Chapter 8: RNA Synthesis and Processing

Lecture 9: Transcription in Bacteria

Learning objectives

- Explain how *E. coli* RNA polymerase initiates transcription
- Diagram a bacterial promoter sequence
- Describe the processes of transcription termination

RNA polymerase

• catalyzes the polymerization of ribonucleoside triphosphates (NTPs) as directed by a DNA template

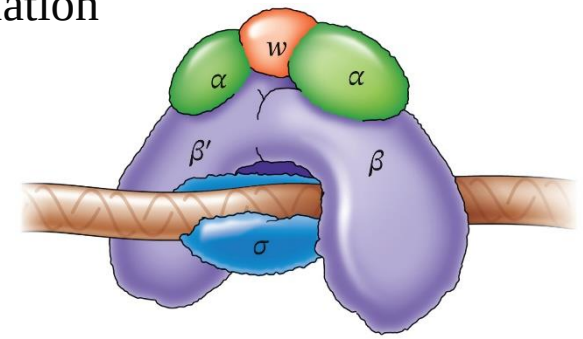
• catalyzes the growth of RNA chains always in the 5’ to 3’ direction

• dose not require a preformed primer to initiate the synthesis of RNA; *de novo* synthesis

***E. Coli* RNA polymerase**

• Core polymerase (2α, β, β’, ω) : RNA synthesizing activity

• σ : required to identify the correct sites for transcription initiation



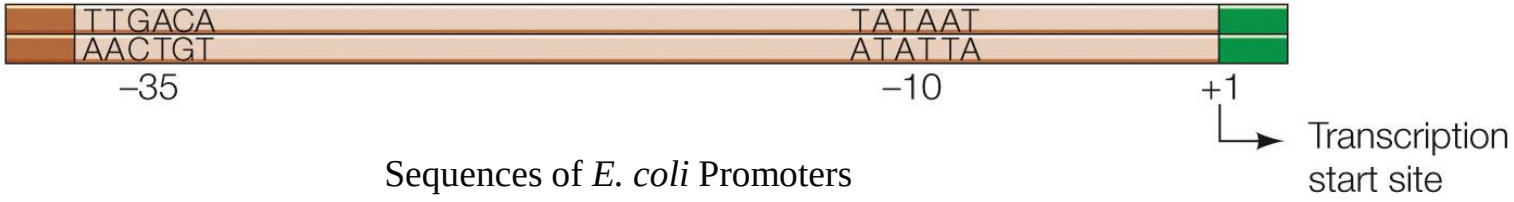
E. coli RNA Polymerase

Bacterial Promoters

: The DNA sequence to which RNA polymerase binds to initiate transcription of a gene

