SOS in Biochemistry, Jiwaji University, Gwalior

M.Sc. II Semester (2019-20)

Paper BCH 201: Fundamentals of Molecular Biology (Unit III)

trp Operon

(A negative repressible operon)

TRYPTOPHAN OPERON

(A Biosynthetic System)

Erythrose-4-phosphate (Pentose phosphate pathway intermediate)

+

Phosphoenol pyruvate (a glycolytic intermediate)

Condensation of PEP with E-4-P

↓

Shikimate

↓

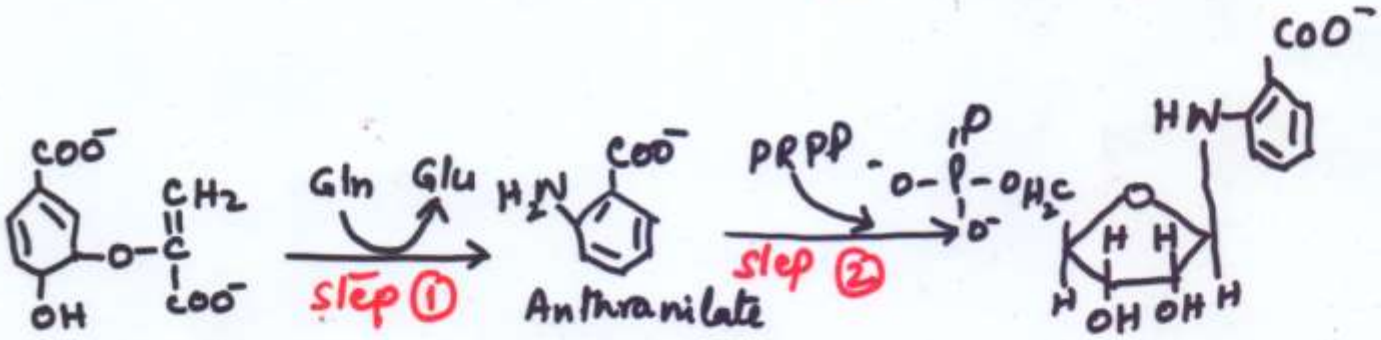
Chorismate

↙ ↘

Prephenate Anthranilate

↙ ↘ ↓

Phe Tyr Trp



Chorismate

Anthranilate

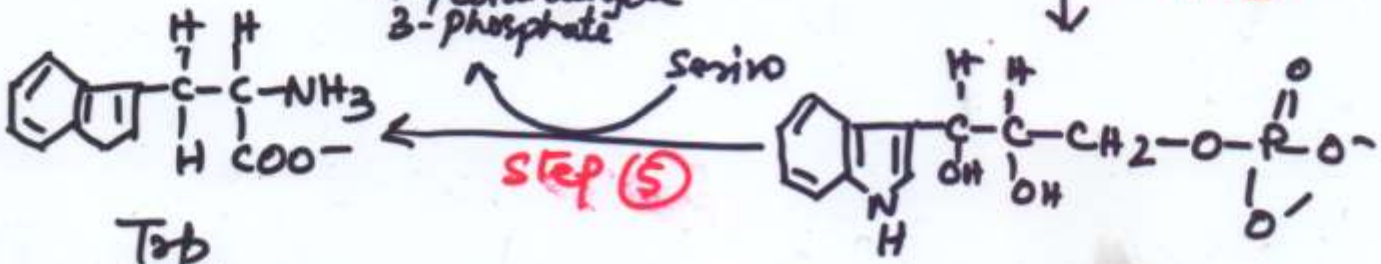
N-5'-phosphoribosyl anthranilate

step ③

1-(o-carboxyphenylamino)-1-deoxyribulose-5-phosphate

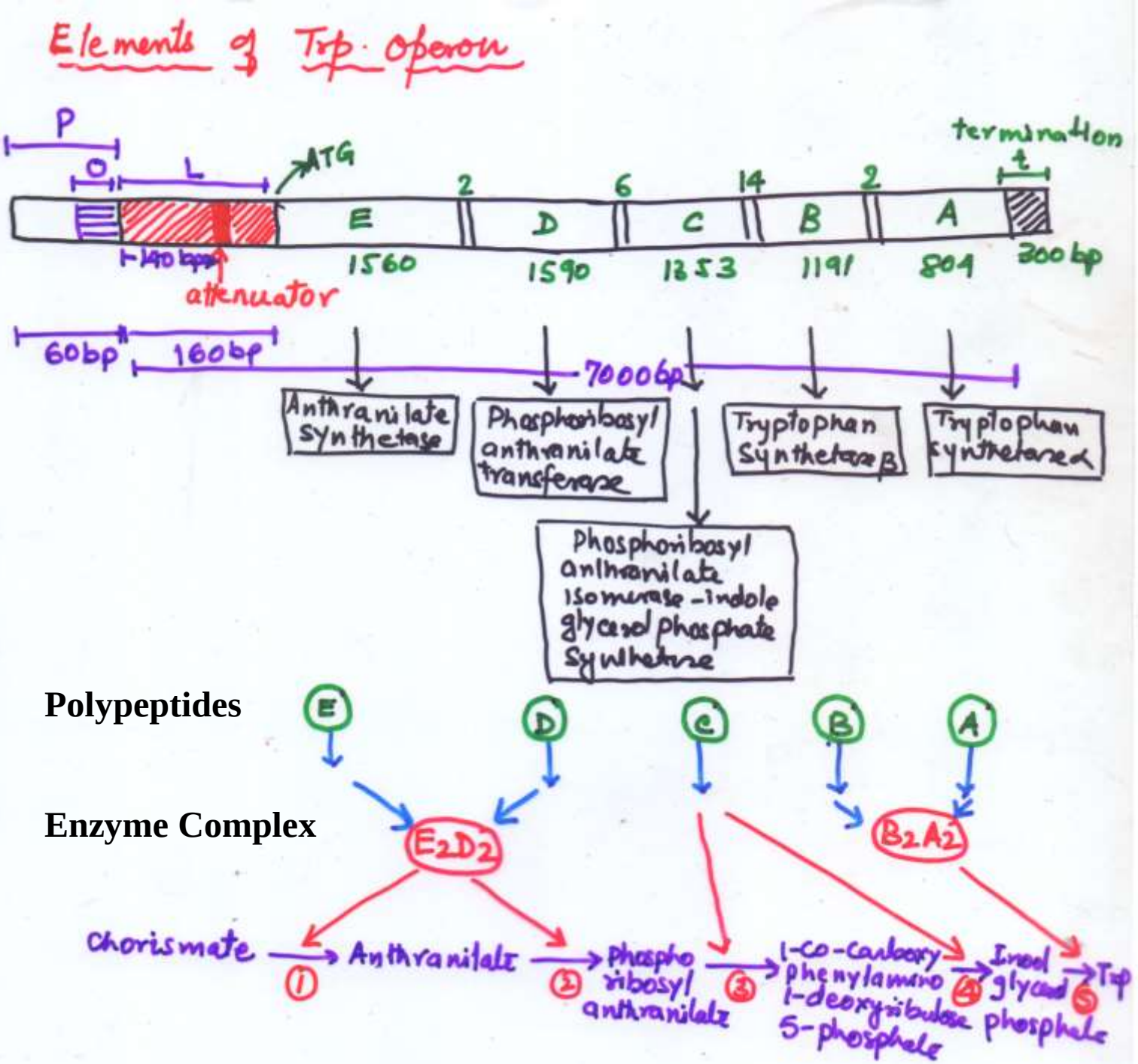
step ④

Synthesis of Trp from Chorismate



Trp

Indole-3-Glycerol phosphate


Elements of Trp. Operon
P
O
L
ATG
termination
t
2
6
14
2
E
D
C
B
A
140 bps
attenuator
1560
1590
1253
1191
804
300 bp
60bp
160bp
7000bp
Anthranilate synthetase
Phosphoribosyl anthranilate transferase
Tryptophan Synthetase B
Tryptophan Synthetase A
Phosphoribosyl anthranilate isomerase - indole glycerol phosphate Synthetase
Polypeptides
Enzyme Complex
E
D
C
B
A
E2D2
B2A2
Chorismate
Anthranilate
Phospho ribosyl anthranilate
1-co-carboxy phenylamino 1-deoxyribulose 5-phosphate
Indol glycerol phosphate
Trp
1
2
3
4
5

Tryptophan Biosynthesis (anabolic pathway)

5 Structural Genes (*a*-*e*)

Promoter/ Operator Region (*p*,*o*)

Regulator Gene (*trpR*)

