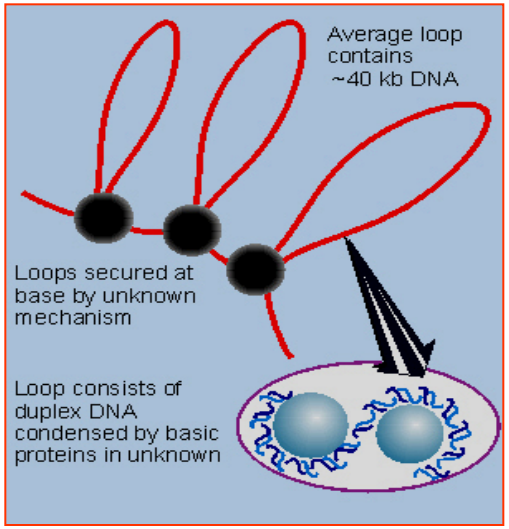
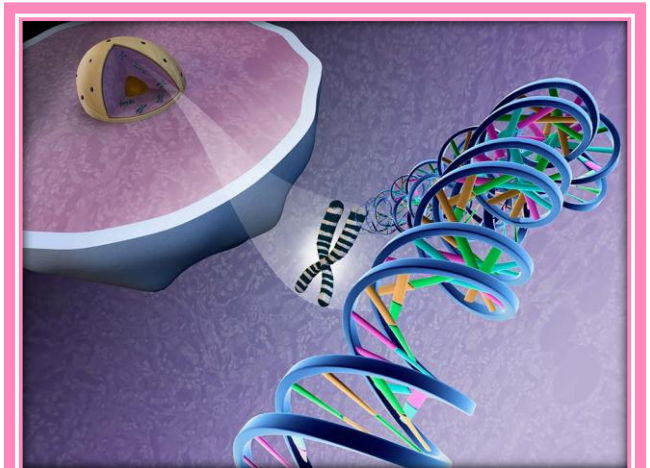
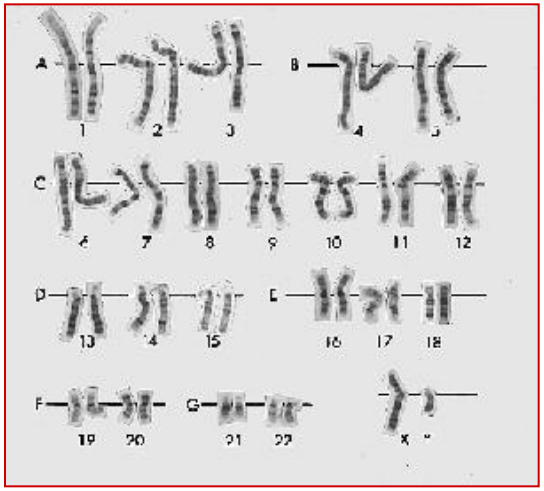
Histone proteins, the nucleosome and chromatin structure

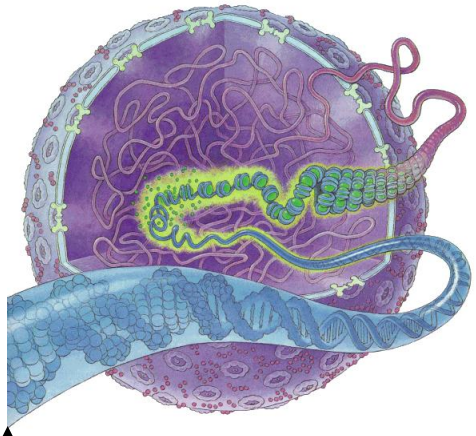




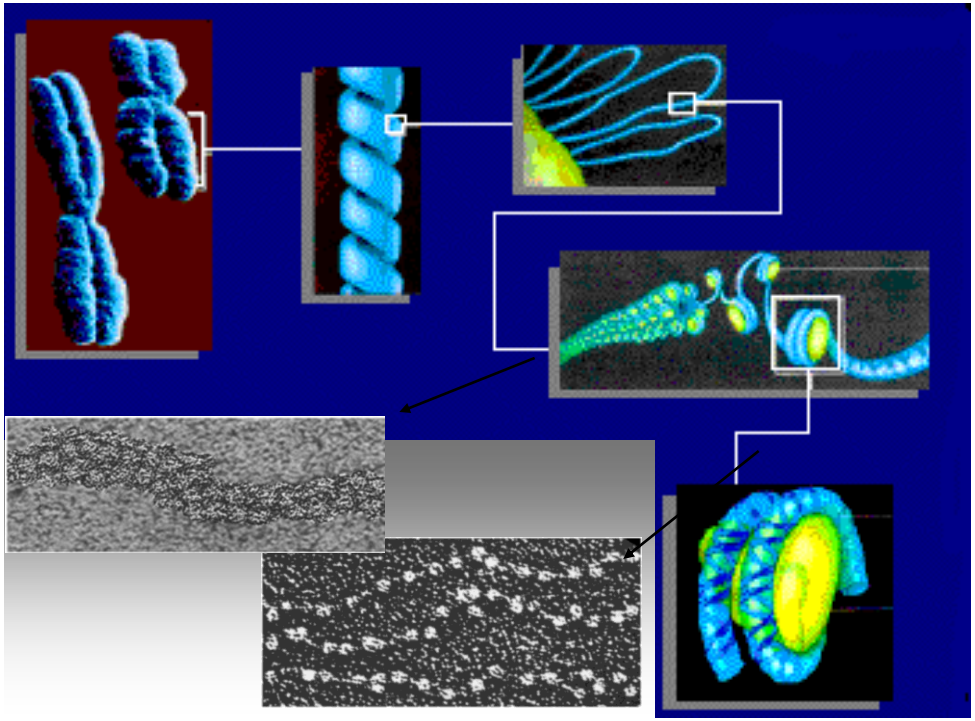


Dr.Rasime Kalkan

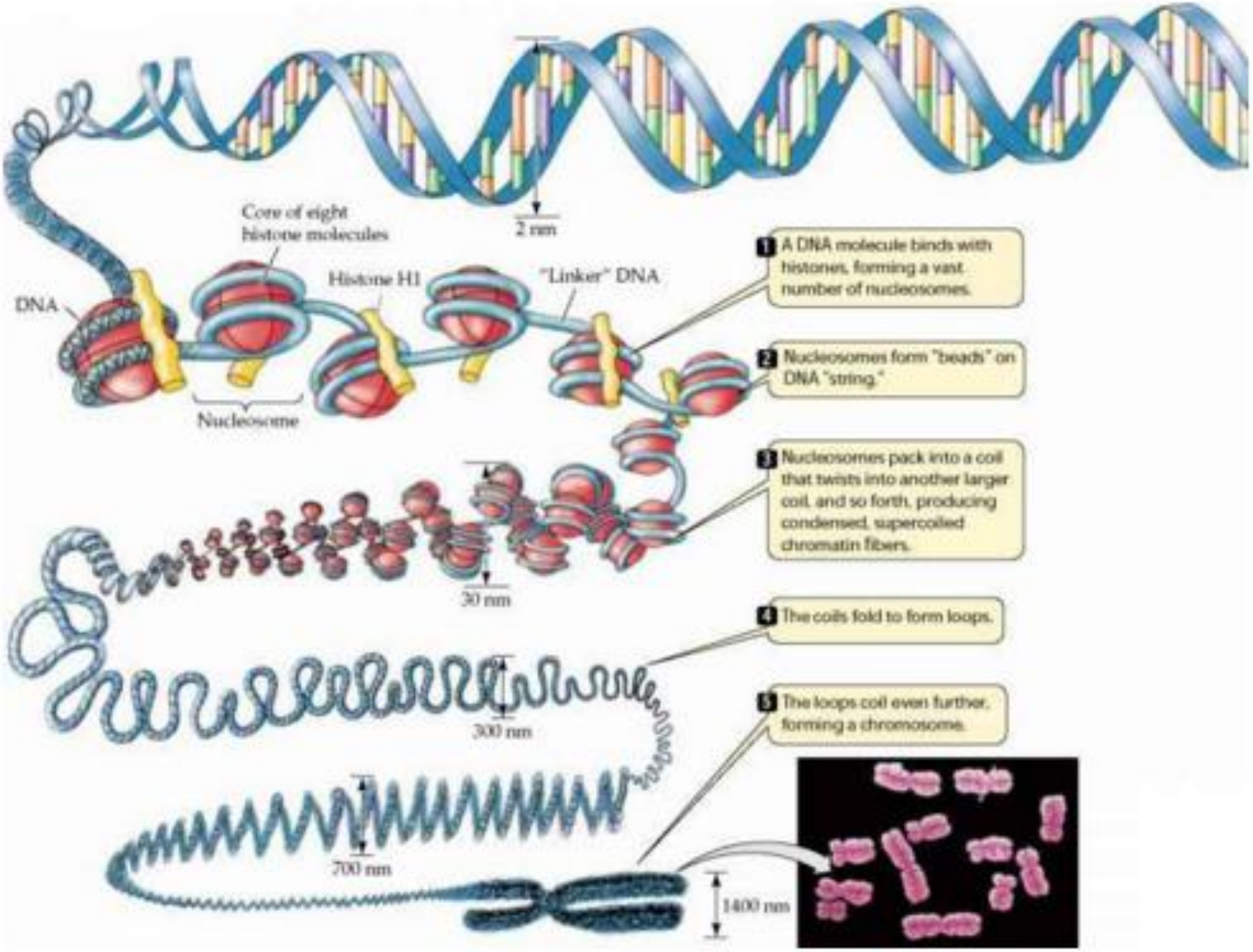
Packing of DNA into chromatin



2 m of DNA in a nucleus with a diameter of 5 to 10 μm.



Chromatin not only packages DNA, but also regulates DNA accessibility through modifications in chromatin structure.


2 nm
Core of eight histone molecules
Histone H1
"Linker" DNA
DNA
Nucleosome
1 A DNA molecule binds with histones, forming a vast number of nucleosomes.
2 Nucleosomes form "beads" on DNA "string."
3 Nucleosomes pack into a coil that twists into another larger coil, and so forth, producing condensed, supercoiled chromatin fibers.
30 nm
4 The coils fold to form loops.
300 nm
5 The loops coil even further, forming a chromosome.
700 nm
1400 nm

