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Program: M.Sc., Microbiology

Course Title : Microbial Genetics & Molecular Biology

Course Code: 24MICCC3

Unit V: Regulation of Gene Expression

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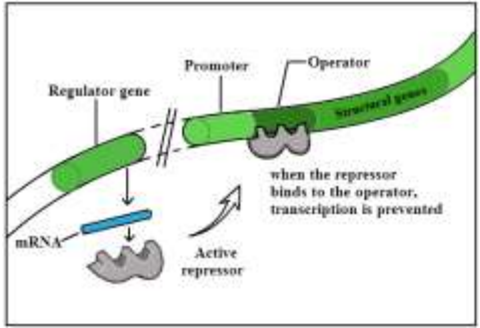
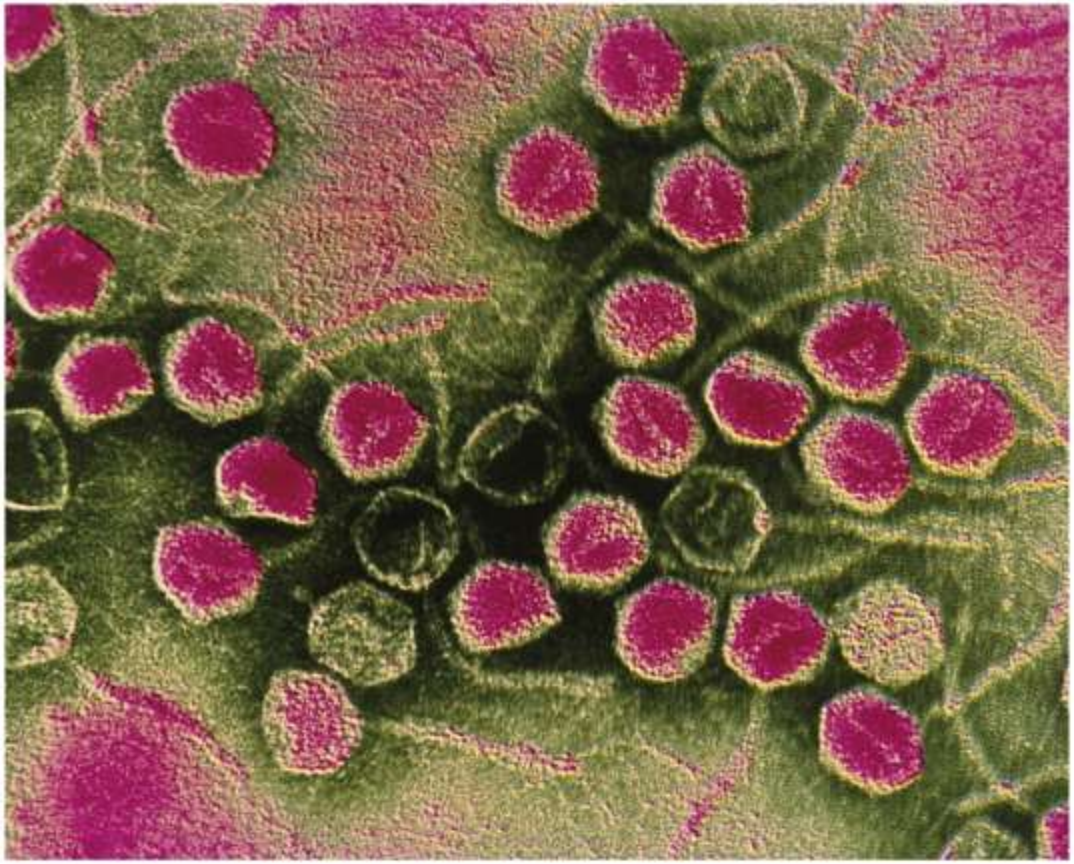


Regulation of Gene Expression in Prokaryotes



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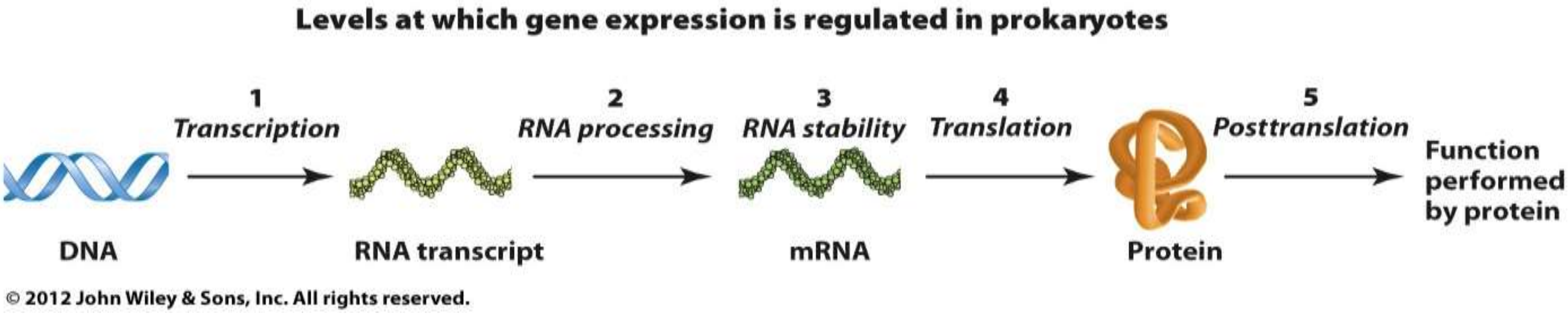
Overview

- Gene Expression
  - Constitutive, Inducible, and Repressible
  - Positive and Negative Control
- Operons:
  - The Lactose Operon in *E. coli*: Induction and Catabolite Repression
  - The Tryptophan Operon in *E. coli*: Repression and Attenuation

- Translational Control of Gene Expression

- Post-Translational Regulatory Mechanisms

Regulation of Gene Expression in Prokaryotes



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- Mechanisms that involve the rapid **turn-on and turn-off** of gene expression in response to environmental changes. **"Plasticity---------adaptability"**

- Preprogrammed **circuits or cascades or system or pathways** of gene

  Expression. **"Proper sequence of events"**

Gene Expression:
Constitutive, Inducible, and Repressible

- **Constitutive:** Genes that specify cellular components that perform housekeeping functions— for example, the ribosomal RNAs and proteins involved in protein synthesis —are expressed constitutively.

- **Non-Constitutive:** Other genes often are expressed only when their products are required for growth.

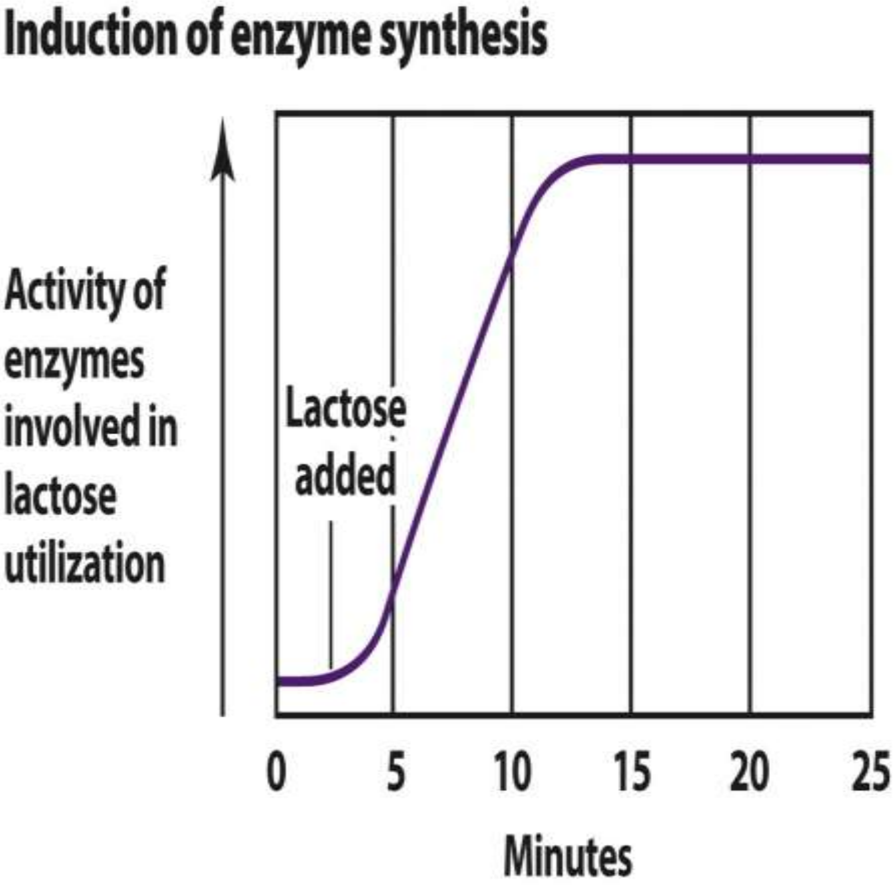
Constitutive Genes

- Gene products (e.g., tRNAs, rRNAs, ribosomal proteins, DNA and RNA polymerase subunits, ATP and GTP, enzymes catalyzing **housekeeping functions**) are essential components of almost all living cells.
- Genes that specify products of this type are **continuously expressed** in most cells.

Non-Constitutive Genes: Inducible and Repressible Genes

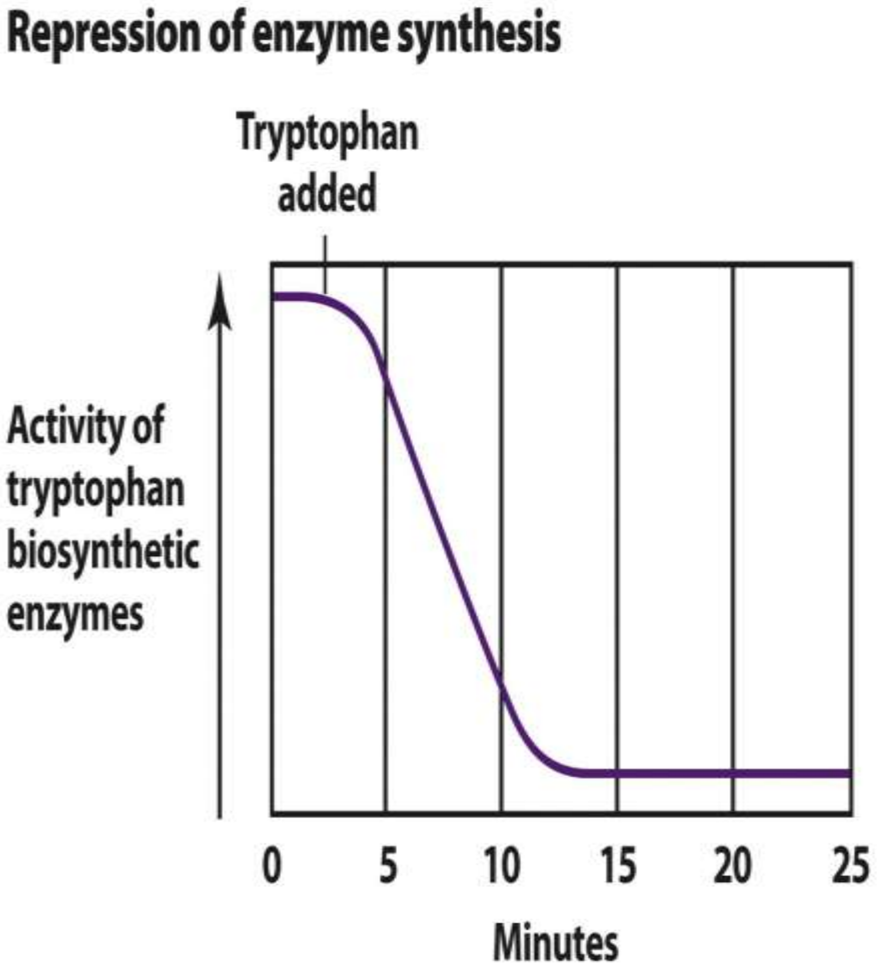
- Other gene products are needed for cell growth only under certain environmental conditions (aerobics and anaerobics).
- Regulatory mechanisms allow the synthesis of these gene products only when they are needed.

