ZOO 203 – MOLECULAR BIOLOGY
UNIT 4: Regulation of Gene Expression in Prokaryotes
(Part- I)

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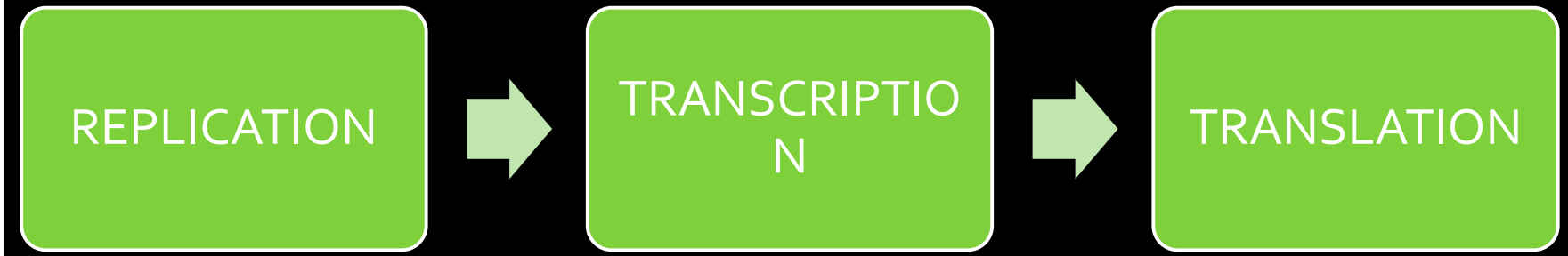
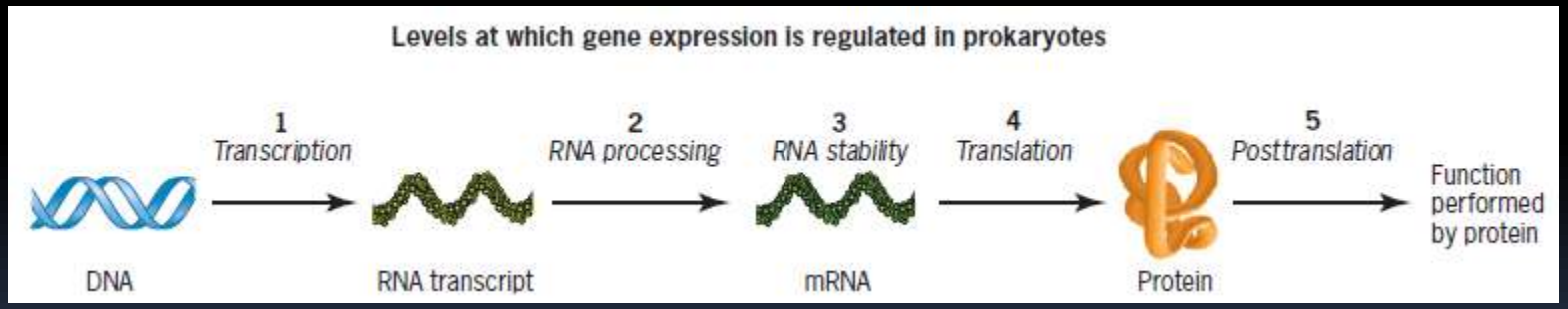
CENTRAL DOGMA IN MOLECULAR BIOLOGY



Gene expression in prokaryotes is regulated at different levels

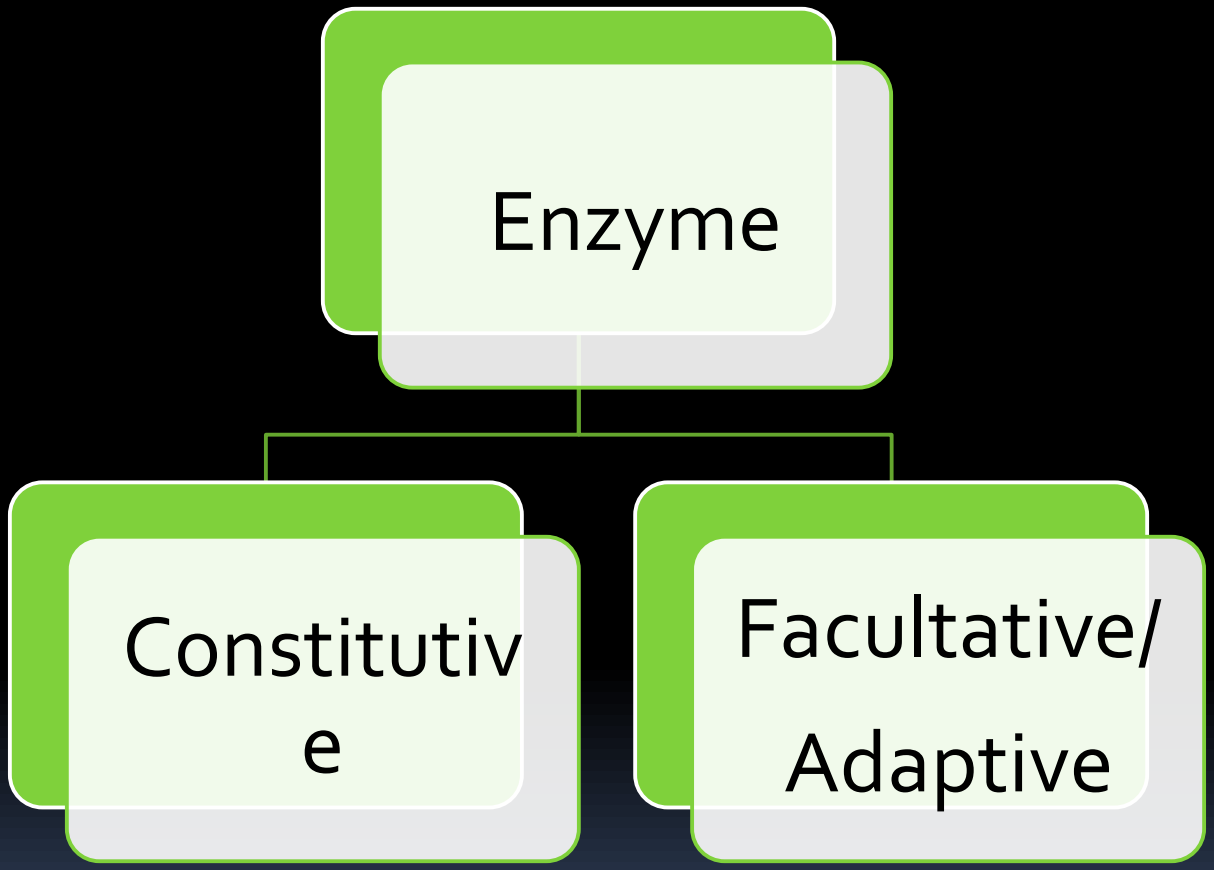
- Transcription
- Processing of m-RNA
- Translation
- Post- Translation



Source : Principles of Genetics by D. Peter Snustad (sixth edition)

Regulation at Transcription level

- Mechanism that involve the rapid turn-on and turn-off of gene expression in response to environmental changes.
- Mechanism referred to as preprogrammed circuits or cascades of gene expression.


Enzyme
Constitutive
Facultative/ Adaptive

Constitutive & Facultative Enzymes

- Enzymes that are produced continuously, regardless of the chemical makeup of the environment, were called **constitutive enzyme.**

- Enzymes that are produced depending on the chemical makeup of the environment for adaptive value is called **adaptive/facultative enzyme.**

Need of regulation

- Synthesis of enzymes require utilization of considerable energy. (ATP or GTP)
- In absence of substrate, production of enzyme is wastage of energy.
- Economical expenditure of energy.

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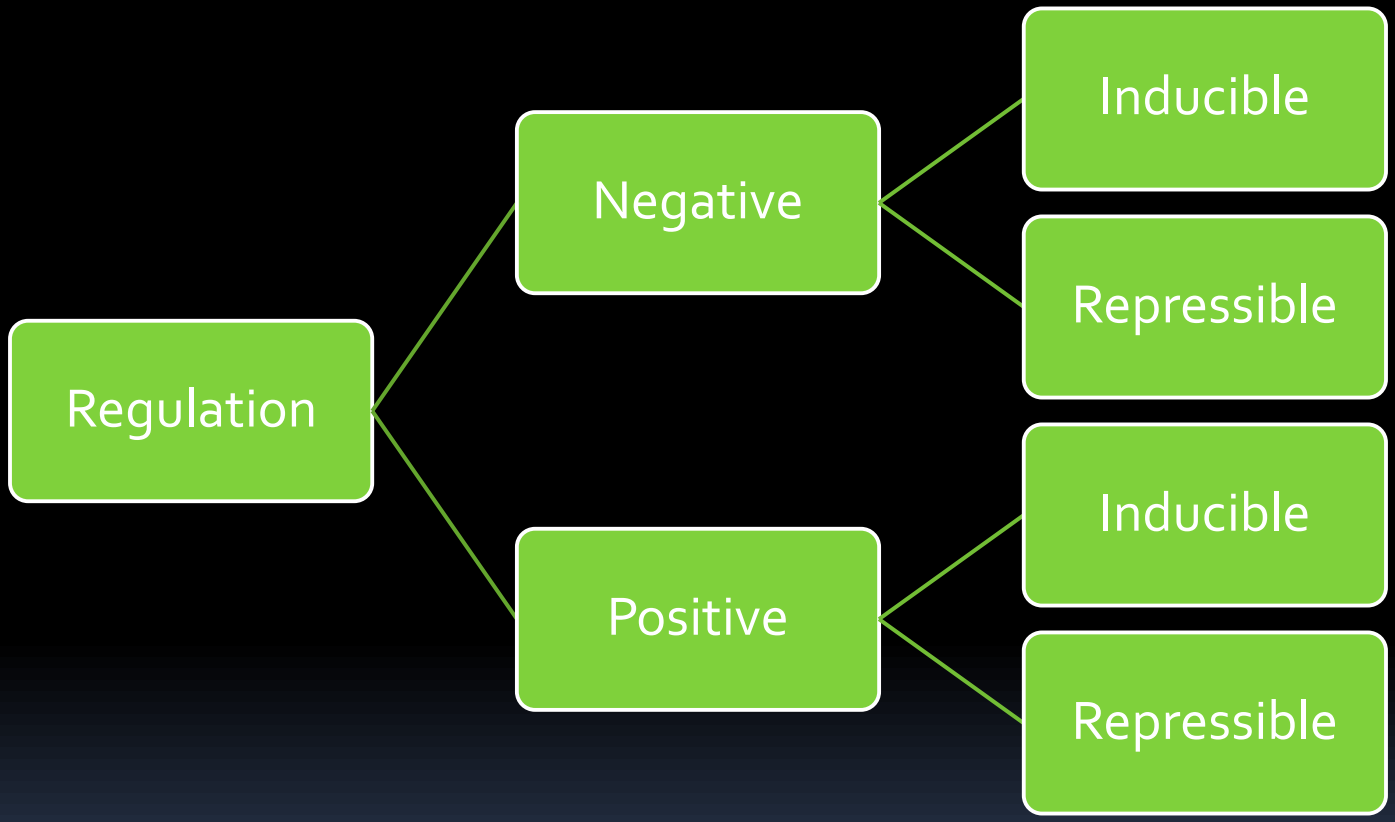
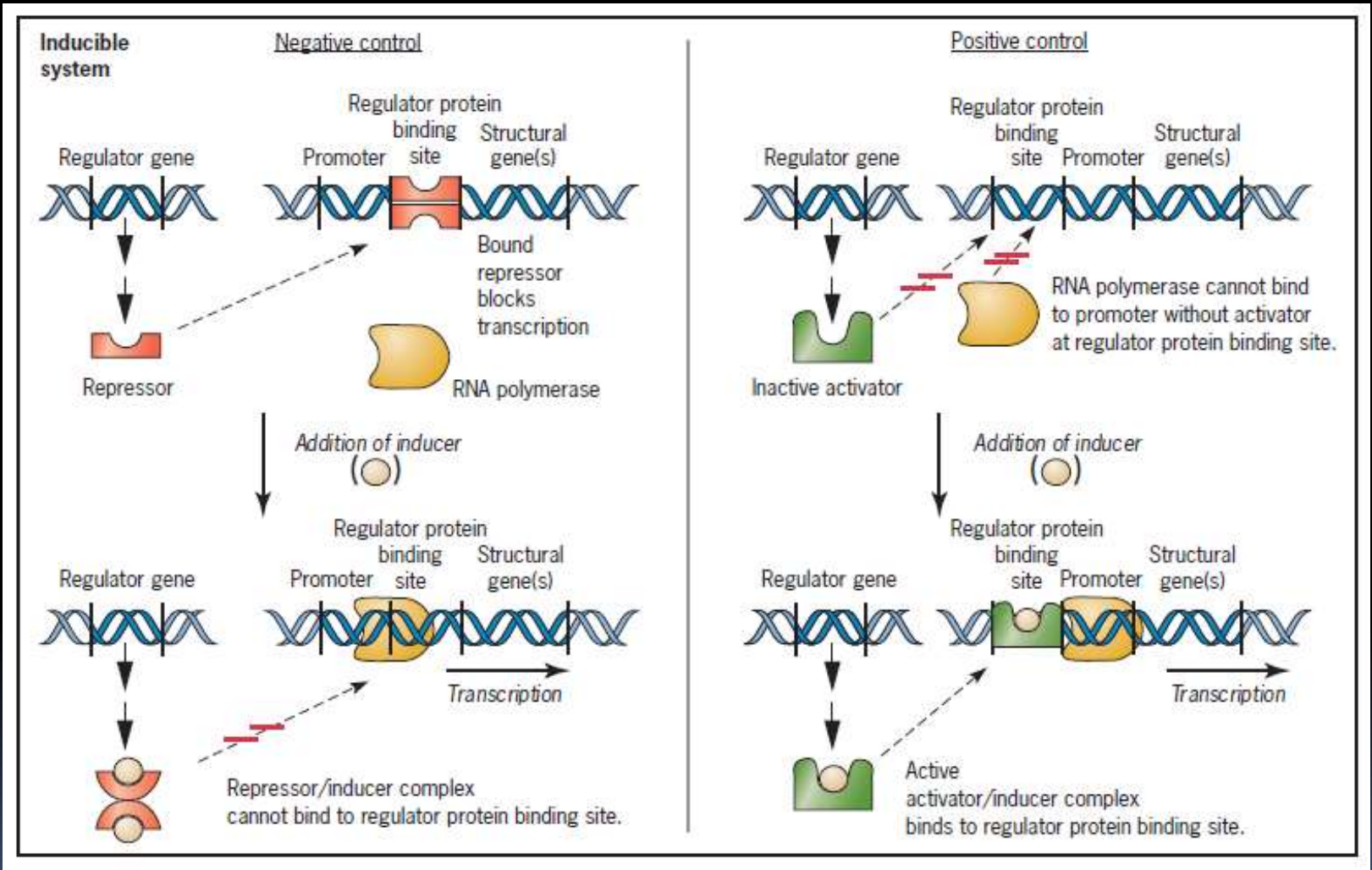

Regulation
Negative
Positive
Inducible
Repressible
Inducible
Repressible

Positive & Negative Control

- In positive regulation a transcription factor(product of regulator gene) is required to bind at the promoter in order to enable RNA polymerase to initiate transcription (turn on)
- In negative regulation a repressor protein (product of regulator gene) binds to an operator to prevent a gene from being expressed(shut off).

Negative and positive control of inducible (a) and repressible (b) gene expression.



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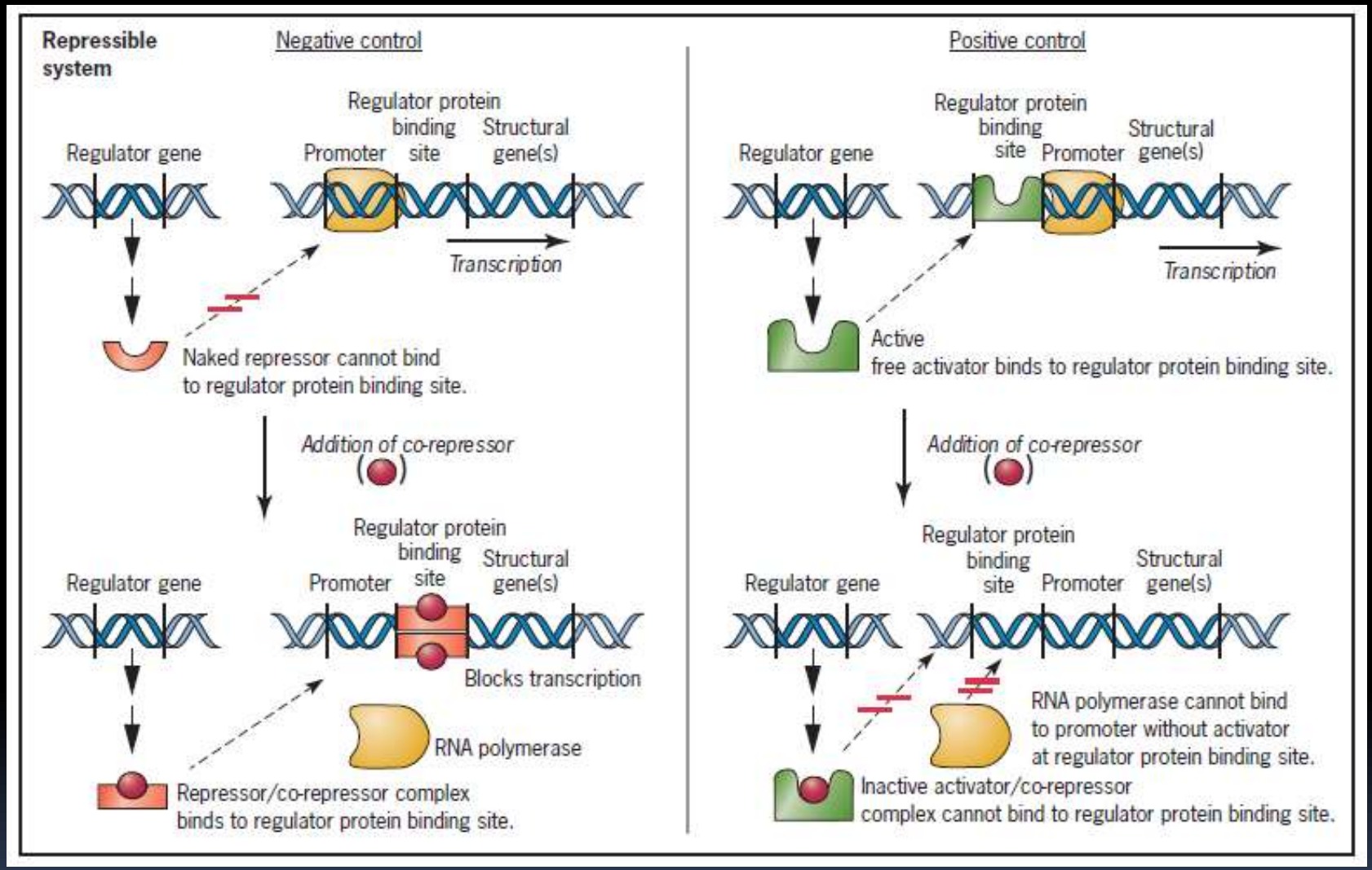


Source : Principles of Genetics by D. Peter Snustad (sixth edition)

- The regulator gene product is required to turn on gene expression in positive control systems and to turn off gene expression in negative control systems.

