

Epigenetics in Toxicology

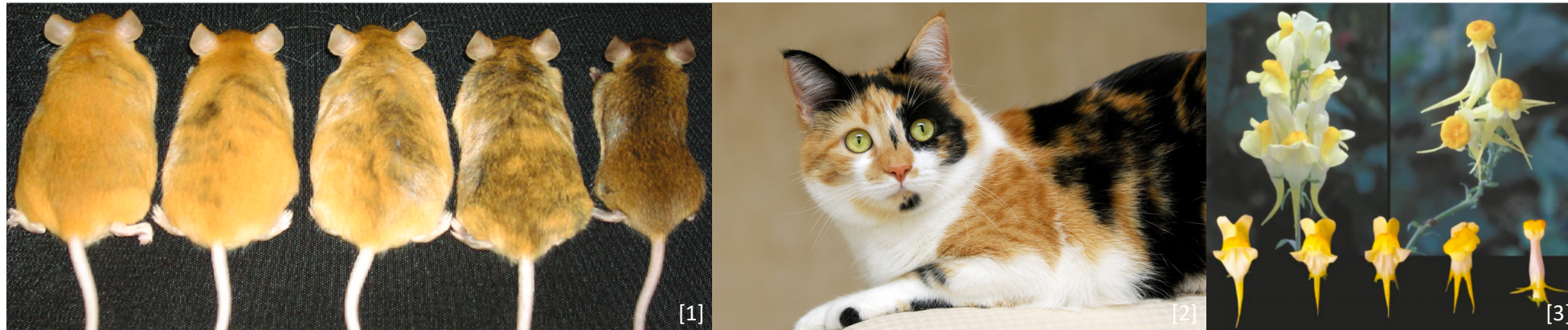


Tobias Sieckmann

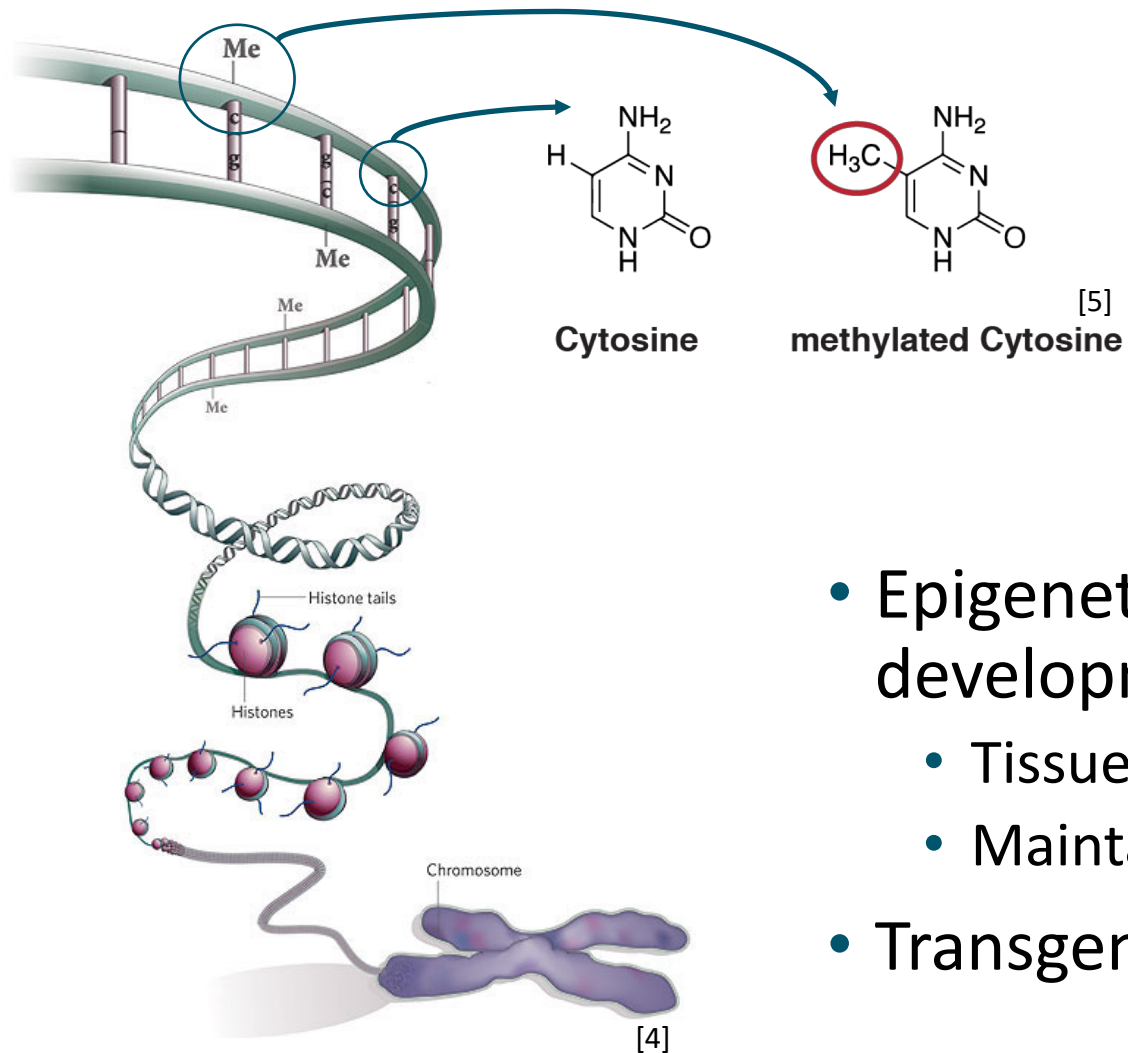
GRK2338 Methods Seminar
14.03.2019

Basics in Epigenetics

- „above“ genetics
- “the study of changes in gene function that are mitotically and/or meiotically heritable and that do not entail a change in DNA sequence” (Wu and Morris 2001)



