MOLECULAR BIOLOGY: TRANSCRIPTION IN PROKARYOTES

Lecture 2
Lecture slides

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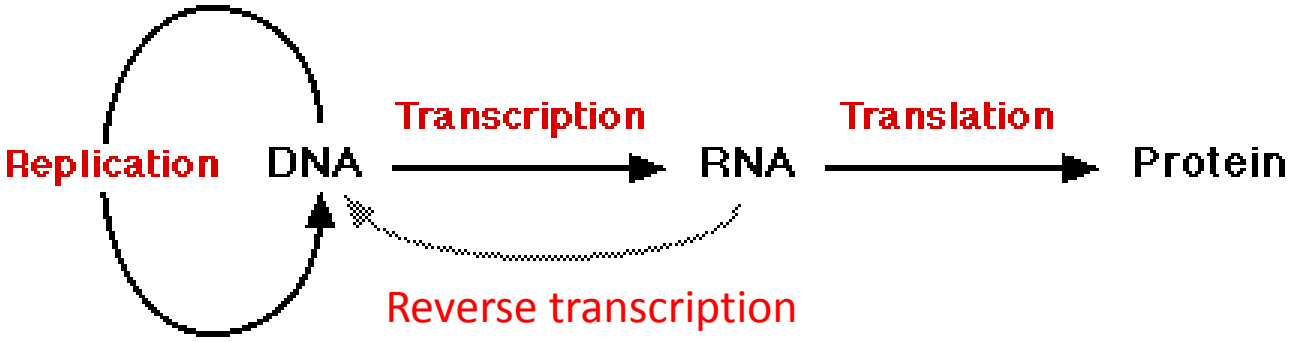
Transcription

- This refers to the synthesis of RNA molecules using DNA strands as the templates so that the genetic information can be transferred from DNA to RNA.
- The synthesis of RNA is the first step of gene expression
- The Central dogma of Molecular Biology said that the flow of genetic information is from DNA to RNA to protein.
- It was later discovered that information can flow from RNA to DNA a process termed Reverse transcription



