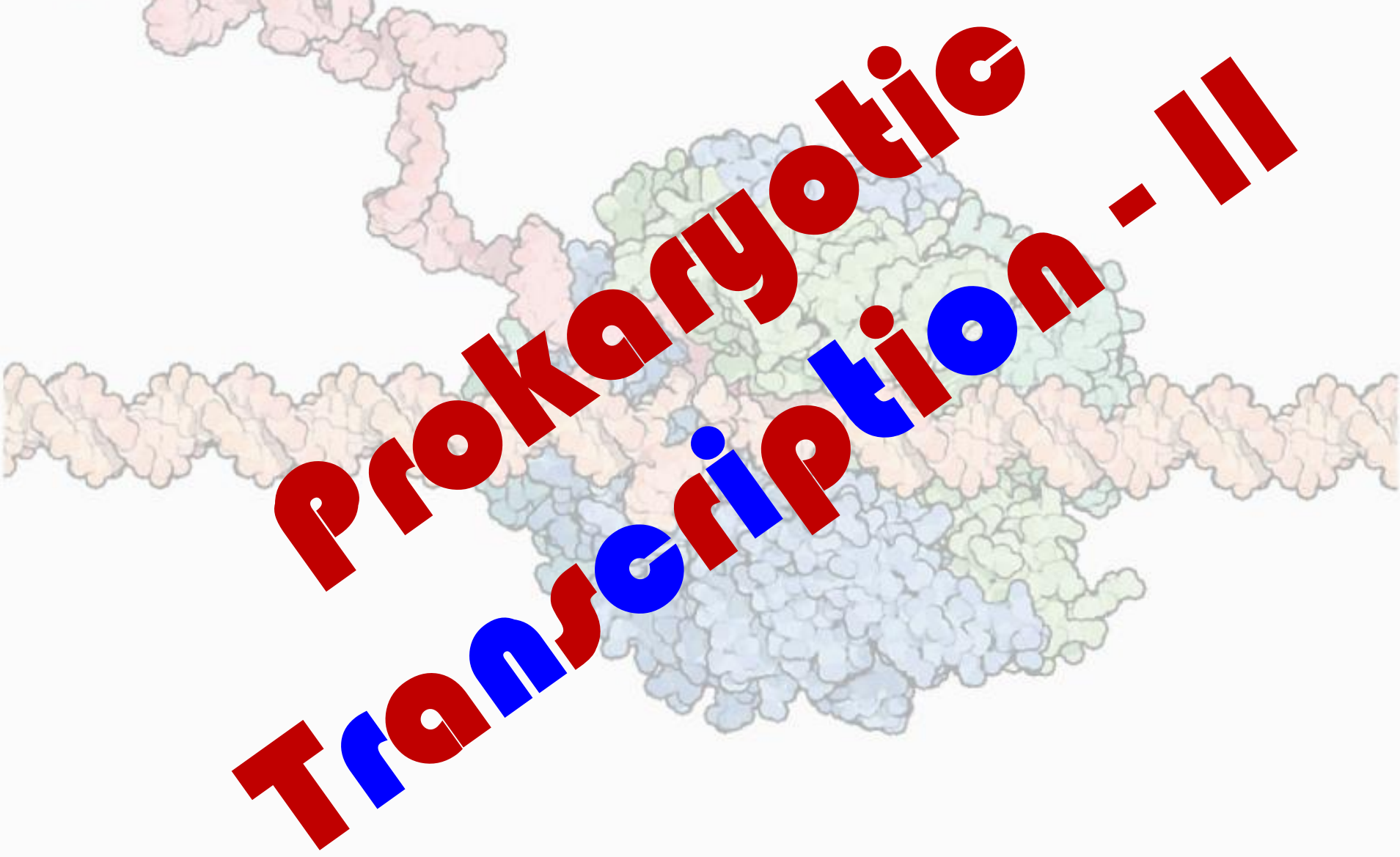
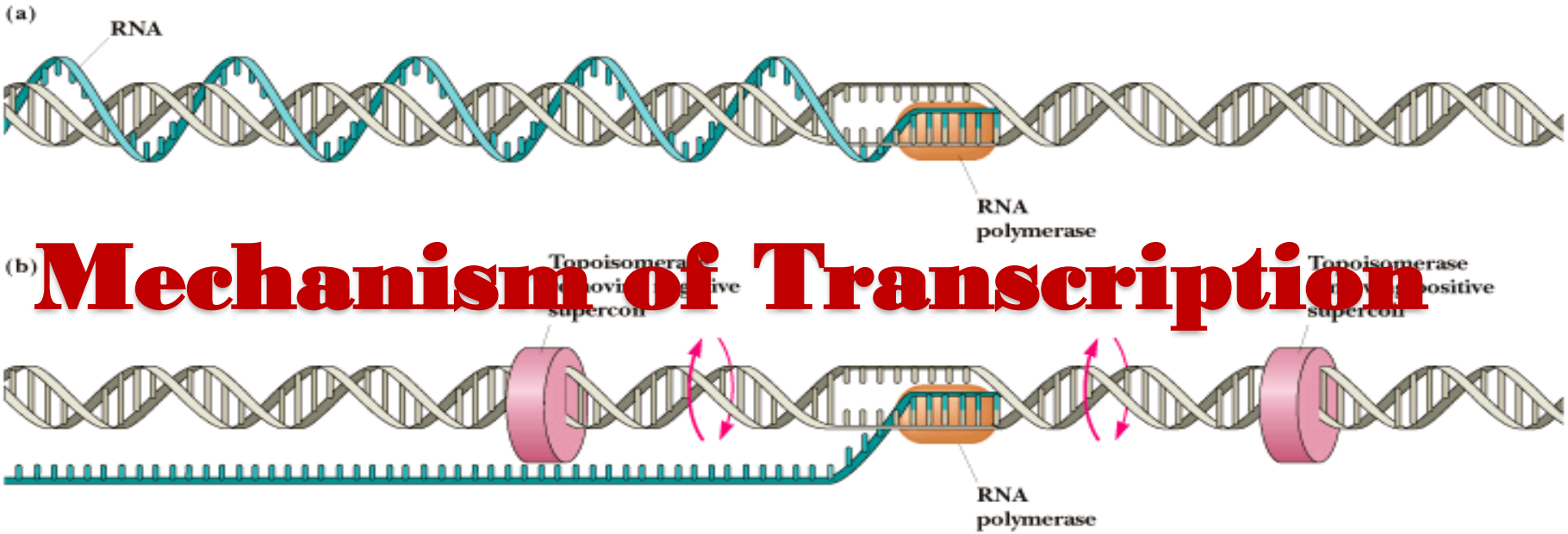
SOS in Biochemistry, Jiwaji University, Gwalior
M.Sc. II Semester (2019-20)
Paper BCH 205: Fundamentals of Molecular Biology (Unit II)



Prokaryotic Transcription - II

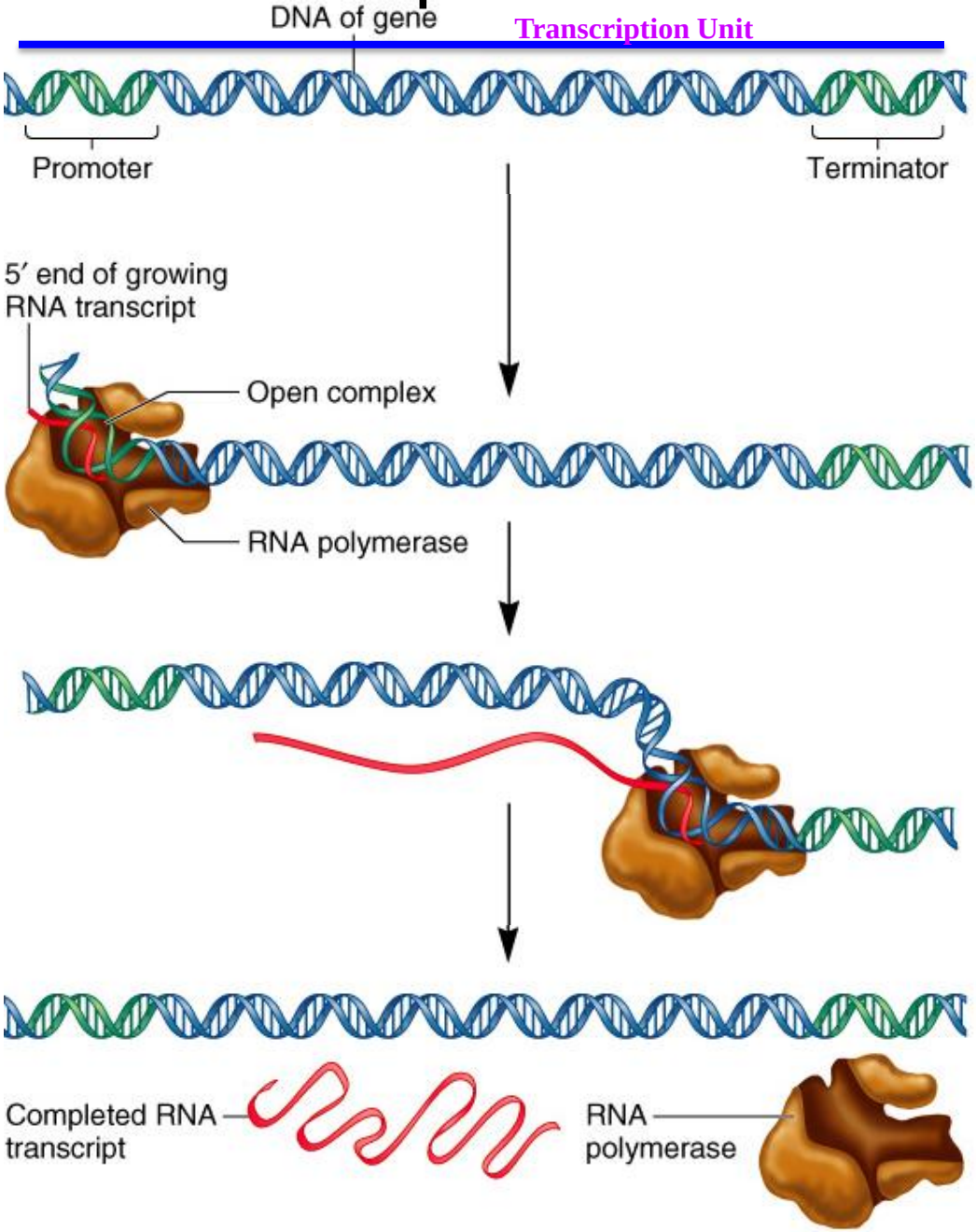
Mechanism of Transcription



The Process of Transcription

- Initiation
  - Where/when most regulation of gene expression occurs
  - Different between proks & euks
- Elongation
  - Essentially same between prokaryotes & eukaryotes
  - Some regulation, more in proks than euks
- Termination
  - Different between proks & euks
  - Some regulation

Transcription Proceeds Through 3 Steps



Initiation

- **Transcription factors & RNA polymerase recognize & bind the promoter**
- **DNA adjacent to the promoter is denatured forming the open promoter complex**

