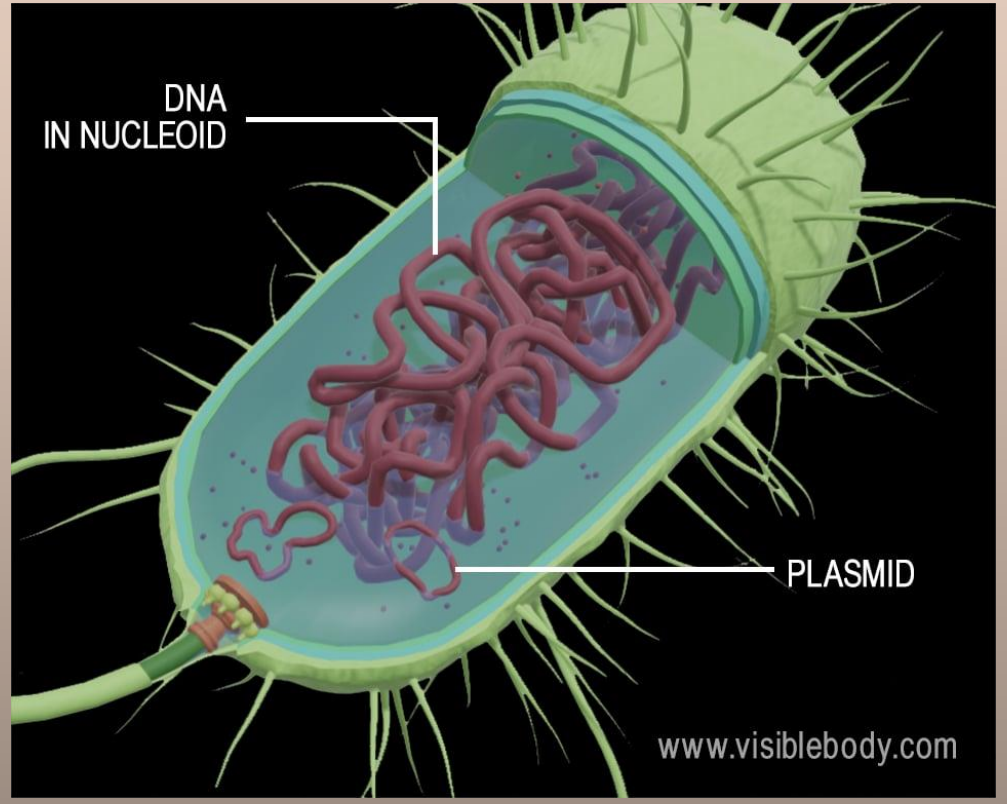
Realization of genetic information. Elements regulating the expression of prokaryotic genes



Lecturer: Senior Lecturer, Department of Molecular Biology and Genetics, PhD, Smekenov I.T.
Subject: Genetic engineering

(Lecture 5)

Lecture Goal:
To understand how genetic information is realized in prokaryotes, focusing on the elements that regulate gene expression and the operon model.

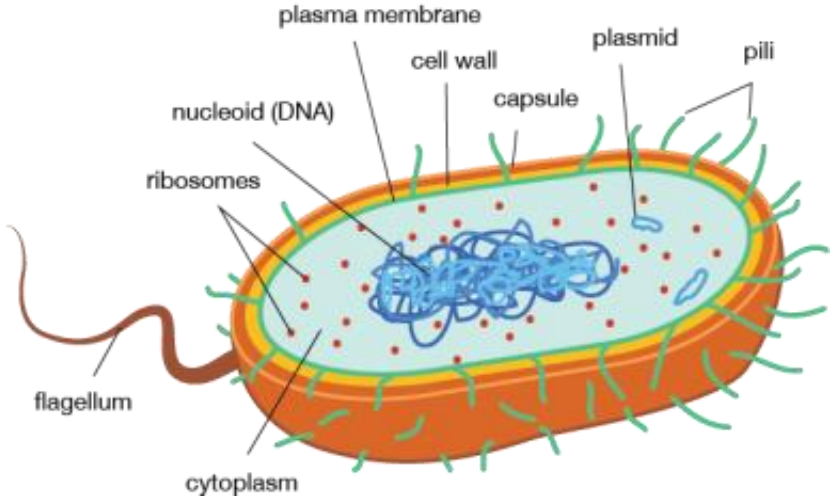
- **Tasks:**

1. Describe the genome of prokaryotes and distinguish between constitutive and inducible enzymes in gene expression.
2. Explain transcriptional regulation in prokaryotes, including the Jacob-Monod operon model, and discuss the value paradox.
3. Compare positive/negative inducible operons with positive/negative repressible operons in regulating gene expression.

Keywords: *Prokaryotic genome, constitutive enzymes, inducible enzymes, transcriptional regulation, Jacob-Monod operon model, operon, value paradox, positive inducible operon, negative inducible operon, positive repressible operon, negative repressible operon*

Genome of prokaryotes

- **Prokaryotes** are unicellular organisms having a single cell, for example— bacteria, blue-green algae and archaea.
- The traditional view has been that an entire prokaryotic genome is contained in a **single circular DNA** molecule. As well as this single 'chromosome', prokaryotes may also have additional genes on independent smaller, circular or linear DNA molecules called **plasmids.**



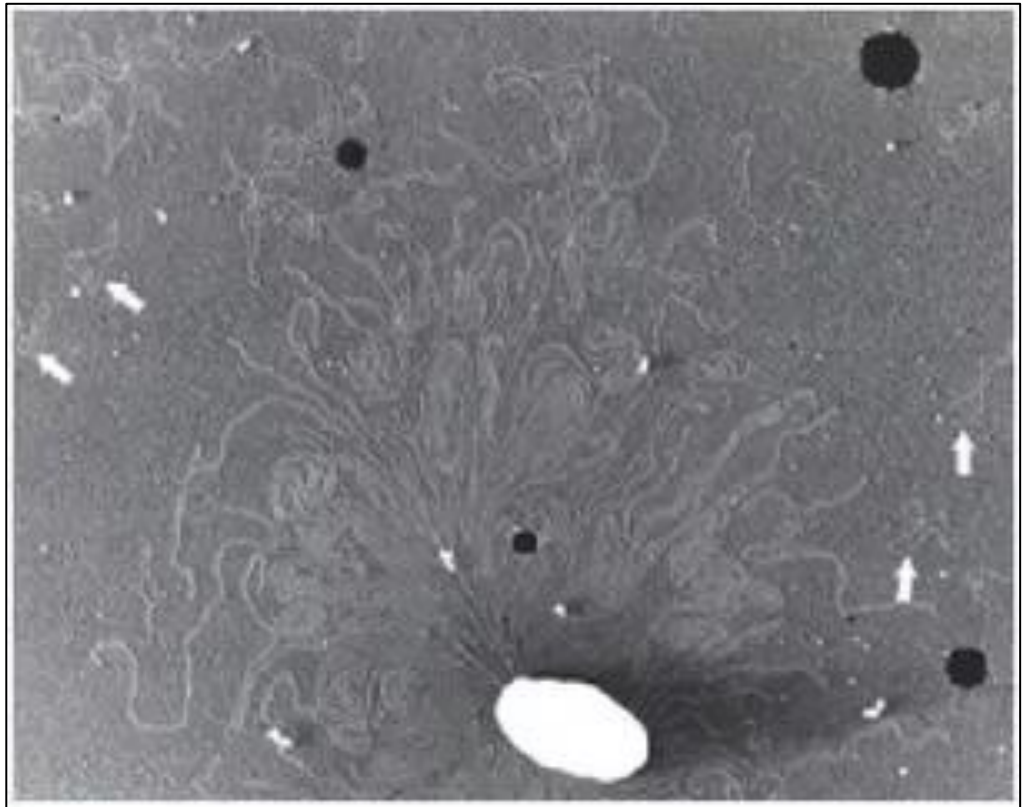
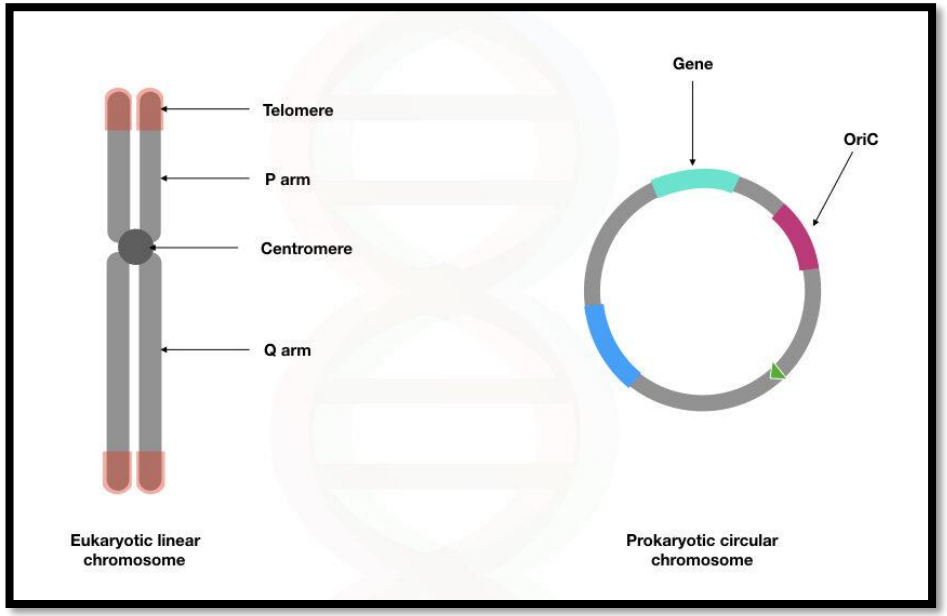


Figure. DNA from a lysed *E. coli* cell. Circular plasmid molecules are indicated by white arrows. The white and black spots are artifacts.

