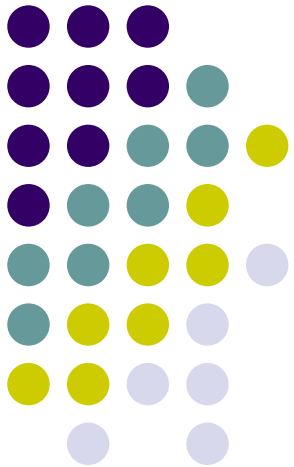
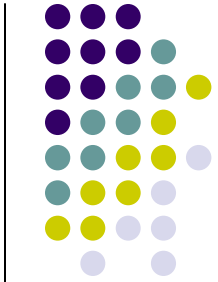
Module 3
Regulation of Gene Expression in Prokaryotes



Recap of last lecture

- Expression of many prokaryotic genes is regulated – either induced (eg: catabolic) or repressed (eg: anabolic)
- Operons are coordinately regulated units of gene expression. May be defined as a unit of expression including structural genes and elements that control their expression; expression of the operon is controlled by other genes whose products interact with these control elements
  - Control region: operator, promoter
  - Polycistronic structural genes
  - Represseor gene (encodes for repressor protein)
  - Effector molecules

Recap of last lecture



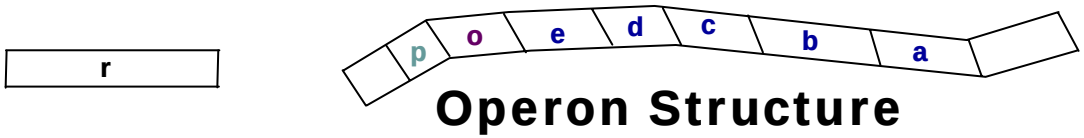
- *Lac* operon encodes for lactose catabolism, and is an inducible catabolic pathway.
- Presence of allolactose changes conformation of the repressor protein, allowing transcription.
- *Lac* is also subject to catabolite repression when glucose concentration is high, via cAMP and CAP interaction.

The *Tryptophan* operon

- The *Tryptophan* (*trp*) operon in *E. coli* controls the enzymes that catalyse the biosynthesis of the amino acid tryptophan-

  5 genes.

  - *trp*E+*trp*D: Anthranilate synthetase
  - *trp*C: Indole glycerolphosphate synthetase
  - *trp*B+*trp*A: Tryptophan synthetase
  - Separate repressor gene (r)



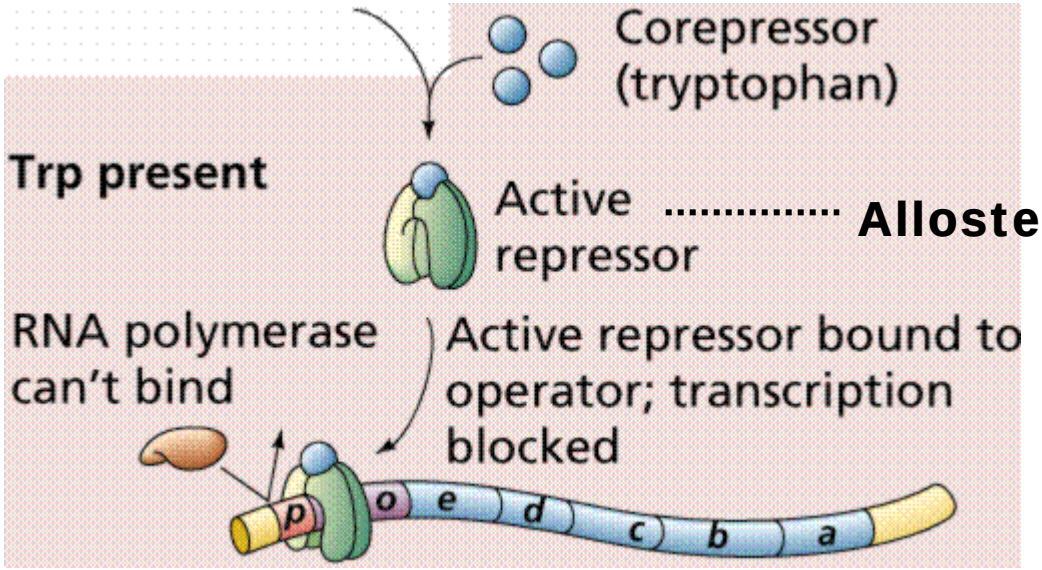
Operon Structure

The *Tryptophan* operon

- *Trp* is regulated by two mechanisms:
  - Repression controls the initiation of transcription
    - Tryptophan present
    - Tryptophan absent
  - Attenuation governs the frequency of early transcript termination.

