

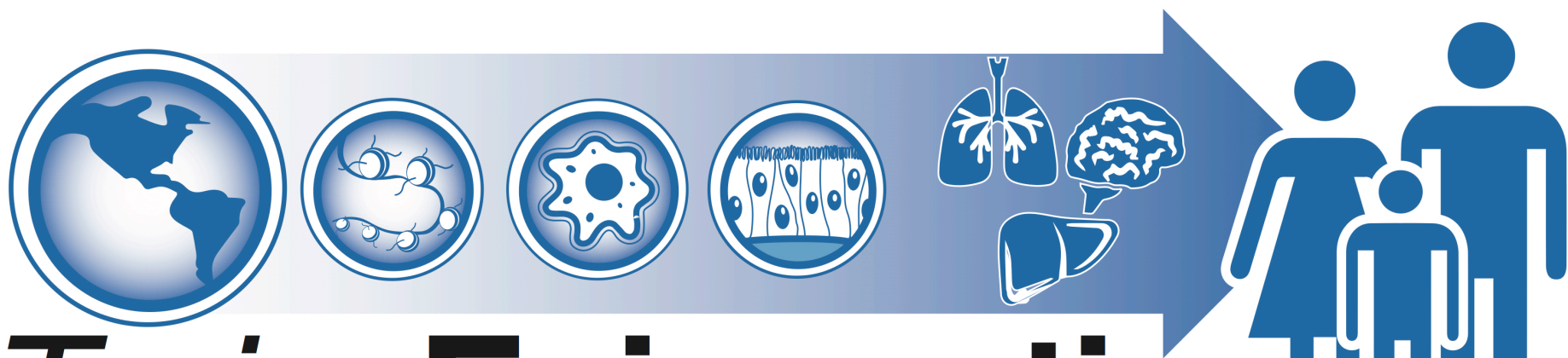
The Epigenome: Regulating Gene Expression Through Chromatin Structure and Function

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U.S. Environmental Protection Agency

Environmental Public Health Division



*Toxico*Epigenetics

The Interface of Epigenetics and Risk Assessment

November 2-4, 2016

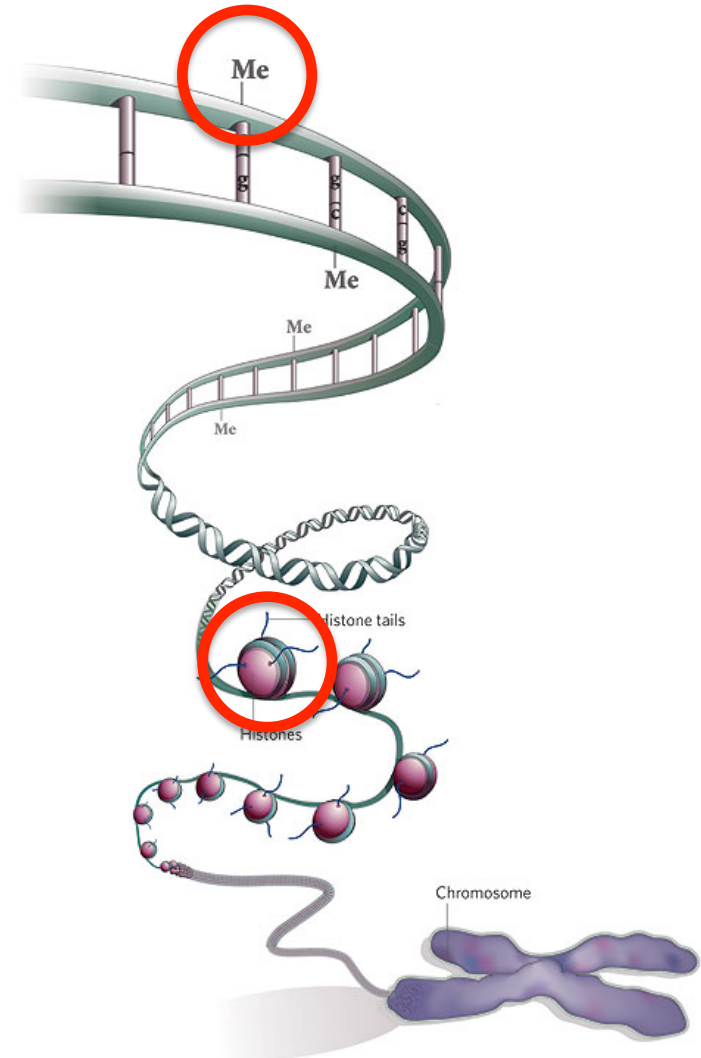
Tyson's Corner, VA

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

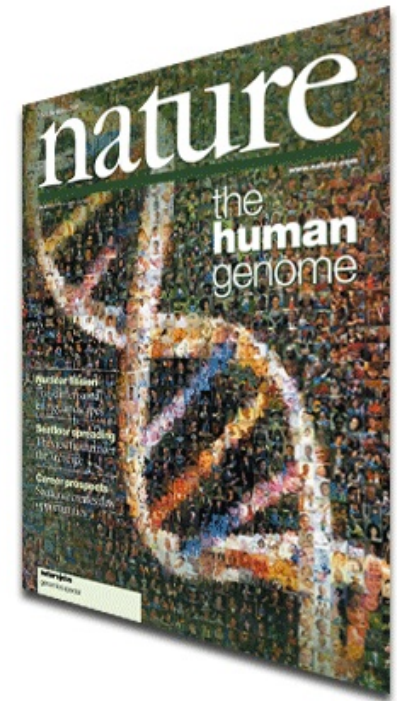
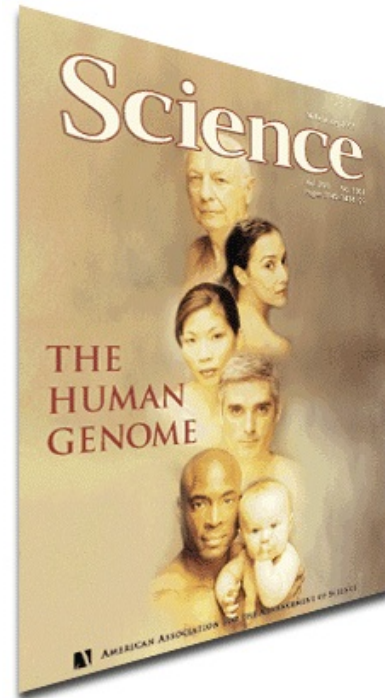


Why is the Epigenome Important in Toxicology and Risk Assessment?

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?

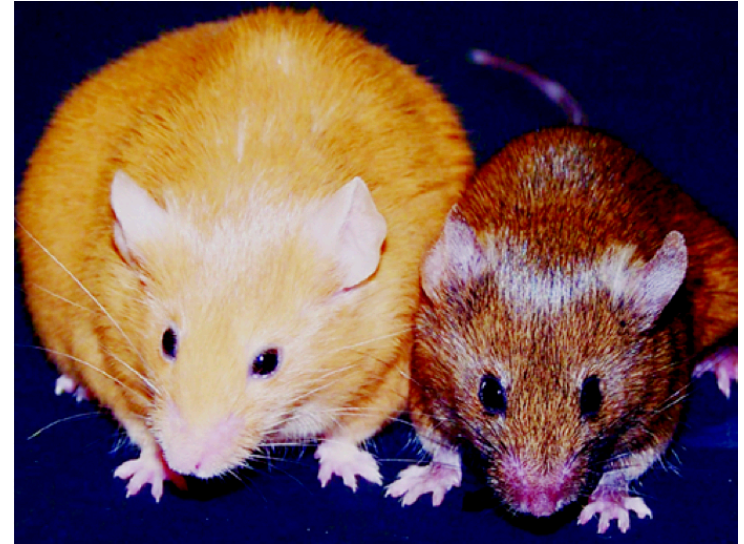
Susceptibility

- 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?