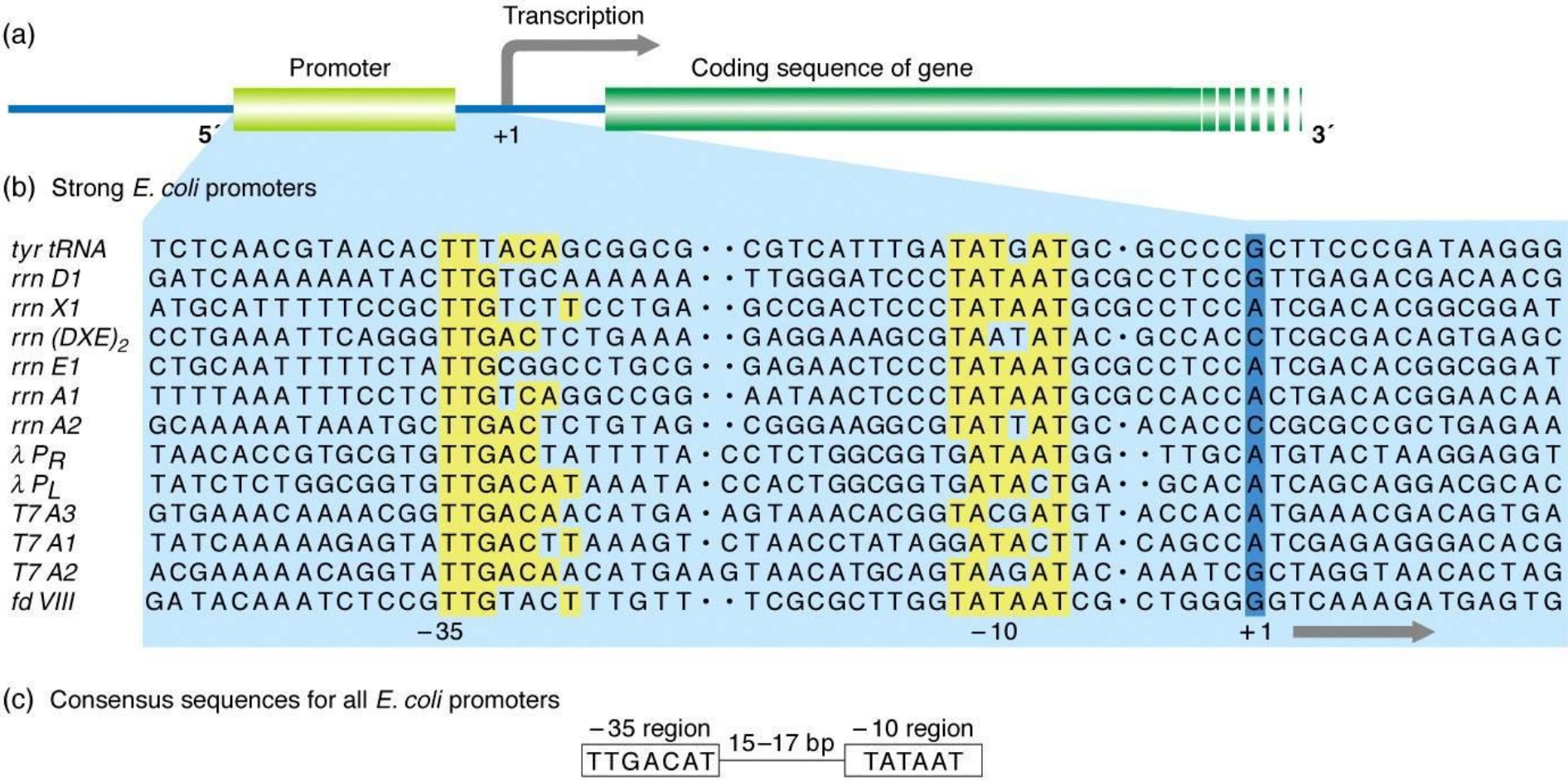
; Consensus sequences (공통서열): -10 element & -35 element

1. Genes with promoters that differ from the consensus sequences are transcribed less efficiently.
2. Mutations introduced in either –35 or –10 consensus sequences have strong effects on promoter function.
3. The sites at which RNA polymerase binds to promoters have been directly identified by footprinting experiments



