Ph. D. course work SEMINAR

Presented by

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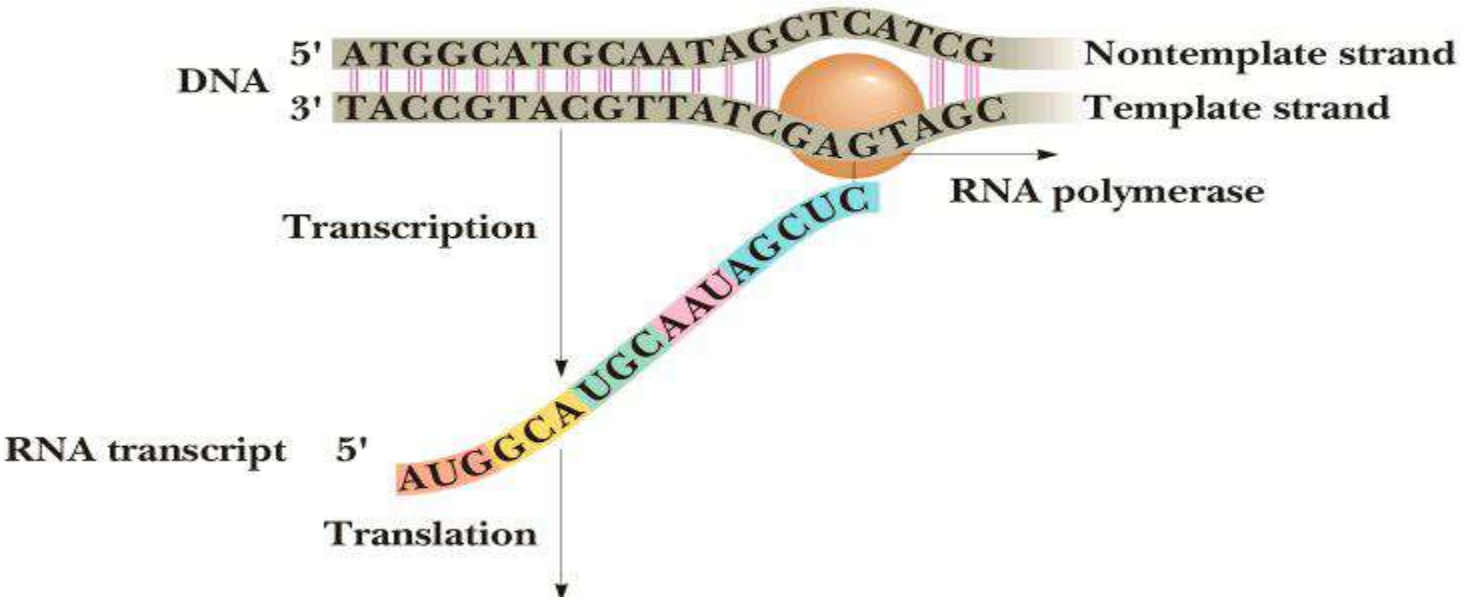
Transcription in Prokaryotes



