.



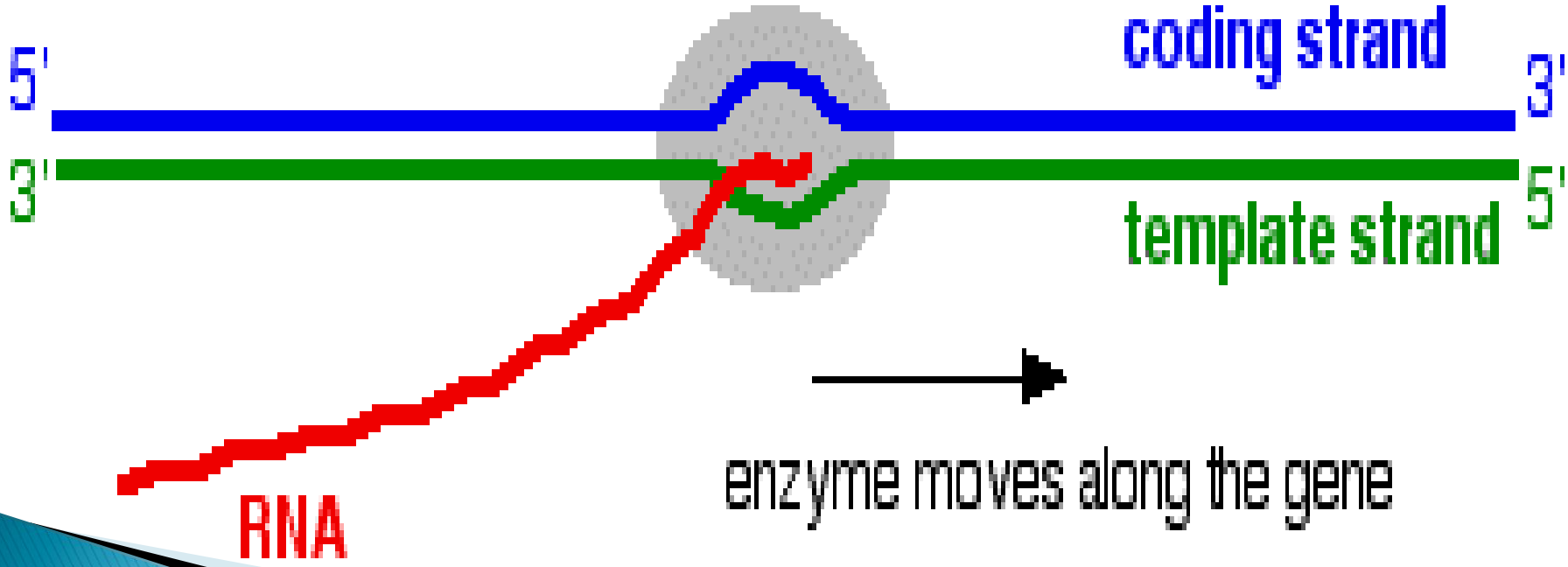
ELONGATION

- RNAP moves along the template
- New nucleotides are added in the nascent RNA according to base pairing.



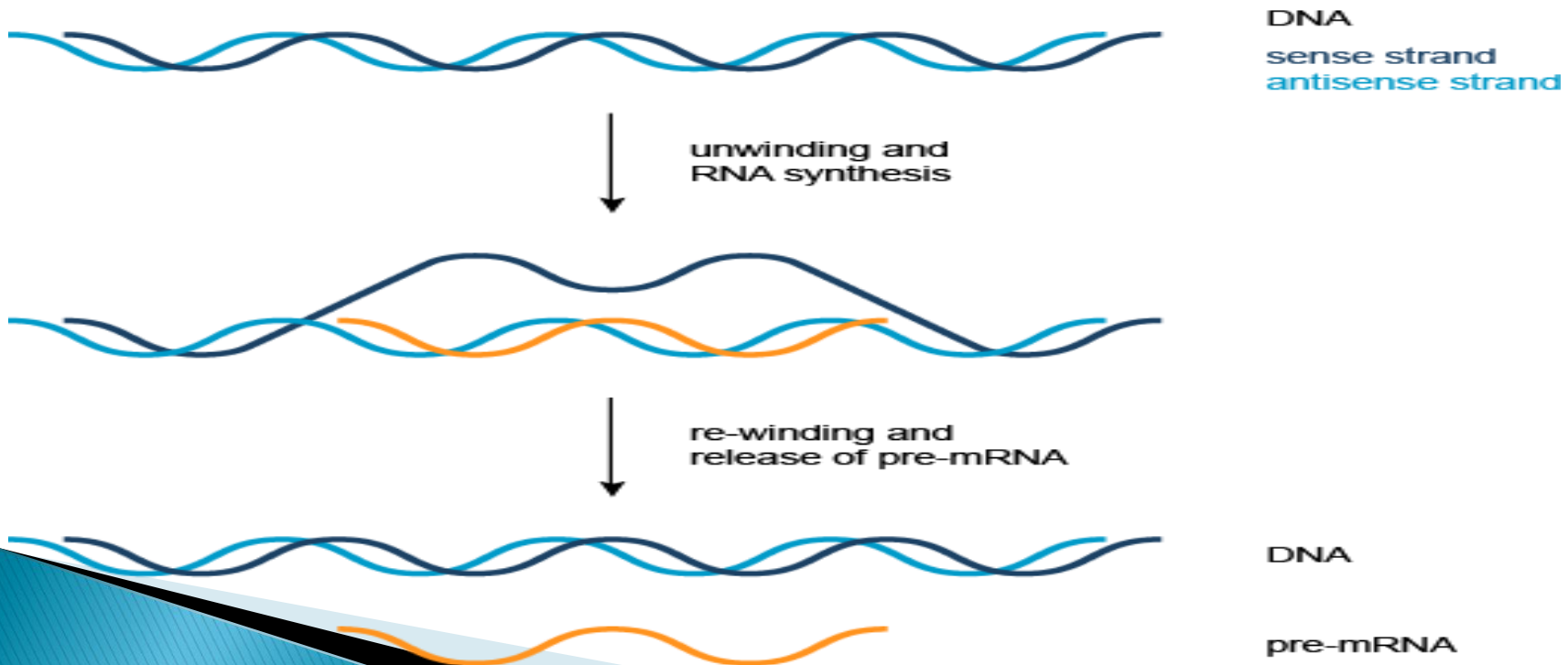
PATHS TAKEN

- Synthesis of mRNA is from the 5 to 3 direction.
- Reading of the DNA is in the 3 to 5 direction.



