

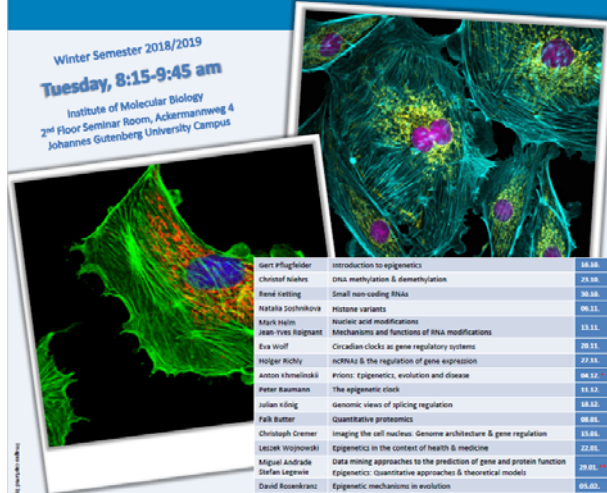
# DNA methylation & demethylation

Lars Schomacher (Group Christof Niehrs)

**imb**  
Institute of  
Molecular Biology

Lecture Series  
**Introduction  
to Epigenetics**

Winter Semester 2018/2019  
**Tuesday, 8:15-9:45 am**  
Institute of Molecular Biology  
2nd Floor Seminar Room, Ackermannweg 4  
Johannes Gutenberg University Campus




Gert Pfugfelder	Introduction to epigenetics	08.10.
Christof Niehrs	DNA methylation & demethylation	15.10.
Rand Letting	Small non-coding RNAs	22.10.
Natalie Dosthikova	Histone variants	29.10.
Mark Helm	Nucleic acid modifications	05.11.
Jean-Yves Hoignat	Mechanisms and functions of tRNA modifications	12.11.
Eva Wolf	Circadian clocks as gene regulatory systems	19.11.
Hoggar Richy	lncRNAs & the regulation of gene expression	27.11.
Anton Khrmel'ski	Prions: Epigenetics, evolution and disease	04.12.
Heinz Baumann	The epigenetic clock	11.12.
Jelena Kling	Genomic views of splicing regulation	18.12.
Falk Butter	Quantitative proteomics	25.12.
Christoph Cremer	Imaging the cell nucleus: Genome architecture & gene regulation	01.01.
Leszek Wojnowski	Epigenetics in the context of health & medicine	08.01.
Miguel Andrade	Data mining approaches to the prediction of gene and protein function	15.01.
Stefan Legewie	Epigenetics: Quantitative approaches & theoretical models	22.01.
David Rosenkrantz	Epigenetic mechanisms in evolution	05.02.

\*\* Change time, when possible at 17:00  
\*\*\* Change location: JGU, Aulastr. 1, Hofsaal A, 55127

The lecture series is intended for Master's students as well as all interested students and scientists.  
Lectures will be held in English.

Please visit [www.imb.de/students/postdocs/lectures](http://www.imb.de/students/postdocs/lectures) for up-to-date information on the lecture series.  
For information, please contact Dr. Gerald Schulten: [gschult@imb.de](mailto:gschult@imb.de), Tel. 06331-28-2460

Participating institutions:



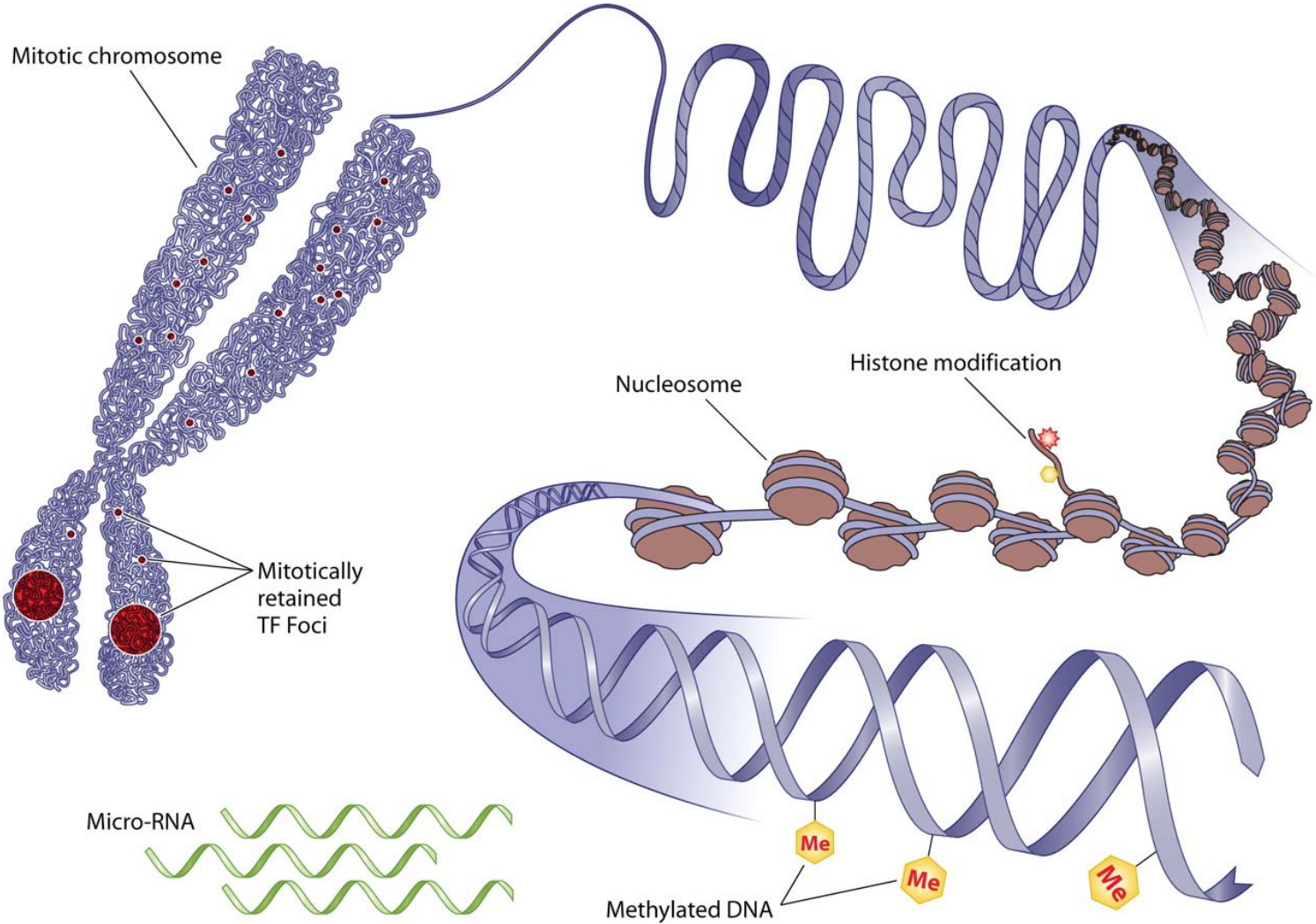
# What is Epigenetics?

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“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.whatisepigenetics.com/fundamentals/>

# Epigenetics at different levels



Zaidi et al., Mol.Cell.Biol., 2010

# Overview

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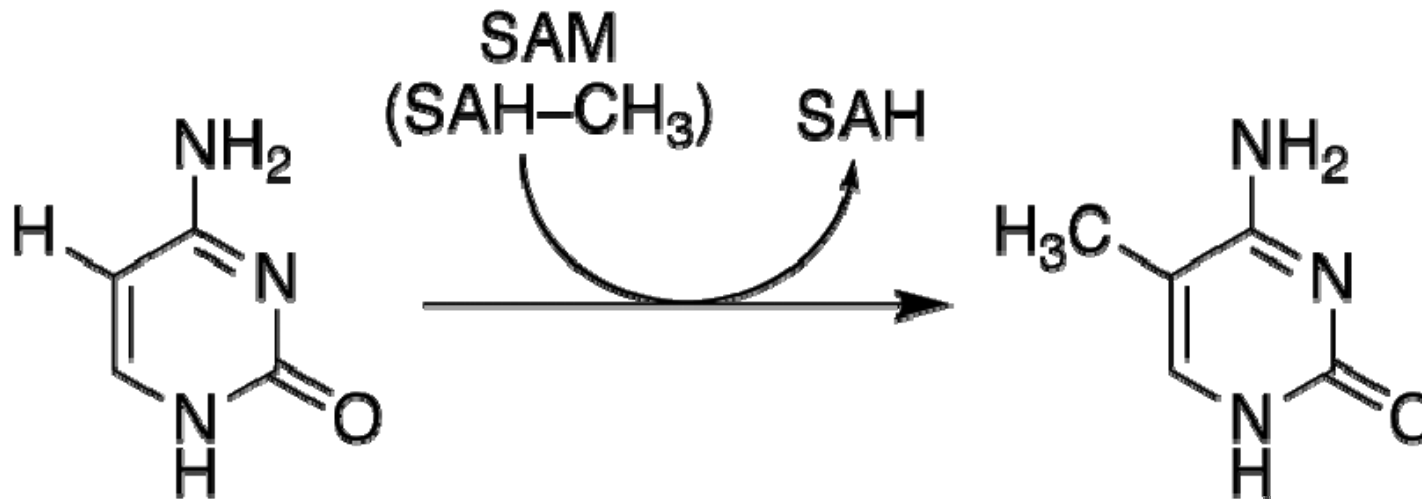
- 1) DNA methylation
- 2) Detection of DNA methylation
- 3) Regulation of gene expression by DNA methylation
- 4) Biological significance of DNA methylation
- 5) Reversibility of DNA methylation

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# 1) DNA methylation

# 5-Methylcytosine

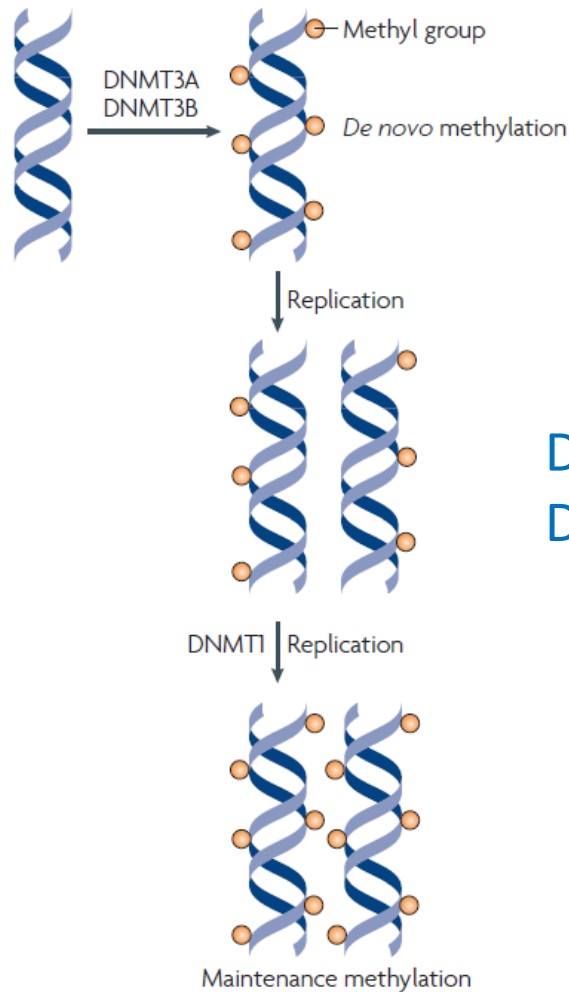
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DNA methyltransferases (DNMTs)