Elongation

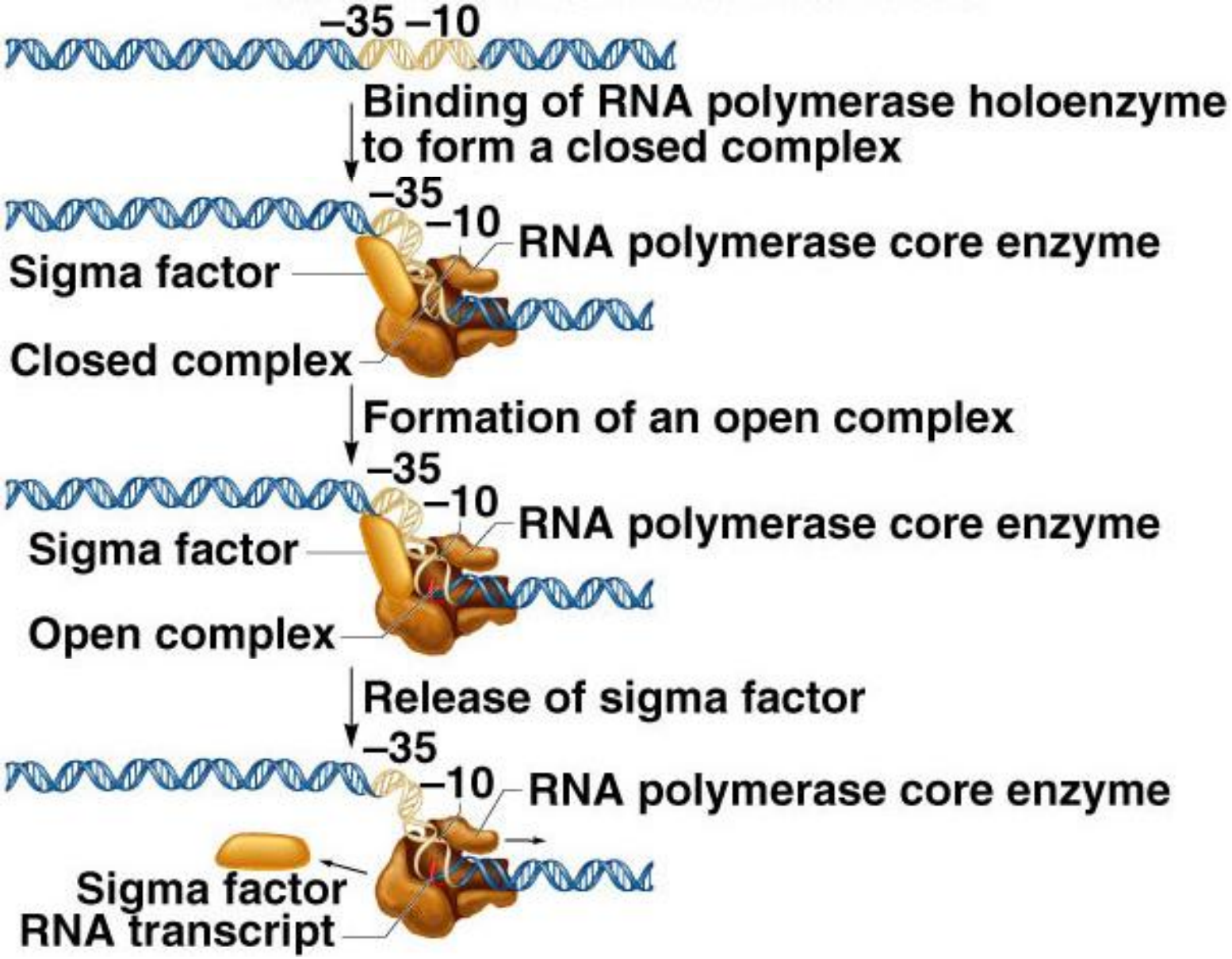
- **RNA polymerase moves along the DNA in synthesizing a RNA transcript. Synthesis is 5'→3' – Only 1 strand of DNA is read as a template.**

Termination

- **A termination signal is reached causing RNA polymerase to dissociated from the DNA**

Prokaryotic Transcription Initiation

Prokaryotic Transcription Initiation



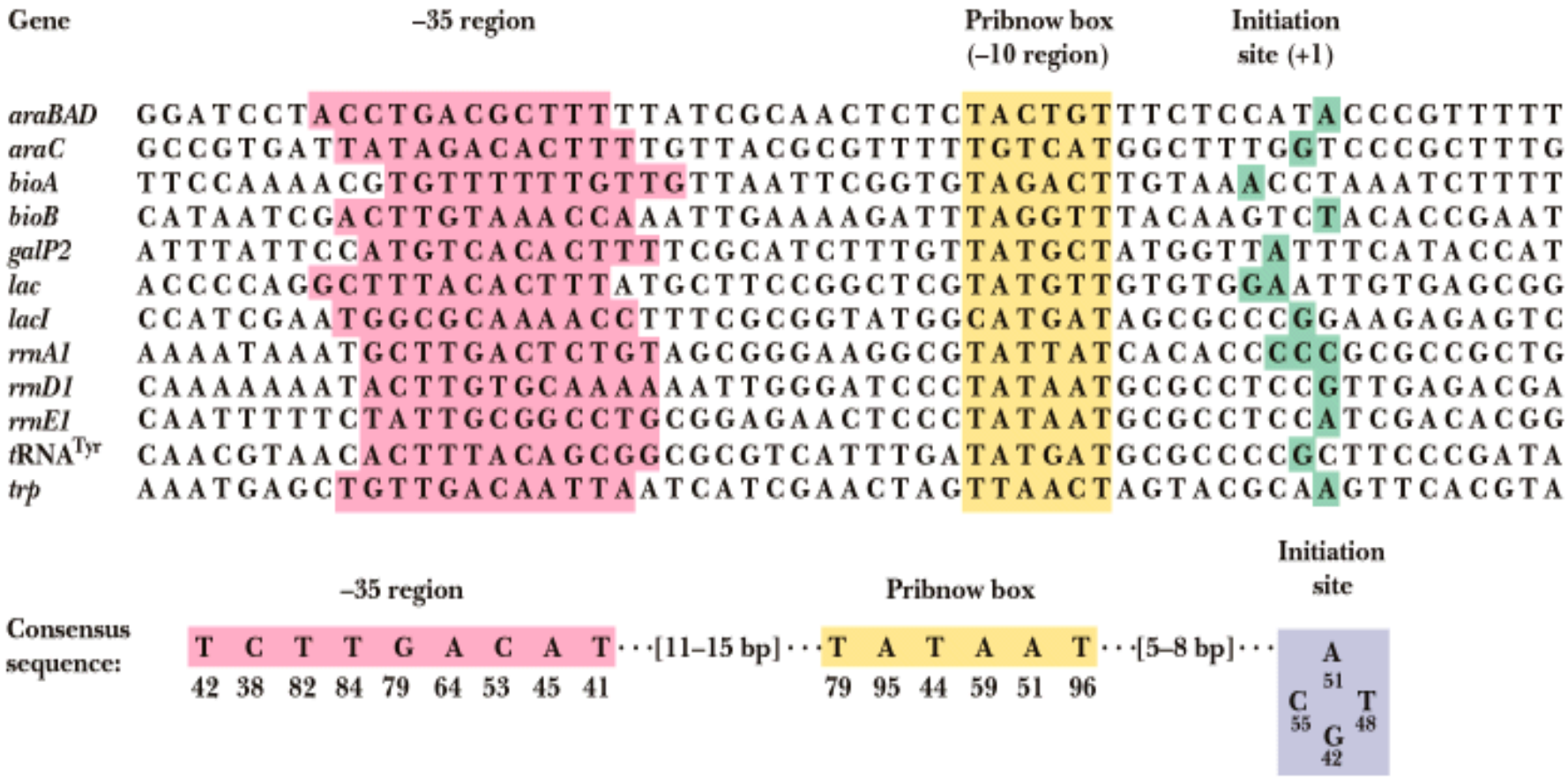
Prokaryotic Promoter

Conserved Sequence:

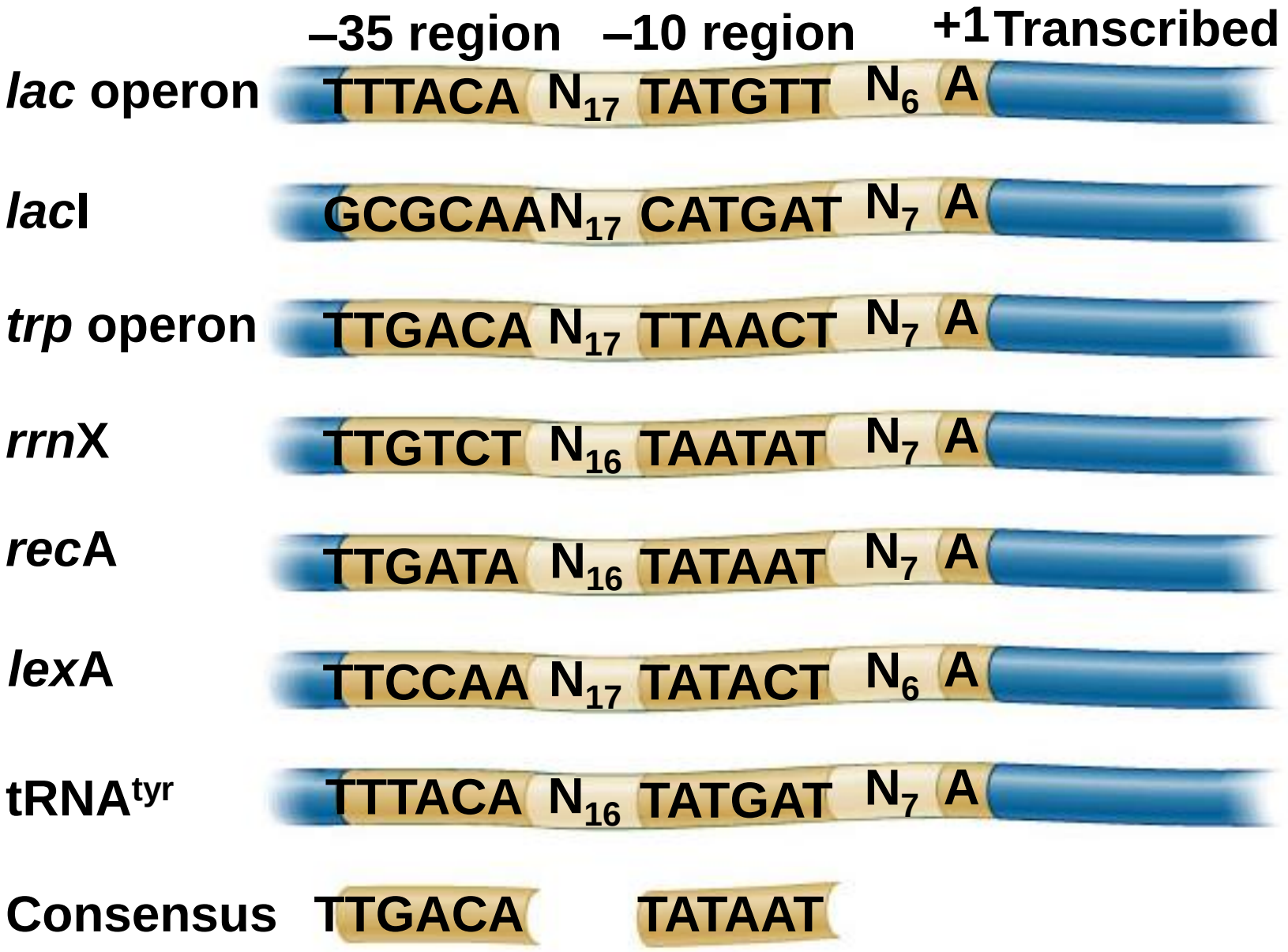
Any essential nucleotide sequence should be present in all the promoter

Consensus Sequence:

