Structure of Chromatin and Chromosome

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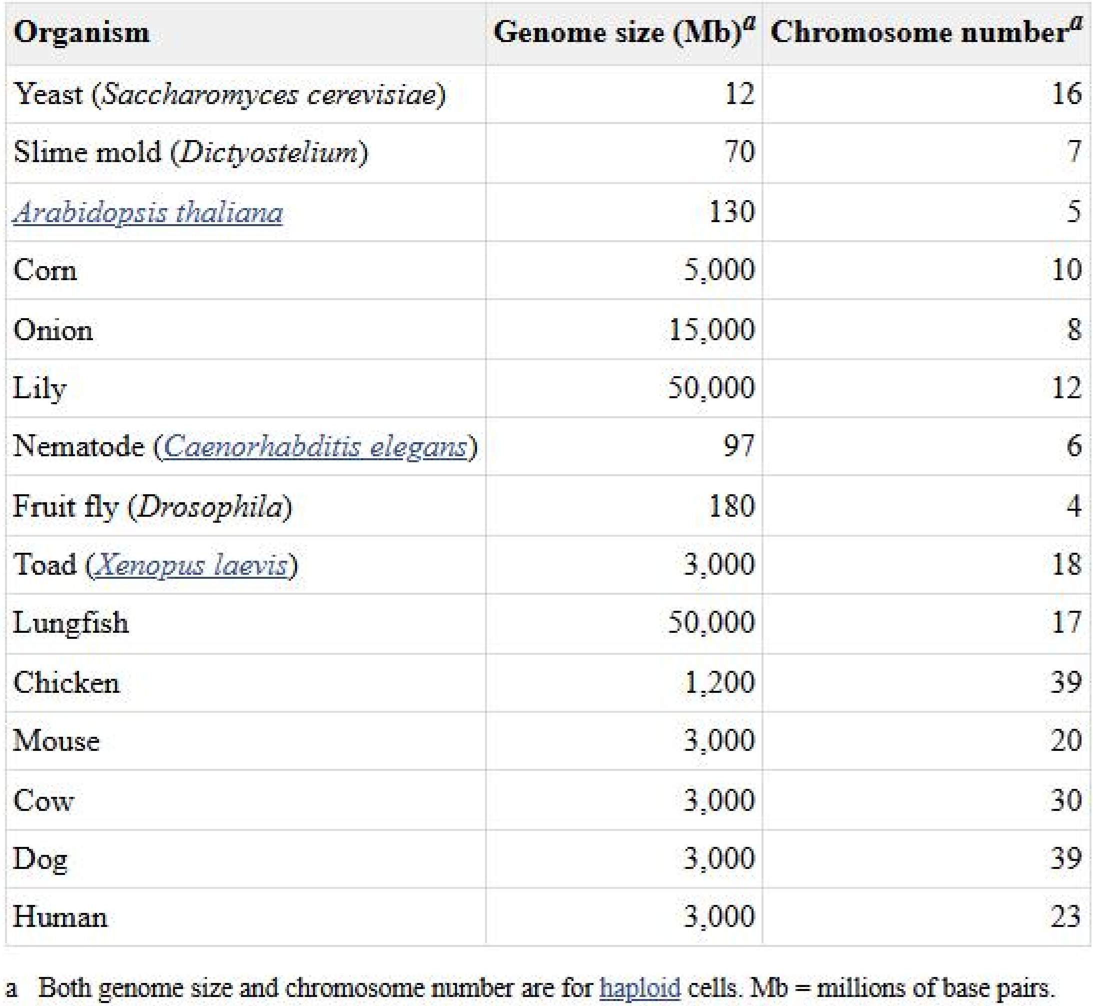
Chromosomes and Chromatin

- The genomes of eukaryotes are composed of multiple chromosomes, each containing a linear molecule of DNA.
- Although the numbers and sizes of chromosomes vary considerably between different species.
- The DNA of eukaryotic cells is tightly bound to small basic protein (histones) that package the DNA in an orderly way in the cell nucleus.