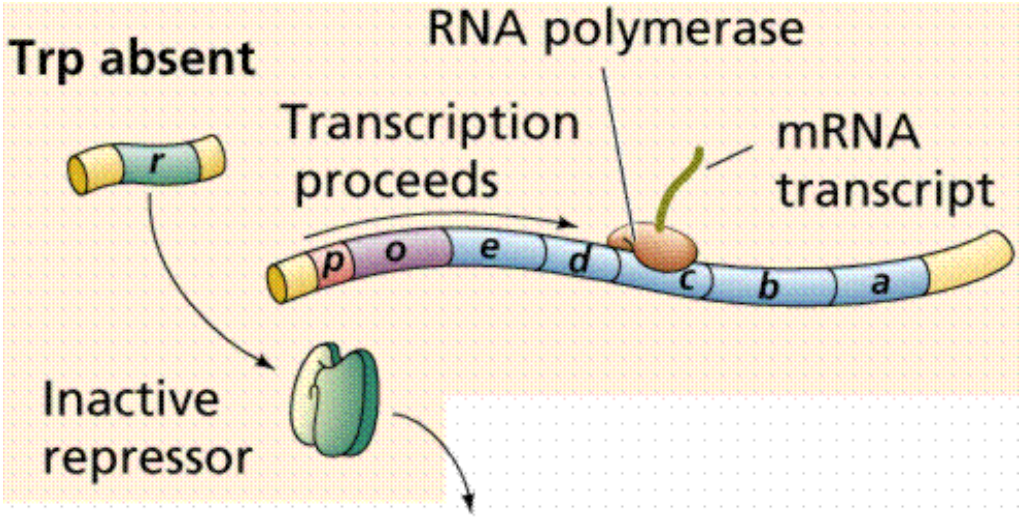
Trytophan present -

- In the presence of tryptophan (corepressor), the repressor binds tryptophan & changes conformation. Repressor / corepressor complex binds to operator DNA, changes conformation, prevents transciption.



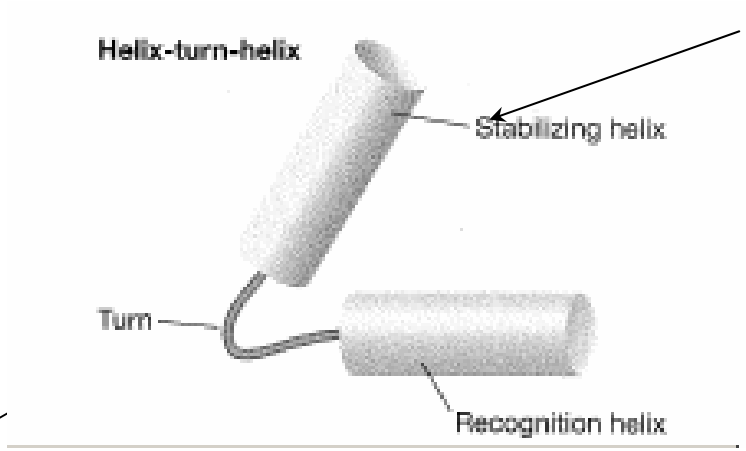
Tryptophan absent -

- In the absence of *trp*, the repressor is inactive, allowing RNA polymerase to bind and transcribe structural genes. Leads to synthesis of tryptophan.



