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Emerging Issues

## Roles for nutrients in epigenetic events

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### Abstract

The field of epigenetics is the study of modifications of DNA and DNA-binding proteins that alter the structure of chromatin without altering the nucleotide sequence of DNA; some of these modifications may be associated with heritable changes in gene function. Nutrients play essential roles in the following epigenetic events. First, folate participates in the generation of *S*-adenosylmethionine, which acts as a methyl donor in the methylation of cytosines in DNA; methylation of cytosines is associated with gene silencing. Second, covalent attachment of biotin to histones (DNA-binding proteins) plays a role in gene silencing and in the cellular response to DNA damage. Third, tryptophan and niacin are converted to nicotinamide adenine dinucleotide, which is a substrate for poly(ADP-ribosylation) of histones and other DNA-binding proteins; poly(ADP-ribosylation) of these proteins participates in DNA repair and apoptosis. Here we present a novel procedure to map nutrient-dependent epigenetic marks in the entire genomes of any given species: the combined use of chromatin immunoprecipitation assays and DNA microarrays. This procedure is also an excellent tool to map the enzymes that mediate modifications of DNA and DNA-binding proteins in chromatin. Given the tremendous opportunities offered by the combined use of chromatin immunoprecipitation assays and DNA microarrays, the nutrition community can expect seeing a surge of information related to roles for nutrients in epigenetic events.

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### 1. Introduction

DNA and DNA-binding proteins make up the bulk of chromatin. DNA-binding proteins comprise a diverse group of compounds including histones, high-mobility group proteins, transcription factors and enzymes that mediate covalent modifications of DNA (see below). Classically, the nucleotide sequence in DNA has been considered as the sole driver of heredity. Consistent with this point of view, phenotypic traits were thought of as being determined by genetic mutations and recombinations. Recently, this point of view has been changed dramatically by epigeneticists. The field of epigenetics is the study of chemical modifications of DNA and DNA-binding proteins that alter the

structure of chromatin without altering the nucleotide sequence of the DNA; some of these modifications may be associated with heritable changes in gene function.

Articles published in the “Emerging Issues” series of the *Journal of Nutritional Biochemistry* are not intended to provide readers with an in-depth review of a given topic. Rather, the purpose of this series is to draw the readers’ attention to recent developments with far-reaching implications for nutrition sciences. Here we will briefly review nutrient-dependent epigenetic events and present a novel tool to study roles for nutrients in epigenetic events: the combined use of chromatin immunoprecipitation (ChIP) assays and DNA microarrays.

### 2. Roles for nutrients in epigenetic events

Most epigenetic mechanisms target DNA and various DNA-binding proteins. In this section, we review three important examples for nutrient-dependent epigenetic events.

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### 2.1. Methylation of DNA

Promoter regions of many genes are rich in CpG dinucleotides [1]. Carbon 5 of cytosine in CpG dinucleotides is a prime target for methylation by DNA methyltransferases (DNMT) [1]. Depending on cell type or tissue, 3–4% of all cytosines in vertebrate DNA and 70% of cytosines in CpG dinucleotides are 5-methylated [1]. The following DNMT have been characterized: DNMT1, DNMT2, DNMT3a, DNMT3b and DNMT3L; catalytic activity has been shown only for DNMT1, DNMT3a and DNMT3b [1]. Methylation of cytosines is a heritable modification of DNA. However, the following mechanisms are available to remove methylation marks *in vivo*: (1) enzyme-mediated removal of methyl groups, although the enzymes involved have not yet been identified, and (2) passive removal of methyl groups if methylation is not maintained at the time of replication [1].

DNMT utilize *S*-adenosylmethionine as a methyl donor [1]. Folate plays a unique role in the generation of *S*-adenosylmethionine [1]. Briefly, methylenetetrahydrofolate reductase catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. The latter donates a methyl group to convert homocysteine to methionine. Methionine adenosyl transferase ligates methionine and adenosine to produce *S*-adenosylmethionine. Methylenetetrahydrofolate reductase deficiency results in hypomethylation of DNA, consistent with a role for folate in DNA methylation [1]. Nutrients other than folate also play roles in one-carbon metabolism: choline and methionine are the major dietary sources of methyl groups, whereas vitamins B-6 and B-12, riboflavin and zinc are coenzymes and cofactors, respectively, for various steps in one-carbon metabolism [1].

Methylation of CpG islands is associated with transcriptional repression and provides a mean to control gene expression. DNA methylation plays a role in allele-specific gene expression (genomic imprinting), heritable transcriptional silencing of parasitic sequence elements and X-chromosome inactivation [1]. Impaired DNA methylation is associated with perinatal death, decreased fertility, abnormal fetal development and tumorigenesis [1].

