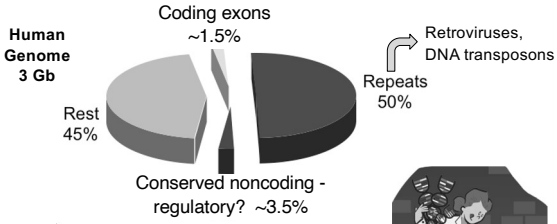
Heterochromatin Formation - It's all about silencing!

GEP 2022

Slides assembled with notes by SCR Elgin, WUSTL

1

Large eukaryotic genomes must be packaged to regulate gene expression while minimizing TE movement.



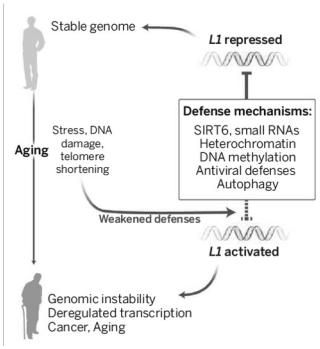
Key Questions:

- Is repetitious DNA junk or garbage ? Junk - used! Provides flexibility?
- How is heterochromatin packaging, & silencing, established & maintained ?

Xiaohui Xie, MIT 2007

2

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.

See "Sleeping dogs of the genome," Gorbunova et al 2014 Science 346: 1187-8.

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?

Napoli et al 1990 Plant Cell 2: 279-289

Parent line (petunia)

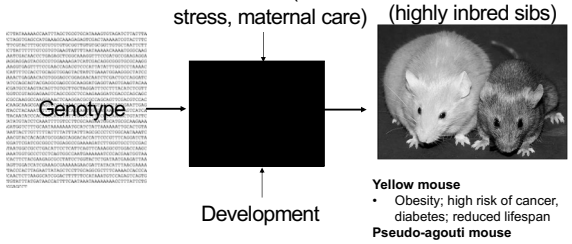
+transgene

+transgene

Slide from N. Riddle; work by Jorgensen lab

4

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



Yellow mouse
- Obesity; high risk of cancer, diabetes; reduced lifespan

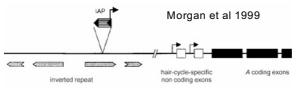
Pseudo-agouti mouse
- Low risk of cancer, diabetes, obesity; prolonged lifespan

Differences? Post synthetic modifications of DNA, histones

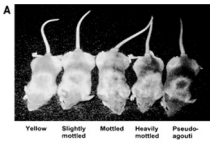
From N. Riddle, T. Wang

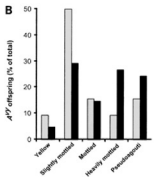
5

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.





Black bars = folate feeding
Waterland and Jirtle 2003
Mol Cell Biol 23: 5293-300.

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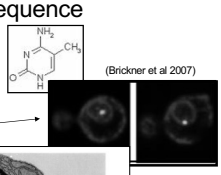
What is epigenetics?

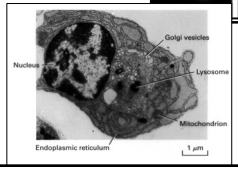
The study of gene expression states that are heritable, through mitosis and/or meiosis, but that are independent of DNA sequence changes.

How is this accomplished?

- DNA modification – 5mC
- Nuclear localization
- Chromatin structure
  - heterochromatin
  - euchromatin
- Environmental impact!



(Brickner et al 2007)



(Lodish et al., Molecular Cell Biology, 4th Edition)

from N. Riddle

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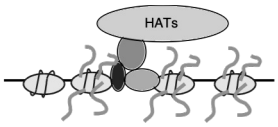
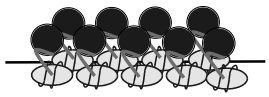
DNA packaging domains





- Euchromatin
  - Less condensed
  - Chromosome arms
  - Unique sequences; gene rich
  - Replicated throughout S
  - Recombination during meiosis
- Heterochromatin
  - Highly condensed
  - Centromeres and telomeres
  - High in repetitious 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

