

Genomics, Bioinformatics & Medicine

<http://biochem158.stanford.edu/>

Epigenetics

<http://biochem158.stanford.edu/Epigenetics.html>



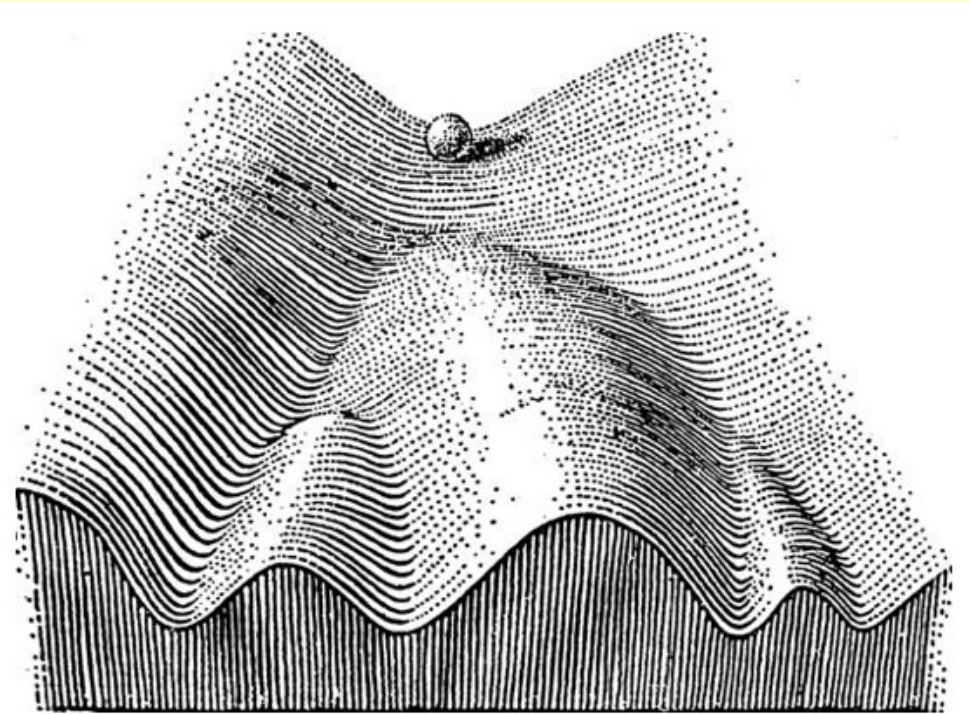
Doug Brutlag

Professor Emeritus of Biochemistry & Medicine
Stanford University School of Medicine

What is Epigenetics?

- C.H. Waddington coined the term epigenetics to mean above or in addition to genetics to explain differentiation.
- How do different adult stem cells know their fate?
 - Myoblasts can only form muscle cells
 - Keratinocytes only form skin cells
 - Hematopoietic stem cells only become blood cells
 - But all have identical DNA sequences.

C.H. Waddington



Waddington's Epigenetic Landscape

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- How can identical twins have different natural hair colors?

Identical Twins with Different Hair Color



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- How can a single individual have two different eye colors?

Mosaicism: An Individual with Two Different Eye Colors



“Diego”

Mosaicism: An Individual with Two Different Eye Colors



“Josie Too”

Mosaicism: An Individual Eye with Two Colors



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- How can identical twin litter mates show different coat colors?

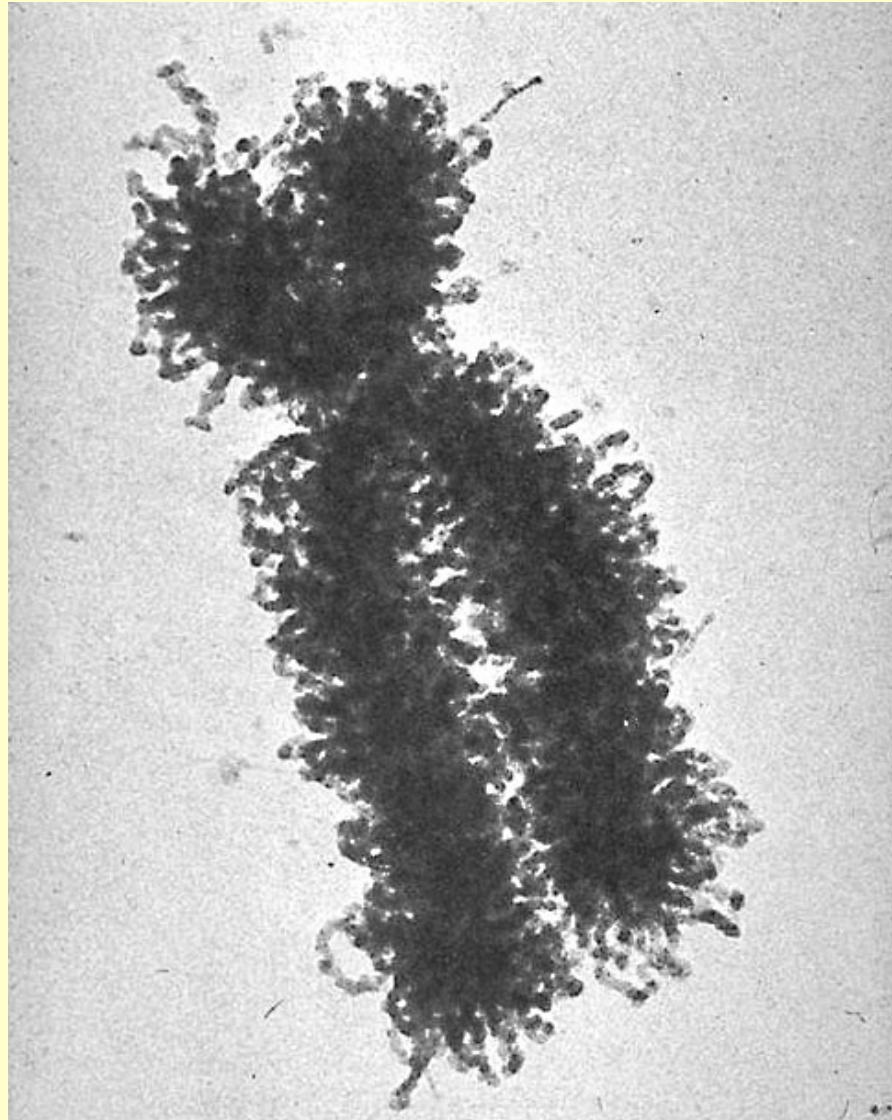
Coat Colors of Genetically Identical Agouti Mice Litter Mates



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- How can a single individual have two different eye colors?
- How can identical twin litter mates show different coat colors?
- How can just paternal or maternal traits be expressed in offspring? This is called genetic imprinting.
- How can females express only one X chromosome per cell?
- How can acquired traits be passed on to offspring?
- Some changes in gene expression that are, in fact, heritable!

