The Epigenome: Regulating Gene Expression Through Chromatin Structure and Function

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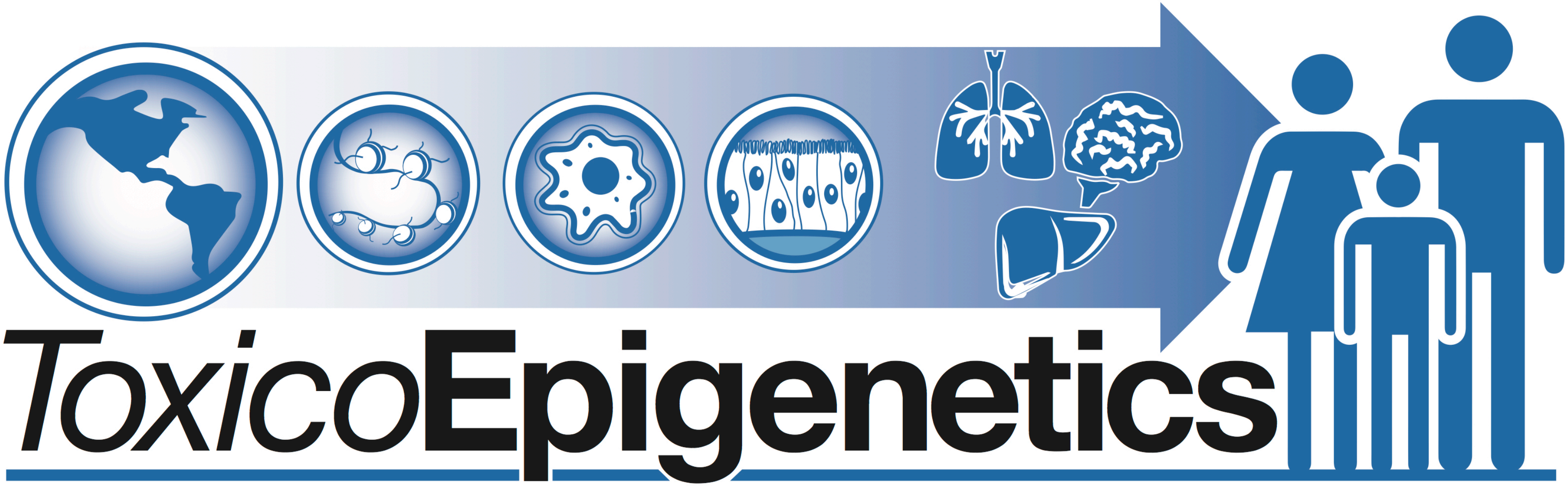


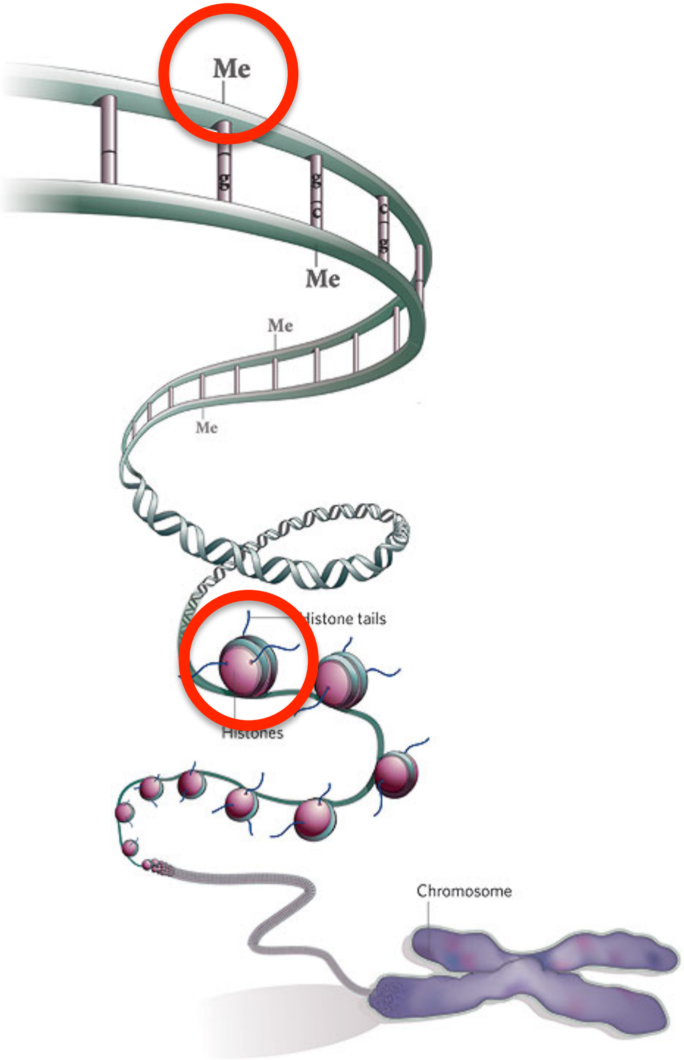
ToxicoEpigenetics

The Interface of Epigenetics and Risk Assessment

November 2-4, 2016
Tyson’s Corner, VA
www.toxicology.org/teg
Registration is Open!

The Epigenome

- Heritable factors that regulate gene expression without a change in DNA sequence
  - Change in phenotype without a change in genotype.
- DNA methylation
- Histone tail modifications
- miRNAs



Nature. **441:** 143-145. 2006

Why is the Epigenome Important in Toxicology and Risk Assessment?

1. Direct role in the regulation of gene expression in response to toxicant/environmental exposure
   - Mediator of exposure effects
   - Biomarker of susceptibility

2. Chromatin modification patterns are responsive to an individual's environment
   - Dynamic and stable
   - Chemical and non-chemical exposures
   - Modifiable risk factors

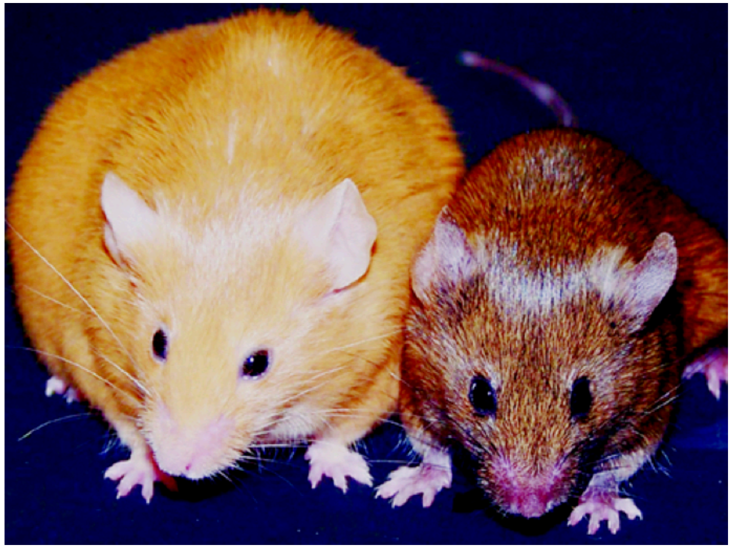
3. Multi- and trans-generational risk
   - How do your parents' and/or grandparents' exposures and lifestyle impact your susceptibility?

Susceptibility

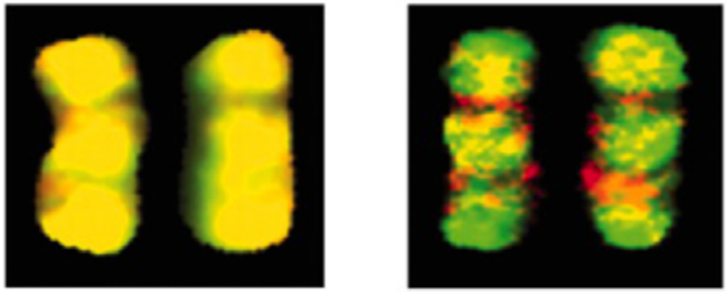
- Traditional susceptibility markers do not faithfully explain inter-individual variability in exposure effects
- Gene variants do not completely explain susceptibility
- What non-genetic mechanisms regulate the response to environmental exposure?

