



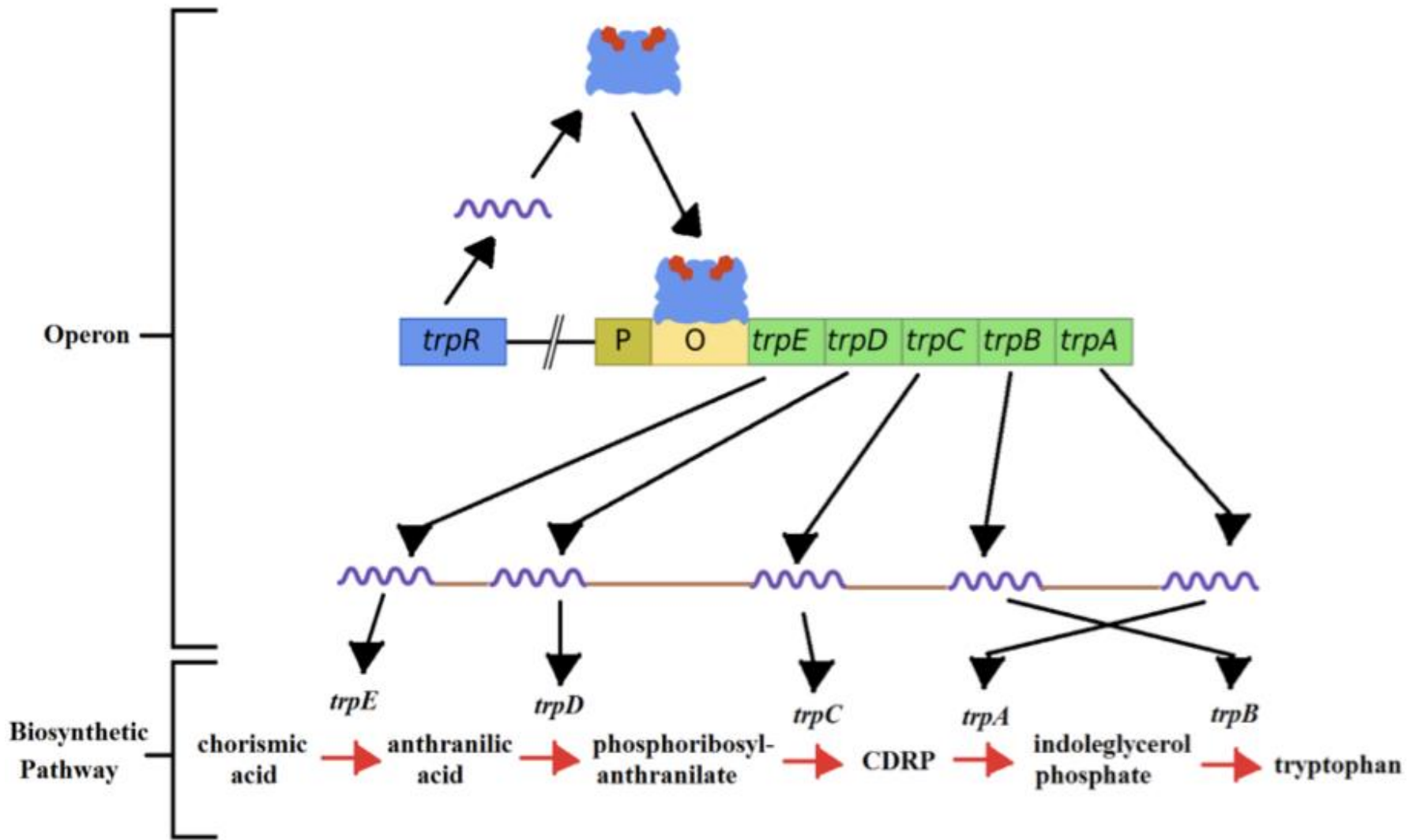
The Tryptophan Operon

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THE TRYPTOPHAN OPERON: A REPRESSIBLE SYSTEM

- The tryptophan operon, in contrast to the lac operon, codes for anabolic enzymes. It consists of 5 structural genes which are transcribed to produce a polycistronic mRNA which codes for 5 different enzymes that systematically convert Chorismic acid (precursor) to tryptophan.
- Thus, when tryptophan is present in adequate quantity, the products of tryptophan operon are not required and the operon remains switched off. It is, therefore, a classical example of a Repressible operon.
- The functions of the 5 structural genes and the regulatory sequences of *trp* operon have been studied in detail by Charles Yanofsky *et. al.*



