ZOO 203 – MOLECULAR BIOLOGY

UNIT 4: REGULATION OF GENE EXPRESSION IN PROKARYOTES (PART- II)

TRYPTOPHAN OPERON

By

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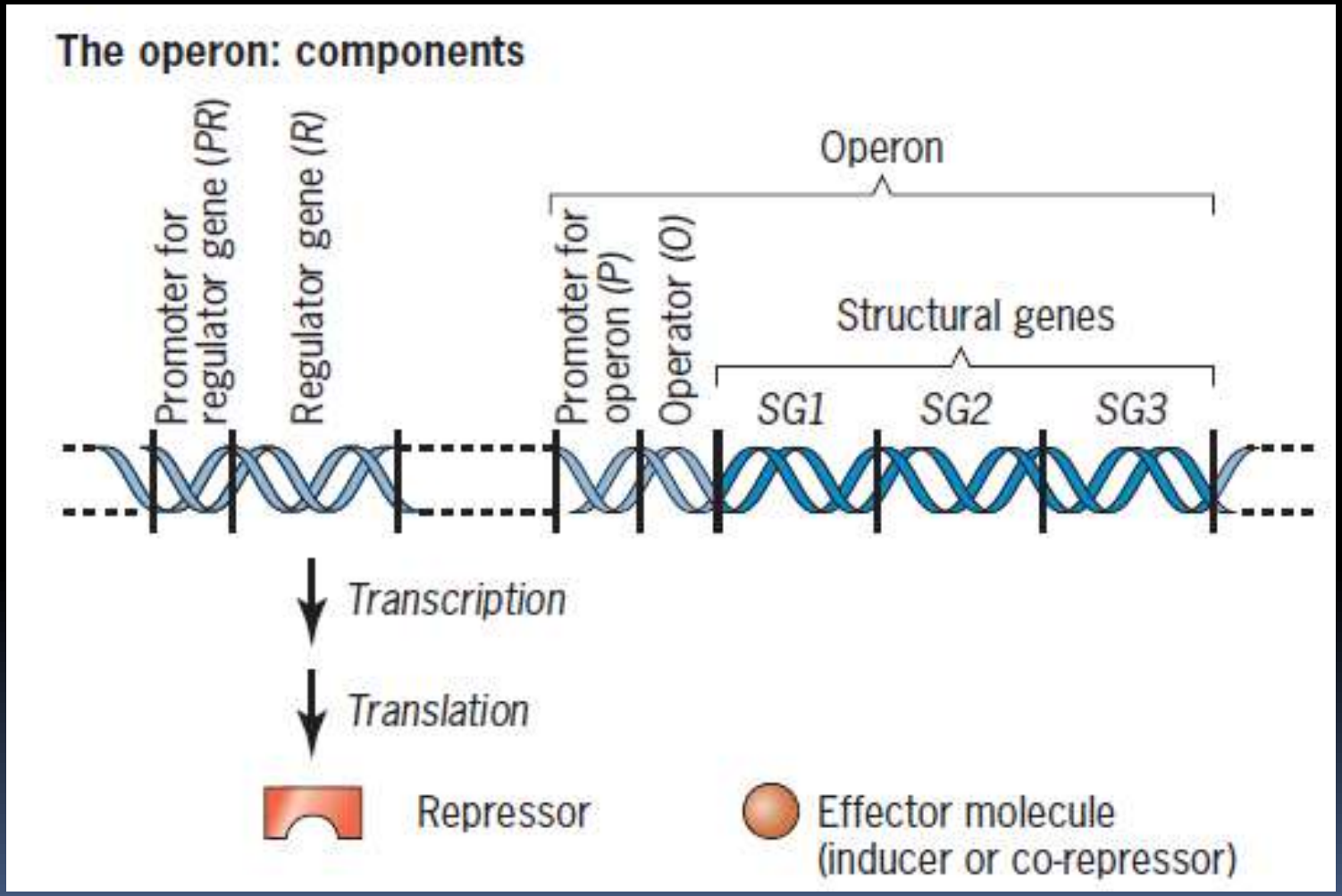
