Genomics & Medicine
http://biochem118.stanford.edu/

Epigenetics
http://biochem118.stanford.edu/16%20Epigenetics.html

Doug Brutlag, Professor Emeritus
Biochemistry and Medicine (by courtesy)
Stanford University School of Medicine

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Epigenetics and DNA Methylation on Henry Stewart Talks

• **Epigenetics** – 22 talks

• **DNA Methylation** – 11 talks

• **Stem Cells** – 10 talks
What is Epigenetics?

• In 1953, 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?
  o Myoblasts can only form muscle cells
  o Keratinocytes only form skin cells
  o Hematopoietic stem cells only become blood cells
  o 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?
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 a single individual have two different eye colors?
• 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!
Human Mitotic Chromosome
DNA in a Human Chromosome

Laemmli: Histone Depleted chromosomes Cell 1977 817-828
https://en.wikipedia.org/wiki/Heparan_sulfate
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

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

\[ \text{DNMTs} \] S-adenosylmethionine

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

\[ \text{...ATTCGTCGCTAG...} \]
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’
Establishment and maintenance

- Replication
- Maintenance methylation
  - Dnmt1
Passive Demethylation of 5-Methyl-Cytosine
Establishment and Maintenance of Cytosine Methylation
5-Methyl Cytosine in DNA

Cytosine methylation
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
5-Methyl Cytosine is Found in Heterochromatic Regions

The distribution of cytosine methylation in mammals

- Heterogeneity visible at cytogenetic scale
- Associated with heterochromatic regions

[Images of DNA with labeled chromosomes]
Cytosine Methylation Maintains Inactive-Condensed Chromatin State

Transcription factors
RNA polymerase

DNA methyltransferase
5-methyl-C

Methyl-CpG
Binding proteins and associated co-repressors

Histone deacetylase

Chromatin compaction
Transcriptional silencing

Transcription blocked
Deacetylation

Cytosine methylation
Methyl-Cytosine

Alex Meissner, Henry Stewart Talks
Histone Modifications in Active and Silent Chromatin
Histone Code
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
Methylated DNA from Zygote to Adult

- **totipotent**
- **pluripotent**
- **multipotent**
- **unipotent**
Methylated DNA from Zygote to Adult

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

Pluripotent cell

Unipotent cell

ctggaggtgcaatggctcttcttctgccttttgatcctttgcttccccatat
ctaggactctagacggtggtggtaagcaacacgtgaggaggtggctgca
ctttccacaccccgacgacgaccccacaggtggctgctttggtggatgctc
tgggaagcagccagctcagtcgctcaagtttctgctcttcacaccgtc
tttcaccacccccacgacagtctctgctccacaccgtcggctgcttcagc
tctcgggtgccccacgtccccatggtctggaacac

cattcataattggttttagctctccagaggtgtcgggtggttggtggtggtggt
cattcataattggttttagctctccagaggtgtcgggtggttggtggtggtggt

ggcttgaagctgctgtaaggacaggccgagaggtgcagttgctgtgctgtg

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

Alex Meissner, Henry Stewart Talks
Nanog and Oct4 Promoter Methylation in Embryonic and Induced Stem Cells

Nanog Promoter

Oct4 Promoter

Embryonic Stem Cells

Embryonic Fibroblasts

Induced Pluripotent Stem Cells
Differentiated Cells can Become Totipotent Again

Nuclear transplantation demonstrates nuclear equivalence

Briggs and King, 1952
Gurdon, 1960s

“Dolly”
Differentiated cells maintain the potential to generate an entire organism

