Heterochromatin Formation - It's all about silencing!

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Slides assembled with notes by SCR Elgin, WUSTL
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1

Silencing is critical in maintaining the somatic genome; loss of silencing is linked to aging.

L1, a retrovirus
- 21% of human genome
- normally silenced
- expression → inflammation; transposition → mutations → cancer.


From N. Riddle; work by Jorgensen lab

3

Repetitious elements are recognized as such and silenced, an epigenetic change

- An extra copy of gene required for pigment production leads to silencing in a stochastic pattern, giving variegation; in extreme cases both copies of the gene are completely silenced (white flower).
- Silencing of repeats is important for genome stability/transposon control
- How is this accomplished?
- What are the consequences for the genes?

Slide from N. Riddle; work by Jorgensen lab

4

What determines phenotypes? It is not just your DNA....its the packaging!
Silencing is critical……

Environment (diet, stress, maternal care) → Phenotype (highly inbred sibs)

Yellow mouse
- Obesity; high risk of cancer, diabetes; reduced lifespan
Pseudo-agouti mouse
- Low risk of cancer, diabetes, obesity; prolonged lifespan

Differences? Post synthetic modifications of DNA, histones

From N. Riddle, T. Wang

5

Methylation and diet: impact on the agouti locus

- Mothers fed BPA (soaks up methyl) → higher IAP expression → yellow coat
- Mothers fed folate (methyl donor) → lower IAP expression → brown coat
- Trait stable at least to the following generation (your grandparents' diet could affect your epigenetics!)
- Very few loci of this type
  - variable, meta-stable mC;
  - impact on neighboring genes.

Morgan et al 1999

6
What is epigenetics?
The study of gene expression states that are heritable, through mitosis and/or meiosis, but that are independent of DNA sequence changes.

How is this accomplished?
• DNA modification – 5mC
• Nuclear localization
• Chromatin structure
  – heterochromatin
  – euchromatin
• Environmental impact!

DNA packaging domains

Euchromatin
• Less condensed
• Chromosome arms
• Unique sequences; gene rich
• Replicated throughout S
• Recombination during meiosis

Heterochromatin
• Highly condensed
• Centromeres and telomeres
• High in repetitive sequences; gene poor
• Replicated in late S
• No meiotic recombination

Transcriptional activators;
RNA pol II
• Hyper-acetylated histone tail

Heterochromatin Protein 1 complex
• Hypo-acetylated histone tail; methylated H3K9

Specific mutations in the core histones have specific impacts on gene regulation

The core histone tails are extensively modified

The histones are extensively modified

Over 100 modified sites characterized
Most in tails, some interior
Impacts: gene expression / silencing
DNA repair, cell cycle
Modifications: add an organic group
add an inorganic group
add a polypeptide
alter an amino acid

Role of acetylation of histone tails in yeast transcription control - Gcn5 is a histone acetyltransferase!
How is this done with precision?
Enzymes often dock and orient using the nucleosome surface

1. acidic patch
2. H3 a1-L1 elbow
3. H2B a1-L1 elbow
4. H2B C-terminal helix

Song Tan, Penn State

The nucleosome as a recognition surface


Inexpensive and easy to culture, short life cycle, easily visible phenotypes: good genetic approaches
Biochemical approaches
Polytene chromosomes: excellent cytology
Simple genome, good reference sequence
PEV – reporter for gene silencing, heterochromatin formation
Metazoan useful for behavioral, developmental, and human disease research

Model system – fruit flies!

*Distribution of Heterochromatin Protein 1
Identified by screening monoclonal antibodies against tight-binding nuclear proteins

Position Effect Variegation in Drosophila
provides a functional assay for mutations in putative chromatin proteins

Wild Type
Inversion

Trans-acting modifiers of PEV:

Su(var):
E(var):

*Heterochromatin-associated gene silencing is dependent on HP1

- some mutations recovered by T. Grigliatti as suppressors of PEV map approximately to HP1a locus;
- sequencing confirmed that mutations in the HP1 gene result in a loss of silencing;
- dosage dependent response.
HP1 sequence from Drosophila, mouse, human and mealy bug identifies conserved chromo & chromo shadow domains

Clark and Elgin, 1992 Nucleic Acids Res. 20:6067

HP1 from mammals can rescue mutations in flies!