The genomes of prokaryotes and eukaryotes differ significantly in structure, organization, and function. Here's a comparison between the two type of cells:

Feature	Prokaryotes	Eukaryotes
Genome Size	Small (thousands to millions of bp)	Large (millions to billions of bp)
Chromosome Structure	Single, circular	Multiple, linear
Plasmids	Common	Rare
Gene Density	High, few introns	Low, many introns and non-coding DNA
Gene Organization	Operons (clusters of genes)	Single genes with introns
Regulation	Mostly at transcriptional level	Complex, at multiple levels
Introns	Rare	Common
Organellar Genomes	None	Mitochondria, chloroplasts
Horizontal Gene Transfer	Frequent	Rare
Replication Origins	Single	Multiple
Telomeres	Not needed (circular chromosomes)	Required (linear chromosomes)



Prokaryotes possess a single chromosome while eukaryotes possess different numbers of chromosomes.

- Number of Genes and Genome Size in Prokaryotes:

Prokaryote	No of Genes	Genome size
E. coli K12	4400	4.64 MB
M.tuberculosis	4000	4.41 MB
A.fulgidus (Archaea)	2500	2.18 MB

- Number of Genes and Genome Size in Eukaryotes:

Eukaryotes	No of Genes	Genome size
H.sapiens	20 to 25,000	3200 MB
A.thaliana	25,500	125 MB
D.melanogaster	13,600	180 MB

Figure 4.3.14.3.1: Genome size ranges (in base pairs) of various life forms. (CC BY-SA 4.0; Abizar).

Sizes of genomes - The C-value paradox

- The **C-value** is the amount of DNA in the haploid genome of an organism.
- The **C-paradox** is the lack of correlation between the physical size of the genome and the complexity of organisms.

Our current understanding of complex genomes reveals several factors that help explain the classic C-value paradox:

- ✓ Introns in genes
- ✓ Regulatory elements of genes
- ✓ Pseudogenes
- ✓ Multiple copies of genes
- ✓ Intergenic sequences
- ✓ Repetitive DNA

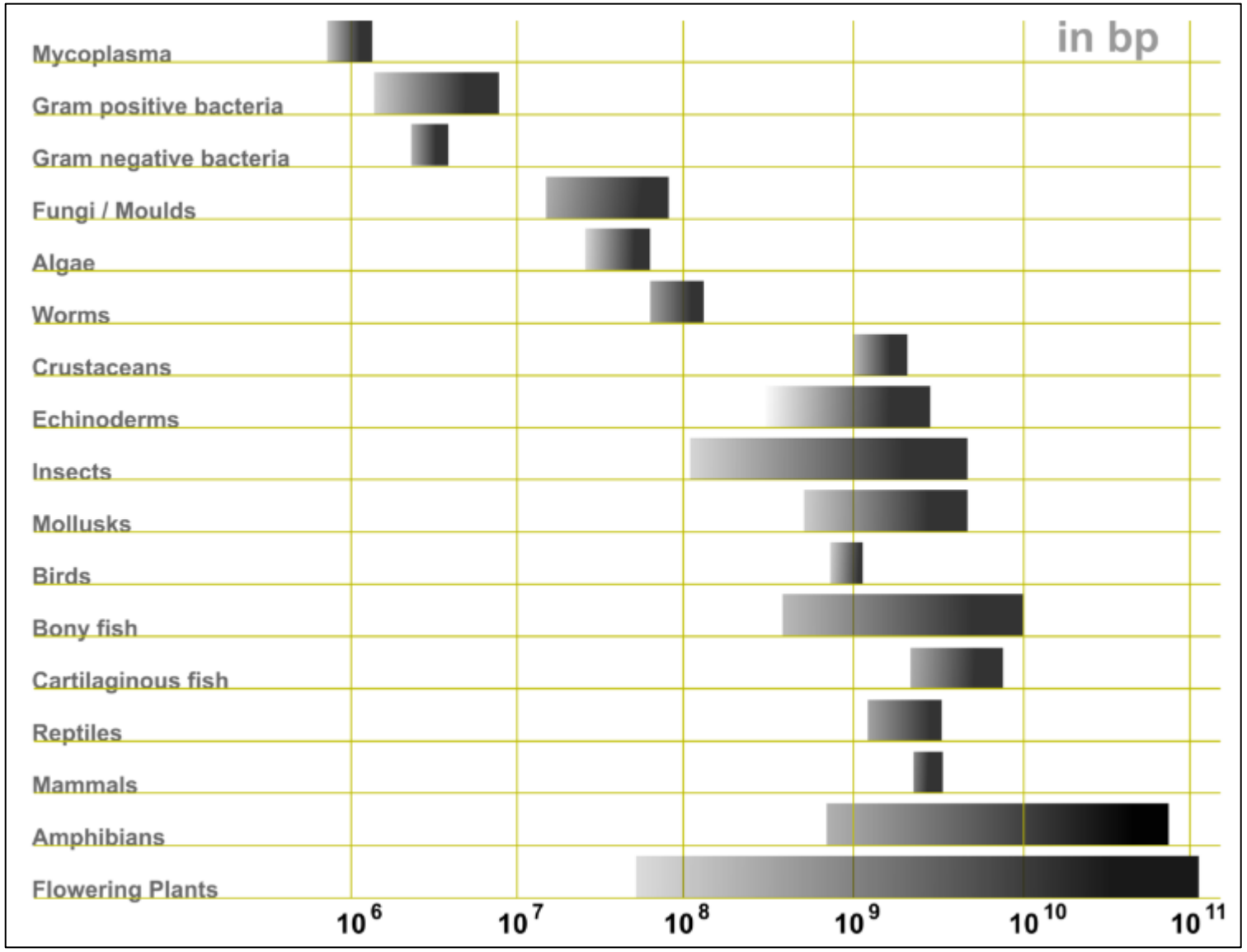


Figure: Genome size ranges (in base pairs) of various life forms.

Prokaryotic Gene Regulation

- ***Gene expression*** refers to the sum of processes that result in a particular level of a specified mRNA and protein in the cell.
- The ***gene expression*** represents the information present in the gene. This is the initial step that produces the mRNAs (protein-coding RNAs) and other functional RNAs, such as rRNA or tRNA. Gene expression is a very basic process and appears in all organisms, which build the macromolecular machinery for an organism.
- ***Gene expression*** is the process by which the information encoded into genomic DNA is used by cells to synthesize a functional gene product through a cascade of steps consisting of transcription, post-transcriptional control, mRNA splicing, translation, and post-translational modifications.

- To understand how **gene expression** is regulated, we must first understand how a gene codes for a functional protein in a cell. The process occurs in both prokaryotic and eukaryotic cells, just in slightly different manners.

- **Prokaryotes** are also characterized by the **conjugation** of *transcription* and *translation processes.*

