



Lecture 17:

Transcription in prokaryotes

Course 371

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.

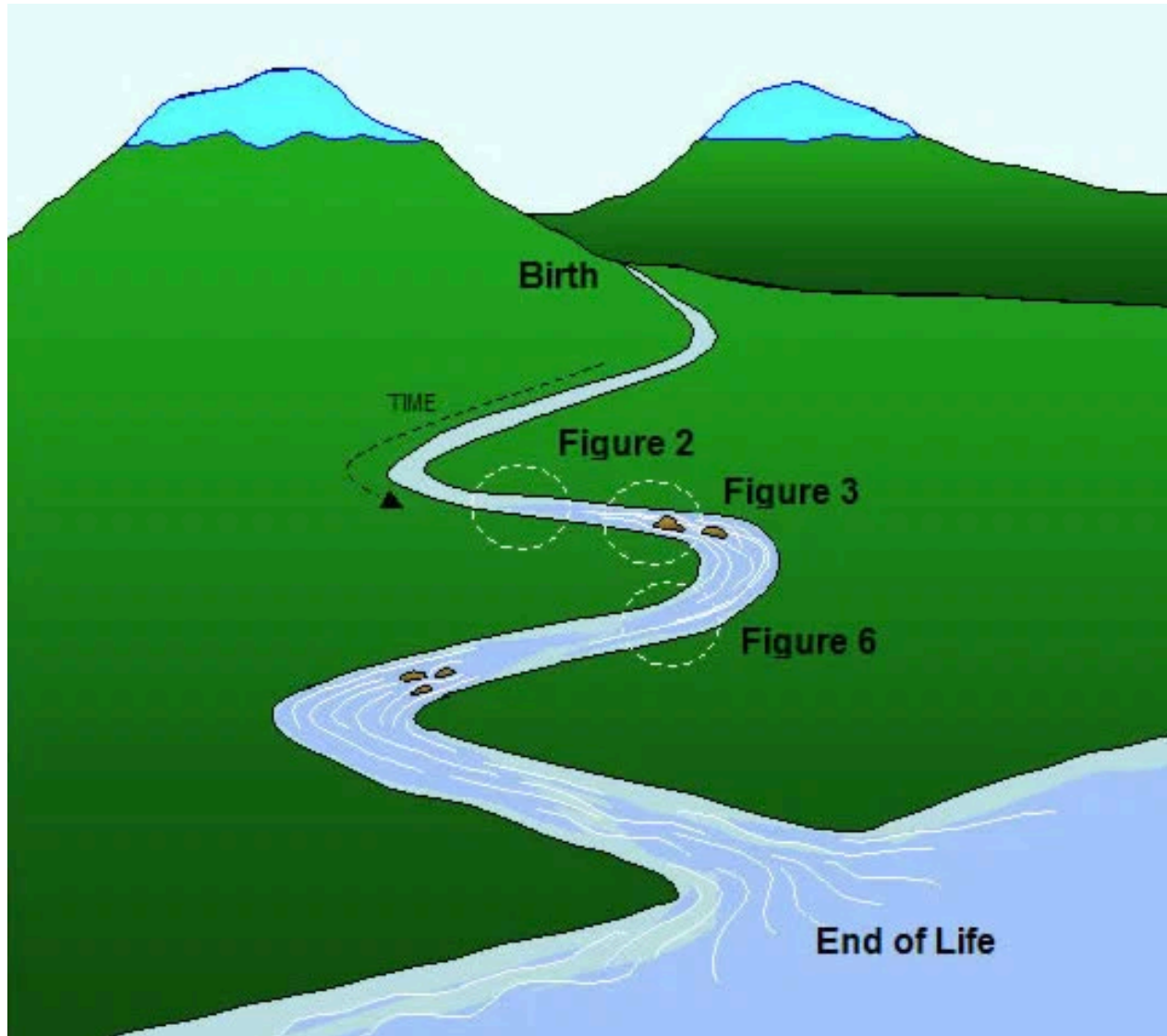
