NAME OF TEACHER	**ROLEE SHARMA**
MOBILE NUMBER	**9336576545**
EMAIL ID	**roleesharma@csjmu.ac.in, roleesh@gmail.com**
DESIGNATION	**Professor**
UNIVERSITY NAME	**C.S.J.M. University, Kanpur**
COLLEGE NAME	**-**
STREAM NAME	**BIOLOGY**
FACULTY NAME	**SCIENCE**
DEPARTMENT NAME	**LIFE SCIENCES**
SUBJECT NAME	**CELL BIOLOGY**
COURSE	**M.Sc. LIFE SCIENCE**
COURSE DURATION	**2 Years**
SUBTOPIC NAME	**DNA Packaging**
CONTENT TYPE	**PDF**
SEARCH KEYWORDS	**Nucleosome, histones, supercoiling**

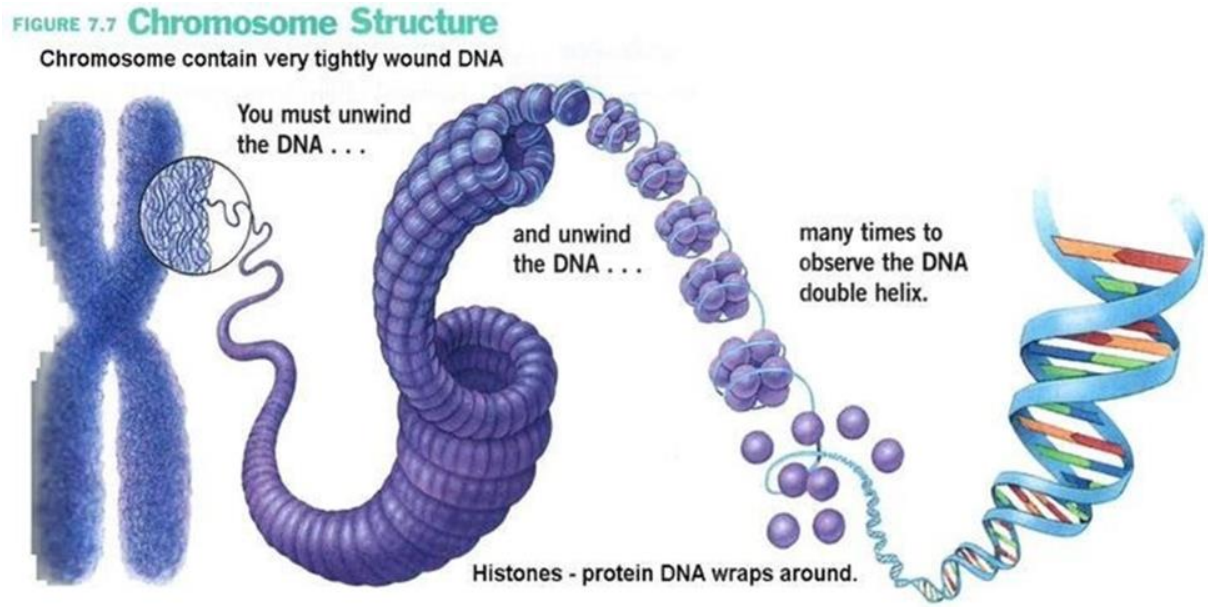
(Note: This E-Content has been prepared as an exclusive reading material for students without any commercial interest. Original contributors are gratefully acknowledged.)

DNA Packaging

Objectives:

To acquaint the students about:

i) Condensation of DNA and involvement of proteins
ii) Wrapping of DNA into nucleosomes
iii) Histone proteins and types
iv) Solenoid model
v) Basic model for chromatin structure
vi) Chromosome remodeling and
vii) Epigenetics.



Each cell within our body contains a huge amount of DNA. The different chromosomes of the human genome contain approximately 3.2×10^9 base pairs of DNA. Since we are diploid organisms, having two sets of each chromosome, the **total amount of DNA in most of our cells** totals 6.4×10^9 base pairs. At 0.33 nm per base pair, this corresponds to an **overall length of approximately 2.1 m**. How can this fit into a nucleus measuring just 5–10 µm across? The answer is that the DNA is highly compacted. It is associated with a number of proteins that results in the wrapping of DNA into **nucleosomes**.

DNA Is Organized into Chromatin in Eukaryotes

During interphase, the genetic material is relatively uncoiled (decondensed) and dispersed throughout the nucleus as **chromatin**. When mitosis begins, the chromatin condenses greatly, and during prophase it is compressed into recognizable chromosomes. As the cell cycle progresses, cells may replicate their DNA and reenter mitosis, whereupon chromatin coils and condenses back into visible chromosomes once again. Thus, now DNA is organized in eukaryotic chromosomes, which are most clearly visible as highly condensed structures during mitosis. This condensation represents a contraction in length of some 10 000-fold for each chromatin fiber.

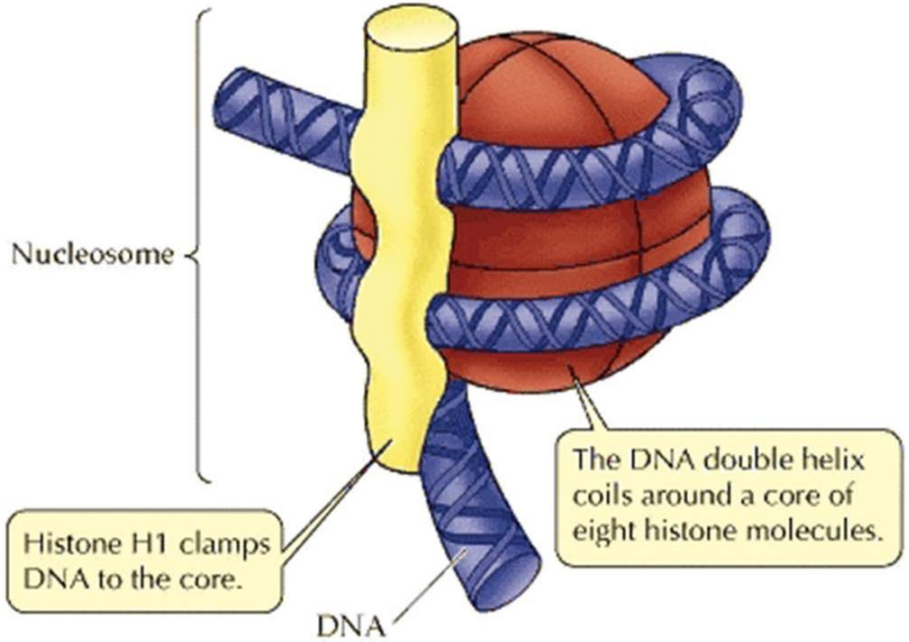
Electron microscopic observations have revealed that chromatin fibres are composed of linear arrays of spherical particles. The particles occur regularly along the axis of a chromatin strand and resemble **beads on a string**. These particles, initially referred to as ***ν*-bodies**, are now called **nucleosomes**.

The proteins associated with DNA in chromatin are divided into basic, positively charged **histones** and less positively charged **non-histones**. Of the proteins associated with DNA, the histones play the most essential structural role. Histones contain large amounts of the positively charged amino acids lysine and arginine, making it possible for them to bind through electrostatic interactions to the negatively charged phosphate groups of the DNA nucleotides.

There are **five different types of histone protein – H1, H2A, H2B, H3 and H4**.
A nucleosome core particle consists of two copies each of histones H2A, H2B, H3 and H4 to form a histone octamer around which ∼150 base pairs of DNA are wrapped in a left-handed superhelix, which completes about 1.7 turns per nucleosome.

