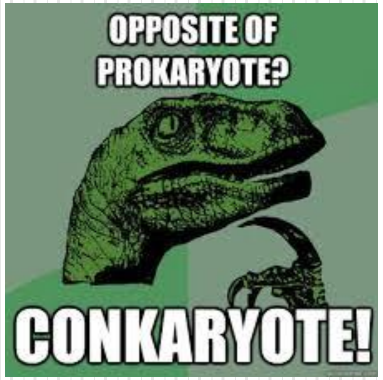
Gene expression in prokaryotes

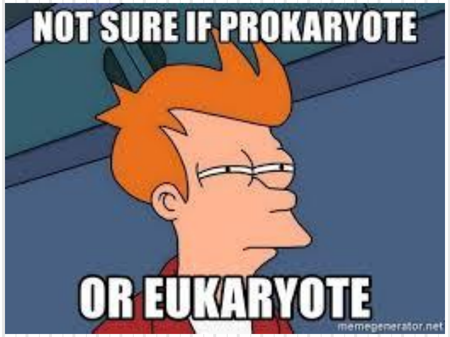
Lecture 16

SLE254 Genetics and Genomics

Concepts of Genetics (12th ed)

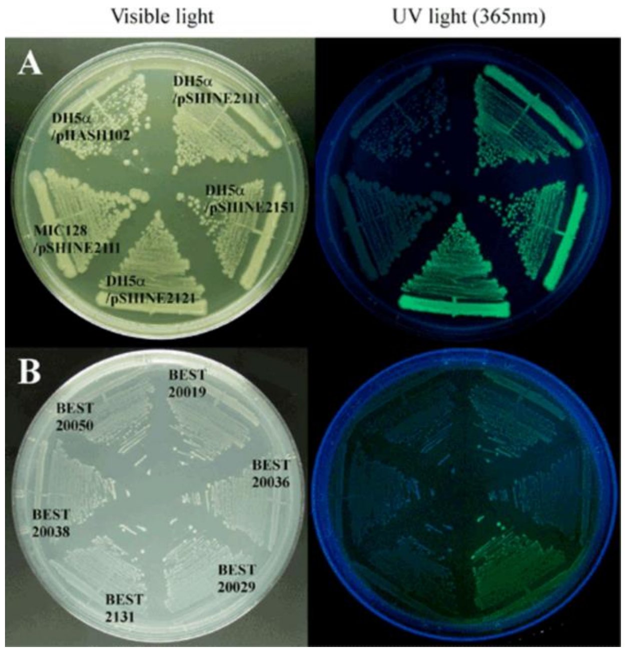
Chapter 16 pages 412-429

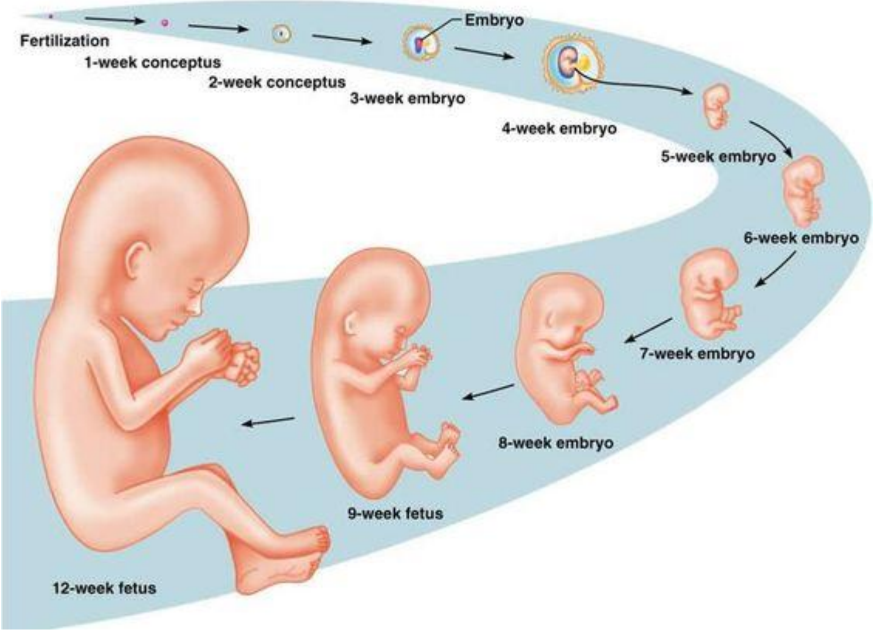




From DNA to gene product: **gene expression is tightly regulated**

- Not all genes are expressed at all times in all situations.





Definitions: positive and negative control

Gene **induction** and **repression** can be negatively or positively controlled:

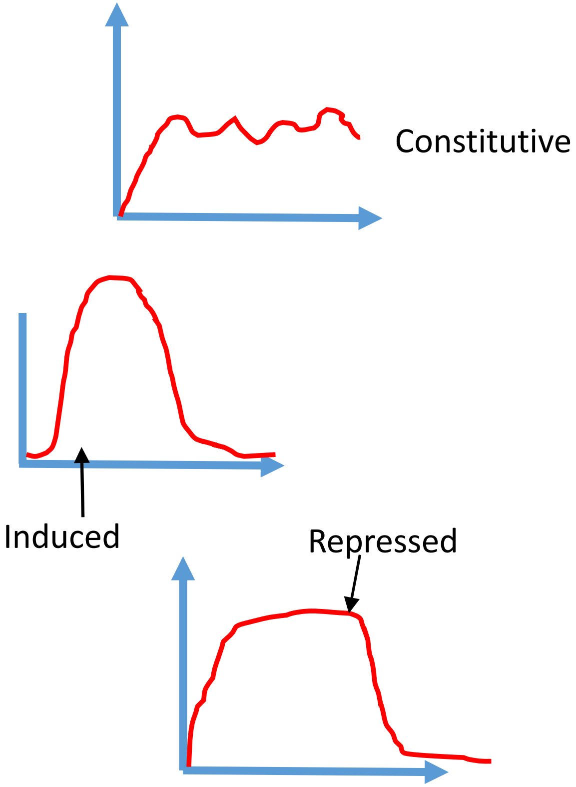
- **Negative control:**
  - When gene expression is actively 'turned off' by a regulatory molecule/complex.

- **Positive control:**
  - Gene expression is actively 'turned on' by a regulatory molecule/complex.

  - *Inducible does not imply positive control, nor does repressible imply negative control*

Inducible/Repressible vs Constitutive expression

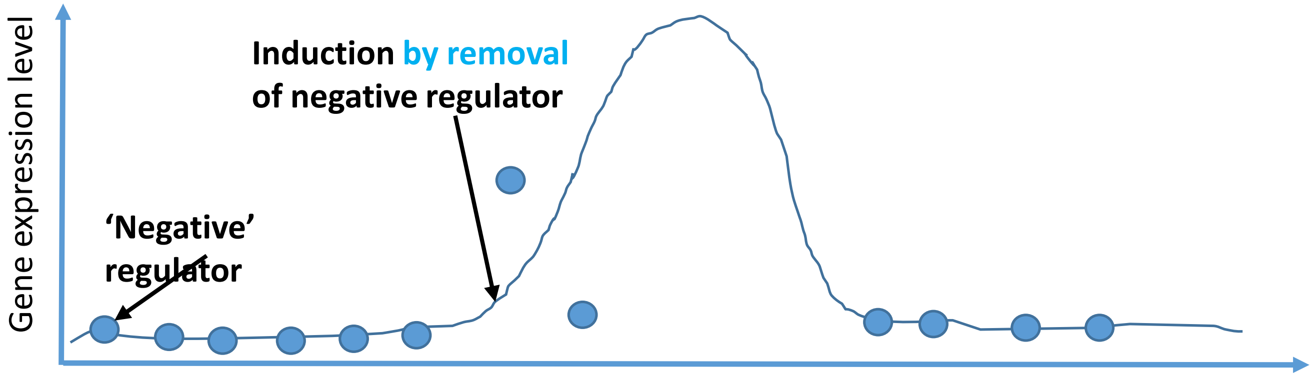
Types of expression	Definition	Type of genes
Constitutive (unregulated)	Genes are always ON	Housekeeping genes
Inducible (regulated)	Genes are only turned on as needed	Structural genes and Enzymes
Repressible (regulated)	Genes are only turned off as needed	Structural genes and Enzymes



Positive and Negative control

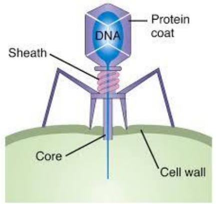
Induction and **repression** can be **negatively** or **positively** controlled:

