LAC OPERON AND FINE STRUCTURE OF GENE

The hereditary units which are transmitted from one generation to the next generation arc called genes. A gene is the fundamental biologic unit, like the atom which is the fundamental physical unit. Mendel while explaining the result of his monohybrid and dihybrid crosses, first of all conceived of the genes as particulate units and referred them by various names such as hereditary factors or hereditary elements. But his concept about the gene was entirely hypothetical and he remained ignorant about the physical and chemical nature of gene.

Even before the rediscovery of Menders laws in 1900, it was already established that chromosomes have a definite role in the inheritance because it was found that chromosomes were the only link between one generation and the next generation and a diploid chromosome set consists of two morphologically similar sets, one is derived from the mother and the other from the father at fertilization. Later on, a parallel behavior among chromosomes and genes was discovered.

Earlier workers proposed various hypotheses to explain the nature of genes. For instance, De Vries postulated one gene one character hypothesis according to which a particular trait of an individual is controlled by a particular gene. Bateson and Punnett proposed the presence or absence theory. According to them, in a cross the character which dominates the other has a determiner, while, the recessive character has no such determiner. But all the theories were discarded by Morgan, who produced the particulate gene theory in 1926. He considered genes as corpuscles, which are arranged in a linear order on the chromosomes and appear like beads on a string. Each gene was supposed to be different from ail others. The particulate theory of gene was widely accepted and supported by cytological observations. But, the discovery of DNA molecule as a sole carrier of genetic informations base altogether discarded the Morgan's theory. Therefore, before defining the gene it will be advisable to consider both the classical as well as modern definitions of gene.

Changing Concept of Gene

The concept of gene has been the focal point of study from the beginning of twentieth century to establish the basis of heredity. The gene has been examined from two main angles, i.e., (1) genetic view, and (2) biochemical and molecular view. These aspects are briefly described below:

(1) A Genetic View

The genetic view or perspective of gene is based mainly on the Mendelian inheritance, chromosomal theory of inheritance and linkage studies. Mendel used the term factors for genes and reported that factors were responsible for transmission of characters from parents to their offspring. Sutton and Boveri (1903) based on the study of mitosis and meiosis in higher plants established parallel behaviour of chromosomes and genes. They reported that both chromosomes and genes segregate and exhibit random assortment, which clearly demonstrated that genes are located on chromosomes. The Sutton- Boveri hypothesis is known as chromosome theory of inheritance.

Morgan based on linkage studies in Drosophila reported that genes are located on the chromosome in a linear fashion. Some genes do not assort independently because of linkage between them. He suggested that recombinants are the result of crossing over. The crossing over increases if the distance between two genes is more. The number of linkage group is the same as the number of chromosomes. The chromosome theory and linkage studies reveal that genes are located on the chromosomes. This view is sometimes called as bead theory. The important points about the bead theory are given below:

1. The gene is viewed as a fundamental unit of structure, indivisible by crossing over. Crossing over occurs between genes but not within a gene.
2. The gene is considered as a basic unit of change or mutation. It changes from one allelic form to another, but there are no smaller components within a gene that can change.

3. The gene is viewed as a basic unit of function. Parts of a gene, if they exist, cannot function.

The chromosome has been viewed merely as a vector or transporter of genes and exists simply to permit their orderly segregation and to shuffle them in recombination. The bead theory is no more valid for any of the above three points. Now evidences are available which indicate that: (1) a gene is divisible (2) part of a gene can mutate, and (3) part of a gene can function.

The Gene is Divisible

Earlier it was believed that gene is a basic unit of structure which is indivisible by crossing over. In other words, crossing over occurs between genes but not within a gene. Now intragenic recombination has been observed in many organisms which indicates that a gene is divisible. The intragenic recombination has following two main features.

1. It occurs with rare frequency so that a very large test cross progeny is required for its detection. Benzer expected to detect a recombination frequency as low as 10^{-6}, the lowest he actually found was 10^{-4} (0.01 x 2 = 0.02%).
2. The alleles in which intragenic recombination occurs are separated by small distances within a gene and are functionally related.

Examples of intragenic recombination include bar eye, star asteroid eye and lozenge eye in Drosophila. The bar locus is briefly described below. Lozenge eye and star asteroid have been discussed under pseudoalleles.

