Chapter 30: Chromatin Structure Is a Focus for Regulation

- **Silencing** describes
  - the repression of gene expression in a localized region,
  - usually as the result of a structural change in chromatin.
- **Heterochromatin** describes
  - regions of the genome that are highly condensed,
  - are not transcribed,
  - and are late-replicating.
- Heterochromatin is divided into two types,
  - constitutive and facultative.

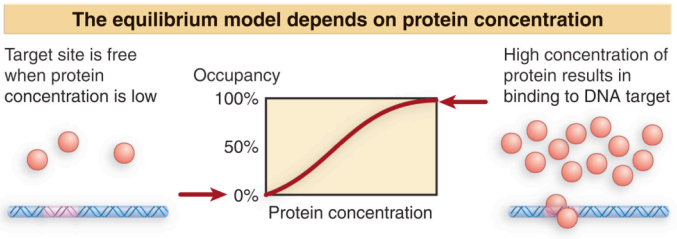
Role of Chromatin in Gene Regulation

- Two broad classes of chromatin:
  - **Euchromatin**: Majority chromatin is in its extended (decondensed) state during interphase, only condenses during mitosis.
  - **Heterochromatin**: Remains highly condensed even in interphase. Accounts for the dark staining regions seen in interphase chromatin. Heterochromatin is further classified as:
    - **Constitutive**: always inactive and condensed: e.g. repetitive DNA, centromeric DNA
    - **Facultative**: can exist in both forms. E.g.: Female X chromosome in mammals.

30.2 Chromatin Remodeling Is an Active Process

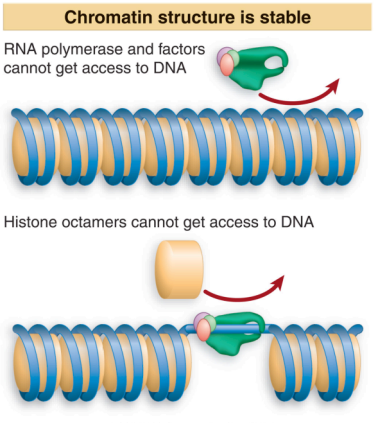
- Chromatin structure is
  - stable and cannot be changed by altering the equilibrium of transcription factors and histones.
- Chromatin remodeling uses
  - energy provided by hydrolysis of ATP to change the organization of nucleosomes.
- **Chromatin remodeling** describes
  - the energy-dependent displacement or reorganization of nucleosomes that occurs in conjunction with activation of genes for transcription.

This does NOT apply when chromatin is involved….

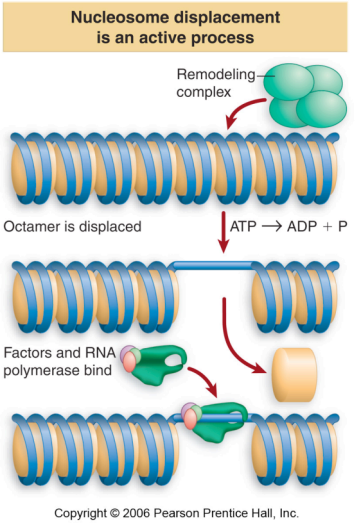


If nucleosomes form at a promoter, transcription factors (and RNA polymerase) cannot bind.

If transcription factors (and RNA polymerase) bind to the promoter to establish a stable complex for initiation, histones are excluded.

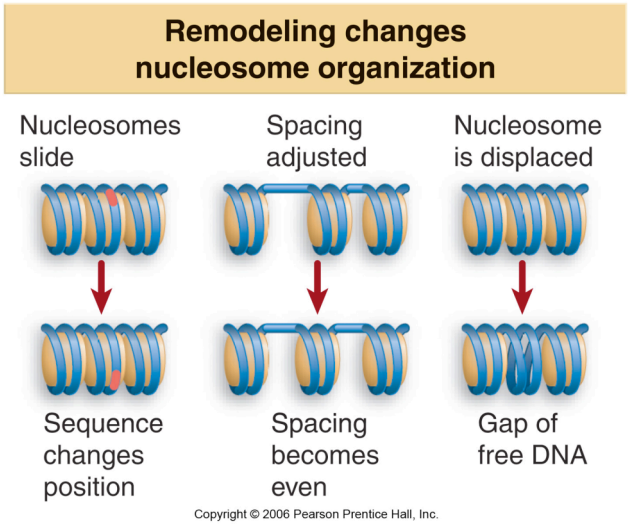


The dynamic model for transcription of chromatin relies upon factors that can use energy provided by hydrolysis of ATP to displace nucleosomes from specific DNA sequences.



