GENE EXPRESSION

The second important characteristic (first is transmission) of the gene is to store and express the genetic information that
will contribute towards the phenotype.
DNA carries information for the synthesis of all proteins required for the function of a cell. A close relationship between genes and enzymes (or gene control the metabolism) was first discovered by a British physician Archibald Garrod in 1909. He observed that certain hereditary diseases in man such as alkaptonuria (black urine disease), albinism (absence of melanin pigment) phcnylketonuria etc. are 'inborn errors of metabolism and are due to the defect in the enzyme that catalyses the conservation of one metabolic substance to another. Thus, the inherited genetic defect is reflected in the deficiency of an enzyme.

Beadle and Tatum's Hypothesis (One gene one enzyme hypothesis)

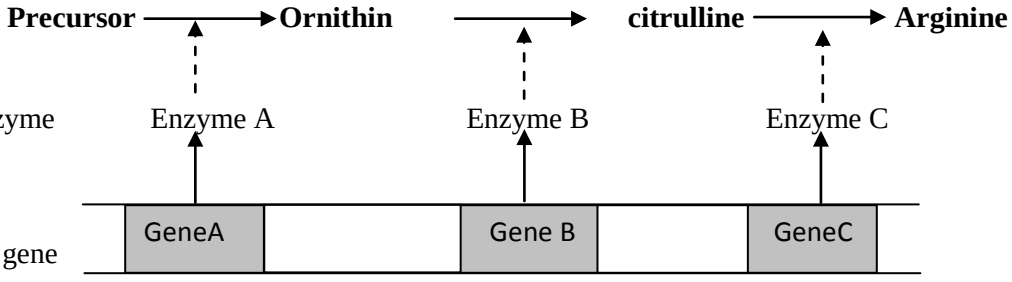
The concept that genes have the information to produce enzymes, or gene metabolism relationship was experimentaly proved by Beadle and Tatum (1948), on the basis of experiments conducted on pink bread mould *(Neurospora crassa).* This mould can normally grow in a simple minimal medium containing salts and sugar, making all other chemicals such as amino acids, purines, pyrimidines etc. through enzyme catalysed reactions. This wild type of the mould is called 'prototroph'. Beadle and Tatum exposed the pink bread mould to X-rays, which can bring about a change in the nucleotide sequence of DNA and thus causes mutation. They found that the mutants were unable to grow on a minimal medium. Each type of mutant required some extra nutrient in the minimal medium for it's normal growth. Such nutritional mutants are called 'auxotrophs'. They obtained different nutritional mutants requiring amino acids ornithine or citrulline or arginine for growth. The mutants could be classified into three types:

(i) some could grow on ornithine -, or citrulline -, or arginine - containing medium,
(ii) some could grow on citrulline - or arginine containing medium, and
(iii) some could grow only on arginine supplemented medium

Effect of medium supplements on the growth of mutants Neurospora crassa

Growth on Medium supplemented with			
Mutant Type	Ornithine	Citrulline	Arginine
I	+	+	+
II	-	+	+
III	-	-	+
+ = growth, - = no growth.			

Biochemical Pathway



This shows that all mutants could grow on arginine supplemented medium suggesting that arginine is the final product of this pathway.
Since some mutants could not grow on ornithine, this compound must be synthesized earlier than citrulline and/or arginine.
Beadle and Tatum taking clue from this growth behaviour of Neurospora and other experiments, proposed the arginine biosynthetic pathway. The precursor compound first gives rise to ornithine, ornithine then gives rise to citrulline and the latter is finally converted into arginine; each of these steps is

- The mutant of **class I,** could grow on ornithine, citrulline or arginine, suggesting that they lacked the capacity to synthesize ornithine, but beyond that they could complete the pathway.
- Similarly, mutants of **class II,** could not convert ornithine to citrulline, but if supplied citrulline could synthesize arginine.
- The last mutants of **class III** could not convert citrulline to arginine and had to be supplied latter for growth.
- Beadle and Tatum reasoned that these defects could arise due to defective enzymes in each case. Since such changes were mutational, they held that one gene controls one enzyme in a pathway leading to their famous **'one gene one enzyme hypothesis'.**

  The hypothesis states that each gene controls the synthesis of a specific enzyme or protein. Beadle and Tatum were awarded with Noble prize for this work in 1958. Their work founded the new science of **'biochemical genetics'.** Beadle and Tatum used **total isolation method** to detect the nutritional mutant of Neurospora.