Environmental Susceptibility

- Genetically identical mice from two mothers with different maternal diets
- Environmental exposure modifies gene expression through the epigenome
  - Modifies susceptibility
  - Mediates effects



Dana Dolinoy and Randy Jirtle

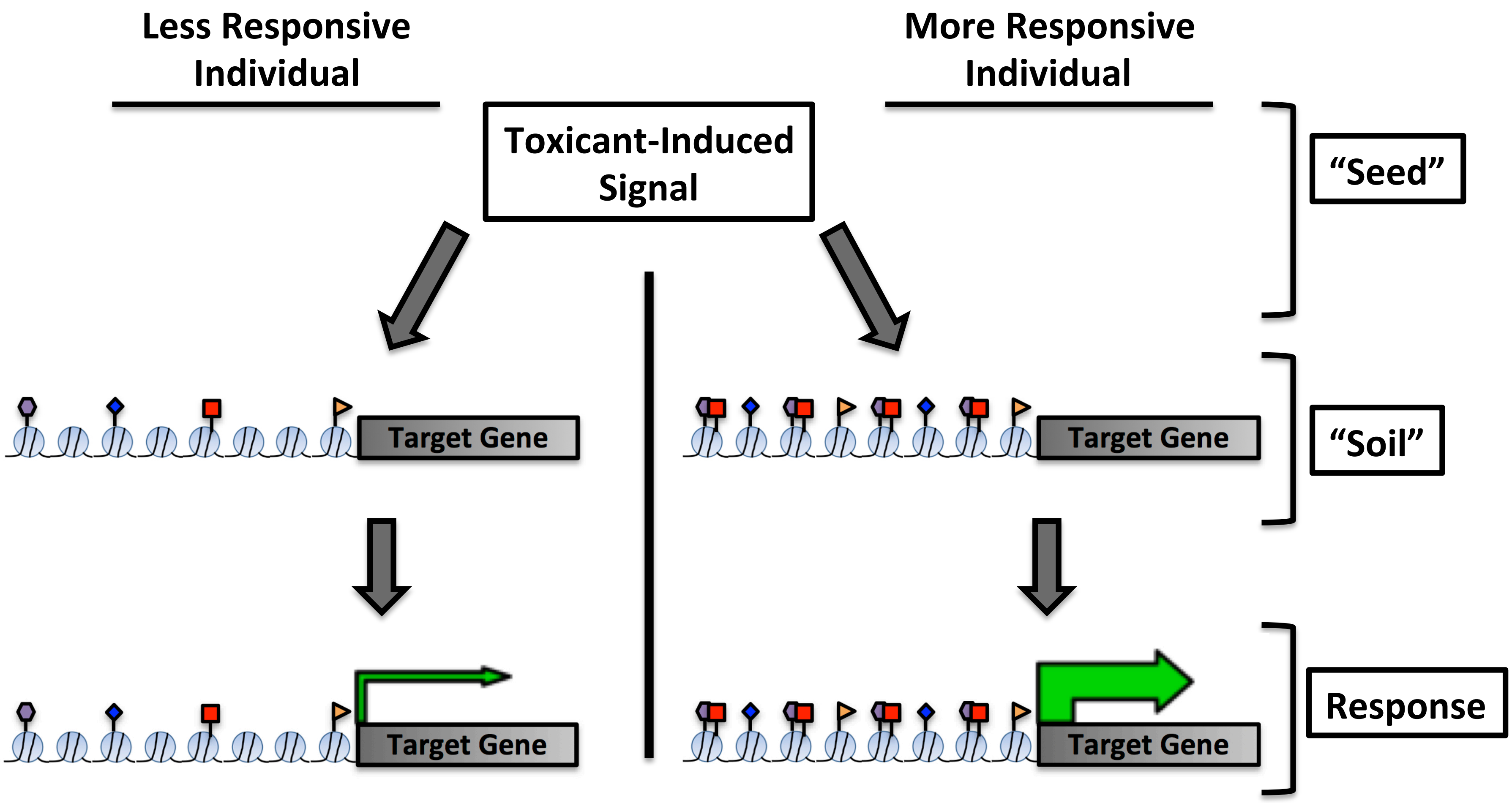


3-year old identical twins

50-year old identical twins

Proc. Natl. Acad. Sci. U.S.A. 2005. **102:**10604-10609.

Epigenetic Seed and Soil Model

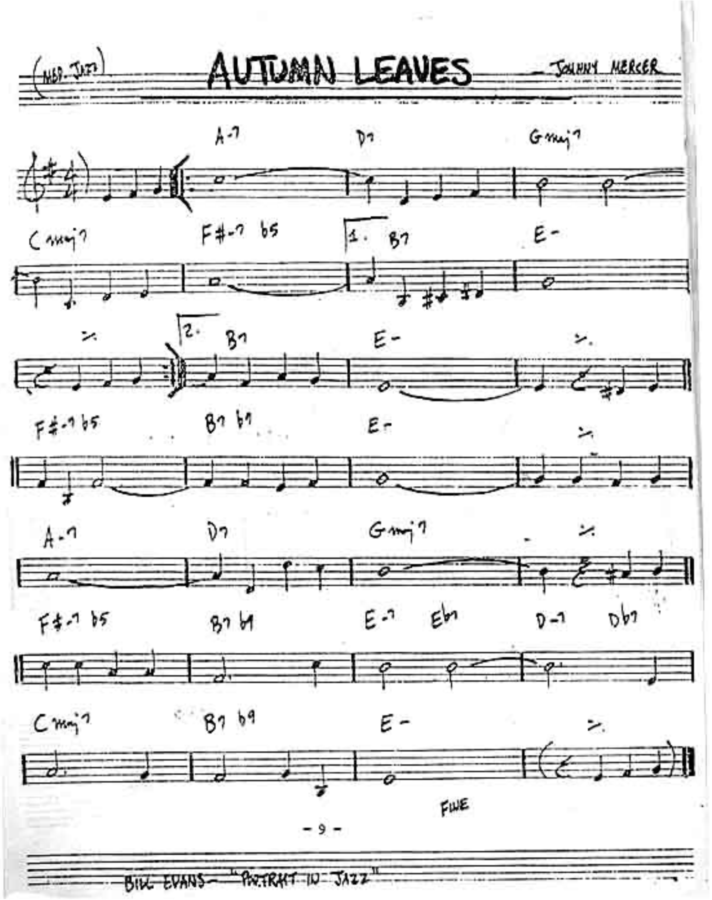


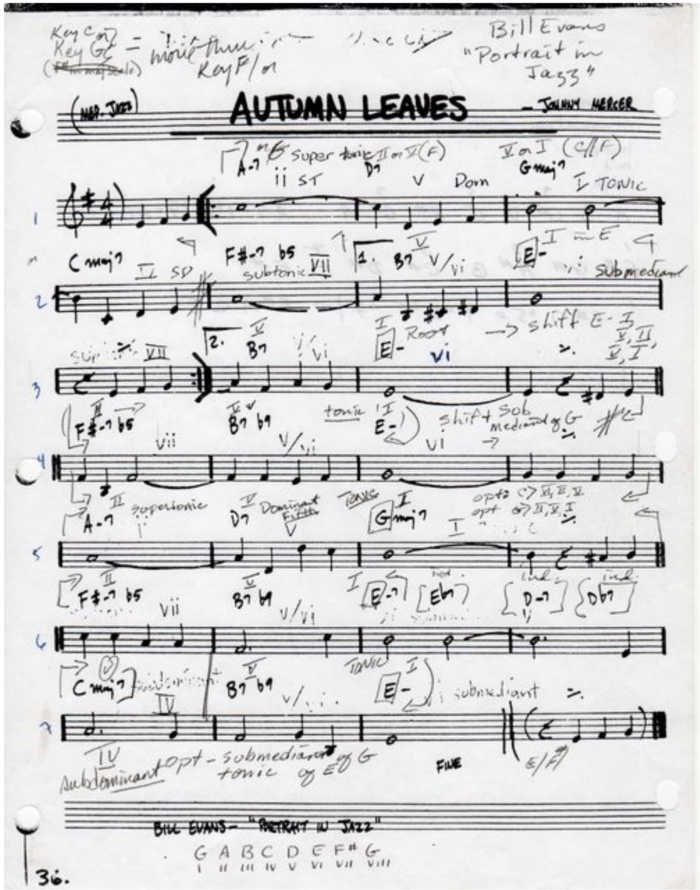
McCullough *et al.* (2016) *Tox. Sci.* **150:**216-224

