

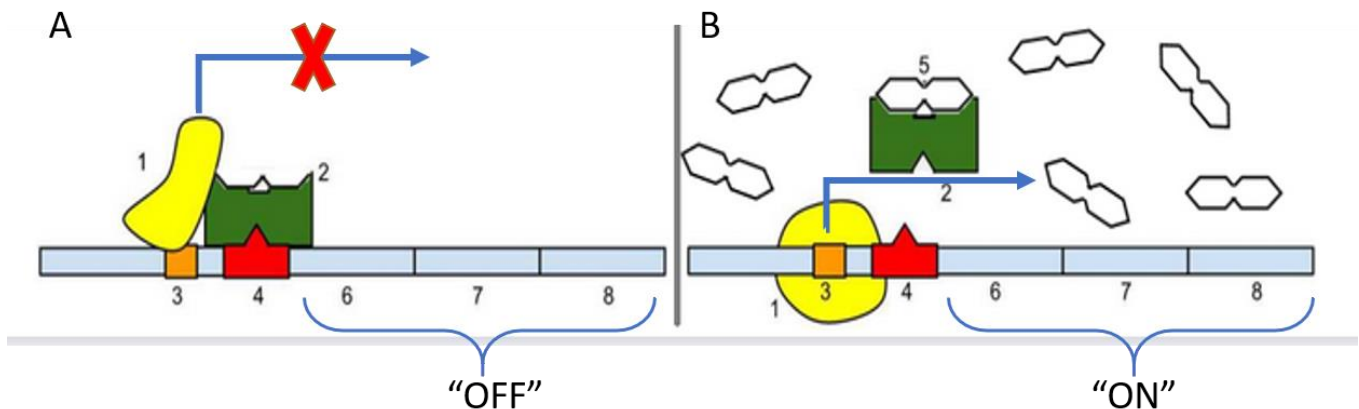
8. Genetic regulation of metabolism in bacteria

In the mid-20th century, French scientists Jacques Monod and Francois Jacob conducted a series of experiments on bacteria that linked metabolism with genes and their regulation. Their work is widely considered to be one of the foundations of molecular biology, and was acknowledged by the 1965 Nobel Prize in Physiology and Medicine.

Lactose is a disaccharide comprised of glucose and galactose

The scientists grew *Escherichia Coli* bacteria on media supplemented with different nutrient sources and noticed that when lactose was present in the media, *E. Coli* produced 200 times more enzyme lactase than in media without lactose (to learn more about lactase, see “Lactase and lactose intolerance” resource).

This has led to a key idea that cells do not waste energy producing something, e.g. an enzyme, that they do not need, so there must be a regulation mechanism allowing bacteria to link lactose presence with the production of proteins needed for metabolising this sugar. Proteins are encoded by genes, so one of the most obvious ways to control protein production is at the DNA level. Below is a schematic depiction of the mechanism by which the lactase gene is regulated in order to only be active when lactose is present. Complete the table by writing the correct number from the diagram next to each label.



Number	Label
	Lactose – a disaccharide of glucose and galactose
	Repressor protein (LacI) – binds operator sequence and blocks transcription
	RNA polymerase – transcribes DNA into mRNA (that then gets translated into protein)
	Genes coding for proteins involved in metabolising lactose (3 numbers), including lactase (also known as beta-galactosidase)
	Operator – a DNA sequence before genes where a repressor binds
	Promoter – a DNA sequence before genes where RNA polymerase has to bind in order to initiate transcription

To delineate this mechanism, Monod and Jacob had to perform experiments with genetic manipulations in *E. Coli*. They mutated different DNA regions in the bacterial chromosome and measured whether lactase is produced. They also conducted so-called genetic complementation experiments: where a wild-type copy of the mutated gene is reintroduced on a plasmid to the mutant cells. Such complementation experiments allowed them to determine whether a regulatory element acts *in cis*, i.e. it needs to be in a particular position within chromosomal DNA (usually adjacent to the DNA it is regulating), or *in trans*, i.e. the position of the regulatory element does not matter.

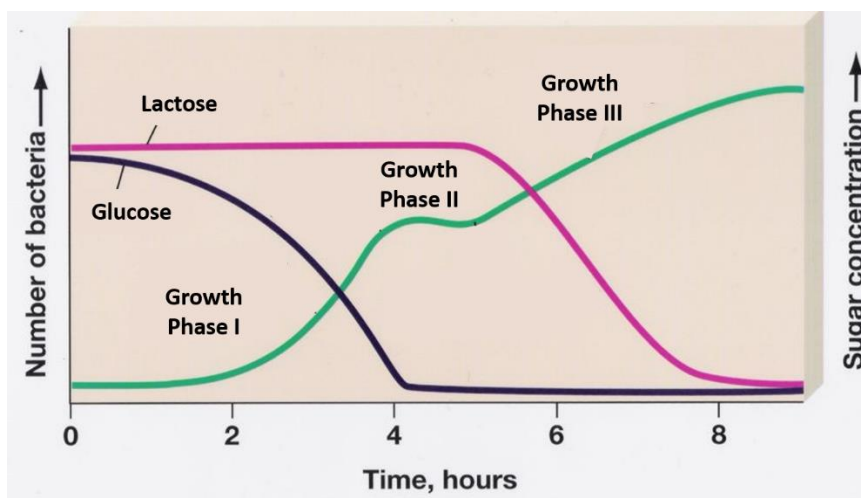
Wild-type – normal, original version of a gene, without mutations; could also be said about a phenotype

Plasmid – an extra DNA molecule separate from the main chromosomal DNA; it is more easily manipulated

Knowing the mechanism from the schematic above, restore what the scientists would have seen in the following cases (marking “+” if lactase would be produced, and “-“ if not).

= lactase produced; “-“ = lactase not produced	Without lactose	With lactose
Without any mutations (wild-type)		
Deactivating mutation in the promoter sequence		
Deactivating mutation in the LacI repressor protein		
Deactivating mutation in the operator sequence		
Deactivating mutation in the LacI repressor protein + a wild-type copy of LacI on a plasmid		
Deactivating mutation in the operator sequence + a wild-type copy of that sequence on a plasmid		

Jacob and Monod also tried to grow *E. Coli* in media with several carbohydrates at the same time, e.g. glucose and lactose. What they saw is schematically represented on the graph below: the growth of bacteria is depicted in green, and the concentration changes of both glucose and lactose throughout the experiment shown.



As you see, there are three distinct growth phases of bacteria. What do you think happens in each one?

To understand why lactose does not get consumed in the first 5 hours of growth, another layer of regulation has to be added to the model described above. See how both glucose and lactose presence can affect lactase production in this video explanation:

<http://highered.mheducation.com/olcweb/cgi/pluginpop.cgi?it=swf::535::535::/sites/dl/free/0072437316/120080/bio27.swf::Combination%20of%20Switches%20-%20the%20Lac%20Operon>

Based on what you have learned from the video, complete the table below:

Carbohydrates in the growing media	cAMP levels: low/high	CRP: active* / inactive**	LacI repressor: active* / inactive**	Lactase: produced / not produced
Glucose only				
Lactose only				
No glucose, no lactose				
Both glucose and lactose				

*active = bound to DNA; **inactive = not bound to DNA

Why do you think bacteria evolved such a system?