- typical human cell: 3-5% of all C's are methylated

# DNA methylation is maintained



## Palindromic methylation

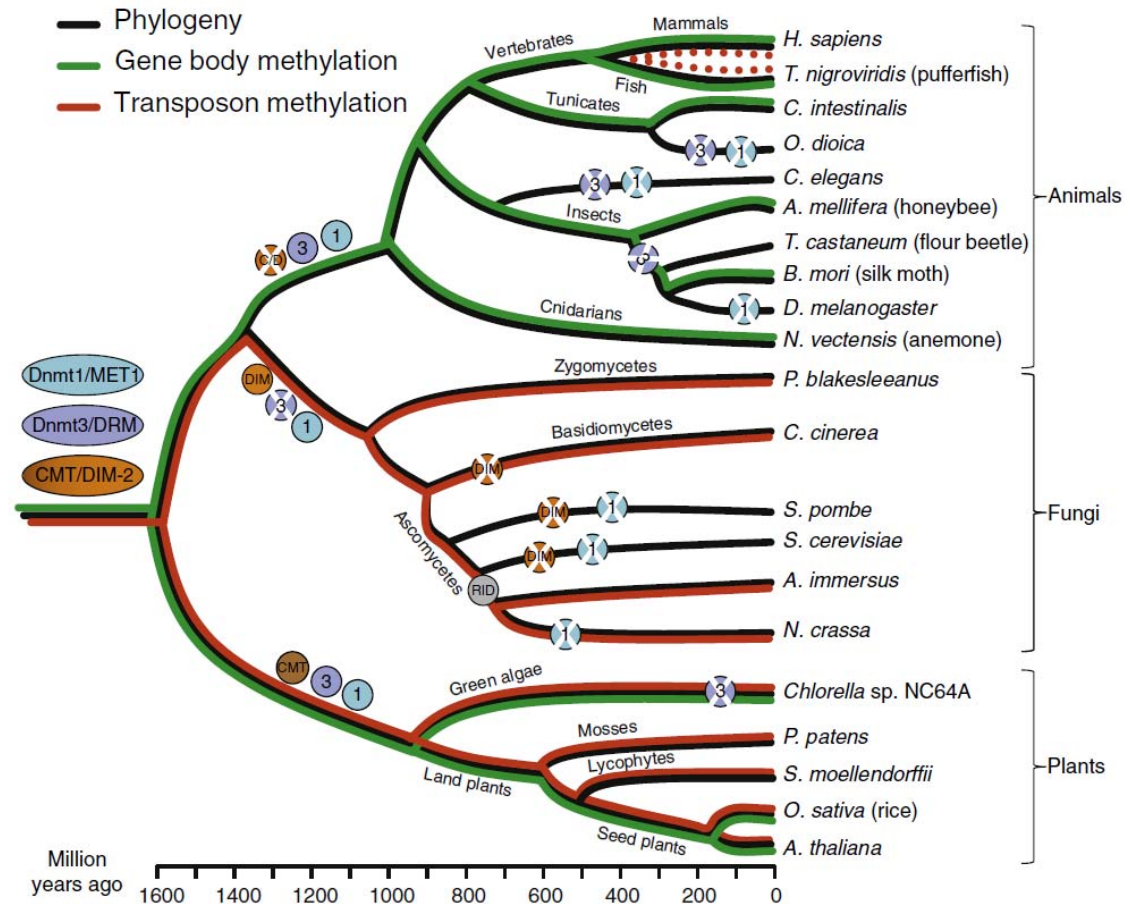
5'-NpNp**CpGp**NpN-3'

3'-NpNp**GpCp**NpN-5'

DNMT3A and DNMT3B = De novo methylation  
DNMT1 = Maintenance methylation

Wu & Zhang, Nat Rev Mol Cell Biol, 2010

# Evolution of DNA methylation

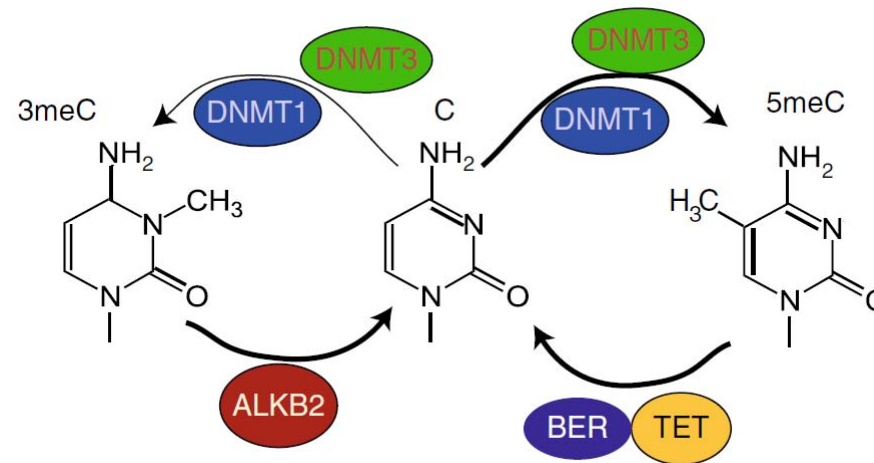


Current Biology

Zemach & Zilberman, Curr. Biol., 2010



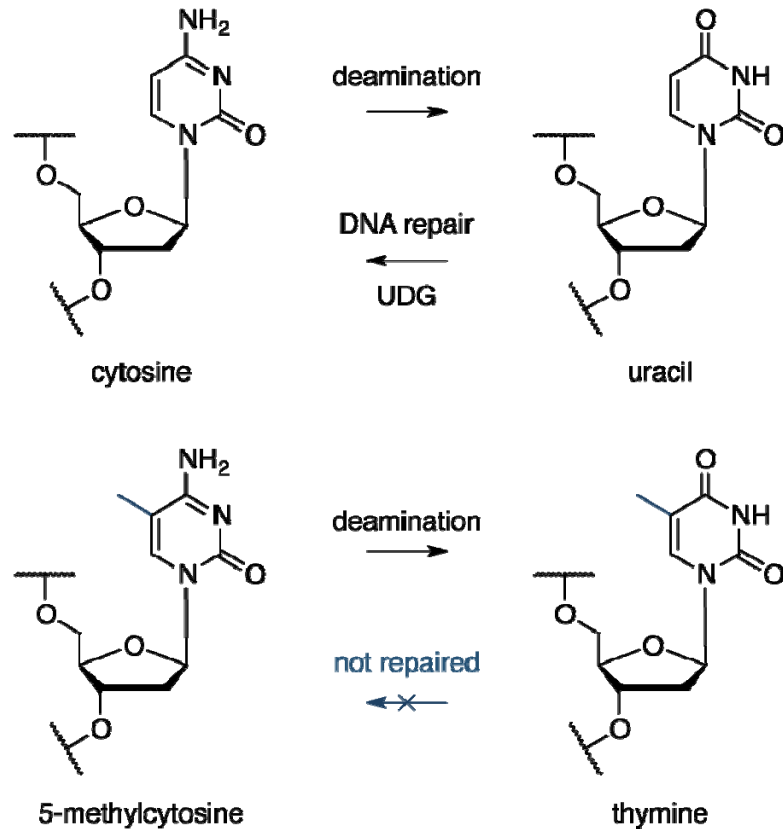
# DNMTs induce DNA damage



Rosic et al., Nat. Genetics, 2018

Possible explanation for independent losses of DNMTs?

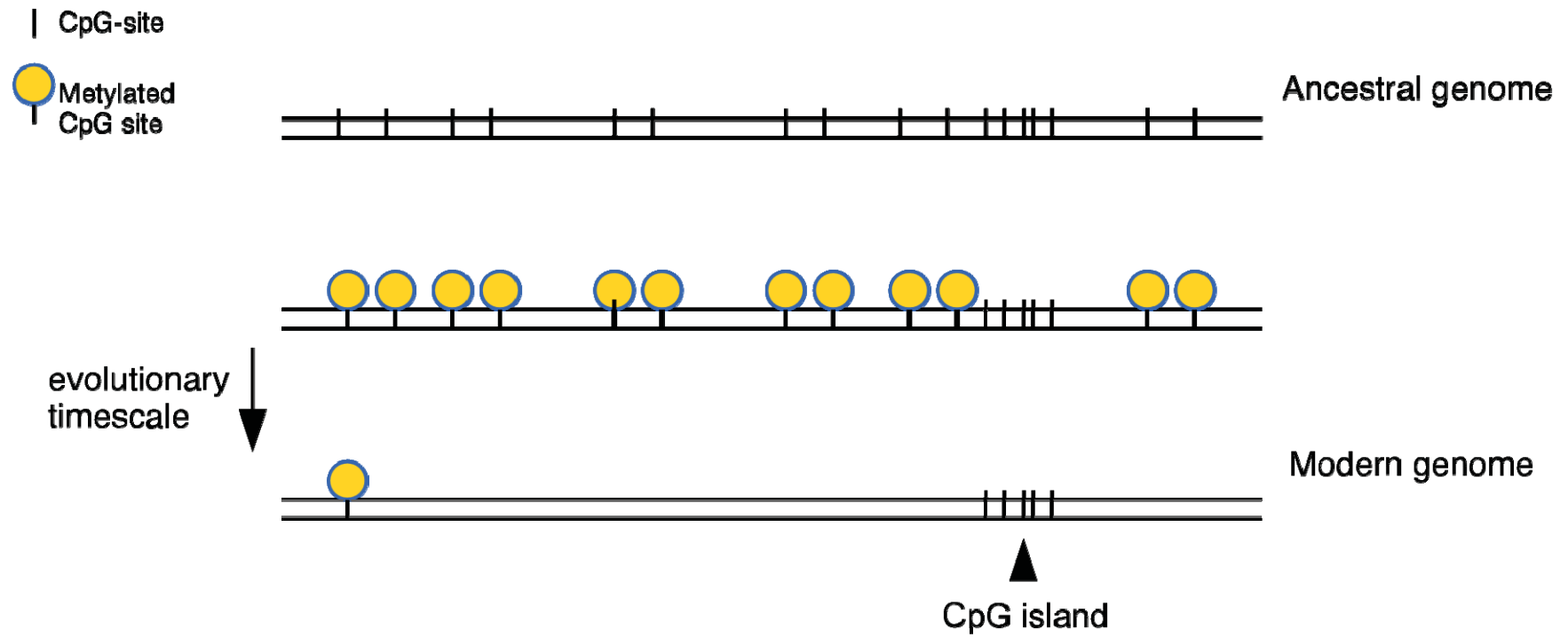
# CpG methylation is mutagenic



- 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



[https://en.wikipedia.org/wiki/CpG\\_site](https://en.wikipedia.org/wiki/CpG_site)

“Erosion rock”

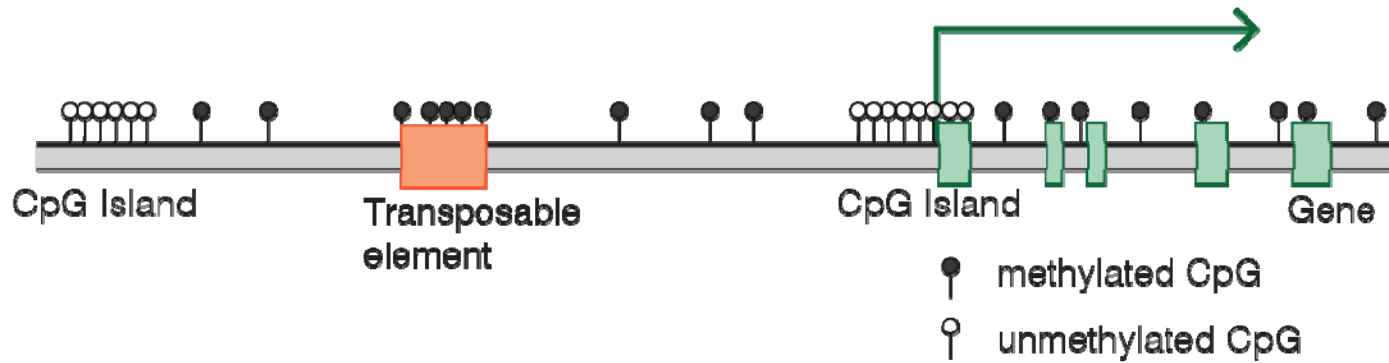
# CpG islands

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- 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

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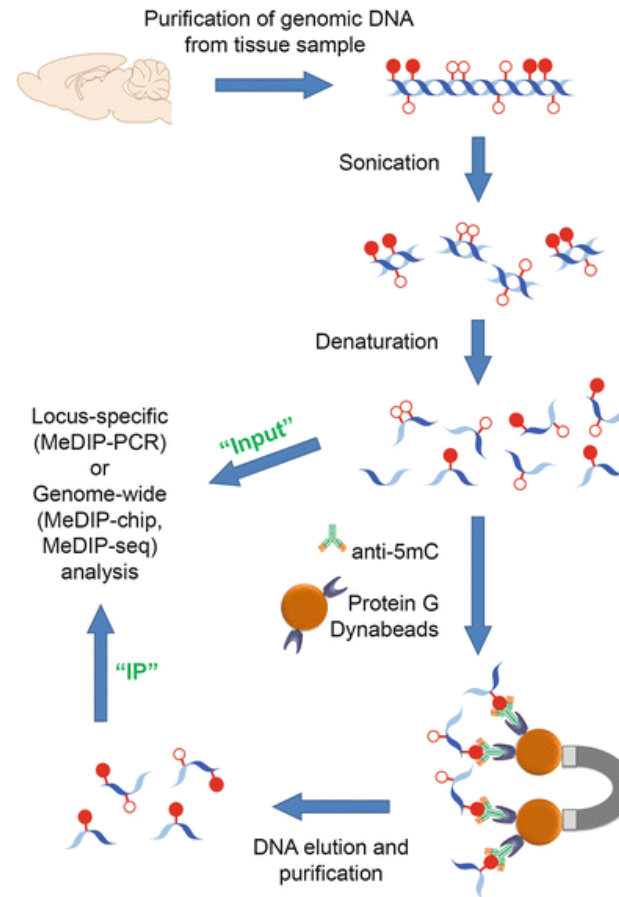
[https://en.wikipedia.org/wiki/DNA\\_methylation](https://en.wikipedia.org/wiki/DNA_methylation)

