The *trp* Operon

(BIOT 4006: Genetics and Molecular Biology)

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Introduction

- Synthesis of the enzymes involved in the biosynthesis of tryptophan is controlled by the trptophan/*trp* operon in *E. coli.*

- Charles Yanofsky and others have studied in detail the working of the five structural genes and the close regulatory elements of the *trp* operon.

- The precursor for biosynthesis of the amino acid tryptophan is “chorismic acid”.

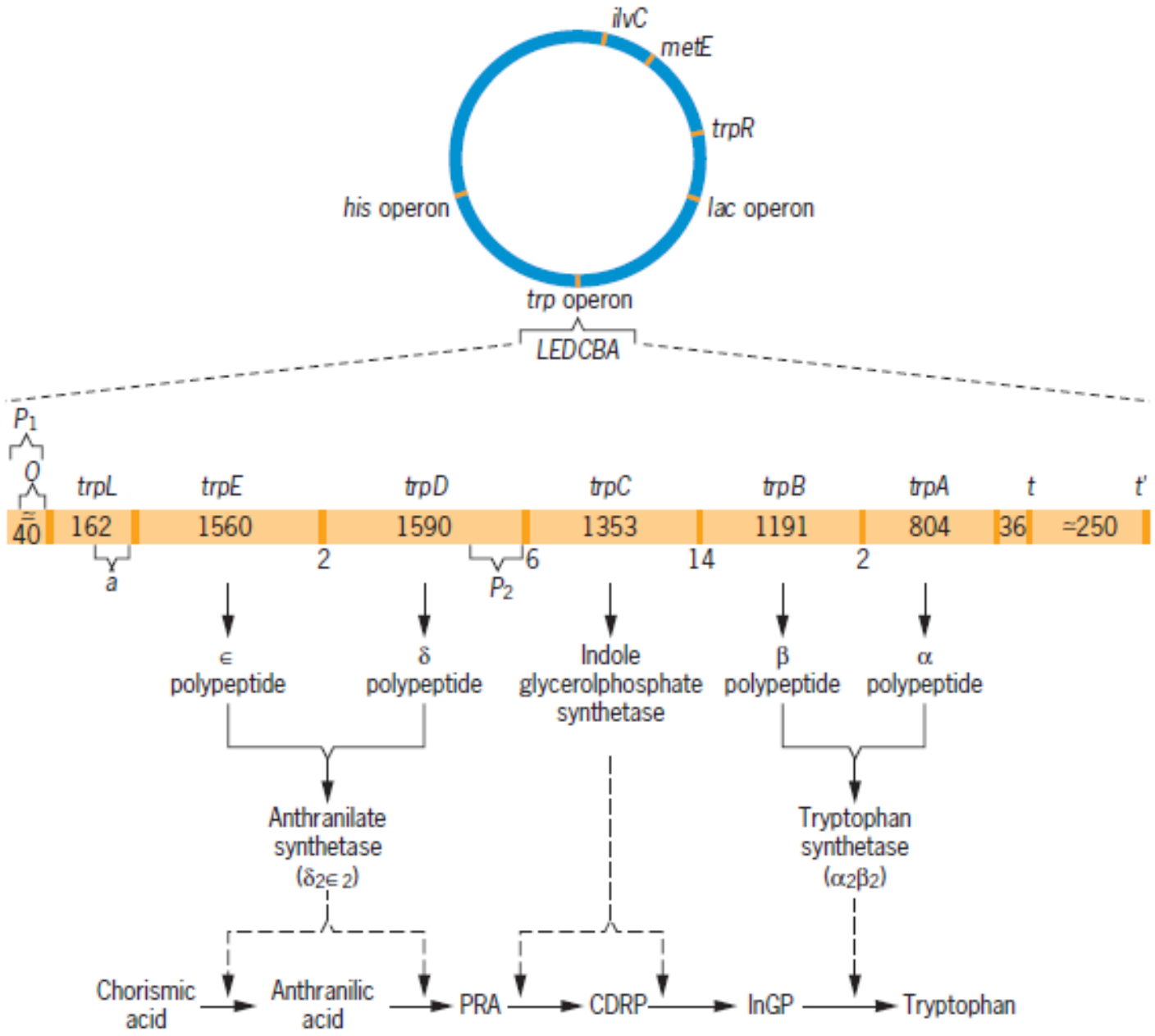
- The five structural genes of the *trp* operon code for enzymes required for conversion of chorismic acid to tryptophan.

