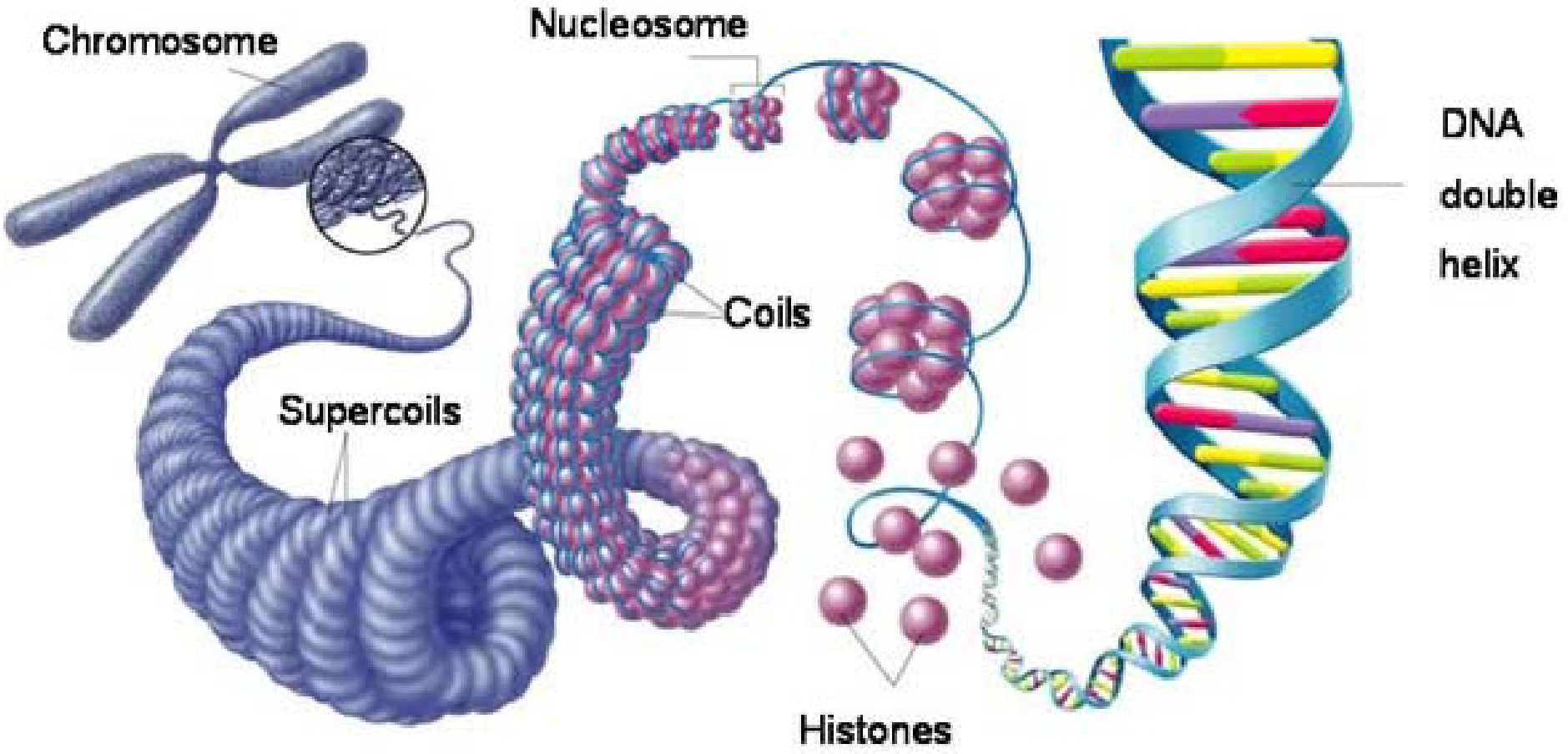
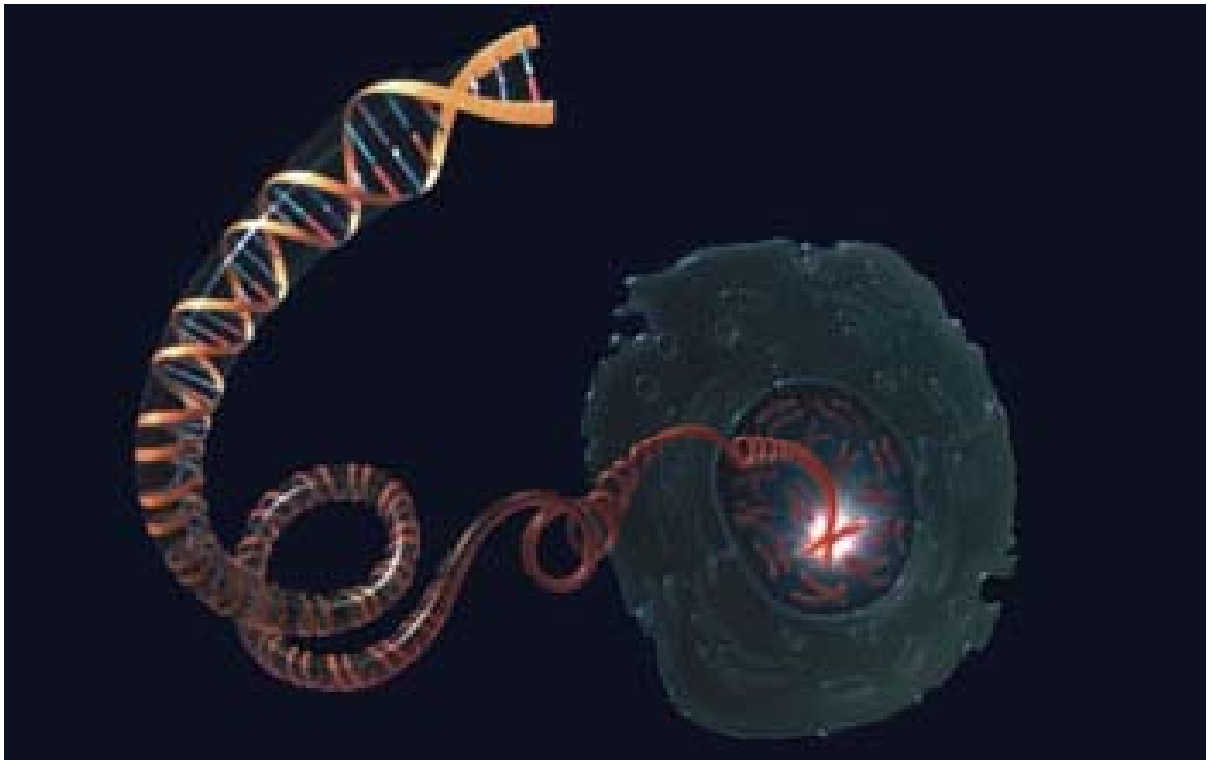
Organization of Eukaryotic Chromosomes



Organization of Eukaryotic Chromosomes

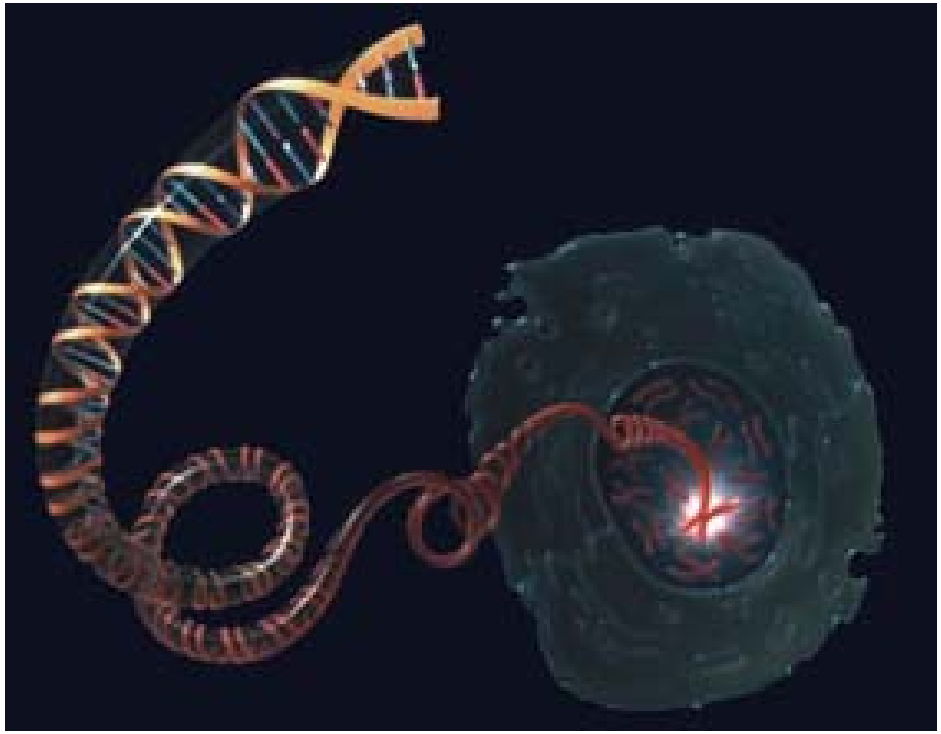
The largest human chromosome in humans contains 0.3 billion base pairs.

