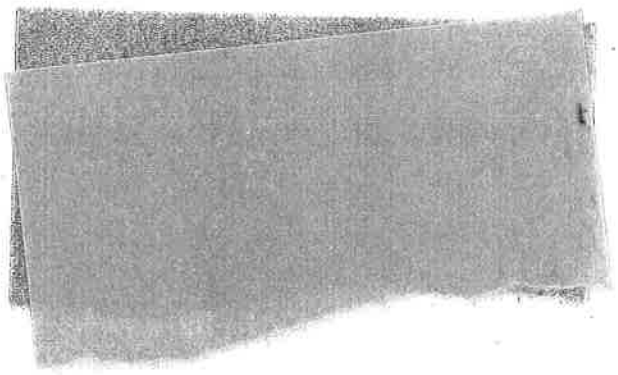
Epigenetic Discoveries



Epigenetics concerns any modifications that physically alter chromatin, without altering the DNA sequence, and as a result, control gene expression. These effects are derived from a variety of sources such as the environment, pollutants, or stressors (Feil & Fraga, 2012). Often times, correlations made between an environmental influence and an experimental result seem intangible. However, they can be understood as an interplay of chemical interactions in the body. These interactions cause the addition or removal of chemical groups on the chromatin, which in turn, affects the transcription of DNA (Jirtle & Skinner, 2007).

One of the most well studied areas of chemical influence is the effect of nutrition on epigenetic gene expression. Nutrition is the fuel by which the body is powered and therefore can influence epigenetic alterations in several ways. Though generally, the nutrients taken in (both the composition and the quantity) influence the chemical status of the body. Studies have increasingly shown that exposure to certain diets can affect chemical modifications to the DNA sequence making certain genes active or inactive (Florean, 2014). One study involving nutrition that has had a major effect on the field of epigenetics is that of the 'Agouti mouse model.' Before the agouti mouse study, the impact that nutrition can have on gene expression was largely unappreciated. However, the results of this study were stepping stones to understanding the challenges of human

susceptibility to certain illnesses. The study also greatly impacted the field of epigenetics as an experiment that legitimized the theory (which was somewhat controversial at the time) of transgenerational inheritance of epigenetic marks (Morgan, Sutherland, Martin, & Whitelaw, 1999).

Waterland, R., & Jirtle, R. (2003). Transposable Elements: Targets for Early Nutritional Effects on Epigenetic Gene Regulation. *Molecular and Cellular Biology, 23*, 5293-5300.

The Agouti gene is a regularly occurring gene in flocculent mammals. It codes for a coat pattern of black hairs with a band of bright yellow wrapped around the middle of each hair, resulting in a brown coat color. However, occasionally a mouse is born with a solid yellow coat. In these cases, instead of the agouti gene being expressed as a single band on an individual hair, it is expressed fully on each hair, producing a yellow mouse. Research conducted in 1994 by Duhl et al. concluded that the agouti gene is associated with both coat pigment and body weight regulation (Duhl, Vrieling, Miller, Wolff, & Barsh, 1994). The normal colored mice tended to have normal body weight, whereas the yellow mice tended to be obese and were overall more likely to develop diabetes and cardiovascular disease. The study showed that this different phenotype was the

result of activity of a transposon, located upstream from the agouti gene. It is well known that most transposons are silenced by CpG methylation. However, CpG sites in the yellow mice were reported to be hypomethylated, when compared to the brown agouti mice (Morgan, Sutherland, Martin, & Whitelaw, 1999). The variance in phenotype between sibling mice provided the foundation for the featured epigenetic experiment, which sought to observe the effects of nutrition on epigenetic mechanisms. For the 'Agouti mouse model' experiment, inbred yellow mice, that had been selectively bred for over 200 generations, were used. This was to refute any past generational effect on offspring phenotype. The female mice were then bred to similarly homozygous mice sires. The researchers assumed that they could manipulate the development of the mouse zygote through nutrition. Pregnant mice were divided into groups, those that would serve as the control, and those that would serve as the experimental group. The experimental group were fed a diet rich in methyl donors (such as folic acid) for two weeks prior to the breeding, while the control group's diet was un-supplemented. The experimental diets were maintained throughout the course of the pregnancy and lactation. When the pups were weaned, they were analyzed (vetted and weighed) and classified by the amount of brown fur they expressed. DNA taken from the pups was processed through a series of steps including: PCR,