Packing of DNA into Chromosomes

Chromatin= nuclear DNA + all the proteins bound to it

Two classes of proteins bind to DNA to form chromosome

1. histones
2. nonhistones

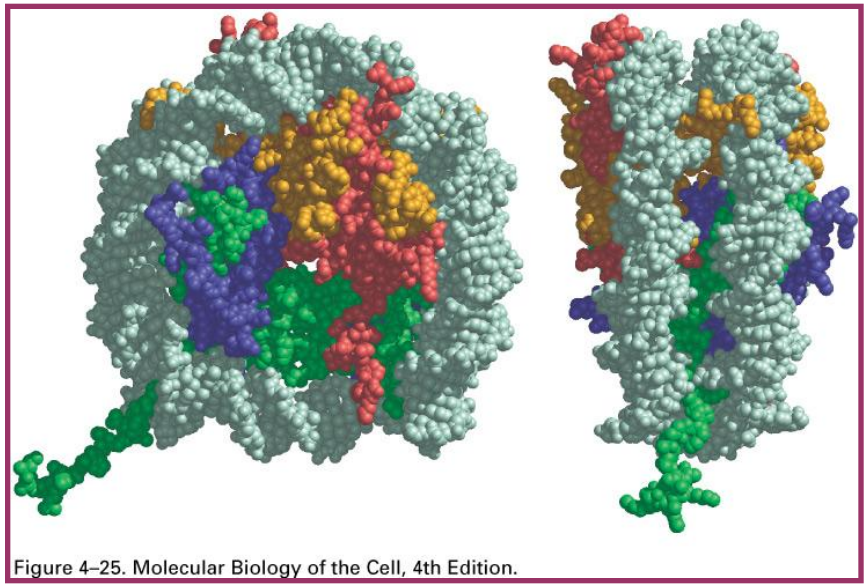
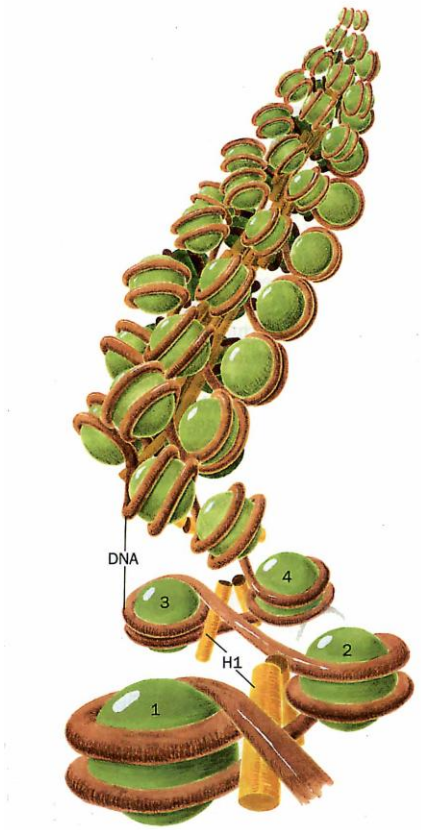


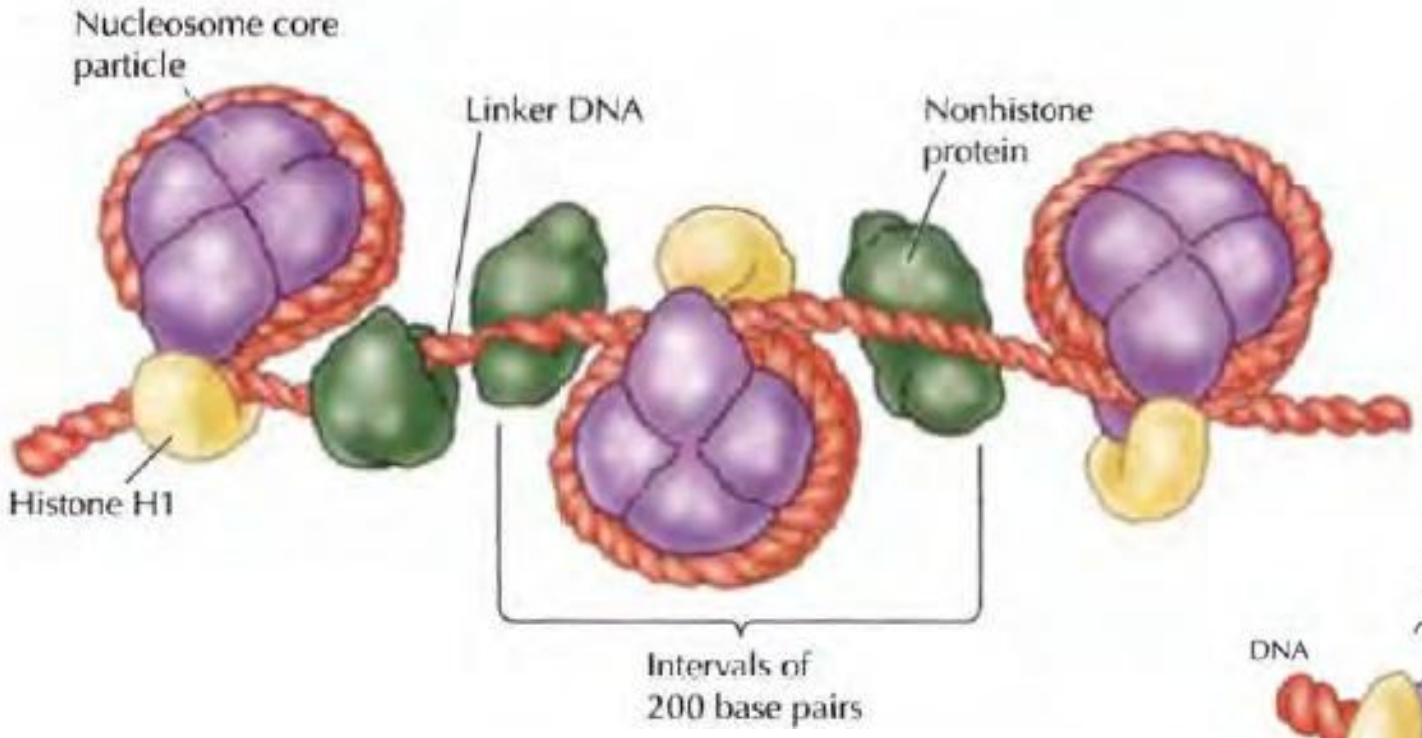
Figure 4–25. Molecular Biology of the Cell, 4th Edition.

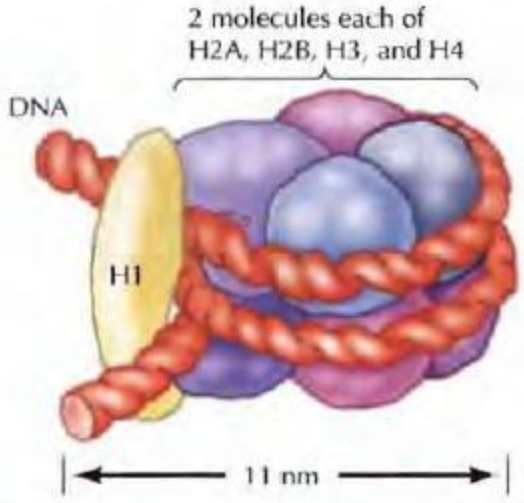


Chromatin

- The complexes between eukaryotic DNA and proteins are called chromatin.
- 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.

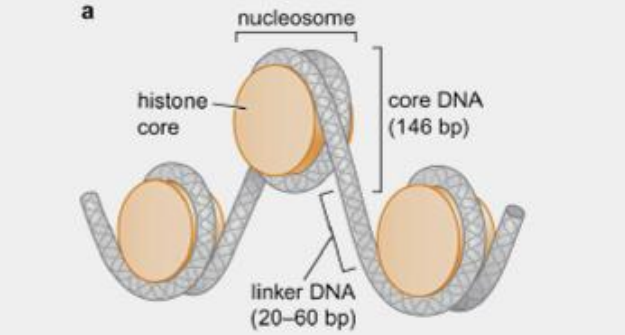
- Nucleosome is the basic structural unit of chromatin.





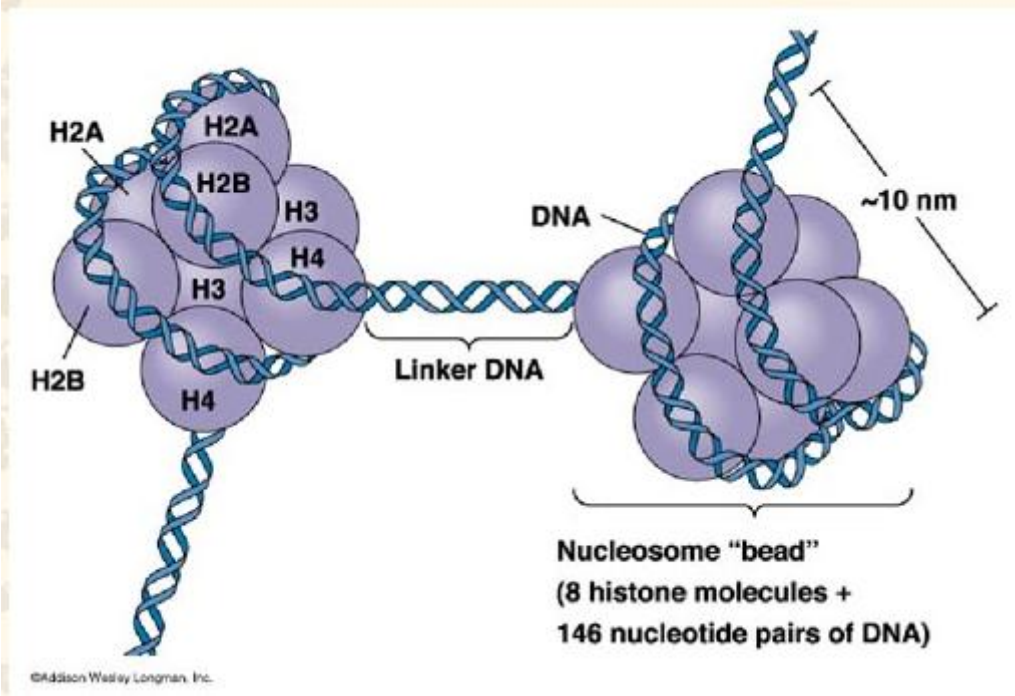
- The nucleosome contains ~200 bp of DNA, organized by an octamer of small, basic proteins into a bead-like structure.
- The protein components are called histones.

- Nucleosomal DNA is divided into the core DNA and linker DNA depending on its susceptibility to micrococcal nuclease.
- Core DNA has an invariant length of 146 bp, and is relatively resistant to digestion by nucleases (H2A, H2B, H3, H4).
- Linker DNA comprises the rest of the repeating unit. Its length varies from as little as 8 bp to as much as 114 bp per nucleosome (H1) .



Histone H1 - linker histone

- H1 linker histone
  - associated with linker DNA between nucleosomes (about one H1 per nucleosome)
    - Binds DNA at entry/exit
    - stimulates folding 10 nm 30 nm fiber
    - repressive effect on transcription
  - H1 binds weaker to acetylated nucleosomes



Nucleosomes or "Beads on a String"

- Histone proteins are responsible for the first level of DNA packing in chromatin
- The fundamental DNA packing unit

H1 and Chromatin

- Treatment of chromatin with trypsin or high salt buffer removes histone H1
- This treatment leaves chromatin looking like "beads-on-a-string"
- The beads named nucleosomes
  - Core histones form a ball with DNA wrapped around the outside
  - DNA on outside minimizes amount of DNA bending
  - H1 also lies on the outside of the nucleosome

Histone H1 and Transcription

- Histone H1 causes further repression of template activity, in addition to that of core histones
- H1 repression can be counteracted by transcription factors
- Sp1 and *GAL4* act as both:
  - Antirepressors preventing histone repressions
  - Transcription activators
- *GAGA* factor:
  - Binds to *GA*-rich sequences in the *Krüppel* promoter
  - An antirepressor – preventing repression by histones

- Interactions between histone H1 molecules appear to play an important role in this stage of chromatin condensation, which is critical to determining the accessibility of chromosomal DNA for processes such as DNA replication and transcription.

