Regulation of Gene Expression

Lac and Trp Operon

Gene Expression

- The expression of a gene is measured by the amount of products form
- Which can be controlled by regulating gene expression at various levels

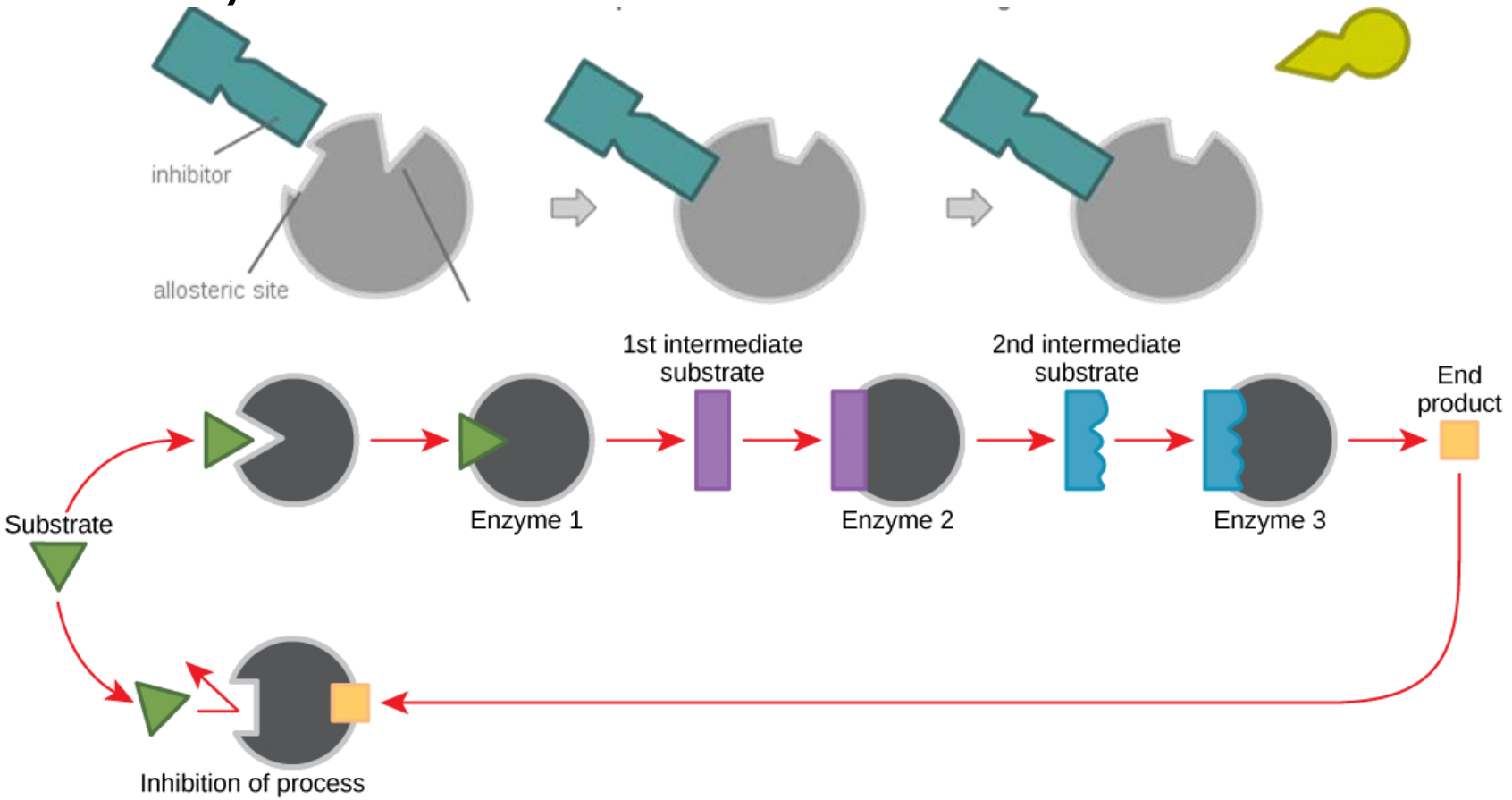
- Watch the video and record ways in which a cell controls the expression of a gene
  - https://www.youtube.com/watch?v=KKR28Y_L4CA

Regulating Gene Expression

- The 3 major levels of metabolic control that we will (have already) looked at are:
  - Transcription: affects whether RNAP can bind to transcribe the gene
  - **Protein activity**: alters protein/enzyme function
  - Chromosome structure: how unwound the chromosome is affects the ability for the information to be read

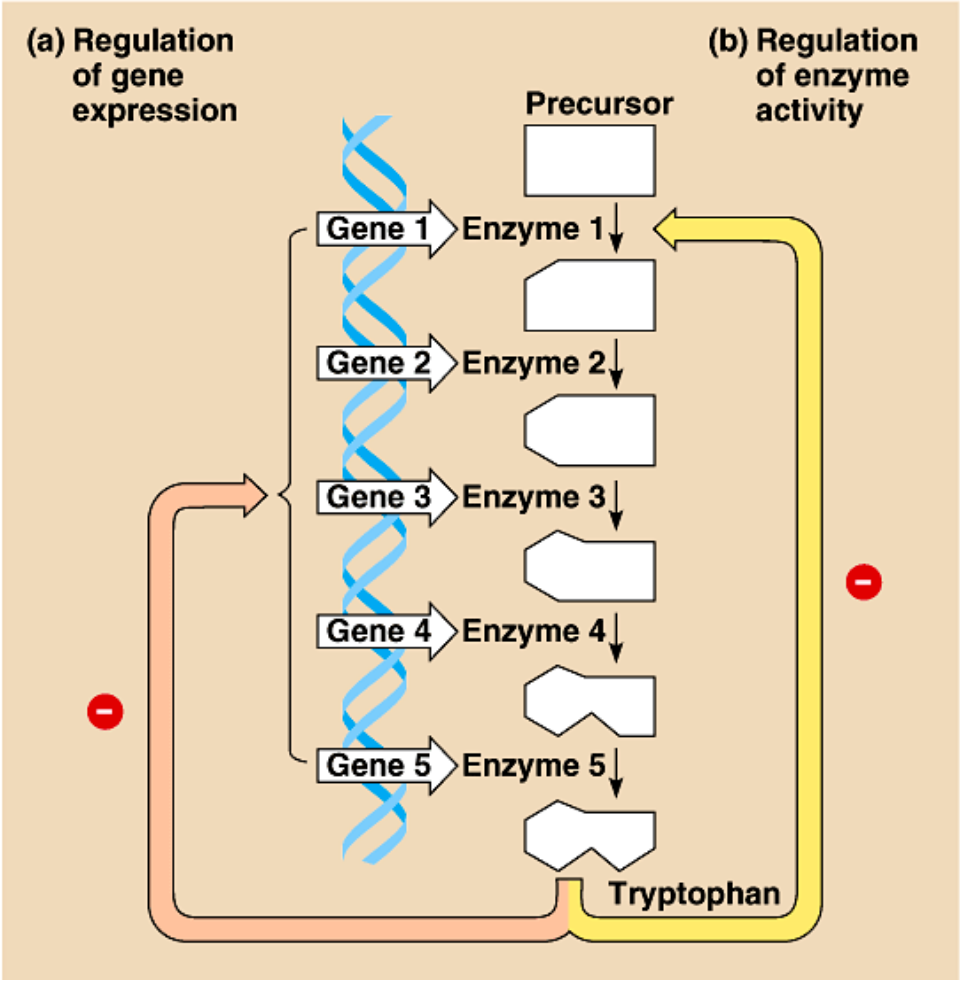
Recall: Enzyme Regulation

- Inhibitors, allosteric regulators, cooperativity
- Why? Feedback inhibition



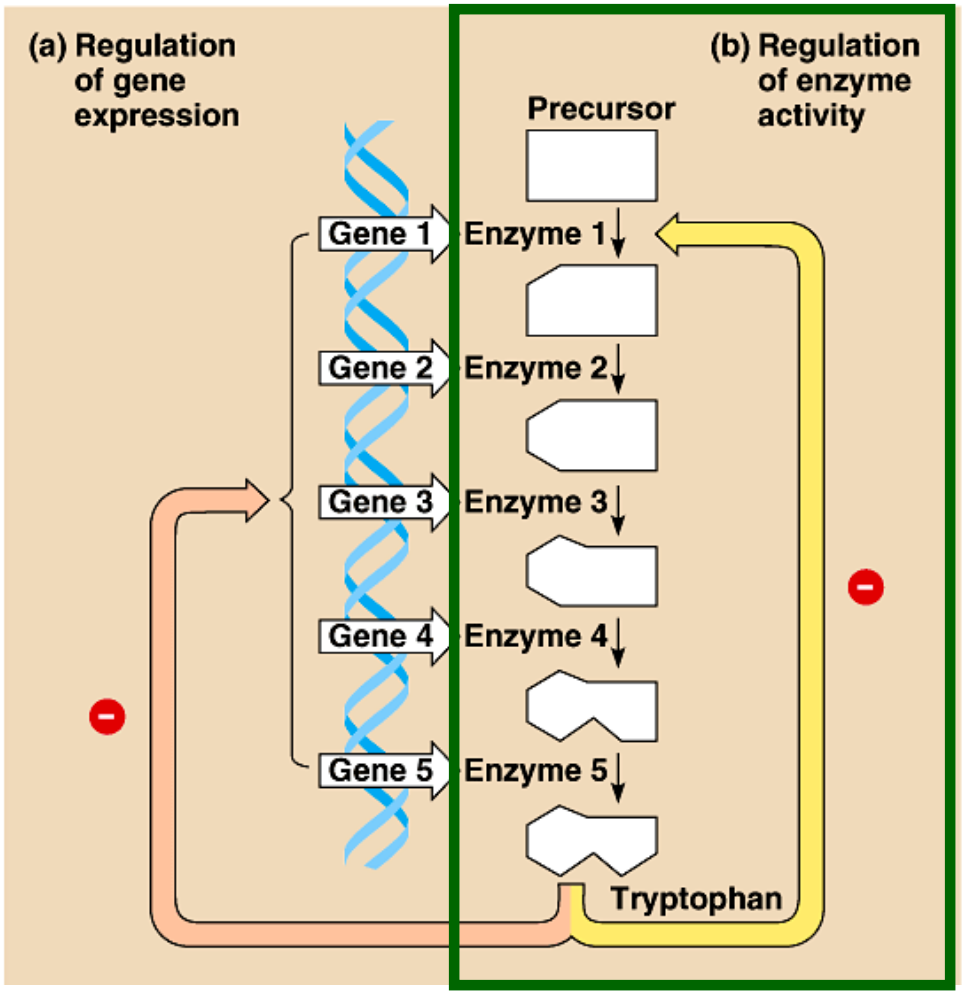
Example: E. coli in human intestine

- No tryptophan present, needs to synthesize
- Tryptophan present, does not need to synthesize
- Feedback inhibition on the production of trp



Control at the Protein Level

- Adjust activity of enzymes already present
- Short term, quick onset regulation



