

## Chromatin and Histones (Pehrson)

### **Big Picture**

- Chromatin represents highly packaged DNA associated with nucleosomes
- Nucleosomes consist of a core DNA wrapped around an octomer of core histones and linker DNA associated with H1 histones
- Histone Variants exist
  - Some are replication dependant
  - Others are replication independent

### **Details**

- Nucleosome is the fundamental subunit of chromatin
  - Contains approx 200bp of DNA
  - Nucleosome associated DNA is divided into core DNA and linker DNA
    - Core DNA is wrapped around octomer of core histones
    - Linker DNA connects one core to another (forming the “bead-like structure”)
  - 2 copies of each core histone per nucleosome
    - H2A, H2B, H3, H4
  - Single H1 interacts with linker DNA (between nucleosomes)
- DNA is coiled into arrays of nucleosomes
  - Crossed-linker models
    - Linker DNA is relatively straight and runs through the middle of fiber
    - Evidence exists that linker DNA is actually straight
  - Solenoid model
    - Require looping or bending of linker DNA
- Organization of histone octamer
  - Kernel of H3-H4 tetramer associated with 2 H2A.H2B dimmers
  - Each histone is extensively interdigitated with its partner
  - N terminal tails extend out of the histone
    - These get modified by methylation, acetylation and phosphorylation to affect transcription
- Some Histone variants have specialized functions
  - CENPA – H3 variant
    - Essential for centromere structure and function
  - H2A.X – H2A variant
    - DNA repair
  - H2A.Z – H2A variant
    - Transcriptional regulation
  - MacroH2A – H2A variant
    - Fusion of H2A gene to nonhistone gene
    - Only in vertebrates

- May have a role in transcriptional repression
    - Preferentially associated with inactive X
- Replacement variants
  - Incorporated into chromatin in the absence of DNA synthesis
  - Likely function is to maintain the integrity of chromatin by replacing damaged or displaced histones

## Chromatin and Gene regulation (Shelley)

### **Big Picture**

- Chromatin has a direct role in transcriptional activation
- Nucleosomes inhibit multiple steps in gene transcription
- Modification of histone tails effect transcriptional regulation

### **Details**

- Chromatin has a direct role in transcriptional activation
  - Chromatin is the physiological template of the genome
  - DNA is associated with histone proteins for packaging
  - ↑ packing seen with ↑ salt
    - 10nm fiber consists of string of nucleosomes
    - 30nm fiber has coiled structure
      - 6 nucleosomes / turn in a radial fashion
  - Histones consist of globular domains and unstructured amino and carboxy terminal tails
  - The unstructured tails emerge from the nucleosome
  - Covalent modifications occur at conserved residues in the N and C terminal extensions
  - Nucleosomes can inhibit multiple steps required for gene transcription
    - Binding of upstream regulatory factors requires accessing nucleosomal DNA and may result in displacement or rearrangement of the histone octamers.
    - Formation of the preinitiation complex at the TATA-box and TSS is also suppressed by the presence of nucleosomes.
    - Elongation of RNAPolIII in inhibited by nucleosome arrays → increasing pausing of engaged polymerases
- Histone covalent modifications
  - 2 models
    - Electrostatic alterations in the histone tails cause nucleosome structural changes
      - In vitro acetylation opens up nucleosome arrays
    - Histone modification specify protein interactions (aka Histone code)
      - Modified residues are “landing platforms” for transcriptional effector proteins
  - N-terminal tails have a role in transcriptional activation
  - Bromodomains vs Chromodomains

- Assays for histone modification enzymes in vitro and in vivo
- HATs and HDACs
- Phosphorylation/acetylation patterns
  - There is evidence for both activation and silencing by acetylation
    - some HATs ↑ silencing
      - ↑acetylation → ↓activation
    - some HDACs ↓ activation
      - ↓ acetylation → ↓activation
- Histone methylation
  - A “permanent mark” to establish epigenetic memory both +ve and –ve
  - Players in heterochromatic epigenetic silencing
    - Su-var-3-9 (enzyme)
    - H3 Lys-9-me (heterochromatin)
    - H3 Lys-4-me
      - Surrounds heterochromatin boundaries/insulators
- Histone code hypothesis

