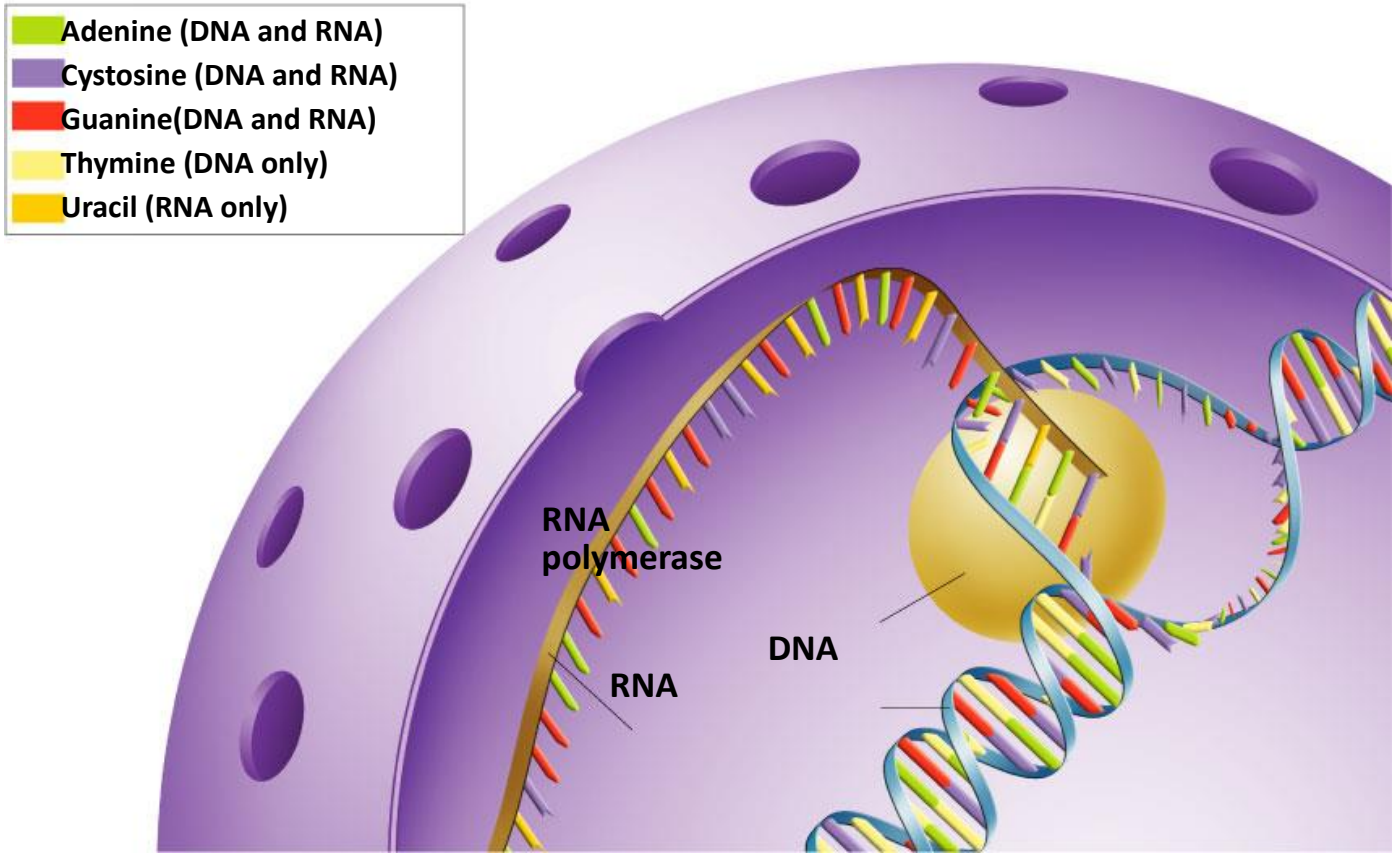
Transcription



Transcription

- DNA is used as a template to make RNA
- RNA is made by RNA polymerase, which follows the base-pairing rule (A-U; G-C).
- The process of transcription occurs in the nucleus (in eukaryotes)
- RNA polymerase separates the two strands of DNA
- Only one of the two DNA strands is used as a template
- The other DNA strand is called the coding strand.
- Three types of RNAs are transcribed from DNA which participate in protein synthesis: mRNA, tRNA and rRNA.

Similarity between replication and transcription

- Both processes use DNA as the template.
- Phosphodiester bonds are formed in both cases.
- Both synthesis directions are from 5′ to 3′.

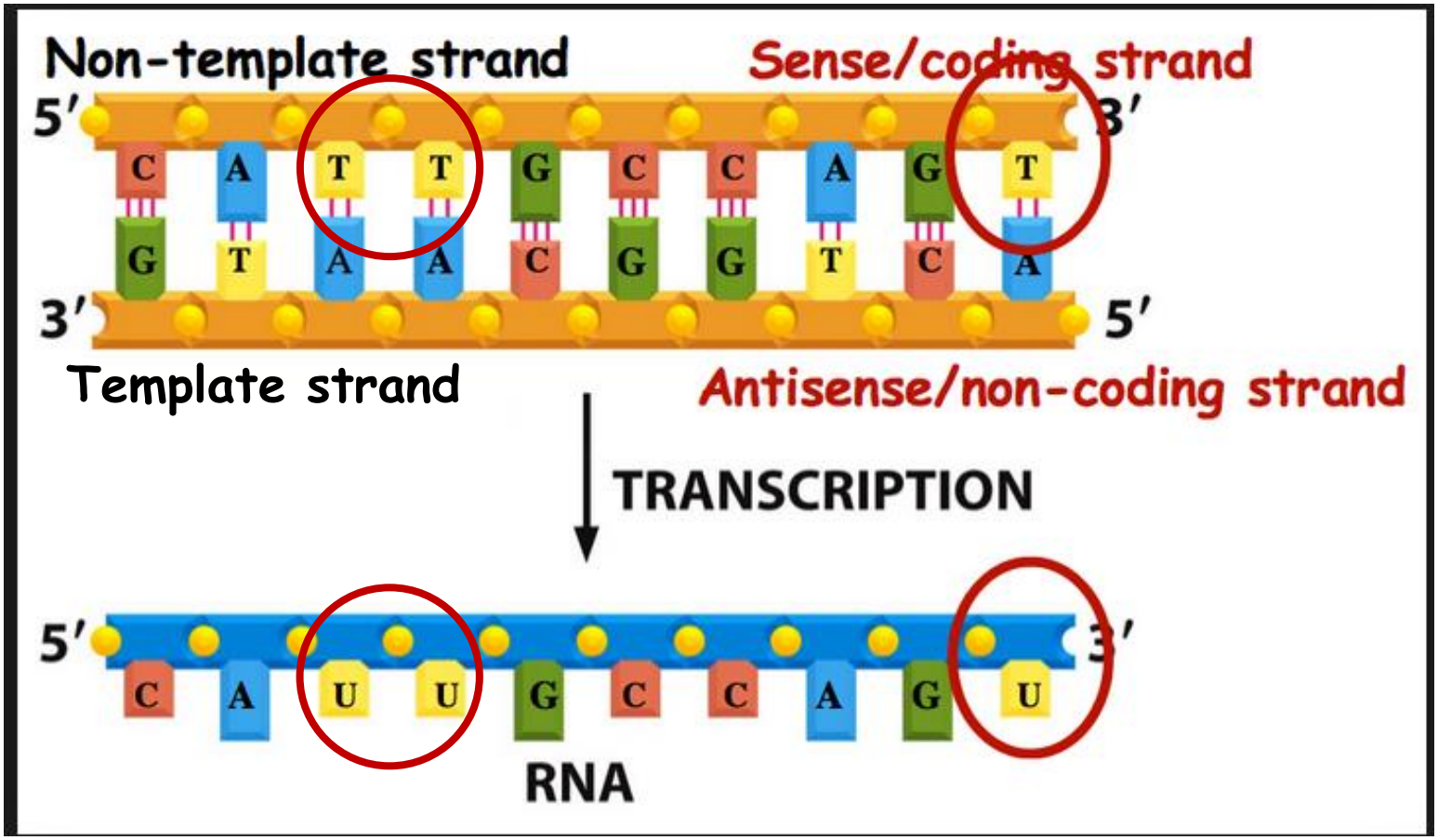
Differences between replication and transcription

	replication	transcription
template	Both Strands	Only one Strand
substrate	dNTP	NTP
primer	yes	no
Enzyme	DNA polymerase	RNA polymerase
product	dsDNA	ssRNA
base pair	A-T, G-C	A-U, T-A, G-C

§ 1.1 Template

The template strand is the strand from which the RNA is actually transcribed. It is also termed as **antisense strand**.

The coding strand is the strand whose base sequence specifies the amino acid sequence of the encoded protein. Therefore, it is also called as **sense strand**.

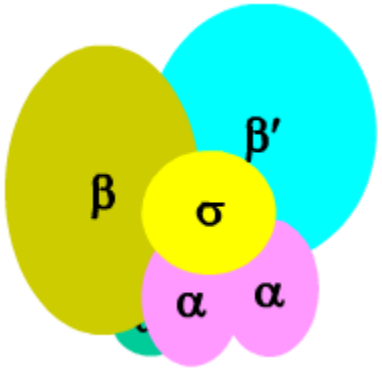

Non-template strand
Sense/coding strand
5′
3′
C A T T G C C A G T
G T A A C G G T C A
3′
5′
Template strand
Antisense/non-coding strand
TRANSCRIPTION
5′
3′
C A U U G C C A G U
RNA

§ 1.2 RNA Polymerase

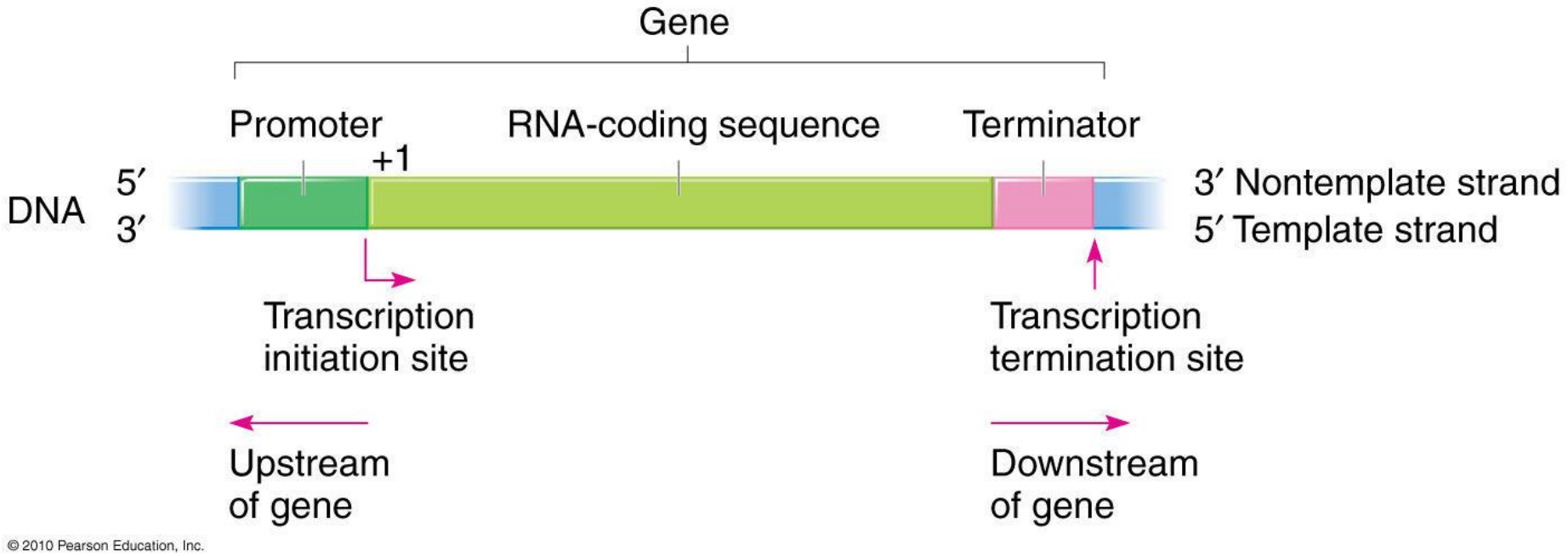
- **The enzyme responsible for the RNA synthesis is DNA-dependent RNA polymerase.**
  - **The prokaryotic RNA polymerase is a multiple-subunit protein of ~480kD.**
  - **Eukaryotic systems have three kinds of RNA polymerases, each of which is a multiple-subunit protein and responsible for transcription of different RNAs.**