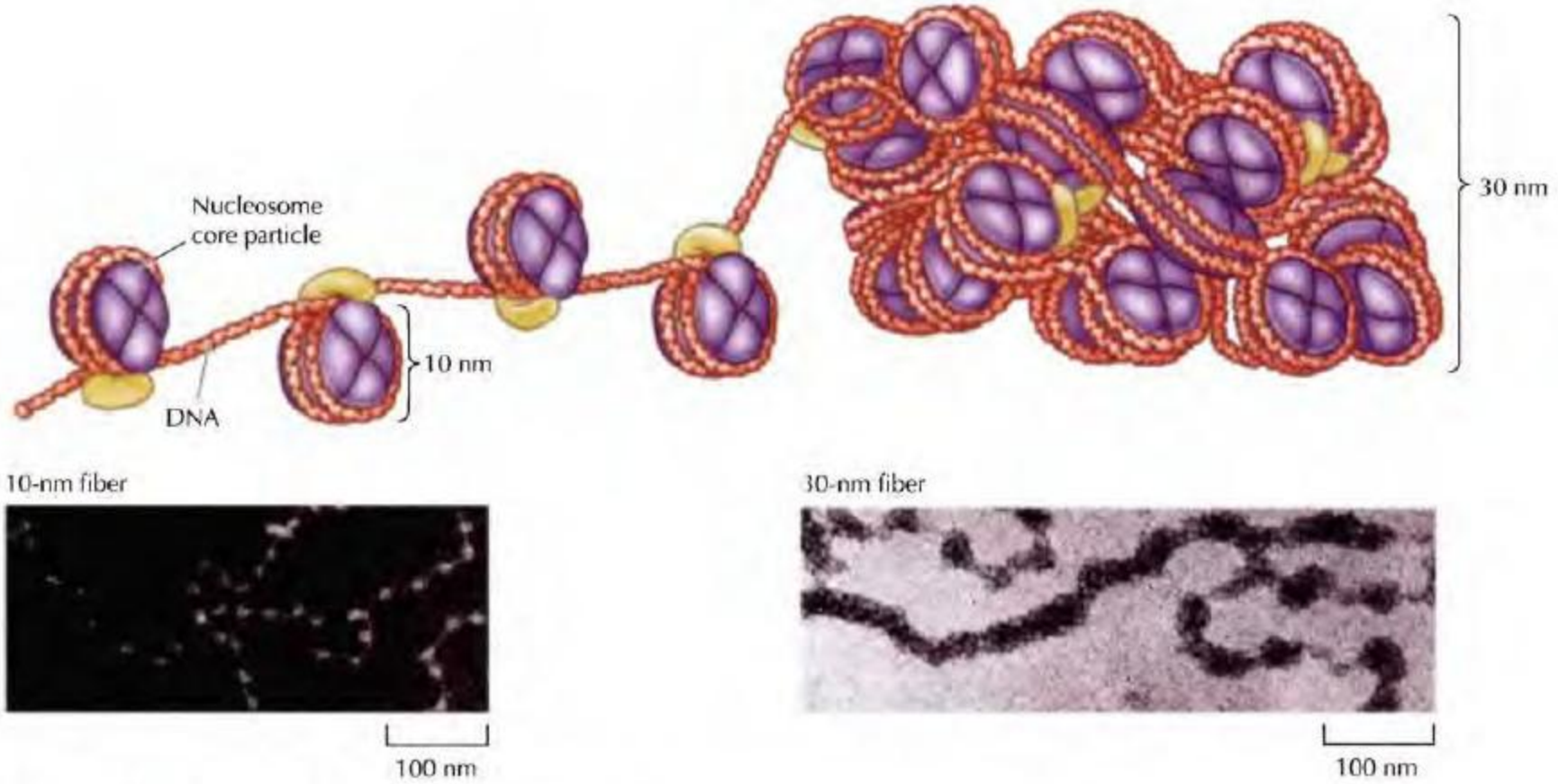
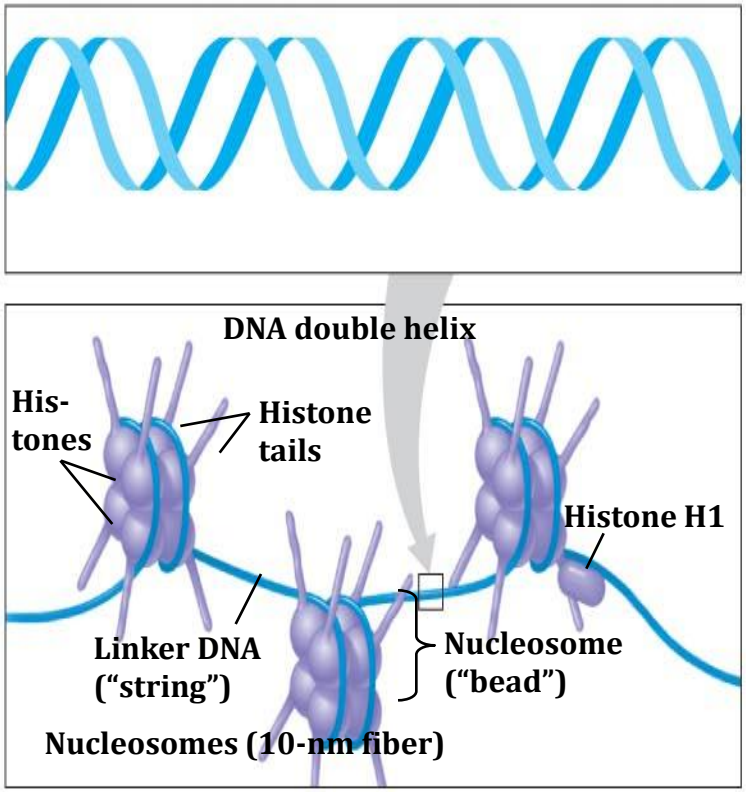
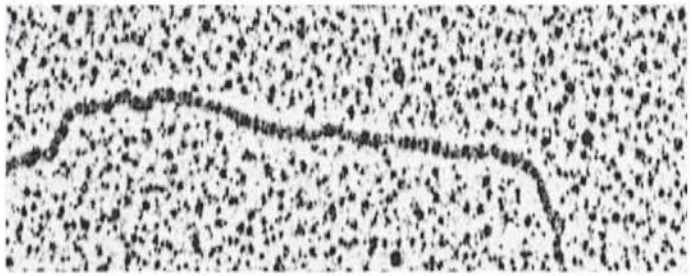
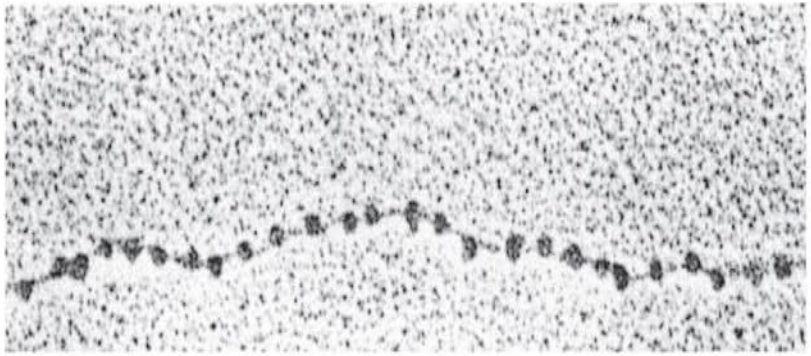


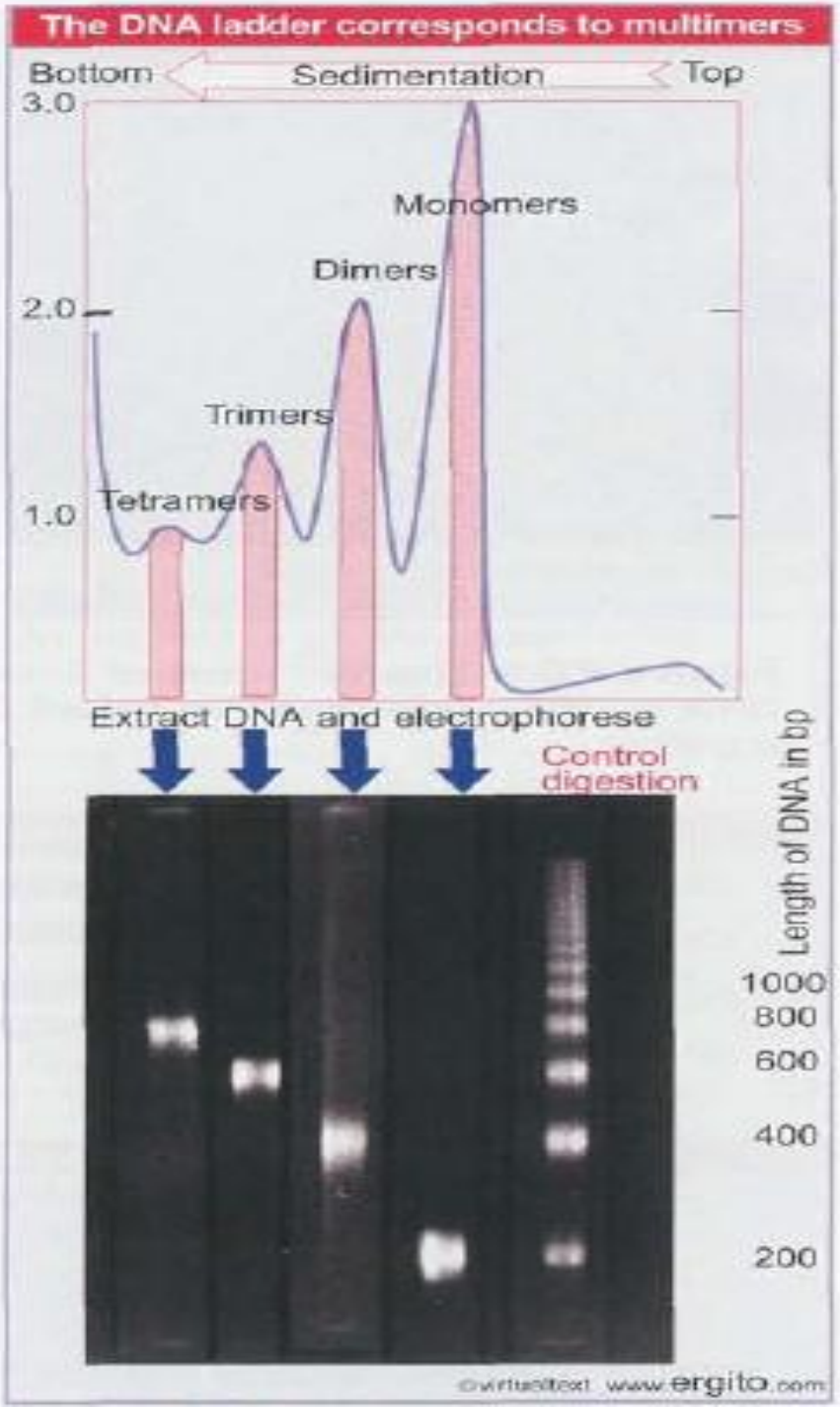
FIGURE 5.13 Chromatin fibers The packaging of DNA into nucleosomes yields a chromatin fiber approximately 10 nm in diameter. The chromatin is further condensed by coiling into a 30-nm fiber, containing about six nucleosomes per turn. (Photographs courtesy of Ada L. Olins and Donald E. Olins, Oak Ridge National Laboratory.)

