Tryptophan Operon and Transcriptional Attenuation

- Transcriptional-attenuation is characterized by the presence of an attenuator within the DNA sequence that results in formation of mRNA-stem loops that prevent further transcription from occurring. The non-functional RNA produced prevents proper transcription.

- Attenuators are characterized by the presence of stop signals within the DNA sequence that can result in either transcriptional-attenuation or translational-attenuation.

- Translational-attenuation is characterized by the misfolding of the Shine-Dalgarno sequence. The Shine-Dalgarno sequence, responsible for ribosomal binding to allow proper translation, is inaccessible because it is folded into a hairpin-loop structure, thus, translation cannot occur.

Attenuation is a regulatory mechanism used in bacterial operons to ensure proper transcription and translation. In bacteria, transcription and translation are capable of proceeding simultaneously. The need to prevent unregulated and unnecessary gene expression can be prevented by attenuation, which is characterized as a regulatory mechanism.

The process of attenuation involves the presence of a stop signal that indicates premature termination. The stop signal, referred to as the attenuator, prevents the proper function of the ribosomal complex, stopping the process. The attenuator is transcribed from the appropriate DNA sequence and its effects are dependent on the metabolic environment. In times of need, the attenuator within the mRNA sequence will be bypassed by the ribosome and proper translation will occur. However, if there is not a need for a mRNA molecule to be translated but the process was simultaneously initiated, the attenuator will prevent further transcription and cause a premature termination. Hence, attenuators can function in either transcription-attenuation or translation-attenuation.

Transcription-attenuation is characterized by the presence of 5'-cis acting regulatory regions that fold into alternative RNA structures which can terminate transcription. These RNA structures dictate whether transcription will proceed successfully or be terminated early, specifically, by causing transcription-attenuation. The result is a misfolded RNA structure

where the Rho-independent terminator disrupts transcription and produced a non-functional RNA product. This characterizes the mechanisms of transcription-attenuation. The other RNA structure produced will be an anti-terminator that allows transcription to proceed.

TRYPTOPHAN OPERON

Bacteria such as *Escherichia coli* (a friendly inhabitant of our gut) need amino acids to survive—because they need to build proteins. One of the amino acids they need is tryptophan.

If tryptophan is available in the environment, *E. coli* will take it up and use it to build proteins. However, *E. coli* can also make their own tryptophan using enzymes that are encoded by five genes. These five genes are located next to each other in what is called the ***trp* operon**.

An **operon** is a set of genes that are transcribed under control of a single promoter, resulting in one long mRNA that contains coding sequences for multiple genes. The operon includes not only the genes, but also the regulatory DNA sequences that control their expression (including the promoter and binding sites for any repressor or activator proteins).

If tryptophan is present in the environment, then *E. coli* bacteria don't need to synthesize it, so transcription of the genes in the *trp* operon is switched "off." When tryptophan availability is low, on the other hand, the operon is switched "on," the genes are transcribed, biosynthetic enzymes are made, and more tryptophan is produced.

The *trp* operon is another example of co- ordinated gene regulation. A more complex regulatory system is used in *E. coli* (where attenuation was originally discovered). The changes in mRNA secondary structure that controls attenuation are determined by the position of the ribosome on mRNA.

The entire tryptophan operon is approximately 7000 bp long.

Transcription of the operon results in the production of a polygenic mRNA for the five structural genes.

Tryptophan is needed itself to inactivate the trp operon, it is called co-repressor.

GENE ORGANIZATION OF THE TRYPTOPHAN OPERON

The *trp* operon includes five genes that encode enzymes needed for tryptophan biosynthesis - coding for the three enzymes that convert chorismic acid to tryptophan along with a promoter **(RNA polymerase binding site) and an** operator **(binding site for a repressor protein). The genes of the *trp* operon are transcribed as a single mRNA.**

- Five structural genes (A-E) occur in the tryptophan operon.
- Promoter & operator regions are closely integrated in the DNA & upstream from the trpE gene.
- Leader region / trpL present between promoter-operator region & trpE, it 162 bp long.
- TrpL is relatively close to trpE, is called attenuator site (att) that plays an important role in the regulation of the tryptophan operon.

