

# Epigenomics

*Mechanisms and Analysis in Human Disease*

**Dr. Christopher G. Bell**

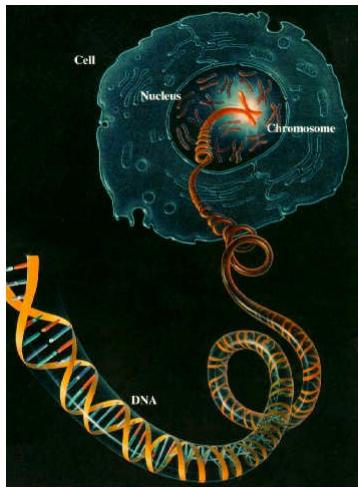
Lecturer in Epigenomics

MRC Lifecourse Epidemiology Unit, Institute of Developmental Sciences, & Biological Sciences, University of Southampton

## Outline

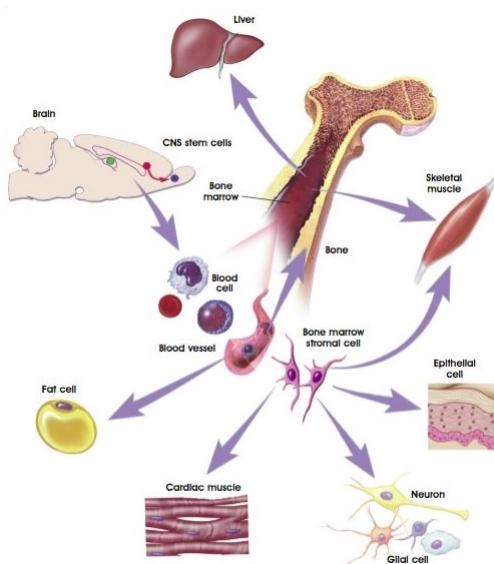
- What is Epigenetics?
- What is Epigenomics
- Chromatin
- DNA Modifications
- Imprinting & X inactivation
- Why & How do we analyses the Epigenome

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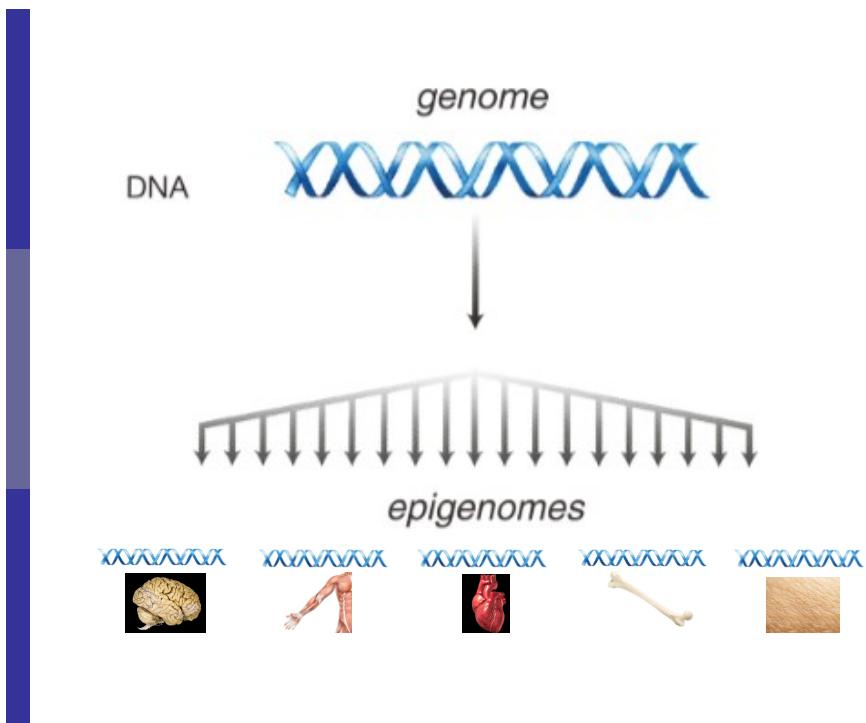
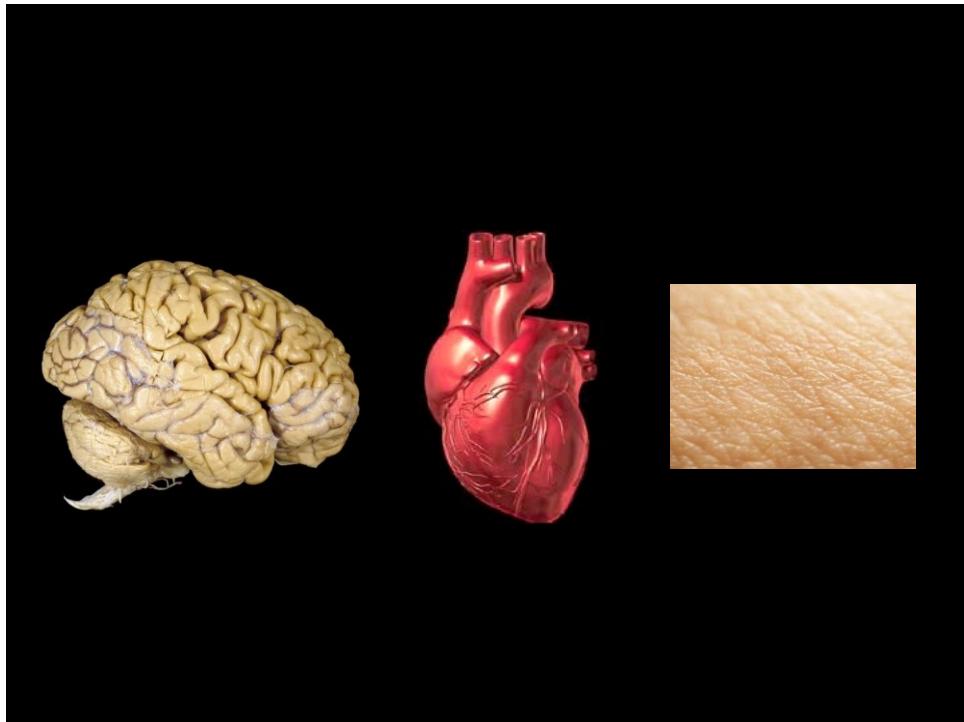
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- All cells
- ~ Same Genome



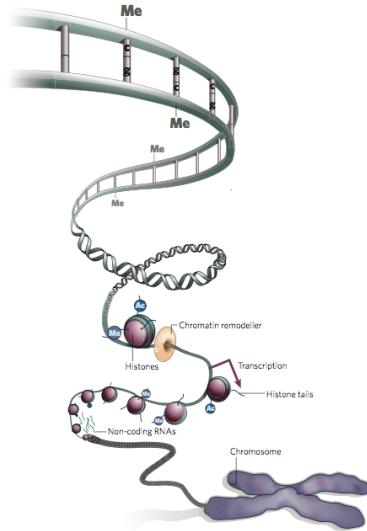
- Multiple Cell Types

stemcells.nih.gov



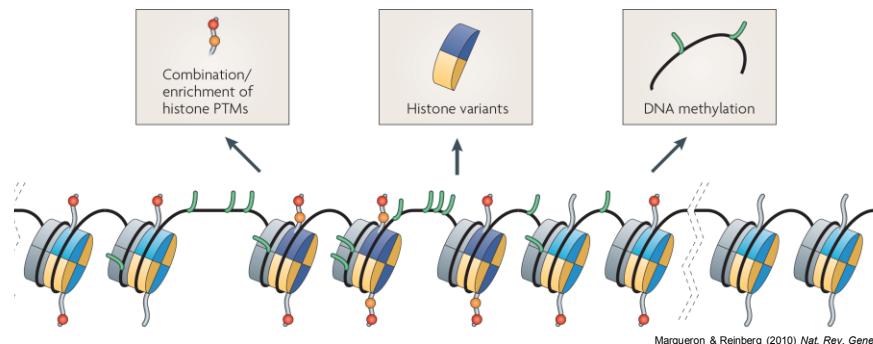
## Epigenetic Mechanisms

- Packaging &
- Chemical Modifications of DNA
  - ⇒ Influences or Indicates Gene Expression
  - ⇒ Enables or Indicates Cell & Organ-Specific Activity