Sequences of *E. coli* Promoters

E. coli Promoters



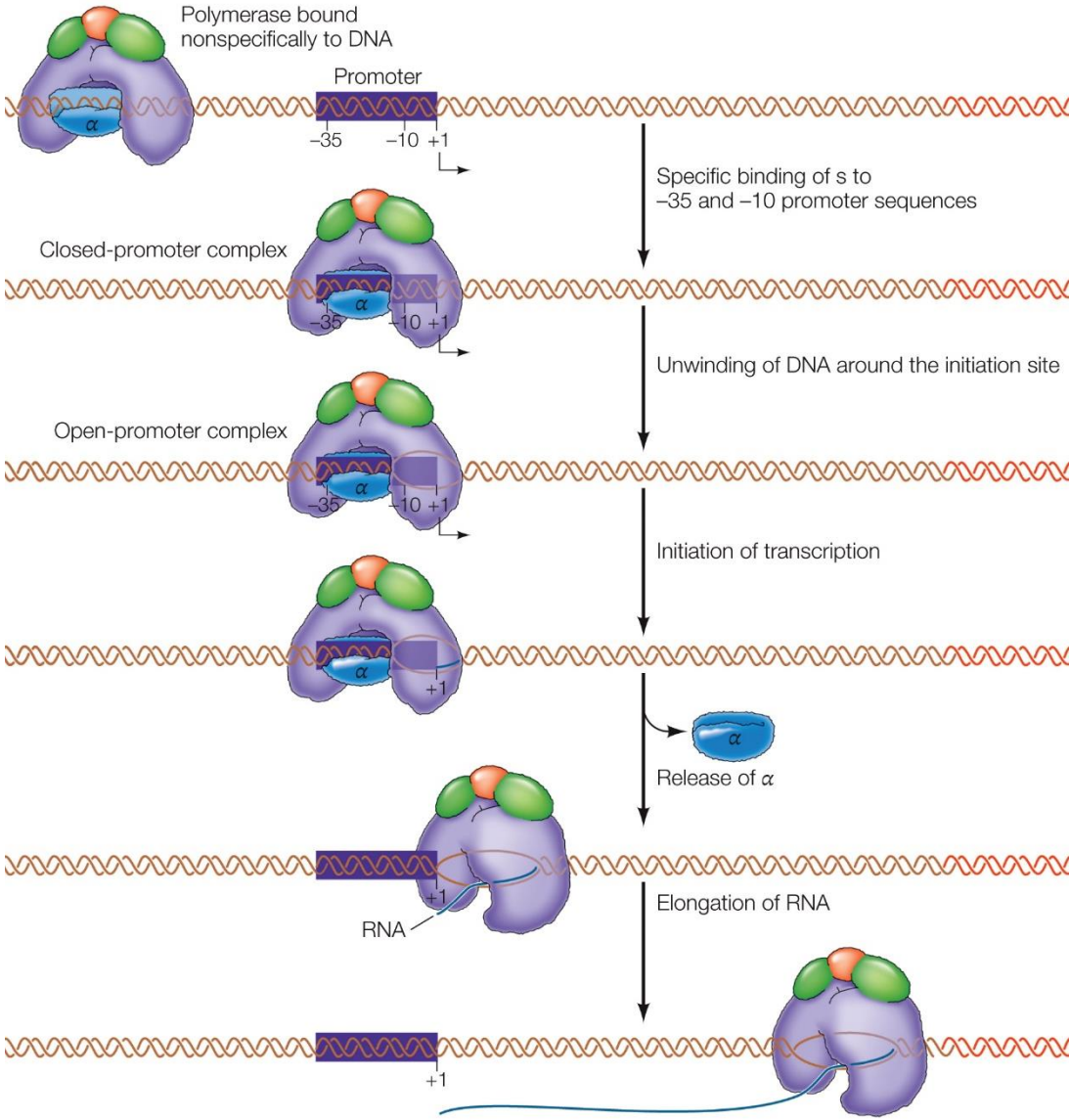
Transcription
by *E. coli* RNA Polymerase

1. RNA polymerase binds nonspecifically to DNA with low affinity

2. σ factor binds specifically to both –35 and –10 sequences and directs RNA polymerase to promoters

3. RNA polymerase unwinds 14 bases of DNA (-12 to +2): closed promoter to open promoter

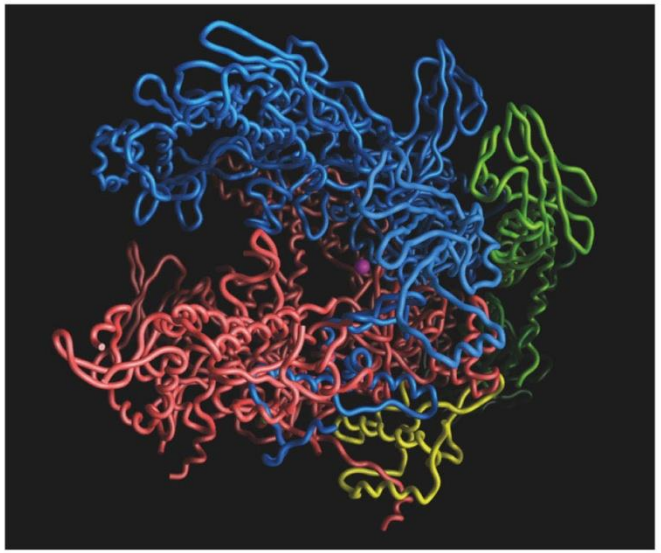
4. After addition of 10 nucleotides, σ is released from the polymerase