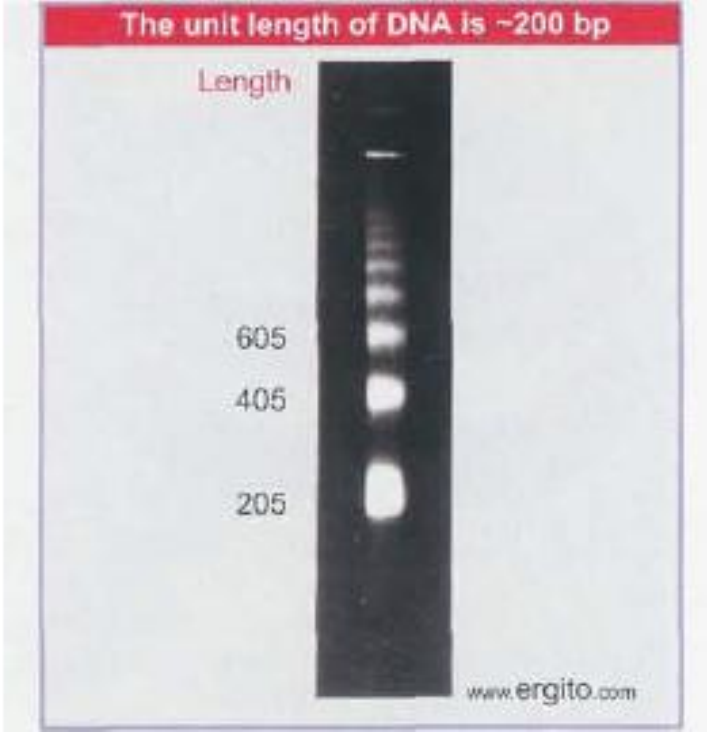






(a)





- When chromatin is digested with the enzyme micrococcal nuclease, the DNA is cleaved into integral multiples of a unit length. Fractionation by gel electrophoresis reveals the "ladder" presented in the above figure.
- When nucleosomes are fractionated on a sucrose gradient, they give a series of discrete peaks that correspond to monomers,dimers, trimers, etc. When the DNA is extracted from the individual fractions and electrophoresed, each fraction yields a band of DNA whose size corresponds with a step on the micrococcal nuclease ladder.

Gene VIII, Benzamin Lewin, Prentice Hall

Packing of DNA into Chromosomes

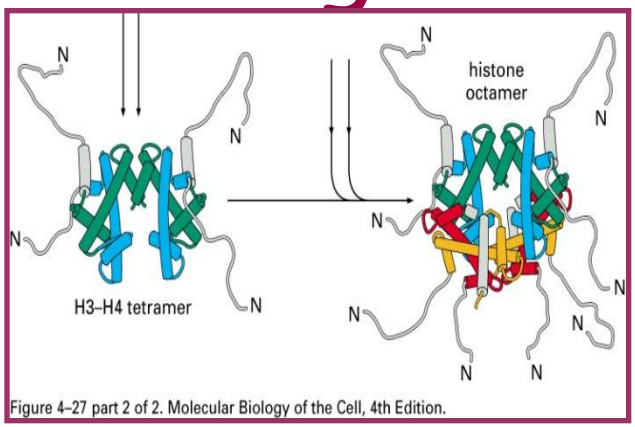


Figure 4–27 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

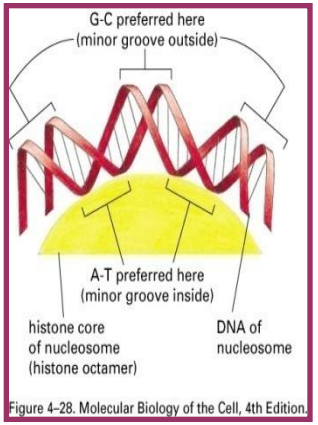


Figure 4–28. Molecular Biology of the Cell, 4th Edition.

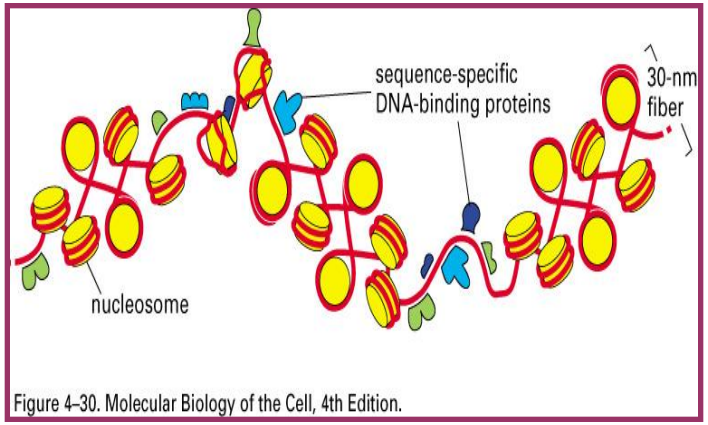
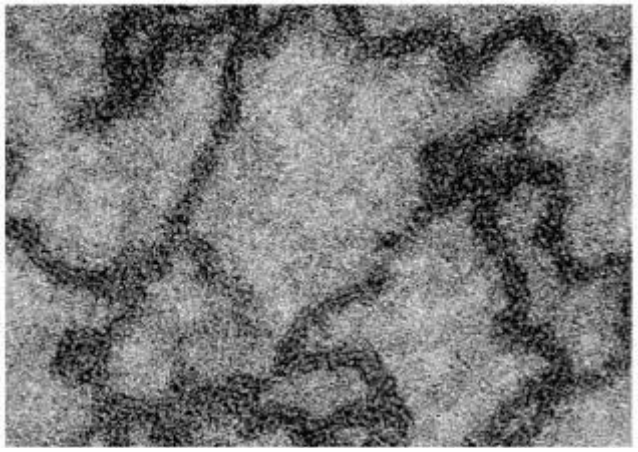


Figure 4–30. Molecular Biology of the Cell, 4th Edition.

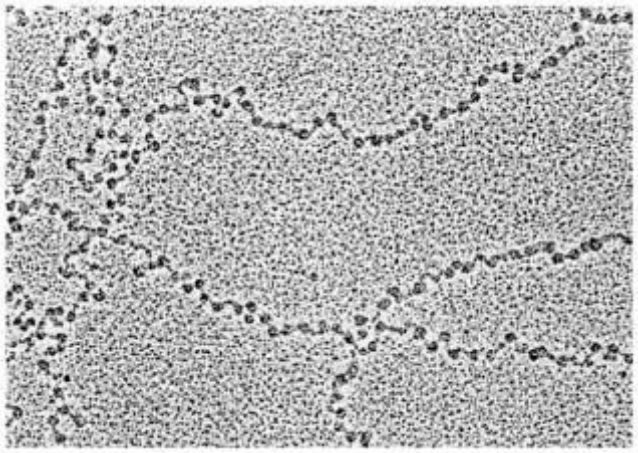
Histones as basic unit of nucleosomes

- Hydrophobic and salt linkages also involved in DNA:histone
- Covalent modifications of N-terminal tail of histones controls aspects of chromatin structure
- Positioning of nucleosomes determined by DNA flexibility and other DNA bound proteins
  - short AT rich regions impart flexibility to DNA
    - bound proteins can facilitate formation of nucleosomes or present obstacle
- Salt bridges between positively charged histones and negatively charges DNA play a major role in stabilizing DNA-histone complex

Appearance of Chromatin Depends on Salt Concentration



Physiological ionic strength
30 nm fiber



Low ionic strength
Beads on a string

from Lodish *et al.*, Molecular Cell Biology, 6th ed. Fig 6-28

- When chromatin is examined in the electron microscope, two types of fibers are seen: the 10 nm fiber and 30 nm fiber.

❑They are described by the approximate diameter of the thread (that of the 30nm fiber actually varies from ~25-30 nm).

❑The 10 nm fiber is essentially a continuous string of nucleosomes.

❑The 10 nm fibril structure is obtained under conditions of low ionic strength and does not require the presence of histone H1.

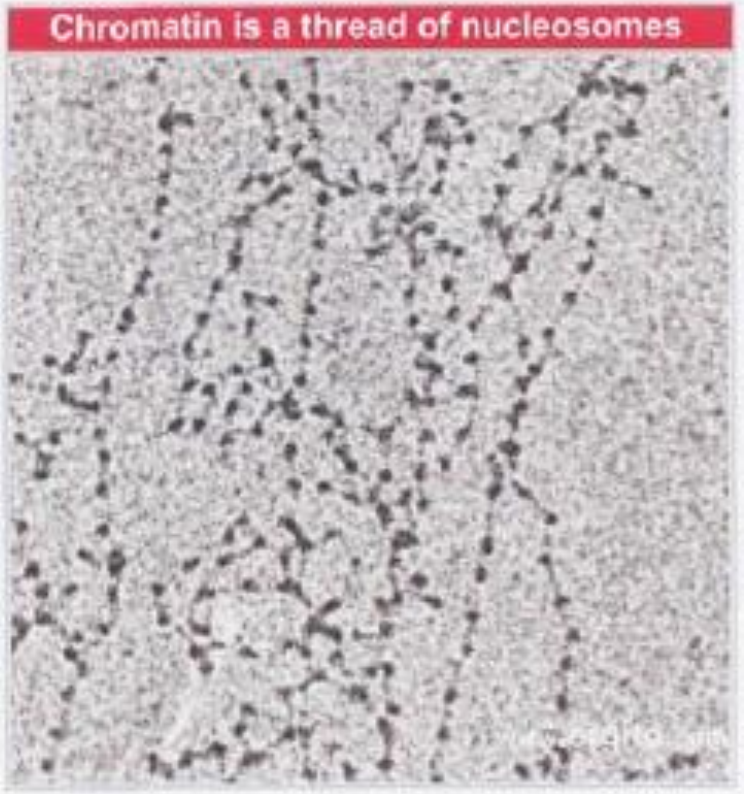


Figure 20.17 The 10 nm fiber in partially unwound state can be seen to consist of a string of nucleosomes. Photograph kindly provided by Barbara Hamkalo.

- When chromatin is visualized in conditions of greater ionic strength the 30 nm fiber is obtained.

❑The fiber can be seen to have an underlying coiled structure.
❑ It has ~6 nucleosomes for every turn, which corresponds to a packing ratio of 40 (that is, each μm along the axis of the fiber contains 40 μm of DNA).

❑The presence of H1 is required. This fiber is the basic constituent of both interphase chromatin and mitotic chromosomes.

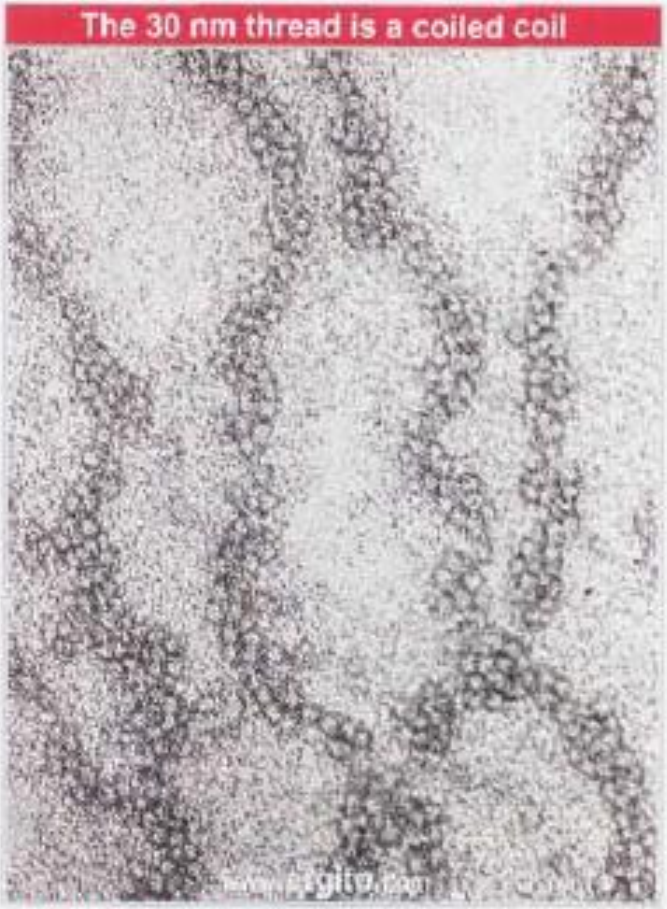
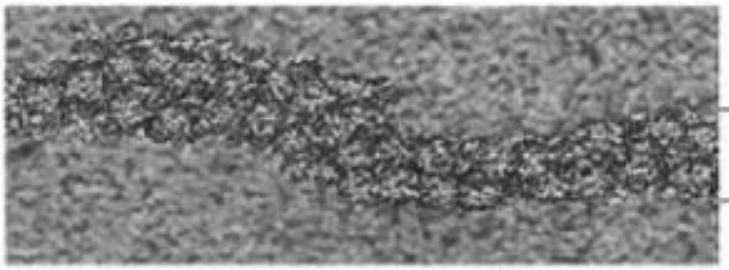
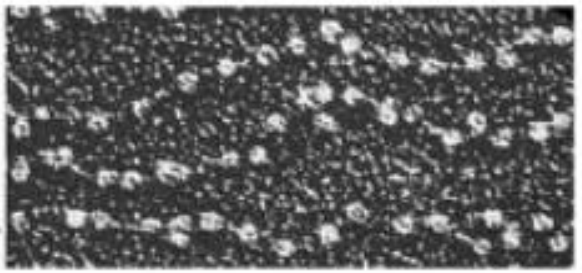