Human Blueprint: The Genome

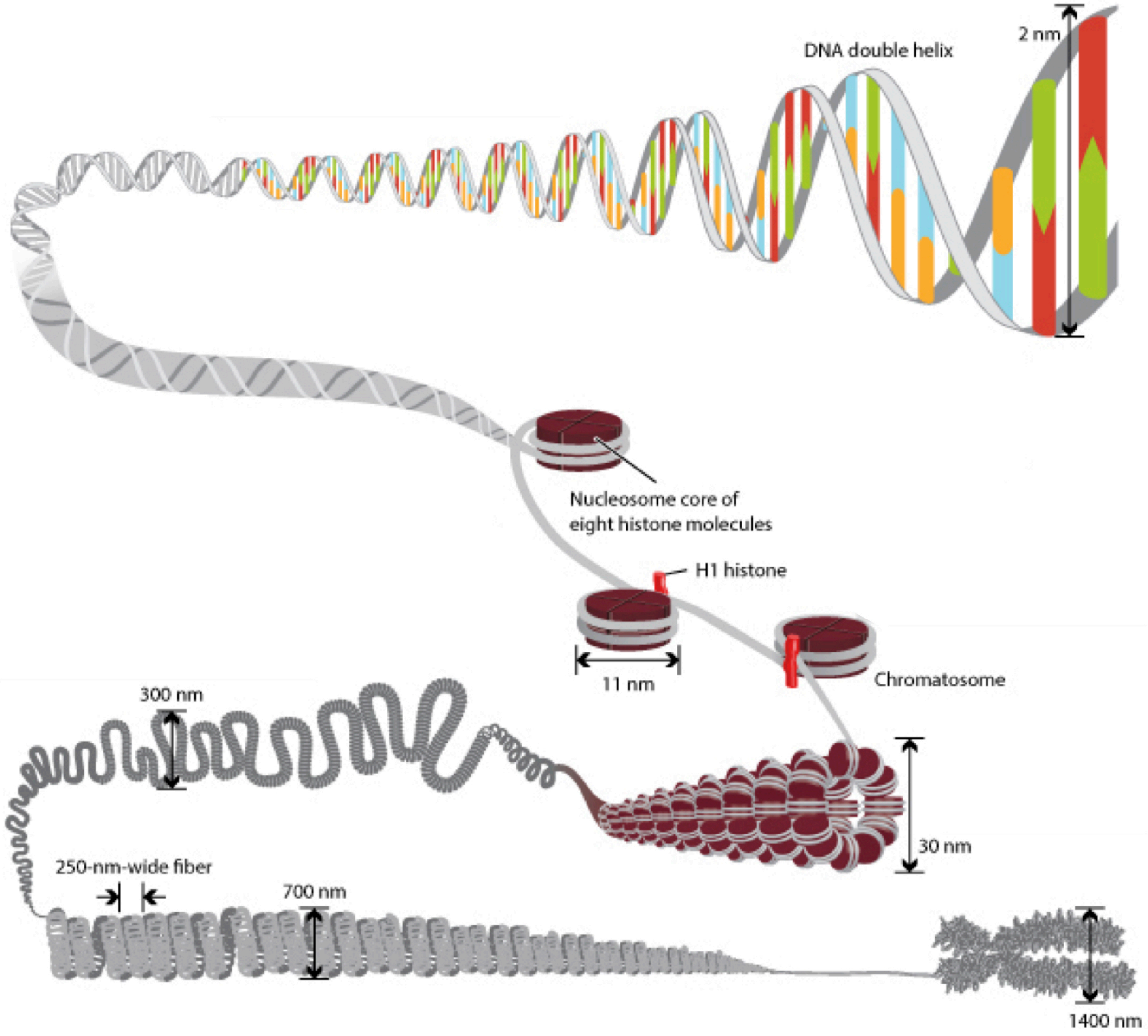
- ~3,234,000,000 base pairs
- Relatively simple code
  - A, C, T, and G
- Encodes ~21,000 genes
- Humans share >99% sequence identity
- Human Genome Project formally completed in 2003

