

Epigenetics and DNA methylation

Bio 5488

Michael Meers

2/2/2026

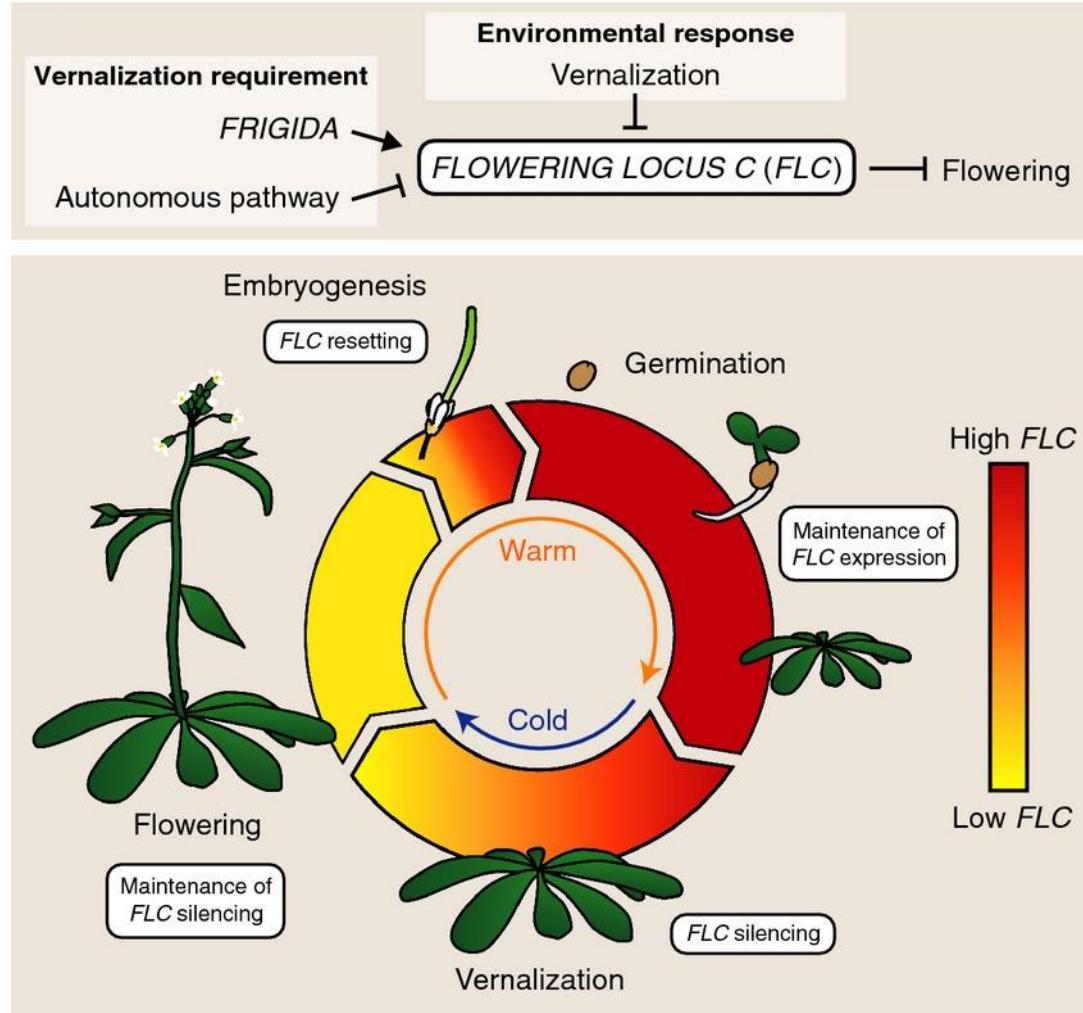
Outline for the next four lectures

1. Intro to epigenetics and DNA modifications
2. Chromatin modifications and accessibility
3. DNA binding proteins
4. 3D genome architecture

What is Epigenetics/Epigenomics?

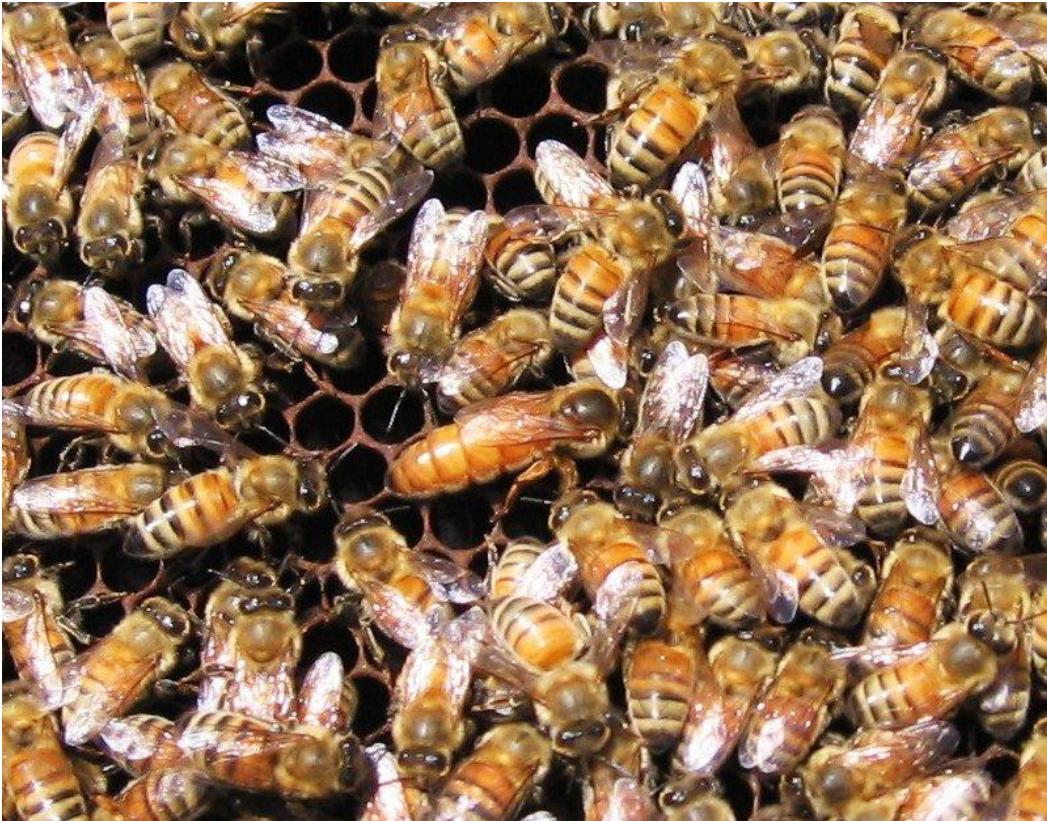
- A mitotically or meiotically **heritable** state of different gene activity and expression (phenotype) that is independent of differences in DNA sequence (genotype) – *based on Conrad Waddington, 1942*
- The sum of the alterations to the chromatin template that collectively establish and propagate different patterns of gene expression (transcription) and silencing from the same genome.
- Epigenetic changes influence the phenotype **without altering the genotype**.

Example: Plant flowering is mediated through vernalization-induced environmental inputs to the FLC locus

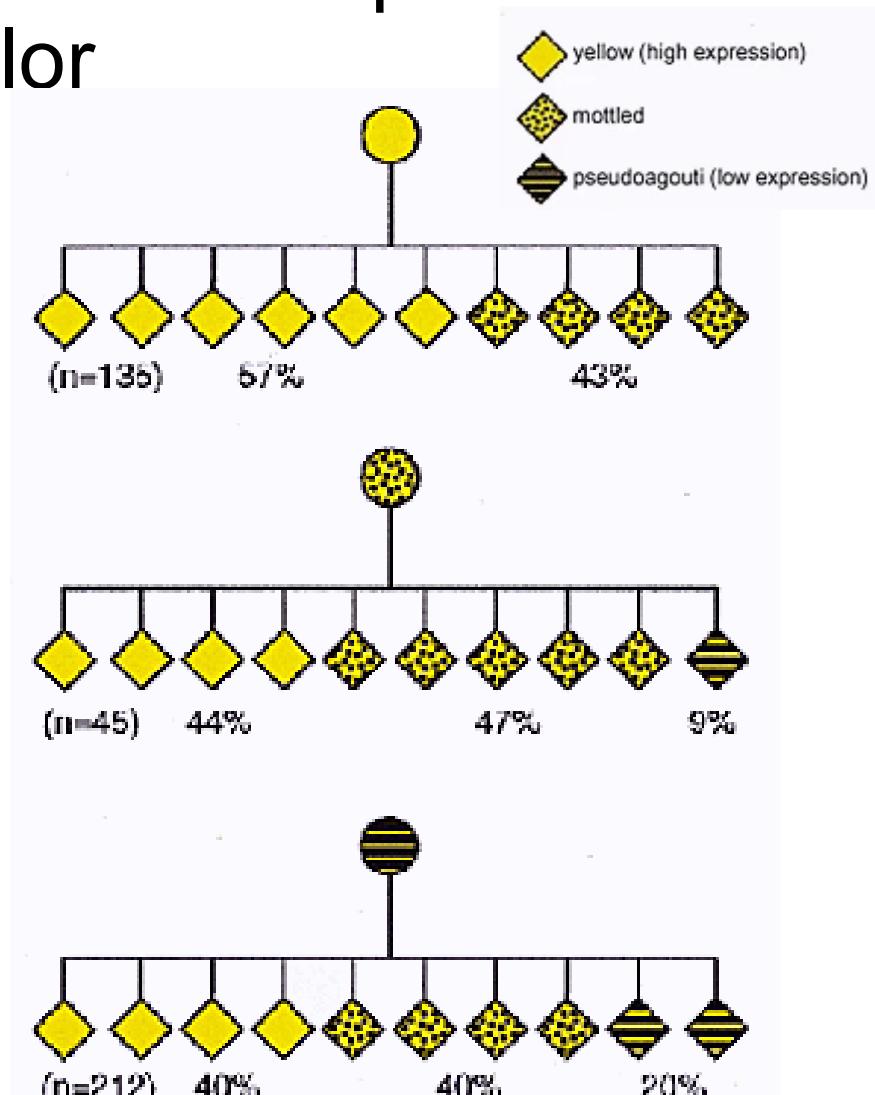


Bastow et al. (Nature 2004)

Example: Early larval diet in honeybees influences adoption of adult queen vs. worker identity

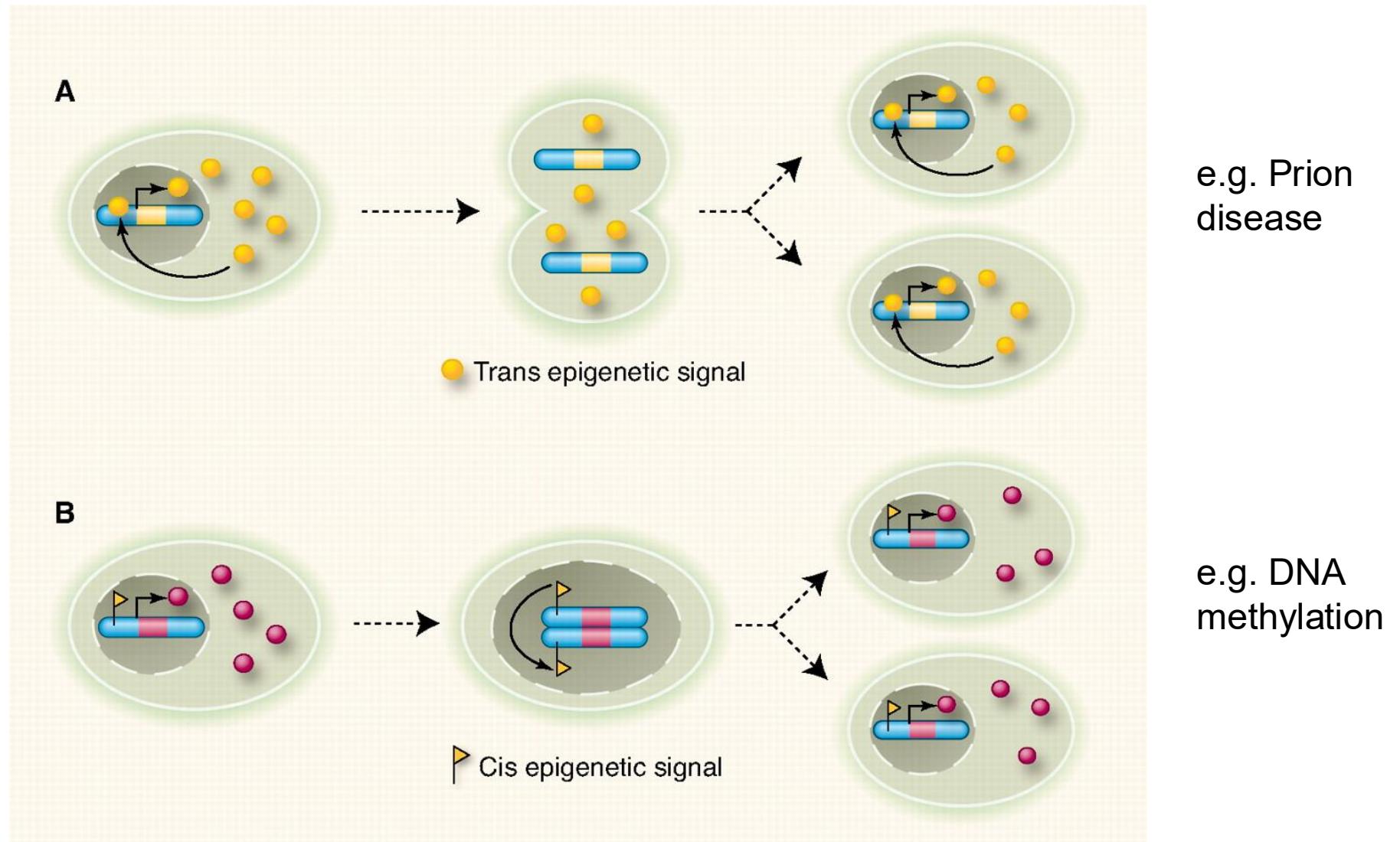


Example: Genetically identical “Agouti” mice adopt maternally inherited variation in hair color

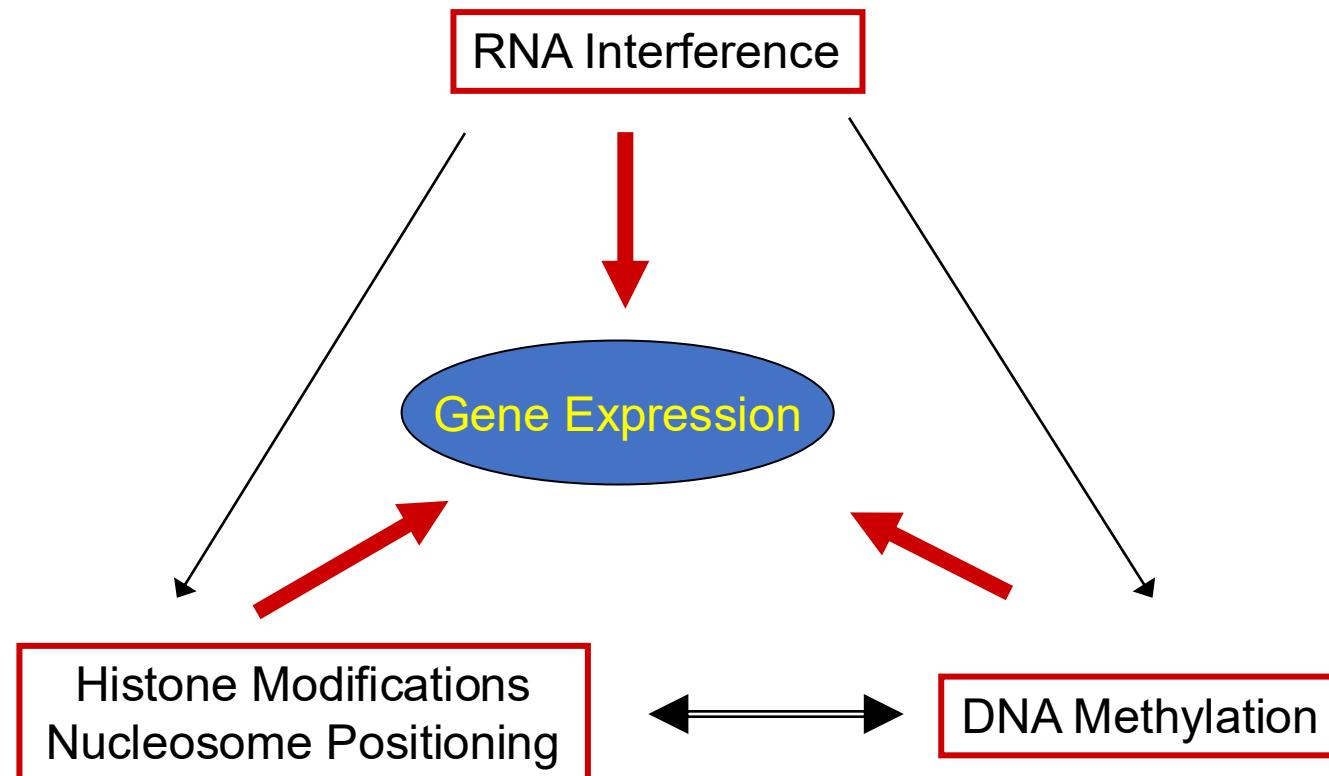


Morgan et al. (Nature Genetics 1999)

Inheritance, broad definition of epigenetics



Epigenetics Mechanisms



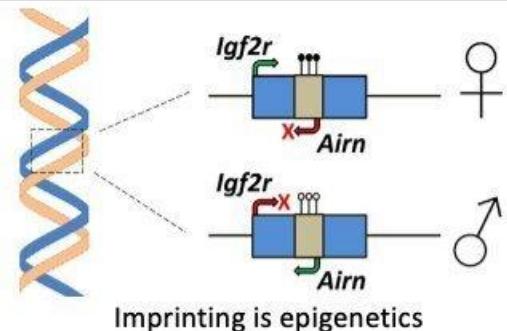
Epigenetic mechanisms

- DNA methylation
 - Normal cells: role in gene expression and chromosome stability
 - Cancer cells: consequences of aberrant hypo- and hyper-methylation
- Histone modification
 - Normal cells - the histone code
 - Cancer cells - consequences of altered histone modifying enzymes
- Interaction between DNA methylation, histone modifications and small RNAs
- Cell/tissue type specificity
- Gene/Environment interaction, disease susceptibility

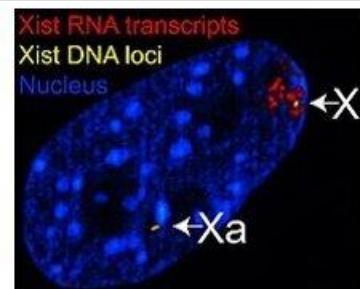
THE EPIGENETICS ALIGNMENT CHART

MECHANISM PURIST
(Epigenetics must be chemical modifications on top of DNA)

