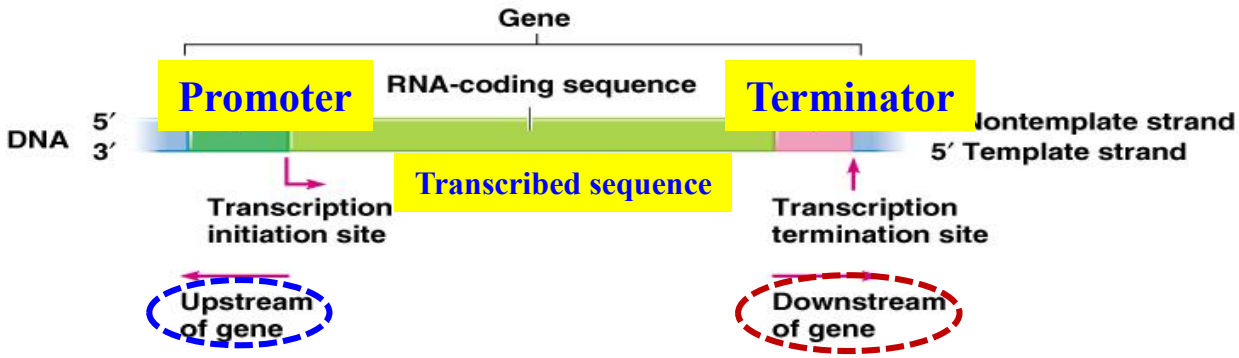
Basic Components of a Gene

Each gene has



BACTERIAL (PROKARYOTIC) TRANSCRIPTION

- Promoters

- DNA sequences that guide RNAP to the beginning of a gene (transcription initiation site).

- RNA Polymerase (RNAP)

- Enzyme for synthesis of RNA.

- Terminators

- DNA sequences that specify then termination of RNA synthesis and release of RNAP from the DNA.

- Reaction (ordered series of steps)

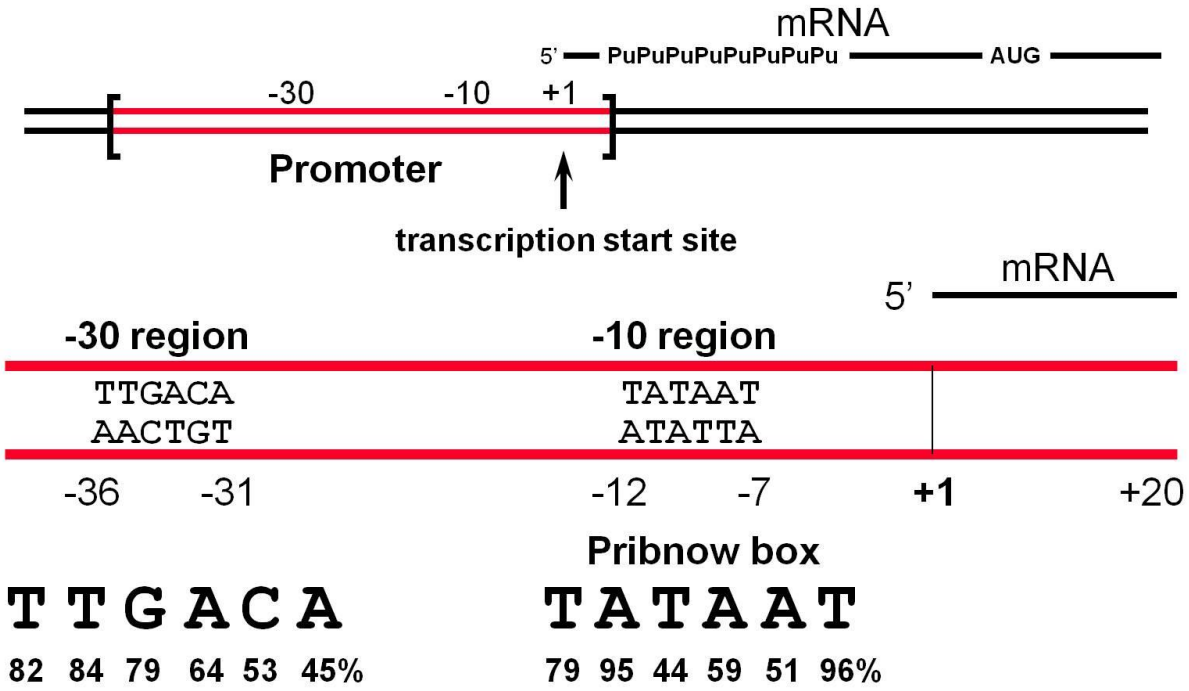
1) Initiation.