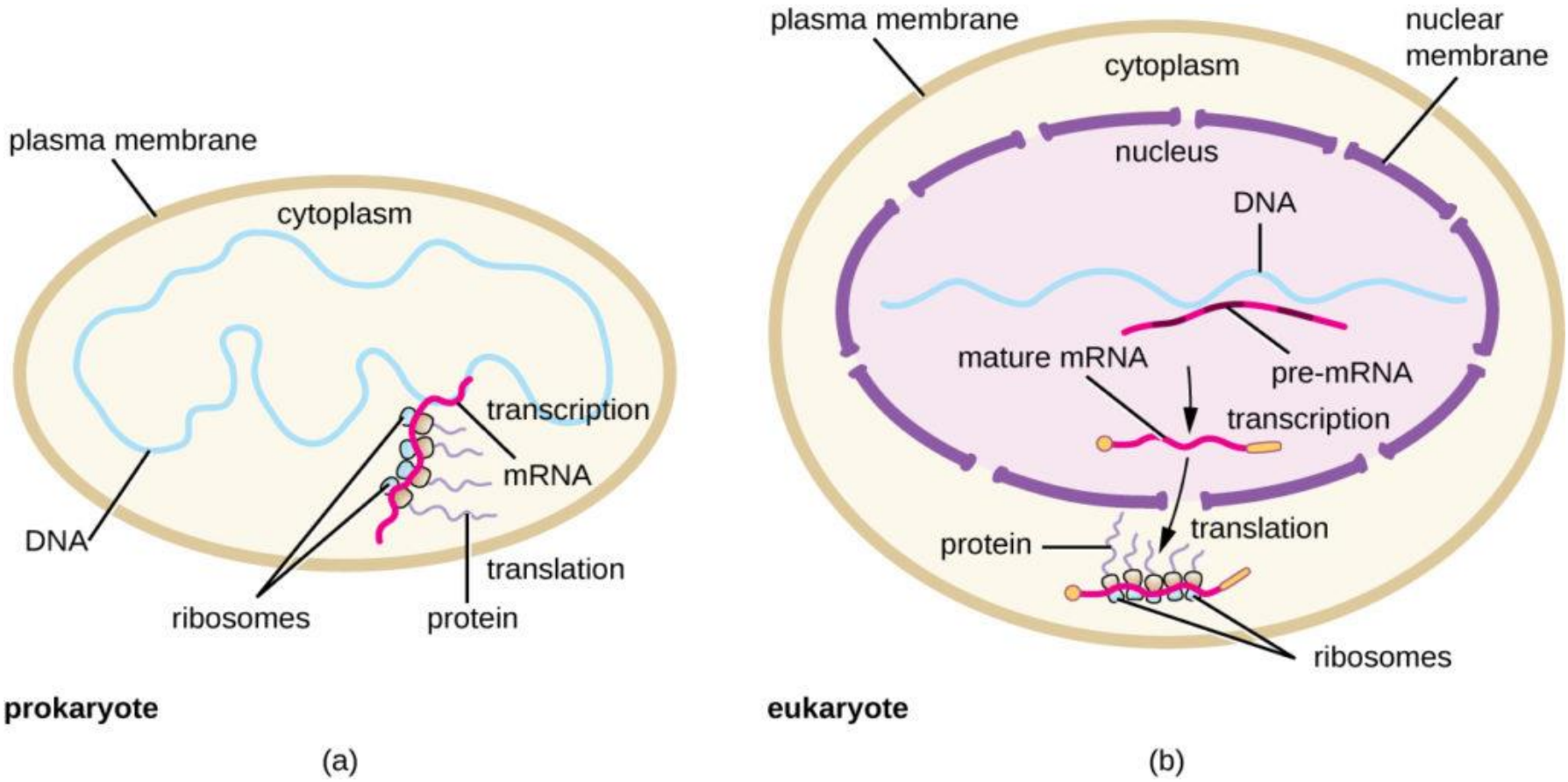
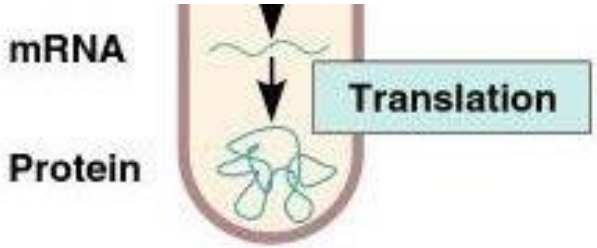
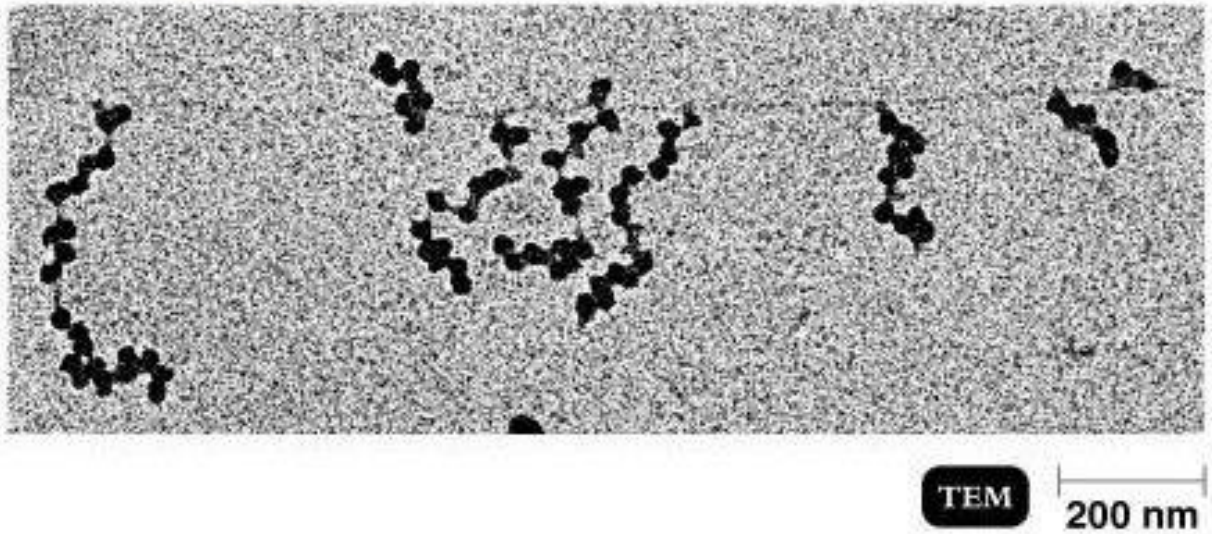
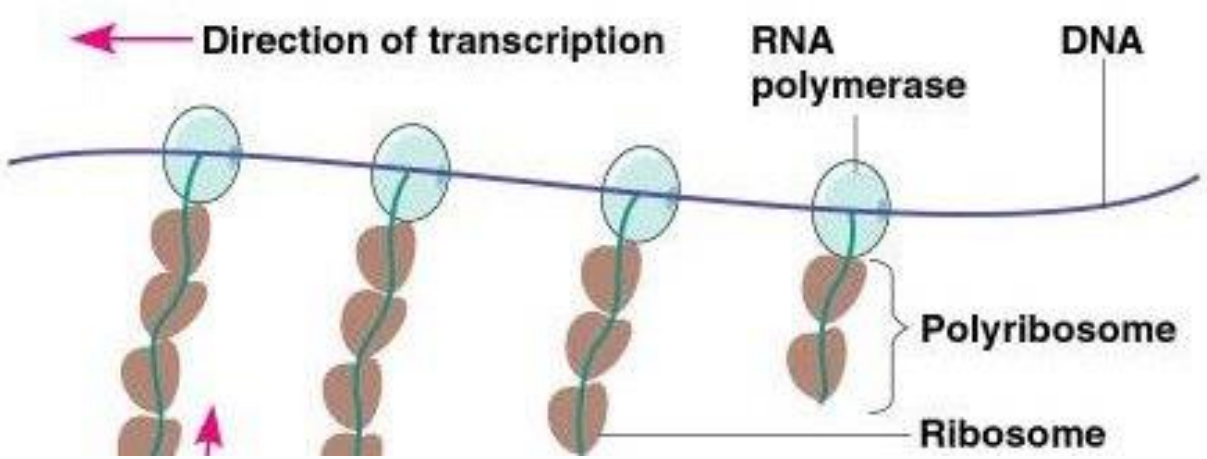


Figure: Prokaryotic transcription and translation occur simultaneously in the cytoplasm, and regulation occurs at the transcriptional level. Eukaryotic gene expression is regulated during transcription and RNA processing, which take place in the nucleus, and during protein translation, which takes place in the cytoplasm. Further regulation may occur through post-translational modifications of proteins.


mRNA
Translation
Protein


TEM
200 nm


Direction of transcription
RNA polymerase
DNA
Polyribosome
Ribosome

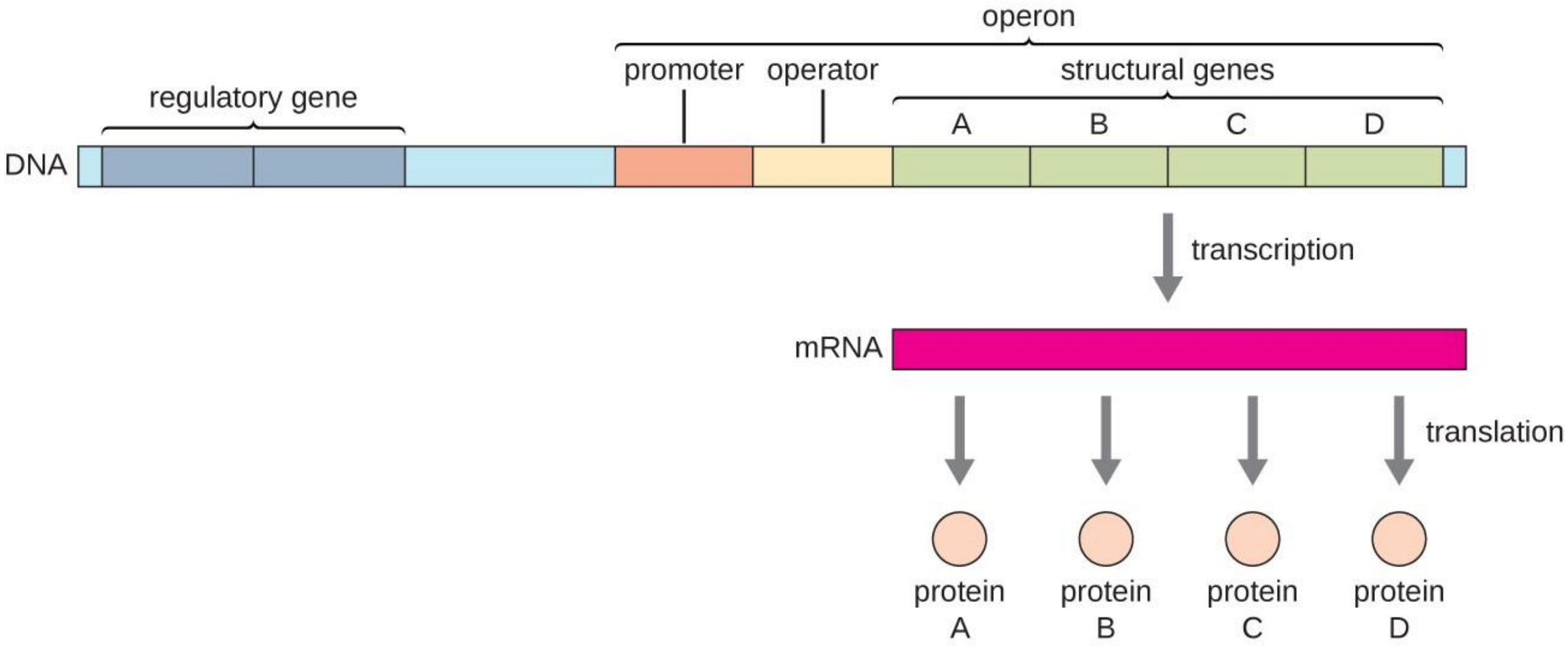
Transcriptional regulation in prokaryotes