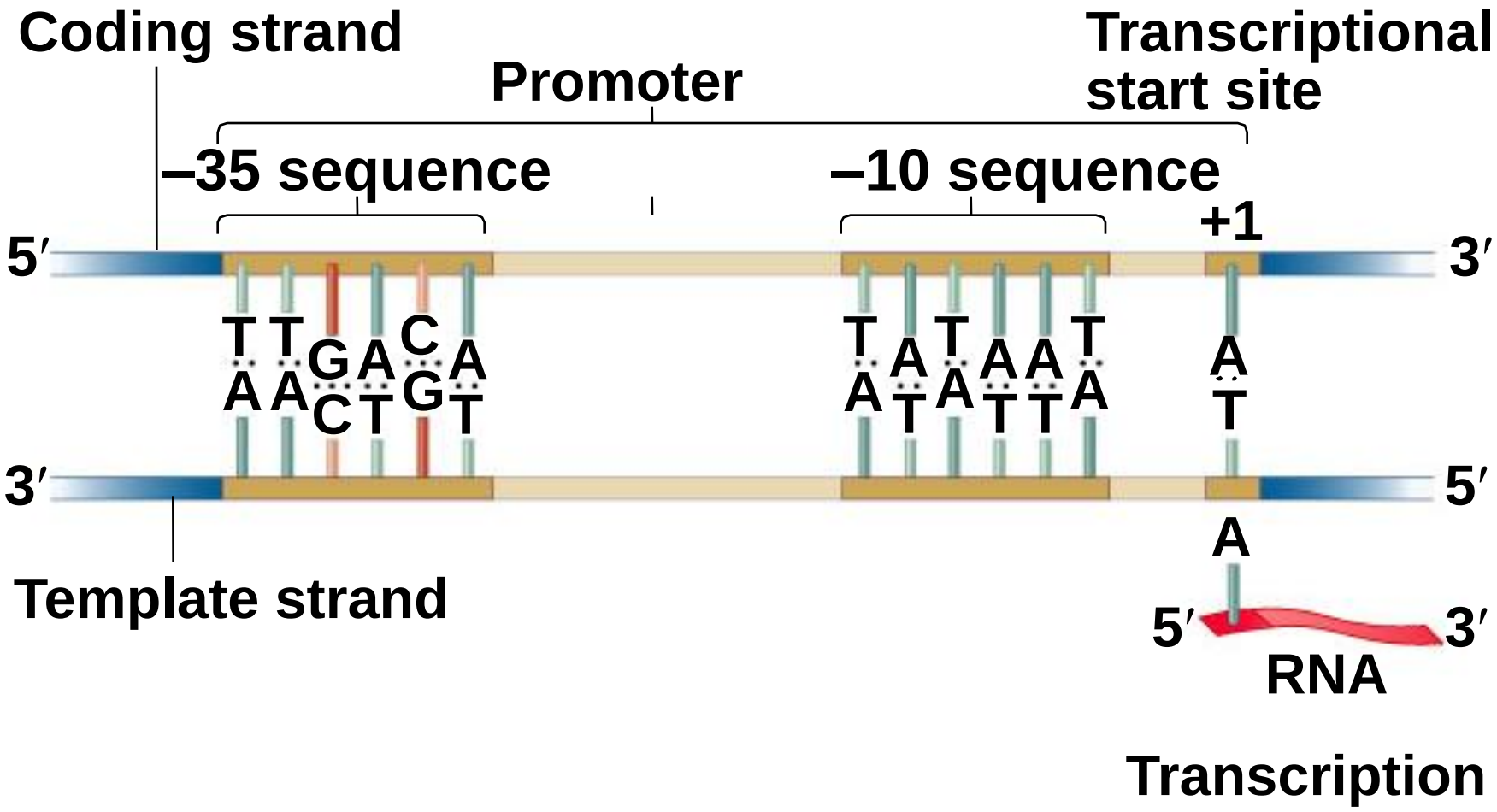
It is defined by aligning all known examples so as to maximize their homology


Gene
–35 region
Pribnow box
(–10 region)
Initiation
site (+1)
araBAD GGATCCTACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCATACCCGTTTTT
araC GCCGTGATTATAGACACTTTTGTTACGCGTTTTTGTCATGGCTTTGGTCCCGCTTTG
bioA TTCCAAAACGTGTTTTTTGTTGTTAATTCGGTGTAGACTTGTAAACCTAAATCTTTTT
bioB CATAATCGACTTGTAAACCAAATTGAAAAGATTTAGGTTTACAAGTCTACACCGAAT
galP2 ATTTATTCCATGTCACACTTTTCGCATCTTTGTTATGCTATGGTTATTTCATACCAT
lac ACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGG
lacI CCATCGAATGGCGCAAAACCTTTCGCGGTATGGCATGATAGCGCCCGGAAGAGAGTC
rrnA1 AAAATAAATGCTTGACTCTGTAGCGGGAAGGCGTATTATCACACCCCGCGCCGCTG
rrnD1 CAAAAAAATACTTGTGCAAAAAATTGGGATCCCTATAATGCGCCTCCGTTGAGACGA
rrnE1 CAATTTTTCTATTGCGGCCTGCGGAGAACTCCCTATAATGCGCCTCCATCGACACGG
tRNA^Tyr CAACGTAACACTTTACAGCGGCGCGTCATTTGATATGATGCGCCCCGCTTCCCGATA
trp AAATGAGCTGTTGACAATTAATCATCGAACTAGTTAACTAGTACGCAAGTTCACGTA
Initiation
site
–35 region
Pribnow box
Consensus
sequence:
T C T T G A C A T ···[11–15 bp]··· T A T A A T ···[5–8 bp]···
42 38 82 84 79 64 53 45 41
79 95 44 59 51 96
A 51
C 55
T 48
G 42

Reaching A Consensus



A Prokaryotic Promoter



Specificity of Promoter Elements

1. **Start Point – A purine (>90% of the time) e.g., CAT**
2. **-10 Box (Element) – Pribnow identified this element required for sequence specific contact of sigma 2.3 & 2.4 regions**

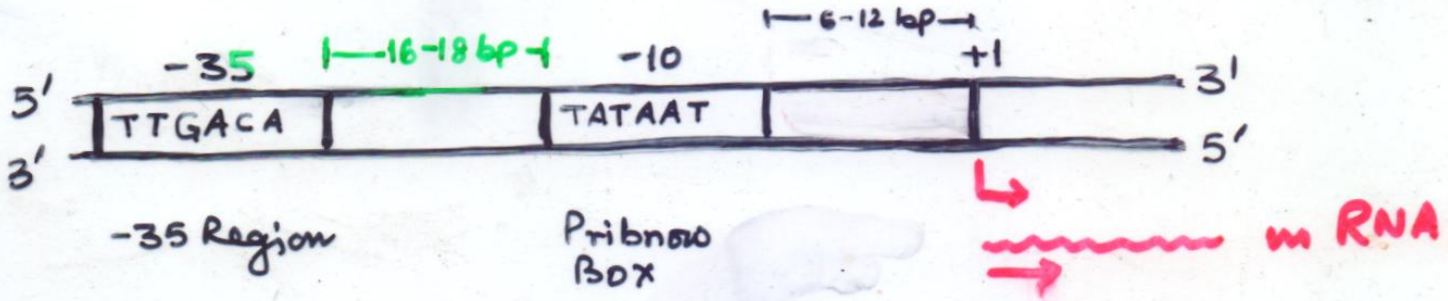
   (can affect the binding or the melting reaction from closed promoter to open promoter)
3. **-35 Box (Element) – Required for sequence specific contact of sigma 4.2 region**

   (can affect initial binding of RNA Polymerase)
4. **UP Element – Increases promoter's strength**

   (e.g., found in promoters of rRNA genes)
5. **Discriminator Region – GGG found in rRNA & tRNA promoters**
6. **Extended -10 Element – TGn sequence found in σ^{70} promoters that lack -35 elements**
7. **Separation between -10 & -35 elements – 16 to 18 bp**

   (can influence strength of a promoter)

DESIGN OF A PROKARYOTIC PROMOTER



Pribnow Box $T_{80\%}$ $A_{95\%}$ $T_{45\%}$ $A_{60\%}$ $A_{50\%}$ $T_{96\%}$

-35 Region $T_{82\%}$ $T_{84\%}$ $G_{78\%}$ $A_{65\%}$ $C_{54\%}$ $A_{45\%}$

* In pribnow box - ① TA conserved in 90% of promoters
② Other three nucleotides show some variations

* The distances between -10 and -35 position is thought to influence the strength of a promoter.
- normally (16-18 bp) i.e in 90% of promoters
- Some times 15 bp & may be 20 bp in some cases

DESIGN OF A PROKARYOTIC PROMOTER

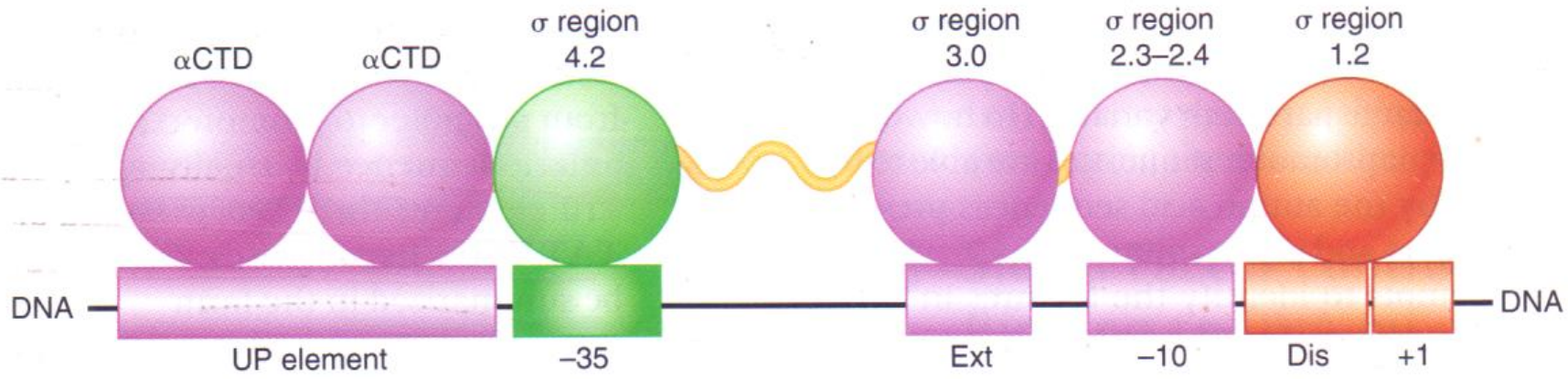
Regions of Sigma Factors

Four main regions that are generally conserved.

N-terminus ---------- C-terminus

1.1 2 3 4

DNA elements & RNA polymerase modules that contribute to promoter recognition by sigma factor



SIGMA (σ) FACTORS

1. A sigma factor is a protein needed only for initiation of RNA synthesis.
2. It is a bacterial transcription initiation factor that enables specific binding of RNA polymerase to gene promoters.
3. The specific sigma factor used to initiate transcription of a given gene will vary. It depends on following two factors:
   a) On gene itself
   b) On the environmental signals needed to initiate transcription of that gene.
4. Every molecule of RNA polymerase holoenzyme contains exactly one sigma factors .
5. The number of sigma factors varies between bacterial species.

