Systems Microbiology

Wednesday Oct 11 - Ch 8 -Brock

Regulation of cell activity

- Transcriptional regulation mechanisms
- Translational regulation mechanisms
- Bacterial genetics

Image removed due to copyright restrictions.
See Figure 8-1 in Madigan, Michael, and John Martinko. *Brock Biology of Microorganisms*. 11th ed. Upper Saddle River, NJ: Pearson Prentice Hall, 2006. ISBN: 0131443291.

Regulation of prokaryotic transcription

1. Single-celled organisms with short doubling times must respond extremely rapidly to their environment.

2. Half-life of most mRNAs is short (on the order of a few minutes).

3. Coupled transcription and translation occur in a single cellular compartment.

Therefore, transcriptional initiation is usually the major control point.

Most prokaryotic genes are regulated in units called operons (Jacob and Monod, 1960)

Operon: a coordinated unit of gene expression consisting of one or more related genes and the operator and promoter sequences that regulate their transcription. The mRNAs thus produced are "polycistronic'—multiple genes on a single transcript.

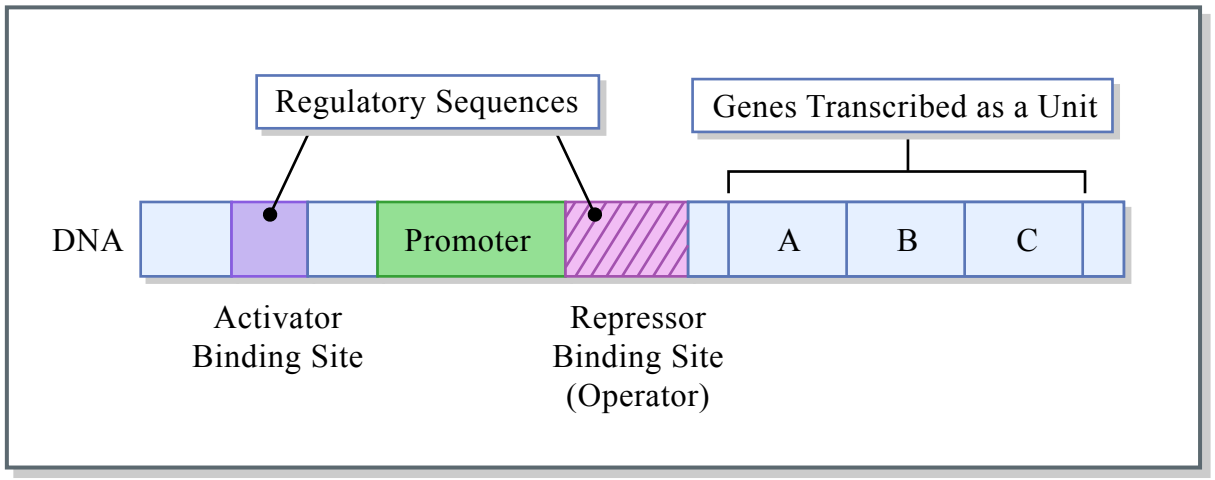


Figure by MIT OCW.

Initiation of transcription begins with promoter binding by RNAP holoenzyme

holoenzyme = RNAP core + Sigma

Diagram of RNA polymerase and transcription removed due to copyright restrictions.

Brock Biology of Microorganisms, vol. 11, Chapter 7

Alternate Sigma Factors

recognize promoters of different architecture – different regulons of genes

Table and graphs removed due to copyright restrictions.
See Ishihama. *Ann Rev Microbiol* 54 (2000): 499-518.

Ishihama, 2000, Ann. Rev. Microbiol. 54:499-518

Transcription factors interact with different components of RNAP in addition to sigma factor…..

Diagram removed due to copyright restrictions.
See Ishihama. *Ann Rev Microbiol* 54 (2000): 499-518.

Ishihama, 2000, Ann. Rev. Microbiol. 54:499-518

Microbiology **151** (2005), 3147-3150; DOI 10.1099/mic.0.28339-0

Genome update: sigma factors in 240 bacterial genomes

Types of sigma factors…

Graphs removed due to copyright restrictions.

Transcription factors

Typically DNA binding proteins that associate with the regulated promoter and either decrease or increase the efficiency of transcription, repressors and activators, respectively - A significant number of regulators do either one depending on conditions

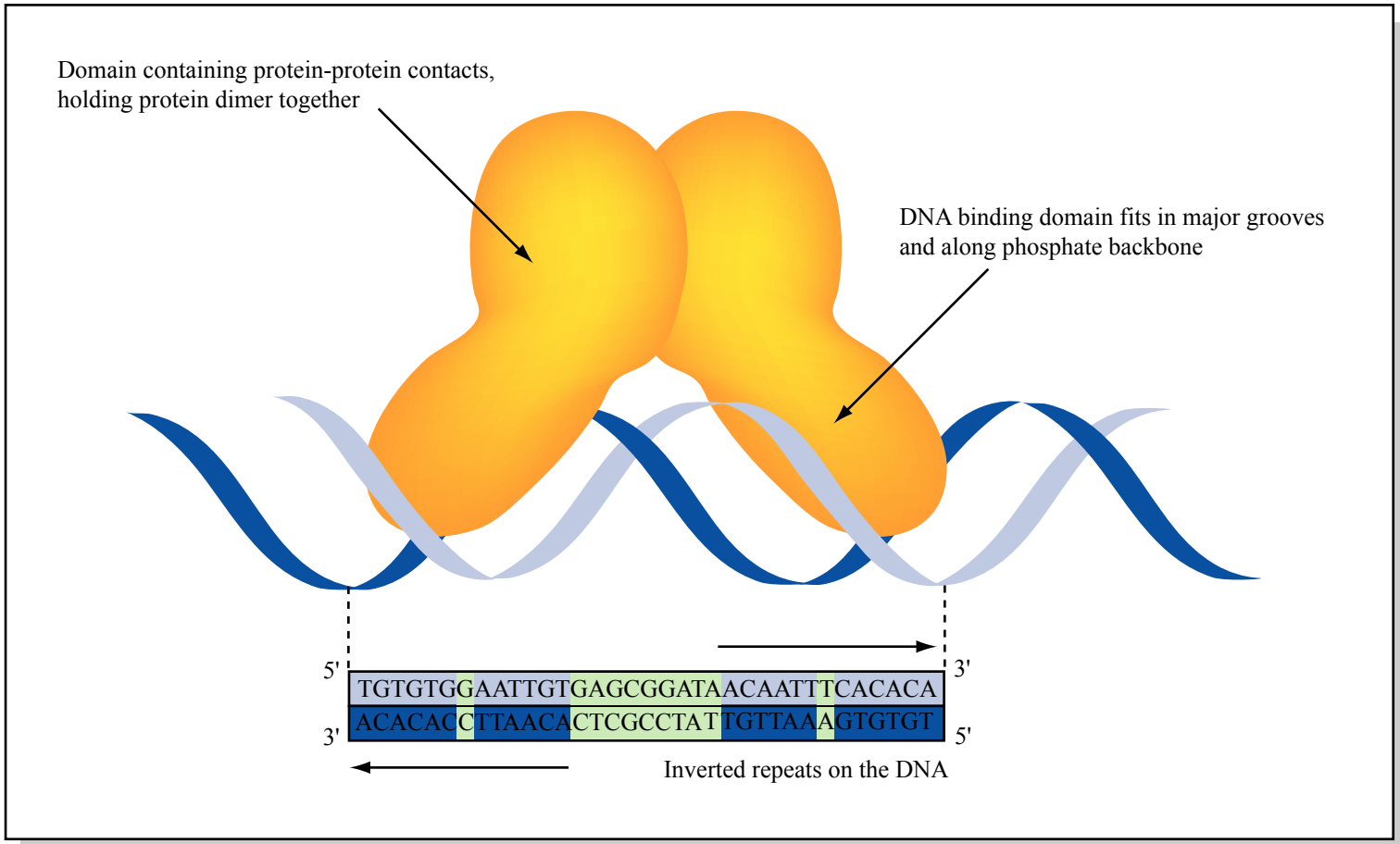


Figure by MIT OCW.

n presence of arginine, arginine biosynthesis genes are **repressed**

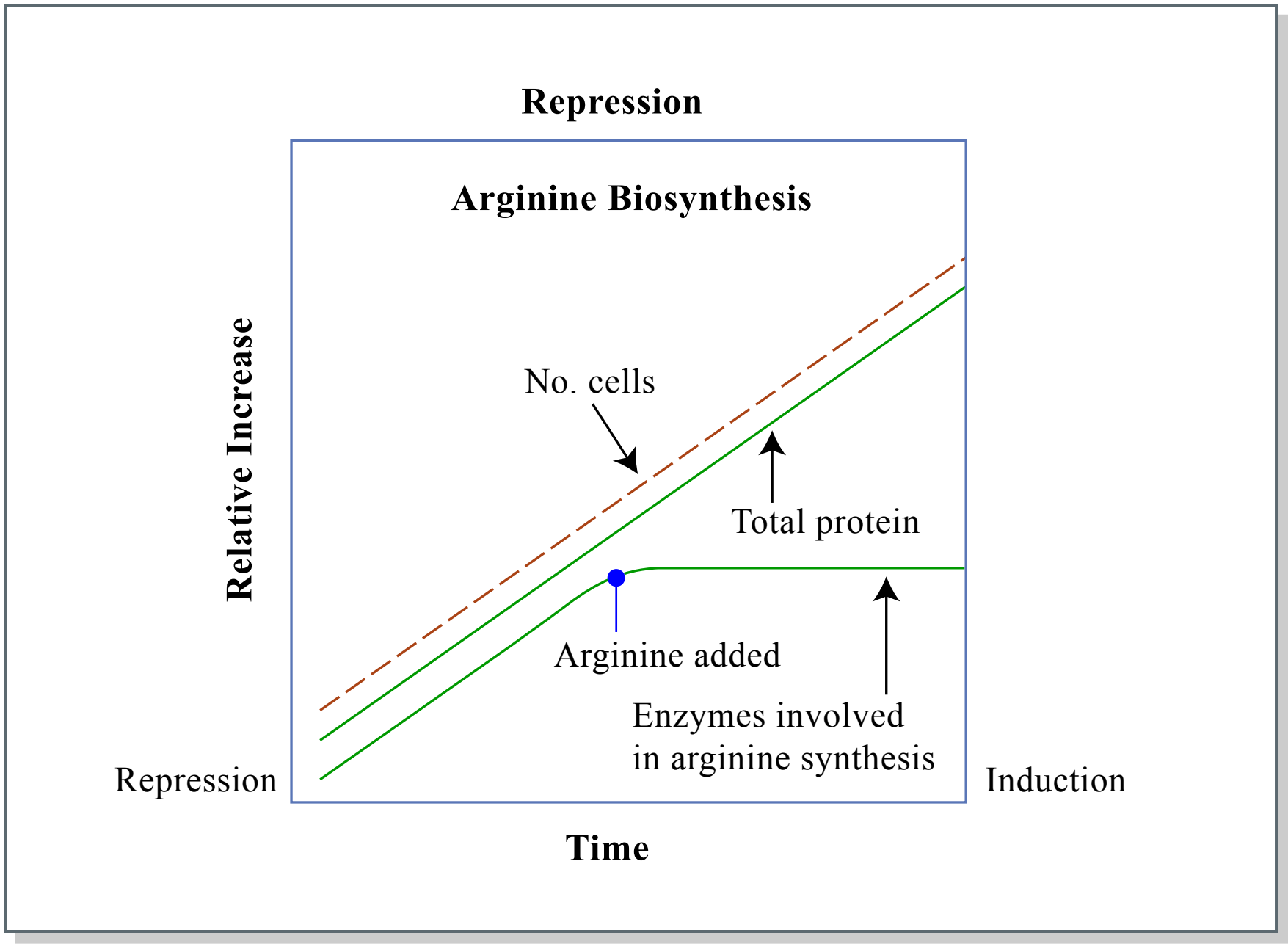


Figure by MIT OCW.