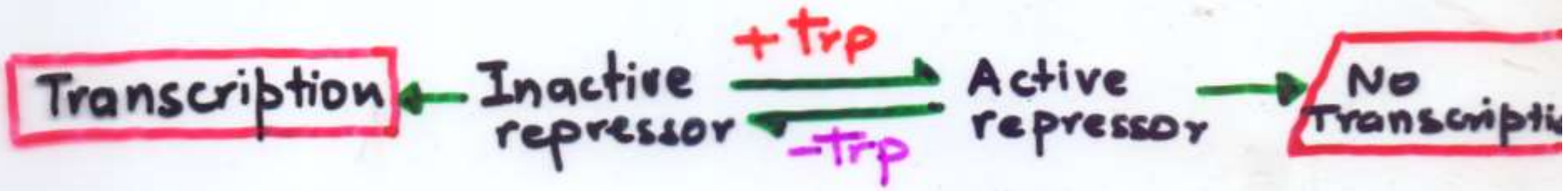
trp Operon

- **A negative repressible operon**
- **Five structural genes**

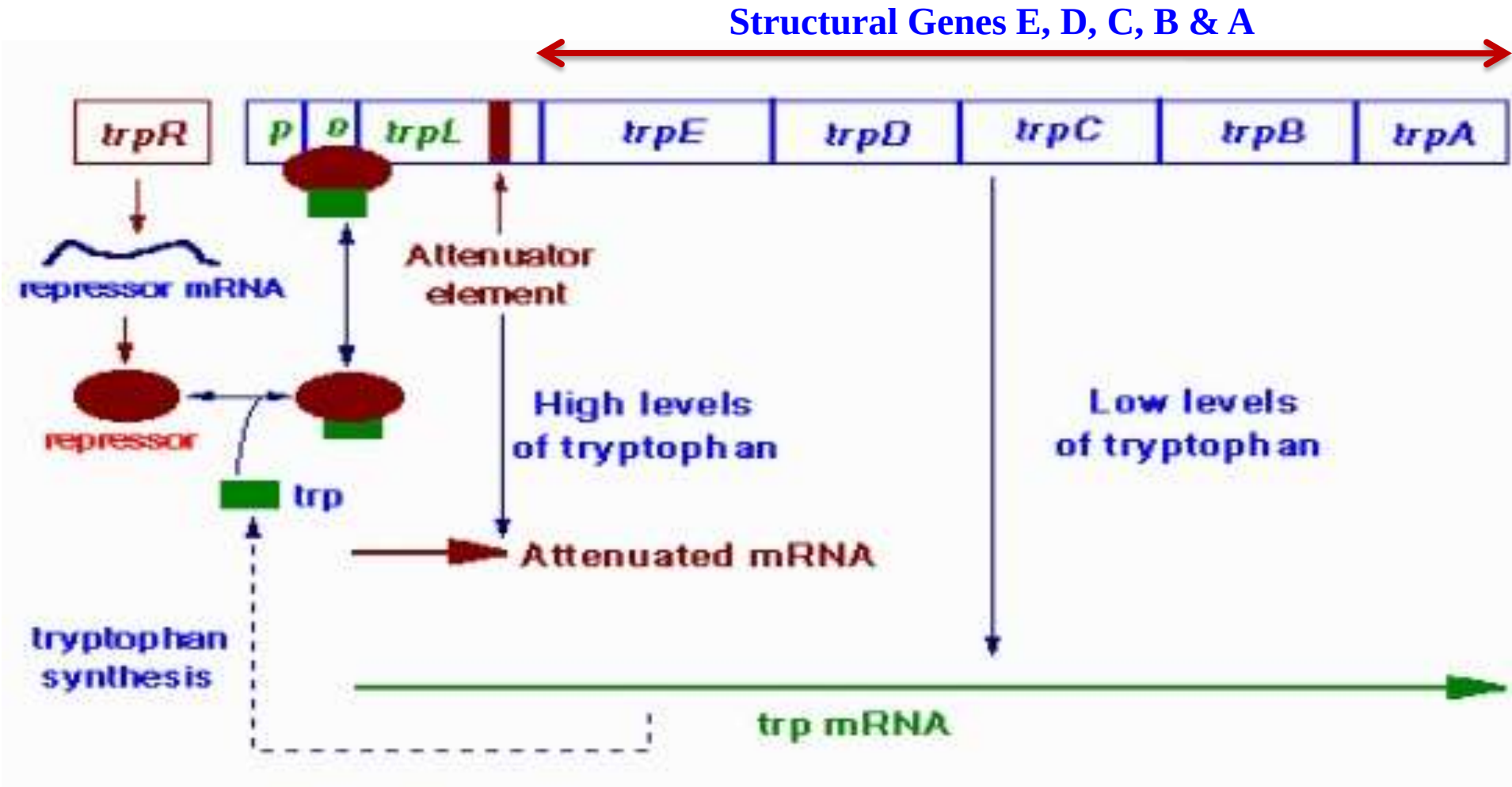
trpE*, *trpD*, *trpC*, *trpB*, and *trpA

(five enzymes together convert chorismate to tryptophan)

