Chapter 15
We Are What We Eat: How Nutritional Compounds Such As Isoflavones Shape Our Epigenome

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15.1. INTRODUCTION

In recent years, the study of epigenetic alterations such as modification of DNA and packaging of DNA into nucleosomes and chromatin has flourished, as we begin to realize that our genome is influenced by our epigenome. It is now recognized that epigenetics plays an important role in several areas of biology, including phenotypic variation (Dolinoy et al., 2006, 2007; Guerrero-Bosagna et al., 2008; Kucharski et al., 2008), carcinogenesis (Laird and Jaenisch, 1996; Warnecke and Bestor, 2000; Liu et al., 2003; Cheng et al., 2004; Frigola et al., 2006; Hinshelwood et al., 2007; Hinshelwood and Clark, 2008), disease etiology (Edwards and Myers, 2007; Hinshelwood and Clark, 2008; Zeisel, 2009), transgenerational transmission of diseases (Anway et al., 2005, 2006a, 2006b, 2008; Anway and Skinner, 2008; Nilsson et al., 2008), and ecology and evolutionary biology (Guerrero-Bosagna et al., 2005; Crews and McLachlan, 2006; Bossdorf et al., 2008). The potential for epigenetic alterations to be influenced by environmental factors, such as diet, has generated a great deal of interest in further understanding the underlying mechanism, especially as slight variations in micronutrient consumption appear to have important consequences in terms of epigenetic alterations and genomic stability. It has been demonstrated that variations in the consumption of micronutrients can affect DNA synthesis and repair, oxidative damage, and maintenance of methylation (Bull and Fenech, 2008). A significant amount of research has been conducted to investigate how maternal nutrition affects epigenetic programming that takes place during early fetal development. Indeed, consumption of trace elements such as arsenic by the pregnant mother has been shown to alter DNA methylation in early development (Waalkes et al., 2004; Vahter, 2007). Dietary compounds have also been implicated in the modulation of histone modifications (Delage and Dashwood, 2008), and consumption of endocrine disruptors or methyl donors has been implicated in changes in DNA methylation, which will be discussed further in the following sections.

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The specific mechanisms by which nutrient consumption affects the epigenome are yet to be fully elucidated and as such the scientific literature is littered with controversy. This controversy is partly due to the fact that DNA methylation can regulate gene expression in opposing ways. DNA methylation can induce gene expression by preventing repressor binding to promoter regions, or DNA methylation can also reduce expression by preventing enhancer binding to promoter regions (Engel et al., 2006; Renaud et al., 2007; Delage and Dashwood, 2008; Ideraabdullah et al., 2008).

In order to fully understand how nutritional compounds affect the epigenome, key questions to be addressed are, what classes of compounds are capable of altering these epigenetic states?, what are the molecular mechanisms of these epigenetic modifications? and what are the ontogenic critical periods that are particularly susceptible to alterations of these epigenetic states? In this chapter, we will review the current literature and discuss the role of diet, in particular flavonoids, on potentially altering the epigenome during development, thereby influencing phenotypic variation and disease susceptibility.

15.2. EPIGENETIC MECHANISMS OF GENE REGULATION

The best-known and studied epigenetic modification to date is DNA methylation. This process of DNA modification constitutes a postreplicative modification in which a methyl group is covalently added to a DNA residue (Laird and Jaenisch, 1996). The chemical reaction of DNA methylation occurs at the carbon 5 of the cytosine ring in 5′- to 3′-oriented CG dinucleotides (known as CpGs) and is catalyzed by the action of DNA methyltransferases (Dnmts) (Singal and Ginder, 1999). The reprogramming of methylation patterns in mammals mainly occurs during two key periods of development, namely, prior to embryo implantation and during development of the germ cell (Reik et al., 2001). However, several additional periods have been described during which there is increased sensitivity to environmental signals, which have the potential to alter methylation states (Edwards and Myers, 2007).