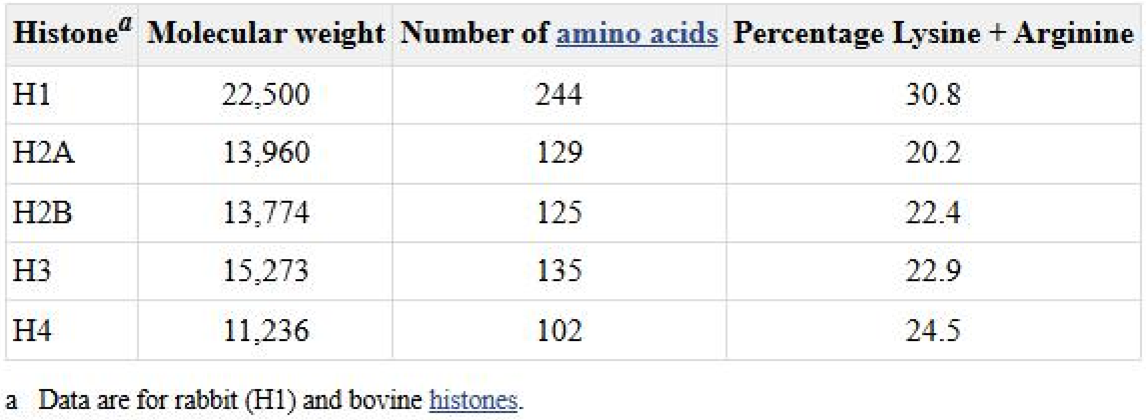


Organism	**Genome size (Mb)**[a]	**Chromosome number**[a]
Yeast (*Saccharomyces cerevisiae*)	12	16
Slime mold (*Dictyostelium*)	70	7
Arabidopsis thaliana	130	5
Corn	5,000	10
Onion	15,000	8
Lily	50,000	12
Nematode (*Caenorhabditis elegans*)	97	6
Fruit fly (*Drosophila*)	180	4
Toad (*Xenopus laevis*)	3,000	18
Lungfish	50,000	17
Chicken	1,200	39
Mouse	3,000	20
Cow	3,000	30
Dog	3,000	39
Human	3,000	23

[a] Both genome size and chromosome number are for haploid cells. Mb = millions of base pairs.

Chromatin

- The complexes between eukaryotic DNA and proteins are called chromatin, which typically contains about twice as much protein as DNA.
- The major proteins of chromatin are the histones—small proteins containing a high proportion of basic amino acids (arginine and lysine) that facilitate binding to the negatively charged DNA molecule.
- There are five major types of histones—called H1, H2A, H2B, H3, and H4—which are very similar among different species of eukaryotes.