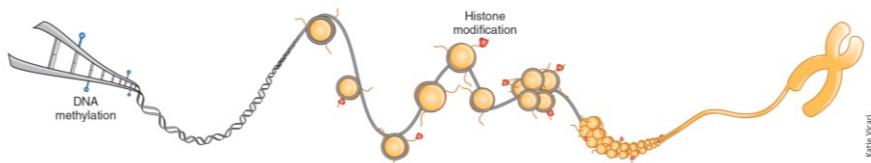
## Epigenetic Mechanisms

1. Chemical modifications of DNA
2. Post Translation Modifications of Histone Tails
3. Histone Variants



## Epigenetics Definition

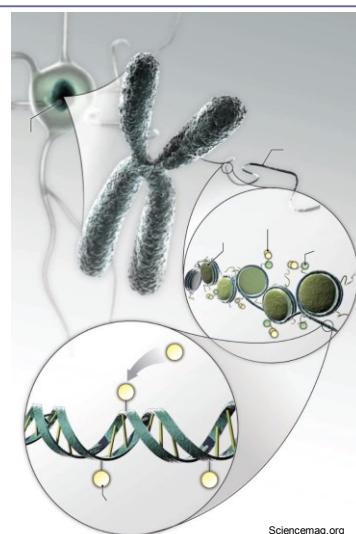
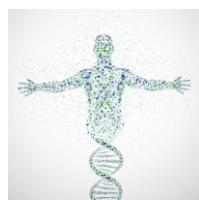
- “Stable heritable information transfer that does NOT require Mutagenic Change of the underlying nucleotide sequence”



Baker (2010) *Nature Methods*

## The Epigenome

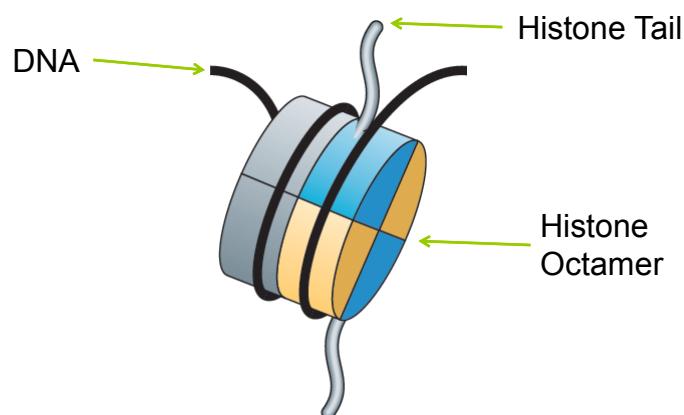
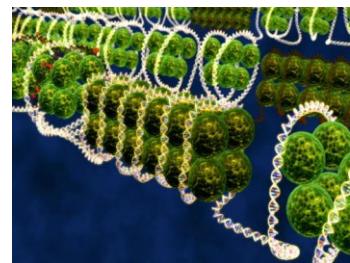
- The Genome-Wide Epigenetic State
- All of the Epigenetic Modifications within the Cell’s Genome



Scienmag.org

## Chromatin

- Packaging Mechanism of Genome
  - Influences Activation
  - & Repression of Loci
- Chromatin
  - Complex of DNA & Histone Protein in Chromosomes
- Basic Structural Unit = **Nucleosome**

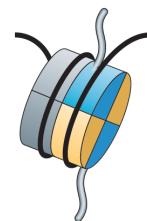


Nucleosome

Margueron & Reinberg (2010) *Nat. Rev. Genet.*

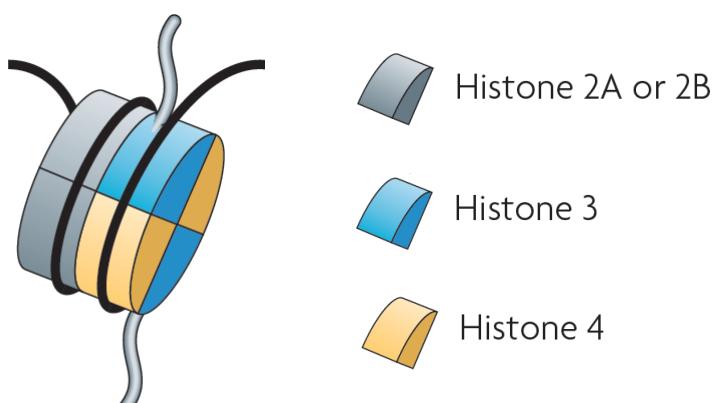
## Nucleosome

- Nucleosome core particle
  - Consists of ~147 base pairs of DNA
    - Wraps 1.67 left-handed superhelical turns
  - Around a Histone Core Octamer
    - Histone =
      - Small highly conserved basic protein
      - Comprise 102–135 amino acids



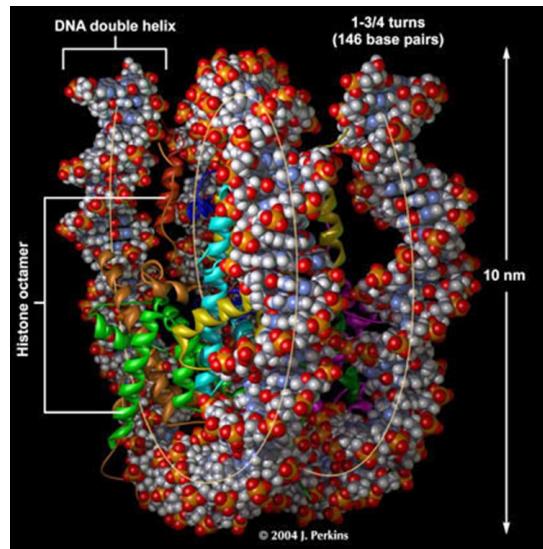
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## Nucleosome – Core Octomer



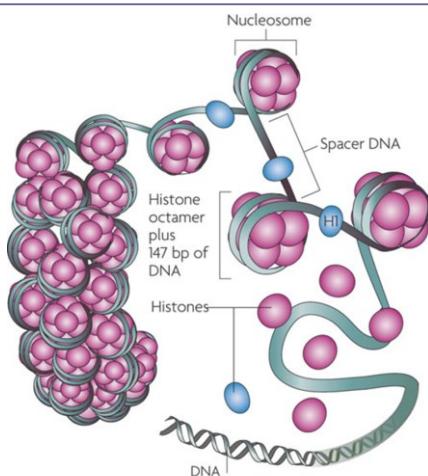
Histone Octamer  
 = 2 copies each of the core histones H2A, H2B, H3, & H4  
 = 2 H2A-H2B dimers and a H3-H4 tetramer

Margueron & Reinberg (2010) *Nat. Rev. Genet.*

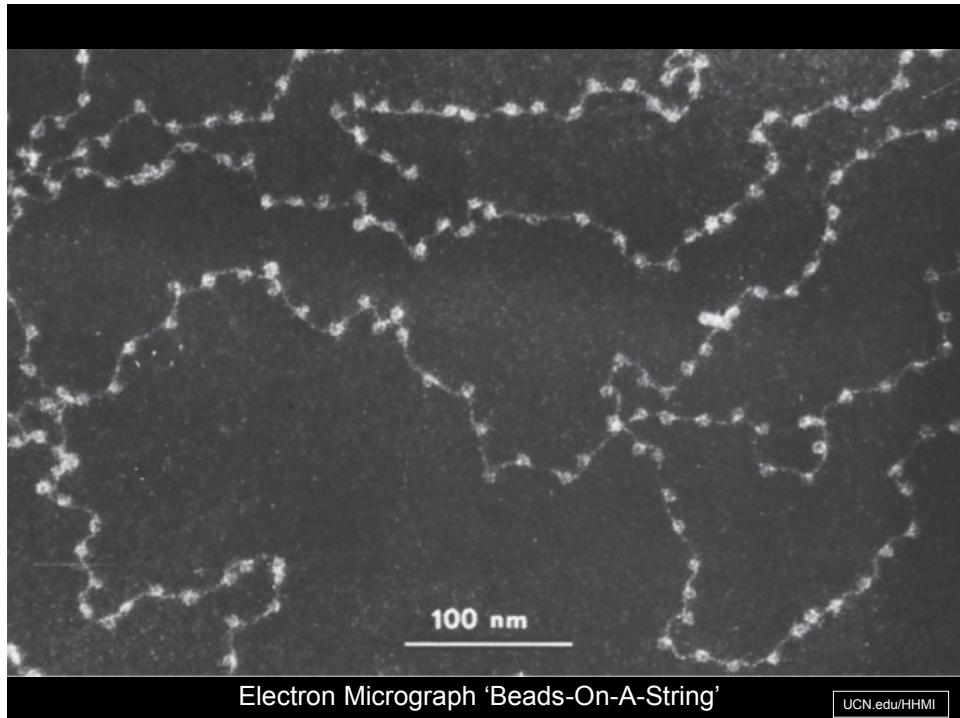


Nucleosome

### 'Beads-on-a String'

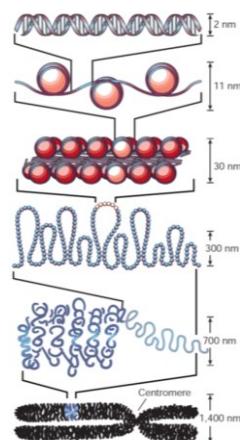


Figueiredo et al. (2009) Nat Rev Micro



## Multiple Hierarchies of DNA folding

- Interphase Chromosome
  - Consist chromatin fibers, organized into long loops
  - Loops of 30 nm chromatin fibre
    - Containing 20–100 kb of DNA per loop
    - Attached to a central scaffold