Structure of Bacterial RNA Polymerase

β and β' subunits of *E.coli* RNA polymerases form a crab claw-like structure that grips the DNA template

→ RNA polymerase remains associated with its template while it continues synthesis of mRNA



Transcription Termination

Termination sequences: a symmetrical inverted repeat of a GC-rich sequence followed by several A residues

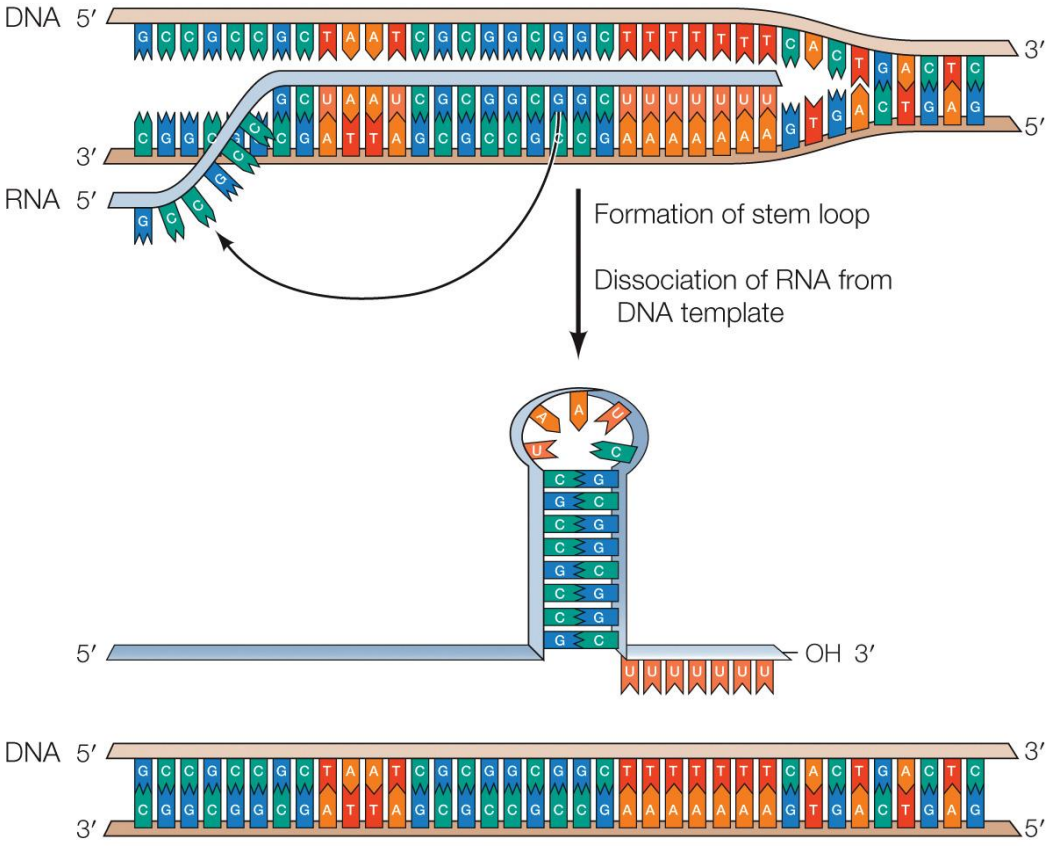
1. Transcription of the GC-rich sequence

→ a stable stem-loop structure by self-complementary base pairing

→ disrupts its association with DNA and terminates transcription

2. Several A residues

→ facilitates the dissociation of the RNA from its template



Eukaryotic RNA polymerases and General transcription factors

Learning objectives

- Summarize the roles of different eukaryotic RNA polymerases
- Distinguish between the binding of bacterial and eukaryotic RNA polymerases to promoters
- Describe the functions of the general transcription factors for RNA polymerase II
- Summarize the organization of promoters transcribed by RNA polymerase I and III

Eukaryotic RNA polymerases and general transcription factors

진핵세포의 전사는 원핵세포 보다 훨씬 더 복잡하게 진행됨

1.원핵세포에서는 1가지 RNA pol에 의해 전사; 진핵세포에서는 3가지 RNA pol이 서로 다른 유전자들을 전사.

