Transcription in Prokaryotes

4 October 2013

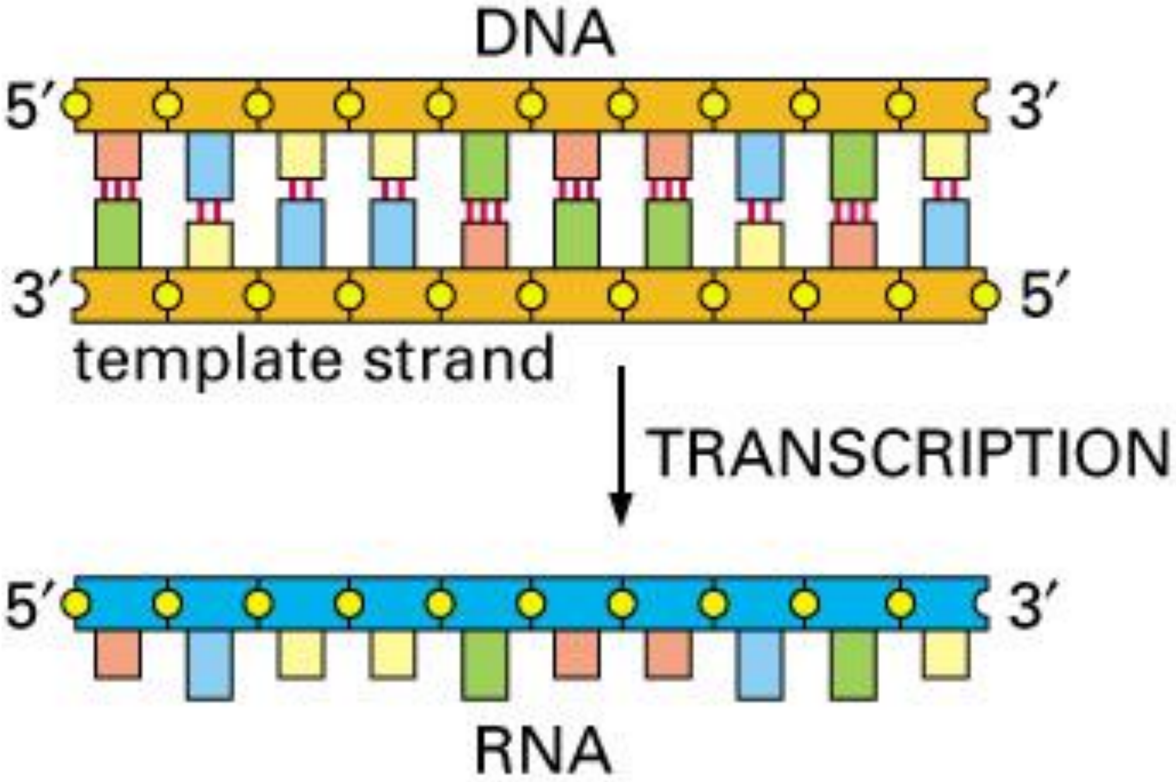
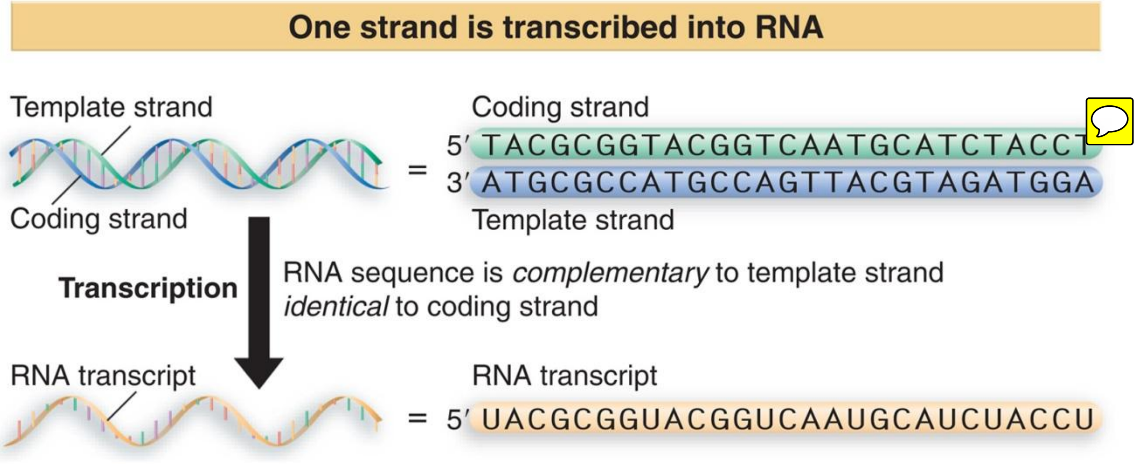


Figure 6–7. Molecular Biology of the Cell, 4th Edition.

