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(From Genetics to) Epigenetics
- relevance for global health

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Breast Cancer Campaign Fellow

Epigenetics Unit, Dept Surgery and Cancer, Imperial College London

Hammersmith Hospital Campus

BREAST CANCER CAMPAIGN BE PART OF THE CURE

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Outline – Learning Objectives

1. What is Epigenetics?
2. What are the different levels of epigenetic regulation?
3. How do we measure the epigenome?
4. What goes wrong in the Epigenome during disease development (eg Cancer)
5. Current research topics
    1. Disease Risk – from GWAS to EWAS
    2. Disease Prognosis – Predictive personalised medicine
    3. Epigenetic drug development
6. Relevance to Global Health
    1. Epigenetics as a mediator of Environmental factors (eg smoking ; dutch famine)
7. Recent controversies – what is an epimutation?
    1. Germline Epimutations.... IGF2
    2. Germline Epimutations.... MLH1
8. Further Reading / Websites

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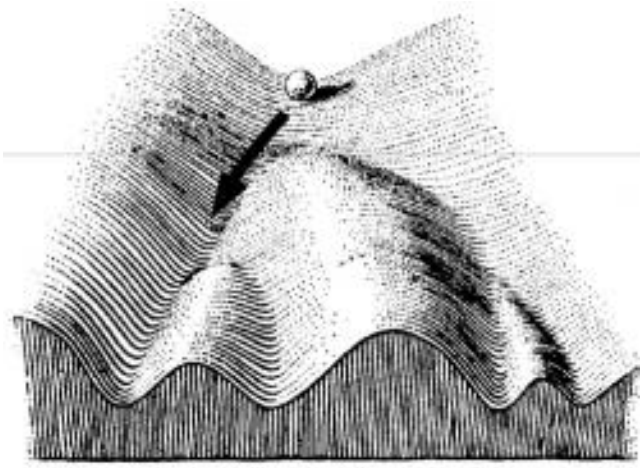
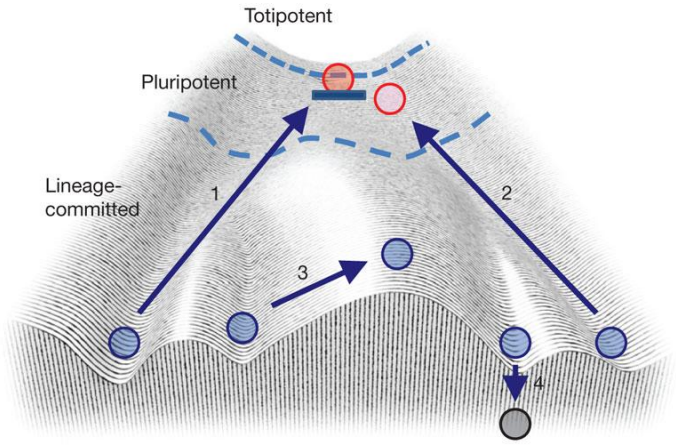
1. What is Epigenetics (Of Maize and Men)

Conrad Waddington (1905-1975) who coined the term 'epigenetics'

Waddingtons Epigenetic Landscape for cell fate (1942)



The Epigenetic Landscape Today



CW: often credited with coining the term epigenetics in 1942 as:
"the branch of biology which studies the causal interactions between genes and their products, which bring the phenotype into being".

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1. What is Epigenetics (Of Maize and Men)

Barbera McClintock (1902 – 1992)
The Nobel Prize in Medicine 1983
("Jumping Genes" / TEs)

Maize

Mice

Men

first scientist to correctly speculate on the basic concept of epigenetics

"[T]he progeny of two (such) sister cells are not alike with respect to the types of gene alteration that will occur...This inactivity or suppression is considered to occur because the genes are 'covered' by other non-genic chromatin materials.... Gene activity may be possible only when a physical change in this covering material allows the reactive components of the gene to be 'exposed' and thus capable of functioning." 1951

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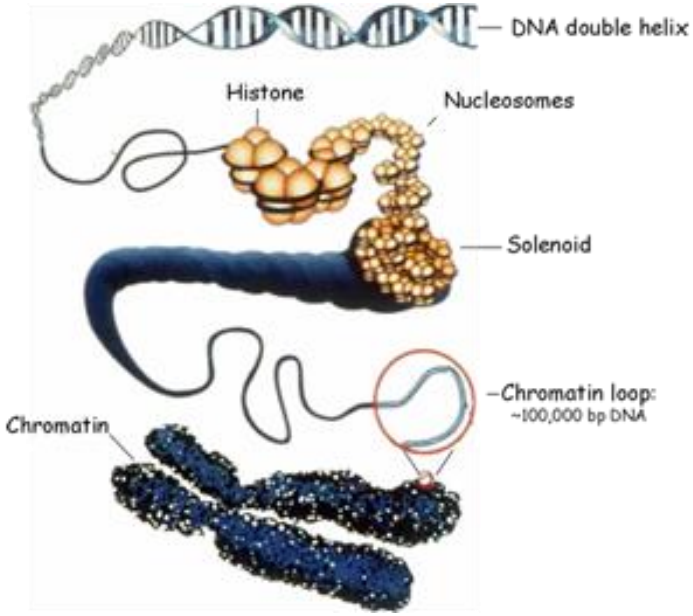
2. What are the different levels of epigenetic regulation?

Genetics = Code for genes
Epigenetics = Code for how much of a gene is made

DNA Methylation	*; Histone modifications*	*; miRNA regulation*
On/Off switch	*; Packaging*	*; Fine tuning dimmer switch*



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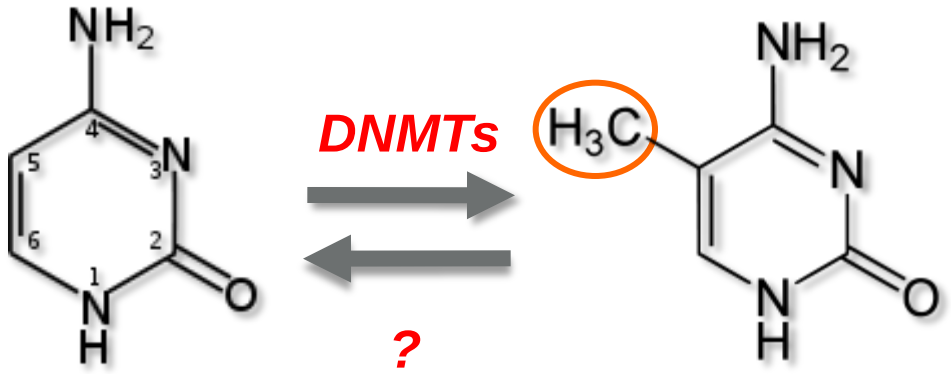
DNA Methylation



C (cytosine)

5'm-C (5'methylcytosine)

- ~4% of cytosines are methylated
- 70% - 80% of CpG cytosines are methylated
- CG are underrepresented in the genome (lost thru evolution)
- Expected Frequency of CG = 4.4%
- Actual Frequency of CG = 1%
- ~60% of genes have CpG island promoters, typically unmethylated

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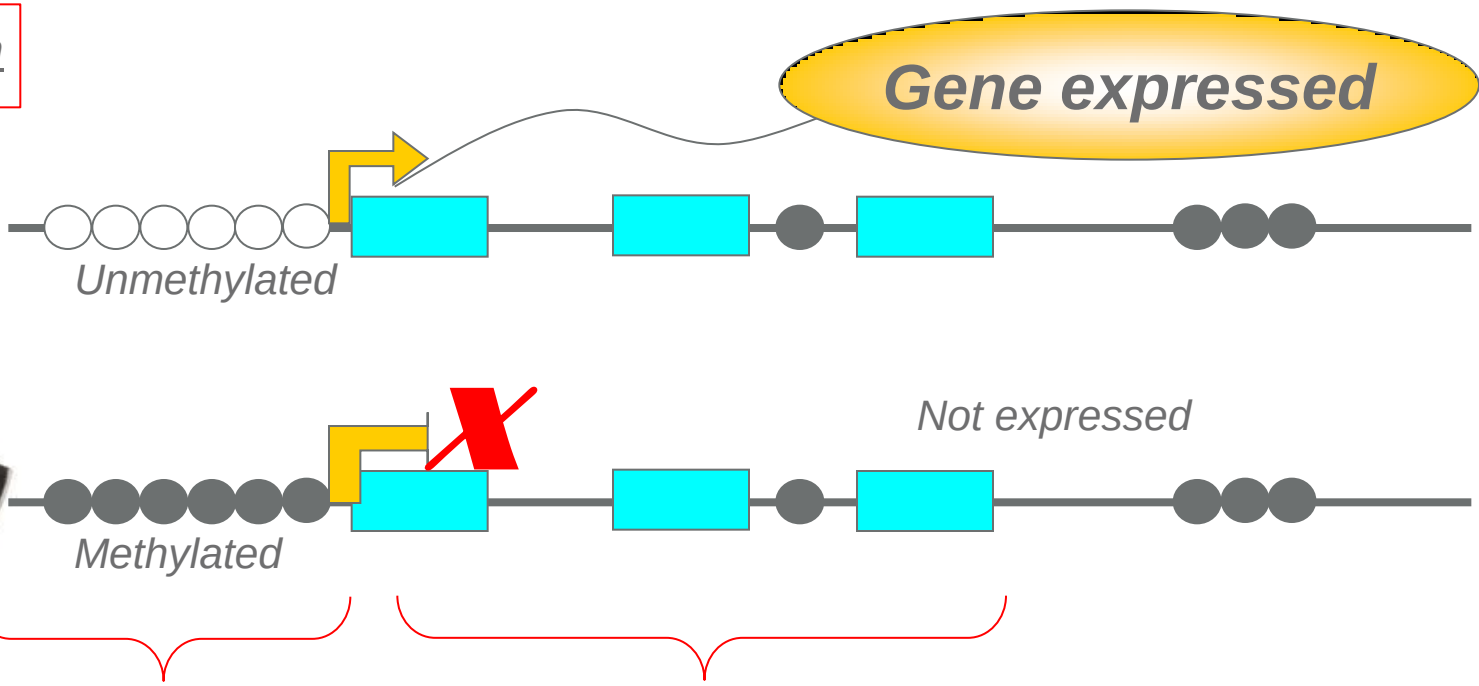
DNA Methylation

Genetics = Code for genes
Epigenetics = Code for how much of a gene is made

DNA Methylation

On/Off switch



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DNA Methylation

DNA Methylation

On/Off switch

Readers

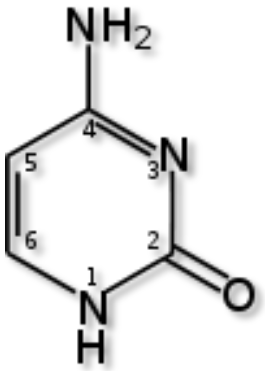
(MBDs, MECP2)

MBD2

MeCP2

Writers

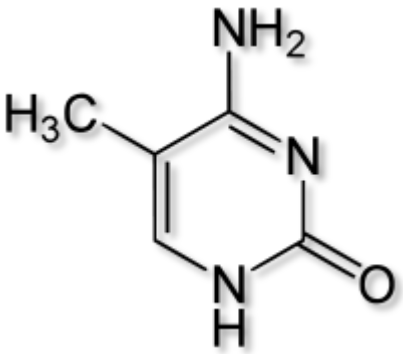
(DNMT1, 3a, 3b)



C

Erasers

(TET1, TET2)



5'm-C

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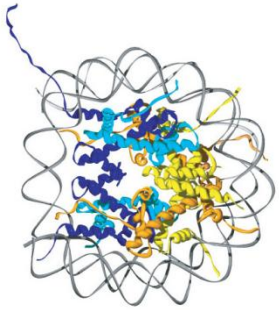
Histones and posttranslational modifications – the marks