2.진핵세포 RNA pol는 promoter에 직접 결합하지 않고 여러 단백질들과의 상호작용을 통하여 전사 개시.

3.진핵세포의 DNA는 histones와 nucleosome을 형성하고 전사를 위해 chromatin이 탈응축되어야 함

Type of RNA synthesized	RNA polymerase
Nuclear genes	
mRNA	II
miRNA	II
lncRNA	II
tRNA	III
rRNA	
5.8S, 18S, 28S	I
5S	III
snRNA and scRNA	II and III[a]
Mitochondrial genes	Mitochondrial[b]
Chloroplast genes	Chloroplast[b]

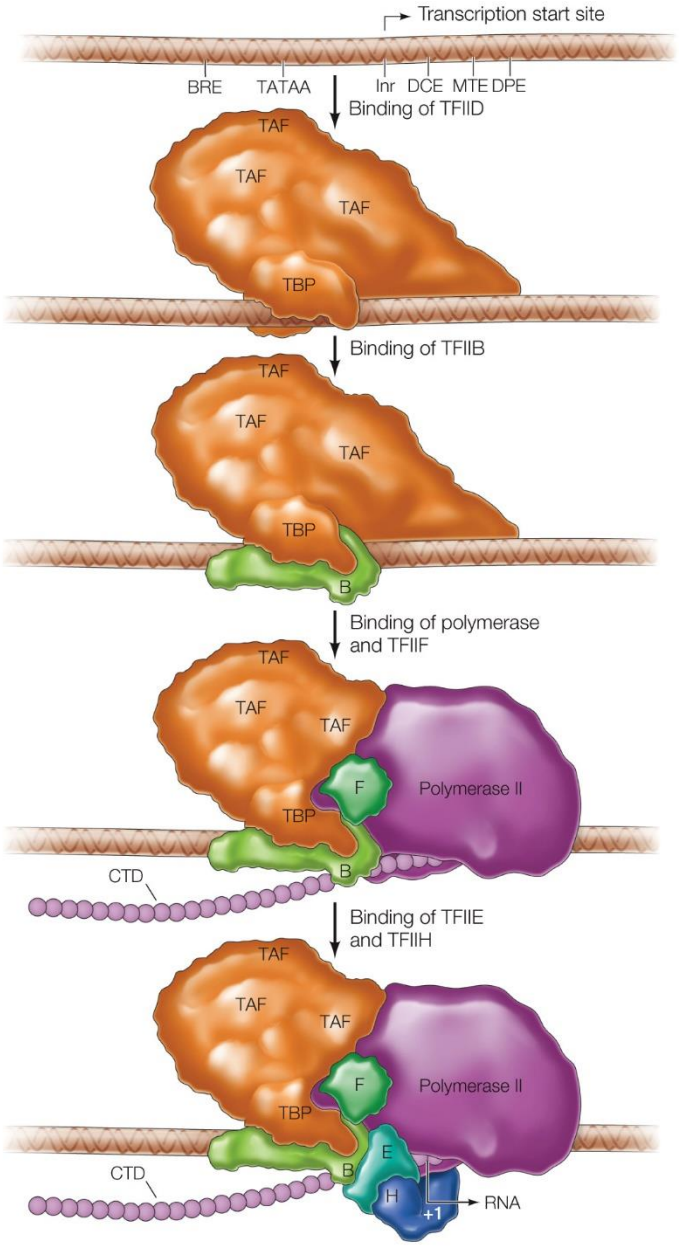
[a]Some small nuclear (sn) and small cytoplasmic (sc) RNAs are transcribed by polymerase II and others by polymerase III.

[b]The mitochondrial and chloroplast RNA polymerases are similar to bacterial enzymes.