genomic sequencing, and methylation assay. The results concluded that the mice which received the methyl rich diet produced a higher percentage of brown offspring than the mice which received a normal diet. Despite the varied phenotypes, the resulting pups were genetically identical. The only perceivable difference in the chromatin of the two mice was that the brown mice had higher rates of methylation on CpG sites, while the yellow mice had hypomethylated CpG sites. Scientists also noted that the additional fat-body trait that accompanied the yellow coat color was removed from the brown offspring, and the pups did not inherit the heightened susceptibility to disease like their parents. The results of the study were also used to validate previous research claiming that methylation on offspring DNA was contributed by methyl marks persisting through the maternal germline (Wolff, Kodell, Moore, & Cooney, 1998). The scientists concluded the paper by highlighting the significance of these results in relation to future medical research. Now, over a decade later it is apparent that the information derived from this study has been instrumental in understanding the epigenetic effects on human health.

Rarely do scientists receive an opportunity to study the effects of nutrition on epigenetic modification in humans. However, one study in the field of epigenetics has produced fruitful evidence that the human epigenome responds

to environmental influence in much the same way as prior experiments conducted on animals. This epigenetic study surrounds the historical *'hongerwinter'* otherwise known as the 'Dutch hunger winter,' which is a longitudinal study that is ongoing even today.

During world war II, in the winter between the years 1944 and 1945, the norther portion of the Netherlands was occupied by German forces. At this time, the Dutch people were supporting the Allies and trying to assist their efforts to end German control of the Netherlands. In retaliation, the German troops formed a blockade that completely cut off the transport of food from Dutch farms to the inhabitants of the most highly populated villages. This war time malevolence resulted in a five-month famine that killed approximately 18,000 people. The citizens who survived did so on government issued rations that were apportioned at soup kitchens in the villages. Prior to the blockade, the Dutch people were reported to be consuming an average of 1600 calories a day, an understandable amount at a time of war. However, once the German soldiers blocked the transport routes, the citizen's estimated food intake dropped to a derisory 400 – 800 calories a day. Many of the Dutch people in these villages died, but some managed to persist through the adversity. Records from the villages even reported many pregnant women had given birth to children during this time.

Later, the records from the famine were used to observe environmental effects of severe environmental cues on epigenetic modifications in fetal development. The study, conducted by a conglomerate of researchers and epidemiologists, found astounding correlations in the survivors. The adults who survived were, for the most part, unaffected by the famine. They resumed normal lives, with normal metabolisms, and typical disease rates, until they eventually passed away at a predictable age for their generation. However, their children, specifically those who were exposed to the famine during early gestation were extraordinarily atypical. The researchers found that these children grew up to have an unusually high susceptibility to many common health risks. This group of exposed individuals became a primary focus of the 'Dutch famine cohort' study.

Heijmans, B., Tobi, E., Stein, A., Putter, H., Blauw, G., Susser, E., Slagboom, E., & Lumey, L. (2008). Persistent epigenetic differences associated with prenatal exposure to famine in humans, *PNAS, 105,* 17046-17049.

Over the years, researchers have monitored the individuals exposed to the famine during early gestation and documented their susceptibility to disease and health risks by comparing each individual to a same sex sibling. This way, they have a control group with whom they can feasibly compare the 'hunger winter'

survivors. The same sex sibling was born either before the famine or after, providing scientists with the ability to observe the effects of similar genetics, and post-natal environments to the results of the experimental group. The study was conducted sixty years after the Dutch hunger winter had concluded, thus scientists could hypothesize the effects of intrauterine environment on adult individuals. The study primarily focused on variance in insulin-like growth factor II (IGF2) because of its known epigenetic regulation through maternal imprinting in differentially methylated regions (DMR). Researchers tested groups of exposed individuals, categorizing them based on what stage of development they would have been in during the famine. They then measured methylation in the participant's IGF2-DMR through a mass spectrometry–based method. Those exposed to the famine during late gestation were insignificantly differential from their unexposed siblings. However, those exposed during early gestation showed a significant variance in the amount of methylation in their IGF2-DMR, compared to the controls. Individuals exposed to the famine in late gestation had lower concentrations of methylation than their same sex sibling. This was suspected to be the result of a suspicion at the time (now confirmed) that epigenetic modifiers are the most malleable in early embryonic development.