2) Elongation.

3) Termination.

Properties of Promoters

- Promoters typically consist of a 40 bp region on the 5'- side of the transcription start site.
- Two consensus sequence elements:
  - The "-35 region", with consensus TTGACA
  - The Pribnow box near -10, with consensus TATAAT - this region is ideal for unwinding.
- **Promoter structure in prokaryotes**



Consensus sequences

RNA Polymerase (RNAP) Has Many Functions

- Scan DNA and identify promoters
- Bind to promoters
- Initiate transcription

- Elongate the RNA chain
- Terminate transcription

- **Thus, RNAP is a multisubunit enzyme**

RNA polymerase structure

- **RNA polymerases are assemblies of several different proteins:**

β' (156 kDa) protein binds to the DNA template and catalytic activity resides in which subunit of RNA polymerase

β (151 kDa) protein links nucleotides a protein holds subunits together

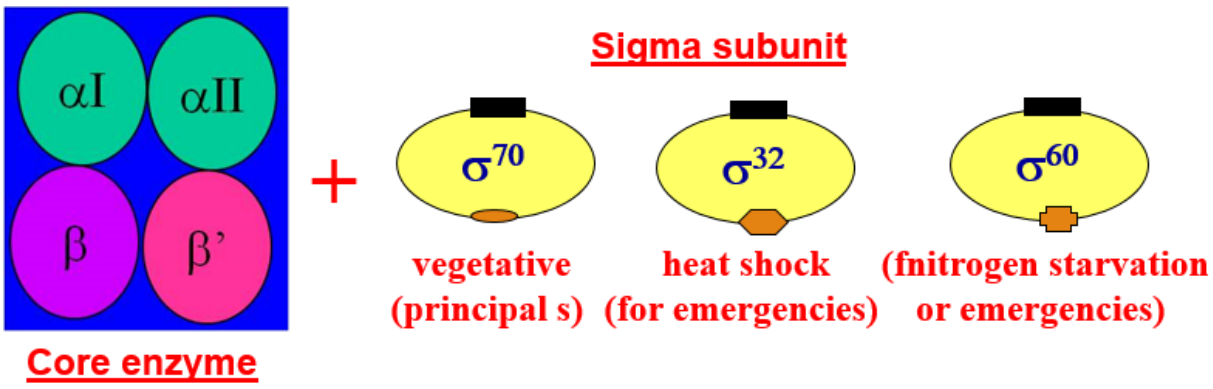
δ protein recognizes specific nucleotide sequences of promoters