RNA-pol of *E. Coli*

subunit	MW	function
α	36512	Determine the DNA to be transcribed
β	150618	Catalyze polymerization
β′	155613	Bind & open DNA template
σ	70263	Recognize the promoter for synthesis initiation



The Process of Transcription



Only a segment of the DNA strand will be used to create each RNA.

- **These segments are called genes.**
- **Each gene starts with a promoter.**
  - **The RNA polymerase binds to the promoter to start building an RNA strand.**
- **Each gene ends with a terminator sequence.**
  - **The RNA polymerase will stop transcribing at the terminator sequence.**

§ 2.1 Transcription of Prokaryotes

- **Initiation phase: RNA-pol recognizes the promoter and starts the transcription.**
- **Elongation phase: the RNA strand is continuously growing.**
- **Termination phase: the RNA-pol stops synthesis and the nascent RNA is separated from the DNA template.**

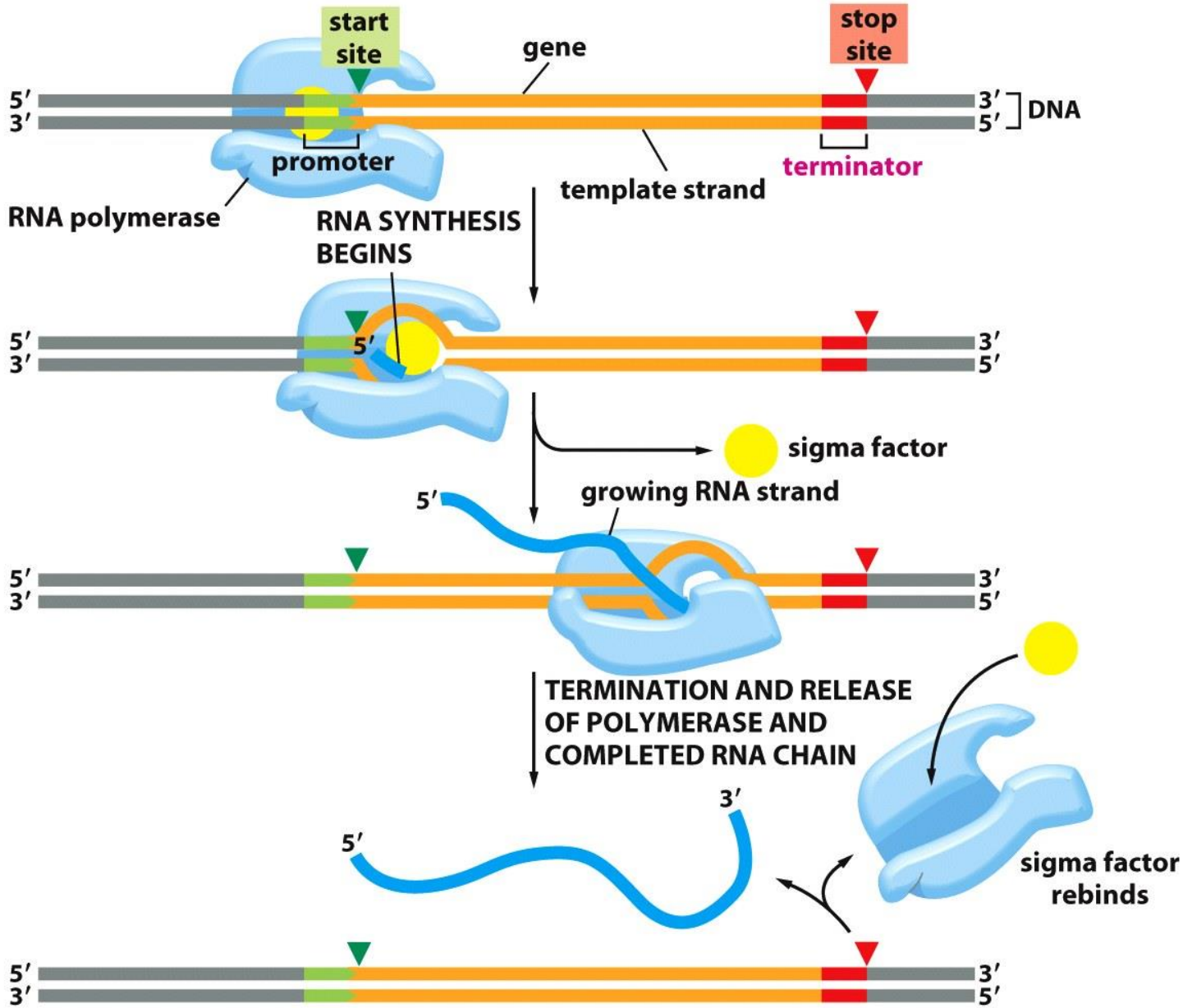
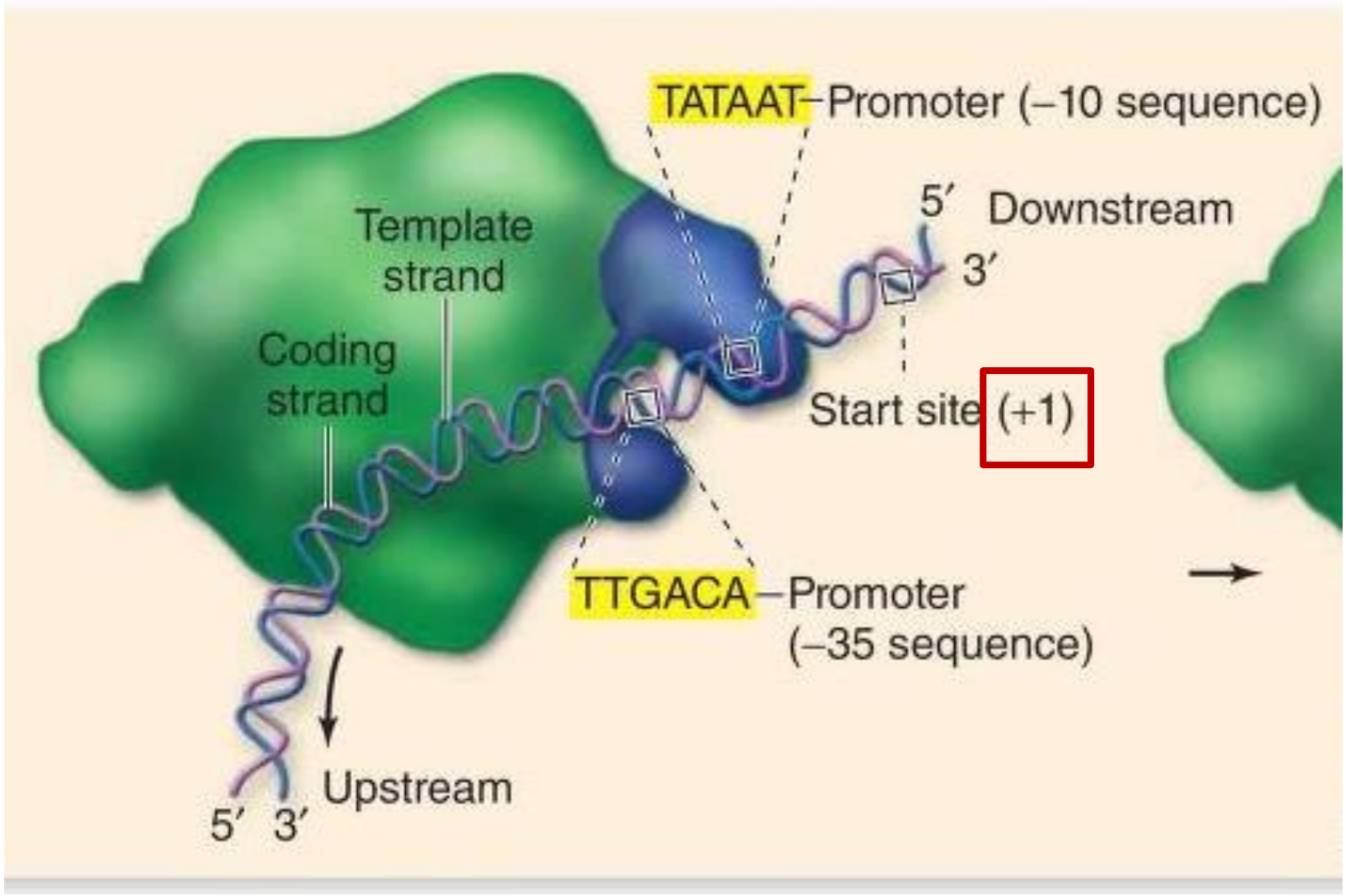
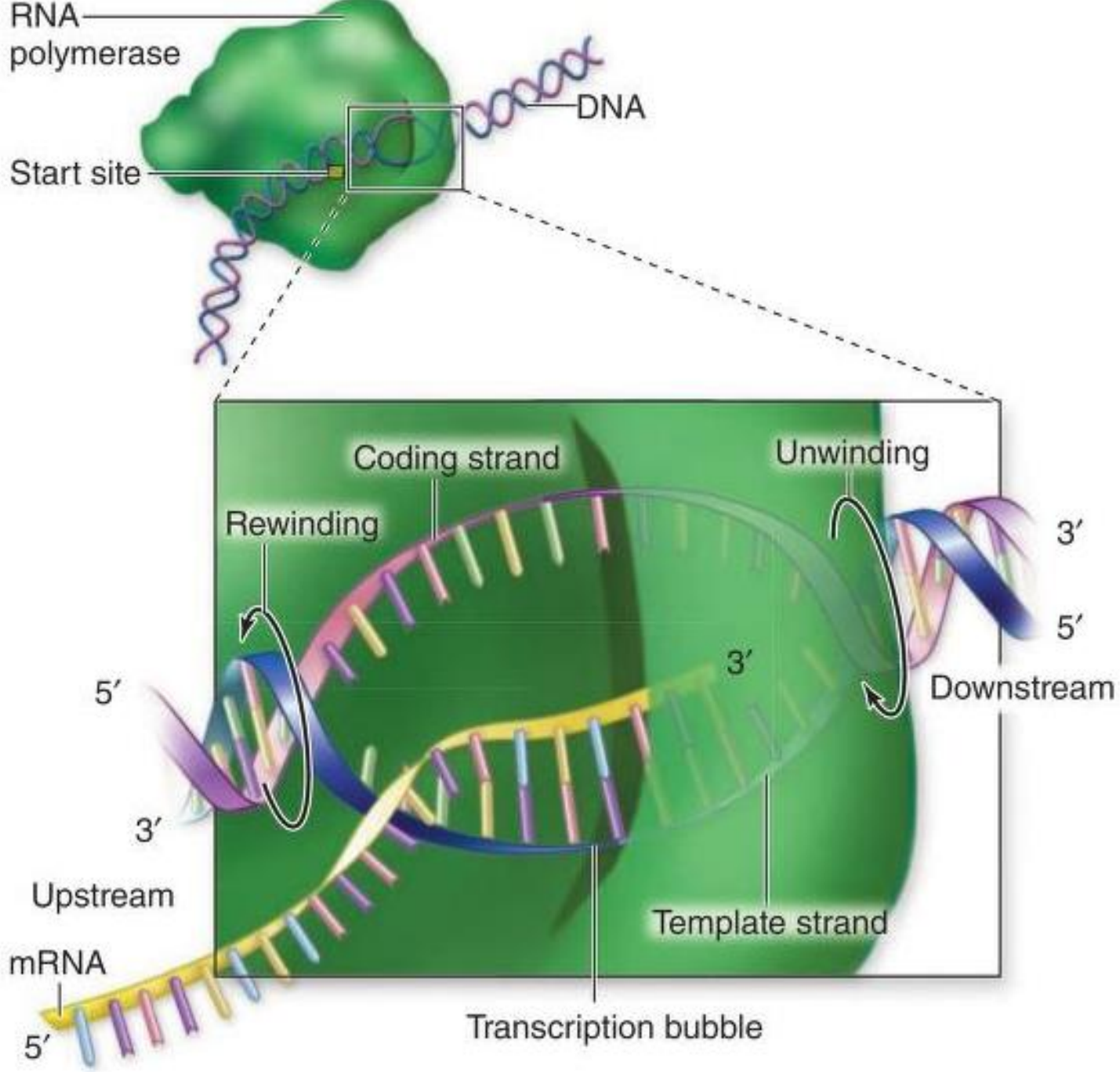


