Epigenetics and Environmental Health
A Step-by-Step Tutorial
Objective of my presentation

• To review:
  – General epigenetic concepts
  – Why we may be interested in epigenetics
  – Environmental influences and epigenetics
Step by step

Step 1
Intro to epigenetics

Step 2
DNA methylation

Step 3
Histone modifications

Step 4
Non-coding RNAs

Step 5
Epigenetics and the environment

Step 6
Epigenome-wide studies

Step 7
Wrap up
Epigenetics glossary

• Glossary enclosed with the seminar invitation
• Compiled from online sources
• General and technical definitions:
  – Epigenetics and the epigenome
  – General concepts
  – Epigenetic mechanisms
    • DNA methylation
    • Histone Modifications
    • Non coding RNAs
Step by step

Step 1
Intro to epigenetics
Gene expression

Central dogma of molecular biology

DNA

5' AGCCTATC...
3' TCGGAGAG...

mRNA

5' AGCCUATC...

PROTEIN (GFP)
Greek “Epi”

Used as a prefix

• Above
• Over
• On
• Upon
• Besides
• In addition to
• Toward
• Among
Greek “Epi”

Prometheus
One of the Titans. He stole fire from the gods and gave it to mankind, and was severely punished for it.

Epimetheus
Prometheus’ brother. He fell in love and married Pandora in spite of the warnings of his more intelligent brother.
Greek “Epi”

Prometheus

PRO-metheus
He who thinks in advance

Epimetheus

EPI-metheus
He who thinks afterwards

Epigenetics intervenes afterwards, i.e. on the DNA sequence, without modifying it
Epigenetics

• Changes in gene expression that:
  -do not depend on the DNA sequence
  -can be stable
    • Through cell division (mitotically stable)
    • Transgenerational inheritance (limited evidence in humans)
  — may persist even in the absence of the conditions that established them (biological memory)

(adapted from Richards, Nat Gen 2006)
A symphonic example

DNA

Phenotype

Epigenetics

Andrea Baccarelli, IOM Meeting, Washington, DC – Feb 26th 2015
Epigenetics & Music Use the Same Markings

markings in **ink** (permanent)

pencil markings (can be erased)
**Epigenetic markings**

**DNA methylation**
Methyl marks added to certain DNA bases **repress** gene transcription.

**Histone modifications**
A combination of different molecules can attach to the ‘tails’ of proteins called histones. These **alter** the activity of the DNA wrapped around them.

**microRNAs**
Small non-coding RNAs that **block translation** of messenger RNAs into proteins.
Epigenetics contribute to tissue differentiation.

**Tissue specificity**

Epigenetic markings are **Tissue Specific**.

Potentially each tissue or cell type has a specific methylation profile.
Step by step

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Central dogma of molecular biology

DNA

mRNA

PROTEIN (GFP)
DNA methylation

The four bases in DNA

Adenine (A)  Cytosine (C)  Guanine (G)  Thymine (T)

Uracil

RNA

Figure 1.4b Genomes 3 (© Garland Science 2007)
Mechanism of DNA methylation

Expert Reviews in Molecular Medicine
©2002 Cambridge University Press
DNA methylation suppresses RNA expression

(more accurately: it is usually associated with suppressed RNA)

DNA methylation

inactive

DNA demethylation

active or poised to be activated

Cytosine-phosphate-Guanine CpG sites

ATCGCTAGCGTGCATTCCGGGATTCCGGCTGGGAGC

Compaction
DNA Methylation maintenance in Arabidopsis Thaliana (thale cress)
Step by step

Step 1
Intro to epigenetics

Step 2
DNA methylation

Step 3
Histone modifications
A severe problem of packaging!

- Human cell has 2m of DNA
- Nucleus is 0.006 mm in diameter

- Two opposing requirements:
  - 1. Compaction
  - 2. Access – Transcription
    - Replication
    - Repair
Chromatin

- Euchromatin –
  - Partially decondensed
  - Transcribed genes

- Heterochromatin –
  - Hypercondensed in interphase
  - Transcriptionally inert
  - Formation of chromosomal structures
    - Centromeres, telomeres
Electron micrographs of “chromatin preparations”

Beads on a string

30-nm fibers
Chromosomal structure

**Nucleosome** – fundamental unit of chromatin

147 bp **DNA** wound 1.75 turns around histones

**Histone octamer:**

2 x (H2A, H2B, H3, H4)
• **Histones**
  – Globular core domain
  – Unstructured N- and C-terminal tails

• **Post-translational modifications:**
  – *Acetylation* – Lys
  – *Methylation* (mono-, di- and tri-) – Lys and Arg
  – *Phosphorylation* – Ser and Thr
  – *Ubiquitination* (mono- and poly-) – Lys
  – *Sumoylation* (Lys); ADP-ribosylation; glycosylation; biotinylation; carbonylation
An Example: Histone acetylation

