Course- B.Sc. (Botany Honours), Part -3

Paper-VI (Group-B), Molecular Biology

Topic- Gene Fine Structure and Gene Regulation.

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Binod Kumar Pandey

Assistant Professor & HOD, Botany Department,

Ram Ratan Singh College, Mokama, Patna.

E.mail- binodkp14@gmail.com

Gene Fine Structure and Gene Regulation

The genetic blueprint contained in the nucleotide sequence can determine the phenotype of an individual. The hereditary units, which are transmitted from one generation to the next generation are called genes. A gene is a fundamental biological unit like atom which is the fundamental physical unit.

Mendel was the first scientist who proposed genes as particulate units and called them hereditary elements or factors. But the concept of gene has undergone a considerable change since Mendel's time.

Fine Structure of Gene:

Benzer, in 1955, divided the gene into recon, muton and cistron which are the units of recombination, mutation and function within a gene. Several units of this type exist in a gene. In other words, each gene consists of several units of function, mutation and recombination. The fine structure of gene deals with mapping of individual gene locus.

This is parallel to the mapping of chromosomes. In chromosome mapping, various genes are assigned on a chromosome, whereas in case of a gene several alleles are assigned to the same locus. The individual gene maps are prepared with the help of intragenic recombination.

Since the frequency of intragenic recombination is extremely low, very large population has to be grown to obtain such rare combination. Prokaryotes are suitable material for growing large population. In Drosophila, 14 alleles of lozenge gene map at four mutational sites which belong to the same locus (Green, 1961). Similarly, for rosy eye in Drosophila, different alleles map at 10 mutational sites of the same locus.

Regulation of Gene Expression in Prokaryotes:

Many prokaryotic genes are regulated in units called operons. Operon is unit of genetic expression consisting of one or more related genes and sequences (gene) controlling them, which includes the operator and promoter sequences that regulate their transcription.

The LAC operon:

It is the operon for utilization and metabolism of lactose in bacteria.

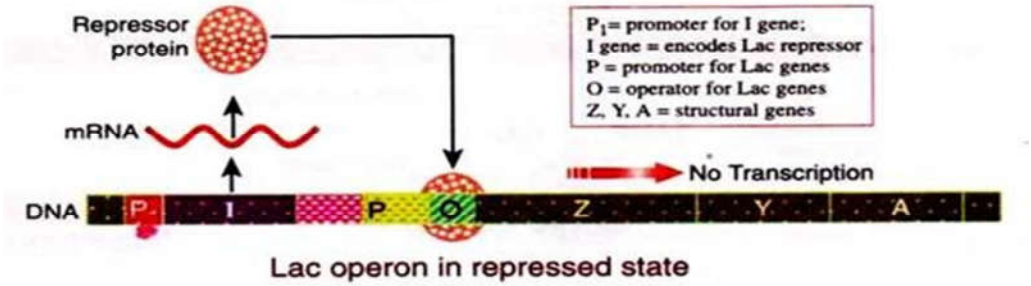
It consists of the following set of genes:

P_I = The promoter gene for regulatory genes
I = The gene for regulatory protein (repressor protein)
P = The promoter sequence for the related genes
O = Operator sequence for these genes
Z = The first gene for utilization of lactose, which forms the enzyme beta-galactosidase
Y = The second gene for the membrane protein galactoside permease
A = The third gene for the enzyme thiogalactoside trans-acetylase

This complete set of sequences (i.e. the operon) helps in switching on/off, the machinery for the utilization of the carbohydrate-lactose by the bacteria E. coli. When glucose is present in the media where the cell is growing, then the lac operon is switched off and when the medium is

devoid of glucose, and instead lactose is present as the sole source of carbon, then the Lac operon becomes operational.

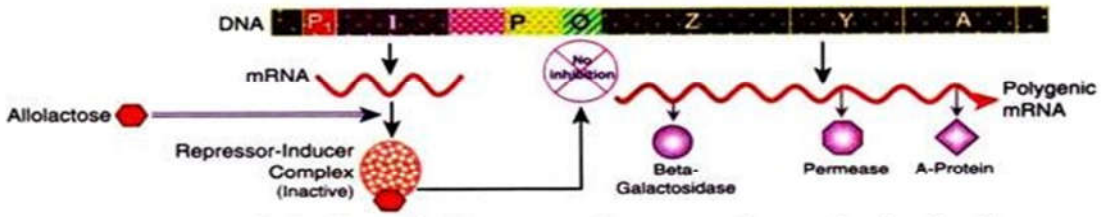
The transcription by RNA polymerase begins at the promoter site i.e. the enzyme binds to the promoter and moves along the DNA towards the structural genes of the operon to transcribe the mRNA for these genes and in this process it passes through the operator region of the operon. Under all circumstances i.e. whether glucose or lactose is to be utilized by the cell, the I gene of the lac operon synthesizes a protein called repressor protein. This protein binds to the operator site in the DNA and thus prevents the movement of the RNA polymerase beyond this point (site), which results in the inhibition of the synthesis of the structural genes Z, Y and A.



