Chromatin Structure

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DNA Packaging Is a Formidable Challenge

- Single DNA molecule in human chromosome ca. 5 cm long
- Diploid genome contains ca. 2 meters of DNA
- Nucleus of human cell ca. 5 µm in diameter
- Human metaphase chromosome ca. 2.5 µm in length
- 10,000 to 20,000 packaging ratio required
Overview of DNA Packaging

NET RESULT: EACH DNA MOLECULE HAS BEEN PACKAGED INTO A MITOTIC CHROMOSOME THAT IS 10,000-FOLD SHORTER THAN ITS EXTENDED LENGTH

Figure 4-55. Molecular Biology of the Cell, 4th Edition.
Packaging in Interphase Nucleus

- Short region of DNA double helix
- "beads-on-a-string" form of chromatin
- 30-nm chromatin fiber of packed nucleosomes
- Section of chromosome in extended form
Chromatin Composition

• Complex of DNA and histones in 1:1 mass ratio

• Histones are small basic proteins
  - highly conserved during evolution
  - abundance of positively charged aa’s (lysine and arginine) bind negatively charged DNA

• Four core histones: H2A, H2B, H3, H4 in 1:1:1:1 ratio

• Linker histone: H1 in variable ratio
Chromatin Fibers

• beads = nucleosomes
• compaction = 2.5X
• low ionic strength buffer
• H1 not required

• physiological ionic strength (0.15 M KCl)
• compaction = 42X
• H1 required
Micrococcal Nuclease Digestion of Chromatin

Figure 4-23 part 1 of 2 Molecular Biology of the Cell 5/e © Garland Science 2008
Stochiometry of Histones and DNA

- 146 bp DNA ca. 100 kDa
- 8 histones ca. 108 kDa
- mass ratio of DNA:protein 1:1
Structure of Core Nucleosome

1.65 left handed turns of DNA around histone octamer
Histone Structure

(A) H2A
N   C
(H2B N  C
H3 N C
H4 N C
N-terminal tail  histone fold

(B) N C

(C) N C

Assembly of a Histone Octamer

Figure 4-26 part 1 of 2 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Figure 4-26 part 2 of 2 Molecular Biology of the Cell 5/e (© Garland Science 2008)
Nucleosomes Are Dynamic

Figure 4-28 Molecular Biology of the Cell 5/e (© Garland Science 2008)
Chromatin Remodeling

Large complexes of ≥ 10 proteins

Use energy of ATP hydrolysis to partially disrupt histone-DNA contacts

Catalyze nucleosome sliding or nucleosome removal
30-nm Chromatin Fiber Structure
Models for H1 and Core Histone Tails in Formation of 30-nm Fiber

Figure 4–31. Molecular Biology of the Cell, 4th Edition.

Figure 4–32. Molecular Biology of the Cell, 4th Edition.
Histone Tails
Covalent Modifications of Histone Tails Control Chromatin Function

- Acetylation (K)
- Phosphorylation (S)
- Methylation (K, R)
- Ubiquitinylation (K)
- Sumoylation (K)

Figure 4-39b Molecular Biology of the Cell 5/e (© Garland Science 2008)
Enzymes that Modify Histones

- Histone acetyltransferases (HATs)
- Histone deacetylases (HDACs)
- Histone methyl transferases (HMTs)
- Histone kinases
Meanings of Histone ‘Code’

Figure 4-44a Molecular Biology of the Cell 5/e (© Garland Science 2008)

Modification state

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“Meaning”

- Heterochromatin formation, gene silencing
- Gene expression
- Gene expression
- Silencing of Hox genes, X chromosome inactivation

Figure 4-44b Molecular Biology of the Cell 5/e (© Garland Science 2008)
Transcriptional Regulators Modify Histone Acetylation

Repressor-directed histone deacetylation

- RPD3
- SIN3
- UME6
- DBD
- URS1
- TATA

Histone N-terminal tail

Deacetylation of histone N-terminal tails

Hexagon = Acetyl group

Figure 7-38a
Molecular Cell Biology, Sixth Edition
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Transcriptional Regulators Modify Histone Acetylation

Activator-directed histone hyperacetylation

- GCN4
- GCN5
- AD
- UAS
- TATA

Hyperacetylation of histone N-terminal tails

= Acetyl group
Histone Code Readers

- Code reader complexes recognize particular marks on chromatin
- Attract additional protein complexes that execute biological function

![Diagram of histone code readers](image)
Formation of Heterochromatin Silences Gene Expression

- **Heterochromatin** - regions of darkly staining chromatin in eukaryotic nuclei
- Transcriptionally silent DNA
- Centromeres, telomeres are heterochromatic
- Genes near heterochromatin show metastable expression patterns
  - position effect variegation in flies
  - telomere position effects in yeast
Formation of Heterochromatin in Mammalian Cells

• Requires specific modification: Histone H3 lysine 9 trimethylation (H3K9Me₃) by H3K9 HMT

• Heterochromatin protein 1 (HP1)
Boundary Elements Prevent Spread of Heterochromatin

Figure 6-34b
Molecular Cell Biology, Sixth Edition
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Histone Variants Have Special Functions

- H3: transcriptional activation
- H3.3: centromere function and kinetochore assembly
- CENP-A: DNA repair and recombination, gene expression, chromosome segregation, transcriptional repression, X-chromosome inactivation
- H2A
- H2AX
- H2AZ
- macroH2A

Figure 4-41 Molecular Biology of the Cell 5/e (© Garland Science 2008)
Centromeres Are Heterochromatric and Contain Specialized Nucleosome

Figure 4–49. Molecular Biology of the Cell, 4th Edition.
Higher Order Packaging

- Short region of DNA double helix
- "beads-on-a-string" form of chromatin
- 30-nm chromatin fiber of packed nucleosomes
- Section of chromosome in extended form
- Condensed section of chromosome
- Entire mitotic chromosome
  - Centromere
Higher Order Packaging
(300-nm fiber)

Mechanism of looping unknown
Mitotic Chromosome Condensation

- Depends on SMC (structural maintenance of chromosomes) proteins, which are conserved from bacteria to man
  - **Condensins** (SMC2 & SMC4)
  - **Cohesins** (SMC1 & SMC3)
  - large proteins with coiled-coiled domains and ATPase domains
Model of Cohesin in Mitotic Chromosomes

Figure 4-73c Molecular Biology of the Cell 5/e (© Garland Science 2008)
Molecular Basis of Cohesion

Cohesin

Nasmyth 2002 Science 297:559