Tryptophan Repressor

- Dimer- a helix turn helix (HTH) protein domain present
- > 250 HTHs known incl. lac & trp repressors
- One tryptophan molecule binds to each monomer of the repressor



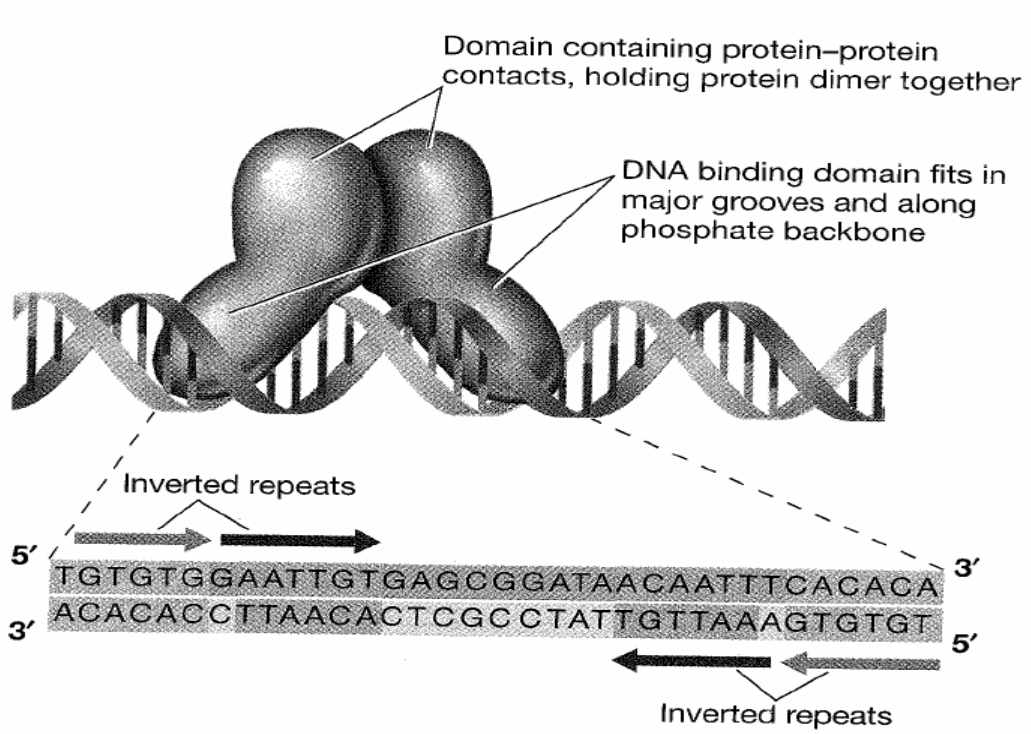
Stabilises the 1st helix by hydrophobic interaction

Interacts specifically with DNA

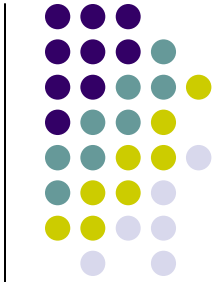
3 aa- 1st aa is typically glycine, assists turning

Tryptophan Repressor

- HTH are homodimeric (2 identical polypep) DNA binding proteins.
- A domain of each polypeptide interacts specifically with A region of the DNA groove (sequence based conformation)- palindromic, inverted repeats