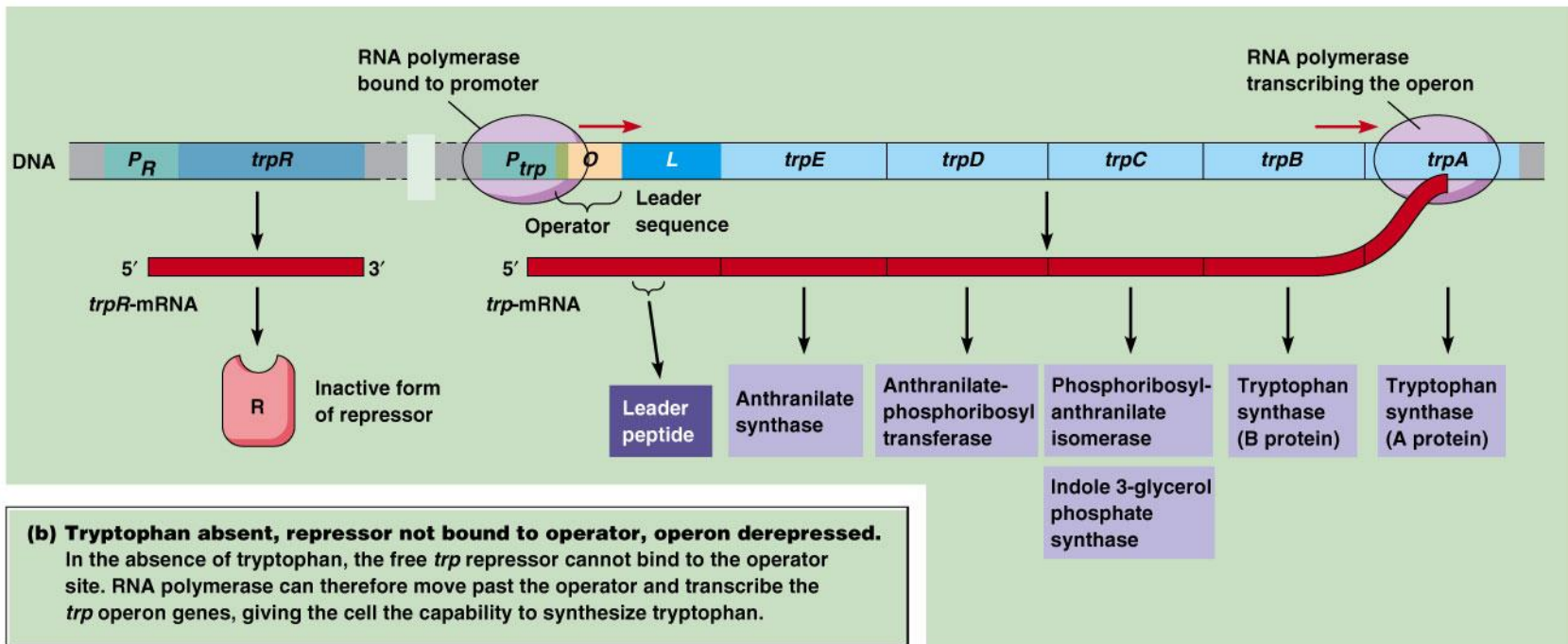
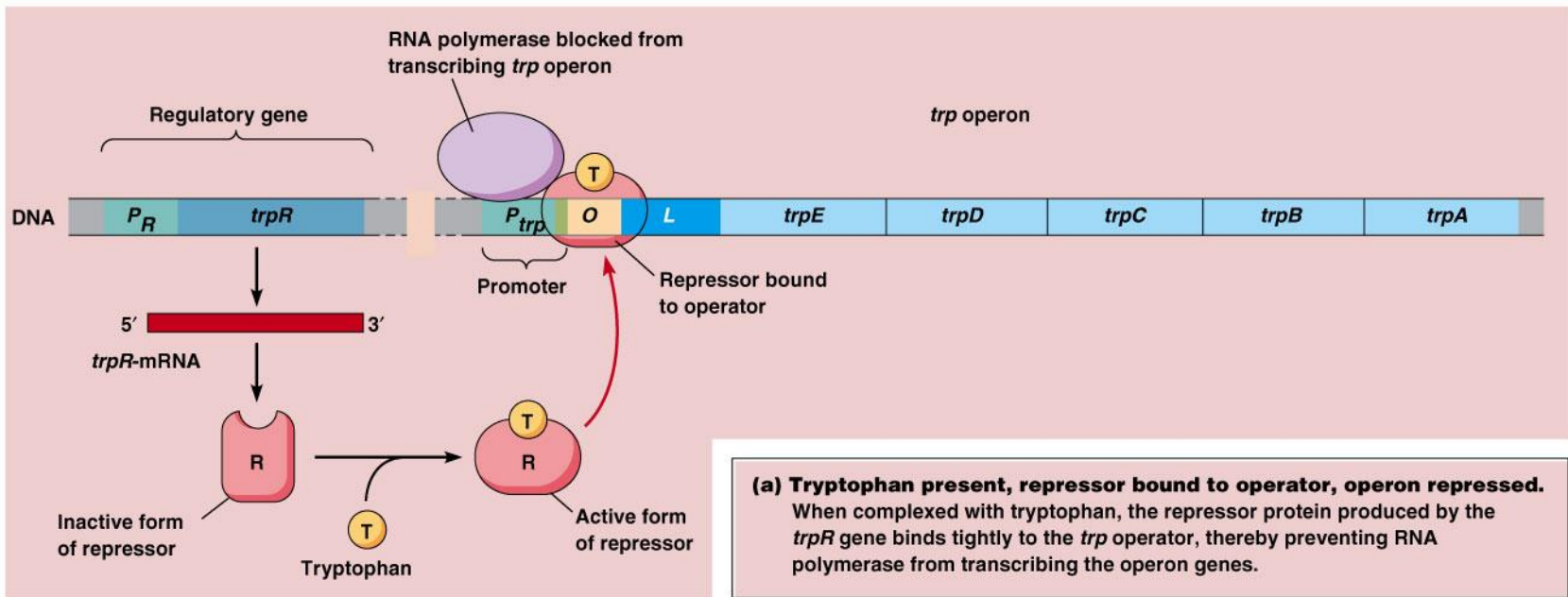
CDRP= Carboxyphenylamino-deoxyribulose-5-phosphate