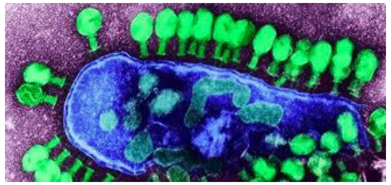
Inducible gene expression regulated under negative control

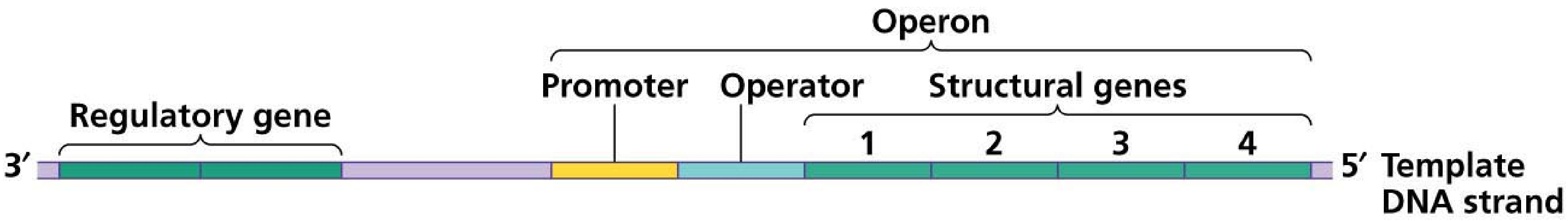


Prokaryote gene expression: Operons

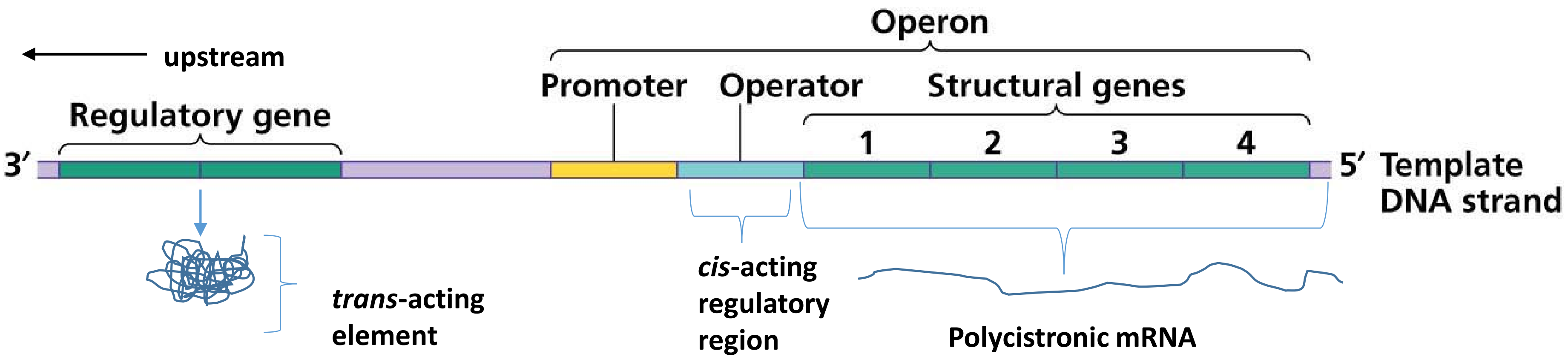
- Operons: are sections of DNA that contain a **cluster of genes under the control of a single promoter.**
- Bacteria organise their genes that code for **similar enzymes and functions in clusters**
- Occur regularly in prokaryotes and only rarely in eukaryotes







Operons: definitions



Promoter: region of DNA to initiate gene transcription

Operator: contain *sequences* that control transcription of the gene cluster: bind activator or repressor molecules

***cis*-acting site:** same strand as gene cluster

Regulatory gene: code for regulatory activator or represser molecules: ***trans-acting elements***

Polycistronic mRNA: one mRNA molecule translated into separate proteins

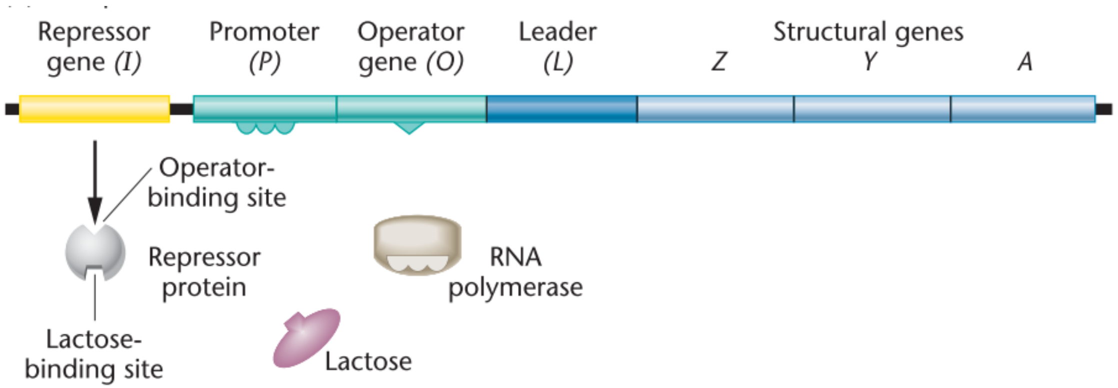
Lac Operon- proof of principal: Lactose metabolism in *E. coli*

- Lac operon: **negative control** of **inducible** gene expression
- **Repressor- operator interaction**
- **No lactose**: repressor gene encodes a molecule that **blocks transcription** of the structural genes

- Blocks the operator, RNA polymerase is inhibited

- **Presence of Lactose:** this sugar binds to the repressor causing a conformational change to the molecule

- Repressor-operator interaction released, promotor free, **structural genes transcribed**