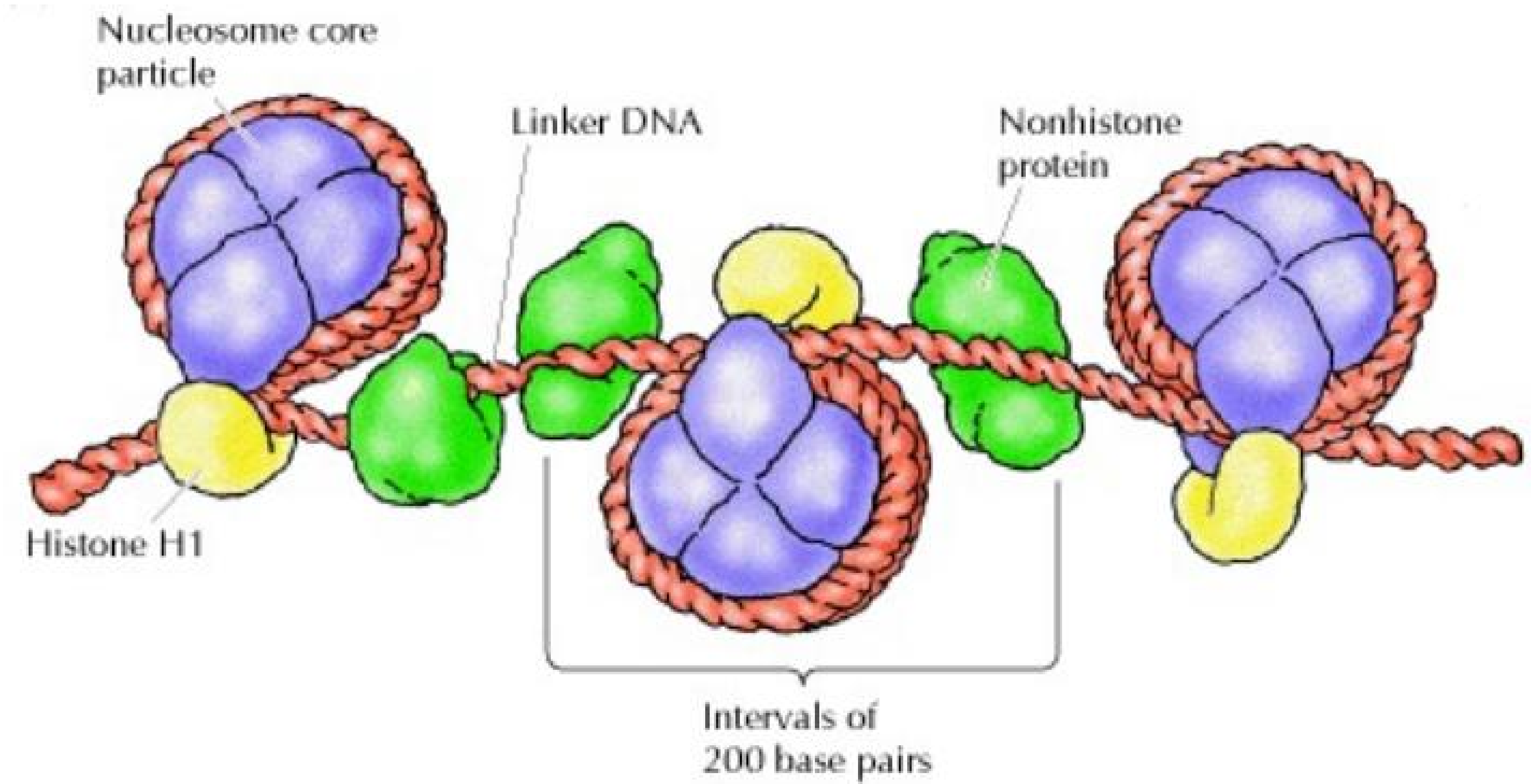


Histone[a]	**Molecular weight**	**Number of amino acids**	**Percentage Lysine + Arginine**
H1	22,500	244	30.8
H2A	13,960	129	20.2
H2B	13,774	125	22.4
H3	15,273	135	22.9
H4	11,236	102	24.5

[a] Data are for rabbit (H1) and bovine histones.

…Chromatin

- The histones are extremely abundant proteins in eukaryotic cells; together, their mass is approximately equal to that of the cell's DNA.
- In addition, chromatin contains an approximately equal mass of a wide variety of nonhistone chromosomal proteins.
- There are more than a thousand different types of these proteins, which are involved in a range of activities, including DNA replication and gene expression.
- Histones are not found in eubacteria (e.g., *E. coli*), although the DNA of these bacteria is associated with other proteins that presumably function like histones to package the DNA within the bacterial cell.
- Archaebacteria, however, do contain histones that package their DNAs in structures similar to eukaryotic chromatin.
- The basic structural unit of chromatin, the nucleosome, was described by Roger Kornberg in 1974.