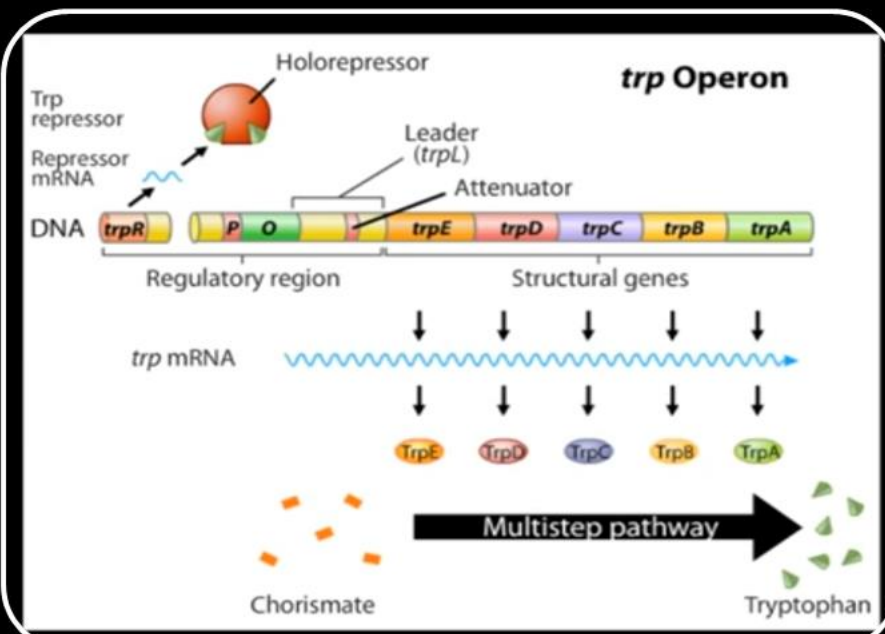
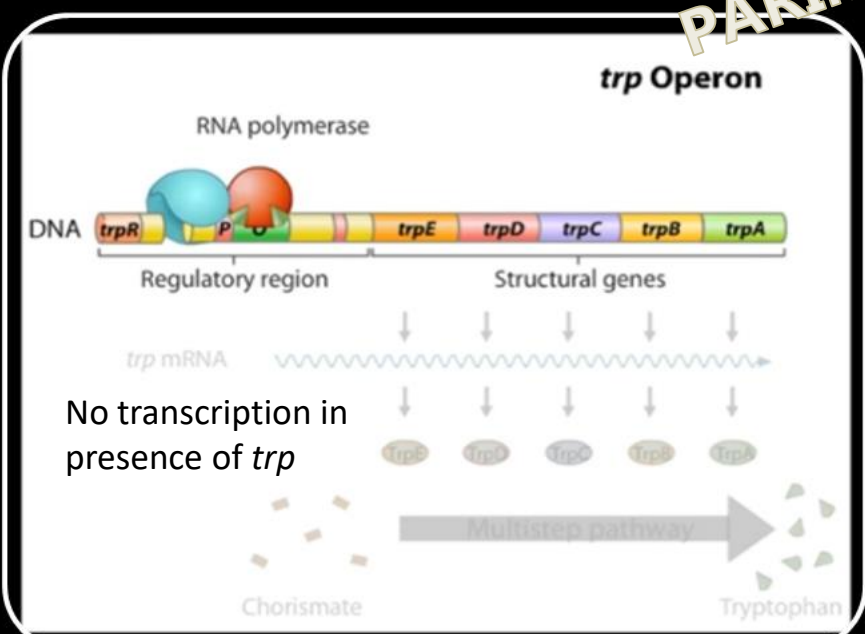
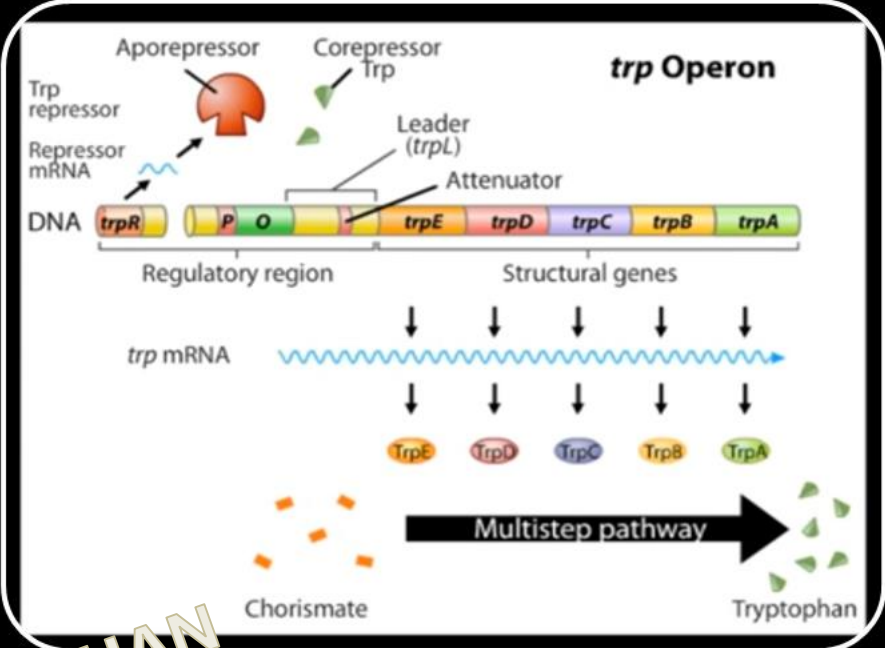
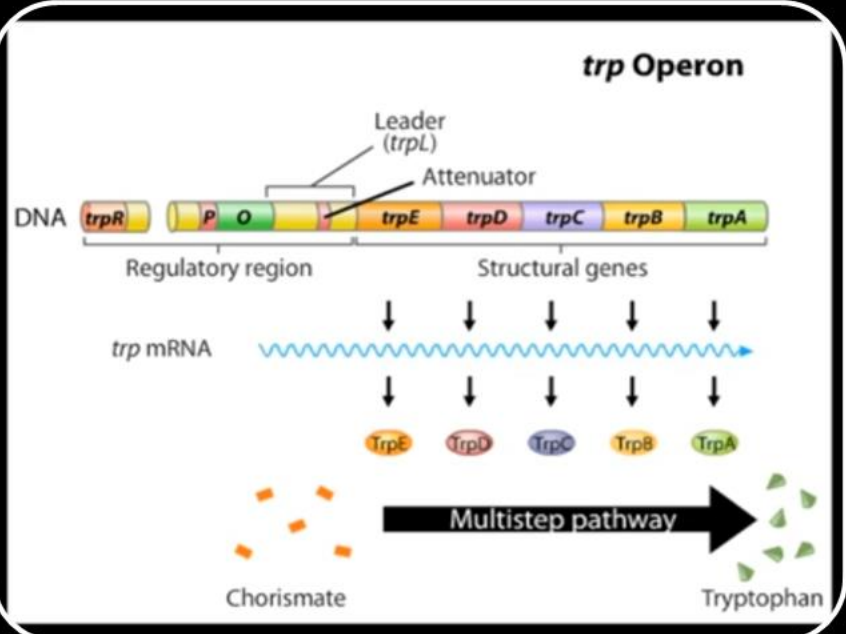
IN THE ABSENCE OF TRYPTOPHAN

