Intro to Epigenetics

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How do specialized cell types with the exact same genotype develop?

Every cell has a distinct epigenetic pattern.
Epigenetics
These “genes” can be turned on

Genetics
This “gene” cannot be turned on
What is chromatin?

A complex of both DNA and proteins that organizes and compacts DNA. This allows a human cell's ~2 meter long DNA (stretched end-to-end) to fit inside a 6 um nucleus.

https://www.nature.com/scitable/topicpage/dna-packaging-nucleosomes-and-chromatin-310/
Nucleosome “bead”
(8 histone molecules + 146 base pairs of DNA)

~10 nm
Histone tail modifications

These modifications have different effects on the DNA-histone interactions, and can open or compact chromatin. There are also proteins that recognize these modifications and can affect expression.

Histone variants and modifications - associated regions & effect on expression

Histone tail modifications

Histone modifications are reversible

Lysine

Acetylation by HATs

Deacetylation by HDs

some residues (lysine) can support multiple methyl groups

e.g. H3K9me3

https://web.stanford.edu/group/gozani/cgi-bin/gozanilab/research/

http://www.web-books.com/MoBio/Free/Ch4G.htm
H2A.B-containing nucleosomes wrap only 118 bp of DNA instead of 146 bp and are less stable.
Nucleosome Remodeling

Figure 4–33. Molecular Biology of the Cell, 4th Edition.
DNA methylation and gene expression
DNA methylation and gene expression

constitutive expression = gene is always on
ectopic expression = gene is on where it's not supposed to be

Dolinoy et al., 2008
DNA methylation and gene expression

constitutive expression = gene is always on
ectopic expression = gene is on where it's not supposed to be

cryptic promoter (active)

constitutive, ectopic expression

cryptic promoter (inactive)

normal expression

Dolinoy et al., 2008
Putting it all together

Chromatin marks and dynamics:

- Active enhancer
  - H3K27ac
  - H3K4me1

- ShmC

- Core promoter

- Active chromatin
  - H3K9ac
  - H3K14ac
  - H3K4me3

- HMTs
- HATs
- DNMTs
- HDACs
- TETs
- SMCs

Closed or poised enhancer
- H3K27me3
- H3K4me1

Inactive chromatin
- H3K27me3
- H3K9me3

DNA topology in the cell:

- Cell 1
- Chromosome territories


Greco and Condorelli (2015). Nat Rev Cardio
Key Techniques Frequently Employed in Epigenetics Research

- **ChIP-seq**: Tells you **WHERE** a protein is **BOUND** on the genome, including histone variants, transcription factors, etc.

- **Bisulfite Sequencing**: Tells you the **PROPORTION** of **METHYLATED** cytosines in your sample
ChIP-seq: Chromatin Immunoprecipitation [coupled to] High throughput Sequencing

Step zero - Native state in the cell, transcription factor is bound to DNA
ChIP-seq

Step One – *crosslink* protein to DNA
ChIP-seq

Step two – **shear** the DNA (aka chromatin)
ChIP-seq

Step three – use antibody (aka immuno) to bind the protein.
ChIP-seq

Step three – use antibody (aka immuno) to bind the protein, wash away unbound DNA.
ChIP-seq

Step four – de-crosslink
Step five – purify DNA (precipitation) and sequence
Example of ChIP seq data

Modified from Michael R. Tallack et al. Genome Res. 2010;20:1052-1063
Histone variants and modifications - associated regions & effect on expression

Bisulfite-sequencing

(reacted as T by transcriptional machinery)
Bisulfite-sequencing

Allele 1 (methylated)

---ACTCCACGG---TCCATCGCT---
---TGAGGTGCC---AGGTAGCGA---

Allele 2 (unmethylated)

---ACTCCACGG---TCCATCGCT---
---TGAGGTGCC---AGGTAGCGA---

Bisulfite treatment
Alkylation
Spontaneous denaturation

---AUTUUAGG---TUUATCGUT---
---TGAGGTGUU---AGGTAGCGA---

---AUTUUAGG---TUUATUGUT---
---TGAGGTGUU---AGGTAGUGA---

Non-methylation-specific PCR
Methylation-specific PCR

Differentiation of bisulfite-generated polymorphisms
Example of bisulfite sequencing data

Wasson et al. (2016) eLIFE
Example of ChIP-seq and Bisulphite Sequencing Data

Harris et al., 2018. Science
Summary of topics in this course

• 9 topics (1/week), 2 papers each
What will you choose?

1. Today
2. DNA methylation
3. Histone methylation / demethylation
4. Polycomb RNA-directed silencing
5. DNA demethylation
6. Enhancers
7. Targeted epigenetics
8. 3D chromatin architecture
9. Phase transition + epigenetics
Week 2 - DNA methylation

  • Looks at mechanism for DNA methylation maintenance after replication

  • One of the first papers published that did whole-genome bisulfite-sequencing to look at DNA methylation genome-wide
Week 3 - Histone methylation/demethylation

  • Important early paper linking a histone modification (H3K9me) to heterochromatin maintenance and gene expression

  • Identified one of the first histone demethylases, showing that histone modifications are reversible
Week 4 - Polycomb

  - Showed that Polycomb group (PcG) proteins (PRC2) methylate H3K27 to silence target genes and establish a repressive chromatin state

  - Showed that Polycomb Repressive Complex 1 (PRC1) creates compacted chromatin through interactions with nucleosomes independently of their histone tail.
Week 5 - RNA-directed silencing

  • Role of piRNAs in maintaining silencing of TEs; piRNA loci help form an 'immune system' against TEs

  • Characterized a specialized Argonaute required for transgenerational maintenance of small-RNA mediated silencing signals, also required for fertility, in C. elegans
Week 6 - RNA-directed silencing

• Extensive Demethylation of Repetitive Elements During Seed Development Underlies Gene Imprinting
  • Showed that DME (a DNA glycosylase) selectively demethylates certain loci in Arabidopsis endosperm, and association with genomic imprinting

• Dual functions of Tet1 in transcriptional regulation in mouse embryonic stem cells
  • Linked Tet1 to maintenance of DNA hypomethylation, gene expression regulation
Week 7 - Enhancers

  - Early work identifying enhancers using whole-genome approaches, also showing that cell-type-specific histone modifications at enhancers correlate with expression

  - Systematically characterized enhancers associated with facial development & variation in humans
Week 8 - Targeted epigenetics

  - Demonstrated that fusion of dCas9 to either Tet1 (removes 5mC) or Dnmt3a (adds 5mC) can be used to cause targeted DNA methylation/demethylation

  - Optimizing CRISPR systems for targeted gene activation/repression via combinatorial manipulation of epigenetic marks & chromatin structure
  • Initial publication of Hi-C, a method for mapping 3D chromatin architecture
  • Demonstrates that CTCF sites are required for looping between CTCF sites and insulation of topologically associating domains through blocking enhancer binding.
  - New potential mechanism for heterochromatin compaction & associated gene silencing, via liquid-liquid phase separation driven by HP1α

  - New model proposing that activation domains (ADs) can form phase-separated droplets via interaction with Mediator, and this phase-separation is associated w/ gene activation