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## 2) Detection of DNA methylation

# Detection of methylation: MeDIP

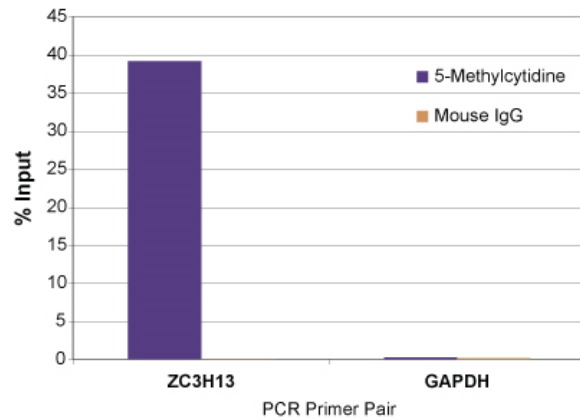
MeDIP: (**M**ethylated **D**NA **I**mmunoprecipitation)



Karpova & Umemori, Epigenetic Methods in Neuroscience Research, 2016

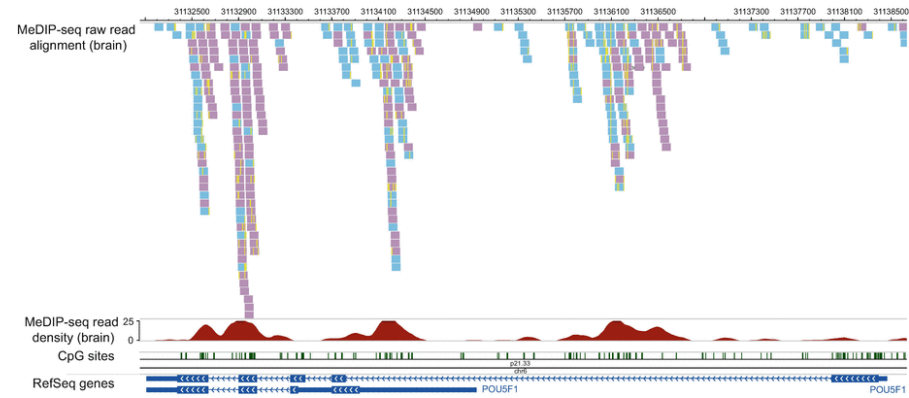
# Detection of methylation: MeDIP

## MeDIP-qPCR



<https://www.activemotif.com/catalog/736/medip>

## MeDIP-seq



Xing et al., DNA Methylation Protocols, 2017

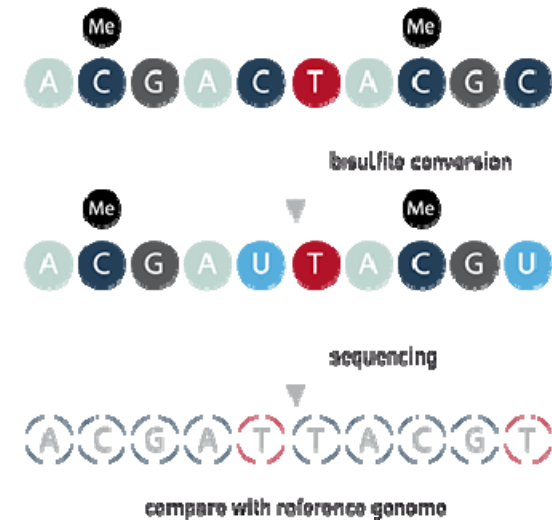
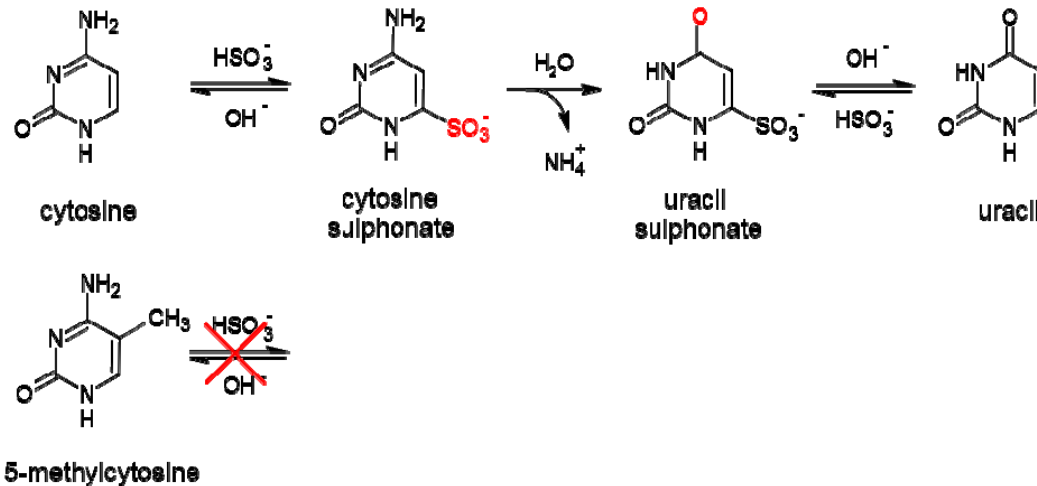


# Detection of methylation: Bisulfite sequencing

Bisulfite conversion

+

Sequencing

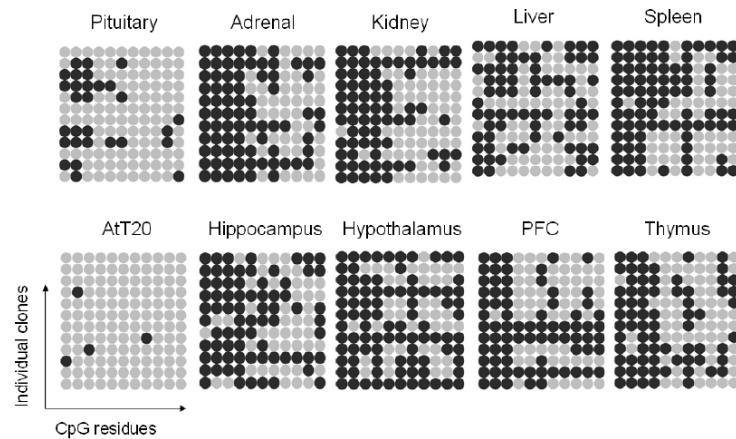


[https://upload.wikimedia.org/wikipedia/commons/b/bc/Bisulfite\\_conversion.svg](https://upload.wikimedia.org/wikipedia/commons/b/bc/Bisulfite_conversion.svg)

<https://www.diagenode.com/en/categories/bisulfite-conversion>

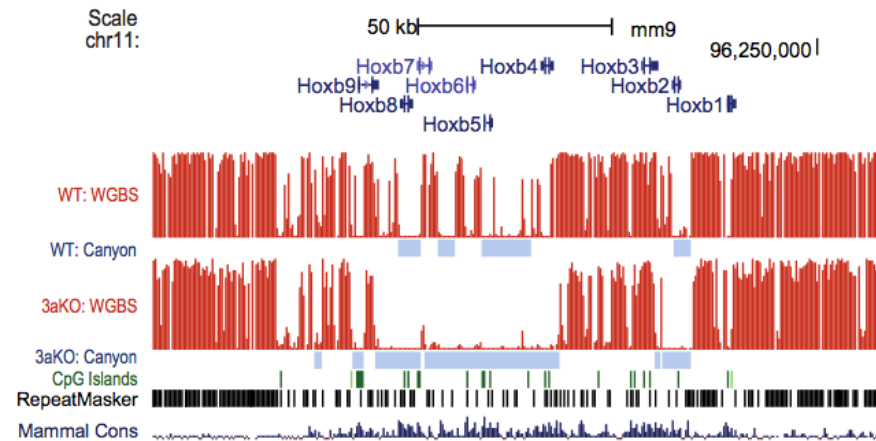
# Detection of methylation: Bisulfite sequencing

## BS amplicon sequencing



[https://www.researchgate.net/publication/269573906\\_Sustained\\_Epi\\_genetic\\_Programming\\_of\\_POMC\\_by\\_Early\\_Life\\_Stress/figures?lo=1](https://www.researchgate.net/publication/269573906_Sustained_Epi_genetic_Programming_of_POMC_by_Early_Life_Stress/figures?lo=1)

## Whole genome BS-seq

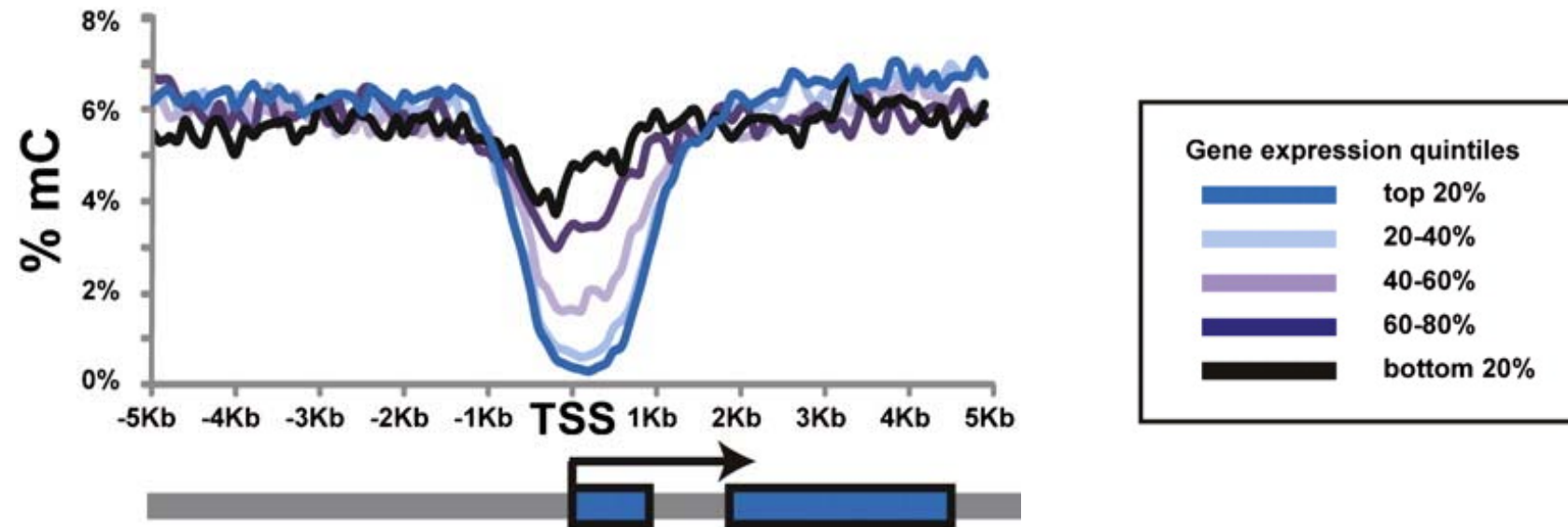


[https://genestack-user-tutorials.readthedocs.io/tutorials/Methylation\\_profiling/](https://genestack-user-tutorials.readthedocs.io/tutorials/Methylation_profiling/)

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# 3) Regulation of gene expression by DNA methylation