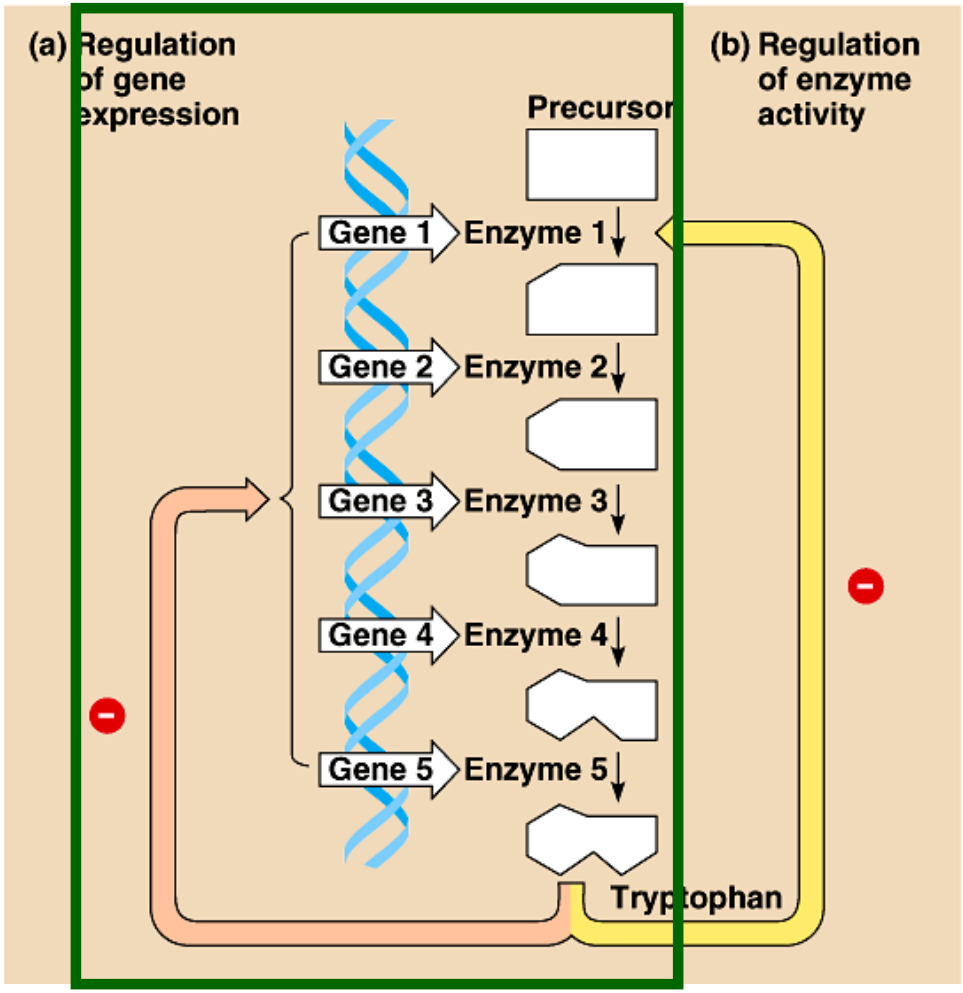
Regulating Gene Expression

(Levels of Metabolic Control)

- **Transcription**: affects whether RNAP can bind to transcribe the gene
  - Genes that are actively being transcribed are **ON**
  - Genes that are not actively being transcribed are **OFF**
- Protein activity: alters protein/enzyme function
- Chromosome structure: how unwound the chromosome is affects the ability for the information to be read

Control at Transcription Level

- Control the expression of genes which affects number of enzymes produced
- Long term, slow onset regulation

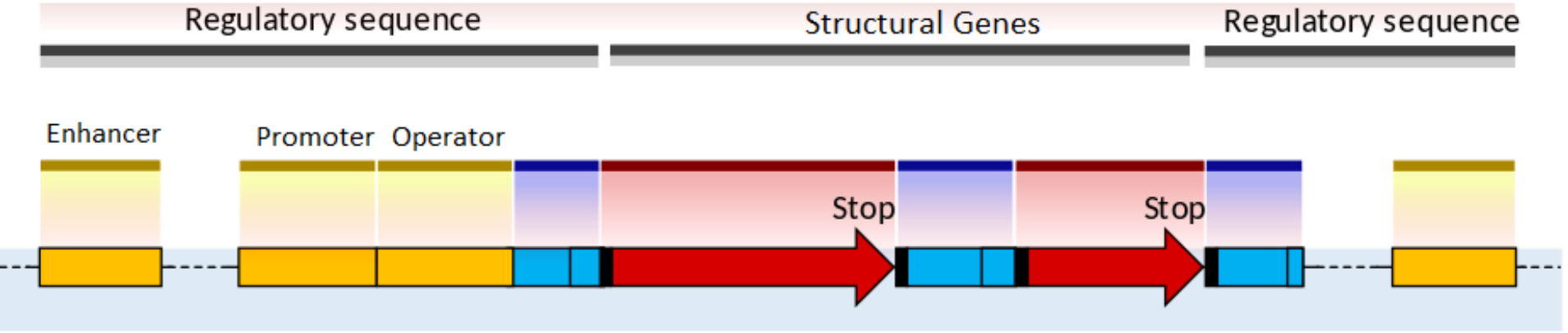


Types of Gene Regulation

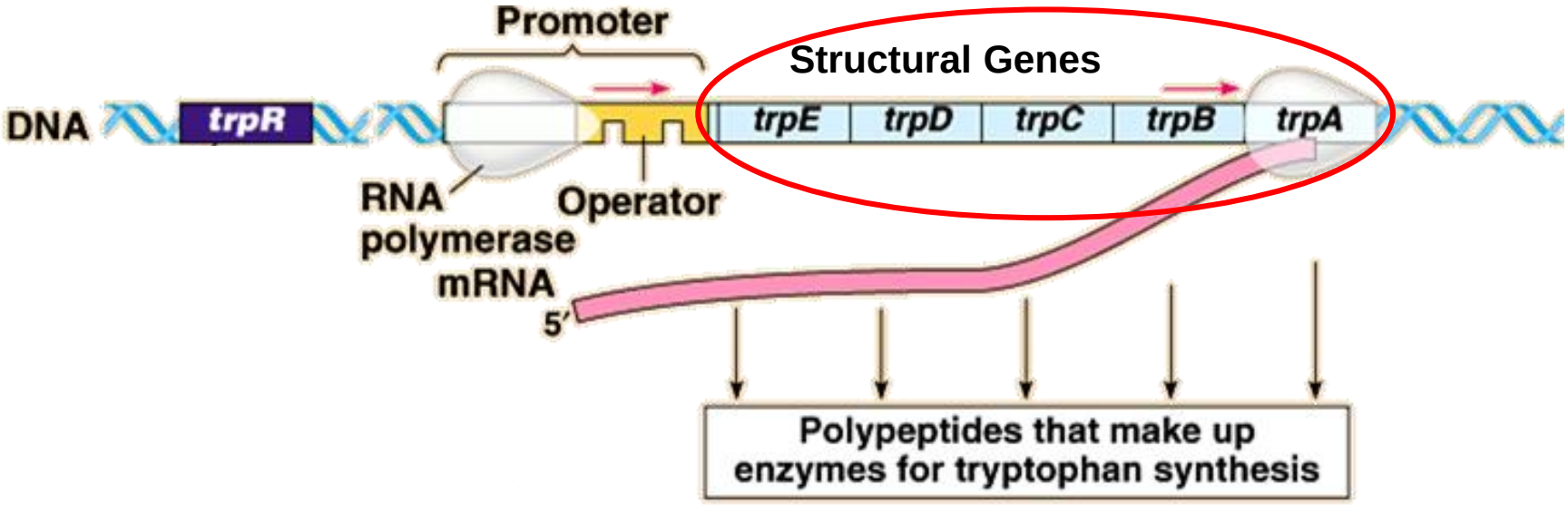
Gene Regulation	POSITIVE	NEGATIVE
Type of regulatory protein		
Regulatory sequence (protein binding site)		
Location of regulatory sequence		
Effect on RNAP		
Effect on transcription		
Effect on gene expression		
Example of regulatory protein in Lac operon		

Anatomy of a Operon

- Operon: a group of genes that share a single promoter
- Genes code for proteins that have **related functions**



Example: *trp* operon



- 5 genes making 5 polypeptides that combine to make 3 enzymes
- Enzymes participate in a sequence of steps to make tryptophan (trp) an amino acid

Fig. 18.20a

Operon Components

- Promoter
  - Region on DNA where RNAP binds to start transcription
  - acts as the on/off switch for genes
- Structural genes
  - Genes to be transcribed by RNAP
  - These genes have related functions
- Regulatory sequences

Regulatory Sequence

- Segment on DNA:
  - where regulatory protein binds
  - capable of increasing or decreasing the expression of a gene (transcription)
- Types of regulatory sequences:
  - Enhancer: activator binds to increase transcription
  - Operator: repressor binds to decrease transcription

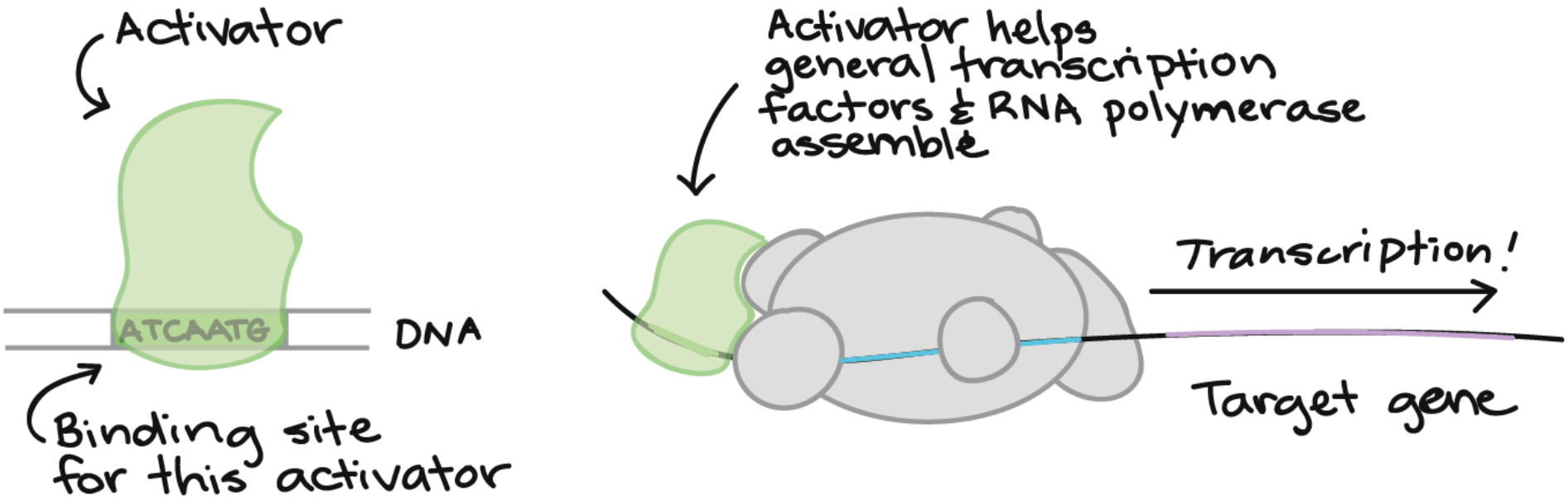
Eukaryotic vs Prokaryotic Gene

- Monocistronic:
  - Eukaryotic genes specify a single protein (but splicing allows for more than variation)
  - Thus each promoter controls one gene
- Polycistronic:
  - Prokaryotic genes with related function are situated in tandem on the DNA
  - Thus one promoter controls more than one gene

Positive Gene Regulation

- Active form of the regulatory protein turns on or increases the transcription of the operon
- Animation of positive and negative regulation of the lac operon: http://highered.mcgraw-hill.com/olc/dl/120080/bio27.swf