- Regulatory gene (*i/TrpR*) transcribes the mRNA but the repressor so translated remains inactive.
- RNA polymerase is thus able to bind at Promoter (p) and transcribes a polycistronic mRNA.
- This results in the synthesis of enzymes required for tryptophan synthesis.
- In this state, the cell has very high levels of these enzymes; *i.e.* nearly 70 times the basal rate.

IN THE PRESENCE OF TRYPTOPHAN

- The regulatory gene transcribes the mRNA to produce a repressor protein which gets activated by the binding of tryptophan which acts as a co-repressor.
- This active complex (holorepressor) binds at the Operator (o).
- RNA polymerase is now not able to bind at the Promoter and thus the structural genes are not transcribed.
- The cell, under such conditions, contains very low levels of tryptophan synthesizing enzymes.





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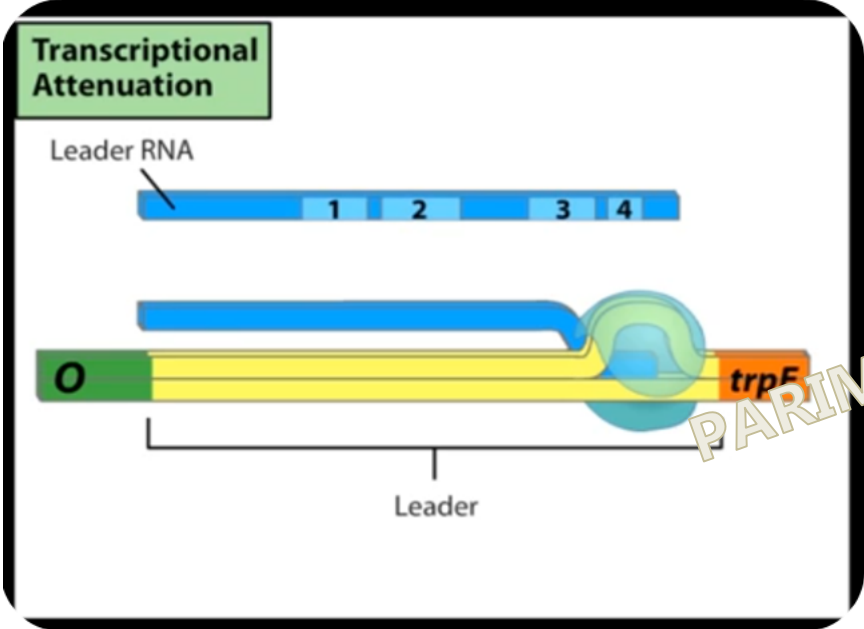
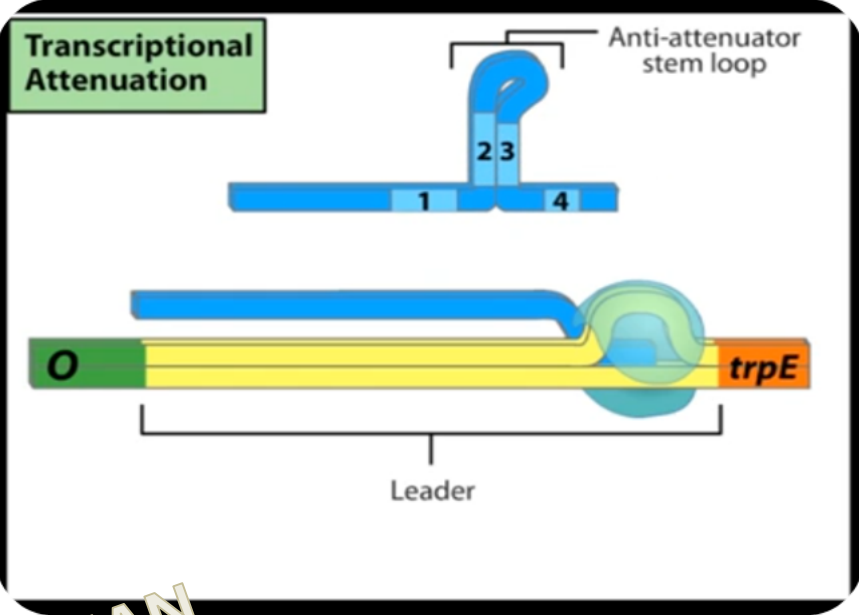
TRANSCRIPTIONAL ATTENUATION

- Sometimes the regulatory gene undergoes mutation (*Trp R* mutation) and encodes for a repressor that remains inactive even in the presence of tryptophan. This leads to constitutive expression of the gene.
- Even under such conditions, about 10-20 times reduction in rate of enzyme synthesis was observed.
- This suggested the existence of a second regulatory mechanism which caused premature termination of transcription. This mechanism is termed as attenuation.
- Two slightly different mechanisms of attenuation have been observed in *E. coli* and *B. subtilis*.