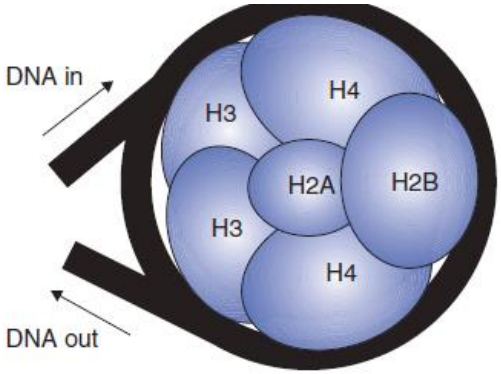
Recent insights into the structure of the nucleosome came in 1997 when Richmond and colleagues were able to solve the X-ray crystal structure of the nucleosome–DNA complex at high resolution. At this resolution, most atoms of each histone are visible, and the precise path the DNA helix as it encircles the histone octamer can be traced. The high-resolution structure also revealed that the amino-terminal ends of some of the histones protrude from the octamer and project away from the nucleosome. The significance of the amino-terminal tails is that they have the potential to interact with adjacent nucleosomes to create nucleosome–nucleosome contacts.

Extensive investigation of the structure of nucleosomes has provided basis for predicting how the chromatin fibre within nucleus is formed, and how it coils up into the mitotic chromosome. The 2 nm DNA double helix is initially coiled into a nucleosome core particle that is about 10 nm in diameter. Approximately 200 base pairs of DNA link each core particle to form the 'beads on a string' seen in electron microscopy images. Histone H1, which is not part of the core octamer, may be located at the site where DNA enters and leaves the nucleosome and possibly functions to seal the DNA around the nucleosome.



Wrapping of DNA around the nucleosome core. The nucleosome is composed of two molecules each of histones H4, H3, H2A and H2B.

In the representation shown here only a monomer of H2A and H2B can be observed, the other monomers being located at the back of the octamer.



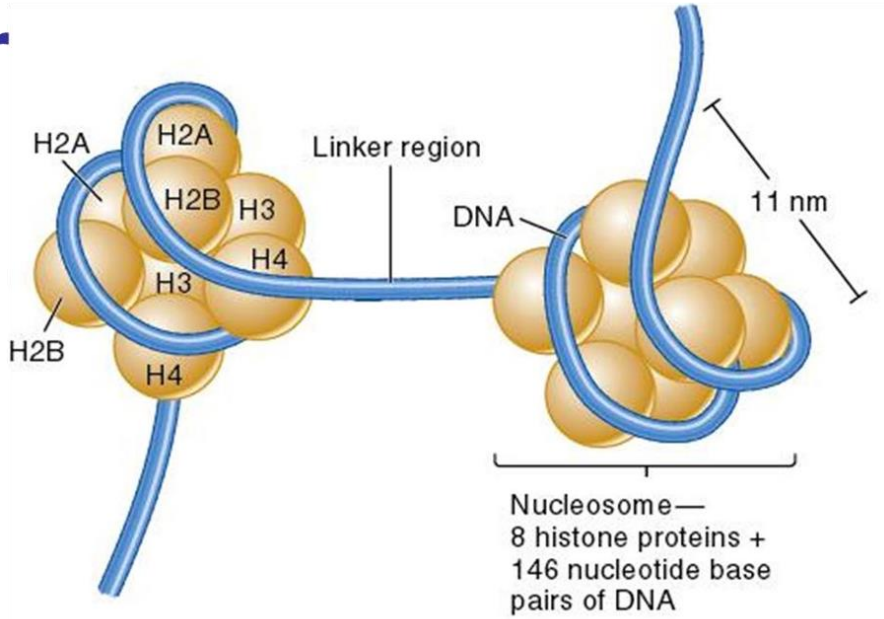
Almost 200 bp of DNA wrap around the octamer core, forming 1.75 turns.

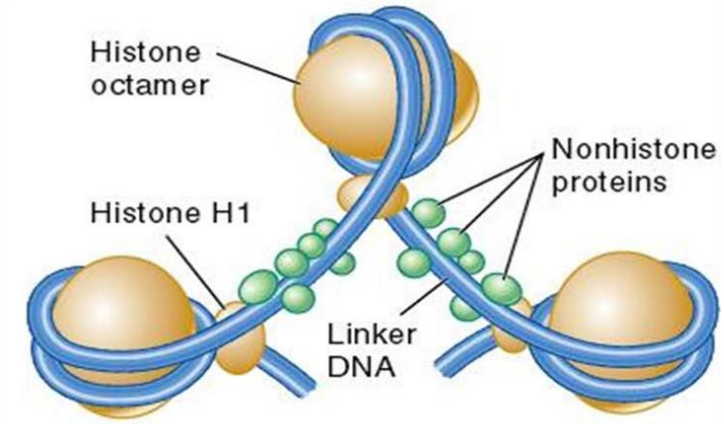
Nucleosomes – 10 nm fiber

- Histone proteins basic (+ charged lysine & arginine) amino acids that bind DNA backbone

- Four core histones in nucleosome
  - Two of each of H2A, H2B, H3 & H4

- Fifth histone, **H1** is the **linker** histone





Histone Proteins

- Histones are highly alkaline proteins found in eukaryotic cell nuclei that package and order the DNA into structural units called nucleosomes.
- They are the chief protein components of chromatin, acting as spools around which DNA winds, and play a role in gene regulation.
- Without histones, the unwound DNA in chromosomes would be very long (a length to width ratio of more than 10 million to 1 in human DNA).
- For example, each human cell has about 1.8 meters of DNA, (~6 ft) but wound on the histones it has about 90 micrometers (0.09 mm) of chromatin, which, when duplicated and condensed during mitosis, result in about 120 micrometers of chromosomes.

Chromatin Structure and Nucleosomes

The genetic material of viruses and bacteria consists of strands of DNA or RNA relatively devoid of proteins. In contrast, eukaryotic chromatin has a substantial amount of protein associated with the chromosomal DNA in all phases of the cell cycle. The associated proteins can be categorized as either positively charged **histones** or less positively charged *nonhistone proteins.*

Of these two groups, the histones play the most essential structural role. Histones contain large amounts of the positively charged amino acids lysine and arginine, making it possible for them to bond electrostatically to the negatively charged phosphate groups of nucleotides. The five main types of histones are as following:

Categories and Properties of Histone Proteins

Histone Type	Lysine-Arginine Content	Molecular Weight (Da)
H1	Lysine-rich	23,000
H2A	Slightly lysine-rich	14,000
H2B	Slightly lysine-rich	13,800
H3	Arginine-rich	15,300
H4	Arginine-rich	11,300

The model for chromatin structure is based on the assumption that chromatin fibers, composed of DNA and protein, undergo extensive coiling and folding as they are condensed within the cell nucleus. Moreover, X-ray diffraction studies confirm that histones play an important role in chromatin structure. Chromatin produces regularly spaced diffraction rings, suggesting that repeating structural units occur along the chromatin axis. If the histone molecules are chemically removed from chromatin, the regularity of this diffraction pattern is disrupted.

