



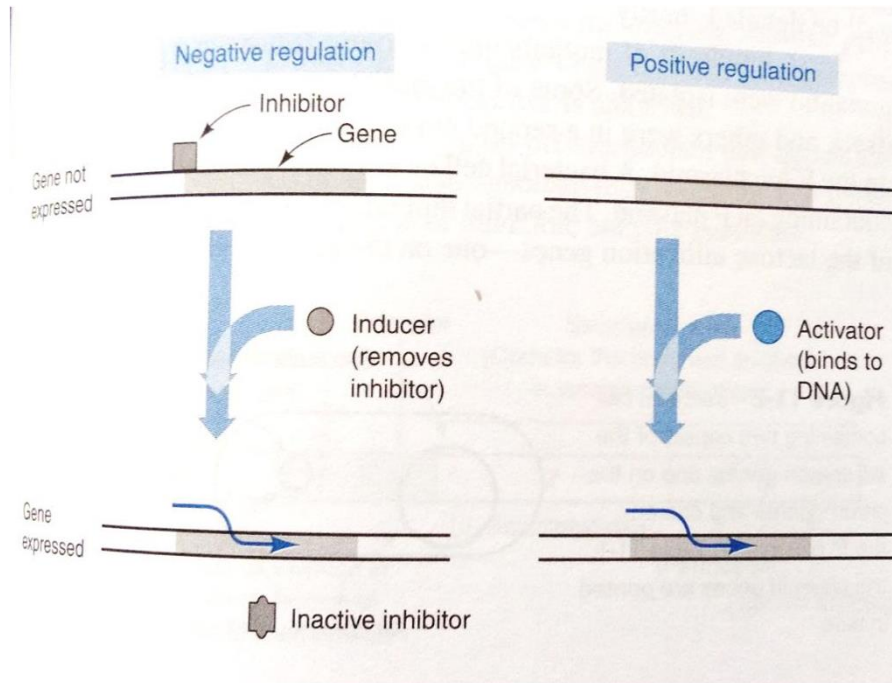
Regulation of gene expression in prokaryotes

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Negative and Positive Regulation of Transcription



Negative regulation: An inhibitor bound to the DNA must be removed before transcription can occur.

Positive Regulation: An activator molecule must bind to the DNA for transcription to occur.

Repressor protein : A specific protein that inhibits the expression of specific gene(s) (found in negatively regulated systems)

Inducer: Is a molecule which is an antagonist of the repressor and is needed to allow transcription in negatively regulated systems.

Activator: A protein which works to increase the frequency of transcription of an operon in positively regulated systems.

Operon

The concept of operon model was first proposed in 1961 by Jacob and Monod.

An operon is a unit of prokaryotic gene expression which included coordinately regulated (structural)genes and control elements which are recognized by regulatory gene products.

- “ **STRUCTURAL genes** code for enzymes involved in a specific biosynthetic pathway whose expression is coordinately controlled.
- “ **CONTROL ELEMENTS**, such as an Operator sequence: Which is a DNA sequence that regulates the transcription of structural genes.
- “ **REGULATORY gene(s)**: Whose products recognize the control elements

Lac Operon (Lactose Operon)

E.coli can use lactose as a source of carbon.

The enzymes required for using the lactose as carbon source are only synthesized when lactose is the sole carbon source