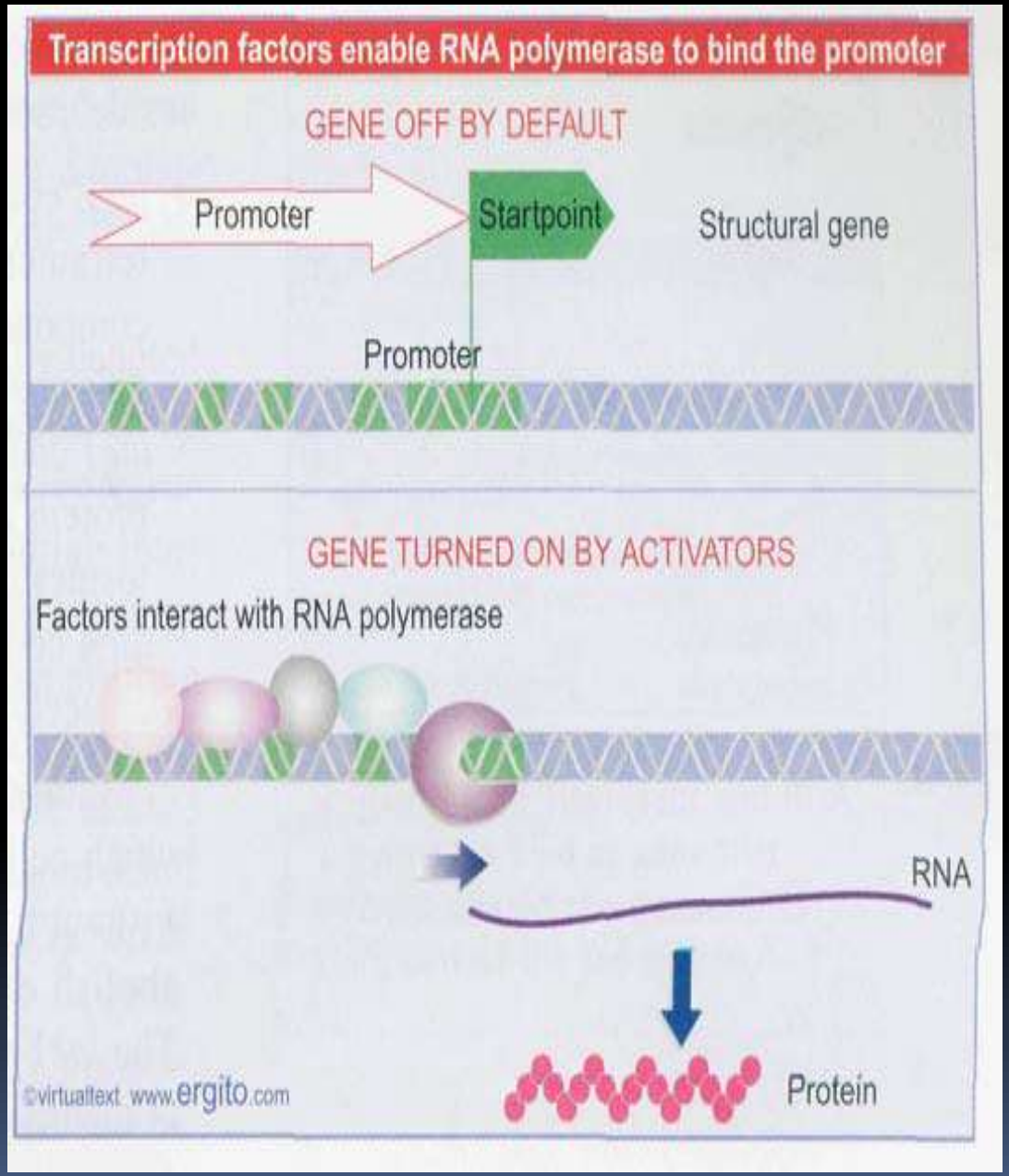
Positive control

In positive control,

Trans-acting factors must bind to cis-acting sites

in order for RNA polymerase to initiate

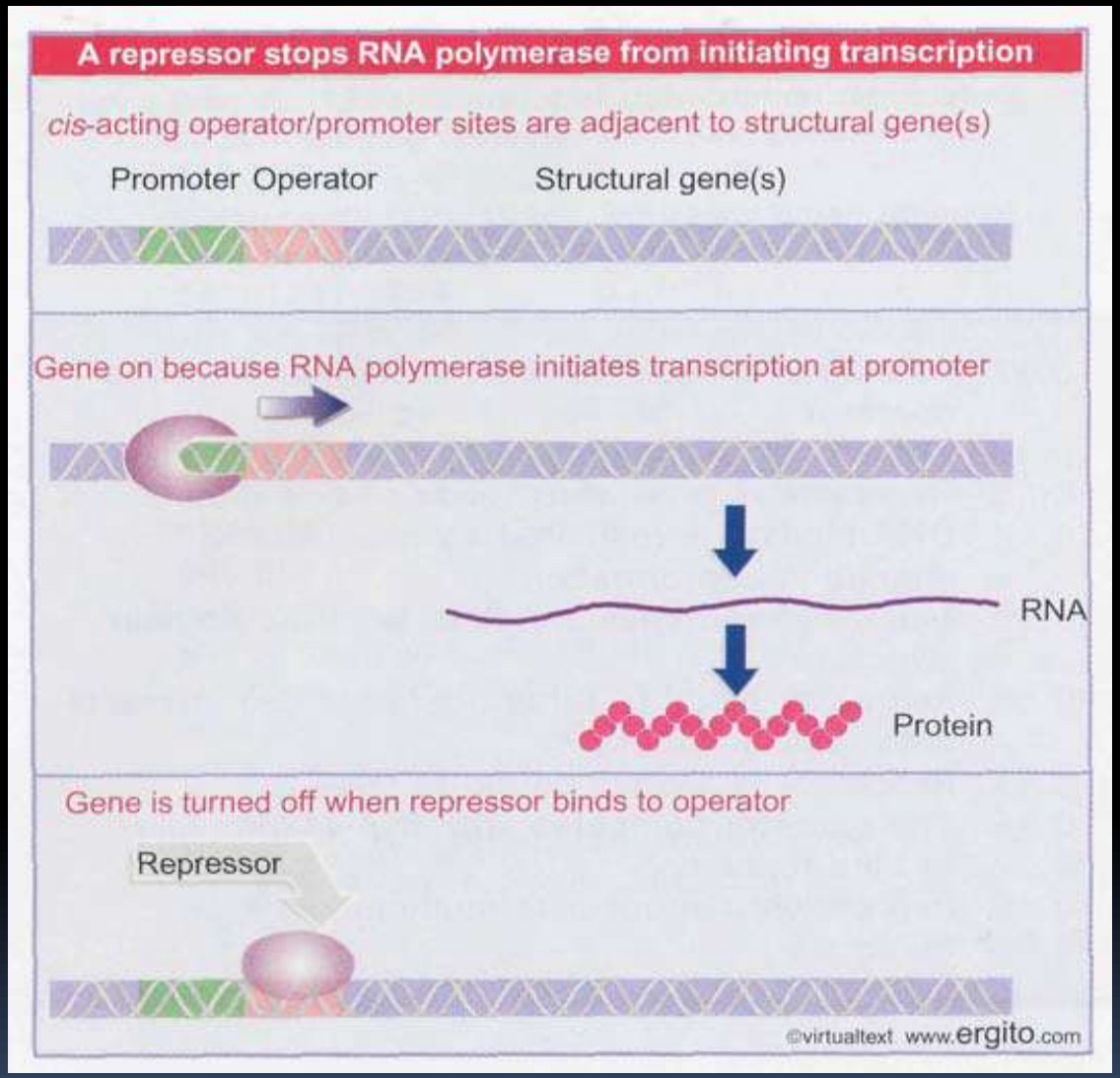
transcription at the promoter



Source: Genes VIII by Benjamin Lewin

Negative control

In negative control, a trans-acting repressor binds to the cis-acting operator to turn off transcription.



Source: Genes VIII by Benjamin Lewin

INDUCIBLE SYSTEM

- Process of turning on the expression of gene in response to a substance in the environment is called INDUCTION.
- Genes whose expression is regulated in this manner are called INDUCIBLE GENE/ if enzymes, are called INDUCIBLE ENZYME.
- Mainly enzymes of catabolic pathway.
- Lactose metabolism pathway.

REPRESSIBLE SYSTEM

- Process of turning off the expression of gene in response to a substance in the environment is called REPRESSION.
- Genes whose expression is regulated in this manner are called REPRESSIBLE GENE/ if enzymes, are called REPRESSIBLE ENZYME.
- Mainly enzymes of anabolic pathway.
- Tryptophan biosynthesis pathway.

THE LAC OPERON MODEL

by

Francois Jacob & Jacques Monod 1961

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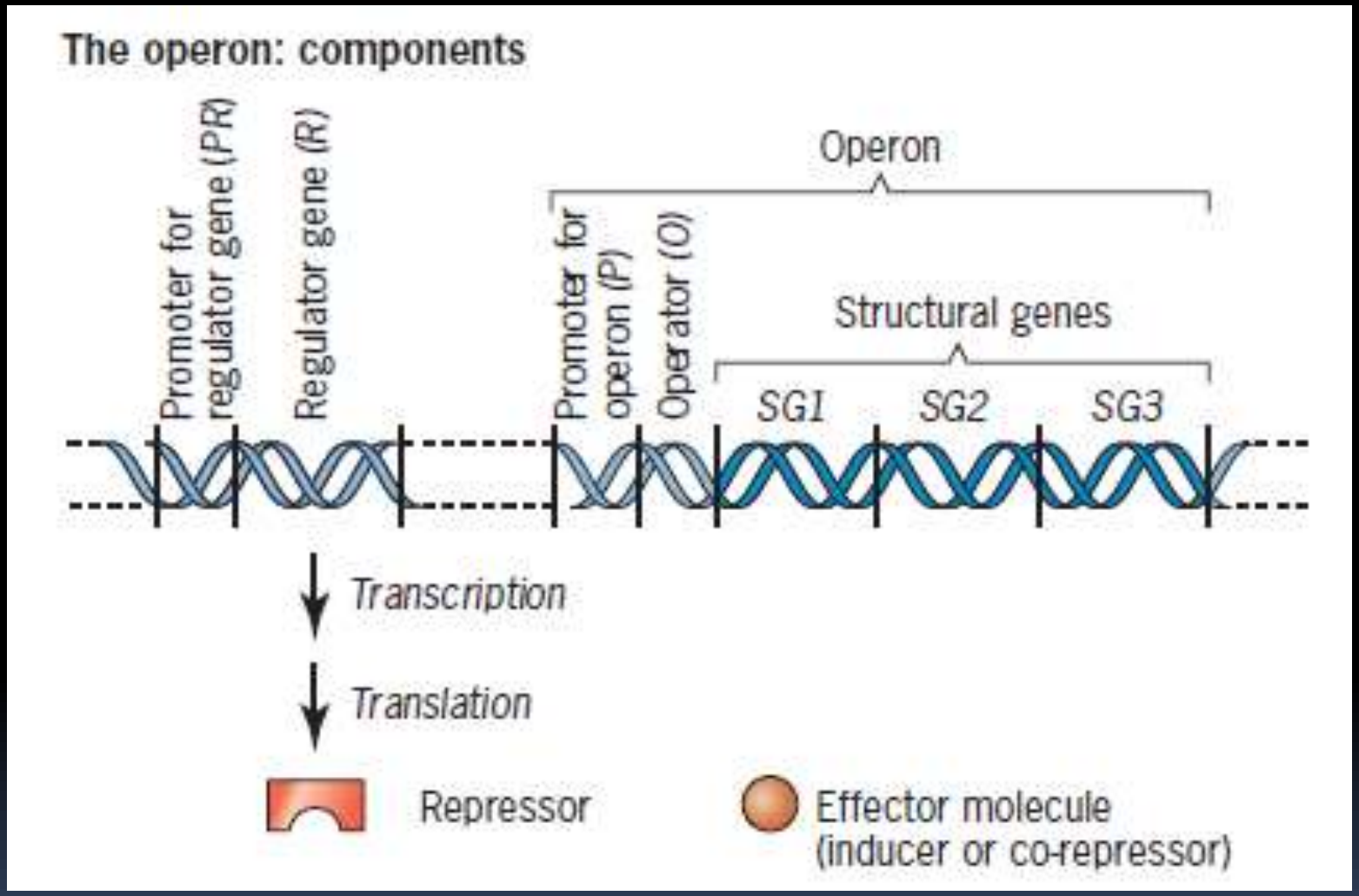

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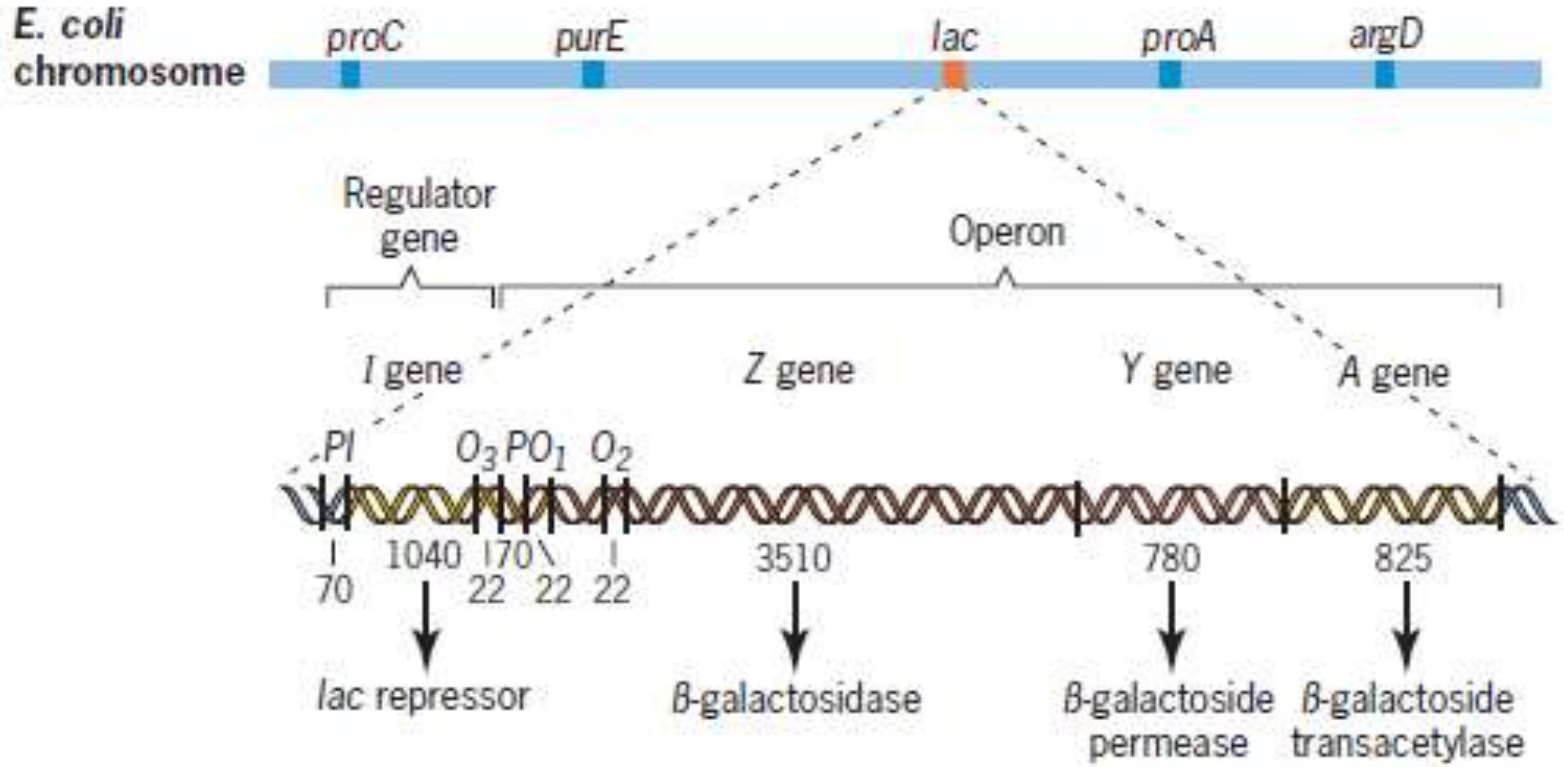
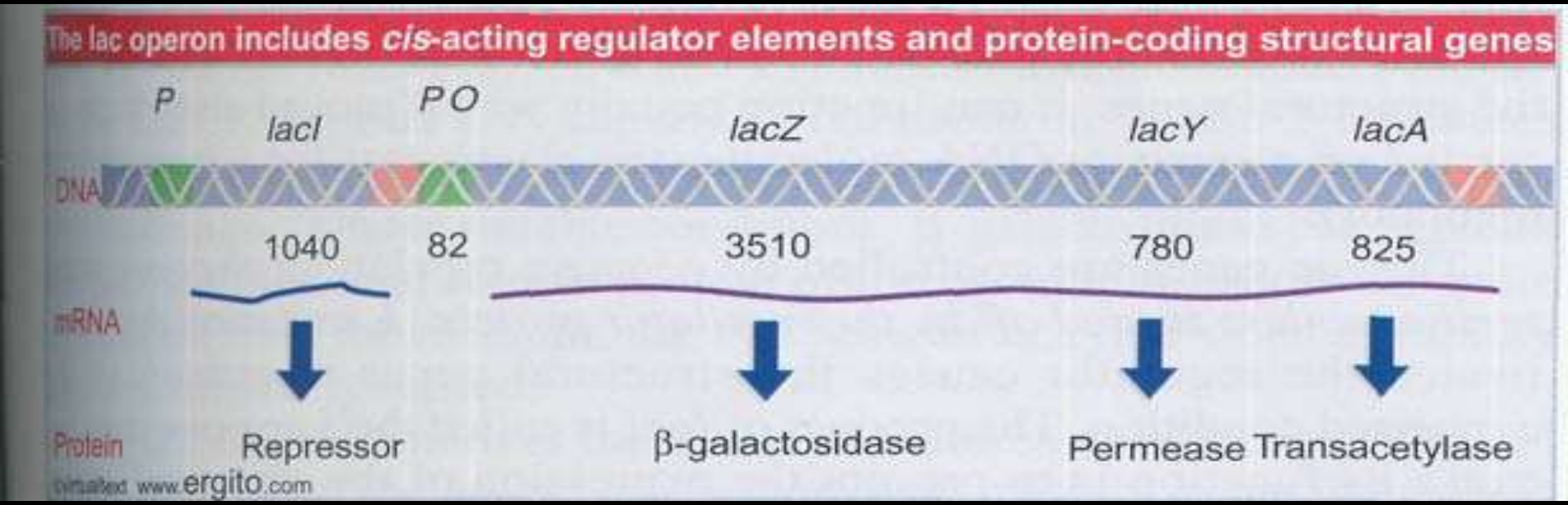


■ **FIGURE 18.5** The *lac* operon of *E. coli*. The *lac* operon consists of three structural genes, *Z*, *Y*, and *A*, plus the promoter (*P*) and three operators (O_1, O_2, *and* O_3). The regulator gene (*I*) is contiguous with the operon in the case of *lac* and has its own promoter (*PI*). The numbers below the various genetic elements indicate their sizes in nucleotide pairs.