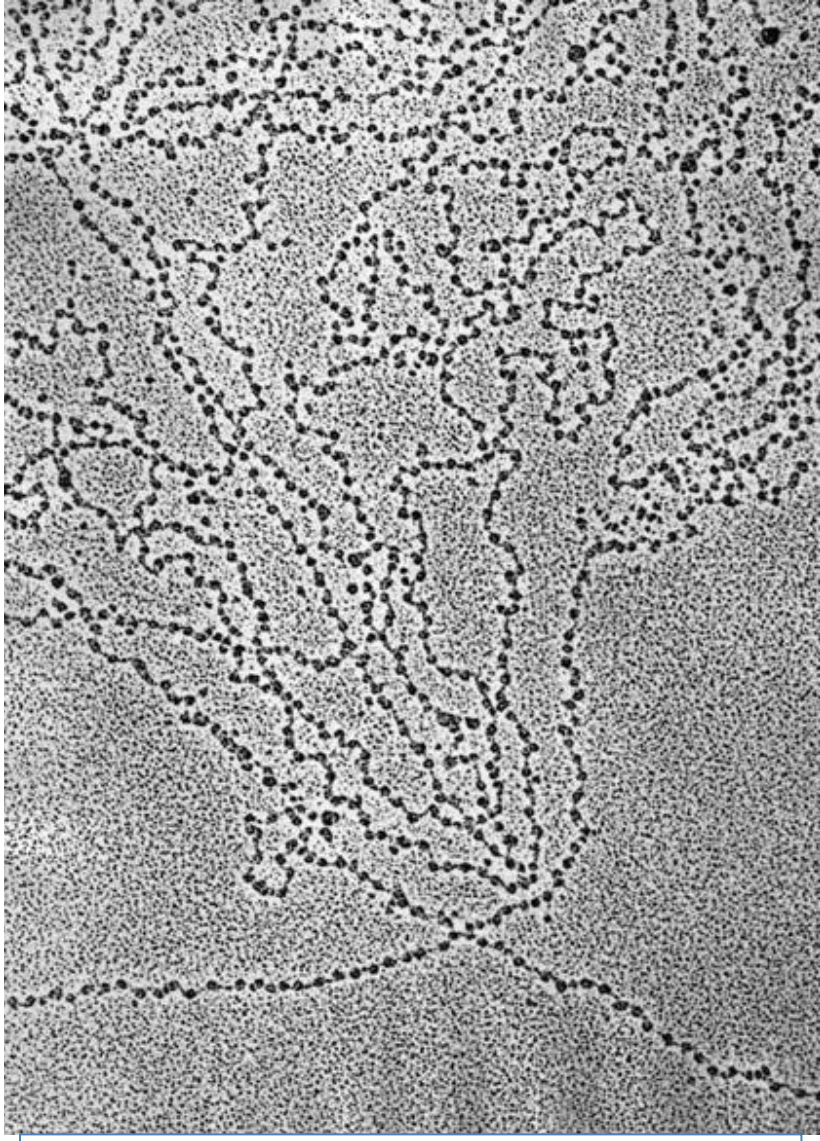
A basic model for chromatin structure was worked out in the mid-1970s. The following observations led to the development of this model:

1. Digestion of chromatin by certain endonucleases, such as micrococcal nuclease, yields DNA fragments that are approximately 200 base pairs in length or multiples thereof. This enzymatic digestion is not random, for if it were, we would expect a wide range of fragment sizes.

Thus, chromatin consists of some type of repeating unit, each of which protects the DNA from enzymatic cleavage except where any two units are joined. It is the area between units that is attacked and cleaved by the endonuclease.

2. Electron microscopic observations of chromatin have revealed that chromatin fibers are composed of linear arrays of spherical particles. Discovered by **Ada and Donald Olins**, the particles occur regularly along the axis of a chromatin strand and **resemble beads on a string**. These particles, initially referred to as *v-bodies* (Greek letter nu), are now called **nucleosomes**.

(Nucleosomes first observed as particles in EM by Don and Ada Olins in 1974).



An electron micrograph revealing nucleosomes appearing as “beads on a string” along chromatin strands derived from *Drosophila melanogaster*

3. Studies of the chemical association between histone molecules and DNA in the nucleosomes of chromatin show that histones H2A, H2B, H3, and H4 occur as two types of tetramers, $(H2A)_2$ - $(H2B)_2$ and $(H3)_2$ - $(H4)_2$.
Roger Kornberg predicted that each repeating nucleosome unit consists of one of each tetramer (creating an octomer) in association with about 200 base pairs of DNA. Such a structure is consistent with previous observations and provides the basis for a model that explains the interaction of histones and DNA in chromatin.
(Nucleosme model – as histone octamers surrounded by DNA - proposed by Kornberg)

4. When nuclease digestion time is extended, some of the 200 base pairs of DNA are removed from the nucleosome, creating what is called a **nucleosome core particle** consisting of 147 base pairs. The DNA lost in the prolonged digestion is responsible for linking nucleosomes together. This **linker DNA** is associated with the fifth histone, H1.

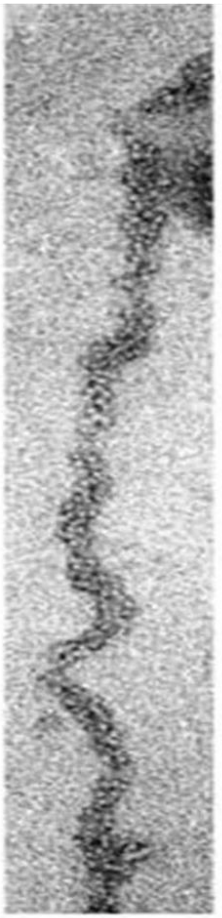
On the basis of this information, as well as on X-ray and neutron-scattering analyses of crystallized core particles by John T. Finch, Aaron Klug, and others, a detailed model of the nucleosome was put forward in 1984, providing a basis for predicting chromatin structure and its condensation into chromosomes.

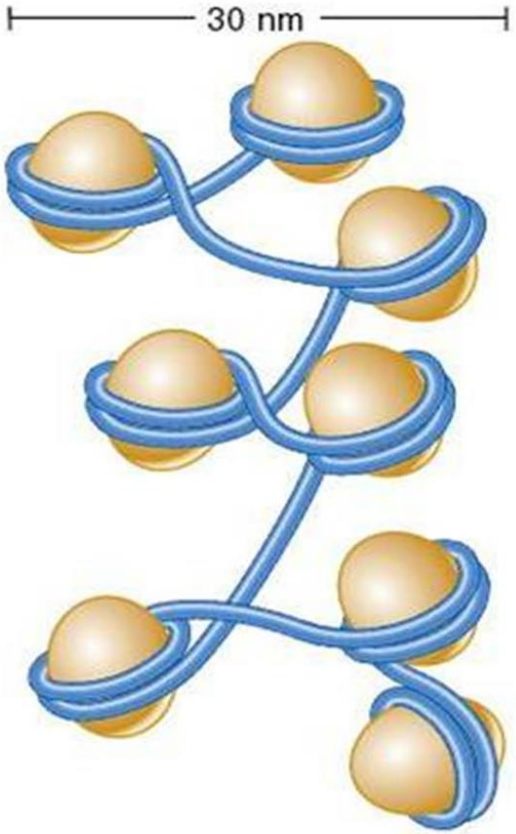
Nucleosomes join to form **30 nm fiber**

- Nucleosomes associate to form more compact structure - the **30 nm fiber**
- Histone H1 plays a role in this compaction

Further Compaction of the Chromosome

- The two events could shorten the DNA about 50-fold
- A third level of compaction involves interaction between the 30 nm fiber and the **nuclear matrix**