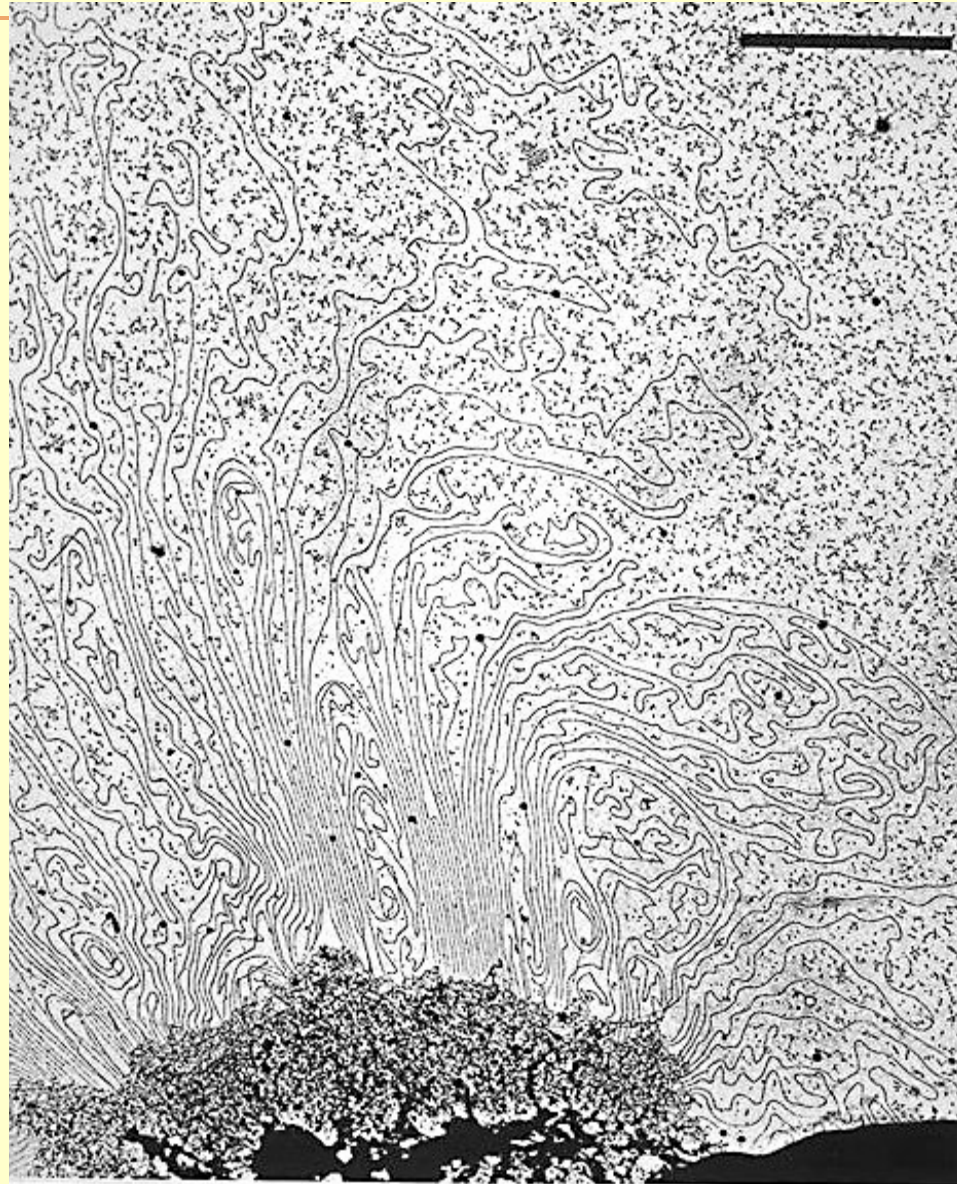
Human Mitotic Chromosome



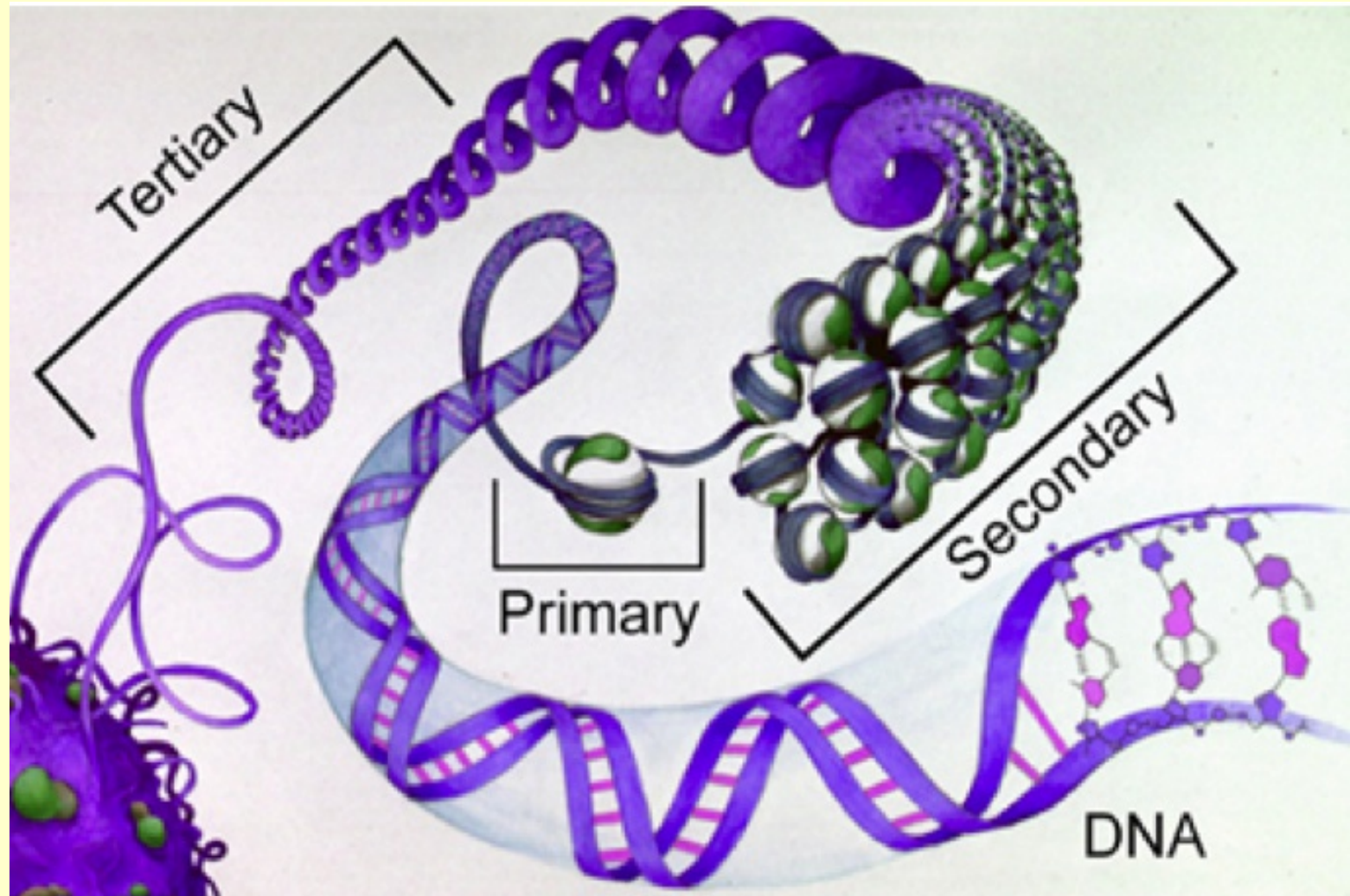
DNA in a Human Chromosome



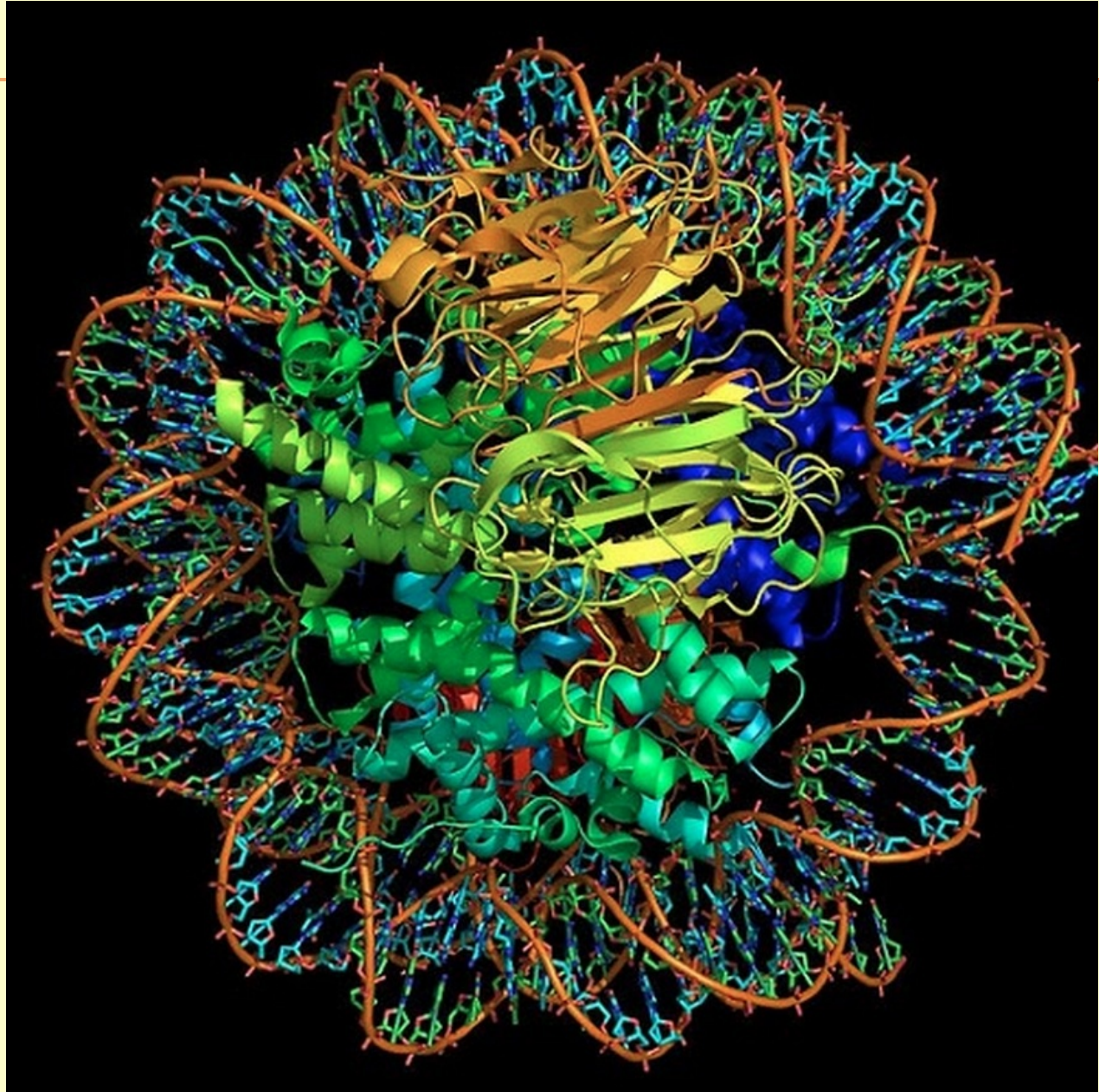
DNA in a Human Chromosome



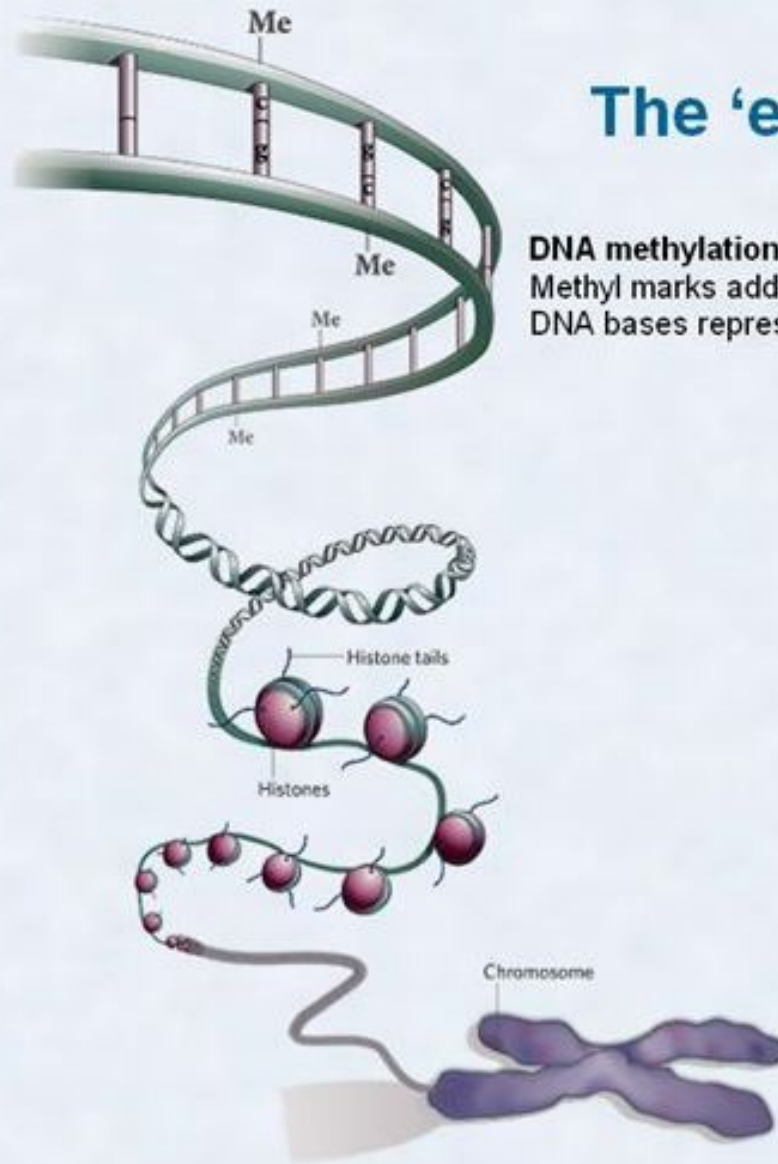
Three Levels of Folding of DNA in Chromatin



Nucleosome Core Structure



DNA Methylation & the Epigenetic Code



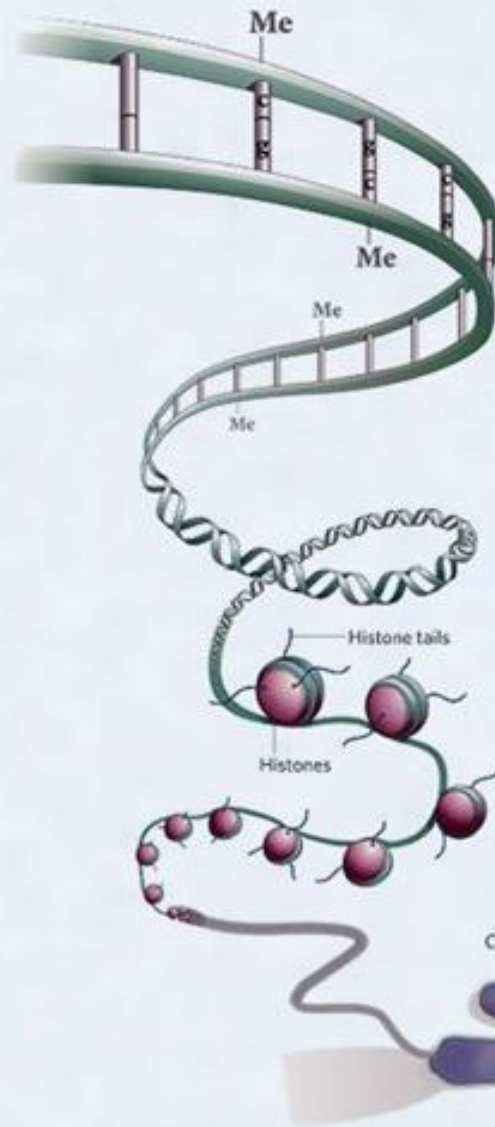
The 'epigenetic' code

DNA methylation

Methyl marks added to certain DNA bases repress gene activity



DNA Methylation & Histone Modifications Form the Epigenetic Code



The 'epigenetic' code

DNA methylation

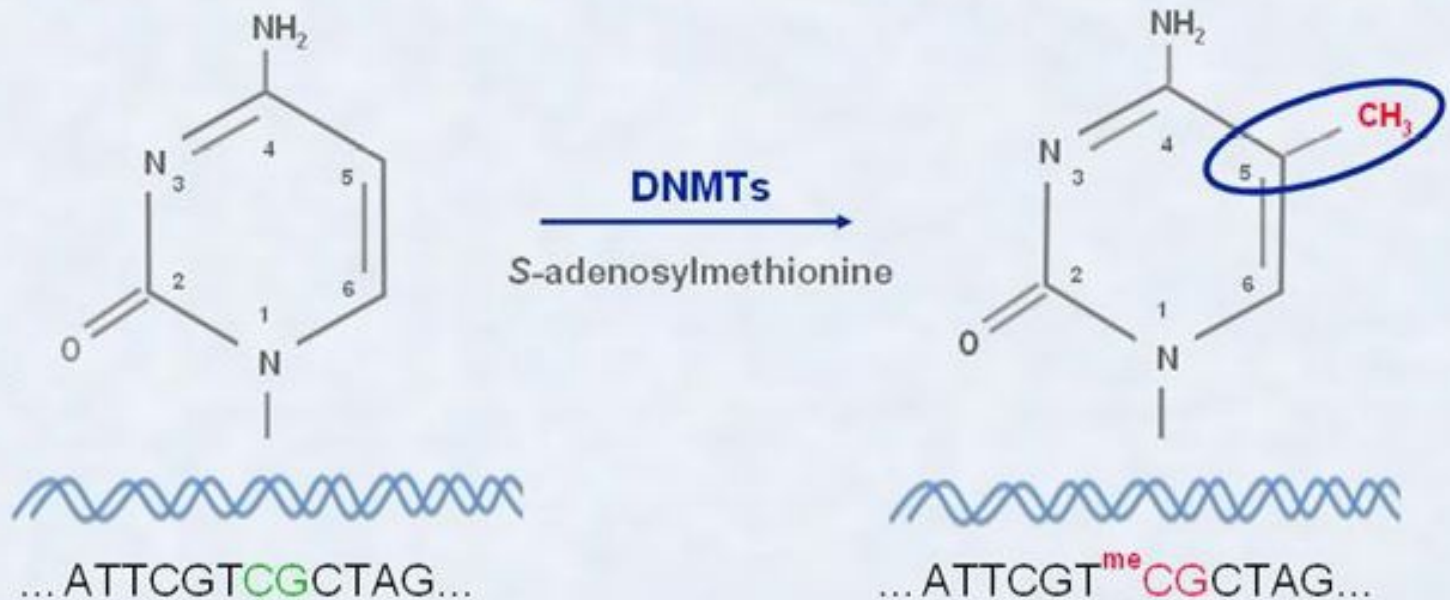
Methyl marks added to certain DNA bases repress gene activity

Histone modification

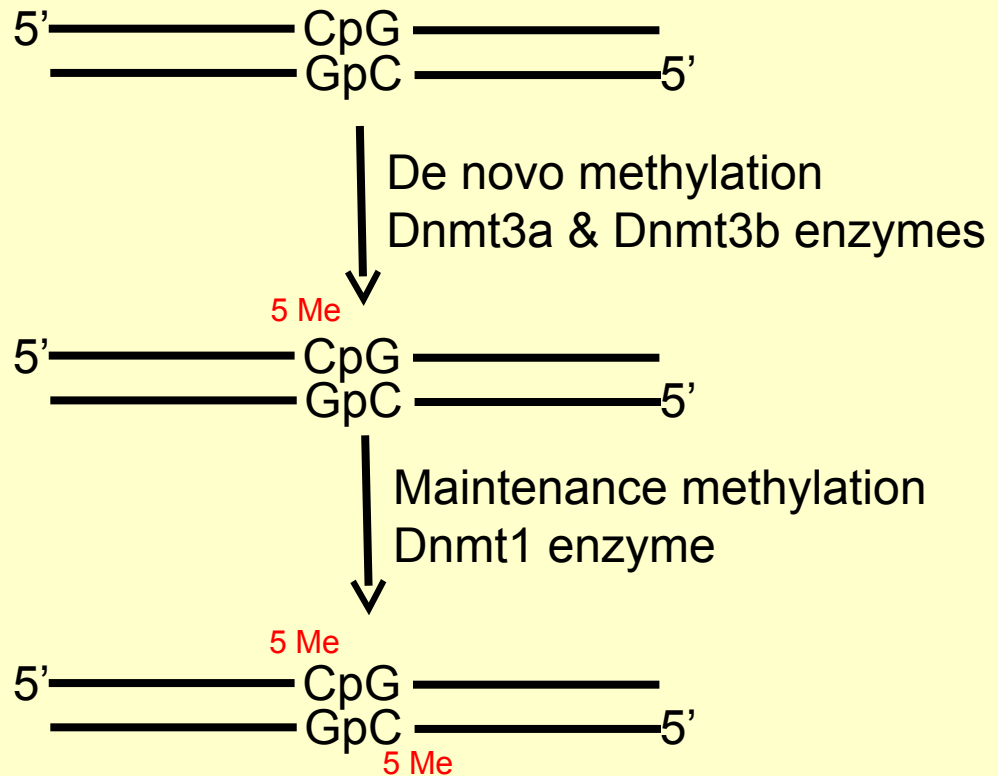
A combination of different molecules can attach to the "tails" of proteins called histones. These alter the activity of the DNA wrapped around them

Methylation of Cytosine in DNA

Cytosine methylation

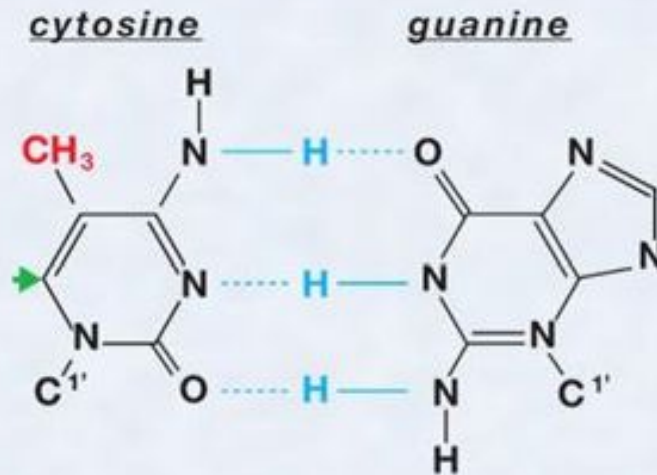


Only Cs in CG sequences are Methylated

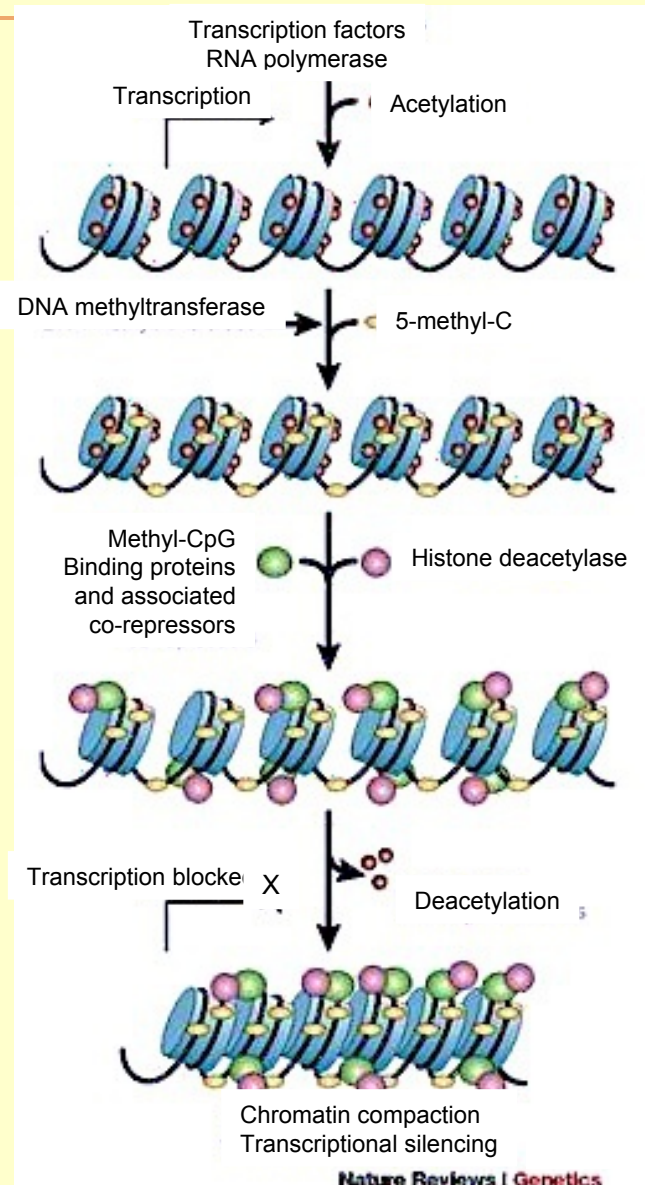


5-Methyl Cytosine in DNA

Cytosine methylation



Cytosine Methylation Maintains Inactive-Condensed Chromatin State

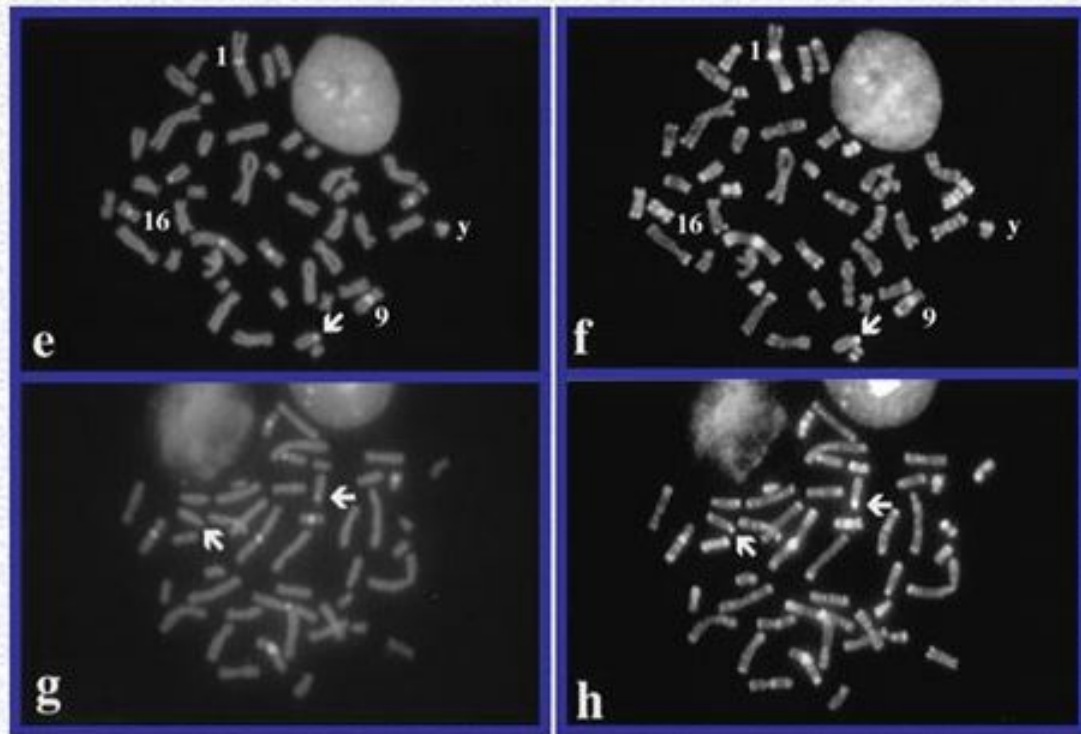


Alex Meissner
Henry Stewart Talks

5-Methyl Cytosine is Found in Heterochromatic Regions

The distribution of cytosine methylation in mammals

- Heterogeneity visible at cytogenetic scale
- Associated with heterochromatic regions

