TRYPTOPHAN OPERON

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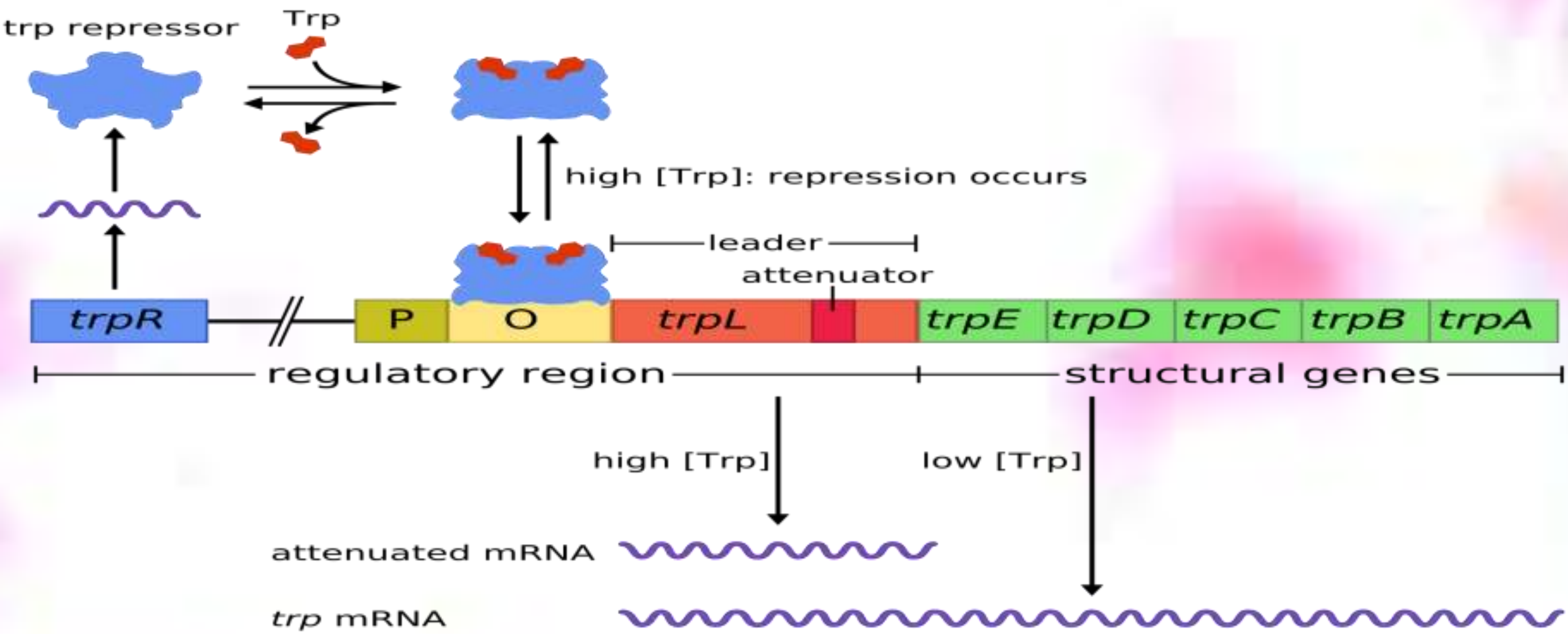
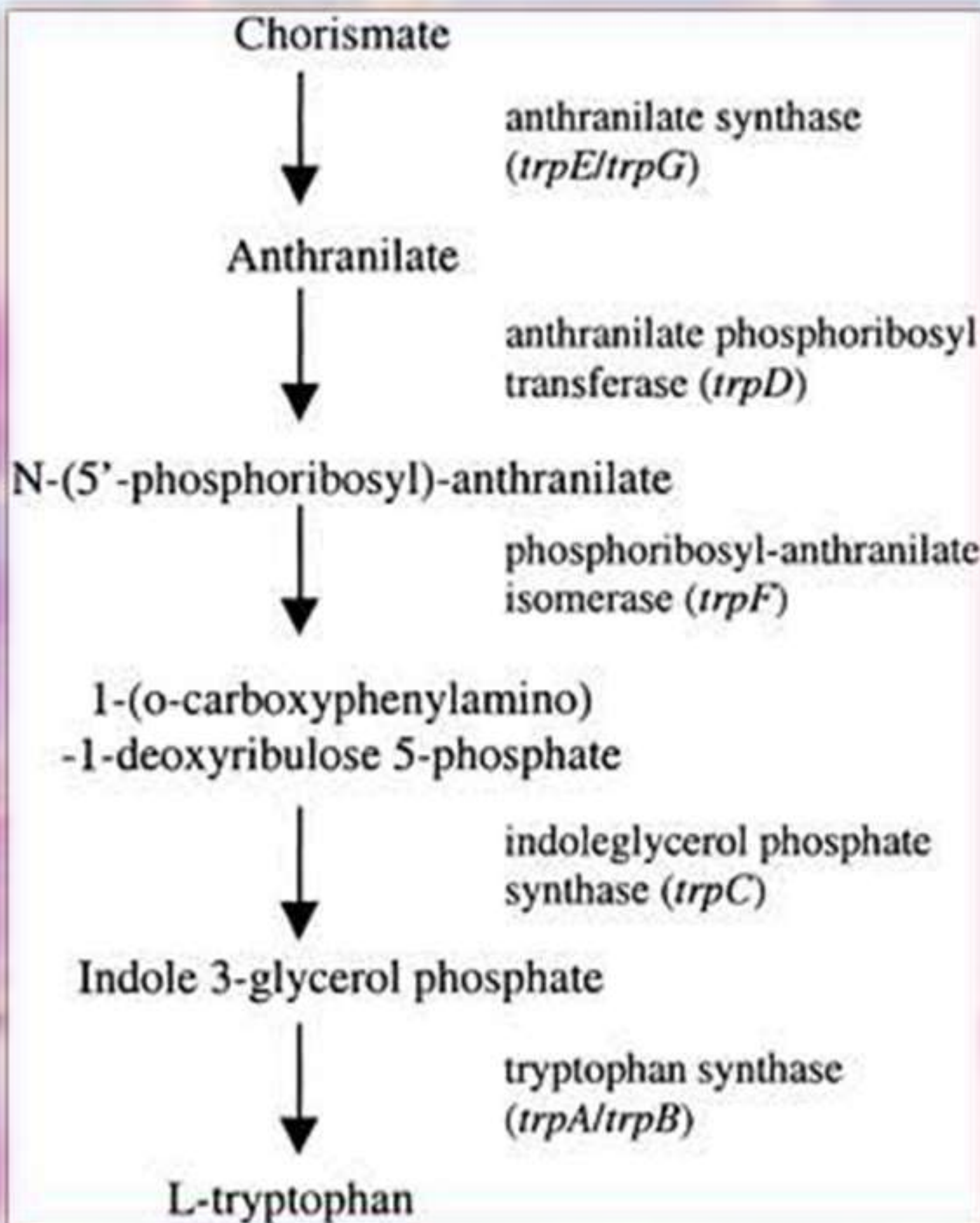
The *trp* Operon

