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Epigenetics and Cancer I

Image: Nicolas Bouvier and Genevieve Almouzni

Constanze Zeller (PhD)

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Overview

1. Definition of Epigenetics

2. Mechanisms of epigenetic regulation

- DNA methylation (enzymes, CpG-islands, binding proteins)
- Histones (modifications, variants and binding proteins)
- RNAi (role in heterochromatin and gene regulation)

3. Epigenetics and Cancer

4. Challenges of 'Epigenetic Therapeutics'

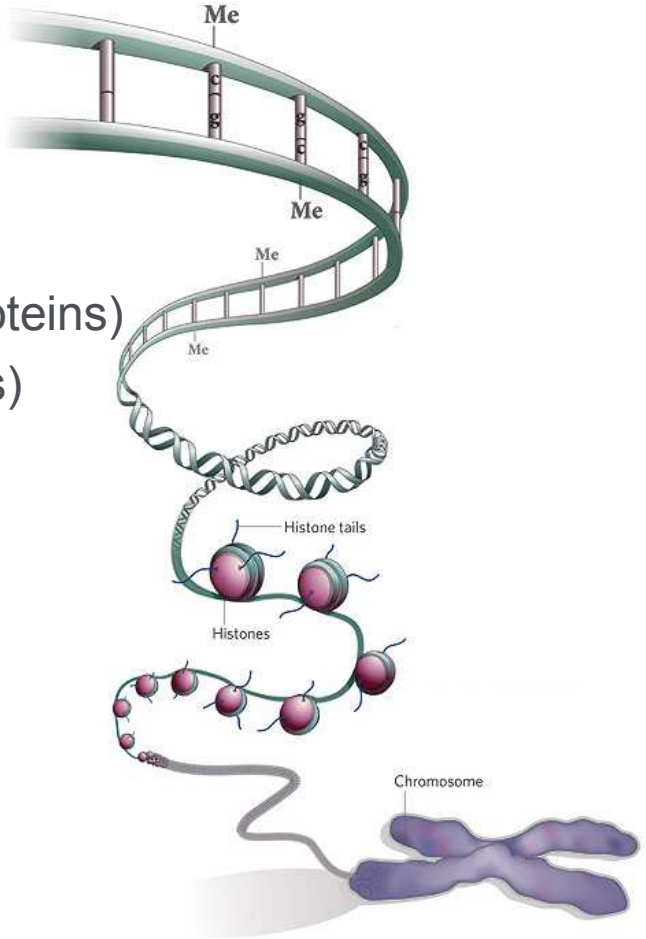

Image: Nature 441, 143-145 (11 May 2006)

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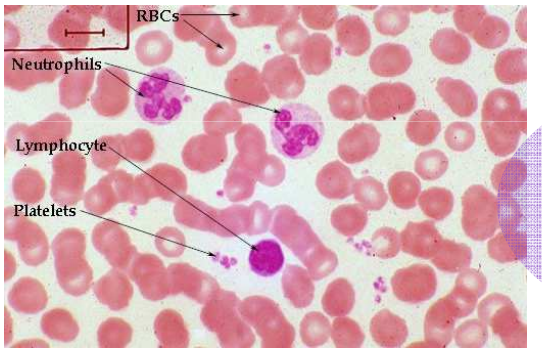
Definition of Epigenetics

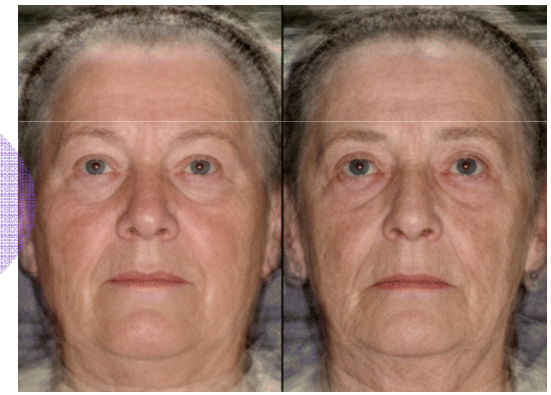
- A change in phenotype that is heritable but does NOT involve a change in DNA sequence



http://greenfield.fortunecity.com/rattler/46/blood.htm

Same genotype – different epigenome

http://www.pbs.org/wgbh/nova



BMJ 2009; 339:b5262

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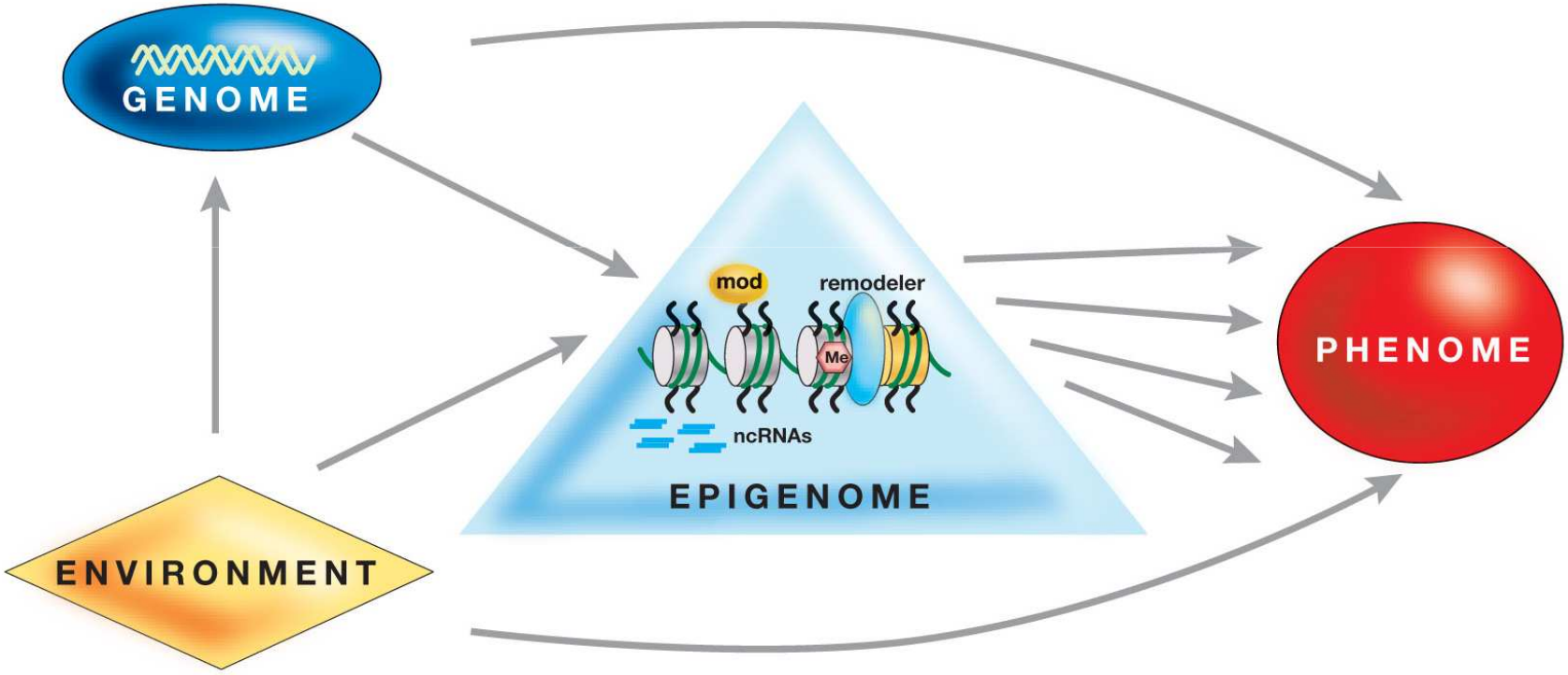
The epigenome



