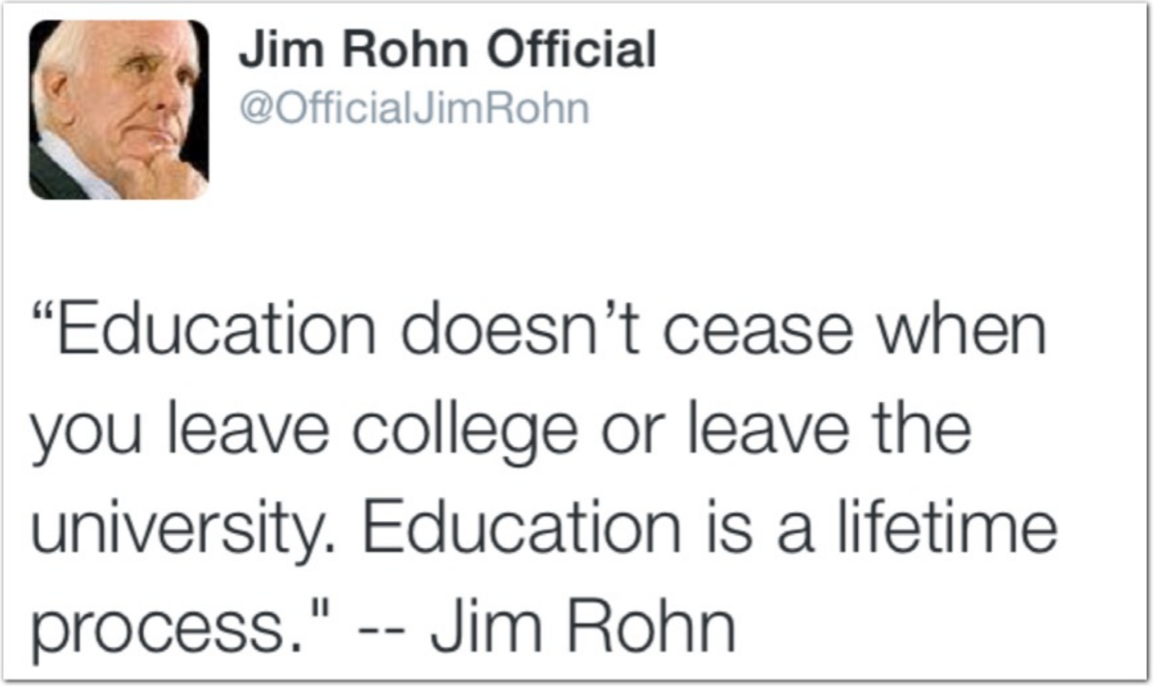
Lecture 12:

Transcription in prokaryotes

Course 281

Lessons for life



Jim Rohn Official
@OfficialJimRohn

“Education doesn’t cease when you leave college or leave the university. Education is a lifetime process." -- Jim Rohn

AIMS

- Understand the transcription process in prokaryotes.
- Understand the steps into transcribing a DNA template into an RNA.
- Understand the gene structure of prokaryotes.
- Understand the promoter structure and the terminator structure.
- Understand how transcription is terminated in prokaryotes.

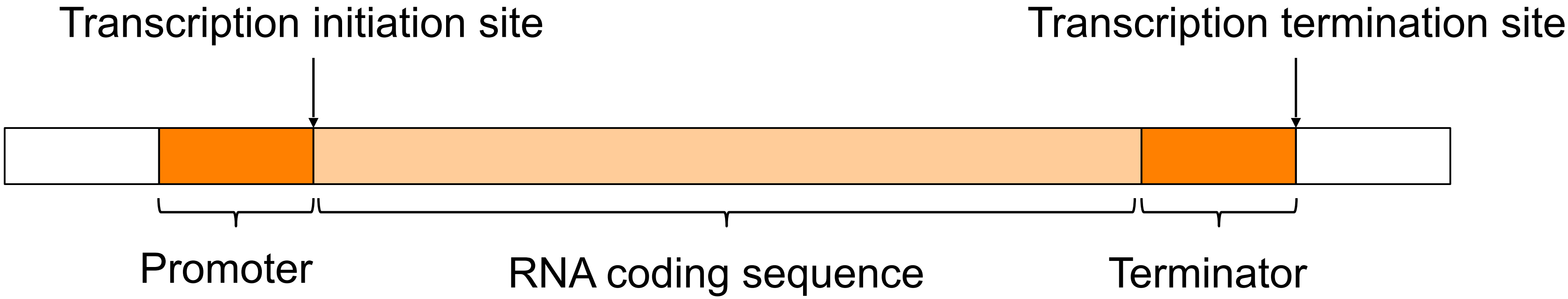
Transcription process

Before we start transcribing DNA,

what get transcribed?

what is the gene structure in prokaryotes?

Prokaryote gene structure



- The region 5’ of the promoter sequence is called **upstream** sequence
- The region 3’ of the terminator sequence is called **downstream** sequence

Prokaryotic gene structure

Genes are composed of three sequence regions:

1. Promoter region
2. RNA coding sequence
3. Terminator region

Prokaryotic gene structure

Genes are composed of three sequence regions:

1. **Promoter region:**
    - Upstream of the sequence that codes for RNA.
    - Site of interaction with RNA polymerase before making RNA.
    - The region that gives the location and direction to start transcribing.

Prokaryotic gene structure

Genes are composed of three sequence regions:

2.**RNA coding sequence:**

- The DNA sequence that will become copied into an RNA molecule (RNA transcript).

Prokaryotic gene structure

Genes are composed of three sequence regions:

3.**Terminator region:**

- The region that tells the RNA polymerase to stop making RNA from DNA template.

Transcription process

The transcription process involves three steps:

1. Initiation
2. Elongation
3. Termination

Transcription process

Where does transcription initiation take place?

Any genomic location?

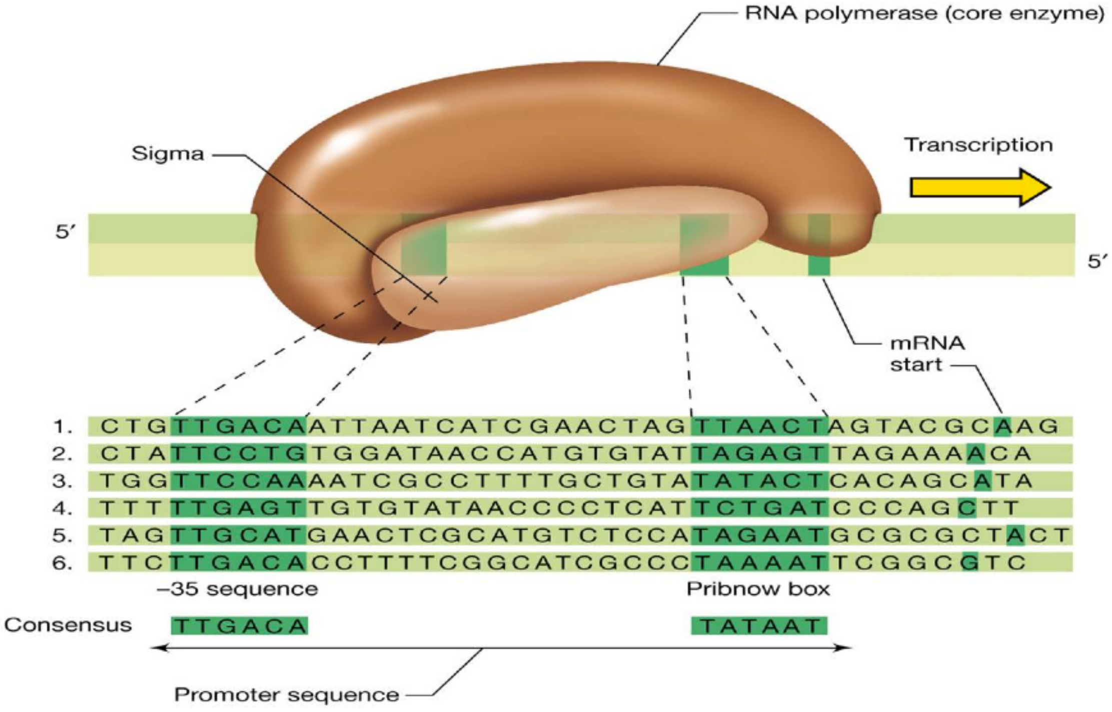
Promoter region

1. Upstream of the transcribed sequence (-).
2. Has a specific sequence at (-10) called:

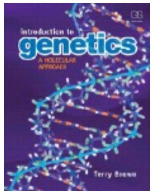
-10 Box (5’ TATAAT3’)

3. Has a specific sequence at (-35) called:

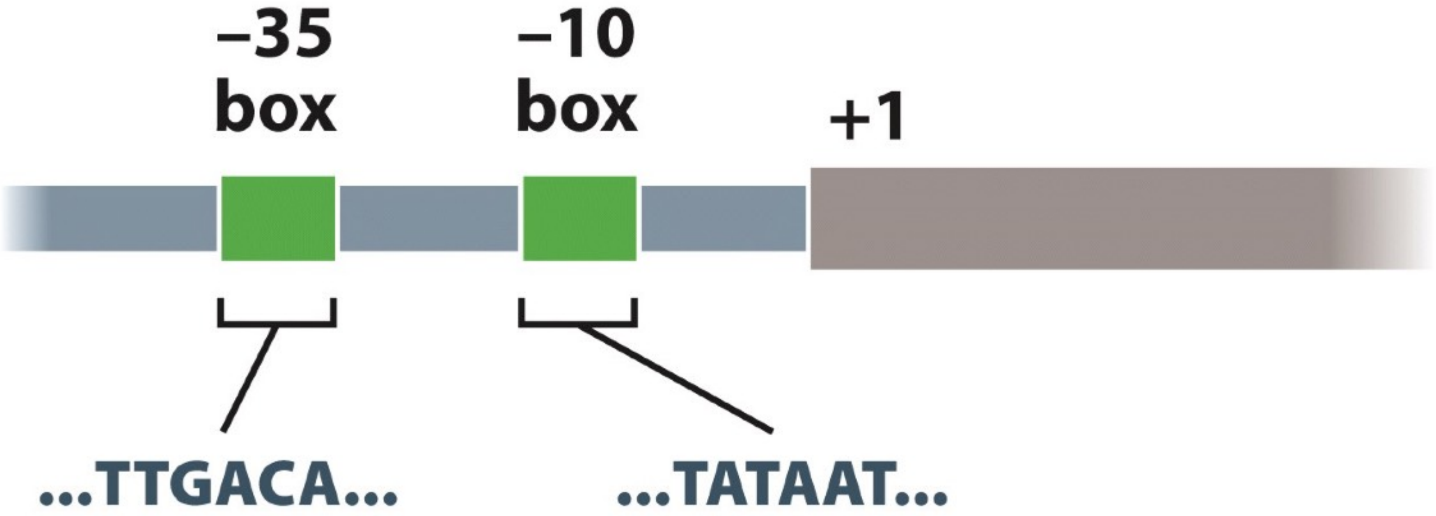
-35 Box (5’ TTAGACA 3’)



- Bacterial RNA polymerases bind to promoter sequences.
- A promoter is a short nucleotide sequence recognized by the bacterial RNA to begin transcription.
- Promoters occur upstream of genes.