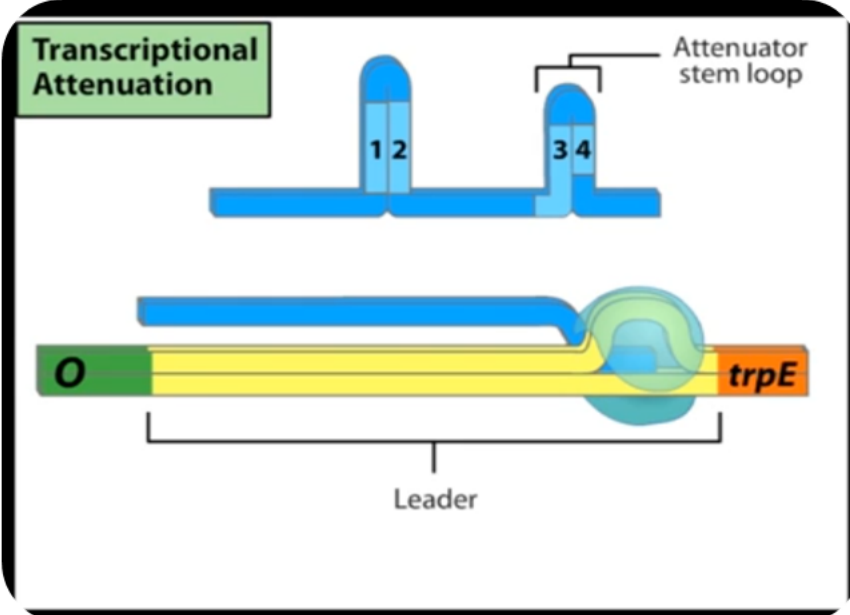
ATTENUATION IN *E. coli*

- In between the Promoter and structural genes, a leader sequence is present within which three sites are found, namely:
 - a) transcription start site
 - b) transcription pause site
 - c) transcription termination site
- The attenuator region contains 4 different segments namely 1, 2, 3 and 4.
- Region 1 and 2 are complementary to each other and base pairing results in a hairpin loop. Also, region 3 can base pair with region 4.
- Complementary base pairing is also possible between regions 2 and 3.
- The pairing between specific regions depends on the concentration of tryptophan and is responsible for regulation of gene expression.

Low
Tryptophan



High
Tryptophan

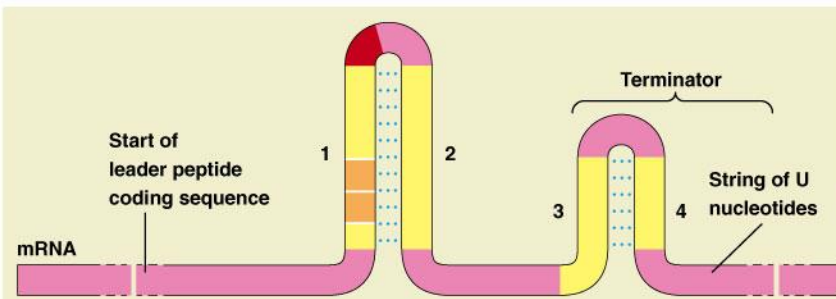


TRYPTOPHAN STARVED CONDITION

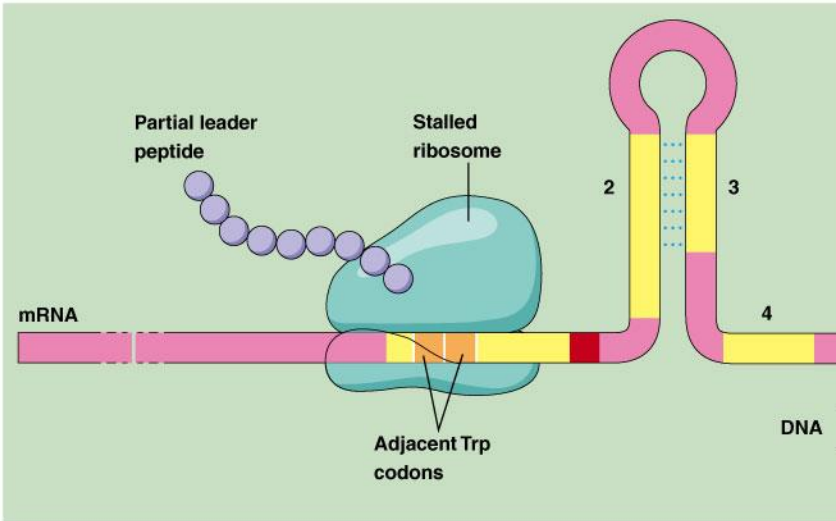
- As the RNA polymerase transcribes the leader sequence, ribosomes assemble on the growing mRNA.
- Low levels of tryptophan result in a deficiency of charged tRNA^{trp}.
- Due to this, the ribosome stalls at one of the two tandem trp codons present in the leader mRNA.
- The stalled ribosome blocks region 1, hindering the formation of 1-2 hairpin loop (attenuator stem loop) and instead, a 2-3 hairpin loop (anti-attenuator stem loop) is formed. Thus, transcription continues.