Epigenetics, Allis, Jenuwein, Reinberg, Caparros, 2009

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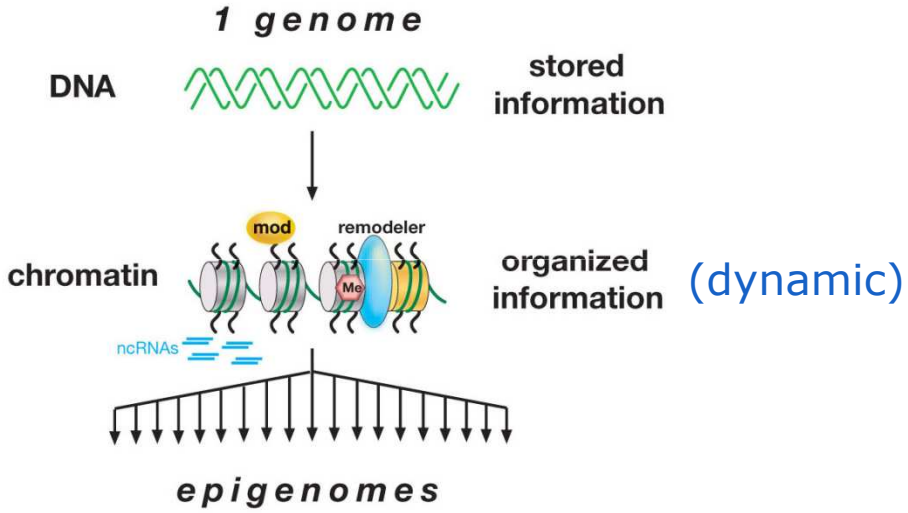
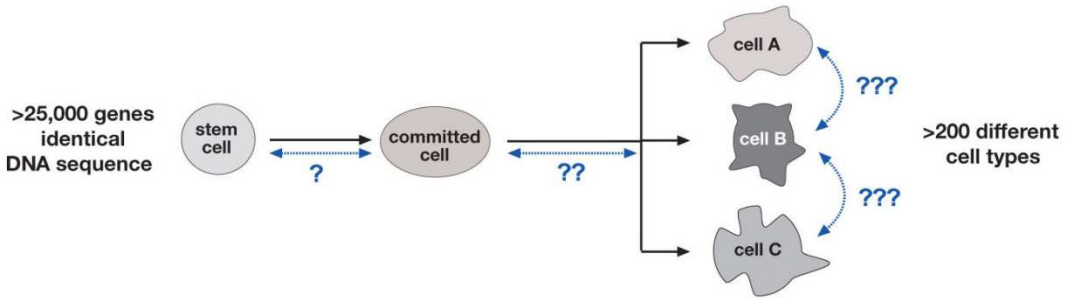
One genome – several epigenomes

Epigenetic Mechanisms control the genome

- DNA methylation
- Histone modifications / variants
- Non coding RNAs





Epigenetics, Allis, Jenuwein, Reinberg, Caparros, 2009

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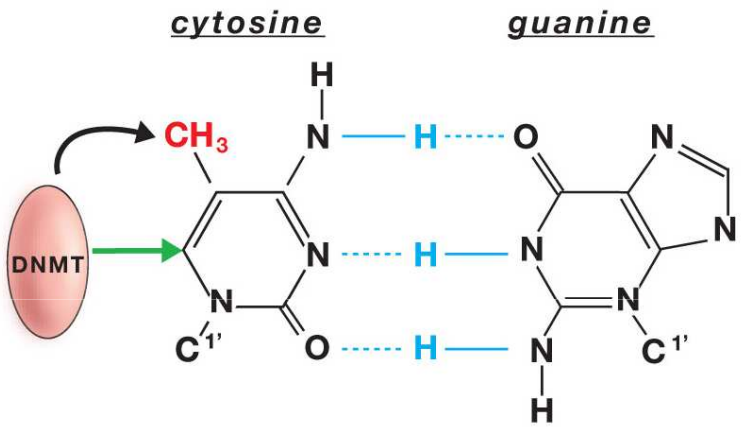
DNA methylation



- Essential for embryonic development
- Gene silencing
- X-inactivation (mammals)
- Imprinting (mammals)
- Stable repression of proviral genomes and retro-transposons
- Tissue specific expression
- Genome integrity

Epigenetics, Allis, Jenuwein, Reinberg, Caparros, 2009

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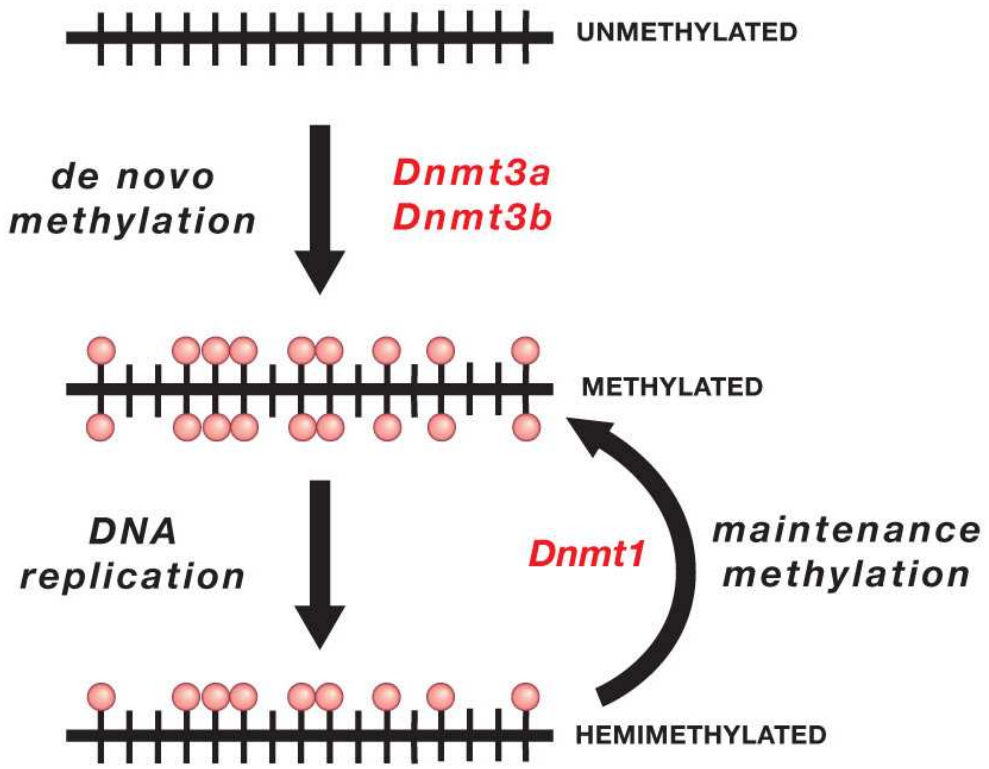
De novo and Maintenance Methylation of DNA