Structural genes

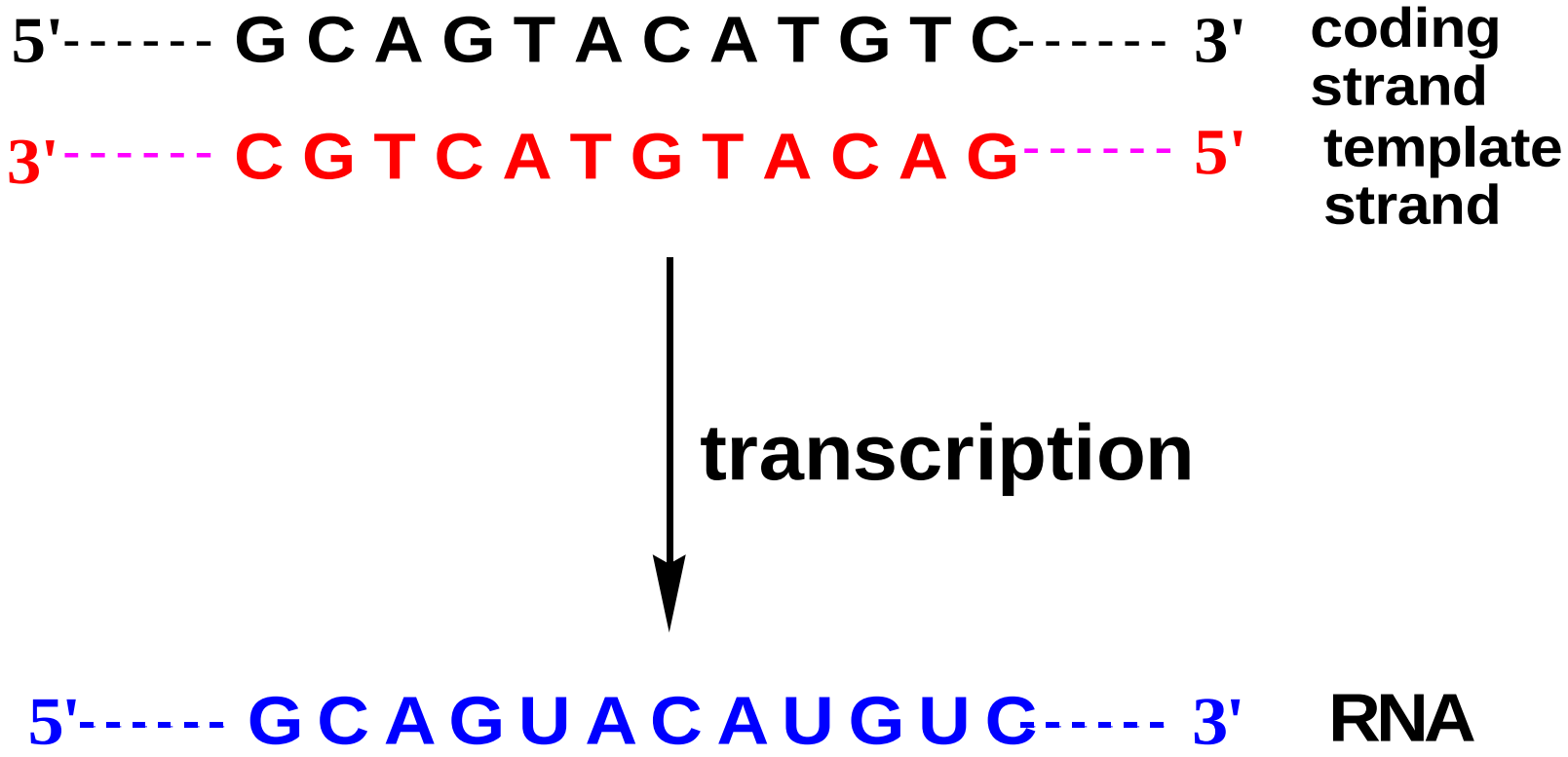
- The whole genome of DNA needs to be replicated, but only small portion of genome is transcribed in response to the development requirement, physiological need and environmental changes.
- DNA regions that can be transcribed into RNA are called **structural genes.**

Features of transcription

- Process of transcription takes place in the nucleus
- Three stages
  - Initiation
  - Elongation
  - Termination
- One strand is used as a template to produce mRNA

-The template strand is the strand from which the RNA is transcribed. It is also termed as **antisense or non-coding or minus** 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 or plus strand**.



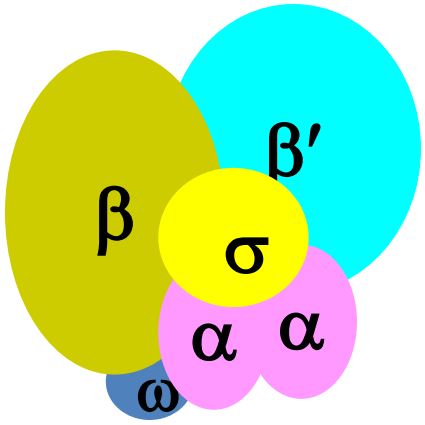
- Only the template strand is used for the transcription, but the coding strand is not.
- This feature is referred to as the asymmetric transcription.

RNA Polymerase

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

•The holoenzyme (catalytically active enzyme) of RNA-polymerase in *E.coli* consists of 6 different subunits: $\alpha_2\ \beta\ \beta'\ \omega\sigma$.

The core enzyme has 5 subunits: $\alpha_2\ \beta\ \beta'\omega$.