Figure 7-9 Essential Cell Biology 3/e (© Garland Science 2010)

Initiation of transcription starts when sigma factor recognizes the promoter.

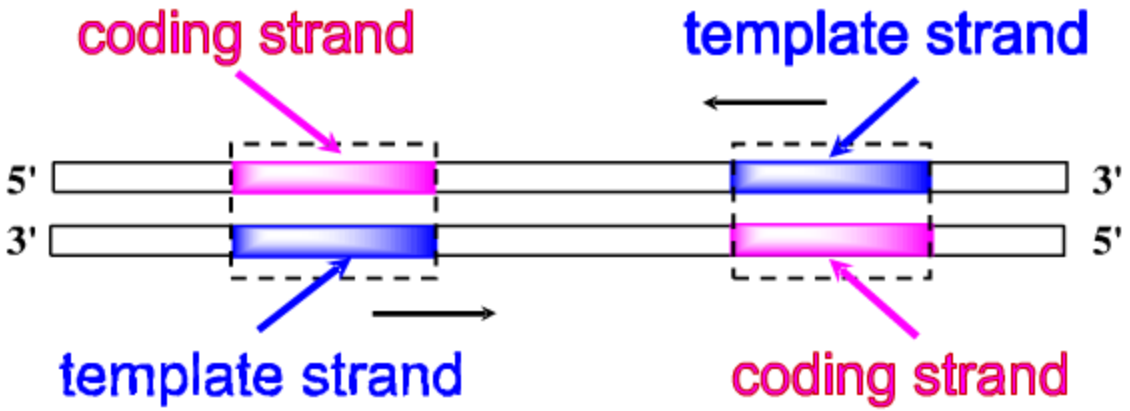



RNA polymerase
DNA
Start site
Coding strand
Unwinding
Rewinding
3′
5′
Downstream
5′
3′
3′
Upstream
Template strand
mRNA
5′
Transcription bubble

Asymmetric transcription

- Only the template strand is used for the transcription, but the coding strand is not.
- Both strands can be used as the templates.
- The transcription direction on different strands is opposite.
- This feature is referred to as the asymmetric transcription.

Depending on the position of the promoter, each strand could be used as a template.



1

5’ ATGGCCTAATGCAATCTG 3’

3’ TACCGGATTACGTTAGAC 5’

2

Considering strand 1 is the template strand and strand 2 is the coding strand, write the mRNA sequence (show the direction of synthesis with an arrow). Draw the locations of the promoter and the Terminator

T

5’ ATGGCCTAATGCAATCTG 3’

3’ UACCGGAUUACGUUAGAC 5’

P

1

5’ ATGGCCTAATGCAATCTG 3’

3’ TACCGGATTACGTTAGAC 5’

2

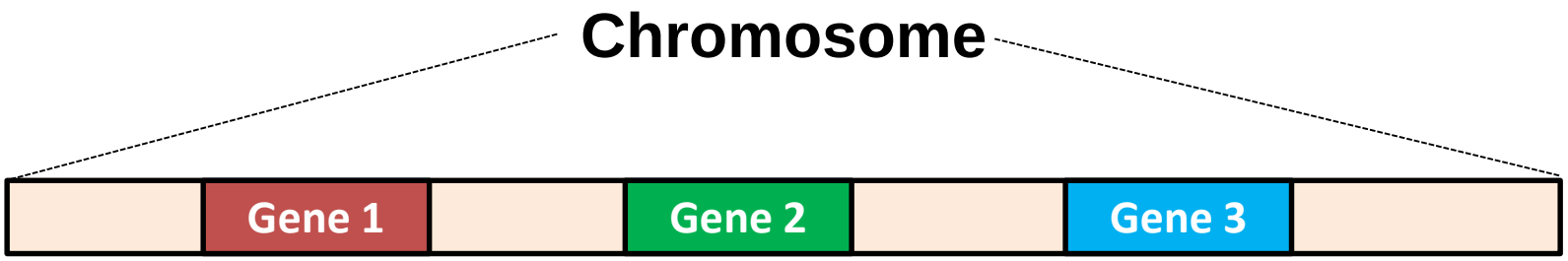
Considering strand 2 is the template strand and strand 1 is the coding strand, write the mRNA sequence (show the direction of synthesis with an arrow). Draw the locations of the promoter and the Terminator

P

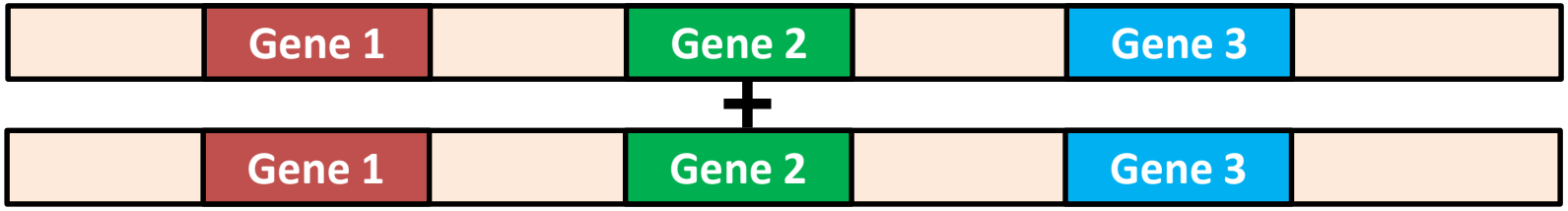
3’ TACCGGATTACGTTAGAC 5’

5’ AUGGCCUAAUGCAAUCUG 3’

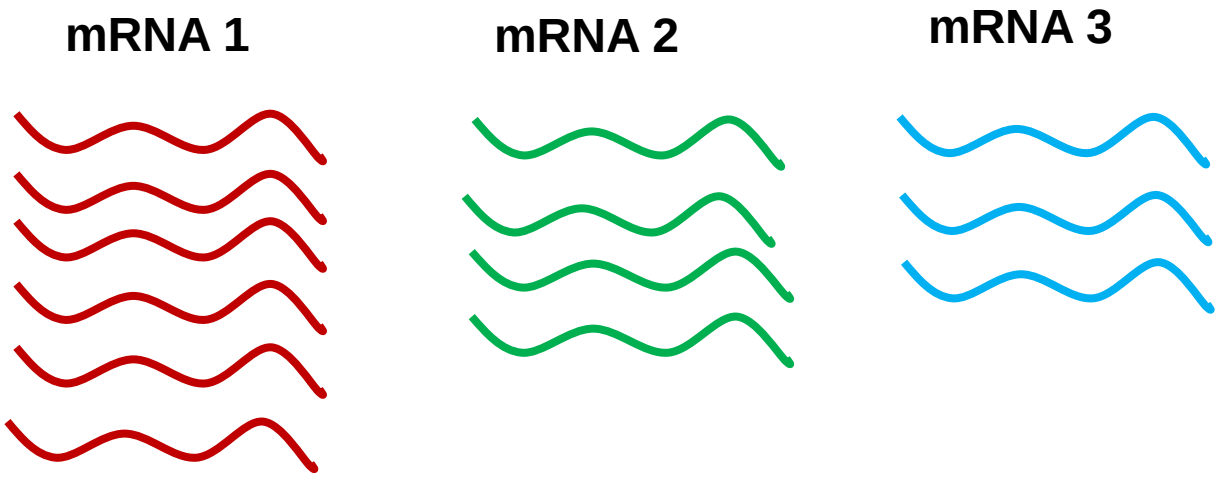
T



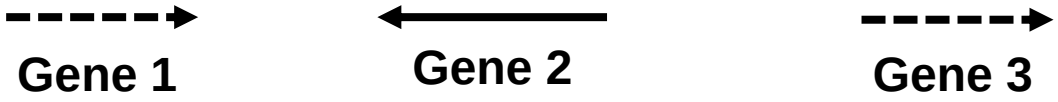
Entire DNA in chromosome is copied (only once per cell cycle) due to DNA Replication