- There are two levels of regulation of this operon:

1. Repression: works at the level of transcription initiation
2. Attenuation: works at the level of transcription termination

Repression of the *trp* operon

- The repression regulation works negatively.
- The *trpR* gene coding for repressor of *trp* operon is distantly located from the operon itself.
- P1 is for primary promoter region and it contains the operator (O) region.
- P2 is a weak promoter found at that end of the *trpD* gene which is more far from the operator. Its function is to increase the basal transcription level of the *trpC, trpB* & *trpA* genes.
- There are two termination regions, namely t and t’ placed downstream to *the trpA* gene. The *trpL* denotes a region for a mRNA leader sequence (162 nucleotides in length).



E. Coli trp operon organization

- The biosynthesis of tryptophan is shown at the bottom.
- The five structural genes coding for enzymes needed for tryptophan biosynthesis are: *trpE, trpD, trpC, trpB & trpA.*
- *trpL* is the regulatory segment.
- The gene lengths and the intergenic distances are shown as bp's.
- PRA: phosphoribosyl anthranilate
- CDRP: carboxyphenylamino-deoxyribulose phosphate
- InGP: indole-glycerol phosphate.

Reference: Principles of Genetics, Sixth Edition, D. Peter Snustad & Michael J. Simmons, Chapter 18

- Tryptophan acts as a co-repressor. In its absence RNA polymerase's binding to the promoter initiates the transcription of the structural genes.

- When tryptophan is present, it forms complex with the repressor (co-repressor/repressor complex).

- This complex pairs with the operator sequence and stops RNA polymerase from initiating the transcription.

- The RNA polymerase activity is around 70 times slower in the presence of tryptophan (repressed state) than in the absence of tryptophan (de-repressed state).

- In *trpR* mutants, there is no functional repressor present but due to attenuation, presence of tryptophan can still cause about 10 fold lowering of the transcription rate.

Attenuation of the *trp* operon

- If a portion of the *trpL* region gets deleted then there is an increase in the rate of transcription of the *trp* operon.
- The repressebility of the *trp* operon doesn't get affected by this deletion.
- This implies that apart from the repression/derepression mechanism, there is some other regulation occuring from the *trpL* sequence.
- "Attenuation" is this second regulatory mechanism and is governed by the trpL sequence known as "attenuator".
- Control of transcription termination, occurring at a position near to the end of mRNA leader sequence, is the event that implies attenuation.