### 2.2. Biotinylation of histones

Histones are small proteins (11 to 22 kDa) that mediate the folding of DNA into chromatin. The following five major classes of histones have been identified in eukaryotic cells: H1, H2A, H2B, H3 and H4 [2]. DNA is wrapped around octamers of core histones, each consisting of one H3–H3–H4–H4 tetramer and two H2A–H2B dimers, to form the nucleosomal core particle. Histone H1 associates with the DNA connecting nucleosomal core particles. Nucleosomes are stabilized by electrostatic interactions between negatively charged phosphate groups in DNA and positively charged  $\epsilon$ -amino groups (lysine residues) and guanidino groups (arginine residues) in histones.

Histones consist of a globular C-terminal domain and a flexible N-terminal tail [2]. The amino terminus of histones protrudes from the nucleosomal surface; lysines, arginines, serines and glutamates in the amino terminus are targets for acetylation, methylation, phosphorylation, ubiquitination, poly(ADP-ribosylation) and sumoylation [2–6]. These modifications play important roles in chromatin structure, regulating processes such as transcriptional activation or silencing of genes, DNA repair and mitotic and meiotic condensation of chromatin.

Recently, a novel posttranslational modification of histones has been identified in human cells: biotinylation of lysine (K) residues [7]. The following biotinylation sites have been identified in human histones: K9 and K13 in histone H2A (unpublished observation); K4, K9 and K18 in histone H3 [8]; and K8 and K12 in histone H4 [7]. Biotinylation of histones is catalyzed by biotinidase [9] and holocarboxylase synthetase [10]. Biotinylation of histones plays a role in processes such as gene silencing, cell proliferation and DNA repair or apoptosis [11]. Different kinds of DNA damage cause distinct changes in histone biotinylation: double-stranded breaks of DNA decrease histone biotinylation [12], whereas formation of thymine dimers in DNA increases biotinylation of histones [13].

### 2.3. Poly(ADP-ribosylation) of histones and other DNA-binding proteins

Double-stranded DNA breaks require repair by either nonhomologous end joining or homologous recombination in order to prevent tumor initiation. If damage is beyond repair capabilities, apoptosis is triggered to prevent propagation of abnormal cells. Covalent modifications of DNA-binding proteins play important roles in DNA repair and apoptosis. Poly(ADP-ribose) polymerase (PARP) binds to the DNA near strand breaks; binding is associated with activation of the polymerase. PARP catalyzes the covalent attachment of linear and branched chains of poly(ADP-ribose) residues on itself (automodification) and other acceptor proteins [14]. For example, poly(ADP-ribosylation) of histones H2A and H2B increases dramatically in response to DNA breaks [5,15]. Poly(ADP-ribose) chains may contain >200 ADP-ribose residues [14]. Poly(ADP-ribosylation) of proteins is a reversible process; degradation of poly(ADP-ribose) chains is mediated by poly(ADP-ribose) glycohydrolase [14]. The half-life of poly(ADP-ribose) in response to DNA damage is <1 min [16].

PARP plays a role in DNA repair, recombination, apoptosis and maintenance of genomic stability [14,16]. PARP interacts with proteins from the DNA excision repair machinery [16]. PARP-null mice are extremely sensitive to genotoxic stressors [16] and are at increased risk for sister chromatid exchanges.

PARP uses NAD as a substrate for generating poly(ADP-ribose) chains [16]. In mammals, nicotinamide adenine dinucleotide (NAD) can be synthesized from either niacin or tryptophan [17,18]. Consistent with a predominant role for