Induction of Genes for Lactose Utilization

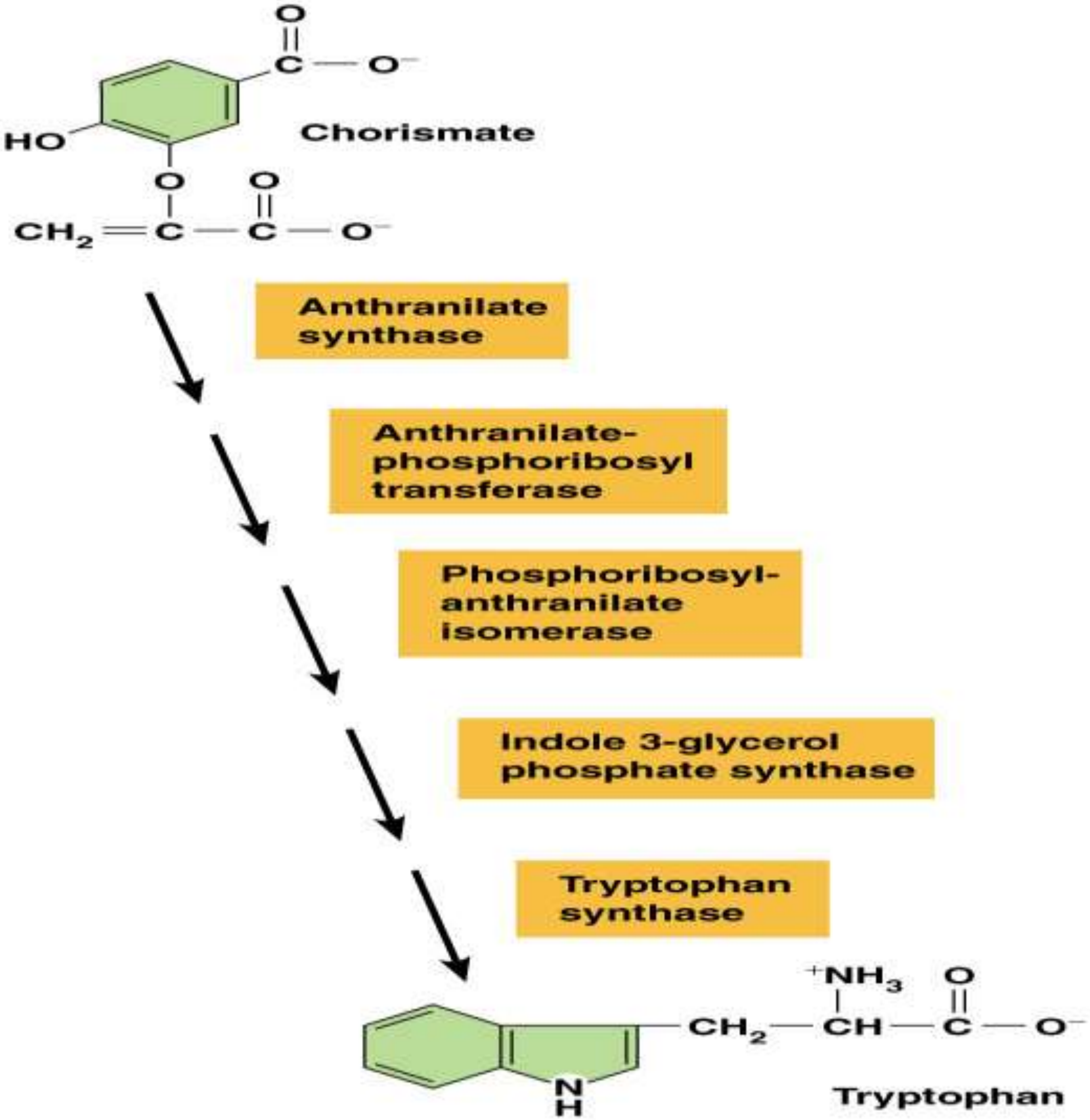


- Gene expression is induced when glucose is absent and lactose is present.
- Induction occurs at the level of transcription and alters the rate of enzyme synthesis.
- Enzymes involved in catabolic pathways are often inducible.

Repression of Genes for Tryptophan Biosynthesis



- Genes are <u>turned on</u> (depressed) in the absence of tryptophan and <u>turned off</u> (repressed) when tryptophan is available.
- Repression occurs at <u>the level of transcription.</u>
- Enzymes involved in <u>anabolic pathways</u> are often repressible.



Chorismate

Anthranilate synthase

Anthranilate-phosphoribosyl transferase

Phosphoribosyl-anthranilate isomerase

Indole 3-glycerol phosphate synthase

Tryptophan synthase

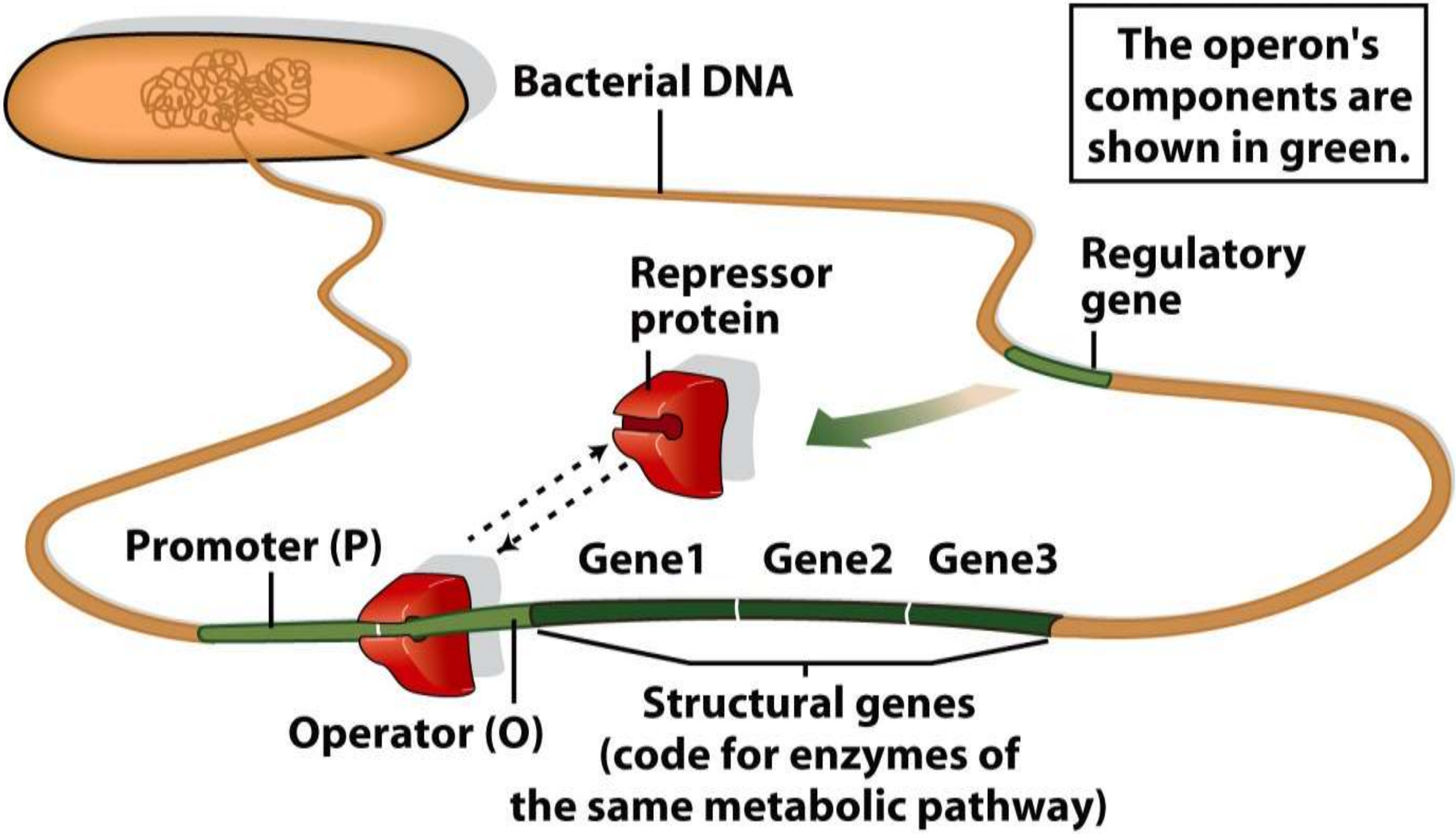
Tryptophan

Gene Expression

The product of a **regulatory gene** is required to **initiate (turn on)** the expression of one or more genes.

The product of a **regulatory gene** is required to **turn off** the expression of one or more genes.

Regulatory gene: Organization of a bacterial operon



Positive and Negative Control Mechanisms

- **Regulator genes** encode products that regulate the expression of other genes.

- In **positive control mechanisms**, the product of the regulator gene is required to **turn on** the expression of structural genes.

- In **negative control mechanisms**, the product of the regulator gene is necessary to **shut off** the expression of structural genes.

- The product of the regulator gene acts (regulator protein) by binding to a site called the regulator protein binding site (*RPBS*) adjacent to the promoter of the structural gene(s).

- In positive control systems, the regulator gene products are called **activators** because they activate transcription of the structural gene(s).

- In negative control systems, the regulator gene products are called **repressors** because they repress transcription of the structural gene(s).

Effector Molecules

- Whether or not a **regulator protein** can bind to the ***RPBS*** depends on the presence or absence of **effector molecules** in the cell.

- **Inducers** are effector molecules involved in induction of gene expression.

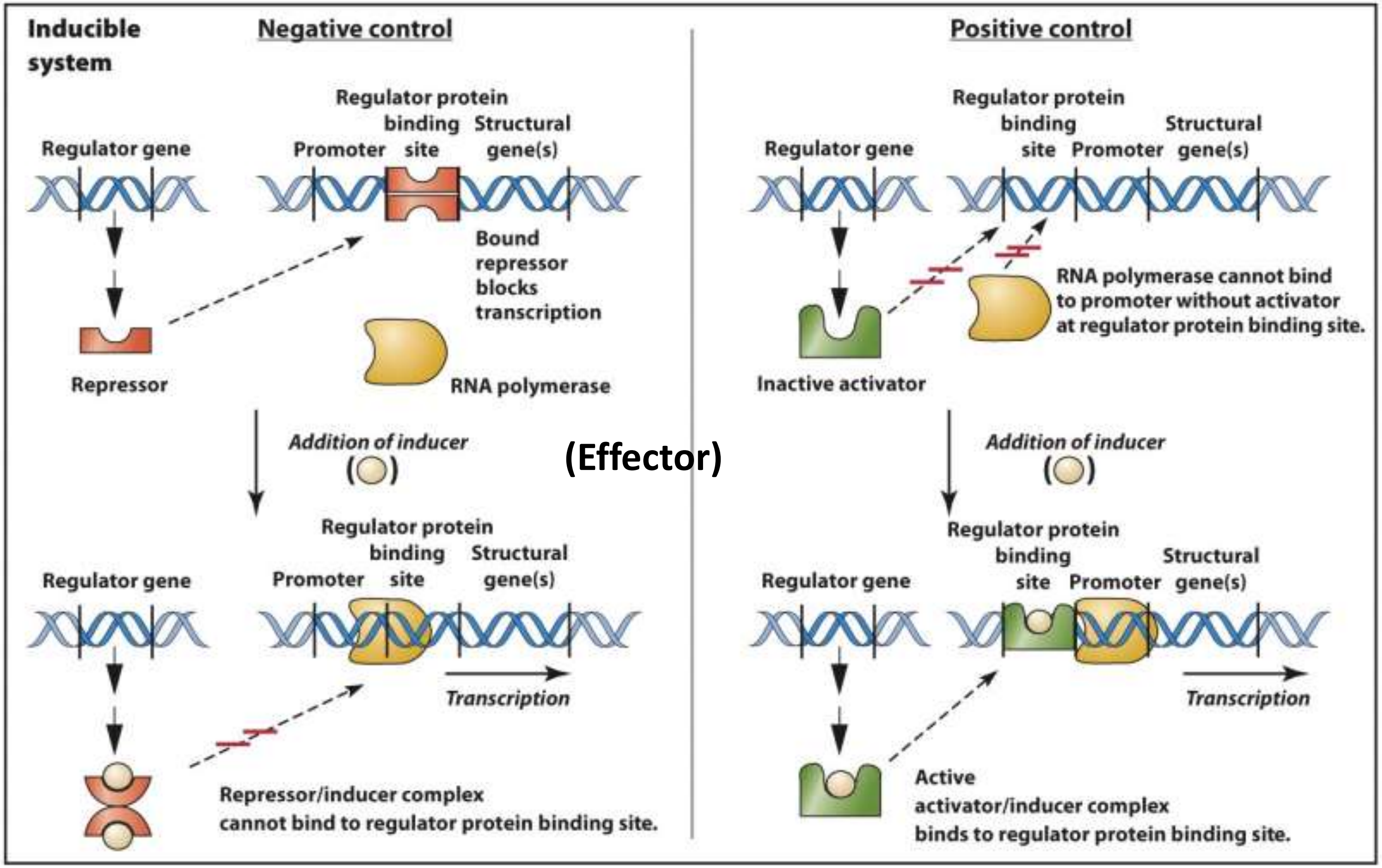
- **Co-repressors** are effector molecules involved in repression of gene expression.

