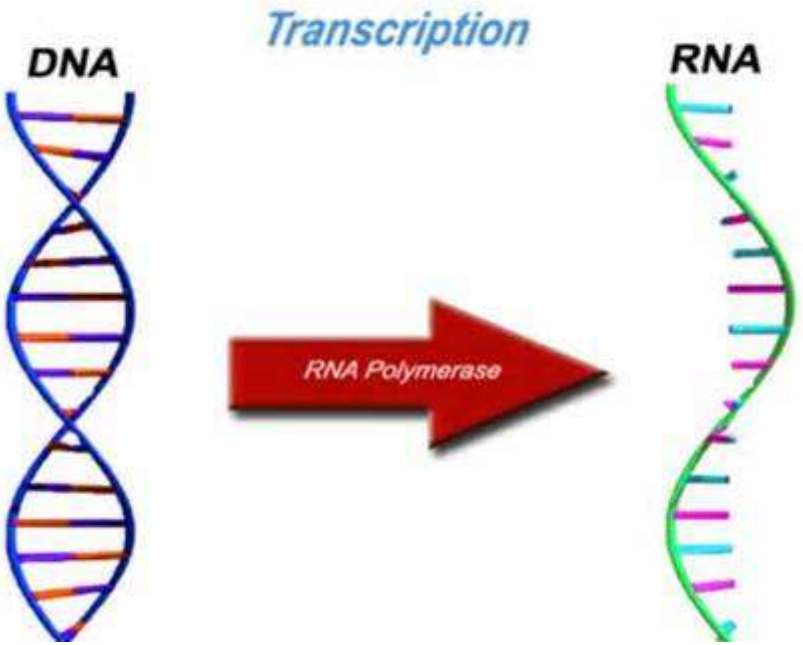
DNA Transcription (Part-1)



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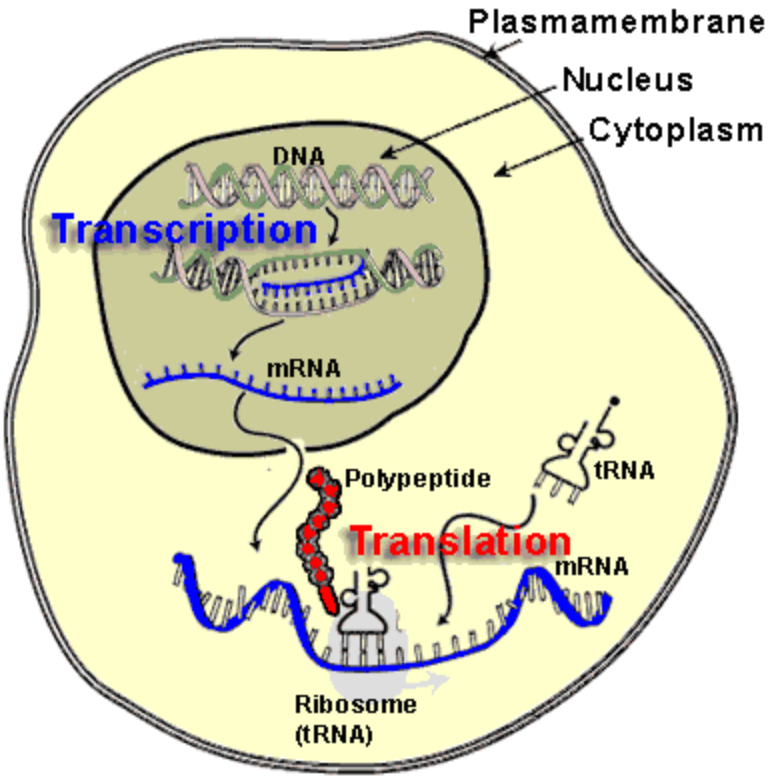
Biochemistry For Medics- Lecture Notes

www.namrata.co

Flow of genetic information



- The genetic information flows from DNA to mRNA and then to the protein synthesizing machinery.

DNA Transcription-Introduction

- The synthesis of an RNA molecule from DNA is called **Transcription.**
- All eukaryotic cells have five major classes of RNA: ribosomal RNA (rRNA), messenger RNA (mRNA), transfer RNA (tRNA), small nuclear RNA and microRNA (snRNA and miRNA).
- The first three are involved in protein synthesis, while the small RNAs are involved in mRNA splicing and regulation of gene expression.

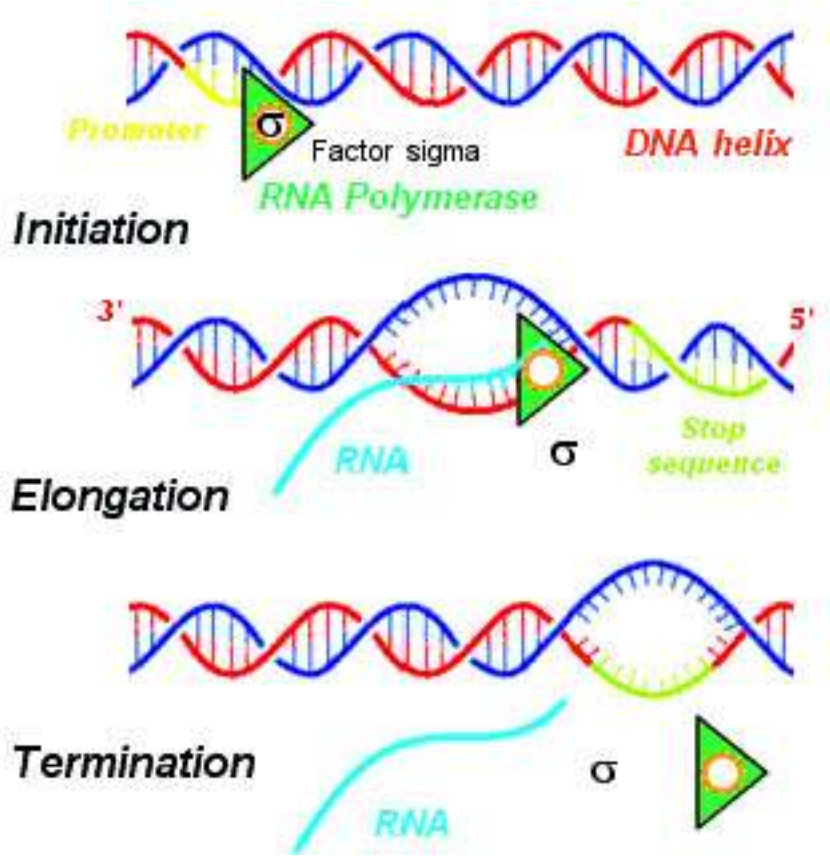
Similarities between Replication and Transcription

The processes of DNA and RNA synthesis are similar in that they involve-

(1) the general steps of initiation elongation, and termination with 5' to 3' polarity;

(2) large, multicomponent initiation complexes; and

(3) adherence to Watson-Crick base-pairing rules.



Differences between Replication and Transcription

(1) Ribonucleotides are used in RNA synthesis rather than deoxy ribonucleotides;