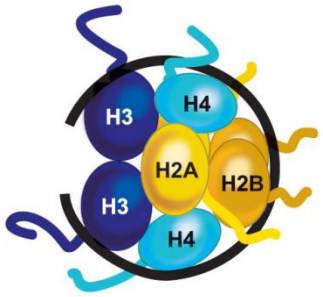


Figure 5. Nucleosome Structure

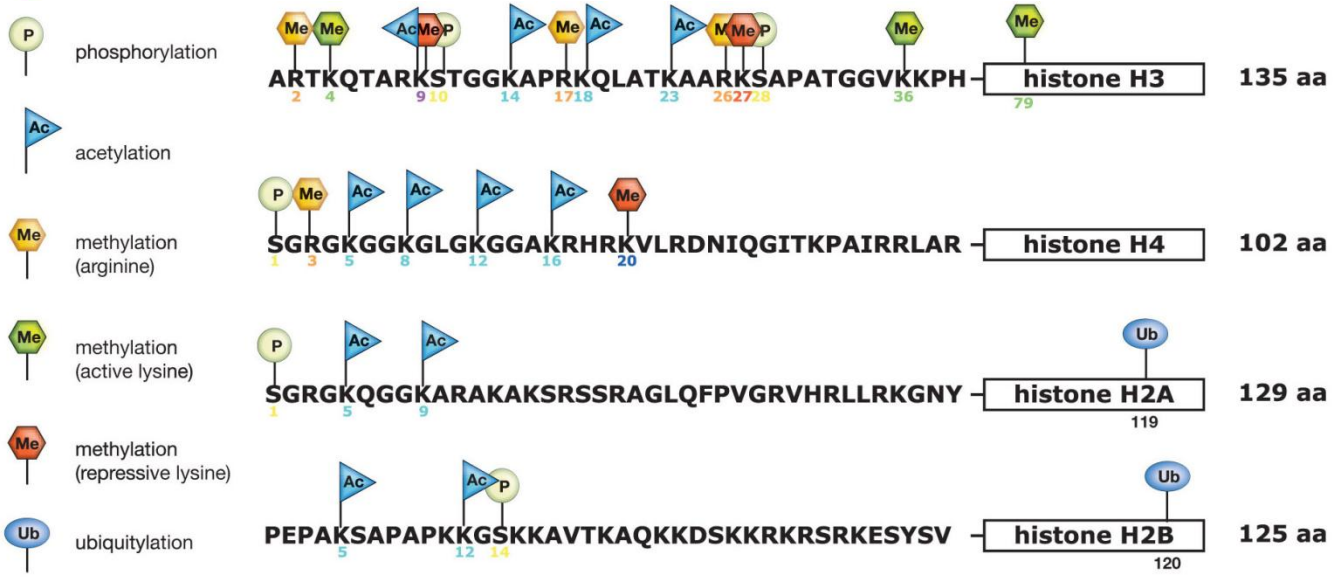
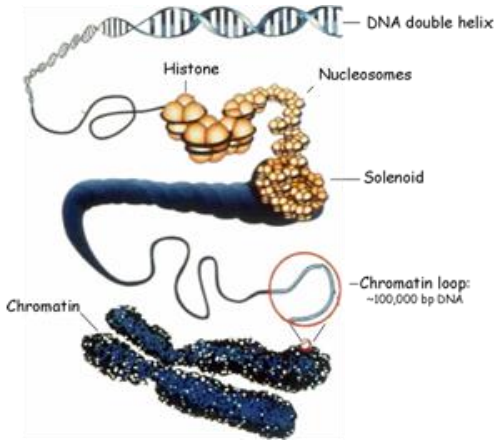
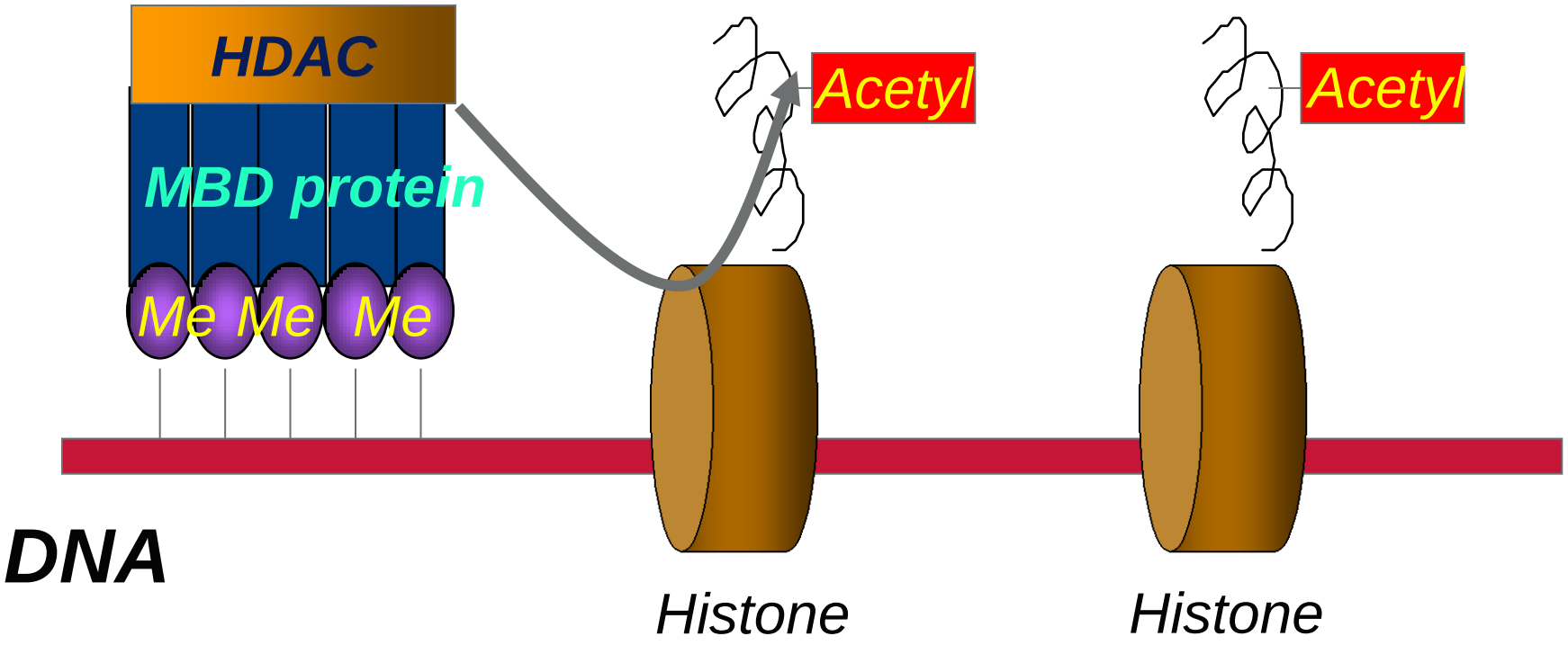
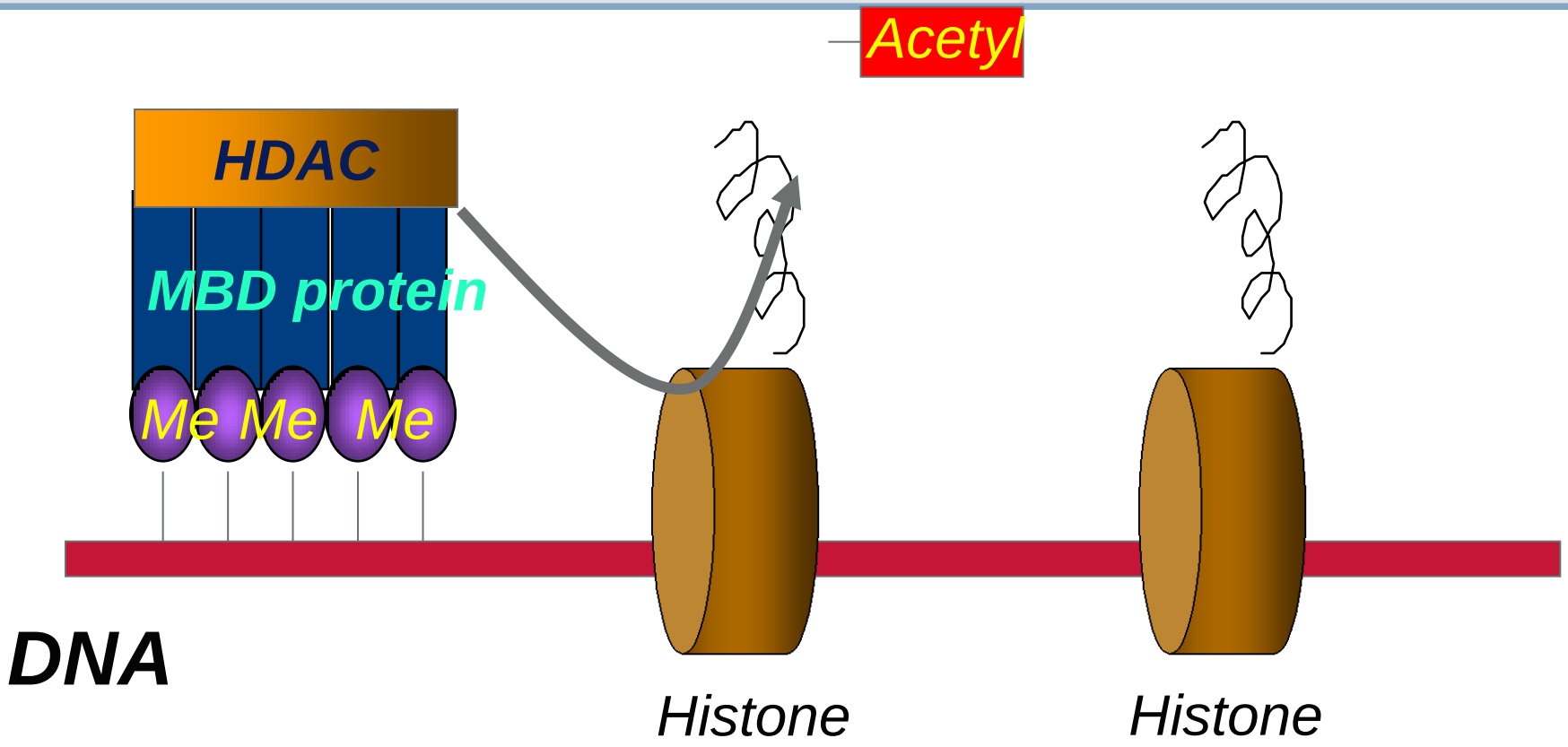


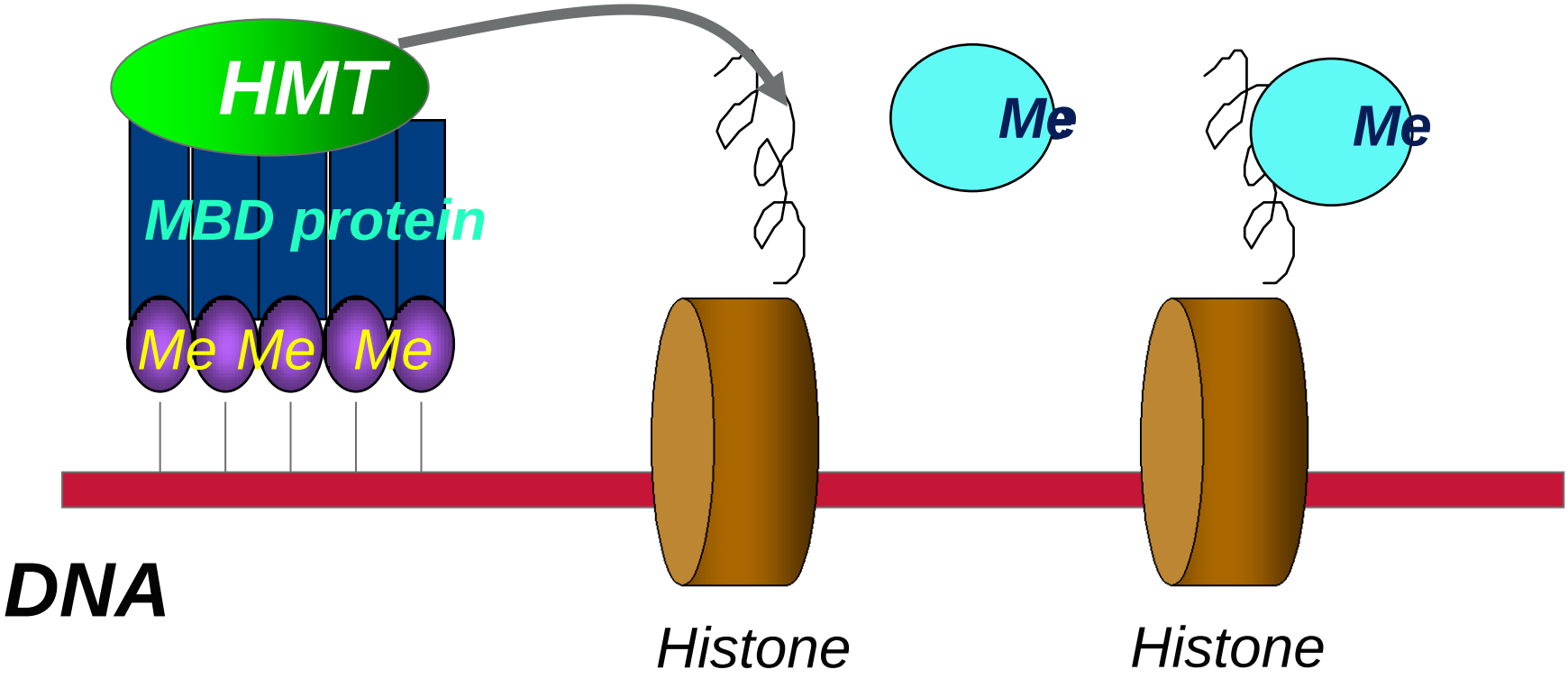
Figure 6. Sites of Histone Tail Modifications

