- Faculty: Physiotherapy
- Class: ١st year
- Semester: Two
- Course Title &code: Genetics and molecular biology(19١-GEN-ME).
- Batch No: (١٥)

RNA Biosynthesis (Transicription)

Learning outcomes:

- ***By the end of this session you should:***
- Define transcription, promotor region, exon, intron, splici ng of exon.
- Locate the transcription process in the cell and the directi on of this process.
- List the RNA biosynthesis requirements.
- Recognize the transcriptional unit contents.
- Describe the steps of mRNA biosynthesis
- Differentiae between different RNA polymerases and thei r roles in transcription in both prokaryotes and eukaryote s.
- Discuss the post-transcription process modifications

Contents:

- Introduction.
- Biosynthesis requirements.
- Transcription unit.
- RNA polymerase enzymes.
- Steps of RNA Biosynthesis.
- RNA processing.
- Summary.

Introduction

- RNA biosynthesis is the process of synthesis of RNA molecule from DNA .
- The process is important for protein synthesis.
- Changes in the synthesis, processing, and splicing of mRNA transcripts may causes a disease.
- The process involves one of the groups of RNA poly merase enzymes and a number of associated protein s.

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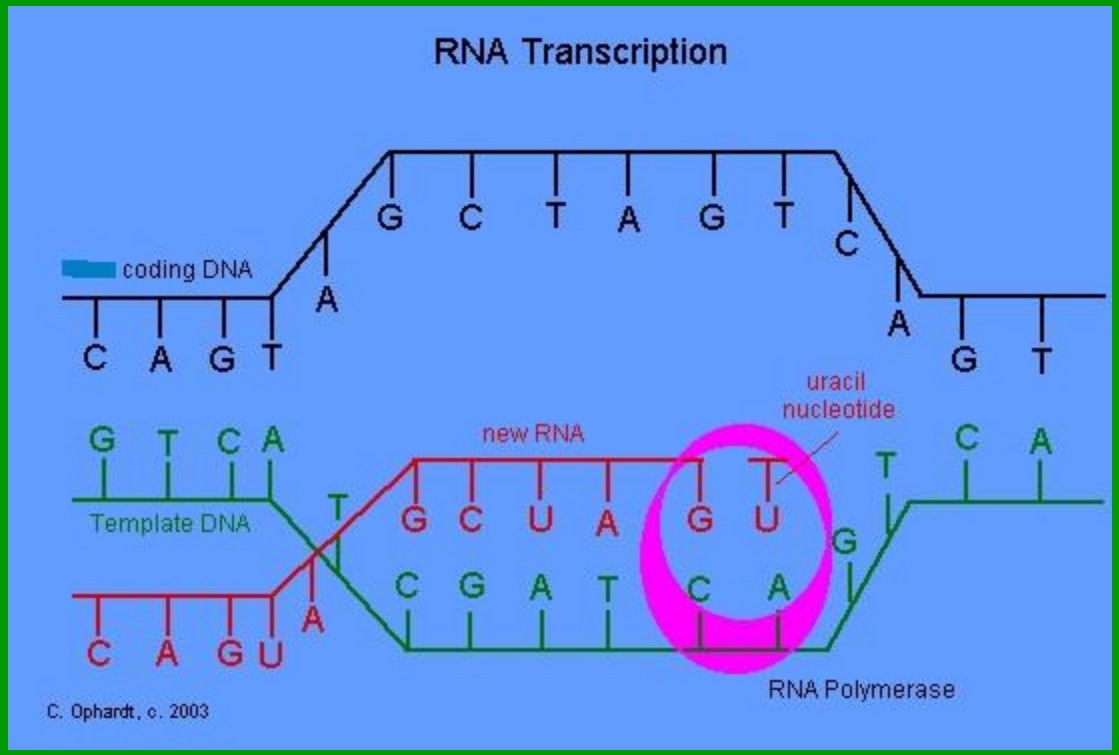
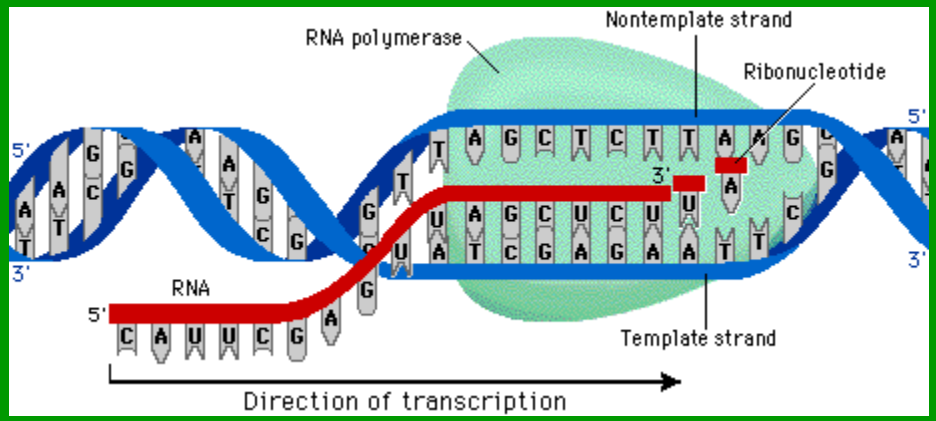
- Transcription occurs on one of the two strand s of DNA.