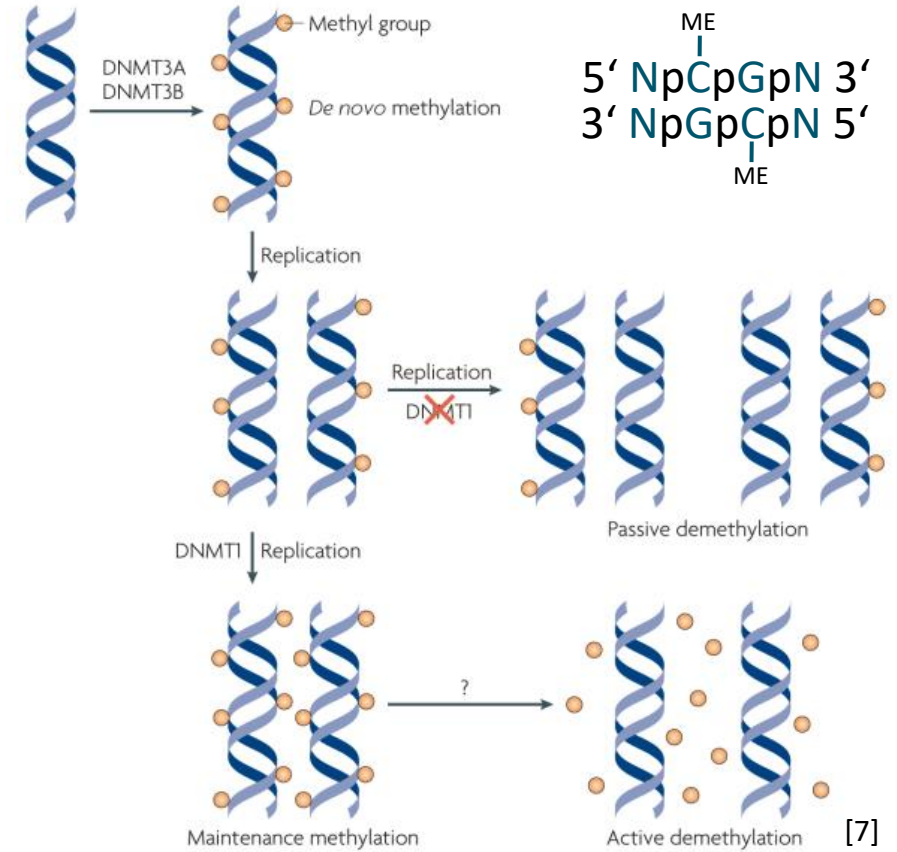
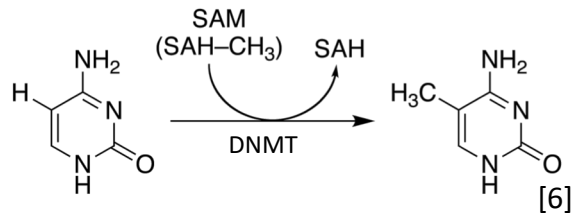
Epigenetics 101



- Three main levels
 - DNA methylation
 - Histone modifications
 - Non-coding RNAs (ncRNAs)
- Epigenetic landscape is established during development
 - Tissue- and cell-type specific
 - Maintained throughout life
- Transgenerational inheritable

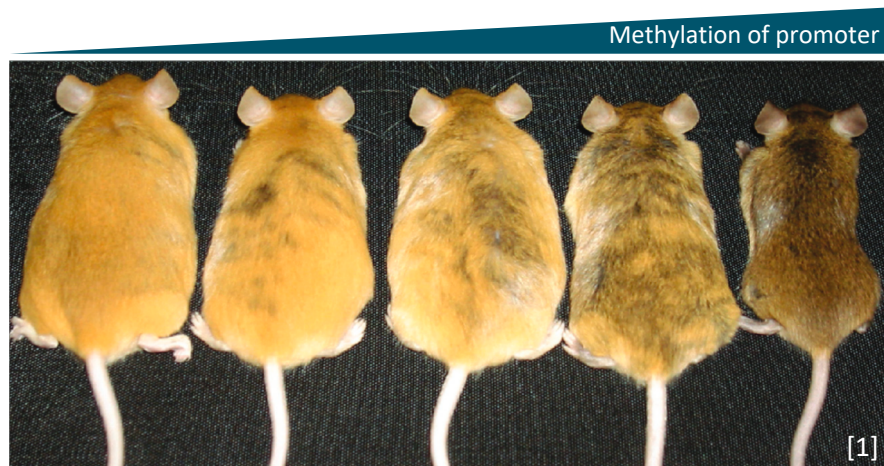
DNA Methylation

- DNA methyltransferases (DNMTs)
- Methylation at CpG dinucleotides
 - Regions with high CpG content are called „CpG islands“ (CGIs)



The Role of 5-methylcytosine (5mC)

- CpGs usually methylated, but not CGIs
 - CGIs close to transcription start site (TSS) are usually unmethylated
 - Methylation of CGI close to TSS can silence a gene



Agouti mouse

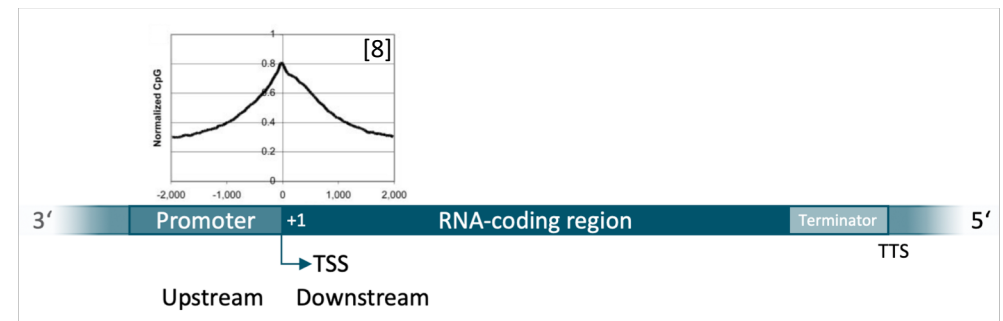
Table 1. Overview of CpG distribution in the human genome

Subset	Length, Mb	GC content	Observed CpG fraction	Normalized CpG fraction
Whole genome	3.1*	0.38	0.009	0.25
1 kb upstream regions	15	0.53	0.042	0.60
1 kb downstream regions	15	0.45	0.013	0.26
Transcription units	930	0.42	0.011	0.26
Exons	45	0.50	0.028	0.45
Introns	880	0.41	0.010	0.24

Length refers to the total length of DNA examined.

*Length given in gigabases.