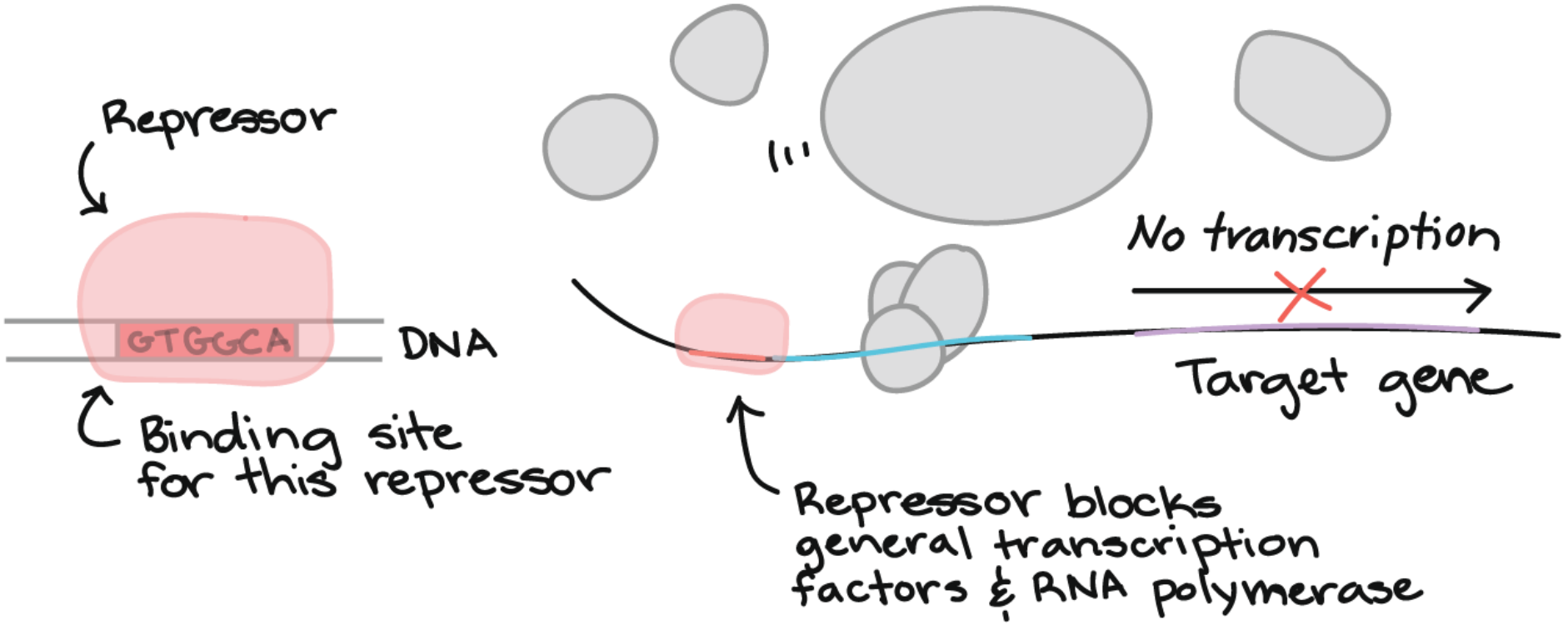
Positive Gene Regulation



Negative Gene Regulation

- Operons are switched off by the active form of the regulatory protein (repressor)
  - Trp operon: repressor activated by corepressor, binds to operator
  - Lac operon: repressor made in active form, binds to operator

Negative Gene Regulation



Overview of Gene Regulation

Gene Regulation

Negative: Repressor protein turns OFF transcription

Positive: Activator protein turns ON transcription

Gene Expression

Repressible Operon
- Has the *ability* to be turned OFF by a **corepressor**
- Gene expression normally ON

Inducible Operon
- Has the *ability* to be turned ON by an **inducer**
- Gene expression normally OFF

Types of Gene Expression

Types of expression	Definition	Type of genes
Constitutive (unregulated)	Genes are always ON	Housekeeping genes (regulatory genes)
Inducible (regulated)	Genes are only turned on as needed	Structural genes
Repressible (regulated)	Genes are only turned off as needed	Structural genes

Gene Expression

- Would anabolic pathways have genes that are inducible or repressible?
- Would catabolic pathways have genes that are inducible or repressible?

- Recall:
  - Anabolism: reactions that build up molecules
  - Catabolism: reactions that breakdown molecules

Anabolic Pathway: Repressible

- Anabolism: synthesis of molecules from simpler subunits
- If an essential nutrient is not present in the environment, bacteria must be able to synthesize the nutrient on its own
- However, bacteria do not need to produce the essential nutrient if it is already present in the environment

Catabolic Pathway: Inducible

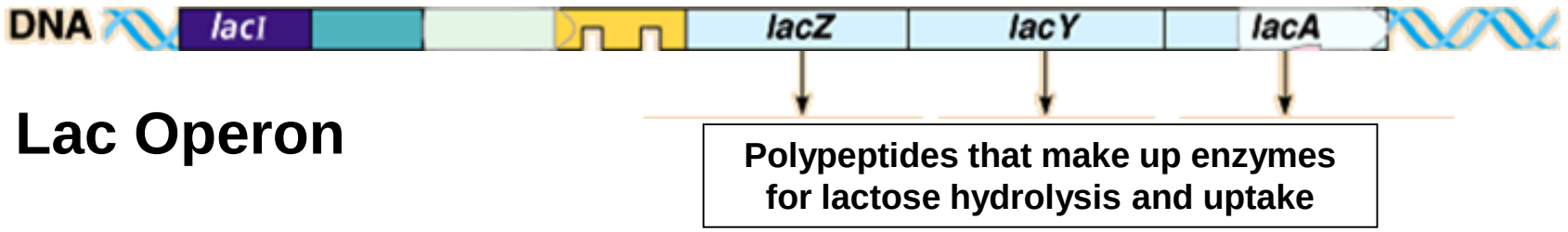
- Catabolism: breakdown of complex molecules into simpler units
- It would be a waste of energy for bacteria to make enzymes for the breakdown of a substance if that substance does not exist
- do not need to catabolize molecules that are not present in its cellular environment

Gene Expression

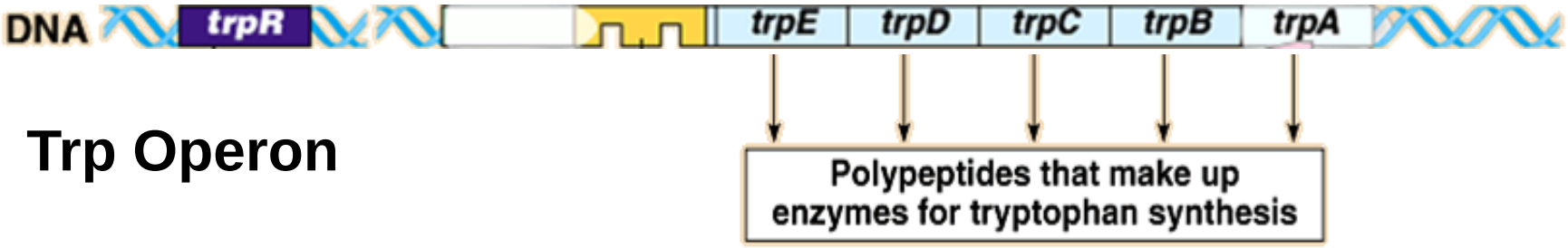
Pathway	Anabolic	Catabolic
Product of genes	Synthesis of nutrient	Breakdown of nutrient
Expression	Repressible	Inducible
Explanation	Gene will turn off when nutrient is present in the environment. Thus no need to synthesize it.	Gene turned on when nutrient being broken down is present.

Examples

- Classify the metabolic pathway: catabolic or anabolic
- Classify the gene expression: inducible or repressible



Lac Operon



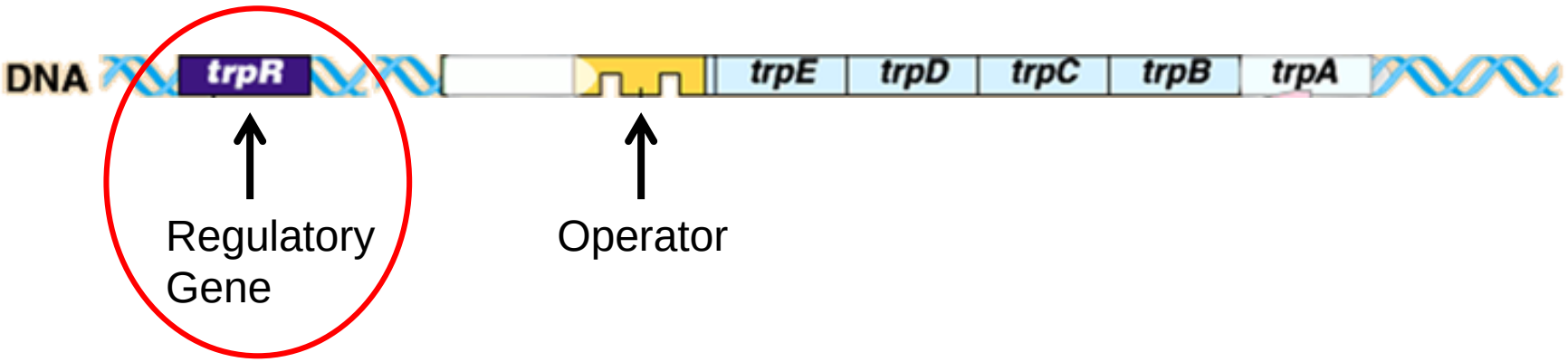
Trp Operon

Components of Operon Regulation

- Regulatory Gene (constitutive)
- Regulatory Protein (repressor, activator)
- Effector (corepressor, inducer)
- Operon
  - Promoter
  - Regulatory sequence (operator, enhancer)
- Structural Genes (repressible, inducible)

Regulatory Gene

- region on DNA that codes for the production of the regulatory protein
- **upstream** of the operon
- **constitutive** expression: transcribed continuously



Regulatory Protein

- **Allosteric**:
  - Has an allosteric site which can bind an effector
  - Two forms: effector bound or unbound
  - Thus alternates between active and inactive forms
- Two types:
  - **Repressor**: **active** form binds to DNA and **blocks** RNAP function by binding to **operator**
  - **Activator**: **active** form binds to DNA and **enhances** RNAP's function by binding to **enhancer**

Negative Gene Regulation

- Operons are switched off by the active form of the regulatory protein
  - Regulatory protein is a repressor

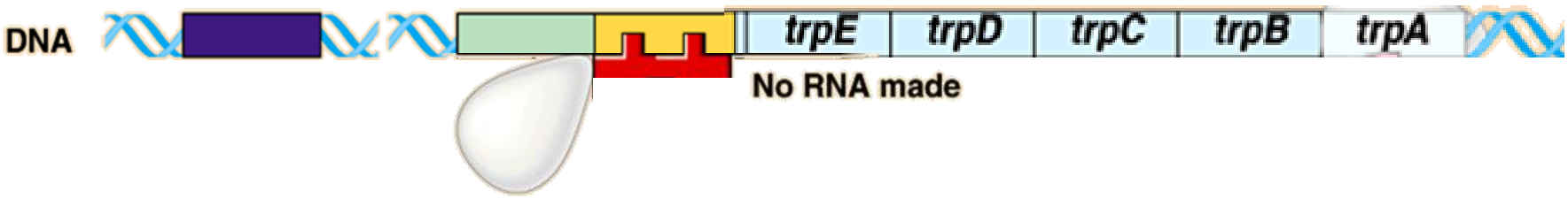
Models of Negative Gene Regulation

- Repressible Operon (e.g. trp)
  - Repressor made in inactive form → activated by corepressor
  - Turns gene off
- Inducible Operon (e.g. lac)
  - Repressor made in active form → inactivated by inducer
  - Turns gene on

Comparing Gene Expression in the two model operons

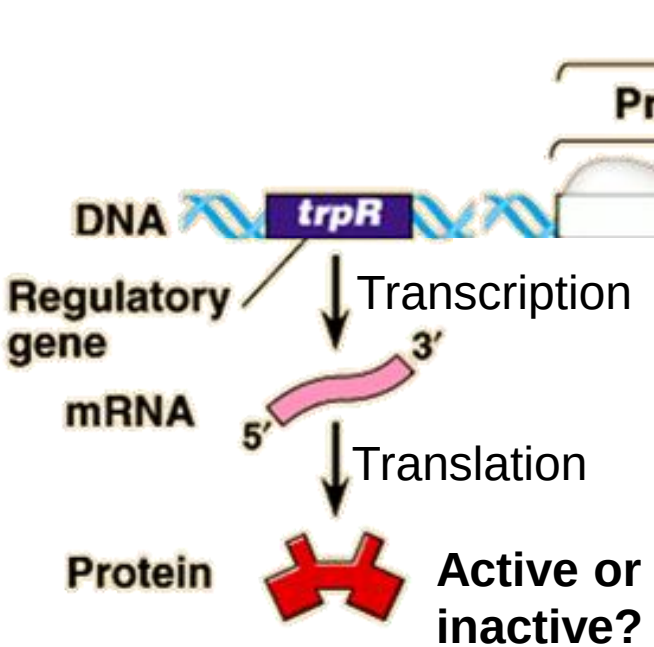
Operon	Trp	Lac*
Regulatory Gene		
Type of regulatory protein		
Name of regulatory protein		
Type of regulation		
Natural state of regulatory protein (active, inactive)		
Type of operon expression		
Type of effector		
Name of effector		

trp operon Regulatory Protein



- Legend:
  - Yellow block = operator
  - Red block = regulatory protein
  - White blob = RNAP
- Given the diagram, is the regulatory protein a repressor or activator?