In mammals established during embryogenesis

Maintained during mitosis by copying mechanism

Form of cellular memory



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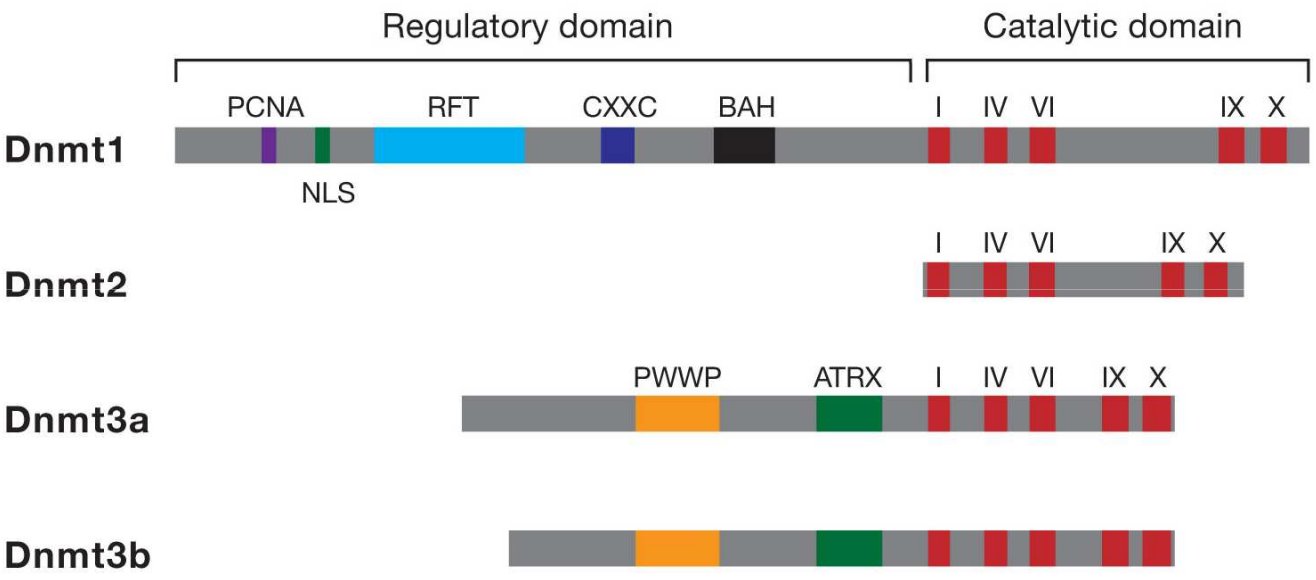
Mammalian DNA Methyltransferases



Major activity:

- **maintenance**
- **weak activity**
- **de novo**
- **de novo**

- Mutations in DNMT3b in humans associated with ICF Syndrome (Immunodeficiency, Centromeric instability and Facial abnormalities)
- Deregulation of DNMT levels can contribute to cancer development

Epigenetics, Allis, Jenuwein, Reinberg, Caparros, 2009

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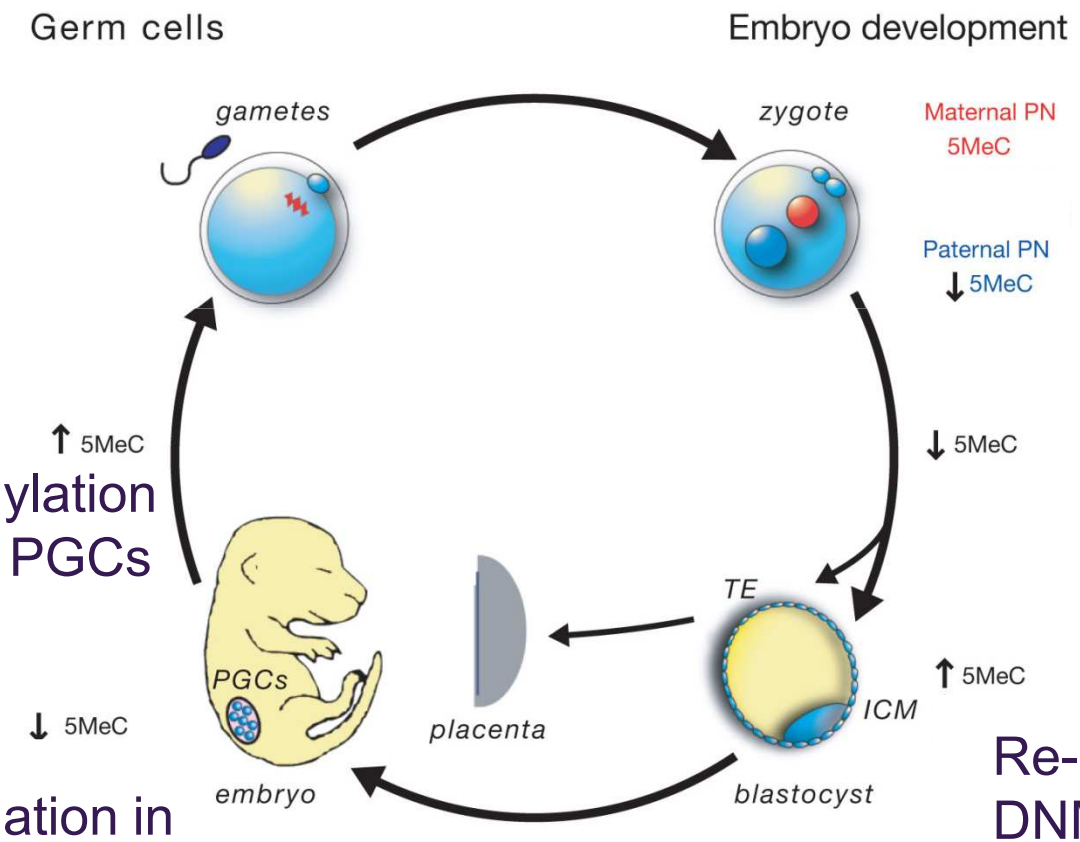
Epigenetic Reprogramming Cycle in Mammalian Development



Active Demethylation

Passive Demethylation

de novo methylation in developing PGCs

Demethylation in primordial germ cells (PGCs)

Re-methylation: DNMT3a + DNMT3b

Epigenetics, Allis, Jenuwein, Reinberg, Caparros, 2009

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DNA Methylation in the Mammalian Genome

- DNA from mammalian somatic tissue is methylated at 70% of all CpG sites (genome stability)
- Key exception: CpG-islands which are usually non-methylated (60% of human genes have CpG-islands)
- Methylation pattern not permanent – it changes throughout life (environmental influences, ageing and oncogenic transformation)
- Problem of 5-methylcytosine being prone to mutagenesis

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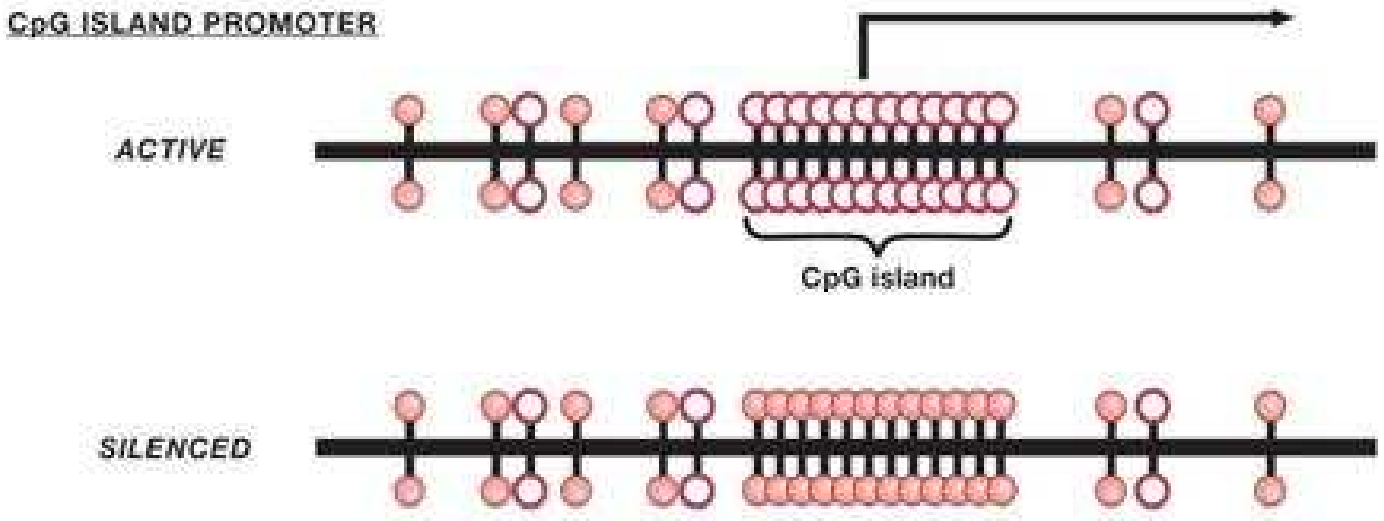
CpG islands