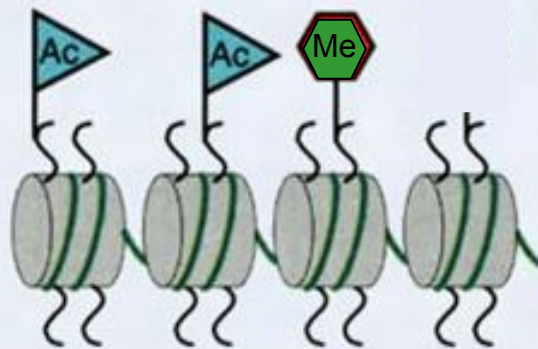


PMID: 9609658

Structure & Epigenetics of Euchromatin versus Heterochromatin

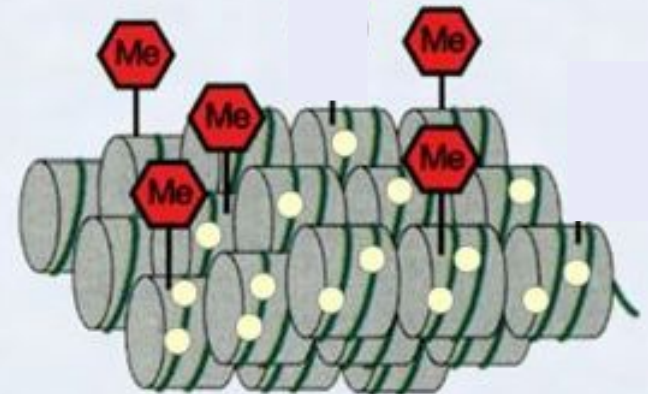
DNA methylation and histone modifications help to compartmentalize the genome into domains of different transcriptional potentials

Euchromatin



- High histone acetylation
- Low DNA methylation
- H3-K4 methylation

Heterochromatin



- Low histone acetylation
- Dense DNA methylation
- H3-K9 methylation

Histone Code



Histone Code

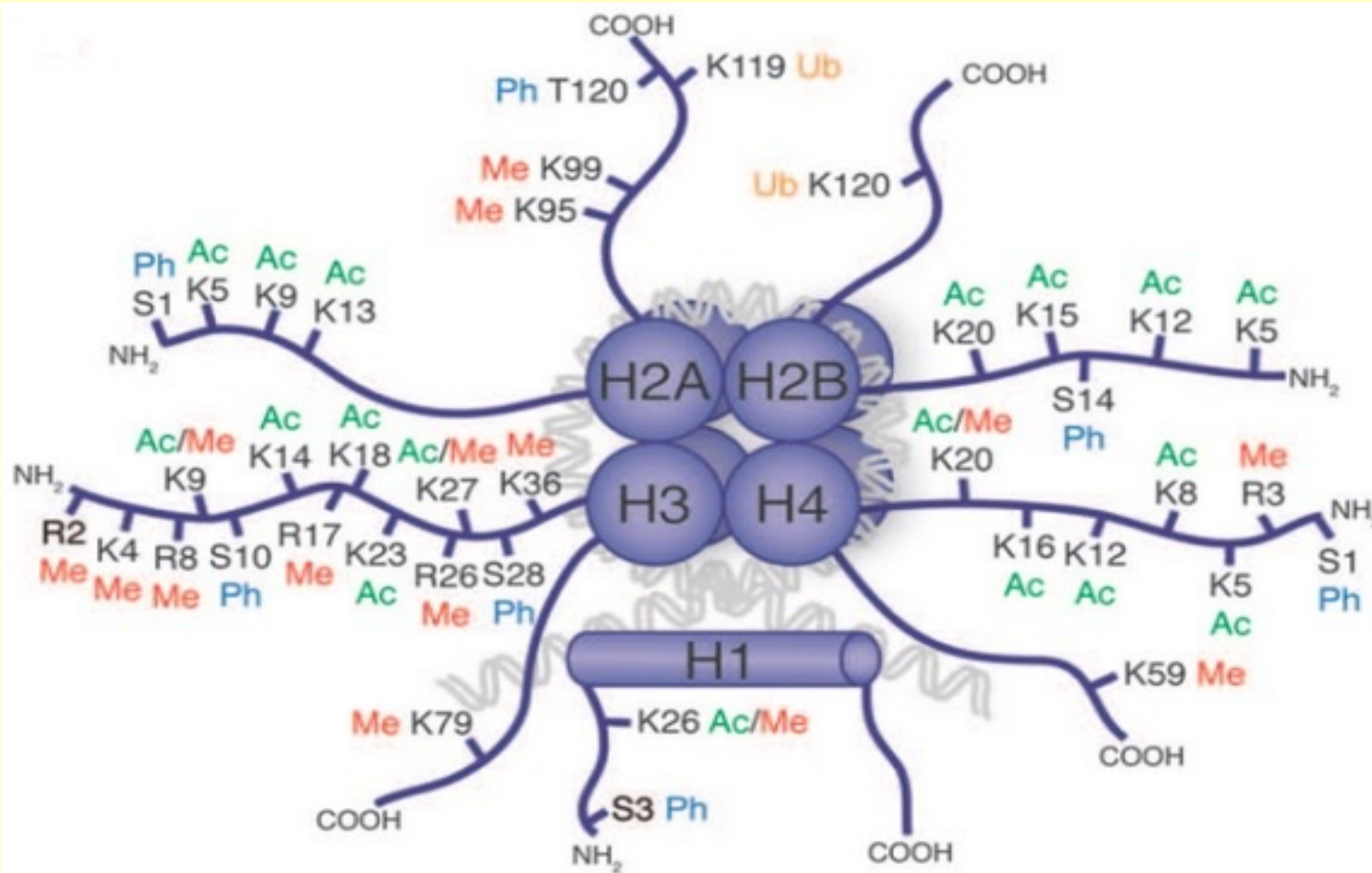


FIGURE 4.

Nucleosome with histone posttranslational modifications (Adapted from 1)

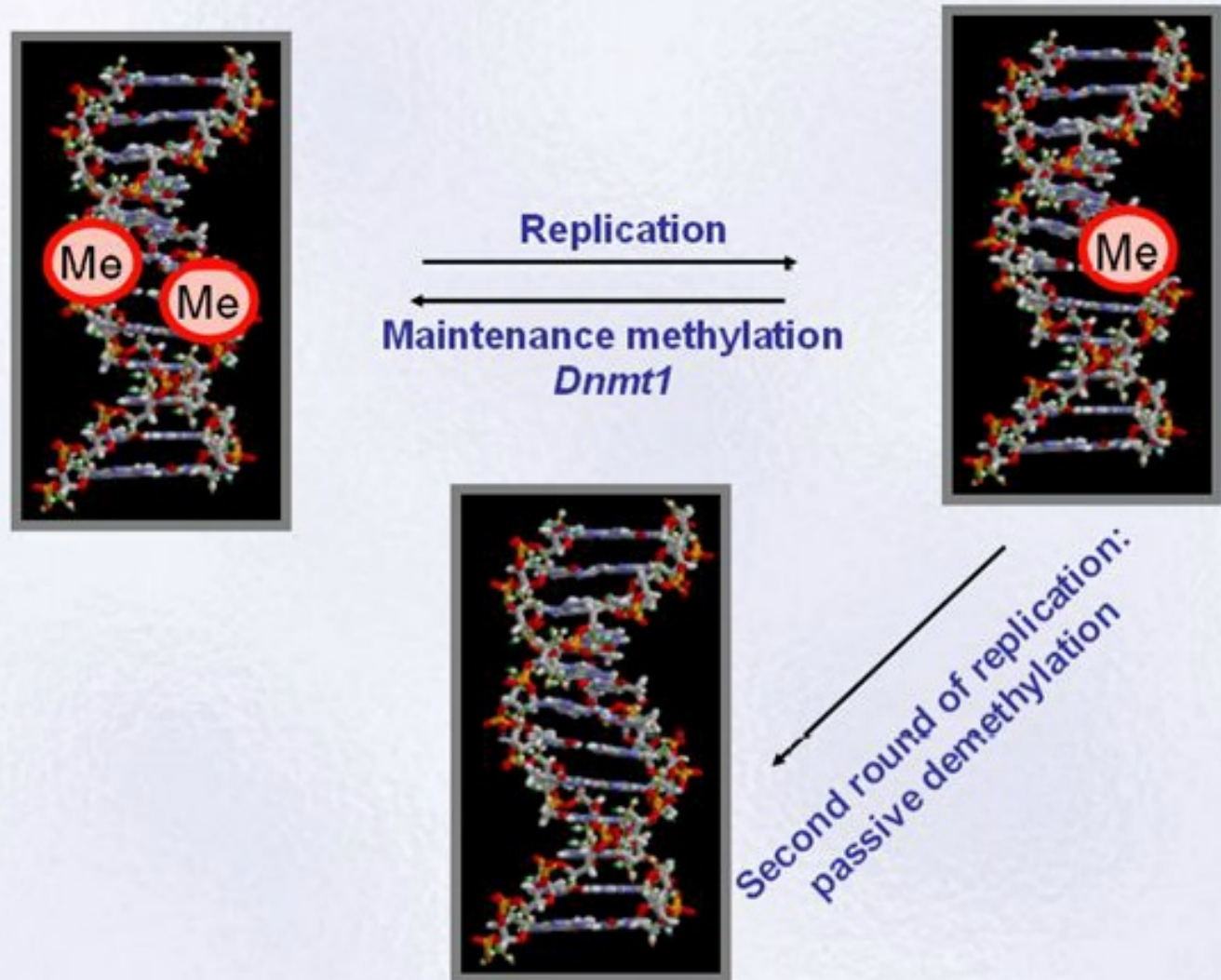
Maintenance of Cytosine Methylation

Establishment and maintenance



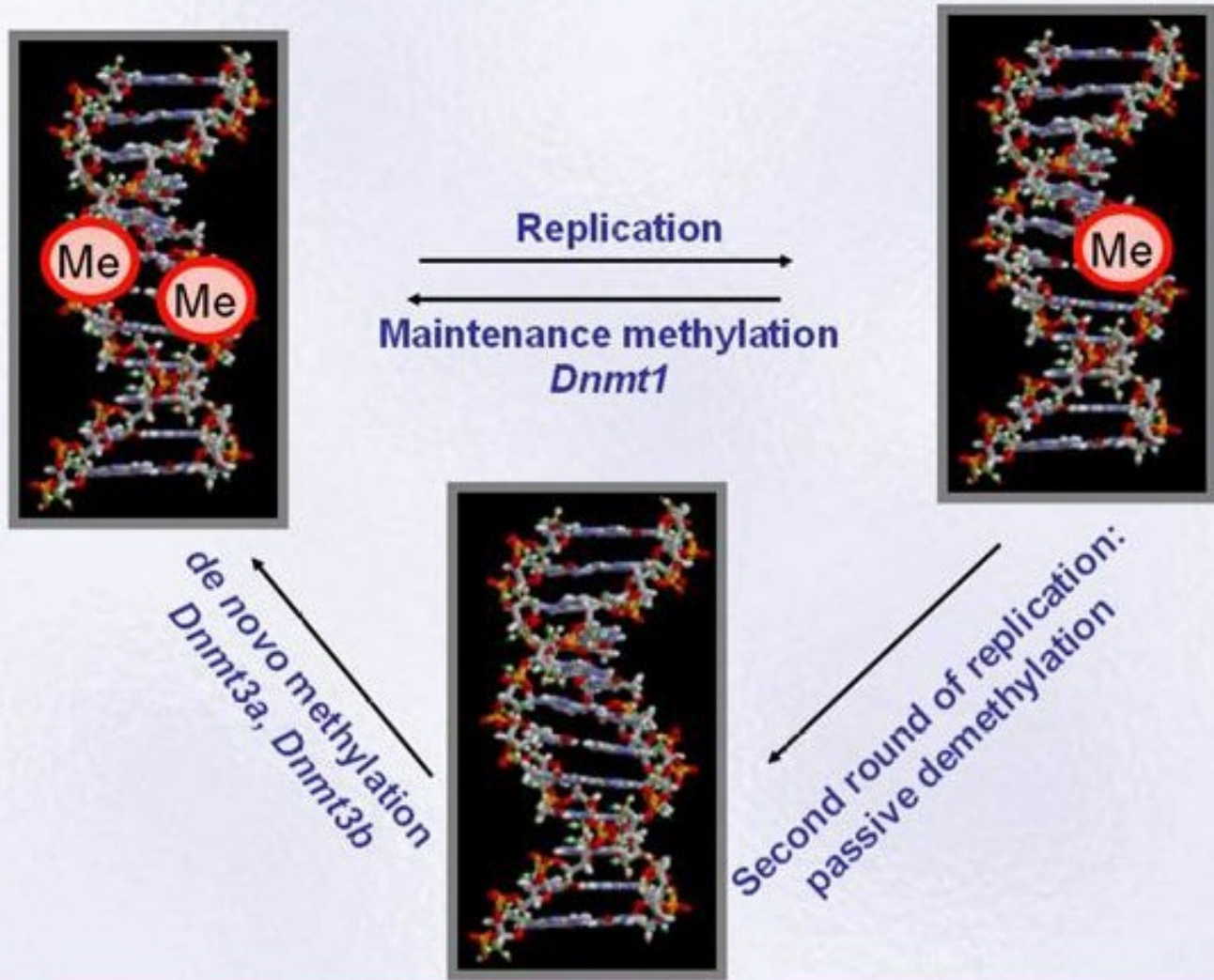
Passive Demethylation of 5-Methyl-Cytosine

Establishment and maintenance



Establishment and Maintenance of Cytosine Methylation

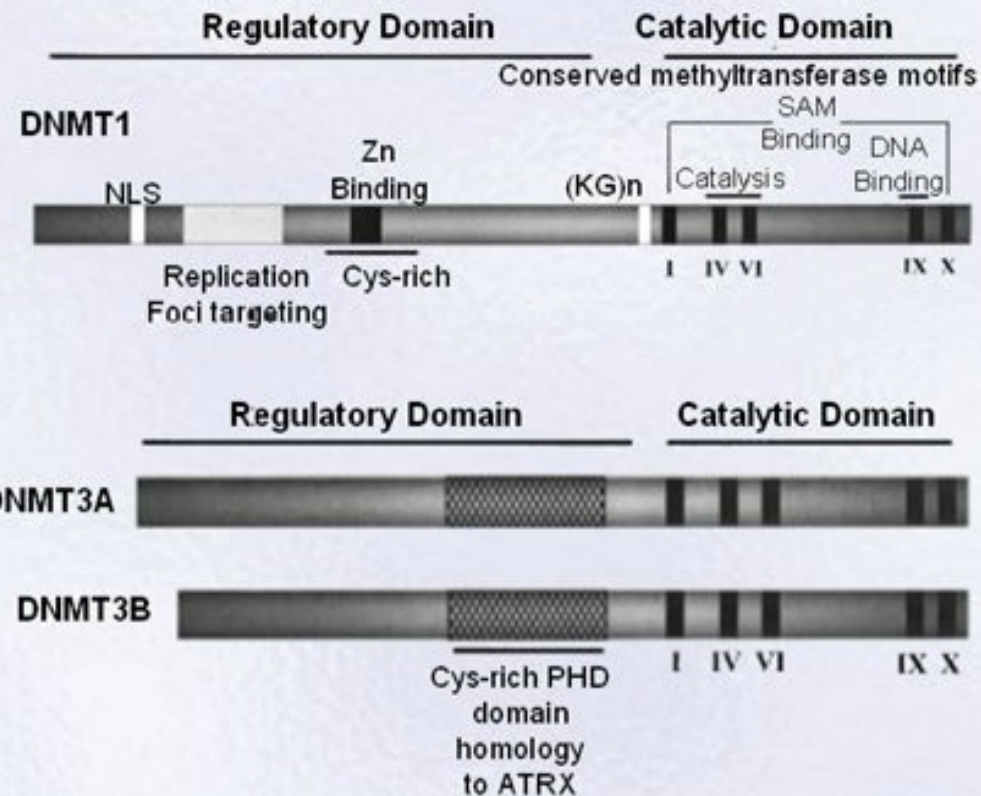
Establishment and maintenance



Some DNA Methyl Transferases are Essential

Mammalian Dnmts are essential

- Dnmt1: embryonic lethal
- Dnmt2: no obvious effect
- Dnmt3a: perinatal death
- Dnmt3b: embryonic lethal
- Dnmt3l: no imprints