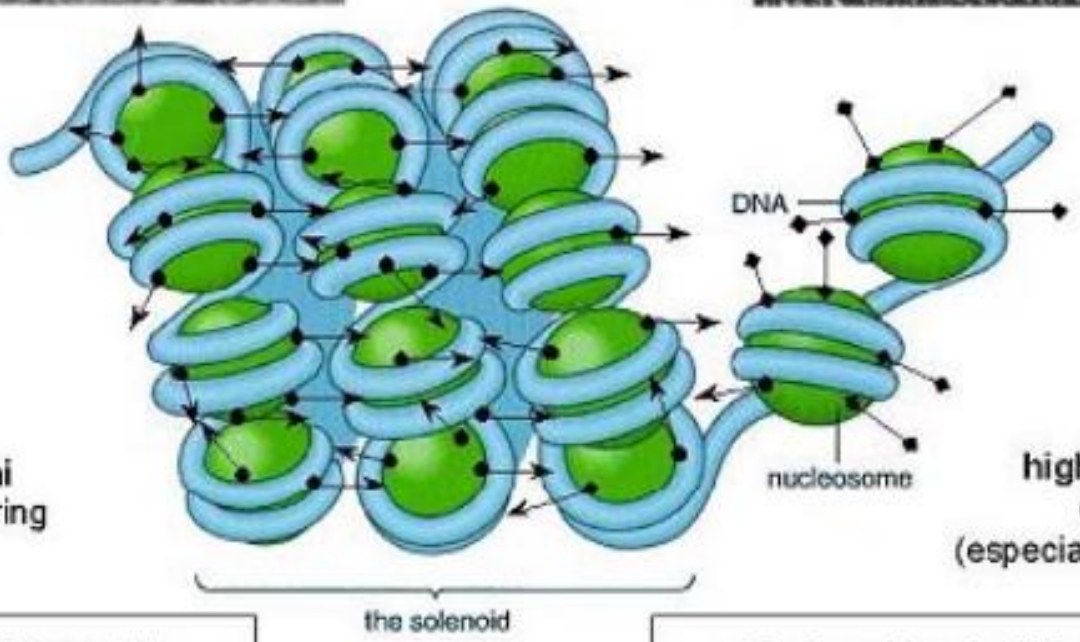
Figure 20.19 The 30 nm fiber has a coiled structure. Photograph kindly provided by Barbara Hamkalo.

Chromatin fibers





30 nm
chromatin fiber



11 nm
(beads)

(+) charged N termini
(bind DNA on neigboring nucleosomes)

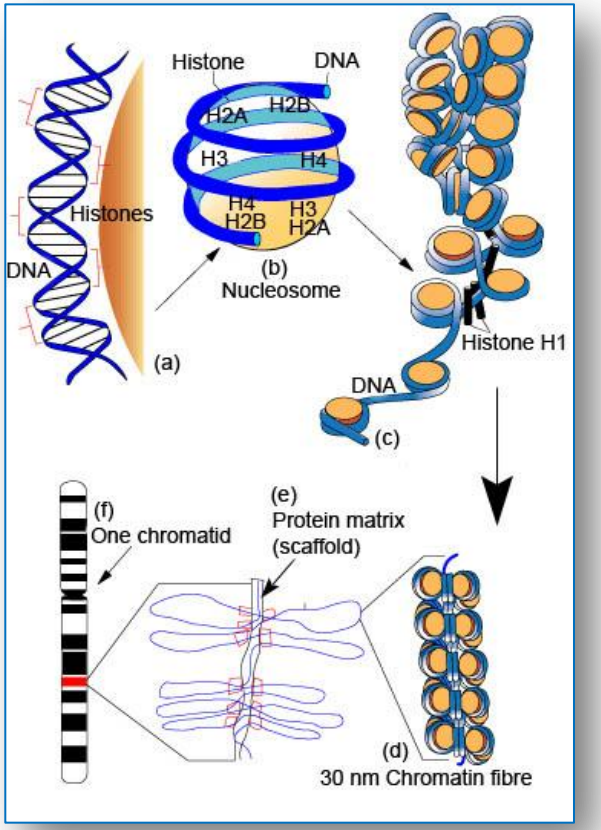
highly acetylated
core histones
(especially H3 and H4)

- HIGH level of histone H1
- NO gene transcription

- Reduced level of histone H1
- Gene transcription possible

Higher-Order Chromosome Structure Involves Loops and Coils

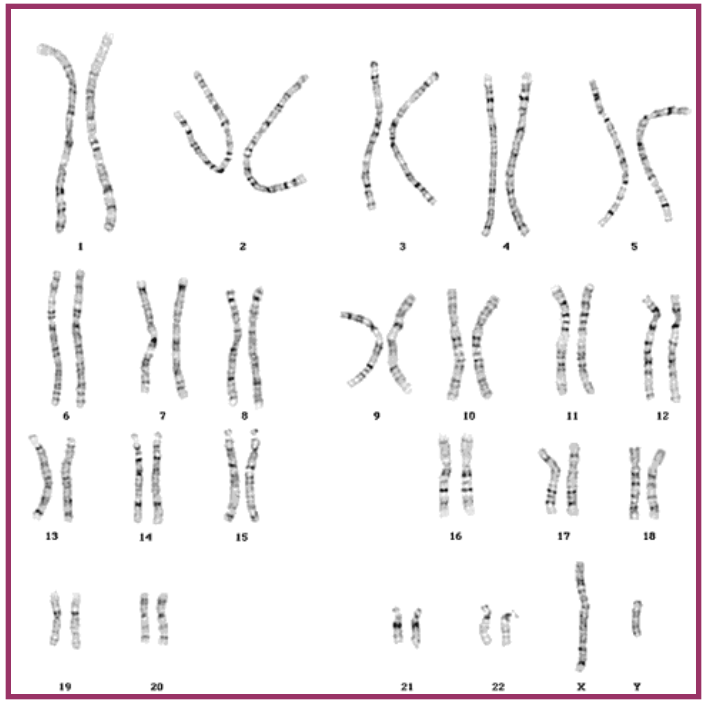
- Inside chromosomes, DNA is much more highly condensed than in the 30 nm filament.
- 30 nm filaments is appear to be organized in loops estimated at 40 to 100 kbp long.
- **chromosomal scaffold:** Proteinaceous residue after extraction of histones from chromosomes, comprised mainly of Structural maintenance of chromosomes (SMC) proteins.
- Regions of the DNA interact with chromosomal scaffold proteins to give a protein core with DNA loops sticking out of it.
- This protein core then coils up to further package the DNA into the chromatids that are visible by light microscopy in metaphase.



Packaging of DNA into Chromosomes

Eucaryotic DNA is packaged into a set of chromosomes

- DNA divided into set of chromosomes
- Chromosome= single DNA molecule and proteins associated with it
- Human DNA 3.2 x 10^9 bases distributed over 24 chromosomes



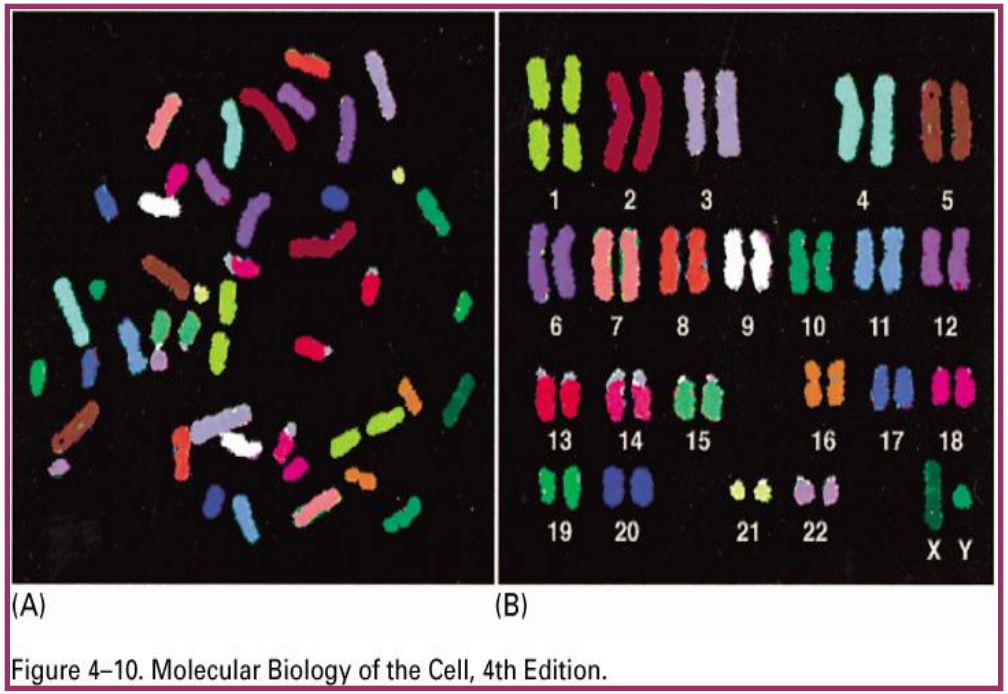
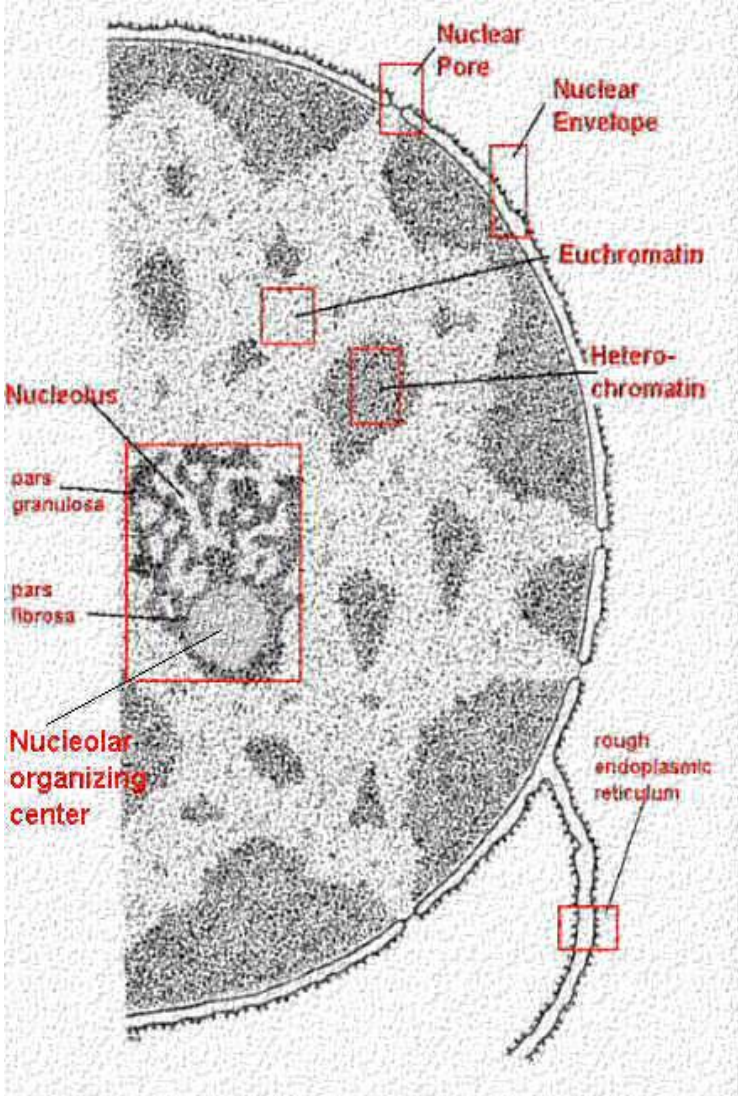


Figure 4–10. Molecular Biology of the Cell, 4th Edition.