- *E.coli* promoter is made of two components, -35 and -10 box.



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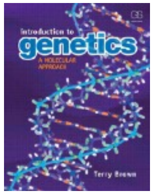

TABLE 4.2 SEQUENCES OF *E. COLI* PROMOTERS

	Sequence	
Promoter	**–35 box**	**–10 box**
Consensus	5′-TTGACA-3′	5′-TATAAT-3′
Lactose promoter*	5′-TTTACA-3′	5′-TATGTT-3′
Tryptophan promoter*	5′-TTGACA-3′	5′-TTAACT-3′

*These are the promoters for the lactose and tryptophan operons, which we will study in Chapter 9.

Table 4.2 Introduction to Genetics (© Garland Science 2012)

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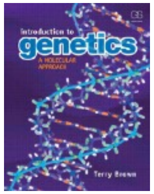
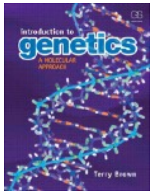


4.3 RECOGNITION SEQUENCES FOR TRANSCRIPTION INITIATION

- RNA polymerase enzymes must transcribe genes and must begin transcription near the start of a gene.

- Initial binding of RNA polymerase to a DNA molecule occur at a specific position, in front (upstream) of the gene to be transcribed.

- RNA polymerase attach **directly** to DNA in bacteria.
- In eukaryotes RNA polymerase attach **indirectly**.



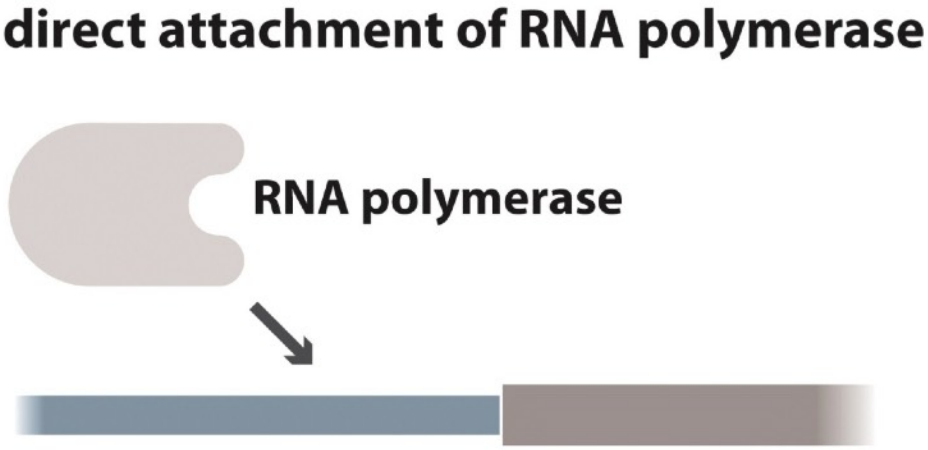
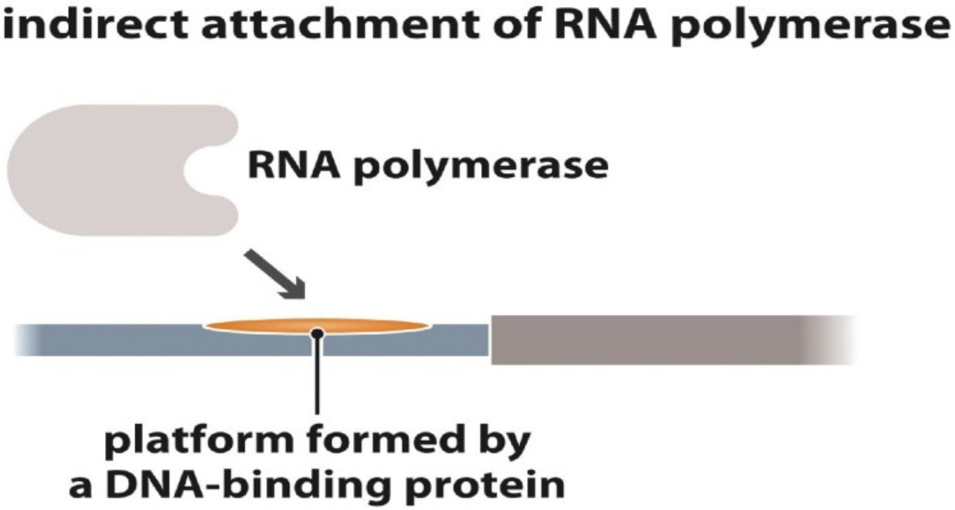


Figure 4.11a Introduction to Genetics (© Garland Science 2012)



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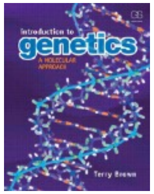


Transcription initiation

1. Sigma factor (σ) binds to promoter sequence (-10, -35 sequence).

2. Core enzyme binds to the sigma factor (σ) and promoter but DNA is still closed.

3. This is called the **closed promoter complex**.

4. Holoenzyme untwist the double strands of DNA.

Transcription initiation

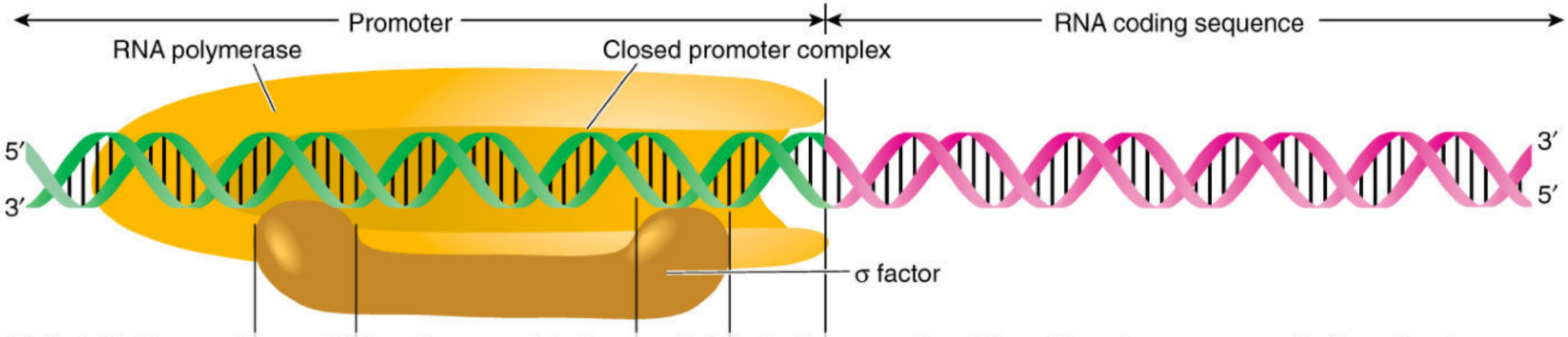
5. Untwisted promoter is called the **open promoter complex**.

6. RNA polymerase binds to -10 sequence and placed in position to start transcribing.

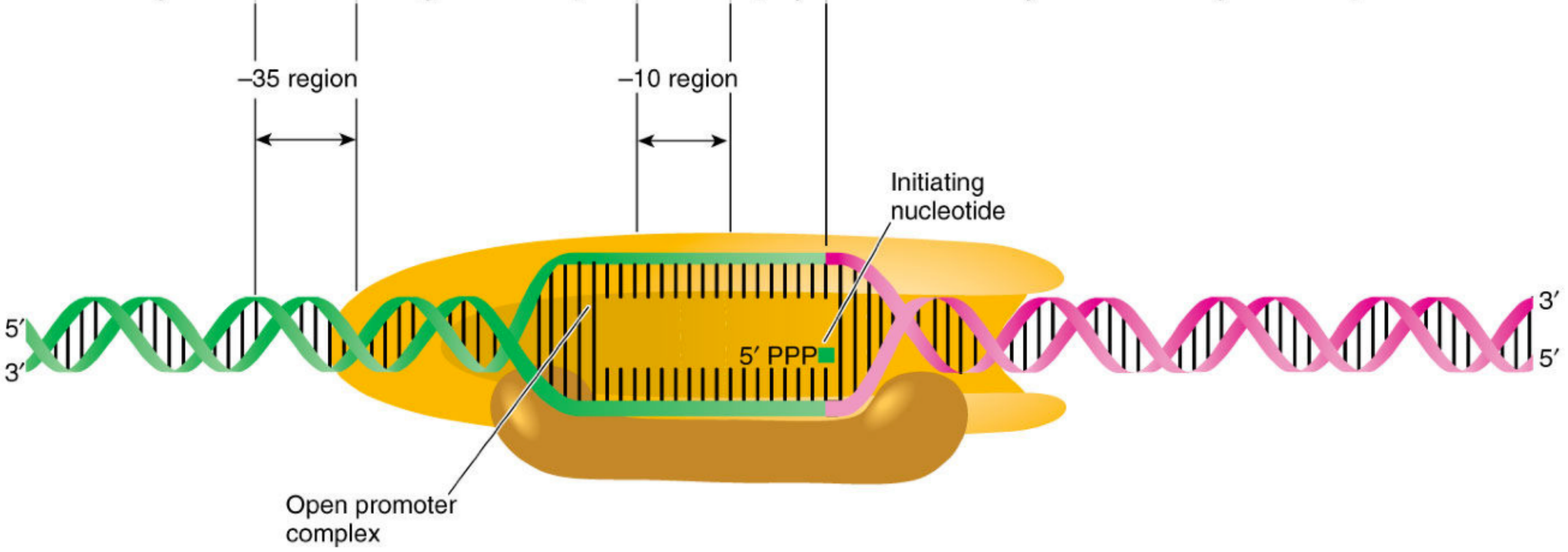
7. Sigma factor (σ) is released so that core enzyme can go forward transcribing.

