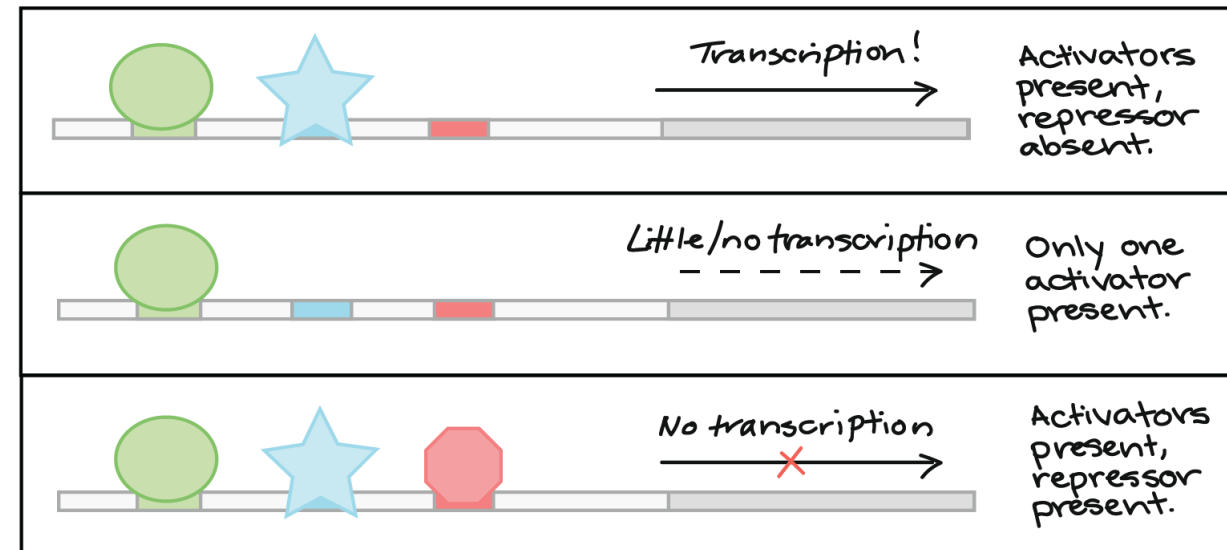
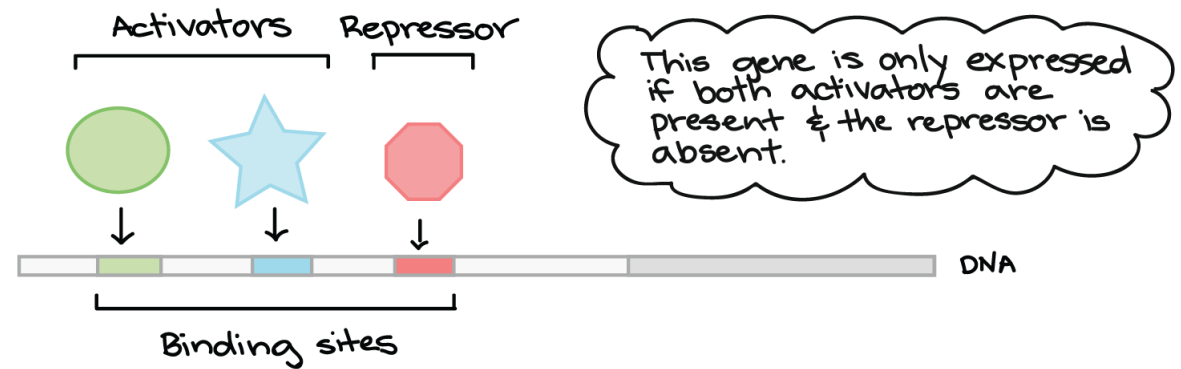


5. Regulation of gene expression- in prokaryotes

Gene expression - the formation of proteins or RNA products

Usually, only a small portion of the genes present in a cell are expressed at a given time

Gene expression is regulated differently in prokaryotes and eukaryotes

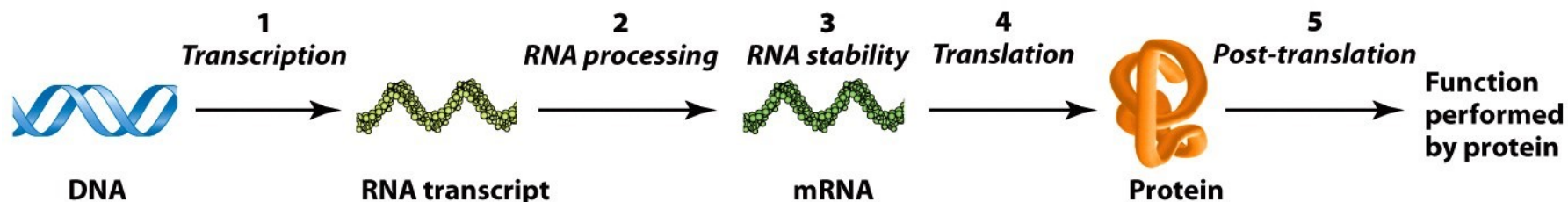


Gene expression is regulated **differently** in prokaryotes and eukaryotes

Level of regulation:

- transcription
- mRNA stability
- translation
- post-translation modification

Levels at which gene expression is regulated in prokaryotes



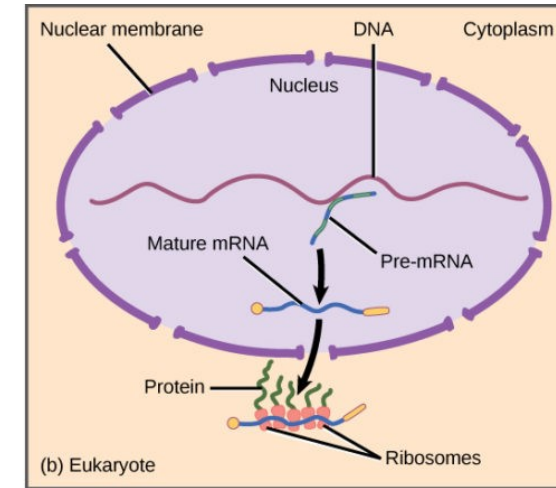
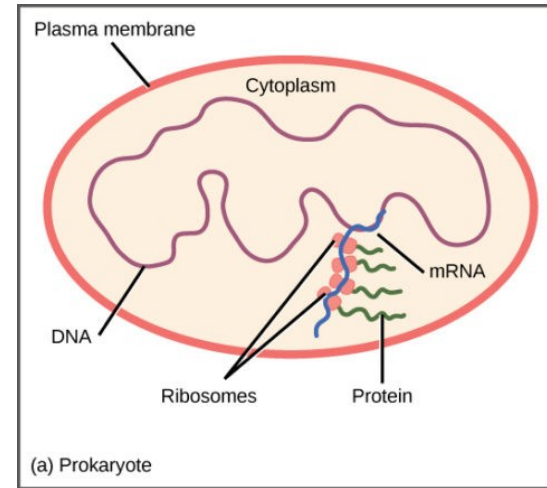
SUMMARY TABLE 18.1 Regulating Gene Expression in Bacteria and Eukaryote

Level of Regulation	Bacteria
Chromatin remodeling	<ul style="list-style-type: none">• Limited packaging of DNA• Remodeling not a major issue in regulating gene expression.
Transcription	<ul style="list-style-type: none">• Positive and negative control by regulatory proteins that act at sites close to the promoter• Sigma interacts with promoter.
RNA processing	<ul style="list-style-type: none">• None documented
mRNA stability	<ul style="list-style-type: none">• Some RNA interference documented
Translation	<ul style="list-style-type: none">• Regulatory proteins bind to mRNAs and/or ribosome and affect translation rate.
Post-translational modification	<ul style="list-style-type: none">• Folding by chaperone proteins• Chemical modification (e.g., phosphorylation) may change activity.



In prokaryotic cells, the control of **gene expression is mostly at the transcriptional level.**

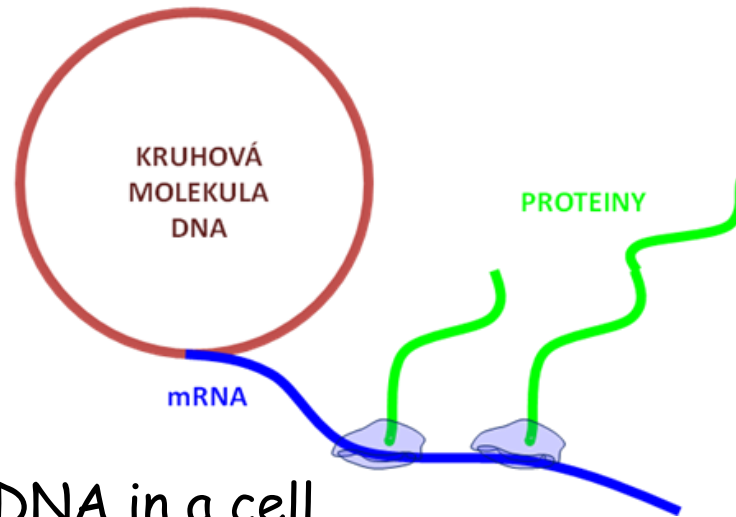
- Prokaryotic organisms are single-celled organisms that lack a cell nucleus, and their DNA therefore floats freely in the cell cytoplasm.
- To synthesize a protein, the processes of **transcription and translation occur almost simultaneously**. When the resulting protein is no longer needed, transcription stops. As a result, the primary method to control what type of protein and how much of each protein is expressed in a prokaryotic cell is the regulation of DNA transcription.
- All of the subsequent steps occur automatically. When more protein is required, more transcription occurs. Therefore, in prokaryotic cells, the control of **gene expression is mostly at the transcriptional level.**



**provide plastic metabolism:
prepare maximum growth and
reproduction under a variety
of conditions**



Basic features of gene expression in prokaryotes



Simpler regulatory mechanisms than eukaryotes

Regulation takes place at the level of **transcription initiation**

The only circular DNA in a cell

DNA is not complexed with histones

The nucleus is not separated from the cytoplasm

Gene transcripts do not contain introns

Translation and transcription take place simultaneously

mechanisms by which gene expression is rapidly turned on or off in response to changes in the environment provide metabolism plasticity: achieving maximum growth and reproduction under a variety of conditions

pre-programmed cascades of gene expression the stimulus affects the expression of a certain gene, the product of which affects the expression of a set of other genes, their products another, etc.



Regulation gene expression in prokaryotes

1. Regulation of transcription in prokaryotes:

- the only type of RNA polymerase
- yet turning genes on and off as needed
- rapidly changing environmental conditions
- signals to start and stop transcription in the form of small molecule substrates
- mediating signal transduction to the promoter by the protein

2. Transcription attenuation

3. Riboswitches - altered secondary structure of mRNA

4. Alternative sigma factors



Constitutive, inducible and repressible gene expression

Constitutive expression

stable (continuous) expression of genes that encode cell components necessary for the maintenance of normal-operational-cellular functions

Eg. expression of genes for rRNA, tRNA, ribosome proteins, RNA polymerases, proteins involved in proteosynthesis, enzymes catalyzing operational functions

constitutive genes are thus expressed in most cells

Constitutive, inducible and repressible gene expression

Inducible / repressible expression

gene expression is increased or decreased as needed

refers to (inducible / repressible) genes whose products are only needed under certain conditions

the synthesis of these genes is under the control of special regulatory systems

constitutive expression of these genes would mean an unnecessary energy load on the cell (evolutionary advantage)



Use of different energy sources in bacteria

several hydrocarbons are usable (eg glucose, sucrose, galactose, arabinose, lactose)

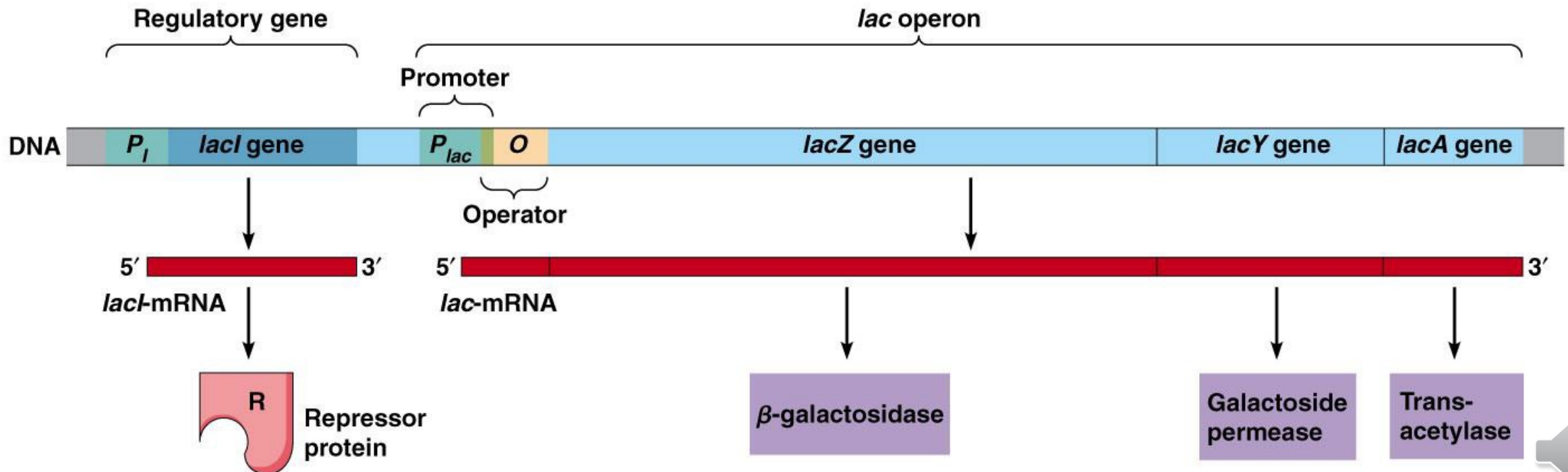
-if available, E. coli prefers the use of **glucose**

- in the presence of lactose and in the absence of glucose, E. coli cells adapt rapidly: initiates the synthesis of two enzymes: β -galactosidase and β -galactoside permease

β -galactoside permease transports lactose into the cell

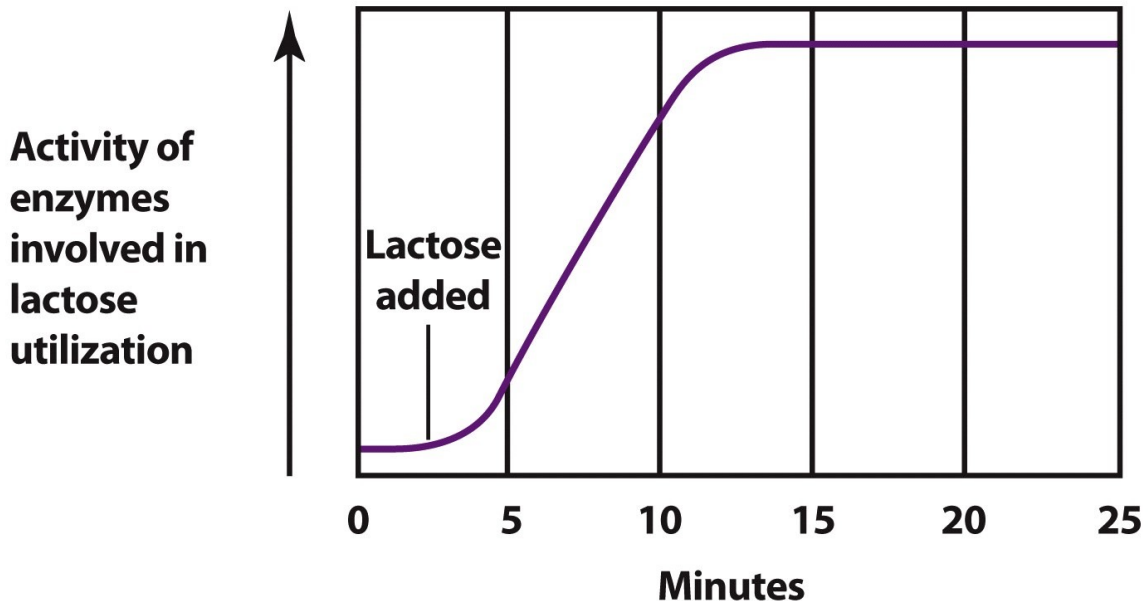
β -galactosidase breaks down lactose into glucose and galactose

in the absence of lactose in the environment, E. coli cells do not synthesize these enzymes