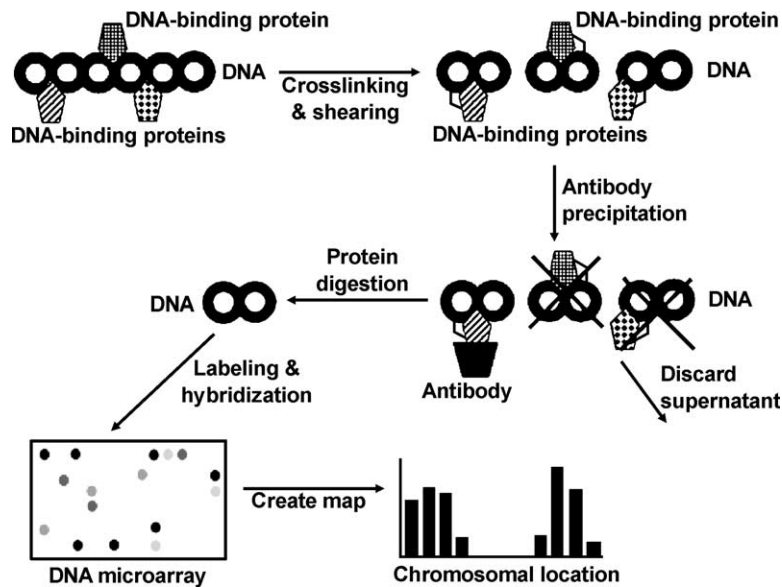


Fig. 1. Creating epigenetic road maps by using ChIP assays in combination with DNA microarrays.

niacin in NAD synthesis, poly(ADP-ribosylation) of proteins correlates with niacin supply in mammals [16]. Niacin supplementation may reduce the frequency of DNA damage in human lymphocytes [16].

### 3. Characterization of epigenetic events by using ChIP assays and DNA microarrays

Nutritional biochemists can benefit greatly from being in full command of tools to investigate epigenetic events. Ground can be lost rapidly in the genomics era by not taking advantage of cutting-edge technology. Recently, a novel analytical procedure that is likely to impact the field of epigenetics dramatically has become available: the combined use of ChIP assays and DNA microarrays [19,20]. This procedure is designed to map epigenetic marks in the entire genome of any given species. This procedure is also an excellent tool to map the enzymes that mediate modifications of DNA and DNA-binding proteins in chromatin. The combination of ChIP assays and DNA microarrays holds great promise for the identification of roles for nutrients in epigenetic events, given the extraordinary power of this new tool.

What is the working principle of ChIP assays/DNA microarrays? Briefly, this procedure uses the following analytical sequence to map epigenetic marks in chromatin (Fig. 1): (1) DNA and proteins in chromatin are cross-linked using formaldehyde, and chromatin is sheared to produce fragments of 500–1500 base pairs in size; (2) chromatin is immunoprecipitated using an antibody that binds the epigenetic mark of interest; the supernatant does not contain the mark of interest and is discarded; (3) DNA is purified from the precipitated chromatin; (4) DNA is chemically labeled (e.g., by using fluorophores) and is hybridized to DNA microarrays; and (5) the epigenetic mark of interest is mapped in the genome, based on its relative enrichment by

antibody precipitation. The information obtained can be used in various ways. For example, the relative enrichment of a given epigenetic mark in a distinct region of chromatin can be correlated with the transcriptional activity of genes residing in that region.

The generation of epigenetic road maps in chromatin depends on the availability of two diagnostic tools: ChIP-grade antibodies for immunoprecipitations and DNA microarrays that contain probes for both coding and noncoding regions in the genomes to be investigated. Nowadays, a relatively large and ever-increasing selection of ChIP-grade antibodies is available from various commercial sources. Also, antibodies can be generated by individual investigators using commercial facilities at reasonable cost. Hence, lack of appropriate antibodies may not be a limiting factor for most researchers who are conducting ChIP assays. In contrast, the current lack of appropriate DNA microarrays hampers studies described above. The vast majority of DNA microarrays contains probes for coding regions in genomes but does not contain probes for noncoding regions. Note that noncoding regions contain the majority of regulatory sequences of genes and, hence, are the prime target for many epigenetic modifications. Likely, an increasing number of DNA microarrays that overcome these early limitations will become available in the near future. Currently, however, investigators will need to rely on using customized DNA microarrays, generated either “in house” or by commercial suppliers [21]. Also, some DNA microarrays that contain probes for both coding and noncoding regions in the human genome are emerging. For example, Affymetrix, Inc.<sup>1</sup> has developed a DNA microarray that contains probes

<sup>1</sup> Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

for coding and noncoding regions from the entire human chromosomes 21 and 22 [22]. Finally, investigators may want to consider using alternative techniques such as “chromatin immunoprecipitation arbitrarily primed PCR assay,” in which immunoprecipitated DNA is amplified using random GC-rich or non-GC-rich primers to allow for random analysis of the entire genome [23].

The experimental procedures outlined here are likely to generate knowledge in areas pertinent to human health. For example, cancer is a manifestation of both abnormal genetic and epigenetic events. The combined use of ChIP assays and DNA microarrays is an excellent approach to investigate anticancer roles for nutrients such as folate, biotin and niacin based on the evidence provided above. Likewise, ChIP assays and DNA microarrays are a unique tool to investigate roles for nutrients in gene silencing during embryonic development.

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