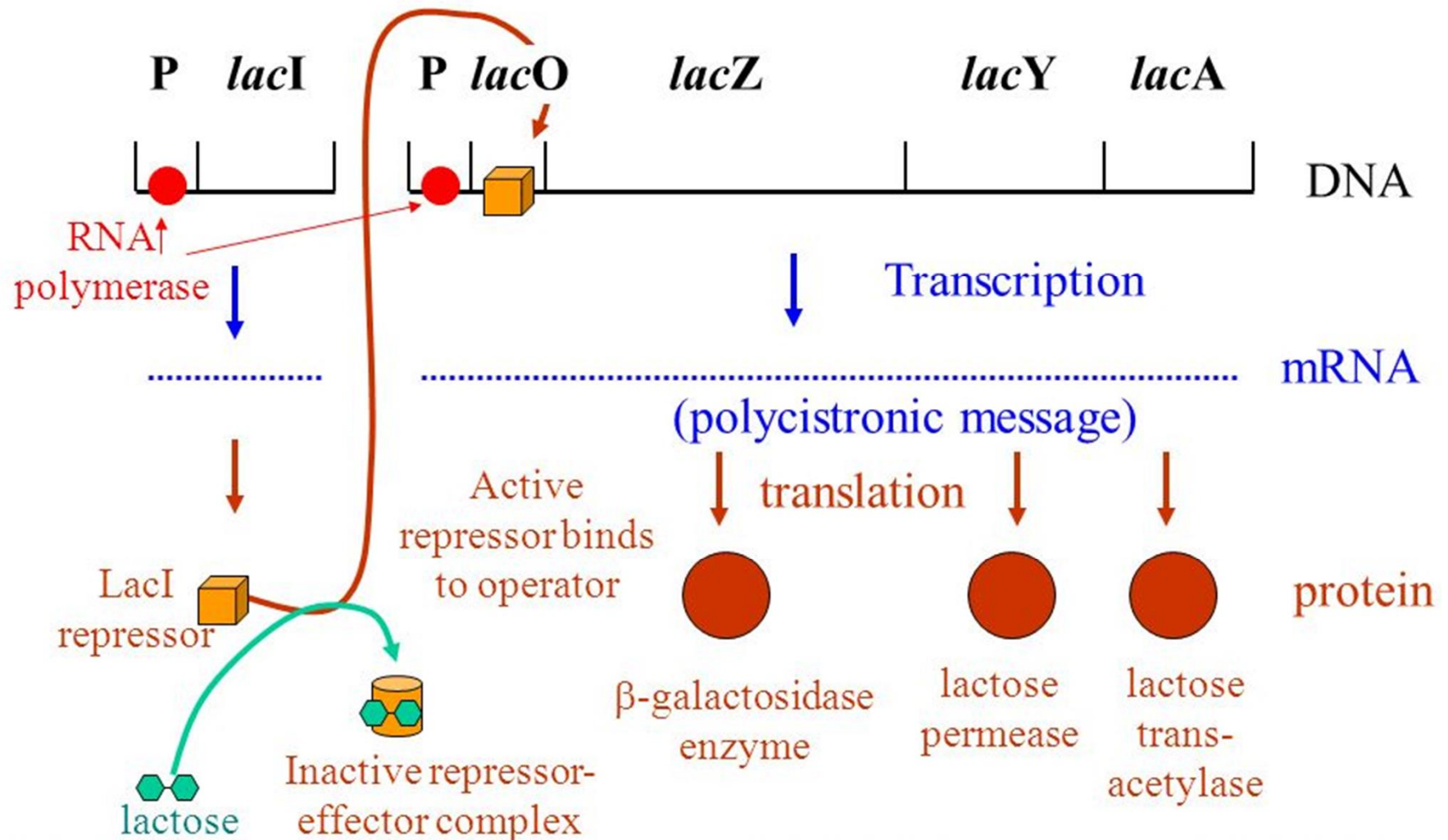
The lac operon consists of 3 structural genes

lacZ: encodes **β -galactosidase** : enzyme responsible for breakdown of lactose into glucose and galactose.

