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Genetic and Epigenomic Footprints of Folate

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Folate and single-carbon metabolism

Folate is an essential micronutrient with a central function in single carbon transfer reactions, and folate status and metabolism is of significant interest to public health. Folate has proven highly successful in the prevention of neural tube defects (Smithells et al., 1977; Smithells et al., 1976; Smithells et al., 1981), so much so that the United States prescribed a mandatory fortification of grain products with folate commencing in 1998, leading to a significantly reduced prevalence of birth defects such as spina bifida. This is a severe and disabling birth defect that comes with significant psychological and financial hardships for afflicted families; prevention with folate has been a clear public health success story (Obican et al., 2010), and a victory for preventive medicine.

While folate is thought to be highly beneficial for the prevention of birth defects, the relationship between folate and cancer is more complex. Folate plays an essential role for genome stability (Fenech, 2001); high folate status may therefore be beneficial in preventing genome instability, a key event of neoplastic transformation (Sieber et al., 2005), while folate insufficiency may actually support early steps of carcinogenesis. Furthermore, high folate levels may in turn promote the cancer progression of already existing neoplasms (Kim, 2003), where it can act as a mitogen. In fact, antifolates are used in cancer chemotherapy (Goldman et al., 2010), and folate is further utilized as a targeting moiety to deliver cytotoxic drugs to tumors that overexpress a folate receptor gene (Low and Kularatne, 2009). Folate metabolism in cancer is therefore not only a target for therapy, but folate conjugates also serve as tools in the fight against cancer. Yet, due to the dual relationship in preventing and promoting neoplastic disease, the benefits of folate fortification, supplementation, and high dietary intake are not unequivocal.

Folate serves to provide a methyl group for two streams of methyl trafficking in the cell: nucleotide synthesis to maintain the nucleotide pool for DNA replication or repair, which ultimately affects genomic stability, and formation of methionine, the precursor for S-adenosyl-methionine, which serves as primary methyl group donor for the majority of methylation reactions in the cell. Methylations of nucleic acids, proteins, and lipids are therefore impacted by folate level. Higher organisms have lost the ability to synthesize folate and must acquire it via diet; typically, leafy green vegetables represent a suitable source. Dietary folate therefore provides a link to maintenance and stability of the genome via the nucleotide synthesis pathway, but also to functional aspects such as the regulation of gene expression, via the methylation of DNA as well as histones. In this review, we will discuss the evolutionary aspects of folate transport and metabolism, examine the

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consequences of genetic defects in folate genes in mouse and man, and explore implications of nutritional methyl donor supply for epigenomics of health and disease.

Genes of the Folate Cycle – Biochemical and Evolutionary Aspects

A simplified schematic overview of the Folate cycle as shown in Figure 1 consists of mechanisms of uptake and transport into the cell, enzymatic trapping and processing of folate for discharge of the methyl group, and recharging of folate with a methyl group from intracellular sources. It should be noted that due to the biochemical complexity, we use the term 'folate' as an umbrella term rather than refer to each of the biochemical derivatives in specific fashion. Several mechanisms of folate uptake from dietary sources exist. Transporter-based means of folate import into the cell are either through the Reduced Folate Carrier (RFC; official gene symbol SLC19A1), or the Proton-coupled Folate Transporter (PCFT; official gene symbol SLC46A1). Transport of Folate into mitochondria is achieved via the Mitochondrial Folate Transporter/Carrier (MFTC; official gene symbol SLC25A32). The genes for these transporter molecules show deep evolutionary conservation within the animal kingdom: they can be detected in genomes of *Pseudocoelomata* such as the nematode *Caenorhabditis elegans*, and within *Coelomata* they are present in both *Protostomia* and *Deuterostomia*. In the branch of *Protostomia*, homologous genes are present in the genome of the fruit fly *Drosophila* melanogaster. In *Deuterostomia*, conserved genes for folate transporters can be detected in Tunicata such as the sea quirt *Ciona intestinalis*, as well as in all higher branches of *Craniata*, as evidenced by the folate transporter genes of *Mus musculus* or *Homo sapiens*. The high conservation of folate transport gene sequences between animal species that diet-dependence on folate is likely of significant evolutionary age.

Following transport into the cell, folate is polyglutaminated by folylpolyglutamate synthase (FPGS), and converted by dihydrofolate reductase (DHFR) to tetrahydrofolate. When required, loading tetrahydrofolate with the crucial single-carbon group is achieved by serine hydroxymethyltransferase 1 (SHMT) in a reaction that converts the amino acid serine – from nutritional or other proteolytic sources – to glycine, and results in the formation of 5,10-methylene tetrahydrofolate. In fact, serine serves as the major source of single-carbon groups in the cell, whereas folate constitutes the essential shuttle vehicle for that methyl group. 5,10-methylene tetrahydrofolate represents a central node from which several directions for single carbon metabolism are possible: (**i**) Diversion of the single-carbon stream towards purine synthesis occurs via MTHFD1 - trifunctional methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 1, methenyltetrahydrofolate cyclohydrolase, formyltetrahydrofolate synthetase – which converts 5,10-methylene tetrahydrofolate through a series of reversible reactions into 10-formyl tetrahydrofolate, the methyl donor substrate for *de novo* purine synthesis. (**ii**) Routing the single carbon stream towards thymidine synthesis happens through the action of thymidylate synthetase (TYMS), which utilizes 5,10-methylene tetrahydrofolate for the conversion of deoxyuridine monophosphate to deoxythymidine monophosphate. (**iii**) Direction of the single-carbon stream towards methionine synthesis ensues in the irreversible reaction catalyzed by MTHFR - methylenetetrahydrofolate reductase (NAD(P)H) – to yield 5-methyl tetrahydrofolate, the methyl donor substrate in the vitamin B12-dependent conversion of homocysteine to methionine via 5-methyltetrahydrofolate-homocysteine methyltransferase reductase (MTRR) and 5-methyltetrahydrofolate-homocysteine methyltransferase (MTR). Through the action of methionine adenosyltransferases (MAT1A, MAT2A, MAT2B), methionine is converted to S-Adenosyl-methionine, the main methyl donor substrate to be channeled towards the major enzymatic methylation reactions in the cell. Synthesis of methionine leaves tetrahydrofolate, as the methyl group has been discharged. (**iv**) Finally, single-carbon recharging of tetrahydrofolate is again achieved by SHMT as mentioned

above. This reaction completes the cycle for replenishment of the methyl donor substrate pool. Methionine is converted to S-Adenosyl-methionine, and execution of a methylation reaction leaves S-adenosyl-homocysteine, which is converted to homocysteine, the methyl acceptor from the folate cycle, but also from the choline/betaine stream of methyl donor supply. All genes that encode enzymes of the folate cycle show deep evolutionary conservation – they are not just present in genomes of the animal kingdom, but can be detected in genomes of primitive *Eukaryota* such as the yeast *Saccharomyces cerevisiae*. This strong evolutionary conservation is not surprising given the fundamental role of the folate cycle for the essential single-carbon biochemistry of the cell.

The picture on folate uptake is, however, not complete without giving consideration to folate receptors. In contrast to folate transporters that are transmembrane proteins, folate receptors are glycolipid-anchored cell surface proteins that bind folates with very high affinity. Transport into the cell occurs by endocytosis, fusion of the resulting vesicle with lysosomes, pH-mediated release of folates from the receptor, and folate entry into the cytoplasm through the membrane-situated folate transporter molecules discussed earlier. Folate receptors are recycled to the cell surface, and essentially serve to critically enrich folates for transporter-based cytoplasmic import (Kamen and Smith, 2004). Four distinct genes for folate receptors can be distinguished in the human genome; currently, three genes are recognized in the mouse genome. In contrast to all the other folate-related genes, folate receptors do not show deep conservation across evolutionary phyla. Rather, it appears that folate receptors are an invention of *Chordata*, as they seem to be missing from genomes of *Protostomia* (e.g. *D. melanogaster*) or *Pseudocoelomata* (e.g. *C. elegans*). Genes for folate receptors can be found as low on the *Chordata* branch as *Tunicata*: species of *Ciona* have folate receptor genes in their genomes; so do all higher branches of *Chordata*. One may speculate that the presence of genes encoding these high affinity receptors for folates permit a highly efficient extraction of folate from nutrition sources. Given the fundamental importance of folate for cell biology, such receptors may have yielded specific evolutionary advantages, and one can imagine that such advantages could have been in the realm of overall genome stability, or in the possible elaboration and expansion of methylation-based genomic regulatory mechanisms.

While the cellular uptake of Folate has received much attention, far less is known about the export of folate from one cell to the next, or to serum or lymph. Uptake of folate is certainly of high importance for the organism, yet not every cell in the mammalian body in need of folate is located at an interface where folate is readily available. Examples for such interfaces are the intestinal epithelium, where folate is imported from the diet; the boundary to blood and lymph, where folate is in circulation, and the contact surface to cerebrospinal fluid. Cells that are not in direct contact with such interfaces would have difficulty acquiring folates, and it was reasonable to assume that folate transport mechanisms between cells must exist, and that cells at folate-uptake interfaces would have mechanisms for export of folates to make it available to other cells in the body. Recent evidence has accumulated indicating that folate export from cells may occur via members of the superfamily of ABC proteins. ABC proteins are ATP-binding cassette (ABC) transmembrane proteins that mediate ATP-dependent transport of various molecules across extra- and intra-cellular membranes. Members of this family implicated in folate export are P-glycoprotein / ABCB1 (Hooijberg et al., 1999; Hooijberg et al., 2004), and multidrug-resistant protein 3 / ABCC3 (Kitamura et al., 2008). It is also thought that folate can leave the cell via the reduced folate carrier SLC19A1, a protein initially thought to be only involved in folate uptake. How folate is transported once it leaves the cell is less clear; the major form of folate circulating in blood is 5-methyl tetrahydrofolate in the monoglutaminated form. Further research is needed to clarify how folate can travel across tissues; this would be of particular interest for

parenchyma of the early developing embryo before establishment of a functional vascular capillary system, or for poorly vascularized tumors.

Genetic Footprints of Folate Pathway Genes

With the success of folate in the prevention of birth defects, folate has taken significant role for public health. Similarly, the role of folate in cancer, either as risk factor or therapeutic target, has brought much attention to this micronutrient. For birth defects as well as cancer, folate is thought to represent a direct interface between nutrition and pathology, with the inferred prospect that therapeutic interventions may be achieved simply via altered nutrition or dietary supplementation. The molecular correlate of this interface is the folate pathway and its genes. Those genes were thought to be primary candidates to cause disease susceptibilities through disturbance of the folate pathway. They have therefore received strong interest in the biomedical research community. Considerable effort has been directed at determining the biological function of these genes for normal development, for birth defect phenotypes, and for contributions to cancer in animal models. Concomitantly, human genetic variations in folate pathway genes were investigated for possible associations to birth defect prevalence, cancer incidence, and roles in aging; the variation MTHFR C677T has received the highest attention in this context. We therefore briefly review the genetic footprint of these genes with respect to mouse model systems as well as human genetics. Up to date, over 5000 sequence variations in folate pathway genes have been described (and are available in detail for each gene at http://www.ensembl.org); these variations are summarized in Table 1.

