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BIO 500H

Dr. Spivey

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Environmental Stimuli and Intragenerational Epigenetics

Epigenetics is the study of any change in gene expression that is not mediated by DNA sequence. There are two broad categories of epigenetic inheritance: intragenerational inheritance and transgenerational inheritance. Intragenerational inheritance consists of those epigenetic changes that are inherited on a cellular level but not on an organismal level. Such intragenerational epigenetic changes can be caused by developmental cues such as hormones (known as cellular inheritance) or by environmental stimuli such as toxicants (known as transcriptional inheritance). The second broad category of epigenetic inheritance, transgenerational inheritance, describes those epigenetic changes which are inherited at an organismal level, and which are often caused by environmental stimuli in a manner similar to intragenerational transcriptional inheritance (D'Urso *et al*. 2014).

The effects of environmental stimuli on epigenetic changes have been studied extensively in recent years for both transgenerational and intragenerational inheritance. Multiple reviews have provided comprehensive summations of the effects of environmental stimuli upon epigenetics within the scope of transgenerational inheritance (Bollati *et al*. 2010). However, few reviews have focused upon environment-induced intragenerational epigenetic changes, although many intragenerational epigenetic changes

have been linked to conditions such as cancer, Alzheimer's disease, and drug addiction (Herceg *et al*. 2007, Feng *et al*. 2013, Mastroeni *et al*. 2011).

This review will summarize current knowledge of the intragenerational epigenetic changes that are induced by two groups of well-studied environmental stimuli: nutritional deficiencies and carcinogens. This survey of intragenerational epigenetic changes will focus upon the mechanisms behind them, the disorders associated with them, and the interactions between them.

MECHANISMS OF EPIGENETIC REGULATION

Before the causes and effects of epigenetic changes are examined, a description of the mechanisms underlying these changes is pertinent. Only those epigenetic mechanisms that are relevant to the intragenerational changes discussed in this review will be described here (for a more extensive discussion of both transgenerational and intragenerational epigenetic mechanisms, see Cedar and Bergman 2009, Watson *et al*. 2014, Zhang and Pradhan 2014, and Zhou *et al*. 2011).

The epigenetic mechanisms that will be described here can be divided into three main layers: DNA methylation, histone modifications, and chromatin condensation (Figure 1). The first layer, DNA methylation, denotes the addition of methyl groups to cytosine residues within a given gene and is usually associated with decreased expression of the affected gene. The second layer, histone modifications, involves the addition or removal of functional groups such as methyl or acetyl groups on the "tails" of histone proteins, which can either increase or decrease gene expression. Finally, chromatin condensation refers to the degree to which DNA is wrapped and folded into secondary

structures, with a higher degree of condensation generally equating to decreased gene expression. These three layers are all closely interrelated in a manner that is still not fully understood, but it is known that changes in one of these layers typically prompts a change in the other layers (Cedar and Bergman 2009, Watson *et al*. 2014). Ultimately, the effects of all of these epigenetic mechanisms are invariably the same: they alter the availability of a given gene to transcription factors and other transcriptional proteins, thereby increasing or decreasing the transcription of that gene (Watson *et al*. 2014).