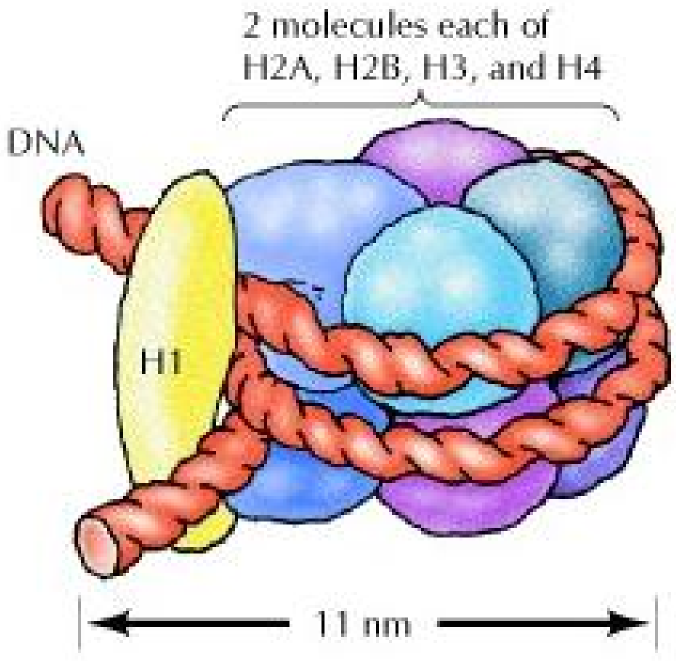
The DNA is wrapped around histones in nucleosome core particles and sealed by histone H1. Nonhistone proteins bind to the linker DNA between nucleosome core particles.

…Chromatin

- Partial digestion of chromatin with nuclease was found to yield DNA fragments approximately 200 base pairs long.
- In contrast, a similar digestion of naked DNA (not associated with proteins) yielded a continuous smear of randomly sized fragments.
- These results suggested that the binding of proteins to DNA in chromatin protects regions of the DNA from nuclease digestion, so that the enzyme can attack DNA only at sites separated by approximately 200 base pairs.
- Consistent with this notion, electron microscopy revealed that chromatin fibers have a beaded appearance, with the beads spaced at intervals of approximately 200 base pairs.
- Thus, both the nuclease digestion and the electron microscopic studies suggested that chromatin is composed of repeating 200-base-pair units, which were called nucleosomes.

Nucleosome

- More extensive digestion of chromatin with micrococcal nuclease was found to yield particles (called **nucleosome core particles**) that correspond to the beads visible by electron microscopy.
- Detailed analysis of these particles has shown that they contain 146 base pairs of DNA wrapped 1.65 times around a histone core consisting of two molecules each of H2A, H2B, H3, and H4 (the core histones).
- One molecule of the fifth histone, H1, is bound to the DNA as it enters each nucleosome core particle.
- This forms a chromatin subunit known as a chromatosome, which consists of 166 base pairs of DNA wrapped around the histone core and held in place by H1 (a linker histone).

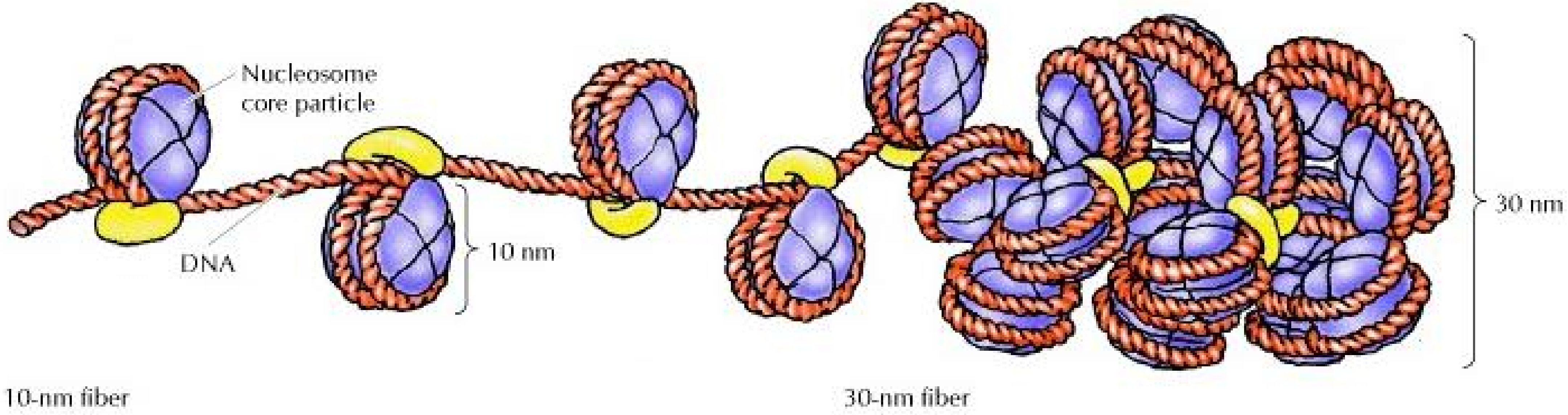


The nucleosome core particle consists of 146 base pairs of DNA wrapped 1.65 turns around a histone octamer consisting of two molecules each of H2A, H2B, H3, and H4. A chromatosome contains two full turns of DNA (166 base pairs) locked in place by one molecule of H1.