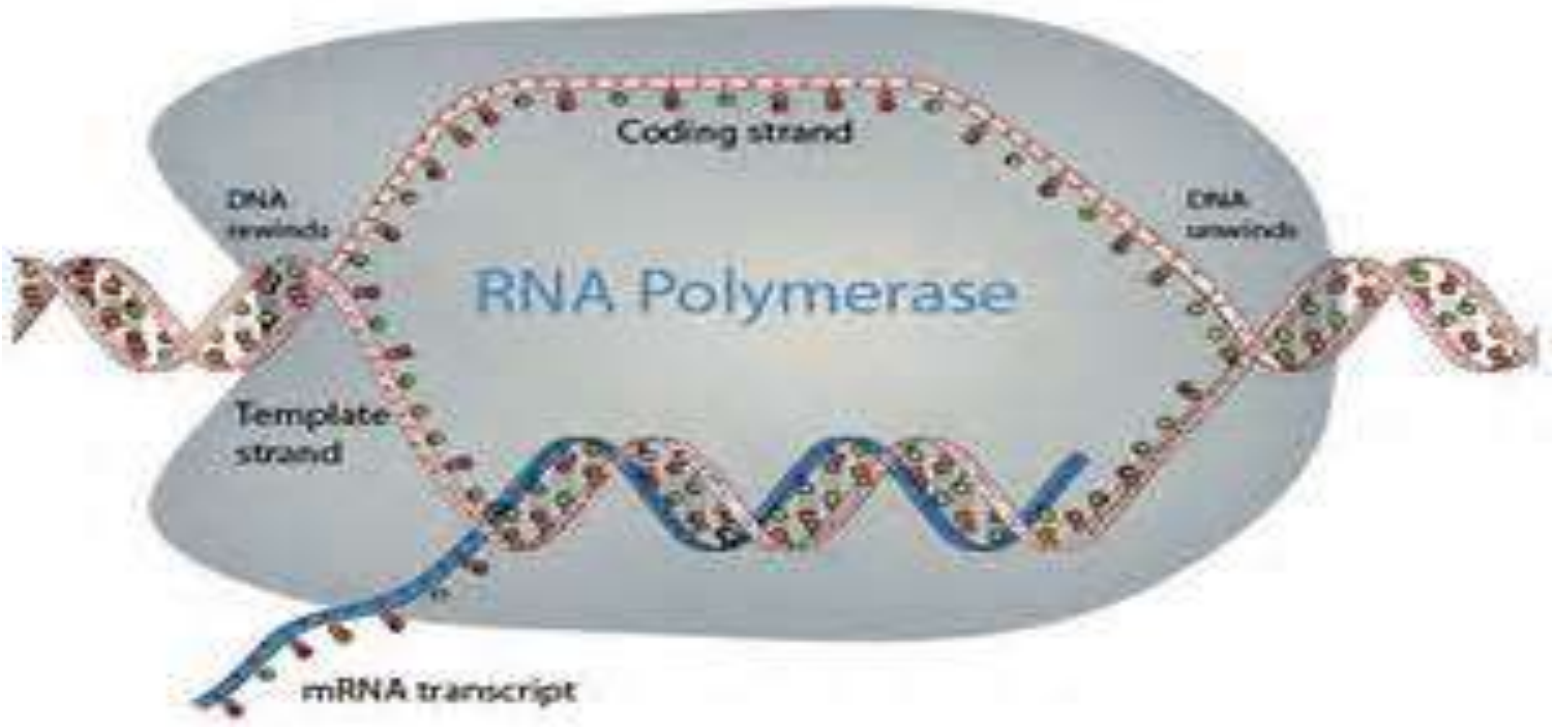
(2) U replaces T as the complementary base pair for A in RNA;

(3) A primer is not involved in RNA synthesis;

(4) Only a portion of the genome is transcribed or copied into RNA, whereas the entire genome must be copied during DNA replication; and

(5) There is no proofreading function during RNA transcription.

Template strand

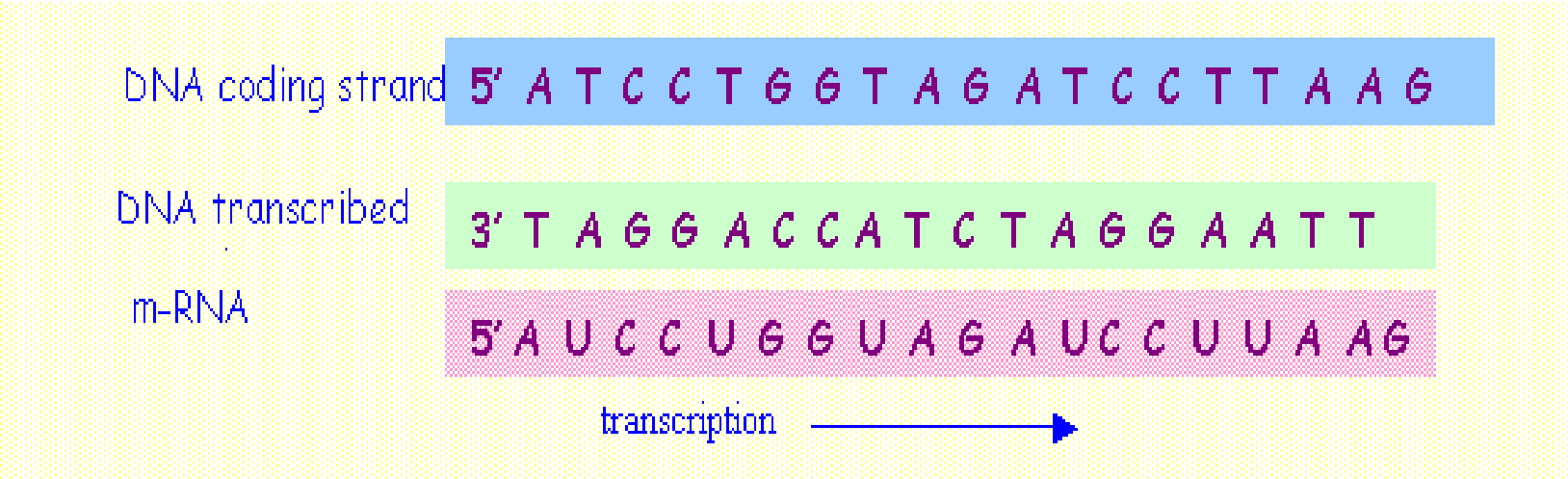


- The strand that is transcribed or copied into an RNA molecule is referred to as the template strand of the DNA.
- The other DNA strand, the non-template strand, is frequently referred to as the coding strand of that gene.

Template strand

- The information in the template strand is read out in the 3' to 5' direction
- The sequence of ribonucleotides in the RNA molecule is complementary to the sequence of deoxy ribonucleotides in template strand of the double-stranded DNA molecule
- In the coding strand (complementary strand) the sequence is same as that of the sequence of nucleotides in the primary transcript.

Template strand (contd.)



With the exception of T for U changes, coding strand corresponds exactly to the sequence of the RNA primary transcript, which encodes the (protein) product of the gene.

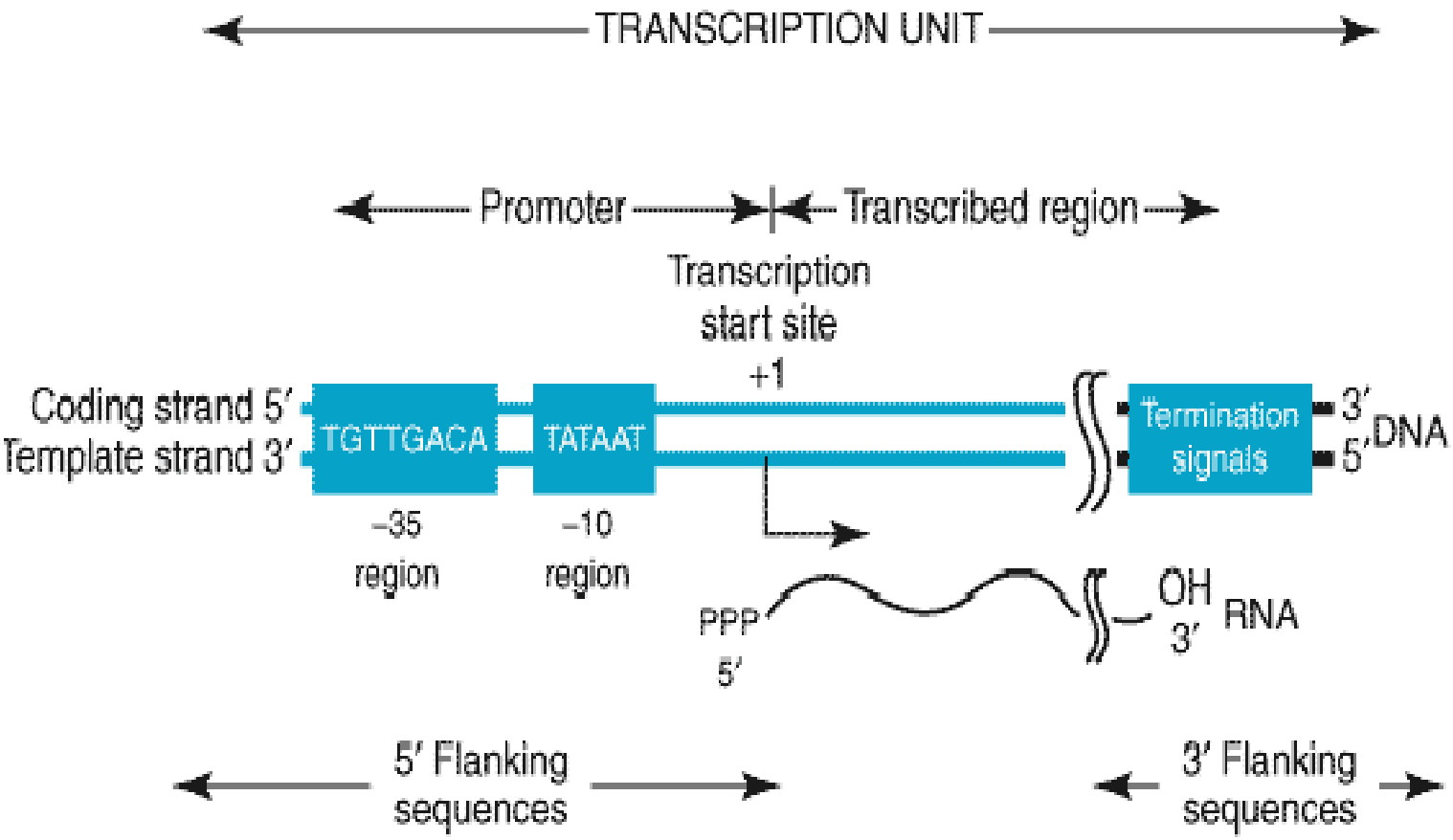
Template strand (contd.)

