An elucidation of the links between hormones, DNA methylation, the microbiome, and disease to restore homeostasis to each component through the genomic engineering of CRISPR microbes

Tatiana Hillman Corresp. 1

1 Biological Sciences, TheLAB, Inc., Los Angeles, California, United States

Corresponding Author: Tatiana Hillman
Email address: thillman@usc.edu

We speculate that there are connections between hormonal changes, the frequency of DNA methylation, and disease. The microbiome may also affect the production of those hormones. Short Chain Fatty Acids as butyrate, propionate, folate, and acetate act as ligands that bind to G-coupled protein receptors. The SCFAs are produced after intestinal microflora ferment glucose from insoluble fiber. When SCFAs bind to G-proteins, a downward cascade is activated, releasing hormones as leptin and PYY, which each control appetite and prevent the formation of type 2 diabetes. When the SCFAs bind G-proteins, a methyl group can be added to a specific and target site of a DNA sequence. For example, folate from Bifidobacterium donates a methyl for synthesizing S-adenosylmethionine or SAM, which then donates a methyl to the enzymes of DNA methylation, acting as a substrate. We presented less arduous ways to measure DNA methylation through a methyl kit, which recording the different levels of DNA methylation can help identify and distinguish between cancer versus non-cancer samples of blood. We reviewed the effects of hormones on DNA methylation. If the microbiome regulates both hormones and DNA methylation, then perhaps through the microbiome diseases can be more readily identified, diagnosed, modeled, and treated. Our purpose for this review, was to find the links between each of the three factors, hormones, DNA methylation, and bacteria, in order to find possible ways to genetically manipulate each into equilibrium to maybe provide alternative diagnosis protocols and treatments for disease.

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Tatiana Hillman[1]

[1]**603 E. University Drive Suite B, 613 Carson, CA 90746**

Corresponding author:
Tatiana Hillman[1]

Email address: thillman@usc.edu

ABSTRACT

We speculate that there are connections between hormonal changes, the frequency of DNA methylation, and disease. The microbiome may also affect the production of those hormones. Short Chain Fatty Acids as butyrate, propionate, folate, and acetate act as ligands that bind to G-coupled protein receptors. The SCFAs are produced after intestinal microflora ferment glucose from insoluble fiber. When SCFAs bind to G-proteins, a downward cascade is activated, releasing hormones as leptin and PYY, which each control appetite and prevent the formation of type 2 diabetes. When the SCFAs bind G-proteins, a methyl group can be added to a specific and target site of a DNA sequence. For example, folate from Bifidobacterium donates a methyl for synthesizing S-adenosylmethionine or SAM, which then donates a methyl to the enzymes of DNA methylation, acting as a substrate. We presented less arduous ways to measure DNA methylation through a methyl kit, which recording the different levels of DNA methylation can help identify and distinguish between cancer versus non-cancer samples of blood. We reviewed the effects of hormones on DNA methylation. If the microbiome regulates both hormones and DNA methylation, then perhaps through the microbiome diseases can be more readily identified, diagnosed, modeled, and treated. Our purpose for this review, was to find the links between each of the three factors, hormones, DNA methylation, and bacteria, in order to find possible ways to genetically manipulate each into equilibrium to maybe provide alternative diagnosis protocols and treatments for disease. Dummy abstract text. Dummy abstract text. Dummy abstract text. Dummy abstract text. Dummy abstract text. Dummy abstract text. Dummy abstract text. Dummy abstract text. Dummy abstract text. Dummy abstract text.

INTRODUCTION

Hormones are chemicals that act as messengers, monitoring response between and on cells and organs. William M. Bayliss and Ernest H. Starling from the London University College found a chemical called secretin, in the intestine, that activated the pancreas to secrete hormones. Thus, the name for hormones became ‘chemical messengers’. There are three subgroups or categories of hormones including: 1. Steroid hormones, 2. Protein, and 3 (4). Peptide hormones. Steroid hormones are fatty and lipid based, meaning they can cross the lipid bilayer of the plasma membrane surrounding a cell. The steroid can then bind to receptor in the cytoplasm, to the nucleus, creating a receptor-hormone structure. Estrogen, progesterone, and testosterone are examples of steroid hormones. These steroids mimic the action of hormones can be placed into two categories called corticosteroids from the adrenal glands and the sex steroids found in the reproductive organs(4). There are five different types of steroids for each group of steroids, which are

named by the kinds of receptors they bind. For the peptide hormones that are not fatty loving and are more lipophobic, so they do not cross the plasma membrane, but they bind to transmembrane proteins and receptors within the plasma membrane of a cell. The receptors they bind are attached to anchored proteins as G proteins (4).