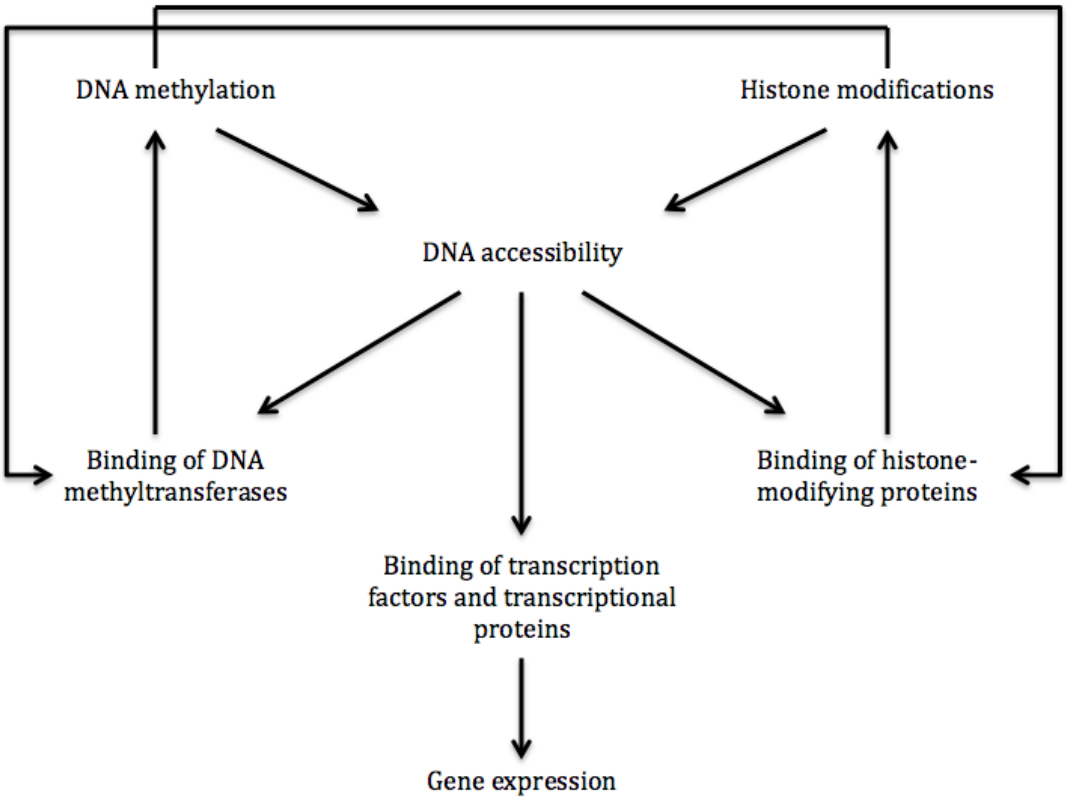

Figure 1 | **Interdependency of epigenetic mechanisms.** The components of the epigenetic mechanisms discussed here. Note the highly-interrelated nature of the mechanisms, as well as the effects on gene expression, which can only come through modification of the binding of transcription factors and transcriptional machinery.

Cytosine methylation and effects on DNA-binding proteins. DNA methylation typically occurs in large "islands" of cytosine residues located in or near the gene's promoter. This modification of cytosines rarely has any significant effects by itself, but rather acts by inhibiting or promoting the binding of specific transcription factors or other DNA-binding proteins to DNA sequences. For example, DNA methylation might directly block or recruit the binding of a transcription factor, or it might act by affecting the binding of histone-modifying proteins, as will be discussed later (Cedar and Bergman 2009, Watson *et al*. 2014).

Histones and histone tail modifications. Histones are protein complexes that interact with ~146 base pairs of DNA. In order for a DNA-binding protein such as a transcription factor to bind to DNA, its binding site must not already be associated with histone proteins. The interactions between histone proteins and DNA are transient interactions, so DNA continually associates and dissociates from histones (This is an oversimplification of DNA-histone interactions; for a full description see Watson *et al*. 2014). The likelihood that a given binding sequence will be dissociated from its histone complex at any given time can be altered by adding or removing functional groups on the histones' tails. (Watson *et al*. 2014). Such histone tail modifications are catalyzed by histone-modifying protein complexes. Whether DNA becomes more or less accessible to DNA-binding proteins depends upon which functional group is added, where this group is added, and how many molecules of this group are added. For example, the addition of an acetyl group generally decreases the association between DNA and histones as the negative charge of the acetyl group repels the negatively-charged phosphate backbone of DNA. Conversely, the removal of an acetyl group typically has the opposite effect of

decreasing the association between DNA and histones. The addition of a positively-charged methyl group can either increase or decrease DNA-histone association, depending on which histone tail residue it is added to, emphasizing the complexity of epigenetic molecular interactions (Watson et al. 2014, Zhang and Pradhan 2014). Consequently, the modification of histone tails can greatly affect the transcription of a given gene by regulating the accessibility of that gene to DNA-binding proteins such as transcription factors and transcriptional machinery (Cedar and Bergman 2009, Watson *et al*. 2014, Zhang and Pradhan 2014).