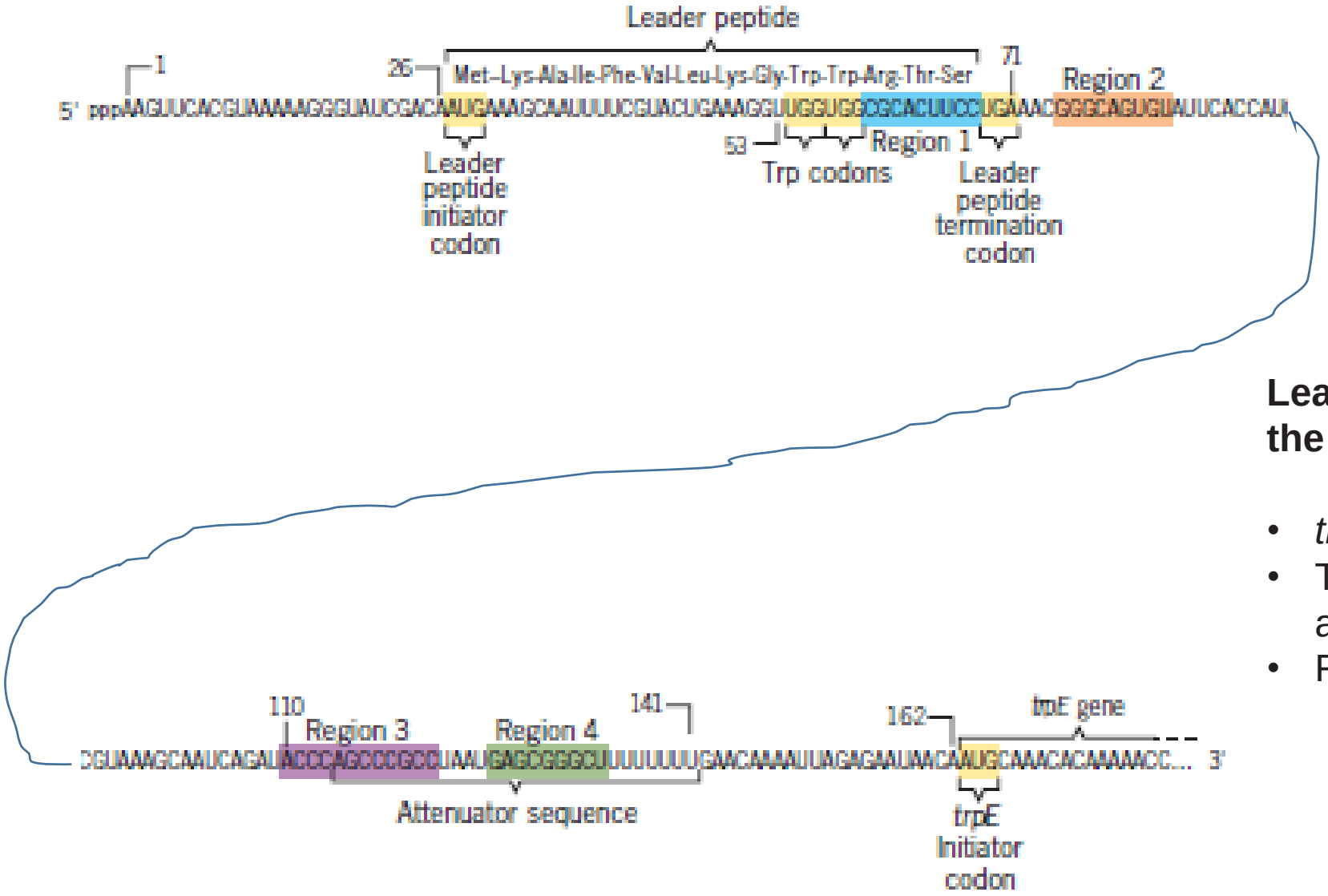
- Presence of tryptophan-charged tRNATrp is a prerequisite for the early termination of transcription of the *trp* operon.

- A truncated trp transcript (140 nucleotides in length) is formed by this attenuation/premature termination.

- There is a nucleotide pair sequence in the attenuator region that is indistinguishable to the termination sequences present at the end of majority of bacterial operons.

- These specific terminator sequences contain several A:T pairs preceded by a G:C rich palallindrome.

- When these terminator sequences are transcribed, a nascent RNA is obtained. It has regions that form a “hairpin” structure by hydrogen bonding. It contains several uracils after the hairpin structure.