[8]



Methylated Cytosine: Complications



- 5mC can undergo spontaneous deamination to thymine
- Role in cancer
 - Hypomethylation (oncogene promoter)
 - Hypermethylation (tumor suppressor promoter)
- Hypomethylation of transposable elements

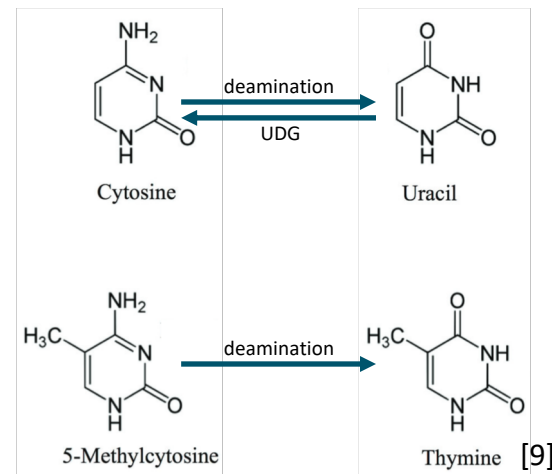
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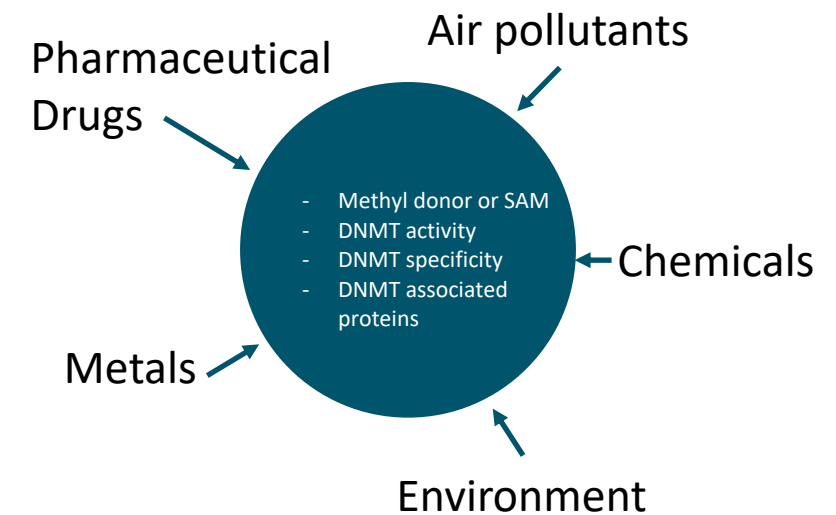
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Toxicology of Cytosine Methylation

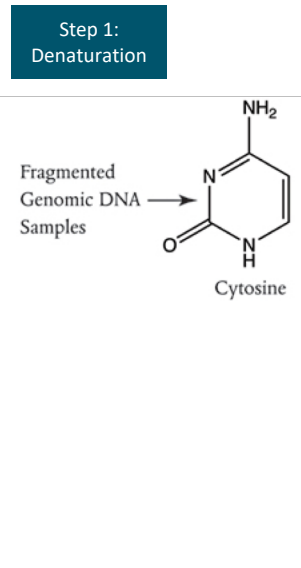


- Antihypertensive hydralazine inhibits DNA methylation
- Acute Cadmium (Cd) exposure can inhibit DNMT → hypomethylation
- Chronic Cd exposure → hypermethylation
- Airborne benzene leads to global hypomethylation (measured in *LINE-1* and *Alu*)



Bisulfite Treatment for 5mC Detection

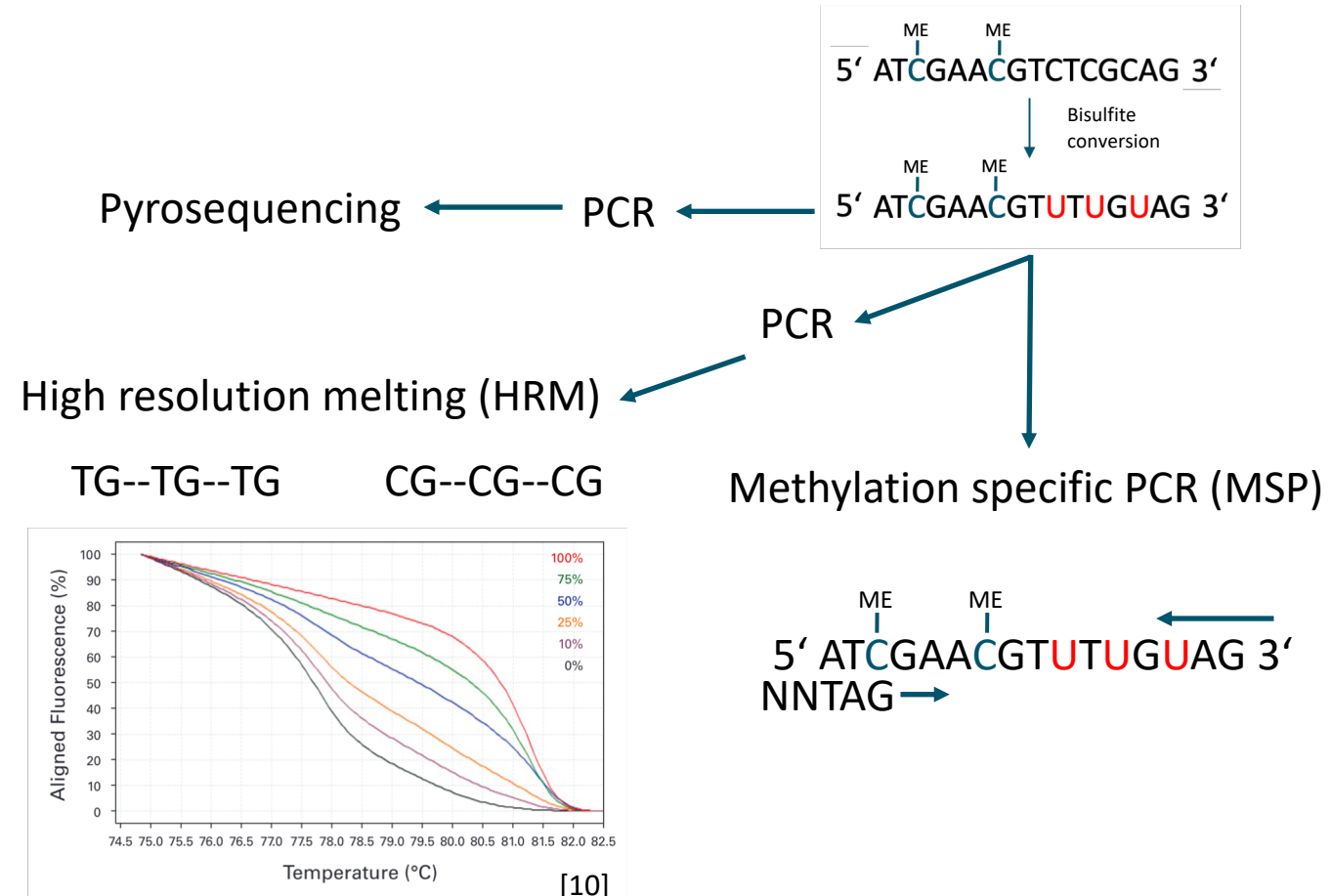
- Bisulfite conversion leads to a C → U conversion, 5mC is not affected
- Suitable for many downstream applications