In addition to DNA methylation, other well-known epigenetic mechanisms include chromatin condensation and histone modifications. Specific regions on chromatin, referred to as facultative chromatin, can be transiently condensed or uncondensed during development, which can lead to variation in gene expression (Wallace and Orr-Weaver, 2005). These chromatin states are susceptible to modification by specific stimuli such as transcriptional repressors, functional RNAs, or accessory factors, which exist in macromolecular complexes with other accessory factors or chromatin proteins (Craig, 2005). Histones, in turn, are susceptible to a variety of posttranslational modifications such as phosphorylation, acetylation, methylation, ubiquitination, sumoylation, ADP ribosylation, glycosylation, biotinylation, and carbonylation (Margueron et al., 2005). It has been suggested that an "epigenetic conversation" exists between histones and DNA, whereby cytosine methylation and histone modification act in synergy to generate a self-reinforcing epigenetic cycle that maintains and perpetuates a repressed chromatin state (Fuks, 2005). Small RNA-regulated gene expression is the newest epigenetic mechanism that has been described to date and refers to the action of several classes of small RNAs, ranging from 20 to 31 nucleotides in length, on regulating gene expression (Kim, 2006). RNA factors, histone methylation, and chromatin-remodeling enzymes appear to all act together with DNA methyltransferases, resulting in the establishment and maintenance of tissue-specific and site-specific methylation patterns (Chen and Riggs, 2005).

15.3. NUTRITION AND THE ENZYMATIC PROCESS OF DNA METHYLATION

Nutrition is a critical component of the environmental influence on the epigenome. This is especially important for the enzymatic process of DNA methylation, which requires the presence of methyl group substrates, commonly derived from the diet. Dietary sources of methyl groups

that can affect DNA methylation include folic acid, betaine, zinc, and vitamin B_{12}. These components ultimately influence the metabolism of methionine and *S*-adenosyl methionine (SAM) (Van den Veyver, 2002). SAM is formed from methyl groups derived from choline, methionine, or methyl-tetrahydrofolate, and is the primary methyl donor for the various methyltransferase enzymes within an organism (Zeisel, 2009). The end product of SAM metabolism is *S*-adenosylhomocysteine (SAH), which forms a negative feedback loop via direct inhibition of these methylation reactions (Hirsch et al., 2008; Piyathilake et al., 2008). There is a correlation between the availability of methyl groups in the diet and levels in an organism. Specifically, it has been shown that the amount of folate in the diet can directly influence the level of these compounds in blood cells (Hirsch et al., 2008).

As mentioned previously, DNA methyltransferase enzymes (Dnmts) catalyze DNA methylation reactions. There are several known Dnmts, each with distinct but at times overlapping roles. Dnmt3A and Dnmt3B are involved in the establishment of methylation patterns during early development, acting as *de novo* methyltransferases on unmethylated DNA (Yokochi and Robertson, 2002). Dnmt1 is in turn responsible for the maintenance of these patterns, since its preferential substrate is hemimethylated DNA (Yoder et al., 1997). The expression of Dnmts is also influenced by nutrition. A recent study suggests a link between dietary folate levels and Dnmt1 expression. An increase in the expression of Dnmt1 was observed in cervical intraepithelial neoplasia samples compared with samples collected prior to mandatory fortification of grain products with folic acid in the United States (Piyathilake et al., 2008). Dnmt1 has also been shown to be reduced in the liver of rat offspring born to protein-restricted mothers (Lillycrop et al., 2007).

Possibly the best-known model for the study of methyl donors in the diet and the effect on DNA methylation status is the agouti mouse model. This model is based on detecting changes in methylation in the mouse A^{vy} allele, which can be easily observed as changes in coat color. Decreasing methylation of the A^{vy} allele, specifically in an intracisternal A particle (IAP) retrotransposon located upstream of the Agouti gene, correlates with a coat color shift from yellow-agouti to yellow (Cooney et al., 2002). Changes in maternal consumption of methyl groups leads to changes in the coat color of offspring, and this coat color can be correlated to the predicted changes in methylation in the A^{vy} allele (Cooney et al., 2002). Methyl donors can also alter the epigenetic state of the A^{vy} allele which exposure *in utero* is transient and during the period of germ line differentiation. This effect, which occurs in the male germ line (Cropley et al., 2006) but not in the female germ line (Waterland et al., 2007), also has implications for the offspring (F2) of those mice exposed to methyl donors *in utero*. Some experiments have taken advantage of other properties of the A^{vy} allele, such as its association with obesity (Yen et al., 1994; Dolinoy et al., 2006). Transgenerational exposure of A^{vy}/a mouse to an ad libitum diet produces amplification of obesity, an effect that is suppressed when the diet is supplemented with extra methyl donors such as folic acid, vitamin B_{12}, betaine, and choline (Waterland et al., 2008).

