GENE EXPRESSION AND REGULATION

GENETIC MATERIAL :

- Earlier gene was regarded as a hypothetical particle. **Chromosomal theory** of **Sutton** and **Boveri** explained that chromosomes are the carriers of genes and are the basis for Mendelian segregation and independent assortment.
- Independently they discovered that chromosomes segregate during gametogenesis due to meiosis, like Mendelian factors.
- These gametes become haploid and the diploid nature is restored in zygote due to random fertilization. Then, which is the genetic material-cither proteins or nucleic acids?
- This was solved with the **transformation experiments** conducted by **Griffith. F.** Griffith 1928 worked on *Diplococcus pneumonias* (now named as *Streptococcus pneumonia*).
- He used type IIR and type III S types *of Diplococcus* for his experimentation. II R is a rough (non-capsulated) and nonvirulent form causing no 'death' when injected to mice.
- Ill S is a smooth (capsulated) and virulent form causing 'death' when injected to mice. These traits are genetically determined. When mice were injected with heat killed III S strain, mice were alive.
- When a mixture of heat killed III S strain and fresh II R strain was injected, the mice died. II R, the nonvirulent was transformed to virulent strain due to the heat killed III S strain.
- Griffith concluded that transforming principle might be polysaccharide capsule or a compound required for capsule synthesis.
- **O.T. Avery, MacLeod** and **McCarty**, 1944 identified the nature of transforming principle in *Diplococcus pneumoniae.*
- They treated the extract of transformed III S strain with different enzymes like protease, amylase, lipase, RNase and DNase and conducted transformation assay tests.
- When the III S filtrate was treated with DNase the transformation did not occur. If filtrate was treated with any other enzyme, transformation occurred.
- They concluded that DNA was the genetic material (a transformation principle or active factor).

FUNCTION OF GENES

- Many genes express whenever the product is required and the expression is regulated. These genes are called **regulated genes.**
- Certain genes are expressed as a function of the interaction between RNA polymerase with promoter without any regulation.
- Such genes are called **constitutive genes.** They are constantly needed for cellular activity.
- Flow of genetic information from DNA to protein through RNA was described by Crick as **central dogma of molecular biology.**

i. Gene expression

- Genes exert their effects on the phenotype. The biological information is contained in the base sequence of DNA.
- Gene expression is the process by which this information is made available to the cell. This has been described by the central dogma.
- This states that information is transferred from DNA to RNA and RNA to a protein. During expression, gene synthesises mRNA molecule, into which information has been transferred in the form of genetic code.
- This synthesis of mRNA from one of the strands of the DNA molecule is called **transcription.**
- The first step in gene expression is **transcription.** Two strands of DNA separate, only one of them acts as **template** for the transcription into mRNA in 5' $\rightarrow$ 3' direction.
- This strand is the noncoding **(antisense)** strand and the other stand of DNA is **coding strand (sense strand).**

- The synthesis of a polypeptide chain or a protein from mRNA is called **translation.** The sequence of amino acids in a polypeptide is determined by the nucleotide sequence of mRNA in accordance with the rules of the genetic code.
- mRNA contains codons and these codons are decoded to respective amino acids in the process of translation to a polypeptide.
- Translation occurs in the ribosomes. The amino acids in polypeptides and the nitrogen bases in DNA of the respective gene are related in a direct linear fashion. So, these two are **colinear.**
- Benzer 1955, proposed the terms cistron, muton and recon for the gene.
- **Cistron** : Cistron is a segment of DNA specifying one polypeptide chain in protein synthesis.
- **Muton** : Muton is the smaller segment of DNA that can be changed in a mutation. It can be as small as one complemental nucleotide pair.
- **Recon** : The smallest segment of DNA that undergoes recombination. It is also as small as one complemental nucleotide pair.

ii. Genetic code

- Genetic code is the relationship between the nucleotide sequence of mRNA and amino acid sequence that constitute a polypeptide chain.
- Codon is a triplet of nucleotides that code for a single amino acid. Anticodon is the triplet of nucleotides in a tRNA molecule that is complementary to nucleotide bases of a codon in a mRNA molecule.

Properties

1. It is same in all the living organisms. So genetic code is **universal.**
2. In triplet codon, group of three ribonucleotides codes for one amino acid only. As each genetic code specifies only one amino acid genetic code is **unambiguous.**
3. One amino acid is specified by more than one codon. So genetic code is called degenerate.
4. The multiple codons specifying one amino acid are grouped together, Hence genetic codes are **ordered.**
5. Most often multiple genetic codes of one amino acid are varying by only the third nitrogenous base.
6. AUG (codes for methionine) is the **initiator codon.** This genetic code initiates transcription of mRNA. UAG (amber), UAA (ochre) and UGA (opal) serve as **terminator codons.** These codons terminate the transcription.
7. Codons are **without punctuation** (comma less) and are written in linear form to compose a mRNA molecule.