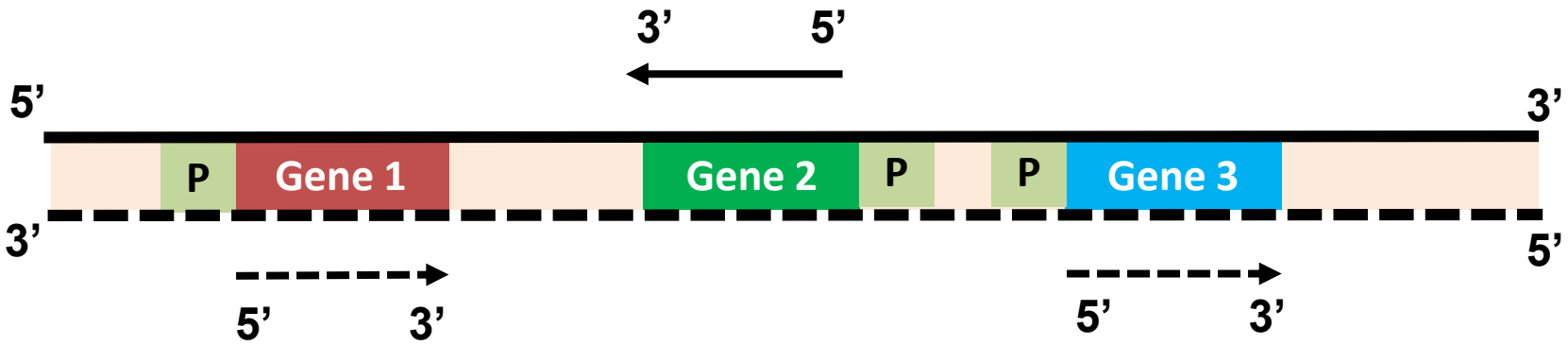


Only Genes are transcribed and they can make many copies

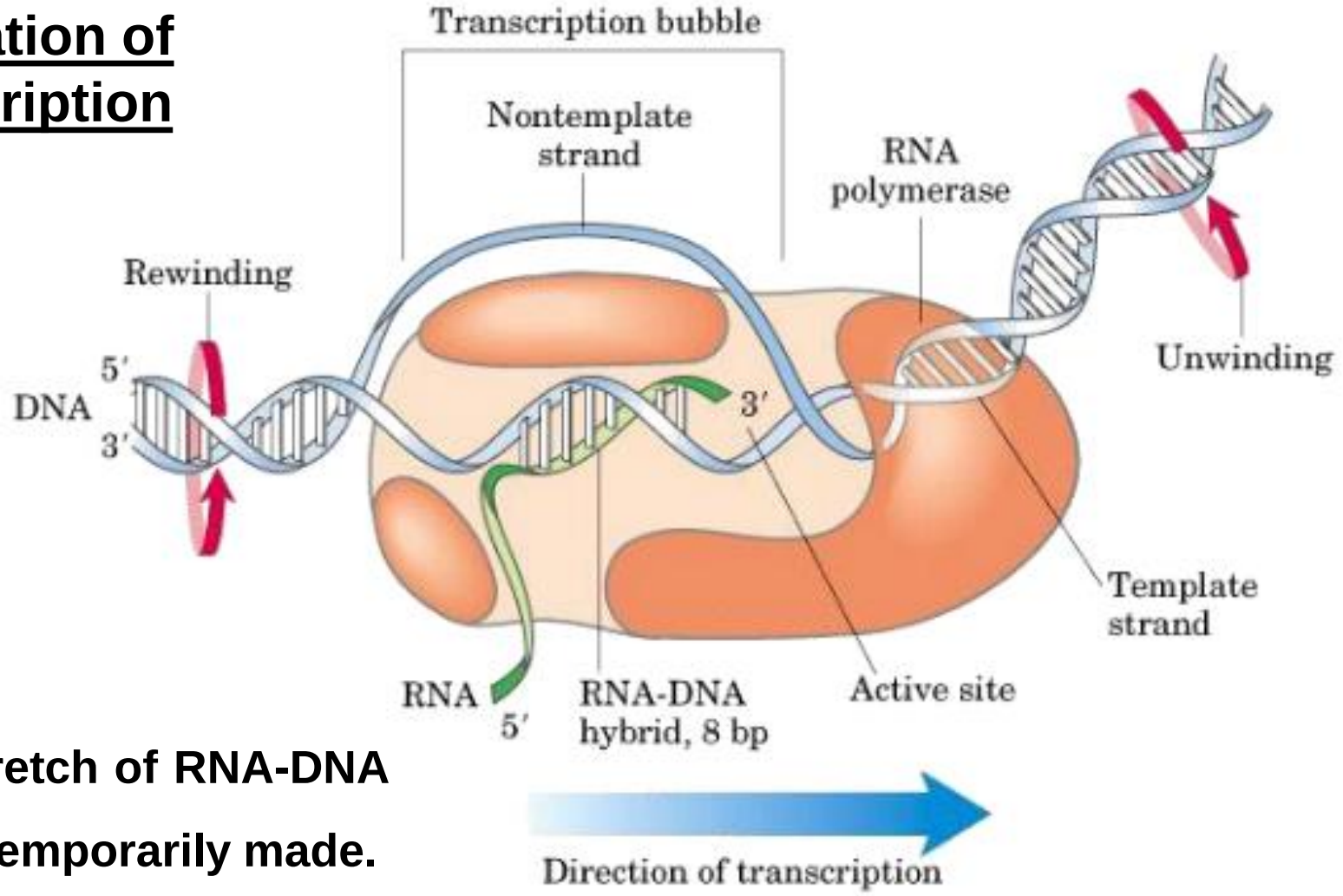


Draw the mRNAs result from transcription of Gene 1, 2 and 3 either with a solid or a broken arrow with proper direction. (Solid arrow, if you consider strand 1 is the template, broken arrow, if you consider strand 2 is the template)





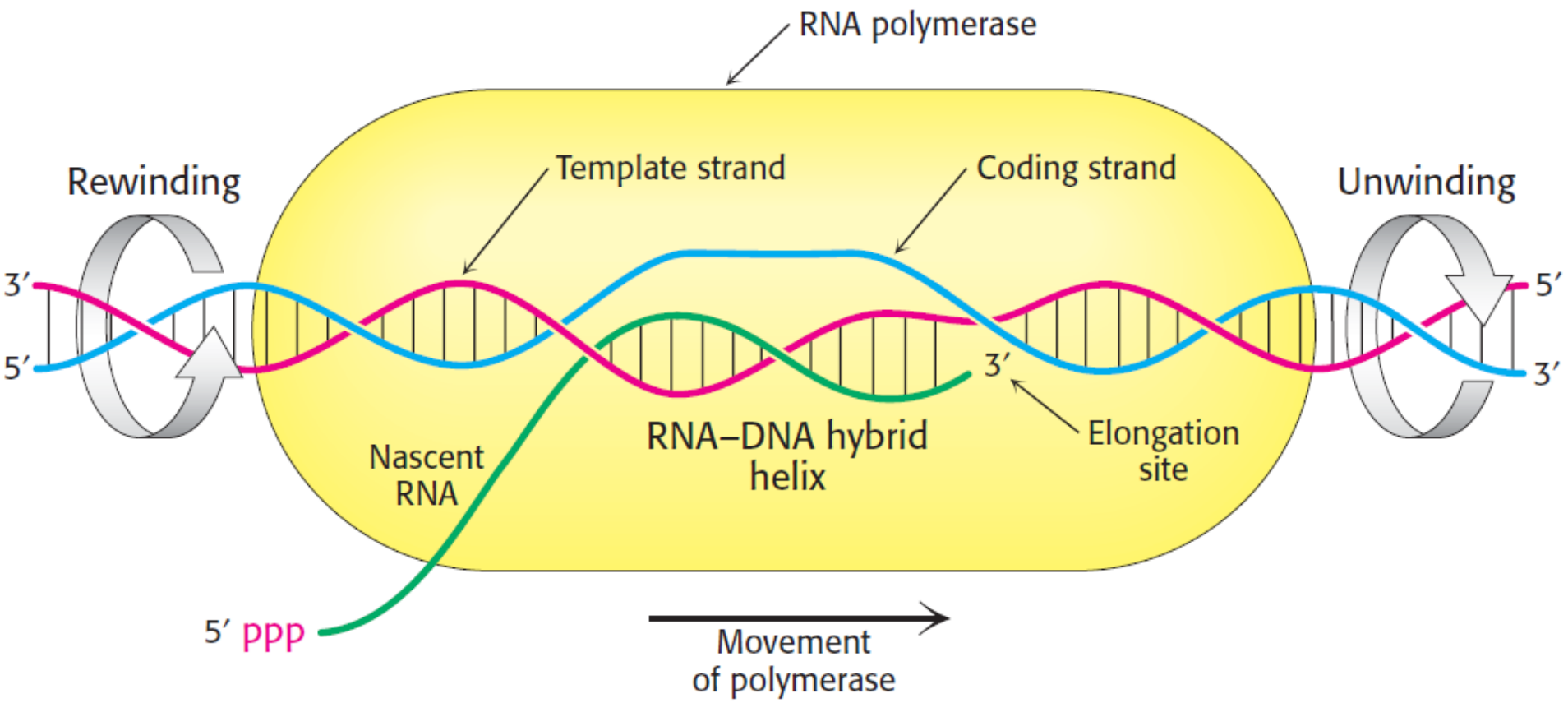
Elongation of Transcription



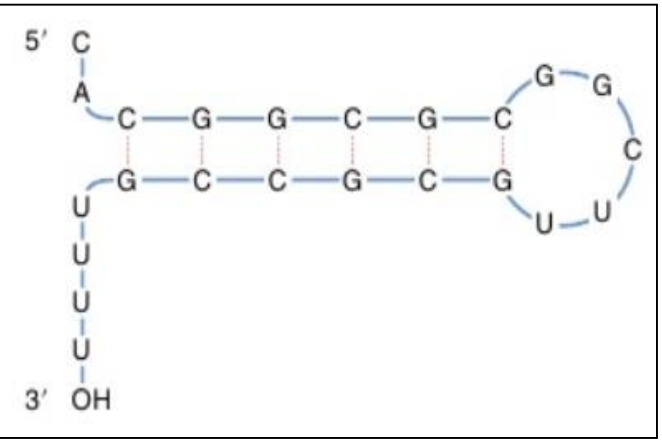
- A short stretch of RNA-DNA duplex is temporarily made.
- Sigma factor leaves
- Transcription bubble moves at a rate of approximately 50 nt/second

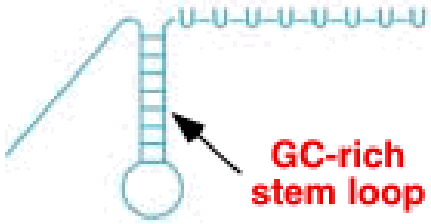
- After the transcription bubble passes, the transcribed DNA rewind.
- RNA polymerases have no proofreading capacity

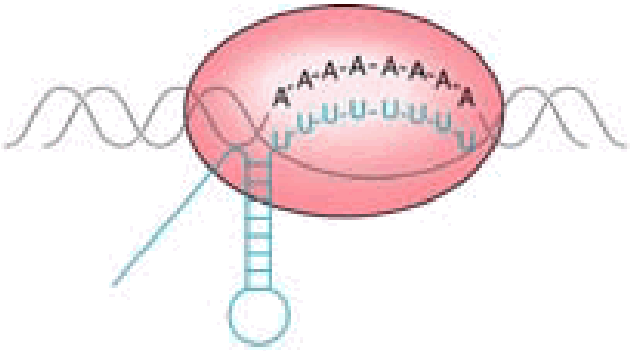
Mechanism for Elongation of transcription