Definitions/Conventions

- Transcription
  - Template strand: 3’ towards 5’ (right to left)
  - Non-template strand (*coding*): 5’ towards 3’ (left to right)



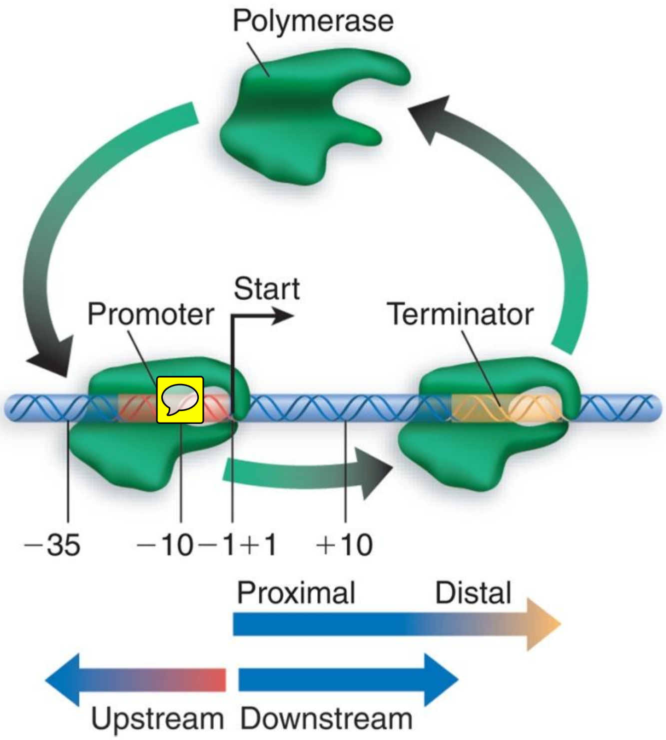
RNA content

Type	% at equilibrium	Synthesis capacity (% of total RNA synthesis)
rRNA	83	58
tRNA	14	10
mRNA	3-5	32

Transcriptional Unit

- Promoter
- Start site (+1)
- Terminator

Promoters and terminators define the unit



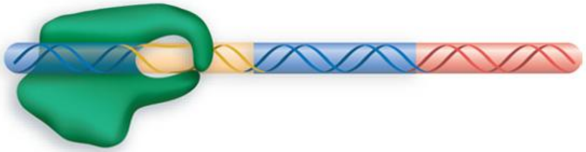
The 4 steps of transcription

- Promoter recognition
- Initiation
- Elongation
- Termination

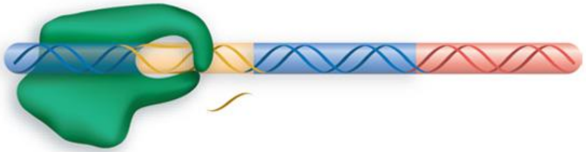
RNA polymerase catalyzes transcription

Template recognition: RNA polymerase binds to duplex DNA

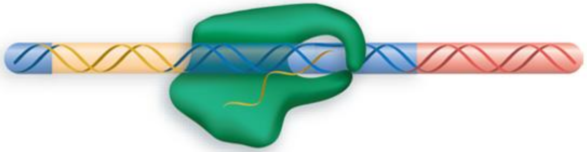
DNA is unwound at promoter



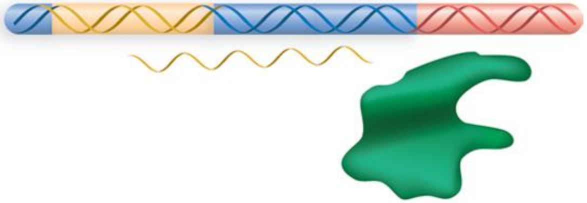
Initiation: Very short chains are synthesized and released



Elongation: Polymerase synthesizes RNA



Termination: RNA polymerase and RNA are released



RNA polymerase

- Makes phosphodiester bonds

RNA polymerase

- Phosphodiester bond formation

$$\text{RNA-OH} + \text{NMP} \rightarrow \text{RNA-NMP} + \text{H}_2\text{0} \quad \Delta G\,' = +\,6.0 \text{ kcal/mol}$$

$$\text{H}_2\text{0} \quad + \text{NTP} \rightarrow \text{NMP} + \text{Ppi} \quad \Delta G\,' = -8.6 \text{ kcal/mol}$$

$$\text{RNA-OH} + \text{NTP} \rightarrow \text{RNA-NMP} + \text{Ppi} \quad \Delta G\,' = -2.6 \text{ kcal/mol}$$

