Gene regulation

Genetics 371B Lecture 27 17 Nov. 1999

Why regulate genes?

Control points:

Two modes of control:

Positive control
Gene **OFF** until activator turns it **ON**

Negative control
Gene **ON** until repressor turns it **OFF**

François Jacob
Jacques Monod

lac operon

E. coli – can metabolize lactose (disaccharide, galactose-o-glucose)

BUT… synthesis of ß-gal is regulated —

Carbon source	**ß-gal enzyme activity/cell**
glycerol	
lactose	

⟹ Lactose is an **inducer** of ß-gal production

[An artificial inducer: isopropyl thiogalactoside, **IPTG**]

Mode of action of inducer?

- **Possibility 1:** Inducer activates already-existing ß-Gal

- **Possibility 2:** Inducer triggers fresh synthesis of ß-Gal

Experiment

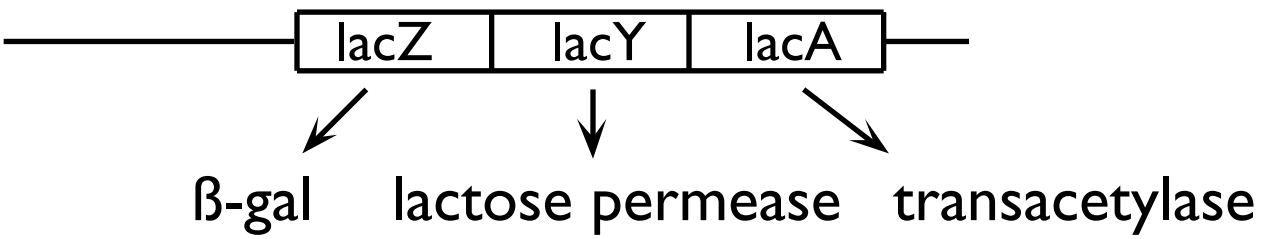
Cells + lactose 

radioactive aminoacids

Control?

From mutational analysis: three linked **structural genes**…

…coordinately regulated



Polar mutations

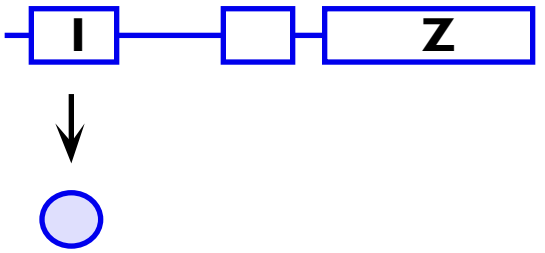
So is transcription of the lac operon under positive control or negative control? How to tell?

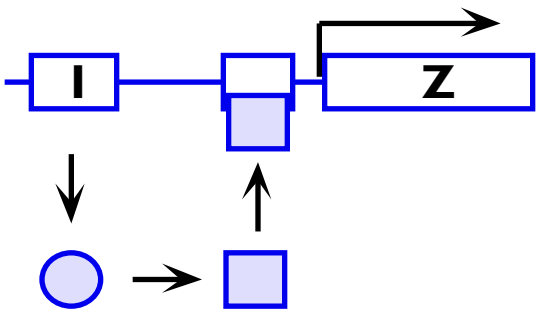
Some mutations: **regulation** affected

strain	ß-gal level in glycerol	lactose
Wildtype		
Mutant 1		
Mutant 2		

lacI map location:

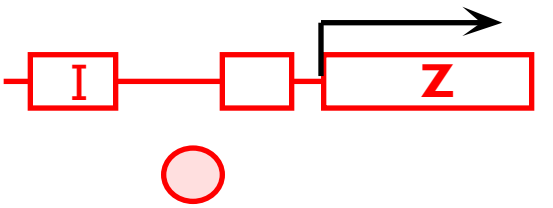
If Positive...



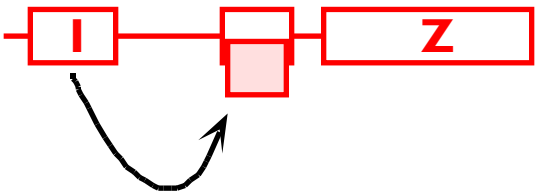


lactose

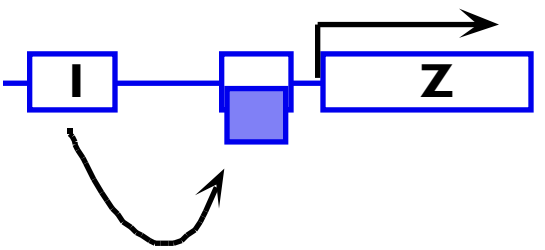
If Negative...

