Gene-Nutrient Interactions and DNA Methylation\(^1,2\)

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ABSTRACT Many micronutrients and vitamins are critical for DNA synthesis/repair and maintenance of DNA methylation patterns. Folate has been most extensively investigated in this regard because of its unique function as methyl donor for nucleotide synthesis and biological methylation. Cell culture and animal and human studies showed that deficiency of folate induces disruption of DNA as well as alterations in DNA methylation status. Animal models of methyl deficiency demonstrated an even stronger cause-and-effect relationship than did studies using a folate-deficient diet alone. Such observations imply that the adverse effects of inadequate folate status on DNA metabolism are mostly due to the impairment of methyl supply. Recently, an interaction was observed between folate status and a common mutation in the gene encoding for methylenetetrahydrofolate reductase, an essential enzyme in one-carbon metabolism, in determining genomic DNA methylation. This finding suggests that the interaction between a nutritional status with a genetic polymorphism can modulate gene expression through DNA methylation, especially when such polymorphism limits the methyl supply. DNA methylation, both genome-wide and gene-specific, is of particular interest for the study of cancer, aging and other conditions related to cell-cycle regulation and tissue-specific differentiation, because it affects gene expression without permanent alterations in DNA sequence such as mutations or allele deletions. Understanding the patterns of DNA methylation through the interaction with nutrients is fundamental, not only to provide pathophysiological explanations for the development of certain diseases, but also to improve the knowledge of possible prevention strategies by modifying a nutritional status in at-risk populations. J. Nutr. 132: 2382S–2387S, 2002.

KEY WORDS: • MTHFR • C677T • folate • DNA methylation • gene expression

Nutrition research has recently highlighted the role of several nutrients in regulating the genome machinery. A number of vitamins and micronutrients are substrates and/or co-factors in the metabolic pathways that regulate DNA synthesis and/or repair and the expression of genes (1). It has been documented that the deficiency of such nutrients may result in the disruption of genomic integrity and alteration of DNA methylation, thus linking nutrition with modulation of gene expression. The discovery of polymorphic enzymes involved in critical steps of nucleic acids metabolic pathways contributed to new insights into the interplay of genetics and nutrition for the phenotypic expression of a defect. The response to a nutrient status seems in many cases to be specific for each genotype, and specific nutrient impairment results in different gene expression, depending on each genotype. The field of gene-nutrient interactions, therefore, seems to be a fascinating model to explain the different response to environmental/diet exposure at the molecular level.

Recently, the interaction between nutrients and DNA methylation has been emphasized. DNA methylation, a characteristic feature of many eukaryotic genomes, consists in the addition of a methyl group at the carbon 5\(^\prime\) position of cytosine within the cytosine-guanine (CpG)\(^4\) dinucleotide (2) in a complex reaction that probably involves the flipping of the cytosine base out of the intact double helix (3). Typically, DNA methylation occurs in CpG-dinucleotide-rich regions,

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necessary for the synthesis of poly-ADP-ribose polymerase (26,27), because it is a key enzyme of the one-carbon metabolism that is responsible for the availability of methyl groups for biological methylation reactions, including that of DNA (8,9). These findings suggest that modulating DNA methylation by nutrition may be a fascinating new field of gene and nutrient interaction. Therefore, it is of considerable interest to identify the factors that determine the patterns of methylation, not only to provide evidence for the mechanisms of several pathological conditions, but also to identify at-risk populations in which to conduct appropriate diet-based interventions (10).

The purpose of this review is to discuss the most recent knowledge about the effects of nutrients on gene expression and integrity, with an emphasis on gene-nutrient interactions in the modulation of DNA methylation.

**DISCUSSION**

**Effect of nutrients on genomic integrity and DNA methylation**

Reduced dietary intake or low tissue/plasma levels of several nutrients have been associated with higher risk for developing cancer. Interestingly, many micronutrients and vitamins are indispensable in DNA metabolic pathways (1,11).

Although most studies have been conducted in vitro and in animal models and there is no clear evidence for the optimal dietary ranges able to protect against DNA damage, roles in maintaining genomic stability have been documented for several nutrients. For example, vitamin C and E deficiencies are known to cause DNA oxidation and chromosome damage (12,13). Vitamin D exerts an antioxidant activity, stabilizes chromosomal structure and prevents DNA double strand-breaks (14). Magnesium is an essential cofactor in DNA metabolism, and its role has been recognized in maintaining high fidelity in DNA transcription (15). Iron may cause DNA breaks (16). A carotenoid-rich diet has been shown to reduce DNA damage (17). Vitamin B-12 deficiency is associated with micronuclei formation (1,18), and reduced transcobalamin II is associated with chromosomal abnormalities (19).