Leader region sequence structure in the *trp* mRNA

- *trpL* sequence: encodes leader peptide
- Two tandem *trp* codons: control of attenuation by tryptophan
- Four shaded regions: hairpin structures

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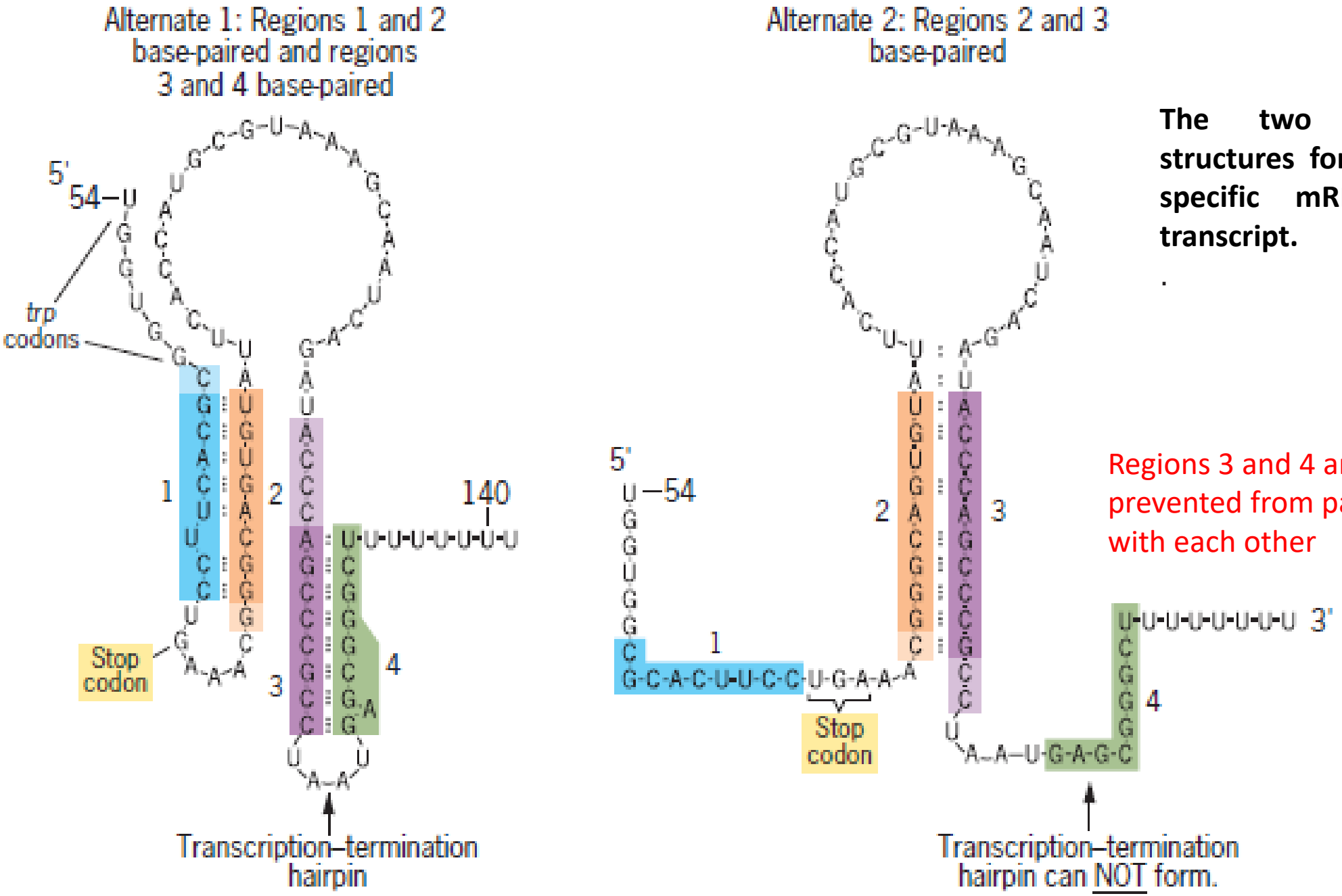

The two different secondary structures formed by the pairing of specific mRNA regions in *trpL* transcript.

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Regions 3 and 4 are prevented from pairing with each other

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