Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2020; 8(1): 780-786

Received: 21-11-2019
Accepted: 25-12-2019

Harshit Kumar
ICAR-Indian Veterinary Research Institute, Bareilly, Uttar Pradesh, India

Anjali Chaudhary
ICAR- National Dairy Research Institute, Karnal, Haryana, India

Amandeep Singh
ICAR-Indian Veterinary Research Institute, Bareilly, Uttar Pradesh, India

Nidhi Sukhija
ICAR- National Dairy Research Institute, Karnal, Haryana, India

Anuradha Panwar
ICAR-Indian Veterinary Research Institute, Bareilly, Uttar Pradesh, India

KA Saravanan
ICAR-Indian Veterinary Research Institute, Bareilly, Uttar Pradesh, India

Ashish Bhaladhare
ICAR-Indian Veterinary Research Institute, Bareilly, Uttar Pradesh, India

Kaiho Kaisa
ICAR-Indian Veterinary Research Institute, Bareilly, Uttar Pradesh, India

Manjit Panigrahi
ICAR-Indian Veterinary Research Institute, Bareilly, Uttar Pradesh, India

Corresponding Author:
Harshit Kumar
ICAR-Indian Veterinary Research Institute, Bareilly, Uttar Pradesh, India

A review on epigenetics: Manifestations, modifications, methods & challenges

Harshit Kumar, Anjali Chaudhary, Amandeep Singh, Nidhi Sukhija, Anuradha Panwar, KA Saravanan, Ashish Bhaladhare, Kaiho Kaisa and Manjit Panigrahi

Abstract
Epigenetics is the sum total of the heritable changes in phenotype, independent and irrespective of the genotype. It works by switching on or off the concerned enzymes. The ongoing research works focus mainly on the revelation of the epigenetics behind the several diseases. Diverse classes of enzymes have been studied duly pertaining to the four main systems running in the background *viz*., DNA methylation processes, histone modifications, chromatin remodeling and nc-RNA silencing, resulting in epigenetic modifications and the eventual constitution of epigenome. The methods used are Combined bisulfite restriction analysis (COBRA), Chromatin Immuno-precipitation method (ChIP) and DNAase I seq.

Keywords: Modifications, methylation, enzyme, sequencing

Introduction
The genomics era has witnessed an ascent in livestock genetics, all through the gain through the advances in DNA technologies. For example, it gave the bead arrays that can genotype 500K SNPs and beyond along the bovine genome. This is a gain in predictive accuracy with respect to genetic merit [1]. This increment accompanied several noteworthy changes in the animal breeding industry, surpassing various the-then thresholds [2, 3]. One of the emerging concepts here is epigenetics. During the process of development, an individual goes through a chain of inbuilt chemical setups that dictate the standard segments of genome, at standard time, at standard phase of development, at standard locations. These inherent biochemical setups inclusive of the factors or stimuli that modify them are grouped and studied under epigenetics.
Conrad Hal Waddington (1907-1975) in 1942 derived the term 'epigenetics' from the Aristotelian term 'epigenesis'. 'Epi' from the Greek roots means 'in addition to'. Epigenetics thus implies 'in addition to genetics'. Waddington defined it as the interaction between the genes and their products which bring the phenotype into being. Evolutionary biologists consider it as the study of transfer of non-genetic information across generations as well [4]. Earlier the edge of heritable considerations was not taken into account. The new and consensus definitions of Epigenetics are mentioned as under: -

1. The collective heritable changes in phenotype arising independent of any change in genotype, and in turn affects how cells read the genes.
2. Heritable phenotypic modifications without alteration of DNA sequence [5]. Trans-generational nature of epigenetics was acknowledged.
3. The structural adaptations of chromosomal region as to register, signal or perpetuate altered activity states [6].
4. The changes in gene function heritable mitotically and/or Meiotically, that cannot be explained by changes in DNA sequence [7].
5. Epigenetics has a regular and natural occurrence, influenced by factors inclusive of the age, diet, stress, drugs, lifestyle, disease state of the subject, pollution and the immediate environment.

It may manifest itself in many forms. They may be the permanent modifications in the differentiation of diverse cell types ranging from brain cells to liver cells, all arising from the same single-celled zygote or, it may have damaging effects that lead to diseases such as cancers.

Mechanism

DNA methylation and Demethylation, histone modifications and non-coding RNA (ncRNA) associated gene silencing are considered currently the three main systems responsible for the initiation and sustenance of epigenetic change. Meanwhile, chromatin remodeling evolved as another system of the epigenetic modifications.

