Methylation introduction

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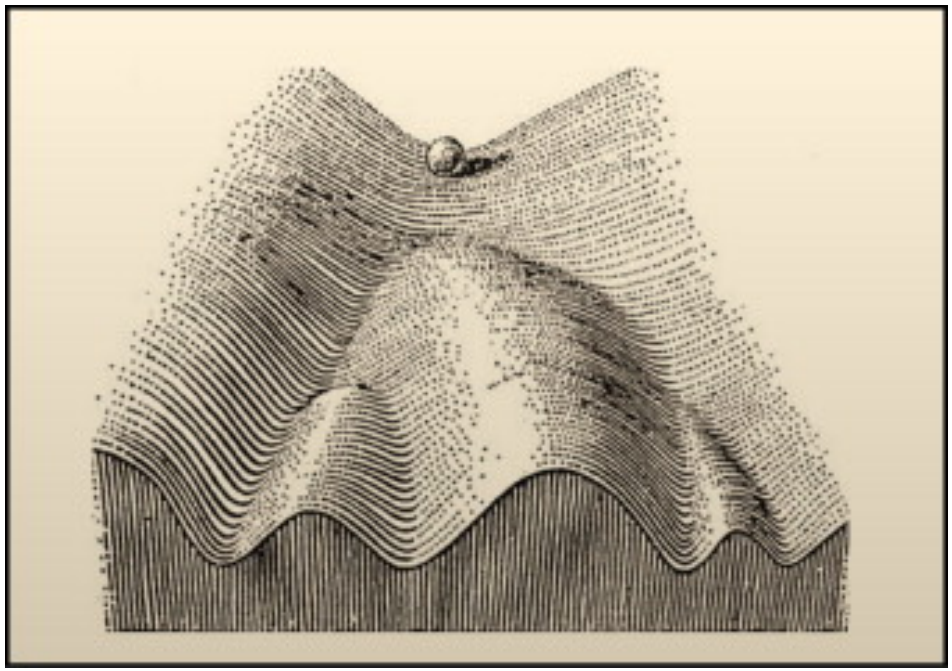
Epigenomics

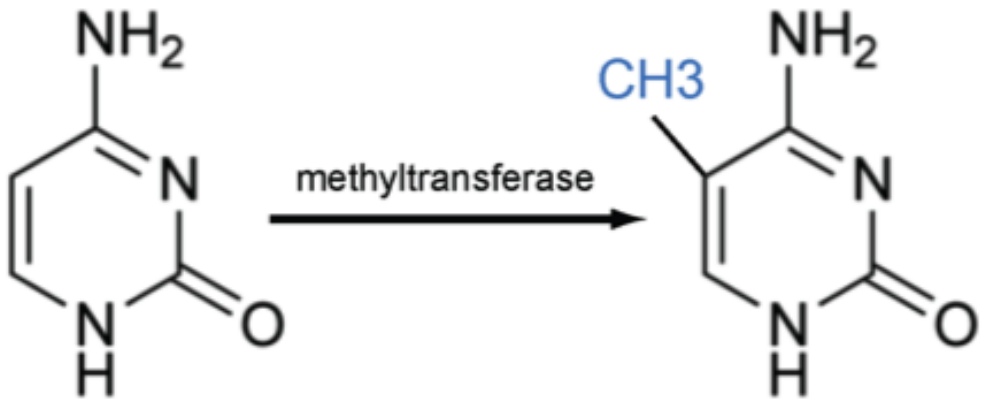
- **Epigenomics** can be defined as the genome-wide investigation of stably heritable phenotypes resulting from changes in a chromosome without alterations in the DNA sequence
- Term coined by Conrad Hal Waddington in 1942

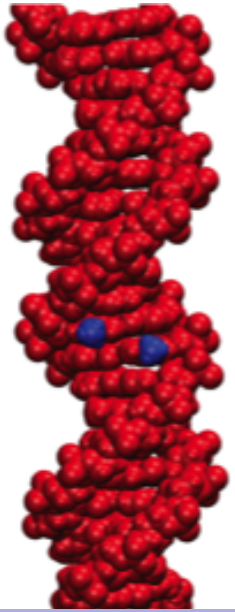


Berger, S. L., Kouzarides, T., Shiekhattar, R., and Shilatifard, A. (2009). An operational definition of epigenetics. Genes Dev. 23, 781–783. doi: 10.1101/gad.1787609

