Nutrigenetics, Nutrigenomics and Precision Nutrition

Nutrient Genetic Regulation of Methylation Potential

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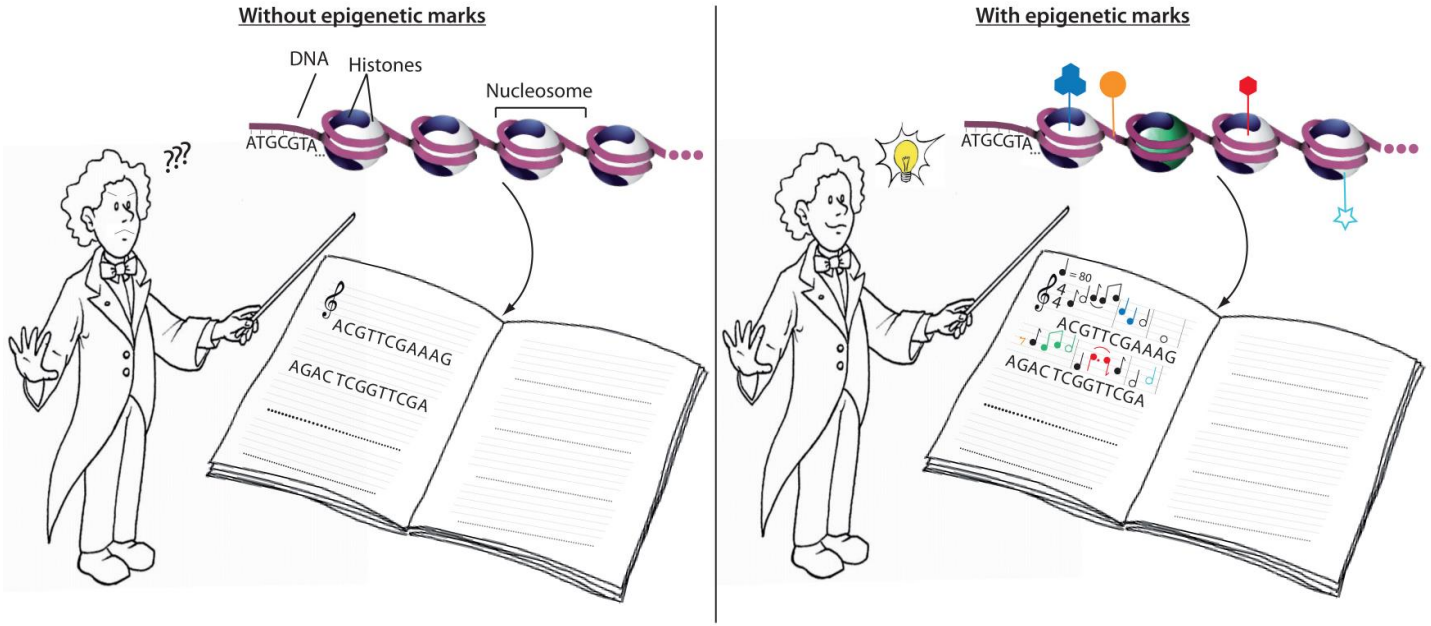
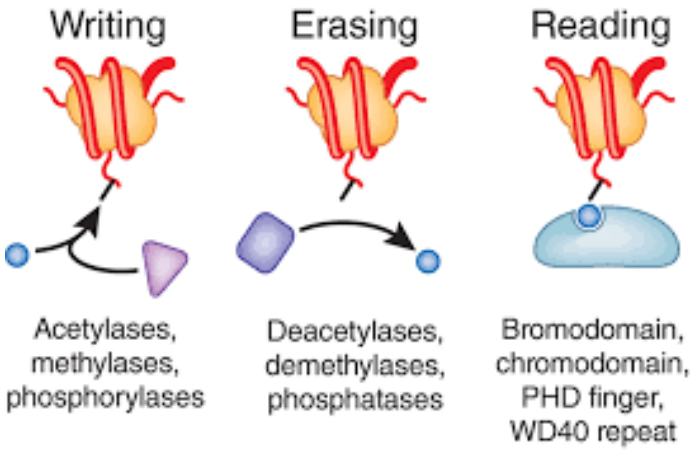
Epigenetics

The study of heritable changes in gene function/cellular phenotype that occur without alterations in the DNA sequence



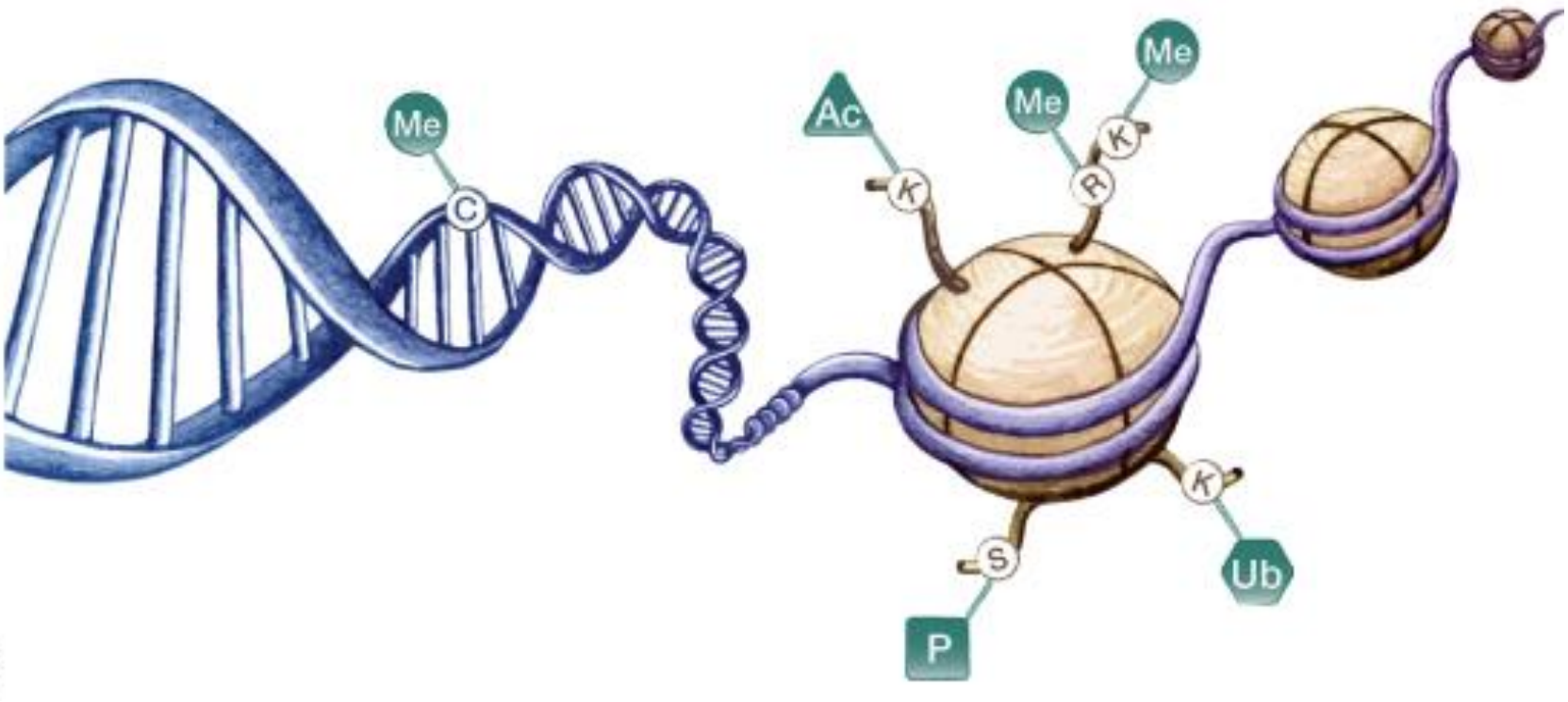
Epigenetic mechanisms

- DNA methylation
- Histone modifications
- Chromatin structure
- Spatial organization of chromosomes
- Regulatory RNAs

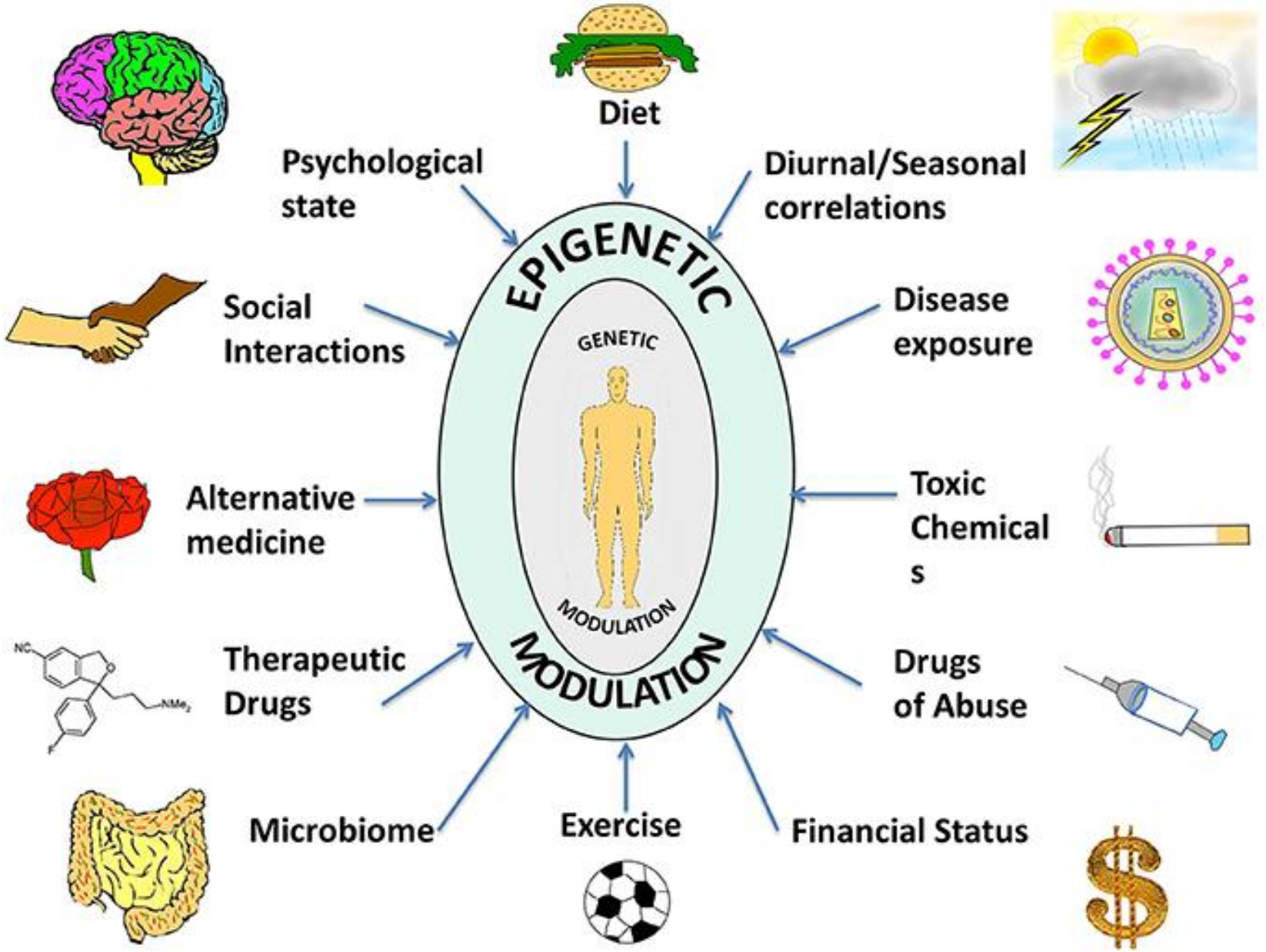


Nature Immunology **11**, 565–568 (2010)