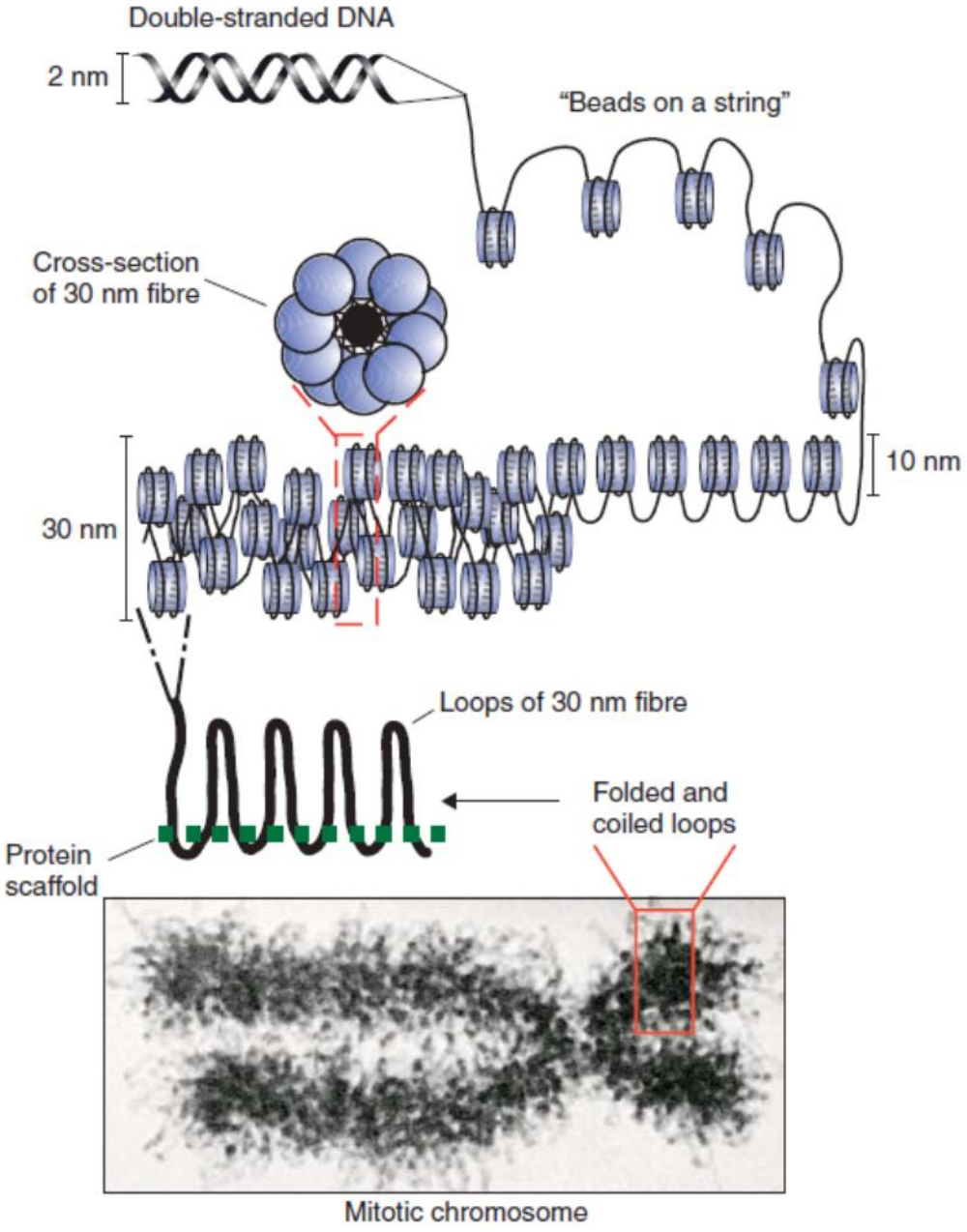




The **formation of nucleosomes represents the first level of packing**, whereby the DNA is reduced to about one-third of its original length. In the nucleus, however, chromatin does not exist in this extended form.

The **10 nm chromatin fibre** is further packed into a thicker 30 nm fibre, which was originally called a **solenoid**. The 30 nm fibre does, however, consist of numerous nucleosomes packed closely together, but the precise orientation and details of the structure are not clear. It has recently been suggested that the 30 nm fibre might adopt a compact helical zig-zag pattern with about four nucleosomes per 10 nm. The formation of the **30 nm fibre** creates a **second level of packaging**, in which the overall length of the DNA is reduced some two fold.

In the transition to the mitotic chromosome, still another level of packing occurs. The 30 nm fibre forms a series of looped domains that further condense the structure of the chromatin fibre. The ***fibres are then coiled into the chromosome arms*** that constitute a **chromatid**, which is part of the metaphase chromosome.
In the overall transition from fully extended DNA helix to the extremely condensed status of the mitotic chromosome, a packaging ratio of about 500:1 must be achieved. The model presented above only accounts for a ratio of about 50:1. The remainder of the packing arises from the coiling and folding of the 30 nm fibre. The tight packing of the DNA into a chromosome presents an enormous challenge to both the replication of DNA and to its **transcription**.



Packaging of DNA into the eukaryotic chromosome.

The 2 nm DNA double helix is wrapped into **nucleosomes**. At some point histone H1 enters the complex, possibly at the DNA entry/exit point on the nucleosome.

The nucleosomes can form extended **10 nm fibres**, which are long arrays of ordered nucleosomes.

The nucleosomes can further condense to form a **30 nm fibre (solenoid)**. This may be a 'zig-zag' array of nucleosomes.

The 30 nm fibre is then wrapped onto a **protein scaffold**, which can be additionally folded and coiled to form the **mitotic chromosome** that is observed under the electron microscope.

Three levels of chromatin organization

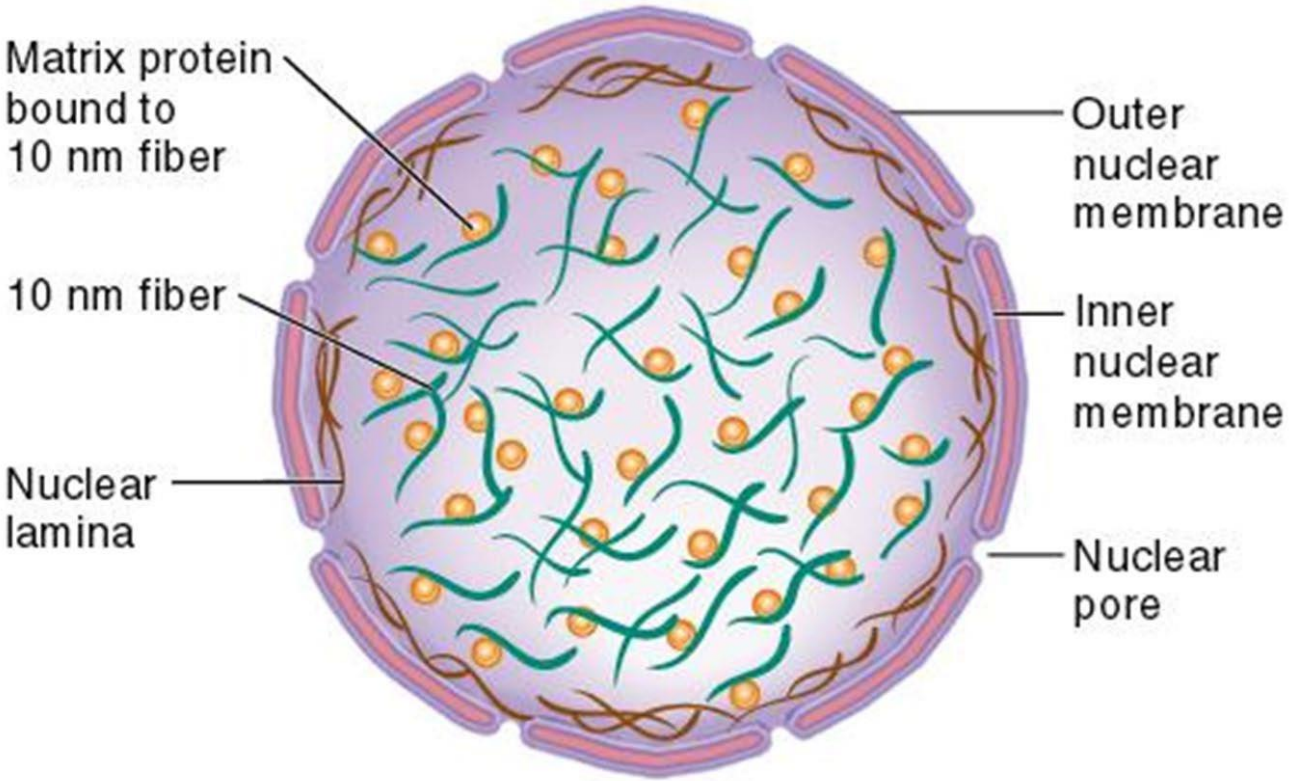
Level 1. DNA wraps around histone proteins forming **nucleosomes**: the "**beads on a string**" structure (euchromatin) **10 nm fiber**.