- In the case of a double-stranded DNA molecule containing many genes, the template strand for each gene will not necessarily be the same strand of the DNA double helix.
- Thus, a given strand of a double-stranded DNA molecule will serve as the template strand for some genes and the coding strand of other genes.

Transcription unit

- **A transcription unit is defined as that region of DNA that includes the signals for transcription initiation, elongation, and termination.**
- **DNA-dependent RNA polymerase** is the enzyme responsible for the polymerization of ribonucleotides into a sequence complementary to the template strand of the gene.
- The enzyme attaches at a specific site—**the promoter**—on the template strand.
- This is followed by initiation of RNA synthesis at the starting point, and the process continues until a termination sequence is reached.

Transcription unit and Primary transcript



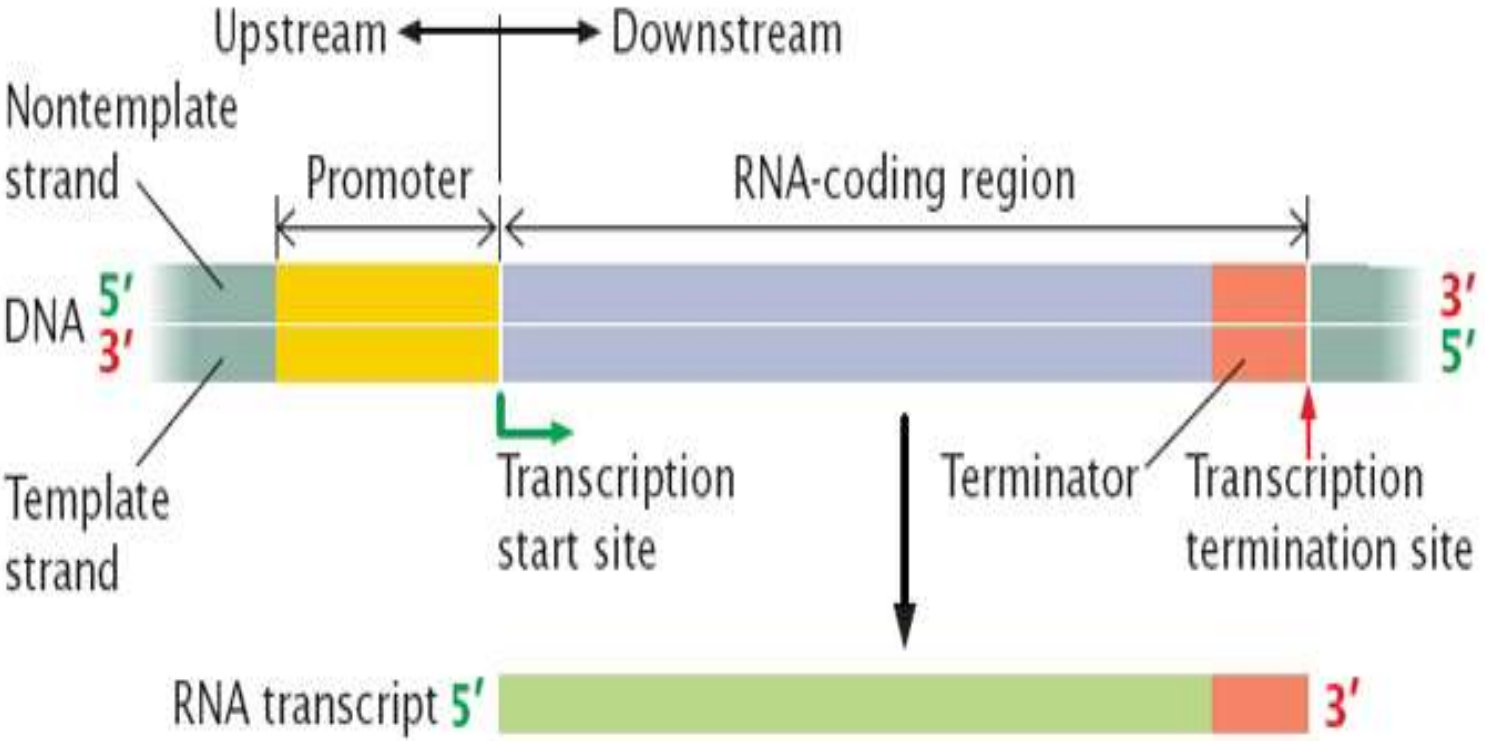
Primary transcript

- The RNA product, which is synthesized in the 5' to 3' direction, is the **primary transcript**.
- In prokaryotes, this can represent the product of several contiguous genes
- In mammalian cells, it usually represents the product of a single gene
- The 5' terminals of the primary RNA transcript and the mature cytoplasmic RNA are identical.
- **The starting point of transcription corresponds to the 5' nucleotide of the mRNA.**

Primary transcript

- This is designated position +1, as is the corresponding nucleotide in the DNA
- The numbers increase as the sequence proceeds *downstream*.
- The nucleotide in the promoter adjacent to the transcription initiation site is designated -1,
- These negative numbers increase as the sequence proceeds *upstream*, away from the initiation site.
- This provides a conventional way of defining the location of regulatory elements in the promoter.

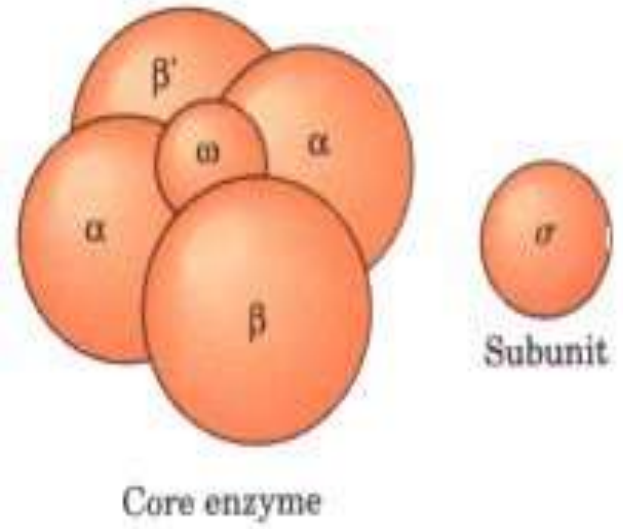
Transcription unit



Bacterial DNA-Dependent RNA Polymerase

The DNA-dependent RNA polymerase (RNAP) of the bacterium *Escherichia coli* exists as an approximately 400 kDa core complex consisting of-

- two identical α subunits,
- similar but not identical β and β ' subunits, and
- an ω subunit and a
- A sigma subunit (σ)
- Beta is thought to be the catalytic subunit.



Bacterial DNA-Dependent RNA Polymerase

- RNAP, a metalloenzyme, also contains two zinc molecules.
- The core RNA polymerase associates with a specific protein factor (the sigma σ factor) that helps the core enzyme recognize and bind to the specific deoxynucleotide sequence of the promoter region to form the preinitiation complex (PIC)
- Bacteria contain multiple factors, each of which acts as a regulatory protein.