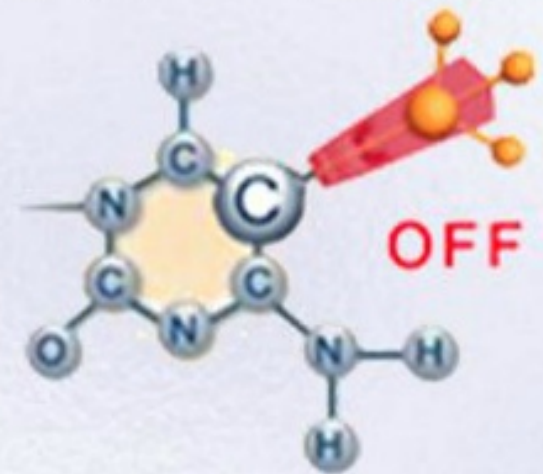


Robertson, KD, *Oncogene* 2002

Some DNA Methyl Transferases are Essential

Cytosine methylation in mammals

- Gene expression
- Chromosomal stability
- Cell differentiation
- Imprinting
- X-Inactivation
- Carcinogenesis
- Aging



Methylated DNA from Zygote to Adult

Zygote



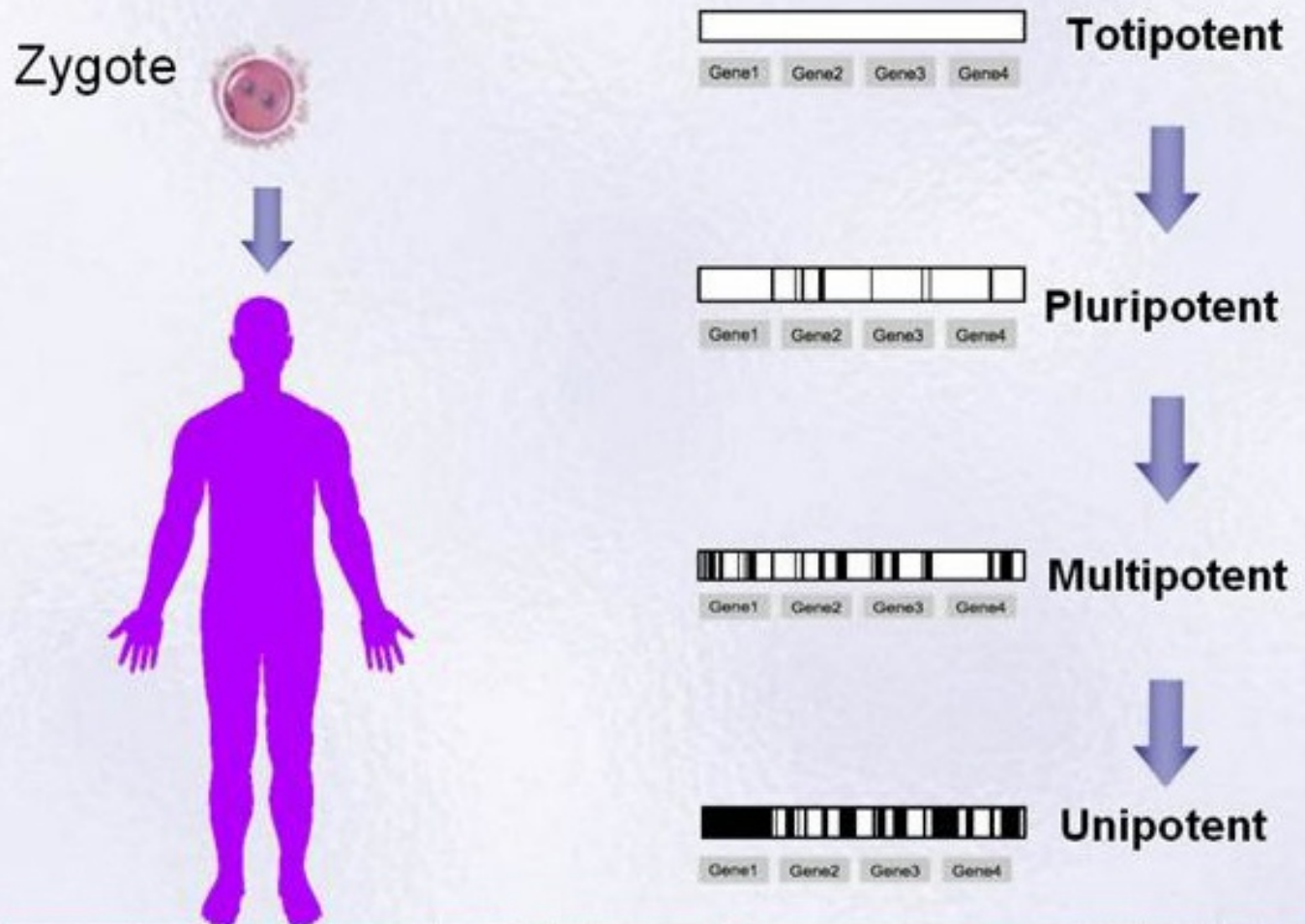
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TTTATTACCAGGATGATCACCATTGGGTACCATTACCAGGATTACACAGTTTTAGATGACCAGT
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```

**How is the diversity of cell types
created and maintained
in multi-cellular organisms?**

```
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ACCAGTAGCTATTAGAGGATTTTAAATTTATTTAGGATTTTATGGGATTGATAAAGGGAGATTTA
ACATAGACATACACACTGTTGATTAGGGAGATAGTGACAGATCCATTACAGCACCATACCATGAT
```

Methylated DNA from Zygote to Adult

Differentiated cells become more restricted in their potential



DNA Methylation Differentiates Totipotent Embryonic Stem Cells from Unipotent Adult Stem Cells

DNA methylation

Pluripotent cell



```
ctggaggtgcaatggctgtcttgtcctggcctt
ggacatgggctgaaatactgggttcacccatat
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```

Unipotent cell



```
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ggacatgggctgaaatactgggttcacccatat
ctaggactctagacgggtgggtaagcaagaact
gaggagtggccccagaaataattggcacacgaa
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cctaggtgagccgtctttccaccaggcccccg
ctcggggtgccaccttccccatggctggacac
```

DNA Methylation Differentiates Totipotent Embryonic Stem Cells from Unipotent Adult Stem Cells

DNA methylation

Pluripotent cell



```
ctggaggtgcaatggctgtcttgtcctggcctt
ggacatgggctgaaatactgggttcacccatat
ctaggactctagacggggtgggtaagcaagaact
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ttctcccacccccacagctctgctcctccacc
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Unipotent cell

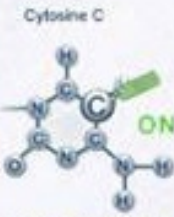



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cattcaatggatgttttaggctctccagaggat
ggctgagtgggctgtaaggacaggccgagaggg
tgcagtgccaacaggctttgtggtgogatgggg
catccgagcaactggtttgtgaggtgtccggtg
acccaaggcaggggtgagaggacctgaaggtt
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tggagacacaggcagatagcgctcgctcagt
ttctcccacccccacagctctgctcctccacc
accaggggggccccggccagaggtcaaggctaga
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```

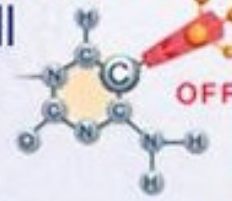

DNA Methylation Differentiates Totipotent Embryonic Stem Cells from Unipotent Adult Stem Cells

DNA methylation

Pluripotent cell

Unipotent cell

≠

ctggaggtgcaatggctgtcttgtcctggcctt
 ggacatgggctgaaatactgggttcacccatat
 ctaggactctagacgggtgggtaagcaagaact
 gaggagtggccccagaaataattggcacacgaa
 cattcaatggatgttttaggctctccagaggat
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Differentiated Cells can Become Totipotent

Nuclear transplantation demonstrates nuclear equivalence

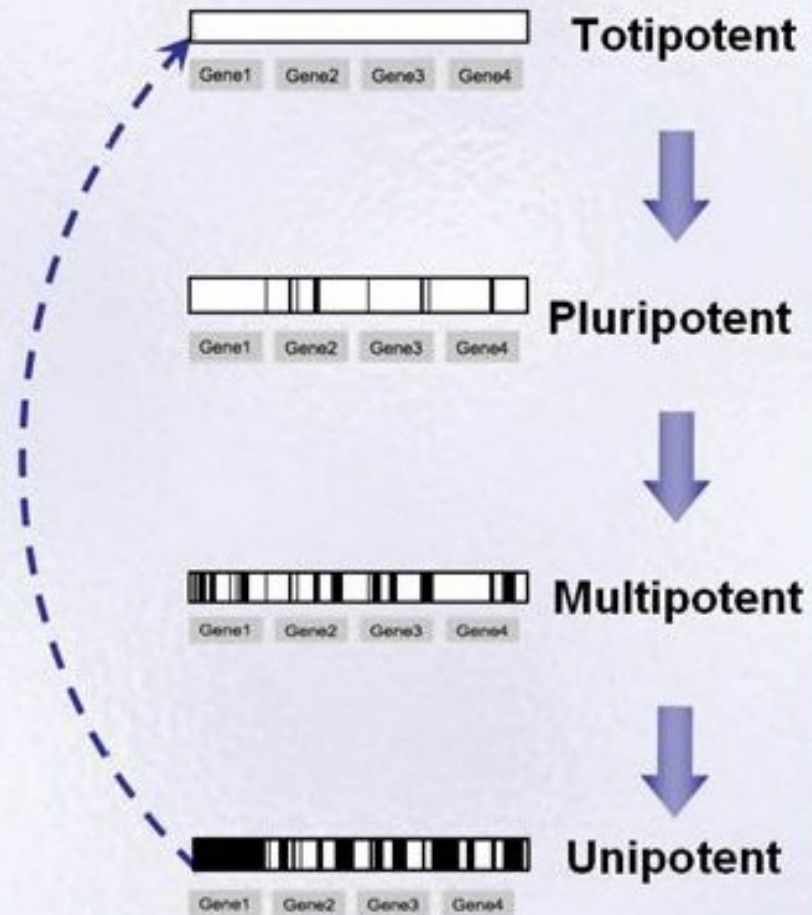


Briggs and King, 1952

Gurdon, 1960s

“Dolly”

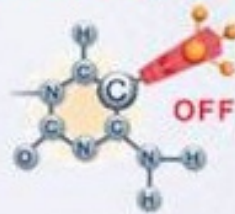
Differentiated cells maintain the potential to generate an entire organism



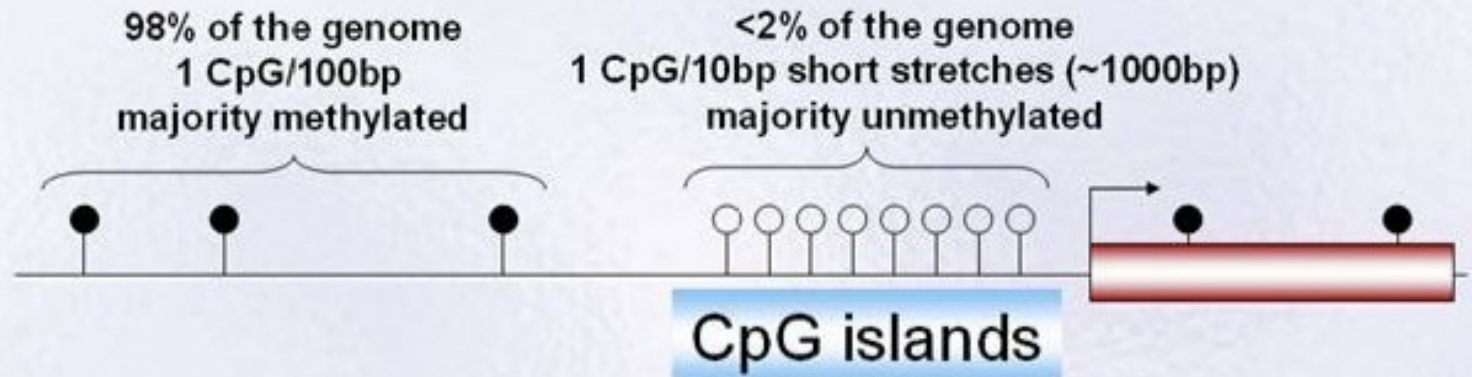
Critical CpG Sequences in CpG Islands Near Promoters

Genomic distribution of DNA methylation

Methyl-Cytosine



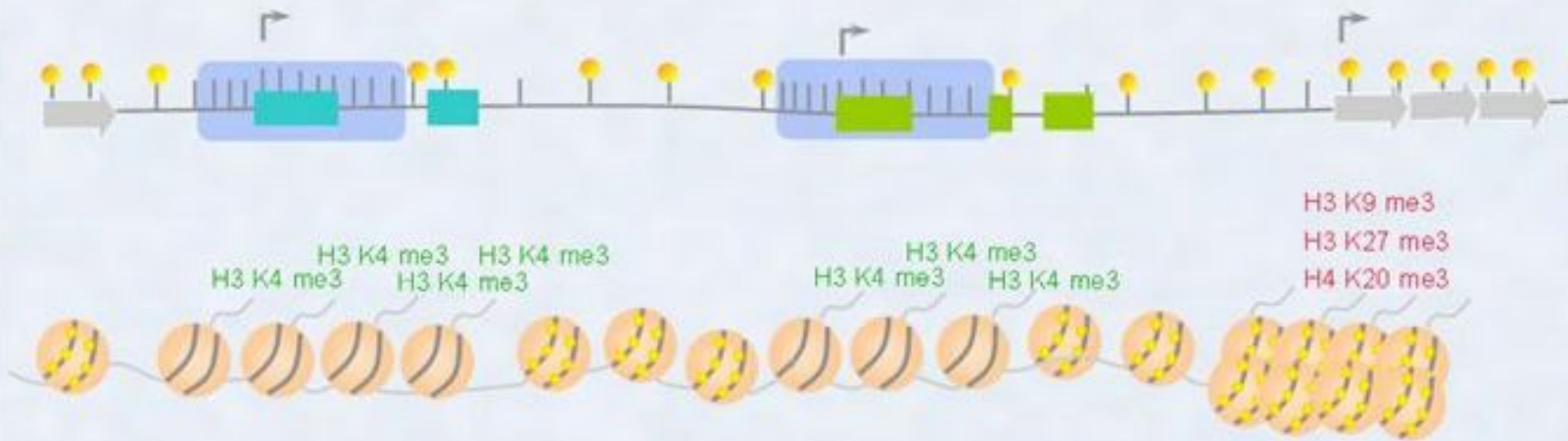
4% of all cytosines are methylated
70-80% of all CpGs are methylated



Organization of the Epigenome

Organization of the 'Epigenome'