RNA Polymerase (465 kDa)

α subunits (2X)

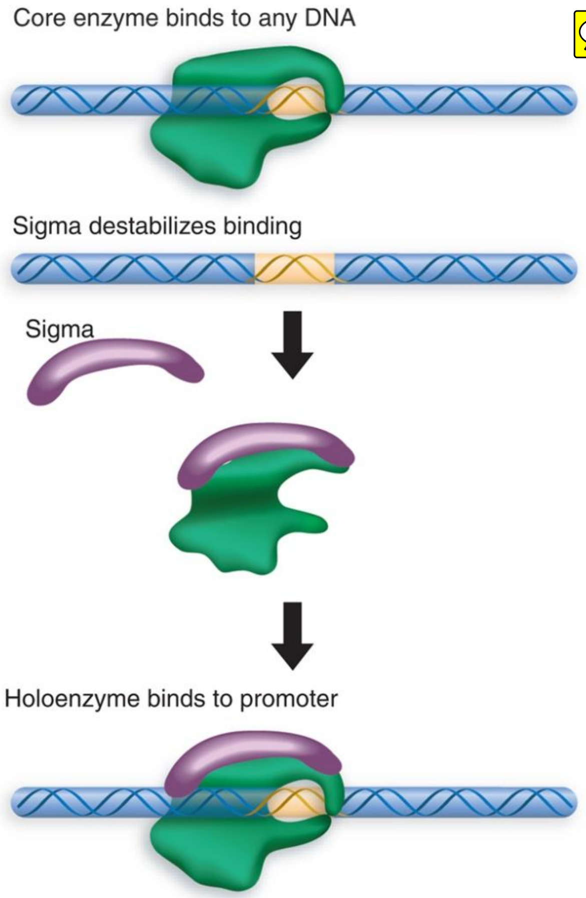
β et β’ subunits Catalytic center

ω subunit

The above subunits constitute the Core Enzyme

σ (sigma) subunit Promoter recognition

Sigma modifies the DNA affinity of the core enzyme



Sigma factor controls specificity

Sigma modifies the DNA affinity of the core enzyme

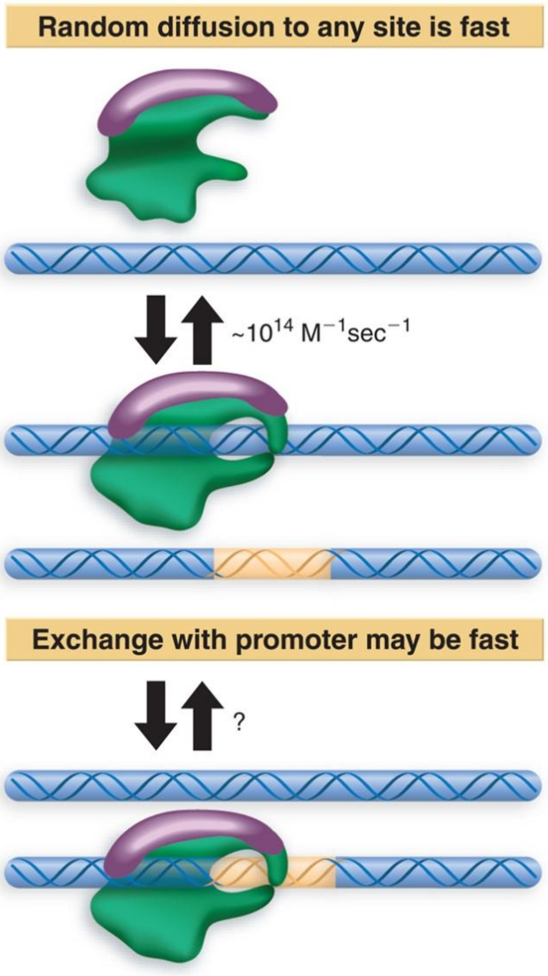
- sigma
  - Reduces the affinity of the core enzyme for sequences that <u>are not</u> promoters.
  - Increases the affinity of the core enzyme for promoter sequences.

How does RNA polymerase find the promoter?