CONTINUED

- As the RNAP moves on the DNA template, the DNA helix unwinds, transcription bubble formed and the nascent RNA formed.



TERMNATION OF TRANSCRIPTION

- Specific signals are recognized by termination protein called Rho factor.
- This occurs to;

1. **Rho-dependent terminators**
2. ***Rho-independent organisms***

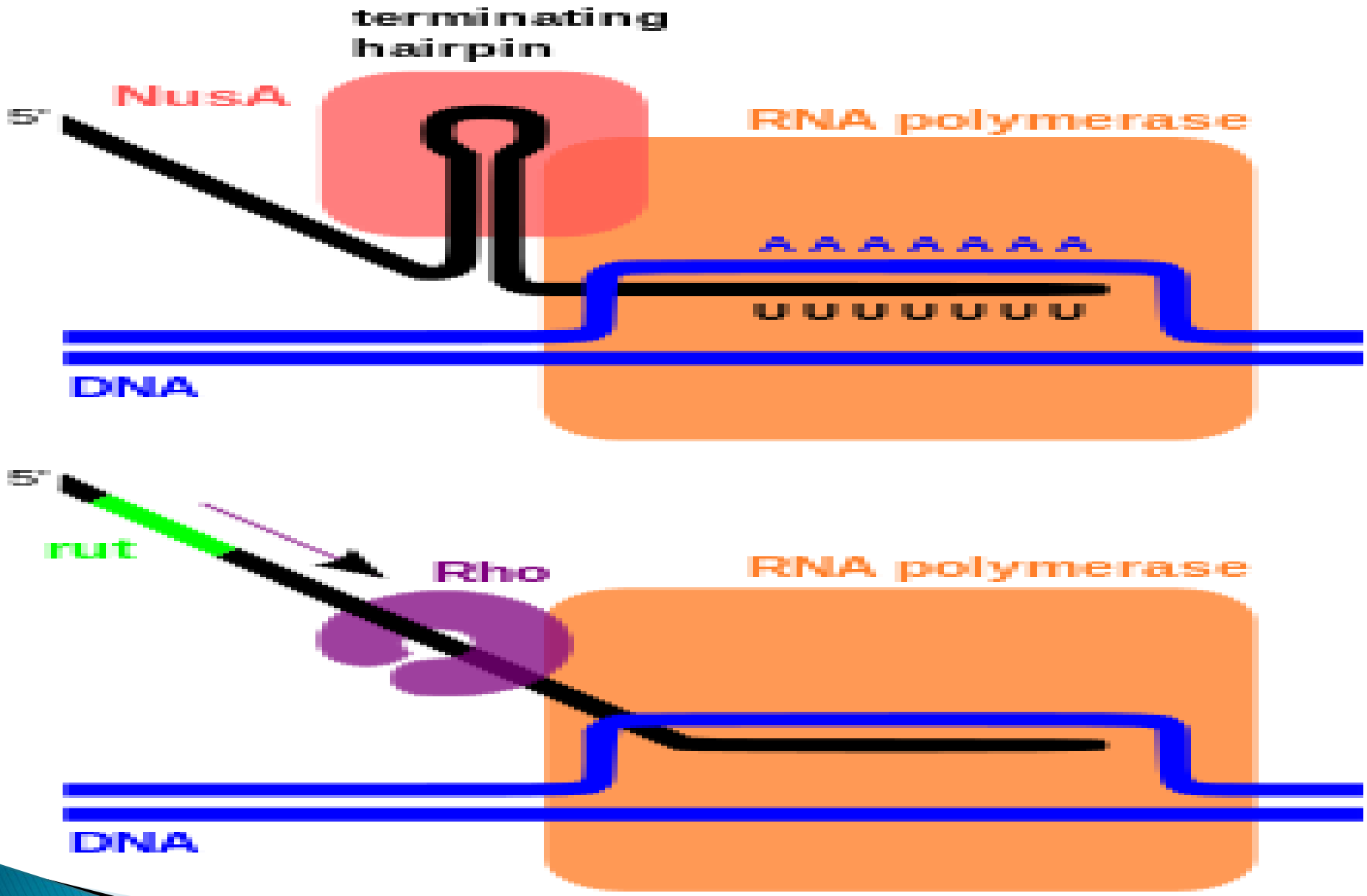
TERMINATION

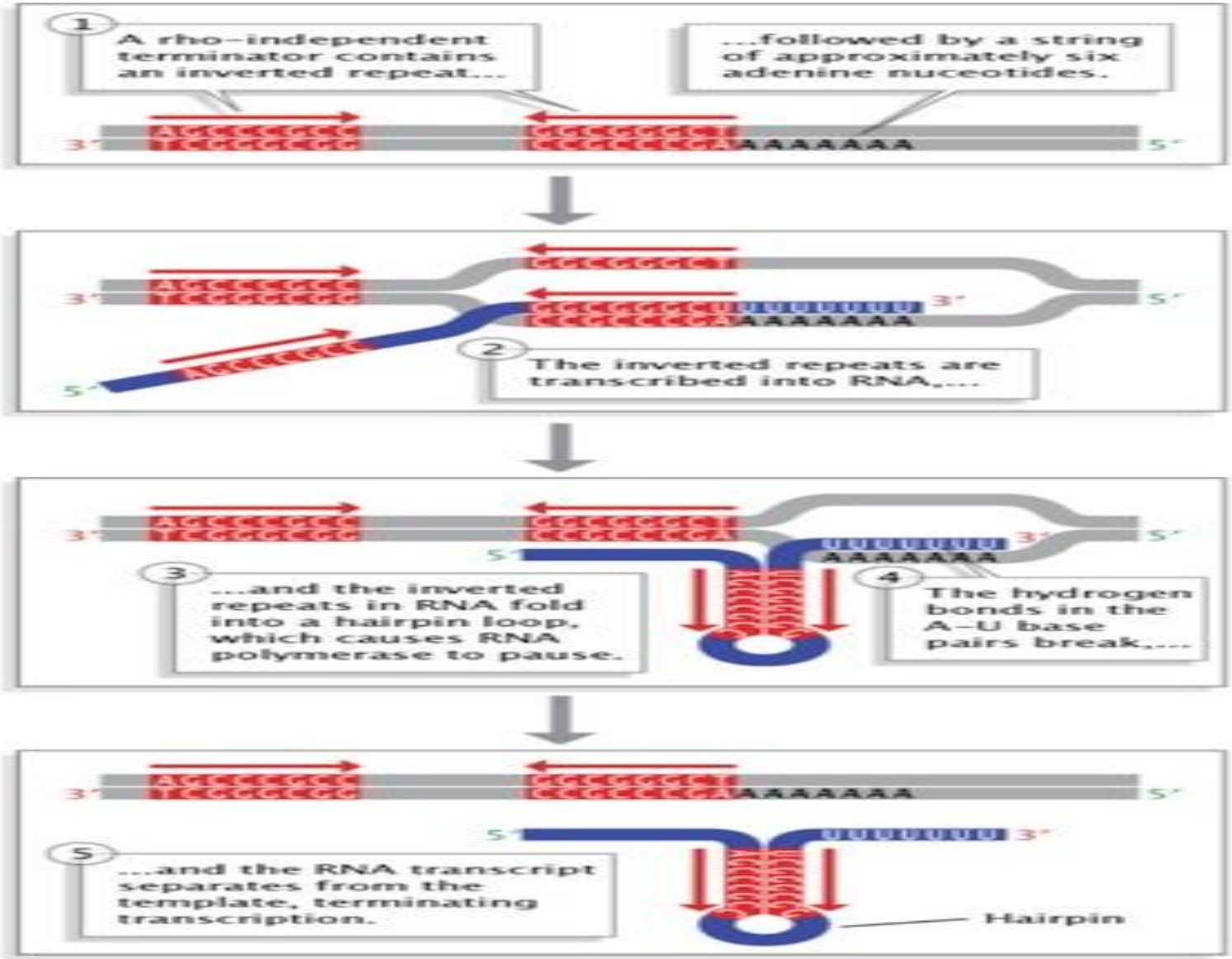
RHO-INDEPENDENT TERMINATION

