



Ph. D. course work SEMINAR

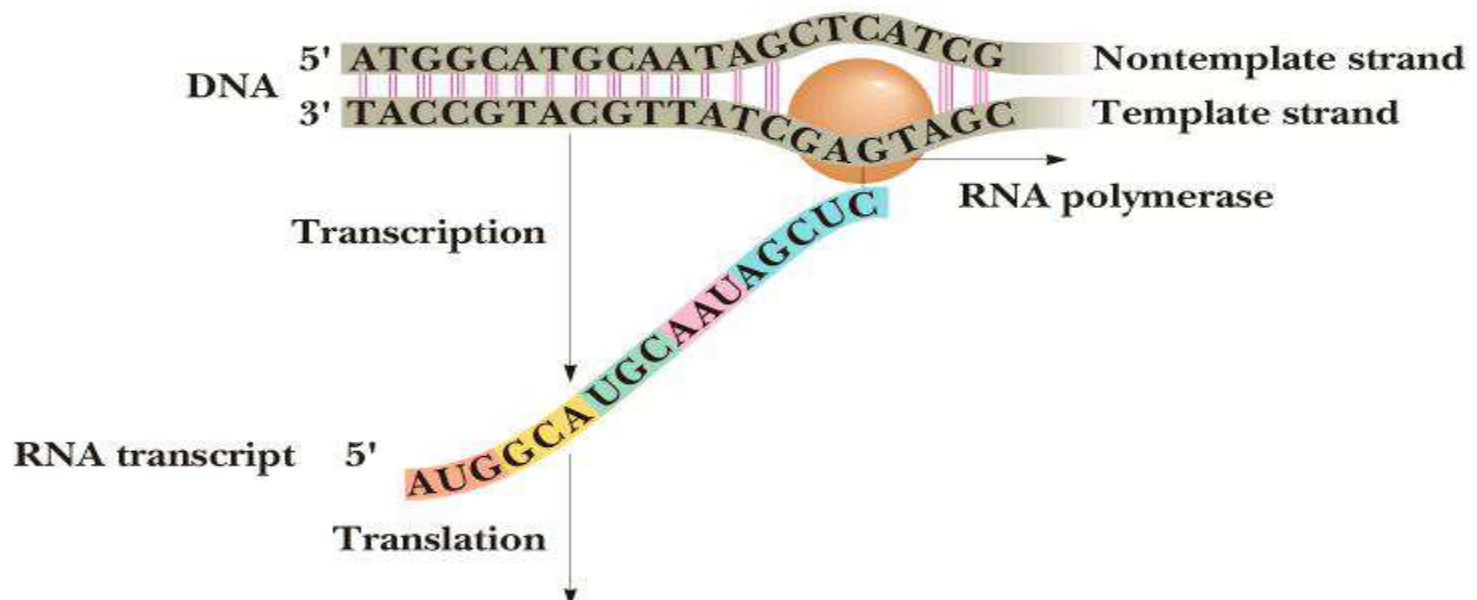
Presented by

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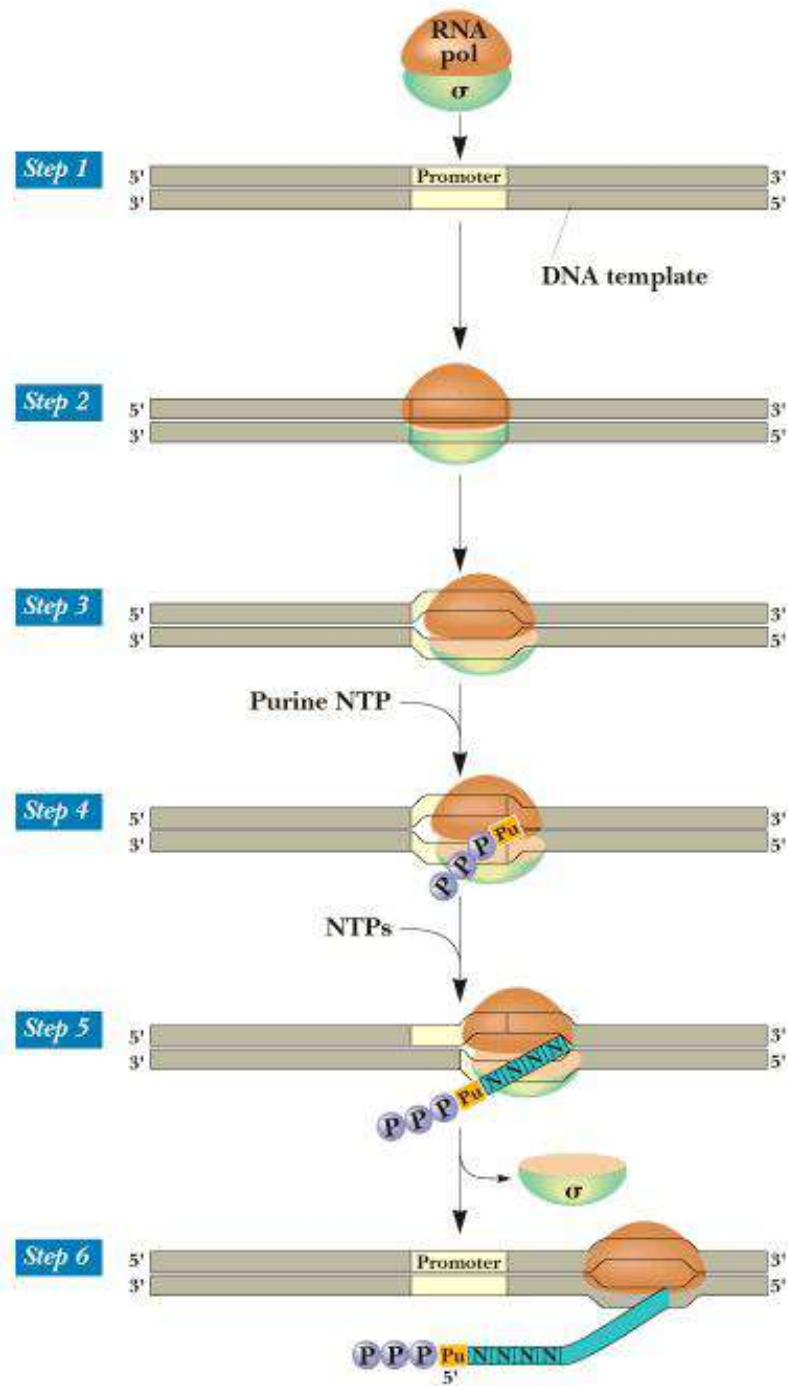
Transcription in Prokaryotes



- *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



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?

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Figure 31.3

Gene	-35 region	Pribnow box (-10 region)	Initiation site (+1)
<i>araBAD</i>	GGATCCTACCTGACGCTTTT	TATCGCAACTCTCTACTGTTT	TCTCCATACCCGTTTTT
<i>araC</i>	GCCGTGATTATAGACACTTTT	TGTTACGCGTTTTTGTTCAT	TGGCTTTGGTCCCGCTTTG
<i>bioA</i>	TTCCAAAACGTGTTTTTTTGTT	GTAAATTCGGTGTAGACTT	TGTAAACCTAAATCTTTT
<i>bioB</i>	CATAATCGACTTGTAACCAAA	ATTGAAAAGATTTAGGTTT	TACAAGTCTACACCGAAT