- The 20 common amino acids are required in large amount for protein synthesis, and E.*coli* can synthesize all of them.
- The genes for the enzymes needed to synthesize a given amino acid are generally clustered in an **operon**_and are expressed whenever existing supplies of that amino acid are inadequate for cellular requirements.
- When the amino acid is abundant the biosynthetic enzymes are not needed and the operon is repressed.



- The E.*coli* tryptophan operon includes **five genes** for the enzymes required to convert **chorismate to tryptophan** .
- The mRNA from the tryptophan operon has a half life of only about **3 min**, allowing the cell to respond rapidly to changing needs for this amino acid.



- The biosynthesis of tryptophan by the enzymes encoded in the *trp* operon is diagrammed.

Regulatory Sequence

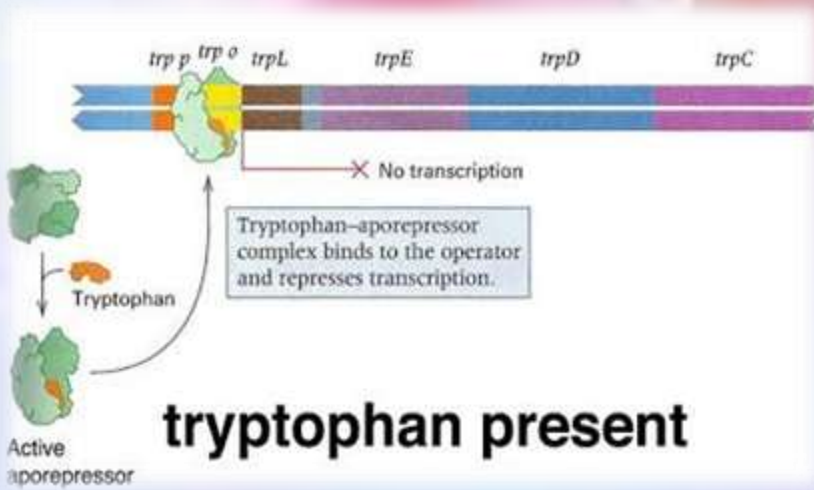
- This Operon is regulated by two mechanisms:

➢ The repressor binds to its operator

➢ The transcription of *trp* mRNA is attenuated.

The *trp* repressor

- The Trp repressor is a homodimer, each subunit containing 107 amino acid residues.
- When tryptophan is abundant it binds to the Trp represor, causing a conformational change that permits the repressor to bind to the *trp* operator and inhibit expression of the *trp* operon.
- The *trp* operator site overlaps the promoter, so binding of the repressor blocks binding of the RNA polymerase.



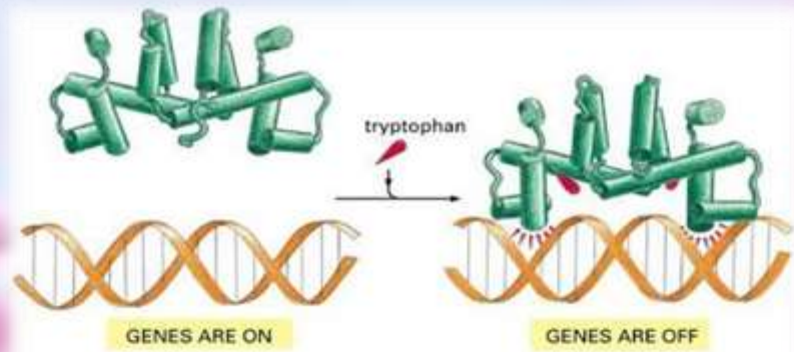
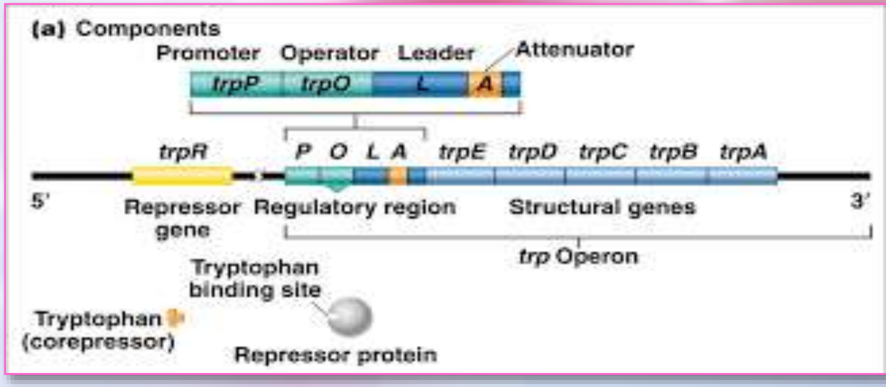