- regions of high CpG density that lack methylation (500 bp – 2kb)
- 60% of human genes have CpG island promoters
- located at promoters or 5’ end of most human genes
- long-term silencing ensured by methylation of CpG island region



Epigenetics, Allis, Jenuwein, Reinberg, Caparros, 2009

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Regulation of Gene Expression by DNA Methylation

- Interference with transcription factor binding
  - several transcription factors recognise CG rich sequence motifs and are unable to bind methylated DNA (Ets-1 or CTCF)

- Attraction of methyl-CpG-binding proteins (MBDs)
  - MeCP2, MBD1, MBD2, MBD3, MBD4, Kaiso
  - binding of MBDs to methyl-CpG provides signal to alter chromatin structure through recruitment of co-repressors (MeCP2, MBD1, MBD2, KAISO)

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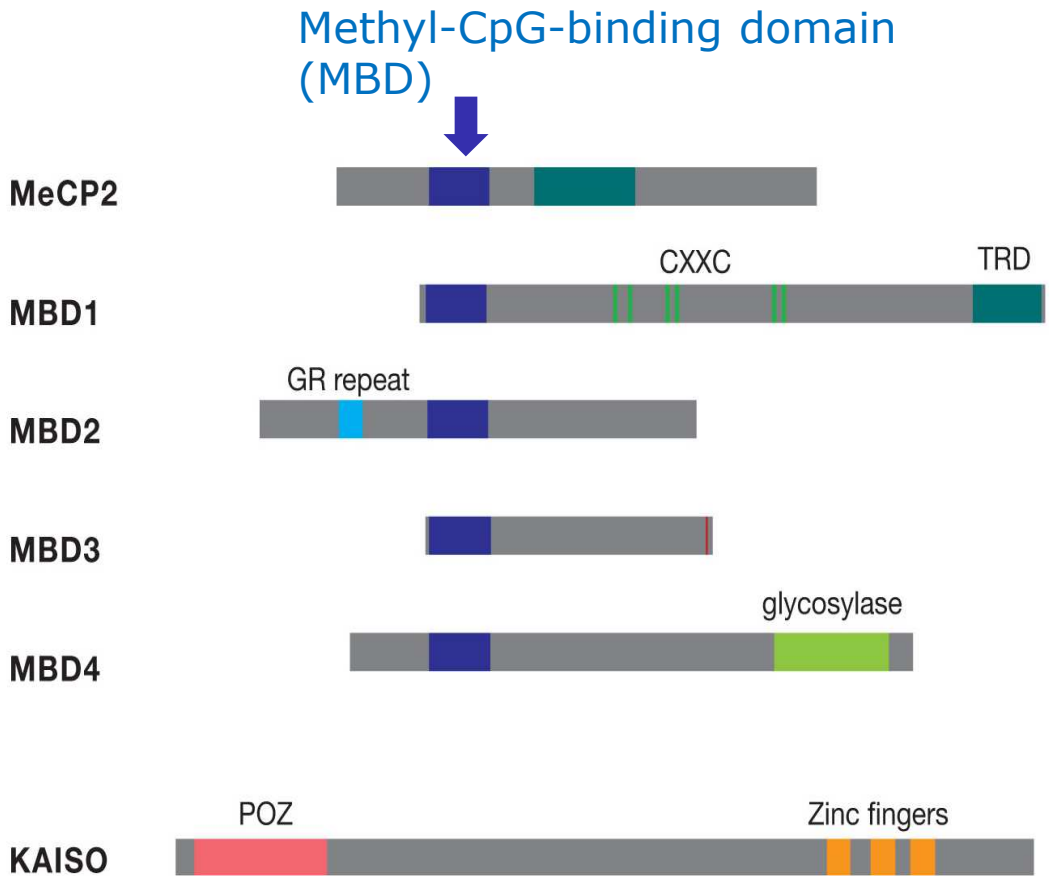
Proteins Binding methyl-CpG



Major activity:

Binds meCpG next to A/T, TR

Binds meCpG via MBD or CXXC, TR

Binds meCpG, TR

No strong binding to meCpG

DNA repair protein binding to meCpG and T:G mismatches

Binds pairs of meCpG, TR

TR= transcriptional repressor

Epigenetics, Allis, Jenuwein, Reinberg, Caparros, 2009

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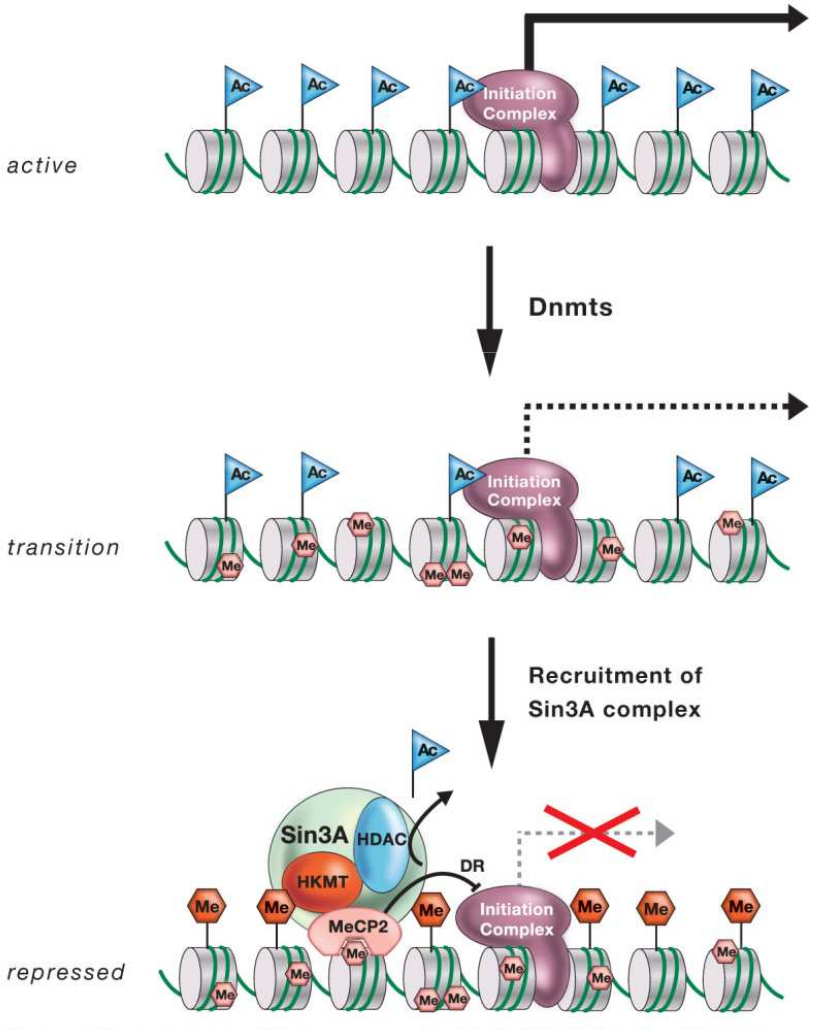
Co-repressor Recruitment via MBD Proteins