PCR Based Techniques



- Direct sequencing of area, 5mC is read as C
- MSP: methylation specific primer is used to detect methylation status on site
- HRM: CG bounds have a higher T_m than TA bounds
 - CG: three hydrogen bonds
 - AT: two hydrogen bonds

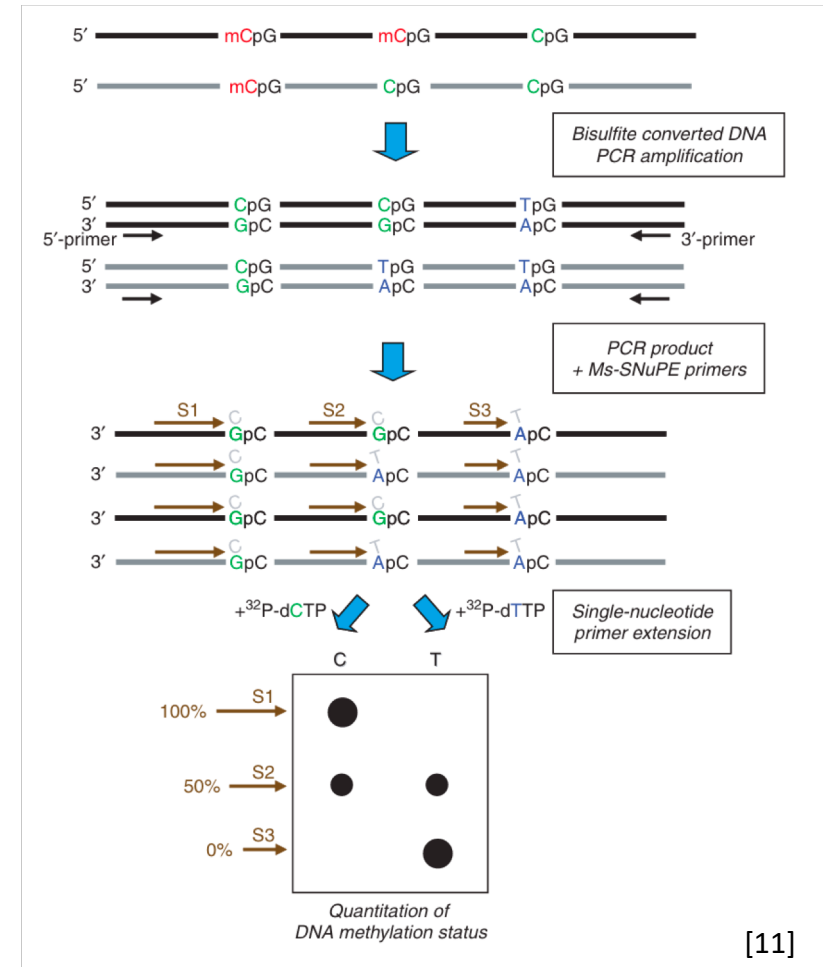


PCR Based Techniques II

- Methylation-sensitive single nucleotide primer extension (MS-SNuPE)
 - Addition of only two labeled nucleotides
- Combined bisulfite restriction analysis (COBRA)
 - e.g. BstUI 5' CGCG 3' → 5' CG 3' + 5' CG 3'
 - Resolving on Gel

5' NNTGTGNN 3'

5' NNCG*CGNN 3'

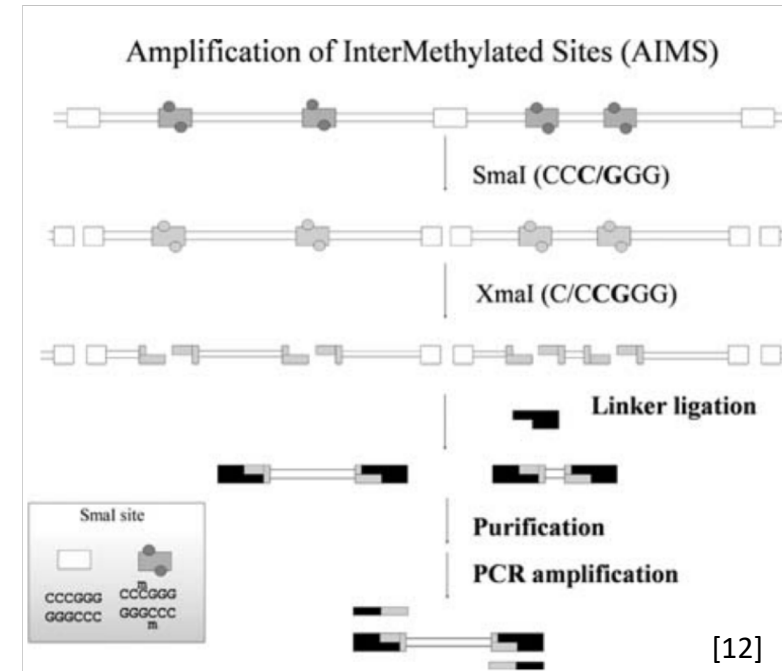
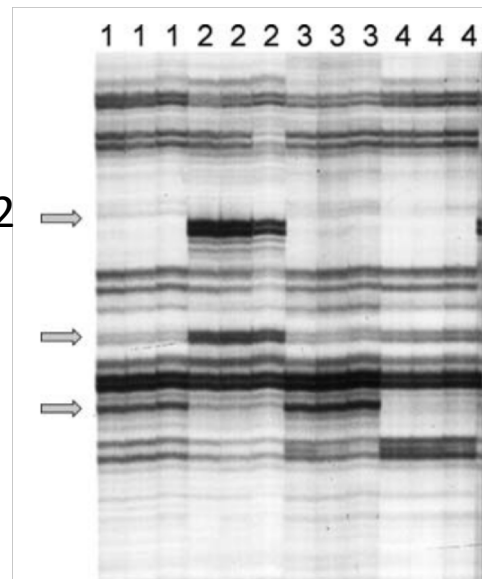


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Whole Genome Methylation Analysis

- AIMS uses isoschizomeric restriction enzymes with different methylation sensitivity
 - Bands can be isolated and further characterized