TIMESCALE PURIST
(Epigenetics must persist across generations of organisms)

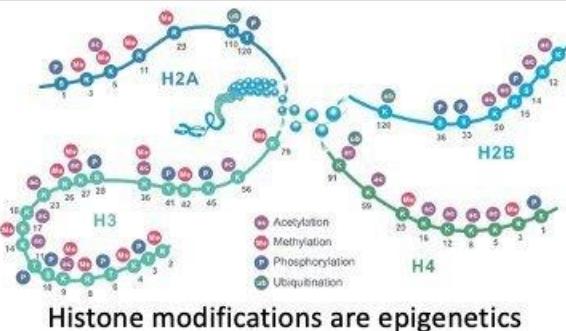


TIMESCALE NEUTRAL
(Epigenetics must persist across cell division)

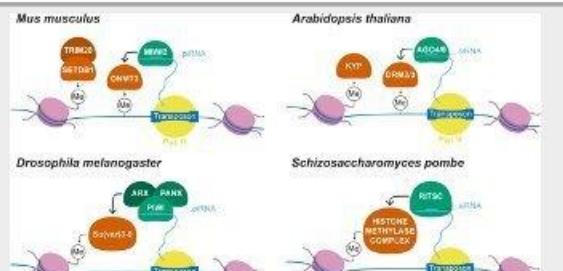


X inactivation is epigenetics

TIMESCALE REBEL
(Epigenetics can persist for any amount of time)



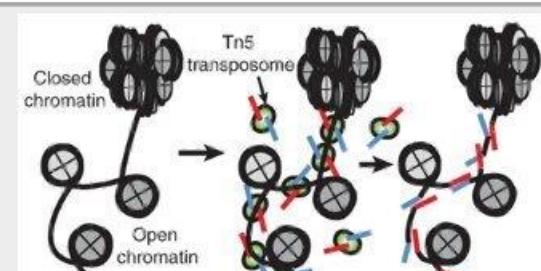
MECHANISM NEUTRAL
(Epigenetics must be functional changes to the genome)



Transposable element silencing is epigenetics

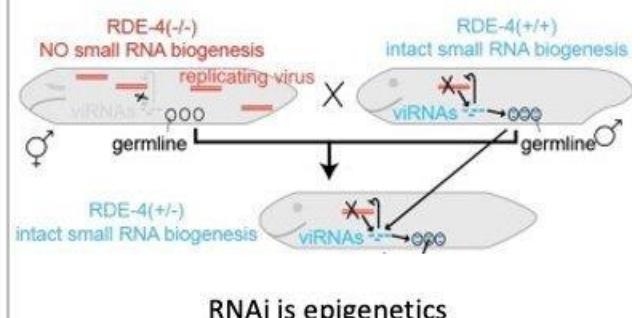


Chromosome positioning is epigenetics

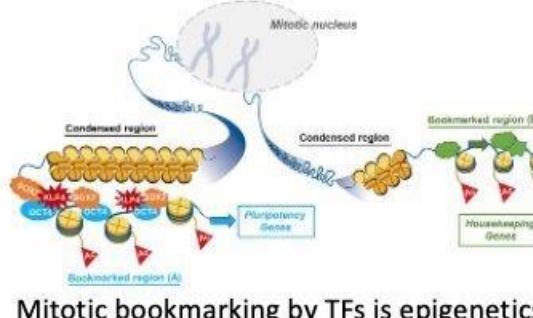


Chromatin accessibility is epigenetics

MECHANISM REBEL
(Epigenetics are any change that doesn't affect nucleotide sequence)



RNAi is epigenetics

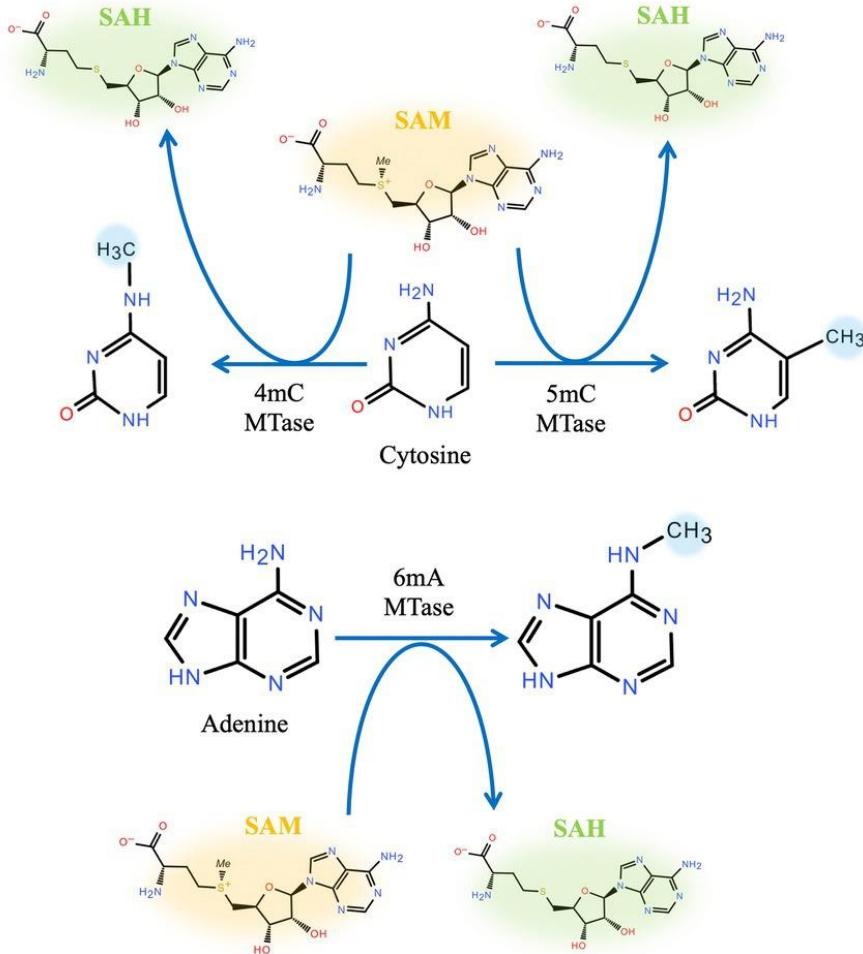


Mitotic bookmarking by TFs is epigenetics



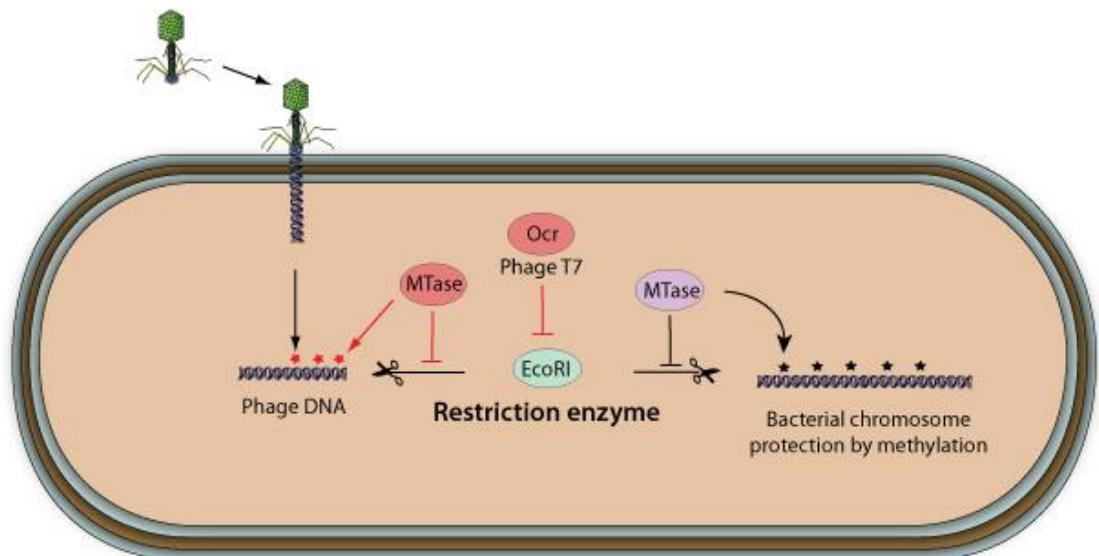
RNAs and proteins are epigenetics

DNA Modifications

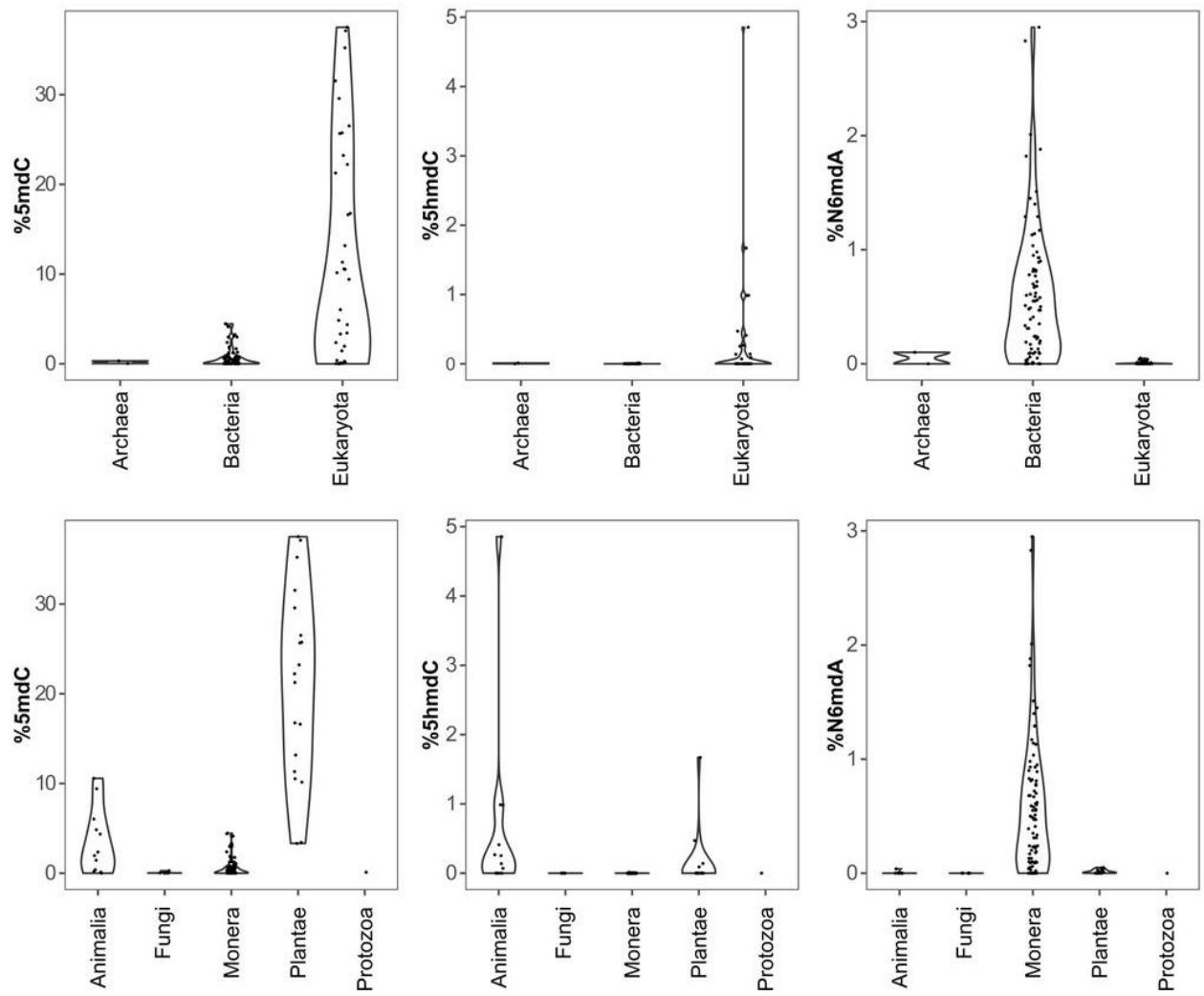


- Frequently DNA methylation (e.g. 5mC, 4mC, 6mA)
- Catalyzed by methyltransferases that consume S-adenosyl methionine (SAM)
- Frequently implicated in heritable changes in organismal phenotypes

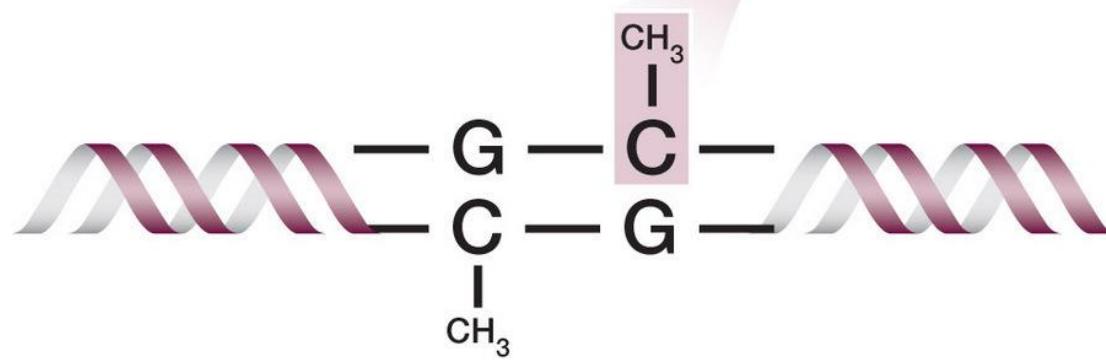
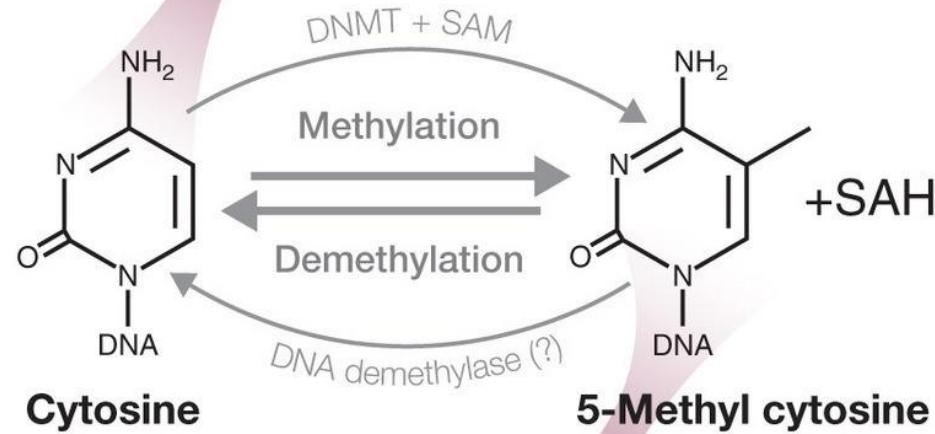
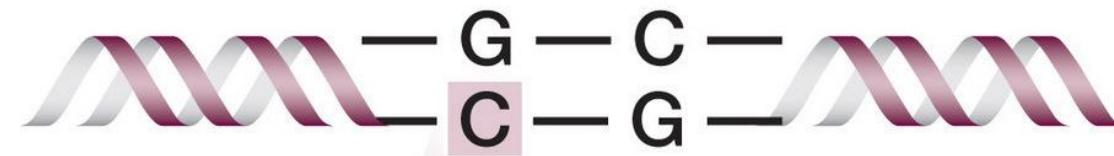
DNA modifications across the tree of life



Many DNA modifications function in **bacterial restriction-modification systems** that evolved for defense against phages

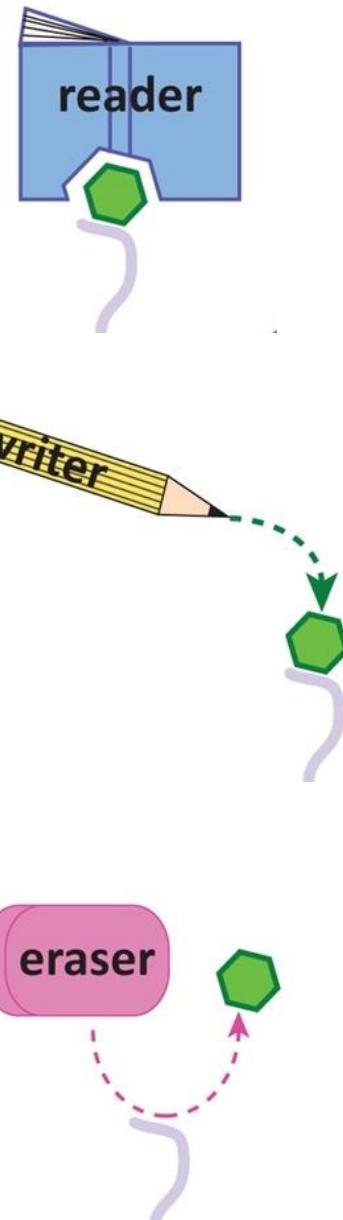


5 methylcytosine: “The 5th base”

