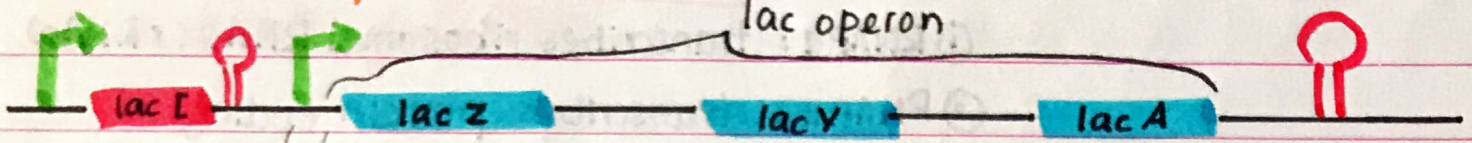
→ The Lac Operon (allows E.coli to utilize lactose as a carbon source)



lac repressor ↓

lacP / lacO. operator

β-galactosidase breaks lactose. ↓ glucose + galactose.

β-galactoside permease brings sugar inside cell

β-galactoside transacetylase ↳ modifies lactose by transfering acetyl group

protein lacI : transcription factor that regulates transcription

β-galactosidase

↓

no lactose : lac repressor on ✓ → binds to operator → RNAP does not recognize lacP →

lacZ, lacY, lacA genes cannot be transcribed

↓

lactose → allolactose → binds to lacI

↓

conformational change cannot bind to lacO ←

RNAP binds to the promoter (lacP)

↓

m-RNA (polycistronic) transcribed (lacZ, lacY & lac A genes) → m-RNA translated in to proteins → lactose transported into cell & metabolized

→ nucleotide sequence for operator site shows nearly perfect inverted repeat ⇒ DNA in this region has a ~twofold axis of symmetry.

↓

Symmetric alignment between operator & repressor.

→ basal expression: lac repressor binds on and off and often allows small amounts of RNA to be made

* lac operon OFF Glucose - High Lactose - Low

↓

lacI bound to operator → DNA loop formed when protein or complex of proteins simultaneously binds to two different sites of DNA

↓

transcriptional repression: bivalent transcrip. factor binds to 2 binding sites & blocks access to RNA polymerase

- low lactose permease
- low β-galactosidase

* catabolite activator protein → activator binding site (CAP) → facilitates transcription.

↓

can bind to CAP bs only if cAMP bound to it ⇒ high glucose = low cAMP

* lac operon ON Glucose - Low Lactose - High allolactose (conformational change) = inducer ✓ + lacI

- high lactose permease + β-galactosidase

↳ DNA loop can also be responsible for transcriptional activation

transcript. factors bind away from site of fixation of RNA polymerase & help recruitment of RNAP and formation of open complex.

low glucose = high cAMP → binds to CAP → binds to CAP binding site

↓

transcription ✓

* this happens ONLY if lactose also present to bind ← to repressor & unblock.

→ Modified inducer

Jacob & Monod wanted to study regulation of lac operon

↓

lactose : degraded by lacz → [lactose] not able to monitor → IPTG : gratuitous inducer

↓

binds lacI → change in conformation BUT non-degradable

inducer (IPTG) always present ← system always ON ←

X-Gal → can be recognized by β-galactosidase & cleave into galactose + colored molecule.

galactosidace present? blue colonies

* at high [IPTG] = system on even if glucose present
  - → IPTG + X-Gal = blue
  - → IPTG = white, lacz transcribed but not visible
  - → X-Gal + high [glucose] = white bc no lacz - no β-galactoside nothing to cleave.

conjugation

MUTATIONS

- → IPTG + X-Gal = $lacz^-$ white
- → X-Gal = blue $lacI^c$ or $lacO^c$, repressor off or mutated operator does not allow repressor to bind.

CIS REGULATION

- O^c only works if copy of WT z^+ on same chromosome
  - copy not mutated

$I^+ O^c z^+$ ✓

- promoters & operators, TF-binding sites, DNA ...

TRANS REGULATION

- I^c mutation always ON

Repressor can diffuse in cell, not fixed. WT lacI can bind to O^+ in mutated copy

- any enzyme that binds DNA trans-acting