The interdependency of histone modifications and DNA methylation. While DNA methylation and histone modifications were initially thought of as separate methods of epigenetic regulation, it has increasingly been found that DNA methylation is actually interdependent with histone modifications. In fact, it is likely that histone modifications help facilitate DNA methylation in embryonic *de novo* methylation (Cedar and Bergman 2009, Watson *et al*. 2014). Histone modifications can exert this influence over DNA methylation by two mechanisms: by altering the accessibility of DNA sequences to DNA methyltransferase proteins (as described above) or by influencing the recruitment of DNA methyltransferases through chaperone proteins like DNMT3L. DNMT3L is usually able to recruit DNA methyltransferases to regions of DNA by binding to the tail of histone H3, but DNMT3L is unable to bind when the fourth lysine in the tail of histone H3 is methylated (H3K4) (Cedar and Bergman 2009). Thus, by indirectly influencing the binding of DNA methyltransferases, histone tail modifications can exert significant control over DNA methylation.

Similarly, DNA methylation exhibits significant control over histone modifications by regulating the binding of histone-modifying protein recruiters to DNA. These recruiter proteins exhibit specificity to either methylated or demethylated cytosines, and thus cannot bind if a given cytosine is in the incorrect methylation state. Without the binding of these chaperones, histone-modifying proteins are not recruited, and the addition or removal of a particular histone modification is inhibited (Cedar and Bergman 2009, Watson *et al*. 2014, Zhang and Pradhan 2014). For example, the chaperone MeCP2, which recruits histone deactetylases, binds to methylcytosine but not cytosine. MeCP2 is therefore only able to facilitate the deacetylation of histone tails if DNA methylation is present (Cedar and Bergman 2009, Watson *et al*. 2014). Vast changes in histone modifications can thereby be facilitated by the influence of DNA methylation, and vice versa.

The interdependency of DNA methylation and histone modifications often lends itself to cooperation between the two mechanisms in order to facilitate long-term inhibition of transcription, especially in the repression of heterochromatic regions, pluripotency genes, and retrotransposons. In the aforementioned case of the chaperone MeCP2, deacetylation of histone tails serves to augment the repressive effects of DNA methylation. DNA methylation reduces the accessibility of a DNA sequence to activating transcription factors and other DNA-binding proteins, while MeCP2 further reduces the DNA sequence's accessibility by triggering the deacetylation of histone tails and thereby increasing the affinity between the DNA sequence and its histones. While reactivation of a gene can occur with DNA methylation or histone deacetylation alone, the combination of both mechanisms greatly reduces the probability that reactivation will occur (Cedar

and Bergman 2009, Watson *et al*. 2014). For this reason, if long-term repression is not needed, short-term transcription regulation can be mediated by the independent action of either DNA methylation or histone modifications, or by transcription factors (Cedar and Bergman 2009, Zhou *et al*. 2011). However, in many cases, the interdependency of DNA methylation and histone modifications greatly complicates and refines epigenetic mechanisms, as will be seen in several of the specific environmental stimuli and mechanisms explored below.

NUTRITION AND METHYL DEFICIENCIES

One-carbon metabolism and SAM. One of the most-studied environmental modulators of epigenetics is nutrition, especially in the context of one-carbon metabolism. One-carbon metabolism refers to the set of reactions by which methyl groups are transferred from one molecule to another in order to facilitate DNA methylation, pyrimidine synthesis, and other cellular pathways. Two of the most important pathways involved in one-carbon metabolism, the folate cycle and the methylation cycle, are responsible for replenishing the body's supply of S-adenosyl methionine (SAM), the primary methyl donor which is essential for DNA methylation. (Figure 2; Anderson *et al*. 2012, Rush *et al*. 2014). One-carbon metabolism and SAM levels are of heightened importance during *in utero* development due to genome-wide erasure and reprogramming of DNA methylation during early development. If SAM levels are insufficient to allow the re-establishment of methylation during early development, lifelong hypomethylation can occur and have pathogenic consequences (Anderson *et al*. 2012, Ciappio *et al*. 2011). Thus, any nutritional deficiency or other

environmental factor that impedes maternal one-carbon metabolism can impede SAM synthesis and have a deleterious effect on DNA methylation in offspring.