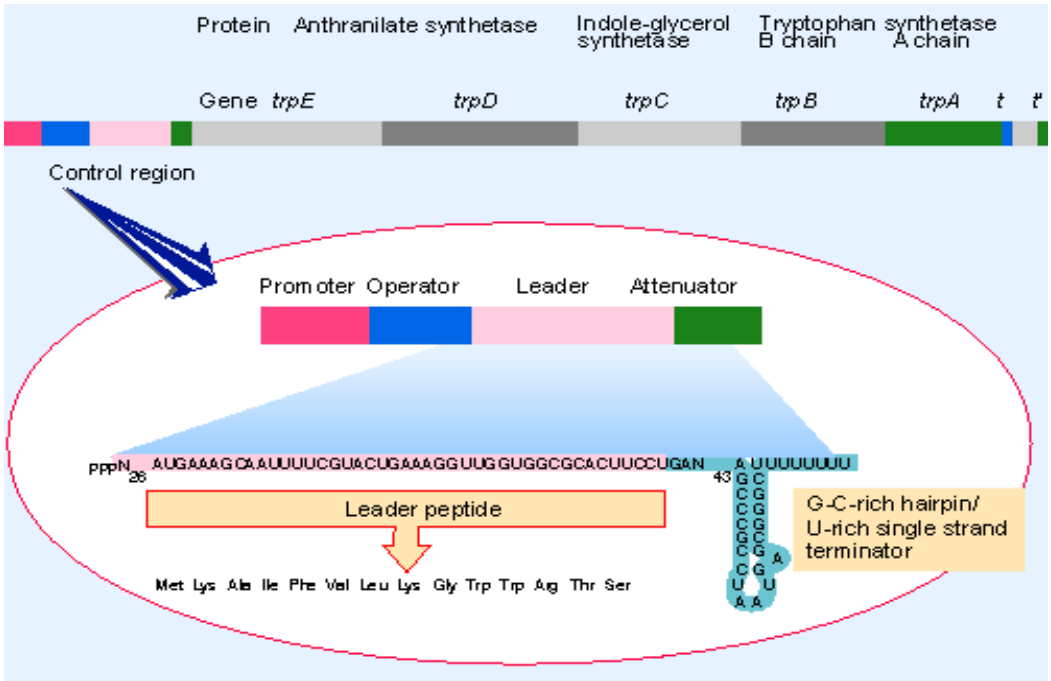


Figure shows that transcription starts at a promoter at the left end of the cluster. *trp* operon expression is controlled by two separate mechanisms. Repression of expression is exercised by a repressor protein (coded by the unlinked gene *trpR*) that binds to an operator that is adjacent to the promoter. Attenuation controls the progress of RNA polymerase into the operon by regulating whether termination occurs at a site preceding the first structural gene.

REGULATION OF TRP OPERON and Attenuation

Two mechanisms regulate expression of the trp operon.

1. Repressor / operator interaction.
2. Transcription termination: Attenuation

EXPRESSION OF TRP OPERON IN THE PRESENCE OF TRYPTOPHAN

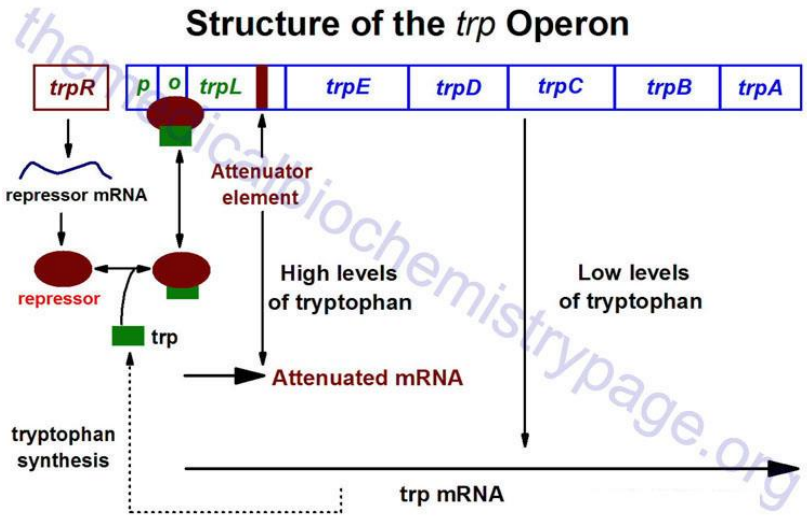
- Regulatory gene for trp operon is trpR located some distance from operon.
- Product of trpR is an aporepressor protein that cannot bind to the operator.
- When tryptophan is abundant in medium it binds to the aporepresser & converts it to an active repressor.
- Active repressor binds to the operator and prevents the initiation of transcription of the trp operon protein – coding genes by RNA polymerase.
- Tryptophan biosynthesis enzymes are not produced.