Another model has been developed recently to study folates and methylation. This model takes advantage of another IAP, located upstream of the promoter of *Axin fused*. In this case, high levels of methylation of *Axin fused* (expressed specifically in the tail) are associated with a straight tail phenotype, while reduced levels of methylation correlate with a kinky tail phenotype (Waterland et al., 2006). In this model, the *Axin fused* DNA methylation-associated tail phenotype is dependent on pre- and postnatal dietary methyl group exposure.

It is interesting to note that these effects are all maternally mediated. It is clear that there are critical periods in which DNA methylation patterns are particularly susceptible to reprogramming. These critical periods include the period from fecundation to blastocyst preimplantation, and also early germ line differentiation (Reik et al., 2001), although other sensitive periods have also been described (Edwards and Myers, 2007). Although epigenetic programming *in utero* has traditionally been thought to be irreversible, a recent study has shown that epigenetic changes mediated by the maternal diet can be reversed after folic acid supplementation in the juvenile–pubertal period (Burdge et al., 2009); however, the mechanism by which this occurs is yet to be determined.

15.4. DIETARY FLAVONOIDS AND EPIGENETIC CHANGES

Flavonoids (or isoflavones) are a class of compounds produced by plants that have estrogenic actions in animals (Liggins et al., 2000); therefore, they are named as phytoestrogens. The physiological effect produced by these agents is known as endocrine disruption. In particular, consumption of these compounds can produce reproductive effects in mammals (Adams, 1981; Adams et al., 1981; Santell et al., 1997; Gallo et al., 1999; Guerrero-Bosagna et al., 2008), including humans (Pino et al., 2000), where transmission of isoflavones from mother to child has also been reported (Franke et al., 2006). In humans, flavonoid consumption may delay breast development (Wolff et al., 2008) and may have a protective effect against breast cancer (Thanos et al., 2006). Nevertheless, this effect would be protective only if the exposure is during childhood/adolescence (Warri et al., 2008; Lee et al., 2009). This protection would occur through upregulation of breast cancer tumor suppressors such as *BRCA1* (Warri et al., 2008). It is interesting to note, however, that the cancer protective effect of flavonoids occurs only in certain organs (Cotterchio et al., 2006). This has been attributed to the action of selective estrogen receptor modulators (SERMs) that lead to tissue-specific agonistic or antagonistic effects of compounds with estrogenic action (McDonnell, 1999).

A possible pathway to explain these cancer protective effects of flavonoids are epigenetic mechanisms such as DNA methylation. Evidence for epigenetic changes produced by exposure to endocrine disrupting chemicals (such as flavonoids) has been increasing in recent years (Guerrero-Bosagna and Valladares, 2007). For example, administration of the phytoestrogens coumestrol and equol to newborn mice increase DNA methylation at the proto-oncogene *H-ras*, resulting in its inactivation (Lyn-Cook et al., 1995). DNA methylation patterns have also shown to be altered in 8-week-old mice after consumption of high doses of the phytoestrogen genistein (Day et al., 2002). It has been hypothesized that phytoestrogens could affect the establishment of methylation patterns in the offspring due to a maternal effect (McLachlan, 2001; Guerrero-Bosagna et al., 2005). The Agouti mouse model has been used as evidence for this effect of phytochemicals. Maternal treatment with bisphenol A (BPA) results in hypomethylation of the A^{vy} allele. However, this hypomethylation is inhibited by maternal dietary supplementation with either methyl donors or genistein (Dolinoy et al., 2007). Recently, in mice, gender-specific changes in *Acta1* gene methylation have been shown as a response to a diet rich in the phytoestrogens genistein and daidzein (Guerrero-Bosagna et al., 2008). An independent study has also shown that neonatal exposure of female mice to high levels of genistein results in tissue-specific hypermethylation in the gene *Nsbp1* in the uterus (Tang et al., 2008).

