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Review paper

Epigenetic modifications and its basic mechanism

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Heritable changes in the plant's phenotype are attributed to genomic sequence change and also by epigenetic variations. These epigenetic variations are involved in controlling plants' developmental processes. Intense and close breeding has reduced the genetic variations in crop increasing their susceptibility to the changing environment. Epigenetic diversity has now emerged as a new source of variation for coping with changing environmental stresses in plants. Epigenetic modifications like DNA methylation, post-translational histone modifications, histone variants, and involvement of non-coding RNAs have played a major role in gene expression and regulation in plants. These epigenetic modifications have created the variability in phenotypic expression by selective turning on and turning off of the genomic sequence. These variabilities are created in plants in response to the environmental factors to which plants are exposed. These phenotypic variations accumulated by epigenetic modification are transferred and expressed in the next generation as they are heritable. DNA methylation and methylation of histone tails on the lysine 4, 9, and 27 positions are among the best-characterized epigenetic marks observed in both plants and animals. These modifications marks have altered the physical state of the DNA. The alternation in the physical state of DNA has changed the way cell reads the genes. This is the potential new area of the research as it creates phenotypic variability in response to stress factors without changing the chemical properties of the DNA. In this paper, we have presented the epigenetic modifications and the way they controlled the gene expression in plants and animals.

Key words: DNA methylation, histone modification, histone variants, epigenetics

INTRODUCTION

The word epigenetics for the first time was coined by Waddington. He described epigenetic as "All those events which lead to the unfolding of the genetic program for development" (Waddington, 2009). Epigenetics made up of epi and genetics where epi stands for above so, epigenetics lies above genetics and it describes those phenomena that classical genetics could not. Epigenetics studies mitotically heritable changes in gene expression that occurs without altering the DNA sequence of an organism (Handy et al., 2011). This mitotic heritability of the epigenetic state helps to maintain cell identity. It is due to epigenetics that different organs have different cell types though they have the same genetic materials. The genes that are expressed in one of the organs are not expressed in another. Epigenetics transfers certain epigenetic marks of the parents to their offsprings (Pang et al., 2017). Epigenetic modifications in the plant genome are vital for the plant's survival against biotic stresses like bacteria, fungus, viruses, insects, and abiotic stresses like heat, cold, nutrient stress, salinity, etc. Genome-wide study of *A. thaliana* suggests that biotic stress in the plants is a major factor shaping epigenome (Dowen et al., 2012). Mutational changes in the regulator of DNA methylation and demethylation can alter the plant susceptibility to pathogen infestation. Mutation of the pol V enzyme involved in the RdDM pathway decrease resistance to necrotic fungal infestation (*Botrytis cinerea* and *Plectosphaerella cucumerina*) (López et al., 2011). Nodulation by symbiotic bacteria in the *Medicago truncatula* requires demethylase enzyme DEM. Several genes are differently methylated during nodulation in a plant (Nagymihály et al., 2017; Satgé et al., 2016). Root infection by cyst nematode in soybean and *Arabidopsis thaliana* have also induced widespread DNA hypomethylation (Dowen et al., 2012). All these biotic factors create variation in phenotypic level which is heritable and can be used in crop improvement. In *A. thaliana,* the induced change in DNA methylation by high salinity stressed is partly transmitted to the progeny (Sanchez & Paszkowski, 2014). Cold treatment of tomato fruits disturbs the DNA methylase DML2. Downregulation of DML2 causes hypermethylation and silencing of the gene responsible for the biosynthesis of flavor volatiles in tomato, explaining the flavor loss of tomato during cold storage (Zhang et al., 2016). Histone variants also affect the genomic stability

and gene expression in a plant. The plant is unable to incorporate the histone variant H2A.Z into nucleosomes when they are grown at elevated temperature and they display a constitutive high-temperature transcriptome (Abel & Duncavage, 2013). Current research on epigenetic mechanisms indicates that DNA methylation, histone posttranslational modifications, and small non-coding RNAs are involved in almost every aspect of plant life including agronomically important traits such as flowering time (Vasudevan, 2019), fruit development, responses to environmental factors, and plant immunity(Álvarez-Venegas & De-la-Peña, 2016). In this article, we have presented the different epigenetic modifications.