- Different models

Diffusion

Exchange



Transcriptional Unit

- Promoter
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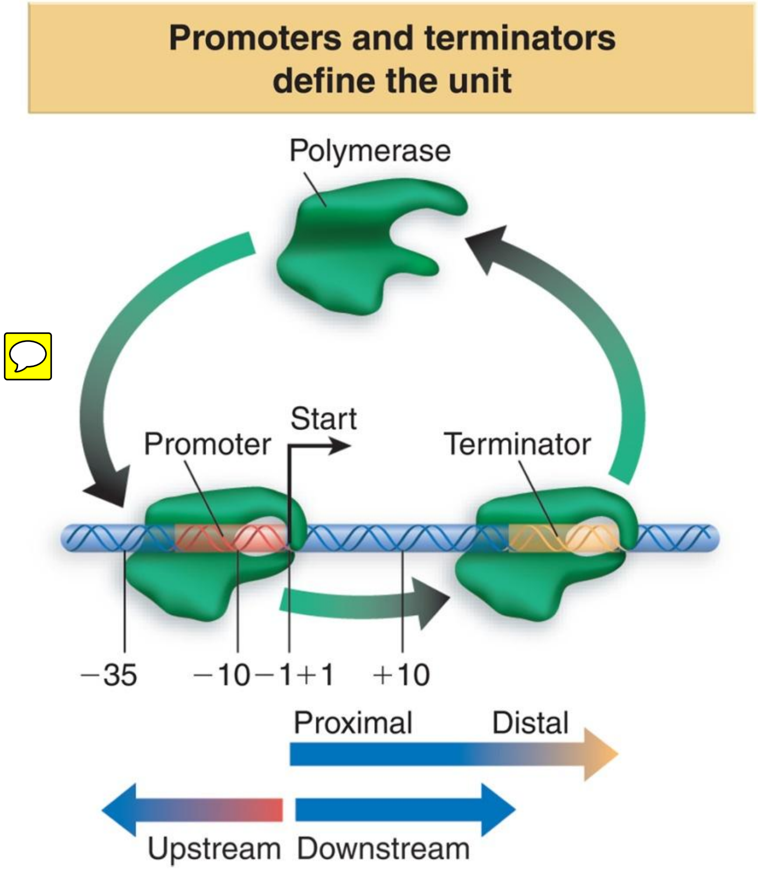

Promoters and terminators define the unit

Promoter

- Two consensus sequences

TTGACA------------------------TATAAT------------ +1

(-35)   16-19 bp   (-10)   5-9 pb

- Structural changes
- Activators and repressors

Consensus Sequence

$T_{80}A_{95}T_{45}A_{60}A_{50}T_{96}$

Mutations in promoters

- “down” mutation:
  - Decreases transcription efficiency.
  - The sequence is further away (differs more) from the consensus sequences.
- “up” mutation (not as frequent)
  - Increases transcription efficiency
  - The sequence is closer to (resembles more closely) the consensus sequence.

Different sigma factors

Factor	Sequence –35	Sequence –10	Used for
σ^{70}	TTGACA	TATAAT	general
σ^{32}	CCCTTGAA	CCCGATNT	heath shock
σ^{E}	?	?	heath shock
σ^{54}	CTGGNA	TTGCA	nitrogen metabolism
σ^{28}	CTAAA	GCCCGATAA	flagellae

DNA winding and unwinding

- Supercoiling.