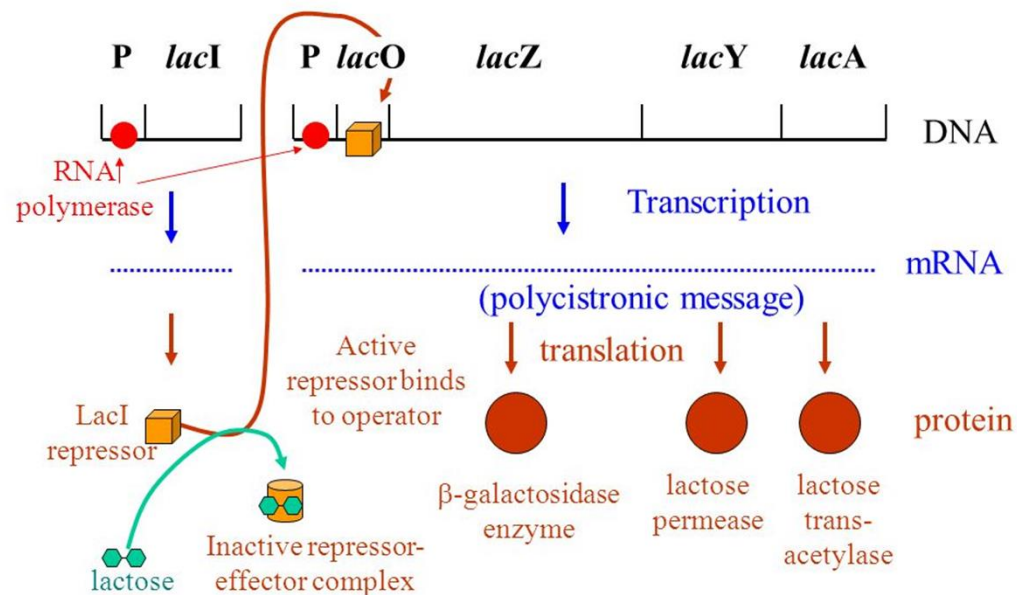
lacY: Galactoside **Permease**: responsible for lactose transport across the bacterial cell wall.

lacA: encodes **tranacetylase**

Lac operon



Lac operon



- “ Three genes are encoded in a single transcription unit **lacZYA**, which has a single promoter **P_{lac}**.
- “ That means the structural proteins are expressed as a polycistronic mRNA
- “ The **lacZYA** transcription unit contains an operator site **O_{lac}**: This site binds a protein called lac repressor, which is a potent inhibitor of transcription when it is bound to the operator.
- “ The lac repressor is encoded by a separate regulator gene **lacI**

- “ **Induction:** In the absence of an inducer, the lac repressor blocks all but a very low level transcription of lacZYA.
- “ When lactose is added to the cells, the low basal level of permease allows its uptake, and β -galactosidase catalyzes the conversion of some lactose to allolactose.
- “ Allolactose acts as an inducer and binds to the lac repressor.
- “ This causes a conformation of the repressor tetramer, reducing its affinity for the lac operator.
- “ The removal of lac repressor from the operator allows the polymerase to rapidly begin transcription of lacZYA genes.