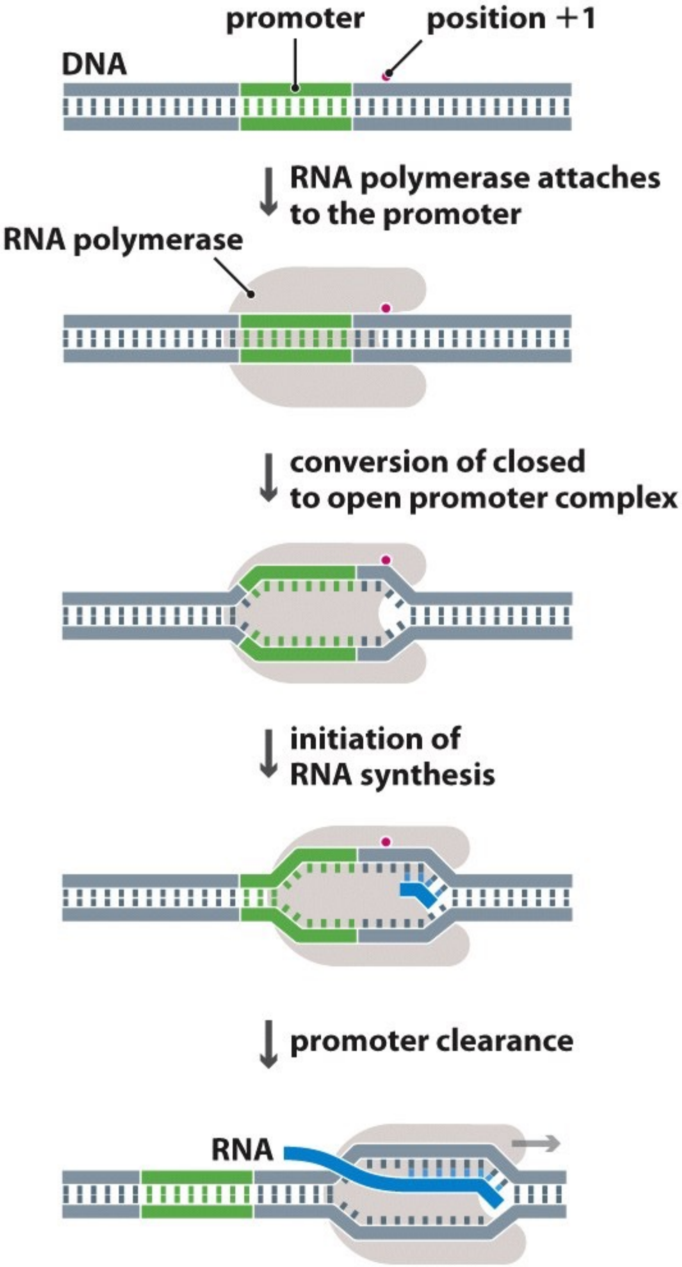
Transcription initiation

a) In initiation, the RNA polymerase holoenzyme first recognizes the promoter at the –35 region and binds to the full promoter.



b) As initiation continues, RNA polymerase binds more tightly to the promoter at the –10 region, accompanied by a local untwisting of the DNA in that region. At this point, the RNA polymerase is correctly oriented to begin transcription at +1.





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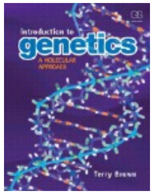

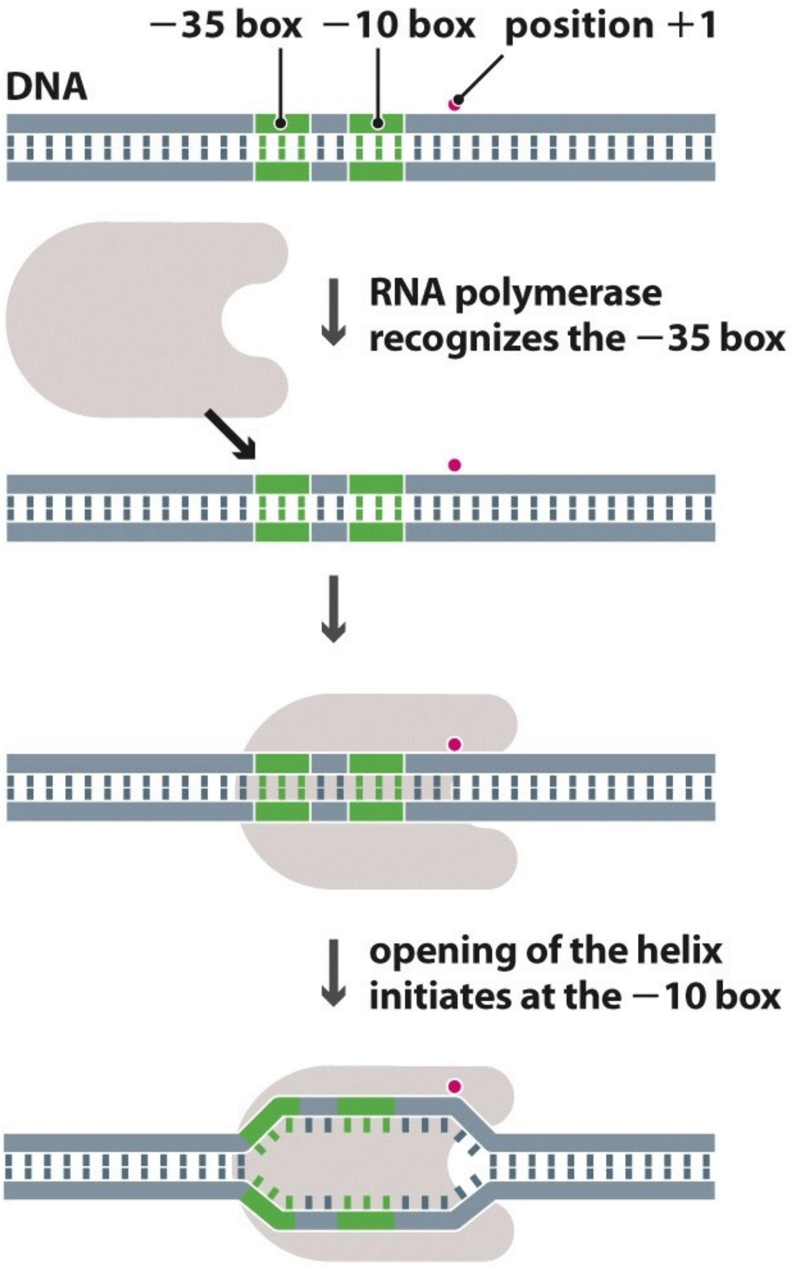

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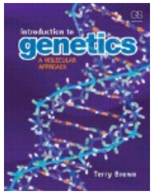


Transcription elongation

1. RNA polymerase (core enzyme) moves along to transcribe the DNA sequence into a single strand RNA of the coding gene.

2. When transcribing, the RNA polymerase interact with DNA sequence forming **transcription bubble**.

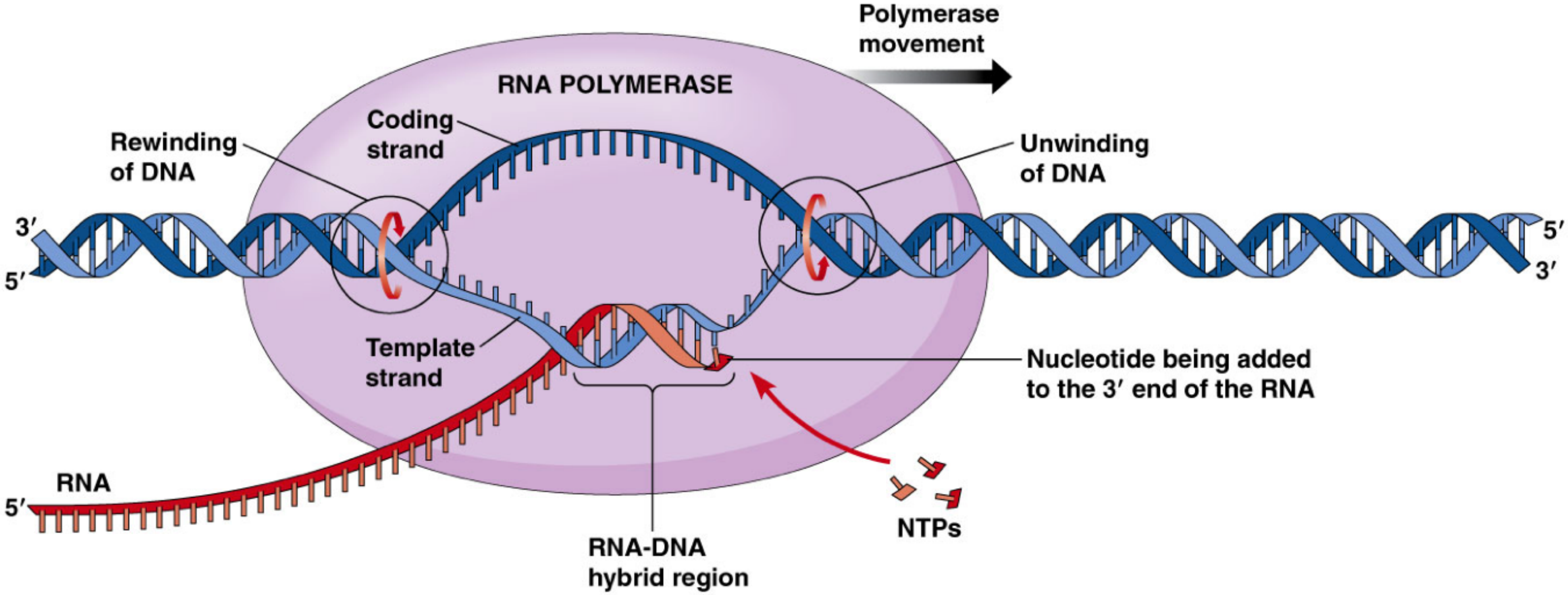
3. DNA double helix is reformed as the RNA polymerase moves forward.