n the presence of lactose, lactose metabolizing genes are **induced**

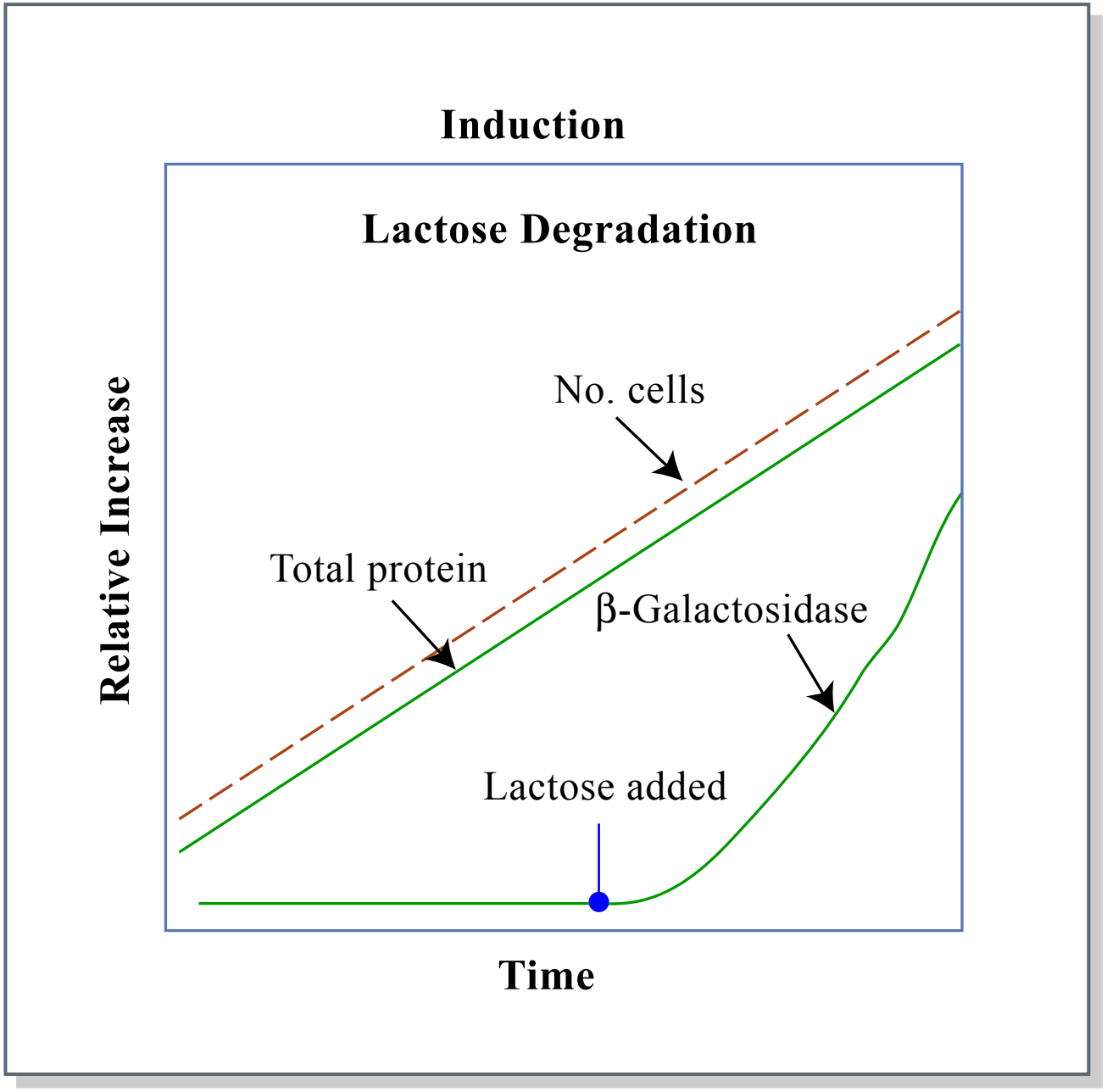
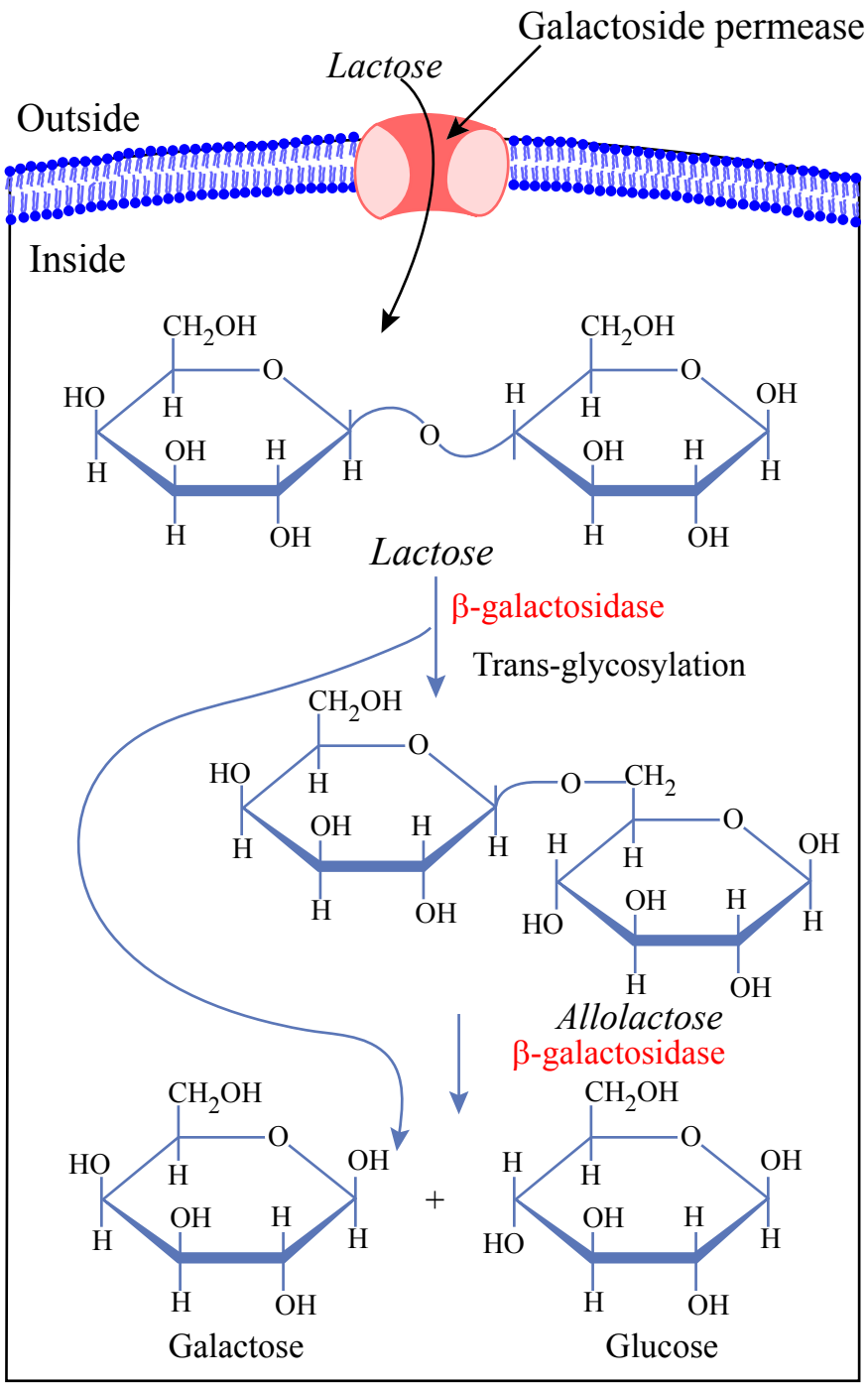
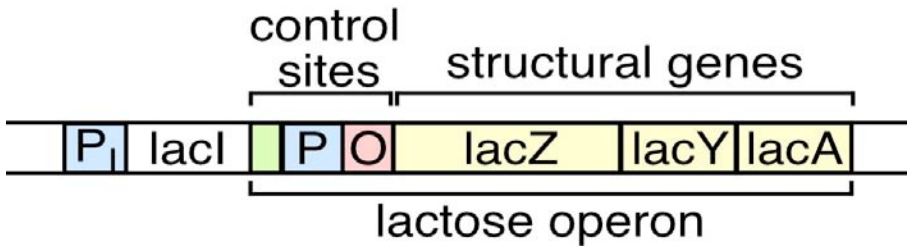


Figure by MIT OCW.

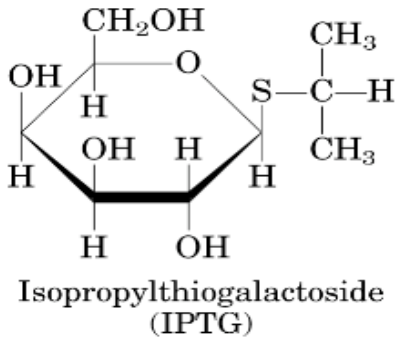
The metabolism of lactose in *E. coli* & the lactose operon



- To use lactose as an energy source, cells must contain the enzyme *β*-galactosidase.
- Utilization of lactose also requires the enzyme lactose permease to transport lactose into the cell.
- Expression of these enzymes is rapidly induced ~1000-fold when cells are grown in lactose compared to glucose.



LacZ: β-galactosidase; Y: galactoside permease;
A: transacetylase (function unknown).
P: promoter; O: operator.
LacI: repressor; P_I and LacI are not part of the operon.



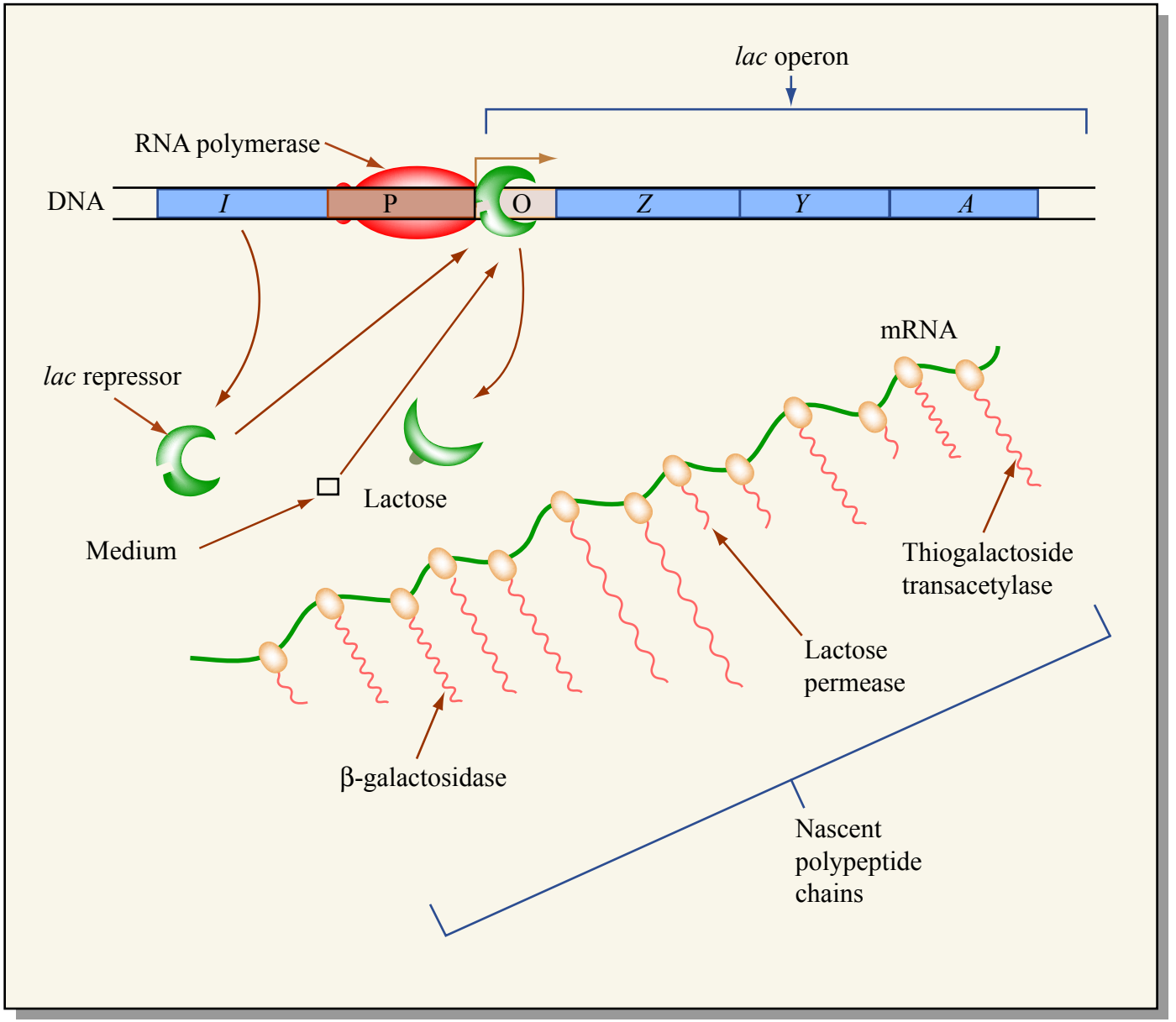
IPTG: non-metabolizable artificial inducer (can't be cleaved)

figure by MIT OCW.

Negative regulation of the *lac* operon

Negative regulation: The product of the *I* gene, the repressor, blocks the expression of the *Z*, *Y*, and *A* genes by interacting with the operator *(O)*.

The inducer (lactose or IPTG) can bind to the repressor, which induces a conformational change in the repressor, thereby preventing its interaction with the operator (O). When this happens, RNA polymerase is free to bind to the promoter (P) and initiates transcription of the lac genes.



How do we know that lacI encodes a trans-acting repressor?

Figure by MIT OCW.

Symmetry matching between the tetramers of lac repressor and the nearly palindromic sequence of the lac operator

Each monomeric unit of lacI is 37-kD

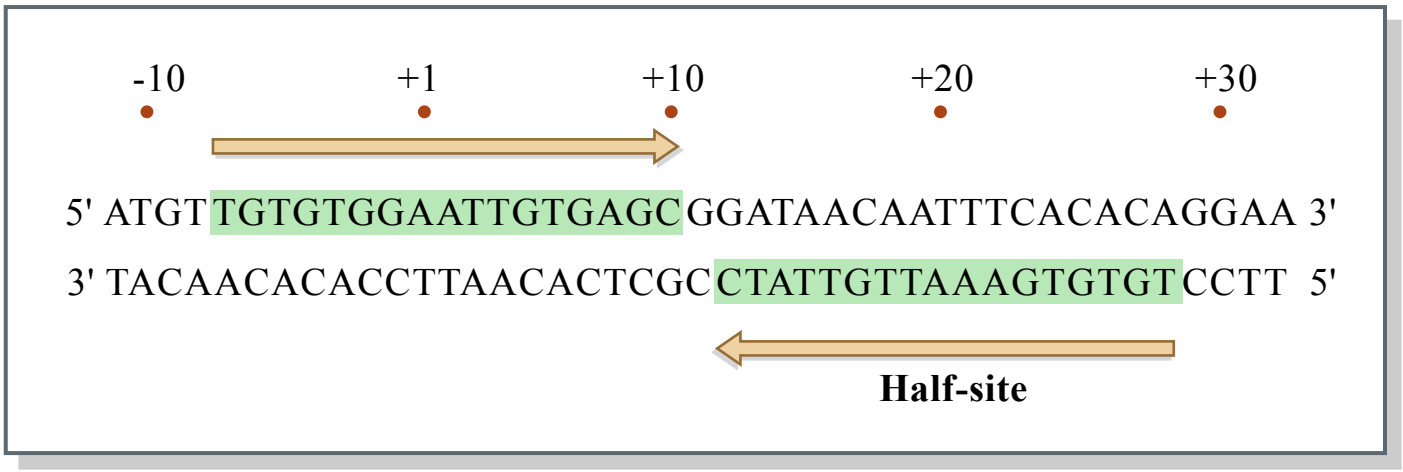


Figure by MIT OCW.

Image of the X-ray structure of lac repressor tetramer bound to two 21-bp segments of DNA removed due to copyright restrictions.

The *lac* operator sequence is a nearly perfect inverted repeat centered around the GC base pair at position + 11.

"Diauxic" growth -
preferential use of one carbon source over another, in sequential fashion

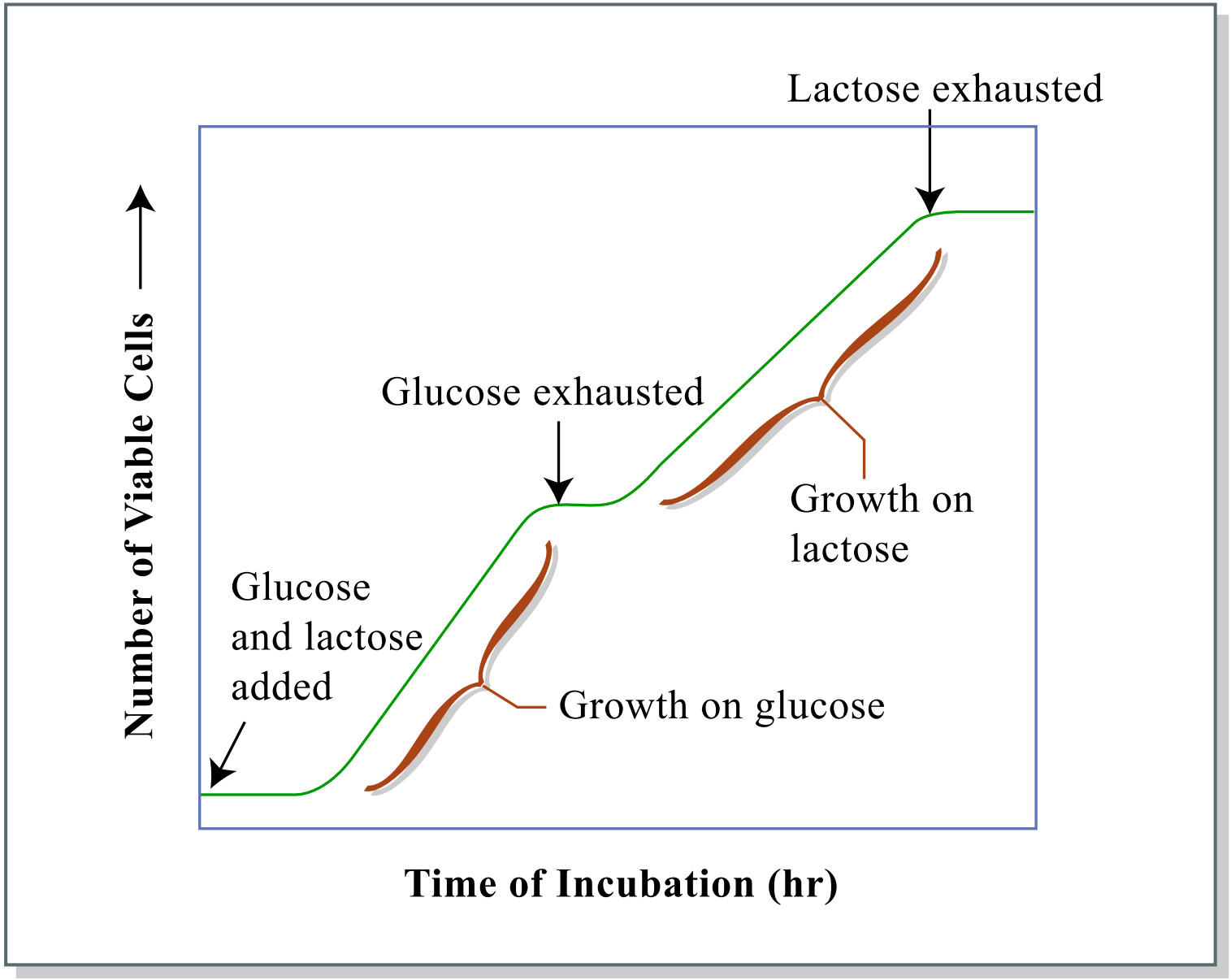


Figure by MIT OCW.