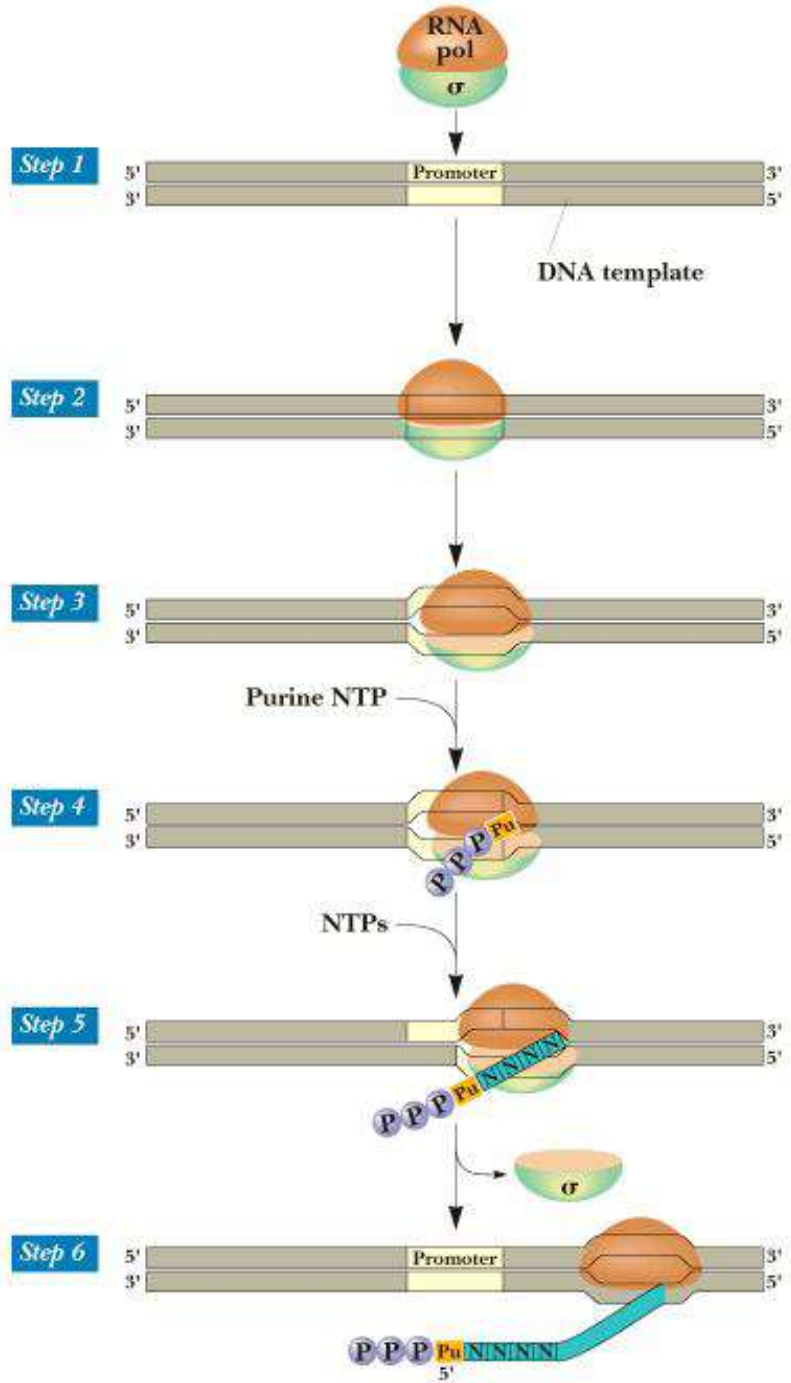
- *Only a single RNA polymerase*

In *E.coli*, RNA polymerase is 465 kD complex, with 2 α, 1 β, 1 β', 1 σ

- β' binds DNA
- β binds NTPs and interacts with σ
- σ recognizes promoter sequences on DNA
- α subunits appear to be essential for assembly and for activation of enzyme by regulatory proteins

Stages of Transcription

- binding of RNA polymerase holoenzyme at promoter sites
- initiation of polymerization
- chain elongation
- chain termination


RNA pol
σ
Step 1
5'
3'
Promoter
3'
5'
DNA template
Step 2
Step 3
Purine NTP
Step 4
Pu
NTPs
Step 5
N N N N
σ
Step 6
Promoter