Transcription elongation

4. Few RNA nucleotides (newly synthesized) form an RNA/DNA hybrid within RNA polymerase.

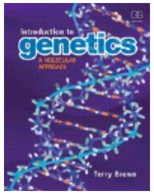
5. As transcription proceed, single strand RNA gets out of the RNA polymerase.

Transcription elongation



4.5 THE ELONGATION PHASE OF TRANSCRIPTION

- Once initiation is achieved, RNA polymerase synthesize transcript.
- Synthesis of an RNA molecule involves polymerization of ribonucleotide subunits.



4.5 THE ELONGATION PHASE OF TRANSCRIPTION

- During transcription,β and Ƴ phosphates are removed from the incoming nucleotide and hydroxyl group is removed from the 3’ carbon of the nucleotide at end of chain.
- This results in loss of Pyrophosphate molecule foe each bond formed.
- RNA transcript is built in 5’→3’ direction.

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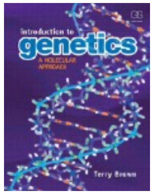

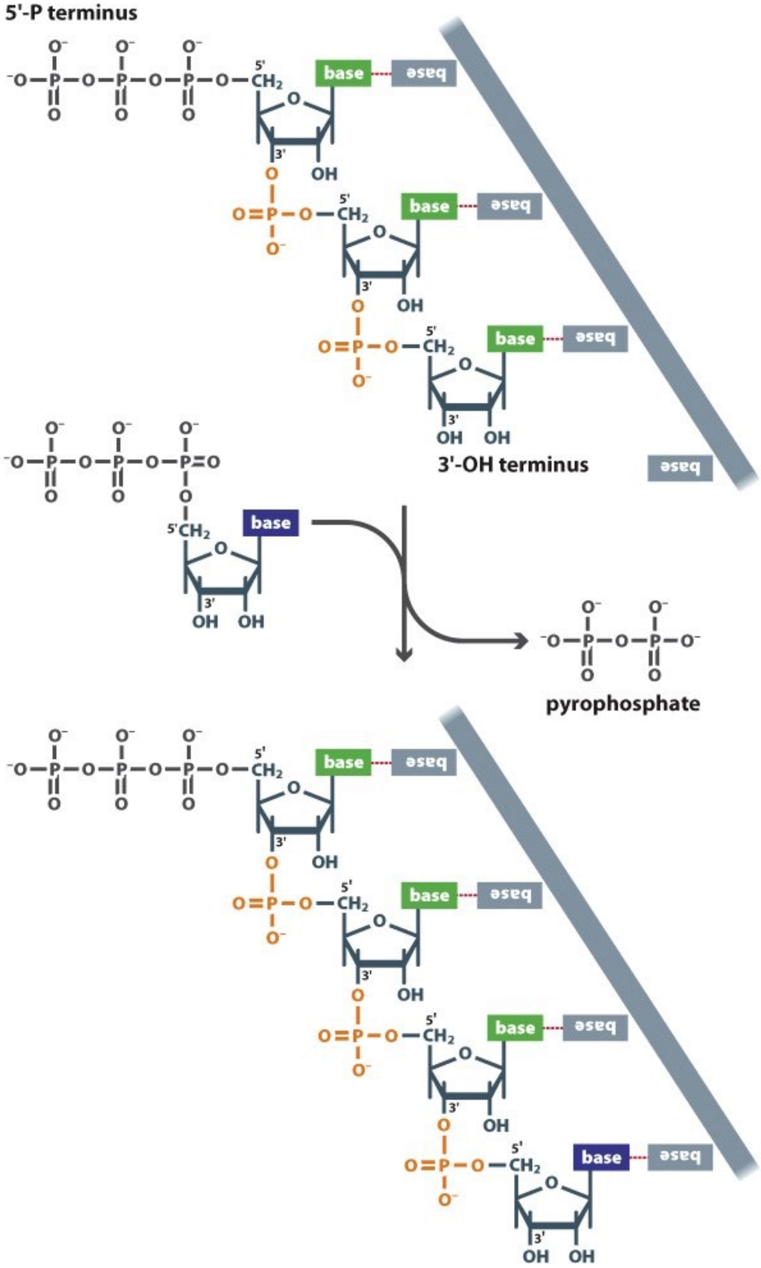

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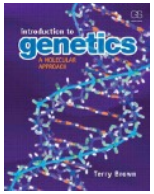
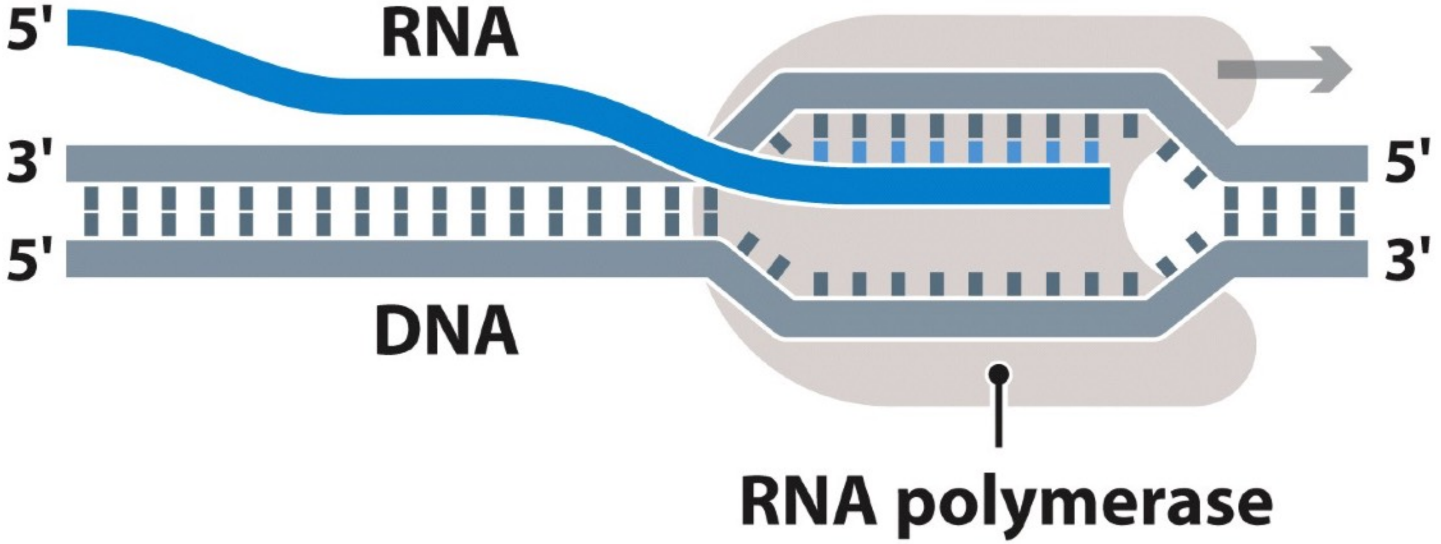


- During the elongation stage of transcription σ leaves the complex.

- The RNA polymerase has to keep a tight grip on both the DNA template and the RNA to prevent the transcription complex from falling apart before the end of the gene is reached.



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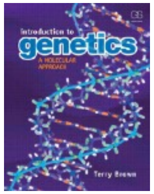

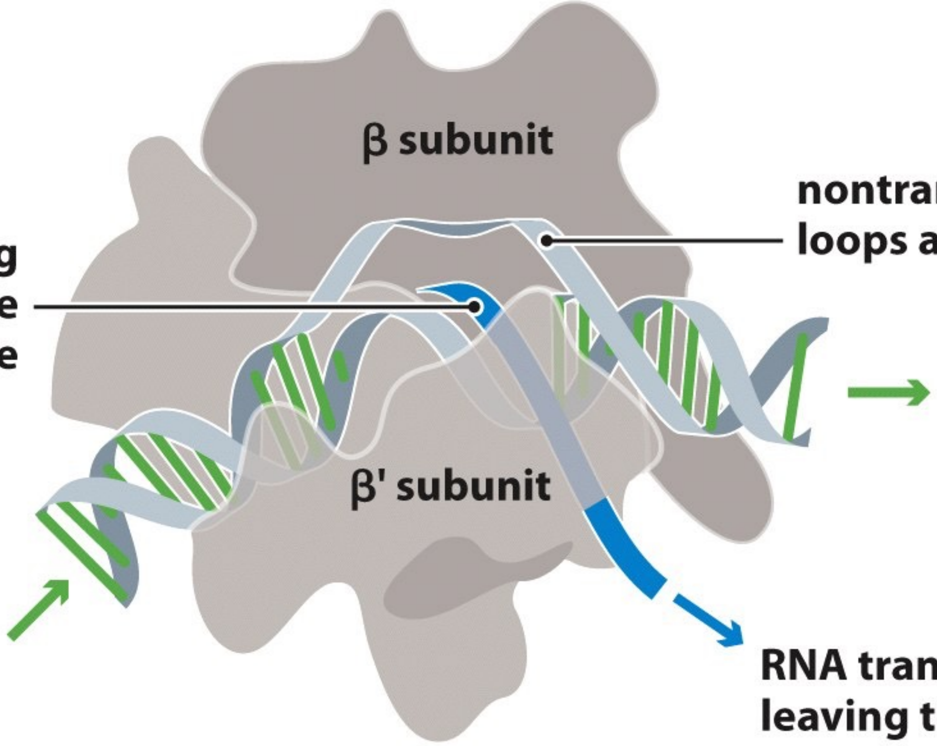

nontranscribed strand of DNA loops away from the active site

RNA being synthesized at the active site

DNA leaving the polymerase

DNA entering the polymerase

RNA transcript leaving the polymerase

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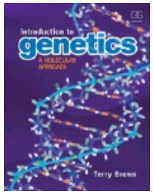


Transcription elongation

Does RNA polymerase have proofreading capabilities?