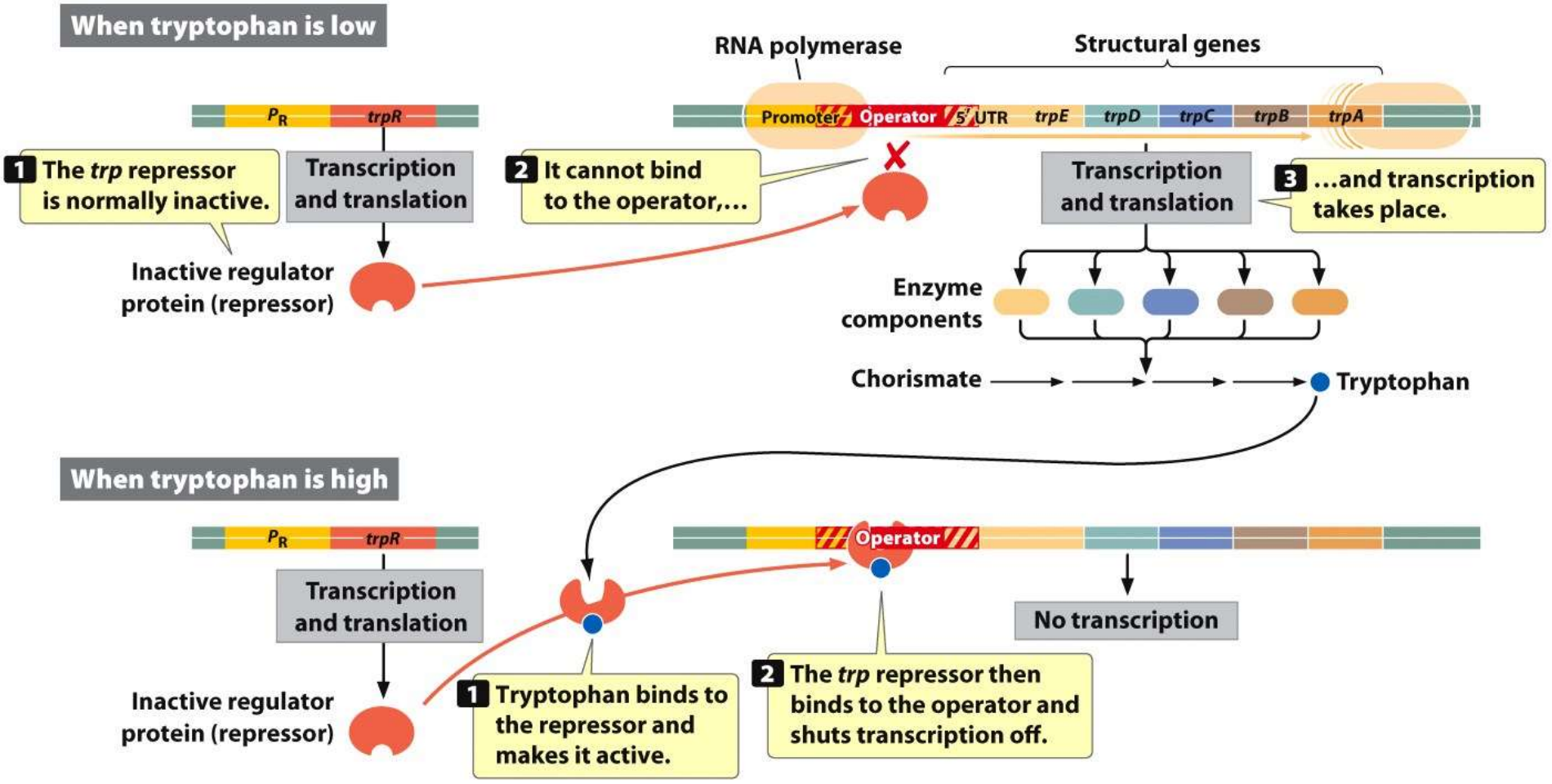
Basic "on-off" Regulatory Mechanism



Organization of *trp* Operon

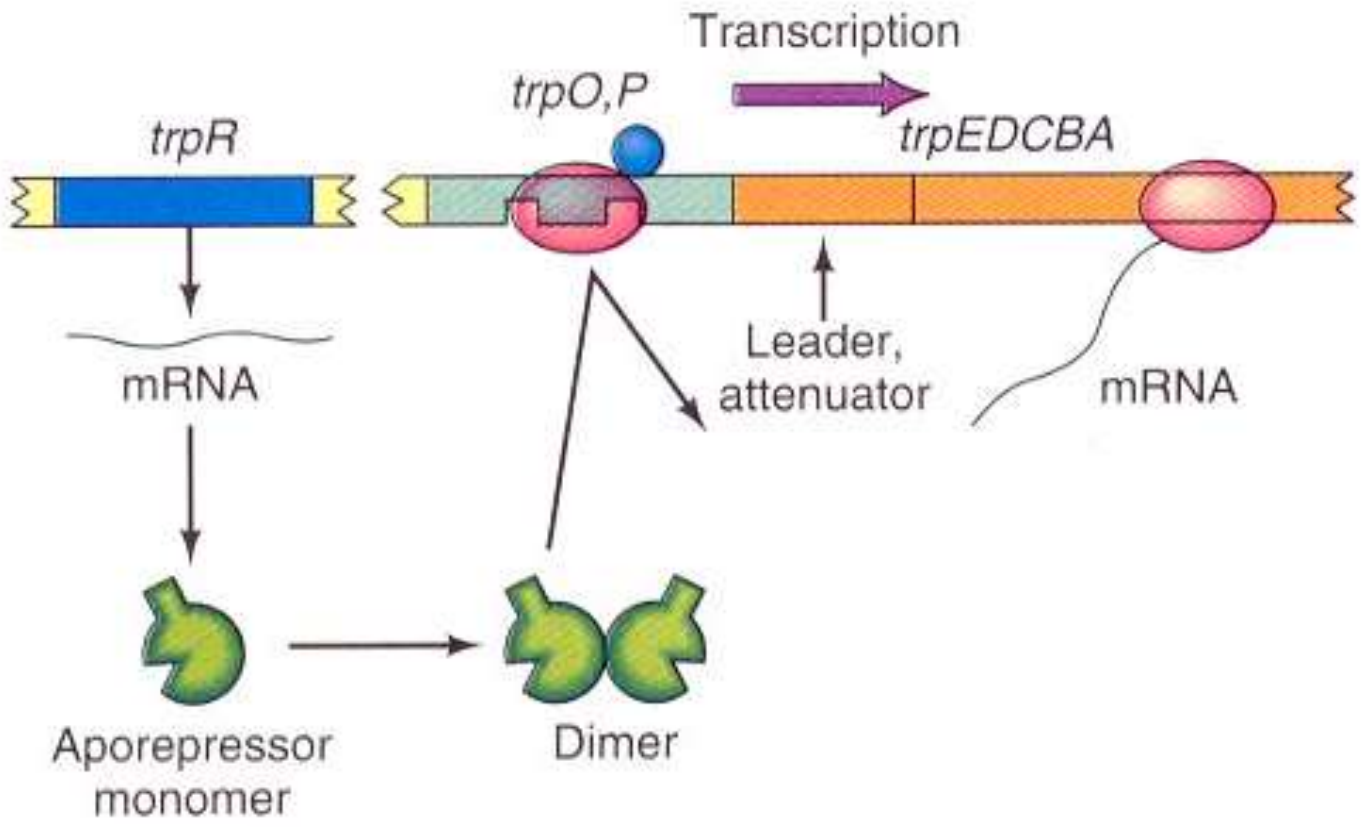


trp Operon of *Escherichia coli*

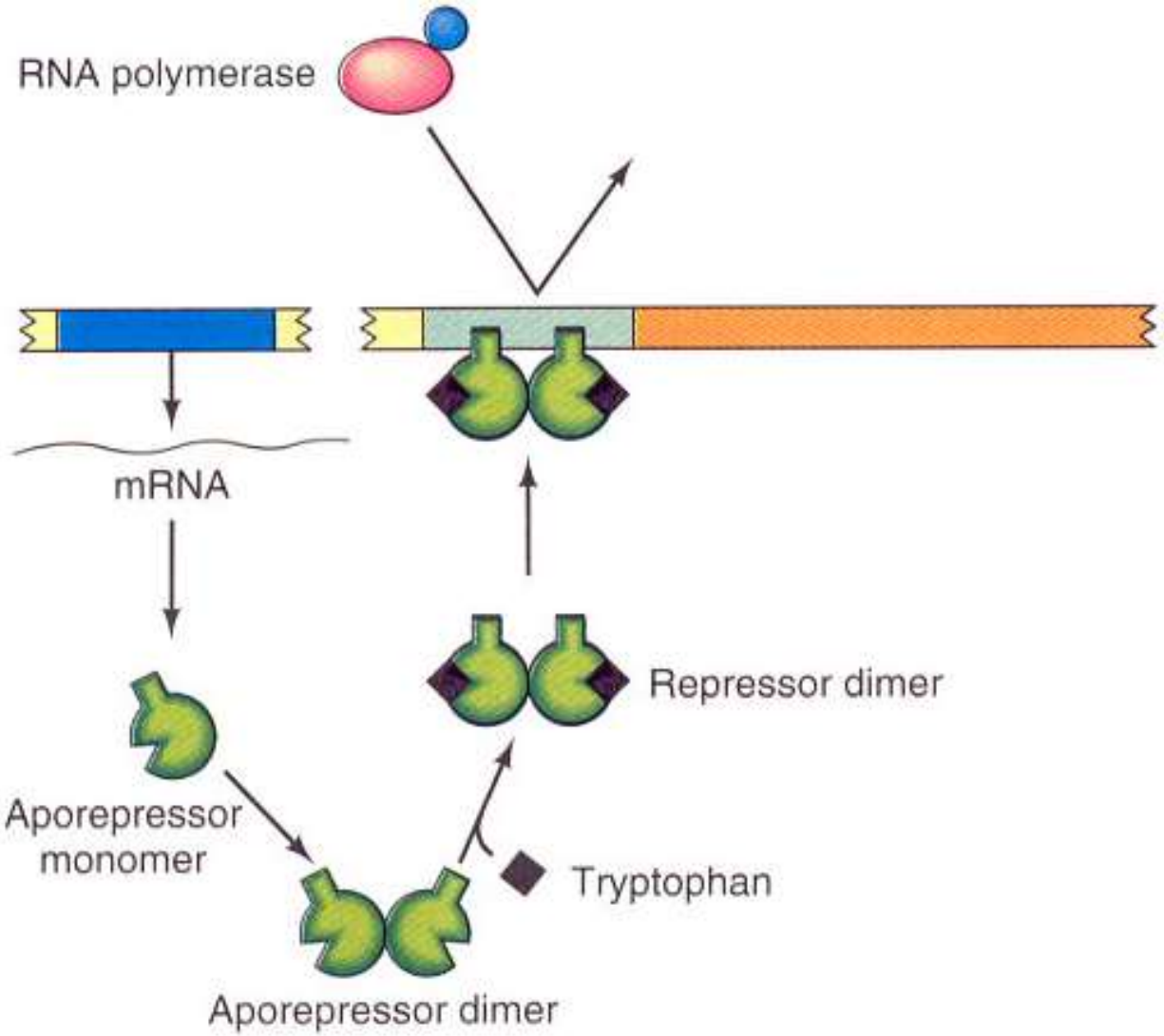


Tryptophan: Effect on Negative Control

Low Tryptophan → no repression



Repression: Tryptophan is a co-repressor → binds inactive apo-repressor converting it to active repressor



1. **Operator site lies within the promoter**

2. **Allosteric transition**

   Allosteric protein-protein whose shape is changed upon binding of a particular molecule → In the new conformation the protein's ability to react to a second molecule is altered

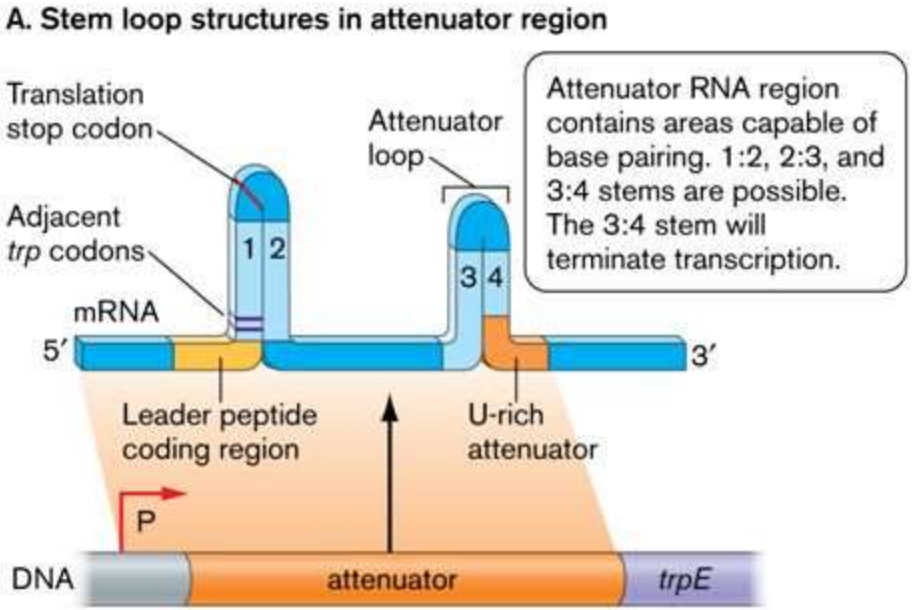
3. ***Trp* operon has another level of control → Attenuation**

4. **Repressor lowers transcription 70-fold (as compared to derepressed state) → attentuation permits another 10-fold control → total dynamic range of control = 700-fold**

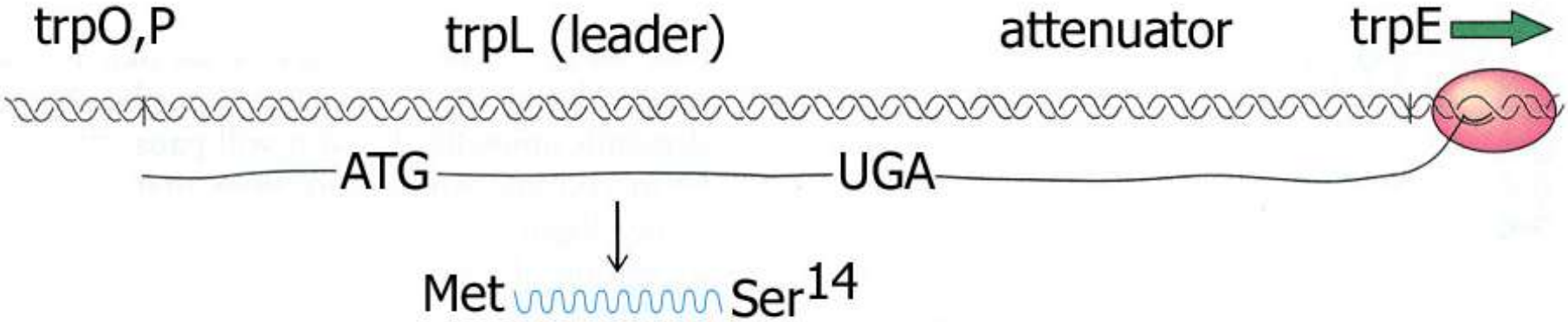
Attenuation of the *trp* Operon

- **Attenuation** is a regulatory mechanism in which translation of a **leader peptide** affects transcription of a downstream structural gene.

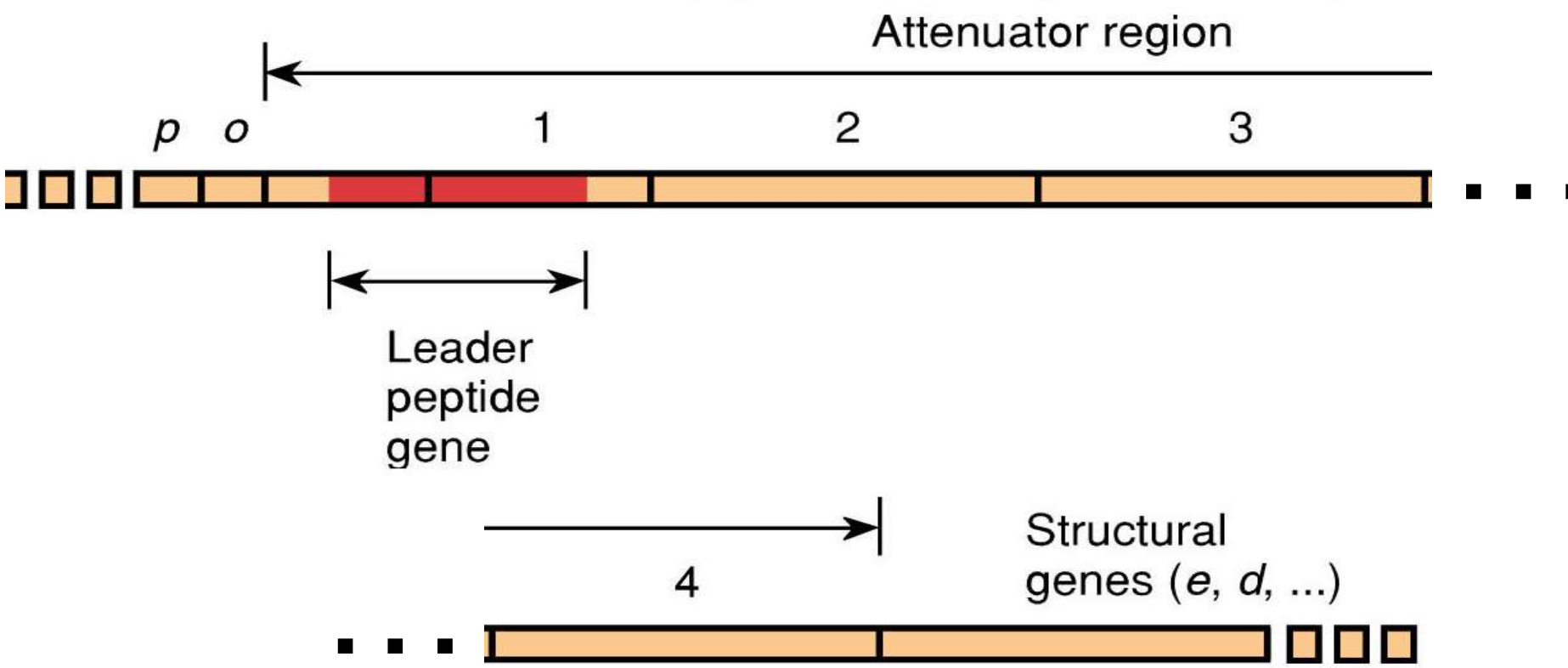
The attenuator region of the *trp* operon has 2 trp codons and is capable of forming stem-loop structures.



