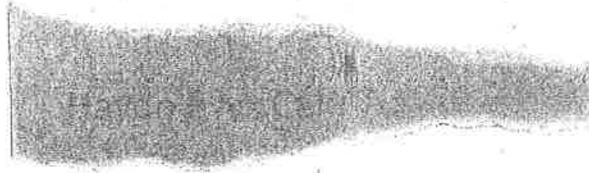
The History of Epigenetics



Principles of Biology 2107 Honors

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Epigenetics is the study of how an organism's genes are expressed, within that organism's established gene sequence (Riddihough & Zahn, 2010) Often described as ' neo-Lamarckian evolution,' (Jablonka & Lamb, 1995) epigenetics focuses on the chemical, environmental, physiological and sometimes psychological circumstances that cause an organism to be unique.

Epigenetics was first introduced into the scientific community following research studies conducted by embryologists in the mid 1800's. During this time, there was much debate about organismal development and the origin of cell variation within fertilized eggs (Felsenfeld, 2014). Decades of research and experiments sought to understand how an embryo can produce new cells, phenotypically different from its own, but nearly a century would pass before a comprehensive theory would be written (Choudhuri, 2011).

In 1942, the first important theory for epigenetics, called canalization, was written. It was developed by British developmental biologist, Conrad Waddington, who was studying embryonic development at the time. Canalization was the speculation that species phenotype was derived from developmental technologies that control for a phenotype, rather than the pure genetic material or environmental pressures (Thomas Flatt, 2005). Waddington's theory explained

that a population, if subject to natural selection, will adjust to maintain a preferred phenotype despite genetic or environmental influence (Siegal & Bergman, 2002). From this theory, Waddington produced his concept of "Epigenetic landscape." He used the metaphor of a landscape, with valleys and crevasse, and a ball to describe different ways genes might be expressed. The simple illustration of a ball traveling across a landscape allowed biologists to conceptualize how a fixed genotype could produce various cells through canalization and developmental influence (Waddington, 1957). Genetic material, like the landscape, remains the same, while gene expression, like the path of the ball, vary depending on factors in the environment. The place where the ball came to rest was the explanation for phenotype (Goldberg, Allis, & Bernstein, 2007). Progress toward uncovering epigenetic mysteries was underway, particularly with the discovery of DNA as the substance responsible for genetic heritability (Avery, Macleod, & McCarty, 1944). However, the greatest contribution to epigenetics came with the discovery of chromatin.

In 1878 Walther Flemming discovered chromatin, a complex of DNA and proteins found in the nucleus of the cell (Flemming, 1880). The mysterious complex was studied by scientists for a hundred and fifteen years before B. M. Turner proposed a correlation between chromatin structure and the transfer of

epigenetic information (Turner, Birley, & Lavender, 1992). Later, the structural arrangements of chromatin were observed more closely, revealing a relationship between the placement of proteins and nucleosomes, to the expression of certain genes. Chromatin structure has since been discovered to be 'remodeled' by changes in developmental and hormonal signals. These signals rearrange the chromatin structure in a way that either block or enable transcriptional proteins to access the DNA (Holde, 1997). Further research surrounding chromatin opened the door for more epigenetic mechanisms to be discovered, leading to the finding of DNA methylation.

DNA methylation is the semi-permanent alteration of DNA by the addition of methyl groups to the DNA molecule (Adrian, 2002) It was discovered years before Conrad Waddington proposed his epigenetic landscape, but it wasn't until 1975 that the association between DNA methylation and gene expression was made (Ginder, Gnanapragasam, & Mian, 2008). Scientists discovered that genes could be suppressed by the addition of methyl groups to bases in a DNA molecule. Proteins attach these methyl groups to DNA bases (typically to the C5 position cytosine bases in mammals, creating 5-methylcytosine) which controls gene expression by blocking the binding of transcription factors (Moore, Le, & Fan, 2013). DNA methylation therefore accounts for which genes the cell perceives as

being 'on' or 'off' and is considered one of three major epigenetic mechanisms that regulate gene expression (Jones & Takai, 2001).