Instructions in the Epigenome

Genome

Epigenome

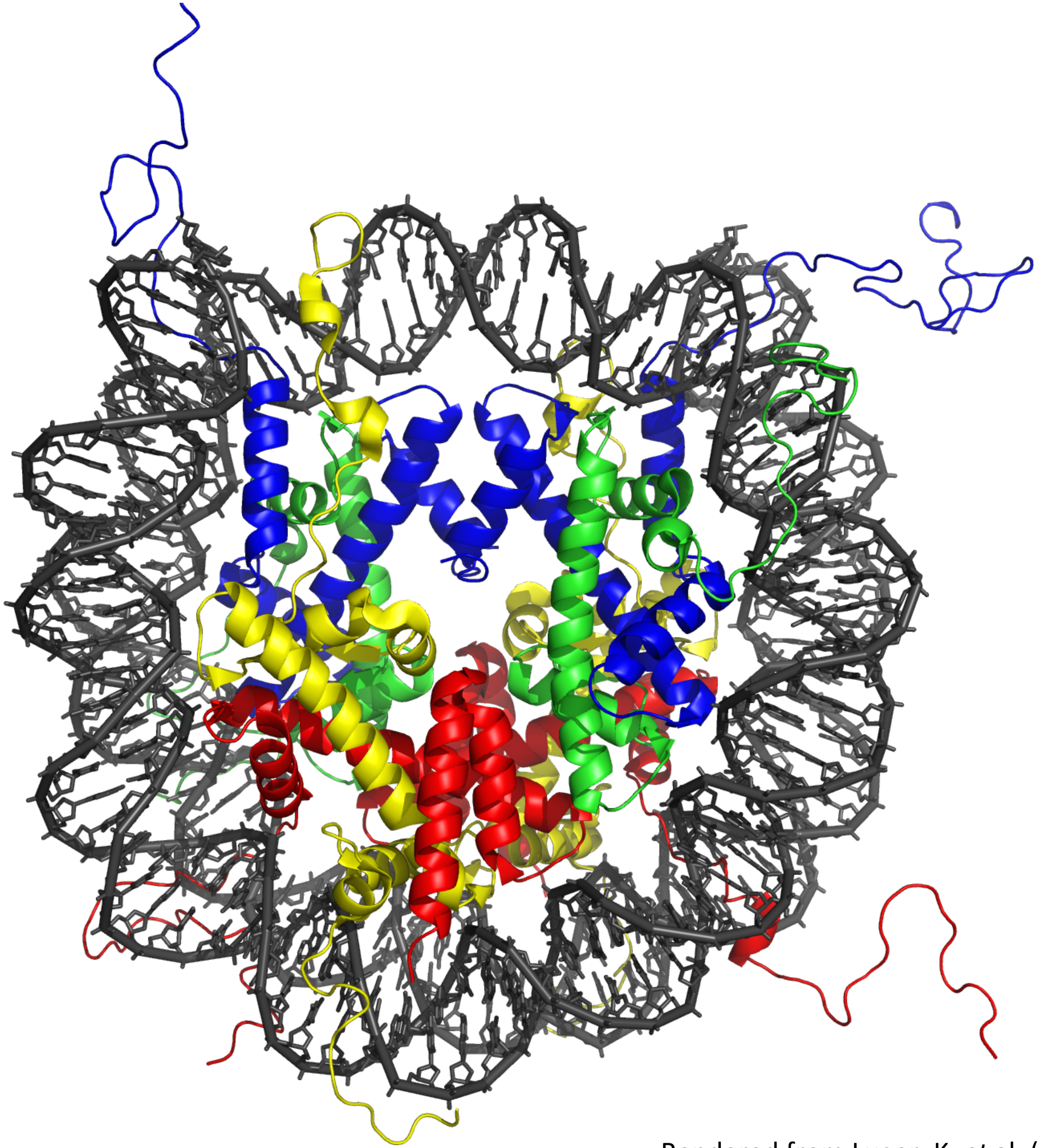


Storage and Accessibility



Adapted from Pierce, B. (2013) *Genetics: A Conceptual Approach, 2nd ed.*

The Fundamental Unit of Chromatin



🟨 **H2A**

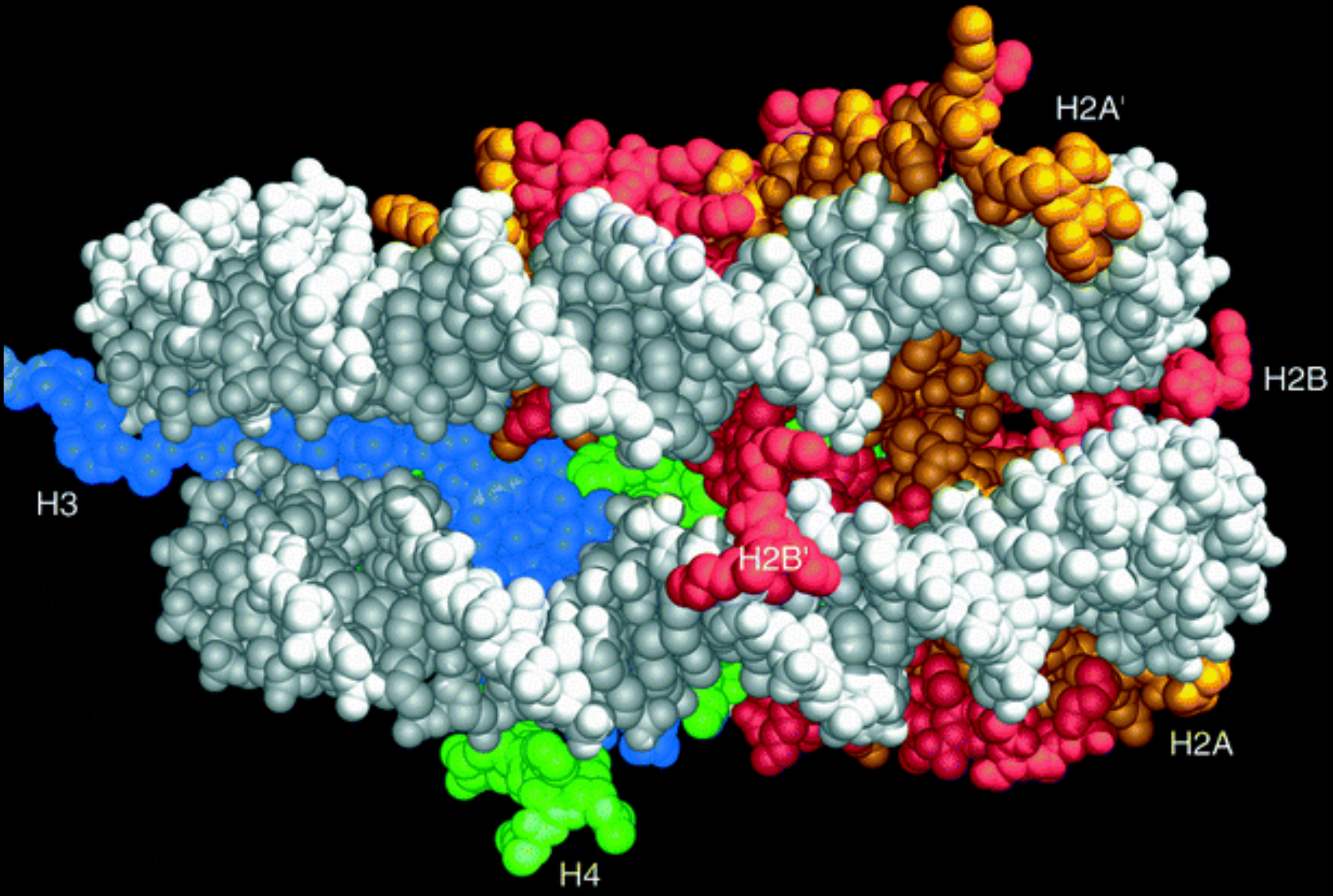
🟥 **H2B**

🟦 **H3**

🟩 **H4**

Rendered from Luger, K. *et* al. (1997) *Nature*. **389:** 251-260

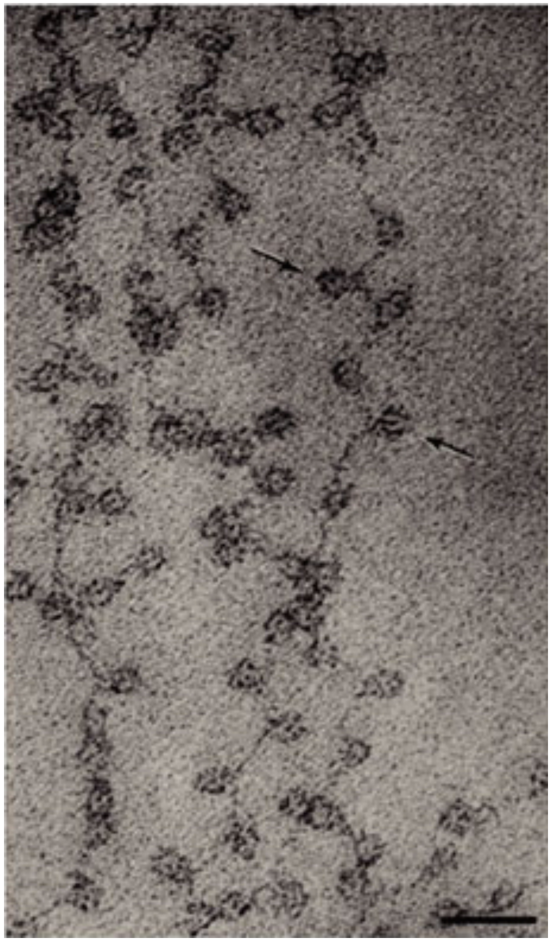
Histone Tail-DNA Interactions



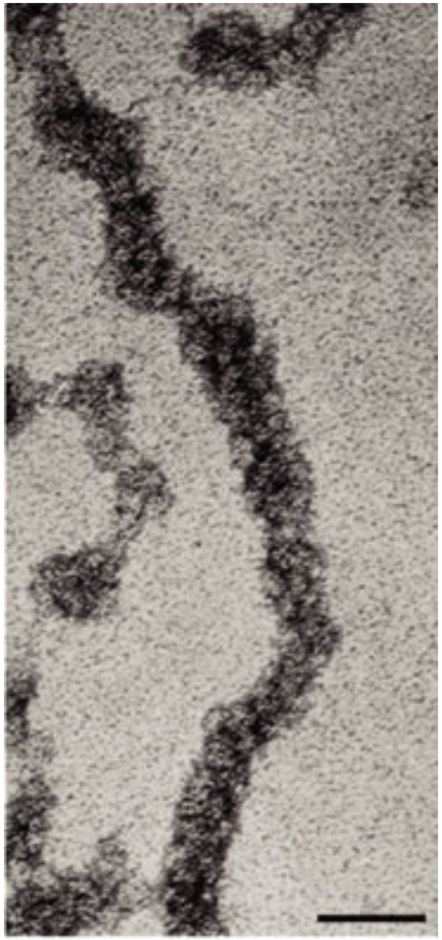
Luger, K. *et* al. (1997) *Nature*. **389:** 251-260