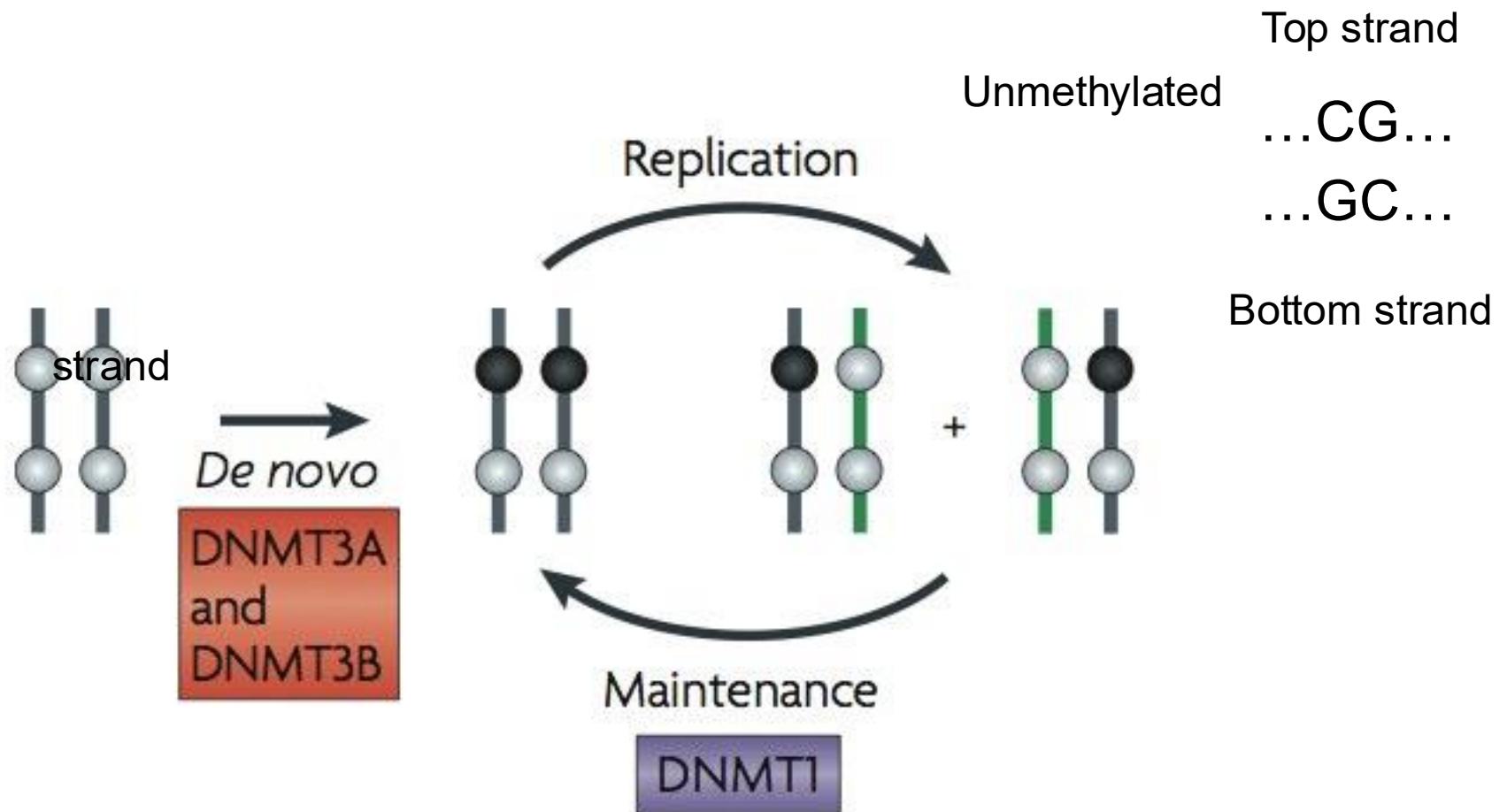


History of DNA 5-mC

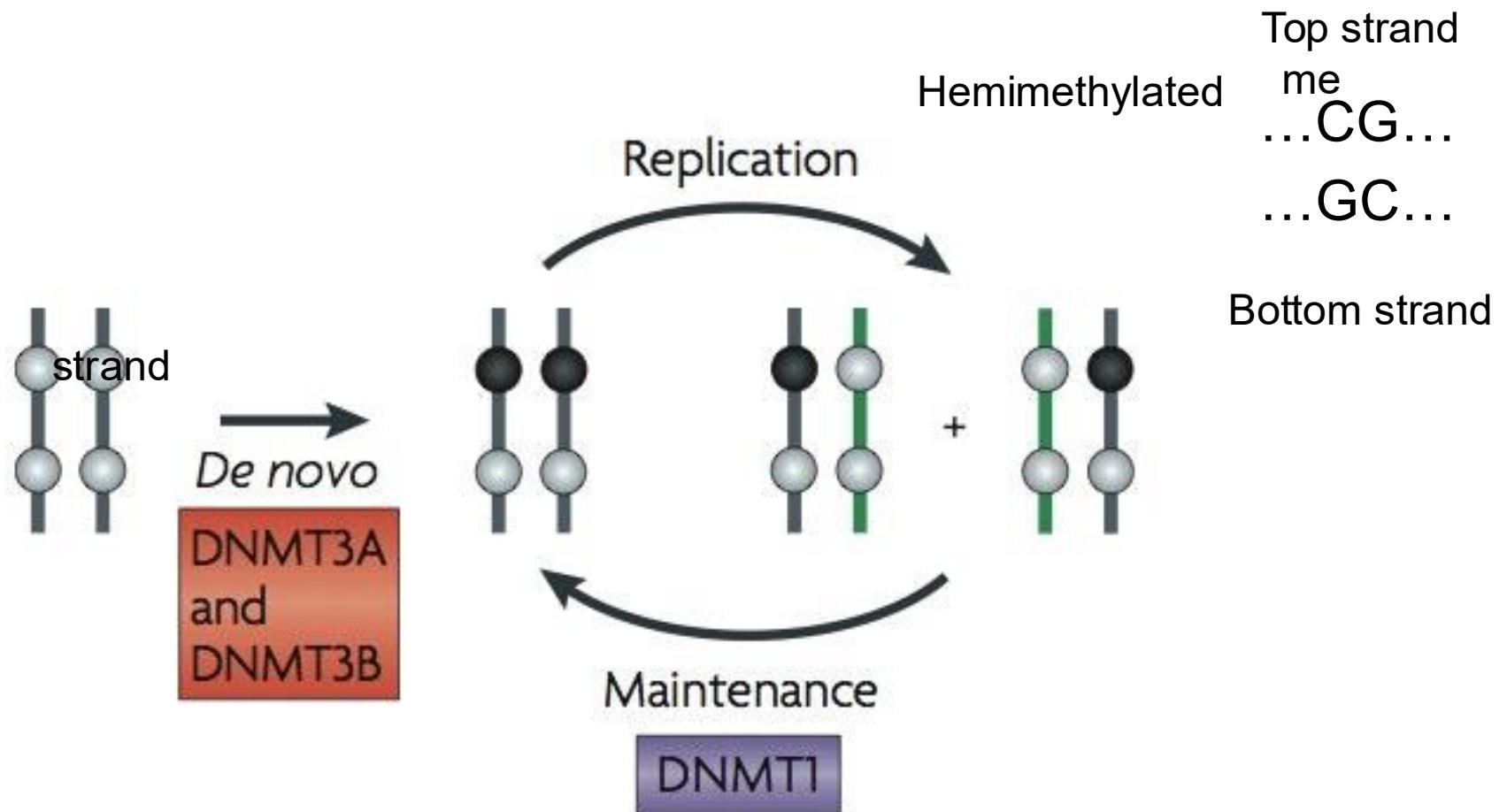
| Year | Event | Scientists |
|------|---|--|
| 1951 | DNA 5-mC first reported | Wyatt |
| 1968 | Activity of a DNA 5-mC writer detected | Kalousek & Morris |
| 1975 | Model for maintaining 5-mC across cell divisions proposed by 2 independent groups | Riggs Holliday & Pugh |
| 1980 | DNA 5-mC is associated with gene repression using 5-azacytidine | Jones & Taylor |
| 1982 | <i>De novo</i> DNA methylation detected | Jahner et al. |
| 1983 | 1 st DNA 5-mC writer, Dnmt1, purified | Bestor & Ingram |
| 1987 | DNA methylation of promoters associated with gene repression | Kovesdi et al. |
| 1989 | 1 st DNA 5-mC reader, MeCP1, discovered | Meehan et al. |
| 1993 | DNA 5-mC is associated with gene repression using dnmt1 knockout mice | Li et al. |
| 1998 | Function of Dnmt3a and Dnmt3b (<i>de novo</i> methylation of proviral DNA and repetitive sequences) determined | Okano et al. |
| 1998 | Additional DNA 5-mC readers, MeCP2, MBD1, MBD2 & MBD4, discovered | Hendrich & Bird |
| 2002 | Function of Dnmt3L (<i>de novo</i> methylation of maternal imprinted genes) determined | Hata et al. |
| 2007 | DNA methylation of gene bodies associated with gene expression | Hellman & Chess |
| 2009 | DNA 5-mC erasers, TET1-3, discovered | Tahiliani et al. Kriaucionis & Heintz |



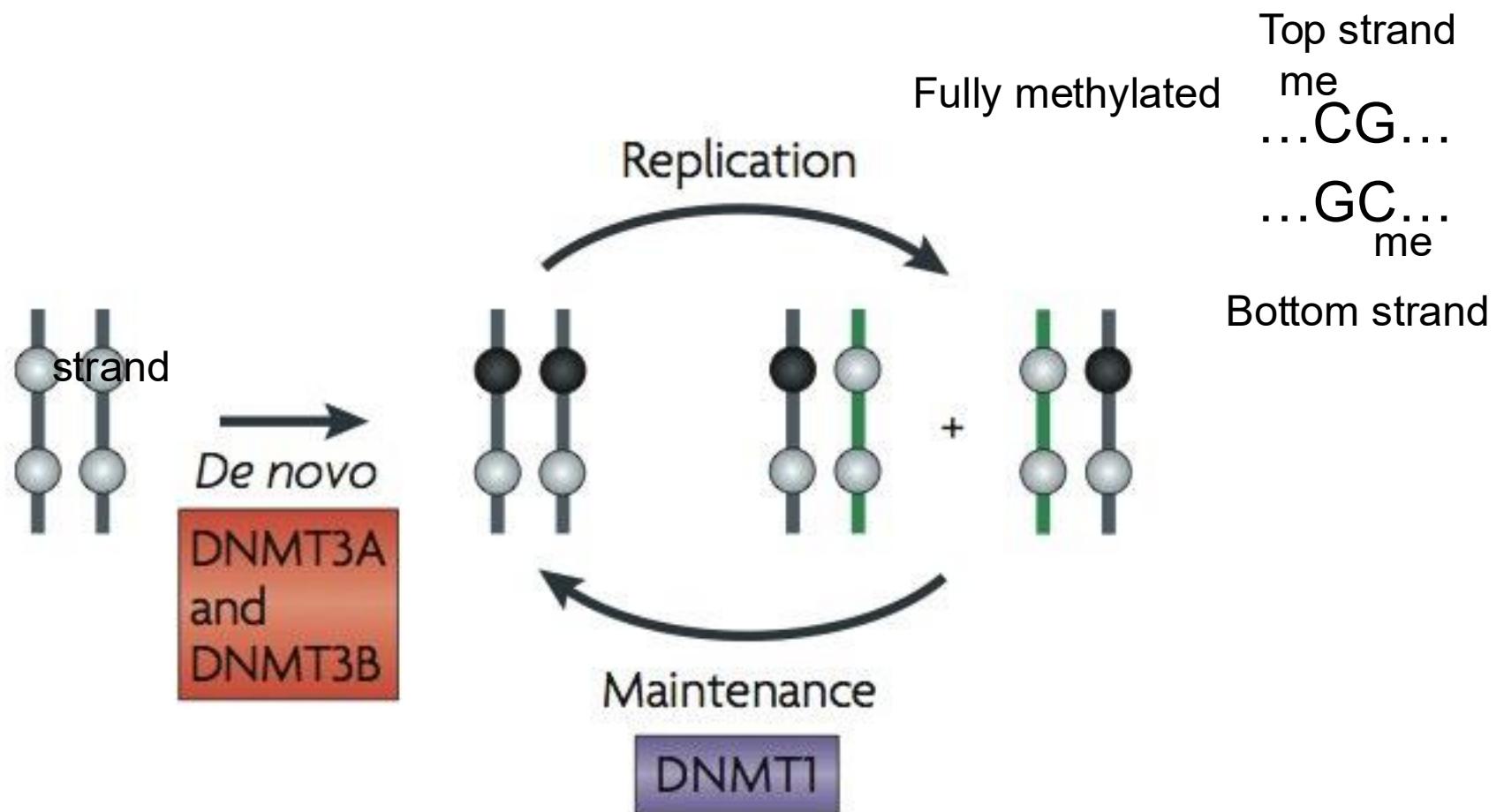
Two classes of DNA methyltransferases (DNMTs)



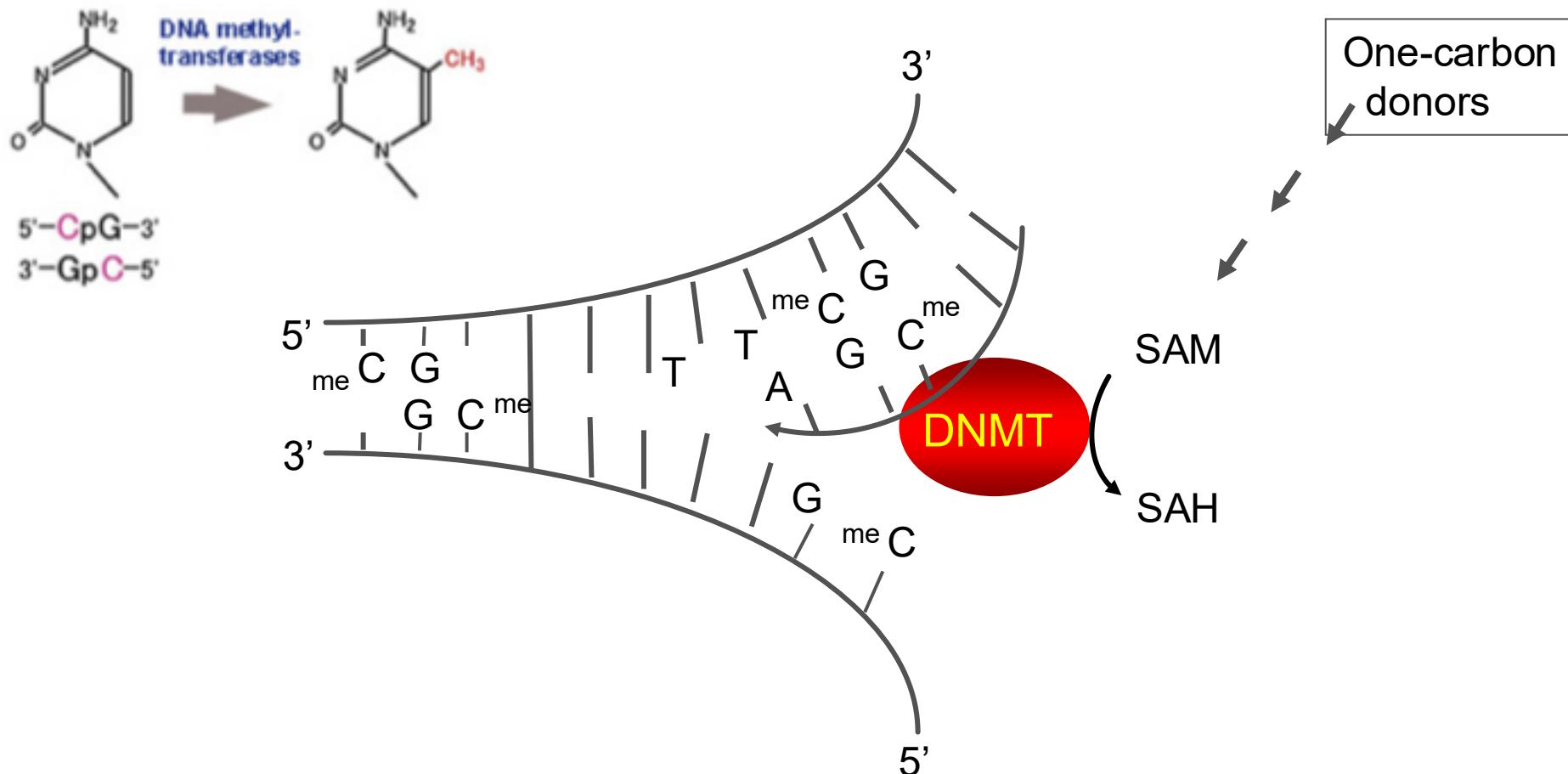
Two classes of DNA methyltransferases (DNMTs)



Two classes of DNA methyltransferases (DNMTs)

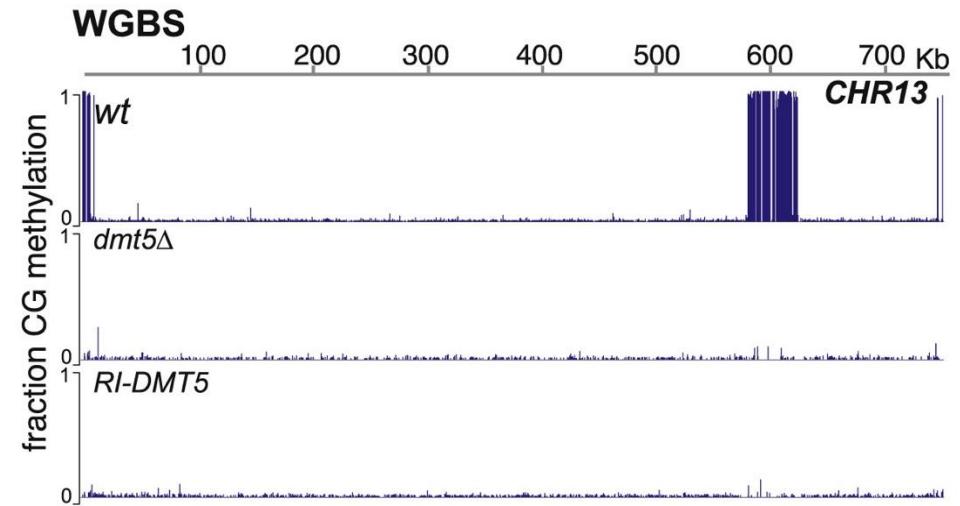
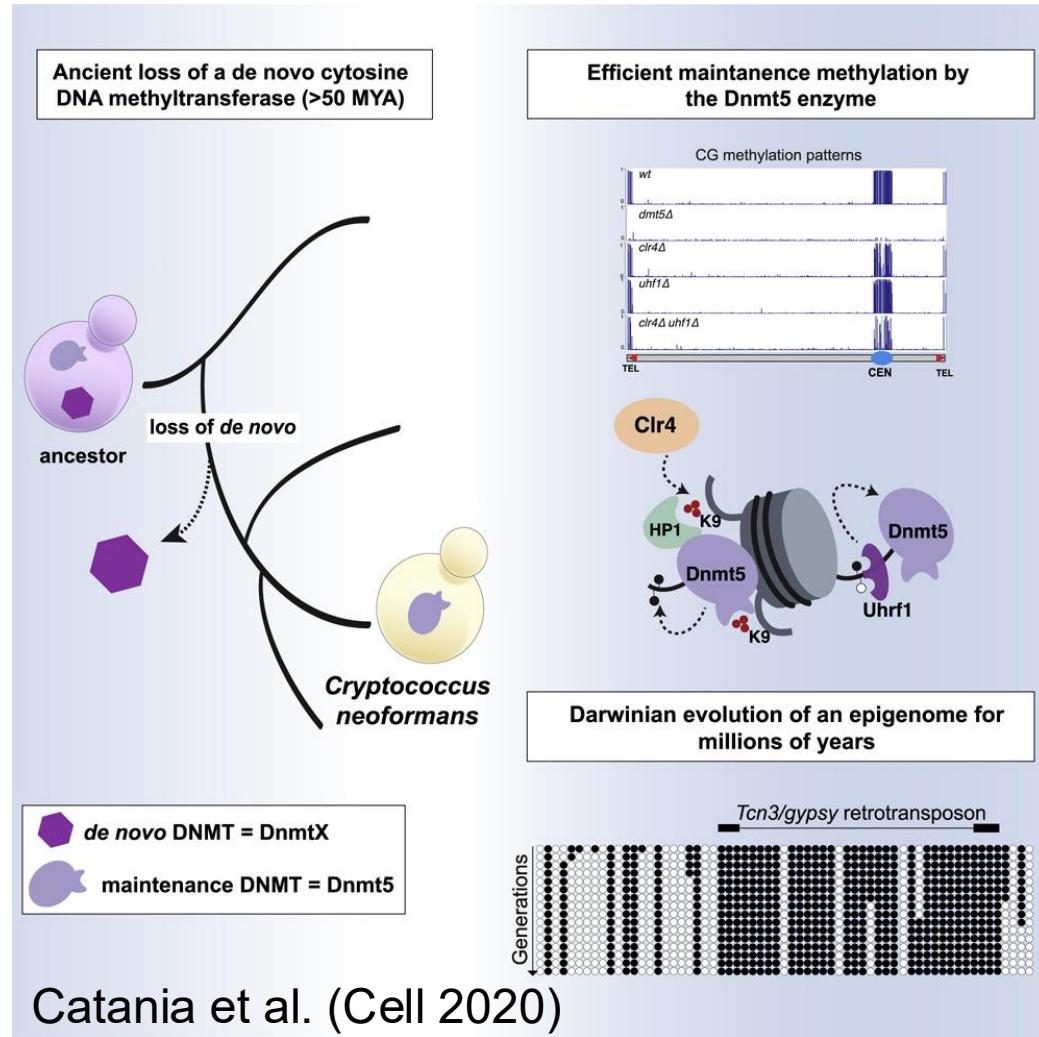