The third epigenetic mark; histone modification, was discovered due to the advancement in the understanding of chromatin. Inside chromatin, there are nucleosomes; structures that consist of DNA wrapped around eight histone proteins (Lennartsson & Ekwall, 2009). The histones in the nucleosomes have tail-like appendages called N-terminus and C-terminus, that protrude out and manage interactions between nucleosomes. Histone modification is a post translational modification that controls gene expression through various processes. These include histone methylation, which is the activation or repression of transcription by the addition of methyl groups to amino acids in the histones (Nakayama, Rice, & Strahl, 2001). Histone acetylation and deacetylation, whereby modification occurs through acetylation or deacetylation of lysine in the N-terminus of the histones (Rice & Allis, 2001). Histone phosphorylation, which occurs in response to extracellular signals indicating damage to the DNA. Histone phosphorylation, which modifies chromatin structure, condenses chromosomes during mitosis, and plays a role in apoptosis (Rossetto, Avvakumov, & Côté, 2012). Lastly, histone ubiquitination, which similarly to histone phosphorylation, functions as an agent of DNA repair (Cao & Yan, 2012). The discovery of the many methods of histone

modification has helped to shape the focus of epigenetic research (Karlića, Chunga, & Lasser, 2010).

The final mechanism responsible for regulating epigenetic gene expression is non-coding RNA (ncRNA). Non-coding RNA are a class of functional proteins that are transcribed, but do not become translated into proteins (Mattick & Makunin , 2006). Scientists discovered that ncRNA can affect gene expression in two ways, through transcriptional gene silencing and post transcriptional gene silencing (Morris, Chan, Jacobsen, & Looney, 2004).

> Only a small proportion of the RNA made in the nucleus of animal and higher plant cells serves as a template for the synthesis of protein. This RNA is characterized by its ability to assume a form which protects it from intracellular degradation. Most of the nuclear RNA, however, is made on parts of the DNA which do not contain information for the synthesis of specific proteins. This RNA does not assume the configuration necessary for protection from degradation and is eliminated. – Henry Harris, 1965 (Harris, 2013)

Although this review was written some years before, the correlation between non-coding RNA and gene regulation was not attributed until the 1980's

when discoveries revealed that, although most of eukaryotes genome is transcribed, only a miniscule portion (believed to be about 1-2%) is translated into proteins (Kornienko, Guenzl, Barlow, & Pauler, 2013). Many different types of ncRNA have been discovered, particularly in higher level eukaryotes, but the ncRNA's that contribute to epigenetic mechanisms seem to be those that regulate interaction with other RNA molecules. A few of these ncRNAs are microRNA (miRNA), Piwi-interacting RNA (piRNA), small interfering RNA (siRNA), and long non-coding RNA (lncRNA). MiRNA and siRNA have their own unique ways of regulating gene expression, but both ultimately control gene silencing by a similar process of interfering with nucleotide sequence of m-RNA molecules (Carthew & Sontheimer, 2009). PiRNA have more complex methods of silencing RNA, which require them to interact with piwi proteins and Heterochromatin Protein 1 (Huang, et al., 2013). LncRNA, which make up the majority of ncRNAs, are classified by their length (200<). As the largest class of ncRNAs, lncRNAs have many methods of influencing gene expression. Some of these methods include transcription mediated silencing, regulation of transcription, post-transcriptional regulation, and chromatin modification (Mercer, Dinger, & Mattick, 2009).

Since the discovery of ncRNA's role in regulating gene expression, knowledge of epigenetic mechanisms has advanced considerably. However, many

ncRNAs and their functions remain mysteriously unintelligible (Larriba & Mazo, 2016).

Recently, epigenetics has made some prolific advances in human disease research. Research is increasingly showing that some diseases are the product of an environment-gene interaction (Portela & Esteller , 2010). One of the first of these studies, conducted by Andrew P. Feinberg and Bert Vogelstein in 1983, discovered a correlation between epigenetic alterations and cancer (Feinberg & Vogelstein, 1983). Since the correlation was made, cancer has been the main target of epigenetic research, through which an incredible amount of knowledge has been gained. Recently scientist have suggested that epigenetic mechanisms play a key role in the development of specific cancers, autoimmune disorders, neurodegenerative diseases, metabolic disorders and psychological disorders (Moosavi & Ardekani, 2016). Such discoveries continue to be made as scientists link more diseases to the activity of epigenetic mechanisms.

Epigenetics is a relatively new science, that is, many new and momentous discoveries are shaping the field through research today. Many universities and medical schools are investing time and resources into epigenetic research. One such discovery that has transcended the so far metaphysical nature of epigenetics

is the applied use of epigenetics in therapy. In epigenetic therapy, drugs and other chemical substances are used to influence the epigenome indirectly by activating epigenetic mechanisms. Epigenetic therapy is predicted to drastically change the way we treat certain diseases (particularly cancers) by enhancing the cells ability to fight off such diseases (Epigenetics and Stand Up To Cancer, n.d.).