Chromatin States

Open - Euchromatin

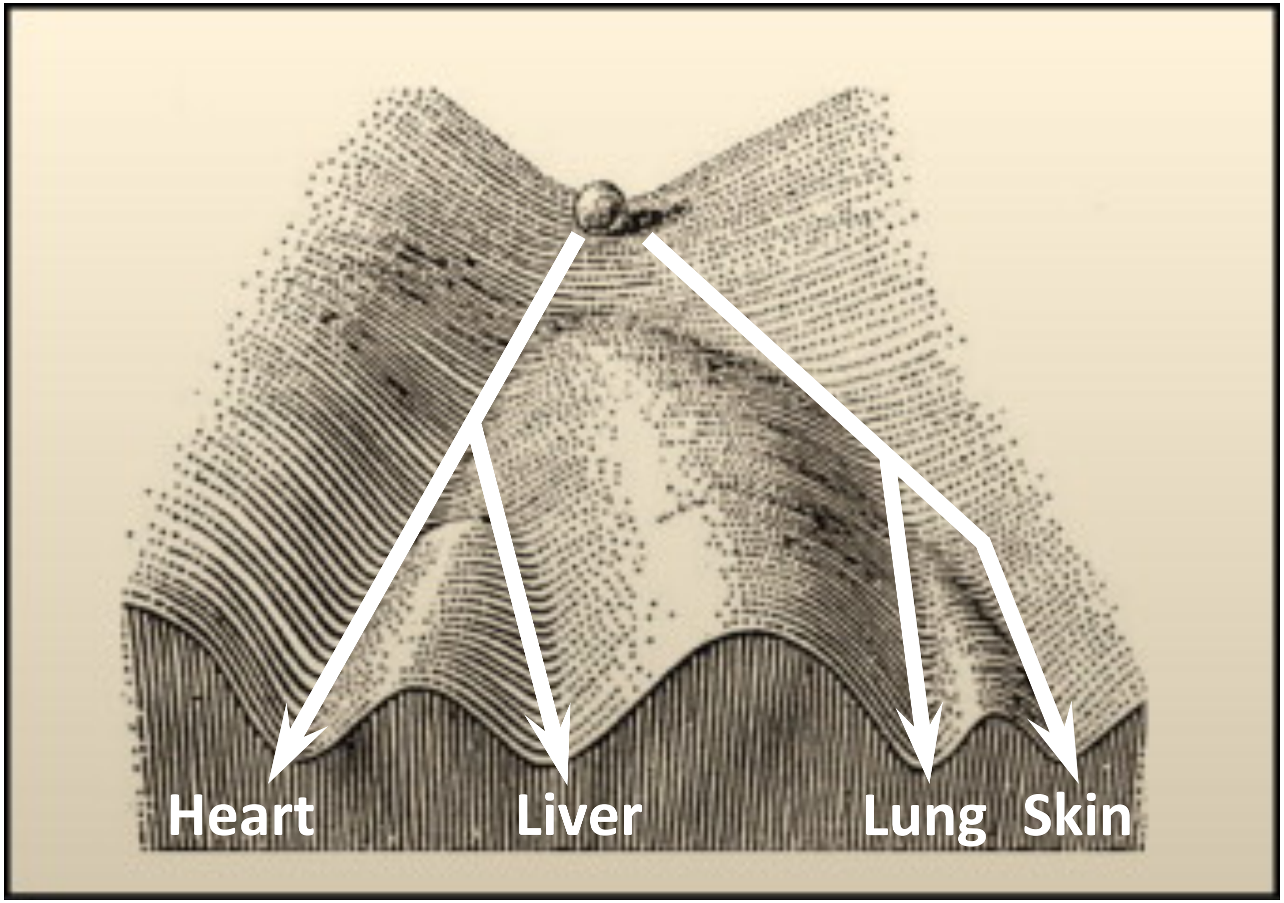


Closed - Heterochromatin

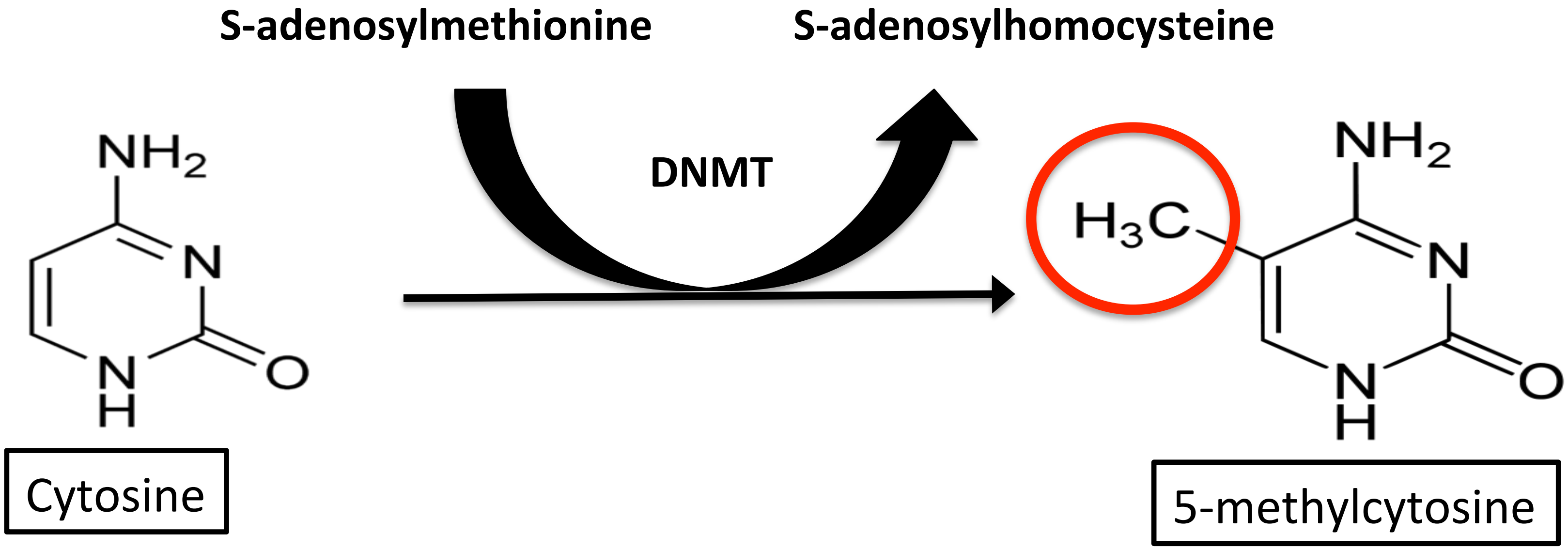


Olins & Olins (2003) *Nat Rev Mol Cell Biol* **4:** 809-814

What Makes Cells Within the Same Individual Different?