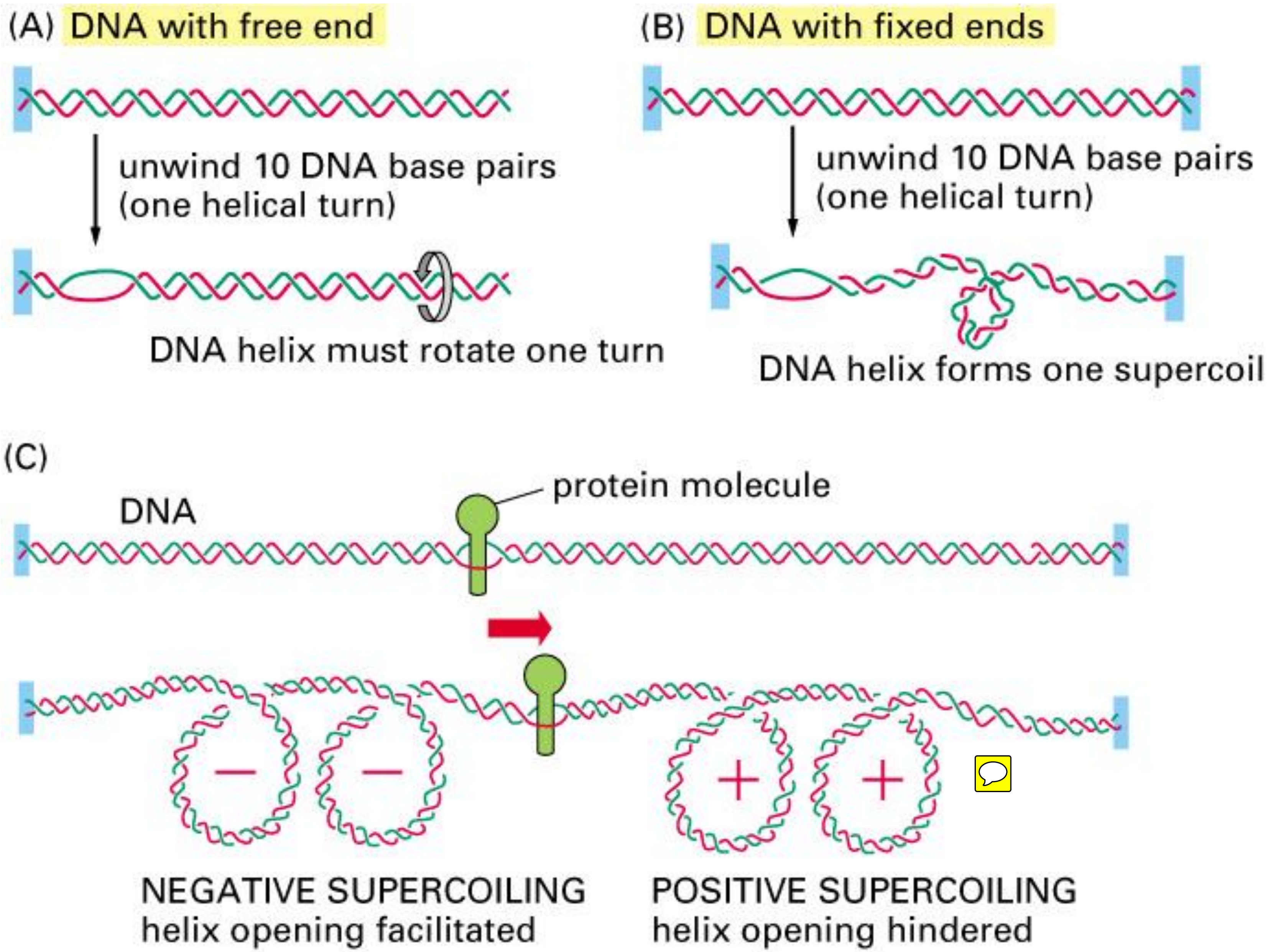
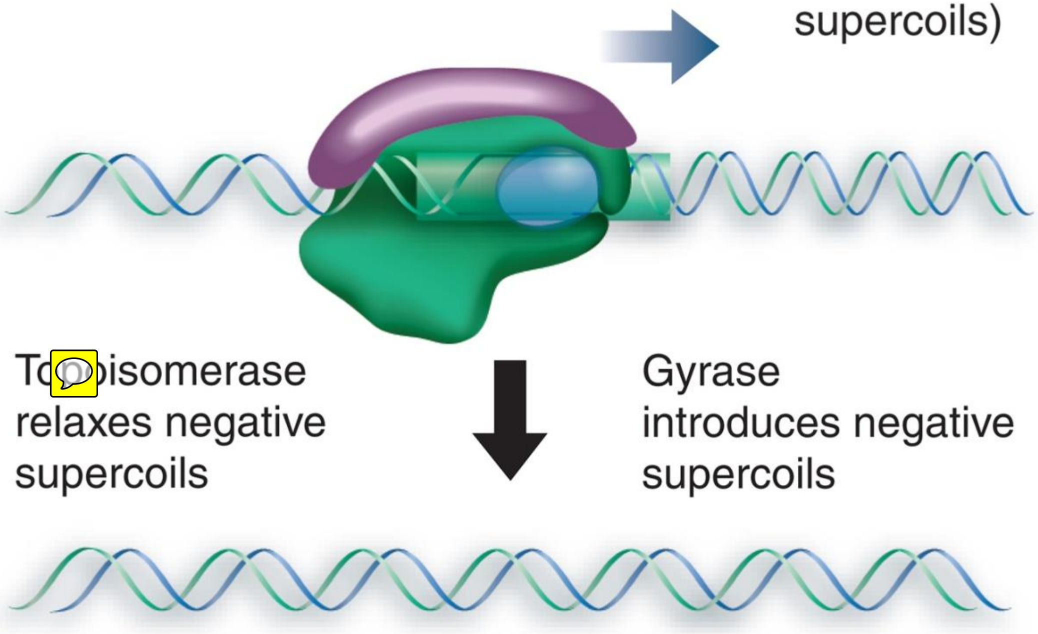


Figure 6–20. Molecular Biology of the Cell, 4th Edition.