Another fascinating facet of epigenetic research currently being studied is that of immunity. The efficacy of immune cells to respond to and fight off health threats such as toxins and infections, have been largely affected by epigenetic mechanisms (Busslinger & Tarakhovsky, 2014). In cells responsible for immune response, abnormal epigenetic modifications alter immunological tolerance and have been recently connected to the development of some autoimmune diseases (Rao & Richardson, 1999). Scientist believe that by understanding the various processes of epigenetic mandated immune systems response, they could develop new strategies for coping with many autoimmune diseases (Fernández-Morera, Calvanese, Rodríguez-Rodero, Menéndez-Torre, & Fraga, 2010).

Epigenetic mechanisms contribute to many factors regulating gene expression and development that allow organisms to be distinct. These distinct characteristics have been a frustrating aspect of biology that we are still exploring

today. There is no doubt that, although we are far from knowing all of the future applications of epigenetics, discoveries surrounding its inception have allowed us to further understand some of the complexities of life.

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Functions of Epigenetics

Prin nors

The name epigenetics comes from the Greek word *Epi-* meaning 'in addition to,' or 'more' and genetics, also Greek inspired, meaning 'origin' (Choudhuri, 2011). The name accurately describes the mechanisms behind epigenetic changes that occur *in addition to* the fixed gene sequence. These changes are sometimes thought of as enigmatic, but in truth, happen regularly in every eukaryotic organism (Biémont, 2011). Epigenetic events, as they are sometimes called, contribute greatly to the activities of eukaryotic cells and are governed by specific biological technologies. From cell differentiation to phenotype, epigenetic mechanisms are now recognized to be key factors in all eukaryotic organismal development (Wilbanks, et al., 2016). For something to be classified as an epigenetic event it must be understood to be a change that alters DNA expression, but not the DNA itself. These events must also contribute to long-term, semi-permanent alterations that are received by other cells and, in some cases, successive generations (EJablonka & Lamb, 1998). Epigenetics could be thought of as an outcome rather than a specific method of alteration, and thus uses several methods of operation. So far, research has revealed four main methods of epigenetic modification. These epigenetic 'marks,' as they are typically called, function as chemical and structural manipulators that influence what sequence of DNA is being expressed at any given time.

Chromatin Structure

The first, and quite frankly, the most inclusive of all the epigenetic mechanisms (in eukaryotic cells) is that of chromatin structure. Chromatin structure acts as an epigenetic modifier by effecting the ability of transcription factors to access the DNA (Margueron & Reinberg, 2010). Chromatin remodeling is the process of changing the chromatin structure by sliding, displacing, or repositioning the nucleosome, making the chromatin more compact (heterochromatin) or less compact (euchromatin) (Becker & Workman, 2013). The compacter the structure the more difficulty transcription factors have accessing the DNA. There are approximately 1600 genes providing 3200 site-specific transcription factors in the human genome (Carlberg & Molnár, 2016). Transcription factors require uncondensed, accessible DNA to carry out their functions. To ensure that important DNA sequences are accessed, chromatin remodeling will take place (Geurtin & Lis, 2013). These shifts in the nucleosomes are usually in response to extracellular signals and are performed by chromatin remodeling complexes. The two main ways that chromatin remodeling can occur is through ATP dependent chromatin remodeling, and histone modification. Histone modification can be considered both a structural manipulation of chromatin and a chemical modification. Therefore, histone modification is

ambiguously classified as a chromatin remodeling epigenetic mechanism, and a chemical classification of its own.

ATP dependent chromatin remodeling requires ant input of energy to function. Energy obtained from the hydrolysis of ATP to ADP is used to physically manipulate the chromatin. This process is enabled by multi-compartment protein complexes called ATP dependent chromatin remodeling complexes. A few of these complexes that have been discovered are ISWI, SWI/SNF, INO80 and NURD/Mi-2/CHD (Tang, Nogales, & Ciferri, 2010). All known ATP dependent complexes contain a subunit protein from the SNF2 (Stannous fluoride) family of proteins (Ryan & Owen-Hughes, 2011). The best characterized of these complexes is SWI/SNF, which is found in yeast. These proteins bind to the nucleosomal DNA through their subunit domain called a translocase domain. Upon the hydrolysis of ATP, SNF2 will direct the energy obtained from hydrolysis, to the contact areas between DNA and histones (Ryan & Owen-Hughes, 2011). The result is that DNA is freed from the tight packaging around the histones. This process is carried out unidirectionally, ensuring that nucleosomes are spaced properly (Tang, Nogales, & Ciferri, 2010).