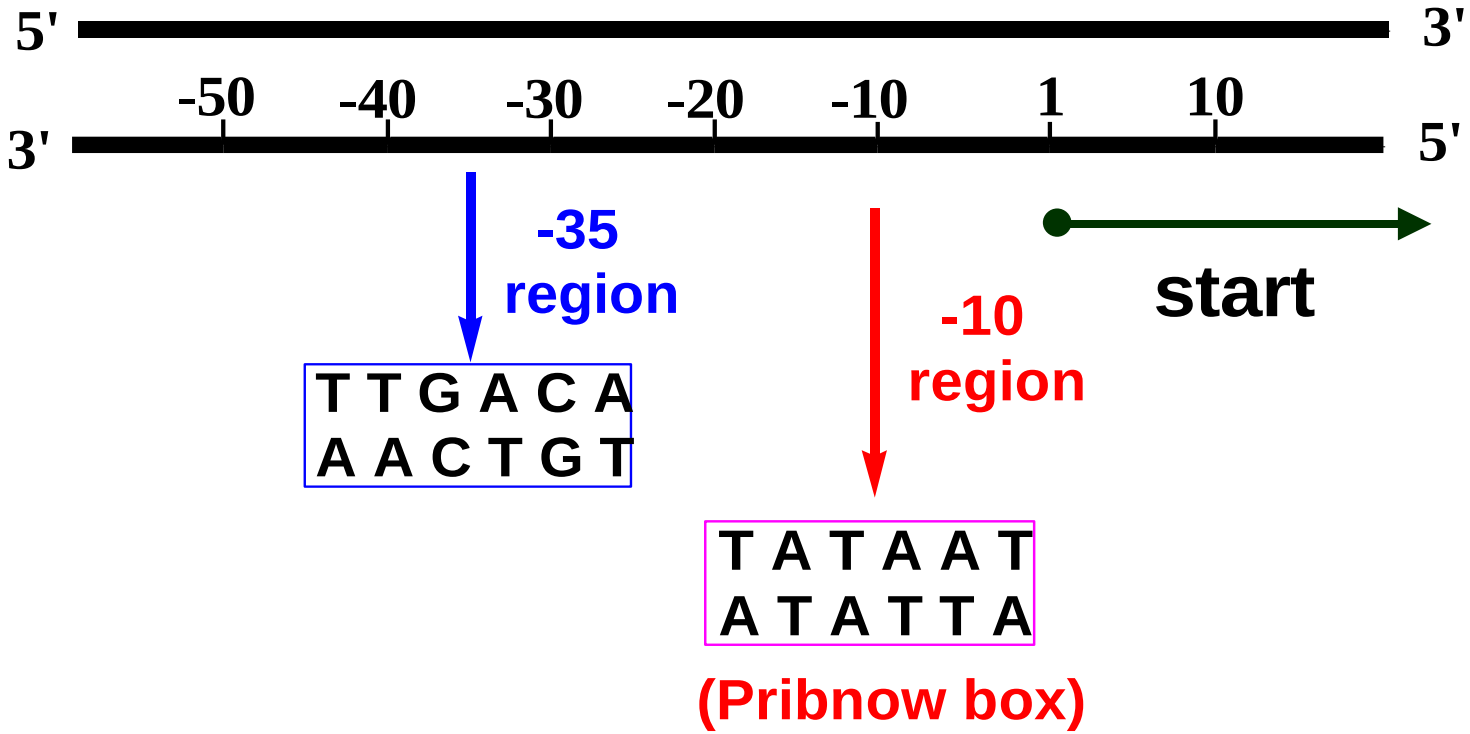
RNA-polymerase of *E. Coli*

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

- **Rifampicin**, a therapeutic drug for tuberculosis treatment, can bind specifically to the β subunit of RNA-polymerase, and inhibit the RNA synthesis in prokaryotes.
- Although mammalian RNA polymerase is different from that of prokaryotes, inhibition of the latter is possible without great toxicity to host. A good therapeutic index for the drug is required.
- RNA-polymerase of other prokaryotic systems is similar to that of *E. coli* in structure and functions.