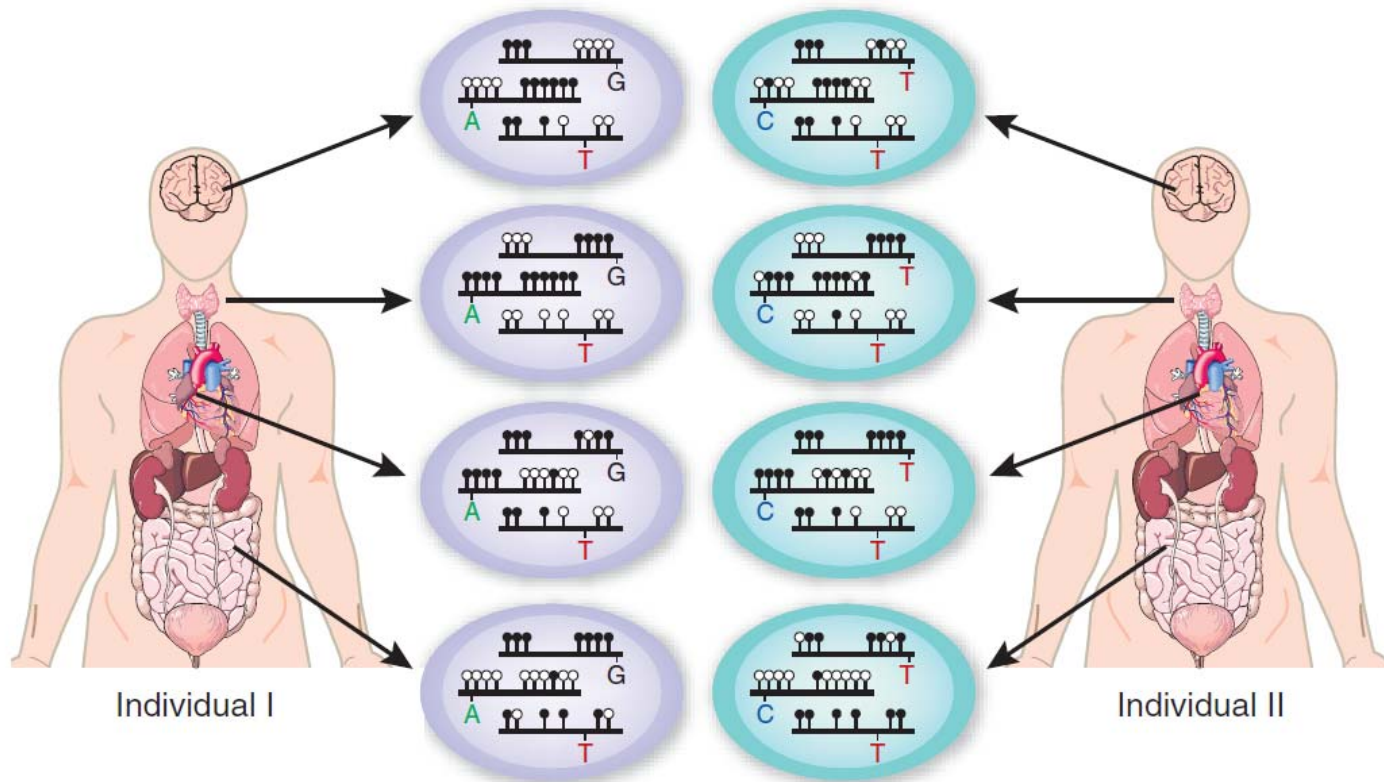
# Methylation and transcription



Laurent et al., Genome Res., 2010

# Tissue-specific methylation differences...

... reflect tissue specific gene expression

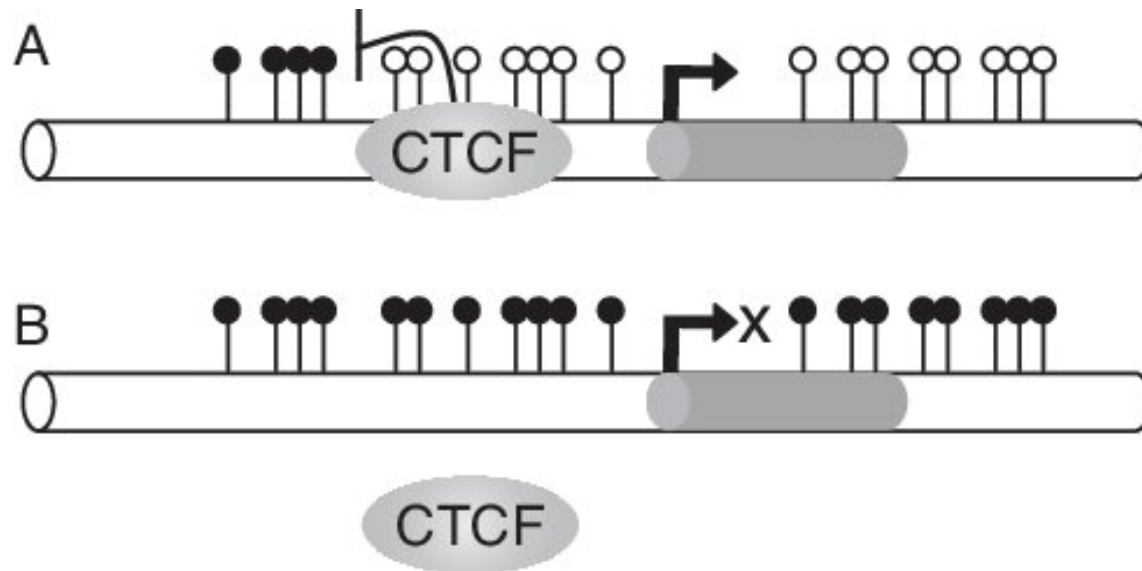


Brena et al., Nat. Genetics, 2006

# Repression by methylation - 1

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Direct interference with DNA binding of TFs

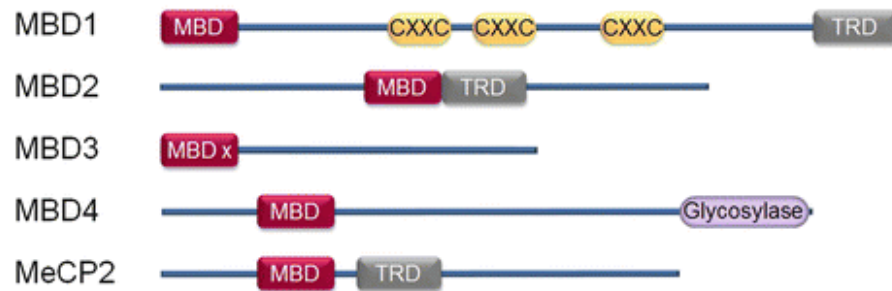


[https://www.researchgate.net/publication/257527016\\_Genetics\\_and\\_Epigenetics\\_of\\_the\\_Multifunctional\\_Protein\\_CTCF/figures?lo=1](https://www.researchgate.net/publication/257527016_Genetics_and_Epigenetics_of_the_Multifunctional_Protein_CTCF/figures?lo=1)

# Repression by methylation - 2

## Recruitment of methyl-CpG binding proteins

### MBD family



### Zinc Finger family



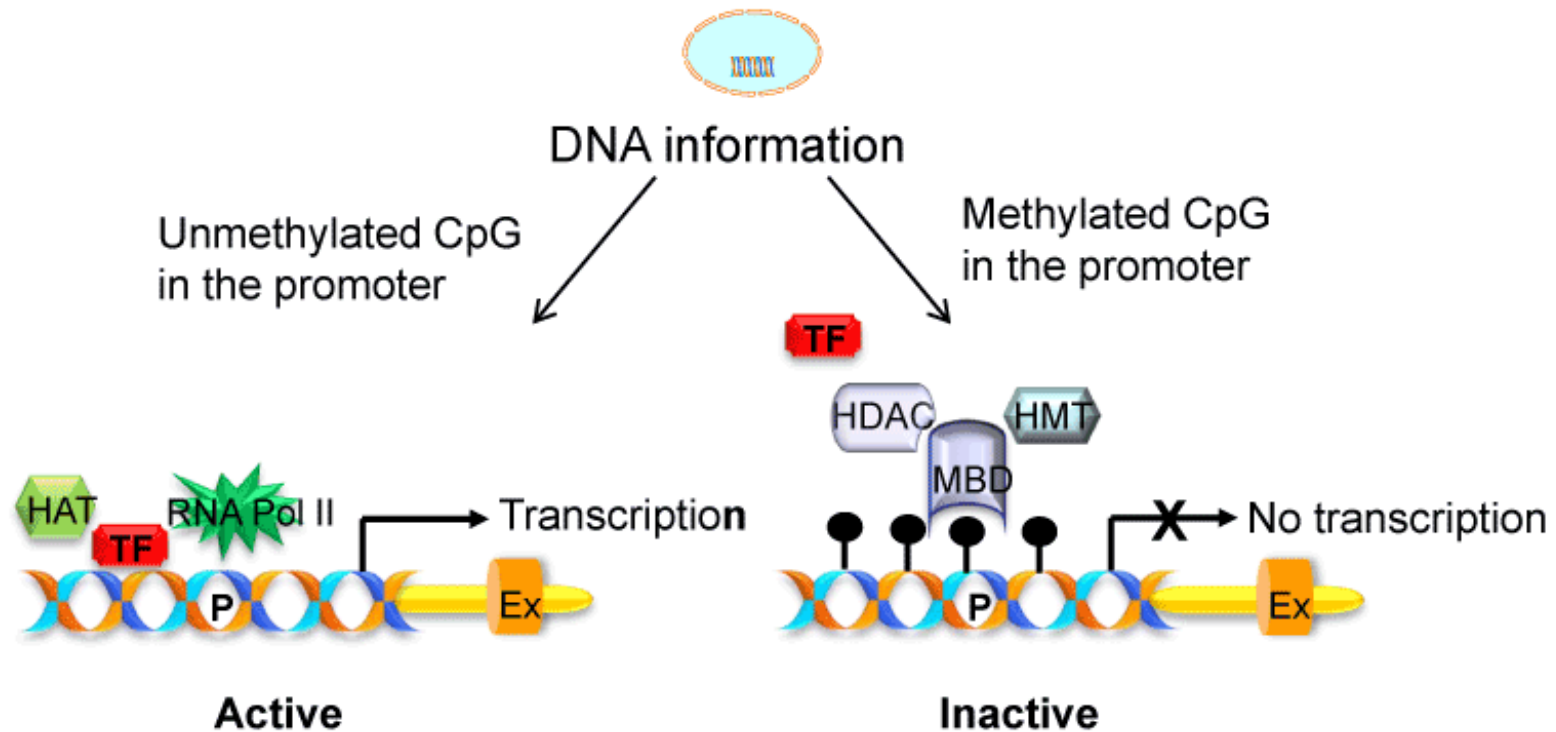
### SRA family



Karpova & Umemori, Epigenetic Methods in Neuroscience Research, 2016

# Repression by methylation - 2

## Consequence of MBD binding



<https://www.omicsonline.org/articles-images/JBABM-04-e108-g001.html>

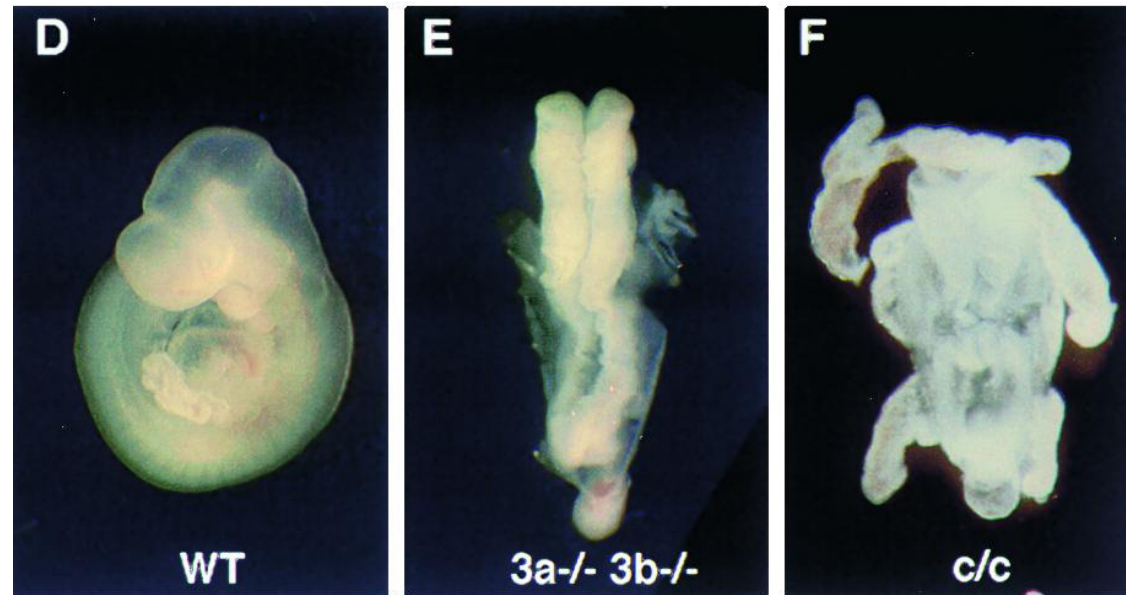


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# 4) Biological significance of DNA methylation

# DNMT-lethality in mice

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Okano et al., Cell, 1999

wildtype

Dnmt3a/b KO

Dnmt1 KO

# Rett syndrome

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<http://www.graceforrett.com/rett-syndrome/r168x/hi-im-kirsty/>