DNA METHYLATION

DNA methylation is the most studied epigenetic modification that almost exclusively occurs in mammals involving the covalent transfer of methyl group to the C5 position of the cytosine base to form 5-methyl cytosine (Moore et al., 2013). Methyl group from SAM (S-Adenosyl Methionine) is added to the Cytosine with the help of an enzyme DNA methyltransferase. DNA methylation occurs in several locations, *i.e.* intergenic region, repetitive elements and CpG island of the genome must frequent being CpG island in case of mammals, which accounts for 98% of the total methylated sites (Lister et al., 2009). DNA methylation at CpG islands leads to the silencing of the gene expression. Methylated CpG (^{me}CpG) in CpG island is associated with the formation of the repressive chromatin structure, and this ^{me}CpG can be bound by methylated CpG binding protein (*e.g.* MeCP 1&2) (Jeziorska et al., 2017). These MeCP proteins have two domains: DNA binding domain and the transcriptional repression domain. Apart, these MeCP proteins recruit other factors that condense chromatin (Lim et al., 2019). CpG methylation has crucial role in imprinting and X-chromosome inactivation. X inactivation is the epigenetic dose compensation mechanism in mammals so that males and females have the same dose of a gene on the X chromosome (Riggs, 1975). The Paternal X chromosome is inactivated in a marsupial, where random inactivation of one of the X chromosome occurs in eutherians (Brown & Chandra, 1973). Random X inactivation occurs at the Gastrulation stage of the embryo, then after this epigenetic stage is mitotically heritable to all of these stages (Iyer et al., 2011). Methylation of CpG islands can impair transcription factor binding, and alter gene expression but the CpG islands in the promoter region of the gene are usually unmethylated. Further studies are needed to determine to what degree DNA methylation of CpG islands regulates gene expression. Inactive DNA is generally highly methylated compared to the DNA that is actively transcribed. DNA methylation is laid down by denovo methyltransferase: DNMT3a, and DNMT 3b in the case of mammals. These enzymes are also involved in the maintenance of DNA methylation. DNA methylation is maintained by DNMT1. This DNMT1 recognizes the hemimethylated DNA during the cell division process and methyl group to the newly synthesized strand. The base pairing property of the DNA allows the base pairing of CpG for the reciprocal maintenance of the methylation in the newly synthesized strand during the subsequent replication cycle (Leonhardt et al., 1992).

DNA methylation at the intergenic regions has very important to maintain genomic integrity. Approximately 45% of the human genome is composed of transposons and viral elements that are silenced by DNA methylation(Michaud et al., 1994). If these elements are expressed in humans then they lead to the disruption of genes and mutation of DNA. DNMT1 null cells (cells lacking DNMT1) display genomic instability. DNA methylation plays a role in silencing cryptic start sites or cryptic splice sites (Greenberg & Bourc'his, 2019). Methylation at the repetitive elements leads to the silencing of the repeats to prevent transposition. Methylated cytosine base changes to thymine causing point mutation which prevents transposition(Bird, 1980). Repetitive regions of mammalian are difficult to study. Recent advancement in this sector shows that the state of these regions may be environmentally sensitive (Law & Holland, 2019). DNA methylation is mitotically heritable and originally thought to be irremovable, except by the failure of DNMT1 to maintain methylation. DNA methylation occurs early in the developmental stage in primordial germ cell development and letter at the specific stage of development. Methylation marks of the DNA can be removed actively or passively. Ten-eleven translocation methylcytosine dioxygenase (TET) proteins are involved in active DNA demethylation (Wu & Zhang, 2014)The methods for studying DNA methylation include the use of methylation-sensitive restriction enzymes, which are sensitive to CpG methylation within their cleavage recognition site (Bird, 1978), bisulfate conversion of unmethylated sequence followed by PCR and sequencing (Y. Zhang et al., 2009) comparative genome hybridization, and microarray analysis (Fazzari & Greally, 2004), use of methylation-specific PCR (Herman, Graff, Myöhänen, Nelkin, & Baylin, 1996). Current research on epigenetic mechanisms indicate that DNA methylation, histone post-translational modifications and small non-coding RNAs are involved in almost every aspect of plant life including agronomically important traits such as flowering time (Vasudevan, 2019), fruit development, responses to environmental factors, and plant immunity(Álvarez-Venegas & De-la-Peña, 2016). Recent studies have found the Activation-Induced Cytidine Deaminase (AID) is also involved in demethylation (Dominguez & Shaknovich, 2014).DNA methylation is crucial for life, essential for viability as DNMT knockout dies in utero.

POST-TRANSLATIONAL HISTONE MODIFICATION

Histone acetylation

The addition of the acetyl group to the N-terminal tails involving amino acids such as lysine or arginine as well as serine, threonine, tyrosine of the histone molecules H3 and H4 is histone acetylation (Bannister & Kouzarides, 2011). Allfrey et al., 1964, reported histone acetylation for the first time. Histone acetylation is regulated by two enzymes, Histone acetyltransferase (HATs) and Histone deacetylase (HDACs). HATs and DHATs increase and decrease the DNA binding ability of histone molecules resulting in condensation and decondensation for gene activation and inactivation (Bannister & Kouzarides, 2011). When the acetyl group is added to the lysine residue positive charge of the histone molecule is neutralized to some extent and the affinity of histone to negatively charged DNA decreases. This provides access to the transcriptional machinery (Sterner & Berger, 2000). Acetylation marks of the histone proteins act as docking sites for other proteins (e.g. bromodomain proteins) that helps in chromosomal remodeling for the transcription of the gene. These bromodomain proteins can detect the acetylated histone inside the cell (Lee & Grant, 2018).

