# E. coli changes gene expression to utilize different nutrient sources





### Lac Operon (Negative Regulation)



# The lac repressor dissociates from the operator sequence upon IPTG (inducer) binding



### **Cis versus Trans**

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Merodiploid with one wild-type gene and one:



# Cis versus Trans Dominant versus Recessive

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Merodiploid with one wild-type gene and one:



# Operator Mutations Reduce Repressor Binding



#### The lac repressor tetramer binds two operators



(Source: Lewis et al., Crystal structure of the lactose operon processor and its complexes with DNA and inducer. Science 271 (1 Mar 1996), f. 6, p. 1251. @ AAAS.)

Fig. 7.12

### The lac operon is also regulated by glucose levels Glucose





Low Glucose => High cAMP => activation of lac operon thru CAP binding site

Figs. 7.16 & 7.13



# cAMP is Needed for Activation



**Catabolite Repression** 

# CAP-cAMP binding creates DNA bending and activates lac operon transcription





#### Figs. 7.17 & 7.19

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(a) Low tryptophan: no repression



(b) High tryptophan: repression



# Trp Operon Anabolic Enzyme)

#### epressor

#### The trp operon attenuator

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Low tryptophan: transcription of trp structural genes



#### Alternative structures of the trp operon attenuator RNA



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# How a Terminator Loop is Formed



#### Negative regulation: Substrate induction



#### (a) Lactose absent, repressor active, operon off

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(b) Lactose present, repressor inactive, operon on

#### Positive regulation of the lac operon



#### Positive regulation of the lac operon



#### <u>No food</u>

• Glucose?

• Low: cAMP high, CRP attaches

• Lactose?

• Low: Repressor attached

Operon is ready to be enhanced but is shu

#### Milk

• Glucose?

• Low: cAMP high, CRP attaches

• Lactose?

High: Repressor removed

Operon is on and enhanced

#### <u>Milkshake</u>

• Glucose?

• High: cAMP low, CRP off

Low: Repressor removed

• Lactose?

### Operon is on but un-enhanced

#### **Powerade**

• Glucose?

• High: cAMP low, CRP off

• Low: Repressor attached

• Lactose?

# Operon is off and un-enhanced

- No food: ready to be enhanced but off
- Milk: enhanced transcription
- Milkshake: un-enhanced transcription
- Powerade: off and un-enhanced

#### Negative regulation: end-product repression



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# LACTOSE OPERON

#### Structural genes

- *lac z, lac y,* & *lac a*
- Promoter
- Polycistronic mRNA
- Regulatory gene
  - Repressor
- Operator
- Operon
- Inducer lactose



# LACTOSE OPERON

#### • Inducer -- lactose

- Absence
  - Active repressor
  - No expression
- Presence
  - Inactivation of repressor
  - Expression
- Negative control



### CATABOLITE REPRESSION (GLUCOSE EFFECT)

• Definition: Control of an operon by glucose

# • Catabolic operons



# MECHANISM OF CATABOLITE REPRESSION

- c-AMP
- CAP (CRP) protein
- CAP-cAMP complex
  - Promoter activation
- Positive control



# MECHANISM OF CATABOLITE REPRESSION

- Glucose $\uparrow$ :cAMP $\downarrow$
- CAP (CRP) protein
- No CAP-cAMP complex
  - No Promoter activation



### REPRESSIBLE GENES - OPERON MODEL

- Definition: Genes whose expression is turned off by the presence of some substance (co-repressor)
  - Tryptophan represses the *trp* genes
- Biosynthetic pathways
  - Co-repressor is typically the end product of the pathway

# **TRYPTOPHAN OPERON**

#### Structural genes

- trp E, trpD, trpC trpB & trpA
- Common promoter
- Regulatory Gene
  - Apo-Repressor
    - Inactive
- Operator
- Leader
- Operon
- Co-repressor
  - Tryptophan



# **TRYPTOPHAN OPERON**

- Co-repressor -tryptophan
  - Absence of tryptophan
    - Gene expression
  - Presence of tryptophan
  - Activates repressor
    Negative control No gene expression



### ATTENUATION

• Definition: Premature termination of transcription

### • Leader region

- Leader transcript
- Translation start
- Translation stop
- Tryptophan codons



# ATTENUATION

- Mutually exclusive mRNA secondary structure
  - region 1 : region 2
  - region 2 : region 3
  - region 3 : region 4 Coupled transcription and
- Coupled transcription and translation



# ATTENUATION



# REGULATION OF ENZYME ACTIVITY

- Feed back inhibition
- Epigenetic modification
  - Post translational modifications
    - Phosphorylation/dephosphorylation
    - Adenylation/deadenylation