After a peptide hormone is linked to a receptor as a ligand, many second messengers in the cell begin cellular actions. The types of second messengers include cyclic AMP, calcium cations, nitric oxide, and protein kinases. Examples of peptide hormones include: insulin,, glucagon, leptin, ADH and Oxytocin (4). Peptides synthesize enzymes within the immune system to degrade foreign materials, antigens, and produce antibiotics. The protein based hormones are the amine hormones made from the amino acids tyrosine and tryptophan. Tyrosine hormones are produced in the Thyroid, controlling metabolism and organ processes. The amines include the Norepinephrine and epinephrine of which these amine hormones are class the "stress hormones" that help to synthesize serotonin and melatonin (4). Short Chain Fatty Acids bind to G protein-coupled receptors as GPR41 and GPR43. These are now called free fatty acid receptors or FFAR3 and FFAR2. For the structure of FFAR2 and FFAR3, these G-protein coupled receptors are connected to G-proteins that are chimeric, which are linked and adjacent to the cytoplasm of the receptor(6). When a SCFA binds to a G-protein, cellular pathways are activated and initiate responses from secondary cascades. In the colon, SCFA stimulate the secretion of anorexigenic hormones as the peptide tyrosine tyrosine or the PYY(6).

A glucagon-like peptide called GLP-1 is also released. The PYY hormone is secreted into the blood after eating food. The SCFAs that are not processed by the liver, re-enter the surrounding blood circulation with a concentration of acetate at 170 umol/l, 4 umol/l for propionate, and 8 umol/l for the butyrate(6). There is a connection between fiber intake in the diet and the different levels of SCFA. The amounts of SCFA are directly proportional to fiber consumption in the diet. Acetate is the SCFA with the largest measure and concentration after fiber intake(6). The SCFA, circulating in the blood, can affect and relate to adjacent organs and tissues. SCFA can help with the catabolism of fat cells, adipocytes, releasing leptin. The concentration of leptin is dependent upon the availability of fat deposits. Leptin can also be transported through the blood-brain barrier, inhibiting the Y/agouti-related peptide, and initiating the POMC/cocaine and the amphetamine-regulated neurons(6). Leptin acts to lessen food intake and appetite through an anorectic effect via the ARC. There is an increased interest in finding and researching the link between the microbiome of the gut and disease. Fungi, viruses, and bacteria interact within the microbiome through an act of symbiosis. Microbiomes inhabit the mouth, the digestive system, the urinary tract, and etc (16).

It is speculated that the microbiome can affect the overall health and condition of the human body.The number of microflora spans into the trillions, preferably 100 trillion, which reside in the digestive tract. The microflora are attached to the mucosal walls of the intestines. It is believed that humans have a commensal interaction with the microbiota of the digestive tract. However, at a molecular level, the process of the interaction, between bacteria to humans, is not well interpreted or understood. For example, innately and native occuring microbiota synthesize end-products, as metabolites, that have a relationship with cells in order to regulate pathways connected to gene expression. The end-products produced alter epigenetics, changes the structure of chromatin, and activates many signaling molecules (16). These metabolites from commensal bacteria, differentiates cells, reduces inflammation, and controls the frequency of apoptotic events.

The epigenetic effects from the microbiota can provide alternative therapy for cancer and other diseases. In the process of DNA methylation a methyl group binds to the 5th carbon of the cytosine nitrogenous base and frequently links to CpG islands of the promoter or start sites of DNA sequences. DNA methyltransferases or DNMTs are enzymes for DNA methylation. However in a worm called C. elegans the position of DNA methylation differs including methylation of the NH2 groups at the sixth position of the adenines (6ma) and at the fourth position of the cytosines (4mc) (10). In bacteria, 4mC and 6mA are used to decipher between foreign DNA and their own DNA (10). These differences are purely used for signaling and epigenetic alteration purposes because they do not interfere with base pairing (10). For DNA methylation to occur the cofactors, alpha-ketoglutarate and oxygen, are needed as intermediate metabolites. Moreover, bacteria can affect and alter DNA methylation within the cells of their host. Through bacterial metabolism and fermentation of fiber, the end-products of this kind of metabolism, folate, butyrate, and acetate, the epigenetics of DNA can be changed. For example, folate from Bifidobacterium donates a methyl for synthesizing S-adenosylmethionine or SAM, which then

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donates a methyl to the enzymes of DNA methylation, acting as a substrate. Another example of environmental changes affecting an organisms epigenetics, is the chemical compound called Glyphosate. Glyphosate has been known as an unharmful substance, yet through experimeatal analysis it has been found to be an environmental toxin that disrupts the homeostasis epigenetically and genetically (18).

Moreover, measuring the strength and endurance of DNA methylation is dependent on more factors as it's ability to silence genetic expression or prevent bacterial transformation.For example, methyl group is removed from the start site of the gene for insulin is when beta-pancreatic cells produce insulin and also upon the specialization of the embryonic stem cells of mice into beta-pancreatic cells (3). The insulin gene is further silenced when a methyl CpG binds to it at the promoter site (3). Also, Caldicellulosiruptor bescii has a highly fortified restriction endonuclease, called CbeI, that cuts unmethylated bases at the five prime end GG/CC to the third prime end. This epigenetic process, within Caldicellulosiruptor bescii, prevents the DNA transformation of several bacterial types and the host's ability to achieve successful transformation depended on its skill to remove restriction sites within the DNA (7). The purpose of our review is to find possible links between hormonal changes and epigenetic modifications, which may cause disease. We believe the changes in the composition of commensal microbiota may influence epigenetic modifications, and the microbiome may serve as a channel for treating different health conditions in relation to hormonal and epigenetic alterations.