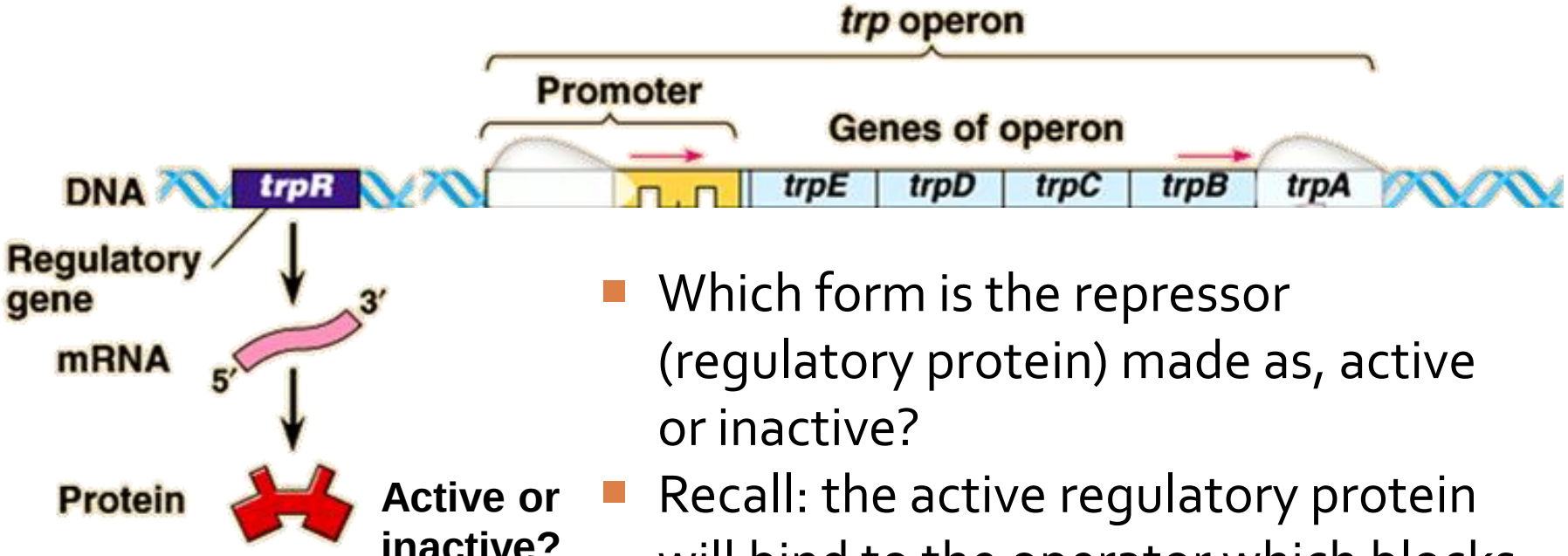
Properties of *trp* Repressor



- Coded by a regulatory gene: trpR
  - Constitutive expression
  - Continuously makes trp repressor
- Allosteric
  - has 2 forms: active or inactive

Fig. 18.20a

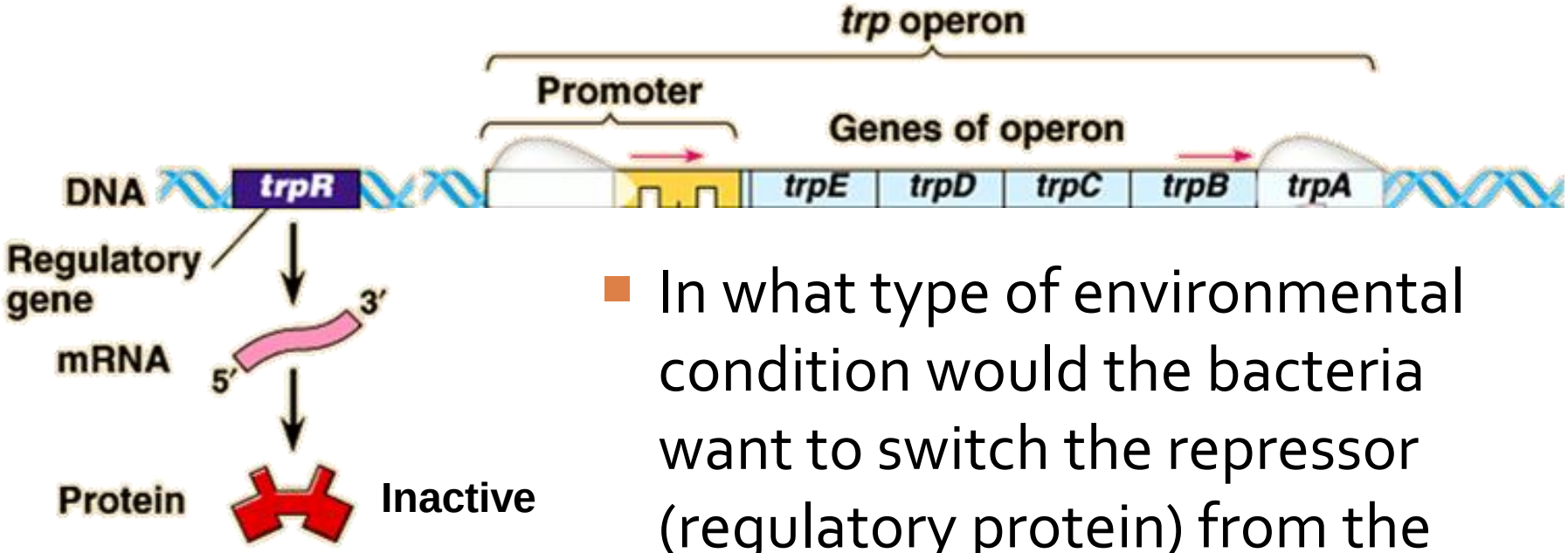
trp operon Regulation



- Which form is the repressor (regulatory protein) made as, active or inactive?
- Recall: the active regulatory protein will bind to the operator which blocks RNAP transcription
- Hint: trp operon produces tryptophan, an amino acid essential to its survival

Fig. 18.20a

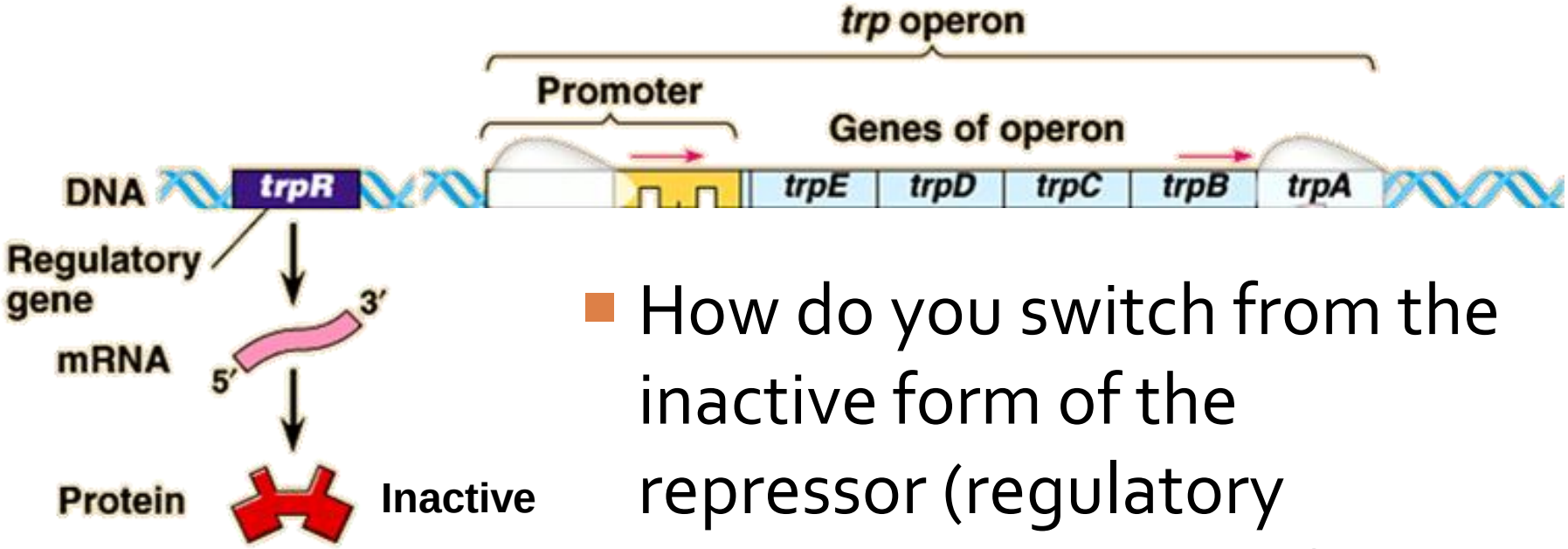
trp operon Regulation



- In what type of environmental condition would the bacteria want to switch the repressor (regulatory protein) from the inactive to the active form?
- In other words, why would the cell want to turn off transcription of these genes?

Fig. 18.20a

trp operon Regulation



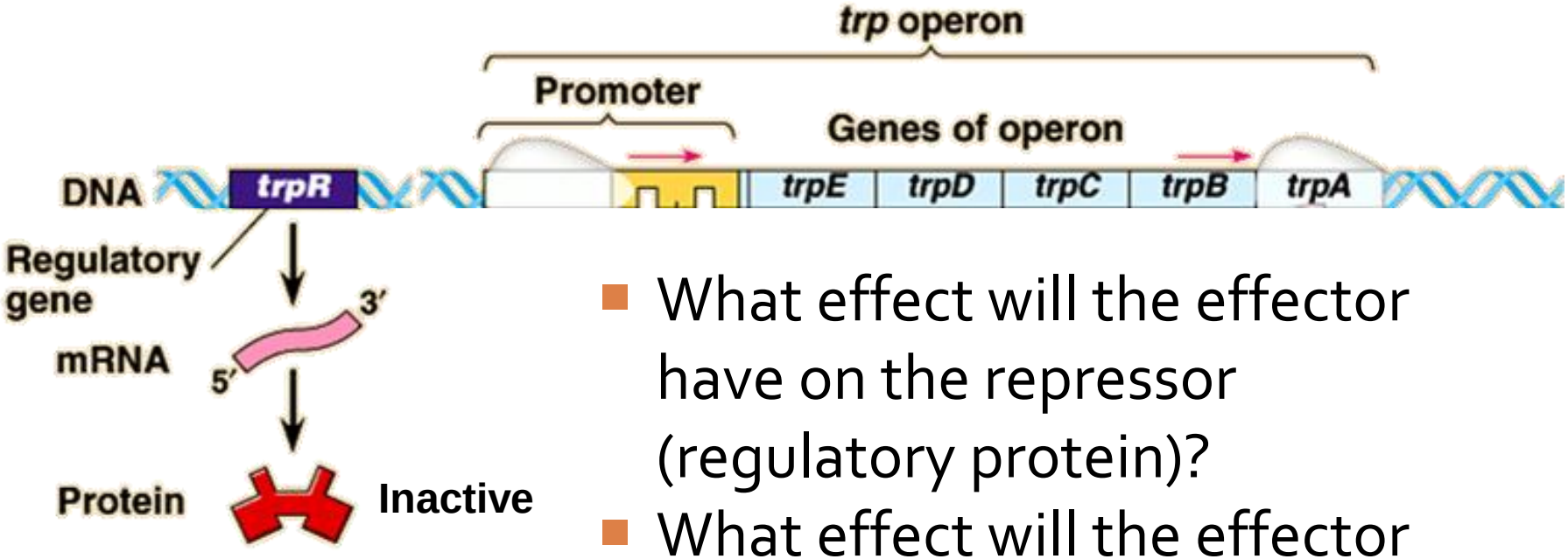
- How do you switch from the inactive form of the repressor (regulatory protein) to the active form?
- How do you turn on the repressor (regulatory protein)?

