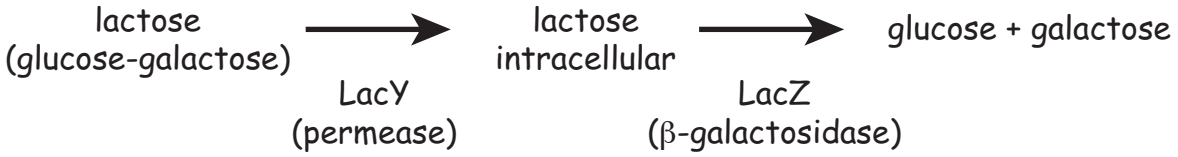
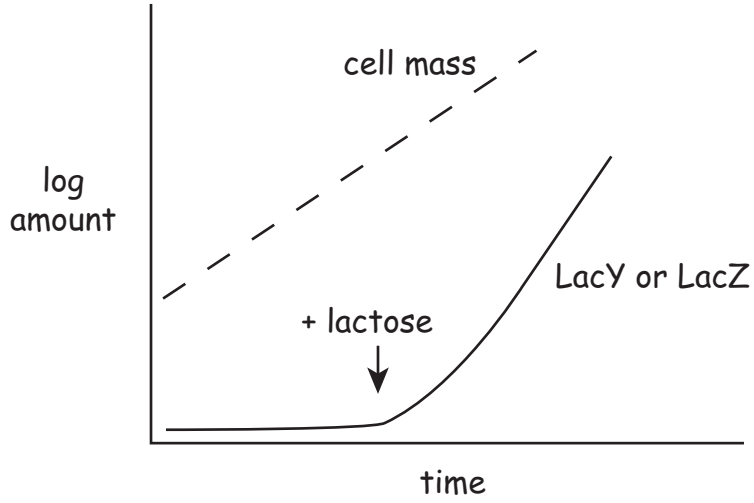
Lecture 16

Gene Regulation

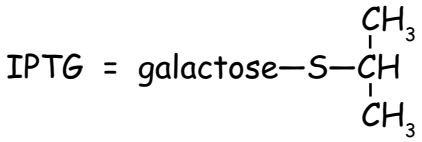
We are now going to look at ways that genetics can be used to study gene regulation. The issue is how cells adjust the expression of genes in response to different environmental conditions. The principles of gene regulation were first worked out by Jacob and Monod studying the *E. coli* genes required for cells to use the sugar lactose as a nutrient.





The logic of the **Lac** operon is that the proteins required to use lactose are only made when their substrate (lactose) is available. This prevents wasteful expression of enzymes when their substrates are notavailable.

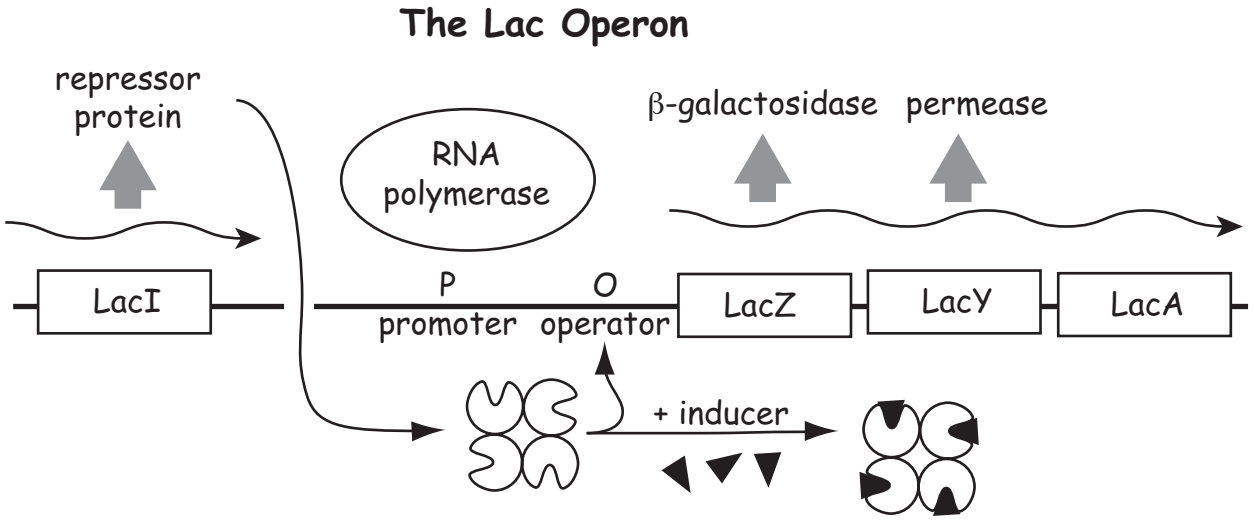
At first, scientists noted that lactose is both an inducer and substrate for the enzymes of the **Lac** operon and they therefore concluded that lactose was somehow acting as a template for the formation of the enzyme. Then compounds were discovered that could act as inducers but were not themselves substrates for the **Lac** enzymes. The classic example of such a "gratuitous inducer" is **IPTG**, which is an effective inducer of **LacZ** expression but isn't hydrolyzed by ß-galactosidase.



