DNA methylation & demethylation

Lars Schomacher (Group Christof Niehrs)
What is Epigenetics?

“Epigenetics is the study of heritable changes in gene expression (active versus inactive genes) that do not involve changes to the underlying DNA sequence — a change in phenotype without a change in genotype — which in turn affects how cells read the genes.”

https://www.whatisepigenedtics.com/fundamentals/
Epigenetics at different levels

Overview

1) DNA methylation
2) Detection of DNA methylation
3) Regulation of gene expression by DNA methylation
4) Biological significance of DNA methylation
5) Reversibility of DNA methylation
1) DNA methylation
5-Methylcytosine

DNA methyltransferases (DNMTs)

- typical human cell: 3-5% of all C’s are methylated
DNA methylation is maintained

*Palindromic methylation*

5′-NpNpCpGpNpN-3′
3′-NpNpGpCpNpN-5′

DNMT3A and DNMT3B = De novo methylation
DNMT1 = Maintenance methylation

Wu & Zhang, Nat Rev Mol Cel Biol, 2010
Evolution of DNA methylation

Zemach & Zilberman, Curr. Biol., 2010
DNMTs induce DNA damage

Possible explanation for independent losses of DNMTs?

Rosic et al., Nat. Genetics, 2018
CpG methylation is mutagenic

- C/5mC undergo spontaneous deamination
- U is efficiently repaired, T not
- 5mC deamination leads to C-T transition mutations
- CpGs are 5x under-represented in genomes of CpG-methylating organisms

https://www.atdbio.com/content/56/Epigenetics
Methylation-dependent genome evolution

CpG-site

Ancestral genome

Methylated CpG site

evolutionary timescale

Modern genome

CpG island

“Erosion rock”

CpG islands

• at least 200 bp long regions with a CpG-density higher 50%

• 70% of promoters located near the transcription start site of a gene (proximal promoters) contain a CpG island

• CpG islands are mostly unmethylated (and escape mutation via deamination)
Mammalian methylation landscape

https://en.wikipedia.org/wiki/DNA_methylation
2) Detection of DNA methylation
Detection of methylation: MeDIP

MeDIP: (Methylated DNA Immunoprecipitation)

- Purification of genomic DNA from tissue sample
- Sonication
- Denaturation
- Locus-specific (MeDIP-PCR) or Genome-wide (MeDIP-chip, MeDIP-seq) analysis
- "IP" reaction
- DNA elution and purification

Karpova & Umemori, Epigenetic Methods in Neuroscience Research, 2016
Detection of methylation: MeDIP

MeDIP-qPCR

MeDIP-seq

Xing et al., DNA Methylation Protocols, 2017
Detection of methylation: Bisulfite sequencing

Bisulfite conversion + Sequencing

https://upload.wikimedia.org/wikipedia/commons/b/bc/Bisulfite_conversion.svg

Detection of methylation: Bisulfite sequencing

BS amplicon sequencing

Whole genome BS-seq


https://genestack-user-tutorials.readthedocs.io/tutorials/Methylation_profiling/
3) Regulation of gene expression by DNA methylation
Methylation and transcription

Laurent et al., Genome Res., 2010
Tissue-specific methylation differences... 

... reflect tissue specific gene expression

Brena et al., Nat. Genetics, 2006
Repression by methylation - 1

Direct interference with DNA binding of TFs

Repression by methylation - 2

Recruitment of methyl-CpG binding proteins

**MBD family**

- **MBD1**
- **MBD2**
- **MBD3**
- **MBD4**
- **MeCP2**

**Zinc Finger family**

- **Kaiso**
- **ZBTB4**
- **ZFP57**

**SRA family**

- **UHRF1**
- **UHRF2**

Karpova & Umemori, Epigenetic Methods in Neuroscience Research, 2016
Repression by methylation - 2

Consequence of MBD binding

DNA information

Unmethylated CpG in the promoter

Methylated CpG in the promoter

Active

Inactive

TF

HAT

RNA Pol II

Transcription

HDAC

HMT

MBD

No transcription

https://www.omicsonline.org/articles-images/JBAM-04-e108-g001.html
4) Biological significance of DNA methylation
DNMT-lethality in mice

wildtype  Dnmt3a/b KO  Dnmt1 KO

Okano et al., Cell, 1999
Rett syndrome

Caused by mutated MeCP2 (methyl-CpG-binding protein 2)

http://www.graceforrett.com/rett-syndrome/r168x/hi-im-kirsty/
Methylation and cancer

- Global hypomethylation
  - Retrotransposons
  - oncogenes

- Local hypermethylation
  - tumor suppressor genes
Genomic imprinting

A maternal and a paternal genome are required for development

http://www.mun.ca/biology/desmid/brian/Biol3530/DEVO_09/ch09f11.jpg

https://www.semanticscholar.org/paper/Genomic-imprinting-in-mammals.-Barlow-Bartolomei/e59220a32087be34ae0c9345d3c3094260f0ca8d/figure/2
Genomic imprinting

imprinted gene

paternal

maternal

DNA methylation

autosomal gene

Promoter

\[\text{w/ no DNA Methylation}\]

\[\text{w/ DNA Methylation}\]

https://slideplayer.com/slide/6330819/
X-chromosome inactivation...