FOLR1

Despite being a rather recent evolutionary invention, the gene for Folate receptor 1 (FOLR1; originally termed Folate receptor alpha in humans, and folate binding protein 1 in the mouse) constitutes an essential gene for mice. Targeted mutation of this gene results in embryonic lethality shortly after gastrulation, and embryos present with severe morphogenetic abnormalities (Piedrahita et al., 1999). Expression of this gene in the mouse embryo has been reported in the vicinity of neural tube closure sites (Saitsu et al., 2003), and most notably, in the visceral endoderm, a tissue that provides critical nutritional support to the embryo at a time when the placenta is yet to be established (Salbaum et al., 2009). Two well-characterized promoters contribute to the expression of this gene (Elwood et al., 1997), and to date this is the only gene in the folate pathway where a transcriptional enhancer element with in vivo activity has been reported (Salbaum et al., 2009). Interestingly, the developmental lethality of the null mutation can be rescued by supplementation with folinic acid (Finnell et al., 2002), indicating a redundancy of folate transport mechanisms. Genetic variations in the human gene are not strongly associated with morbidities such as neural tube defects (Barber et al., 2000; Barber et al., 1998). However, loss-of-function mutations in the human FOLR1 gene have been detected in patients afflicted with cerebral folate transport deficiency (Steinfeld et al., 2009); the lack of functional FOLR1 expression in the choroid plexus presents a plausible mechanism how the folate deficit in cerebrospinal fluid may be incurred in these patients.

FOLR2

Mice with a targeted mutation of the Folr2 gene are viable, but respond to exposure to valproic acid or arsenic (Spiegelstein et al., 2005) with a higher rate of developmental defects. Expression in the developing embryo occurs in the cartilaginous anlagen of the skeleton (Kappen et al., 2004). Human genetic variation in this gene has received little attention; this is also the case for FOLR3 and FOLR4.

SLC19A1 – Reduced folate carrier

The human sequence variant G80A of the SLC19A1 gene conveys increased risk for acute lymphoblastic leukemia (Laverdiere et al., 2002), and for Alzheimer's disease (Bi et al., 2009). In mice, a targeted mutation of the SLC19A1 gene leads to early developmental lethality (Ma et al., 2005; Zhao et al., 2001); death of homozygote mutant embryos can be postponed by supplementation with folinic acid in a dose-dependent manner; however, perinatal lethality of the mutation cannot be overcome by supplementation (Zhao et al., 2001). SLC19A1 is a widely expressed gene (Maddox et al., 2003). Besides regulating intracellular concentrations of folates, this carrier also can transport methotrexate (Dixon et al., 1994), a chemotherapeutic drug that acts as antifolate by blocking dihydrofolate reductase and several other enzymes in the folate pathway.

SLC46A1 – Proton-coupled folate transporter

Loss of function in the human SLC46A1 gene due to a mutation that results in skipping exon 3 during mRNA splicing leads to hereditary familial folate malabsorption (Qiu et al., 2006), a lack of adequate folate uptake from the gastrointestinal tract. Mice homozygous for a targeted mutation of this gene are viable, but display elevated levels of homocysteine, and exhibit severe hematopoietic deficits (Jakubowski et al., 2009; Salojin et al., 2011). Consistent with a role in *intestinal* absorption of folate, supplementation via intraperitoneal injection is indeed successful where *oral* supplementation fails to rescue the hematopoietic phenotype (Salojin et al., 2011). Parenteral supplementation of human infants afflicted by SLC46A1 mutations (Zhao et al., 2007) with folinic acid (Atabay et al., 2010; Borzutzky et al., 2009) is an effective treatment that permits normal development of these children.

SLC25A32 – mitochondrial folate transporter; FPGS - folylpolyglutamate synthase

Currently, mouse strains bearing mutations in these two genes are not available, and the consequences of human genetic variations have not been explored.

DHFR – dihydrofolate reductase

Similar to the previous two genes, mouse mutations for this gene have not been reported. Mutations in the human DHFR gene however, in particular A458T (Cario et al., 2011) and C238T (Banka et al., 2011), have recently been found as the cause for severe DHFR deficiency; these mutations were associated with megaloblastic anemia and cerebral folate deficiency. Therapeutic intervention could be achieved by treatment with folinic acid (Banka et al., 2011), a 5-formyl derivative of tetrahydrofolic acid that can be converted to other reduced folates while bypassing DHFR. DHFR is a target for antifolate cancer chemotherapy with compounds such as methotrexate (Rajagopalan et al., 2002), and folinic acid is used as part of a methotrexate chemotherapy regimen in order to save normal, non-transformed cells in the body from the effects of methotrexate. In that context, the sequence variant C829T is of note, revealing the regulation of DHFR by microRNAs. It renders a binding site for the miR-24 microRNA ineffective (Mishra et al., 2007), leading to increased expression of DHFR and subsequent methotrexate resistance. DHFR is also a target in the fight against the malaria parasite *Plasmodium falciparum*, where several mutations in DHFR have been detected that render *P. falciparum* resistant to anti-malaria drugs (Sridaran et al., 2010).

MTHFD1; MTHFD2

A single nucleotide polymorphism in the human MTHFD1 gene has shown genetic association with several folate-dependent pathologies. Homozygosity at G1958A appears to constitute a maternal risk for neural tube defects (Parle-McDermott et al., 2006) and heart defects in the infant, severe placental abruption, and late pregnancy loss (Parle-McDermott

et al., 2005). Loss of the MTHFD1 gene via a gene-trap mutation in mice also has severe consequences, leading to embryonic lethality (MacFarlane et al., 2009). A targeted mutation in the MTHFD2 gene, which encodes the mitochondrial version of the enzyme, also leads to embryonic death at mid-gestation (Di Pietro et al., 2002), thereby revealing an essential developmental function for folate metabolism in mitochondria.

MTHFR – methylene tetrahydrofolate reductase

A mutation in the human MTHFR gene (C677T) represents the most common genetic cause for elevated homocysteine levels. This sequence variant yields a hypomorphic allele of the gene (Frosst et al., 1995), with the T configuration showing significantly reduced - but not absent - enzyme activity. The result is hyperhomocysteinemia, a condition thought to lead to vascular pathology (Austin et al., 2004). The MTHFR C677T mutation has received attention in many lines of investigation. It is associated with increased risk for neural tube defects (Munoz et al., 2007; van der Put et al., 1995) and further pregnancy complications (Nurk et al., 2004), increased risk for gastric cancer (Boccia et al., 2008), and decreased risk for childhood acute lymphoblastic leukemia (Yan et al., 2011) and colon cancer (Le Marchand et al., 2005); no association to prostate cancer (Collin et al., 2009) or lung cancer (Mao et al., 2008) was found. Recent metaanalyses have called some of these risk associations into question (Boyles et al., 2006; Vollset et al., 2007; Wang et al., 2010). Furthermore, the relationship of this mutation to congenital heart defects (van Beynum et al., 2007) or coronary heart disease (Klerk et al., 2002) has been questioned (Lewis et al., 2005). MTHFR C677T may affect the risk for migraine (Liu et al., 2010; Schurks et al., 2008), and a link to autism-spectrum disorder has been postulated (Goin-Kochel et al., 2009; Pasca et al., 2009). Mice lacking the MTHFR gene (Chen et al., 2001) display delayed development, impaired growth, and increased morbidity and mortality in the early postnatal period. Interestingly, supplementation of these mice with betaine, a methyl donor derived from choline that is capable of fueling the methylation reaction from homocysteine to methionine, can at least partially rescue the mortality phenotype, and can ameliorate neuronal proliferation and differentiation deficits associated with the lack of MTHFR (Schwahn et al., 2004). Furthermore, MTHFR deficiency can be protective against the adverse developmental effects brought about by excessively high folate intake (Pickell et al., 2011).

MTHFR catalyzes an irreversible reaction, directing the single carbon stream towards generation of S-Adenosyl-methionine and general methylation reactions; lack of MTHFR activity is therefore more likely to compromise that aspect rather than purine or pyrimidine synthesis. Hence, one may speculate that phenotypes resulting from MTHFR deficiency are likely to involve a methylation deficit, potentially in the epigenome, in the pathogenesis.

MTR - methionine synthase

Human patients with a deficiency in the MTR gene show altered levels of methionine and homocysteine (Watkins et al., 2002), and are affected by megaloblastic anemia that is sometimes associated with neural dysfunction and mental retardation (Zavadakova et al., 2002). The mutation MTR A2756G, which may constitute a gain-of-function allele, exhibits an association with elevated risk for prostate cancer (Collin et al., 2009). Mice lacking the MTR gene die in utero (Swanson et al., 2001); homozygous embryos survive past the implantation stage, but succumb a short time after that. This suggests that the human mutation may not be as severe as the null mutation of the mouse model, and that residual MTR activity may be retained in humans carrying a genetic burden at the MTR locus.

MTRR- methionine synthase reductase

Mutations of the human MTRR gene, encoding an enzyme necessary for the activation of MTR, are associated with a higher risk for birth defects (Zhu et al., 2003), including neural

tube defects (van der Linden et al., 2006). The mutation A66G shows association to decreased risk for childhood acute lymphoblastic leukemia (Gast et al., 2007). Mice carrying a hypomorphic allele at the MTRR locus exhibit hyperhomocysteinemia (Elmore et al., 2007). Such mice are viable, but are burdened by adversely affected cardiac development and reduced overall reproductive success.

SHMT - serine hydroxymethyltransferase

Heterozygosity at SHMT1 C1420T in humans appears to confer a lower risk for childhood acute lymphoblastic leukemia (Collin et al., 2009; Vijayakrishnan and Houlston, 2010), and a weak genetic association was detected between the SHMT1 C1420T and increased risk for prostate cancer (Collin et al., 2009). Mice lacking the SHMT1 gene encoding the cytoplasmic version of the SHMT enzyme (MacFarlane et al., 2008) appear healthy, but suffer from abnormalities in hepatic levels of S-Adenosyl-methionine, as well as uracil incorporation into genomic DNA; furthermore, these mice show neural tube defects in response to low maternal folate status (Beaudin et al., 2011). For the mitochondrial version of the enzyme encoded by the SHMT2 gene, the KOMP repository reports targeted embryonic stem cells, but mice have not yet been generated as of yet.

Deficiencies in many of the genes of the folate cycle are associated with embryonic lethality. Although specific phenotypes differ and manifest at various stages of embryogenesis, it is noteworthy that such phenotypes can be found in almost all aspects of the folate metabolism. Hematopoietic phenotypes, where they have been characterized, appear dependent on the folate moiety itself, as no such phenotypes can be observed downstream of MTR where the methyl group is carried by molecules other than folate. Together, the existing mouse mutations in folate pathway genes underscore the essential position of the folate pathway for functional cell biology. The picture available from human mutations, however, is more complex, as several instances exist where a single mutation can confer increased risk for one type of morbidity, but also decreased rick for another type of morbidity. The molecular mechanisms that mediate those risks are thought to involve two general realms, namely DNA synthesis and repair for cell proliferation, and regulation of gene expression via the epigenome.

Folate and the Epigenome

With its role for methylation reactions, the relationship between Folate and the epigenome has received increased attention in recent years. The epigenome – the combination of DNA methylation, histone modification, transcription factor function, and non-coding RNA expression– constitutes domains in the genome that permit gene transcription. The term is derived from the classical definition of epigenetics – generation of different and stable phenotypes without changes in the underlying DNA sequence (Van Speybroeck, 2002); the epigenome represents the molecular instantiations of this concept. Interest in the biological role of epigenetic modifications has intensified with the development of technologies that allow assessment of epigenetic modifications on a genome-wide scale (Bock et al., 2010; van Steensel and Henikoff, 2003) rather than at single genes – hence the term epigenomics. The connection to folate comes from the fact that the various molecular modalities of the epigenome rely on methylation reactions to define the status of the respective epigenomic marks, or to regulate activities of epigenomic factors. While these methylation reactions all use S-Adenosyl-methionine as the direct methyl donor, the supply of S-Adenosyl-methionine – and thereby the efficacy of the pertinent methylation reaction – is dependent on the rate of replenishment of methionine from homocysteine; this reaction, in turn, depends mainly on the availability of 5-methyl-tetrahydrofolate, or to some extent on the choline/betaine pathway. This dependence establishes a direct link between nutrient

availability and epigenetic modifications, and hence, regulation of gene expression and phenotypes of health and disease.