Source : Principles of Genetics by D. Peter Snustad (sixth edition)



- The lac operon occupies -6000 bp of DNA.
- At the left the lacI gene has its own promoter and terminator.
- The end of the lacI region is adjacent to the promoter, P.
- The operator, O occupies the first 26 bp of the transcription unit.
- The long LacZ gene starts at base 39, and is followed by the LacY and LacA genes and a terminator

Source: Genes VIII by Benjamin Lewin

Repressor – A regulator gene product

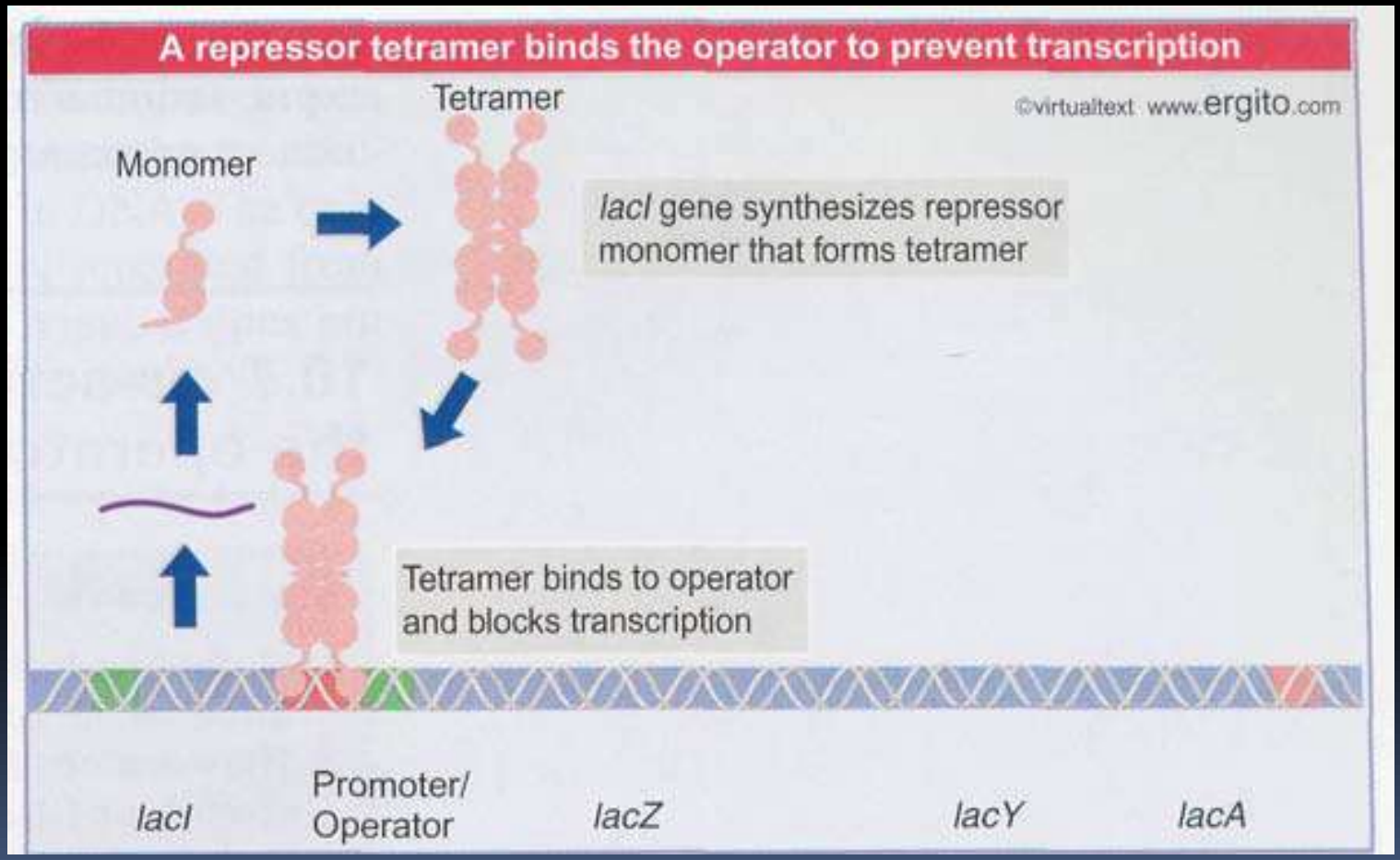
- The product of *lac I is called the Lac repressor*
- trans-acting products
- diffusible protein product
- a regulator gene codes for a protein that controls transcription by binding to particular site(s) on DNA(RBS).
- repressor is a tetramer of identical subunits of 38 kD each.
- It is transcribed into a monocistronic mRNA

DUAL ROLE :

Repressor has two binding sites, one for the operator(DNA binding site) and another for the inducer.

- Can prevent transcription by binding operator
- Can recognize inducer

Repressor maintains the *Lac* operon in the inactive condition by binding to the operator



Source: Genes VIII by Benjamin Lewin

Promoter

- A promoter serves to initiate transcription only of the gene or genes physically connected to it on the same stretch of DNA.
- promoters and terminators are cis-acting elements

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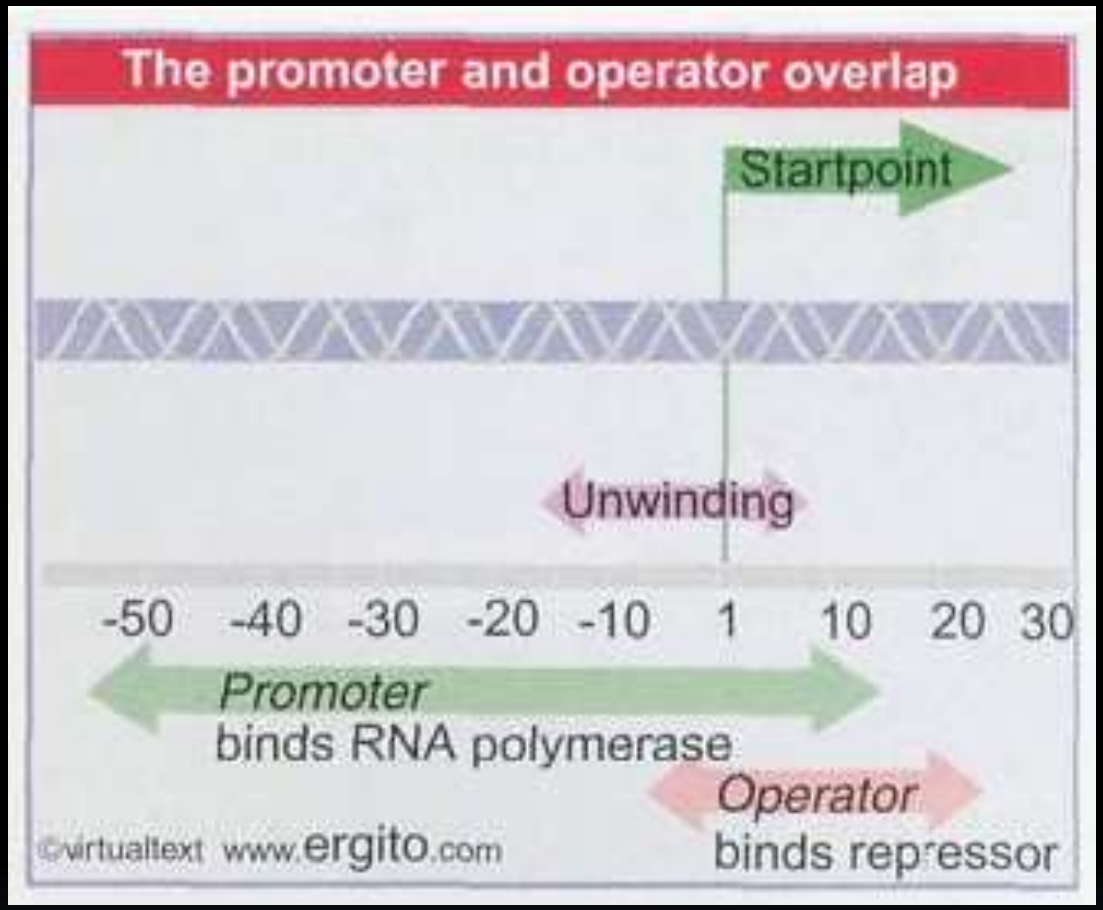
Operator

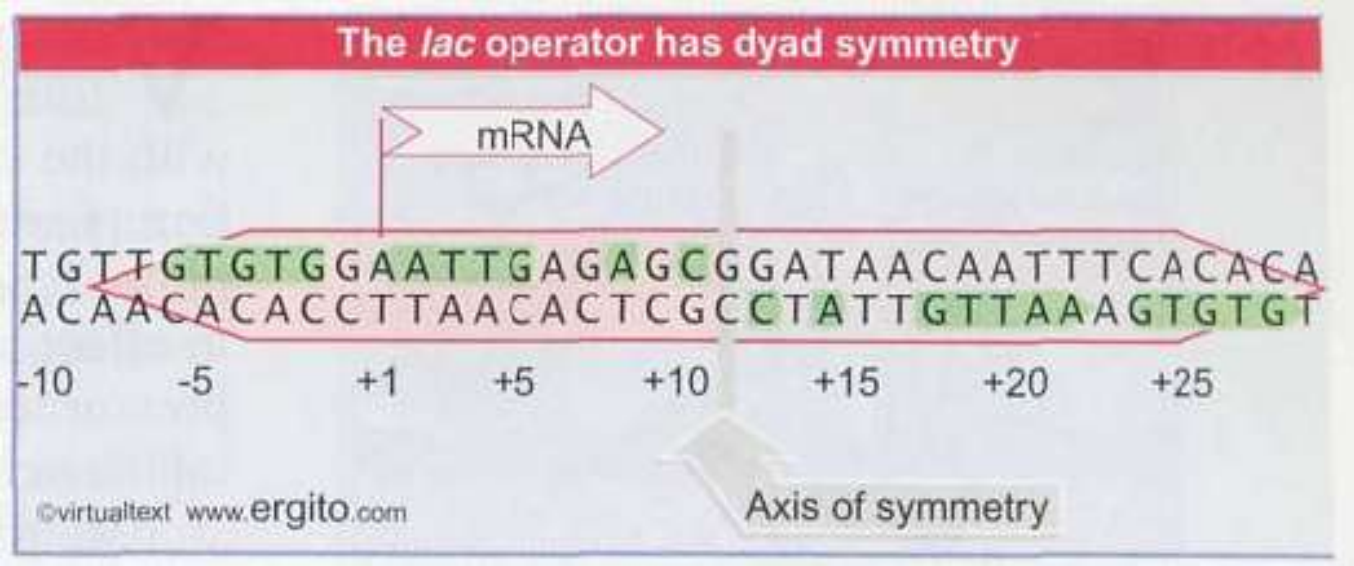
- cis-acting regulatory element (sequences that function exclusively within the DNA)
- The operator extends from position -5 just upstream of the mRNA start point to position +21 within the transcription unit. So it overlaps the right end of the promoter
- Contain palindromic sequence (inverted repeat)



Repressor & RNA polymerase bind at sites that overlap around the transcription startpoint of *lac* operon

Source: Genes VIII by Benjamin Lewin

The lac operator has a symmetrical sequence. The sequence is numbered relative to the start point for transcription at + 1. The pink arrows to left and right identify the two dyad repeats. The green blocks indicate the positions of identity



