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The Importance of Epigenetic Phenomena in Regulating Activity of the Genetic Material

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The Importance of Epigenetic Phenomena in Regulating Activity of the Genetic Material

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HPR 401

Dr. Joel Chandlee

2 May 2013

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INTRODUCTION TO EPIGENETICS

Epigenetics is the study of factors that affect gene expression in a heritable way, but that do not alter the nucleotide sequence of DNA. It can be described as a phenomenon that determines the final functionality of a locus or chromosome without changing the underlying DNA sequence (Goldberg, Allis, and Bernstein 2007). Instead of altering the DNA sequence, epigenetic factors affect how and when genes are expressed. The initial discovery and subsequent study of epigenetic phenomena have redefined how the scientific community now views the function of genes. It was once thought that the DNA sequences inherited from each parent served as a set blueprint of an individual's life. With the expanding understanding of epigenetic influence of gene expression, this is no longer the case. Through experimental analysis of epigenetic phenomena, different environmental factors have been shown to affect the DNA and how genes function.

The long debate of whether an individual's traits are the product of nature or nurture may be finally settled. The DNA inherited through nature cannot be changed, but environmental factors are able to play a role and affect how DNA, specifically genes are expressed. One of the first people to have proposed the idea of inheritance of acquired traits was a French scientist named Jean-Baptiste Lamarck. He hypothesized a theory of heredity involving the "inheritance of acquired traits." A classic example of Lamarck's was the acquisition of a giraffe's long neck (Figure 1). He hypothesized that originally giraffes had a much shorter neck. Acquisition of the longer neck was driven by the need to obtain nutrients from leaves on much higher trees. Giraffes stretched their necks to reach the higher leaves and the trait was then passed down to their descendants. Hence, the theory of "inheritance of acquired traits."

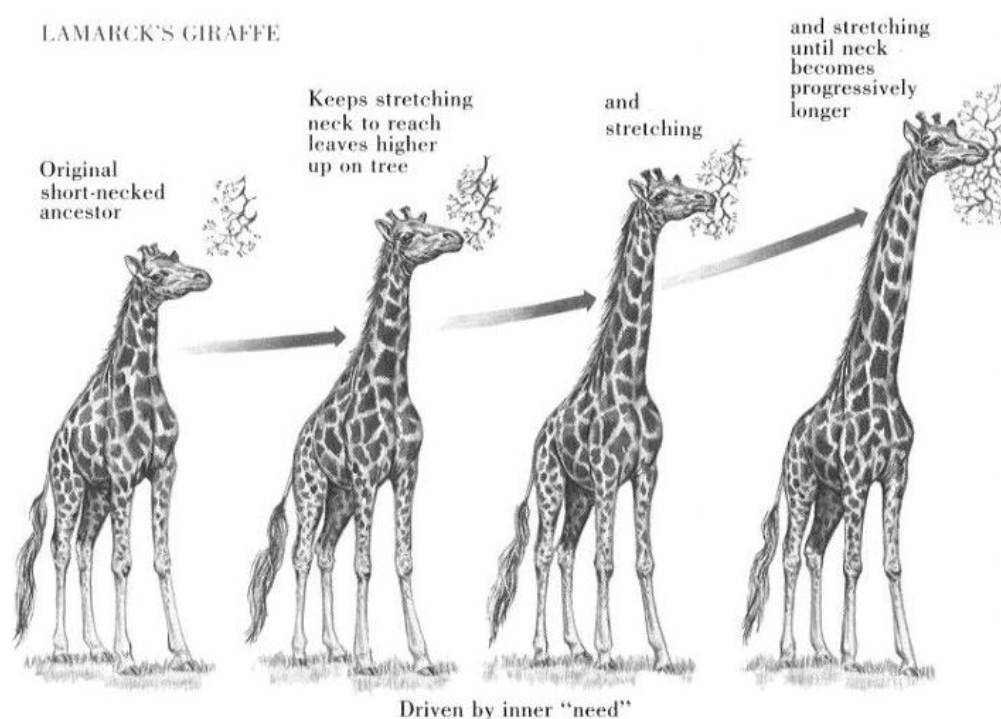


Figure 1. Lamarck's Giraffes

Jean-Baptiste Lamarck explained how acquired traits could be passed down from one generation to the next with the giraffe's long neck. It is thought that the giraffe's long neck that we see in present day is due to an environmental need to access leaves on higher trees. This need drives the giraffe's neck to lengthen over time and this trait is then passed down through generations. Reprinted from website: <https://benchprep.com/blog/ap-biology-evolution-part-2/>

The first epigenetic mechanism to be understood was DNA methylation (Figure 2). DNA methylation occurs through methyl groups being attached to the DNA building blocks, specifically the cytosine bases of nucleotides of the genome. The attachment of the methyl group has the ability to turn genes off (Goldberg, Allis, and Bernstein 2007). The product of the methylation is 5-methylcytosine and is produced through the activity of DNA methyltransferase (DNMT) enzyme. DNMTs commonly catalyze regions of DNA that are rich in cytosine bases, known as CpG islands.