Regulation of the *lac* operon involves more than a simple on/off switch provided by lacI/lacO

Observation: Glucose is a preferred sugar for *E. coli*, which uses glucose and ignores lactose in media containing both sugars. In these cells, β-galactosidase level is low, suggesting that derepression at the operator site is not enough to turn on the lac operon. This phenomenon is called catabolite repression.

This involves regulation of cyclic AMP (cAMP) levels, and its interaction with the Catabolite Activator Protein, CAP.

cAMP-CAP complex activates *lac* gene expression.

Cooperative binding of cAMP-CAP and RNAP on the *lac* promoter

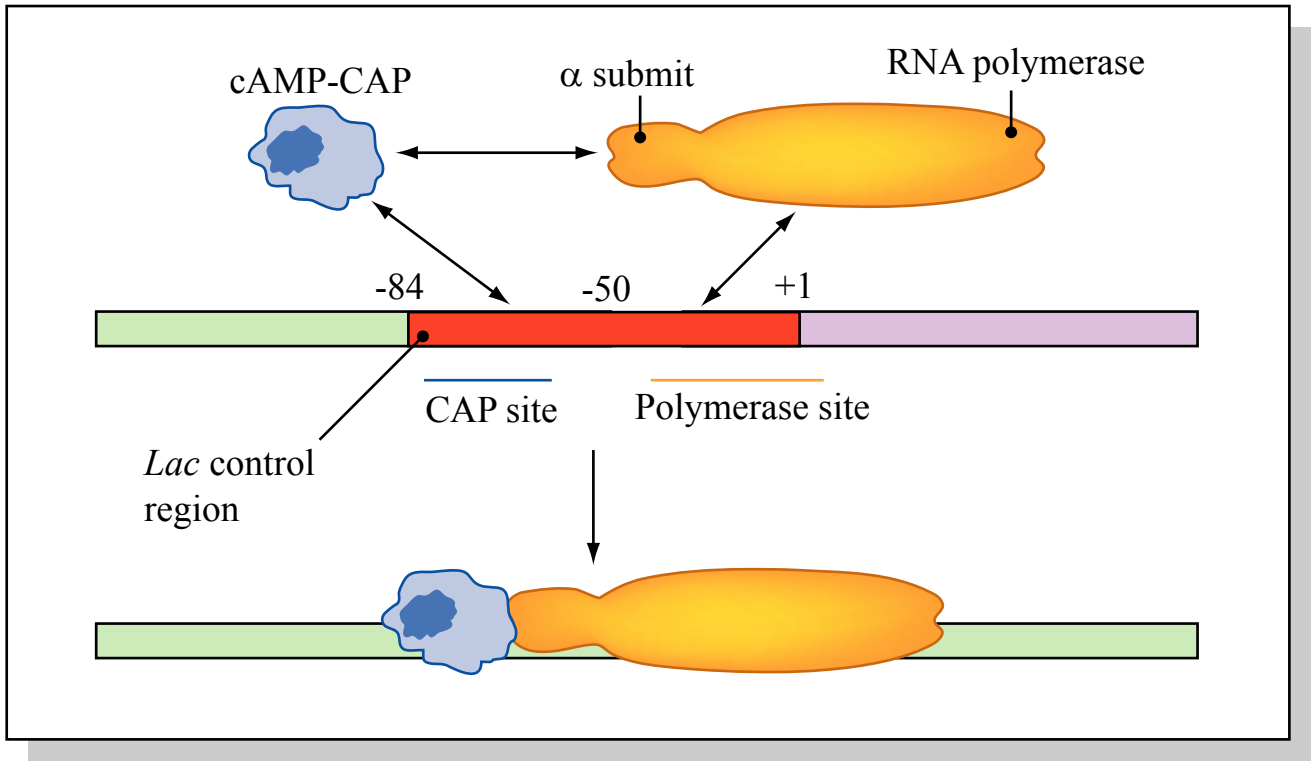
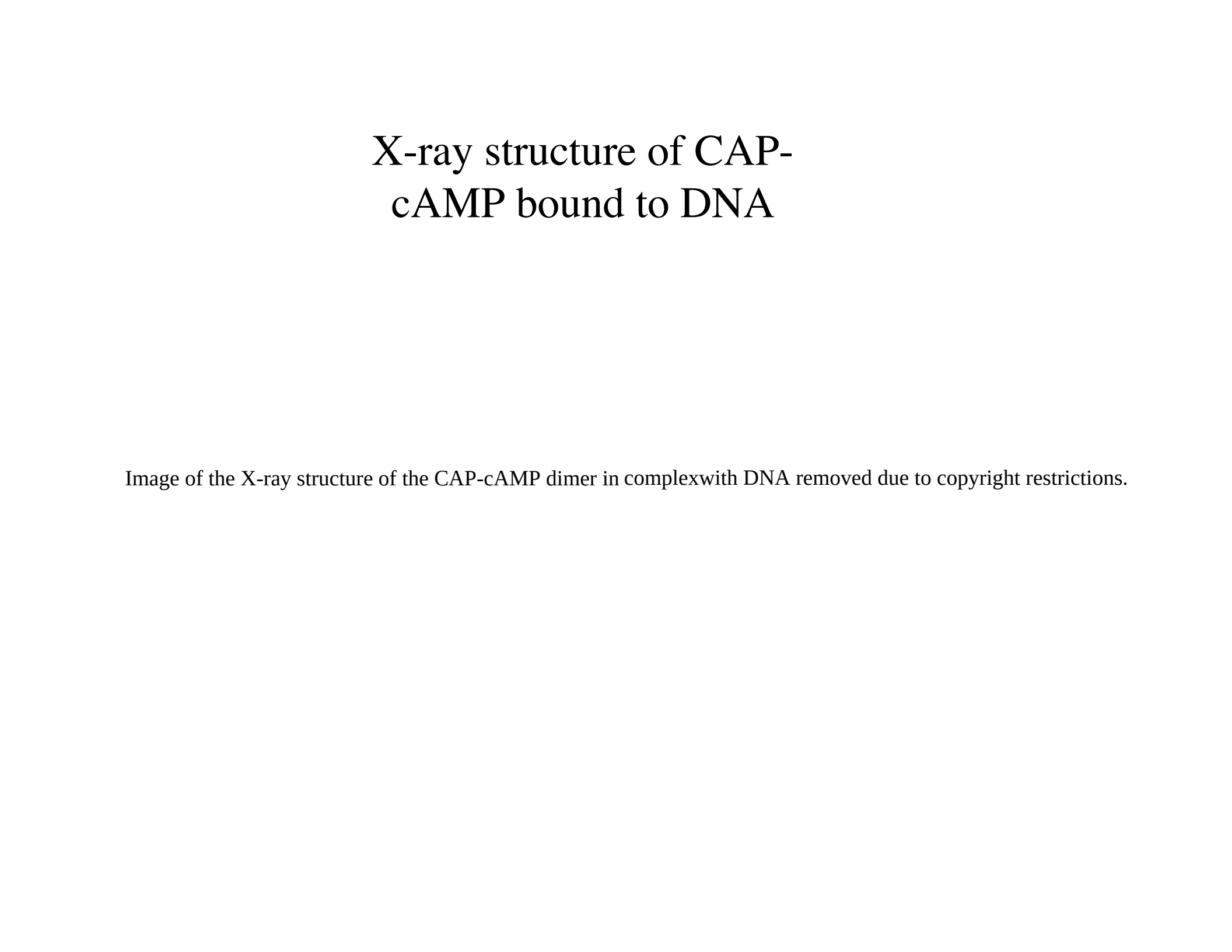


Figure by MIT OCW.

cAMP-CAP contacts the α-subunits of RNAP and enhances the binding of RNAP to the promoter.

X-ray structure of CAP-cAMP bound to DNA



Catabolite control of the *lac* operon

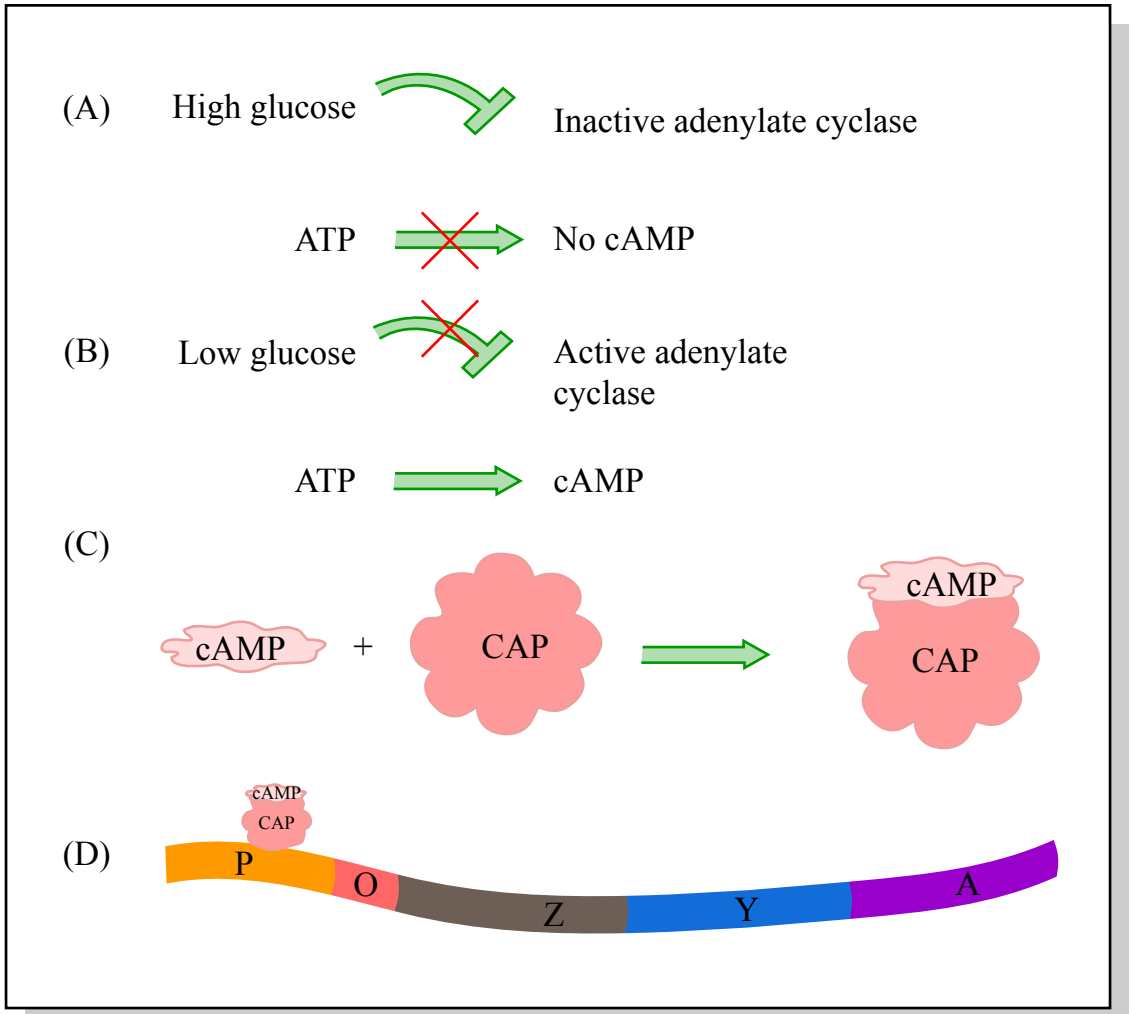


Figure by MIT OCW.

CAP sites are also present in other promoters. cAMP-CAP is a global catabolite gene activator.

(a) Under conditions of high glucose, a glucose breakdown product inhibits the enzyme adenylate cyclase, preventing the conversion of ATP into cAMP.

(b) As E. coli becomes starved for glucose, there is no breakdown product, and therefore adenylate cyclase is active and cAMP is formed.

(c) When cAMP (a hunger signal) is present, it acts as an allosteric effector, complexing with the CAP dimer.