Lysine

Acetylation by HATs

Deacetylation by HDs
Histone modifications

types and functions

<table>
<thead>
<tr>
<th>Modification State</th>
<th>“meaning”</th>
</tr>
</thead>
<tbody>
<tr>
<td>unmodified</td>
<td>gene silencing?</td>
</tr>
<tr>
<td>acetylated</td>
<td>gene expression</td>
</tr>
<tr>
<td>acetylated</td>
<td>histone deposition</td>
</tr>
<tr>
<td>methylated</td>
<td>gene silencing/heterochromatin</td>
</tr>
<tr>
<td>phosphorylated</td>
<td>mitosis/meiosis</td>
</tr>
<tr>
<td>phosphorylated/acetylated</td>
<td>gene expression</td>
</tr>
<tr>
<td>higher-order combinations</td>
<td>?</td>
</tr>
<tr>
<td>unmodified</td>
<td>gene silencing?</td>
</tr>
<tr>
<td>acetylated</td>
<td>histone deposition</td>
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<tr>
<td>acetylated</td>
<td>gene expression</td>
</tr>
</tbody>
</table>

**Ac** - acetyl (lysine), **Me** - methyl (lysine), **P** - phosphoryl (Ser or Thr)
Step by step

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Non coding RNAs
Gene expression

DNA

Non coding RNAs

mRNA

5'...AGCCTATC...
3'...TCGGAGAG...

5'...AGCCUATC...

PROTEIN (GFP)
Meet the microRNAs (miRNAs)

- Small non-coding RNAs
  - 20-22 nt in length
- block the translation of messenger RNAs into proteins
miRNAs

• May regulate >30% of human genes
• miRBASE Release 21.0 (Sep 2014) has 28,645 entries
  – from 223 species
  – in humans: 1,881 precursors, 2,588 mature miRNAs
• Discovery of new miRNAs is ongoing ...

• Source: miRBASE database
  http://www.mirbase.org/cgi-bin/browse.pl?org=hsa
Other non-coding RNA types

• Non-coding RNA = miRNAs?
• Other types of non-coding RNAs
  – PIWI-interacting RNAs (piRNAs)
  – small nucleolar RNAs (snoRNAs)
  – promoter-associated small RNAs (PASRs)
  – transcriptional start sites associated (TSSa-RNA)
  – transcribed ultraconserved regions (T-UCRs)
  – promoter upstream transcripts (PROMPTS)
  – large intergenic non-coding RNAs (lincRNAs)
Step by step

Step 1: Intro to epigenetics
Step 2: DNA methylation
Step 3: Histone modifications
Step 4: Non coding RNAs
Step 5: Epigenetics and the environment
**Environment, genetics, epigenetics**

**ENVIRONMENTAL EXPOSURES**

**GENETICS**
- **E→G**
  - DNA DAMAGE/ MUTATIONS
  - HIGHLY PERSISTENT
  - TRANSMITTED TRANSGENERATIONALLY (if in germline)

- **G × E**
  - GENETIC MAKE UP
  - HIGH/LOW SUSCEPTIBILITY
  - TRANSMITTED TRANSGENERATIONALLY

**EPIGENETICS**
- **E→Epi**
  - EPIGENETIC CHANGES
  - REVERSIBLE
  - TRANSMITTED TRANSGENERATIONALLY (limited human evidence)

- **Epi × E**
  - EPIGENETIC MAKE UP
  - HIGH/LOW SUSCEPTIBILITY
  - TRANSMITTED TRANSGENERATIONALLY (on selected genes)

**HEALTH EFFECTS**

Adapted from Bollati & Baccarelli, Heredity 2010
Concentrated Ambient Particle (CAP) exposure
Effects of fine CAPs on Blood pressure and DNA methylation

Effect on Blood Pressure

Effect on Blood DNA methylation

Differences of fine CAP exposure vs control

Bellavia et al. JAHA 2013
Parental olfactory experience influences behavior and neural structure in subsequent generations.

Dias & Ressler, Nature Neuroscience 2014
(Graphics adapted from Szyf Nature Neuroscience 2014)
Dias & Ressler’s experiment

• Offspring mice inherited conditioned fear to acetone odor
  – The father mouse experienced odor in conjunction with electric shock (after repeated experience, the mouse was conditioned to get a fear reaction upon exposure to odor alone)
  – The offspring mouse experienced fear to the acetone odor although never exposed to electric shock

• Experiment repeated with IVF to exclude any behavioral transmission through mothers

• Altered DNA methylation in an odorant gene found in the mouse sperm
When Brian Dias became a father last October, he was, like any new parent, mindful of the enormous responsibility that lay before him. From that moment on, every choice he made could affect his newborn son's physical and psychological development. But, unlike most new parents, Dias was also aware of the influence of his past experiences — not to mention those of his parents, his grandparents and beyond.

Where one's ancestors lived, or how much they valued education, can clearly have effects that pass down through the generations. But what about the legacy of their health: whether they smoked, endured famine or fought in a war?

As a postdoc in Kerry Ressler's laboratory at Emory University in Atlanta, Georgia, Dias had spent much of the two years before his son's birth studying these kinds of questions in mice. Specifically, he looked at how fear associated with a particular smell affects the animals and leaves an imprint on the brains of their descendants.