#	Species	Types of sigma factors
1	*E. coli*	07
2	*B. subtilis*	18
3	*Streptomyces coelicolor*	60

E. coli Sigma Factors

(recognize promoters with different consensus sequences)

Subunit/gene	Size (# aa)	Approx. # of promoters	Promoter sequence recognized
Sigma 70 (*rpoD*)	613	1000	TTGACA-16 to 18 bp-TATAAT
Sigma 54 (*rpoN*)	477	5	CTGGNA-6 bp-TTGCA
Sigma S (*rpoS*)	330	100	TTGACA-16 to 18 bp-TATAAT
Sigma 32 (*rpoH*)	284	30	CCCTTGAA-13 to 15 bp-CCCGATNT
Sigma F(*rpoF*)	239	40	CTAAA-15 bp-GCCGATAA
Sigma E (*rpoE*)	202	20	GAA-16 bp-YCTGA
Sigma FecI (*fecI*)	173	1–2	?

Anti-sigma Factors

1. In the regulation of gene expression in prokaryotes, anti-sigma factors and inhibit transcriptional activity.
2. It has been found in a number of bacteria including *E.coli.*
3. Anti sigma factors are antagonists to the sigma factors which regulate number of cellular processes including flagellar production, stress response, transport and cellular growth etc.

Example : 70 Rsd in *E.coli*

Anti-anti-sigma Factors

1. **The anti-sigma factor antagonist is an anti-anti-sigma factor.**
2. **It relieves inhibition of sigma factor activity by the anti-sigma factor.**

Example : SpoIIAA protein in *Bacillus subtilis*

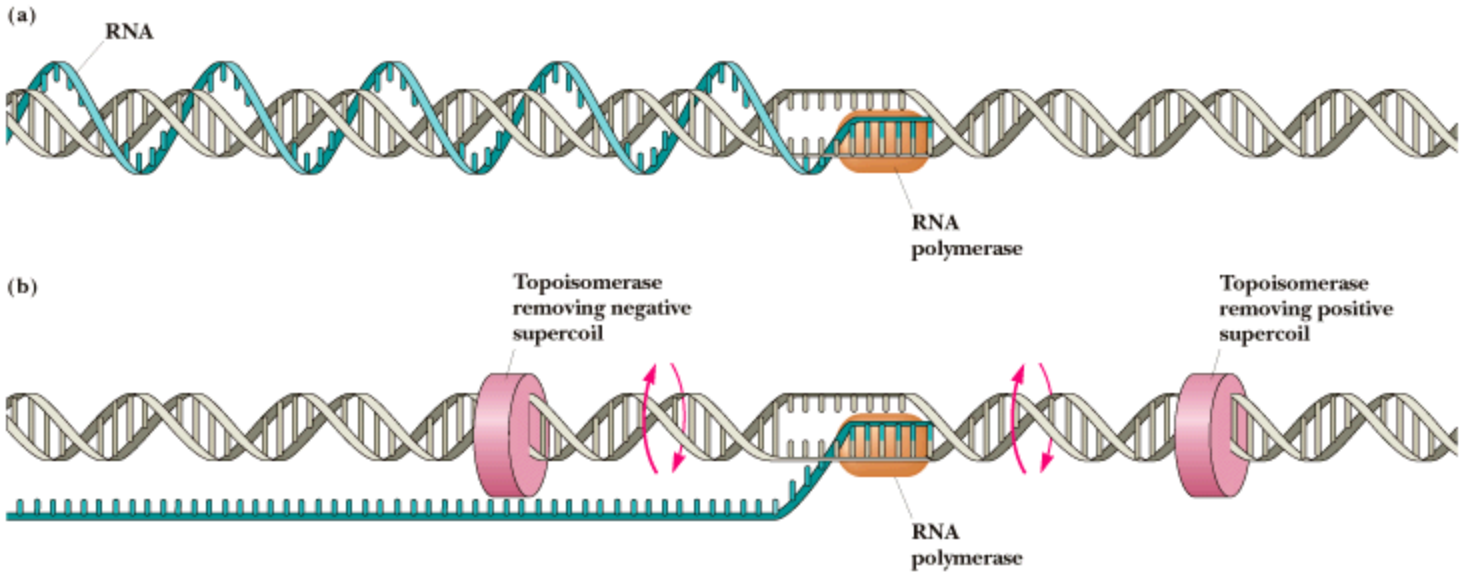
Chain Elongation

Core polymerase – with 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**

Garrett & Grisham: Biochemistry, 2/e
Figure 31.6

Chain Elongation



Functions of elongating RNA Polymerase

1. Unwind & Re-wind DNA
2. Holds separated strands of DNA
3. Catalysis of phosphodiester bond formation between two nucleotides
4. Monitor the progress of catalysis
5. To fix problems that occur during the process

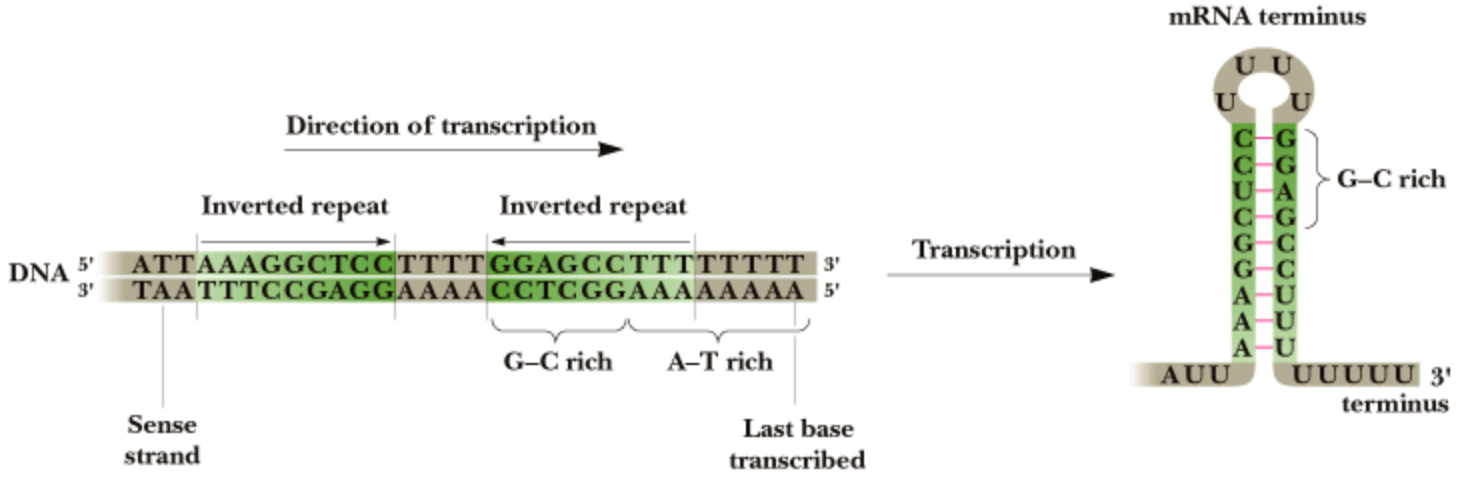
Termination

Chain Termination

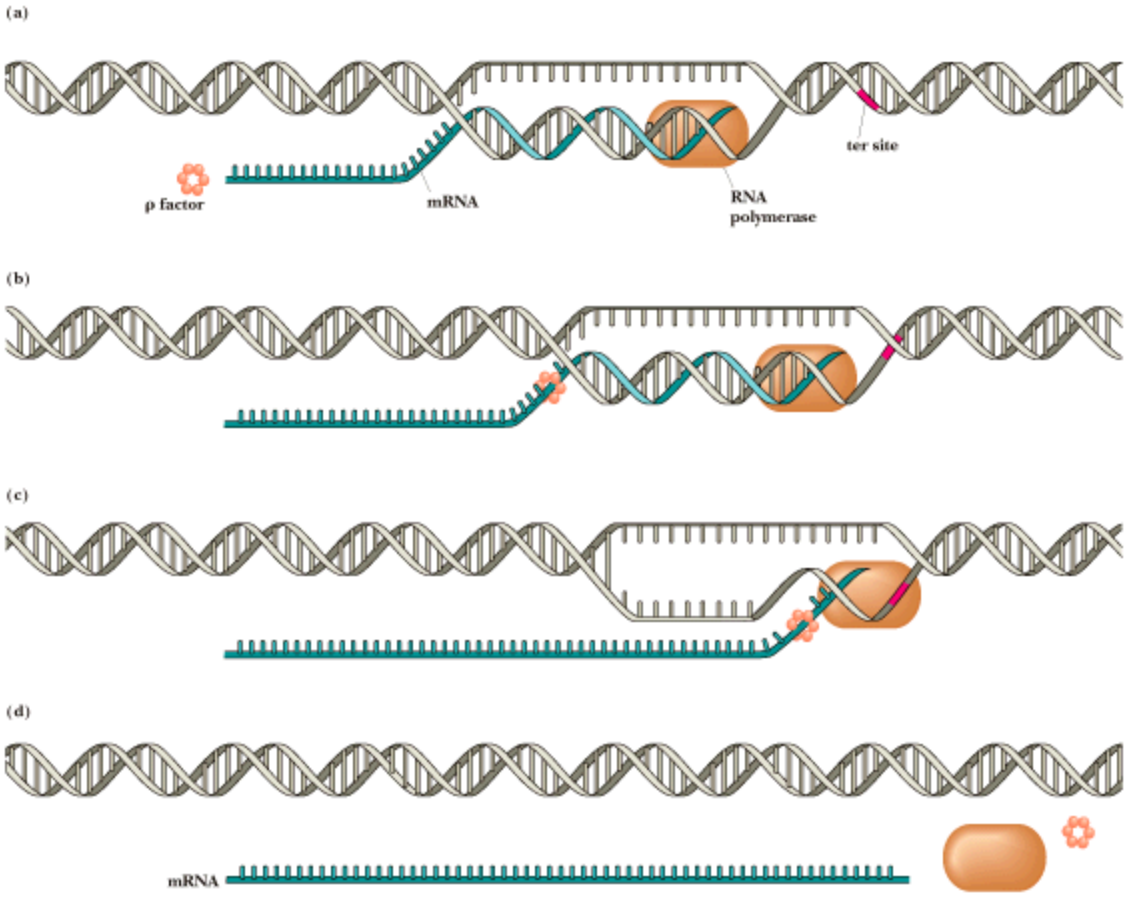
By two mechanisms

1. **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
2. **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

Garrett & Grisham: Biochemistry, 2/e
Figure 31.7



Garrett & Grisham: Biochemistry, 2/e
Figure 31.8



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Rho Dependent Termination in Prokaryotes