DNA methylation

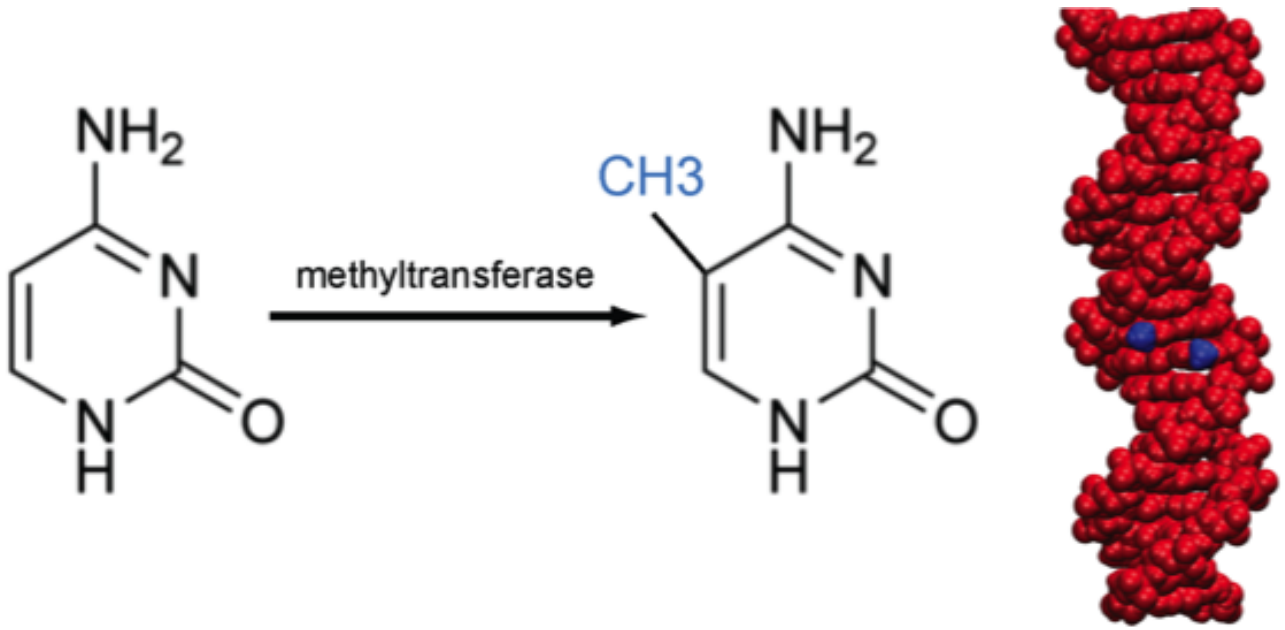
- DNA methylation is a type of chemical modification of DNA which involves the addition of a methyl group to the number 5 carbon of the cytosine (5C), to convert cytosine to 5-methylcytosine (5mC).
- The most well characterized epigenetic mechanism.
- In humans, DNA methylation occurs in cytosines that precede guanines (hence, CpG)
- Invertebrates (Drosophila, yeast) do not exhibit cytosine methylation





DNA modifications

- Other nucleotides (Adenine, Guanine, Thymine) can also be modified
- Mainly in bacteria, but genomes of eukaryotes may contain base modifications on bases other than cytosine, such as methylated adenine or guanine



Sood AJ, Viner C, Hoffman MM. 2016. "**DNAmod: the DNA modification database.**" bioRxiv 071712. https://www.pmgenomics.ca/hoffmanlab/proj/dnamod/

CpG Sites and CpG islands

- CpG sites are not randomly distributed in the genome - the frequency of CpG sites in human genomes is 1% (~28 million CpGs), which is less than the expected (~4-6%).
- Around 60-90% of CpGs are methylated in mammals.
- DNA methylation frequently occurs in repeated sequences, and may help to suppress transcription from these sequences, and aid chromosomal stability.

CpG Sites and CpG islands

- There are regions of the DNA that have a higher concentration of CpG sites ($> 60\%$), named the CpG islands, which tend to be located in the promoter regions of many genes.
- Between 200-1000 bp in length
- Usually not methylated.
- Less than 10% of CpGs occur in CG-dense regions

non-CG methylation

- Embryonic stem cells have ~25% of non-CG methylation (mCHG and mCHH, where H=A, C, T).
- non-CG methylation correlates with gene expression in ESCs.
- non-CG methylation is on anti-sense strand of gene bodies, and correlates with increased *intronic* transcription in ESCs.
- non-CG methylation is depleted in ehancers in ESCs.

Lister, Ryan, Mattia Pelizzola, Robert H. Dowen, R. David Hawkins, Gary Hon, Julian Tonti-Filippini, Joseph R. Nery, et al. "Human DNA Methylomes at Base Resolution Show Widespread Epigenomic Differences." Nature 462, no. 7271 (November 19, 2009): 315–22. https://doi.org/10.1038/nature08514.