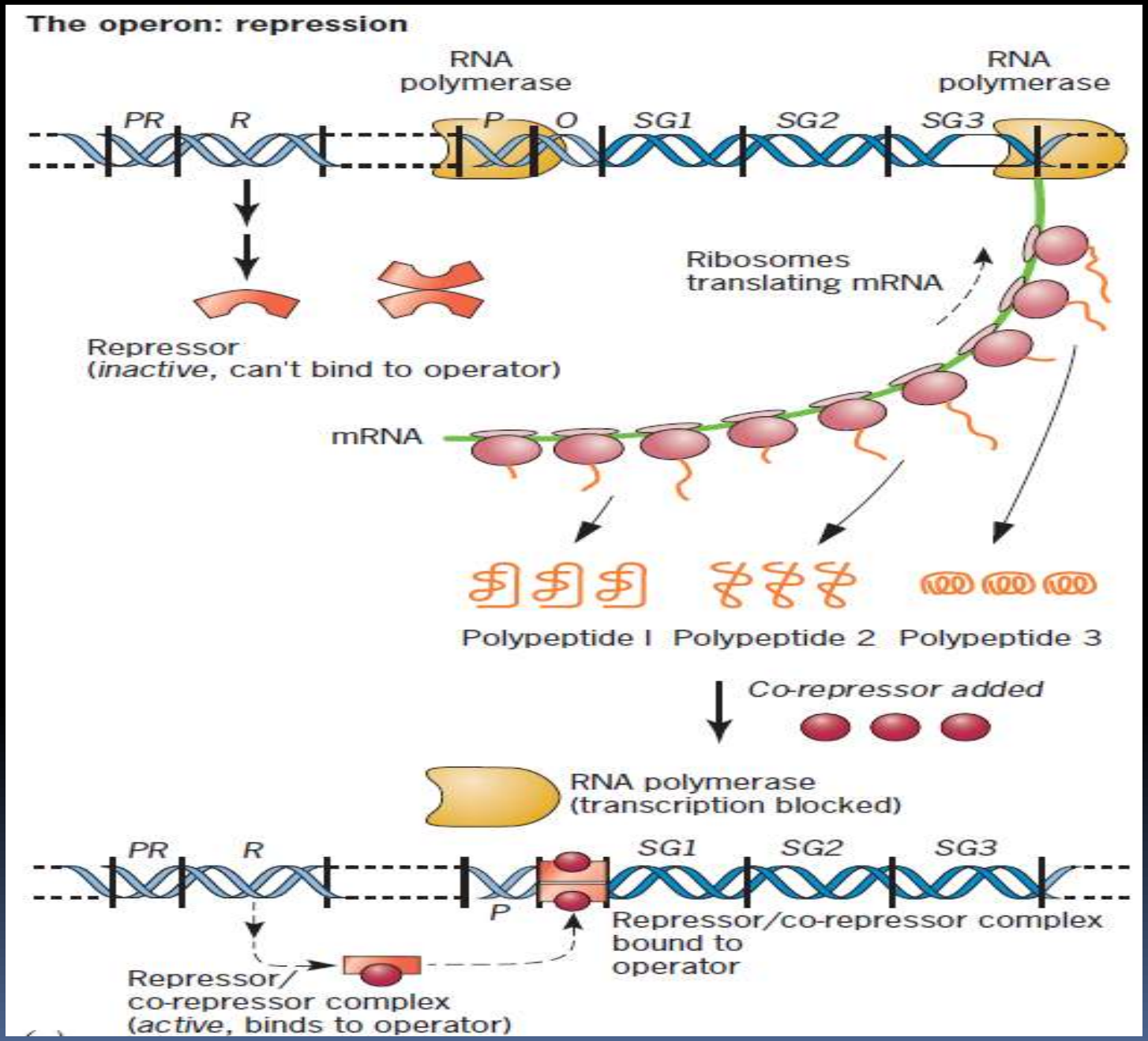
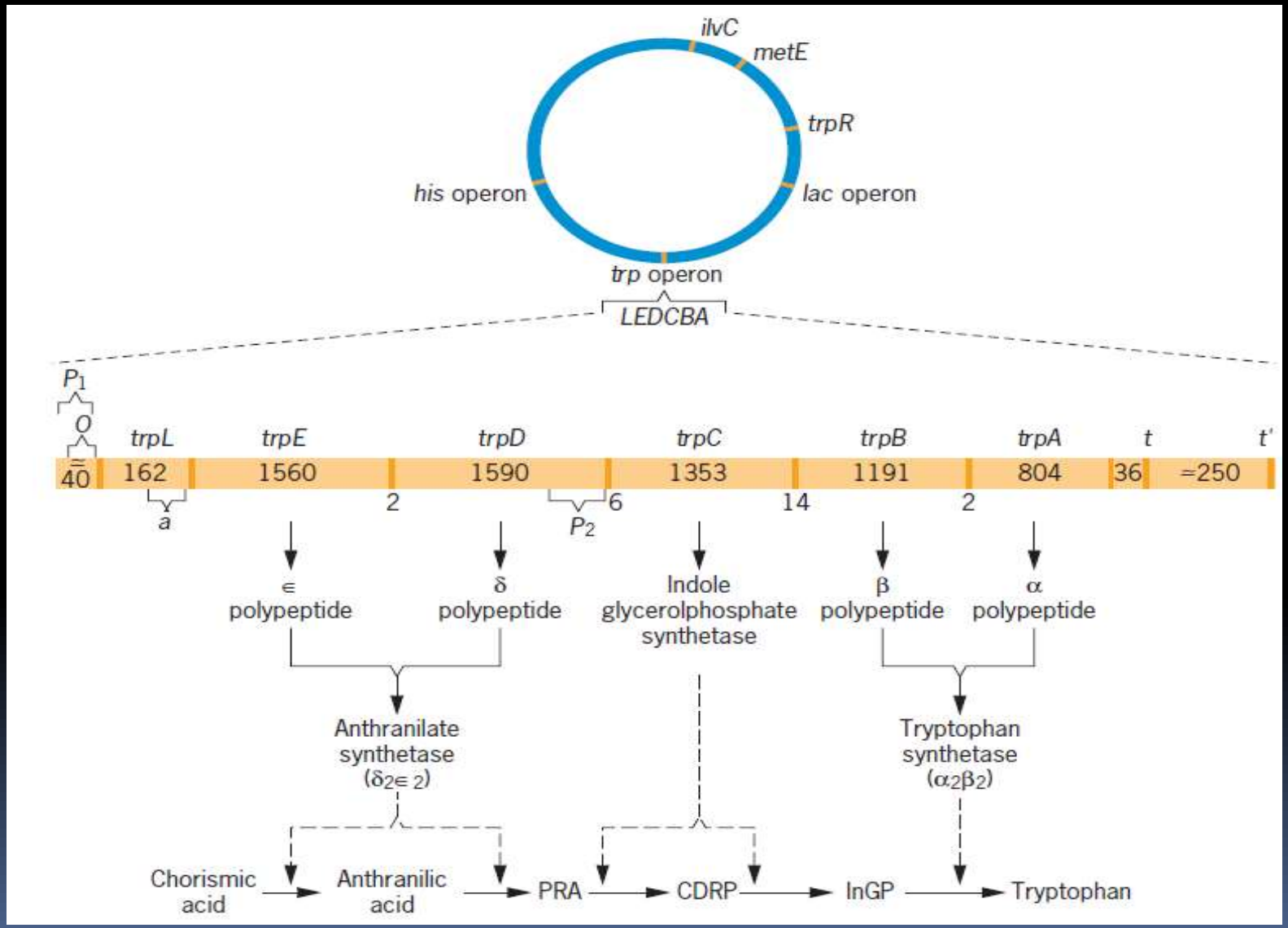
Components of an operon: one or more structural genes (three, *SG1, SG2, and SG3) and the adjoining operator (O) and promoter (P) sequences*