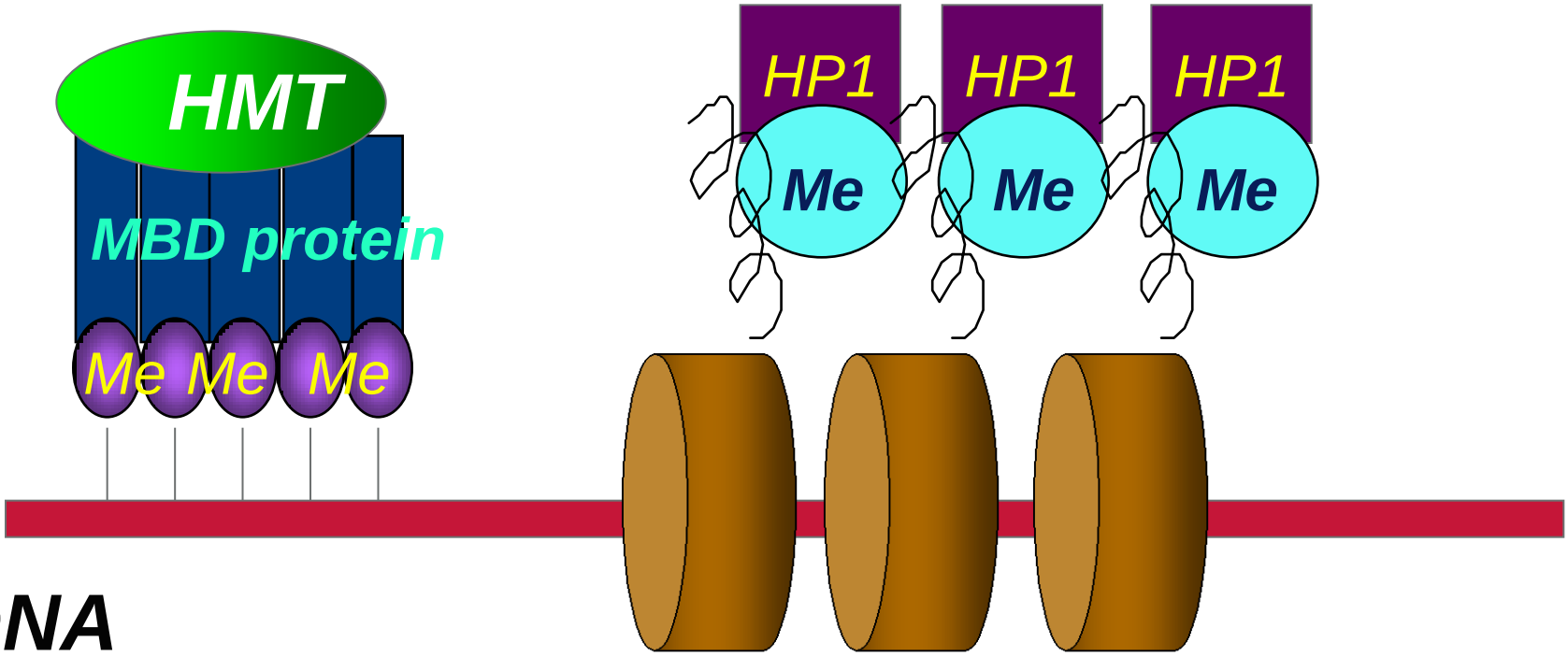


Epigenetics, Allis, Jenuwein, Reinberg, Caparros, 2009


HDAC
MBD protein
Me Me Me
Acetyl
Acetyl
DNA
Histone
Histone


Acetyl
HDAC
MBD protein
Me Me Me
DNA
Histone
Histone


HMT
MBD protein
Me Me Me
Me
Me
DNA
Histone
Histone


HMT
MBD protein
Me Me Me
HP1
HP1
HP1
Me
Me
Me
DNA

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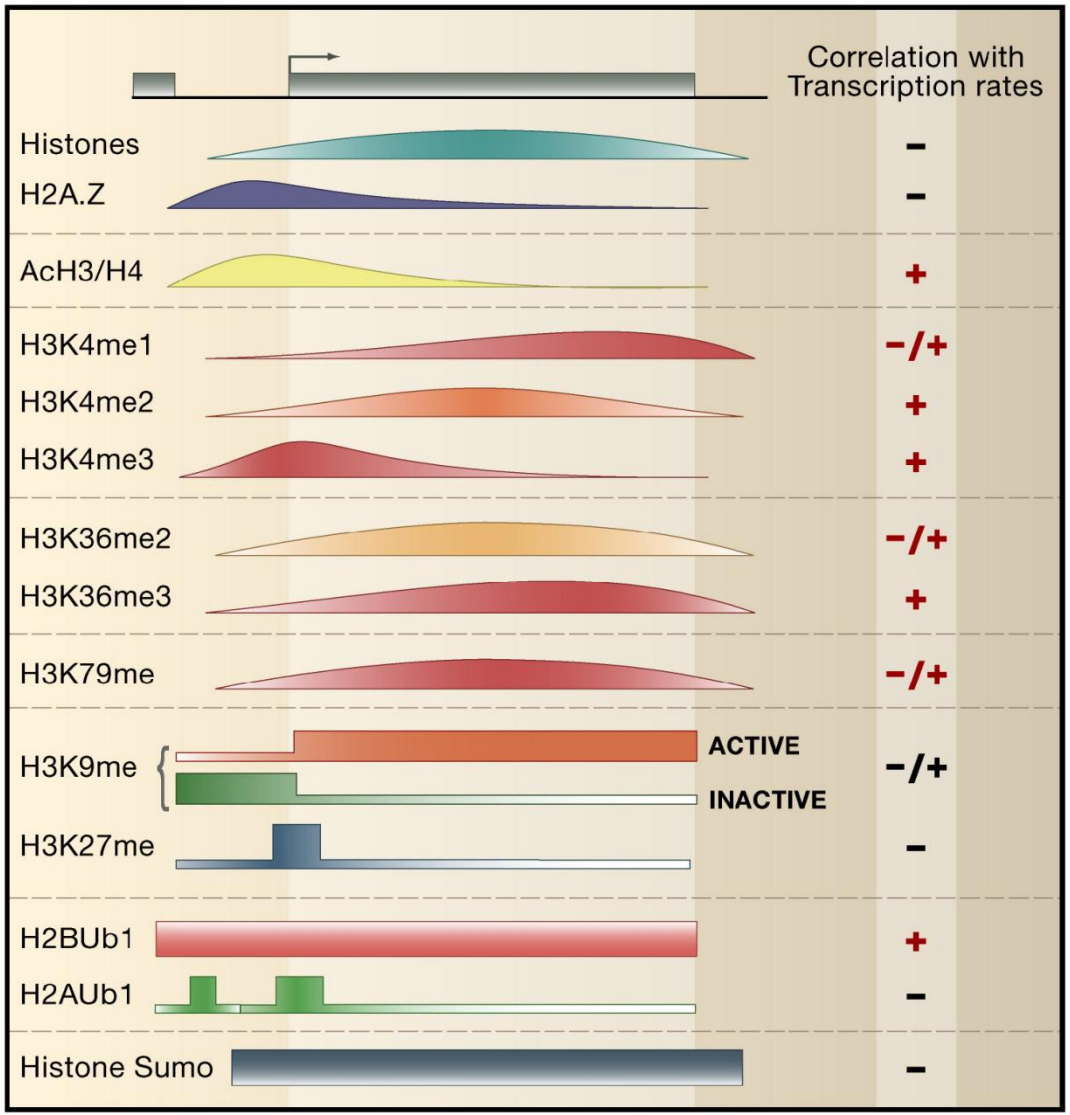
Histone Modifications – Correlation with transcription



Key Marks to Remember:

H3 + H4 acetylation *= active gene*

H3K4me3 *= active gene*

H3K9me (promoter) *= silent gene*
H3K9me (gene) *= active gene*

H3K27me3 (promoter) *= silent gene*

Li et al Cell 2007 128:p707-719

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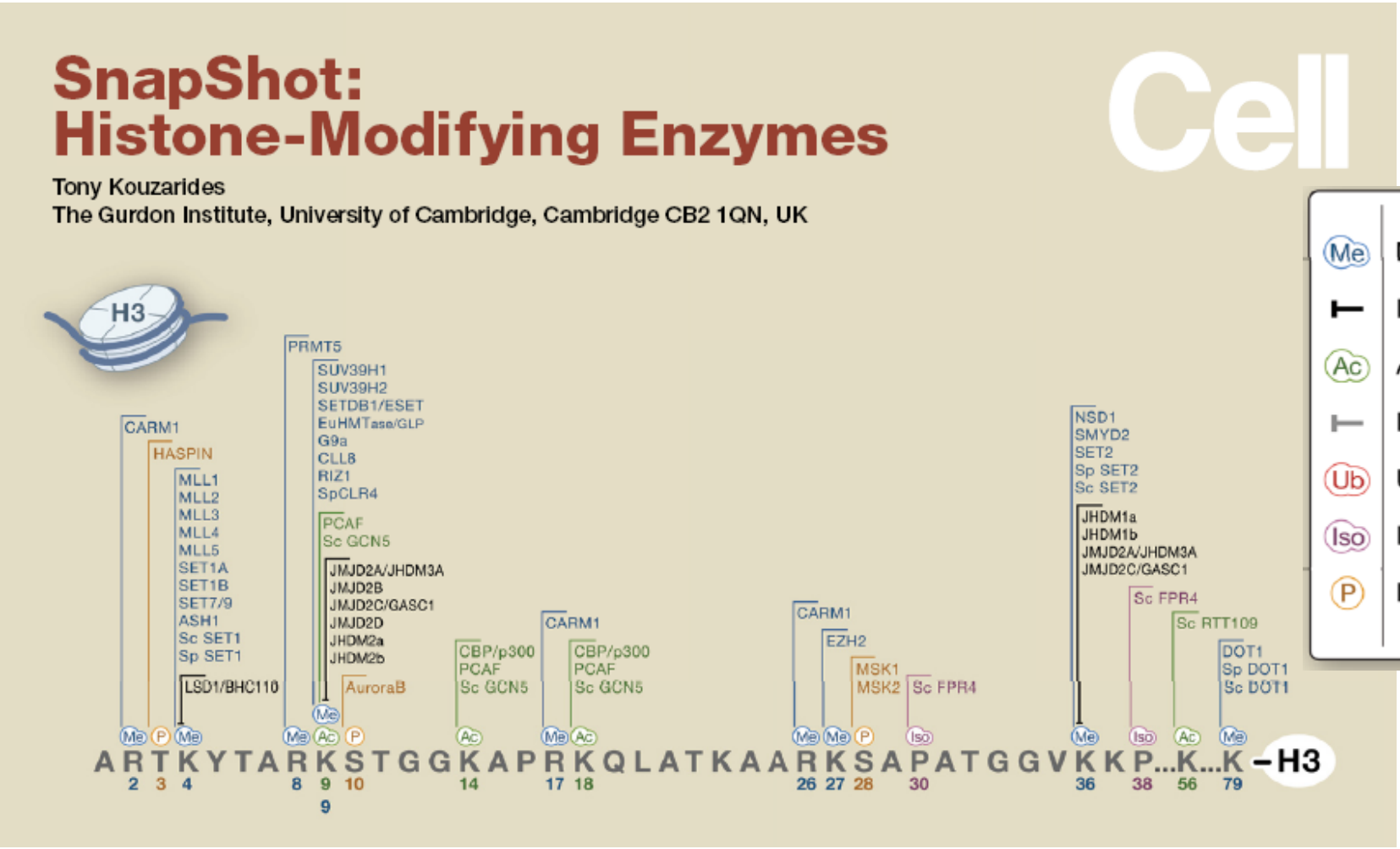
Histone Modifications – the readers / writers / erasers



Kouzarides, T. Snapshot: Histone Modifying enzymes. Cell 2007,128: p802.e1-802.e2

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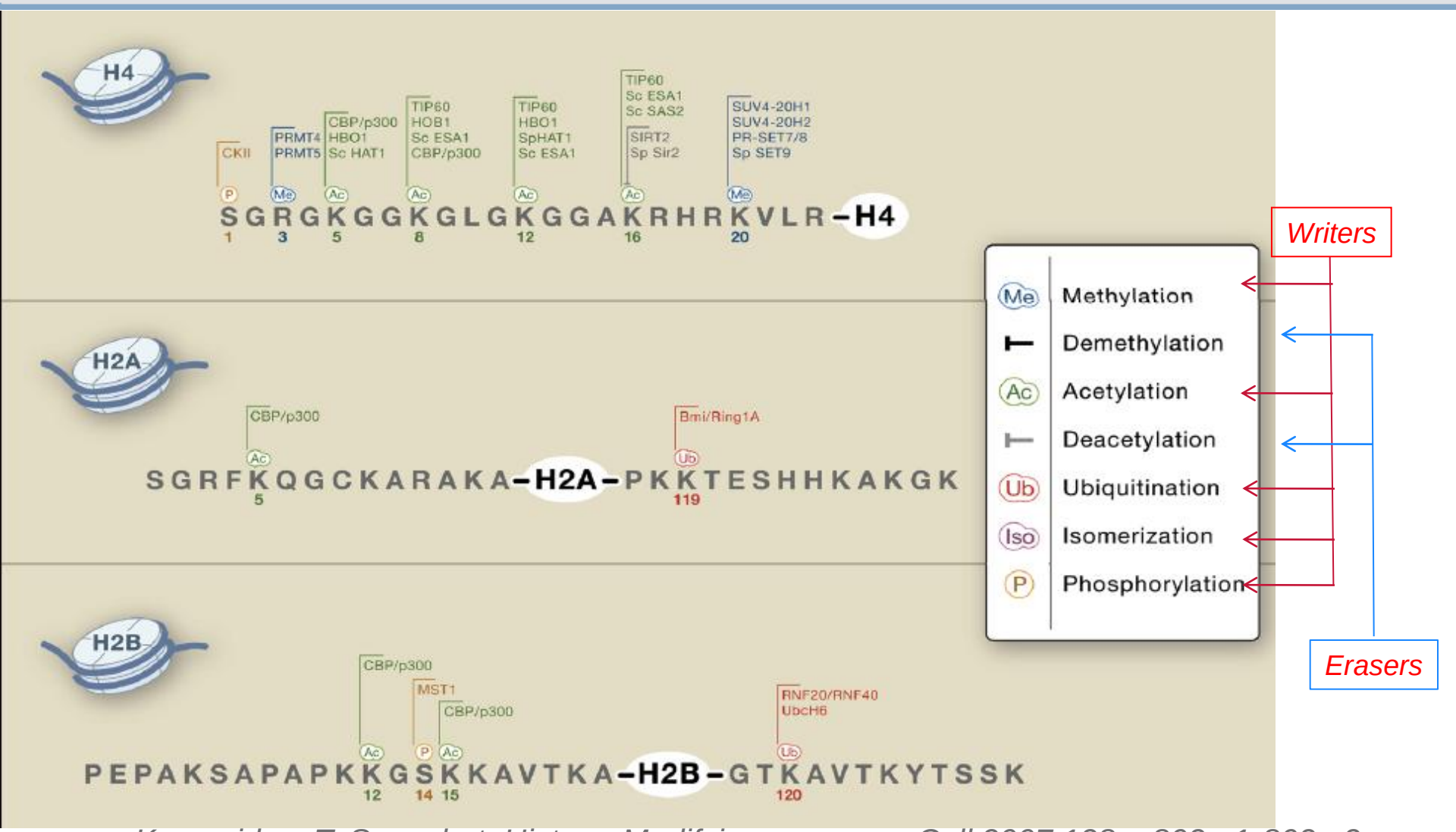
Histone Modifications – the readers / writers / erasers



Kouzarides, T. Snapshot: Histone Modifying enzymes. Cell 2007,128: p802.e1-802.e2

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Non-coding RNAs / microRNAs / RNAi

The Nobel Prize in Physiology or Medicine 2006
Andrew Z. Fire, Craig C. Mello

The Nobel Prize in Physiology or Medicine 2006

Nobel Prize Award Ceremony

Andrew Z. Fire

Craig C. Mello

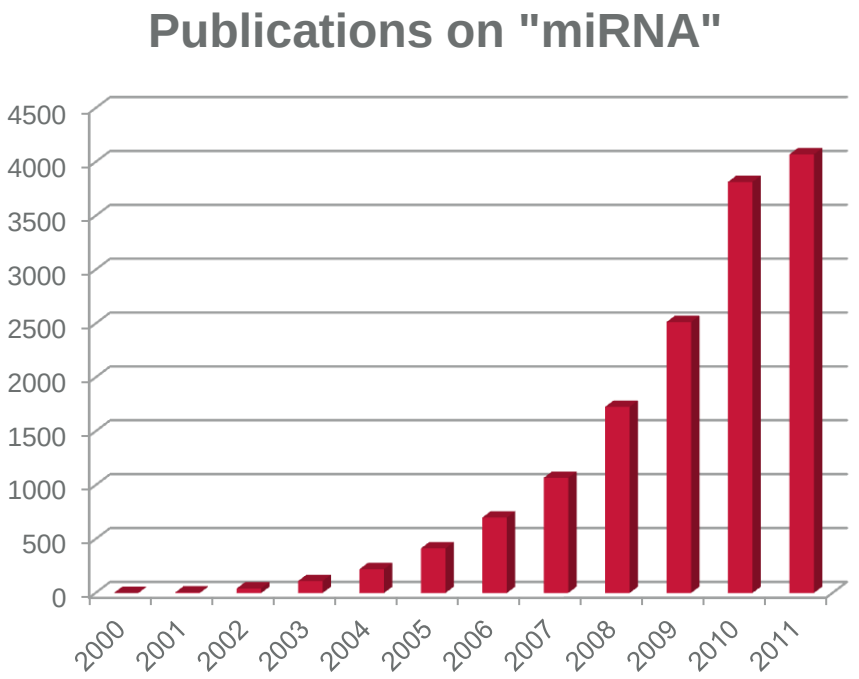
Photo: L. Cicero

Andrew Z. Fire

Photo: J. Mottern

Craig C. Mello

The Nobel Prize in Physiology or Medicine 2006 was awarded jointly to Andrew Z. Fire and Craig C. Mello *"for their discovery of RNA interference - gene silencing by double-stranded RNA"*