3 types of remodeling changes in chromatin

- Remodeling complexes can cause
- nucleosomes to slide along DNA:
  - Histone octamers may slide along DNA
  - Changing the relationship between the nucleic acid and the protein
  - Alters the position of a particular sequence on the nucleosomal suface
- can reorganize the spacing between nucleosomes:
  - Results in alteration of the position of a particular sequence on the nucleosomal suface
- can displace nucleosomes from DNA:
  - Most extensive change
  - Histone octamer(s) may be displaced entirely from DNA



30.3 There Are Several Chromatin Remodeling Complexes

- **SWI/SNF** is
  - a chromatin remodeling complex;
  - it uses hydrolysis of ATP to change the organization of nucleosomes.
- The SWI/SNF, RSC, and NURF remodeling complexes all are very large;
- they are classified into groups according to the ATPase subunits.

There are several types of remodeling complexes

Type of complex	SWI/SNF	ISW	Other
Yeast	SWI/SNF RSC	ISW1 ISW2	
Fly	dSWI/SNF (Brahma)	NURF CHRAC ACF	
Human	hSWI/SNF	RSF hACF/WCFR hCHRAC	NuRD
Frog			Mi–2

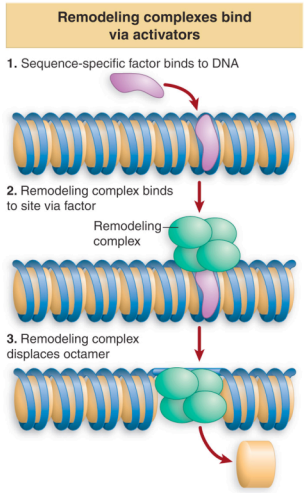
30.4 Nucleosome Organization May Be Changed at the Promoter

- A remodeling complex does not itself have specificity for any particular DNA target site, but must be recruited by a component of the transcription apparatus.
  - Activators, transcription factors
  - Repressors?
- The factor may be released once the remodeling complex has bound.
- The MMTV promoter requires a change in rotational positioning of a nucleosome to allow an activator to bind to DNA on the nucleosome.

Hypothesis:

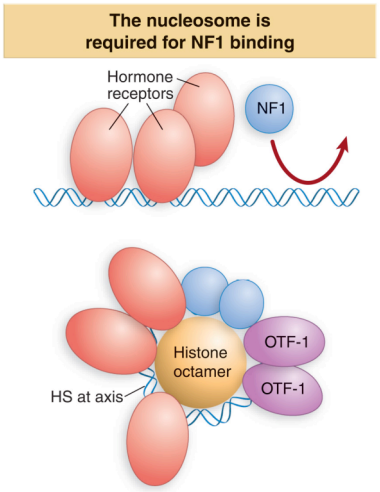
A remodeling complex binds to chromatin via an activator (or repressor).

Does anyone have a problem with this?



Hormone receptor and NF1 cannot bind simultaneously to the MMTV promoter in the form of linear DNA,

but can bind when the DNA is presented on a nucleosomal surface.



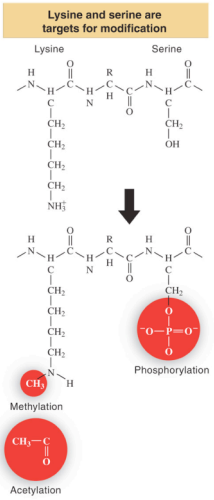
30.5 Histone Modification Is a Key Event

- Histones are modified by
  - acetylation of lysine,
  - and methylation of lysine and arginine.
- The target amino acids are located
  - in the N-terminal tails of the histones.
- Phosphorylation occurs on serine.

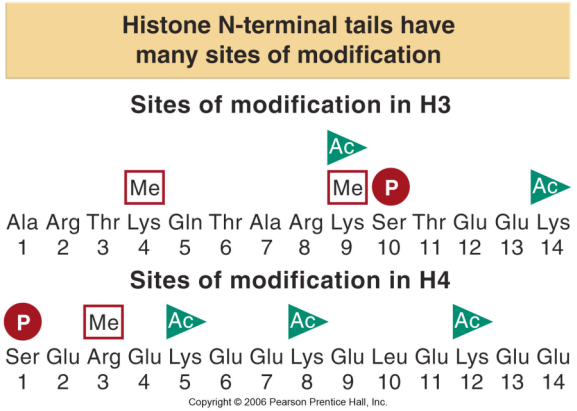
Acetylation of lysine or phosphorylation of serine reduces the overall positive charge of a protein.