Normal Cells

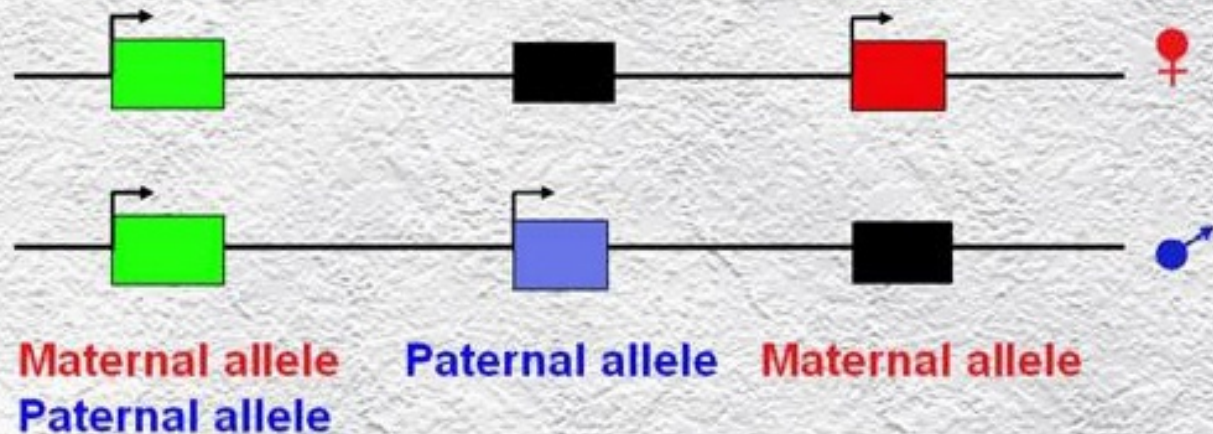


Transcriptional potential

Epigenetic Imprinting

Genomic imprinting

The unequal expression of the maternal and paternal alleles of a gene



- Imprinted or marked with their gametic (parental) origin

Epigenetic Imprinting of H19 & Igf2 Loci

H19 and Igf2 imprinted locus



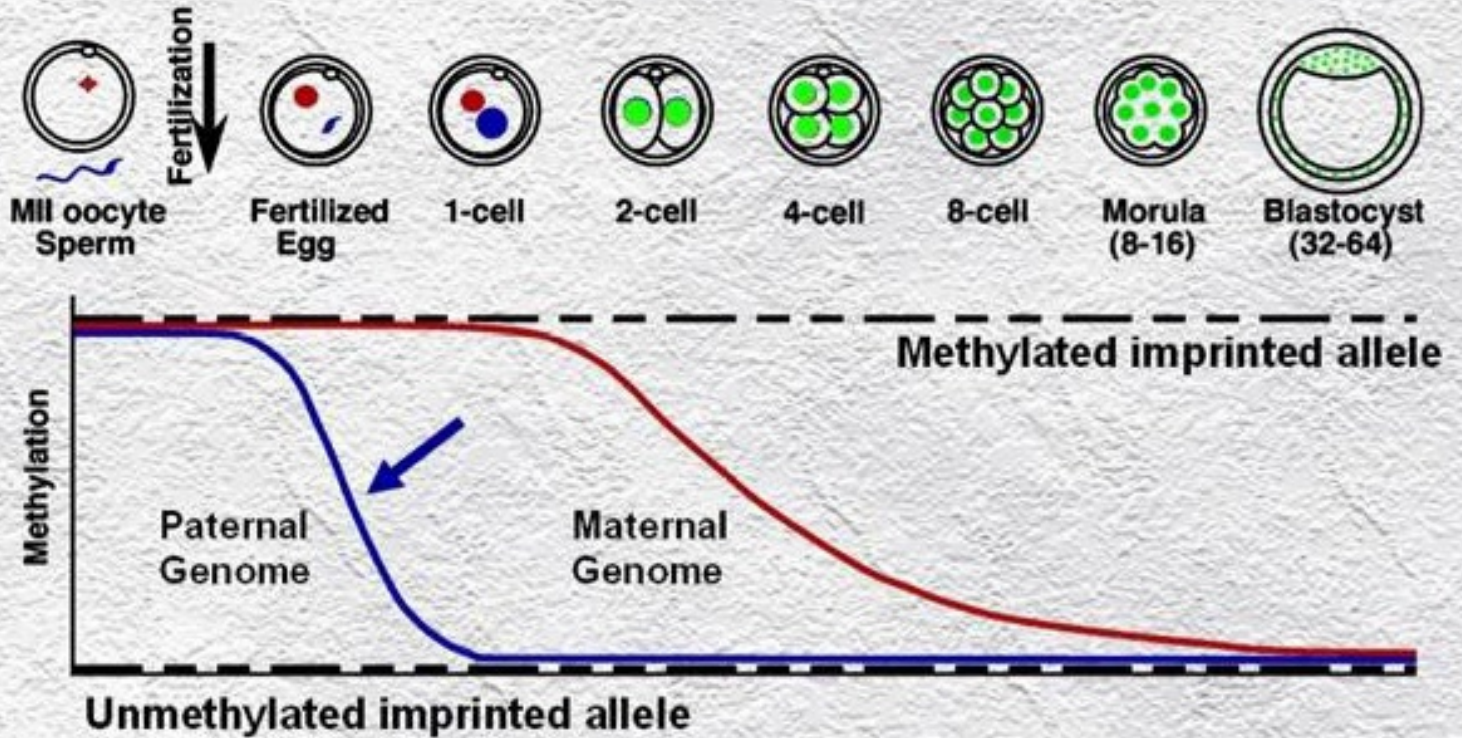
Insulator Model for the Imprinting of H19 & Igf2 Loci

Insulator model for the control of imprinted gene expression at the *H19/Igf2* locus



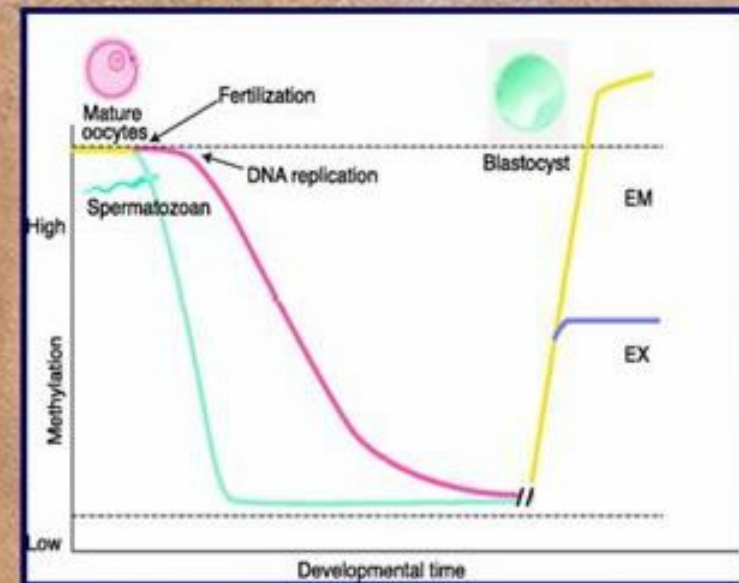
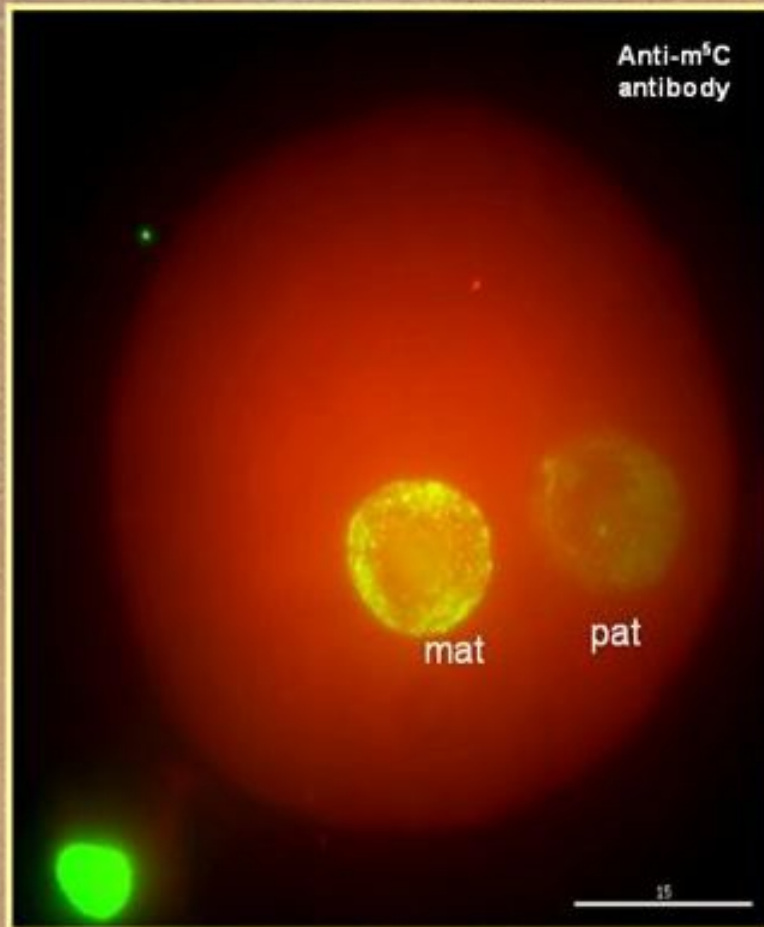
Methylation Changes During Development

Methylation Changes During Mouse Preimplantation Development

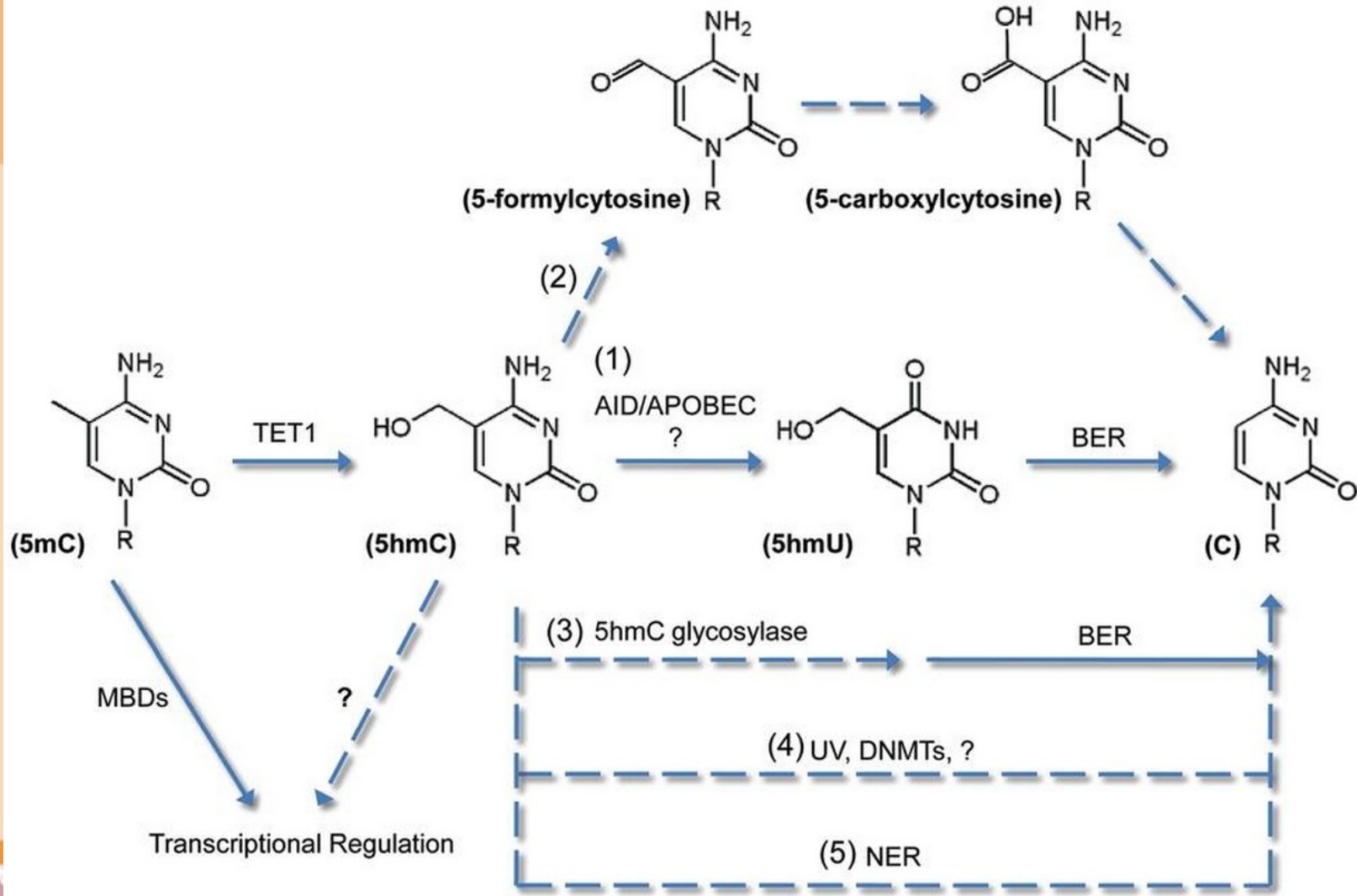


Demethylation of the Paternal Genome

De-methylation of the paternal pronucleus in the one-cell embryo of mouse

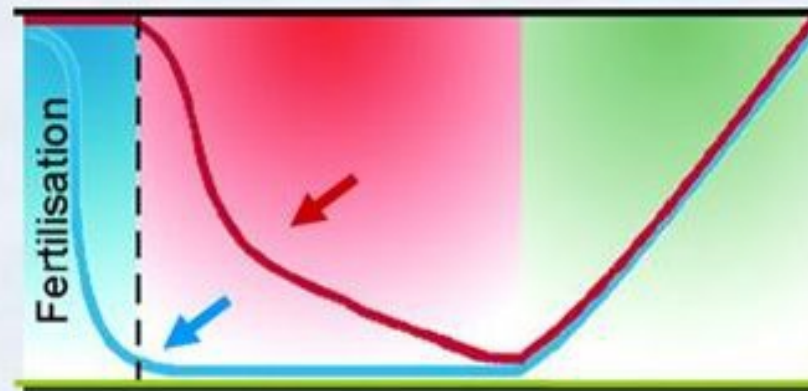
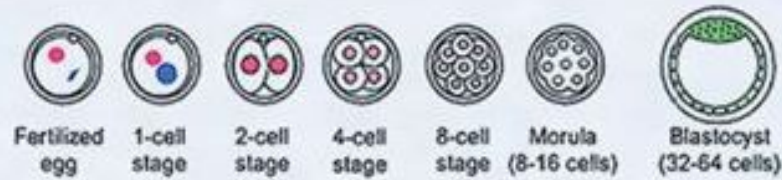