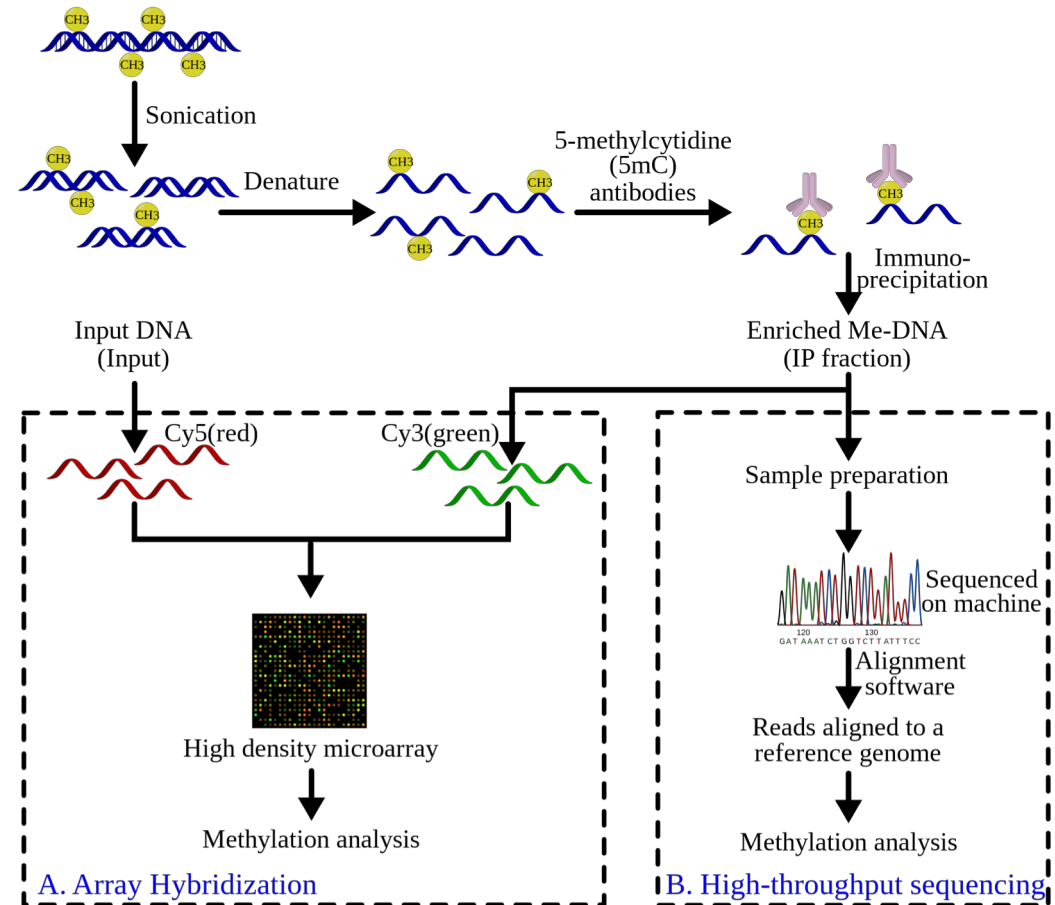
Hypermethylation in 2



Whole Genome Methylation Analysis II



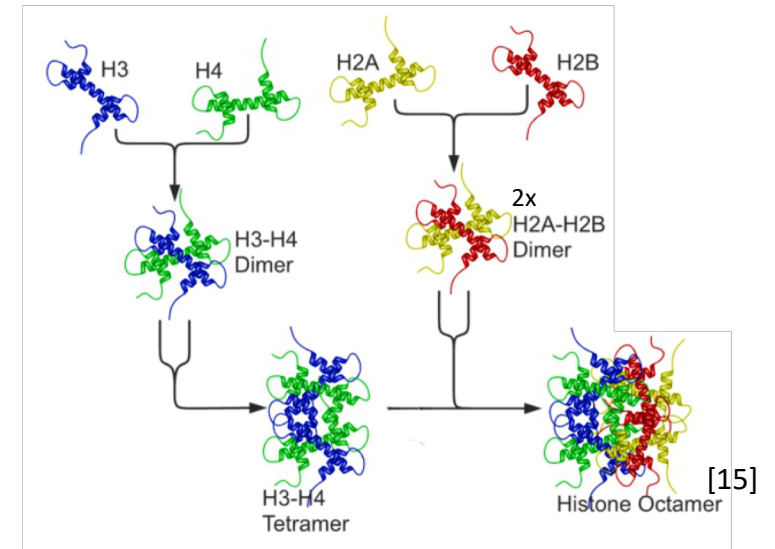
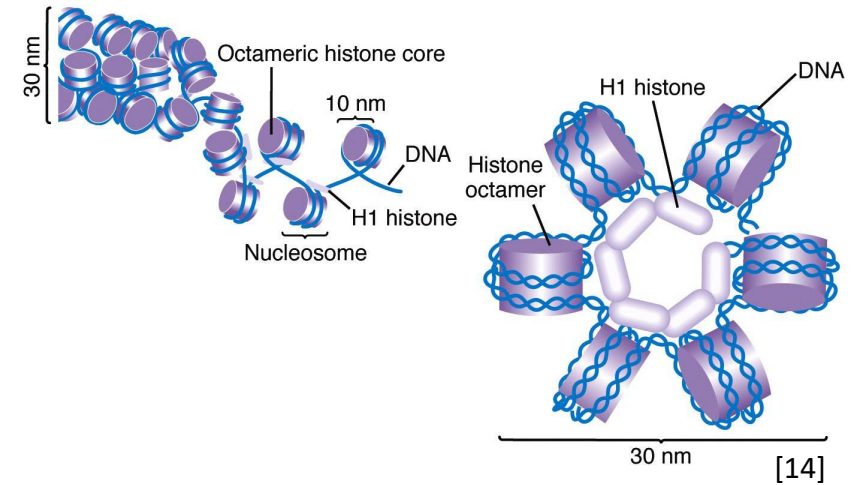
- MeDIP: Methylated DNA immunoprecipitation
 - Specific anti-5mC antibody
 - Subsequent downstream analysis
 - MeDIP-chip
 - MeDip-seq



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Histone Structure and Modifications