DNA methylation

DNA methylation has long been thought of as the main conduit to establish, maintain, and transmit epigenetic information. Methylation of cytosine residues in the CpG dinucleotide sequence forms a covalent, stable alteration of DNA that is generally associated with silencing of gene transcription (Comb and Goodman, 1990; Nan et al., 1997). This methylation mark is established by de novo DNA methyltransferases during development (Goll and Bestor, 2005), remains on the DNA through mitosis, and serves as template for the maintenance DNA methyltransferase DNMT1 to establish the correct cell type-specific pattern of methylation marks on the as of yet unmethylated daughter strand after passage of the replication fork. In this fashion, epigenetic marks can be transmitted through mitosis, and maintained over many cell generations. DNA methylation was long believed to be irreversible and permanent (Razin and Riggs, 1980). However, recent findings support the notion that DNA methylation is in fact reversible (Ito et al., 2010; Ma et al., 2009), and active DNA demethylation may occur through the base-excision repair mechanism (Hajkova et al., 2010). Folate deficiency and the resulting lack of methyl donors for DNA methylation may lead to passive loss of the DNA methylation mark based on a ‘failure to maintain’ mechanism, and thereby to a loss or a change of epigenetic information. This is of particular interest in situations where rapid cell proliferation occurs, requiring high activity from the Dnmt1 maintenance DNA methyltransferase after each mitotic division, with a concomitantly high demand for methyl donor substrates. Examples for such situations are (i) embryonic development, with its rapid cell cycles during growth of the developing embryo; (ii) haematopoiesis; (iii) intestinal cell regeneration; (iv) the swift cell proliferation necessary to mount a successful immune response to e.g. a pathogen challenge; or (v) the proliferation of transformed cells in the progression of cancer. Failure of the maintenance DNA methyltransferase to keep up with demand due to low levels of S-Adenosyl-methionine under methyl-donor deficiency would result in hemi-methylated DNA sites following the first mitotic cycle, and in absence of template information for the next cycle of maintenance DNA methylation, with under- or un-methylated DNA as consequence. In this fashion, epigenetic information in form of DNA methylation patterns can quickly be diluted or lost altogether. It is generally thought that DNA hypomethylation may lead to a loss of gene silencing, and to ectopic gene expression.

Histone methylation

Histone modifications are another realm of the epigenome where methylation reactions take center stage. The N-termini of histones H3 and H4 form so-called histone tails that are amenable to covalent modifications. Methylation of lysine residues is one prominent modification, however, acetylation, phosphorylation, ubiquitination, and ATP-ribosylation occur as well. Collectively, these modifications are referred to as the ‘histone code’ (Jenuwein and Allis, 2001); in adhering to the context of folate and methylation reactions, we will limit our discussion to histone methylation. In contrast to DNA methylation, where presence of the methylation mark is normally associated with transcriptional silencing, the situation for histone methylation is more complex. Depending on the specific nature of the methylation mark, histone methylation can either be associated with active transcription, or with gene silencing; in fact, many proteins with histone modifying activities were originally described as transcriptional co-activators or co-repressors (Lee et al., 2006; Spencer et al., 1997; Xu et al., 1999). In this context, it is not only important which lysine residue on the histone tail carries the mark, but the extent of methylation also plays a role: mono-, di-, and trimethylation of the terminal amino group of the respective lysine are possible. While discussion of each histone methylation mark would go beyond the scope of this review, a

few paradigmatic examples serve to illustrate the complexity. Trimethylation at lysine 4 of histone H3 (H3K4me3) occurs primarily at promoters of actively transcribed genes (Eissenberg and Shilatifard, 2010; Shilatifard, 2008), whereas monomethylation at the same residue is more likely to be found at DNA sequences with transcriptional enhancer function (Heintzman et al., 2009). Trimethylation at lysine 36 of histone H3 is also associated with active transcription (Krogan et al., 2003); this mark is typically found within the transcribed body of a gene, and appears to be enriched on exons. In contrast, trimethylation at lysine 27 of histone H3 is associated with repression of transcription (Peters et al., 2003; Plath et al., 2003); in similar fashion, trimethylation at lysine 9 of histone H3 is concomitant with silencing of transcription (Lachner et al., 2001), and subsequent formation of heterochromatin. Those two trimethylation marks are of particular interest, since acetylation at these specific lysine residues is typically correlated with transcriptional activation. Histone H3 lysine 27 acetylation is a mark for transcriptional enhancer activity (Lachner et al., 2001), and changing the nature of the modification at lysine 27 of histone H3 can in fact serve as a regulatory switch from transcriptional activation to silencing. Therefore, alteration of these histone marks can have profound effects on transcriptional output, and consequently on the phenotype of a given cell. Transcriptional effects of histone modifications are the result of a balance between transcription-activating methylation marks, transcription-silencing methylation marks, and transcription-activating acetylation marks. Histone acetylation status can be affected by the supply of Acetyl-CoA dependent on metabolism and nutrition (Wellen et al., 2009). Likewise, histone methylation status may be influenced by dietary methyl donor supply (Davison et al., 2009; Piyathilake et al., 2010). These histone modifications are therefore a direct target of nutrition, with epigenetic consequences for the organism.

The mechanism for the transmission of histone methylation marks through mitosis is not as well understood as the transmission of DNA methylation information. It is thought to employ an analogous ‘read and write’ style mechanism, since the enzymes that generate the respective histone methylation marks tend to remain associated with the DNA replication fork to regenerate the histone mark after the replication fork has passed and nucleosomes are reassembled (Salbaum and Kappen, 2011). Similar to the passive loss of DNA methylation, histone methylation marks are susceptible to mitotic dilution or complete loss due to a ‘failure to maintain’ under conditions of limited supply of methyl donors. In addition, histone methylation marks are subject to active enzymatic removal by histone demethylases (Agger et al., 2008).

The role of folate for histone methylation, however, extends beyond that of being the methyl carrier towards methylation reactions. A recent finding revealed a new relationship between folate and histone methylation, as folate was found to be an enzymatic cofactor for the histone demethylase LSD1 (official gene symbol KDM1A, lysine (K)-specific demethylase 1A) (Luka et al., 2011). Specifically, tetrahydrofolate can serve as an acceptor for formaldehyde that is generated during the oxidative demethylation of histone tails. Such a reaction would not only serve to trap and convert formaldehyde, a toxic compound, but also recharge tetrahydrofolate with a single-carbon moiety that can then be used for future methylation reactions. Mouse embryos lacking Kdm1a fail to gastrulate, resulting in embryonic lethality at 7.5 days of gestation (Wang et al., 2009). Loss of the LSD1 homologue in C.elegans can lead to transgenerational effects (Katz et al., 2009), underscoring the contribution of histone methylation marks to epigenetic information.

Transcription factors

Regulation of epigenomic activities by methylation is not only restricted to DNA and histone methylation. Recent evidence demonstrates that transcription factors can also be regulated in their activity by direct methylation. Transcription factors are not always

discussed in the context of the epigenome, but rather in the framework of direct transcriptional regulation (Salbaum and Kappen, 2011). However, they also fit the definition of epigenetics that we use here – generation of differential and stable phenotypes without changes in the genomic DNA sequence. Transcription factors establish stable phenotypic differences between cell types (Tapscott, 2005), and transcriptional activation of gene loci can be propagated through mitosis. The context between methylation and transcription factors was originally established by the finding that transcriptional co-activators or co-repressors – proteins that associate with transcription factors into larger complexes and thereby modulate transcriptional output – have histone-modifying activities (Xu et al., 1999), and establish or modify histone acetylation or histone methylation in the immediate vicinity of the genomic binding site for the transcription factor complex. By now, it is also established that transcription factor proteins can be directly methylated (Stark et al., 2011; Yamagata et al., 2008), which affects the transcriptional output driven by these factors. What is not clear at this time, however, is whether this methylation-dependent regulatory mechanism has general applicability, or whether it is restricted to specific transcription factors. It is therefore also difficult to estimate how a methyl donor-deficiency may influence this regulatory mechanism, and how broad the effect on overall transcriptional output may be.

Non-coding RNA

Finally, non-coding RNAs are subjected to methylation reactions as well. RNA methylation has long been described, with tRNA as the prime example. Recent results suggest that methylation of tRNA (Goll et al., 2006) – interestingly by Dnmt2 (originally considered a DNA methyltransferase, but recently renamed TRDMT1 for tRNA aspartic acid methyltransferase 1) - may afford protection from ribonuclease degradation, thereby extending the half-life and functionality of each molecule. Less is known how the epigenetic function of non-coding RNAs, such as microRNAs or chromatin-associated long non-coding RNAs - both associated with reduced transcriptional output (Malecova and Morris, 2010) - may be impacted by methylation reactions. However, generation of microRNAs and small interfering RNAs in plants, production of small interfering RNAs in *D. melanogaster*, and biosynthesis of RNAs of the Piwi-interacting family of small non-coding RNAs (Siomi et al., 2011) in mammalian germ cells includes a methylation reaction at the 3'end (Huang et al., 2009). How these methylation reactions impact the epigenetic function of small RNAs represents an emerging field of biological inquiry. Yet, Piwi-interacting small RNAs also serve to illustrate the intricate relationship between the different molecular modalities of the epigenome in general and the various methylation reactions in particular, as methylated piRNAs direct DNA methylation in the process of transcriptional silencing of retrotransposons in developing mammalian germ cells (Kuramochi-Miyagawa et al., 2008).

Methylation reactions therefore play a fundamental role for the different molecular modalities of the epigenome, and contribute in a significant fashion to the way the epigenome shapes the output of genomic information in the form of gene expression. The majority of methylation reactions in the epigenome tend to be associated with reduced transcriptional output, and even transcriptional silencing of large regions of the genome; however, the correlation between particular histone methylation marks and transcriptionally active promoters or enhancers argues against such a categorical view.

All the respective enzymes that catalyze methylation reactions in the epigenome critically depend on sufficient supply of their methyl donor substrate, typically S-Adenosyl-methionine. Folate feeds into this methyl donor supply, and therefore folate levels are thought to have a profound effect on the efficacy of methylation reactions. Yet, it is important to note that while the Folate pathway is the major source for replenishing S-

Adenosyl-methionine levels in the cell for future methylation reactions, it is not the sole source. The Choline/Betaine route of methyl donor supply also feeds into the cellular pool of S-Adenosyl-methionine (Zeisel, 2011), and may interact with folate deficiency (Kim et al., 1994; Maloney et al., 2007). When considering epigenomic effects with respect to nutritional folate deficiency, or genetic deficits affecting enzymes of the folate cycle, such effects must be interpreted in the context of the entire methyl donor stream, and not isolated pathways.

Epigenomic Footprint of Folate

Historically, the biological relevance of folate for epigenetic effects has been derived from the view that folate is critical for the supply of methyl groups in the cell, and epigenetic effects are mediated principally by DNA methylation. Therefore, the parsimonious assumption was that folate levels would essentially constitute the rate-limiting factor for DNA methylation by dictating the status of the methyl donor pool. Based on such a feed-forward model, a direct and positive correlation between folate levels and DNA methylation status could be postulated: folate deficiency results in reduced DNA methylation; conversely, high folate status leads to increased DNA methylation, and hence, folate status determines epigenetic events. Since folate status is dependent on nutrition, this would comprise a direct conduit between nutrition, epigenetics, and the resulting phenotypes. In addition, the prevailing view for a long time was that DNA methylation was essentially a stable modification, and therefore epigenetic events, once established, were nearly permanent or at least very long-lasting biological phenomena. Such epigenetic events could only be changed due to insufficient means to maintain DNA methylation patterns, such as in the case of folate deficiency. As consequence, the loss of DNA methylation was thought to lead to increased levels of gene expression, which in the case of oncogenes may contribute to the development of cancer. However, this picture has dramatically changed in recent years.