DNA Methylation is Heritable



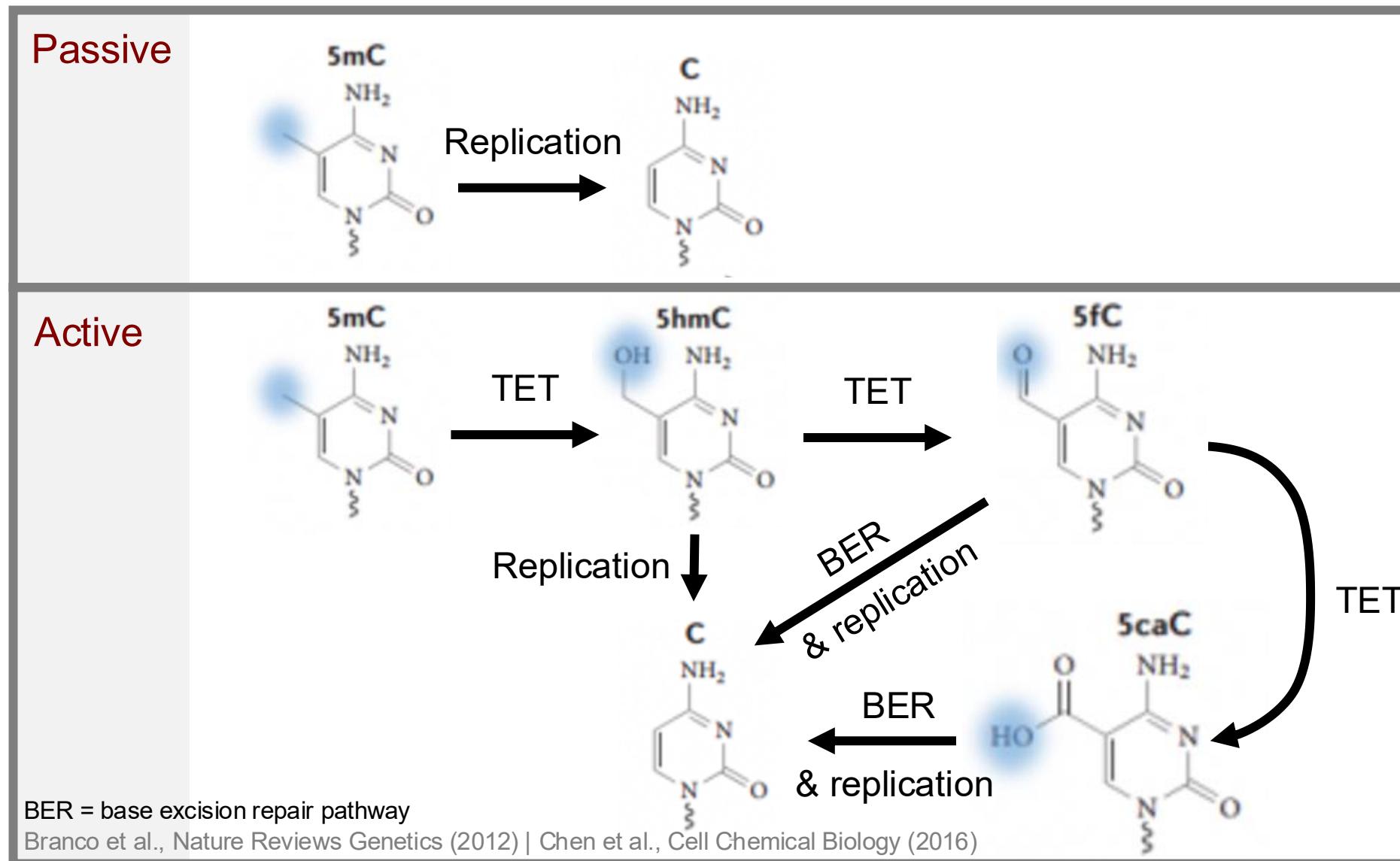
DNMT: DNA methyltransferase
SAM: S-adenosyl-methionine
SAH: S-adenosyl-L-homocysteine

DNA methylation patterns can persist for millions of years without *de novo* methyltransferases!



“We propose that an **epigenome** has been propagated for >50 million years through a process analogous to Darwinian evolution of the genome.”

DNA demethylation pathways



Function of DNA Methylation in Mammalian System

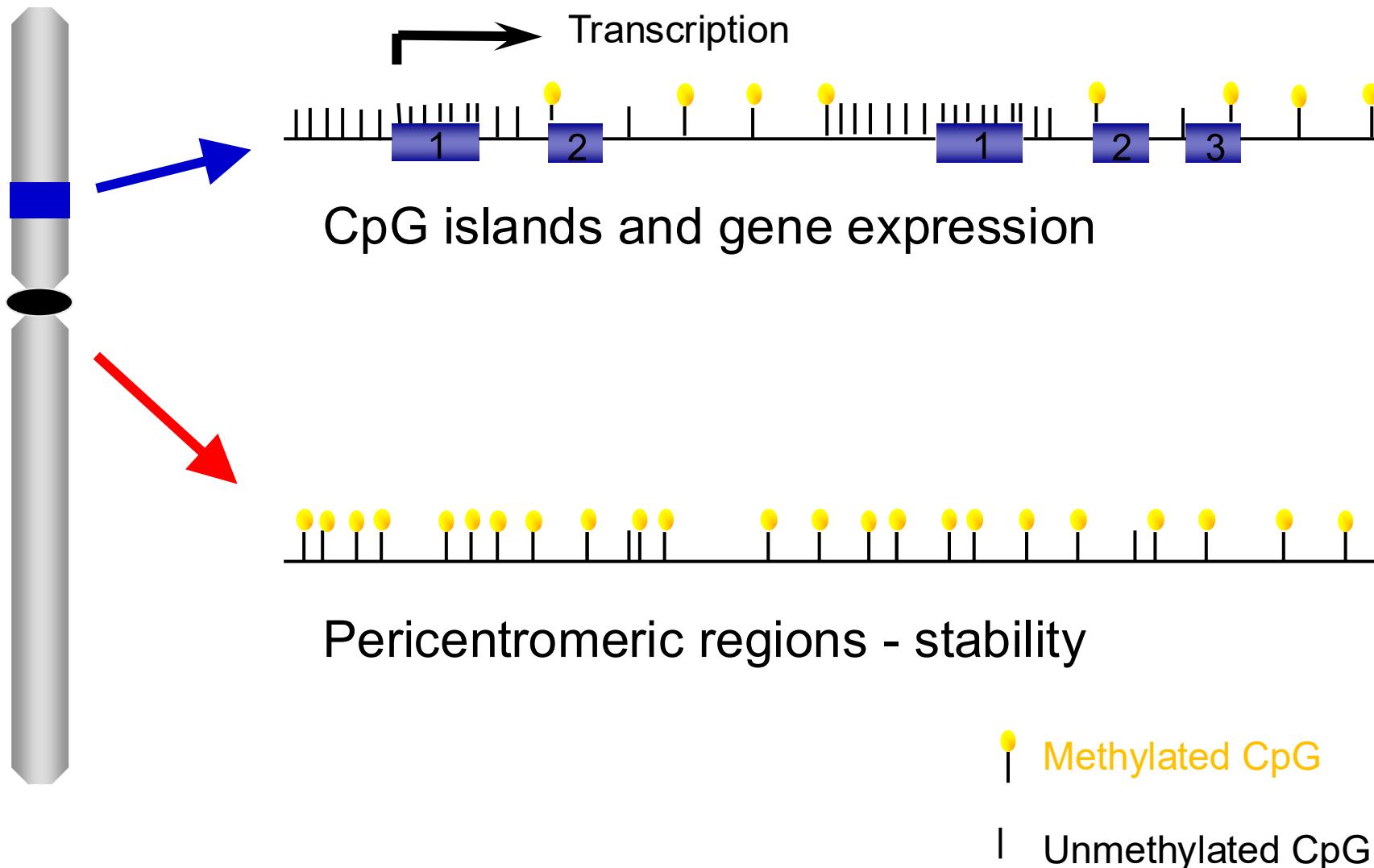
- Host defense - endogenous parasitic sequence (repeats, etc.)
- Imprinting
- X chromosome inactivation
- Heterochromatin maintenance, chromosome stability, telomere length
- Gene expression controls

Normal pattern and function of DNA methylation

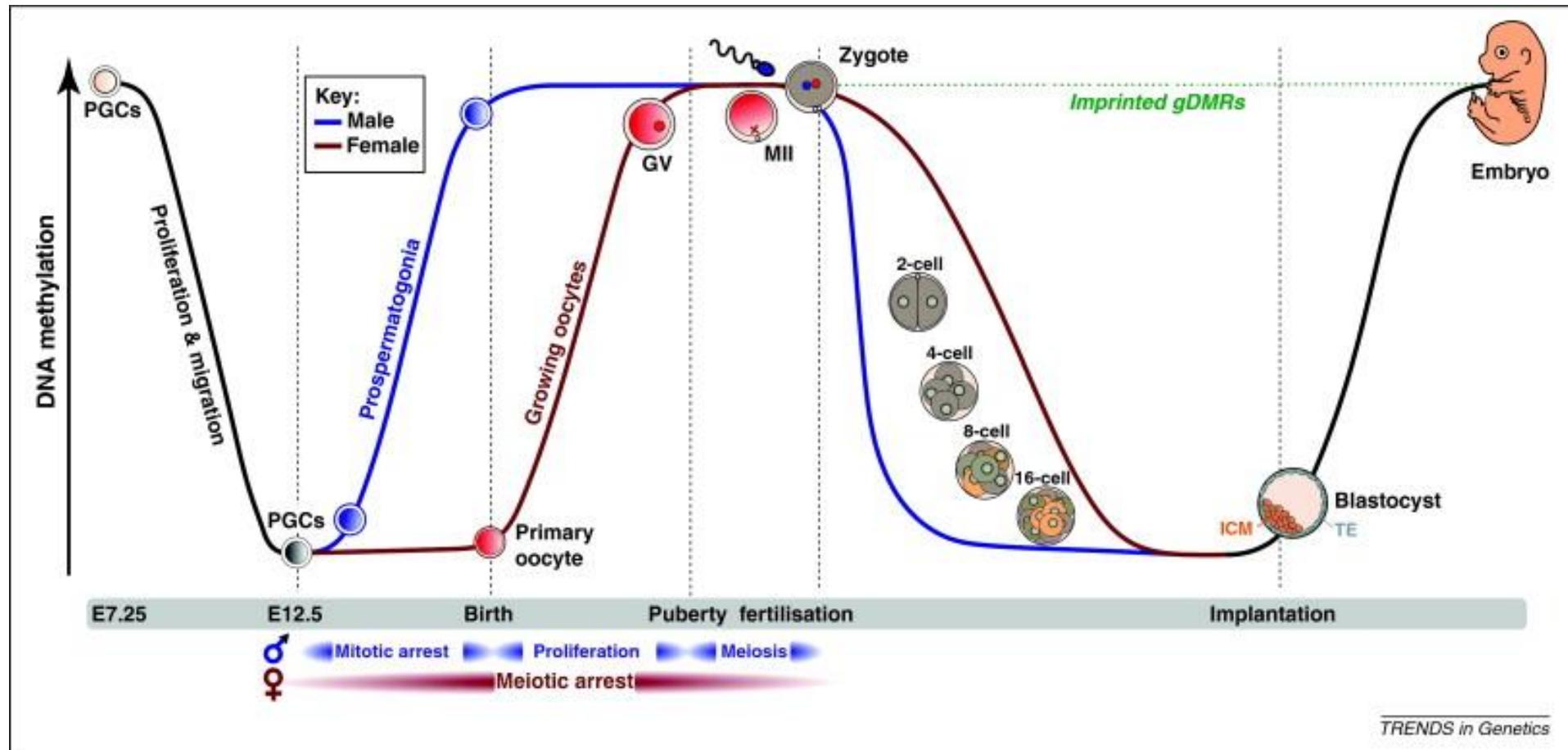
DNA methylation is not distributed evenly in the mammalian genome

- In human somatic cells, 60%-80% of all CpGs (~1% of total DNA bases) are methylated
 - Most methylation is found in “repetitive” elements
- “CpG islands”, GC-rich regions that possess a high density of CpGs, remain methylation-free
 - The promoter regions of ~70% of genes are embedded in CpG islands

Normal Patterns of DNA Methylation

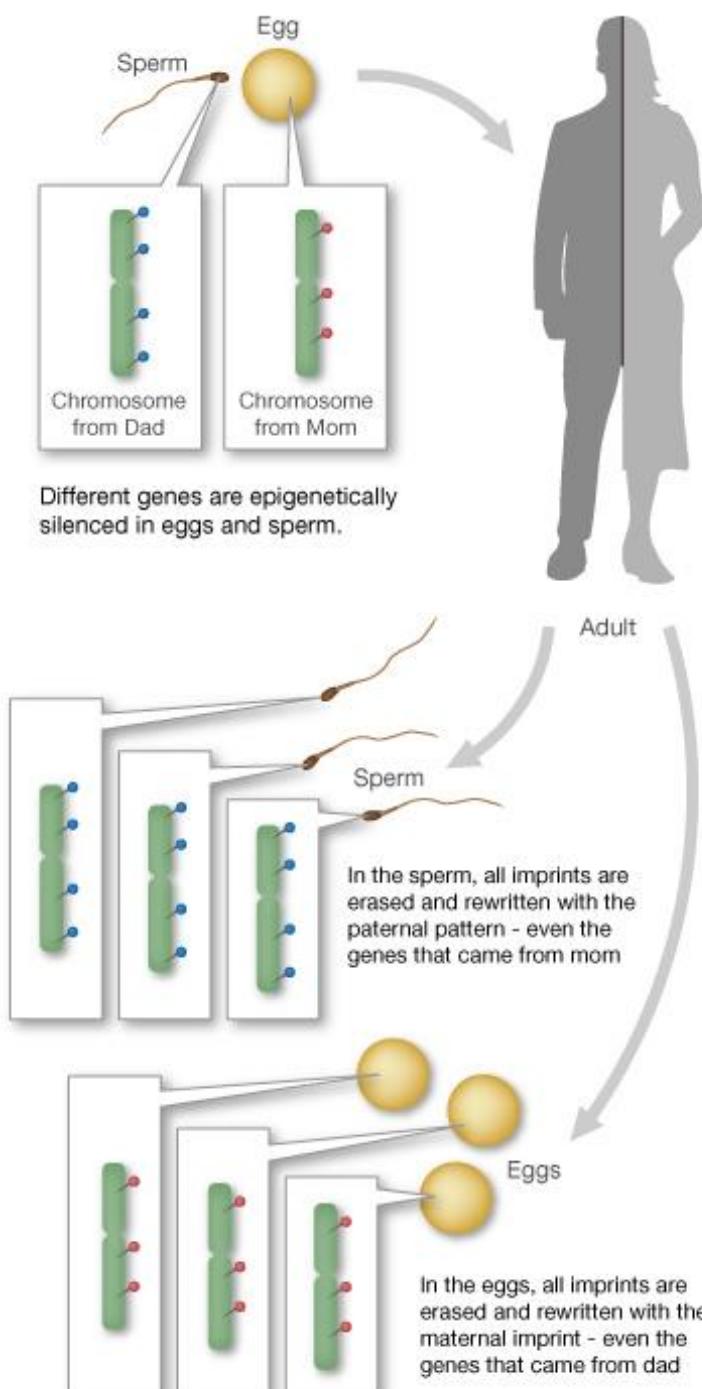


DNA methylation changes during developmental epigenetic reprogramming



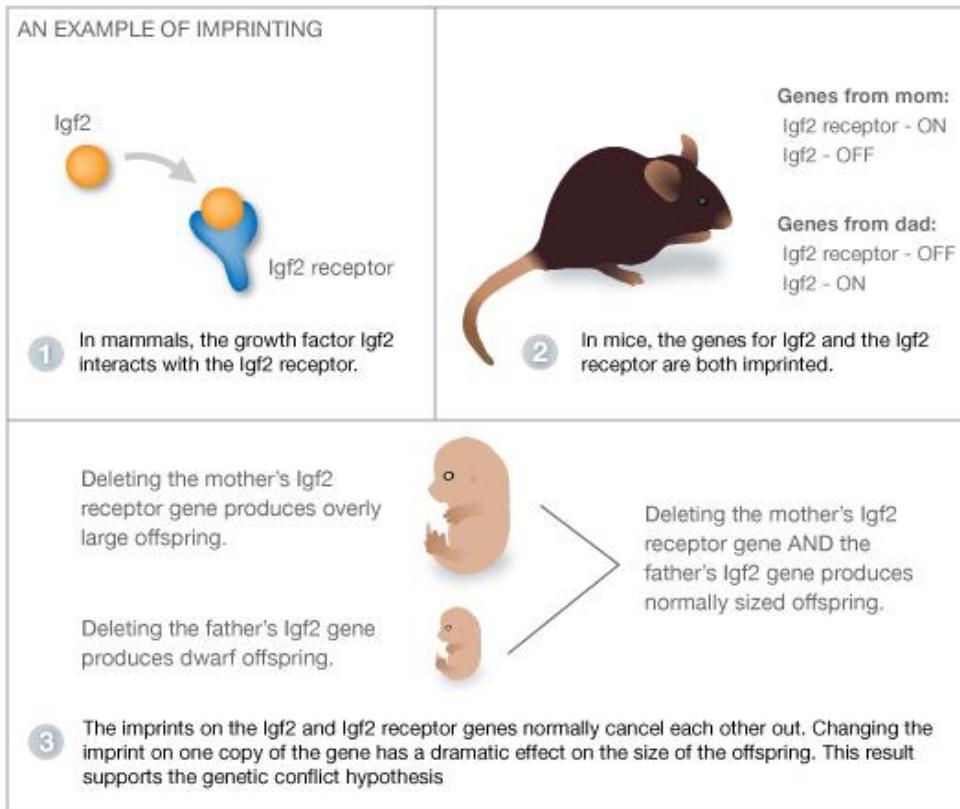
Genomic imprinting

- Imprinting is unique to mammals and flowering plants. In mammals, about 1% of genes are imprinted.
- For imprinted genes, one allele is expressed and the other is silent.
- This is typically controlled epigenetically. The expressed alleles are unmethylated and associated with loosely packed chromatin.
- Imprinted genes bypass epigenetic reprogramming.
- Imprinting is required for normal development



Why imprinting?