  Modification of Beadle and Tatum's Hypothesis - One gene one enzyme hypothesis held that a gene has information to produce one enzyme. The enzymes are proteins, but all proteins are not enzymes. Recently some RNAs have also been found to manifest enzyme activity. Proteins are complex molecules and may be composed of one or more polypetide chains. For example, haemoglobin consists of four polypetide chains-2a **and** 2^ **chains.** Thus, more than one gene may control the synthesis of a protein. It is now held that one gene is responsible for the formation of one polypetide chain. Therefore, one gene one enzyme hypothesis has been replaced by **'one gene one polypetide hypothesis'.** Further modification came when one gene was identified as a **functional unit or cistron** and the same was called **'one cistron-one polypeptide hypothesis'.**

TYPES OF GENE

All the genes do not play the same role nor all genes are active all the time. With regard to their role and activity, the genes are of following types:

Jumping genes -

It is a segment of DNA which moves from one chromosome to another chromosome wihtin the genome of an indivisual. McClintock (1983) got nobel prize for the discovery of jumping gene in maize. Two transposable controling elements (Ds) and activator (Ac), which can jump to any chromosome from their original location on chromosome 9. Also in bacteria plasmid transposone carry gene for antibiotic resistance (ampieilline).

Other Examples :

Transposable elements (TE) in Drosophila- As much as 10% of the genome consist of transposons, most important

of these are **copia like element, Fold back (FB)** (for eye colour), and P and I element (for sterlity). Ty - element in yeast.

- **Overlapping gene** - A few genes in certain bacteria and animal viruses code for two different polypeptides. These are called overlapping genes. For example-in ϕ x 174 virus, SV-40 virus.

- **Constitutive genes (House-keeping genes)** - These genes are expressed constantly, because their products are constant needed for cellular activity, e.g. genes for glycolysis, gene of ATPase enzyme.

- **Non-constitutive genes (Smart gene or Luxary gene)** - These genes remain silent and are expressed only when the gene product is needed. They are switched 'on' or 'off according to the requirement of cellular activities. Non-constitutive genes are of two types; **inducible and repressible.** The inducible genes are switched-

  on in presence of a chemical substance called **inducer,** required for the functioning of gene activity. The repressible genes continue to express themselves till a chemical, often an end product of the metabolism inhibits or represses their activity. Such type of inhibition is called **feed back inhibition** or **feed back repression.**

- **Homeotic genes** - Homeotic gene regulates the organ differentiation in embryo.

  Homeobox related to transcription of homeotic gene.

If mutation takes place in homoetic gene organ formation is disturbed.

- Pseudoallele - Gene which is located on non homologous chromosome or gene which is located on different locus on homologous chromosome produces almost same phenotype called as pseudoallele.
  Pseudo allele is non allelic gene produced identical phenotype in cis-trans position, eg. Duplicate gene
- Isoallele - If several alleles exhibit same phenotype then they are said to Isoallele. In Drosophila allele W^{+c} W^{+s} W^{+s} —> produce red eye colour
  Hybrid vigour/Heterosis - Superiority of offsprings over it's parents is called as Hybrid vigour & it develops due to Heterozygosity.
- Hybrid vigour can be maintained for long time in vegetaively propagated crops.
- Hybrid vigour can be lost by inbreeding (selfing) because inbreeding induces the Homozygosity in offsprings. Loss of Hybrid vigour due to inbreeding, is called as inbreeding depression.

☞ Inbreeding depression mainly related to cross pollinated crops.

REGULATION OF GENE EXPRERSION

- The mechanism which stimulates the expression of certain genes and inhibits that of others is called regulation of gene expression.
- It is possible only if the organism has a mechanism of regulating gene activity by allowing some to function and others to restrain their activity through switching on and switching off system. This means, the genes are turned 'on' or 'off as per requirement. \
- A set of genes is 'switched on' when enzymes are required to metabolise a new substrate. The enzymes produced by these genes metabolise the substrate.
- The molecules of metabolite that come to switch on of the genes are termed as inducers and the phenomenon is called induction.
- Similarly, certain genes which are in their 'switch on' state, continue to synthesise a metabolite till the later is produced in amount more than required or else, it is supplied to the cell from outside. In other words, certain genes continue to express themselves till the end product of inhibits or repress their expression. Inhibition by an end product is known as 'feed back repression'.