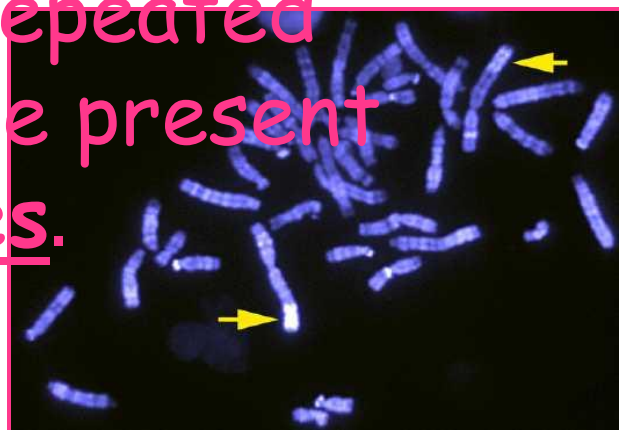
Chromatin compaction influences activity of DNA in transcription

- Some regions of chromatin are very densely packed with fibers, displaying a condition comparable to that of the chromosome at mitosis.
- **Heterochromatin ; transcriptionally silent**

- Condensed chromatin can no longer be used as a template for RNA synthesis, so transcription ceases during mitosis.



- Genes that are actively transcribed are in a more decondensed state that makes the DNA accessible to the transcription machinery.
- 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**.



Classes of heterochromatin

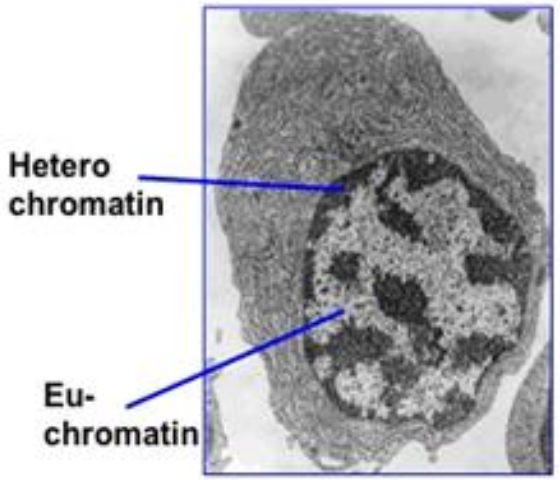
- Constitutive heterochromatin remains condensed in all cells of the organism

1. Relatively resistant to decondensation in interphase
2. Contains relatively simple, serially repeated DNA sequences (i.e. satellite DNA)
3. Found adjacent to centeromeres in most eukaryotes, and some conserved telomeric repeats

- Facultative heterochromatin is condensed only in some cells, but not in others

1. Does not contain large amounts of highly repeated DNA sequences
2. Does not stain differentially in mitotic DNA

Euchromatin



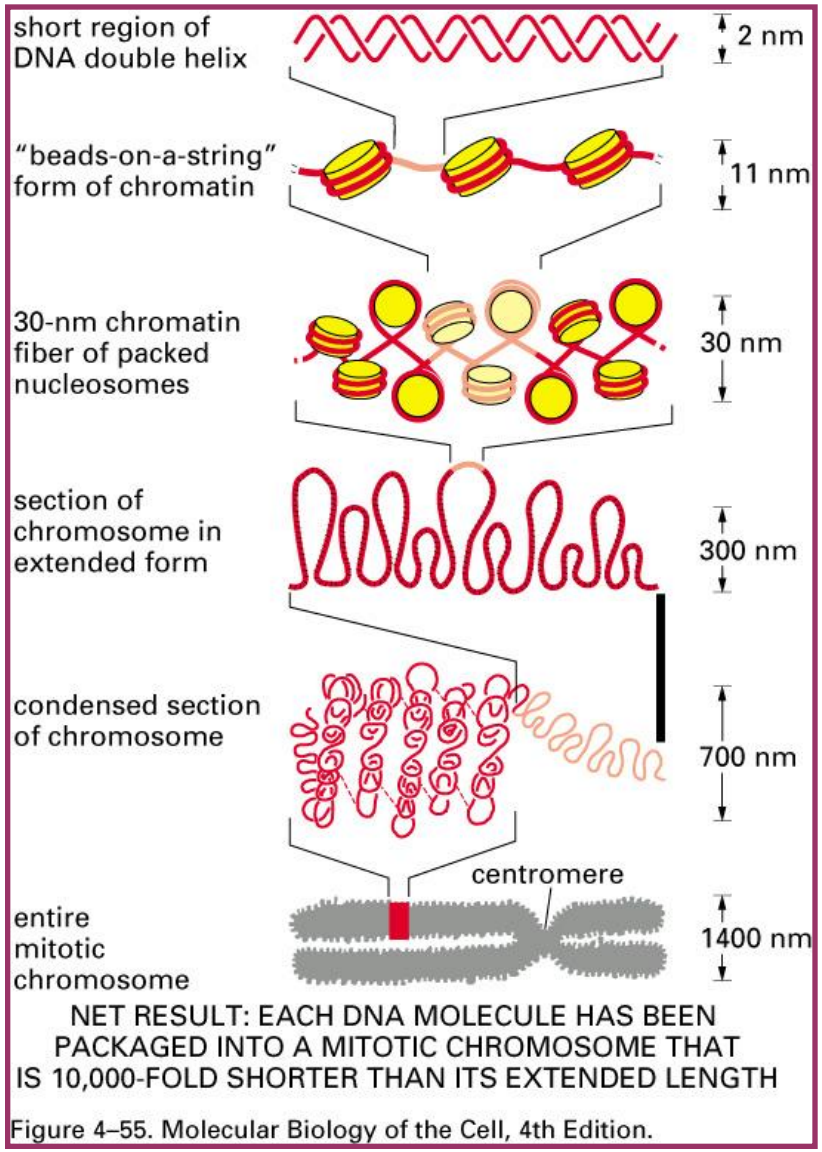
- Less condensed than in the mitotic chromosome and relatively dispersed appearance in the nucleus, and occupies most of the nuclear region
- Composed of all types of chromatin structures- 30 nm fibers, loops, etc
- 90% of chromatin
- **Euchromatin - transcriptionally active.**

- In interphase (nondividing) cells, most of the chromatin (called euchromatin) is relatively decondensed and distributed.
- During this period of the cell cycle, genes are transcribed and the DNA is throughout the nucleus . replicated in preparation for cell division.

Packing of DNA into Chromosomes -Summary-

- The nucleosome provides the first level of organization, giving a packing ratio of ~6.
- The second level of organization is the coiling of the series of nucleosomes into a helical array to constitute the fiber of diameter ~30 nm that is found in both interphase chromatin and mitotic chromosomes.
- In chromatin this brings the packing ratio of DNA to ~40. The structure of this fiber requires additional proteins, but is not well defined.
- The final packing ratio is determined by the third level of organization, the packaging of the 30 nm fiber itself. This gives an overall packing ratio of ~ 1000 in euchromatin, cyclically interchangeable with packing into mitotic chromosomes to achieve an overall ratio of ~10,000. Heterochromatin generally has a packing ratio -10,000 in both interphase and mitosis.