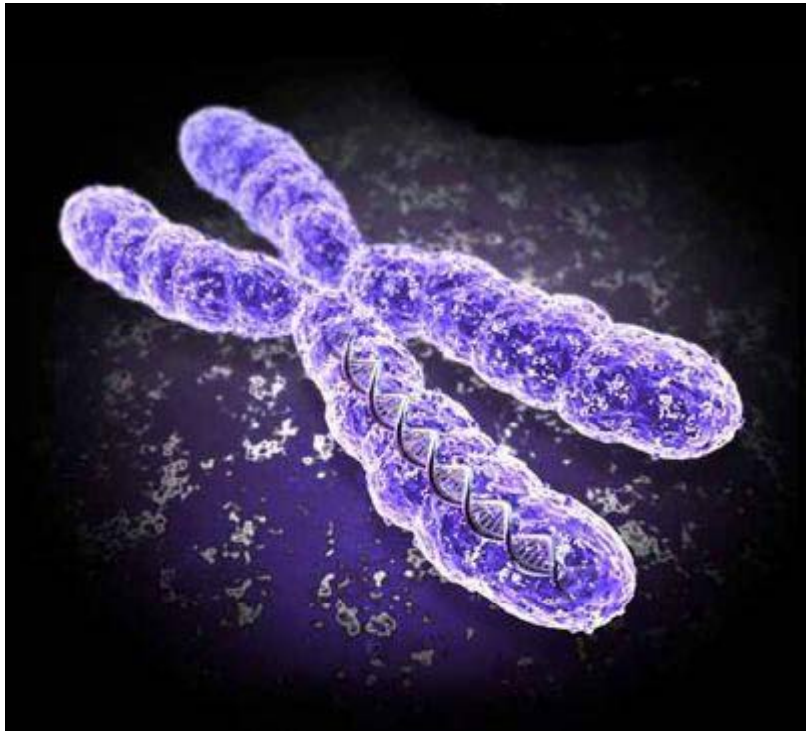
If it is in normal confirmation, it would constitute a chromosome of greater than 10 cm long.

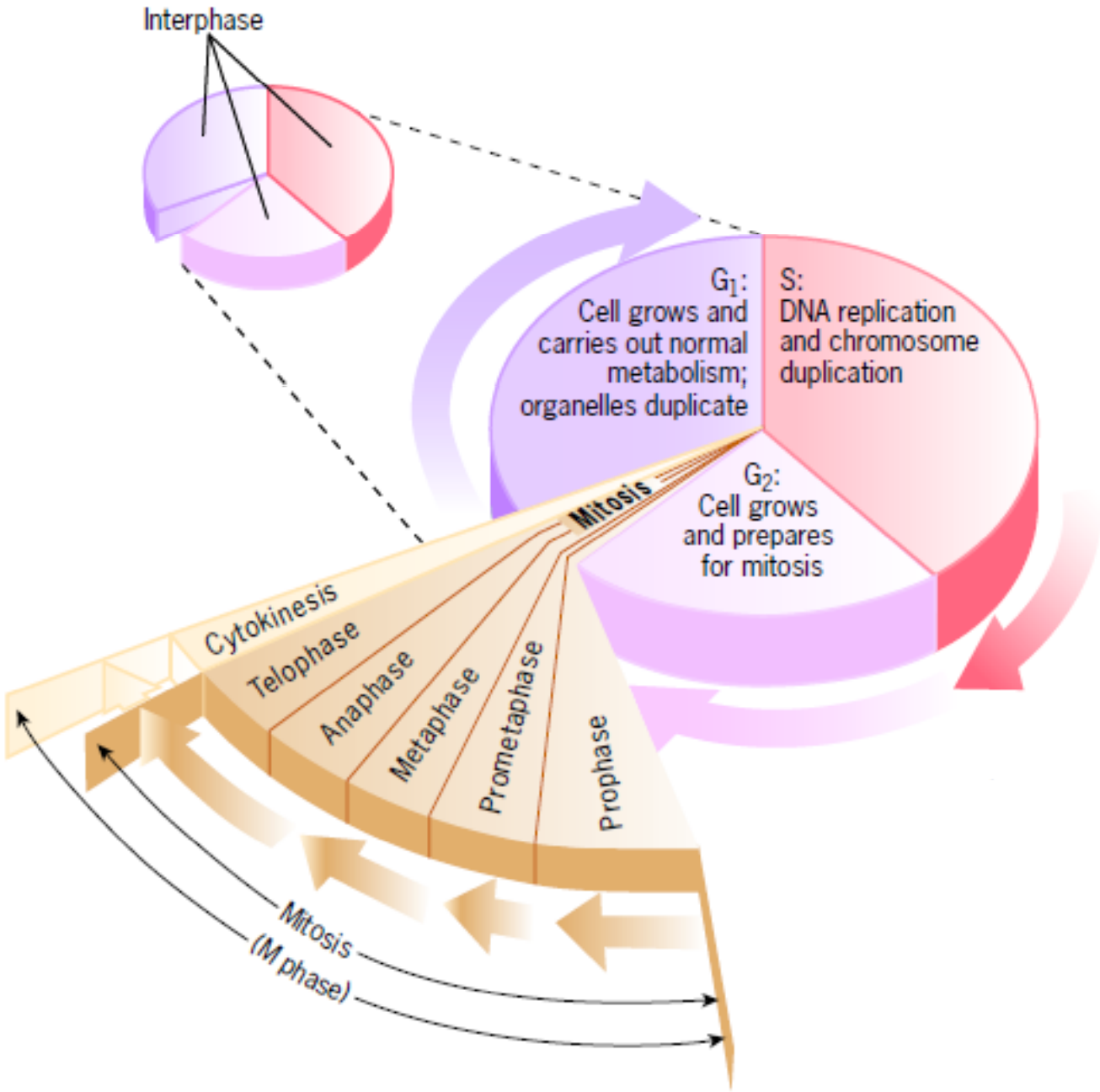


How is it possible to fit all 46 chromosomes into a nucleus that is only 10μm in diameter and, at the same time, maintain the DNA in a state that is accessible to enzymes and regulatory proteins?

The answers lie in the remarkable manner in which a DNA molecule is packaged in eukaryotic cells.





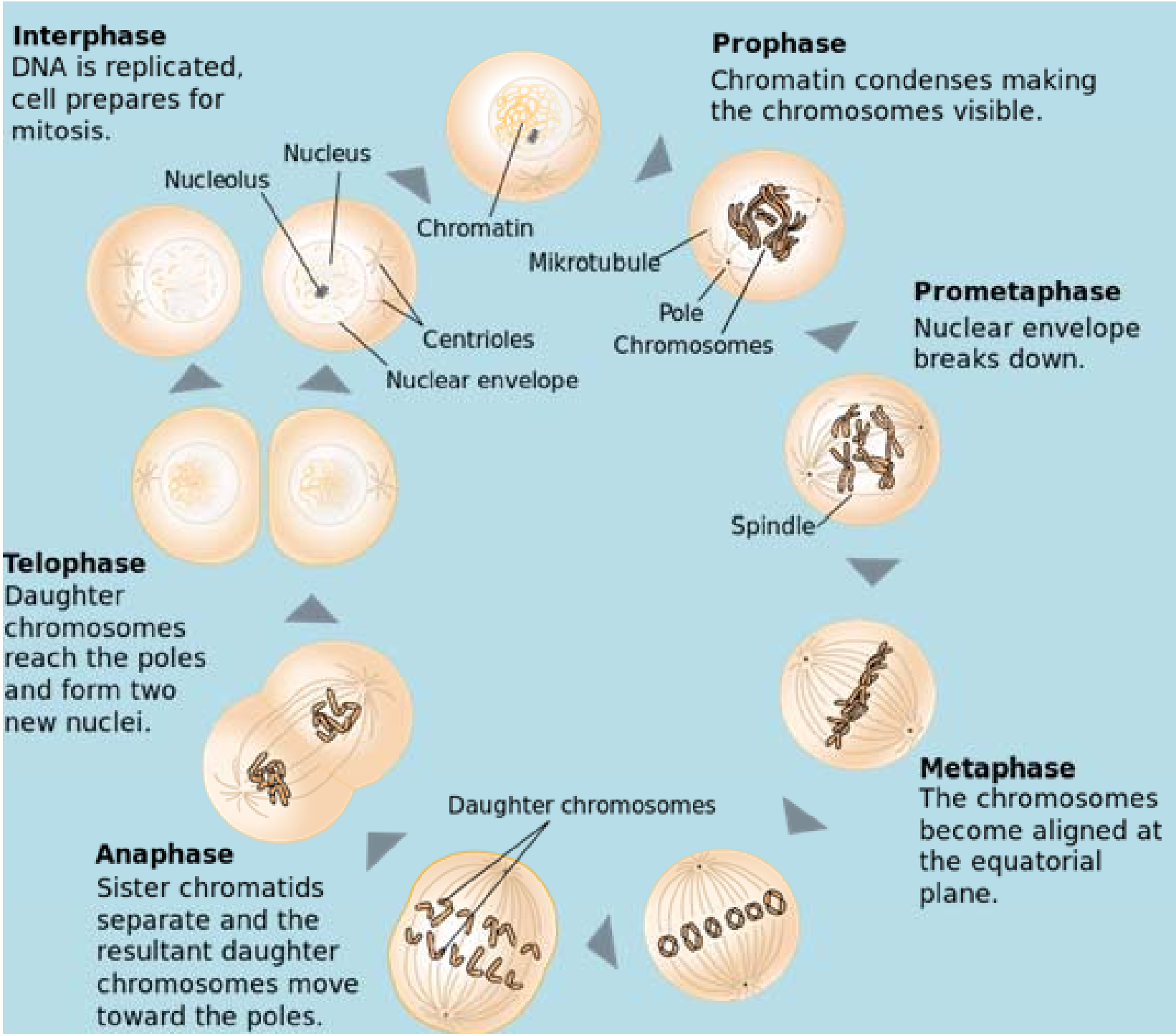

Interphase
G1:
Cell grows and carries out normal metabolism; organelles duplicate
S:
DNA replication and chromosome duplication
G2:
Cell grows and prepares for mitosis
Mitosis
Prophase
Prometaphase
Metaphase
Anaphase
Telophase
Cytokinesis
Mitosis
(M phase)

Organization of Eukaryotic Chromosomes

Each eukaryotic chromosome consists of a single, extremely long molecule of DNA.