Prokaryotic promoter

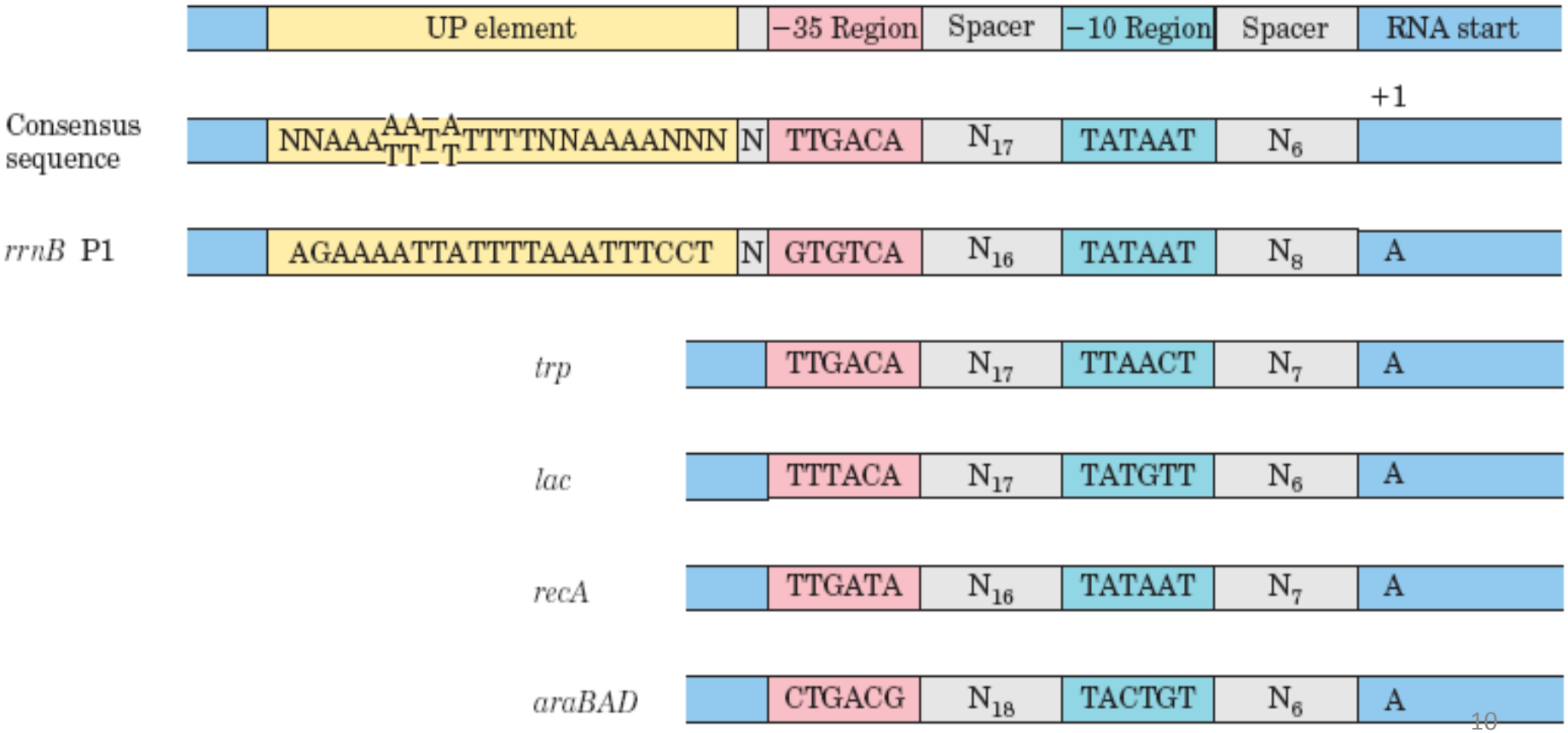
- The promoter is the DNA sequence that RNA-pol can bind. It is the key point for the transcription control.



