All proteins produced by total set of genes are not needed by any organism at one time. However, at all times in the life cycle every cell contains same set of genes. So, there must have some regulatory system which would allow a particular set of genes to express at any given time. A variety of mechanisms are known which regulates gene expression at different levels including transcription, RNA processing and translation.

Operon model

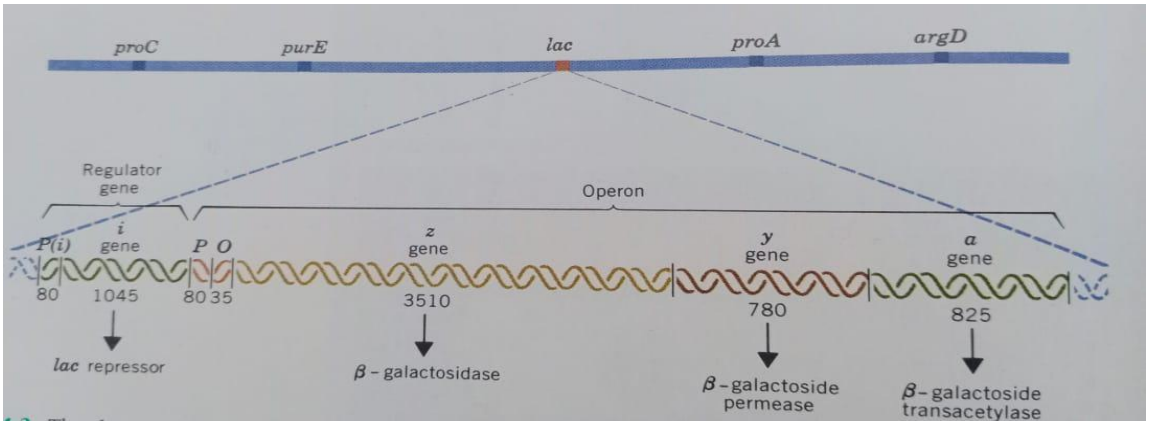
F. Jacob and J. Monad in 1961, on the basis of their study on the inducible system for the synthesis of β-galactosidase enzyme in *E. coli*, proposed a model in order to explain the induction or repression of enzyme synthesis. The model is popularly known as operon model. An operon is a unit of coordinated control of protein synthesis. This unit consists of a promoter, an operator, a number of structural genes.

lac operon

Negative control of *lac* operon:

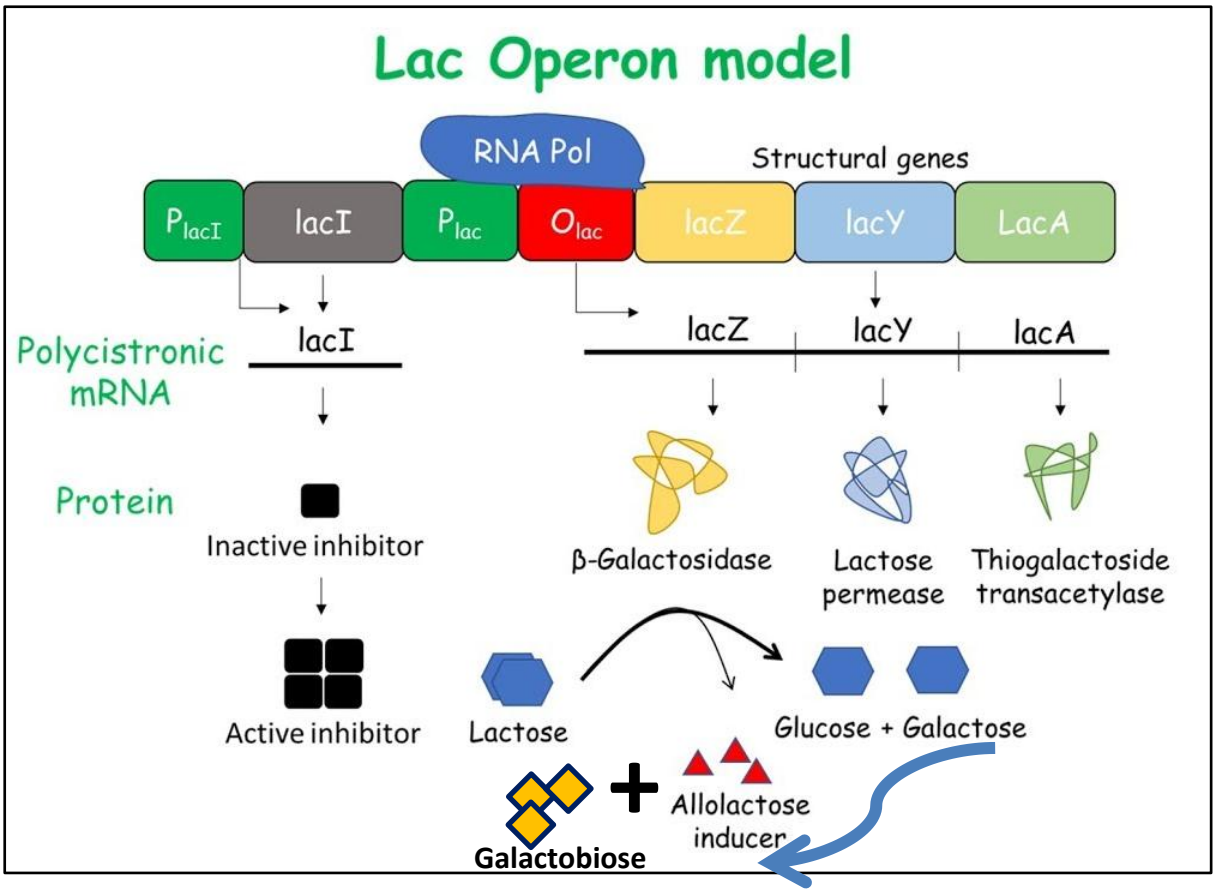
When lactose is not supplied to *E. coli* cell, the enzyme β-galactosidase hydrolyse lactose into glucose and galactose. When lactose is not supplied to *E. coli* cell, presence of the β-galactosidase is hardly detected, but as soon as lactose is added, production of the enzyme β-galactosidase increases as much as 10,000 times. The enzyme quantity again falls down as quickly as lactose is removed. Such enzymes, whose synthesis can be induced by adding the substrate (inducer, often also called as effector molecule), are known as inducible enzymes and the genetic systems responsible for the synthesis of such an enzyme are known as inducible systems. That means in the absence of 'inducer' the genes responsible for β-galactosidase do not function. Molecules called repressor check the activity of the genes. Active repressor molecule may be rendered inactive by addition of inducer.

lac operon is the mostly studied operon. *lac* operon contains a promoter, an operator, and three structural genes-z, y, and a – coding for the enzymes β-galactosidase, β-galactoside permease and β-galactoside transacetylase respectively. β-galactoside permease pumps lactose inside the cell, where β-galactosidase cleaves it into glucose and galactose. The function of the β-galactoside transacetylase is not clear but it transfers an acetyl group from acetyl CoA to β-galactosides and this acetylation probably gives an advantage for utilizing certain non-metabolizable analogues of β-galactoside, because it results in detoxification and excretion.



The *lac* regulator gene, designated as *i* gene, codes for a repressor protein of 360 amino acids in length. The active form of the lac repressor is in tetramer form that contains four copies of the *i* gene product. In the absence of inducer (lactose), the repressor binds to the *lac* operator sequence, preventing RNA polymerase from binding to the promoter and transcribing the structural genes. If lactose is added, the repressor is rendered inactive, so that it cannot attach on operator gene, and the synthesis of mRNA takes place.

Since in the repressed state of lac operon, *E. coli* cells will not synthesize β-galactoside permease, one can question how lactose enters the cell. It is believed that a minimal amount (basal level) of enzyme is always present in the cell to start the metabolic pathway. It has been shown that lactose is not the real inducer of lac operon, but rather it is an anti-inducer, since it binds to repressor to increase its affinity for repressor. On the other hand, the bound protein in inactive repressor was allolactose. It has also been shown that while β-galactosidase breaks lactose into glucose and galactose, a side reaction changes galactose into allolactose and galactobiose. Therefore, in the regulation of *lac* operon, lactose is taken up and a part of it, through galactose, is converted into allolactose, and then allolactose binds to the repressor, causing it to be released from the operator, in so doing, it induces transcription of the z, y, and a structural genes, and out-weighs the anti-inducing effect of lactose.