The modified sites are concentrated in the N-terminal tails of the histones

"the histone tails" are the 20 N-terminal amino acids of the histone proteins that extend from the nucleosome between the turns of DNA



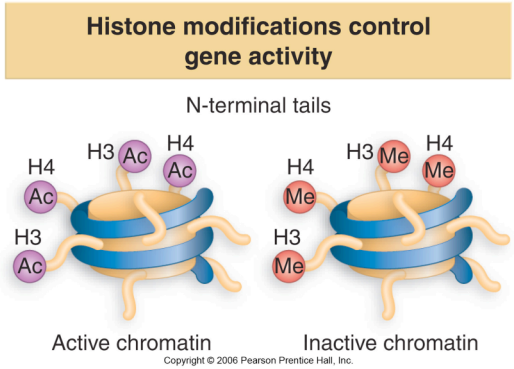
The N-terminal tails of histones H3 and H4 can be acetylated, methylated, or phosphorylated at several positions.



IN GENERAL:

Acetylation of H3 and H4 is associated with active chromatin,

Methylation is associated with inactive chromatin.

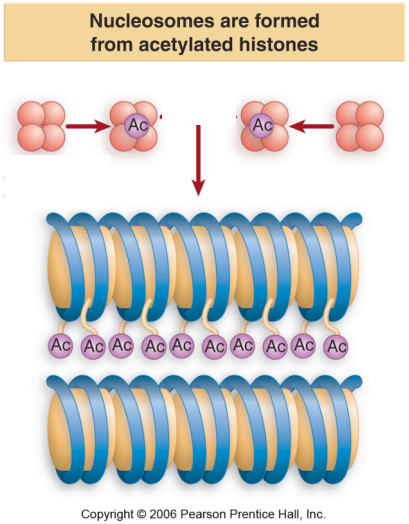


30.6 Histone Acetylation Occurs in Two Circumstances

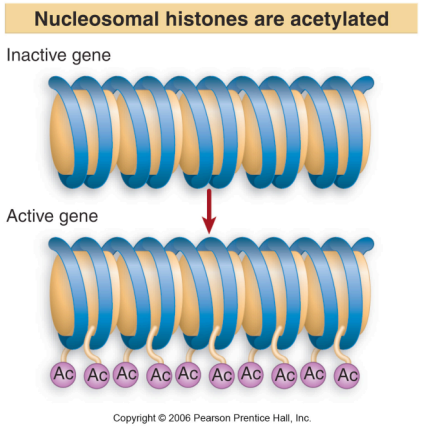
- Histones are acetylated transiently at replication.
- Histone acetylation is associated with activation of gene expression.
- All the core histones can be acetylated, but the major targets for acetylation are lysines in the N-terminal tails of histones H3 and H4

Acetylation at replication occurs on histone tails before the histones are incorporated into nucleosomes.

Acetyl groups are removed soon after incorporation into nucleosomes



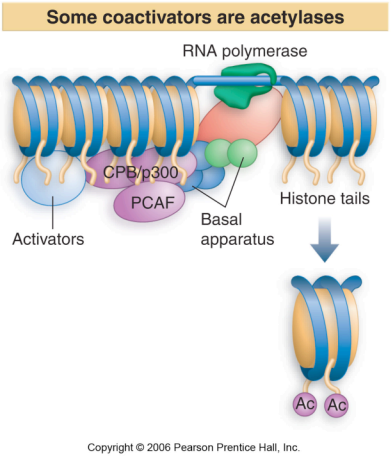
Acetylation associated with gene activation occurs by directly modifying histone tails in nucleosomes.



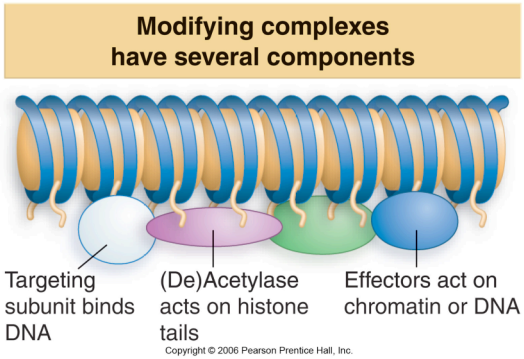
30.7 Acetylases Are Associated with Activators

- **Histone acetyltransferase (HAT)** enzymes modify histones by
  - addition of acetyl groups;
  - some transcriptional coactivators have HAT activity
- **Histone deacetylase (HDAC)** enzymes remove
  - acetyl groups from histones;
  - they may be associated with repressors of transcription.
- Deacetylated chromatin may have a more condensed structure.
- Transcription activators are associated with histone acetylase activities in large complexes
- Histone acetylases vary in their target specificity.
- Acetylation could affect transcription in a quantitative or qualitative way.