(i) Epigenetics and plasticity

The first aspect of this change is the recognition that epigenetic events are not permanent, but epigenetic marks are actually subject to plasticity beyond the passive ‘failure-to-maintain’ mechanisms through mitotic dilution. Active DNA demethylation can occur via the base excision repair pathway (Hajkova et al., 2010), and histone demethylation may ensue though specific enzymes (Agger et al., 2008). Less is known about non-coding RNA or transcription factor methylations, but cellular turnover may erase these marks. Very strong corroboration for epigenomic plasticity has arisen from nutritional studies. Mice exposed to methyl-donor diets show diet-dependent DNA methylation patterns at specific gene loci, together with differences in gene expression (Waterland et al., 2006; Waterland and Jirtle, 2003; Wolff et al., 1998). Choline in the diet can affect DNA methylation, histone methylation, as well as expression of histone methyltransferases such as G9a (Davison et al., 2009). High levels of methyl donors in the diet affect both DNA and histone methyltransferases, with concomitantly higher levels of DNA and histone methylation (Waterland, 2006). Dietary methyl deficiencies can alter histone methylation as well (Dobosy et al., 2008). Besides nutritional studies, it is thought that the capacity for epigenomic plasticity is the basis for induction of pluripotency via a specific set of transcription factors (Takahashi and Yamanaka, 2006). These examples demonstrate that epigenetic structures can be actively changed, and not just by degrading the epigenetic marks through deficient maintenance mechanisms.

(ii) Relationship between epigenetic marks and transcription

The second aspect of change is in regard to the nature and diversity of epigenetic modifications. As discussed previously, epigenetics is no longer viewed as being synonymous with DNA methylation, but the concept of the epigenome has expanded to several molecular dimensions. While methylation reactions play a central role in setting the various epigenetic marks in each of the realms, the categorical idea that methylation status is negatively correlated with transcription does not extend through all epigenomic modalities: in the world of histones, methylation status can be associated with active transcription *or* with silencing of gene expression, depending on the specific methylation mark (see above). While there may be a positive correlation between methyl donor supply and methylation levels, it means that subsequent conclusions about the transcriptional status – and thereby about potential phenotype - of a cell are no longer straightforward.

(iii) Folate status and DNA methylation

To complicate matters further, experimental evidence on the relationship between folate status and DNA methylation suggested higher levels of complexity than would be predicted on the basis of a simple feed-forward model. The consequences of folate deficiency can range from global DNA hypomethylation (Balaghi and Wagner, 1993), to global DNA hypomethylation coupled with DNA hypermethylation at specific genes or promoters (Jhaveri et al., 2001; Pogribny and James, 2002), to global DNA hypermethylation (Sohn et al., 2003; Song et al., 2000). Hypermethylation in particular is in stark contrast to predictions for folate deficiency if one follows the simple supply model; one potential explanation for this conundrum is the induction of DNA methyltransferases in response to low methyl-donor levels (Ghoshal et al., 2006). This may reveal why there are increased levels of methylation; it does not explain why promoters or CpG islands, typically regions of low or no DNA methylation, are no longer protected, and are subjected to de novo methylation. The picture is further complicated by findings that folate effects can be tissue-specific (Linhart et al., 2009), and that not just the extent, but also the duration of folate deficiency can have consequences on DNA methylation (Kim, 2005); evidence has also been obtained that DNA methylation may in fact not change at all in response to folate status (Crott et al., 2008; Duthie et al., 2000; Kim et al., 1995; Protiva et al., 2011) despite folate-responsive changes in gene expression. It appears that a linear feed-forward model from folate level, to methyl donor status, to DNA methylation, and finally to gene expression does not suffice to explain the various experimental observations, and that more complex regulatory relationships must be considered.

(iv) Folate and the regulation of transcription

In contrast to the historical view that folate affects gene expression solely via DNA methylation, it would appear that changes in gene transcription may in fact precede changes at the level of DNA methylation. A recent study on folate supplementation, deficiency, and repletion in human subjects at risk for colon cancer reported significant effects of folate on the regulation of gene expression, but neither genomic nor promoter methylation were affected under conditions of high or low folate (Protiva et al., 2011). Interestingly, the experiments provided support for the notion that high folate status may constitute a risk factor for colorectal carcinogenesis by affecting proinflammatory pathways. It should be noted that the altered transcriptome can exert immediate effect on cellular phenotype and function.

We suggest that in this scenario, it is conceivable that changes in DNA methylation, rather than being the primary means of translating folate effects, are the final outcome of a multilayered regulatory circuitry that may engage other molecular realms of the epigenome, such as e.g. histone methylation (Piyathilake et al., 2010) or microRNAs (Davis and Ross,

2008; Shookhoff and Gallicano, 2010) in order to manifest the effects of folate. Evidence for this concept has emerged recently in a report on the effects of folate on histone methylation at the Hes1 and Neurog2 gene loci (Ichi et al., 2010). In mouse embryos with Pax3 deficiency, histone H3 K27 dimethylation, a repressive epigenomic mark, is increased at the promoters for both genes, whereas treatment with Folate reduced the respective methylation marks to normal levels. This result demonstrated that Folate is capable of modulating epigenetic chromatin marks. However, the fact that an *increase* in folate lead to a *decrease* of the methylation status underscores the notion that the feed-forward model is insufficient to explain such a change, and that the regulatory mechanism underlying this methylation change are more complex. It has been proposed that in this scenario, folate acts through microRNAs to increase expression of the lysine demethylase KDM6B, which then mediates the reduction of histone H3K27 dimethylation levels. As more such studies are conducted, one would expect a clearer picture how folate affects epigenome and transcriptome, and ultimately cellular phenotypes.

We therefore suggest that the relationship between folate and the epigenome is far more complex that previously anticipated, yet, it has significant ramifications for biology of health and disease. It I therefore necessary to explore that relationship in more detail, taking advantage of genome-wide tools that have become available in recent years.

A Roadmap for Folate and the Epigenome

One particular caveat to interpretations of the relationship between folate and the epigenome is that many results were obtained before the advent of genome-wide technologies. Such studies initially measured global DNA methylation levels (Kim et al., 1995; Piyathilake et al., 2000) – e.g. as overall content of 5-methyl-cytosine, but without positional information in the genome - or resorted to candidate gene studies of gene- or promoter-specific DNA methylation (Pogribny et al., 1995). Technological limitations dictated this approach, as methods that would have allowed gene-specific attributions in a whole-genome context were not available. While candidate gene approaches were successful in unraveling the role of folate and DNA methylation in specific paradigms, the lack of a whole-genome context made generalizable biological interpretations difficult. One such example is the methyl donordependent transcription change at the Av^{Y} allele: methylation patterns change at an IAP element (intracisternal A-particle) near the gene. As a remnant of a viral sequence with a long terminal repeat (LTR) element that can activate transcription, it can override the normal regulatory machinery at the gene (Waterland and Jirtle, 2003). Similarities have been detected at the $Axin^{Fu}$ allele (Waterland et al., 2006). With approximately 8000 IAP elements with intact LTRs in the mouse genome (Qin et al., 2010), IAP methylation-based mechanisms may affect many more genes, and therefore may account for a significant fraction of methyl donor-conferred phenotypic changes. Although the studies on Av^{Y} and Ax^{Fu} were paradigmatic for this mechanism, the general conclusion has been questioned recently (Cropley et al., 2010), and it will require a whole-genome context in order to determine the entire epigenomic footprint of methyl-donor diets on IAPs so that a correlation to the respective phenotypes can be made. Furthermore, IAPs are thought to be a feature of rodent genomes, and mechanistic parallels to the human genome need to be established. We therefore propose that it is necessary to make the whole-genome context a primary focus for new investigations of molecular consequences of folate status.

Genome-wide technologies to determine changes in gene expression patterns in response to folate status are well established. Microarray hybridization has been used extensively to characterize transcriptomic responses in various tissues and cell types (Garcia-Crespo et al., 2009; Gelineau-van Waes et al., 2008; Jhaveri et al., 2001; Katula et al., 2007); folate-responsive gene expression incorporates common themes such as proliferation,

inflammation and apoptosis in folate-responsive transcriptomic changes. The focus of such experiments was primarily on known protein-coding genes. Genome-wide technologies to assess the epigenomics footprints of folate – mostly based on next-generation sequencing - have become available, not just for DNA methylation (Bock et al., 2010; Bormann Chung et al., 2010), but also for histone methylation (Barski et al., 2007; Barski and Zhao, 2009; Mikkelsen et al., 2007), as well as other epigenomics modalities. In addition, new sequencing-based methods (Mortazavi et al., 2008) now make it possible to overcome the prior restriction of expression analyses to known protein-coding genes, and permit genome-wide assessments of the entire transcriptome, including antisense and non-coding RNAs (Kuchen et al., 2010), in relation to folate status.

To get a better general understanding of the effects of folate - on epigenome, transcriptome, and ultimately cellular phenotypes - it will be necessary to conduct epigenomic studies in conjunction with transcriptomic assessments, and apply several of these new genome-wide technologies within the same paradigm. Only the combination of such approaches will allow the concomitant definition of folate-sensitive target genes and reveal how the epigenome landscape of those target genes is affected by folate status, and how the epigenome through such targets manifests phenotype. While this appears as an ambitious goal that will require substantial efforts and resources, we believe that only such integrative studies will reveal the true epigenomic footprint of folate, and establish the biological context that is necessary to interpret folate effects upstream of gene expression with reference to DNA methylation, histone modifications non-coding RNAs, and transcription factors. Furthermore, such analyses will permit ‘downstream’ correlations of this epigenomic footprint to functional outcomes beyond transcription - at various ‘–omics’ levels, all the way to assessments for health and disease. We suggest that such an experimental approach will be highly productive, bear many surprises, and lead to novel insights into the physiology and pathophysiology of folate.