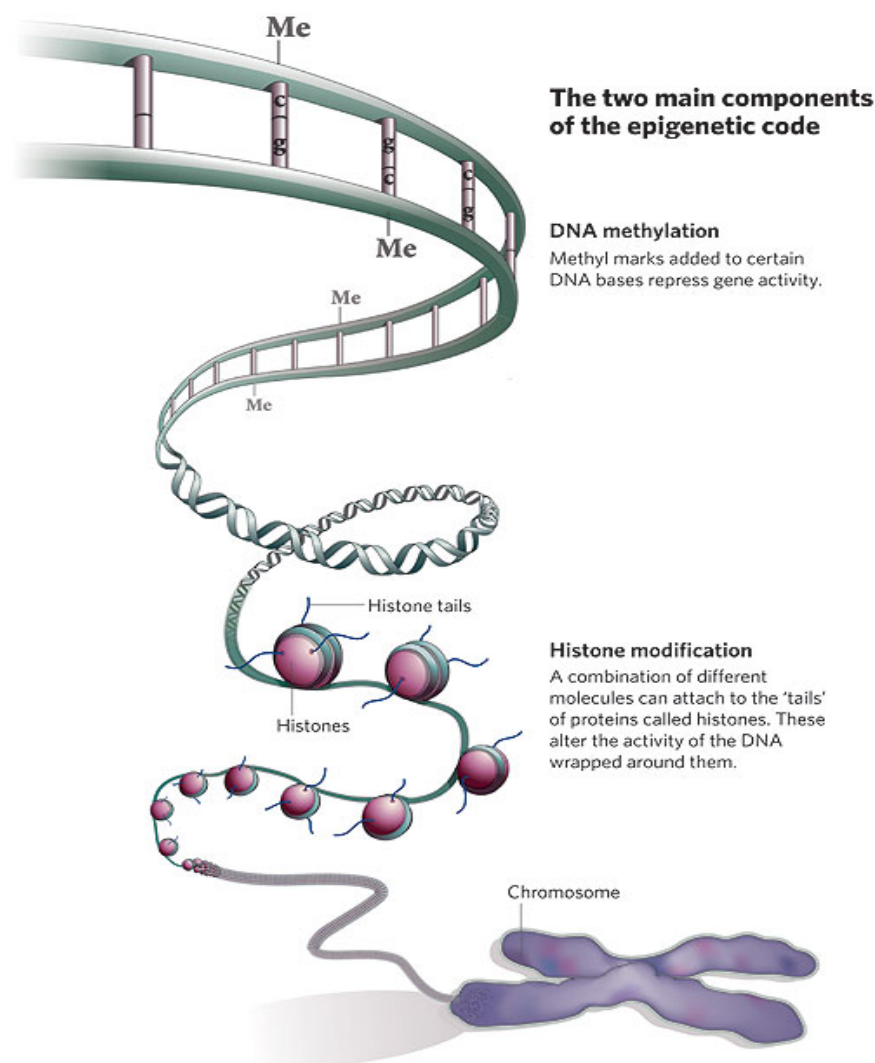


Figure 2. The Two Main Components of the Epigenetic Code

The first component and the best understood is DNA methylation. Methylation occurs at the cytosine bases of DNA strands converting the cytosine bases to 5-methylcytosines. The second component is histone modification. The histone proteins that DNA is wrapped around have histone 'tails' that when bound to can affect gene expression. Reprinted from website: <http://cerch.org/research-programs/chamacos/chamacos-epigenetics/>

An enzyme attaches methyl groups to the carbon 5' position of specific cytosine residue. Methyl groups are usually added to cytosine in CpG dinucleotides. The pattern of methylation is symmetric, meaning a methyl group is present on both strands of DNA in the same context. In the entire genome 70-80% of all CpG dinucleotides are methylated (Griffiths et al. 2010). Most unmethylated CpG islands are located near gene promoters. DNA methylation patterns can be passed from one generation to the next. Due to the semiconservative replication of DNA, it leads to one strand of the daughter DNA to be methylated.

The enzymes responsible for DNA methylation are most commonly classified by their ability to methylate DNA *de novo* or to maintain previously established methylation patterns after replication. DNMT3 is a family of DNA methyltransferases that methylate CpG sites *de novo*. The two best known members of DNMT3 are DNMT3a and DNMT3b. Both are believed to be essential for the survival of mammals. In a knockout experiment performed with mice, the absence of either one was fatal (Robertson, K.D. 2001). DNMT1 is the primary enzyme responsible for the maintenance of DNA methylation. It is often referred to as ‘maintenance’ methyltransferase. After methylation is started by DNMT3 and replication has occurred, DNMT1 copies the methylation pattern to the other strand (Figure 3).