Positive control of *lac* operon:

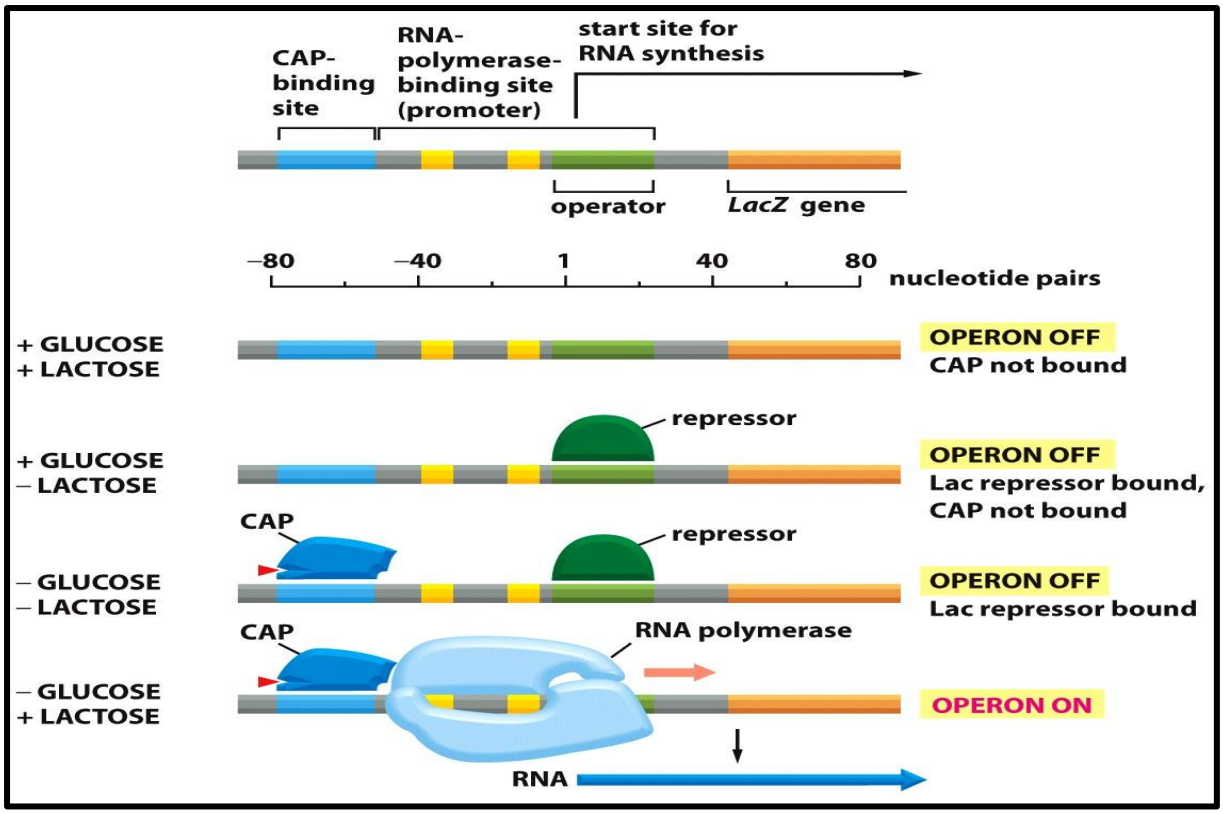
There are two proteins which are known to be involved in the regulation of lactose operon: 1. *lac* repressor and 2. Catabolite gene activator (cga) protein, also known as CAP (Cyclic AMP Protein or Catabolite Activator Protein), or CRP (Cyclic AMP Receptor Protein). While repressor binds to operator site, cga protein binds to cga site. Moreover, repressor exercises a control by checking RNA polymerase to travel through operator, but cga protein exercises a positive control.