Bar Eye in Drosophila

The first case of intragenic recombination was recorded in Drosophila for bar locus which controls size of eye. The bar locus contains more than one unit of function. The dominant bar gene in Drosophila produces slit like eye instead of normal oval eye. Bar phenotype is caused by tandem duplication of 16A region in X chromosome, which results due to unequal crossing over. The flies with different dose of 16A region have different types of eye as follows:

1. Single 16A region → Wild type oval eye
2. Double 16A region → Bar eye small in size
3. Triple 16A region → Double bar or ultrabar eye very small in size

The homozygous bar eye (B/B) produced both wild and ultra bar types though at a low frequency which indicated intragenic recombination in the bar locus but the frequency was much higher than that expected due to spontaneous mutations.

Part of a Gene Can Function

It was considered earlier that gene is the basic unit of function and parts of gene, if exist, cannot function. But this concept has been outdated now. Based on studies on rll locus of T4 phage, Banzer (1955) concluded that there are three sub divisions of a gene, viz., recon, muton and cistron. These are briefly described below:

Recon

Recons are the regions (units) within a gene between which recombinations can occur, but the recombination cannot occur within a recon. There is a minimum recombination distance within a gene which separates recons. The map of a gene is completely linear sequence of recons.

Muton

It is the smallest element within a gene, which can give rise to a mutant phenotype or mutation. This indicates that part of a gene can mutate or change. This disproved the bead theory according to which the entire gene was a mutate or change.

Cistron

It is the largest element within a gene which is the unit of function. This also nocked down the bead theory according to which entire gene was the unit of function. The name cistron has been derived from the test which is performed to know whether two mutants are within the same cistron on in different cistrons. It is called cis-trans test which is described below.

Cis – Trans Test

When two mutations in trans position produce mutant phenotype, they are in the same cistron. Complementation in trans position (appearance of wild type) indicates that the mutant sites are in different cistrons. There is no complementation between mutations within a ciston.

It is now known that some genes consist of only one cistron ; some consist of two or even more. For example, the mutant miniature (m) and dusky (dy) both decrease wing size in Drosophila and map in the same part of X chromosome. But when brought together in dy +/+m heterozygote, the phenotype is normal which indicates that the locus concerned with wing size is composed of at least two cistrons.

(2) A Biochemical View

It is now generally believed that a gene is a sequence of nucleotides in DNA which controls a single polypeptide chain. The different mutations of a gene may be due to change in single nucleotide at more than one location in the gene. Crossing over can take place between the altered nucleotides within a gene. Since the mutant nucleotides are placed so close together, crossing over is expected within very low frequency. When several different genes which affect the same trait are present so close that crossing over is rare between them, the term complex locus is applied to them. Within the nucleotide sequence of DNA, which represents a gene, multiple alleles are due to mutations at different points within the gene.

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 order 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 materials 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.

Genes can be classified in various ways. The classification of genes is generally done on the basis of (1) dominance, (2) interaction, (3) character controlled, (4) effect on survival, (5) location, (6) movement, (7) nucleotide sequence, (8) sex linkage, (9) operon model, and (10) role in mutation. A brief classification of genes on the basis of above criteria is presented below

Classification and brief description of genes

Classification of genes	**A brief description**
Based on Dominance	
Dominant genes	Genes that express in the F_1
Recessive genes	Genes whose effect is suppressed in F_1
Based on Interaction	
Epistatic gene	A gene that has masking effect on the other gene controlling the same trait.
Hypostatic gene	A gene whose expression is masked by another gene governing the same trait
Based on Character Controlled	
Major gene	A gene that governs qualitative trait. Such genes have distinct phenotypic effects.
Minor gene	A gene which is involved in the expression of quantitative trait. Effect of such genes cannot be easily detected.

Based on Effect on Survival	
Lethal gene	A gene which leads to death of its carrier when in homozygous condition. It may be dominant or recessive.
Semi lethal gene	A gene that causes mortality of more than 50% of its carriers.
Sub-vital gene	A gene that causes mortality of less than 50% of its carriers.
Vital gene	A gene that does not have lethal effect on its carriers.
Based on Location	
Nuclear genes	Genes that are found in nuclear genome in the chromosomes.
Plasma genes	Genes that are found in the cytoplasm in mitochondria and chloroplasts. Also called cytoplasmic or extranuclear genes.
Based on Position	
Normal genes	Genes that have a fixed position on the chromosomes. Most of the genes belong to this category
Jumping genes	Genes which keep on changing their position on the chromosome of a genome. Such genes have been reported in maize.
Based on Nucleotide sequence	
Normal genes	Genes having continuous sequence of nucleotides which code for a single polypeptide chain.
Split gene	A gene having discontinuous sequence of nucleotides. Such genes have been reported in some eukaryotes. The intervening sequences do not code for amino acids.
Pseudo genes	Genes having defective nucleotides which are non-functional. These genes are defective copies of some normal genes.
Based on Sex Linkage	