Transcription, unmethylated promoter

DNA methylation of promoter

Binding of MeCP2 to methyl-C and recruitment of co-repressors

Transcriptionally inactive

Epigenetics, Allis, Jenuwein, Reinberg, Caparros, 2009

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DNA Methylation – Summary I

- Stable modification of DNA
- Established and maintained via DNMTs
  - DNMT1: maintenance
  - DNMT3a + DNMT3b: *de novo*
- Central role in development, X-inactivation, imprinting, genome stability, tissue-specificity, repression of provirus/retrotransposons/repeats, silencing of genes
- Reversible: epigenetic reprogramming during embryogenesis
- 70% of CG sites methylated except CpG islands
- Regulates gene expression via binding of MBDs or interference with TFs

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Epigenetic Determinants of Cancer

- Heritable deregulation of genes
- Usually genes involved controlling cell division and apoptosis
- Interplay of oncogenes (dominant) and tumour suppressor genes (recessive) results in formation of cancer

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- Gene activation (oncogenes) via:

  mutation, translocation, duplication or gene switched on

- Gene inactivation (tumour suppressors) via:

  mutation, gene loss or gene being switched off

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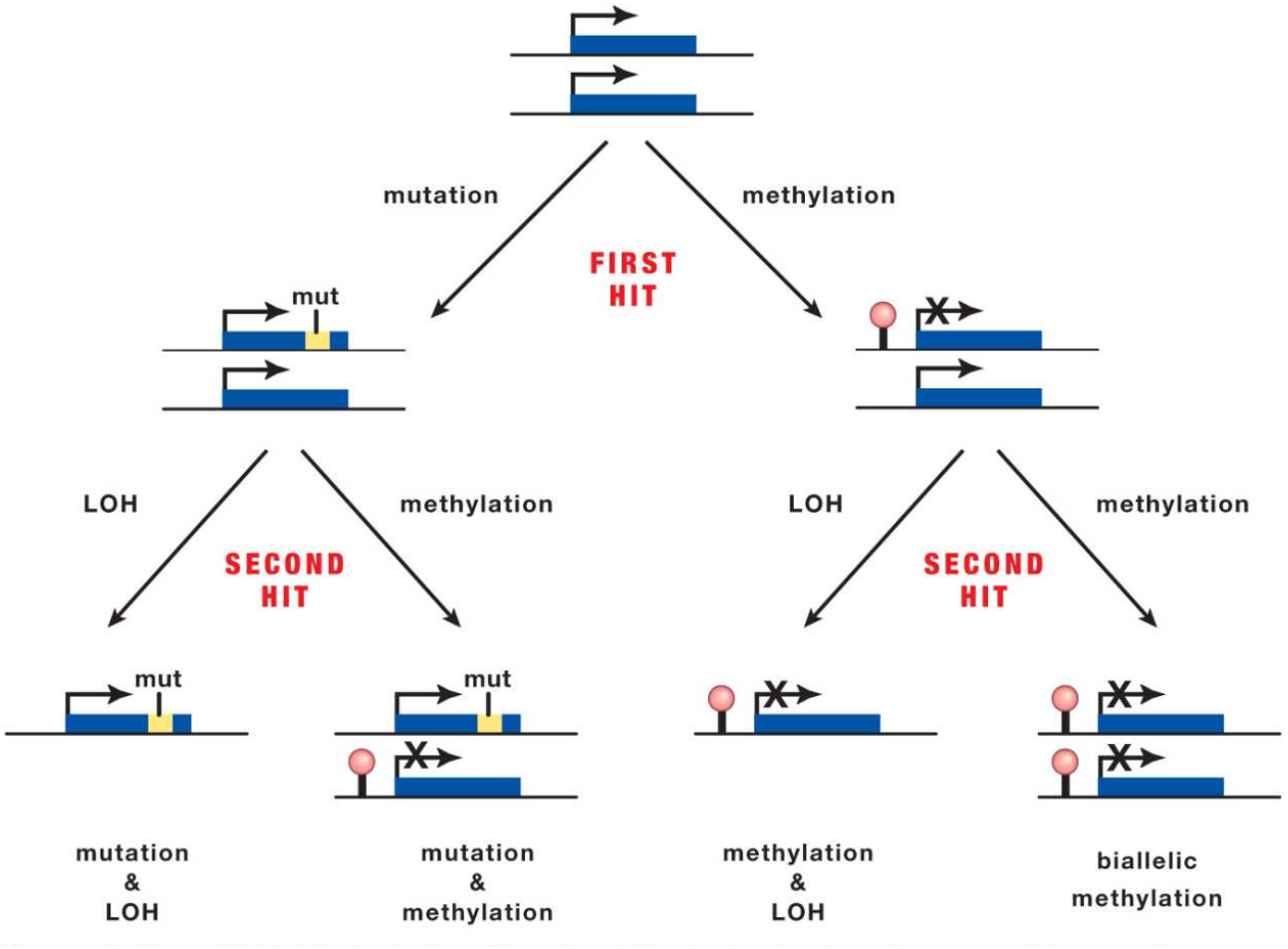
DNA Methylation Can Inactivate Tumour Suppressor Genes



Epigenetics, Allis, Jenuwein, Reinberg, Caparros, 2009

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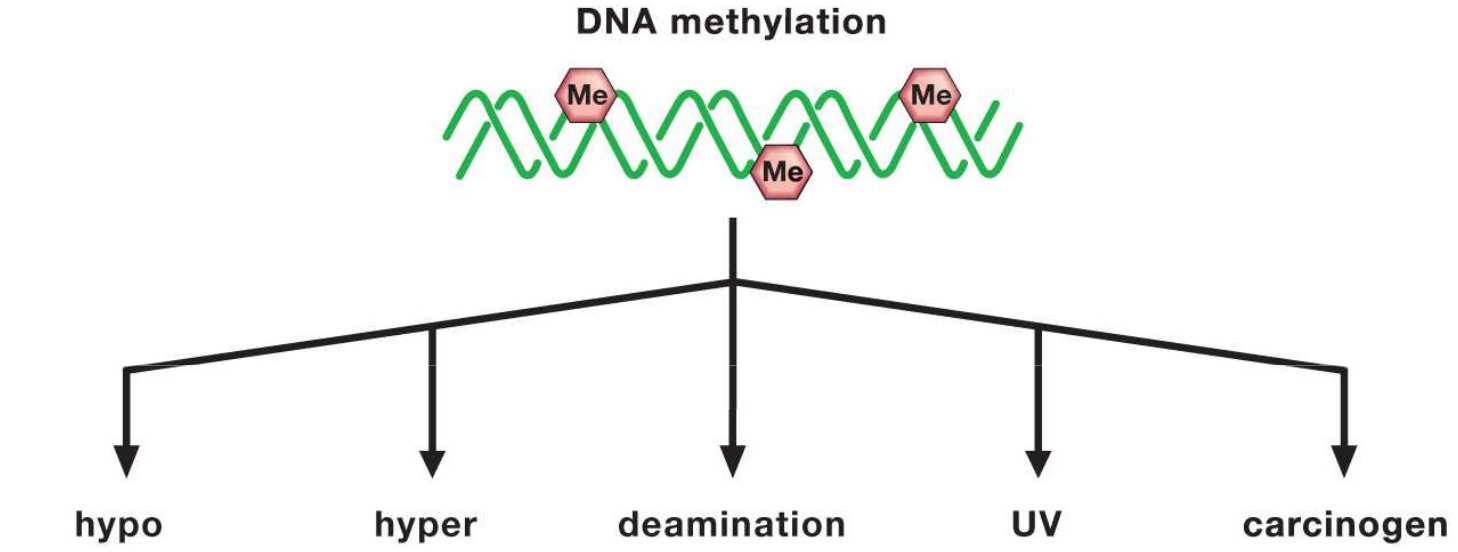
Alterations in DNA Methylation Can Lead to Cancer