Only when cyclic AMP (c-AMP) molecule activates cga protein, the later will allow RNA polymerase to bind. A protein such as CAP which interacts with DNA and RNA polymerase to assist in transcription initiation is called apoinducer.

It has also been shown that this c-AMP – cga protein system is influenced by the level of glucose concentration. It is therefore sometimes described as glucose sensitive system which is common to several glucose sensitive operons in *E. coli*, example- operons for degradation of lactose, galactose, arabinose, maltose etc. In all cases glucose is one of the catabolic products of biosynthetic pathway and if accumulates, it is injurious. Therefore, whenever the

concentration of glucose increases beyond a certain level, it leads to reduction of transcription.

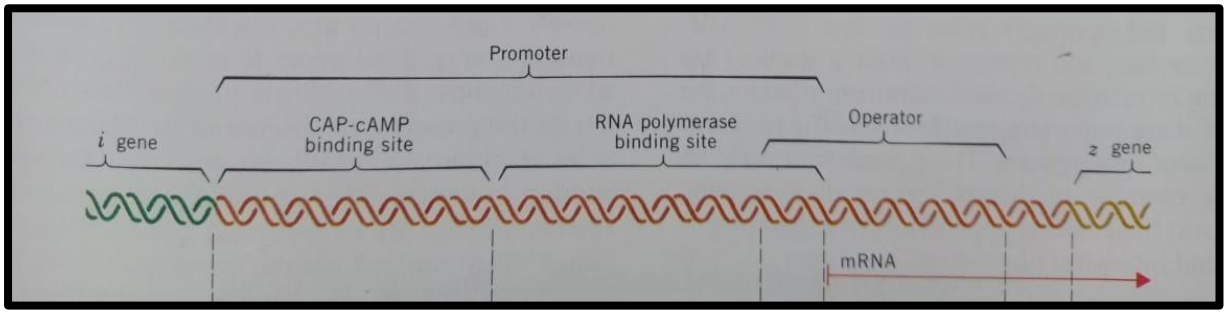
Actually, intracellular concentration of c-AMP is influenced by glucose level of the cell. High concentration of glucose cause sharp decrease in the intracellular concentration of c-AMP. How glucose controls the c-AMP concentration is not clear. This may achieve by reducing the level of c-AMP by glucose, either through checking the synthesis of adenocyclase, the enzyme that catalyzes the formation of c-AMP from ATP or by removing c-AMP, by making a complex with it. Whatever the mechanism, the presence of glucose results in a decrease in the intracellular concentration of c-AMP. In the absence of (or in the presence of a low concentration of) c-AMP, CAP cannot bind to the *lac* operon promoter. In turn, RNA polymerase cannot bind efficiently to the *lac* promoter in the absence of bound CAP.



Thus, if glucose is present in the cell, the induction of *lac* operon is prevented. This phenomenon is called catabolite repression. This occurs to assure the cell that glucose is metabolised when present, in preference to other, less efficient energy sources. Catabolite repression of the *lac* operon is now known to be mediated via positive control of transcription

by a regulatory protein called CAP (for Catabolite Activator Protein) and a small effector molecule called c- AMP.

The *lac* promoter contains two separate binding sites: 1. One for RNA polymerase and 2. One for the CAP - c-AMP complex.



The CAP – c-AMP complex must be bound to its binding site in the lac promoter in order for the operon to be induced. The CAP – c-AMP complex thus exerts positive control over the transcription of the *lac* operon. It has an effect exactly opposite to that of repressor binding to an operator. Although the precise mechanism by which CAP-cAMP complex stimulates RNA polymerase binding to the promoter is still uncertain, its positive control of lac operon transcription is firmly established by both *in vivo* and *in vitro* experiments. CAP is known to function as a dimer, thus, like the *lac* repressor, it is multimeric in its functional state.

Only the CAP-cAMP complex binds to the lac promoter, in the absence of c-AMP, CAP cannot bind. Thus c-AMP acts as the effector molecule, determining the effect of CAP on lac operon transcription.

Therefore, transcription in *lac* operon requires that 1. Cyclic AMP activates cga protein – a positive control and that 2. *lac* repressor is inactivated by inducer – a negative control.