Sex linked genes	Genes which are located on sex or X-chromosomes.
Sex limited genes	Genes which express in one sex only
Sex influenced genes	Genes whose expression depends on the sex of individual e.g., gene for baldness in humans.
Based on Operon Model	
Regulator gene	A gene found in lac operon of E.Coli which directs synthesis of a repressor
Operator gene	In lac operon, a gene which control the function of structural genes.
Promotor gene	A gene in lac operon of E.Coli which initiates mRNA synthesis
Structural genes	The genes in lac operon of E.Coli which control the synthesis of protein through mRNA.
Based on role in Mutation	
Mutable genes	Genes which exhibit higher mutation rate than others e.g., which eye gene is Drosophila.
Mutator genes	Genes which enhance the natural mutation rate of other genes in the same genome e.g., dotted gene in maize.
Antimutator genes	Genes which decrease the frequency of natural mutation of other genes in the same genome. Such genes are found in bacteria and bacteriophages.

More about Genes

There are some genes which are different from normal genes either in terms of their nucleotide sequences or functions. Some examples of such genes are split gene, jumping gene, overlapping gene and pseudo gene. A brief description of each of these genes is presented below:

Split Genes

Usually a gene has a continuous sequence of nucleotides. In other words, there is no interruption in the nucleotide sequence of a gene. Such nucleotide sequence codes for a particular single polypeptide chain. However, it was observed that the sequence of nucleotides was not

continuous in case of some genes; the sequences of nucleotides were interrupted by intervening sequences. Such gene with interrupted sequence of nucleotides is referred to as split genes or interrupted genes. Thus, split genes have two types of sequences, viz., normal sequences and interrupted sequences.

1. Normal Sequence. This represented the sequence of nucleotides which are included in the mRNA which is translated from DNA of split gene These sequences code for a particular polypeptide chain and are known as exons.
2. Interrupted Sequence: The intervening or interrupted sequence of split gene are known as introns. These sequences do not code for any peptide chain. Moreover, interrupted sequences are not included into mRNA which is transcribed from DNA of split genes. The interrupted sequences are removed from the mRNA during processing of the same. In other words, the intervening sequences are discarded in mRNA as they are non-coding sequences. The coding sequences or exons are joined by ligase enzyme.

The first case of split gene was reported for ovalbumin gene of chickens. The ovalbumin gene has been reported to consist of seven intervening sequences.. Later on interrupted sequences (split genes) were reported for beta globin gene of mice and rabbits, tRNA genes of yeast and ribosomal genes of Drosophila.

The intervening sequences are determined with the help of R loop technique. This technique consists of hybridization between mRNA and DNA of the same gene under ideal conditions, i.e., at high temperature and high concentration of formamide. The mRNA pairs with single strand of DNA. The non-coding sequences or intervening sequences of DNA make loop in such pairing. The number of loops indicates the number of interrupted sequences and the size of loop indicates length of the intervening sequence. These loops can be viewed under electron microscope. The ovalbumin gene has seven interrupted sequences (introns) and eight coding sequences (exons). The beta globin gene has been reported to have two intervening sequences, one 550 nucleotides long and the other 125 nucleotides long.

The intervening sequences are excised during processing to form mature mRNA molecule. Thus, about half of the ovalbumin gene is discarded during processing. Earlier it was believed that there is colinearity (correspondence) between the nucleotide sequence and the sequence of amino acids which it specifies. The discovery of split genes has disproved the concept of colinearity of genes. Now colinearity between genes and their products is considered as a chance rather than a rule. Split genes have been reported mostly in eukaryotes.

Jumping Genes

Generally, a gene occupies a specific position on the chromosome called locus. However in some cases a gene keeps on chaining its position within the chromosome and also between the chromosomes of the same genome. Such genes are known as jumping genes or transposons or transposable elements. The first case of jumping gene was reported by Barbara Mc-Clintock in maize as early as in 1950. However, her work did not get recognition for a long time like that of Mendel. Because she was much ahead of time and this was an unusual finding, people did not appreciate it for a long time. This concept was recognized in early seventies and McClintock was awarded Nobel Prize for this work in 1983.