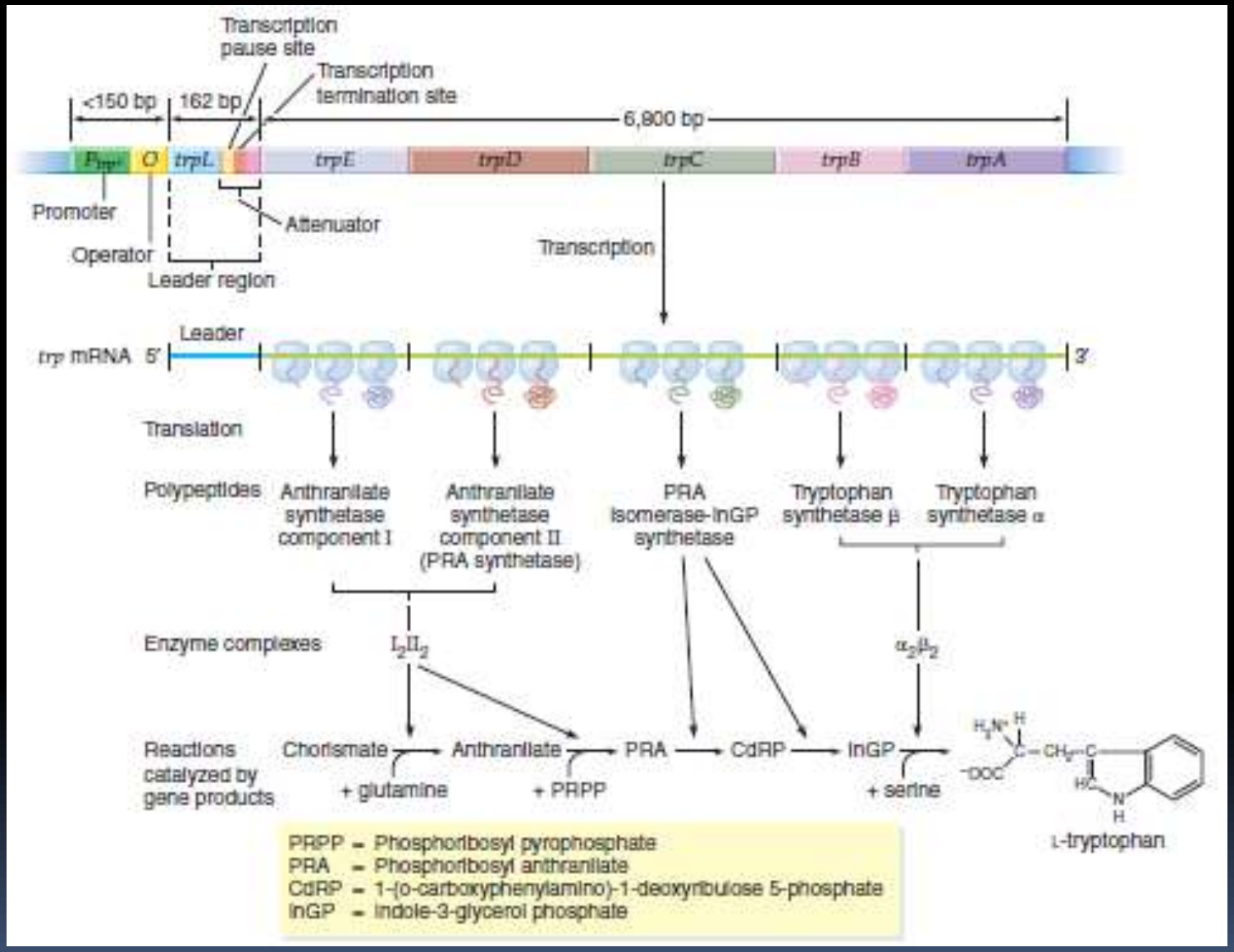


- The trp operon of *E. coli* is a negative repressible operon
- analyzed in detail by Charles Yanofsky and colleagues


The operon: repression
RNA polymerase
RNA polymerase
PR
R
P
O
SG1
SG2
SG3
Ribosomes translating mRNA
Repressor (inactive, can't bind to operator)
mRNA
Polypeptide I
Polypeptide 2
Polypeptide 3
Co-repressor added
RNA polymerase (transcription blocked)
PR
R
SG1
SG2
SG3
P
Repressor/co-repressor complex bound to operator
Repressor/ co-repressor complex (active, binds to operator)

Organization of the trp (tryptophan) operon in *E. coli*.




Transcription pause site
Transcription termination site
<150 bp
162 bp
6,900 bp
trpL
trpE
trpD
trpC
trpB
trpA
Promoter
Operator
Attenuator
Leader region
Transcription
trp mRNA 5'
Leader
3'
Translation
Polypeptides
Anthranilate synthetase component I
Anthranilate synthetase component II (PRA synthetase)
PRA isomerase-InGP synthetase
Tryptophan synthetase β
Tryptophan synthetase α
Enzyme complexes
I_2II_2
$\alpha_2\beta_2$
Reactions catalyzed by gene products
Chorismate
Anthranilate
PRA
CdRP
InGP
+ glutamine
+ PRPP
+ serine
L-tryptophan
PRPP = Phosphoribosyl pyrophosphate
PRA = Phosphoribosyl anthranilate
CdRP = 1-(o-carboxyphenylamino)-1-deoxyribulose 5-phosphate
InGP = Indole-3-glycerol phosphate

Two regulatory mechanisms are involved:

- repressor–operator interaction
- other determines whether initiated transcripts include the structural genes or are terminated before those genes are reached

- The rate of transcription of the trp operon in absence of tryptophan (derepressed state) is 70 times the rate that occurs in presence of tryptophan (repressed state)

In trpR mutants, which lack a functional repressor, the rate of synthesis of the tryptophan biosynthetic enzymes is still reduced about tenfold by the addition of tryptophan to the medium.

This additional reduction in trp operon expression is caused by attenuation

- Attenuation can reduce transcription of the trp operon by a factor of 8 to 10. Thus, repression and attenuation together can regulate the transcription of the trp operon by a factor of about 560 to 700.

ATTENUATION

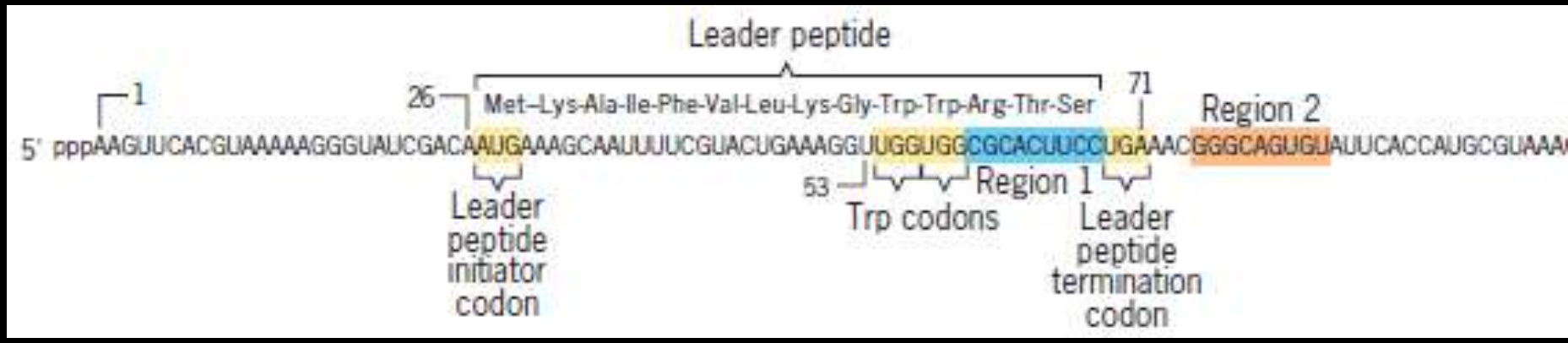
- The regulation of the trp operon other than repression is called **attenuation**
- the sequence within trpL that controls this phenomenon is called the **attenuator**
- Attenuation controls the termination of transcription at a site near the end of the mRNA leader sequence.
- This "premature" termination of trp operon transcription occurs only in the presence of tryptophan-charged tRNA Trp.
- When this premature termination or attenuation occurs, a truncated (140 nucleotides) trp transcript is produced.