Lac operon

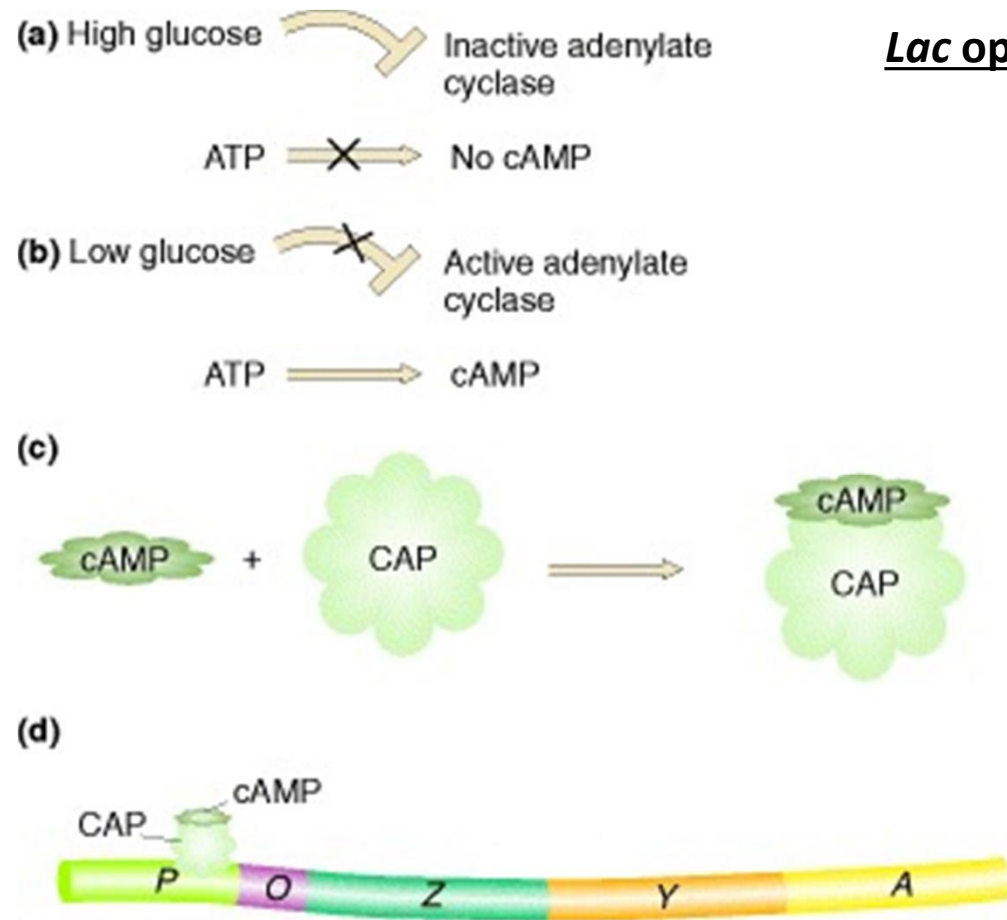
- “ The addition of lactose or a synthetic inducer such as isopropyl- β -D-thiogalactopyranoside (IPTG) very rapidly stimulates the transcription of lactose operon.
- “ The subsequent removal of inducer leads to an almost immediate inhibition of this induced transcription, Since the free lac repressor rapidly occupies the Operator site.

Catabolite repression of the *lac* operon

- “ An additional control system is superimposed on the repressor-operator system. This system exists because cells have specific enzymes that favor glucose uptake and metabolism.
- “ If both lactose *and* glucose are present, synthesis of β -galactosidase is not induced until all the glucose has been utilized. Thus, the cell conserves its metabolic machinery (that, for example, induces the *lac* enzymes) by utilizing any existing glucose before going through the steps of creating new machinery to metabolize the lactose.
- “ Studies indicate that in fact some catabolic breakdown product of glucose (no exact identity is yet known) prevents activation of the *lac* operon by lactose, so this effect was originally called [catabolite repression](#).