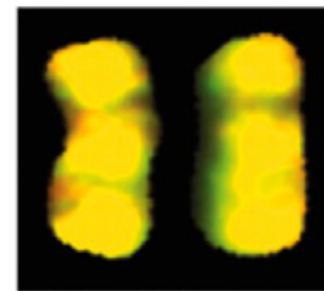


Environmental Susceptibility

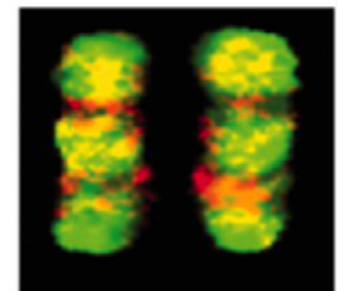
- Genetically identical mice from two mothers with different maternal diets
- Environmental exposure modifies gene expression through the epigenome
 - Modifies susceptibility
 - Mediates effects



Dana Dolinoy and Randy Jirtle

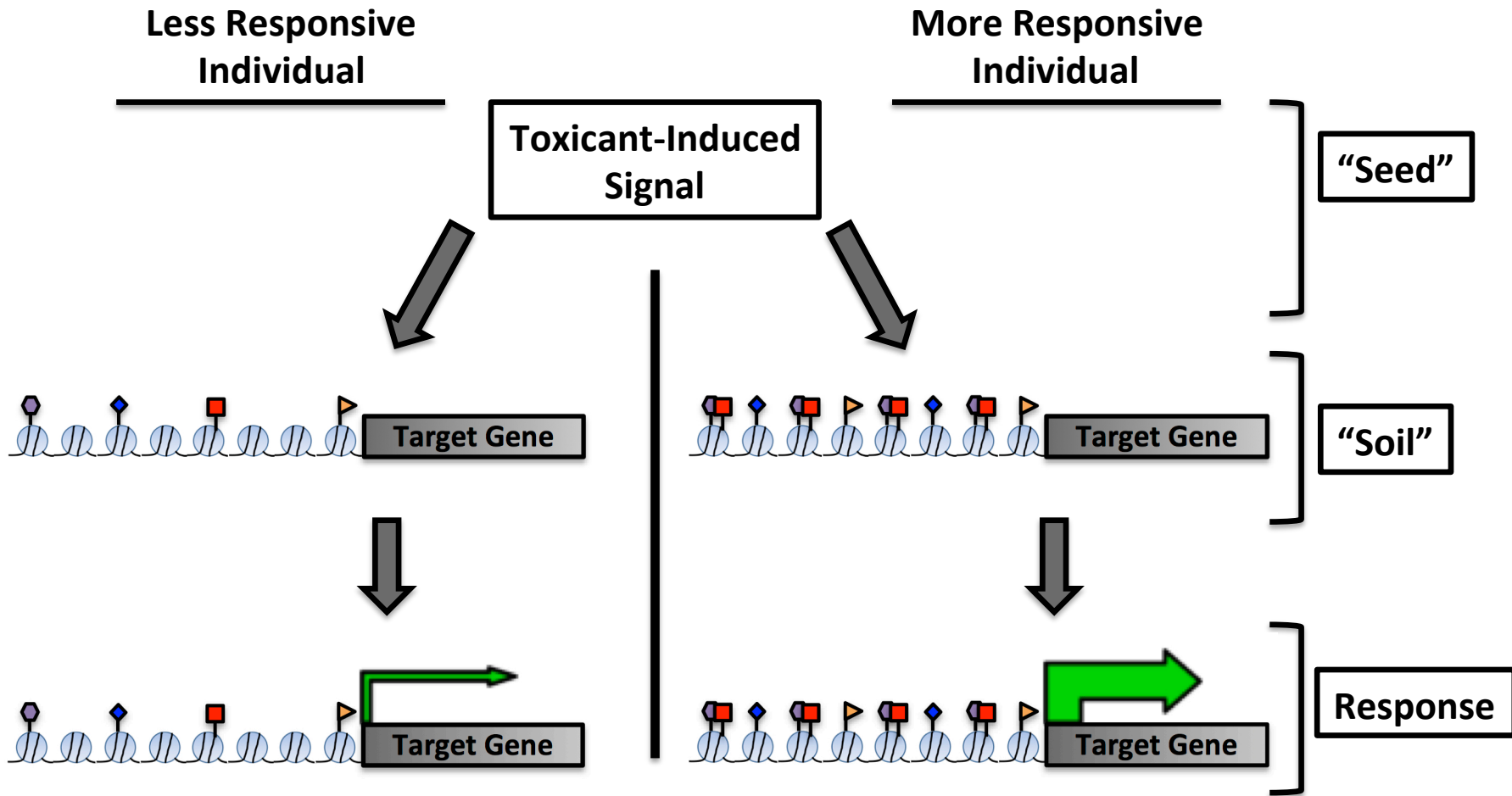


3-year old
identical twins



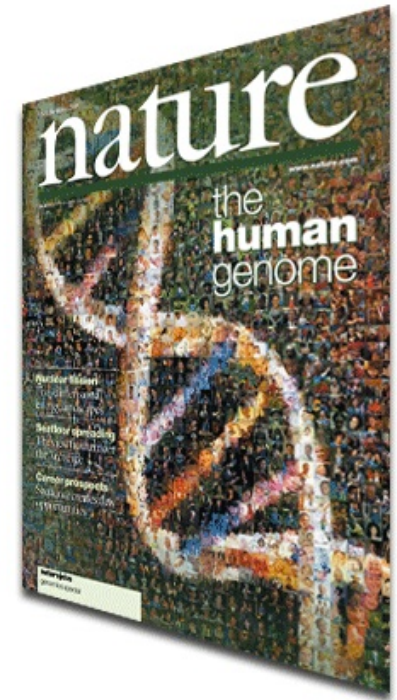
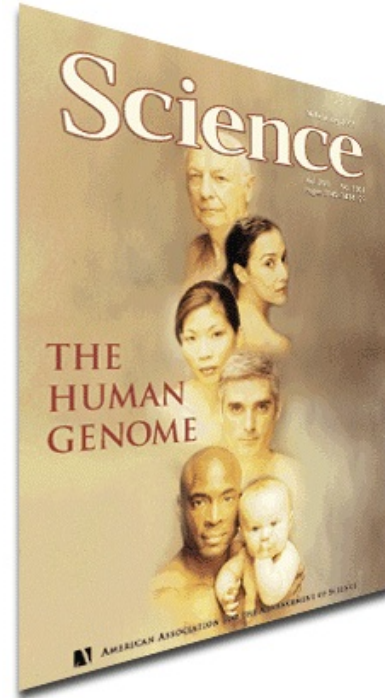
50-year old
identical twins

Epigenetic Seed and Soil Model



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

AUTUMN LEAVES - JULIUS MERGER

(1950 Jazz)

Key: G major (initially), E minor (later)

Chords: A-7, D7, Gmaj7, Cmaj7, F#-7 b5, B7, E-, F#-7 b5, B7 b9, E-, A-7, D7, Gmaj7, F#-7 b5, B7 b9, E-7, Eb7, D-7, Db7, Cmaj7, B7 b9, E-

- 9 -

BILL EVANS - "PORTRAIT IN JAZZ"

Epigenome

AUTUMN LEAVES - JULIUS MERGER

(1950 Jazz)

Bill Evans "Portrait in Jazz" 1954

Key: G major = 7: i: ii: iii: iv: v: vi: vii: viii: Bill Evans "Portrait in Jazz" 1954

Key: F#m

Annotations: A-7 (ii ST), D7 (V), Gmaj7 (I TONIC), Cmaj7 (I), F#-7 b5 (ii ST), B7 (V), E- (I), F#-7 b5 (ii ST), B7 b9 (V), E- (I), A-7 (ii ST), D7 (V), Gmaj7 (I), F#-7 b5 (ii ST), B7 b9 (V), E-7 (I), Eb7 (II), D-7 (III), Db7 (IV), Cmaj7 (I), B7 b9 (V), E- (I)