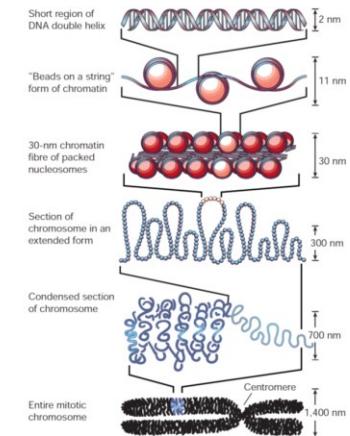


Felsenfeld & Groudine (2003) *Nature*

## Multiple Hierarchies of DNA folding

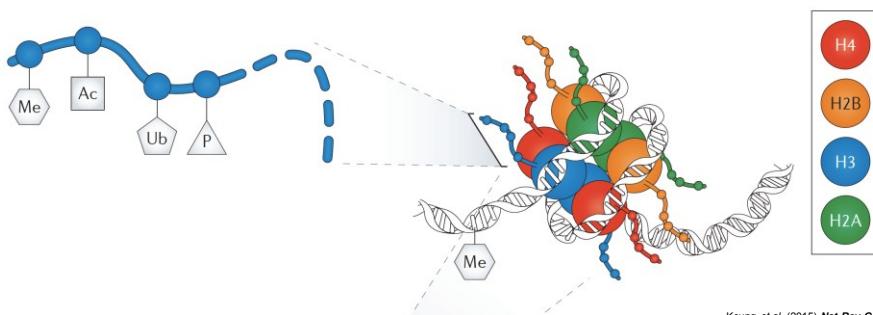
- During cell division
  - Chromosomes ↑condense

 Metaphase chromosome



Felsenfeld & Groudine (2003) *Nature*

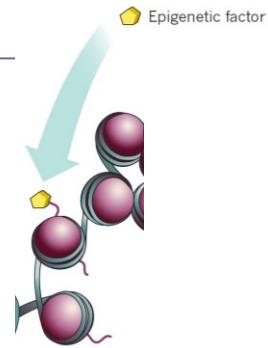
## Histone Tail Modifications



Keung et al. (2015) *Nat Rev Genet*

Histone Tail Modifications form Histone Code

## Histone Code

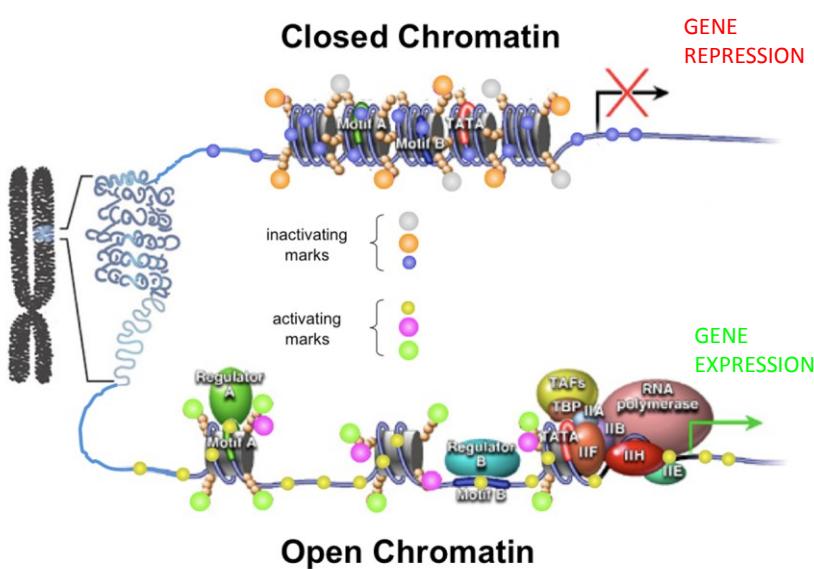


### ▫ Histone Tails

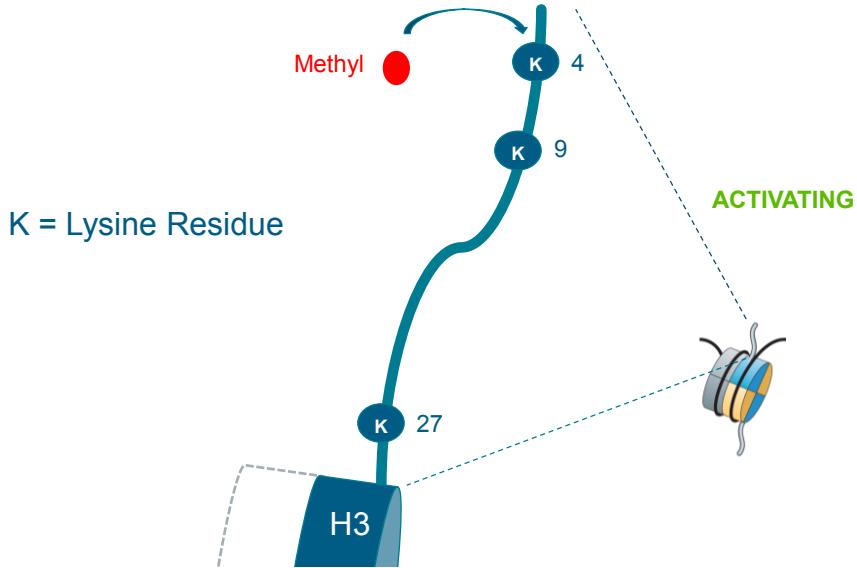
- Post-Translational Modifications
- Influence Gene Expression
  - Activating Marks
  - Repressive Marks

### ▫ Modifications

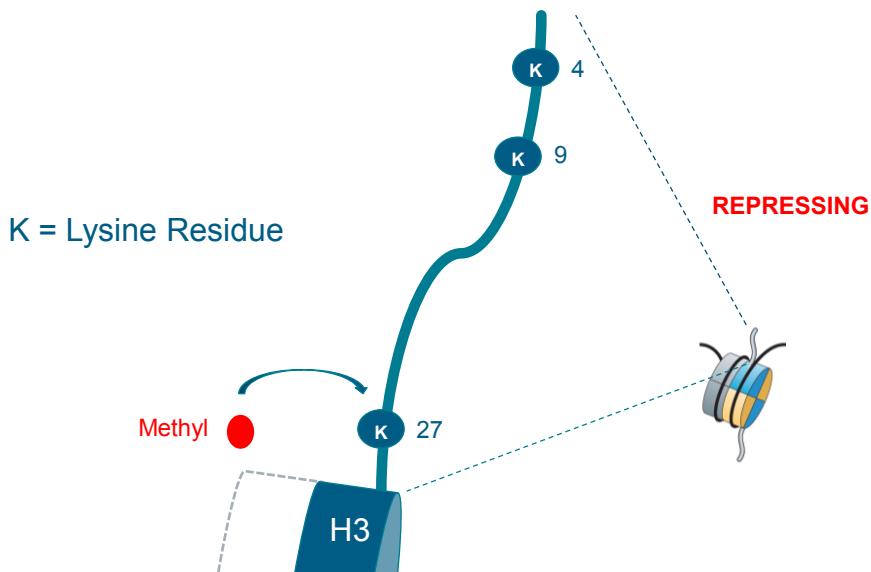
- Create or Prevent binding of
  - Specific Chromatin Remodelling Factors
- Influence Nucleosome mobility & function
- Scaffold for the recruitment of regulatory proteins