Transcription process

Before we start transcribing DNA,

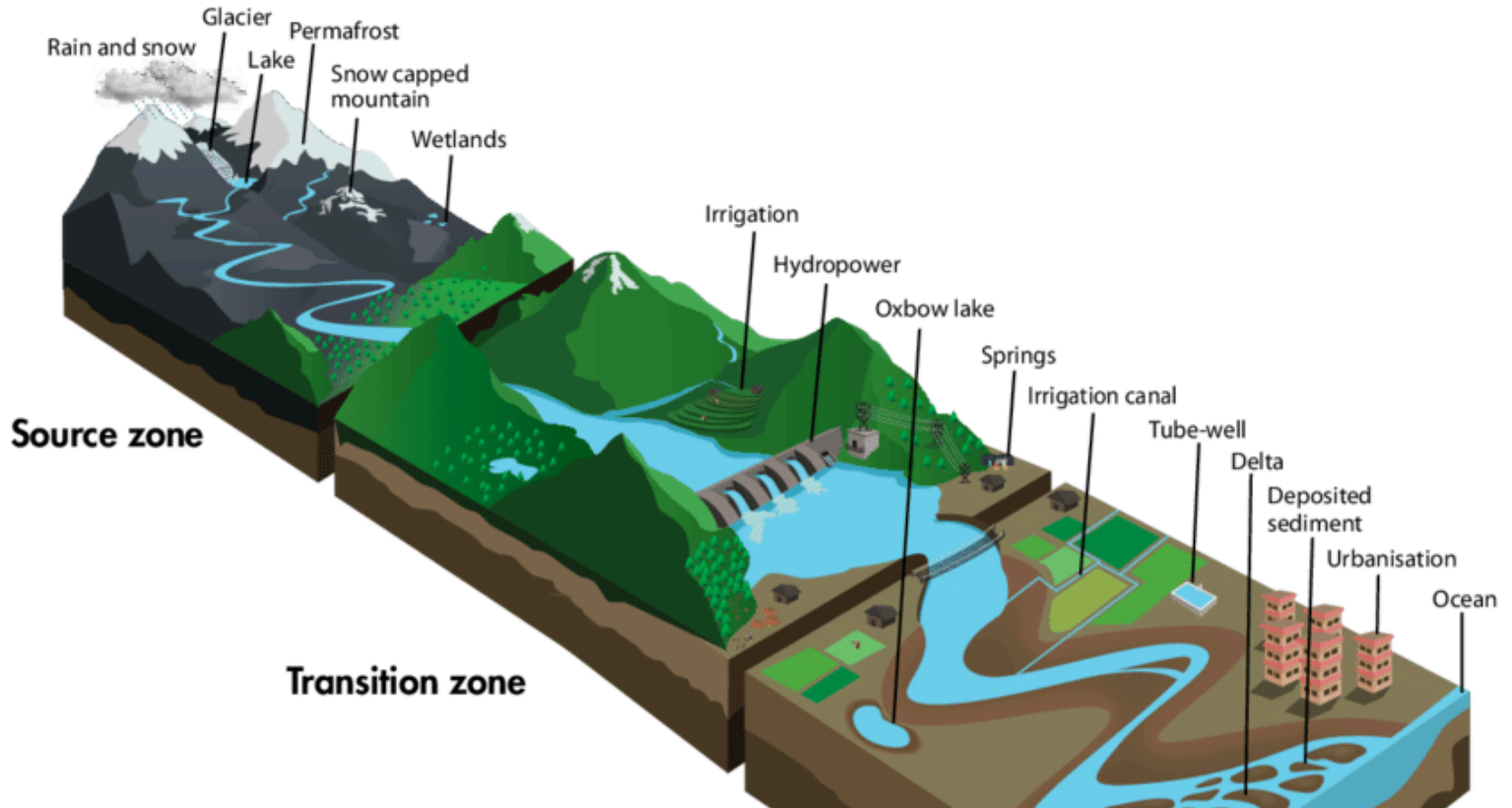
what get transcribed?

what is the gene structure in prokaryotes?