DNA Methylation



S-adenosylmethionine

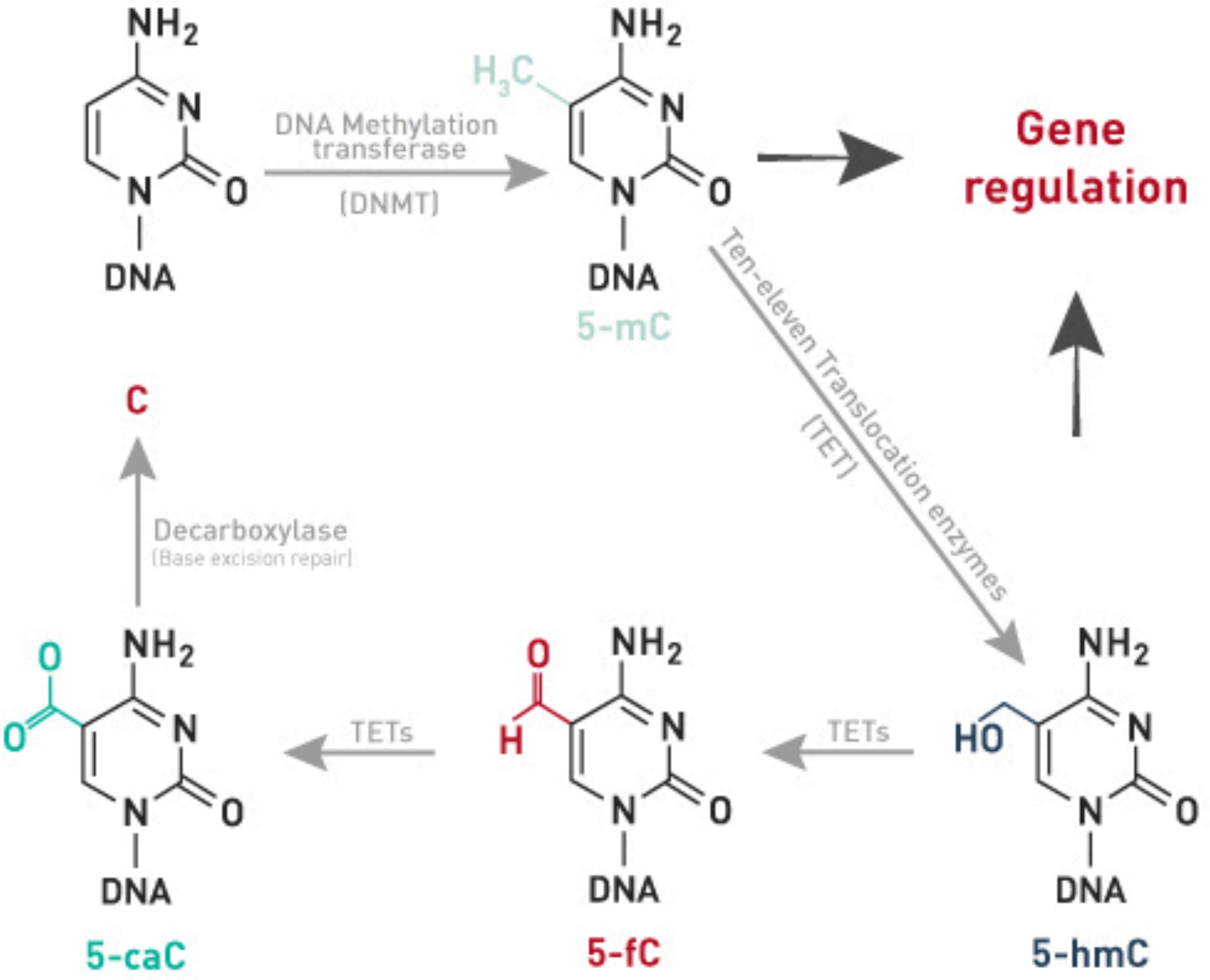
S-adenosylhomocysteine

DNMT

Cytosine

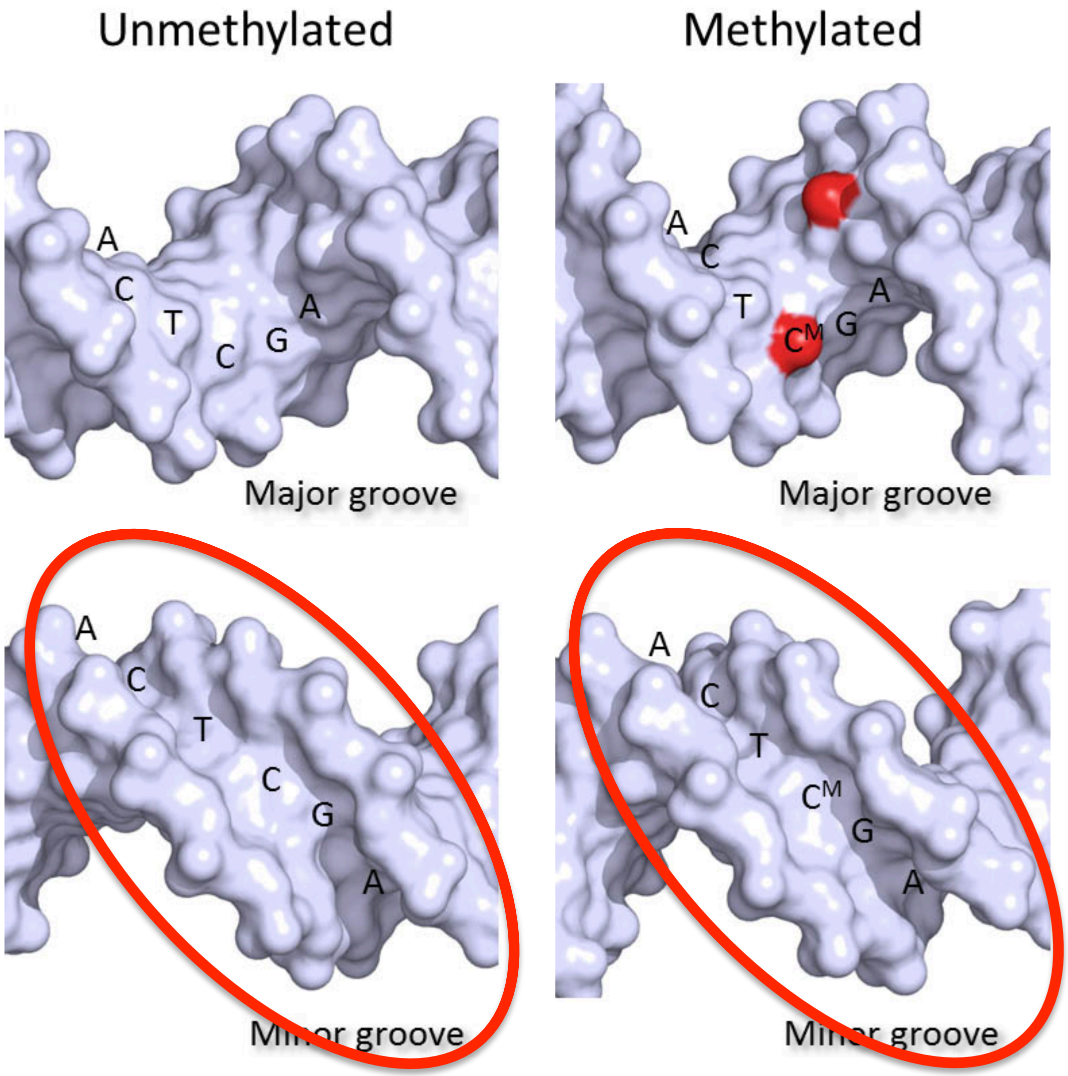
5-methylcytosine

Oxidation of 5-methylcytosine



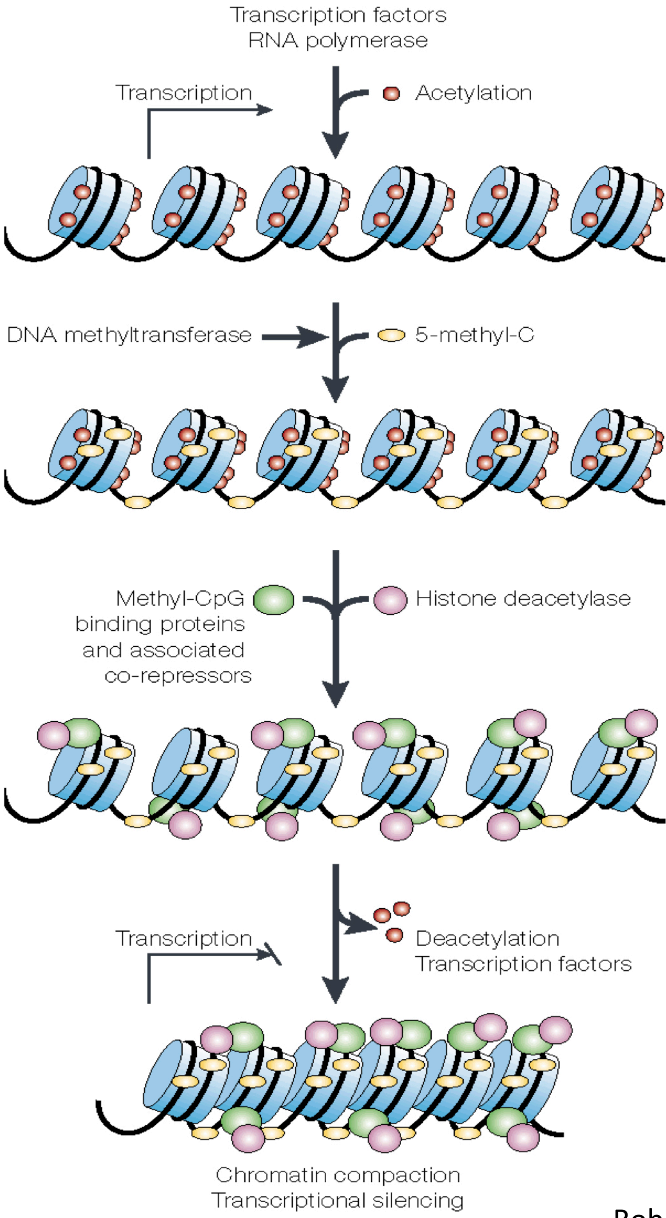
www.diagenode.com

DNA Methylation Alters DNA Structure



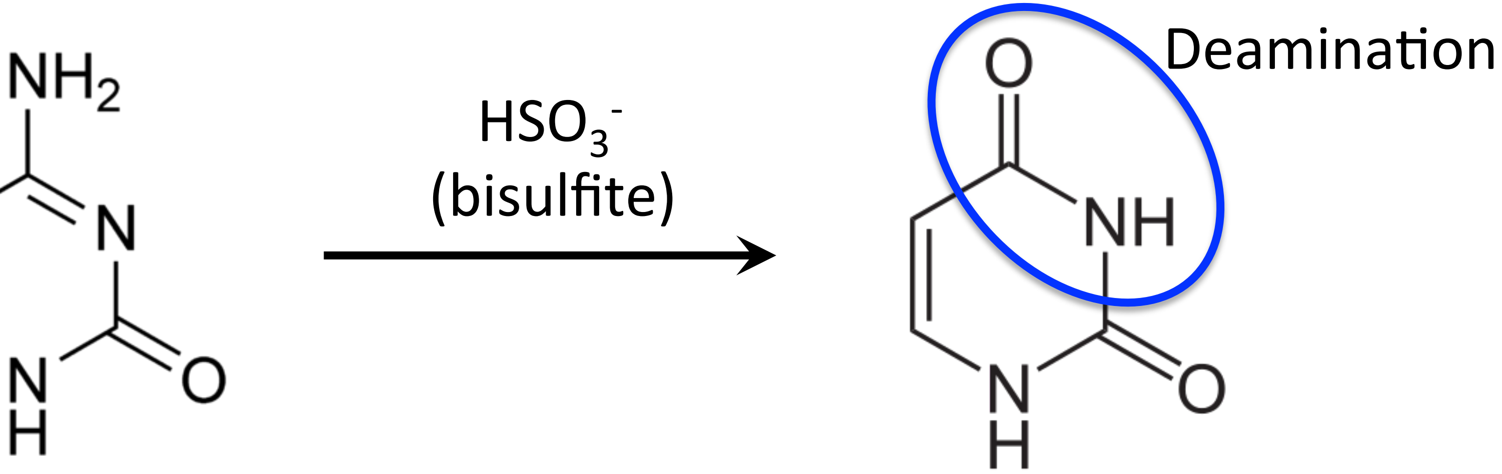
Lazarovici *et al*. (2013) *Proc Nat Acad Sci USA*. **110:** 6376-6381

DNA Methylation in Gene Silencing



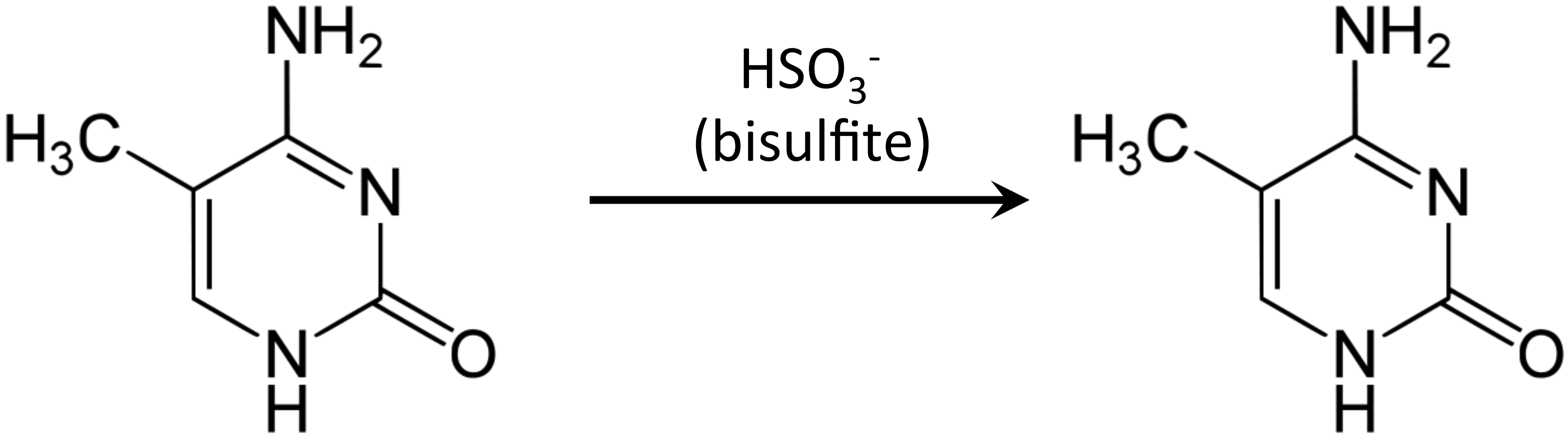
Robertson & Wolfe (2000) *Nat Rev Genetics* **1:** 11-19