…Nucleosome

- The packaging of DNA with histones yields a chromatin fiber approximately 10 nm in diameter that is composed of chromatosomes separated by linker DNA segments averaging about 80 base pairs in length.
- In the electron microscope, this 10-nm fiber has the beaded appearance that suggested the nucleosome model.
- The 10 nm fiber or beads on a string conformation is the most basic formation.
- Packaging of DNA into such a 10-nm chromatin fiber shortens its length approximately sixfold.
- The chromatin can then be further condensed by coiling into 30-nm fibers.
- Interactions between histone H1 molecules appear to play an important role in this stage of chromatin condensation (solenoid structure, which consists of 6 nucleosomes linked together by the Histone H1 linker proteins).

Solenoid Structure



The packaging of DNA into nucleosomes yields a chromatin fiber approximately 10 nm in diameter. The chromatin is further condensed by coiling into a 30-nm fiber, containing about six nucleosomes per turn.

Euchromatin

- The extent of chromatin condensation varies during the life cycle of the cell.
- In interphase (nondividing) cells, most of the chromatin (called euchromatin) is relatively decondensed and distributed throughout the nucleus (Figure 4.11).
- During this period of the cell cycle, genes are transcribed and the DNA is replicated in preparation for cell division.
- Most of the euchromatin in interphase nuclei appears to be in the form of 30-nm fibers, organized into large loops containing approximately 50 to 100 kb of DNA.
- About 10% of the euchromatin, containing the genes that are actively transcribed, is in a more decondensed state (the 10-nm conformation) that allows transcription.
- Chromatin structure is thus intimately linked to the control of gene expression in eukaryotes.