Level 2. Multiple histones wrap into a **30 nm fiber** consisting of nucleosome arrays in their most compact form (heterochromatin).

Level 3. Higher-level DNA packaging of the 30 nm fiber into the **metaphase chromosome** (during mitosis and meiosis).

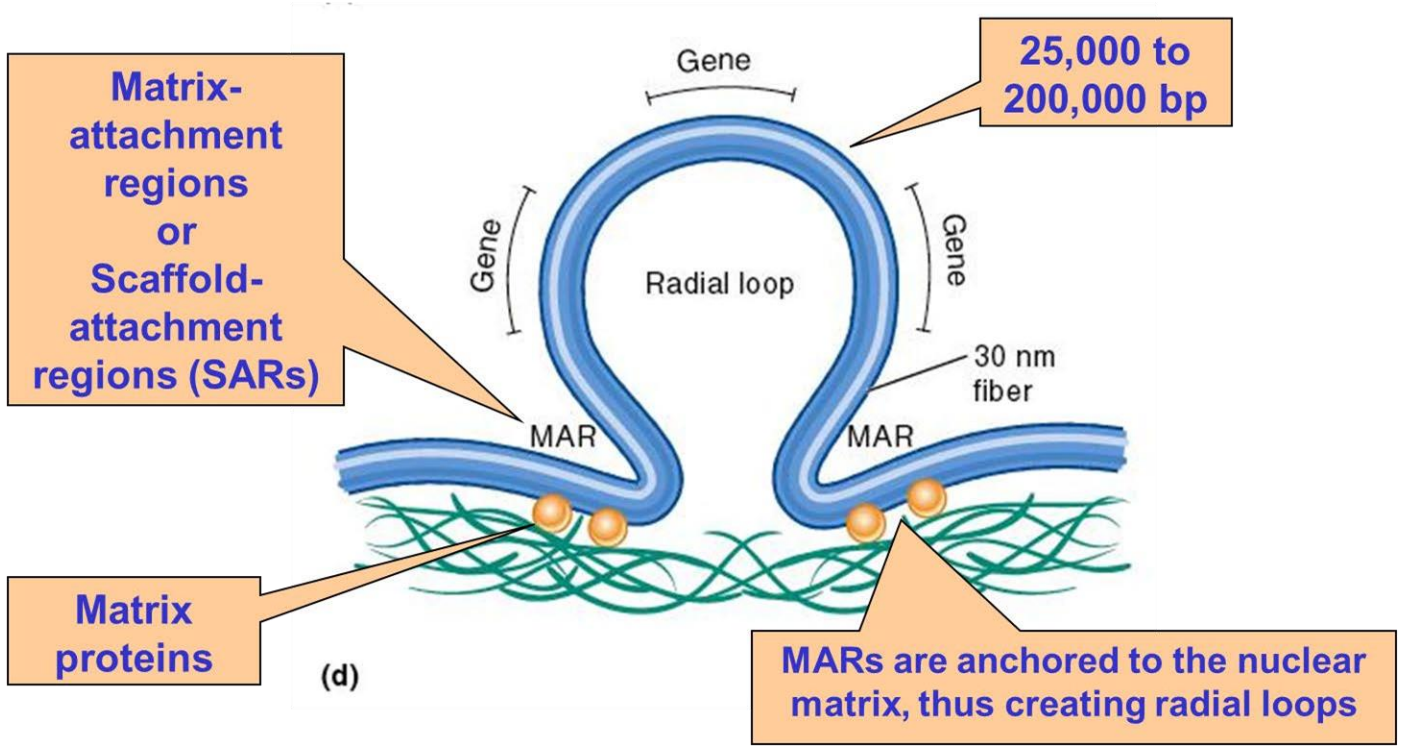
Nuclear Matrix Association

- Nuclear matrix composed of two parts
  - Nuclear lamina
  - Internal matrix proteins
    - 10 nm fiber and associated proteins



DNA Loops on Nuclear Matrix

- The third mechanism of DNA compaction involves the formation of radial loop domains