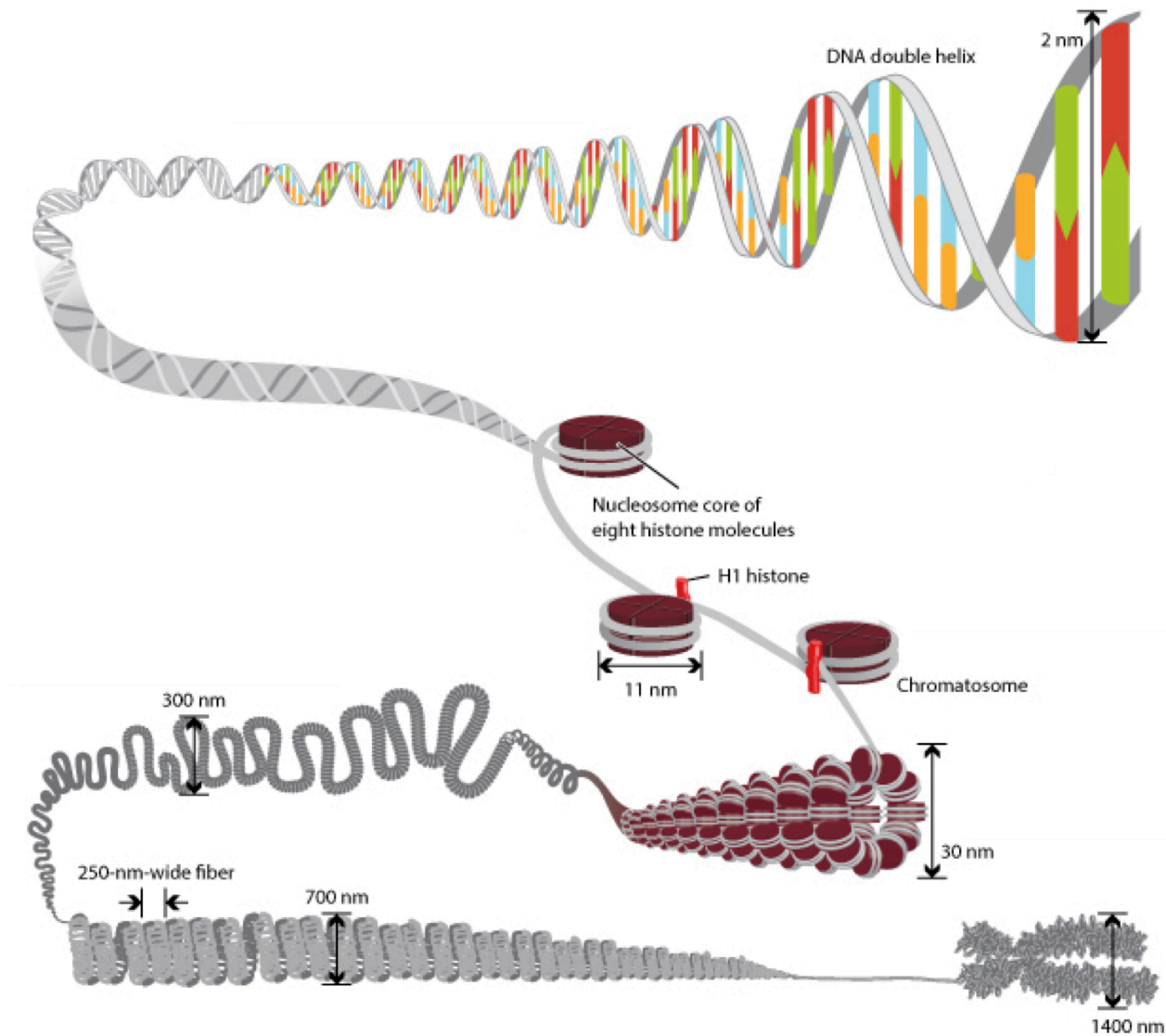
IV Subdominant opt - submediant of G tonic of Eb

Bill Evans - "Portrait in Jazz"

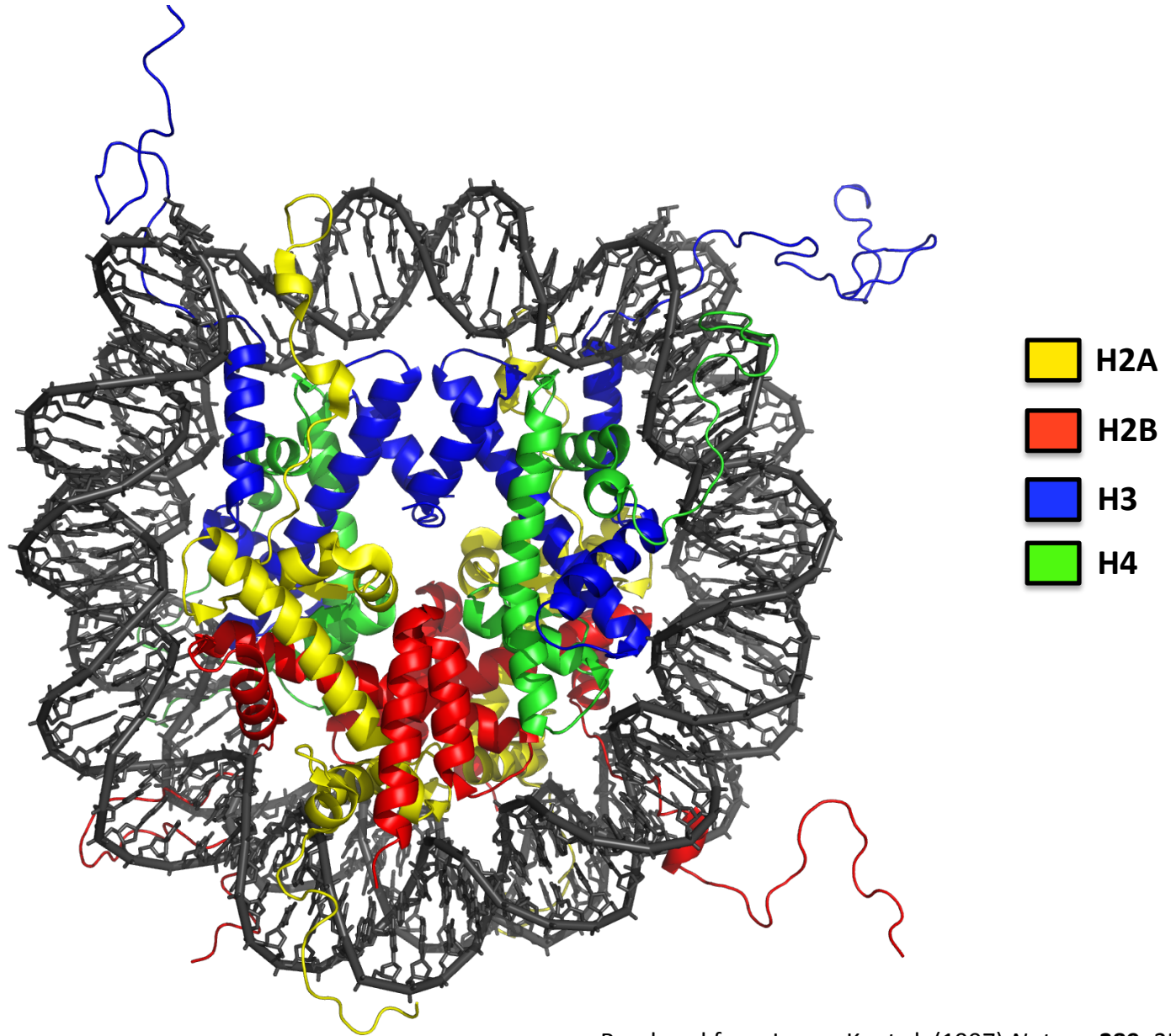
G A B C D E F# G
I II III IV V VI VII VIII

36.