RNA biosynthesis requirements

- The process requires the following:
- (1)Transcription unit.

(2) Ribonucleotide triphosphates (ATP, GTP, CTP and UTP)

(3) RNA polymerase enzymes.

Transcription unit

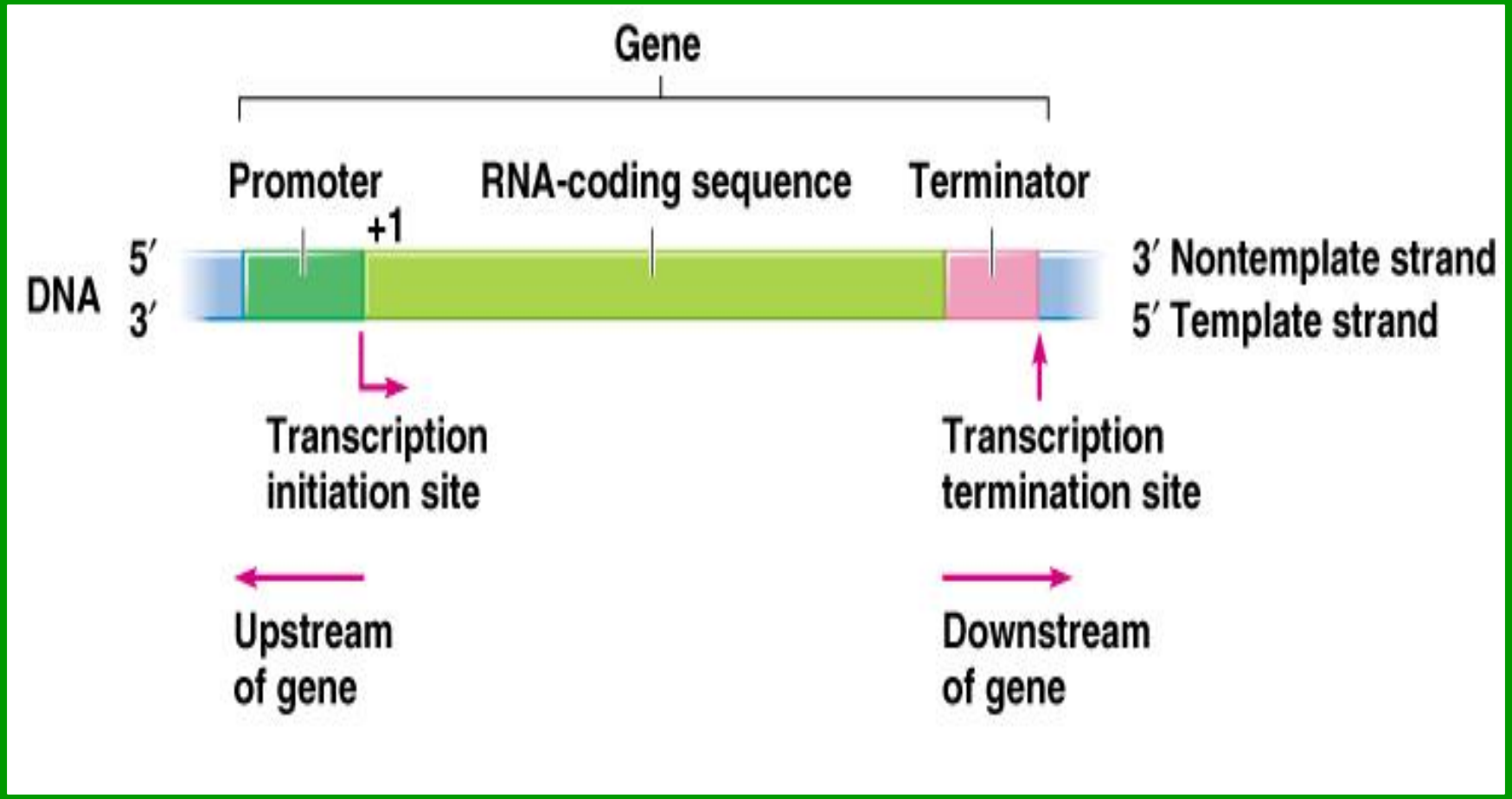
- It is the part of DNA which is the site of transcripti on.
- This unit includes:

(1) promoter region..

(2)Transcription region.

(3)Termination region

.(4) Enhancers.


Gene
Promoter
RNA-coding sequence
Terminator
+1
DNA
5′
3′
3′ Nontemplate strand
5′ Template strand
Transcription initiation site
Transcription termination site
Upstream of gene
Downstream of gene

Promoter region

- It is a certain sequence of bases located at the beginning of the transcription unit.
- It is important for initiation of transcription to occur.
- It can be recognized by RNA polymerase.