Epigenetic modifications, or “tags,” such as **DNA methylation** and histone modification, alter **DNA** accessibility and **chromatin** structure, thereby regulating patterns of **gene expression**

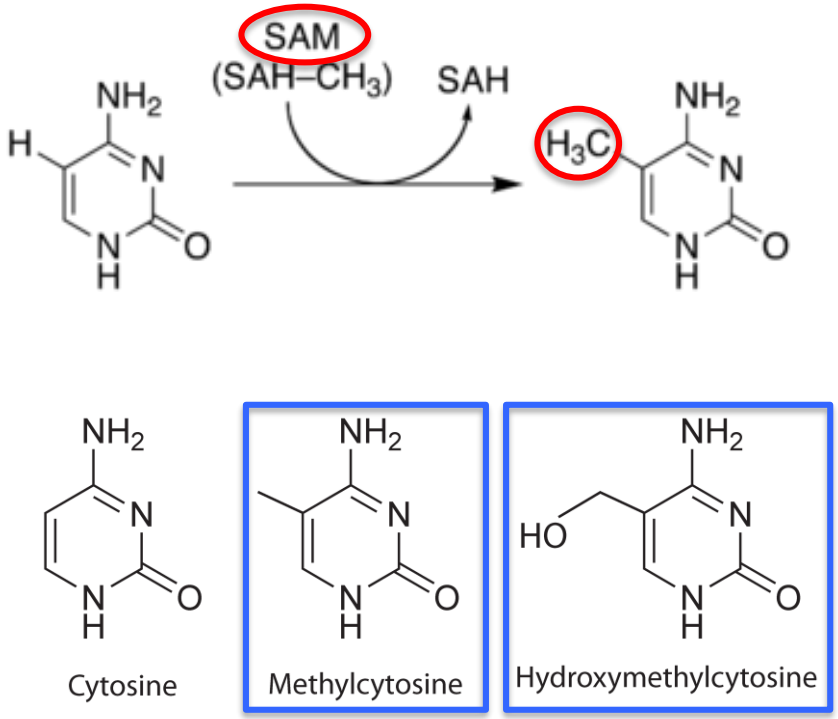


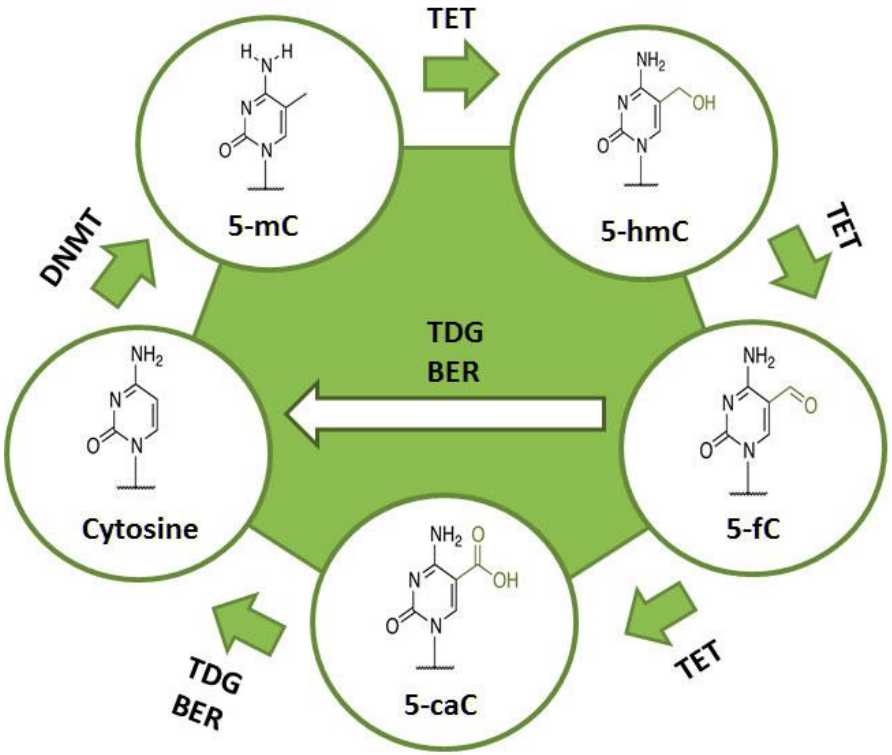
https://www.promega.com/resources/product-guides-and-selectors/protocols-and-applications-guide/epigenetics/



http://dx.doi.org/10.3389/fcell.2014.00049

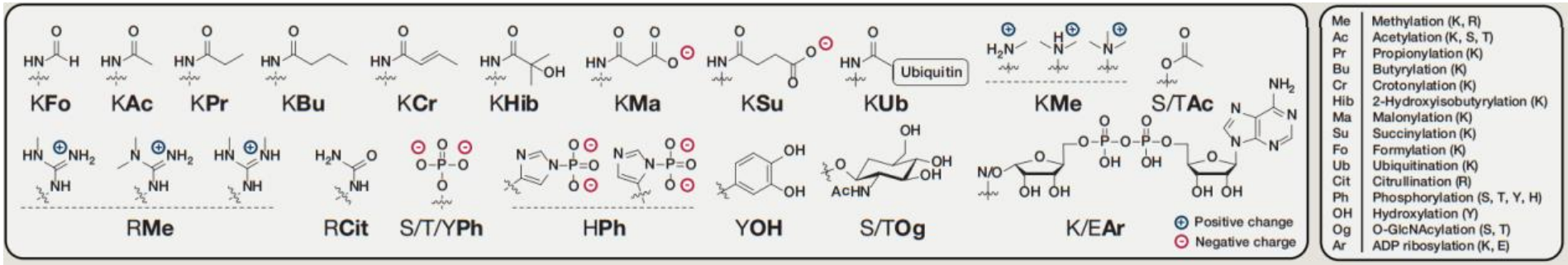
DNA Methylation at CpG dinucleotides





Histone modifications

- Involve lysine, arginine, serine, threonine, thyrosine or histidine residues.
- Methylation, acetylation and phosphorylation are most common and most studied.
- Over a dozen of other modifications were found.



https://www.tumblr.com/search/histone%20modification

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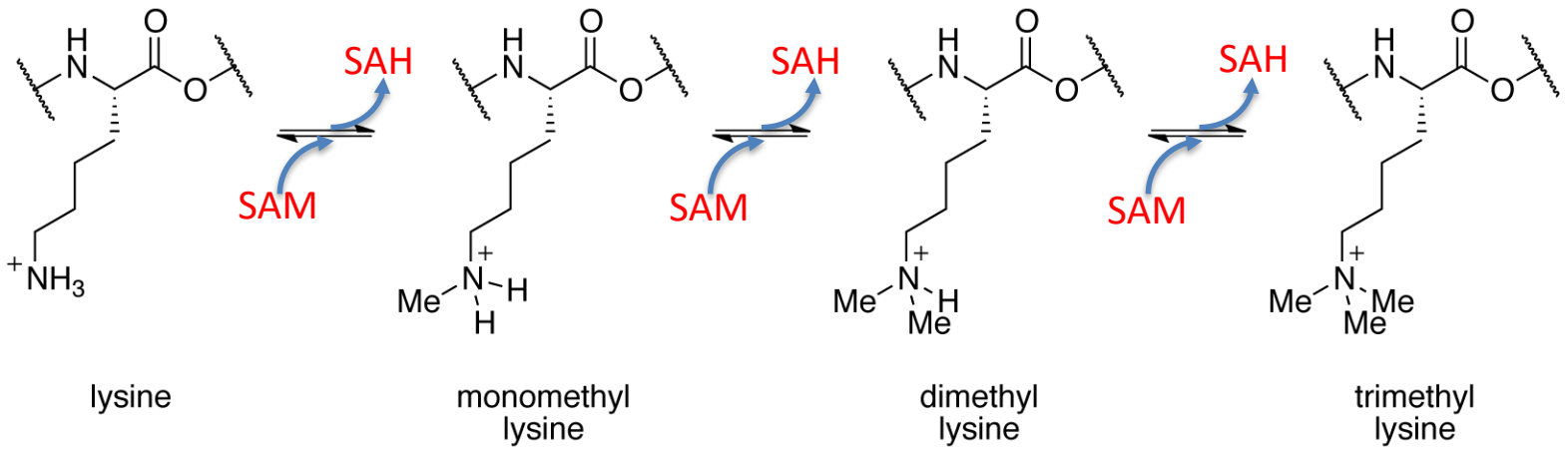
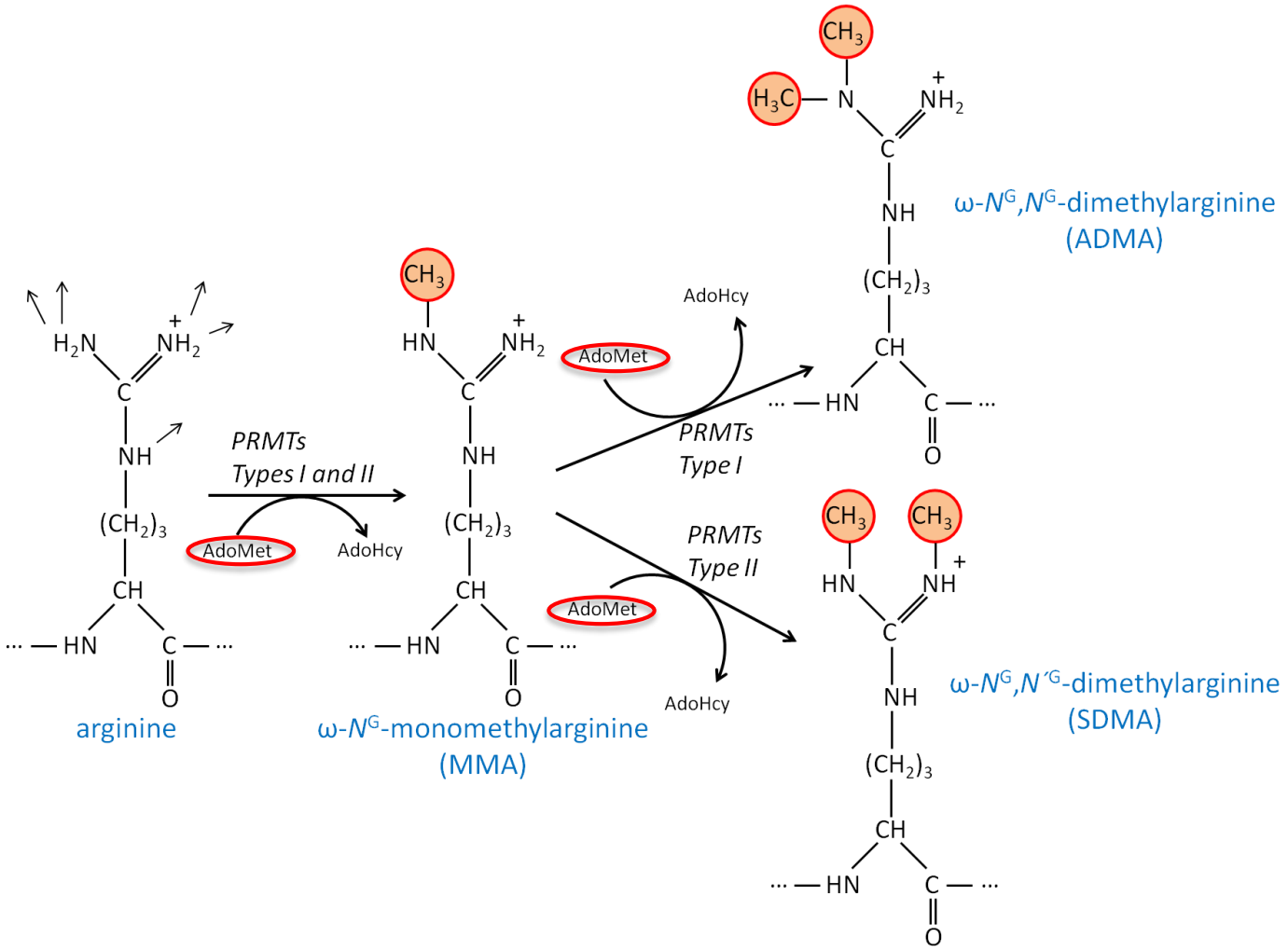
Histone Modifications