DNA methylation

Methylation of DNA makes gene inactive and non-functional, hence down regulated. Methylation is the most studied method for controlled gene expression [8]. DNA methylation refers to the addition of a methyl group (-CH3) to nucleotides, thus silencing gene expression [9]. The DNA methylation patterns are distinct for each cell type, thus giving cell type identity [10]. Enzymes involved herein are the DNMTs (DNA Methyl transferase). DNMTs are of several types. Firstly, *de novo* DNMTs, which are essential during gametogenesis and early embryogenesis for specific chromosomal DNA methylation and designated as DNMT 3a and 3b – *de novo* methyl transferase. Further, maintenance DNMTs, i.e. DNMT 1 which conserves the methylation patterns after each DNA replication cycle and DNMT 2 which carries enzymatic modifications in DNA and RNA methylation [11].

DNA methylation is very common modification which takes place by enzymatic modifications in nucleotide by addition of methyl groups to the cytosines at CpG islands, called the repetitive elements and imprinted genes [12]. It is noteworthy that the extent of methylation, in terms of hypermethylation or hypermethylation, is linked to different physiological or pathological processes. Generally, hypermethylation leads to gene silencing, while hypomethylation leads to gene expression [13].

Histone modifications

In eukaryotes, there are two types of nuclear proteins *viz*., histone proteins and non-histone proteins (NHPs). The most abundant nuclear proteins are histone proteins (consisting of two rows of each core histone *viz*. H2A, H2B, H3, H4), organized into a histone octamer with the DNA wrapped around it forming the nucleosome. Histone tails gain epigenetic chromatin tags required for post transcriptional gene regulation. Post-translational modifications of histone tails include, ribosylation, methylation, acetylation, phosphorylation, ubiquitination and SUMOylation. Array of biological phenomenon is associated with these histone modifications for gene regulation and expression. Acetylation and deacetylation are enzyme reversible (HAT and HDAC). Acetylation of histone tails leads to more functionality of genes by increasing its accessibility to different enzymes, while decreasing affinity of histone to DNA.

In contrast, methylation of histone tails leads to non-functionality by making the chromatin more condensed. Acetylation cycle is maintained by 2 complementary principal enzymes in the run *viz*., histone acetyl transferases (HAT) and histone deacetylase (HDAC). Methylation silencing occurs at *Lys* and *Arg* residue by HMTase. Phosphorylation occurs on serine residue associated with transcriptional activation and SUMOylation occurs at lysine residues, linking the covalent attachment of other small ubiquitin associated modifier proteins i.e., SUMO proteins to certain lysine residues. SUMO proteins are also involved in various cellular processes like as programmed cell death and stress related responses. The linking or delinking of SUMO proteins to other proteins modulates their functionality. Histone tags and associated modifications are easier to induce or remove through enzymes, thus this phenomenon of histone coloration is a dynamic process and relatively less enduring [15].

RNA-associated silencing

Turning on and off of genes can also be done by various arrays of non-coding RNAs (ncRNAs) seen as RNA interference, caused by noncoding RNAs both long and short or antisense transcripts (RNAi) [17, 18, 19, 20]. RNA modulates gene expression by heterochromatin formation, DNA methylation or histone tagging. MicroRNAs are evolving as the major key factors for post-transcriptional modifications and for directing modifications in gene expression. They play a significant role in physiological and pathological states of body. Non-coding RNA activities, including small RNAs, microRNAs and large RNAs, modulate the translation, transcription or protein structure, thereby affecting protein activity [21, 22].

A non-coding RNA i.e., ncRNA is a well-designed RNA molecule which is transcribed from DNA but it is not translated any further. Epigenetics correlated ncRNAs molecules include these i.e., micro RNAs, short interfering RNAs, Piwi-interacting RNAs and long non coding RNAs. ncRNA operates at both i.e., transcriptional and post-transcriptional levels [23,24]. These ncRNAs which are having epigenetic interests can further be divided into two main categories i.e., the short ncRNAs which are less than 30 nucleotides and other is the long ncRNAs which is more than 200 nucleotides. The 3 important sub classes of short non-coding RNAs are microRNAs (miRNAs), short interfering RNAs (siRNAs), and Piwi-interacting RNAs (piRNAs). Both chief groups play an important role in heterochromatin organization, histone modifications, DNA methylation leveling and gene silencing [25].

Long non-coding RNA: Transcripts more than 100bp to 200bp in size. Long ncRNAs function in chromatin remodeling, transcriptional regulation, post-transcriptional regulation, and as precursor for siRNAs [26].

Short non-coding RNAs: Involved in development and gene regulation.

MicroRNAs: These are primarily 20-22bp long and are involved in negative control of their target gene expression post transcriptionally. MicroRNAs (miRNAs) bind to a target messenger RNA with a complementary sequence either to induce cleavage or degradation, or to block translation done via feedback mechanism that involves chromosome methylation [27, 28].