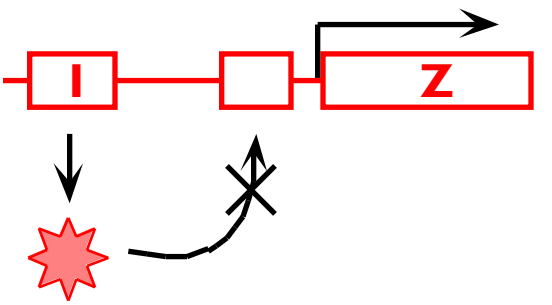


lactose



I^- constitutive mutants

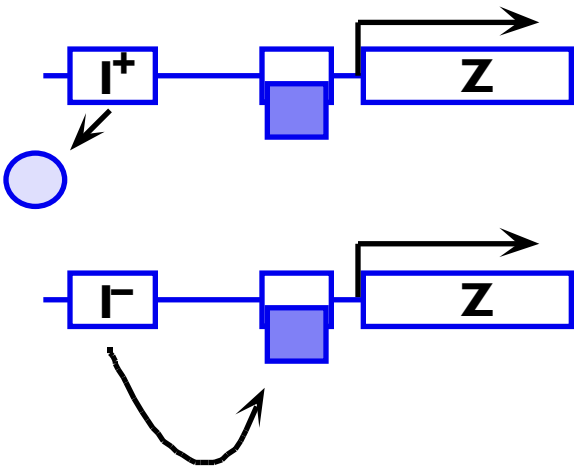


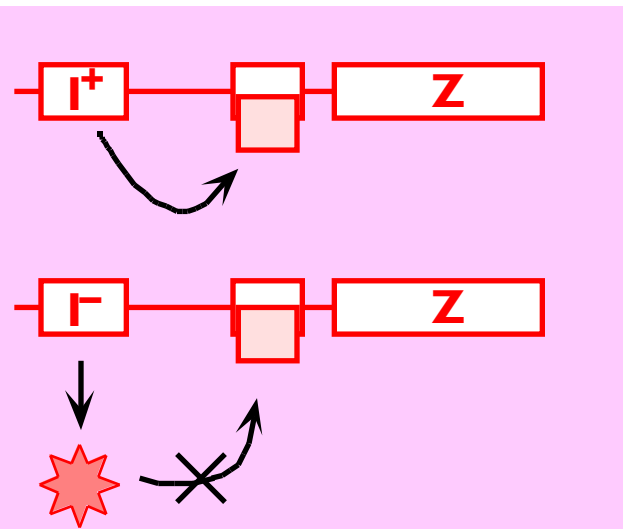


To distinguish between these two possibilities: does the I^- mutation act as a **dominant** or a **recessive** mutation?

Positive

Negative





BUT… these are bacteria

How to get "diploids" to test dominant vs recessive?

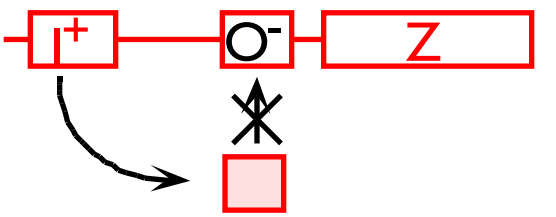
– partial diploid $\begin{bmatrix} I^+ Z^+ \\ I^- Z^+ \end{bmatrix}$

Implicit in the model: repressor acts in **trans**

"Super repressor" $lacI^s$:

Target of the repressor? **Operator** sequence, or lacO

Predicted phenotype of lacO mutation?



Challenge: lacO is small (24 bp) relative to lacI (1080 bp)
How to avoid getting mainly $lacI^-$ mutants?

lacO acts in cis; $lacO^c$ is cis-dominant

– it matters whether lacZ is "attached" to O^+ or O^c

$I^+ O^+ Z^-$
$I^+ O^c Z^+$

$I^+ O^c Z^-$
$I^+ O^+ Z^+$