Transcription changes DNA structure



(Negative supercoils)

Transcribing DNA

Overwound (positive supercoils)

Topoisomerase relaxes negative supercoils

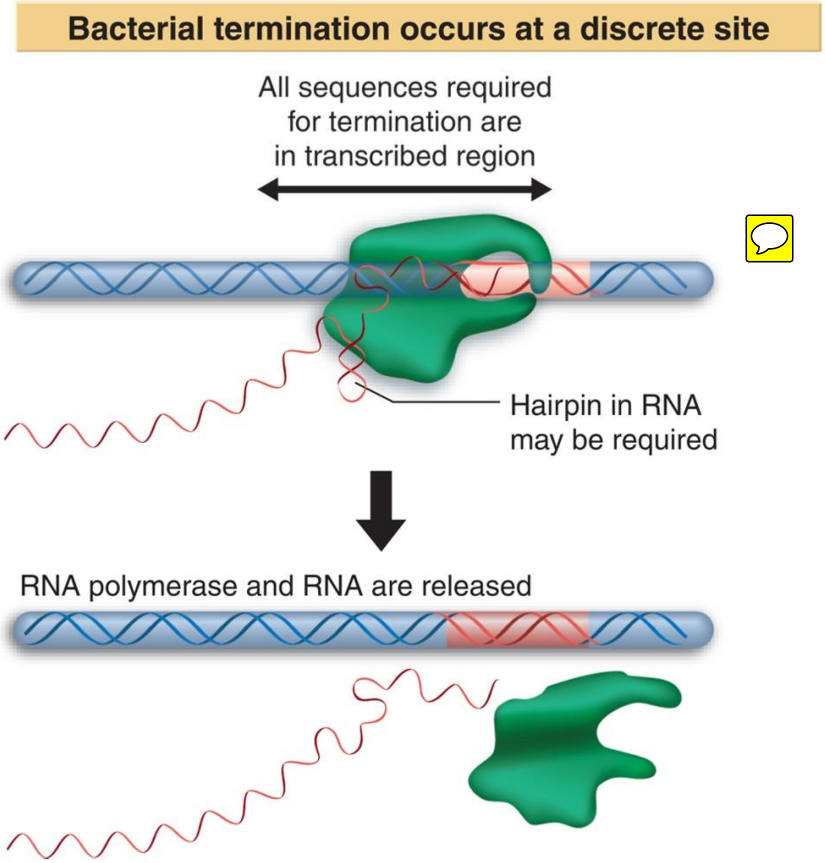
Gyrase introduces negative supercoils

Duplex DNA (10.4 bp/turn)

DNA winding and unwinding

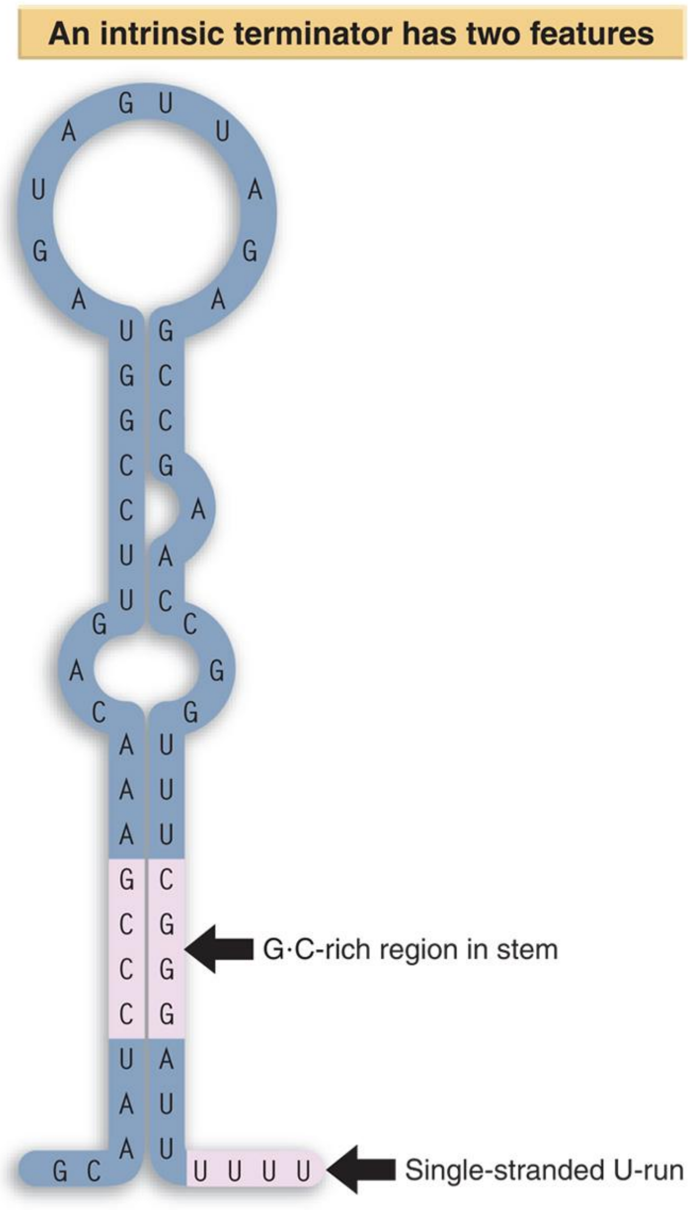
- DNA gyrase introduces negative supercoiling.
- Topoisomerase 1 decreases negative supercoiling.