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

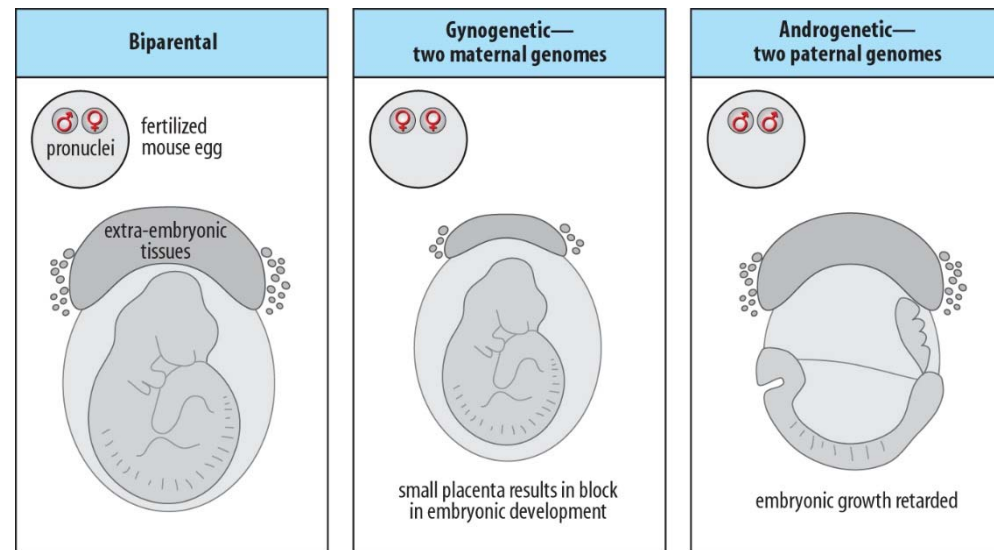
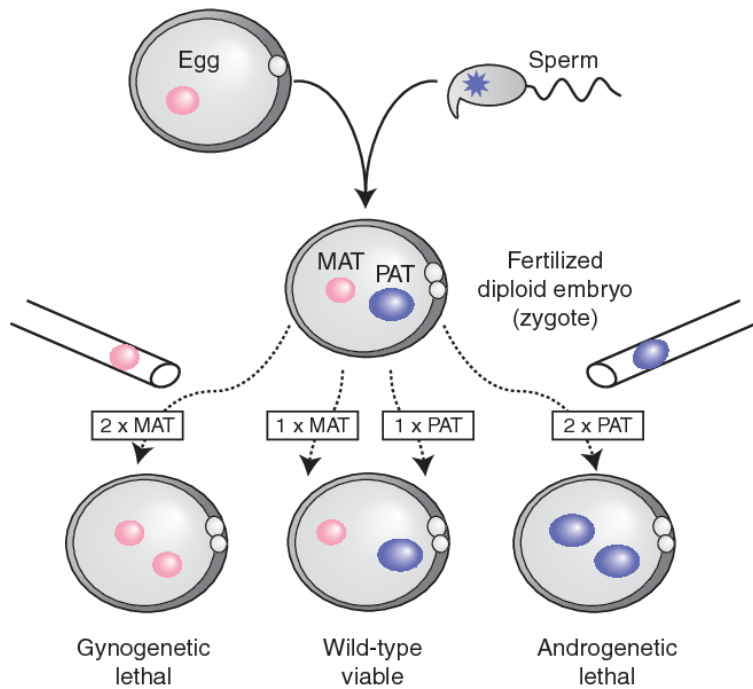
# Methylation and cancer

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- Global hypomethylation
  - Retrotransposons
  - oncogenes
  
- Local hypermethylation
  - tumor suppressor genes

# Genomic imprinting

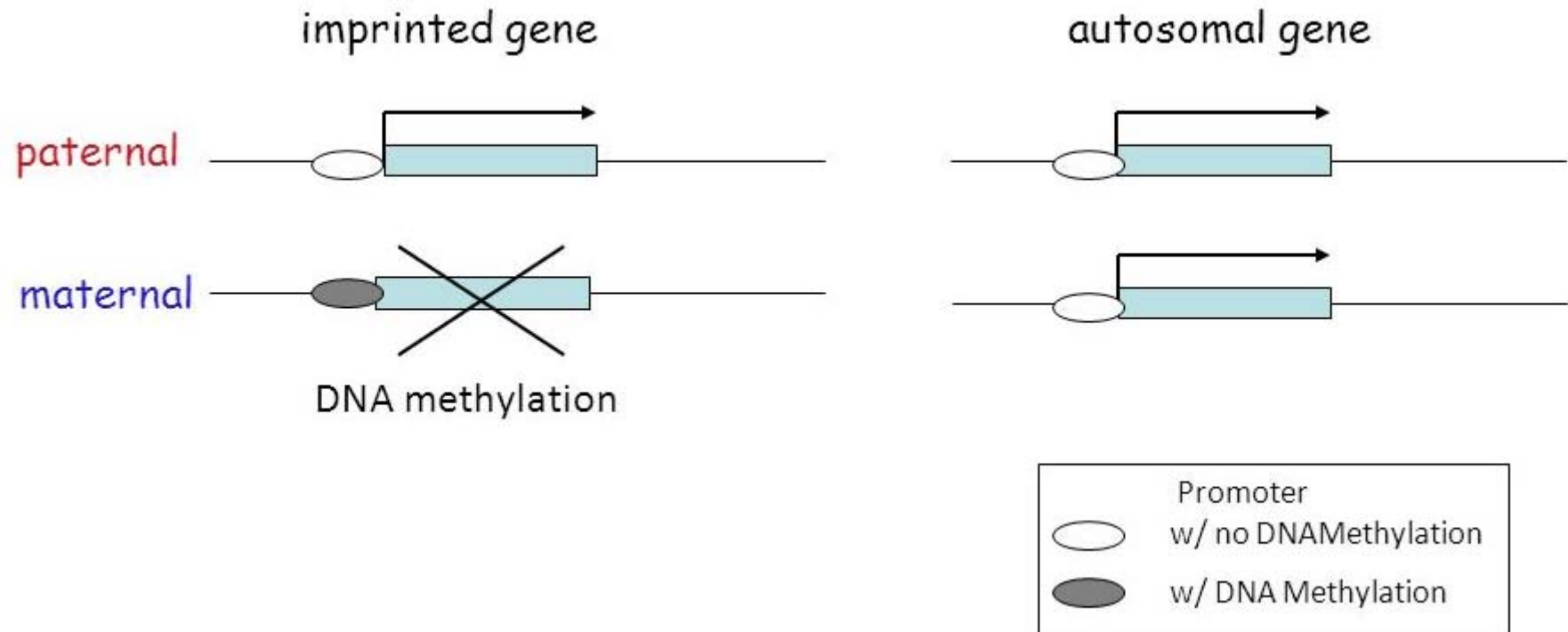
A maternal and a paternal genome are required for development



[http://www.mun.ca/biology/desmid/brian/BIOL3530/DEVO\\_09/ch09f11.jpg](http://www.mun.ca/biology/desmid/brian/BIOL3530/DEVO_09/ch09f11.jpg)

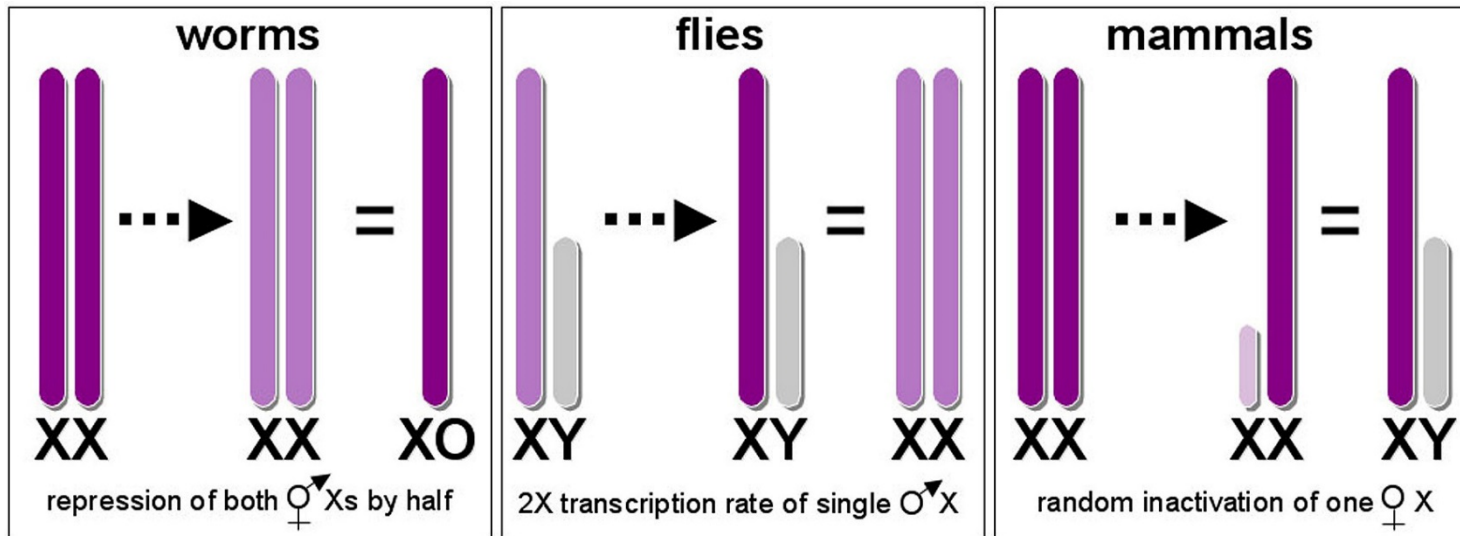
<https://www.semanticscholar.org/paper/Genomic-imprinting-in-mammals.-Barlow-Bartolomei/e59220a32087be34ae0c9345d3c3094260f0ca8d/figure/2>

# Genomic imprinting



<https://slideplayer.com/slide/6330819/>

# X-chromosome inactivation...

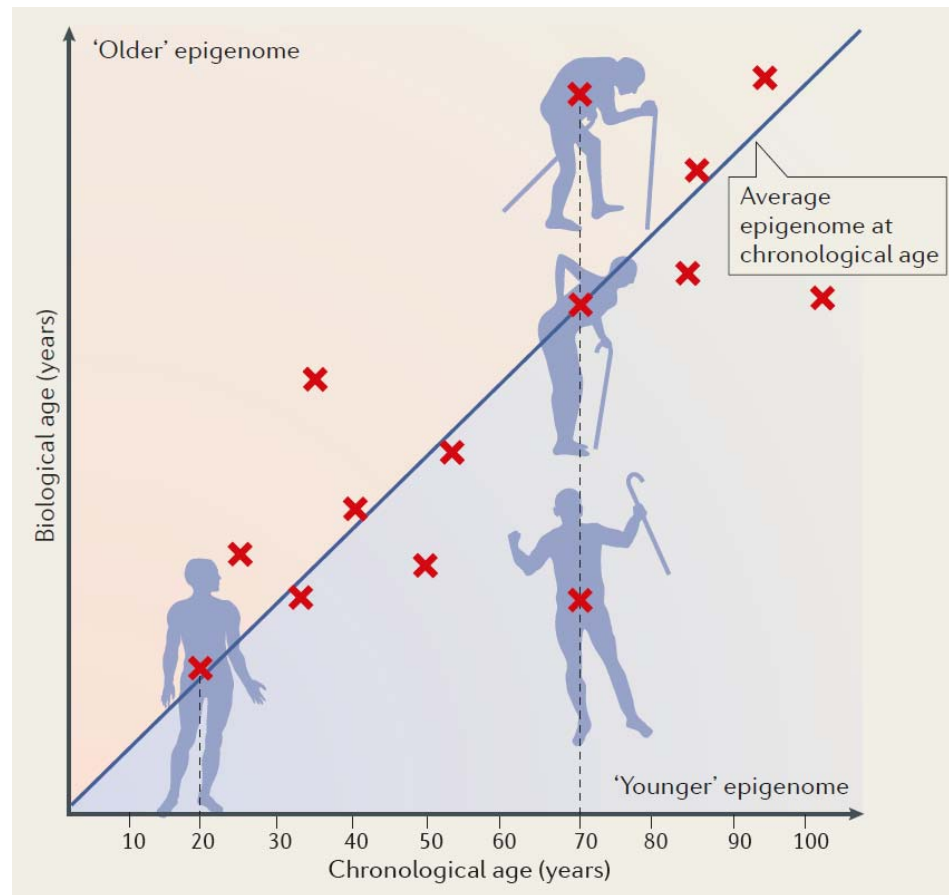


[http://www.wormbook.org/chapters/www\\_dosagecomp/dosagecomp.html](http://www.wormbook.org/chapters/www_dosagecomp/dosagecomp.html)