- **The -35 region of TTGACA sequence is the recognition site and the binding site of RNA-pol.**
- **The -10 region of TATAAT is the region at which a stable complex of DNA and RNA-pol is formed.**

Consensus sequence

- A DNA sequence consisting of the residues that most commonly occur at each position in a set of similar sequences



Transcription Process in Prokaryotes

- Three phases:

  -initiation,

  -elongation, and

  - termination.
- The prokaryotic RNA polymerase can bind to the DNA template directly in the transcription process.
- **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.

Initiation

- RNA-pol recognizes the TTGACA region, and slides to the TATAAT region, then opens the DNA duplex.
- The first nucleotide on RNA transcript is always purine triphosphate. GTP is more often used than ATP.
- The initiating NTP (the iNTP) and guanosine 5’-diphosphate 3’-diphosphate (ppGpp)
- The pppGpN-OH structure remains on the RNA transcript until the RNA synthesis is completed.
- The three molecules form a transcription initiation complex.

- No primer is needed for RNA synthesis.
- The σ subunit falls off from the RNA-pol once the first 3′,5′ phosphodiester bond is formed.
- The core enzyme moves along the DNA template to enter the elongation phase.

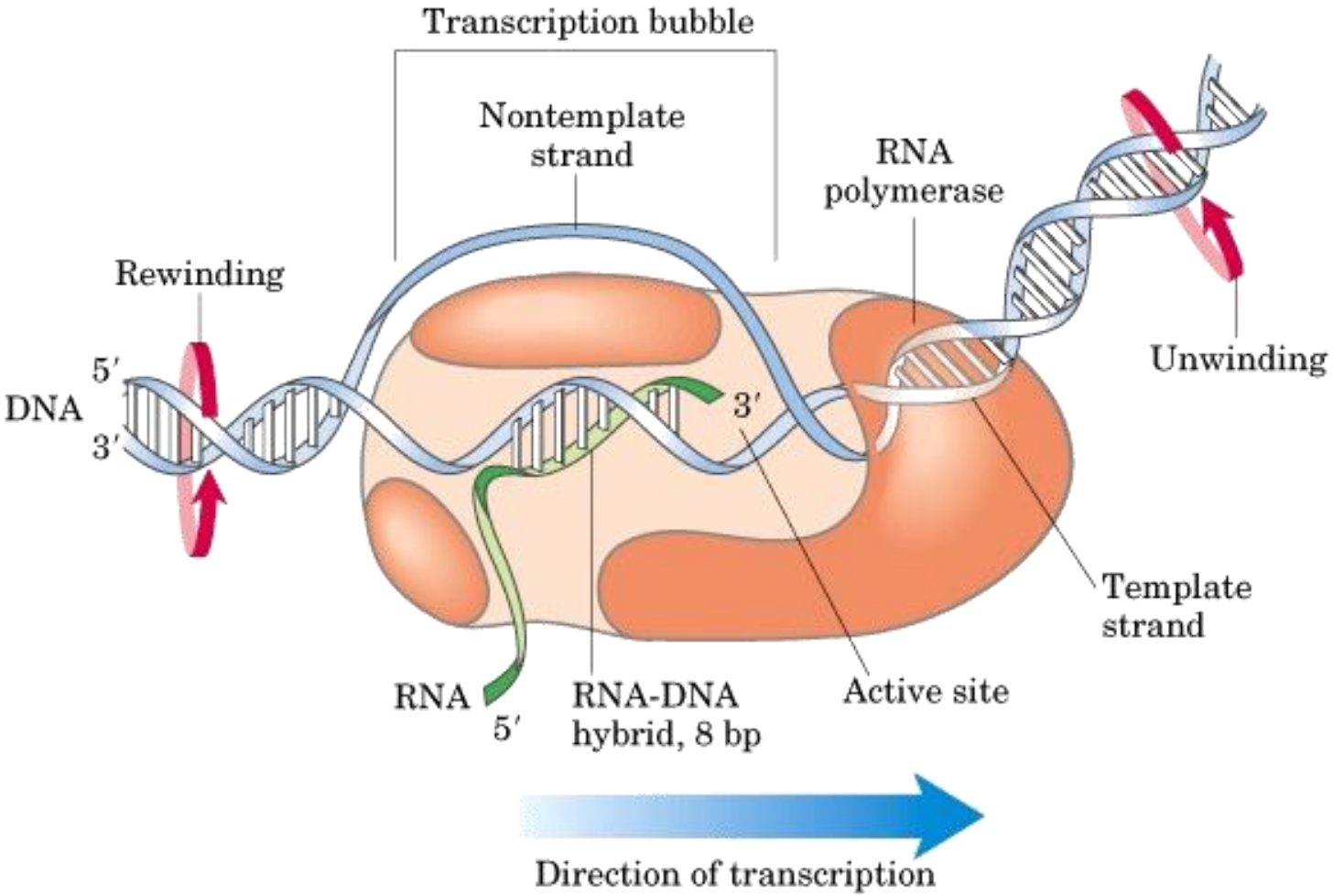
Elongation

- The release of the σ subunit causes the conformational change of the core enzyme. The core enzyme slides on the DNA template toward the 3′ end of the coding strand (non-template strand) or 5’ end of template strand.
- Free NTPs are added sequentially to the 3′ -OH of the nascent RNA strand.

$$(NMP)_n + NTP \longrightarrow (NMP)_{n+1} + PPi$$

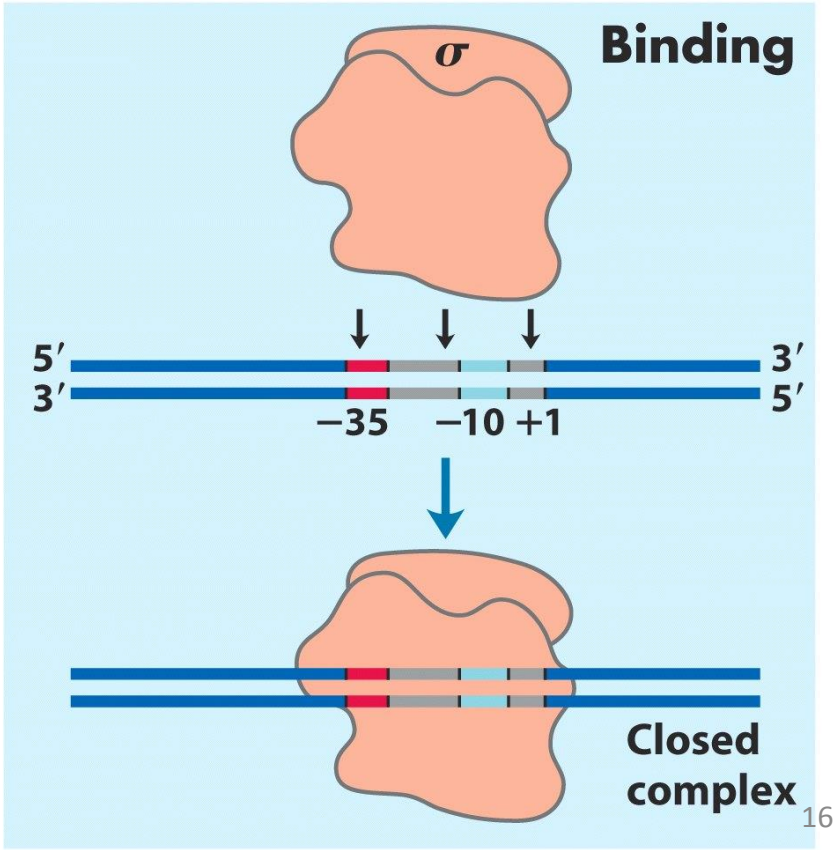
RNA strand substrate elongated RNA strand

- RNA-pol, DNA segment of ~40nt and the nascent RNA form a complex called the **transcription bubble.**
- The 3′ segment of the nascent RNA hybridizes with the DNA template, and its 5′ end extends out of the transcription bubble as the synthesis is progressing.

