Transcript and Related Materials

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Notes

Positive and negative regulation of the lac operon

Emil Duclaux a colleague of Louis Pasteur was the first scientist to discover the ability of microorganisms to adapt to their environments by adjusting enzyme levels. He found that the fungus *Aspergillus niger* would produce invertase, the enzyme that hydrolyzes sucrose only when grown in the presence of sucrose. Further examples of adaptations were discovered subsequently and by 1930 H. Karstrom could divide enzymes into two categories 1) Adaptive enzymes that formed only in the presence of their substrates and 2) Constitutive enzymes that are always produced in the cell.

The control of metabolism by regulation of enzyme activity helps to conserve energy and raw material as well as to maintain a balance between the amounts of various proteins and to adapt to long term environmental changes.

The lactose operon is a regulatory mechanism that is found in *E. coli* and which was first studied by Jacques Monod in 1942. Lac operon is an operon or a group of genes with a single

promoter that encode genes for the transport and metabolism of lactose in *E.coli* and other bacteria.

Structure of the lactose operon:

The lac operon is made up of structural genes and regulatory sequences. The regulatory sequences are the promoter (P) and the operator (O) which are present upstream of the structural genes. The three structural genes denoted as Z, Y and A are present immediately after the operator . The lac Z gene encodes for the enzyme β galactosidase which is a tetramer and breaks the complex β galactoside into its component sugars eg. Lactose is cleaved into glucose and galactose . The lac Y gene encodes for the enzyme β galactoside permease which is a membrane bound protein constituent of the transport system which transports β galactoside into the cell and the lac A gene encodes for the enzyme β galactoside transacetylase which transfers an acetyl group from acetyl coA to the β galactoside respectively. In addition to these genes there is yet another gene lac I gene which encodes for the lactose repressor protein and its promoter sequence both of which are located at some distance upstream of the lac operon .

The lac operon is under dual control that is positive as well as negative control.

Negative regulation of the lac operon:

By default the lac operon is always turned off. The transcription of the lac operon is controlled by the repressor protein which is a product of the lac I gene. This repressor protein is a product of the lacI gene . It is a tetramer made up of two dimers. It has three different regions: a N terminal DNA binding domain, a hinge and the core of the protein. The DNA binding domain contains two short α helical regions which will bind to the major groove of the DNA. The inducer binding site and the regions which are responsible for multimerisation are located in the core region. The lac repressor protein binds to the operator region .There are multiple palindromic sequences present in the operator region and each repeat is called as a half site of the operator. Each DNA binding domain in the repressor protein will bind to one half site of the operator hence two DNA binding domains bind to the full operator. Since there are four DNA binding domains in the repressor it can bind to two operators simultaneously , one

being the primary operator region situated before the promoter and another operator present either upstream or downstream, this results in bending of the DNA segment containing the promoter region. As a result the RNA polymerase enzyme cannot bind to the promoter region and thus transcription and subsequently translation cannot occur thus switching off the operon. However repressor molecule cannot affect transcription if it has already begun.

Effect of allolactose on the repressor protein:

Inducer is also called as a co-repressor and is generally a small molecule. In case of the lac operon the natural inducer is not lactose but a by product of the lac Z enzyme and is called as allolactose. When the inducer molecule binds to the inducer binding site in the core region of the repressor molecule it results in conformational changes in the repressor and as a result decreases the affinity of the repressor to the operator . As a result the repressor can no longer remain bound to the operator. The operator is now vacant and subsequently the RNA polymerase can bind to the promoter region without hindrance and proceed with the mechanism of transcription and translation thus switching on the operon.

Positive regulation of the lac operon:

The functioning of the lac operon is also regulated by the catabolite activator protein (CAP) or cyclic AMP receptor protein(CRP)and the small cyclic nucleotide 3',5'-cyclic adenosine monophosphate (cAMP). The lac promoter contains a CAP site to which the CAP must bind before the RNA polymerase binds to the promoter and begins transcription. However the CAP can bind to the CAP site only when it is bound to the cAMP. When the CAP cAMP complex binds to the CAP site the RNA polymerase binds very firmly to the promoter and transcription can take place optimally.

Relation of glucose to cAMP levels:

cAMP levels in the cell is inversely proportional to the concentration of glucose. When the concentration of glucose in the extracellular environment is high then it is taken into the

cell in the form of glucose- 6- PO_4. The PO_4 group is donated by phosphoenol pyruvate transferase system (PTS) and in the process the PTS gets dephosphorylated. So if the concentration of glucose is high then the concentration of dephosphorylated PTS also increases, however this in turn adversely affects the concentration of cAMP in the cell. PTS activates the enzyme adenyl cyclase which is responsible for converting ATP to cAMP so if the concentration of dephosphorylated PTS increases in the cell it cannot activate adenyl cyclase thus decreasing the concentration of cAMP in the cell.

Paradoxes of the lac operon:

There are several paradoxes in the lac operon.

The lac operon contains the structural gene lac Z coding for the enzyme β galactosidase which metabolizes lactose and it also contains the lac Y gene which codes for the enzyme β galactoside permease which transports lactose into the cell. Therefore when the operon is switched off then how does the inducer that is allolactose which is obtained by the action of β galactosidase on lactose enter the cell in order to start the induction of the operon?

The answer lies in the fact that the lac operon always has a basal level of expression ensuring that minimal amounts of lac Z and lac Y enzymes are always present in the cell which are enough to start the process of induction. The genes of the lac operon are always expressed at a residual level hence it is also referred to as a leaky operon. In addition to this some inducer can also enter the cell through another uptake system.