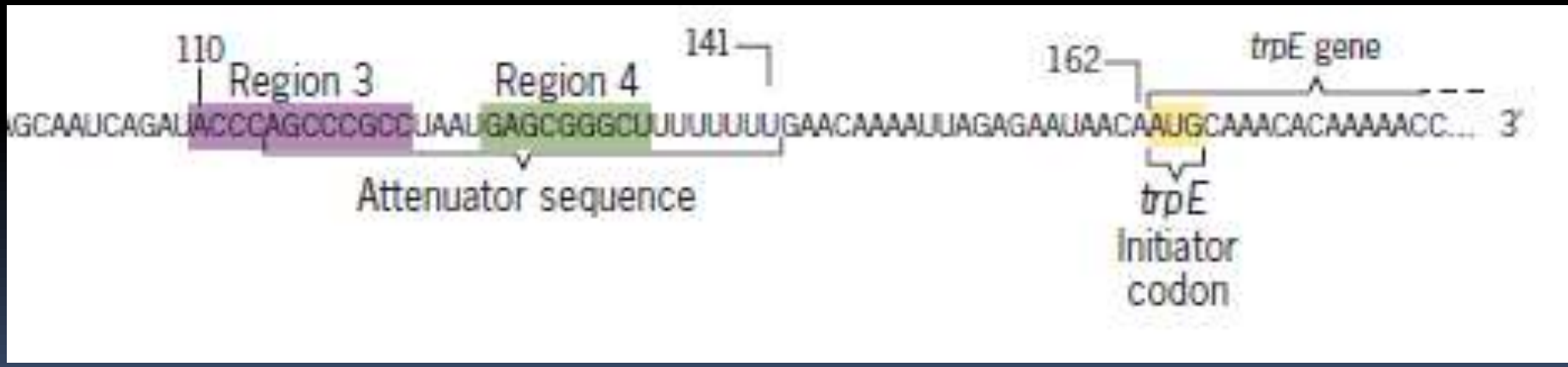
- *The principle of attenuation is that some external event controls the formation of hairpin needed for intrinsic termination*

Attenuator region:

- It has a nucleotide-pair sequence essentially identical to the *transcription–termination signals* found at the ends of most bacterial operons
- signals contain a G:C-rich palindrome followed by several A:T base pairs.
- Transcription of these termination signals yields a nascent RNA with the potential to form a hydrogen-bonded hairpin structure followed by several uracils

Regulatory components of the *trpL* region





4 regions of Leader sequence:

- (1) nucleotides 60–68
- (2) nucleotides 75–83
- (3) nucleotides 110–121
- (4) nucleotides 126–134

Alternate secondary structures formed by the trpL mRNA—

either region 1 will pair with region 2 and region 3 with region 4, forming a transcription–termination hairpin

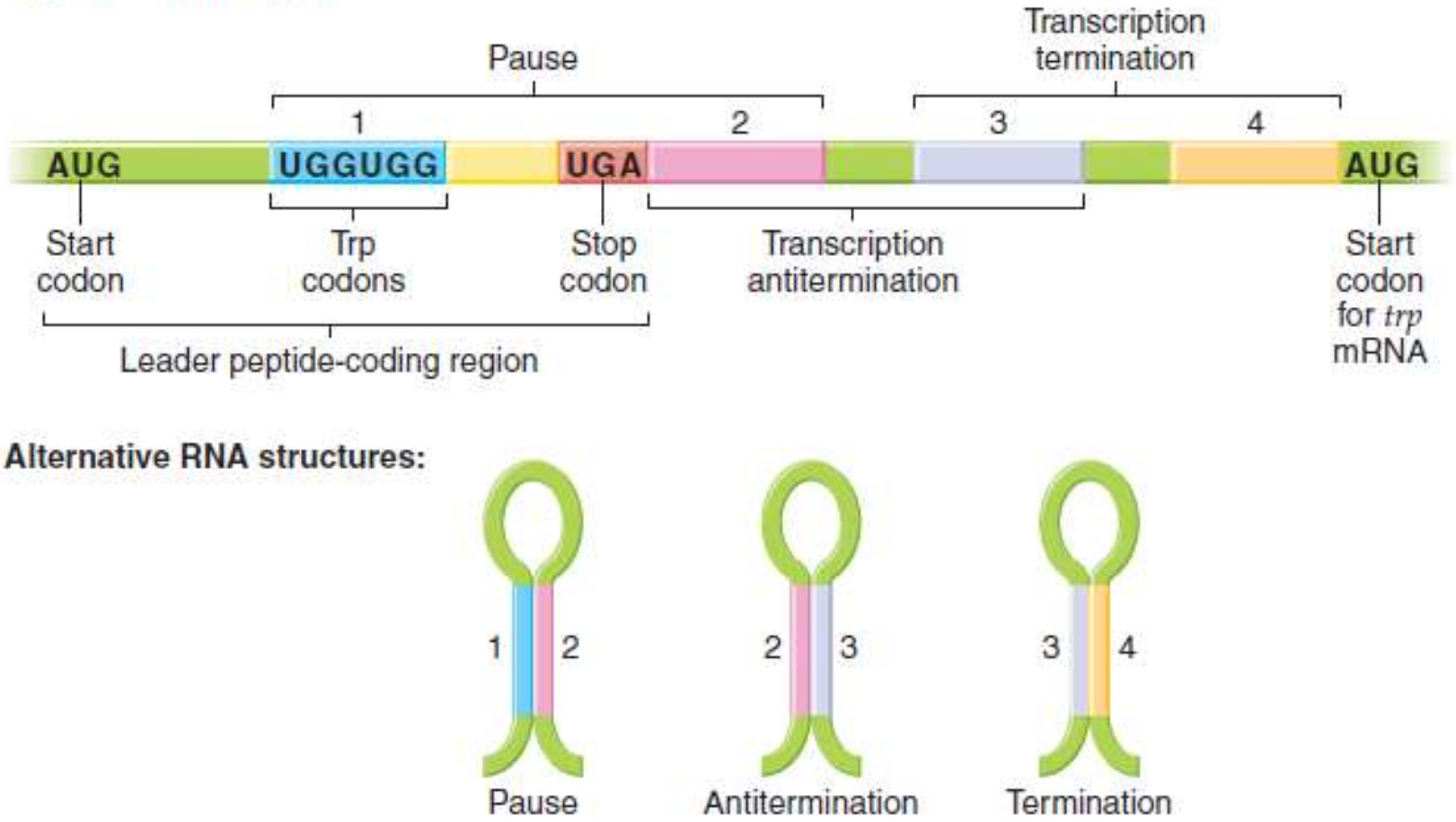
or **region 2 will base-pair with region 3, preventing region 3 from pairing with region 4**

The concentration of tryptophan in the cell determines which of these structures will form during the transcription of the trp operon.