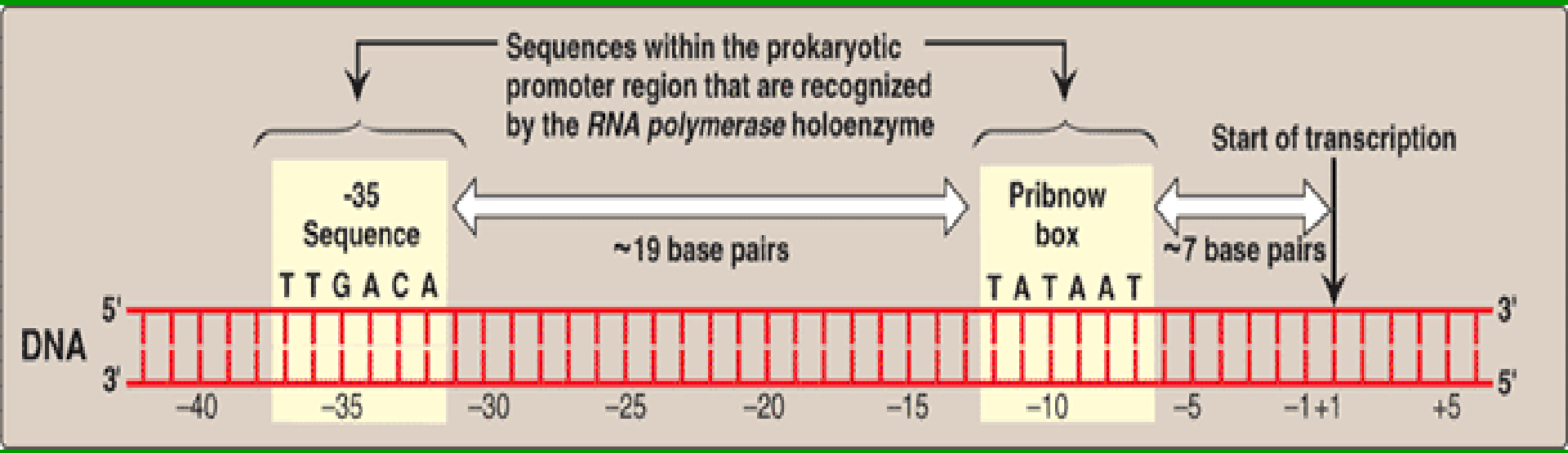
It is composed of:

(1)Prinbow box which is stretch of 6 nucleotides (TATA AT), locate about 10 bases to the left of the transcription initiation site.

(2)A second nucleotide stretch (TTGACA) that located a bout 35 bases to the left of the transcription initiation sid e.

(3) 19 bases (nucleotide) in between two stretches.

(4) Both prinbow box and (TTGACA) are regions that can be recognized by RNA polymerase.


Sequences within the prokaryotic promoter region that are recognized by the RNA polymerase holoenzyme
Start of transcription
-35 Sequence
T T G A C A
~19 base pairs
Pribnow box
T A T A A T
~7 base pairs
DNA
5'
3'
3'
5'
–40
–35
–30
–25
–20
–15
–10
–5
–1 +1
+5

Transcription &Termination regions

- Transcription region is the part of DNA that is to be transcribed into RNA molecule.
- Termination region is the region of DNA.. locat ed at the end of the transcribed DNA.

- Enhancers are specific segments present i n DNA which control and increase the rate of transcription in eukaryotes

Transcription factors

: TF Ⅱ D, TF Ⅱ A, TF Ⅱ B, TF Ⅱ F, TF Ⅱ E, TF Ⅱ H, TF Ⅱ J bind to the DNA around the TATA box and form a platform for RNA polymerase Ⅱ to bind. TF binding in a specific order .

RNA polymerase enzymes

- RNA polymerase (RNAP) catalyzes the polymerization of ribonucleotides into an RN A sequence that is complementary to the temp late strand of the gene.

- The RNA transcript has the same polarity (5′ to 3′) as the coding strand but contains U rat her than T .