The roles of nutrients in DNA methylation, especially in genome-wide methylation, have also been described. Zinc deficiency can reduce the utilization of methyl groups from S-adenosylmethionine (SAM) in rat liver and results in genomic DNA hypomethylation as well as histone hypomethylation (20,21). Dietary deficiency in selenium decreased genomic DNA methylation in Caco-2 cells and in the rat liver and colon (22,23). Vitamin C deficiency has been associated with DNA hypermethylation in lung cancer cells (24,25). Interestingly, niacin, precursor of NAD+, is required to maintain the unmethylated state of CpG dinucleotides by inhibiting the enzymatic DNA methylation (26,27), because it is necessary for the synthesis of poly-ADP-ribose polymerase-1, which converts histone H1 to poly-ADP-riboseylated forms. The poly-ADP-ribosylated forms of histone H1 are responsible for the enzymatic inhibition of DNA methylation (26,27).

Nevertheless, folate and/or methyl group dietary supply provides the most compelling data for the interaction of nutrients and DNA methylation, because these dietary elements are directly involved in DNA methylation via one-carbon metabolism. The sole metabolic function of all coenzymatic forms of folate is to transfer one-carbon units. Within the scope of this function is the synthesis of SAM, universal methyl donor for several biological methylation reactions, and the de novo deoxynucleoside triphosphate synthesis. Methionine is regenerated from homocysteine by methionine synthase in a reaction in which 5-methyltetrahydrofolate (methyl THF) serves both as a cofactor and as a substrate. The reduced availability of methyl-THF, the main circulating form of folate, decreases the biosynthesis of SAM, thus limiting the availability of methyl groups for methylation reactions. Therefore, not only can dietary folate depletion decrease genomic DNA methylation in both human (28,29) and animal models (30), but, as described in the study by Rampersaud and colleagues, a folate replete diet also may restore the DNA methylation status (29).

**Gene-nutrient interactions in one-carbon metabolism**

Methylenetetrahydrofolate reductase (MTHFR; EC 1.5.1.20) is considered a key enzyme in the one-carbon metabolism because it catalyzes the irreversible conversion of 5,10-methylenetetrahydrofolate to methyl-THF (31). In 1988 Kang and colleagues identified a variant of the MTHFR that causes enzyme thermolability and reduced activity (32). The mutant enzyme was associated with elevated plasma homocysteine levels, which is to be expected because the conversion of homocysteine to methionine is impaired (31). Not only did the mild hyperhomocysteinemia appear as an indicator of altered one-carbon metabolism, but the higher levels of this sulfur-containing amino acid also were recognized to be an independent risk factor for cardiovascular disease (33,34).

The thermolabile variant of the MTHFR is due to a common missense mutation, a cysteine-to-thymine transition at base pair 677 (C677T) (35) that results in an alanine-to-valine substitution in the MTHFR amino acid sequence. The prevalence of the valine-valine substitution is rather common, with a frequency in homoygous persons of up to 20% in certain populations (35,36,37). By determining plasma total homocysteine levels, a strong gene-nutrient interaction was demonstrated in the phenotypic expression of this polymorphism in MTHFR (37,38). Only those affected homozygous persons with inadequate folate status, as indicated by blood folate levels, showed elevated plasma homocysteine concentrations (38). An intermediate effect has also been observed in heterozygous persons (37). These findings contributed to the opening of a new field of interest for both nutrition and genetics, especially because the relationship of the MTHFR polymorphism with plasma folate levels was implicated as the likely link between the C677T genetic defect and cardiovascular disease (34) as well as neural tube defects (39).

The MTHFR C677T polymorphism also provides a paradigm of gene-nutrient interaction in carcinogenesis (40,41). The mutant thymine/thymine (TT) genotype is associated with a lower risk of developing colorectal cancer; however, this protective effect is observed only in persons with adequate folate status. Among those persons with low systemic folate status, the protection associated with the mutation is eliminated (42) and an even higher risk of developing colorectal cancer is reported (43).

The biological significance of the MTHFR C677T mutation is predominantly related to the reduced availability of methyl-THF. Also consistent with this concept is the recent
observation that the distribution of different coenzymatic forms of folate is altered in MTHFR TT homozygotes (44). The red blood cells (RBC) of TT homozygous mutants show variable amounts of formylated tetrahydrofolate polyglutamates at the expense of methylated tetrahydrofolates. In contrast, cells from the cytosine/cytosine (CC) wild-type persons contain exclusively methylated tetrahydrofolate derivatives (44).