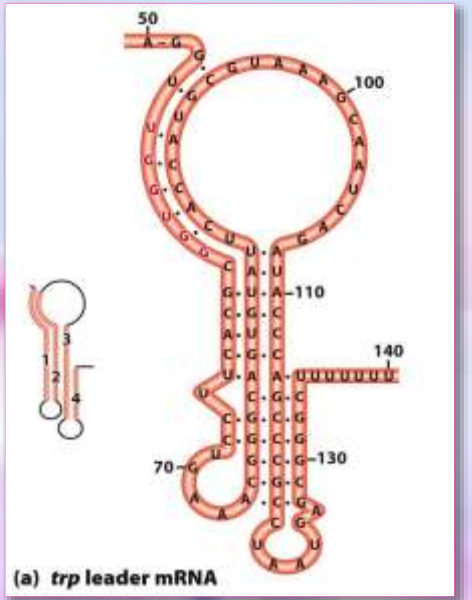
Figure 7–35. Molecular Biology of the Cell, 4th Edition.

The binding of tryptophan to the tryptophan repressor protein changes the conformation of the repressor. It is an helix-turn-helix protein.

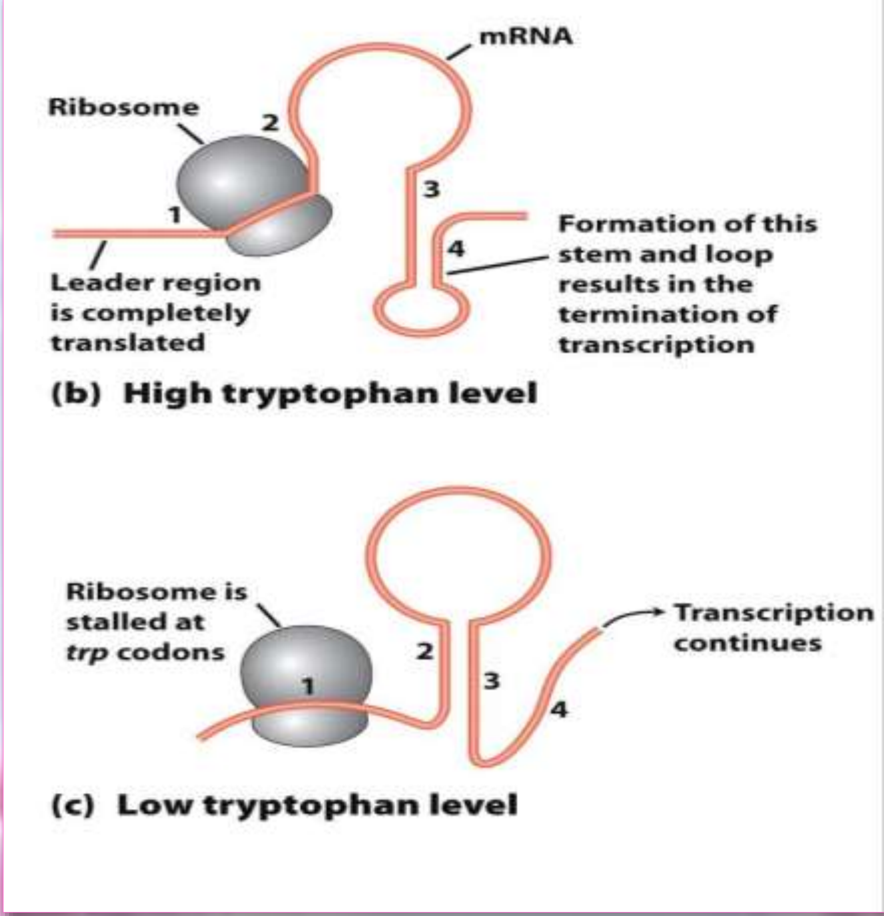
Transcriptional Attenuation

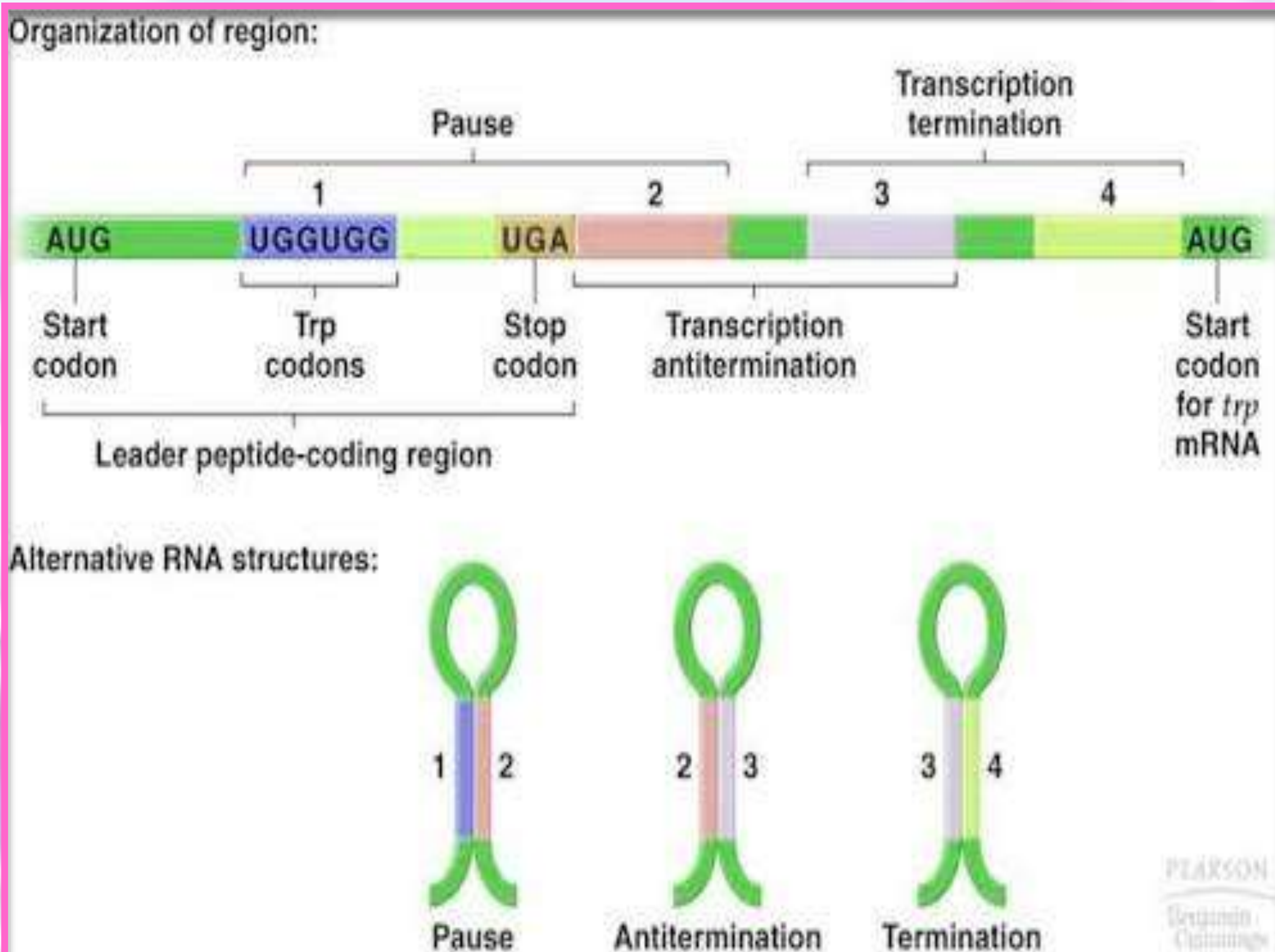
- It is a second regulatory process, in which transcription is initiated normally but is abruptly halted before the operon genes are transcribed.
- The *trp* operon attenuation mechanism uses signals encoded in four sequences within a 162 nucleotide **leader region** at the 5’ end of the mRNA, preceding the initiation codon of the first gene.





