

Epialleles: The Missing Link in Field of Genetics

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Introduction:

Variation in the degree of methylation of a gene among individuals, results in unique traits that are passed down through generation is known as Epialleles (Kalisz and Purugganan, 2004) or rather than persistent nucleotide mutations, alleles differ from one another in the patterns of methylation of the gene's DNA nucleotides is known as Epialleles. The science dealing with study of heritable changes (changes that can be passed down to an organism's offspring) in gene expression which does not involve changes to the underlying DNA sequence *i.e.*, a change in phenotype without a change in genotype is known as Epigenetics. An Epigenome consisting of a record of chemical changes to the DNA and histone proteins of an organism. A change in gene expression is resultant of DNA bases modification, histone proteins and/or non-coding RNA biogenesis modification.

Epigenetics play an important role in plant defence mechanism in response to various stresses that arise either biotic (e.g., pathogen, insects) or abiotic (e.g., salinity, draught, cold, toxicity of chemicals, pH, heat) factors (Singroha and Sharma, 2019). So, in order to protect or adapt them in adverse or changing environmental condition, plants modify themselves at molecular level. These molecular changes are provided by epigenetic regulations which improve the survival of plants by changing their tolerance level to various stresses (Richards, 2006; Hirayama and Shinozaki, 2010). Now it is clear that heritable phenotypic variation does not need to be based on DNA variation (Richards, 2006; Bossdorf *et al.*, 2008).

The term, “Epigenetic” was given by Conrad Waddington, known as Father of Epigenetic, in the year of 1942. He explained how asset of DNA sequences could dynamically give rise to a complex organism.

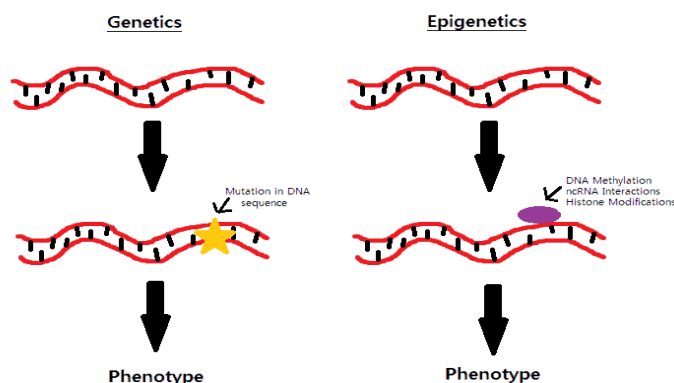
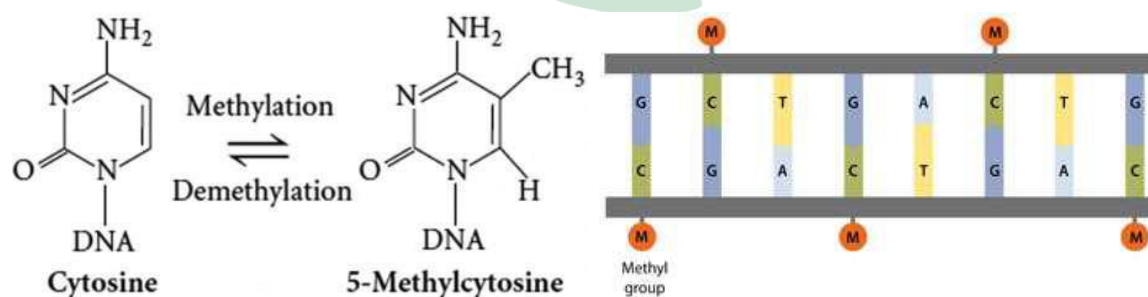


Fig. 1: Difference between the genetics and epigenetics

Regulatory mechanisms controlling Epigenetics:

There is main three types of regulatory mechanisms observed for the control of epigenetic phenomenon viz., DNA methylation, Histone modification and RNA mediated interference (Singroha and Sharma, 2019).

1. DNA methylation: DNA methylation is a chemical modification, catalyzed by enzyme cytosine methyltransferases. In that chemical alteration in which addition of a methyl group (-CH₃) is carried out in a DNA sequence onto the cytosine residue in a sequence specific manner, primarily within CpG dinucleotide (Bewick *et al.*, 2016; Niederhuth and Schmitz, 2017). The added methyl group provides platform for attachment of various protein complexes that alters or modifies the histone scaffolds which will result in altered gene expression. It occurs in Cytosine ring at fifth carbon position. It leads to formation of 5-methyl cytosine. Based on target site, methylation is of two type viz., symmetrical (CG & CHG) and asymmetrical (CHH). In plants, cytosine is methylated at CpG, CpHpG and CpHpH sites, where H represents any nucleotide. In other words, plants have symmetrical and asymmetrical type of methylation (Miura *et al.*, 2001).



In plants, some genes are methylated and that are generally to CG within gene, while in case of transposable elements (TE), it is methylated at CG, CHG and CHH positions. Gene silencing is resulted as a consequence of methylation in TE and promoter region of gene, whereas gene expression is induced as a consequence of methylation inside gene (Simon *et al.*, 2005). A methylated cytosine (methyl cytosine) in gene affects the gene expression while that in repetitive sequences (TE) prevent it from compromising normal genome function (Miura *et al.*, 2001; Simon *et al.*, 2005). Increasing in methylated gDNA reduces gene expression which enables plant to conserve energy for the sake of biotic or abiotic stresses. In contrast, the reduction in methylation of resistance-related genes favors chromatin activation and the expression of novel genes, which provides long-term or permanent resistance for stress.