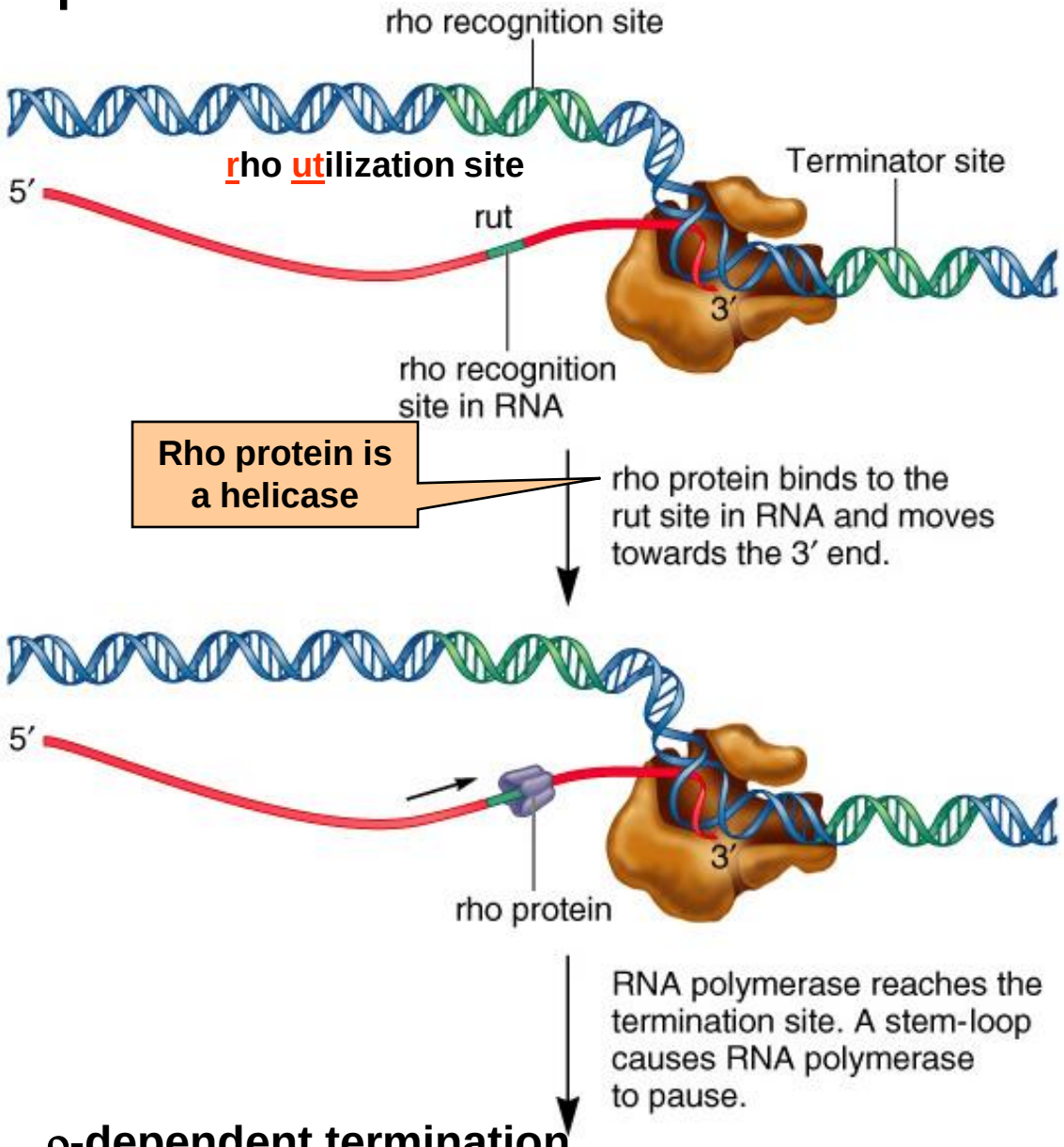


Figure 12.8 **ρ-dependent termination**

Rho Dependent Termination in Prokaryotes

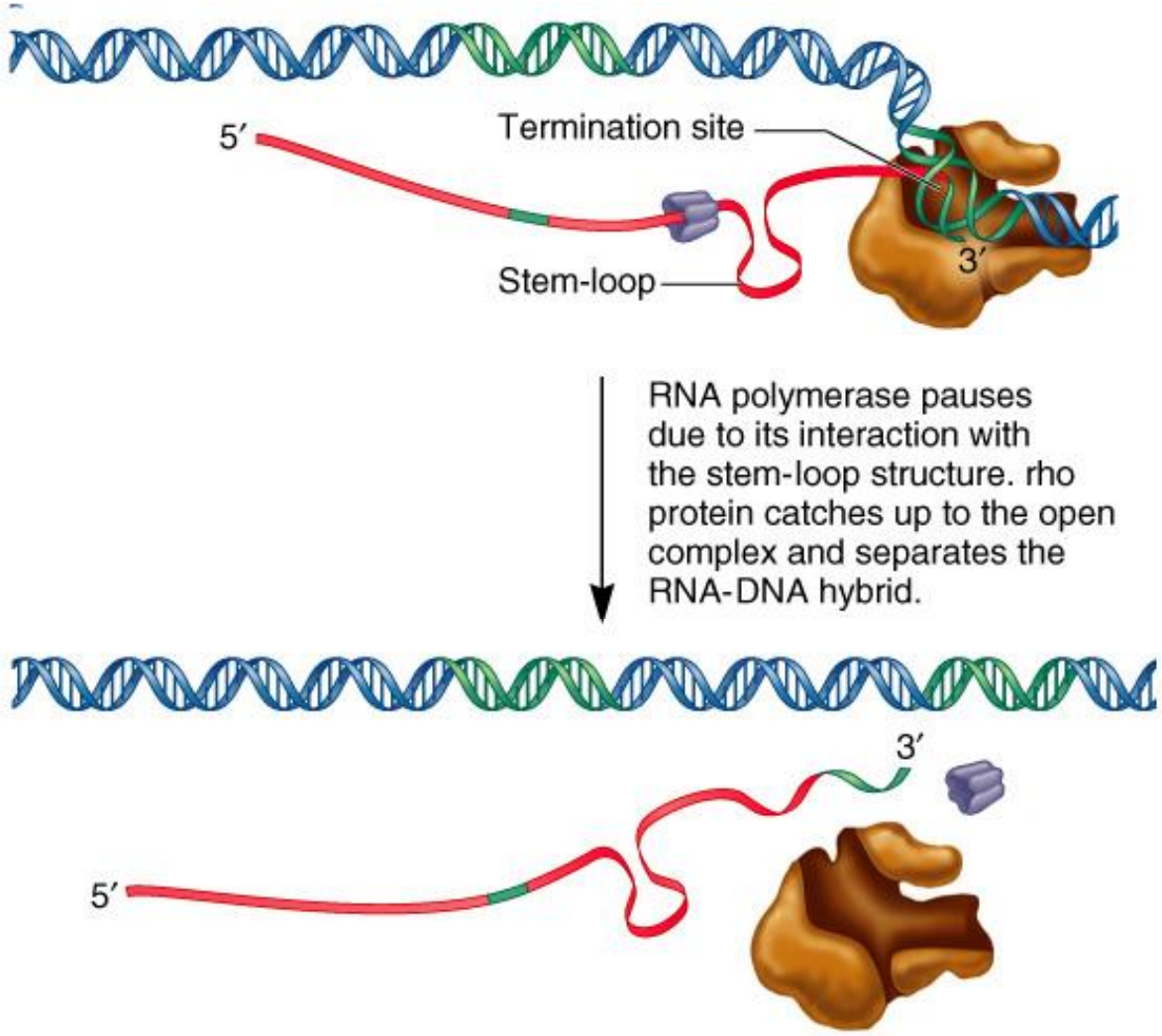


Figure 12.8 **ρ-dependent termination**

Rho Independent Termination in Prokaryotes

- ρ-independent termination requires two sequences in the RNA
  - A stem-loop structure upstream of 7-9 U residues

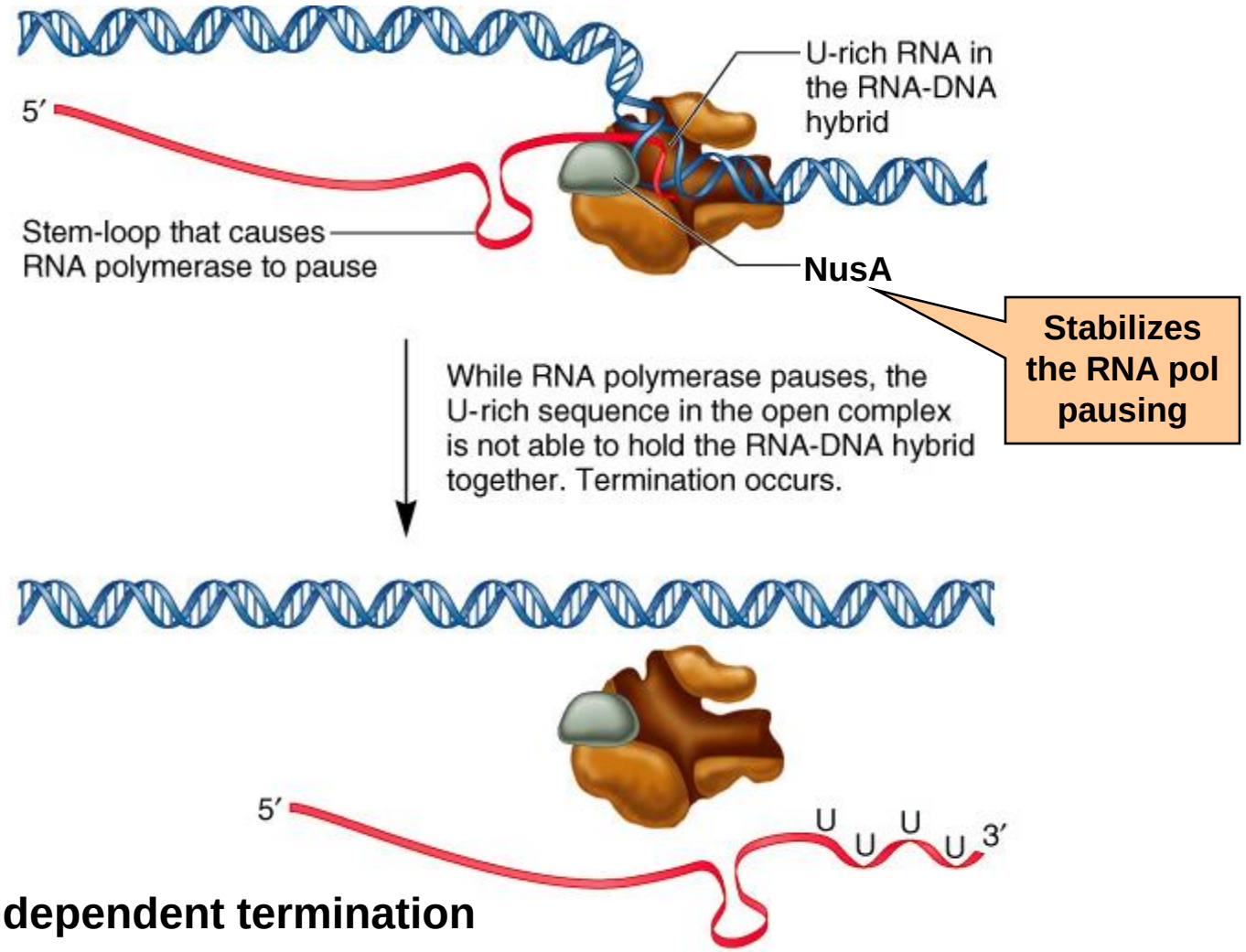


Figure 12.9 **ρ-independent termination**

Key Concept

1. There are two classes of terminators: Those recognized solely by RNA polymerase itself without the requirement for any cellular factors are usually referred to as "intrinsic terminators." Others require a cellular protein called rho and are referred to as "rho-dependent terminators."
2. Intrinsic termination requires recognition of a terminator sequence in DNA that codes for a hairpin structure in the RNA product.
3. The signals for termination lie mostly within *sequences already transcribed* by RNA polymerase, and thus termination relies on scrutiny of the template and/or the RNA product that the polymerase is transcribing.