Yeast RNA pol II의 구조

General transcription factors and initiation of transcription by RNA polymerase II

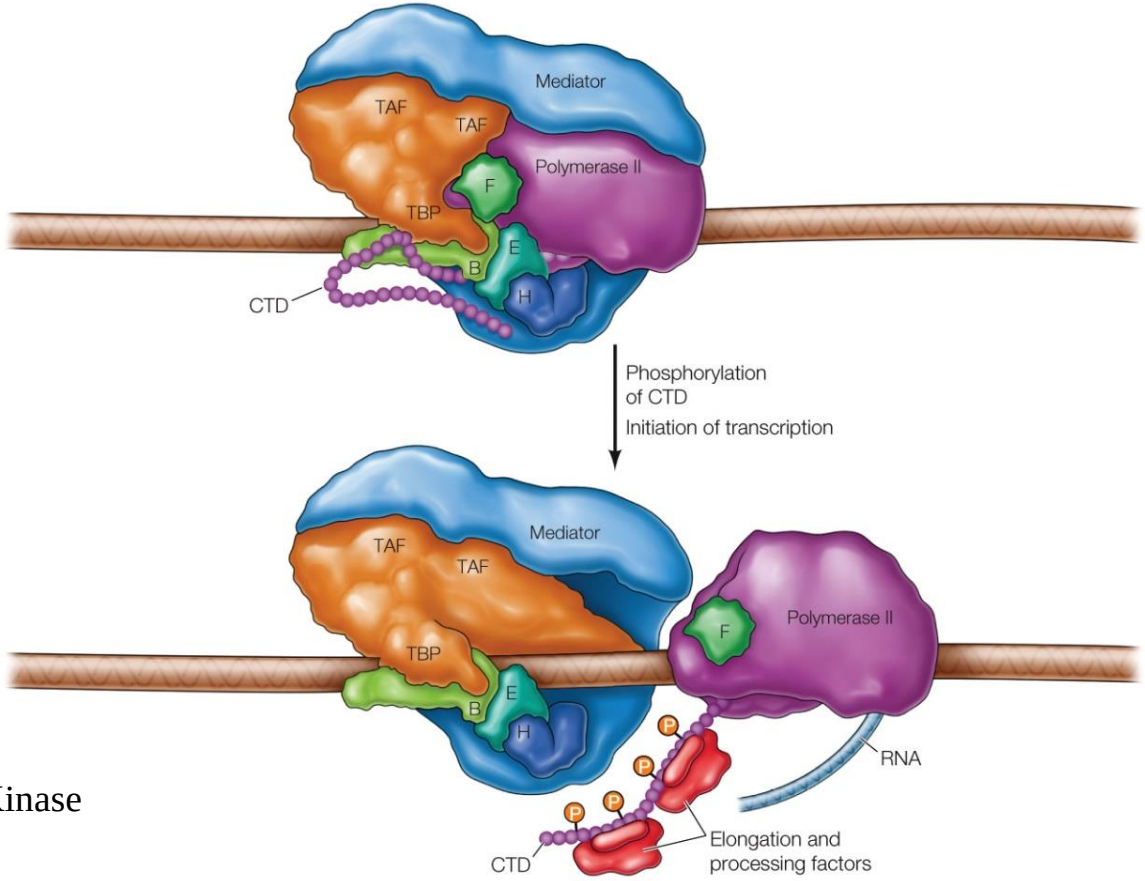
- Unlike prokaryotic RNA polymerase, RNA polymerase II requires initiation factors that are not associated with the polymerase
- General transcription factors are proteins involved in transcription from all polymerase II promoters.
- Sequence elements include the **TATA box** which resembles the –10 sequence element of bacterial promoters.
- The first step is binding of general transcription factor TFIID, composed of multiple subunits, including the **TATA-binding protein (TBP)** and 14 other polypeptides, called **TBP-associated factors (TAFs)**.
- Sequential assembly of TFIIB, TFIIF/Pol II, and then two additional factors (TFIIE and TFIIH) completes formation of the preinitiation complex.