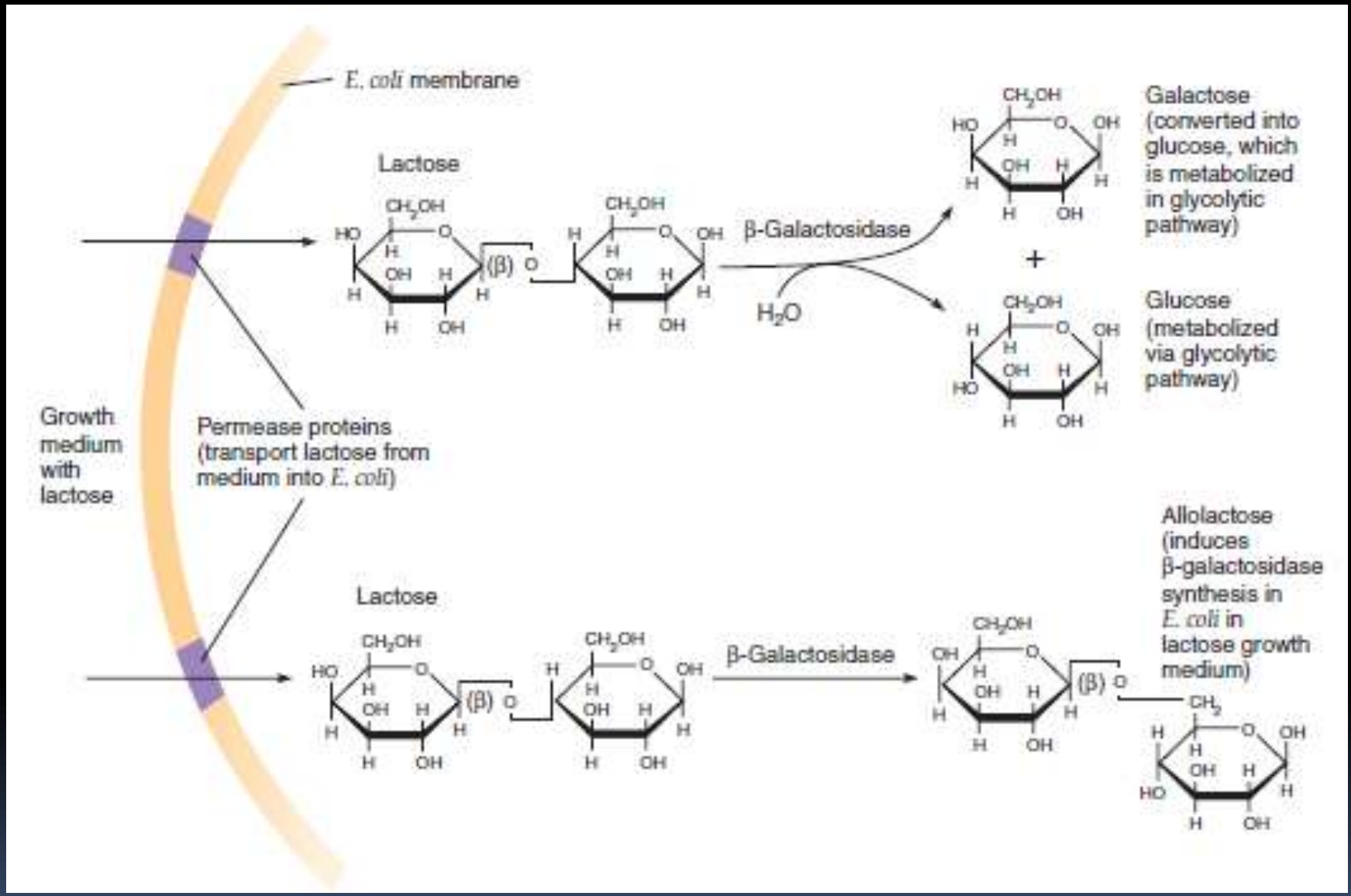
Source: Genes VIII by Benjamin Lewin

Structural Genes

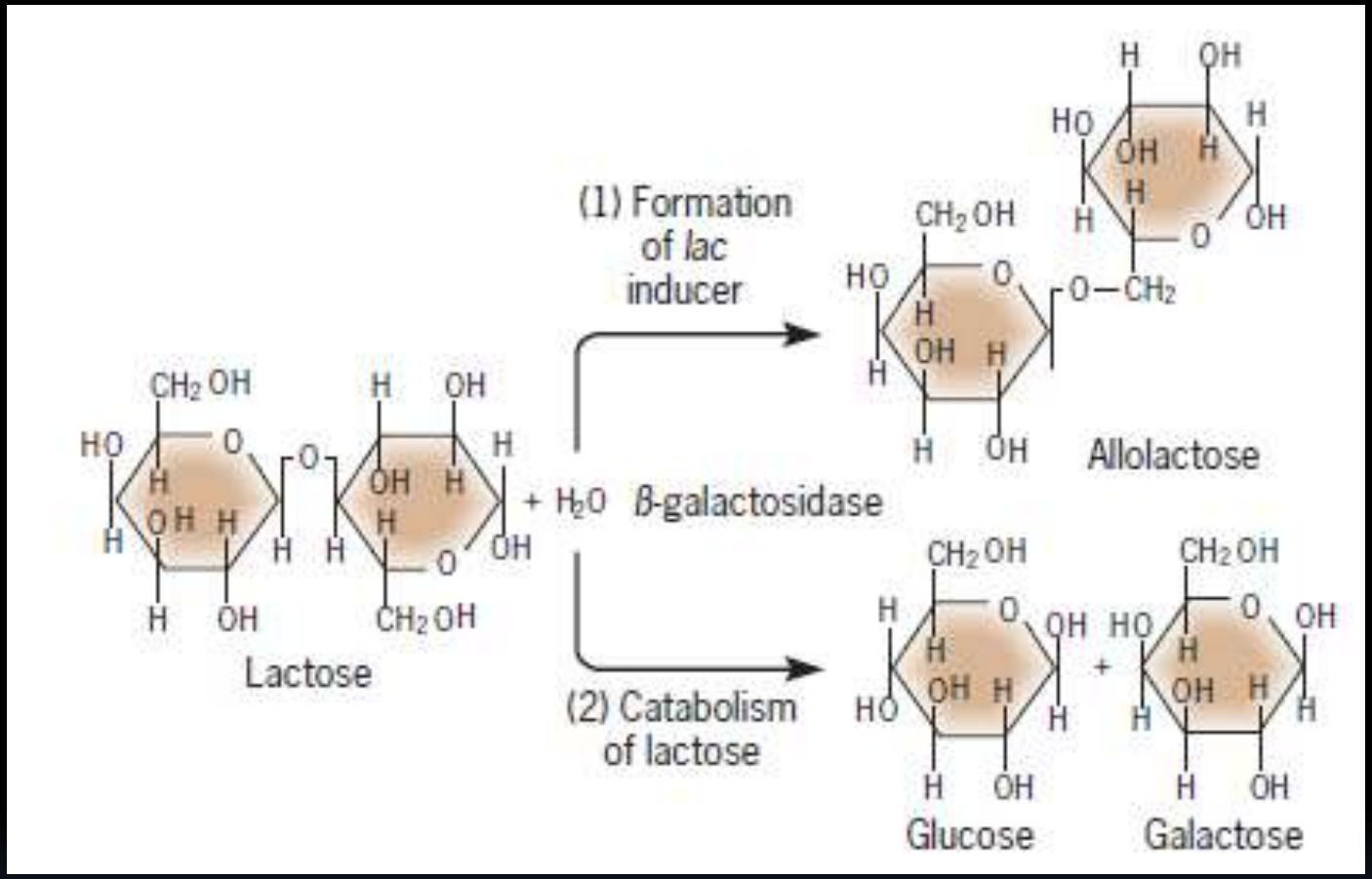
- Clustering of structural genes
- Coordinately controlled by means of interactions at a single promoter.
- Genes for the enzymes of metabolic pathway are organized into cluster.
- The cluster is transcribed into a single polycistronic mRNA from a promoter.

Role of Structural genes

- Lac Z codes for the enzyme β-galactosidase, whose active form is a tetramer of-500 kD. The enzyme breaks a β-galactoside into its component sugars. For example, lactose is cleaved into glucose and galactose (which are then further metabolized).



Source : iGENETICS: A Molecular Approach by Peter J. Russell



reactions catalyzed by β-galactosidase

Source : Principles of Genetics by D. Peter Snustad (sixth edition)

- lac Y codes for the β-galactoside permease, a 30 kD membrane-bound I protein constituent of the transport system. This transports β-galactosides into the cell.

- lac A codes for β-galactoside transacetylase, an enzyme that transfers an acetyl group from acetyl-CoA to β-galactosides.

Inducer

- Small molecules that cause the production of enzymes able to metabolize them are called inducer.
- An inducer functions by converting the repressor protein into an inactive form.
- Repressor is inactivated by an allosteric interaction in which binding of inducer at its site changes the properties of the DNA-binding site.
- Reduces affinity of repressor for the operator

- Gratuitous inducer:

Molecules that induce enzyme synthesis but are not metabolized.

eg - IPTG

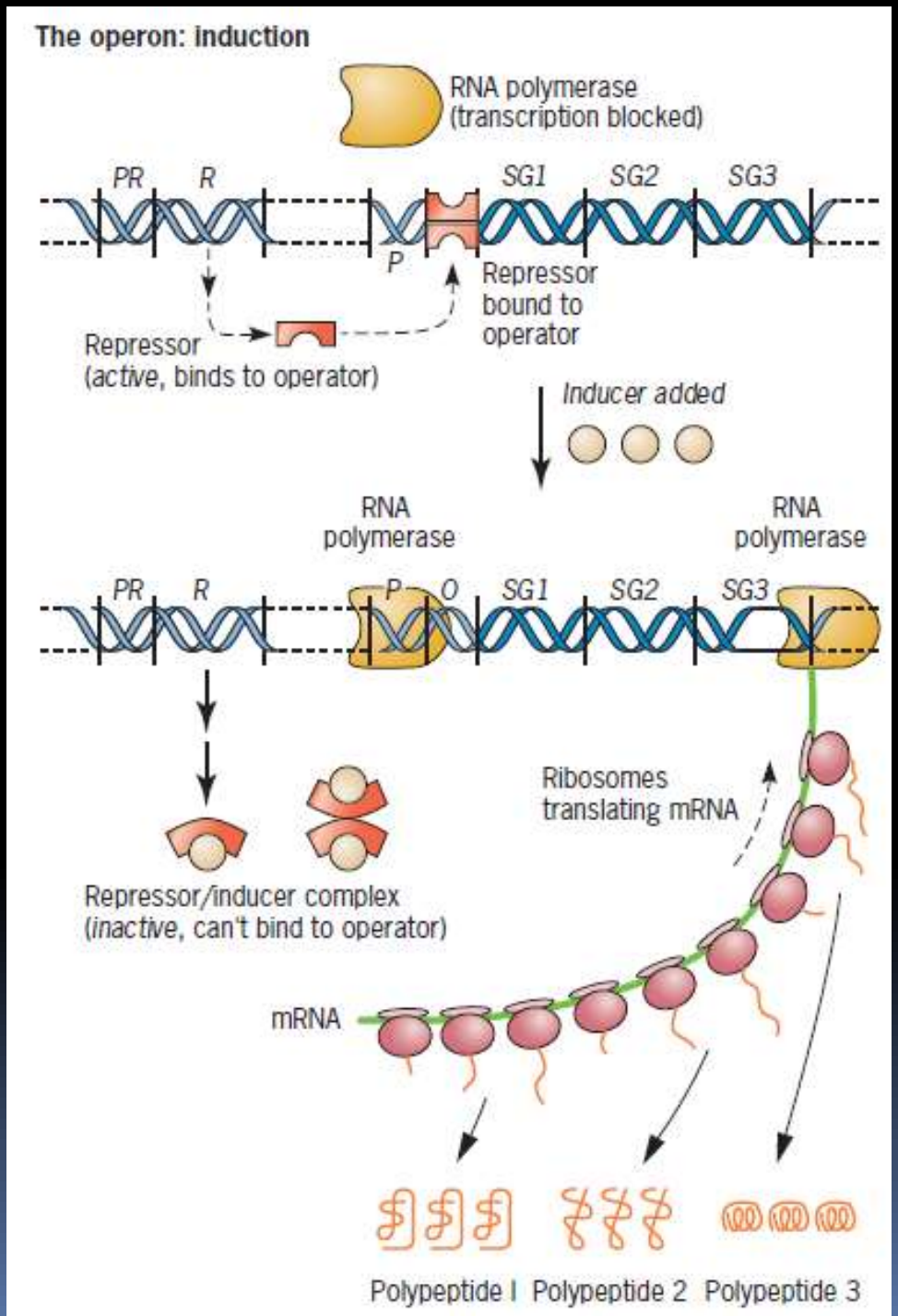
- Corepressor:

Molecules that prevent the production of enzymes able to synthesize them are called corepressor.

eg - Tryptophan

Process

- In the absence of an inducer, the genes are not transcribed, because repressor protein is in an active form that is bound to the operator.
- When the repressor binds at the operator, it prevents RNA polymerase from initiating transcription at the promoter.
- when an inducer is added, the repressor is converted into an inactive form that leaves the operator. Then transcription starts at the promoter and proceeds through the genes to a terminator located beyond the 3' end of lacA.



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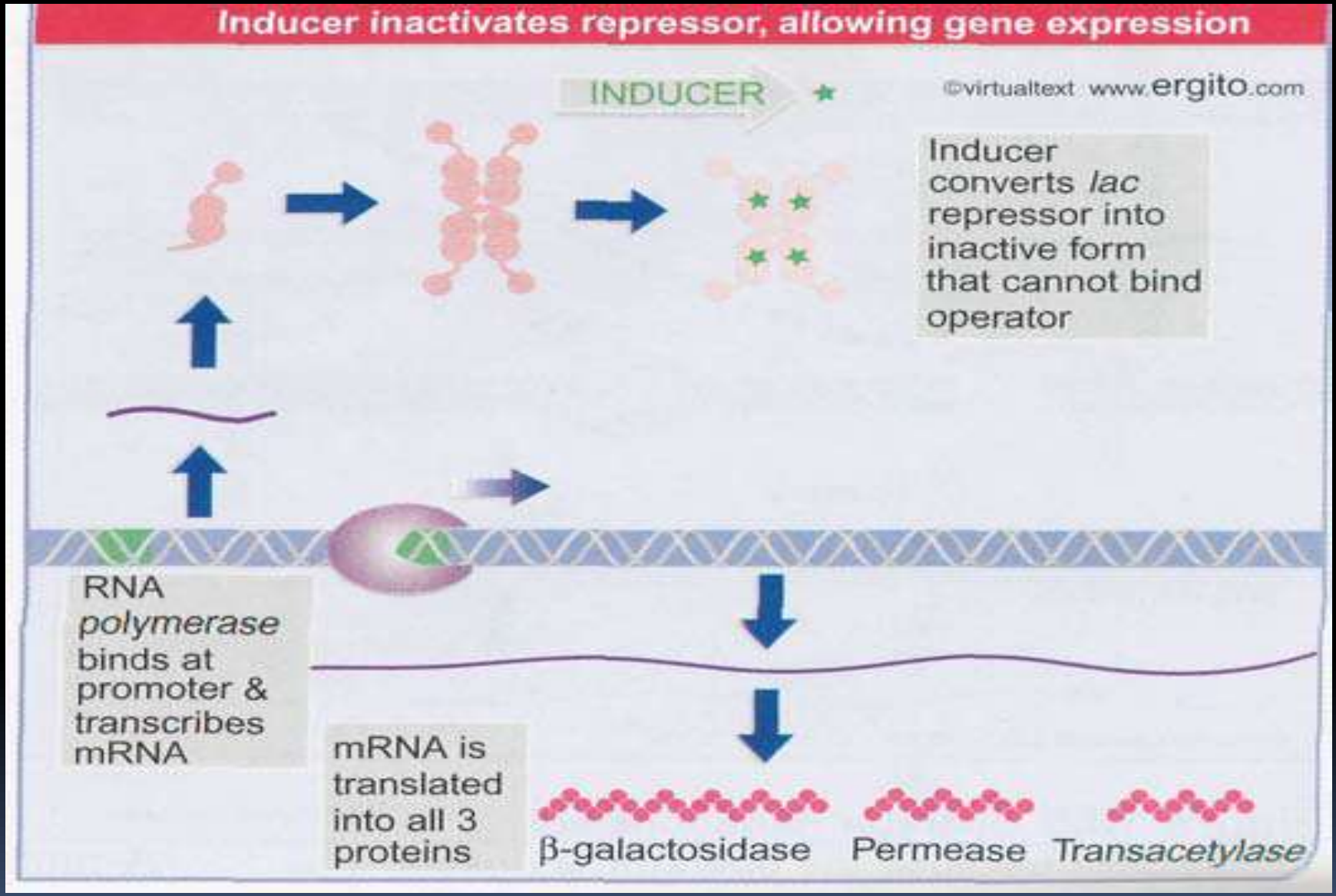

Source: Genes VIII by Benjamin Lewin

STRUCTURE OF REPRESSOR PROTEIN AND MECHANISM OF REPRESSION

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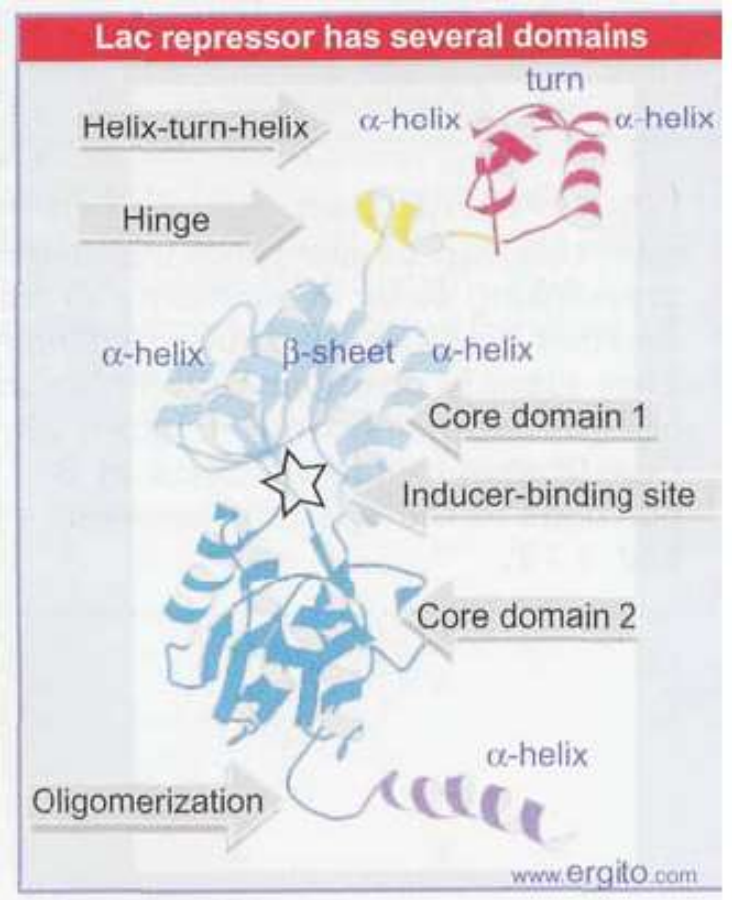
Structure of Repressor monomer

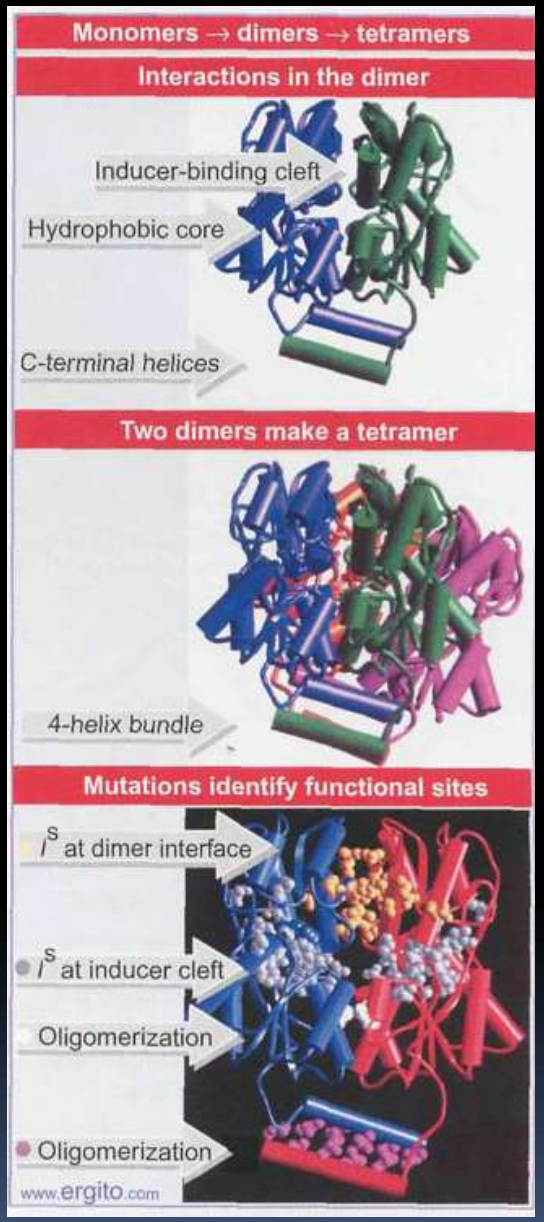


Source: Genes VIII by Benjamin Lewin

STRUCTURE OF DIMER AND TETRAMER

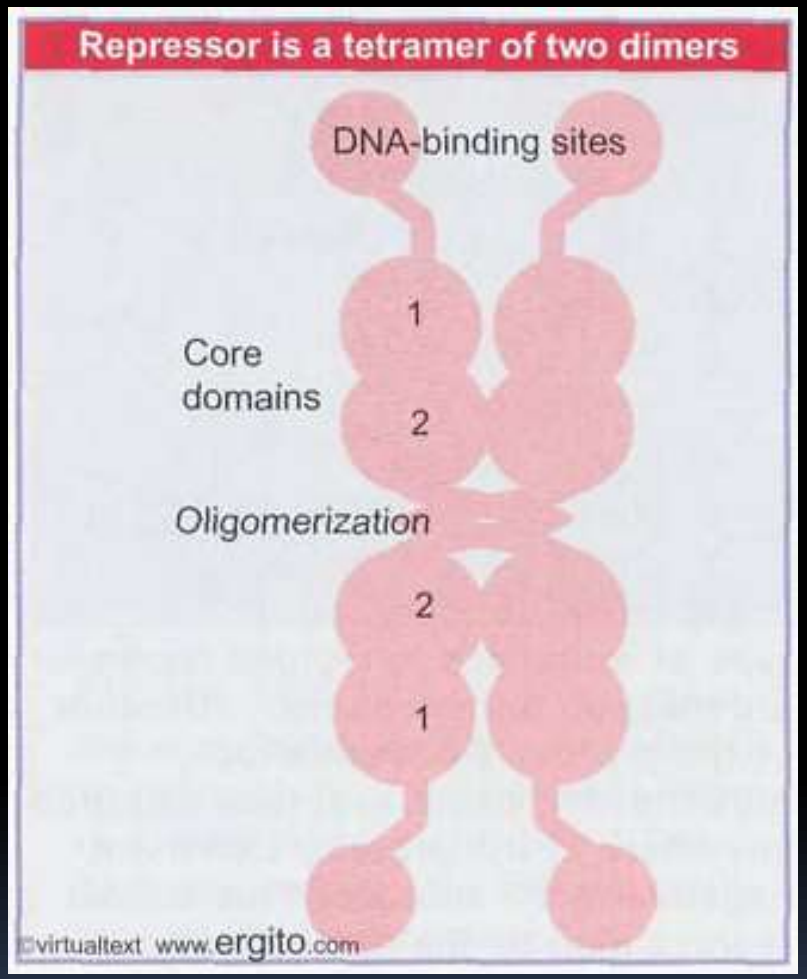
C-terminus- an α-helix that contains two leucine heptad repeats. This is the oligomerization domain.