Mammalian DNA-Dependent RNA Polymerases

Mammalian cells possess three distinct nuclear DNA-Dependent RNA Polymerases

- RNA polymerase I is for the synthesis of r RNA
- RNA polymerase II is for the synthesis of m RNA and miRNA
- RNA polymerase III is for the synthesis of tRNA/5S rRNA, snRNA

Prokaryotic transcription

Steps of RNA Synthesis-

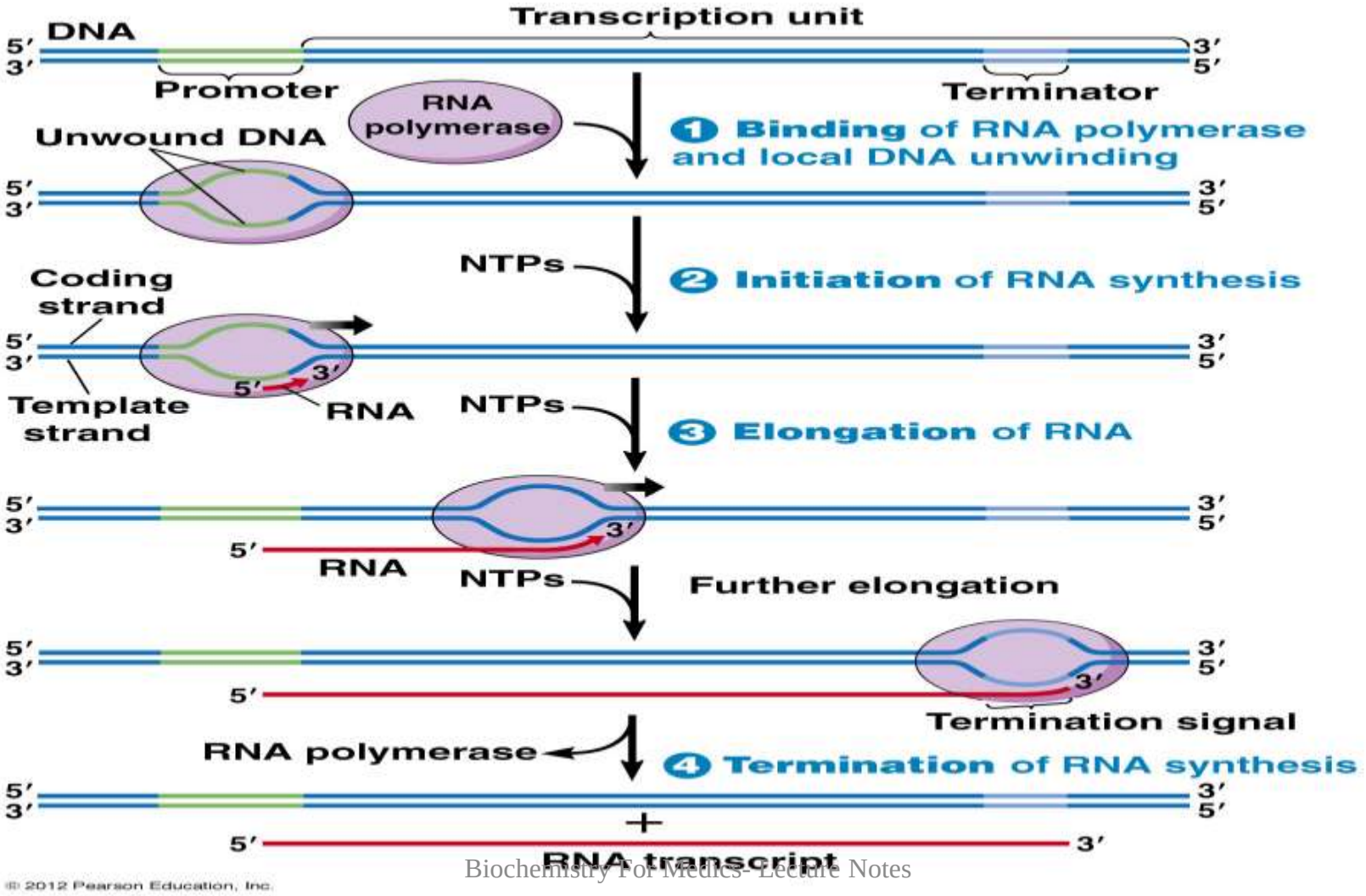
The process of transcription of a typical gene of E. Coli can be divided in to three phases-

i) Initiation

ii) Elongation

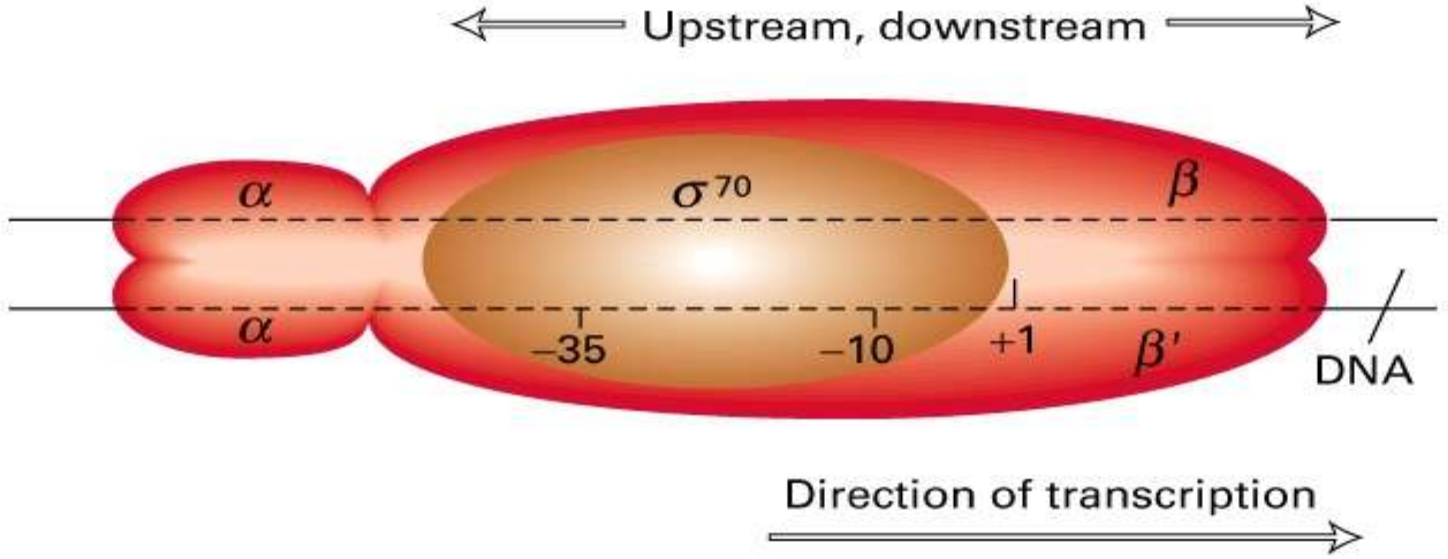
iii) Termination

Overview of Prokaryotic DNA Transcription



i) Initiation of Transcription

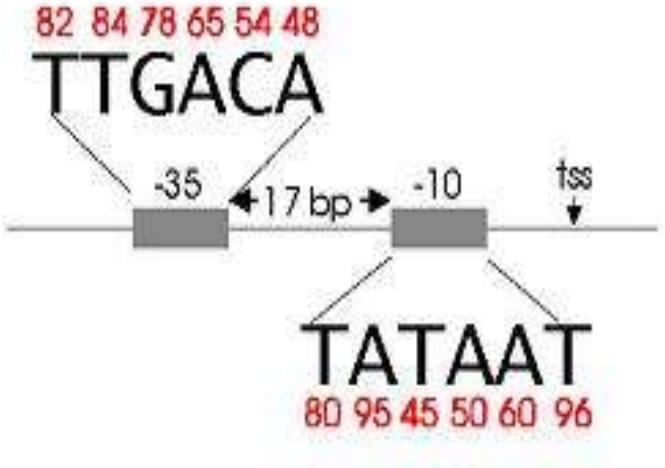
- Initiation of transcription involves the binding of the RNA polymerase holoenzyme to the promoter region on the DNA to form a **preinitiation complex, or PIC**
- Characteristic "Consensus" nucleotide sequence of the prokaryotic promoter region are highly conserved.



Structure of bacterial prokaryotic promoter region

Pribnow box

- This is a stretch of 6 nucleotides (5'- TATAAT-3') centered about 8-10 nucleotides to the left of the transcription start site.



-35 Sequence

- A second consensus nucleotide sequence (5'- TTGACA-3'), is centered about 35 bases to the left of the transcription start site.