Nature Biotechnology **28**, 1057–1068 (2010)

Lysine methylation



Arginine methylation

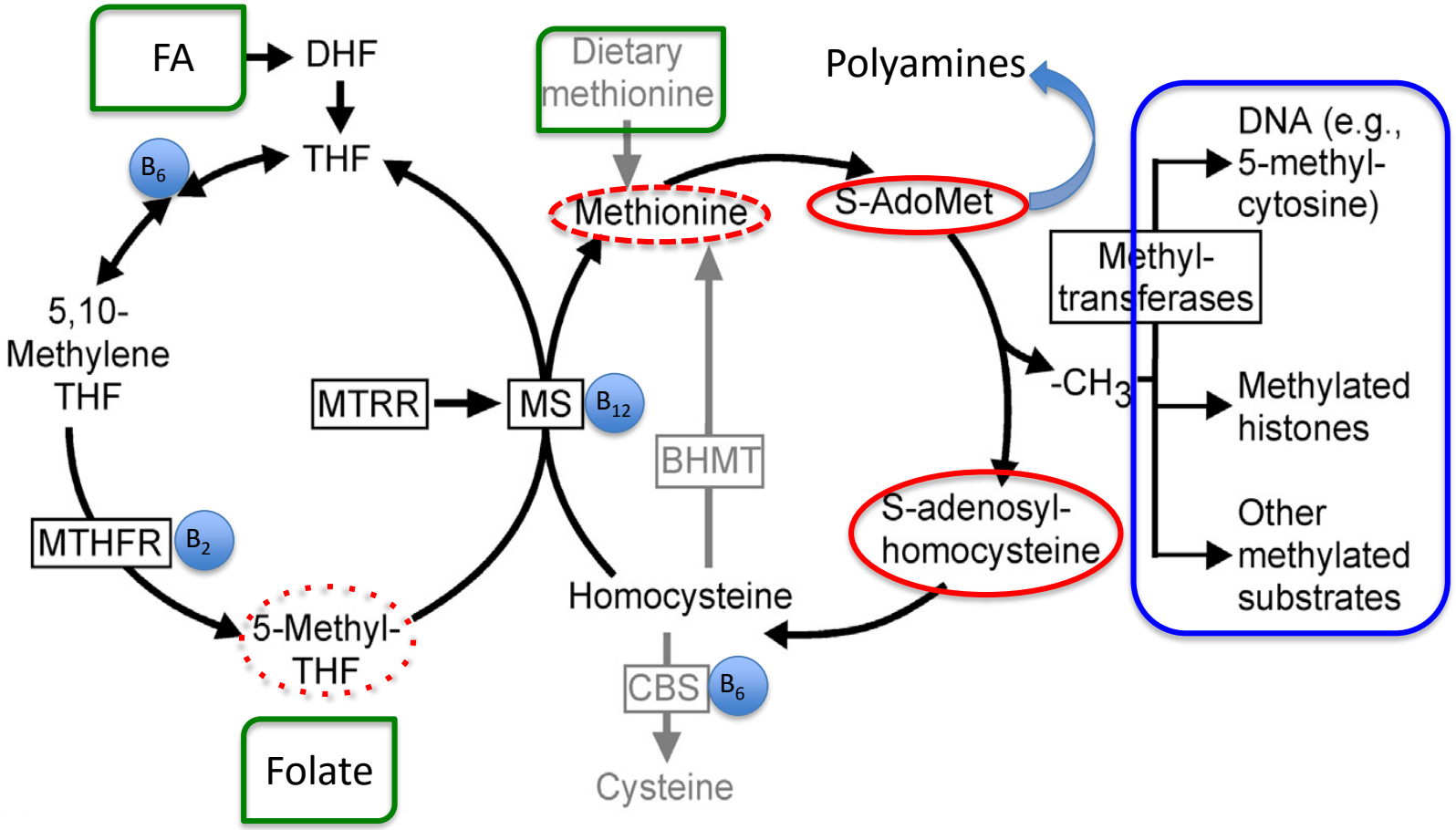


http://www.intechopen.com/books/methylation-from-dna-rna-and-histones-to-diseases-and-treatment/deciphering-protein-arginine-methylation-in-mammals

S-adenosylmethionine = SAM

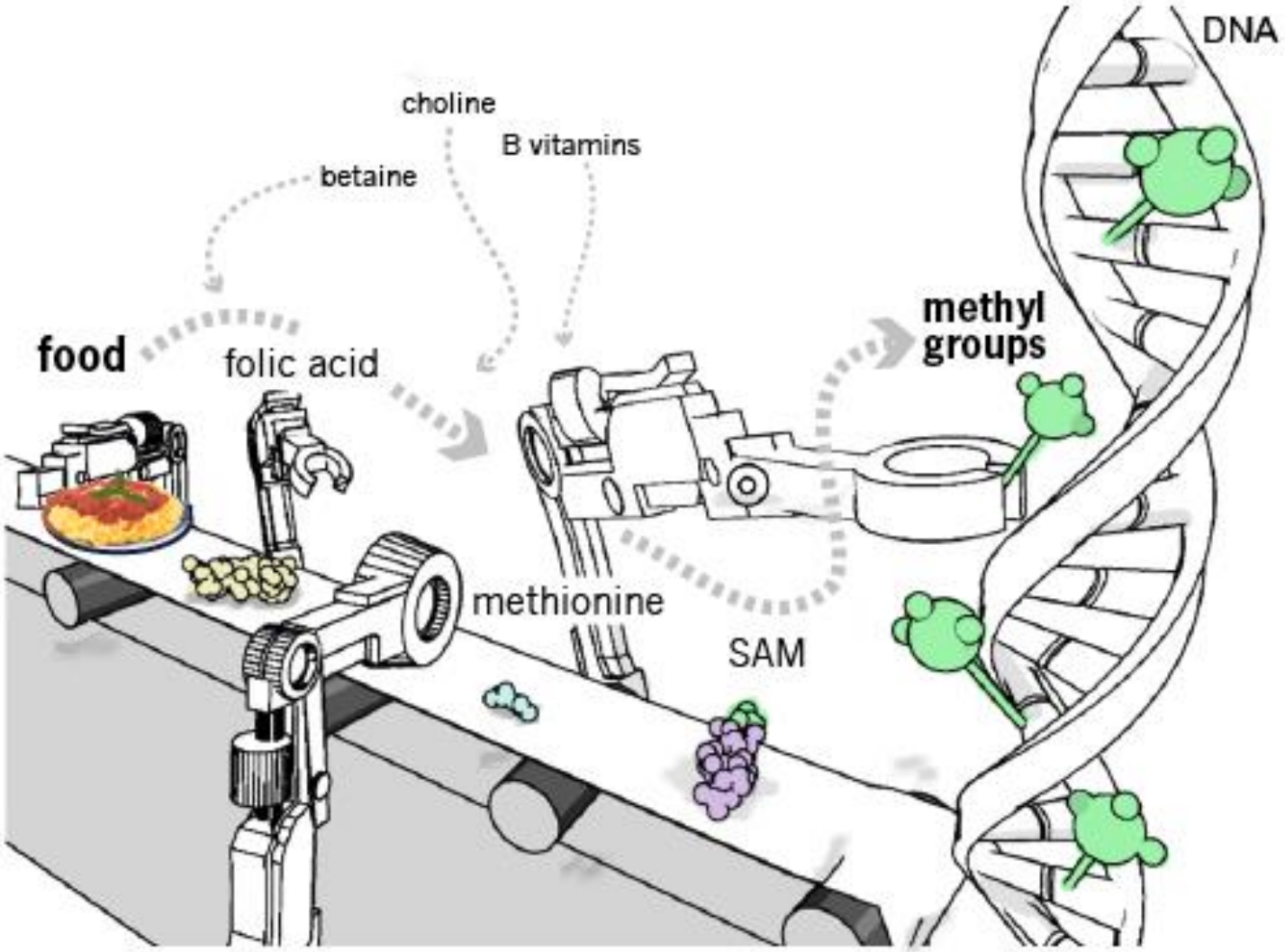
- Is a universal methyl group donor.
- Made from an essential amino acid methionine.
- Used by over a 100 of different enzymes for methyl transfer reactions.
- Converts into S-adenosylhomocysteine in the course of the reaction.
- S-adenosylhomocysteine (SAH) is a potent inhibitor of methyltransferases.
- The SAM/SAH ratio determines the ability of methyltransferases to attach methyl groups to their substrates.