REFERENCES

Agger K, Christensen J, Cloos PA, Helin K. The emerging functions of histone demethylases. Curr Opin Genet Dev. 2008; 18:159–168. [PubMed: 18281209]

Atabay B, Turker M, Ozer EA, Mahadeo K, Diop-Bove N, Goldman ID. Mutation of the proton-coupled folate transporter gene (PCFT-SLC46A1) in Turkish siblings with hereditary folate malabsorption. Pediatr Hematol Oncol. 2010; 27:614–619. [PubMed: 20795774]

Austin RC, Lentz SR, Werstuck GH. Role of hyperhomocysteinemia in endothelial dysfunction and atherothrombotic disease. Cell Death Differ. 2004; 11(Suppl 1):S56–s64. [PubMed: 15243582]

Balaghi M, Wagner C. DNA methylation in folate deficiency: use of CpG methylase. Biochem Biophys Res Commun. 1993; 193:1184–1190. [PubMed: 8323540]

Banka S, Blom HJ, Walter J, Aziz M, Urquhart J, Clouthier CM, Rice GI, de Brouwer AP, Hilton E, Vassallo G, et al. Identification and characterization of an inborn error of metabolism caused by dihydrofolate reductase deficiency. Am J Hum Genet. 2011; 88:216–225. [PubMed: 21310276]

Barber R, Shalat S, Hendricks K, Joggerst B, Larsen R, Suarez L, Finnell RH. Investigation of folate pathway gene polymorphisms and the incidence of neural tube defects in a Texas hispanic population. Mol Genet Metab. 2000; 70:45–52. [PubMed: 10833330]

Barber RC, Shaw GM, Lammer EJ, Greer KA, Biela TA, Lacey SW, Wasserman CR, Finnell RH. Lack of association between mutations in the folate receptor-alpha gene and spina bifida. Am J Med Genet. 1998; 76:310–317. [PubMed: 9545095]

Barski A, Cuddapah S, Cui K, Roh TY, Schones DE, Wang Z, Wei G, Chepelev I, Zhao K. High-resolution profiling of histone methylations in the human genome. Cell. 2007; 129:823–837. [PubMed: 17512414]

Barski A, Zhao K. Genomic location analysis by ChIP-Seq. J Cell Biochem. 2009; 107:11–18. [PubMed: 19173299]

Beaudin AE, Abarinov EV, Noden DM, Perry CA, Chu S, Stabler SP, Allen RH, Stover PJ. Shmt1 and de novo thymidylate biosynthesis underlie folate-responsive neural tube defects in mice. Am J Clin Nutr. 2011; 93:789–798. [PubMed: 21346092]

Bi XH, Zhao HL, Zhang ZX, Zhang JW. Association of RFC1 A80G and MTHFR C677T polymorphisms with Alzheimer's disease. Neurobiol Aging. 2009; 30:1601–1607. [PubMed: 18258338]

Boccia S, Hung R, Ricciardi G, Gianfagna F, Ebert MP, Fang JY, Gao CM, Gotze T, Graziano F, Lacasana-Navarro M, et al. Meta- and pooled analyses of the methylenetetrahydrofolate reductase C677T and A1298C polymorphisms and gastric cancer risk: a huge-GSEC review. Am J Epidemiol. 2008; 167:505–516. [PubMed: 18162478]

Bock C, Tomazou EM, Brinkman AB, Muller F, Simmer F, Gu H, Jager N, Gnirke A, Stunnenberg HG, Meissner A. Quantitative comparison of genome-wide DNA methylation mapping technologies. Nat Biotechnol. 2010; 28:1106–1114. [PubMed: 20852634]

Bormann Chung CA, Boyd VL, McKernan KJ, Fu Y, Monighetti C, Peckham HE, Barker M. Whole methylome analysis by ultra-deep sequencing using two-base encoding. PLoS One. 2010; 5:e9320. [PubMed: 20179767]

Borzutzky A, Crompton B, Bergmann AK, Giliani S, Baxi S, Martin M, Neufeld EJ, Notarangelo LD. Reversible severe combined immunodeficiency phenotype secondary to a mutation of the proton-coupled folate transporter. Clin Immunol. 2009; 133:287–294. [PubMed: 19740703]

Boyles AL, Billups AV, Deak KL, Siegel DG, Mehltretter L, Slifer SH, Bassuk AG, Kessler JA, Reed MC, Nijhout HF, et al. Neural tube defects and folate pathway genes: family-based association tests of gene-gene and gene-environment interactions. Environ Health Perspect. 2006; 114:1547–1552. [PubMed: 17035141]

Cario H, Smith DE, Blom H, Blau N, Bode H, Holzmann K, Pannicke U, Hopfner KP, Rump EM, Ayric Z, et al. Dihydrofolate reductase deficiency due to a homozygous DHFR mutation causes megaloblastic anemia and cerebral folate deficiency leading to severe neurologic disease. Am J Hum Genet. 2011; 88:226–231. [PubMed: 21310277]

Chen Z, Karaplis AC, Ackerman SL, Pogribny IP, Melnyk S, Lussier-Cacan S, Chen MF, Pai A, John SW, Smith RS, et al. Mice deficient in methylenetetrahydrofolate reductase exhibit hyperhomocysteinemia and decreased methylation capacity, with neuropathology and aortic lipid deposition. Hum Mol Genet. 2001; 10:433–443. [PubMed: 11181567]

Collin SM, Metcalfe C, Zuccolo L, Lewis SJ, Chen L, Cox A, Davis M, Lane JA, Donovan J, Smith GD, et al. Association of folate-pathway gene polymorphisms with the risk of prostate cancer: a population-based nested case-control study, systematic review, and meta-analysis. Cancer Epidemiol Biomarkers Prev. 2009; 18:2528–2539. [PubMed: 19706844]

Comb M, Goodman HM. CpG methylation inhibits proenkephalin gene expression and binding of the transcription factor AP-2. Nucleic Acids Res. 1990; 18:3975–3982. [PubMed: 1695733]

Cropley JE, Suter CM, Beckman KB, Martin DI. CpG methylation of a silent controlling element in the murine Avy allele is incomplete and unresponsive to methyl donor supplementation. PLoS One. 2010; 5:e9055. [PubMed: 20140227]

Crott JW, Liu Z, Keyes MK, Choi SW, Jang H, Moyer MP, Mason JB. Moderate folate depletion modulates the expression of selected genes involved in cell cycle, intracellular signaling and folate uptake in human colonic epithelial cell lines. J Nutr Biochem. 2008; 19:328–335. [PubMed: 17681772]

Davis CD, Ross SA. Evidence for dietary regulation of microRNA expression in cancer cells. Nutr Rev. 2008; 66:477–482. [PubMed: 18667010]

Davison JM, Mellott TJ, Kovacheva VP, Blusztajn JK. Gestational choline supply regulates methylation of histone H3, expression of histone methyltransferases G9a (Kmt1c) and Suv39h1 (Kmt1a), and DNA methylation of their genes in rat fetal liver and brain. J Biol Chem. 2009; 284:1982–1989. [PubMed: 19001366]

Di Pietro E, Sirois J, Tremblay ML, MacKenzie RE. Mitochondrial NAD-dependent methylenetetrahydrofolate dehydrogenase-methenyltetrahydrofolate cyclohydrolase is essential for embryonic development. Mol Cell Biol. 2002; 22:4158–4166. [PubMed: 12024029]

Dixon KH, Lanpher BC, Chiu J, Kelley K, Cowan KH. A novel cDNA restores reduced folate carrier activity and methotrexate sensitivity to transport deficient cells. J Biol Chem. 1994; 269:17–20. [PubMed: 8276792]

Dobosy JR, Fu VX, Desotelle JA, Srinivasan R, Kenowski ML, Almassi N, Weindruch R, Svaren J, Jarrard DF. A methyl-deficient diet modifies histone methylation and alters Igf2 and H19 repression in the prostate. Prostate. 2008; 68:1187–1195. [PubMed: 18459101]

Duthie SJ, Narayanan S, Brand GM, Grant G. DNA stability and genomic methylation status in colonocytes isolated from methyl-donor-deficient rats. Eur J Nutr. 2000; 39:106–111. [PubMed: 10918992]

Eissenberg JC, Shilatifard A. Histone H3 lysine 4 (H3K4) methylation in development and differentiation. Dev Biol. 2010; 339:240–249. [PubMed: 19703438]

Elmore CL, Wu X, Leclerc D, Watson ED, Bottiglieri T, Krupenko NI, Krupenko SA, Cross JC, Rozen R, Gravel RA, et al. Metabolic derangement of methionine and folate metabolism in mice deficient in methionine synthase reductase. Molecular genetics and metabolism. 2007; 91:85–97. [PubMed: 17369066]

Elwood PC, Nachmanoff K, Saikawa Y, Page ST, Pacheco P, Roberts S, Chung KN. The divergent 5' termini of the alpha human folate receptor (hFR) mRNAs originate from two tissue-specific promoters and alternative splicing: characterization of the alpha hFR gene structure. Biochemistry. 1997; 36:1467–1478. [PubMed: 9063895]

Fenech M. The role of folic acid and Vitamin B12 in genomic stability of human cells. Mutat Res. 2001; 475:57–67. [PubMed: 11295154]

Finnell RH, Spiegelstein O, Wlodarczyk B, Triplett A, Pogribny IP, Melnyk S, James JS. DNA methylation in Folbp1 knockout mice supplemented with folic acid during gestation. J Nutr. 2002; 132:2457S–2461S. [PubMed: 12163711]

Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJ, den Heijer M, Kluijtmans LA, van den Heuvel LP, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nature genetics. 1995; 10:111–113. [PubMed: 7647779]

Garcia-Crespo D, Knock E, Jabado N, Rozen R. Intestinal neoplasia induced by low dietary folate is associated with altered tumor expression profiles and decreased apoptosis in mouse normal intestine. J Nutr. 2009; 139:488–494. [PubMed: 19176749]

Gast A, Bermejo JL, Flohr T, Stanulla M, Burwinkel B, Schrappe M, Bartram CR, Hemminki K, Kumar R. Folate metabolic gene polymorphisms and childhood acute lymphoblastic leukemia: a case-control study. Leukemia. 2007; 21:320–325. [PubMed: 17136115]

Gelineau-van Waes J, Maddox JR, Smith LM, van Waes M, Wilberding J, Eudy JD, Bauer LK, Finnell RH. Microarray analysis of E9.5 reduced folate carrier (RFC1; Slc19a1) knockout embryos reveals altered expression of genes in the cubilin-megalin multiligand endocytic receptor complex. BMC Genomics. 2008; 9:156. [PubMed: 18400109]

Ghoshal K, Li X, Datta J, Bai S, Pogribny I, Pogribny M, Huang Y, Young D, Jacob ST. A folate- and methyl-deficient diet alters the expression of DNA methyltransferases and methyl CpG binding proteins involved in epigenetic gene silencing in livers of F344 rats. J Nutr. 2006; 136:1522–1527. [PubMed: 16702315]

Goin-Kochel RP, Porter AE, Peters SU, Shinawi M, Sahoo T, Beaudet AL. The MTHFR 677C-->T polymorphism and behaviors in children with autism: exploratory genotype-phenotype correlations. Autism Res. 2009; 2:98–108. [PubMed: 19455642]

Goldman ID, Chattopadhyay S, Zhao R, Moran R. The antifolates: evolution, new agents in the clinic, and how targeting delivery via specific membrane transporters is driving the development of a next generation of folate analogs. Curr Opin Investig Drugs. 2010; 11:1409–1423.

Goll MG, Bestor TH. Eukaryotic cytosine methyltransferases. Annu Rev Biochem. 2005; 74:481–514. [PubMed: 15952895]

Goll MG, Kirpekar F, Maggert KA, Yoder JA, Hsieh CL, Zhang X, Golic KG, Jacobsen SE, Bestor TH. Methylation of tRNAAsp by the DNA methyltransferase homolog Dnmt2. Science. 2006; 311:395–398. [PubMed: 16424344]

Hajkova P, Jeffries SJ, Lee C, Miller N, Jackson SP, Surani MA. Genome-wide reprogramming in the mouse germ line entails the base excision repair pathway. Science. 2010; 329:78–82. [PubMed: 20595612]

Heintzman ND, Hon GC, Hawkins RD, Kheradpour P, Stark A, Harp LF, Ye Z, Lee LK, Stuart RK, Ching CW, et al. Histone modifications at human enhancers reflect global cell-type-specific gene expression. Nature. 2009; 459:108–112. [PubMed: 19295514]

Hooijberg JH, Broxterman HJ, Kool M, Assaraf YG, Peters GJ, Noordhuis P, Scheper RJ, Borst P, Pinedo HM, Jansen G. Antifolate resistance mediated by the multidrug resistance proteins MRP1 and MRP2. Cancer Res. 1999; 59:2532–2535. [PubMed: 10363967]

Hooijberg JH, Jansen G, Assaraf YG, Kathmann I, Pieters R, Laan AC, Veerman AJ, Kaspers GJ, Peters GJ. Folate concentration dependent transport activity of the Multidrug Resistance Protein 1 (ABCC1). Biochem Pharmacol. 2004; 67:1541–1548. [PubMed: 15041471]

Huang Y, Ji L, Huang Q, Vassylyev DG, Chen X, Ma JB. Structural insights into mechanisms of the small RNA methyltransferase HEN1. Nature. 2009; 461:823–827. [PubMed: 19812675]

Ichi S, Costa FF, Bischof JM, Nakazaki H, Shen YW, Boshnjaku V, Sharma S, Mania-Farnell B, McLone DG, Tomita T, et al. Folic acid remodels chromatin on Hes1 and Neurog2 promoters during caudal neural tube development. J Biol Chem. 2010; 285:36922–36932. [PubMed: 20833714]

Ito S, D'Alessio AC, Taranova OV, Hong K, Sowers LC, Zhang Y. Role of Tet proteins in 5mC to 5hmC conversion, ES-cell self-renewal and inner cell mass specification. Nature. 2010; 466:1129–1133. [PubMed: 20639862]

Jakubowski H, Perla-Kajan J, Finnell RH, Cabrera RM, Wang H, Gupta S, Kruger WD, Kraus JP, Shih DM. Genetic or nutritional disorders in homocysteine or folate metabolism increase protein N-homocysteinylation in mice. The FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2009; 23:1721–1727.