...is concomitant with hypermethylation of the inactive X-chromosome

# Methylation and aging

Horvath's epigenetic clock: predictor of biological age



Benayoun et al, Nat. Rev. Mol. Cell Biol., 2015

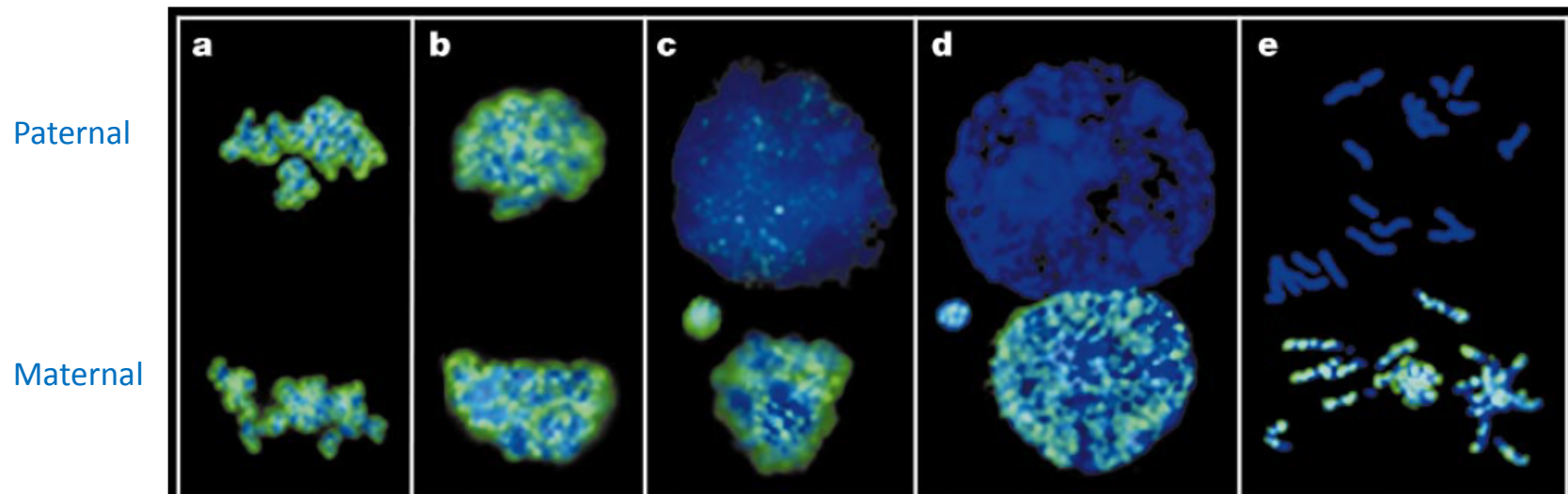


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# 5) Reversibility of DNA methylation