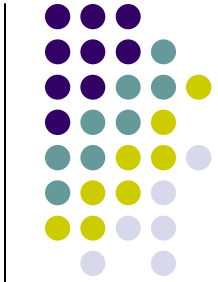


Tryptophan Repressor



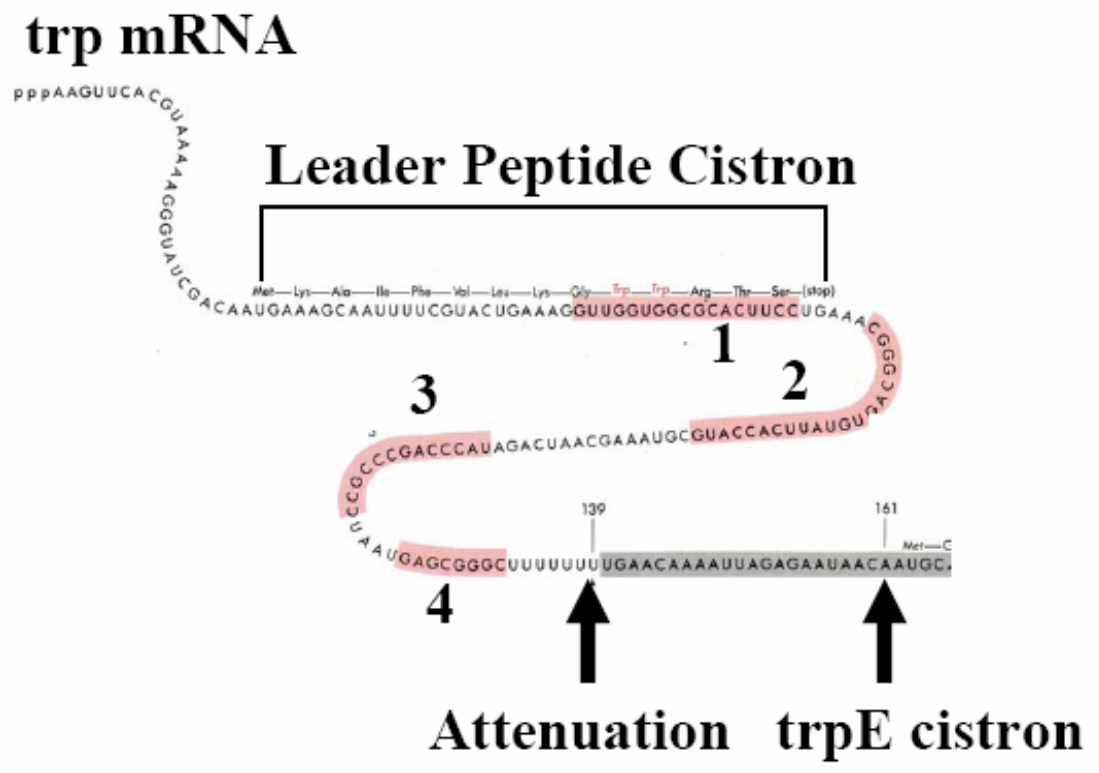
http://www.maxanim.com/genetics/Tryptophan%20Repressor/Tryptophan%20Repressor.htm