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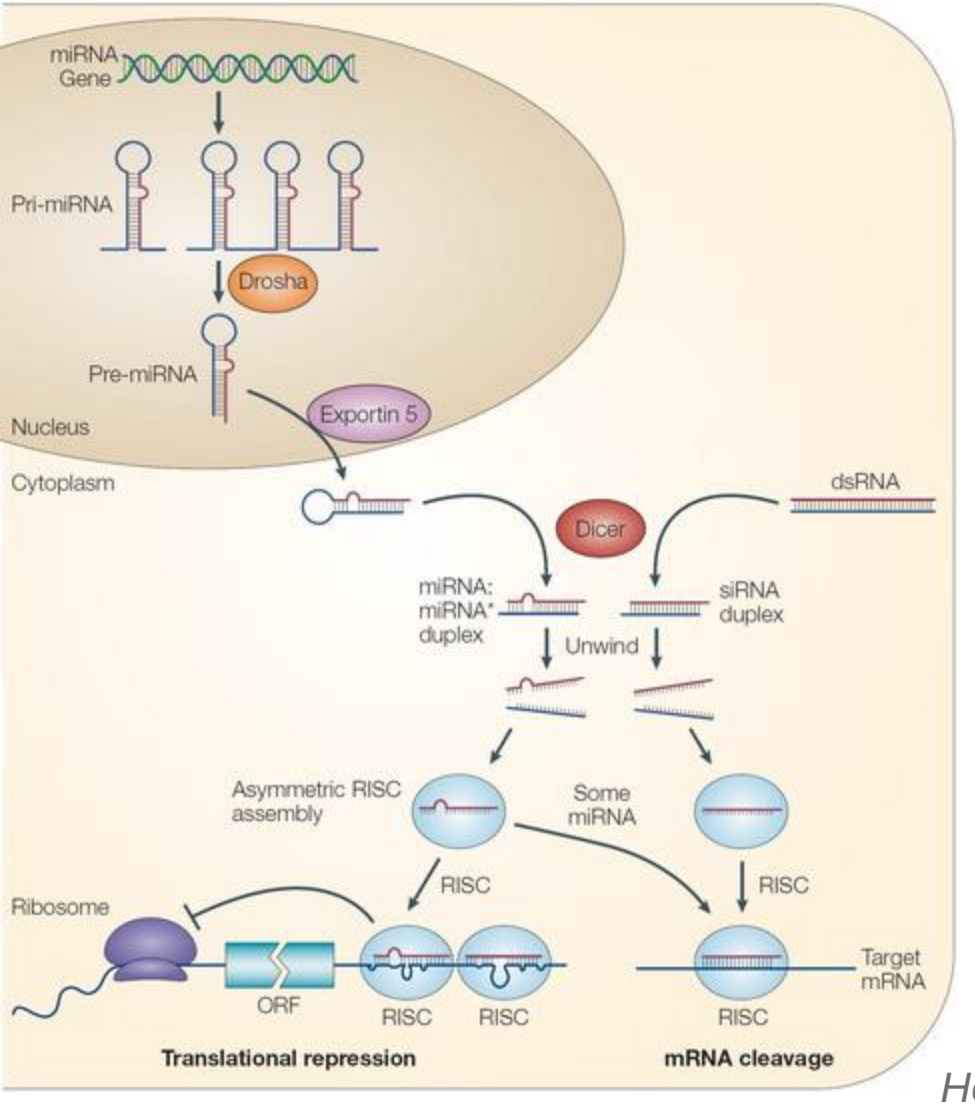
Non-coding RNAs / microRNAs



- *A new mechanism for gene regulation*
- *RNA which is not used for making proteins (non-coding RNA) can be chopped up and used to inhibit protein-coding RNAs*

He and Hannon Nat Rev Genet. 2004, 5(7):522-31

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A Quick Half-time Summary

- Epigenetics is the study of gene regulation by mechanisms including:
  - DNA methylation
  - Histone Modifications
  - Non-coding RNA
- DNA methylation is important at the promoter of genes
  - Methylated = inactive ; Unmethylated = active gene
- There are many Histone Modification combinations
  - Many different writers / erasers
  - Key histone marks include:
    - Active Genes === H3 + H4 acetylation plus H3K4me3
    - Silent Genes === H3K9me (promoter) plus H3K27me3 (promoter)

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3. How do we measure the Epigenome

DNA Methylation

Gene Specific:

1) Sodium Bisulphite Conversion
2) PCR amplification
3) Quantification - Pyrosequencing

Genome-wide:

1) Sodium Bisulphite Conversion
   A. Microarray
   B. Highthroughput Sequencing
2) Enrichment by Enzymes
   A. Microarray
   B. Highthroughput Sequencing
3) Enrichment by Antibody / Protein
   A. Microarray
   B. Highthroughput Sequencing

Histone Modifications

Gene Specific:

1) Chromatin Immunoprecipitation
   1) Mod-Specific Antibody
   2) PCR for gene

Genome-wide:

1) Chromatin Immunoprecipitation
   A. Microarray
   B. Highthroughput Sequencing

ncRNA

Gene Specific:

1) RNA extraction
   A. qRT-PCR

Genome-wide:

1) RNA extraction
   A. Microarray
   B. Sequencing

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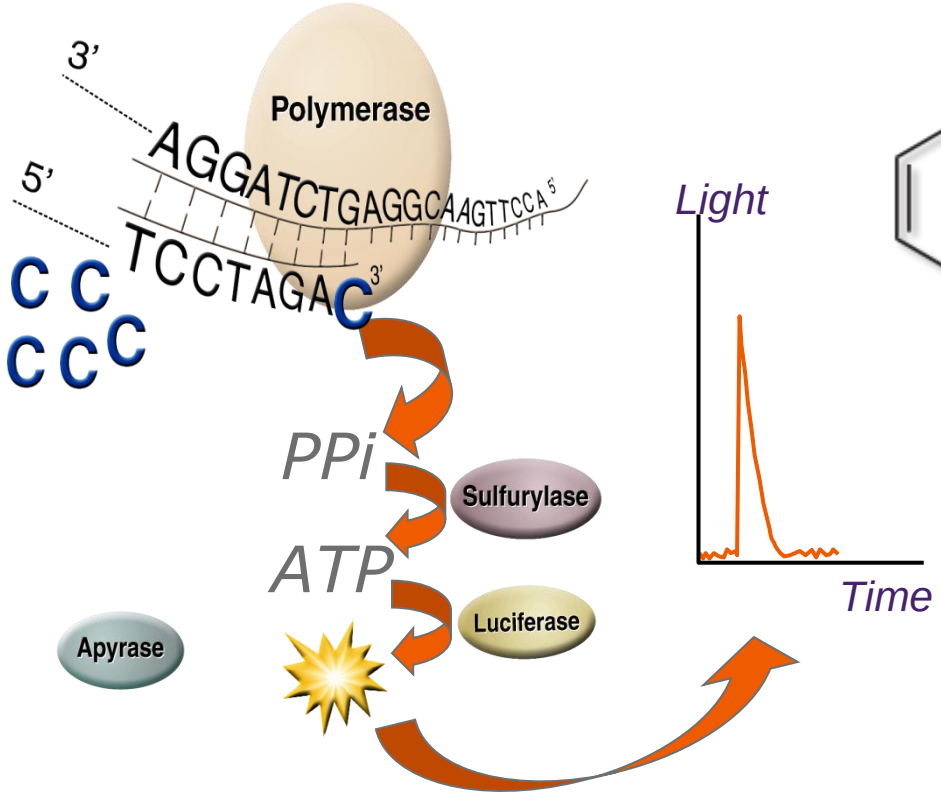
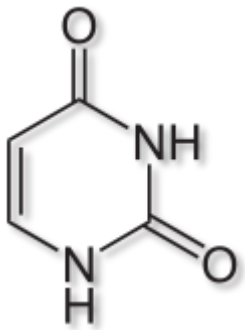
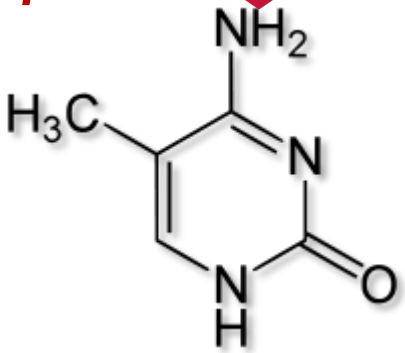
Pyrosequencing for DNA methylation

"Sequencing by Synthesis"
Ronaghi et al., Science (1998)



C → **U (T)** + **Sodium Bisulphite** → **5'm-C**





Measure the different levels of T vs C

QIAGEN

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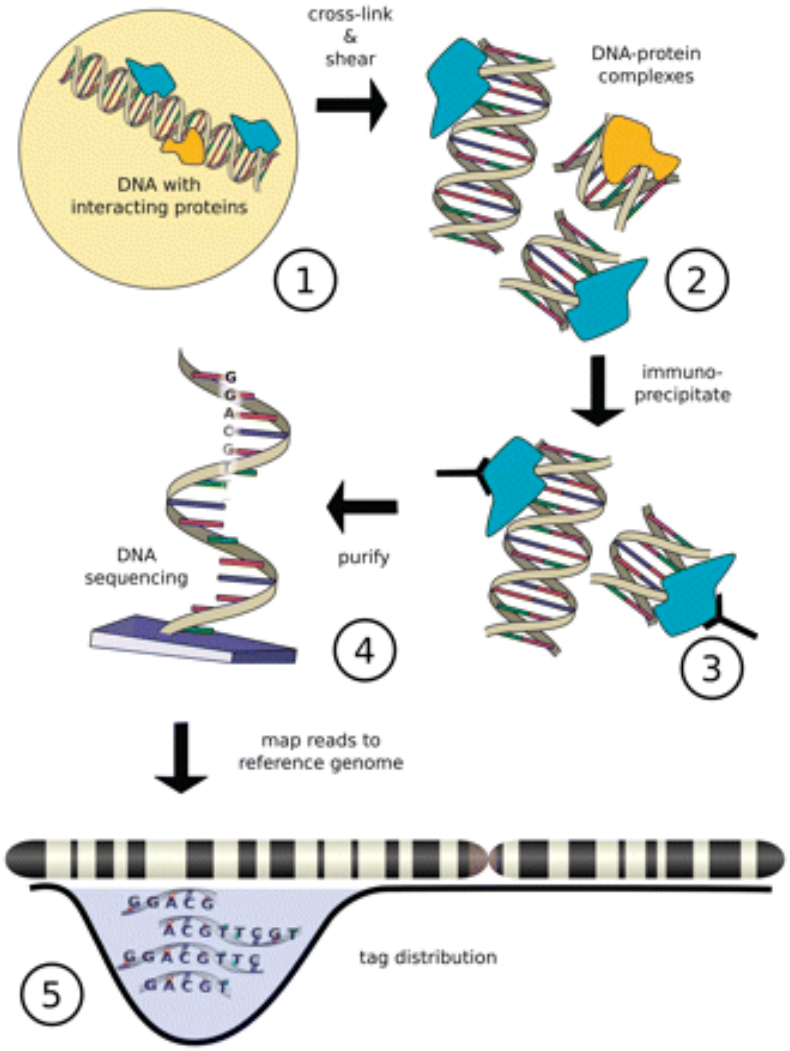
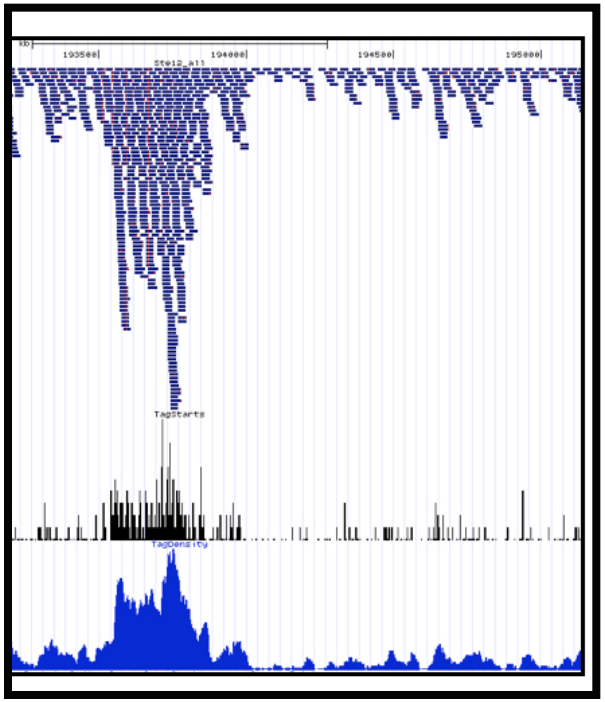
ChIP – seq

"Chromatin Immunoprecipitation followed by next generation sequencing"





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4. What goes wrong in the Epigenome in Cancer

1. DNA methylation
   - Gene specific hypermethylation (eg RASSF1, MLH1)
   - Genome-wide hypomethylation (4% down to 2-3% of all cytosines)