Small interfering RNAs: The processing of long dsRNA via dicer enzyme forms siRNAs. Their mode of action is similar to that of microRNAs. They are mainly used in gene silencing [29].

Piwi interacting RNAs: Piwi-RNAs are named so because of their interaction with the piwi family of proteins. They are primarily involved in chromatin regulation and suppression of transposon activity in germline and somatic cells [30]. piRNAs that are antisense to expressed transposons, form complexes with Piwi-proteins, and then target and cleave the transposon. This cleavage generates additional piRNAs which further

target and cleave additional transposons. This cycle continues producing piRNAs in enough quantities which leads to transposon silencing [31].

Chromatin remodeling

Chromatin is the multifaceted complex of important histone proteins which are having DNA wrapped around them like a yarn ball. This special kind of packaging can accommodate whole of the DNA into the nucleus itself. The complex molecular organization can be modified by chemical groups like as acetyl components, histone-associated enzymes and non-coding RNAs like as miRNAs and siRNAs [32]. These chemical modifications change the structural organization of chromatin complex, thus further upsetting or enhancing gene expression. In general context, genetic functions of densely packed chromatin are hindered, although the lightly folded chromatin inclines to be functional and gets expressed easily. Certain combinations of these chemical modifications establish the popular "histone code". Which can be read and inferred by many different cellular factors, thus establishing their transcriptional activation or repression as required by cell [32]. For instance, a quite common epigenetic modification of euchromatin is triple methylation at certain lysine residues of histone proteins of class H3 i.e., K4, K36, and K79 and a very high level of histone acetylation, whereas heterochromatin is characteristically enriched in tri-methylation of various other lysine residues of H3 like as i.e., K9, K20, and K27 [33, 34].

Causal factors

Epigenetor: A signal or some sort of induction is always required for every biological process to start and execute the requisite function. Likewise, some triggers or signals are always required for the epigenetic modifications [36]. Epigenetor works directly or indirectly by inducing changes in the vicinity of the cell to modify an epigenetic phenotype. Epigenetic inducers can be pathogens, diet, radiations, toxin, hormones, etc.

Nutrients: Nutrients may cause reversal of epigenetic processes either by stimulating or inhibiting the enzymes that catalyze DNA methylation or histone modification or by altering the availability of substrates required for enzymatic reactions leading to dynamic regulation of gene expression. Besides perinatal period, adulthood is also the time prone to phenotypic plasticity, contributing largely to developmental programming. Li *et al*. (2016) [37] revealed that the folic acid through *in-ovo* feeding improves hatchability, feed conversion ratio (FCR), expression of MTHFR and MTRR genes, IgG and IgM concentrations due to improved folate metabolism and immune-boost of the bird. The molecular mechanism involved in this is splenic expression up-regulation due to histone methylation of IL2 and IL4 promoters and inhibition on the IL6 promoter [38, 39, 40].

Obeid (2013) [41] reported about the direct delivery of methyl group donors through diet. The methyl donors in feed are methionine, folate, choline, vitamin B6, vitamin B12 and others. Deficiency of any one of the members of methyl group donors may be compensated by the availability of other member. Deficiency of methyl group donors in diet may increase the risk of some metabolic as well as production diseases in birds [42]. The process of DNA methylation improves lean muscle growth in poultry (positively influences the expression of a protein that increases the growth of pectoralis muscles of broilers) as well as egg production (positively influences the dry matter intake). Ratriyanto *et al*. (2009) [43] reported the beneficial effects of methyl donor supplementation in poultry feed on intestinal cells and gut microflora. Hence, the nutri-epigenetics approach provides a molecular foundation for understanding the relation between the feed given throughout the life, the subsequent epigenetic alterations and its role in improving the production in livestock and poultry [44, 45].

Environmental factors: Environmental factors may also induce epigenetic modifications. Environmental agents, such as chemical toxins or radiations, may enter the cells of a tissue and interact with the genetic material. Environmental stress factors are also thought to produce epigenetic factors though this needs more research to be done [46]. The immune system is also subjected to epigenetic modification by environmental factors. Bacterial infection in the bovine udder inhibits lactation, independent of circulating prolactin levels by epigenetic modification.

Children born during the period of the Dutch famine (1944-1945) had increased rates of coronary heart disease and obesity due to maternal exposure to famine during early pregnancy compared to those not exposed to famine. Less DNA methylation of the insulin-like growth factor II (IGF2) gene, an epigenetic locus was linked to it. In similar studies, higher incidence of schizophrenia was reported among the adults that were prenatally exposed to famine conditions [47].