- The DNA of prokaryotes is organized into a circular chromosome supercoiled in the nucleoid region of the cell cytoplasm. Proteins that are needed for a specific function, or that are involved in the same biochemical pathway, are encoded together in blocks called **operons**.
- An **operon -** a functional unit of the genome in prokaryotes, which includes cistrons that encode jointly or sequentially working proteins and are united under one (or several) promoters.
- **The concept** of an operon for prokaryotes was proposed in 1961 by the French scientists Jacob and Monod, for which they received the Nobel Prize in 1965.

François Jacob

Jacques Lucien Monod

REGULATORY ELEMENTS OF THE PROKARYOT GENOME



• **Operons** according to the number of **cistrons** are divided into **mono-, oligo- and polycistronic**, containing, respectively, only one, several or many cistrons (genes).

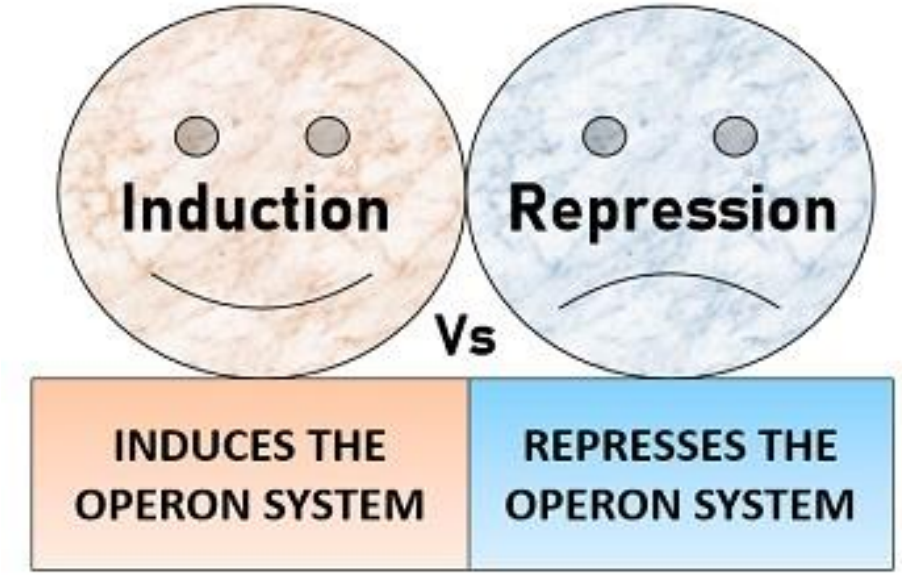
The operon principle of gene organization in prokaryotes

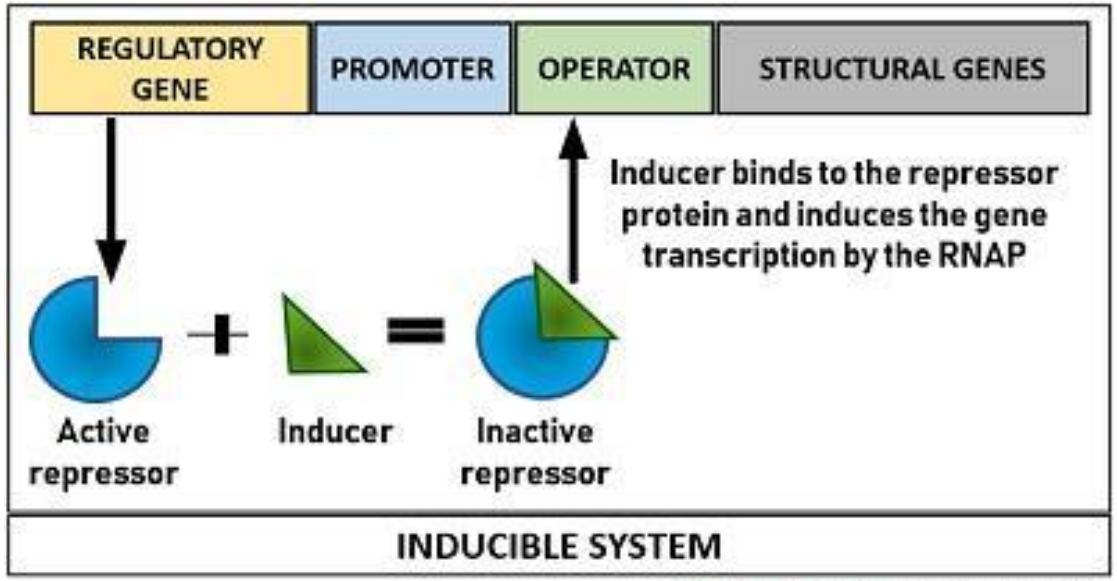
Advantages of the operon organization of prokaryotic genes::

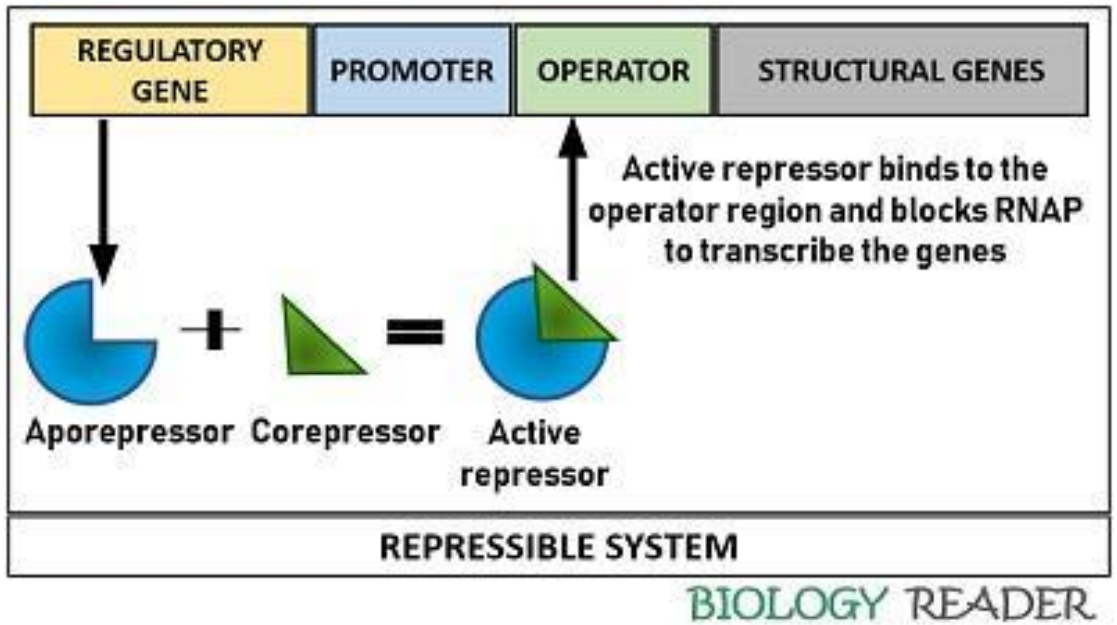
- compactness
- quick response to environmental changes: the synthesis of essential enzymes starts and stops at any moment.
- activity regulation coordination: all genes are expressed or not expressed in unison

Types of regulation of gene activity in prokaryotes

- ***Repression*** and ***induction*** of protein synthesis in prokaryotes implement the principles of adaptation to changing conditions of existence and cellular economy: enzymes appear in cells when there is a need for them, and cease to be produced if the need disappears.