Induction of genes for the use of **lactose**

Induction of enzyme synthesis



- expression of **both genes** is rapidly induced in the absence of glucose and in the presence of **lactose**
- The process of turning on gene expression by a substance delivered to the environment in which a cell grows is called **induction**
- the genes whose expression is thus regulated are called **inducible**
- induction occurs at the level of transcription and changes the number of molecules of the respective proteins (not the activity of already existing proteins)
- typical inducible enzymes are enzymes involved in **catabolic (degradation) pathways**



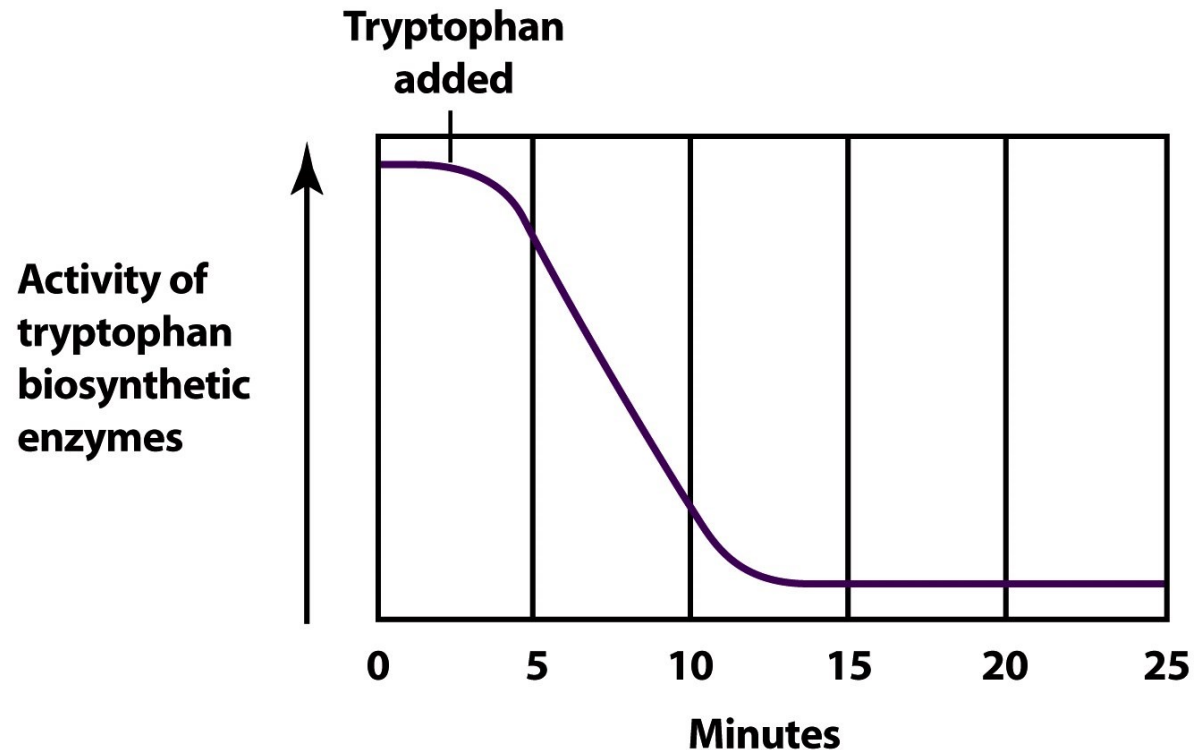
Repression of genes for tryptophan synthesis

- bacteria can synthesize **most of the organic molecules necessary for their growth (amino acids, purines, pyrimidines, etc.)**
- eg in the E. coli genome there are **5 genes encoding enzymes involved in tryptophan biosynthesis**
- their expression is necessary in an **environment lacking tryptophan (to ensure proteosynthesis)**
- in the environment with tryptophan the expression of these genes is unnecessary, **regulatory mechanisms ensure their repression (attenuation)**
- in the absence of tryptophan, **derepression occurs (expression of the relevant genes is switched on)**



Repression of genes for tryptophan synthesis

Repression of enzyme synthesis



- repression occurs at the **transcriptional level**
- enzymes involved in **anabolic (synthetic) processes are often repressible**
- repression is not the same as **negative feedback (the product of the biosynthetic pathway inhibits the activity of the first enzyme of the pathway)**



Summary

TEST

- in prokaryotes, genes providing operational functions (rRNA, tRNA, ribosome proteins) are **constitutively expressed**; other genes are usually expressed depending on the need for their products
- genes that encode enzymes of **catabolic pathways are often expressed inducibly**; only in the presence of appropriate substrates
- the expression of genes encoding enzymes of **anabolic (synthetic) pathways is usually switched off in the presence of the end product of this pathway; they are repressible**
- gene expression can be regulated at many levels, but regulation of transcription is most common



Positive and negative regulation of gene expression

- the cell has **regulatory proteins** that can induce or inhibit the expression of one or more genes
- they are encoded by **regulatory genes**
- **positive regulatory** mechanisms turn on the expression of structural genes
- **negative regulatory** mechanisms turn off the expression of structural genes
- in both cases **inducible and repressible systems** can be applied



Activators and repressors

- regulatory proteins bind to DNA at a regulator protein binding site (RPBS) adjacent to the **structural gene promoter**
- in positive regulatory systems, regulatory proteins are termed **activators** because, upon binding to RPBS, they activate the transcription of structural genes
- in negative regulatory systems, regulatory proteins are called **repressors** because, after binding to RPBS, they repress the transcription of structural genes

Effector molecules

- the binding of a regulatory protein to the RPBS site depends on the presence of **effector molecules**
- effector molecules are usually small molecules (amino acids, sugars, etc.)
- effector molecules involved in the induction of gene expression are termed **inducers**
- effector molecules involved in the repression of gene expression are termed **corepressors**



Basic concepts

- **repressor** is a protein encoded by regulatory genes that bind to DNA to stop transcription of an operon.
- **operator** is a DNA sequence outside the operon to which a specific repressor protein regulating the functionality of a structural gene binds.
- **operon** is a unit that coordinates and regulates gene activity in prokaryotes - controlling protein synthesis. It contains regulatory elements and genes encoding proteins.
- **inducer** is a compound that leads to the production of a larger amount of a given protein (enzyme) in a cell, by direct binding to the regulatory sequences of its gene.
- **promoter** is the 5' -non-coding sequence of the gene to which RNA polymerase binds.
- **regulator**: any substance that is involved in the regulation of molecular processes
- **regulatory protein**: the self-entity involved in regulation usually binds to the promoter
- **allosteric effector**: compound that leads to the production of conformation changes of protein



- **Activator** protein that binds to prokaryotic operators to increase transcription
- **catabolite activator protein (CAP)** protein that complexes with cAMP to bind to the promoter sequences of operons which control sugar processing when glucose is not available
- **inducible operon** operon that can be activated or repressed depending on cellular needs and the surrounding environment
- lac operon - operon in prokaryotic cells that encodes genes required for processing and intake of lactose-
- negative regulator- protein that prevents transcription
- Operator- region of DNA outside of the promoter region that binds activators or repressors that control gene expression in prokaryotic cells
- Operon- collection of genes involved in a pathway that are transcribed together as a single mRNA in prokaryotic cells
- positive regulator- protein that increases transcription
- Repressor- protein that binds to the operator of prokaryotic genes to prevent transcription
- transcriptional start site- site at which transcription begins
- trp operon- series of genes necessary to synthesize tryptophan in prokaryotic cells
- Tryptophan- amino acid that can be synthesized by prokaryotic cells when necessary



Participants in gene expression regulation:

regulator:

any substance that is involved in the regulation of molecular processes

regulatory protein

own entity involved in regulation, they mostly bind to the promoter

allosteric effector

low-molecular substance, which changes its binding to the regulatory protein and its affinity for the regulated region

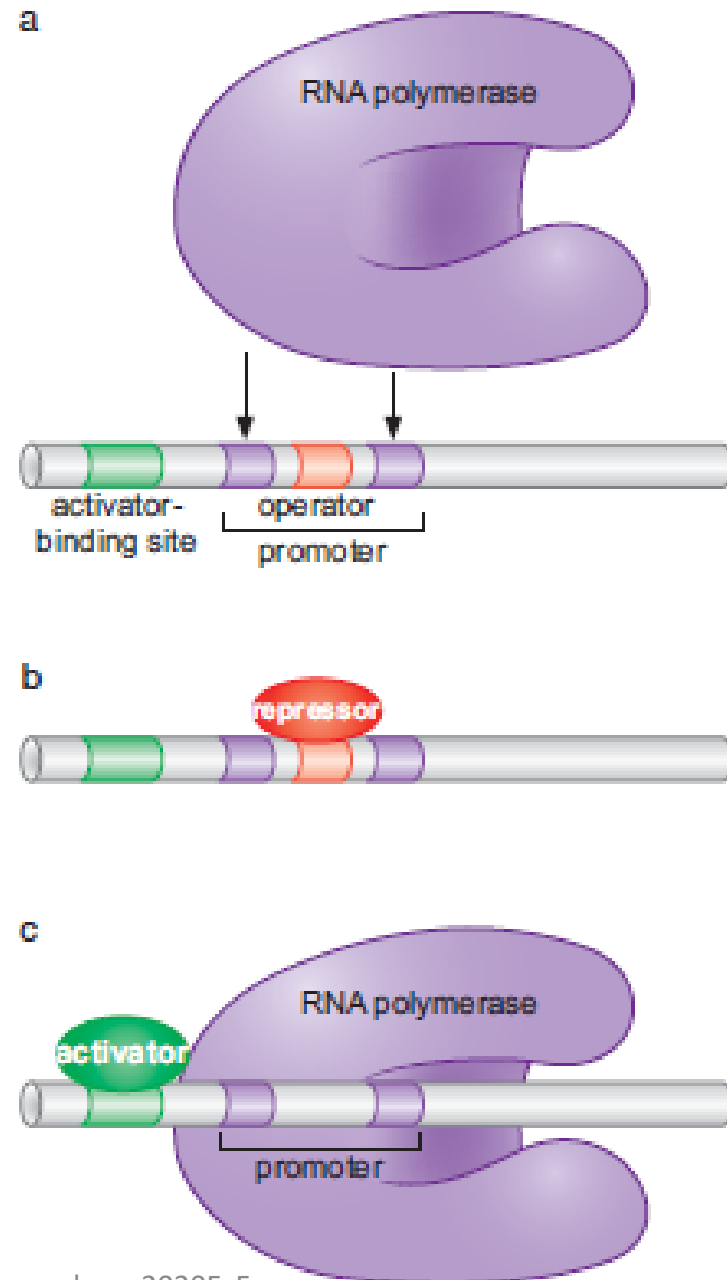
Types of regulators:

positive regulator

induces transcription, translation

negative regulator

stops transcription, translation

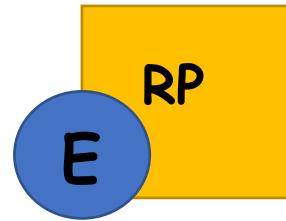


Activation by recruitment of RNA polymerase. (a) In the absence of both activator and repressor, RNA polymerase occasionally binds the promoter spontaneously and initiates a low level (basal level) of transcription. (b) Binding of the repressor to the operator sequence blocks binding of RNA polymerase and so inhibits transcription. (c) Recruitment of RNA polymerase by the activator gives high levels of transcription. RNA polymerase is shown recruited in the closed complex (see Fig. 13-3). It then spontaneously isomerizes to the open complex and initiates transcription. If both the repressor and activator are present and functional, the action of the repressor typically overcomes that of the activator. (This case is not shown in the figure.)



Allosteric effect: interaction of the effector with the regulatory protein

- change in the conformation of the **regulatory protein**
- destruction or creation of a binding site for the regulatory region
- interaction of the **effector** with the regulatory protein



change in conformation of a regulatory protein



creation or destruction of a binding site for the regulatory region



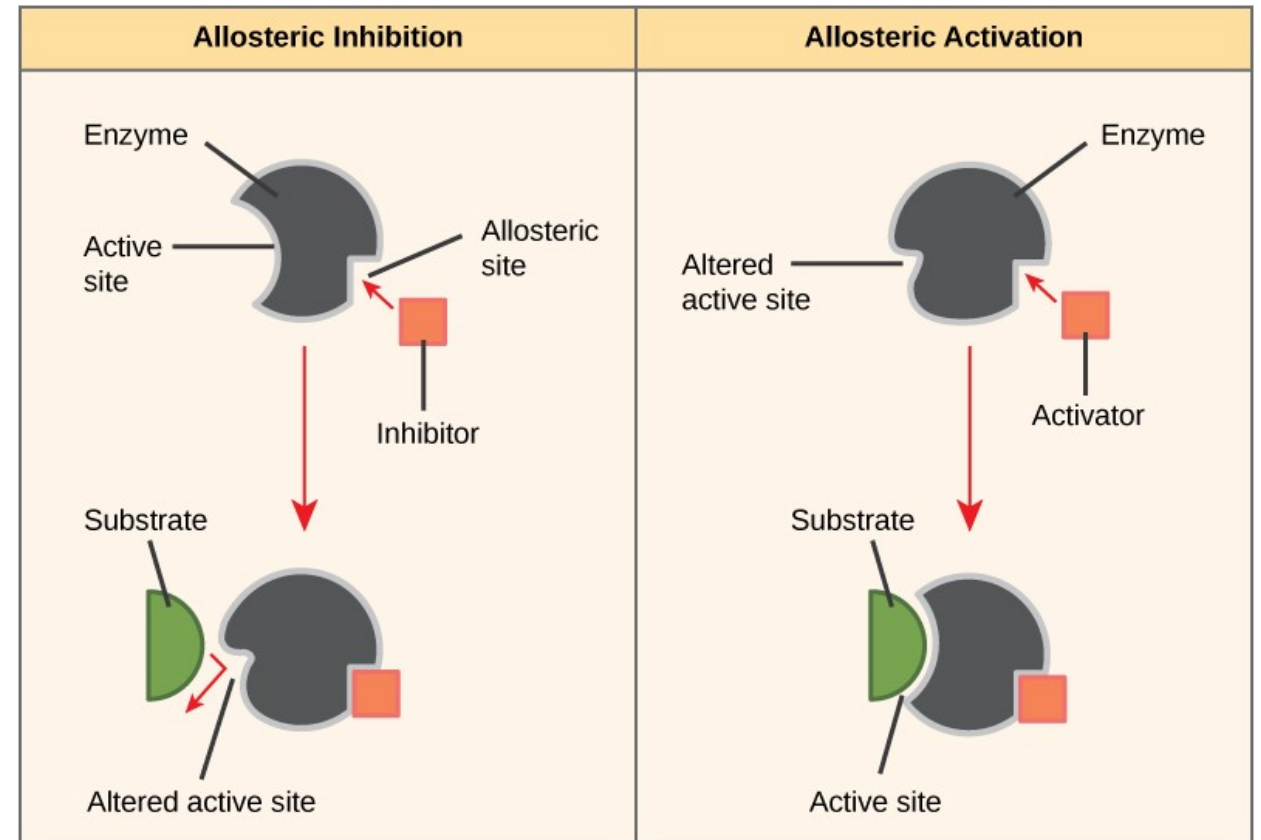
Types of allosteric effectors:

negative allosteric effector

prevents binding of the regulatory protein to the regulatory region

positive allosteric effector

allows binding of the regulatory protein to the regulatory region



Types of regulatory proteins:

negative

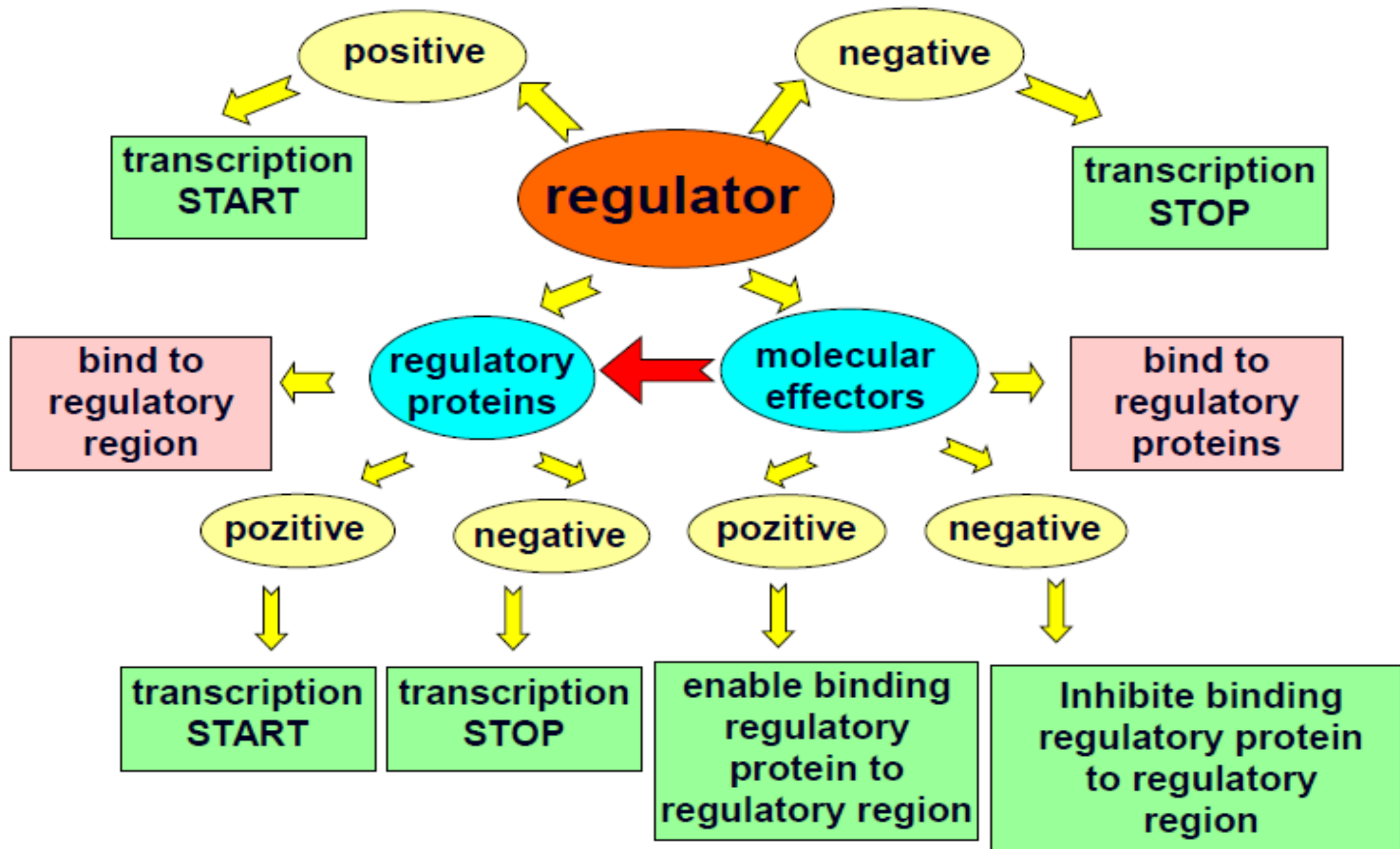
their binding to the regulatory region prevents RNA polymerase from transcribing the transcription unit

<http://teachmephysiology.com/basics/enzyme-activity/enzyme-inhibition/>

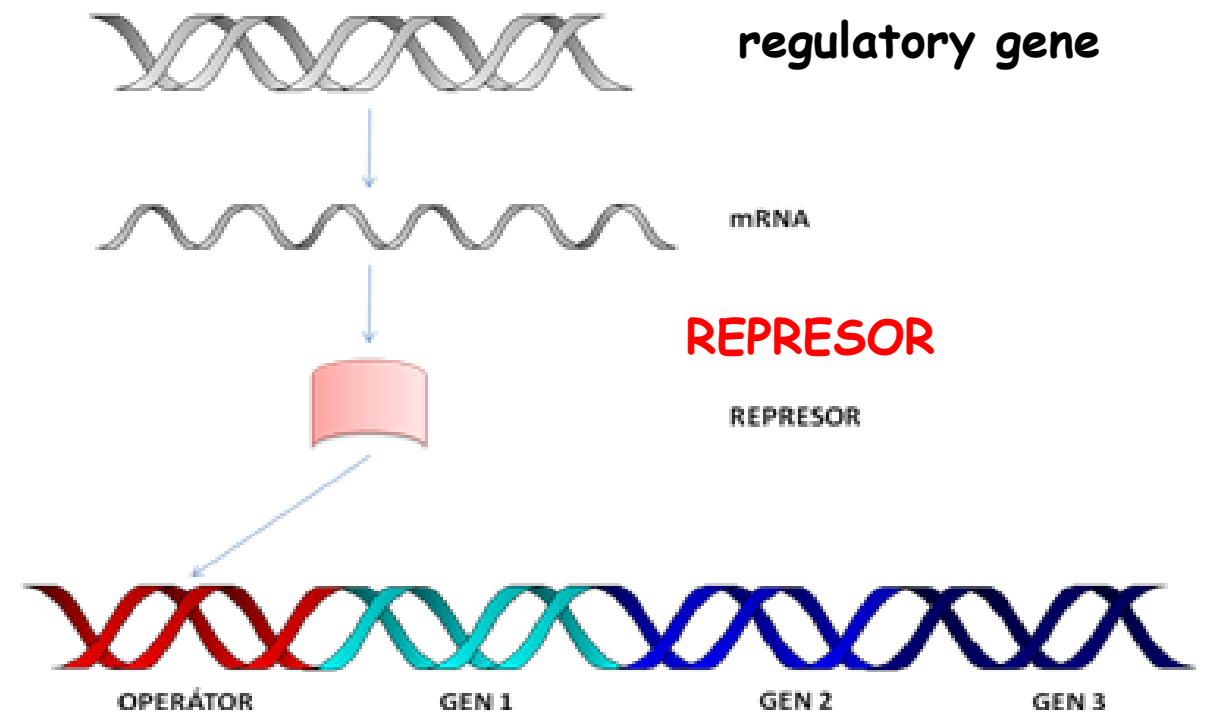
positive

their binding to the regulatory region allows RNA polymerase to transcribe the transcription unit
transcription activators





RNA polymerase regulation by repressor - negative control



repressor is encoded by a regulatory gene

After synthesis, the repressor diffuses to the promoter and binds in a region called the **operator** (usually part of the promoter)

The repressor blocks RNA polymerase binding to the promoter

mRNA synthesis does not occur



The repressor is controlled by two mechanisms

Induction

inductor is a small molecule that binds to a repressor, changes its conformation, and causes disconnection from the operator

Transcription can begin

Inducers: small molecules of nutrients or their metabolites

Corepression

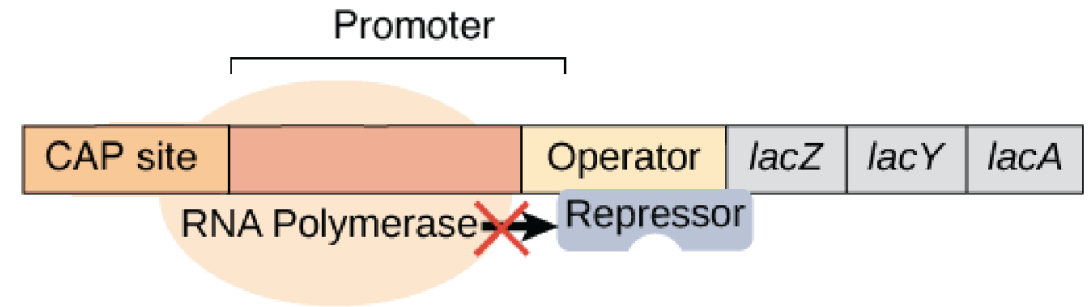
repressor is not active unless the **corepressor** is attached to it. The repressor-corepressor complex binds to DNA and prevents RNA polymerase binding

There is no transcription

Corepressors: small molecules of nutrients or their metabolites

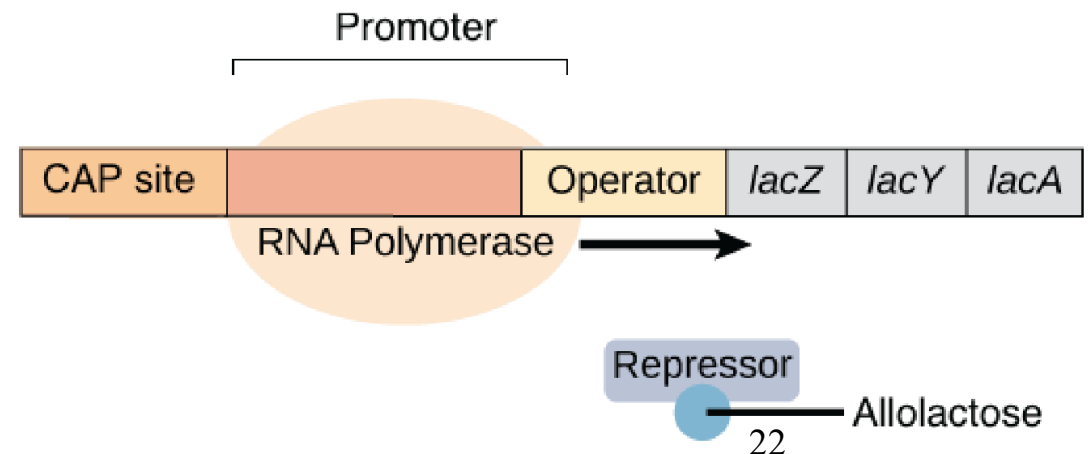
No lactose:

When lactose is absent, the *lac* repressor binds tightly to the operator. It gets in RNA polymerase's way, preventing transcription.

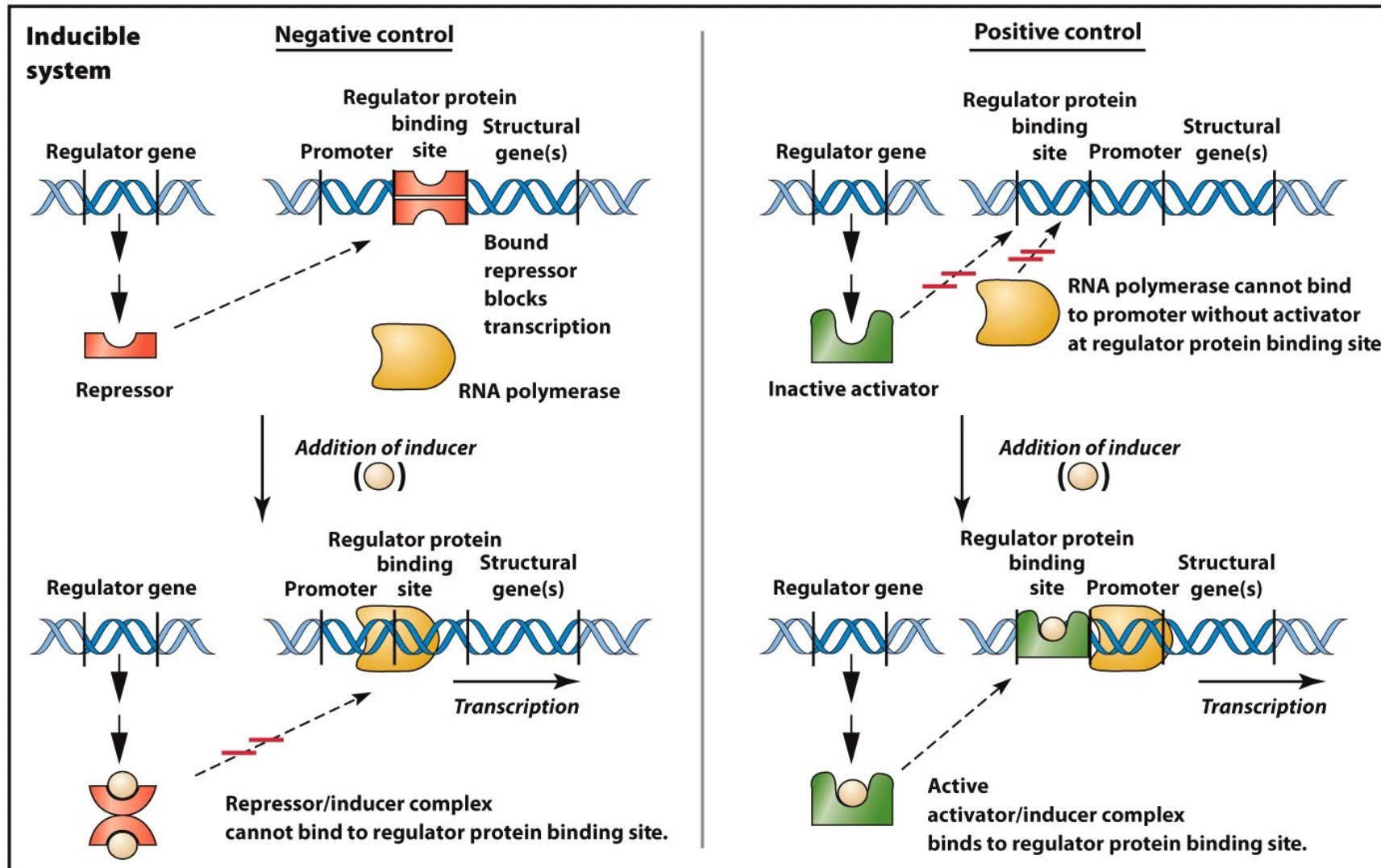


With lactose:

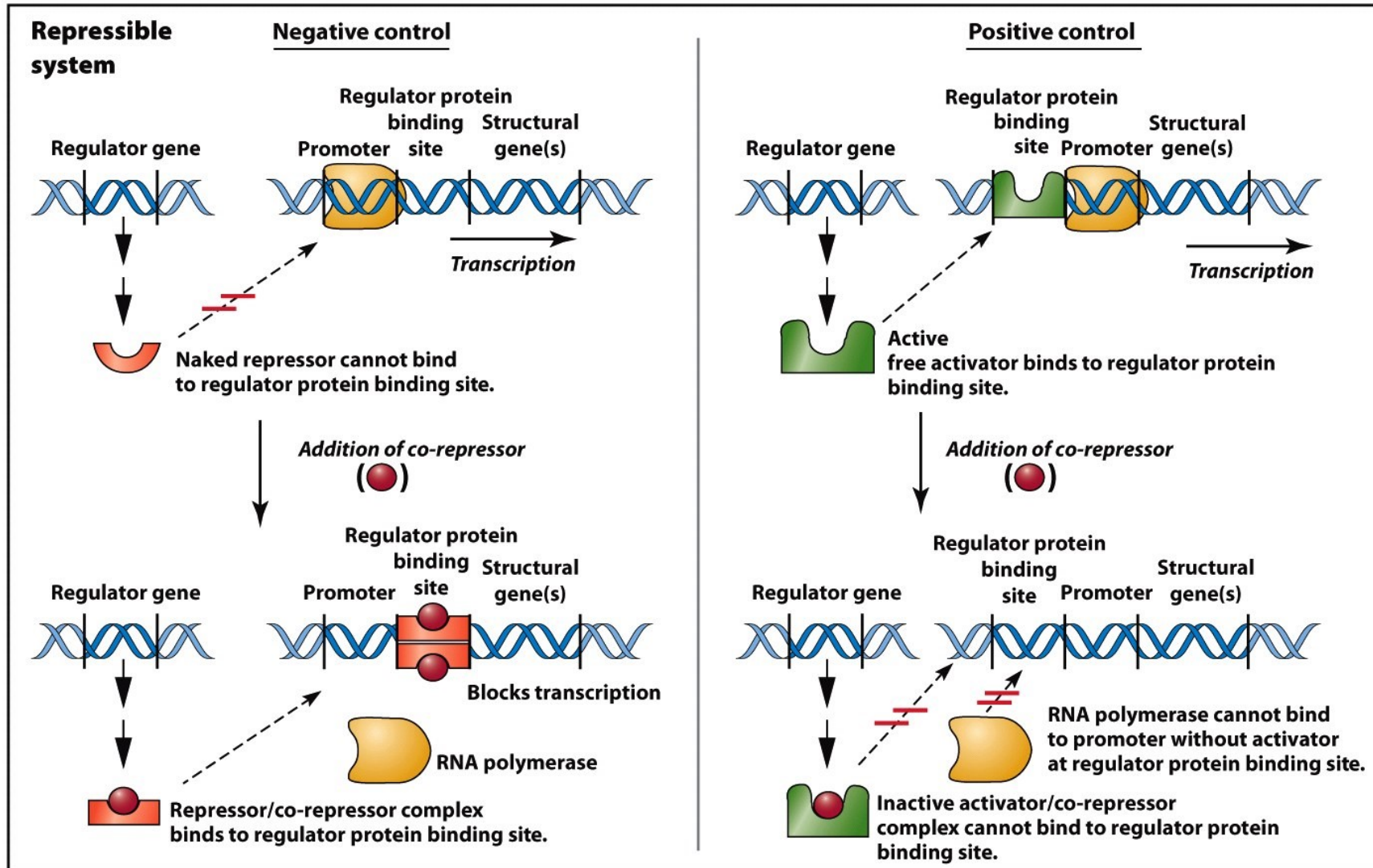
Allolactose (rearranged lactose) binds to the *lac* repressor and makes it let go of the operator. RNA polymerase can now transcribe the operon.