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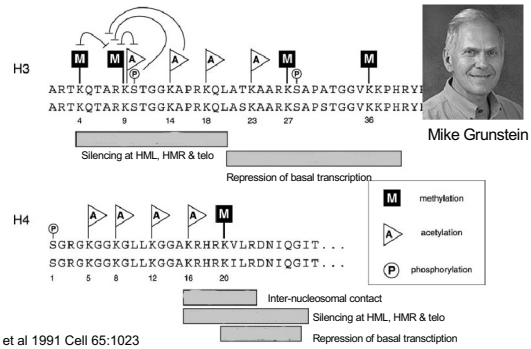
Specific mutations in the core histones have specific impacts on gene regulation



Mike Grunstein

Durrin et al 1991 Cell 65:1023
Recent work by Duronio in flies

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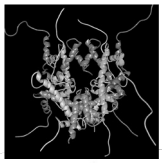
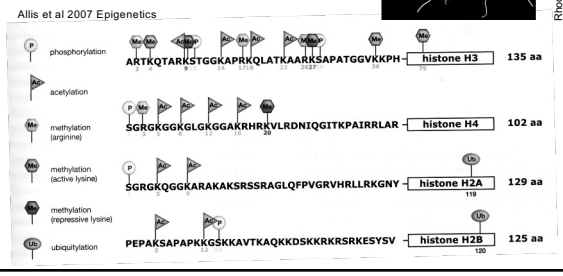
The core histone tails are extensively modified

Tails are accessible, outside of core
Histone modifications are reversible-
Examples: HAT / HDAC
HMT / KDM
(writers, erasers; readers)



Rhodes, Nature (1997) **389**, 231-233

Allis et al 2007 Epigenetics



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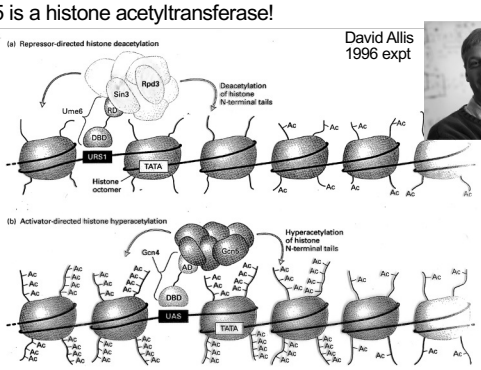
Role of acetylation of histone tails in yeast transcription control - Gcn5 is a histone acetyltransferase!

David Allis
1996 expt



Lodish et.al., Molecular Cell Biology, 4th Ed

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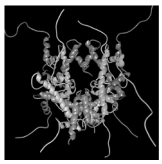
The histones are extensively modified

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



Rhodes, Nature (1997) **389**, 231-233

ac	acetylation	mal	malonylation
ar1	mono-ADP-ribosylation	me1	monomethylation
bio	biotinylation	me2	dimethylation
but	butyrlyation	me3	trimethylation
cit	citrullination	og	*O*-GlcNAcylation
cr	crotonylation	oh*	hydroxylation
for	formylation	ox*	oxidation
gt*	glutathionylation	ph	phosphorylation
hib	2-hydroxyisobutyrylation	su	SUMOylation
iso	isomerization	ub	ubiquitination

Zhao & Garcia 2015 CSHL Perspect Bio 7:a025064.

Nomenclature: H3K9me3

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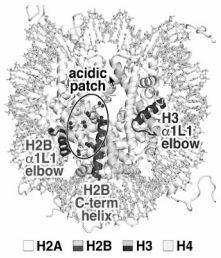
How is this done with precision?