Turning the operon "on" and "off"

Trp Operator: This stretch of DNA is recognized by a regulatory protein known as the ***trp* repressor**. When the repressor binds to the DNA of the operator, it keeps the operon from being transcribed by physically getting in the way of RNA polymerase, the transcription enzyme.

The *trp* repressor protein is encoded by a gene called *trpR*. This gene is not part of the *trp* operon, and it's located elsewhere on the bacterial chromosome, where it has its own promoter and other regulatory sequences.

The *trp* repressor does not always bind to DNA. Instead, it binds and blocks transcription only when tryptophan is present. When tryptophan is around, it attaches to the repressor molecules and changes their shape so they become active. A small molecule like trytophan, hich switches a repressor into its active state, is called a **corepressor**.



EXPRESSION OF THE TRP OPERON IN THE ABSENCE OF TRYPTOPHAN

- Under severe tryptophan starvation trp genes are expressed maximally and controlled by attenuation.
- This is accomplished by a mechanism that controls the ratio of the transcripts include five structural gene
- 140 bp transcripts at the attenuator site terminate within the trpL region.
- The short transcript terminated by which process called attenuation

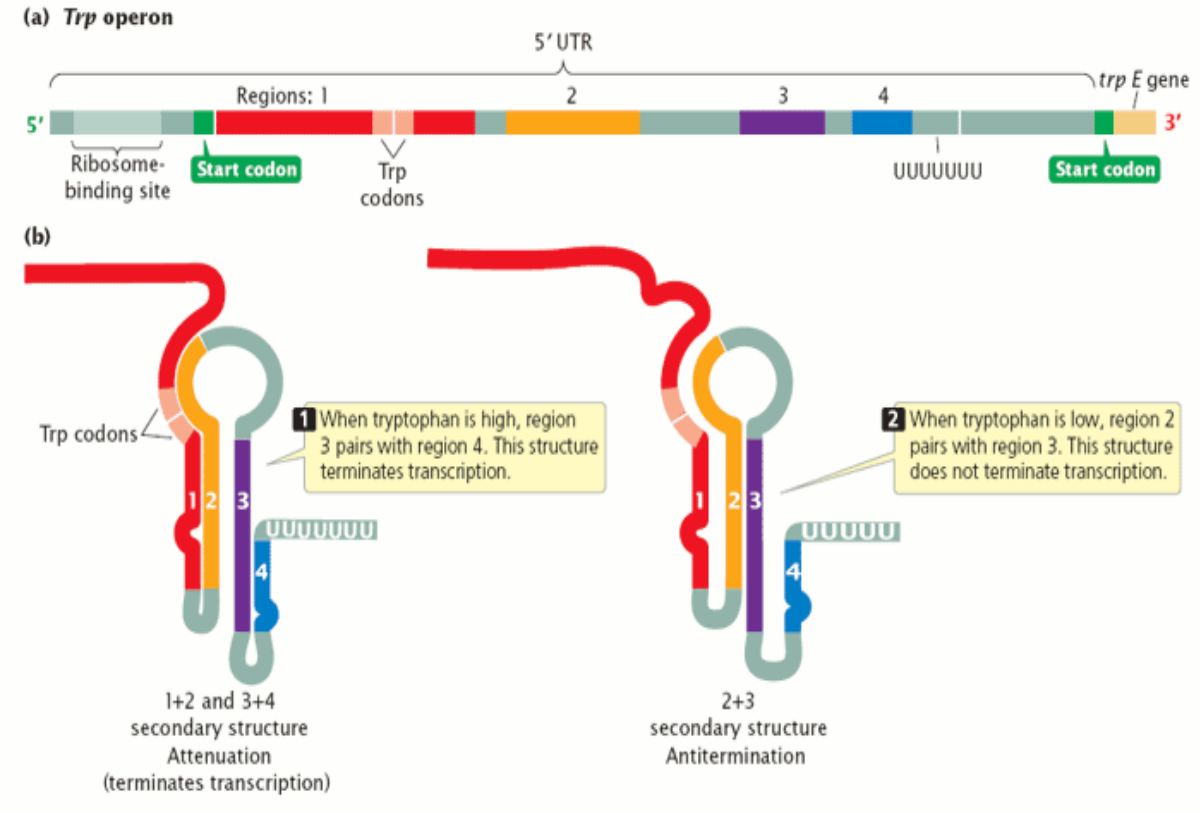
TRYPTOPHAN OPERON: A CLASSIC MODEL FOR ATTENUATION

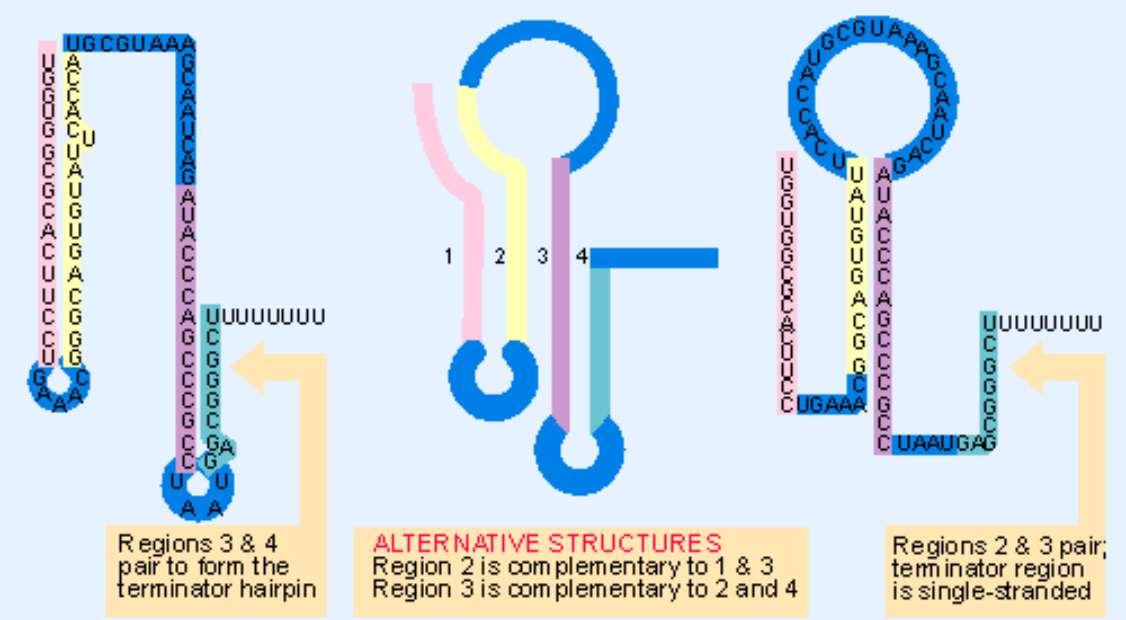
When levels of tryptophan are high, attenuation causes RNA polymerase to stop prematurely when it's transcribing the *trp* operon.