Micronutrient deficiency and SAM-related hypomethylation. A commonly-studied cause of SAM deficiencies is dietary deficiency of the micronutrients that are essential to one-carbon metabolism, such as vitamin B_{12} and folate. *In vitro*, human adipocytes deficient of vitamin B_{12} exhibit hypomethylation and associated overexpression of the cholesterol-regulating genes *LDLR* and *SREBF1* (Adaikalakoteswari *et al*. 2015). In addition, maternal vitamin B_{12} deficiency is correlated with *IGF2* promoter hypomethylation in newborns' cord blood (Ba *et al*. 2011). Furthermore, such *IGF2* hypomethylation can be reduced by supplementing maternal diet with folate (Steegers-Theunissen *et al*. 2009). General genomic hypomethylation is also seen in the offspring of mice fed a folate-deficient diet. Importantly, continuation of this folate-deficient diet in postnatal offspring did not affect DNA methylation, emphasizing the heightened importance of methyl donors during fetal epigenetic reprogramming (McKay *et al*. 2011). The hypomethylation observed in these instances of micronutrient deficiency appears to be pathogenic in many cases: *IGF2* overexpression is associated with various cancers; overexpression of *LDLR* and *SREBF1* leads to excessive cholesterol synthesis; and the impairment of one-carbon metabolism by micronutrient deficiency in general is associated with increased prevalence of neural tube defects and other diseases and defects (Adaikalakoteswari *et al*. 2015, Anderson *et al*. 2012, Rush *et al*. 2014, Steegers-Theunissen *et al*. 2009). The diversity of these epigenetic changes and associated diseases illustrates the significance of one-carbon metabolism on epigenetic health: a lack of nutrients can affect not only the tissues that are traditionally associated

with certain nutrients, but any tissue as SAM levels become insufficient and DNA methylation is impaired throughout the body.

Protein deficiency and DNA methylation. Protein deficiency can also impair one-carbon metabolism and lead to DNA methylation changes through two mechanisms: micronutrient deficiency and amino acid deficiency. Because the micronutrients required for one-carbon metabolism must be obtained from proteinaceous dietary sources, protein-deficient diets can have the side effect of one-carbon micronutrient deficiency and resultant SAM deficiency (Rush *et al*. 2014). Additionally, protein deficiency can lead to a deficiency of amino acids required to produce cellular proteins, including those required for methylation reactions (Rees *et al*. 2000, Rush *et al*. 2014). Unexpectedly, protein deficiencies often manifest in DNA hypermethylation rather than hypomethylation, suggesting that one-carbon micronutrient deficiency is not the main determinant of methylation changes in the case of protein deficiency (Rees *et al*. 2000, Rush *et al*. 2014, Sandovici *et al*. 2011). However, as of the time of this writing no studies have been performed to isolate the effects of amino acid deficiency from those of micronutrient deficiency. Further studies should examine amino acid deficiency in the absence of micronutrient deficiency, *i.e.* in the presence of micronutrient supplementation.

Nutrition-related histone modifications. Although the investigation of nutrition-modulated histone modifications is in very early stages compared to the study of such changes in DNA methylation, several *in murine* studies have provided early evidence for nutritional modulation of histone modifications. For example, maternal choline deficiency *in murine* is associated with decreased H3K9me1 and H3K9me2 levels in

hippocampal neural progenitor cells. However, other histone methylation marks are not affected by such choline deficiency (Mehedint *et al*. 2010). Similarly, *in murine* maternal protein deficiency correlates with increased H3K27me3 and H3K9me2 levels, but decreased H3K4me1 and H3ac levels (Sandovici *et al*. 2011). Such specificity in histone methylation/demethylation and the inclusion of changes in histone acetylation shows that– unlike micronutrient-correlated changes in DNA methylation– changes in histone modifications are not directly caused by a lack of methyl donors. Instead, it is likely that the complex interactions between changes in DNA methylation, the binding of histone-modifying protein recruiters, and altered gene expression lead to this selective histone demethylation. In support of this idea, choline-related H3K9me1 and H3K9me2 deficiencies *in vitro* were associated with altered expression of the histone-modifying protein recruiter REST and reduced binding of the histone methyltransferase G9a (Mehedint *et al*. 2010). In light of the prevalence and complexity of the histone interactions seen in other areas of epigenetics, it is likely that much more evidence to support such interactions will develop as more nutritional epigenetics research is performed.