Lac Operon: negative inducible expression

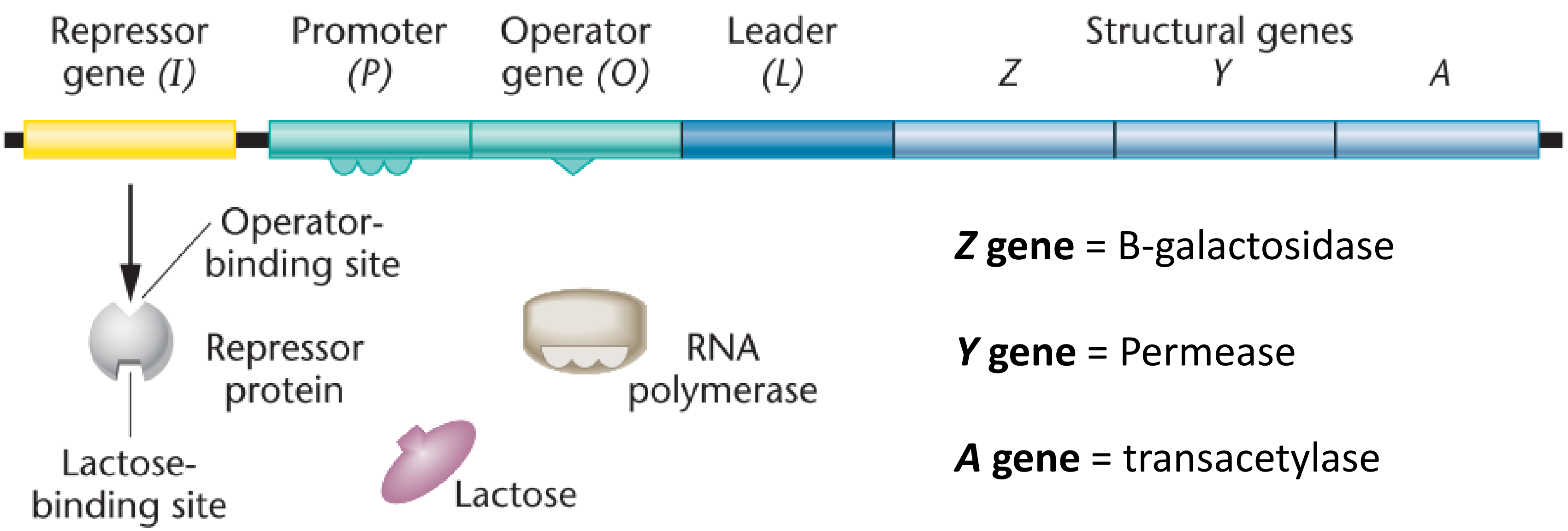


Figure 16.5:a

***Z* gene** = B-galactosidase

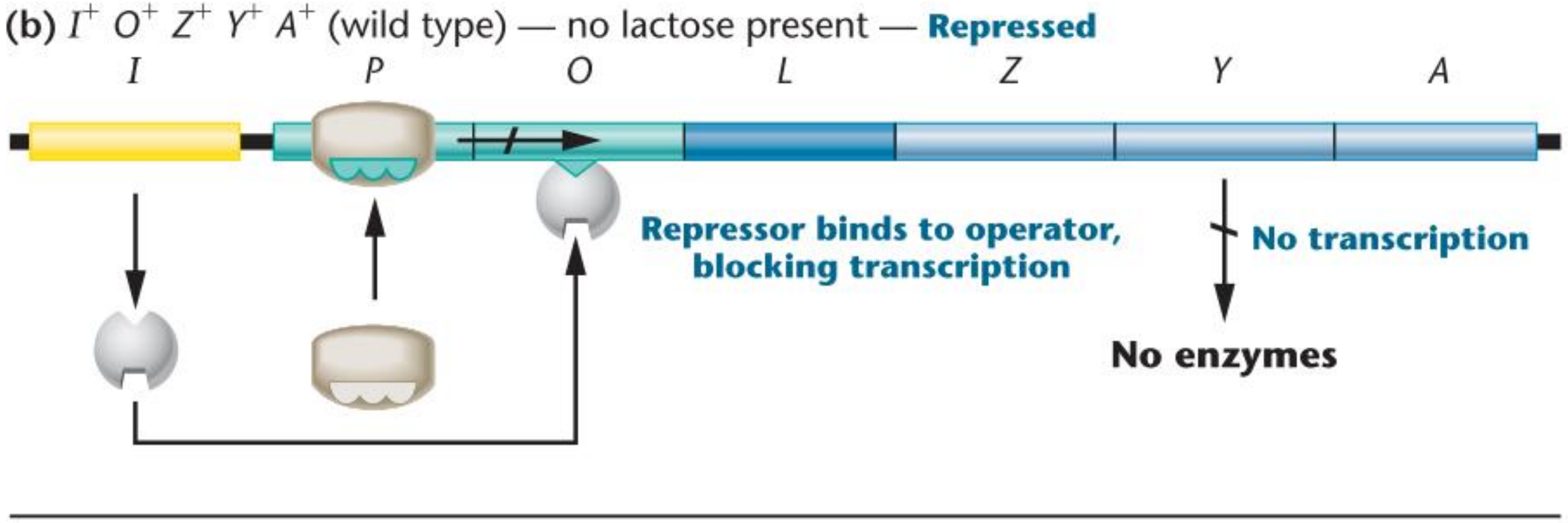
***Y* gene** = Permease

***A* gene** = transacetylase

***I* gene** = repressor protein acts as a trans-acting regulator.

***O* gene** = non-coding region containing cis-acting binding sites.

Negatively controlled gene that is *switched off* by a repressor gene



Absence of lactose: the repressor molecule binds to the operator which inhibits RNA polymerase from attaching and polymerising the transcript

Figure 16.5:b

Negatively controlled gene that is induced by the environment and the removal of a repressor = *negative inducible operon*

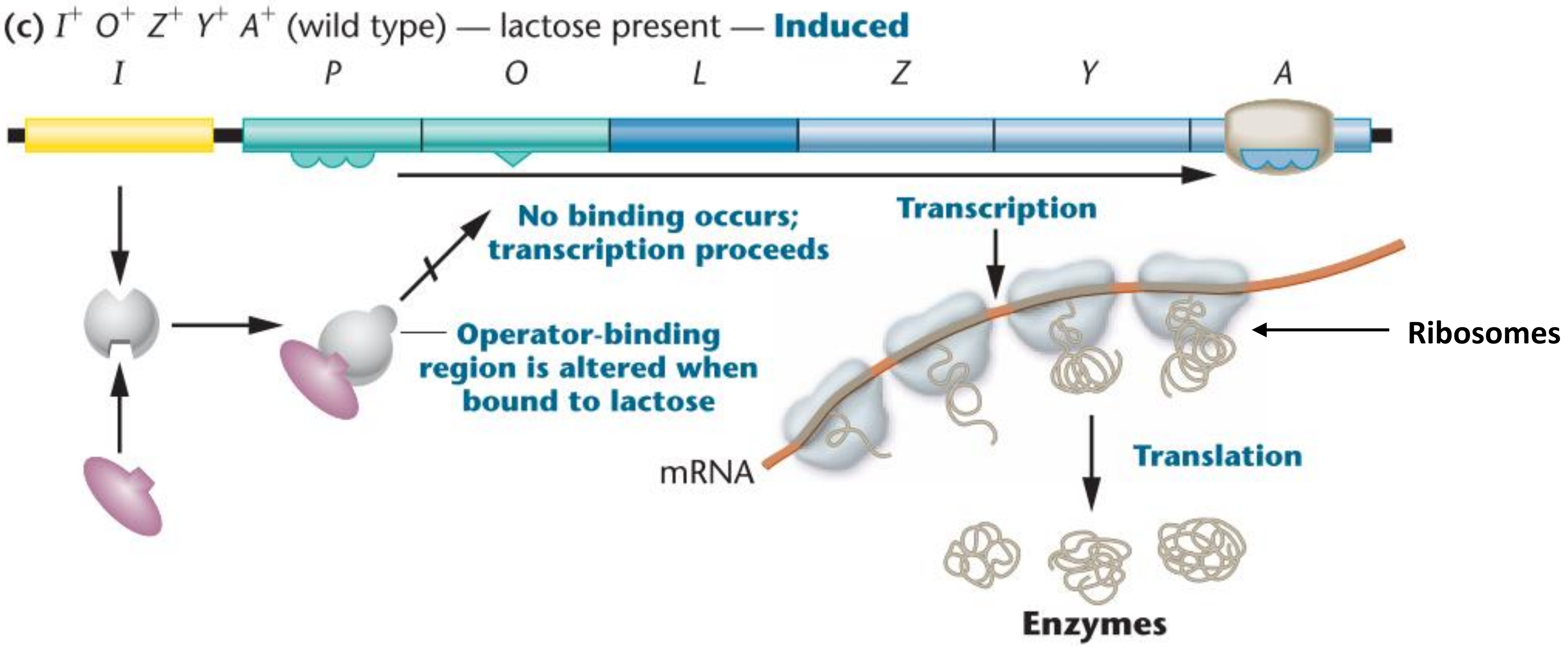
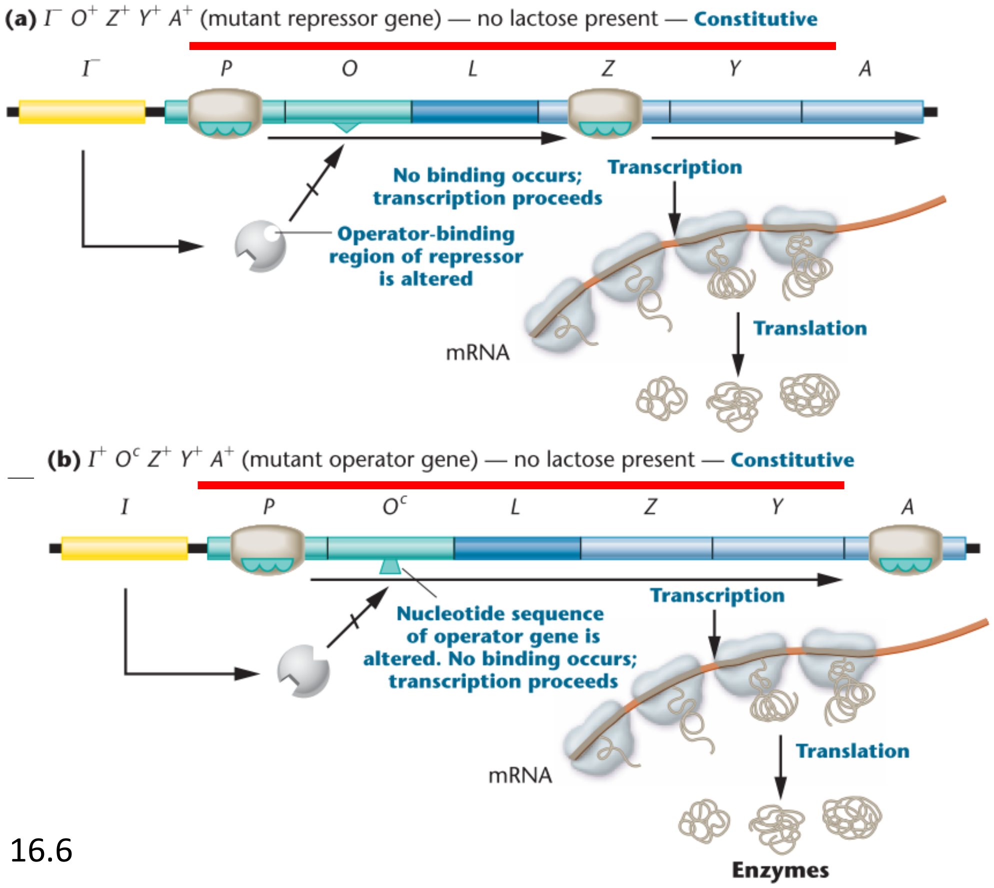