Allosteric Transitions

- Effector molecules bind to regulator gene products (regulator protein) and cause conformational changes in protein structure called **allosteric transitions.**

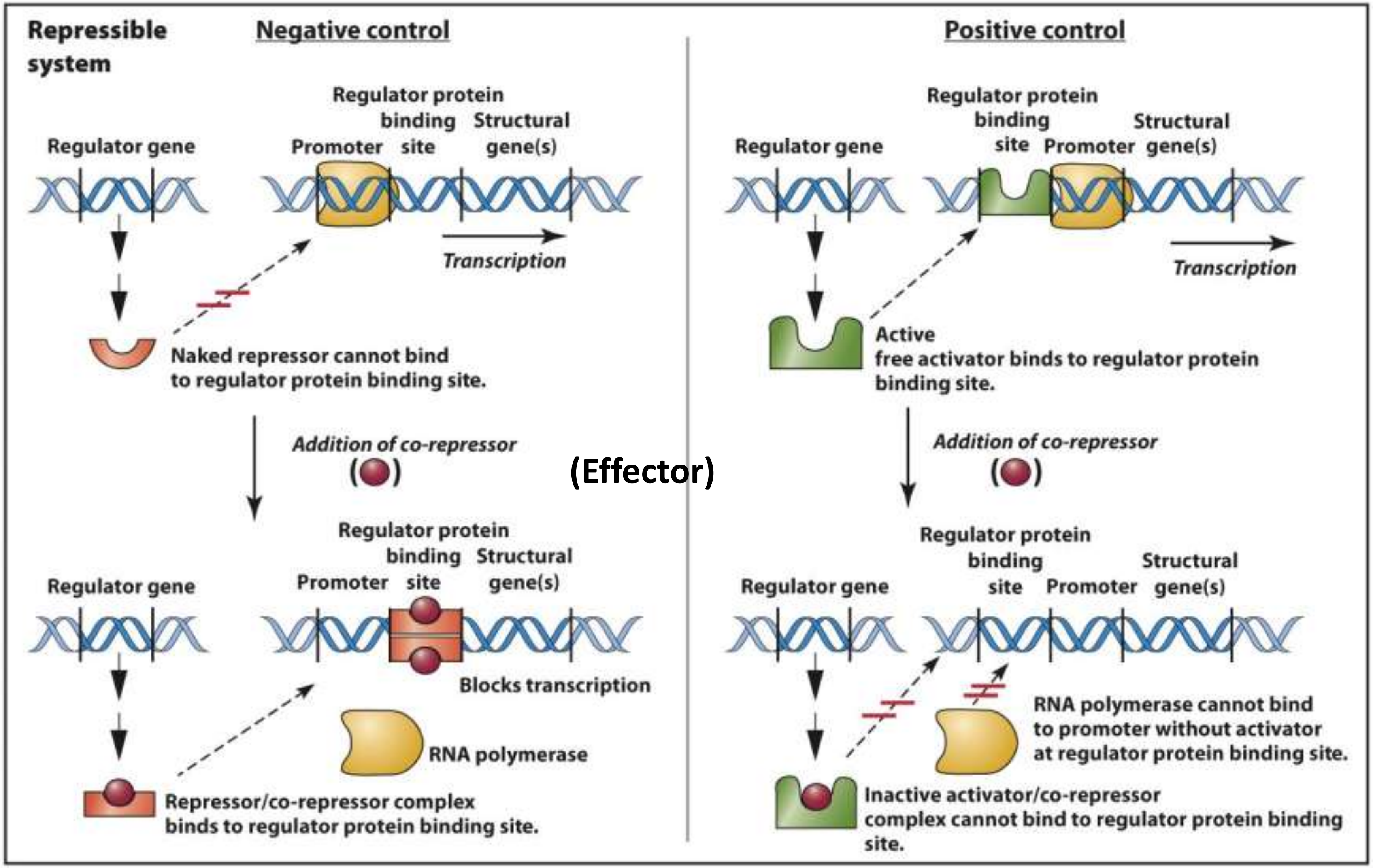
- Allosteric transitions caused by the binding of effector molecules to activators and repressors alter their ability to bind to the *RPBS.*

Regulation of Inducible Systems



(Effector)

Regulation of Repressible Systems



(Effector)

Regulatory Mechanisms

- In a **positive control mechanism**, the activator is involved in **turning on** gene expression.

- In a **negative control mechanism**, the co-repressor is involved in **turning off** gene expression.

- With both positive and negative control mechanisms, whether gene expression is **inducible or repressible** depends on whether the **free regulator protein/** or **the regulator protein/ effector molecule complex** binds to the *RPBS*.

Operons

Units required for Gene Expression

↓

Sequence of DNA

In prokaryotes, the **operon** includes structural genes, the operator and the promoter.

Components of the Operon Model

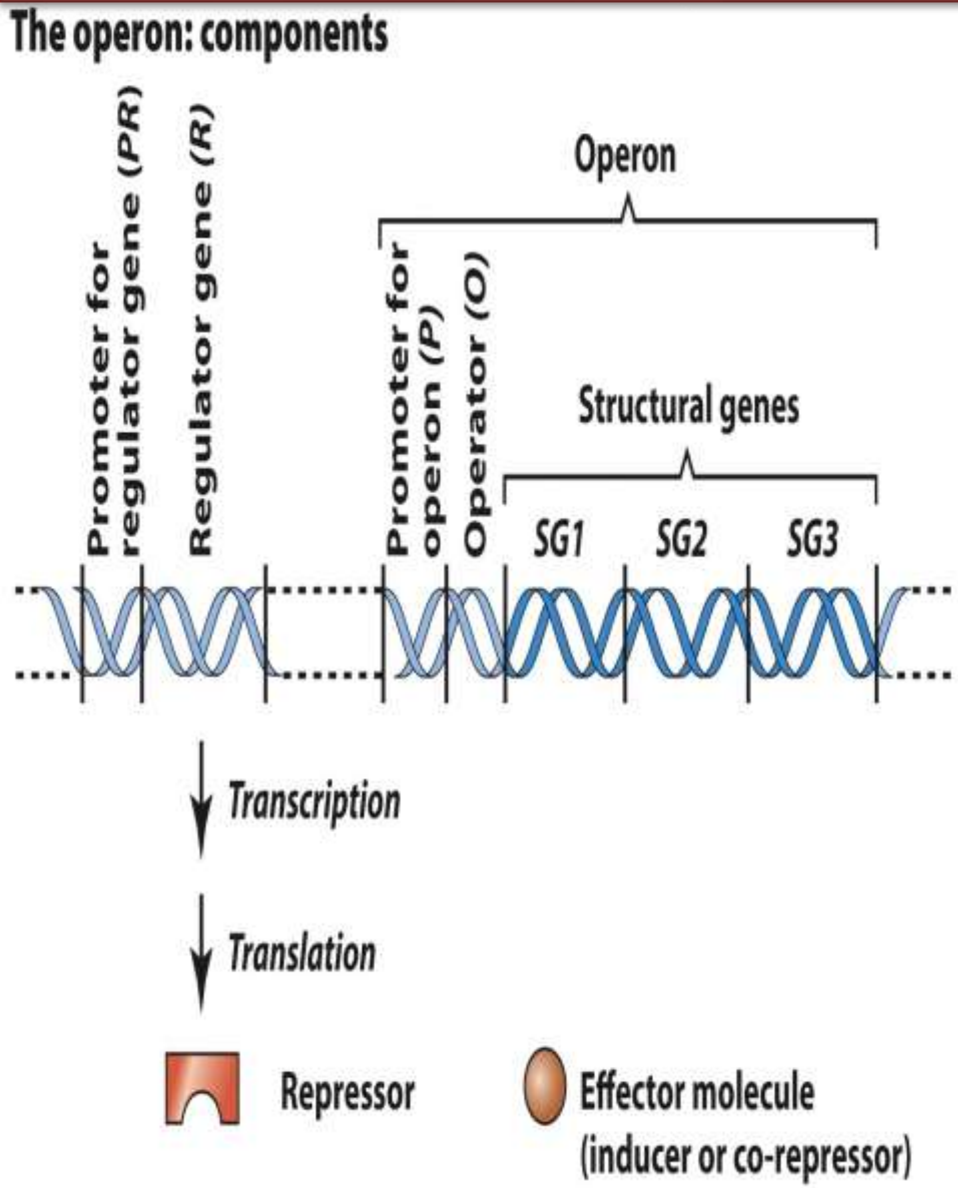
- The repressor gene encodes a **repressor**.
- The repressor binds (under appropriate conditions) to the **operator**. Binding is regulated by the presence or absence of the **effector molecule** (inducer or co-repressor).

- The **promoter** is the site of transcription initiation for the structural gene(s).
- Transcription of the **structural gene(s)** is regulated by binding of the repressor to the operator.

Control of Gene Expression in Bacteria

- The Bacterial Operon
  - An **operon** is a functional complex of genes containing the information for enzymes of a metabolic pathway. It includes:
    - **Structural genes** – code for the enzymes and are translated from a single mRNA (**Polycistronic)**.
    - **Promoter** – where the RNA polymerase binds.
    - **Operator** – site next to the promoter , where the regulatory protein can bind.
    - A **repressor** (~proteins) which binds to a specific DNA sequence to determine whether or not a particular gene is transcribed.
    - The **regulatory gene** encodes the repressor protein