Future of nutritional epigenetics. Micronutrient deficiencies impair the one-carbon metabolism cycles and lead to DNA hypomethylation and often-pathogenic phenotypes. However, it is clear from nutrient-related histone modifications and protein deficiency-related DNA hypermethylation that many epigenetic changes are not caused by a simple deficiency in methyl donors, but rather involve interactions between proteins, genes, and epigenetic mechanisms. Therefore, further research into the relationship between nutrition and epigenetics will need to consider such interactions between

different epigenetic modifications and their effects on gene expression and protein binding, rather than focusing only upon methyl-donor pathways.

CARCINOGENS

Carcinogens have been widely linked to genetic mutations and changes in gene expression for decades. More recently, many carcinogens which were thought to be already well-understood by conventional molecular and genetic mechanisms have been shown to have additional epigenetic actions. Changes in DNA methylation and histone modifications have been discovered to possess major roles in both new and previously-established carcinogenic pathways. Here, the newfound epigenetic mechanisms of previously-characterized carcinogens present in cigarette smoke, fossil fuel emissions, and alcohol will be discussed.

Benzo(a)pyrene and retrotransposons. Benzo(a)pyrene (BaP), a carcinogen that is present in the smoke given off by cigarettes and fossil fuels, was already well-known to contribute to lung and esophageal tumorigenesis by inhibiting DNA repair and disrupting transcription regulation (Lu and Ramos 1998, Teneng *et al*. 2011). However, as focus upon epigenetics increased, BaP was also found to affect DNA methylation and histone modifications on the *LINE-1* retrotransposon. In the presence of BaP, repressive DNA methylation is lost on the promoter of the *LINE-1* retrotransposon, expression of *LINE-1*'s two transcripts occurs, and *LINE-1* is reinserted throughout the genome (Box 3; Teneng *et al*. 2011). This reinsertion potentially disrupts both coding sequences and regulatory regions throughout the genome, leading to mass disruptions in gene expression and/or mutations (Alves *et al*. 1996). Associated with the loss of methylation along

LINE-1 is the BaP-induced degradation of the maintenance methyltransferase DNMT1 by proteasomes. It is therefore likely that the targeting of DNMT1 for destruction by BaP is responsible for the observed hypomethylation and reactivation of *LINE-1* (Teneng *et al*. 2011). Thus, unlike the methyl shortage that is seen in some nutritional deficiencies, it is a shortage of DNA methyltransferase that is responsible for hypomethylation in the case of BaP.

NNK metabolism and cancer. Another tobacco-associated carcinogen, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), was previously known to cause cancer by forming mutation-causing DNA adducts as well as by activating cell surface receptors that lead to inhibited apoptosis and increased proliferation (Jalas *et al*. 2005, Hecht 2003). However, with the advancement of epigenetics, NNK has more recently been linked to DNA hypermethylation and resultant miRNA dysregulation, as well as DNA repair-disabling histone modifications (Shen *et al*. 2014, Watanabe *et al*. 2012). In the lungs, NNK is metabolized into its active, carcinogenic form (NNAL) by hydroxylation reactions catalyzed by *CYPA3*; after this conversion NNAL proceeds to form DNA adducts and cause lung cancer (Jalas *et al*. 2005). *CYAP3* is down-regulated by miR-126, and in lung cancer, miR-126's host gene, *EGFL7*, is hypermethylated and resultantly silenced (Kalscheuer *et al*. 2008, Watanabe *et al*. 2012). Thus, a positive feedback loop is established in which NNK increases the expression of its own catalyst via epigenetic changes, potentially compounding the effects of NNK and accelerating carcinogenesis (Kalscheuer *et al*. 2008).

Alcohol-mediated carcinogenesis. As with BaP and NNK, much of what is known of alcohol's carcinogenesis was non-epigenetic in nature until recent years (Seitz

and Stickel 2007). Ethanol and its derivative, acetaldehyde, were previously shown to have a variety of carcinogenic effects through such mechanisms as inhibiting DNA repair, causing chromosomal instability, inducing DNA point mutations, and allowing the exchange of genetic material between sister chromatids. (Kayani and Parry 2010, Seitz and Stickel 2007). A comprehensive review of these pathways can be found elsewhere (Seitz and Stickel 2007).