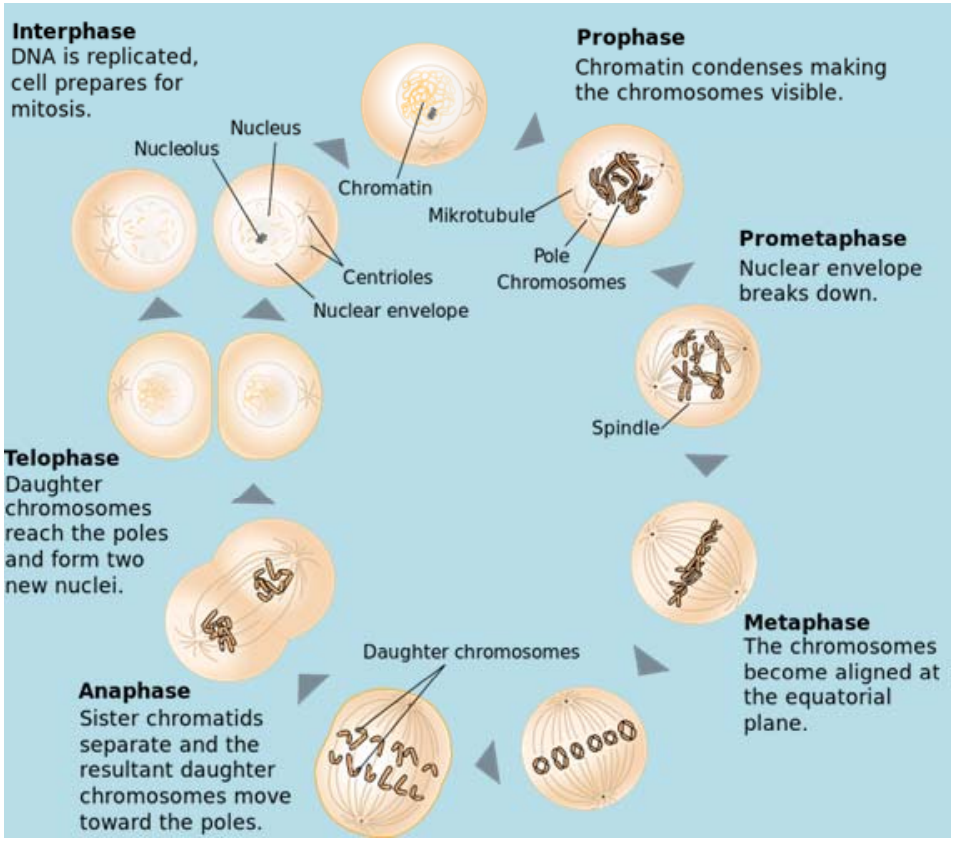
For all of this DNA to fit into the nucleus, tremendous packing and folding are required

The chromosomes are in an elongated, relatively uncondensed state during interphase of the cell cycle.


Interphase
DNA is replicated, cell prepares for mitosis.
Nucleus
Nucleolus
Chromatin
Centrioles
Nuclear envelope
Prophase
Chromatin condenses making the chromosomes visible.
Mikrotubule
Pole
Chromosomes
Prometaphase
Nuclear envelope breaks down.
Spindle
Metaphase
The chromosomes become aligned at the equatorial plane.
Daughter chromosomes
Anaphase
Sister chromatids separate and the resultant daughter chromosomes move toward the poles.
Telophase
Daughter chromosomes reach the poles and form two new nuclei.

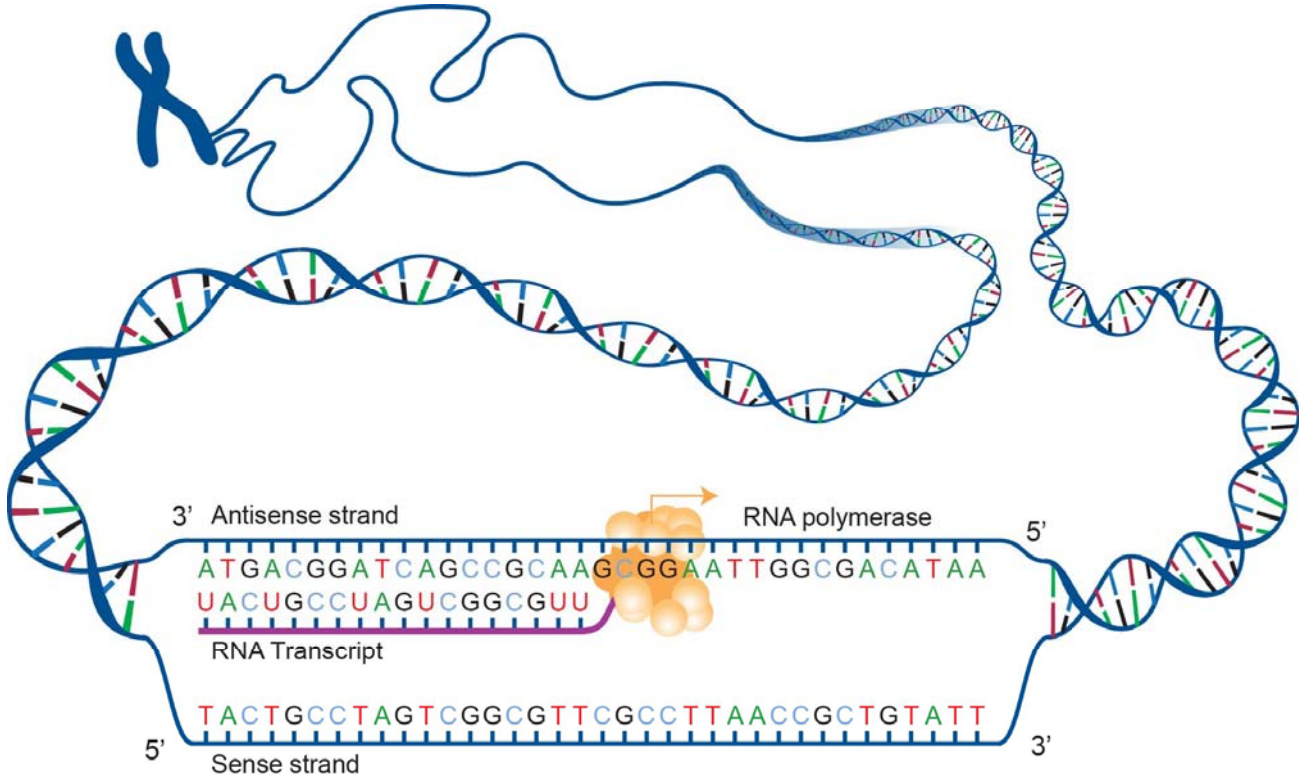
Although the DNA of interphase chromosomes is less tightly packed than the DNA of mitotic chromosomes, it is still highly condensed

In the course of the cell cycle, the level of DNA packing changes; chromosomes progress from a highly packed state to a state of extreme condensation.



DNA packing also changes locally in replication and transcription, when the two nucleotide strands must unwind so that particular base sequences are exposed.

Thus, the packing of eukaryotic DNA is not static but changes regularly in response to cellular processes.



Chromatin

Eukaryotic DNA in the cell is closely associated with proteins. This combination of DNA and protein is called chromatin.

There are two kinds of proteins are associated with DNA; histone and nonhistone proteins.