Methylation cycle



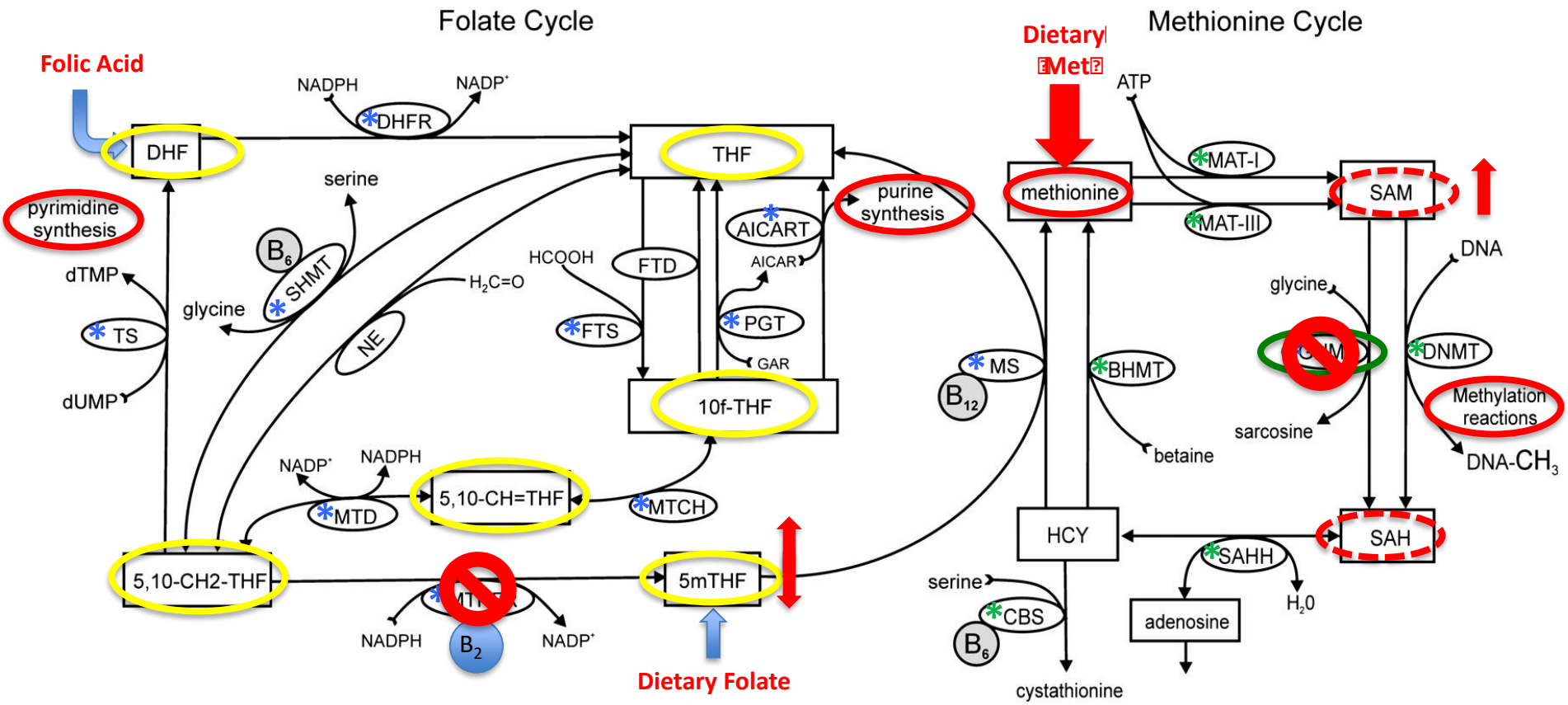
Cell 155, 81–93, 2013

Nutrition and epigenetics



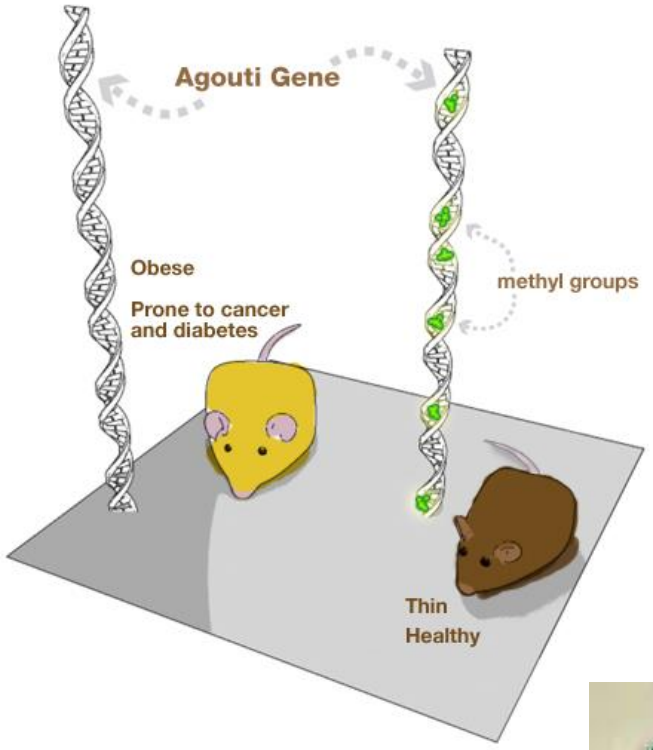
learn.genetics.utah.edu/content/epigenetics/nutrition/

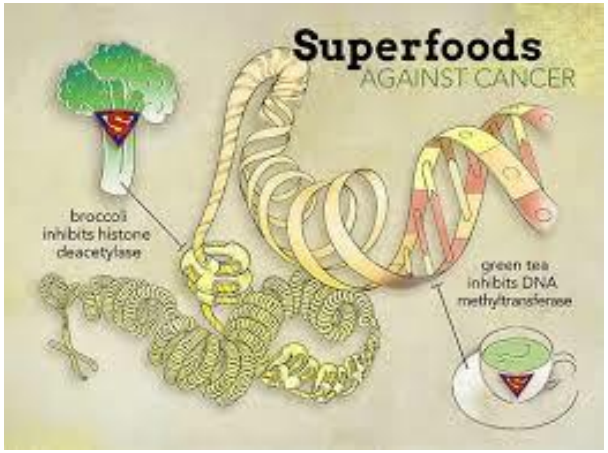
Folate metabolism, diet and methylation



Boyles AL, Billups AV, Deak KL, Siegel DG, Mehltretter L, Slifer SH, Bassuk AG, Kessler JA, Reed MC, Nijhout HF, George TM, Enterline DS, Gilbert JR, Speer MC, NTD Collaborative Group - (2006)

Diet and epigenome





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Plasma SAM and DNMT polymorphisms are associated with peripheral blood LINE-1 methylation

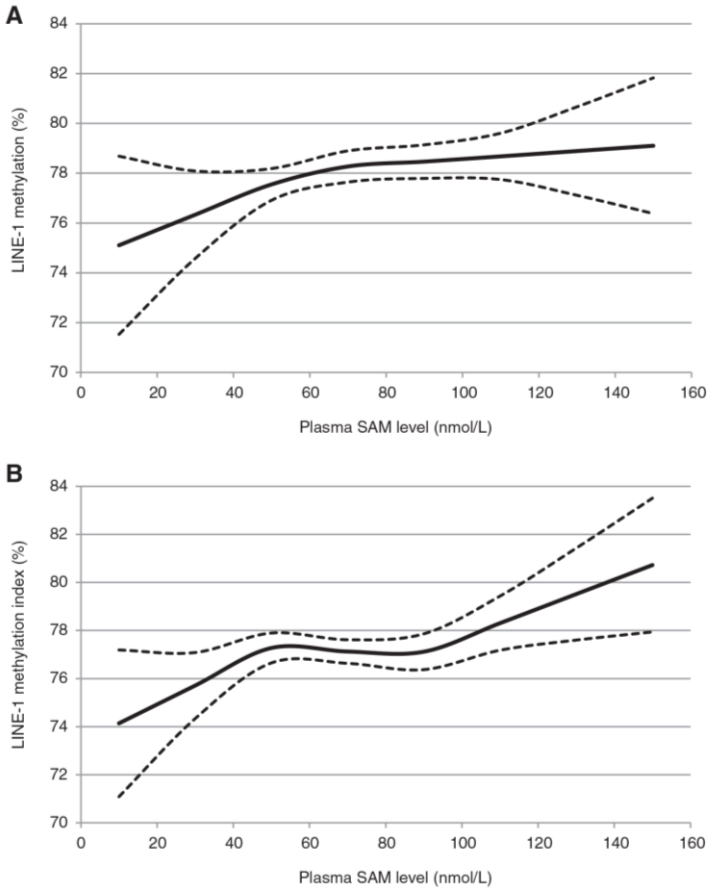


Figure 1. LINE-1 methylation and plasma SAM level in men (A) and women (B).

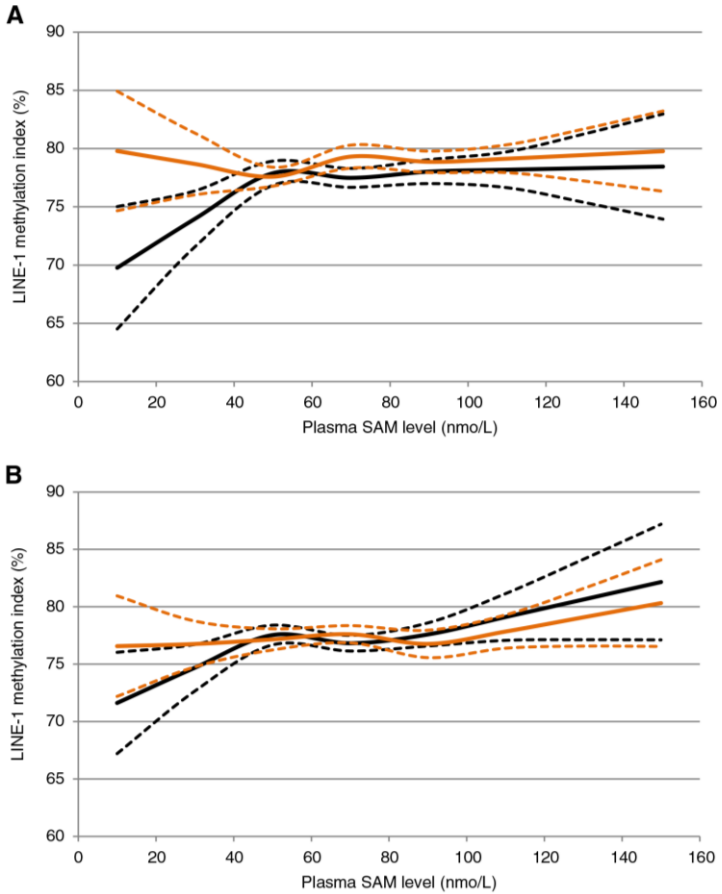


Figure 2. LINE-1 methylation and plasma SAM by DNMT1 rs2114724 genotype in men (A) and women (B). Black line: the wild type (CC), red line: variant genotypes (CT and TT).