Later on transposable elements were reported in the chromosome of E. coli and other prokaryotes. In E.coli, some DNA segments were found moving from one location to other location. Such DNA segments are detected by their presence at such a position in the nucleotide sequence, where they were not present earlier. The transposable elements are of two types, viz, insertion sequence and transposons.

1. Insertion Sequence. There are different types of insertion sequences each with specific properties. Such sequences do not specify for protein and are of very short length. Such sequence has been reported in some bacteria bacteriophages and plasmids.
2. Transposons. These are coding sequences which code for one or more proteins. They are usually very long sequences of nucleotides including several thousand base pairs.

Transposable elements are considered to be associated with chromosomal changes such as inversion and deletion. They are hot spots for such changes and are useful tools for the study of mutagenesis. In eukaryotes, moving DNA segments have been reported in maize, yeast and Drosophila.

Overlapping Genes

Earlier it was believed that a nucleotide sequence codes only for one protein. Recent investigations with prokaryotes especially viruses have proved beyond doubt that some nucleotide sequences (genes) can code for two or even more proteins. The genes which code for more than one protein are known as overlapping genes. In case of overlapping genes, the complete nucleotide sequence codes for one protein and a part of such nucleotide sequence can code for another protein. Overlapping genes are found in tumor producing viruses such as ϕ X 174, SV 40 and G4, in virus ϕ X 174 gene A overlaps gene B. In virus SV 40, the same nucleotide sequence codes for the protein VP 3 and also for the coboxyl – terminal end of the protein VP2. In virus G4, the gene A overlaps gene B and gene E overlaps gene D. The gene of this virus also contains some portions of nucleotide sequences which are common for gene A and gene C.

Pseudogenes

There are some DNA sequences, especially in eukaryotes, which are non-functional and defective copies of normal genes. These sequences do not have any function. Such DNA sequences or genes are known as pseudogenes. Pseudogenes have been reported in humans, mouse and Drosophila. The main features of pseudogenes are given below :

1. Pseudogenes are non functional or defective copies of some normal genes. These genes are found in large numbers.
2. These genes being defective cannot be translated.
3. These genes do not code for protein synthesis, means they do not have any significance.
4. The well known examples of pseudogenes are alpha and beta globin pseudogenes of mouse.

LAC OPERON CONCEPT

History

The term ‘‘operon’’ was first proposed in a short paper in the Proceedings of the French Academy of Sciences in 1960. From this paper, the so-called general theory of the operon was developed. This theory suggested that all genes are controlled by means of operons through a single feedback regulatory mechanism: repression. Later, it was discovered that the regulation of genes is a much more complicated process. Indeed, it is not possible to talk of a general regulatory mechanism, as there are many, and they vary from operon to operon. Despite modifications, the development of the operon concept is considered one of the landmark events in the history of molecular biology.

Components of operon

The structural genes

The structural genes form a single long polycistronic m RNA molecule and the number of structural genes corresponds to the number of proteins. Each structural gene is controlled independently and transcribe mRNA molecule separately, this, depends on substrate to be utilized. Example: In lac operon three structural genes (Z, Y, A) are associated with lactose utilization. Beta-galactosidase is the product of lac Z that cleaves beta (1-4) linkage of lactose & releases the free monosaccharides. The enzyme permease (a product of lacy) facilitates the lactose the entry inside the bacterium. The enzyme transacylase is a product of lac A where no definite role has been assigned. The lac operon consists of a promoter (p) operator (o) together with structural genes. The lac operon cannot function in the presence of sugars other than lactose.

The operator gene

The operator gene is present adjacent to lac Z gene. The operator gene overlaps the promoter region. The lac repressor protein binds to the operator invitro & protect part of the promoter region from the digestion of DNase. The repressor protein binds to the operator & forms an operator

–repressor complex which in turn physically blocks the transcription of Z, Y & A genes by preventing the release of RNA polymerase to begin transcription.

The promoter gene

The promoter gene is long nucleotide &continuous with the operator gene. The promoter gene lies between the operator &regulator gene, like operators the promoter region consists of palindromic sequences of nucleotides (i.e show 2 fold geometry from a point). These palindromic sequence are recognized by such proteins that have symmetrically arranged subunits. This section of two fold symmetry is present on the CRP site(c-AMP receptor protein site that binds to a protein called CRP).the CRP is encoded by CRP gene, it has been shown experimentally that CRP gene binds to cAMP (c AMP found in e.coli & other organisms) molecule & form a cAMP CRP complex. This complex is required for transcription because it binds to promoter& enhances the attachment of RNA polymerase to the promoter therefore it increases the transcription &translation process.

