Imperial College London

Epigenetics and Cancer I

Constanze Zeller, PhD

Department of Surgery and Cancer
Imperial College London
e mail: c.zeller@imperial.ac.uk

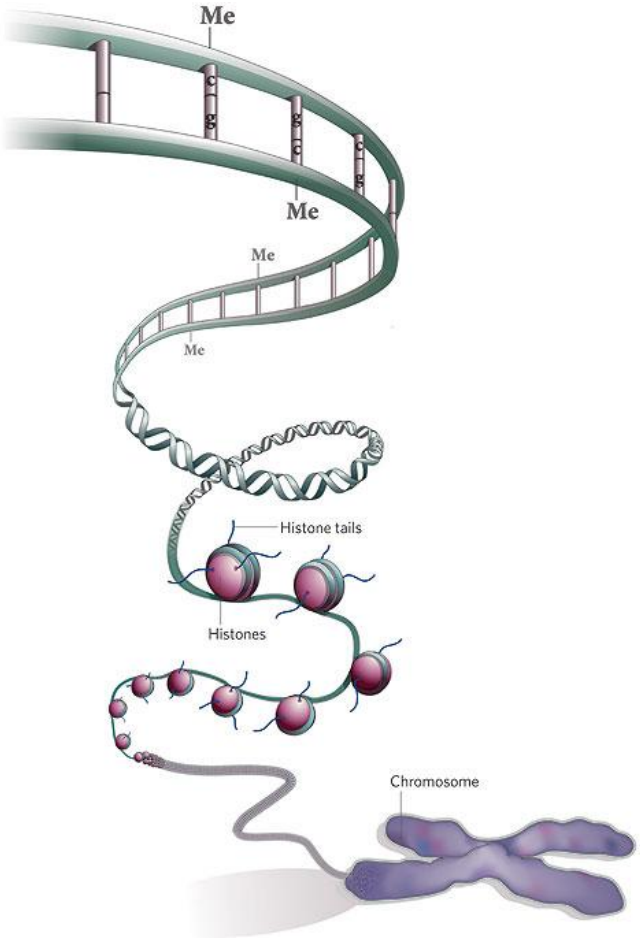


Image: Nature 441, 143-145 (11 May 2006)

Imperial College London

Learning Outcomes

After this lecture you will be better able to:

1. define ‘Epigenetics’

2. state one major mechanism of epigenetics: DNA methylation (enzymes, CpG-islands, binding proteins)

3. describe how DNA methylation changes contribute to cancer

4. give example of an ‘Epigenetic Therapy’ being used for cancer treatment

5. list challenges of ‘Epigenetic Therapeutics’

Imperial College London

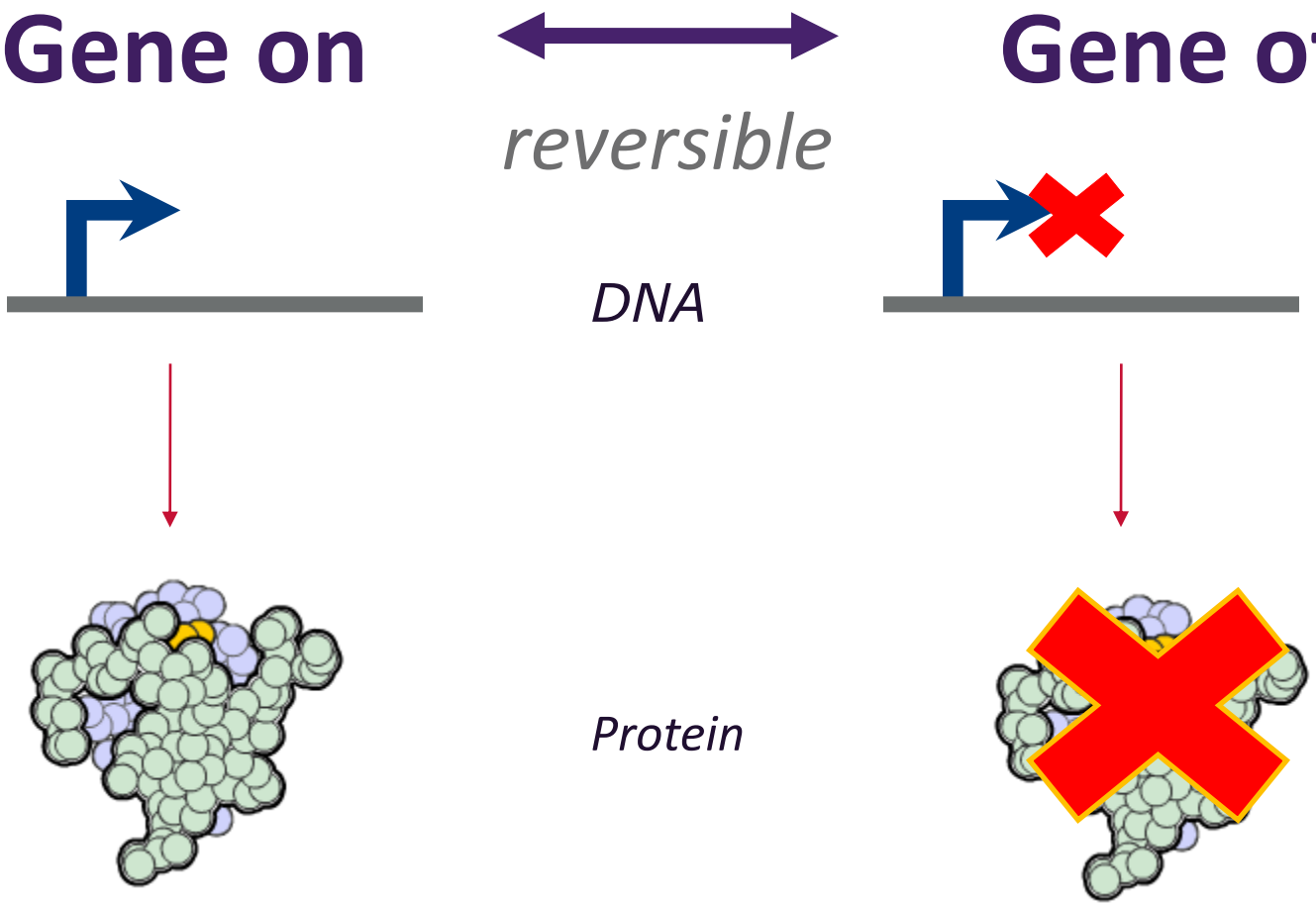
Definition of Epigenetics

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

Same genome – different epigenome

Imperial College London

Gene expression is reversible



Protein acts to prevent cells from dividing

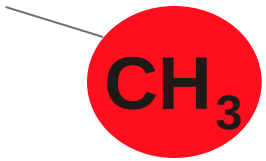
NO protein to prevent cells from dividing

Epigenetics

"A heritable change in gene expression not involving a change in DNA sequence"

- **DNA methylation**:

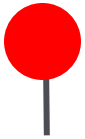
addition of methyl groups to Cytosine in DNA



Adenine(A)

Guanine(G)

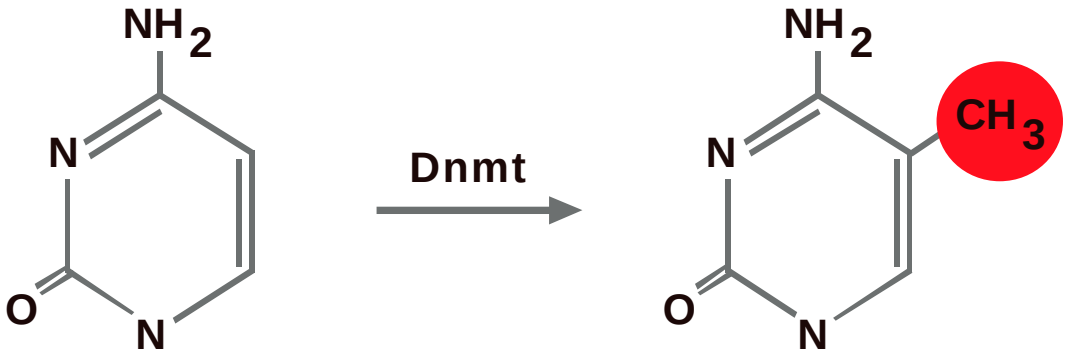
Thymine(T)



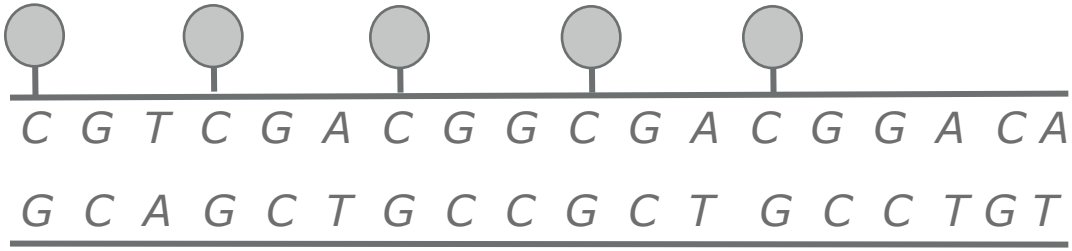
Cytosine(C)

Imperial College London

(cytosine-) DNA methylation

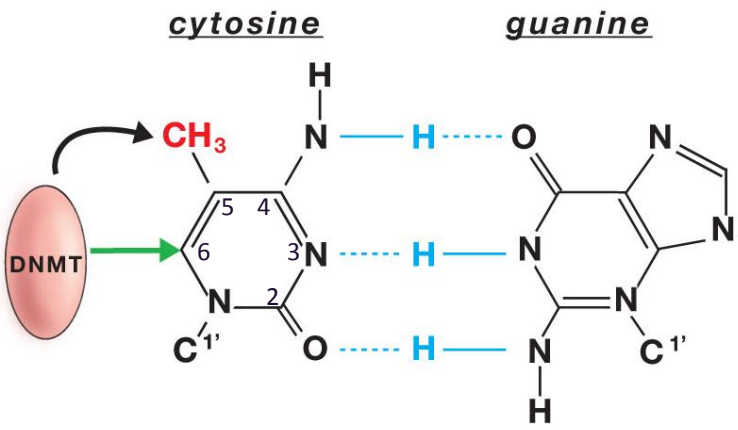


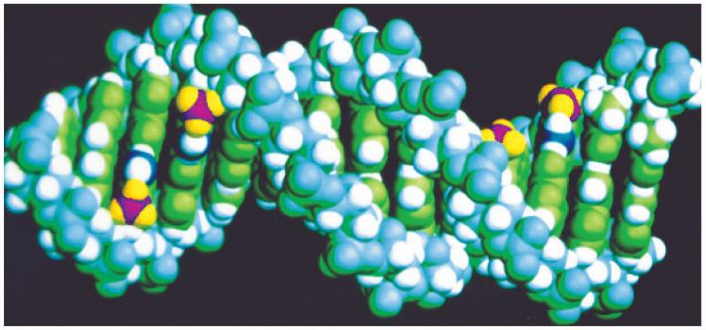
- is catalyzed by DNA methyltransferases (DNMTs)
- occurs at Cytosine followed by a Guanine (CpG):