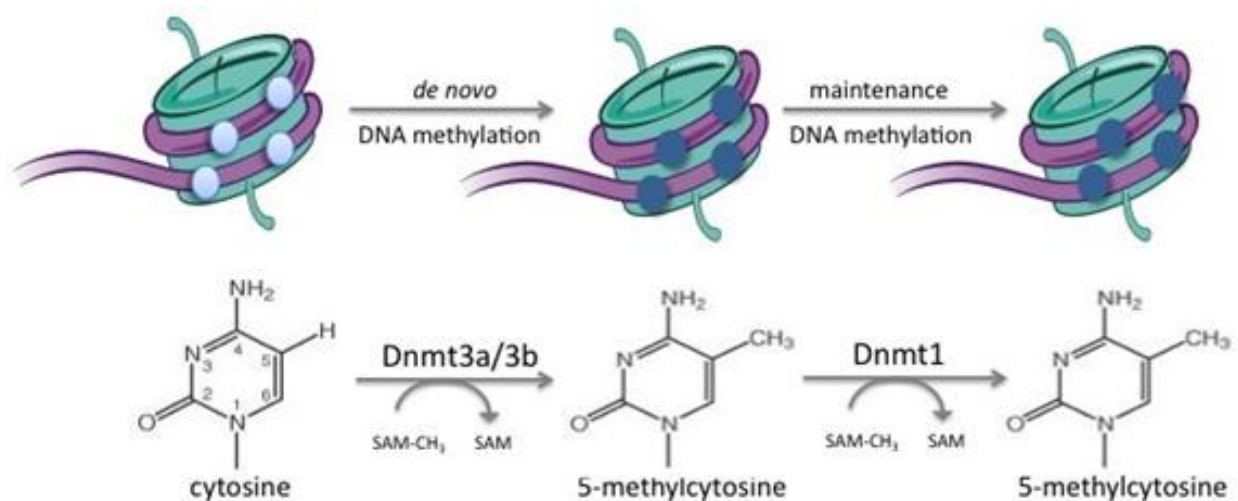


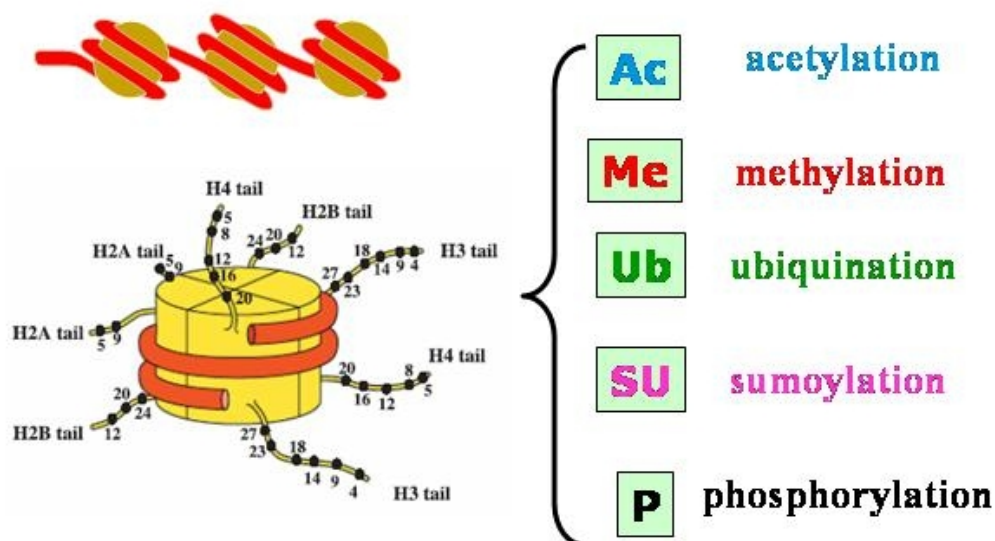
Figure 3. DNA Methyltransferases

DNMT3a/3b methylates the 5' position of cytosine *de novo*. Following DNA replication, DNMT1 maintains the pattern of DNA methylation. Reprinted from website: <http://www.scrb.harvard.edu/lab/550/research>

The exact role played by DNA methylation in regulating gene expression is still not fully understood. DNA methylation to the correct sites is important for cell differentiation and embryonic development. It has been proven that the methylation of promoters correlates with low levels or no transcriptional activity (Goldberg, Allis, and Bernstein 2007). Methylation levels have also been observed to be different among organisms and in different tissue, as well. DNA methylation is important in cell differentiation and embryonic development. Errors in

DNA methylation may lead to harmful consequences, such as cancer, autoimmune disease, and a range of birth defects.