A terminating hairpin forms on the nascent mRNA interacting with the NusA protein to stimulate release of the transcript from the RNA polymerase complex (top).

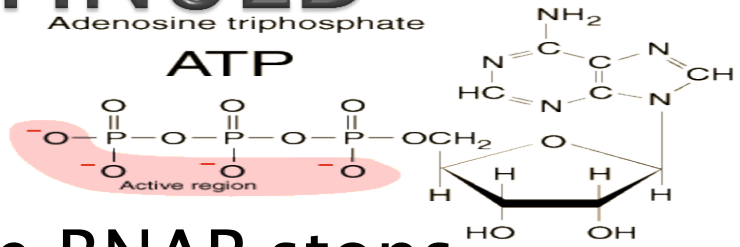
RHO-DEPENDENT TERMINATORS

- The Rho protein binds at the upstream rut site, translocates down the mRNA, and interacts with the RNA polymerase complex to stimulate release of the transcript.(bottom)

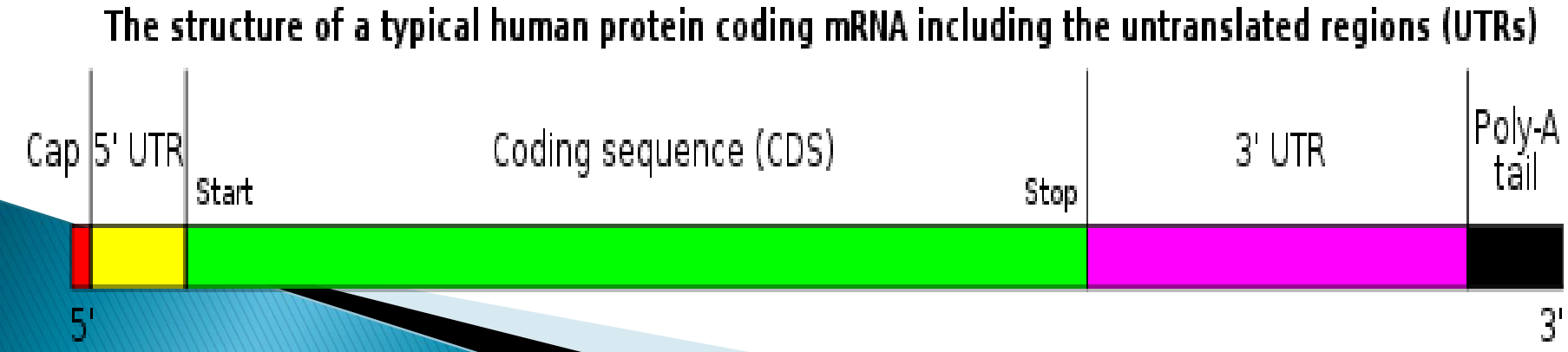

terminating
hairpin
NusA
5'
RNA polymerase
A A A A A A A A
U U U U U U U U
DNA
5'
rut
Rho
RNA polymerase
DNA