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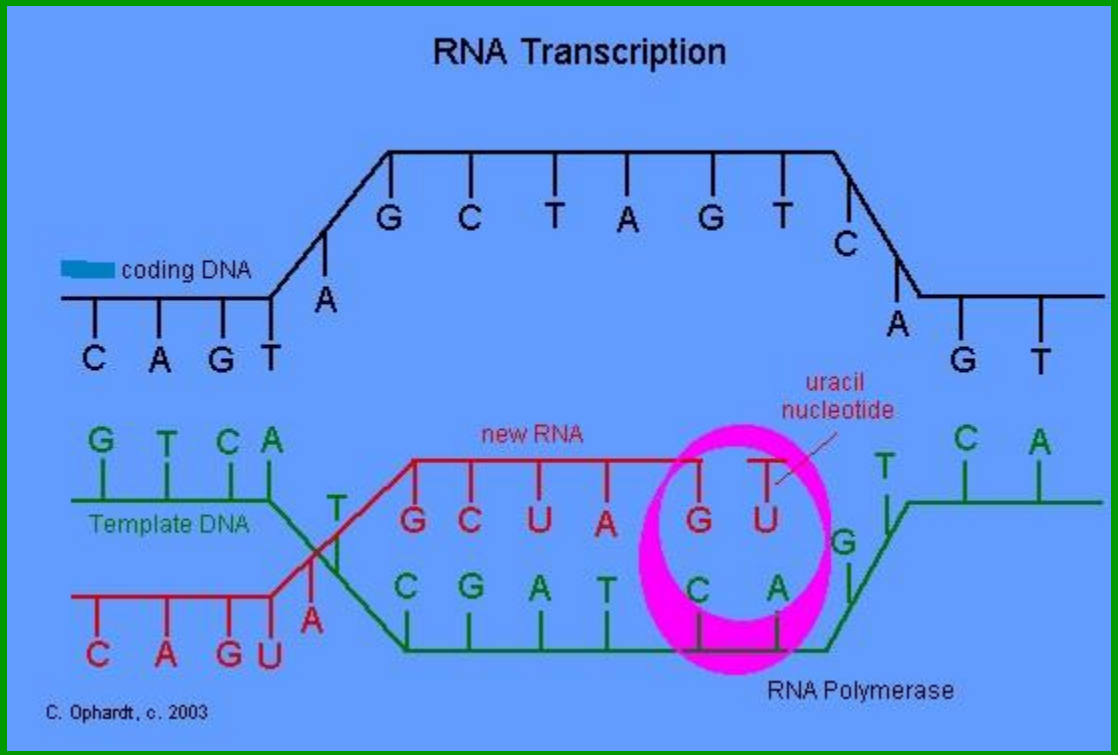
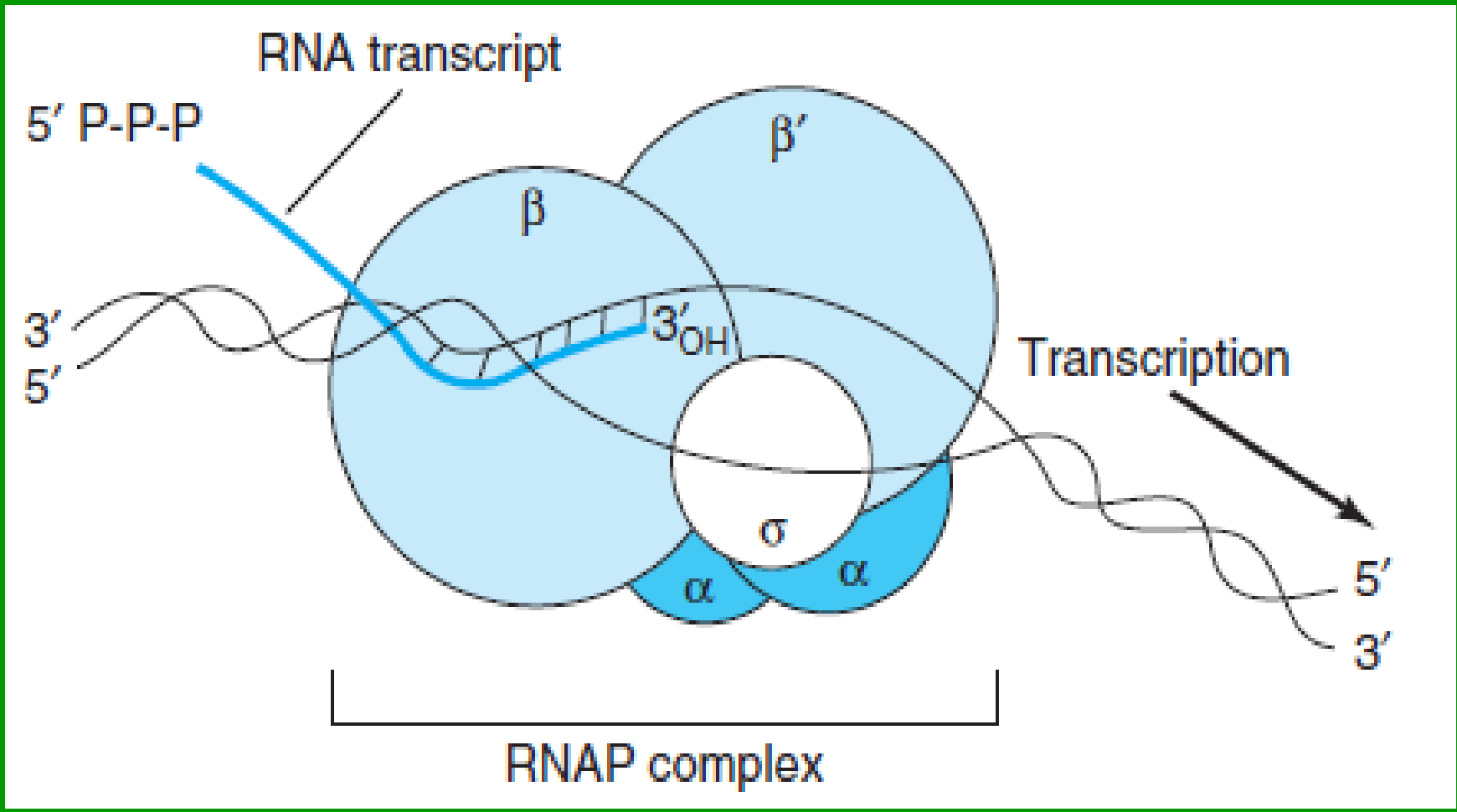

RNA Transcription
coding DNA
G C T A G T C
C A G T A A G T
uracil nucleotide
new RNA
G T C A
Template DNA
T
G C U A G U
C G A T C A
T C A
G
C A G U A
RNA Polymerase
C. Ophardt, c. 2003

Prokaryotic RNA polymerase enzymes

- The holo enzyme of the RNA polymerase consists of:
- (1) The core molecule of 4 sub unites, 2 α sub unites the other 2 are β′ & β subunits .