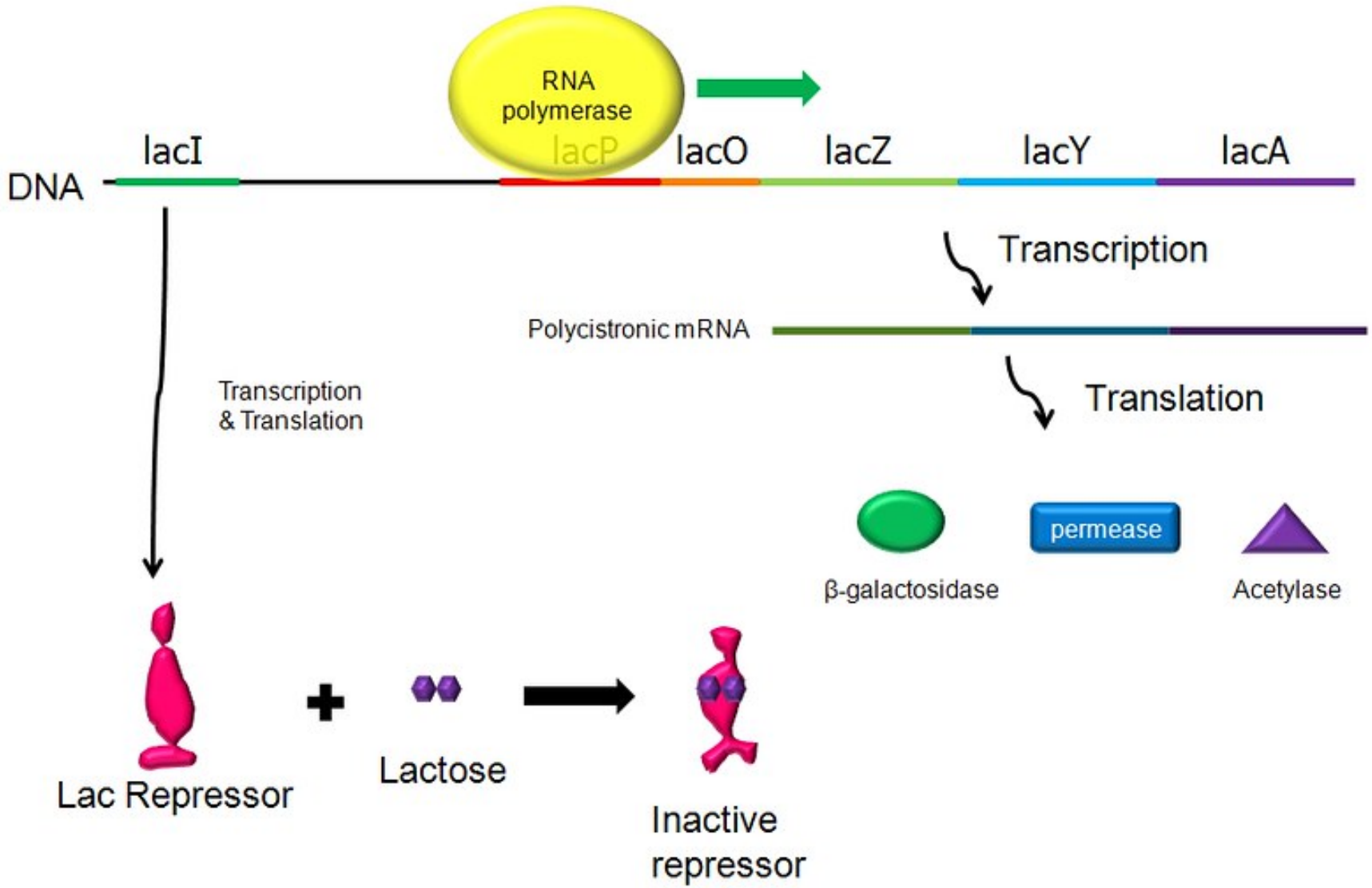

REGULATORY GENE
PROMOTER
OPERATOR
STRUCTURAL GENES
Inducer binds to the repressor protein and induces the gene transcription by the RNAP
Active repressor
Inducer
Inactive repressor
INDUCIBLE SYSTEM
BIOLOGY READER


REGULATORY GENE
PROMOTER
OPERATOR
STRUCTURAL GENES
Active repressor binds to the operator region and blocks RNAP to transcribe the genes
Aporepressor
Corepressor
Active repressor
REPRESSIBLE SYSTEM
BIOLOGY READER

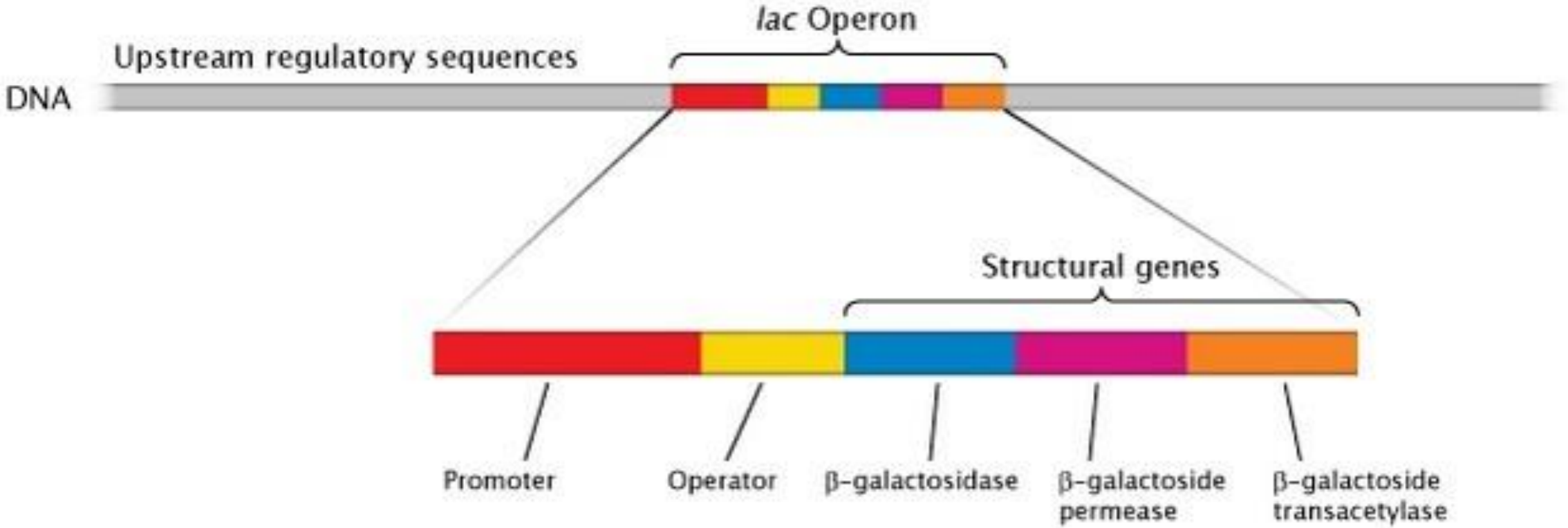
Comparison Chart

Properties	Induction	Repression
Meaning	The meaning of induction system states that it will induce the gene expression via an inducer	The meaning of repression system states that it will suppress the gene expression via a corepressor
Regulation	The operon system regulates the synthesis of enzymes that are stimulated by the addition of inducer	The operon system suppresses the enzyme synthesis, which is facilitated by the existing end-product or corepressor molecules
Key element	Inducer or anti-repressor	Corepressor or effector molecule
Mechanism	Inducer inactivates the repressor protein by preventing the attachment of a repressor to the operator region	Corepressor activates the apo-repressor protein (inactive) and allows the attachment of an active repressor to the operator region
Effect on the control system	Mediates movement of the RNA polymerase along the control system, i.e. promoter and operator region	Blocks the movement of RNA polymerase along the promoter and operator region
Effect on structural genes	It initiates the expression of structural genes	It inhibits the expression of structural genes
Overall impact	An induction system activates or turns on the whole operon system through an adequate supply of the inducer metabolites	A repression system terminates or switches off the entire operon system under an adequate level of corepressor molecules
Operon system	The genetic system regulated by the presence or absence of an inducer is called inducible operon	The genetic system regulated by the presence or absence of a corepressor is called repressible operon
Enzyme	Enzymes synthesis stimulated by the addition of inducer metabolites are termed as inducible enzymes	Enzymes synthesis inhibited by the addition of corepressor molecules are termed as repressible enzymes
Example	Lactose operon	Tryptophan operon
Metabolic pathway	It operates a catabolic synthesis	It operates an anabolic synthesis