2. Histone Modifications
   - Active vs Inactive histone marks
   - Polycomb group gene silencing (H3-K27-me3)

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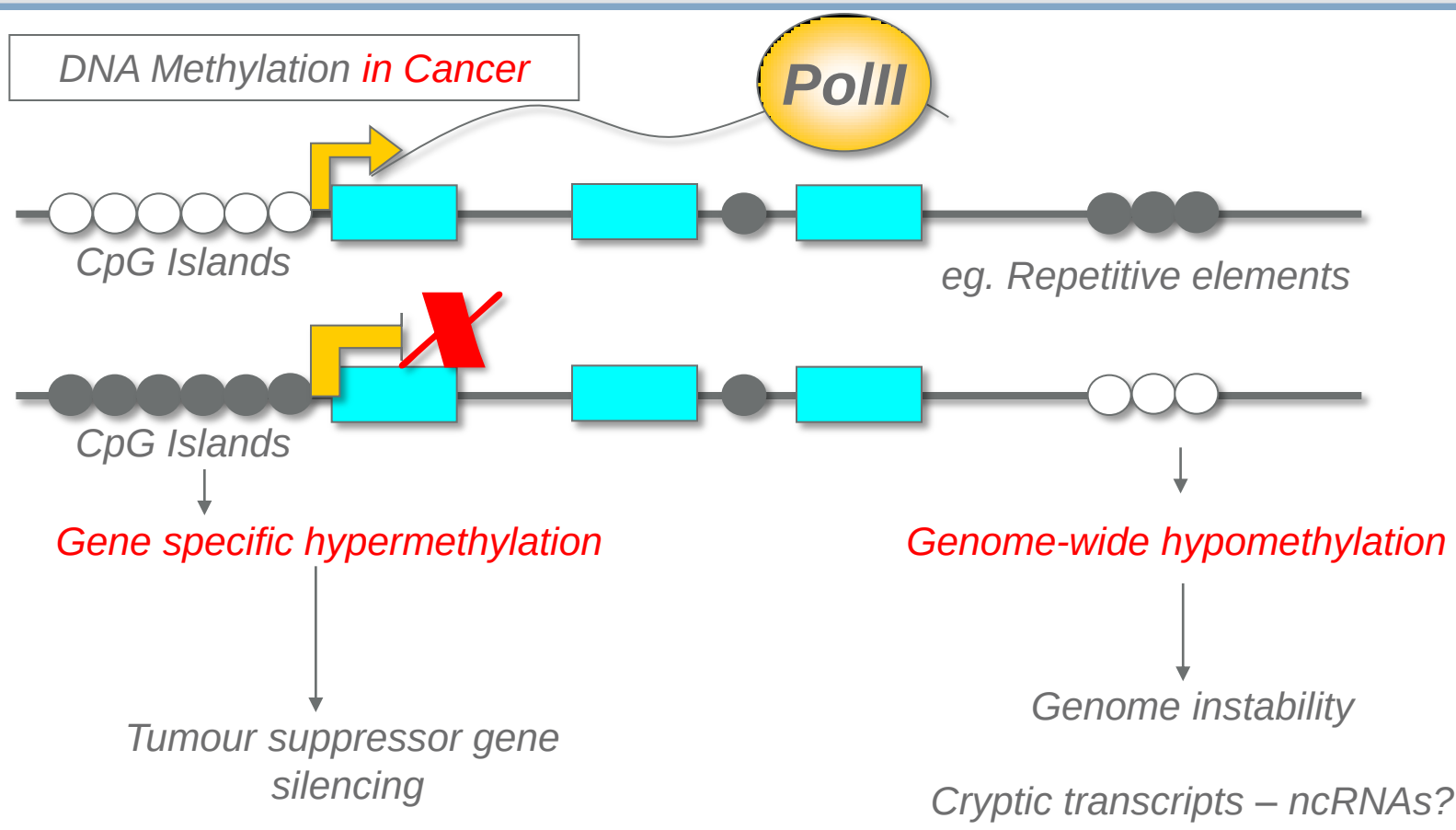
DNA Methylation



Wild and Flanagan, BBA Reviews on Cancer 2010
Wild et al, Carcinogenesis 2010

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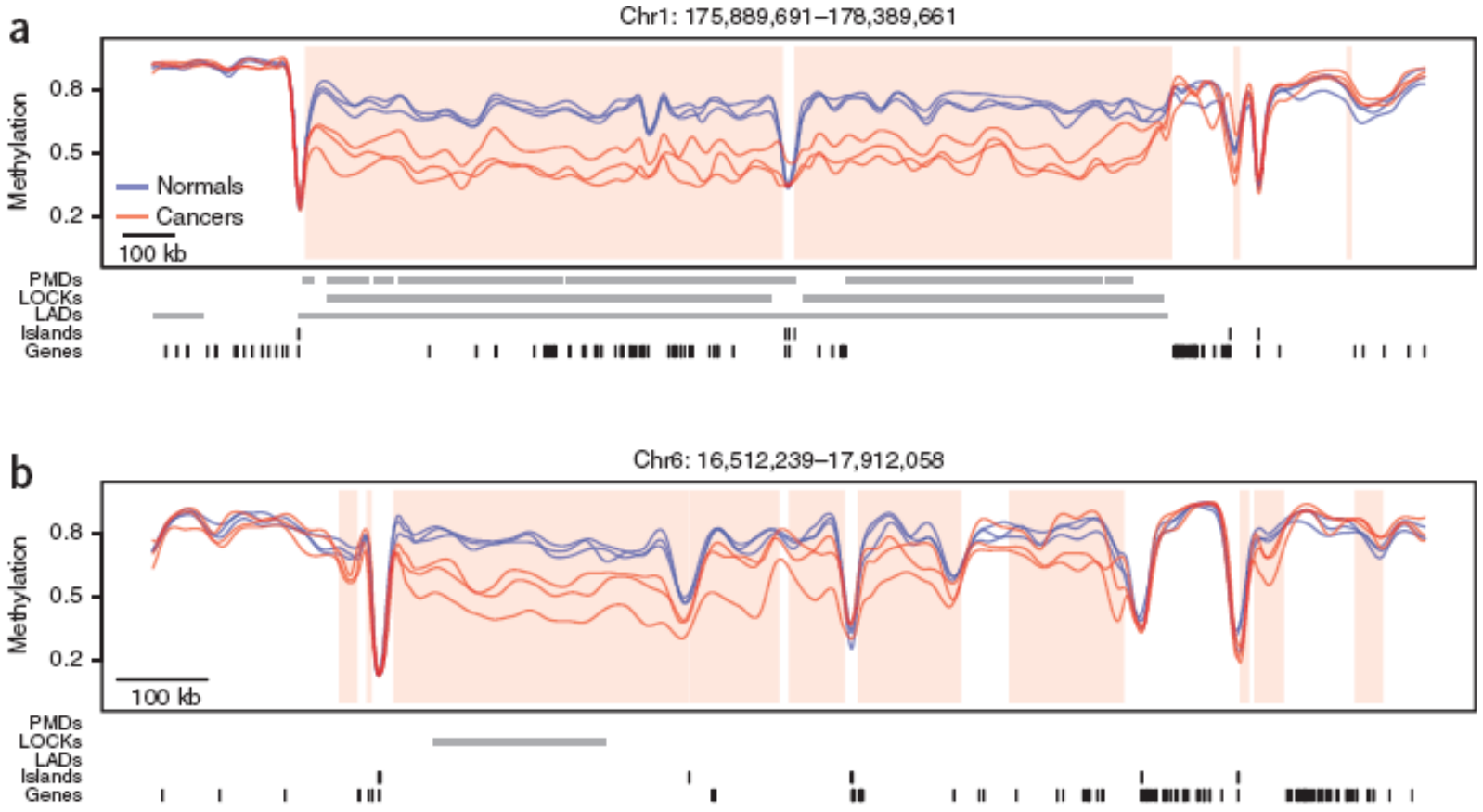
Genome-wide Hypomethylation in Cancer occurs in Blocks



Hansen et al, ***Nat Genet****. 2011; 43(8):768-75.*

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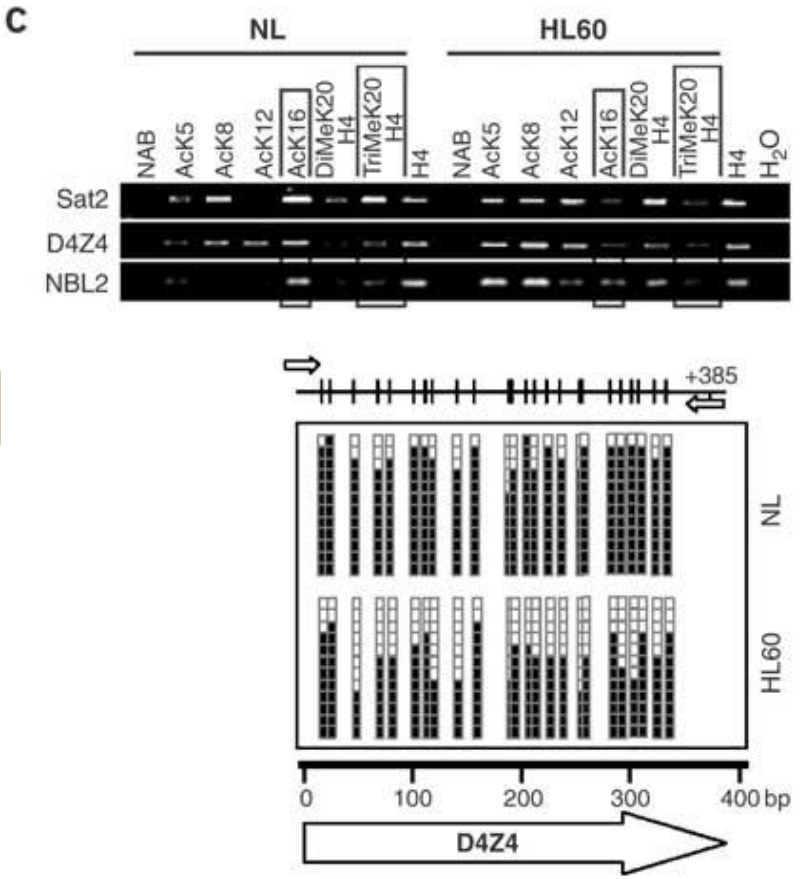
Characteristic Histone Modifications in Cancer

Loss of acetylation at Lys16 and trimethylation at Lys20 of histone H4 is a common hallmark of human cancer

Fraga et al, *Nature Genetics* ***37****, 391 - 400 (2005)*



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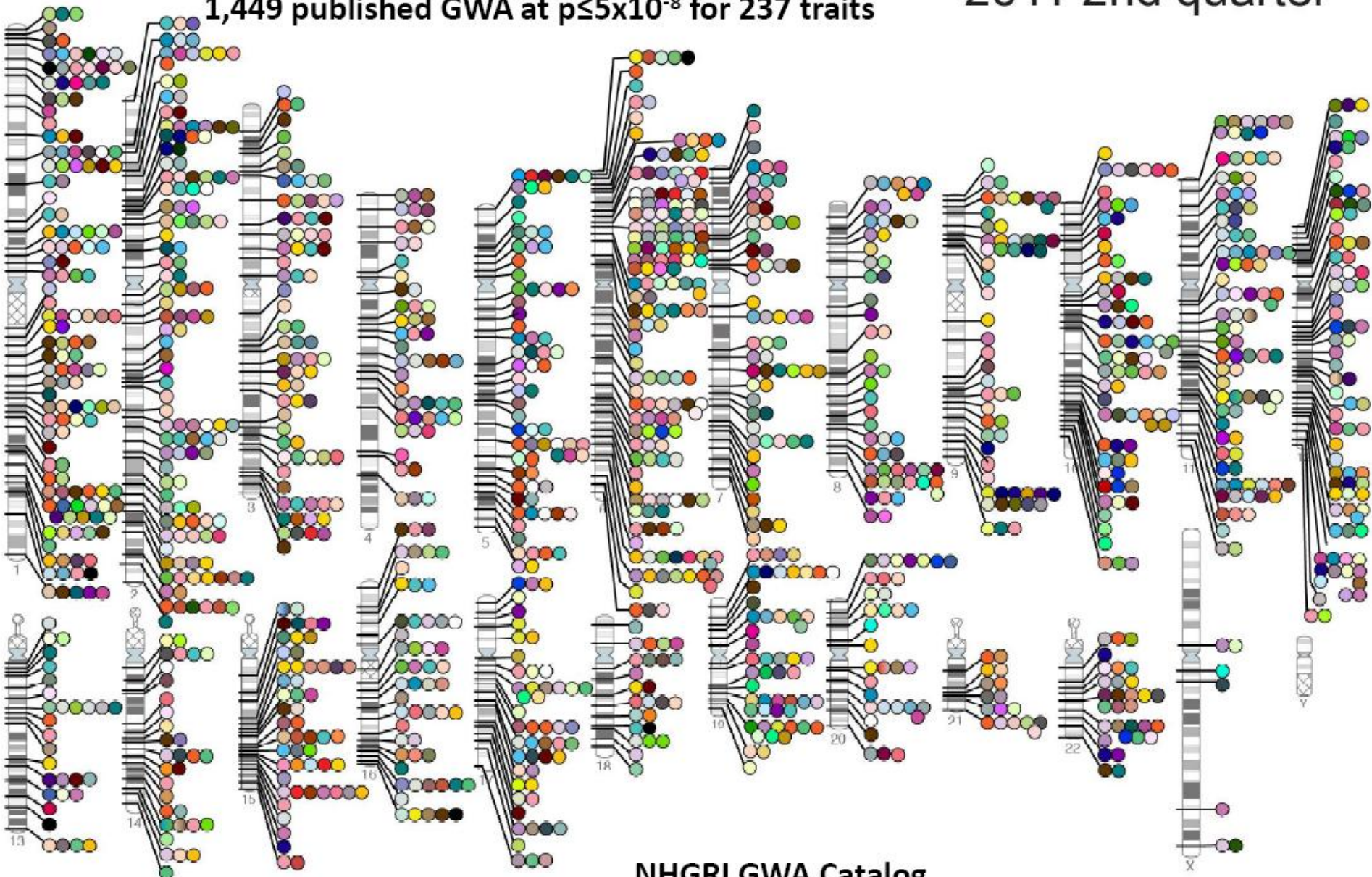
5. Current Research topics

1. Disease Risk – from GWAS to EWAS
2. Disease Prognosis – Predictive personalised medicine
3. Epigenetic drug development


Published Genome-Wide Associations through 06/2011,
1,449 published GWA at p≤5x10-8 for 237 traits
2011 2nd quarter
NHGRI GWA Catalog
www.genome.gov/GWAStudies