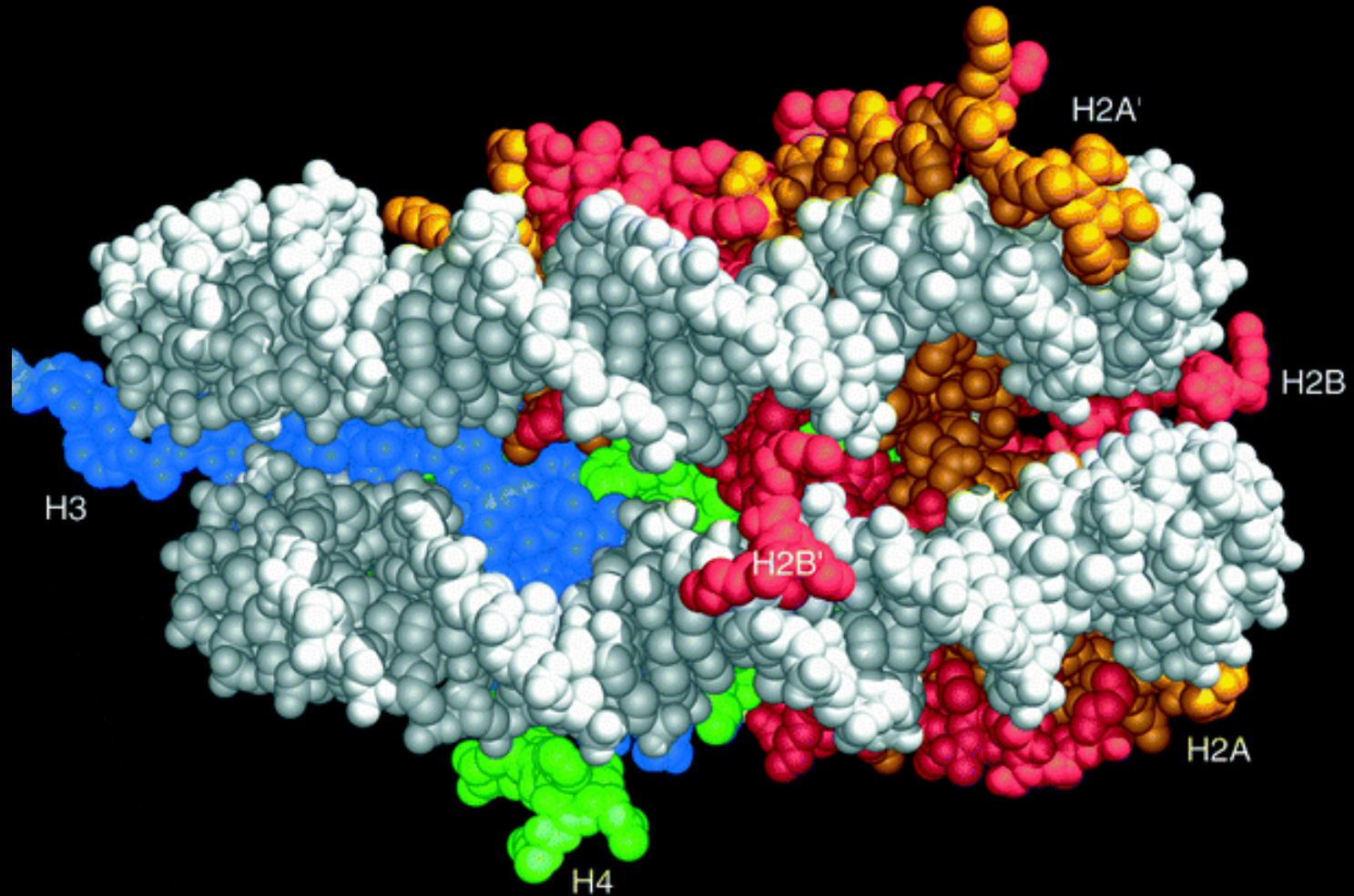
Storage and Accessibility



The Fundamental Unit of Chromatin

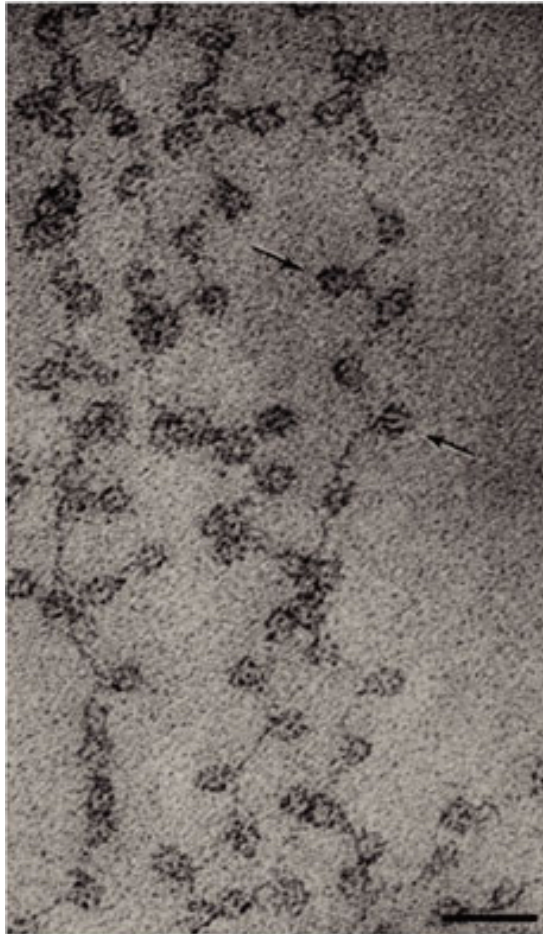


Histone Tail-DNA Interactions

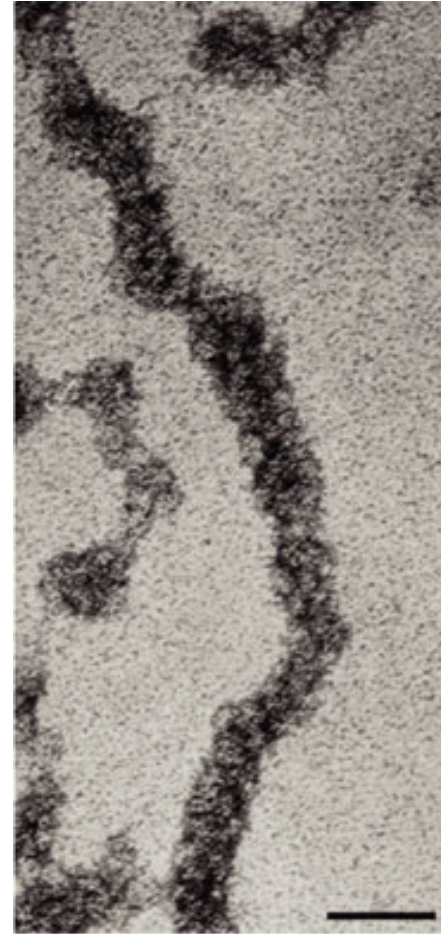


Chromatin States