Trp operon attenuation



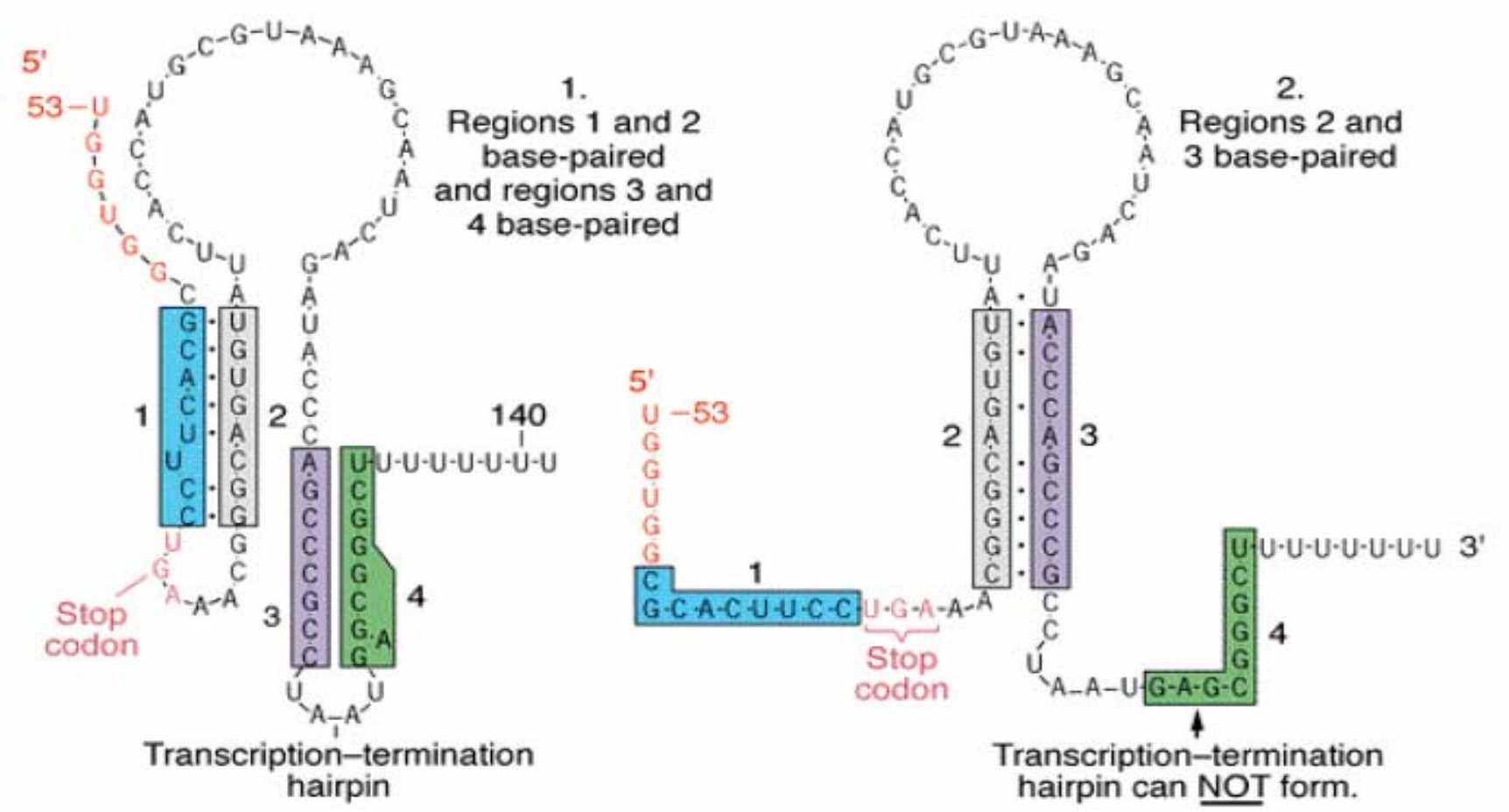
- Transcriptional attenuation is a second, separate control mechanism. Attenuator controlled.
- There is an attenuator region between the operator DNA and structural genes in the *trp* operon.
- Features:
  - RNA from the attenuator region is called the leader transcript.