Abdominal aortic aneurysm
Acute lymphoblastic leukemia
Adhesion molecules
Adiponectin levels
Age-related macular degeneration
AIDS progression
Alcohol dependence
Alopecia areata
Alzheimer disease
Amyloid A levels
Amyotrophic lateral sclerosis
Angiotensin-converting enzyme activity
Ankylosing spondylitis
Arterial stiffness
Asparagus anosmia
Asthma
Atherosclerosis in HIV
Atrial fibrillation
Attention deficit hyperactivity disorder
Autism
Basal cell cancer
Behcet's disease
Bipolar disorder
Biliary atresia
Bilirubin
Bitter taste response
Birth weight
Bladder cancer
Bleomycin sensitivity
Blond or brown hair
Blood pressure
Blue or green eyes
BMI, waist circumference
Breast cancer
Calcium levels
Cardiac structure/function
Cardiovascular risk factors
Carnitine levels
Carotenoid/tocopherol levels
Celiac disease
Celiac disease and rheumatoid arthritis
Cerebral atrophy measures
Chronic lymphocytic leukemia
Chronic myeloid leukemia
Cleft lip/palate
Coffee consumption
Cognitive function
Conduct disorder
Colorectal cancer
Corneal thickness
Coronary disease
Creutzfeldt-Jakob disease
Crohn's disease
Crohn's disease and celiac disease
Cutaneous nevi
Cystic fibrosis severity
Dermatitis
DHEA-s levels
Diabetic retinopathy
Dilated cardiomyopathy
Drug-induced liver injury
Drug-induced liver injury (amoxicillin-clavulanate)
Endometrial cancer
Endometriosis
Eosinophil count
Eosinophilic esophagitis
Erectile dysfunction and prostate cancer treatment
Erythrocyte parameters
Esophageal cancer
Essential tremor
Exfoliation glaucoma
Eye color traits
F cell distribution
Fibrinogen levels
Folate pathway vitamins
Follicular lymphoma
Fuch's corneal dystrophy
Freckles and burning
Gallstones
Gastric cancer
Glioma
Glycemic traits
Hair color
Hair morphology
Handedness in dyslexia
HDL cholesterol
Heart failure
Heart rate
Height
Hemostasis parameters
Hepatic steatosis
Hepatitis
Hepatocellular carcinoma
Hirschsprung's disease
HIV-1 control
Hodgkin's lymphoma
Homocysteine levels
Hypospadias
Idiopathic pulmonary fibrosis
IFN-related cytopeni
IgA levels
IgE levels
Inflammatory bowel disease
Insulin-like growth factors
Intracranial aneurysm
Iris color
Iron status markers
Ischemic stroke
Juvenile idiopathic arthritis
Keloid
Kidney stones
LDL cholesterol
Leprosy
Leptin receptor levels
Liver enzymes
Longevity
LP (a) levels
LpPLA(2) activity and mass
Lung cancer
Magnesium levels
Major mood disorders
Malaria
Male pattern baldness
Mammographic density
Matrix metalloproteinase levels
MCP-1
Melanoma
Menarche & menopause
Meningococcal disease
Metabolic syndrome
Migraine
Moyamoya disease
Multiple sclerosis
Myeloproliferative neoplasms
Myopia (pathological)
N-glycan levels
Narcolepsy
Nasopharyngeal cancer
Natriuretic peptide levels
Neuroblastoma
Nicotine dependence
Obesity
Open angle glaucoma
Open personality
Optic disc parameters
Osteoarthritis
Osteoporosis
Otosclerosis
Other metabolic traits
Ovarian cancer
Pancreatic cancer
Pain
Paget's disease
Panic disorder
Parkinson's disease
Periodontitis
Peripheral arterial disease
Personality dimensions
Phosphatidylcholine levels
Phosphorus levels
Photic sneeze
Phytosterol levels
Platelet count
Polycystic ovary syndrome
Primary biliary cirrhosis
Primary sclerosing cholangitis
PR interval
Progranulin levels
Progressive supranuclear palsy
Prostate cancer
Protein levels
PSA levels
Psoriasis
Psoriatic arthritis
Pulmonary funct. COPD
QRS interval
QT interval
Quantitative traits
Recombination rate
Red vs.non-red hair
Refractive error
Renal cell carcinoma
Renal function
Response to antidepressants
Response to antipsychotic therapy
Response to carbamazepine
Response to clopidogrel therapy
Response to hepatitis C treat
Response to interferon beta therapy
Response to metaformin
Response to statin therapy
Restless legs syndrome
Retinal vascular caliber
Rheumatoid arthritis
Ribavirin-induced anemia
Schizophrenia
Serum metabolites
Skin pigmentation
Smoking behavior
Speech perception
Sphingolipid levels
Statin-induced myopathy
Stroke
Sudden cardiac arrest
Suicide attempts
Systemic lupus erythematosus
Systemic sclerosis
T-tau levels
Tau AB1-42 levels
Telomere length
Testicular germ cell tumor
Thyroid cancer
Thyroid volume
Tooth development
Total cholesterol
Triglycerides
Tuberculosis
Type 1 diabetes
Type 2 diabetes
Ulcerative colitis
Urate
Urinary albumin excretion
Urinary metabolites
Uterine fibroids
Venous thromboembolism
Ventricular conduction
Vertical cup-disc ratio
Vitamin B12 levels
Vitamin D insuffiency
Vitiligo
Warfarin dose
Weight
White cell count
White matter hyperintensity
YKL-40 levels

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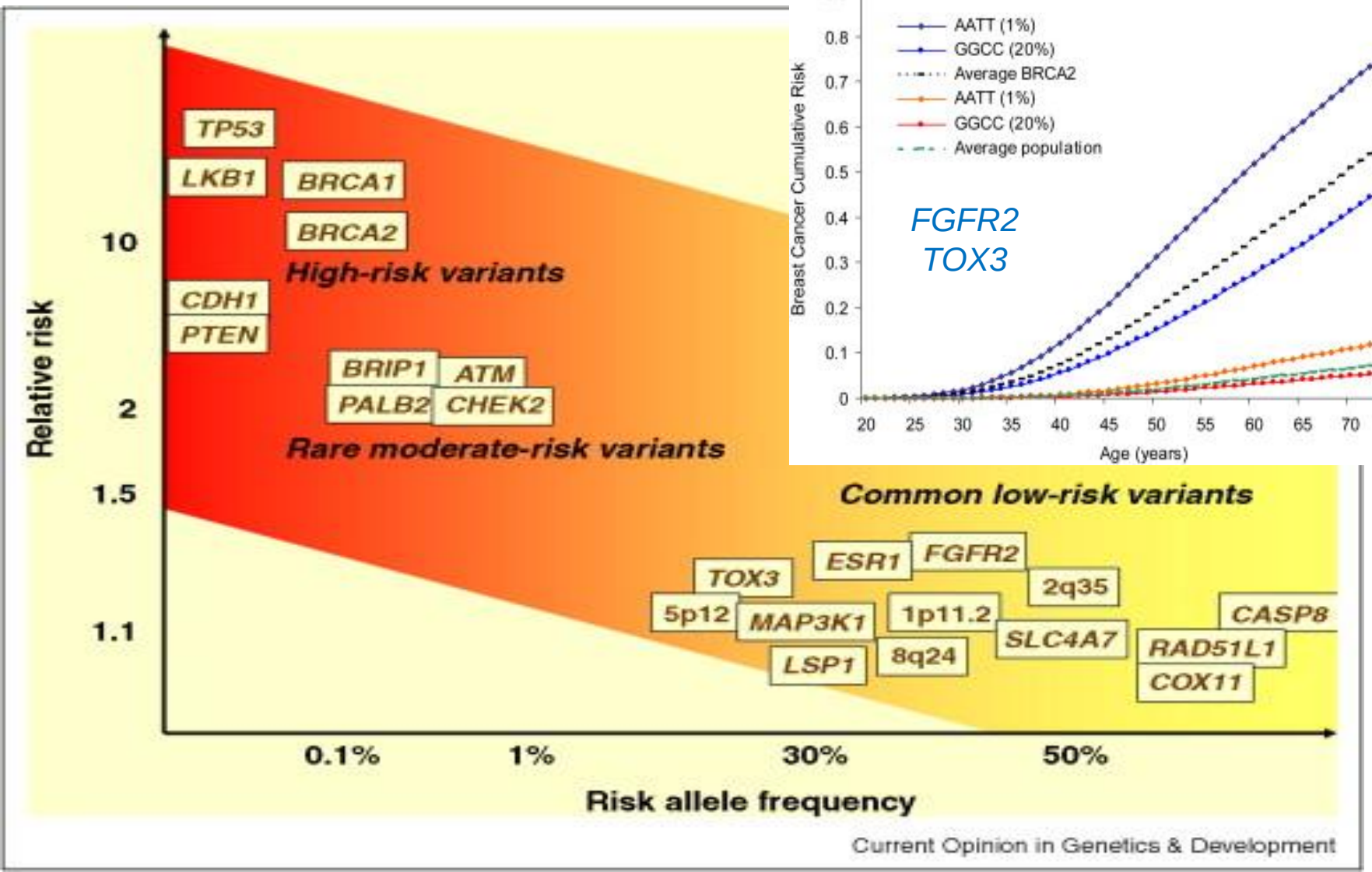
GWAS – Breast Cancer



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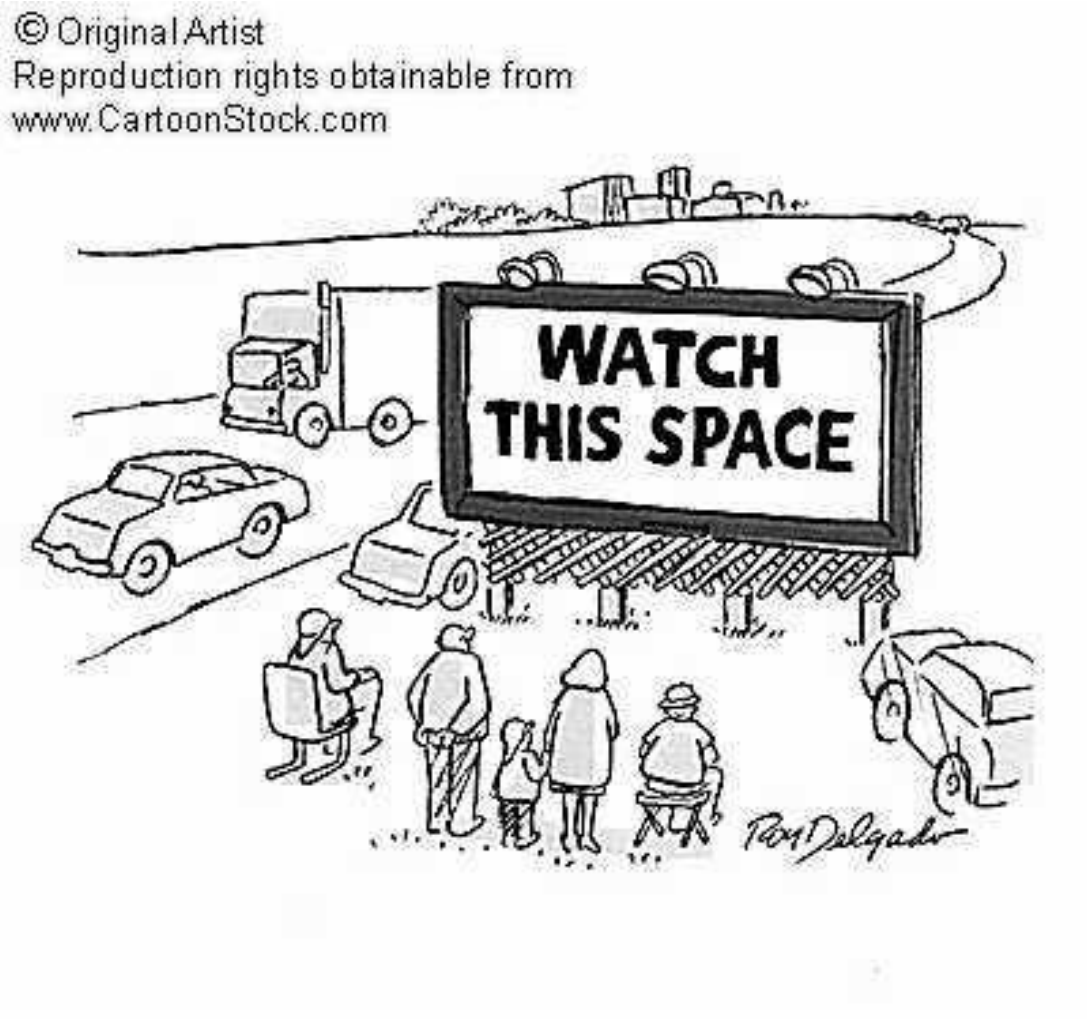
EWAS – Epigenome-wide Association Studies



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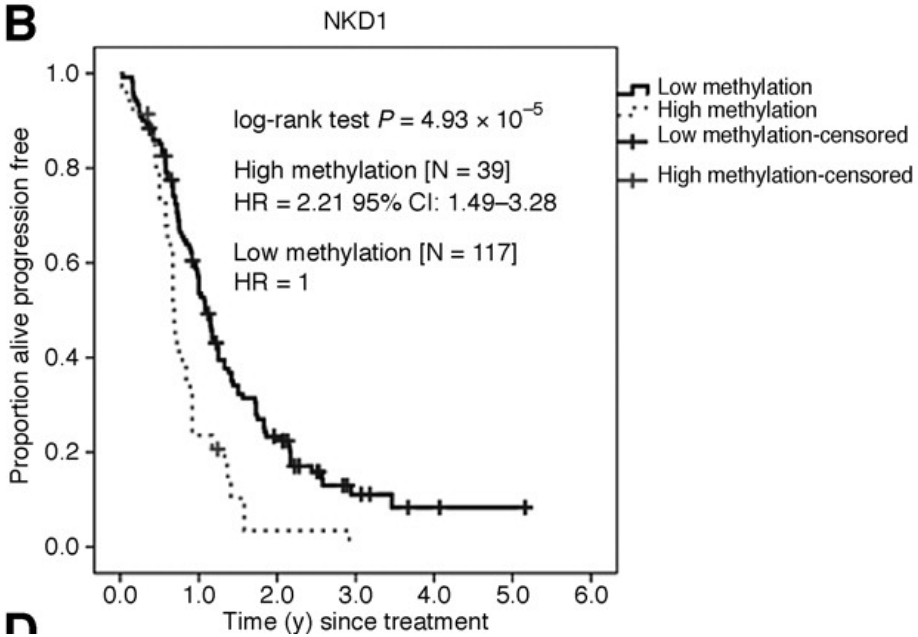
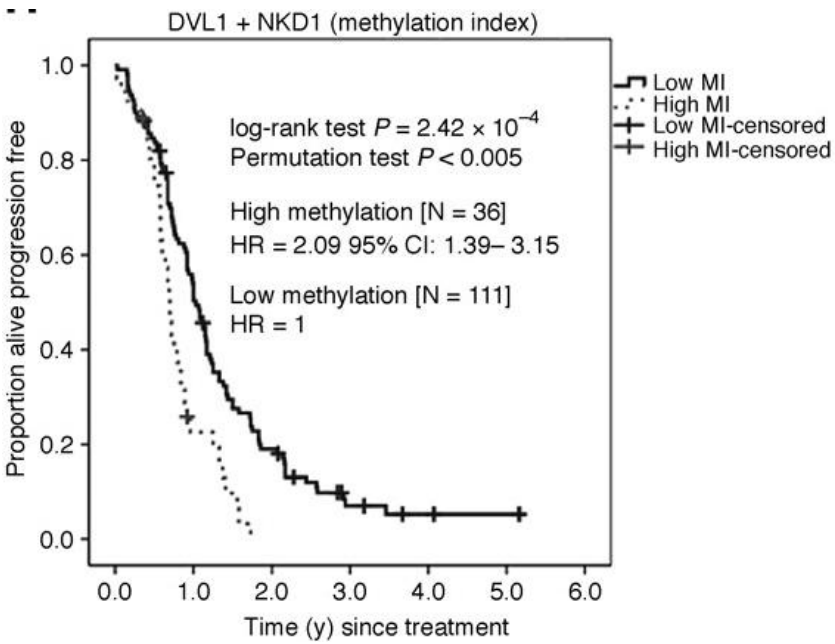
Disease Prognosis – Predictive Personalised Medicine





Hazard Ratio of 2.09, means patients with high methylation for both genes are twice as likely to have poorer prognosis:
Progression free survival (<1 year) vs (>1.5 years)

Dai et al, (2011) Clin Cancer Res. 17(12):4052-62.

