Chromatin Structure and its Effects on Transcription
What is the point?

- DNA is not naked. Histones and other proteins organized it into a fantastically compact unit.

- The presence of nucleosomes can interfere with the binding of TFs to enhancers and with the preinitiation complex to the promoter. = Repression

- When other proteins (simple TFs) are bound to the DNA they can prevent the histones from binding. This is competitive inhibition of histone binding. Called de-repression or antirepression in the book by Weaver.
Eukaryotes contain many copies of each histone gene.  
10-20 in mice, 100X in Drosophila
A) **General information about the histones**

1) **Basic**
   - Histones rich in K and R which gives them a basic charge at neutral pH.
   - Positive charge at neutral pH means that they are resistance to acid extraction (this is unusual) and migrate in the opposite direction during electrophoresis than most proteins.
   - Eukaryotes contain many copies of each histone gene. 10-20 in mice, 100X in Drosophila
   - Positive charge helps them interact with the negatively charged DNA.

2) **Histones are evolutionarily ancient and strongly conserved molecules**
   - Histones evolved very early during the history of life on our planet. In fact, histone H3 and H4 are the most conserved proteins known. In most organisms, their amino acid sequence is identical or only slightly changed. For example, histone H4 from cows and from Mendel's pea plant are identical in all but 2 amino acids. Furthermore, these two changes are conservative amino acid changes. This indicates that the sequence of histone H4 has not significantly changed in the 10^9 years since plants and animals diverged (Freifelder and Malacinski, Essentials of Molecular Biology 2nd ed).

3) **Compaction**
   - A chromosome is much more compact that the DNA molecule it contains. For example, if stretched out the DNA in just a single human chromosome would be about 10 cm long. A human cell contains about 2m of DNA. But a eukaryotic cell is only about 3 - 10 um in diameter and the nucleus is smaller than this.

4) **Interaction with DNA**
   - DNA wraps around histone core 1.65 times
   - The amount of DNA that interacts with the core is 147 bp
   - Histones H-bind to the DNA
   - Histones organize DNA into a nucleosome structure
   - Linker joins the nucleosomes to each other - core + linker = ~200 bp (length of the linker is species dependent)
5) Nucelosome (fig. from Tsankova et al 2007)

6) Compaction
Naked -> Beads on a string -> 30 nm fiber -> etc

7) Histone H1

8) 30 nm fiber and the Tetranucleosome

9) Loops ! as regulatory units

It is only recently that we have started to think about chromosome shapes and organization as a regulatory mechanism. Manipulation of chromosome conformation may be the most important regulatory mechanism. Subnuclear localization is also an important manipulation (for instance - heterochromatin is often associated with the nuclear envelope).
Nucleosome

- DNA wraps around the histone core 1.75 times
- Core DNA contains 147 base pairs
- Hydrogen bonding binds DNA to the core

Compaction

DNA
Isolated patches.
The Nucleosome
Genes under active transcription.
"Beads-on-a-String"

The 30nm Fibre
Less active genes.
The Solenoid
During interphase.
PR 25

Active Chromosome
During cell division.
The Metaphase Chromosome

Double helix
Add core histones.
Beads On A string
Add histone H1.
zig-zag
Add further scaffold proteins.
Snake
Add further scaffold proteins.
The Solenoid
PR 25
700 nm fiber

Probably involve SARS

Figure from http://upload.wikimedia.org/wikipedia/commons/4/4b/Chromatin_Structures.png
Two competing models

solenoid model

zig-zag model  tetrasome model

Images from Watson et al
Molecular Biology of the Gene
6th edition
Histone H1

- H1 binding stimulates formation of 30 nm fiber
- H1 histones pull adjacent nucleosomes into close proximity
Tetranucleosome Fig 13.7
Tetranucleosome

http://www.nature.com/nature/journal/v436/n7047/suppinfo/nature03686.html
35-80 kb loops
Each loop is very independent (e.g. independently supercoiled).
Histones repress transcription

• Activators are responsible for much of the variation in levels of transcription of different genes.

• Why? See above!
Histones represses transcription

Natural break on runaway transcription

Naked DNA can be transcribed. Histones repress transcription.

A difference between prokaryotes and eukaryotes?