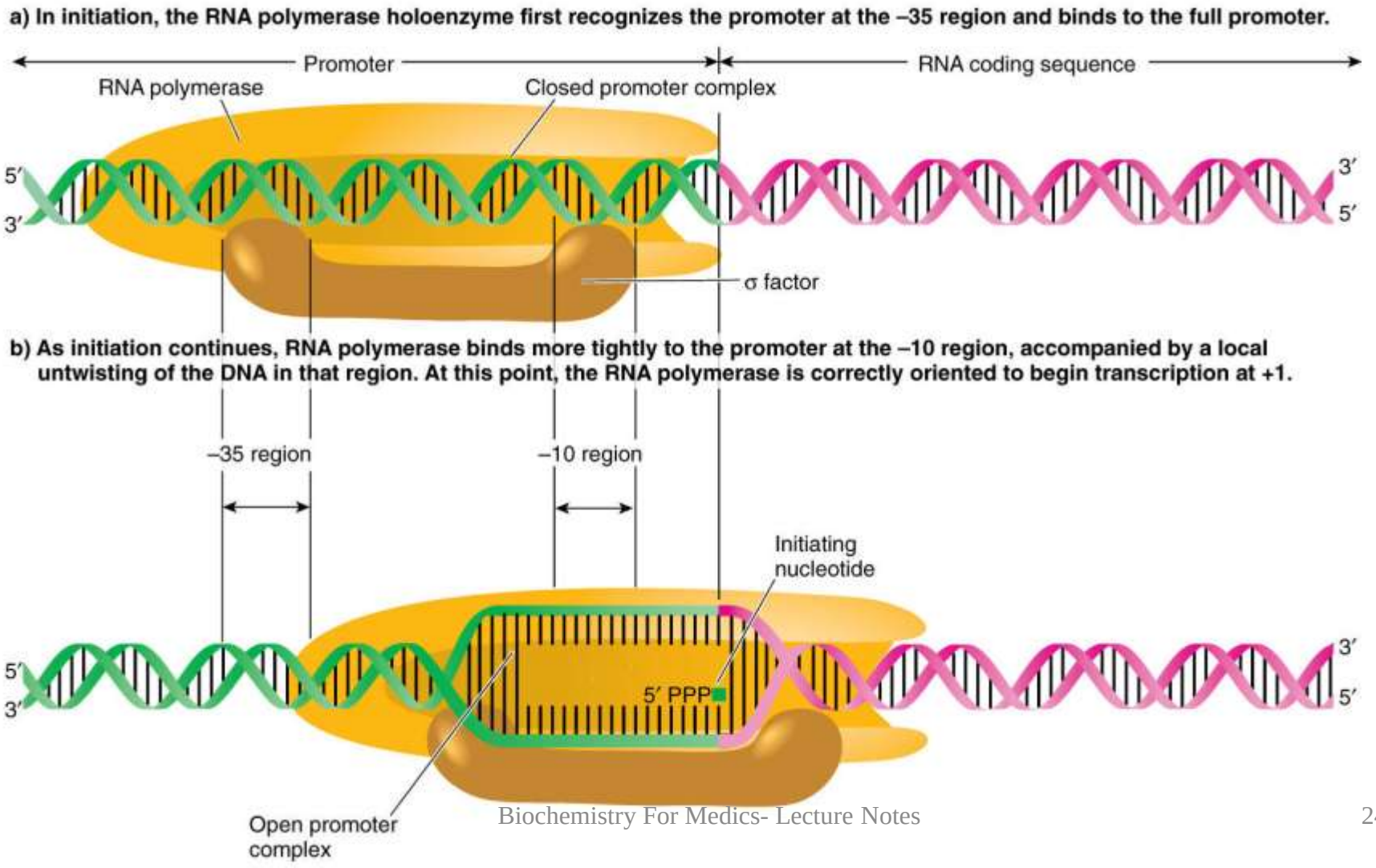
i) Initiation of Transcription (contd.)

- Binding of RNA-polymerase (RNAP) to the promoter region is followed by a conformational change of the RNAP, and the first nucleotide (almost always a purine) then associates with the initiation site on the subunit of the enzyme.
- In the presence of the appropriate nucleotide, RNAP catalyzes the formation of a phosphodiester bond, and the nascent chain is now attached to the polymerization site on the subunit of RNAP.

i) Initiation of Transcription (contd.)

- In both prokaryotes and eukaryotes, a purine ribonucleotide is usually the first to be polymerized into the RNA molecule.
- After 10–20 nucleotides have been polymerized, RNAP undergoes a second conformational change leading to **promoter clearance.**
- Once this transition occurs, RNAP physically moves away from the promoter, transcribing down the transcription unit, leading to the next phase of the process, elongation.

i) Initiation of Transcription (contd.)

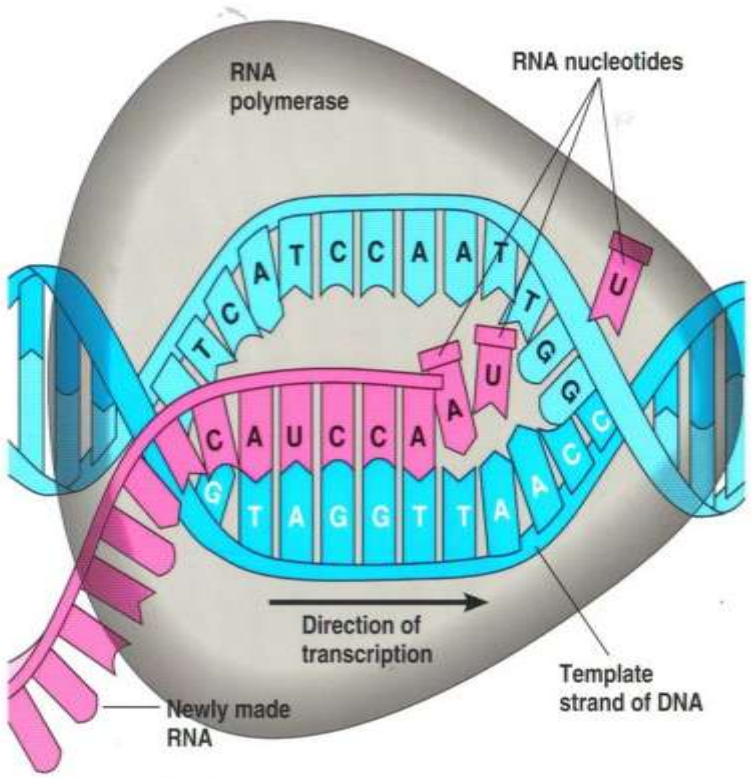


II) Elongation step of Transcription

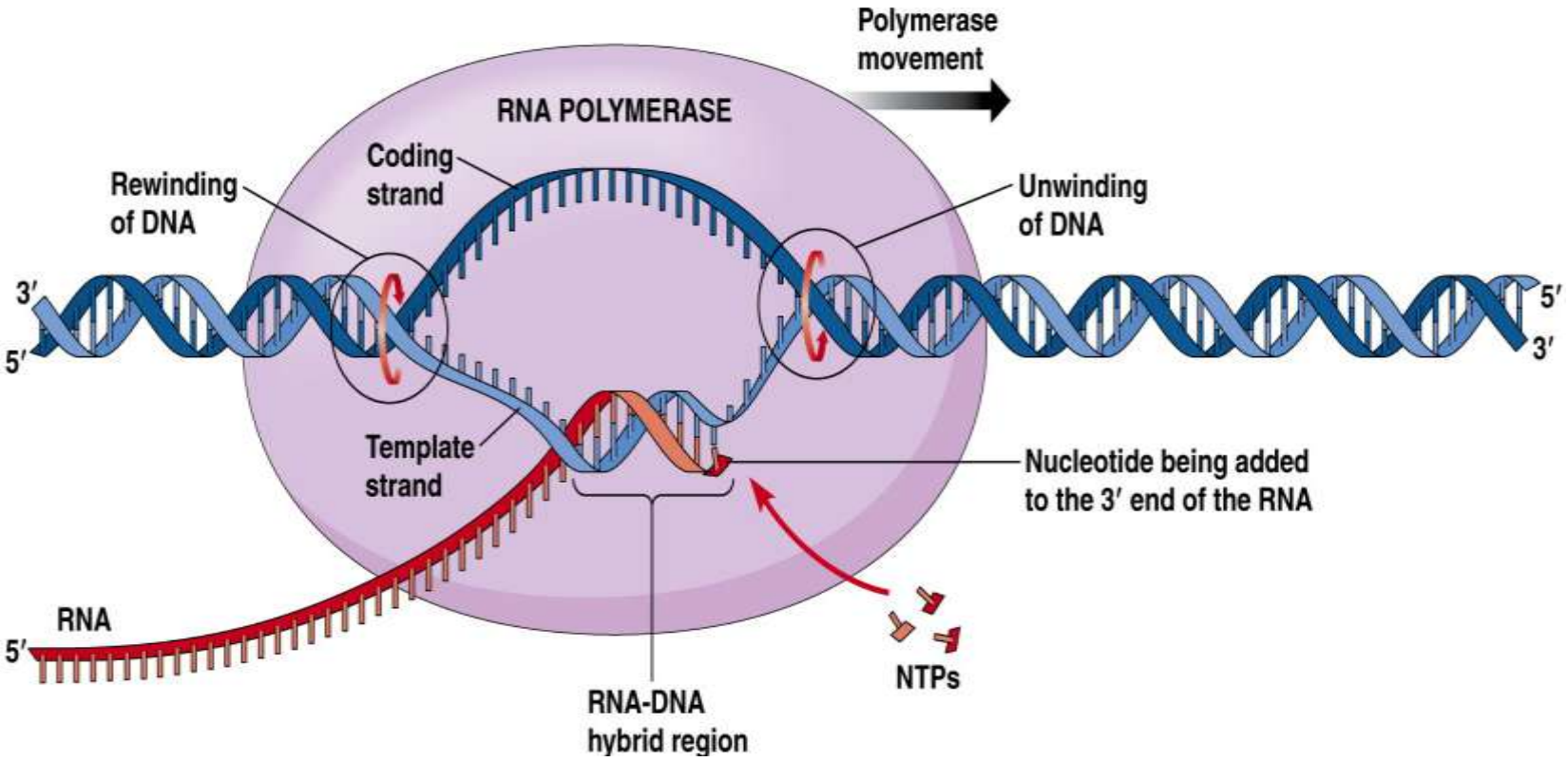
- As the elongation complex containing the core RNA polymerase progresses along the DNA molecule, DNA unwinding must occur in order to provide access for the appropriate base pairing to the nucleotides of the template strand.
- The extent of this transcription bubble (i.e., DNA unwinding) is constant throughout and is about 20 base pairs per polymerase molecule.