Lac operon in repressed state

Thus, when the cell is utilizing glucose as the only carbon source, the lac operon is switched off. Then, if the cell shifts over to the utilization of lactose as the carbon source then lactose is first converted to allolactose by the enzyme beta-galactosidase (which is always present in the cell in a few copies, irrespective of glucose or lactose is being utilized), and this allolactose acts as a positive modulator or inducer for the lac operon.

Here the allolactose binds to the repressor protein present at the operator site resulting in the release of the repressor protein from the operator site thereby permitting the enzyme RNA polymerase to pass freely through this operator site from the promoter site and thus transcribe all three structural genes Z, Y, & A.



Induction of the Lac operon in response to a molecular signal

The activity of the lac operon is not only dependent upon the binding and release of repressor molecule (with modulator) but it is also cAMP dependent. When glucose is low in the media/cell, then the cellular cAMP concentration increases. This increased amount of cAMP results in its binding at a particular site (sequences) on the promoter.

The promoter site can be divided into two parts:

(1) The site for the binding of RNA polymerase

(2) The site for a protein called catabolite gene activator protein (CAP).

The RNA polymerase can bind to the promoter site only if the CAP is bound to the promoter sequence and CAP can bind to the promoter only if cAMP is bound to it and cAMP binds to CAP only when its cellular concentration increases, which occurs when the cell is devoid of glucose and hence this facilitates the utilization of these sugars and the presence of lactose converts it to allolactose.

This acts as a positive modulator for switching on the lac operon genes by releasing the repressor protein from the operator site and producing the products of the three structural genes which produces:

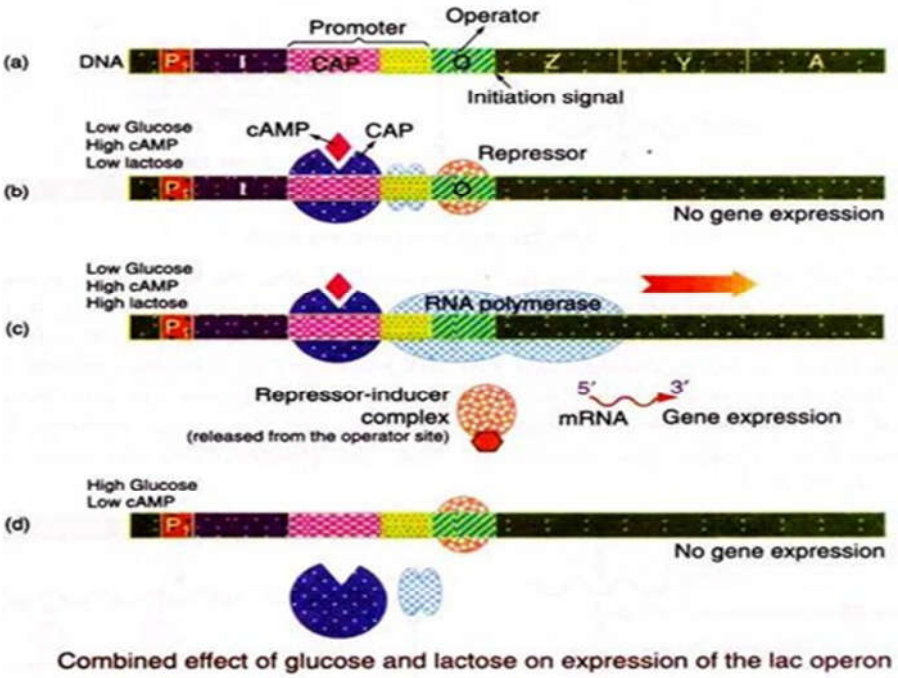
(1) The membrane protein β-galactoside permease, that enhances the uptake of lactose by the cells

(2) β-galactosidase which hydrolysis lactose to allolactose and then to glucose and galactose

(3) The enzyme thiogalacatosidase-transacetylase, whose function is unknown.

When glucose is again available to the cell the cAMP concentration decreases in the cytosol, resulting in its release from the CAP, this in turn results in the release of CAP from promoter site, which in turn results in release of the enzyme RNA polymerase from the promoter site and further prevents its binding to promoter.

This again results in the diminished synthesis of the structural genes, one of which is beta-galactosidase, that results in low production of allolactose (or no synthesis of allolactose), this is turn results in the repressor protein (formed from I gene) being devoid of the modulator and thus is free to bind at the operator site thereby prevent the movement of RNA polymerase and thus resulting in the inhibition of lac operon.



Combined effect of glucose and lactose on expression of the lac operon

Each and every metabolite has got its own operon, with different number of structural genes and whenever the genes for that metabolite are required it is switched on by a similar mechanism as that of the lac operon and switched off whenever not required.

The other operons and their details are as under:

Operon for	No. of structural genes	Function
His operon	9	Enzymes required in synthesis of histidine
Leu operon	4	Conversion of alpha-keto-isovalerate to leucine
Ara operon	4	Transport and utilization of the carbohydrate arabinose

All of the operons found in the bacteria do not function only by completely switching on or off their genes. Some operons function at differential rates depending upon the need of the cell by a mechanism called the transcription attenuation i.e. slowing down of the rate of synthesis of enzymes, ex. those enzymes involved in the synthesis of amino acids (His).