The Operon Model



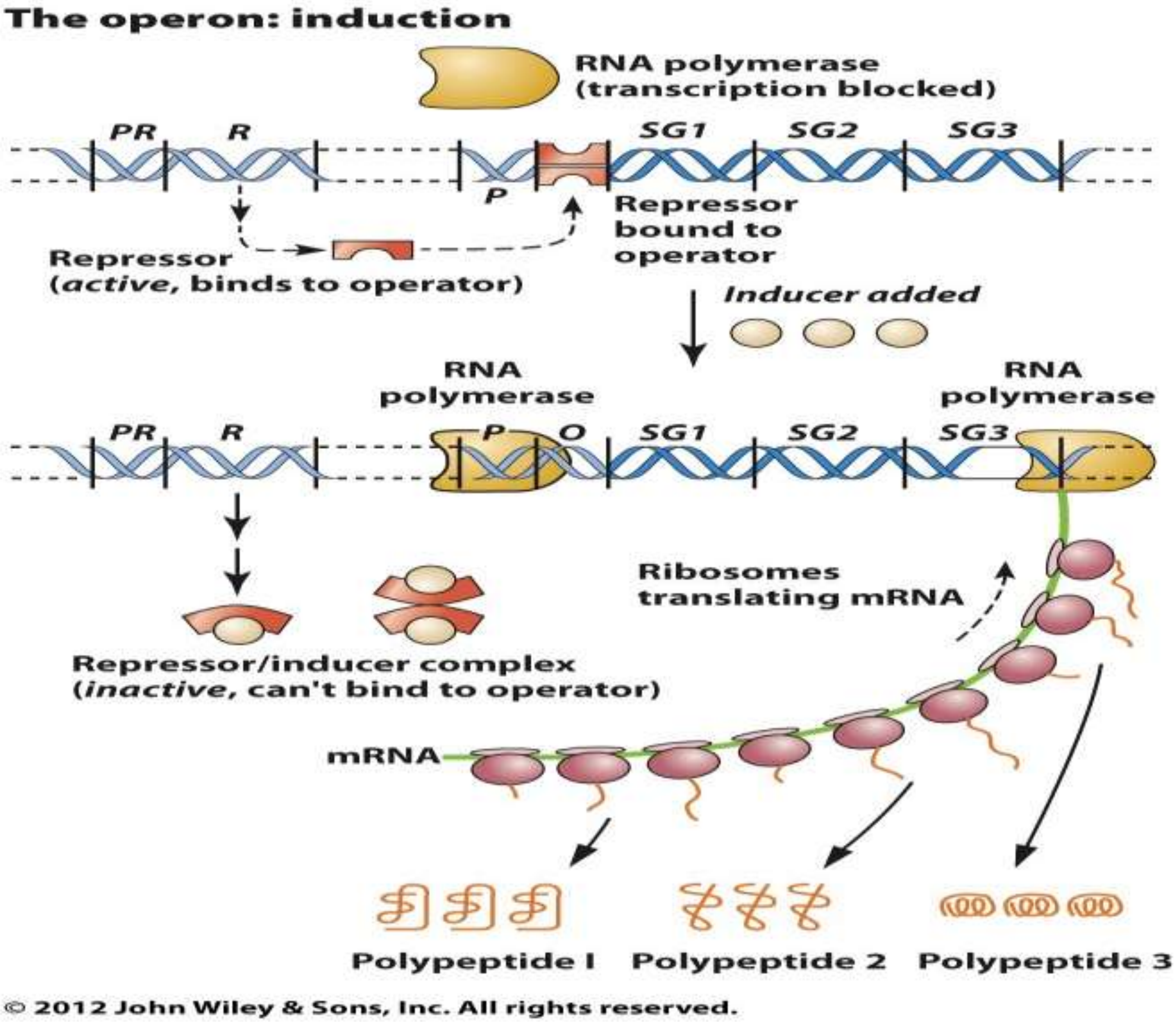
Each operon contains

--several contiguous structural genes,

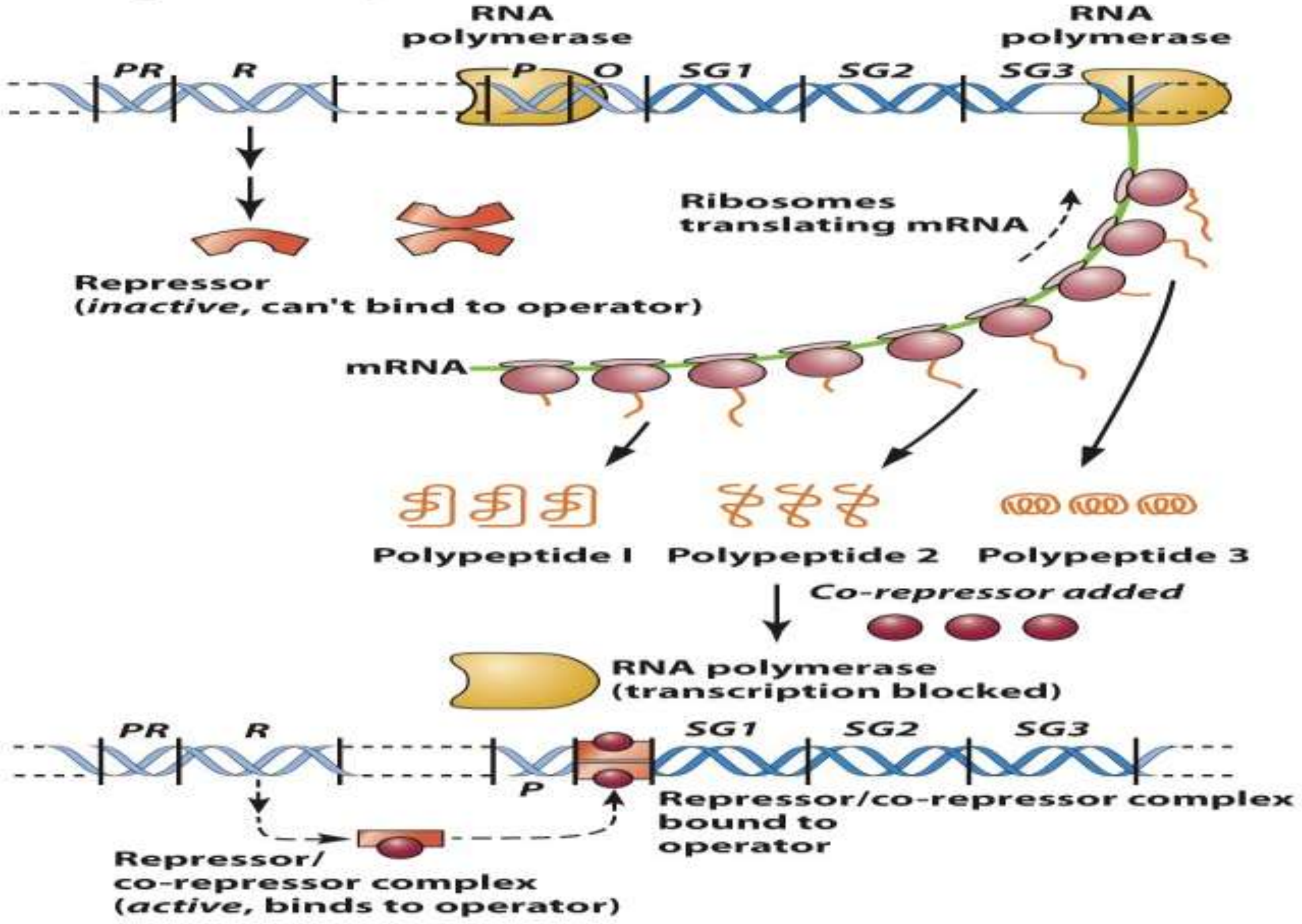
--a promoter

--an operator

An Inducible Operon



A Repressible Operon



The operon: repression

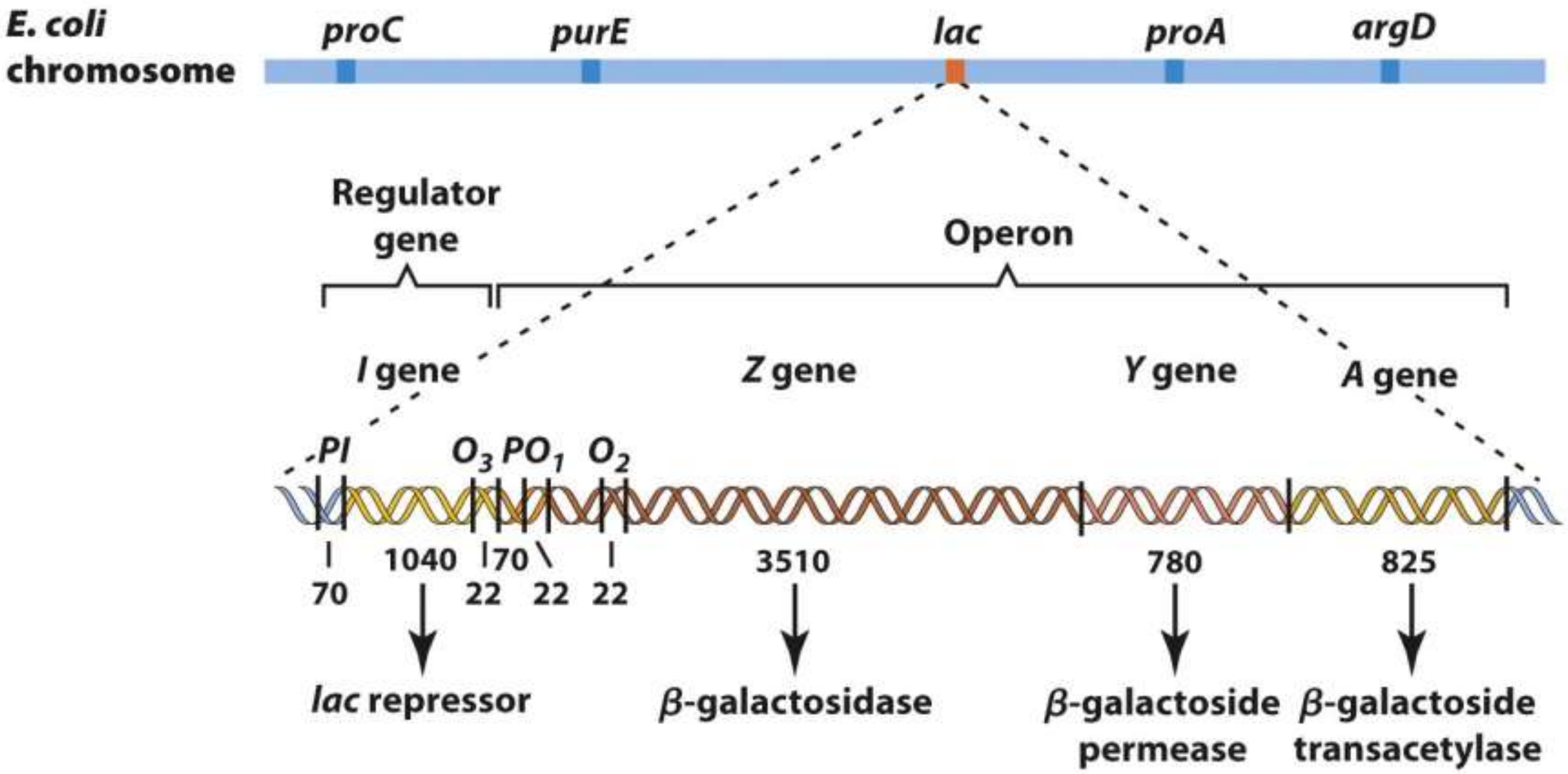
The Structural Genes of an Operon

- A **single mRNA transcript** carries the coding information of an entire operon.
- **Operons** containing more than one structural gene are **multigenic.**
- **All structural genes** in an operon are **co-transcribed** and therefore are coordinately expressed.

The Lactose (lac) Operon in *E. coli:* **Induction and Catabolite Repression**

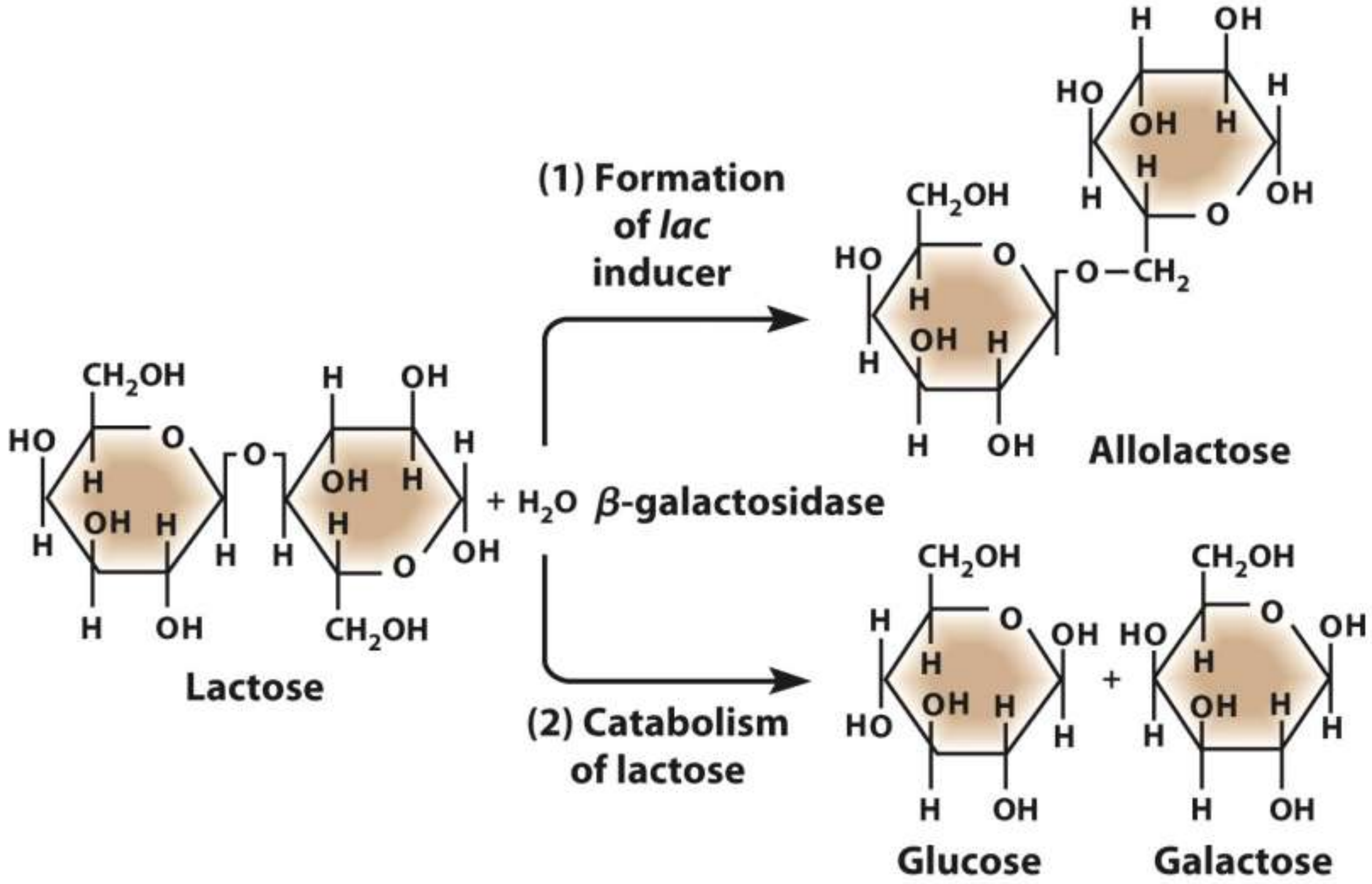
The structural genes in the ***lac* operon** are transcribed only when lactose is present and glucose is absent.

The *lac* Operon of *E. coli*



Structural genes

Reactions Catalyzed by β-Galactosidase



Induction of the *lac* Operon

- In the absence of inducer, the repressor binds to the *lac* operator and represses transcription of the structural genes.

- When the repressor binds to inducer, it is released from the operator, and transcription of the structural genes is turned on.

- The inducer, allolactose, is derived from lactose in a reaction catalyzed by β-galactosidase.

- The ***lac I*** gene encodes a repressor.

Catabolite Repression (Glucose effect)

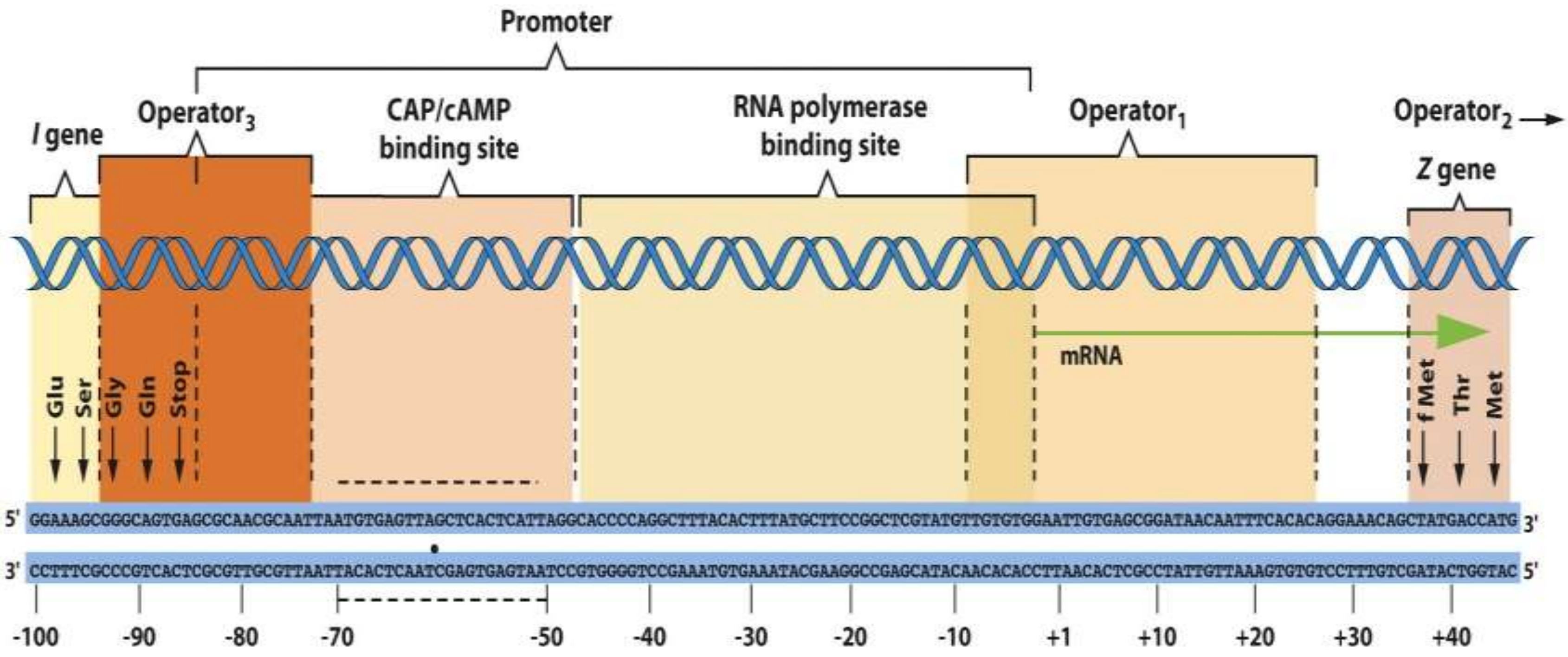
High glucose… low induction of lac operator

- The *lac* promoter has two components
  - The RNA polymerase binding site
  - A binding site for catabolite activator protein (CAP)

- Binding of CAP to the promoter activates transcription of the *lac* operon from being induced when glucose is absent.