- prok require active repression
- Histones act as basal repressors. Euk require activation to remove basal repression

Figure 18.13
Weaver 4th edition
Moving them can be an act of activation!!!
- Remodeling means to move them. Modifying them can make them moveable.

The cell does this

Example given: swi/snf proteins
Histone acetylation

- amino groups of lysine side chains
- unacetylated histones tend to repress transcription
- acetylated histones tend to activate transcription
- Histone acetyl transferase (HAT)
- Histone deacetylase (HDAC)
HAT type A

- Nuclear, involved in regulating gene expression
- Have bromodomain (HAT B’s do not)
  - Binds acetylated lysines. So HAT A’s can recognize partially acetylated histone tails.
    - Think of it like this: the work of one HAT can attract other HATs.
- Examples p55, Gcn5p, CBP/p300, TAFII250

- What mechanism does this suggest? In the context of TAFII250 and core promoter recognition?
HAT type B

- cytoplasmic, acetylate newly synthesized histones H3 and H4 so that they can be assembled into a nucleosome.
- These acetylations will later be removed in the nucleus.
- no bromodomain
- NOT thought to be used in the regulation of gene expression.
Acetylation continued

- Acetylation of histone tails neutralizes some of the positive charge, causing them to relax their grip on the DNA.

- Reduces nucleosome cross-linking. That is; the interaction between histones in neighboring nucleosome.
  eg. basic N-terminal tail of H4 in one nucleosome and an acidic pocket in H2A-H2B dimer in the next nucleosome
Acetylation continued

• Also some TFs recognize acetylated histones. eg. TAFII250 has a double bromodomain and recognizes low level acetylated histones. Once bound it is a HAT and increases acetylation.

• low level acetylation of histones occurs in inactive chromatin.
Glucocorticoid receptor (GR) → GRE → Deacetylated histones → CBP (Coactivator) → Acetylase → Acetylated histones → TATA → TBP → TAFii250 → Other general transcription factors (see Fig. 11.19) → RNA Pol II

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Deacetylation

- Most eukaryotic repressors recruit corepressors
- Most corepressors complexes include histone deacetylases
- Example, NCoR/SMRT in mammals
(a) Repressor-directed histone deacetylation

Repressor proteins (e.g., Ume6) recruit the Sin3-Rpd3 complex, which leads to deacetylation of histone N-terminal tails.

(b) Activator-directed histone hyperacetylation

Activator proteins (e.g., Gcn4) recruit the Gcn5 complex, which leads to hyperacetylation of histone N-terminal tails.
Normal chromatin
Basal levels of histone acetylation and transcription

Transcriptional corepressors:
histone deacetylases

Repressive chromatin
Deacetylated histones and no transcription

Transcriptional coactivators:
histone acetyltransferases

Active chromatin
High levels of histone acetylation and transcription

Transcriptional corepressors: histone deacetylases

Repressive chromatin: Deacetylated histones and no transcription
Controlling DNA accessibility

• Histones are repressors - by regulating how tightly histones bind the DNA we regulated access to transcriptional control regions.

• Acetylation helps to make DNA more accessible but by itself it is not enough. Also need additional chromatin remodeling.

• Acetylation is a form of chromatin modification that can make remodeling easier.
Chromatin remodeling

- Clearest definition

- ATP-dependent mobilization movement of nucleosomes so that DNA is accessible.
Chromatin remodeling

- List of protein complex families
  SWI/SNF - SwitchSniff
  ISWI - iimitation switch
  CHD - Chromodomain and helicase-like domain ATPases (NuRD)
  INO80

- Produce nucleosome free regions around enhancers & promoters
Constitutive heterochromatin,

Facultative heterochromatin,

Euchromatin
Additional levels of organization that are regulatory in nature
Figure 13.13

30 nm fiber

Loop of 30-nm chromatin fiber

Chromosome scaffold

45-80 kb loops
Insulators

- Provides a barrier through which activation or repression cannot pass.
- Some also block the encroachment of condensed chromatin.
- Some do one, some the other.
- What we know is based on very few examples.
- Probably many different types exist.
- There are probably many different mechanisms.
One way that they are thought to work

Haini Cai & Ping Shen
Figure 13.13

30 nm fiber

45-80 kb loops