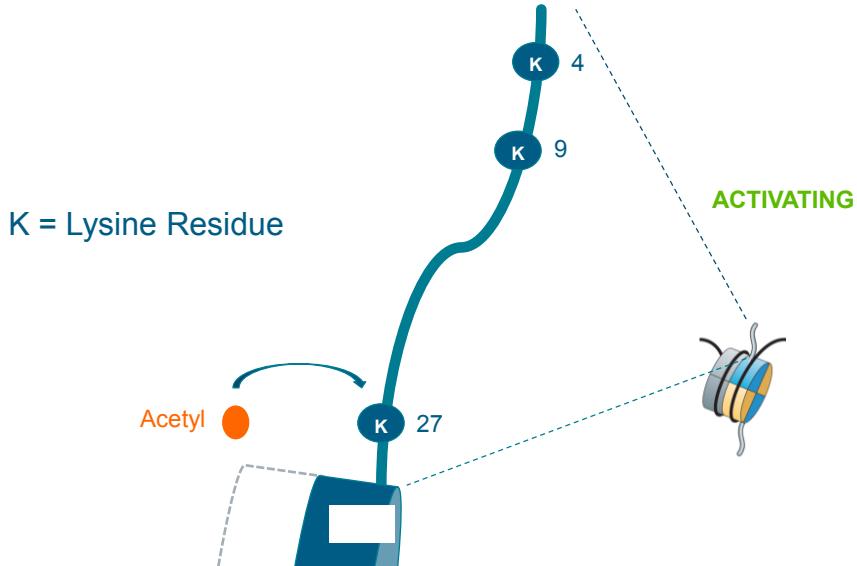
## Histone Tail Modifications



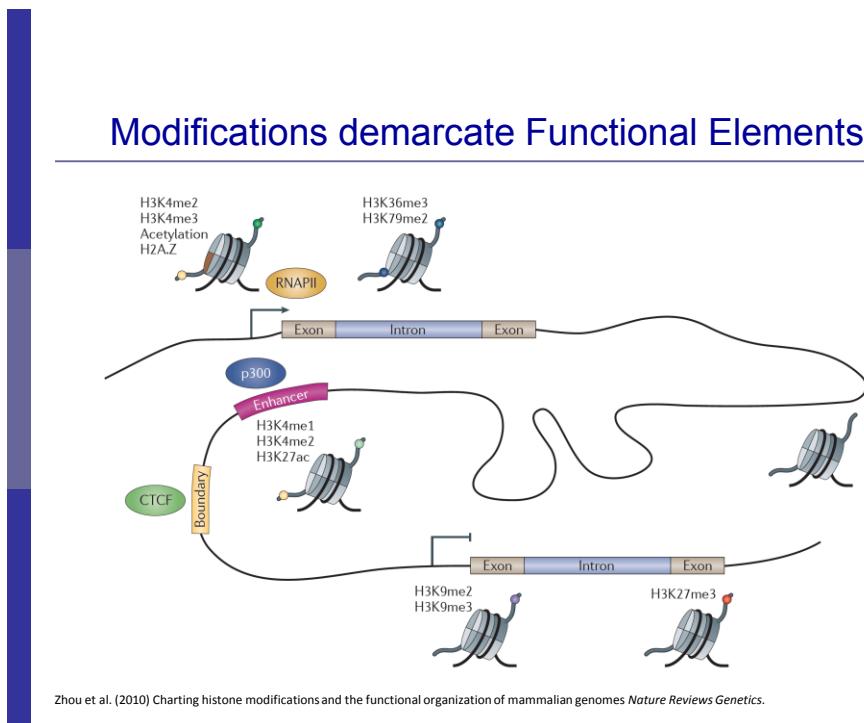
## Histone Tail Modifications



## Histone Tail Modifications



## Modifications demarcate Functional Elements

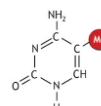
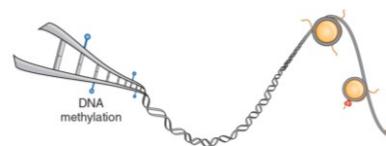


Zhou et al. (2010) Charting histone modifications and the functional organization of mammalian genomes *Nature Reviews Genetics*.

## Modifications of DNA

- DNA methylation

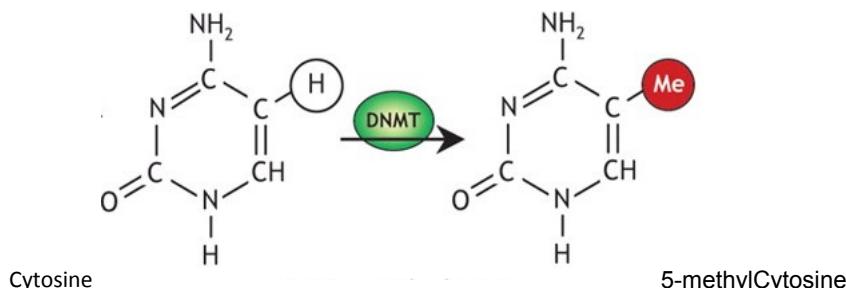
- Methylcytosine (5mC)
  - Addition of Methyl group onto 5 Carbon of Cytosine
    - Highly Stable Mark
    - Most Common
    - Most Studied



- Additional Rarer DNA Modifications

- Hydroxymethylcytosine(5hmC)
- Formylcytosine (5fC)
- Carboxylcytosine (5cC)

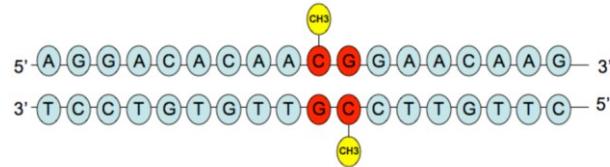
## DNA methylation



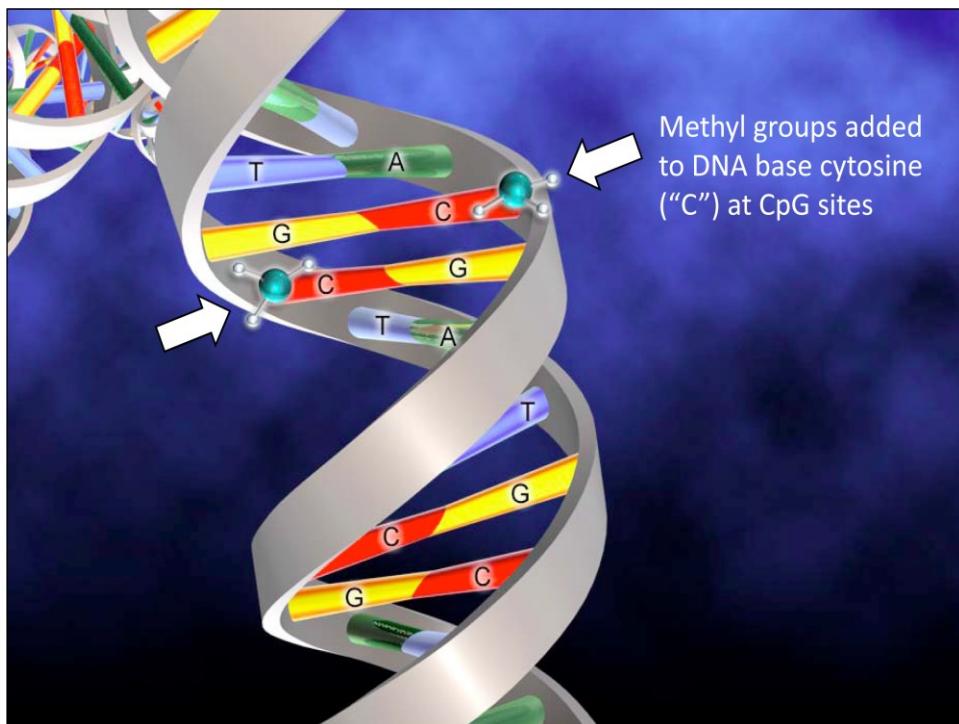
DNA Methyltransferase (DNMT) Enzyme

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## DNA Methylation

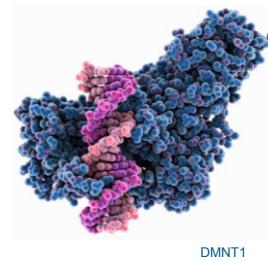


- Occurs usually at a Cytosine followed by Guanine base
- Palindromic Motif
  - C then G from 5' to 3' on both strands
  - = CpG dinucleotide

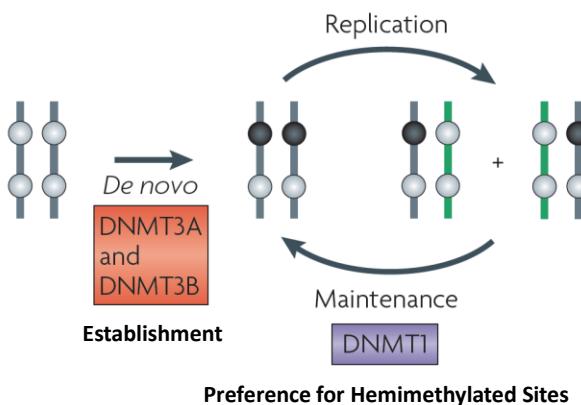


## DNA Methyltransferase

- Enzyme that catalyzes addition of Methyl group to DNA
- DNMT1
  - Maintenance Enzyme
  - Recognises hemimethylated DNA
  - Post replication
- DNMT3 (A & B)
  - *De Novo* Enzyme
  - Establishes Methylation