Structure of the lactose operon

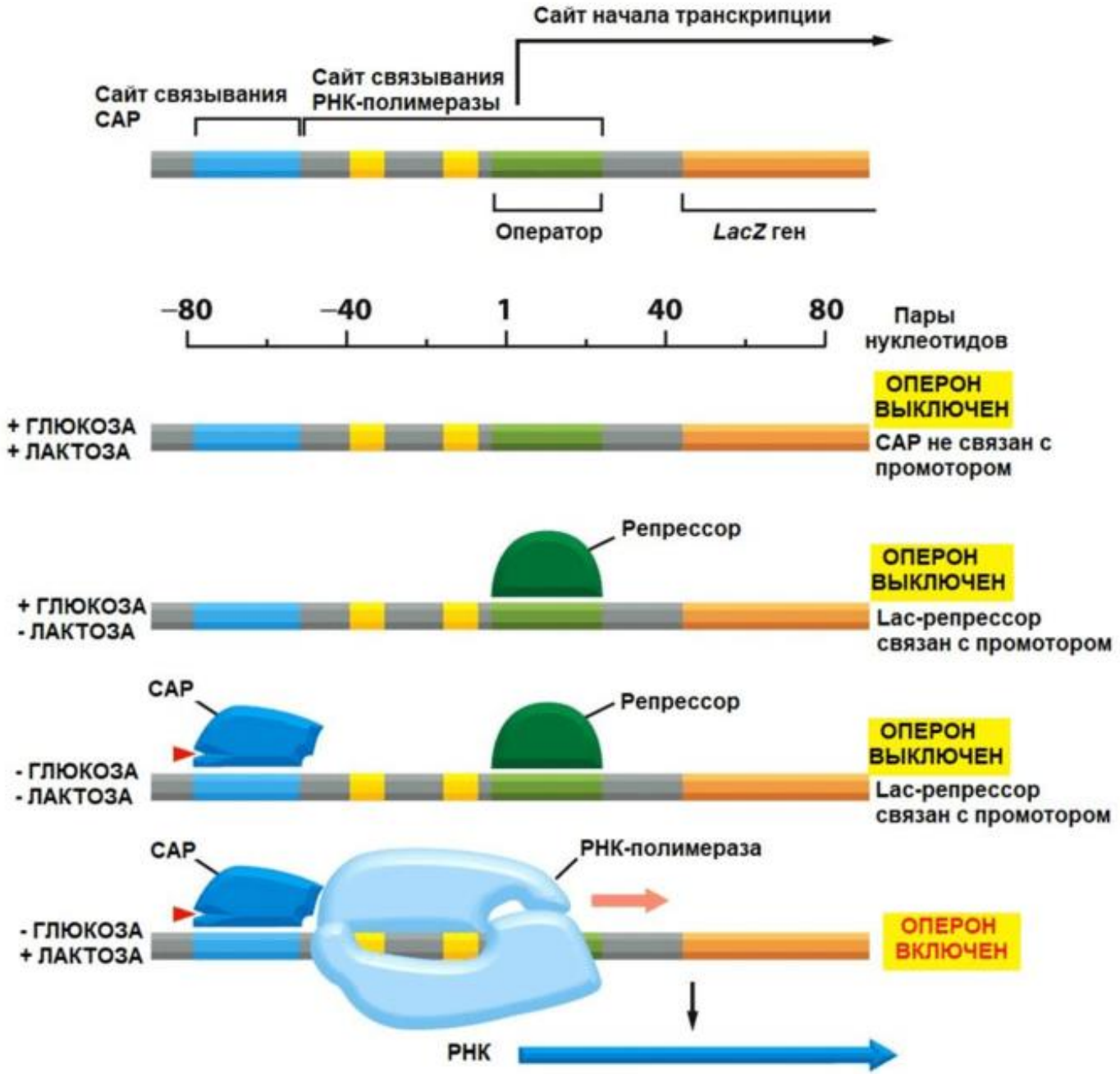


Operon structure



Expressed genes can be divided into the following categories:

- **constitutive**, present in cells in constant quantities, regardless of the metabolic state of the body;
- **induced**, their concentration under normal conditions is low, but can increase by a factor of 100 or more if, for example, a substrate of such an enzyme is added to the cell culture medium;
- **repressed**, i.e. enzymes of metabolic pathways, the synthesis of which stops when the end product of these pathways is added to the growth medium.

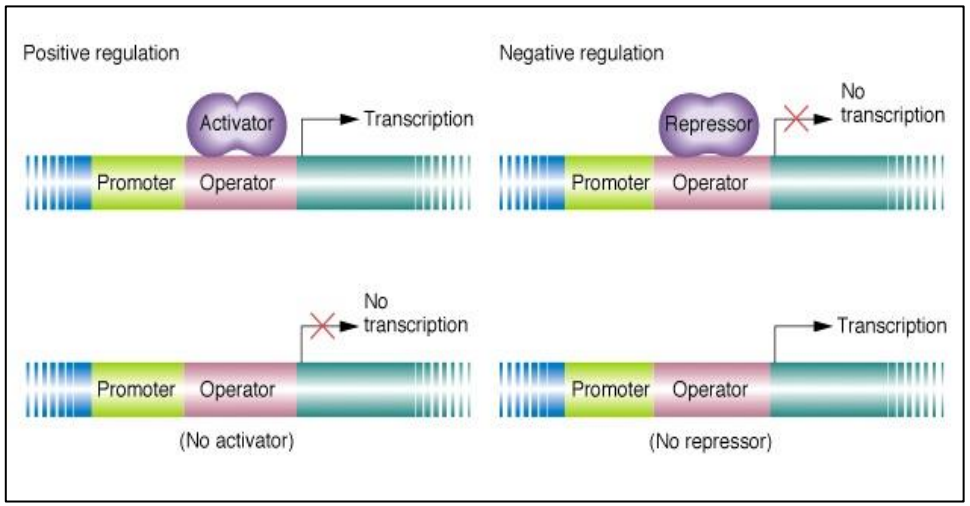


CAP - Catabolite Activator Protein.

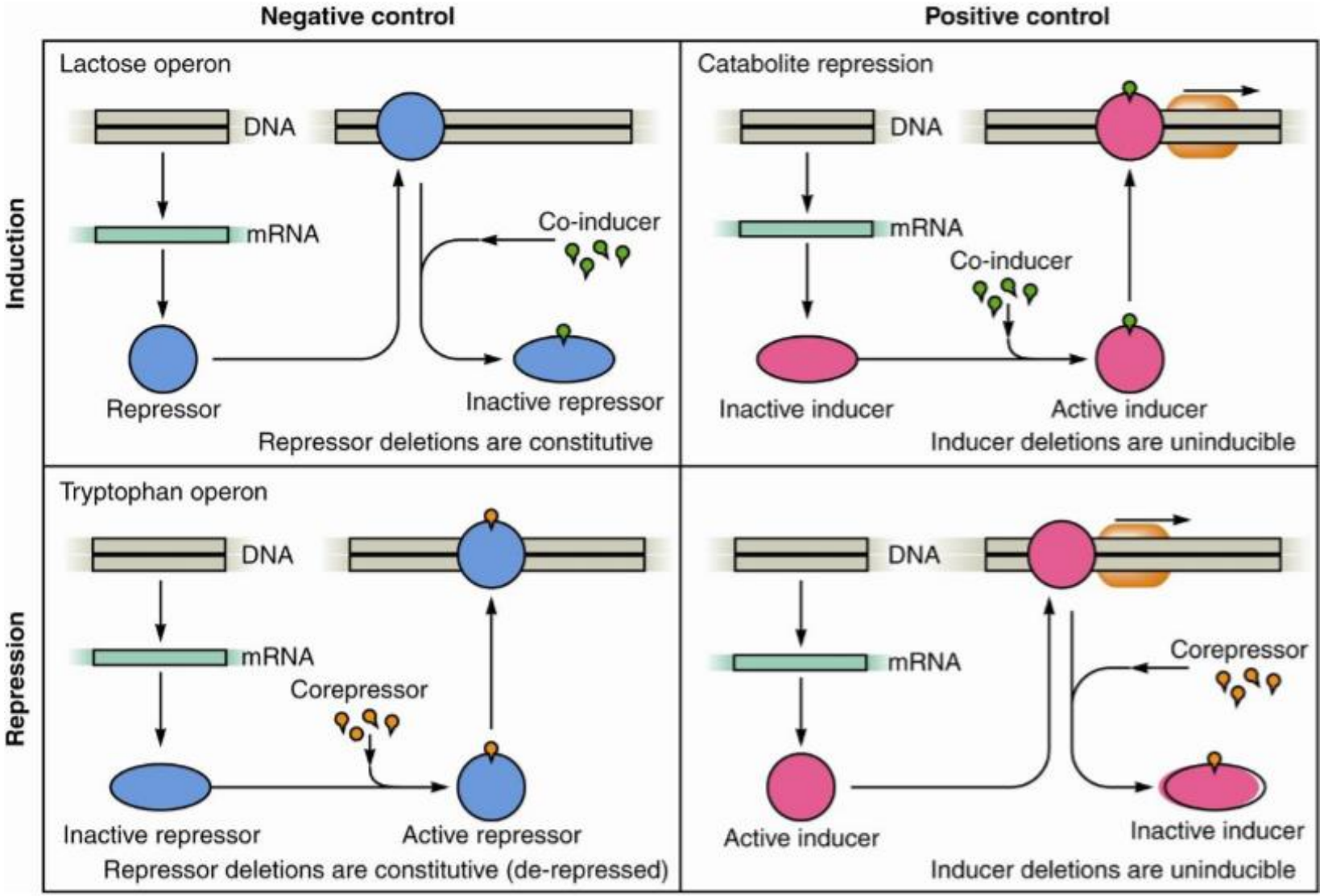
The following schemes of transcriptional regulation in prokaryotes are distinguished:

1) negative induction (Jacobeau and Monod);
2) positive induction;
3) positive repression;
4) negative repression.