Euchromatin

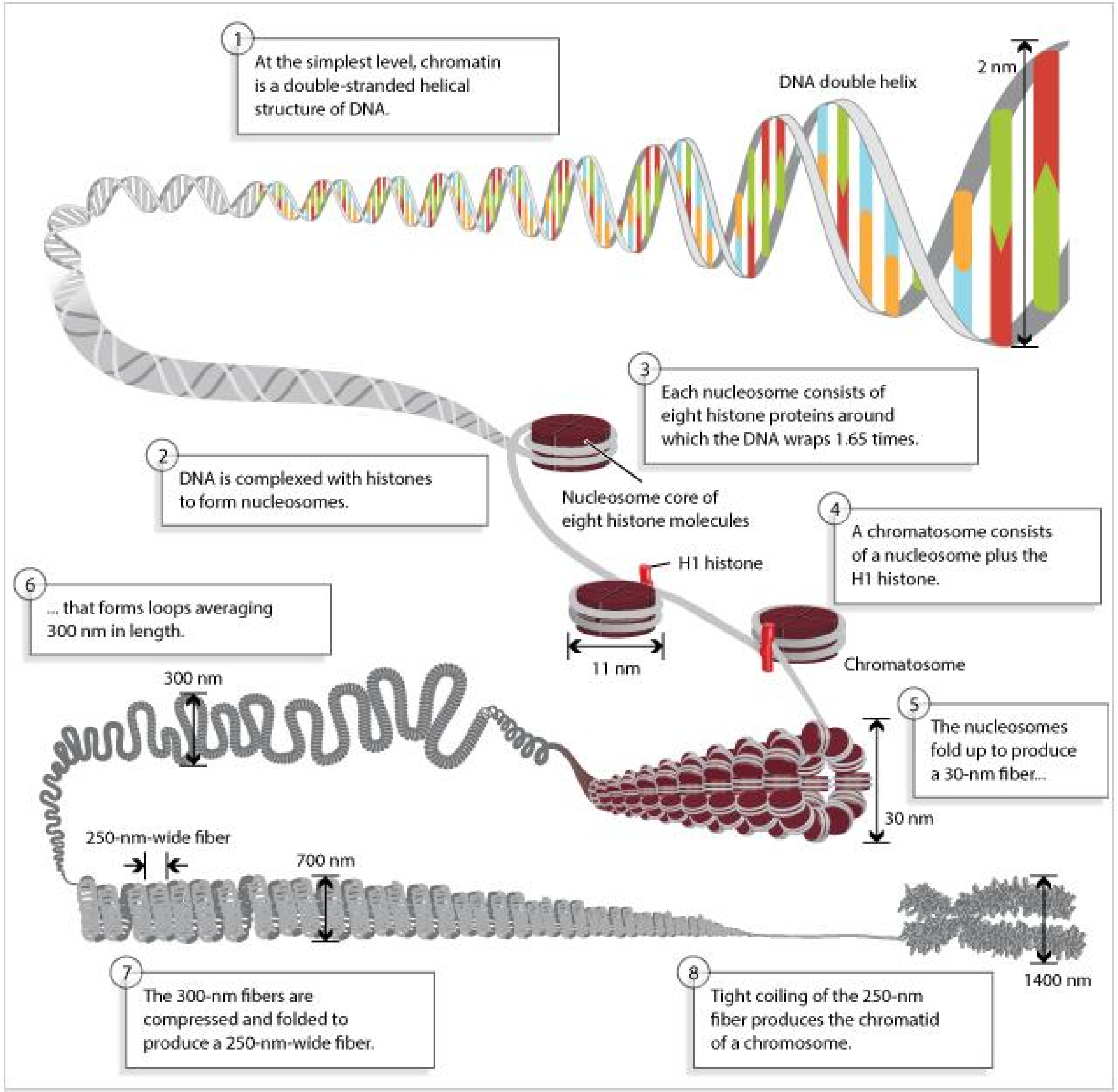
- Processes such as transcription and replication require the two strands of DNA to come apart temporarily, thus allowing polymerases access to the DNA template.
- However, the presence of nucleosomes and the folding of chromatin into 30-nanometer fibers pose barriers to the enzymes that unwind and copy DNA.
- It is therefore important for cells to have means of opening up chromatin fibers and/or removing histones transiently to permit transcription and replication to proceed.
- Generally speaking, there are two major mechanisms by which chromatin is made more accessible:
  - Histones can be enzymatically modified by the addition of acetyl, methyl, or phosphate groups (Fischle *et al*., 2005).
  - Histones can be displaced by chromatin remodeling complexes, thereby exposing underlying DNA sequences to polymerases and other enzymes (Smith & Peterson, 2005).
- It is important to remember that these processes are reversible, so modified or remodeled chromatin can be returned to its compact state after transcription and/or replication are complete.

Heterochromatin

- In contrast to euchromatin, about 10% of interphase chromatin (called heterochromatin) is in a very highly condensed state that resembles the chromatin of cells undergoing mitosis.
- Heterochromatin is transcriptionally inactive and contains highly repeated DNA sequences, such as those present at centromeres and telomeres.
- As cells enter mitosis, their chromosomes become highly condensed so that they can be distributed to daughter cells.
- The loops of 30-nm chromatin fibers are thought to fold upon themselves further to form the compact metaphase chromosomes of mitotic cells, in which the DNA has been condensed nearly 10,000-fold.
- Such condensed chromatin can no longer be used as a template for RNA synthesis, so transcription ceases during mitosis.
- Electron micrographs indicate that the DNA in metaphase chromosomes is organized into large loops attached to a protein scaffold.
- This level of compaction is achieved by repeatedly folding chromatin fibers into a hierarchy of multiple loops and coils.
- Exactly how this is accomplished is unclear, but the phosphorylation of histone H1 may play a role.

Figure 1: Chromosomes are composed of DNA tightly-wound around histones.