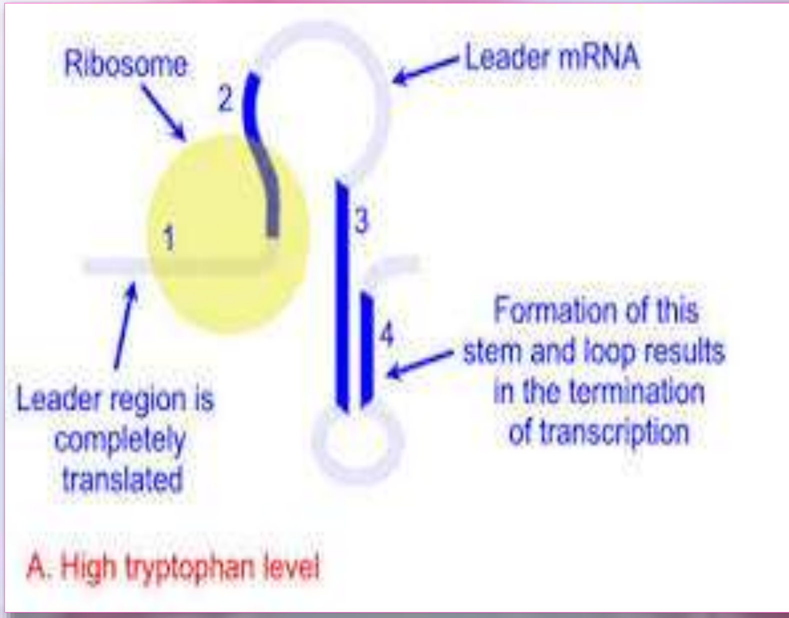
- Within the leader lies a region known as **attenuator**, made up of sequences **3 and 4**.
- These sequences base pair to form a G≡C rich stem and loop structure closely followed by a series of U residues.
- The attenuator structure act as a transcription terminator.
- If sequence 2 and 3 base pair the attenuator structure cannot form and transcription continues into the *trp* biosynthetic genes.



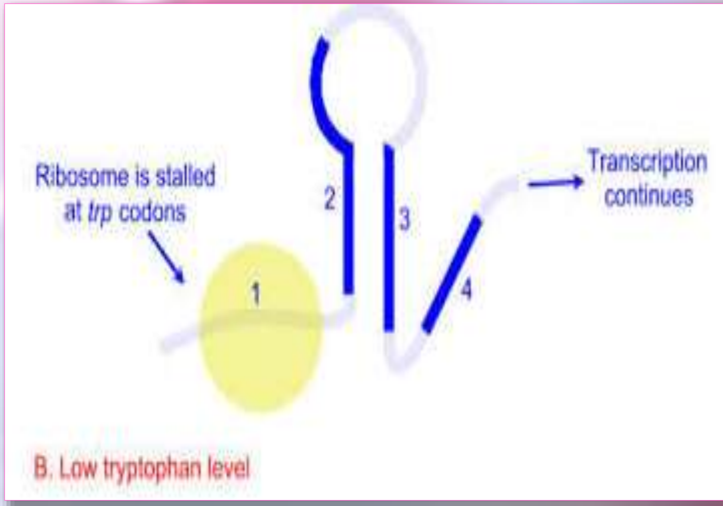

Organization of region:
Pause
Transcription
termination
1
2
3
4
AUG
UGGUGG
UGA
AUG
Start
codon
Trp
codons
Stop
codon
Transcription
antitermination
Start
codon
for trp
mRNA
Leader peptide-coding region
Alternative RNA structures:
1
2
2
3
3
4
Pause
Antitermination
Termination

What mechanism determines whether sequence 3 pairs with sequence 2 or with sequence 4 ?

- **When tryptophan concentrations are high**, concentrations of charged tryptophan tRNA are also high. This allows translation to proceed rapidly past the two trp codons of sequence 1 and into sequence 2, before sequence 3 is synthesized by RNA polymerase.
- In this situation, sequence 2 is covered by the ribosome , and unavailable for paring to sequence 3 when sequence 3 is synthesized; the attenuator structure sequence 3 and 4 forms and transcription halts.



- **When tryptophan concentrations are low**, the ribosome stalls at the two Trp codons in sequence 1 because the charged tryptophan tRNA is unavailable.
- Sequence 2 remains free while sequence 3 is synthesized, allowing these two sequences to base pair and permitting transcription to proceed.



Summary