Fig. 18.20a

Effector

- any molecule that can regulate the activity of a **protein**
- **Corepressor**:
  - activates the repressor
  - causing regulatory protein to bind to the operator
  - inactivates gene expression
- **Inducer**:
  - inactivates the repressor
  - prevents regulatory protein from binding to the operator
  - activates gene expression

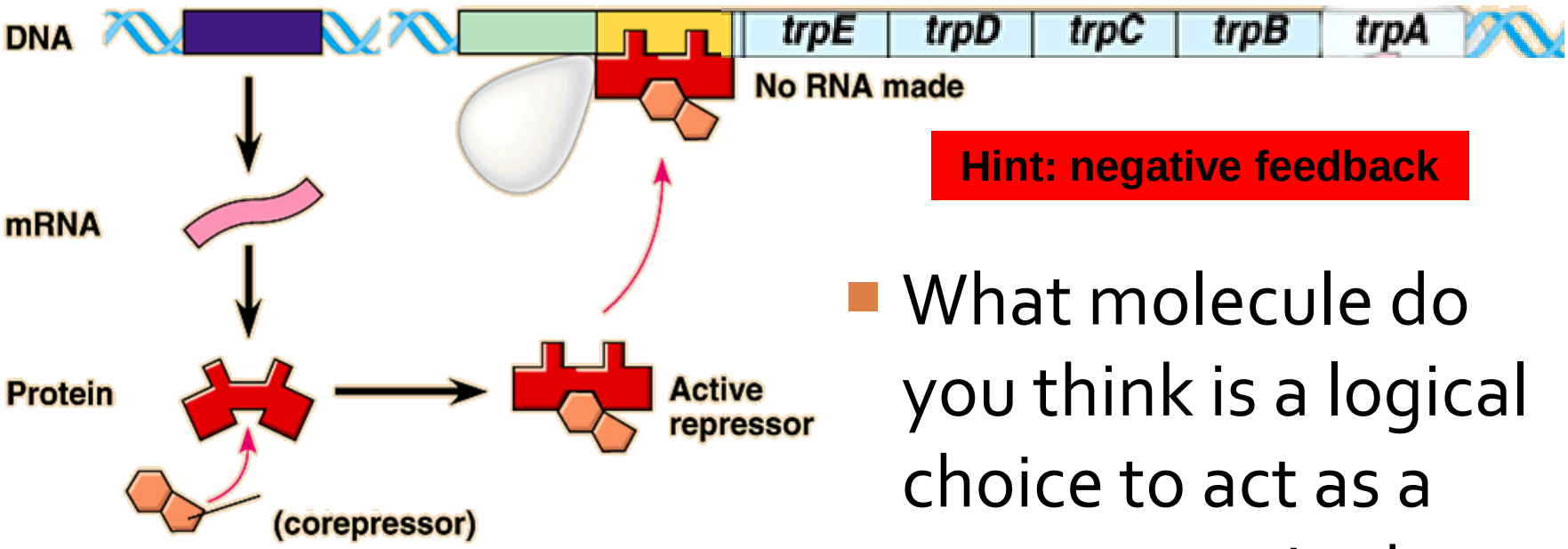
trp operon Regulation



- What effect will the effector have on the repressor (regulatory protein)?
- What effect will the effector have on gene expression of the trp operon?
- Is the effector a corepressor or inducer?

Fig. 18.20a

Effector: Corepressor



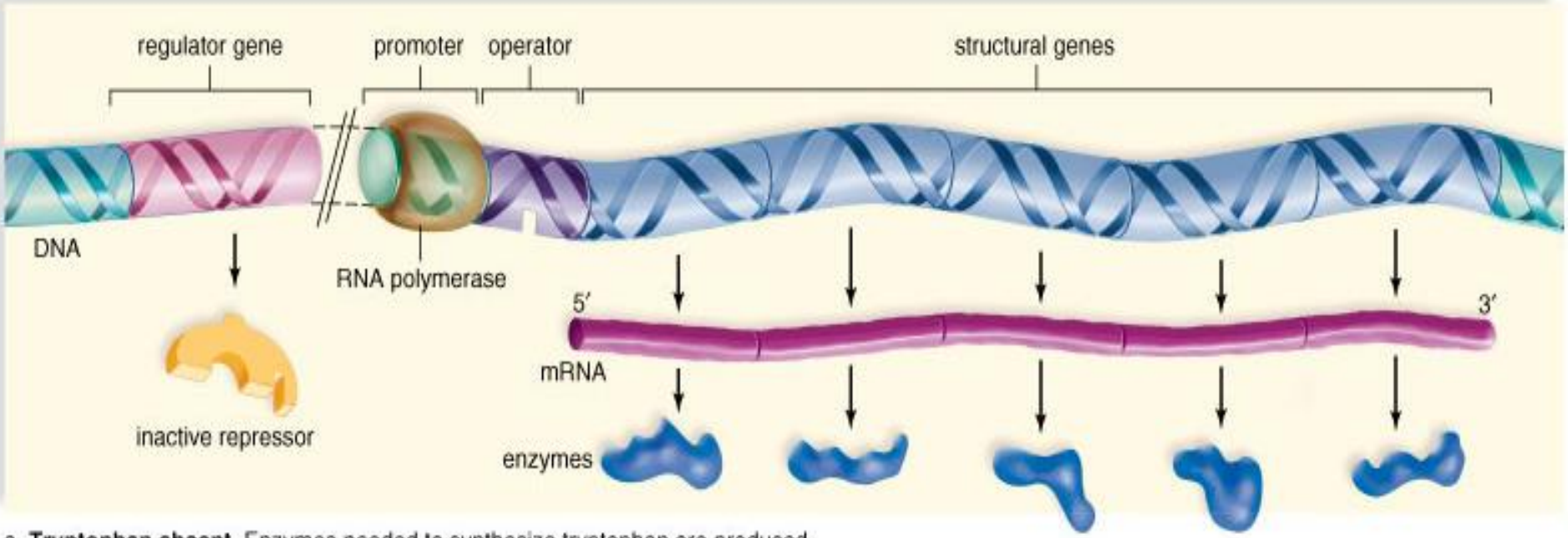
Hint: negative feedback

- What molecule do you think is a logical choice to act as a corepressor in the trp operon?

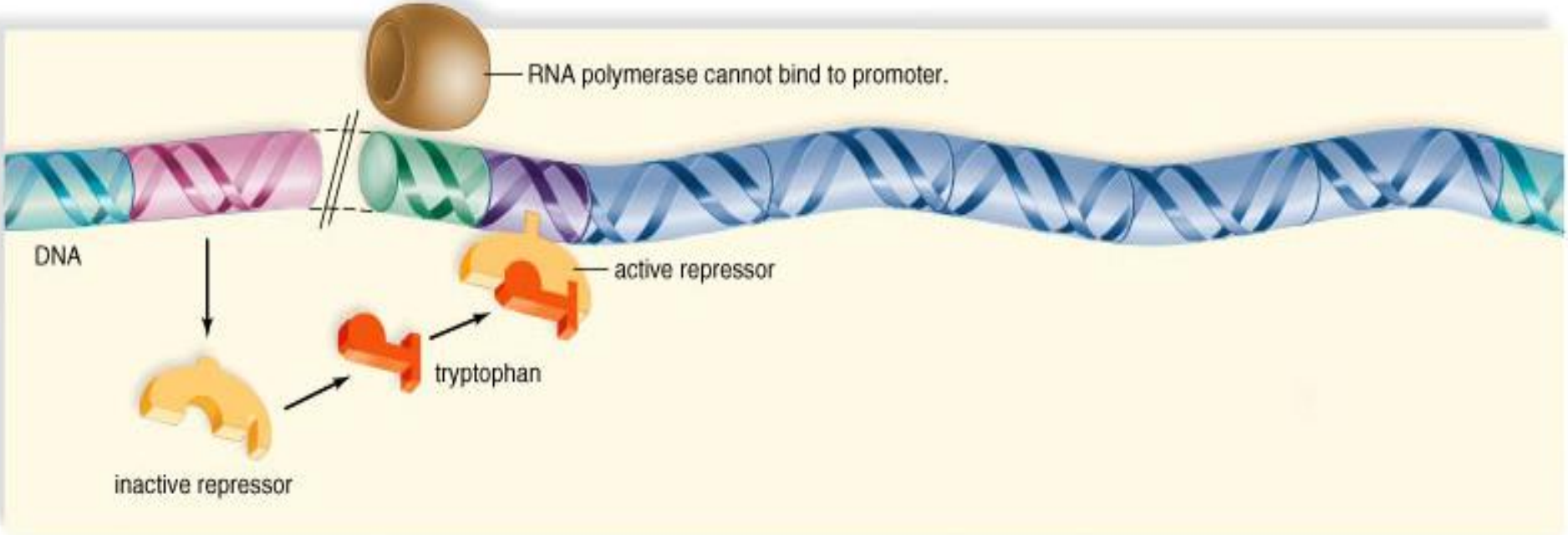
Fig. 18.20b

trp operon Regulation Summary

- Effector molecule: tryptophan (trp)
  - Corepressor
- Regulatory protein: trp repressor
  - Made inactive, RNAP can transcribe, trp made
  - Activated by trp (corepressor)
- Active trp repressor binds to operator
  - Prevents RNAP from transcribing genes
  - Stops trp production



a. **Tryptophan absent.** Enzymes needed to synthesize tryptophan are produced.



b. **Tryptophan present.** Presence of tryptophan prevents production of enzymes used to synthesize tryptophan.

Repressible operon

- Gene is normally "on"
- Transcription is inhibited when a molecule binds allosterically to a regulatory protein
- Negative feedback / feedback inhibition
- **Anabolic** pathways
- Example: Trp operon is turned off when tryptophan (corepressor) binds to repressor

trp operon animation

Animation

- http://highered.mcgraw-hill.com/olc/dl/120080/bio26.swf
- http://www.as.wvu.edu/~dray/219files/TrpAttenuation.mov (ignore information on attenuation)

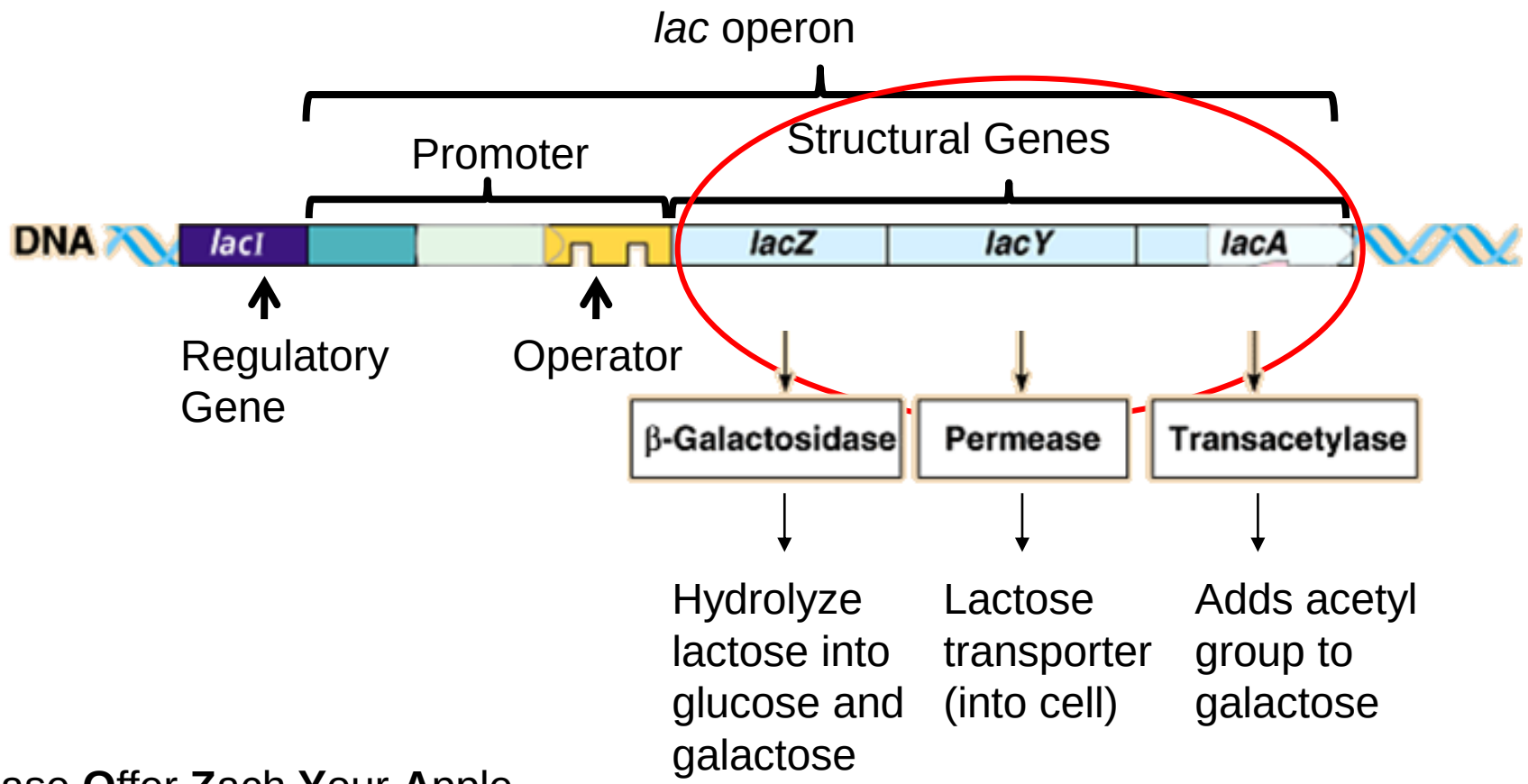
Tutorial

- http://bcs.whfreeman.com/thelifewire/content/chp13/1302002.html

lac operon Simulation

- WebSite:
  - Spongelab.com
  - Explore
  - Biology
  - Games and Simulation
  - “Gene Machine: The Lac Operon”