Hormones: Hormones also influence the epigenetic makeup of a cell and hence the organism, whether animal or poultry. Melatonin and/or its metabolites, are considered as methyl transferase inhibitors as they competitively inhibit methyl transferase due to their structural identity with them. These are involved in epigenetic modifications in various vital life processes.

Methods for studying Epigenetic Modifications

The methylation sites in the particular DNA base sequence in the genome are detected to know the epigenetic alterations and the corresponding diagnostic marker for diseases such as cancer. However ordinary genetic tests cannot detect methylated regions. In recent years, various methods have been developed to detect methylation. The most practiced method is bisulfite sequencing which determines the DNA sequence through bisulfite preparation of a DNA fragment to change the base. Recently, identification of DNA methylation is based on second-generation sequencing on a genome-wide basis. Such technologies detect different DNA methylation with different levels of coverage and resolution. The third-generation technologies for sequencing bring deeper and more accurate knowledge on the epi-genomic base modifications and may help to develop specific bead-arrays for their use in livestock [48].

The noteworthy methods that have evolved along with time in the scientific world for the purpose of quantification of epigenetic effects in an organism are mentioned as under:

Combined bisulfite restriction analysis (COBRA) method

A common technique for DNA methylation evaluation includ es the use of sodium bisulfite chemistry to differentially conv ert unmethylated cytosine residues to uracil, while unmodified methylated cytosines are left. It is based upon unmodified cyt osine quantification.

Methylated cytosines can then be detected using specific dow nstream methods of nucleic acid analysis, including PCR, qP CR and sequencing.
In identifying and quantifying the level of methylation in a pa rticular genomic region, this preparatory technique is used to measure epigenetic modifications.

Chromatin immuno precipitation method (ChIP)
ChIP is a preparatory method requiring the use of highly speci fic antibodies to the DNA-binding proteins.
This is usually followed by several techniques of nucleic acid analysis, including PCR, qPCR, sequencing and microarray h ybridization.
It can aid in assessing the association of certain proteins with different genomic regions and the identification of genome re gions associated with specific modifications of the histone.

DNase I Seq
Digital DNase and DNase Seq are two methods of generating short DNA strands by nuclease digestion from nuclear-accessible genomic regions. These short DNA strands are isolated and examined by next-generation sequencing to provide detailed sequence information on the accessible genomic stretches for nuclease digestion [49, 50, 51]. Researchers practice these techniques to associate structural alterations between DNA samples on a genome-wide scale. Moreover, other methods include:
a. Small RNA expression
b. Methylated DNA immuno precipitation Seq (Me DIP-Seq)
c. Methylation-sensitive RE Seq.

Challenges in epigenetics for livestock breeding
Recent advances in epigenomics include genome-wide next-generation sequencing, genomic dynamic imaging, quantitative proteomics, and computational analysis. They combined facilitated fine-detailed mapping of DNA methylation and its derivatives (e.g. 5hmC), captured histone modifications in single cells, and also contributed significantly to chromatin accessibility studies such as chromosome conformation capture (3C) capture technologies [52, 53].
Subsequently, these are significant, and these extreme effects are assumed to include the unbalanced expression of imprinted genes. There are also many imprinted human and mouse genes printed in sheep (Table 1). In addition, many imprinted genes were detected in numerous species including cow, sheep, dog, pig, rabbit, chicken, opossum, lab opossum, human, mouse and rat (Table 1). Actually there are about 60 (26.3 percent) confirmed imprinted genes in livestock (cow, horse, dog, pig, chicken, Table 1) and most of them are found in pigs and cows. Most importantly, due to its variation in complex production traits, there is growing interest in the role of certain imprinted genes, such as IGF2, in livestock. For example, deposition of muscle mass and fat in pigs as well as meat and milk production in beef and dairy cattle, respectively [54, 55].

Table 1: List of imprinted genes by species

Organism	Imprinted	Paternally expressed	Maternally expressed	Other[a]
Cow	20	12	8	–
Sheep	16	6	8	2 (1× isoform dependent, 1× unknown)
Dog	1	–	1	–
Pig	22	14	6	2 (1× tissue dependent, 1× biallelic)
Rabbit	1	1	–	–
Chicken	–	–	–	–
Opossum	2	1	1	–
Lab Opossum	6	2	4	–
Human	97	61	29	7 (4× isoform dependent, 2× random, 1× unknown)
Mouse	124	50	62	12 (5× isoform dependent, 7× unknown)
Rat	6	3	3	–
Wallaby	5	4	1	–