Creation and maintenance of DNA methylation

- In humans, DNA is methylated by three enzymes, DNA methyltransferase DNMT1, DNMT3a, DNMT3b.
- DNMT1 is the maintenance methyltransferase that is responsible for copying DNA methylation patterns to the daughter strands during DNA replication.
- DNMT3a and 3b are the *de novo* methyltransferases that, in combination with DNMT3L, set up DNA methylation patterns early in development.

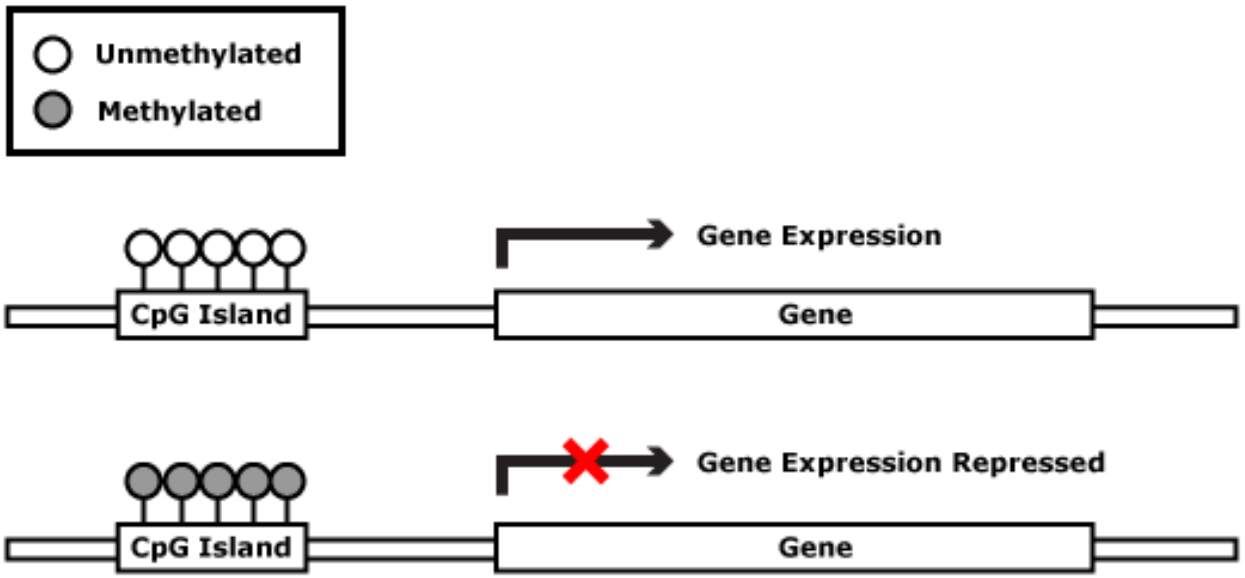
Removal of DNA methylation

- Loss of 5mC can be achieved **passively** by dilution during replication or exclusion of DNMT1 from the nucleus.
- Ten-eleven translocation (TET) family of proteins can **actively** convert 5-methylcytosine (5mC) into 5-hydroxymethylcytosine (5hmC) in vertebrates - demethylation
- Iterative oxidations of 5hmC catalysed by TET result in 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC). 5caC mark is excised from DNA by G/T mismatch-specific thymine-DNA glycosylase (TDG), which as a result returns cytosine residue back to its unmodified state

Guo, Junjie U., Yijing Su, Chun Zhong, Guo-li Ming, and Hongjun Song. "Hydroxylation of 5-Methylcytosine by TET1 Promotes Active DNA Demethylation in the Adult Brain." Cell 145, no. 3 (April 29, 2011): 423–34. https://doi.org/10.1016/j.cell.2011.03.022.

Roles of DNA methylation

- Transcriptional gene silencing
- Maintain genome stability
- Embryonic development
- Genomic imprinting
- X chromosome inactivation (females)



http://learn.genetics.utah.edu/content/epigenetics/imprinting/

Factors associated with changes in DNA methylation

- Aging (developmental stage)
- Diet
- Inflammatory patterns
- Environmental exposures
- Smoking
- Alcohol