Enzymes often dock and orient using the nucleosome surface

Nucleosome targets



1. acidic patch
2. H3 α1-L1 elbow
3. H2B α1-L1 elbow
4. H2B C-terminal helix

Song Tan, Penn State

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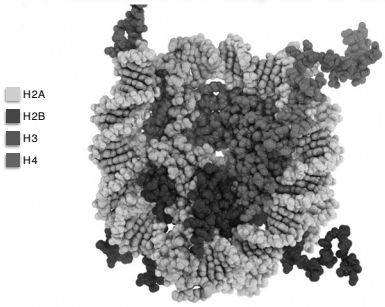
The nucleosome as a recognition surface



Song Tan, Penn State

Luger et al, Nature, 1997; Davey et al, JMB, 2002; Richmond and Davey, Nature, 2003

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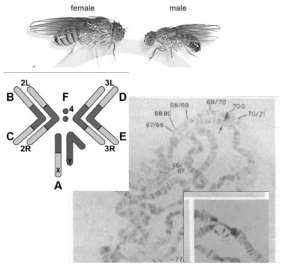
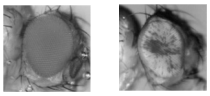
Model system – fruit flies!



Mary Lou Pardue, MIT

euchromatin heterochromatin



expressed silenced

- 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

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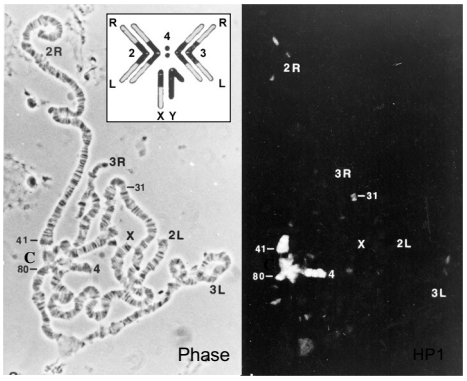
*Distribution of Heterochromatin Protein 1

Identified by screening monoclonal antibodies against tight-binding nuclear proteins



James et al, 1989, Europ J Cell Bio 50: 170

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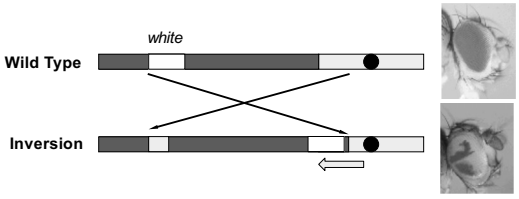
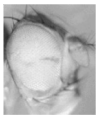
Position Effect Variegation in *Drosophila*

provides a functional assay for mutations in putative chromatin proteins



Trans-acting modifiers of PEV:

Su(var): 

E(var): 

Photos: Elgin lab

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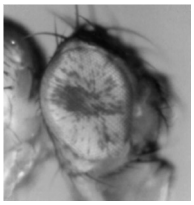
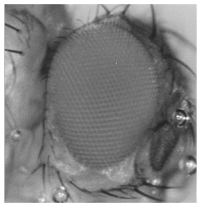
*Heterochromatin-associated gene silencing is dependent on HP1



Mutations in → gene for HP1a



- 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.

Eissenberg et al, 1990, PNAS 87: 9923

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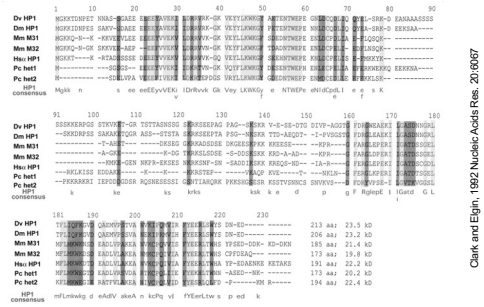
*HP1 sequence from Drosophila, mouse, human and mealy bug identifies conserved chromo & chromo shadow domains



HP1 from mammals can rescue mutations in flies!

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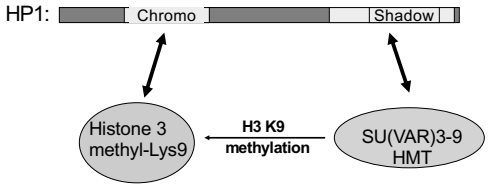
HP1 interacts with both the modified histone H3K9me2/3 *and* the modifying enzyme

(It is a reader that binds to its writer; first example!)