Transcription elongation

RNA polymerase has proofreading capabilities (3’ – 5’)

1. Removing 1 nucleotide and adding correct one
2. Removing several nucleotides and adding new ones

Transcription termination

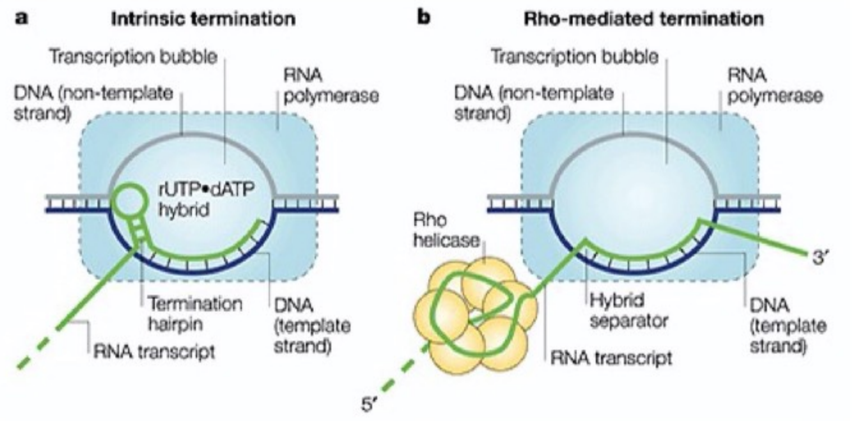
There are specific signals for the termination of transcription (stop).

Terminators are:

1. Rho-independent terminator (type 1 terminator)

2. Rho-dependent terminator (type 2 terminator)

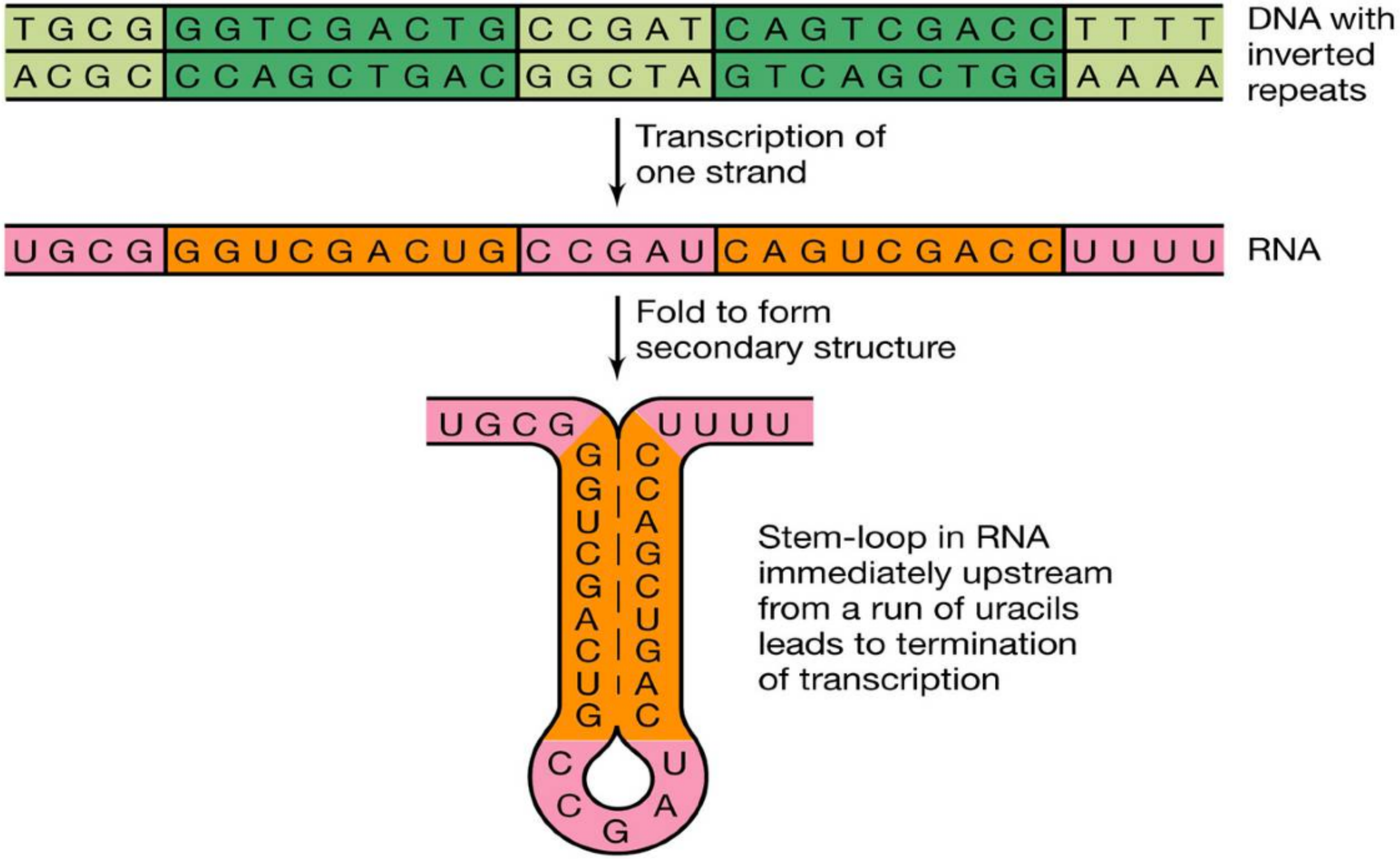
Goofy analogy



Taboulah is a parsley dependent salad whereas Fatoosh is a parsley independent salad

Termination (Rho-independent terminator) - type 1 terminator

- Sequences with the DNA code consisting of inverted repeats upstream of termination point.

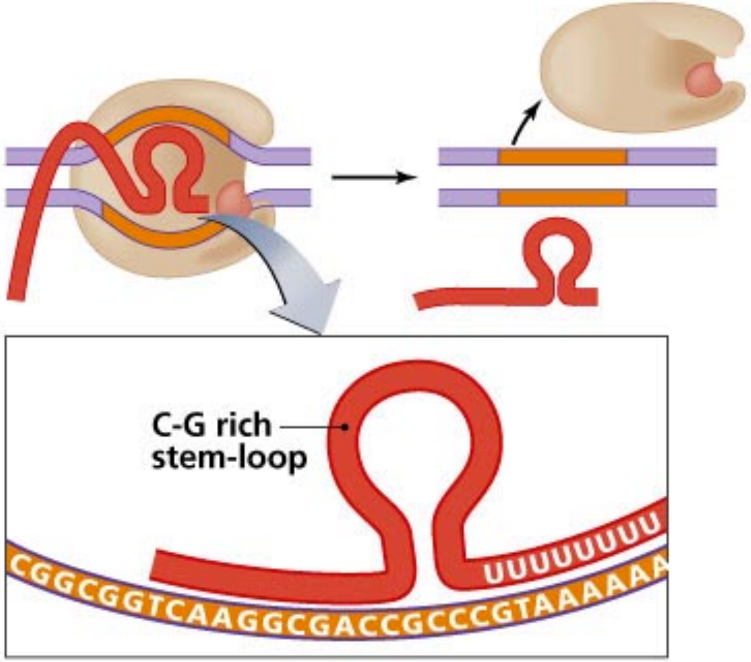


Termination (Rho-independent terminator) - type 1 terminator

- RNA moves pass the inverted repeats and transcribes the termination sequence.

- Because of the inverted repeat arrangement ➔ RNA synthesized forms a hairpin loop structure.