Inducible systems

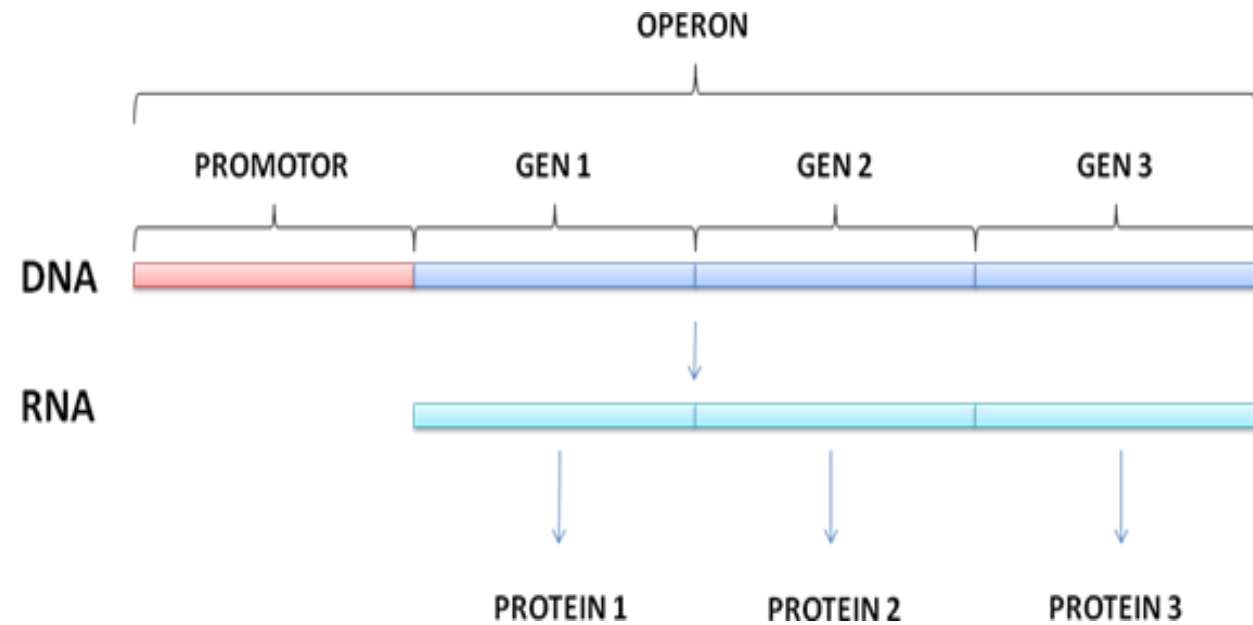


Repressible systems



Operon theory

Structural genes in bacteria are often grouped into operons



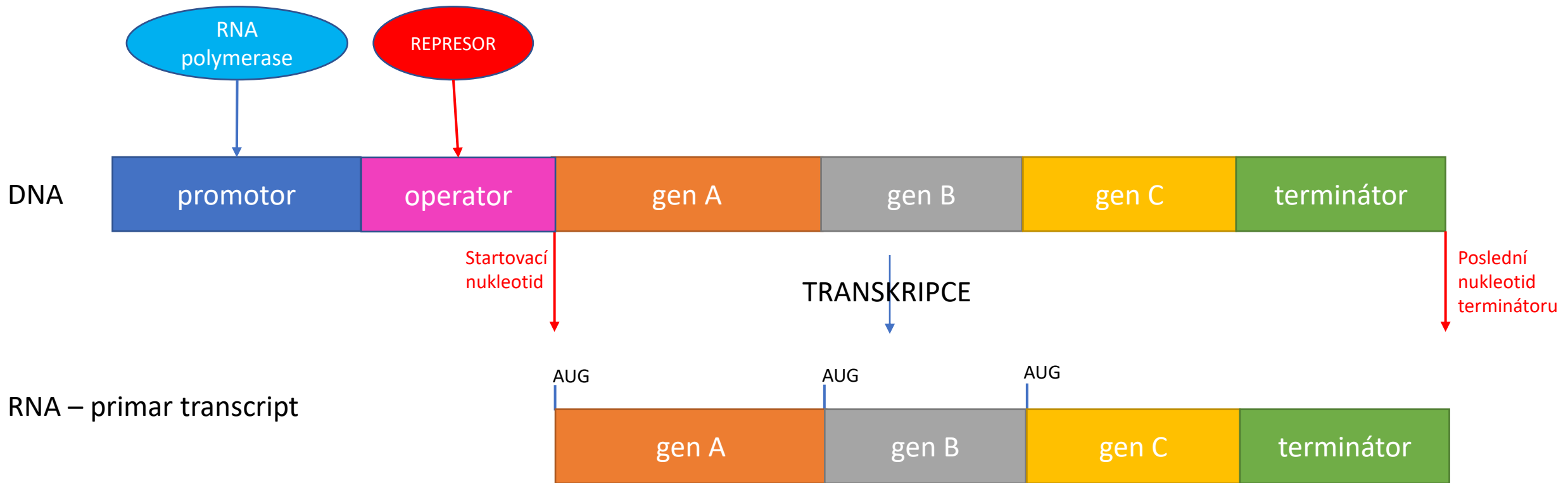
Operon

- Includes **structural genes** for metabolically coupled proteins
- The genes in the operon are usually expressed in a coordinated way (they are either all "off" or all "on")
- The transcription product is **polycistronic mRNA**
- Transcription is regulated by a single promoter
- RNA polymerase is regulated - positively or negatively



Operon regulation - positive and negative operon regulation:

Operon



- **THE PROMOTOR MAY OVERLAPP WITH THE OPERATOR**
- **The size of the individual regions does not correspond to reality, the genes are in fact much larger than the regulatory regions**

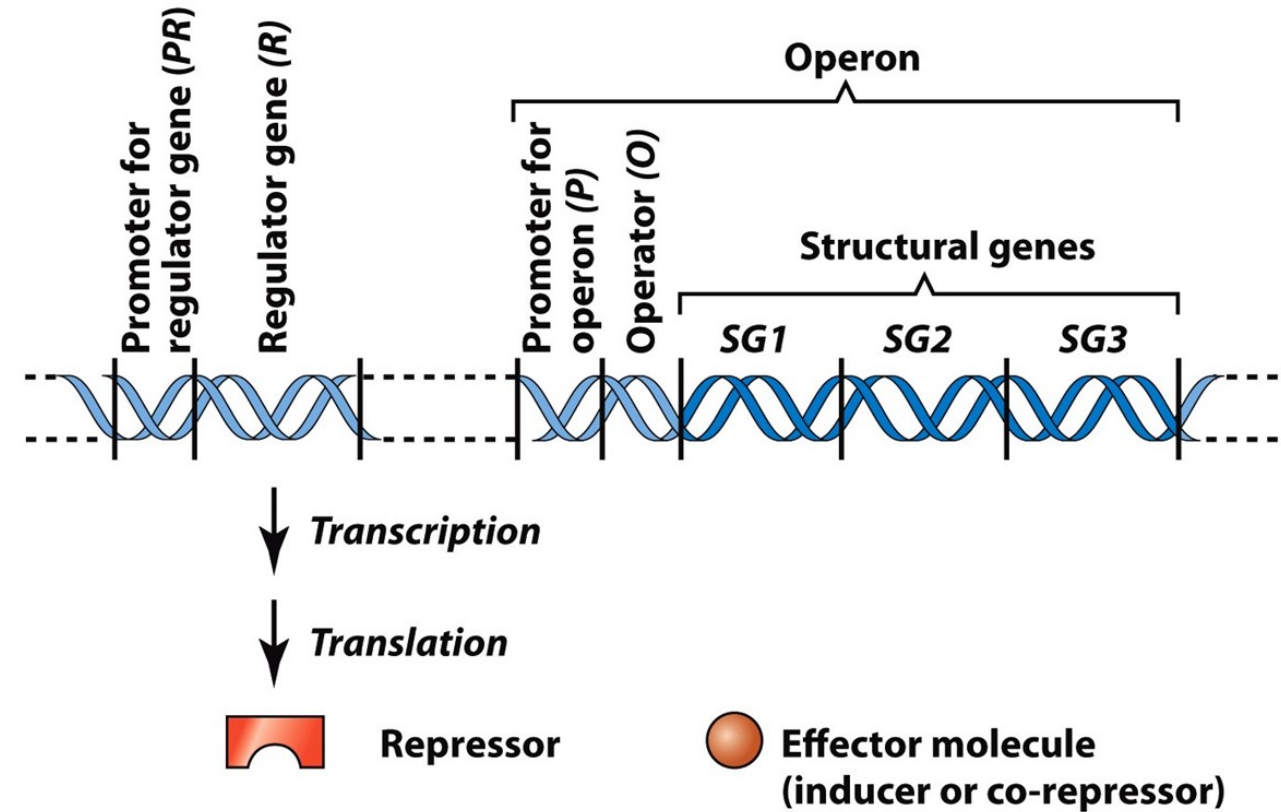


Operon regulation - positive and negative operon regulation:

1) Negative regulation of the operon:
is the essence of **enzyme induction and repression**
binding of the active repressor to the operator stops transcription

2) Positive regulation of the operon:
is the essence of **catabolic repression**
binding of CAP to a promoter in the presence of an inducer induces transcription

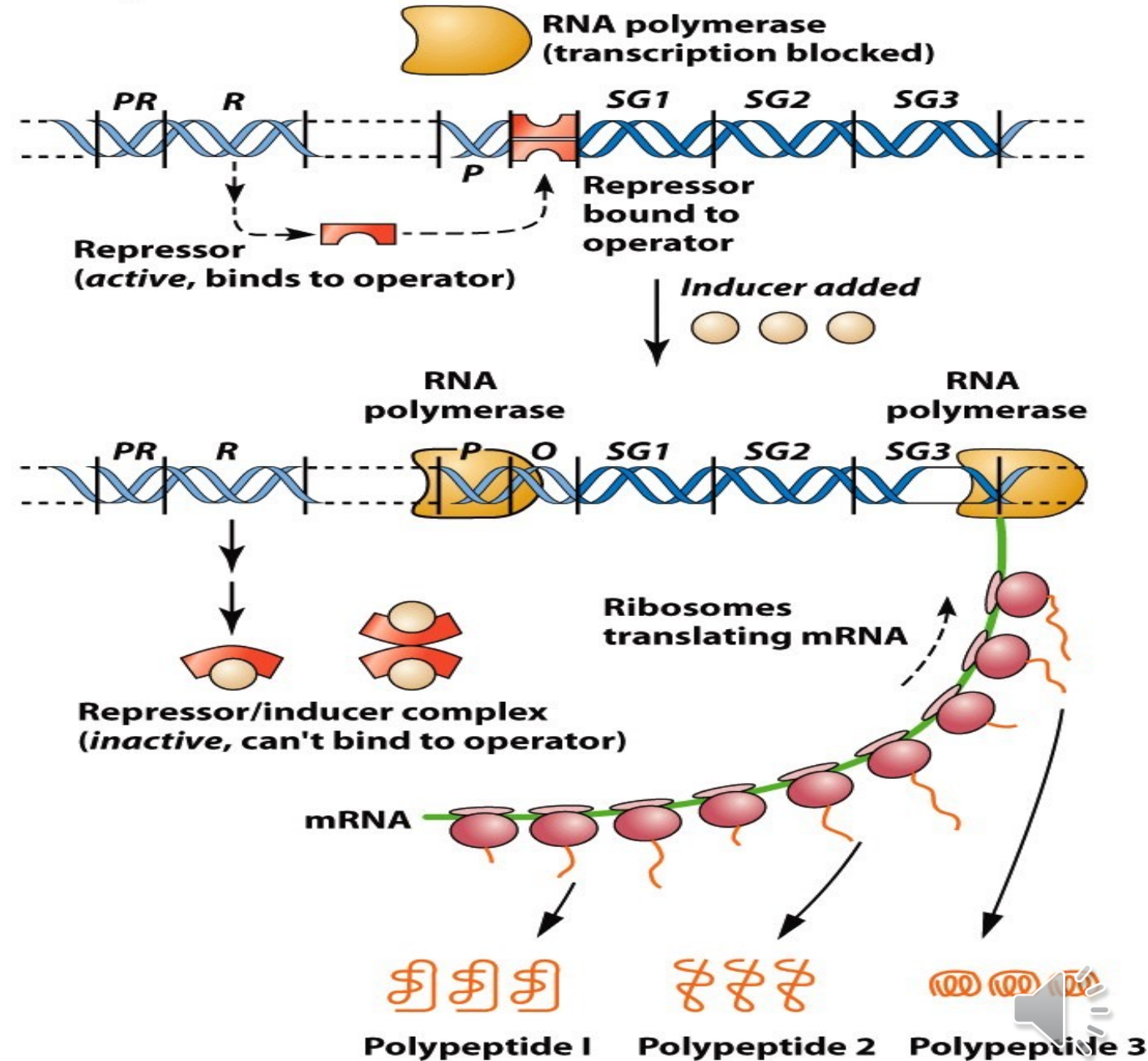
The operon: components



Inducible operon

- free repressor binds to the operator and thus sterically prevents RNA polymerase from initiating transcription of structural genes - transcription turned off
- by binding the inducer, the repressor is inactivated - it loses the ability to bind to the operator - transcription is switched on

The operon: induction



➤ **Enzyme induction (negative regulation):**

most often refers to the induction of enzymes induced by inducers

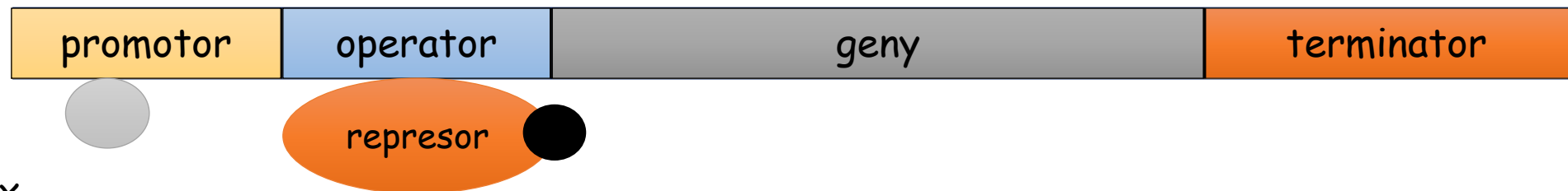
In general, genes, proteins, enzymes...