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

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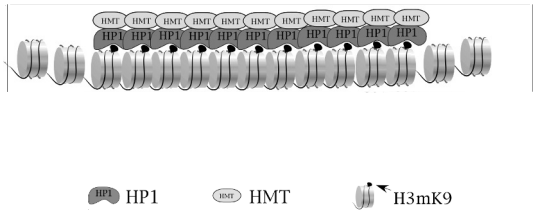
Model for spreading of heterochromatin (and epigenetic inheritance):

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



Elgin summary

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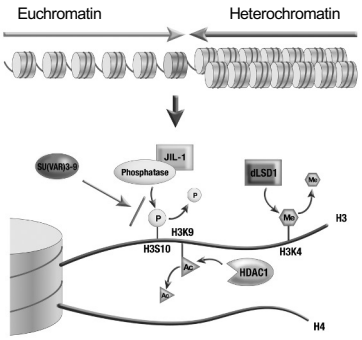
PEV transition: loss of euchromatin marks



-and probably others!

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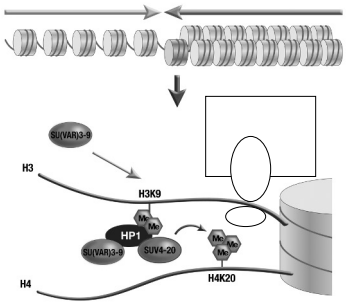
Establishing silencing: gain of heterochromatin marks



And additional proteins are required –

flies have 3 different H3K9 HMTs; HP1a has ~30 interacting partners

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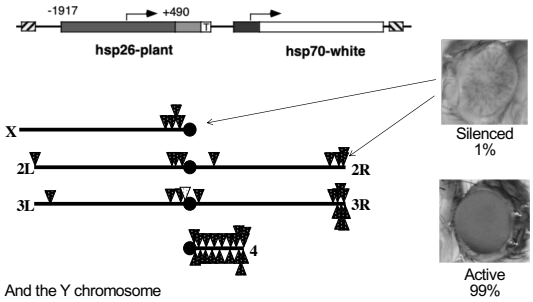
*Transposition of a P element reporter allows sampling of heterochromatin domains



And the Y chromosome

Wallrath and Elgin, 1995

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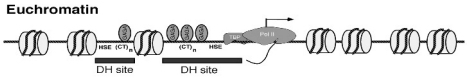
*Assessing chromatin structure- same gene, different environments

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



What happens to the same gene in heterochromatin?

Elgin lab

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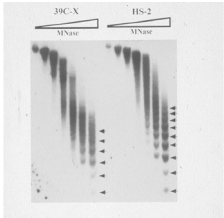
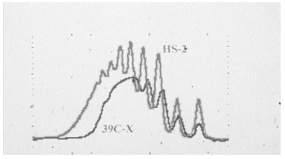
*The nucleosome array associated with the silenced transgene in heterochromatin shows more regular spacing and sharper MNase cleavage sites





Sun et al, 2001, Mol Cell Biol 21: 2867

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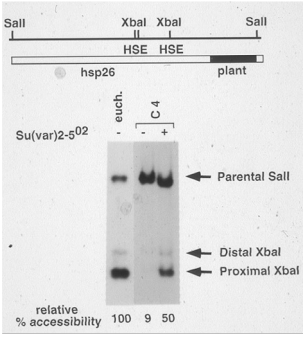
*Loss of accessibility in the heterochromatic *hsp26* transgene is reversed in an HP1 mutant background



Cryderman et al 1999, Nucleic Acids Res 27: 3364

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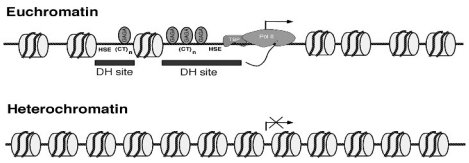
*Assessing chromatin structure- same gene, different environments

Analysis based on nuclease digestion of chromatin



The euchromatic *hsp26* transgene:
- DH sites: accessibility at the TSS, upstream regulatory region
- irregular nucleosome array

The heterochromatic *hsp26* transgene:
- loss of DH sites
- regular nucleosome array

Elgin lab

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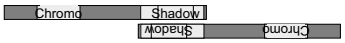
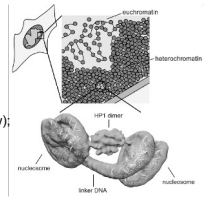
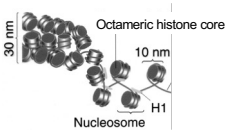
What might define the heterochromatin fiber?