1 SURVEY METHODOLOGY

Research questions include: How can DNA methylation be measured? How can changes in genetic expression affect the output of hormones? What is the link between epigenetics and endocrinology? How can bacteria be re-engineered to alter DNA expression? Search strategy included: the search terms hormones the microbiome and disease hormones and DNA methylation, measuring DNA methylation, bacteria and DNA methylation, and the CRISPR Genome Engineering. The resources included: Plos one, American Journal of clinical Nutrition, Engineering and Technology, Journal Integrative Medicine, Nature Biotechnology, and Nature Reviews Genetics. For the study selection, the discussion and the introduction for each article was screened according to its relation to the scope of the research questions and topics. 5 research articles were matched and assigned for each search term. Examples: 1.CRISPR Genome Engineering Hsu, P. D., Lander, E. S.,and Zhang, F. (2014). Development and applications of CRISPR-Cas9 for genome engineering. Cell, 157(6), 1262-1278. 2.Bacteria and DNA Methylation Barres, R.,and Zierath, J. R. (2011). DNA methylation in metabolic disorders–. The American journal of clinical nutrition, 93(4), 897S-900S. 3.Hormones and DNA Methylation, Bharati, P.,and Rai, D. V. (2018). The Modulatory Effects of Hormones on Sato, Rajo and Tamo Guna. Engineering And Technology Journal, 3(01), 384-388. 4.Hormones, the Microbiome and Disease Bull, M. J.,and Plummer, N. T. (2014). Part 1: The human gut microbiome in health and disease. Integrative Medicine: A Clinician's Journal, 13(6), 17. 5.Measuring DNA Methylation matched with Chung, D., Farkas, J., Huddleston, J. R., Olivar, E.,and Westpheling, J. (2012). Methylation by a unique alpha-class N4-cytosine methyltransferase is required for DNA transformation of Caldicellulosiruptor bescii DSM6725. PloS one, 7(8), e43844. Articles were excluded due to their title and abstract description, which was not fitting for the research questions being reviewed. For example, 19 of the full-text research articles were high-quality with a clear link to the research questions. Four articles were low-quality and less aligned with the central idea and focus of the review. The four articles of low-quality were more about women's reproductive health than hormones direct effect on genetic expression. Quality Assessment Criteria and data synthesis included: 7 studies included in the introduction, 14 citations from articles in the body paragraphs, 3 Studies included in the conclusion, 19 full-text articles of high-quality, and 4 full-text articles of low-quality. We arranged the sources and each study, according to the themes and research terms, which 8 major points from each study were annotated and outlined with 4 main points were outlined, from each study, from the introduction, results, and discussion for each term.

2 HORMONES AND THE MICROBIOME

The gut-brain axis combines the communication systems of the neural, hormonal, and immunological signaling pathways of the gut and the brain (5). The metabolites produced in the microbiome of the gut are able to travel to the brain via the gut-brain axis. The signals sent through the gut-brain axis are bidirectional with sensors traveling from the gut to the brain and vice versa (5). Diet is the greatest

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influencer and environmental stimulus for differentiating the composition of the gut microbiota. However, it has been suggested that sex hormones may be linked and relate to the microbiota (14). For example, diabetic mice without displaying any obesity, but could develop type 1 diabetes, showed that males had a higher level of immunity to developing the disease when compared to female mice (14). It was proven that type-1 diabetes prevalence was lower in males due to the early-life composition and colonization of gut microbes in males.The good bacteria and healthy microbiota seemed to propagate more readily within the breast milk of mothers who experienced vaginal birth versus a cesarean-section(14). Lactobacilli within the vaginal area prevent infection through releasing lactic acid, peroxide, bacteriocins, and by out competing other bacteria (9). At the start of the menstrual cycle, the amount of estrogen is low, but the composition of the microbiome remains balanced even through hormonal imbalances during puberty and the menstrual cycle(9).

As an infant grows with age, the infants born through a C-section exhibit more antibiotic resistance genes within their microbiota. Healthy and good bacteria as Bifidobacterium longum help the dendritic cells within the Peyer's patch mature and differentiate with assisting in developing T cells in the thymus. The good microbes can propagate signals that monitor T cells and invariant natural killer T cells (21). The microbial signals can trigger the direction of these T cells towards pathogens, releasing a great amount of cytokines to initiate or inhibit many immune responses. The prolonged use of antibiotics can wipe out an entire taxa of microbes even the good bacteria (21). This loss of biodiversity leads to the pathogenic bacteria becoming more dominant and profuse. There is a recovery period, but the recovery of the microflora may be lengthy without enough of the healthy microbes to block the spread of pathogens, gaining access and momentum in causing infection (21).