Tet Proteins Modify 5-Methyl-Cytosine Leading to Removal by DNA Repair



Methylation Changes During Development

Reprogramming the DNA methylome

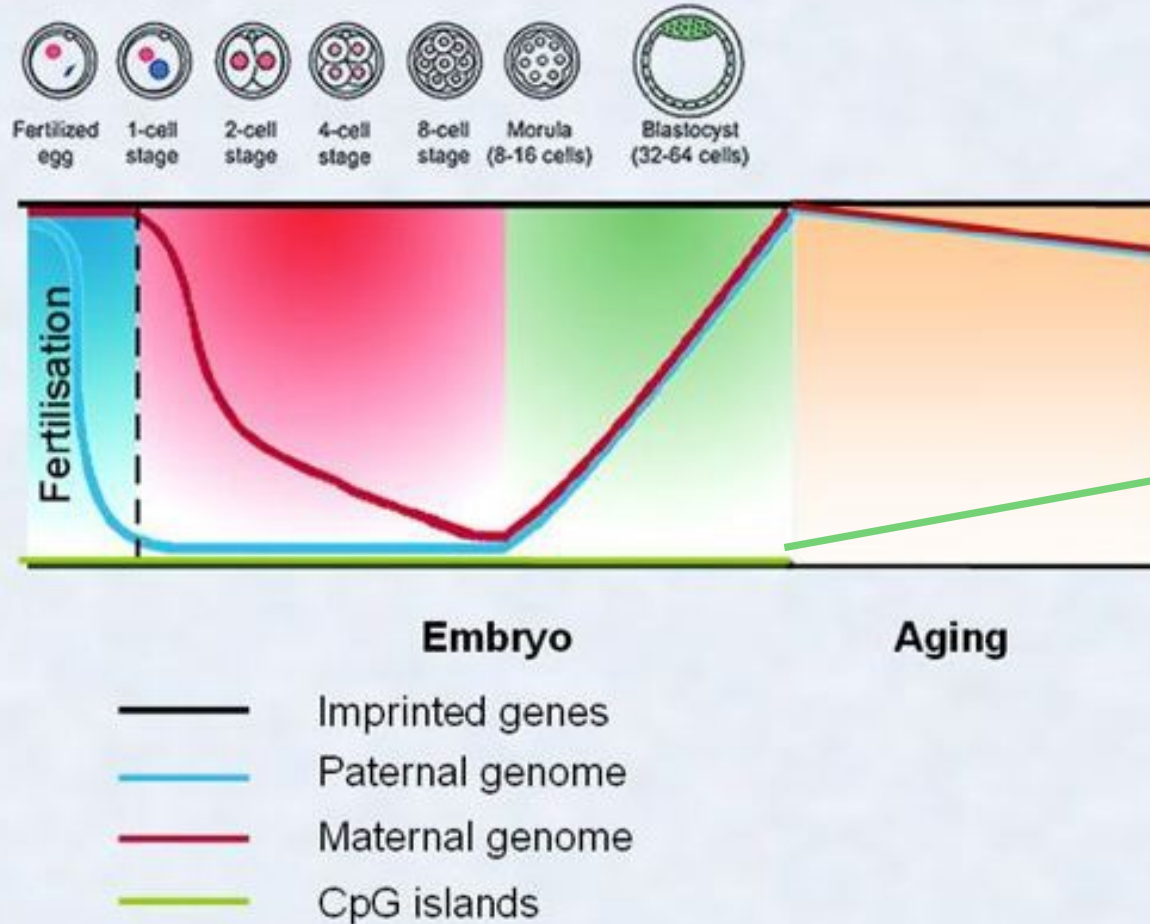


Embryo

- Imprinted genes
- Paternal genome
- Maternal genome
- CpG islands

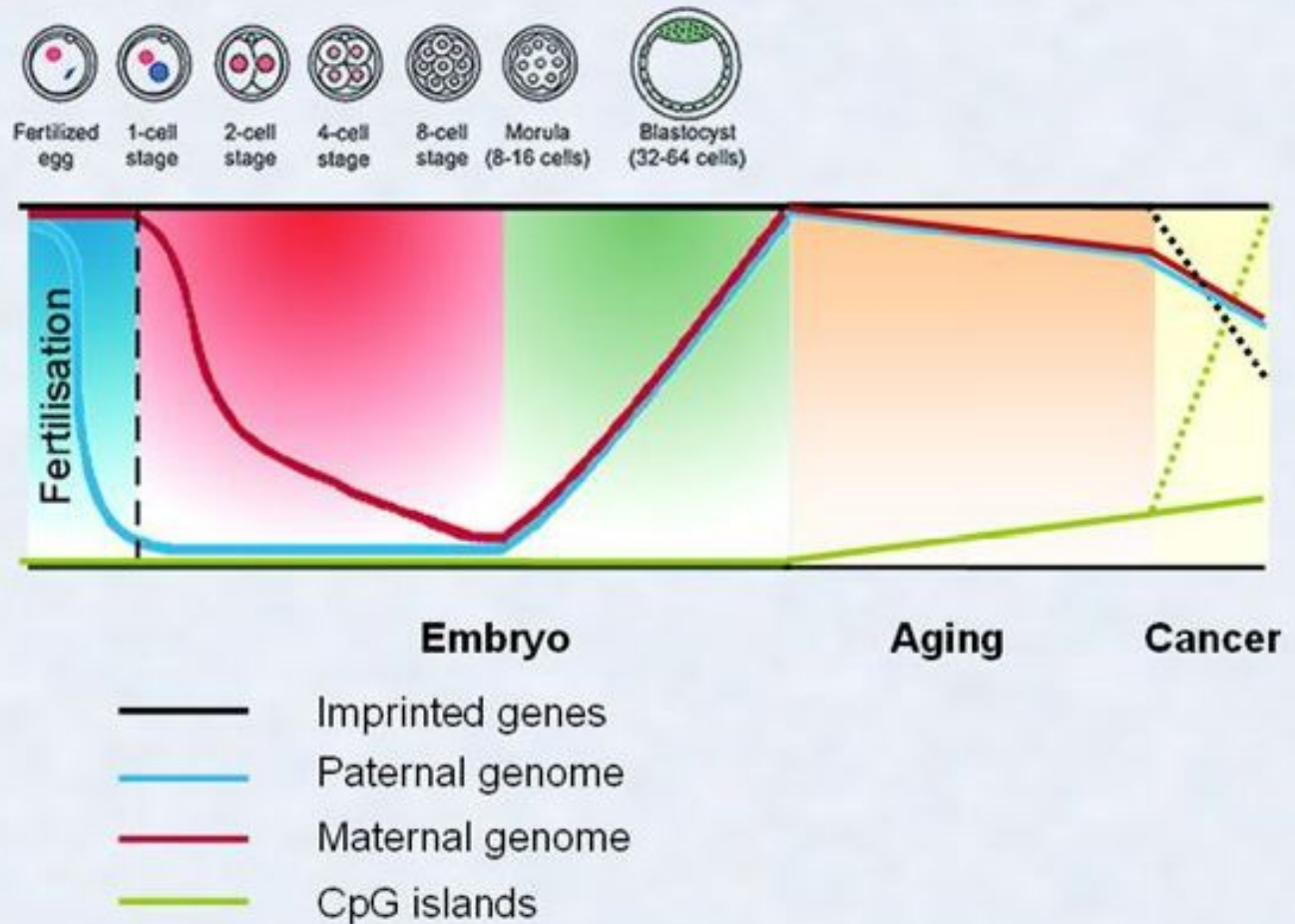
Methylation Changes During Development

Reprogramming the DNA methylome

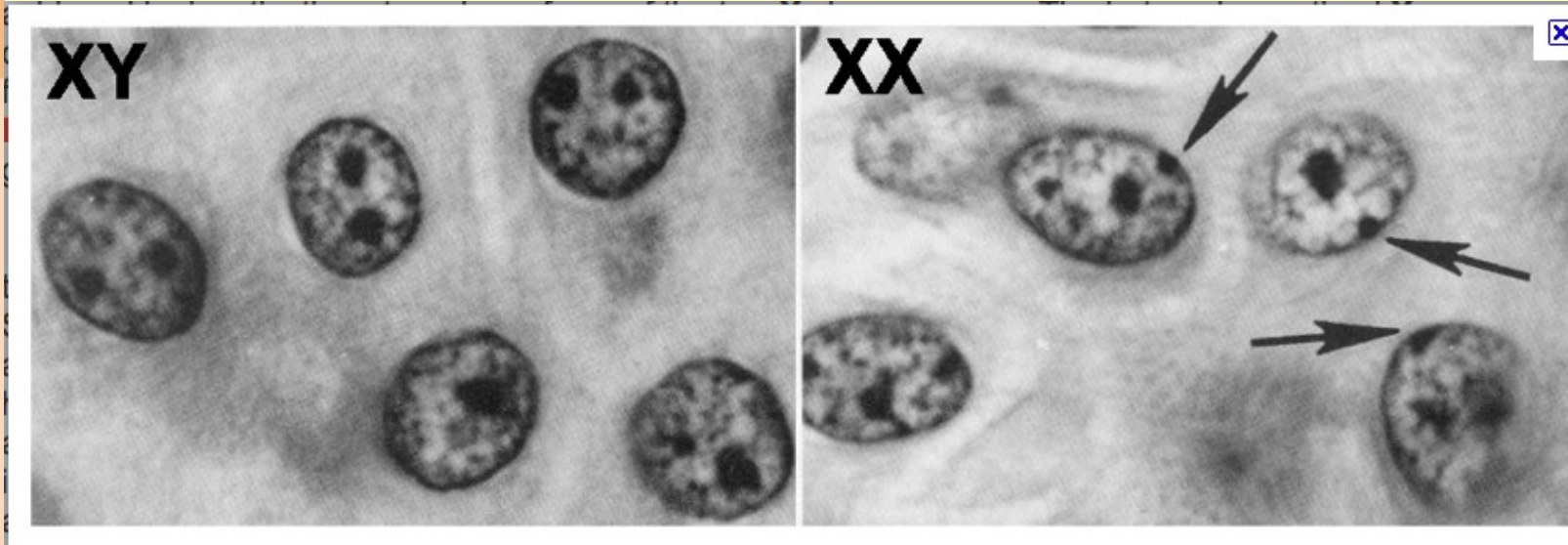


Methylation Changes During Development

Reprogramming the DNA methylome



X Chromosome Inactivation: Barr Bodies

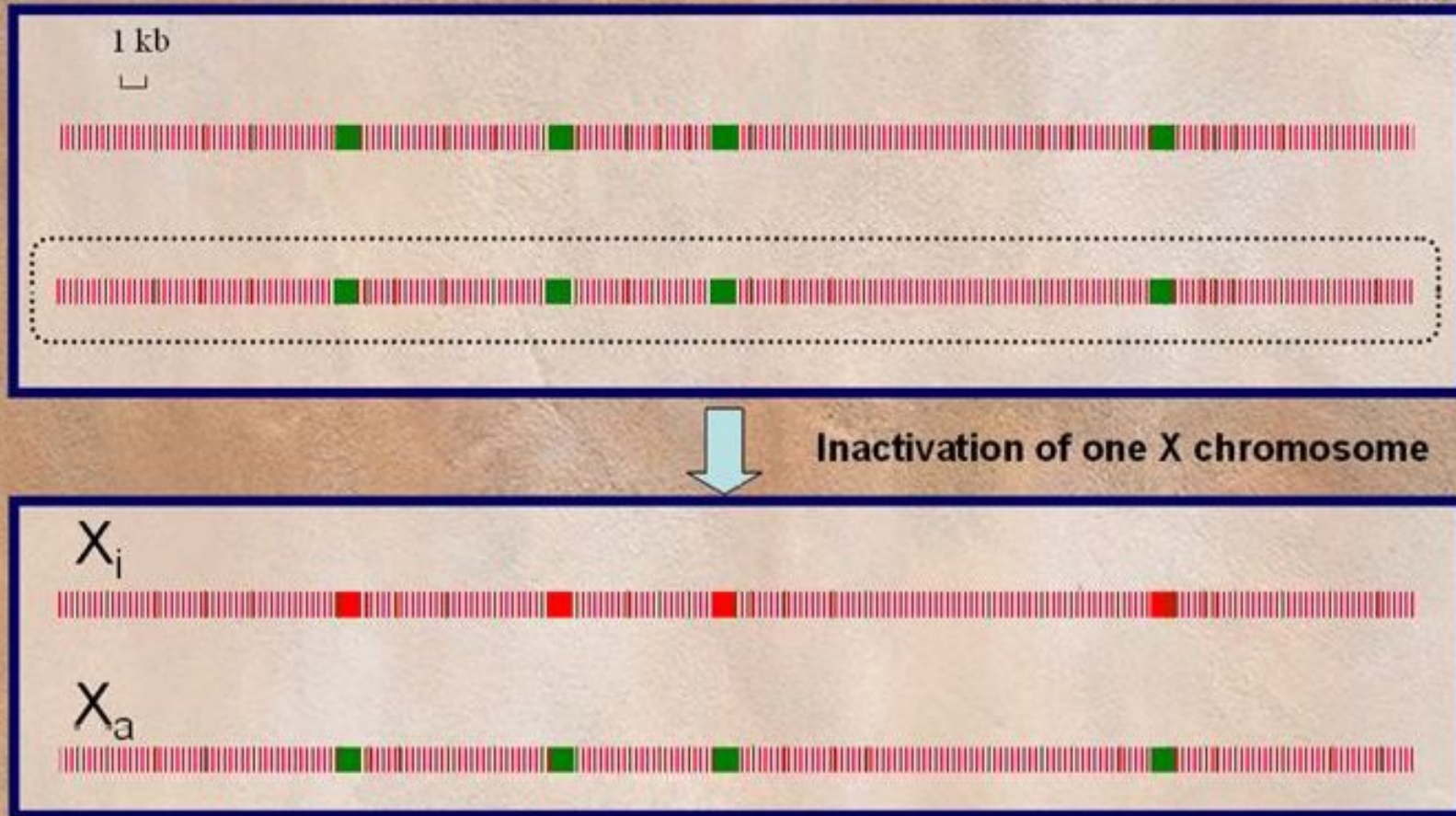


Barr, M. L., Bertram, E. G., (1949), A Morphological Distinction between Neurons of the Male and Female, and the Behavior of the Nucleolar Satellite. *Nature*. **163** (4148): 676-7.

Lyon, M. F., (2003), The Lyon and the LINE hypothesis. *j.semcd* 14, 313-318. (Abstract)

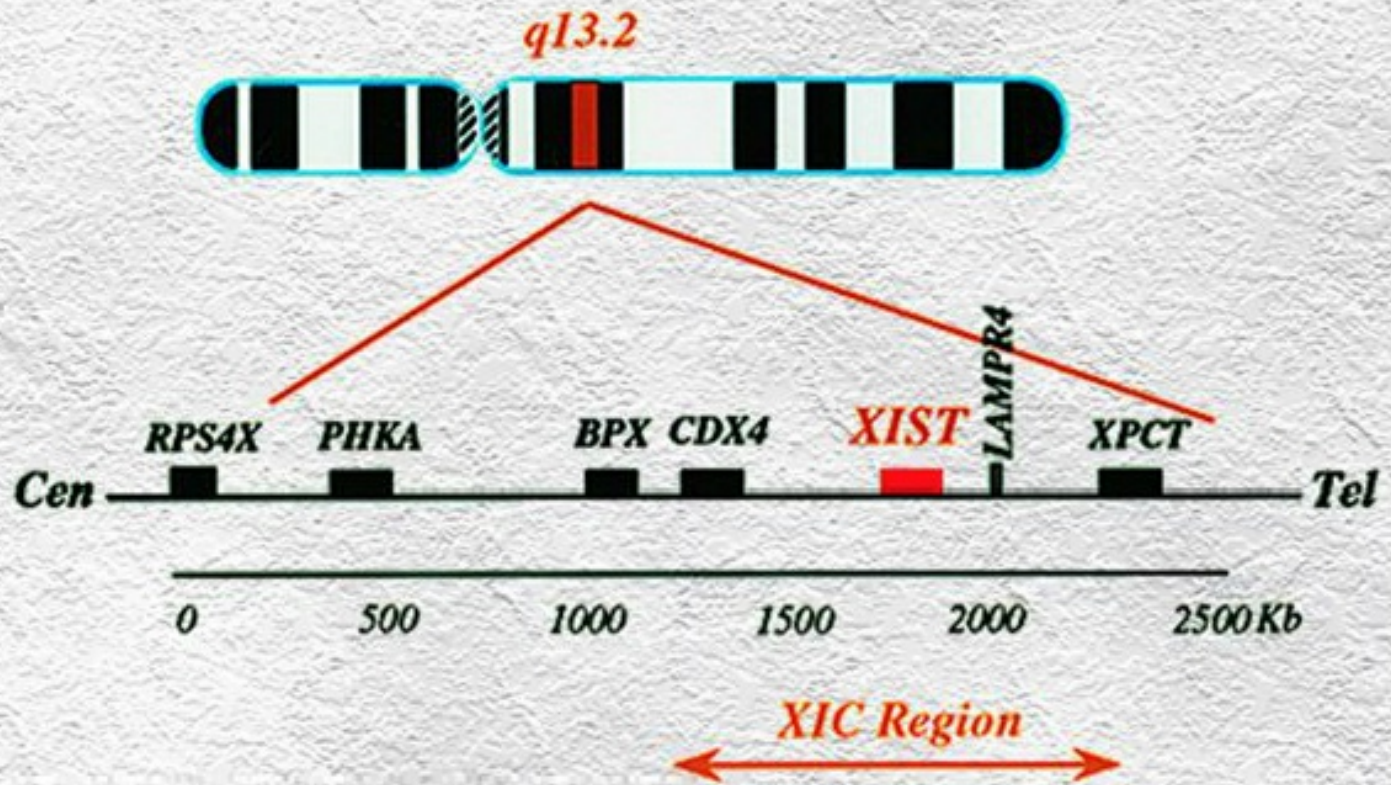
X Chromosome Inactivation: CG Island Methylation

De novo methylation of CpG islands on the inactive X chromosome



XIC Region

The XIC region on the human X chromosome



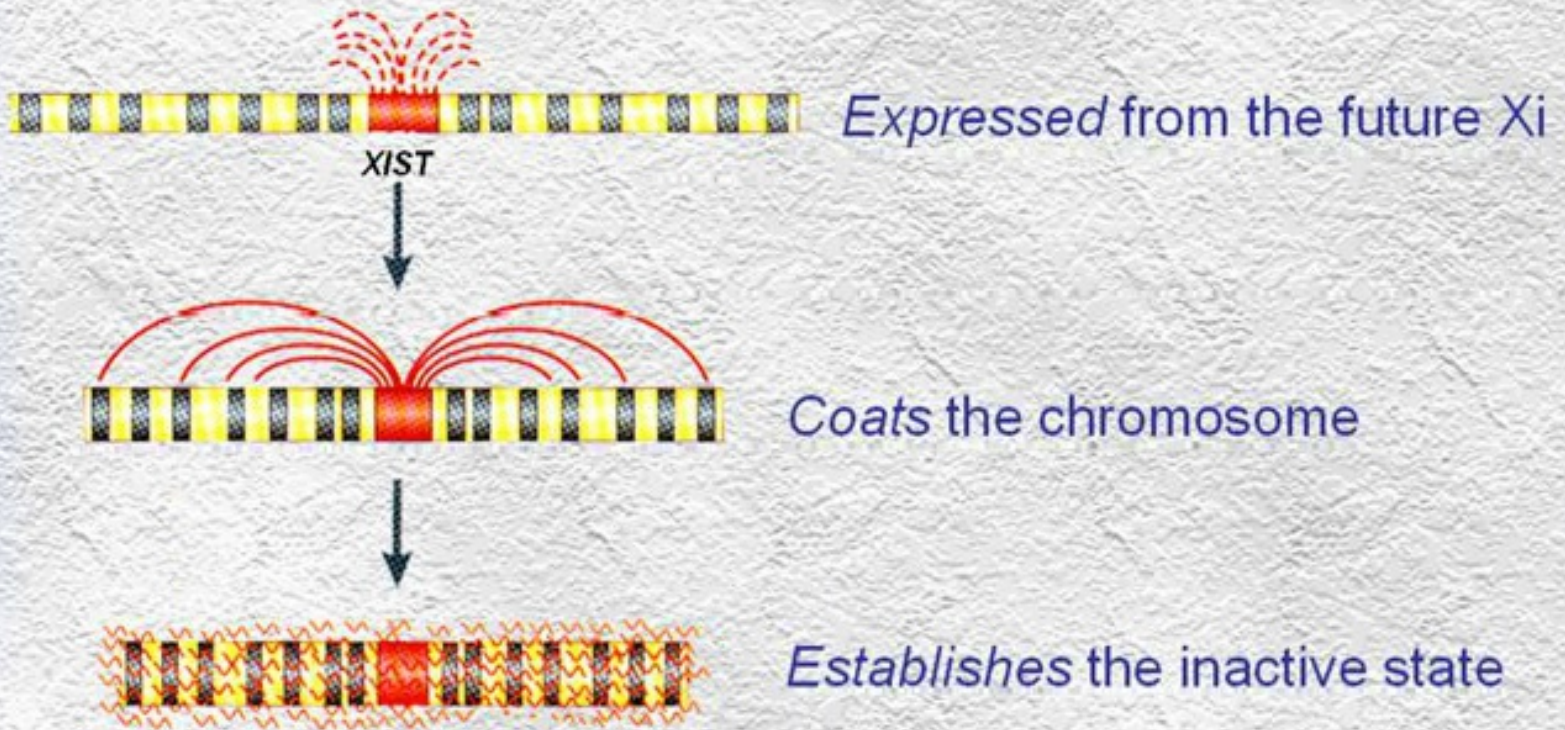
Characteristics XIST Gene

Characteristics of *XIST*

- Located in the XIC
- Transcribed *only* from the *inactive* X
- 20kb cDNA with no ORF, remains intranuclear, surrounding the *Barr body*
- *The* XIC gene responsible for Cis inactivation