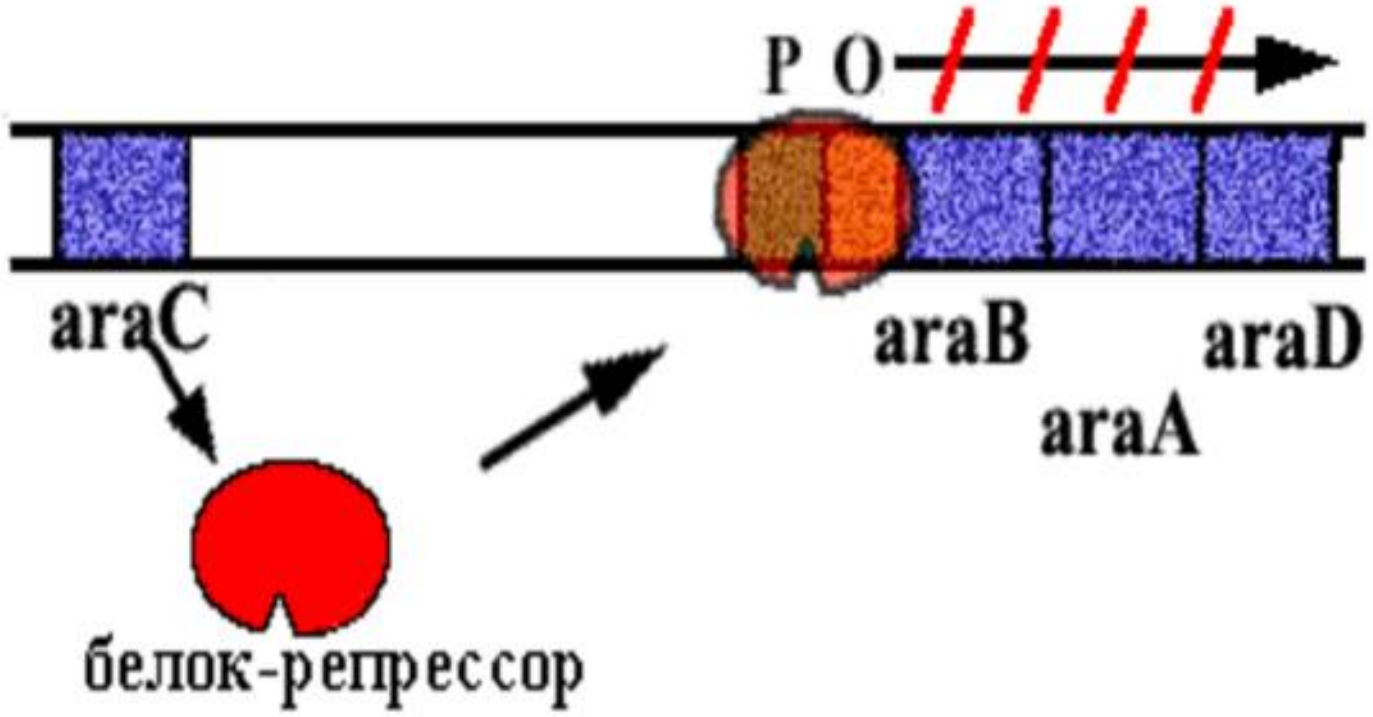
	Regulatory protein is present	Example of regulatory protein	Mutate regulatory gene to lose function
Positive control	Operon ON	Activator	Operon OFF
Negative control	Operon OFF	Repressor	Operon ON



Positive/negative inducible operons and positive/ negative repressible operons

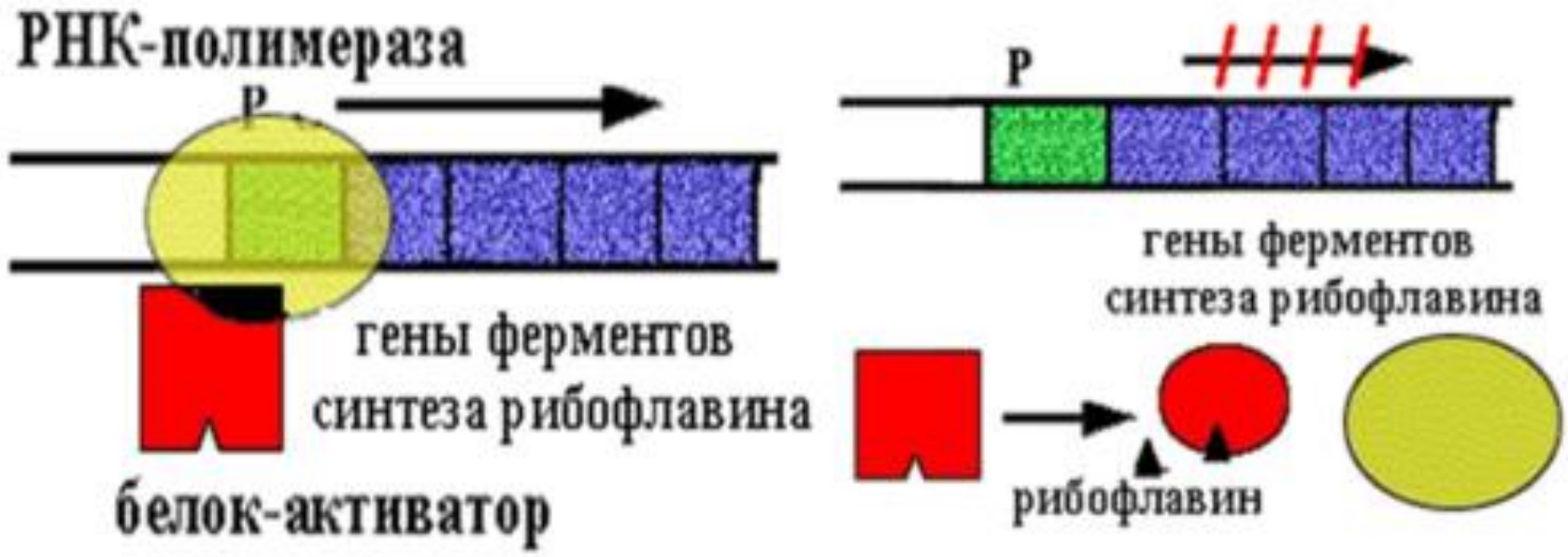


Positive induction scheme - arabinose opera (Aga-operon) Escherichia coli



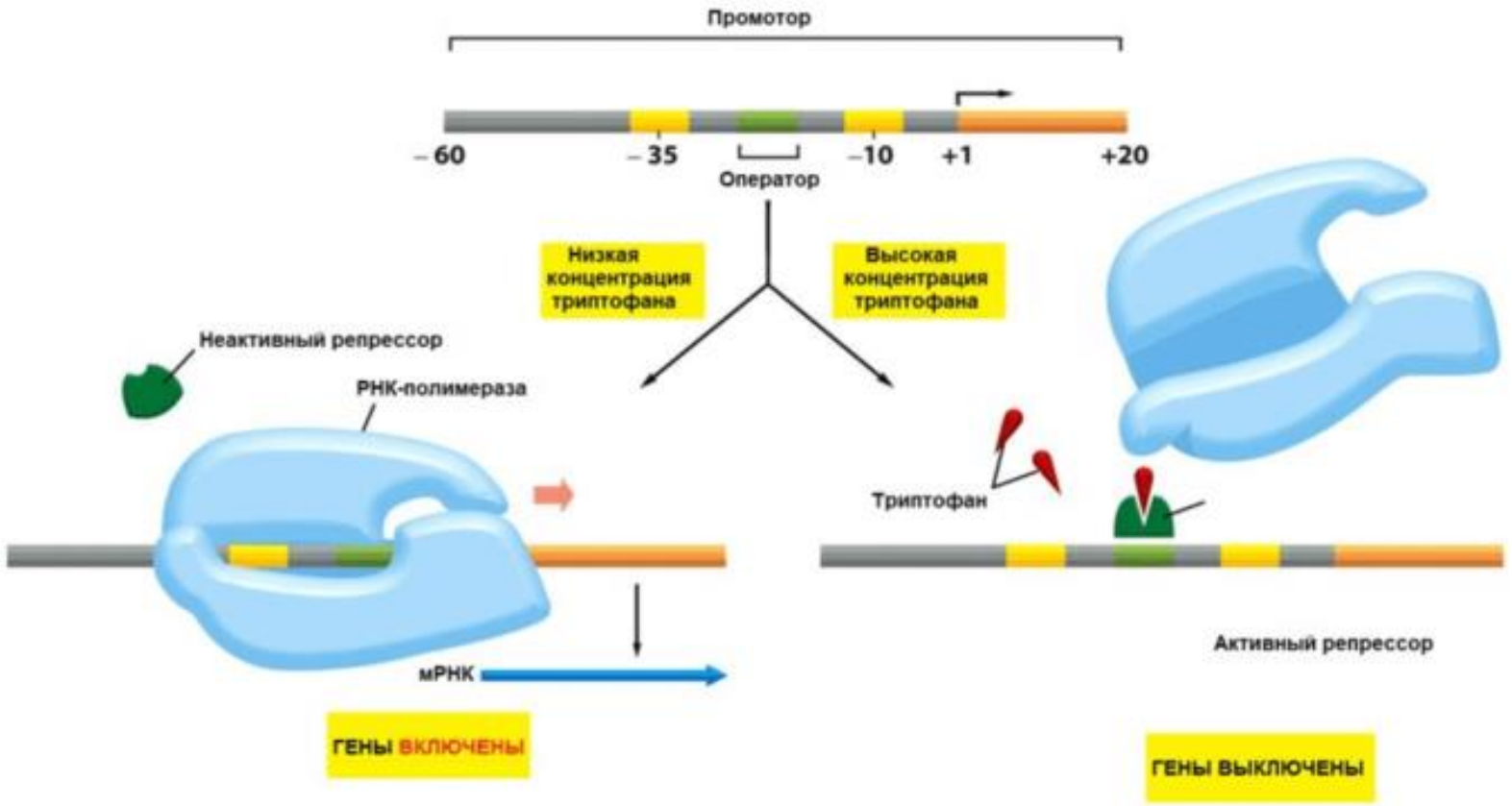
This operon has 3 cistrons that code for enzymes that break down arabinose sugar. Normally, the operon is closed. The repressor protein is linked to the operator. When arabinose enters the cell, it interacts with the repressor protein. The repressor protein changes conformation and turns from a repressor into an activator that interacts with the promoter and facilitates the landing of RNA polymerase on the promoter.