- β', β and a often very similar from one bacterial species to another

$\beta\beta'\sigma \rightleftharpoons \beta\beta' + \sigma$

holoenzyme — core polymerase — sigma factor

RNA POLYMERASE STRUCTURE

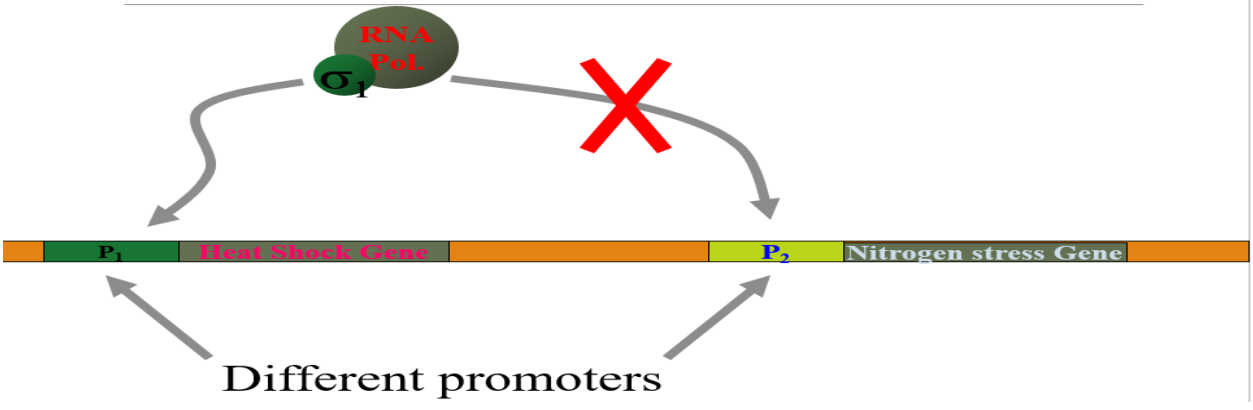


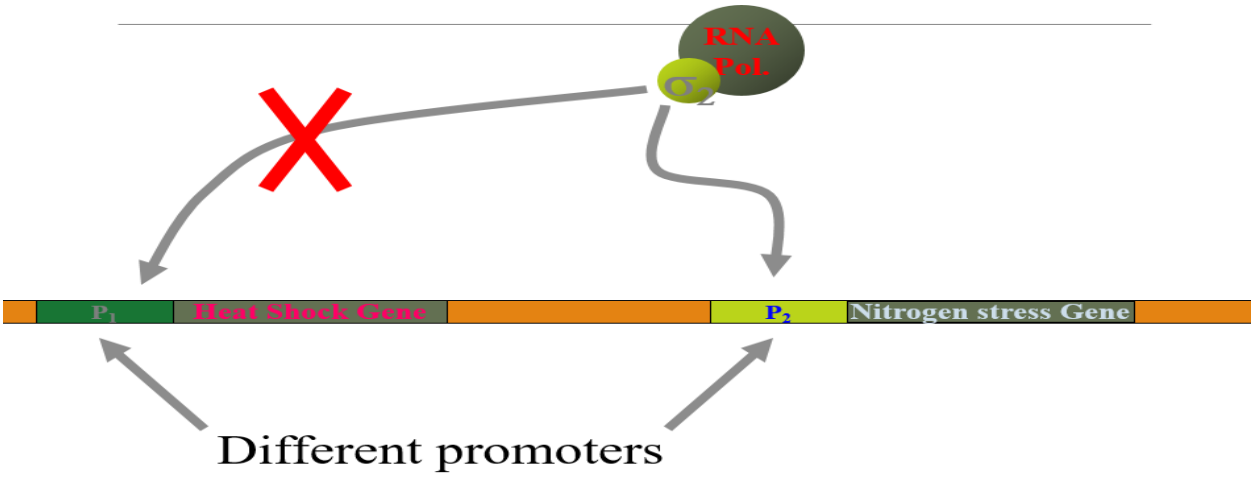
- In *E.coli*, RNA polymerase is a 465 kD complex, with 2 α, 1 β, 1 β', 1 σ (holoenzyme).
- Core enzyme is 2 α, 1 β, 1 β′ (can transcribe but it can't find promoters).
- α subunits appear to be essential for assembly and for activation of enzyme by regulatory proteins.

The function of sigma (σ) factor

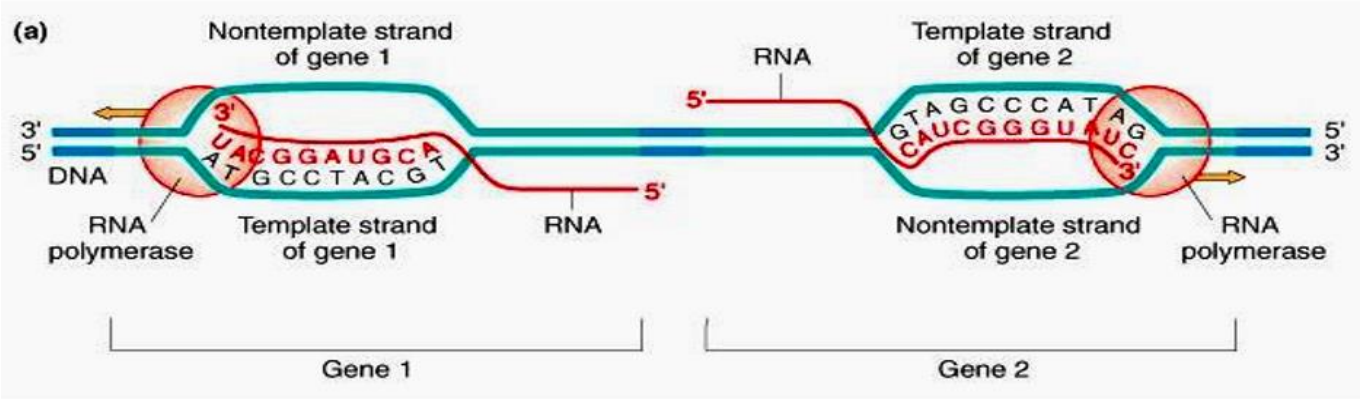
- the sigma subunit of RNA polymerase is an **"initiation factor"**
- there are several **different sigma** factors in *E. coli* that are specific for different sets of genes.
- Different σ subunits allow recognition of different types of promoters thus the type of genes transcribed can be modulated by altering the types of σ subunits which attach to RNA polymerase.
- For example, σ^{32} turns on gene expressions for genes associated with heat shock.