Lac operon

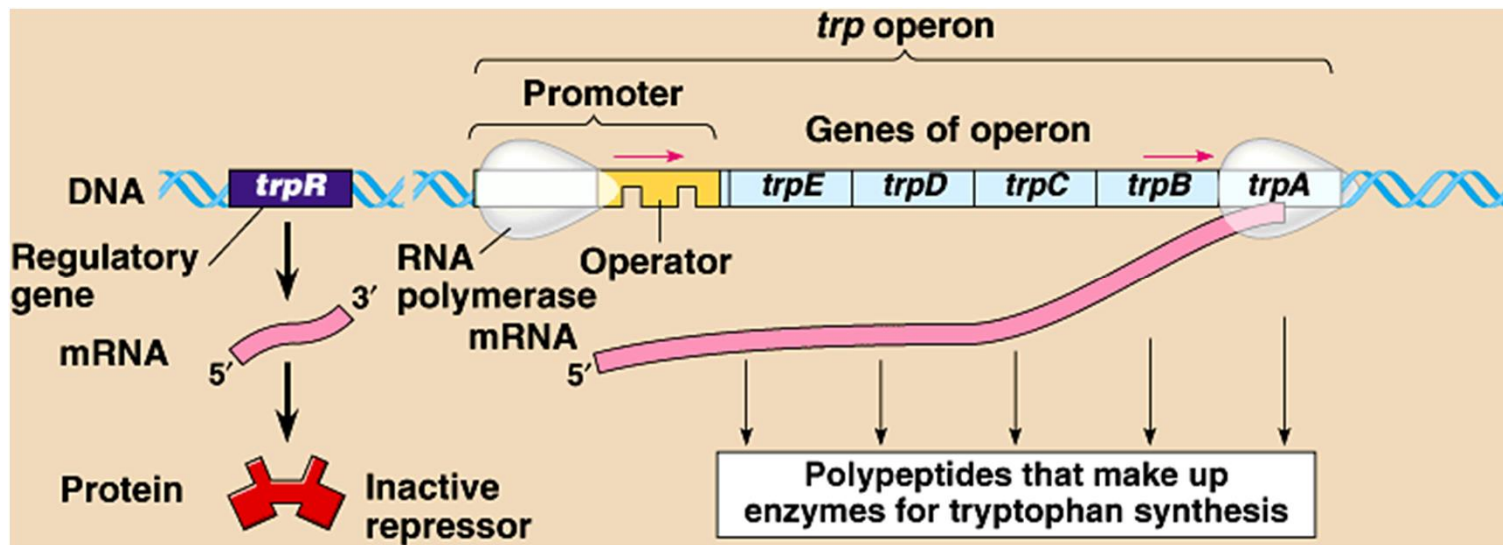
Catabolite repression of the lac operon

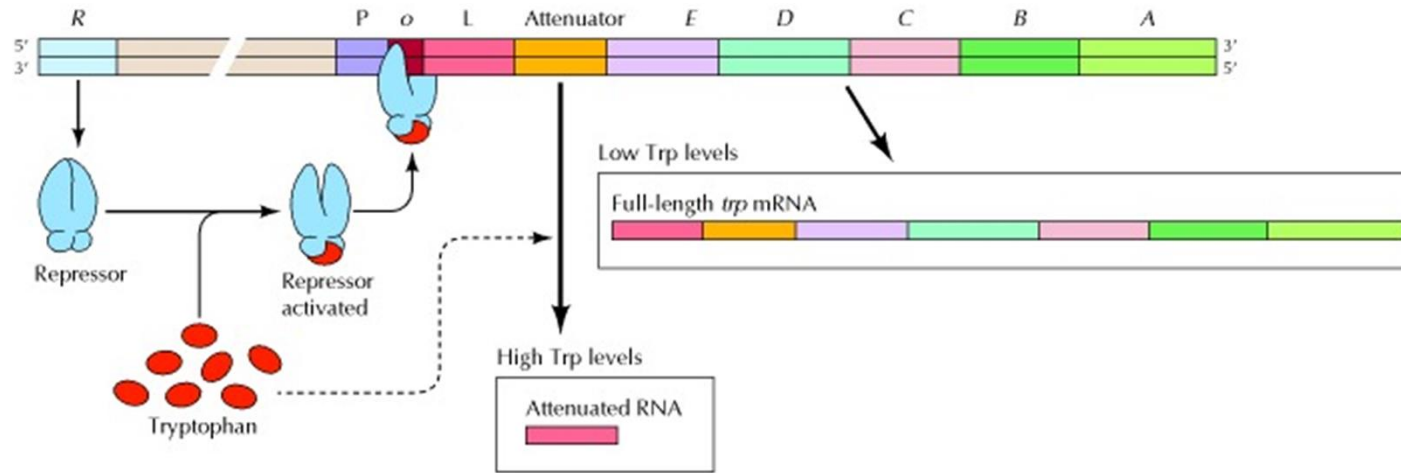


The operon is inducible by lactose to the maximal levels when cAMP and CAP form a complex. (a) Under conditions of high glucose, a glucose breakdown product inhibits the enzyme adenylate cyclase, preventing the conversion of ATP into cAMP. (b) Under conditions of low glucose, there is no breakdown product, and therefore adenylate cyclase is active and cAMP is formed. (c) When cAMP is present, it acts as an allosteric effector, complexing with CAP. (d) The cAMP-CAP complex acts as an activator of *lac* operon transcription by binding to a region within the *lac* promoter. (CAP, [catabolite activator protein](#); cAMP, [cyclic adenosine monophosphate](#).)

trp operon

- “ *trp* operon encodes five structural genes whose activity is required for **tryptophan biosynthesis**.
- “ The operon encodes a single transcription unit which produce a 7kb transcript.