(d) The cAMP-CAP complex (not CAP alone) 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)

Positive and negative regulation of the *lac* operon

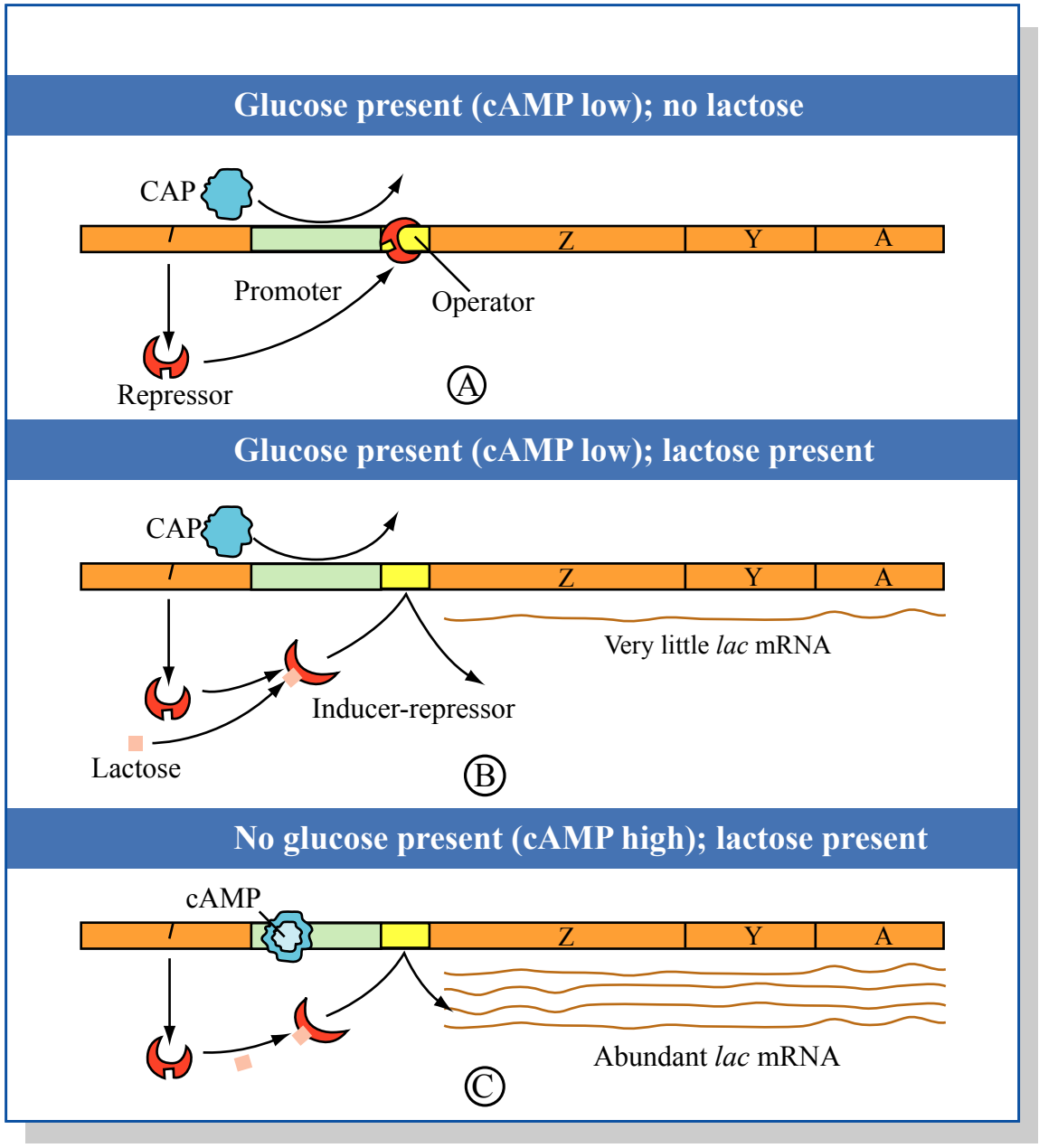


Figure by MIT OCW.

Transcriptional termination can be an important target for regulation

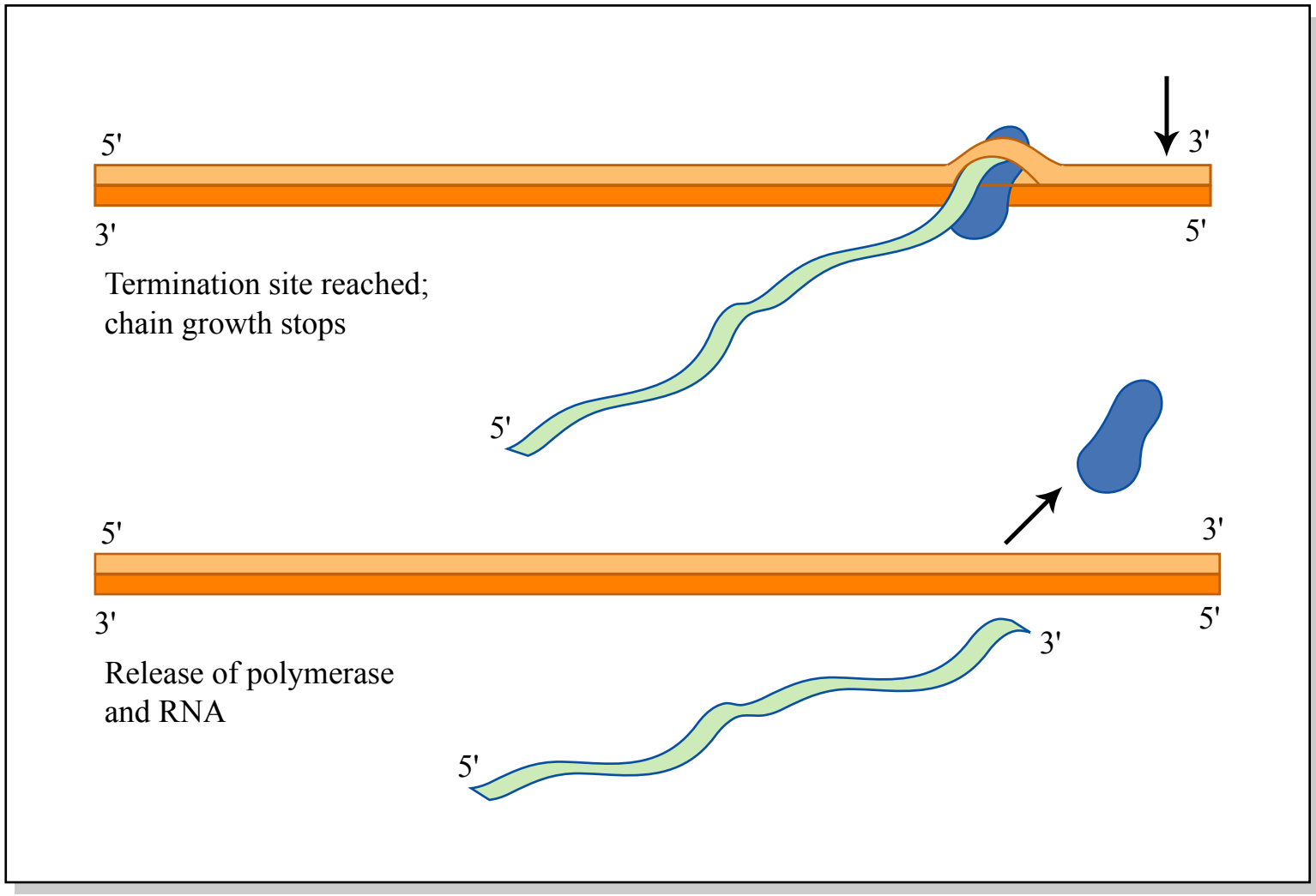


Figure by MIT OCW.

The tryptophan *trp* operon: two kinds of negative regulation

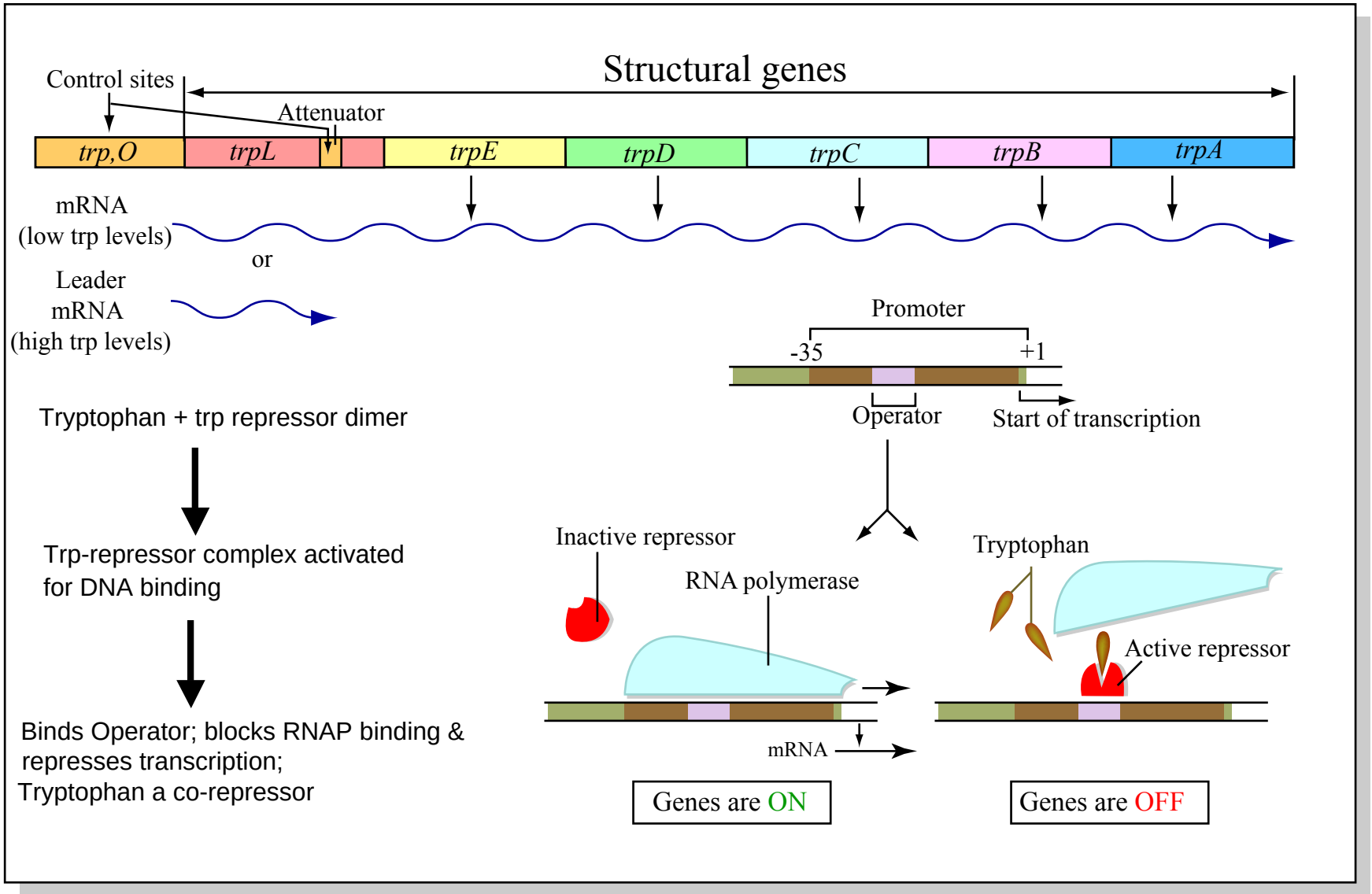


Figure by MIT OCW.

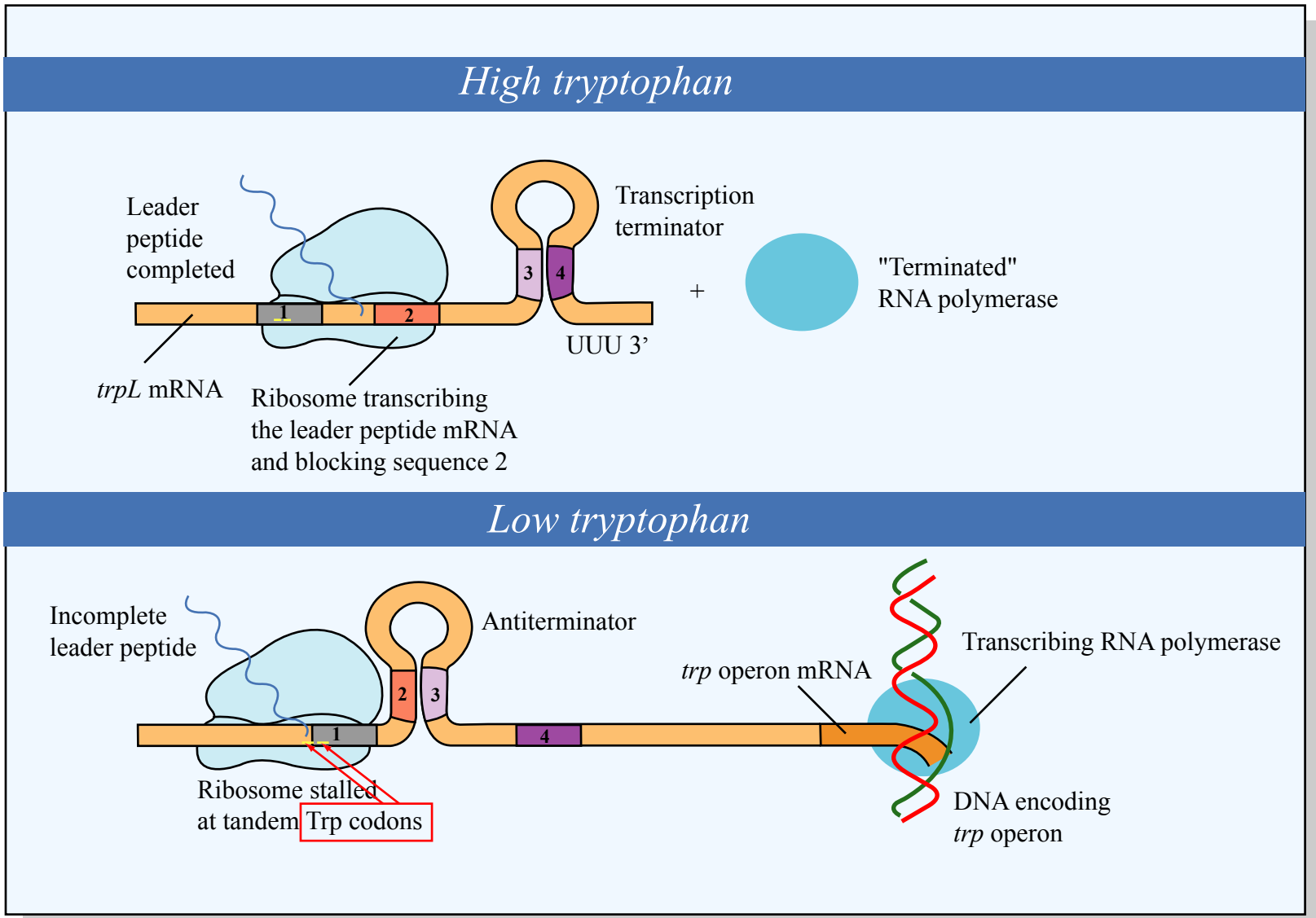

Image of trp and DNA removed due to copyright restrictions.

Many genes terminate transcription at sequences downstream of the coding sequence

Attenuation and the Tryptophan Operon

Attenuation is mediated by the tight coupling of transcription and translation

- **The ribosome translating the *trp* leader mRNA follows closely behind the RNA polymerase that is transcribing the DNA template.**
- **Alternative conformation adopted by the leader mRNA.**



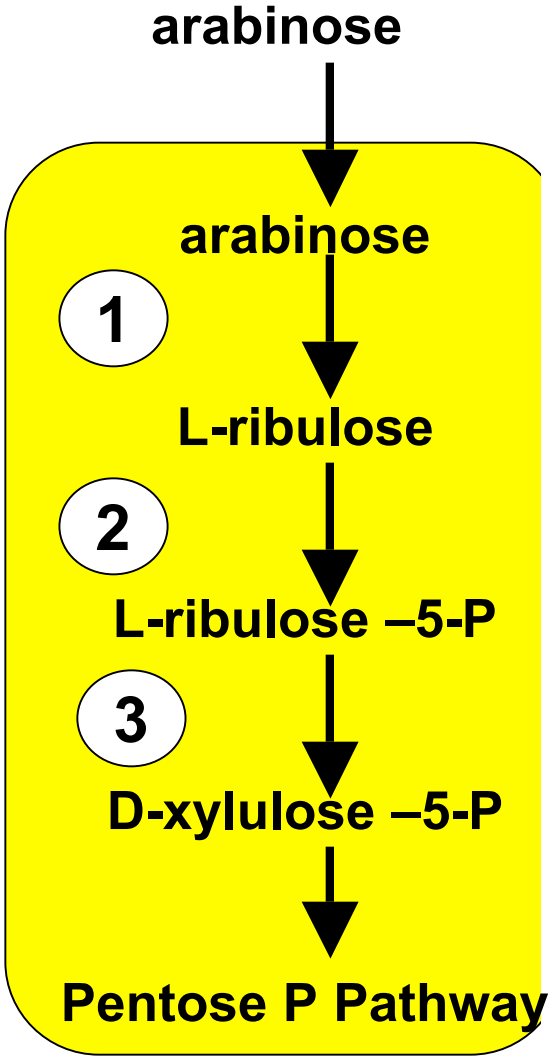
- **The stalled ribosome is waiting for tryptophanyl-tRNA.**
- **The 2:3 pair is not an attenuator and is more stable than the 3:4 pair.**

Figure by MIT OCW.