Components: *lac* operon

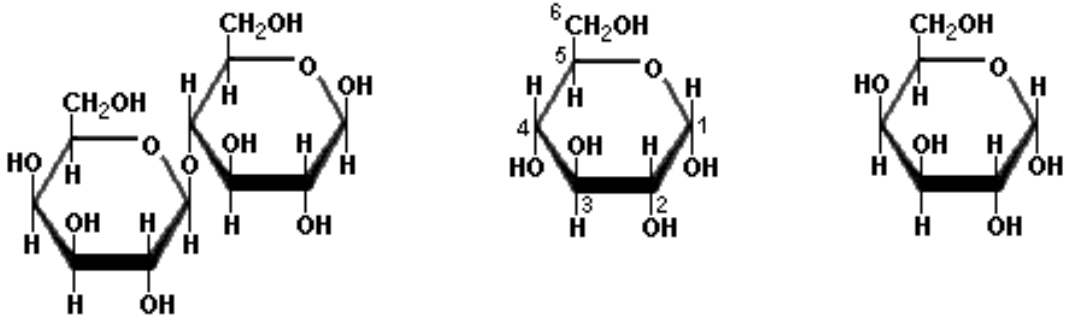


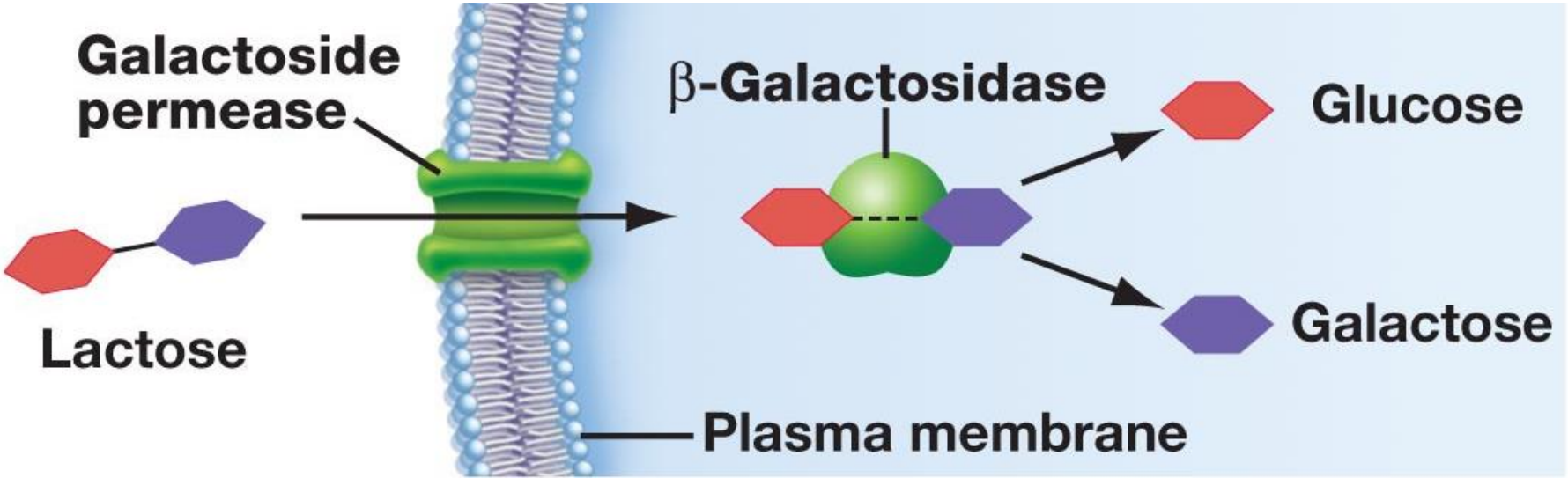
Please **O**ffer **Z**ach **Y**our **A**pple

Fig. 18.21

β-galactosidase

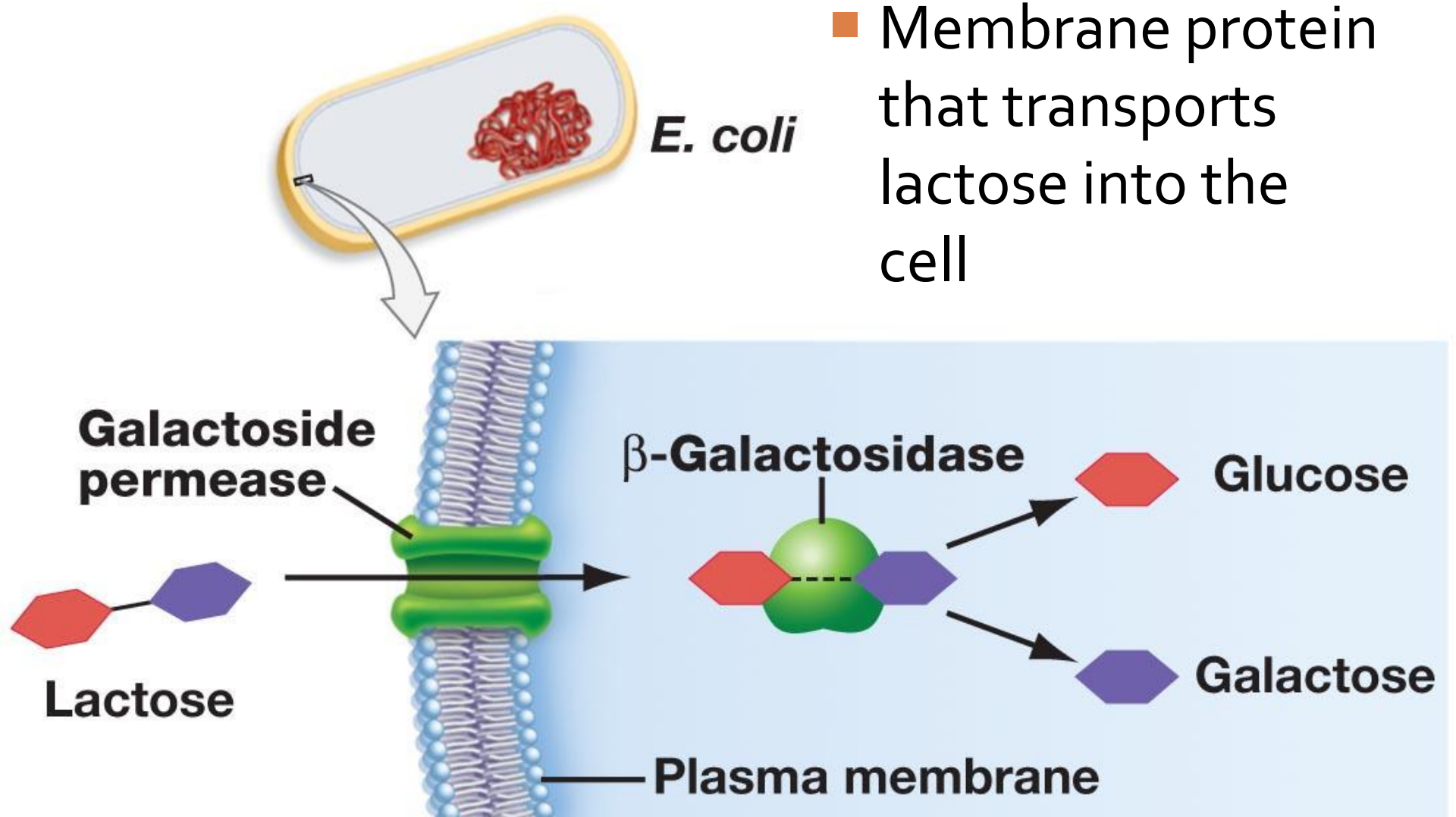
- Catalyzes the hydrolysis of lactose
- Lactose = glucose + galactose





Permease

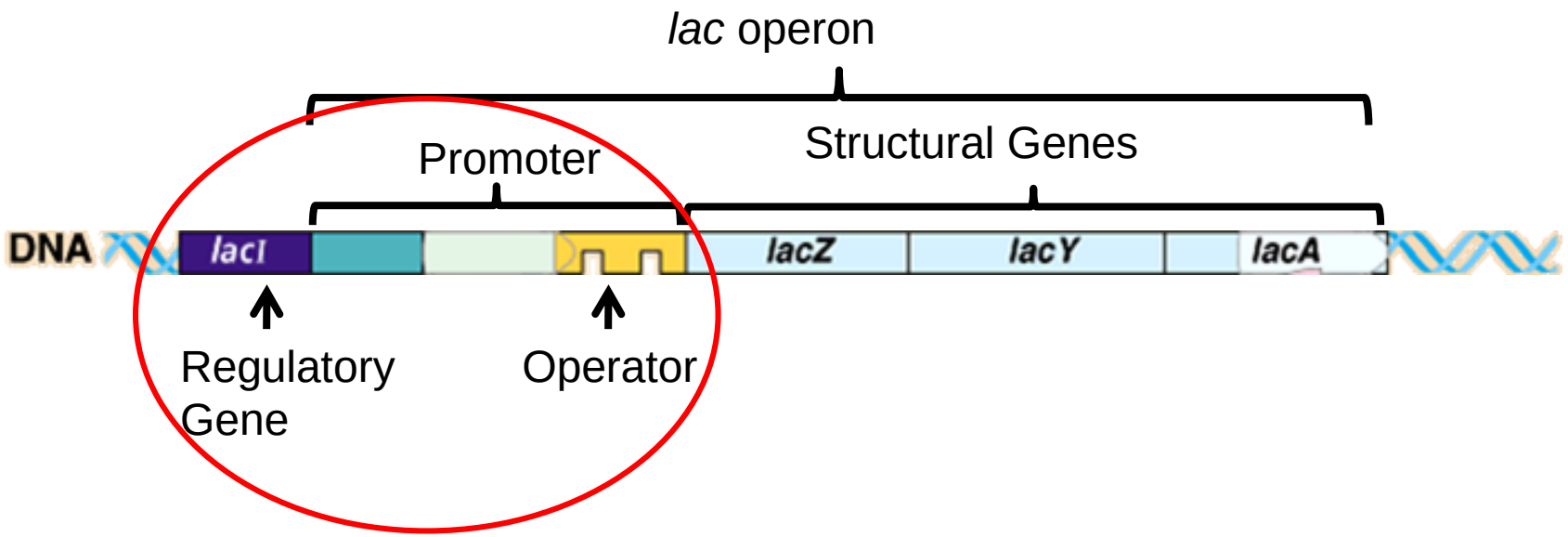
- Membrane protein that transports lactose into the cell



Structural Genes: lac operon

Enzyme	Gene	Function
β-galactosidase	Lac Z	Lactose hydrolysis into glucose and galactose
Permease	Lac Y	Membrane protein that transports lactose into the cell
Transacetylase	Lac A	Adds acetyl group to galactose (significance in lactose metabolism unclear)