1
A rho-independent terminator contains an inverted repeat...
...followed by a string of approximately six adenine nuceotides.
3'
AAAAAAA
5'
2
The inverted repeats are transcribed into RNA,...
3
...and the inverted repeats in RNA fold into a hairpin loop, which causes RNA polymerase to pause.
4
The hydrogen bonds in the A–U base pairs break,...
5
...and the RNA transcript separates from the template, terminating transcription.
Hairpin

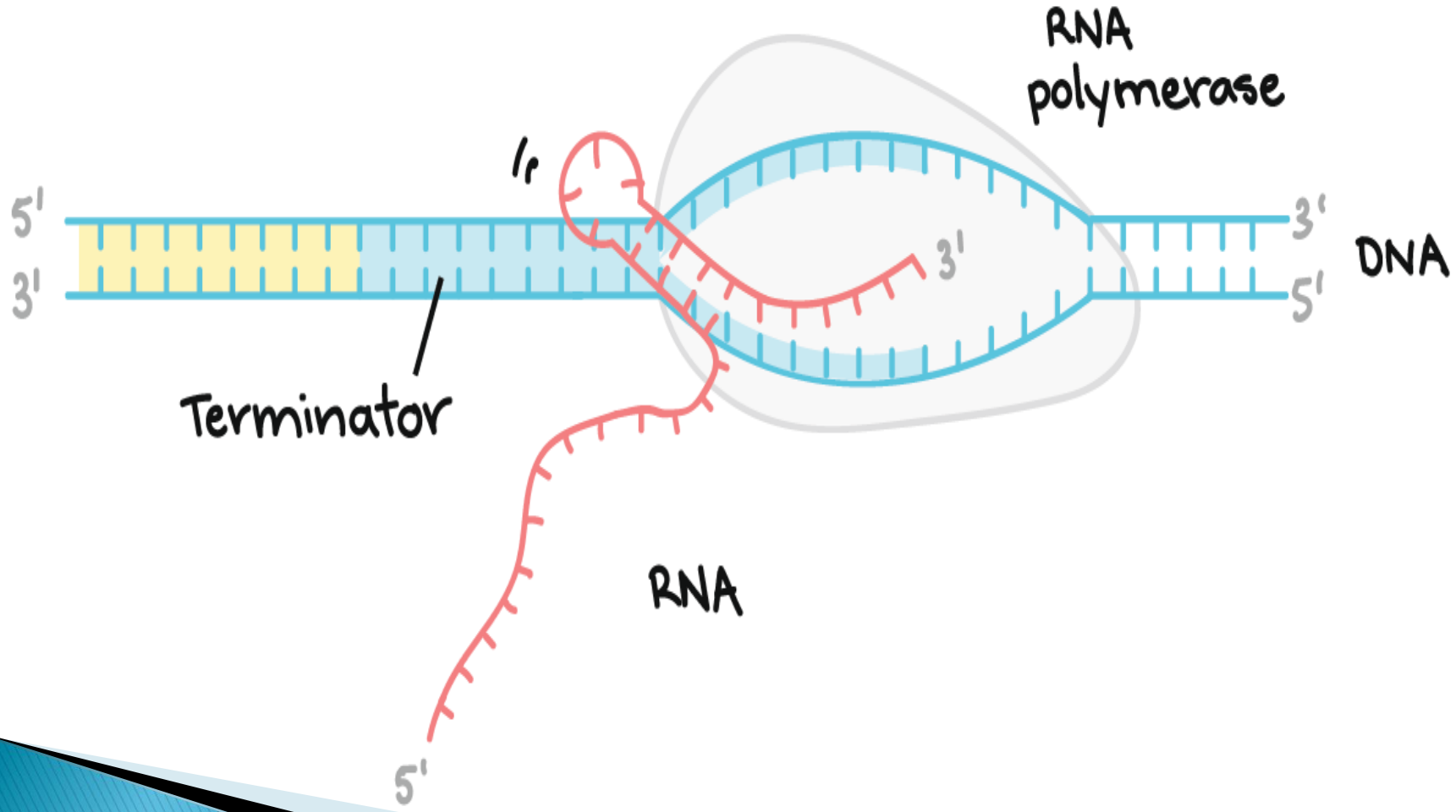
TERMINATION CONTINUED



- Its ATP dependent.
- When it attaches to DNA, the RNAP stops moving, so the enzyme dissociates from DNA and consequently newly 4med mRNA is released.