Alex Meissner, Henry Stewart Talks
Organization of the Epigenome

Normal Cells

H3 K4 me3  H3 K4 me3  H3 K4 me3
H3 K4 me3  H3 K4 me3  H3 K4 me3

Transcriptional potential
Methylation Changes During Development

Methylation Changes During Mouse Preimplantation Development

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

Methylated imprinted allele

Unmethylated imprinted allele

Paternal Genome

Maternal Genome

Marisa Bartolomei, Henry Stewart Talks
Demethylation of the Paternal Genome

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

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

Methylation Changes During Development
Methylation Changes During Development

Reprogramming the DNA methylome

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

Aging
Methylation Changes During Development

Reprogramming the DNA methylome

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

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

Aging
Cancer

Paula Vertino, Henry Stewart Talks

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

\[ X_i \]

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

XIC Region
Characteristics of 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
• If transcribed (at critical time) it invariably inactivates its X by modifying chromatin
Xist Works in Cis

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

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

Agouti Genes in Mice

Agouti viable yellow (A^{vy})

Emma Whitelaw, Epigenetic regulation of phenotype. Henry Stewart Lectures
Methylation of A^vy Agouti Genes in Mice

Emma Whitelaw, Epigenetic regulation of phenotype. Henry Stewart Lectures
Environment influences this process

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)
Environment can Affect Epigenetics

- Feed pregnant mice folic acid, vitamin B12, choline, betaine the offspring have more methylation of agouti yellow promoter.
- Mothers who lick offspring decreased stress in offspring and decreased methylation of promoters.
- Stress also increase methyl state of a number of promoters including the promoter of the glucocorticoid receptor.
- Pattern of epigenetics can be passed on from mother to offspring.
Hongerwinter 1944

- German’s blocked food to Belgium and Holland 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.
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 H3K4 and H3K27 methyl).
• 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.
Henry Stewart Talks: DNA Methylation

Introduction
1. DNA methylation during development (38 mins)
   Prof. Howard Cedar – Hebrew University, Hadassah Medical School, Israel

The Nuts and Bolts of DNA Methylation
2. DNA methylation patterns in mammals (54 mins)
   Prof. John Greally – Albert Einstein College of Medicine, USA
3. Proteins that Bind Methylated DNA (31 mins)
   Dr. Pierre-Antoine Defossez – CNRS, Paris, France

DNA Methylation and Normal Physiology
4. Genomic Imprinting (25 mins)
   Prof. Marisa Bartolomei – University of Pennsylvania, USA
5. DNA methylation and stem cells (32 mins)
   Dr. Alexander Meissner – Harvard University, USA
Henry Stewart Talks: Epigenetics

The Notion of Epigenetics

1. Epigenetics: A Historical Overview (24 mins)
   Dr. Robin Holliday – National Institute for Medical Research, Mill Hill, London, UK

2. Cytoplasmic Epigenetics: Proteins Acting as Genes (36 mins)
   Dr. Reed Wickner – National Institutes of Health, USA

3. Cytoplasmic Epigenetics: Inheritance by Cytoplasmic Continuity (43 mins)
   Prof. Philippe Silar – University of Paris, France
   Dr. Fabienne Malagnac – University of Paris, France

Epigenetics: Paradigms

4. A Historical Perspective on Ideas on X-Chromosome Inactivation (34 mins)
   Dr. Mary Lyon – Mammalian Genetics Unit, Medical Research Council, UK
Chromatin and Epigenetics

8. Introduction to Chromatin Structure (41 mins) [Video]
   Prof. Karolin Luger – Colorado State University, USA

9. Histone Dynamics, Heritability and Variants (36 mins) [Video]
   Dr. Genevieve Almouzni – Curie Institute/CNRS, France

10. Dynamic chromatin: ATP-dependent chromatin remodeling machines (56 mins) [Video]
    Prof. Bradley Cairns – University of Utah School of Medicine, USA

11. Epigenetic Information in Gene Expression and Cancer (34 mins) [Video]
    Prof. Siavash Kurdistani – University of California, Los Angeles, USA

12. Gene Silencing by Polycomb Complexes (27 mins) [Video]
    Prof. Yi Zhang – University of North Carolina at Chapel Hill, USA