Imperial College London

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

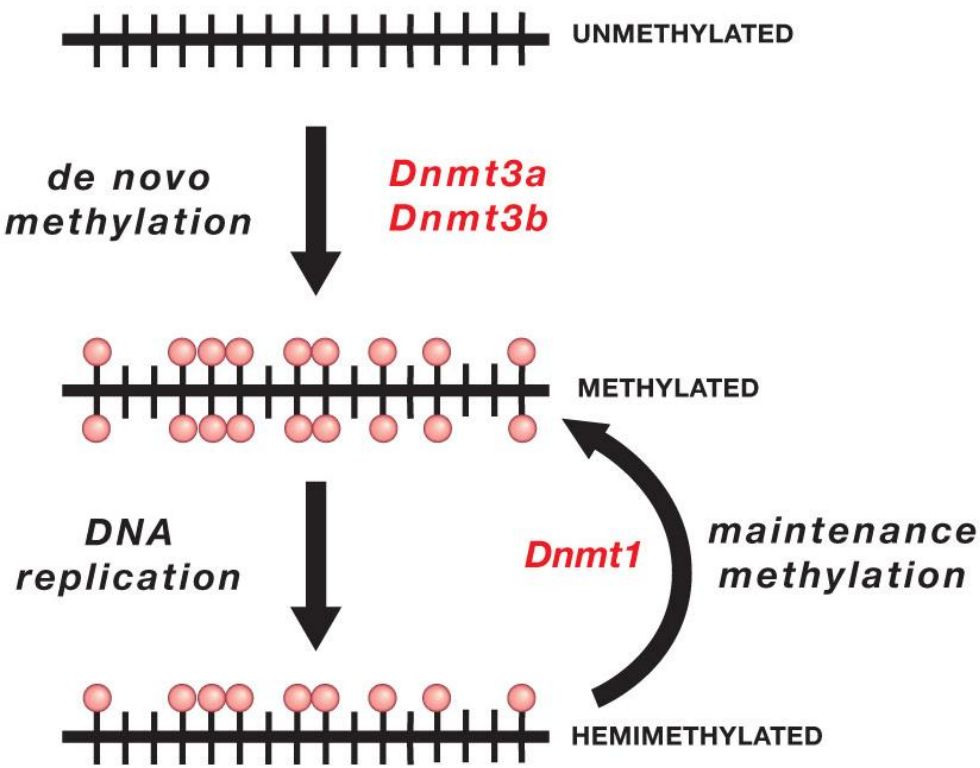
Imperial College London

De novo and Maintenance Methylation of DNA

In mammals established during embryogenesis

Maintained during mitosis by copying mechanism

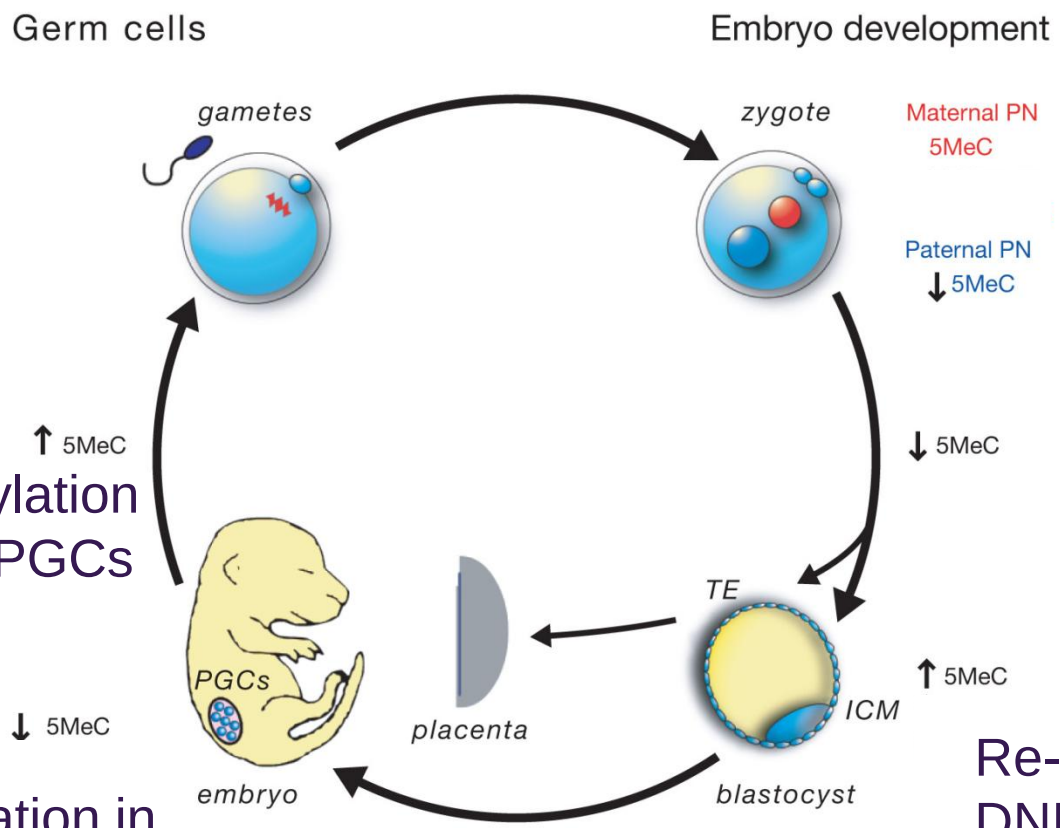
Form of cellular memory



Epigenetics, Allis, Jenuwein, Reinberg, Caparros, 2009

Imperial College London

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

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

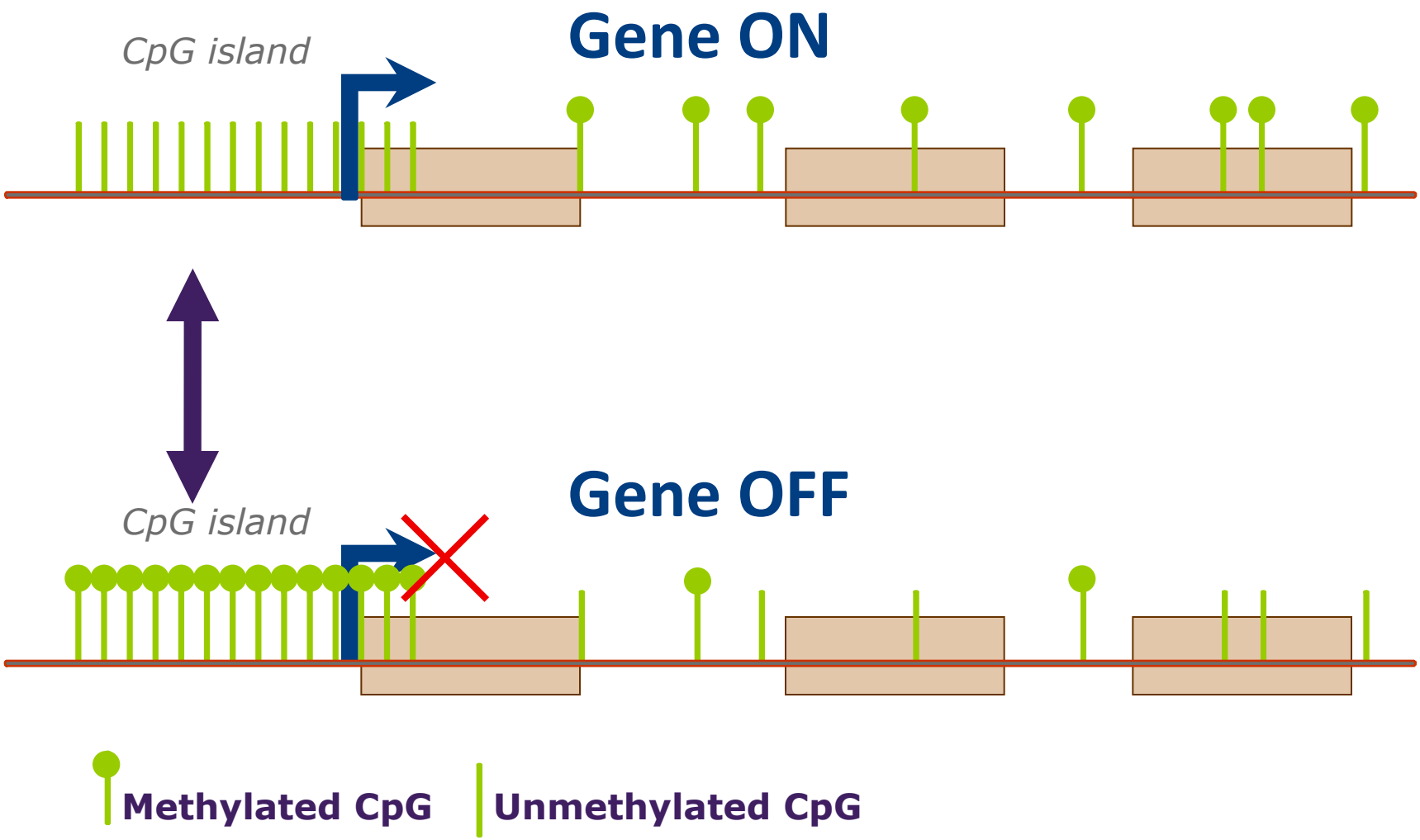
Imperial College London

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

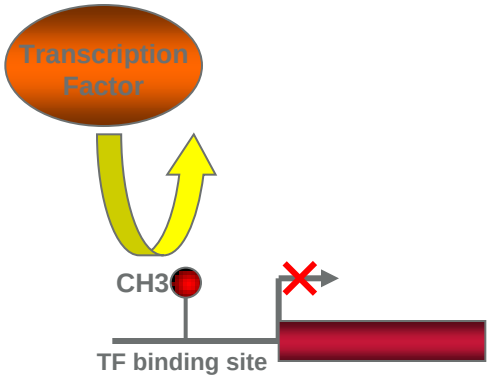