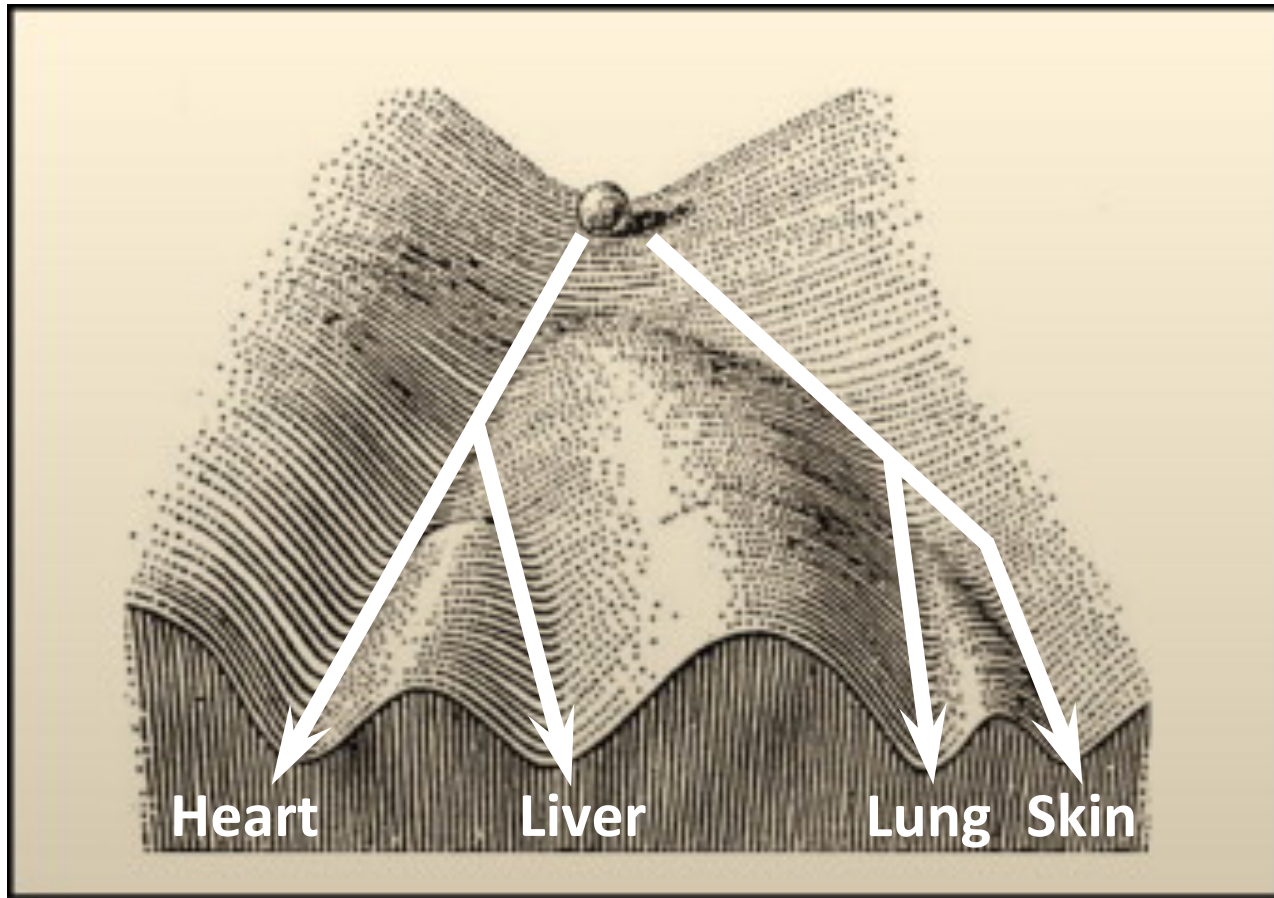
Open - Euchromatin



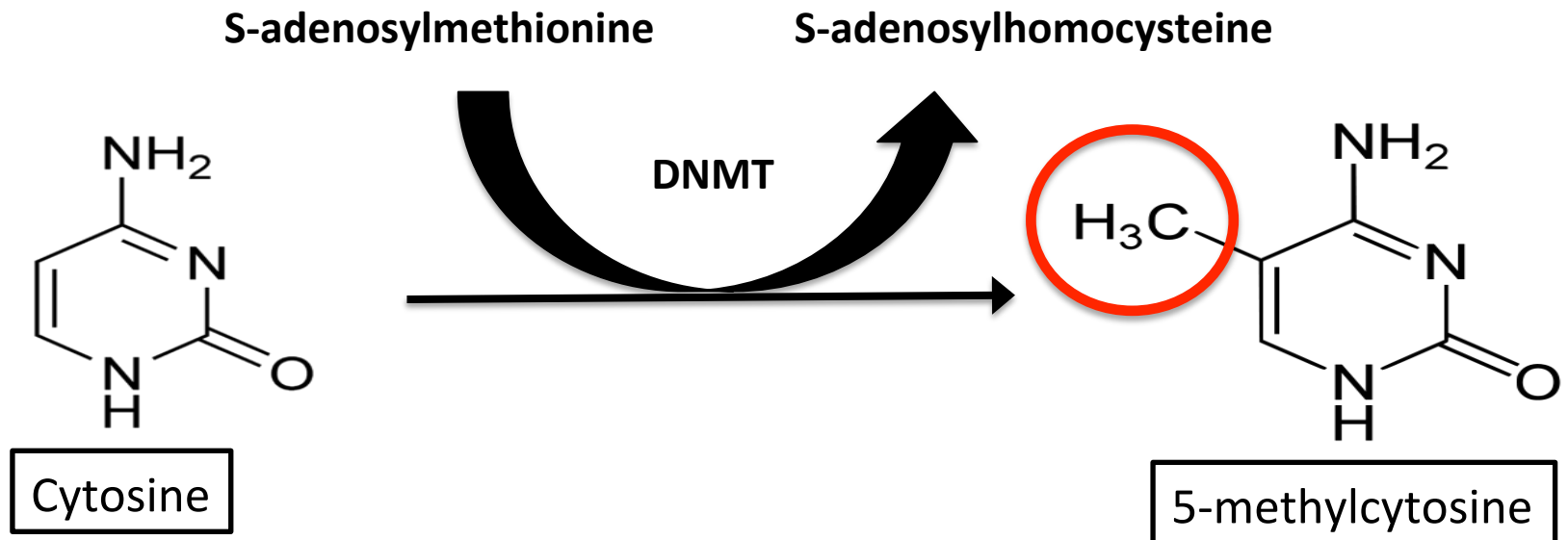
Closed - Heterochromatin



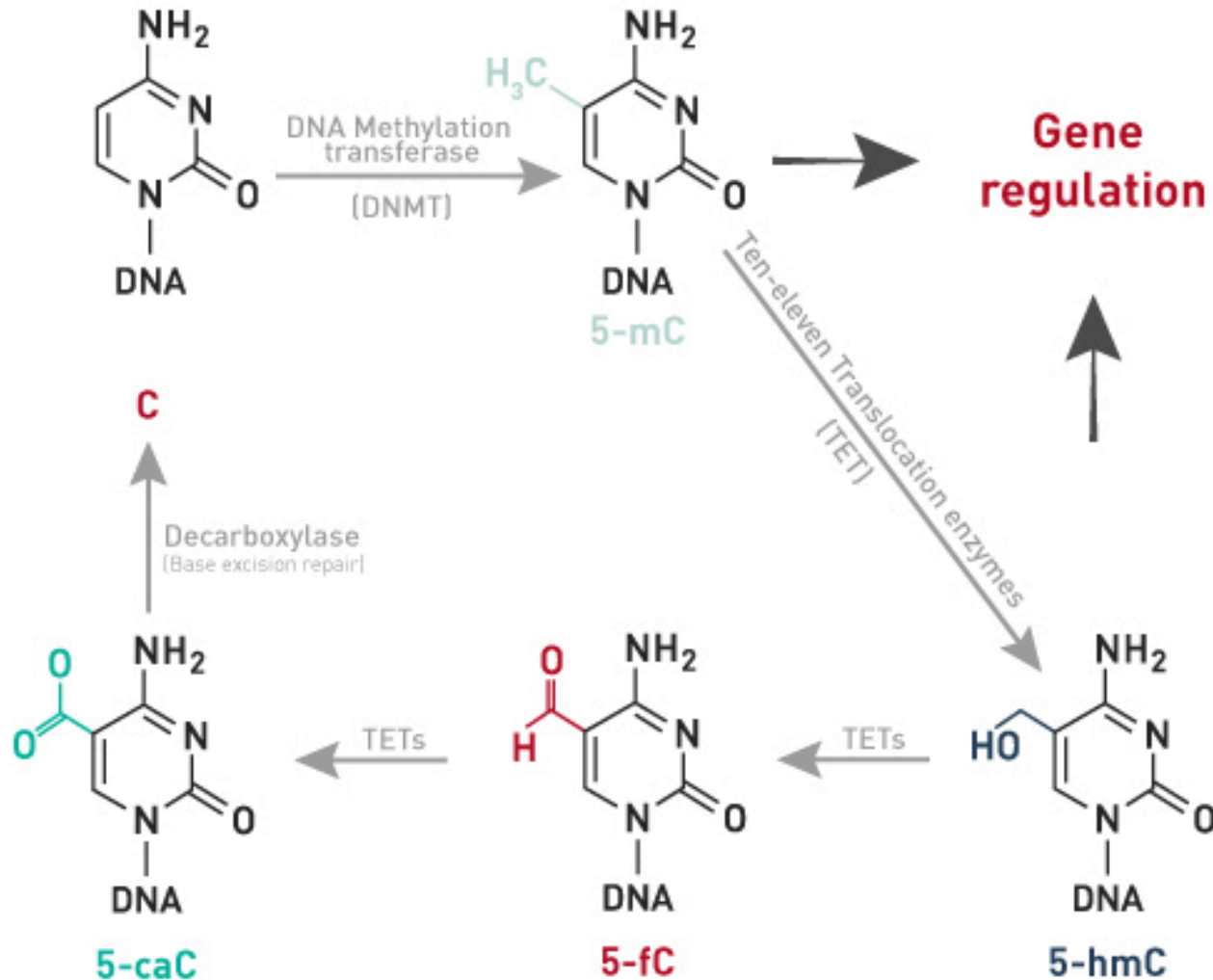
What Makes Cells Within the Same Individual Different?



