RAMA UNIVERSITY

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FACULTY OF AGRICULTURAL SCIENCES

AND ALLIED INDUSTRIES

GENE REGULATION

Each cell of a living organism contains thousands of genes. But all genes do not function at a time. Genes function according to requirements of the cell. Genes control the phenotypic expression of various characters through the production of specific enzymes. Enzymes are special proteins which catalyse chemical reactions. Thus, there exists an on-off system which regulates protein synthesis in all living cells. The precise study of this on-off mechanism is called regulation of gene action or regulation of gene expression or regulation of protein synthesis.

There are two types of gene regulation, viz, (1) negative regulation, and (2) positive regulation. In negative regulation, this system and inhibitor is present in the cell, which prevents transcription by inactivating the promoter. This inhibitor is known as repressor. For initiation of transcription, an inducer in required. Inducer acts as antagonist of the repressor. In the negative regulation, absence of product increases the enzyme synthesis and presence of the product decreases the synthesis. Positive Control In positive regulation, this system, an effectors molecule (which may be a protein or a molecular complex) activates the promoter for transcription. In a degradative system, either negative or positive mechanism may operate. In a biosynthetic pathway negative mechanism usually operates.

It is essential to define various terms which are commonly used in connection with regulation of gene expression. A brief description of important terms is presented below:

Repressor: In operon, protein molecules which prevents transcription. The process of inhibition of transcription is called repression.

Inducer: The substance which allows initiation of transcription (i.e., lactose in lac operon). Such process is known as induction.

Corepressor: A combination of repressor and metabolite which prevents protein synthesis. Such process is termed as corepression.

Inducible enzyme: An enzyme whose production is enhanced by adding the substrate in the culture medium. Such system is called inducible system.

Repressible enzyme: An enzyme whose production can be inhibited by adding an end product. Such system is known as repressible system.

Constitutive enzyme: An enzyme whose production is constant irrespective of metabolic state of the cell.

Negative control: Inhibition of transcription by repressor through inactivation of promoter e.g in lac operon.

Positive control: Enhancement of transcription by an effector molecule through activation of promoter.

Effector: The molecule which acts as an inducer or corepressor in the operon model of E.coli

The Operon Model: The operon refers to a group of closely linked genes which act together and code for various enzymes of a particular biochemical pathway. In other words, operon is a model which explains about the one-off mechanism of protein synthesis in a systematic manner. The operon model of gene regulation was proposed by Jacob and Monod in 1961. They were awarded Nobel prize for this discovery in 1965. The operon model was developed working with lactose region (lac region) of the human intestine bacteria E.coli.

The gene regulation was studied for degradation of the sugar lactose. The operon model consists of seven main components, viz, (1) structural genes, (2) operator gene, (3) promotor gene, (4) regulator gene, (5) repressor, (6) corepressor, and (7) inducer.

LAC OPERON CONCEPT

Components of operon:

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 corepressor. 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 coordinated 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.

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 biosynthetic 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.

Changing Concept of Gene

(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 mutant 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.

(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.

CLASSIFICATION OF GENES

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.

1. Based on Dominance

- Dominant genes: Genes that express in the F1
- Recessive genes: Genes whose effect is suppressed in F1

2.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

3.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.

4.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.

5.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.

6. 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.

7. 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 nonfunctional. These genes are defective copies of some normal genes.

8. 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.

9. Based on Operon Model

- Regulator gene : A gene found in lac operon of E.Coli which directs synthesis of a repressor In lac operon.
- Operator gene: 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.

10. 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.

Jumping Genes

Generally, a gene occupies a specific position on the chromosome called locus. However in some cases a gene keeps on changing 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.

IMPORTANT QUESTIONS:

1. Define gene regulation in prokaryotes and eukaryotes.
2. Describe lac operon, positive control and negative control mechanism.
3. Describe fine structure of genes.
4. Discuss in detail about types/ classes of genes with examples.