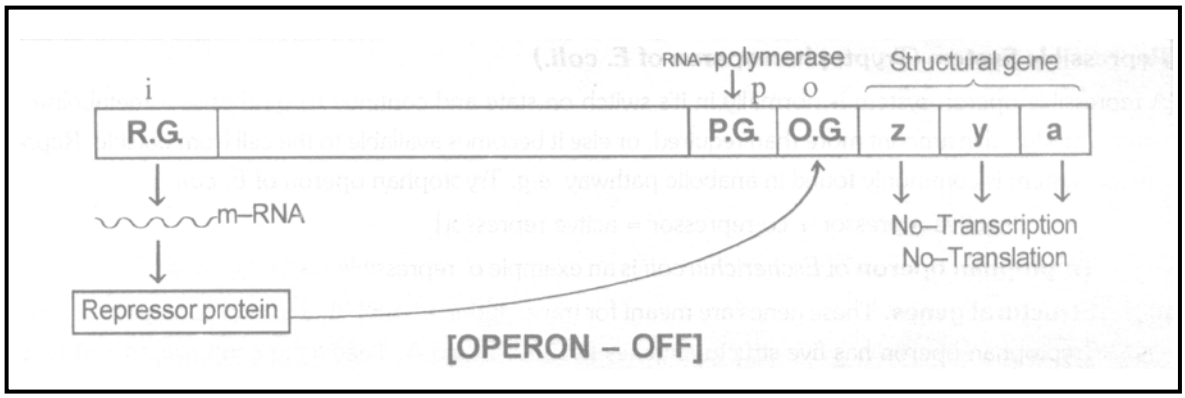
OPERON CONCEPT

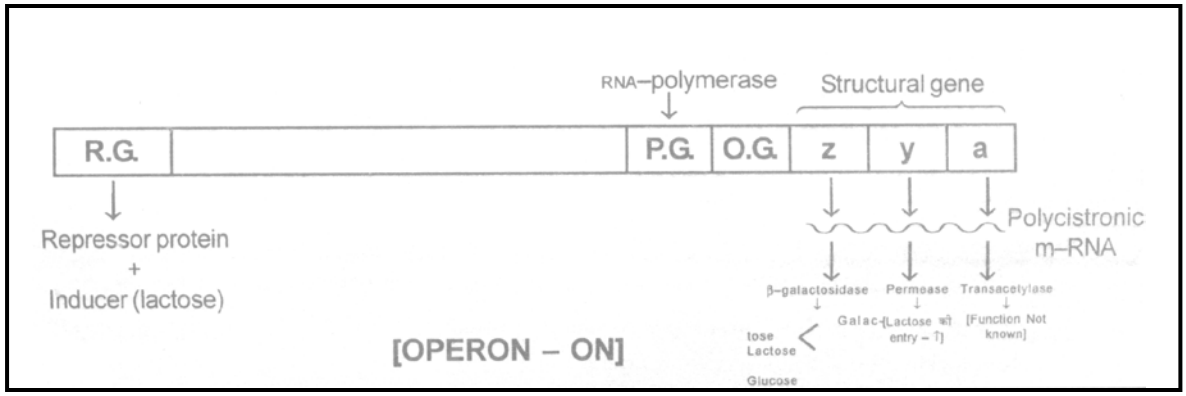
- In 1961, two French microbiologist Francis Jocob and Jacques Monad at the Pasteur Institute in Paris, proposed a mechanism called operon model for the regulation of gene action in *E. coli.*
- *An operon is a part of genetic material or DNA, which acts as a single regulated unit having one or more structural genes-an operator gene, a promoter gene, a regulator gene.*
- Operons are of two types (i) inducible (ii) repressible.

1. Inducible System (Lac operon of £. *coli)*

- An inducible operon system normally remains in switched off condition and begins to work only when the substance to be metabolised by it is present in the cell. Inducible operon system generally occurs in catabolic pathways, *e.g.* Lac operon of *E. coli.*

Active represser + inducer = inactive represser





An inducible operon system consists of four types of genes

(i) **Structural genes** - These genes synthesise mRNAs, which in turn synthesise polypeptide or enzyme *over* the ribosomes. An operon may *have* one or more structural genes. Each structural gene of an operon is called cistron. The lac operon (lactose operon) of *Escherichia coll* contains three structural genes (Z, Y and A). These genes occur adjacent to each other and thus are linked. They transcribe a polycistronic mRNA molecule (a single stretch of mRNA covering all the three genes), that helps in the synthesis of three enzymes-p" galactosidase, lactose permease and transacetylase.