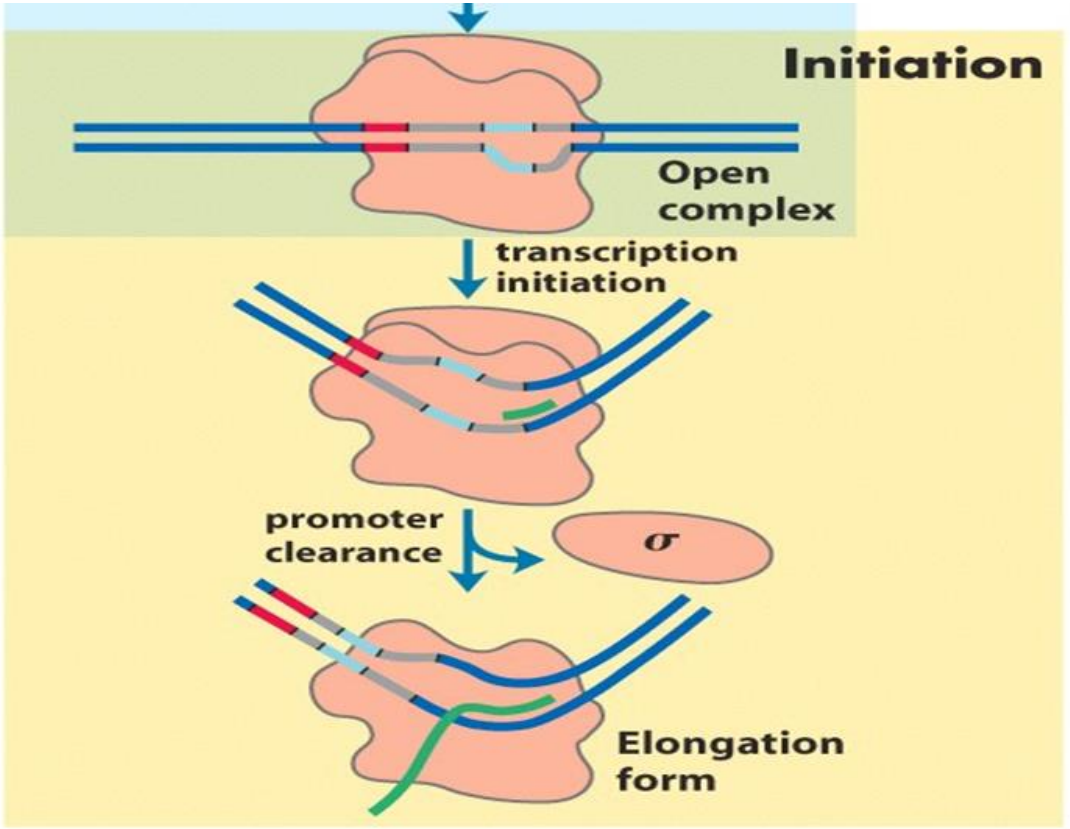


Binding Step of Transcription

- The initial interaction of RNA-pol with the promoter leads to a formation of a closed complex, in which the promoter is stably bound but not unwound.