  - Contains 4 regions than may form stem-loop structures, with three possible pairings: 1-2, 3-4, and 2-3.
  - Stem-loop 3-4 followed by 8 U's: this is a typical ρ-independent transcription termination signal.
  - Encodes a leader peptide – 14 aa's, with two adjacent trp residues.

Trp operon attenuation



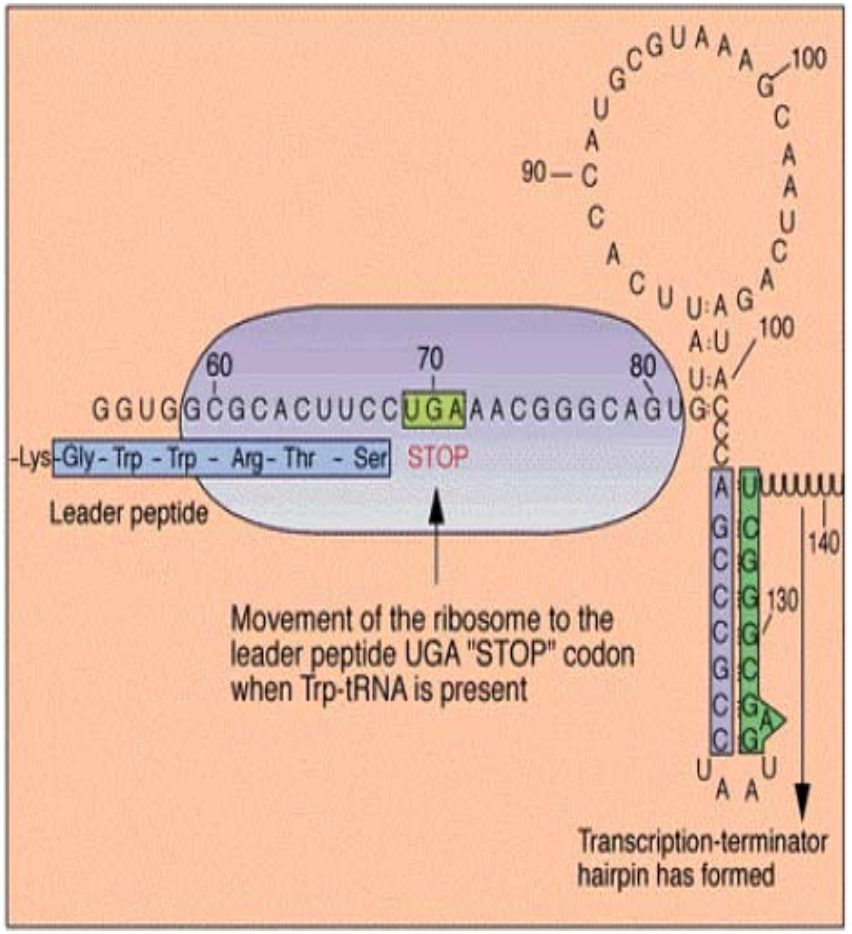
Picture adapted from: Watson et al Molecular Biology of the Gene, Benjamin/Cummings Pub Co