Positive repression scheme — riboflavin synthesis operon in *Bacilus subtilis*



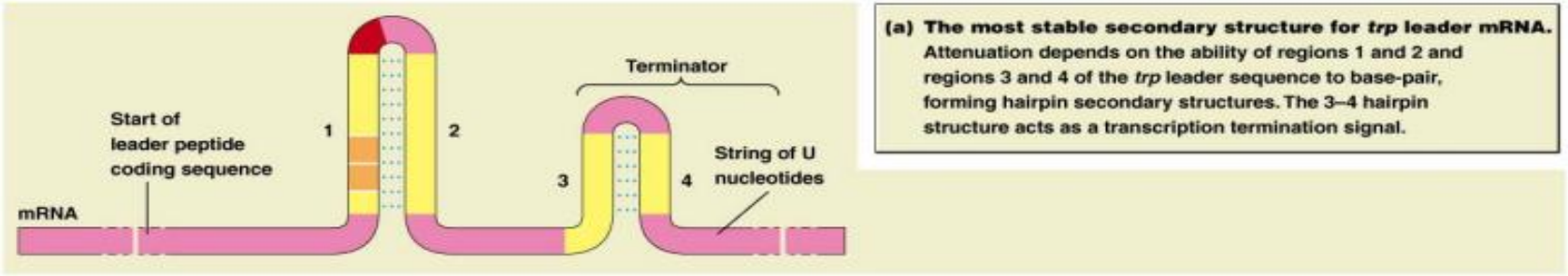
The operon contains cistrons of riboflavin synthesis enzymes. There is an activator protein that ensures the landing of RNA polymerase on the promoter. Normally, the operon is open. N molecules of riboflavin are formed. The N + 1st molecule (extra) interacts with the activator, and it loses its ability to activate the binding of RNA polymerase to the promoter.

Scheme of negative repression by the operation of tryptophan synthesis in *Escherichia coli*

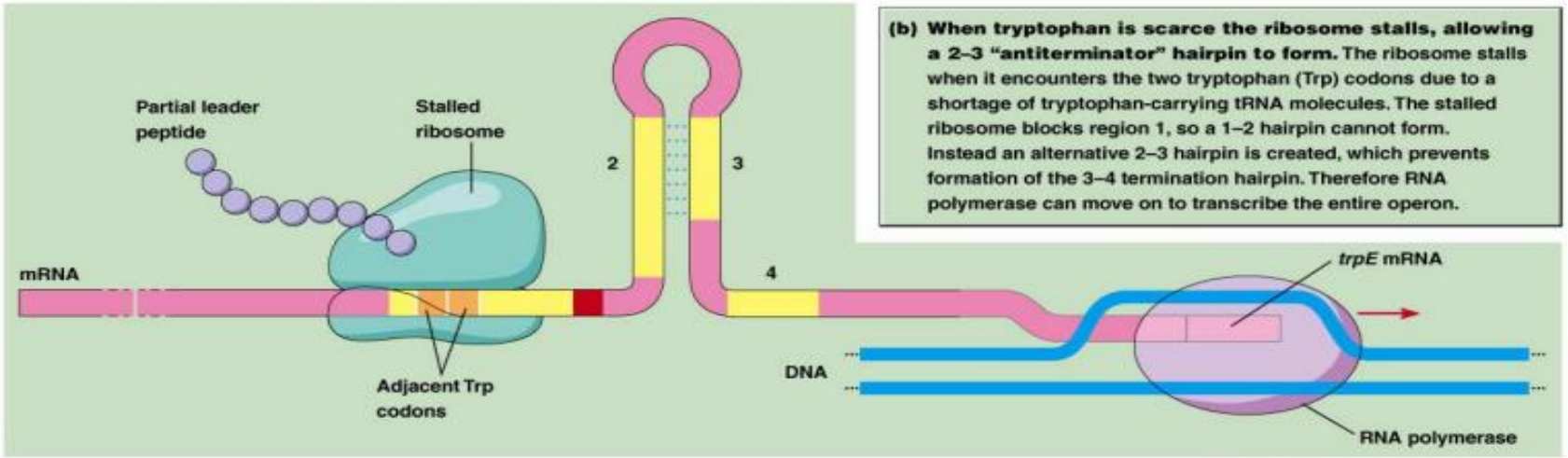


Negative repression, because the repressor protein "turns off" the operon. There are 5 cistrons in the operon, which encode the enzymes of a sequential chain of tryptophan synthesis reactions. Normally, the operon is turned on. The repressor protein is inactive (in the form of an apo-repressor), it is not able to sit on the operator. A cell needs N molecules of tryptophan. The N+1 molecule interacts with the apo-repressor. It changes conformation, sits on the operator and RNA synthesis stops.

Репрессия синтеза белков. Триптофановый оперон.

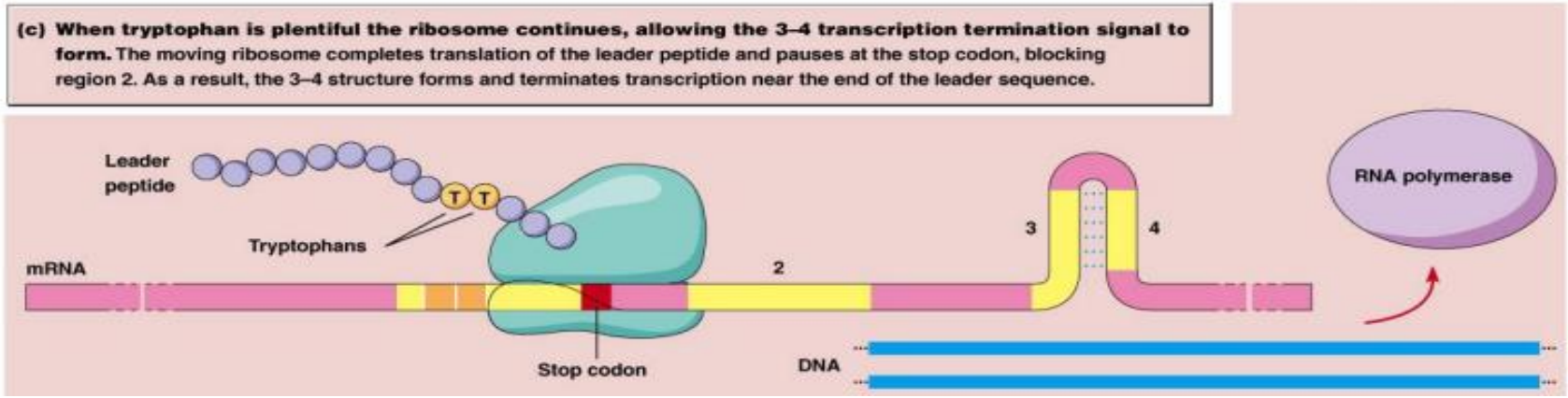


(a) The most stable secondary structure for *trp* leader mRNA. Attenuation depends on the ability of regions 1 and 2 and regions 3 and 4 of the *trp* leader sequence to base-pair, forming hairpin secondary structures. The 3–4 hairpin structure acts as a transcription termination signal.



(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.



В лидерном пиптиде фенилаланинового оперона среди 15 остатков 7 остатков фенилаланина, а в лидерном пиптиде гистидинового оперона — 7 подряд остатков гистидина.

Most E. coli structural genes have two binding sites for RNA polymerase. One of them is usually a nucleotide sequence

TATAAT

ATATTA (TATA-box, or Pribnov box),

and the other –

TTGAC

AACTG.

The TATA box and the TTGAC sequence are located 10 (region -10) and 35 (region -35) nucleotides before the site of transcription initiation, respectively (nucleotide +1)

- Последовательность Шайна — Дальгарно — сайт связывания рибосом на молекуле мРНК прокариот, обычно на расстоянии около 10 нуклеотидов до стартового кодона AUG
- Исследована австралийскими учёными Джоном Шайном и Линн Дальгарно.
- Консенсусом является последовательность из шести нуклеотидов AGGAGG
- Комплементарная последовательность CCUCCU (анти-Шайна — Дальгарно) располагается на 3'-конце молекулы 16S рибосомной РНК. Комплементарное взаимодействие между последовательностями Шайна — Дальгарно и анти-Шайна — Дальгарно служит для помещения старт-кодона мРНК в Р-сайт рибосомы для начала биосинтеза белка
- Мутации в последовательности Шайна — Дальгарно снижают эффективность трансляции.