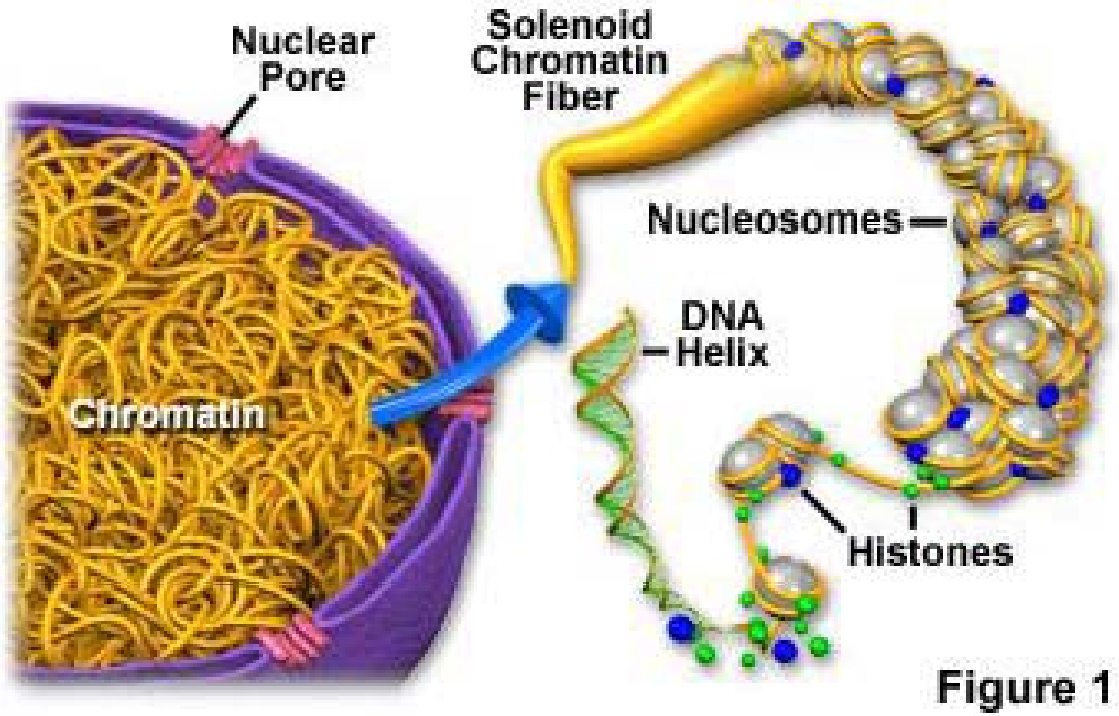
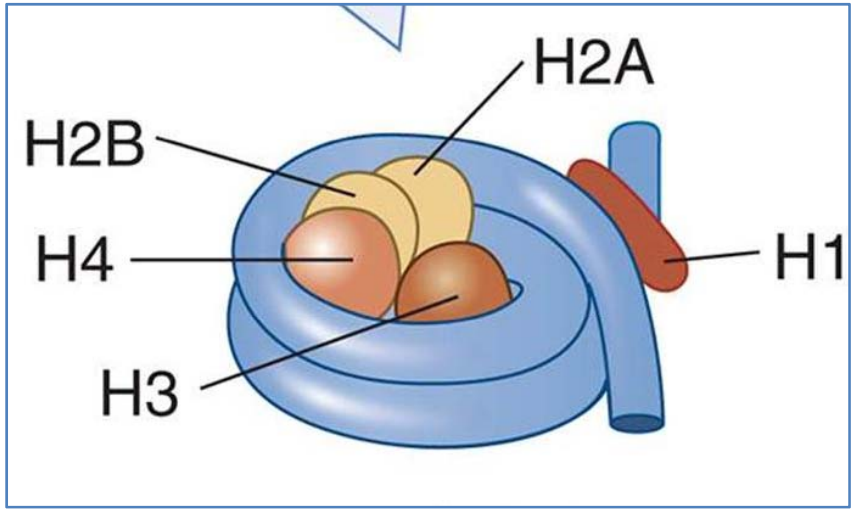
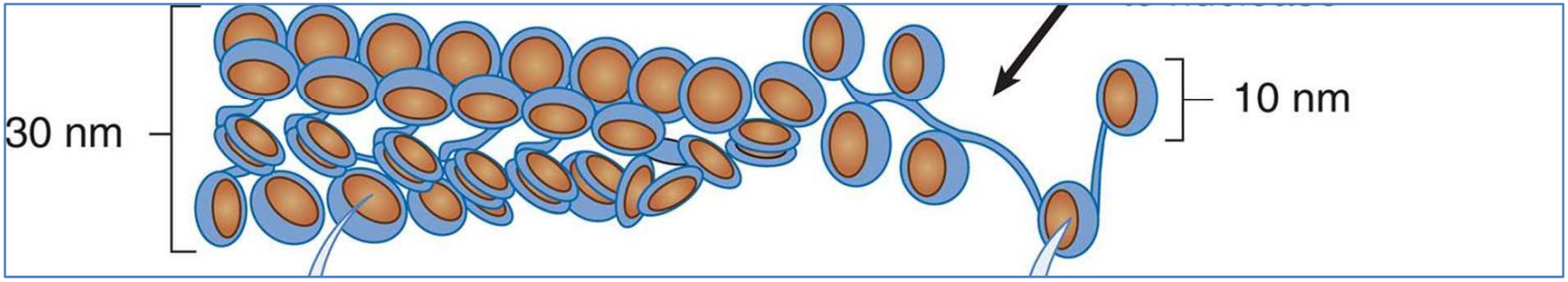


Figure 1

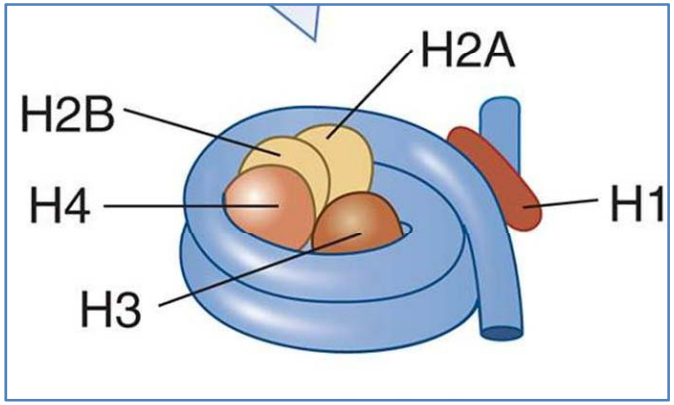
The most abundant proteins in chromatin are the histones, which are small, positively charged proteins of five major types: H1, H2A, H2B, H3, and H4.





All histones have a high percentage of arginine and lysine- positively charged amino acids- that give the histones a net positive charge.

The positive charges attract the negative charges on the phosphates of DNA; this attraction holds the DNA in contact with the histones.



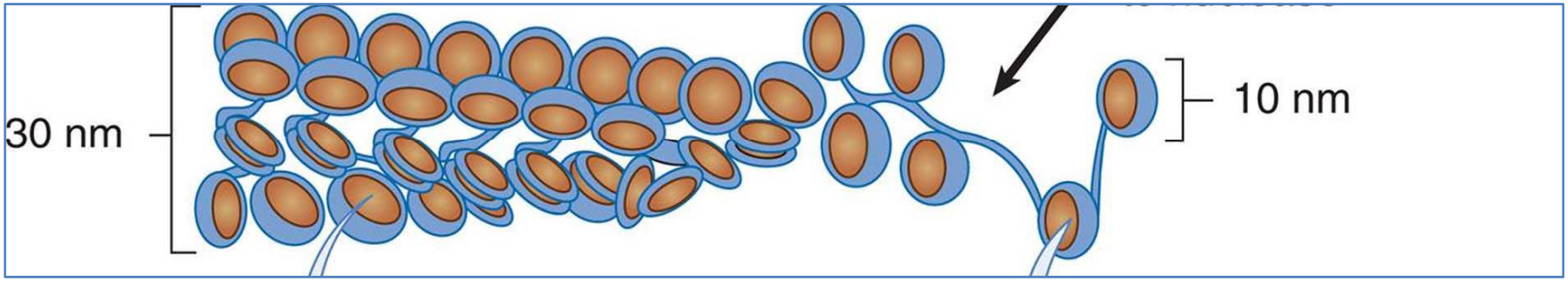


Table 11.1 Characteristics of histone proteins

Histone Protein	Molecular Weight	Number of Amino Acids
H1	21,130	223
H2A	13,960	129
H2B	13,774	125
H3	15,273	135
H4	11,236	102

