DNA packaging summary

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A severe problem of packaging

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   \[
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   \]
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2. A typical cell $= 10$ µm $= 10 \times 10^{-6}$ m

3. Therefore the DNA must be compacted $\sim 10^4$-fold
   
   This is like fitting an 11-mile-long string into a 6-foot box
Visualizing chromatin breakdown products

Electron micrographs of “chromatin” preparations

1. Beads-on-a-string (low salt)  2. 30-nm fiber (0.15 M KCl)

Beads contained “histones”: H1, H2A, H2B, H3, and H4

Packaging proteins discovered by DNase I digests of nuclei

1. Isolate nuclei
2. Partial digest with DNase I (nonspecific endonuclease)
3. Run agarose gel.
4. Result: Ladder of multiples of 150-200 bp
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Conclusion: Something is protecting the DNA!
Proteins = H1, H2A, H2B, H3 & H4

5. Treat with more DNase I.
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   Conclusion: DNA is wrapped around the outside of the nucleosome.

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Crystal structure of the nucleosome

Ribbon drawings

“Top” view

“Side” view of 1/2 nucleosome

147 bp of DNA wrapped almost twice around 8 core histones: (H2A, H2B, H3, H4)₂

Space-filling drawing

Carolyn Luger and Tim Richmond
The histone fold

Ribbon drawing

Simple.
Conserved.
Adopted by all 4 “core” histones (H2A, H2B, H3 and H4).

Sequence schematic

H3-H4 tetramer binds two H2A-H2B dimers to form the histone octamer
The DNA is not smoothly bent

Water mediates many histone:PO$_4$ contacts
Histone “tails” stick out between the gyres of DNA

Post-translational chemical modification of the tails controls function. Modification patterns comprise the “histone code”.

Examples of the “histone code” for H3/H4

<table>
<thead>
<tr>
<th>N-terminal tail</th>
<th>modification state</th>
<th>“meaning”</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ac</td>
<td>unmodified</td>
<td>gene silencing?</td>
</tr>
<tr>
<td>Ac</td>
<td>acetylated</td>
<td>gene expression</td>
</tr>
<tr>
<td>Me</td>
<td>acetylated</td>
<td>histone deposition</td>
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<tr>
<td>Me</td>
<td>methylated</td>
<td>gene silencing/heterochromatin</td>
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<tr>
<td>Me</td>
<td>phosphorylated</td>
<td>mitosis/meiosis</td>
</tr>
<tr>
<td>Ac, Me</td>
<td>phosphorylated/acyetylated higher-order combinations</td>
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</tbody>
</table>

Ac - acetyl (lysine), Me - methyl (lysine), P - phosphoryl (Ser or Thr)
Hypo-acetylated histones in heterochromatin

Sir proteins (Sir 1, 3, 4) bind cooperatively to de-acetylated chromatin, tightly packaging the 30-nm filament.
Sir2 is a histone deacetylase. The combined actions of the packaging proteins and the deacetylase spread heterochromatin (and silence packaged genes).

Replication distributes nucleosomes to the daughters

Experiment: replicate mixed chromatin and nucleosome-free templates in vitro

Results:
Replisome copied through nucleosomes! Nucleosomes were found only on the daughters of the chromatin template. No nucleosomes moved to the daughters of the nucleosome-free template.
Levels of chromatin structure

**Euchromatin:** transcribed and less condensed

“Loops” of 30-nm fibers seen at interphase

**Heterochromatin:** more condensed, genes silenced, replicated later in S phase.

Loops and scaffold

Electron micrograph of “histone-depleted” chromosome
DNA amplification produces visible “bands” in staining pattern of fly “polytene” chromosomes

Functional DNA sites -- Telomere & Centromere

Schematic and electron micrograph of X chromosome.
• Telomeres protect the ends.
• Centromere is at the primary constriction. It mediates chromosome cohesion, spindle attachment and chromosome segregation.
Eukaryotic Cell Division

Kinetochore mediates chromosome-microtubule attachments

Kinetochore mediates attachment to the spindle

Schematic drawing. Centromere (DNA segment) is at the primary constriction. The kinetochore is a huge, complicated protein complex with several layers. The outer layer provides attachment sites for microtubules.
Centromeres contain special DNA sequences that assemble kinetochores. Does transcription promote replacement of centromeric histone H3?

A model for the yeast kinetochore. Yeast kinetochore assembly and architecture. Speculative model for the organization of known components. Protein complexes are drawn approximately to scale.
Kinetochore complexity & structural challenge

The Kinetochore Puzzle
The Yeast Complex Dam1 Solution

Westermann et al. (2006) Nature
**Dam1: Mechanism and structure**

Model: Dam1 rings slide along shrinking microtubules!

**YACS = yeast artificial chromosomes**

50-1000 Kbp

DNA LIGATION AND YEAST CELL TRANSFORMATION

ARTIFICIAL YEAST CHROMOSOME WITH INSERTED HUMAN DNA

Needed to sequence the human genome

Figure 8-32 part 2 of 2. Molecular Biology of the Cell, 4th Edition.
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High resolution scanning immunogold electron micrograph. Yellow = phosphorylated H3 in the pericentromeric region.