Figure 16.5:c

1. Loss of function **mutations** in these *trans*- or *cis*-sites: **always expressed**

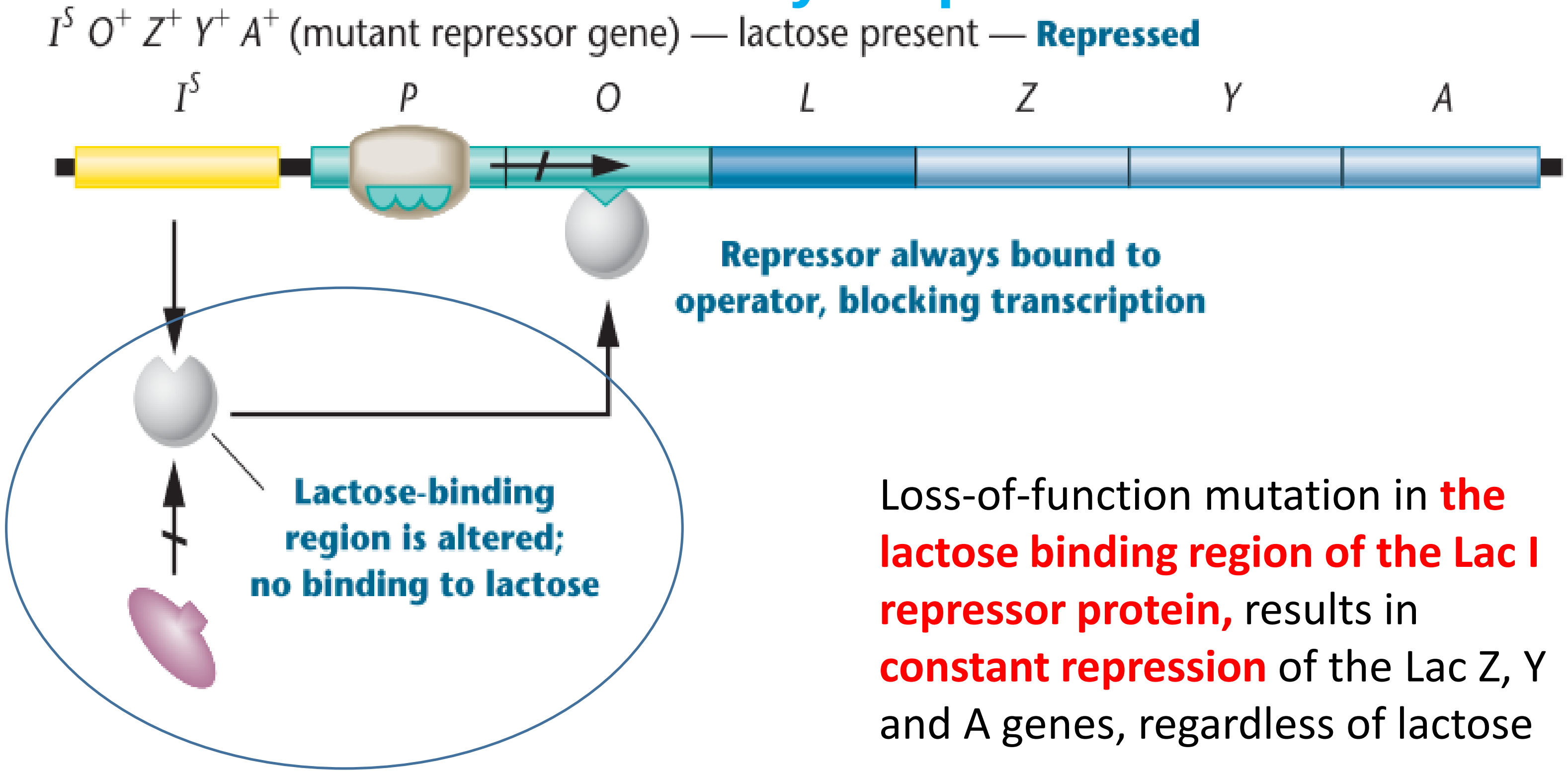


Mutant repressor gene

Mutant operator gene

Figure 16.6

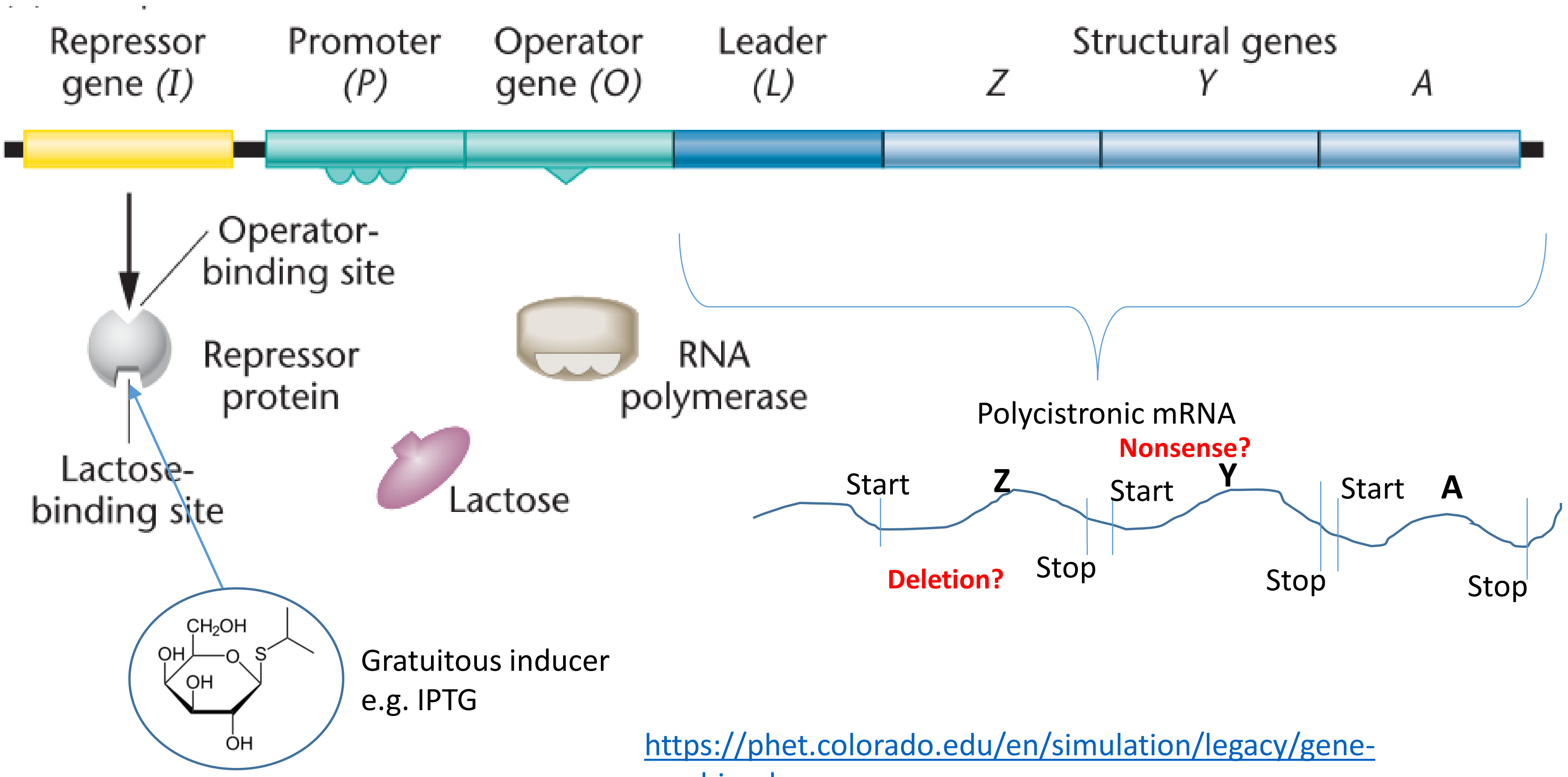
2. Loss of function mutations in these *trans*- or *cis*-sites: **always repressed**



Loss-of-function mutation in **the lactose binding region of the Lac I repressor protein,** results in **constant repression** of the Lac Z, Y and A genes, regardless of lactose presence.