Trp operon attenuation



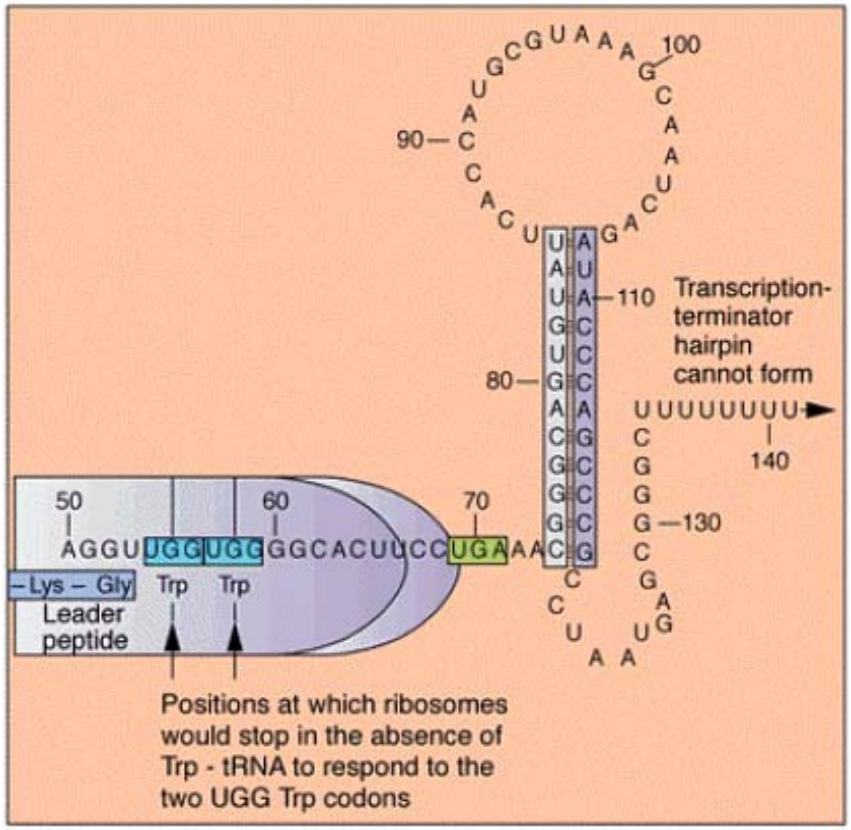
Trp operon attenuation

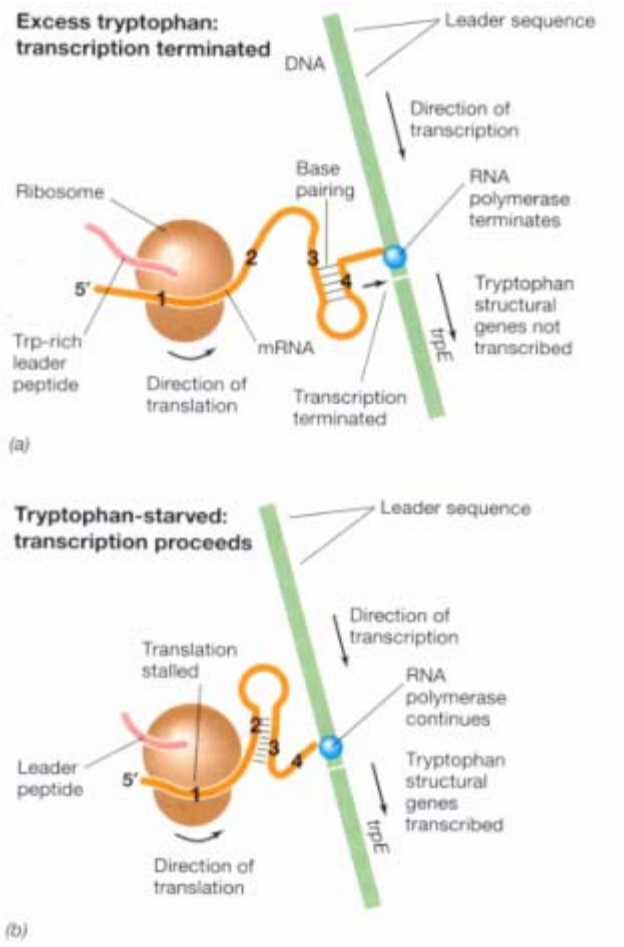
- **The leader sequence allows regulation of the operon via preferential formation of the stem-loop structures.**
- **When tryptophan is high, the leader RNA is <u>transcribed</u>, ribosome binds and <u>translates</u> leader peptide covering regions 1 & 2, and then 3 & 4. Transcription terminates via ρ-independent mechanism due to termination stem-loop formed between 3 & 4 and adjacent U's . Structural genes not transcribed due to early termination (attenuation).**



Trp operon attenuation

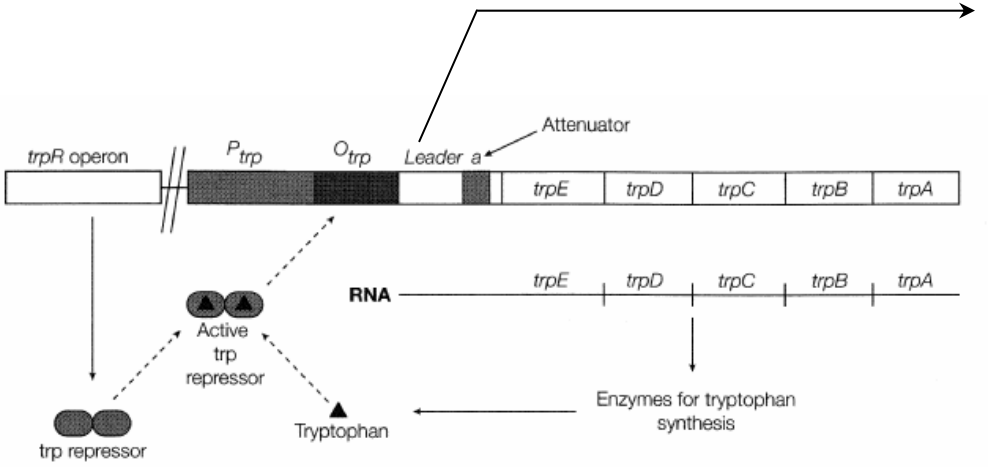
- When tryptophan is low, the leader RNA is transcribed, but the ribosome is stalled at the trp codons in leader peptide due to absence of trp-tRNA. Region 2/3 stem-loop forms, preventing formation of the 3/4 transcription termination stem-loop. This allows expression of the *trp* operon structural genes.