The effect of dietary phytochemicals on the epigenome is not just limited to DNA methylation. It has been shown that in prostate cancer, genistein can have a protective effect via histone demethylation and/or acetylation and chromatin remodeling of tumor suppressor genes, resulting in their activation (Kikuno et al., 2008; Majid et al., 2008). A very complete study of the role of genistein on the repression of breast cancer in human cultured cancerous and precancerous cells has been published by Li et al. (2009). The authors showed that genistein promotes hypomethylation of the E2F-1 sites in the *hTERT* (human telomerase reverse transcriptase) promoter, which leads to increasing binding of E2F-1 and inhibition of *hTERT* transcription. Moreover, they found that genistein also reduced expression of Dnmt1, Dnmt3a, and Dnmat3b in these breast cancer cells and changed methylation in H3K9 and H3K4 histones at the *hTERT* promoter (Li et al., 2009).

15.5. A PROPOSED MECHANISM FOR ENDOCRINE-MEDIATED FLAVONOID ACTION ON EARLY MAMMALIAN EMBRYOS

Considering the direct effects of flavonoid consumption on mothers and the indirect epigenetic effects on the developing embryo, a necessary question is about the mechanisms implicated

in this mother-to-embryo flavonoid action. In previous reports (Guerrero-Bosagna et al., 2005, 2008) we proposed that this flavonoid action could occur either directly, through the presence of flavonoids in uterine secretions, or indirectly, mediated by other compounds secreted in the uterine epithelia such as 4-OH-17β-estradiol, responding to circulating levels of flavonoids. It is not known whether flavonoids can act directly upon the developing embryo. Nevertheless, recent evidence supports the fact that intrauterine secretions are indeed affected by the maternal consumption of isoflavones. For example, the known cause of infertility in female mice that consumed the flavonoid genistein could be that uteri of genistein-treated females are not capable of supporting normal implantation (Jefferson et al., 2009). In fact, maternal genistein consumption is able to alter uterine wet weight and gene expression in the offspring, in which, a particularly striking change is observed in the expression of the estrogenic marker *complement-C3* gene in juveniles (Moller et al., 2009).

The relationship between maternal flavonoids and the machinery of DNA methylation in the preimplantational embryo could be mediated by *c-fos* expression. Interestingly, previous evidence shows that both Dnmt1 expression and activity can be directly upregulated by *c-fos* (Bakin and Curran, 1999) or *v-fos* (Ordway et al., 2004), and that induction of *c-fos* is attributed to membrane-mediated estrogen actions (Das et al., 2000). Studies in pancreatic β cells show that this is an alternative mechanism to the classical estrogen response involving receptors α and β (Nadal et al., 2000). Therefore, membrane-mediated estrogenic actions would first induce c-fos and then activate the Dnmt1 enzyme. This indirect and membrane-mediated mechanism of *c-fos* activation could also occur in blastocysts. For example, the activation of latent preimplantational blastocysts due to the uterine synthesis of the catecholestrogen 4-OH-17β-estradiol can also occur via a pathway distinct from the classical nuclear estrogen receptors (Paria et al., 1998). In this indirect pathway, levels of 4-OH-17β-estradiol increase with the epithelial growth factor (EGF) receptor (Paria et al., 1998). Interestingly, increasing the expression of EGF receptor would also be correlated to the activation of *c-fos* (Kamiya et al., 1996). A direct induction of *c-fos* by estrogen via an estrogen receptor element (Hyder et al., 1992) has also been shown in different cell types (Allen et al., 1997; Garcia et al., 2000). Recently, a study in lactotrophs showed that estrogens have no effect in the induction of *c-fos* caused by EGF (Chen et al., 2009), a finding that gives support to the idea that a Dnmt1 induction mediated by the *fos* pathway is not induced by the classical estrogen receptors α and β but would be initiated by the membrane receptor suggested by Nadal et al. (2000), which would respond to uterine cathecol estrogens (Paria et al., 1998). In summary, there is a possibility that estrogenic actions could induce *c-fos*, either directly, through an estrogen receptor element in the gene, or indirectly, through membrane-mediated reactions. Figure 15.1 summarizes the possible pathways via which an estrogenic stimulus could influence DNA methylation in developing embryos.