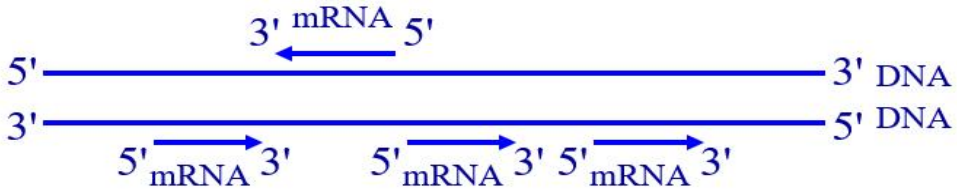
Example of sigma (σ) factor





The template strand can reside on either strand of the DNA
***so*….genes can be transcribed left to right or right to left**





THREE STEPS TO TRANSCRIPTION:

1. **Initiation**
2. **Elongation**
3. **Termination**

✓ Occur in both prokaryotes and eukaryotes.

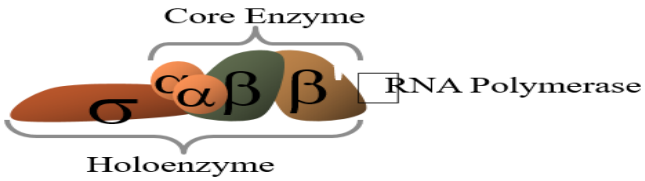
✓ Elongation is conserved in prokaryotes and eukaryotes.

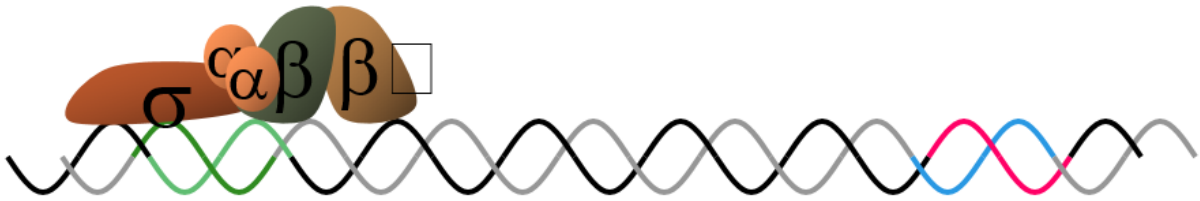
✓ Initiation and termination proceed differently.

✓ STEP 1: INITIATION

1. RNA polymerase combines with sigma factor to create RNA polymerase holoenzyme
   - ✓ Recognizes promoters and initiates transcription.
   - ✓ Sigma factor required for efficient binding and transcription.
   - ✓ Different sigma factors recognize different promoter sequences.
2. RNA polymerase holoenzyme binds promoters and untwists DNA (melting)
   - ✓ Binds loosely to the -35 promoter (DNA is ds.)
   - ✓ Binds tightly to the -10 promoter and untwists
3. Different types and levels of sigma factors influence the level and dynamics of gene expression.

General Model For Transcription





RNA polymerase holoenzyme and promoter form **"Closed promoter complex"** (DNA not unwound).

Polymerase unwinds about 12 base pairs to form **"Open promoter complex"**.

