Field Guide to Methylation Methods

Methylation and Cellular Processes

Cytosine methylation (5-mC) has a significant effect on gene expression and chromatin remodeling. Cytosine methylation and hydroxymethylation (5-hmC) regulate spatial and temporal gene expression, and are critical for embryonic development and cellular differentiation processes.

- **Modulation of gene expression.**
  Cytosine methylation in promoter regions often reduces gene expression. 88% of active promoters are associated with unmethylated CpGs in mammals. Hydroxymethylation often correlates with increased gene expression.

- **Change in chromatin structure.**
  Heterochromatin formation is initiated by hypermethylation. Methyl binding domain (MBD) proteins specific for 5-mC recruit histone-modifying and chromatin-remodeling proteins. Abnormal hypomethylation of heterochromatin (normally hypermethylated) is linked to chromosomal instability and loss of imprinting. Controlled changes in chromatin structure are responsible for selective X chromosome inactivation and suppression of transposable elements.

- **Cellular differentiation.**
  Methylation change usually drives one-way differentiation; that is, differentiated cells do not typically revert to stem cells.

- **Onset of diseases.**
  Perturbation of any of the above processes may result in disease.

Glossary

5-Methylcytosine (5mC) Formed by addition of -CH3 to N5 position of cytosine. 70–80% of CpG dinucleotides are methylated (high proportion in repetitive elements). In plants, cytosine methylation is observed at CpG, CpHpG, and CpHpH (H = nucleotide other than guanine).

5-Formylcytosine Oxidation product of 5-hmC. Detected in mouse embryonic stem cells.

Base modification Addition of methyl- or other groups to nucleic acid bases. In addition to 5-methylcytosine, other common modifications are 7-methylguanosine (5'-cap for RNA), and 6-methyladenosine (common RNA modification.)

CpG island Defined as regions > 500 bp, > 55% GC and expected/observed CpG ratio of > 0.65. 40% of gene promoters contain islands.

CpG shelves ~4Kb from islands.

CpG shores ~2Kb from islands, > 75% of tissue-specific differentially methylated regions found in shores. Methylation in shores shows higher correlation with gene expression than CpG islands.

Differentially methylated regions (DMR) Cell-, tissue-, and condition-specific differences in methylation.

Enhancer A short region of DNA that can activate transcription and is often regulated by methylation.

Expected/observed CpG ratio The human genome contains 25% of the expected number of C-G pairs due to spontaneous deamination of meC to T over evolutionary time scales.

Genomic imprinting An epigenetic process causing genes to be expressed only from one of the parental chromosomes.

Hypermethylation Most cytosines are methylated

Hypomethylation Most cytosines do not have 5-mC. Euchromatin and active gene promoters are hypomethylated.

Methylation Enzymes

Mammalian

DNMT1 (DNA methyltransferase) isoforms methylate hemimethylated CpGs, maintaining methylation patterns during DNA replication.

DNMT3a and DNMT3b, de novo methyltransferases that set up DNA methylation patterns early in development; can methylate unmethylated and methylated DNA.

DNMT3L unknown; facilitates de novo methyltransferase activity.

TET (Ten eleven translocation) enzymes involved in methyl group oxidation with the production of 5-hydroxymethylcytosine as an intermediate.

Plant

DRM2 (RNA directed DNA methylation) homologous to DNMT3.

MET1 (methyltransferase 1) homologous to DNMT1.

CMT3 unique to plants. Function for other methyl transferases unknown.

Bacteria

Dam (DNA adenine methyltransferase), methylates ‘A’ in GATC, key role in mismatch repair, DNA replication timing, and gene regulation. Independent of restriction modification systems.

Dcm (DNA cytosine methylase) produces 5-mC in CCAG and CCTGG sites.

EcoKI methylates adenine in AAC(N6)GTGC and GCAC(N6)GTT.