- “ The *trp* repressor: gene product of *trpR* operon , specifically interacts with the operator site of the *trp operon*. *Trp* repressor is a dimer of two subunits.
- “ Only when the tryptophan binds to the repressor, it will bind to the operator of *trp operon*.
- “ Tryptophan, the end product of the enzymes encoded by the *trp* operon, therefore acts as a co-repressor and inhibits its own synthesis through **end-product inhibition**.
- “ The repressor reduces transcription initiation by around 70 fold

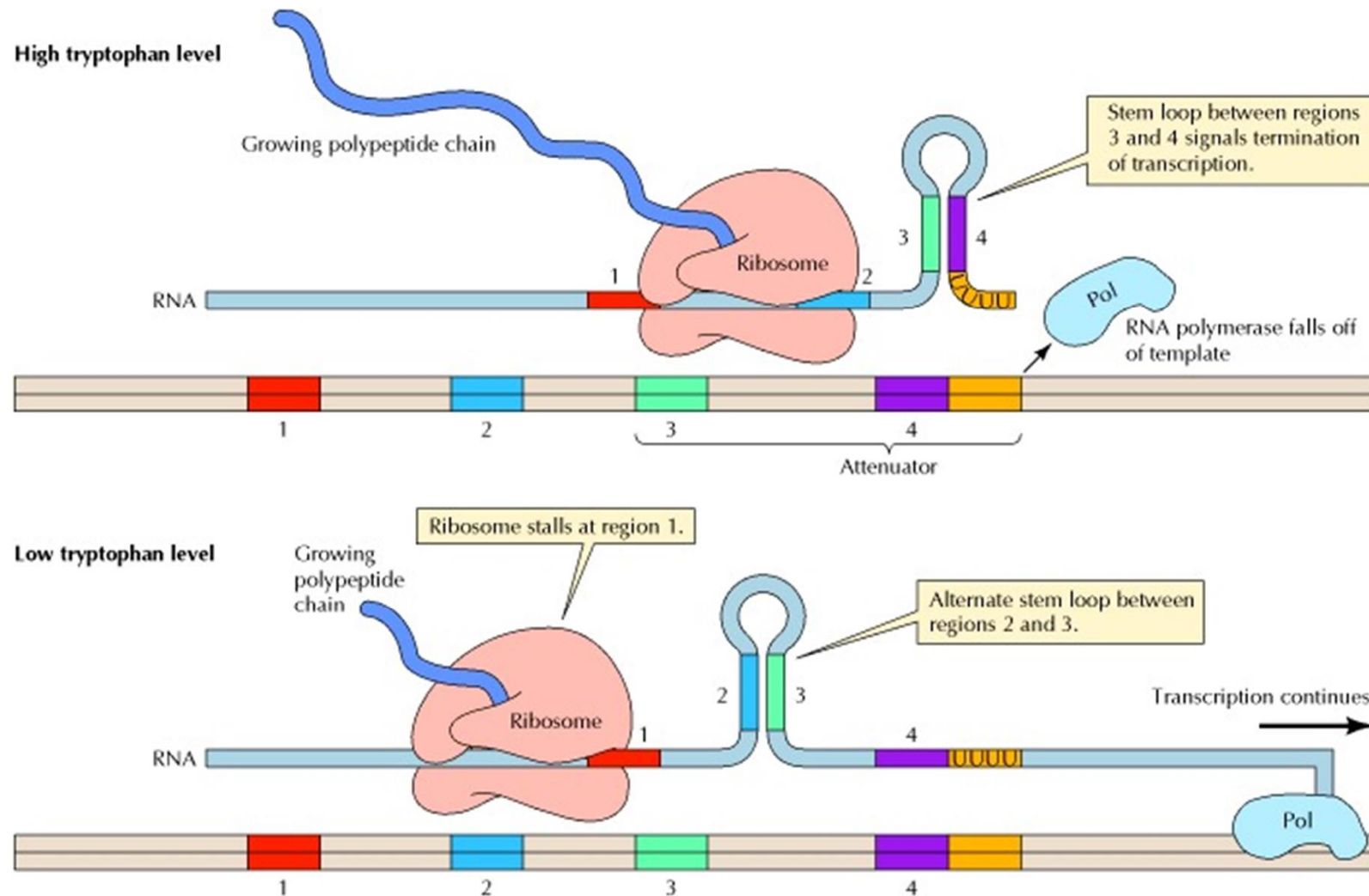
Transcriptional regulation through attenuator