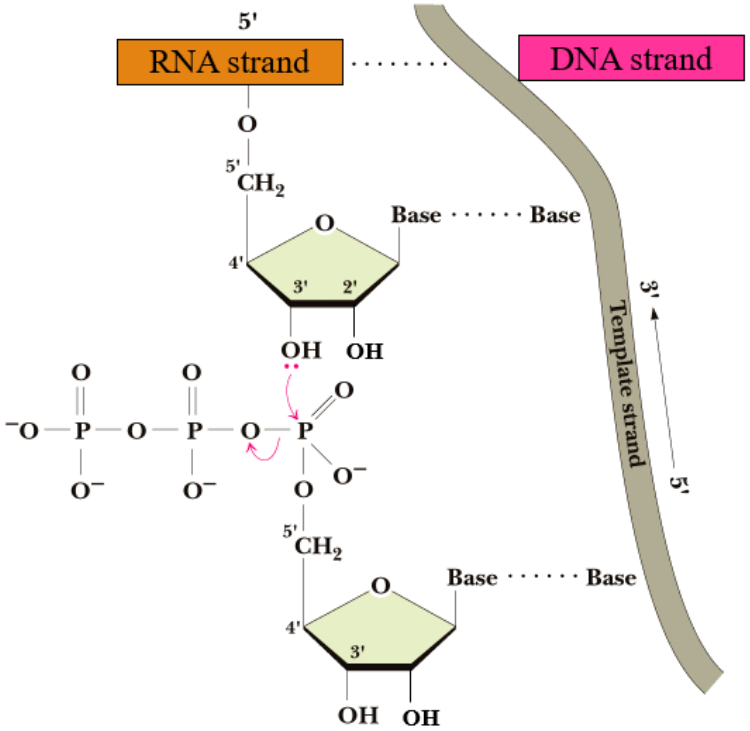
STEP 2: ELONGATION

Core polymerase - no sigma

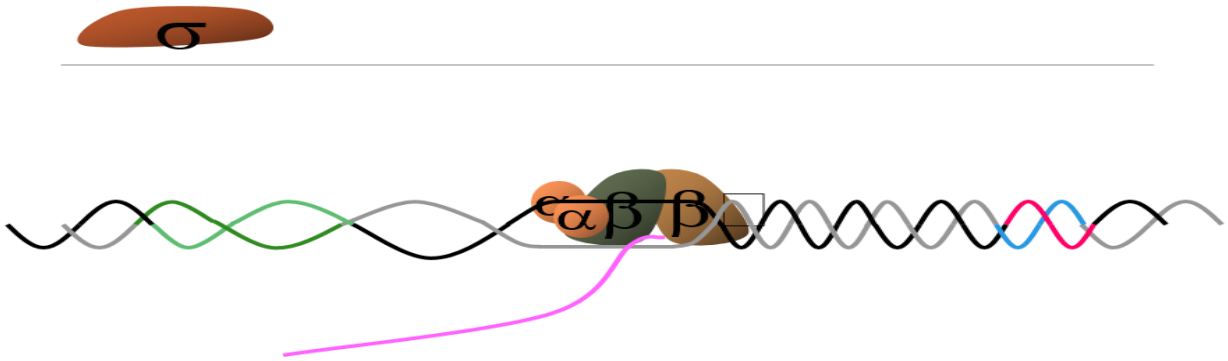
- **Polymerase is pretty accurate - only about 1 error in 10,000 bases.**
- **Even this error rate is OK, since many transcripts are made from each gene.**
- **Elongation rate is 20-50 bases per second - slower in G/C-rich regions and faster elsewhere.**
- **Topoisomerases precede and follow polymerase to relieve supercoiling.**

RNA Synthesis is in the 5' to 3' Direction

Subsequent hydrolysis of PPi drives the reaction forward



RNA has polarity (5' phosphate, 3' hydroxyl)



STEP 3: TERMINATION

Two types of terminator sequences occur in prokaryotes:

1. Type I (ρ-dependent) Involves ρ factor proteins, believed to break the hydrogen bonds between the template DNA and RNA.

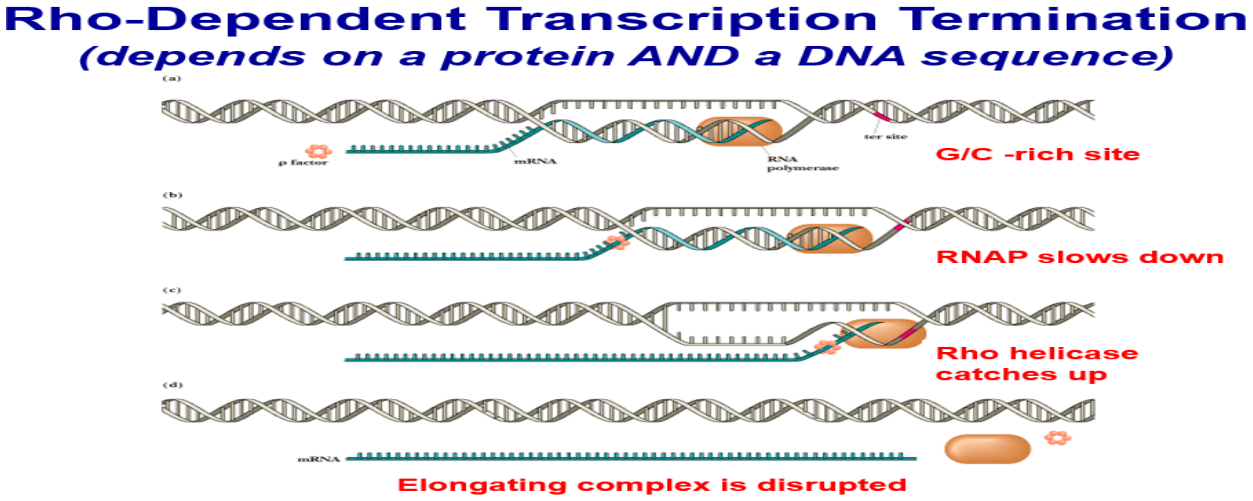
2. Type II (ρ-independent) inverse repeat forms a hairpin loop and is believed to physically destabilize the DNA-RNA hybrid.