➤ **inducible enzymes**

➤ **constitutive enzymes**

➤ their synthesis does not depend on the presence of an inducer

➤ they are formed in the cell in a constant amount



inducer-inactivates the repressor



gene transcription takes place, induced enzymes are formed



➤ **Enzyme induction (negative regulation):**

most often refers to the induction of enzymes induced by inducers

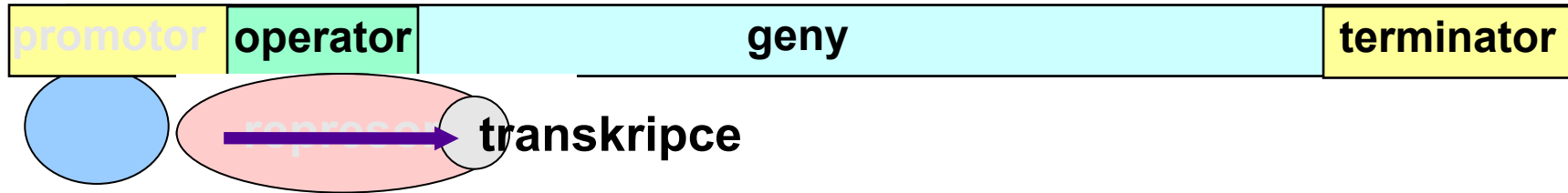
In general, genes, proteins, enzymes...

inducible enzymes

constitutive enzymes

their synthesis does not depend on the presence of an inducer

they are formed in the cell in a constant amount



inaktivní komplex

induktor

transkripce genů probíhá - tvoří se indukovatelné enzymy



1) Example of induction

Induction of the **lac operon** by **lactose** in *E. coli*

Enzymes for glucose metabolism by glycolysis are **constitutively produced** in *E. coli*

When lactose is added, the cells adapt and begin to produce other enzymes encoded by the **lac operon**

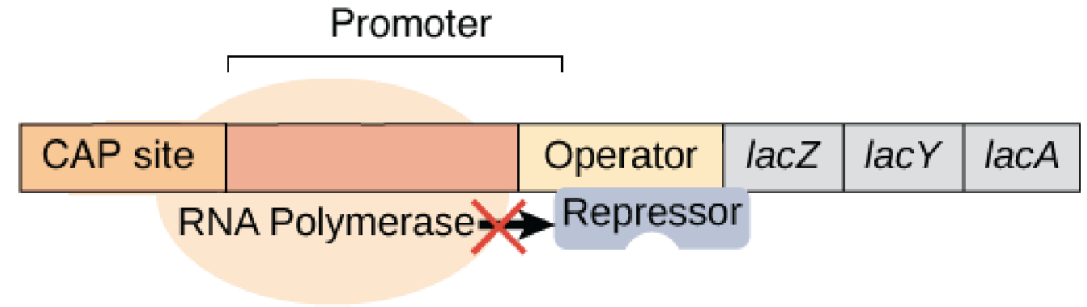
Allolactose (an isomer of lactose formed spontaneously) serves as an inducer, binds to and inactivates the repressor.

RNA polymerase can bind to the promoter and transcribe structural genes in the **lac operon** to produce polycistronic RNA, which encodes three other enzymes (β -galactosidase, permease, and transacetylase)

* The process takes place when there is a **lack of glucose** in the cell

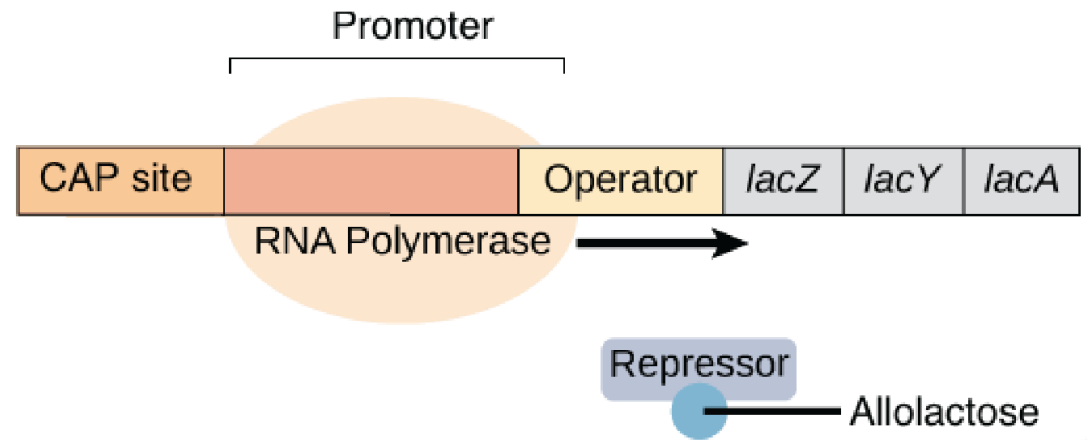
No lactose:

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With lactose:

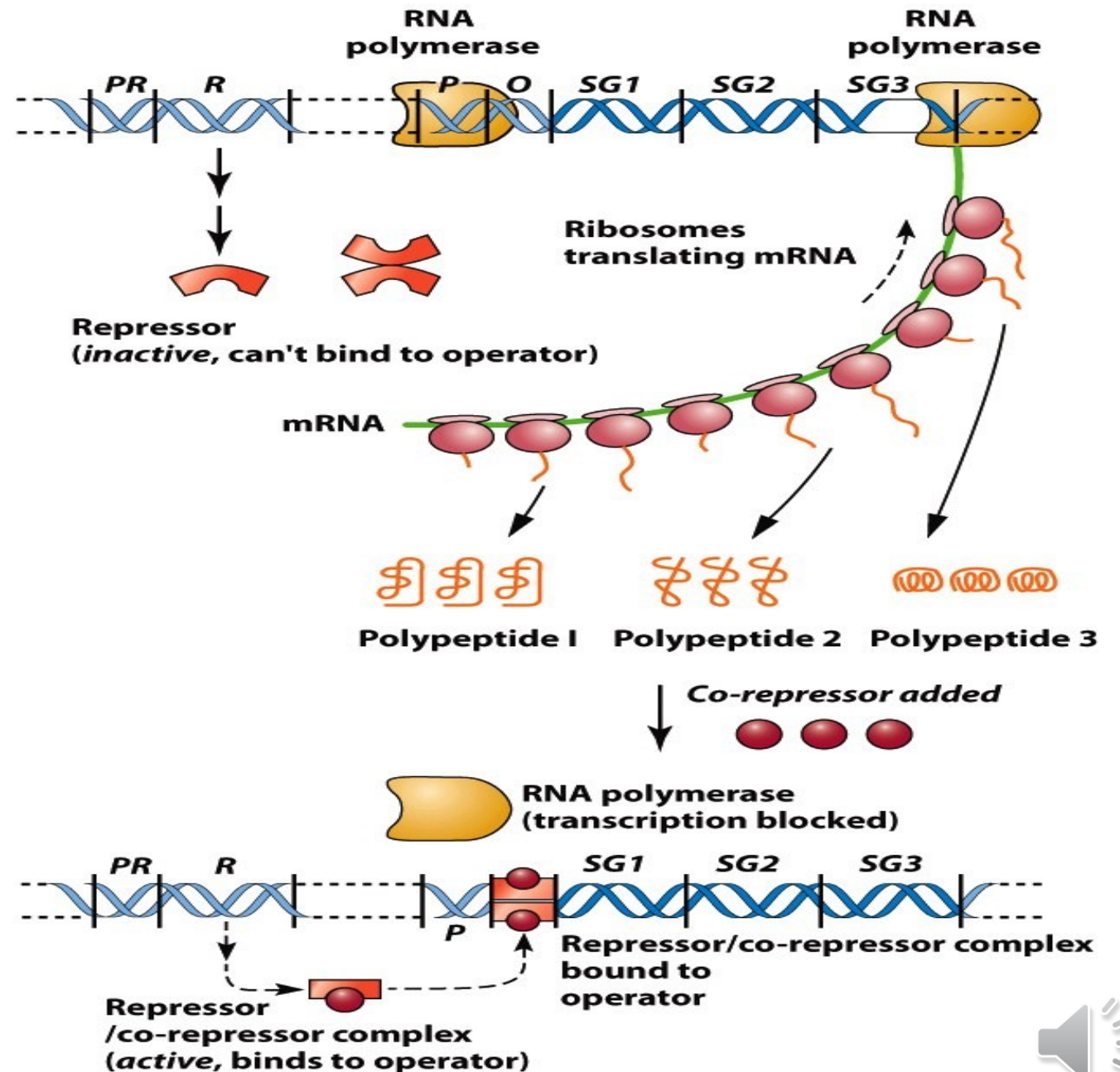
Allolactose (rearranged lactose) binds to the *lac* repressor and makes it let go of the operator. RNA polymerase can now transcribe the operon.



Repressible operon

- free repressor cannot bind to operator - transcription enabled
- the binding of the corepressor to the repressor restores the ability of the repressor to bind to the operator - transcription disabled

The operon: repression



2) Enzyme repression: corepression

- it most often concerns enzymes of biosynthetic pathways
- the synthesis of these enzymes is suppressed by a specific metabolite of a given metabolic pathway, which accumulates in a critical amount and stops further synthesis
- synthesis resumes as soon as the metabolite concentration falls below a critical value

Example of corepression

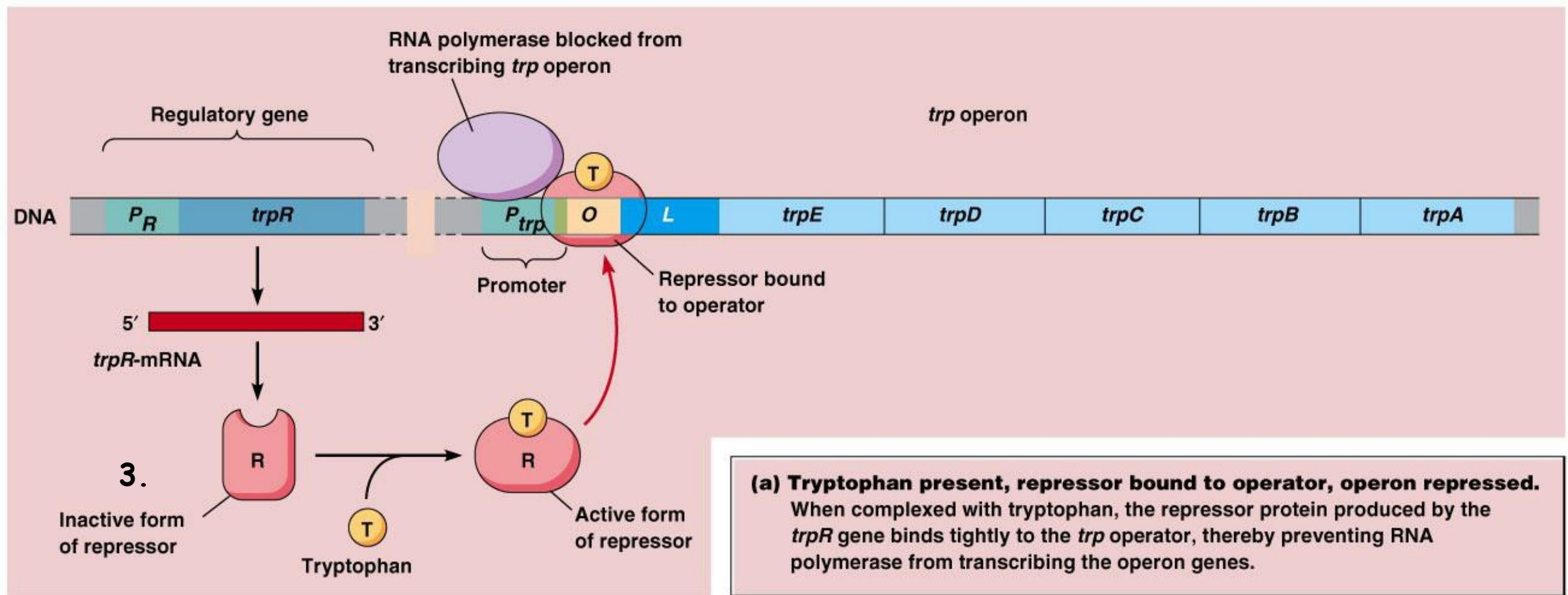
Corepression of trp operon (tryptophan synthesis in E.coli)

The genes for tryptophan synthesis enzymes (5 enzymes in total) are concentrated in the *trp operon*

Tryptophan is a corepressor, it binds to an inactive repressor, it changes its conformation.

The **tryptophan-repressor** complex inhibits operon transcription.





Corepression of trp operon (tryptophan synthesis in E.coli)

The genes for tryptophan synthesis enzymes (5 enzymes in total) are concentrated in the **trp operon**

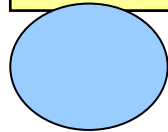
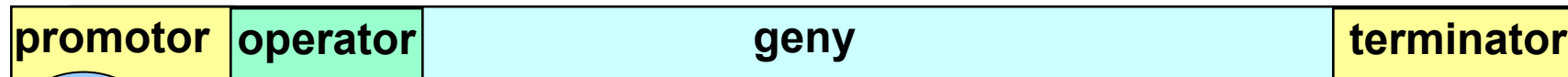
Tryptophan is a corepressor, it binds to an inactive repressor, it changes its conformation.

The **tryptophan-repressor** complex inhibits operon transcription.

<http://www.mhhe.com/biology/scarr/bio4241/chapter13.htm>

➤ Enzyme repression: corepression

repressor



corepressor – activatione
repressor

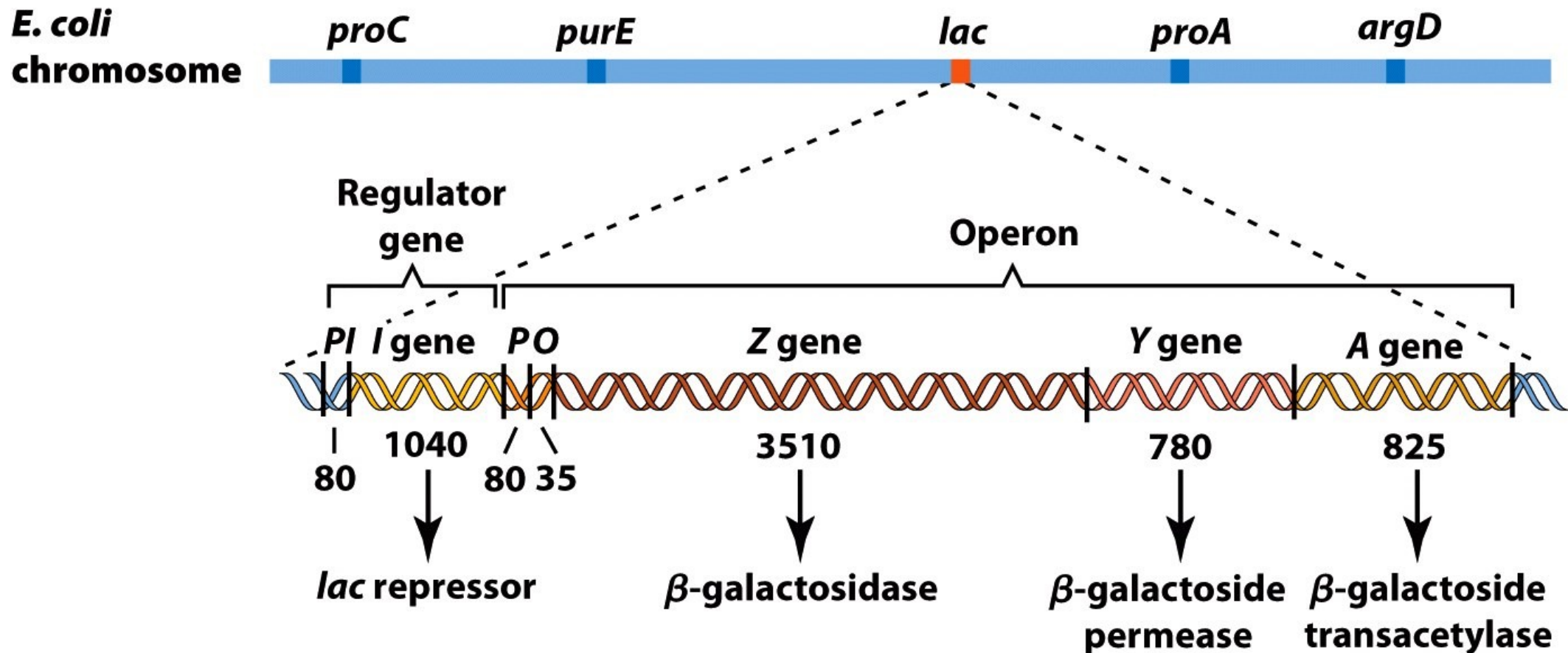


gene transcription does not take place - corepressor synthesis stops



Lactose operon in *E. coli*: induction and catabolic repression

The structural genes of the *lac* operon are transcribed only in the presence of lactose and in the absence of glucose