Histone Modification

Histone modification is another method of epigenetic regulation of gene expression. Histone modification is the post-translational alteration of histone proteins. These changes effect gene expression by altering chromatin structure through the employment chemicals. These chemicals modify the histones, which in turn, alters the structural conformation of chromatin (Bannister & Kouzarides, 2011). However, unlike ATP dependent chromatin remodeling, an input of energy is not required. The histones themselves are comprised of eight histone proteins (two copies of H2A, H2B, H3, and H4) and associate with H1 proteins that act as supports that hold together the chromatin structure. Chemical modifications of histones influence transcriptional activation or inactivation of genes. This process is instructed through chemical modifications that occur on the histone tails (Strahl & Allis, 2000). These modifications are ascribed to the histone tails (called N-terminals) via enzymes that add chemical marks to the tails. Other enzymes are responsible for removing the chemical marks on the N-terminals (Turner, 2002).

Histone acetylation is the most well-documented of the of the chemical histone modifications. Acetyl groups are added to the lysine in n-terminals of the histones by a family of enzymes called histone acetyltransferases, otherwise

known as HATs (Brownell & Allis, 1996). HATs use Acetyl co-enzyme A as a co-substrate. The HAT enzyme will remove the acetyl group from Acetyl-CoA, later transferring it to one of the lysine residues on the histone tails (Takahashi, McCaffery, Irizarry, & Boeke, 2006). HAT can be classified into two categories; type A, which is found in the nucleus and regulates the acetylation of histones; and type B, which is found in the cytoplasm and functions in the acetylation of newly synthesized, pre-nucleosomal histones. Type b HATs are not well understood and appear to have no direct instrumentation on transcriptional activity. However, type A HATs do directly affect transcription and can be further classified in families called GNAT, MYST, and p300/CBP proteins (Fukuda, Sano, Muto, & Horikoshi, 2006). The GNAT (Gcn5-related N-acetyltransferase) family which includes Nut1, Hpa2, PCAF, Elp3, Hat1, and Gcn5, is classified by the homology sequence in addition to the structure and function of the enzymes. The GNAT family of histone acetylation enzymes is recognized as the most influential in eukaryotic gene expression (Vetting, et al., 2005). GNAT enzymes are diverse and function to catalyze the transfer of an acetyl group from Acetyl CoA to lysine tails in H3 and H4. However, unlike other classes of HATs, some GNAT enzymes are more liberal in their substrate specificity and will use Succinyl CoA or Myristoyl CoA as their acetyl donor (Vetting, et al., 2005). GNATs typically interact

with bromodomains which are reader proteins that recognize acetyl-lysine residues and have been shown to influence gene transcription (Sanchez, Meslamani, & Zhou, 2014). MYSTs are another class of HATs that influence gene regulation. The MYST family is comprised of Morf, Sas2, Ybf2 (Sas3), and Tip6. Unlike GNATs, MYSTs are characterized by their chromodomain proteins and zinc fingers (Avvakumov & Coˆ te, 2007). MYSTs are considered the most diverse of the HAT families and, although their homologous sequence is similar to GNATs, carry out broader range of functions (Sapountzi & Co ˆte , 2011). P300 and Creb binding proteins are members of a family of HAT proteins that assist the acetylation of histones by acting as coactivators of transcription factors (Vo & Goodman, 2001). These proteins have a similar structure and function and are typically addressed together within the domain 'p300/CBP.' P300 and CBP are currently the least understood of the HATs and many of their functions and methods of acetylation remain a mystery (Sun, Man, Tan, Nimer, & Wang, 2015). The histone's innate charge is the reason that they are able to influence gene expression. Histones are positively charged which plays a key role in the transcriptional accessibility of negatively charged DNA. When the histone tails have received a negatively charged acetyl group, the DNA wrapped around the histones becomes loosened, thus leading to greater accessibility by transcription

factors (Verdone, Caserta, & Di Mauro, 2005). The genes that were previously inaccessible to transcription factors are now transcriptionally activated and therefore can be read. Histone acetylation can also be reversed through a process called histone deacetylation. Histone deacetylation, which is carried out by the enzyme histone deacetylase (HDAC), removes the acetyl groups from the histone tails leading to the tightening of DNA around the histones (Seto & Yoshida, 2014). The tightening of the chromatin prevents transcriptional activation, thereby suppressing gene expression.