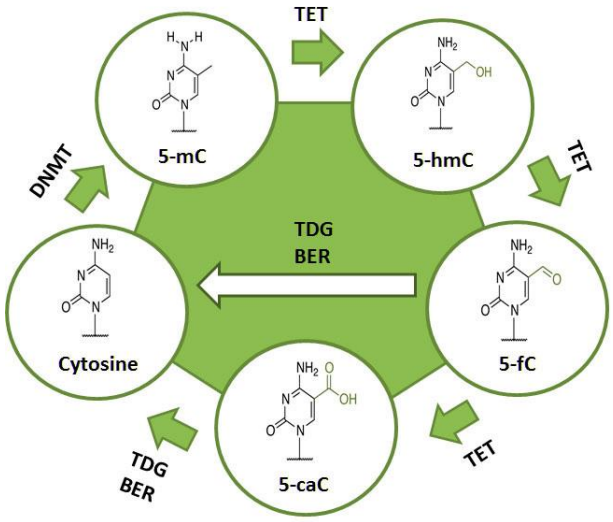
BMC Cancer 2013, 13:389

Diet and DNA demethylation

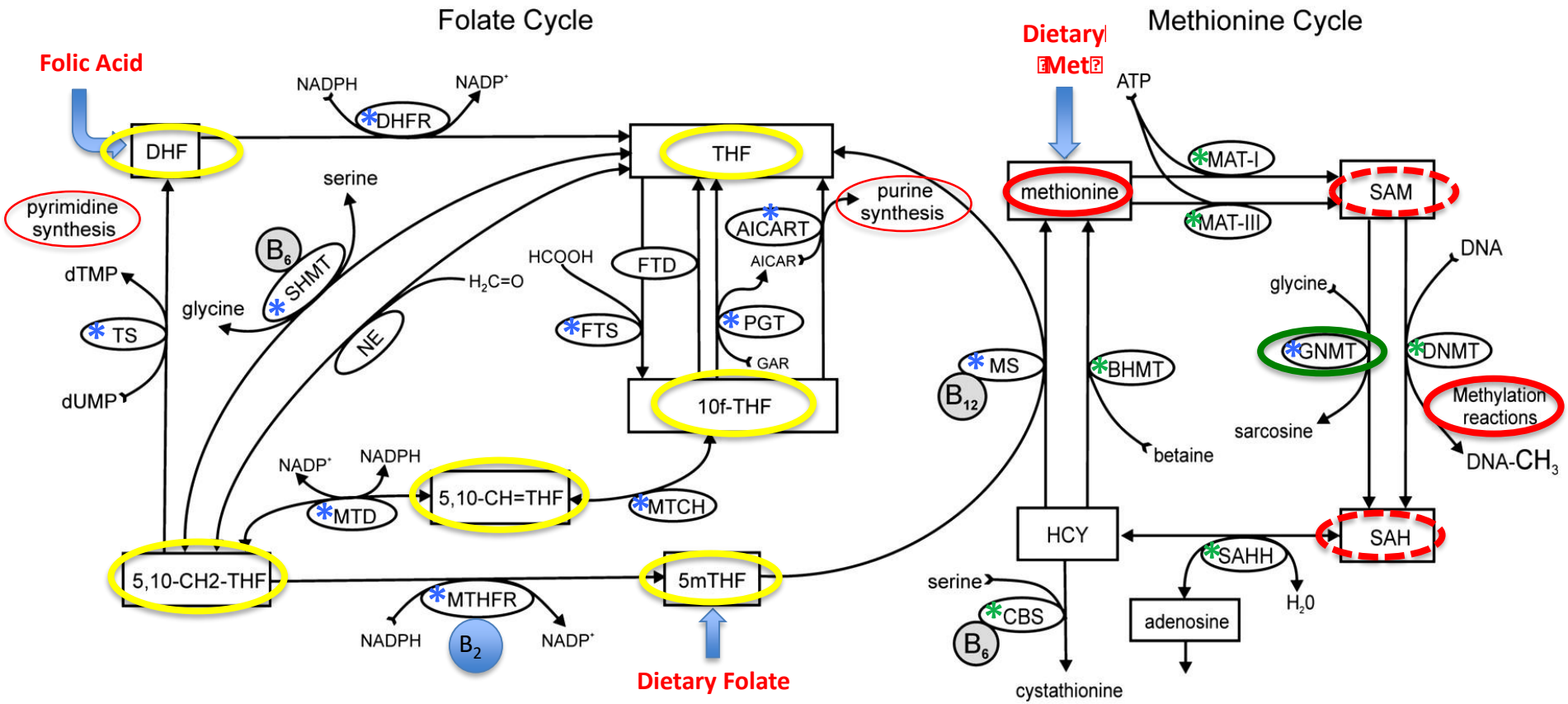
=

DNMT3A knockdown by RNAi

- TET family of enzymes (dioxygenases) are involved in DNA demethylation.
- Require Fe^{2+} and α-ketoglutarate (TCA cycle intermediate) as co-substrates.



Folate & methyl group metabolism



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Methionine Adenosyltransferase (MAT)

MAT1A: 44 polymorphisms; 1 non-synonymous: E238K

30 mutations causing protein deficiency, R264H – most frequent.

MAT2A: 74 polymorphisms; 2 non-synonymous: A11V and I205V

No information on the mutations effect on SAM/SAH.

MAT2B: 44 polymorphisms; 2 non-synonymous: P261S and T291I

No apparent functional consequences of change in amino acid sequence.

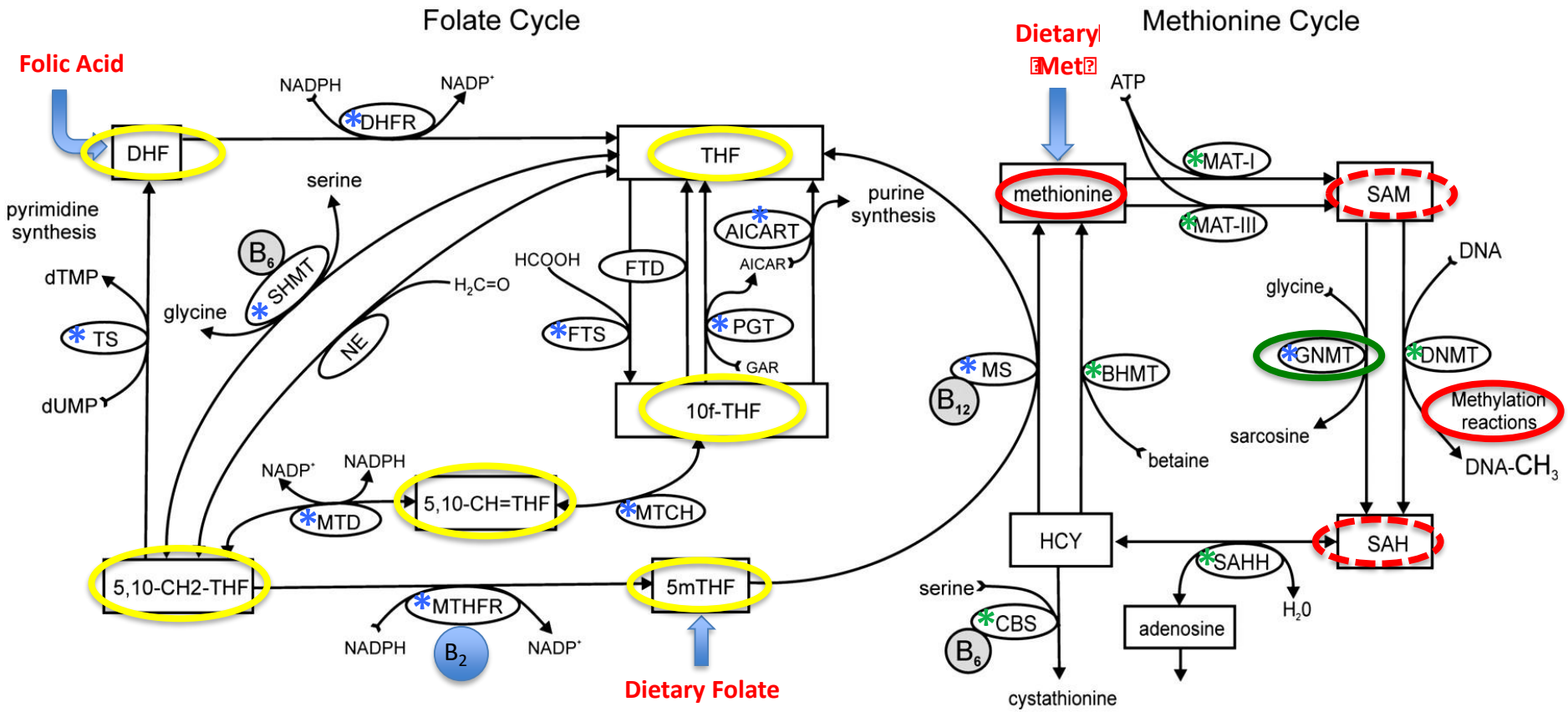
MAT1A knockout in mice resulted in ~7.5-fold plasma methionine increase. Hepatic SAM reduced 4 fold and SAH was unchanged.

Human MAT Deficiency

64 patients known today
~50% show some CNS abnormalities.
Have hypermethioninemia and low SAM.
No information on epigenetic modifications.

MAT1A/MAT2A and MATI/III:MATII ratios positively correlate with apoptosis and global DNA methylation in human HCC.

Methyl groups flow



Genetic variability of folate enzymes

Folate enzyme	SNP Entries	In Del
MTHFR	2,107	78
DHFR	2,276	132
MTR	7,143	325
MTRR	4,164	7
SHMT	3,436	1
TS	57,874	95
MTHFD	4,605	219
AICART	164,986,643	138
GNMT	1795	23
ALDH1L1	5,643	11
CBS	2,371	1

Functional relevance?

GNMT variations

Major regulator of the methylation potential!