***Table 12.1* Calf Thymus Histones**

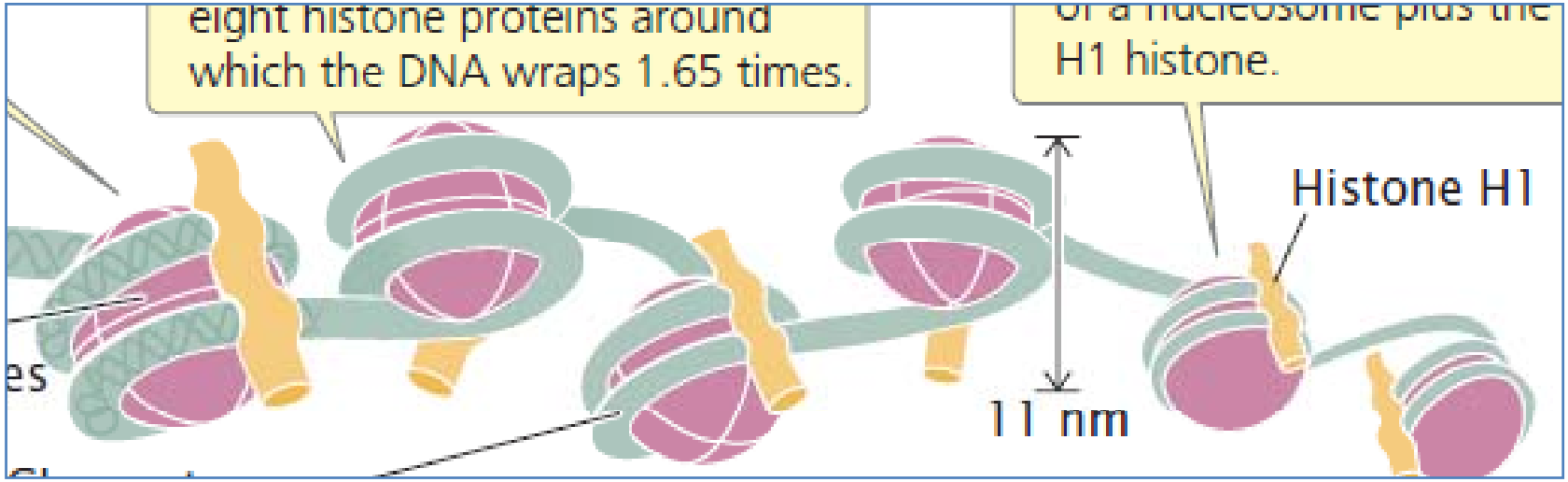
Histone	Number of residues	Mass (kDa)	%Arg	%Lys	UEP* ($\times 10^{-6}$ year)
H1	215	23.0	1	29	8
H2A	129	14.0	9	11	60
H2B	125	13.8	6	16	60
H3	135	15.3	13	10	330
H4	102	11.3	14	11	600

*Unit evolutionary period: the time for a protein's amino acid sequence to change by 1 percent after two species have diverged.

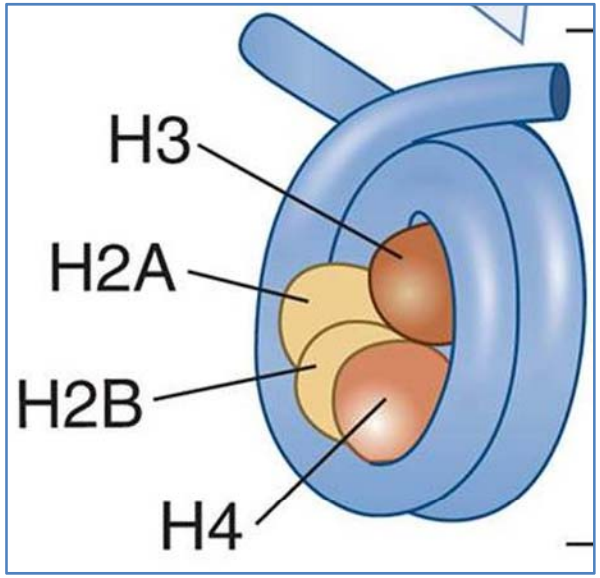
Nucleosomes

Nucleosome is the lowest level of chromosome organization.

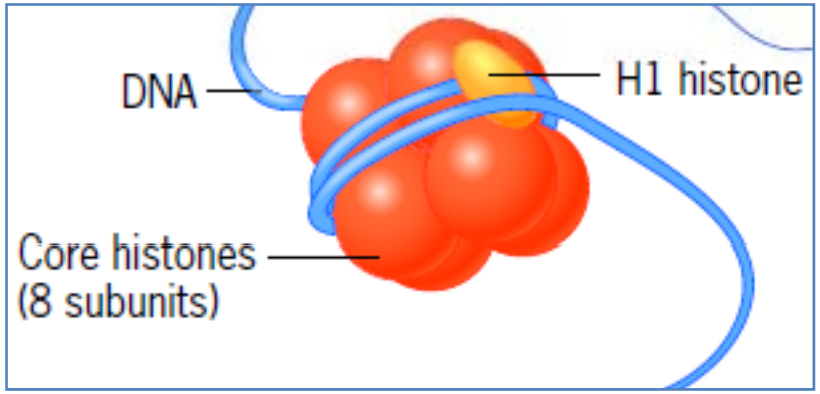
DNA and histones are organized into repeating subunits, called nucleosomes.

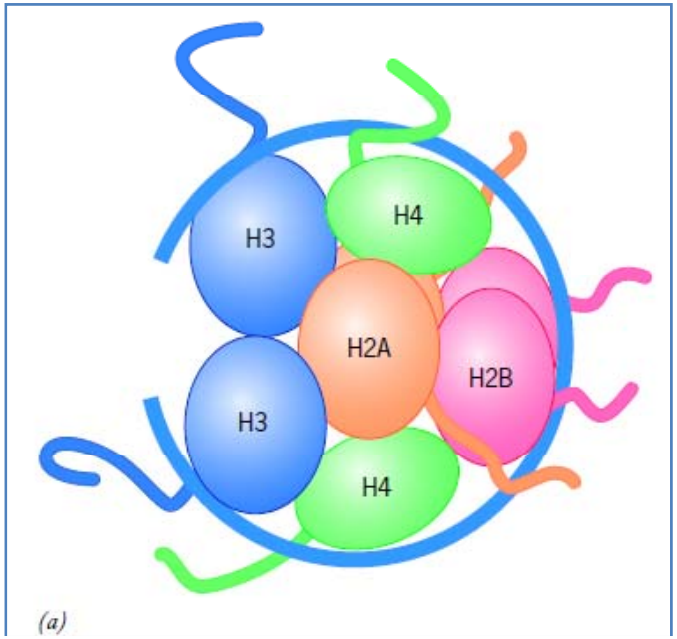


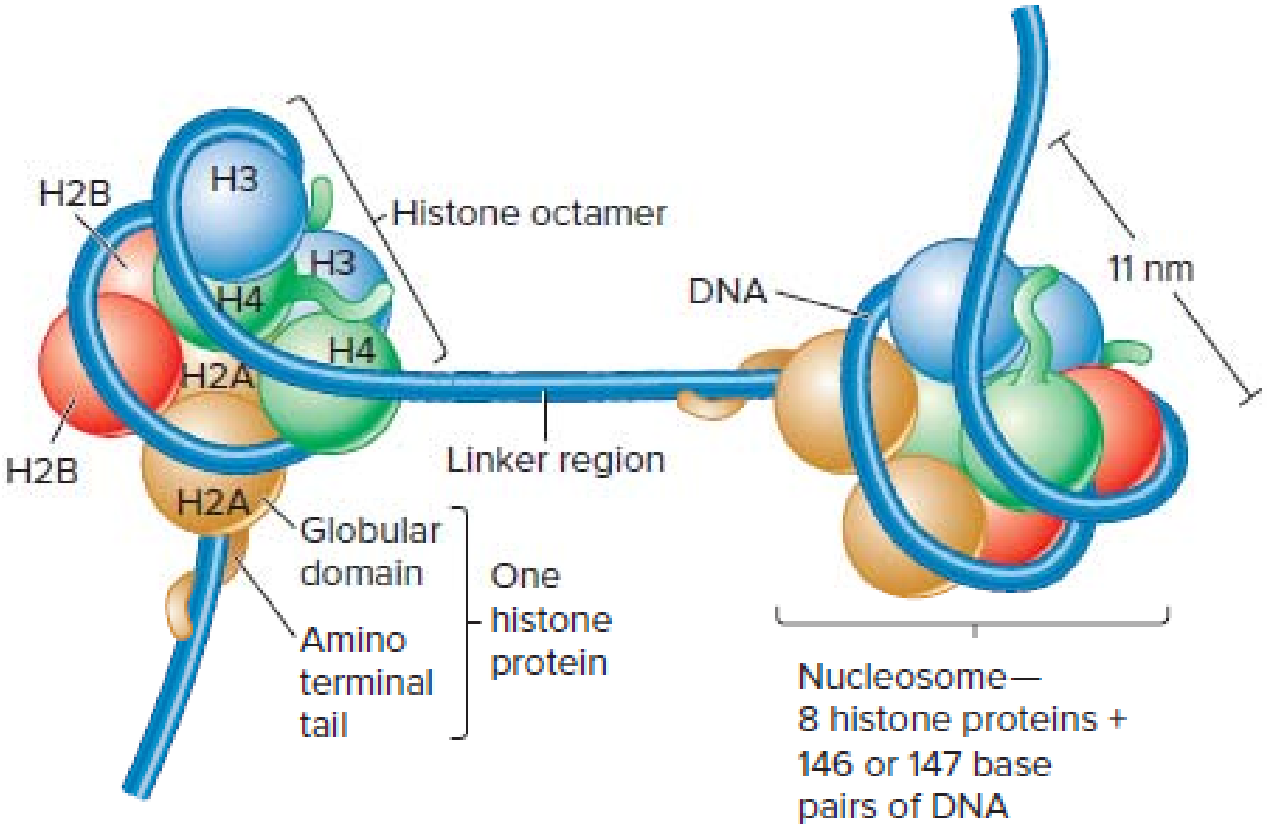
Each nucleosome contains a 146 base pairs of DNA wrapped almost twice around a disk shaped complex of eight histone molecules (histone core).