A second epigenetic mechanism is histone modification. Histones are chromosomal proteins that are important for DNA packaging into the chromosome structure. Strings of DNA wrap around the histone proteins to create the nucleosome complex that is packaged into the chromosome. There are five different types of histone modifications: methylation, acetylation, phosphorylation, ubiquitination, and sumoylation. Histone modifications occur at the N-terminal end of histones (Figure 4). The two main modes of modifications are acetylation and methylation. Acetylation of the lysine residue generally activates the chromatin by loosening the DNA around the histone and making the gene more readily available to be transcribed. Deacetylation is associated with the heterochromatin, which is a more condensed form of the chromatin that inhibits DNA transcription for occurring (Simmons 2008). Methylation can cause either activation or inactivation depending on where methyl groups attach to and when the modification happens. The genomic patterns and locations of modifications can be inherited and affect gene expression. Methylation at lysine nine of histone three (H3K9; see Figure 4) causes silencing of corresponding genes, but methylation at lysine 4 of histone three (H3K4; see Figure 4) will activate the corresponding genes (Simmons 2008).



The figure illustrates nucleosome models and major posttranslational modifications which play essential roles in gene expression regulation and disease processes

Figure 4. Histone Tail Modifications

The five types of histone modifications occur at the N-terminal end of the histone tails. The tails are targeted sites for modifications to the histones that affect gene expression. Reprinted from website: http://www.integratedhealthcare.eu/1/en/histones_and_chromatin/1497/

Certain environmentally induced chemical changes in histones are self-perpetuating and the altered gene function they cause can be handed down to descendants either through daughter cells or individual offspring. Environmental exposure can affect the function of these genes. The part of the genome susceptible to these epigenetic changes is known as the epigenome. Some of these effects are heritable. Epigenetic changes can switch genes on or off and determine which proteins are transcribed. Cells are differentiated by what genes are turned on or off.

The study of epigenetics has major importance in the biological sciences. It is incorporated into many major research studies currently underway. Understanding how cells execute the instructions in their individual genomes will hopefully provide important clues about

what goes wrong in diseases, such as cancer. It may also pave way for new therapeutic approaches. Learning about epigenetic marks that cause a change in the expression of genes may lead to ability to undo some of the epigenetic marks that cause harm one day in the future, as well. Epigenetics is key to better understanding disease, such as cancer, behavior, and stem cells/development.

HUMAN EPIGENOME PROJECT

In 2003 The Human Epigenome Project was launched by the Human Epigenome Consortium. The international members of the Consortium include the Wellcome Trust Sanger Institute, Epigenome AG, and Centre National de Genotypage. The Consortium was comprised of both private and public institutions working together. The goal of the project was to identify, catalogue, and interpret the genome wide DNA methylation patterns of all human genes in all major tissues. Each tissue type and disease state has a distinct cytosine methylation pattern. Therefore, different methylation variable positions (MVPs) correspond to different tissue types and disease states. MVPs become epigenetic markers for tissue types and diseases. It was hoped that the knowledge gained from the Human Epigenome Project would greatly enhance our understanding and diagnosis of diseases.

The Human Epigenome Consortium first started the Human Epigenome Pilot Project in 2000. The aim of the pilot study was to identify and catalog the MVPs in major histocompatibility complex (MHC) of seven different tissue types. MHC is a cell surface molecule encoded by a large gene family. The scientists at the Consortium focused on 150 expressed genes rather than the entire MHC region. For each of the gene, scientists looked at the promoter region that regulated whether genes get turned on or off and the most CpG rich region where methylation of cytosine occurs. The result of the three-year pilot study was fruitful.

Scientists identified and cataloged over 100,000 methylation sites. The differences in MVPs of the different tissue types were also identified.

The method used in both the pilot study and the full scale Human Epigenome Project was a bisulfite treatment coupled with PCR amplification (Figure 5). Originally, DNA methylation activity was recognized using enzymes. This gave an incomplete restriction cutting to limitation of the region of study and required Southern blot technologies that required DNA sample with a high molecular weight (Esteller 2006). The new method was more efficient and accurate. DNA sequences treated with bisulfite resulted in unmethylated cytosine being converted to thymine. Methylated cytosines were unaffected and stayed the same.

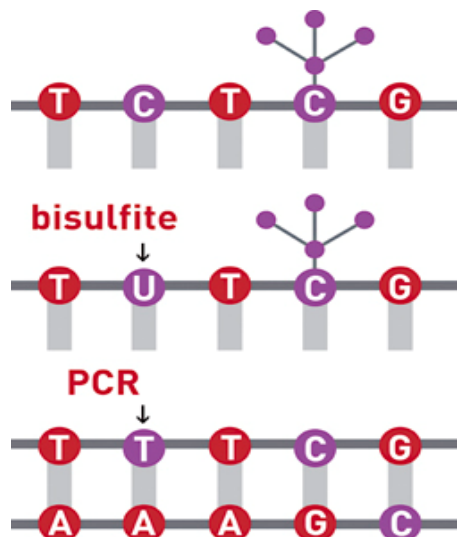


Figure 5. Bisulfite Treatment

Methylated cytosines treated with bisulfite is not altered. However, cytosine bases on the same strand of DNA that is not methylated is converted to uracil. PCR amplification further converts the uracil to a thymine and unmethylates the 5-methylcytosine. The resulting cytosines allowed scientists to identify methylation patterns. Reprinted from website: <http://www.diagenode.com/en/applications/bisulfite-conversion.php>