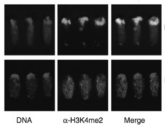
HP1a can dimerize, cross-linking 2 nucleosomes
- Here (right) linker DNA free, allowing ACF remodeling
- *In vivo* preferentially X-links alternating nucleosomes (below);
- → heterochromatin uses 2-start helical fibers;
  also stabilized by H1 ("linker histone");
  some satellites = 2 nu (Drosophila 378 bp repeat)
- ~20 kb segments required for segregated mass (modeling)



Machida et al 2018 Cell 69: 385-397
Cryo-EM of reconstituted nu-dimers



Segregated heterochromatin mass



Elgin & Reuter 2013 CSH Perspect Biol

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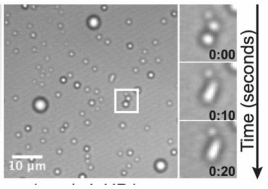
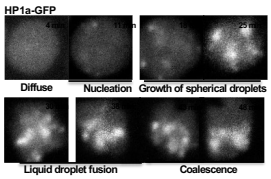
Drosophila HP1a exhibits liquid phase separation properties *in vitro* and *in vivo*.

Strom et al. Nature 2017

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* HP1a forms liquid droplets *in vitro*



Heterochromatin establishment in *Drosophila* embryos; HP1 masses display characteristics of liquid droplets.

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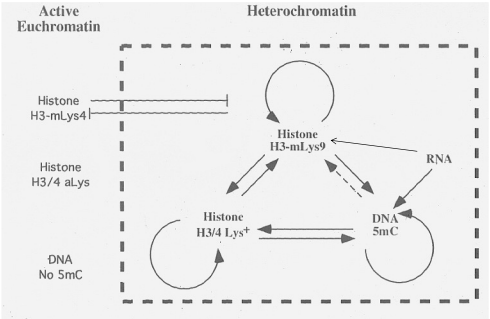
Feedback loops to maintain heterochromatin



[Richards & Elgin, 2002]

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

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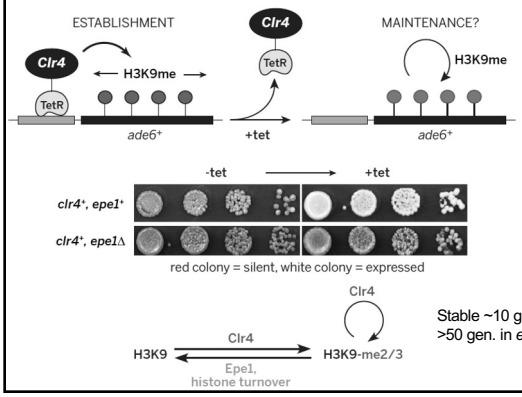
Histone modification patterns can be inherited



Ragunathan et al 2015 Science 348: 90.

Stable ~10 gen. *epe1*+, >50 gen. in *epe1Δ*

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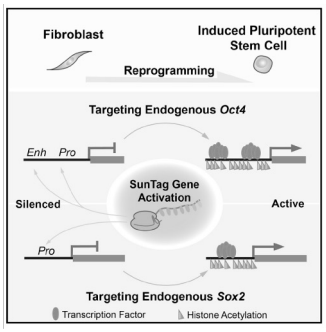
Applications: reversal of the differentiated state

Use localized histone acetylation to allow expression of key regulators



Liu et al, 2018, Cell Stem Cell 22: 1-10

The SunTag system uses dCas9 fused with HAT p300 core. Activation of either Oct4 or Sox2 transcription factors can drive reprogramming of iPSCs