Bisulfite Conversion



Cytosine

Uracil



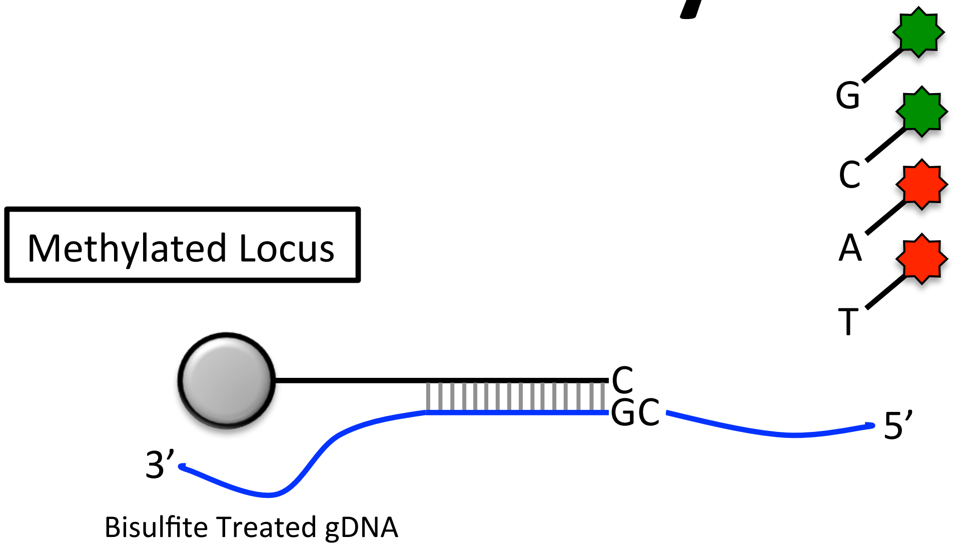
5-methylcytosine

5-methylcytosine

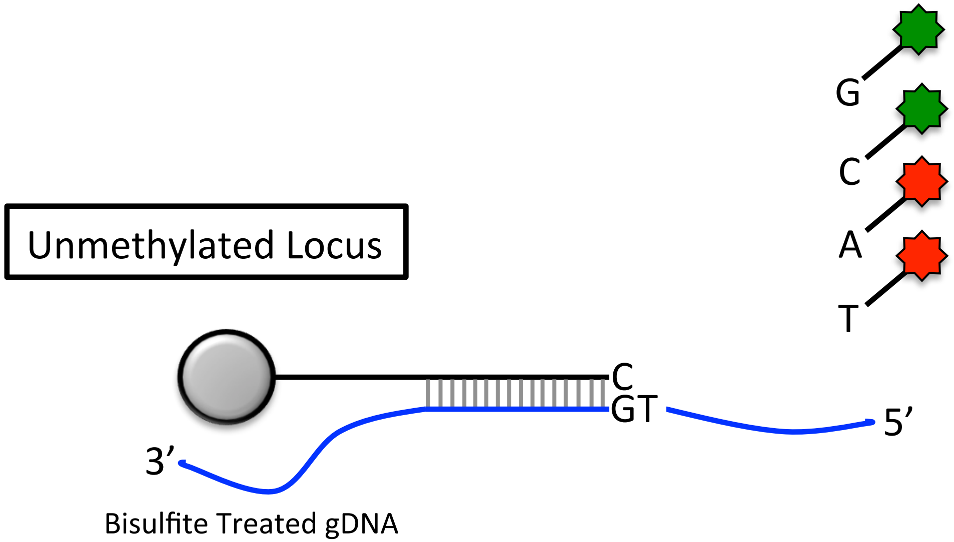
DNA Methylation Bead Array

- 27K/450K/850K CpG sites
- **Sample types**
  - Fresh or FFPE
  - ≥250-500 ng gDNA
- **Identification of differentially methylated regions (DMRs)**
  - Measures ratio of methylated and unmethylated signal at each locus
- **Advantages**
  - Large data volume
- **Limitations**
  - Large data volume = complex analysis
  - Requires ≥250 ng sample DNA
  - Does not discriminate between 5mC and 5hmC

Methylated Locus

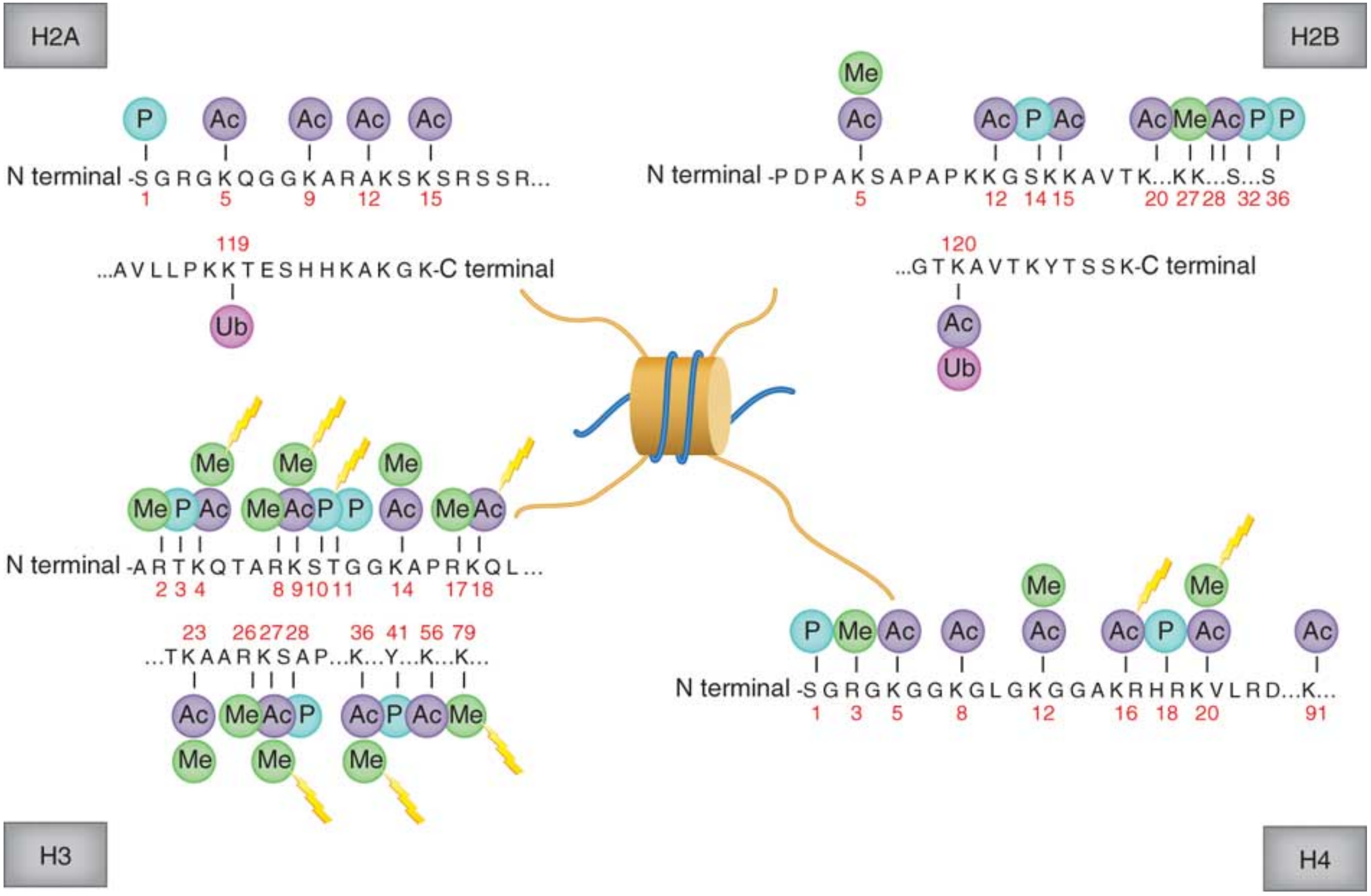


Unmethylated Locus



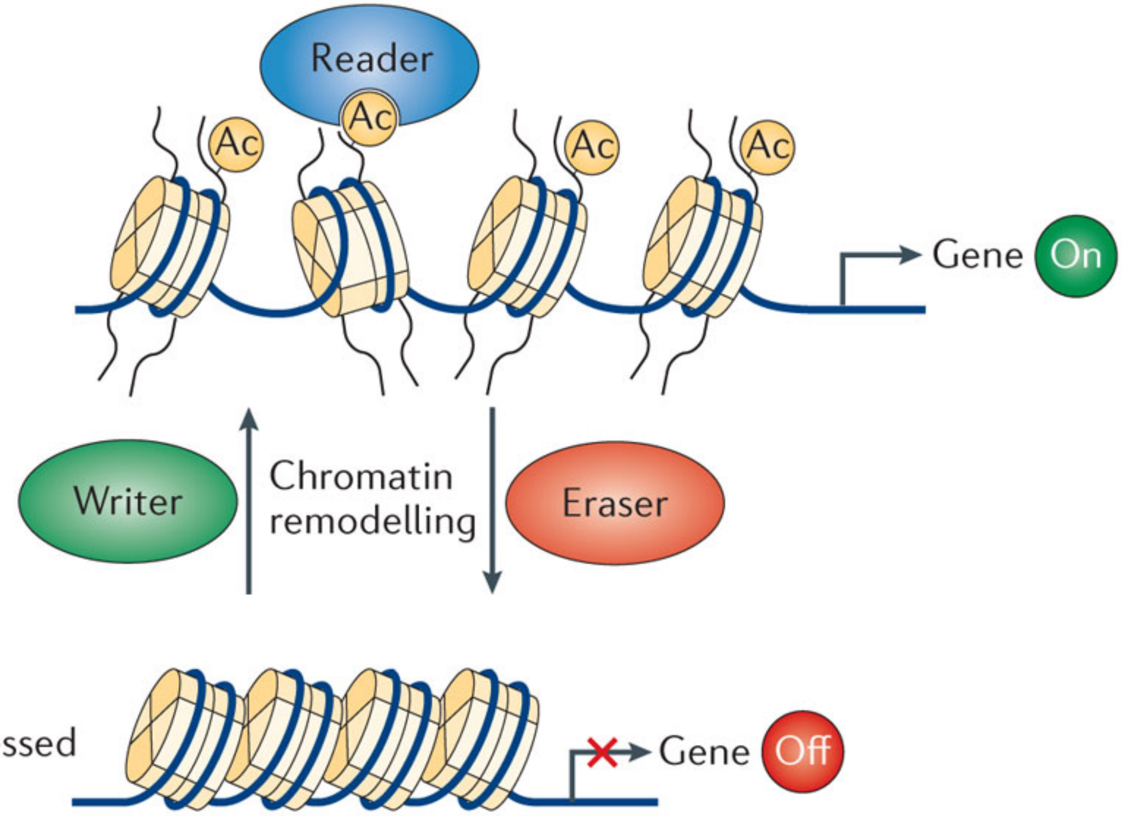
The “Histone Code”