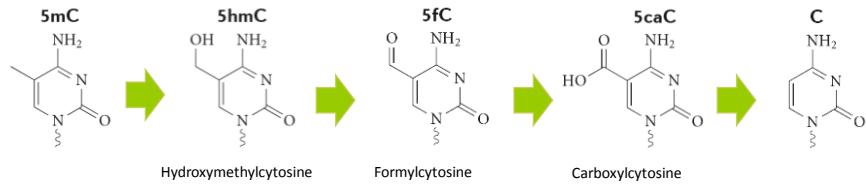


## Establishment & Inheritance of DNA methylation



Jones & Liang (2009) *Nature Reviews Genetics*

## Potential Pathways of DNA demethylation



- Active Removal
  - Oxidised to 5hmC (TET Enzyme)
  - 5hmC further oxidised to 5fC & 5caC
  - Base Excision Repair Machinery → Unmodified Cytosine
    - Deformation Or TDG cleaves
- Passive Removal
  - Hemimethylated DNA is not methylated by DNMT1 during replication

Branco & Reik (2012) *Nature Reviews Genetics*

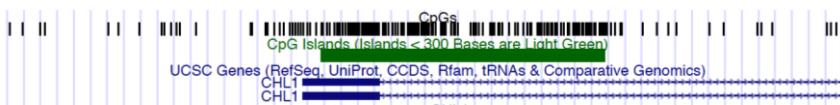
## CpG Genome Distribution

- Non-random distribution of CpGs
  - Majority are sparse and methylated
- Clusters of CpGs (only ~7% of total)

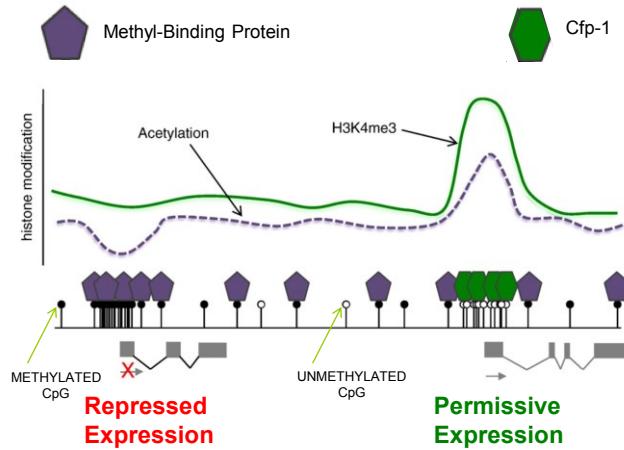
### CpG Islands

Located at the Promoter region of majority of genes

- Typically Unmethylated



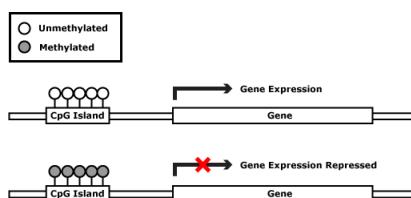
## CpG Dinucleotide Signalling Molecule



Bird (2011) The Dinucleotide CG as a Genomic Signalling Module. *J. Mol. Biol.*

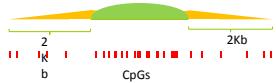
## DNA methylation at CpG Islands

- Represses Gene Expression
- Involved in
  - Cell-Specific Differences in Transcription
  - X-inactivation
  - Genomic imprinting
  - Improper Gene silencing in Cancer cells



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## CpG Island ‘Shores’

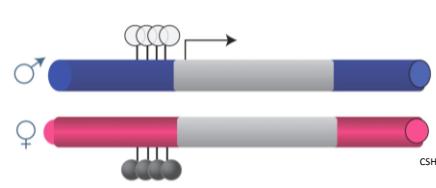
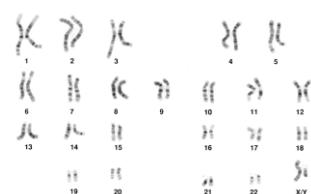


- CpG island ‘shores’ – Dynamic and ↑ Function
  - Tissue-specific (Izirary et al.)
  - Cancer-specific
  - Reprogramming-specific (Doi et al.)
  - Species-specific (Wilson et al.)
    - Inter-Primate comparison

Izirary et al. (2009) *Nat Genet*  
 Doi et al. (2009) *Nat Genet*.  
 Wilson et al. (2014) *Genome Med*

## Imprinting

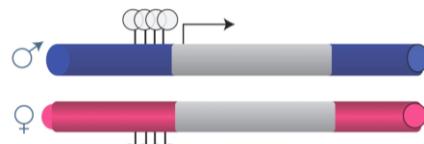
- Inherit 2 copies of each gene
  - One Maternal
  - One Paternal
- Only one expressed
  - Specific to parent-of-origin
- Other silenced by DNA methylation.
  - Parent-of-Origin Specific DNA Methylation



## Imprinting

- By convention:

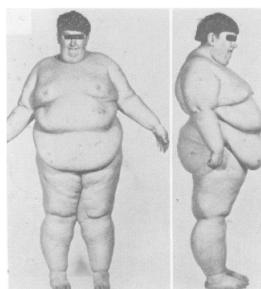
- Imprinting refers to the Gene that is Silenced NOT the Gene Expressed
- ∴ Where the precise DNA methylation occurs



Maternally Imprinted Gene

## Imprinting Disorders

- Prader Willi Syndrome
  - Maternal Imprinted Gene Disorder



- Angelman Syndrome
  - Paternal Imprinted Gene Disorder

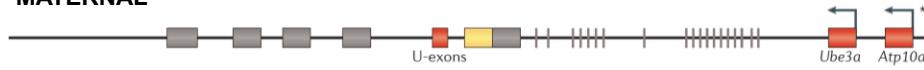


Both in Same Genetic Locus on Chromosome 15  
with Complex Maternal and Paternal Imprinted Genes

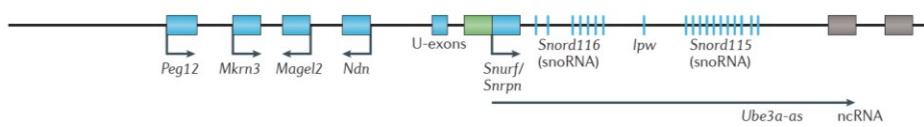
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## Imprinted Locus Chr15 *Snrpn* Cluster

### MATERNAL



### PATERNAL



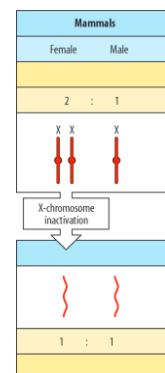
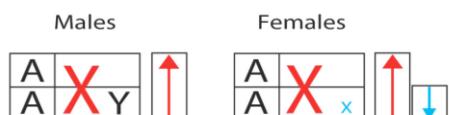
■ Maternally expressed gene    ■ Paternally expressed gene    ■ Repressed gene    ■ Unmethylated ICR (active)    ■ Methylated ICR (inactive)

Peters (2014) Nat Rev Genet

## X Inactivation

### Epigenetic Dosage Compensation Mechanism

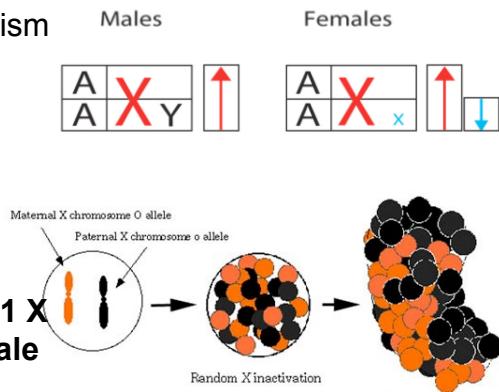
- As Females have 2 X Chromosomes
- Males only 1
- Random Switch-Off



### Hypermethylation of 1 X chromosome in Female

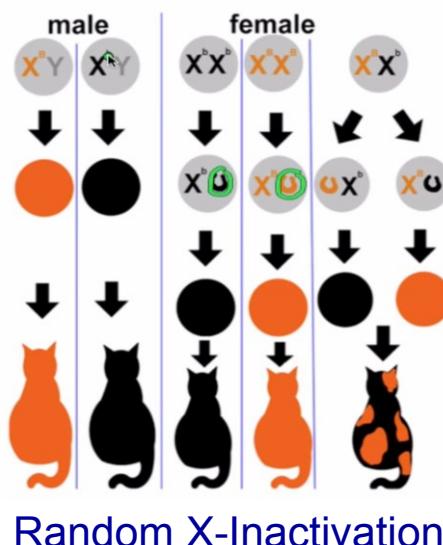
## X Inactivation

- Epigenetic Dosage Compensation Mechanism
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  - Males only 1
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**Hypermethylation of 1 X chromosome in Female**