(2) sigma factor (σ) enables polymerase to recognize promoter region on DNA and helps the core enzyme to attach tightly to the promoter site.

RNA polymerase enzymes



Eukaryotic RNA polymerase enzymes

- There are three species of RNA polymerase enzyme in eukaryotic cells.
- (1) RNA polymerase I: Which synthesizes the large ribosomal RNAs in the nucleolus.
- (2) RNA Polymerase II: which synthesizes messenger RNA and recognizes the promoter region.
- (3) RNA polymerase III: which synthesizes the small RNAs including the tRNA and the small ribosomal RNA.

Steps of RNA Biosynthesis

- There are three steps of RNA biosynthesis:

(1) Initiation .

(2) Elongation.

(3) Termination.

Initiation

- RNA polymerase holo enzyme binds with the promo ter area :

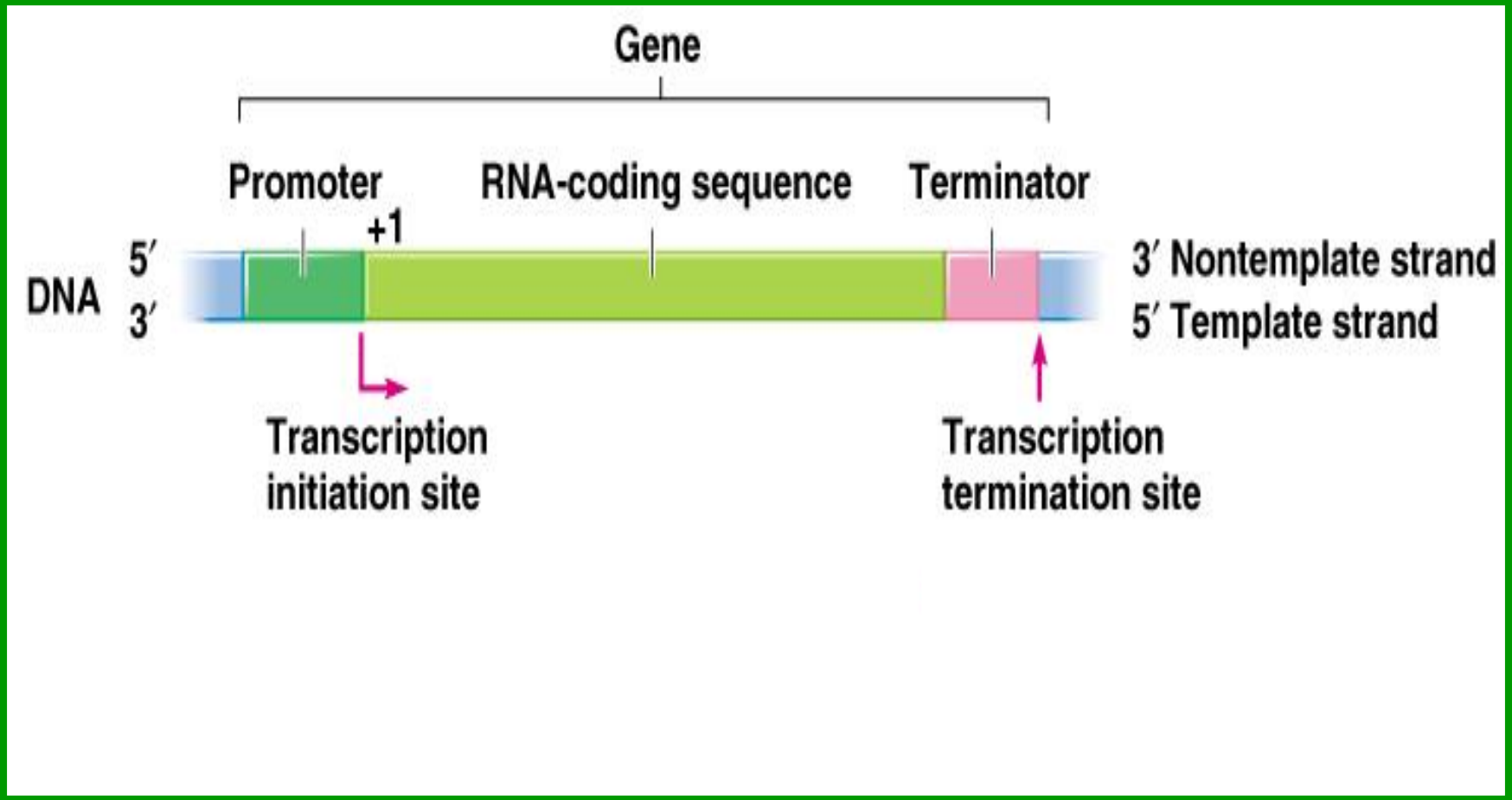
  (1) Sigma σ sub unite enables polymerase to recogn ize promoter region on DNA.

  (2) β′ subunit binds to the DNA template.