- The Genetic Conflict Hypothesis
 - Many imprinted genes are involved in growth and metabolism.
 - Paternal imprinting favors the production of larger offspring, and maternal imprinting favors smaller offspring
- Imprinted genes are under greater selective pressure.
 - No back up!
 - Any variation in single gene is expressed
 - Closely related species have different imprinting patterns
 - Liger and Tigon
- Imprinted genes are sensitive to environmental signals.



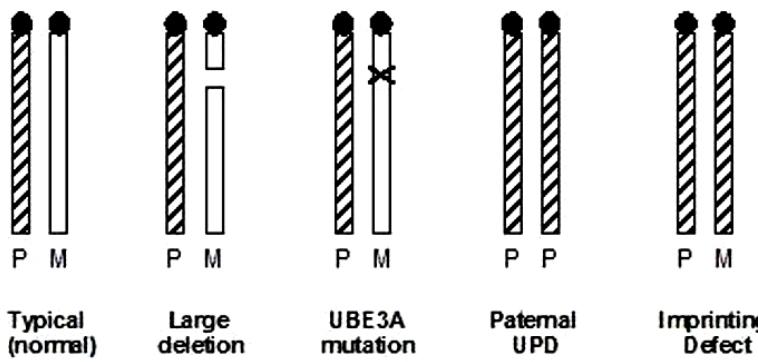
Imprinting Diseases: Angelman and Prader-Willi Syndromes



Chr15 deletion, ~4Mb

Loss of maternal contribution
UBE3A
Angelman Syndrome

Loss of paternal contribution
SNRPN
Prader-Willi Syndrome



Mechanisms of gene silencing by methylation

Direct mechanism:

Inhibition of transcription factor binding (eg.CTCF, UBF)

Not a universal mechanism since not all transcription factor binding sites contain CG dinucleotides

Indirect mechanism:

Inhibition mediated by methyl-CpG binding proteins MeCP1/ MeCP2

Recruitment of corepressor complexes including histone deacetylases (HDAC)

Change in chromatin conformation

Rett Syndrome

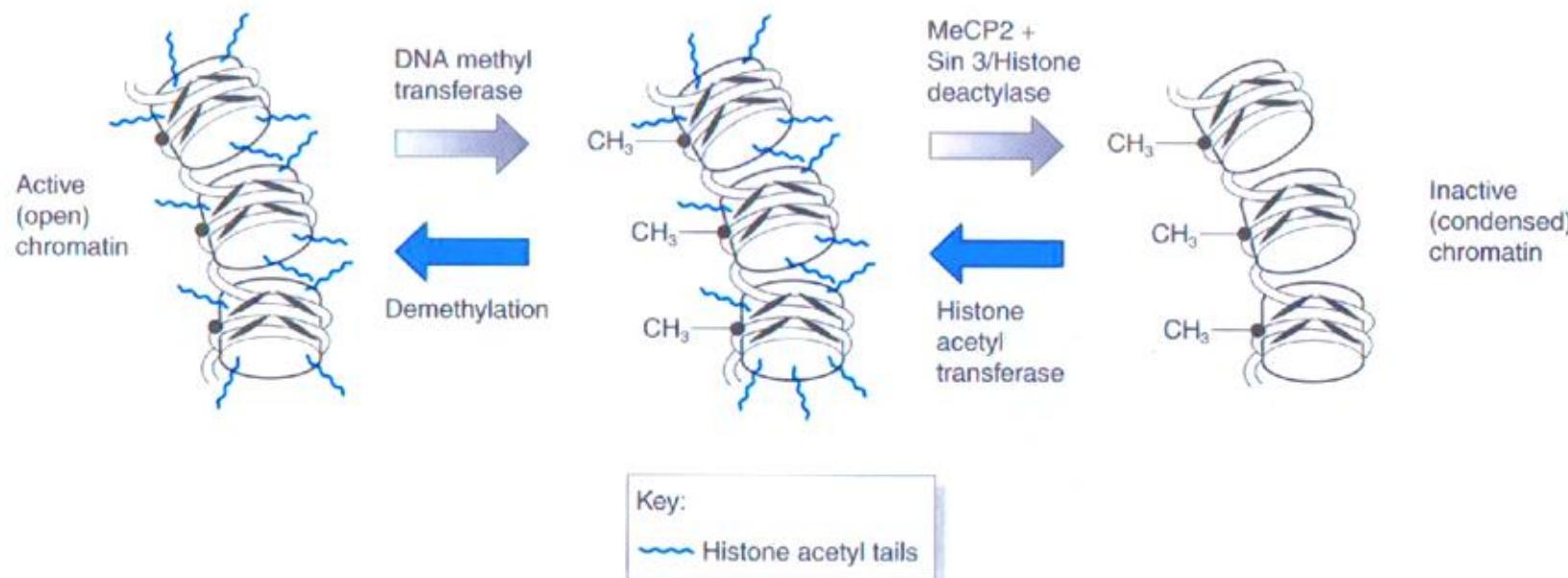


Nature Genetics (1999) 23:127-128

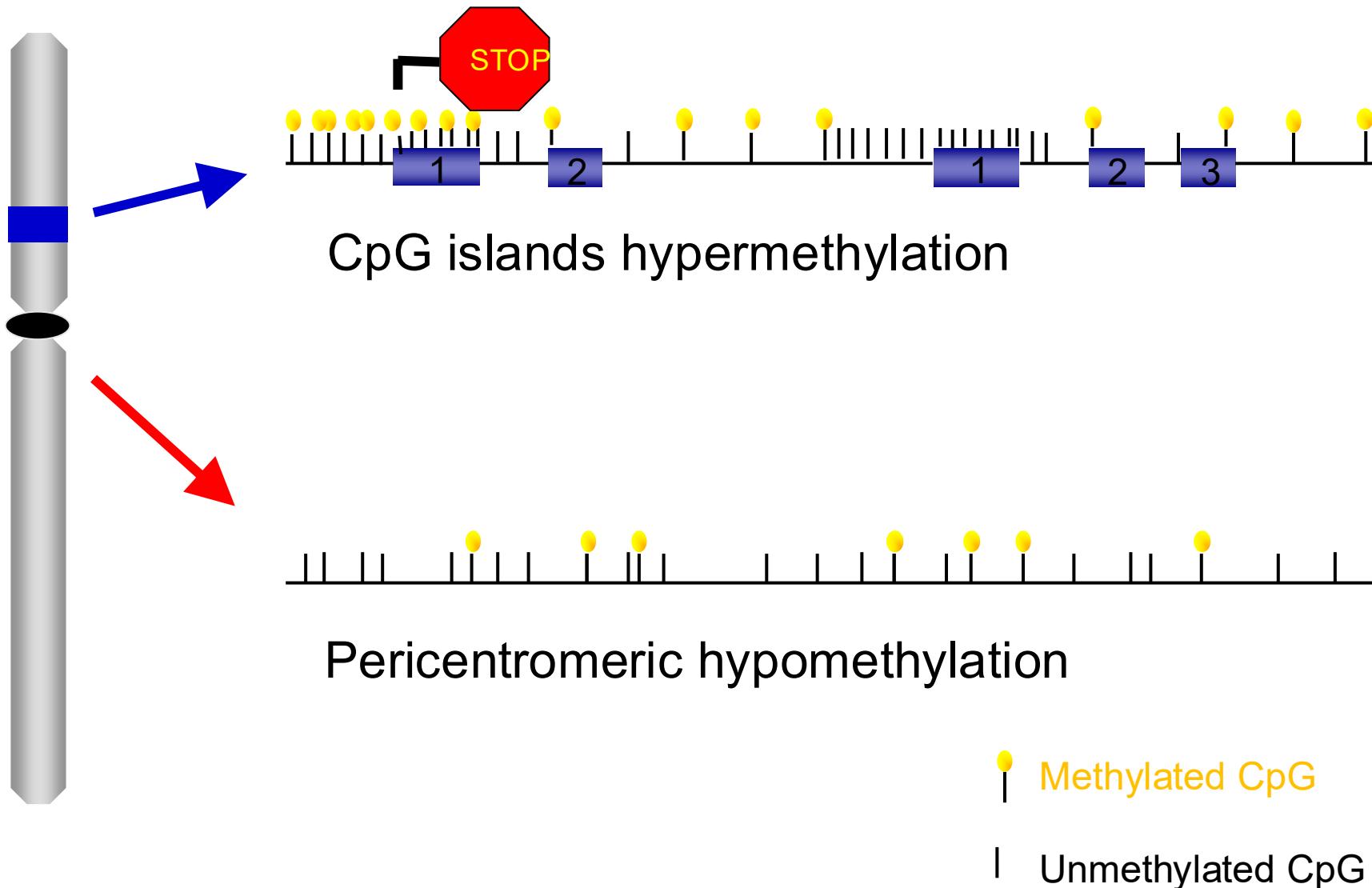
- X-linked trait
- Mainly girls affected
- Normal at birth
- At 6-18 months, begin losing purposeful movement
- Persistent wringing of hands
- Loss of speech, gait
- Severe intellectual disability

Rett's is due to defect in MeCP2

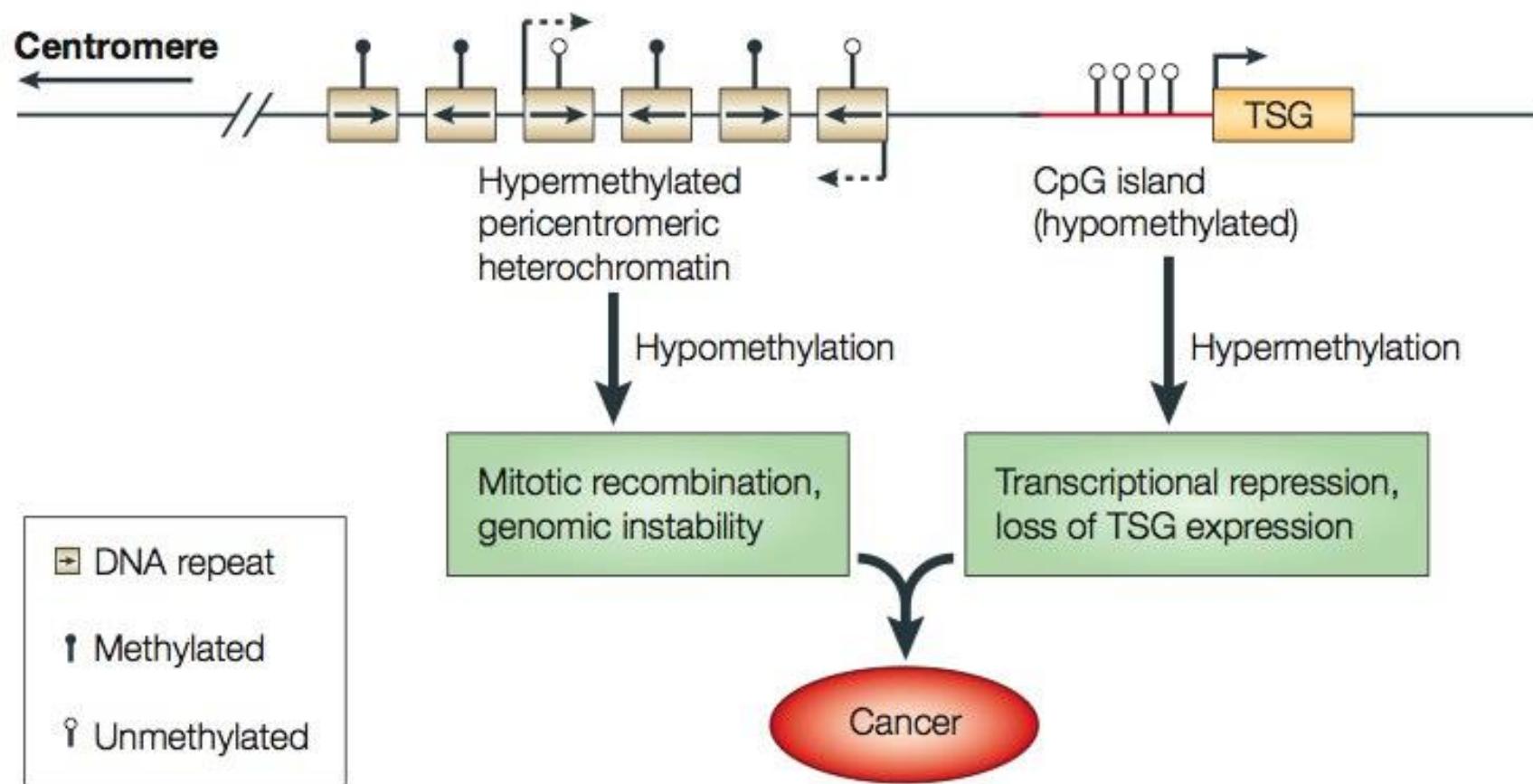
- Methyl-cytosine binding protein 2 (MeCP2) binds methylated DNA and recruits binding of a histone deacetylase
- Normal role is tightening chromatin packing, leading to gene silencing



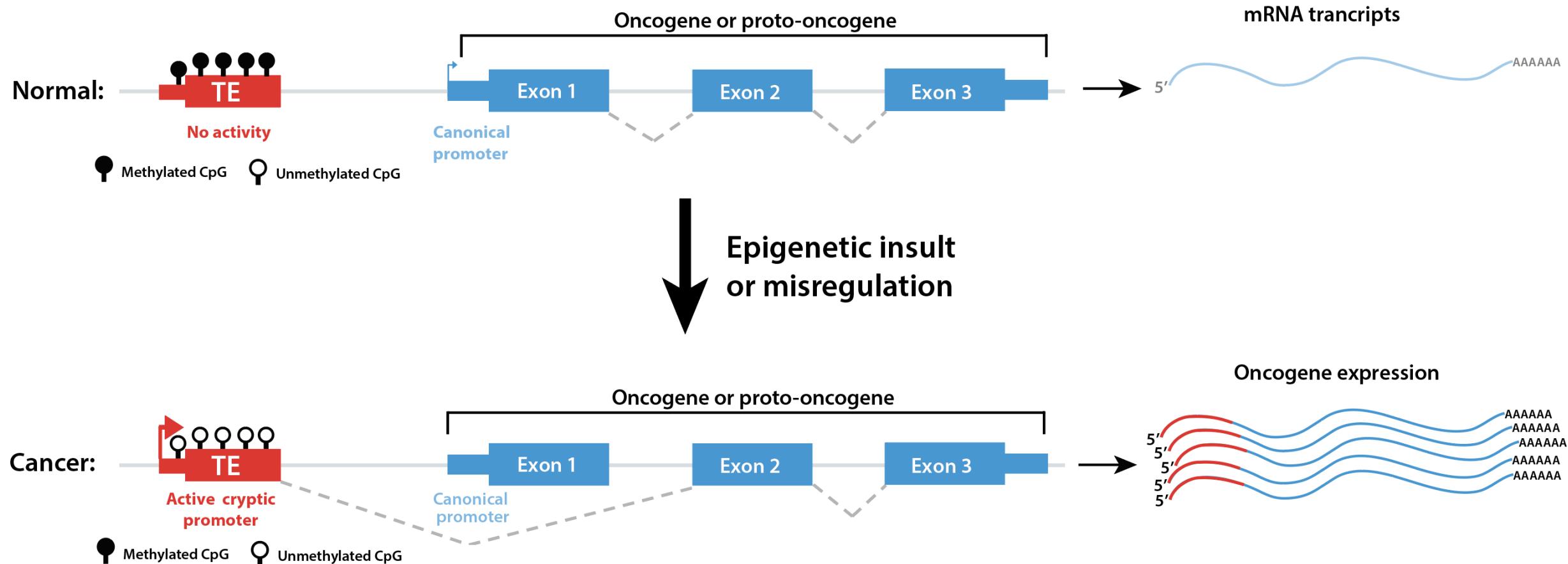
Aberrant DNA Methylation in Cancer



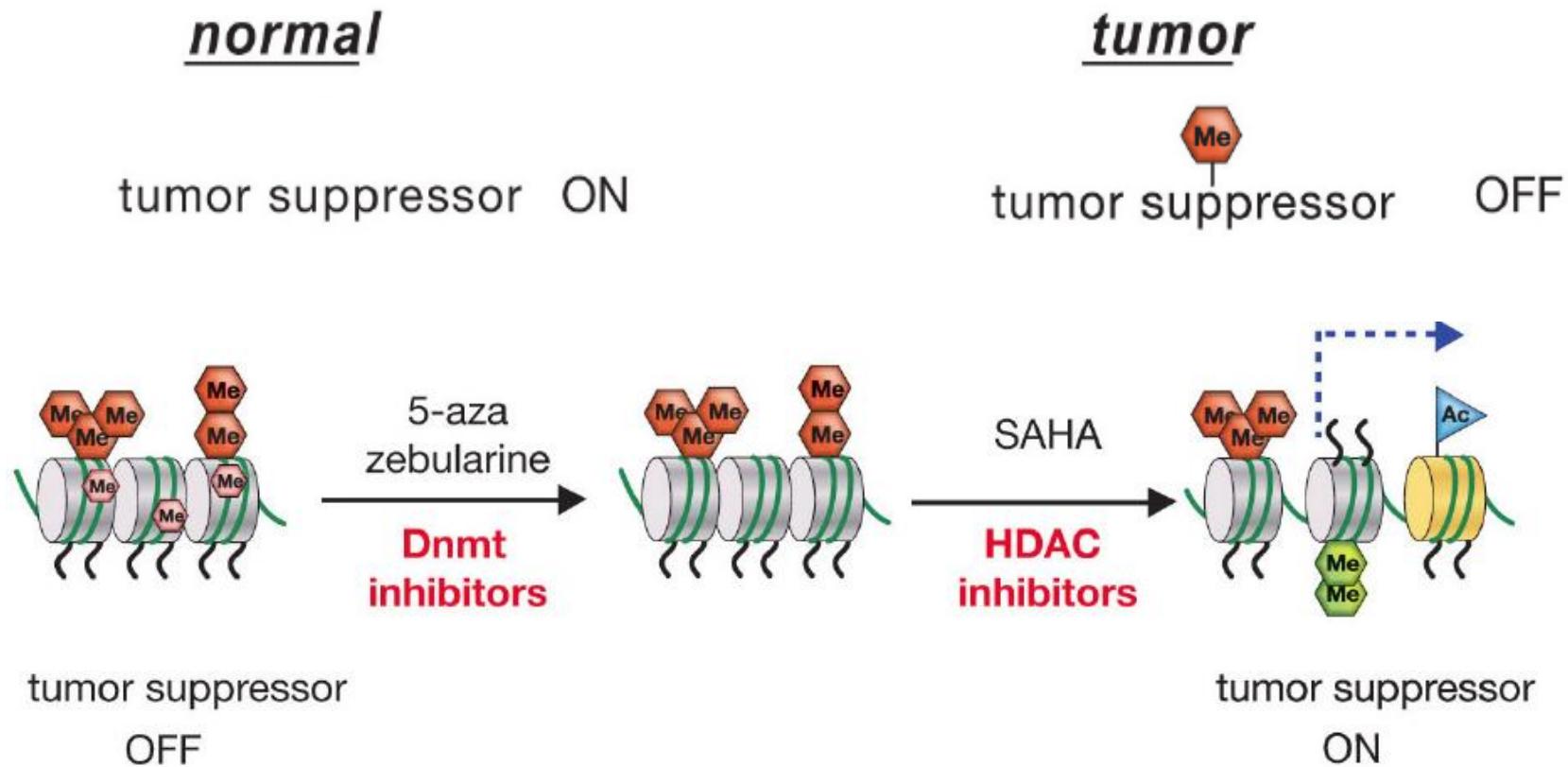
Different regions of the genome are hypermethylated or hypomethylated in cancer