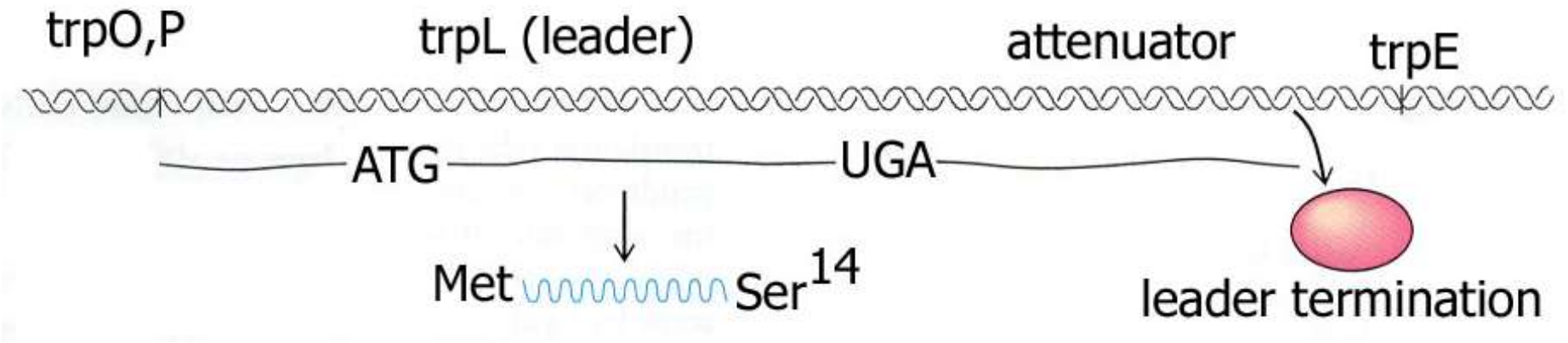
Low tryptophan: transcription of *trp* operon genes→ RNA polymerase reads through attenuator.



Attenuator Region of Trp Operon



High tryptophan: attenuation, premature termination → attenuator causes premature termination of transcription

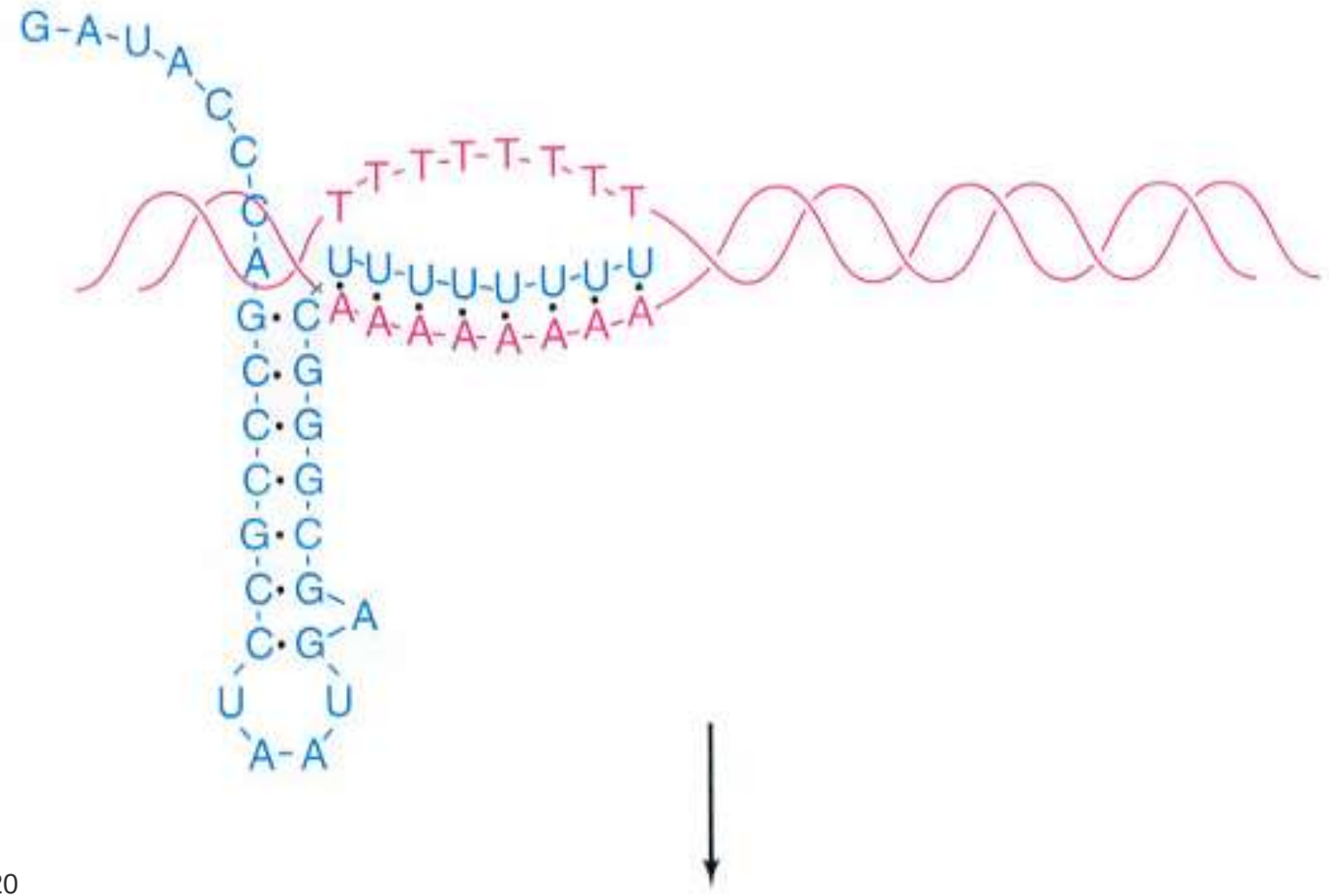


1. Attenuator region contains transcription stop signal (terminator) → not STOP codon!

2. The terminator consists of an inverted repeat followed by string of eight A-T pairs.

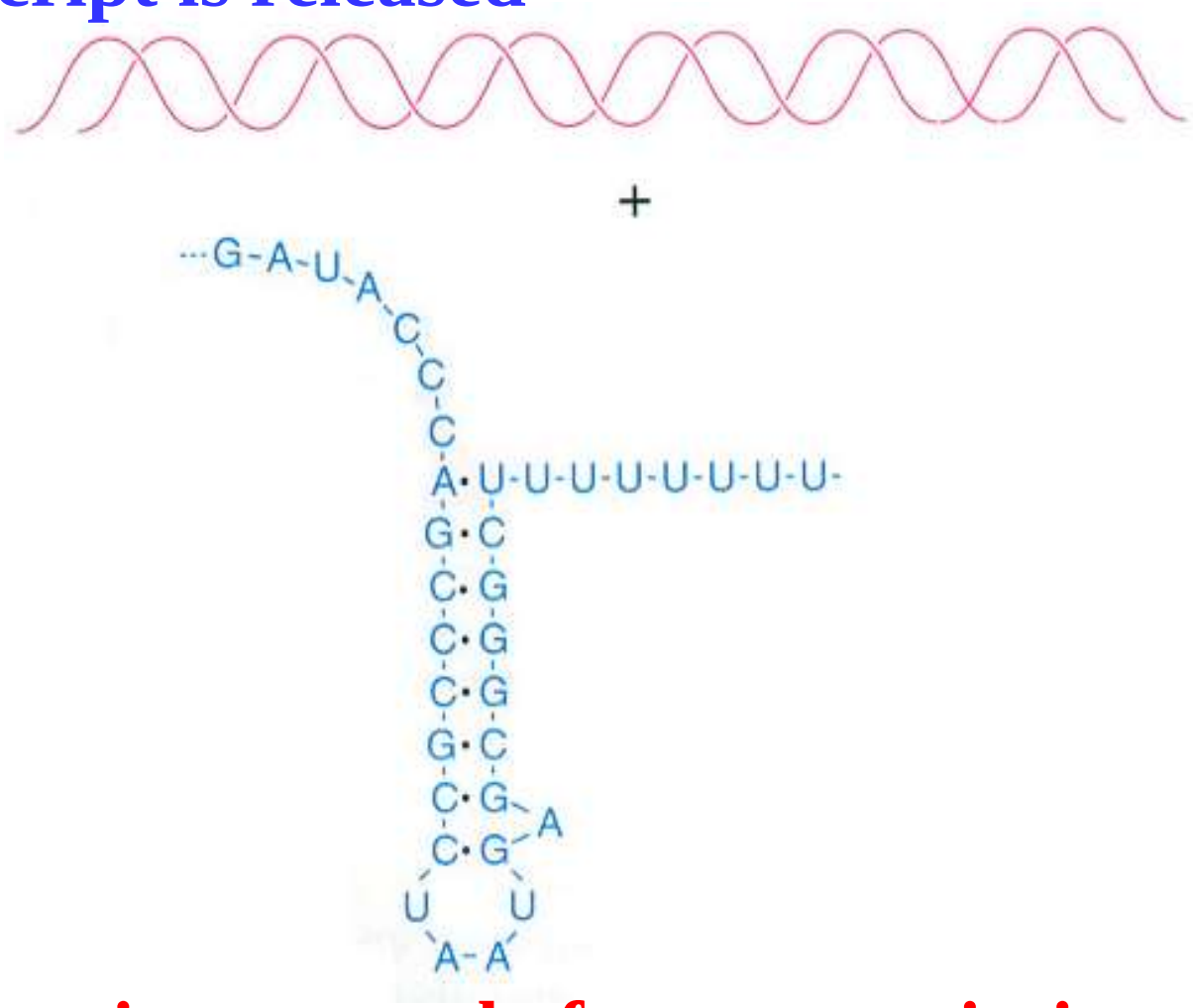
3. The inverted repeat forms a hairpin loop.