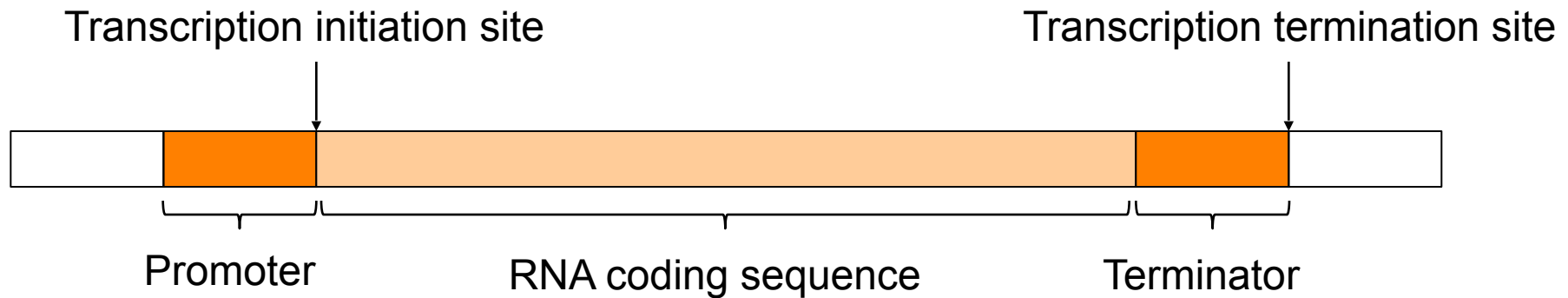
Transcription process



Transcription process



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

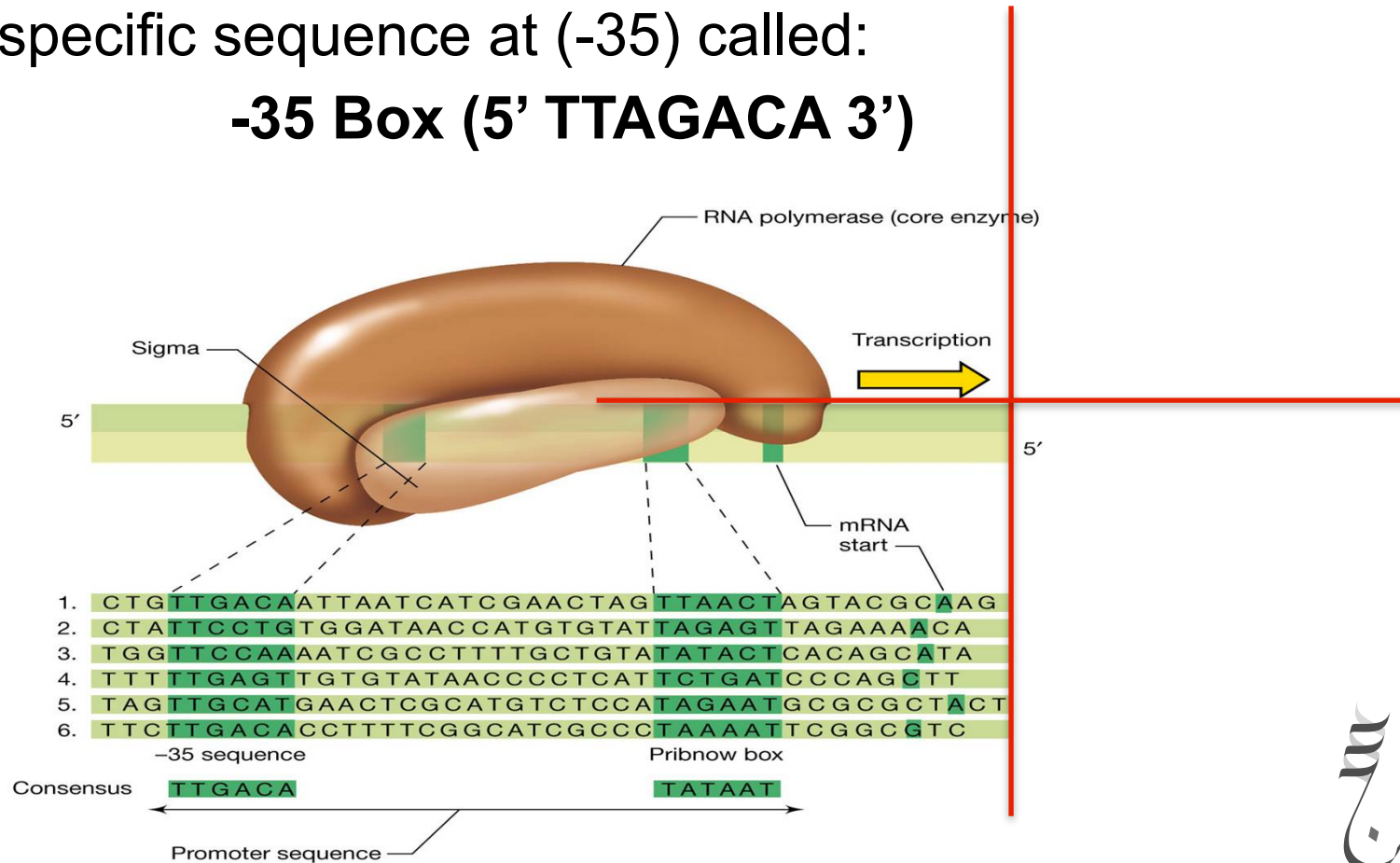
Transcription process

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' TTGACA 3')



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



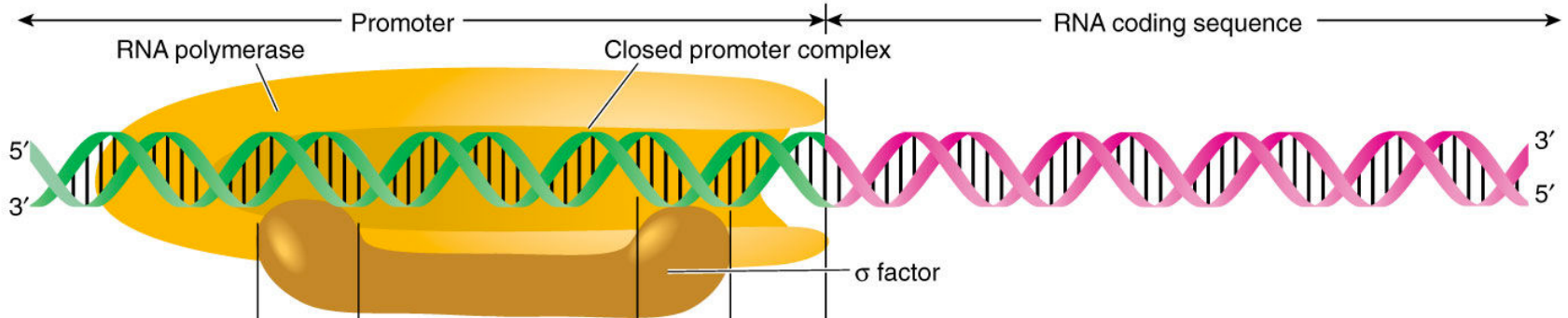
5. Untwisted promoter is called the **open promoter complex**.