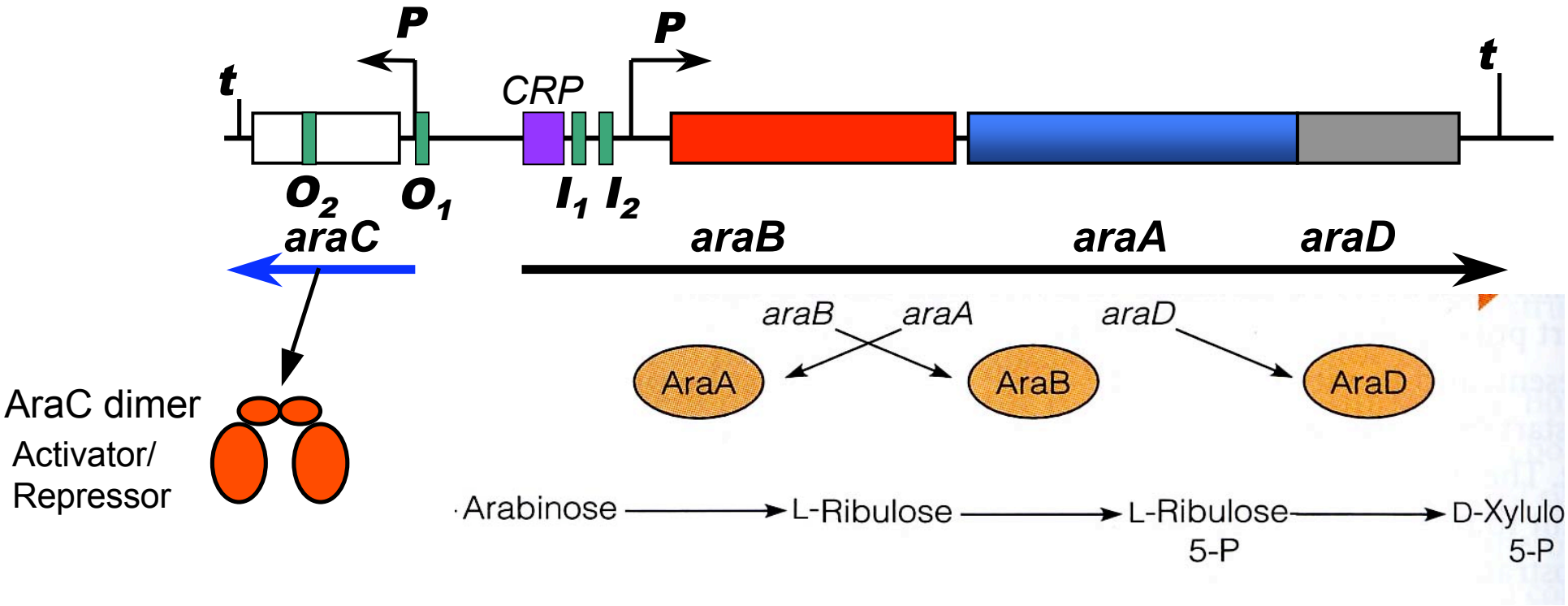
The arabinose (ara) operon

- 3 genes encoding enzymes of the **arabinose degradation** pathway
  - (1) *ara*A => L-arabinose isomerase
  - (2) *ara*B => L-ribulose kinase
  - (3) *ara*D => L-ribulose 5-phosphate

- Regulatory elements
  - *ara*O1, *ara*O2
  - *ara*I (I for inducer)
  - P_{BAD} promoter



The arabinose (*ara*) operon of *Escherichia coli*



Interaction of AraC with O and I sites determines transcription at the Ara locus

Key features of Arabinose regulation

1. **Arabinose is a positive regulator of transcription.**

2. **In the absence of arabinose AraC binds regulatory sites I_1 and O_2. This *represses* AraBAD operon transcription (DNA looping).**

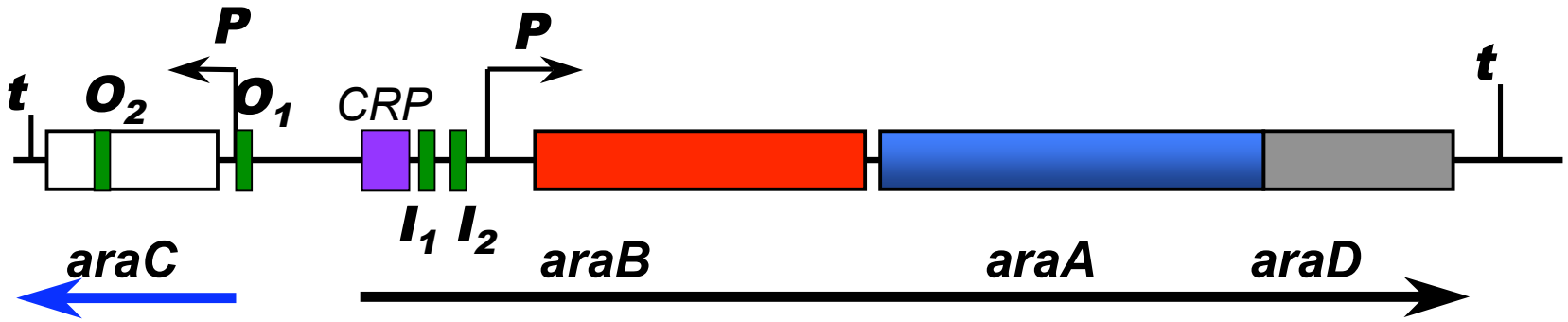
3. **AraC levels are autoregulated by excess AraC binding to O_1**

4. **When arabinose is bound to AraC, AraC binds I_1 and I_2.**

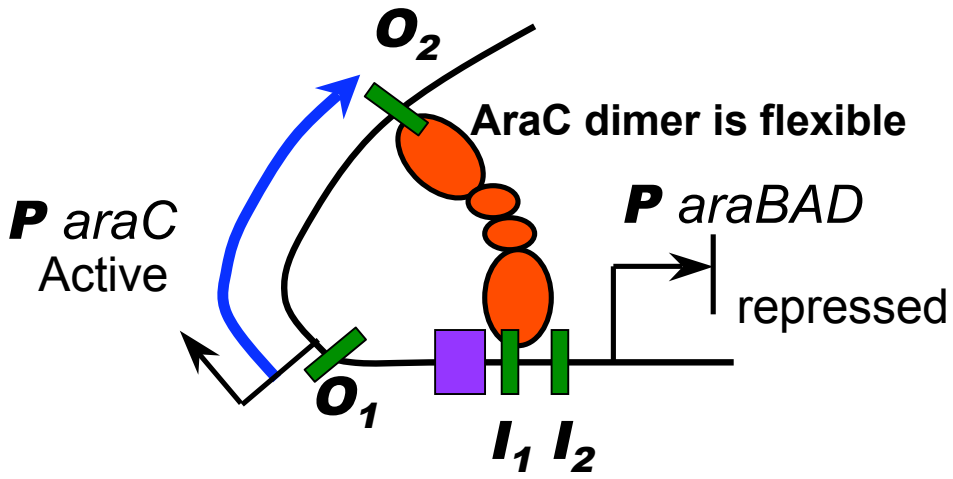
5. **When *Glucose is absent*, cAMP levels rise and cAMP-CRP bind adjacent to I1.**

Together these events trigger AraBAD expression.

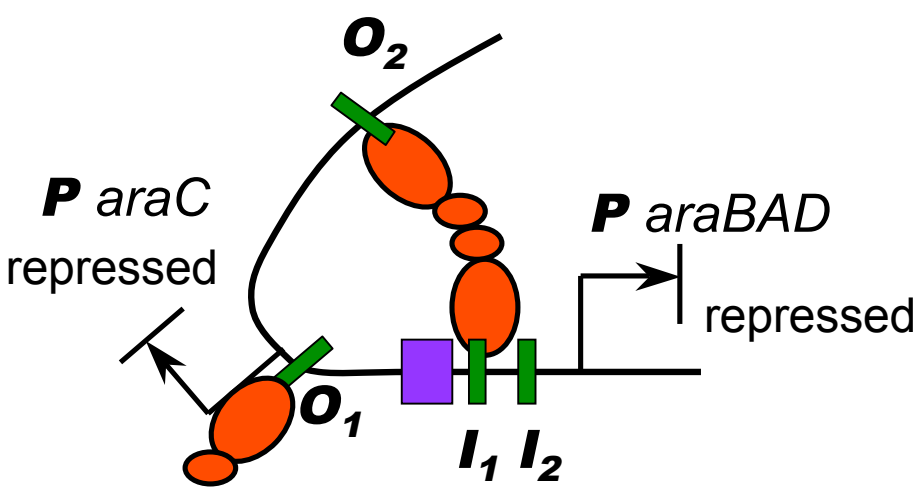
ara Transcriptional control-1



1. No L-arabinose

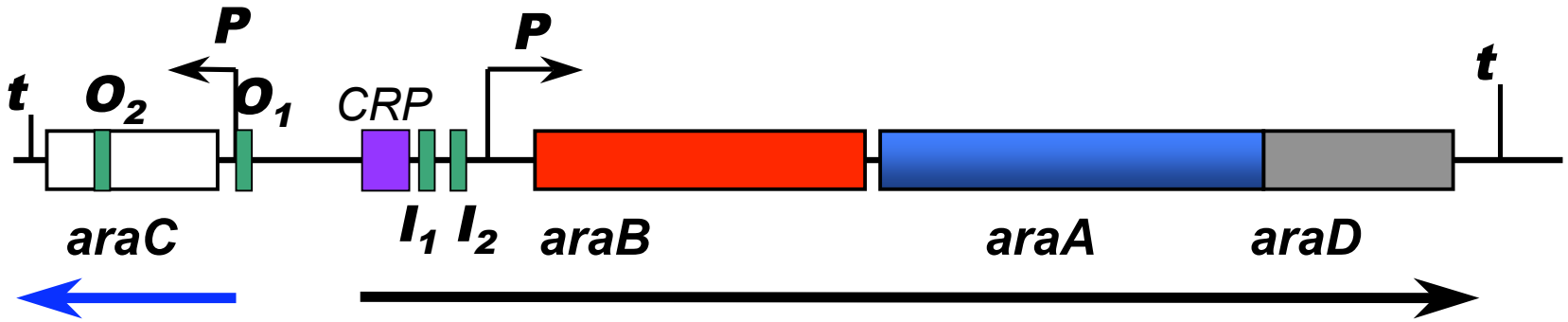


AraC binds to O_2 and I_1 repressing *P araBAD*



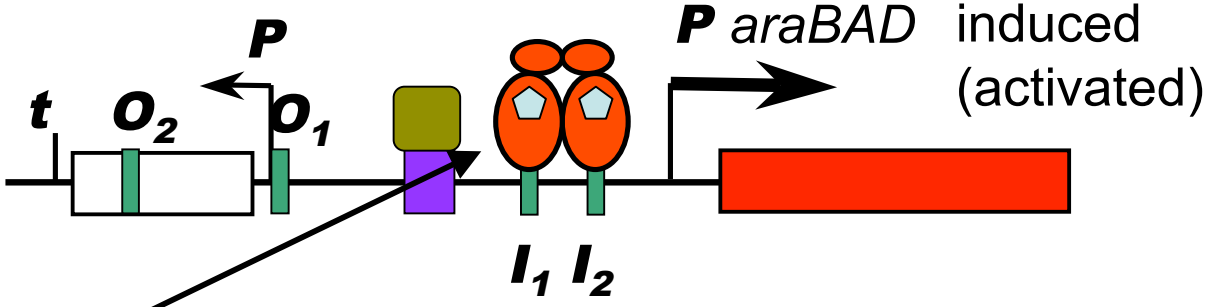
Excess AraC binds to O_1 regulating cellular levels of AraC protein

ara Transcriptional control-2



2. + *Inducer* L-arabinose

+ cAMP-CRP



AraC binds to I_2 and I_1 activating *P araBAD*

Differences between the arabinose and lactose operons

1. **AraC can act as both a repressor and activator of *AraBAD* expression. *This is an example of positive control.***

2. **AraC can regulate its own synthesis by repressing its own transcriptio This is a common feature of many genes.**

3. ***Ara* operon provides an example of regulation at a distance by DNA looping.**

Common mechanisms of regulation of transcription, with variations on a theme…

	lac	ara	trp
Regulation	negative positive	negative, positive, auto-	negative attenuat
Operators	lacO1, O2, O3	araO1, O2, I	trpO
CRP-site	+	+	-
Regulator (location)	lacI upstream	araC upstream	trp R distant
Effector	allolactose (activator)	arabinose (activator)	tryptophan (co-repressor)