15.6. EPIGENETICS AND NUTRITIONAL EPIDEMIOLOGIC STUDIES

An interesting and necessary approach to study epigenetic modifications that correlate with nutrition is at a population level. This approach requires the study of epigenetic mechanisms in epidemiological studies. In nature, an example of how nutrition can influence populations comes from honeybees. Using Dnmt3 RNA interference it was found that reduced levels of Dnmt3 were associated with an increased proportion of queens compared with worker bees, an effect that mimics the consumption of royal jelly (Kucharski et al., 2008). This example highlights the extent to which nutritional components may influence the epigenetic make-up of organisms and how this may affect whole populations. Given the recent national implementation of folic acid supplementation of foods in several countries including the United States, Canada, Costa Rica, and Chile, and active debate in several European countries plus Australia and New Zealand to

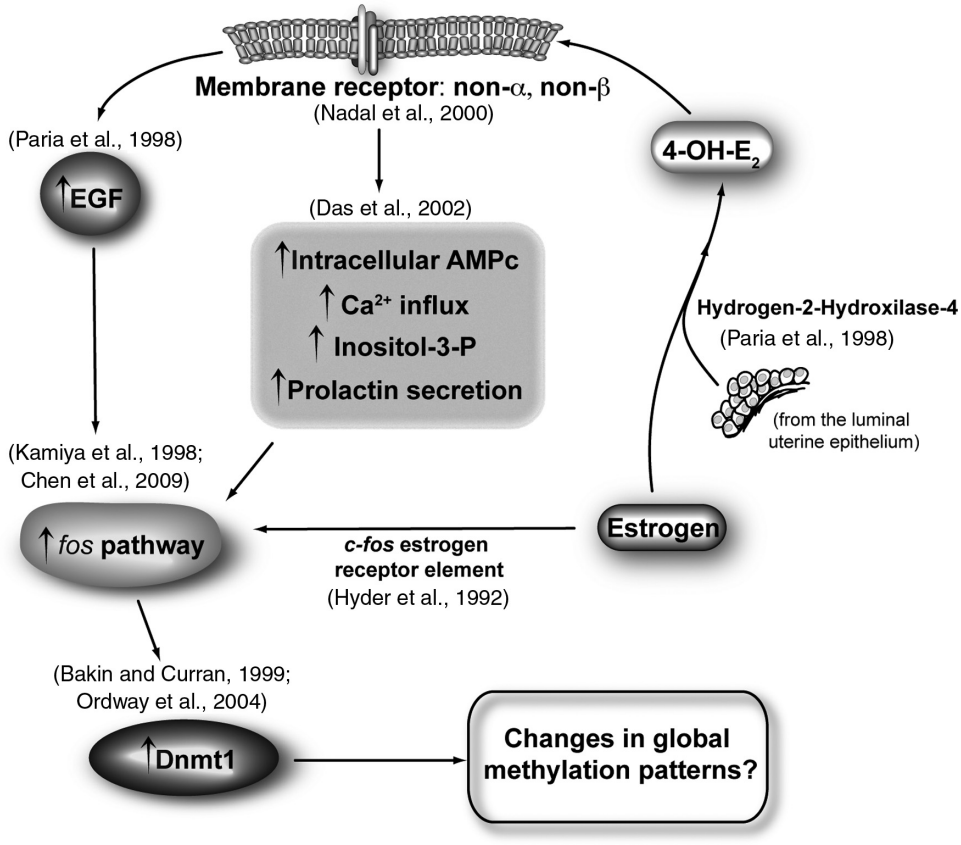

Figure 15.1. Possible pathways via which an estrogenic stimulus could influence DNA methylation in developing embryos.

proceed with national strategies of folic acid fortification (Kim, 2007), a deeper understanding of how nutrition affects the epigenome is paramount.