The Human Epigenome Project demonstrated great promise in advancing the current understanding and diagnosis of disease. Mapping out the epigenome of normal and disease state cells allows for comparison and analysis of the origin of methylation patterns. Application of the results of the project can also extend to therapeutic approaches. Endeavors to reverse harmful

methylation events can be pursued if sites of methylation of disease states can be located. From current applications to possible future ones, it is evident the mapping of the human epigenome is essential to fully understand the epigenetic phenomena.

ENVIRONMENTAL STRESS/TOXINS AND NUTRITION

Lamarck's theory of inheritance of acquired traits through generations can be witnessed in behavioral and developmental studies. Epigenetic mechanisms allow parents to pass information about their environment to their offspring. The ancestral environment has the potential to influence the phenotype of offspring. Of the various epigenetic mechanisms, DNA methylation is the most common one in which information about the ancestral environment may be passed on.

There are two general types of environmental factors that can affect descendants, stress/toxins and nutrient availability. Stressful environments have the potential to have an affect on future generations even after the initial stress has subsided (Rando 2012). A stressful environment can include social defeat, DNA damaging stress, and environmental toxins. Two studies have been performed suggesting that environmental stress on the parent does indeed have an affect on the offspring. The first case involved injecting a high concentration of an endocrine disruptor vinclozolin into a pregnant female rat. The offspring were observed to have diminished fertility over three to four generations. Males had an increased testicular apoptosis and altered behaviors in the male germ line. In the second case early fly embryos were heat shocked or placed under osmotic pressure. This environmental stress caused derepressed heterochromatin in both the parent and the progeny. The male offspring with derepressed heterochromatin were bred with a control wild type female. The offspring of this breeding produced male offspring with derepressed heterochromatin that was derived from an unstressed female.

Ancestral diet has been linked to offspring phenotype (Rando 2012). Epidemiological studies link maternal undernutrition to increase risk of various diseases, specifically type II diabetes and obesity in children. During the harsh winter of 1944-45 Dutch children suffered from in utero starvation. The children were noted to have an increased rate of diabetes, cardiovascular disease, and obesity later in life. The parents' diet effect on the offspring was experimentally proven with rodent studies (Figure 6). In the case of agouti mice, a female pregnant mouse was fed a methyl rich diet. Her offspring had a brown coat color, were of healthier weight and size, and had a lower risk for diseases. When fed a methyl poor diet the offspring had a yellow coat color that is linked to obesity and higher risk of disease. Overall, the offspring produced by the parent when fed a methyl rich diet were much healthier and lived a longer life (Wolff, Ralph, Moore, and Cooney 1998).



Figure 6. Agouti Mice

The offspring of a female agouti mouse varies in physical appearance and health. From left to right, the ones further to the left have a more yellow coat color (agouti) and far more obese. These offspring developed within the mother while she was fed a methyl poor diet. Going down the line to the right the offspring have a visible decrease in size and the coat shows brown coloring. These offspring developed within the mother while she was fed a more methyl rich diet. This correlation between offspring health and mother's diet indicates the effect of diet on the next generation. Reprinted from: website: <https://www.caymanchem.com/app/template/Article.vm/article/2182>

There is also evidence suggesting the transmission of disease caused by diet may be sex specific. Epidemiological data from human population studies have shown that food availability in paternal grandparents may affect obesity and cardiovascular disease risks two generations later (Rando 2012). The grandsons' were affected by their paternal grandfather and not by their paternal grandmother. The reverse is true for the granddaughters who are only affected by the diet of their paternal grandmother and not their paternal grandfather. In both cases malnutrition during early adulthood around the age of nineteen of the paternal grandparent caused an increased mortality risk in the grandchildren. However, poor nutrition in early adolescence around the age of ten of the grandparent had a more positive effect on the grandchildren. The grandchildren showed a decreased mortality risk.

In a study done with mice, male mice were subjected to preconception fasting. Offspring of the male mice had an altered glucose metabolism. Male rats that were instead fed a diet high in fat produced daughters with varied pancreatic phenotype, decreased glucose tolerance, and a decreased number of islet cells. Male mice fed a low protein diet from the time of weaning to sexual maturity sired offspring with decreased hepatic levels of cholesterol esters and altered hepatic expression of lipid/cholesterol biosynthesis genes (Rando 2012).