Histone methylation is the process by which a methyl group is added to histone tails on the arginine or lysine residues. Unlike histone acetylation, histone methylation can either encourage or discourage transcription by the placement location of the methyl group, and its interaction with surrounding molecules. The enzymes that facilitate this process are a class known as histone methyltransferase, or HMTs (Zhang & Reinberg, 2001). The exact enzyme that interacts with the histone will be either arginine methyltransferase, or lysine methyltransferases depending on the respective residue. Lysine residue interactions will occur through the enzyme lysine methyltransferase (KMT) which are further divided into SET (Su(var)3-9, Enhancer of Zeste, Trithorax; and the non-SET domains (Dillon , Zhang, Trievel, & Cheng, 2005). Arginine residue

interactions occur through arginine methyltransferase (RMT). Both of these HMT enzymes receive their methyl group via the co-factor S-Adenosyl methionine (SAM) (Bannister, Schneider, & Kouzarides, 2002). Arginine and lysine can be mono- or di-methylated, but lysine is independent in its ability to be tri-methylated (Epigenetic Regulation, 2016). Histone methylation primarily occurs on the H3 and H4 histones. Some of the most common sites of histone methylation are H3K4, H3K48, and H3K79, which have been shown to contribute to gene activation. Other sites including H3K9 and H3K27, when methylated, have been connected to gene inhibition (Epigenetic Regulation, 2016).

Histone phosphorylation is only now being thoroughly studied by scientists. This mechanism of histone modification is not well understood, but seems to contribute to a diverse array of processes in the cell (Banerjee & Chakravarti , 2011). Phosphorylation of the histones occurs on either thyronine, tyrosine, or serine, though serine is preferred. Histone phosphorylation is carried out by kinase enzymes which attach a phosphate from an ATP molecule to the hydroxyl group of the target amino acid (Bannister & Kouzarides, 2011). Histone phosphorylation seems to play a role in DNA damage repair as well as regulation of apoptosis, but its function is primarily related to chemical interactions that influence the acetylation or methylation of histones (Rossetto, Avvakumov, &

Côte, Histone phosphorylation, 2012). An example of this relationship has been seen in the phosphorylation of H3S10 which has been shown to enhance the transcription of an acetylated H3K9. Phosphorylation can also control prior chemical modifications, as was observed in the removal of a methyl group on H3K9 following the phosphorylation of H3T11 and H3T6 (Rossetto, Avvakumov, & Côté, 2012). Histone phosphorylation is reversible and is executed by phosphatase enzymes which remove the phosphate group from the H3 tails.

Histone ubiquitination is the process of adding ubiquitin- a relatively large, versatile protein, typically associated with protein degradation- to the histone tails of histone proteins. Histone ubiquitination is carried out by the enzyme Ubiquitin ligase. Ubiquitin tends to prefer the lysine residues of H2A and H2B and will add a single ubiquitin to their histone tails (Cao & Yan, 2012). Polyubiquitinated histones have been reported, though they are usually in constructed to signal DNA damage repair. Histone ubiquitination has also been correlated with attracting proteins which will enhance or inhibit transcription. An example of this inhibition is seen in H2B-K120 which, once mono-ubiquitinated, will stimulate the methylation of H3K4 (Epigenetic Regulation, 2016). Ubiquitinated histones have also been shown to cause di-methylation in H3K4 during meiosis. This meiotic function of ubiquitin has been proposed by

epigeneticists to contribute to epigenetic memory in post-meiotic cells (Jason, Moore, Lewis, Lindsey, & Ausio, 2002). De-ubiquitination in yeast cells has also been reported recently. The process is achieved by large protein complexes like Spt-Ada-Gcn5-Acetyltransferas (SAGA); SAGA-like (SILK); and SAGA altered, Spt8 absent (SALSA) (Köhler, Schneider, Cabal, Nehrbass, & Hurt, 2008). A de-ubiquitinating enzyme called Ubp8 is present in both SAGA and SILK, and has been attributed to the removal of ubiquitin from H2b (Daniel, et al., 2003).