This study has provided many insights into epigenetic mechanisms in early embryonic development. Further studies have shown that the famine group have a higher risk for obesity, are three times as likely as their siblings to contract cardiovascular disease, and they have a higher risk of developing schizophrenia (Heijmans,a, et al., 2008). These findings have been crucial to understanding the affects of environment on development.

Cancer has been the primary focus of most epigenetic research for the past few decades. The reason for this is the landmark study by Andrew Feinberg and Vogelstein, which corelated the epigenetic mechanism of DNA methylation to tumor growth.

Feinberg, A. & Vogelstein, R. (1983). Hypomethylation Distinguishes Genes of Some Human Cancers From Their Normal Counterparts. *Nature, 301,* 89-92.

Around the time of the study, researchers were beginning to believe that cancer was the result of aberrant differentiation in cells (A Look at the Origins of Cancer Epigenetics, 2013). However, this claim was largely base on animal models and the results were inconsistent when using different techniques of the time. Feinberg and Vogelstein's study recorded a correlation between the epigenetic modification of DNA methylation and cancerous tissues. The study focused on the

location and comparison of methylation patterns in tissues, rather than the amount of methylation present. In their experiment, they analyzed the difference between cancerous tissues and the adjacent normal tissues in individuals who were known to have tumors but had not yet received treatment. To do this, they employed specific restriction enzymes that were known to cut along the nucleotides, but were blocked by DNA methylation. This method allowed the scientists to record if the target gene in the cancerous, and non-cancerous tissues were methylated. They used southern blotting technique to interpret the DNA sequence of the fragments collected. The results they received from the experiment were significant. Cancerous tissue was significantly less methylated than comparable tissues in the body. The methylation differences were present in all but one of the comparisons run, and in some cases, the methylation in normative tissue was 9-fold that found in the cancerous tissues. Steinberg and Vogelstein ended their paper by addressing the need to expand their research to different cancer types, as well as a need to increase our understanding of epigenetic mechanisms. Their results were without a doubt profound, and have influenced various disciplines' research surrounding cancer.

One of the most recent topics in the field of epigenetics are epigenome modifications and their correlation with intellectual disorders. Only within the past few years have scientists begun to speculate about the possibility of epigenetic abnormalities being the source of many intellectual disabilities (Bokhoven, 2011). Most of these disabilities certainly fit the criteria for an epigenetic disorder; not entirely genetic, and not entirely environmental. The association was purely hypothetical, until a study conducted by Pier et al. (1991) discovered the correlation between histone acetylation and an intellectual disability called Fragile X syndrome.

Pieretti, M., Zhang, F., Fu, Y., Warren, S., Oostra, B., Caskey, T., & Nelson, D. (1991). Absence of Expression of the FMR-1 Gene in Fragile X Syndrome, *Cell, 66,* 817-822.

Fragile X syndrome (FXS) is currently recognized as the most common cause of autism, and certainly the most well studied. FXS is characterized be severe mental retardation and morphological abnormalities. The study pointed out an abnormality in histone acetylation found in individuals with fragile X syndrome, which until that time was only associated with an aberrant expansion of the FMR1 gene (Rousseau, Labelle, Bussières, & Lindsay, 2011). The expansion of the FMR1

gene is caused by multiple CGG repeats which is the characteristic mutation of FXS. The study conducted by Pieretti et al. revealed that this mutation is further coupled with epigenetic mechanisms that determine the scope of the disorder. For the experiment, researchers used a reverse transcriptase-PCR method to analyze FMR1 transcripts in individuals with FXS. They also used restriction endonucleases that were unable to cut methylated DNA to track methylation patterns on FMR1 gene. Results showed that the FMR1 gene of normal individuals were transcriptionally active and unmethylated, whereas those with FXS had hyper-methylated FMR1 genes. The FMR1 gene was also hypo-methylated in FXS carriers. Methylation patterns control the accessibility of transcription factors to DNA. Areas of the genome containing a high percentage of methyl groups are typically considered 'silenced,' whereas genes with a low concentration of methyl groups are normally expressed. These results suggested a correlation between the expression of FMR1 and the presence of the syndrome. The researchers concluded their paper by stating that the experiment would need further research and replication to fully understand the contribution of DNA methylation on FXS. Almost prophetically, their experiment has been replicated several times and has greatly contributed to the discovery of DNA methylation in other intellectual disorders.