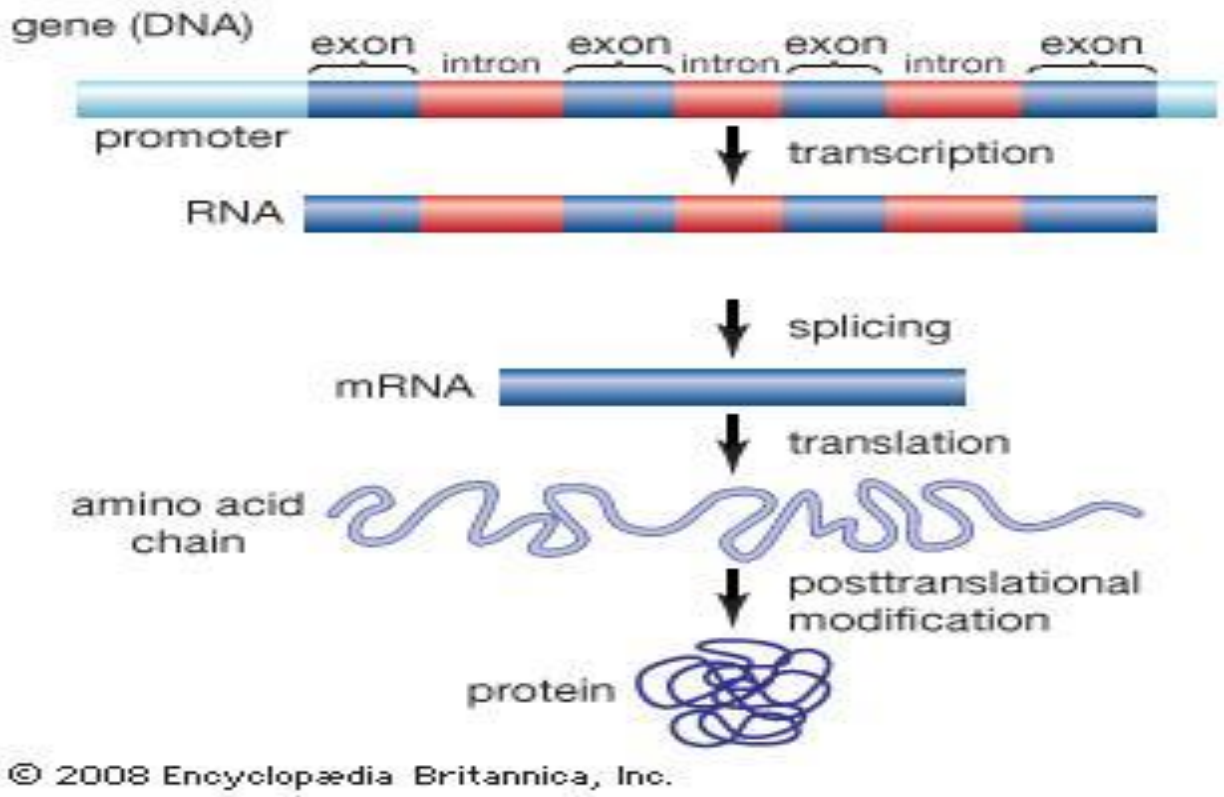


TERMINATION



POST TRANSCRIPTION PROCESSES

- The mRNA formed and released from DNA is a primary transcript with heteronuclear mRNA or hnRNA.



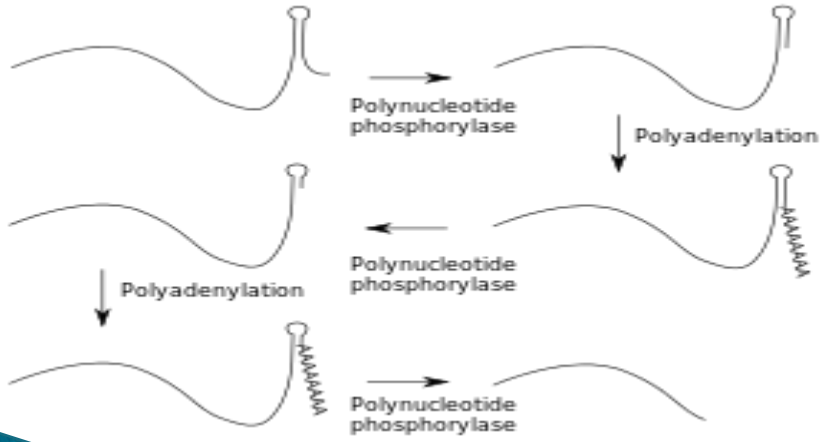
CONTINUED

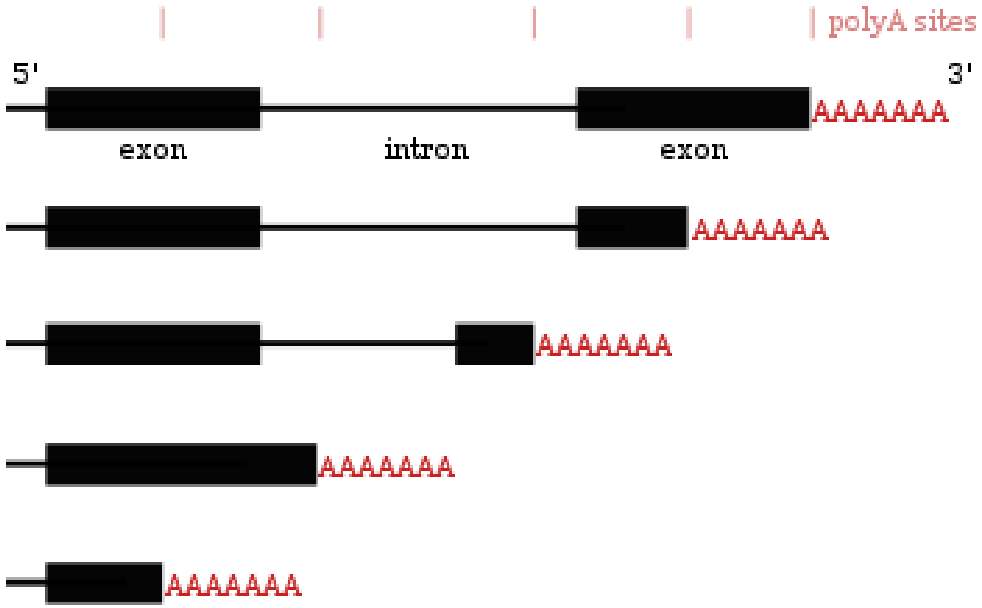
- In mammals, it undergoes extensive processing to become a mature mRNA.ie