4. When RNA polymerase reaches string of U's…



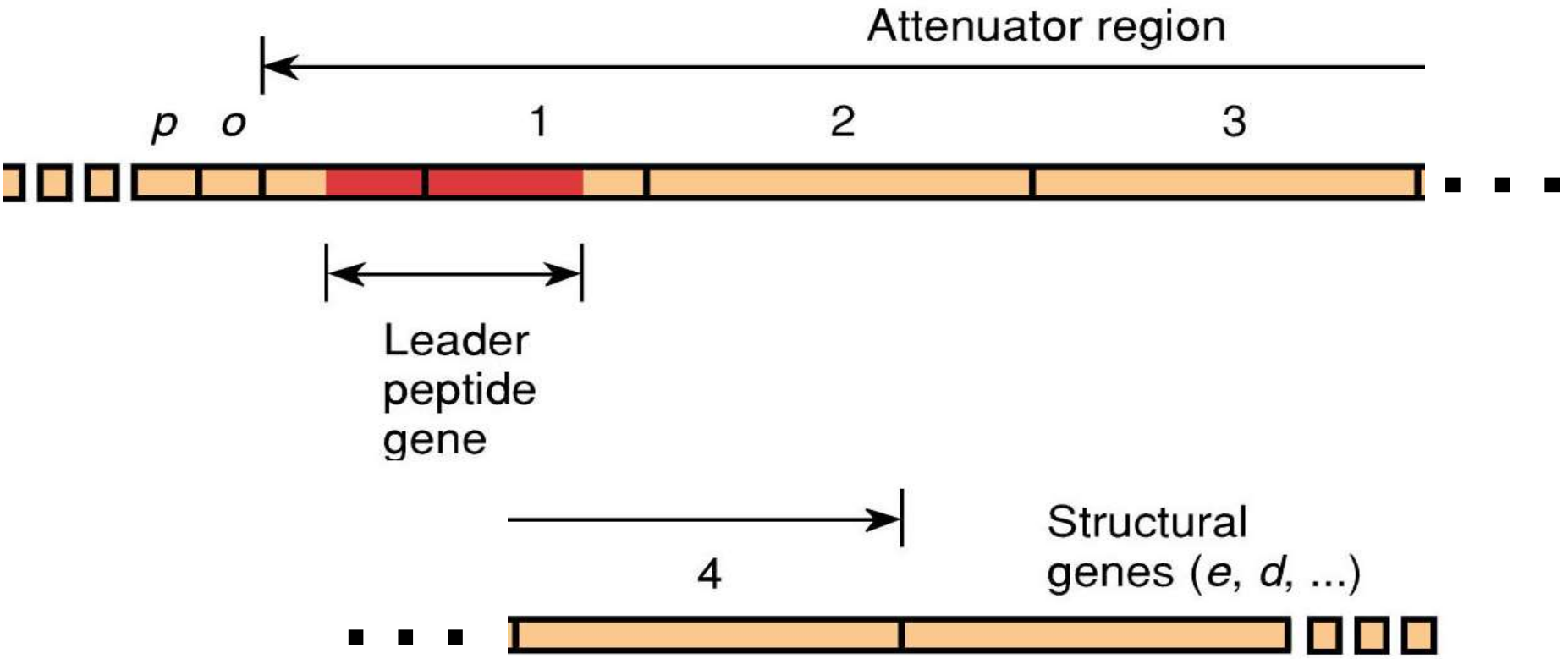
…the polymerase pauses, the hairpin forms

→ Transcript is released



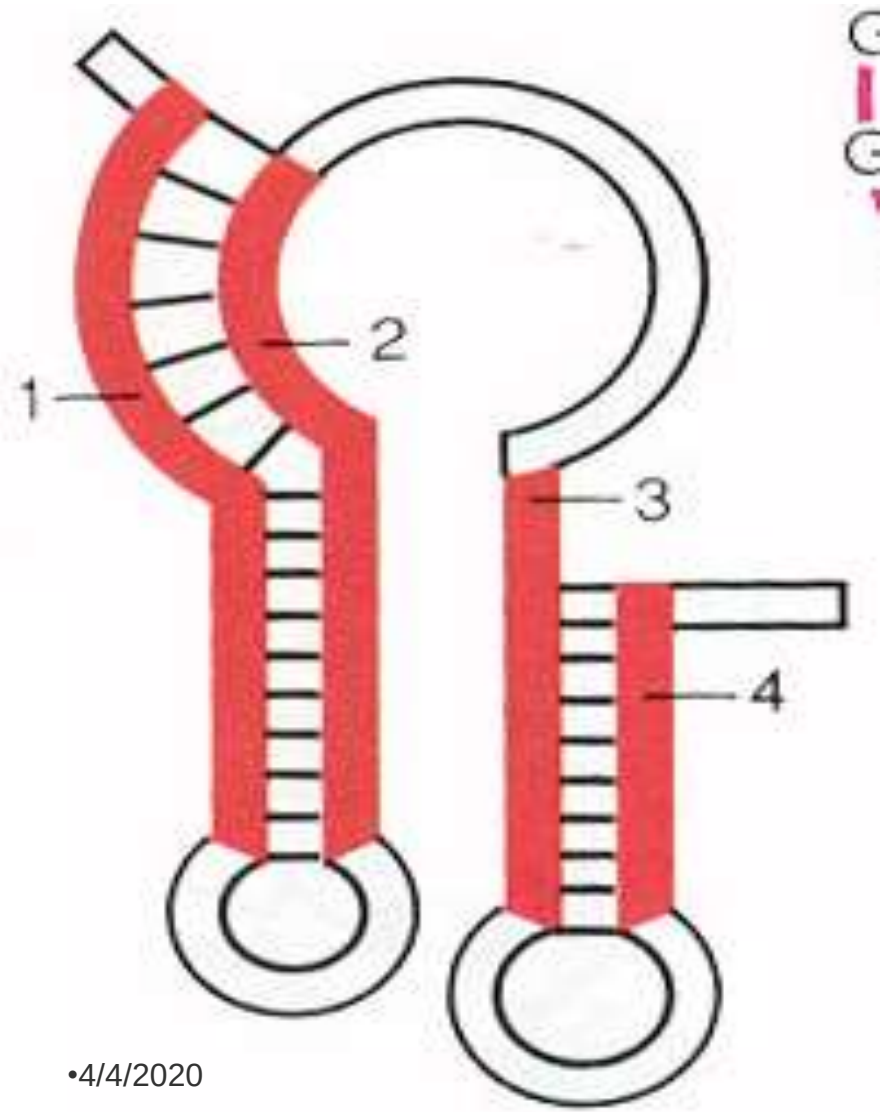
→ Termination occurs before transcription reaches the *trp* structural genes

Mechanism of Attenuation

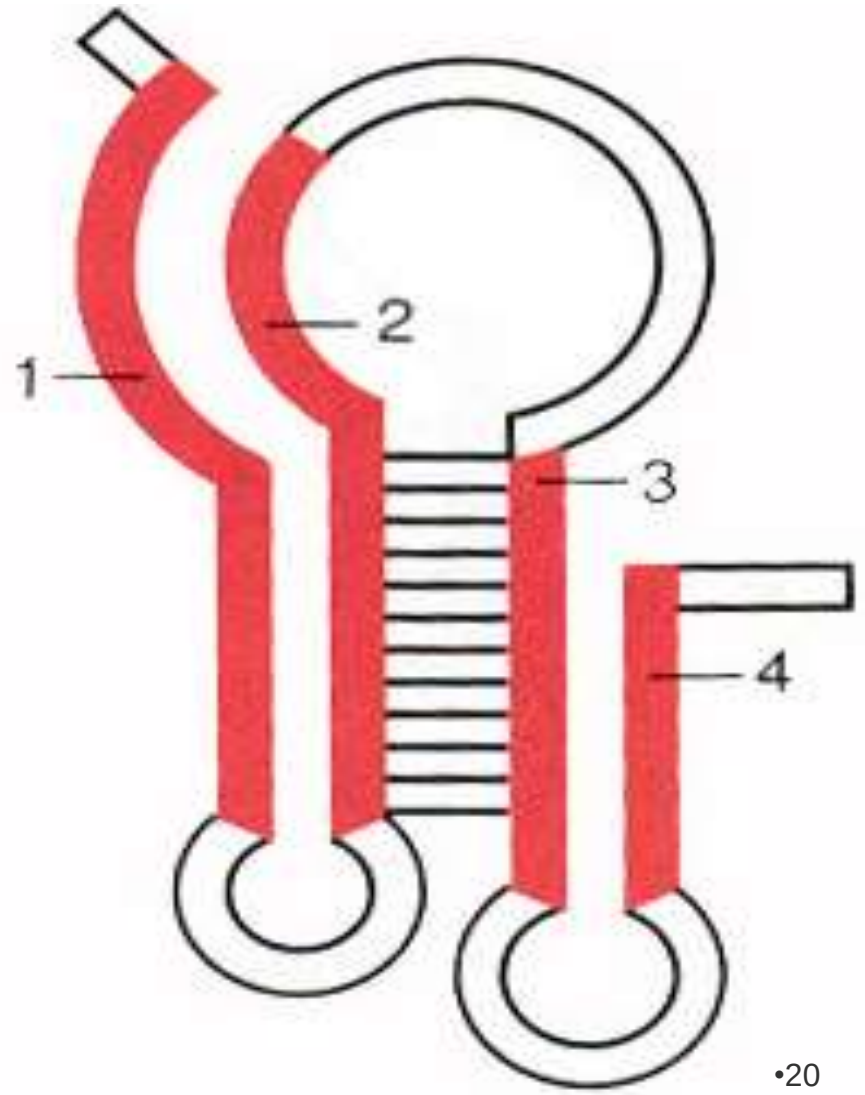


Key insight: mRNA produced from attenuator region can fold into two different secondary structures