DNA methylation. DNA methylation is a method by which gene expression is controlled, and is considered the most influential mechanism in the discipline of epigenetics. Unlike the previous chemical modifications, DNA methylation is much more permanent in nature (Bird, 2002). DNA methylation is considered a paramount epigenetic regulator in vertebrates, and is normally associated with gene silencing. This silencing occurs through the binding of a methyl group to specific cytosine or arginine bases on the DNA (Jones & Takai, 2001). The methyl group is transported by a class of enzymes called DNA methyltransferase (DNMT). These DNMT enzymes receive the methyl group from a SAM (S-adenosyl methionine) and transfer the methyl group onto the DNA, where it will bind. The DNMTs are divided into two classes based on the time of their interaction with the DNA. Initial DNA methylation is consummated by the *de novo*

methyltransferases called DNMT3a and DNMT3b (Okano, Bell, Haber, & Li, 1999). DNMT3 enzymes donate the founding methyl mark to the cytosine or arginine residues. Another DNMT enzyme called DNMT1 is required to maintain the methyl marks on DNA strands that are produced from meiosis. DNMT1's job is to recognize hemi-methylated DNA, which is daughter DNA that has yet to obtain the properties of its parent strand (Hirasawa, et al., 2008). During cell division, the parent strand of DNA will contain the methyl mark, the daughter strand on the other hand, will not. DNMT1 will seek out and methylate the target region of the daughter strand, fully replicating the methyl configuration of the parent DNA. Once the cell has divided, the same methyl mark will be present in the DNA of both the parent and daughter cell (Klose & .Bird, 2006). As stated before, DNMTs prefer the nitrogenous base cytosine and will typically interact with it rather than arginine. However, even though cytosine is the predominant recipient of methyl groups, only about 1 percent of all cytosine nucleotides are methylated (Ehrlich, et al., 1982). This estimate changes when cytosine occurs next to guanine, there cytosine methylation is present 70-80 percent of the time (Chen & Riggs, 2011). These hot spots for methylation are specific areas of the DNA called CpG islands. CpG islands (cytosine and guanine connected through a phosphate bond) are characterized by their sequence of base pairs. CpG islands are classified as an area

containing 200 or more nucleotides, an above average occurrence of cytosine and guanine, and higher rates of CpG dinucleotides (Bird A. , 1986). When cytosine becomes methylated, it is converted into 5-methylcytosine. Hypermethylated areas of DNA tend to be inactive, while areas lacking methyl groups, or hypomethylated regions, are considered active (Tate & Bird, 1993). This is due to the association between methylated CpG (meCpG) and binding proteins called MeCP1 and MeCP2. The MeCP1 and MeCP2 are also methylated and will bind to the meCpG islands (Turek-Plewa & P.P., 2005). MeCPs contain an Methyl-CpG-binding domain that has been shown to inactivate genes by recruiting histone deacetyl transferase (HDAC), or other chromatin remodeling proteins. Both of these methods will condense chromatin, thereby preventing transcription (Meehan & Stancheva, 2001). Recently, DNA demethylation has garnered a great deal of attention in the field of biology. DNA demethylation is a phenomenon that is still in the infancy of its documentation. Scientists are uncertain of how enzyme facilitated demethylation is performed, but substantial evidence is pointing to demethylation as a source of epigenetic reprograming in cells (Ooi & Bestor, 2008). DNA demethylation has been confirmed to occur during two stages of mammalian development. The first follows the inception of the newly formed zygote, and the second takes place in the embryonic primordial germ cells

(Oswald, et al., 2000). After fertilization, the paternal genome DNA is demethylated. This process is rapid and globally demethylates the paternal genome. The maternal genome on the other hand is passively demethylated, relying on replication in the first few cell cycles to gradually remove prior methyl marks. Methylation is relatively low in both sets of parental DNA by the time the zygote reaches the blastocyst stage (Sanz, Kota, & Feil, 2010). Later, when the embryo has been implanted, the DNA is fervently methylated by *de novo* methyltransferase. This process will eventually account for the 70 percent of methylated CpG islands in the offspring's genome (Sanz, Kota, & Feil, 2010). The global demethylation of the mammalian zygote is made possible by oxidative Ten-eleven translocation proteins (Pastor, Aravind, & Rao, 2014). Currently the Ten-eleven translocation (Tet) enzymes are the focus of most practical DNA demethylation theorizing. These enzymes have been shown to catalyze the oxidation 5-methylcytosine. Tet enzymes function to demethylate DNA by adding a hydroxyl group to a methylated cytosine, thereby converting it from 5-methylcytosine to 5-hydroxylmethylcytosine (5hmC) (Kohli & Zhang, 2013). The ways in which 5-hydroxylmethylcytosine can cause epigenetic reprograming is through actively or passively removing the methyl groups from the genome. Through the passive method, maintenance enzymes cannot copy the 5hmC, and

therefore methyl marks will eventually disappear. The active method of demethylation seems to remove the methyl group all together by base excision repair, resulting in a perceivably unmodified cytosine (Chen & Riggs, 2011). Scientist are hoping to someday harness the mechanisms of DNA demethylation and employ its reprograming properties to reverse diseases.