Regulation of Transcription in Prokaryotes

Regulation by Three Mechanisms

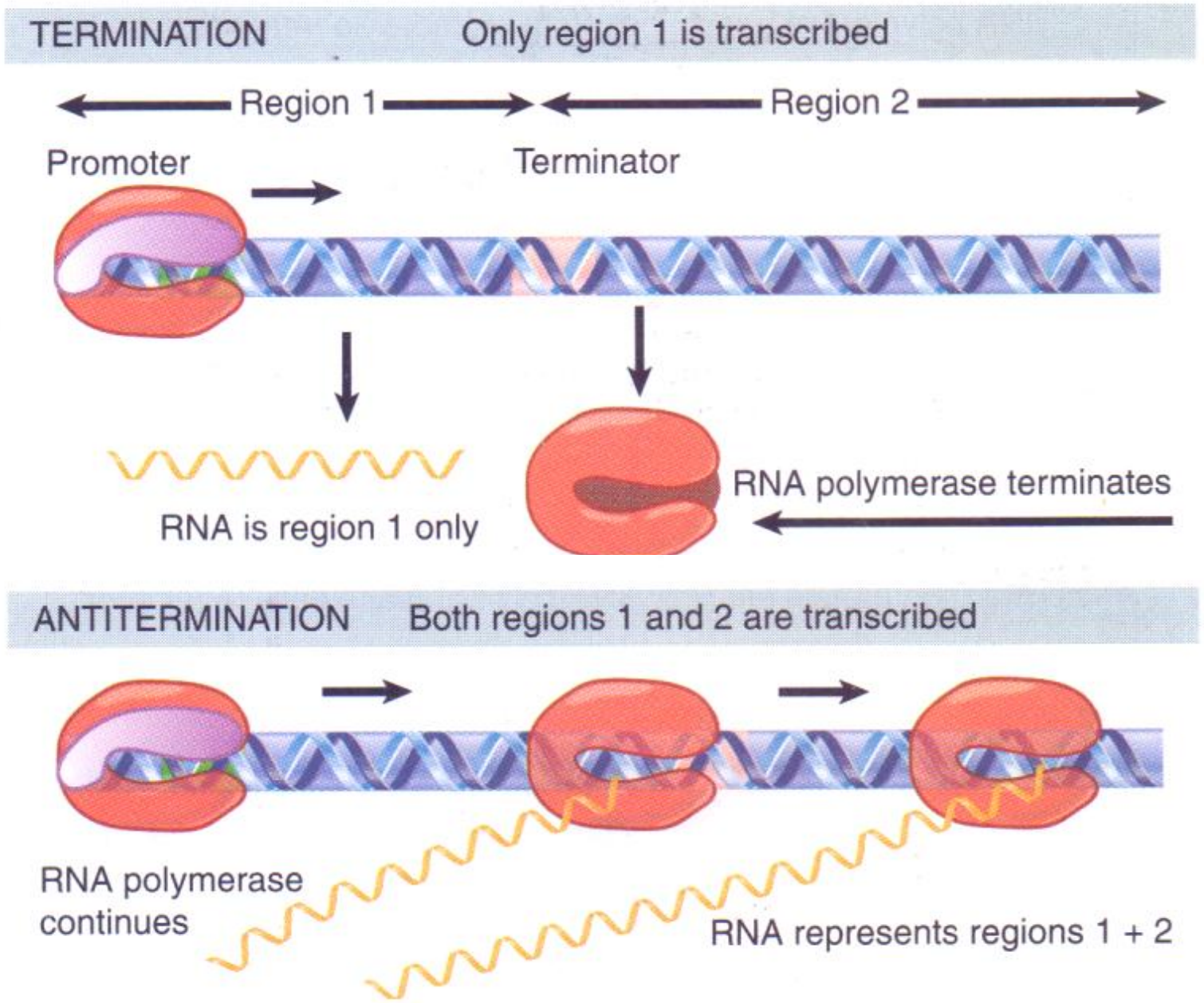
1. Anti-termination Control
2. Attenuation Control
3. Control by Proteins
   - a) Negative Control by Repressors
     - e.g., Lac repressor
   - b) Positive Control by Activators
     - e.g., Catabolite Gene Activator Protein
   - c) Control by Sigma Factors

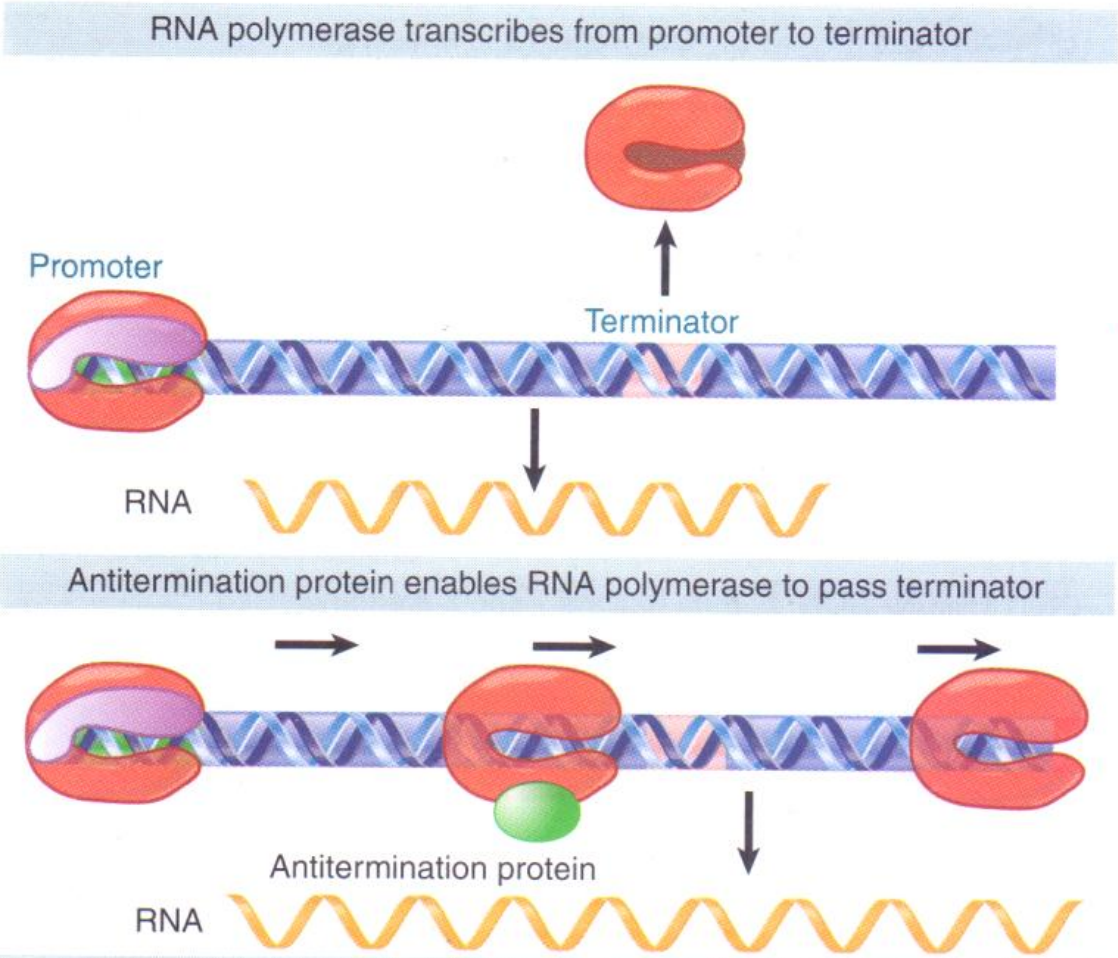
Anti-termination Control

Anti-termination is used as a mechanism for control of transcription in both phage and bacterial operons.

Anti-termination refers to modification of the enzyme, which allows it to read past a terminator into genes that lie downstream.

Anti-termination Control





Antitermination proteins act on specific terminators

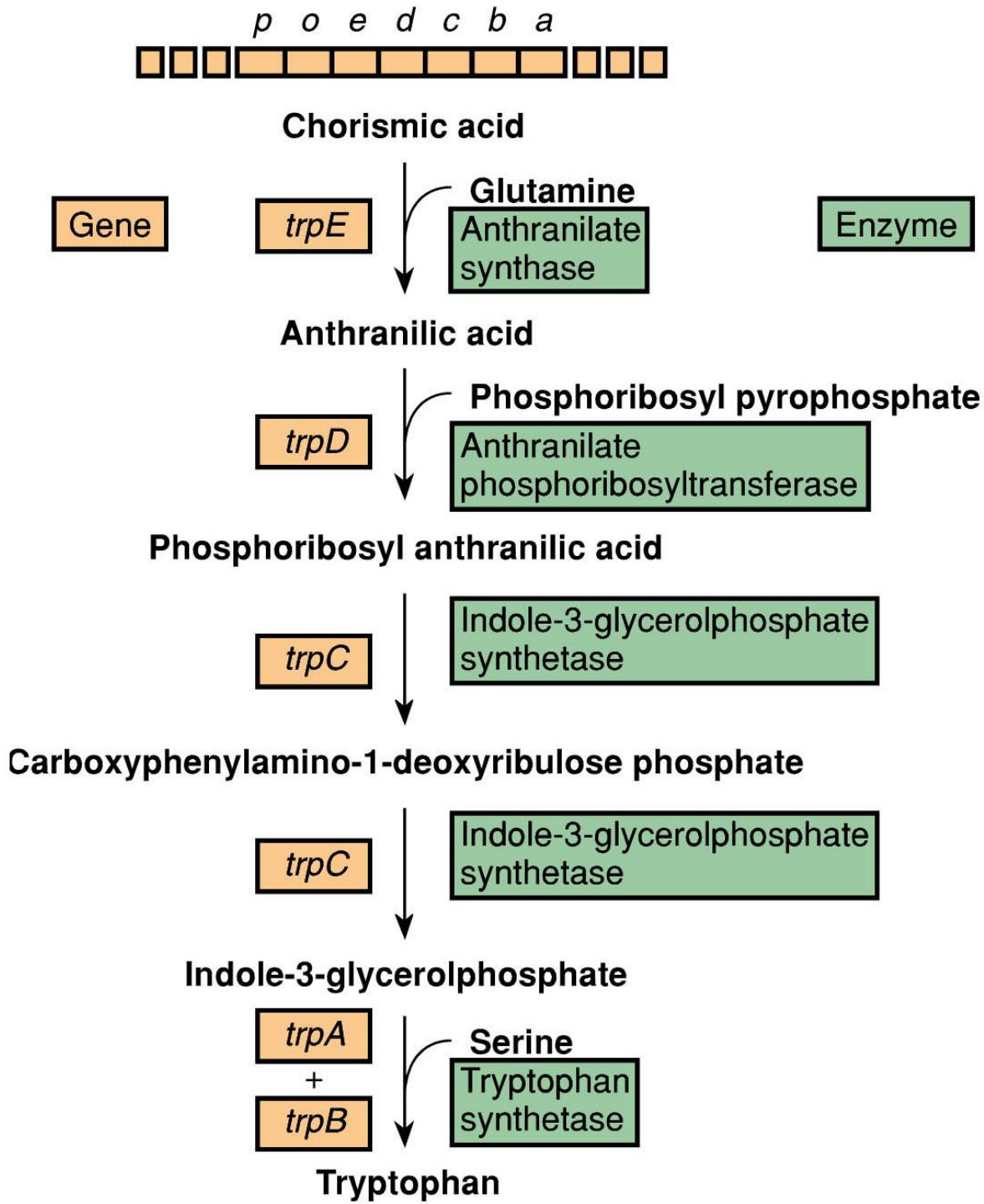
Transcription unit	Promoter	Terminator	Antitermination Protein
Immediate early	P_L	t_L	pN
Immediate early	P_{R1}	t_{R1}	pN
Late	$P_{R'}$	$t_{R'}$	pQ

FIGURE 19.44 An antitermination protein can act on RNA polymerase to enable it to read through a specific terminator.