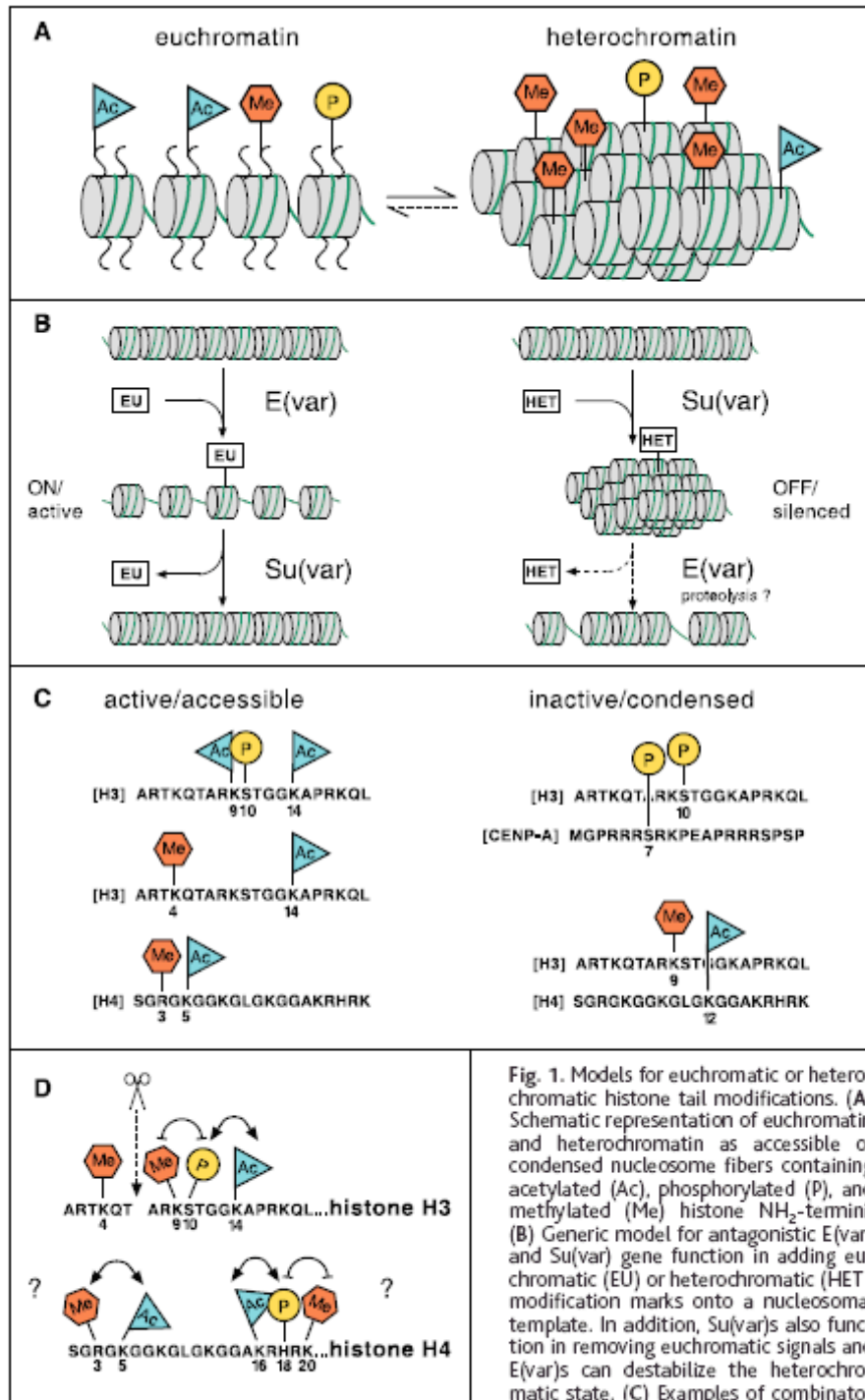


Fig. 1. Models for euchromatic or heterochromatic histone tail modifications. (A) Schematic representation of euchromatin and heterochromatin as accessible or condensed nucleosome fibers containing acetylated (Ac), phosphorylated (P), and methylated (Me) histone NH<sub>2</sub>-termini. (B) Generic model for antagonistic E(var) and Su(var) gene function in adding euchromatic (EU) or heterochromatic (HET) modification marks onto a nucleosomal template. In addition, Su(var)s also function in removing euchromatic signals and E(var)s can destabilize the heterochromatic state. (C) Examples of combinatorial modifications in histone NH<sub>2</sub>-termini

that are likely to represent "imprints" for active or inactive chromatin. Single-letter abbreviations for amino acid residues: A, Ala; E, Glu; G, Gly; H, His; K, Lys; L, Leu; M, Met; P, Pro; Q, Gln; R, Arg; S, Ser; and T, Thr. (D) Proposed synergistic (connected arrowheads) or antagonistic (blocked oval line) modifications in histone H3 and H4 NH<sub>2</sub>-termini. The arrow with the scissors indicates possible proteolytic cleavage of the H3 NH<sub>2</sub>-terminus.