<i>galP2</i>	ATTTATTCCATGTGACACTTT	TTCGCATCTTTGTTATGCT	TATGGTTATTTTCATACCAT
<i>lac</i>	ACCCAGGCTTTACACTTTAT	GCTTCCGGCTCGTATGTT	TGTGTGGAATTGTGAGCGG
<i>lacI</i>	CCATCGAAATGGCGCAAAAC	CTTTCGCGGTATGGCATG	ATAGCGCCCGGAAGAGAGTC
<i>rmA1</i>	AAAATAAATGCTTGACTCTGT	AGCGGGAAGGCGTATTAT	CACACCCCGCGCGCGCTG
<i>rmD1</i>	CAAAAAAATACTTGTGCAAAA	AATTGGGATCCCTATAAT	TGCGCCTCCGTTGAGACGA
<i>rmE1</i>	CAATTTTTCTATTGCGGCCT	GCGGAGAACTCCCTATAAT	TGCGCCTCCATCGACACGG
<i>tRNA^{Tyr}</i>	CAACGTAAACACTTTACAGCG	GGCGTCATTTGATATGAT	TGCGCCCCGCTTCCCGATA
<i>trp</i>	AAATGAGCTGTTGACAAATTA	ATCATCGAACTAGTTAACT	AGTACGCAAGTTTACGTA
Consensus sequence:	T C T T G A C A T ... [11-15 bp] ...	T A T A A T ... [5-8 bp] ...	A C ₅₅ T ₄₈ G ₄₂
	42 38 82 84 79 64 53 45 41	79 95 44 59 51 96	