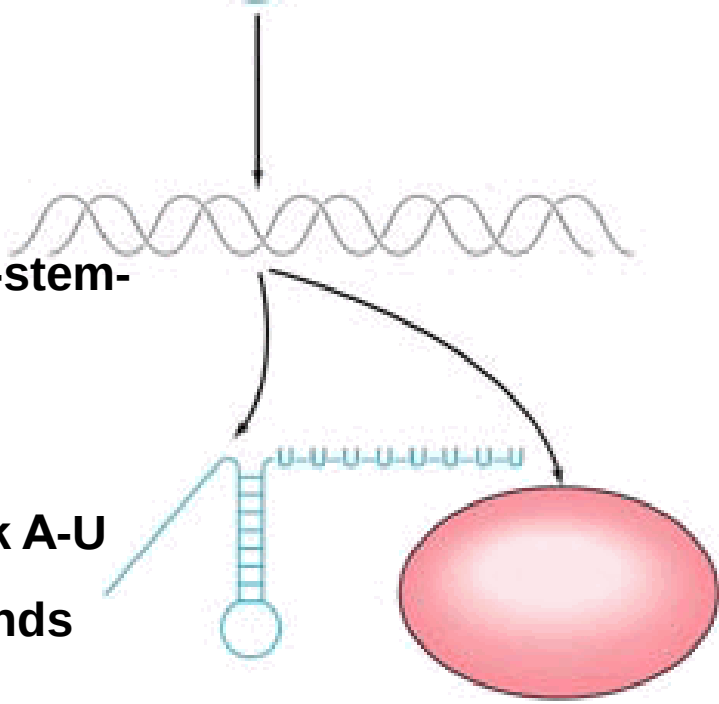


Termination of Transcription









- **RNA transcript at the termination site forms a GC-stem-loop, followed by a series of U ribonucleotides.**
- **The hairpin causes the polymerase to pause immediately after it's synthesis, whereas the weak A-U base-pairs can not hold the RNA-DNA hybrid strands during the long pause.**
- **The RNA strand dissociates from the DNA, polymerase releases the DNA and transcription stops.**

RNA Editing

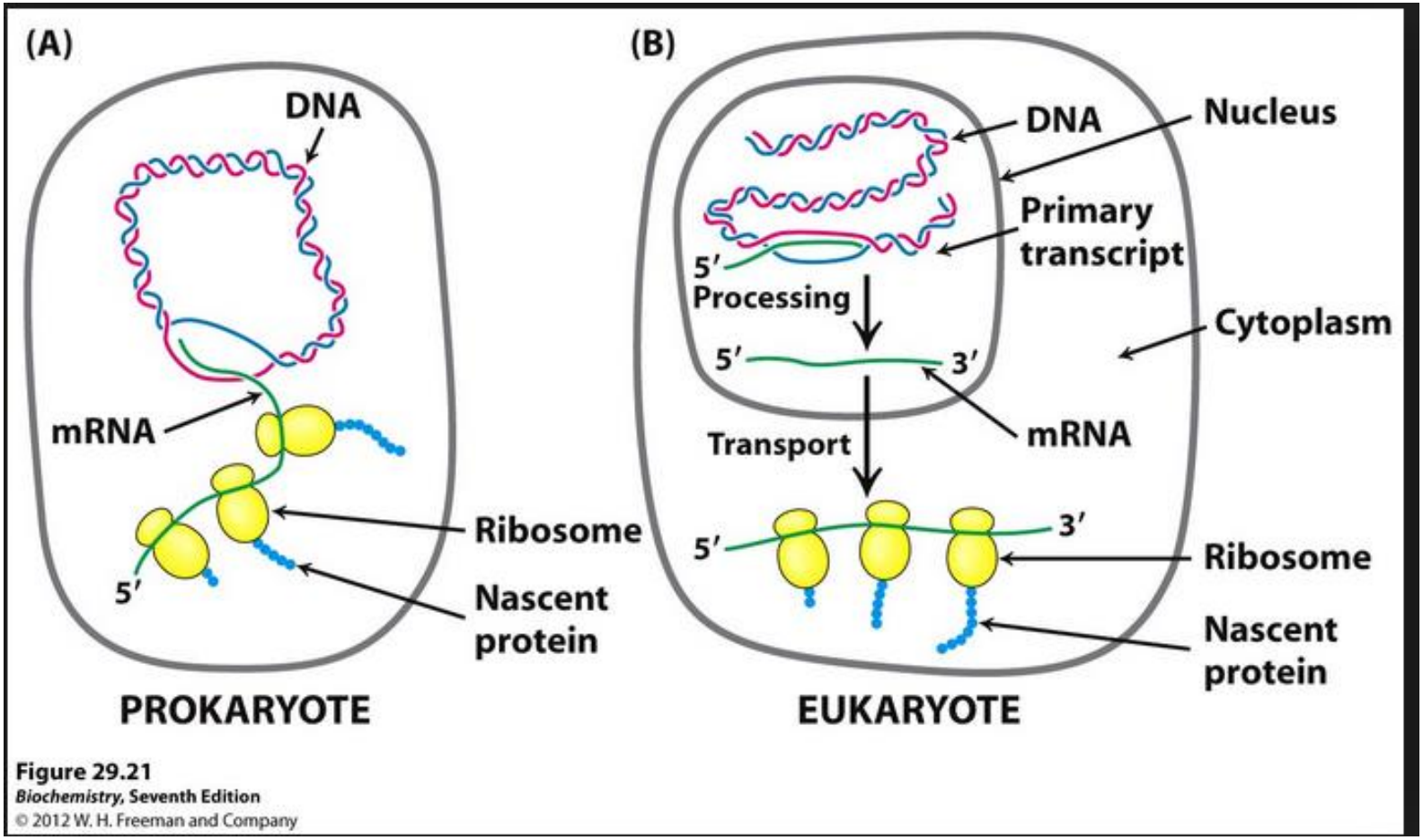


Figure 29.21
Biochemistry, Seventh Edition

Modification of the Primary Transcript in Eukaryotes

- **Primary transcripts are larger than matured mRNA by several fold.**

- **Modification includes**
  - **Capping at the 5′- end**
  - **Tailing at the 3′- end**
  - **mRNA splicing**
  - **RNA edition**

5’ Capping

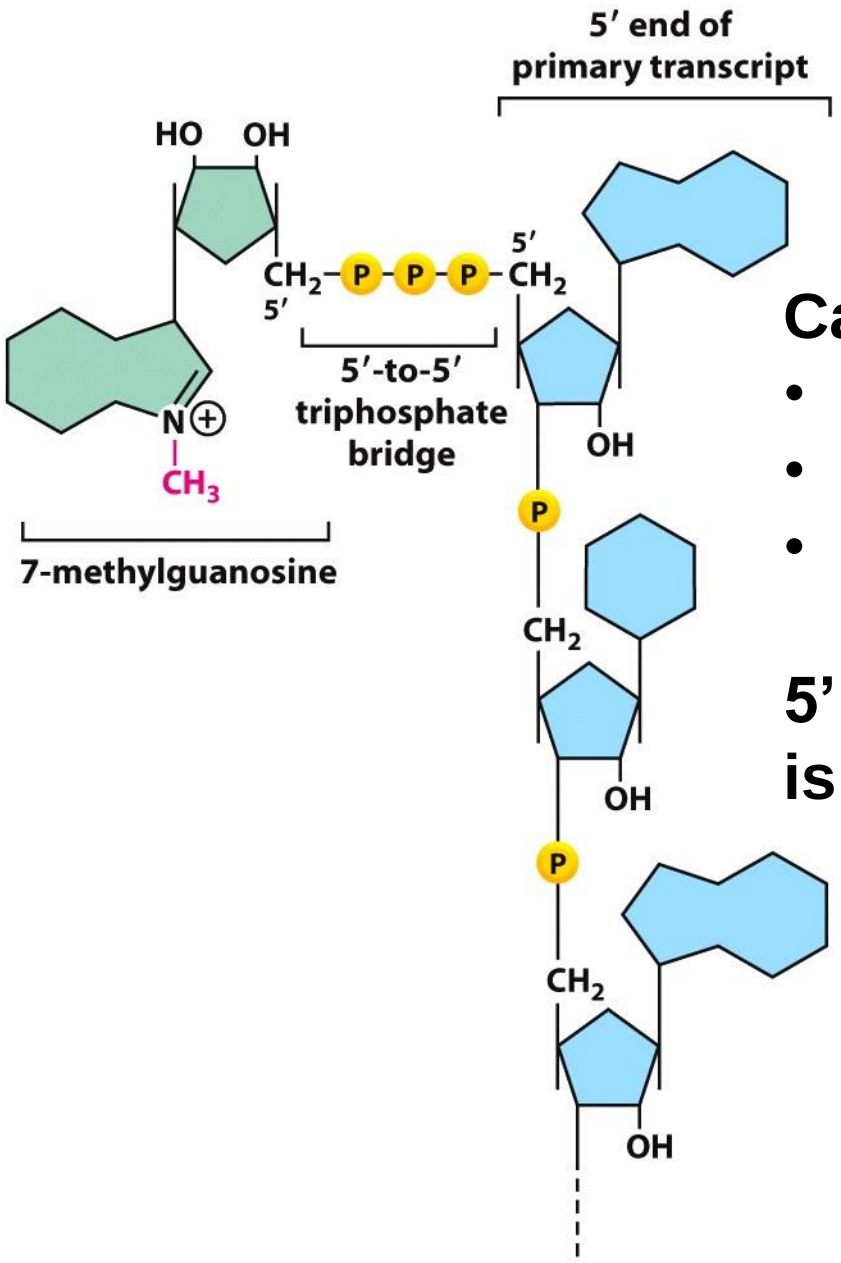


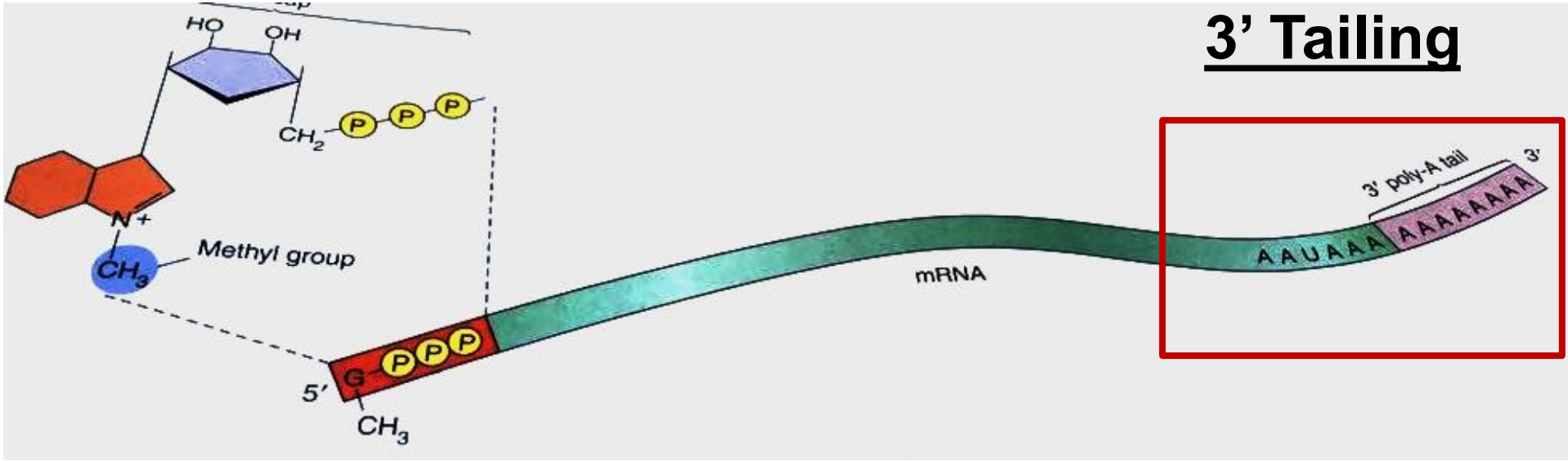
Figure 7-16b Essential Cell Biology 3/e (© Garland Science 2010)