The histone core of each nucleosome consists of two copies H2A, H2B, H3, and H4





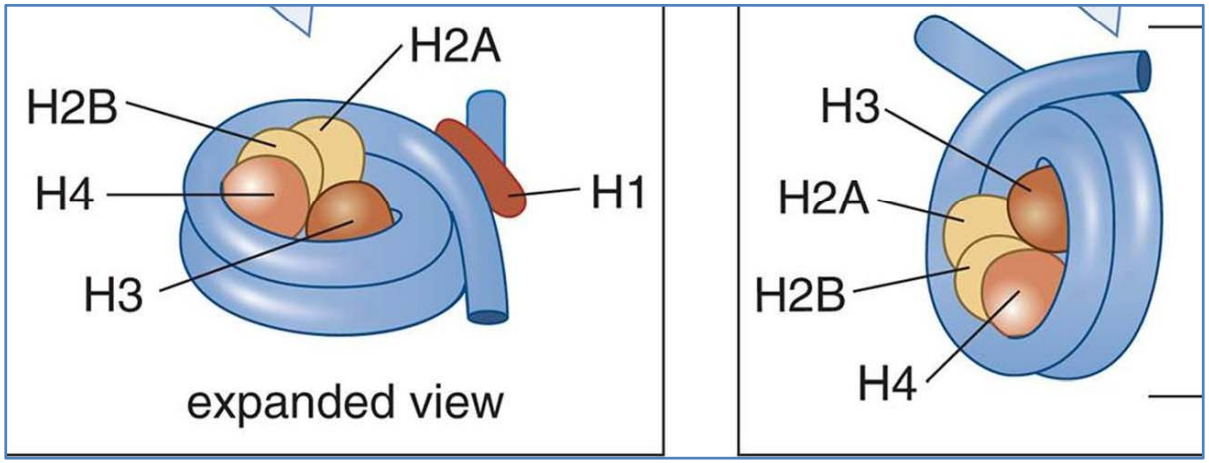


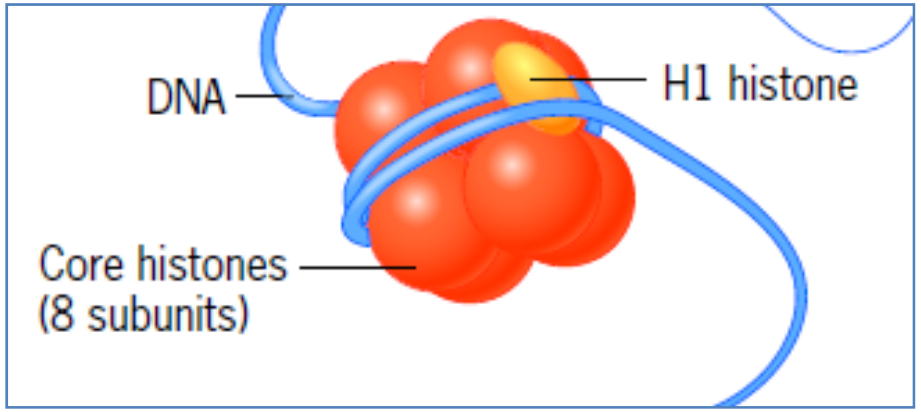
(a) Nucleosomes showing core histone proteins

The remaining histone, H1 resides outside the nucleosome core.

H1 binds to 20 to 22 bp of DNA where the DNA joins and leaves the octamer and helps to lock the DNA into place, acting as a clamp around the nucleosome.

Together, the core particle and its associated H1 histone are called the chromatosome.

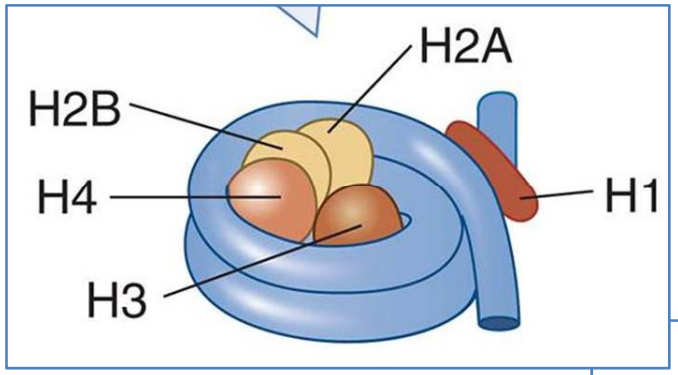


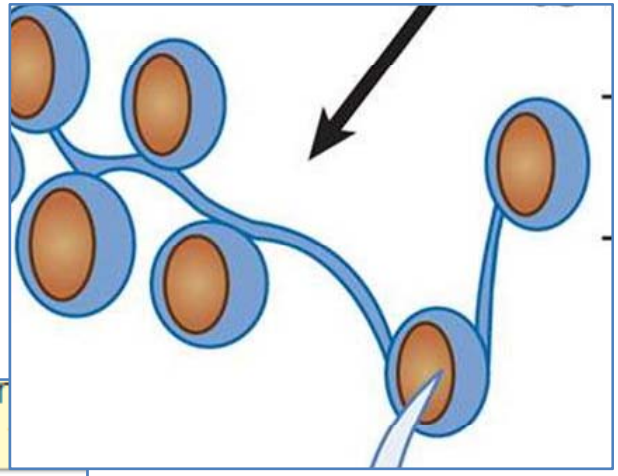


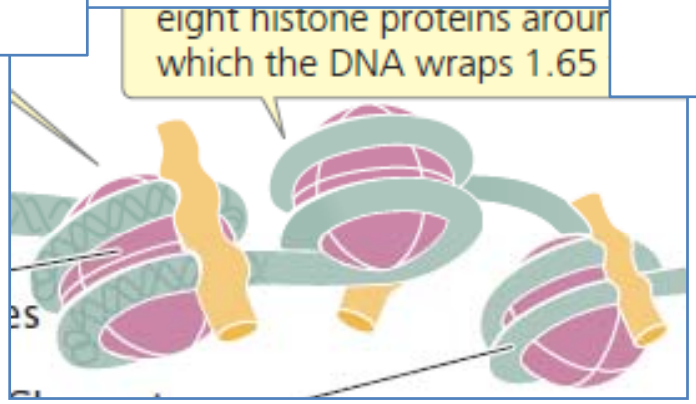
Each chromatosome encompasses about 167 bp of DNA.

Chromatosomes are located at regular intervals along the DNA molecule and are separated from one another by linker DNA of 30 to 40 bp.

linker DNA is the piece of DNA that connects adjacent chromatosomes.