  (3) β subunit binds to the nucleotide substrates

- Binding of RNA polymerase to DNA template leads to local separations (un winding) of doub le helix into sense ands anti sense strands.
- Template strand = antisense (-) strand, nonte mplate strand = sense(+) strand, coding stran d
- The first nucleotides of RNA at the initiation site is always Purine.

initiation


Gene
Promoter
RNA-coding sequence
Terminator
+1
DNA
5′
3′
3′ Nontemplate strand
5′ Template strand
Transcription initiation site
Transcription termination site

Elongation

- At the sense strand, formation of RNA begins at 5′ e nd by the core enzyme with release of sigma factor.
- Then the elongation of RNA occurs from 5′ to 3′ en d anti parallel to the template.
- The nucleotide building blocks are 5′ ribonucleoside triphosphate (ATP,GTP,CTP and UTP).
- They are inserted in the RNA molecules according t o the pairing rule i.e. A.U, G.C, T.A and C.G.

- Pyrophosphate (PPi) is released when each nuc leotide is added to growing chain .

  GTP GMP +PPi.

- RNA polymerase forms bond between the 3′ OH of one ribose sugar and 5′ OH of next ribo se .

- The process of the elongation of RNA chain c ontinues until a terminating region is reached.

- Initiation: σsubunit of RNA holoenzyme rec ognize and bind -35 box→
- a closed (DNA remains as a double helix) promoter complex is formed →
- DNA double helix partially dissociate at th e -10 box to give an open promoter compl ex →
- σsubunit dissociate leaving core enzyme →
- the first two nucleotides bind to DNA and fi rst phosphodiester bond forms

The RNA polymerase moves along the DNA molecule melting and unwinding the double helix as it progresses.

RNA molecule is growing by adding ribonucl eo-tide that is paired with DNA template.

Termination

Termination involves release of transcript and the core enzyme and initiate another round of transcription.

Termination

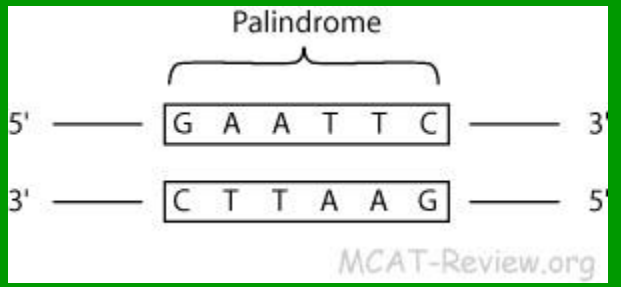
- Termination region on DNA can be recognized by:

(a) rho (p) factor which may be required for
the release of both RNA strand and RNA polymerase

(b)) RNA polymerase enzyme itself
(rho independent termination).

- Termination results from one of the following processes:

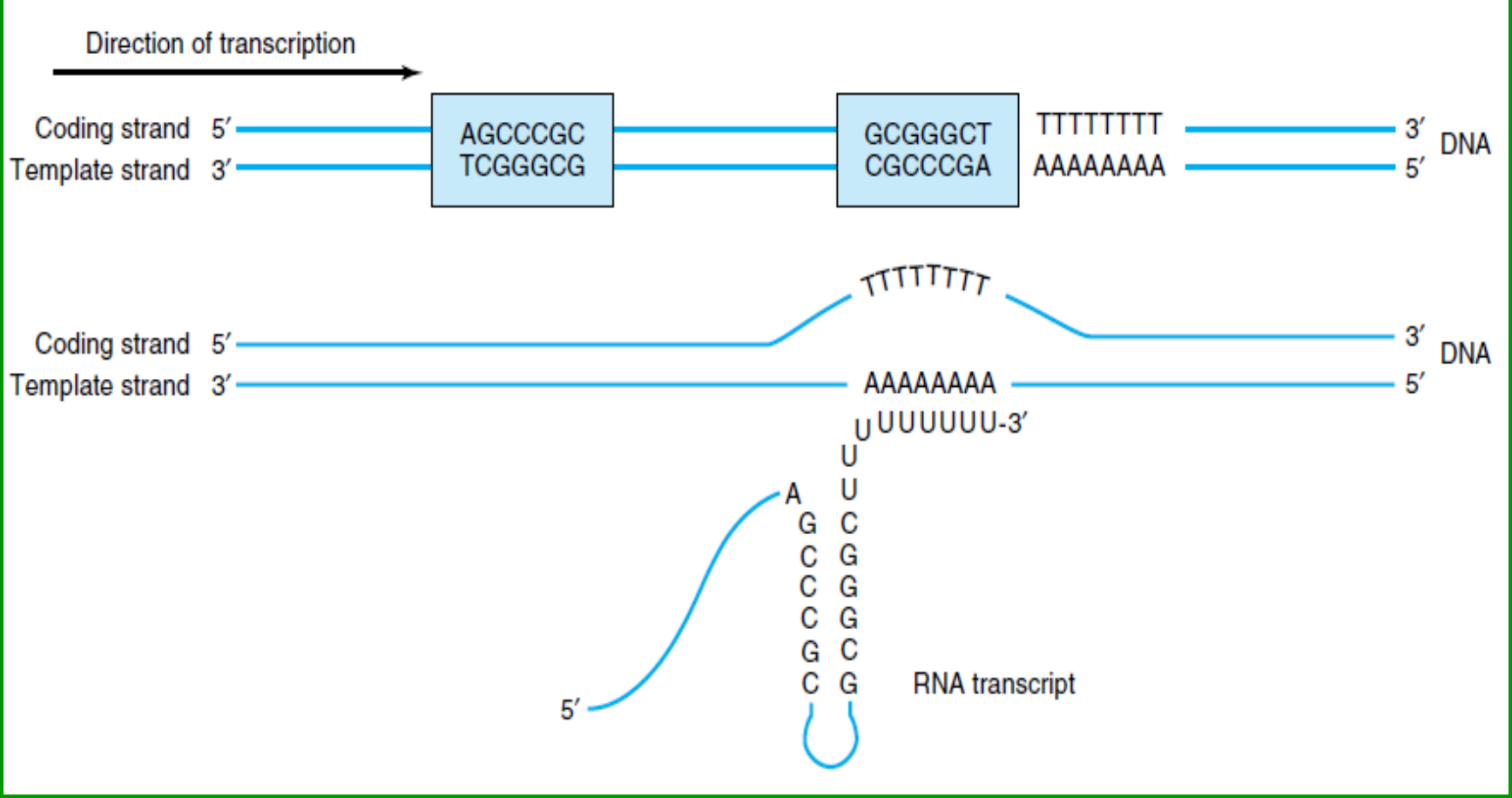
(a) Binding of rho factor to polymerase enzyme.
(b) Slowing down of RNA polymerase at the
termination site (palindrome)