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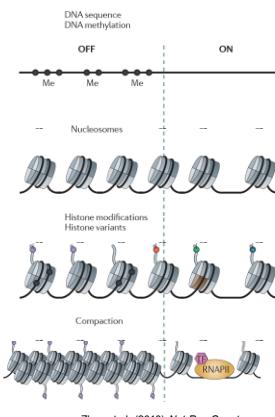




## Summary – So Far

### ▫ Epigenetic Modifications

- Packaging and Direct Modifications of DNA
  - Includes:
    - Histone Tail Modifications
    - DNA modifications
- Influence or Indicates Gene Expression
- Role in:
  - Cell-Specific Activity
  - Imprinted Genes
  - X inactivation

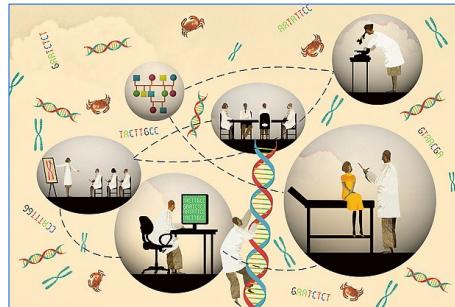


Zhou et al. (2010) *Nat Rev Genet*

## Analysing the Epigenome

- Can we detect Functional Aberration?

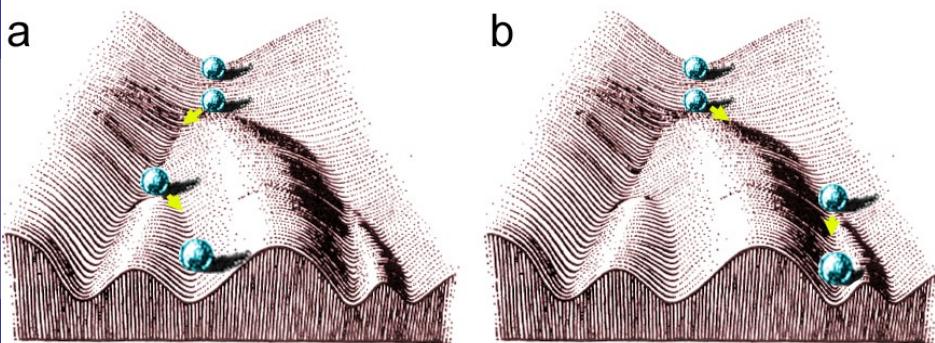
- Aetiology - Prevent
- Predict - Focus
- Diagnose
- Prognostic
- Therapeutic Options



- ↑ Understanding

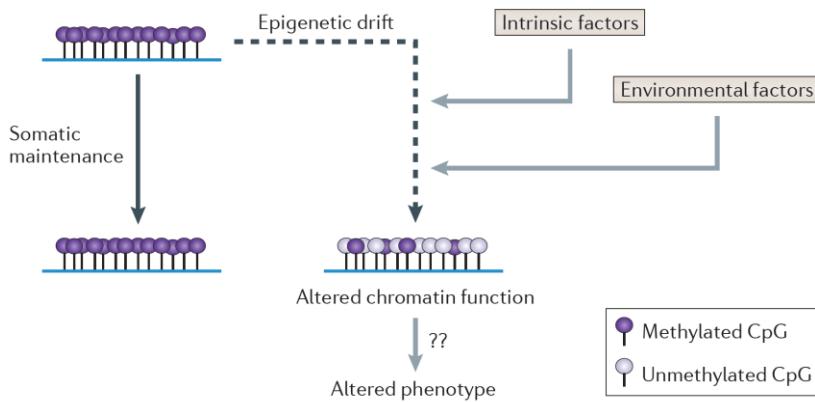
- Technological
- Statistical
- Biological
- Intrinsic/External Factors influencing Genome Function
  - Non Fixed Nature = Potential + Difficulty

## Waddington's Epigenetic Landscape



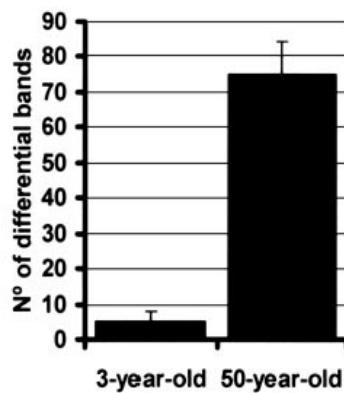
In 1957, Conrad Waddington proposed the concept of an Epigenetic Landscape to represent the process of cellular decision-making during Development

## 'Epigenetic Drift' with Age

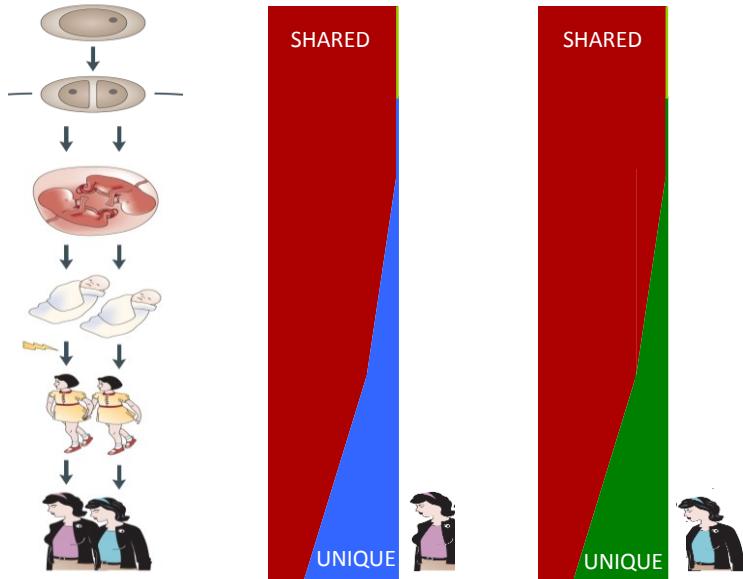


Feil & Fraga. (2012) *Nature Reviews Genetics*

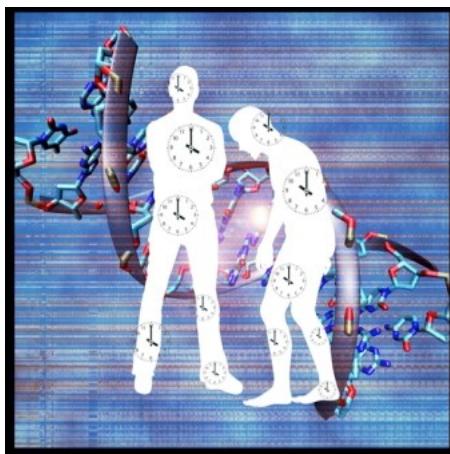
## Differences arising during Identical Twins Lifetimes



Fraga et al (2005) PNAS



Mill & Heijmans (2013) *Nat Rev Genet*

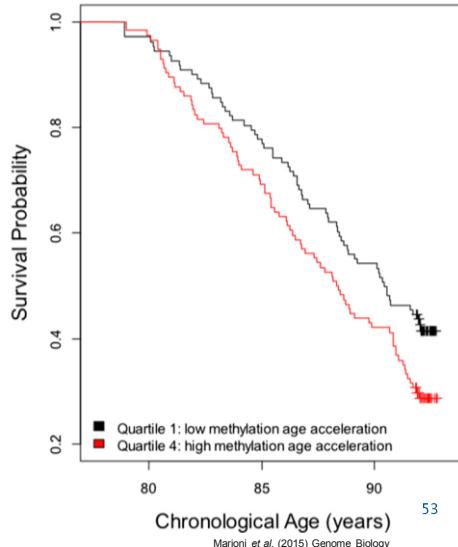


Horvath (2013) *Genome Biology*

- The ‘Epigenetic Clock’
  - Horvath (2013)
  - DNA methylation data to predict age

## Predict Mortality Risk

- Accelerated Discordance
  - DNA methylation Predicted Age
    - versus
  - Actual Chronological Age
  
- = Risk factor
  - All-Cause Mortality in later life
  - \*Accounting for Known Risk Factors



## External Exposures

- Any Outside Stimulus
  - That body can Detect
  - Potential to cause Epigenetic Modifications
  
- Which Exposures?
  - Affect which epigenetic marks?
  - What are the mechanisms and downstream effects?