The repressor (regulator) gene

Regulator gene determines the transcription of structural gene. It is of two types-active & inactive repressor. It codes for amino acids of a defined repressor protein. After synthesis, the repressor molecules are diffused from the ribosome & bind to the operator in the absence of an induces. Finally the path of RNA polymerase is blocked & m RNA is not transcribed consequently; no protein synthesis occurs .this type of mechanism occurs in inducible system of active repressor. Moreover when an inducer is present it binds to repressor proteins 7forms an inducer – repressor complex. Due to formation of complex the repressor undergoes changes in the confirmation of shape 7 becomes inactive consequently the structural genes can synthesize the polycistronic m RNA and later synthesize enzyme.

In contrast in the reversible system the regulator gene synthesis repressor protein that is inactive & therefore fails to binds to

operator, consequently ,proteins are synthesized by the structural genes .however the repressor protein can be activated in the presence of an co-repressor. the co-repressor together with repressor proteins forms the repressor-co repressor complex. This complex binds to operator gene & blocks the protein synthesis

Types of operon

1. Lactose (Lac) operon

The regulatory mechanism of operon is responsible for the utilization of lactose as a carbon source that is why it is called as lac operon. the lactose utilizing system consists of 2 types of components i.e the structural genes (lacZ, lacy, lacA) the products of which are required for transport and metabolism of lactose &regulatory genes (lacI, lacP, lacO).these two components together comprises of lac operon .one of the most key features is that operon provides a mechanism for the co-ordinated expression of structural genes controlled by regulatory genes. Operon shows polarity i.e. the genes Z, Y, A synthesize equally qualities of 3 enzymes beta-galactosidase by lac Z, permease by lac Y & acetylase by lac A. These are synthesized in an order i.e. beta-galactosidase at first and acetylase in the last.

Regulation of lac operon

Regulation of the lac operon by repressor is called negative control. The lac operon is also under positive control by CRP (or cAMP Receptor Protein; also known as CAP or catabolite activator protein). CRP or CAP is now thought to be bound to its lac binding site at all times (even during repression). During induction, the inducer (either the natural inducer, allolactose, or the synthetic inducer, IPTG, binds to the lac repressor. Inducer-bound repressor does not bind to operator sites. This allows RNA polymerase to bind to the promoter and start transcribing the lac operon.

Negative (lac repressor) ----------Bound to DNA----------Not bound to DNA

(Type of Control) (Operon off) (Operon on)

Positive(CRP protein) ---------- Bound to DNA----------Not bound to DNA
(Type of control) (Operon on) (Operon off)

2. Tryptophan (Trp) operon

The tryptophan operon of E.coli is responsible for the synthesis of the amino acids tryptophan regulation of this operon occurs in such a way that when tryptophan is present in the growth medium, Trp operon is not active but, when adequate trp is present, the transcription of the operon is inhibited, however when its supply is insufficient transcription occurs, the Trp is quite different from the lac operon in that trp acts directly in the repression system rather than as an inducer. Moreover since the trp operon encodes a set of bio-synthetic caranabolic rather than a catabolic enzyme neither glu nor c AMP –CAP has a role in the operon activity.

Regulation of Trp Operon

Trp is synthesized in 5 steps each required a particular enzyme.in E.coli chromosome the genes encoding these enzymes are located adjacent to one another in the same order as they are used in the bio-synthetic pathway they are translated from a single polycistronic m RNA molecule. These genes are called TrpE, TrpP, TrpC, TrpB, TrpA, The TrpE gene is the first one translated. Adjacent to the Trp E gene are the promoter, the operator &2 region, called the leader and the attenuated which are designated as TrpL & TrpA respectively .the repressor gene TrpR is located quite for from the gene cluster. The regulatory protein of the repressor system o the TrpR operon is the product of the TrpR gene. mutations either in this gene or in the operator cause constitute initiation of transcription of Trp-m RNA on the lac operon. This regulatory protein is called Trp apo repressor &it does not bind to the operator, unless Trp is present. The apo repressor &the tryptophan molecule joins together to form an active trp repressor which binds to the operator. The reaction scheme is as follows:

Apo repressor (no trp) ----------active repressor (transcription occurs)

Apo repressor+trp----------active repressor +operator---------- inactive operator (transcription does not occur)