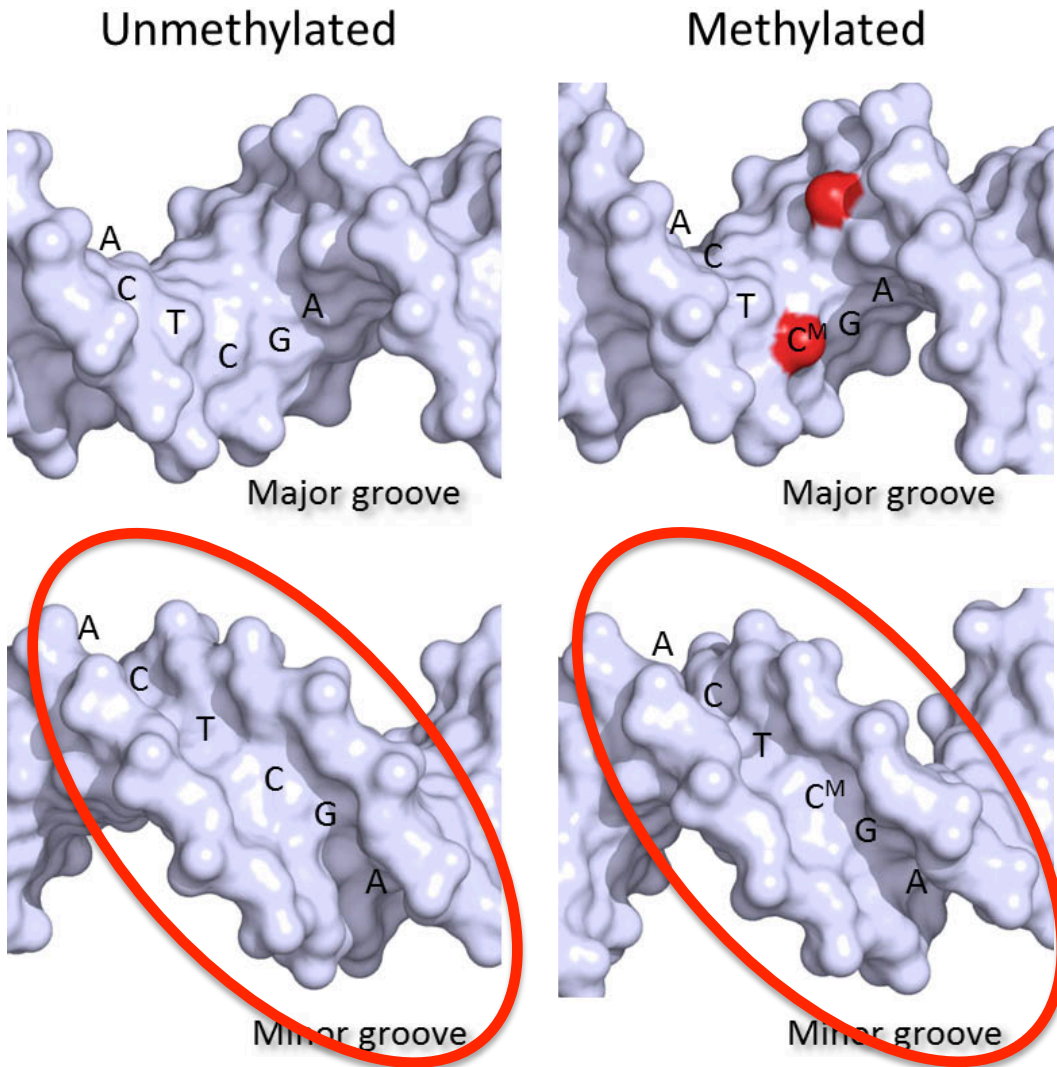
DNA Methylation



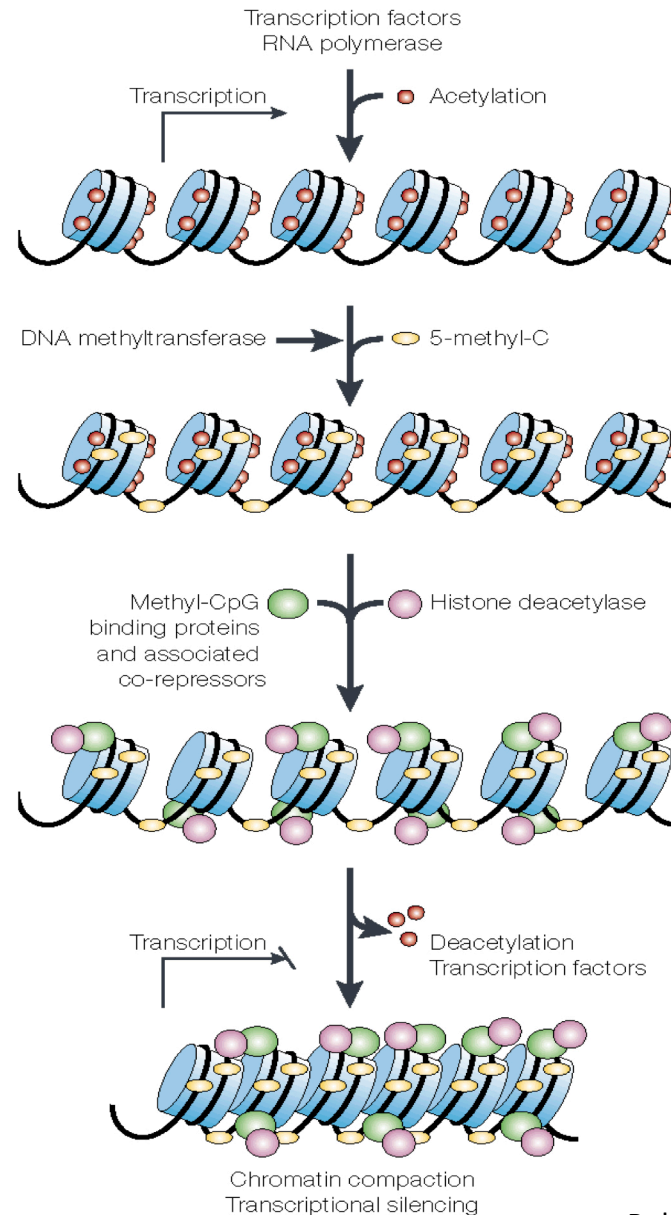
Oxidation of 5-methylcytosine



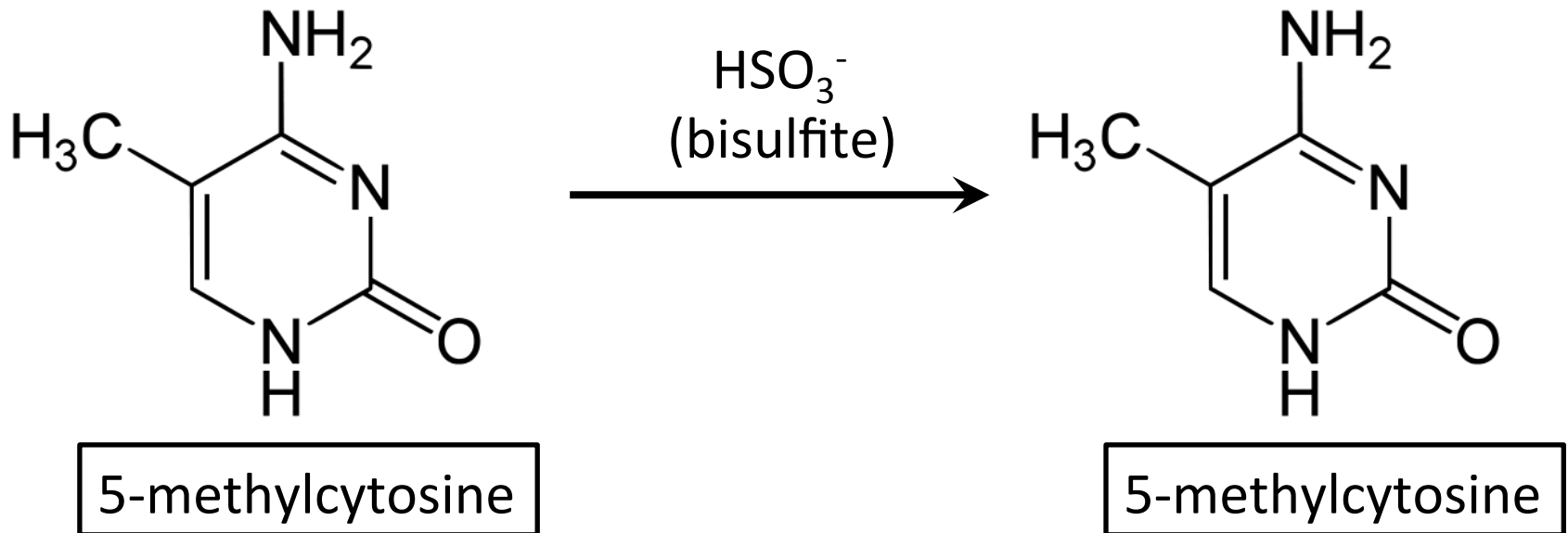
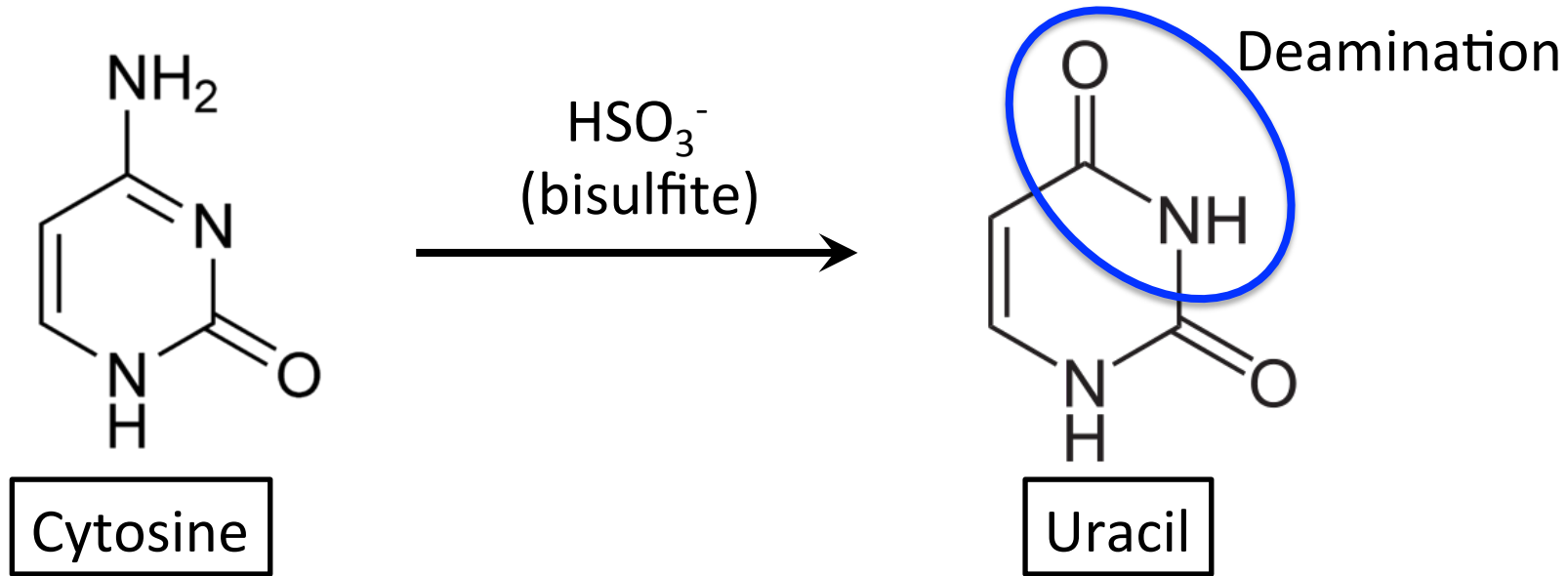
DNA Methylation Alters DNA Structure



DNA Methylation in Gene Silencing



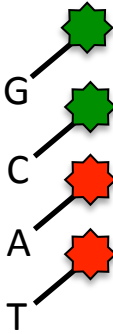
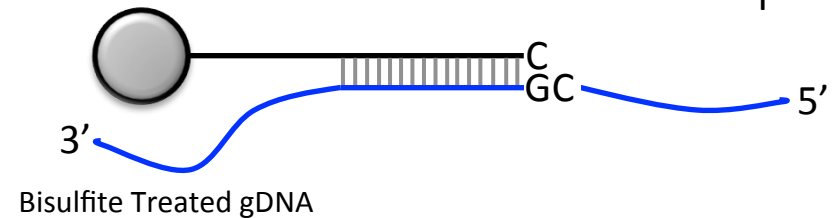
Bisulfite Conversion



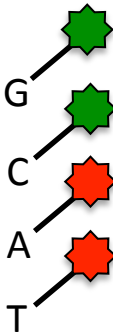