Coactivators (PCAF and CPB/p300) have HAT activities that acetylate the tails of nucleosomal histones.



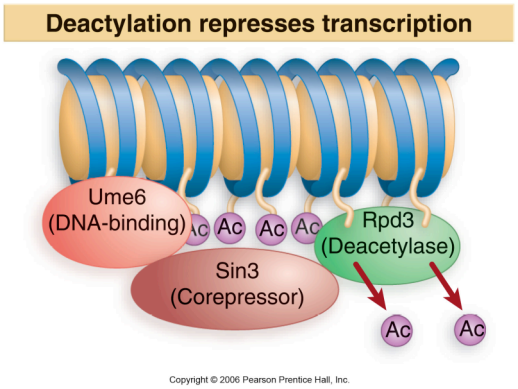
- Complexes that modify chromatin structure or activity have targeting subunits that determine their sites of action:
  - HAT or HDAC enzymes that acetylate or deacetylate histones,
  - effector subunits that have other actions on chromatin or DNA.



30.8 Deacetylases Are Associated with Repressors

- Histone Deacetylation is associated with repression of gene activity.
- Histone Deacetylases (HDAC) are present in complexes with repressor activity.

A repressor complex contains three components:
a DNA-binding subunit,
a corepressor, and a histone deacetylase.



30.9 Methylation of Histones and DNA Is Connected

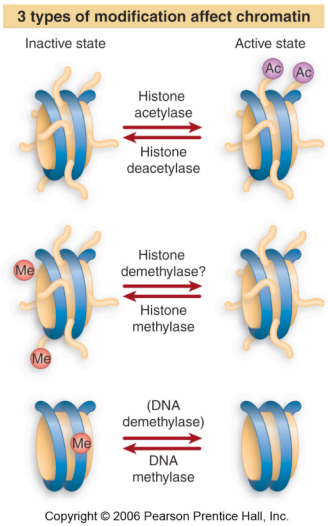
- Methylation of both DNA and histones is a feature of inactive chromatin.
- The two types of methylation event may be connected.
- Methylation of histones can occur on 2 lysines in the H3 tail and an arginine in the H4 tail.
- DNA methylation generally occurs on the cytosine of CpG sequences
- A methylated CpG may cause a histone methyltransferase to bind and methylate histone tails

30.10 Promoter Activation Is an Ordered Series of Events

- The remodeling complex may recruit the acetylating complex.
- Acetylation of histones may be the event that maintains the complex in the activated state.
- Active chromatin is
  - acetylated on the histone H3 and H4 tails
- Inactive chromatin is
  - methylated on 9Lys of histone H3 tails
  - methylated on cytosines of CpGs

Acetylation of histones activates chromatin

Methylation of DNA and histones inactivates chromatin.

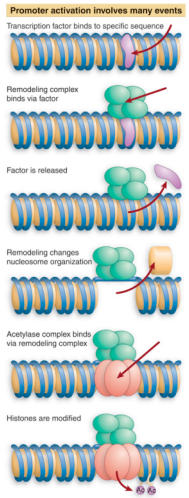


Promoter activation involves:

• binding of a sequence-specific activator

• recruitment and action of a remodeling complex,

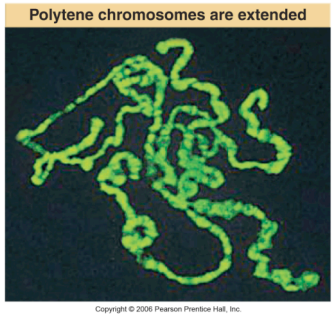
• recruitment and action of an acetylating complex.

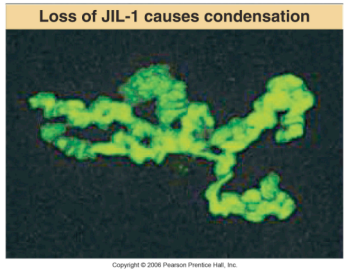


30.11 Histone Phosphorylation Affects Chromatin Structure

- Most histones are targets for phosphorylation
  - but the consequences are less well characterized than for acetylation or methylation.
- Histones are phosphorylated in 2 circumstances:
- Cyclically during the cell cycle
  - Histone H1 is phosphorylated during mitosis
  - Role in cell division?
- In association with chromatin remodeling
  - Loss of Histone H3 is phosphorylation causes chromosome condensation
  - Loss of euchromatin

Polytene chromosomes of flies that have no JIL-1 kinase (no phosphorylation at histone H3) have abnormal polytene chromosomes that are condensed instead of extended.