Only a short leader mRNA is made, one that does not encode any tryptophan biosynthesis enzymes. Attenuation works through a mechanism that depends on coupling the translation of the leader mRNA that is still in the process of being transcribed.

- **The mRNA transcript of the leader region includes a sequence that can be translated to produce a short polypeptide.**
- **Just before the stop codon of the transcript, two adjacent codon for tryptophan present that play an important role in attenuation.**
- **There are four regions of the leader peptide mRNA that can fold & form secondary structures by complementary base pairing.**
- **Pairing of region 1 & 2 results in a transcription pause signal, pairing of region 3 & 4 results in a termination of transcriptional signal, and pairing of region 2 & 3 results anti termination signal for transcription to continue.**
- **The position of the ribosome on the leader transcript plays an important role in the regulation of transcription termination at the attenuator.**

Leader mRNA organization for attenuation:





WHEN TRYPTOPHAN ABSENT IN THE MEDIUM

- If the cells are starved for tryptophan the amount of trp- tRNA molecules (charged tryptophenyl tRNA) decrease dramatically because very few tryptophan molecules are available for aminoacylation of the tRNA.
- A ribosome translating on leader transcript stalls at the tandem trp codons in region 1 because next specified amino acid in the peptide is in short supply, the leader peptide cannot be completed.
- Since the ribosome now covers region 1 of the attenuator region, the 1 — 2 pairing cannot happen
- RNA region 2 will pair with RNA region 3, this 2-3 pairing gives antitermination signal, so transcription continues