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

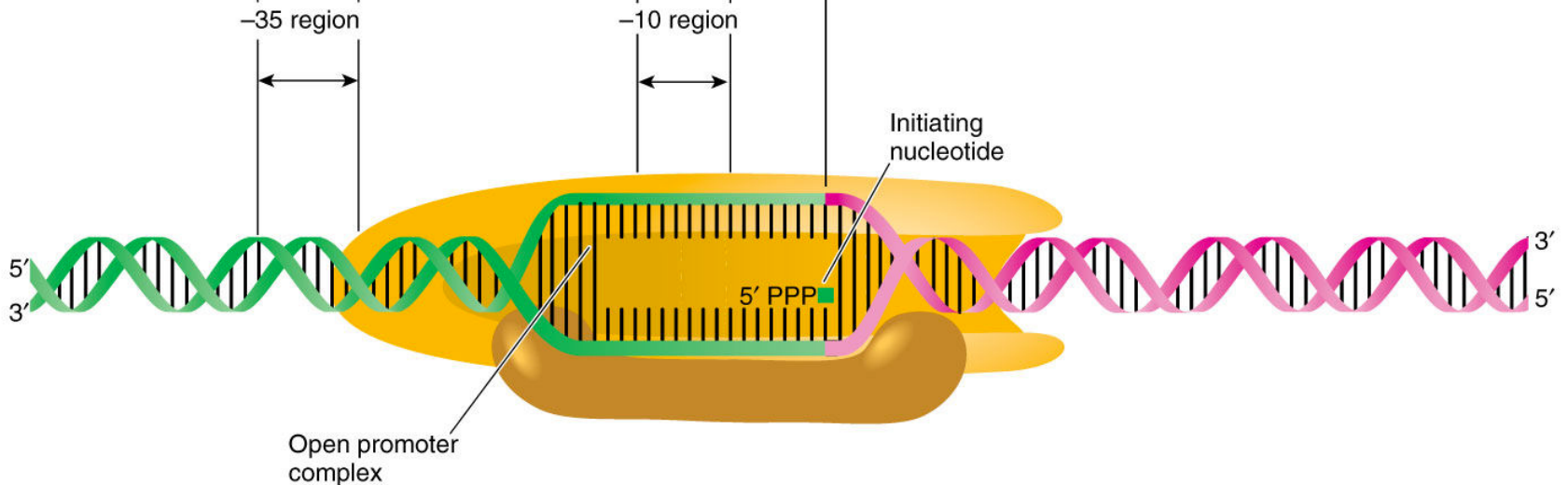
7. Sigma factor (σ) is released so that core enzyme can go forward transcribing.

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.



Transcription elongation



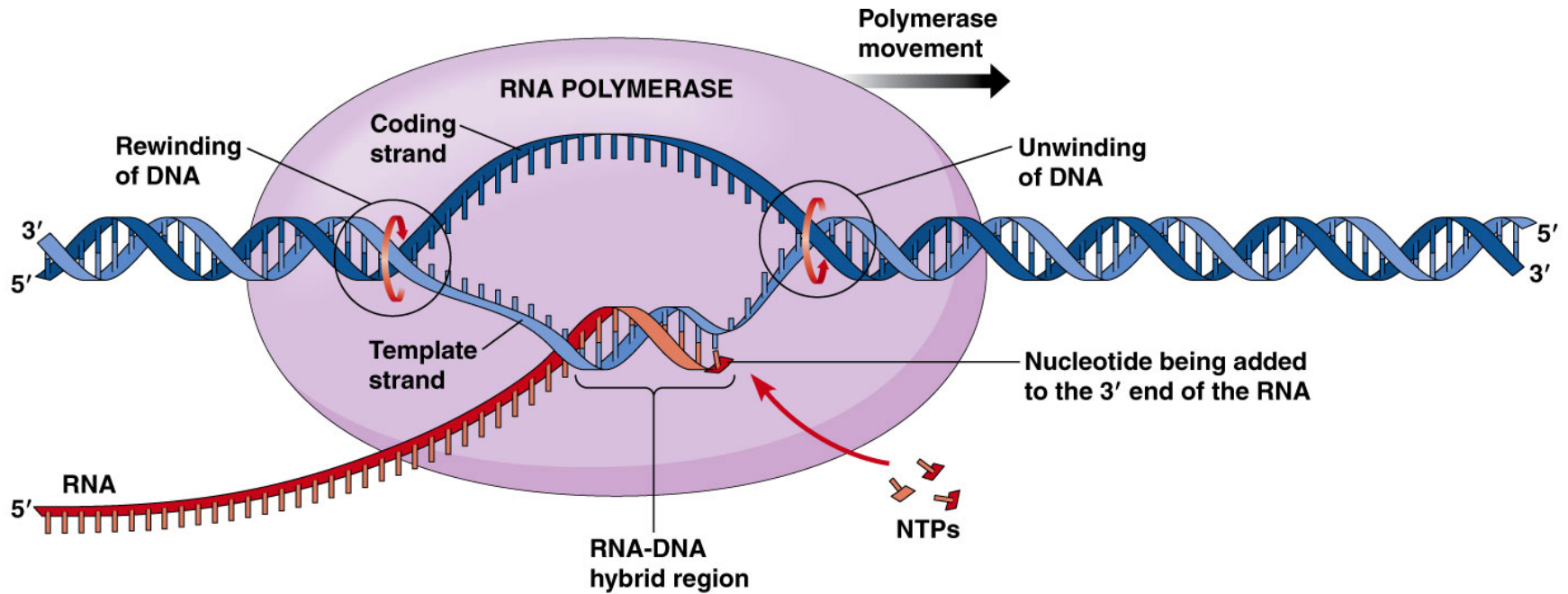
1. RNA polymerase (core enzyme) moves along to transcribe the DNA sequence into a single strand RNA of the coding gene.
2. When transcribing, the RNA polymerase interact with DNA sequence forming **transcription bubble**.
3. DNA double helix is reformed as the RNA polymerase moves forward.