Figure 16.7

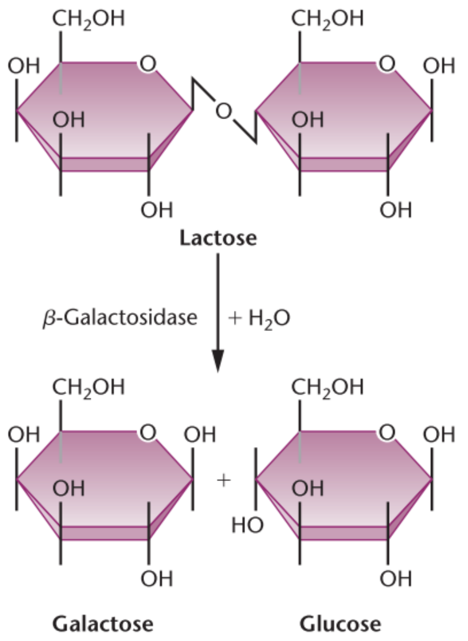
Lac Operon



https://phet.colorado.edu/en/simulation/legacy/gene-machine-lac-operon

Function of the Lac operon

Lactose Disaccharide



Monosaccharides

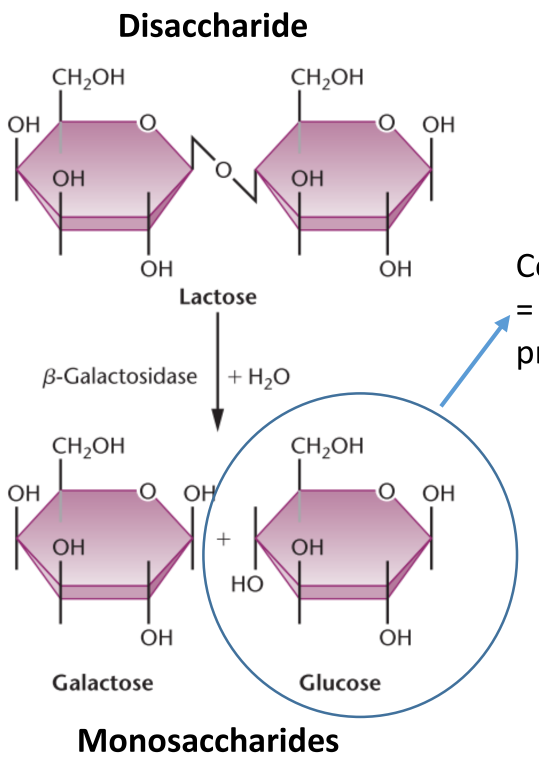
Figure 16.2

Lac Z gene encodes the enzyme **B-galactosidase**, cleaves the disaccharide lactose into its monosaccharide components glucose and galactose.

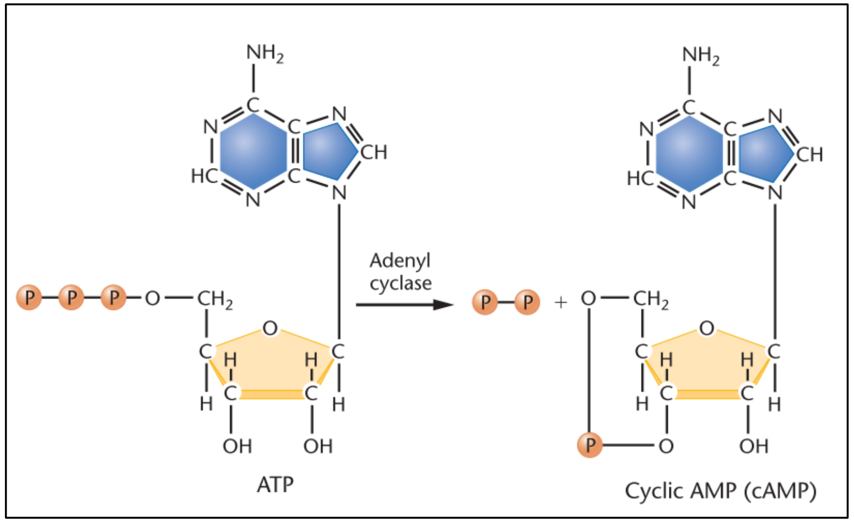
Lac Y gene encodes for the enzyme **permease**, which allows lactose to enter bacterial cells.

Lac A: involved in the removal of waste by-products from the digestion of lactose.

Selective gene regulation: *E. coli* 😍 glucose over lactose



Cellular Respiration
= Energy
production

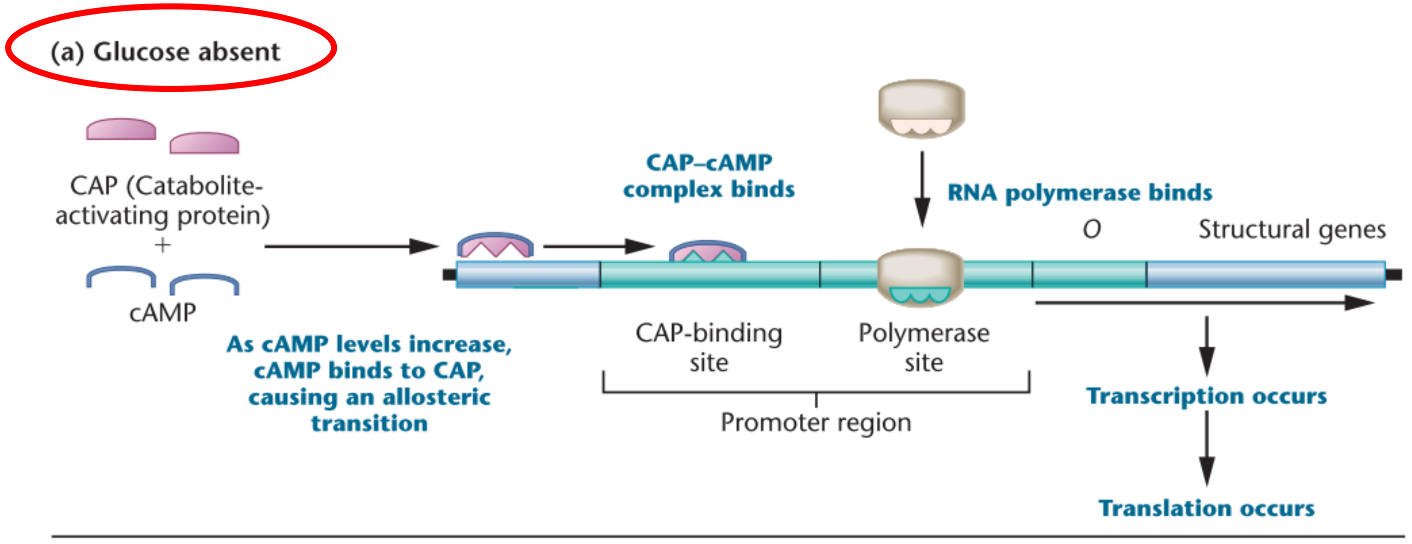


Decrease in glucose stimulates cAMP production via Adenyl cyclase

cAMP is a derivative molecule of ATP used for signalling

Positive control over the Lac operon: Catabolite-Activating Protein CAP

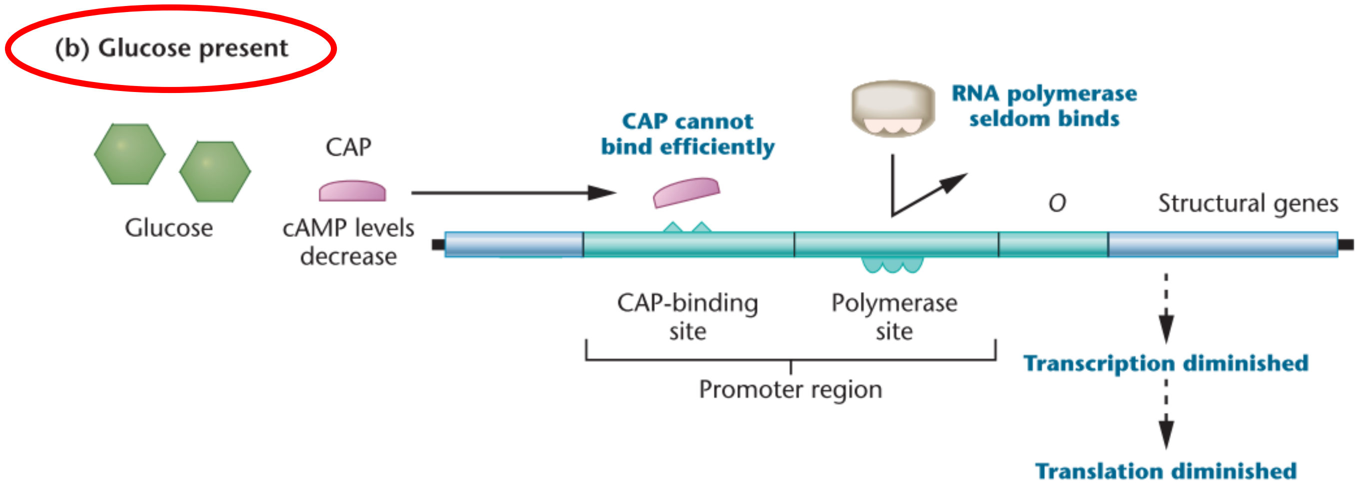
Regulation of the lac operon is more complex and depends on the preferred sugar source glucose