Imperial College London

DNA methylation in normal cells



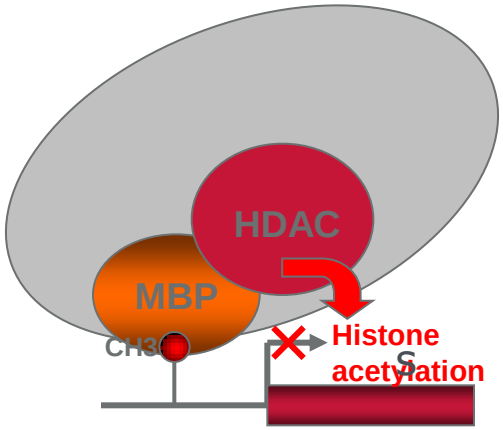
Imperial College London

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)

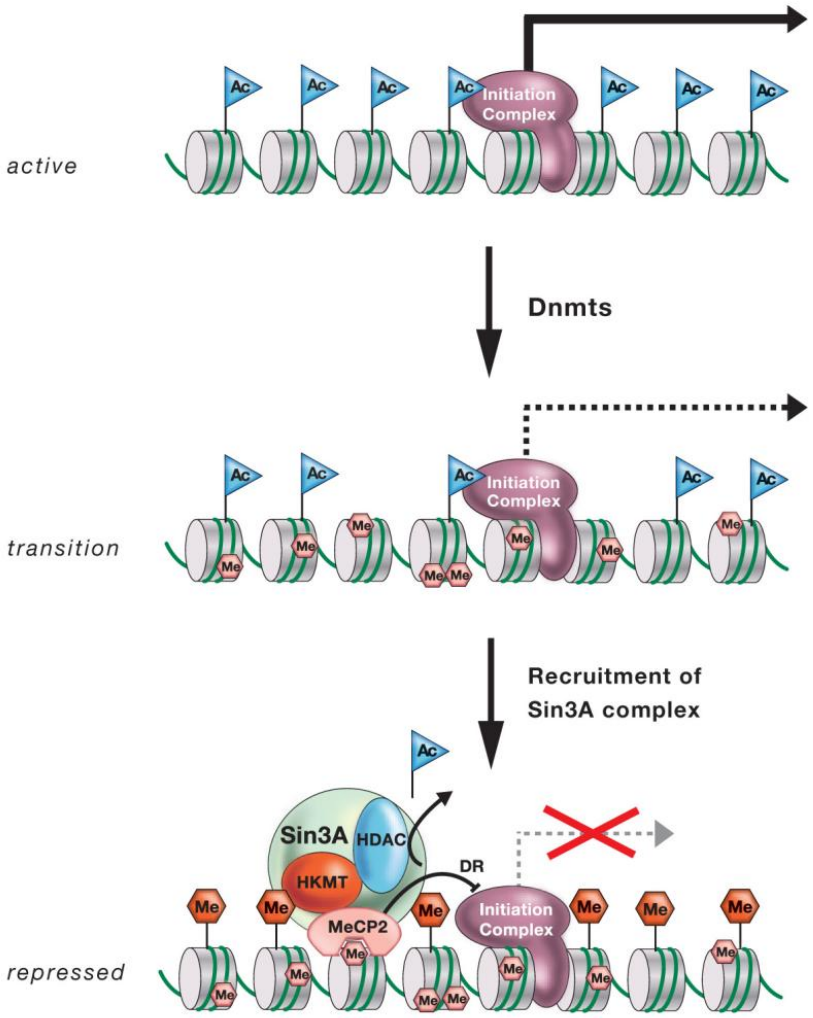


» **Attraction of methyl-CpG-binding proteins (MBDs)**

- 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)

Imperial College London

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

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

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

---

- Gene activation (oncogenes) via:

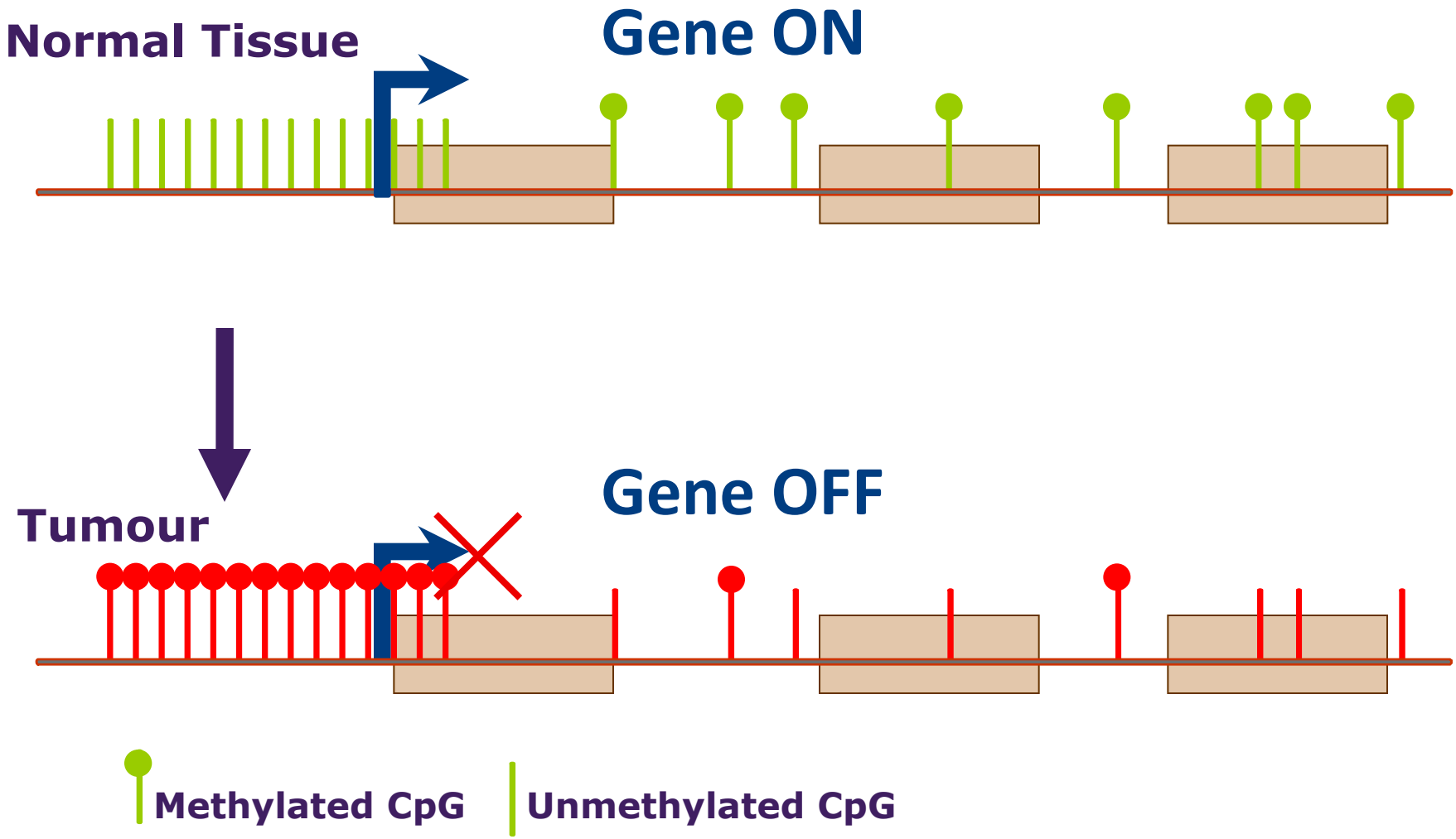
  mutation, translocation, duplication or gene switched on