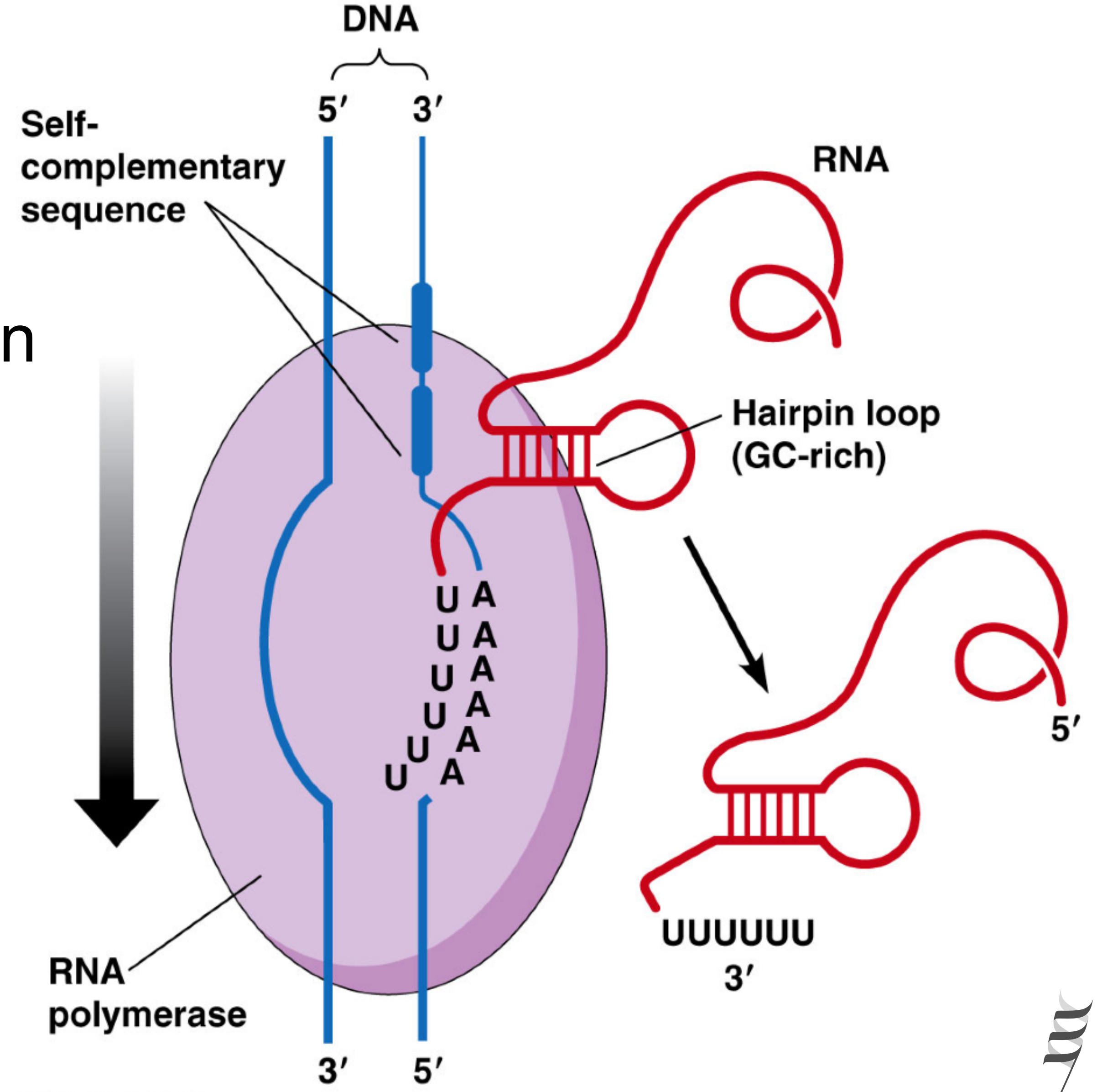
- Hairpin loop makes the RNA polymerase slow down and eventually stops.



(c) Termination of transcription

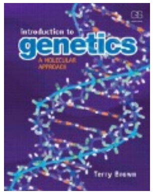
Termination (Rho-independent terminator) - type 1 terminator

- RNA polymerase can not continue attached to DNA.
- RNA polymerase dissociate.

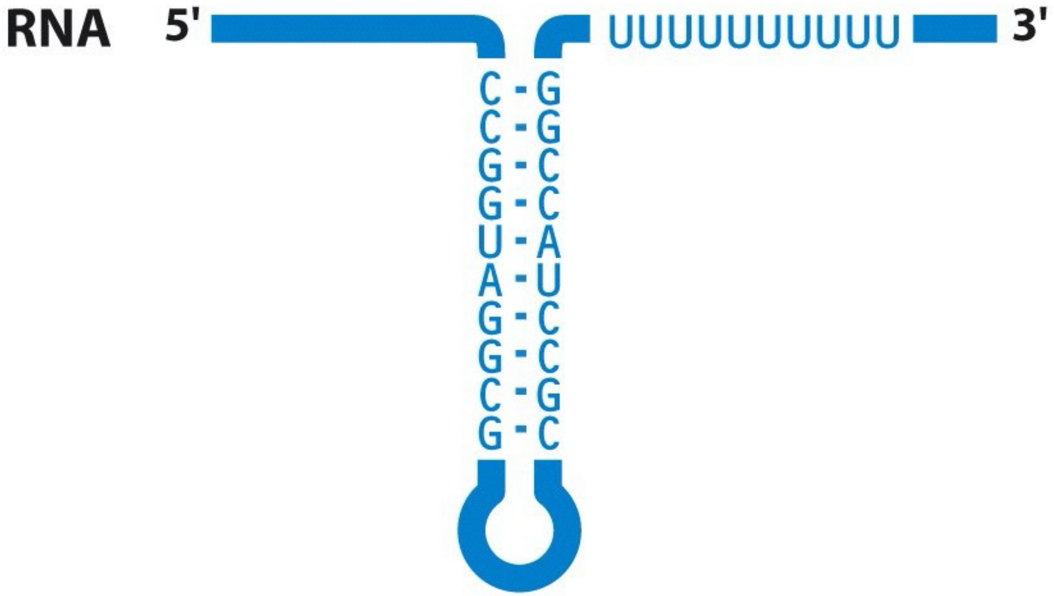


- Bacteria use two distinct strategies for transcription termination, termination site is marked by an **inverted repeat in the DNA sequence.**

- **An inverted repeat is a segment** of DNA or RNA in which a sequence is followed by its reverse complement.



- An inverted repeat in a double-stranded DNA molecule can give rise to a hairpin loop in a single-stranded RNA molecule.



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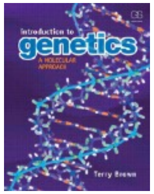
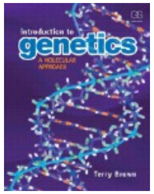


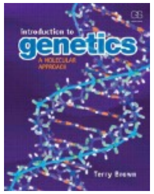
- At about half the positions in *E. coli* at which transcription terminates, the inverted repeat is followed by a run of deoxyadenosine nucleotides in the non-transcribed strand.

- These are called **intrinsic terminators**, and the hairpin loops are relatively stable.

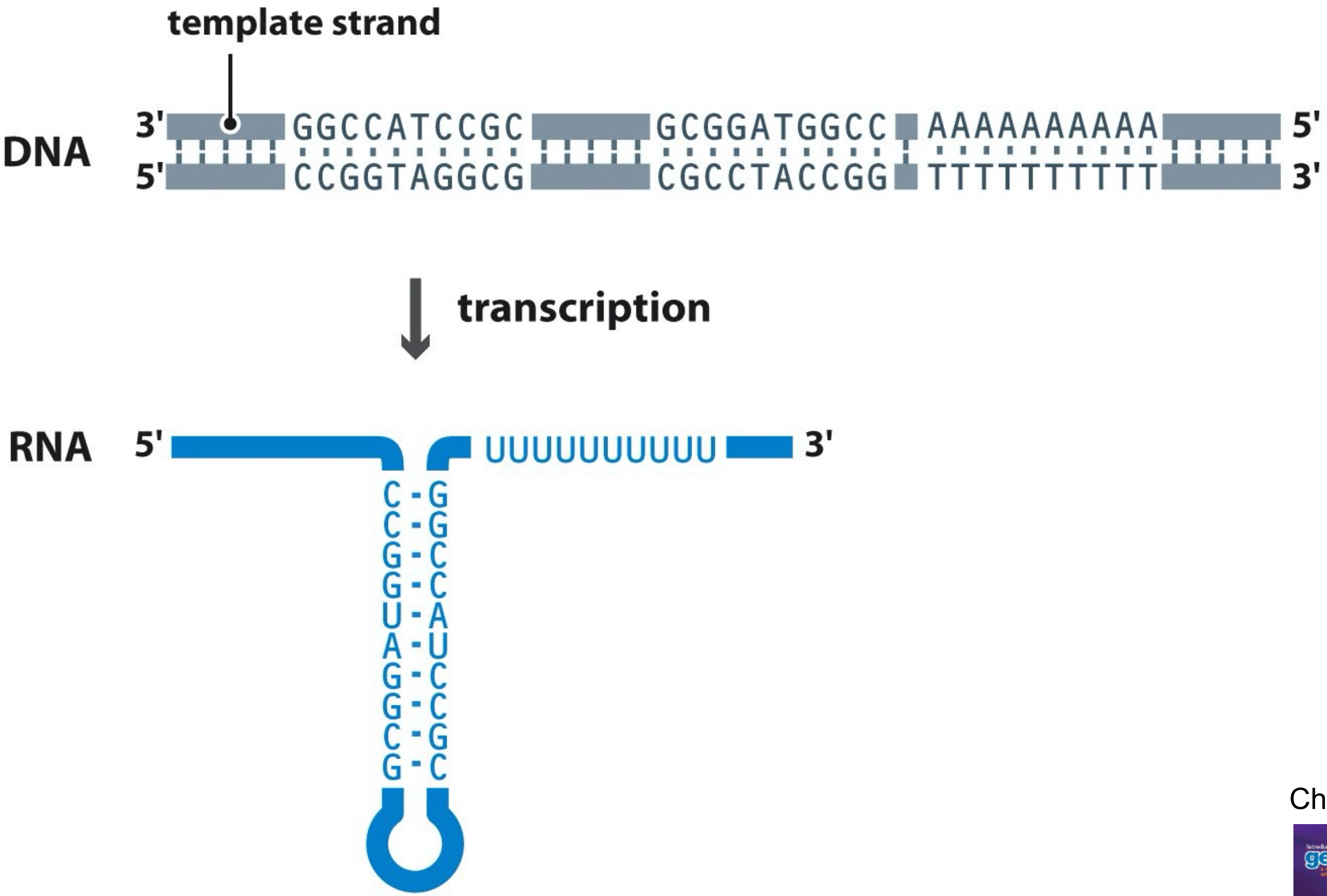


- Reducing the number of contacts between the template DNA and transcript, and weakening the overall DNA–RNA interaction.