- Under lactose inducible conditions RNApol binding is not efficient unless **CAP is present**
- CAP exerts **positive control** by binding to the promotor and facilitating transcription
- CAP binding requires the molecule **cAMP** (cyclic adenosine monophosphate)
- **Low levels of glucose = high levels of cAMP**

Figure 16.8:a

Positive control over the Lac operon: Catabolite-Activating Protein CAP



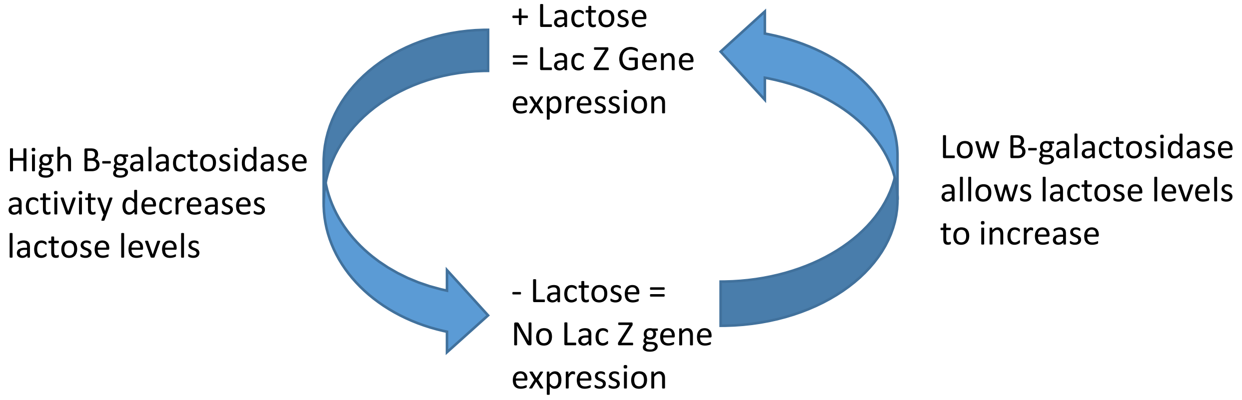
- Presence of glucose: cAMP levels decrease
- The presence of glucose inhibits the enzyme adenyl cyclase decreasing cAMP
- CAP cannot form the **cAMP-CAP complex** for positive control of the lac operon
- CAP can't bind to promoter, Lac operon down-regulated

Figure 16.8:b

Self-regulation/limiting gene expression

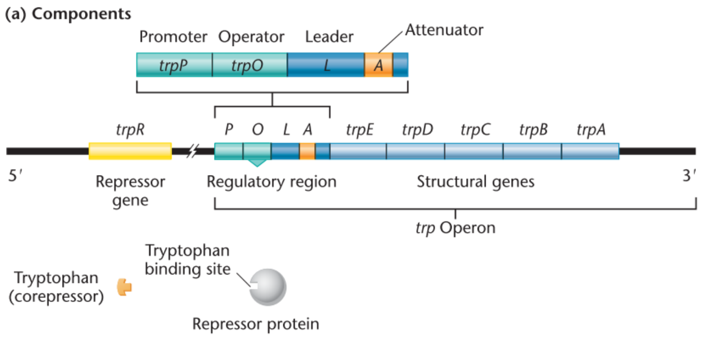
- Many genes in both prokaryotes and eukaryotes are **self-limiting**
  - This is when the gene products that they produce play a role in inactivating transcription of the genes that encode them.

- This can sometimes be referred to as negative-feedback or be part of a negative-feedback loop.



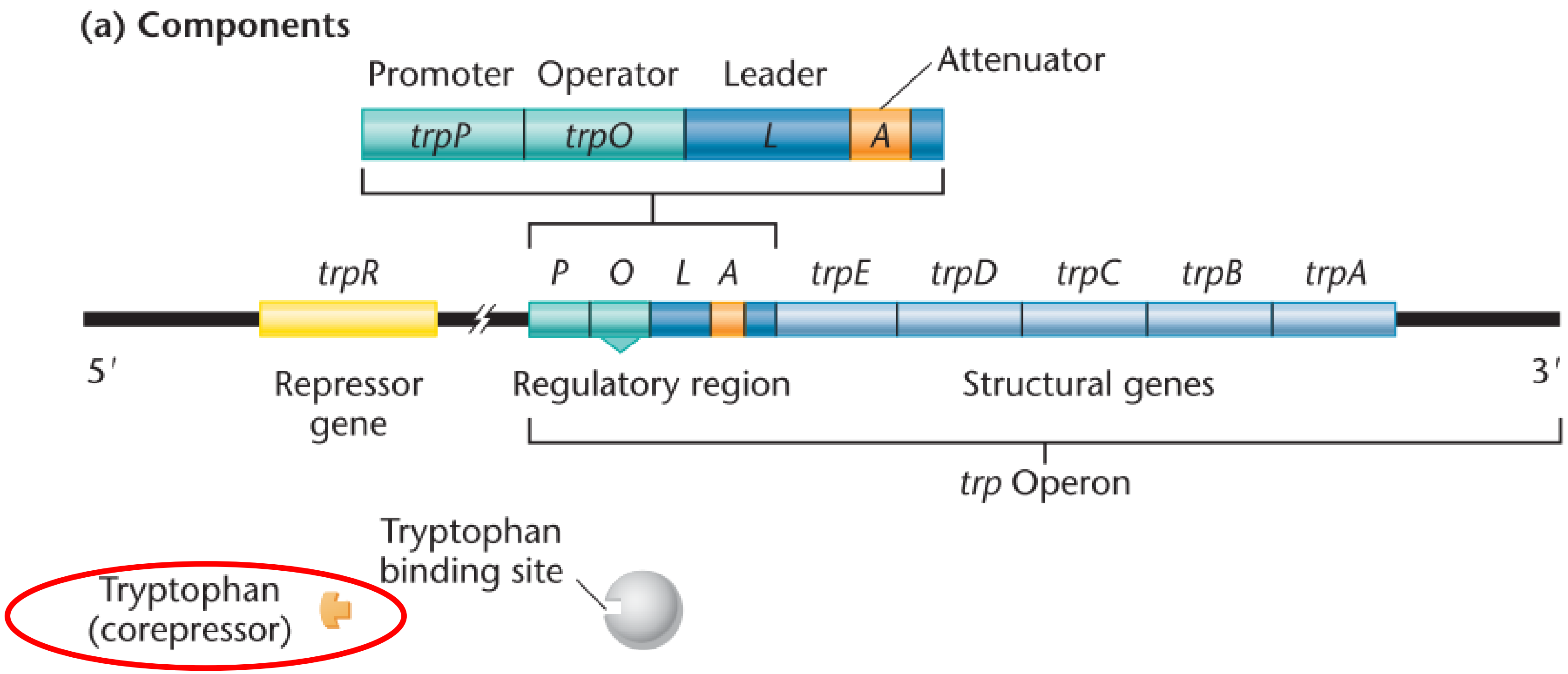
Self limiting **repressible operon**: tryptophan (*trp*) operon