Despite the importance of understanding the consequences of epigenetic modification from a population perspective, epidemiological studies involving DNA methylation are still in their infancy. Most of these studies only evaluate global methylation (Axume et al., 2007a, 2007b; Pilsner et al., 2007), which will only undercover drastic changes in methylation patterns and underestimate local gene changes that may be a determinant for the establishment of a phenotype. In addition, although the timing of exposure to dietary methyl supplements is a critical determinant of phenotypic outcome, it is often not considered. DNA methylation patterns are established early during development (Reik et al., 2001) and once established are maintained for the life of the individual by the action of Dnmt1 (Li et al., 1993; Bestor, 2000). Therefore in the adult, drastic changes in global methylation patterns should not occur in a short space of time. If, however, there is modification of dietary methyl groups, over the lifetime of an individual the activity of Dnmt1 may be affected, which will lead to changes in global methylation patterns as the individual ages. This is highlighted by the study discussed above in which the expression of Dnmt1 was higher in cervical intraepithelial neoplasia after the introduction of dietary folate in the United States (Piyathilake et al., 2008).

One study that considered gene-specific methylation was performed by Van Den Donk et al. (2007a). In this study, subjects with previous colorectal adenomas were treated for a period of 6 months with folic acid and vitamin B_{12} dietary supplementation. Analysis of methylation in the genes *O-MGMT* and *hMLH* in rectal biopsies showed no major changes in methylation after the intervention, even though significant increases in erythrocyte folates were observed (Van Den Donk et al., 2007a). The same group also investigated methylation in the *MTHFR* (methylenetetrahydrofolate reductase) gene, which encodes an important enzyme in folate metabolism, and correlated this to folate intake and genotype of the individual. Results demonstrated that folate consumption was inversely correlated with promoter methylation in colorectal adenomas; however, there was a positive correlation between folate intake and the occurrence of adenomas without promoter methylation (Van Den Donk et al., 2007b).

An independent study that also investigated gene-specific methylation analysis was performed by Hirsch et al. (2008). In this study SAM and SAH concentrations, SAM/SAH ratio, methylation in the promoter region of the *ec-SOD* (extra cellular superoxide dismutase) gene, and *ec-SOD* activity were assessed in healthy men who were categorized in quintiles according to their folate status. Previously, it was shown that high *ec-SOD* expression in the arterial wall correlated with prevention of oxidation of cellular proteins and low-density lipoproteins, and with inactivation of nitric oxide (an endothelium-derived relaxing factor) (Fattman et al., 2003). Hirsch et al. (2008) showed that serum folate concentration in the highest quintile was associated with increased erythrocyte SAM and SAH concentrations, but not with SAM/SAH ratio, nor with changes in *ec-SOD* methylation. These types of studies are a very important first step in applying gene-specific methods of measuring DNA methylation; however, further studies need to be done in which the epigenetic status of more genes that associate with SAM/SAH metabolism are interrogated.

Methylation of the *MnSOD* gene has been studied by Thaler et al. (2009). In this study, methylation of the *MnSOD* promoter in samples from the buccal mucosa was determined to be reduced in vegetarian people compared with age-matched and older omnivores. This reduction in methylation correlated with increased expression of *MnSOD* (Thaler et al., 2009). These findings have important implications when considering certain features of vegetarian diets. These diets are low in vitamin B_{12}, which reduces the methionine content (Geisel et al., 2005), but are rich in flavonoids, which, as shown above, is an important factor in triggering specific methylation patterns. In fact, there are marked differences in isoflavone consumption between Asian and occidental countries, being much higher in the former (Mulligan et al., 2007).

An epidemiologic study that incorporates both gene-level methylation analysis and also timing of exposure have been performed by Heijmans et al. (2008). The methylation status of the *Igf2* imprinted gene was analyzed in individuals who were exposed during gestation to the caloric restriction imposed by the Nazi regime in Holland, a period known as the "Dutch Hunger Winter." The methylation of *Igf2* was shown to be reduced in adults that were *in utero* during the "Dutch Hunger Winter" (Heijmans et al., 2008).