Non-coding RNA. Non-coding RNA is one of the most complex of all the epigenetic mechanisms. Non-coding RNAs (ncRNAs) are functional RNA molecules that are transcribed but never translated into proteins (Peschansky & Wahlestedt, 2014). This epigenetic mechanism manages gene expression at both the transcriptional and post-transcriptional level. NcRNAs have only recently been recognized as a major proponent of eukaryotic gene expression. It is estimated that 98 percent of higher level eukaryotic RNA is transcribed into ncRNC (Matsumoto, et al., 2016). While these ncRNAs are not translated into proteins, they do impact gene expression in alternative ways. Studies have recently shown that ncRNAs control expression by up-regulating or down-regulating transcription (Kaikkonen , Lam, & Glass, 2011). Non-coding RNAs are classified into one of two

categories determined by their size; long non-coding RNAs and small regulatory RNAs.

The first, long-non-coding RNAs (lncRNA), are characterized by their length, which is over 200 nucleotides long. LncRNAs share many of the same structural features as mRNA. Some of these features include polyadenylated tails, a 5-prime cap, and a typically spliced structure (Li, Zhu, & Luo, 2016). LncRNAs are found almost exclusively in the nucleus and are assumed to have primarily nuclear functions. Cytoplasmic lncRNAs have recently been discovered, though these variants are rare and the reason for their peculiar localization is not well understood (Zhang, et al., 2014). LncRNAs are incredibly efficient in their ability to bind to DNA and mRNA. This is thought to be due to the lncRNA's length and ability to fold into complex shapes, which allow it to be less selective about the specific sequence it will bind to. This characteristic of the lncRNA allow it to contribute to many diverse functions in the cell (Geisler & Coller, 2013). One of the most researched of these functions is lncRNA initiated chromatin remodeling (Mercer & Mattick, 2014). LncRNA effects chromatin structure by employing other epigenetic mechanisms to chemically or structurally alter the chromatin. Epigenetic machinery, like DNA methyltransferase (DNMT) and other chromatin remodeling complexes, latch onto the lncRNA molecule and are guided to target

sequences of DNA. LncRNA acts as a vessel and enables the modifiers to travel down the chromatin where they will ultimately bind. The interaction between these proteins and the chromatin could result in any number of epigenetic modifications (Rinn & Chang, 2012). LncRNAs have been reported to do this in both *cis* and *trans* in which the sequence that they bind to is directly, or indirectly affected by the enzyme. Two lncRNAs that use a somewhat similar *cis or trans* function are *Xist* and *HOTAIR. Xist* is a lncRNA transcribed from the X-chromosome that codes for the mammalian female's inactive X-chromosome. The X-chromosomes are expressed equally within males and females, but the second X-chromosome in females is silenced. The reason for this is *Xist,* which allows the chromosome to be condensed down to an inaccessible state, making the whole chromosome inactive. Xist will spread along the chromosome, while simultaneously binding to many chromosome remodeling complexes. These proteins will facilitate the methylation and deacetylation of DNA along the X-chromosome. Therefore, transcription of this lncRNA will result in the chromosome being coated is *Xist* and methylated, which effectively silences the X-chromosome (Chatterjee & Eccles, 2015). HOTAIR is another lcnRNA that has been extensively researched. HOTAIR is transcribed from the HOXC gene. The HOX genes regulate spatial body develoment and are comprised of four proteins

positioned in a cluster. The HOTAIR lncRNA will remain associated with HOC, but when expressed, will then act *in trans* to modify HOXD by employing histone modifying complexes. Like in the case with *Xist*, the result is epigenetic mediated silencing. However, in both of these lncRNAs, as well as many others, the exact process is still relatively mysterious (Rinn J. , 2014).