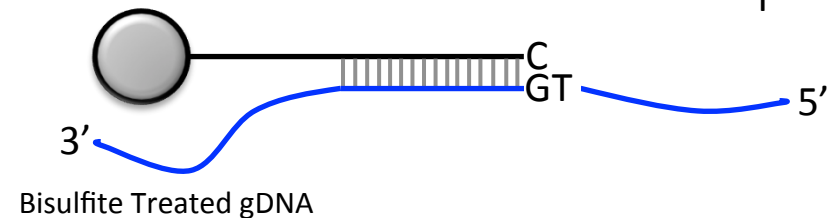
DNA Methylation Bead Array

- 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

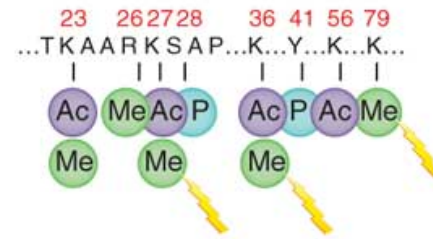
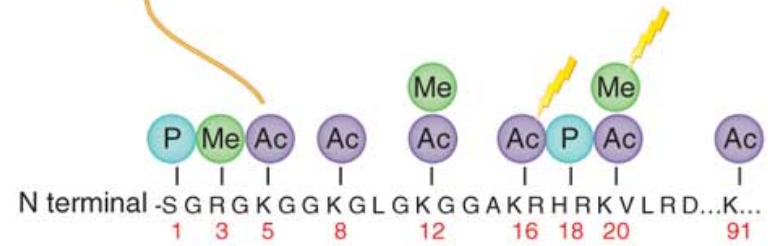
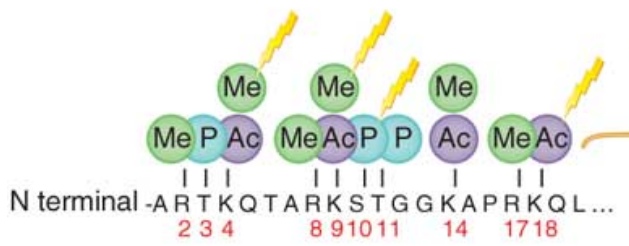
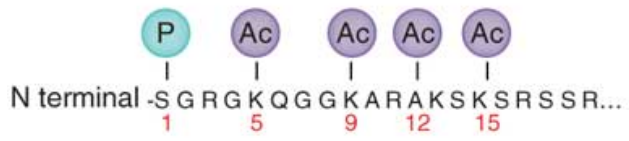


The "Histone Code"

>130 Unique histone modifications

H2A

H2B

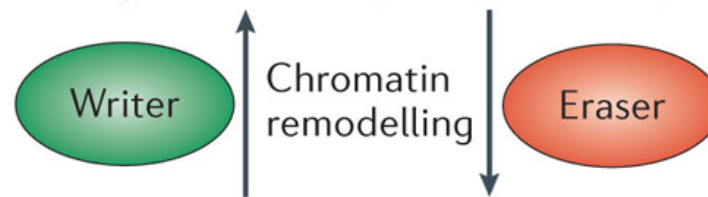
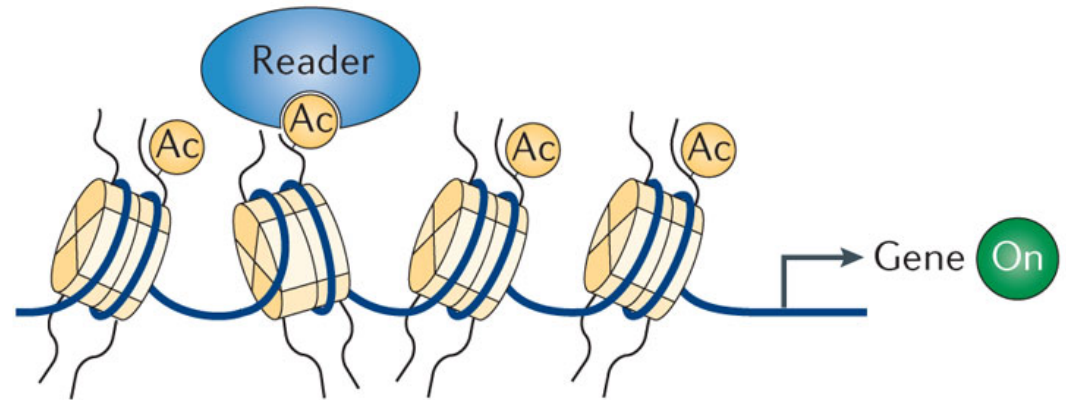