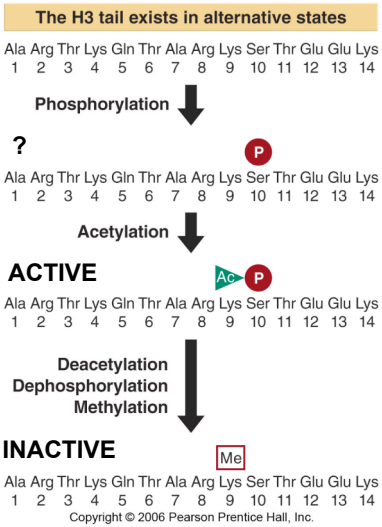


30.12 Some Common Motifs Are Found in Proteins That Modify Chromatin

- Several motifs are characteristic of nonhistone proteins, and identify their functions.
- The **chromo domain** is found in nonhistone chromatin proteins and is involved in protein–protein interactions
- The **SET domain** is part of the active site of histone methyltransferase enzymes
- The **bromo domain** is found in a variety of proteins that interact with chromatin and is used to recognize acetylated sites on histones.

Multiple modifications in the H3 tail affect chromatin activity.

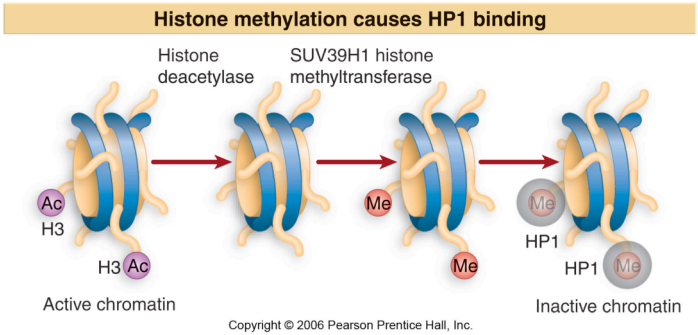
Phosphorylation of 10Ser and methylation of 9Lys are mutually exclusive (they cannot happen together)



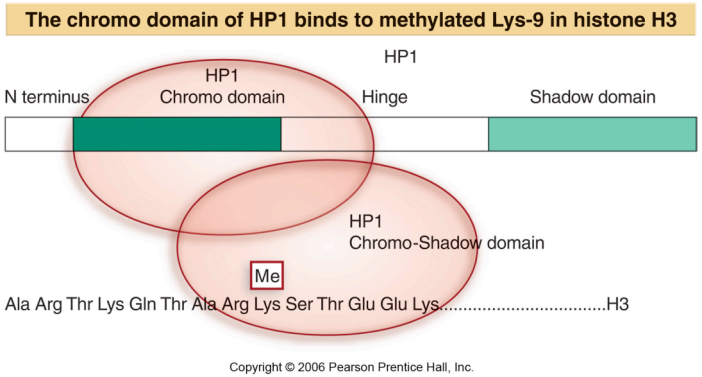
30.13 Heterochromatin Depends on Interactions with Histones

- HP1 is
  - the key protein in forming mammalian heterochromatin,
  - acts by binding to methylated H3 histone
- RAP1 initiates
  - formation of heterochromatin in yeast
  - by binding to specific target sequences in DNA
- The targets of RAP1 include
  - telomeric repeats
  - and silencers at *HML* and *HMR*.
- RAP1 recruits
  - SIR3/SIR4,
  - which interact with the N-terminal tails of H3 and H4.

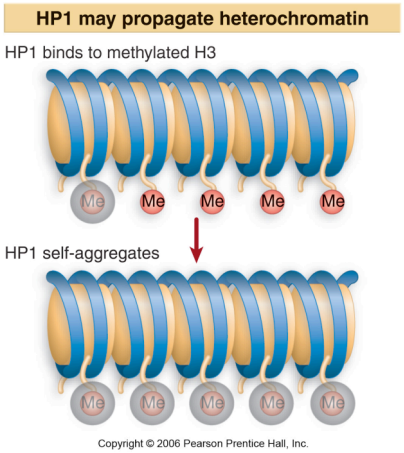
- SUV39H1 is a histone methyltransferase that acts on ^{9}Lys of histone H3 tail.
- HP1 binds to the methylated histone.



Methylation of histone H3 tail creates a binding site for HP1.



Binding of HP1 to methylated histone H3 tail forms a trigger for silencing because further molecules of HP1 aggregate on the nucleosome chain.



Formation of heterochromatin is initiated when Rap1 binds to DNA.

Sir3/4 bind to Rap1 and also to histones H3/H4.

The complex polymerizes along chromatin and may connect telomeres to the nuclear matrix.