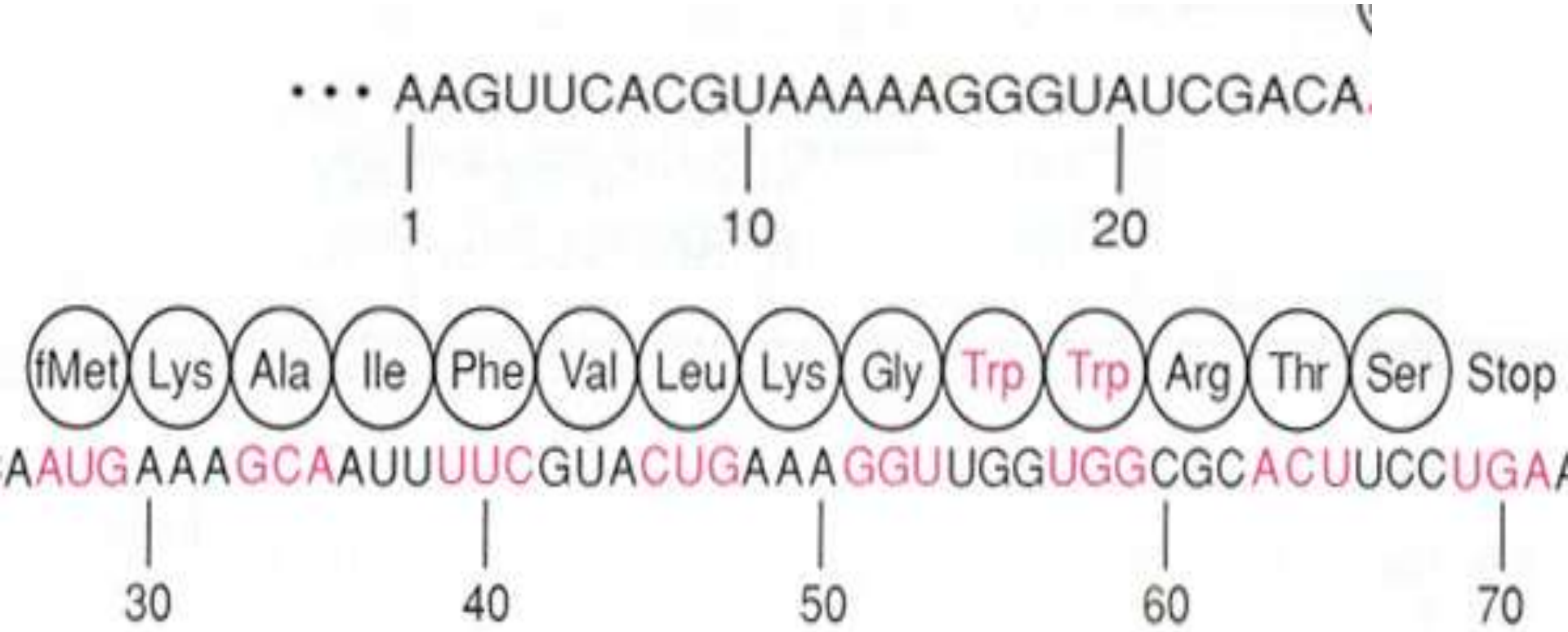
Stem loops: 1-2, 3-4



Stem loop: 2-3



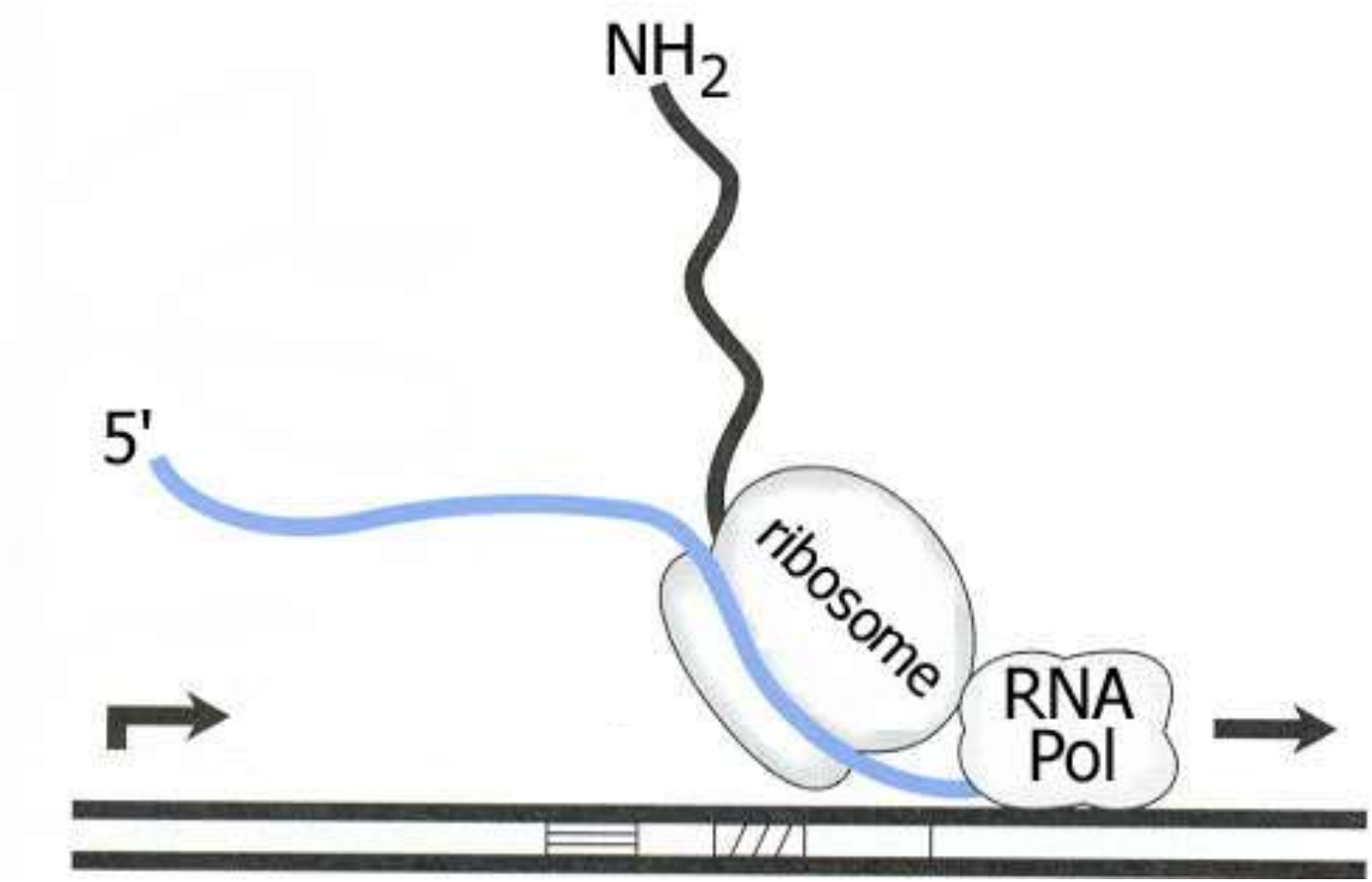
The Importance of the Leader Region



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

-trp is a "rare" amino acid

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



•2. Thus, as soon as *trp* leader region is transcribed, translation begins.

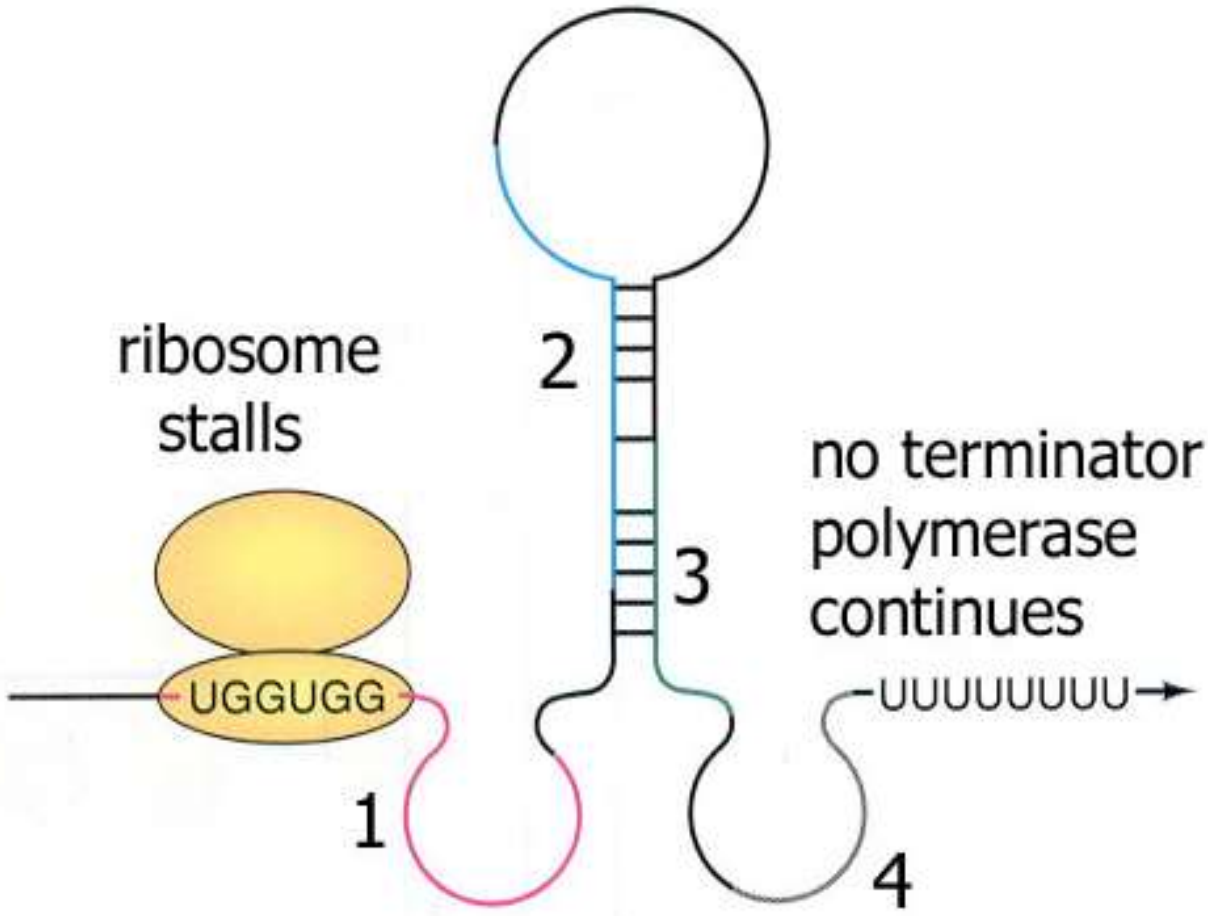
•Consider LOW Trp Conditions

•3. During low tryptophan concentration, ribosome will stall at trp sites.

•4. The trp site is right in the middle of region 1 of the attenuator

•→ Meanwhile RNA polymerase continues to transcribe

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



•1. Stem loop structure 2-3 does not result in transcriptional termination → whole operon mRNA made.