H3

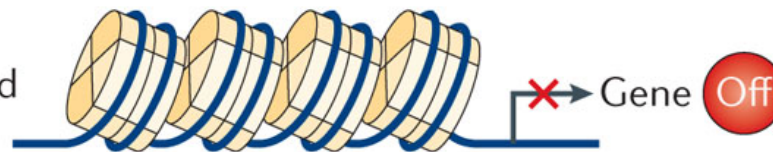
H4

Histone Acetylation

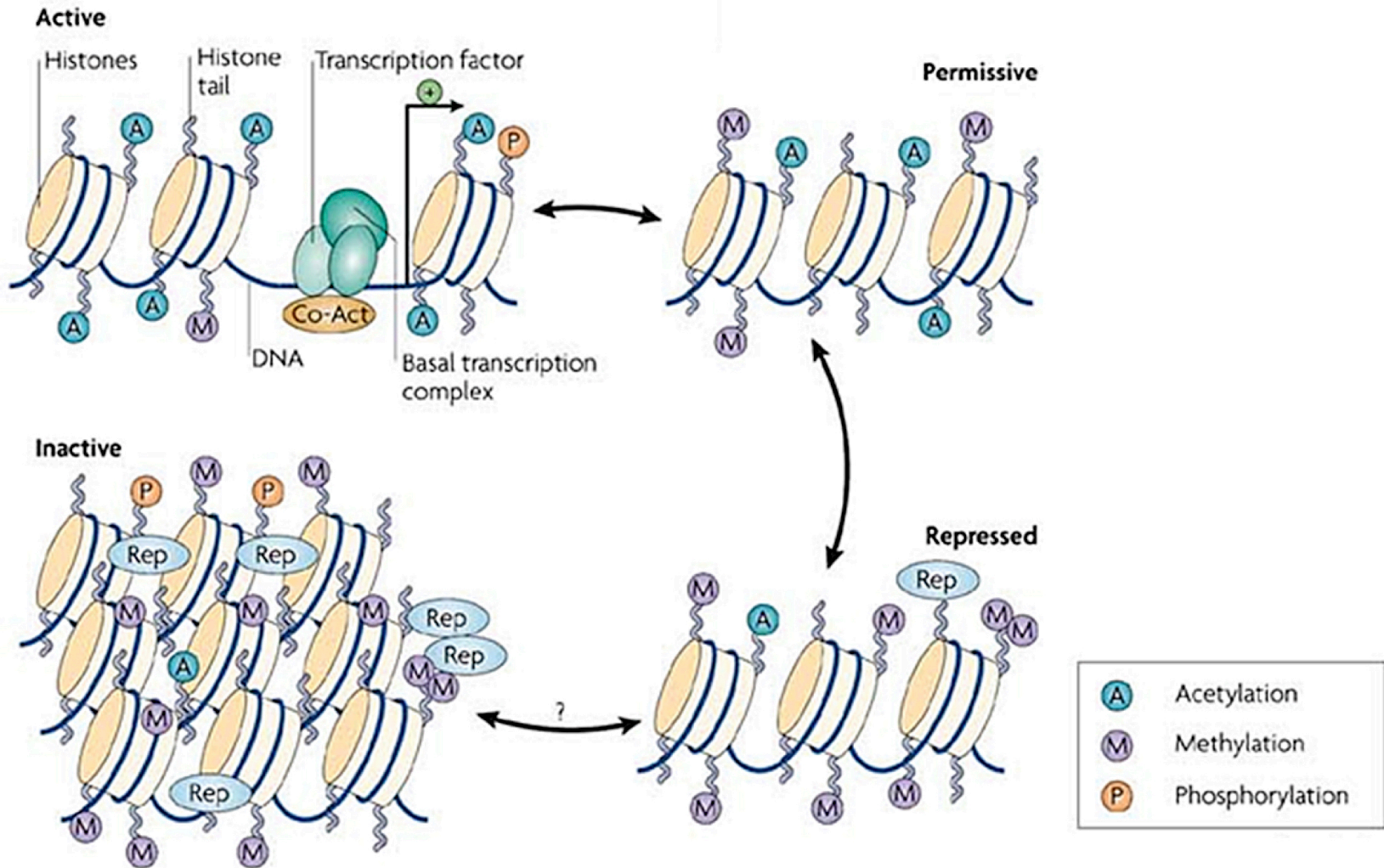
Acetylated chromatin
Open and transcriptionally active



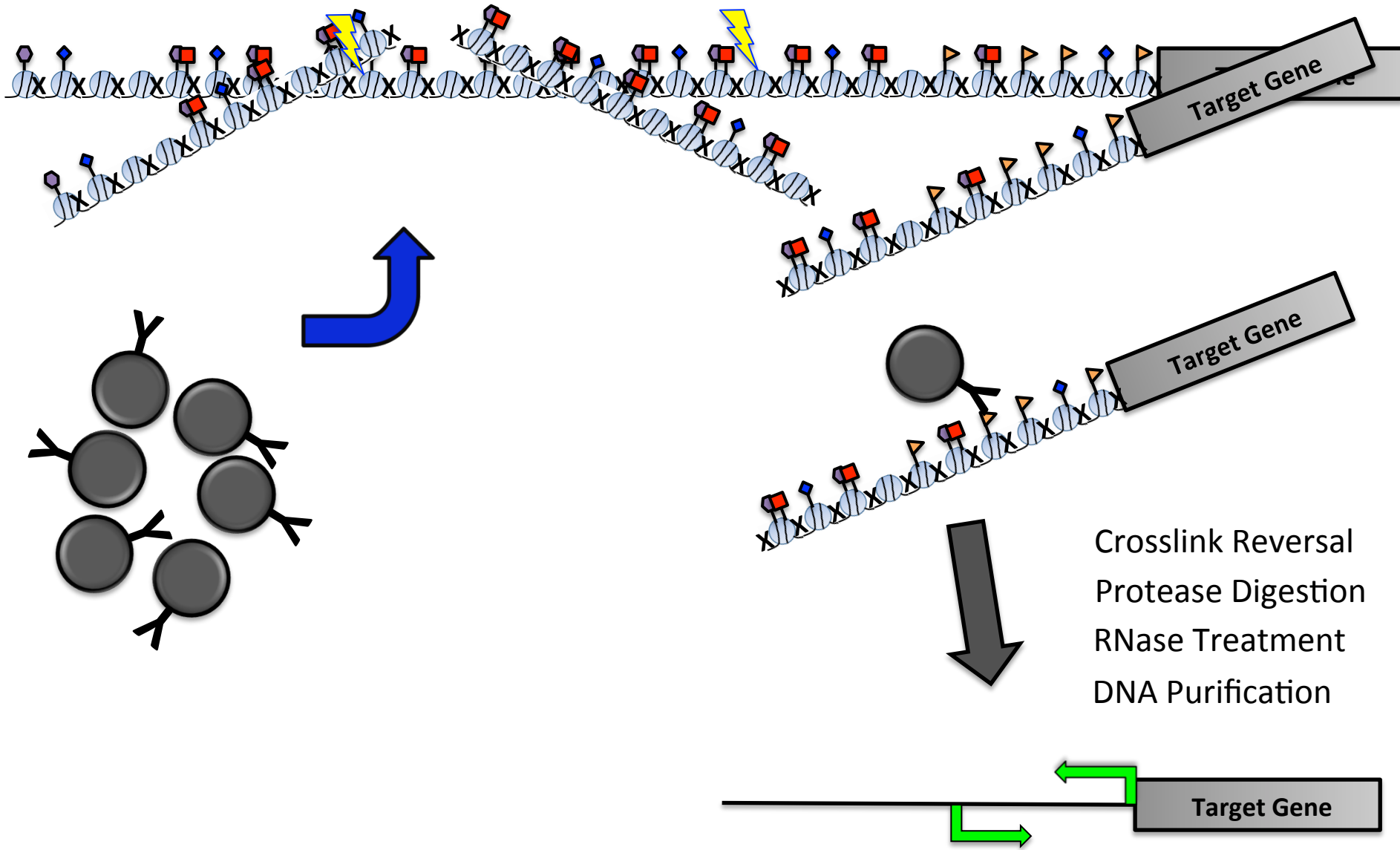
Deacetylated chromatin
Compact and transcriptionally repressed



Chromatin States



Chromatin Immunoprecipitation (ChIP)



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?**