3 HORMONES AND DNA METHYLATION

To quantify the time and the shifting of seasons, the requirement of changes in behavior, neural, hormonal, and genomic plasticity is necessary. The photoperiod or the change of hours in a day triggers the time for mating and reproduction (19). In vertebrates, the light and dark cycle encapsulates the circadian rhythm that is needed to secret the neurotransmitter, melatonin. The melatonin released monitors the hormones in the reproductive pathway of the neuroendocrine system. As the summer season transitions into autumn, more melatonin is secreted, and initiates signaling in the thalamus, hypothalamus, and the pituitary glands (19). In vertebrates that breed more frequently with a shorter day and photoperiod, the amount of melatonin decreases. The hormone found in the thyroid causes amplified signaling during a photoperiod is called T4. In the hypothalamus T4 is metabolized by the enzyme, deiodinase, into triiodothyronine (19). During the Winter, the absorption of dio3 is elevated, blocking T3 signaling and inhibiting the release of gonadotropin. However, in the spring and summer months dio2 is increased with more production of T3, stimulating the release of gonadotropin.

DNA methylation adds a methyl group to the CpG sites in mammalian DNA loci. DNA methylation in the promoter and start sites of genes block transcription (19). Methylation is an ideal source of epigenetic change of behavioral function and in physical structure since its allows for a timely and reversible control of genetic expression. When seasons or photoperiods change, the methylation within the promoter site of the gene dio3 is altered as well. The gene of dio3 is responsible for transporting photoperiod signals to the brain, to the reproductive, and to the brain's endocrine system (19). Reversible methylation cycles were found in the dio3 promoter site. Thus, methylation can affect monitor phenotypic and behavioral changes.

DNA methylation is significant for determining dio3 expression, which is affected by day length, melatonin, and the shifting of seasons. The deiodinase mRNA expression dependency on the photoperiod of reversible methylation is an important stage in regulating hormones in the brain for reproduction in birds and in mammals (19). During the summer, the activity of the dio3 promoter increases with more methylation to decrease dio3 expression in birds and mammals. Less dio3 expressed is followed with a greater production of the catabolism of the prohormone T4 from the hypothalamus. This then, stimulates the reproductive hormone called T3. When the season changes to decrease the day length, more melatonin is synthesized, which then lowers regulation of dnmt3b expression, lessening the methylation in the dio3 promoter (19). Then, dio3 expression is amplified, inhibiting the hypothalamic signaling of T3. Hormones can affect the different levels of methylation of specific promoter DNA sequences. For example, testosterone released increases methylation at CpG sites of steroid sensitive genes as vasopressin and on estrogen transmembrane proteins receptors.

Cortisol is a hormone responsible for exerting a stress response by binding to a receptor called the

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glucocorticoid receptor. Cortisol is produced in response to immune signals, reproductive, metabolic, and cardiovascular stress. To remedy the stress, DNA methylation is changed within the promoter site of the gene NR3C1 called the 1F promoter site (11). DNA methylation can silence many differentiated tissues with alternative first exons within the NR3C1 gene. More DNA methylation in exon 1F is linked to more perinatal and prenatal stress within the brain, cord blood, and in the placenta. More stress raises the amount of glucocorticoid by inhibiting the expression of placental HSD11B2 and 11bHSD expression. In rats, increased methylation of CpG sites occurs in the promoter sites of Hsd11b2. Pre-eclampsia and pregnancy issues in pregnant women may be caused by epigenetic changes in DNA methylation in response to raised levels of cortisol and hormonal signaling (11).

Antimicrobial peptides found in keratinocytes in the epidermis of the skin can protect against microbial infection in many tissues and at many sites of entry by microbes. Skin infections caused by Streptococcus, Staphylococcus aureus, and some other viruses increase and proliferate as the production of antimicrobial peptides become dysfunctional and disabled (15). However, vitamin D in high concentrations acting in compliance with PTH/PTHrP to intervene in the immune protection from skin infection. PTH increases in production and in activity when vitamin D is less available through continuing the equilibrium and calcium. The PTH/PTHrP increases the expression of cathelicidin in mice and human cells. DNA methylation monitors the expression of cathelicidin. Activating PTH1R, increases the suppression through DNA methylation (15). The promoter site of the cathelicidin in human skin and hair cells is methylated, increasing CAMP expression that mimics effects caused by PTH/PTHrP. A decreased in vitamin D may lead to a higher production of PTH, increasing a release of antimicrobial peptides that contribute to the innate immunity. More availability of PTH prevents skin infections by a normal diet of vitamin D, producing mBD4, when there is a lack of 1,25-D3 by producing CD14 when vitamin D3 and PTH are present (15).