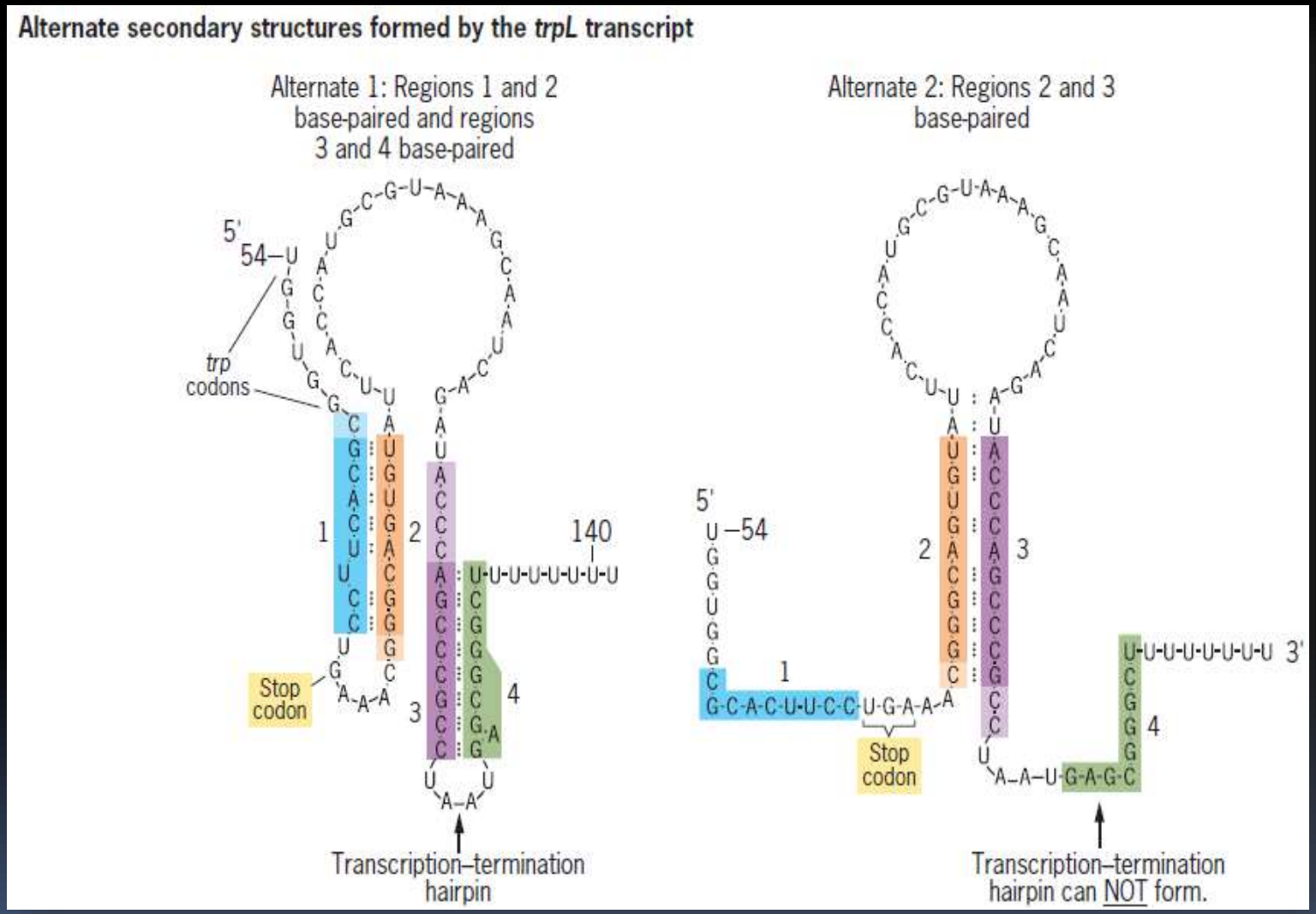
Crucial three signals :

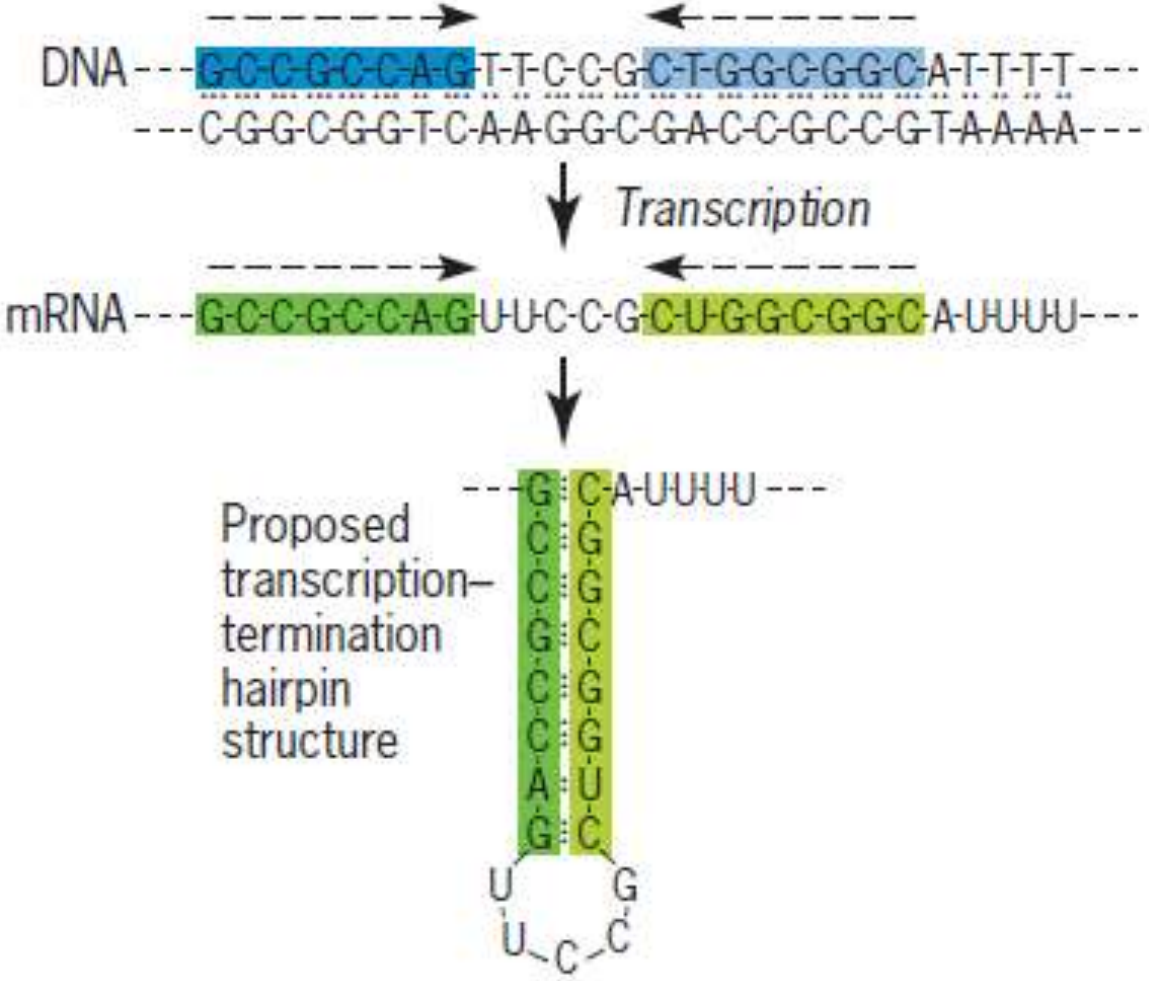
- The pairing of regions 1 and 2 results in the formation of a transcription pause signal
- that of 3 and 4 is a termination of transcription signal
- the pairing of 2 and 3 forms an antitermination signal for transcription to continue.