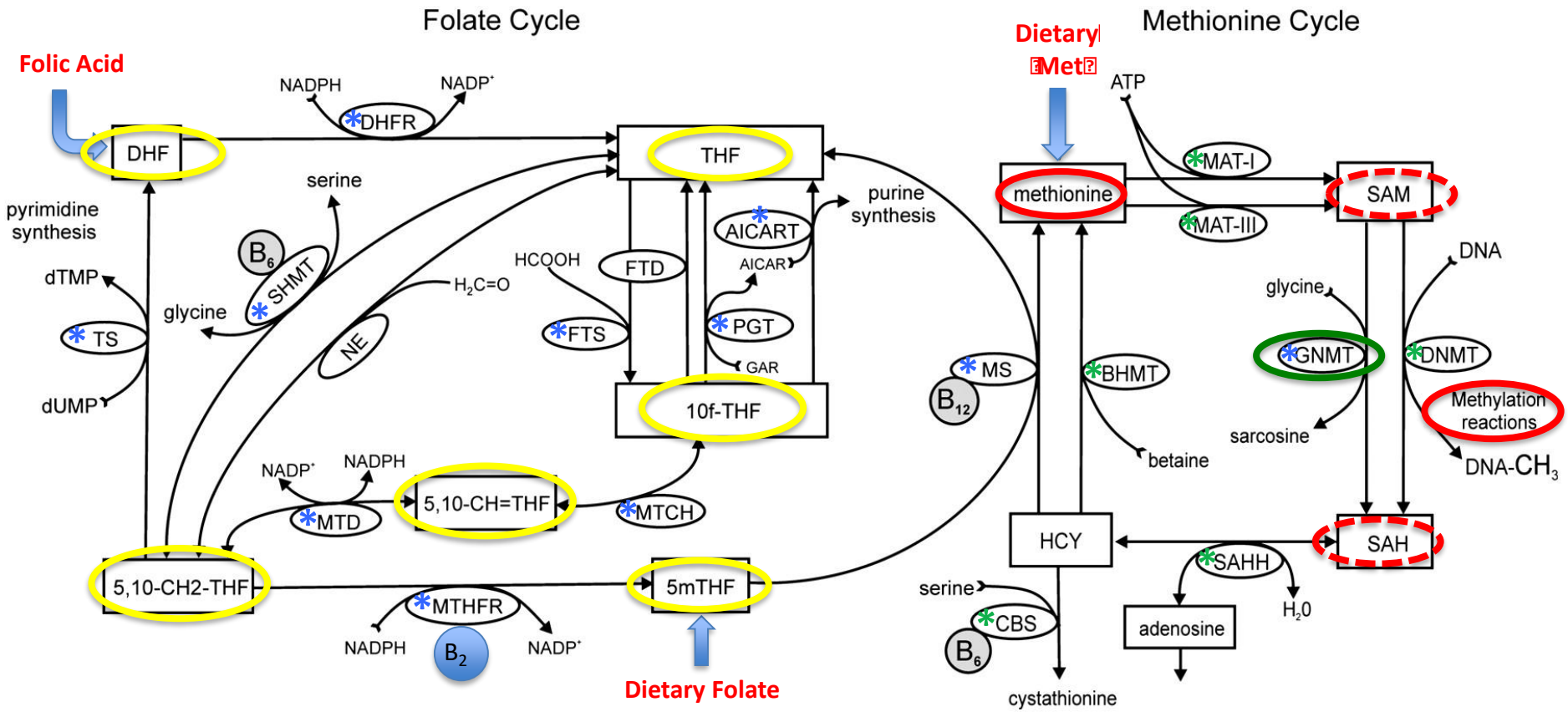
Total 1795 SNPs identified

65 SNPs causing an amino acid substitution

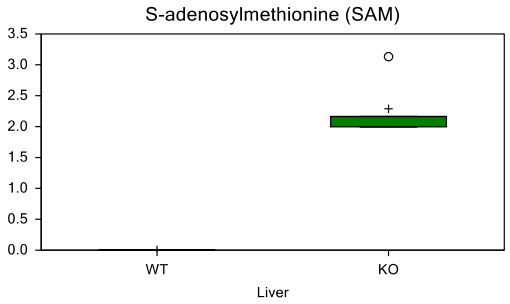
Relatively few SNPs will cause change in enzyme activity, but might affect regulation.

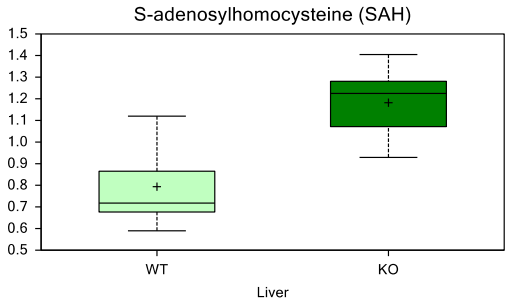
SNPs discovered in the non-coding regions could affect expression levels.

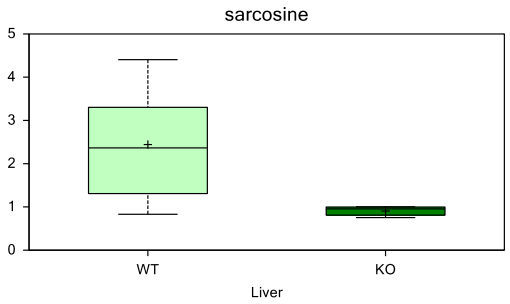
Methyl groups flow



GNMT deficiency: mouse model







SAM/SAH ratio is increased by a factor of more than 200

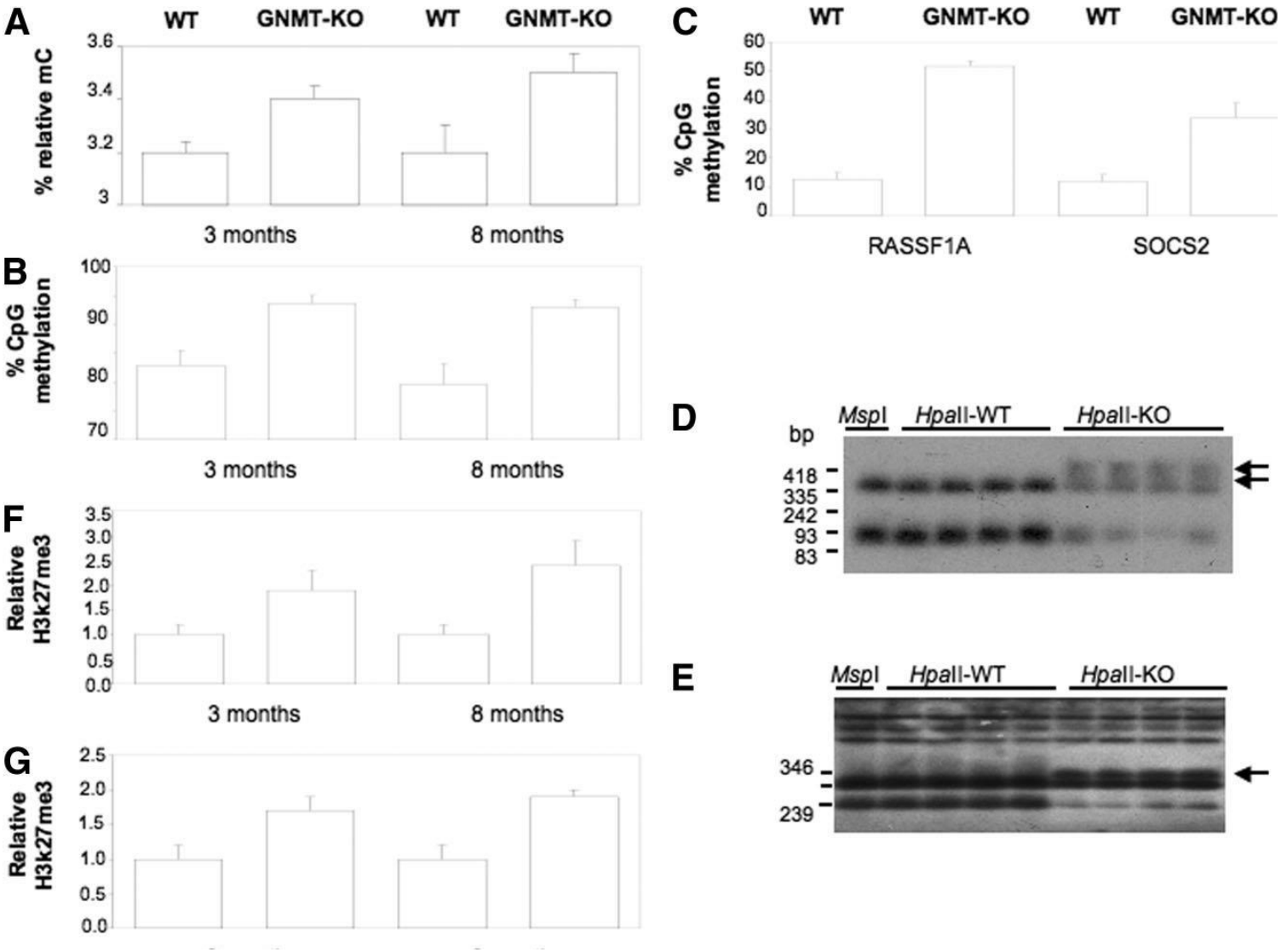
Phenotype:

Liver steatosis and fibrosis develops by 3 month.

At 6 month 100% mice develop multifocal hepatocellular carcinomas.

No significant signs of inflammation at either 3 or 8 months.

GNMT deficiency: mouse model



Hepatology, 47, 1191-1199, 2008

GNMT deficiency: human studies

- Rare disorder (3 patients reported so far)
- GNMT mutants show some residual activity (30-1%)
- High levels of plasma methionine and SAM (>20 times)
- Normal to slightly elevated SAH
- Betaine and dimethylglycine elevated (>10 times)

Phenotype

Mild liver disfunction and hepatomegaly

Partially corrected by dietary methionine restriction

No info on DNA/histone methylation in tissues

GNMT polymorphisms

C1289T : Higher tHcy upon folate restriction for TT versus CC or CT genotype

rs9296404 : associated with the difference of pre- and post-methionine load tHcy concentrations ($p=1.6x10^{-63}$)

rs10948059 : TT genotype associated with 1.62 fold increase of prostate cancer risk compared with CC genotype

STRP1 : ≥16GAs/≥16GAs genotype associated with lower risk of prostate cancer (OR=0.68) than <16GAs/<16GAs genotype

Methionine Synthase (MTR)

A2756G (rs1805087) - change of aspartate to glycine

- effects of the substitution are not clear
- associated with a lower methylation of its own gene

Methionine synthase reductase (MTRR)

A66G (rs1801394) = Ile22Met

- a trend for association with a lower SAM/SAH ratio
- indications for association with a lower Hcy levels
- associated with increased risk for NTDs
- associated with a differentially methylated CpGs in ADAMTS2 and C3orf55
- Knockdown in mice → ~ 3-fold reduction in protein mRNA levels, ~ 3-fold increase of tHcy and tissue-specific DNA hypomethylation.
- *Epigenetic changes were inherited, resulting in methylation and developmental phenotypes in the WT grand-progeny.*

Cell, 155, 81-93, 2013

(MTHFR)
5,10-Methylene-THF Reductase

5,10-Methylene-THF → 5-Methyl-THF

Common variant: C677T = A222V
rs1801133

~ 5-20% of population has TT genotype resulting in mild MTHFR deficiency

Associated with mild hyperhomocysteinemia, lower plasma and red blood cell folate levels, and global DNA hypomethylation.