- Gene inactivation (tumour suppressors) via:

  mutation, gene loss or gene being switched off

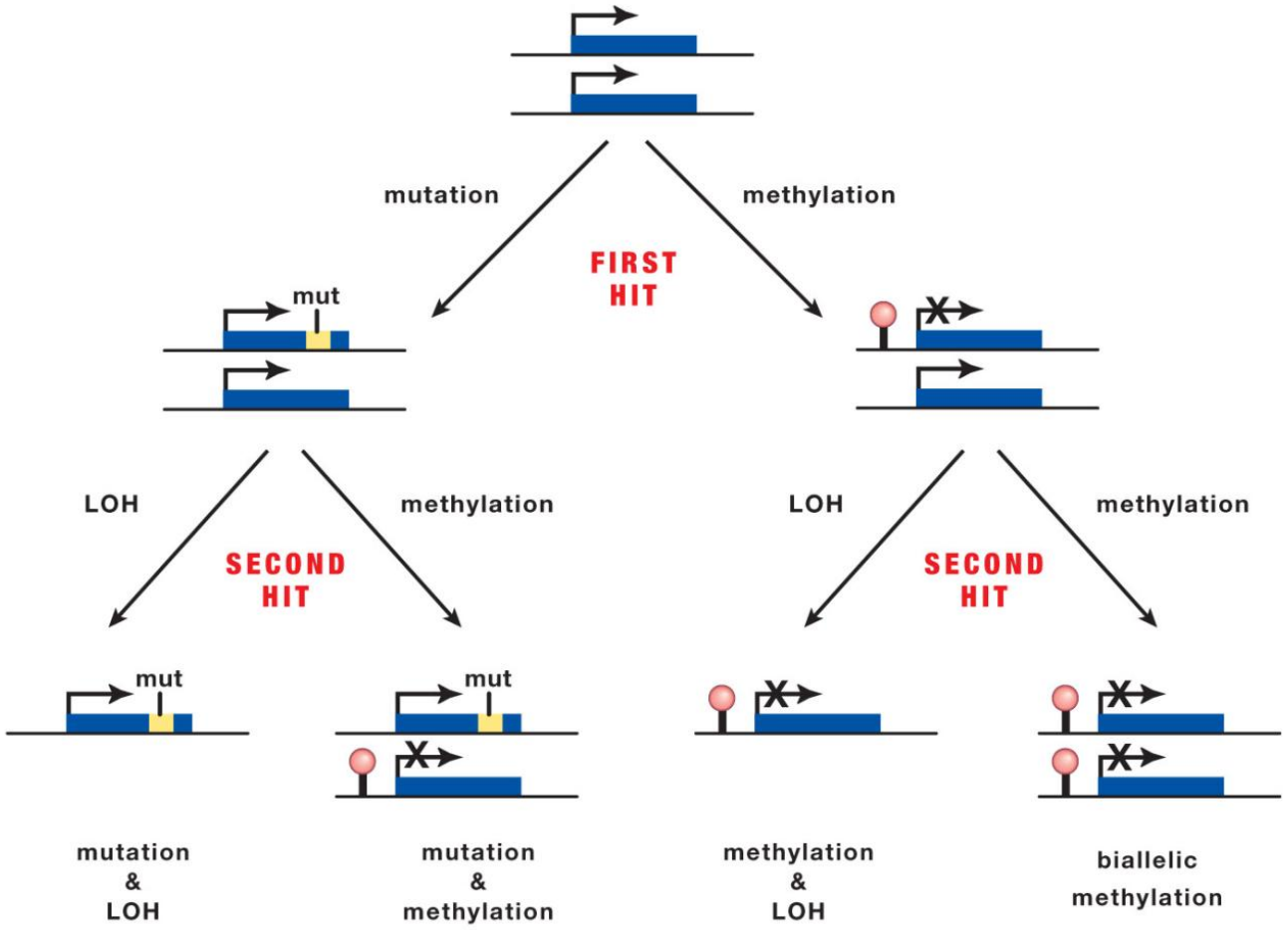
Imperial College London

DNA methylation and Cancer



Imperial College
London

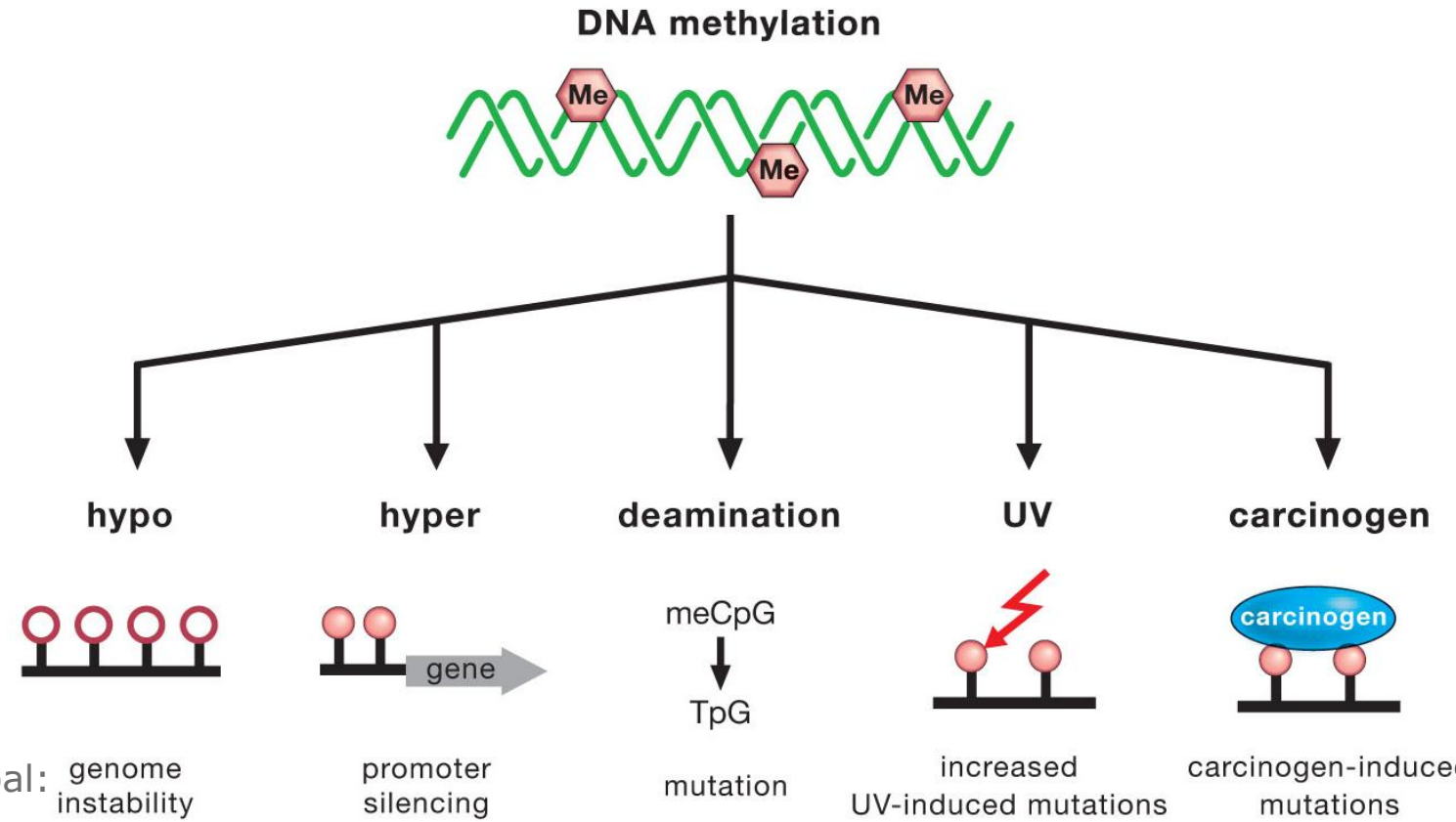
DNA Methylation Can Inactivate Tumour Suppressor Genes



Epigenetics, Allis, Jenuwein, Reinberg, Caparros, 2009

Imperial College London

Alterations in DNA Methylation Can Lead to Cancer



- global: genome instability
- local: **activation of oncogenes**

promoter silencing

mutation

increased UV-induced mutations

carcinogen-induced mutations

