How DNA Is Packed In The Cell: Chromosomes, Genes, Nucleosomes

Brian D. Strahl
Department of Biochemistry & Biophysics
UNC-School of Medicine
I. Chromatin organization
   • The DNA packaging problem
   • Histones and nucleosome core particle
   • Chromatin folding and nuclear organization
   • Euchromatin vs Heterochromatin

II. Factors that influence chromatin organization and gene function
   • Histone post-translational modifications (PTMs) and the ‘histone code’
   • Histone variants
   • DNA methylation

III. Tools and technologies leading the charge in chromatin research
   • Modification-specific antibodies and chromatin immunoprecipitation
   • High-throughput microarray/DNA sequencing technologies
   • Proteomics and mass spectrometric analyses
The DNA packaging problem

<table>
<thead>
<tr>
<th>Organism</th>
<th>Multiplication Factor</th>
<th>Base Pairs</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. Coli (Chlamydia trachomatis)</td>
<td>1X</td>
<td>1 million base pairs</td>
</tr>
<tr>
<td>Yeast genome</td>
<td>12X</td>
<td>12 million base pairs</td>
</tr>
<tr>
<td>Fruit fly genome</td>
<td>122X</td>
<td>122 million base pairs</td>
</tr>
<tr>
<td>Human genome</td>
<td>3400X</td>
<td>3.4 billion base pairs</td>
</tr>
</tbody>
</table>

If our strands of DNA were stretched out in a line, the 46 chromosomes making up the human genome would extend more than six feet (~ 2 meters).
A Matter of Fitting In!

8850 meters (~5.5 miles)


Pipet tip image: Biologix Research

0.0043 meters (0.17 inches)

(slide provided by Raymond Reeves)
How is DNA packaging achieved?
Organization of eukaryotic chromatin

- DNA double helix
- Histones
- Nucleosomes
- Solenoid
- Chromatin loop: ~100,000 bp DNA
- Chromatin
First order of DNA compaction
Nucleosomes are the building blocks of chromatin.
Histone structure

2/3 of chromatin mass is protein
95% of chromatin protein are histones

- N-terminal tail
- Histone fold

- Regulatory domain
- Involved in higher-order packing

H2A
H2B
H3
H4

H1

“Tail” domain
- Regulatory domain
- Involved in higher-order packing

“Globular” domain
- Histone-histone interactions
- DNA wrapping
Nucleosome organization

H3-H4 tetramers build a “wall” that is “capped” by H2A-H2B dimers
Blue = H2A/H2B
White = H3/H4
Second order of DNA compaction
Secondary Structure

- H1: essential for the solenoid structure
Third order of DNA compaction
Histone-depleted metaphase chromosome

Loops of DNA

Protein scaffold
Histone-depleted metaphase chromosome

Scaffold/Matrix attachment regions
A condensed metaphase human chromosome

Figure 1-14
An electron micrograph of a human chromosome. Chromosome XII from a HeLa cell culture. (Courtesy of Dr. E. Du Praw.)
Heterochromatin lies against the nuclear envelope in patches and is broken up at the site of the nuclear pore.
Heterochromatin vs. Euchromatin

- Highly condensed
- Repetitive sequences
- Replicates later in the cell cycle
- Transcriptionally OFF

- Decondensed
- Single copy sequences (genes)
- Replicates early in the cell cycle
- Transcriptionally ON
Outline

I. Chromatin organization
   - The DNA packaging problem
   - Histones and nucleosome core particle
   - Chromatin folding and nuclear organization
   - Euchromatin vs Heterochromatin

II. Factors that influence chromatin organization and gene function
   - Histone post-translational modifications (PTMs) and the ‘histone code’
   - Histone variants
   - DNA methylation

III. Tools and technologies leading the charge in chromatin research
   - Modification-specific antibodies and chromatin immunoprecipitation
   - High-throughput microarray/DNA sequencing technologies
   - Proteomics and mass spectrometric analyses
Molecular mechanisms that influence chromatin structure and function

1. Chromatin remodeling complexes (e.g. Swi/Snf)
2. Histone modifications
3. Histone variants (e.g. H2A.Z, CENP-A, etc.)
4. DNA methylation
Molecular mechanisms that influence chromatin structure and function

1. Chromatin remodeling complexes (e.g. Swi/Snf)
2. Histone modifications
3. Histone variants (e.g. H2A.Z, CENP-A, etc.)
4. DNA methylation
Histone Modifications