The oligomerization helices of four monomers associate to maintain the tetrameric structure



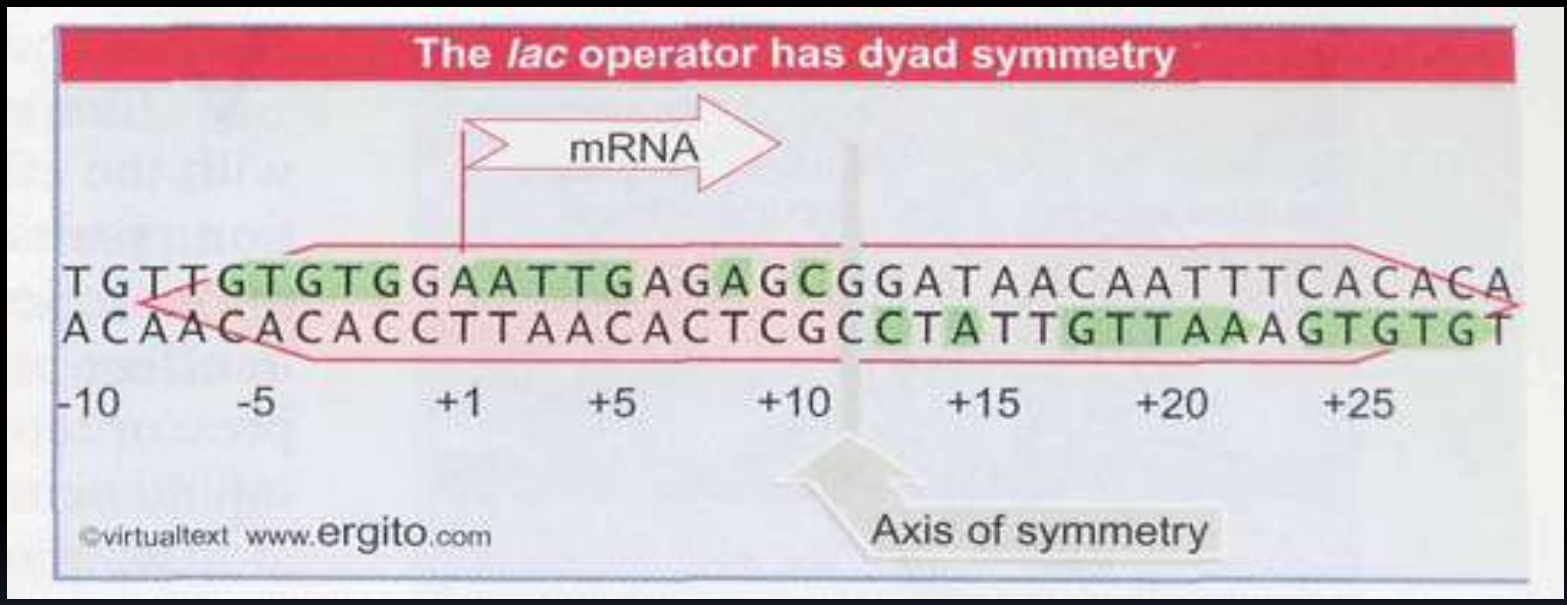
Source: Genes VIII by Benjamin Lewin

STRUCTURE OF REPRESSOR TETRAMER

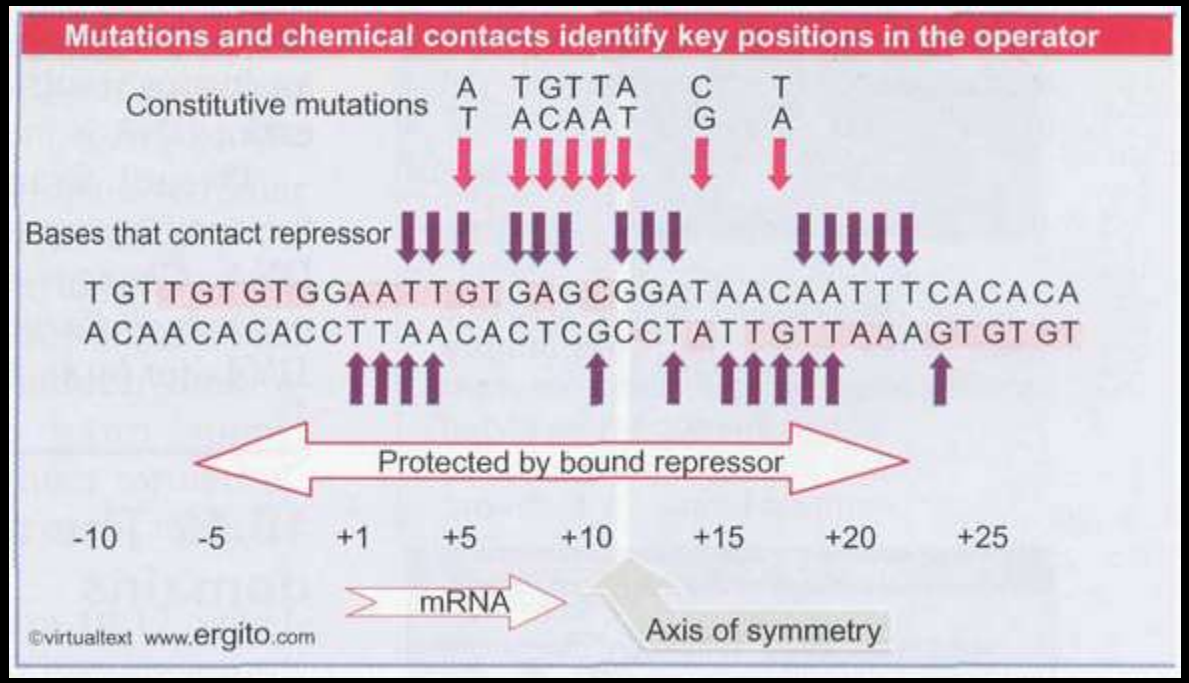


Source: Genes VIII by Benjamin Lewin

- How does the repressor recognize specific sequence of Operator DNA?

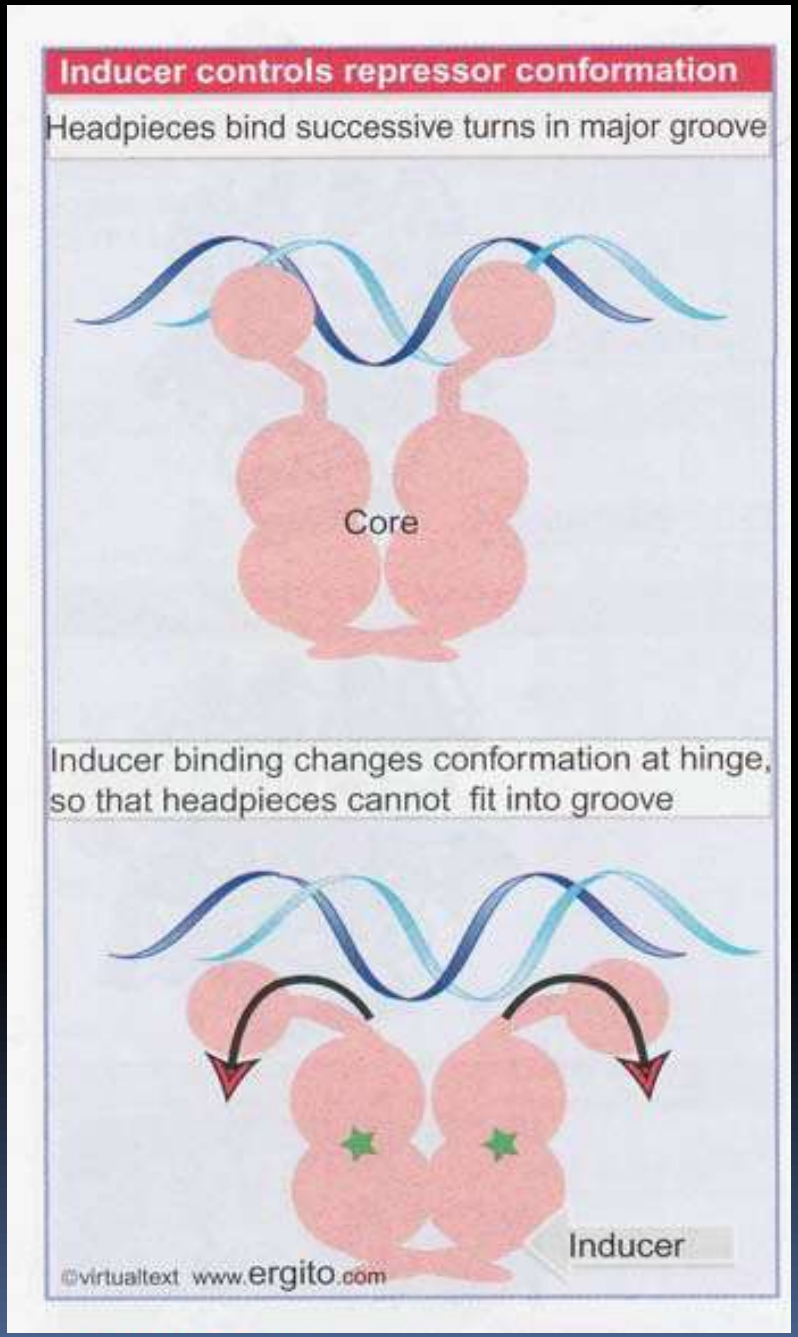


Source: Genes VIII by Benjamin Lewin



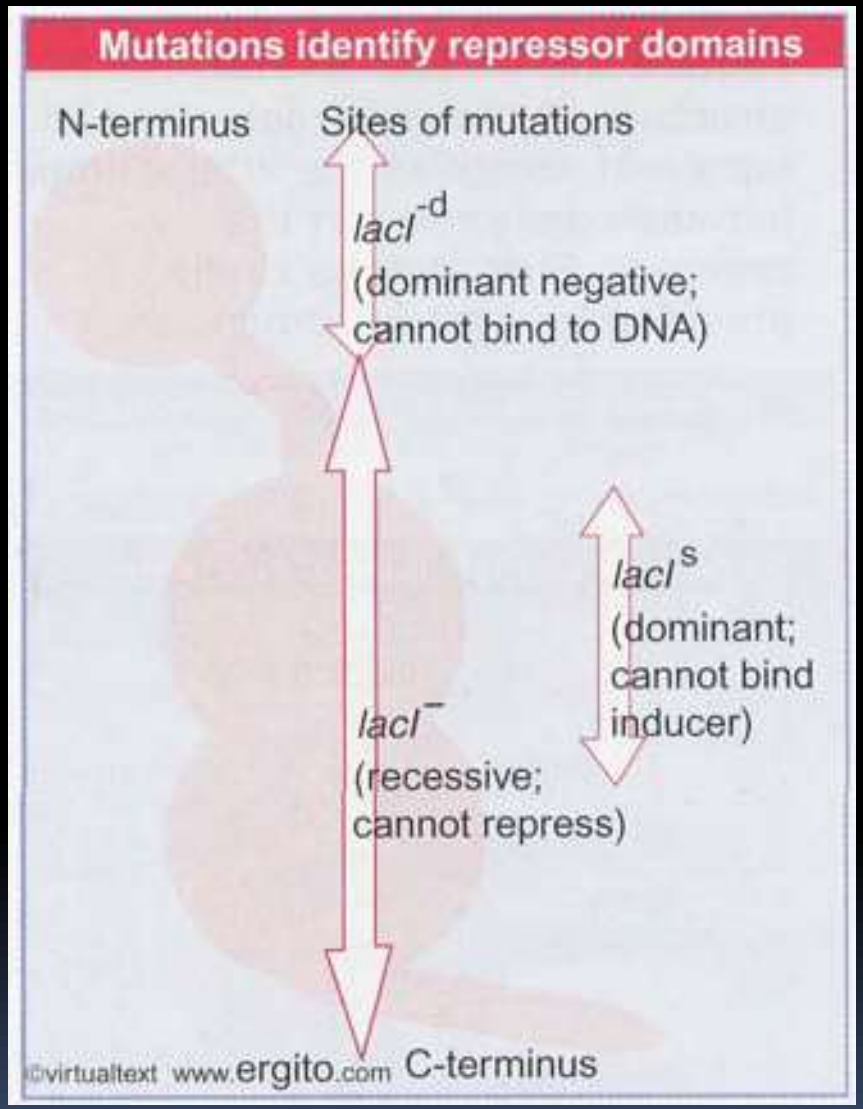
Source: Genes VIII by Benjamin Lewin

DNA-binding is regulated by an allosteric change in conformation



Source: Genes VIII by Benjamin Lewin

Locations of three mutations in lac repressor protein



Source: Genes VIII by Benjamin Lewin

How does inducer binding release repressor from operator?

- The equilibrium model - calls for repressor bound to DNA to be in rapid equilibrium with free repressor. Inducer would bind to the free form of repressor, and thus unbalance the equilibrium by preventing reassociation with DNA.
- Inducer bind directly to repressor protein complexed with the operator. inducer binding produces a change in the repressor that makes it release the operator.

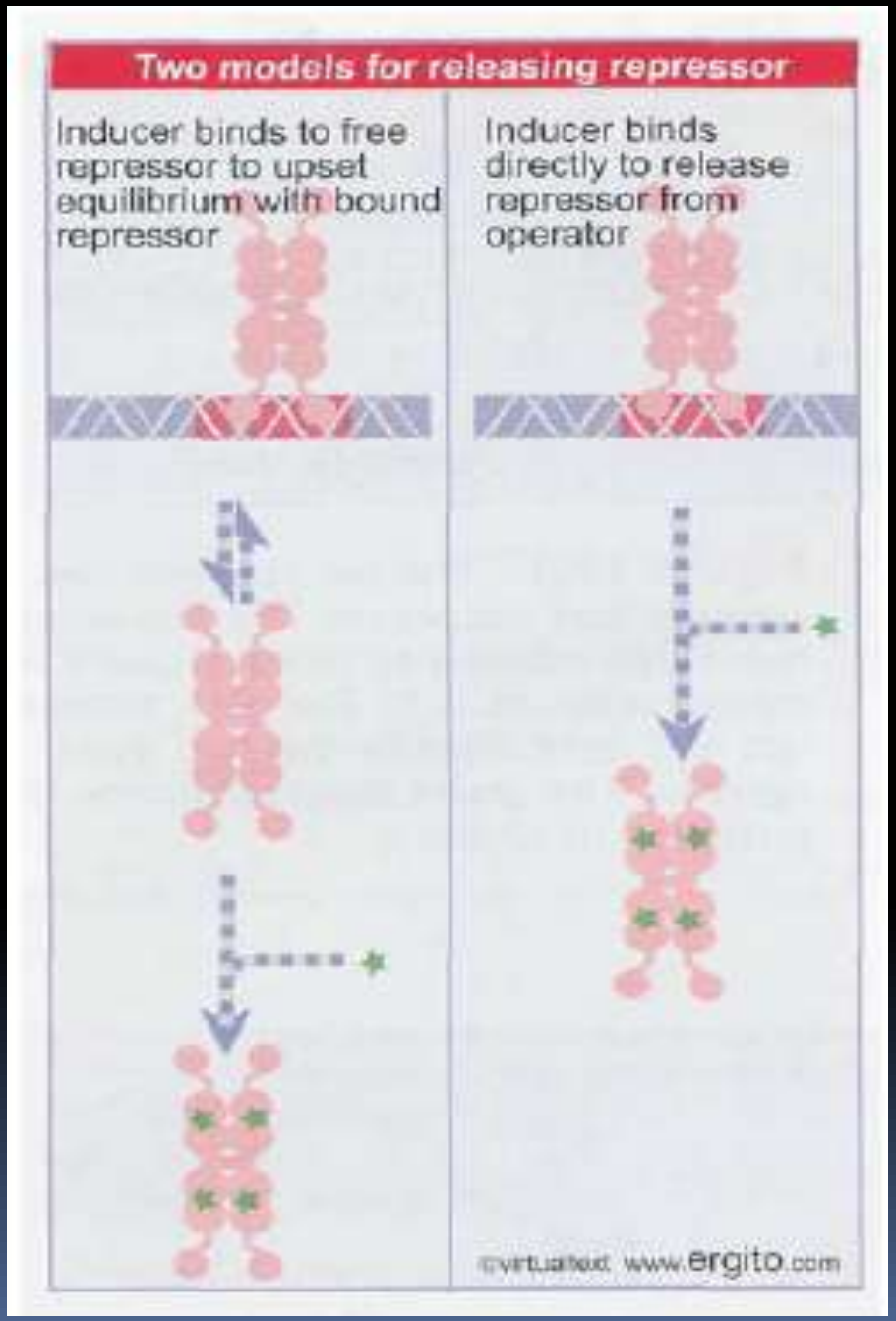


Figure 10.14 Does the inducer bind to free repressor to upset an equilibrium (left) or directly to repressor bound at the operator (right)?