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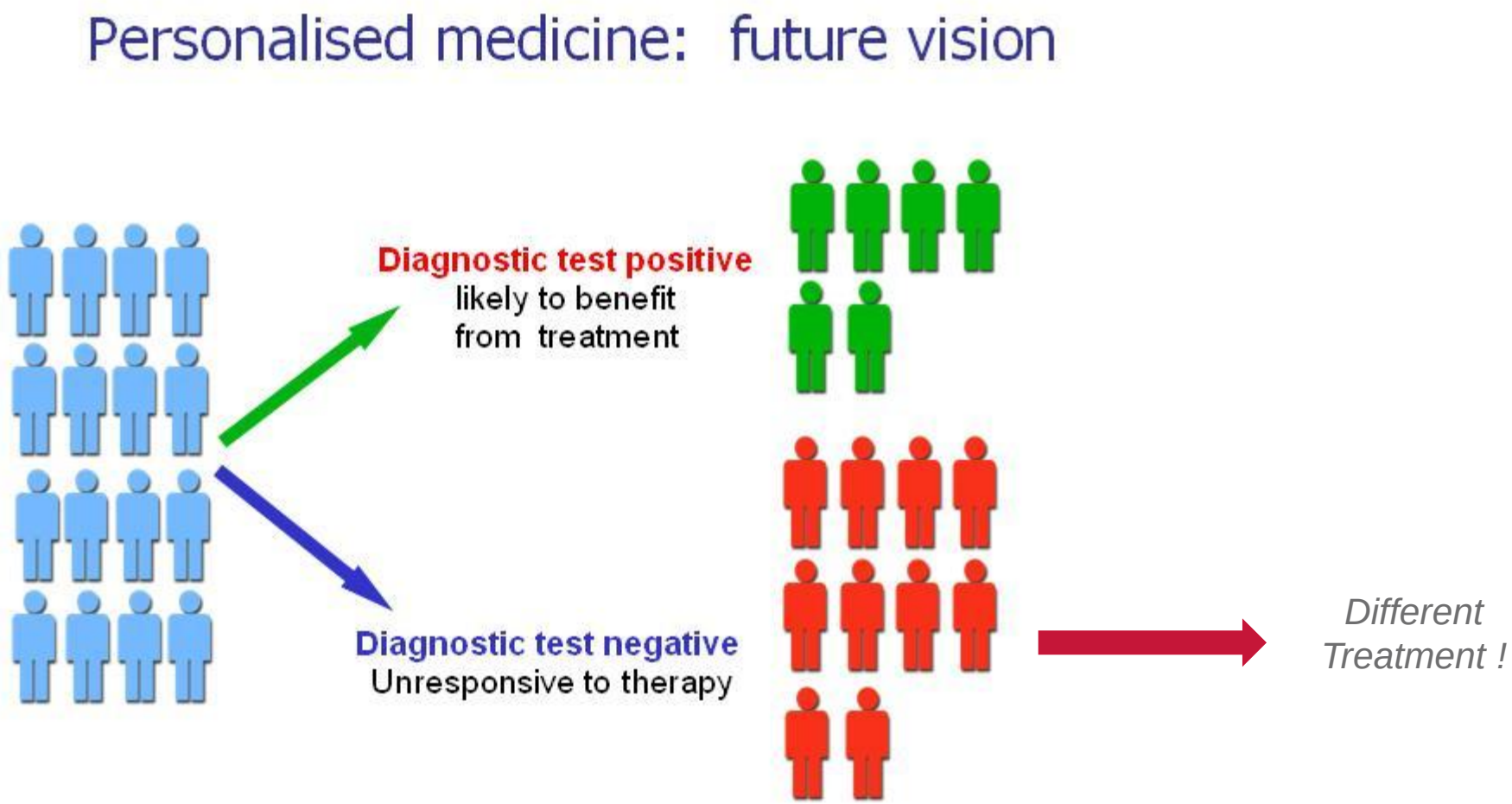
Personalised Medicine



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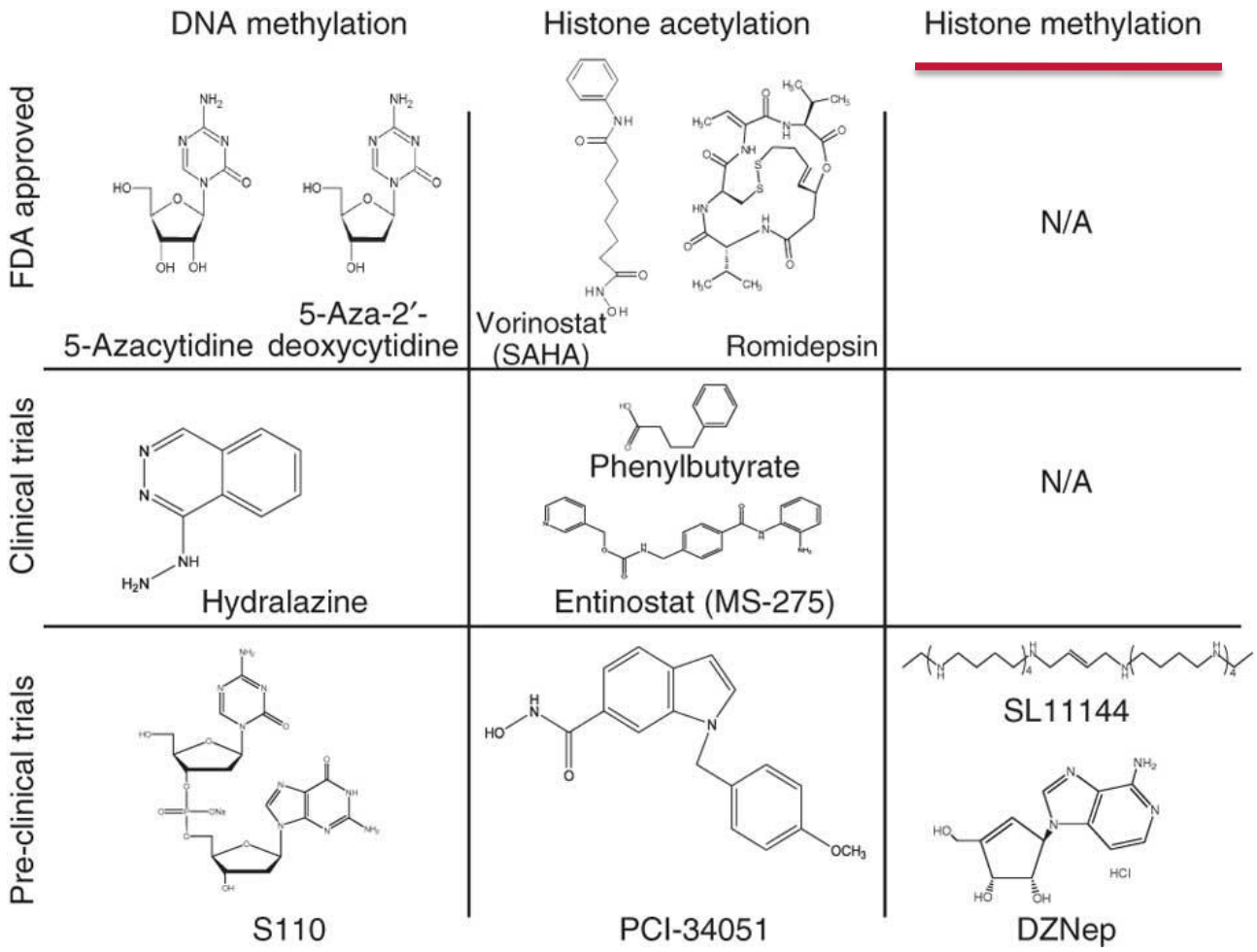
Epigenetic Drug Development



Kelly et al, Epigenetic modifications as therapeutic targets (2010) **Nature Biotechnology** 28, 1069–1078

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6. Relevance to Global Health

1. Epigenetic mechanisms as a mediator of Environmental factors

2. Epigenetic traits are reversible (as are environmental factors)

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Interactions - DNA methylation and cancer risk factors

Environmental sensitivity

- Prenatal environment
  - » Famine exposure, Folic acid use (Tobi et al HMG 2009,Steegers-Theunissen et al Plos One 2009)
- Adult methylome
  - Smoking, Diet (Breitling AMHG 2011, Zhang Journal of Nutrition 2010)
- Cancer methylome
  - Alcohol and folate (Christensen et al Plos genetics 2010)

Age sensitivity

- Some genes (360/27000) are associated with age (Rakyan et al Genome Res 2010)
- Methylation variability between monozygotic twins increases with age (Fraga et al PNAS 2005)

Genetic background

- Allele specific methylation (ASM) with underlying genetic variation (Meaburn et al Egenetics 2011)
- DNA methylation patterns are more similar in monozygotic than dizygotic twins (Kaminsky Nature genetics 2009)

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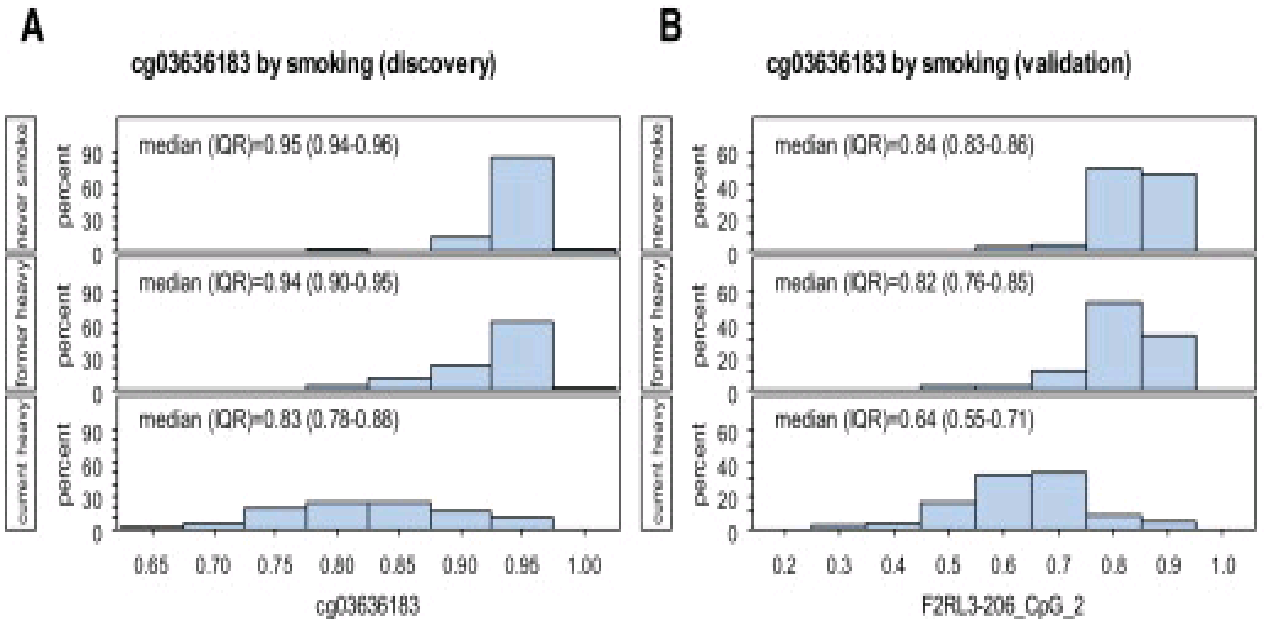
Smoking associated DNA methylation

Breitling et al AJHG April 2011

Never Smokers

Former Smokers

Current Smokers



1. *DNA methylation detected in blood DNA shows who is a smoker and who is not.*
2. *Epigenetic traits are reversible – look at the Former smokers.*

F2RL3 gene locus

27K array $P = 2.68 \times 10^{-31}$

EpiTyper $P = 6.33 \times 10^{-34}$

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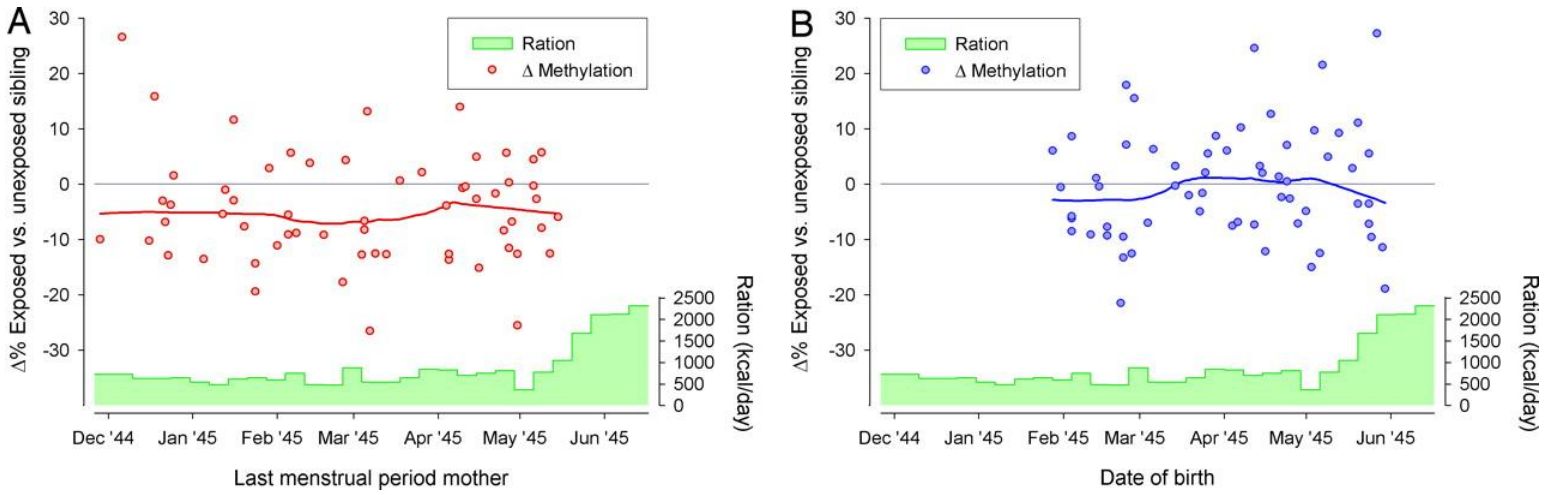
Dutch Famine

Heijmans BT, et al Persistent epigenetic differences associated with prenatal exposure to famine in humans. Proc Natl Acad Sci U S A. 2008;105(44):17046-9.