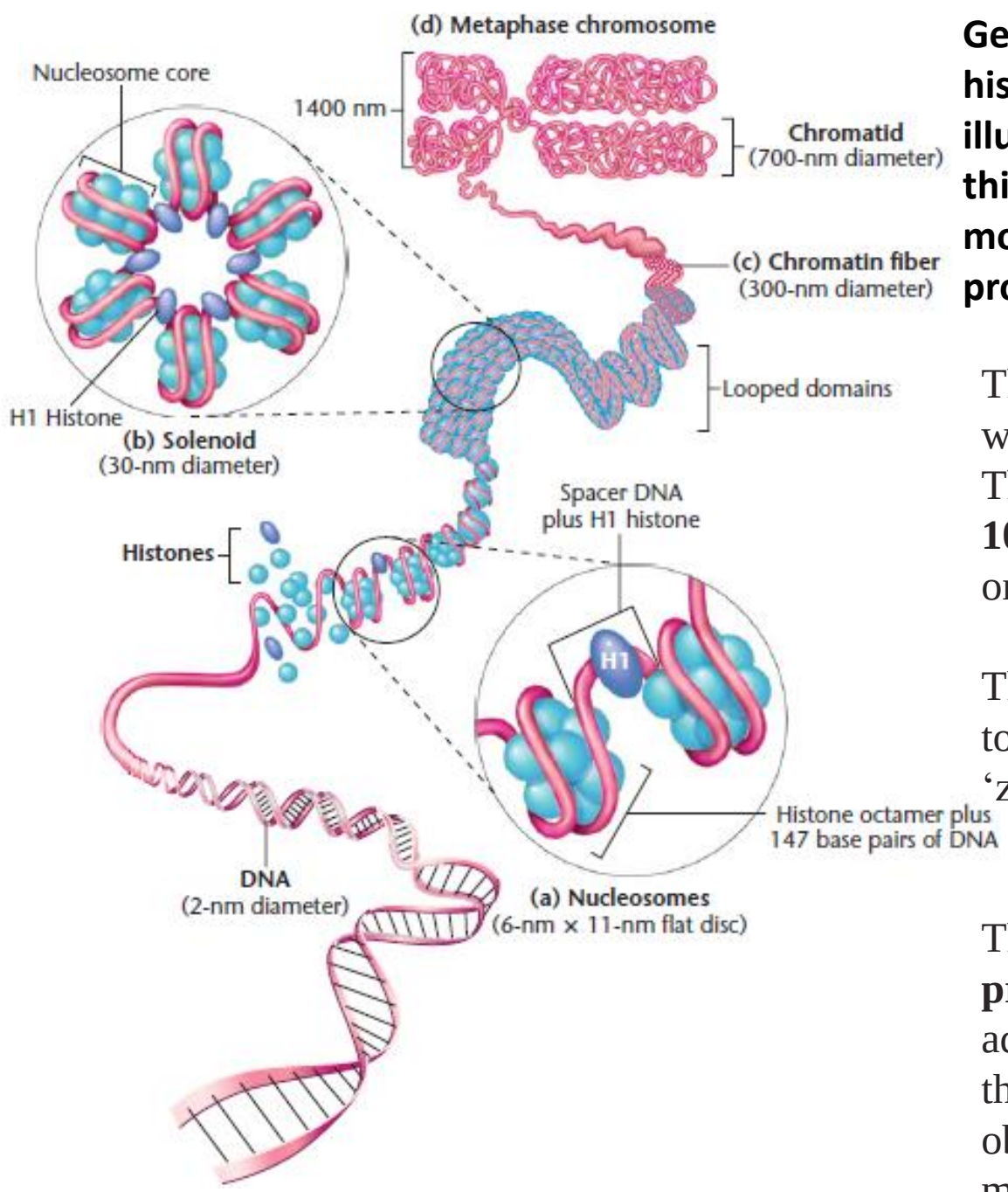


In this model, a ***147-bp*** *length* **of** the **2-nm-diameter DNA molecule** coils around an octamer of histones in a *left-handed superhelix* that completes about *1.7 turns per nucleosome*. Each nucleosome, ellipsoidal in shape, measures about **11 nm** at its longest point [Figure 12–9a]. Significantly, the formation of the **nucleosome** represents the **first level of packing**, whereby the DNA helix is reduced to about *one-third* of its original length by winding around histones.

In the nucleus, the chromatin fiber seldom, if ever, exists in the extended form that is, as an extended chain of nucleosomes. Instead, the 11-nm diameter fiber is further packed into a thicker, **30-nm-diameter** structure that was initially called a ***solenoid*** [Figure 12–9b]. This thicker structure, which is dependent on the presence of histone H1, consists of numerous nucleosomes coiled around and stacked upon one another, creating a **second level of packing**. This provides a *six-fold increase in compaction of the DNA*. It is this structure that is characteristic of an uncoiled chromatin fiber in interphase of the cell cycle. In the transition to the mitotic chromosome, still further compaction must occur.

The 30-nm structures are folded into a series of ***looped domains,*** which further condense the **chromatin fiber** into a structure that is **300 nm in diameter** [Figure 12–9c].

These *coiled chromatin fibers* are then compacted into the **chromosome arms** that constitute a **chromatid**, one of the longitudinal subunits of the metaphase chromosome [Figure 12–9d]. While Figure 12–9 shows the chromatid arms to be **700 nm in diameter**, this value undoubtedly varies among different organisms.
At a value of 700 nm, a pair of sister chromatids comprising a chromosome measures about 1400 nm.



Packaging of DNA into eukaryotic chromosome

General model of the association of histones and DNA to form nucleosomes, illustrating the way in which each thickness of fiber may be coiled into a more condensed structure, ultimately producing a metaphase chromosome.

The 2 nm DNA double helix is wrapped into **nucleosomes**.
The nucleosomes can form extended **10 nm fibres**, which are long arrays of ordered nucleosomes.

The nucleosomes can further condense to form a **30 nm fibre**. This may be a 'zig-zag' array of nucleosomes.

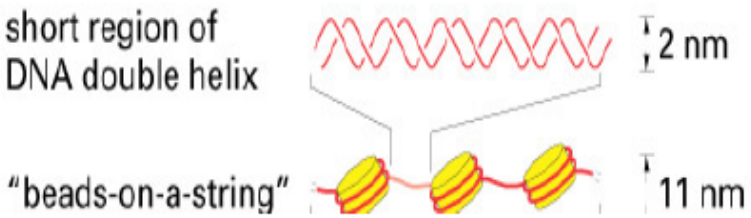
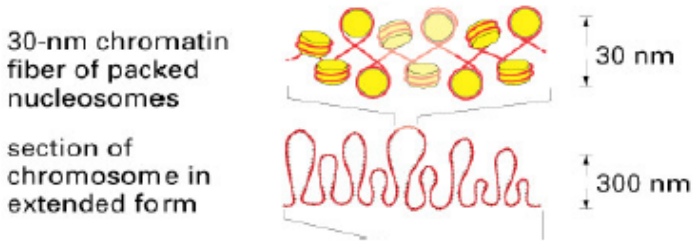
The 30 nm fibre is then wrapped onto a **protein scaffold**, which can be additionally *folded and coiled* to form the **mitotic chromosome** that is observed under the electron microscope.

The importance of the organization of DNA into chromatin and of chromatin into mitotic chromosomes can be illustrated by considering that a human cell stores its genetic material in a nucleus about 5 to 10 mm in diameter. The haploid genome contains more than 3 billion base pairs of DNA distributed among 23 chromosomes. The diploid cell contains twice that amount. At 0.34 nm per base pair, this amounts to an enormous length of DNA (as stated earlier, almost 2 meters)!

One estimate is that the DNA inside a typical human nucleus is complexed with roughly 25 * 10^6 nucleosomes.

In the overall transition from a fully extended DNA helix to the extremely condensed status of the mitotic chromosome, a packing ratio (the ratio of DNA length to the length of the structure containing it) of about 500 to 1 must be achieved. In fact, our model accounts for a ratio of only about 50 to 1. Obviously, the larger fiber can be further bent, coiled, and packed to achieve even greater condensation during the formation of a mitotic chromosome.

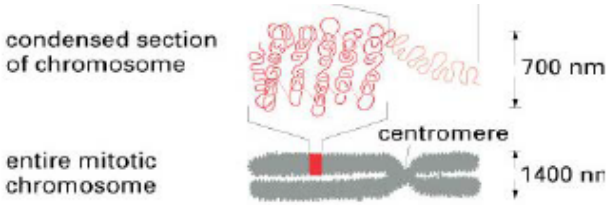
Table 2: The hierarchies of chromosomal organization

Levels	Summary	Schematic representation
The first level of packing	Winding of DNA around a protein core to produce a "bead-like" structure called a **nucleosome**. This gives a packing ratio of about 6. This structure is invariant in both the euchromatin and heterochromatin of all chromosomes. The protein core is composed of 8 histone proteins, two each of H2A, H2B, H3 and H4. Histone H1 forms the linker between to nucleosomes. 146 bp of DNA is wrapped around each nucleosome.	
The second level of packing	Coiling of beads in a helical structure called the **30 nm fiber** that is found in both interphase chromatin and mitotic chromosomes. This structure increases the packing ratio to about 40. This appears to be a solenoid structure with about 6 nucleosomes per turn. This gives a packing ratio of 40, which means that every 1 μm along the axis contains 40 μm of DNA. The stability of this structure requires the presence of the last member of the histone gene family, histone H1. Because experiments that strip H1 from chromatin maintain the nucleosome, but not the 30 nm structure, it was concluded that H1 is important for the stabilization of the 30 nm structure.	