[a]Other: non-paternal or maternal form of allele expression in the zygote

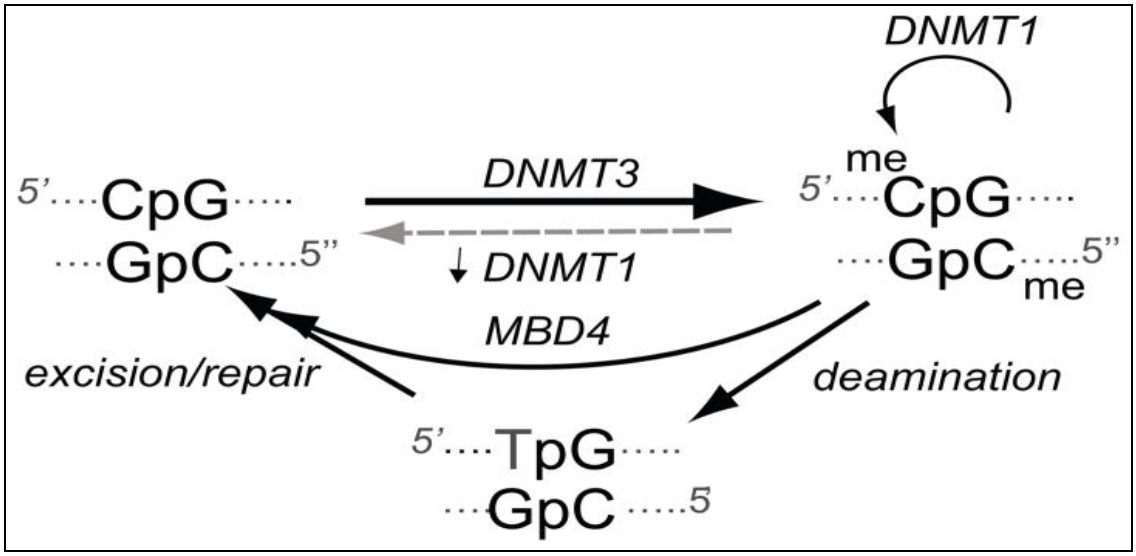

Fig 1: DNA Methylation [14]