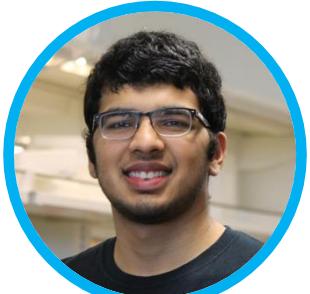
Epigenetic mutation upregulate oncogenes



“Epigenetic cancer therapy”



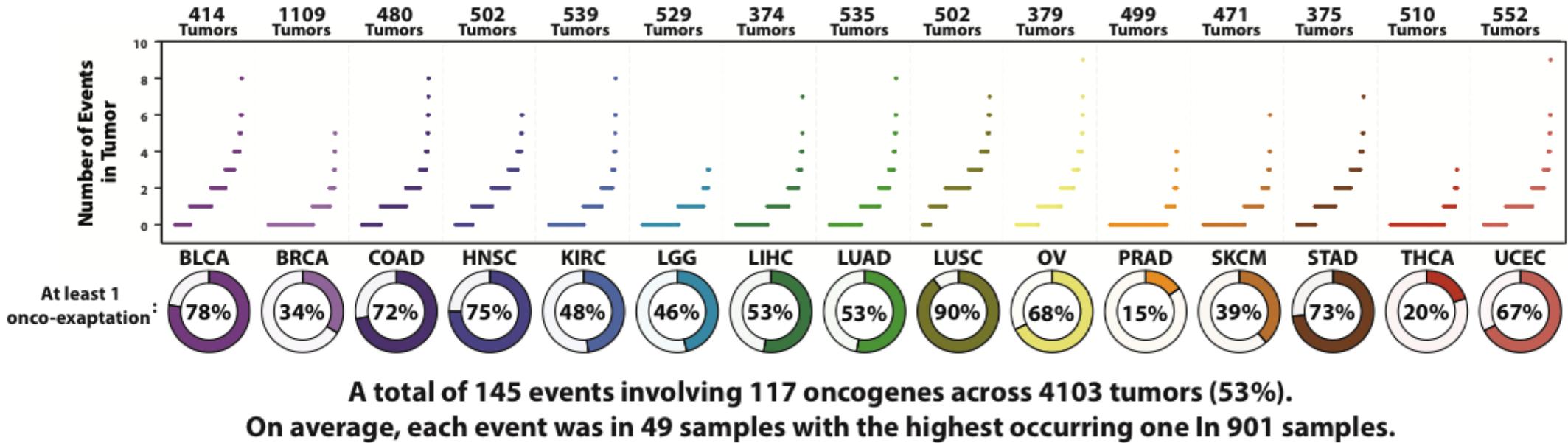
50% tumors had at least one oncoexaptation



Nakul

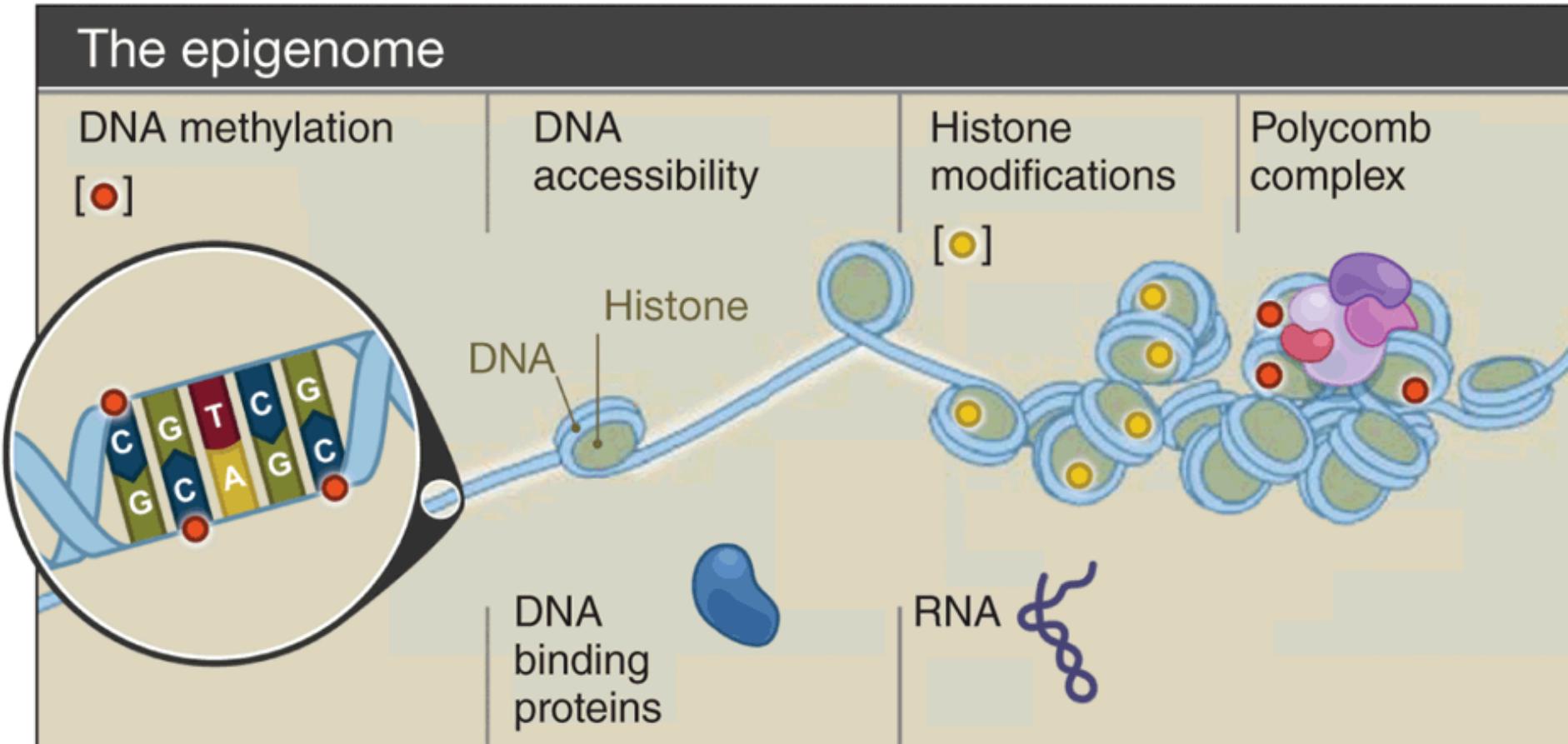


Josh



The Epigenome

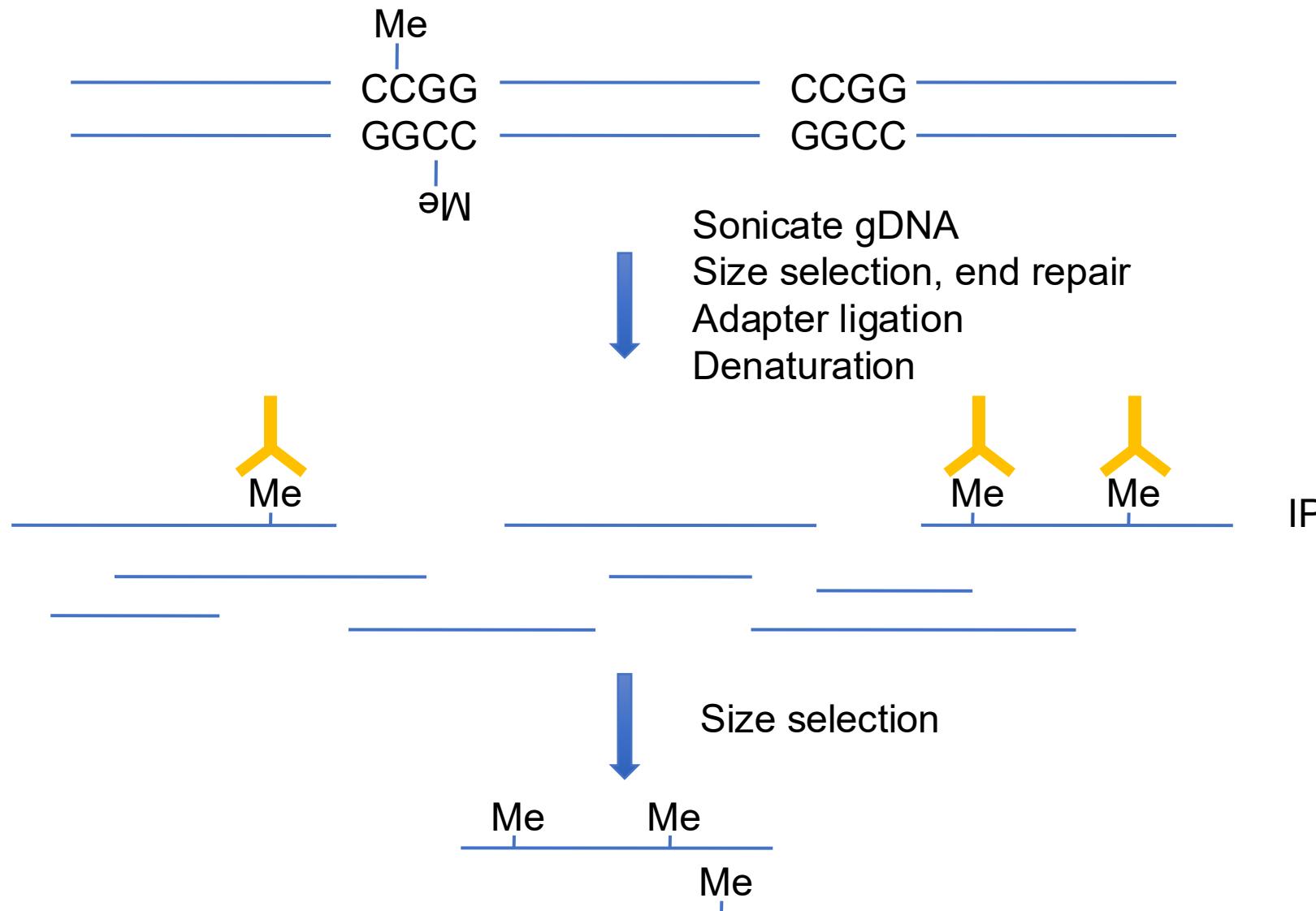
- While epigenetics often refers to the study of single genes or sets of genes, epigenomics refers to more global analyses of epigenetic changes across the entire **genome**.



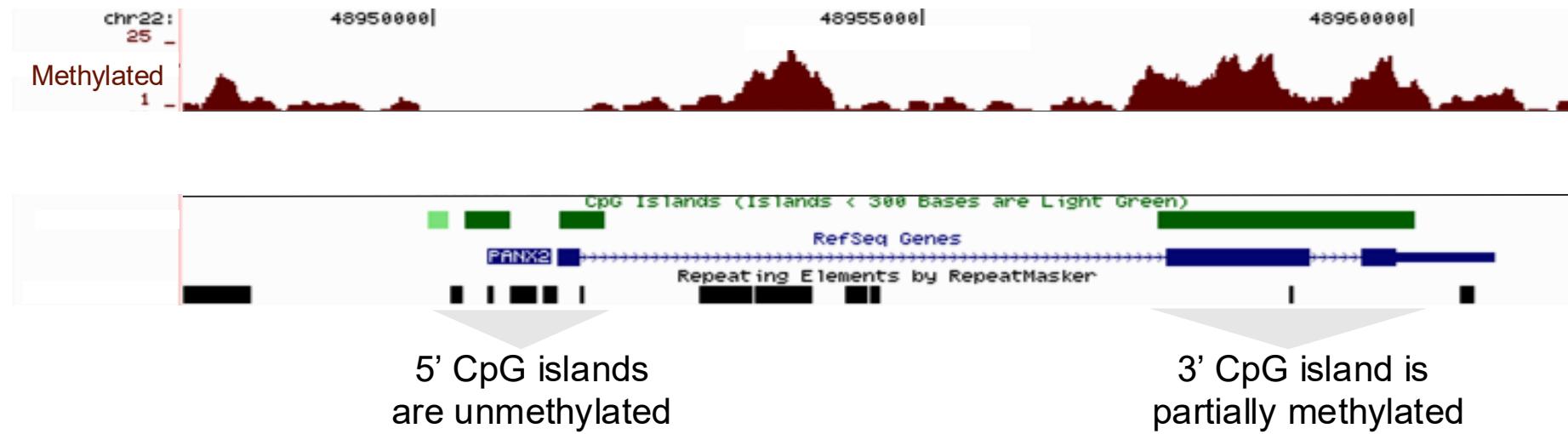
How to detect epigenetic marks?

