Genome organization: from nucleotides to chromatin

Dr Saeb Aliwaini
• Chromosomes seem to appear out of nowhere at the beginning of mitosis and disappear once again when cell division has ended.

• An average human cell contains about 6.4 billion base pairs of DNA divided among 46 chromosomes (the value for a diploid, unreplicated number of chromosomes).

• Each unreplicated chromosome contains a single, continuous DNA molecule; the larger the chromosome, the longer the DNA it contains.

• Each base pair is about 0.34 nm in length so ........
• ≈2 m of unpacked DNA must fit in a less than 10 micron diameter space.

• The diversity of mechanisms for packaging very long molecules of DNA into very small cellular spaces is truly remarkable.
Eukaryotic genome

- Linear DNA → chromatin, what is chromatin?

- Chromatin is a DNA with its associated proteins.

1- A histone complex called a nucleosome.

2- Beads-on-a-string

3- A zig-zagging string of chromatin.

4- Loop domains.

5- Finally the metaphase chromosome.

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Table 3.2 Diversity of DNA-based genome organization.

<table>
<thead>
<tr>
<th>Genome</th>
<th>Form</th>
<th>Size (kb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eukaryotes</td>
<td>ds linear</td>
<td>$10^4$ to $10^6$</td>
</tr>
<tr>
<td>Bacteria</td>
<td>ds circular</td>
<td>$10^3$</td>
</tr>
<tr>
<td>Plasmids</td>
<td>ds circular (some ds linear)</td>
<td>2–15</td>
</tr>
<tr>
<td>Mammalian DNA viruses</td>
<td>ss linear, ds linear, ds circular</td>
<td>3–280</td>
</tr>
<tr>
<td>Bacteriophage</td>
<td>ss circular, ds linear</td>
<td>~50</td>
</tr>
<tr>
<td>Chloroplast DNA</td>
<td>ds circular (or ds linear?)</td>
<td>120–160</td>
</tr>
</tbody>
</table>
| Mitochondrial DNA           | ds circular (some ds linear)     | Animals: 16.5
                                                                                   Plants: 100–2500

ds, double-stranded; ss, single-stranded.
In eukaryotes

- **Genome** = genomic DNA, chromosomal DNA, or nuclear DNA

The eukaryotic genome is located in the cell nucleus
Histones

- Two main types divided into classes:

Both have high content of the basic amino acids arginine and lysine.

1- The highly conserved core histones (MW 11,000–16,000 Da).

DA = $1.66053921(73) \times 10^{-27}$ kg

present in the nucleosome as an octamer composed of a dimer of histones H2A and H2B at each end and a tetramer of histones H3 and H4 in the center, around which 146 bp of genomic DNA is wound.

- Cow histone H4 differs from pea H4 in only two places!

Why are histones so highly conserved?

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<table>
<thead>
<tr>
<th>Histone</th>
<th>Number of residues</th>
<th>Mass (kDa)</th>
<th>%Arg</th>
<th>%Lys</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>215</td>
<td>23.0</td>
<td>1</td>
<td>29</td>
</tr>
<tr>
<td>H2A</td>
<td>129</td>
<td>14.0</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>H2B</td>
<td>125</td>
<td>13.8</td>
<td>6</td>
<td>16</td>
</tr>
<tr>
<td>H3</td>
<td>135</td>
<td>15.3</td>
<td>13</td>
<td>10</td>
</tr>
<tr>
<td>H4</td>
<td>102</td>
<td>11.3</td>
<td>14</td>
<td>11</td>
</tr>
</tbody>
</table>
• In addition to the four “conventional” core histones discussed above, several alternate versions of the H2A and H3 histones are also synthesized in most cells.

• H2A.X .......

• H2A.X becomes phosphorylated at sites of DNA-strand breakage and may play role in recruiting the enzymes that repair the DNA.

• Research area......
1-nucleosome

- All four core histones contain an extended histone-fold domain at the carboxyl (C) terminal end to bind other histons and DNA

- The contacts at sites where the minor groove

- Every 10 bp.