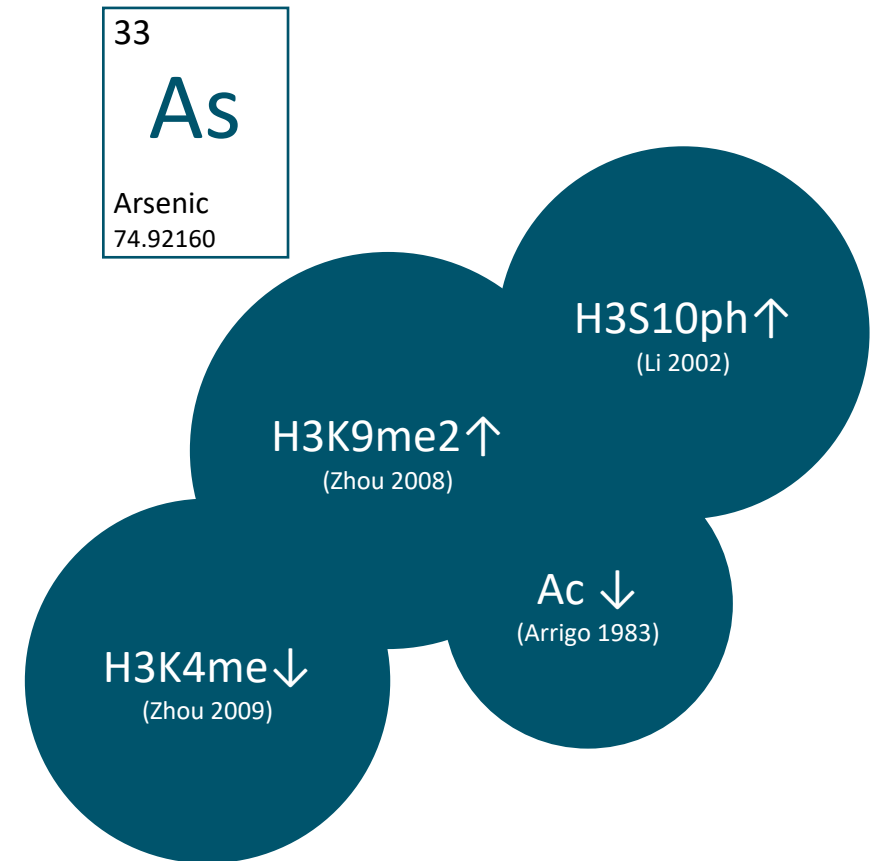
- H2A, H2B, H3 and H4 core histones
- H1 linker histone for 30 nm structure
- Core histones with helix-turn-helix motif
 - Binding of DNA
- N-terminus of histone can be modified
 - Methylation (Me), Acetylation (Ac), Ubiquitination (Ub), Phosphorylation (P)
 - Nomenclature: e.g. H3K4me3
 - Histone 3, Lysin 4, trimethylation



Toxicology of Histone Modifications

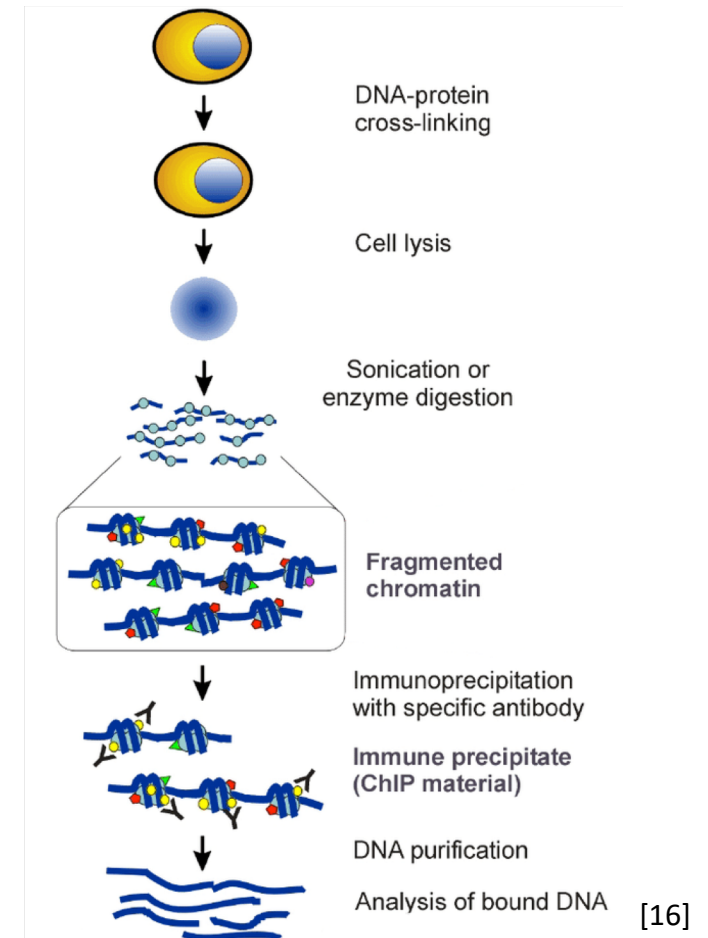


- Methylation at K or R residues
 - Activating and silencing function
- Acetylation usually at K residues
 - Mostly activates transcription
- Phosphorylation
 - Activates transcription
- Ubiquitination at K residues
 - H2A and H2B Ub activates transcription