TWINNING PHENOMENON

Environmental factors such as diet and stress/toxins can also explain what is known as the twinning 'phenomenon'. Identical twins who are born with the same genome may exhibit phenotypical differences as they age. The epigenetic changes to the genome induced by a range of environmental factors are now believed to be a probable origin of variation between identical twins. As young children twins are more likely to be exposed to the same environment and even fed the same diet. However, over time the lifestyles of the twins are inevitably going to vary

from one another. Scientists have compared the chromosomes of a spectrum of identical twins from the ages of three to fifty, and found there to be more variation in DNA methylation between the chromosomes of older twins than younger ones (Fraga et al. 2005). There is a noticeable difference in pattern of epigenetic modifications and in varying degrees as identical twins progress in their lifetime (Figure 7). Environmental influences contribute to epigenetic modifications that may explain the twinning phenomenon.

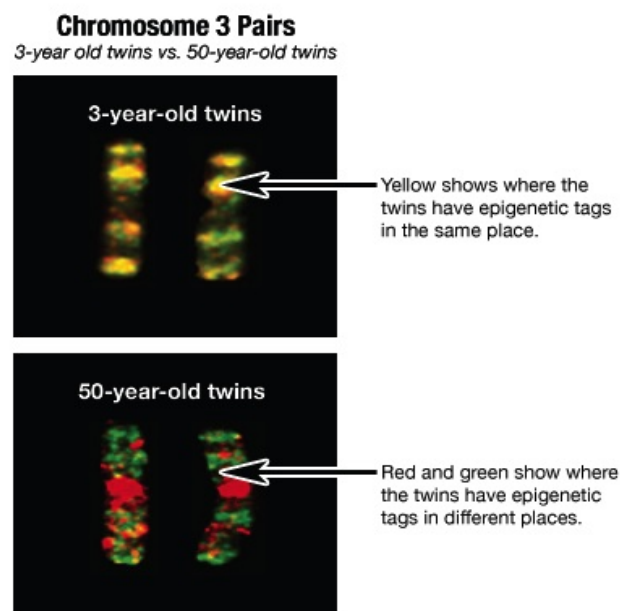


Figure 7. Comparison of Chromosome 3 Between 3-Year-Old Twins and 50-Year-Old Twins

In the top picture of the above figure the chromosome of the 3-year-old twins are nearly identical. The epigenetic tags of the chromosome are highlighted in yellow and occur in the same location across the two chromosomes. In the bottom picture of the 50-year-old twins the epigenetic tags are highlighted in red and green. The red and green epigenetic tags do not occur in the same location across the two chromosomes. The older set of twins show differences in the epigenetic tags compared to the younger twins. The differences among the older twins can be attributed to the different environmental influences experienced throughout their lifetimes from different lifestyles. Reprinted from website: <http://learn.genetics.utah.edu/content/epigenetics/twins/>

Environmental factors have a hand in how the genome information is executed. It does not only affect the individual directly but generations down the line as well. The environment experienced by a parent or grandparent can ultimately affect the life of the descendants. The epigenetic changes to identical genomes can produce different phenotypes as observed with the

twinning phenomenon. If and when these epigenetic modifications occur is key to understanding development, behavior, and other life functions.

STEM CELLS

Stem cells have become a key interest in scientific research due to their ability to differentiate into any given cell type. These cells are defined by their ability to differentiate into diverse specialized cell types. This ability is known as pluripotency. However, as a cell divides it loses its pluripotency and is directed toward a more specified cell lineage (Figure 8). This loss of pluripotency can be attributed to epigenetic modifications. Recent data imply that epigenetic mechanisms can confer stability to the steady state gene expression patterns of stem cells and direct the terminal fate of cells (Mohn and Schubeler 2009). Hence, the study of epigenetic phenomena plays an essential role in stem cell pluripotency.

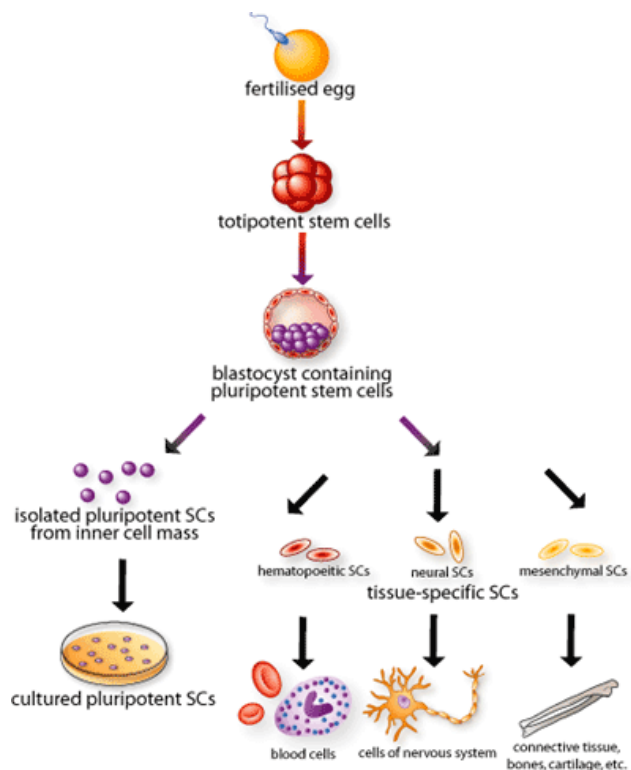


Figure 8. Stem Cells and Loss of Pluripotency

A fertilized egg produces totipotent stem cells that have the ability to differentiate to any cell type. As cell division occurs, epigenetic modifications restrict the cells to a specific cell lineage. These epigenetic modifications that turn on and off certain genes allows for the production of specific cells such as hematopoietic, neural, and mesenchymal stem cells. Reprinted from website: <http://www.scq.ubc.ca/stem-cell-bioengineering/>