Xist Works in Cis

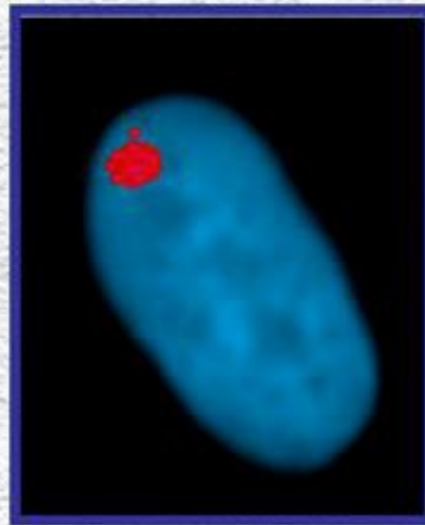
How *XIST* silences the future inactive X



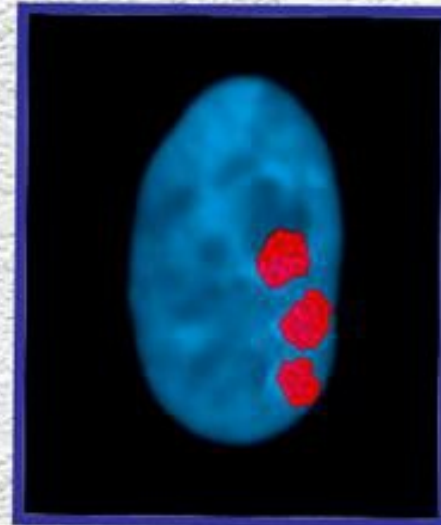
After Avner

Only one X is active

46, XX female



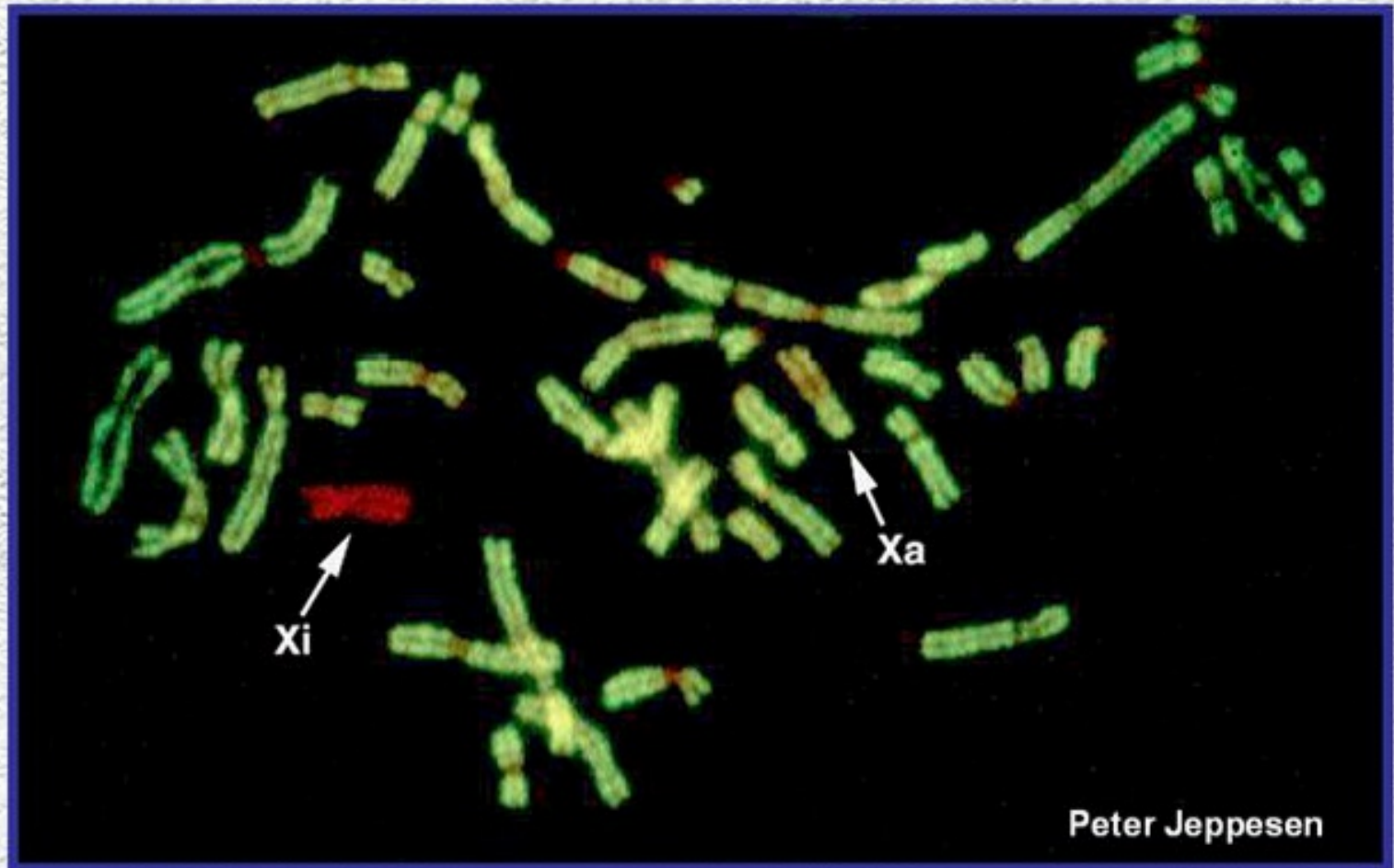
49, XXXXY male



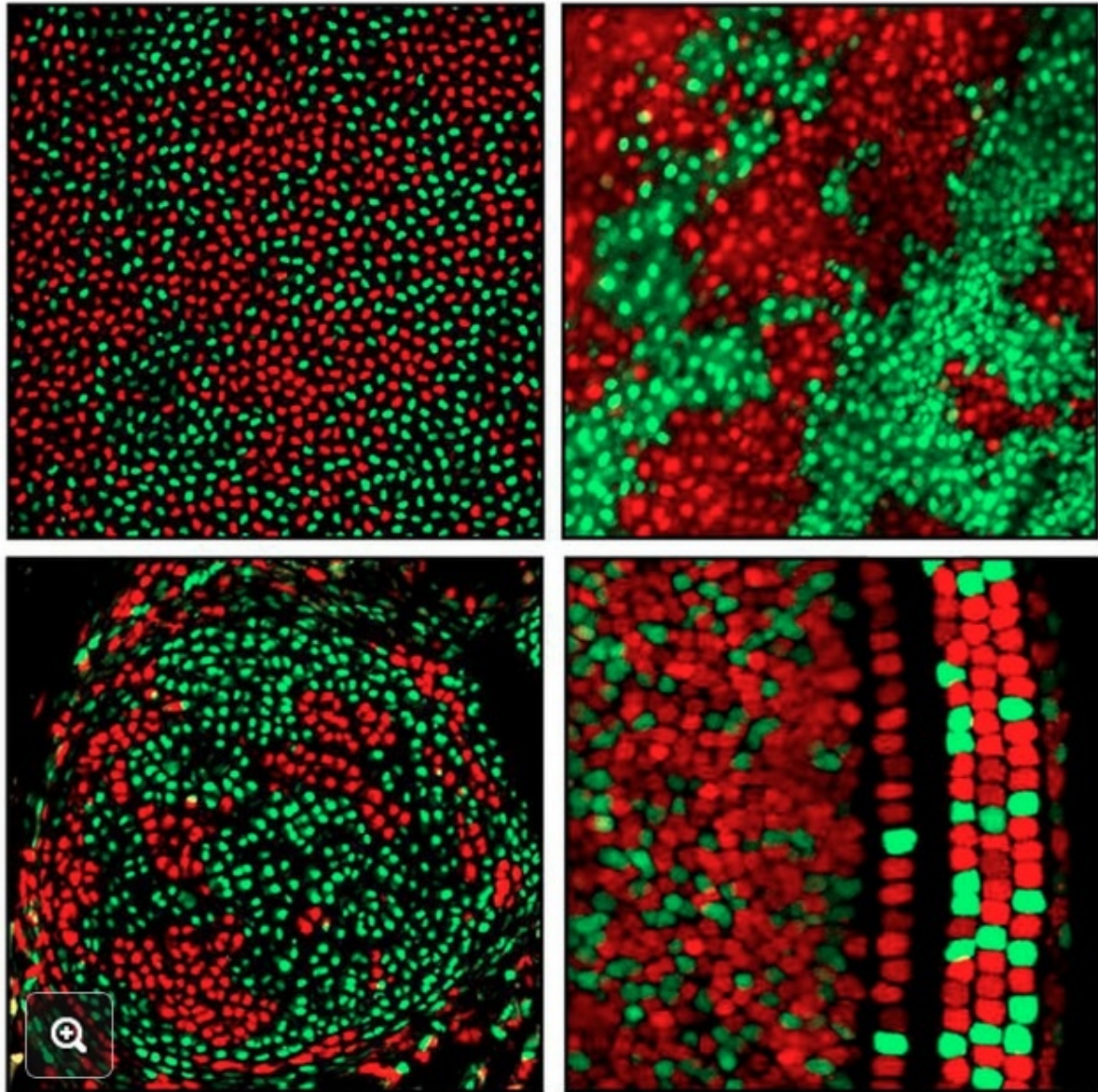
Barr bodies visualized by XIST RNA FISH

Inactive X has unacetylated histone H4

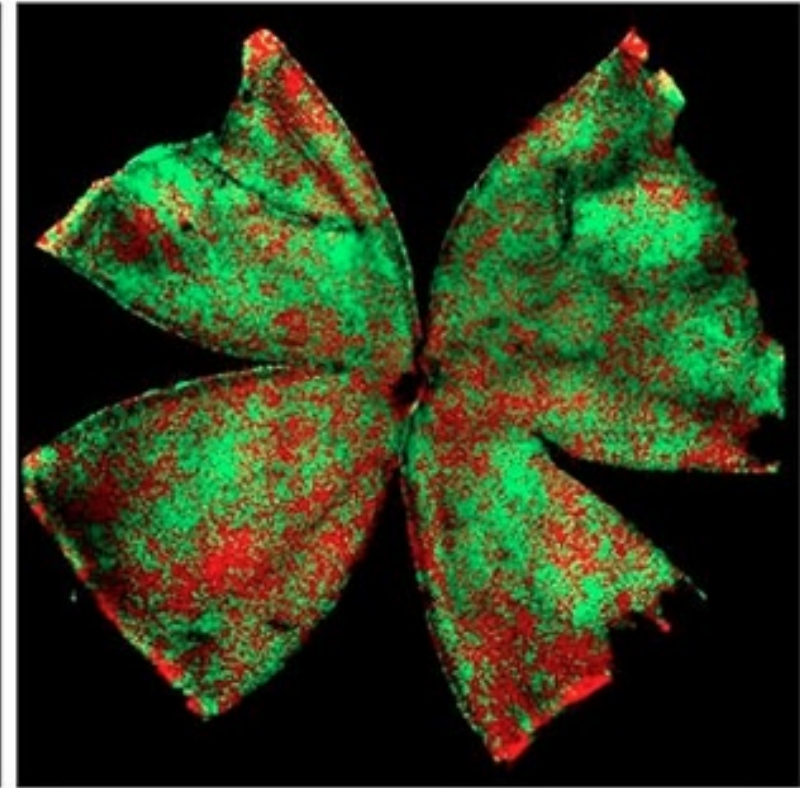
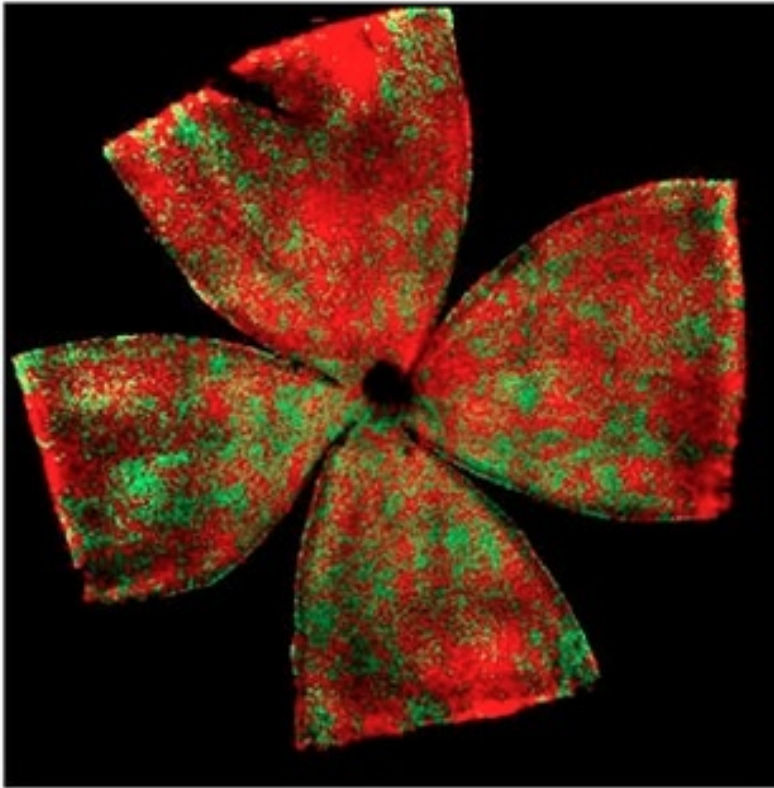
**Inactive X has inactive chromatin:
unacetylated histone H4**



Female X chromosome Mosaicism (cornea, skin, cartilage & inner ear)



Female X chromosome Mosaicism Left and Right Retina



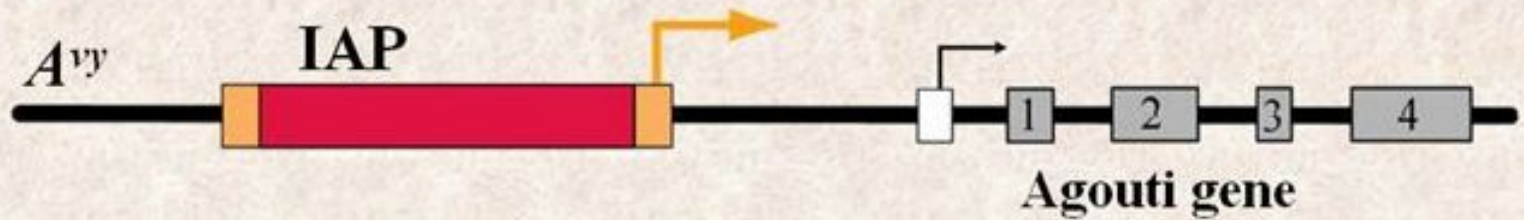
Distinguishing features of Xi and Xa

Table 1. Distinguishing features of chromatin on inactive and active X chromosomes

Feature	Xi	Xa	References
Barr body formation	+	-	[30]
XIST expression/association	+	-	[3-6]
CpG islands methylation	+	-	[43,44]
Methylated H3 K-9/27	+	-	[59,61,62,65]
Methylated H3 K-4	-	+	[59,60]
Histone tail acetylation	-	+	[55-58]
Elevated levels mH2A1/2	+	-	[69,71,72]
Elevated levels histone H1	+	-	[49]
Elevated levels HMG-I/Y	+	-	[49]
Elevated levels of HP1	+	-	[49]
H2A-Bbd presence	-	+	[68]
Replication timing	Late	Early	[94,95]