II) Elongation step of Transcription

- RNA polymerase has associated with it an "unwindase" activity that opens the DNA helix.
- Topo isomerase both precedes and follows the progressing RNAP to prevent the formation of super helical complexes.
- Base pairing rule is followed during the incorporation of ribonucleotides



II) Elongation step of Transcription



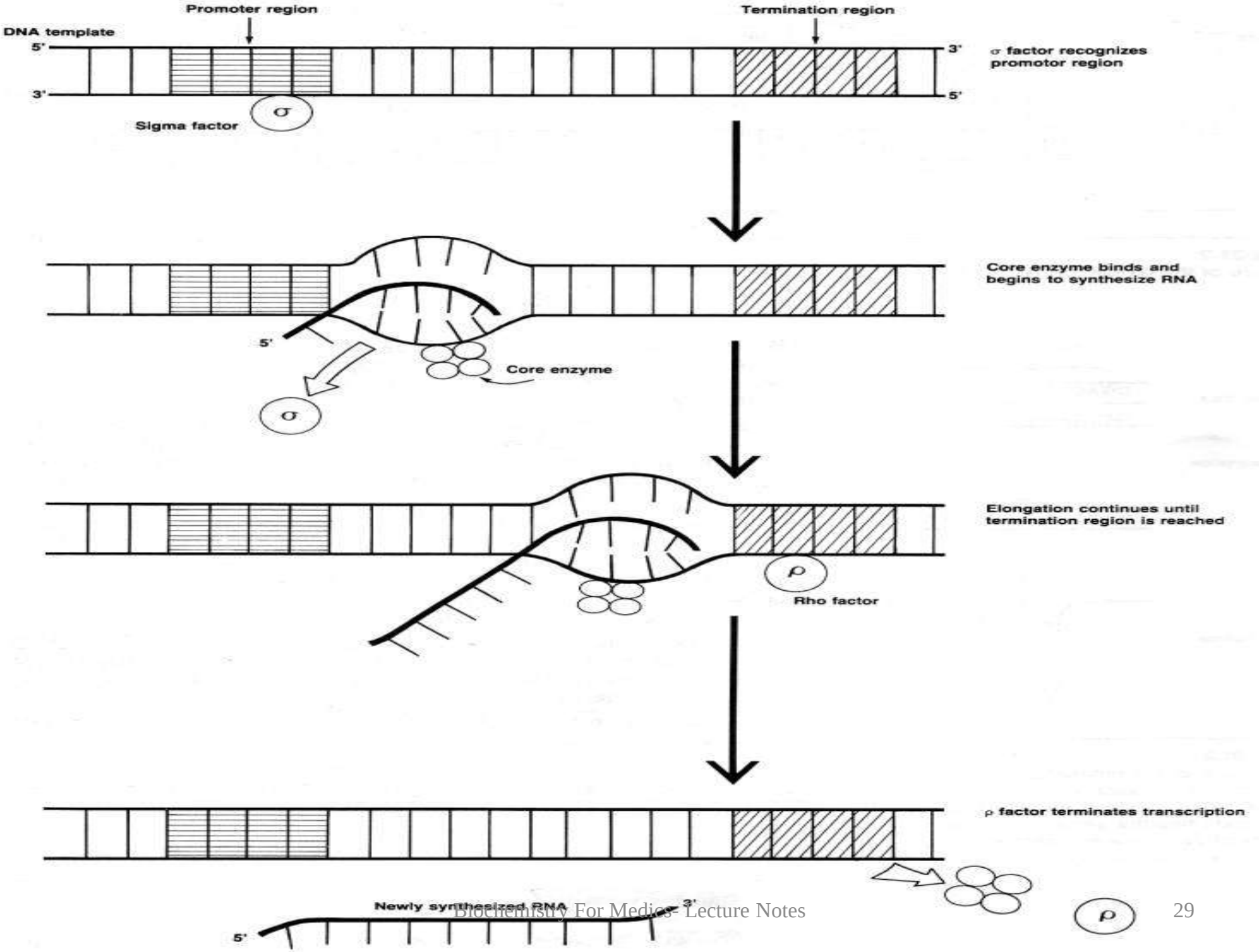
III) Termination of transcription

Termination of the synthesis of the RNA molecule in bacteria is of two types-

a) Rho (ρ) dependent termination-

•The termination process is signaled by a sequence in the template strand of the DNA molecule—a signal that is recognized by a termination protein, the rho (ρ) factor.

•Rho is an ATP-dependent RNA-stimulated helicase that disrupts the nascent RNA-DNA complex.


Promoter region
Termination region
DNA template
5'
3'
3'
5'
Sigma factor
σ factor recognizes promotor region
Core enzyme binds and begins to synthesize RNA
5'
Core enzyme
Elongation continues until termination region is reached
Rho factor
ρ factor terminates transcription
Newly synthesized RNA
5'
3'

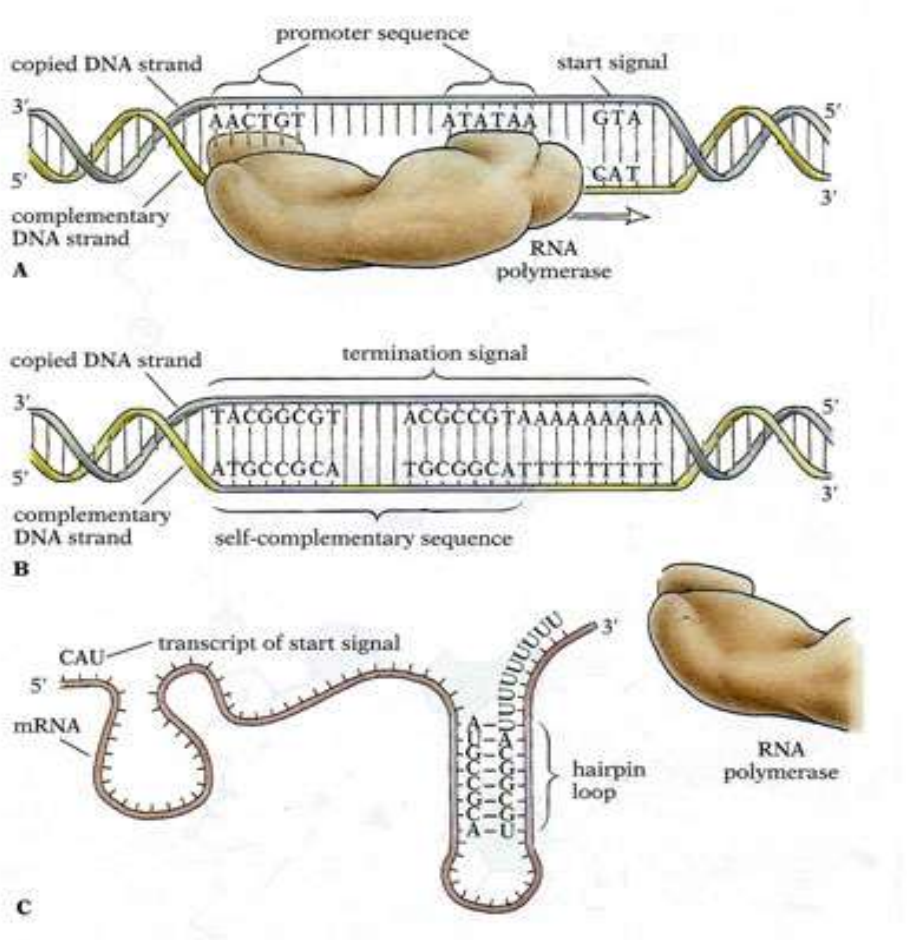
III) Termination of transcription (contd.)

b) Rho independent termination

- This process requires the presence of intrachain self complementary sequences in the newly formed primary transcript so that it can acquire a stable hair pin turn that slows down the progress of the RNA polymerase and causes it to pause temporarily.
- Near the stem of the hairpin, a sequence occurs that is rich in G and C.
- This stabilizes the secondary structure of the hair pin.