•2. What happens to the stalled ribosome?

- •(i) Since the genes in the operon have their own start sites other ribosomes can come and translate those proteins

- •(ii) Stalled ribosome can eventually either incorporate trp-tRNA (+ 3 more a.a. before reaching stop codon) or dissociate from mRNA

•At HIGH Trp Conditions

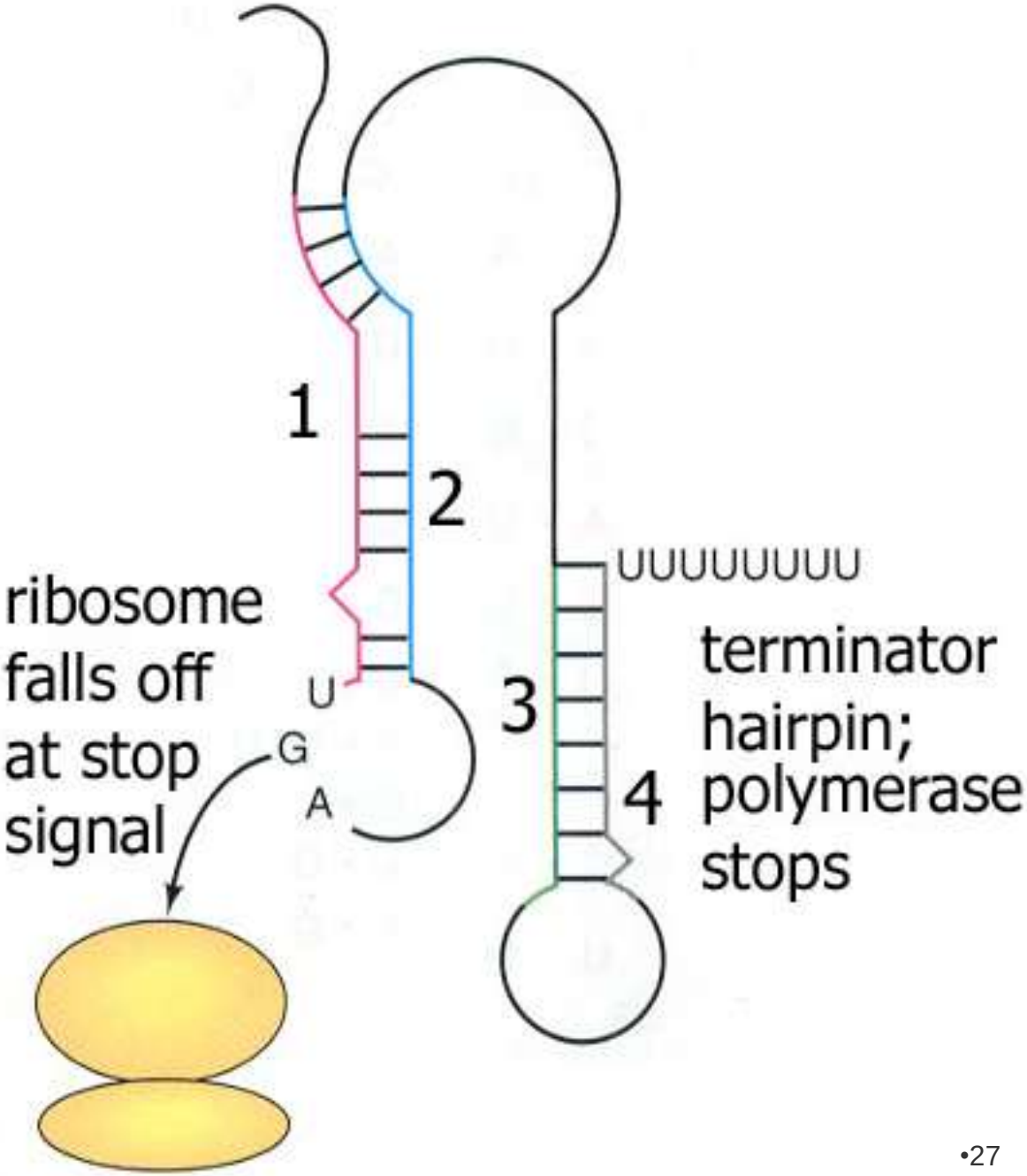
•1. When high levels of Trp-tRNA are present the two tryptophan codons do not represent a barrier translation → ribosome breezes through.

•2. Ribosome continues through element 1 (no stalling) and reaches stop signal (UGA)

•3. With no ribosome → stem loops 1-2/2-3 form on the mRNA → halting transcription before polymerase has chance to reach trp structural genes.

•Effect on ribosome and transcription at <u>HIGH Trp levels</u>

•Note: the 14 amino acid leader peptide is synthesized



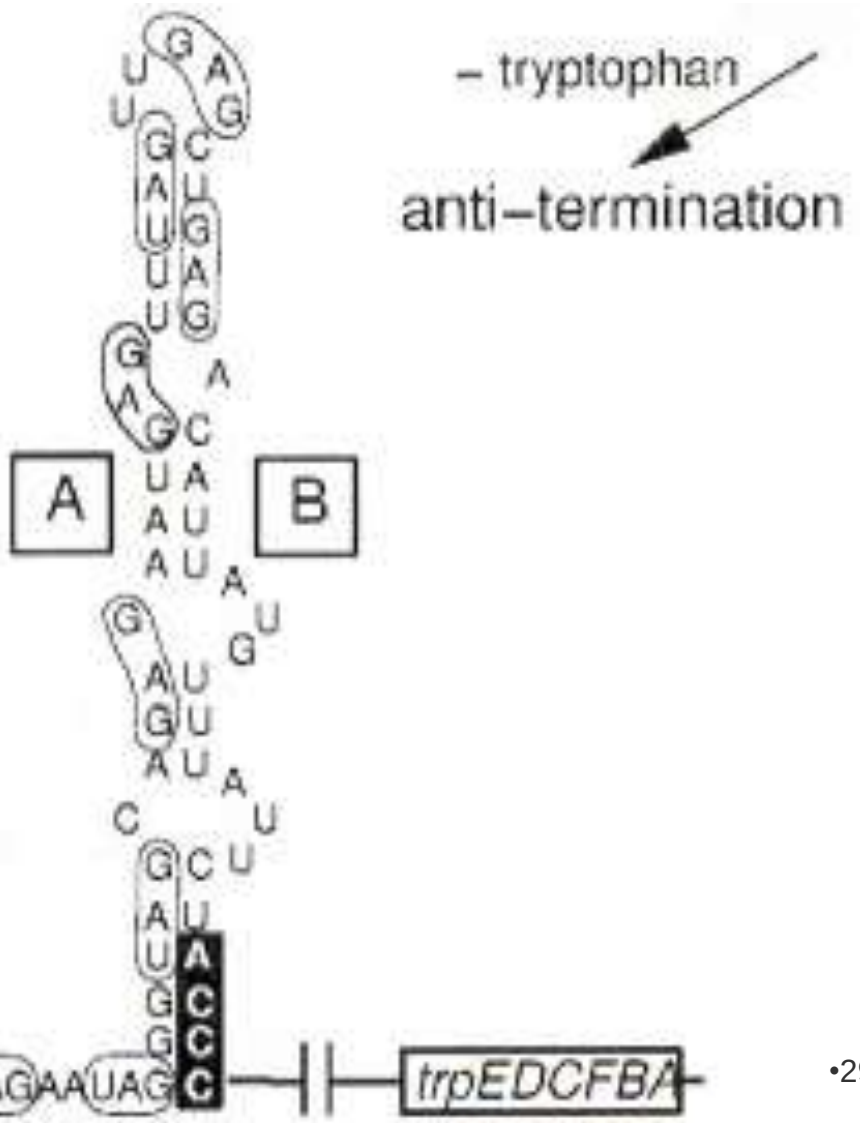
•-This mechanism involves: transcriptional-translational coupling.

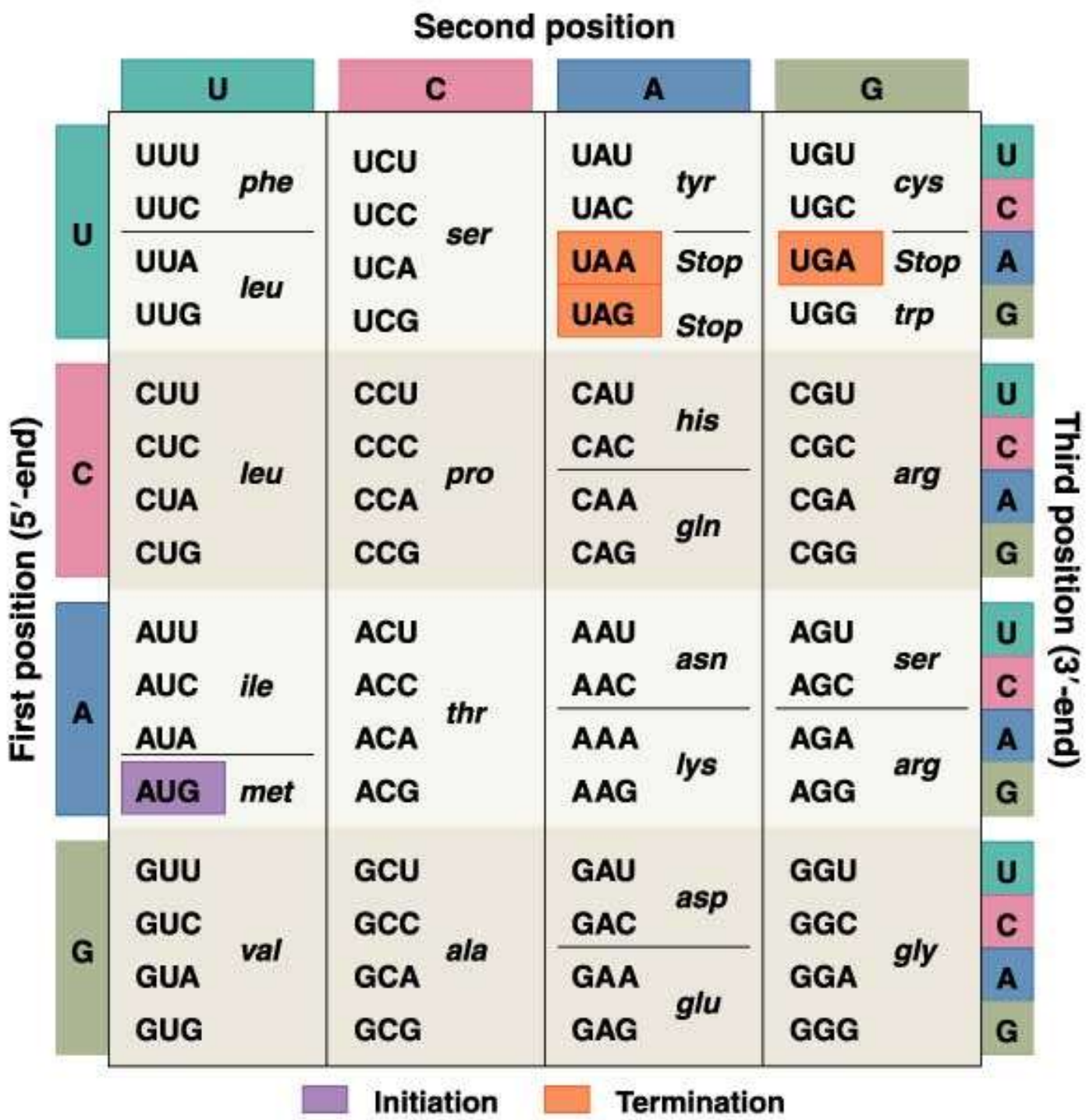
•-Relies on rate of transcription & translation to be comparable → if RNA polymerase >> ribosome, it might pass through attenuator region before ribosome had a chance to stall at the tryptophan codons.