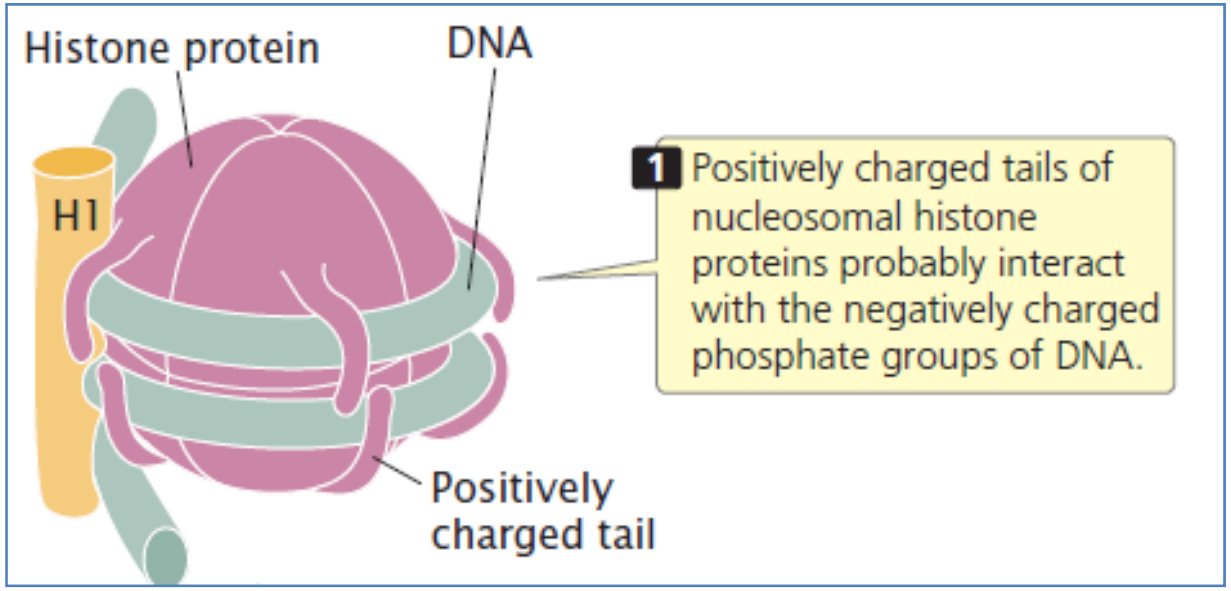




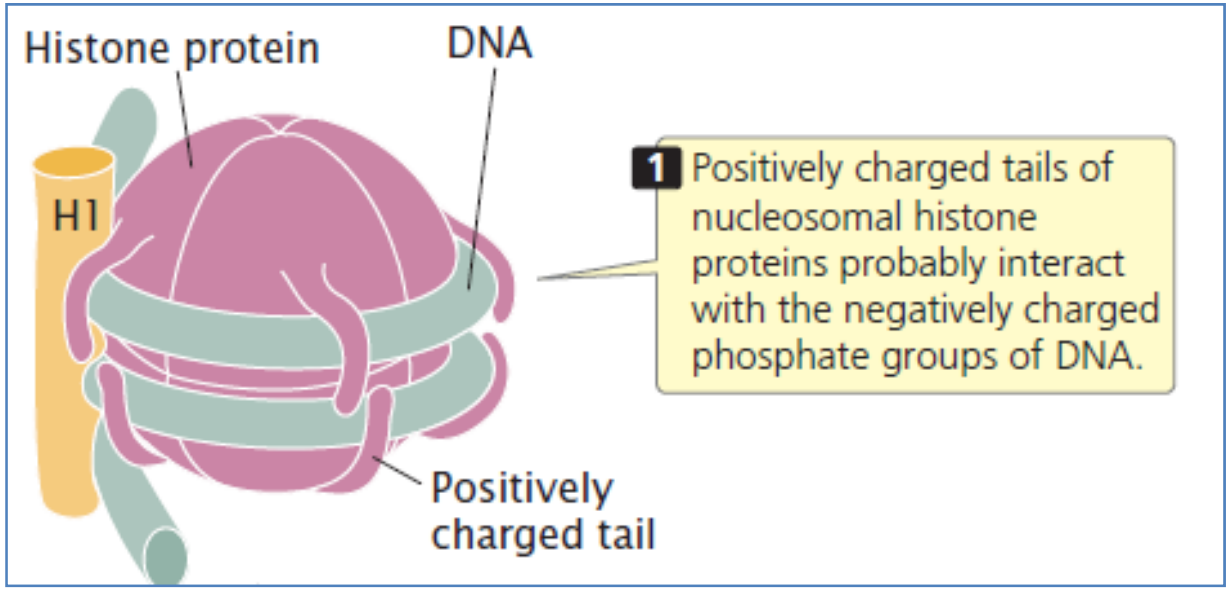


Together the H1 protein and the histone octamer interact with about 168 (167) base pairs of DNA and interconnected by linker DNA (30-40 bps), which together appears like "beads on a string"

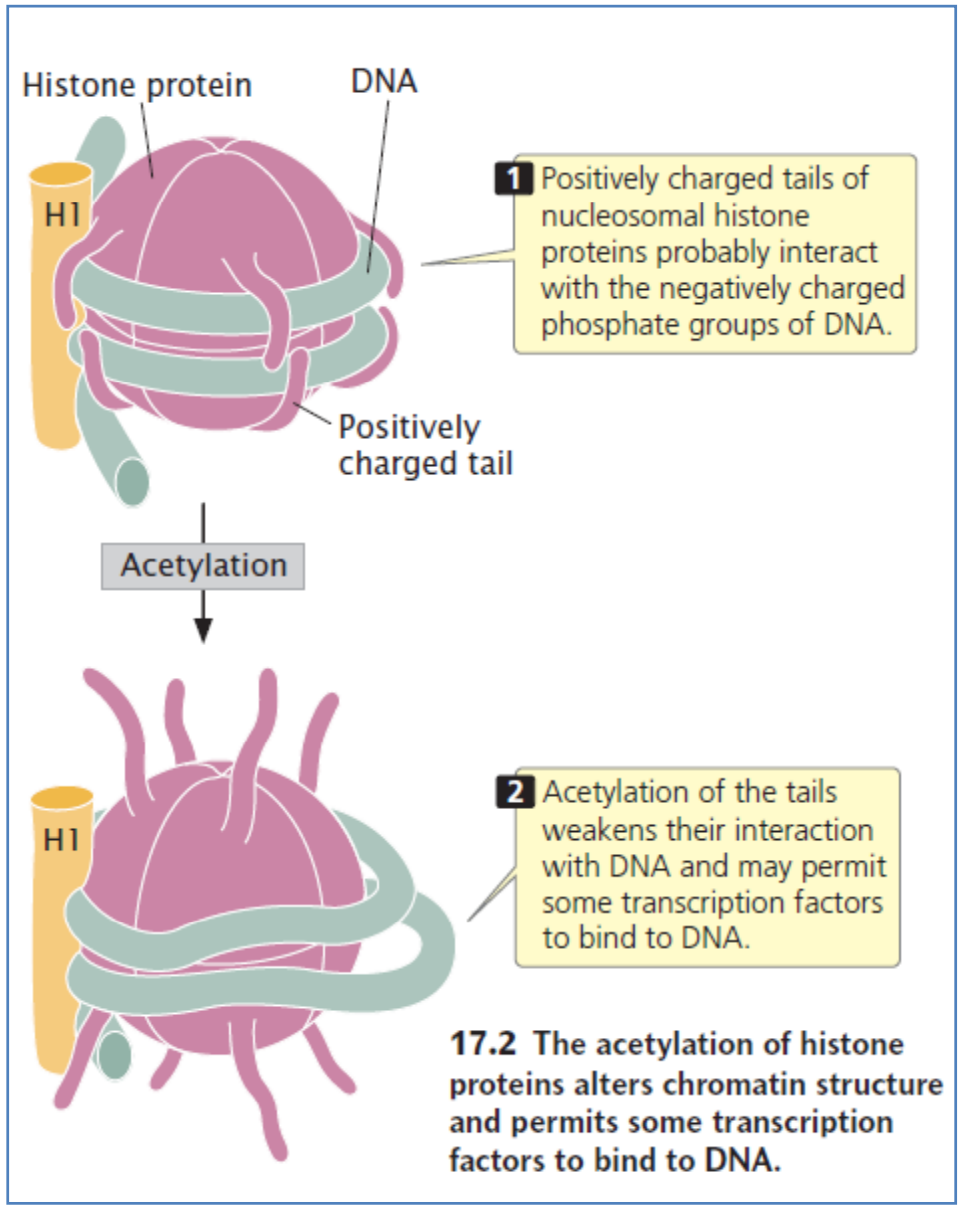
Each of the histone proteins that make up the nucleosome core particle has a flexible "tail," containing from 11 to 37 amino acids, that extends out from the nucleosome.



Positively charged amino acids in the tails of the histones interact with the negative charges of the phosphates on the DNA, keeping the DNA and histones tightly associated.