- CAP binds to the promoter only when cyclic AMP (cAMP) is present at sufficient concentrations.

Organization of the *lac* Operon Promoter-Operator Region

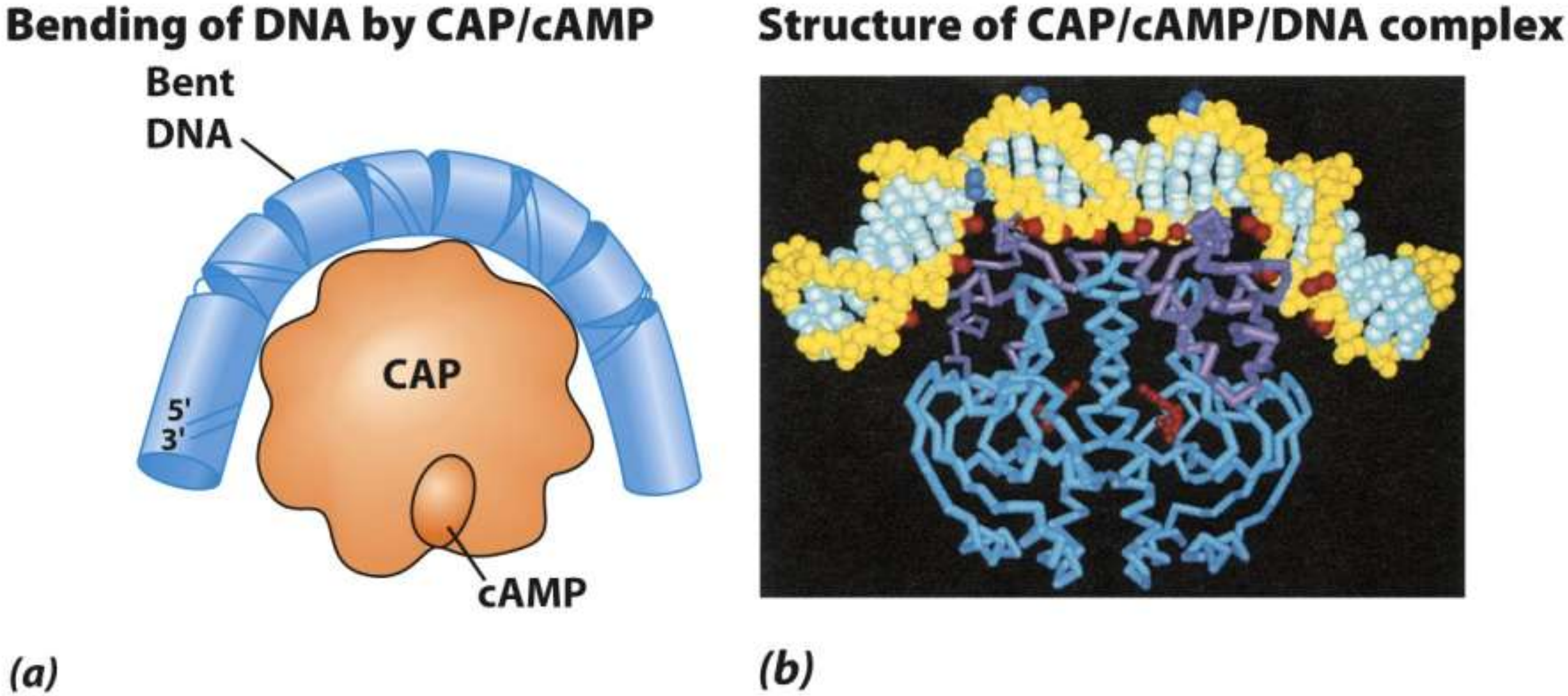


CAP Exerts Positive Control of the *lac* Operon; cAMP is the Effector

- When glucose is present
  - Adenylcyclase is inactive.
  - cAMP levels are low.
  - CAP cannot bind to the *lac* operon.
  - The *lac* structural genes cannot be induced at high levels.

- When glucose is absent
  - Adenylcyclase is active.
  - cAMP levels are high.
  - CAP/cAMP binds to the *lac* operon.
  - The *lac* structural genes can be induced.

Binding of CAP/cAMP to DNA



Bending of DNA by CAP/cAMP

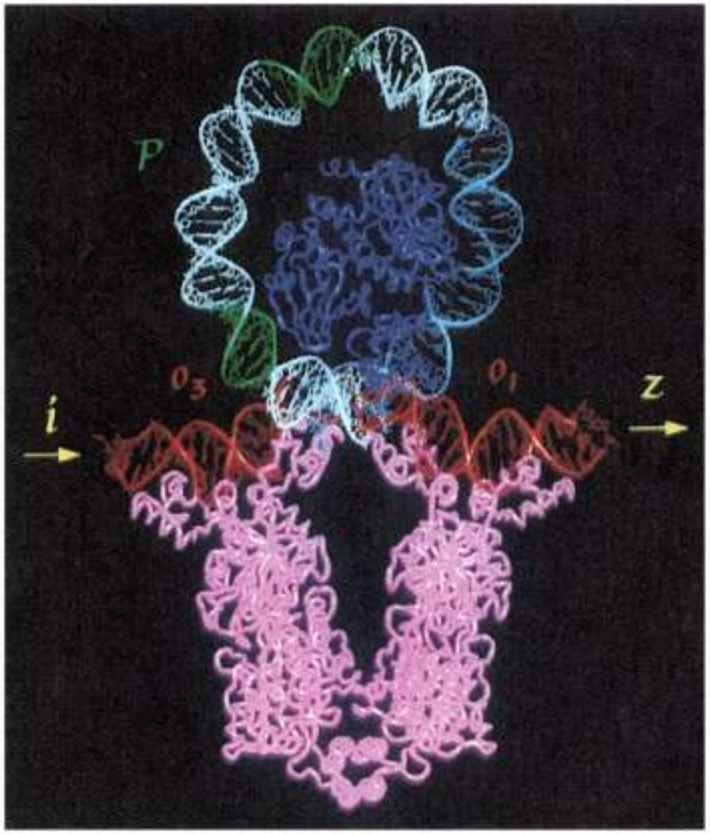
Structure of CAP/cAMP/DNA complex

(b) Courtesy T.A. Steitz, Yale University

Bending and wrapping of DNA by Cap/cAMP: specific sequence is required for this protein-DNA interaction.

Binding of the *lac* Repressor to DNA

Structure of the *lac* repressor/O_1-O_3 operator DNAs/CAP/cAMP complex



Courtesy Ponzy Lu and Mitchell Lewis, University of Pennsylania. Lewis et al. 1966. *Science*, 271:1247–1254, Fig. 6.

--Specific binding site for the repressor (tetramer)

--they bind O1 or (O2 or O3) forming a DA loop

- *The* E. coli lac *operon* *<u>is a negative inducible and catabolite repressible system</u>; the three structural genes in the lac operon are transcribed at high levels only in the presence of lactose and the absence of glucose.*

- *<u>In the absence of lactose,</u> the* lac *repressor binds to the* lac *operator and prevents RNA polymerase from initiating transcription of the operon.*

- *<u>Catabolite repression</u> keeps operons such as* lac *encoding enzymes involved in carbohydrate catabolism from being affected (induced) in the presence of glucose, the preferred energy source.*

- *The binding of the CAP/cAMP complex to its binding site in the* lac *promoter bends the DNA and makes it more accessible to RNA polymerase.*

- *The* lac *repressor binds to two operators-either* O_1 *and* O_2 *or* O_1 *and* O_3*-simultaneously and bends the DNA into a hairpin or a loop, respectively.*

The Tryptophan Operon in *E. coli*: Repression and Attenuation

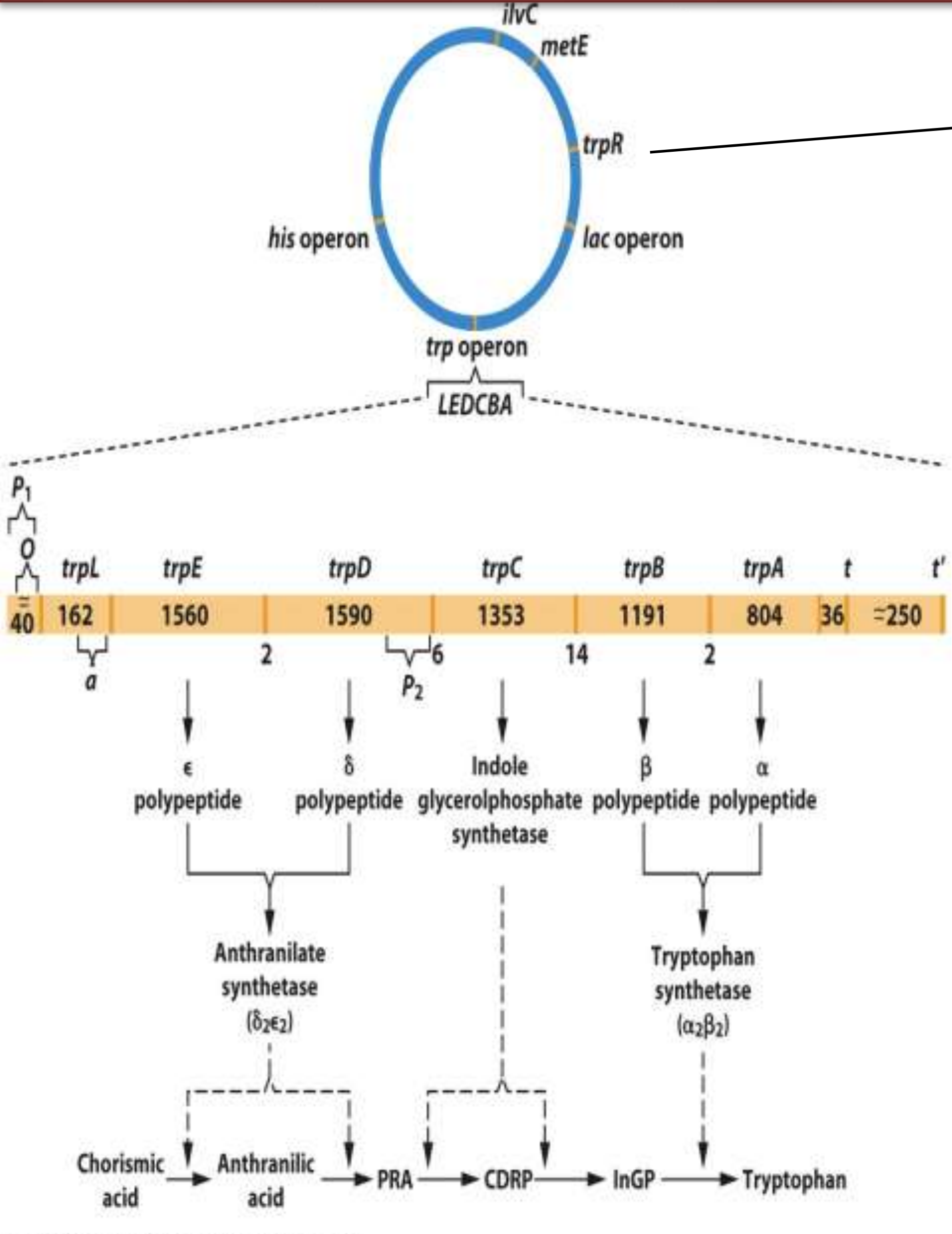
It controls the synthesis of the enzymes required for the synthesis of trytophan

The structural genes in the tryptophan operon are transcribed only when **tryptophan is absent or present in low concentrations**.

How is it regulated?

The expression of the genes in the *trp* operon is regulated by repression of transcriptional initiation and by attenuation (premature termination) of transcription when tryptophan is prevalent in the environment.