TRYPTOPHAN ADEQUATE

- Under conditions of presence of sufficient amount of tryptophan, charged tRNA^{trp} is high and the ribosome does not stop at the trp codons.
- Instead, it moves along and encounters the stop codon present in the leader sequence.
- In this manner, region 2 is blocked by the ribosome leading to the formation of 3-4 hairpin loop.
- RNA polymerase discontinues the transcription process and attenuation of tryptophan operon is achieved.

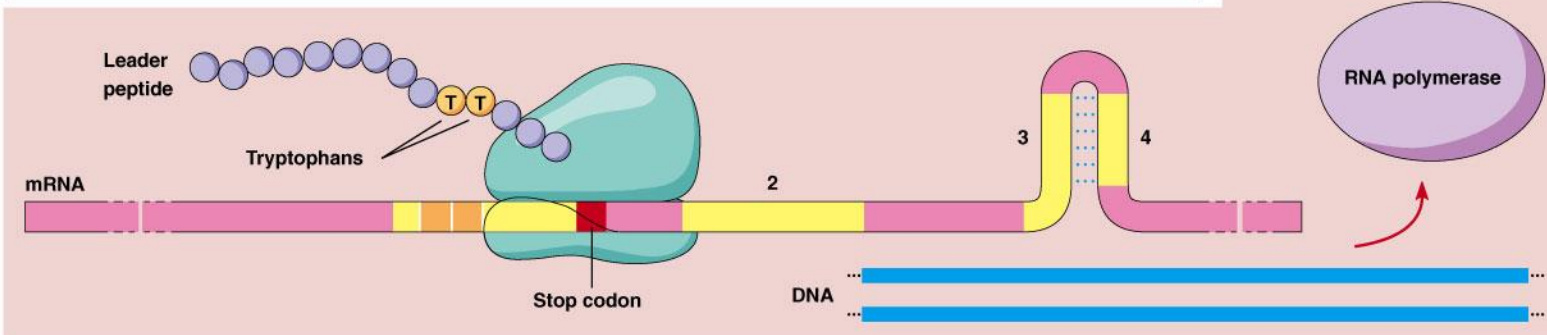


(a) The most stable secondary structure for *trp* leader mRNA. Attenuation depends on the ability of regions 1 and 2 and regions 3 and 4 of the *trp* leader sequence to base-pair, forming hairpin secondary structures. The 3–4 hairpin structure acts as a transcription termination signal.

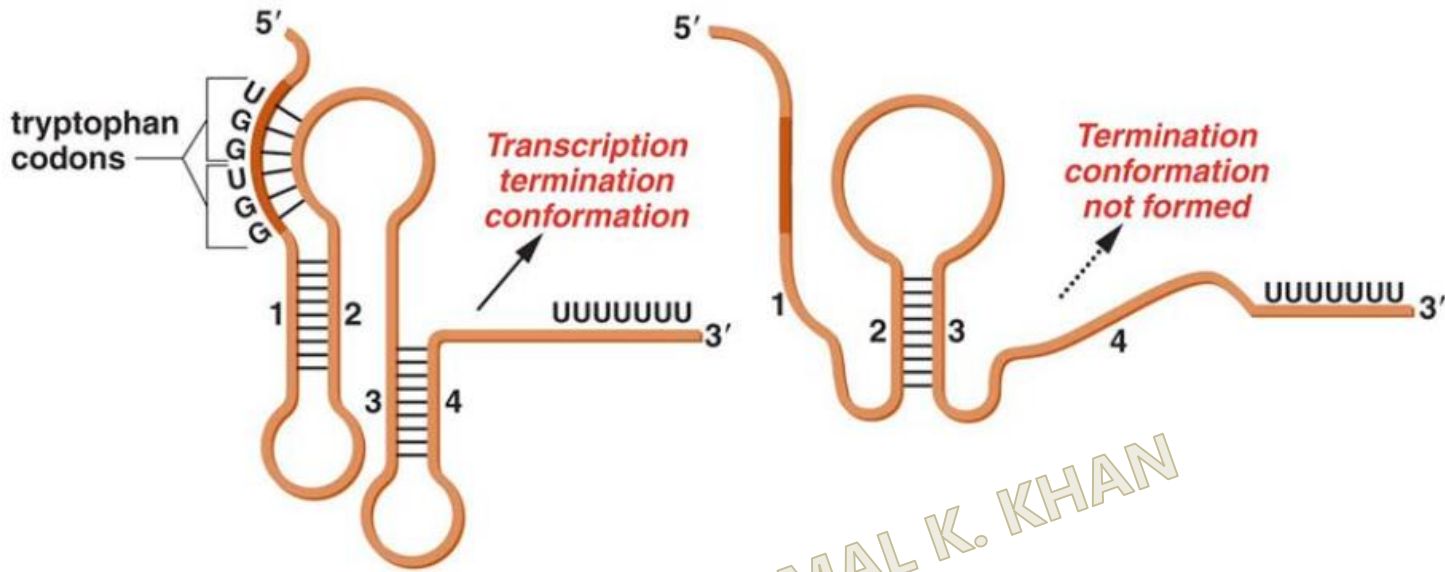


(b) When tryptophan is scarce the ribosome stalls, allowing a 2–3 “antiterminator” hairpin to form. The ribosome stalls when it encounters the two tryptophan (Trp) codons due to a shortage of tryptophan-carrying tRNA molecules. The stalled ribosome blocks region 1, so a 1–2 hairpin cannot form. Instead an alternative 2–3 hairpin is created, which prevents formation of the 3–4 termination hairpin. Therefore RNA polymerase can move on to transcribe the entire operon.