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



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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

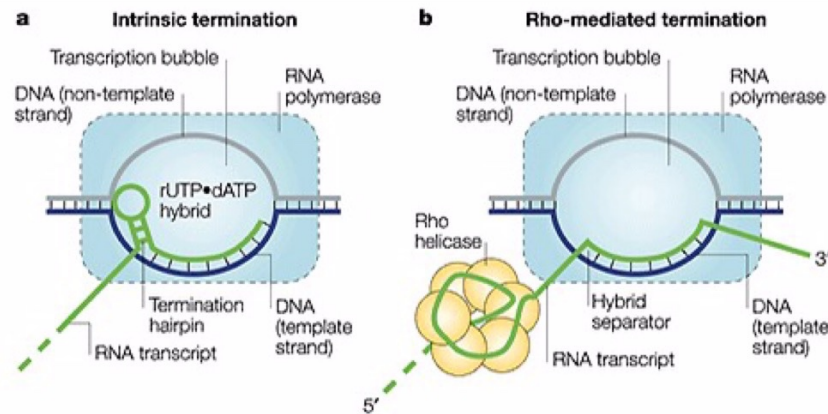
Transcription termination

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)

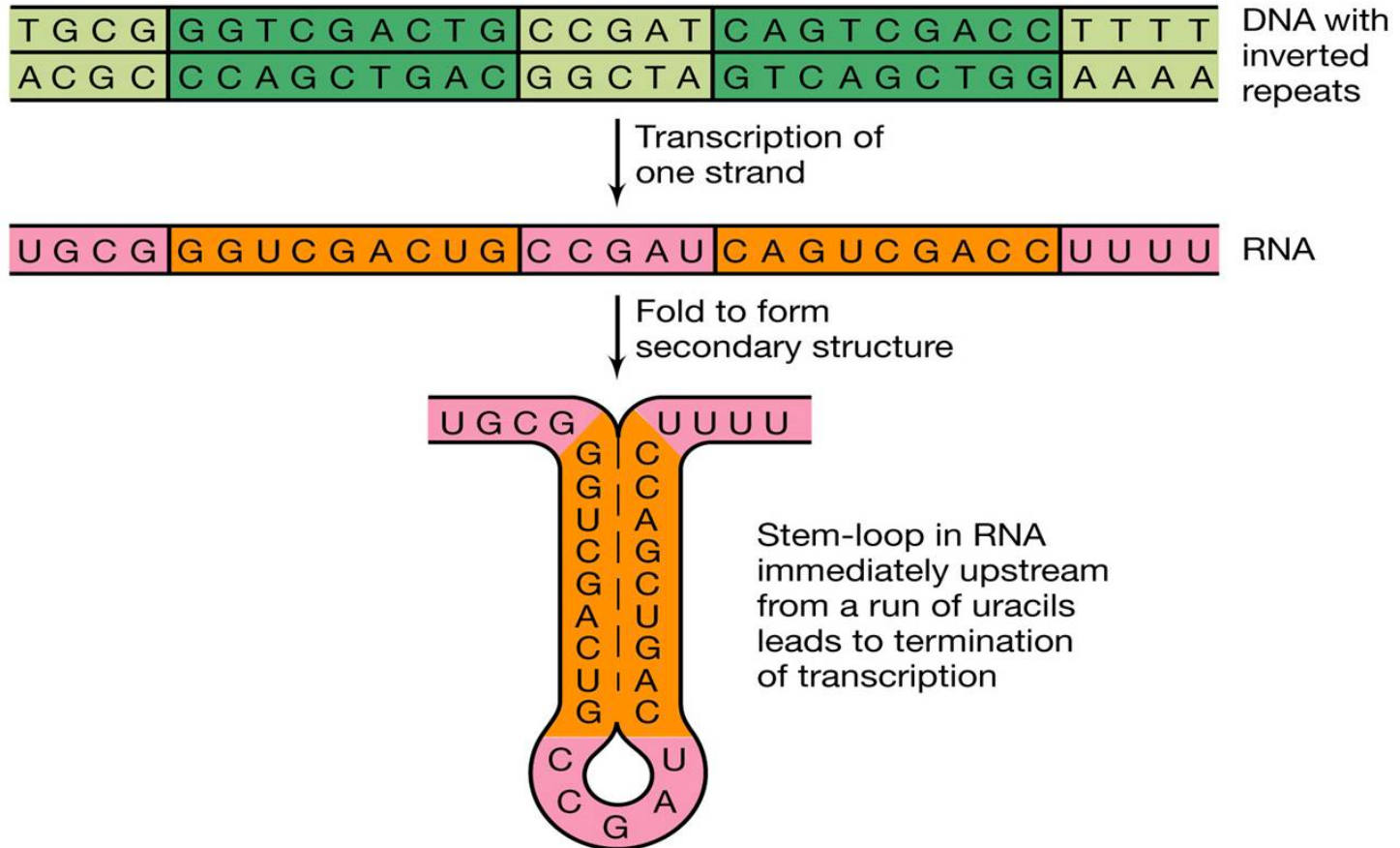
Goofy analogy



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

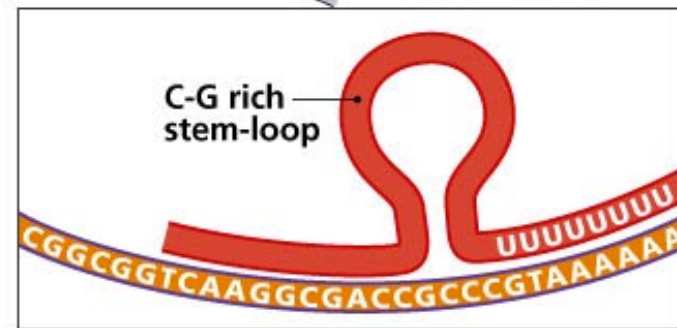
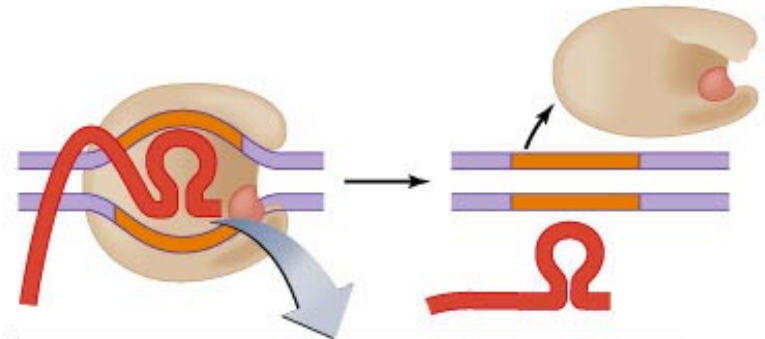