Histone methylation

Histone methylation unlike histone acetylation does not alter the electrostatic state but rather indirectly influences the gene transcription through the recruitment and binding of different regulatory proteins to chromatin (Alaskhar et al., 2018). Histone methylation may activate or deactivate gene transcription depending upon the location of the methylated residue. Methylated histone is recognized by chromodomain-containing proteins inside the cell. Histone methylation and demethylation are mediated by enzymes histone methyltransferase (HMTs) which includes lysine methyltransferase (KMTs) and arginine methyltransferase (PRMTs) and demethylation of histone by histone

demethylase (HDMs) (Youn, 2017). Histone methylation at lysine residue 4, 36, 79 (H3K4me, H3K36me, H3K79me) activates the gene whereas methylation at H3K9, H3K27, H4K20 are involved in the gene inactivation (Whetstine, 2010).

Histone phosphorylation

There is an addition of the (32) P- phosphate by the facilitation of enzymes phosphoryl transferase (phosphorylase) on serine, threonine, or tyrosine residue of the histone tail in histone phosphorylation. Histone phosphorylation for the first time discovered in 1967 (Gutierrez & Hnilica, 1967). Phosphate has negative charges thereby producing repulsion between histone and wrapped DNA consequently opening the nucleosome for transcriptional machinery (Watson & Higgins, 2016). Histone phosphorylation is mainly involved in DNA repair, transcription regulation, chromatin remodeling, apoptotic responses (Cao & Dang, 2018). Phosphorylated histones work with other histone modifications. Preexisting histone phosphorylation can recruit other histone-modifying enzymes, promoting other modifications. H3K14 is acetylated by the HAT Gcn5 acetyltransferase when there is the phosphorylation of H3S10, and it promotes transcription (Henry et al., 2003).

Histone ubiquitination

The ubiquitination of the histone is one of the post-translational modifications regulating cellular functions in cell signaling pathways in the case of eukaryotes (Alaskhar et al., 2018; Jason et al., 2002)**.** Histone ubiquitination was first observed in histone H2A. H2A and H2B are the most abundant ubiquitinated protein found in the nucleus of the cell (Cao & Yan, 2012). The C-terminal end of the H2A and H2B is the site for the mono-ubiquitylation. Histone molecule is ubiquitinize by histone ubiquitin ligase and ubiquitin is removed by deubiquitinatizing enzymes (DUBs). Mono-ubiquitination of the histone has a critical role in the translocation of proteins, DNA damage signaling, and transcriptional regulation. The ubiquitination of H2A is often associated with gene silencing whereas H2B ubiquitination (H2Bub)activates transcription initiation and elongation (Weake, 2014). There occurs cross-talk between histone ubiquitination and other histone modifications. Recent studies reveal the relation between H2Bub and H3-K4 methylation (Chandrasekharan, Huang, & Sun, 2009). Also, the mono-ubiquitination of H3 histone can induce H3 acetylation (X. Zhang et al., 2017).

HISTONE VARIANTS

Histone variants are non-canonical variants of histone with one or more amino acid differences from the conventional histones (Henikoff & Smith, 2015). Histone with varying stabilities that alters the functions of the nucleosome. Different variants exist for H2A, H3 not for the core histone H4 (Szenker, Boyarchuk, & Almouzni, 2014). Each histone variant has specific properties that differ from the canonical histones, and carry out a diverse role on replication, transcription, and heterochromatin formation (Talbert & Henikoff, 2017). Histone variant H2A.X is involved in the DNA repair, double-strand break, and is a universal histone variant that is highly conserved (Turinetto & Giachino, 2015; Weyemi et al., 2016; Yuan et al., 2010). H2A.X histone variant is used as a marker of the double-stranded break. H2A.X histone variant is involved in genomic stability and is known as a tumor repressor (Weyemi et al., 2016). Cell lacking H2A.X histone variant shows defective proliferation and sensitivity to the environmental and DNA damaging agents (genotoxic stress)(Celeste et al., 2003). H3.3 is the most conserved histone variant similar to canonical histone H3. Five variants of H3 have been identified in mammals. Among these H3.3 has a potential role in the transcription and transmission of the epigenetic state of the genome (Szenker et al., 2011). Variant H3.3 is associated with actively expressed genes of plants and animals and found enriched near transcription end sites (TES) of genes (Almouzni, 2011).