A common mutation that results in an altered activity of methionine synthase (5-methyltetrahydrofolate–homocysteine S-methyltransferase; EC 2.1.1.13), another important enzyme of the homocysteine/methionine metabolic pathway, has been described as interacting with levels of vitamin B-6 (45). This polymorphism, an adenine-to-guanine transition at nucleotide position 2,756 which results in substitution of glycine for aspartic acid at amino acid position 919, has been observed to cause elevation of plasma total homocysteine levels in persons with low levels of vitamin B-6 (45). However, more evidence is needed to demonstrate a clear gene-nutrient interaction in determining the biochemical expression of this genotype.

### Effect of folate and MTHFR gene interaction in genomic DNA methylation

A recent study investigated whether the mutant MTHFR, in association with folate status, affected methylation of DNA. (8). It was observed that subjects homozygous for the MTHFR C677T polymorphism possessed a lower degree of genomic DNA methylation in peripheral lymphocytes compared with the CC wild-type persons (8) and also that there was an inverse correlation between RBC folate and DNA methylation status. Because of the small number of subjects and the indirect method used to assess genomic DNA methylation, a large cohort of persons was subsequently studied using a newly developed quantitative and highly specific liquid chromatography/electrospray ionization-mass spectrometry assay in which the previous observations were reproduced and extended (9). The results showed that genomic DNA methylation in peripheral blood mononuclear cells directly correlated with folate status and inversely correlated with plasma homocysteine levels. MTHFR TT genotypes had a diminished level of DNA methylation compared with those with the CC wild-type. When analyzed according to folate status, however, only the TT subjects with low levels of folate accounted for the diminished DNA methylation (as shown in a model in Fig. 1). Moreover, in TT subjects, DNA methylation status correlated with the methylated proportion of RBC folate and was inversely related to the formylated proportion of RBC folates that are known to be solely represented in TT persons (9). These findings indicate that the MTHFR C677T polymorphism influences DNA methylation status through an interaction with folate status.

### Nutrients and gene-specific DNA methylation

**Gene-specific DNA methylation at the promoter region.** Approximately one-half of human genes have CpG islands in their 5'-promoter regions or within their first exons (46). CpG islands usually are unmethylated (47), and the methylation of these CpG-rich sequences induces inhibition of their expression. The patterns of DNA methylation, therefore, distinguish at a molecular level the genes to be expressed selectively (48). Alterations in DNA methylation have been described as regulating a differentiation path in a particular tissue. DNA in germ line cells usually is fully methylated, and demethylation usually is observed in a tissue-specific fashion, except that most of the housekeeping genes usually are maintained in a completely unmethylated state in both the germ line and in tissue-specific sites (49).

Although the exact molecular mechanism by which DNA methylation represses the transcription is not yet clear, data demonstrating an active role of promoter methylation in gene silencing are quite convincing. In vitro methylation of promoter-reporter constructs inhibits their subsequent expression in transfected cells (50). Demethylation by 5-aza-deoxycytidine, a DNA methyltransferase inhibitor, leads to re-expression of previously methylated genes (51). Homozygous embryos with a germline deletion of the DNA methyltransferase 1 (Dnmt1; EC 2.1.1.37) gene, on which the prototypical mammalian cytosine DNA methyltransferase is encoded, re-express a number of genes, including the normally silent alleles of several imprinting genes and the abundant but normally repressed endogenous retroviral sequences that are methylated and silent in heterozygous littermates (52).

The mechanism for the CpG island-associated gene silencing seems to involve the link of specific methylated DNA binding proteins, followed by the recruitment of a silencing complex that includes histone deacetylases (53,54). The de novo methylation, by itself, has a minimal effect on gene expression. However, methylated DNA recruits methyl-binding proteins, which also attracts a protein complex that con-
histsone deacetylases. Through the action of methyl-binding proteins and histone deacetylases, the DNA structure changes to a compact, condensed chromatin configuration that results in permanent inhibition of messenger RNA and protein production (53). This conformation makes the DNA refractory to nuclease or restriction endonuclease digestion and leads also to the loss of deoxyribonuclease (DNase)-I-hypersensitive sites. On the other hand, unmethylated CpG islands possess a nuclease-sensitive chromatin structure that differs from the bulk of the methylated genome (56). In carcinogenesis, hypermethylation of CpG islands in promoter region clearly is associated with transcriptional silencing of gene expression, which has an important role as an alternative mechanism by which tumor suppressor genes are inactivated without mutation or allele deletion. On the other hand, hypomethylation of CpG islands is associated with the gene activation, which also is an important mechanism by which protooncogenes are activated.