DNA methylation and cancer

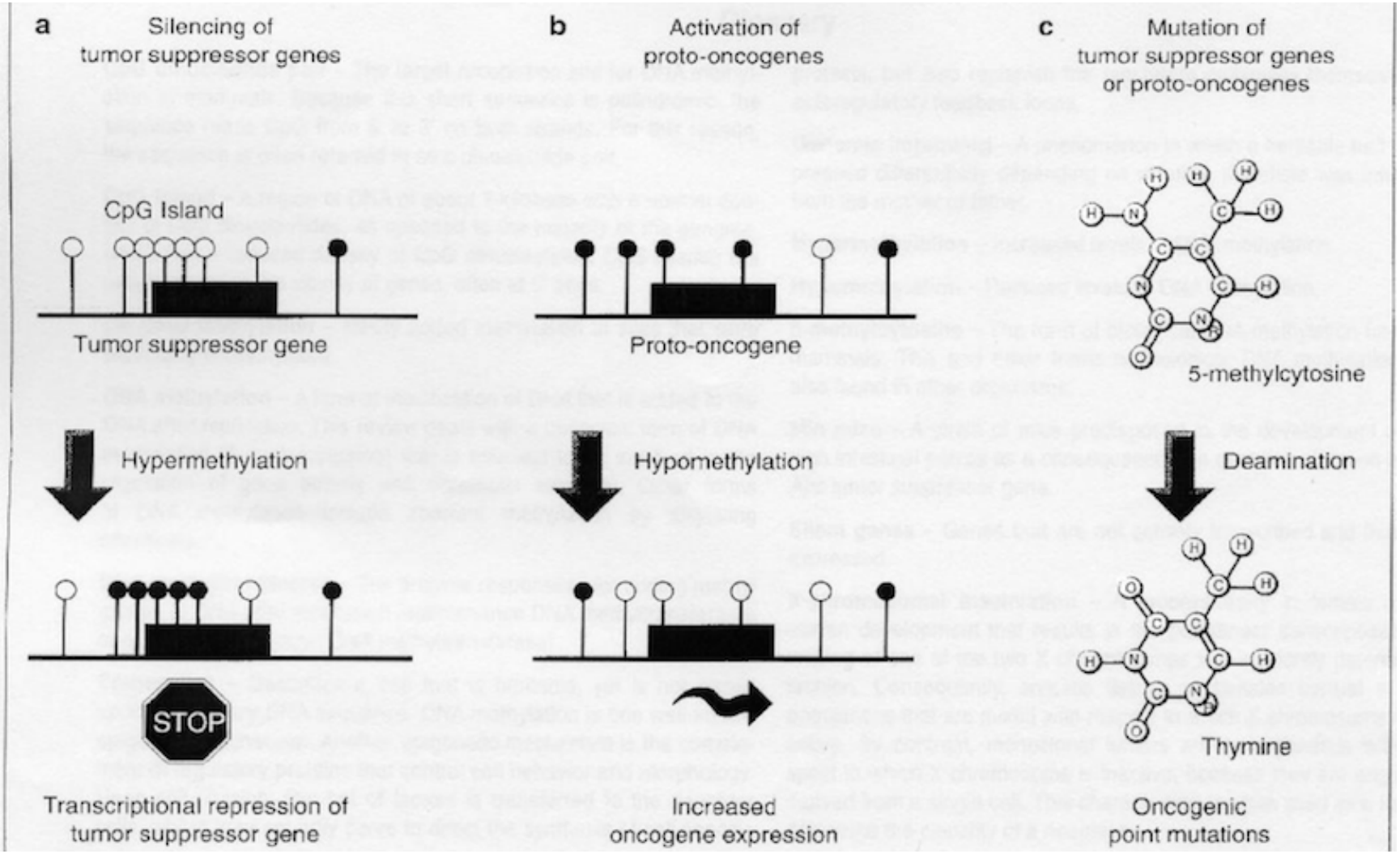
Hypomethylation – decrease methylation levels

- A lower level of DNA methylation in tumors was one of the first epigenetic alterations to be found in human cancer. (Feinberg AP, et al., 1983).
- Demethylation of the promoter region of proto-oncogenes will activate normally repressed gene expression
- Global hypomethylation of DNA sequences that are normally heavily methylated may result in:
  - Chromosomal instability
  - Increased transcription from transposable elements
  - An elevated mutation rate due to mitotic recombination

DNA hypermethylation

Hypermethylation – increase methylation levels

- Hypermethylation of the CpG islands in the promoter regions of tumor-suppressor genes is a major event in the origin of many cancers.
- Hypermethylation of promoters can inactivate tumor-suppressor genes, affect genes involved in the cell cycle, DNA repair, and the metabolism of carcinogens, all of which are involved in the development of cancer.
- The profiles of hypermethylation of the CpG islands in tumor-suppressor genes are specific to the cancer type.



Laird PW "**Oncogenic mechanisms mediated by DNA methylation.**" Mol Med Today. 1997
http://www.cell.com/moltod/pdf/S1357-4310(97)01019-8.pdf

Application of DNA methylation assays

Early diagnosis

- Detection of CpG-island hypermethylation in biological fluids and serum

Prognosis

- Hypemethylation of specific genes
- Whole DNA methylation profiles

Prediction

- CpG island hypermethylation as a marker of response to chemotherapy

Prevention

- Developing DNMTs inhibitors as chemopreventive drugs to reactive silenced genes