Jenuwein T, Allis CD. Translating the histone code. Science. 2001; 293:1074–1080. [PubMed: 11498575]

Jhaveri MS, Wagner C, Trepel JB. Impact of extracellular folate levels on global gene expression. Mol Pharmacol. 2001; 60:1288–1295. [PubMed: 11723236]

Kamen BA, Smith AK. A review of folate receptor alpha cycling and 5-methyltetrahydrofolate accumulation with an emphasis on cell models in vitro. Adv Drug Deliv Rev. 2004; 56:1085–1097. [PubMed: 15094208]

Kappen C, Mello MA, Finnell RH, Salbaum JM. Folate modulates Hox gene-controlled skeletal phenotypes. Genesis. 2004; 39:155–166. [PubMed: 15282741]

Katula KS, Heinloth AN, Paules RS. Folate deficiency in normal human fibroblasts leads to altered expression of genes primarily linked to cell signaling, the cytoskeleton and extracellular matrix. J Nutr Biochem. 2007; 18:541–552. [PubMed: 17320366]

Katz DJ, Edwards TM, Reinke V, Kelly WG. A C. elegans LSD1 demethylase contributes to germline immortality by reprogramming epigenetic memory. Cell. 2009; 137:308–320. [PubMed: 19379696]

Kim YI. Role of folate in colon cancer development and progression. J Nutr. 2003; 133:3731S–3739S. [PubMed: 14608107]

Kim YI. Nutritional epigenetics: impact of folate deficiency on DNA methylation and colon cancer susceptibility. J Nutr. 2005; 135:2703–2709. [PubMed: 16251634]

Kim YI, Christman JK, Fleet JC, Cravo ML, Salomon RN, Smith D, Ordovas J, Selhub J, Mason JB. Moderate folate deficiency does not cause global hypomethylation of hepatic and colonic DNA or c-myc-specific hypomethylation of colonic DNA in rats. Am J Clin Nutr. 1995; 61:1083–1090. [PubMed: 7733033]

Kim YI, Miller JW, da Costa KA, Nadeau M, Smith D, Selhub J, Zeisel SH, Mason JB. Severe folate deficiency causes secondary depletion of choline and phosphocholine in rat liver. J Nutr. 1994; 124:2197–2203. [PubMed: 7965204]

Kitamura Y, Hirouchi M, Kusuhara H, Schuetz JD, Sugiyama Y. Increasing systemic exposure of methotrexate by active efflux mediated by multidrug resistance-associated protein 3 (mrp3/abcc3). J Pharmacol Exp Ther. 2008; 327:465–473. [PubMed: 18719291]

Klerk M, Verhoef P, Clarke R, Blom HJ, Kok FJ, Schouten EG. MTHFR 677C-->T polymorphism and risk of coronary heart disease: a meta-analysis. JAMA. 2002; 288:2023–2031. [PubMed: 12387655]

Krogan NJ, Kim M, Tong A, Golshani A, Cagney G, Canadien V, Richards DP, Beattie BK, Emili A, Boone C, et al. Methylation of histone H3 by Set2 in Saccharomyces cerevisiae is linked to transcriptional elongation by RNA polymerase II. Mol Cell Biol. 2003; 23:4207–4218. [PubMed: 12773564]

Kuchen S, Resch W, Yamane A, Kuo N, Li Z, Chakraborty T, Wei L, Laurence A, Yasuda T, Peng S, et al. Regulation of microRNA expression and abundance during lymphopoiesis. Immunity. 2010; 32:828–839. [PubMed: 20605486]

Kuramochi-Miyagawa S, Watanabe T, Gotoh K, Totoki Y, Toyoda A, Ikawa M, Asada N, Kojima K, Yamaguchi Y, Ijiri TW, et al. DNA methylation of retrotransposon genes is regulated by Piwi family members MILI and MIWI2 in murine fetal testes. Genes Dev. 2008; 22:908–917. [PubMed: 18381894]

Lachner M, O'Carroll D, Rea S, Mechtler K, Jenuwein T. Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. Nature. 2001; 410:116–120. [PubMed: 11242053]

Laverdiere C, Chiasson S, Costea I, Moghrabi A, Krajinovic M. Polymorphism G80A in the reduced folate carrier gene and its relationship to methotrexate plasma levels and outcome of childhood acute lymphoblastic leukemia. Blood. 2002; 100:3832–3834. [PubMed: 12411325]

Le Marchand L, Wilkens LR, Kolonel LN, Henderson BE. The MTHFR C677T polymorphism and colorectal cancer: the multiethnic cohort study. Cancer Epidemiol Biomarkers Prev. 2005; 14:1198–1203. [PubMed: 15894672]

Lee DY, Northrop JP, Kuo MH, Stallcup MR. Histone H3 lysine 9 methyltransferase G9a is a transcriptional coactivator for nuclear receptors. J Biol Chem. 2006; 281:8476–8485. [PubMed: 16461774]

Lewis SJ, Ebrahim S, Davey Smith G. Meta-analysis of MTHFR 677C->T polymorphism and coronary heart disease: does totality of evidence support causal role for homocysteine and preventive potential of folate? BMJ. 2005; 331:1053. [PubMed: 16216822]

Linhart HG, Troen A, Bell GW, Cantu E, Chao WH, Moran E, Steine E, He T, Jaenisch R. Folate deficiency induces genomic uracil misincorporation and hypomethylation but does not increase DNA point mutations. Gastroenterology. 2009; 136:227–235. e223. [PubMed: 18992744]

Liu A, Menon S, Colson NJ, Quinlan S, Cox H, Peterson M, Tiang T, Haupt LM, Lea RA, Griffiths LR. Analysis of the MTHFR C677T variant with migraine phenotypes. BMC Res Notes. 2010; 3:213. [PubMed: 20663228]

Low PS, Kularatne SA. Folate-targeted therapeutic and imaging agents for cancer. Curr Opin Chem Biol. 2009; 13:256–262. [PubMed: 19419901]

Luka Z, Moss F, Loukachevitch LV, Bornhop DJ, Wagner C. Histone Demethylase LSD1 Is a Folate-Binding Protein. Biochemistry. 2011; 50:4750–4756. [PubMed: 21510664]

Ma DK, Jang MH, Guo JU, Kitabatake Y, Chang ML, Pow-Anpongkul N, Flavell RA, Lu B, Ming GL, Song H. Neuronal activity-induced Gadd45b promotes epigenetic DNA demethylation and adult neurogenesis. Science. 2009; 323:1074–1077. [PubMed: 19119186]

Ma DW, Finnell RH, Davidson LA, Callaway ES, Spiegelstein O, Piedrahita JA, Salbaum JM, Kappen C, Weeks BR, James J, et al. Folate transport gene inactivation in mice increases sensitivity to colon carcinogenesis. Cancer Res. 2005; 65:887–897. [PubMed: 15705887]

MacFarlane AJ, Liu X, Perry CA, Flodby P, Allen RH, Stabler SP, Stover PJ. Cytoplasmic serine hydroxymethyltransferase regulates the metabolic partitioning of methylenetetrahydrofolate but is not essential in mice. J Biol Chem. 2008; 283:25846–25853. [PubMed: 18644786]

MacFarlane AJ, Perry CA, Girnary HH, Gao D, Allen RH, Stabler SP, Shane B, Stover PJ. Mthfd1 is an essential gene in mice and alters biomarkers of impaired one-carbon metabolism. J Biol Chem. 2009; 284:1533–1539. [PubMed: 19033438]

Maddox DM, Manlapat A, Roon P, Prasad P, Ganapathy V, Smith SB. Reduced-folate carrier (RFC) is expressed in placenta and yolk sac, as well as in cells of the developing forebrain, hindbrain, neural tube, craniofacial region, eye, limb buds and heart. BMC Dev Biol. 2003; 3:6. [PubMed: 12887734]

Malecova B, Morris KV. Transcriptional gene silencing through epigenetic changes mediated by non-coding RNAs. Curr Opin Mol Ther. 2010; 12:214–222. [PubMed: 20373265]

Maloney CA, Hay SM, Rees WD. Folate deficiency during pregnancy impacts on methyl metabolism without affecting global DNA methylation in the rat fetus. The British journal of nutrition. 2007; 97:1090–1098. [PubMed: 17433124]

Mao R, Fan Y, Jin Y, Bai J, Fu S. Methylenetetrahydrofolate reductase gene polymorphisms and lung cancer: a meta-analysis. J Hum Genet. 2008; 53:340–348. [PubMed: 18340404]

Mikkelsen TS, Ku M, Jaffe DB, Issac B, Lieberman E, Giannoukos G, Alvarez P, Brockman W, Kim TK, Koche RP, et al. Genome-wide maps of chromatin state in pluripotent and lineage-committed cells. Nature. 2007; 448:553–560. [PubMed: 17603471]

Mishra PJ, Humeniuk R, Longo-Sorbello GS, Banerjee D, Bertino JR. A miR-24 microRNA binding-site polymorphism in dihydrofolate reductase gene leads to methotrexate resistance. Proc Natl Acad Sci U S A. 2007; 104:13513–13518. [PubMed: 17686970]

Mortazavi A, Williams BA, McCue K, Schaeffer L, Wold B. Mapping and quantifying mammalian transcriptomes by RNA-Seq. Nat Methods. 2008; 5:621–628. [PubMed: 18516045]

Munoz JB, Lacasana M, Cavazos RG, Borja-Aburto VH, Galaviz-Hernandez C, Garduno CA. Methylenetetrahydrofolate reductase gene polymorphisms and the risk of anencephaly in Mexico. Mol Hum Reprod. 2007; 13:419–424. [PubMed: 17439956]

Nan X, Campoy FJ, Bird A. MeCP2 is a transcriptional repressor with abundant binding sites in genomic chromatin. Cell. 1997; 88:471–481. [PubMed: 9038338]

Nurk E, Tell GS, Refsum H, Ueland PM, Vollset SE. Associations between maternal methylenetetrahydrofolate reductase polymorphisms and adverse outcomes of pregnancy: the Hordaland Homocysteine Study. Am J Med. 2004; 117:26–31. [PubMed: 15210385]

Obican SG, Finnell RH, Mills JL, Shaw GM, Scialli AR. Folic acid in early pregnancy: a public health success story. FASEB J. 2010; 24:4167–4174. [PubMed: 20631328]

Parle-McDermott A, Kirke PN, Mills JL, Molloy AM, Cox C, O'Leary VB, Pangilinan F, Conley M, Cleary L, Brody LC, et al. Confirmation of the R653Q polymorphism of the trifunctional C1-synthase enzyme as a maternal risk for neural tube defects in the Irish population. Eur J Hum Genet. 2006; 14:768–772. [PubMed: 16552426]