- transcription factors???

- The charged tails at the amino (N) terminal end contain the bulk of the lysine residues for post-translational modifications

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• four heterodimers: two H2A-H2B dimers and two H3-H4 dimers
nucleosome

2- linker histones > 20,000 Da.

The linker histone H1 (or alternative forms such as histone H5 and H1) occurs between core octamers, where the DNA enters and exits the nucleosome.
1- Nucleosomes

• A **nucleosome core particle** = The core octamer of histones (H2A, H2B, H3, and H4)x2 + approximately 146 bp of supercoiled DNA + the linker histone H1 (outside the core) negatively charged DNA
• Together the H1 protein and the histone octamer interact with about 168 base pairs of DNA
• DNA wraps nearly twice around the positively charged histones in 1.67 left-handed superhelical turns
• The major interactions between DNA and the core histones appear to be electrostatic in nature (ionic bonds).
• Histones can be removed from DNA by high salt on concentration.
• With a nucleotide-nucleotide spacing of 0.34 nm the 200 base pairs of a single nucleosome would stretch nearly ---- nm if fully extended.

• The packing ratio of the DNA of nucleosomes is approximately 7:1.

• The ( 10-nm ) diameter nucleosome is the lowest level of chromatin organization

• **What are the higher Levels of Chromatin Structure?**
2-Beads-on-a-string: the 10 nm fiber

- The beads-on-a-string appearance of chromatin can be visualized by electron microscopy as a 10–11 nm fiber after low salt extraction

- The beads represent DNA wrapped around the histone core octamer

- The string represents the DNA double helix

The linker histone is not required for this level of packing.
3- 30-nm fiber

- When chromatin is released from nuclei and prepared at physiologic ionic strength, a fiber of approximately 30-nm thickness is observed
Two models

The models differ in the relative positioning of nucleosomes within the fiber.

Recent research favors the “zig-zag”

The DNA-packing ratio an additional 6-fold
What types of links

- Linker histone
- Tails of cores can also bind to the adjacent cores and linkers DNA!
- These types of interactions are thought to mediate the folding of the nucleosomal filament into a thicker fiber
- In fact, chromatin fibers prepared with H4 histones that lack their tails are unable to fold into higher-order fibers
4- The (80–100 nm) fibers or loop Domains

• The next stage in the hierarchy of DNA packaging is thought to occur as the 30-nm chromatin fiber is gathered into a series of large, supercoiled loops, or domains, that may be compacted into even thicker (80–100 nm) fibers

• Matrix proteins: Type II Topoisomerase and other proteins to make these loops

• At G0 this structure is the most common.
Mitotic chromosome is the ultimate

1µm of the chromosome ----- 1 cm DNA

The processes is not very clear yet!
Condensine and Cohesin

- Looping by supercoiling
- Topoisomerases + ATP— +ve supercoiling
- **Condensine is a target for Cdks**
- Rode like structure and two mirror –image “sister chromatids” after replication.
- Cohesin
• Condensin compresses one sister into itself, has intra molecular activates, whereas cohesin adheres two different sister chromatids, has intermolecular activates
Heterochromatin and Euchromatin

• After mitosis has been completed, most of the chromatin returns to its diffuse interphase condition.

• But 10 percent of the chromatin, however, generally remains in a condensed, compacted form.
Chromatin that remains compacted during interphase is called **heterochromatin** to distinguish it from **euchromatin**, which returns to a dispersed state.

Little transcriptional activity for heterochromatin.

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Heterochromatin

- little transcriptional activity for hetero chromatin

- 2 types:

  1- **Constitutive heterochromatin**

  remains in the compacted state in all cells at all times, represents DNA that is permanently silenced
**position effect**

- When genes that are normally active move into a position adjacent to heterochromatin (result of transposition or translocation), they become transcriptionally silenced.

- The spread of heterochromatin along the chromosome is apparently blocked by specialized barrier sequences (*boundary elements*)
Heterochromatin

2- Facultative heterochromatin

inactivated during certain phases of an organism’s life or in certain types of differentiated cells