DNA methylation directly and significantly related to RBC folate levels in TT, but not in CC individuals

C677T (A222V) MTHFR variant

Mutant protein displays the same catalytic properties as the WT enzyme

222V variant releases cofactor FAD ~3 times faster, is more prone to dissociation and more thermolabile than the wild type

5-methyl-THF and SAM protect MTHFR from activity loss

Yamada et al, 2001

MTHFR C677T clinical relevance

Associated with increased risk for:

- cardiovascular disease
- neural tube defects
- cleft lip and palate
- thrombosis
- schicsophrenia
- adverse pregnancy outcomes

May have protective effect against some cancers

MTHFR polymorphisms

A1298C (rs1801131)
causes Glu 429 to Ala substitution
also affects enzyme activity, but to a lesser extent

1298CC individuals have normal Hcy levels,
but compound heterozygotes for the 1298C and 677T variants have biochemical profile and increased Hcy similar to 677TT genotype

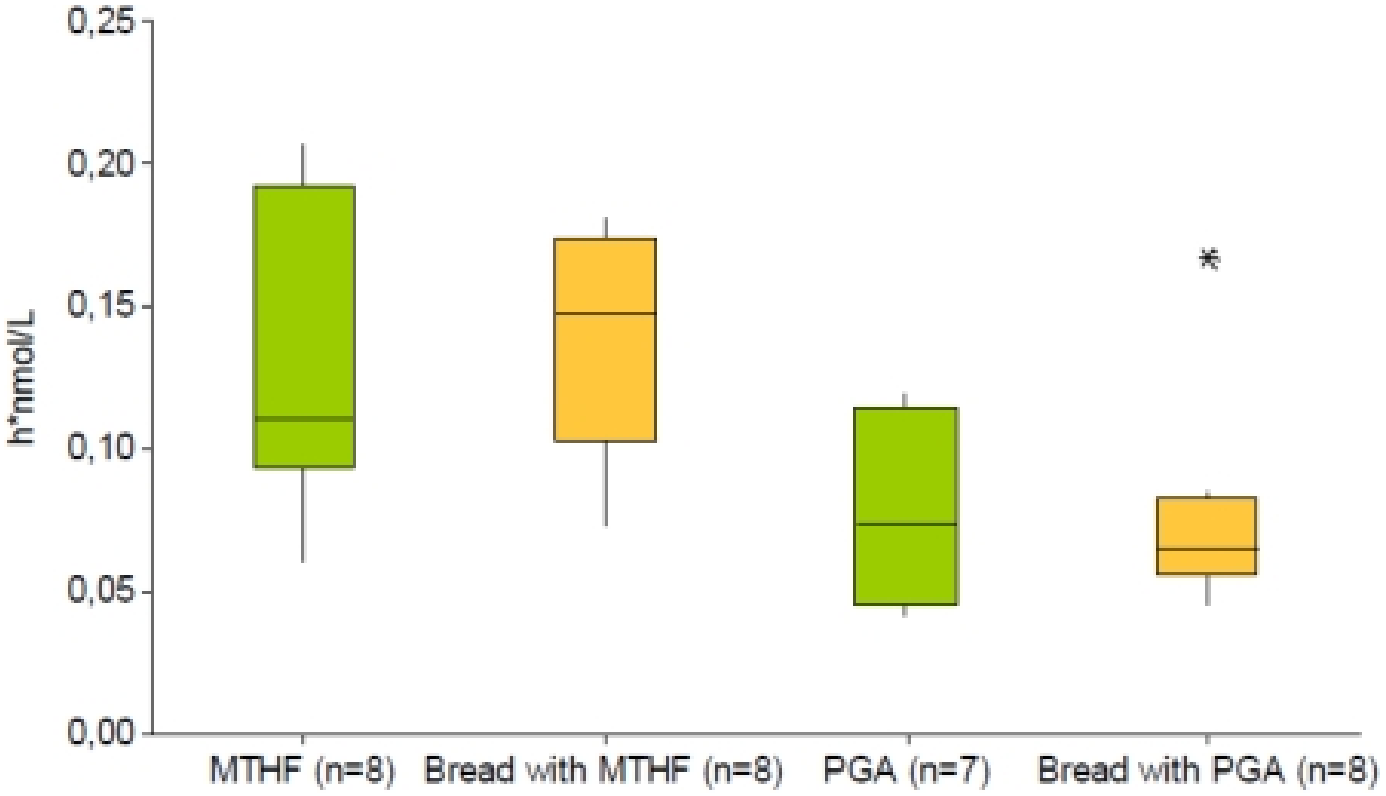
C677T (A222V) MTHFR variant

Mild MTHFR deficiency may be corrected by low dose of folic acid.

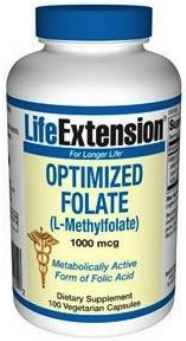
5-methyl-THF but not folic acid rescues severe MTHFR deficiency in mice (*Li et al, 2008*).

5-methyl-THF increases plasma folate more efficiently than folic acid in women with both wt and 677T MTHFR (*Prinz-Langenohl et al, 2009*)

Absorption of a single 200 µg dose of 5-methyl-THF or folic acid



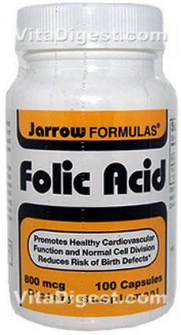
Ohrvik V.E, Witthoft C.M., 2011



Should we supplement with 5-methyl-THF?

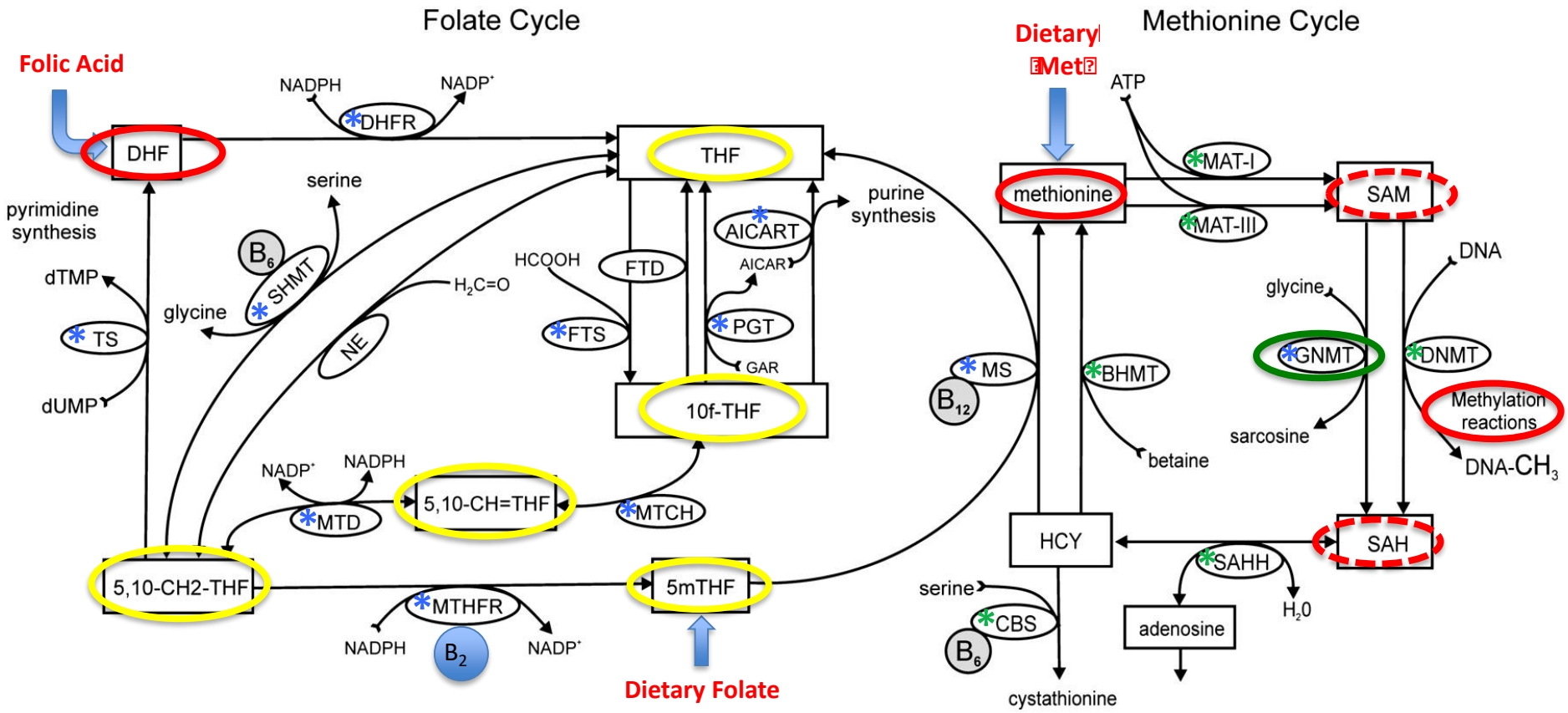
These suggestions should be interpreted with caution…

Stover et al, 2015



Should we supplement with 5-formyl-THF?

DHFR polymorphisms



DHFR polymorphisms

19bp Del in intron-1, 60 bp from the splice donor site

Biological effects of the deletion are controversial.

Associated with increased unmetabolized folic acid in plasma (intake >500 µg/d) and decreased red blood cell folate (intake <250 µg/d).

Associated with adenomatous polip occurrence, but reduces risk with high folate intake.

Deletion diminishes capacity of the enzyme to reduce folic acid.

(*Kalmbach et al, 2008*)

DHFR mutations

L80F and D153V - reduced enzyme activity

Biological effects: hematological abnormalities, low CSF folate levels, neurological problems.

Folinic acid (5-formyl-THF), but not the folic acid, was able to by-pass the enzyme deficiency and correct the pathologic effects of mutation.

MTHFD1 polymorphisms

rs1950902 : R134K

In dehydrogenase/cyclohydrolase domain, may affect the 10-formyl-THF/ 5,10-methylene-THF levels.

G1958A (rs2236225) : R653Q

Located in the synthetize domain, reduces both protein stability and metabolic activity.

In mice - reduced purine biosynthesis and caused increased rate of developmental defects without change in methylation-related metabolites.

In humans - associated with promoter CpG islands hyper-methylation of seven genes in breast cancer tumors.

Increased risk of developing choline deficiency in women on low choline diet.