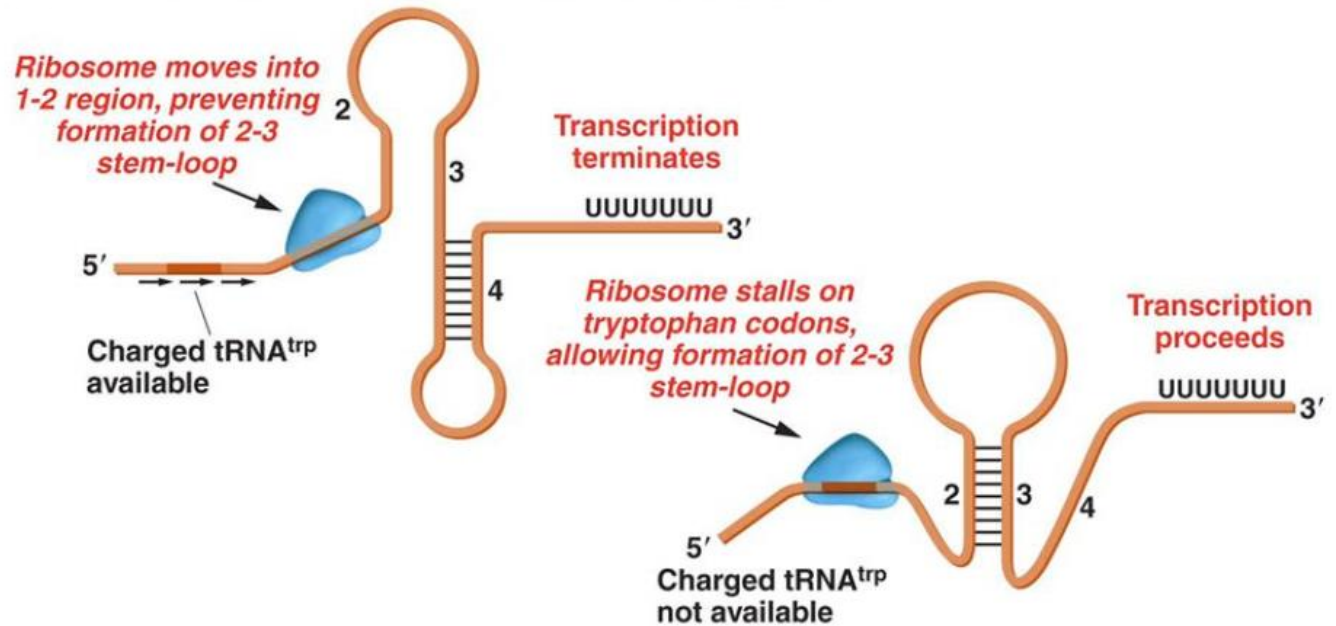
(c) When tryptophan is plentiful the ribosome continues, allowing the 3–4 transcription termination signal to form. The moving ribosome completes translation of the leader peptide and pauses at the stop codon, blocking region 2. As a result, the 3–4 structure forms and terminates transcription near the end of the leader sequence.



Two potential stem-loop structures can form within the *trp* leader



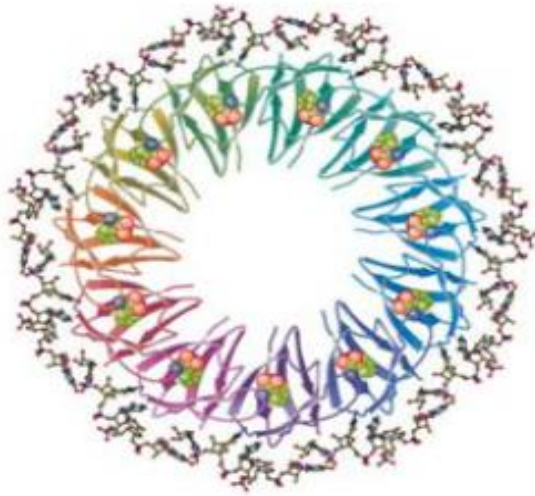
Position of ribosome determines which stem-loop forms