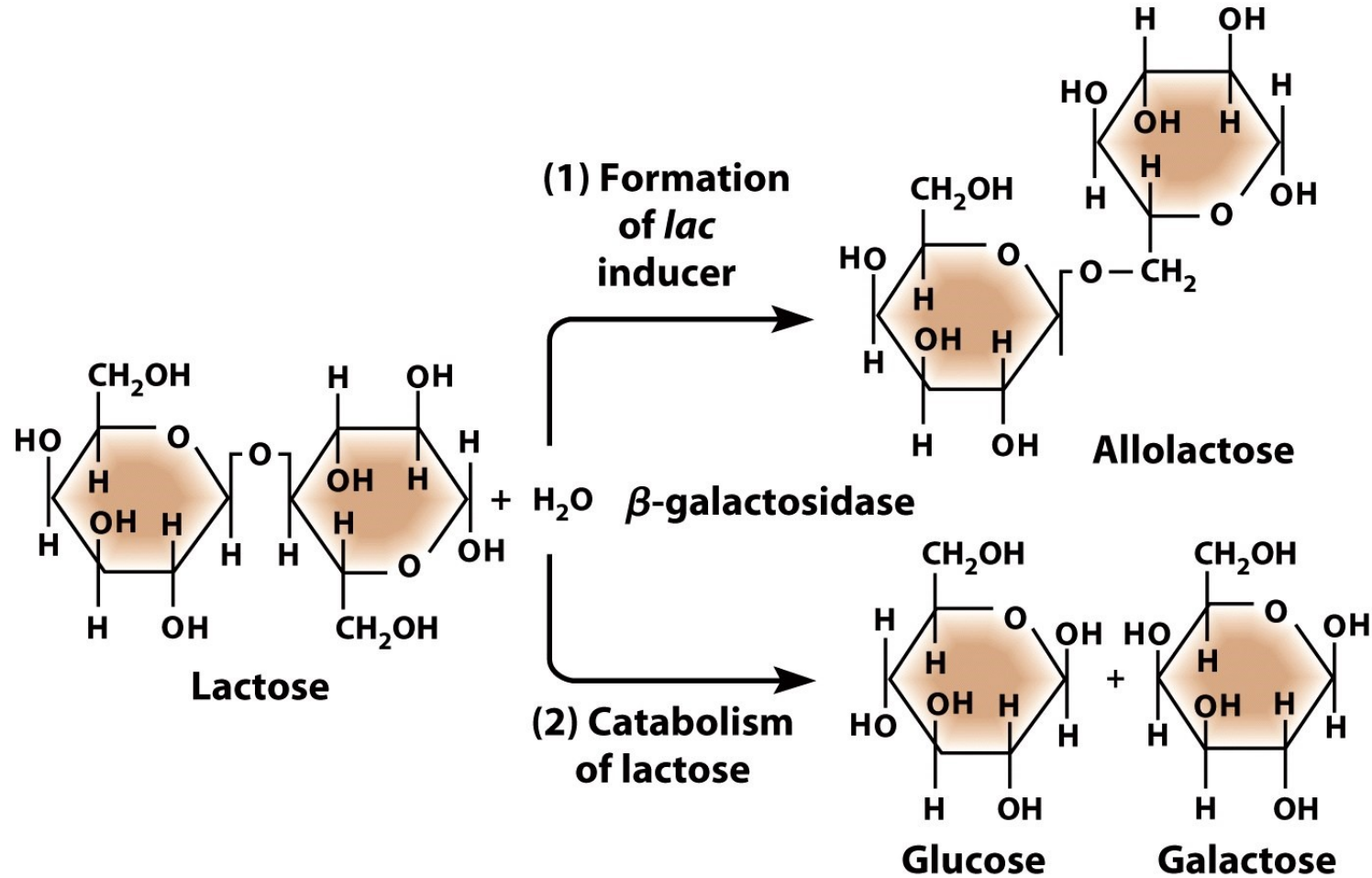


Lac operon induction

- the *lac I* gene encodes a **repressor**
- in the absence of an inducer, the repressor binds to the lac operator and blocks the transcription of structural genes
- the inducer is allolactose, which is formed from lactose by a β -galactosidase catalysed reaction
- by binding of allolactose to the repressor, the repressor is released from the operator, the transcription of structural genes is thus switched on



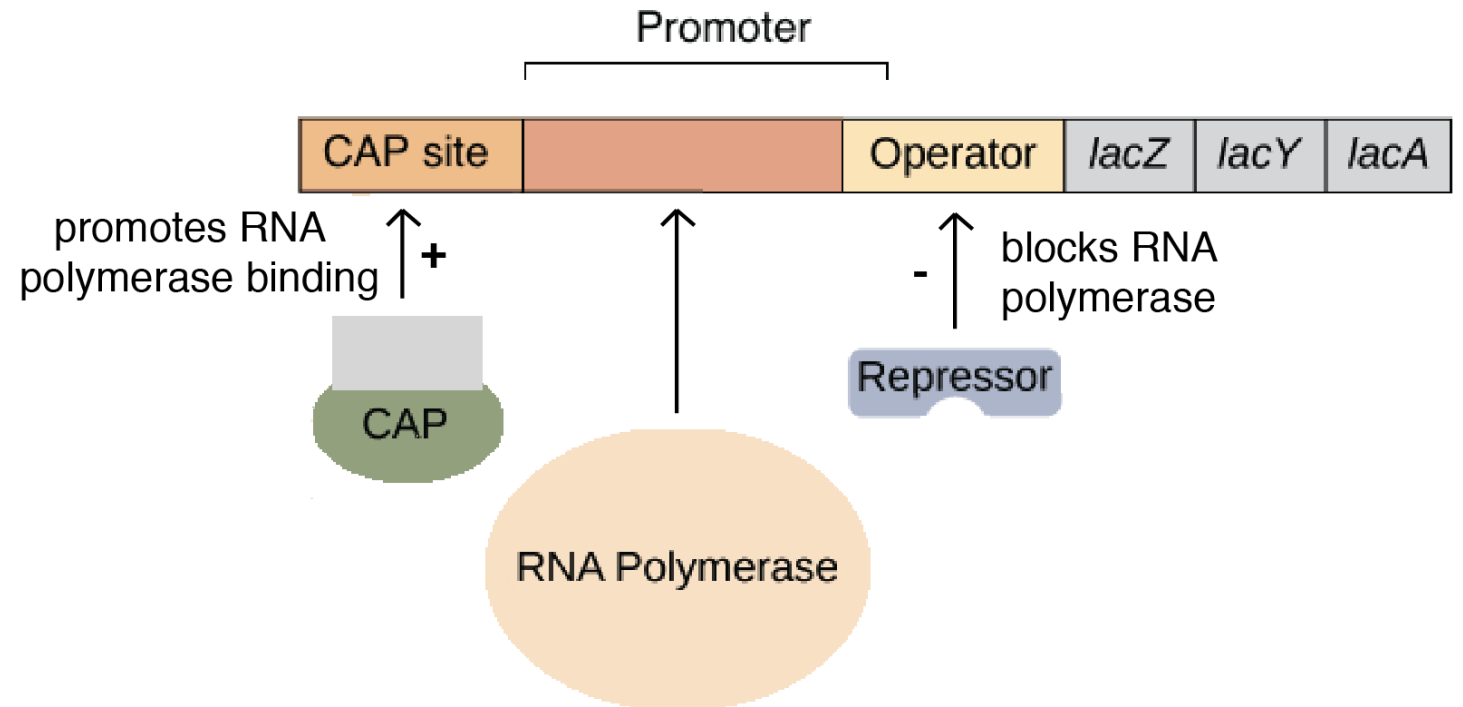
Reaction catalyzed by β -galaktosidase



Structure of the *lac* operon

- The *lac* operon contains three genes: **lacZ**, **lacY**, and **lacA**. These genes are transcribed as a single mRNA, under control of one promoter.
- Genes in the *lac* operon specify proteins that help the cell utilize lactose. *lacZ* encodes an enzyme that splits lactose into monosaccharides (single-unit sugars) that can be fed into glycolysis. Similarly, *lacY* encodes a membrane-embedded transporter that helps bring lactose into the cell.
- The *lacZ* gene encodes an enzyme called **β -galactosidase**, which is responsible for splitting lactose (a disaccharide) into readily usable glucose and galactose (monosaccharides).
- The *lacY* gene encodes a membrane protein called **lactose permease**, which is a transmembrane "pump" that allows the cell to import lactose.
- The *lacA* gene encodes an enzyme known as a **transacetylase** that attaches a particular chemical group to target molecules. It's not clear if this enzyme actually plays any role in lactose breakdown.

The *lac* operon:



In addition to the three genes, the *lac* operon also contains a number of regulatory DNA sequences. These are regions of DNA to which particular regulatory proteins can bind, controlling transcription of the operon.

the promoter is the binding site for RNA polymerase, the enzyme that performs transcription.

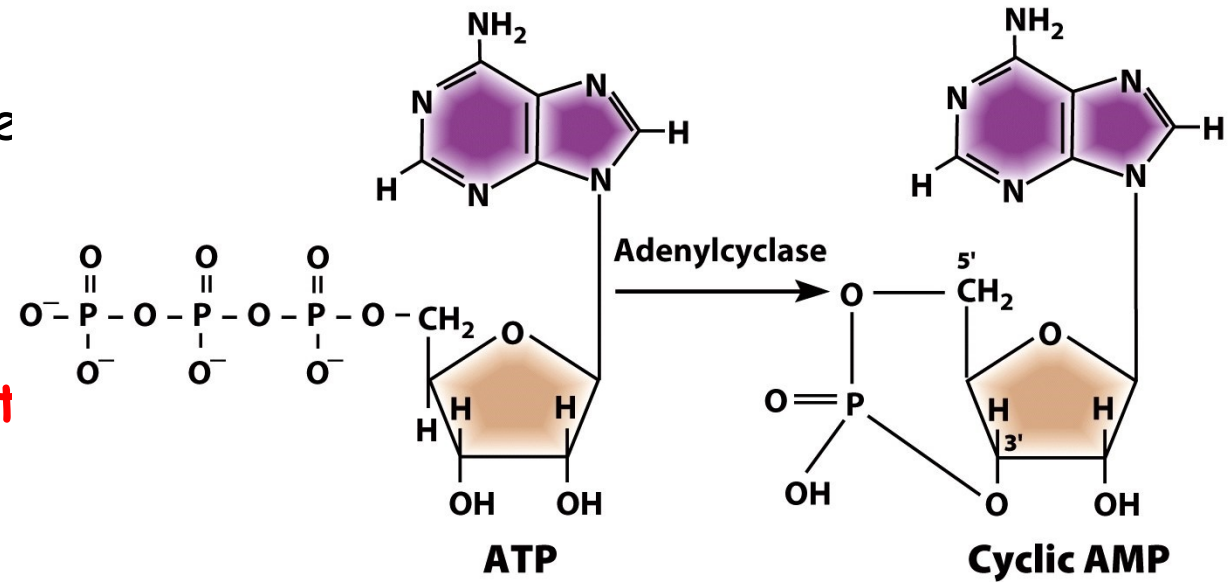
The **operator is a negative regulatory** site bound by the lac repressor protein. The operator overlaps with the promoter, and when the lac repressor is bound, RNA polymerase cannot bind to the promoter and start transcription.

The **CAP binding site is a positive regulatory site** that is bound by catabolite activator protein (CAP). When CAP is bound to this site, it promotes transcription by helping RNA polymerase bind to the promoter.



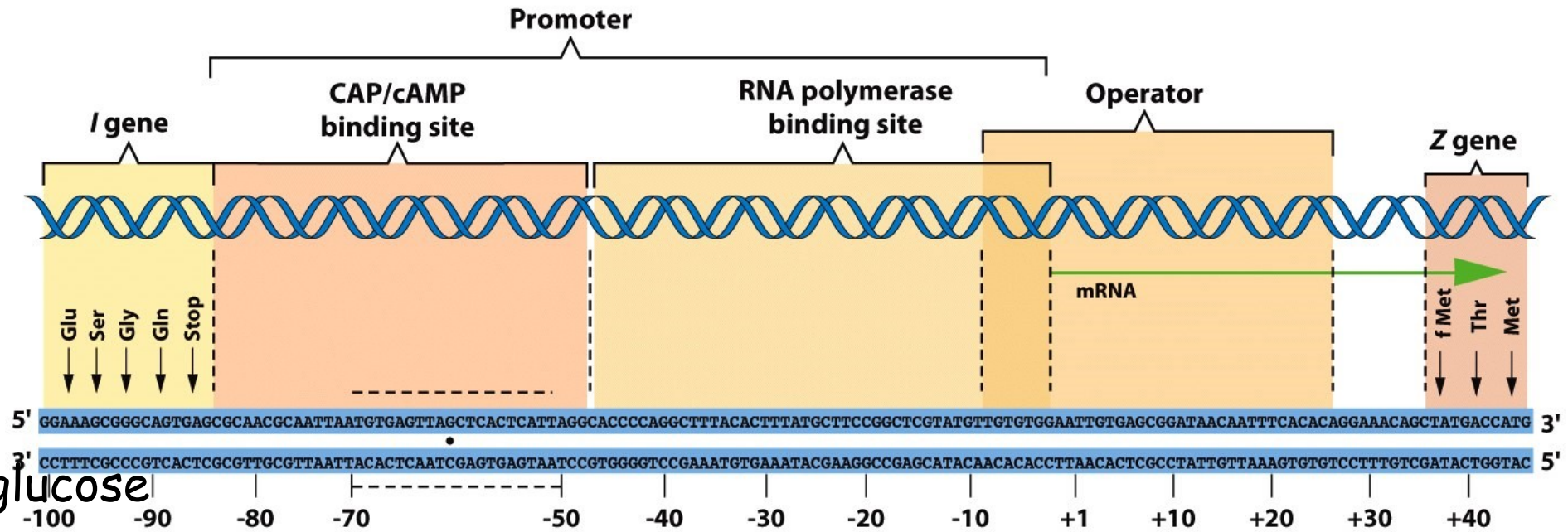
Catabolic repression

- **glucose** prevents the **induction of the lactose operon**: this ensures the preferential use of glucose instead of less efficient energy source
- The lac promoter has two components
- RNA polymerase binding site
- **catabolite activator protein (CAP) binding site**
- binding of the CAP to the promoter activates transcription of the lac operon
- CAP binds to the promoter only in the presence of a sufficient level of cyclic AMP (cAMP) - cAMP functions as an effector molecule
- the level of cAMP is under the control of glucose (**glucose prevents the activation of adenylate cyclase**, ie the enzyme that catalyzes the formation of cAMP)



Organization of the lac operon in the promoter-operator

CAP positively regulates the lac operon, cAMP is an effector



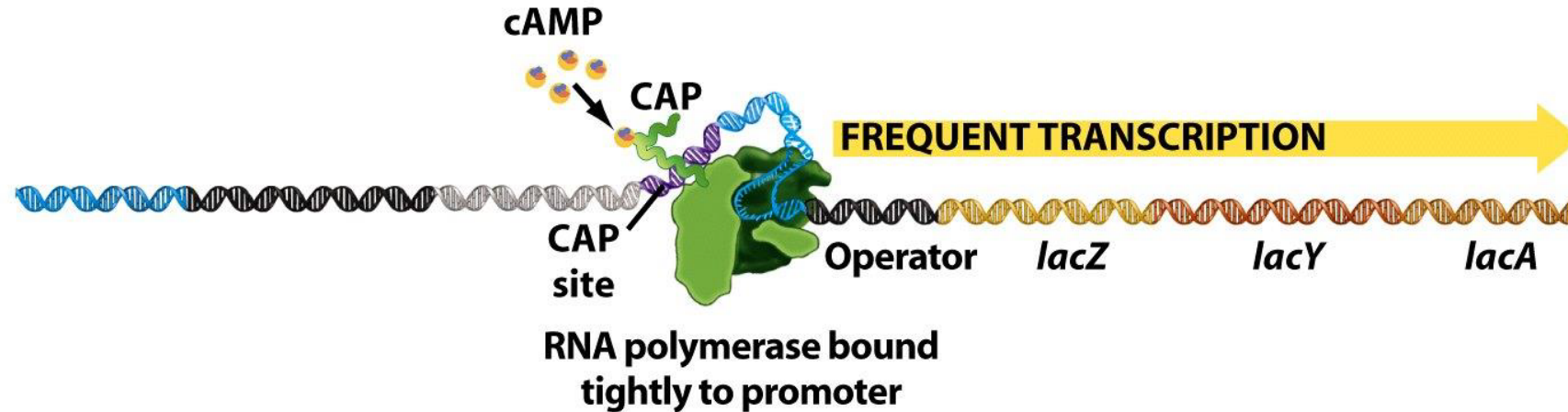
- In the presence of glucose
- adenylate cyclase is inactive
- cAMP levels are low
- CAP cannot bind to a lac operon
- the structural genes of the lac operon are not expressed
- operator
- In the absence of glucose
- adenylate cyclase is active
- cAMP levels are high
- CAP / cAMP binds to the lac operon
- the structural genes of the lac operon are expressed



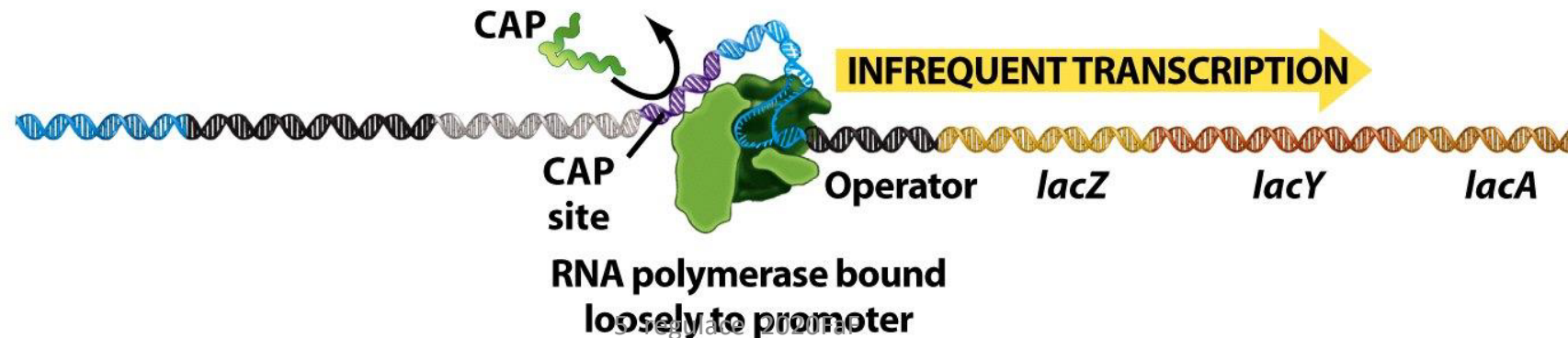
➤ Enzyme induction:

Catabolite activator protein (CAP; also known as [cAMP receptor protein](#), CRP) is a trans-acting [transcriptional activator](#) that exists as a [homodimer](#) in solution.