Each of these studies have contributed to the scope of epigenetic research considerably. In addition to assisting in the formal inauguration of epigenetics into biology, they have shaped the direction of current genetic research. The diverse origins of epigenetic alterations and the substantial effects they can have on organism phenotype would not have been truly appreciated without the knowledge gained from these studies. Future research will certainly profit from this information, leading to further understanding of epigenetic mechanisms and their effects on biological systems.

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Personal Reflections on Epigenetics

Epigenetics is a recently cultivated branch of biology that has become of increasing interest to scientists. Although it is a relatively new discipline, research derived from epigenetic experiments have provided insights into some of the complex and undefined mysteries of biology. Some of the discoveries made from these studies have impacted areas such as organismal development, disease susceptibility, and inheritance. Without a doubt the contributions made to all of biology by the young field are vast, and furthermore, they are growing.

On one hand, the study of epigenetics seems somewhat contained. The discipline is concentrated solely on the semi-permanent changes to an organism's genetic material without altering the gene sequence. However, the scope of this seemingly confined focus is proving to be almost undefinably inclusive. Elements effecting epigenetic mechanisms have been observed to be caused by nutritional imbalances, psychological stress, physiological stress, as well as many other factors. In this regard, epigenetics is reshaping preexisting fields through its ubiquitous inclusivity. Knowledge gained through future studies could cause this field to become an important contributor to human health research. So far, some of the most important epigenetic studies have already led to the creation of new treatments or therapies for diseases, and an intensified concentration on epidemiological research. But possibly the greatest impact these findings have

made is in raising awareness that genetic determinism can be affected by more than just the DNA sequence.

This aspect of epigenetics is incredibly exciting and shows a great deal of promise for discovery as research continues. However, the products of epigenetic discoveries may benefit other facets of biology more than the discipline of epigenetics independently. Rather than a new discipline of its own, I believe that the greatest contributions made by epigenetics will be those that are taken and used by other areas of biology. Understanding the epigenetic mechanisms involved in controlling gene expression could improve currently established fields. These fields could certainly benefit from the incorporation of epigenetic concepts into their study. As more research is conducted and more knowledge is gained, groups that specialize in development, inheritance, and disease will predictably use this information to evolve the applications of their field.

Another reason epigenetics could be more affective working with other concentrations is because it is inherently constrained to a somewhat intangible study. Although some scientists have been able to manipulate the mechanisms driving epigenetic change, these alterations seem incredibly precarious to control. The most important quality allowing traditional genetics to be wieldable by

scientists is its near permanence. The incredibly malleable nature of epigenetic alterations could prove to be the limiting factor of its potential use. Small almost undetectable changes in environment have been shown to have a substantial impact on the epigenome. This could be a powerful tool for future applications, or this transitory quality could make it difficult, if not impossible, to be controlled. This of course provides a problem for applied science. If epigenetics is ever to be used, it will need to be changeable exclusively by intentional methods.

The reasons to expand and explore epigenetic mechanisms are substantial and out way the questionable practicality of the field. Some of the most ambiguously defined paradoxes in biology could be caused by epigenetic mutations and controls. Aging, disease susceptibility, and cell differentiation are all promising areas that are predicted to be associated with epigenetic alterations. Therefore, the potential to control these facets of human life is incredibly motivating. If the mechanisms involved in deciding the fate of a cell were to be controllable, complex problems like aging and cancer could be removed. However, these mechanisms would need to be much more stable than they are currently proving to be. If scientists were to program epigenetic alterations, these would need to persist through changes in the organism itself and its environment.

Epigenetics, until recently, has been a purely metaphysical science. But current advances in the field have shown that there may be a potential use, outside of basic discovery, for the new field. However, it is my personal belief that most of these uses will occur outside of epigenetics and within a field more suited for applied biology. In this way, I think that epigenetics can play a vital role as a future, theory driving, precursor to applied science. As research accrues, epigenetics will certainly earn its place among the standard areas of biology, as a sponsor, if not as a player.