Initiation

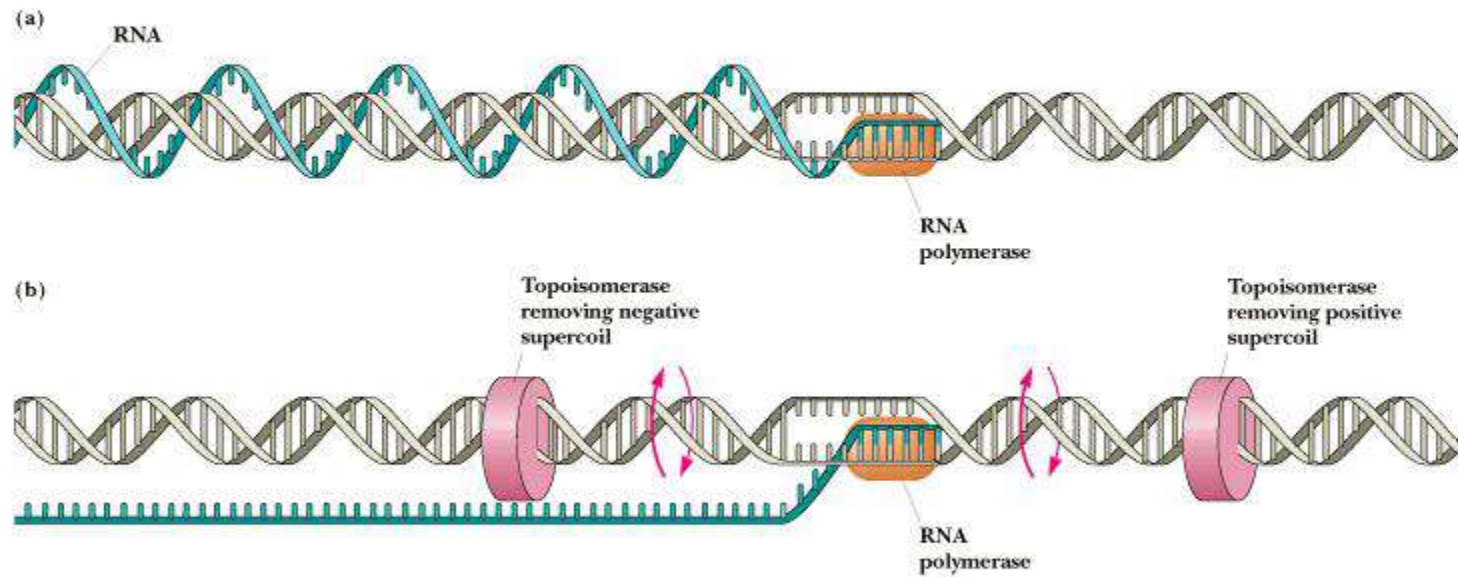
- RNA polymerase has two binding sites for NTPs
 - **Initiation site** prefers to binds ATP and GTP (most RNAs begin with a purine at 5'-end)
 - Elongation site binds the second incoming NTP
 - 3'-OH of first attacks alpha-P of second to form a new phosphoester bond (eliminating PP_i)
 - When 6-10 unit 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