The Microbe-Associated Molecular Pattern or MAMPS triggers immunity in plants by binding to the plant innate immune system's pattern recognition of receptors or to PRRs. After MAMPS binds to PRRs an immune response is signaled to activate MAMP-triggered immunity or MTI. This prevents the spread and proliferation of pathogenic microbes in plants (20). The hormone called salicylate found in plants produces a barrier of immunity against biotrophic and hemibiotrophic pathogenic bacteria. Other hormones as Jasmonate, JA, and ET, increase immunity against necrotrophic pathogens. The hormones of SA,JA, and ET must remain balanced and in homeostasis in order to maintain plant immunity. When there is an imbalance between these three plant hormonal-signaling pathways, the MTI is enormously hindered (20).

4 MEASURING DNA METHYLATION

Mutations in the gene bodies bring about cancer when there is much methylation in tumor suppressor genes as ITP53, which codes for translation of p53. In the transcription start sites of various genes of tumor suppressor genes in retinoblastoma are methylated, resulting in skin cancerous growths (13). So, CpG site genes have been used as an epigenetic label in vertebrates where methylation is genetically succeeded to the next generation of offspring through the mitosis of somatic cells.

An enzyme identifies the methylated palindromes, translating the number of DNA methylation sites through DNA linkage proteins. DNA methylation suppresses and blocks the expression of genes. DNA methylation in the transcriptional start sites that precede and are adjacent to the actual encoding regions of genes are blocked. However, methylation in the regions of gene bodies, activate continued DNA transcription, lengthening the DNA sequence. This elongation of DNA sequences can have a profound effect on splicing (13). Transcription factors modulate the expression of genes. For example, polycomb proteins inhibit CGI promoters. Moreover, genes located in the methylated parts of CGI promoters are mostly in the loci coding for long-term stability. One-third of pathogenic DNA mutations are caused by and found in methylated cytosine bases. Methylated cytosine bases have highest chance and frequency of producing a point mutation with the cytosines changing into thymines (13).

The methylation of cytosine is profuse through the genome mainly forming in the CpG dinucleotides. The methylation of cytosines inhibit the linkage and interaction between transcription proteins and bases that are methylated. Bisulfite sequencing measures DNA methylation. Cytosine bases are deaminated into uracil when DNA is exposed to sodium bisulfite (2). After the DNA has been administered with the sodium bisulfite, the 5-methylcytosine DNA residues remain unaltered. To quantify the frequency and percent of methylation, the ratio of c/c+t are recorded and numbered at each DNA base. Therefore, to

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eliminate this arduous task, Akalin et al. (2012) presented a digital software program to interpret and categorize data points from methylation quantification assays in a document format. With the methyl kit, images of DNA methylation can be developed (2). Single-molecule real-time (SMRT) of DNA sequencing can automatically detect the sites of DNA methylation. The SMRT method was used to detect 49,311 6-methyladenines (m6A) and 1,407 5-methylcytosines (m5C) from pathogenic Escherichia coli (8).

Changes in DNA methylation can identify many types of human diseases. For example, changes in DNA methylation can be found in the blood of cancer patients. Also, the mothers of children with illnesses within hearts have multiple sites in their white blood cells with alterations of DNA methylation and frequency (17). There are different levels of DNA methylation for each category of cells. For example, monocytes have methylated CpG sites that lose methylation, which quicken the differentiation of the myeloid process. However, white blood cells that live longer than 20 days, have more amounts of methylation because they need to maintain cell memory (17). B cells present foreign pathogenic proteins, antigens, and antibodies. However, B cells differ from other lymphocytes and tend to remain separate from other white blood cells. B cells have highly unmethylated CpG sites in areas of high amounts of CpG islands with 5 aCTORs, but the CpG sites in introns carried more methylation (17). Adalsteinsson et al. (2012) observed differences in methylation between two cells called MNCs and PMNCs, which reside in blood (1). Each white blood cell has a different frequency of methylation and a majority about 40 percent of all 23,000 CpG sites are more methylated within diverging cell types (1).

5 CANCER AND DNA METHYLATION

Pluripotent stem cells can be identified and classified by biomarkers, which were somatically inherited types of epigenetic alterations in a cell. DMRs are called differential methylated DNA regions. These DMRs can be observed in order to classify and divide the many different types of cell lines. Through hematopoiesis, stem cells that inhabit the bone marrow can continue to produce many subtypes of blood cells (12). Using DMRs as biomarkers, can allow for the identification of white blood cells, which can help to determine and mimic various conditions of disease. Housman et al. (2012) suggested methods to quantify the amount of DMRs and then analyze these data points for approximating the measure of white blood cells in blood. Measuring the amount of DNA methylation can help to quantify the proportion of leukocyte dispersal and allotment (12). DNA methylation found in blood can be used as epigenetic markers for the discovering of disease in clinics and abroad as in local or global epidemics. Assaying the blood through DNA methylation arrays can help to separate samples of cancer from non-cancer control groups. The DNA methylation arrays can characterize the different types of cancer as ovarian, bladder, and pancreatic cancers (12).