Transcription Termination



Transcription Termination

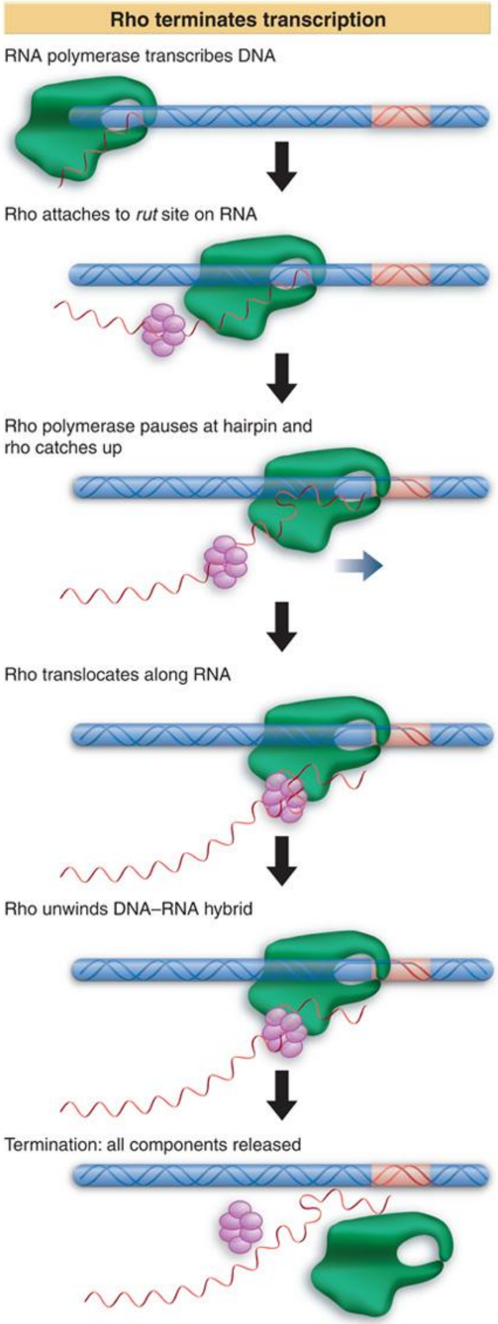
- Intrinsic terminator



An intrinsic terminator has two features

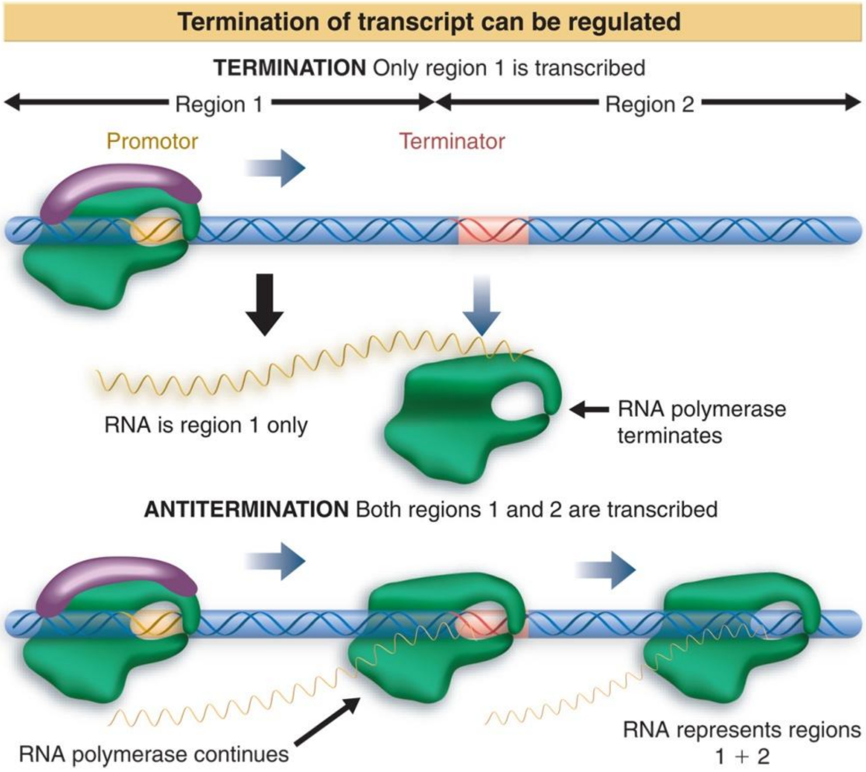
Transcription Termination

- Rho-dependent terminators
  - Rho: 46 kDa proteins that forms hexamers.
  - Termination sequence (for Rho binding): 50 to 90 nt.
  - Has ATPase activity and helicase activity that separates the RNA-DNA hybrid.


Rho terminates transcription
RNA polymerase transcribes DNA
Rho attaches to *rut* site on RNA
Rho polymerase pauses at hairpin and rho catches up