There are a few studies that suggest that certain nutrients can affect gene expression by altering methylation of promoter regions. In a rat model of hepatocellular carcinoma, a choline-deficient diet induced hypomethylation of CpG sites of the c-myc gene as well as overexpression of this gene (57). Jhaveri and colleagues reported that the H-cadherin gene showed hypermethylation of 5’sequences and downregulation of this gene in response to folate depletion in human nasopharyngeal carcinoma KB cells (58).

**Gene-specific DNA methylation at the coding region.** Because local cytosine methylation of a particular sequence can directly interfere with the binding of certain transcription factors (59), hypermethylation of the coding region can decrease the gene transcription. Conversely, hypomethylation of the coding region also can increase the gene transcription by enhancing the binding of transcription factors. Pogribny and colleagues evaluated the gene-specific alterations of DNA methylation during hepatocarcinogenesis with chronic dietary methyl deficiency (60). The authors reported the progressive loss of methyl groups at most CpG sites at both coding and noncoding strands in hepatic DNA during the early phase of folate/methyl deficiency. After tumor formation, the majority of cytosines became remethylated. In the preneoplastic nodules, the level of p53 mRNA was increased and associated with hypomethylation in the coding region, whereas in tumor tissue, p53 mRNA was decreased and was associated with relative hypermethylation. This observation suggests that a folate/methyl-deficient diet induces liver cancer by affecting the methylation status of the p53 gene coding region and by consequent alteration of p53 gene transcription. In other methyl-deficient animal studies, increased levels of mRNA for c-fos, c-Ha-ras and c-myc were correlated with hypomethylation at specific sites within these genes (61,62). These observations suggest that nutrients may affect gene transcription by exon-specific DNA methylation.

**Hypomethylation of the coding regions of critical genes can lead to instability either because this region becomes more susceptible to endogenous nucleases (63) or because the site of hypomethylation is likely to undergo enzymatic deamination to uracil (64,65).** The latter situation is particularly prone to occur in conditions where intracellular S-adenosylmethionine levels are low, such as in folate depletion (65). In a folate-deficient rat model, Kim and colleagues observed that hypomethylation of hypermutable sites (exon 5 through 8) on the p53 gene was related to increased DNA strand breaks in the same region (66). Furthermore, hypomethylation within the exon 8 of the colonic p53 gene was shown to be induced in an animal model of chemical carcinogenesis (67), and hypomethylation of this site in peripheral mononuclear cell DNA was highly associated with the development of lung cancer in a nested case-control study (68). Cell culture studies indicate that these foci of aberrant methylation may serve as initiators of mutations (64) and may induce susceptibility to breakage of the DNA backbone (69) by increased uracil insertion and strand breaks (70).

Collectively, these studies suggest that folate deficiency might induce DNA strand breaks and subsequent mutations through exon site hypomethylation. On the other hand, increasing levels of dietary folate effectively overrode the induction of hypomethylation in a dose-responsive manner in an animal model of chemical carcinogenesis (67), which suggests that folate supplementation can reduce gene disruption by reversing the site-specific DNA hypomethylation.

This review discusses recent data in the field of gene-nutrient interactions and DNA methylation, a fundamental epigenetic feature of DNA that affects gene expression and genomic integrity (Fig. 2). Several nutrients are involved in the maintenance of DNA metabolism, however most convincing data indicate a critical role for folate, an essential vitamin for DNA metabolism because it is involved in both DNA synthesis/repair and DNA methylation. The observation of an interaction between a common mutation in MTHFR, a key enzyme of the one-carbon metabolic pathway, and DNA methylation provides the basis for research on the potential role of nutrients in modulating an epigenetic feature of DNA as well as in possible future prevention strategies.

**LITERATURE CITED**


G. (1988) Intermediate homocysteinemia: a thermolabile variant of methyl-


methylation of DNA. Cancer 89: 1712

squamous cell carcinomas of the lung and larynx is associated with global

dna methylation. J. Nutr. 130: 2903

arsenic affect DNA methylation in vitro in Caco-2 cells and in vivo in rat liver and

1716.

1295.

199.

113.


53. Welch, G. N. & Lonsalaco, J. (1998) Homocysteine and atherothrombo-