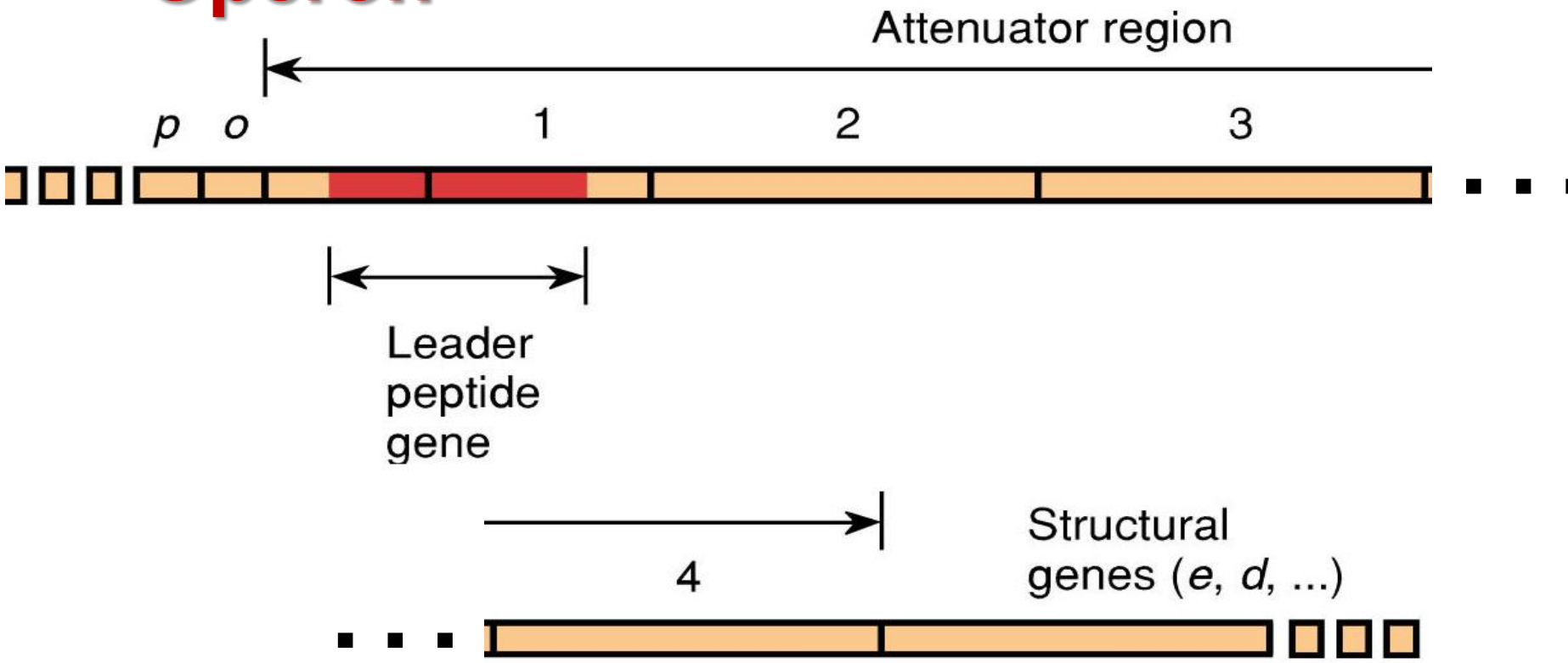
Key Concept

1. An antitermination complex allows RNA polymerase to read through terminators.
2. Phage lambda uses antitermination systems for regulation of both its early and late transcripts, but the two systems work by completely different mechanisms.
3. Binding of factors to the nascent RNA links the antitermination proteins to the terminator site through an RNA loop.
4. Antitermination of transcription also occurs in rRNA operons.

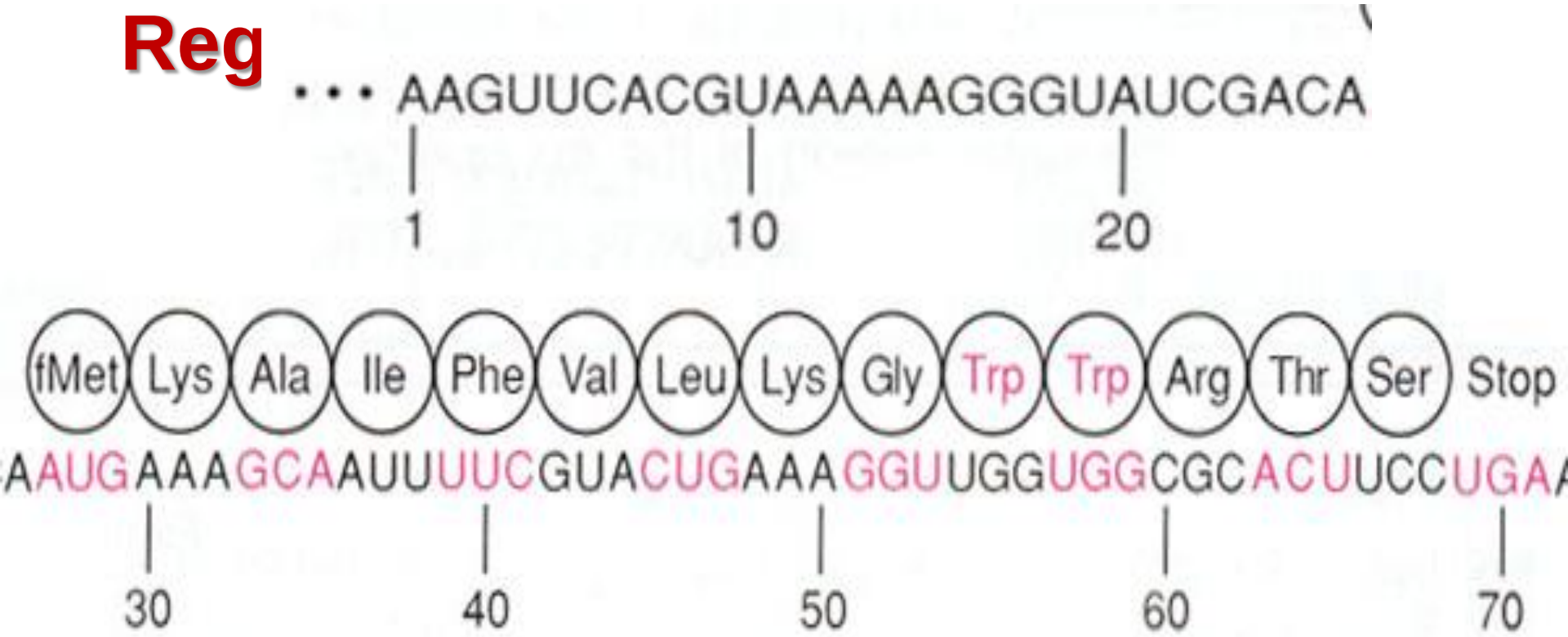
trp operon


p o e d c b a
Chorismic acid
Gene
trpE
Glutamine
Anthranilate synthase
Enzyme
Anthranilic acid
trpD
Phosphoribosyl pyrophosphate
Anthranilate phosphoribosyltransferase
Phosphoribosyl anthranilic acid
trpC
Indole-3-glycerolphosphate synthetase
Carboxyphenylamino-1-deoxyribulose phosphate
trpC
Indole-3-glycerolphosphate synthetase
Indole-3-glycerolphosphate
trpA
+
trpB
Serine
Tryptophan synthetase
Tryptophan