Pallindromes

termination occurs at pallindromes, such tha t a stem-loop structure of RNA (Fig.) will for m after transcription.

Termination

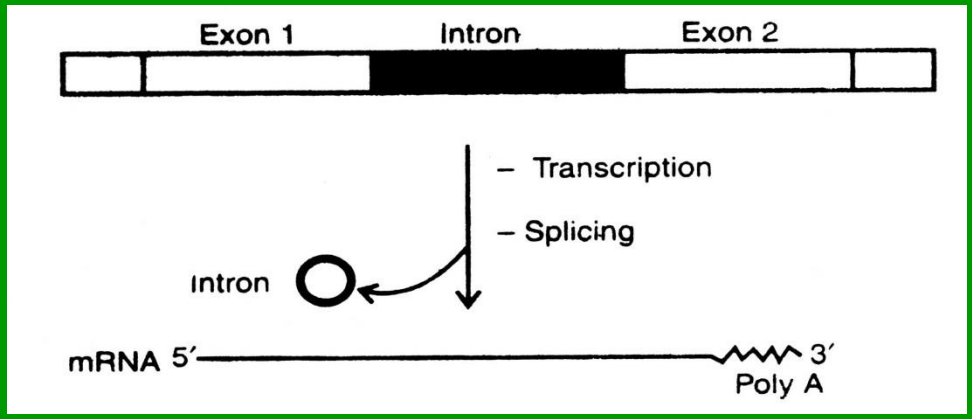


Transcription in eukaryotes

Similar to prokaryotes, but are more comple x in initiation, termination does not involve st em-loop structure and transcription carried o ut by three RNA polymerases.

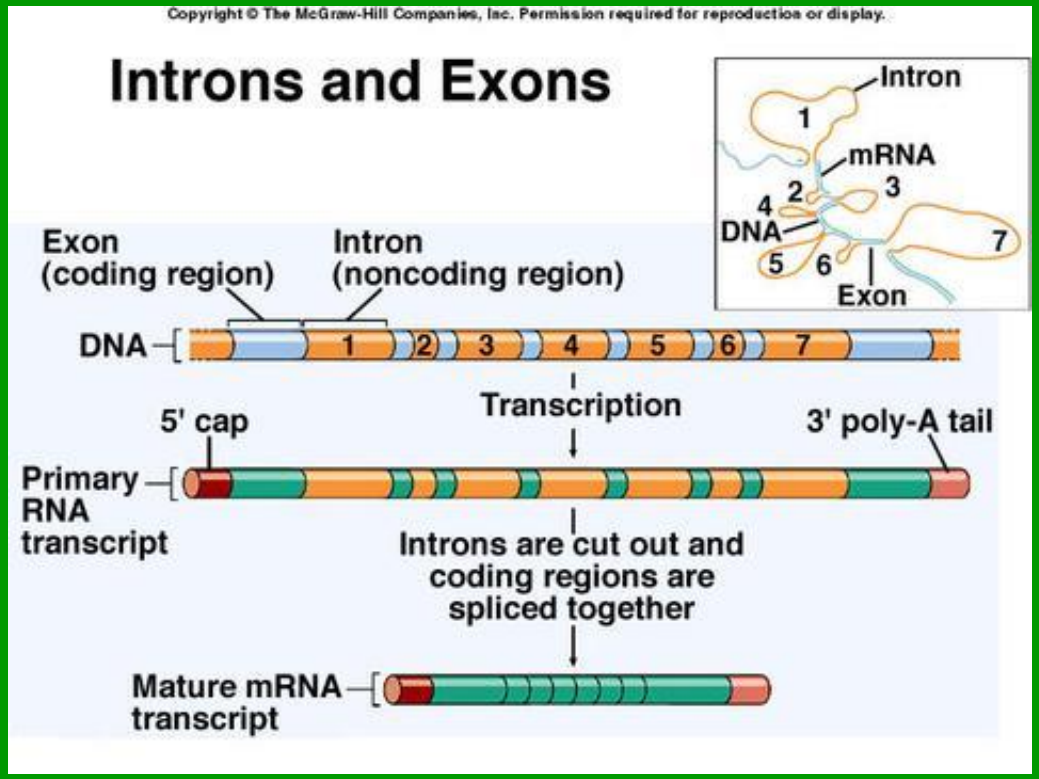
Processing of RNA

- Eukaryote pre-mRNAs often have intervening introns that must be removed during RNA processing.
- intron = non-coding DNA sequences between exons in a gene.
- exon = expressed DNA sequences in a gene, code for amino acids.