	No Folic Acid (n = 34)	Yes Folic Acid (n = 86)	*P*-value
Complete DMR	0.474 (0.007)	0.495 (0.004)	0.014

Steegers-Theunissen et al, PlosONE 2009

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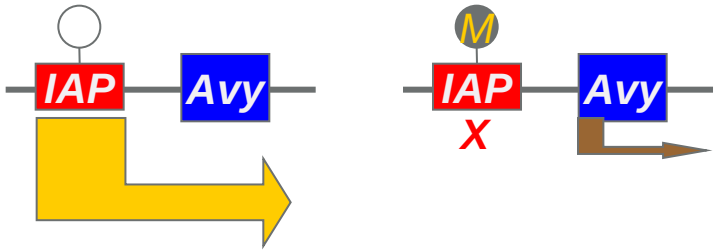
Obesity

Heritable transmission of epigenetic traits (eg Agouti mice)



Morgan et al Nature Genetics 23, 314 - 318 (1999)

J Physiol 587.20 (2009) pp 4963–4976

J Physiol Biochem, 65 (1), 1-10, 2009

Hypothalamic proopiomelanocortin promoter methylation becomes altered by early overfeeding: an epigenetic model of obesity and the metabolic syndrome

Andreas Plagemann[1], Thomas Harder[1], Matthias Brunn[1], Anja Harder[2], Katharina Roepke[1], Manon Wittrock-Staar[1], Thomas Ziska[1], Karen Schellong[1], Elke Rodekamp[1], Kerstin Melchior[1] and Joachim W. Dudenhausen[1]

Wang *et al. BMC Medicine* 2010, **8**:87
http://www.biomedcentral.com/1741-7015/8/87

BMC Medicine

RESEARCH ARTICLE Open Access

Obesity related methylation changes in DNA of peripheral blood leukocytes

Xiaoling Wang[1*], Haidong Zhu[1], Harold Snieder[1,5], Shaoyong Su[6], David Munn[2,4], Gregory Harshfield[1,3], Bernard L Maria[2,3,4], Yanbin Dong[1], Frank Treiber[1], Bernard Gutin[1], Huidong Shi[4]

High fat diet-induced obesity modifies the methylation pattern of leptin promoter in rats

F.I. Milagro*, J. Campión*, D.F. García-Díaz, E. Goyenechea, L. Paternain and J.A. Martínez

1. *Diet / Obesity have a direct impact on an individuals epigenome.*

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7. Recent Controversies

1. Recent controversies
   - Germline Epimutations.... IGF2 (Cui et al) ; MSH2 (Chan et al) ; MLH1 (Suter et al)

observed a very small fraction of hypermethylated *MLH1* sequences in DNA extracted from FACS-sorted spermatozoa. If these always occurs *de novo* in each generation[7]. If we did not know about the unstable CGG repeat, *FMR1* methylation might be mistaken

(2007).
6. Morgan, H.D., Sutherland, H.G., Martin, D.I. & Whitelaw, E. *Nat. Genet.* **23**, 314–318 (1999).
7. Malter, H.E. *et al. Nat. Genet.* **15**, 165–169 (1997).

Heritable germline epimutation is not the same as transgenerational epigenetic inheritance

To the Editor:

'Heritable germline epimutati
atypical epigenetic state that oc
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In the absence of this, there is always an alternative explanation for any finding of a heritable germline epimutation. An underlying genetic change could direct the re-establishment of an atypical epigenetic state each generation; that is, a DNA variant could predispose in *cis* to methylation. Although Chan and colleagues

Suter and Martin reply:

We agree[1] that the findings of Chan and co-workers[2] are likely to result from a germline genetic aberration that predisposes to somatic epimutation in *cis*. But we disagree with the criticisms of work on the *MLH1* germline epimutations[3,4]. Whitelaw and co-workers[5] conflate a variety of different phenomena; Hortshemke[6] dismisses the evidence that the *MLH1* epimutation is present in germline cells.

Nature Genetics 39, 576 (2007)

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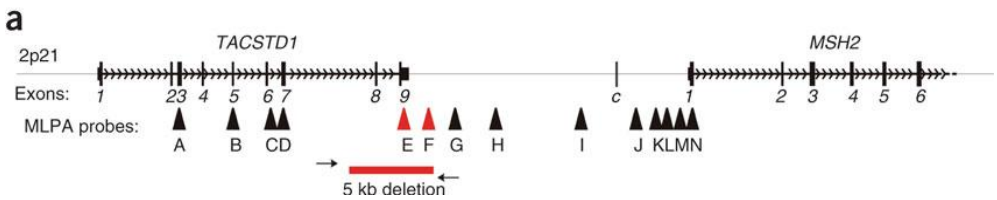
Genetic mutations define Epimutations

nature genetics

Heritable somatic methylation and inactivation of *MSH2* in families with Lynch syndrome due to deletion of the 3′ exons of *TACSTD1*

Marjolijn J L Ligtenberg[1,2,6], Roland P Kuiper[1,6], Tsun Leung Chan[3,4,6], Monique Goossens[2], Konnie M Hebeda[2], Marsha Voorendt[1], Tracy Y H Lee[3], Danielle Bodmer[1], Eveline Hoenselaar[1], Sandra J B Hendriks-Cornelissen[2], Wai Yin Tsui[3], Chi Kwan Kong[5], Han G Brunner[1], Ad Geurts van Kessel[1], Siu Tsan Yuen[3,4], J Han J M van Krieken[2], Suet Yi Leung[3,4] & Nicoline Hoogerbrugge[1]

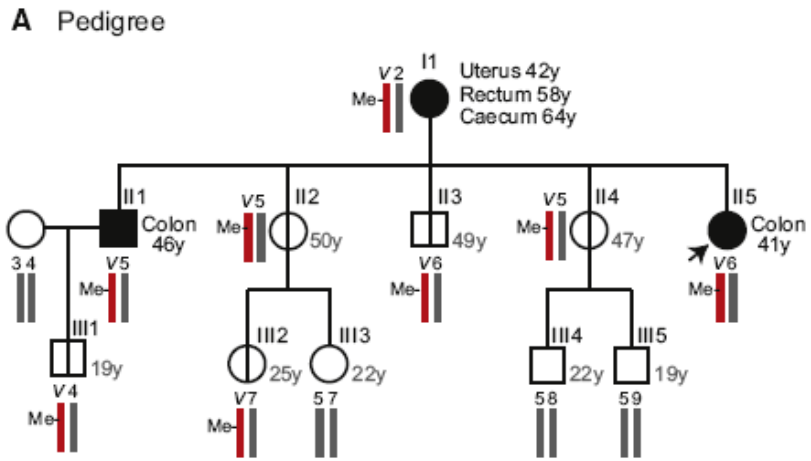


Cell PRESS

Cancer Cell
Article

Dominantly Inherited Constitutional Epigenetic Silencing of *MLH1* in a Cancer-Affected Family Is Linked to a Single Nucleotide Variant within the 5′UTR

Megan P. Hitchins,[1] Robert W. Rapkins,[1] Chau-To Kwok,[1] Sameer Srivastava,[1] Justin J.L. Wong,[1] Levon M. Khachigian,[2] Patsie Polly,[3] Jack Goldblatt,[4] and Robyn L. Ward[1,*]



Ligtenberg et al, Nat Genet

Hitchins et al; Cancer Cell. 2011 Aug 16;20(2):200-13.

Another Quick Summary

- Best method to detect DNA methylation uses Sodium Bisulfite conversion and quantitation (eg Pyrosequencing)
- Best method to detect Histone modifications is ChIP – seq

- In Cancer – lots of things go wrong with the Epigenetic patterns
  - Lots of cancer risk factors cause Epigenetic modifications

- Epigenetics has a role in lots of Research Areas (Risk, Personalised medicine, drug development)

- Epigenetics plays an important part in Global Health
  - Environmental factors
  - Smoking
  - Obesity

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8. Further Reading / Websites

1: Heijmans BT, Tobi EW, Stein AD, Putter H, Blauw GJ, Susser ES, Slagboom PE,Lumey LH. Persistent epigenetic differences associated with prenatal exposure to famine in humans. Proc Natl Acad Sci U S A. 2008 Nov 4;105(44):17046-9.

2: Santos-Rosa H, Caldas C. Chromatin modifier enzymes, the histone code and cancer. Eur J Cancer. 2005 Nov;41(16):2381-402. Epub 2005 Oct 13. Review.

3: McCabe MT, Brandes JC, Vertino PM. Cancer DNA methylation: molecular mechanisms and clinical implications. Clin Cancer Res. 2009 Jun 15;15(12):3927-37.

4: Suter CM, Martin DI, Ward RL. Germline epimutation of MLH1 in individuals with multiple cancers. Nat Genet. 2004 May;36(5):497-501.

And other papers mentioned on slides!

Wikipedia

Google

Pubmed

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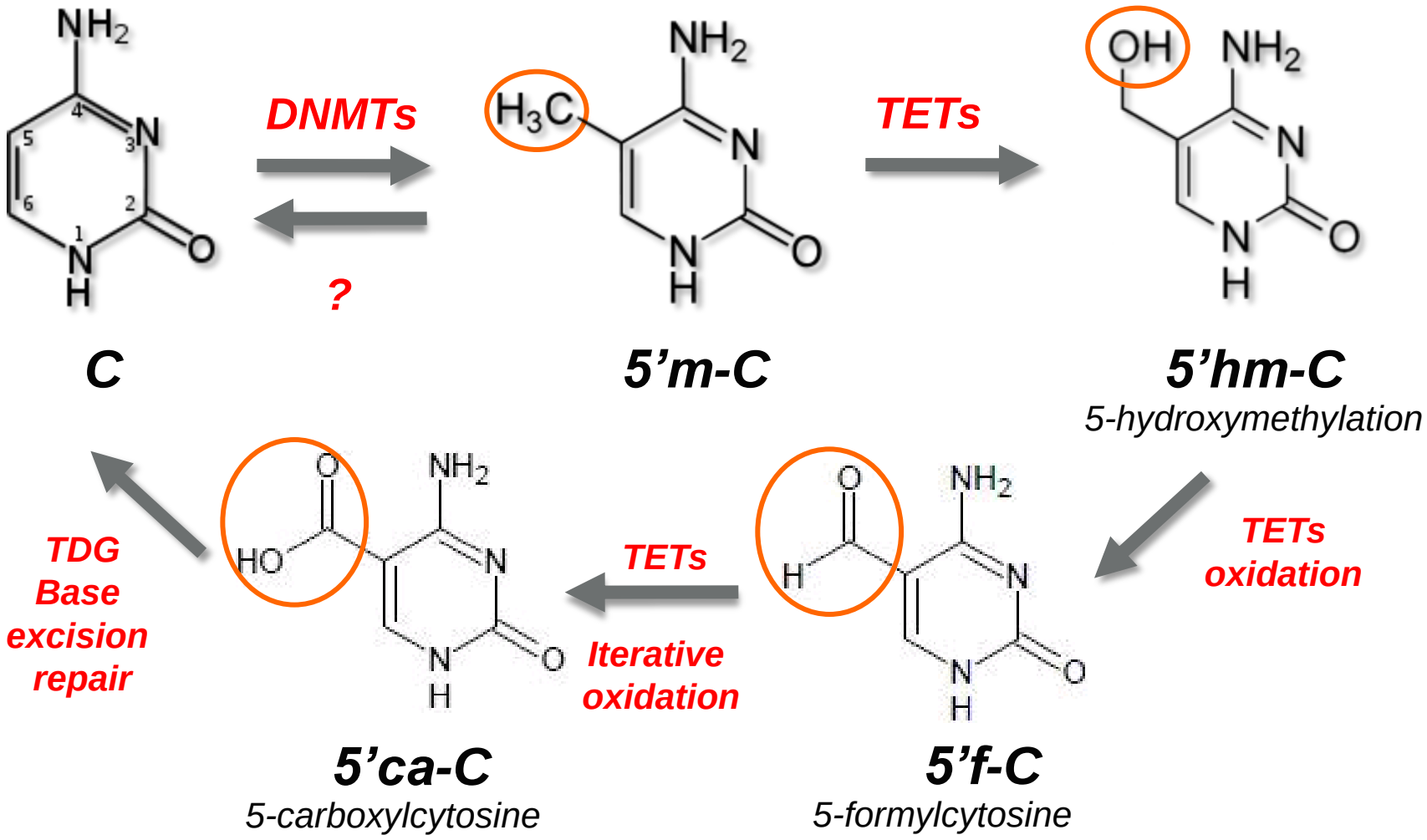
DNA Demethylation demystified ! Sept 2011



Ito et al Science Sept 2011; He et al Science Sept 2011; Maiti et al JBC Oct 2011

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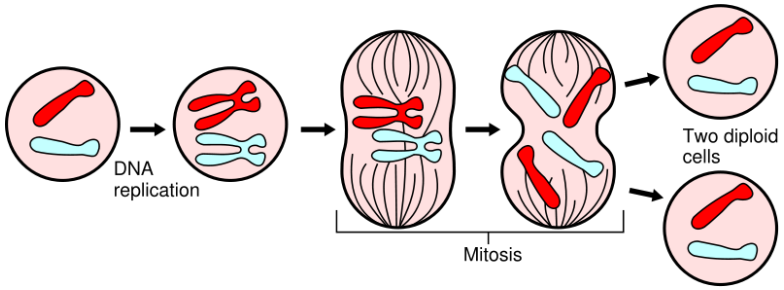
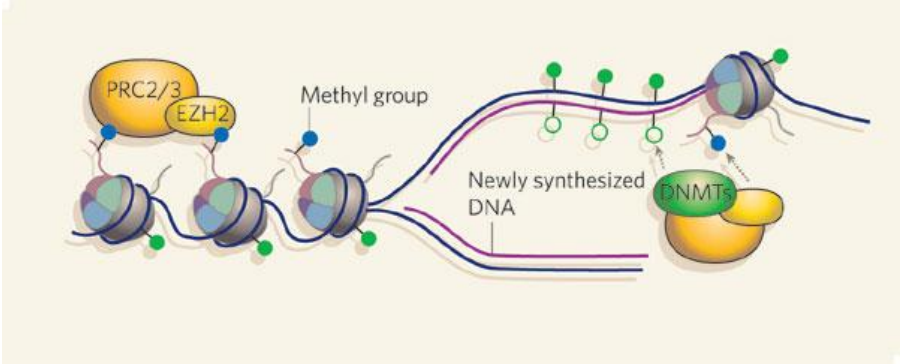
Meiotic heritability vs Mitotic heritability

Mitosis – DNA methylation is copied





Meiosis – DNA methylation is reprogrammed