III) Termination of transcription (contd.)

- Beyond the hair pin, the RNA transcript contains a strings of Us, the bonding of Us to the corresponding As is weak.
- This facilitates the dissociation of the primary transcript from DNA.



III) Termination of transcription (contd.)

- After termination of synthesis of the RNA molecule, the enzyme separates from the DNA template.
- With the assistance of another factor, the core enzyme then recognizes a promoter at which the synthesis of a new RNA molecule commences.

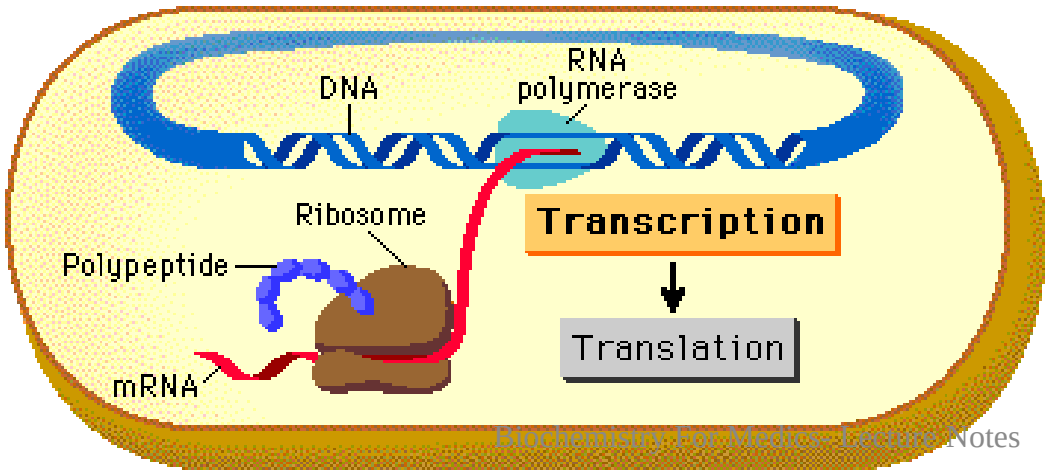
Eukaryotic transcription

- The general process of transcription can be applied to both prokaryotic cells and eukaryotic cells.
- The basic biochemistry for each is the same; however, the specific mechanisms and regulation of transcription differ between prokaryotes and eukaryotes.
- Transcription of eukaryotic genes is far more a complicated process than prokaryotes.

Prokaryotic versus Eukaryotic Transcription

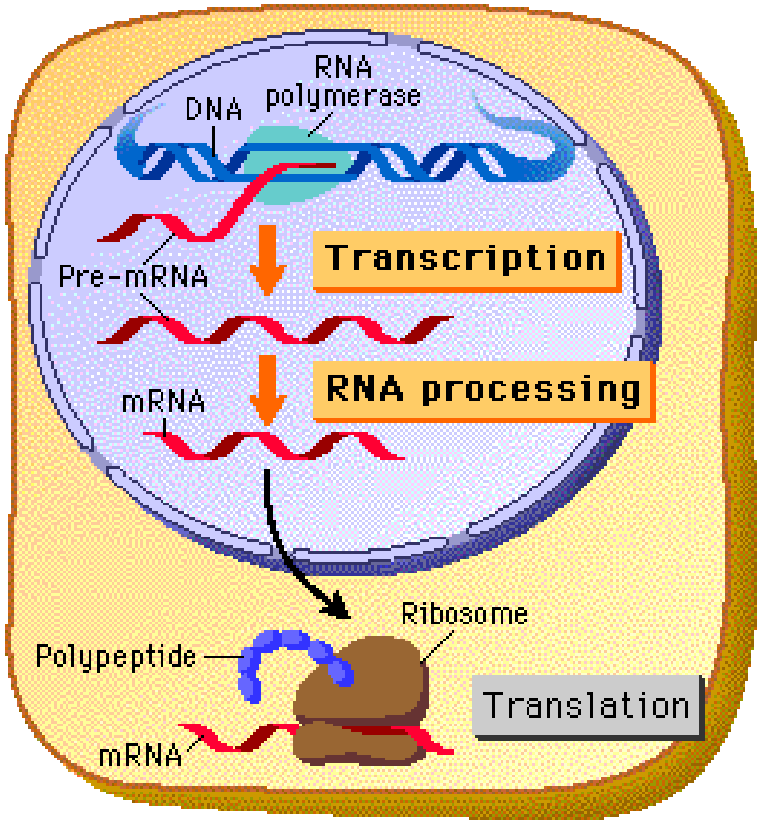
1) Location

- In prokaryotes (bacteria), transcription occurs in the cytoplasm.
- Translation of the mRNA into proteins also occurs in the cytoplasm



Prokaryotic versus Eukaryotic Transcription

- In eukaryotes, transcription occurs in the cell's nucleus, mRNA then moves to the cytoplasm for translation.



Prokaryotic versus Eukaryotic Transcription

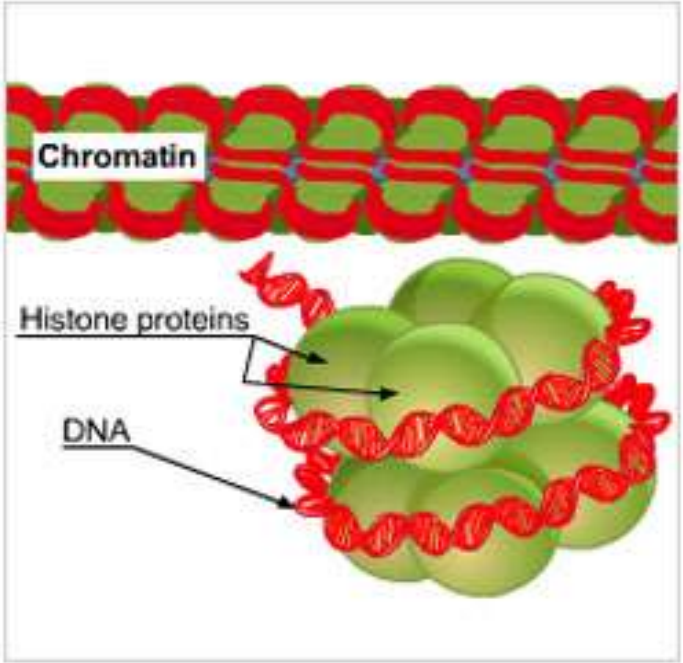
2) Genome size

- The genome size is much larger in eukaryotes,
- Greater specificity is needed for the transcription of eukaryotic genes.

Prokaryotic versus Eukaryotic Transcription

3) Chromatin Structure

- DNA in prokaryotes is much more accessible to RNA polymerase than DNA in eukaryotes.
- Eukaryotic DNA is wrapped around proteins called histones to form structures called nucleosomes
- Eukaryotic DNA is packed to form chromatin .
- While RNA polymerase interacts directly with prokaryotic DNA, other proteins mediate the interaction between RNA polymerase and DNA in eukaryotes



Prokaryotic versus Eukaryotic Transcription

4) RNA polymerases

- There are three distinct classes of RNA polymerases in eukaryotic cells. All are large enzymes with multiple subunits. Each class of RNA polymerase recognizes particular types of genes.
- RNA polymerase I- Synthesizes the precursor of the large ribosomal RNAs (28S, 18S and 5.8S).
- RNA polymerase II - Synthesizes the precursors of messenger RNA and small nuclear RNAs(snRNAs).
- RNA polymerase III- Synthesizes small RNA, including t RNAs, small 5S RNA and some snRNAs.