SHMT polymorphisms

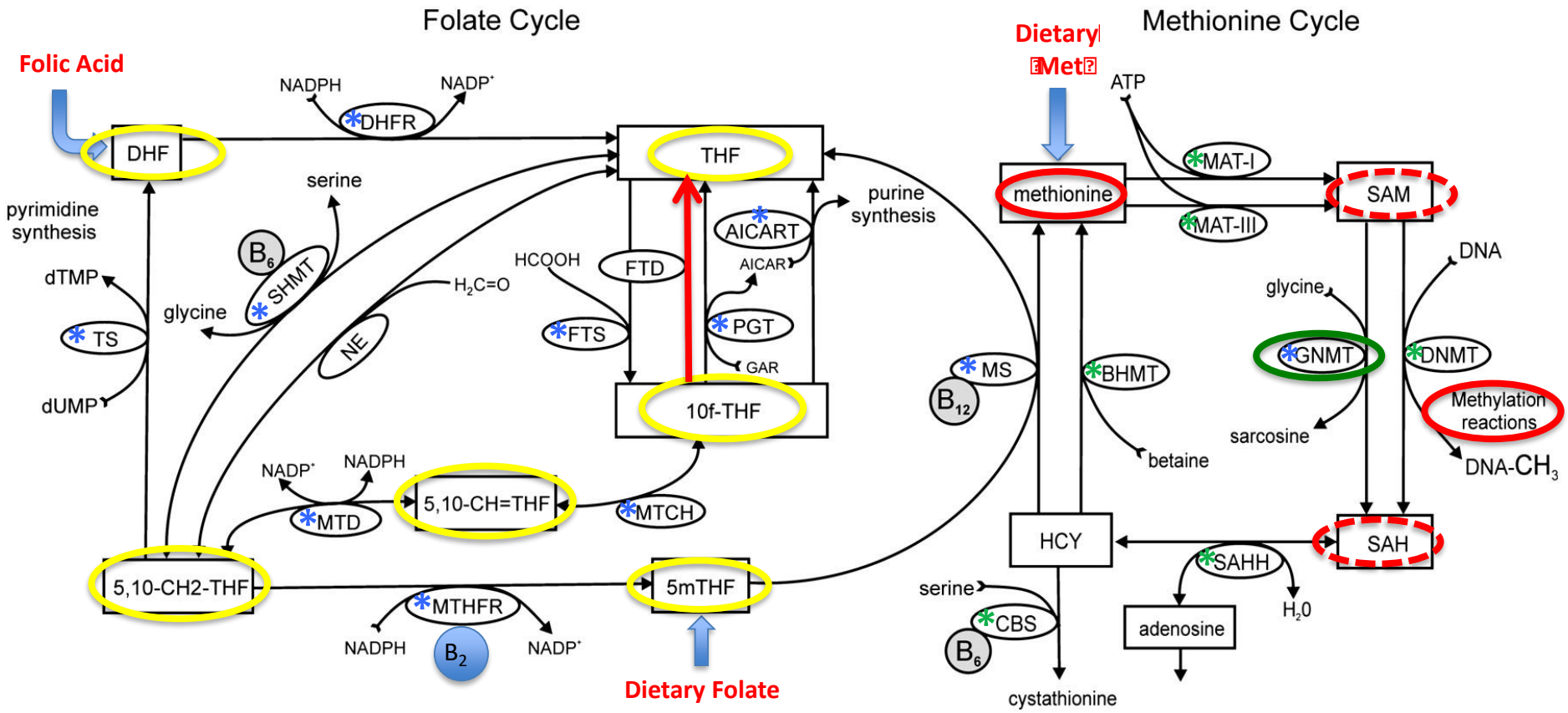
C1420T : rs1979277

Negatively correlates with plasma Hcy

Affects CpG island methylation of Bcl-2/adenovirus E1B 19 kDa-Interacting protein 3 (BNIP3) in duct cell carcinoma.

Increases frequency of cytogenetic damage in patients exposed to tobacco-specific carcinogen 4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanone [NNK].

AlDH1L1 polymorphisms



ALDH1L1 polymorphisms

rs2276731 : T/C substitution in intronic region, likely affecting expression/ regulation of the enzyme. ≥1 variant alleles correlated with higher global DNA methylation, methylation of its own gene and negatively correlated with its own expression. Shown to be associated with increased risk of breast cancer.

rs2002287 : T/C also in the intronic region, shown to associate with decreased risk of breast cancer.

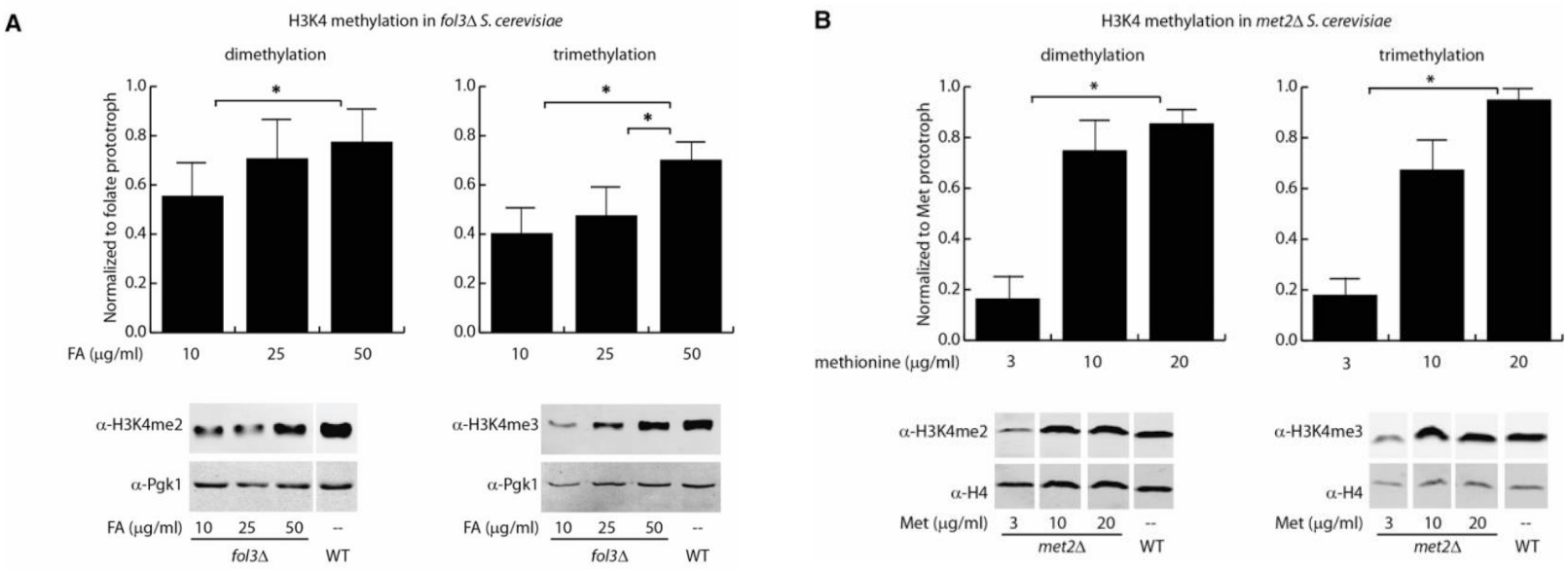
rs4646750 : V812I substitution and in linkage disequilibrium with the two polymorphisms above.

rs2276724 : G481S substitution, in linkage disequilibrium with the first polymorphisms.

rs2886059 : F330V substitution, in linkage disequilibrium with the first polymorphisms.

Nutritional control and histone methylation

From yeast

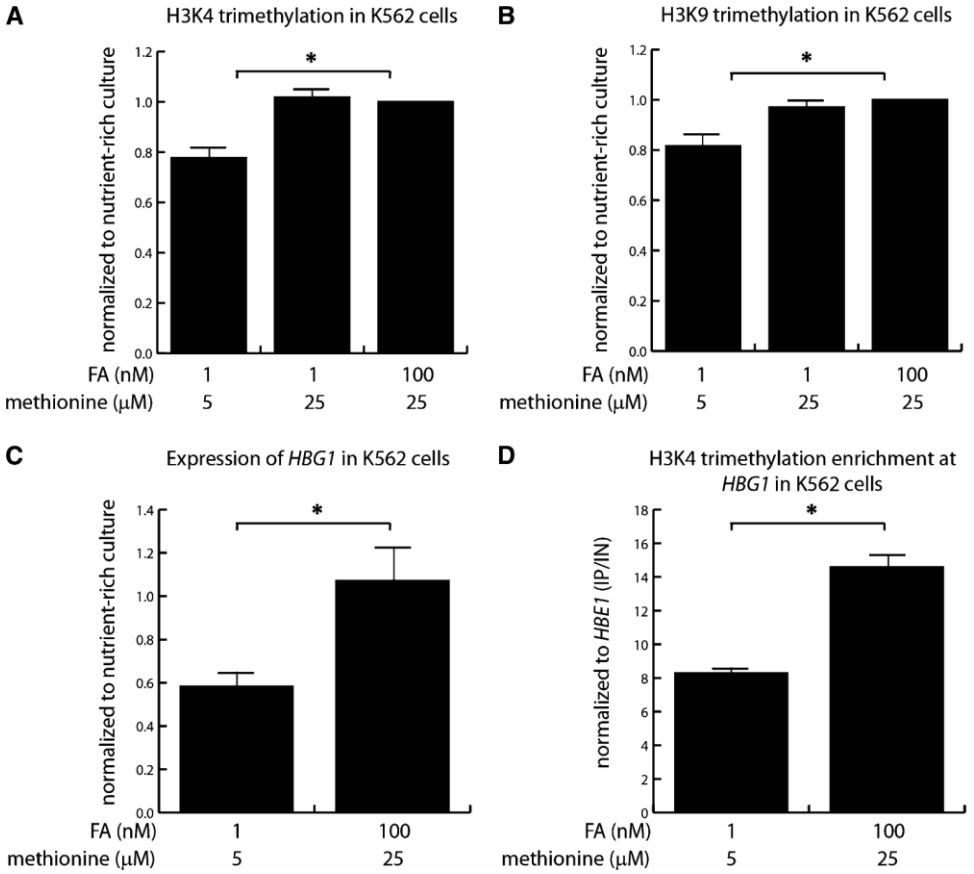


to

Genetics, 195, 831–844, 2013

Nutritional control and histone methylation

Humans



Genetics, 195, 831–844, 2013

Nutritional control and histone methylation

- Nutritional status of eukaryotic cells is linked to histone methylation at least at some sites with epigenetic potential.
- A hierarchy of sensitivities to nutritional limitation exists.
- A metabolic triage mechanism allows cells to compromize less essential histone methylation to preserve SAM for vital functions.
- The triage mechanism does not involve transcriptional or translational control of histone methyltransferases.

Nutrition and histone demethylation

- Histone lysine demethylase 1 (LSD1) utilizes FAD to oxidize metyl group and remove it from lysine residues.
- Second class of lysine demethylases (JHDM) uses a Fe^{2+} and α-ketoglutarate sytem to oxidize N-methyl bond.
- Formaldehyde is produced in both reactions.
- LSD1 was shown to bind THF tightly and specifically.
- The bound THF is believed to scavenge formaldehyde produced in demethylation reaction and protect the enzyme from damage.

Epigenetic role of folate itself?

Additional regulators

Vitamins

Transporters

Energy metabolism