Packing of DNA into Chromosomes

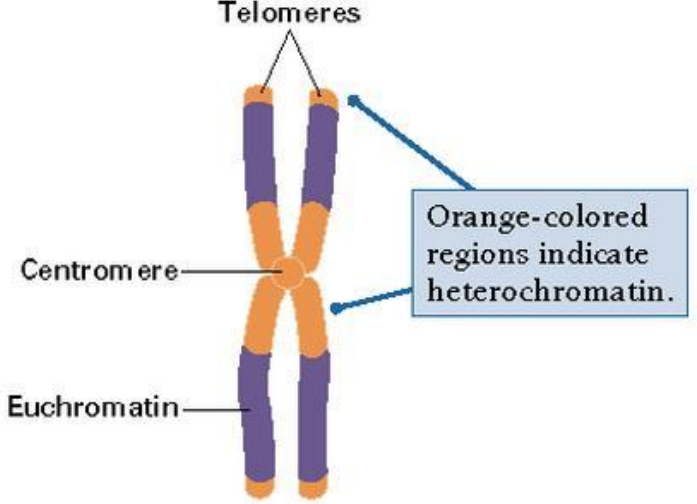


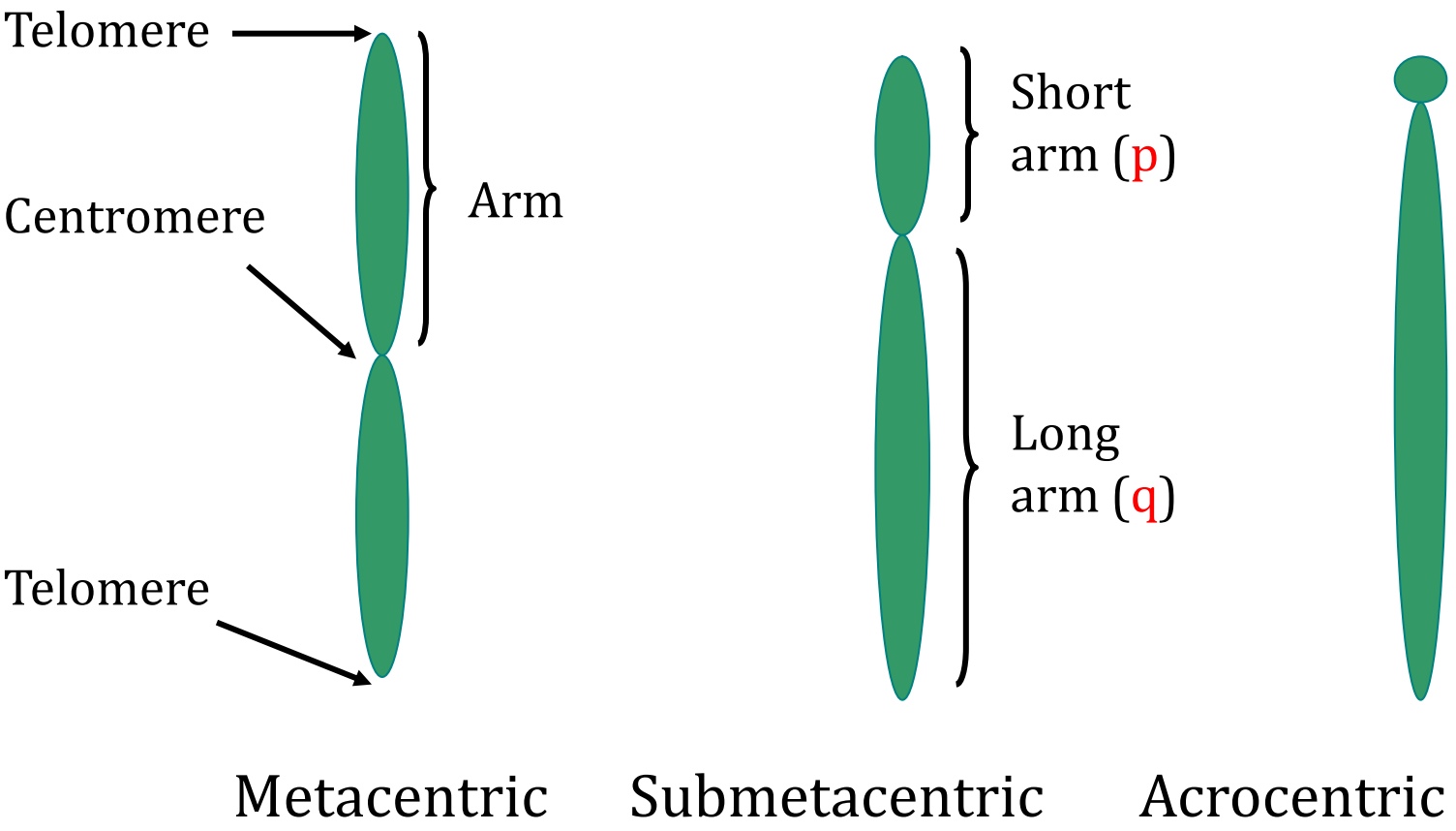
5 levels of Chromosomal Packaging

Chromatin

Condensed Chromosomes

Chromosome Morphology



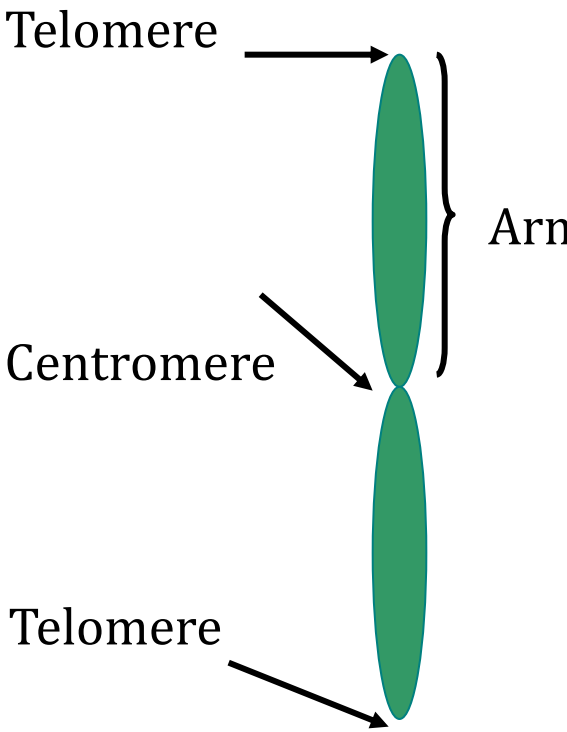


Centromeres

- 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.

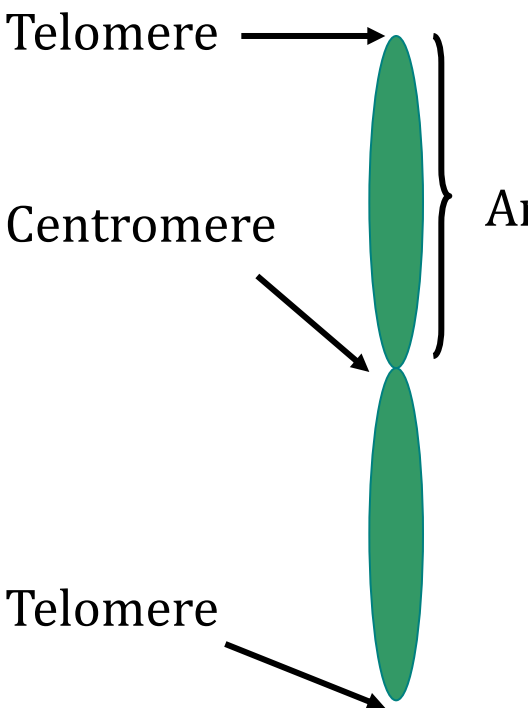
- They consist of specific DNA sequences to which a number of centromere associated proteins bind, forming a specialized structure called the kinetochore.

- 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.



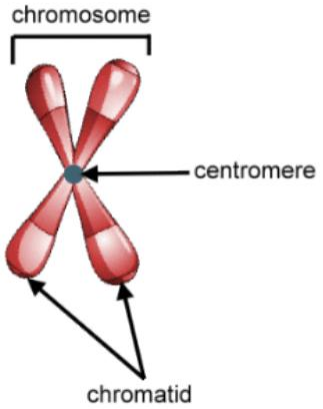
Telomeres

- The sequences at the ends of eukaryotic chromosomes, called telomeres.
- 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.



- Chromatid

- one of two parallel strands in a duplicated chromosome. "sister chromatids



- Chromosome

-Condensed parts of nucleoporin complex

-tightly wound, condensed form of DNA.

-Observed during M-phase

-The most important function of chromosomes is to carry genes- the functional units of heredity

- Chromatin

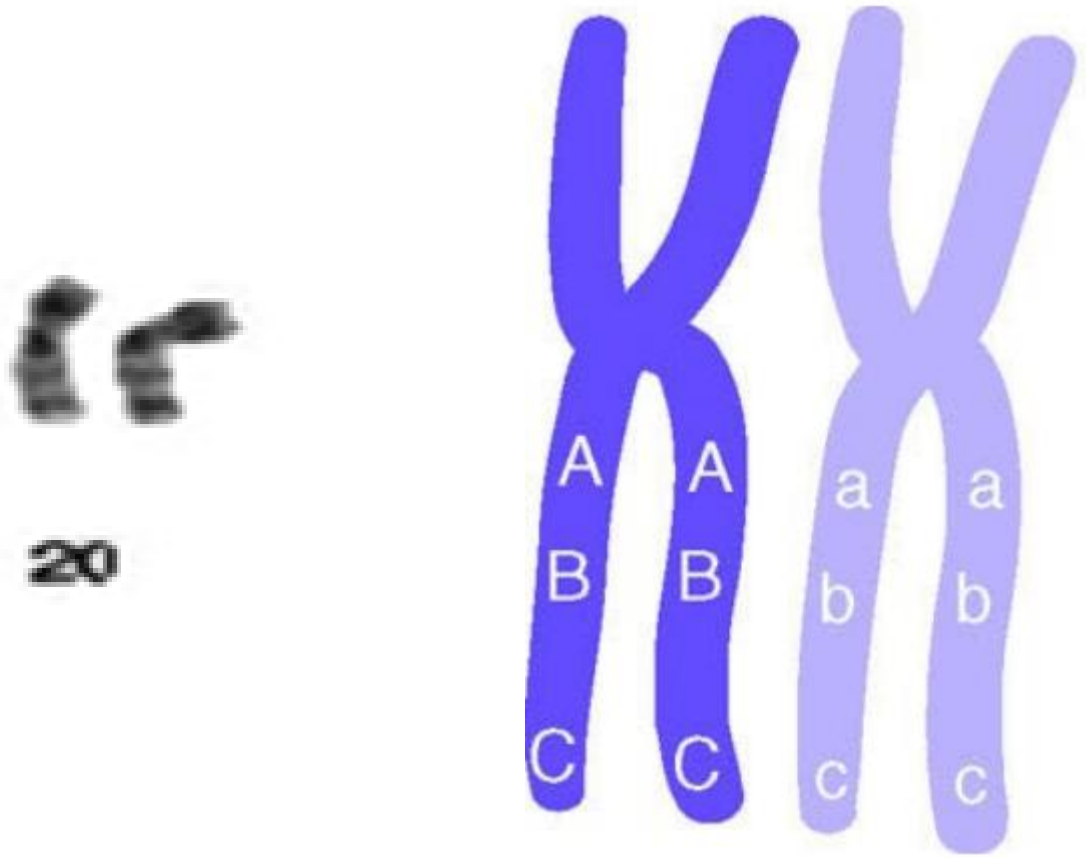
-Is the chromosomal material in its decondensed, threadlike state

-Uncondensed part od nucleoporin complex

-Chromatin observed in interphase

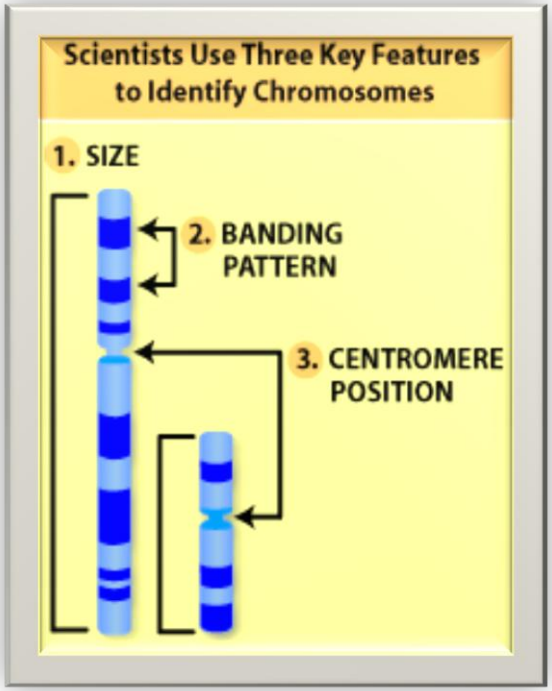
- Homologous Chromosomes

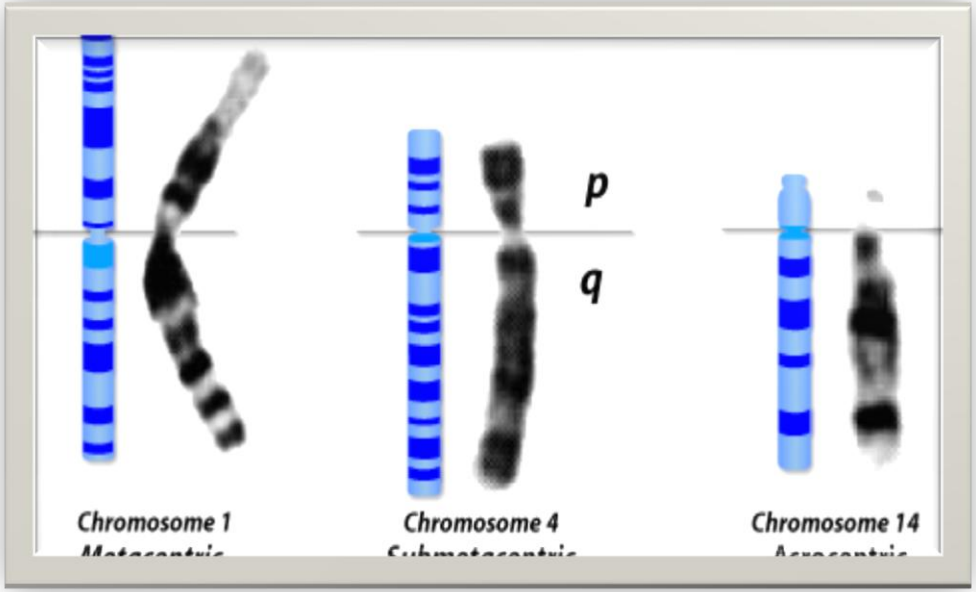
- pairs of unattached chromosomes with the same genes in the same place.