Organization of the *trp* Operon



TrpR: Repressor gene

O: operator region
P1 and P2: promoter regions

Transcription from TrpL to TrpA

Transcription from TrpC to TrpA

t and t′: Transcription termination sequence

Absence of tryptophan (W): -derepressed state-

RNA pol binds to P regions and transcribes

Presence of tryptophan (W): -repressed state-

Repressor binds to P regions and preventing RNA pol binding

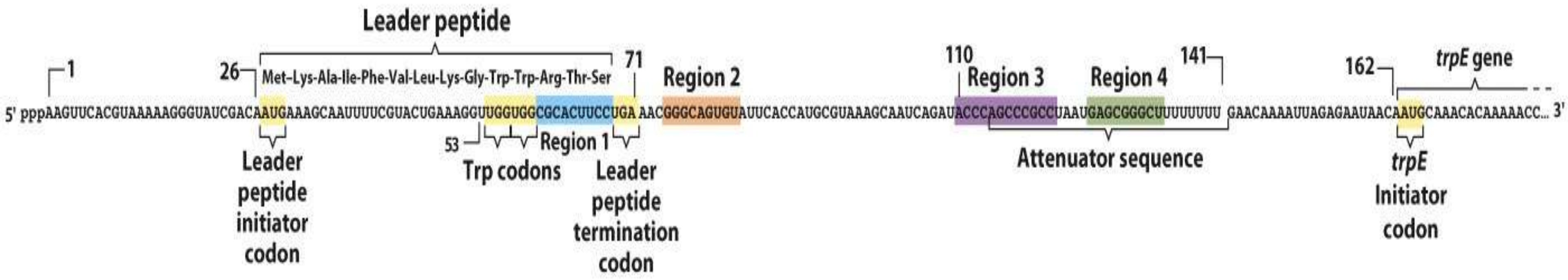
TrpR mutants: ? ..still ….. repressed…

New independent mechanism of regulation

(nucleotide sequences located in trpL and the amount of Trp)

The Leader Peptide and **Attenuator Sequence**

Regulatory components of the *trpL* region



Attenuation

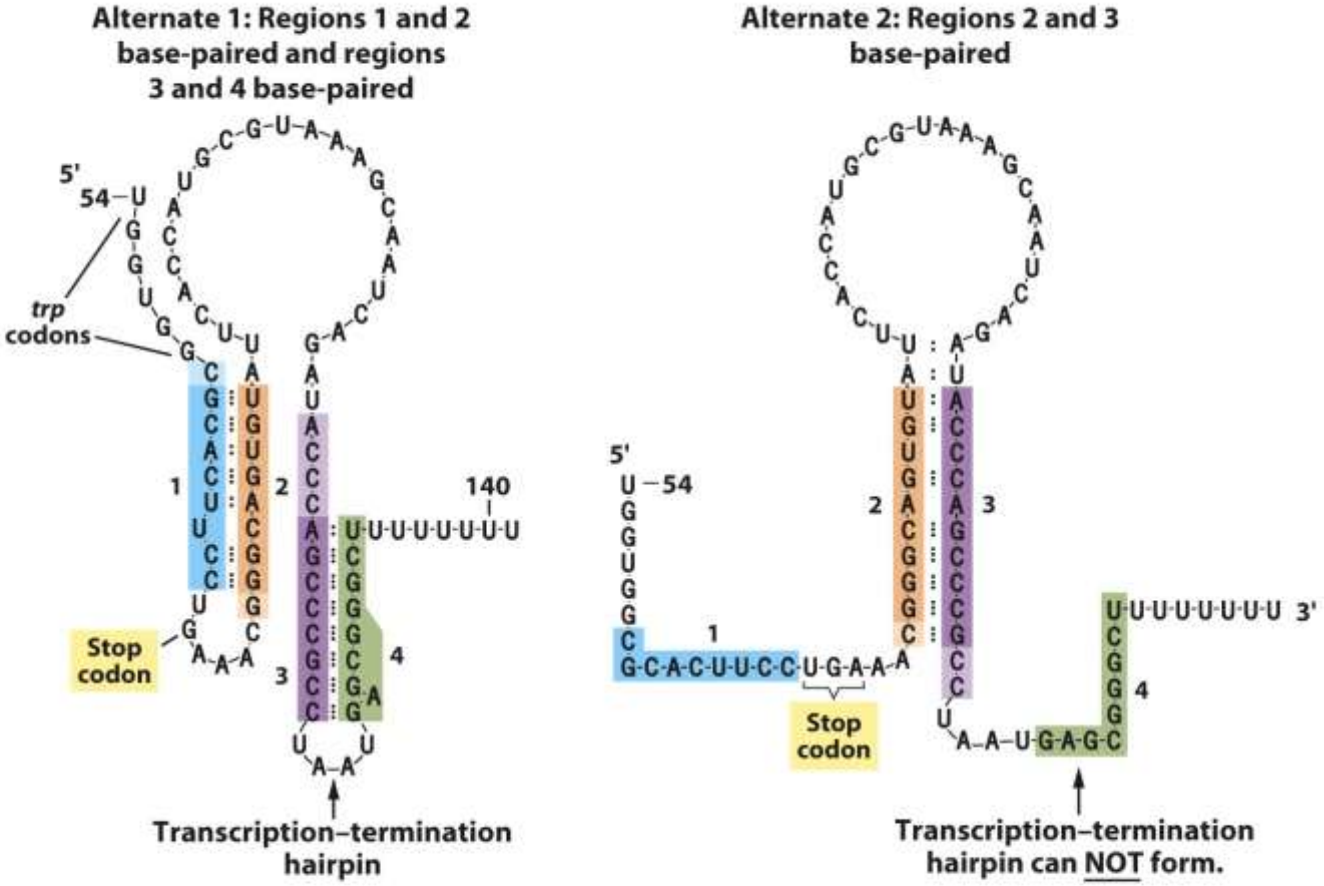
-occurs by controlling the termination of transcription close the mRNA of the leader sequence. It contains G:C rich regions followed by several A:T bps.

-needs trp (or trp charged-tRNA^{W}).

-produces small transcritp.

Alternate Secondary Structures of the *trpL* Transcript

Alternate secondary structures formed by the *trpL* transcript

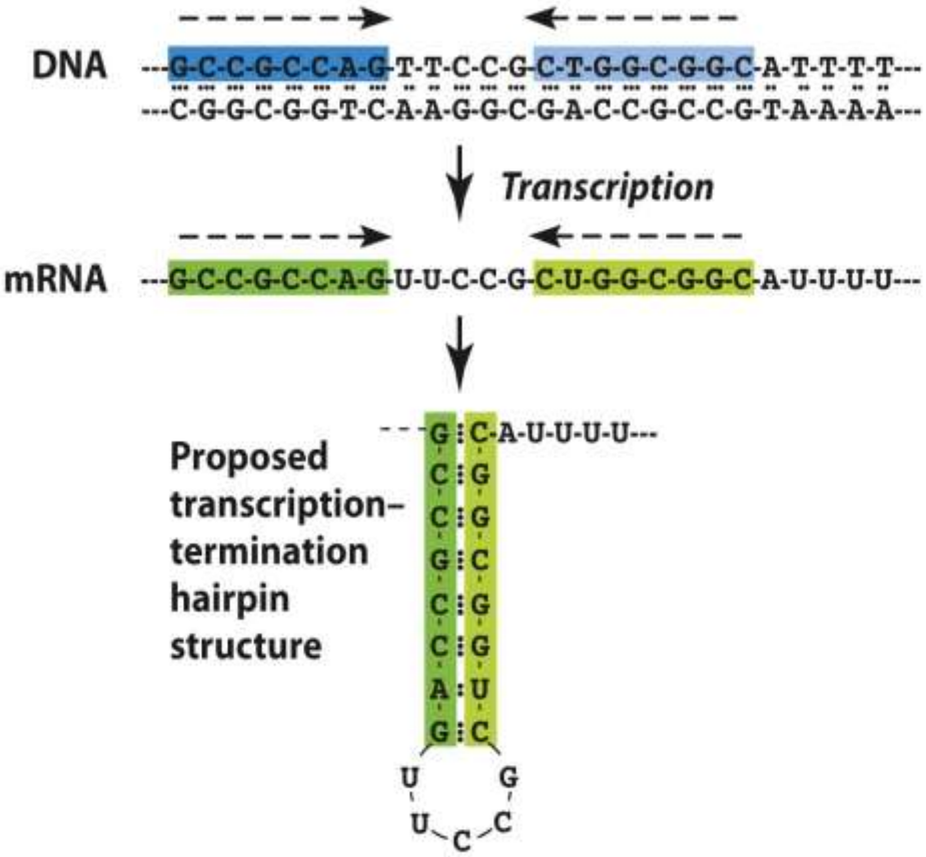

Alternate 1: Regions 1 and 2 base-paired and regions 3 and 4 base-paired

Alternate 2: Regions 2 and 3 base-paired

Transcription–termination hairpin

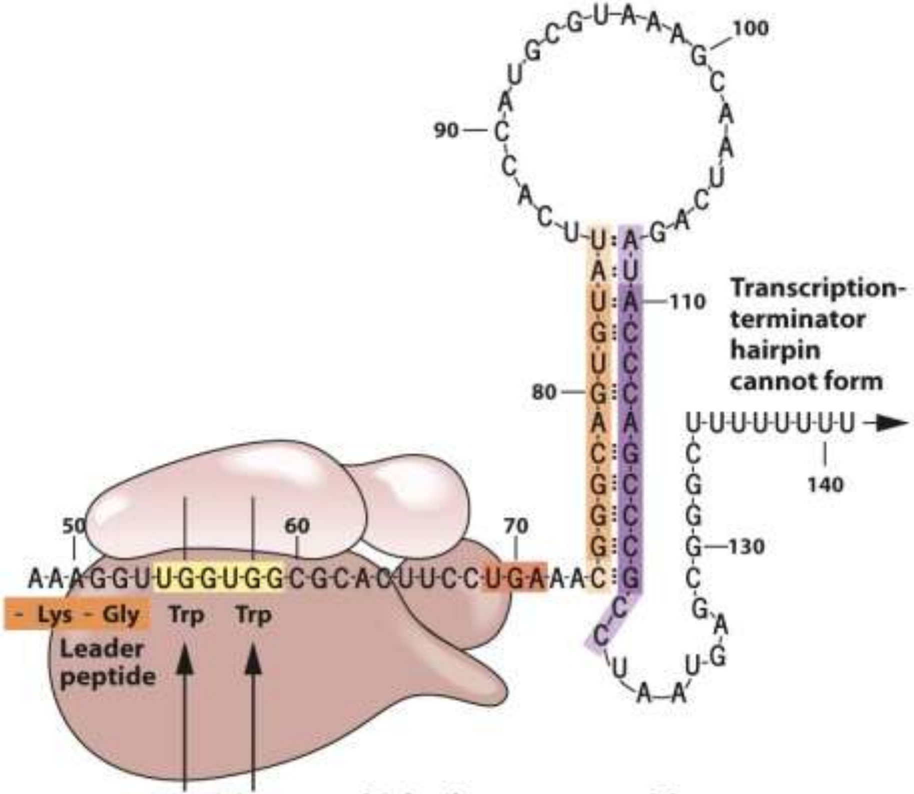
Transcription–termination hairpin can NOT form.

Transcription-Termination Sequence *t* Forms a Transcription-Termination Hairpin



Structure of *trp* operon transcription–termination sequence *t* and formation of the transcription–termination hairpin.

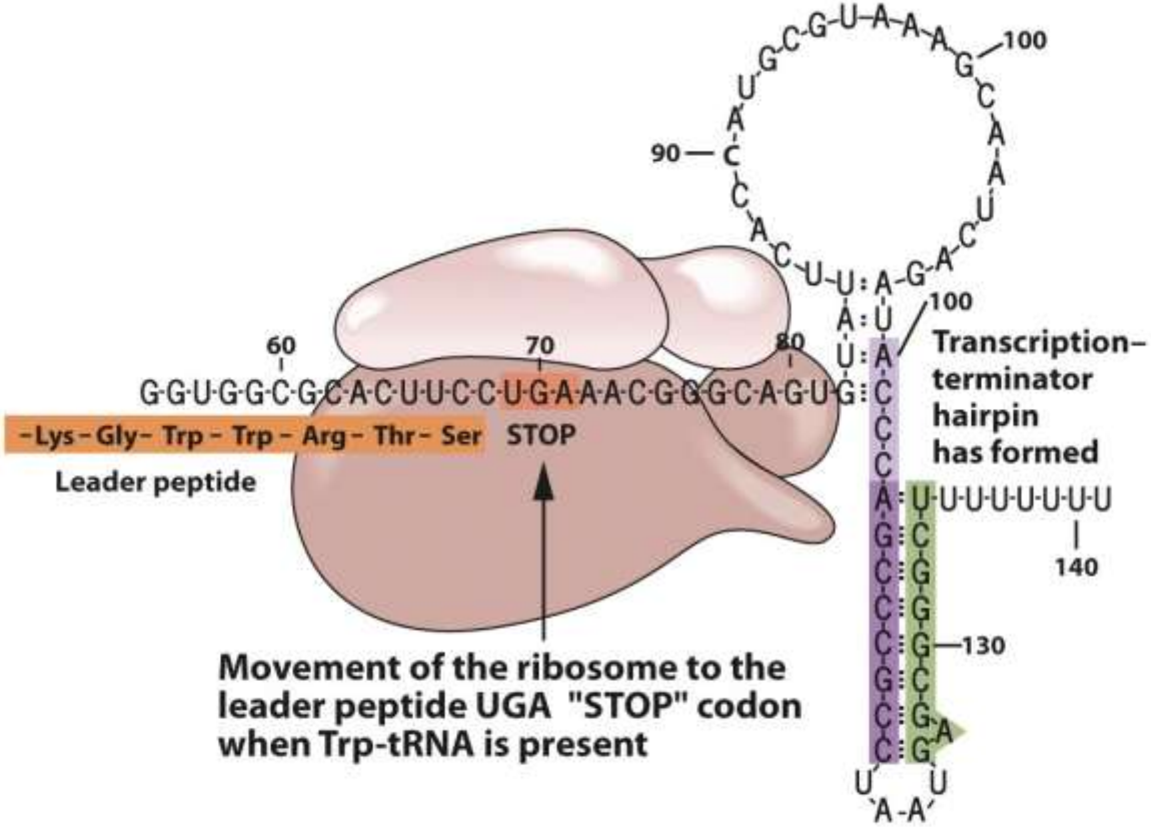
When Tryptophan Levels are Low, Transcription Proceeds through the Entire *trp* Operon



Positions at which ribosomes would stop without sufficient Trp-tRNA to respond to the two UGG Trp codons

With low levels of tryptophan, translation of the leader sequence stalls at one of the Trp codons. This stalling allows leader regions 2 and 3 to pair, which prevents region 3 from pairing with region 4 to form the transcription–termination hairpin. Thus transcription proceeds through the entire *trp* operon.