Prokaryotic versus Eukaryotic Transcription

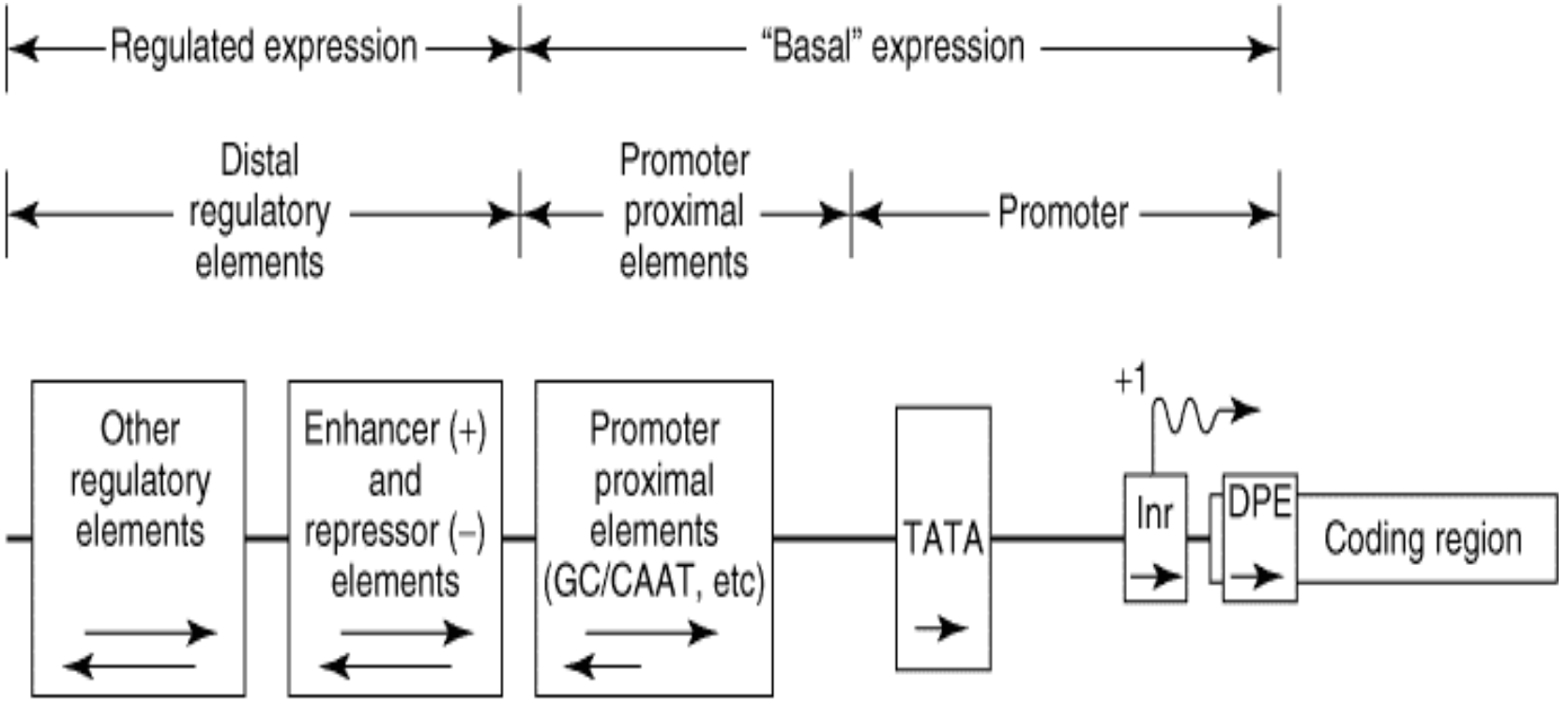
5) Promoter regions

- Eukaryotic promoters are more complex.
- Two types of sequence elements are promoter-proximal and distal regulatory elements.
- There are two elements in promoter proximal ,One of these defines **where transcription is to commence** along the DNA, and the other contributes to the mechanisms that control **how frequently** this event is to occur.
- Most mammalian genes have a TATA box that is usually located 25–30 bp upstream from the transcription start site.

Prokaryotic versus Eukaryotic Transcription

- The consensus sequence for a TATA box is TATAAA, though numerous variations have been characterized.
- Sequences farther upstream from the start site determine how frequently the transcription event occurs.
- Typical of these DNA elements are the GC and CAAT boxes, so named because of the DNA sequences involved.
- Each of these boxes binds a specific protein.
- Distal regulatory elements enhance or decrease the rate of transcription.
- They include the enhancer/ silencer regions and other regulatory elements.

Prokaryotic versus Eukaryotic Transcription

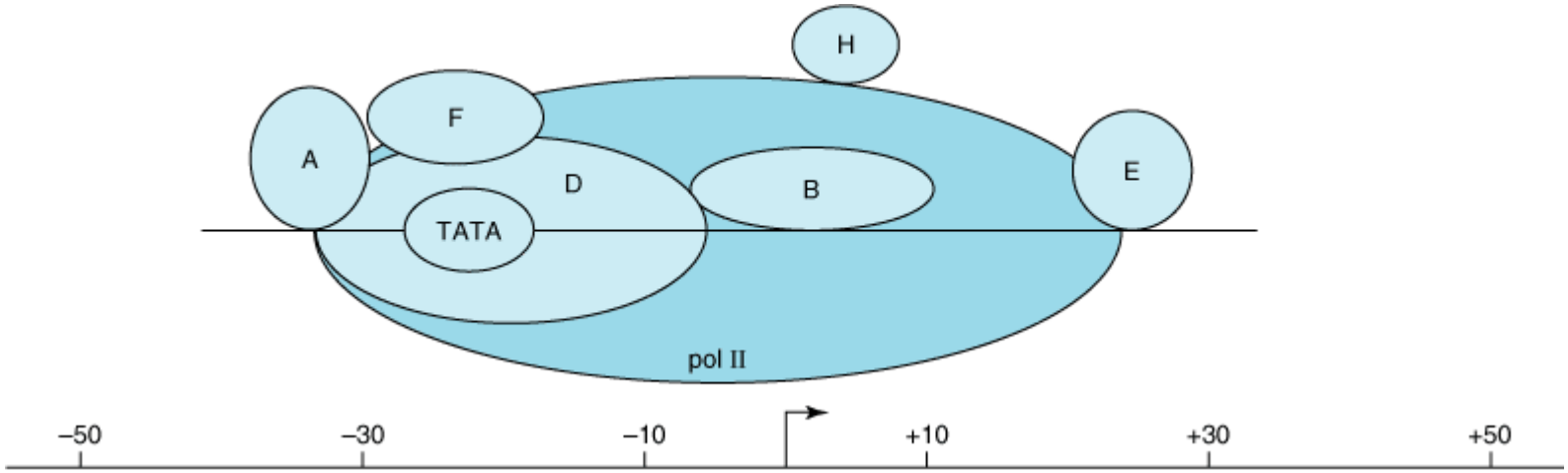


Prokaryotic versus Eukaryotic Transcription

6) Promoter identification

- In contrast to the situation in prokaryotes, eukaryotic RNA polymerases alone are not able to discriminate between promoter sequences and other regions of DNA
- The TATA box is bound by 34 kDa **TATA binding protein (TBP),** which in turn binds several other proteins called **TBP-associated factors (TAFs).**
- This complex of TBP and TAFs is referred to as TFIID

Prokaryotic versus Eukaryotic Transcription



- Formation of the basal transcription complex begins when TFIID binds to the TATA box.
- It directs the assembly of several other components by protein-DNA and protein-protein interactions. T
- The entire complex spans DNA from position -30 to +30 relative to the initiation site.

Prokaryotic versus Eukaryotic Transcription

- Binding of TFIID to the TATA box sequence is thought to represent the first step in the formation of the transcription complex on the promoter.
- Another set of proteins—co activators—help regulate the rate of transcription initiation by interacting with transcription activators that bind to upstream DNA elements

Prokaryotic versus Eukaryotic Transcription

7) Enhancers and Repressors

- A third class of sequence elements can either increase or decrease the rate of transcription initiation of eukaryotic genes
- These elements are called either enhancers or repressors (or silencers), depending on which effect they have.

Prokaryotic versus Eukaryotic Transcription

- They have been found in a variety of locations both upstream and downstream of the transcription start site and even within the transcribed portions of some genes.
- In contrast to proximal and upstream promoter elements, enhancers and silencers can exert their effects when located hundreds or even thousands of bases away from transcription units located on the same chromosome.
- **Hormone response elements** (for steroids, T_3, retinoic acid, peptides, etc) act as—or in conjunction with—enhancers or silencers

Prokaryotic versus Eukaryotic Transcription

7) Termination of transcription

- The **signals for the termination of transcription** by eukaryotic RNA polymerase II are very poorly understood.

8) Processing of primary transcript

- mRNA produced as a result of transcription is not modified in prokaryotic cells. Eukaryotic cells modify mRNA by RNA splicing, 5' end capping, and addition of a polyA tail.
- Most eukaryotic RNAs are synthesized as precursors that contain excess sequences which are removed prior to the generation of mature, functional RNA.

Transcription summary

- To revise the concepts follow the links
- http://highered.mcgraw-hill.com/sites/0072507470/student_view0/chapter3/animation__mrna_synthesis__transcription___quiz_1_.html
- http://telstar.ote.cmu.edu/biology/animation/DnaTranscription/transcription_simple.html
- http://bcs.whfreeman.com/thelifewire/content/chp12/1202001.html