WHEN TRYPTOPHAN PRESENT IN THE MEDIUM

- When enough tryptophan is present, the ribosome can translate the trp codons then the ribosome continues to the stop codon for the leader peptide.
- Ribosome is then covering part of RNA region 2
- Region 3 is then able to pair with region 4 when it is transcribed.
- The bonding of region 3 with region 4 results termination signal for transcription
- The 3-4 pairing structure is called the attenuator.
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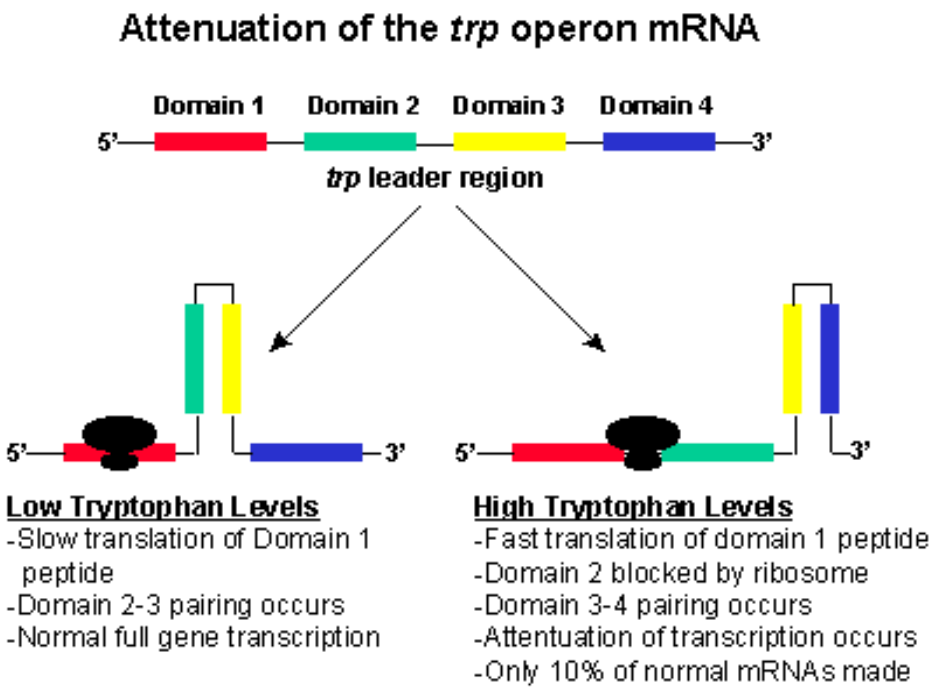
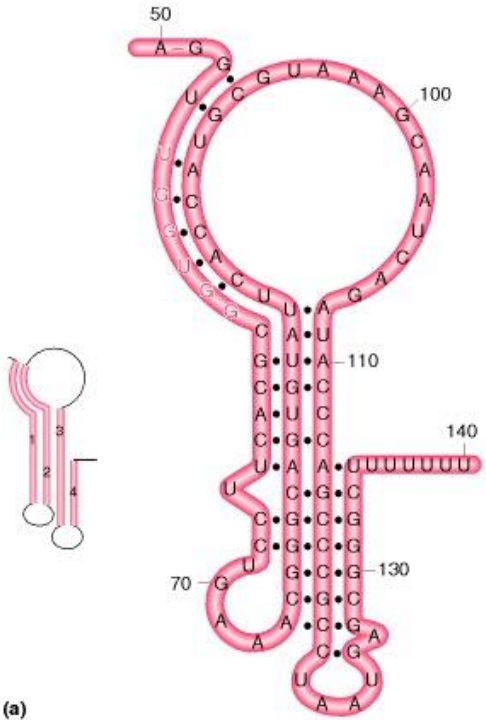
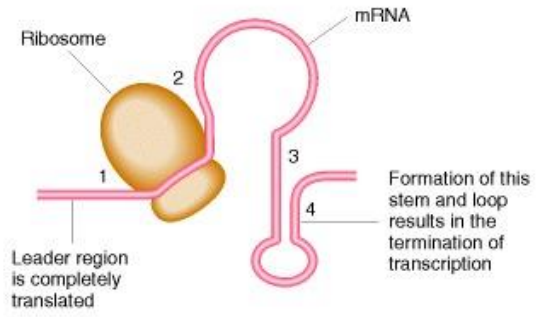


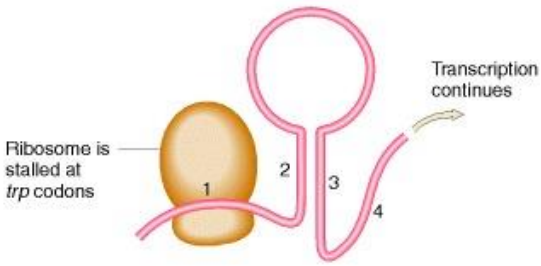
Ribosome Stalling Positions on leader mRNA at High & Low tryptophan levels, respectively and subsequent regulation of premature transcriptional termination of leader before entering into structural genes, known to be attenuation.



(a)



(b) High tryptophan level



(c) Low tryptophan level