- Acetylation
- Phosphorylation
- Methylation
- ADP-ribosylation
- Ubiquitination
- Sumoylation
Histone Modifications

- Acetylation
- Phosphorylation
- Methylation
- ADP-ribosylation
- Ubiquitination
- Sumoylation
Histone acetylation and chromatin structure

(Adapted from Wade & Wolffe - Current Biology, 1997)
Bromodomain-containing proteins can bind to acetylated histones
Epigenetic 'Toolkit'

Histone Code ‘readers’

(Figure from Abcam)
Histone Modifications

- Acetylation
- Phosphorylation
- **Methylation**
- ADP-ribosylation
- Ubiquitination
- Sumoylation
Histone Modifications

- Acetylation
- Phosphorylation
- **Methylation**
- ADP-ribosylation
- Ubiquitination
- Sumoylation
Histone H3 methylation

- Set1/MLL
- SUV39/ESET/G9a
- EZH2
- Set2/NSD1
- Dot1

H3

N-ARTKQTARKSTGGKAPRKQLATKAAR

Gene activation

Gene repression
Heterochromatin
X-inactivation

Gene repression
X-inactivation

Gene activation

Globular domain

K79
Staining of female metaphase chromosomes with site-specific methyl H3 antibodies

methyl (Lys 9) H3

methyl (Lys 4) H3

(Taken from Boggs BA et al. - Nat Genet., 2002)
Roles of H3 lysines 4 and 9 methylation

(K4 Me) K9 Me

On

SUV39H1 methylase

Spreading of silenced and HP1-coated heterochromatin

Off

(Taken from Bannister et al. - Nature, 2001)
Post-translational modifications decorate histones

- H3

ARTKYTARKSTGGGKAPRKQLATKAARKSAPSTGGVKKP...K...TK

- H4

SGRGKGGKGLGKGGAKRHRKVL

- H2A

SGRFKQGCKARAKA - PKKTESHHHKAKGK

- H2B

PEPAKSAPAPPKGSKKAVTKA - GTKAVTKYTSSK

Phosphorylation
Ubiquitylation
Methylation*
Acetylation
Molecular mechanisms that influence chromatin structure and function

1. Chromatin remodeling complexes (e.g. Swi/Snf)
2. Histone modifications
3. Histone variants (e.g. H2A.Z, CENP-A, etc.)
4. DNA methylation
## Histone Variants

<table>
<thead>
<tr>
<th>Histones</th>
<th>Features</th>
<th>Assembled by (organism)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Archaeal histones</td>
<td>Ancestral histone fold proteins without tails found in singly wrapped tetrameric units that comprise nucleosome particles.</td>
<td>Unknown</td>
</tr>
<tr>
<td>H2A, H2B</td>
<td>Canonical core histones encoded by replication-coupled genes.</td>
<td>FACT (yeast, <em>Drosophila</em>)</td>
</tr>
<tr>
<td>H2AZ (HTZ1)</td>
<td>H2A variant found in nearly all eukaryotes that has a diverged self-interaction domain.</td>
<td>SWR1 (yeast), Tip60 (<em>Drosophila</em>)</td>
</tr>
<tr>
<td>macroH2A</td>
<td>Vertebrate-specific H2A variant with a C-terminal globular domain. Enriched on the mammalian inactive X-chromosome.</td>
<td>Unknown</td>
</tr>
<tr>
<td>H2A-Bbd</td>
<td>Vertebrate-specific H2A variant that is widely distributed. Relatively deficient on the inactive X-chromosome.</td>
<td>Unknown</td>
</tr>
<tr>
<td>H2AX</td>
<td>H2A form with an SQ[E/D] $\Theta$ ($\Theta$ = hydrophobic) C-terminal motif that becomes serine phosphorylated at sites of double-stranded breaks.</td>
<td>INO80 (yeast)</td>
</tr>
<tr>
<td>H3, H4</td>
<td>Canonical core histones encoded by replication-coupled genes.</td>
<td>CAF-1 (plants, animals, fungi)</td>
</tr>
<tr>
<td>H3.3 (H3.2 in plants)</td>
<td>H3 variant that replaces H3 and differs at position 31 and at a few residues on helix 2 that allow deposition outside of replication.</td>
<td>HIRA (mammals)</td>
</tr>
<tr>
<td>Packaging histones</td>
<td>Core and linker histone variants adapted for tight packaging of DNA in sperm and pollen in some organisms.</td>
<td></td>
</tr>
</tbody>
</table>