- Endonucleases cleavage
- Poly A tail
- 5 capping
- Methylation
- Removal of introns
- Splicing of exons

POLY A TAIL AT 3 END

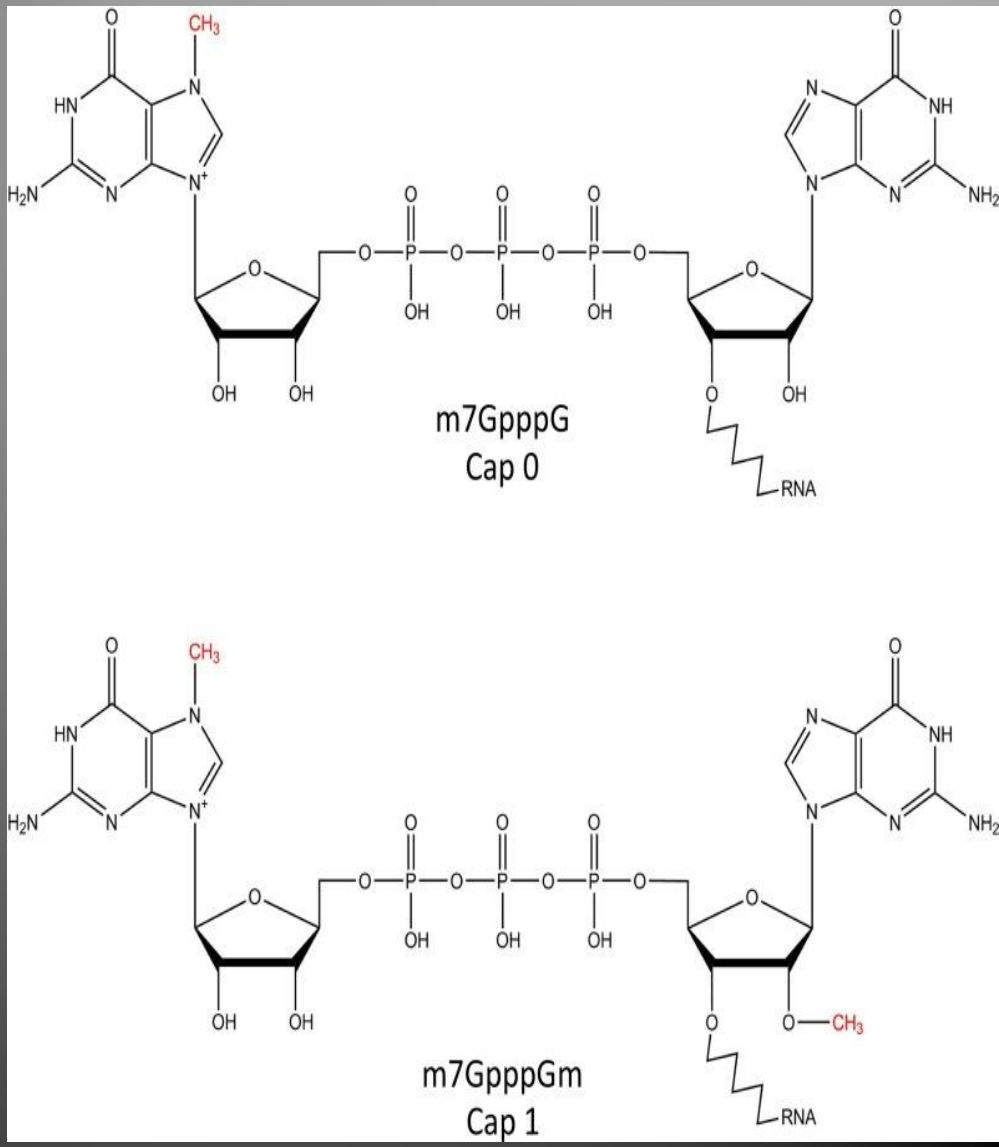
- The 3 terminus is polyadenylated in the nucleoplasm.
- Poly A tail may be 20 to 250 nucleotides long
- This tail protects the mRNA from attacks by the 3 exonucleases.