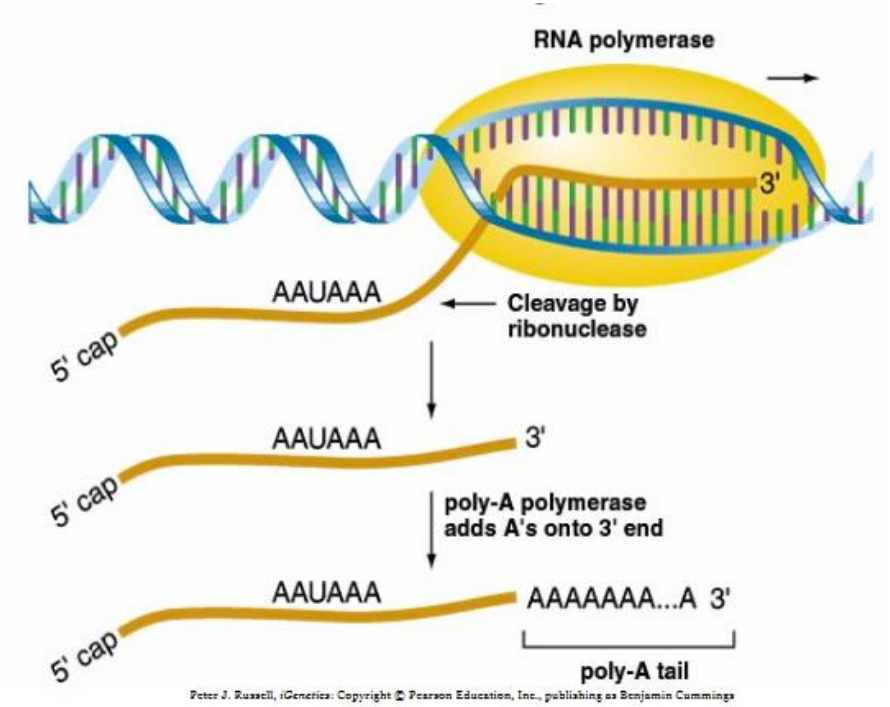
Capping helps in:

- **protection of mRNA**
- **Transport from the nucleus**
- **Initiation of translation**

5’ cap is added while transcription is still on progress.

3’ Tailing





A series of Adenines at the 3’ end of the RNA transcript is added.

Poly-A tail protects the mRNA from degradation and helps in transport

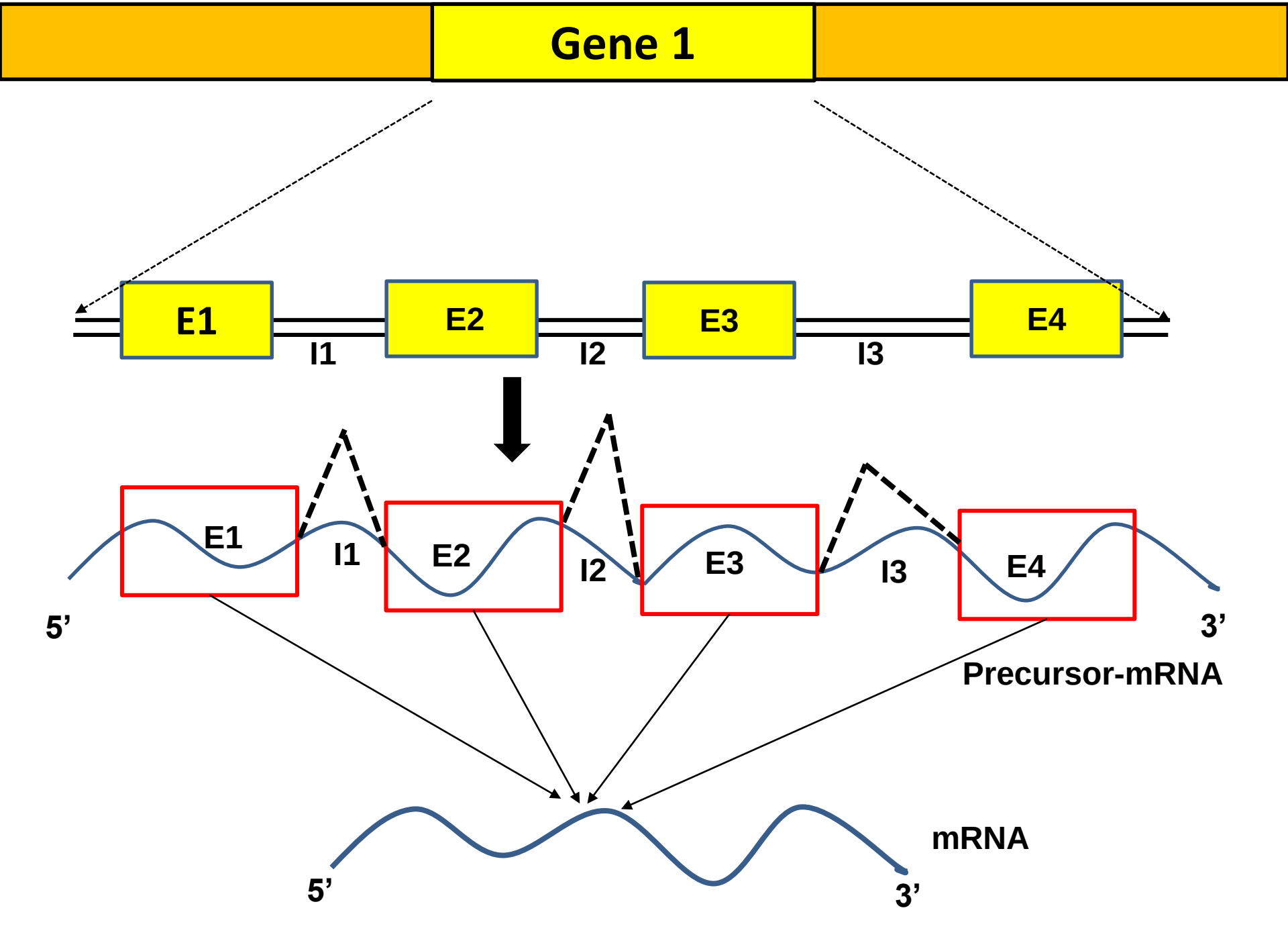
RNA Splicing

- Eukaryotic genes contain introns and exons. The introns do not code for proteins. Exons code for proteins.

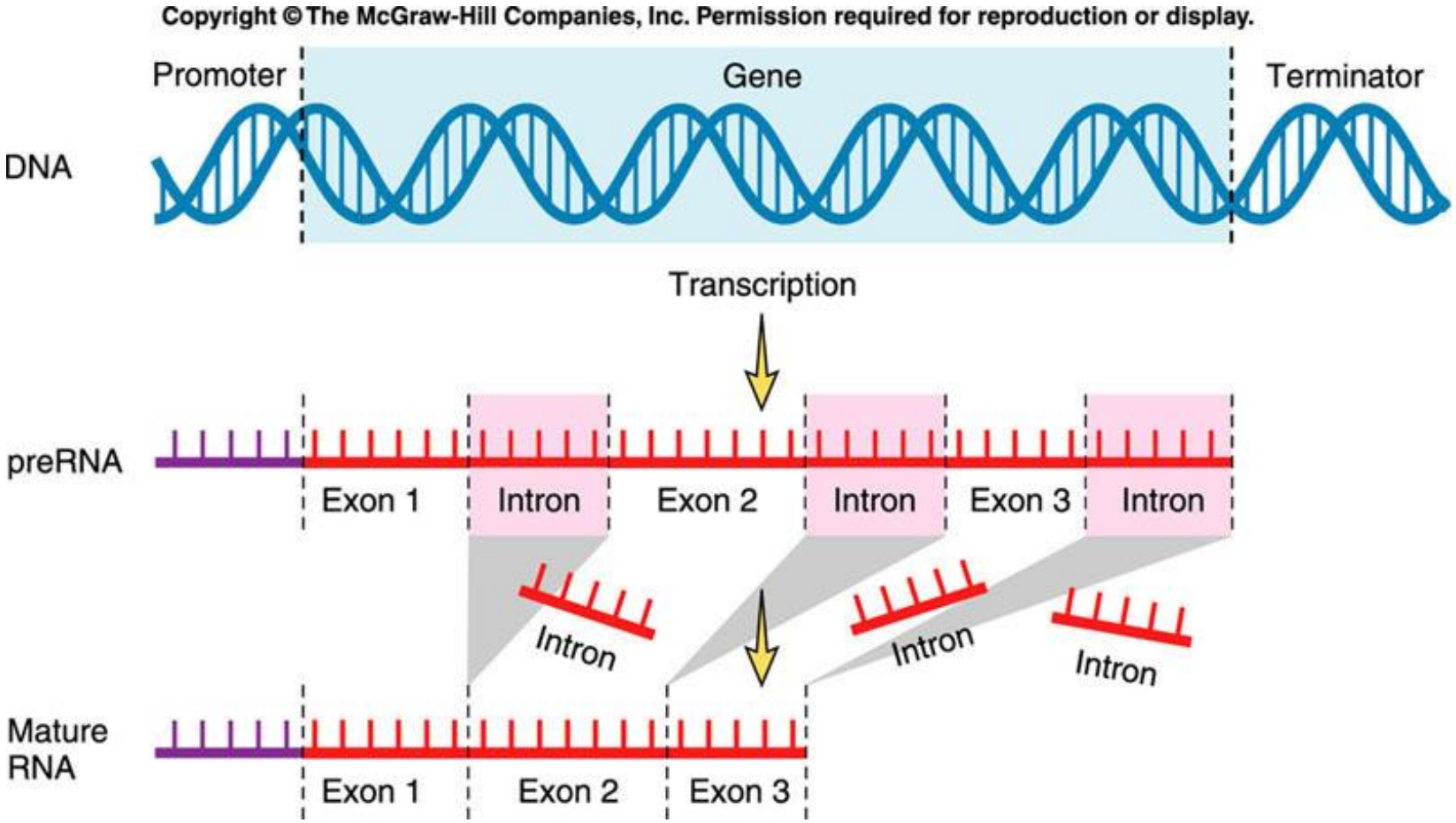
- When pre-mRNA is made, both the introns and exons are copied from the DNA. The introns are cut out and the exons are then spliced together to form the final mRNA.