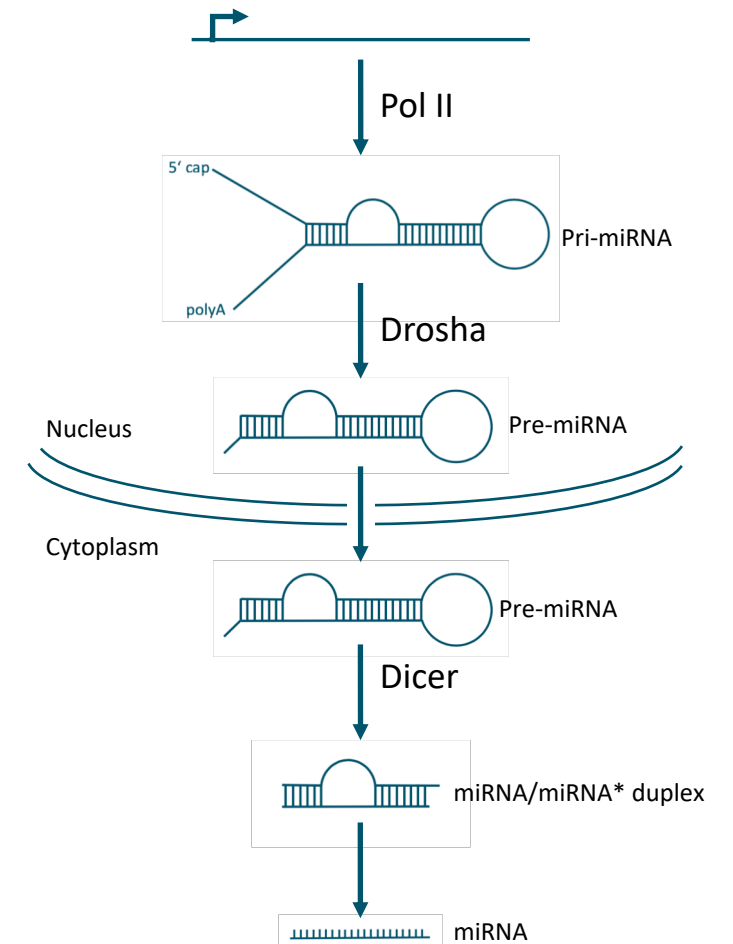
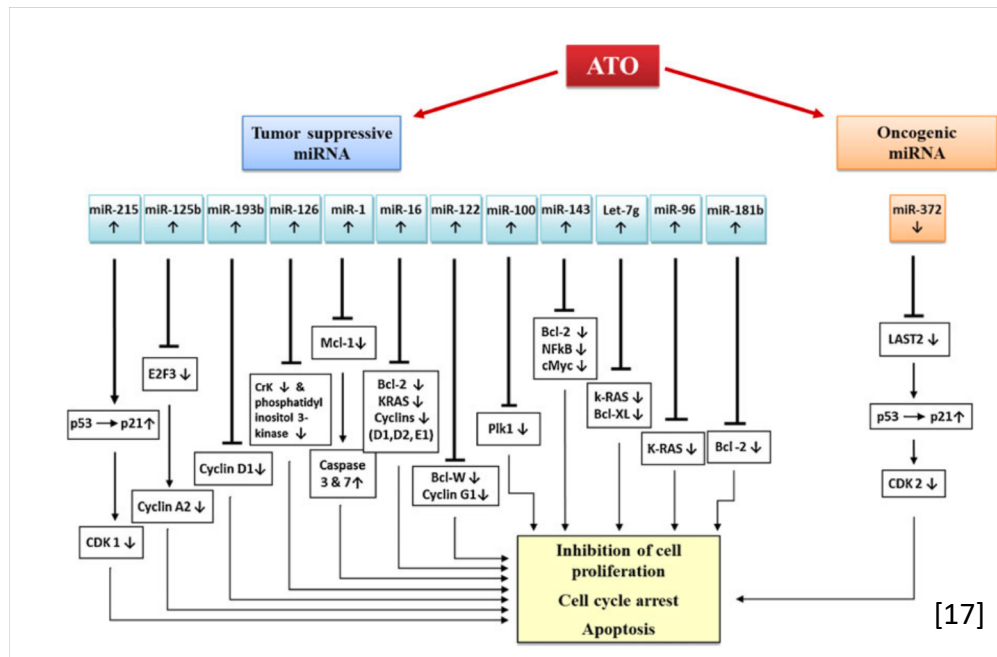
Detection of Histone Modifications

- Chromatin Immuno-precipitation (ChIP)
 - Subsequent analysis by PCR
- ChIP-chip (ChIP-on-chip)
 - Subsequent analysis by DNA microarray
- ChIP-Seq
 - Subsequent sequencing of DNA
 - Comparison with whole-genome sequence database



Non-coding RNAs

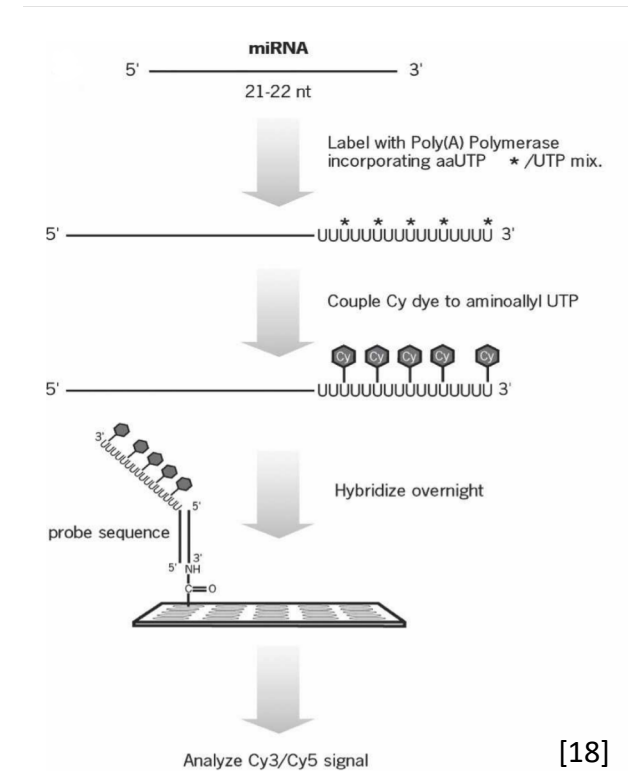
- miRNAs ca. 21-23 nt
 - Interfering with mRNA (complement to 3' UTR)



miRNA microarrays, In situ hybridization



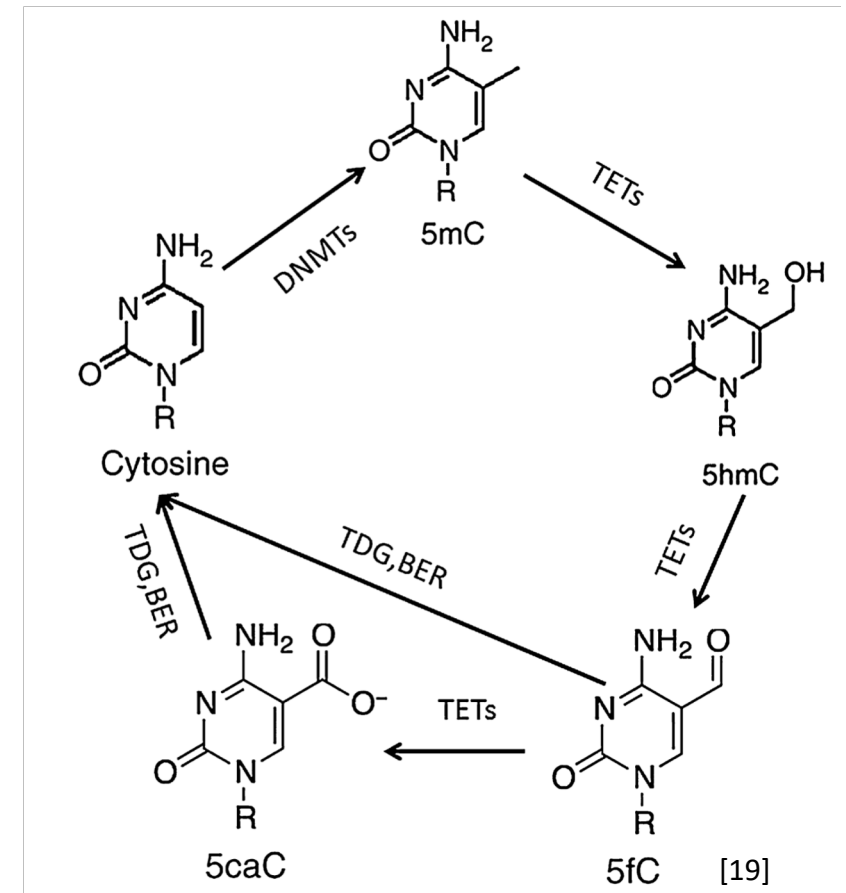
- miRNA array
 - Isolation using TRIzol or extraction kit
 - Labeling and hybridisation to probe
- RT-qPCR
 - Using Poly(A) polymerase and subsequent RT
 - Use of a Poly(A) reverse primer and a specific forward primer



Outlook, what's new?