- global: genome instability; promoter silencing; mutation; increased UV-induced mutations; carcinogen-induced mutations
- local: **activation of oncogenes**

Epigenetics, Allis, Jenuwein, Reinberg, Caparros, 2009

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Role of DNA Methylation in Cancer

- Loss of methylation (hypomethylation) leads to genomic instability /activation of oncogenes:
  - » Hypomethylation *HOX11* proto-oncogene in leukemias
- Aberrant promoter CpG-island methylation (hypermethylation) leads to gene silencing (alternative to mutation):
  - » e.g. *BRCA1* in sporadic breast cancer
- Mutagenesis of 5meC major factor:
  - » e.g. 50% of all *p53* mutations occur at sites of cytosine methylation (in sporadic colorectal cancer)
- UV-light also increases formation of pyrimidine dimers when 5meC present :
  - » Higher mutation rate in *p53* gene in sunlight exposed skin due to increased formation of pyrimidine dimers
- 5meC favours formation of carcinogenic adducts:
  - » benzo (a) pyrene in cigarette smoke $\rightarrow$ increased mutation rate at CpG site in lungs of smokers

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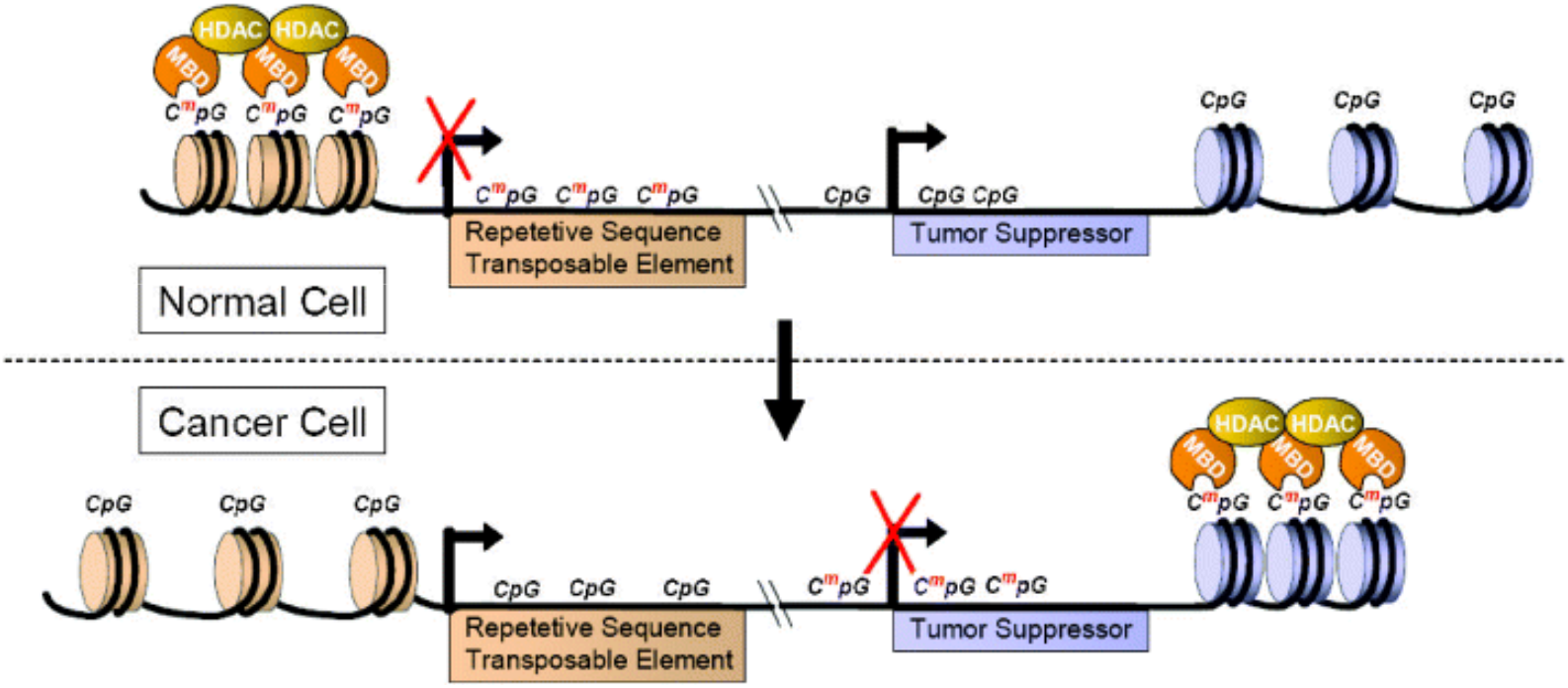
Hypermethylated Gene Promoters and Hypomethylated Repeats in Cancer



Hypomethylation of repeats

Hypermethylation of gene promoters

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Tumour Suppressor Genes Methylated and Epigenetically Silenced in Cancer

Cell cycle: *Rb, $p16^{INK4a}$, $p15^{INK4a}$, $p14^{ARF}$*

Signal transduction: *RASSF1, APC*

Apoptosis: *DAPK, Caspase 8*

DNA repair: *MLH1, MGMT, BRCA1*

Senescence: *TERT, TERC*

Invasion/metastasis: *TIMP-3, E-cadherin*

APC = adenomatous polyposis coli
DAPK = death-associated protein kinase
MGMT = O-6-methylguanine-DNA methyltransferase

1. *Jones & Baylin. Cell 2007;128:683–92*
2. *Teodoridis et al., Drug Resistance Updates 2004;7:267-278*