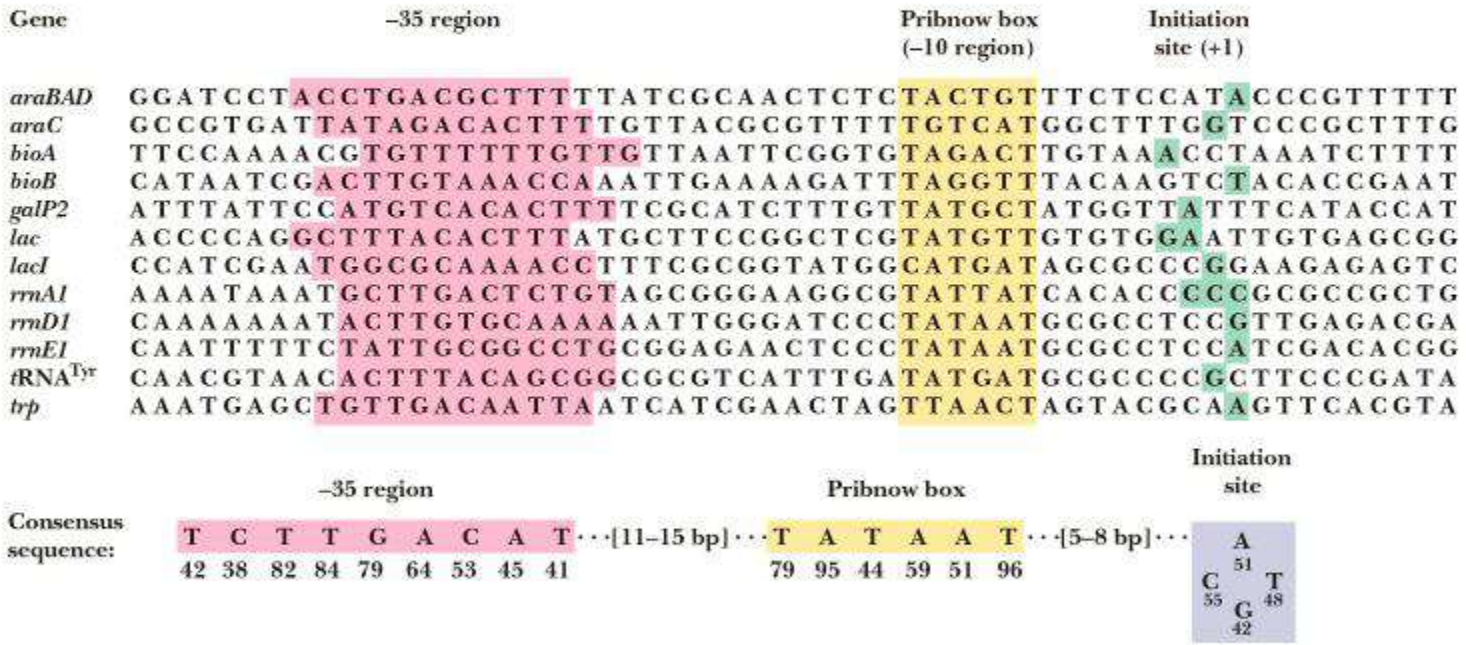
Binding of polymerase to Template DNA

- Polymerase binds nonspecifically to DNA with low affinity and migrates, looking for promoter
- Sigma subunit recognizes promoter sequence
- RNA polymerase holoenzyme and promoter form "closed promoter complex" (DNA not unwound) - $K_d = 10^{-6}$ to 10^{-9} M
- Polymerase unwinds about 12 pairs to form "open promoter complex" - $K_d = 10^{-14}$ M

Properties of Promoters

- Promoters typically consist of 40 bp region on the 5'-side of the transcription start site
- Two consensus sequence elements:
- The "-35 region", with consensus TTGACA - sigma subunit appears to bind here
- The Pribnow box near -10, with consensus TATAAT - this region is ideal for unwinding - why?

Garrett & Grisham: Biochemistry, 2/e
Figure 31.3



Initiation

- RNA polymerase has two binding sites for NTPs
- Initiation site prefers to binds ATP and GTP (most RNAs begin with a purine at 5'-end)
- Elongation site binds the second incoming NTP
- 3'-OH of first attacks alpha-P of second to form a new phosphoester bond (eliminating PP_i)
- When 6-10 unit oligonucleotide has been made, sigma subunit dissociates, completing "initiation"
- Note rifamycin and rifampicin and their different modes of action

Elongation

Core polymerase - no sigma

- Polymerase is 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 (why??) and faster elsewhere
- Topoisomerases precede and follow polymerase to relieve supercoiling