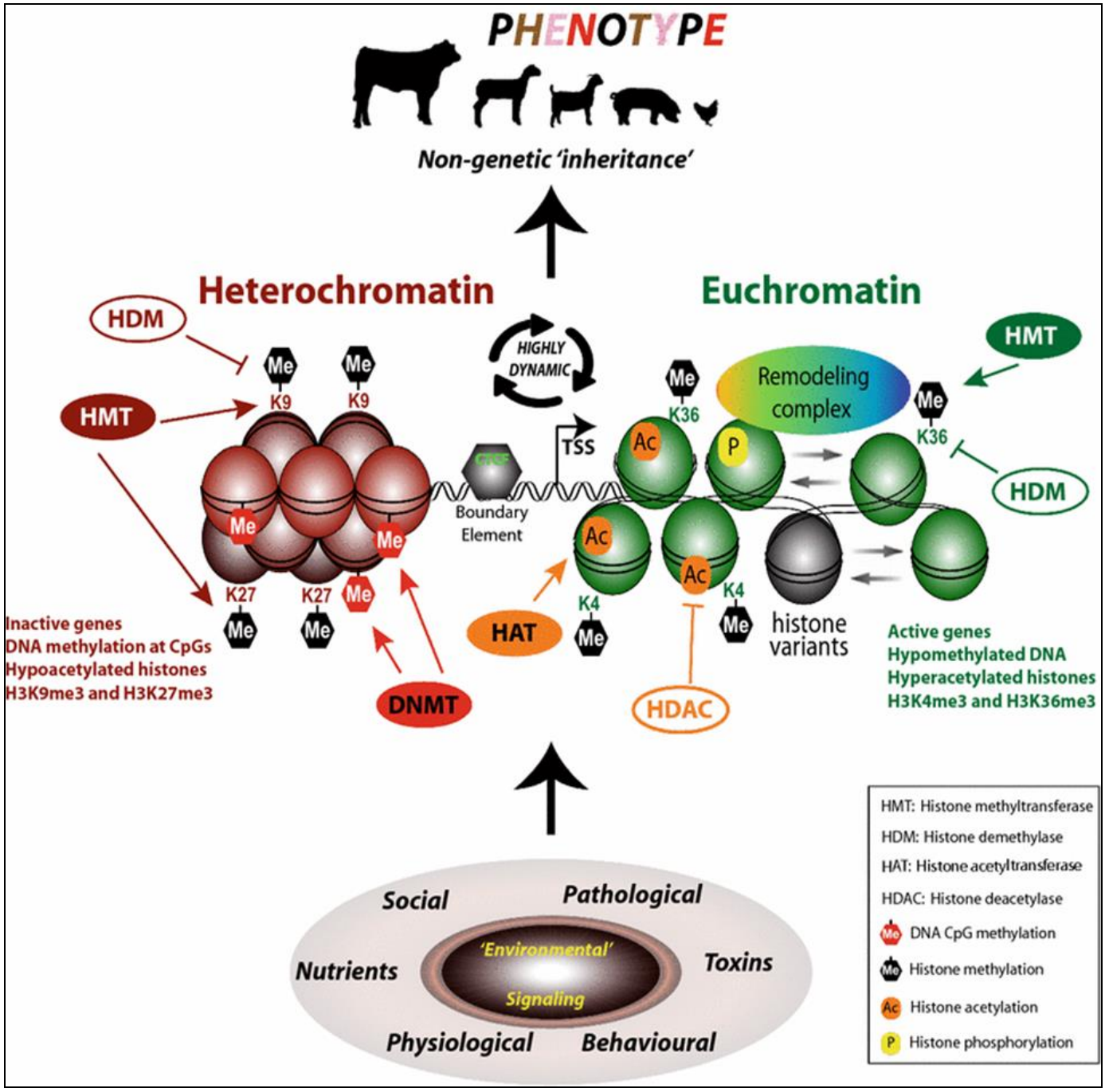

Fig 2: Histone Modification [16]

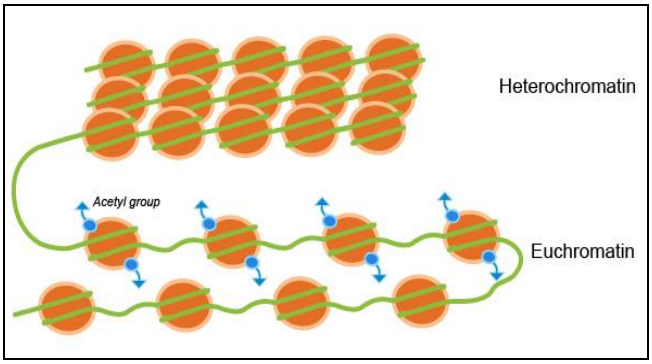

Fig 3: Representation of condensed heterochromatin and relaxed euchromatin. Acetylation of histones, for example, can impact chromatin accessibility and alter gene expression [35].

Conclusion

Epigenetics manifests by switching on or off of the enzymes, bringing about phenotypic differences with or without alteration of genetic sequence. These epigenetic alterations form basis of many diseases such as, cancers which are heritable in nature. Most of these changes are due to the unbalanced expression of imprinted genes causing variation in complex production traits. So, causes of such diseases are to be identified and accordingly precautions may be taken. The recent advances in epigenomics include genome-wide next-generation sequencing, dynamic imaging of genomic loci, quantitative proteomics and computational analyses.

Authors' Contributions: All authors have contributed equally in production of this manuscript

Acknowledgments

The authors would wish to acknowledge financial support provided by ICAR, New Delhi in terms of fellowships to

scholars. Authors also wish to acknowledge Directors of ICAR-IVRI, Bareilly and ICAR-NDRI, Karnal for providing infrastructural facility to review this study.

Competing Interests

The authors declare that they have no competing interests.