Epigenetics, Allis, Jenuwein, Reinberg, Caparros, 2009

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 → increased mutation rate at CpG site in lungs of smokers

Imperial College London

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*

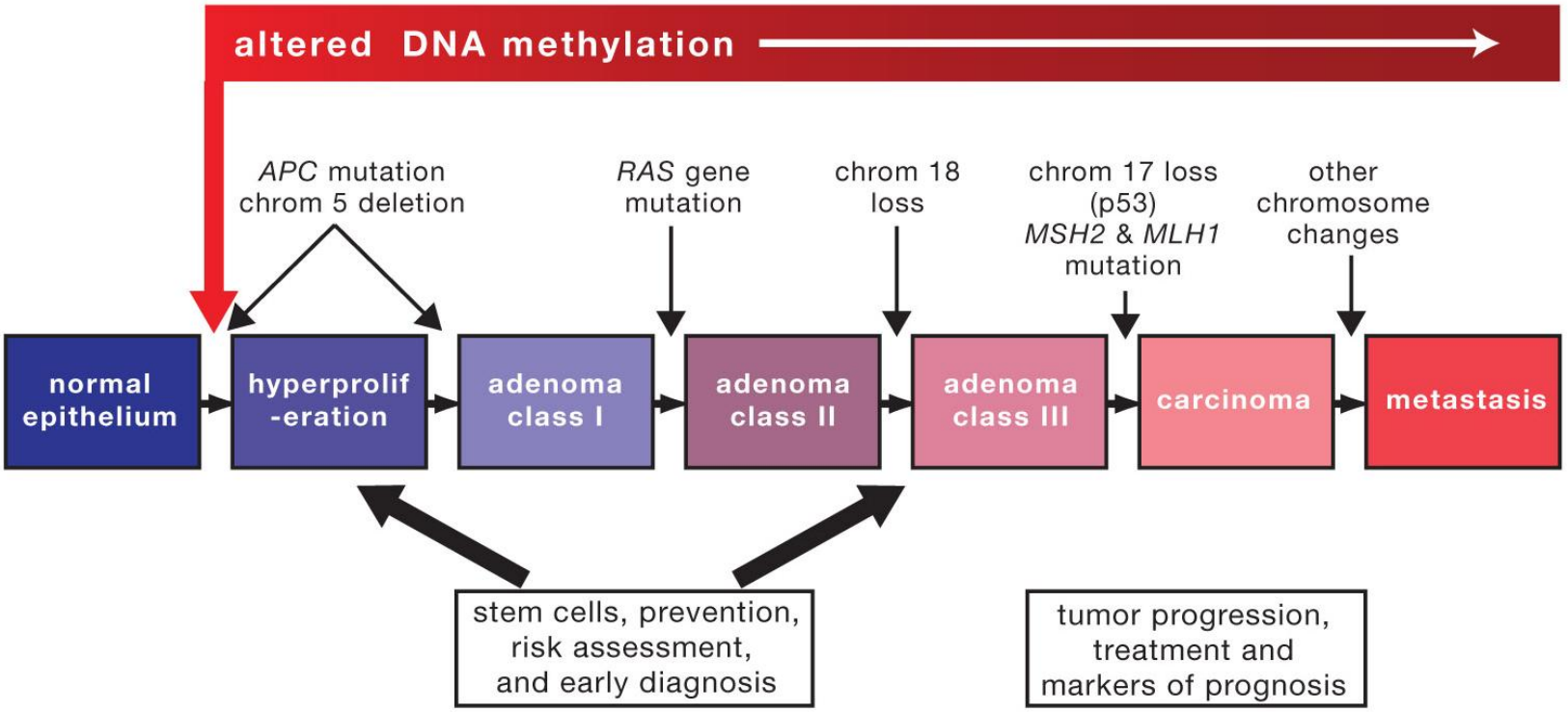
APC = adenomatous polyposis coli
DAPK = death-associated protein kinase
MGMT = O-6-methylguanine-DNA methyltransferase

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

Imperial College London

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

Imperial College London

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)