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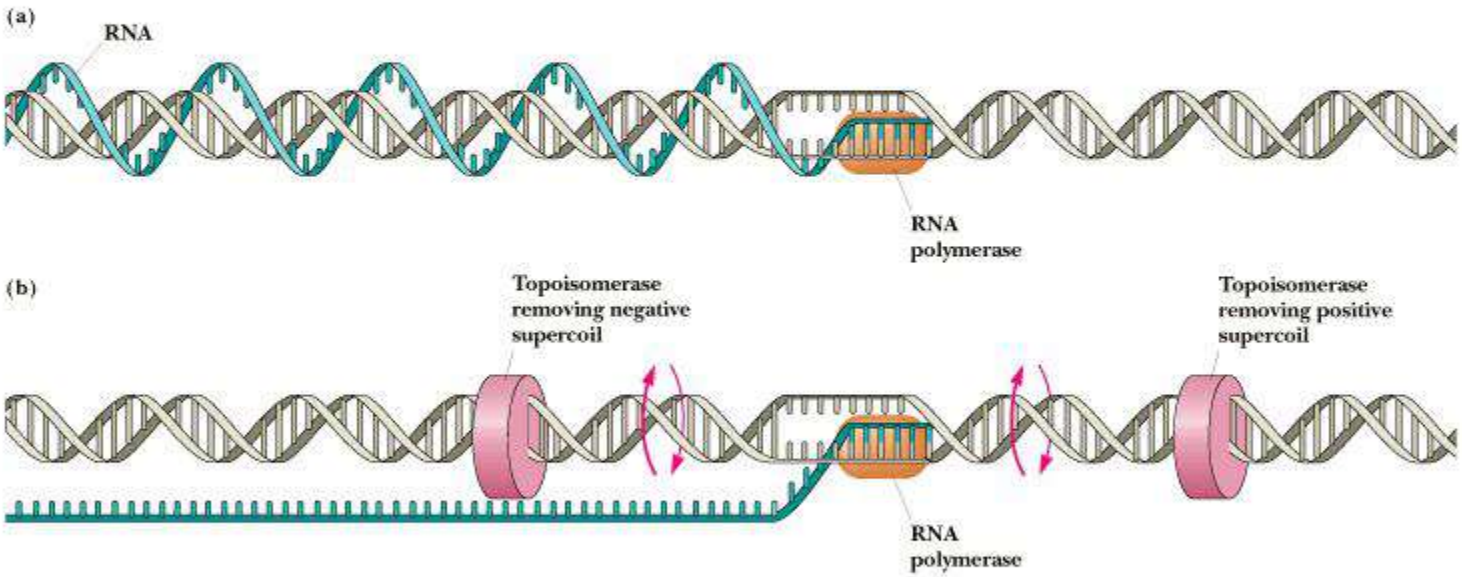
Figure 31.6



Termination

Two mechanisms

Rho - the termination factor protein

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

Specific sequences - termination sites in DNA

- inverted repeat, rich in G:C, which forms a stem-loop in RNA transcript
- 6-8 As in DNA coding for Us in transcript