*(Table from Henikoff and Ahmad, *Annu. Rev. Cell Dev. Biol, 2005)*
Molecular mechanisms that influence chromatin structure and function

1. Chromatin remodeling complexes (e.g. Swi/Snf)
2. Histone modifications
3. Histone variants (e.g. H2A.Z, CENP-A, etc.)
4. DNA methylation
DNA methylation

Occurs in:
(1) select organisms and (2) usually at CpG dinucleotide residues

1. Organisms found in:
   - Humans
   - Mice
   - Frogs
   - Flies* (low levels and CpT)

2. Occurs on Cytosine:

![Chemical Structure of 5-Methylcytosine]
How DNA methylation regulates gene repression?

A) By sterically blocking the binding of transcription factors (e.g. E2F, NF-kB, CTCF)
B) & C) By recruiting chromatin modifying activities
D) By affecting RNA Polymerase II transcription

(figure from Klose & Bird, Trends Biochem Sci., 2006)
Outline

I. Chromatin organization
   • The DNA packaging problem
   • Histones and nucleosome core particle
   • Chromatin folding and nuclear organization
   • Euchromatin vs Heterochromatin

II. Factors that influence chromatin organization and gene function
   • Histone post-translational modifications (PTMs) and the ‘histone code’
   • Histone variants
   • DNA methylation

III. Tools and technologies leading the charge in chromatin research
   • Modification-specific antibodies and chromatin immunoprecipitation
   • High-throughput microarray/DNA sequencing technologies
   • Proteomics and mass spectrometric analyses
Histone modification-specific antibodies have enabled the study of chromatin!

methyl \((\text{Lys} \, 9)\, H3\)  
methyl \((\text{Lys} \, 4)\, H3\)

(Taken from Boggs BA et al. - Nat Genet., 2002)
The ChIP-chip procedure

Crosslink Chromatin with Formaldehyde

Shear Chromatin by Sonication

Hybridize To Microarray

Reverse Crosslinks

Recover Input DNA

Amplify, Label Green

Incubate with Antibody

Reverse Crosslinks

Recover IP DNA

Amplify, Label Red

Hybridize To Microarray

(P)SAMPLE (REFERENCE)

(Provided by Jason Lieb, UNC)
Solexa Sequencing (Illumina)

DNA (0.1-1.0 ug)

Sample preparation

Cluster growth

Sequencing

Image acquisition

Base calling
ChIP-Seq

• Follow standard ChIP procedure

• Identify uniquely aligned sequences in human genome
Mass spectrometry is a vital tool in combinatorial PTM discovery

A. Bottom-up MS

H3 $\xrightarrow{RP-HPLC}$ H3 $\xrightarrow{Trypsin}$ $\xrightarrow{RP-HPLC}$ MS

B. Top-down MS

H3 $\xrightarrow{RP-HPLC}$ H3 $\xrightarrow{HILIC}$ MS

(HPILC: hydrophilic interaction liquid chromatography)
Mass Spectrometry technologies have revealed novel histone ‘marks’ and specific histone codes

Regulated nucleosome mobility and the histone code

Michael S Cosgrove¹, Jef D Boeke²,³ & Cynthia Wolberger¹,⁴
Mass spectrometry is a vital tool in combinatorial PTM discovery

NATURE METHODS | VOL.4 NO.6 | JUNE 2007 |

Brief Communications

Pervasive combinatorial modification of histone H3 in human cells

Benjamin A Garcia¹, James J Pesavento², Craig A Mizzen¹,³ & Neil L Kelleher¹,⁴

Research

Molecular & Cellular Proteomics 8.10

High Throughput Characterization of Combinatorial Histone Codes*§

Nicolas L. Young‡, Peter A. DiMaggio§, Mariana D. Plazas-Mayorca¶, Richard C. Baliban§, Christodoulos A. Floudas§, and Benjamin A. Garcia‡,¶,||
SILAC-based approaches are unlocking identification of novel effector proteins

(Stable isotope labeling by amino acids in cell culture)
Bromodomain-containing proteins can bind to acetylated histones

(Taken from E. Pennisi - Science, 2000)
Semi-synthetic modified nucleosomes explore multivalent engagements in chromatin

Native chemical ligation (NCL) and Expressed protein ligation (EPL) (Kent/Cole/Muir labs)

Methyl-lysine analogue (MLA) (Shokat et al.)
Thank you!