Parle-McDermott A, Mills JL, Kirke PN, Cox C, Signore CC, Kirke S, Molloy AM, O'Leary VB, Pangilinan FJ, O'Herlihy C, et al. MTHFD1 R653Q polymorphism is a maternal genetic risk factor for severe abruptio placentae. Am J Med Genet A. 2005; 132:365–368. [PubMed: 15633187]

Pasca SP, Dronca E, Kaucsar T, Craciun EC, Endreffy E, Ferencz BK, Iftene F, Benga I, Cornean R, Banerjee R, et al. One carbon metabolism disturbances and the C677T MTHFR gene polymorphism in children with autism spectrum disorders. J Cell Mol Med. 2009; 13:4229–4238. [PubMed: 19267885]

Peters AH, Kubicek S, Mechtler K, O'Sullivan RJ, Derijck AA, Perez-Burgos L, Kohlmaier A, Opravil S, Tachibana M, Shinkai Y, et al. Partitioning and plasticity of repressive histone methylation states in mammalian chromatin. Mol Cell. 2003; 12:1577–1589. [PubMed: 14690609]

Pickell L, Brown K, Li D, Wang XL, Deng L, Wu Q, Selhub J, Luo L, Jerome-Majewska L, Rozen R. High intake of folic acid disrupts embryonic development in mice. Birth Defects Res A Clin Mol Teratol. 2011; 91:8–19. [PubMed: 21254354]

Piedrahita JA, Oetama B, Bennett GD, van Waes J, Kamen BA, Richardson J, Lacey SW, Anderson RG, Finnell RH. Mice lacking the folic acid-binding protein Folbp1 are defective in early embryonic development. Nat Genet. 1999; 23:228–232. [PubMed: 10508523]

Piyathilake CJ, Johanning GL, Macaluso M, Whiteside M, Oelschlager DK, Heimburger DC, Grizzle WE. Localized folate and vitamin B-12 deficiency in squamous cell lung cancer is associated with global DNA hypomethylation. Nutr Cancer. 2000; 37:99–107. [PubMed: 10965526]

Piyathilake CJ, Macaluso M, Celedonio JE, Badiga S, Bell WC, Grizzle WE. Mandatory fortification with folic acid in the United States appears to have adverse effects on histone methylation in women with pre-cancer but not in women free of pre-cancer. Int J Womens Health. 2010; 1:131–137. [PubMed: 21072283]

Plath K, Fang J, Mlynarczyk-Evans SK, Cao R, Worringer KA, Wang H, de la Cruz CC, Otte AP, Panning B, Zhang Y. Role of histone H3 lysine 27 methylation in X inactivation. Science. 2003; 300:131–135. [PubMed: 12649488]

Pogribny IP, James SJ. De novo methylation of the p16INK4A gene in early preneoplastic liver and tumors induced by folate/methyl deficiency in rats. Cancer Lett. 2002; 187:69–75. [PubMed: 12359353]

Pogribny IP, Poirier LA, James SJ. Differential sensitivity to loss of cytosine methyl groups within the hepatic p53 gene of folate/methyl deficient rats. Carcinogenesis. 1995; 16:2863–2867. [PubMed: 7586211]

Protiva P, Mason JB, Liu Z, Hopkins ME, Nelson C, Marshall JR, Lambrecht RW, Pendyala S, Kopelovich L, Kim M, et al. Altered folate availability modifies the molecular environment of the human colorectum: implications for colorectal carcinogenesis. Cancer Prev Res (Phila). 2011; 4:530–543. [PubMed: 21321062]

Qin C, Wang Z, Shang J, Bekkari K, Liu R, Pacchione S, McNulty KA, Ng A, Barnum JE, Storer RD. Intracisternal A particle genes: Distribution in the mouse genome, active subtypes, and potential roles as species-specific mediators of susceptibility to cancer. Mol Carcinog. 2010; 49:54–67. [PubMed: 20025072]

Qui A, Jansen M, Sakaris A, Min SH, Chattopadhyay S, Tsai E, Sandoval C, Zhao R, Akabas MH, Goldman ID. Identification of an intestinal folate transporter and the molecular basis for hereditary folate malabsorption. Cell. 2006; 127:917–928. [PubMed: 17129779]

Rajagopalan PT, Zhang Z, McCourt L, Dwyer M, Benkovic SJ, Hammes GG. Interaction of dihydrofolate reductase with methotrexate: ensemble and single-molecule kinetics. Proc Natl Acad Sci U S A. 2002; 99:13481–13486. [PubMed: 12359872]

Razin A, Riggs AD. DNA methylation and gene function. Science. 1980; 210:604–610. [PubMed: 6254144]

Saitsu H, Ishibashi M, Nakano H, Shiota K. Spatial and temporal expression of folate-binding protein 1 (Fbp1) is closely associated with anterior neural tube closure in mice. Dev Dyn. 2003; 226:112–117. [PubMed: 12508232]

Salbaum J, Finnell R, Kappen C. Regulation of Folate receptor 1 gene expression in the visceral endoderm. Birth Defects Research Part A: Clin Mol Teratol. 2009 *in press*.

Salbaum JM, Kappen C. Diabetic embryopathy: A role for the epigenome? Birth defects research Part A, Clinical and molecular teratology. 2011

Salojin KV, Cabrera RM, Sun W, Chang WC, Lin C, Duncan L, Platt KA, Read R, Vogel P, Liu Q, et al. A mouse model of hereditary folate malabsorption: deletion of the PCFT gene leads to systemic folate deficiency. Blood. 2011; 117:4895–4904. [PubMed: 21346251]

Schurks M, Zee RY, Buring JE, Kurth T. Interrelationships among the MTHFR 677C>T polymorphism, migraine, and cardiovascular disease. Neurology. 2008; 71:505–513. [PubMed: 18672474]

Schwahn BC, Laryea MD, Chen Z, Melnyk S, Pogribny I, Garrow T, James SJ, Rozen R. Betaine rescue of an animal model with methylenetetrahydrofolate reductase deficiency. Biochem J. 2004; 382:831–840. [PubMed: 15217352]

Shilatifard A. Molecular implementation and physiological roles for histone H3 lysine 4 (H3K4) methylation. Curr Opin Cell Biol. 2008; 20:341–348. [PubMed: 18508253]

Shookhoff JM, Gallicano GI. A new perspective on neural tube defects: folic acid and microRNA misexpression. Genesis. 2010; 48:282–294. [PubMed: 20229516]

Sieber O, Heinimann K, Tomlinson I. Genomic stability and tumorigenesis. Semin Cancer Biol. 2005; 15:61–66. [PubMed: 15613289]

Siomi MC, Sato K, Pezic D, Aravin AA. PIWI-interacting small RNAs: the vanguard of genome defence. Nat Rev Mol Cell Biol. 2011; 12:246–258. [PubMed: 21427766]

Smithells RW, Ankers C, Carver ME, Lennon D, Schorah CJ, Sheppard S. Maternal nutrition in early pregnancy. Br J Nutr. 1977; 38:497–506. [PubMed: 201270]

Smithells RW, Sheppard S, Schorah CJ. Vitamin dificiencies and neural tube defects. Arch Dis Child. 1976; 51:944–950. [PubMed: 1015847]

Smithells RW, Sheppard S, Schorah CJ, Seller MJ, Nevin NC, Harris R, Read AP, Fielding DW. Apparent prevention of neural tube defects by periconceptional vitamin supplementation. Arch Dis Child. 1981; 56:911–918. [PubMed: 7332338]

Sohn KJ, Stempak JM, Reid S, Shirwadkar S, Mason JB, Kim YI. The effect of dietary folate on genomic and p53-specific DNA methylation in rat colon. Carcinogenesis. 2003; 24:81–90. [PubMed: 12538352]

Song J, Sohn KJ, Medline A, Ash C, Gallinger S, Kim YI. Chemopreventive effects of dietary folate on intestinal polyps in ApcMsh2-/- mice. Cancer Res. 2000; 60:3191–3199. [PubMed: 10866310]

Spencer TE, Jenster G, Burcin MM, Allis CD, Zhou J, Mizzen CA, McKenna NJ, Onate SA, Tsai SY, Tsai MJ, et al. Steroid receptor coactivator-1 is a histone acetyltransferase. Nature. 1997; 389:194–198. [PubMed: 9296499]

Spiegelstein O, Gould A, Wlodarczyk B, Tsie M, Lu X, Le C, Troen A, Selhub J, Piedrahita JA, Salbaum JM, et al. Developmental consequences of in utero sodium arsenate exposure in mice with folate transport deficiencies. Toxicol Appl Pharmacol. 2005; 203:18–26. [PubMed: 15694460]

Sridaran S, McClintock SK, Syphard LM, Herman KM, Barnwell JW, Udhayakumar V. Anti-folate drug resistance in Africa: meta-analysis of reported dihydrofolate reductase (dhfr) and dihydropteroate synthase (dhps) mutant genotype frequencies in African Plasmodium falciparum parasite populations. Malar J. 2010; 9:247. [PubMed: 20799995]

Stark GR, Wang Y, Lu T. Lysine methylation of promoter-bound transcription factors and relevance to cancer. Cell Res. 2011; 21:375–380. [PubMed: 21151202]

Steinfeld R, Grapp M, Kraetzner R, Dreha-Kulaczewski S, Helms G, Dechent P, Wevers R, Grosso S, Gartner J. Folate receptor alpha defect causes cerebral folate transport deficiency: a treatable neurodegenerative disorder associated with disturbed myelin metabolism. Am J Hum Genet. 2009; 85:354–363. [PubMed: 19732866]

Swanson DA, Liu ML, Baker PJ, Garrett L, Stitzel M, Wu J, Harris M, Banerjee R, Shane B, Brody LC. Targeted disruption of the methionine synthase gene in mice. Mol Cell Biol. 2001; 21:1058–1065. [PubMed: 11158293]

Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006; 126:663–676. [PubMed: 16904174]

Tapscott SJ. The circuitry of a master switch: Myod and the regulation of skeletal muscle gene transcription. Development. 2005; 132:2685–2695. [PubMed: 15930108]

van Beynum IM, den Heijer M, Blom HJ, Kapusta L. The MTHFR 677C->T polymorphism and the risk of congenital heart defects: a literature review and meta-analysis. QJM. 2007; 100:743–753. [PubMed: 17965089]

van der Linden IJ, den Heijer M, Afman LA, Gellekink H, Vermeulen SH, Kluijtmans LA, Blom HJ. The methionine synthase reductase 66A>G polymorphism is a maternal risk factor for spina bifida. J Mol Med. 2006; 84:1047–1054. [PubMed: 17024475]

van der Put NM, Steegers-Theunissen RP, Frosst P, Trijbels FJ, Eskes TK, van den Heuvel LP, Mariman EC, den Heyer M, Rozen R, Blom HJ. Mutated methylenetetrahydrofolate reductase as a risk factor for spina bifida. Lancet. 1995; 346:1070–1071. [PubMed: 7564788]

Van Speybroeck L. From epigenesis to epigenetics: the case of C.H. Waddington. Ann N Y Acad Sci. 2002; 981:61–81. [PubMed: 12547674]

van Steensel B, Henikoff S. Epigenomic profiling using microarrays. Biotechniques. 2003; 35:346–350. 352–344, 356–347. [PubMed: 12951776]

Vijayakrishnan J, Houlston RS. Candidate gene association studies and risk of childhood acute lymphoblastic leukemia: a systematic review and meta-analysis. Haematologica. 2010; 95:1405–1414. [PubMed: 20511665]