(a) When cAMP is present, it binds to CAP. The cAMP-CAP complex binds to DNA at the CAP site and increases binding of RNA polymerase to promoter. Transcription occurs frequently.

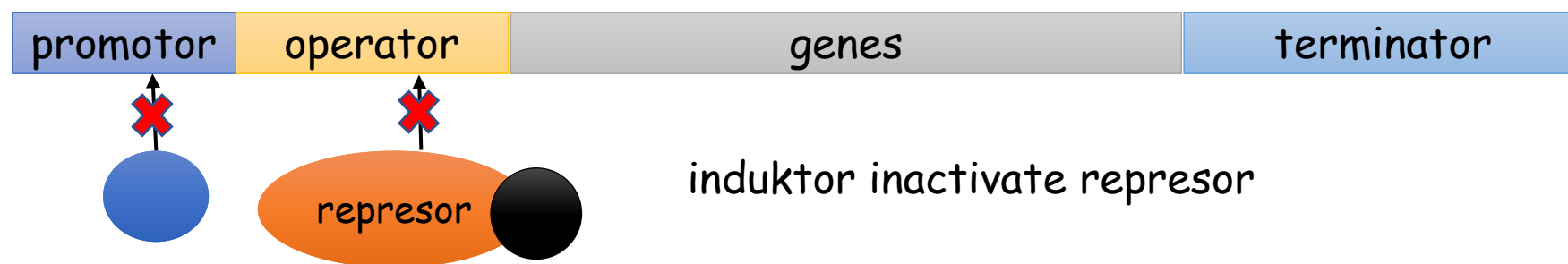


(b) When cAMP is absent, CAP does not bind to DNA. RNA polymerase does not bind the promoter efficiently, and transcription occurs rarely.

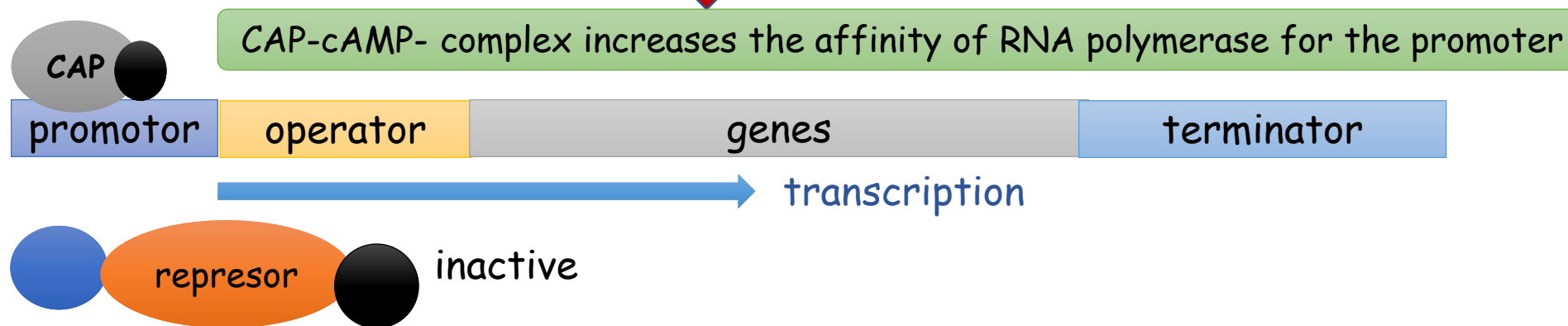


➤ 2) Catabolic repression:

- the substrate suppresses the synthesis of inducible enzymes even in the presence of an inducer
- Example: Galactose suppresses β -galactosidase synthesis even in the presence of lactose as an inducer



RNA polymerase has a low affinity for the promoter - it does not bind and transcription occurs at a low frequency



↑ affinity of RNA polymerase for promoter-binding transcription is ongoing



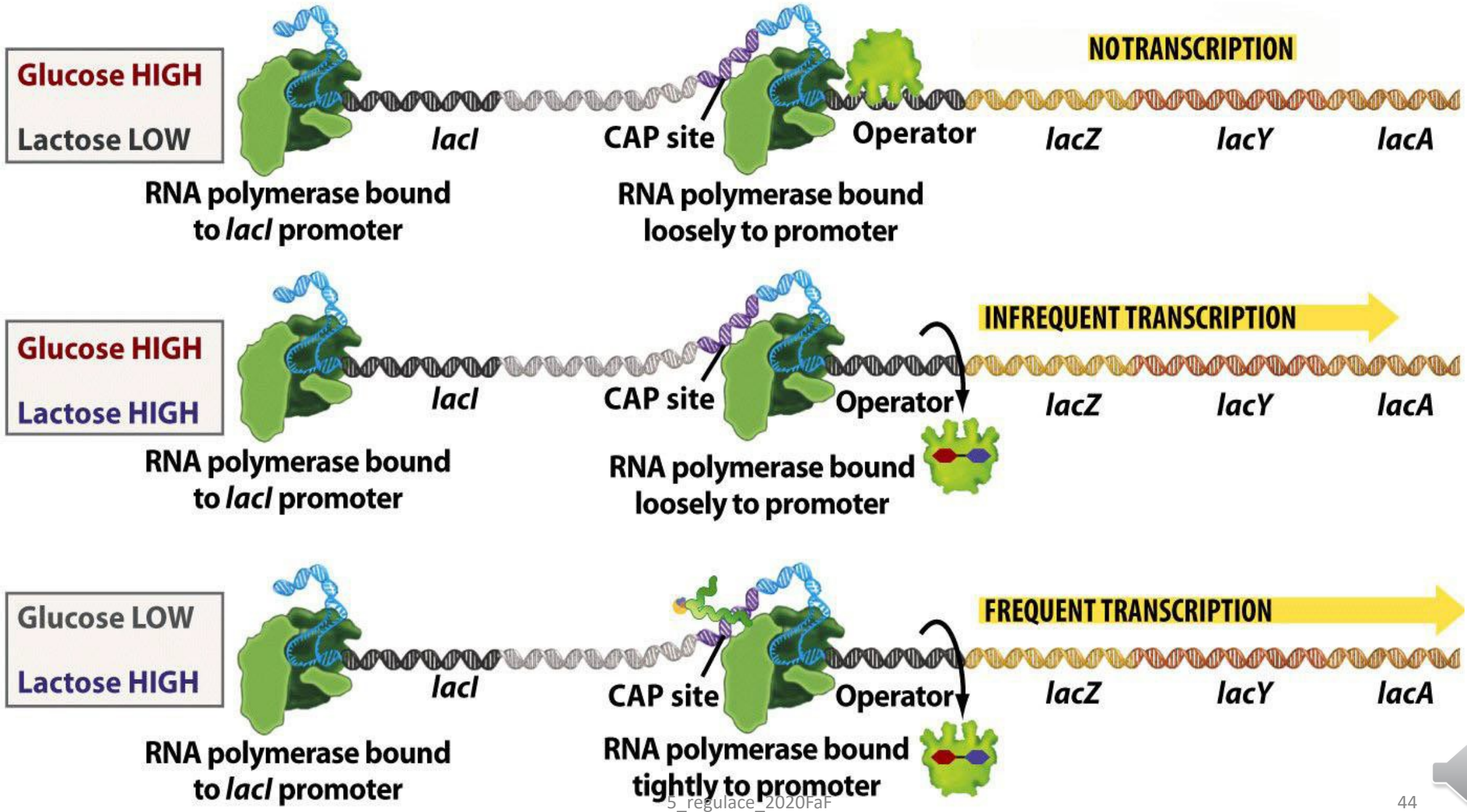


Figure 17-10 Biological Science, 2/e

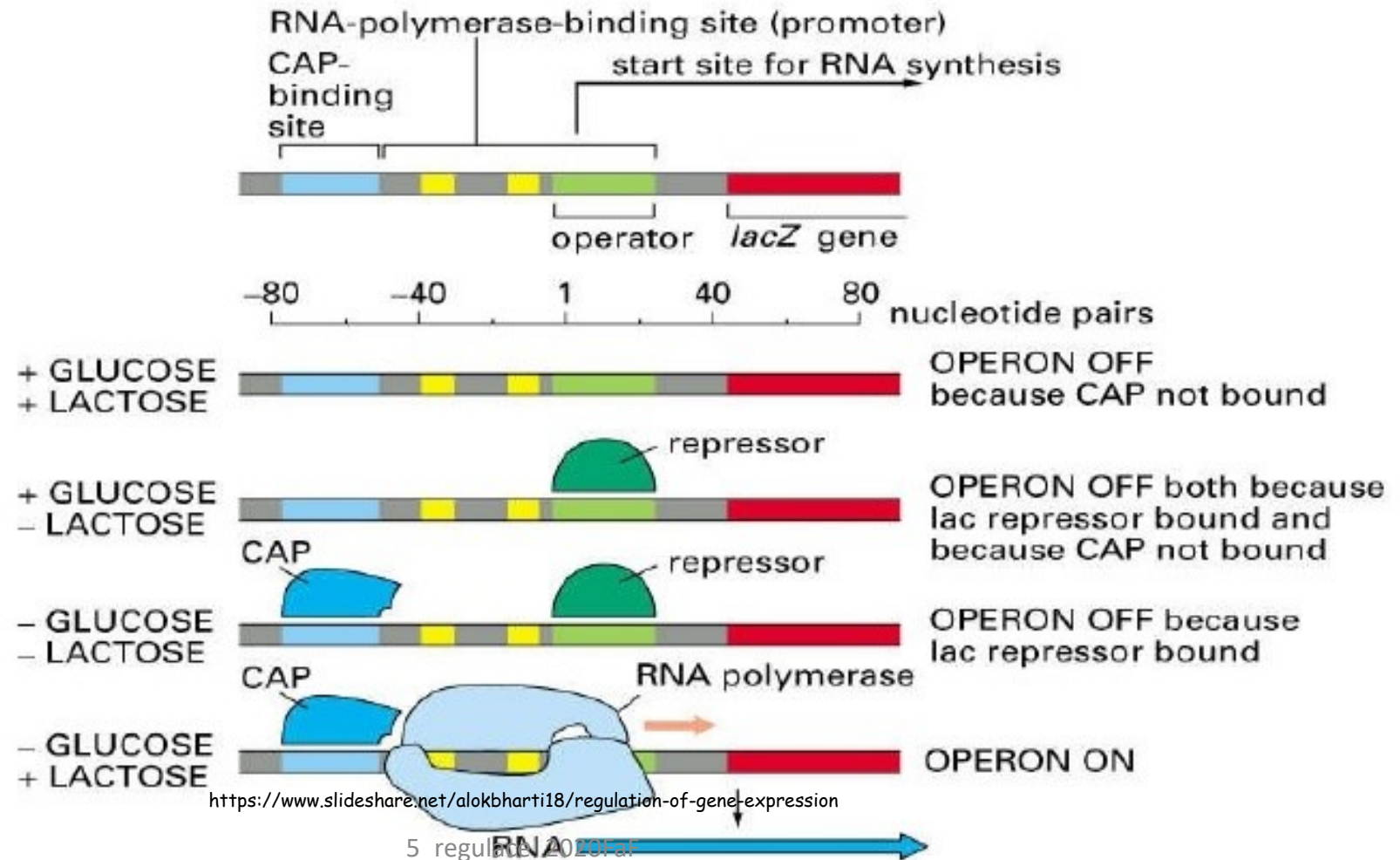


5_regulace_2020FaF

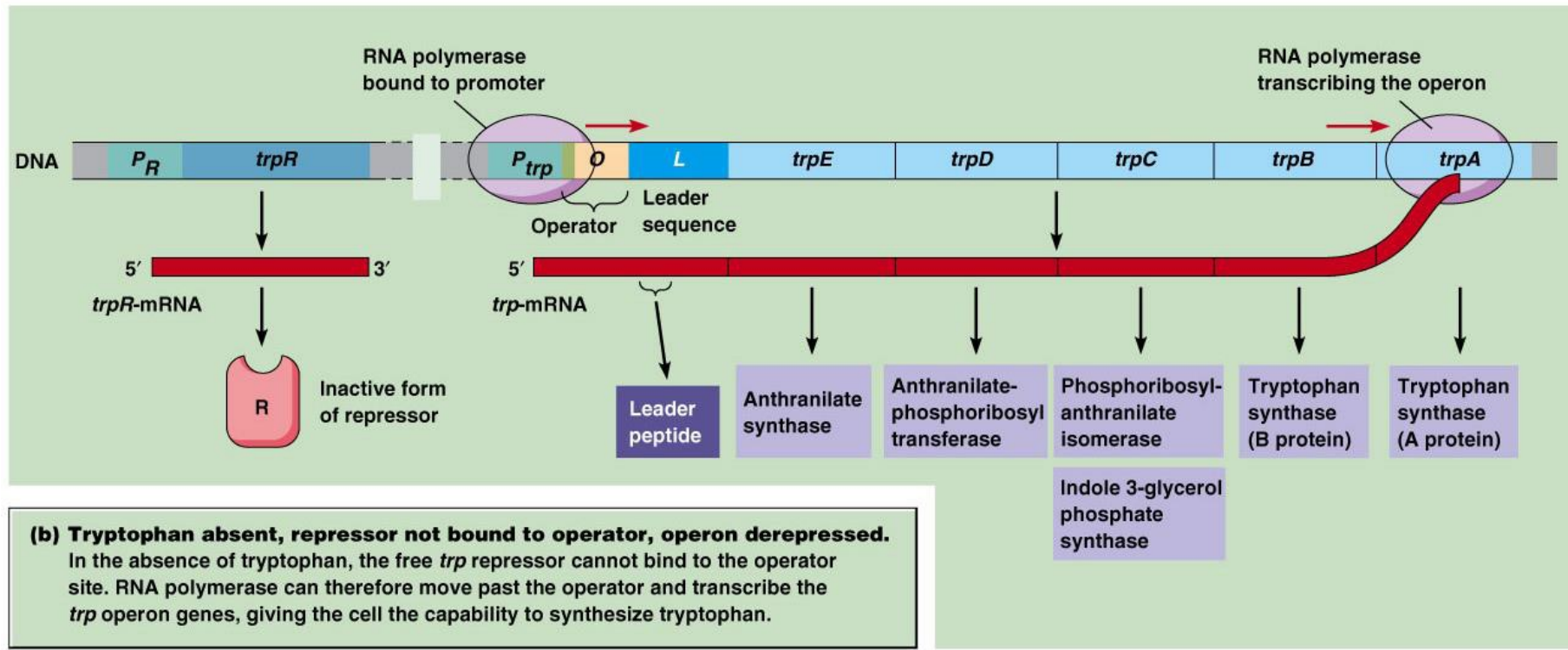
➤ Regulation- lac operonu:

Combinatory Regulation of Lac Operon:

CAP: catabolite activator protein; breakdown of lactose when glucose is low and lactose is present

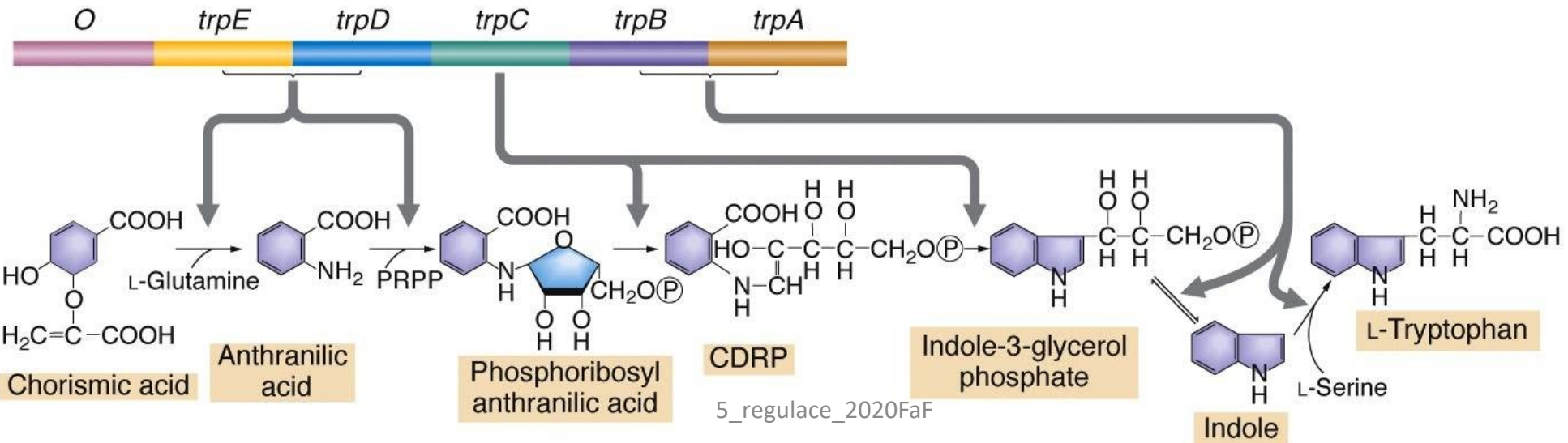


1.



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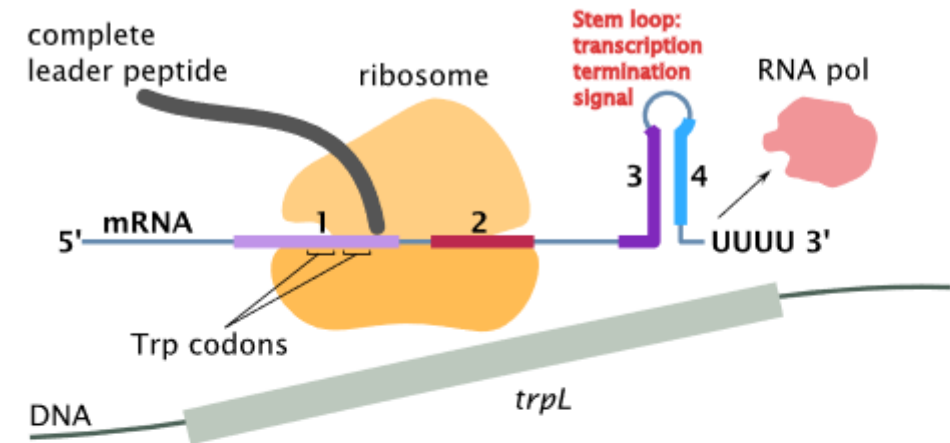
2.



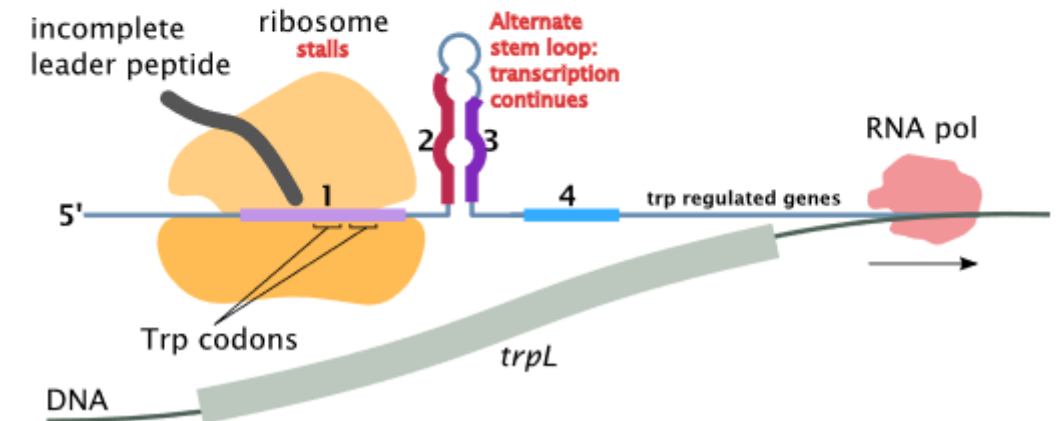
Attenuation

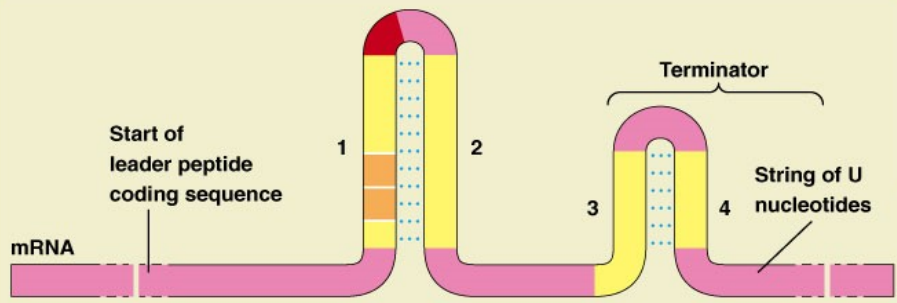
- Attenuation means the premature termination (interruption) of transcription by a change in the secondary structure of the mRNA. It occurs when a cell does not need a transcription product. Part of the operon is an area called the attenuator.
- In prokaryotes, both transcription and translation take place at the same time. Imagine that behind the RNA polymerase, which produces mRNA for tryptophan synthesis proteins, the ribosome is already moving and forming these proteins. As RNA polymerase approaches the 5' end of the transcript, many codons for tryptophan appear.
- An example is the trp operon that produces tryptophan:

High level of tryptophan

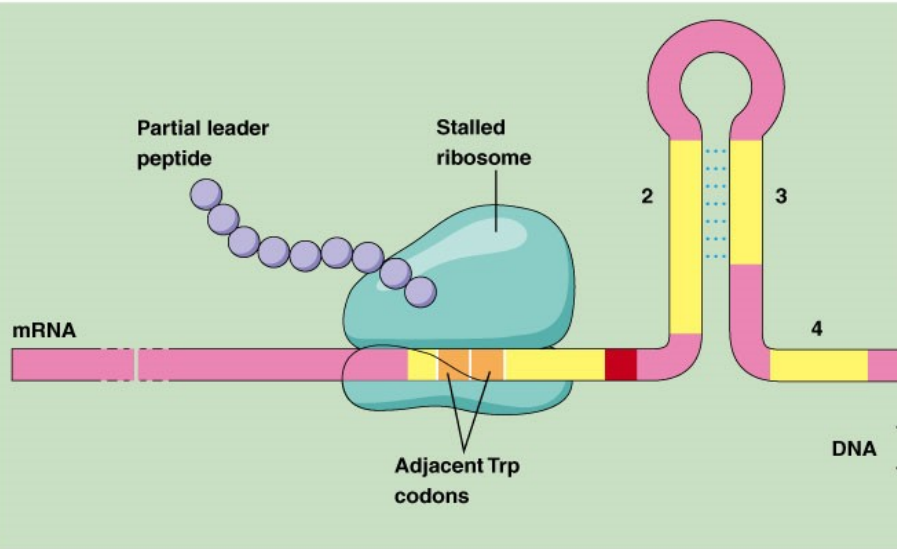


Low level of tryptophan



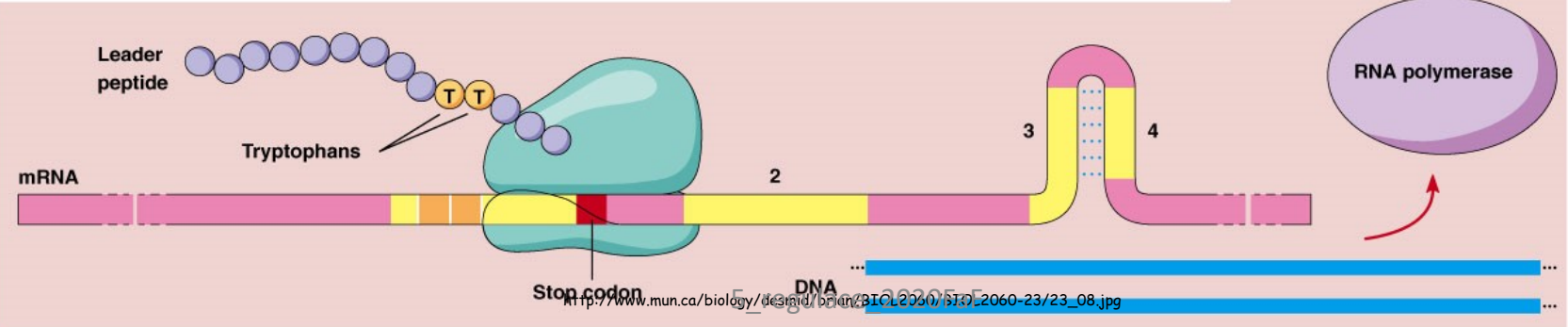


(a) The most stable secondary structure for *trp* leader mRNA. Attenuation depends on the ability of regions 1 and 2 and regions 3 and 4 of the *trp* leader sequence to base-pair, forming hairpin secondary structures. The 3-4 hairpin structure acts as a transcription termination signal.



(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.

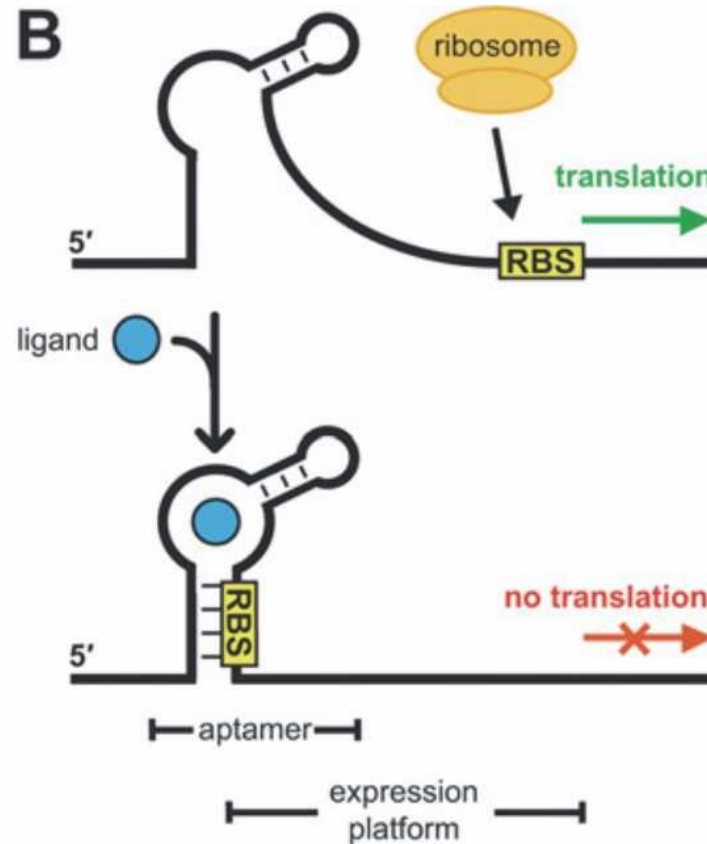
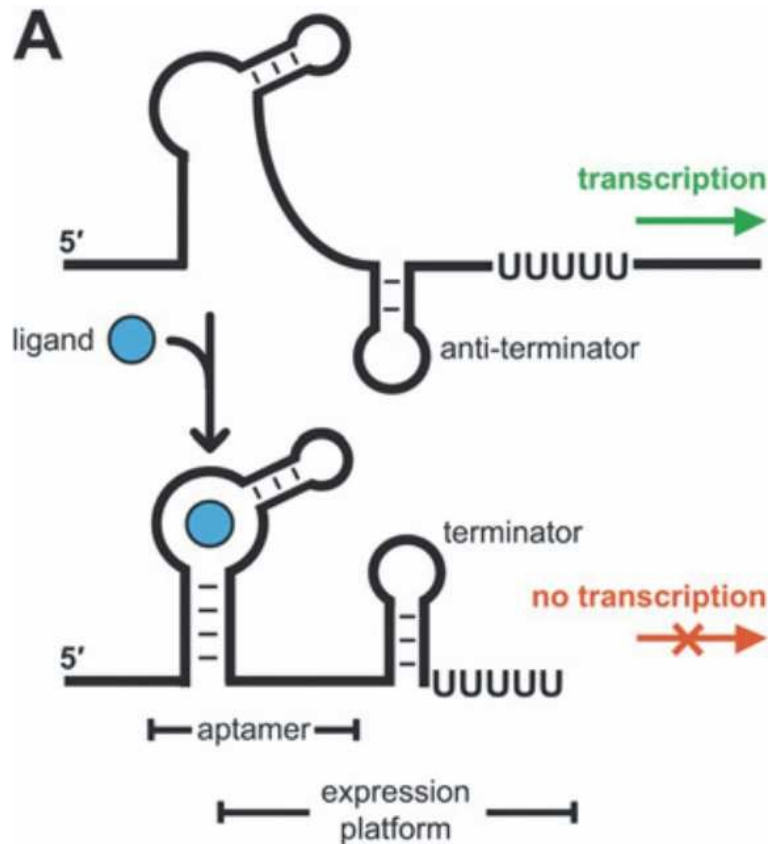


Riboswitch:

TEST

secondary structure of mRNA capable of binding any ligand

Binding of the ligand to the mRNA changes its secondary structure - "on" or "off" gene expression



riboswitch is a regulatory segment of a [messenger RNA](#) molecule that binds a [small molecule](#), resulting in a change in [production](#) of the [proteins](#) encoded by the mRNA. Thus, an mRNA that contains a riboswitch is directly involved in regulating its own activity, in response to the concentrations of its [effector](#) molecule. The discovery that modern organisms use RNA to bind small molecules, and discriminate against closely related analogs, expanded the known natural capabilities of RNA beyond its ability to code for [proteins](#), [catalyze reactions](#), or to bind other RNA or protein [macromolecules](#).

Transkripce
Translace



Alternative sigma factors:

- It recognizes the promoters of all transcription units
- Composed of 5 types of subunits
- 2x α
- 40 kDa - maintains the stability of the complex
- 1x β
- 155 kDa - Key for rNTP binding to enzyme
- 1x β'
- 160 kDa - Key for template DNA template binding
- 1x ω
- 160 kDa - regulatory and stabilization roles
- 1x σ
- 85 kDa - the so-called σ -factor - Key for binding to the promoter - several "interchangeable" variants, each providing binding to a different type of promoter
- The rate of polymerization is about 15-20 nt / s - depends on the presence of σ -factor, ribosomes and the like

Sigma factor (σ factor) is required to initiate protein transcription in bacteria. It is a bacterial transcription initiation factor that allows specific binding of RNA polymerase (RNAP) to gene promoters.

Prokaryotic RNA polymerase