When Tryptophan Levels are high enough, Transcription Stops at the Attenuator Sequence



Transcription–terminator hairpin has formed

Movement of the ribosome to the leader peptide UGA "STOP" codon when Trp-tRNA is present

In the presence of sufficient tryptophan, translation proceeds past the Trp codons to the termination codon and disrupts the base-pairing between leader regions 2 and 3. This process leaves region 3 free to pair with region 4 to form the transcription-termination hairpin, which stops transcription at the attenuator sequence.

- *The* E. coli trp *operon is* *a negative repressible system; transcription of the five structural genes in the* trp *operon is repressed in the presence of significant* ***concentrations of tryptophan****.*

- *Operons such as* trp *that encode enzymes involved in amino acid biosynthetic pathways often are controlled by a* ***second regulatory mechanism*** *called attenuation.*

- *Attenuation occurs by* ***the premature termination of transcription*** *at a site in the mRNA leader sequence (the sequence 5’ to the coding region) when tryptophan is prevalent in the environment in which the bacteria are growing.*

Translational Control of Gene Expression

The regulation of gene expression is **often fine-tuned** by modulating either the frequency of initiation of translation or the rate of polypeptide chain elongation.

mRNA are multigenic (several genes)

E. Coli Lac operon mRNA (3 enzymes)

Post-Translational Regulatory Mechanisms

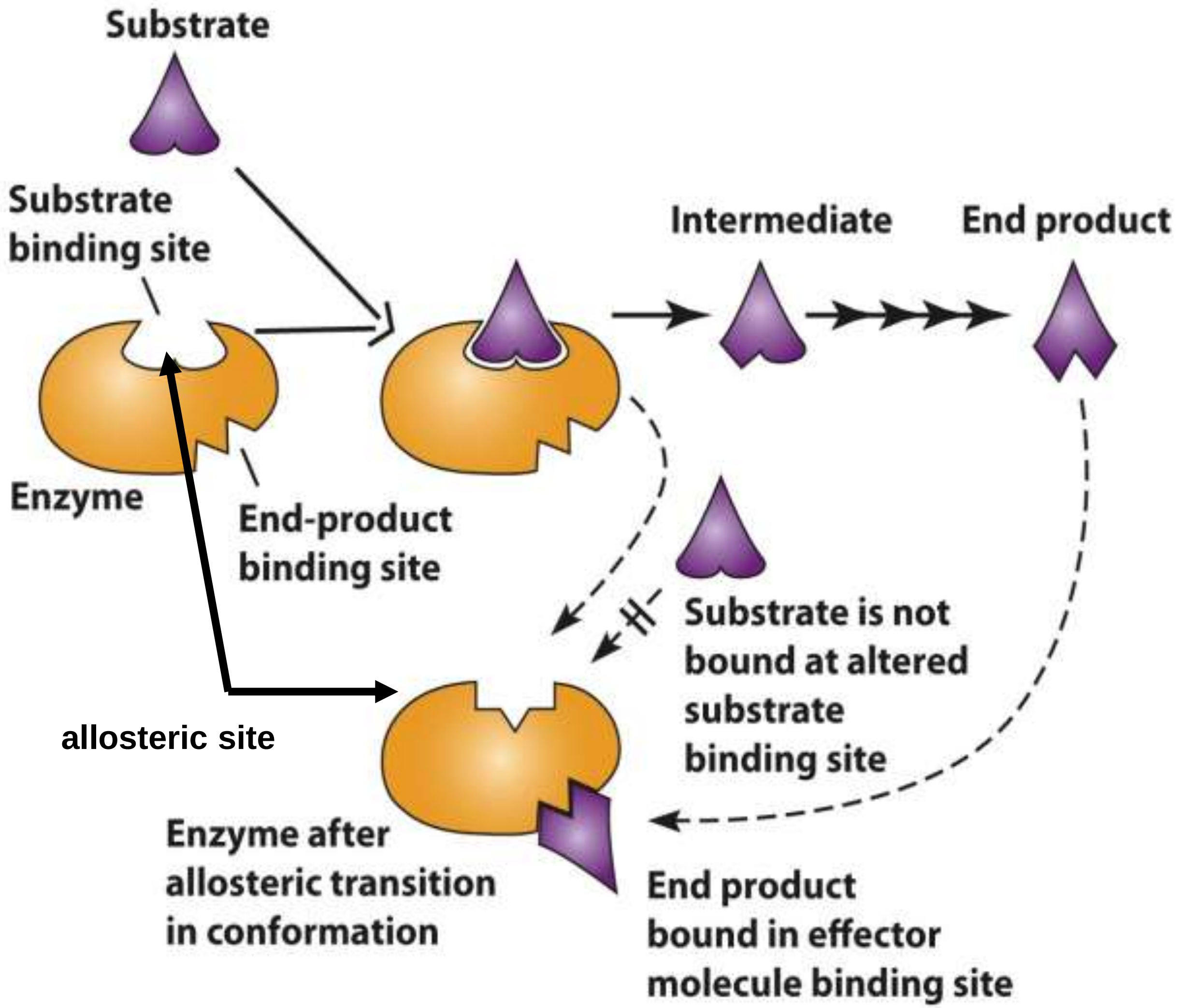
Feedback (control) inhibition occurs when **the product** of a biosynthetic pathway inhibits the activity of the **first enzyme in the pathway**, rapidly shutting off the synthesis of the product.

Rate Limiting

Feedback Inhibition

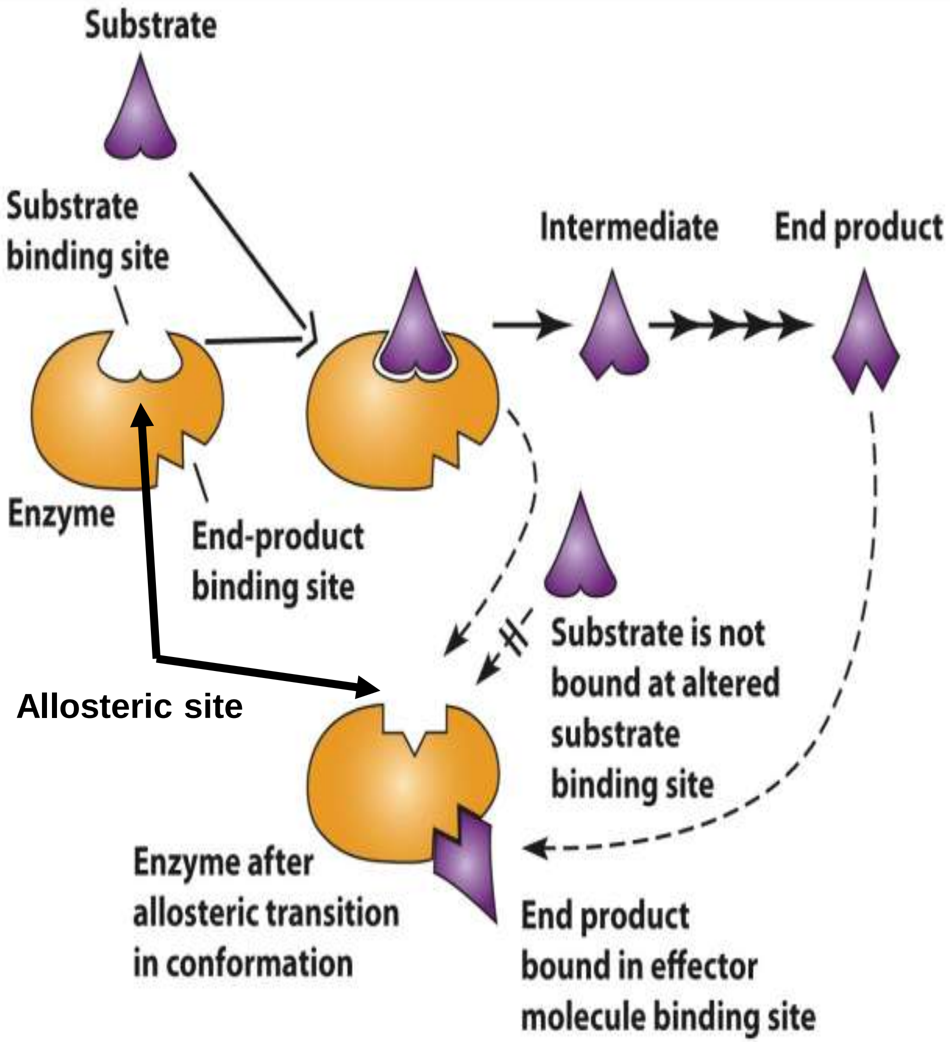
(End-product inhibition)

Trytophan inhibition



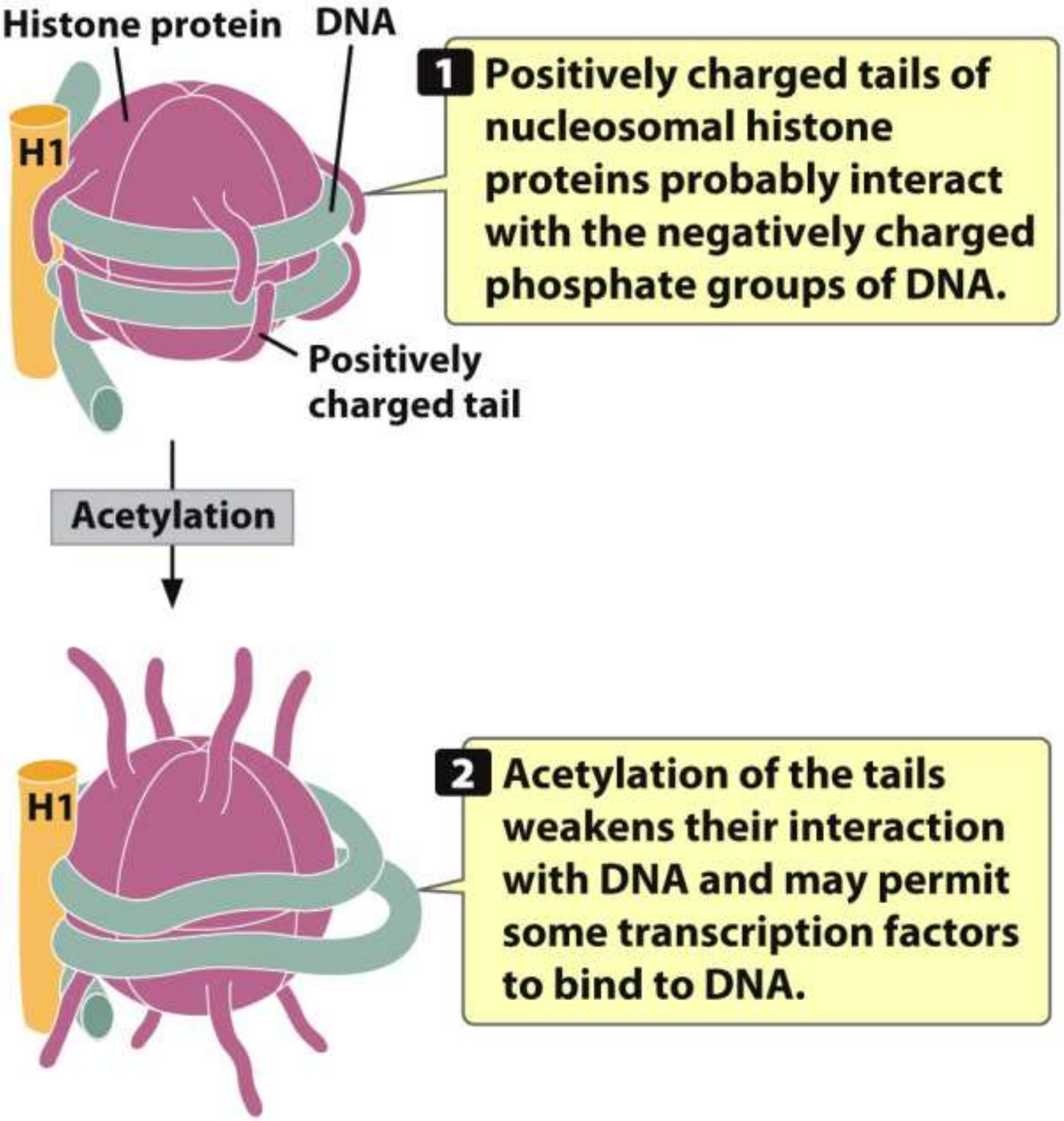
Allosteric Transitions

- Allosteric transitions are conformational changes that occur upon binding of a cofactor to an enzyme.
- Binding of an end product to an enzyme can result in an allosteric transition that **reduces enzymatic activity (inhibition)**
- Allosteric transitions can also result in enzyme activation when binding of a substrate or cofactor **increases enzymatic activity**.