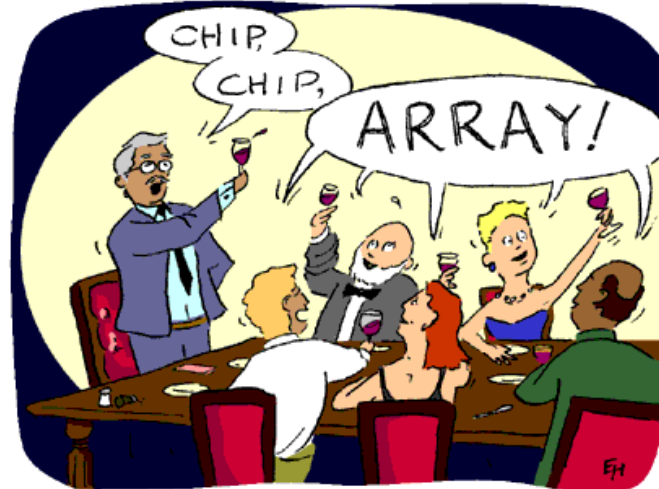
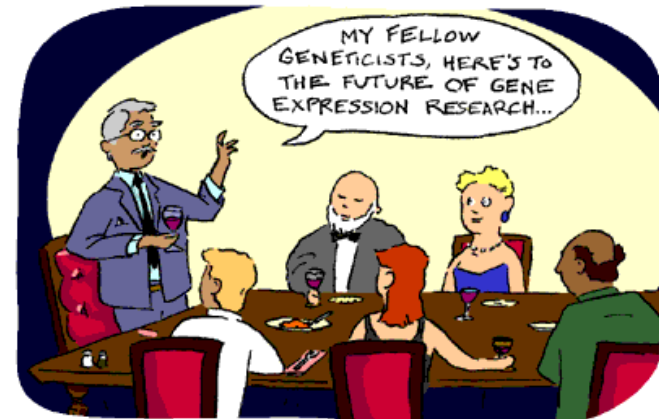
- Other bases found in the genome
 - 5-hydroxymethylcytosine (5hmC)
 - 5-formylcytosine (5fC)
 - 5-carboxylcytosine (5caC)



Take Home Messages / Questions?



If they ask you anything you don't know, just just say it's due to epigenetics.



References (Pictures)



1. Dolinoy DC, Weidman JR, Waterland R A et al. Maternal genistein alters coat color and protects Avy mouse offspring from obesity by modifying the fetal epigenome. *Environ. Health Perspect.* 114(4), 567–572 (2006).
2. <https://www.mskcc.org/blog/what-epigenetics-and-why-everyone-talking-about-it> (14.02.19)
3. [https://www.cell.com/current-biology/fulltext/S0960-9822\(99\)80490-4](https://www.cell.com/current-biology/fulltext/S0960-9822(99)80490-4) (14.02.19)
4. https://en.wikipedia.org/wiki/DNA_methylation#/media/File:DNA_methylation.png (14.02.19)
5. https://embryology.med.unsw.edu.au/embryology/images/2/2f/Epigenetics_cartoon.jpg (14.02.19)
6. https://de.wikipedia.org/wiki/5-Methylcytosin#/media/File:Cytosine_5-methylation.png (15.02.19)
7. Wu, Susan C., and Yi Zhang. "Active DNA demethylation: many roads lead to Rome." *Nature reviews Molecular cell biology* 11.9 (2010): 607.
8. Saxonov, Serge, Paul Berg, and Douglas L. Brutlag. "A genome-wide analysis of CpG dinucleotides in the human genome distinguishes two distinct classes of promoters." *Proceedings of the National Academy of Sciences* 103.5 (2006): 1412-1417.
9. Based on: Do, Hongdo, et al. "Reducing artifactual EGFR T790M mutations in DNA from formalin-fixed paraffin-embedded tissue by use of thymine-DNA glycosylase." *Clinical chemistry* 63.9 (2017): 1506-1514.
10. <https://international.neb.com/applications/epigenetics/dna-methylation-analysis/bisulfite-conversion#tabselect4> (21.02.19)
11. Gonzalgo, Mark L., and Gangning Liang. "Methylation-sensitive single-nucleotide primer extension (Ms-SNuPE) for quantitative measurement of DNA methylation." *Nature protocols* 2.8 (2007): 1931.
12. Jorda, Mireia, et al. "Analysis of DNA methylation by amplification of intermethylated sites (AIMS)." *DNA Methylation*. Humana Press, 2009. 107-116.
13. https://en.wikipedia.org/wiki/Methylated_DNA_immunoprecipitation#/media/File:MeDIP.svg (27.02.19)
14. https://www.mun.ca/biology/scarr/Histone_Protein_Structure.html (27.02.19)
15. https://de.wikipedia.org/wiki/Histon#/media/File:Nucleosome_structure-2.png (27.02.19)
16. Collas, Philippe, and John Arne Dahl. "Chop it, ChIP it, check it: the current status of chromatin immunoprecipitation." *Front Biosci* 13.17 (2008): 929-943.
17. Ghaffari, Seyed H., et al. "Alteration in miRNA gene expression pattern in acute promyelocytic leukemia cell induced by arsenic trioxide: a possible mechanism to explain arsenic multi-target action." *Tumor Biology* 33.1 (2012): 157-172.
18. Shingara, Jaclyn, et al. "An optimized isolation and labeling platform for accurate microRNA expression profiling." *Rna* 11.9 (2005): 1461-1470.
19. Wang, Jingyu, et al. "5-Hydroxymethylcytosine and disease." *Mutation Research/Reviews in Mutation Research* 762 (2014): 167-175.

Title Slide: <https://pixabay.com/vectors/dna-dna-icon-icon-2316536/> (11.03.19)

Last Slide: <https://www.promega.de/resources/pubhub/cartoons/> (11.03.19)