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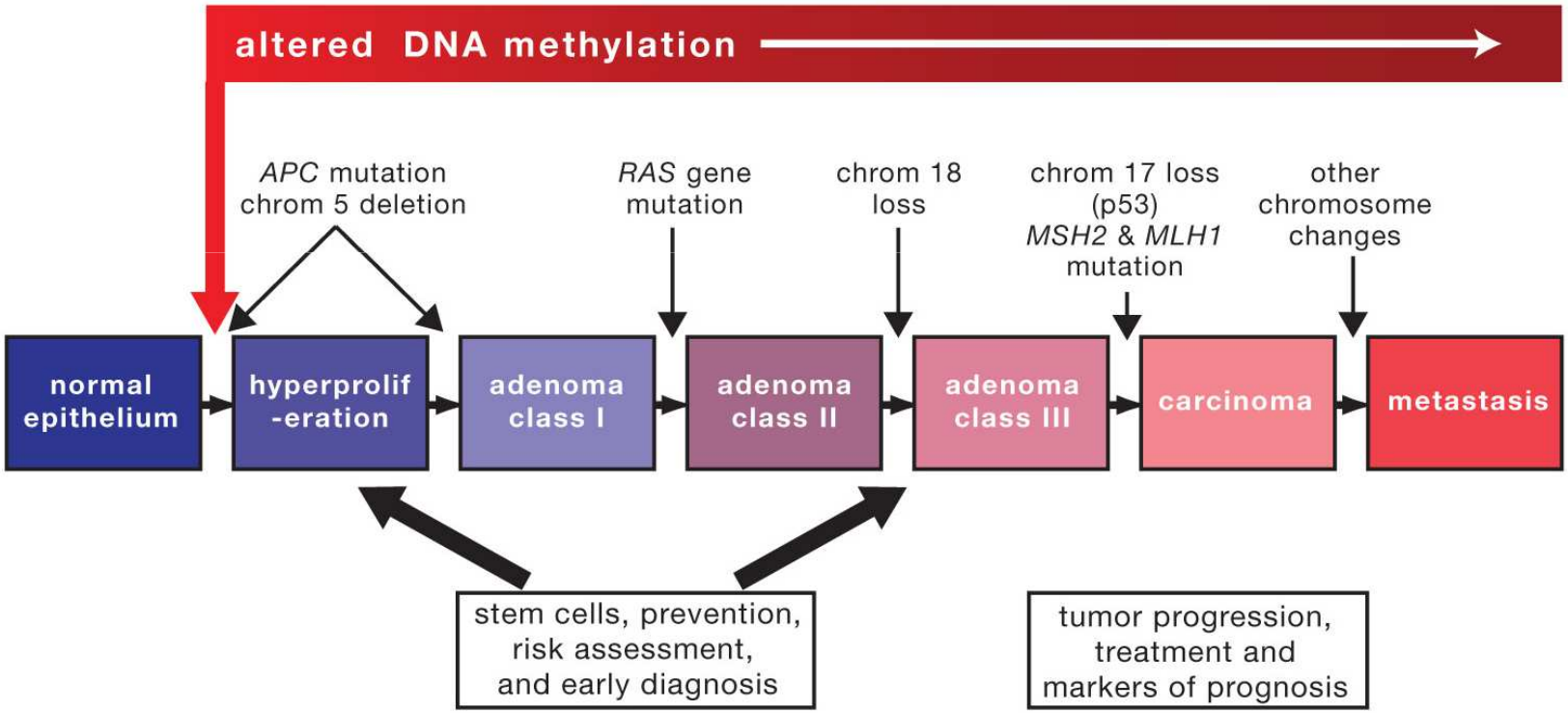
Early role for abnormal DNA-CH_3 in tumour progression

Modified model of colon cancer evolution (Kinzler and Vogelstein, 1997):



Interaction of genetic and epigenetic events

Epigenetics, Allis, Jenuwein, Reinberg, Caparros, 2009

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Clinical significance of DNA methylation

- **Need for biomarkers:**
  - screening (early detection of cancer in the general or at risk populations)
  - diagnostic (definition of tumour type, stage and grade)
  - prognostic (Identification of the likely clinical disease course)
  - prediction of therapeutic response
    (patient enrichment to maximize likely benefit from individual therapies)

- **Stable epigenetic modification of DNA detectable in:**
  - body fluids (plasma, urine, sputum)
  - paraffin embedded samples

- **Therapeutic implications:**
  - targeted therapies using demethylating agents (DNMT inhibitors)

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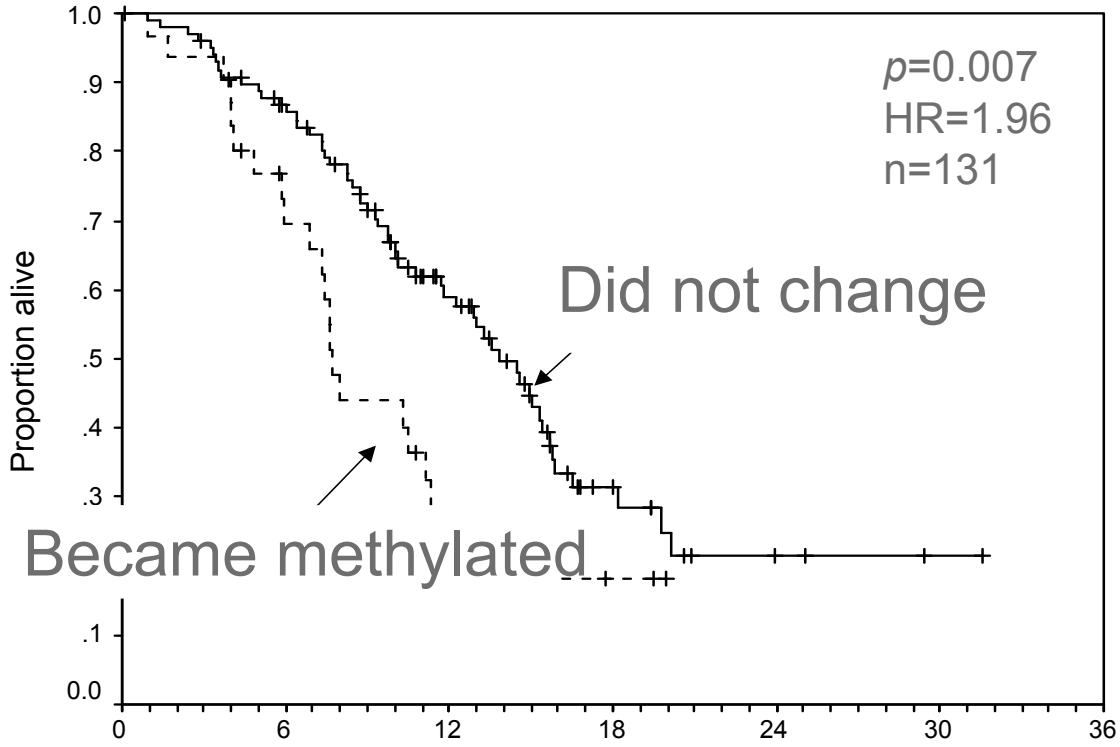
Acquired *MLH1* CpG island methylation detected in plasma DNA predicts patient survival in ovarian cancer patients

Gifford *et al.* Clin Cancer Res (2004):



Number At risk:-							
	Did not become methylated	100	81	41	11	3	1	0
	Became methylated	31	19	7	2	0	0	0