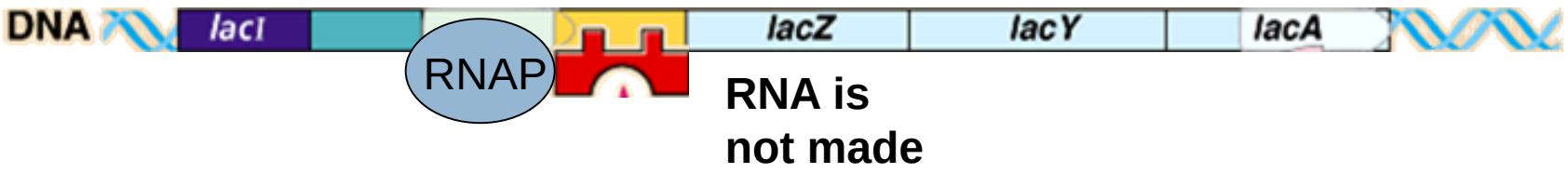
lac operon Regulation



Please **O**ffer **Z**ach **Y**our **A**pple

Fig. 18.21

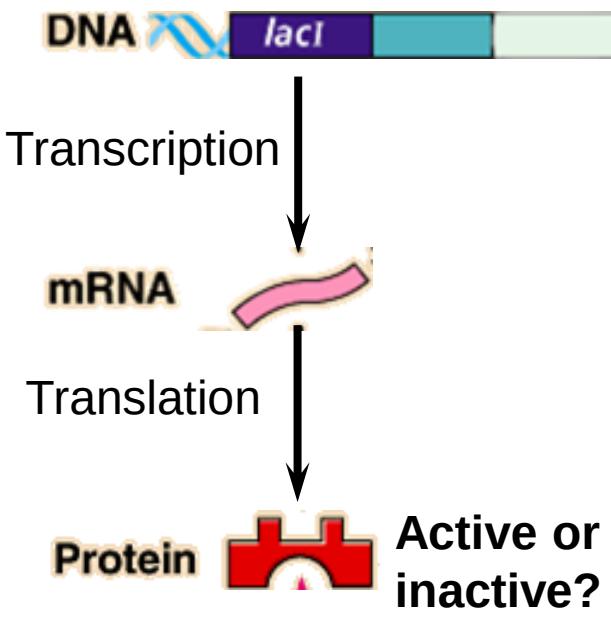
lac operon Regulatory Protein



- Legend:
  - Yellow block = operator
  - Red block = regulatory protein
  - Blue oval = RNAP
- Is the regulatory protein a repressor or activator?

Fig. 18.21a

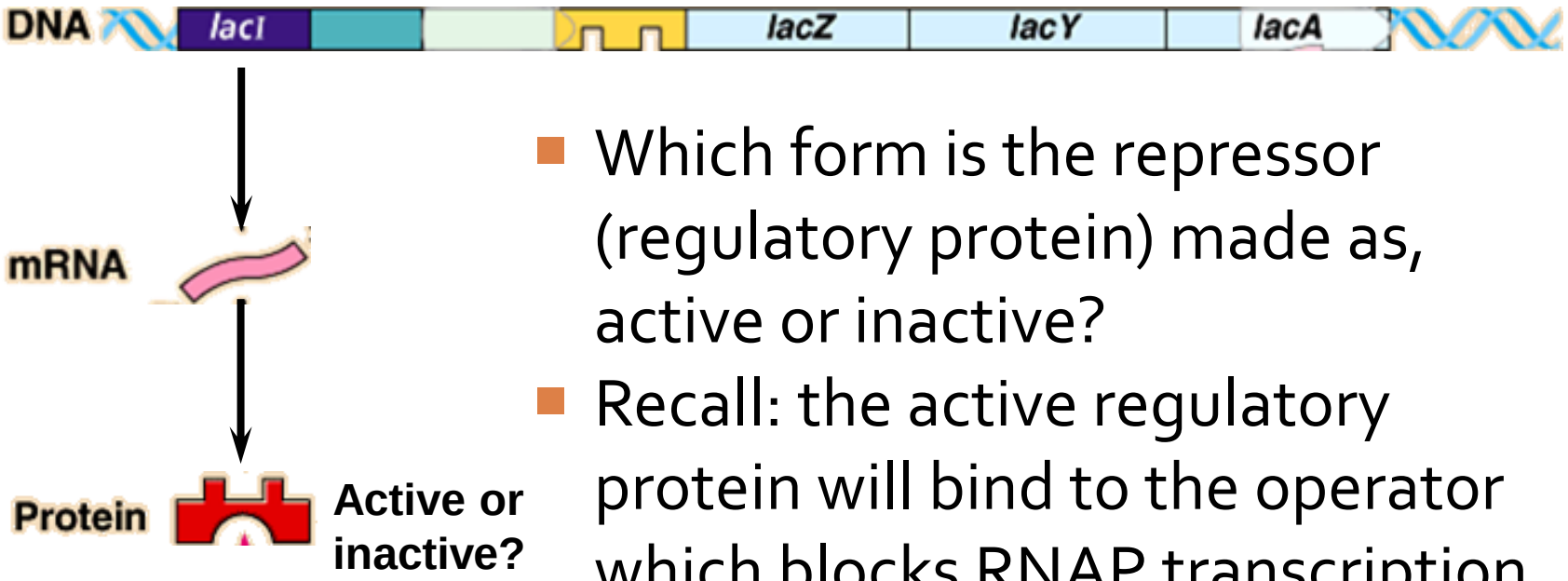
Properties of *lac* Repressor



- Coded by the regulatory gene: LacI
  - Constitutive expression
  - Continuously makes lac repressor
- Allosteric
  - has 2 forms: active or inactive

Fig. 18.21a

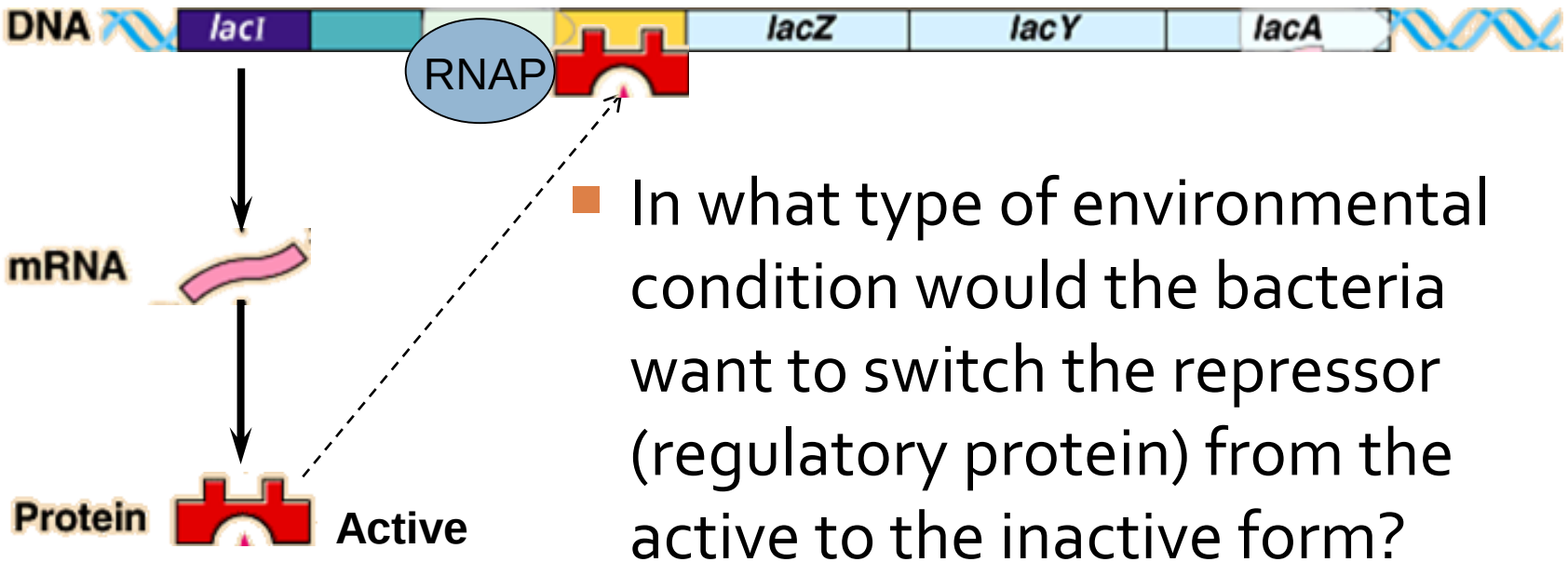
lac operon Regulation



- Which form is the repressor (regulatory protein) made as, active or inactive?
- Recall: the active regulatory protein will bind to the operator which blocks RNAP transcription
- Hint: lac operon produces enzymes that breakdown lactose

Fig. 18.21a

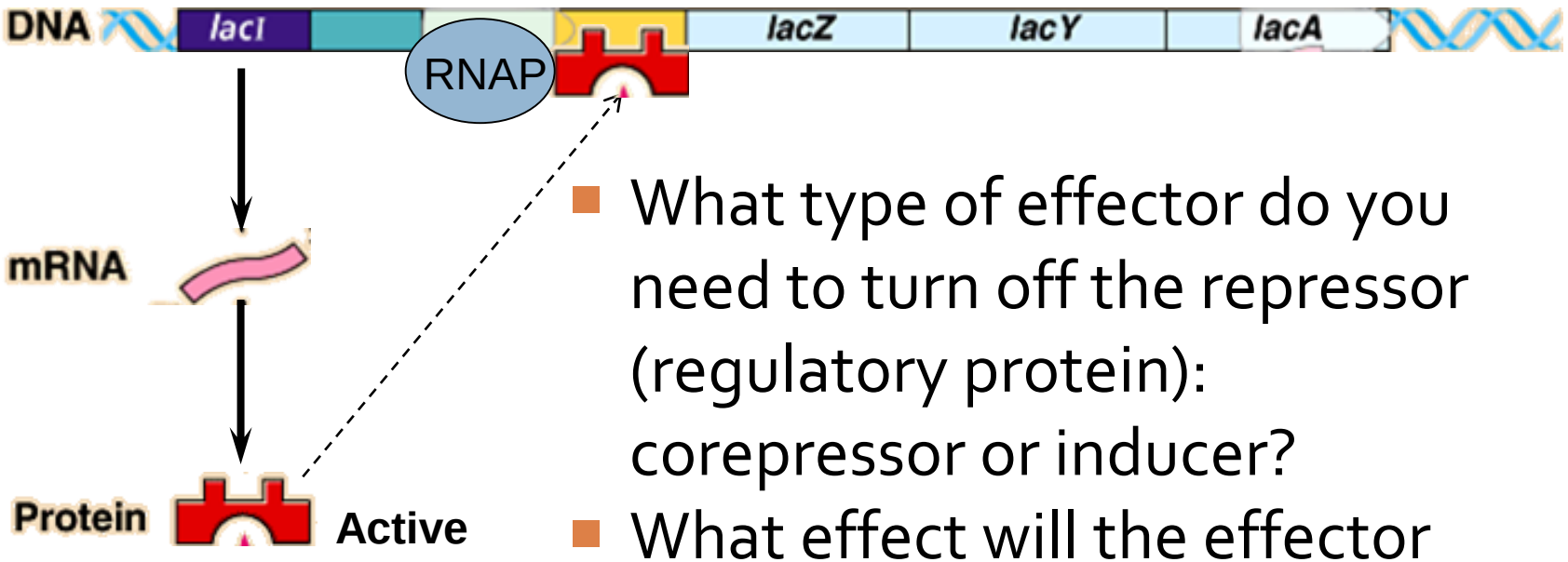
lac operon Regulation



- In what type of environmental condition would the bacteria want to switch the repressor (regulatory protein) from the active to the inactive form?
- In other words, why would the cell want to turn on transcription of these genes?