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Figure 31.7

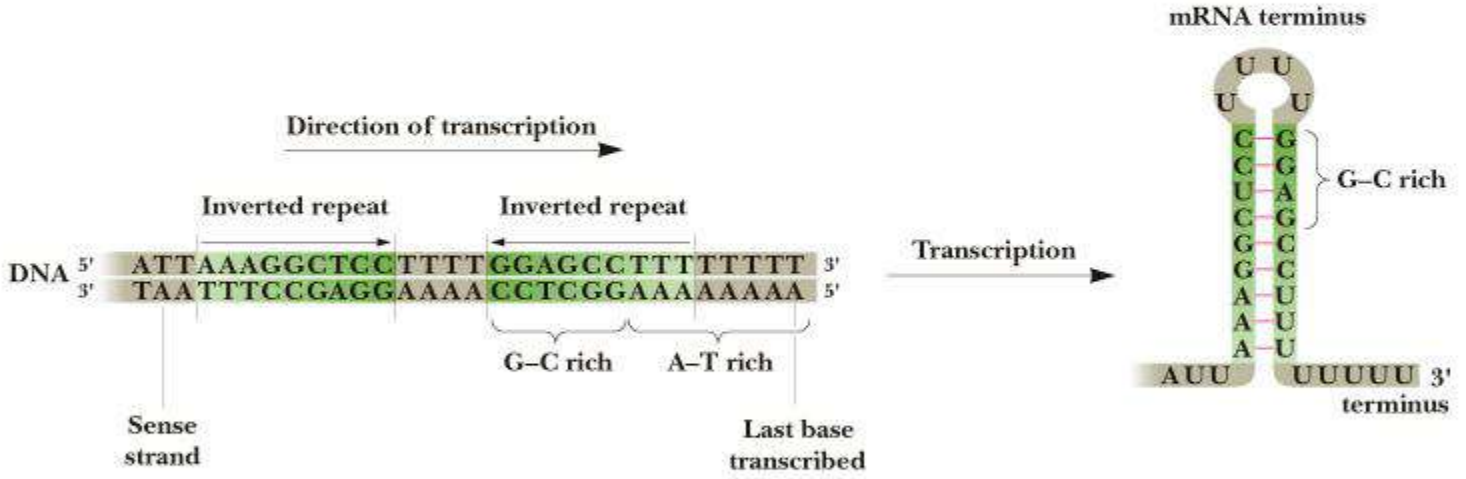

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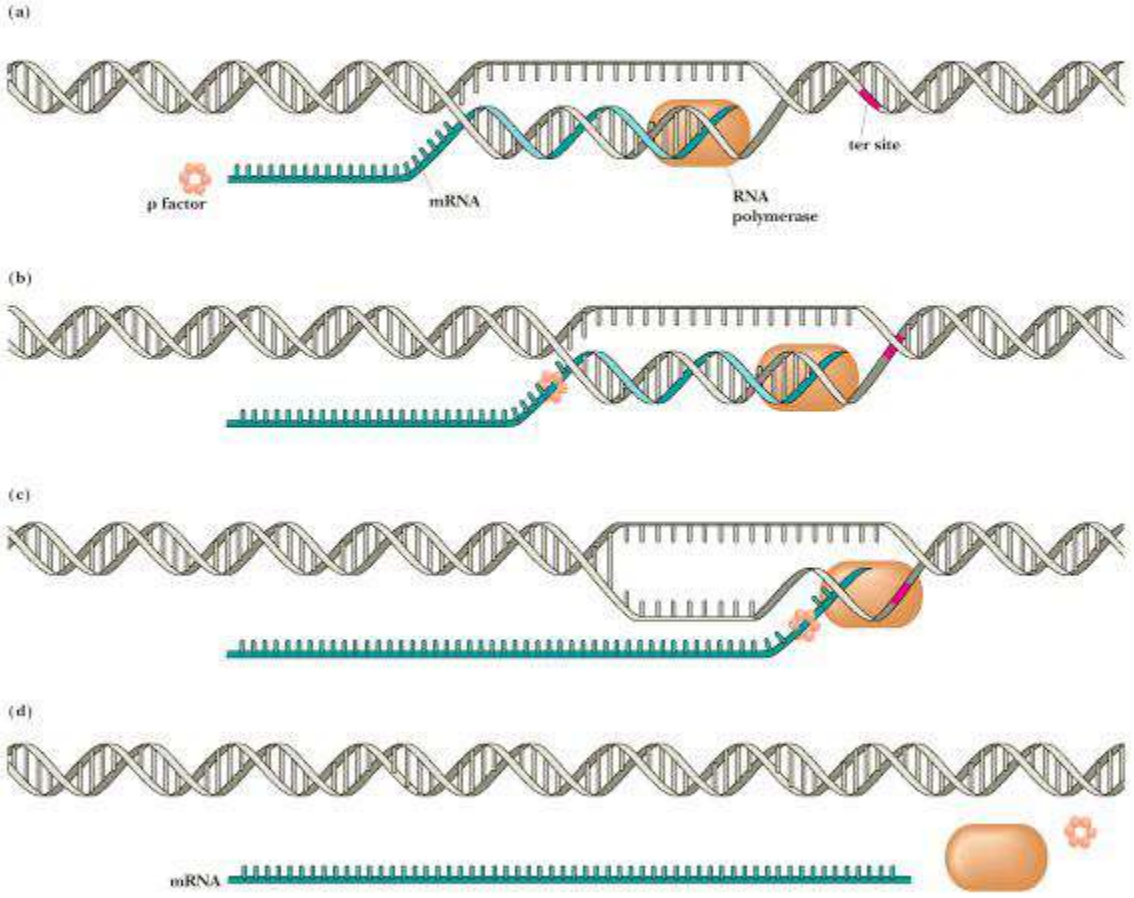
Figure 31.8



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Your Queries Please!!!

THANK YOU