6 CONCLUSION

Many bacteria within the microbiome have been identified, but their operational methods of function are still poorly understood (16). The unresolved question include: how do microbes contribute to the pathogenesis and the physiological changes of disease? We attempted to highlight a few links between the hormones that cause disease through epigenetic means that can be induced by the dynamic changes in composition within the microbiome. We attempted to interpret the links between hormones, commensal bacteria, and DNA methylation more cohesively. We wanted to review specific literature to uncover a series of hormonal and bacterial, altering the epigenetics of DNA sequences, events that occur naturally and simultaneously in the human body and in nature. We noted the effects of neural, hormonal, and immunal signals within the gut-brain axis, being manipulated by metabolites from the microbiome. The effects of sex hormones from a changing microbiome, may cause type 2 diabetes. The microflora affect on the sex hormones, produced during the menstrual cycle does seem to change the composition of the microflora. Also, for women who had a C-section versus a vaginal birth, have more differentiated microflora, increasing the strength of T-cell protection against antigens and foreign material.

We also explored the effect of DNA methylation on hormones and in the reverse of the process. We found seasonal changes as a source of altering the propagation of hormonal signals. In the spring and summer some vertebrates T3 hormonal signals are blocked, leading to more genetic expression of dio3. In some vertebrate animals, more dio3 signals the release of gonadotropin, amplifying reproductive activity. In other animals longer photoperiod in the summers increases methylation in the promoter site of dio3, increasing T3 hormone production. In addition, pregnant women release more of the hormone cortisol

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when under much stress. More cortisol is released when methylation is increased at the promoter site of the gene NR3C1. Next, we summarized a procedure for quantifying DNA methylation levels.

The methyl kit as a computer program can more deeply analyze data form DNA methylation measurement experiments. Recording the levels of DNA methylation can give insight into the identification of possible cells developing into cancerous cells. For example, in patients with cancer there are multiple changes in DNA methylation found in their blood. Therefore, changes in DNA methylation levels can help to identify different types of human diseases. DNA methylation found in blood can be used as biomarkers to later identify and categorize diseases and chronic conditions. For example, assays of blood, using DNA methylation arrays, can distinguish between cancer and non-cancer samples from patients as well.

For future research, we want to explore the question of rather bacteria can be engineered to correct hormonal imbalances, reverse, or induce epigenetic changes as DNA methylation. We found possibilities through the genomic engineering of CRISPR microbes. Genomic engineering targets and recognizes epigenetic marks for altering the genome, producing new transcripts (12B). The function of genes and the monitoring factors of genetic expression need to be analyzed with greater precision. This will allow for the output of new drug targets to fix pathogenic mutations, and initiate more alternatives of treatment as for human gene therapy. The total genome of a eukaryotic cell consists of approximately billions of nucleic acid and nitrogenous bases (12B). Due to the high number and density of bases in eukaryotic cells, eukaryotic DNA is difficult to change or alter. One possible method was homologous recombination, which combined repair templates, consisting of homologous sequences to bind to the donor site. Homologous recombination allowed for the propagation of knock-in/knock-out animal models, to change the germ line of stem cells. However, the level of combination events are infrequent, with less consistency (12B).

The remedies for this issue, of HR include: RNA-guided endonucleases called Cas9 from the immune system of microbes. Cas9 originates from the immune system of microbes as a possible replacement for the tedious and inaccurate procedures of HR. Cas9 from the CRISPR or from the Clustered Regularly interspaced Short Palindromic Repeats can be easily attached to any gene locus, using short RNA guides (12B). The CrisPR nuclease Cas9 can be bound to a short guiding RNA, which then targets specific nucleic-acid sequences through Watson-Crick base-pairing (Fig.2.C). The RNAs are similar to the sequences of phages that occur innately in many CRISPR microbes as an immune response for the invading of foreign viruses (12B). The CRISPR Cas9 nucleases allow for less cumbersome and oversized proteins, giving easier access to Cas9 targets, and are precise with accurate identification. The CRISPR Cas9 can more correctly identify and then cleave through a site-specific nuclease. CRISPR was developed from studying and researching bacterial and archaeal diversity. Microbes have loci that are associated with genes called CRISPR-associated or Cas genes. To derive Cas into an array of sequence repeats they must be bound to spacers. The spacers originate from sequences from foreign nucleic acid material (12B). Cas genes from CRISPR arrays are transcribed as a ssRNA or single RNA strand, and then are cleaved into more CRISPR ssRNAs and are shorter in length, which are called crRNAs. After Cas genes are transcribed, the proteins are translated into Cas enzymes. These Cas enzymes directly cleave target nucleic acids through mimicking phagocytic degradation.In 2006, it was hypothesized that the CRISPR spacers were small RNAs that chaperoned and assisted with the elimination and phagocytosis of RNA end products of transcripts from viruses (12B).