- Resulting A–U base pairs have only two hydrogen bonds each, compared with three for each G–C pair. The net result is that detachment of the transcript.



synthesis of an intrinsic terminator



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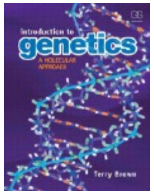
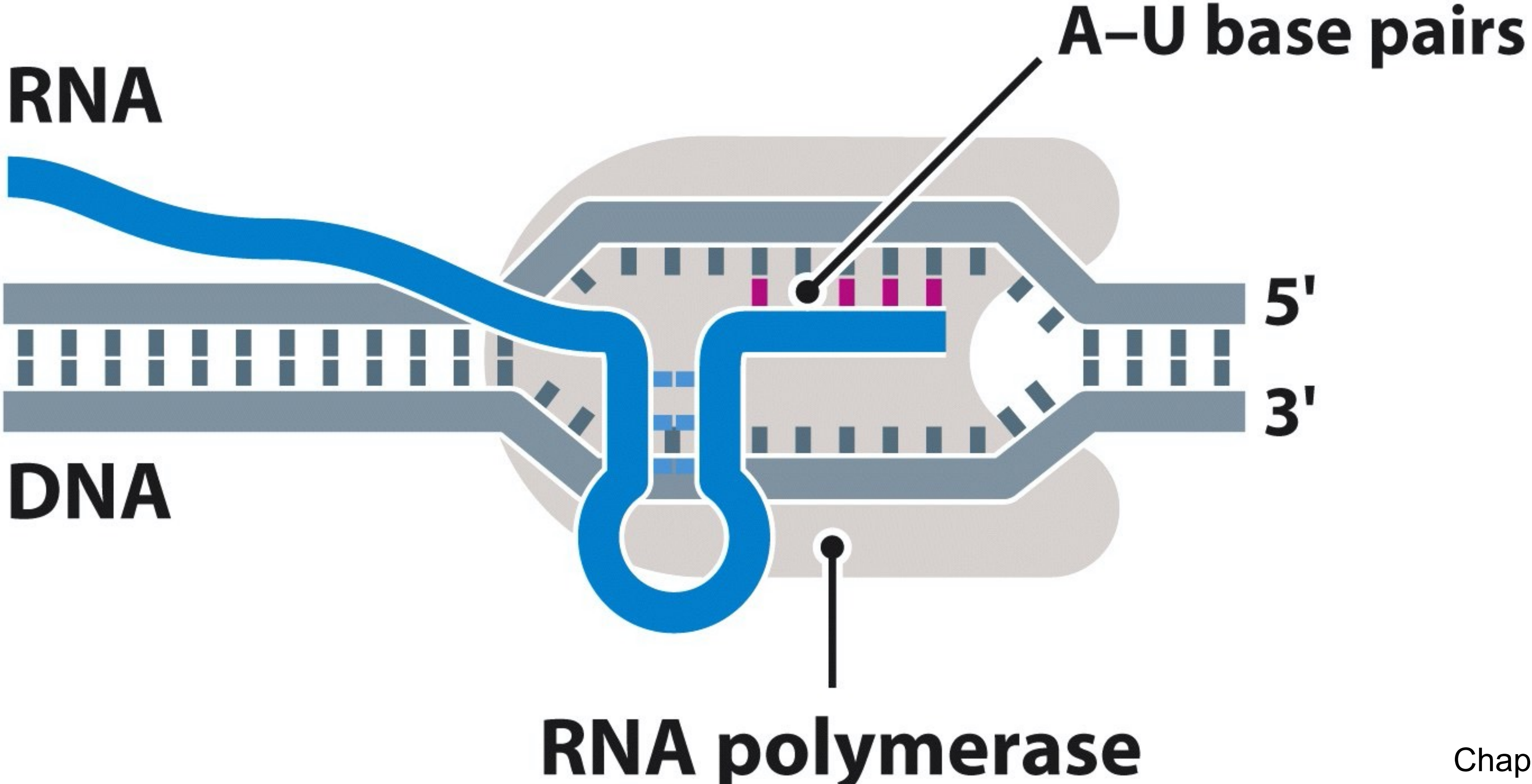


the role of an intrinsic terminator



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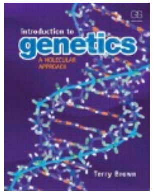
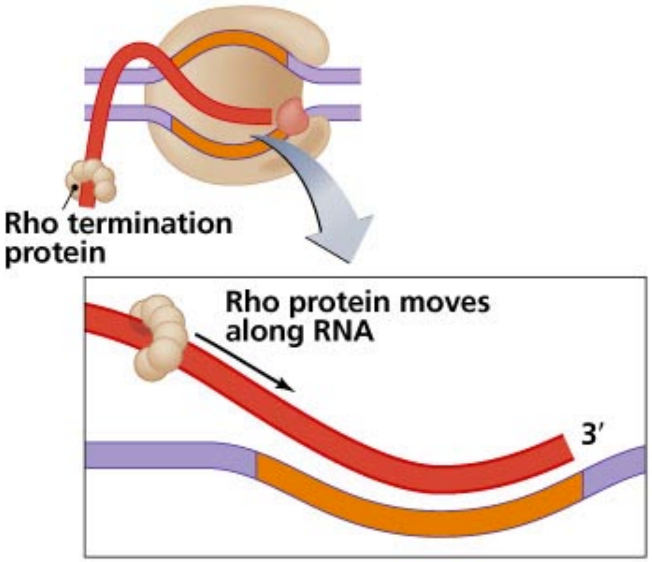


Termination (Rho-dependent terminator) – type 2 terminator

- Terminator is a sequence rich in C (C-rich) and poor in G (G-poor).
- Terminator **DOES NOT** form a hairpin loop.
- Rho (protein) binds to C-rich sequence upstream of the termination site.

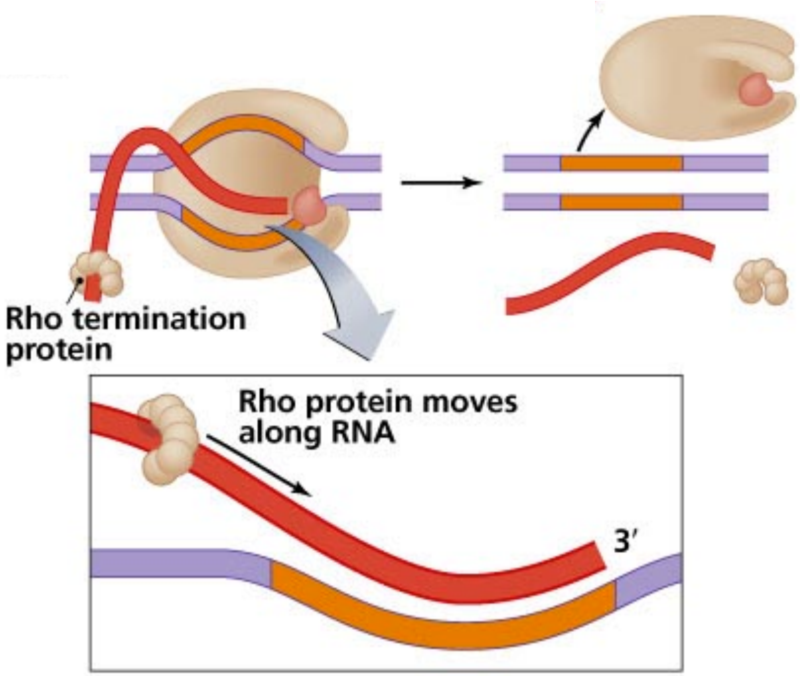


Termination (Rho-dependent terminator) – type 2 terminator

- Rho, which is a helicase, moves to the location of the RNA polymerase.

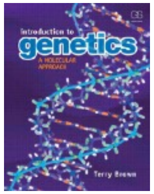
- Rho unwinds the DNA/ RNA hybrid and the RNA transcript is released.

- RNA polymerase and Rho dissociate.



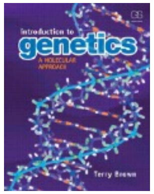
- The second type of bacterial termination signal is **Rho-dependent.**

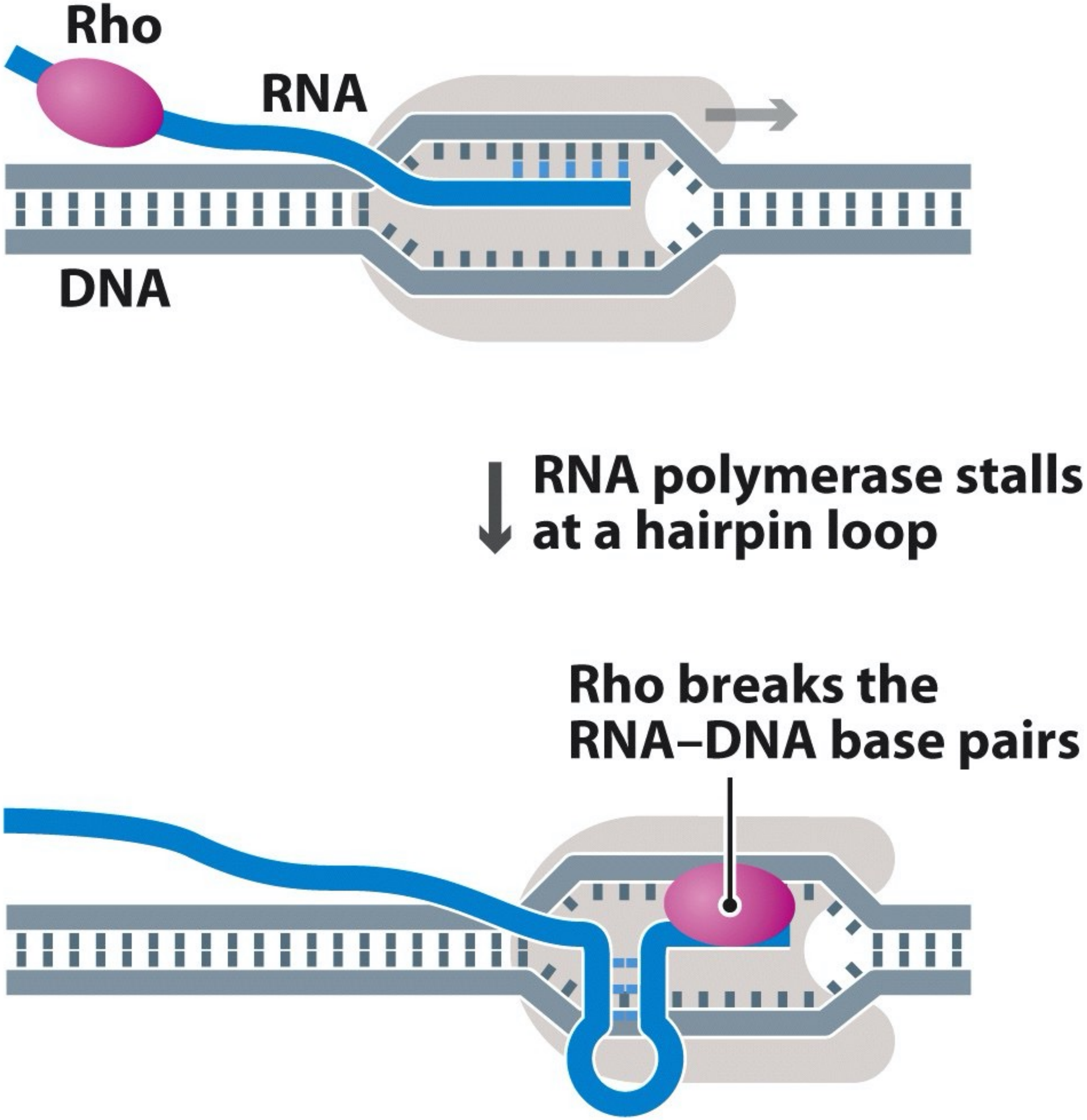
- **Signals** of this type usually include an inverted repeat as seen at intrinsic terminators.