More recently, new mechanisms of ethanol's action were discovered with the increased study of epigenetics. Chronic alcohol consumption in rats has been linked to increased H3K9 acetylation in the liver, lungs, spleen, and testes (Kim *et al*. 2006, Oliva *et al*. 2009). In the liver, alcohol-mediated H3K9 hyperacetylation has further been linked to decreased nuclear proteasome activity and increased p300 activity (Oliva *et al*. 2009). p300 is a histone acetyltransferase coactivator and has been strongly implicated in liver, breast, prostate, and colorectal cancers (Li *et al*. 2011). These changes in p300 activity and acetylation were concomitant with the divergent expression of 1,300 genes, including genes in such well-established tumor suppressor and oncogenic pathways as the Wnt, TGFβ, Notch, insulin signaling, and apoptosis pathways (Oliva *et al*. 2009). Thus, alcohol consumption has been linked to multiple carcinogenic pathways via induced changes in histone acetylation. Ethanol and acetaldehyde have been linked to many other epigenetic pathways in both cancer and liver disease, including histone phosphorylation. For a full review of ethanol-induced epigenetic changes, see Shukla *et al*. 2013.

Implications of environment-induced epigenetic carcinogenesis. From such examples as these, it becomes apparent that epigenetics holds great potential for increasing understanding of carcinogens—even carcinogens that have already been

extensively studied in non-epigenetic contexts. Herceg *et al*. proposed that this re-understanding of conventional carcinogens is due to the dependence of genetic and molecular mechanisms upon epigenetic mechanisms– the genetic and molecular mechanisms that were previously known are the products of development, which is largely regulated by epigenetics (Herceg *et al*. 2007). As methods for studying epigenetics continue to improve, the understanding of these and other carcinogens will continue to improve and lead to novel treatment and prevention techniques.

THE FUTURE OF ENVIRONMENTAL EPIGENETICS

Notably, there is often substantial interplay between the nutrition- and carcinogen-related epigenetic pathways discussed above. For example, carcinogens in cigarette smoke such as NNK not only cause the hypomethylation of genes like *EGFL7*, but also disrupt one-carbon metabolism and induce generalized global DNA hypomethylation via SAM shortage (Drake *et al*. 2015, Kalscheuer *et al*. 2008, Watanabe *et al*. 2012). This concurrence implies a number of potential explanations, including the possibility that the disruption of one-carbon metabolism by NNK may contribute to the observed *EGFL7* hypomethylation and associated carcinogenesis. The causation could also be reversed: *EGFL7* hypomethylation may contribute to the disruption of one-carbon metabolism by any number of pathways that are regulated by *EGFL7*/miR-126. Finally, the two effects of NNK may be entirely separate phenomena, amongst many other explanations. The discernment of the exact relationship between the carcinogenic and one-carbon metabolic effects of NNK will require extensive research and perfectly illustrates the added complexity that arises from environmental epigenetics. Interactions

between multiple different environmental stimuli and epigenetic pathways further complicate the already-complex web of interactions that exists between protein binding, genotypes, and other genomic factors. This complexity necessitates that further study into environmental epigenetics will be a momentous undertaking that will likely require the development of new molecular and computational techniques. However, as is shown in the examples described above, the study of intragenerational environmental epigenetics will also allow for new understanding of molecular mechanisms for a variety of pathways– many of which were already thought to be understood– and thereby the potential prevention of and treatment for a variety of diseases.

BOX 1: CpG islands are regions of DNA that are at least 200 bp in length and contain a higher prevalence of cytosines/guanines than would be typically expected (usually >50%) (Zhang and Pradhan 2014). While most transcriptionally-significant DNA methylation occurs in CpG islands, another commonly methylated motif has recently been identified: the CpG desert (Skinner and Guerrero-Bosagna 2014). CpG deserts possess greatly decreased prevalence of CpGs in a region of 500-2000 bp (<15% CpG prevalence, as compared to CpG islands' >50% CpG prevalence) (Skinner and Guerrero-Bosagna 2014, Watson *et al*. 2014). However, the CpGs that are present appear in small, concentrated clusters in gene promoters. Skinner and Guerrero-Bosagna found that >97% of the differentially-methylated regions (DMRs) associated with a variety of environmental toxicants were located within CpG deserts rather than islands and were heritable (Skinner and Guerrero-Bosagna 2014). However, causative connections to transcription have not yet been found, and the biological significance of these CpG deserts remains unknown, but promising.