Gene Regulation in Eukaryotic Cells Takes Place at Multiple Levels

- **Chromatin remodeling**
  - Chromatin-remodeling complexes: bind directly to DNA sites and reposition nucleosomes
- **Histone modification**
  - Addition of methyl groups to the histone protein tails
  - Addition of acetyl groups to histone proteins



1 Positively charged tails of nucleosomal histone proteins probably interact with the negatively charged phosphate groups of DNA.

2 Acetylation of the tails weakens their interaction with DNA and may permit some transcription factors to bind to DNA.

Figure 12.13

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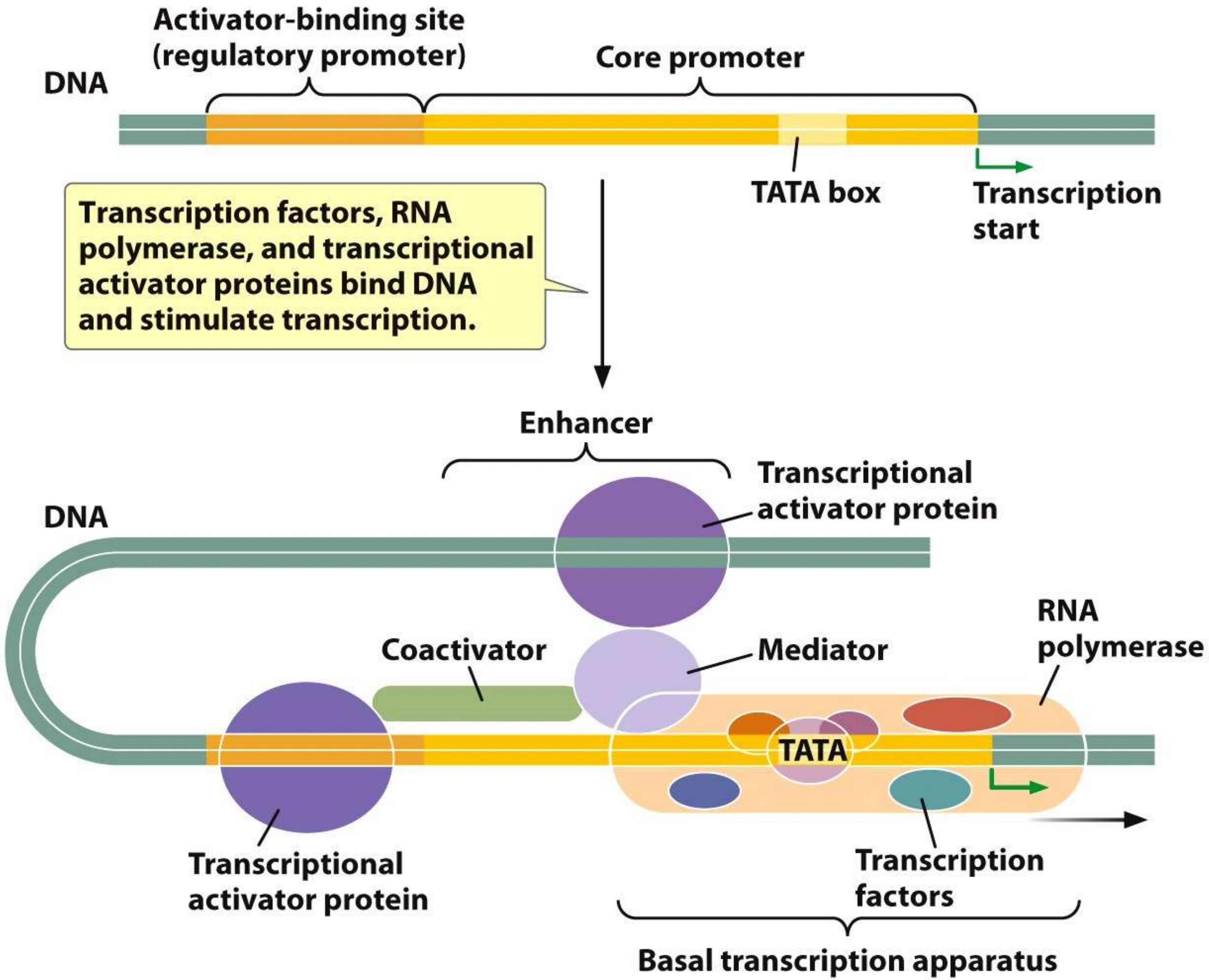
Gene Regulation in Eukaryotic Cells Takes Place at Multiple Levels

- **DNA Methylation**
- DNA methylation of cytosine bases adjacent to guanine nucleotides (CpG)–CpG islands

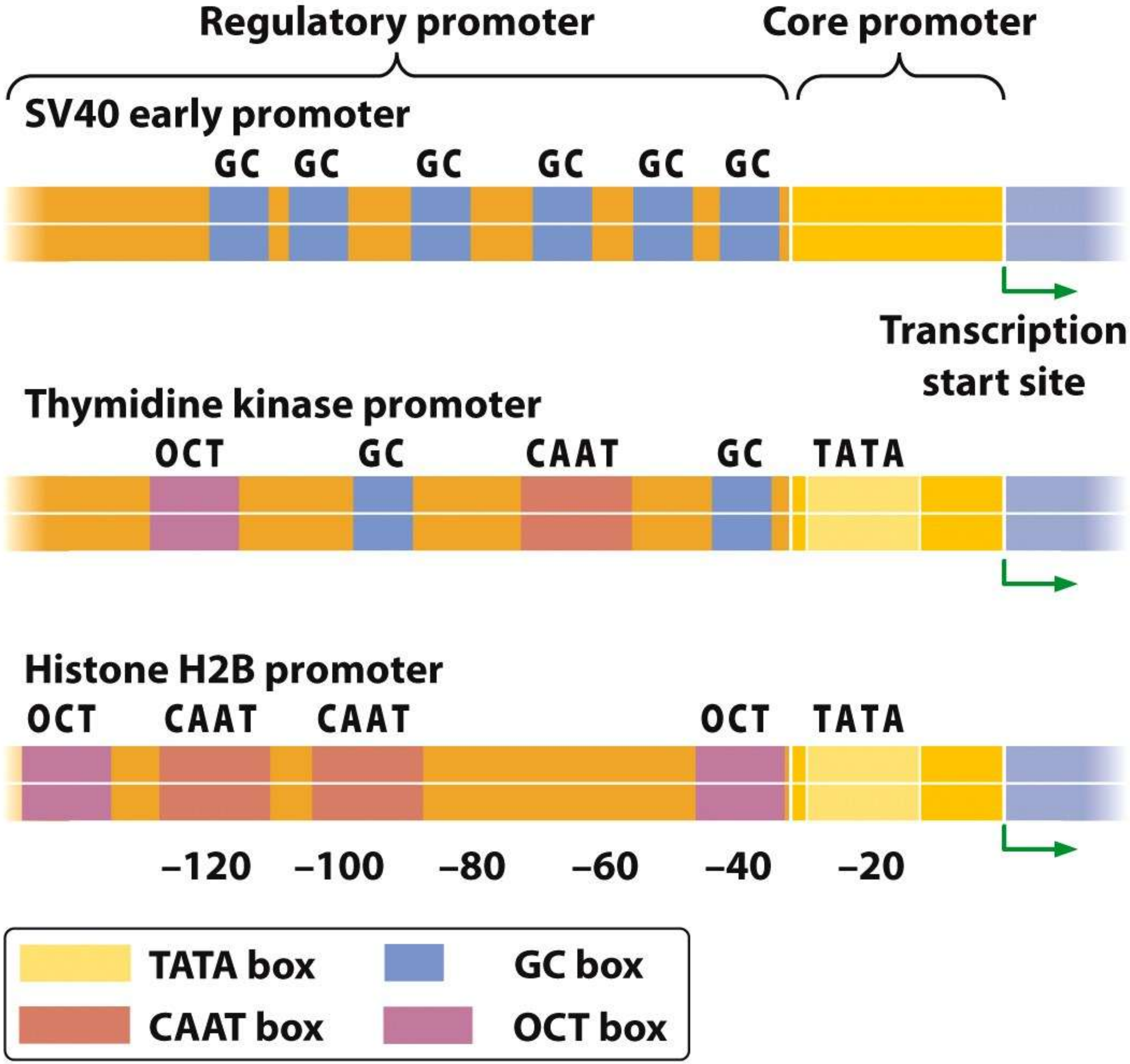
Gene Regulation in Eukaryotic Cells Takes Place at Multiple Levels

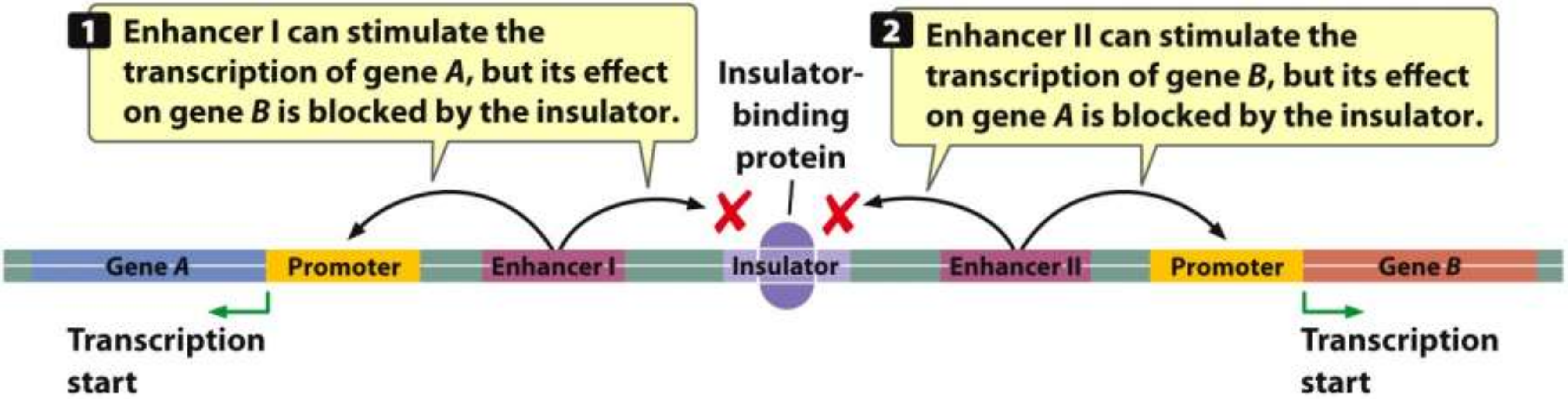
- **Transcriptional activators and repressors**
  - Bind to *silencers*

  - **Enhancers and Insulators**
  - **Enhancer:** DNA sequence stimulating transcription from a distance away from promoter
  - **Insulator:** DNA sequence that blocks or insulates the effect of enhancers



Transcription factors, RNA polymerase, and transcriptional activator proteins bind DNA and stimulate transcription.


Regulatory promoter
Core promoter
SV40 early promoter
GC GC GC GC GC GC
Transcription start site
Thymidine kinase promoter
OCT GC CAAT GC TATA
Histone H2B promoter
OCT CAAT CAAT OCT TATA
–120 –100 –80 –60 –40 –20
TATA box
GC box
CAAT box
OCT box



1 Enhancer I can stimulate the transcription of gene *A*, but its effect on gene *B* is blocked by the insulator.

2 Enhancer II can stimulate the transcription of gene *B*, but its effect on gene *A* is blocked by the insulator.

Figure 12.17

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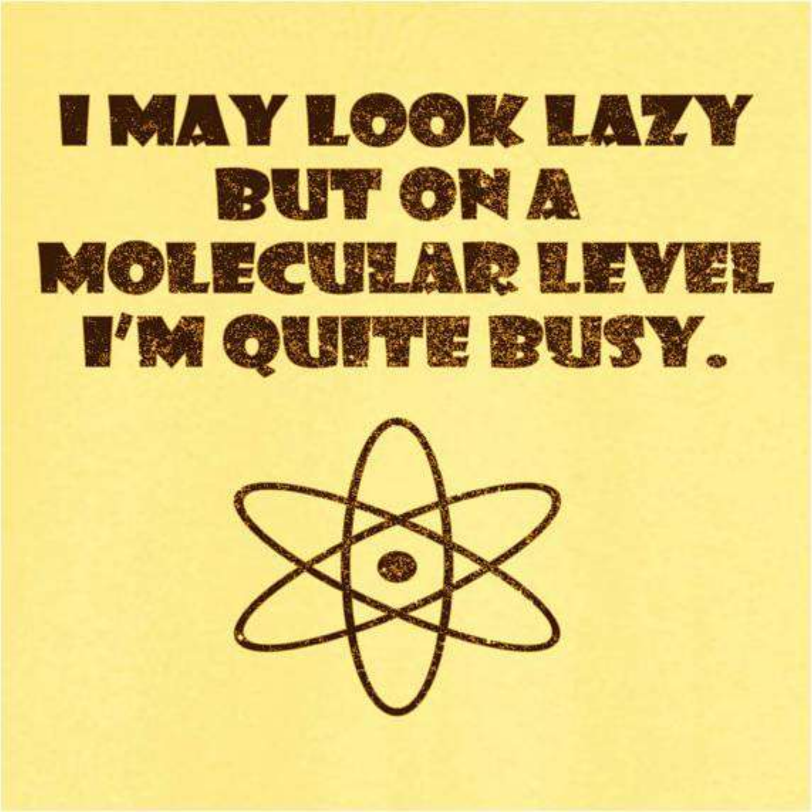

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