“Flipping promoters” Flagellar phase variation

Flagellar
Phase variation

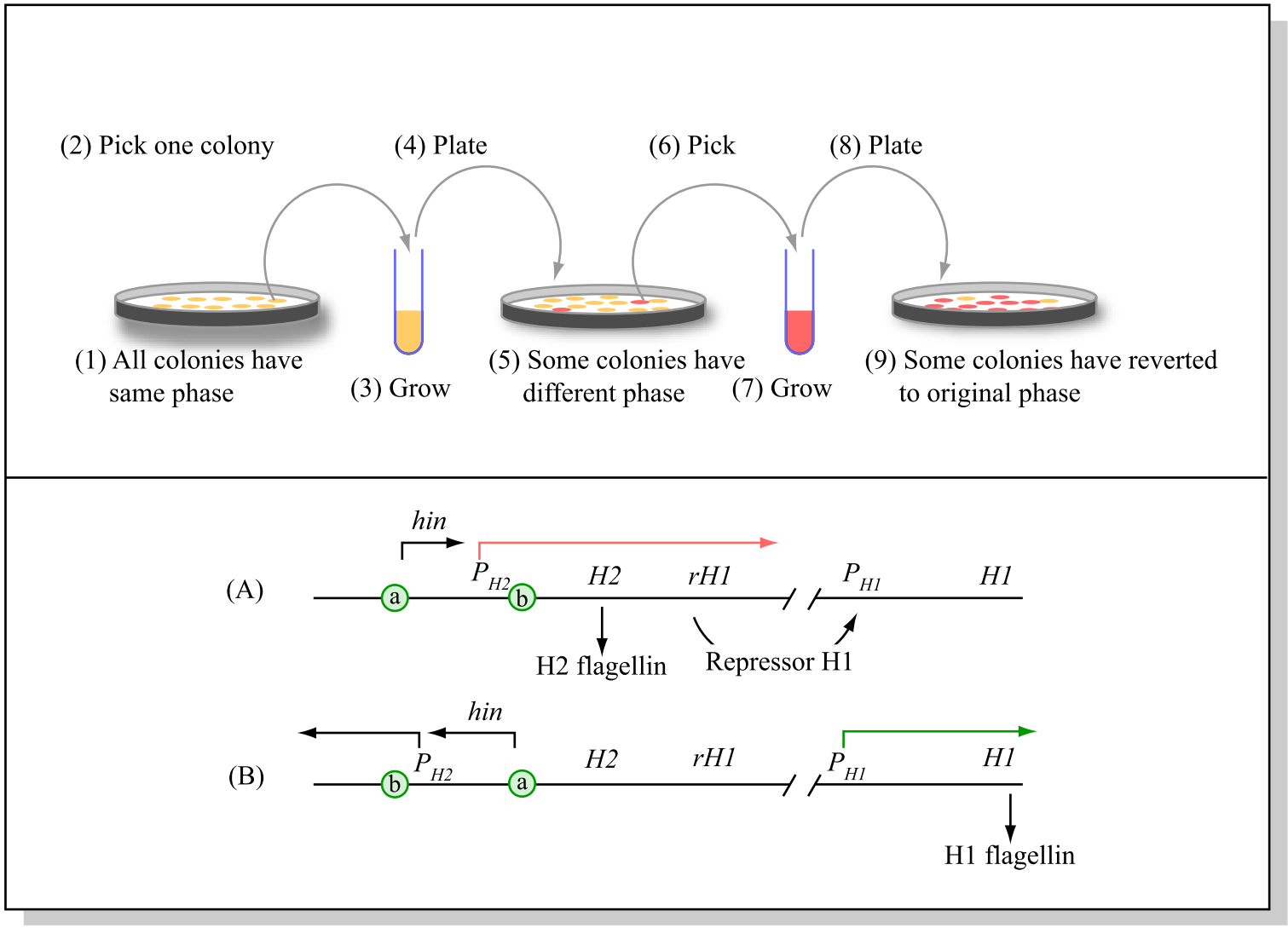
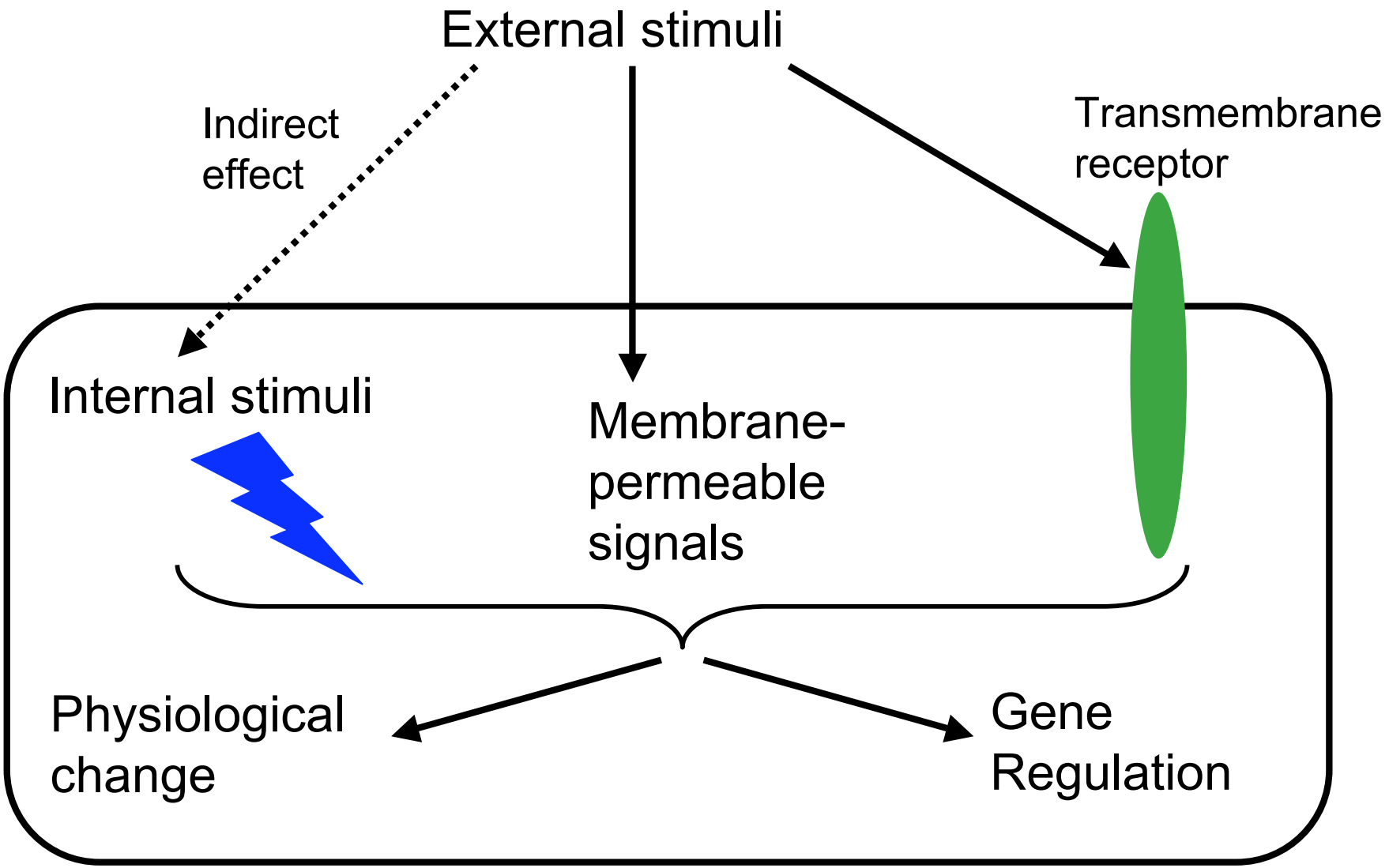


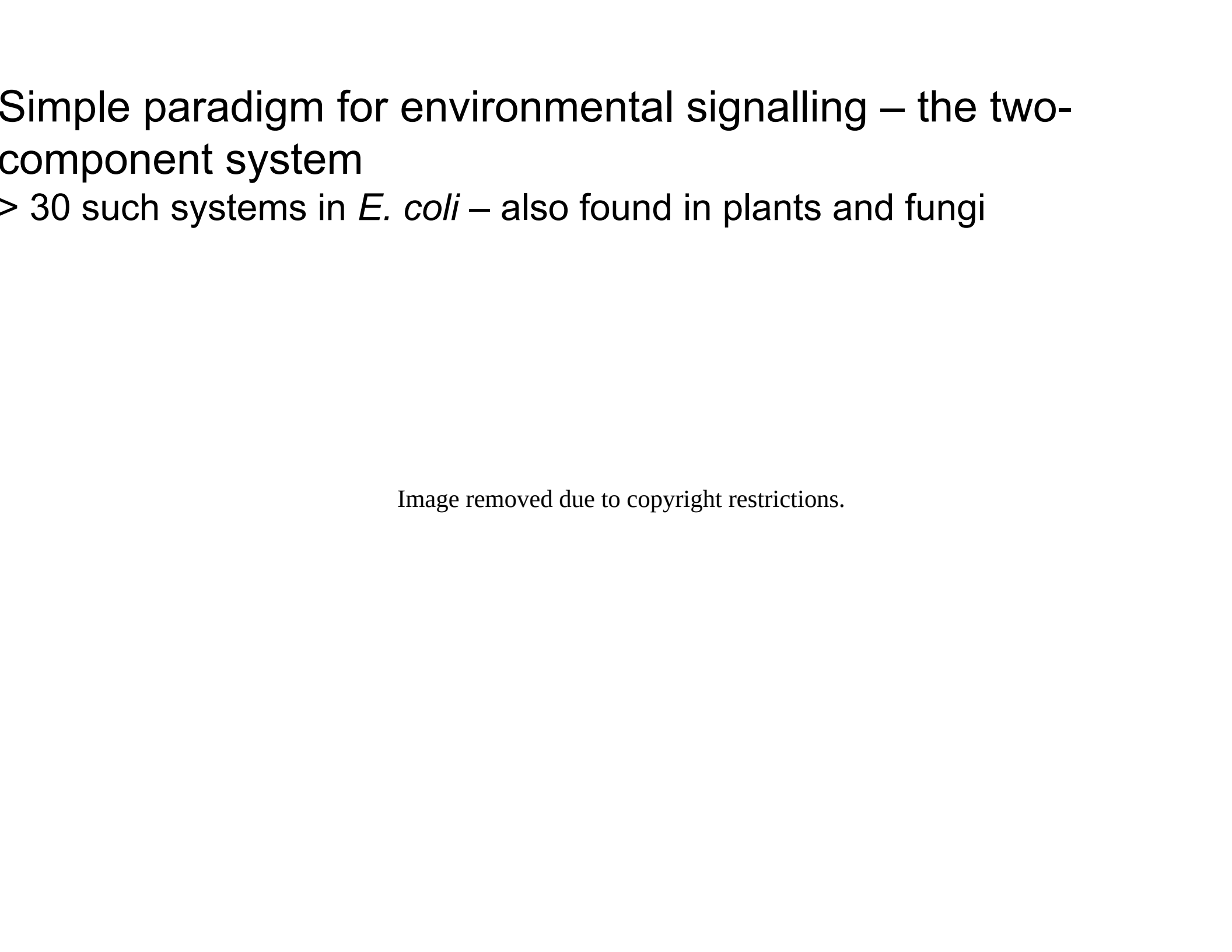
Figure by MIT OCW.

Environmentally-responsive adaptation



Simple paradigm for environmental signalling – the two-component system

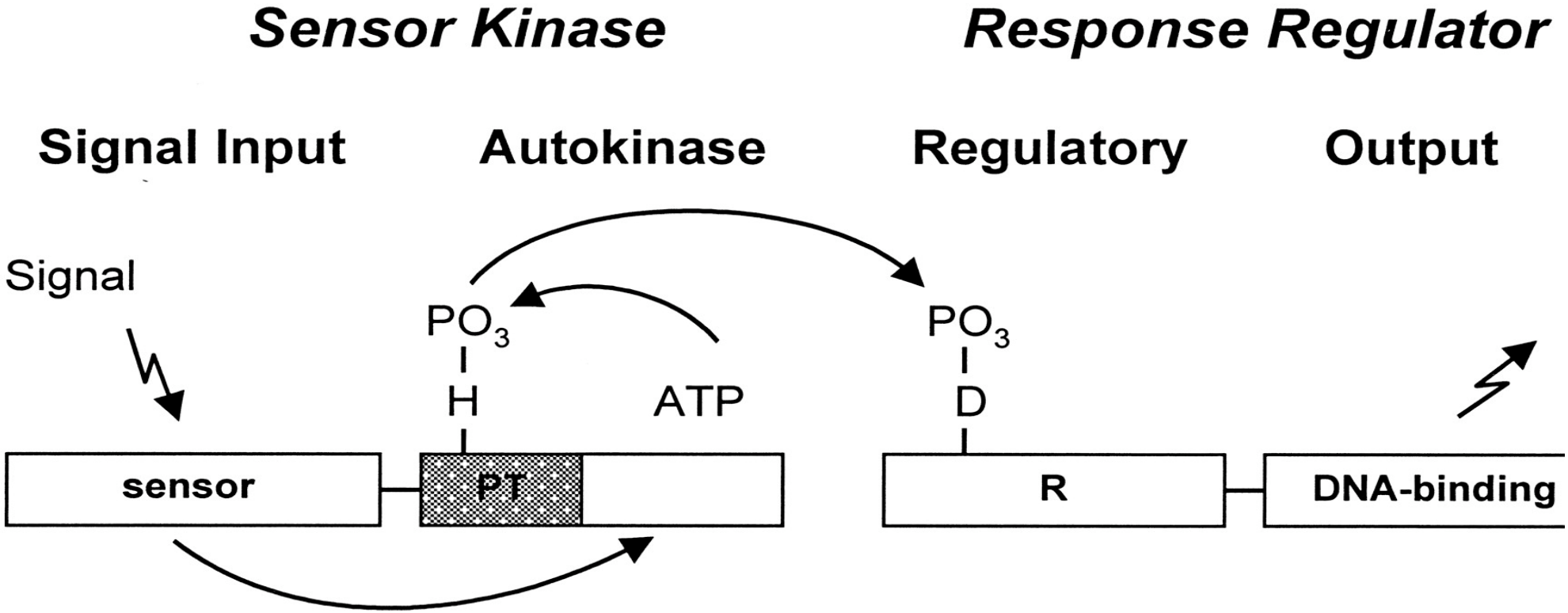
> 30 such systems in *E. coli* – also found in plants and fungi



Basic model for a two component-regulatory system

Sensor histidine kinase (HK) – may or may not be transmembrane – phosphorylates itself

Response regulator (RR) – often, but not always affects gene expression – phosphorylated by HK



Hoch and Varughese, 2001, J. Bacteriol. 183:4941-4949

The PhoR/PhoB two-component regulatory system in E. coli

Diagram removed due to copyright restrictions.

In response to low phosphate concentrations in the environment and periplasmic space, a phosphate ion dissociates from the periplasmic domain of the **sensor** protein PhoR. This causes a conformational change that **activates** a protein kinase transmitter domain in the cytosolic region of PhoR. The activated transmitter domain transfers an ATP γ-phosphate to a histidine in the transmitter domain. This phosphate is then transferred to an aspartic acid in the **response regulator** PhoB. Phosphorylated PhoB then activate transcription from genes encoding proteins that help the cell to respond to low phosphate, including phoA, phoS, phoE, and ugpB.

Two-components alone aren't always sufficient – phospho relays are through multiple protein modulators are a common regulatory mechanism

Diagram removed due to copyright restrictions.

Stock et al. 2000, Ann. Rev. Genet 69:183-215

'phosphorylation cascades'

Allow response to wide range of chemical and physical stimuli

Diagram removed due to copyright restrictions.