Initiation and Elongation Steps of Transcription



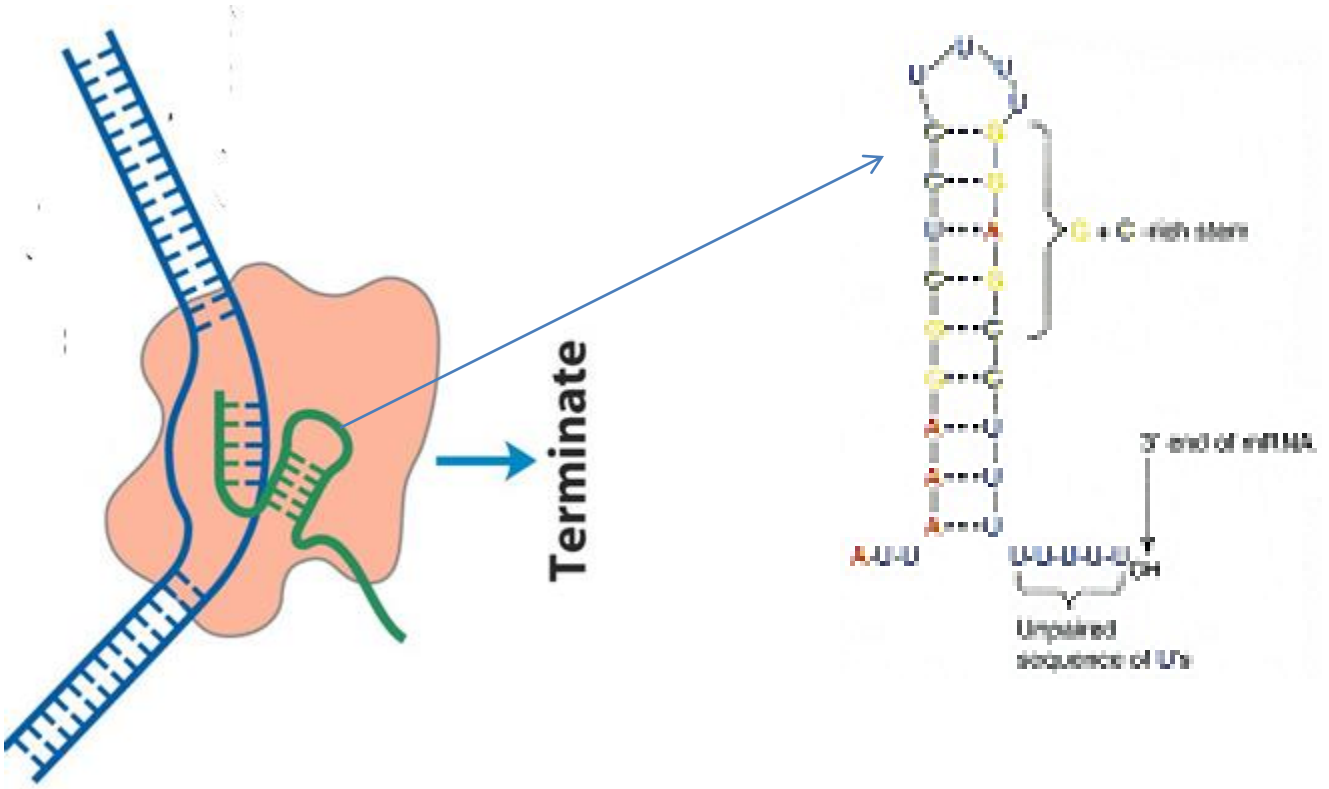
- A 12 to 15 bp region of DNA is then unwound to form an open complex, followed by transcription initiation and promoter clearance.
- Once elongation commences, the σ subunit is released and the polymerase leaves the promoter and becomes committed to elongation of RNA.

Termination

- Transcription stops at sequences called terminators.
- Example:Rho(***ρ)*-independent terminators** have two distinguishing features:

1. The first is a region that produces an RNA transcript with self-complementary sequences, permitting the formation of a hairpin structure centered 15 to 20 nucleotides before the projected end of the RNA strand.
2. The second feature is a highly conserved string of three A residues in the template strand that are transcribed into U residues near the 3’ end of the hairpin.

- When a polymerase arrives at a termination site with this structure, it pauses.
- Formation of the hairpin structure in the RNA disrupts several A=U base pairs in the DNA-RNA hybrid segment and may disrupt important interactions between RNA and the RNA polymerase, facilitating dissociation of the transcript.



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	double strands	single 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