- *E. coli* can produce enzymes necessary for the **biosynthesis of amino acids**
- Five contiguous genes on the *E. coli* chromosome code for tryptophan synthesis, the *trp* operon
- In the **presence of *tryptophan*** all enzymes are **repressed**
- The repressor molecule alone cannot bind to the operator
- The repressor molecule contains a binding site for tryptophan, changing conformation and binding to the operator
- Tryptophan is a **co-repressor** negatively regulating it's own production



negative repressible operons

Self limiting operon: tryptophan (*trp*) operon



negative repressible operons

Figure 16.2:a

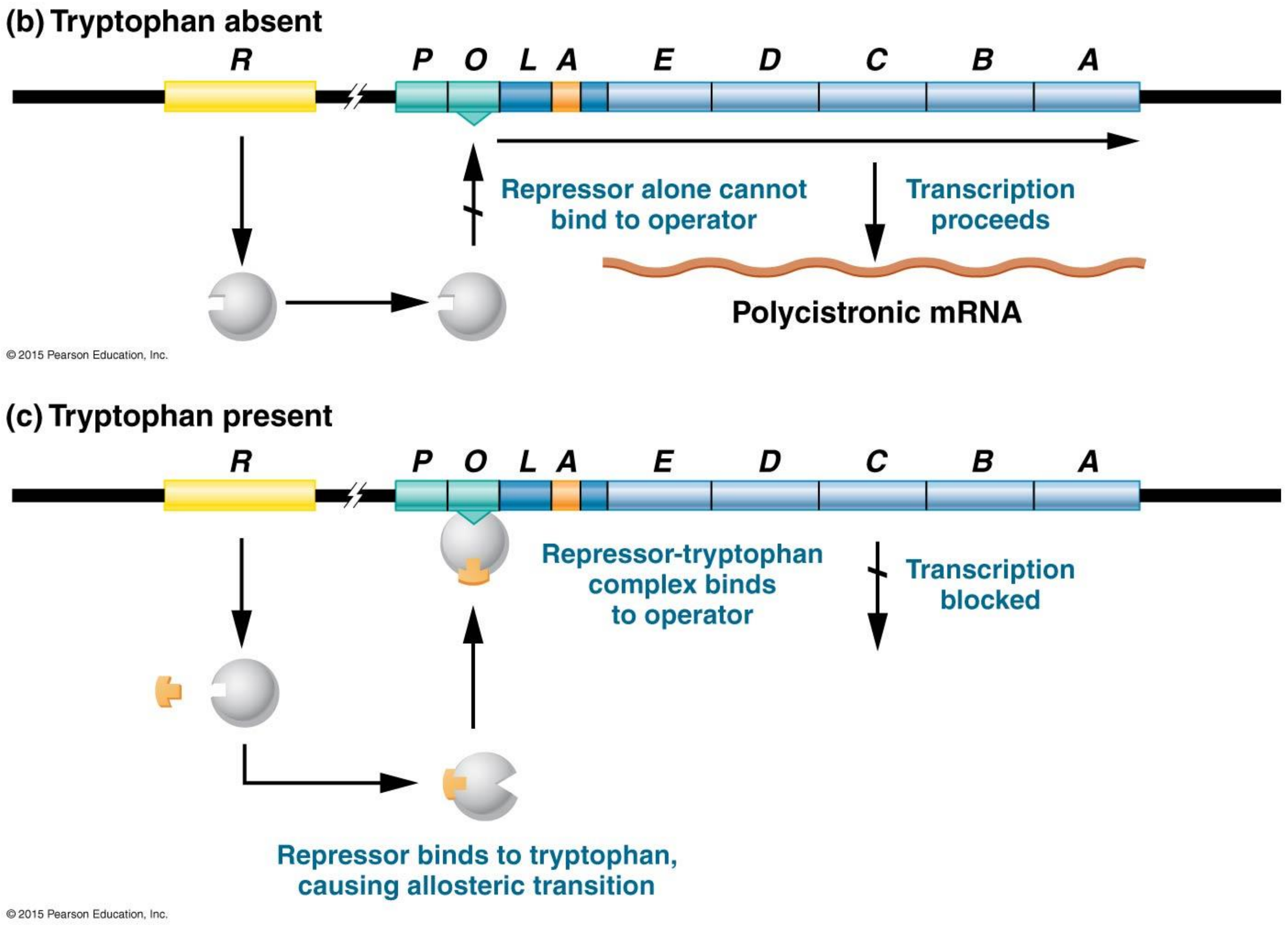

(b) Tryptophan absent
R
P O L A E D C B A
Repressor alone cannot bind to operator
Transcription proceeds
Polycistronic mRNA
© 2015 Pearson Education, Inc.
(c) Tryptophan present
R
P O L A E D C B A
Repressor-tryptophan complex binds to operator
Transcription blocked
Repressor binds to tryptophan, causing allosteric transition
© 2015 Pearson Education, Inc.

Figure 16.2

Summary: inducible *vs* repressible operon

1. In the **absence of lactose** the *lac* operon is **repressed** by a repressor molecule

2. The **presence of lactose** forms a complex with the repressor molecule which stops it binding to the operator, **transcription proceeds**

3. In the **absence of tryptophan** the *trp* operon is **activated** as the repressor cannot bind alone

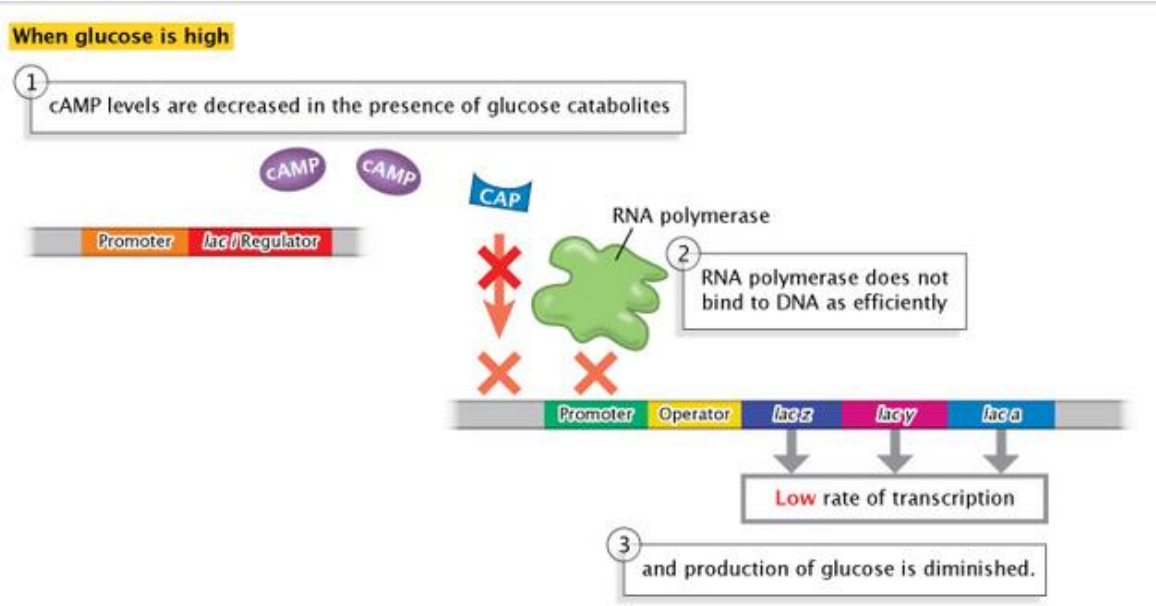
4. The **presence of tryptophan** forms a complex with the repressor molecule that binds to the operator, **transcription is diminished**

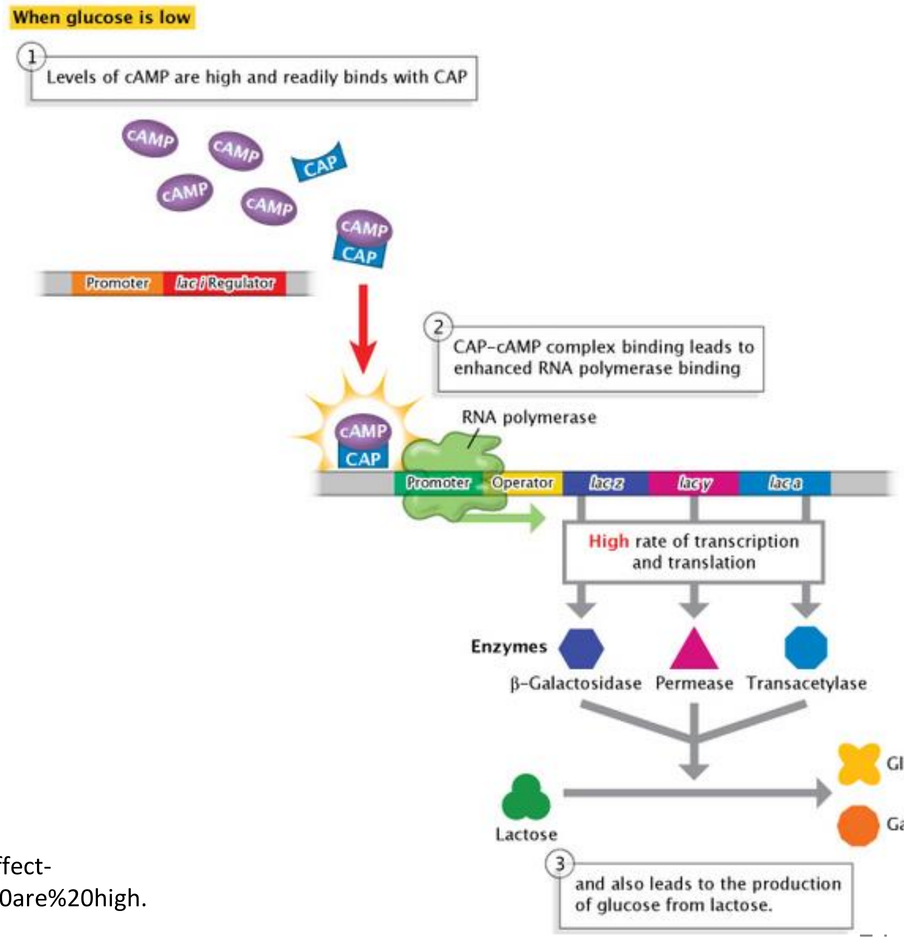
Recap: Greater levels of complexity negative and positive control of the lac operon: lactose metabolism is regulated by *glucose catabolites:*