Fig. 18.21a

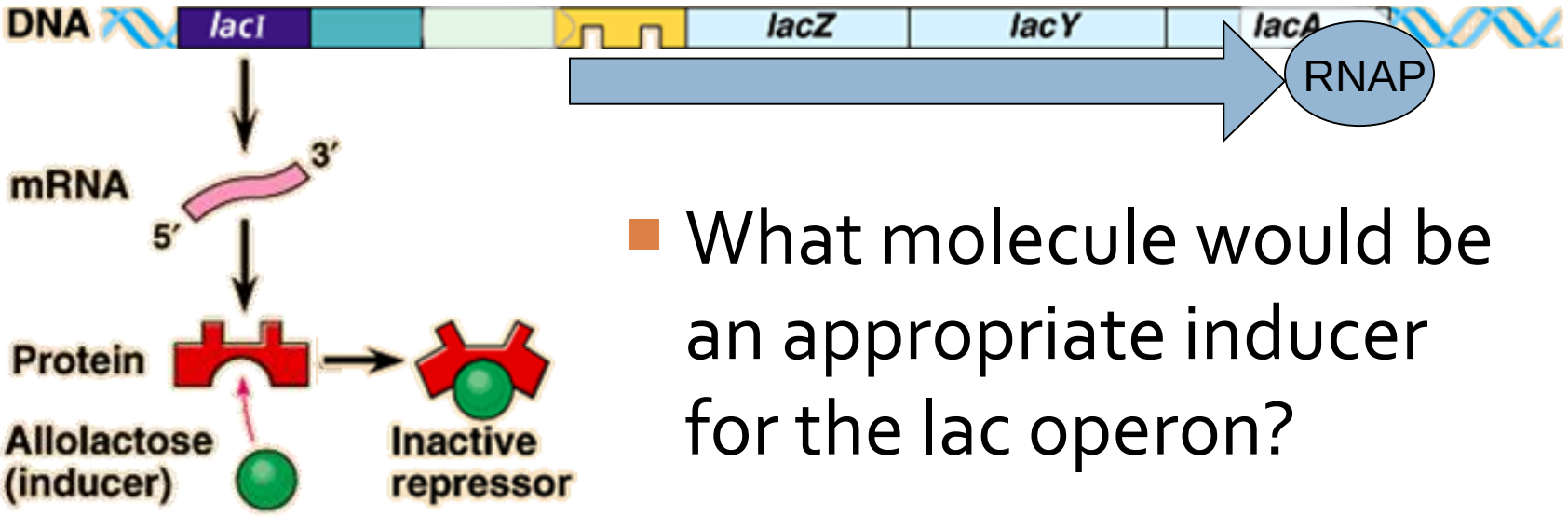
lac operon Regulation



- What type of effector do you need to turn off the repressor (regulatory protein): corepressor or inducer?
- What effect will the effector have on the on gene expression of the lac operon?

Fig. 18.21a

Effector: Inducer



- What molecule would be an appropriate inducer for the lac operon?

Fig. 18.21a

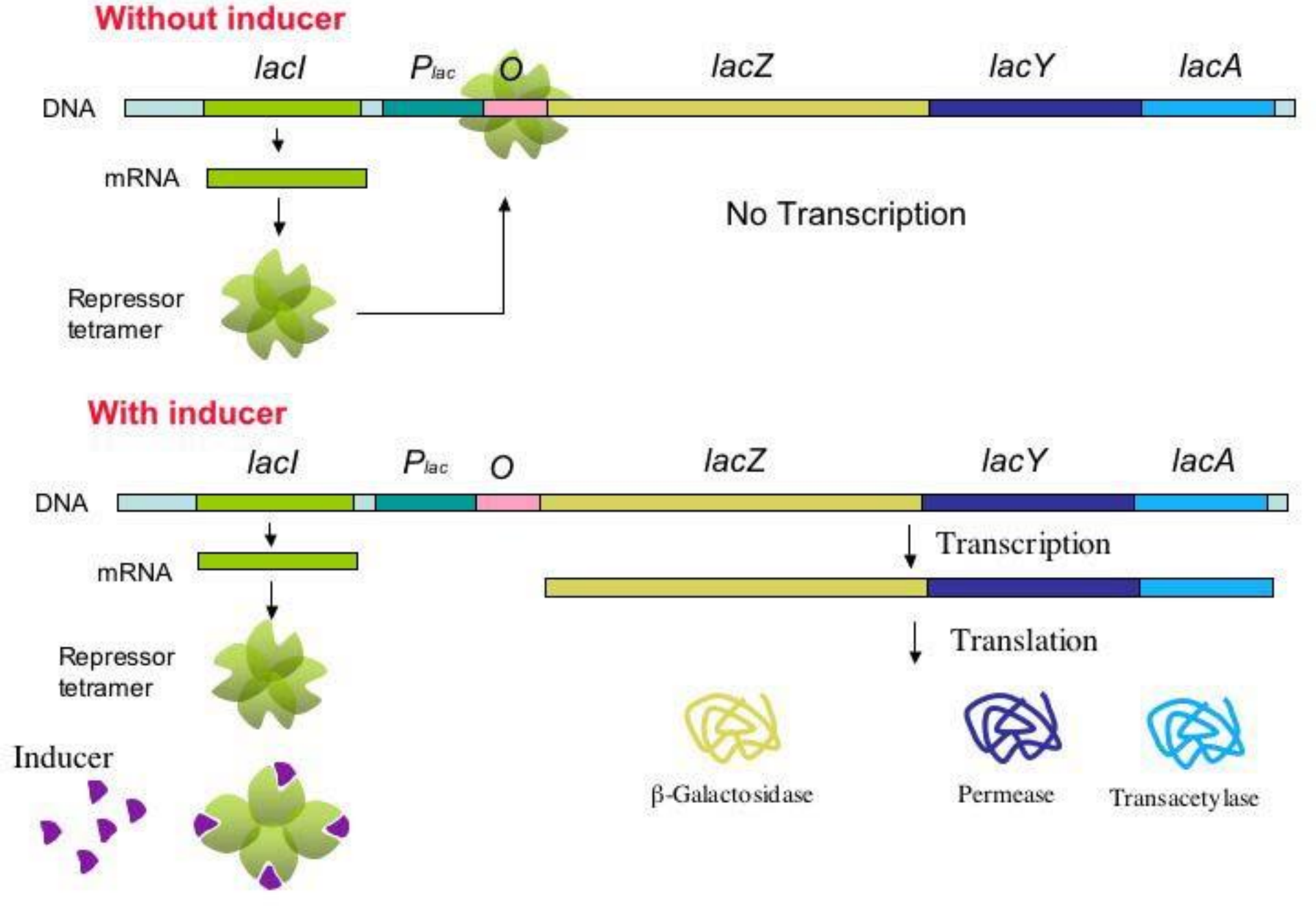
lac operon Regulation Summary

- Effector molecule: allolactose
  - An isomer of lactose
  - An inducer
- allolactose binds to lac repressor (regulatory protein)
  - Inactivates lac repressor preventing it from binding to the operator
  - Allows RNA polymerase to transcribe genes
  - Produces enzyme β-galactosidase which hydrolyzes lactose

lac operon Regulation Summary

- E. Coli cells mainly use glucose as a source of energy
- When living in a system with lactose, bacteria can hydrolyze lactose to produce glucose
- The lac operon is only turned on when lactose is present

The Lac Operon



Inducible operon

- Gene normally "off"
- Transcription is stimulated when a molecule binds allosterically to a regulatory protein
- Proteins made "on demand"
- **Catabolic** pathway (breakdown substances)
- Example: Lac operon turned on when allolactose (inducer) binds to repressor

lac operon animation

Animation

- http://www.youtube.com/watch?v=oBwtxdI1zvk&feature=related
- http://vcell.ndsu.nodak.edu/animations/lacOperon/movie-flash.htm
- http://www.dartmouth.edu/~cbbc/courses/movies/LacOperon.html (a little low tech)

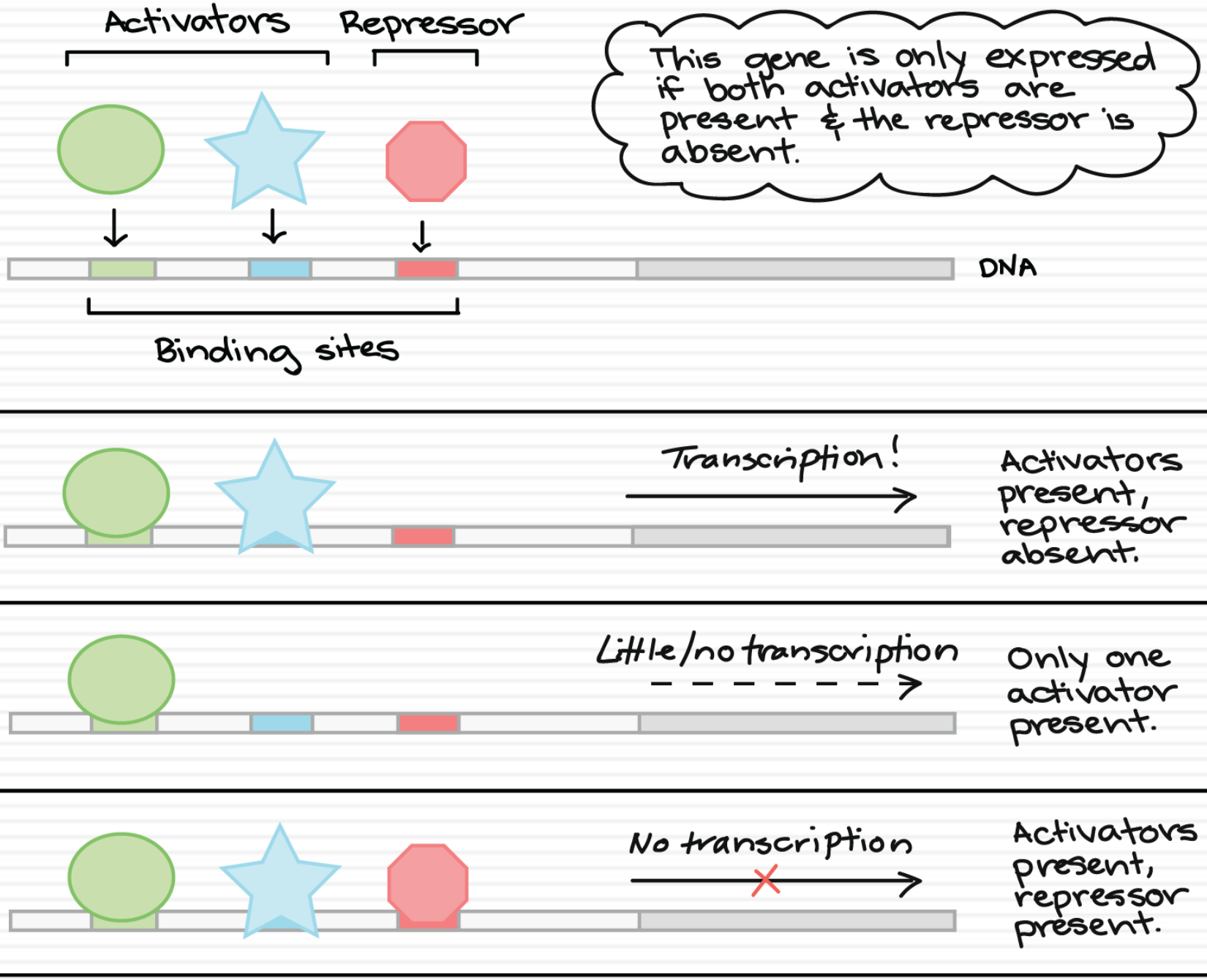
Tutorial

- http://bcs.whfreeman.com/thelifewire/content/chp13/1302001.html
- http://www.sumanasinc.com/webcontent/animations/content/lacoperon.html (looks the same as above)

Comparing Gene Expression

Operon	Trp	Lac*
Regulatory Gene		
Type of regulatory protein		
Name of regulatory protein		
Type of regulation		
Natural state of regulatory protein (active, inactive)		
Type of operon expression		
Type of effector		
Name of effector		

*The Lac operon also has positive gene regulation with an activator. We won't study that detail in this course. But you do need to understand positive gene regulation.



Overview of Gene Regulation

Gene Regulation

Negative: Repressor protein turns OFF transcription
e.g. lac repressors on lac operon

Positive: Activator protein turns ON transcription
e.g. cAMP on lac operon

Gene Expression

Repressible Operon
•Has the *ability* to be turned OFF by a **corepressor**
•Gene expression normally ON
•E.g. trp corepressor

Inducible Operon
•Has the *ability* to be turned ON by an **inducer**
•Gene expression normally OFF
•e.g. allolactose inducer

Thought Question

- In positive gene regulation how would a repressible and inducible operon look?
- Consider these questions:
  - Is the gene being expressed or not?
  - Would the activator (regulator protein) normally be bound or unbound to the enhancer (regulatory sequence)?
  - Would the activator be made active or inactive?
  - What would be a logical effector to activate or inactivate the activator (regulatory protein)?