Table 1   Chemicals and pollutants	
Compound	Species
Tobacco smoke	Human
Particulate air pollution	Human, Mouse
Asbestos	Human
Bisphenol A (BPA)	Mouse
Diethylstilbestrol (DES)	Mouse
Metal ions (such as chromium, cadmium, nickel, arsenic and methylmercury)	Multiple species
Vinclozolin	Mouse, rat
Methoxychlor	Mouse
Silica	Human
Benzene	Human
Di- and trichloroacetic acid, trichloroethylene	Mouse

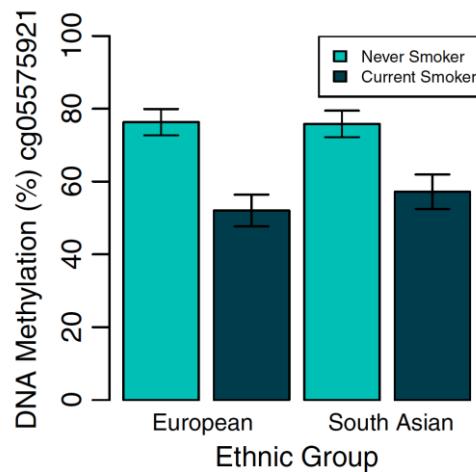
Feil & Fraga (2012) *Nat Rev Genet*

## Tobacco Smoking

- **AHRR = Aryl Hydrocarbon Receptor Repressor**
  - Maternal – *In utero* effects
  - Passive Effects
  - Ex-smoker Effects



Joubert et al. (2012) EHP

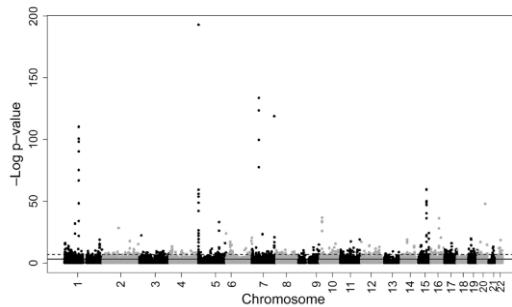


### Aryl Hydrocarbon Receptor Repressor (*AHRR*)

Elliot et al. (2014) Clinical Epigenetics

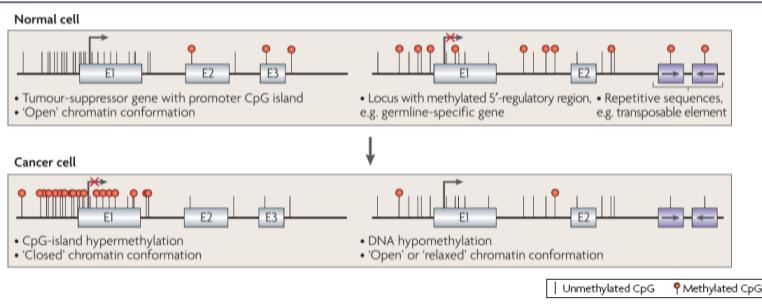
## In Utero Effects

- Meta-analysis Epigenome Wide Association Study
  - 13 cohort (6,685 individuals)
  - Methylation Variation Relevant to Diseases influenced by Maternal Smoking
    - Incl. Asthma and Orofacial Clefts
  - & can persist into childhood



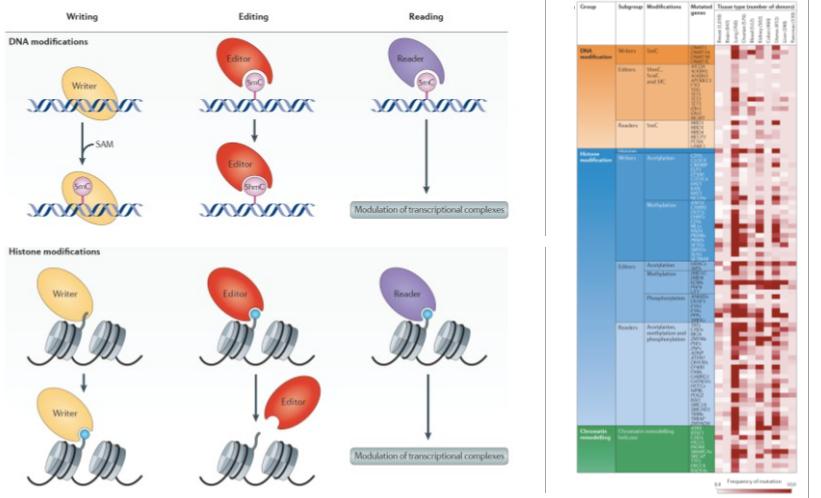
Jorbert et al. (2016) DNA methylation in Newborns and Maternal Smoking in Pregnancy: Genome-Wide Consortium Meta-analysis. *AJHG*

## Major Epigenetic Aberrations in Cancer



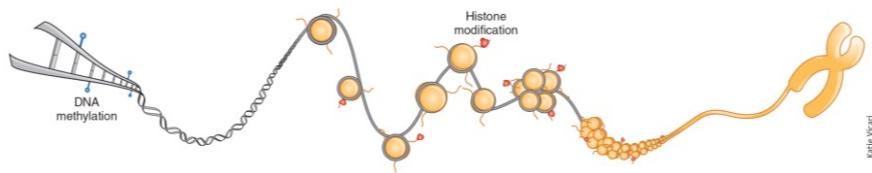
- Epigenomic Disruption
  - Global hypomethylation
    - Repetitive sequence
  - Locus hypermethylation
    - Tumour-Suppressor Gene Silencing
  - Histone-modification imbalance