Four regions of the *trp* operon leader mRNA and the alternative secondary structures they can form by complementary base pairing.



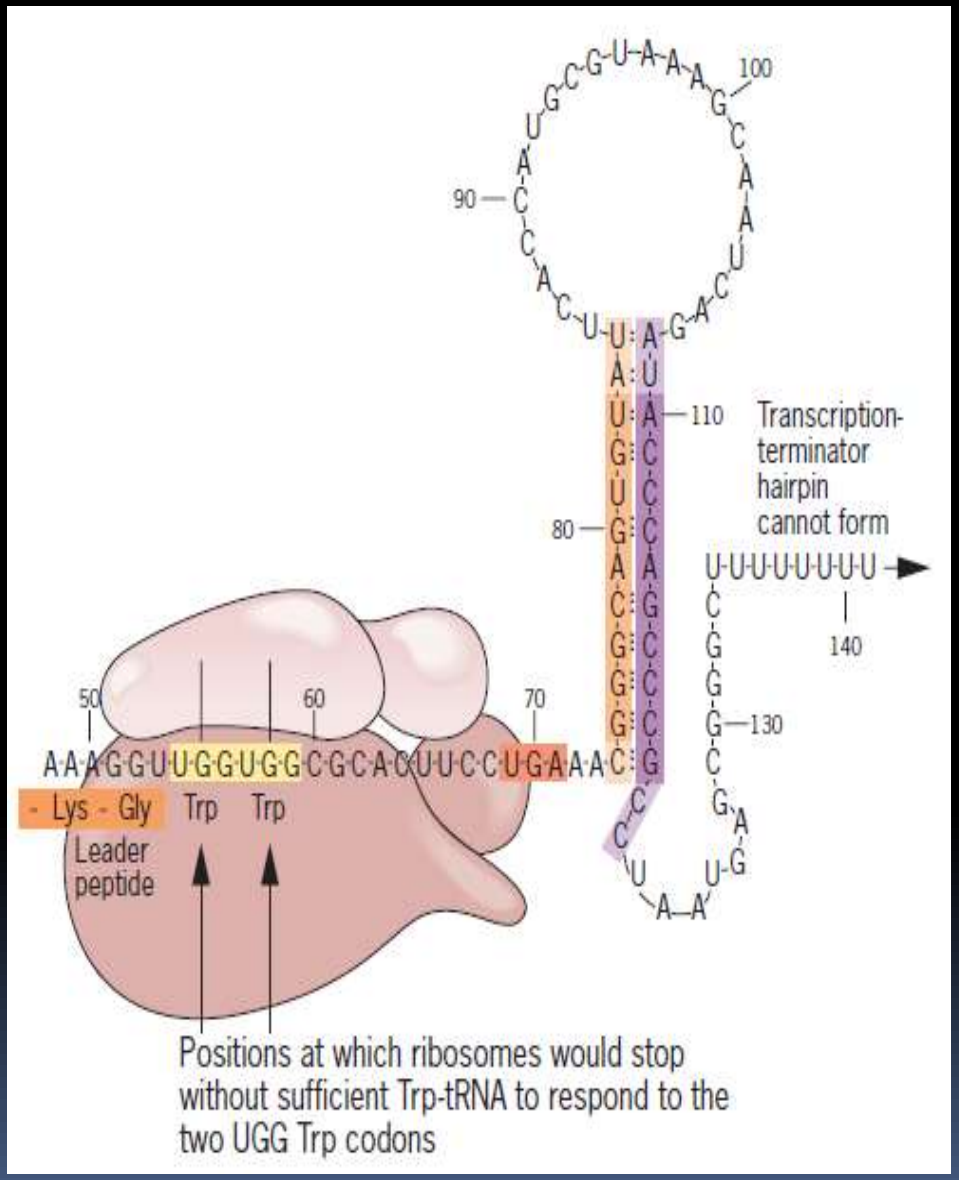
Mechanism :


Alternate secondary structures formed by the trpL transcript
Alternate 1: Regions 1 and 2 base-paired and regions 3 and 4 base-paired
5'
54
trp codons
1
2
3
4
140
Stop codon
Transcription–termination hairpin
Alternate 2: Regions 2 and 3 base-paired
5'
54
1
2
3
4
Stop codon
3'
Transcription–termination hairpin can NOT form.