The final level

The fiber is organized in loops, scaffolds and domains that give a final packing ratio of about 1000 in interphase chromosomes and about 10,000 in mitotic chromosomes.

The final level of packaging is characterized by the 700 nm structure seen in the metaphase chromosome. The condensed piece of chromatin has a characteristic scaffolding structure that can be detected in metaphase chromosomes. This appears to be the result of extensive looping of the DNA in the chromosome.



NET RESULT: EACH DNA MOLECULE HAS BEEN PACKAGED INT
CHROMOSOME THAT IS 10,000-FOLD SHORTER THAN ITS EXTEN

Further Compaction of the Chromosome

Heterochromatin *vs* Euchromatin

Compaction level of interphase chromosomes is not uniform

- Euchromatin
  - Less condensed regions of chromosomes
  - Transcriptionally active
  - Regions where 30 nm fiber forms radial loop domains

- Heterochromatin
  - Tightly compacted regions of chromosomes
  - Transcriptionally inactive (in general)
  - Radial loop domains compacted even further

Feulgen stain: a staining technique discovered by Robert Feulgen

- used to identify chromosomal material or DNA in cell specimens
- **Higher stain – heterochromatin**

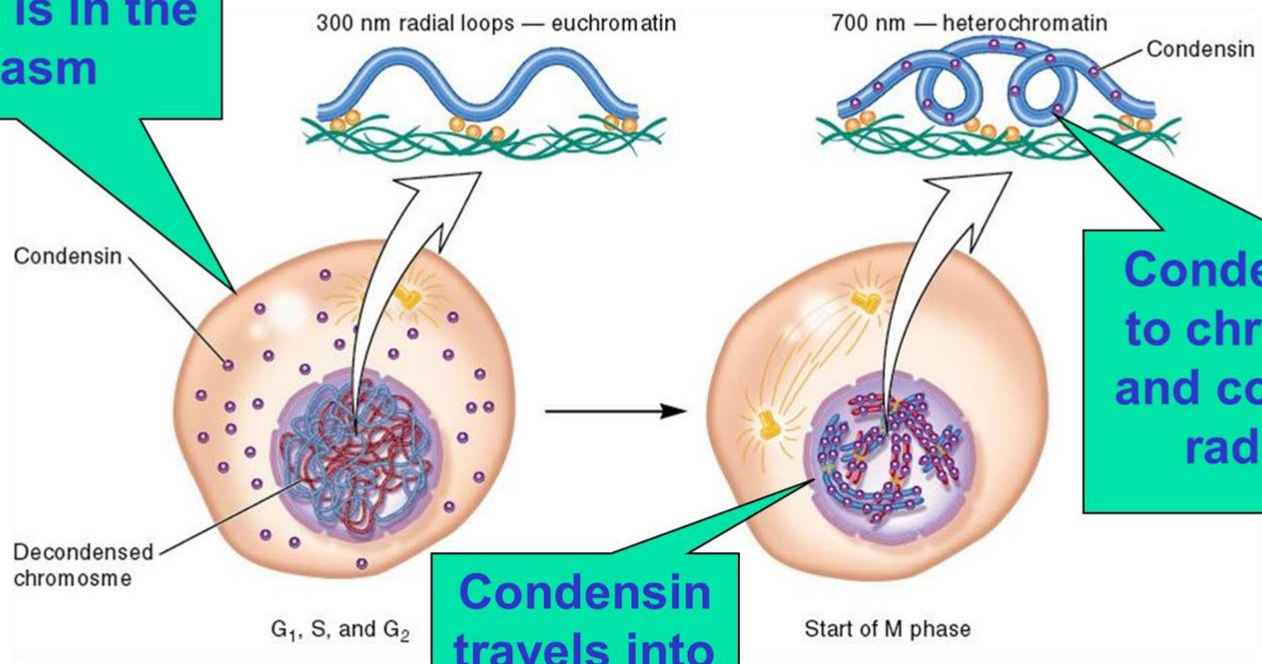
Metaphase Chromosomes

- Condensed chromosomes are referred to as metaphase chromosomes
- During prophase, the compaction level increases
- By the end of prophase, sister chromatids are entirely heterochromatic
- These highly condensed metaphase chromosomes undergo little gene transcription
- In metaphase chromosomes, the radial loops are compacted and anchored to the nuclear matrix scaffold

Chromosome Condensation

During interphase, condensin is in the cytoplasm

The number of loops has not changed
However, the diameter of each loop is smaller