RNA polymerase II preinitiation complex

Eukaryotic RNA polymerase II/Mediator complexes and transcription initiation

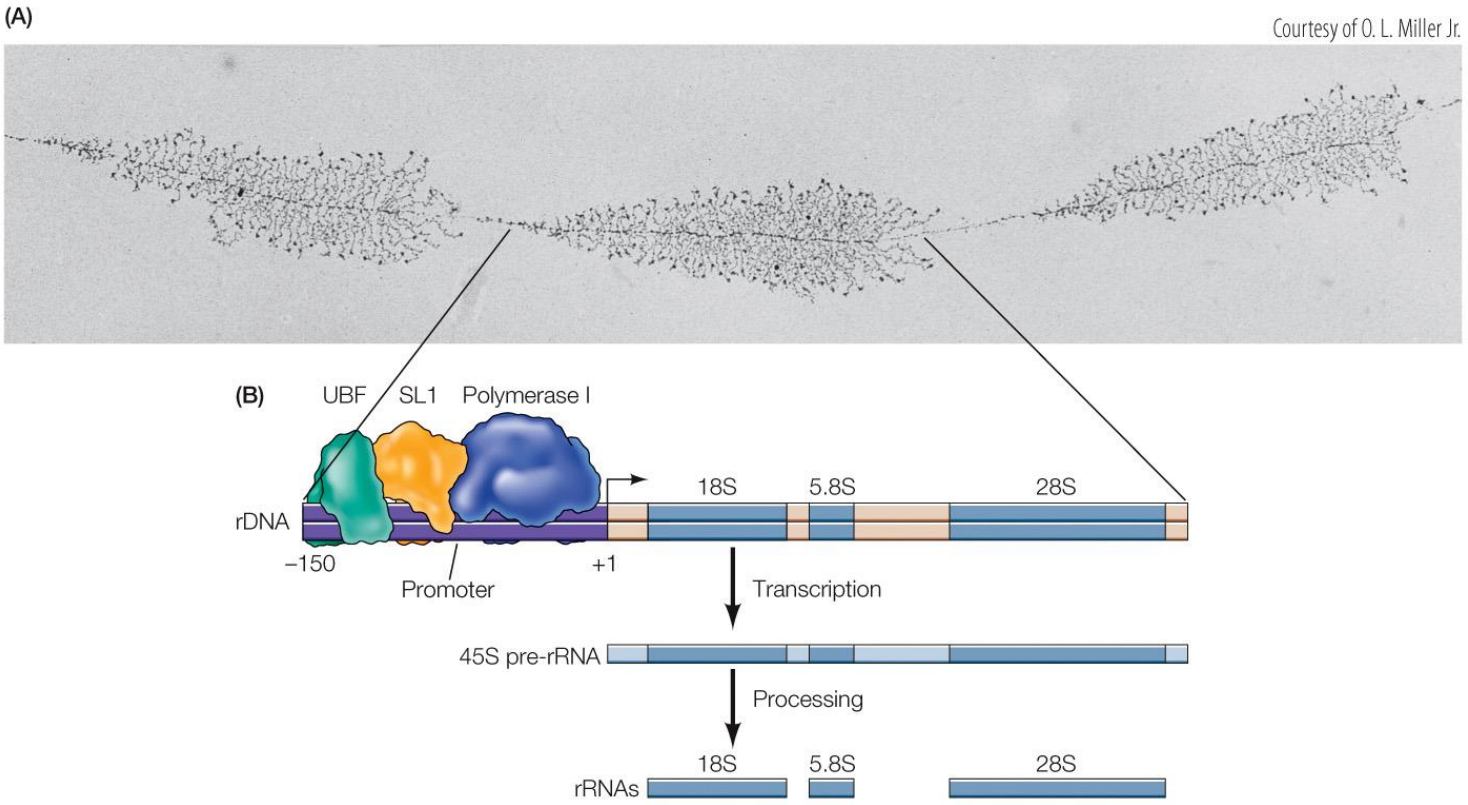
- Within a cell, additional factors are required to initiate transcription.
- These include **Mediator,** a large protein complex of more than 20 subunits; it interacts with both general transcription factors and RNA polymerase.
- Mediators play a key role in linking the general TFs to the gene-specific TFs that regulate gene expression.