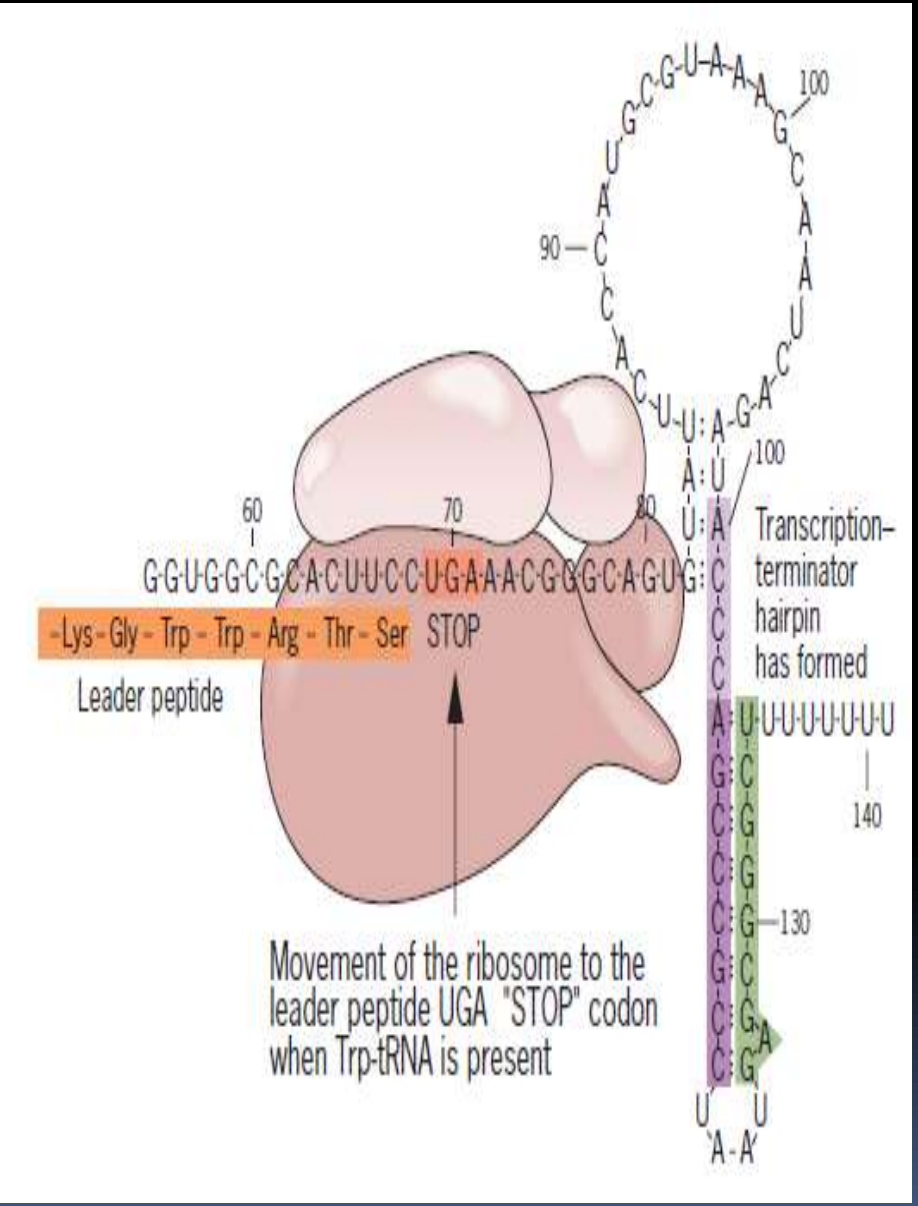


Structure of *trp* operon transcription–termination sequence *t* and formation of the transcription–termination hairpin.

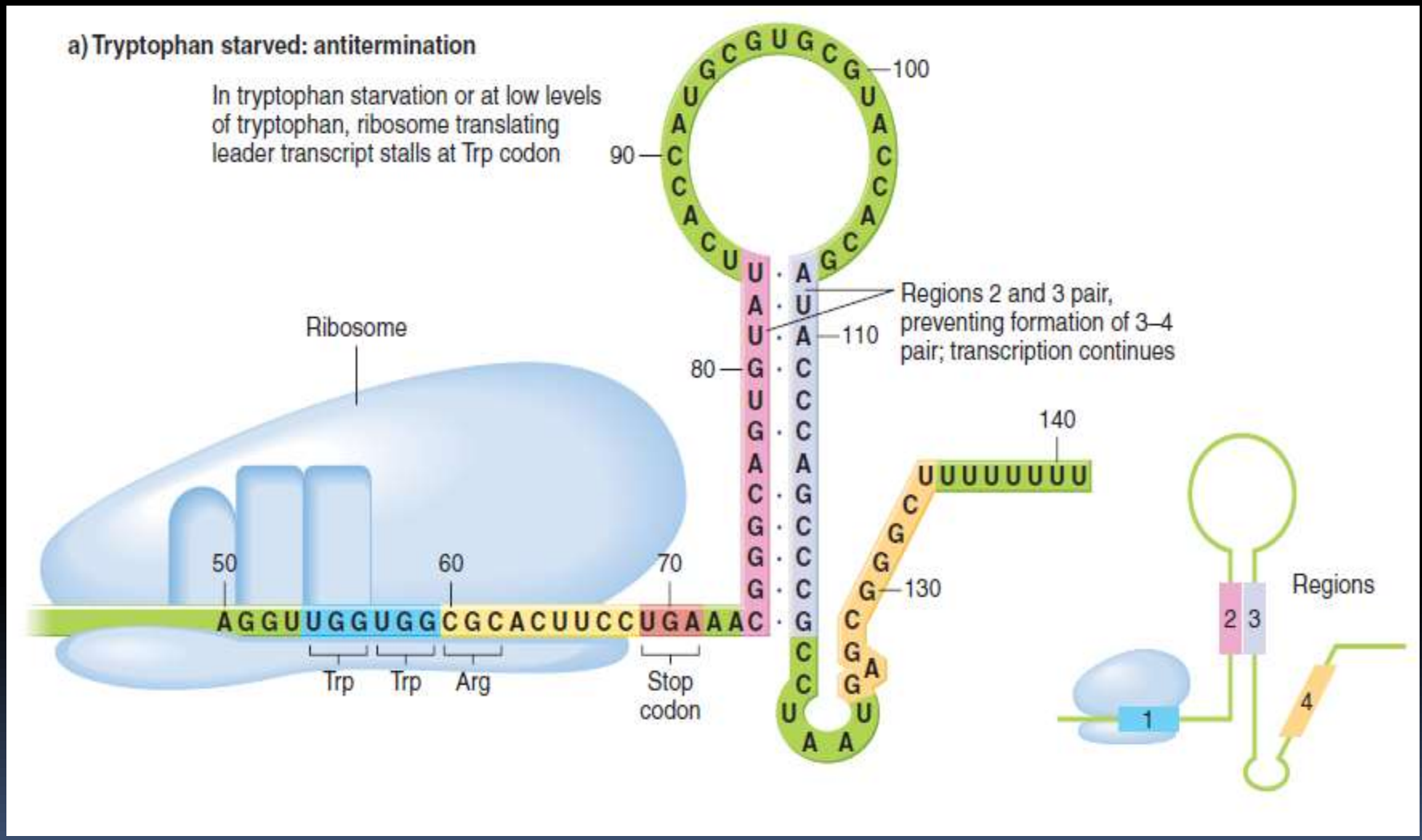
- With low levels of tryptophan, translation of the leader sequence stalls at one of the Trp codons. This stalling allows leader regions 2 and 3 to pair, which prevents region 3 from pairing with region 4 to form the transcription–termination hairpin. Thus transcription proceeds through the entire *trp operon.*