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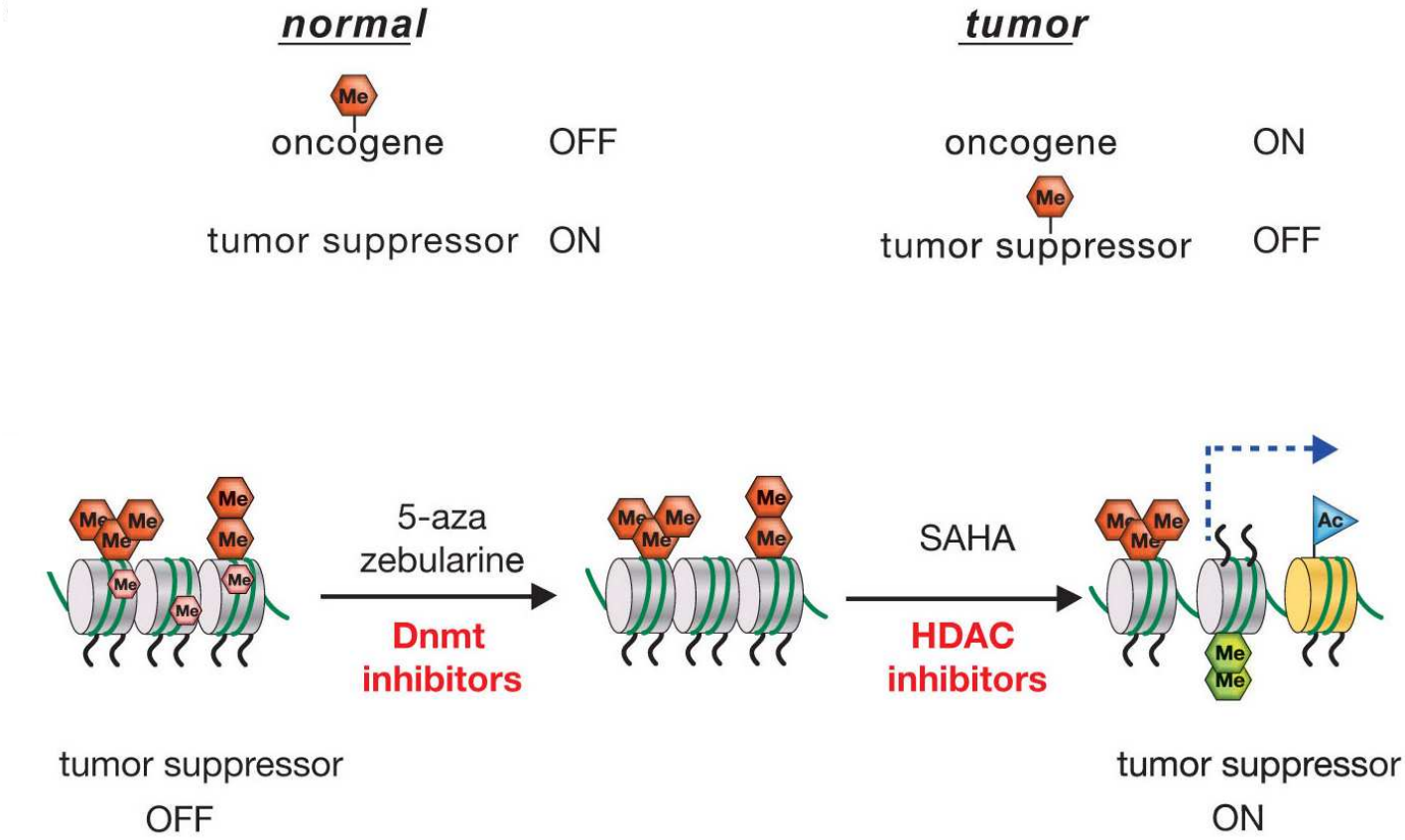
Unlike Genetic Change, Epigenetic Marks are Reversible



- **abnormal epigenetic change can be readily reversed using small molecule inhibitors of epigenetic maintenance**

Epigenetics, Allis, Jenuwein, Reinberg, Caparros, 2009

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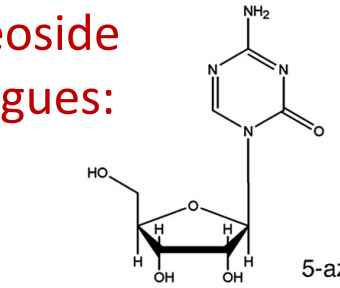
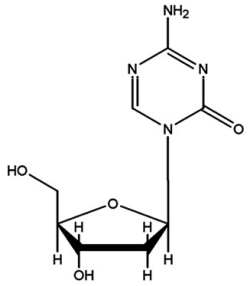
Types of DNMT inhibitors

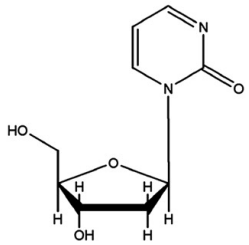
Nucleoside analogues:



5-azacytidine

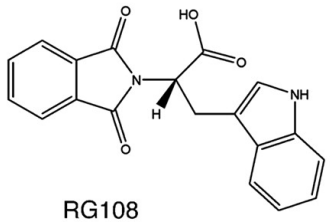


5-aza-2'-deoxycytidine



zebularine

Non-nucleoside analogues:



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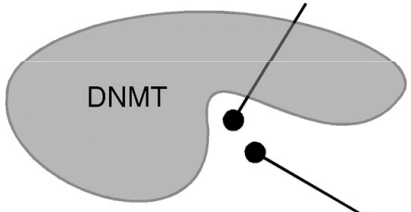
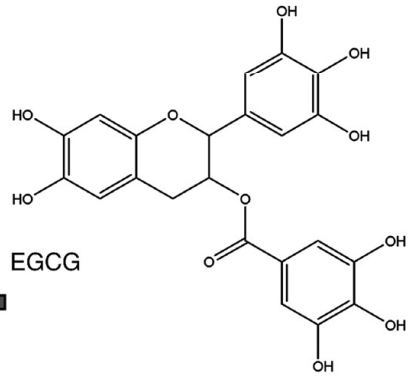
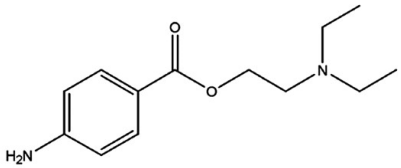




EGCG



procaine

- e.g. catalytic site inhibitors of DNMTs

Lyko & Brown. J Natl Cancer Inst 2005;97:1498-1506

- incorporate into DNA during replication
- sequester DNMTs by covalent binding

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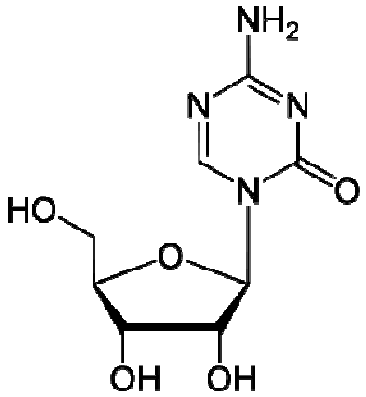
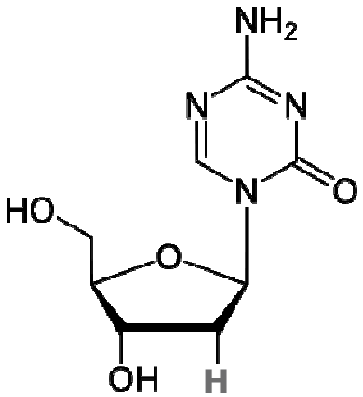
5'- Azacytidine and Decitabine



5'-Azacytidine

(Vidaza)



Decitabine

(5'-Aza-2'-deoxycytidine)

- Both agents have DNA hypomethylating activity and both are reported to have cytotoxic activity
- The relationship between these activities and clinical responses has not been established
- Greater demethylating activity at lower less toxic doses

Baylin, Nat Clin Pract Oncol 2005;2:S4–S11

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Epigenetic Therapies

DNA Methyltransferase (DNMT) Inhibitors

- Azacytidine: approved in the EU for the treatment of patients with higher-risk MDS, CMML and AML
- Decitabine: approved in the USA for the treatment of patients with MDS

Challenges

- Limited effectiveness in solid tumours
- Off-target effects
- Lack of gene specificity
- Uncertain mechanism of action in responsive tumours
- Delivery

AML= acute myeloid leukemia
CMML=chronic myelomonocytic leukemia
MDS=myelodysplastic syndrome

Graham et al. Eur J Cancer. 2009

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Conclusions

- Aberrant DNA methylation and epigenetic silencing is widespread in cancer and affects genes involved in all the hallmarks of cancer

  - genome-wide hypomethylation

  - local CpG island hypermethylation

- Epigenetic silencing can be reversed by small molecules, but there is a need for more specific drugs

- DNA methylation is a rich source of prognostic and predicitive biomarkers that can readily be applied in a clinical setting