- Chromosomal DNA is packaged inside microscopic nuclei with the help of histones.
- These are positively-charged proteins that strongly adhere to negatively-charged DNA and form complexes called nucleosomes.
- Each nuclesome is composed of DNA wound 1.65 times around eight histone proteins.
- Nucleosomes fold up to form a 30-nanometer chromatin fiber, which forms loops averaging 300 nanometers in length.
- The 300 nm fibers are compressed and folded to produce a 250 nm-wide fiber, which is tightly coiled into the chromatid of a chromosome.



① At the simplest level, chromatin is a double-stranded helical structure of DNA.

② DNA is complexed with histones to form nucleosomes.

③ Each nucleosome consists of eight histone proteins around which the DNA wraps 1.65 times.

④ A chromatosome consists of a nucleosome plus the H1 histone.

⑤ The nucleosomes fold up to produce a 30-nm fiber...

⑥ ... that forms loops averaging 300 nm in length.

⑦ The 300-nm fibers are compressed and folded to produce a 250-nm-wide fiber.

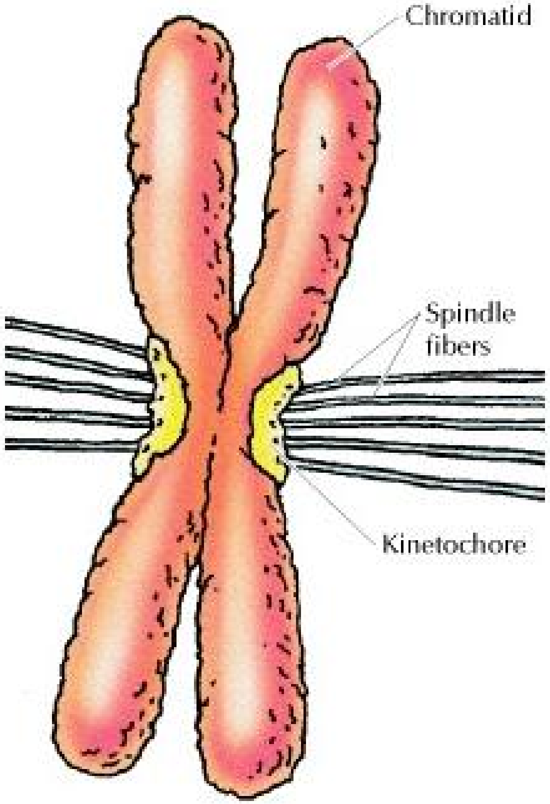
⑧ Tight coiling of the 250-nm fiber produces the chromatid of a chromosome.

Centromeres

- The centromere is a specialized region of the chromosome that plays a critical role in ensuring the correct distribution of duplicated chromosomes to daughter cells during mitosis.
- The cellular DNA is replicated during interphase, resulting in the formation of two copies of each chromosome prior to the beginning of mitosis. As the cell enters mitosis, chromatin condensation leads to the formation of metaphase chromosomes consisting of two identical sister chromatids.
- These sister chromatids are held together at the centromere, which is seen as a constricted chromosomal region.
- As mitosis proceeds, microtubules of the mitotic spindle attach to the centromere, and the two sister chromatids separate and move to opposite poles of the spindle.
- At the end of mitosis, nuclear membranes re-form and the chromosomes decondense, resulting in the formation of daughter nuclei containing one copy of each parental chromosome.

…Centromeres

- The centromeres thus serve both as the sites of association of sister chromatids and as the attachment sites for microtubules of the mitotic spindle.
- They consist of specific DNA sequences to which a number of centromere-associated proteins bind, forming a specialized structure called the kinetochore.
- The binding of microtubules to kinetochore proteins mediates the attachment of chromosomes to the mitotic spindle.
- Proteins associated with the kinetochore then act as “molecular motors” that drive the movement of chromosomes along the spindle fibers, segregating the chromosomes to daughter nuclei.