- Assays
  - Gene Activation and Chromatin Accessibility Assay aka indirect end labeling (
    - What's the relationship between gene activation and chromatin disruption?

- Is there a nucleosome structure between proteins and DNA?
- Grow cells in media as either induced or uninduced
  - High phosphate = gene off (uninduced)
  - Low phosphate = gene on (induced)
- Digest yeast cell wall to allow permeability of enzymes
- Partially digest with ClaI, MNAse, DNAse, etc
  - Histone sensitive
  - Choose enzyme that cuts probe to put it on the end
    - Necessary for the end labeling
- Isolate DNA with deproteinase treatment
- Completely digest with selected restriction enzyme
- Southern blot with radiolabeled probe to end of promoter
  - Probe position that is cut by restriction enzyme so that it lies at the end
    - Necessary for the end labeling
  - look for deletion of restriction sites due to histones present when gene is off
- Histone Modification Assay
  - What histone is modified on induction?
  - Incubate
    - peptides, histones or nucleosomes
    - radiolabeled donor (ex SAM or acetyl CoA)
    - source of enzyme
  - control is stain of histone proteins
  - experiment is xray of hot proteins
    - look for protein that lines up
- Histone Modification Assay
  - What particular HAT modifies a given histone (from Histone Modification Assay)
  - Completely enrich gel with protein of interest (ie histone)
  - Cast gel and crosslink to immobilize protein
  - Run gel with extract including potential HATs through protein gel with labeled acetylCoA buffer
  - Look for substrate to light up as acetylated
  - Compare to control gel without substrate (ie no protein of interest) and staining for all proteins
- Order of Chromatin Remodeling Assay (Berger 2)
  - ChIP analysis using antibodies to both enzymes (ex HAT and ATP-dependant remodeler) at non-induction and induction
    - Do a time course after induction to determine which enzyme gets there first
  - To prove do epistasis analysis
    - redo ChIP in KO strains of each enzyme with antibodies to the other functional enzyme

- whichever KO yields a negative result in recruitment of the second enzyme is the one that gets to the promoter first

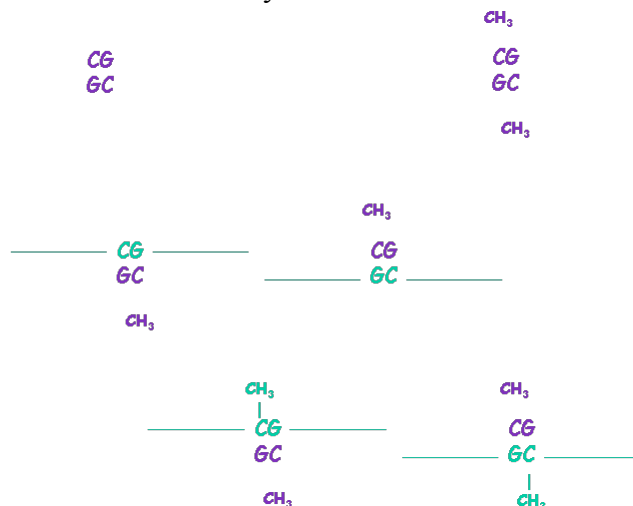
## DNA Modification (Marissa 1):

### Big Picture

- DNA methylation is implicated in normal and abnormal cell functioning
  - Maintenance methylation
  - Denovo methylation
- DNAMTs are responsible for methylation mainly on CG sites
- Eukaryotic DNAMTs are a more specialized form of prokaryotic DNAMTs
  - Have NLS and other eukaryotic specific domains
- Many DNAMTs
  - Establish a normal equilibrium with some inducing and some repressing the 2 types of methylation
- DNAMT interferes with transcription
  - Direct by inhibiting TFs from binding
  - Indirect by having MBPs bind which then inhibits other TFs from binding

### Details

- Bacterial DNA Methyl Transferases
  - DNA Adenine Methyltransferase (Dam)
  - DNA Cytosine Methyltransferase (Dcm)
- Eukaryotic DNA methylation
  - 1% bases methylcytosine
    - 60-90% CpGs are methylated at C
- Methylation places a heavy mutational load on the genome
  - Methylated C → T (spontaneous deamination)
    - No good repair
- Maintenance vs de novo methylation