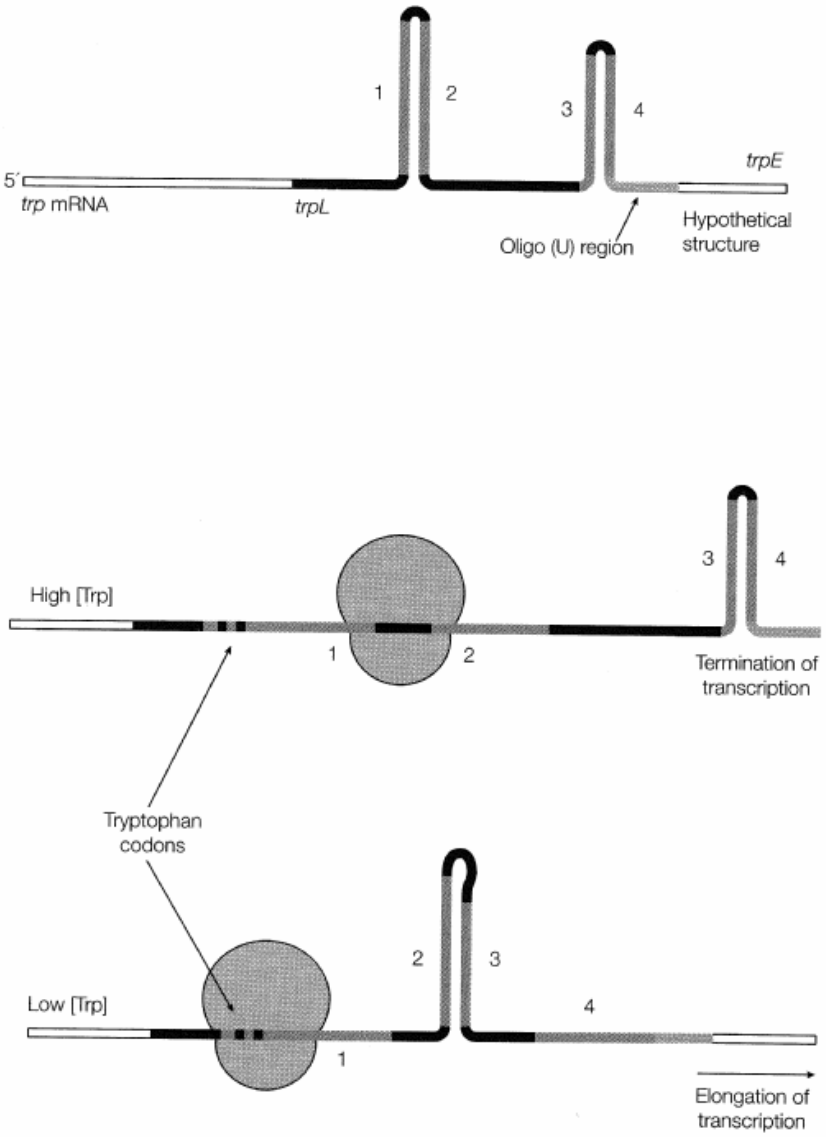


Mechanism of attenuation. Control of transcription of tryptophan operon structural genes by attenuation in *Escherichia coli*. The leader peptide is encoded by regions 1 and 2 of the mRNA. Two regions of the growing mRNA chain are able to form double-stranded loops, shown as 2:3 and 3:4. (a) Under conditions of excess tryptophan, the ribosome translates the complete leader peptide, and so region 2 cannot pair with region 3. Regions 3 and 4 then pair to form a loop that terminates RNA polymerase. (b) If translation is stalled because of tryptophan starvation, loop formation via 2:3 pairing occurs, loop 3:4 does not form, and transcription proceeds past the leader sequence.

Summary of Trp gene regulation



Structure of Trp operon and function of the Trp repressor



Transcriptional attenuation in the Trp operon

Trp operon regulation overview

- Expression of the *trp* operon is regulated by both transcriptional repression and transcriptional attenuation. Other anabolic operons (eg amino acid synthesis) are controlled in a similar way, or by one mechanism alone. For example, the *his* operon is regulated by attenuation alone, and the leader peptide contains seven consecutive histidines (compared to the two tryptophans in the *trp* leader peptide).