•1. Attenuation response controlled by *trp* RNA-binding attenuation protein (TRAP)

•2. Protein assists in translational termination.

•Absence of *trp* transcription proceeds



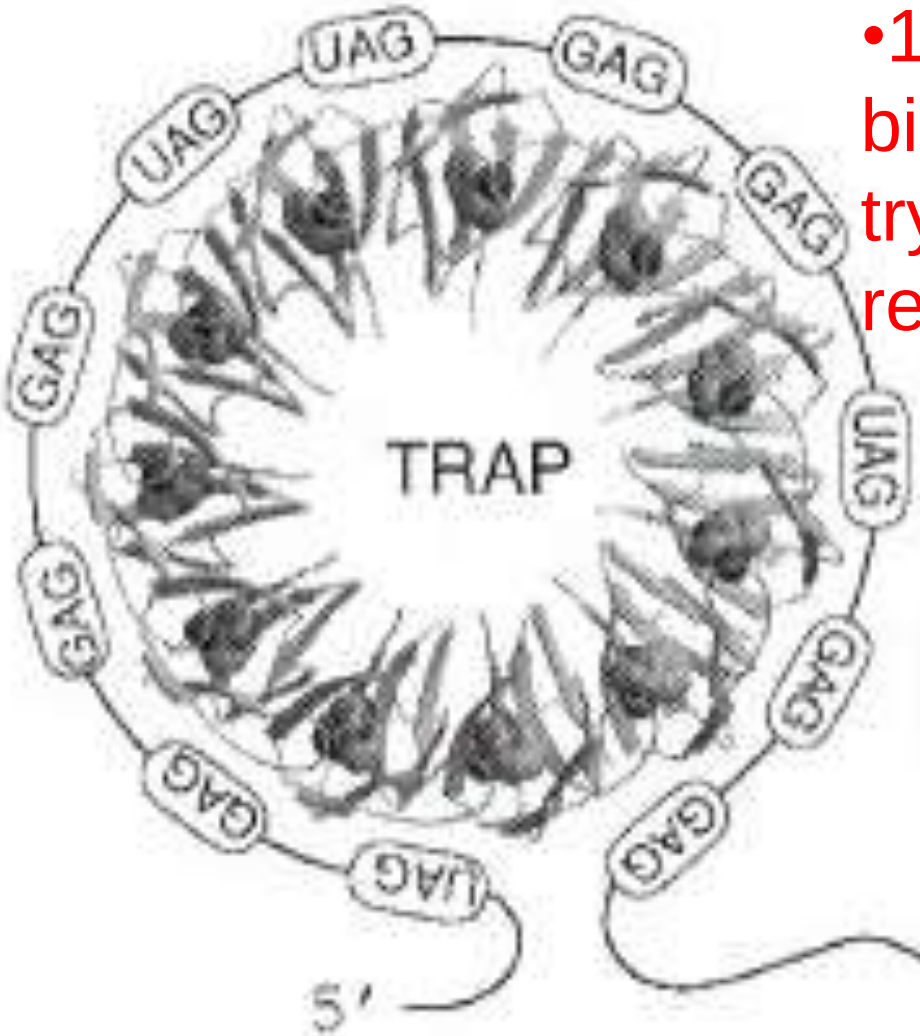

Second position
U
C
A
G
First position (5′-end)
Third position (3′-end)
U
C
A
G
UUU phe
UUC
UUA leu
UUG
UCU ser
UCC
UCA
UCG
UAU tyr
UAC
UAA Stop
UAG Stop
UGU cys
UGC
UGA Stop
UGG trp
CUU leu
CUC
CUA
CUG
CCU pro
CCC
CCA
CCG
CAU his
CAC
CAA gln
CAG
CGU arg
CGC
CGA
CGG
AUU ile
AUC
AUA
AUG met
ACU thr
ACC
ACA
ACG
AAU asn
AAC
AAA lys
AAG
AGU ser
AGC
AGA arg
AGG
GUU val
GUC
GUA
GUG
GCU ala
GCC
GCA
GCG
GAU asp
GAC
GAA glu
GAG
GGU gly
GGC
GGA
GGG
Initiation
Termination

•2. Trp-TRAP binds leader sequences by recognizing 11 triplet codons.

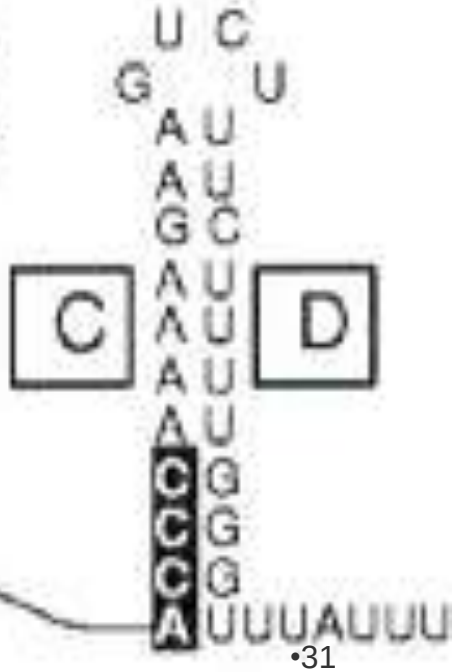
•3. Blocks anti-termination formation.

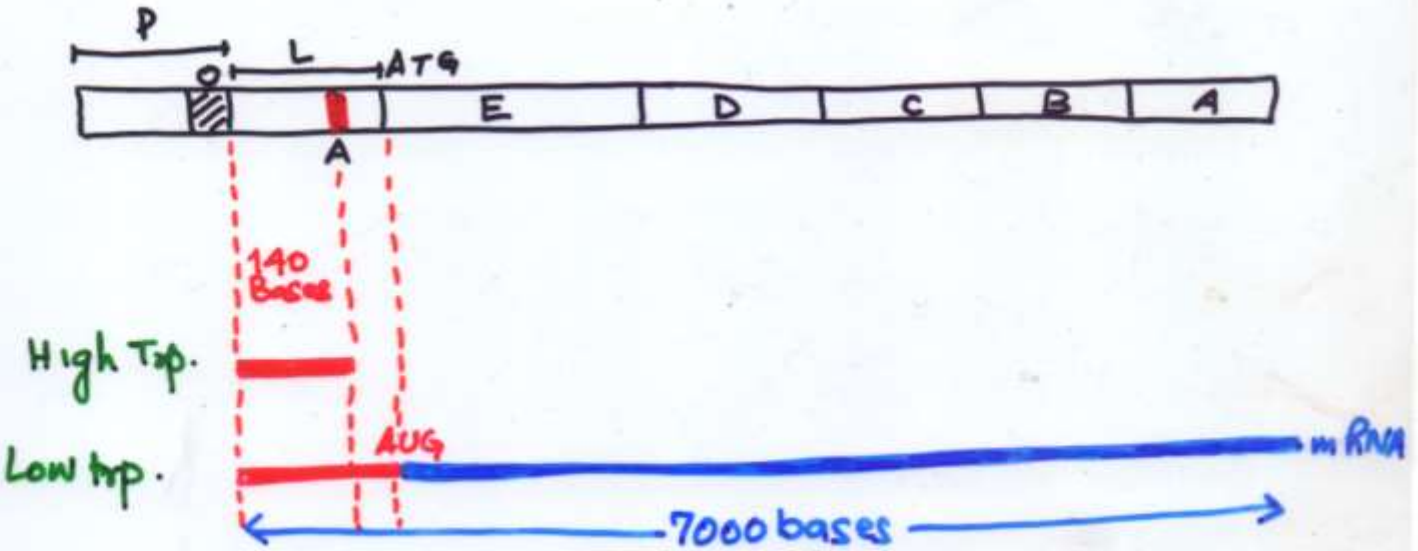
•4. Allows formation of termination loop

•5. Result: translational termination occurs



•1. TRAP binds 11 tryptophan residues.





Amino Acid sequence of leader polypeptide chain

1) Met–Lys–Ala–Ile–Phe–Val–Leu–Lys–Gly–Trp–Trp–Arg–Thr–Ser–Stop
(Trp leader)

2) Met–Lys–Arg–Ile–Ser–Thr–Thr–Ile–Thr–Thr–Thr–Ile–Ile–Thr–Thr
(Thr leader)

3) Met–Lys–His–Ile–Pro–Phe–Phe–Phe–Phe–Ala–Phe–Phe–Phe–Thr–Phe–Pro–Stop
(Phe leader)

4) Met–Thr–Asp–Val–Gln–Phe–Lys–His–His–His–His–His–His–His–Pro–Asp
(His leader)

The following table shows the amino acid sequences of some leader peptides

Operon	Leader Length	Sequence
trp	14	MKAIFVLKGWWRTS
pheA	16	MKHIPFFFAFFFTFP
his	16	MTRVQFKHHHHHHHHPD
thr	21	MKRISTTITTTTITITTQNGAG
leu	28	MSHIVRFTGLLLLNAFIVRGRPVGGIQH
ilv	32	MTALLRVISLVVISVVVIIIPPCGAALGRGKA

(1) The number of sensing codons reflects the abundance of tRNAs for those amino acids in the cell.

(2) The *thr* and *ilv* operons code for enzymes that are required in the biosynthesis of more than one amino-acid as indicated