References

1. Van Raden PM. Efficient methods to compute genomic predictions. Journal of dairy science. 2008; 91(11):4414-23.
2. Ibañez-Escriche N, Gonzalez-Recio O. Promises, pitfalls and challenges of genomic selection in breeding programs. Spanish Journal of Agricultural Research. 2011; 9(2):404-13.
3. Gonzalez-Recio Oscar, Toro Miguel-A, and Bach Alex. Past, present, and future of livestock epigenetics applied to livestock breeding. Frontiers in Genetics. 2015; 6:305.
4. Youngson NA, Whitelaw E. Tran's generational epigenetic effects. Annual Review of Genomics and Human Genetics. 2008; 9:233-57.
5. Ho DH, Burggren WW. Epigenetics and transgenerational transfer: a physiological perspective. Journal of Experimental Biology. 2010; 213(1):3-16.
6. Bird A. Perceptions of epigenetics. Nature. 2007; 447(7143):396.
7. Feil R, Fraga MF. Epigenetics and the environment: emerging patterns and implications. Nature reviews genetics. 2012; 13(2):97.
8. Quina AS, Buschbeck M, Di Croce L. Chromatin structure and epigenetics. Biochemical pharmacology. 2006; 72(11):1563-9.
9. Cooney CA, Dave AA, Wolff GL. Maternal methyl supplements in mice affect epigenetic variation and DNA methylation of offspring. J Nutr. 2002; 132:2393-400.
10. Meaney MJ, Szyf M. Environmental programming of stress responses through DNA methylation: life at the interface between a dynamic environment and a fixed genome. Dialogues in clinical neuroscience. 2005; 7(2):103.
11. Schaefer M, Pollex T, Hanna K, Tuorto F, Meusburger M, Helm M *et al*. RNA methylation by Dnmt2 protects transfer RNAs against stress-induced cleavage. Genes & development. 2010; 24(15):1590-5.
12. Holmes R, Soloway PD. Regulation of imprinted DNA methylation. Cytogenetic and genome research. 2006; 113(1-4):122-9.
13. Luczak MW, Jagodziński PP. The role of DNA methylation in cancer development. Folia histochemical et cytobiologica. 2006; 44(3):143-54
14. Zeric D. Importance of epigenetics in animal breeding: Genomic imprinting. Bachelor Thesis, Faculty of Veterinary Medicine and Animal Science, Swedish University of Agricultural Sciences, 2012.
15. Stuppia L, Franzago M, Ballerini P, Gatta V, Antonucci I. Epigenetics and male reproduction: the consequences of paternal lifestyle on fertility, embryo development, and children lifetime health. Clinical epigenetics.2015; 7:120.
16. Tuiskula-Haavisto M, De Koning D-J, Honkatukia HM, Schulman FN, Mäki-Tanila A, Vilkki A *et al*. Quantitative trait loci with parent-of-origin effects in chicken. Genet. Res. 2004; 84:57-66.
17. Fresard L, Morrisson M, Brun MJ, Collin A, Pain Bertrand, Minivielle F *et al*. Epigenetics and phenotypic Variability: Some interesting insights from birds. Genetic Selection Evolution. 2013; 45:16.
18. Yu Ying, and Song Jiuzhou. Epigenetics and Animal Health. John Wiley and sons. 2012; 1:8-131.
19. Krebs Jocelyn E, Goldstein Elliott S, Kilpatrick Stephen T. Lewin's Genes X. 10, Jones and Bartlett Publishers, Canada, 2011, 829-846.
20. Nayak Nibedita, Bhanja Subrat, Firdous Ahmad, Sheikh Mehra, Manish Goel, Akshat Sahu *et al*. Role of epigenetic modifications in improving the thermo-tolerance, growth and immuno-competence in poultry: current status and future applications. Indian Journal of Poultry Science. 51. 1. 10.5958/0974-*8180.2016.00015.5. 2016
21. Amrutkar SA. Evaluation of Frizzle, Naked Neck and Normal Plumaged broilers under tropical stress conditions using Functional Genomics and Epigenetic tools. Thesis submitted to Deemed University, IVRI, Izatnagar, Bareilly, U.P., India, 2012
22. Bhanja SK, Goel A, Pandey N, Mehra M, Majumdar S, Mandal AB. In ovo carbohydrate supplementation modulates growth and immunity-related genes in broiler chickens. Journal of Animal Physiology and Animal Nutrition. 2015; 99:163-73.
23. Bhanja SK, Hotowy A, Mehra M, Sawosz E, Pineda L, Vadalasetty KP *et al*. In ovo administration of silver nanoparticles and/ or amino acids influence metabolism and immune gene expression in chicken embryos. International Journal of Molecular Sciences. 2015; 16:9484-503.
24. Bhanja SK, Mandal AB, Goswami TK. Effect of in ovo injection of amino acids on growth, immune response, development of digestive organs and carcass yields of broiler. Indian Journal of Poultry Science. 2004; 39:212-18
25. Bhanja SK, Sudhagar M, Goel A, Pandey N, Mehra M, Agarwal SK *et al*. Differential expression of growth and immunity related genes influenced by in ovo supplementation of amino acids in broiler chickens. Czech Journal of Animal Science. 2014; 59:399-408
26. Goel A, Bhanja SK, Mehra M, Mandal AB, Pande V. In ovo trace element supplementation enhances expression of growth genes in embryo and immune genes in post-hatch broiler chickens. Journal of the Science of Food and Agriculture, doi: 10.1002/jsfa.7438. Epub, 2015.
27. Pathik D, Wadhwa MD, Buss C, Entringer S, Swanson JM. Developmental origins of health and disease: brief history of the approach and current focus on epigenetic mechanisms. Seminars in Reproductive Medicine. 2009; 27:358- 68.
28. Soren SK. Effect of incubational thermal stress on tolerance to heat or cold exposure and epigenetic changes in broiler chicken. Thesis submitted to Deemed University, IVRI, Izatnagar, Bareilly, and Uttar Pradesh, India, 2014.
29. Waddington CH. The epigenotype. Endeavour.1942; 1:18-20.
30. Waddington CH. towards a Theoretical Biology. In The Basic Ideas of Biology, Edinburgh University Press, Edinburgh, Scotland, 1962, 1-32.
31. Yamazaki Y, Mann MR, Lee SS, Marh J, McCarrey JR, Yanagimachi R *et al*. Reprogramming of primordial germ cells begins before migration into the genital ridge, making these cells inadequate donors for reproductive