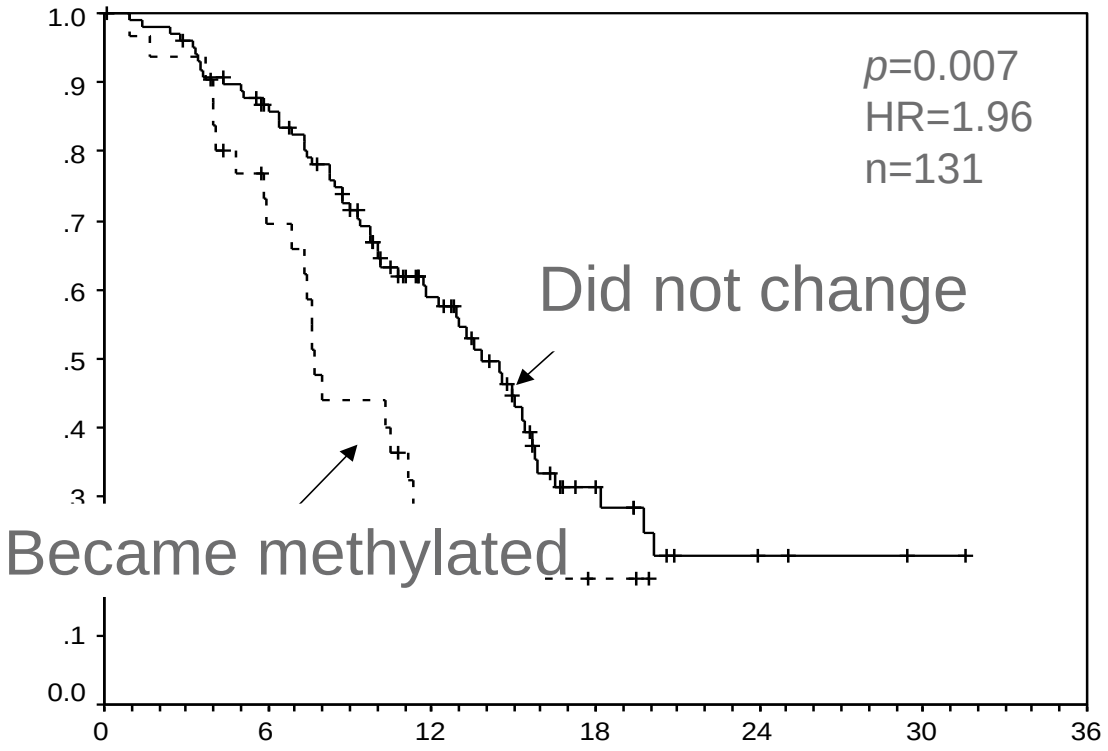
- **Stable epigenetic modification of DNA detectable in:**
  - body fluids (plasma, urine, sputum)
  - paraffin embedded samples

- **Therapeutic implications:**
  - targeted therapies to reverse aberrant DNA methylation

Imperial College London

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

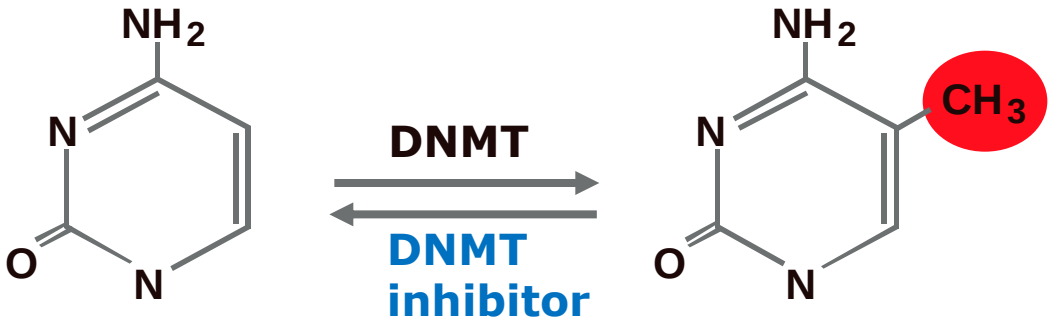
Gifford *et al.* Clin Cancer Res (2004):



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

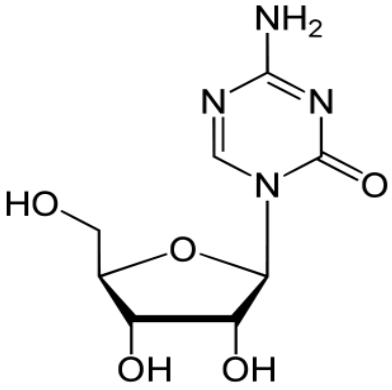
Imperial College London

Epigenetic therapy

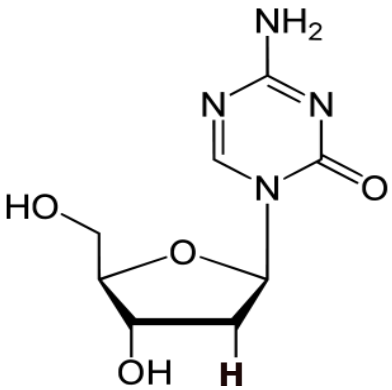


- unlike oncogenic mutations abnormal epigenetic change can be reversed using DNMT inhibitors

Approved DNMT inhibitors:



5-Azacytidine

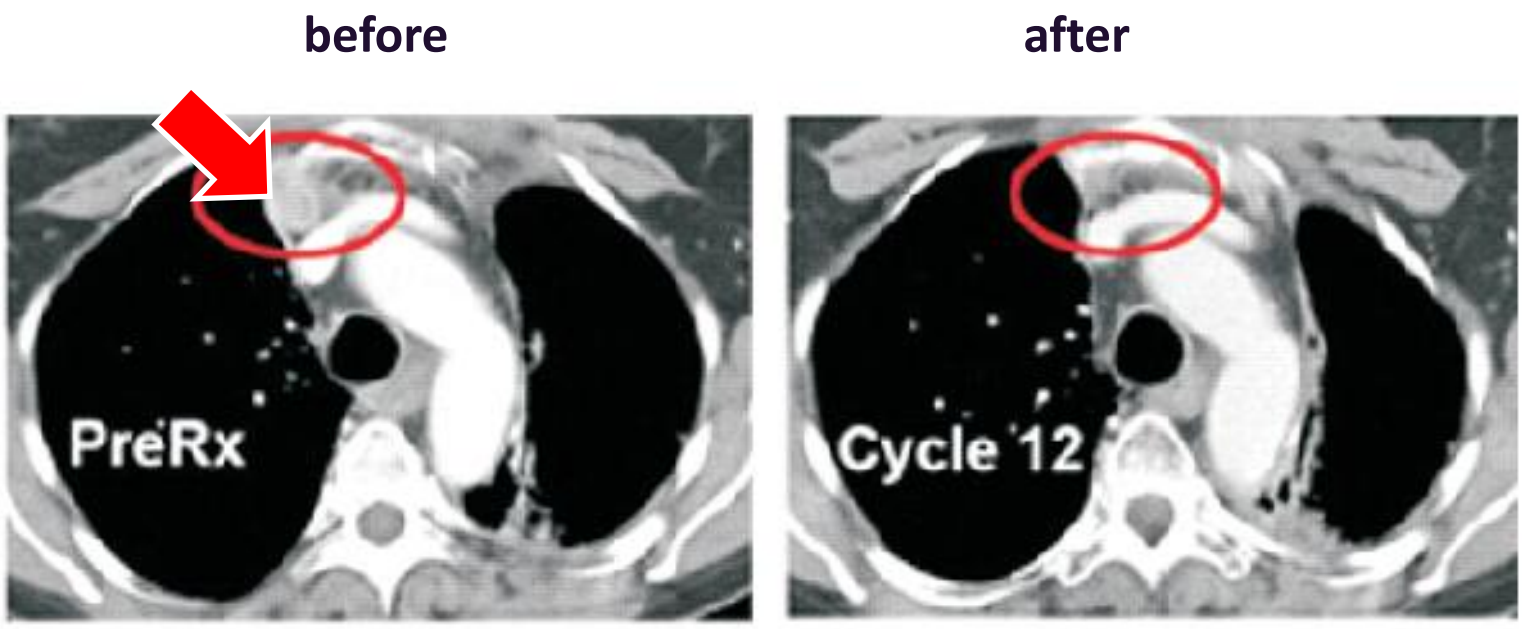


5-Aza-2'-deoxycytidine

Nucleoside analogues

Imperial College London

Treatment with epigenetic therapies leads to tumour regression

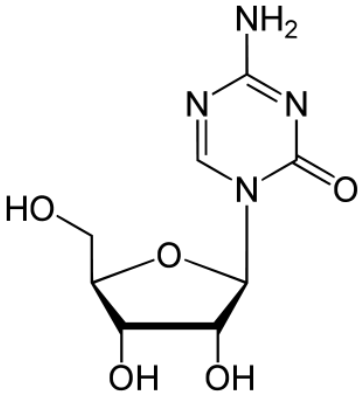


Therapy: combined low-dose 5-Azacytidine and entinostat in advanced lung cancer

Juergens et al., 2011, Cancer Discovery

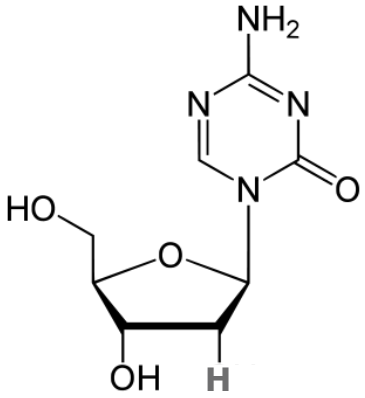
Imperial College London

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 and antitumour effects at lower less toxic doses

Baylin, Nat Clin Pract Oncol 2005;2:S4–S11;
Tsai et al., Cell, 2012

Imperial College London

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

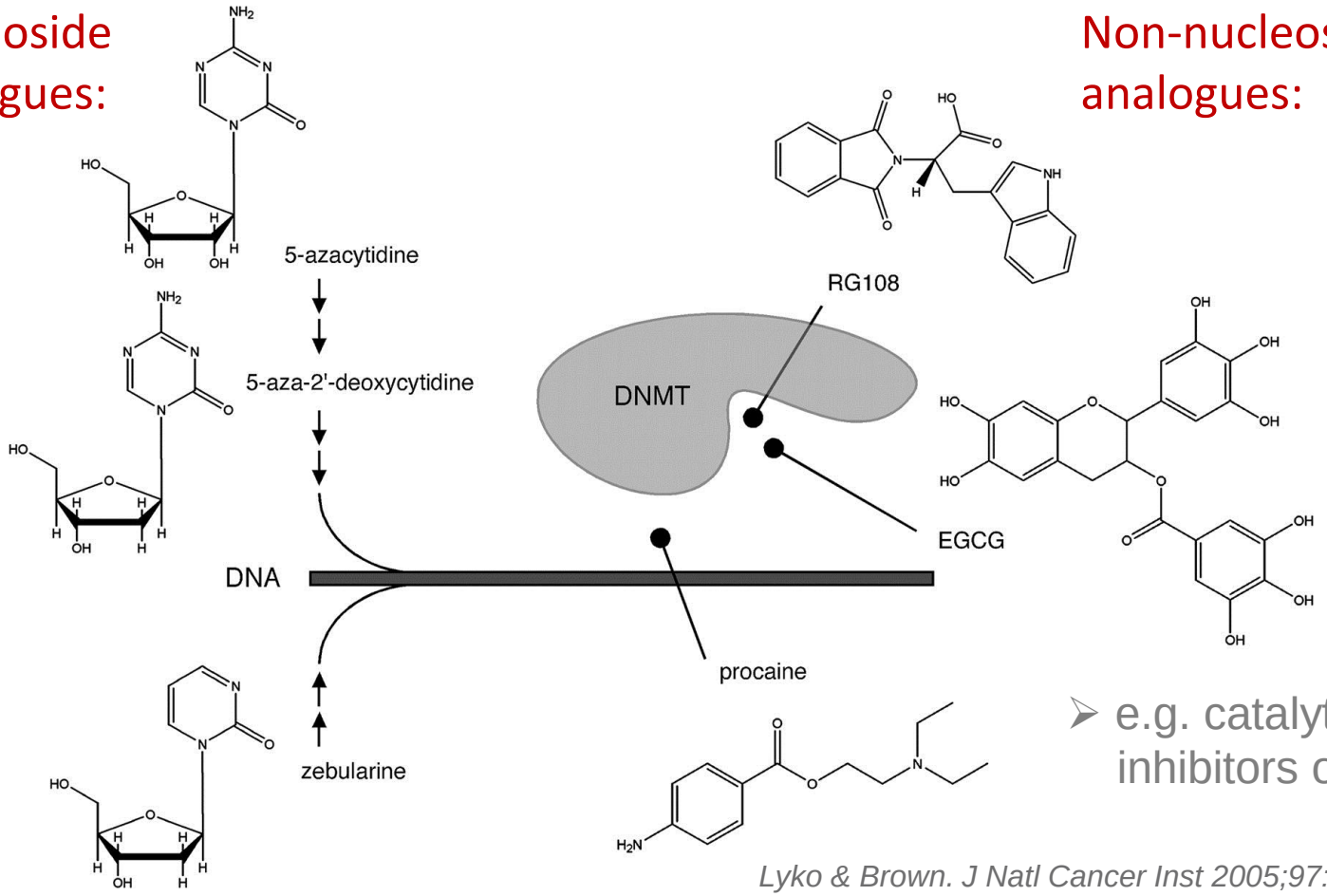
AML= acute myeloid leukemia
CMML=chronic myelomonocytic leukemia
MDS=myelodysplastic syndrome

Graham et al. Eur J Cancer. 2009

Imperial College London

Types of DNMT inhibitors

Nucleoside analogues:



Non-nucleoside analogues:

- 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

Imperial College London

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