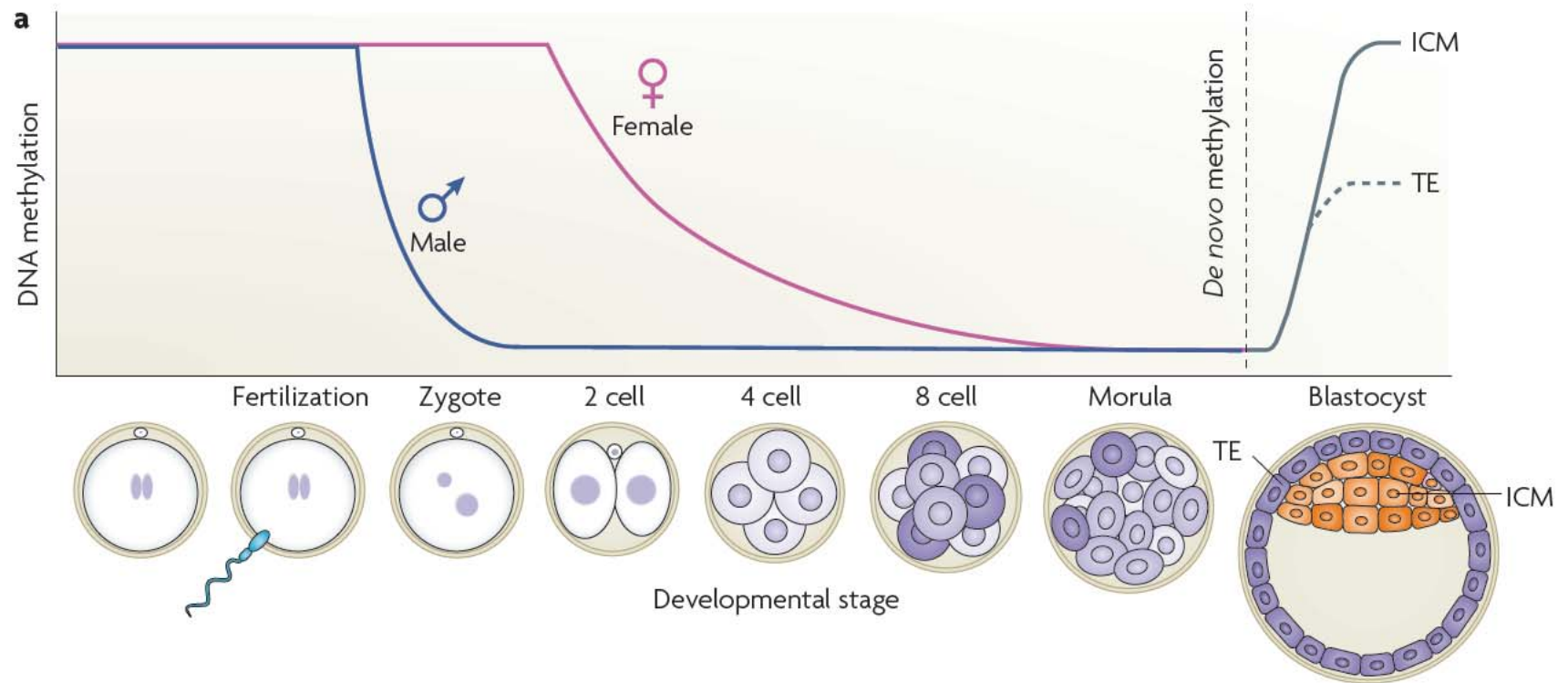
# Pronuclear DNA demethylation

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Mayer et al., Nature, 2000

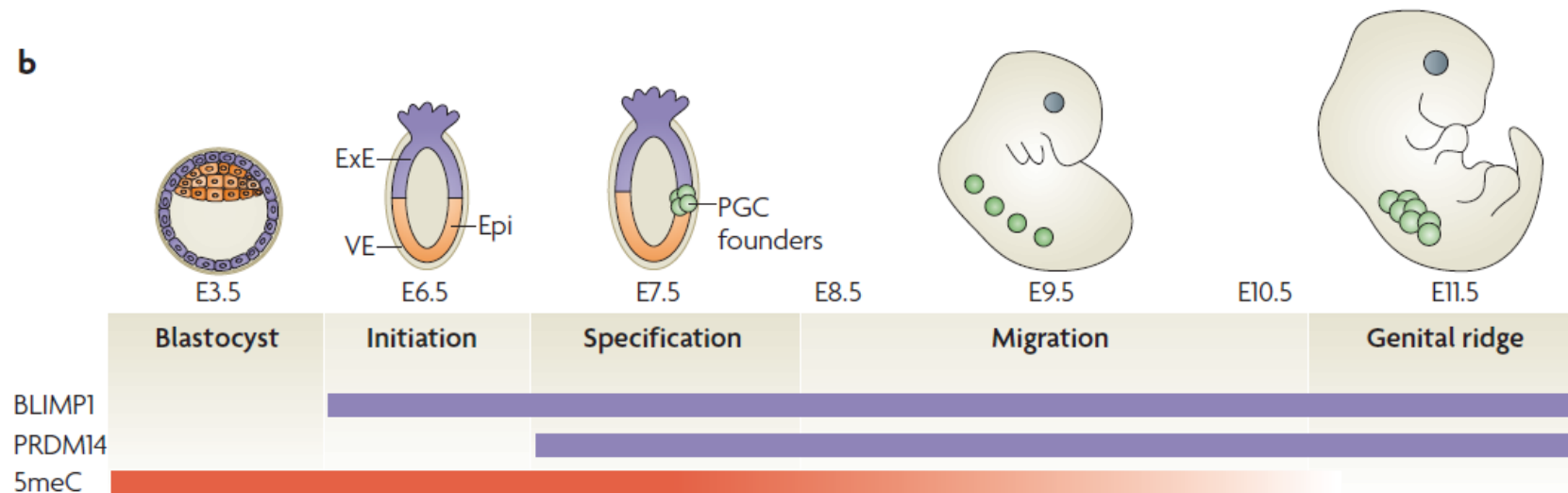
# Pronuclear demethylation



Wu & Zhang, Nat Rev Mol Cell Biol, 2010

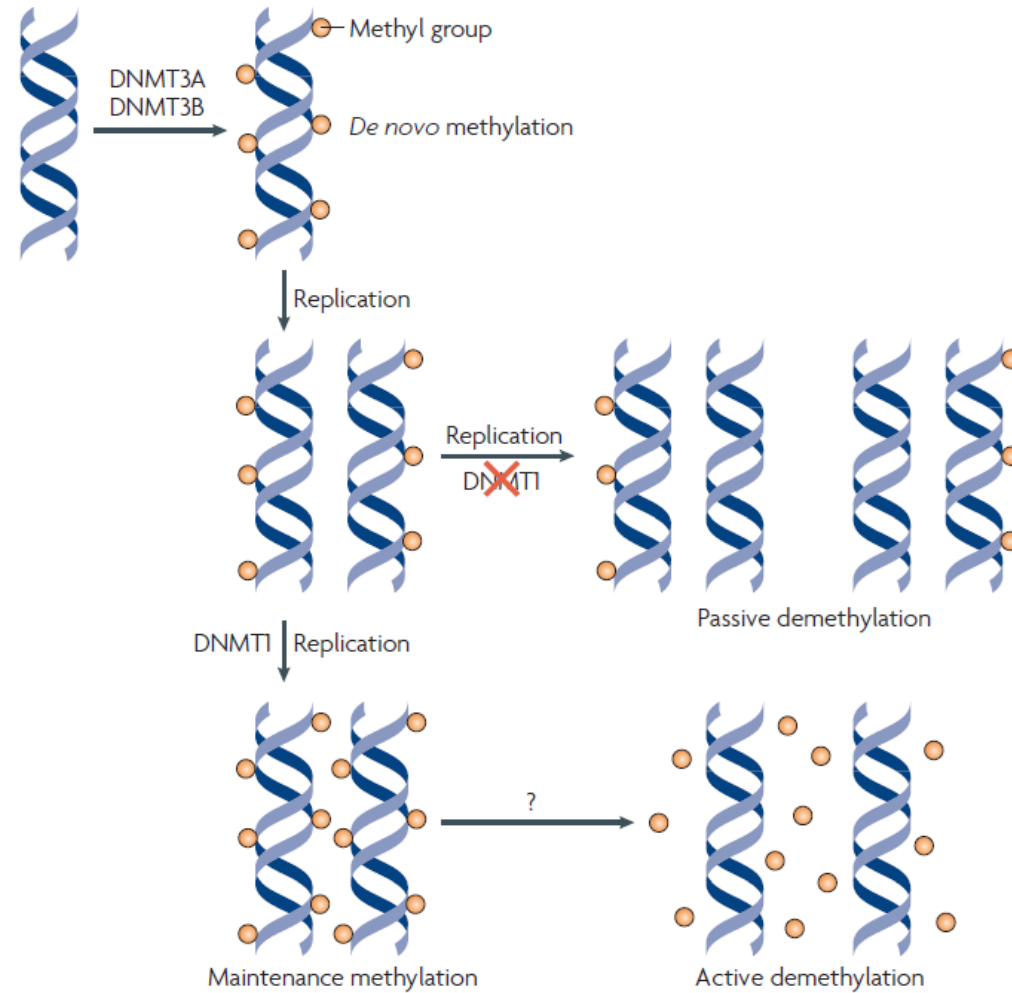
# Primordial germ cell demethylation

b



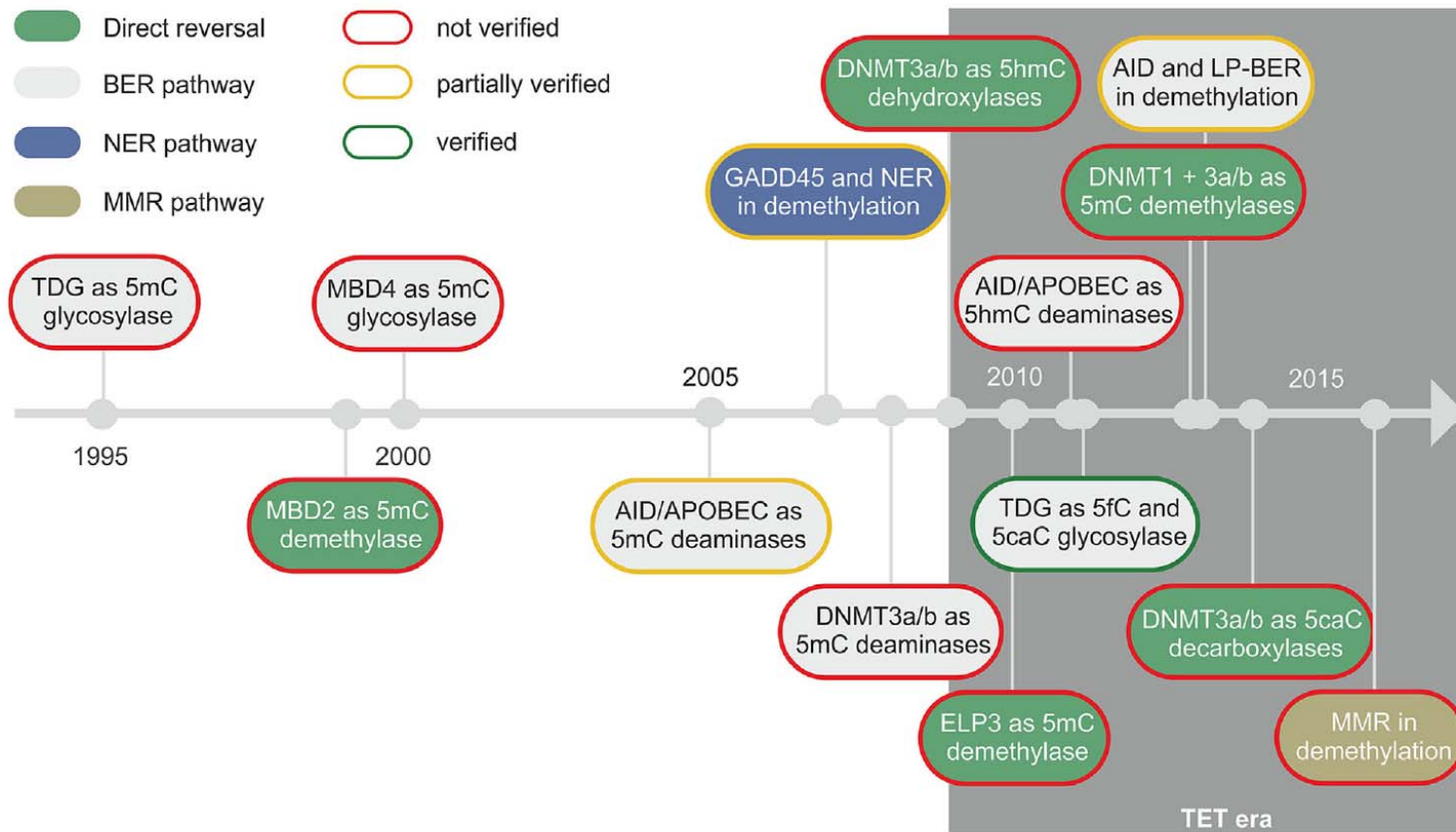
Wu & Zhang, Nat Rev Mol Cell Biol, 2010

# DNA demethylation



Wu & Zhang, Nat Rev Mol Cell Biol, 2010

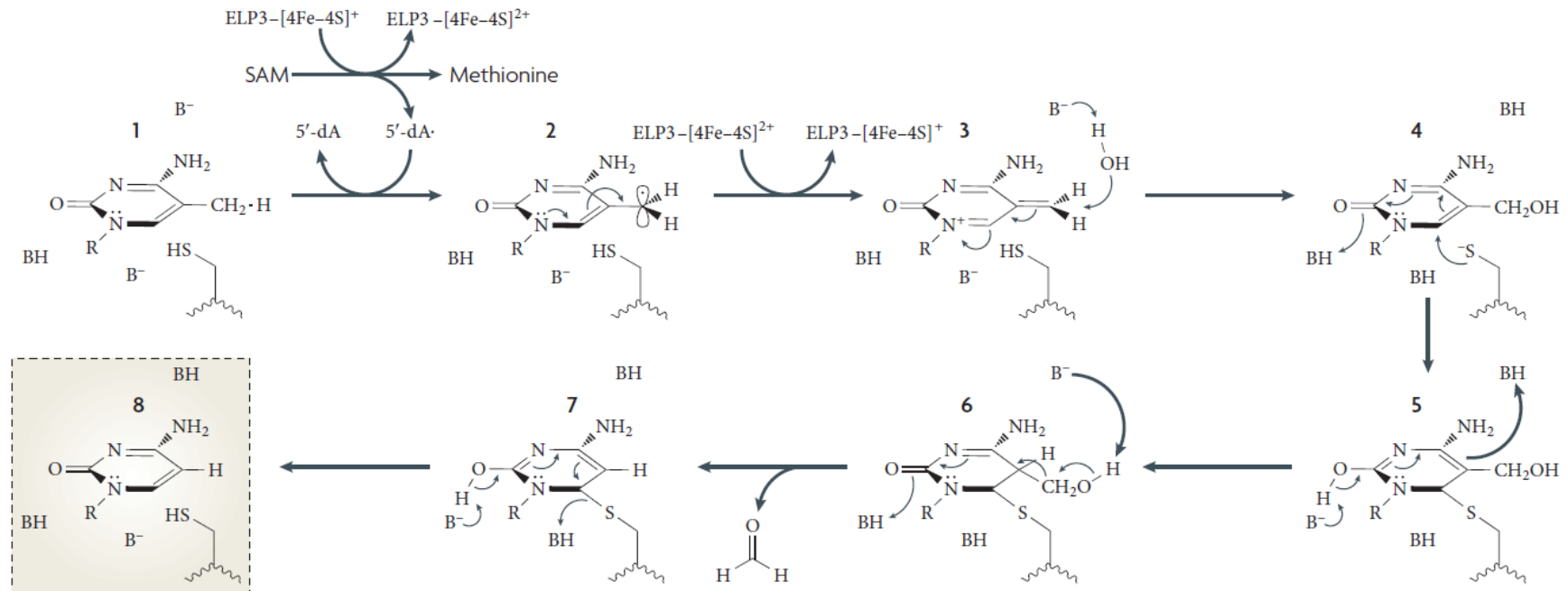
# Active DNA demethylation



Schomacher & Niehrs, Bioessays, 2017

# Radical-mediated demethylation

## 1. Direct removal of methyl group

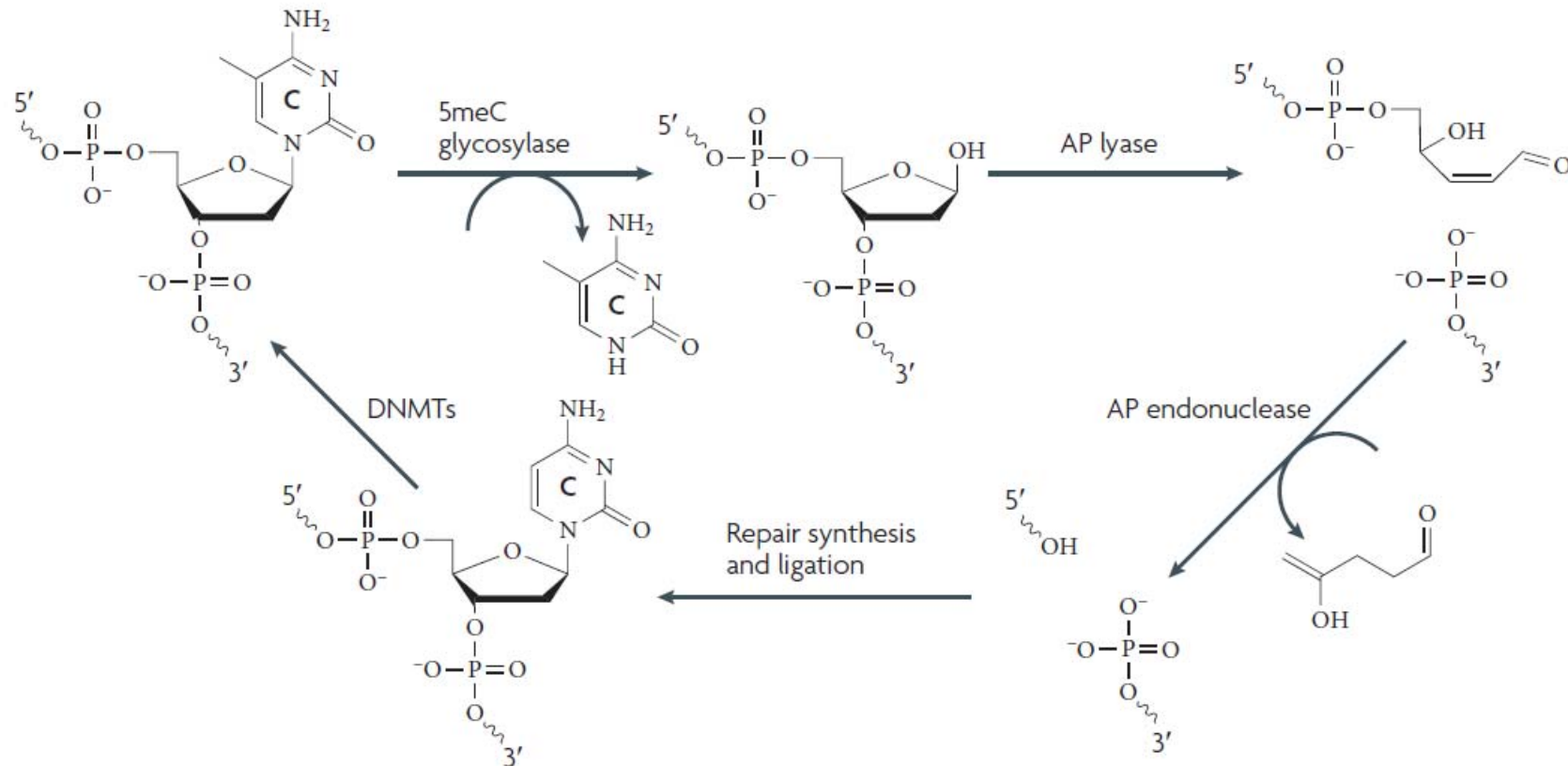


Wu & Zhang, Nat Rev Mol Cel Biol, 2010

- not biochemically verified!

# BER-mediated demethylation

## 2. Excision of unedited 5mC



- only in plants

Wu & Zhang, Nat Rev Mol Cell Biol, 2010



# NER-mediated demethylation

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## 2. Excision of unedited 5mC

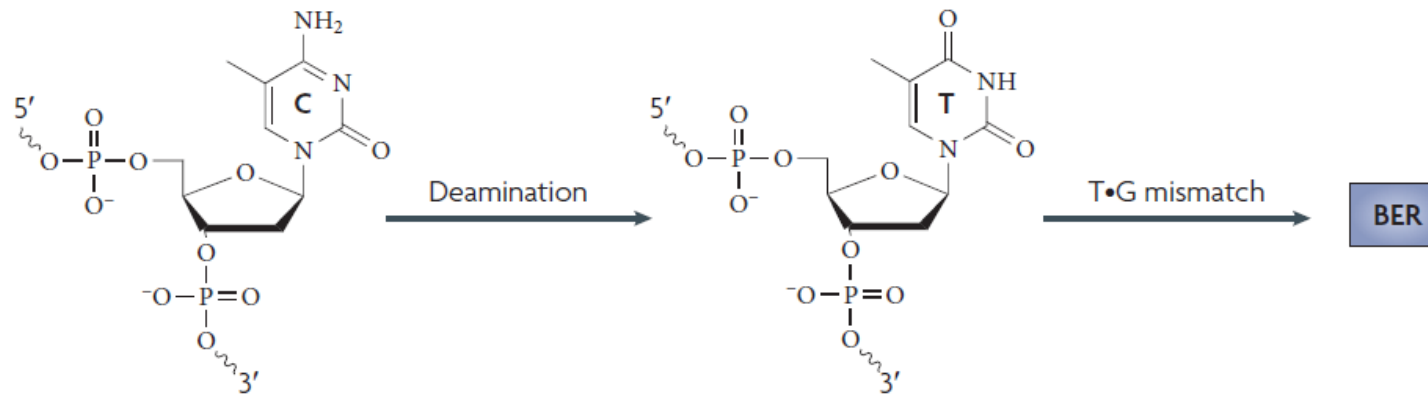


Niehrs & Schäfer, Trends Cell Biol, 2012

- not biochemically nor *in vivo* verified

# Deamination and BER demethylation

## 3. Excision of edited 5mC - 1



Wu & Zhang, Nat Rev Mol Cell Biol, 2010

- not biochemically nor *in vivo* verified

# Breakthrough in active demethylation

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

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

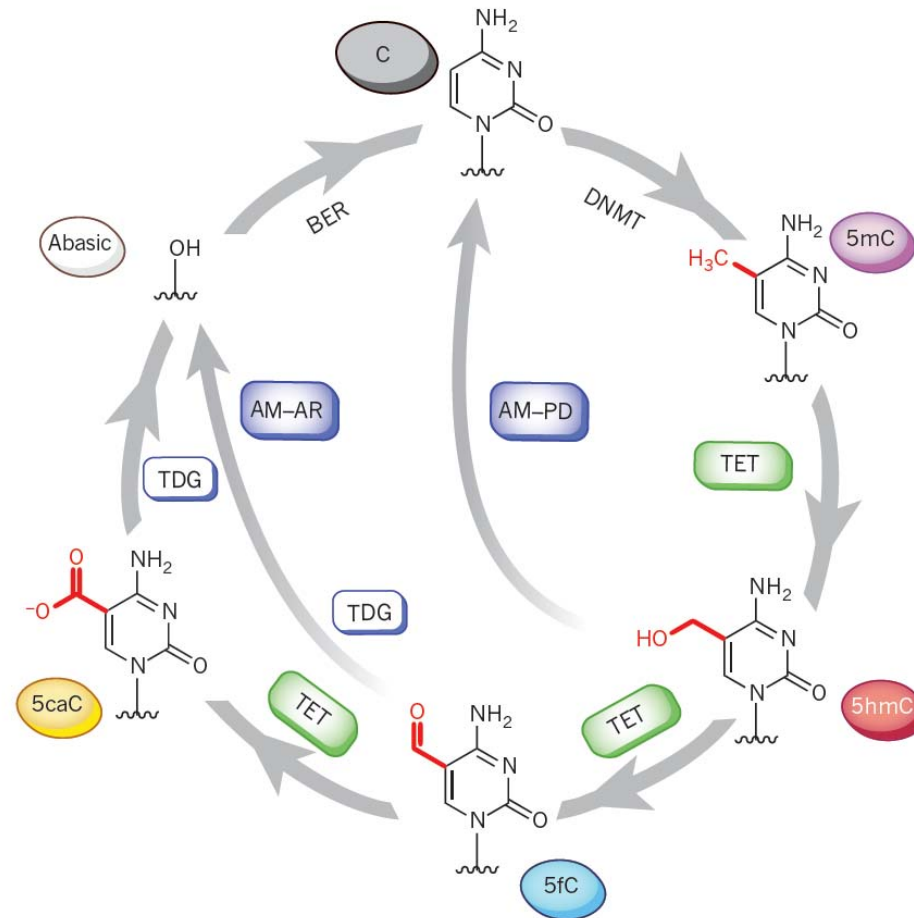
## **The Nuclear DNA Base 5-Hydroxymethylcytosine Is Present in Purkinje Neurons and the Brain**