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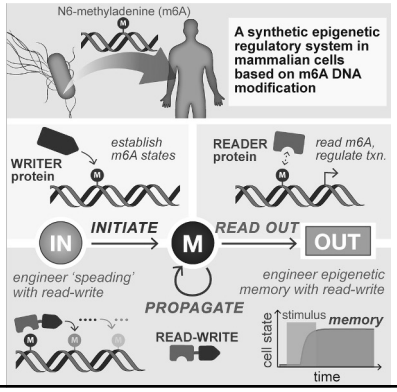
Engineering epigenetic regulation using synthetic read-write modules for histone modifications



Park et al 2019 Cell 176: 227-38

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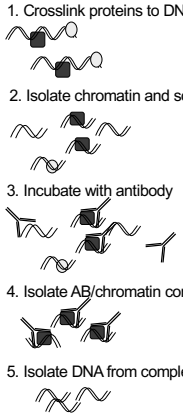
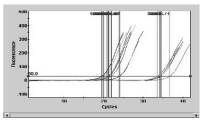
Chromatin Immuno-precipitation – ChIP → mapping chromatin structure

1. Crosslink proteins to DNA
2. Isolate chromatin and sonicate
3. Incubate with antibody
4. Isolate AB/chromatin complexes
5. Isolate DNA from complexes



qPCR



ChIP-chip

ChIP-seq

N Riddle

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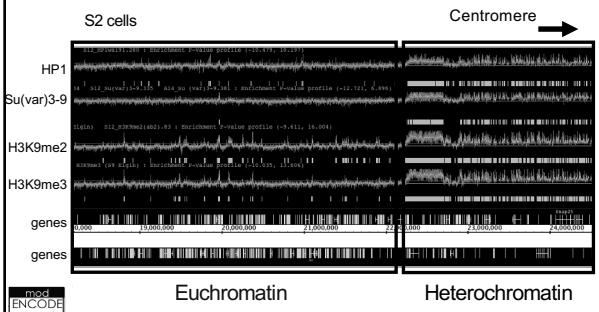
*ChIP-chip mapping of chromosomal proteins shows the distinct differences between heterochromatin and euchromatin



modENCODE

Euchromatin / heterochromatin transition point from Flybase
Riddle et al, 2011, Genome Research 21: 147-63.

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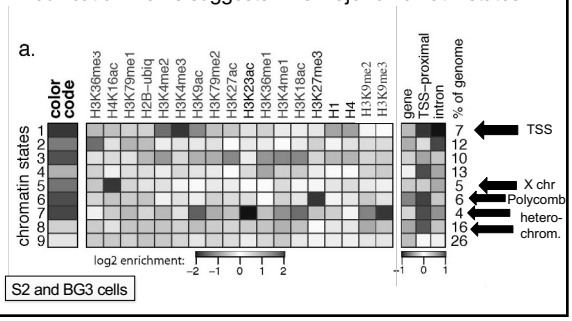
Karchenko et al, 2011, Nature 471: 480-5.

*Clustering chromatin fragments according to histone modification marks suggests nine major chromatin states



S2 and BG3 cells

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Histone modifications are critical for setting levels of gene expression.

- 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.

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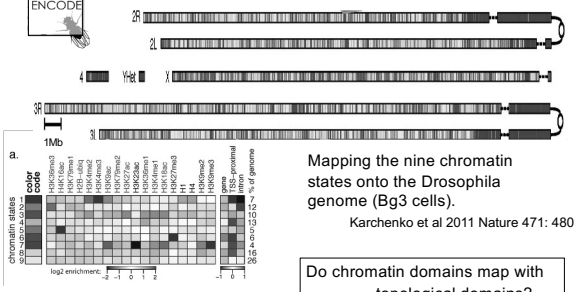
*Chromatin states identify large-scale genomic domains



Mapping the nine chromatin states onto the Drosophila genome (Bg3 cells).

Karchenko et al 2011 Nature 471: 480

Do chromatin domains map with
- topological domains?
- Hi-C (contact) domains?

Probably – single cell analysis

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Summing up…..

Much of the genome is packaged in heterochromatin.

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!

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