Termination (Rho-independent terminator) - type 1 terminator

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



Termination (Rho-independent terminator) - type 1 terminator

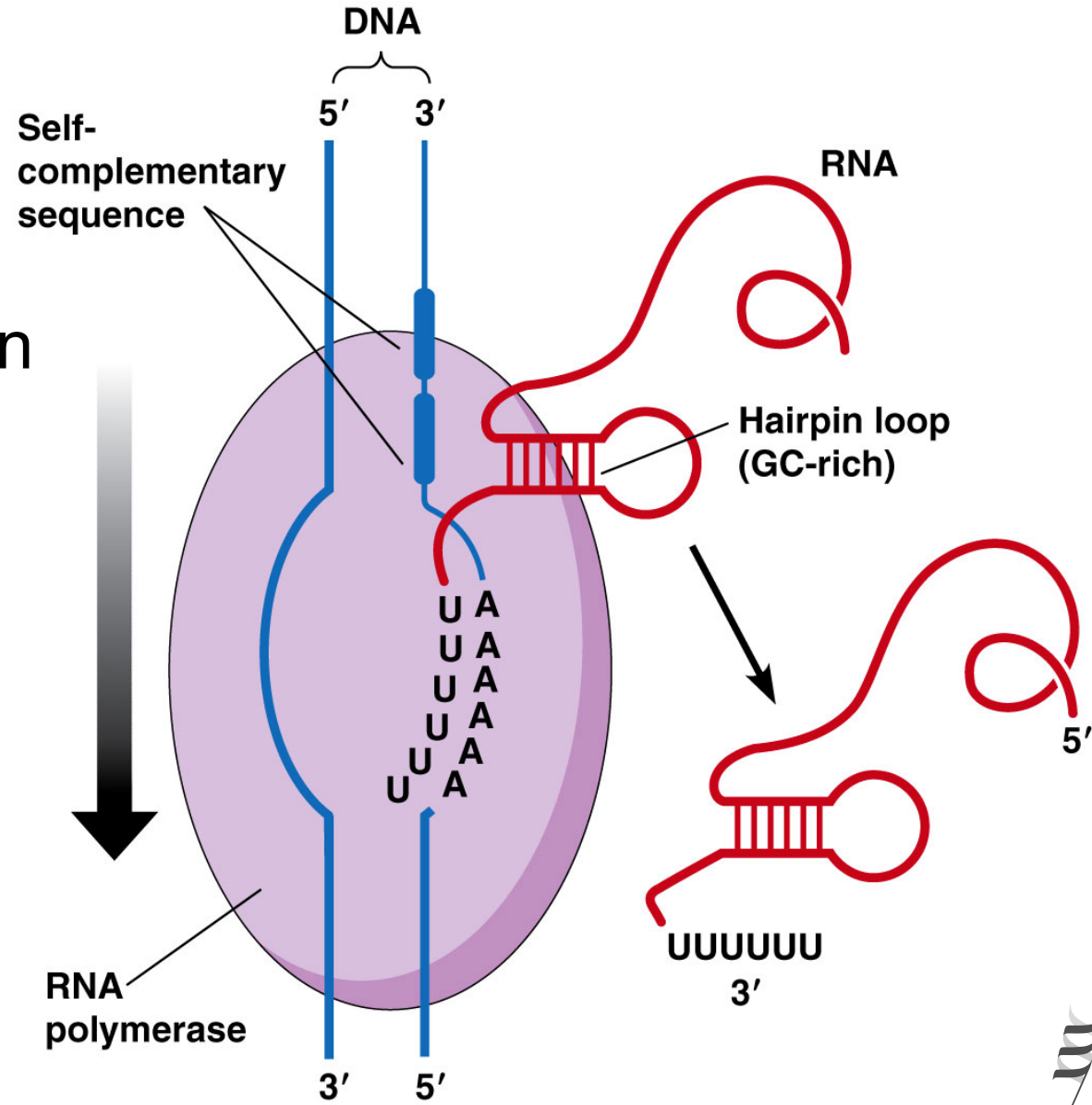
- RNA moves past the inverted repeats and transcribes the termination sequence.
- Because of the inverted repeat arrangement → RNA synthesized forms a hairpin loop structure.
- Hairpin loop makes the RNA polymerase slow down and eventually stops.