Attenuation:

Transcription attenuation is a process in which transcription is initiated normally but is abruptly halted before the complete operon genes are transcribed. The frequency with which transcription is attenuated depends upon the cellular concentration of that particular amino acid for which the operon is meant for.

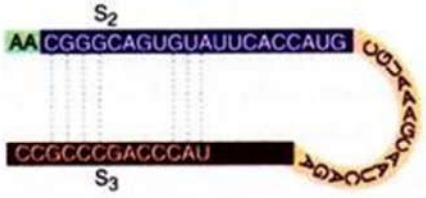
Attenuation of His operon:

In bacteria, transcription and translation are closely coupled. The rate at which RNA is transcribed and the rate at which that protein is translated is almost the same. Most of the transcribed RNAs for amino acid metabolism in the cell contain various complementary intra base pairing sequences. For example the following is the part of RNA that is being transcribed for His operon, which is also simultaneously being translated.

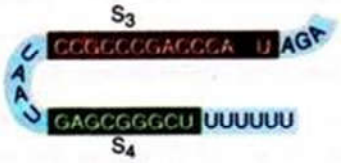
Complementary intra-base pairing sequences of His operon (shown in dark)

The sequence 2 and 3 are complementary and can base pair with each other. Likewise sequences 3 and 4 are also complementary and can also base pair with each other. If 2 and 3 bases pair, then transcription can proceed normally and if 3 and 4 bases pair the transcription is terminated, just like the termination of transcription due to appearance of a hair pin structure in DNA. The base pairing between the sequences 2 & 3 or 3 & 4 is dependent upon the rate of translation of the mRNA, which in turn is dependent upon the concentration of His-tRNA^{His} that reflects the concentration of histidine in the cell.

If the concentration of His-tRNA^{His} is more and the rate of translation is very fast such that it passes the 2nd site before site 3 is transcribed, then this results in the site 3 base pairing with site 4 as soon as it is transcribed resulting in the termination of transcription.



Intrachain base pairing between S_2 and S_3 in mRNA do not affect translation



Intrachain base pairing between S_3 and S_4 in mRNA attenuating translation

On the other hand when the His-tRNA^{His} concentration is low, the rate of translation is very slow and thus the process of translation does not pass the 2nd site on mRNA by the time site (sequences) 3rd is transcribed then this result in the continuation of transcription because this will result in the 2 & 3 sites base pairing and so site 3 is not free for base pairing with site 4. Thus this results in a continuous operation of His operon.

Regulation of Gene Expression in Eukaryotes:

The genes in eukaryotes are also regulated in more or less the same manner as that of prokaryotes, but the regulation is mostly positive and very rarely negative regulation is seen. In higher eukaryotes the regulation of gene expression is solely by positive modulation and negative inhibition of the genes/operon is totally absent.

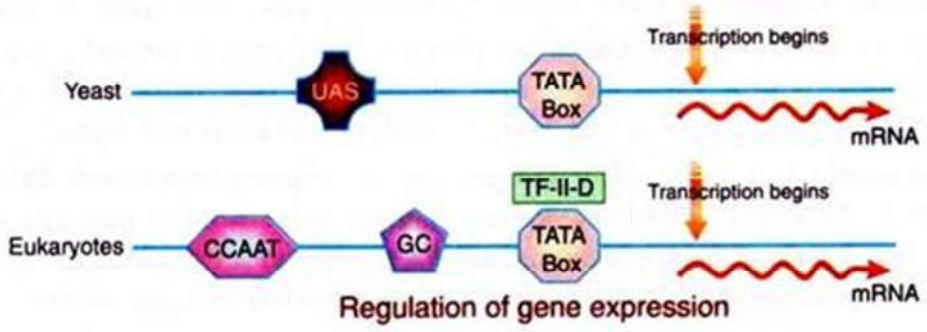
However in yeast some genes are regulated by negative modulation. Further, there is a physical separation between the process of transcription and translation is eukaryotes as transcription takes place in the nucleus and translation occurs in the cytosol.

Mechanism:

The gene regulation is only by positive regulation. Most of the genes are normally inactive in eukaryotes i.e. RNA polymerases cannot bind to the promoters. The cells synthesize only the selected group of activator proteins needed to activate transcription of the small subset of genes required in that cell.

There are at least five regulatory sites for RNA polymerase promoter sites in higher eukaryotes designated as (a) TATA box (b) GC box and (c) CAT box. In yeast there are two types of promoter sequences i.e. TATA box and UAS i.e. upstream activator sequence.

These sequences are the binding sites for the transcription factors called TF-II-D that is required for RNA polymerase binding. Each of these sequences are recognised and bound specifically by one or more regulatory proteins called transcription factors. These regulatory sequences are about 1000 bases away form the main gene, thus to activate the main gene a protein-protein interaction is required which can reach the main gene sequence.



Regulation of gene expression