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

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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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• Modern definition is non-sequence dependent inheritance.

• How can identical twins have different natural hair colors?
Identical Twins with Different Hair Color
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- How can identical twins have different natural hair colors?
- 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 Liter Mates
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• How can identical twins have different natural hair colors?
• 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!
What is Epigenetics?
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

Histones
Histone tails
Chromosome
DNA Methylation & Histone Modifications Form 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

\[
\text{ATTCGTCGCTAG...} \rightarrow \text{ATTCGTCGCTAG...}
\]

DNMTs

S-adenosylmethionine

\[
\text{ATTCGTCGCTAG...} \rightarrow \text{ATTCGTCGCTAG...}
\]
Only Cs in CG sequences are Methylated

5’-CpG-GpC-5’

De novo methylation
Dnmt3a & Dnmt3b enzymes

5’-CpG-GpC-5’

Maintenance methylation
Dnmt1 enzyme

5’-CpG-GpC-5’
5-Methyl Cytosine in DNA

Cytosine methylation

![Diagram of Cytosine methylation]
Cytosine Methylation Maintains Inactive-Condensed Chromatin State

Transcription factors
RNA polymerase

Transcription

Acetylation

DNA methyltransferase

5-methyl-C

Methyl-CpG Binding proteins and associated co-repressors

Histone deacetylase

Transcription blocked

Deacetylation

Chromatin compaction
Transcriptional silencing

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

John Greally, Henry Stewart Talks  
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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 H3: 135 aa
- H3-K4
- H3-K9
- H3-K27

Histone H4: 102 aa
- H4-K16
- H4-K20
FIGURE 4.
Nucleosome with histone posttranslational modifications (Adapted from 1)
Maintenance of Cytosine Methylation

Establishment and maintenance

Replication

Maintenance methylation

$Dnmt1$
Passive Demethylation of 5-Methyl-Cytosine

Establishment and maintenance

Replication

Maintenance methylation

Dnmt1

Second round of replication: passive demethylation
Establishment and Maintenance of Cytosine Methylation

Establishment and maintenance

Replication

Maintenance methylation
Dnmt1

Second round of replication:
passive demethylation

de novo methylation
Dnmt3a, Dnmt3b

Alex Meissner, Henry Stewart Talks
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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

How is the diversity of cell types created and maintained in multi-cellular organisms?
Differentiated cells become more restricted in their potential.

Zygote

Totipotent

Pluripotent

Multipotent

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

Alex Meissner, Henry Stewart Talks
DNA Methylation Differentiates Totipotent Embryonic Stem Cells from Unipotent Adult Stem Cells

Alex Meissner, Henry Stewart Talks
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

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

98% of the genome
1 CpG/100bp
majority methylated

<2% of the genome
1 CpG/10bp short stretches (~1000bp)
majority unmethylated

CpG islands
Organization of the Epigenome
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

Marisa Bartolomei, Henry Stewart Talks
Insulator model for the control of imprinted gene expression at the $H19/\text{Igf2}$ locus
Methylation Changes During Development

Methylation Changes During Mouse Preimplantation Development

- MII oocyte
- Sperm
- Fertilized Egg
- 1-cell
- 2-cell
- 4-cell
- 8-cell
- Morula (8-16)
- Blastocyst (32-64)

Methylation

- Paternal Genome
- Maternal Genome

Unmethylated imprinted allele

Methylated imprinted allele
Demethylation of the Paternal Genome

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

(mat) (pat)

[Graph showing developmental stages and methylation levels]

Anti-m\(^5\)C antibody

Adrien Bird, Henry Stewart Talks
Tet Proteins Modify 5-Methyl-Cytosine Leading to Removal by DNA Repair

Methylation Changes During Development

Reprogramming the DNA methylome

- Fertilized egg
- 1-cell stage
- 2-cell stage
- 4-cell stage
- 8-cell stage
- Morula (8-16 cells)
- Blastocyst (32-64 cells)

Embryo
- Imprinted genes
- Paternal genome
- Maternal genome
- CpG islands
Methylation Changes During Development

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Aging
- CpG islands
Methylation Changes During Development

Reprogramming the DNA methylome

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

Aging

Cancer

Lyon, M. F., (2003), The Lyon and the LINE hypothesis. *j.semcdb* 14, 313-318. (Abstract)
X Chromosome Inactivation: CG Island Methylation

De novo methylation of CpG islands on the inactive X chromosome

Inactivation of one X chromosome

\[ X_i \]

\[ X_a \]
The XIC region on the human X chromosome

XIC Region

Barbara Migeon, Henry Stewart Talks

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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
How XIST silences the future inactive X

Expressed from the future Xi

Coats the chromosome

Establishes the inactive state

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
Female X chromosome Mosaicism (cornea, skin, cartilage & inner ear)
Female X chromosome Mosaicism
Left and Right Retina
## Distinguishing features of Xi and Xa

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

Agouti viable yellow ($A^{vy}$)

$A^{vy}$  IAP

1  2  3  4

Agouti gene

Emma Whitelaw, Henry Stewart Talks

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Epigenetic Inheritance

Transgenerational epigenetic inheritance at $A^v$y

Paternal

Maternal

Morgan et al. (1999) Nature Genetics, 23:314-318
Methylation of Agouti Genes in Mice

Methylation at the $A^{vy}$ allele

![Diagram showing methylation at the $A^{vy}$ allele]

Yellow: 27% mCpG

Pseudoagouti: 69% mCpG

Emma Whitelaw, Henry Stewart Talks

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Reprogramming of $A^{vy}$ Allele in Development

$A^{vy}$ allele is reprogrammed in early development.

Diagram showing changes in methylation from gametes through zygote, blastocyst, and 10.5 stages.
Environment can Influence Epigenetic Changes

Can environment influence these processes?

They are what she ate...

Normal Diet

Modified Diet

Adding vitamin B12, folic acid, choline and betaine

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.
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.

We invite you to explore the research content, the News & Views, the video and other associated material.
### Epigenome Roadmap

[www.nature.com/collections/vbqgtr](http://www.nature.com/collections/vbqgtr)

#### Thread 1
1. Annotation of the non-coding genome

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

#### Thread 3
3. Epigenomic changes during differentiation and development

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

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

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#### Research Papers

**Nature**
- Conserved epigenomic signals in mice and humans reveal immune basis of Alzheimer's disease
  - Elizabeta Gjoneska, Andreas R. Pfening, Hansrued Metzly, Gerald Quan, 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 Wijstunga, 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
  - Highlight associated threads

**Nature Communications**
- Epigenomic footprints across 111 reference epigenomes reveal tissue-specific epigenetic regulation of lincRNAs
  - Viren Amin, R. Alan Harris, Vitor Onudic, Andrew R. Jackson, Tim Charnecki et al.
  - Highlight associated threads

**Nature Biotechnology**
- Intermediate DNA methylation is a conserved signature of genome regulation
  - Gillen Elliott, Chiho Hong, Xiaoyun Xing, Xin Zhou, Daoqin Li et al.
  - Highlight associated threads

**Nature Communications**
- Large-scale imputation of epigenomic datasets for systematic annotation of diverse human tissues
  - Jason Ernst, Manolis Kellis
  - Highlight associated threads
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.