Enriching for methylated DNA targets

MeDIP-seq and MBD-seq

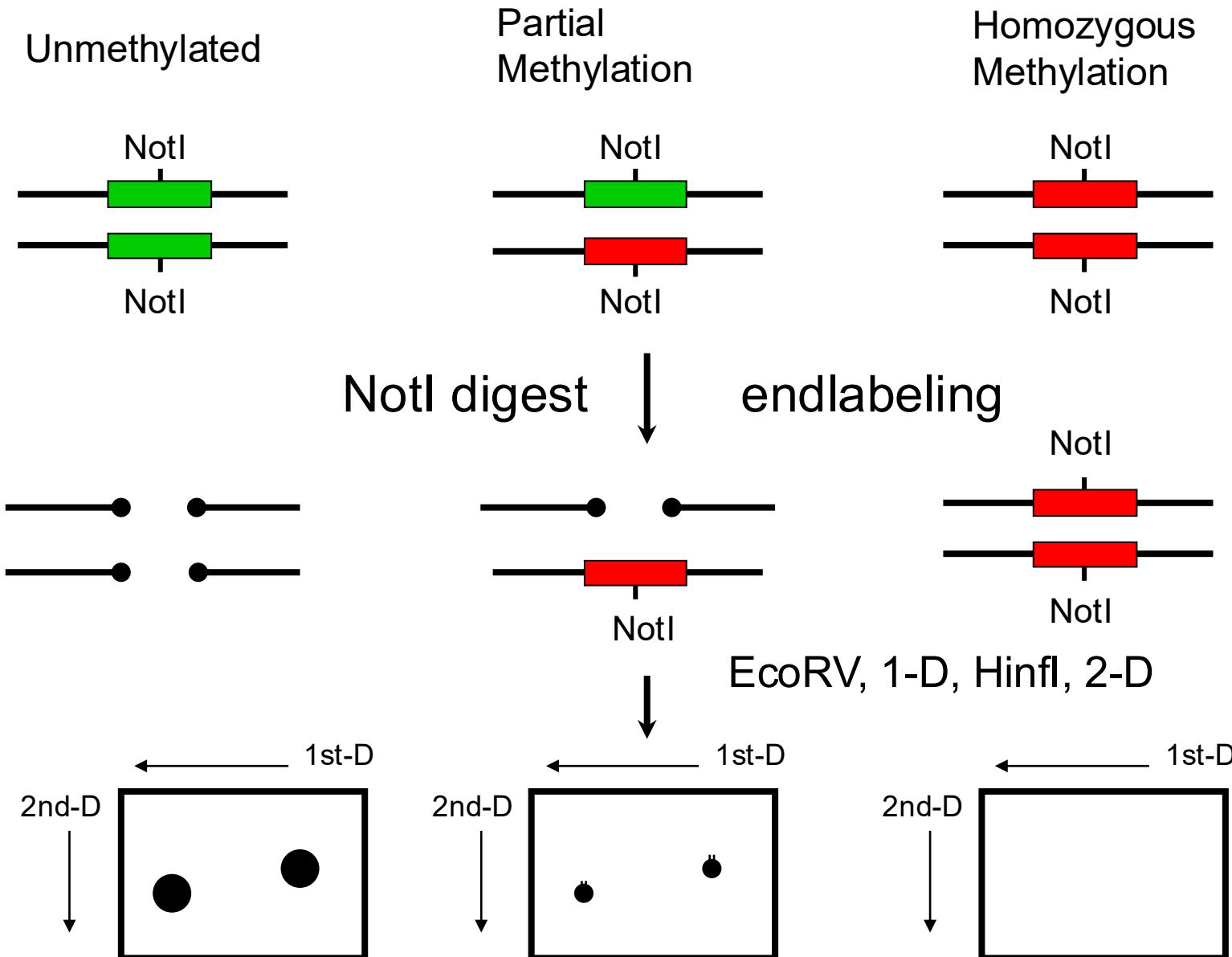


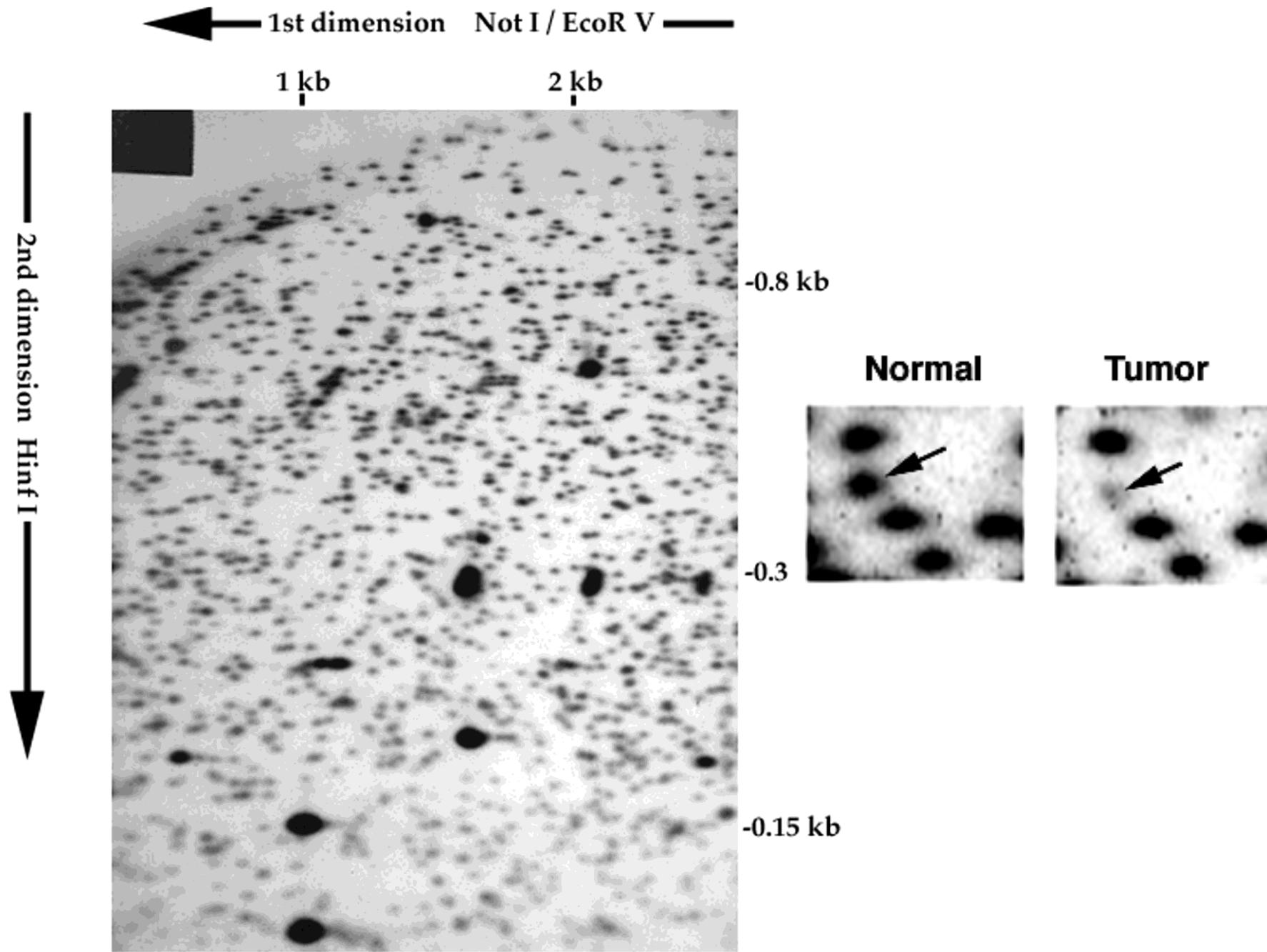
Typical MeDIP data on a genome browser



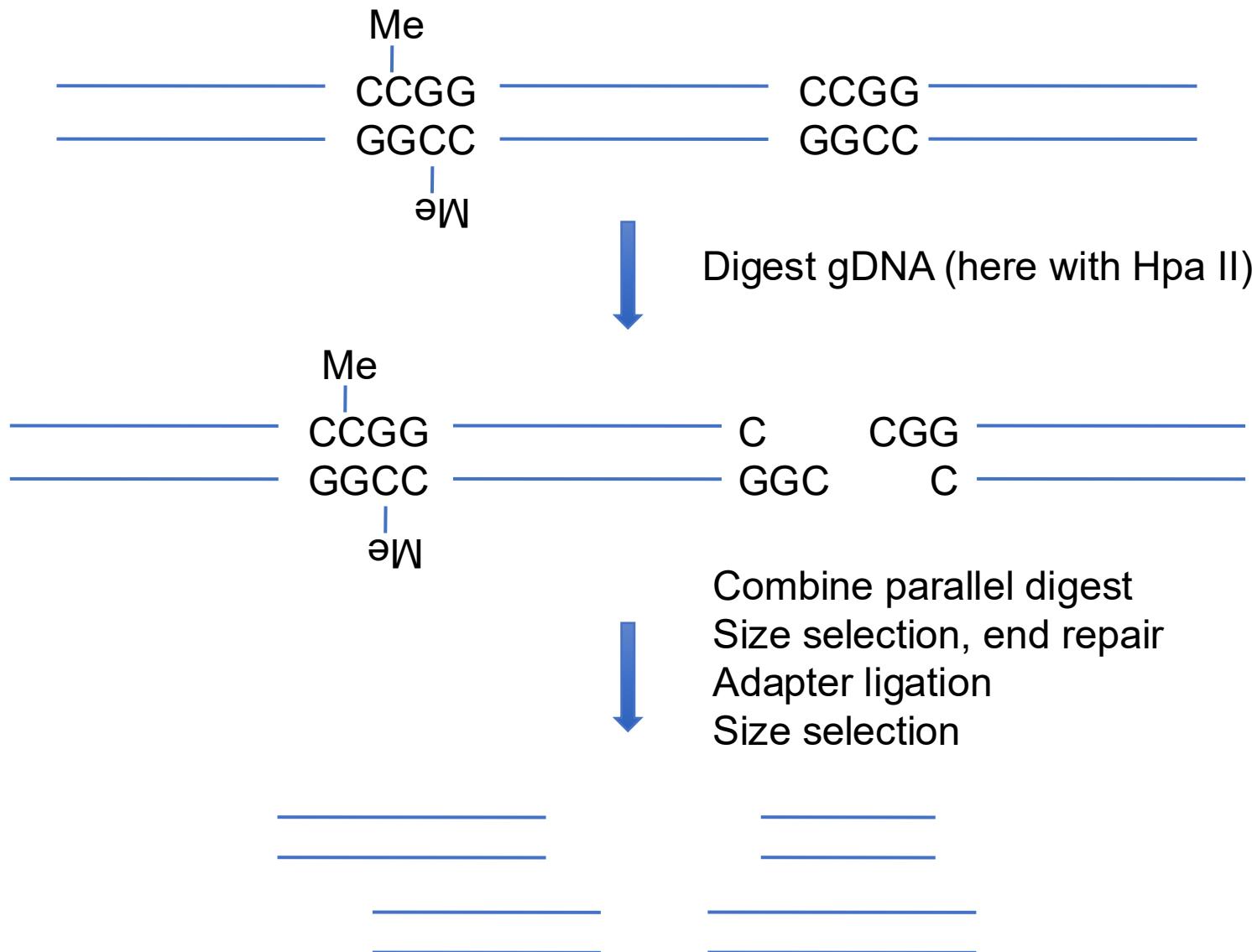
Taking advantage of methylation
dependent restriction enzymes

Restriction Landmark Genome Scanning (RLGS)

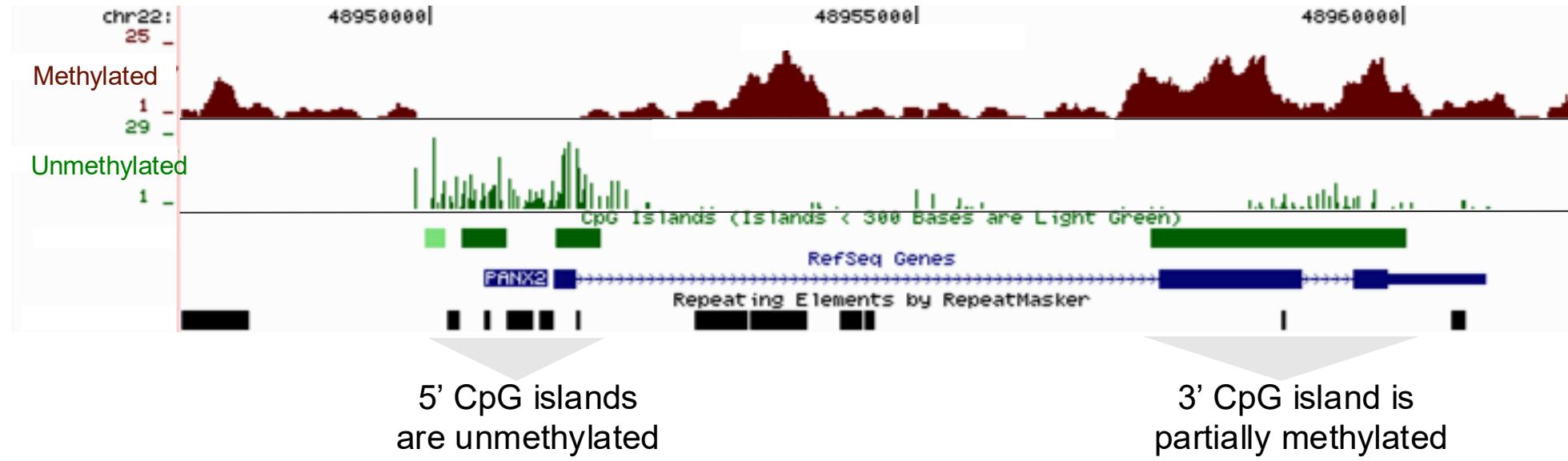




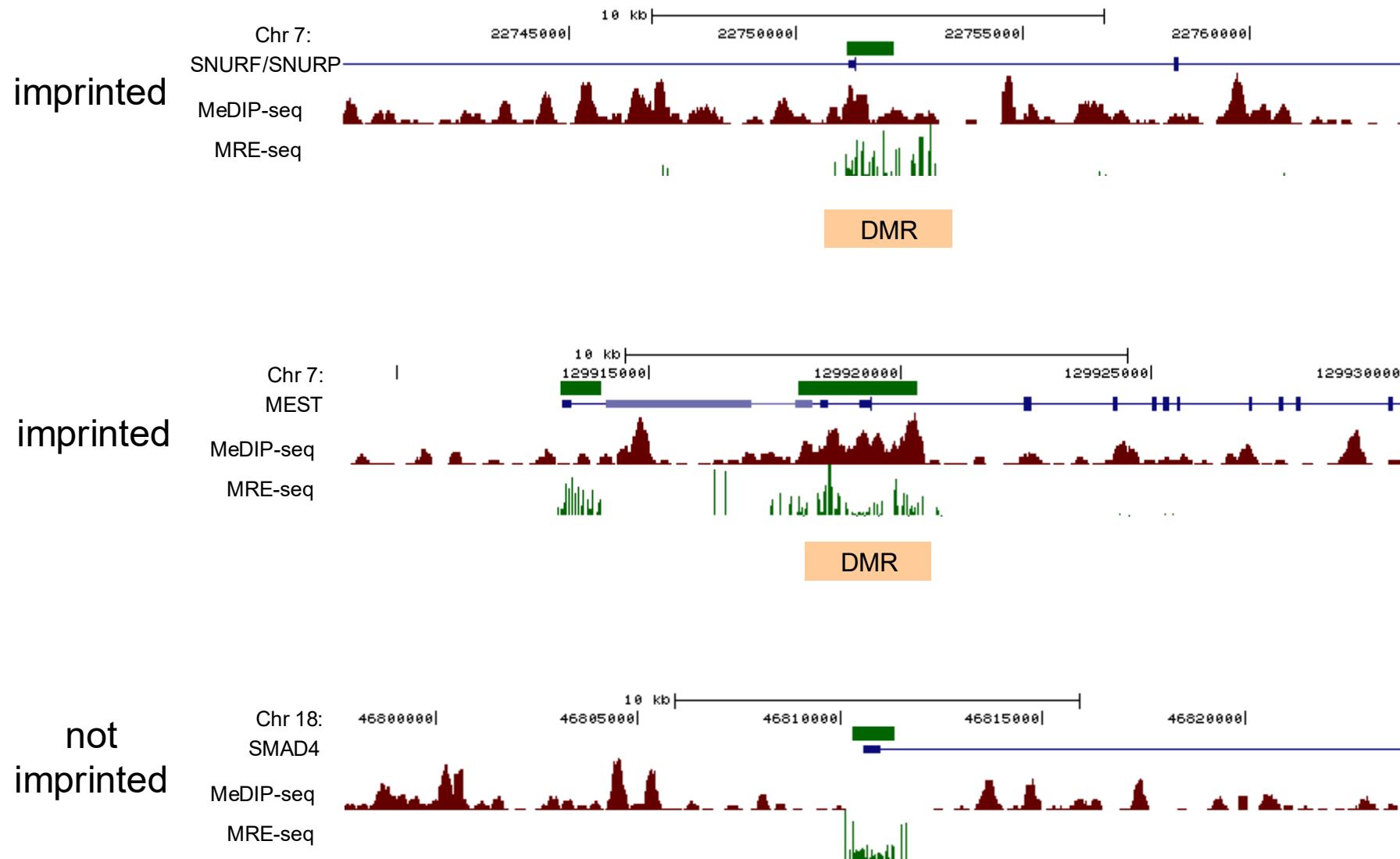
MRE-seq



Typical MeDIP/MRE data on a genome browser

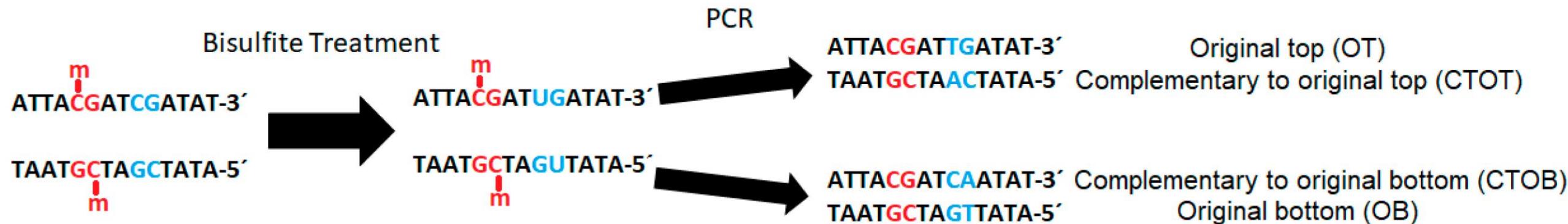
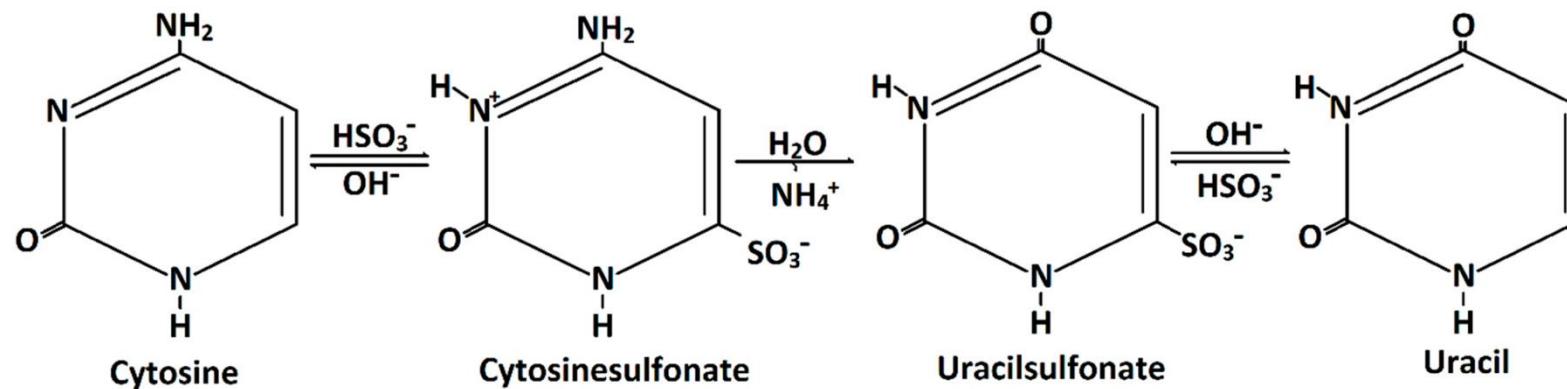


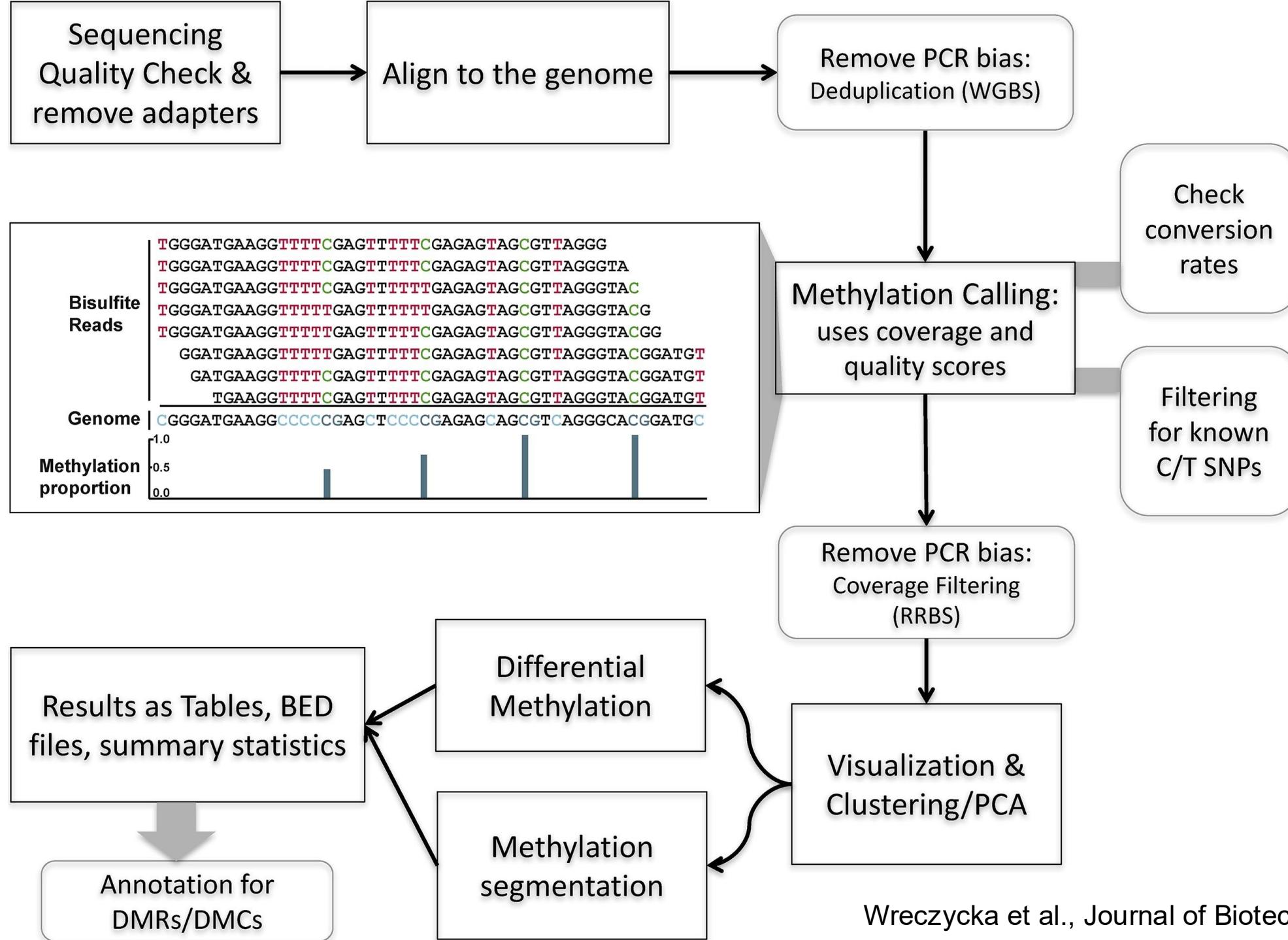
Allele-specific methylation at imprinted genes