>130 Unique histone modifications



Nat. Med. **17(3):** 330-339. 2011

Histone Acetylation



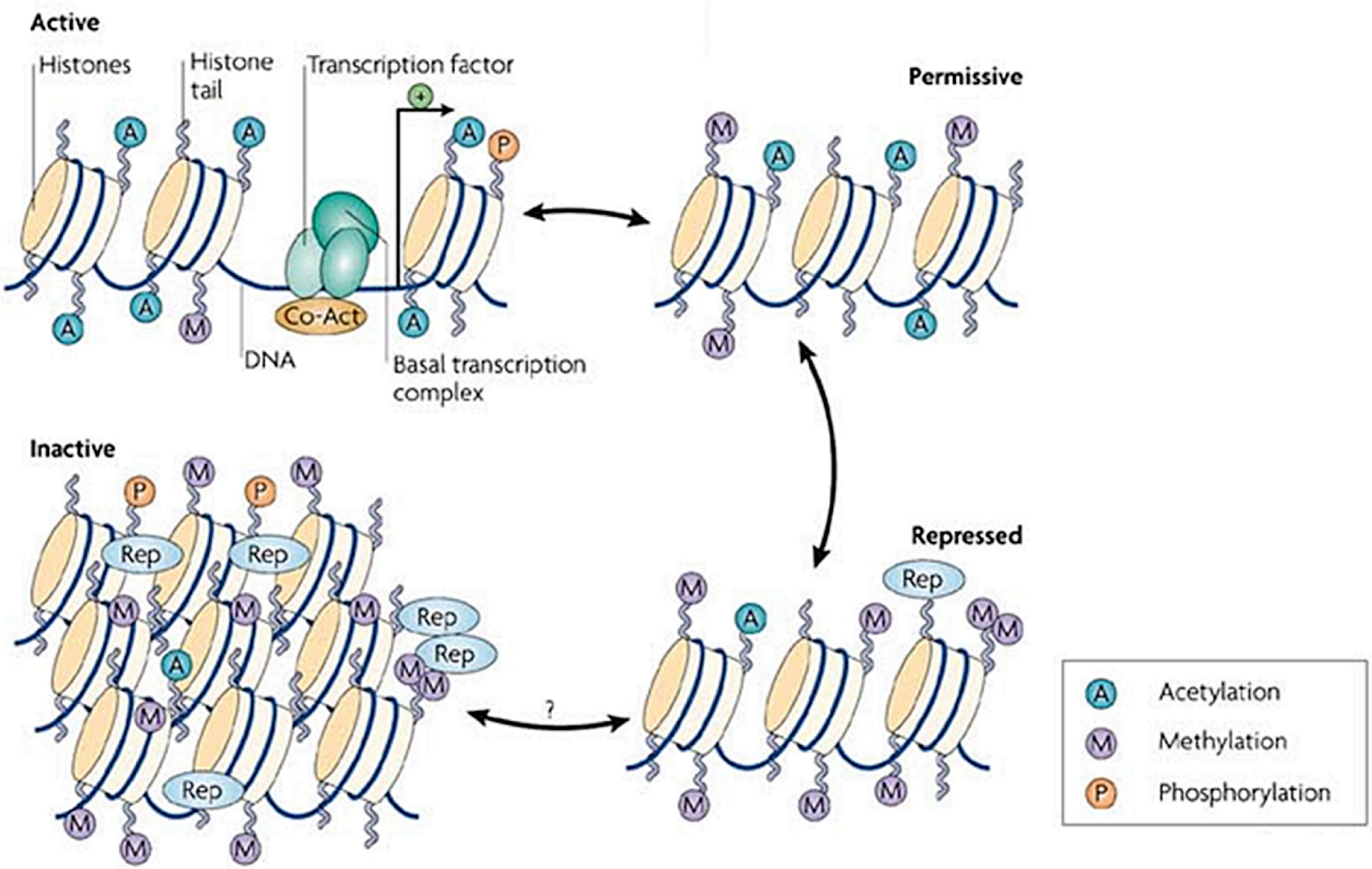
Acetylated chromatin
Open and transcriptionally active

Deacetylated chromatin
Compact and transcriptionally repressed

Nature Reviews | **Molecular Cell Biology**

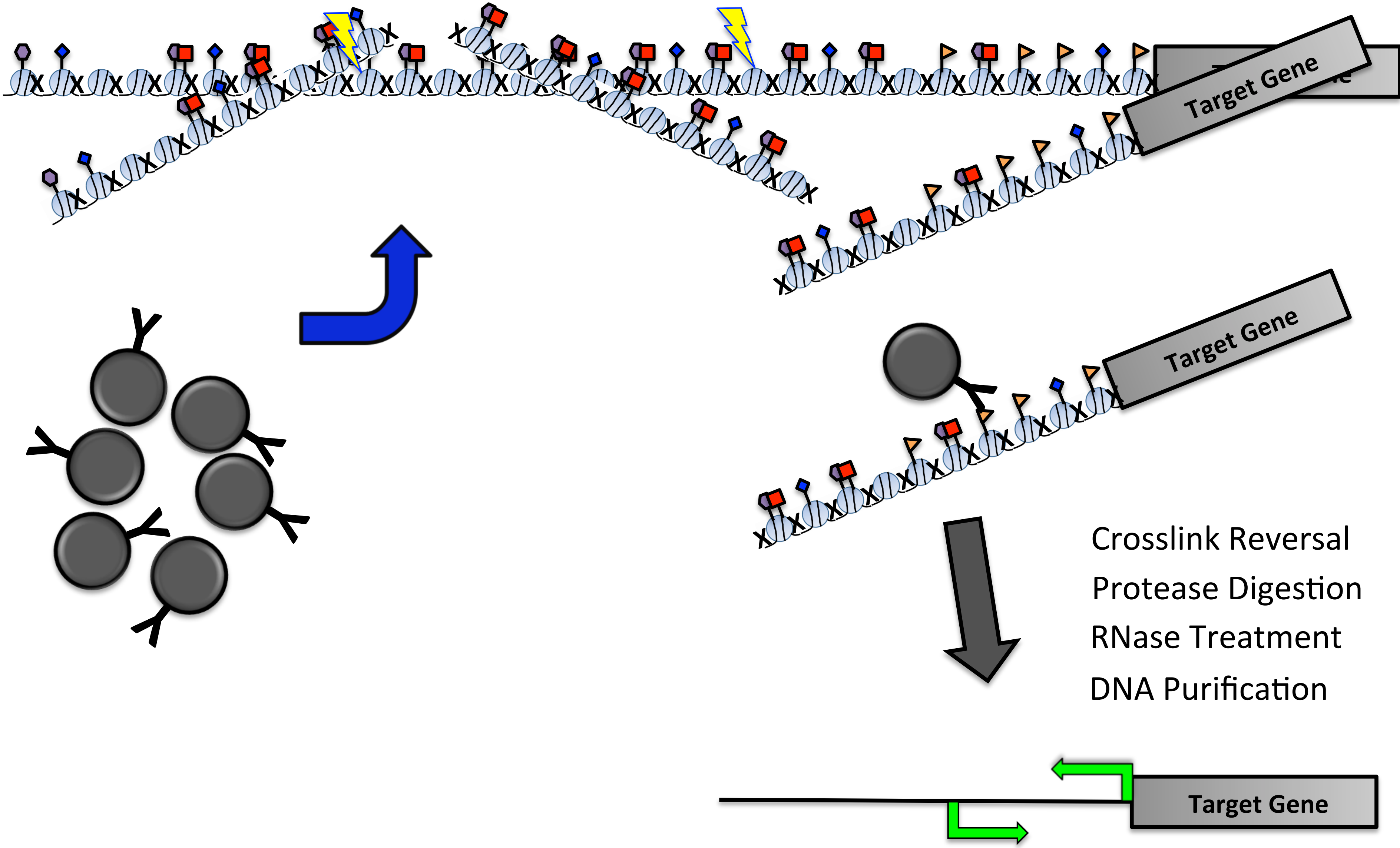
Verdin & Ott (2015) *Nat Rev Cell Biol*. **16:** 258-264

Chromatin States



Adapted from Tsankova *et al.* (2007) *Nat Rev Neurosci*. **8(5):** 355-67

Chromatin Immunoprecipitation (ChIP)



Crosslink Reversal

Protease Digestion

RNase Treatment

DNA Purification

Studying the Epigenome

- DNA Methylation
  - Methyl enrichment
  - Bisulfite-PCR/seq
  - DNA methylation array
  - RRBS
  - MeDIP
- Methyl oxidation products
- Histone Modifications
  - ChIP-PCR/seq
- Chromatin structure
  - ATAC-seq
  - MNase/DNase-seq
  - FAIRE-seq

So, what’s available to help you explore the role of the epigenome in your toxicology research program?