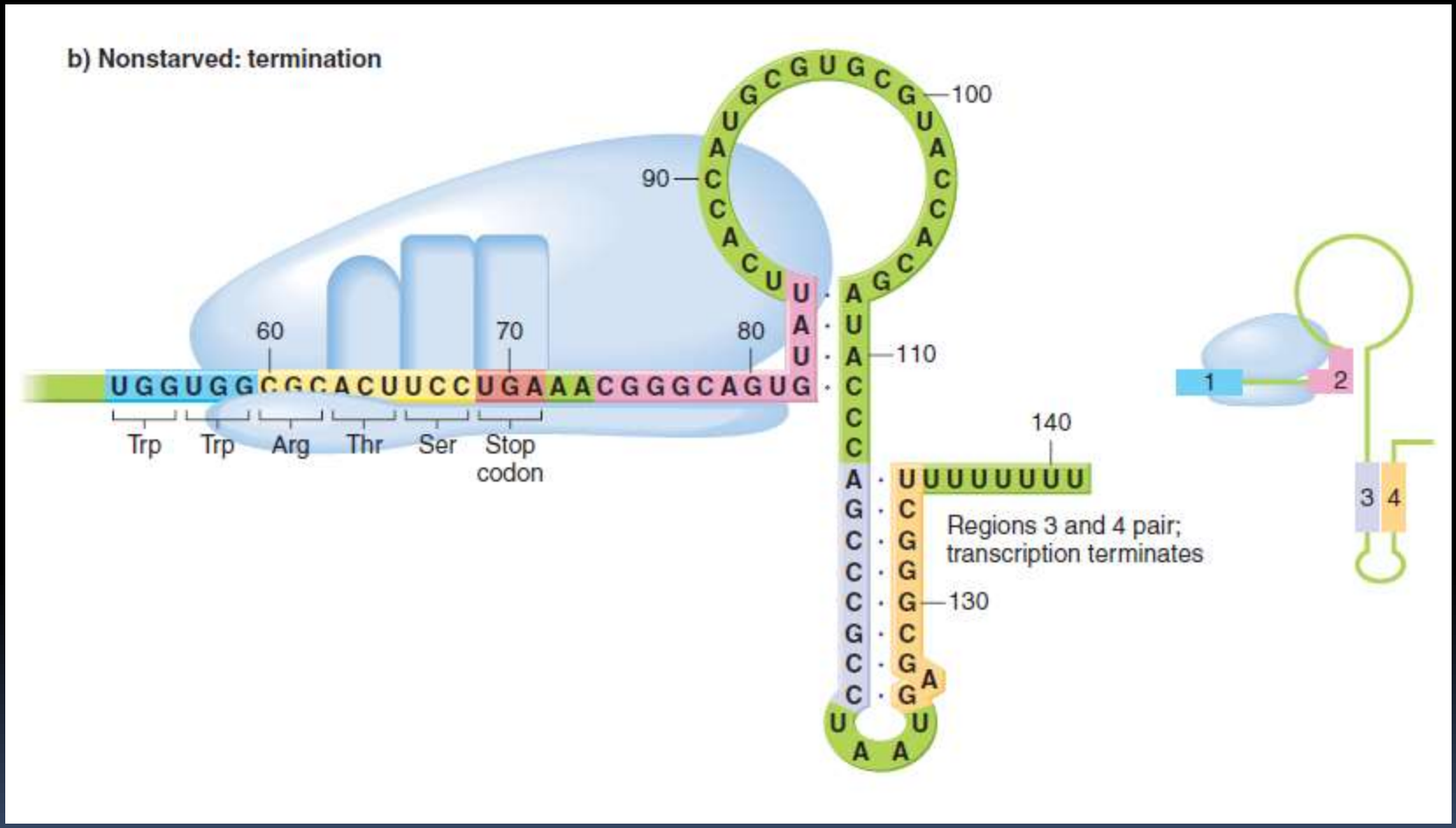


- In the presence of sufficient tryptophan, translation proceeds past the Trp codons to the termination codon and disrupts the base pairing between leader regions 2 and 3. This process leaves region 3 free to pair with region 4 to form the transcription-termination hairpin, which stops transcription at the attenuator sequence.



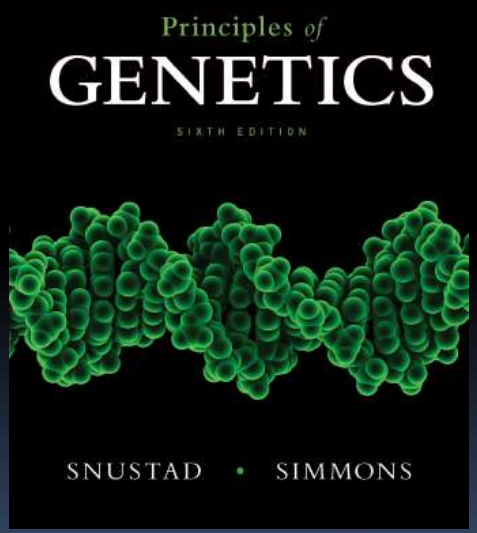
Model for attenuation in the *trp* operon of *E. coli*.

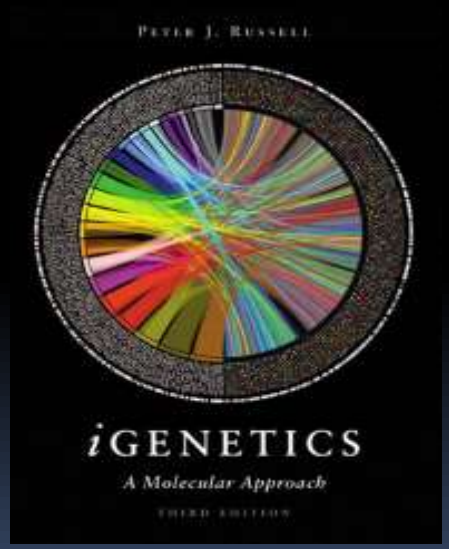


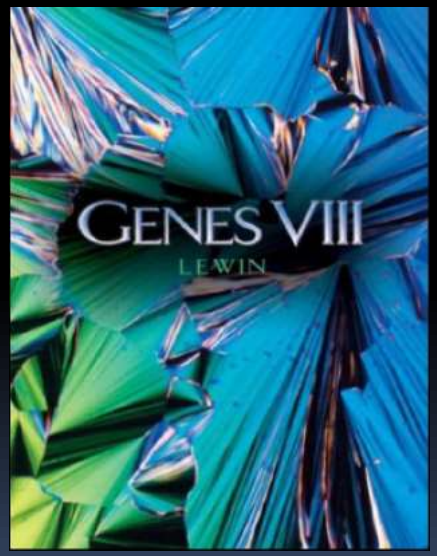

b) Nonstarved: termination
60
70
80
90
100
110
130
140
UGGUGGCGCACUUCCUGAAACGGGCAGUG
Trp
Trp
Arg
Thr
Ser
Stop codon
UUUUUUUU
Regions 3 and 4 pair; transcription terminates
1
2
3
4

REFERENCES:

I. Principles of Genetics by D. Peter Snustad
II. iGENETICS: A Molecular Approach by Peter J. Russell
III. Genes VIII by Benjamin Lewin
IV. Genes IX by Benjamin Lewin







Thank you for your patience