Source: Genes VIII by Benjamin Lewin

HOW INDUCER WORK?

- Binding of 2 molecules of inducer is adequate to release repressor.
- Then what is the requirement of Tetrameric repressor?
- To establish full REPRESSION.
- What is the mystery behind it?
- There are 2 other operators apart from the main operator.

- Operator 1 is the main operator. Operator 2 is situated downstream 410bp & Operator 3 is situated upstream 88bp.

- Lac repressor binds simultaneously to O1 & to one of the other operator(O2/03).Causing the DNA between them to form a LOOP.

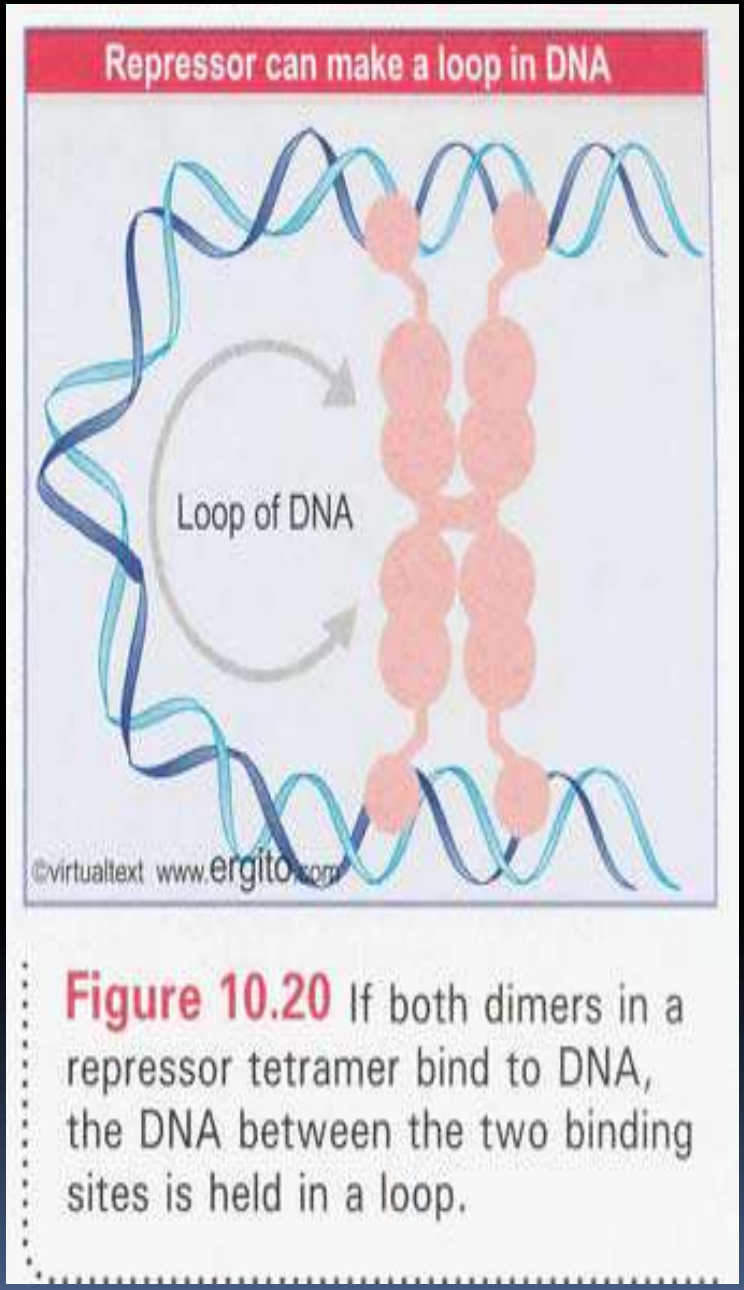


Figure 10.20 If both dimers in a repressor tetramer bind to DNA, the DNA between the two binding sites is held in a loop.

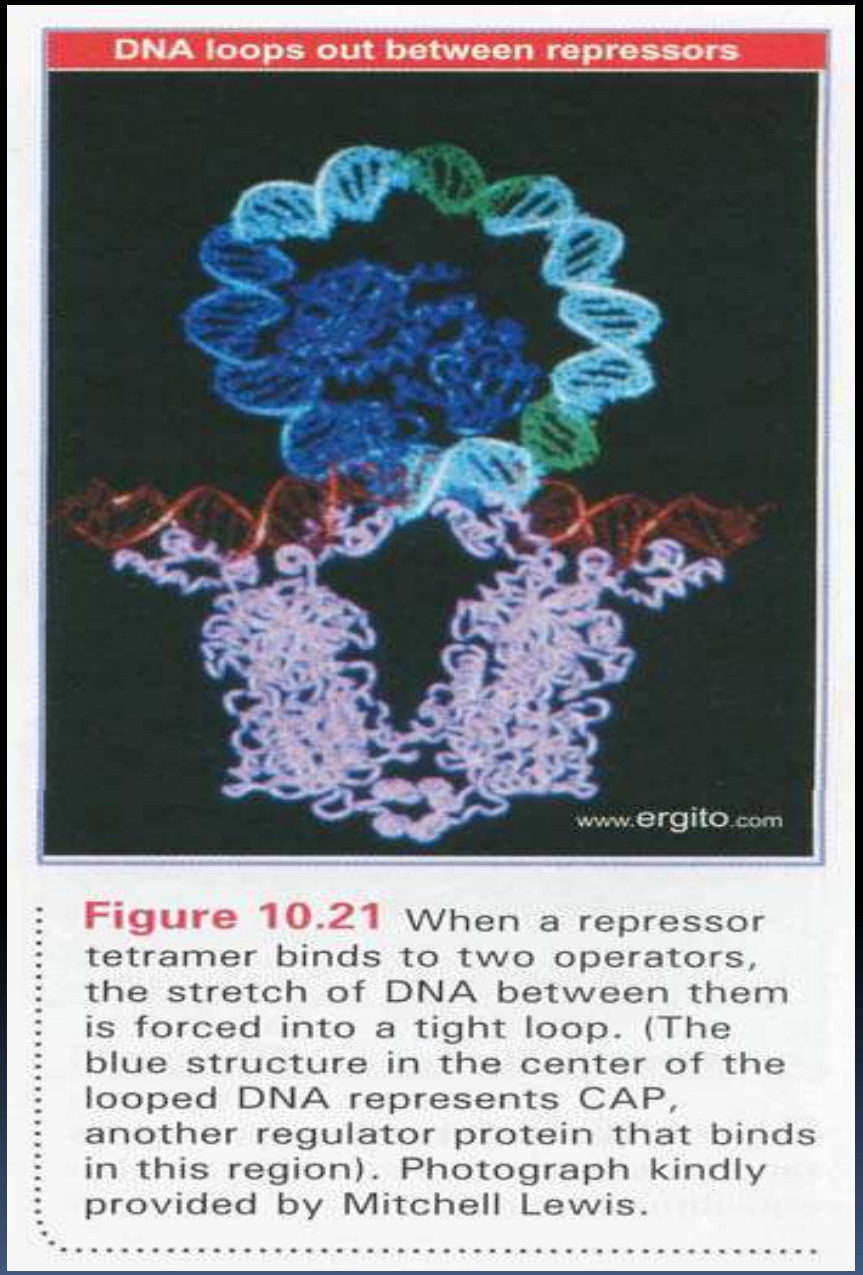
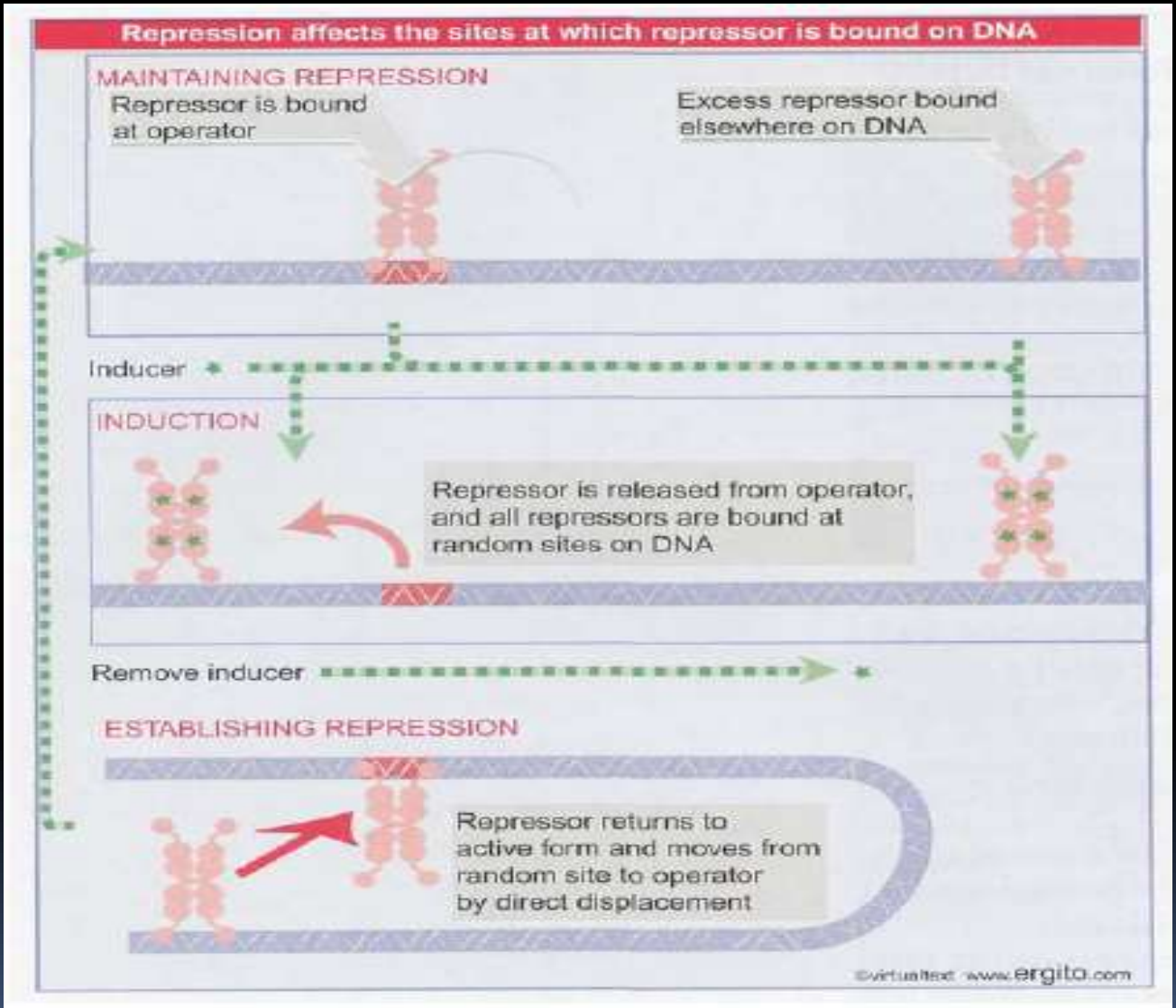


Figure 10.21 When a repressor tetramer binds to two operators, the stretch of DNA between them is forced into a tight loop. (The blue structure in the center of the looped DNA represents CAP, another regulator protein that binds in this region). Photograph kindly provided by Mitchell Lewis.

Source: Genes VIII by Benjamin Lewin

- Binding at the additional operators affects the level of repression.
- The two proteins may be bound to DNA simultaneously, and the binding of repressor actually enhances the binding of RNA polymerase. But the bound enzyme is prevented from initiating transcription
- The complex of RNA polymerase-repressor-DNA is blocked at the closed stage.
- When inducer is added, the repressor is released and the closed complex is converted to an open complex that initiates transcription.

Repressor is always bound to DNA



Source: Genes VIII by Benjamin Lewin

The effect of induction is therefore to change the distribution of repressor on DNA,
rather than to generate free repressor

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Mutation in operator gene o^c

- Mutations in the operator cause constitutive expression of all three *lac* structural genes.
- They are cis-acting and affect only those genes on the contiguous stretch of DNA.

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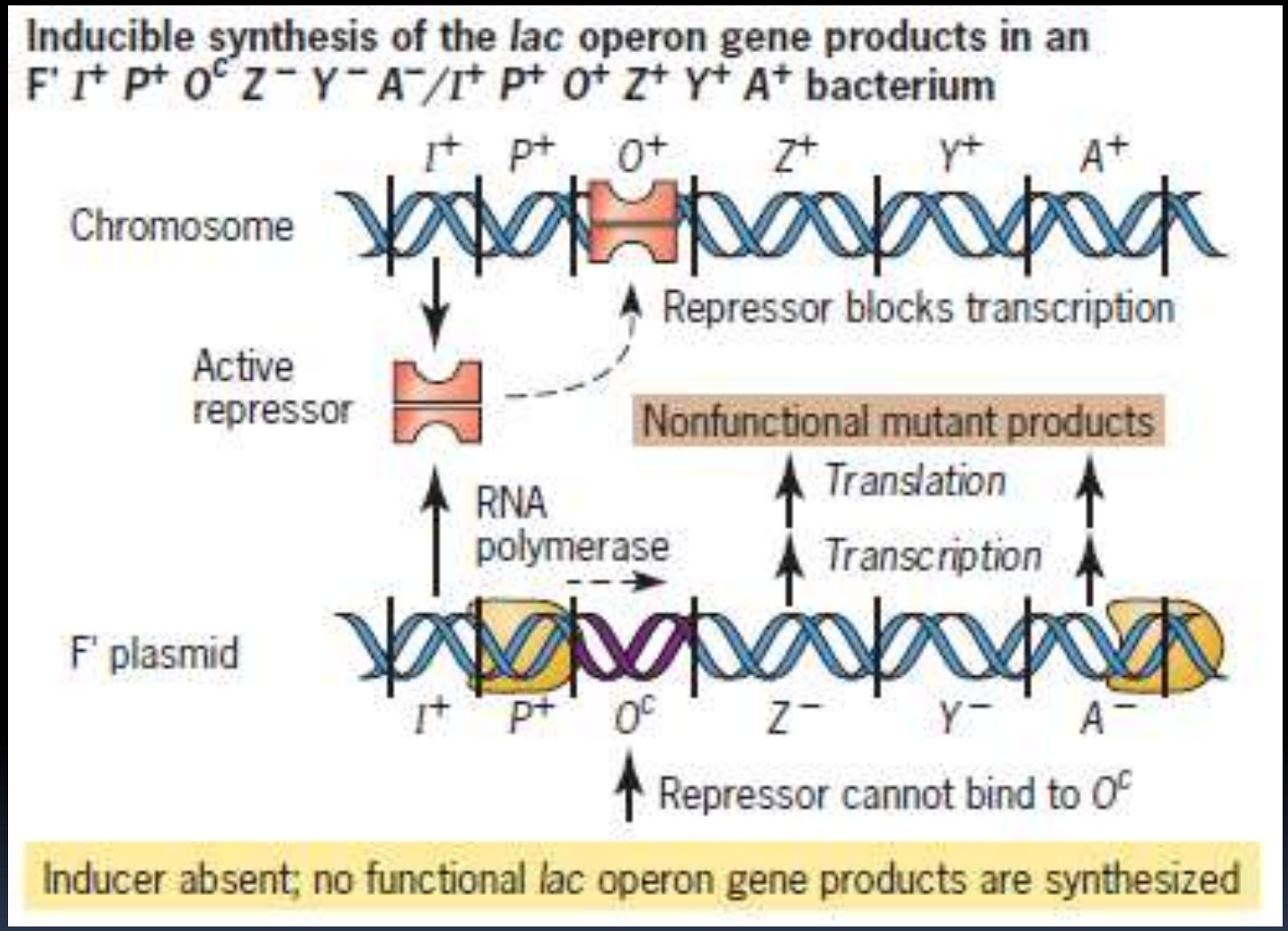

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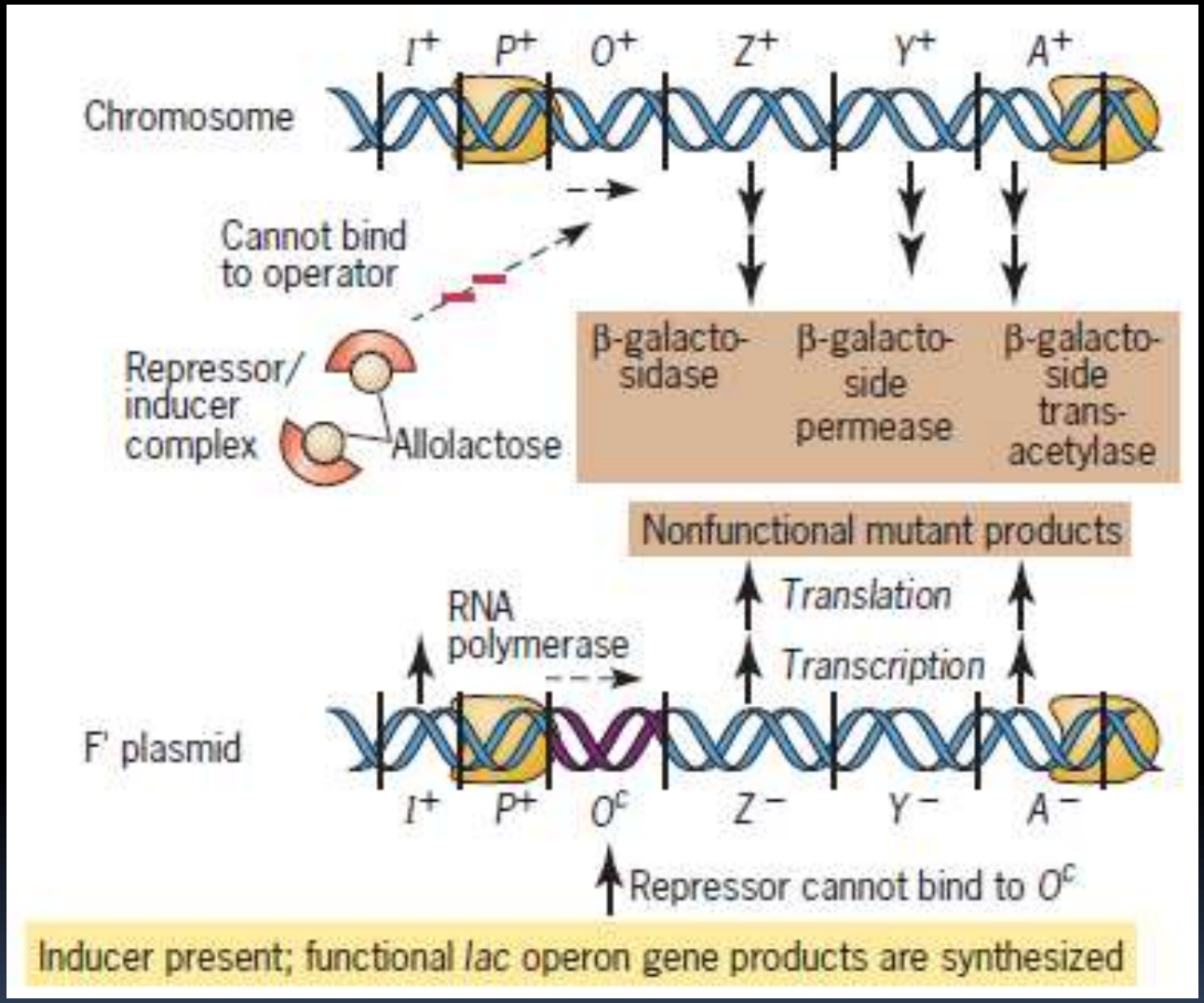