Selective gene regulation: *E. coli* ☺ glucose over lactose

If you grow this bacteria in a medium **with both glucose and lactose**, is that it selectively turns off lactose metabolism and uses straight glucose for its cellular respiration. The products of breaking down of glucose prevent the signalling molecule cAMP.

When glucose levels start getting **low,** the lac operon is **activated positively by high levels of cAMP** and lactose is back!



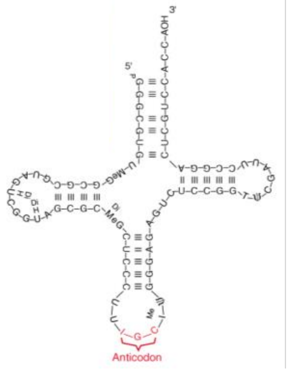


https://www.nature.com/scitable/topicpage/positive-transcription-control-the-glucose-effect-1009/#:~:text=The%20lac%20operon%20is%20therefore,signal%20molecule%20cAMP%20are%20high.

Fine tuning gene expression regulation by RNA secondary structure

- The lac repressor and CAP proteins are examples of **DNA-binding regulatory proteins** that interact with the operator regions to regulate gene expression
- Prokaryote gene regulation also occurs through interactions of regulatory molecules with **specific regions of the mRNA**
- This occurs **after transcription initiation**
- Causes alterations to the **secondary structure of mRNA**

- Leads to termination of transcription or repressed translation



Gene expression regulation by RNA secondary structure- definitions

Leader sequence: transcription proceeds along the leader sequence prior to the first structural gene- **5’UTR**

Attenuation: leader sequence contains an **attenuator sequence**, which can cause transcription or translation to stop and an attenuated (non-functional) mRNA or protein is formed.

Riboswitches: leader sequence contains a **binding site**, which if bound by a ligand (simple or complex binding molecule) can cause transcription to terminate.

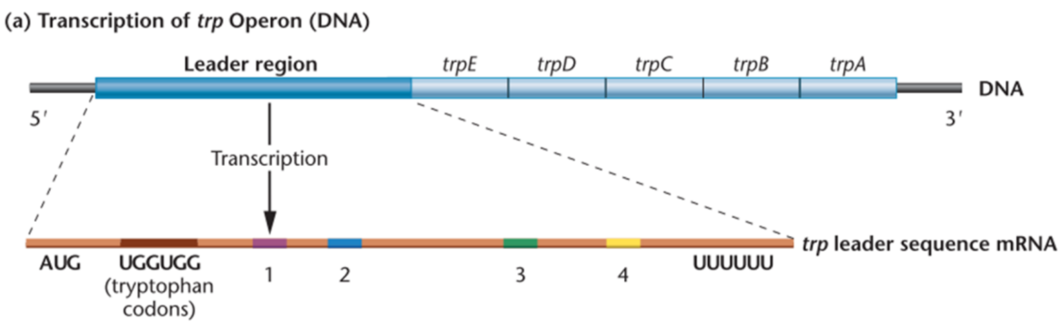
Tryptophan attenuation

***Is a way to control gene expression using the trp RNA as it is being transcribed.**

***Note that transcription and translation occurs at the same time**

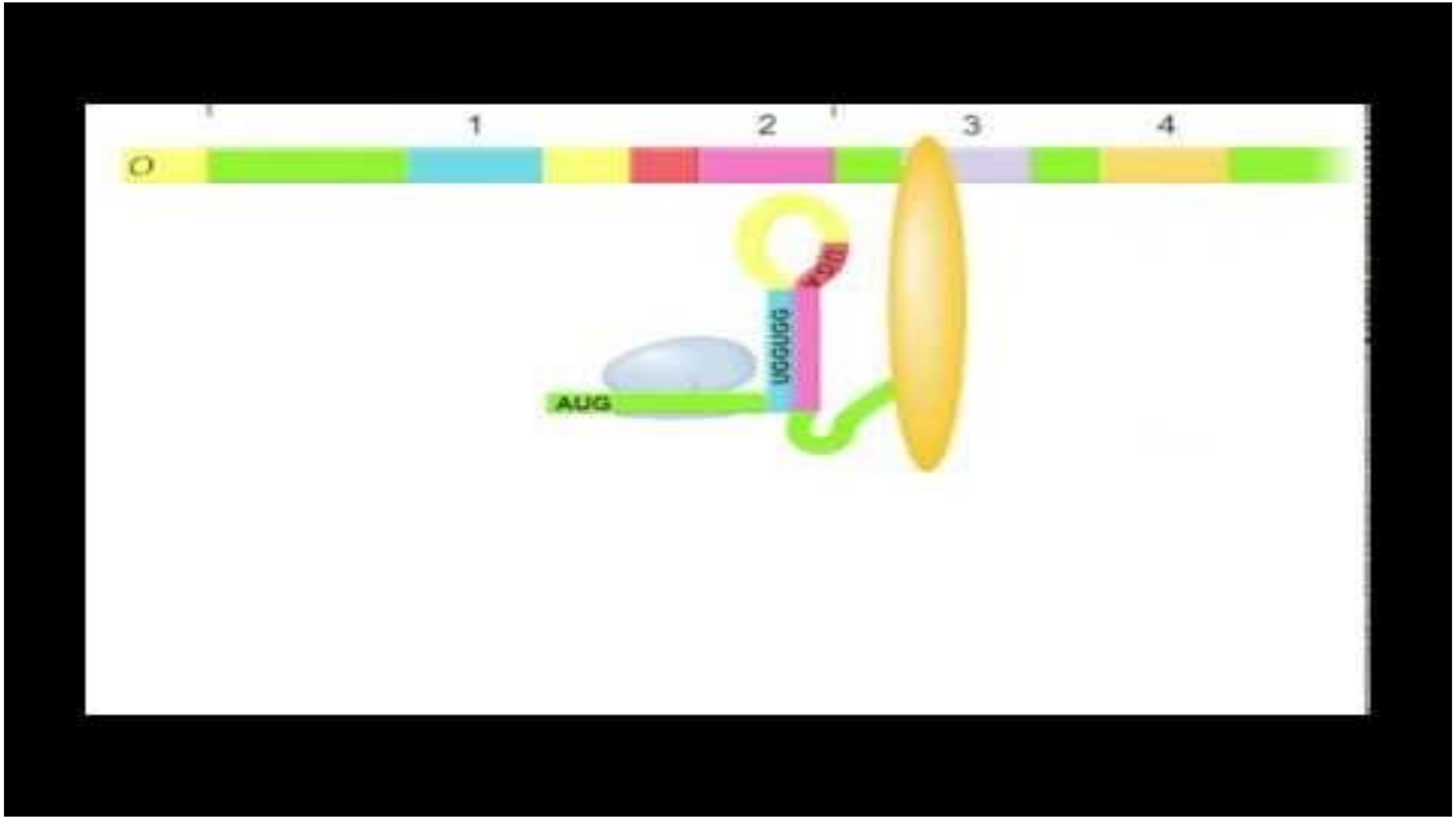
***Even when trp is present it transcribes but terminates- why?**

***The leader sequence!! Codes for regulatory RNA that forms regulatory loops**



This is the leader sequence blown up

https://youtu.be/XspyeURhrcQ



Riboswitches: controlling gene expression by RNAs encoded by the leader sequence

- Riboswitches are RNAs encoded by the leader sequence again, that can control transcription via anti-terminator or terminator structure.
- This time however it involves ligand binding