(c) Termination of transcription

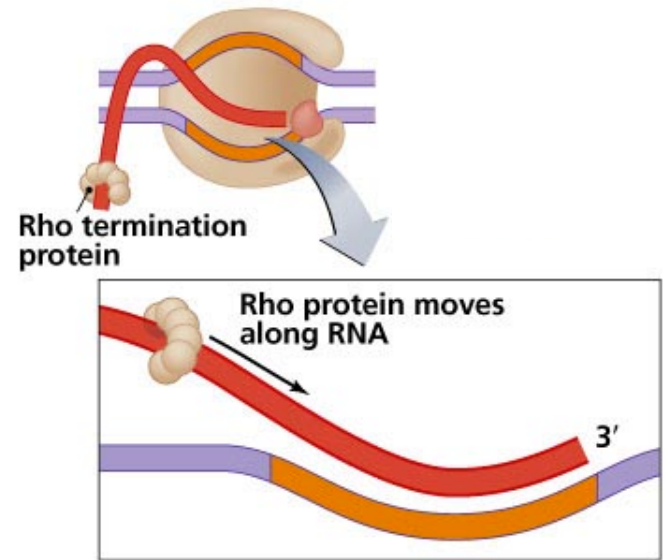
Termination (Rho-independent terminator) - type 1 terminator

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



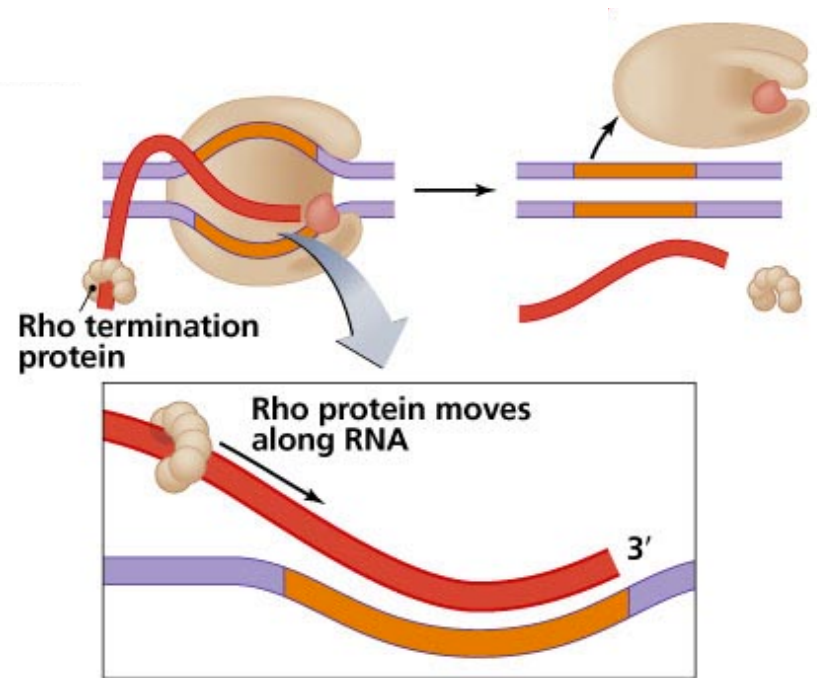
Termination (Rho-dependent terminator) – type 2 terminator

- 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.



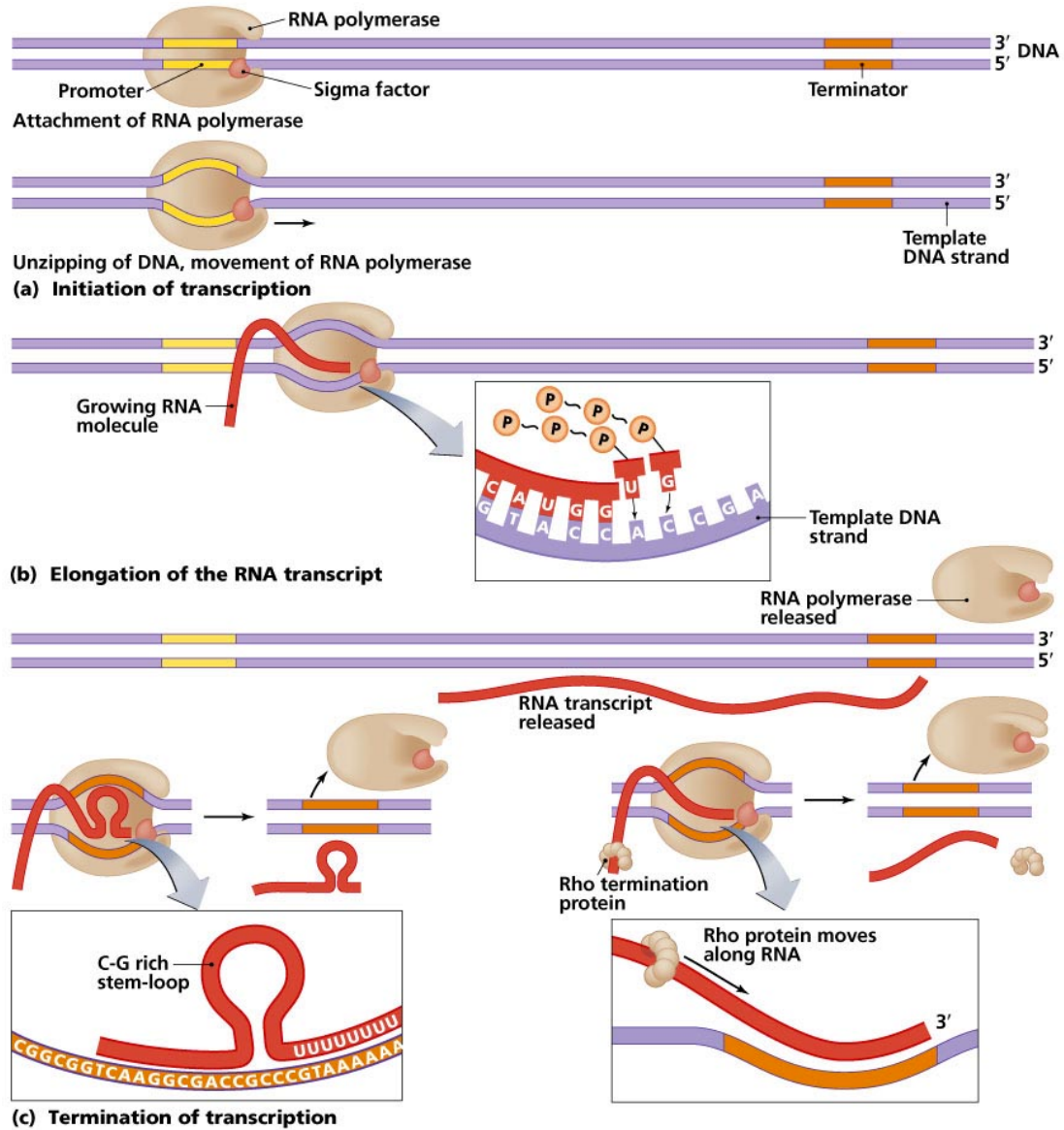
Termination (Rho-dependent terminator) – type 2 terminator