- “ Initially it was thought that the repressor was responsible for all the transcriptional regulation of *trp* operon.
- “ It was later observed that the deletion of a sequence between the operator and *trpE* gene coding region results in increase levels of both basal and activated (de-repressed) transcription.
- “ This site is termed as attenuator.

The attenuator

- “ This site lies at the end of the transcribed leader sequence. The attenuator is a rho-independent terminator site which has a short GC-rich palindrome followed by eight successive U residues.
- “ If this sequence is able to form a hairpin structure, it acts as a highly efficient transcription terminator. Then only a 140bp transcript is synthesized.

Leader RNA structure



- “ The leader sequence contains four regions of complementary sequence which can form different base paired RNA structures
- “ The attenuator is the product of the base pairing of sequences 3 and 4
- “ Sequences 1 and 2 also form a second hairpin.
- “ However sequence 2 is also complementary to sequence 3.
- “ If 2:3 form a hairpin structure then 3:4 attenuator hairpin will not form.
- “ Leader peptide encode 14 amino acids. 11th and 12th codons of this leader RNA encode successive tryptophan residues.
- “ The function of leader peptide is to determine tryptophan availability and to regulate transcription termination.

- “ Tryptophan is a rare amino acid therefore, under the conditions of low tryptophan availability, the ribosome would be expected to pause at the site.
- “ Transcription and translation are highly coupled in E.coli. So translation can occur while mRNA being synthesized.
- “ The availability of tryptophan (the ultimate product of *trp* operon) is sensed through its requirement in translation of leader peptide.
- “ In the presence of high levels of tryptophan, the ribosomes proceed along the message slightly behind the site of transcription. Under these conditions, the mRNA regions designated 3 and 4 hybridize to form a stem-loop structure that signals the termination of transcription. In the presence of low levels of tryptophan, however, the ribosomes stall at region 1 of the mRNA, which contains two adjacent codons for tryptophan. In this case, since region 2 is not bound to a ribosome, it is free to form an alternative stem-loop structure by hybridizing to region 3. This hybridization prevents formation of the 3-4 stem loop, and transcription is able to continue past the attenuator sequence.

References:

1. Essentials of Molecular biology, by Freifelder/Malacinski
2. An introduction to Genetic Analysis, by Griffiths
3. The Cell, by Cooper GM
4. Molecular biology of the Cell, by Bruce Alberts

Online resources for further reading:

Regulation of gene expression in prokaryotes: Negative regulation and positive regulation, Transcriptional activator, transcriptional repressor, *lac* operon and *trp* operon

<https://www.ncbi.nlm.nih.gov/books/NBK26872/>

<https://www.ncbi.nlm.nih.gov/books/NBK9850/>

For any other topics of interest in the subject Molecular biology can be found at the following UGC MOOCS portal.

http://ugcmoocs.inflibnet.ac.in/ugcmoocs/view_module_ug.php/75

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