- Some mRNA may be cut and spliced in different ways to produce different mRNA molecules. This allows for a single gene to produce different forms of mRNA.

- Intron sequences may be involved in regulation of expression of genes.

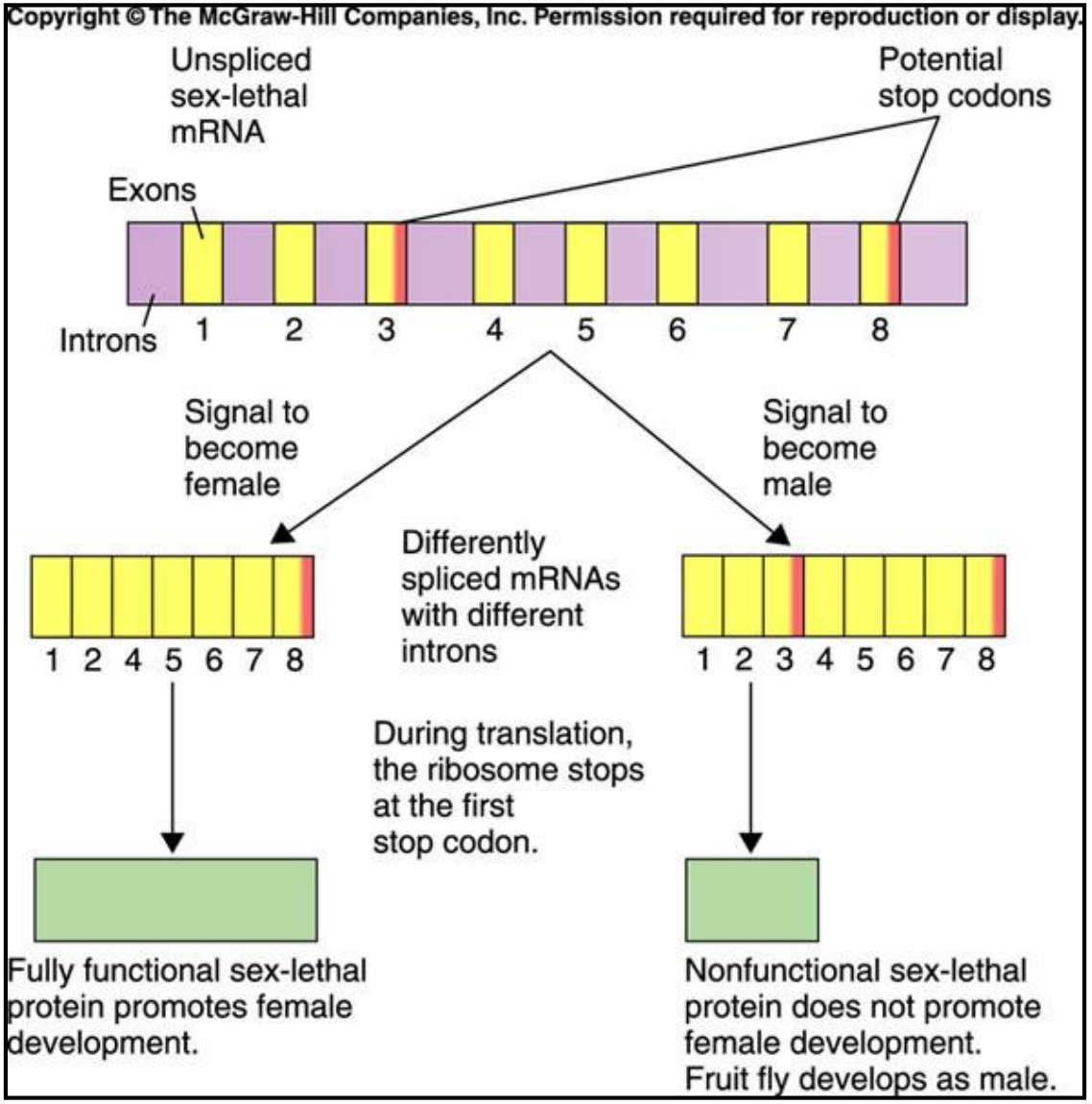

Gene 1
E1
I1
E2
I2
E3
I3
E4
E1
I1
E2
I2
E3
I3
E4
5’
3’
Precursor-mRNA
mRNA
5’
3’

Transcription of mRNA in Eukaryotic Cells

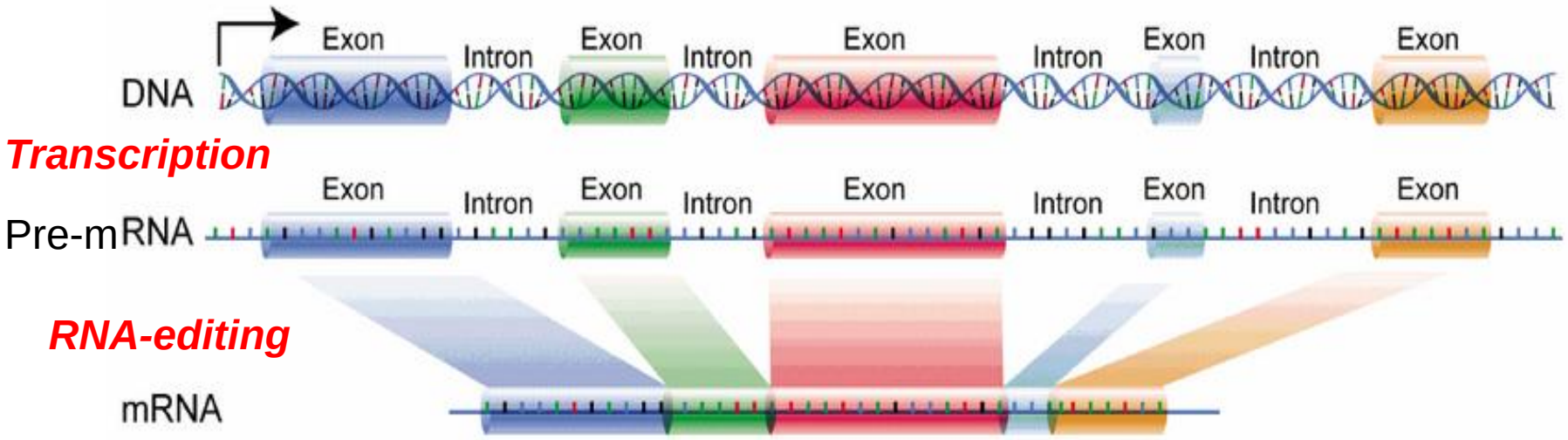


Alternative Splicing

Different combinations of exons from a single gene can be joined to build a number of different mRNAs for a number of different proteins.

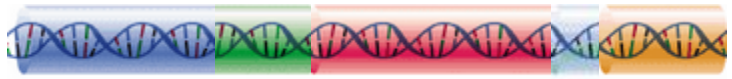


Reverse Transcription



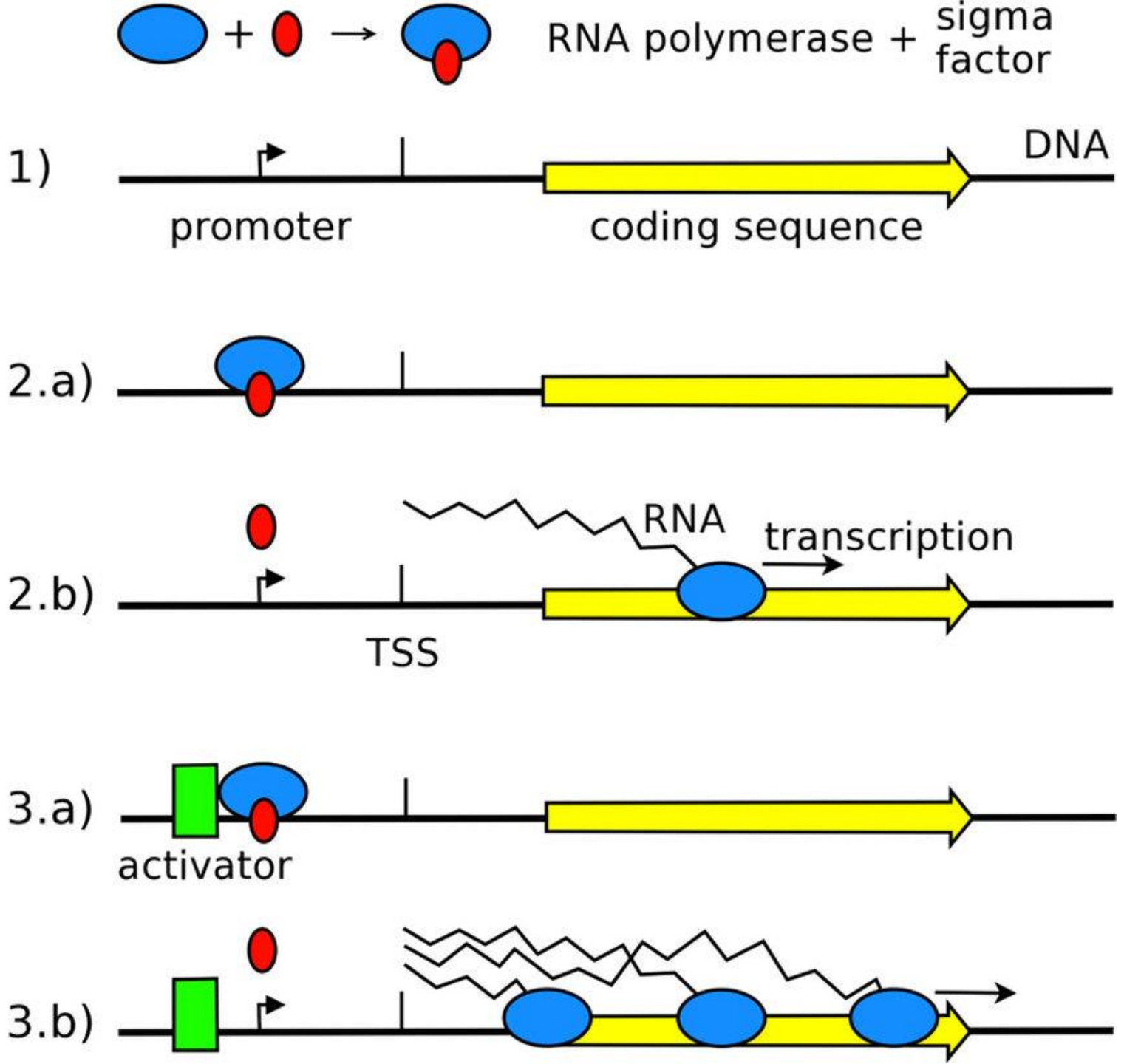
Reverse Transcription

(by Reverse Transcriptase)