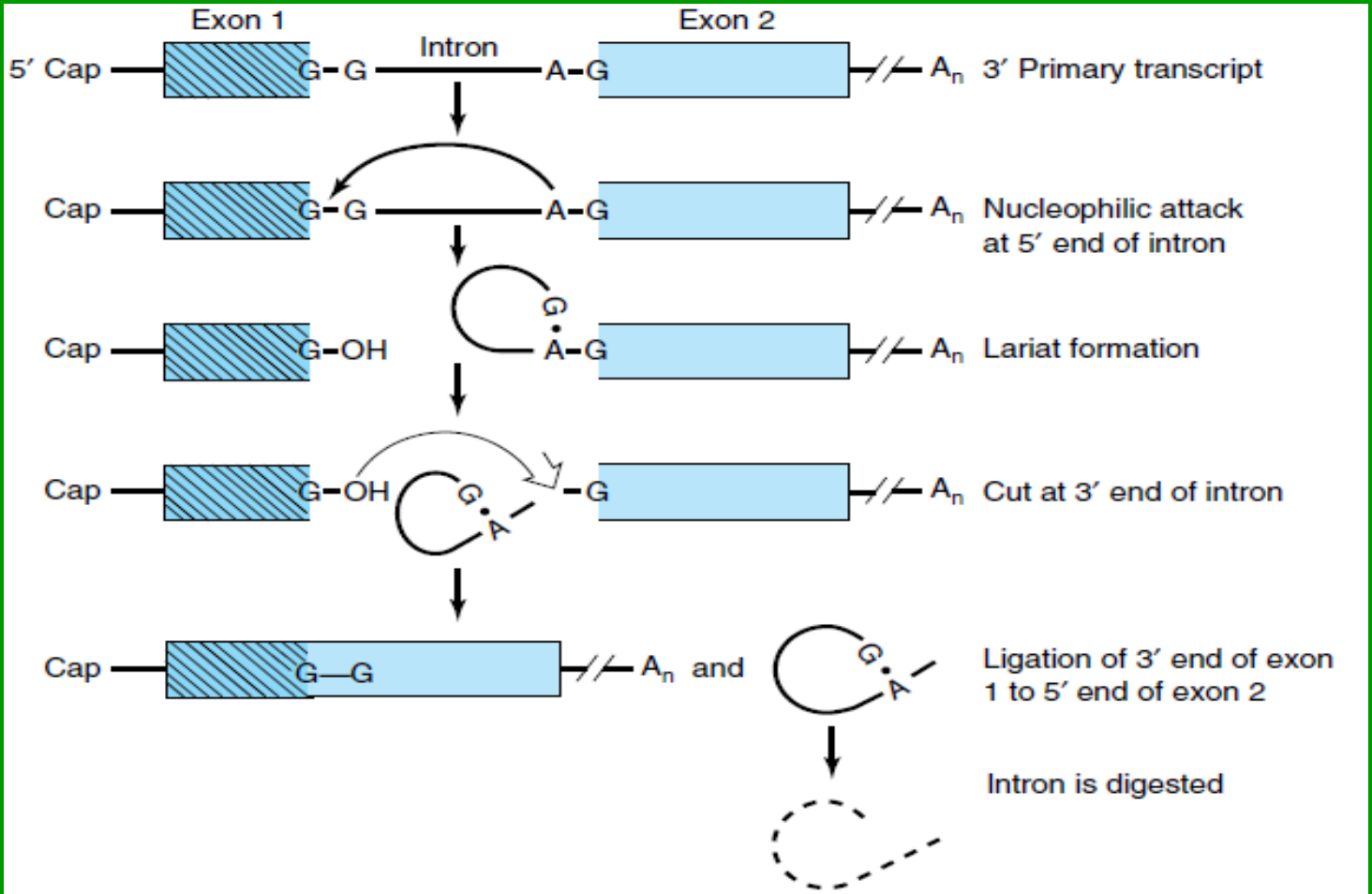


mRNA splicing of exons and removal of introns

- Splicing is mediated by spliceosomes, complexes of small nuclear RNAs (snRNAs) and proteins, that cleave the intron at the 3' end and join the exons.
- Introns are degraded by the cell.



The processing of the primary transcript t o mRNA



Summary

- RNA is synthesized from a DNA template by the enzyme RNA polymerase.
- There are three distinct nuclear DNA-dependent RNA poly merases in mammals:

  RNA polymerases I, II, and III. these control the transcripti onal function.
- Transcription exhibits three phases: initiation, elongation, a nd termination.
- During the process of RNA , pre-mRNAs often have interve ning introns that must be removed and exons that are joined .