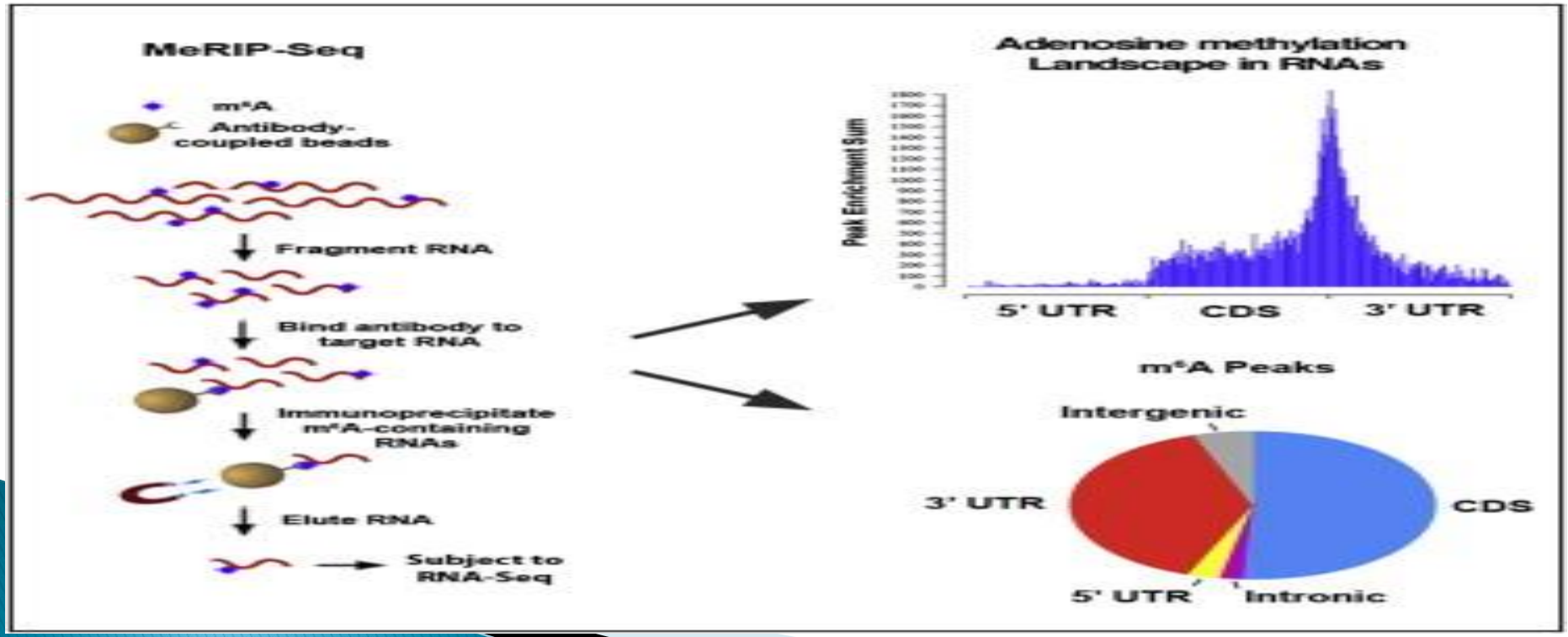
CAPPING AT THE 5 END

- Eukaryotic mRNA are capped at the 5 terminus by 7methyl guanosine triphosphate.
- The cap is useful in recognition of mRNA by translating machines.



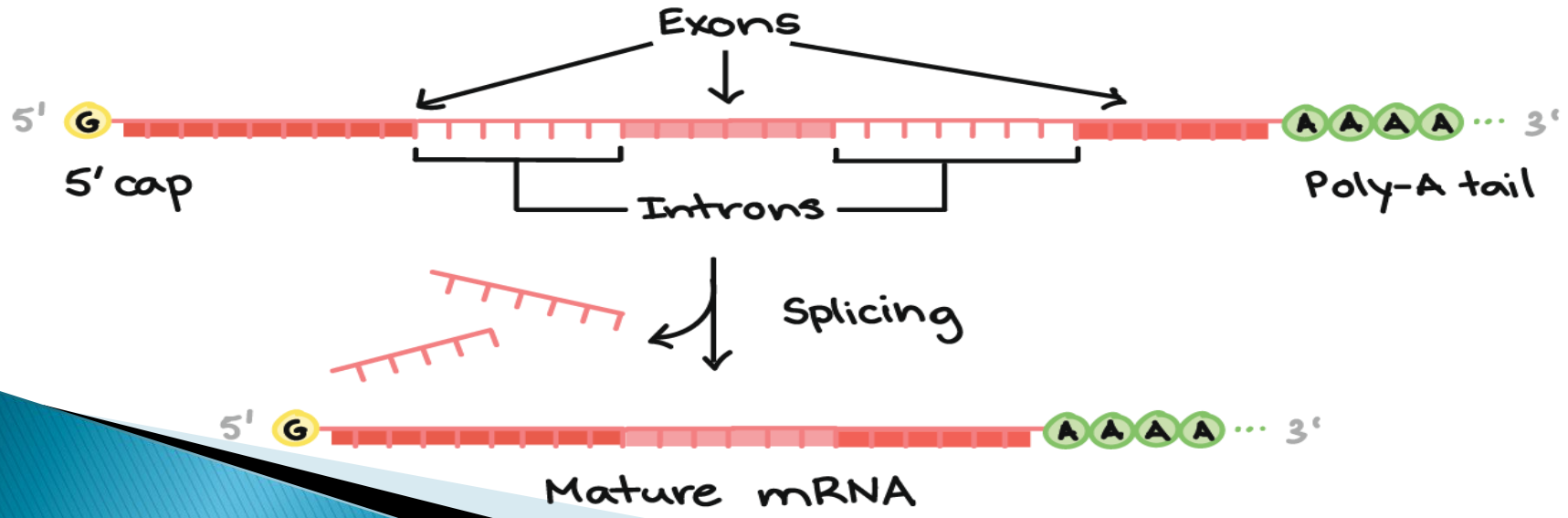
METHYLATION

- Methylation of the N6 of the adenine residues and 2-hydroxyl groups of ribosome are common occur in the cytoplasm.