Many variations on the basic theme exist and the more they are studied the more permutations are observed

Stock et al. 2000, Ann. Rev. Genet 69:183-215

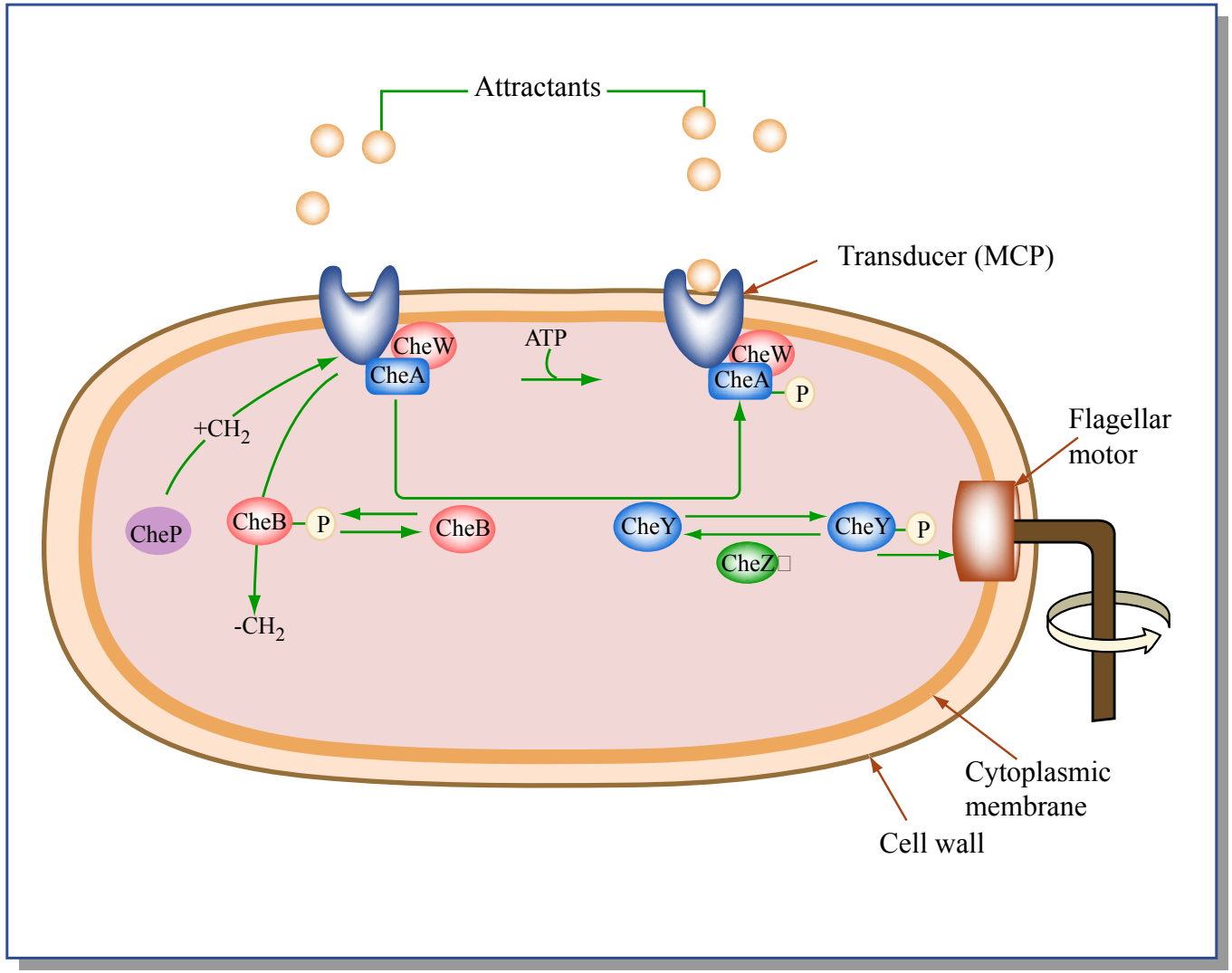
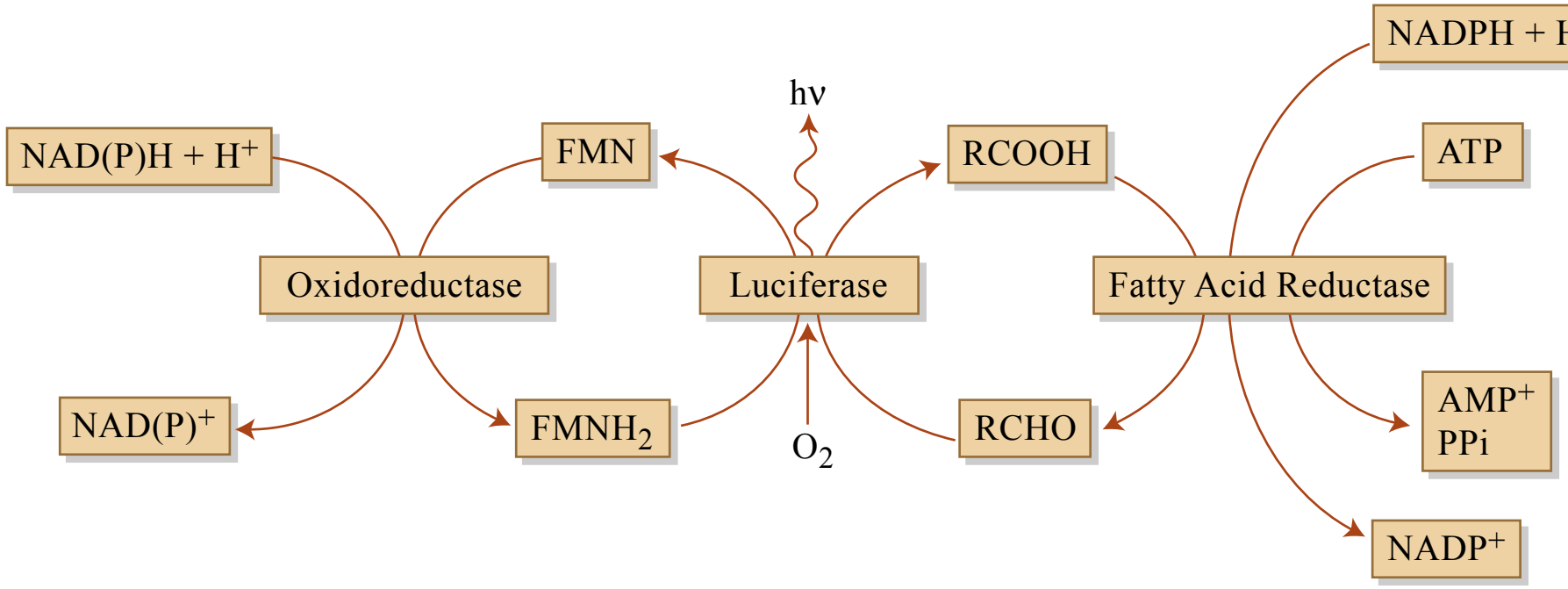


Figure by MIT OCW.

Quorum Sensing

Cell, Vol. 32, 773–781, March 1983, Copyright © 1983 by MIT

Bacterial Bioluminescence: Isolation and Genetic Analysis of Functions from Vibrio fischeri



Substrates, Products and Pathways Involved in the Bacterial Bioluminescence Reaction

Figure by MIT OCW.

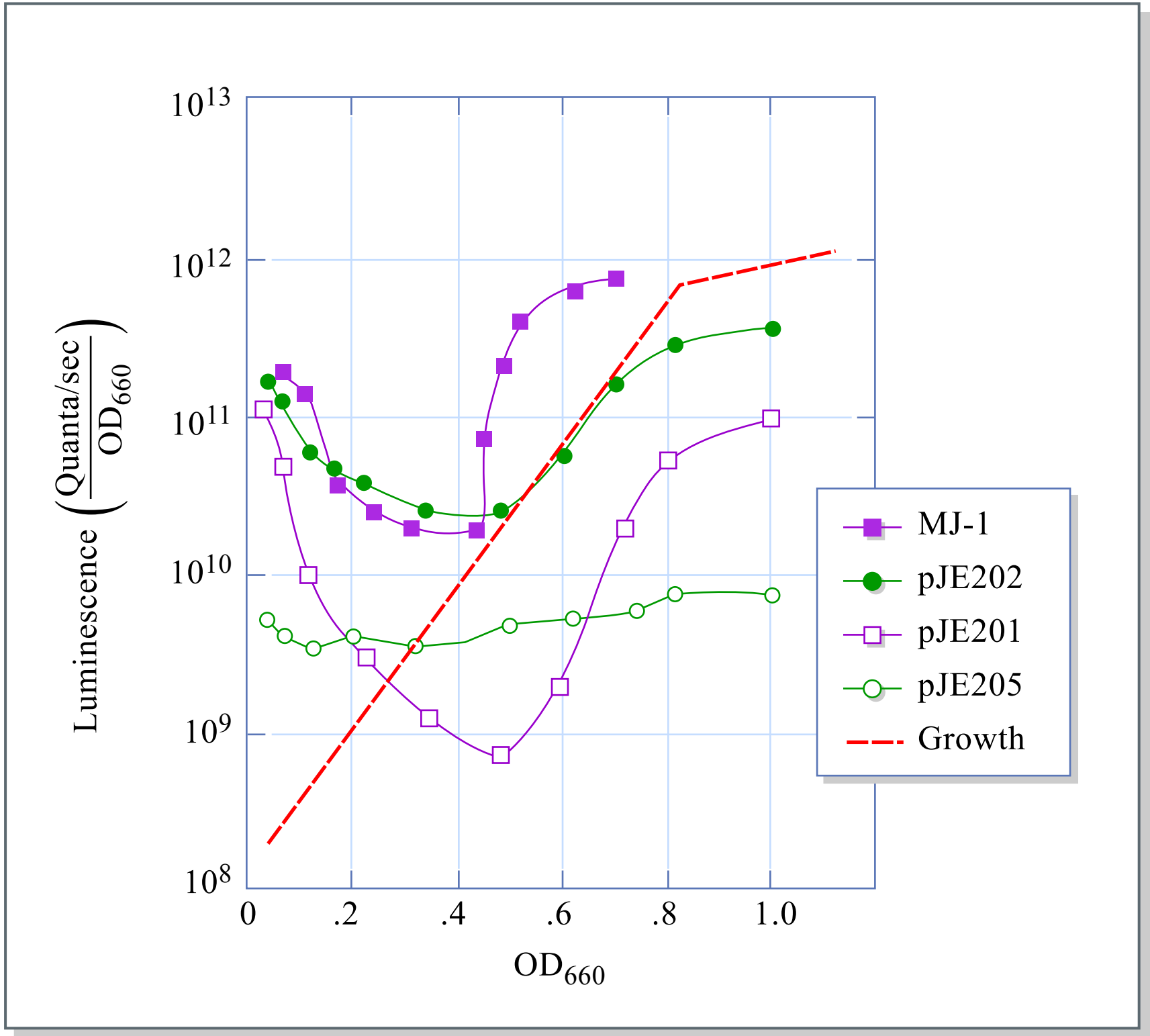


Figure by MIT OCW.

Nucleic Acids Res. 1987 December 23; 15(24): 10455–10467.

Nucleotide sequence of the regulatory locus controlling expression of bacterial genes for bioluminescence

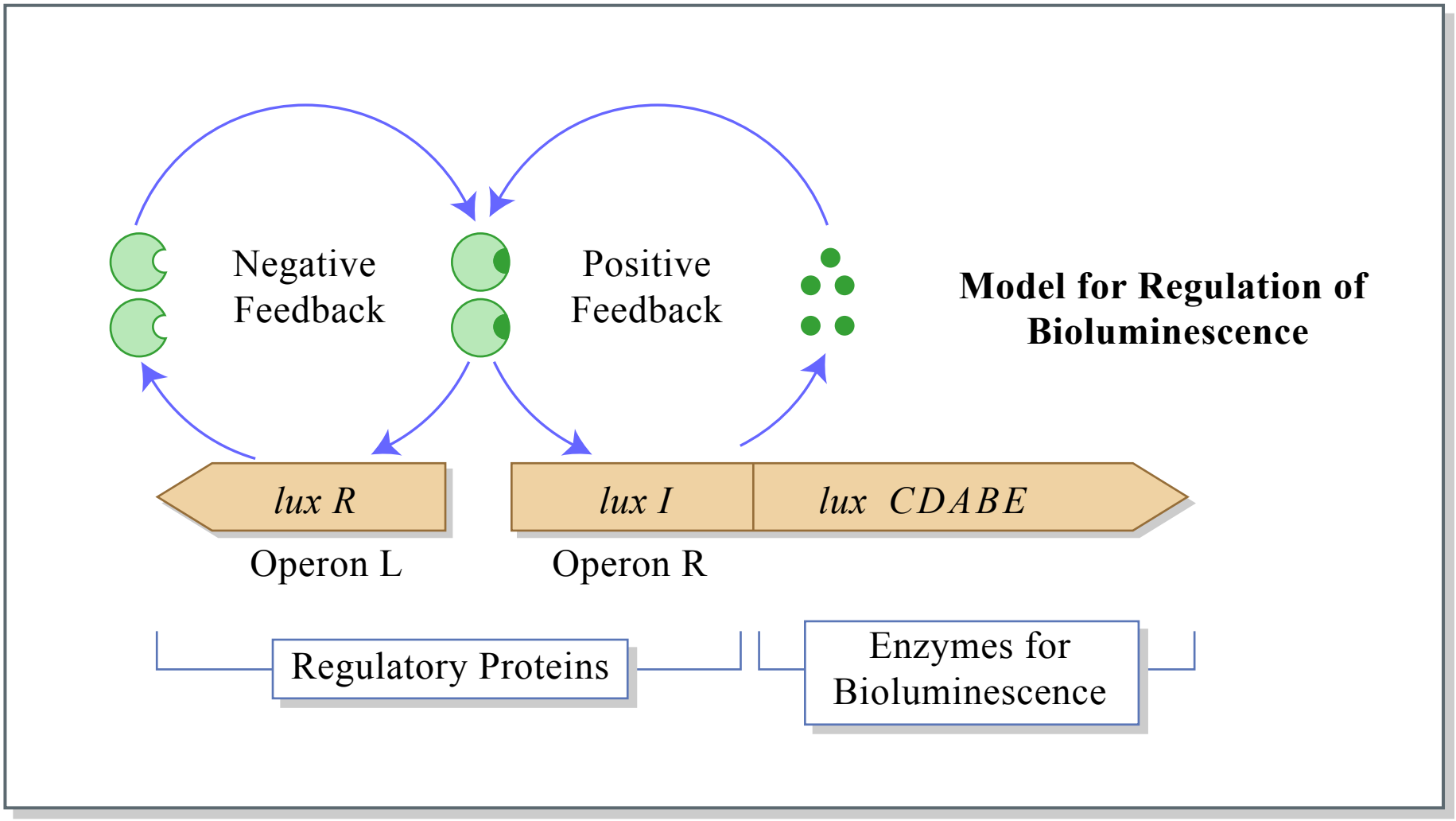


Figure by MIT OCW.