HP1 interacts with both the modified histone H3K9me2/3 and the modifying enzyme (It is a reader that binds to its writer; first example!)

HP1: Chromo Shadow

Histone 3 methyl-Lys9

H3 K9 methylation

SU(VAR)3-9 HMT

(SU(VAR)3-9 identified in screen by Reuter; H3 interaction first shown from work in mammals – Jenuwein, Kouzarides; demonstrated in flies by Imhof.)

Model for spreading of heterochromatin (and epigenetic inheritance):

create a system that can recognize a histone modification mark and can generate that same mark.

Elgin summary

PEV transition: loss of euchromatin marks

Establishing silencing: gain of heterochromatin marks

And additional proteins are required – flies have 3 different H3K9 HMTs; HP1a has ~30 interacting partners

*Transposition of a P element reporter allows sampling of heterochromatin domains

And the Y chromosome

Wallrath and Elgin, 1996
*Assessing chromatin structure—same gene, different environments*

**Analysis:**
- MNase cuts between nucleosomes; DNase I and restriction enzymes cut hypersensitive sites

**Euchromatin**

What happens to the same gene in heterochromatin?

**Loss of accessibility in the heterochromatic hsp26 transgene is reversed in an HP1 mutant background**

The nucleosome array associated with the silenced transgene in heterochromatin shows more regular spacing and sharper MNase cleavage sites


What might define the heterochromatin fiber?

HP1α can dimerize, cross-linking 2 nucleosomes
- Here (right) linker DNA free, allowing ACF remodeling
- In vivo preferentially X-links alternating nucleosomes (below)
- → heterochromatin uses 2-start helical fibers; also stabilized by H1 ("linker histone")
- some satellites = 2 nu (Drosophila 378 bp repeat)
- ~20 kb segments required for segregated mass (modeling)

Drosophila HP1α exhibits liquid phase separation properties in vitro and in vivo.

Dynamic, liquid-like assembly
- Fusion of "droplets" / coalescence
- Sensitive to disruption of weak hydrophobic interactions
- Dependent on intrinsically disordered regions—N terminus, linker
- Selective permeability (inert probe exclusion)
- Allows high concentration and high mobility of components

Purified Drosophila HP1α forms liquid droplets in vitro

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Impact of mutation H3K9R (S-phase expressed gene cluster) in flies:
• Nucleosome occupancy in heterochromatin decreased; ~2% flies survive
• Gene expression similar; but increased expression, mobilization of transposons

Histone modification patterns can be inherited

Applications: reversal of the differentiated state
Use localized histone acetylation to allow expression of key regulators

ChIP Immuno-precipitation – ChIP → mapping chromatin structure
Clustering chromatin fragments according to histone modification marks suggests nine major chromatin states

- H3/H4 acetylation is associated with the active state
- HATs are activators; HDACs are silencers
- H4K16 acetylation is associated with dosage compensation
- H3K9 di- and tri-methylation are found in constitutive silent domains (centromeres, telomeres)
- H3K27 tri-methylation is associated with developmentally regulated silencing (Pc complex) (This is NOT facultative heterochromatin!)
- H3K4 di- and tri-methylation occurs at the 5' end of active genes (HS site), and H3K36me3 over the gene body
- Enhancers are marked by H3K4me1 and H3K27ac (HS sites)
- Chromatin modification states switch to achieve gene regulation, controlling frequency of transcription.

Chromatin states identify large-scale genomic domains

- Heterochromatic structure differs -
  - in a loss of accessibility, loss of 5' HS sites;
  - in having a more ordered nucleosome array & fiber;
  - in having increased nucleosome stability.
- Heterochromatin is marked by -
  - hypoacetylated H4, H3K9me2/3, HP1a, [methylated DNA].
  - Epigenetic inheritance and spreading are achieved by -
    - a protein (ex. HP1a) or protein complex that interacts with a modified histone and with the modifying enzyme; this can now be achieved with synthetic systems.
  - The genome can be subdivided into different states based on histone modifications -
    - specific patterns associated with transcription start sites, enhancers, transcript elongation;
    - two types of silencing domains: H3K27me3 (Polycomb) and H3K9me2/3 (HP1a)
    - and more!