The existence of compounds such as IPTG shows that recognition of the inducer is a separate molecular event from lactose breakdown.

The next major finding was the discovery of **LacI⁻** mutants. **LacI⁻** mutants are constitutive, meaning that they always express β-galactosidase at high levels regardless of whether there is an inducer present or not. **LacI⁻** mutants have apparently lost a component of the machinery the cell uses to turn off β-galactosidase expression.

The regulatory system turns out to be quite simple and by isolation of mutants and simple genetic tests Jacob and Monod were able to figure out the following scheme:



The idea is that the inducer has a net positive effect on expression because the inducer is a negative regulator of the repressor, which is itself a negative regulator of the gene for β-galactosidase.

We will now consider how regulatory mutants can be analyzed genetically. We will use as examples different mutations in the **Lac** system but the genetic tests are very general and can be applied to most regulatory systems.

Dominance test

	β-galactosidase -IPTG	β-galactosidase +IPTG	Interpretation
$I^+ Z^+$	-	+	
$I^- Z^+$	+	+	**I^-** is constitutive
$I^- Z^+ / F' I^+ Z^+$	-	+	**I^-** is recessive
$I^+ Z^-$	-	-	**Z^-** is uninducible
$I^+ Z^- / F' I^+ Z^+$	-	+	**Z^-** is recessive
$I^+ Z^- / F' I^- Z^+$	-	+	**I^-** and **Z^-** mutations complement each other i.e. the mutations are in different genes.

A second type of constitutive mutant inactivates the operator site and is known as a **LacO^c** mutation. **LacO^c** mutations are dominant as revealed in tests of the appropriate merodiploids:

	-IPTG	+IPTG	Interpretation
O^c Z$^+$	+	+	**O^c** is constitutive
O^c Z$^+$ / F' O$^+$ Z$^+$	+	+	**O^c** is dominant

You might think that on the basis of a dominance test we could tell whether we have a **LacO^c** or a **LacI$^-$** mutation. However, life is not so simple, because it is possible to find **LacI$^-$** mutations that are dominant. Such mutations are known as **LacI^{-d}**. They are dominant because the repressor protein is a tetramer and **LacI^{-d}** mutant subunits can combine with normal subunits and interfere with their function.

	-IPTG	+IPTG	Interpretation
I^{-d} Z$^+$	+	+	**I^{-d}** is constitutive
I^{-d} Z$^+$ / F' I$^+$ Z$^+$	+	+	**I^{-d}** is dominant

We will now consider a new genetic test that will let us distinguish **LacO^c** (operator constitutive) from **LacI^{-d}** (dominant repressor negative) mutations.

Cis/trans test

	-IPTG	+IPTG	Interpretation
I$^+$ O$^+$ Z$^+$	-	+	
I^{-d} Z$^+$ / F' I$^+$ Z$^-$ (cis)	+	+	**I^{-d}** is dominant in cis or in trans with **Z$^+$**; Therefore we say it is "trans-acting".
I^{-d} Z$^-$ / F' I$^+$ Z$^+$ (trans)	+	+	
O^c Z$^+$ / F' O$^+$ Z$^-$ (cis)	+	+	**O^c** is dominant *only* in cis with **Z$^+$**; Therefore we say it is "cis-acting".
O^c Z$^-$ / F' O$^+$ Z$^+$ (trans)	-	+	

If a mutation is cis-acting we take this as evidence that the mutation affects a site on DNA like an operator. If a mutation is trans-acting we take this as evidence that the mutation affects a diffusible gene product such as a repressor.