There is evidence that chromatin based regulatory mechanisms and transcription factors may have an important role in transcriptional programs that in turn affects epigenetic modifications (Mohn and Schubeler 2009). How exactly epigenetic modifications work to change the chromatin structure of DNA is not perfectly understood, but it is believed to work by directly blocking transcription factors or by establishing a higher order chromatin structure. Transcription factors are proteins that turn genes on or off. One of the best-studied cases of higher order chromatin structure is the acetylation of histone H4Lys16 (H4K16). The modification made to the histone structure can prevent higher order chromatin structure from forming. A higher order chromatin structure is known to make DNA less accessible to transcription factors. Acetylation of H4K16 allows transcription factors to more easily bind to the DNA.

Besides acetylation, another significant form of histone modification identified in stem cell research is methylation of Lys4 of histone H3 (H3K4me) at CpG islands. During stem cell differentiation *de novo* methylation of CpG rich sequences coincides with the loss of H3K4 methylation (Mohn and Schubeler 2009). Methylation of H3K4 creates a signal that is recognized by multiprotein complex that is involved in the activation of certain genes. There is an apparent difference in the methylation profile at CpG rich versus CpG poor promoters. CpG promoters have di- and tri- methylated H3K4 when the genes are actively transcribed. CpG rich promoters are methylated at H3K4 independently of transcriptional activity of the genes. Methylation of H3K4 blocks DNA methylation from occurring. In the absence of H3K4 methylation, DNA methylation takes place. These two occurrences are mutually exclusive. It is with the absence of methylation of H3K4 can DNA methylation occur at CpG islands and shut down pluripotency to allow for cell differentiation.

DNA methylation has a direct effect on the pluripotency of stem cells. Methylation to certain regions of the DNA sequence has the ability to stabilize the shut down of pluripotency during cell differentiation. In vertebrates specifically, the regions targeted by DNA methylation are known as CpG regions. Recent studies have shown that DNA methylation at CpG rich sequences is low in stem cells (Mohn and Schubeler 2009). It is not until cellular differentiation does hypermethylation occur at CpG island promoters and CpG rich sequences outside of the promoter regions. The opposite is also true, very little demethylation is detected during cell differentiation. This is an indication that DNA methylation mediated gene repression increases during cell differentiation. There is enough genetic and molecular proof that suggests that DNA methylation can shut down pluripotency and activate cell differentiation in stem cell.

CANCER

Epigenetic modifications have a tremendous effect on the determination and stability of normal and disease biological states of organisms. An area of study where these implications have had a direct affect is cancer research. The changes to gene expression caused by DNA methylation and histone modifications have been known to lead to cancerous states of cells. The cancer epigenome is defined by specific patterns of DNA methylation and histone modification.

The DNA methylation profile of cancer cells consist of hypomethylation, less than normal amount of methylation, on the genome wide scale and hypermethylation, over abundance of methylation, at the CpG island promoter regions (Figure 9). CpG islands are short sequences that are rich in cytosine and guanine bases one after the other. The most recognized form of DNA methylation of tumor cells is hypermethylation of tumor suppressor genes such as the following; CDKN2A (cyclin-dependent kinase inhibitor 2A), MLH1 (mutL homolog-1), BRCA1 (breast cancer-associated-1) and VHL (von Hippel-Lindau tumor suppressor) (Rodriguez-

Paredes and Esteller 2011). The hypermethylation of these tumor suppressor genes causes them to lose their functionality and allow for the production of tumor cells to start and continue without an end.