Chromosomes can be differentiated by their characteristics such as size, position of the centromere and banding pattern.

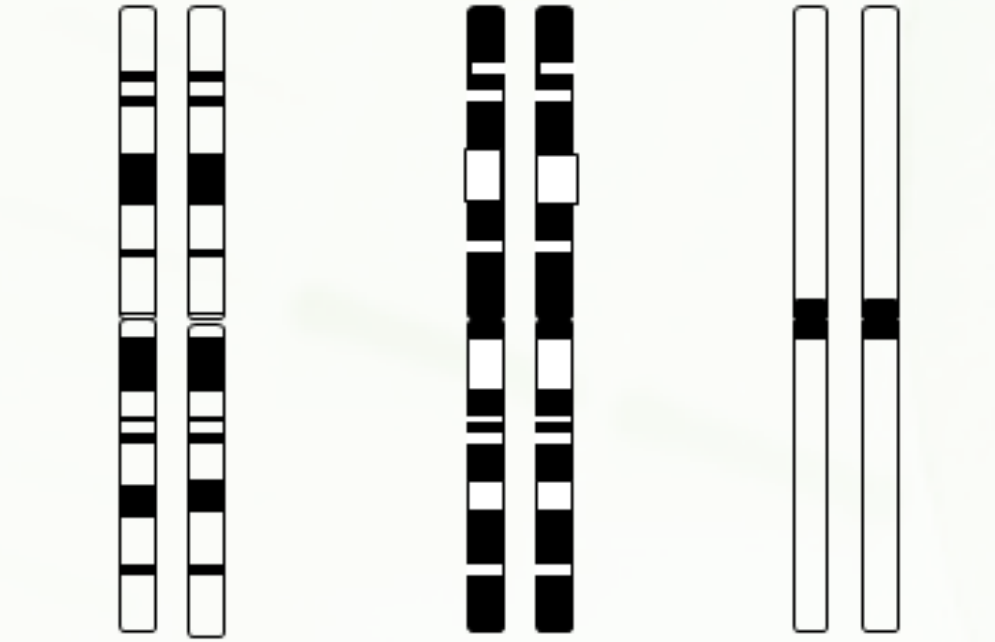
probes can be localized on a highly defined genetic map. The resolution of this map is about one chromosome band that corresponds to a size of 5-10 Mega base pairs (Mbp).





Visualizing Metaphase Chromosomes (Banding)

- Giemsa-, reverse- or centromere-stained metaphase chromosomes



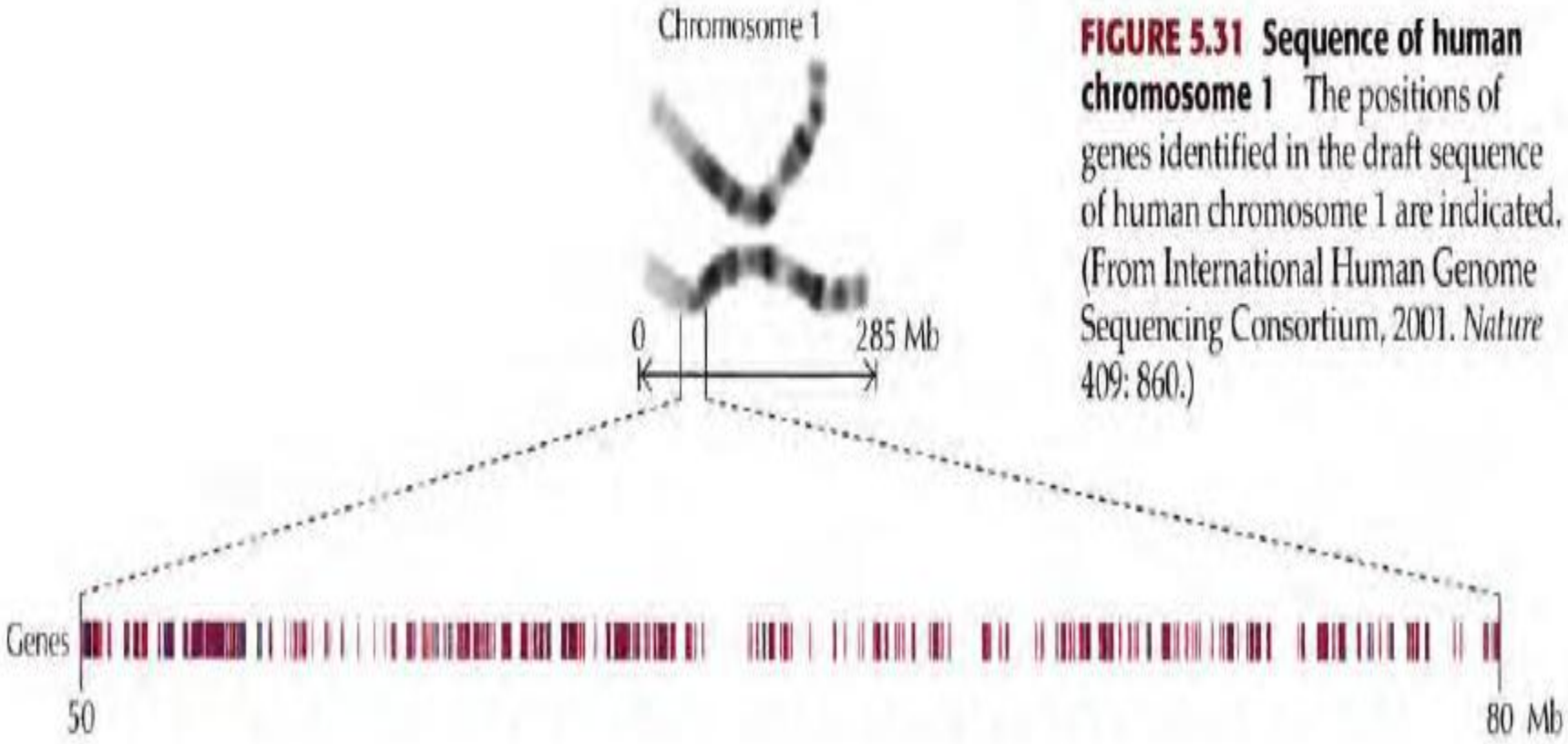
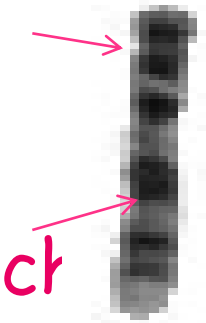


FIGURE 5.31 Sequence of human chromosome 1 The positions of genes identified in the draft sequence of human chromosome 1 are indicated. (From International Human Genome Sequencing Consortium, 2001. *Nature* 409: 860.)

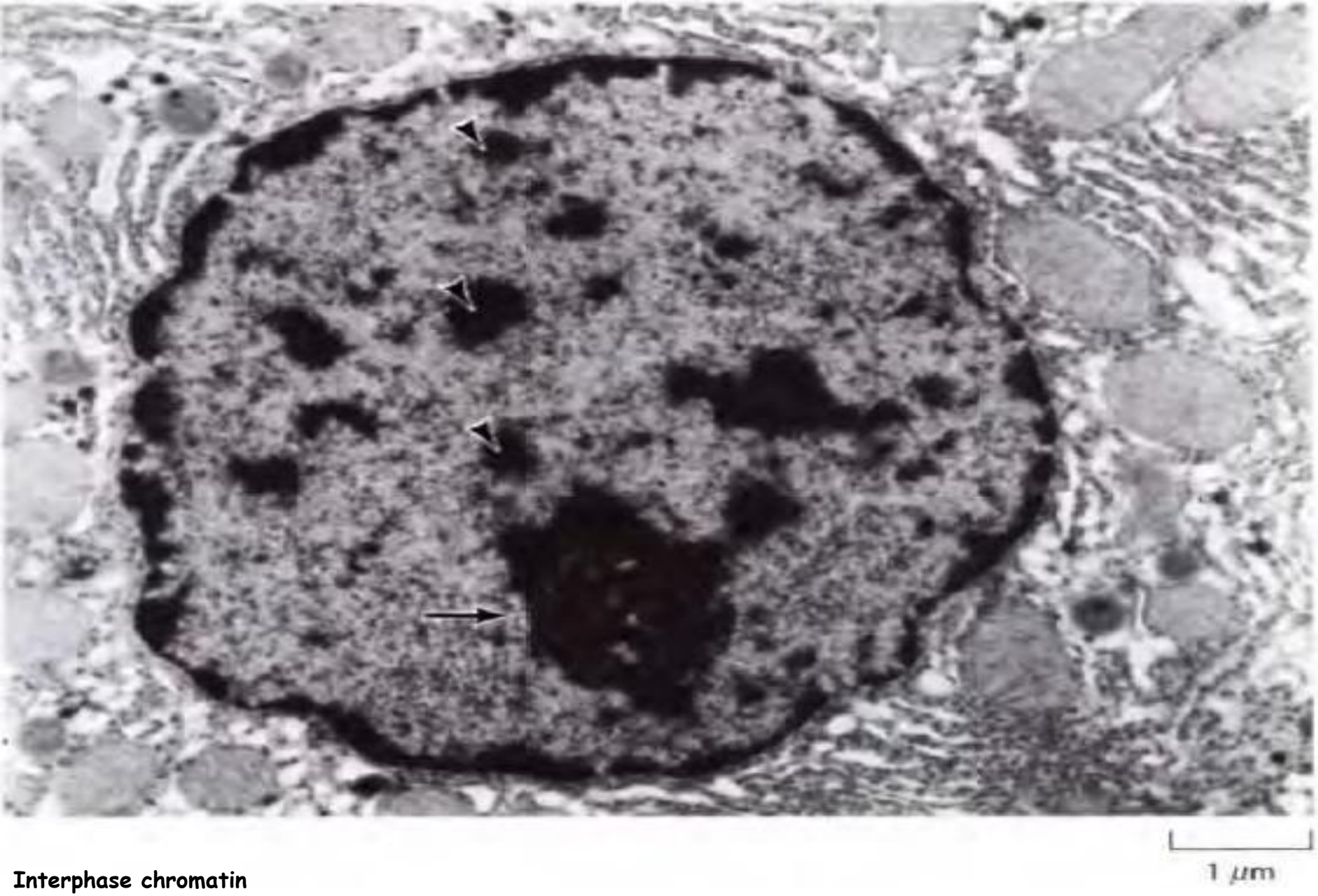
- Several staining techniques yield characteristic patterns of alternating light and dark chromosome bands, which result from the preferential binding of stains or fluorescent dyes to AT-rich versus GC-rich DNA sequences.

- These bands are specific for each chromosome and appear to represent distinct chromosome regions.

- Heterochromatic regions, which tend to be rich with adenine and thymine (AT-rich) DNA and relatively gene-poor, stain more darkly in G-banding.
- Euchromatin less condensed chromatin —which tends to be rich with guanine and cytosine (GC-rich) and more transcriptionally active— incorporates less Giemsa stain, and these regions appear as light bands in G-banding



- Genes can be localized to specific chromosome bands by *in situ* hybridization, indicating that the packaging of DNA into metaphase chromosomes is a highly ordered and reproducible process.



Interphase chromatin

Electron micrograph of an interphase nucleu::.. The euchromatin is distributed throughout the nucleus. The heterochromatin is indicated by arrowheads and the nucleolus by an arrow.

(Courtesy of Ada L. Olins and Donald E. Oli ns, Oak Ridge National Laboratory.)lpm