Attenuator Region of Trp Operon



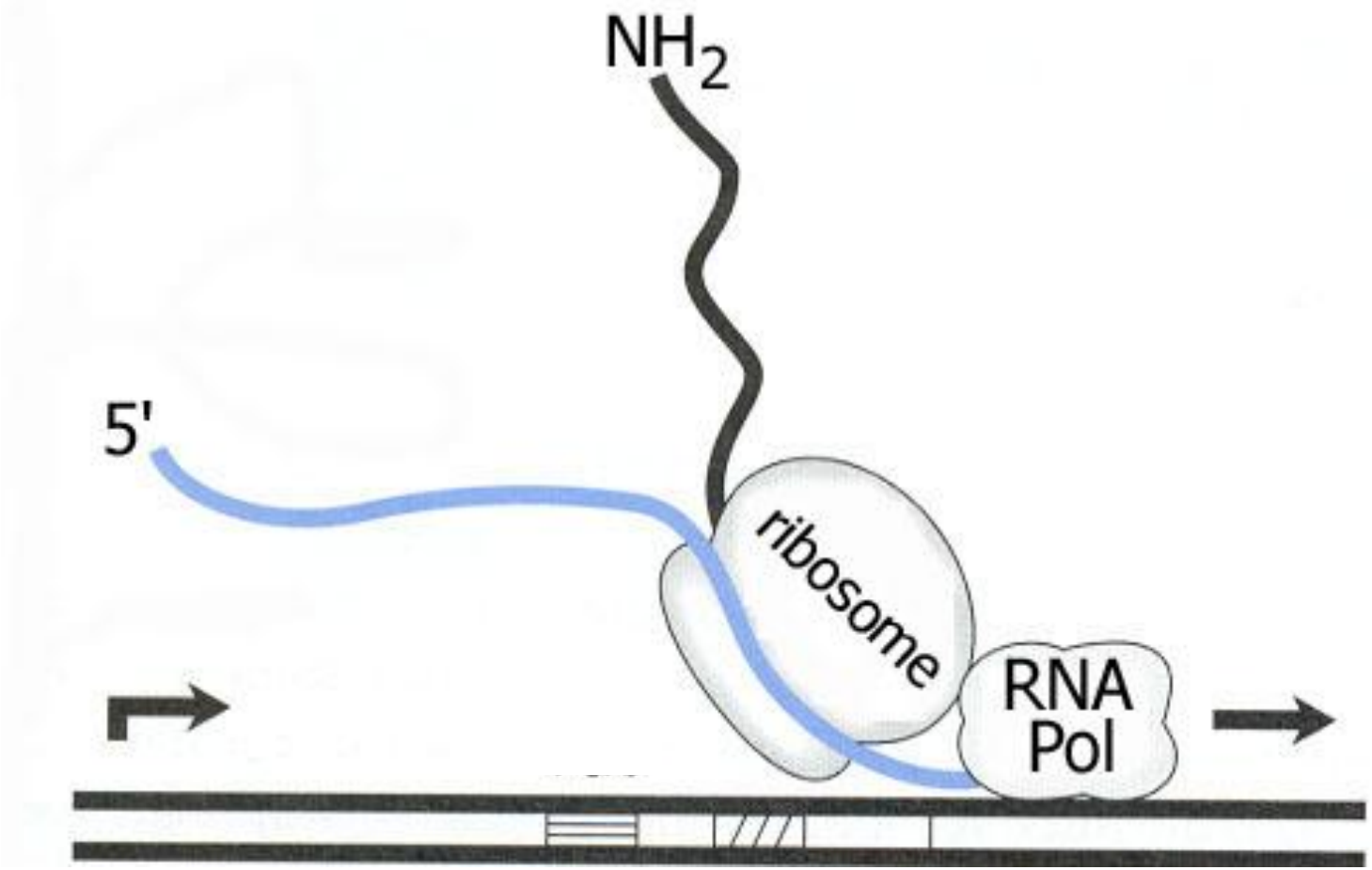
The Importance of the Leader Reg



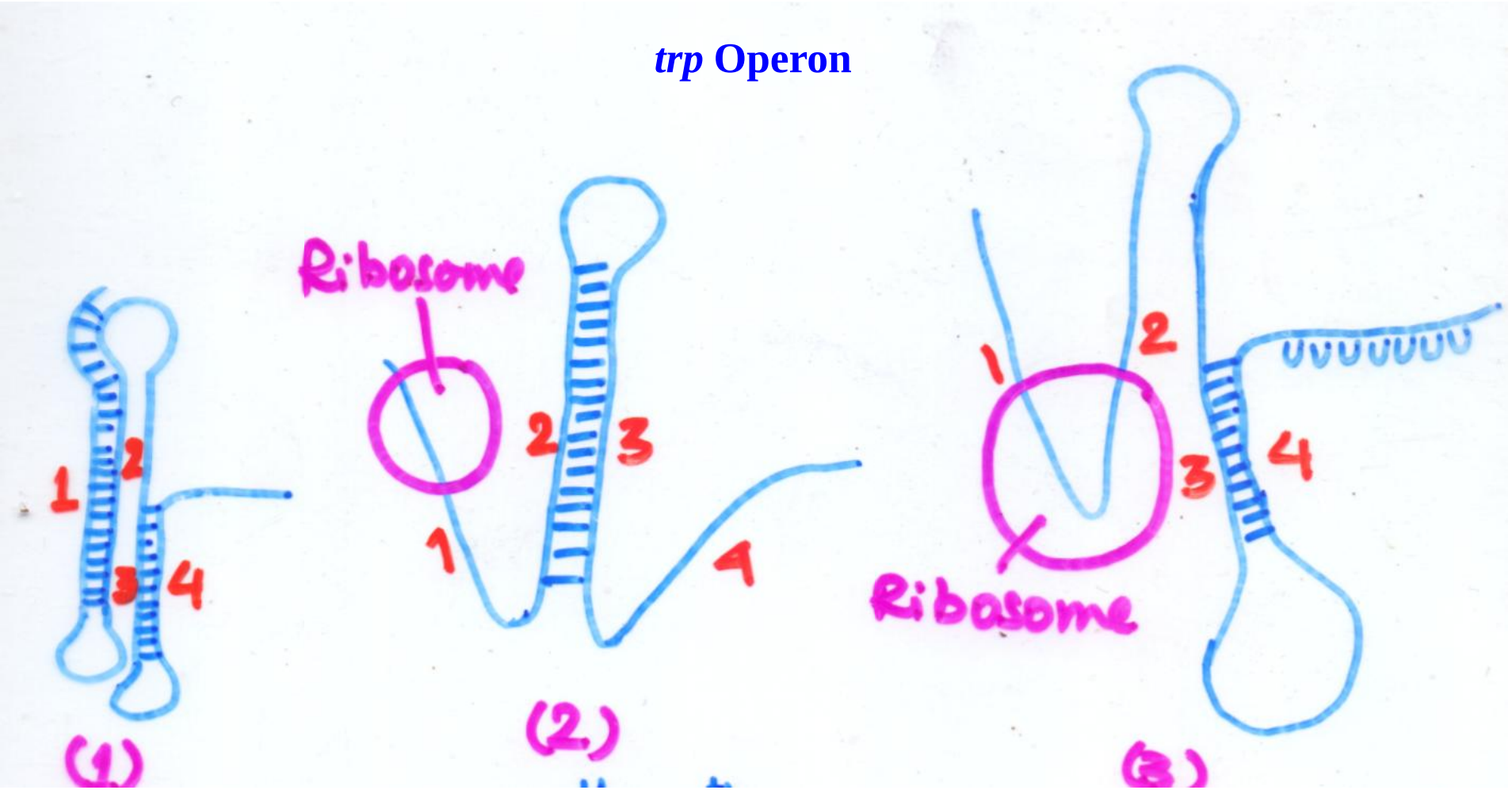
-the 14 amino acid peptide formed from the leader sequence has 2 tryptophans.

-trp is a "rare" amino acid

Recall that in bacteria, translation typically occurs almost simultaneously with transcription.



Attenuation Control



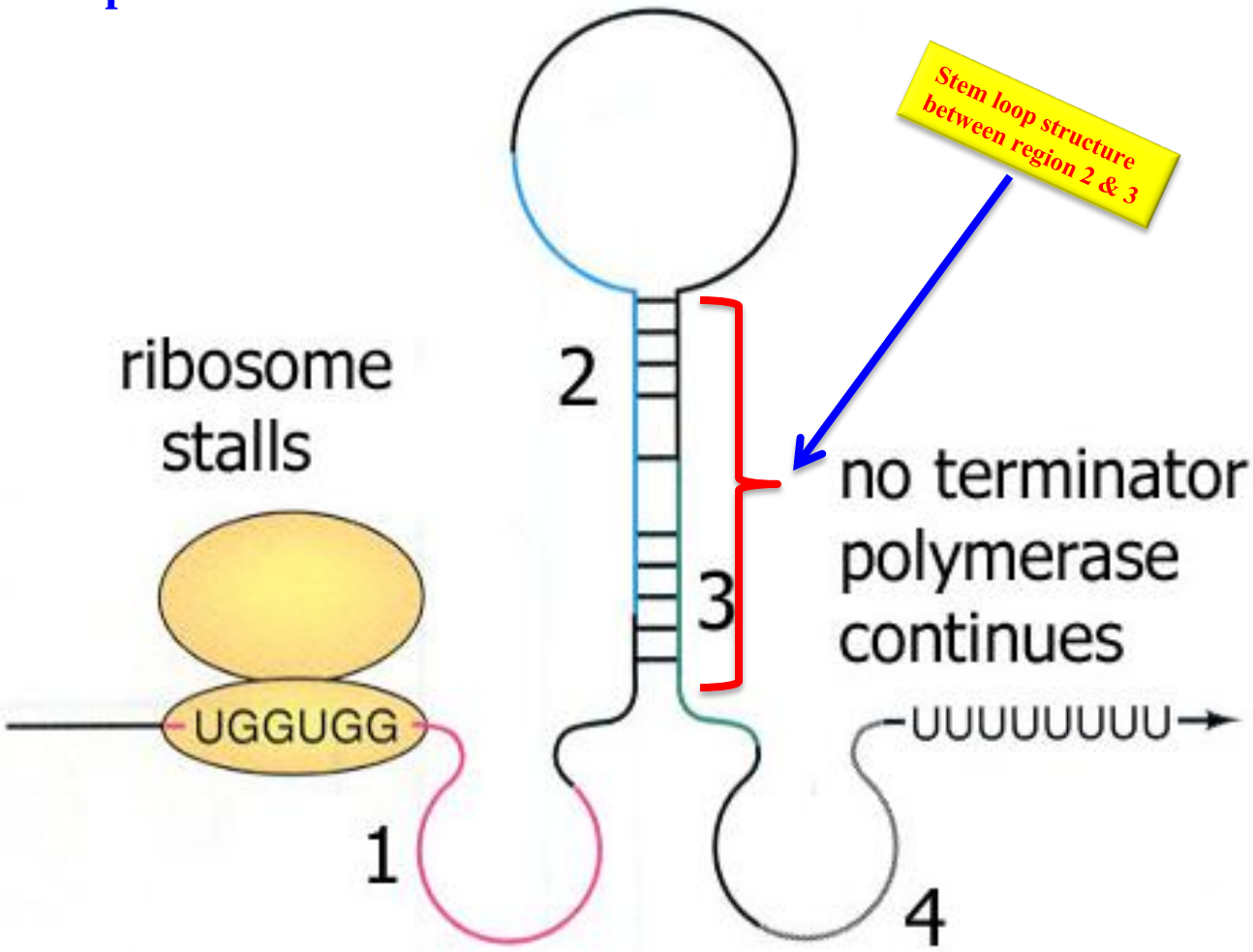
Free mRNA base pairs between 1&2 and between 3 & 4 (NO trp)

Continuation of transcription at low conc of trp (2 & 3 will base pair)

- **At high trp conc ribosome will block 1 & 2 segment**
- **Hence, 3 & 4 will base pair and due to 7 Us, chain will terminate**

At low Trp level in the cytoplasm

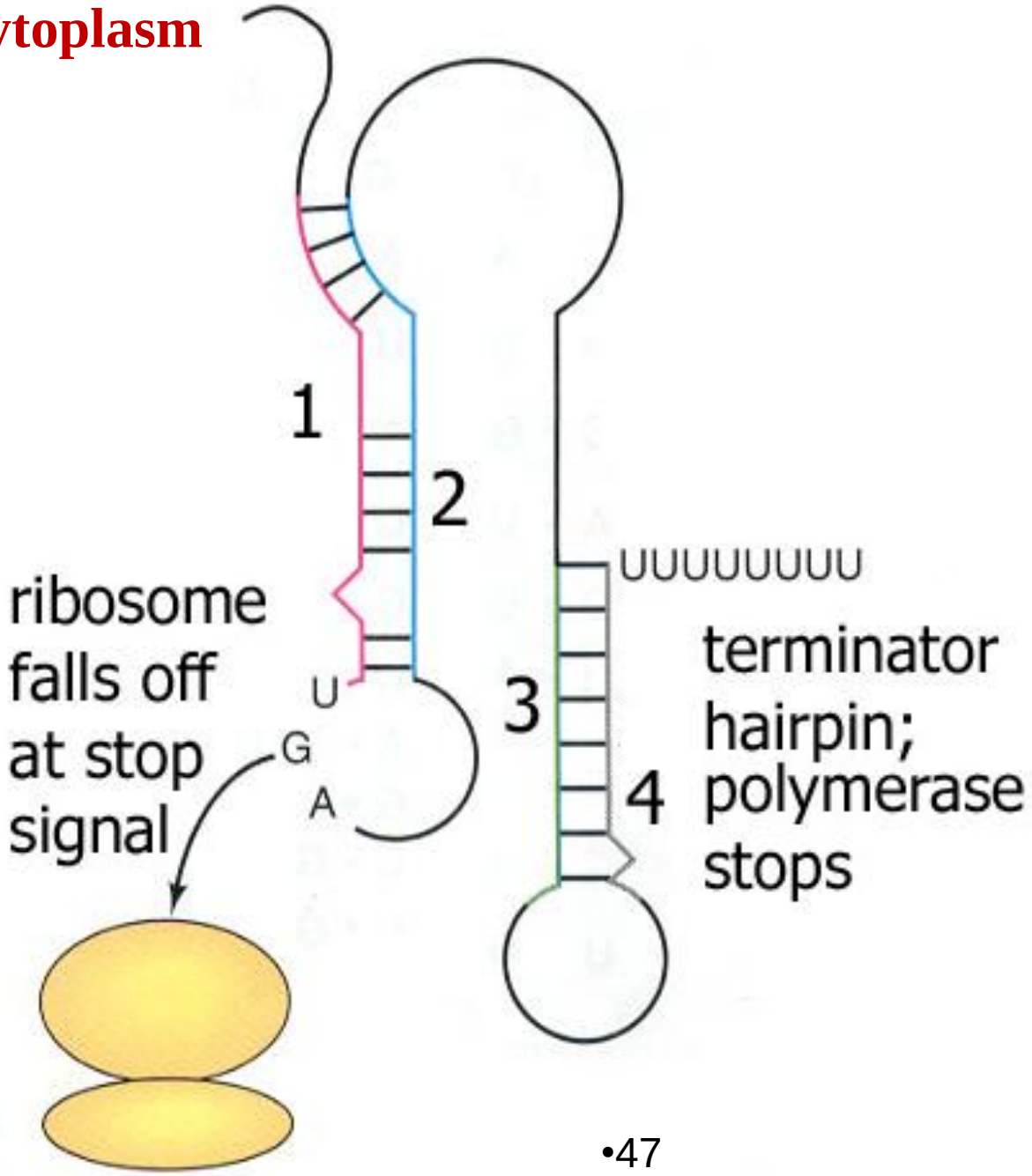
The stalled ribosome prevents the formation of stem loops 1-2/3-4 and promote the formation of stem loop structure 2-3



At high Trp level in the cytoplasm

Effect on ribosome and transcription at HIGH Trp levels

Note: the 14 amino acid leader peptide is synthesized



Control by Proteins

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