## Enrichment for Mutations in Global Epigenomic Modifying Genes

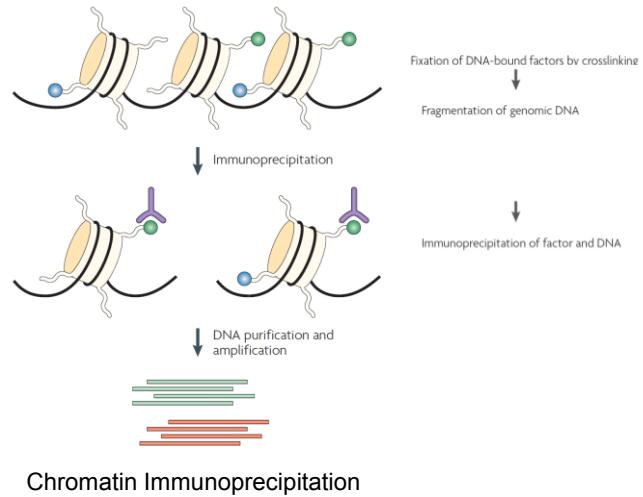


Plass et al. (2013) *Nat Rev Genet*

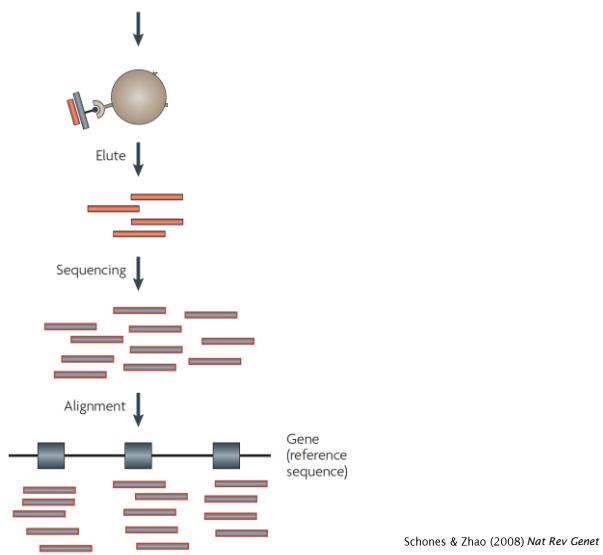
## Analysing the Epigenome



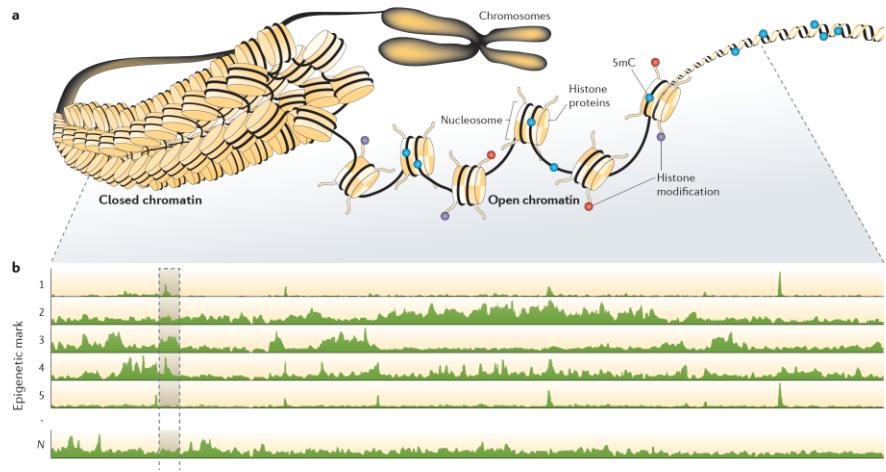
## Analysing Histone Tail Modifications

Schones & Zhao (2008) *Nat Rev Genet*

## Chromatin Immunoprecipitation (ChIP-Seq)

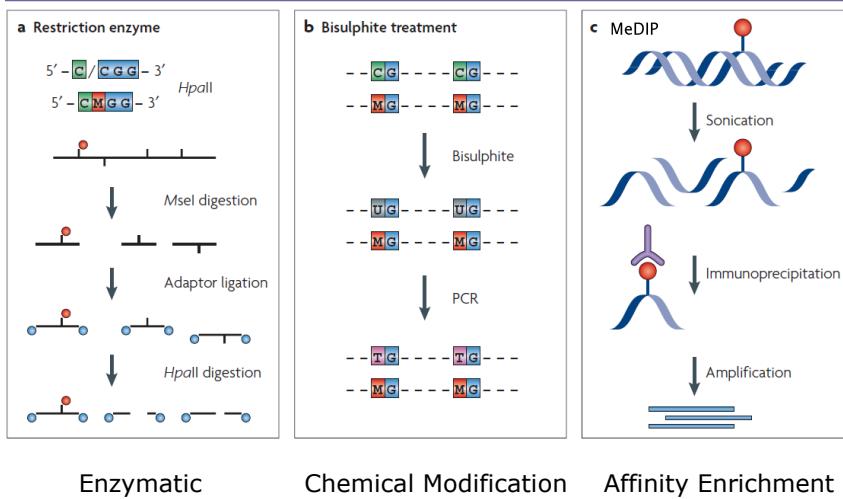
Schones & Zhao (2008) *Nat Rev Genet*

## Chromatin Maps



Taudt et al. (2016) *Nat Rev Genet*

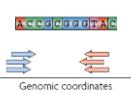
## Methods for Assessing DNA Methylation



## All 3 Methods can be followed by:

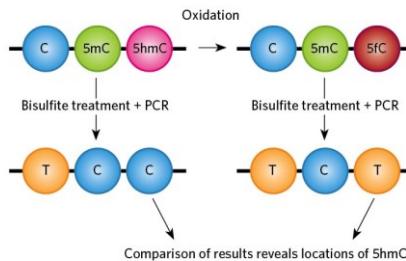


- Locus-Specific
  - Single Locus
- Array Based
  - High Throughput
  - Limited Genome
    - MRE-chip/ BiS-chip / MeDIP-chip



- 2<sup>nd</sup> Gen. Sequencing
  - ~ Genome-wide
  - MRE-seq/ BiS-seq / MeDIP-seq

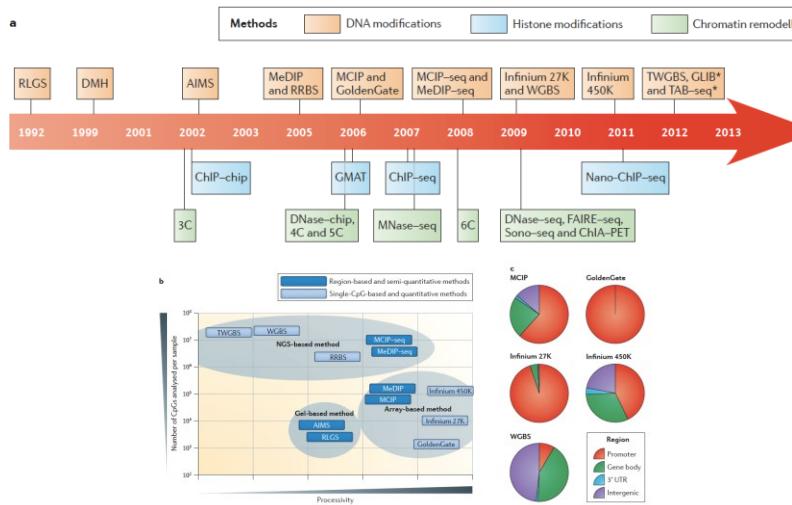
## Oxidative BiSulphite Sequencing



- oxBS-seq method
  - **Selectively** oxidises 5hmC to 5fC
  - Bisulphite converts both unmodified C and 5fC to T after PCR

Booth et al. (2012) *Science*

## Technology Advance



Plass et al. (2014) *Nat Rev Genet*

## First Human DNA Methylome

Vol 462 | 19 November 2009 | doi:10.1038/nature08514

nature

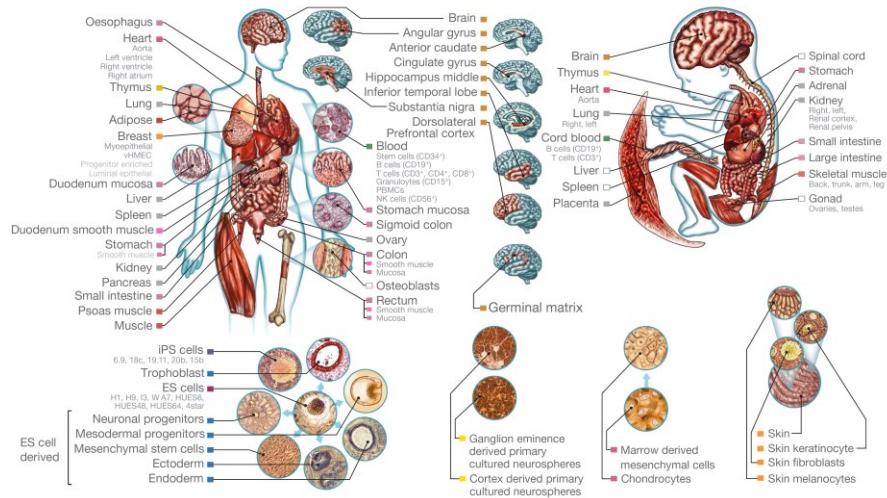
## ARTICLES

### Human DNA methylomes at base resolution show widespread epigenomic differences

Ryan Lister<sup>1\*</sup>, Mattia Pelizzolla<sup>1\*</sup>, Robert H. Downen<sup>1</sup>, R. David Hawkins<sup>2</sup>, Gary Hon<sup>2</sup>, Julian Tonti-Filippini<sup>4</sup>, Joseph R. Nery<sup>1</sup>, Leonard Lee<sup>2</sup>, Zhen Ye<sup>2</sup>, Que-Minh Ngo<sup>2</sup>, Lee Edsall<sup>2</sup>, Jessica Antosiewicz-Bourget<sup>3,6</sup>, Ron Stewart<sup>3,6</sup>, Victor Ruotti<sup>3,6</sup>, A. Harvey Millar<sup>4</sup>, James A. Thomson<sup>5,6,7,8</sup>, Bing Ren<sup>3,5</sup> & Joseph R. Ecker<sup>1</sup>

= Whole Genome BiSulphite-seq

## Epigenomics Roadmap Project



2015

# BLUEPRINT

epigenome

BLUEPRINT  
publications  
in Cell



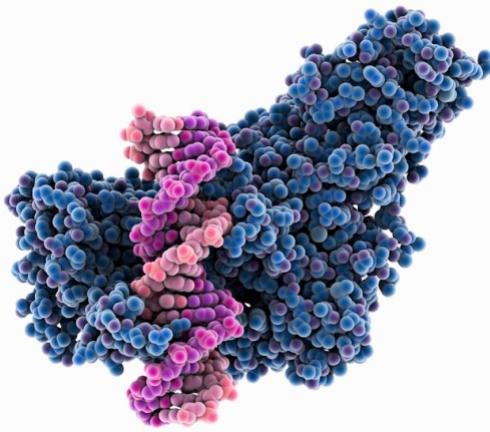
2016

## Conclusion

- Measure the Epigenome to understand:
  - Development
  - Ageing
  - Environmental Impacts on Disease Risk
  - Disease Pathology
- Chromatin Assessment
  - Chromatin Immunoprecipitation
- DNA Methylome Assessment
  - Enzymatic/ Chemical Conversion/ Affinity
    - Arrays/Sequencing/Capture
- Future: Direct Analysis

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## Questions



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