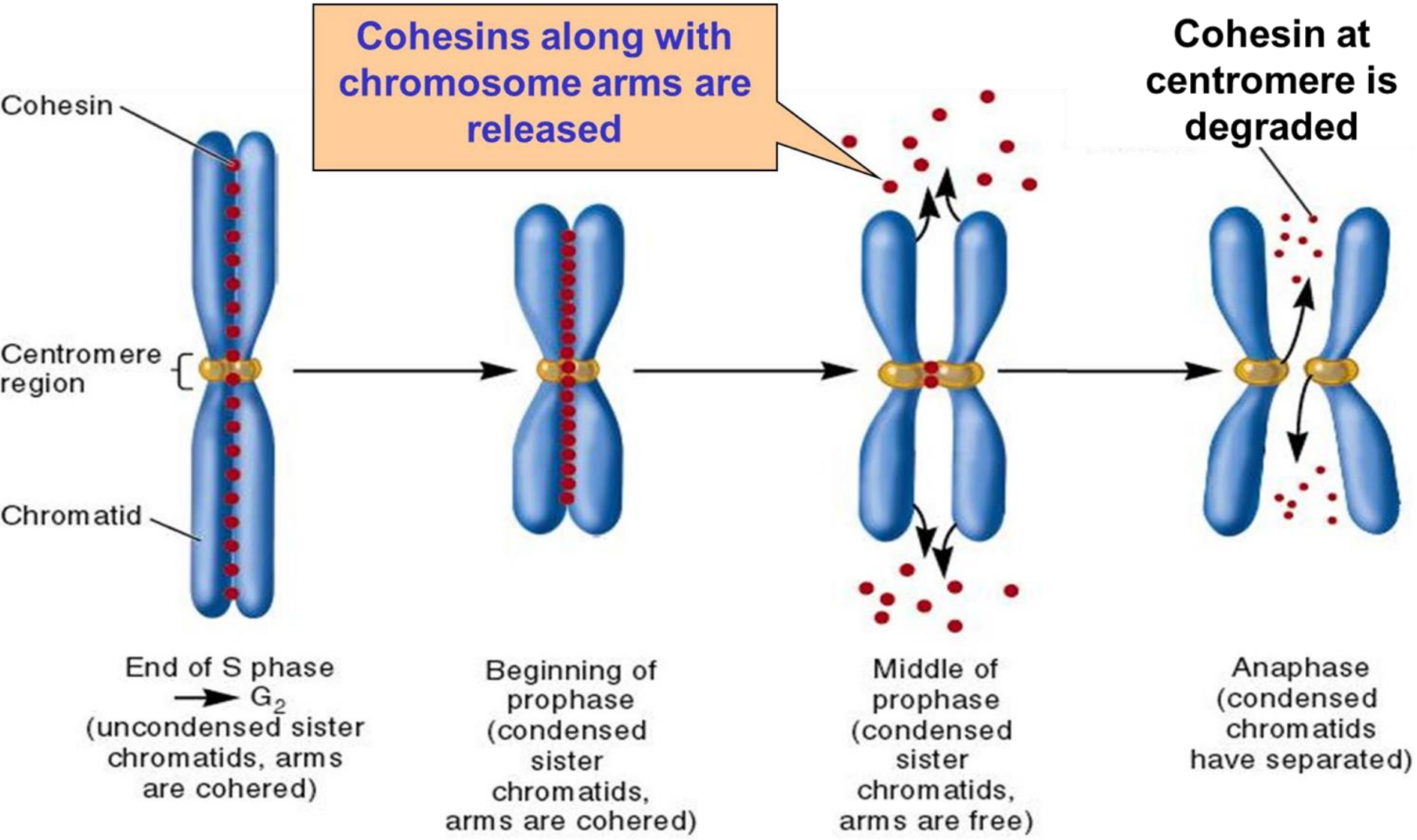


Condensin binds to chromosomes and compacts the radial loops

Condensin travels into the nucleus

The condensation of a metaphase chromosome by condensin.

Chromosomes During Mitosis



The alignment of sister chromatids via cohesin

Chromatin Remodeling

While this discovery helped solve the structural problem of how the huge amount of DNA is organized within the eukaryotic nucleus, it brought another problem to the fore: *In the chromatin fiber, complexed with histones into nucleosomes, which may be further folded into several more levels of compaction, the DNA is inaccessible to interaction with important nonhistone proteins.*

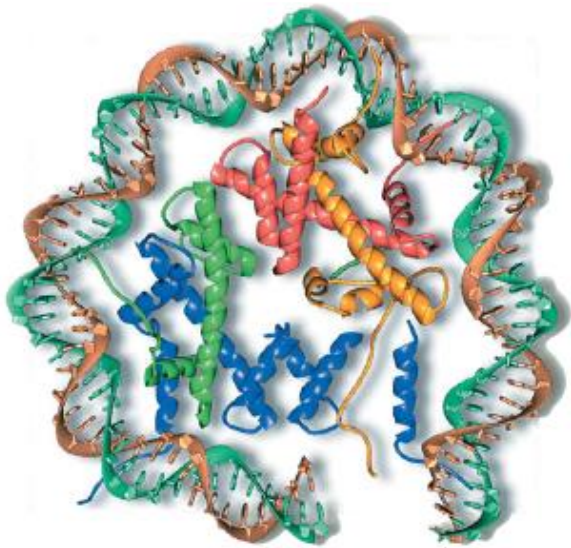
For example, the various proteins that function in enzymatic and regulatory roles during the processes of replication and transcription must interact directly with DNA. To accommodate these protein–DNA interactions, chromatin must be induced to change its structure, a process now referred to as **chromatin remodeling**. To allow replication and gene expression, chromatin must relax its compact structure and expose regions of DNA to regulatory proteins, but there must also be a mechanism for reversing the process during periods of inactivity.

Insights into how different states of chromatin structure might be achieved began to emerge in 1997, when **Timothy Richmond** and members of his research team were able to significantly improve the level of resolution in X-ray diffraction studies of nucleosome crystals, from 7 A in the 1984 studies to 2.8 A in the 1997 studies.

At this resolution, most atoms are visible, thus revealing the subtle twists and turns of the superhelix of DNA encircling the histones. Recall that the double-helical ribbon in the figure represents 147 bp of DNA surrounding four pairs of histone proteins. This configuration is essentially repeated over and over in the chromatin fiber and is the principal packaging unit of DNA in the eukaryotic nucleus.

By 2003, Richmond and colleagues achieved a resolution of 1.9 A that revealed the details of the location of each histone entity within the nucleosome. Of particular relevance to the discussion of chromatin remodeling is the observation that there are unstructured **histone tails** that are *not* packed into the folded histone domains within the core of the nucleosomes but instead protrude from it. For example, tails devoid of any secondary structure extending from histones H3 and H2B protrude through the minor groove channels of the DNA helix.

Other tails of histone H4 appear to make a connection with adjacent nucleosomes. The significance of histone tails is that they provide potential targets along the chromatin fiber for a variety of chemical modifications that may be linked to genetic functions, including chromatin remodeling and the possible regulation of gene expression.



The nucleosome core particle derived from X-ray crystal analysis at 2.8 A resolution. The double-helical DNA surrounds four pairs of histones.

Several of these potential chemical modifications are now recognized as important to genetic function.

One of the most well-studied histone modifications involves **acetylation** by the action of the enzyme *histone acetyltransferase (HAT).* The addition of an acetyl group to the positively charged amino group present on the side chain of the amino acid lysine effectively changes the net charge of the protein by neutralizing the positive charge. Lysine is in abundance in histones, and geneticists have known for some time that acetylation is linked to gene activation. It appears that high levels of acetylation open up, or remodel, the chromatin fiber, an effect that increases in regions of active genes and decreases in inactive regions. In a well-studied example, the inactivation of the X chromosome in mammals, forming a Barr body, histone H4 is known to be greatly underacetylated.

Two other important chemical modifications are the **methylation** and **phosphorylation** of amino acids that are part of histones. These chemical processes result from the action of enzymes called *methyltransferases* and *kinases,* respectively. Methyl groups can be added to both arginine and lysine residues in histones, and this change has been correlated with gene activity. Phosphate groups can be added to the hydroxyl groups of the amino acids serine and histidine, introducing a negative charge on the protein. During the cell cycle, increased phosphorylation, particularly of histone H3, is known to occur at characteristic times. Such chemical modification is believed to be related to the cycle of chromatin unfolding and condensation that occurs during and after DNA replication. It is important to note that the above chemical modifications (acetylation, methylation, and phosphorylation) are all reversible, under the direction of specific enzymes.

Interestingly, while methylation of histones *within nucleosomes* is often positively correlated with gene activity in eukaryotes, methylation of the nitrogenous base cytosine *within polynucleotide chains of DNA,* forming **5-methyl cytosine**, is usually negatively correlated with gene activity. Methylation of cytosine occurs most often when the nucleotide cytidylic acid is next to the nucleotide guanylic acid, forming what is called a **CpG island**.

Methylation can have a positive or a negative impact on gene activity.

A great deal more work must be done, however, to elucidate the specific involvement of chromatin remodeling during genetic processes. In particular, the way in which the modifications are influenced by regulatory molecules within cells will provide important insights into the mechanisms of gene expression.

What is clear is that the dynamic forms in which chromatin exists are vitally important to the way that all genetic processes directly involving DNA are executed.

In addition, chromatin remodeling is an important topic in the discussion of **epigenetics**, the study of modifications of an organism's genetic and phenotypic expression that are *not* attributable to alteration of the DNA sequence making up a gene.

Disclaimer

All the original contributors of the concept and findings published elsewhere are gratefully acknowledged while preparing the E-content for the purpose of student reading material in convenient form for biochemistry and allied discipline.

References

- William S. Klug, Michael R. Cummings, Charlotte A. *Concepts of Genetics*. 10th ed. Pearson, 2012.
- Reece, Richard J. Analysis of genes & genomes. 5th ed. John Wiley & Sons Ltd, England, 2004.
- NPTEL Swayam online platform.
- Open Access Online Resources