The second class of ncRNA, small regulatory ncRNAs, are further classified into three categories; miRNA, siRNA, piRNA. Each of these small RNA molecules differ in their function (Mattick & Makunin, 2006). However, they all share correspondence with a family of proteins called Argonauts. Micro RNAs are small regulatory RNA that seem to influence post-transcriptional gene silencing. There are over 460 miRNAs coded for in the human genome (Chuang & Jones, 2007). Each one begins as a primary RNA (pri-miRNA) and is transcribed by RNA polymerase II, before undergoing various processing methods. Pri-miRNAs fold in half, forming a loop structure on one end and sections of unpaired bases down its length. The pri-miRNA shape acts as a substrate for nuclear enzyme (Drosha), which is simultaneously interacting with a binding protein called DGCR8 (Oxford University Press, 2014). This protein complex cleaves pri-miRNA, forming a smaller transcript called pre-miRNA. Exportin 5, which mediates nuclear export, transports the pre-miRNA into the cytoplasm (Oxford University Press, 2014). In

the cytoplasm, the pre-miRNA will encounter Dicer, which further cleaves the RNA molecule. After this final cleavage, the resulting miRNA will interact with the Argonaut protein which will remove the passenger strand of RNA. The miRNA can now bind to the mRNA. The miRNAs, together with the Argonaut, bind to mRNAs and down-regulate their translation. MiRNAs bind to mRNA with complementary sequence, and as a result the sequence will be silenced. This can happen through the recruitment of other proteins that will bind to the Argonaut-miRNA complex, also known as an RNA-induced silencing complex (RISC), hindering the ribosomal translation of mRNA into proteins. This interaction with other proteins is thought to destabilize the mRNA molecule, although there is very little data as of now (Djuranovic, Nahvi, & Green, 2012).

Another kind of small regulatory ncRNA is small interfering RNA. Unlike miRNAs, siRNAs have a peculiar origin. These ncRNA are not encoded in the genome and are typically obtained through exogenesis by viruses or repetitive elements in the genome (Carthew & Sontheimer, 2009). Again, contrary to miRNA, siRNA can be immediately cleaved by Dicer. SiRNA are then bound to the Argonaut protein and the passenger strand removed (Oxford University Press, 2014). The siRNA RISC complex will bind to a complementary sequence on the mRNA. Once the siRNA has paired with the mRNA molecule, a 'slicing'

mechanism is activated that further cleaves the mRNA molecule (Oxford University Press, 2014). These final strands of MRNA are then broken down by the exosome complex, preventing translation (Carthew & Sontheimer, 2009).

Piwi-interacting RNAs are another kind of ncRNA molecule. PiRNAs have been recognized as playing a major role in the mediation of transposons. The primary way in which piRNAs mediate transposons is through the germline, with a particularly strong influence on spermatogenesis (Watanabe & Lin, 2014). PiRNAs are thought to manage genome and stem cell stability. Although piRNA are now known to be present in both vertebrates and invertebrates, most of our information comes from studies on *Drosophila melanogaster*. However, it appears that the sequence of piRNA varies significantly from species to species. For this reason, many of the functions of piRNA remain a mystery (Simonelig, 2011). One proposed reason for the prevalence of piRNA in the germline is that many other epigenetic modifications happen during this time. PiRNAs are produced in genomic sites called piRNA clusters. These clusters have a high rate of transposable elements and the piRNAs made in these clusters will be homologous to the transposons (Yamanaka, Siomi, & Siomi, 2014). The piRNA is bound to a sub family of the Argonaut proteins called piwi-proteins (PIWI1, PIWI2, PIWI4, MIWI1, MIWI2, and MIWI4) (Simonelig, 2011). Each piwi-protein has its own specific

function. When a transposon is transcribed, piwi-precursors are made in what is considered primary processing. These pi-precursors are then cleaved and bind to the piwi-proteins (Simonelig, 2011). The piRNA secondary processing is termed Ping-Pong amplification. Ping-pong amplificaton is a cyclical process that signals the production of more piRNA than transposon elements (Czech & Hannon, 2016). This process will then lead to one of two results. First, the degradation of transposon RNA, which is carried out similarly to the RNA degradation by siRNAs. The second, which is an indirect method of managing transposons through the implementation of DNA methylation. This second process is rare and only occurs through the mammalian piwi-protein Miwi2. Through this process Miwi2 will bind to the piRNA, import it back into the nucleus, and direct DNA methylation to the target transposon (Watanabe & Lin, 2014).

The epigenetic mechanisms that we currently know of are numerous and complex, often working in concert with one another to produce a desired variation. Some of these marks are well understood, while others remain obscure in their functions. Undoubtedly, we will see many more ways in which these biological technologies influence gene expression as the field of epigenetics expands.

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