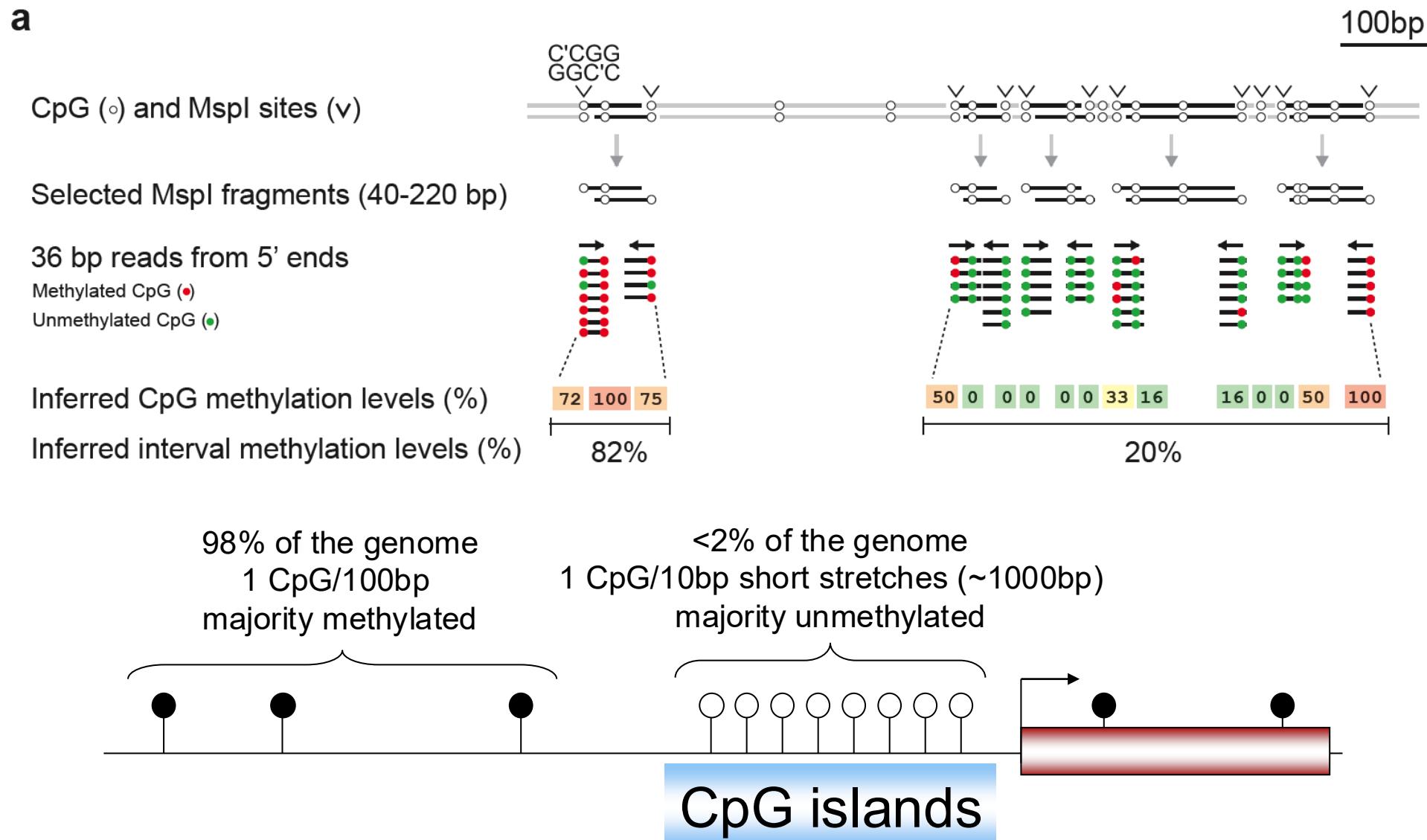
Chemical/enzymatic protection of methylated bases

Bisulfite sequencing





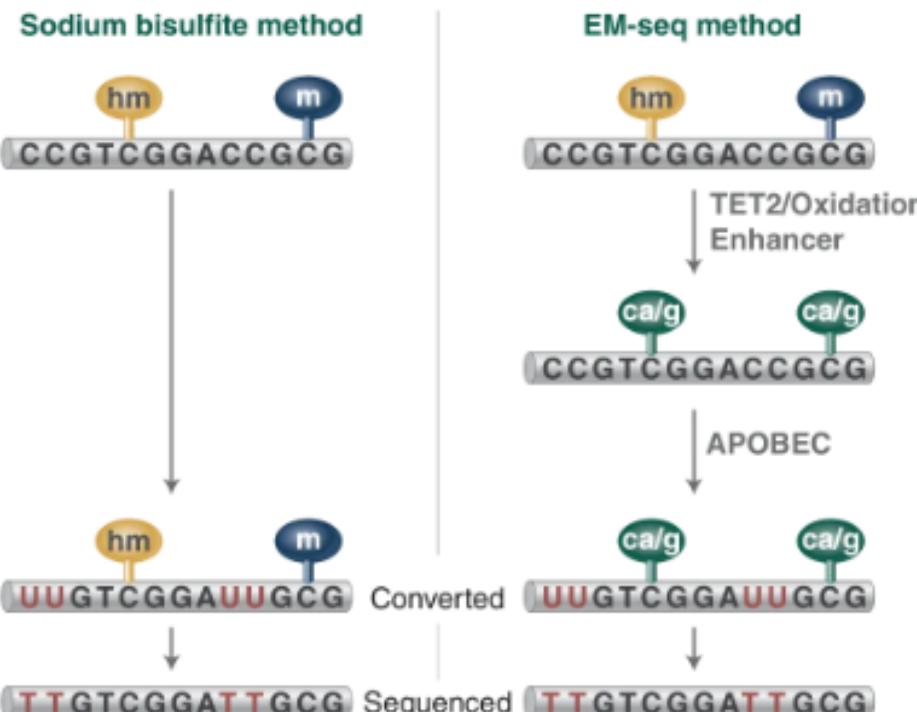
RRBS: Reduced Representations Allow Enrichment of CpG Dinucleotides



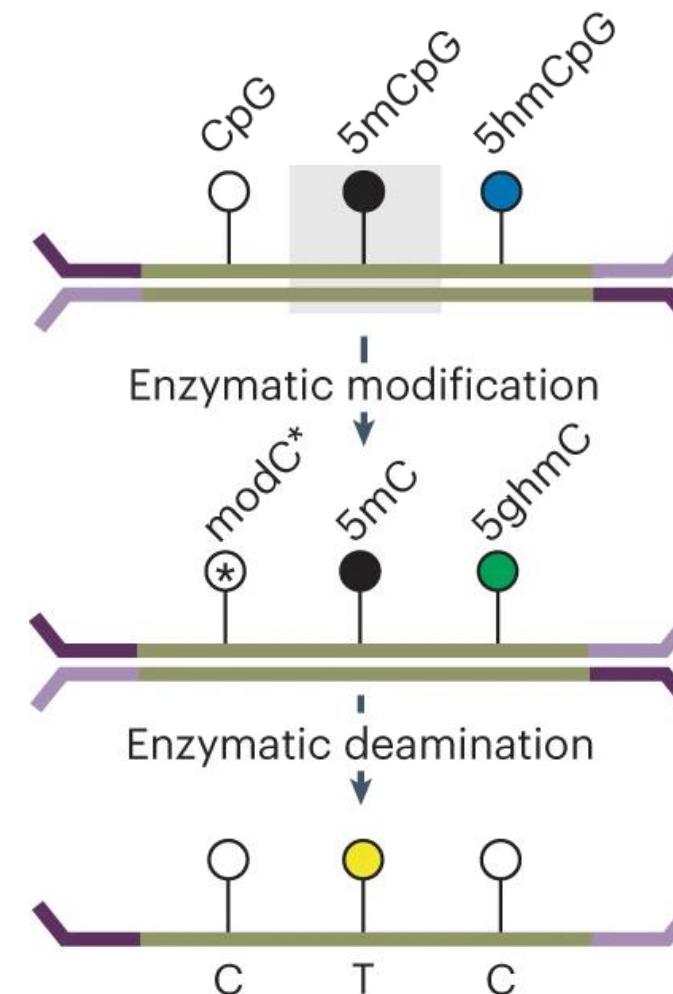
DNA BEFORE BISULFITE

DNA AFTER BISULFITE

Enzymatic alternatives to bisulfite conversion: TET or deaminase enzymes



New England Biolabs

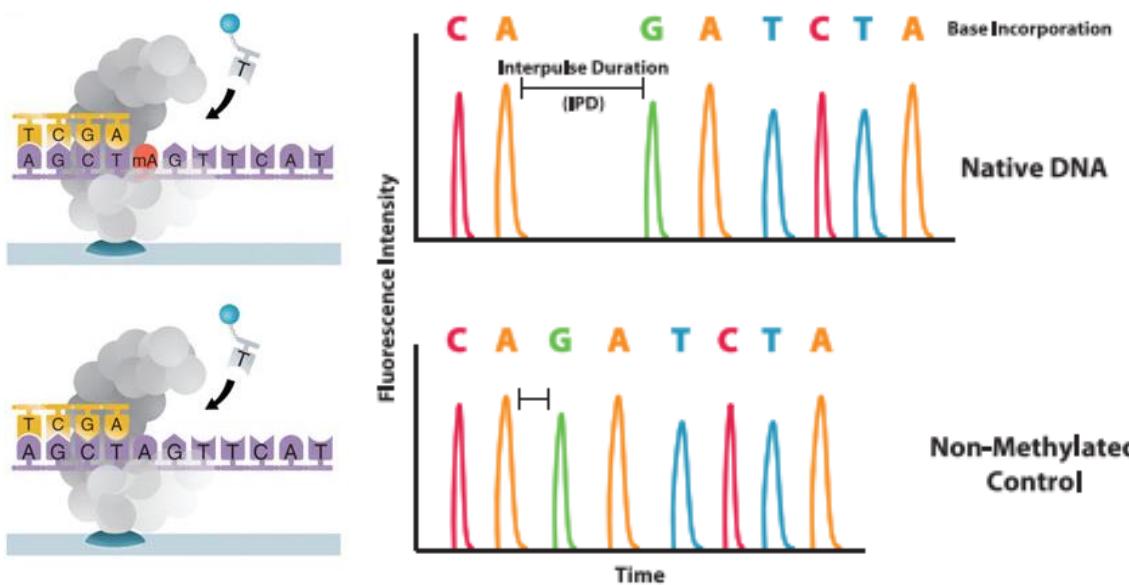


Wang et al. *Nat Chem Biol.* (2023)

Direct detection of modified nucleotides

Single-molecule real-time (SMRT) sequencing

SMRT sequencing discriminates between different bases by analyzing variations in polymerase kinetics



- **Pros**

- Single-base resolution
- Measures absolute levels of many modified nucleotides
- “Raw” DNA is used
- Long reads

- **Cons**

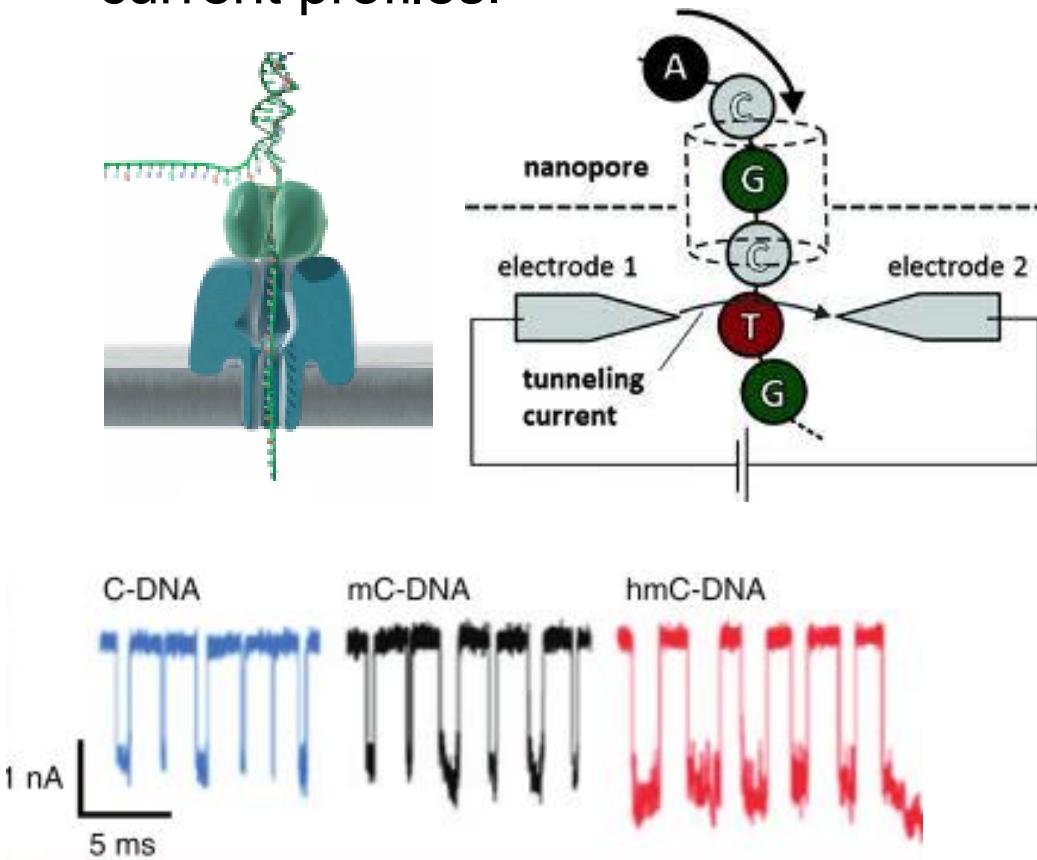
- Suboptimal accuracy
- Low throughput

Genome-wide detection of cytosine methylation by single molecule real-time sequencing

O. Y. Olivia Tse^{a,b,1} , Peiyong Jiang^{a,b,1} , Suk Hang Cheng^{a,b,1}, Wenlei Peng^{a,b}, Huimin Shang^{a,b}, John Wong^c , Stephen L. Chan^{d,e} , Liona C. Y. Poon^f, Tak Y. Leung^f, K. C. Allen Chan^{a,b,e}, Rossa W. K. Chiu^{a,b} , and Y. M. Dennis Lo^{a,b,e,2}

Nanopore sequencing

Nanopore amperometry methods can discriminate between C, 5-mC, and 5-hmC due to differences in current profiles.



- **Pros**

- Single-base resolution
- Measures absolute levels of many modified nucleotides
- “Raw” DNA is used
- Long reads

- **Cons**

- Suboptimal accuracy
- Low throughput

Article

Telomere-to-telomere assembly of a complete human X chromosome

<https://doi.org/10.1038/s41586-020-2547-7>

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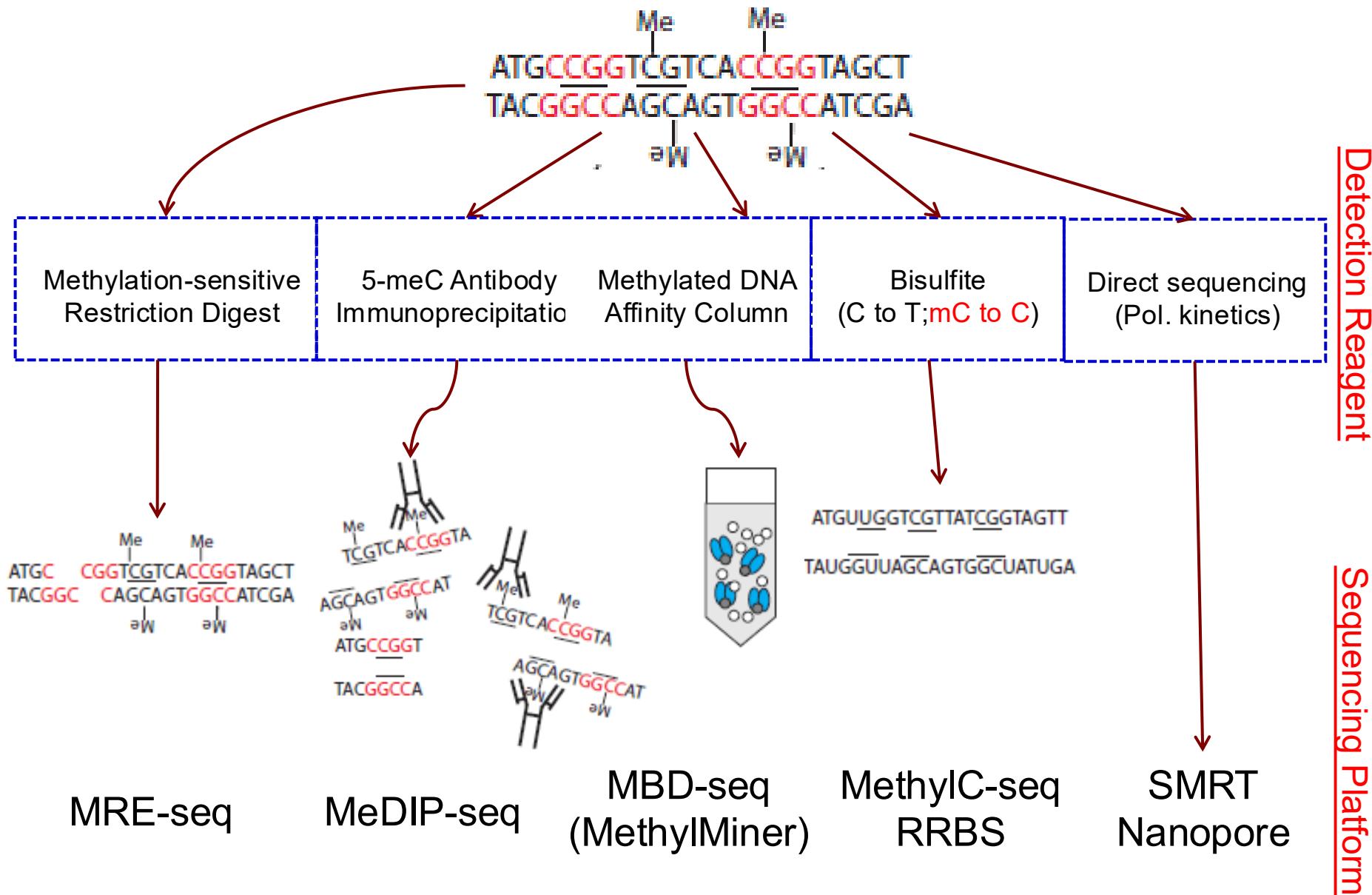
Published online: 14 July 2020

Open access

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Modern DNA Methylomics



Summary

- Epigenetics is the heritable (conventionally across cell cycles or generations) acquisition of phenotype differences in genetically identical cells/individuals
- DNA modifications (particularly 5mC methylation) can heritably influence gene expression outcomes that change phenotypes
- DNA modifications can be read and quantified via direct (e.g. SMRT-sequencing) or indirect (e.g. bisulfite, restriction digest) genomics methods