Lecture 17

Until now we have been considering mutations that lead to constitutive synthesis of ß-galactosidase. It is also possible to get mutations that are uninducible. For example, a mutation in the promoter (**$LacP^-$**) is uninducible.

	-IPTG	+IPTG	Interpretation
$P^- Z^+$	-	-	P^- is uninducible
$P^- Z^+ / F' P^+Z^+$	-	+	P^- is recessive
*$P^- Z^+ / F' P^+Z^-$	-	-	P^- is cis-acting
$P^- Z^- / F' P^+Z^+$	-	+	

*Note that this experiment can also be viewed as a complementation test that shows that **$LacP^-$** and **$LacZ^-$** are mutations in the same gene. This fits with our primary definition of a gene as the DNA segment needed to make a protein, since the promoter is certainly needed for protein expression.

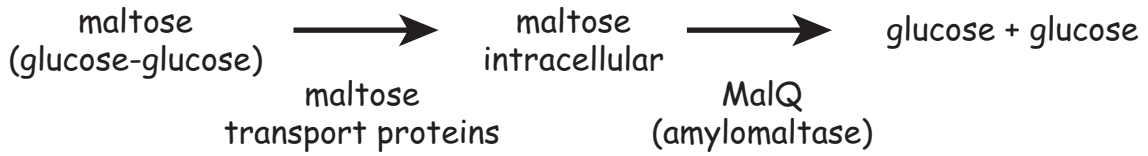
Promoter mutants in **Lac** operon can be distinguished from simple **$LacZ^-$** mutations since promoter mutations affect the **LacY** and **LacA** genes as well.

I^S designates a "super repressor" which binds to the operator DNA but won't bind inducer.

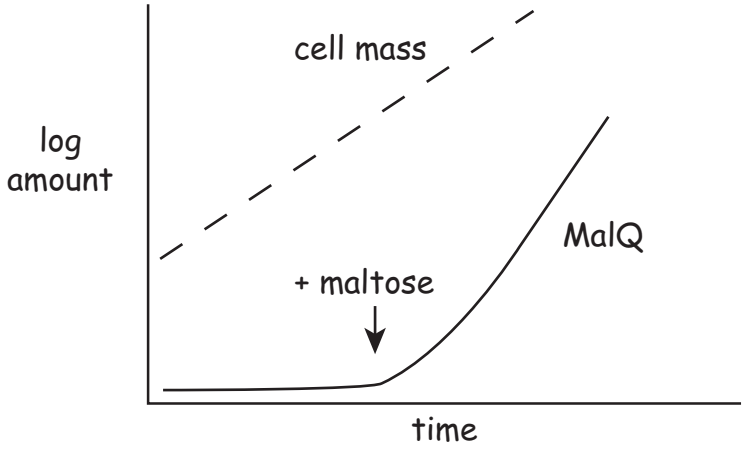
	-IPTG	+IPTG	Interpretation
$I^S Z^+$	-	-	I^S is uninducible
$I^S Z^+ / F' I^+ Z^+$	-	-	I^S is dominant

Positive regulation.

Now we will consider how a different *E. coli* operon is regulated. The **Mal** operon encodes several genes necessary to take up and degrade maltose; a disaccharide composed of two glucose residues.



Much like the **Lac** operon, the products of the **Mal** operon are induced when maltose is added to cells. Thus, maltose acts as an inducer.

