## DNA Packaging in Chromatin & Chromosomes

<table>
<thead>
<tr>
<th>Model</th>
<th>Diameter (A)</th>
<th>Packing Ratio</th>
<th>Packing Ratio</th>
<th>Model</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Naked DNA</strong></td>
<td>20</td>
<td>20A dia.</td>
<td>10 bp/turn</td>
<td>1</td>
</tr>
<tr>
<td><strong>Chromatin</strong> (100 A fiber)</td>
<td>100</td>
<td>100A dia.</td>
<td>~80 bp/turn</td>
<td>6-7</td>
</tr>
<tr>
<td><strong>Chromatin</strong> (300 A fiber)</td>
<td>300</td>
<td>300A dia.</td>
<td>6 ν, 1200 bp/turn</td>
<td>~40</td>
</tr>
<tr>
<td><strong>Domains (loops)</strong></td>
<td>20-100</td>
<td>20-100 kb/loop</td>
<td>~700</td>
<td>vague</td>
</tr>
</tbody>
</table>

### Electron Micrograph of Chromatin Fibers

*(rat thymus nucleus)*

Olins et al., J. Cell Biol, (1975) 64, 528-537
Chromatin Structure

Metaphase Chromosomes Appear to Be Organized in Domains
Chromosome condensation during spermatogenesis suggests clustering of adjacent domains in “rosettes”

Hamkalo et al., 1978

One Model of Chromosome Organization

Griffiths et al., Introduction to Genetic Analysis, 2000
“A eukaryotic chromosome made out of self-assembling 70A units, which could perhaps be made to crystallize, would necessitate rewriting our basic textbooks on cytology and genetics! I have never read such a naïve paper purporting to be of such fundamental significance. Definitely it should not be published anywhere!”

- Anonymous review of paper submitted by C.F.L. Woodcock, 1973, showing EM pictures of nucleosome arrays
CHAP. XV: Cautions in viewing Objects. 
“Beware of determining and declaring your Opinion suddenly on any Object, for Imagination often gets the Start of Judgment…. Pass no Judgment upon Things over-extended by Force, or contracted by Dryness, or in any Manner out of their natural State, without making suitable Allowances.”

From “The Microscope Made Easy” by Henry Baker, 1742

Establishing the nucleosome model. - a paradigm shift, 1973-1974

1. Electron microscopy - images
2. Micrococcal nuclease digestion patterns
3. Knowledge of histone:histone interactions
Preparation of Defined Lengths of Chromatin

Sucrose gradient fractionation of micrococcal nuclease digestion products

- Top of gradient is on the right
- Bottom of gradient is on the left
- Fractions collected from shaded areas

Polyacrylamide gel electrophoresis of purified DNA

- Right lane: unfractionated digest
- Left lanes: DNA purified from sucrose gradient peaks

Electron Micrographs of Fractions from Sucrose Gradient

Monomer fraction

Dimer fraction

Trimer fraction

Tetramer fraction

Finch et al., PNAS (1975) 72, p3321
Ribbon Model of the Four Histones

The Histone Octamer

The complete histone octamer in the absence of DNA.

The view is down the superhelix axis.

Color code:
- H2A
- H2B
- H3
- H4


Arents et al., PNAS (1991) 88, 10148-52
The Structure of the Nucleosome Core

Resolution: 2.8 Å
Half of the nucleosome structure is shown
One turn of the DNA helix is visible (73 bp)
View is down the superhelix axis
Protein - DNA contact: white hooks


Histone “Footprints” and the Axis of the DNA Supercoil in the Nucleosome

Axis of the DNA path in the nucleosome (not full width)

Arents et. al., PNAS (1993) 90, 10489-93
Stoichiometry:

1. Core particle:
   - 147 bp DNA
   - histone octamer
     - tetramer \([H3 + H4]^2\)
     - 2 dimers \([H2A + H2B]\)

2. Nucleosome (repeating subunit)
   - 167 bp DNA (2 turns) plus ~50 bp linker
   - histone octamer
     - tetramer \([H3 + H4]^2\)
     - 2 dimers \([H2A + H2B]\)
     - 1 H1 (histone 1)

Role of Acetylation of Histone Tails in Yeast Transcription Control
Boundary Assay

---scs---E---P---lacZ---R---scs---
---E---scs---P---lacZ---R---scs---
---scs---R---P---lacZ---R---scs---
---scs---R---P---lacZ---E---scs---
---scs---R---P---lacZ---scs---E---
---scs---P---lacZ---E---scs---

P = promoter       scs = boundary
E = enhancer       R = random spacer
The Structure of the Nucleosome Core

The Structured Tails of Histones


Preparation of Defined Length of Chromatin

Finch et al., PNAS (1975) 72, 3321
Nucleosome

Griffiths et al., Introduction to Genetic Analysis, 2000

Eukaryotic Cell

Lodish et al., Molecular Cell Biology, 4th Edition
Organization of Mating-Type (MAT) Locus in Yeast

Lodish et al., Molecular Cell Biology, 4th Edition