- 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.

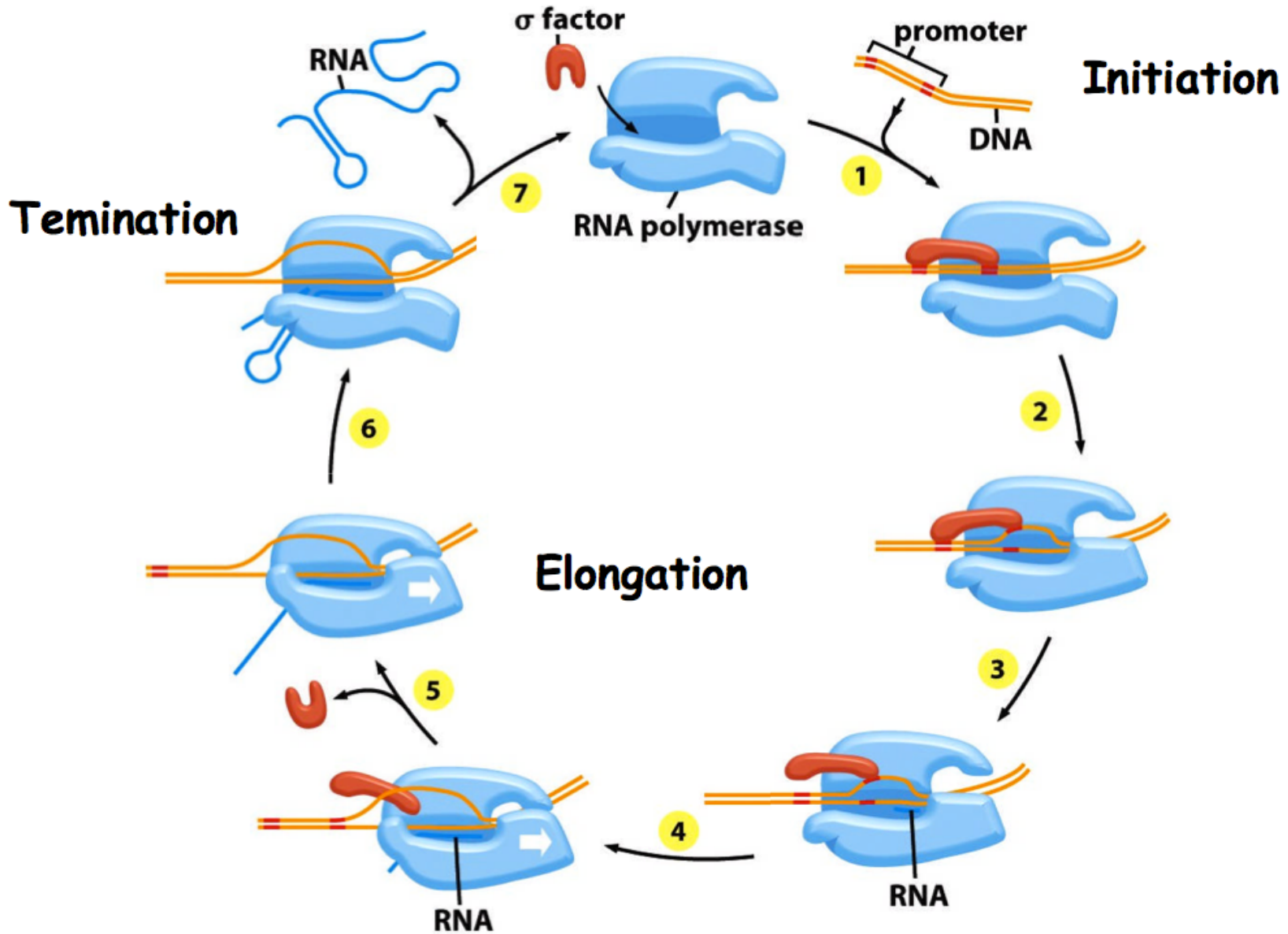


Does Rho need energy to function?

Summary



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

Upstream

Hairpin loop

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