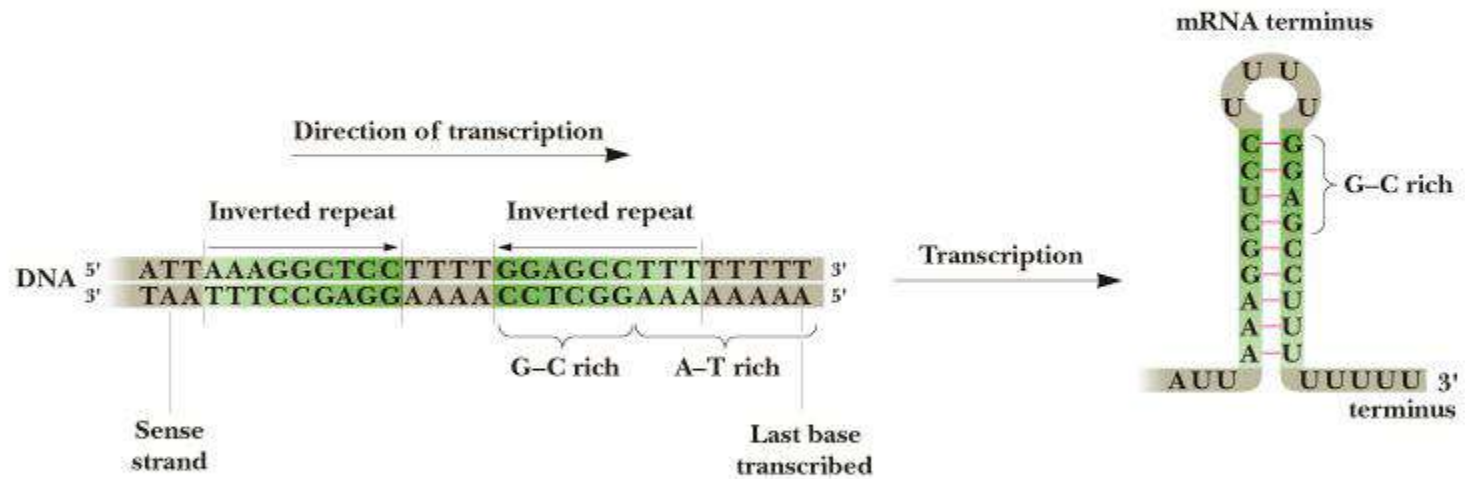
Rho - the termination factor protein

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

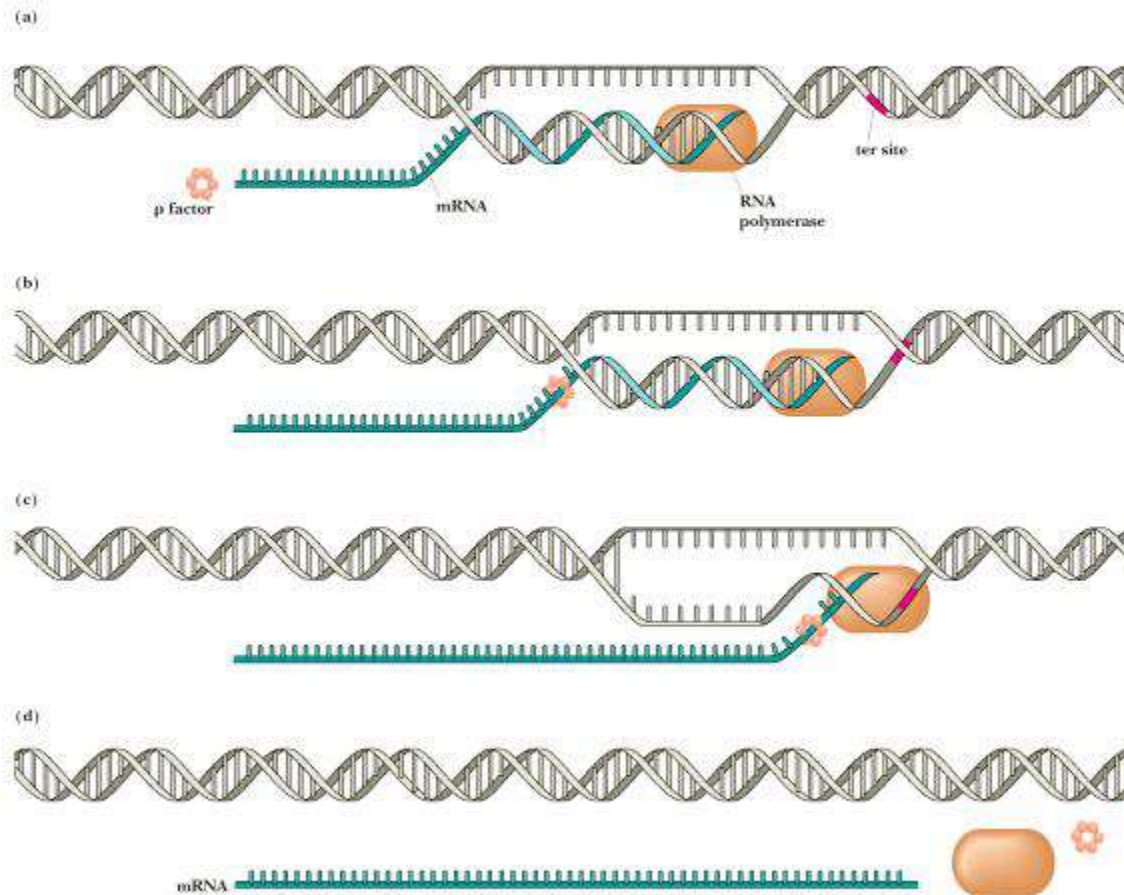
Specific sequences - termination sites in DNA

- 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





Your Queries Please!!!



THANK YOU