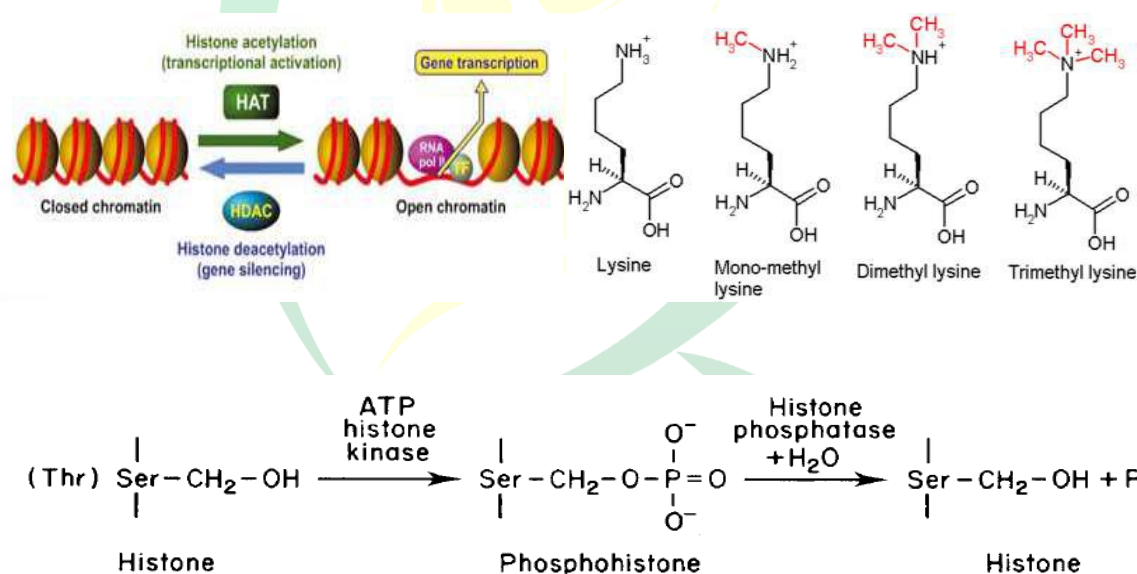
- 2. Histone modification:** A modification in histone protein also plays a major role in field of epigenetic. Histone is a type of protein that is one of the structural components of DNA organization in eukaryotes and it has several variants. In genome organizations, nucleosome is composed of two copies of each type of histone protein, *viz.*, H2A, H2B, H3 and H4 forming a histone octamer around which 147 bp of DNA sequence is wrapped. The N terminal of histone protein is affected by chemical alteration such as acetylation or methylation. Such type of alteration is associated with suppression or activation of gene (Heet *et al.*, 2011; Roudier *et al.*, 2011).

Histone acetylation/deacetylation: Addition of acetyl group to the N terminal lysine of histones known as acetylation leads to transcriptional activation of DNA sequence (Fuchs *et al.*, 2006) which causes reduction in the net positive charge of histone protein and as a result, the electrostatic force of attraction between the negatively charged DNA and positively charged histone protein reduces which leads to the loosening of chromatin and transcriptional activation of DNA (Shahbazian and Grunstein, 2007). This addition of acetyl group is catalyzed by an enzyme histone acetyltransferase (HATs).

Histone methylation: In case of histone methylation, amino acids arginine and lysine undergo methylation changes. Based on nature of amino acid residue methylation, it can either activate or deactivate gene. A different type of arginine and lysine in different histones protein undergoes different type of methylation changes. The amino acid residues undergone may be one, two or three methylated. In case of arginine, it is

methylated at one or two places whereas in case of lysine, it undergoes mono, di and tri methylation. Methylation affects the hydrophobicity of the histone and hence may change histone DNA interactions or may create binding site for various proteins which restricts the binding of transcription machinery and prevents transcription. Histone lysine methyltransferases (HKMT) and protein arginine methyltransferases (PRMT) catalyze methylation of lysine and arginine residues respectively (Kanno *et al.*, 2010).

Histone phosphorylation: This alteration i.e., histone phosphorylation is catalyzed by the enzyme protein kinase in which phosphorus molecules is added on serine, threonine and tyrosine residues mainly in the N terminal of histone tail. The process of phosphorylation increases the negative charge on histone protein, as a resultless interaction between DNA and histones that leads to chromatin de-condensation. Whereas in case of dephosphorylation, it is catalyzed by enzyme phosphatase and which increases positive charge followed by chromatin condensation.



3. **RNA mediated interference:** This process of epigenetic modification is also known as post transcriptional gene silencing (PTGS). This RdDM (RNA directed DNA methylation) is *de novo* in which methylation occurs in cytosine residues mainly within region of RNA-DNA with sequence identity. A RdDM pathway specifically methylate CHH sequence because CHH methylation at many silenced loci is dependent on RNA-guided *de novo* methylation, but it has ability to methylate all sequence as symmetrical



methylation is maintained through each DNA replication followed by maintenance methylation (Matzke *et al.*, 2009).

Methods to study epigenetic modifications:

1. DNA methylation:

- a. Bisulfite sequencing
- b. Methylation Sensitive Amplification Polymorphism (MSAP)
- c. By affinity purification

2. Histone modification:

- a. Chromatinimmunoprecipitation(ChIP)

3. Non-coding RNA

- a. Deep sequencing

Different functions regulated by the epigenetic mechanism:

1. It plays an important role in gene silencing
2. Silencing of repetitive elements and transposable element (TE)
3. Establishment and maintenance of imprinted genes
4. Specific DNA methylation patterns, chromatin modifications and correction of chromatin organization
5. It controls gene and tissue-specific epigenetic patterns
6. It involved in plant abiotic stress management

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