- Maintenance
  - DNAMT1

- Prefers hemi-methylated residues
    - N-term **suppresses** denovo methylation
    - specific to eukaryotic function and contains NLS
  - De-novo
    - DNAMT3a and DNAMT3b **promotes** denovo activity
- Effects of methylation on Tx
  - Direct
    - Some TFs have CGs in their recognition sequence therefore if methylated, physically can't bind
      - AP2, CREB/ATF, E2F, NF-KB,
  - Indirect
    - Methyl binding proteins MBPs inhibit transcription
      - **MeCP2**, Mbd1, Mbd2, Mbd3, Mbd4, Kaiso
    - MeCP2
      - Interacts with Sin3a and HDAT
- Is the methylation causing a direct or indirect interference with tx?
  - Assay tx by primer extension over time
  - If you see ↓ tx over time → indirect
    - Because MBPs take time to bind

## DNA Modification II

### Big Picture

- DNA Methylation is essential
  - Bac use it to identify old vs new residues
  - Eukaryotes
    - Imprinting
    - Help organize heterochromatin to not interfere with euchromatic
    - Developmental control of expression

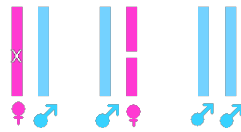
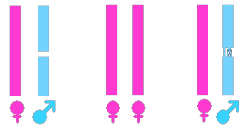
### Details

- Imprinted disease

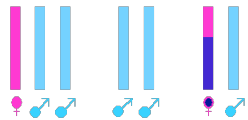


- Hallmark of imprinted disease is parent of origin specific inheritance of mutation
- Human disorders involving methylation
  - ICF syndrome

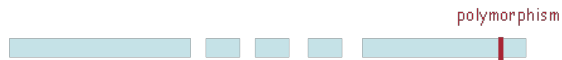
- Rett Syndrome
  - MeCP2 mutations
  - Predominantly females
    - Phynotypic severity depends on Random X inactivations
- Prader-Willi/Angelman



- Beckwith-Wiedemann



- Is a particular gene imprinted?
  - Use F1 progeny between an inter-species cross
    - SNPs allow you to distinguish where each gene came from
  - RNase protection assay using F1 hybrid animals
  - If imprinted, you will see one or the other bands
  - If not imprinted, you see both bands



Legend – Always male X female

- Epigenetic modifications
  - Modifications that don't change the sequence
  - Candidates
    - DNA Methylation
    - Chromatin
    - Asynchronous Replication
    - Repetitive elements
- Insulators
  - Barriers
    - Prevent spread of heterochromatin into expressed region
  - Enhancer blockers
    - Block promoter from engaging enhancer

## Invitro Methylation Assays (Marissa 2):

- Is a particular sequence methylated?
- Restriction Enzymes (Genomic southern methylation assay)
  - use 2 restriction enzymes to assay
  - MspI – insensitive to methylation status
  - HpaII – sensitive to methylation status, does not cleave methylated
  - probe with labeled probe to cover region you want to know if is methylated
  - will see methylated sequence show up as missing band on MspI versus HpaII
- Retroviral Transfection
  - Introduce retrovirus coding for probe into cell (ie unmethylated DNA)
  - Look for denovo methylation with restriction enzymes
- Tritiated SAM
  - Nuclear extract (alter enzymes as needed to test mutations on proteins per se)
  - Tritiated Sam (hot)
  - Oligo probe (cold)
  - Look for labeled probe
- Control?
  - Methylated prope
  - Unmethylated probe

## ChIP (Kadesh 3, Berger 1)

- What proteins are bound to DNA?
- Snapshot at level of DNA
- Crosslink proteins to DNA (treat with formaldehyde)
  - Freezes everything
- Sonicate to cut DNA into fragments (or use MNase)
- IP with antibody your interested in (ex antiTBP)
  - Pulls down TBP and associated proteins b/c crosslinking freezes everything
- Reverse cross links
- PCR to see if your protein is bound to your DNA of interest (ie promoter)
- Basically 2 purifications

- IP purifies your protein away from all the proteins
  - Has complex mix of DNA
- PCR purifies dna of interest
- Controls
  - Preimmune to show Ab specificity (ex rabbit IgG/input)
  - Loading control to show same amt of DNA present
  - Nonspecific gene to show gene induction specificity ((ex housekeeping GAPDH)