BOX 2: Retrotransposons code for DNA-binding proteins and reverse transcriptases that allow them to self-proliferate by reinserting themselves into the genome after transcription. Early research into epigenetics revealed that prevention of such reinsertion, which can cause severe mutations if the retrotransposon is inserted into a coding or regulatory sequence, is typically achieved by extensive DNA methylation along the retrotransposon's promoter. However, if the hypermethylation of the retrotransposon's promoter is lost, expression of protein products and the reinsertion of the retrotransposon can occur (Alves *et al*. 1996, Teneng *et al*. 2011).

REFERENCES

Adaikalakoteswari A., Finer S., Voyais P., McCarthy C., Vatish M., *et al*. Vitamin B_{12} insufficiency induces cholesterol biosynthesis by limiting s-adenosylmethionine and modulating the methylation of SREBF1 and LDLR genes. *Clin Epigenetics*. **7**:14 (2015).

Alves G., Tatro A., & Fanning T. Differential methylation of human LINE-1 retrotransposons in malignant cells. *Gene*. **176,** 3944 (1996).

Anderson O., Sant K., & Dolinoy D. Nutrition and epigenetics: an interplay of dietary methyl donors, one-carbon metabolism and DNA methylation. *J Nutr Biochem*. **23**, 853-859 (2012).

Ba Y., Yu H., Liu F., Geng X., Zhu C., *et al*. Relationship of folate, vitamin B_{12} and methylation of insulin-like growth factor-II in maternal and cord blood. *Eur J Clin Nutr*. **65**, 480-485 (2011).

Bollati V., & Baccarelli A. Environmental epigenetics. *Heredity*. **105,** 105–112 (2010).

Cedar, H., & Bergman, Y. Linking DNA methylation and histone modification: patterns and paradigms. *Nat. Rev. Genet*. **10**, 295–304 (2009).

Ciappio E., Mason J., & Crott J. Maternal one-carbon nutrient intake and cancer risk in offspring. *Nutr Rev*. **69**(10), 561-571 (2011).

D'Urso, A. & Brickner, J. Mechanisms of epigenetic memory. *Trends Genet*. **30**(6), 230–236 (2014).

Drake A., O'Shaughnessy P., Bhattacharya S., Monteiro A., Kerrigan D., *et al*. *In utero*

exposure to cigarette chemicals induces sex-specific disruption of one-carbon metabolism and DNA methylation in the human fetal liver. *BMC Medicine*. **13**:18 (2015).

Hecht S. Tobacco carcinogens, their biomarkers and tobacco-induced cancer. *Nat Rev Cancer*. **3,** 733–44 (2003).

Herceg, Z. Epigenetics and cancer: towards an evaluation of the impact of environmental and dietary factors. *Mutagenesis* (2007). **22**(2), 91-103 (2007).

Feng J., & Nestler E. Epigenetic mechanisms of drug addiction. *Current Opinion in Neurobiology*. **23,** (2013).

Jalas J., Hecht S., & Murphy S. Cytochrome P450 enzymes as catalysts of metabolism of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, a tobacco specific carcinogen. *Chem. Res. Toxicol.* **18,** 95–110 (2005).

Kalscheuer S., Zhang X., Zeng Y., & Upadhyaya P. Differential expression of microRNAs in early-stage neoplastic transformation in the lungs of F344 rats chronically treated with the tobacco carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. *Carcinogenesis*. **29,** 2394–2399 (2008).

Kayani M., & Parry J. The in vitro genotoxicity of ethanol and acetaldehyde. *Toxicol in Vitro*. **24,** 56–60 (2010).

Kim J. & Shukla S. Acute in vivo effect of ethanol (binge drinking) on histone H3 modifications in rat tissues. *Alcohol Alcohol.* **41,** 126–32 (2006).

Li M., Lou R., Chen J., Cao Y., Lu J., *et al.* High expression of transcriptional coactivator p300 correlates with aggressive features and poor prognosis of hepatocellular carcinoma. *J Transl Med*. **9,** 5 (2011).