Rho translocates along RNA
Rho unwinds DNA–RNA hybrid
Termination: all components released

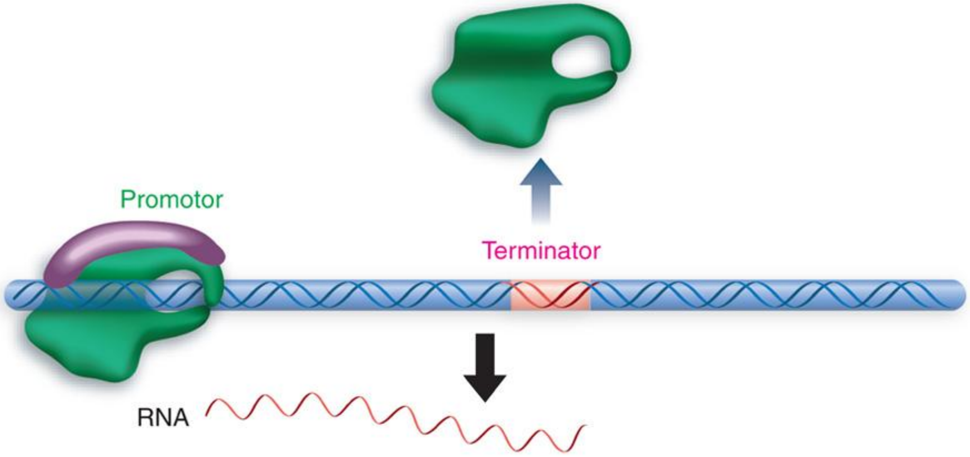
Transcription Termination

- Anti-termination

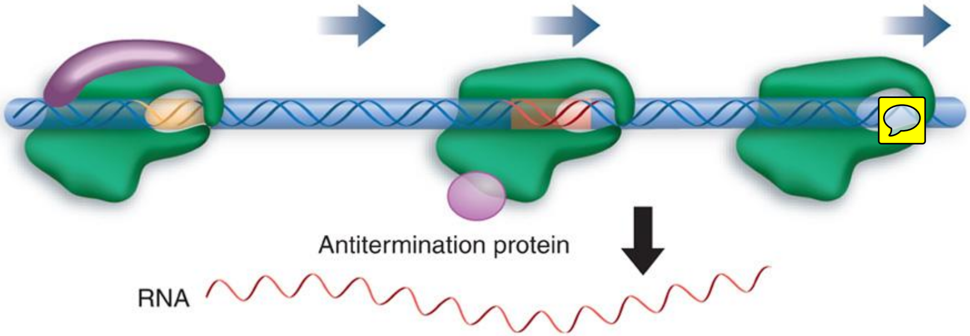


Antitermination extends the transcription unit

RNA polymerase transcribes from promoter to terminator



Antitermination protein enables RNA polymerase to pass terminator



Antitermination proteins act on specific terminators

Transcription unit	Promotor	Terminator	Antitermination protein
Immediate early	P_L	t_L	pN
Immediate early	P_{R1}	t_{R1}	pN
Late	$P_{R'}$	$t_{R'}$	pQ