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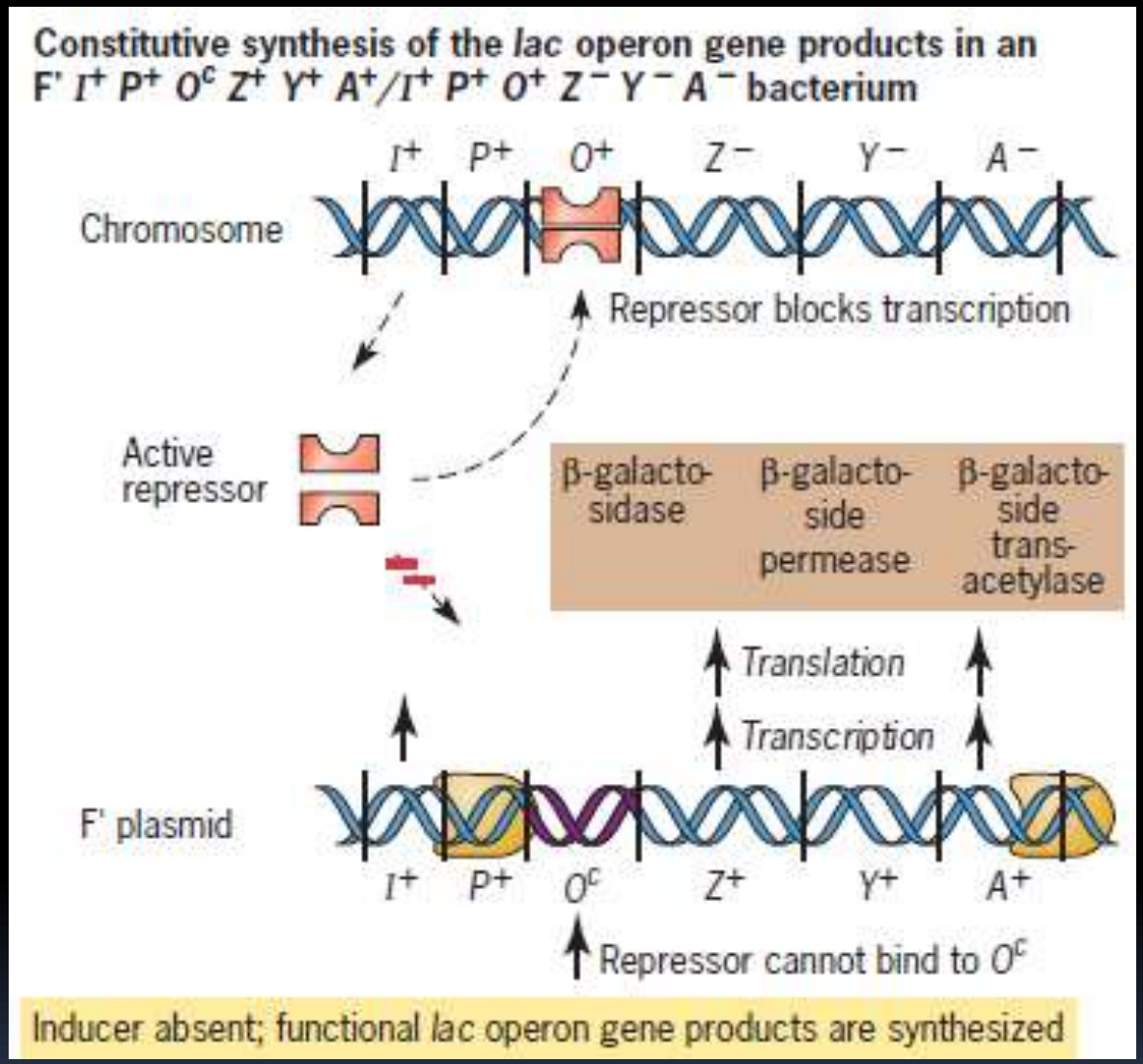

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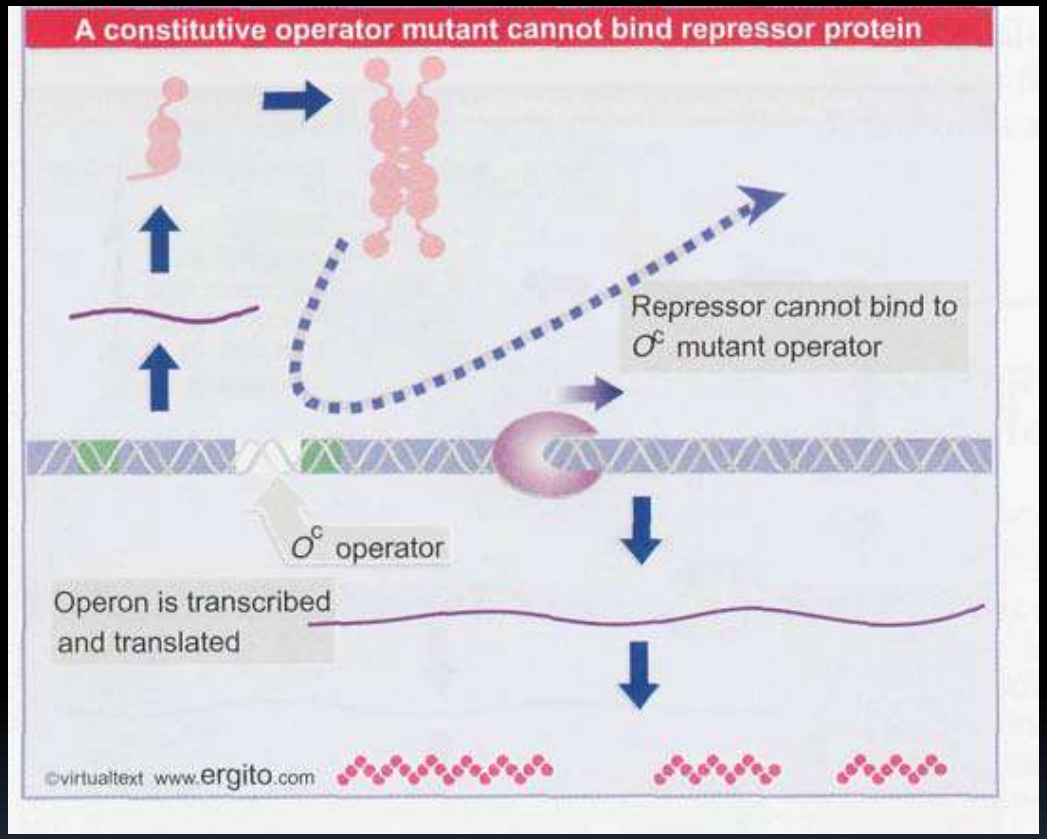
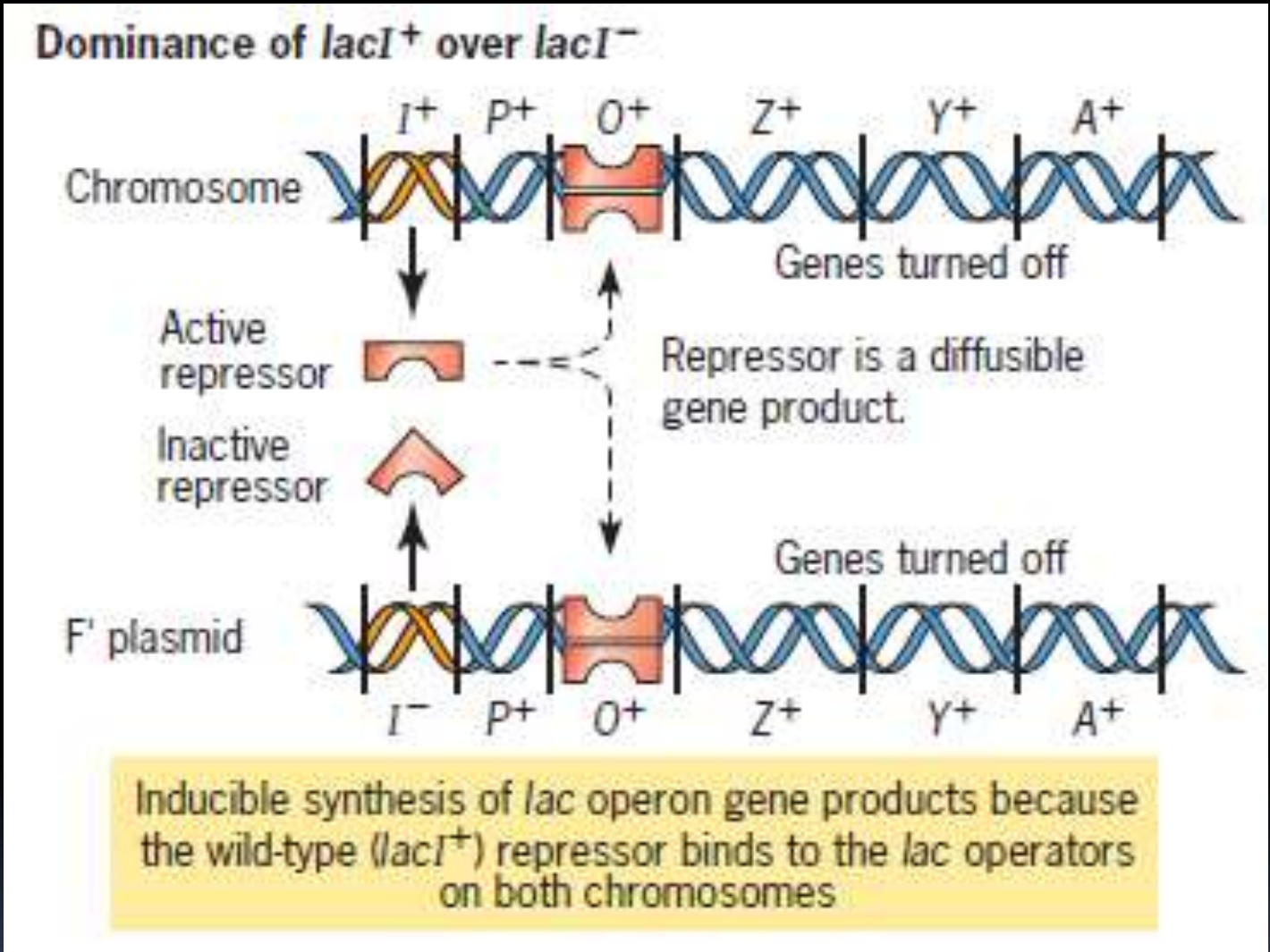
The o^c mutation is cis-dominant



Source: Genes VIII by Benjamin Lewin

Mutation in repressor gene

- Mutations in the *lacI* gene are trans-acting and affect expression of all lacZYA clusters in the bacterium.
- Mutations that eliminate *lacI* function cause constitutive expression and are recessive.
- Mutations in the DNA-binding site of the repressor are constitutive because the repressor cannot bind the operator.
- Mutations in the inducer-binding site of the repressor prevent it from being inactivated and cause uninducibility.



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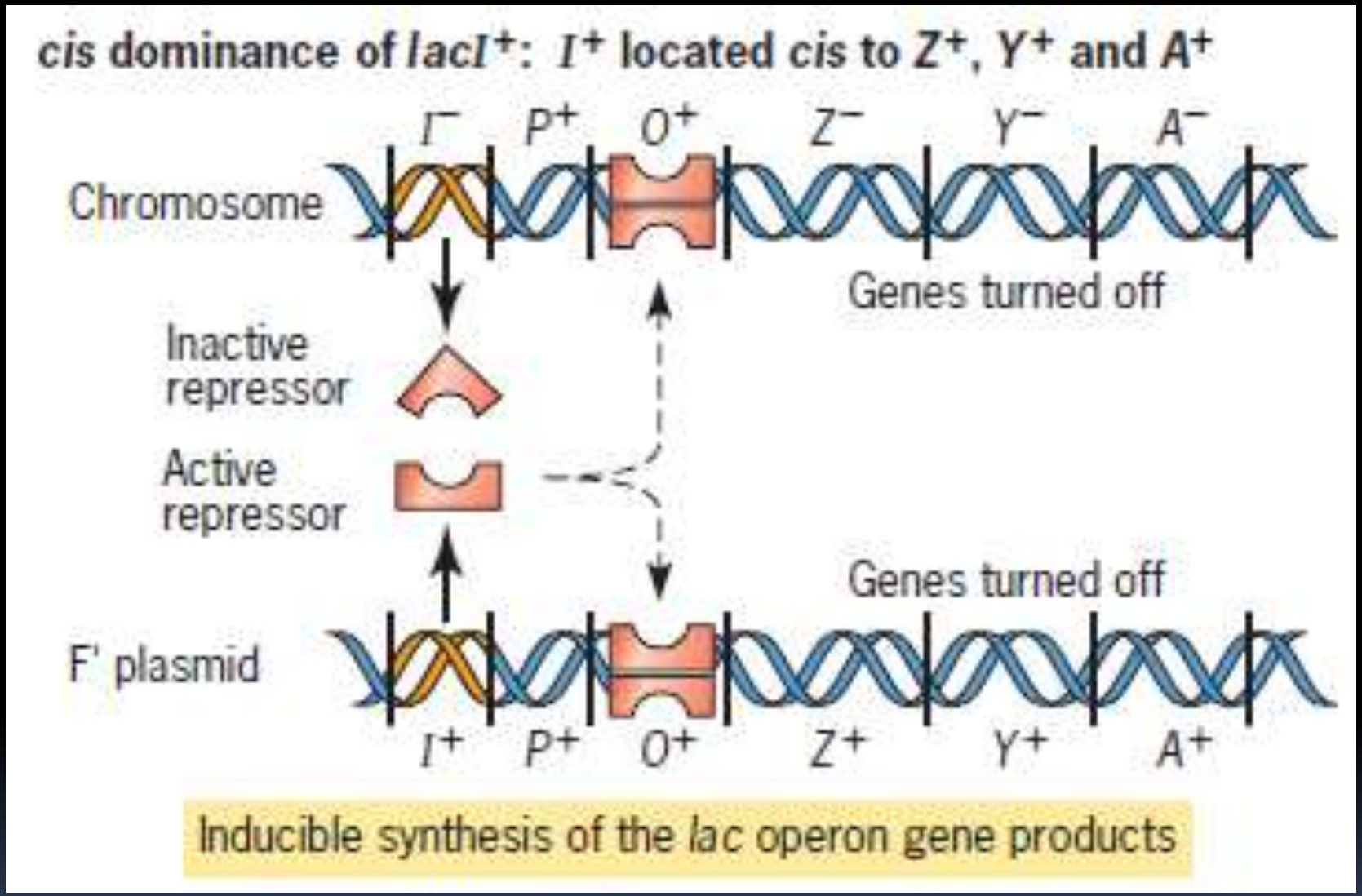