Lu K., & Ramos K. Identification of Genes Differentially Expressed in Vascular Smooth Muscle Cells following Benzo[A]Pyrene Challenge: Implications for Chemical Atherogenesis. *Biochemical and Biophysical Research Communications*. **253,** 828-833 (1998).

Mastroeni D., Grover A., Delvaux E., Whiteside C., Coleman P., *et al.* Epigenetic mechanisms in Alzheimer's disease. *Neurobiology of aging*. **32,** 1161–80 (2011).

McKay J., Waltham K., Williams E., & Mathers J.. Folate depletion during pregnancy and lactation reduces genomic DNA methylation in murine adult offspring. *Genes Nutr*. **6**, 189-196 (2011).

Mehedint M., Niculescu M., Craciunescu C., & Zeisel S. Choline deficiency alters global histone methylation and epigenetic marking at the Re1 site of the calbindin 1 gene. *FASEB J*. **24**, 184-195 (2010).

Oliva J., Dedes J., Li J., French S., & Bardag-Gorce F. Epigenetics of proteasome inhibition in the liver of rats fed ethanol chronically. *World Journal of Gastroenterology*. **15,** 705 (2009).

Rees W., Hay S., Brown D., Antipatis C., & Palmer R. Maternal protein deficiency causes hypermethylation of DNA in the livers of rat fetuses. *J Nutr.* **130**, 1821-1826 (2000).

Rush E., Katre P., & Yajnik C. Vitamin B12: one carbon metabolism, fetal growth and programming for chronic disease. *Eur J Clin Nutr*. **68**, 2-7 (2014).

Sandovici I., Smith N., Nitert M., Ackers-Johnson M., Uribe-Lewis S., *et al*. Maternal diet and aging alter the epigenetic control of a promoter-enhancer interaction at the *Hnf4a* gene in rat pancreatic islets. *PNAS*. **108**(13), 5449-5454 (2011).

Seitz H., & Stickel F. Molecular mechanisms of alcohol-mediated carcinogenesis. *Nature Reviews Cancer*. **7,** 599–612 (2007).

Shen Y., Zhang S., Huang X., Chen K., Shen J., *et al.* Involvement of p53 Mutation and Mismatch Repair Proteins Dysregulation in NNK-Induced Malignant Transformation of Human Bronchial Epithelial Cells. *BioMed Research International*. **2014,** (2014).

Shukla S., & Lim R. Epigenetic effects of ethanol on the liver and gastrointestinal system. *Alcohol Res*. **35,** 47–55 (2013).

Skinner M. & Guerrero-Bosagna C. Role of CpG deserts in the epigenetic transgenerational inheritance of differential DNA methylation regions. *BMC Genomics*. **15**, 692–697 (2014).

Steegers-Theunissen R., Obermann-Borst S., Kremer D., Lindemans J., & Siebel C. Periconceptional maternal folic acid use of 400 μg per day is related to increased methylation of the *IGF2* gene in the very young child. *PloS ONE*. **4**(11), e7845 (2009).

Teneng I., Montoya-Durango D., Quertermous J., Lacy M., & Ramos K. Reactivation of L1 retrotransposon by benzo(a)pyrene involves complex genetic and epigenetic regulation. *Epigenetics*. **6,** 355-367 (2011).

Watanabe K., Noriko E., Hamano E., Sunohara M., Kawakami M., *et al.* Genome structure-based screening identified epigenetically silenced microRNA associated with invasiveness in non-small-cell lung cancer. *International Journal of Cancer*. **130,** 2580–2590 (2012).

Watson J., Gann A., Baker T., Levine M., Bell S., Losick R., & Harrison S. *Molecular*

Biology of the Gene. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, seventh edition, (2014).

Provides an extremely thorough and current overview of genetic molecular mechanisms, with chapters 8 and 18-21 focusing upon mechanisms related to epigenetics.

Zhang P., & Pradhan S. Mammalian epigenetic mechanisms. *IUBMB Life*. **66**(4), 240–256 (2014).

Zhou V., Goren A., & Bernstein B. Charting histone modifications and the functional organization of mammalian genomes. *Nat. Rev. Genet*. **12**, 7–18 (2011).