NON-CODING RNAs

They are RNAs that are not translated into proteins. It includes siRNA, miRNA, piRNA, lncRNA, and many more. These RNAs have a great role in gene regulation.

miRNA

MicroRNAs are small non-coding endogenous RNA first discovered in *C. elegans* and plants (Lee et al., 1993; Peng & Croce, 2016). There are more than 1000 miRNA genes discovered in mammals, each being 19-24bp long. Each mature miRNA may silence many genes. miRNA mostly interacts with 3'UTR of the target RNA (Lee et al., 1993). Gene regulation by miRNA mainly occurs by two processes *i.e.* imperfect homology and perfect homology. When there is imperfect homology between mi RNA and target RNA then there occurs translational repression, whereas perfect homology leads to cleavage of the target RNA regulating gene expression in eukaryotes (Henikoff & Smith, 2015). However interaction of miRNA with other regions of target RNA like 5' UTR, promoter, coding sequence have also been reported with their role in activating gene expression in a certain condition (O'Brien et al., 2018). The dysregulation of miRNA has been associated with the development of cancer (Gebert & MacRae, 2019).

siRNA

siRNA is derived from a long double-stranded RNA molecule that may arise from the different sources: virus replication, gene transcription, transposons activity that are proce=ssed by the dicer into 19-24 long base pair sequence. These 19-24bp long sequences are loaded into argonaut protein. These RNA doesn't depend upon the drosha for processing (Nozawa & Kinjo, 2016). Recent studies have revealed that siRNA is involved in gene silencing through DNA methylation and modification of histone in cells. siRNA-mediated epigenetic control is found in the model plant, Arabidopsis which requires specific protein AGO4 for the accumulation of siRNA, and DNA and histone methylation (Guo et al., 2016).

piRNA

piRNA also knows as piwi interacting RNA are of 26-31nt long. Under physiological conditions, they bind to the Piwi protein. piRNA comes from a long single chain precursor molecule which indicates that their function is independent of the dicer enzyme activity (Nozawa & Kinjo, 2016). piRNAs are divided into two large subclusters: pachytene piRNA cluster and pre-pachytene piRNA cluster. Pachytene piRNA cluster occurs during meiosis and continuous to express through the haploid spermatid stage. Per-pachytene piRNA cluster appears mainly in a premeiotic germ cell (Calcagno et al., 2019). These piRNAs regulate the transcription activity of the cell by effectively recruiting the HP1a proteins to specific genomic loci to repress RNA polymerase II. The involvement of pi proteins, MILI, and MIWI 2 in silencing transposons (LINE-1 and IPA) in testis has been revealed. The deletion of one of these pi proteins reduces the level of transposon methylation (Calcagno et al., 2019; Huang et al., 2013)

LncRNA

LncRNA is a long non-coding regulatory RNA, which is generally >200nt long and located in the nucleus or cytoplasm (Cao, 2014). The Source of the LncRNA may be the disruption of the transcriptional reading frame of the protein-encoding gene, replication of the non-coding gene by retrotransposition, resulted from the chromosomal reorganization or may have arisen from the insertion of the transposable elements into a gene in such a way that produces non-encoding RNA (Nozawa & Kinjo, 2016). LncRNA is involved in the regulation of transcriptional and translational products (Cao, 2014). Their critical role in epigenetic modifications like chromatin remodeling, post-transcriptional processing has been identified (Chen & Carmichael, 2010). X-chromosome inactivation via genomic imprinting is the most studied epigenetic regulation of LncRNA (Koerner et al., 2009).

CONCLUSION

With the advancement of genetic tools and biotechnical methods, several methods for detecting epigenetic modification have evolved. The methods for studying DNA methylation include the use of methylation-sensitive restriction enzymes, bisulfate conversion of unmethylated sequence followed by PCR and sequencing, comparative genome hybridization, and many more. These different techniques of detecting epigenetic modifications have helped us to understand and exploit epigenetic. Different non-coding RNAs which were earlier known as junk of the genome have been found to have regulatory mechanisms regulating the different functions of the different genes. Variations occurring in these modifications need to be understood to fully understand the regulation of the genes.

AUTHOR CONTRIBUTIONS

The initial idea about the reviewed topic was proposed by Kushal Bhattarai. All authors have contributed equally during initial manuscript preparation and the work of further reviewing and editing of manuscript till publication was done by Kushal Bhattarai.

COMPETING INTERESTS

The authors declare they have no conflict of interest. The manuscript has not been submitted for publication in other journal.

ETHICS APPROVAL

Not applicable.

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