15.7. DNA METHYLATION AND DISEASES

The epigenetic and developmental basis of several diseases is well characterized and widely accepted as contributing to the incidence and pathogenesis of disease (Godfrey et al., 2007; Hanson and Gluckman, 2008). However, when we consider the implications of nutrition in epigenetic modifications, the obvious assumption is that nutrition will also have a significant effect on some of these diseases. Indeed, some studies have suggested a link between cancer incidence and folate intake, which would likely help to protect against cancer via maintenance of methylation (James et al., 2003; Pogribny et al., 2006) or via prevention of hypermethylation in certain gene promoters such as tumor suppressor genes (Bhave et al., 1988; Dizik et al., 1991; Nan et al., 2005).

In general terms, cancer is a disease of an aging population. Since changes in the epigenome have been implicated in carcinogenesis, it follows that epigenetic modifications may also be influenced by age. Previously it has been shown that the *p16* gene product is a tumor suppressor, and loss of function secondary to hypermethylation is associated with human colorectal carcinogenesis (Wiencke et al., 1999). Keyes et al. (2007) evaluated the effect of aging combined with folate intake on the expression and methylation of *p16* in the mouse colon. Results of this study demonstrated that, interestingly, both promoter methylation and expression of *p16* increase with age. Moreover, this increase in methylation and expression was more striking in mice that were methyl-supplemented (Keyes et al., 2007).

Hyperhomocysteinemia is a condition that is associated with atherosclerosis and an elevation in plasma homocystein (a precursor of SAM). Patients with hyperhomocysteinemia are at an increased risk of developing cardiovascular disease (Becker et al., 2003). Nutritional factors, in particular consumption of methyl donors and DNA methylation during early arteriosclerosis, have been implicated in the development of hyperhomocysteinemia (Zaina et al., 2005). Specifically, Devlin et al. (2007) have shown that hyperhomocysteinemia is associated with hypermethylation and silencing of liver *Fads2* (involved in elongation of essential fatty acids) in mice. This evidence, together with changes in liver fatty acids, may help to explain the pathogenesis of hyperhomocysteinemia (Devlin et al., 2007).

15.8. CONCLUDING REMARKS

Throughout the genome, specific regions of DNA are normally methylated whereas other regions are normally unmethylated. This "normal" pattern of genomic DNA methylation is important for the appropriate expression of genes and homeostasis of the organism (Singal and Ginder, 1999; Bestor, 2000; Jones and Takai, 2001). It is becoming clear that dietary consumption of some compounds significantly influences specific methylation patterns of the genome. It seems, however, that the individual effect can be tissue and gene specific and also dependent on the timing of consumption in relation to the ontogeny of an organism. Therefore the coordinated action of these dietary compounds would be critical for the establishment of a specific epigenotype and associated phenotype. Moreover, since this epigenetic map is affected by nutrition, cultural aspects in relation to diet would also define epigenotypes associated with different human populations.

Several nutritional compounds are known to influence the establishment of epigenetic patterns in mammals and some of these are differentially consumed by human populations. Of these nutritional compounds that are known to produce changes in methylation, special emphasis should be given to methyl groups and flavonoids. Methyl groups interact with the already well-known methyl cycle, which include SAM and SAH metabolism. Nevertheless, the action of flavonoids on DNA methylation is still unknown. Here we propose a mechanism in which this action could be taking place. This mechanism could be mediated by a nonclassical estrogenic pathway, which includes membrane receptor actions of uterine secretions on preimplantational blastocysts.

The role of epigenome modifications in the development of human disease will be the focus of ongoing research. The relationship between epigenetic, genetic, and developmental causes of disease will gain importance in future studies. These relationships may shed some light on the epidemiology of human disease, with specific focus on the epigenetic patterns of populations. This understanding will likely lead to new prevention strategies and recommendations for specific diseases, from both an epidemiological and political levels. Finally, the mechanisms by which specific methylation patterns are established and how exposure to certain substances, including nutritional compounds, can trigger changes in methylation in one region of the genome and not another remain to be elucidated.

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