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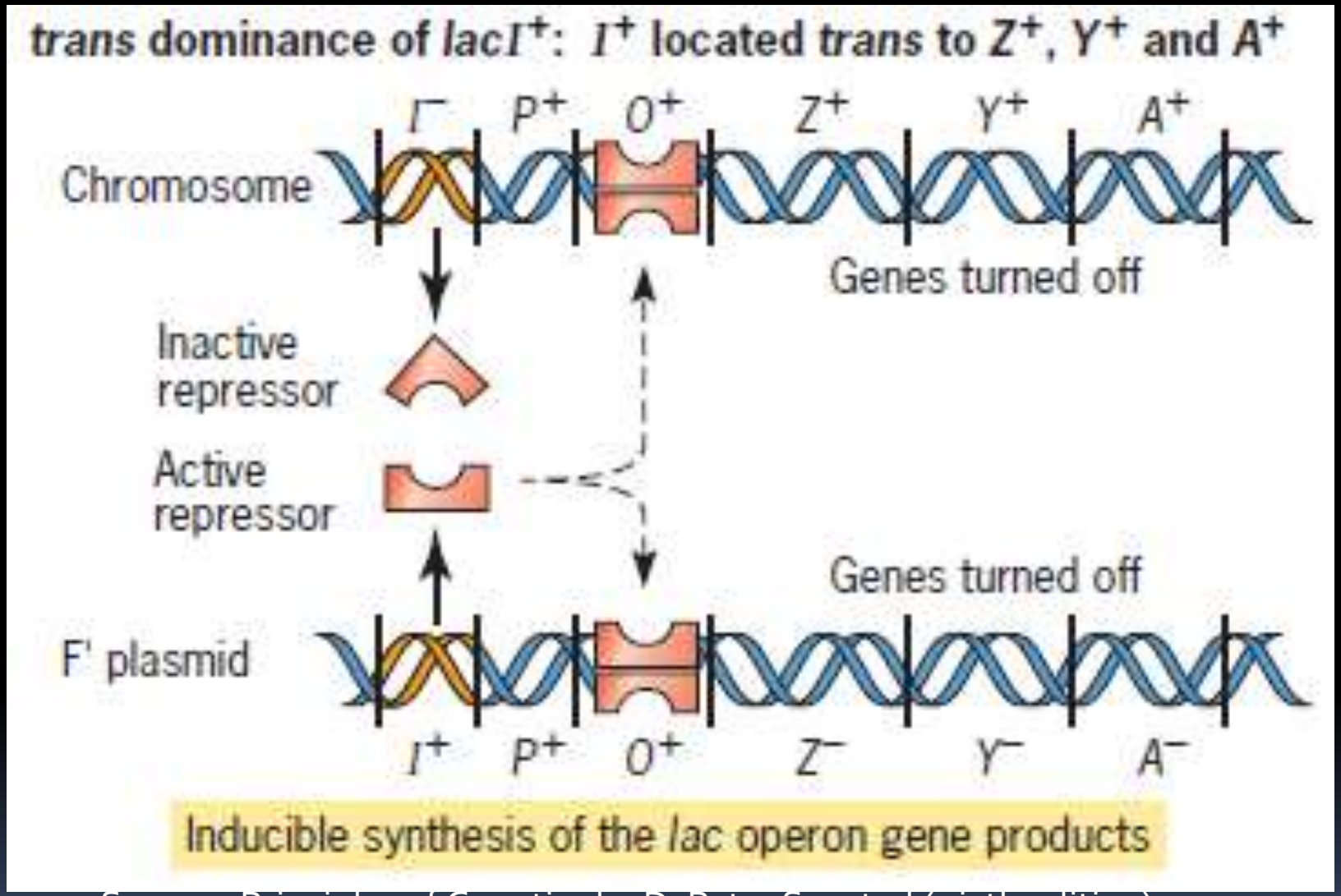


Source : Principles of Genetics by D. Peter Snustad (sixth edition)

Mutation in promoter

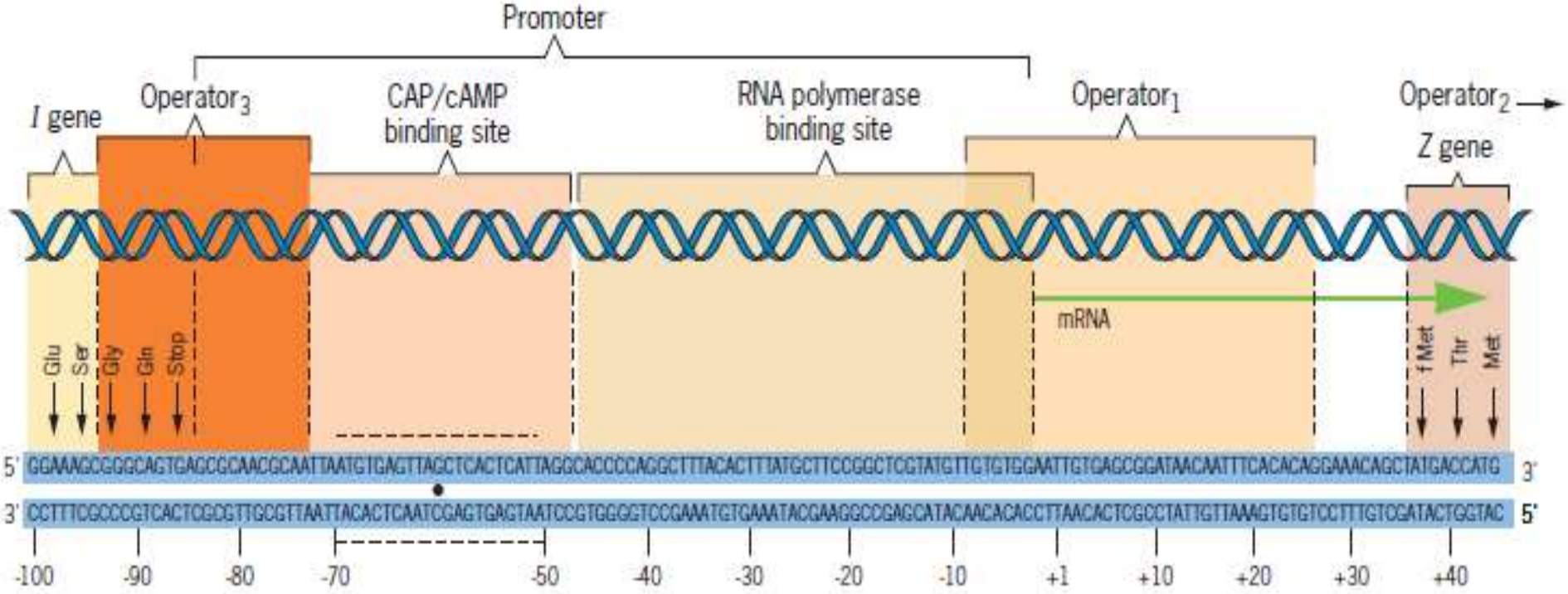
- Mutations in the promoter are uninducible and cis-acting

Mutation in structural genes

- Mutations in either lacZ or lacY can create the lac genotype, in which cells cannot utilize lactose. (The genotypic description "lac‟ without a qualifier indicates loss-of-function.)
- The lacZ mutations abolish enzyme activity, directly preventing metabolism of lactose.
- The lacY mutants cannot take up lactose from the medium.
- No defect! is identifiable in lacA cells, which is puzzling. It is possible that the acetylation reaction gives an advantage when the bacteria grow in the presence of certain analogs of β-galactosides that cannot be metabolized, because the modification results in detoxification and excretion.

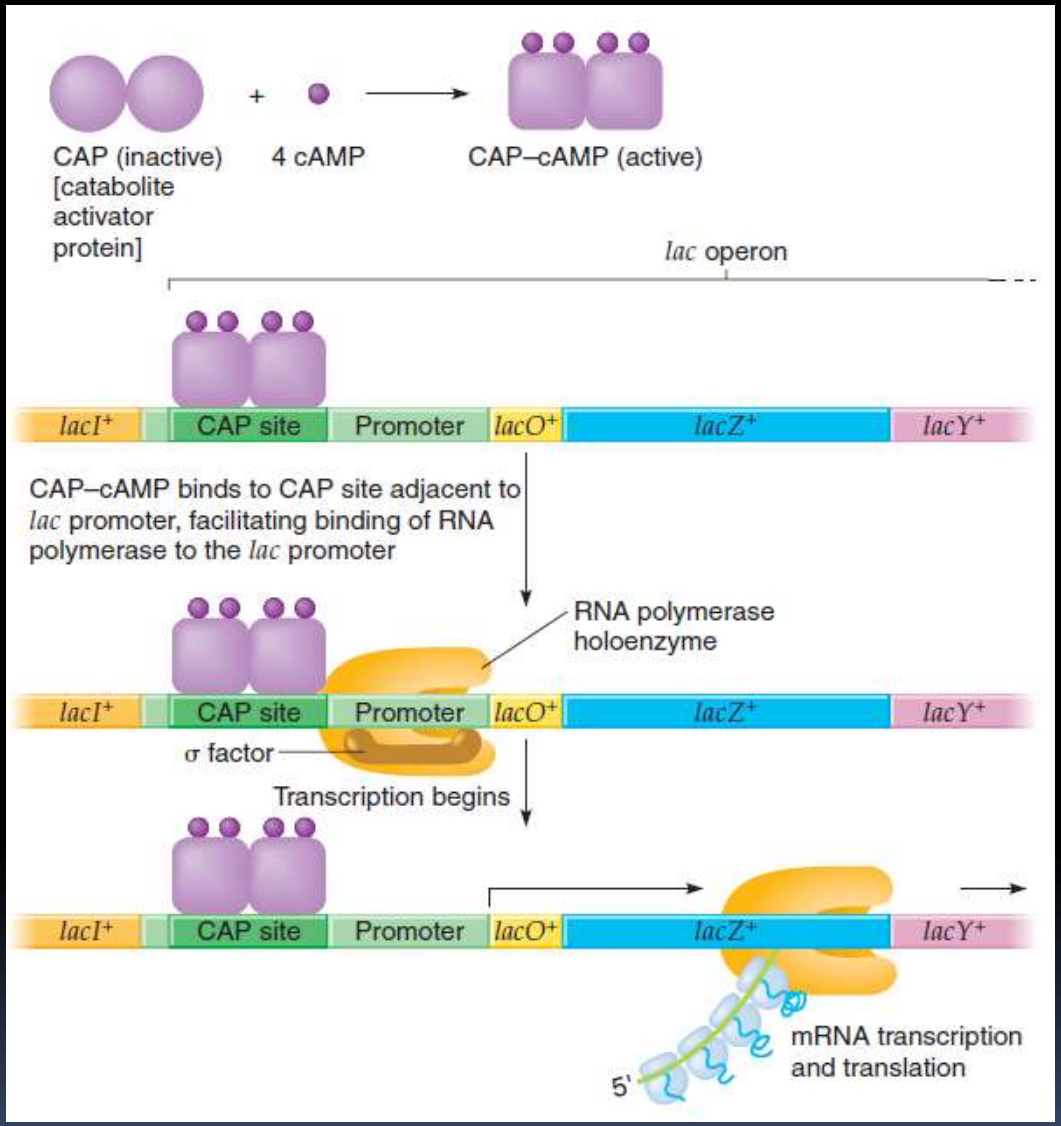
CATABOLITE REPRESSION

- CAP (catabolite activator protein) / CRP
- cyclic AMP (adenosine-3, 5-monophosphate; abbreviated cAMP)



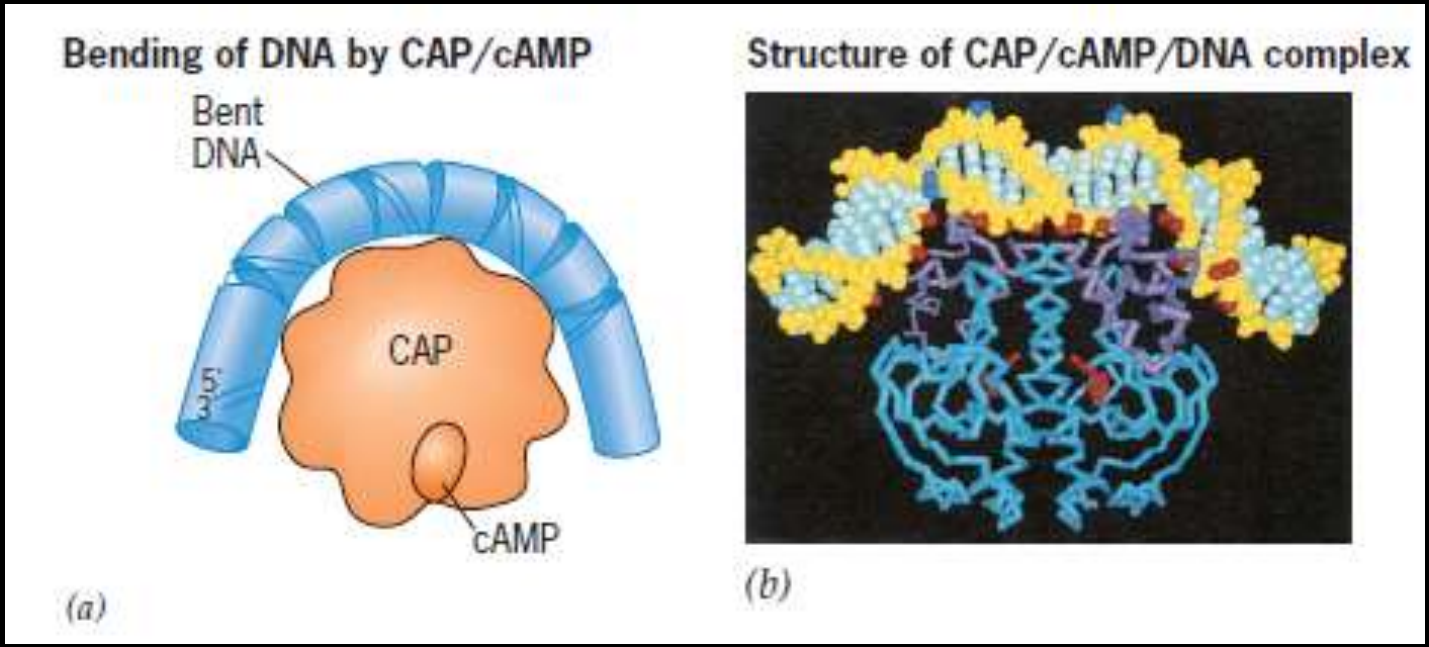
■ **FIGURE 18.10** Organization of the promoter–operator region of the *lac* operon. The promoter consists of two components: (1) the site that binds the CAP/cAMP complex and (2) the RNA polymerase binding site. The adjacent segments of the *lacI* (repressor) and *lacZ* (β-galactosidase) structural genes and the *lac* operators O_1 and O_3 are also shown. Operator O_2 is located downstream (centered at position +412) in the *lacZ* gene. The horizontal line labeled mRNA shows the position at which transcription of the operon begins (the 5′ end of the *lac* mRNA). The numbers at the bottom give distances in nucleotide pairs from the site of transcript initiation (position +1). The dot between the two nucleotide strands indicates the center of symmetry of an imperfect palindrome.

Source : Principles of Genetics by D. Peter Snustad (sixth edition)



Source : iGENETICS: A Molecular Approach by Peter J. Russell

- How does the binding of CAP/cAMP stimulate transcription of the lac structural genes?



The interaction of CAP/Camp with its binding site in the Lac promoter

Source : Principles of Genetics by D. Peter Snustad (sixth edition)

Solve It

1. $I^+ P^+ O^+ Z^+$
2. $I^- P^+ O^+ Z^+$
3. $I^+ P^+ O^c Z^+$
4. $I^- P^+ O^c Z^+$
5. $I^+ P^+ O^c Z^-$
6. $\dfrac{F' I^+ P^+ O^c Z^-}{I^+ P^+ O^+ Z^+}$
7. $\dfrac{F' I^+ P^+ O^+ Z^-}{I^+ P^+ O^c Z^+}$
8. $\dfrac{F' I^+ P^+ O^+ Z^+}{I^- P^+ O^+ Z^-}$
9. $\dfrac{F' I^+ P^+ O^c Z^-}{I^- P^+ O^+ Z^+}$
10. $\dfrac{F' I^- P^+ O^+ Z^-}{I^- P^+ O^c Z^+}$

Q17.1 In the laboratory, you are given 10 strains of *E. coli* with the following *lac* operon genotypes, where $I = lacI$ (the Lac repressor gene), $P = P_{lac}$ (the promoter), $O = lacO$ operator), and $Z = lacZ$ (the β-galactosidase gene):

For each strain, predict whether β-galactosidase will be produced (a) if lactose is absent from the growth medium and (b) if lactose is present in the growth medium. Glucose is absent from the medium in every case. (*Note*: In the partial diploid strains (6–10), one copy of the *lac* operon is in the host chromosome and the other copy is in the extrachromosomal *F* factor.)

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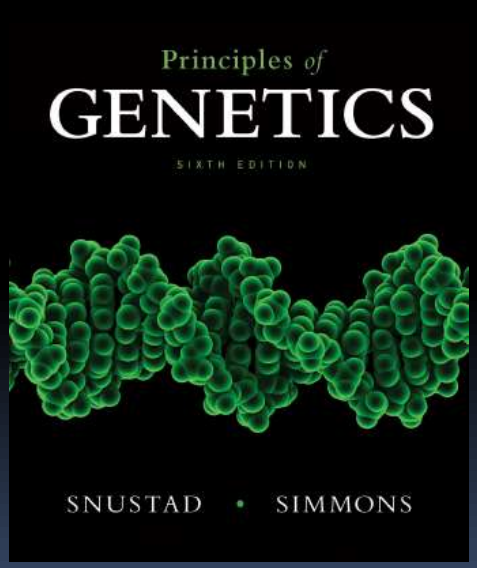
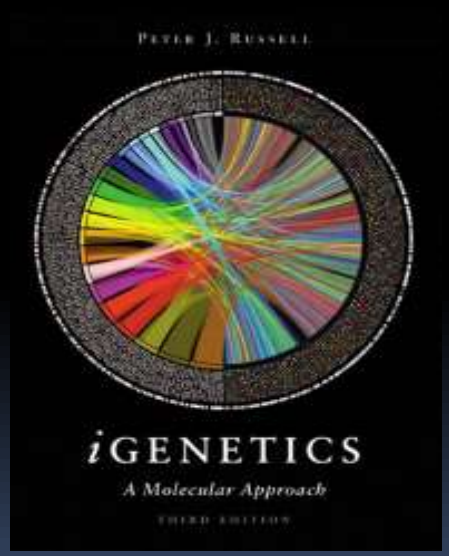
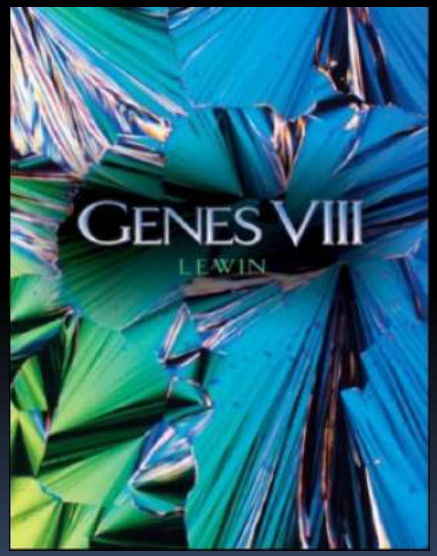
REFERENCES:

I. Principles of Genetics by D. Peter Snustad
II. iGENETICS: A Molecular Approach by Peter J. Russell
III. Genes VIII by Benjamin Lewin
IV. Genes IX by Benjamin Lewin







Thank you for your patience

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