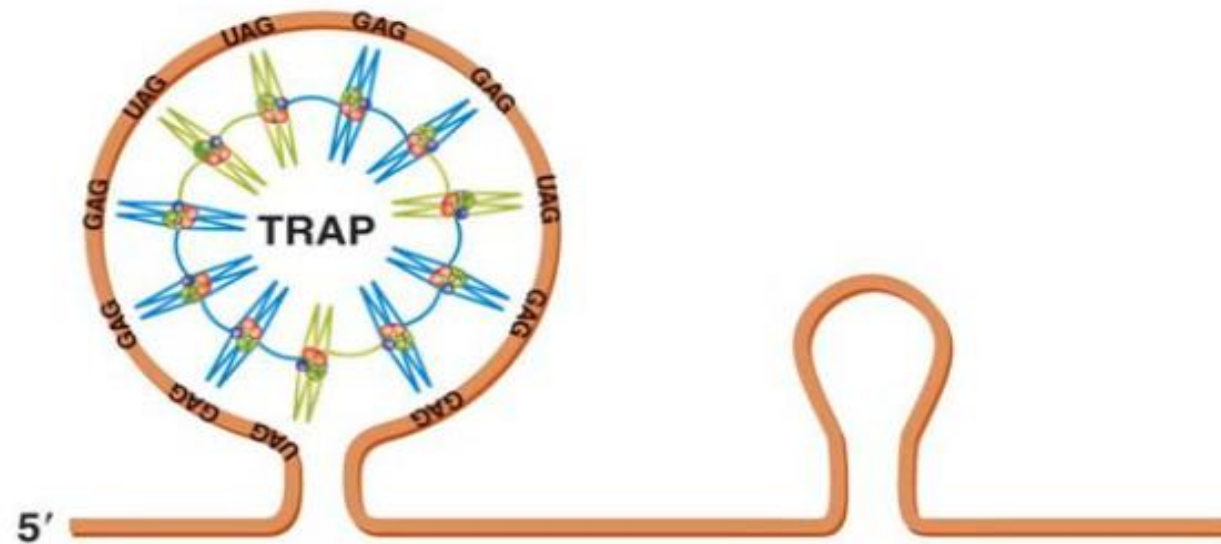
ATTENUATION IN *B. subtilis*

- *B. subtilis* uses a different mechanism to establish attenuation in Trp operon.
- It involves the protein TRAP (Tryptophan RNA binding attenuator protein) which consists of 11 subunits, each capable of binding one molecule of tryptophan.
- In addition to TRAP, another protein called anti-TRAP protein is involved in the attenuation process which remains sensitive to uncharged tRNA^{trp} and can bind to tryptophan bound TRAP.
- The *B. subtilis* trp operon, therefore, has two ways to sense trp concentration. Through TRAP it senses concentration of tryptophan itself and through anti-TRAP leader, it senses concentration of uncharged tRNA^{trp}.

(a)



(b)



**Terminator hairpin
(Tryptophan abundant)**

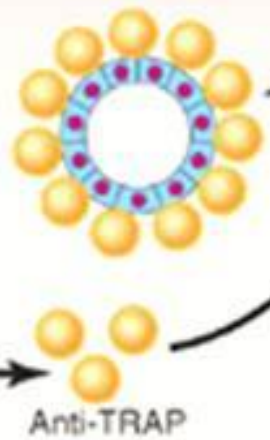
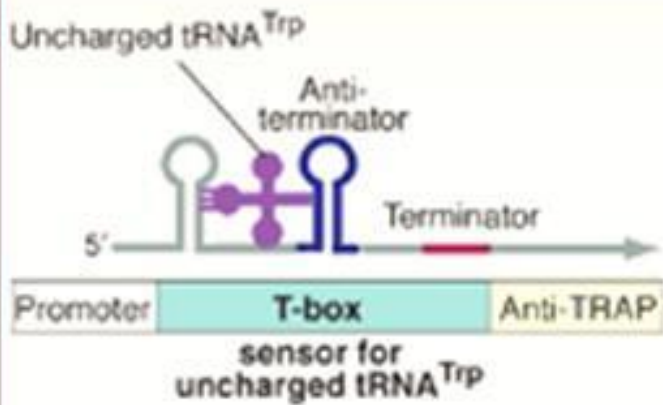
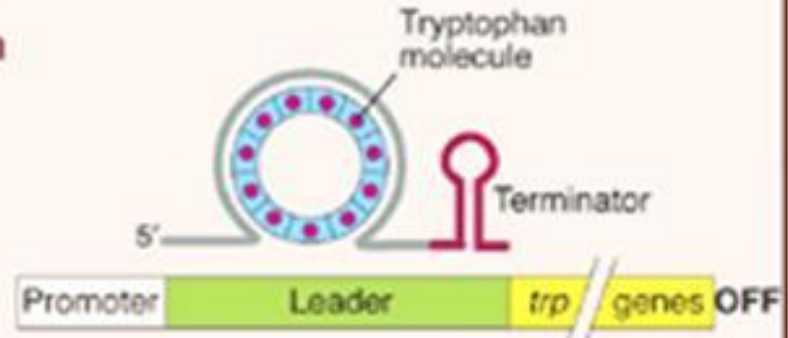
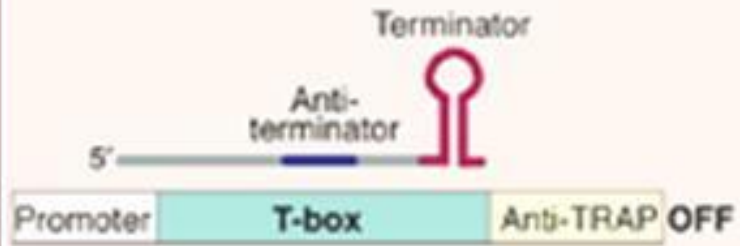
ATTENUATION THROUGH TRAP

- When trp is abundant, trp bound TRAP molecule binds to 5' leader sequence of RNA transcript that contains 11 tandem codons for trp, and causes it to form a terminator loop. This attenuates transcription of trp operon.
- In contrast, when trp is scarce, it does not bind to TRAP which is subsequently unable to bind with the trp leader sequence.
- Without TRAP, the leader sequence doesn't form a terminator loop and instead forms an anti-terminator loop, defeating attenuation.

CONTROL THROUGH ANTI-TRAP

- The gene for synthesis of anti-TRAP molecules also consists of a leader sequence which contains a region called T-box.
- The leader transcript may fold alternatively to form either a terminator or anti-terminator depending on the amount of uncharged $tRNA^{trp}$.
- If concentration of trp is high, the concentration of uncharged $tRNA^{trp}$ decreases. Under such conditions, anti-TRAP leader forms a terminator loop and the gene is turned off. Anti-TRAP molecules will, thus, not be formed.
- If the concentration of trp is low, a rise in concentration of uncharged $tRNA^{trp}$ is seen. This uncharged $tRNA^{trp}$ binds to anti-TRAP leader and stabilizes the anti-terminator loop. The anti-TRAP gene remains turned on and anti-TRAP molecules are formed.
- These anti-TRAP molecules bind to trp bound TRAP & prevent its binding to Trp leader transcript which then forms an anti-terminator loop & transcription of trp operon continues.

High tryptophan



Low tryptophan

A microscopic image showing several rod-shaped bacteria, likely Bacillus subtilis, stained with a blue dye. The bacteria are scattered across the frame, with some in sharp focus and others blurred in the background. Each bacterium has a textured, granular surface and several thin, hair-like flagella extending from its ends. The background is a dark, uniform blue.

Thank you