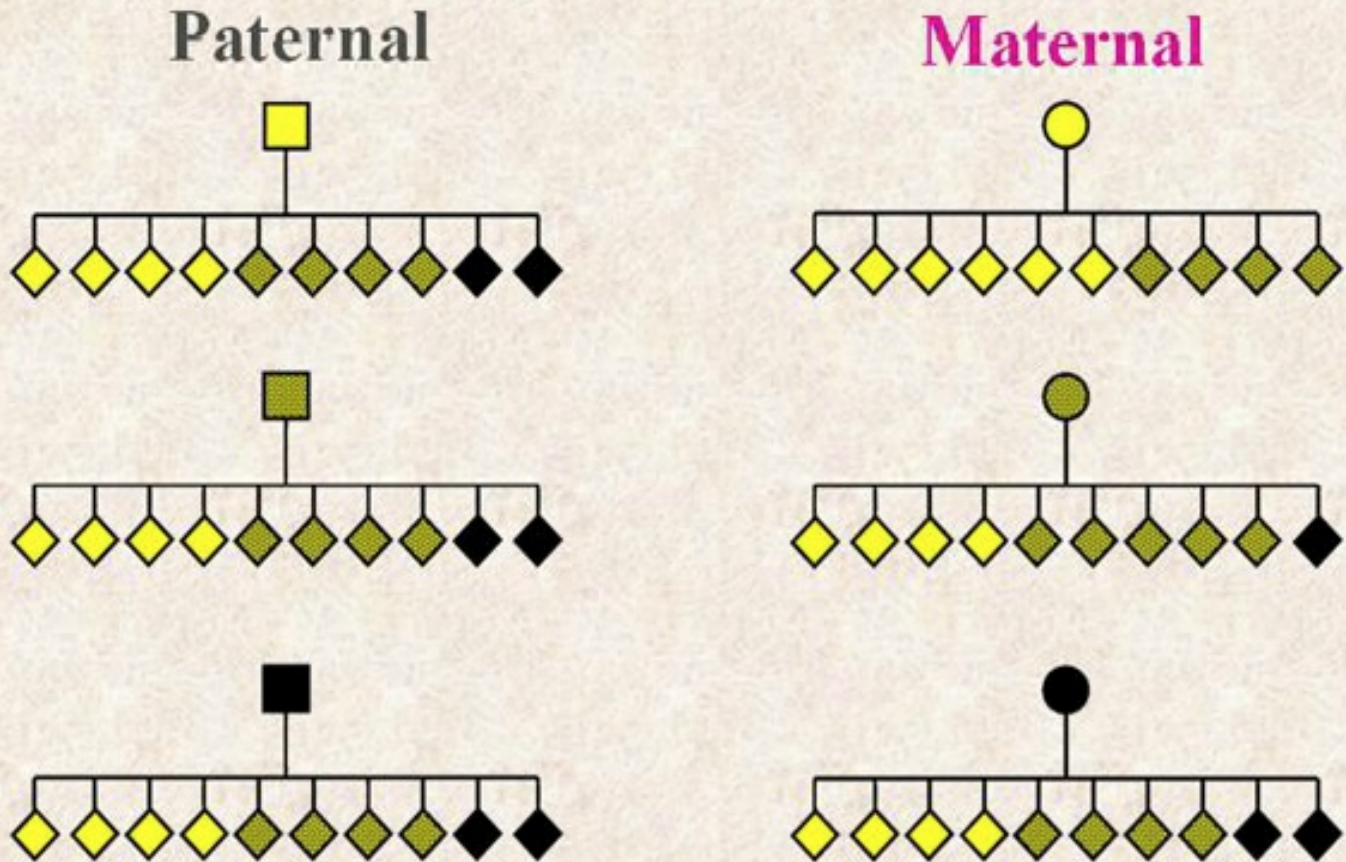
Agouti Genes in Mice

Agouti viable yellow (A^{vy})



Epigenetic Inheritance

Transgenerational epigenetic inheritance at A^{vy}



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

Methylation of Agouti Genes in Mice

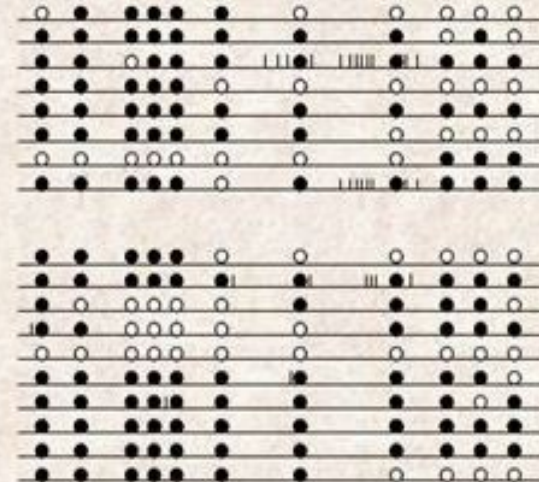
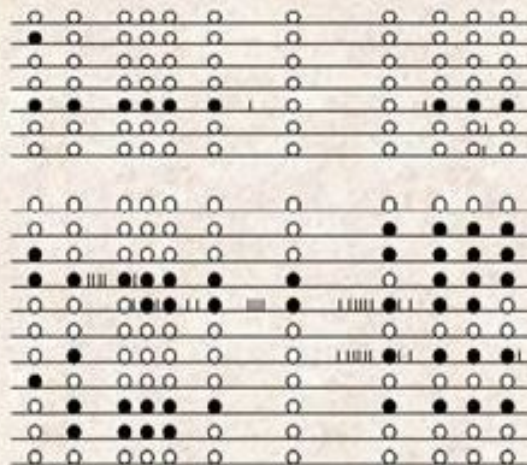
Methylation at the A^{vy} allele



Yellow

Pseudoagouti

27%
mCpG

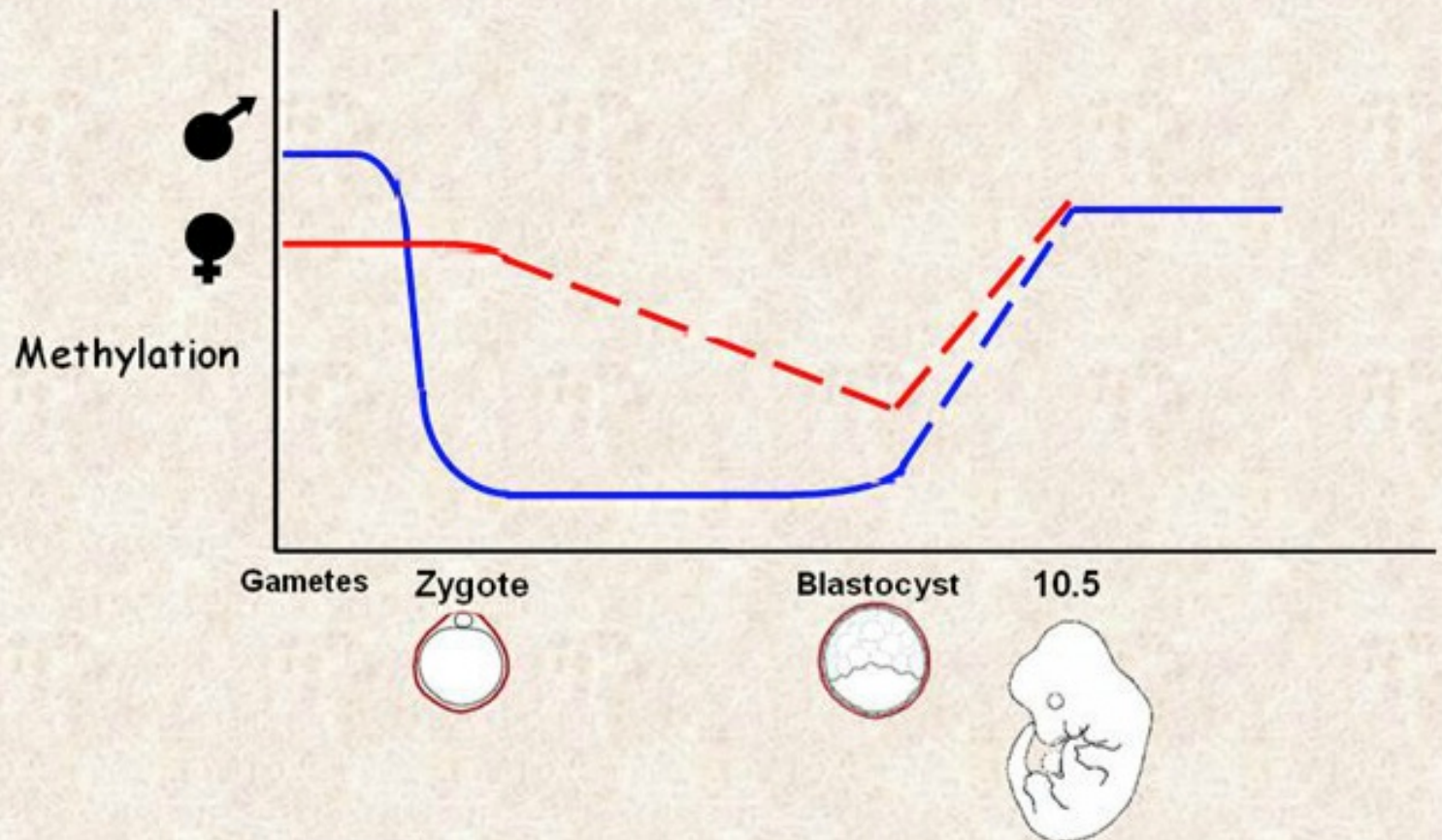


69%
mCpG

Reprogramming of A^{vy} Allele in Development

A^{vy} allele

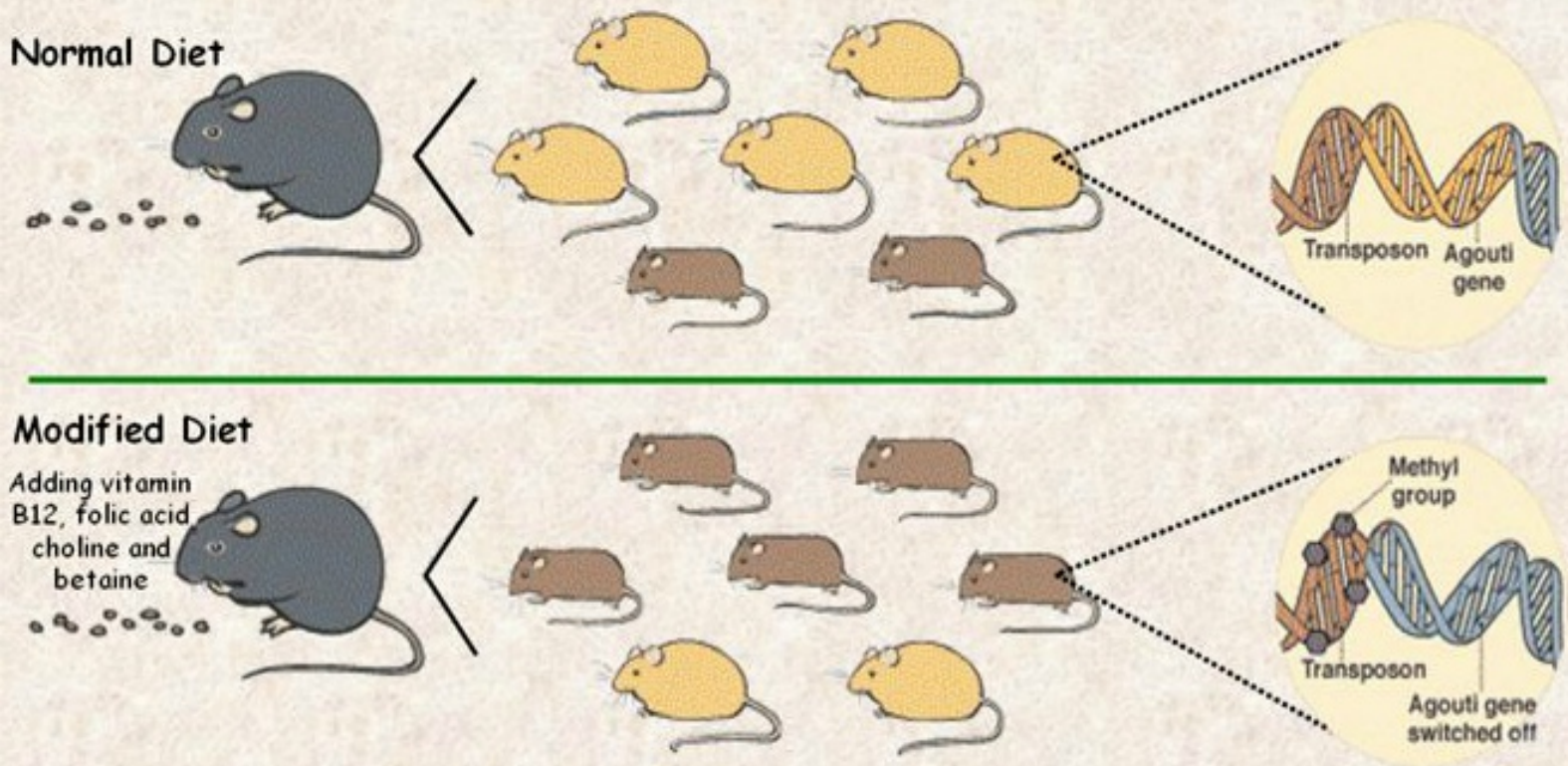
is reprogrammed in early development



Environment can Influence Epigenetic Changes

Can environment influence these processes?

They are what she ate...



Source: Waterland & Jirtle, Mol Cell Biol (2003)
Also Wolff & Cooney, Faseb J (1998)

Hongerwinter 1944

- German's blocked food to the Dutch in the winter of 1944.
- Calorie consumption dropped from 2,000 to 500 per day for 4.5 million.
- Children born or raised in this time were small, short in stature and had many diseases including, edema, anemia, diabetes and depression.
- The Dutch Famine Birth Cohort study showed that women living during this time had children 20-30 years later with the same problems despite being conceived and born during a normal dietary state.



Epigenome Roadmap

<http://www.nature.com/collections/vbqgtr>

Epigenome Roadmap



Home | Research | Threads | News and Multimedia | Additional research | Sponsor



Welcome to the Epigenome Roadmap! Here, we have collected research papers describing the main findings of the NIH Roadmap Epigenomics Program, the aim of which was to systematically characterize epigenomic landscapes in primary human tissues and cells. The papers are complemented by eight threads each of which highlights a topic that runs through more than one paper. Threads are designed to help you explore the wealth of information collectively published across several Nature Publishing Group journals. Each thread consists of relevant paragraphs, figures and tables from across the papers, united around a specific theme.

Produced with exclusive support from:

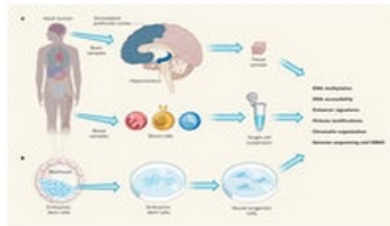
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the video and other associated material.

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Beyond the genome



Nature | News and Views

Epigenomics: Roadmap for regulation



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Epigenome: The symphony in your cells

Epigenome Roadmap

<http://www.nature.com/collections/vbqgtr>

Thread articles

Research papers

THREAD 1

1. Annotation of the non-coding genome

[Highlight referenced papers ▶](#)

THREAD 2

2. Relationship between different epigenomic marks: DNA accessibility and methylation, histone marks, and RNA

[Highlight referenced papers ▶](#)

THREAD 3

3. Epigenomic changes during differentiation and development

[Highlight referenced papers ▶](#)

THREAD 4

4. Regulatory models: networks, motifs, modules, sequence drivers and predictive models

[Highlight referenced papers ▶](#)

THREAD 5

5. Interpreting variation: GWAS, cancer, genotype, evolution and allelic

[Highlight referenced papers ▶](#)



Nature

Conserved epigenomic signals in mice and humans reveal immune basis of Alzheimer's disease

Elizabeta Gjoneska, Andreas R. Pfening, Hansruedi Mathys, Gerald Quon, Anshul Kundaje *et al.*

[◀ Highlight associated threads](#)



Nature Communications

The meta-epigenomic structure of purified human stem cell populations is defined at cis-regulatory sequences

N. Ari Wijetunga, Fabien Delahaye, Yong M. Zhao, Aaron Golden, Jessica C. Mar *et al.*

[◀ Highlight associated threads](#)



Nature

Genetic and epigenetic fine mapping of causal autoimmune disease variants

Kyle Kai-How Farh, Alexander Marson, Jiang Zhu, Markus Kleinewietfeld, William J. Housley *et al.*

[◀ Highlight associated threads](#)



Nature Communications

Epigenomic footprints across 111 reference epigenomes reveal tissue-specific epigenetic regulation of lincRNAs

Viren Amin, R. Alan Harris, Vitor Onuchic, Andrew R. Jackson, Tim Charnock *et al.*

[◀ Highlight associated threads](#)



Nature Communications

Intermediate DNA methylation is a conserved signature of genome regulation

GiNell Elliott, Chibo Hong, Xiaoyun Xing, Xin Zhou, Daofeng Li *et al.*

Nature Biotechnology

Large-scale imputation of epigenomic datasets for systematic annotation of diverse human tissues

Jason Ernst, Manolis Kellis

Summary of Epigenetic Gene Regulation

- Patterns of DNA methylation in adult cells parallels cell fate, chromatin structure and gene activation.
- Most DNA methylation is removed at fertilization and re-established during embryogenesis.
- Imprinted genes keep their parental pattern of methylation giving rise to parental patterns of expression.
- Patterns of histone modifications parallel DNA methylation.
- Methylated gene regions are genetically inactive, highly condensed and special histone modifications.
- Active gene regions have little DNA methylation and distinctive histone modifications (acetyl groups and H3K4methyl).
- X chromosome inactivation in females is correlated with extensive CG island methylation on one chromosome, condensation, inactivation and Barr body formation.
- Alterations in gene and CG island methylation patterns are seen in aging and in cancer.
- Most CG islands are not methylated except for X chromosome inactivation and tumor suppressors in cancer.