(ii) **Operator gene** - It lies adjacent to the structural genes and directly controls the synthesis of *mRNA over* the structural genes. It is switched off by the presence of a repressor. An inducer can take away the represser and switch on the gene that directs the structural genes to transcribe.

(iii) **Promoter gene** - This gene is the site for initial binding of RNA polymerase. When the operator gene is turned on, the enzyme RNA polymerase moves over it and reaches the structural genes to perform transcription.

(iv) **Regulator gene** - It produces a repressor that binds to operator gene and stops the working of the operator gene.

(v) **Repressor** - It is a protein, produced by the regulator gene. It binds to the operator gene so that the transcription of structural gene stops. Repressor has two allosteric site (1) operator gene (2) effective molecule (inducer/corepressor)

(vi) **Inducer** - It is a chemical (substrate, hormone or some other metabolite) which after coming in contact with the repressor, forms an **inducer repressor complex.** This complex cannot bind with the operator gene, which is thus switched on. The free operator gene allows the structural gene to transcribe mRNA to synthesise the enzymes.

The inducer for lac operon of *Escherichia coli* is lactose (in fact allolactose an isomer of lactose). When the sugar lactose is added to the culture of *E. coli*, a few molecules of lactose gets into the bacterial cells by th... action of the enzyme *permease*, *a* small amount of this enzyme is present in the cell even when the operon is not working. These few lactose molecules are then converted into an active form which acts as an inducer and binds

to the repressor protein. The inducer repressor complex fails to join with the operator, which is turned on. The three genes are expressed as three enzymes to metabolise lactose. **Allolactose is real inducer of lac operon.** Gratuitous inducer —> Some molecules resemble with natural inducer but are not metabolize by the enzyme. Example : **Isopropyl thio galactoside (IPTG)**: This resembles lactose and thus has the property of induction. Such inducer which induces enzyme synthesis, without getting metabolized are called **gratuitous inducer.**

2. Repressible System (Tryptophan operon of £. colt.)

- A repressible operon system is normally in it's switch on state and continue to synthesise a metabolise till the latter is produced in amount more than required, or else it becomes available to the cell from outside. Repressible

operon system is commonly found in anabolic pathway, *e.g.* Tryptophan operon of *E. coli.*

[Inactive represser + co-repressor = acitve represser]

Tryptophan operon of *Escherichia coli* is an example of repressible system. It consists of the following:

(i) Structural genes. These genes are meant for transcription of *mRNA,* which in turn synthesise enzyme. Tryptophan operon has five structural genes E, D, C, B and A. They lie in continuation and synthesise enzymes for five steps of tryptophan synthesis.

(ii) Operator gene (trp O). It lies adjacent to the structural genes and controls the functioning of the structural genes. Normally, it is kept switched on, because the apo-repressor produced by the regulator gene does not bind to it. The operator gene is switched off when a co-repressor is available alongwith apo-repressor.

(iii) Promoter gene (trp P). It marks the site at which the RNA polymerase enzyme binds. When the operator gene is switched on, it moves from promotor gene to structural genes for transcription.

(iv) Regulator gene (trp R). It produces a regulatory protein called apo-repressor for (Inactive represser) possible blocking the activity of operator gene.

(v) **Apo-repressor**. It is a regulatory protein synthesised by regulator gene. When a co-repressor substrate is available in the cell, the apo-repressor combines with the co-repressor to form a apo-repressor co-repressor complex. This complex binds with the operator gene and switches it off. Presence of apo-repressor alone, the operator gene is kept switched on because, by itself the apo-repressor is unable to block the working of operator gene.

(ui) Co-repressor. It is an end product of reactions catalysed by enzymes produced by the structural genes. In the presence of tryptophan some molecules of tryptophan act as co-repressor, co-repressor-bind with

inactive represser, co-repressor repressor complex bind with-operator region and prevent the binding of RNA polymerase to the promoter, the trp-operon is off.