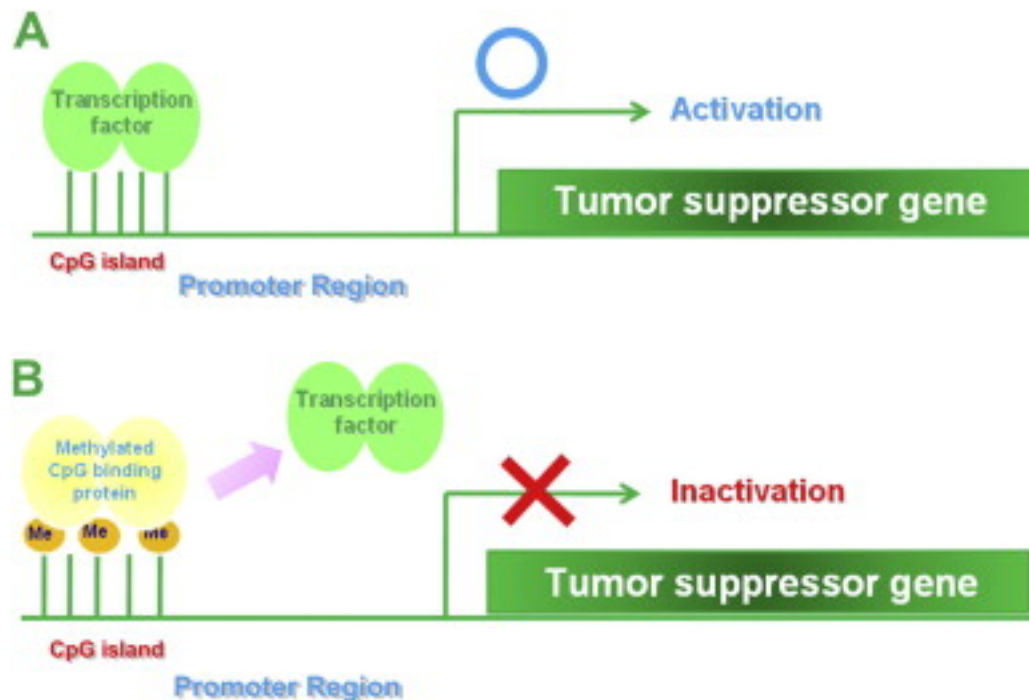


Figure 9. Hyper Methylation of Tumor Suppressor Gene

The top half of the figure shows genetic material within a healthy individual. The transcription factor is allowed to bind to the CpG island of the promoter region and turns on the tumor suppressor gene. The bottom half of the figure shows hypermethylation of the CpG island. Hypermethylation blocks the transcription factor of the tumor suppressor gene from binding and activating the tumor suppressor gene. With the tumor suppressor gene turned off tumor cells are allowed to be constantly produced. Reprinted from website: <http://www.sciencedirect.com/science/article/pii/S2186495012000077>

Besides DNA methylation, histone modifications have been also known to lead to cancer. Histone modifications take place on the N-terminal tail of the histone proteins during posttranslational modification. There are five main types of histone modifications that may occur, methylation, ubiquitination, sumoylation, acetylation, and phosphorylation. The best characterized form of histone modification associated with cancer is global reduction of the trimethylation of H4K20 (H4K20me3) and acetylation of H4K16 (H4K16Ac), along with DNA

hypomethylation, at repeat sequences in many primary tumors, tumors where tumor progression began and continue to yield a cancerous mass (Rodriguez-Paredes and Esteller 2011).

In the field of oncology, the study of epigenetics can better expand the knowledge of cancer treatment. In the past ten years tumor detection has made great progress, along with the help of the Human Epigenome Project. As the first project to undertake the task of mapping out the epigenome it set the groundwork for others to do so as well. Researchers have been able to map out the epigenome of tumor cells and normal ones. By comparing the epigenome of the two biological states it is possible to see where the variation and possible origins of disease may lie. In the case of different classes of human neoplasia, tumorous conditions, it is the unique profile of the hypermethylated CpG islands that define the various tumor types. Hypermethylation of CpG islands serve as biomarkers for tumor detection. However, hypermethylation of CpG islands does not necessarily infer cancer states since premalignant and or precursor lesions have an identical epigenetic marker, but are helpful in early detection (Rodriguez-Paredes and Esteller 2011).

CONCLUSIONS

The first epigenetic drug produced was for the treatment of cancer, specifically leukemia and lymphoma. Currently there are four FDA approved epigenetic drugs on the market, in which two are for DNMT inhibitors and the other two for HDAC inhibitors. The first two work to prevent methylation occurrence at CpG island sites that promote the tumor suppressor genes to deactivate and start tumorigenesis. By inhibiting these DNMTs tumor suppressor genes are allow to functionally normally and prevent tumor formation. HDAC inhibitors prevent differentiation and continued cell growth and differentiation in tumor cells and enhance the sensitivity of chemotherapy (Rodriguez-Paredes and Esteller 2011). Even though there are productions of

epigenetic drugs available more information and knowledge is needed to better understand epigenetic relations to cancer in order to better our ability to fight cancer.

The emergence of epigenetics as field of study can help to answer questions that were once eluded scientists and could not be explained by traditional genetics. For decades DNA has been thought to be the blueprint of life, but the emergence of epigenetics shatters this idea. Epigenetic modifications through DNA methylation and histone modification have the ability to manipulate how the genes in DNA can be expressed. The study of epigenetics has been incorporated into various other studies of life, such as research in disease, behavior, and stem cell/development. We have only just scraped the surface on the topic of epigenetics, there is still much we don't know and more to learn about this phenomena.

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