GENE REGULATION IN PROKARYOTES (OPERON CONCEPT)

- The studies of bacterial genetics indicate that all genes not only specify the structure of an enzyme but some of them also regulate the expression of other genes.
- These genes are called **regulator** genes. This concept of gene regulation has been studied by **Francois Jacob** and **Jacques Monod** in 1961 in *E. coli.* They proposed the **operon hypothesis.**
- According to the operon hypothesis gene regulation in prokaryotes and bacteriophages involves **structural genes,** the **operator,** the **promoter,** the **regulator genes,** repressor **proteins** and an **inducer.**

Lac operon of *E. coli* :

- A genetic unit that consists of one or more **"structural genes"** (cistrons that code for polypeptides) and an adjacent **"operator-promoter"** region that controls the transcriptional activity is called **operon.**
- The lac operon of *E. coli* has the following :

1. Structural genes:

- The lactose operon of *E. coli* is composed of three structural genes Z, Y and A. 'Z' gene transcribes mRNA for a single long polypeptide chain to form the enzyme β**-galactosidase.**
- This enzyme catalyses the hydrolysis of lactose into glucose and galactose. 'Y' gene transcribes the mRNA that translates the permease protein.
- It is located in the bacterial cell membrane arid affects the uptake of lactose from external medium.
- 'A' gene specifies the enzyme **transacetylase** which catalyses the acetylation of β-**galactoside.**

2. The Operator region:

- Operator lies immediately left to the structural genes. When the operator is 'on', the structural genes transcribe mRNA. When it is 'off, the structural genes can't function.
- Operator is the **target** for the attachment of repressor protein produced by the regulator gene.

The promoter region:

- The actual site of transcription initiation is known as **promoter** region. It lies left to the operator region. mRNA transcription by the structural gene is catalysed by an enzyme **RNA polymerase.**
- This enzyme first binds to the promoter region and then moves along the operator region and structural genes.

4. Regulator gene and repressor protein:

- The regulator gene transcribes an mRNA for the protein called **repressor.**
- This lac repressor regulates the expression of structural genes by binding to the operator region.
- In the absence of lactose, repressor binds to the operator and blocks the path of RNA polymerase and prevents the expression of structural genes.

5. Inducer:

- Lactose is an inducer of lac operon. This inducer binds to the repressor and changes its configuration, such that it can not bind to the operator. RNA polymerase path way is cleared allowing the expression of structural genes.
- **Such molecules that induce the expression of any operon by binding to the** repressor **are called inducers.**

GENE EXPRESSION IN EUKARYOTES :

- Unlike the prokaryotes in eukaryotes genes have stretches of bases, that code for amino acids called **exons** and that do not code for amino acid called **introns**. These eukaryotic genes are called **split genes.**
- Heterogenous nuclear mRNA (hn RNA) is transcribed from the split genes. The unwanted RNA regions in the hnRNA are removed and the regions coding for amino acids are joined together. This process is called gene splicing.
- It is directed by RNA protein complexes called spliceosomes that remove complementary nitrogen bases of introns in eukaryotic **hn RNA.**

CONCEPTS OF GENE ACTION :

i. One gene and one enzyme hypothesis

- **Beadle and Tatum, 1958** proposed that single mutation interferes with the synthesis of single enzyme. Thus each gene in an organism controls the synthesis of a specific enzyme.
- **One gene-one enzyme,** or **one gene-one phenotype** relationship is best explained by the experiment on muntants *of Neurospora crassa.*
- This can normally survive on a minimal medium containing sugar, organic acids and vitamin biotin. **G.W. Beadle and E.L.Tatum** produced mutants in *Neurospora* crassa by exposing the conidia to X rays and ultraviolet rays.
- These mutants required additional nutrients in the minimal medium for their survival. They are therefore known as nutritional mutants or auxotrophs.
- The extra nutrients are required because the mutants produced could not synthesise the amino acid arginine from the precursors available in the minimal medium.
- While the wild type *N.crassa* could synthesise arginine from the precursors available in the minimal medium through intermediate compounds such as ornithine and citrulline.
- They also observed that the mutants are of 3 types.
- Mutant 'a' required arginine supplemented medium; Mutant 'b' required citrulline or arginine added medium and mutant V is requiring ornithine or citrulline or arginine containing medium.
- These differences, they argued, are due to the lack of suitable specific enzyme in each mutant for the required conversion.
- They attributed the absence of enzyme to the changes caused by mutation that caused the loss of respective gene. It is therefore contended that one gene controls synthesis of one enzyme.

ii. One cistron - one polypeptide hypothesis

- One gene-one enzyme hypothesis was redefined as **one cistron-one polypeptide hypothesis by V.M. Ingram** *et al.*
- One cistron in an organism specifies the synthesis of one polypeptide chain and not the complete enzyme or protein molecule.
- Hence for synthesis of an enzyme or protein, polycistronic gene is required, e.g. The gene for haemoglobin synthesis is polycistronic with α_1, α_2, β_1 and β_2 cistrons.
- Any mutation in β cistron leads to the synthesis of **sickle cell haemoglobin** (amino acid, glutamic acid, at 6^{th} place of normal haemoglobin is replaced by valine). Since cistron is recognised as a unit of function, it is called **one cistron - one polypeptide hypothesis.**