The mode for dismantling viruses' foreign genetic material resembles iRNA processing. The CRISPR Cas enzymes then follow these spacers of CRISPR arrays directly to the target site and to the sequences of foreign and viral DNA (Fig. 2). The natural purpose of the CRISPR process was observed in Streptococcus thermophilus from Danisco yogurt (Fig. 1). Also, it was viewed in the type II CRISPR system as an adaptive immune response that was DNA- centered. The CRISPR spacers determine the orientation of the bond between Cas enzymes and its target DNA (12B). The Cas enzymes, in return, regulate the amount of spacers generated, and the Cas enzymes monitor the frequency of active phage immune responses. Type I of CRISPR immune responses is found in E.coli bacteria, which are transcribed then reformed into small crRNAs. The crRNAs with the spacers are guided into nuclease cleavage of DNA target sites.

The type III level of the CRISPR system found in Staphylococcus epidermis inhibits the transfer of plasmids between bacterial cells, called conjugation, revealed the Cas enzymes specifically and exclusively target DNA and not RNA (12B). The protospacer adjacent motifs or PAMs lead the type II Cas9 enzymes of cleavage to the selected targets of DNA. The three subunits of a CRISPR cas9 nuclease includes: a Cas9, crRNA, and a tracrRNA. The Cas9 focuses on the degradation of site-specific DNA.

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The tracrRNAs are non-coding trans regions of RNA. Cas9 is extracted from Streptococcus thermophilus or from Streptococcus pyogenes. The Cas9 is attached to crRNAs for cutting specified DNA. The single guide RNA is assembled by binding the crRNA, with the target sequence, to a tracrRNA. To target several genes simultaneously, multiple guide RNAs can be composed and combined (12B).

Cas9 has epigenetic effects that place or remove epigenetic tags at certain regions of DNA. However, there is still a need for limiting the cross communication between target DNA and naturally occuring epigenetic complexes. To solve this problem of crosstalk, the epigenetic enzymes of bacteria can be isolated and used. Currently, Cas9 has been isolated and implemented from the Streptococcus pyogenes bacteria, labeled SpCas9. The use of SpCas9 has altered genomes in human cell lines, mammals, bacteria, fruit flies, invertebrates as roundworms, domesticated animals as a pig, yeast, zebrafish, agricultural plants, and in mice (Fig. 4). The SpCas9 is bonded to crRNA and to tracrRNA or to a heterogenous and chimeric sgRNA. The crRNA or the sgRNA has 20 nucleotide guiding DNA sequences that can pair with the matching chosen and specific site of DNA (12B). The matching DNA target site in eukaryotic cells must have a protospacer adjacent motif are a PAM located downstream of the target DNA sequence.

Also other possible ways to manipulate genetic expression in various conditions is through the use of non-coding RNAs and metagenomics. Non-coding RNAs or microRNAs, miRNAs, cause transformation in development and within the endocrine system in response to biological and abiotic stressors (23). The miRNAs from bacteria help give plants a protective immunity through regulating many plant hormone signaling cascades. Hormonal-signaling pathways as auxin, ABA, and jasmonic acid are influenced and altered by miRNAs. The miRNAs as miR160, miR167, miR390, and miR393 monitor the genes within the auxin signalling pathway with ARFs and auxin receptors(23). The four miRNAs, miR160, miR67, miR390, and miR393, monitor auxin signaling, preventing the spread of pathogens. For example, miR393 releases from the bacteria called flg22 to block the expression of auxin receptor genes (23). Metagenomics was introduced in the 1990s to isolate and clone DNA from microbes without a need for cell culture procedures. Metagenomics included a type of analysis for microbial genomes (22). The study of metagenomics can identify and increase our understanding of the consistency and structure of the microbiome that resides in women's reproductive organs and system. Focusing unto analyzing the metagenomics of women's reproductive system can ameliorate women's overall health (22). Finding the biomarkers for genes of different microbial categories and types can be implemented to foresee the risk factors of certain diseases, bettering women's health.

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Figure 1(on next page)

Three Major Components of the CRISPR System

The arrangement of 3 major components of the CRISPR system in prokaryotic cells.

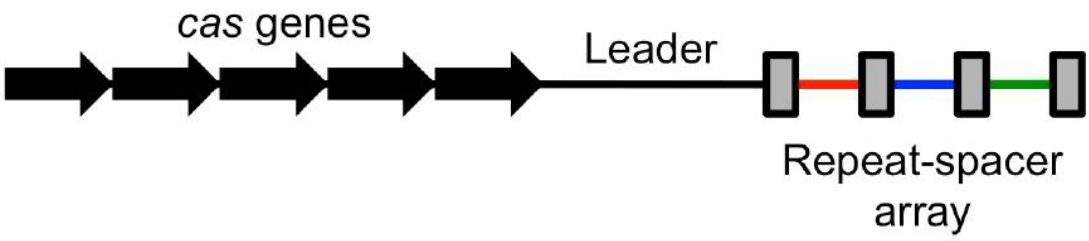

Figure 1: The arrangement of three major components of the Clustered regularly-interspaced short palindromic repeats (CRISPR) in Prokaryotic DNA.

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Figure 2(on next page)

Overview of the CRISPR-Cas9 System

The CRISPR/Cas9 system can target a specific gene sequence.