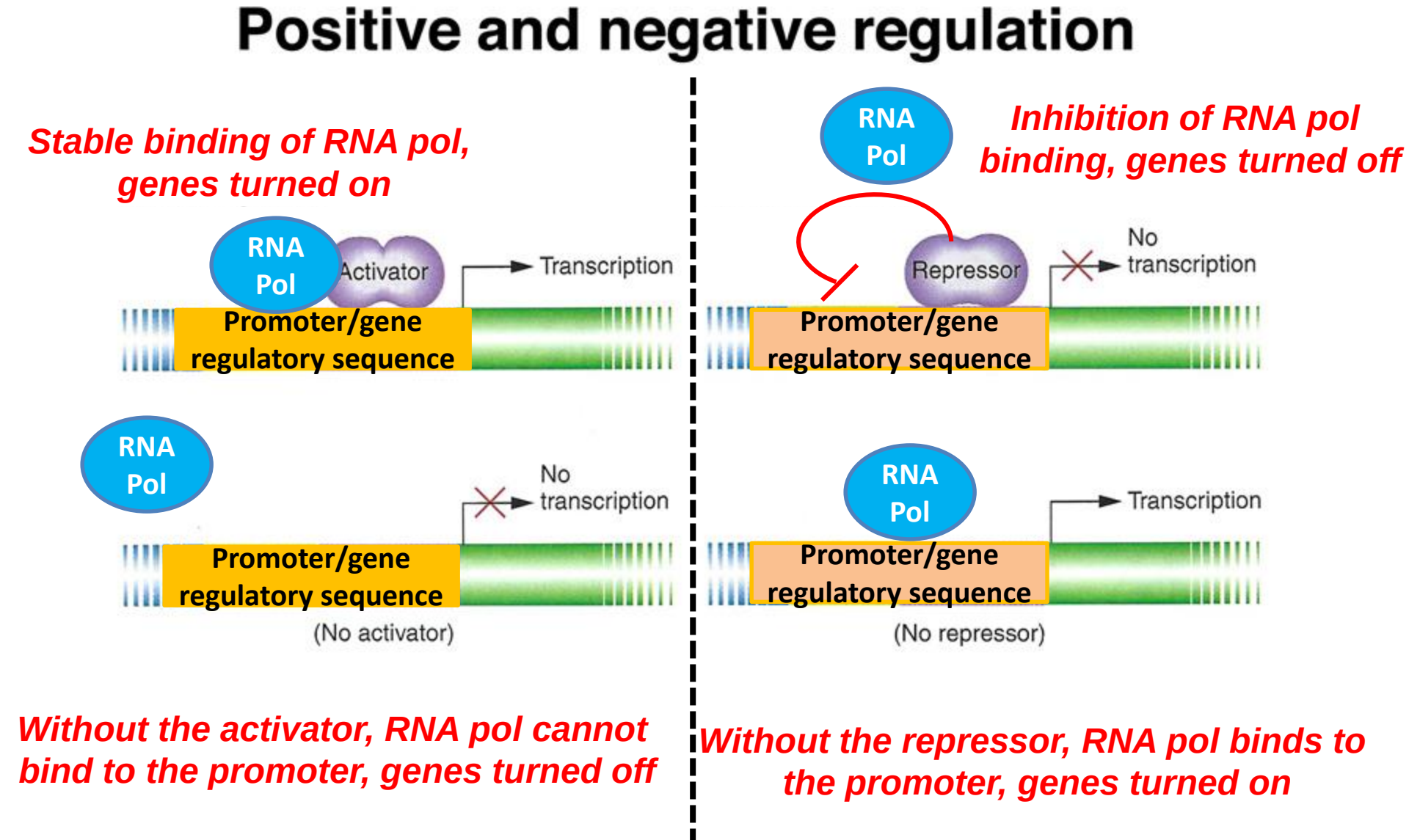


c-DNA

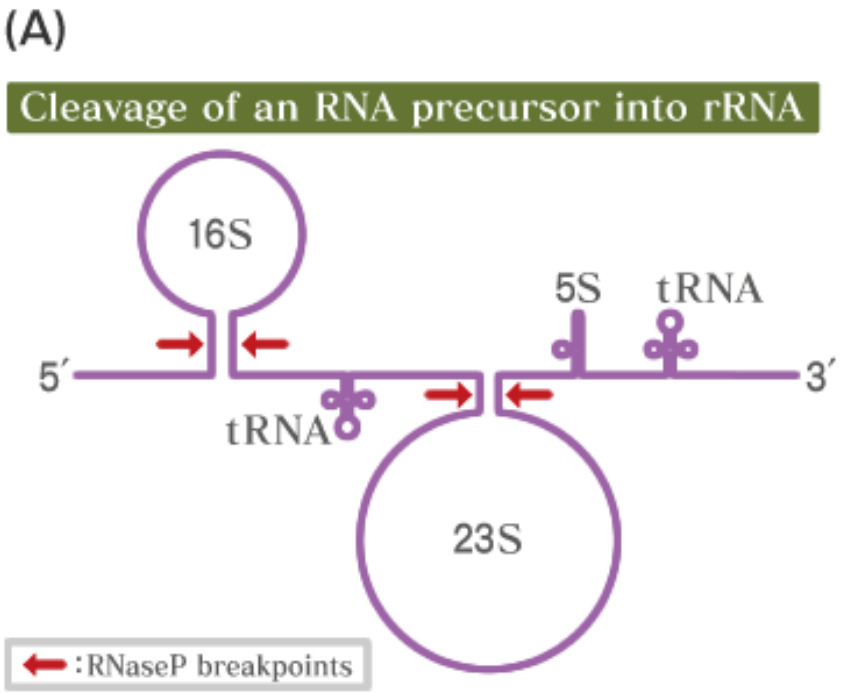
(complementary DNA)

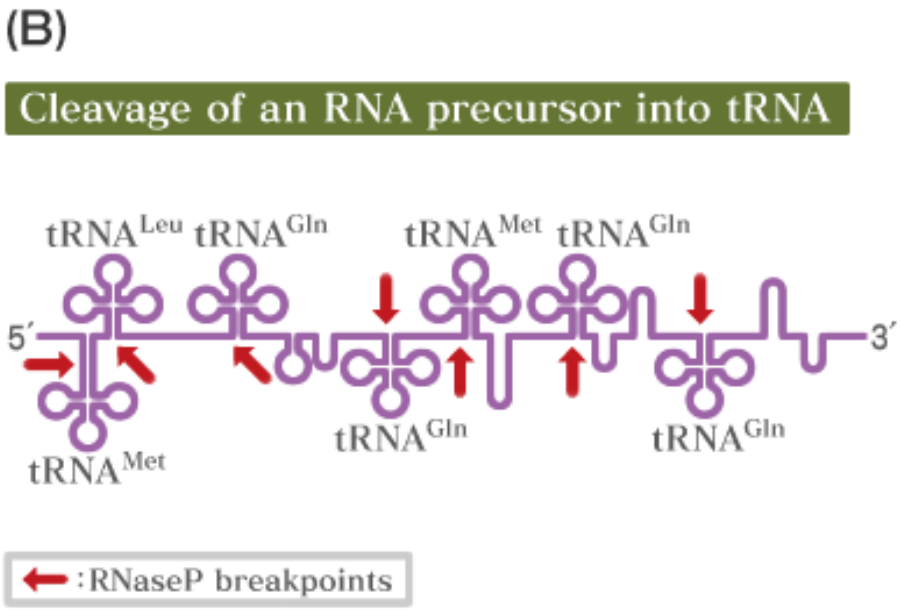

+
RNA polymerase +
sigma factor
1)
DNA
promoter
coding sequence
2.a)
2.b)
RNA
transcription
TSS
3.a)
activator
3.b)

Activators and repressors of transcription

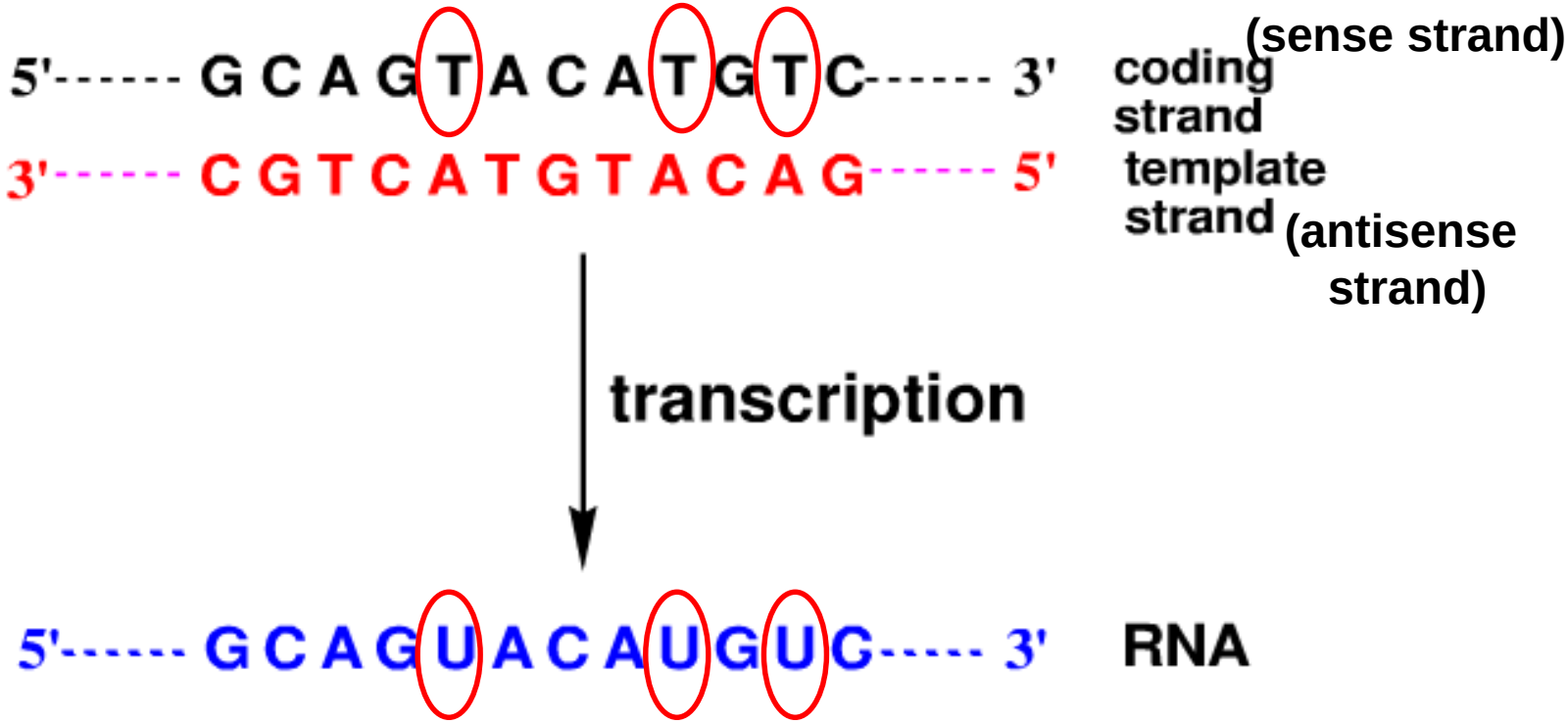


Synthesis of tRNA ans rRNA





Extra Slides


5'------ G C A G T A C A T G T C ------ 3'
(sense strand)
coding strand
3'------ C G T C A T G T A C A G ------ 5'
template strand
(antisense strand)
transcription
5'------ G C A G U A C A U G U C ----- 3'
RNA