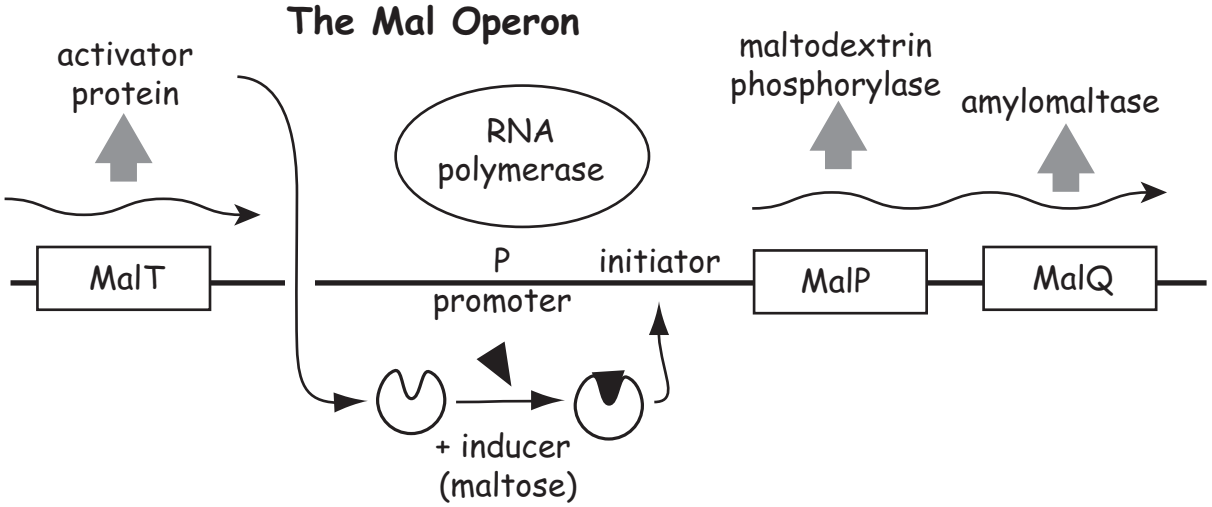


When mutants that affect the regulation of the **Mal** operon were isolated, the most common type consisted of uninducible mutations in a gene known as **MalT**. We can apply dominance tests and cis-trans tests to **MalT** mutations with the following results:

	maltase activity		
	-maltose	+maltose	Interpretation
Mal$^+$	–	+	Maltose induces **Mal** operon
MalT$^-$	–	–	**MalT$^-$** is uninducible
MalT$^-$ / F' MalT$^+$	–	+	**MalT$^-$** is recessive
MalT$^-$ MalQ$^+$ / F' MalT$^+$ MalQ$^-$	–	+	**MalT** is trans-acting
MalT$^-$ MalQ$^-$ / F' MalT$^+$ MalQ$^+$	–	+	

From this table it looks as if the **MalT$^-$** trait is not expressed either in cis or in trans. Because **MalT$^-$** is recessive, it makes more sense to consider the properties of the dominant **MalT$^+$** allele in the cis/trans test. Viewed in this way, the **MalT$^+$** trait is expressed in both cis and trans and therefore **MalT** is considered to be trans-acting.

This behavior is different from any of the **Lac** mutations that we have discussed. The interpretation is that **MalT** encodes a diffusible gene product (not a site on DNA) that is required for activation of transcription of the **Mal** operon. This type of gene is usually called an activator. As shown in the diagram below, maltose binds to the **MalT** activator protein causing a conformational change in **MalT** allowing it to bind near to the promoter and to stimulate transcription. Note that the genes required for maltose uptake are located in an operon elsewhere on the chromosome, but these genes are also regulated by **MalT**.



This model requires a site, called the initiator, which is where the activator binds near the promoter to activate transcription. If you think about how mutations in an initiator site should behave in dominance and cis/trans tests, you will see why in practice it is difficult to distinguish initiator site mutations from promoter mutations.

It is also possible to isolate "super activator" mutants that will bind to the initiator site and activate transcription regardless of whether the inducer maltose is present. Such alleles of the **MalT** gene are called **MalT^{c}** and their properties are given below.

	-maltose	+maltose	Interpretation
MalT^{c}	+	+	**MalT^{c}** is constitutive
MalT^{c} / F' MalT^{+}	+	+	**MalT^{c}** is dominant
MalT^{c} MalQ^{+} / F'MalT^{+} MalQ^{-}	+	+	**MalT^{c}** is trans-acting
MalT^{c} MalQ^{-} / F'MalT^{+} MalQ^{+}	+	+	

For a multimeric activator it should also be possible to isolate activator^{-d} mutants that will interfere with the binding of wild-type subunits to the initiator site. Actually **MalT^{-d}** mutants have not been isolated, probably because **MalT** is a monomer.