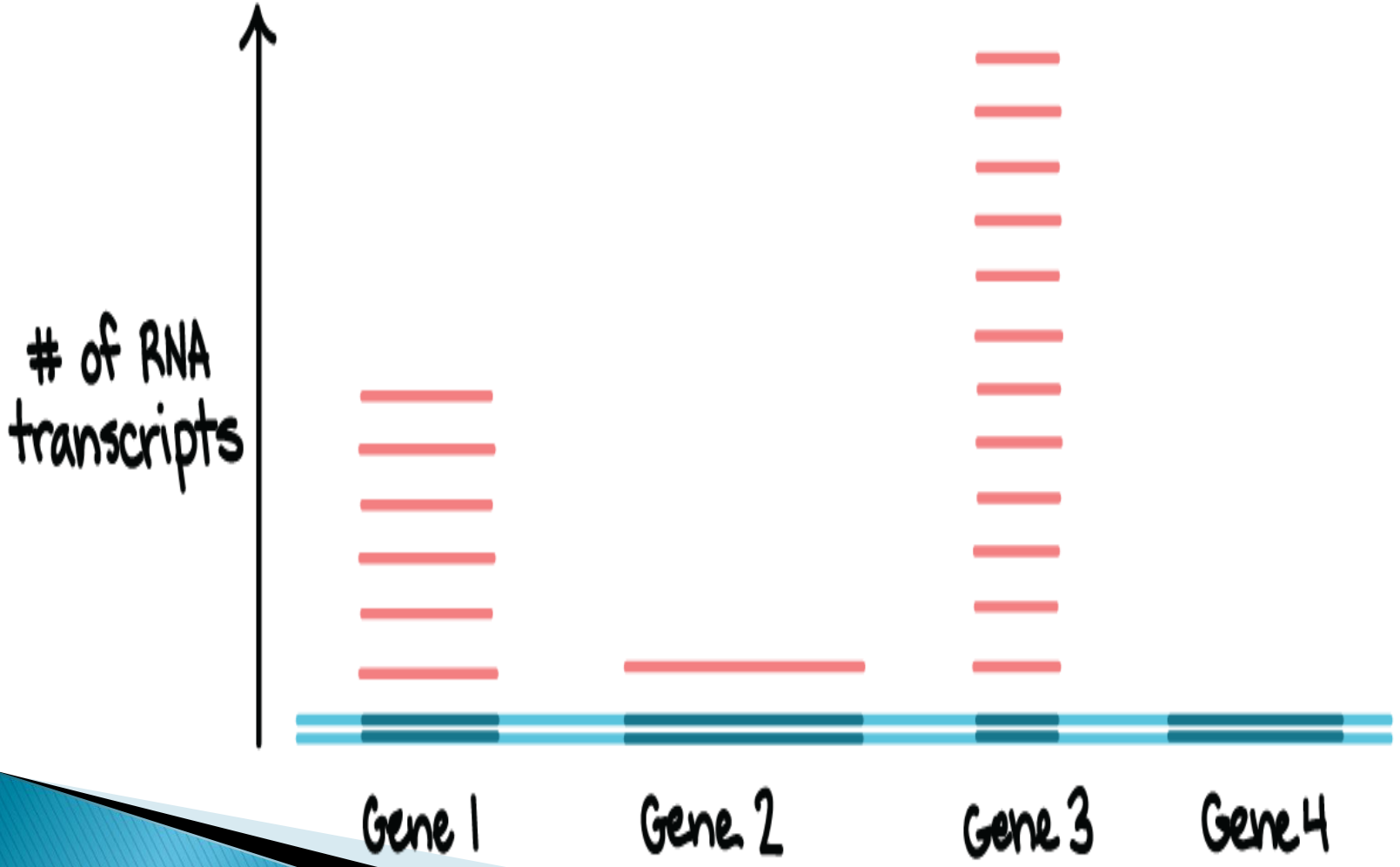


REMOVAL OF INTRONS

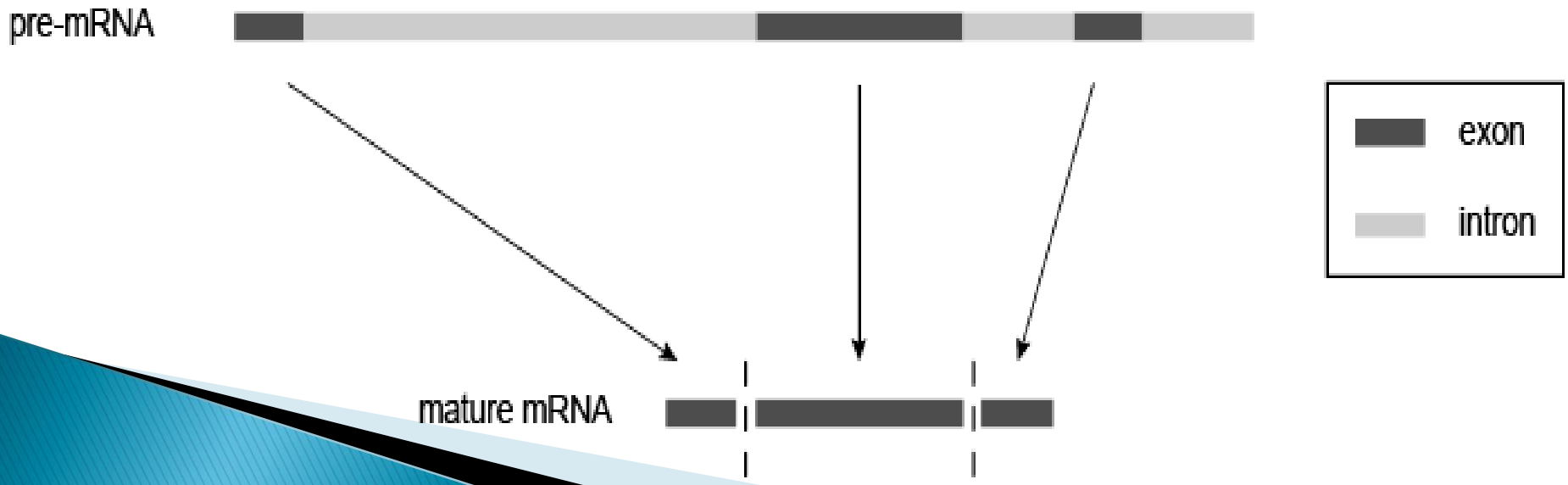
- The primary transcript are more than 10^7
- The molecular weight of a mature mRNA is about 1-2(10^6).Hence large portions of hnRNA are cleaved off.



EXONS AND INTRONS



- The primary transcript contains coding regions(exons) and the non coding region the introns.
- The introns are cleaved and the exons are combined to form a mature mRNA, it occurs in the nucleus and splicing needs energy.



OVERVIEW OF TRANSCRIPTION