Dias had been exposing male mice to acetophenone — a chemical with a sweet, almond-like smell — and then giving them a mild foot shock. After being exposed to this treatment five times a day for three days, the mice became reliably fearful, freezing in the presence of acetophenone even when they received no shock.

Ten days later, Dias allowed the mice to mate with unexposed females. When their young grew up, many of the animals were more sensitive to acetophenone than to other odours, and more likely to be startled by an unexpected noise during exposure to the smell. Their offspring — the 'grandchildren' of the mice trained to fear the smell — were also jumpier in the presence of acetophenone. What's more, all three generations had larger-than-normal 'M71 glomeruli,' structures where acetophenone-sensitive neurons in the nose connect with neurons in the olfactory bulb. In the January issue of Nature Neuroscience, Dias and Ressler suggested that this hereditary transmission of environmental information was the result of epigenetics — chemical changes to the genome that affect how DNA is packaged and expressed without altering its sequence.

Biologists first observed this 'transgenerational epigenetic inheritance' in plants. Tomatoes, for example, pass along chemical markings that control an important ripening
A lingering smell?

Studies in animals have shown that stressful experiences can be passed onto offspring, often in the form of a general anxious or stress-sensitive phenotype. A new study now shows that highly specific experiences can also be inherited by subsequent generations, in terms of behaviour and anatomy, and that this transmission occurs through parental gametes.

Dias and Ressler trained male mice (F0 mice) to associate mild footshocks with one of two odours: ace-anatomical changes might underlie the behavioural effects; however, the two could not be directly correlated because they were assessed in different sets of animals.

Interestingly, the male offspring of F1-Ace males and F1-Prop males (that is, F2 males) also showed increased sensitivity to Ace and Prop, respectively. Moreover, like their fathers, F2-Ace males had larger M71-specific glomeruli. These findings suggest that a specific olfactory experience had sensitivity to Ace was not socially mediated and can also occur through the maternal line.

The authors reasoned that if F0-Ace mice transmit their olfactory experience through gametes, then DNA in sperm of F0-Ace males might show epigenetic changes in the gene encoding the M71 receptor (Olfr151). Indeed, Olfr151 was hypomethylated in both F0-Ace sperm (compared with F0-Prop sperm) and F1-Ace sperm. Interestingly, however.
Disease programming throughout the lifecourse

Figure adapted from Fleisch, Wright & Baccarelli, J Mol Endocrinol, 2012
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Epigenome-wide studies
Some nomenclature
(DNA methylation used as example)

• Candidate gene (gene-specific) approach
  • A priori knowledge $\rightarrow$ candidate genes
  • test for association with exposure/risk factor
  • test for association with disease/phenotype

• Global (average) level of methylation (5mC content)
  • Average methylation of all CpG sites across the genome
  • test for association with exposure/risk factor
  • test for association with disease/phenotype

• Epigenome-wide approach (EWAS)
  • Agnostic approach $\rightarrow$ entire genome
  • test for association with exposure/risk factor
  • test for association with disease/phenotype
Examples (DNA methylation)

• Candidate gene approach
  – Participant #1’s blood has 26% methylation in the IL6 promoter (N.B.: any other region of interest can be targeted, e.g., CpGi shore, shelf, etc.)

• Global methylation approach
  – Participant #1’s blood has 4.5% methylation (i.e., 4.5% of all cytosines found in blood are methylated; no information on where the methylated cytosines are located)

• Genome-wide approach
  – Methylation in Participant #1’s blood is measured at a high number of CpG sites (e.g, if we use Illumina Infinium 450K beadchip → we will get ≈486,000 numbers [one for each CpG site] for Participant #1’s blood)
GWAS/EWAS

• Genetics
  – Genome wide association studies
  – We study single nucleotide polymorphisms (SNPs) or other differences (e.g., insertion, deletion, copy number variations)

• Epigenetics
  – Epigenome wide association studies
  – We study DNA methylation, histone modifications, etc.
  – Screen for 100Ks to millions of individual features (e.g., CpG sites)
The 450K BeadChip covers a total of 77,537 CpG Islands and CpG Shores (N+S)

<table>
<thead>
<tr>
<th>Region Type</th>
<th>Regions</th>
<th>CpG sites covered on 450K BeadChip array</th>
<th>Average # of CpG sites per region</th>
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<tbody>
<tr>
<td>CpG Island</td>
<td>26,153</td>
<td>139,265</td>
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<td>N Shore</td>
<td>25,770</td>
<td>73,508</td>
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<td>S Shore</td>
<td>25,614</td>
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<td>Total</td>
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The 450K BeadChip covers a total of 20,617 genes
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Wrap up
Conclusions

• Epigenetics is all about control of gene expression
  – Relatively stable
  – Biological programming
  – Influenced by the environment

• Epigenetics investigates different mechanisms
  – Not limited to those presented here

• Expanding research on how environmental toxicants may reprogram the epigenome and affect human health
Some readings