Skirmantas Kriaucionis and Nathaniel Heintz\*

Science, 15. May 2009

# Oxidative DNA demethylation

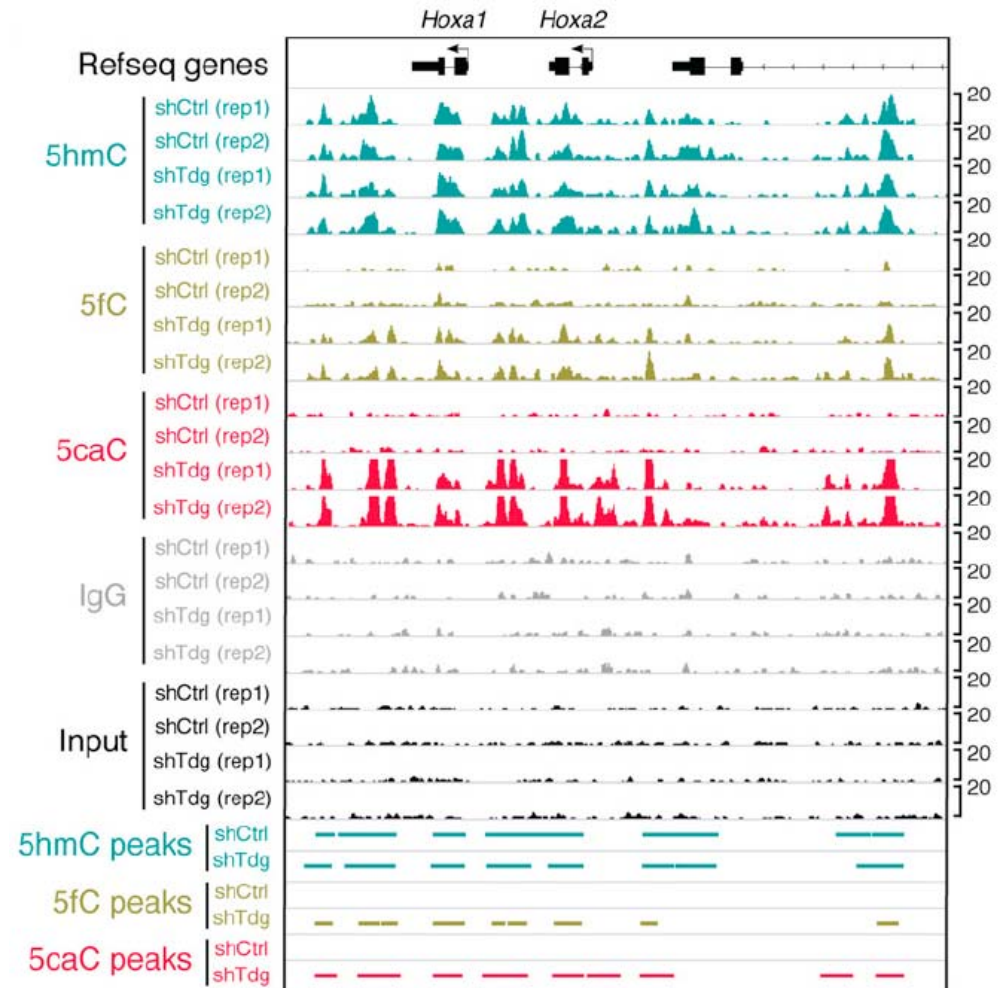
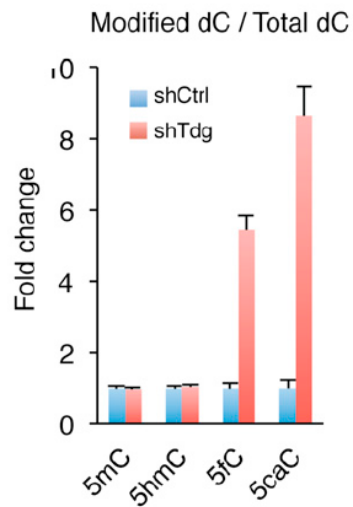
## 3. Excision of edited 5mC - 2



Kohli & Zhang, Nature, 2013

# Oxidative demethylation *in vivo* - 1

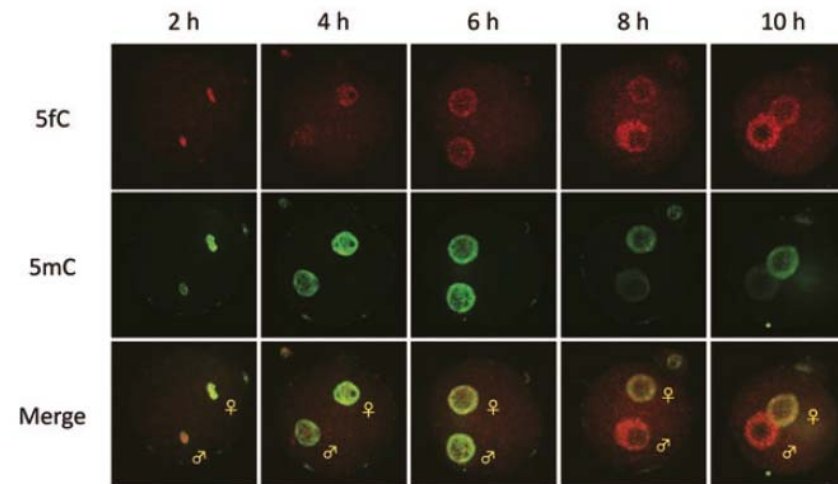
## mESCs



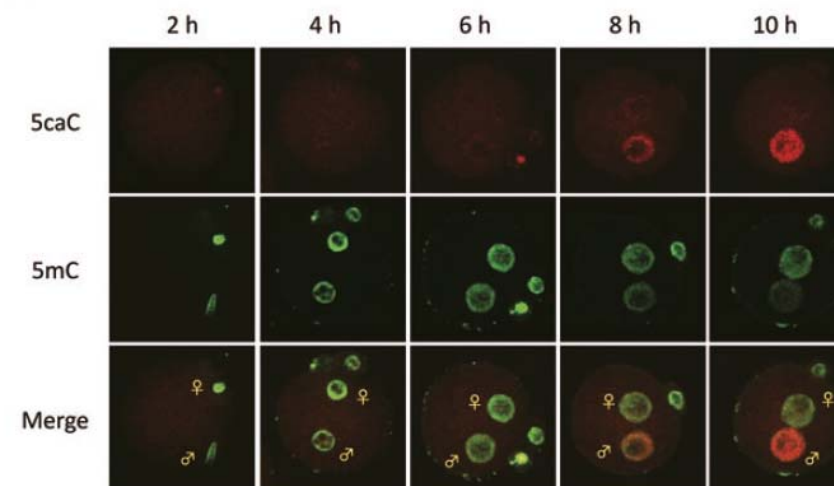
Shen et al., Cell, 2013

# Oxidative demethylation *in vivo* - 2

Zygote

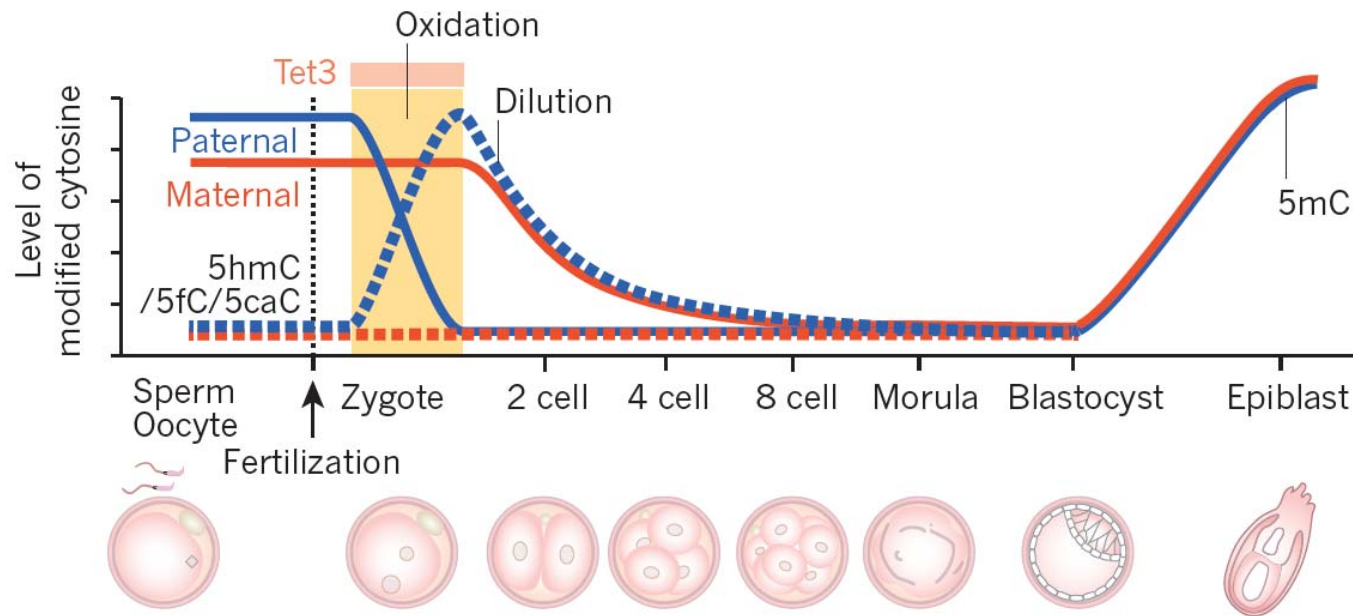


C



Inoue et al., *Cel Res*, 2011

# Model: global demethylation in zygotes



Kohli & Zhang, Nature, 2013

# Summary

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- 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



# Literature: DNA Methylation

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## Functions of DNA methylation: islands, start sites, gene bodies and beyond

*Peter A. Jones*

NATURE REVIEWS | GENETICS

484 | JULY 2012 | VOLUME 13

## DNA METHYLATION AND HUMAN DISEASE

*Keith D. Robertson*

NATURE REVIEWS | GENETICS

VOLUME 6 | AUGUST 2005 | 597

## DNA methylation: roles in mammalian development

*Zachary D. Smith and Alexander Meissner*

204 | MARCH 2013 | VOLUME 14

[www.nature.com/reviews/genetics](http://www.nature.com/reviews/genetics)

# Literature: DNA demethylation

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## Active DNA demethylation: many roads lead to Rome

*Susan C. Wu and Yi Zhang*

NATURE REVIEWS | MOLECULAR CELL BIOLOGY VOLUME 11 | SEPTEMBER 2010 | 607

## Reversing DNA Methylation: Mechanisms, Genomics, and Biological Functions

Hao Wu<sup>1,2,3,4</sup> and Yi Zhang<sup>1,2,3,4,\*</sup>

Cell 156, January 16, 2014 ©2014 Elsevier Inc. 45

## REVIEW

doi:10.1038/nature12750

## TET enzymes, TDG and the dynamics of DNA demethylation

Rahul M. Kohli<sup>1,2</sup> & Yi Zhang<sup>3,4,5,6,7</sup>

472 | NATURE | VOL 502 | 24 OCTOBER 2013

## TET-mediated active DNA demethylation: mechanism, function and beyond

Xiaoji Wu<sup>1-5</sup> and Yi Zhang<sup>1-4</sup>

NATURE REVIEWS | GENETICS