Chain Termination

Two mechanisms

1) Rho-dependent the termination factor protein

- rho is an ATP-dependent helicase
- it moves along the RNA transcript, finds the "bubble", unwinds it and releases the RNA chain



TWO MECHANISMS

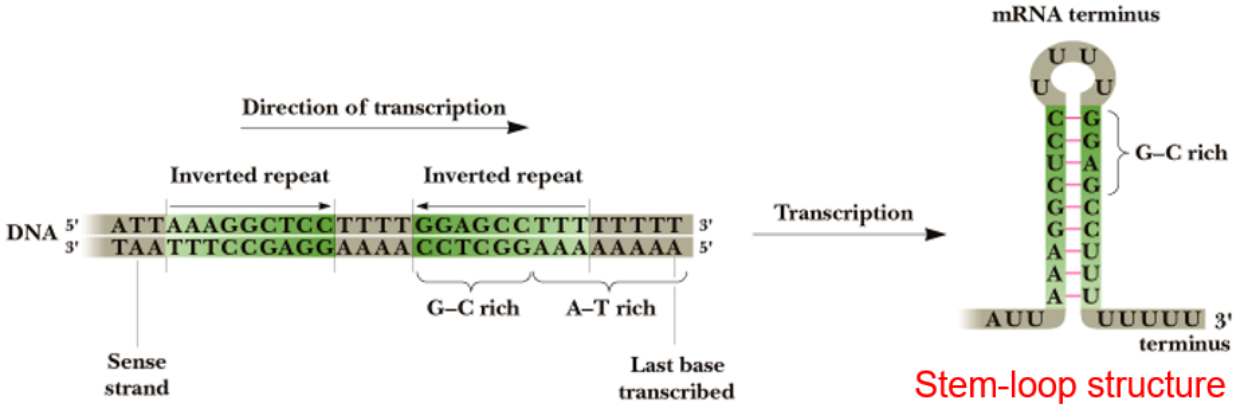
2) RHO-INDEPENDENT

Termination sites in DNA:

- **inverted repeat, rich in G:C, which forms a stem-loop in RNA transcript**
- **6-8 A's in DNA coding for U's in transcript**

Rho-Independent Transcription Termination

(depends on DNA sequence - NOT a protein factor)



LECTURE 3 QUESTIONS SHEET

1. Which protein mentioned below can reverse central dogma?
a) Ribosome
b) Restriction Endonuclease
c) Reverse Transcriptase
d) RNA Polymerase

2. The catalytic activity resides in which subunit of RNA polymerase?
a) β′ (156 kDa)
b) β (151 kDa)
c) α (37 kDa)
d) σ70 (70 kDa)

3. What is the direction in which the transcript produced by RNA polymerase grows?
a) 3'->5' direction on 3'->5' strand
b) 5'->3' direction on 5'->3' strand
c) 3'->5' direction on 5'->3' strand
d) 5'->3' direction on 3'->5' strand

4. In an experiment you use RNA polymerase without its sigma factor for transcription. What will be the result that you observe?
a) More transcription
b) Less transcription
c) More specific transcription
d) More random transcription

5. Which of these is the 1st event to take place during transcription initiation?
a) Formation of a closed initiation complex
b) Formation of open initiation complex
c) Formation of absorptive transcript
d) Promoter clearance

6. The catalytic unit of RNA polymerases when placed properly during initiation is just over _____
a) -1 site
b) 0 site
c) +1 site
d) – 10 site

WITH OUR BEST WISHES