Vollset SE, Igland J, Jenab M, Fredriksen A, Meyer K, Eussen S, Gjessing HK, Ueland PM, Pera G, Sala N, et al. The association of gastric cancer risk with plasma folate, cobalamin, and methylenetetrahydrofolate reductase polymorphisms in the European Prospective Investigation into Cancer and Nutrition. Cancer Epidemiol Biomarkers Prev. 2007; 16:2416–2424. [PubMed: 18006931]

Wang J, Hevi S, Kurash JK, Lei H, Gay F, Bajko J, Su H, Sun W, Chang H, Xu G, et al. The lysine demethylase LSD1 (KDM1) is required for maintenance of global DNA methylation. Nat Genet. 2009; 41:125–129. [PubMed: 19098913]

Wang J, Zhan P, Chen B, Zhou R, Yang Y, Ouyang J. MTHFR C677T polymorphisms and childhood acute lymphoblastic leukemia: a meta-analysis. Leuk Res. 2010; 34:1596–1600. [PubMed: 20409583]

Waterland RA. Assessing the effects of high methionine intake on DNA methylation. J Nutr. 2006; 136:1706S–1710S. [PubMed: 16702343]

Waterland RA, Dolinoy DC, Lin JR, Smith CA, Shi X, Tahiliani KG. Maternal methyl supplements increase offspring DNA methylation at Axin Fused. Genesis. 2006; 44:401–406. [PubMed: 16868943]

Waterland RA, Jirtle RL. Transposable elements: targets for early nutritional effects on epigenetic gene regulation. Mol Cell Biol. 2003; 23:5293–5300. [PubMed: 12861015]

Watkins D, Ru M, Hwang HY, Kim CD, Murray A, Philip NS, Kim W, Legakis H, Wai T, Hilton JF, et al. Hyperhomocysteinemia due to methionine synthase deficiency, cblG: structure of the MTR gene, genotype diversity, and recognition of a common mutation, P1173L. American journal of human genetics. 2002; 71:143–153. [PubMed: 12068375]

Wellen KE, Hatzivassiliou G, Sachdeva UM, Bui TV, Cross JR, Thompson CB. ATP-citrate lyase links cellular metabolism to histone acetylation. Science. 2009; 324:1076–1080. [PubMed: 19461003]

Wolff GL, Kodell RL, Moore SR, Cooney CA. Maternal epigenetics and methyl supplements affect agouti gene expression in Avy/a mice. FASEB J. 1998; 12:949–957. [PubMed: 9707167]

Xu L, Glass CK, Rosenfeld MG. Coactivator and corepressor complexes in nuclear receptor function. Curr Opin Genet Dev. 1999; 9:140–147. [PubMed: 10322133]

Yamagata K, Daitoku H, Takahashi Y, Namiki K, Hisatake K, Kako K, Mukai H, Kasuya Y, Fukamizu A. Arginine methylation of FOXO transcription factors inhibits their phosphorylation by Akt. Mol Cell. 2008; 32:221–231. [PubMed: 18951090]

Yan J, Yin M, Dreyer ZE, Scheurer ME, Kamdar K, Wei Q, Okcu MF. A metaanalysis of MTHFR C677T and A1298C polymorphisms and risk of acute lymphoblastic leukemia in children. Pediatr Blood Cancer. 2011

Zavadakova P, Fowler B, Zeman J, Suormala T, Pristoupilova K, Kozich V, Zavad'akova P. CblE type of homocystinuria due to methionine synthase reductase deficiency: clinical and molecular studies and prenatal diagnosis in two families. J Inherit Metab Dis. 2002; 25:461–476. [PubMed: 12555939]

Zeisel SH. Nutritional genomics: defining the dietary requirement and effects of choline. J Nutr. 2011; 141:531–534. [PubMed: 21270363]

Zhao R, Min SH, Qiu A, Sakaris A, Goldberg GL, Sandoval C, Malatack JJ, Rosenblatt DS, Goldman ID. The spectrum of mutations in the PCFT gene, coding for an intestinal folate transporter, that are the basis for hereditary folate malabsorption. Blood. 2007; 110:1147–1152. [PubMed: 17446347]

Zhao R, Russell RG, Wang Y, Liu L, Gao F, Kneitz B, Edelmann W, Goldman ID. Rescue of embryonic lethality in reduced folate carrier-deficient mice by maternal folic acid supplementation reveals early neonatal failure of hematopoietic organs. J Biol Chem. 2001; 276:10224–10228. [PubMed: 11266438]

Zhu H, Wicker NJ, Shaw GM, Lammer EJ, Hendricks K, Suarez L, Canfield M, Finnell RH. Homocysteine remethylation enzyme polymorphisms and increased risks for neural tube defects. Molecular genetics and metabolism. 2003; 78:216–221. [PubMed: 12649067]

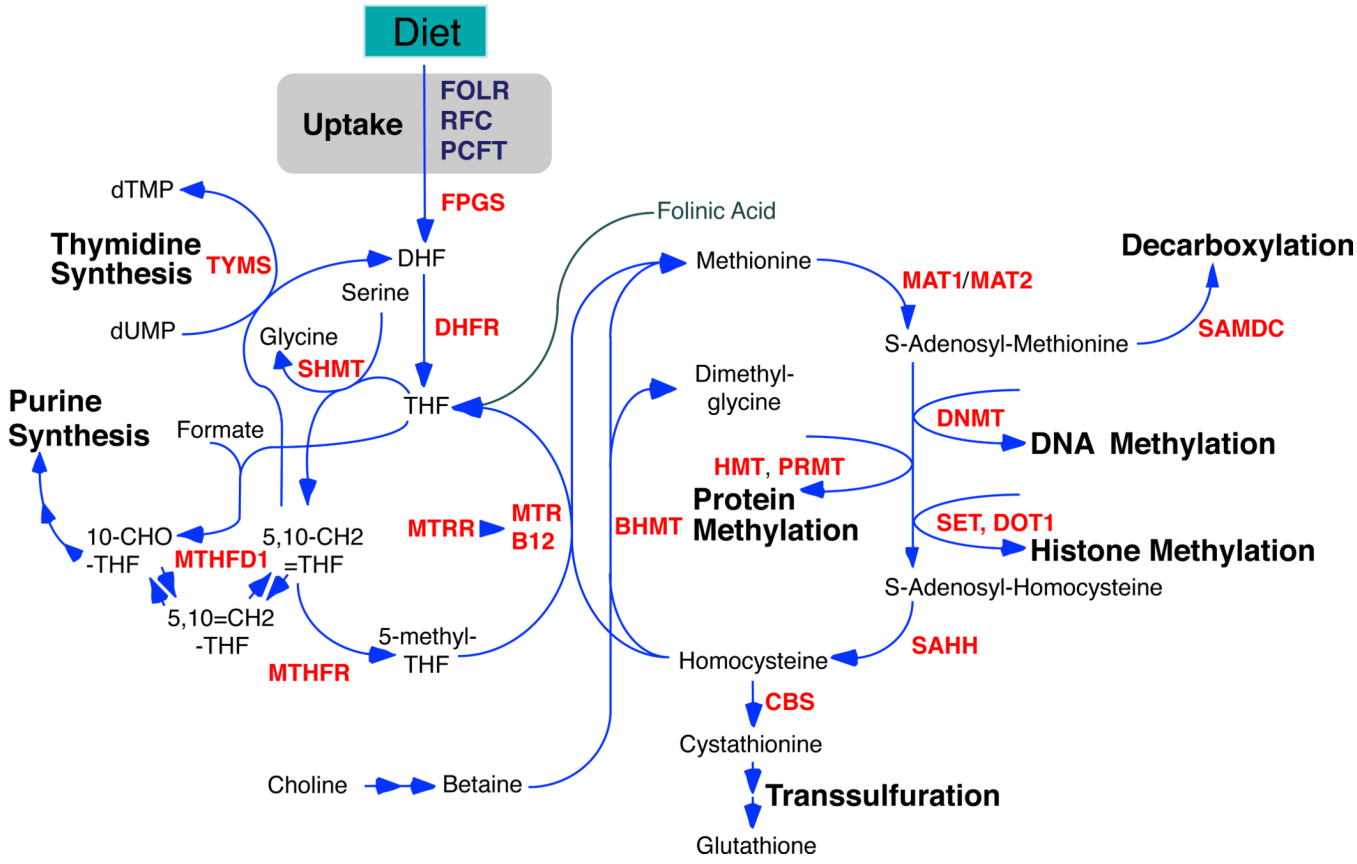

Figure 1.

Schematic representation of core aspects of the folate cycle. Abbreviations: FOLR, folate receptor; RFC, reduced folate carrier; PCFT, proton-coupled folate transporter; FPGS, folylpolyglutamate synthase; DHF, dihydrofolate; DHFR, dihydrofolate reductase; THF, tetrahydrofolate; SHMT, serine hydroxymethyl transferase; TYMS, thymidylate synthase; dTMP, deoxythymidine monophosphate; dUMP, deoxyuridine monophosphate; 5,10-CH2=THF, 5,10-methylene tetrahydrofolate; 5,10=CH2-THF, 5,10-methenyl tetrahydrofolate; 10-CHO-THF, 10-formyl tetrahydrofolate; MTHFD1, methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 1, methenyltetrahydrofolate cyclohydrolase, formyltetrahydrofolate synthetase; MTHFR, methylenetetrahydrofolate reductase; 5-methyl-THF, 5-methyl tetrahydrofolate; MTRR, methionine synthase reductase; MTR, methionine synthase; B12, vitamin B12; BHMT, betaine homocysteine methyltransferase; MAT1, methionine adenosyltransferase II; MAT2, methionine adenosyltransferase II; SAMDC, S-adenosylmethionine decarboxylase; DNMT, DNA methyltransferase; HMT, histone methyltransferase; PRMT, protein arginine methyltransferase; SET, DOT1, histone H3 methyltransferase; SAHH, S-adenosylhomocysteine hydrolase; CBS, choline betaine synthetase.

Table 1

Mutations in genes of the folate cycle

VARIATION	FOLR1	F0LR2	F0LR3	F0LR4	SLC19A1	SLC46A1	SLC25A32	FPGS	DHFR	MTHFD1	MTHFR	MTRR	MTR	SHMT
Essential splice site	0	0	7	0	0	2	0	0	0	2	0	0	4	0
Stop gained	0	0	0	0	0	0	0	0	0	0	4	6	0	0
Stop lost	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Complex in/del	0	0	5	0	0	0	0	0	0	0	0	0	0	0
Frameshift coding	0	0	5	0	0	6	0	0	0	1	0	2	12	0
Non-synonymous coding	20	49	8	4	25	26	8	23	3	31	82	61	64	27
Splice site	3	0	1	0	13	0	6	1	0	6	14	12	17	16
Partial codon	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Synonymous coding	8	3	27	0	40	5	0	25	3	4	77	62	38	8
Coding unknown	0	0	3	0	0	0	0	0	0	0	4	30	0	0
Within mature miRNA	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Intronic	50	66	37	2	366	20	111	407	57	58	295	577	325	163
NMD transcript	0	0	39	0	0	0	95	0	0	0	0	480	0	0
Within non-coding gene	0	0	10	0	100	0	5	308	29	0	0	213	110	0
Upstream/5 kb	2	4	16	2	13	2	8	6	18	1	11	31	13	3
Downstream/5kb	0	15	7	0	15	0	0	23	14	2	17	30	18	3
Regulatory region	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Intergenic	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5 prime UTR	10	9	6	1	17	0	8	0	30	2	76	22	12	10
3 prime UTR	8	12	14	0	92	94	66	43	73	5	151	199	124	46
ALL	98	158	133	7	622	154	206	608	210	106	717	1106	680	260