cloning. Proceedings of the National Academy of Sciences, USA. 2003; 100:12207-12.
32. Yadav L and Maurya P. Epigenetics: new relation of health and nutrition. Asian Journal of Bio Science. 2014; 9:288-92.
33. Oswald J, Engemann S, Lane N, Mayer W, Olek A, Fundele R *et al*. Active demethylation of the paternal genome in the mouse zygote. Current Biology. 2000; 10:475-78.
34. Nätt D, Rubin CJ, Wright D, Johnsson M, Beltéky J, Andersson L *et al*. Heritable genome-wide variation of gene expression and promoter methylation between wild and domesticated chickens. BMC Genomics.2012; 13:59.
35. Brien FD, Cloete S, Fogarty NM, Greeff JC, Hebart ML, Hiendleder S *et al*. A review of the genetic and epigenetic factors affecting lamb survival. Animal Production Science. 2014; 54:667-93.
36. Carrell DT. Epigenetics of the male gamete. Fertility and Sterility. 2012; 97:267-74.
37. Li Q, Li N, Hu X, Li J, Du Z, Chen L, *et al*. Genome-wide mapping of DNA methylation in chicken. PloS One. 2011; 6(5):e19428.
38. Ueda T, Abe K, Miura A, Yuzuriha M, Zubair M, Noguchi M *et al*. The paternal methylation imprint of the mouse H19 locus is acquired in the gonocyte stage during foetal testis development Genes to Cells. 2000; 5:649-59.
39. Rajendar S, Avery K, Agarwal A. Epigenetics, spermatogenesis and male infertility. Mutation Research. 2011; 727:62-71.
40. Zhang X, Ho S. Epigenetics meets endocrinology. Journal of Molecular Endocrinology. 2011; 46:11-32.
41. Obeid TVL, Parmentier HK, Lammers A. Transgenerational epigenetic effects on innate immunity in broilers: An underestimated field to be explored? Poultry Science. 2013; 92:2904-13.
42. Goddard ME, Hayes BJ, Meuwissen TH. Genomic selection in livestock populations. Genetics research. 2010; 92(5-6):413-21.
43. Ratriyanto JM. Epigenetics in spermatogenesis. Molecular and Cellular Endocrinology. 2009; 306:33-36.
44. Loscalzo J, Handy DE. Epigenetic modifications: basic mechanisms and role in cardiovascular disease (2013 Grover Conference series). Pulmonary circulation. 2014; 4(2):169-74.
45. Janmeda M, Ramani UV, Pandya GM, Tyagi K, Kharadi VB, Brahmkshtri BP *et al*. Epigenetics: Regulation of Gene expression. International Journal of Science, Environment and Technology. 2017; 6(2):1390-6.
46. Ptak C, Petronis A. Epigenetic approaches to psychiatric disorders. Dialogues in clinical neuroscience. 2010; 12(1):25.
47. Doherty R, Couldrey C. Exploring genome wide bisulfite sequencing for DNA methylation analysis in livestock: a technical assessment. Front Genet. 2014; 5:126.
48. Kubiak M, Lewandowska MA. Can chromatin conformation technologies bring light into human molecular pathology? Acta Biochim Pol. 2015; 62:483-9.
49. Ferguson-Smith AC, Cattanach BM, Barton SC, Beechey CV, Surani MA. Embryological and molecular investigations of parental imprinting on mouse chromosome 7. Nature. 1991; 351:667-70.
50. Neugebauer N, Räder I, Schild HJ, Zimmer D, Reinsch N. Evidence for parent-of-origin effects on genetic variability of beef traits. J Anim Sci. 2010; 88:523-32.
51. Reik W, Dean W, Walter J. Epigenetic reprogramming in mammalian development. Science. 2001; 293:1089-93.
52. Jirtle LR, Weidman RJ. Imprinted and more equal. Am Sci. 2007; 95:143-9.
53. McQueen HA, McBride D, Miele G, Bird AP, Clinton M. Dosage compensation in birds. Curr Biol. 2001; 11:253-7.
54. Reik W, Walter J. Genomic imprinting: parental influence on the genome. Nat Rev Genet. 2001; 2(1):21.
55. Feil R. Environmental and nutritional effects on the epigenetic regulation of genes. Mutat. Res. 2006; 600:46-57.