...is concomitant with hypermethylation of the inactive X-chromosome
Methylation and aging

Horvath’s epigenetic clock: predictor of biological age

5) Reversibility of DNA methylation
Pronuclear DNA demethylation

Mayer et al., Nature, 2000
Pronuclear demethylation

Figure a shows a graph of DNA methylation levels during early development, with stages including Fertilization, Zygote, 2 cell, 4 cell, 8 cell, Morula, and Blastocyst. The graph indicates a decrease in DNA methylation from fertilization to the blastocyst stage, highlighting the process of pronuclear demethylation. The diagram is attributed to Wu & Zhang, Nat Rev Mol Cell Biol, 2010.
Primordial germ cell demethylation

Wu & Zhang, Nat Rev Mol Cel Biol, 2010
DNA demethylation

Wu & Zhang, Nat Rev Mol Cel Biol, 2010
Active DNA demethylation

- Direct reversal
- BER pathway
- NER pathway
- MMR pathway

<table>
<thead>
<tr>
<th>Event</th>
<th>Year</th>
</tr>
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<tbody>
<tr>
<td>TDG as 5mC glycosylase</td>
<td>1995</td>
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<tr>
<td>MBD2 as 5mC demethylase</td>
<td>1995</td>
</tr>
<tr>
<td>MBD4 as 5mC glycosylase</td>
<td>2000</td>
</tr>
<tr>
<td>AID/APOBEC as 5mC deaminases</td>
<td>2005</td>
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<tr>
<td>GADD45 and NER in demethylation</td>
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<tr>
<td>DNMT3a/b as 5mC dehydroxylases</td>
<td>2010</td>
</tr>
<tr>
<td>DNMT1 + 3a/b as 5mC demethylases</td>
<td>2010</td>
</tr>
<tr>
<td>AID/APOBEC as 5hmC deaminases</td>
<td>2010</td>
</tr>
<tr>
<td>TDG as 5fC and 5caC glycosylase</td>
<td>2010</td>
</tr>
<tr>
<td>DNMT3a/b as 5caC decarboxylases</td>
<td>2015</td>
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<td>MMR in demethylation</td>
<td>2015</td>
</tr>
</tbody>
</table>

Schomacher & Niehrs, Bioessays, 2017
Radical-mediated demethylation

1. Direct removal of methyl group

- not biochemically verified!
BER-mediated demethylation

2. Excision of unedited 5mC

• only in plants

Wu & Zhang, Nat Rev Mol Cel Biol, 2010
NER-mediated demethylation

2. Excision of unedited 5mC

- not biochemically nor *in vivo* verified
Deamination and BER demethylation

3. Excision of edited 5mC - 1

- not biochemically nor in vivo verified

Wu & Zhang, Nat Rev Mol Cel Biol, 2010
Breakthrough in active demethylation

Conversion of 5-Methylcytosine to 5-Hydroxymethylcytosine in Mammalian DNA by MLL Partner TET1

Mamta Tahiliani,1 Kian Peng Koh,1 Yinghua Shen,2 William A. Pastor,1 Hozefa Bandukwala,1 Yevgeny Brudno,2 Suneet Agarwal,3 Lakshminarayan M. Iyer,4 David R. Liu,2* L. Aravind,4* Anjana Rao1*

The Nuclear DNA Base 5-Hydroxymethylcytosine Is Present in Purkinje Neurons and the Brain

Skirmantas Kriaucionis and Nathaniel Heintz*

Science, 15. May 2009
Oxidative DNA demethylation

3. Excision of edited 5mC - 2

Oxidative demethylation in vivo - 1

mESCs

Shen et al., Cell, 2013
Oxidative demethylation \textit{in vivo} - 2

Zygote

\[ \text{Inoue et al., Cel Res, 2011} \]
Model: global demethylation in zygotes

Summary

- Cytosine methylation...
  - ...occurs mostly within CpG dinucleotides
  - ...is heritable to progeny
  - ...has partially been lost during eukaryotic evolution
  - ...increases genome instability
  - ...can be monitored by e.g. MeDIP and bisulfate sequencing
  - ...leads to gene silencing and heterochromatin
  - ...plays important roles in development and disease
  - ...is actively erased by oxidative DNA demethylation
Functions of DNA methylation: islands, start sites, gene bodies and beyond

Peter A. Jones

DNA METHYLATION AND HUMAN DISEASE

Keith D. Robertson

DNA methylation: roles in mammalian development

Zachary D. Smith and Alexander Meissner
Literature: DNA demethylation

Active DNA demethylation: many roads lead to Rome
Susan C. Wu and Yi Zhang

REVIEW
TET enzymes, TDG and the dynamics of DNA demethylation
Rahul M. Kohli and Yi Zhang
472 | NATURE | VOL 502 | 24 OCTOBER 2013

Reversing DNA Methylation: Mechanisms, Genomics, and Biological Functions
Hao Wu and Yi Zhang
Cell 156, January 16, 2014 ©2014 Elsevier Inc.

TET-mediated active DNA demethylation: mechanism, function and beyond
Xiaojie Wu and Yi Zhang

www.imb.de