TFIIH=1. helicase, 2. Kinase

Transcription by RNA polymerase I

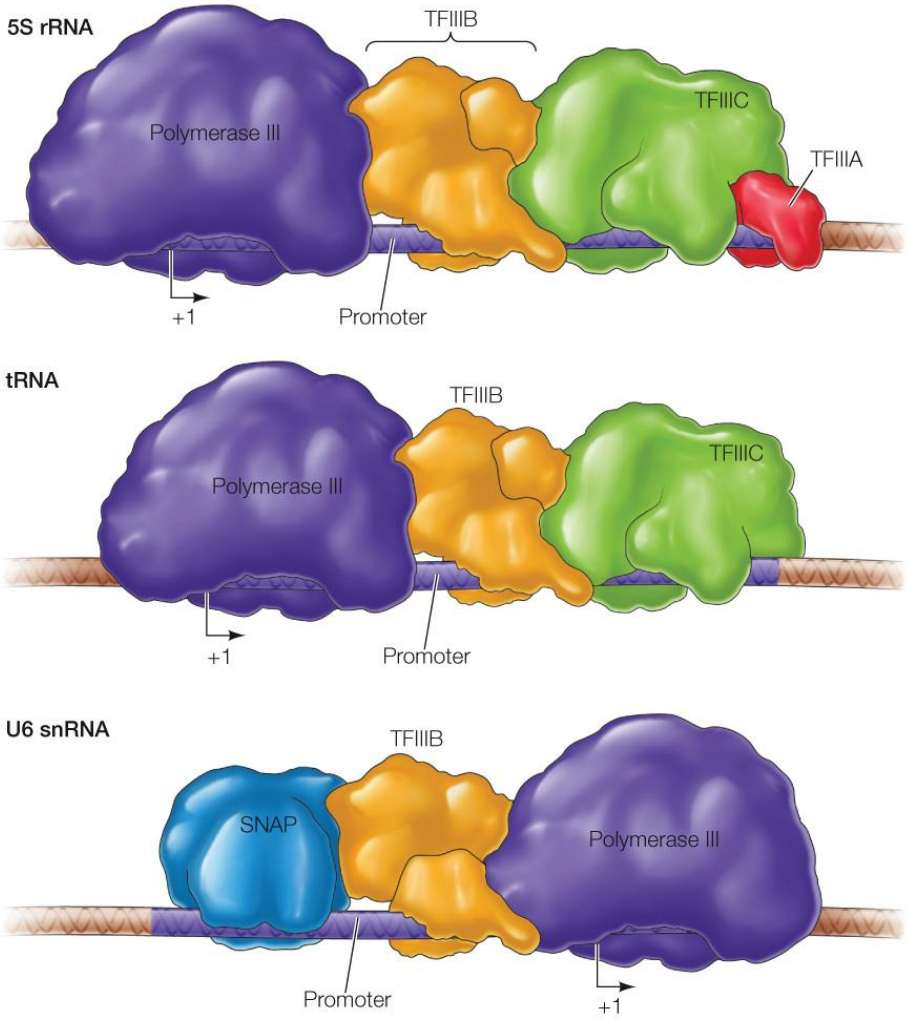
- Two transcription factors, UBF (upstream binding factor), and SL1 (selectivity factor 1), bind cooperatively to the rDNA promoter and recruit RNA polymerase I to form an initiation complex.
- Transcription yields a large RNA molecule (45S pre-rRNA), which is then cleaved into 28S, 18S, and 5.8S rRNAs



Transcription of the ribosomal RNA gene

Transcription by RNA polymerase III

- The promoters of 5S rRNA and tRNA genes are downstream of the transcription initiation site: Internal promoter.
- Transcription of the 5S rRNA gene is initiated by the binding of TFIIIA, followed by the binding of TFIIIC, TFIIIB, and the polymerase.
- Promoters of tRNA and U6 snRNA genes do not contain binding sites for TFIIIA and are recognized by other factors (TFIIIC and SNAP, respectively) that recruit TFIIIB and RNA polymerase



snRNA activating protein (SNAP)