- Termination requires the activity of a protein called Rho, which attaches to the transcript and moves along the RNA toward the polymerase.

- If the polymerase continues to synthesize RNA, then it keeps ahead of the pursuing Rho, but at the termination signal the polymerase stalls.





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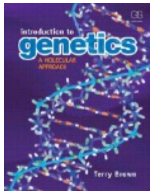
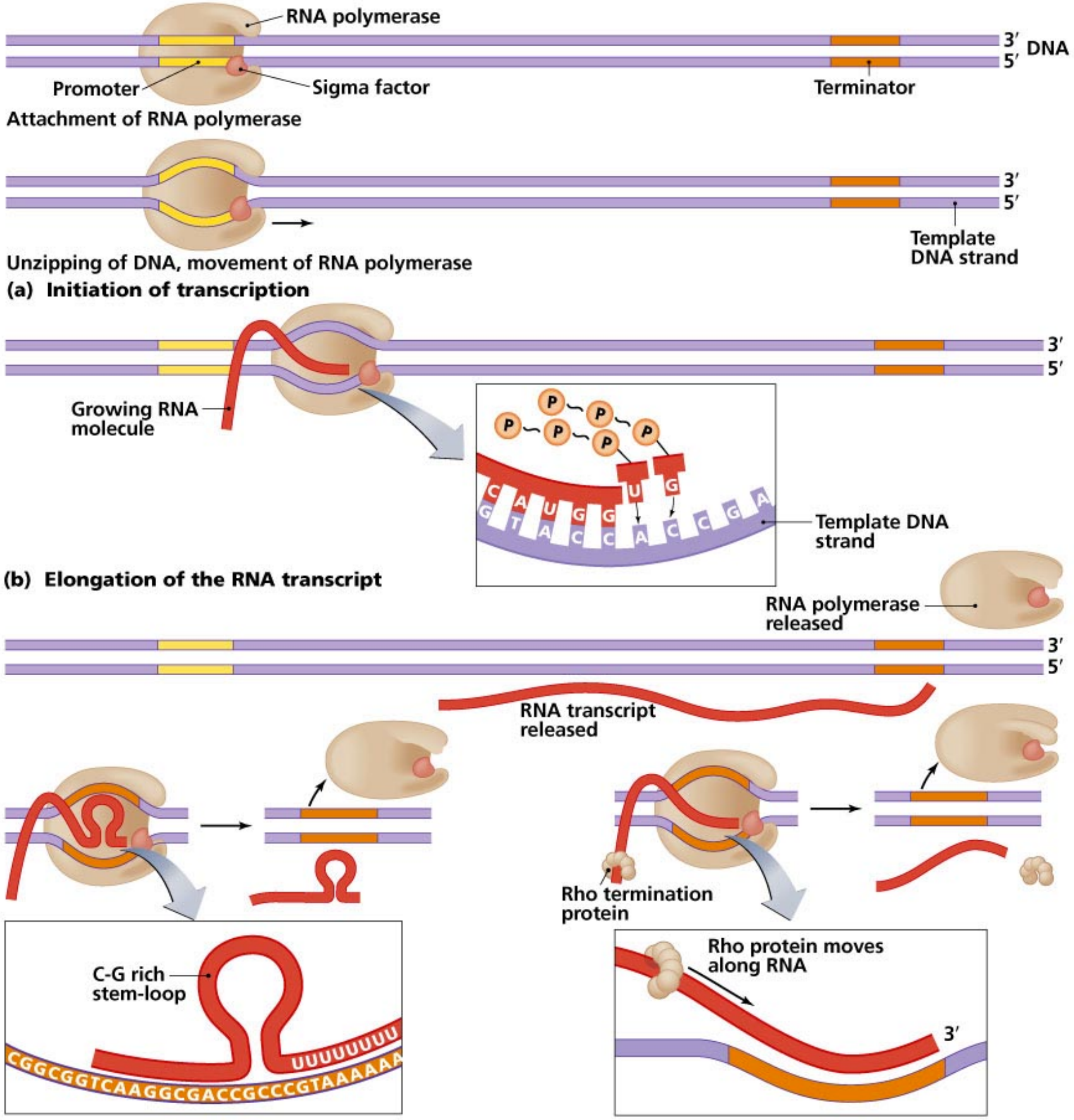


Termination (Rho-dependent terminator) – type 2 terminator

Does Rho need energy to function?

Summary

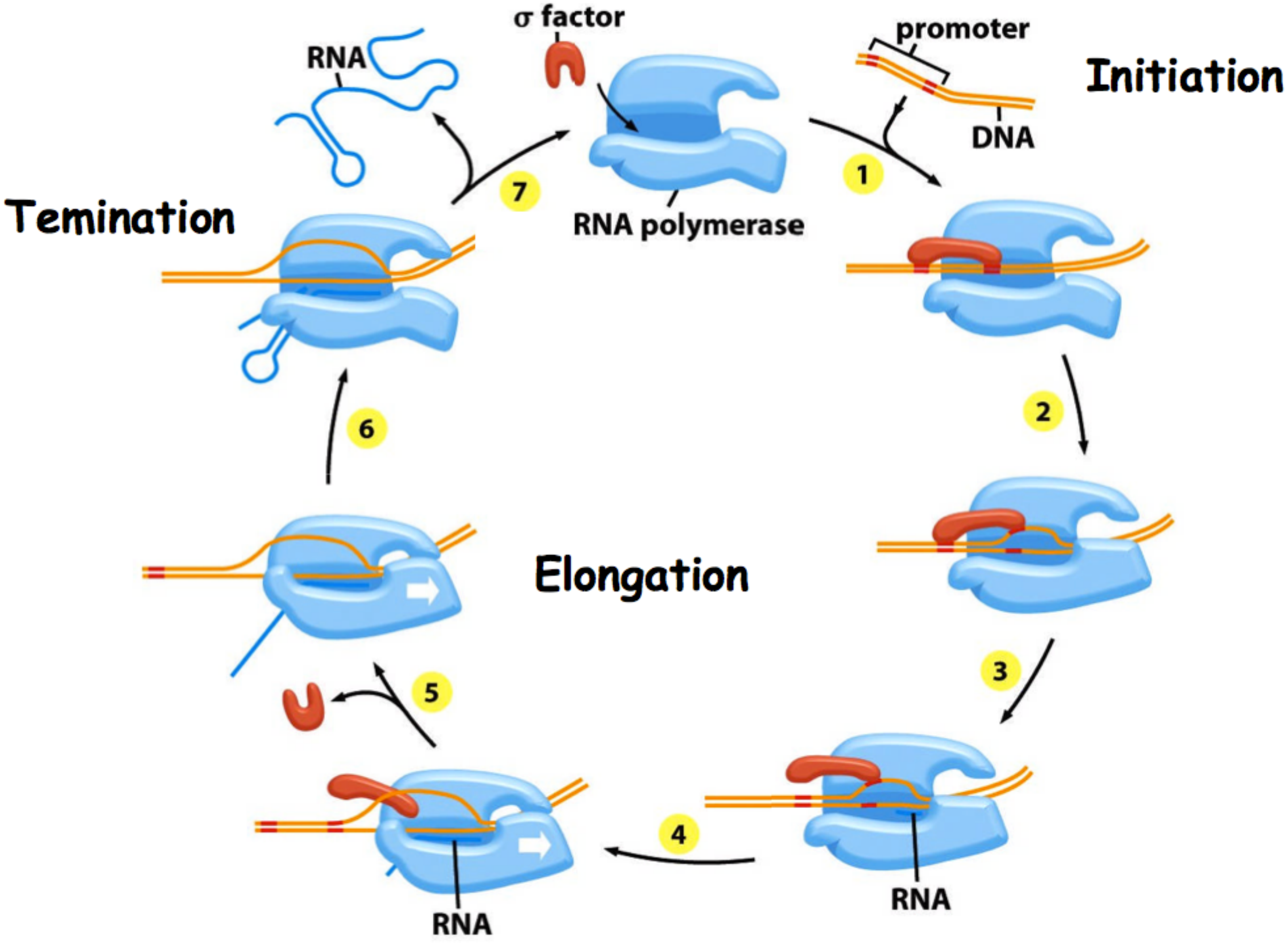


(a) Initiation of transcription

(b) Elongation of the RNA transcript

(c) Termination of transcription

Transcription in prokaryotes once again



To know

Promoter

-35 box

Sigma factor

Downstream

Transcription initiation

Terminator

-10 box

Rho-dependent termination

Rho-independent termination

RNA polymerase

Transcription

RNA polymerase proofreading

Transcription bubble

Transcription termination

Core enzyme

Hairpin loop

Upstream

Initiation site

Termination site

Transcription elongation

Holoenzyme

RNA coding sequence

Expectations

- You know the three steps involved in the transcription of prokaryotic gene(s).
- You know the sequence of events into transcription and what biochemical elements involved in the process.
- You know the terminology and the names of the enzymes and sequence regions.

For a smile


There they go, "expressing" themselves again
www.biocomicals.com