…Centromere

- Centromeric DNA sequences have been defined best in yeasts.
- the centromere sequences of the well-studied yeast *Saccharomyces cerevisiae* are contained in approximately 125 base pairs consisting of three sequence elements: two short sequences of 8 and 25 base pairs separated by 78 to 86 base pairs of very AT-rich DNA.
- The centromeres of *S. pombe* span 40 to 100 kb of DNA; they are approximately a thousand times larger than those of *S. cerevisiae*.
- They consist of a central core of 4 to 7 kb of single-copy DNA flanked by repetitive sequences.
- The *Drosophila* centromere spans 420 kb, most of which (more than 85%) consists of two highly repeated satellite DNAs with the sequences AATAT and AAGAG.
- The remainder of the centromere consists of interspersed transposable elements, which are also found at other sites in the *Drosophila* genome, in addition to a nonrepetitive region of AT-rich DNA.

Telomeres

- The sequences at the ends of eukaryotic chromosomes, called telomeres, play critical roles in chromosome replication and maintenance.
- Telomeres were initially recognized as distinct structures because broken chromosomes were highly unstable in eukaryotic cells, implying that specific sequences are required at normal chromosomal termini.
- This was subsequently demonstrated by experiments in which telomeres from the protozoan *Tetrahymena* were added to the ends of linear molecules of yeast plasmid DNA.
- The addition of these telomeric DNA sequences allowed these plasmids to replicate as linear chromosome-like molecules in yeasts, demonstrating directly that telomeres are required for the replication of linear DNA molecules.

…Telomeres

- The telomere DNA sequences of a variety of eukaryotes are similar, consisting of repeats of a simple-sequence DNA containing clusters of G residues on one strand.
- For example, the sequence of telomere repeats in humans and other mammals is AGGGTT, and the telomere repeat in *Tetrahymena* is GGGGTT.
- These sequences are repeated hundreds or thousands of times, thus spanning up to several kilobases, and terminate with an overhang of single-stranded DNA.
- Recent results suggest that the repeated sequences of telomere DNA form loops at the ends of chromosomes, thereby protecting the chromosome termini from degradation.

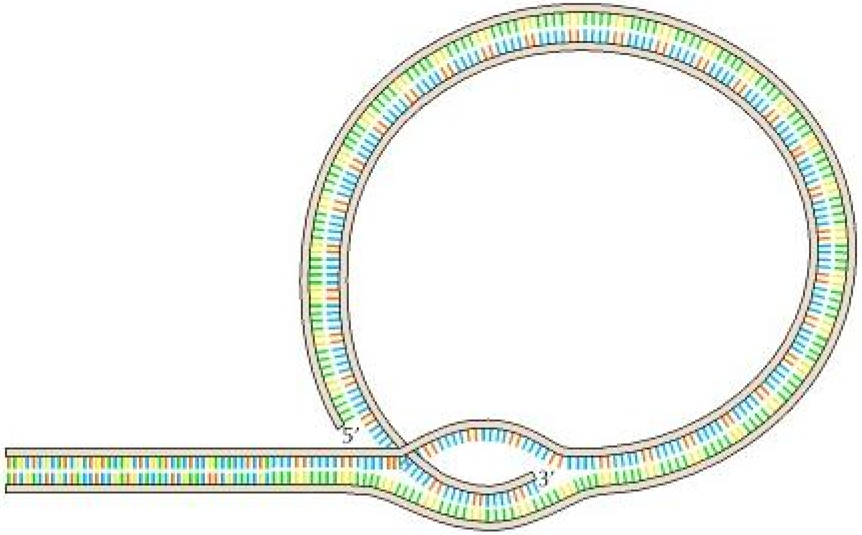


Figure 4.19 Structure of a telomere

…Telomeres

- Telomeres play a critical role in replication of the ends of linear DNA molecules.
- DNA polymerase is able to extend a growing DNA chain but cannot initiate synthesis of a new chain at the terminus of a linear DNA molecule.
- Consequently, the ends of linear chromosomes cannot be replicated by the normal action of DNA polymerase.
- This problem has been solved by the evolution of a special mechanism, involving reverse transcriptase activity, to replicate telomeric DNA sequences.
- Maintenance of telomeres appears to be an important factor in determining the lifespan and reproductive capacity of cells, so studies of telomeres and telomerase have the promise of providing new insights into conditions such as aging and cancer.