Vibrio fischeri and luminescence :

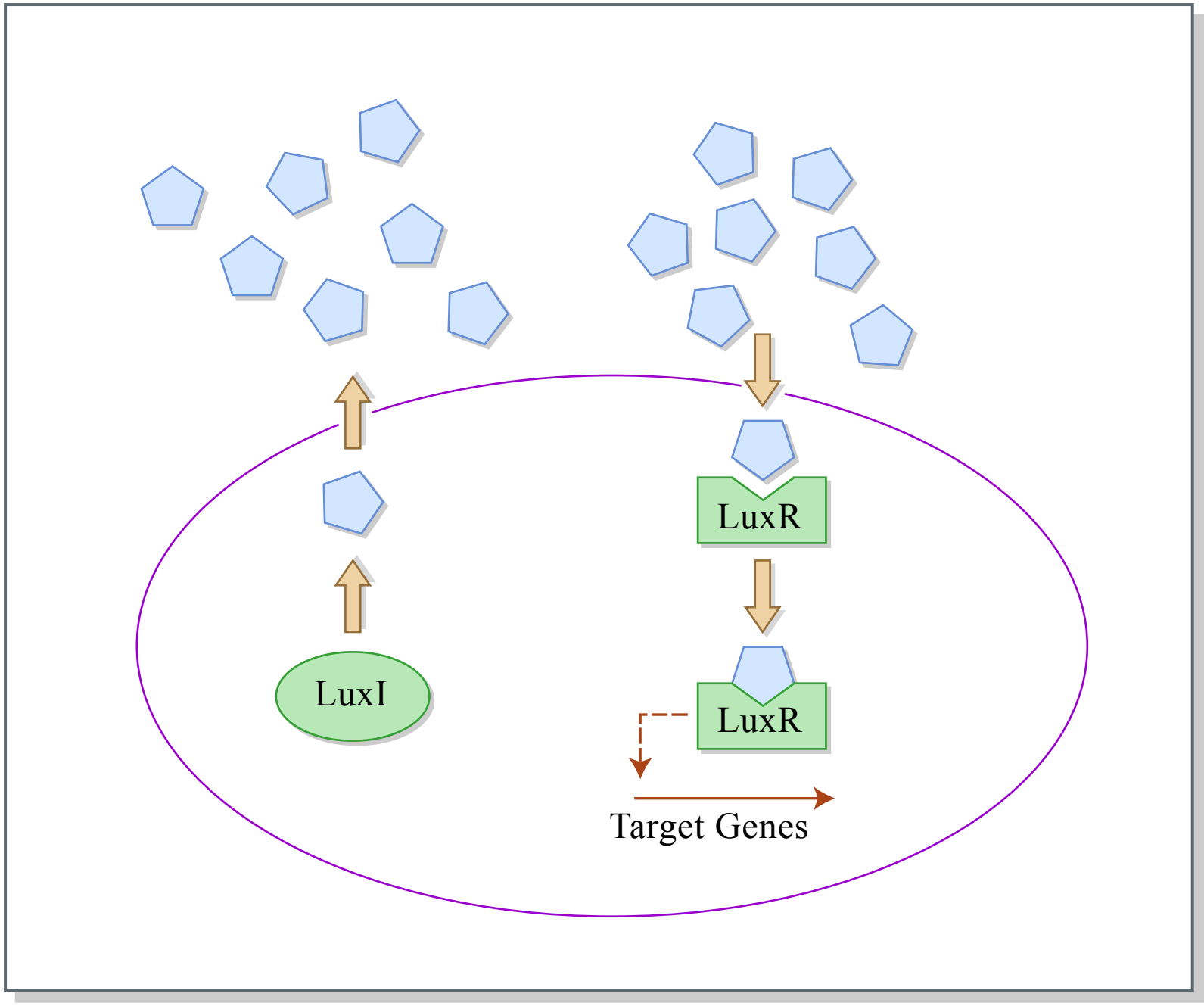
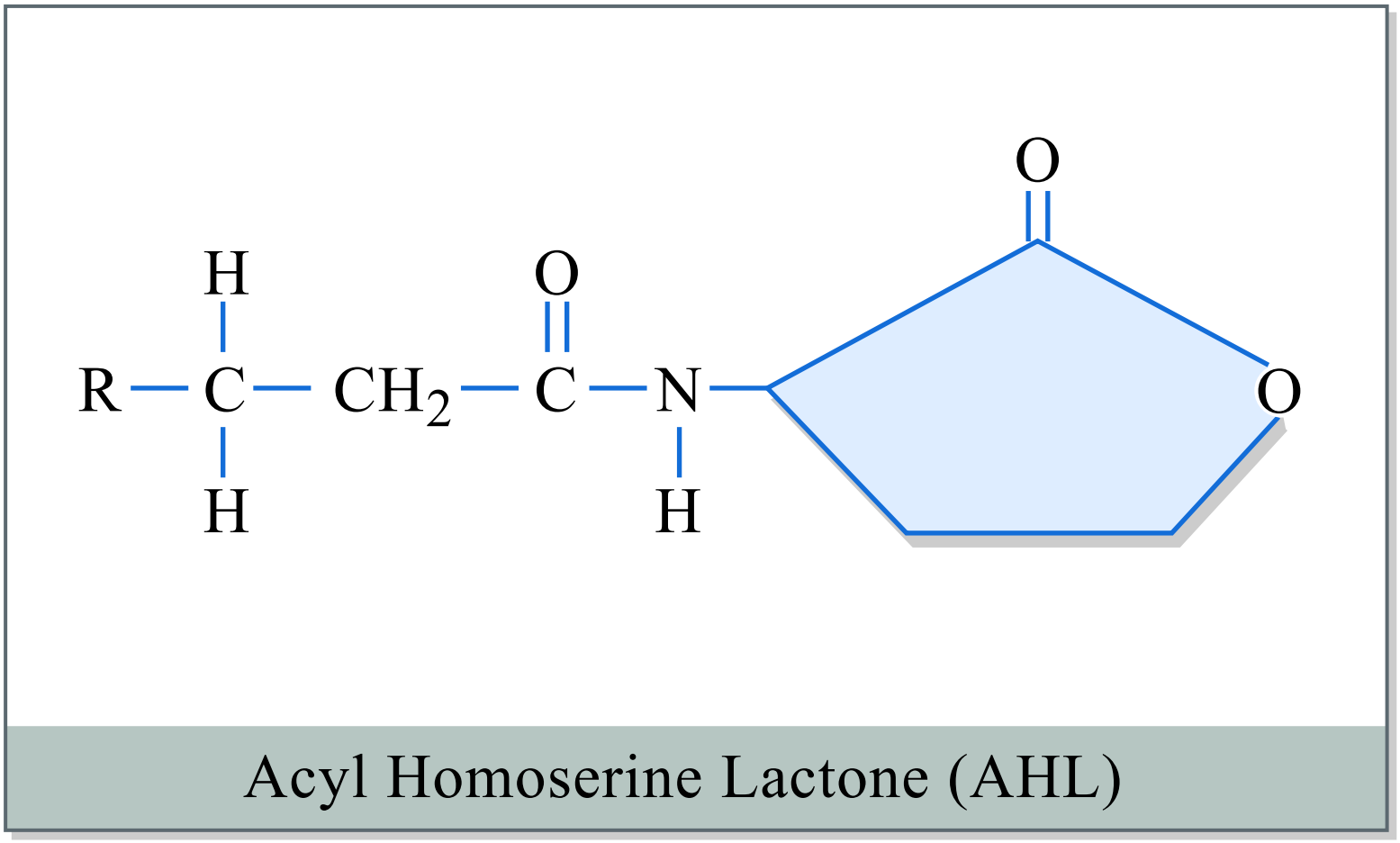


Figure by MIT OCW.

Quorum Sensing



Acyl Homoserine Lactone (AHL)

Figure by MIT OCW.

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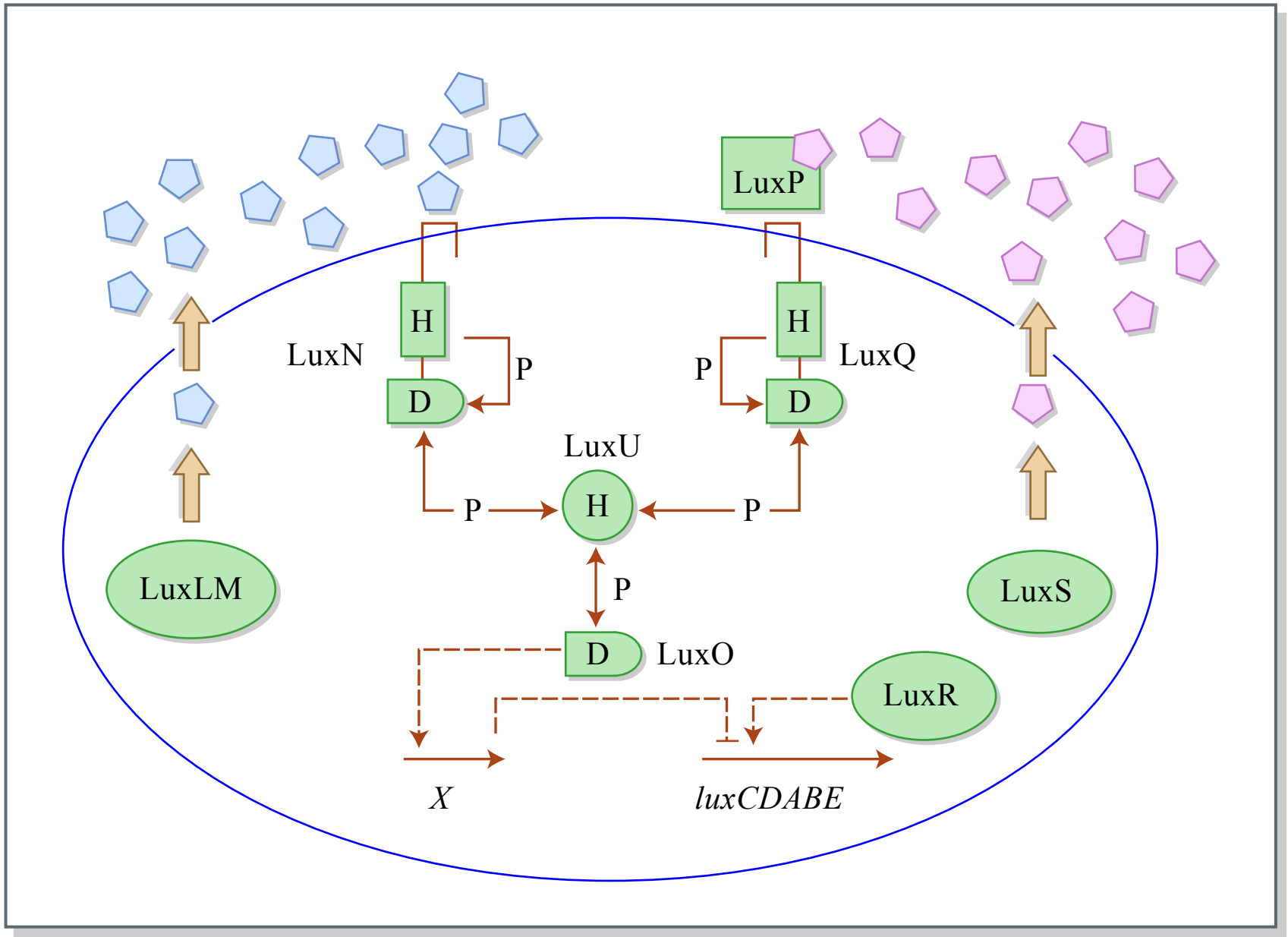
See Figure 8-22b in Madigan, Michael, and John Martinko. *Brock Biology of Microorganisms.* 11th ed. Upper Saddle River, NJ: Pearson Prentice Hall, 2006. ISBN: 0131443291.

Examples of bacteria that use acylated homoserine lactones

Bacteria	Function
Vibrio fischeri	luminescence
Aeromonas hydrophila	proteases
Agrobacterium tumefaciens	conjugation
Burkholderia cepacia	siderophores
Chromobacterium violaceum	antibiotics
Erwinia chrysanthemi	pectinase
Pseudomonas aereofaciens	phenazines
Pseudomonas aeruginosa	biofilms, etc
Rhizobium etli	number of nodules
Yersinia pseudotuberculosis	agreggation and motility

Variations and Complications :
Vibrio harveyi



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See Figure 8-1 in Madigan, Michael, and John Martinko. *Brock Biology of Microorganisms*. 11th ed. Upper Saddle River, NJ: Pearson Prentice Hall, 2006. ISBN: 0131443291.

The "stringent response"
A type of translational global control

When bacteria are starved of nutrients, they immediately shut down gene expression and other metabolic activities.

1. **Total RNA synthesis is reduced to ~ 10% of normal levels.**

2. **There is a massive >10-fold reduction in rRNA and tRNA transcription.**

3. **Protein synthesis decreases.**

(The unusual nucleotides ppGpp and pppGpp accumulate during the stringent response).

Stringent response in *E. coli*

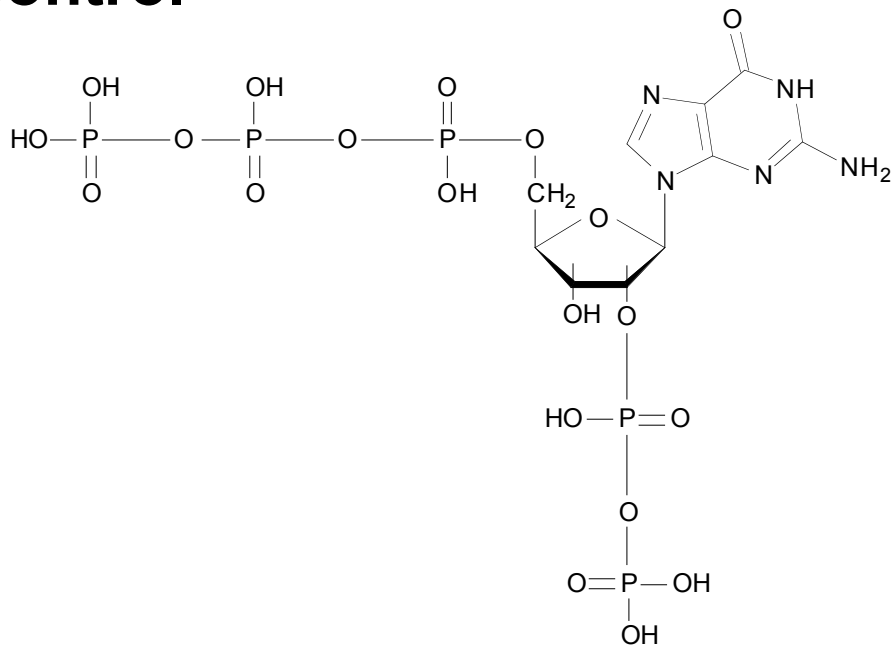
1. Binding of an uncharged tRNA to the A-site

2. Binding of RelA to the 30S subunit

3. Synthesis of ppGpp

4. Downregulation / inhibition of transcription

The “stringent response”
A type of translational global control



The ppGpp inhibits the elongation phase of transcription.

The stringent factor RelA is a pppGpp synthetase that is associated with ~ 5% of ribosomes.

RelA protein produces one pppGpp every time the A site is occupied by an uncharged tRNA.

The "stringent response"
A type of translational global control

Ribosomal protein L11 undergoes a conformational change when an uncharged tRNA binds.

This activates RelA stringent factor.

The unusual nucleotides ppGpp and pppGpp accumulate during the stringent response.

Total RNA synthesis is reduced to ~ 10% of normal levels.
There is a massive >10-fold reduction in rRNA and tRNA transcription.
Protein synthesis decreases.

(The SpoT protein degrades ppGpp so that normal gene expression can resume rapidly when conditions improve.)

Review of gene regulation in bacteria.

With genes that are expressed constitutively,
promoter strength determines the level of expression.

DNA-binding proteins can switch genes on and off.

Repressors switch genes off.
[They prevent RNA pol from gaining access to promoters].

Activators switch genes on.
[They enable RNA pol to bind to promoters]

Activity of repressors and activators can be influenced by
small molecules, temperature, phosphorylation etc.

RNA secondary structure can control gene expression.

With attenuation, AA-tRNA availability influences early termination.

With riboswitches, small molecules influence early termination or translation intiation.

Alternative sigma factors bring about global changes in gene expression.

The stringent response shuts down gene expression when times are tough.

Promoter inversion can affect gene expression in pathogenic bacteria.