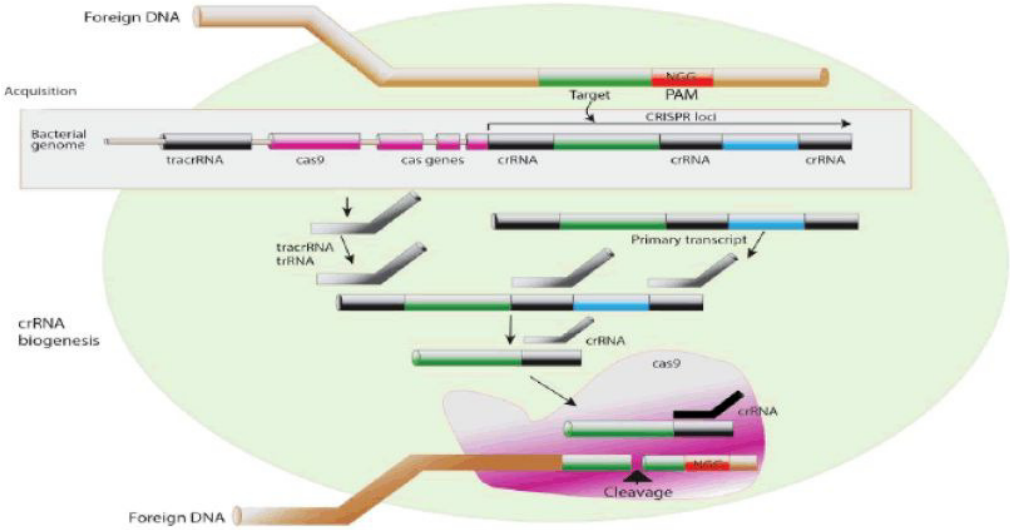

Figure 2: Overview of the CRISPR-Cas9 system: The CRISPR/Cas9 system that can target a specific gene sequence Step 1A) Acquisition of target sequence: insertion of new foreign DNA sequence (called spacer) into the CRISPR locus. Step 2B) Expression: transcription of the CRISPR locus and processing of CRISPR RNA. Step 3C) Silencing/Degradation: detection and degradation of mobile genetic elements by CRISPR RNA and Cas protein(s).

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Figure 3(on next page)

Applications of the CRISPR System

Genome editing in Eukaryotic organisms

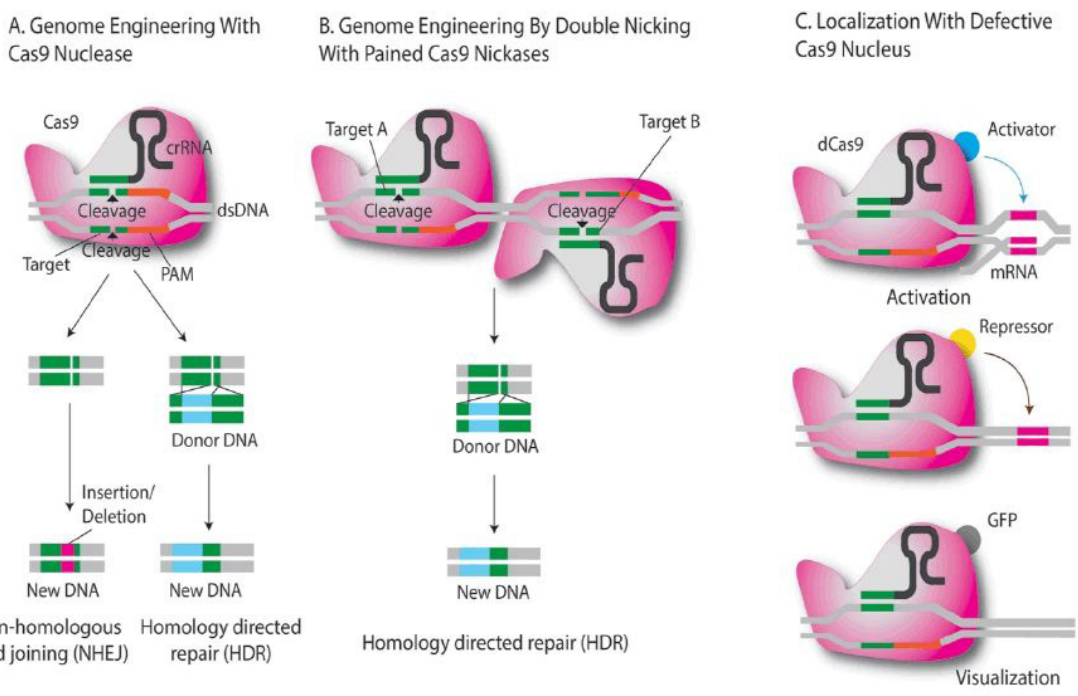

Figure 4: Major applications of the CRISPR system by genome editing in eukaryotic organisms. (A) Genome engineering with Cas9 nuclease, (B) Genome engineering by double nicking with pained Cas9 nickases, (C) Localization with defective Cas9 nucleus.

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