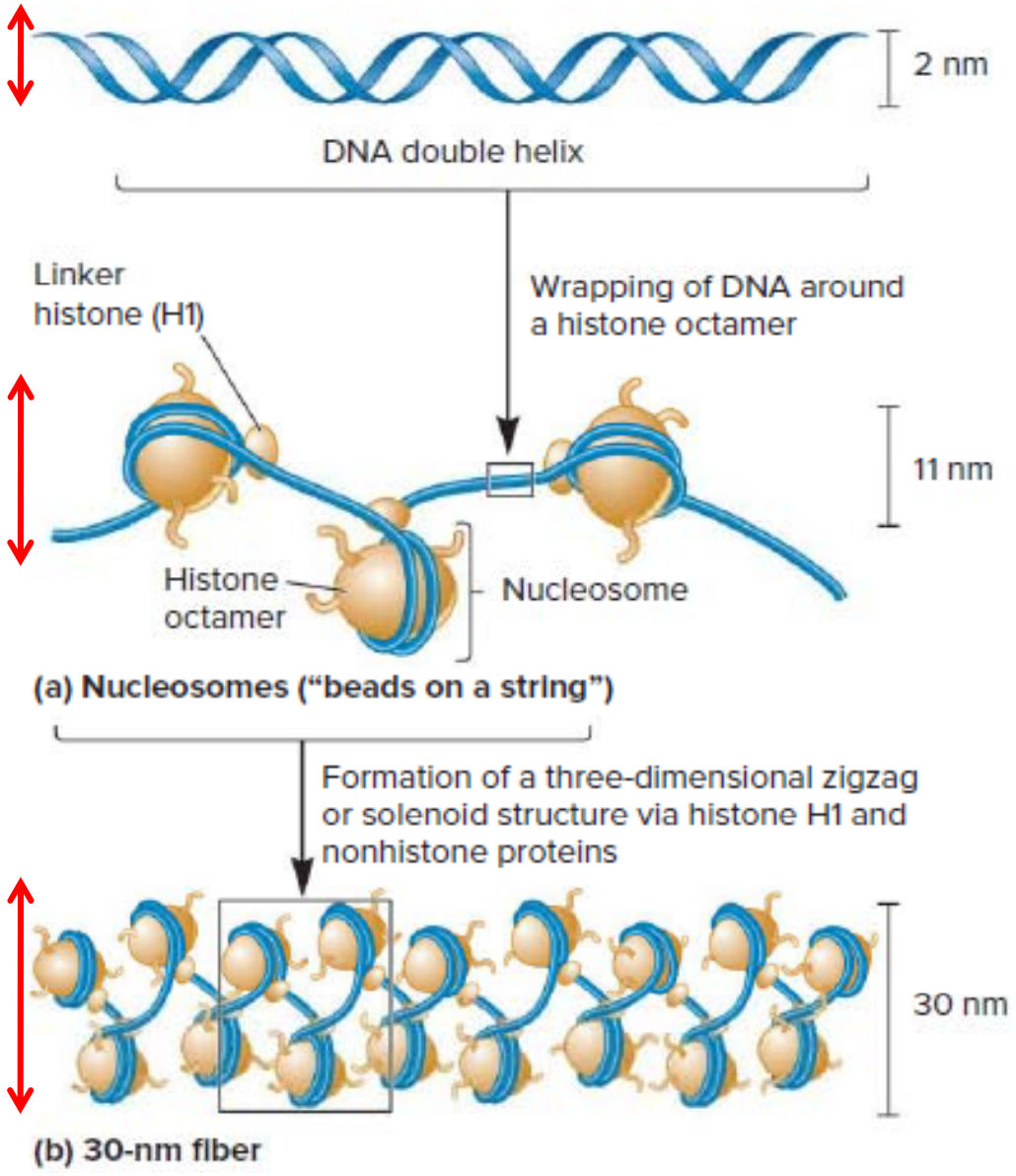


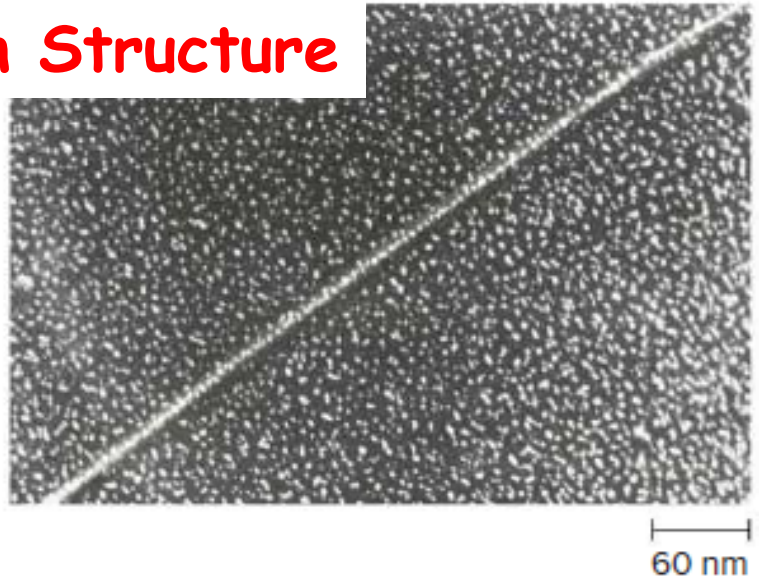
Chemical modifications of the histone tails bring about changes in chromatin structure that are necessary for gene expression.

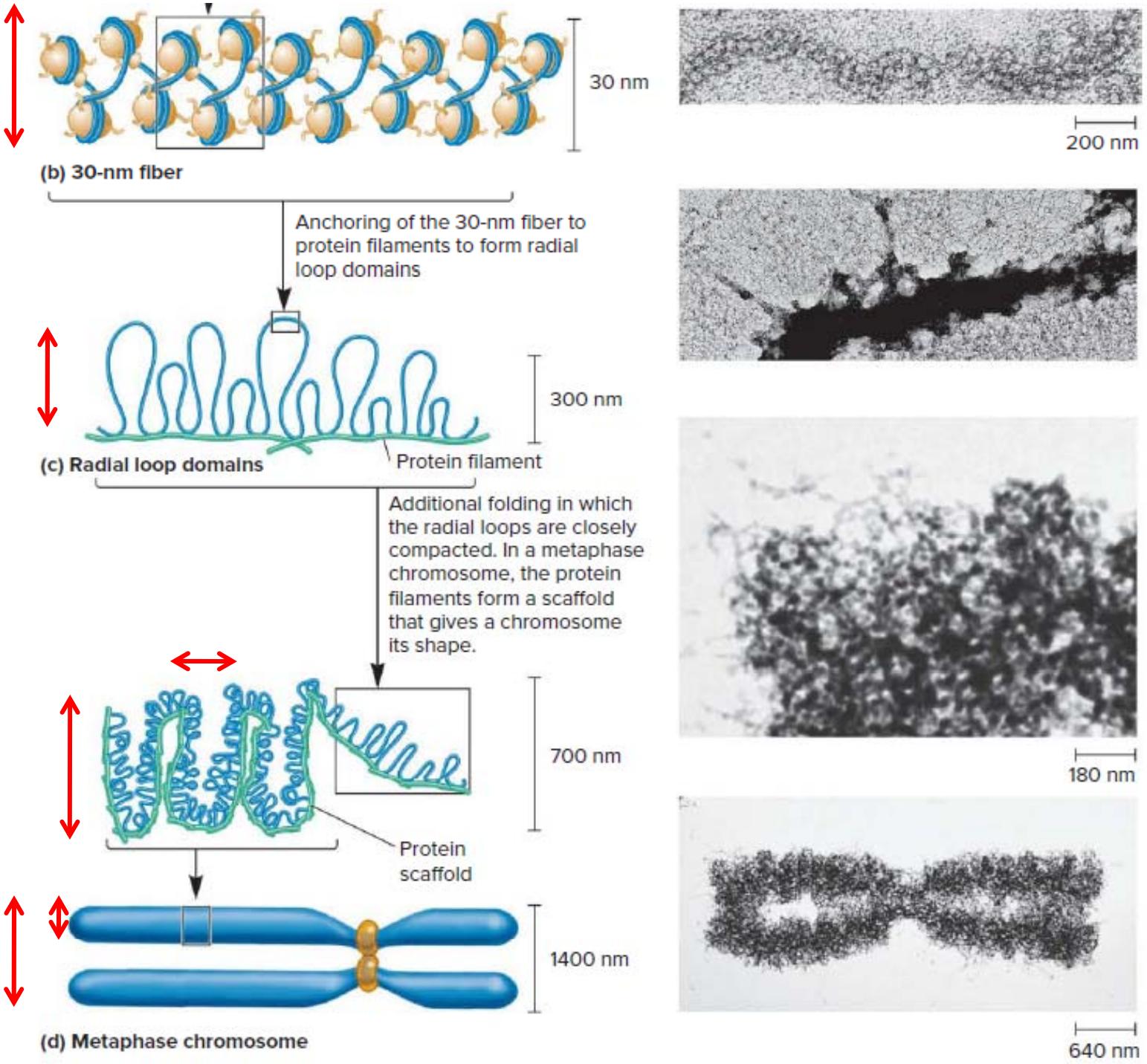


17.2 The acetylation of histone proteins alters chromatin structure and permits some transcription factors to bind to DNA.

Higher Levels of Chromatin Structure



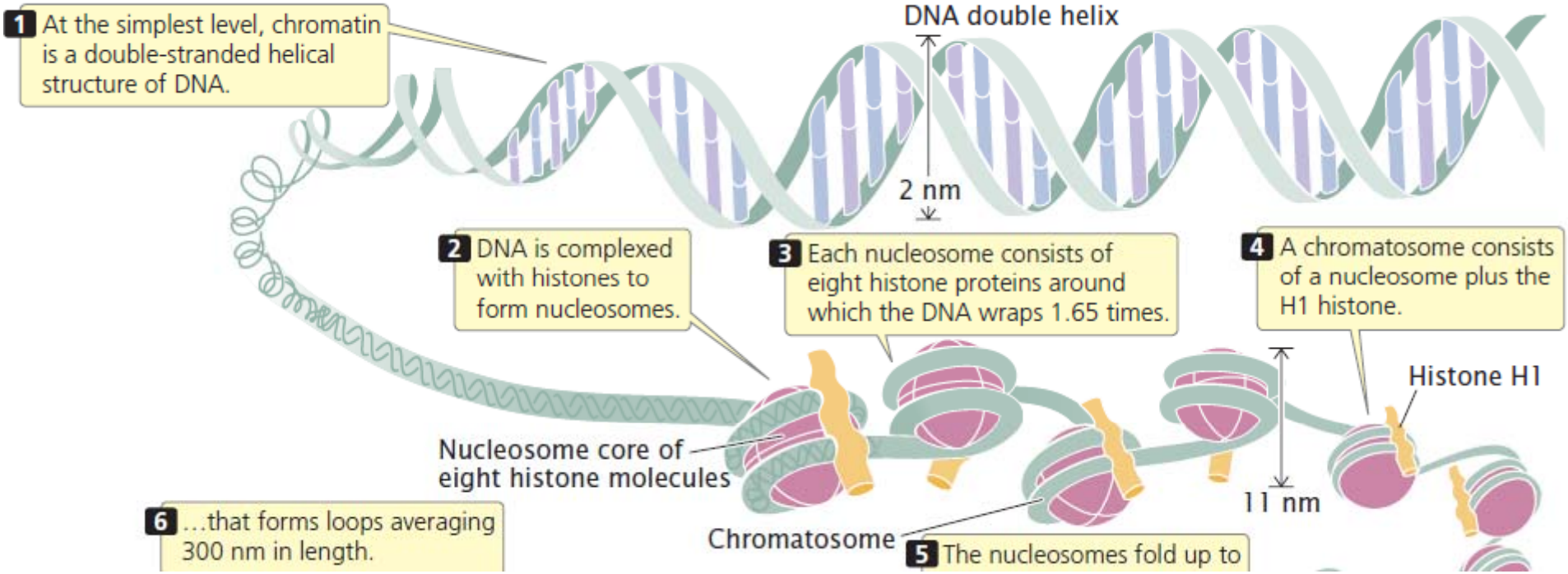


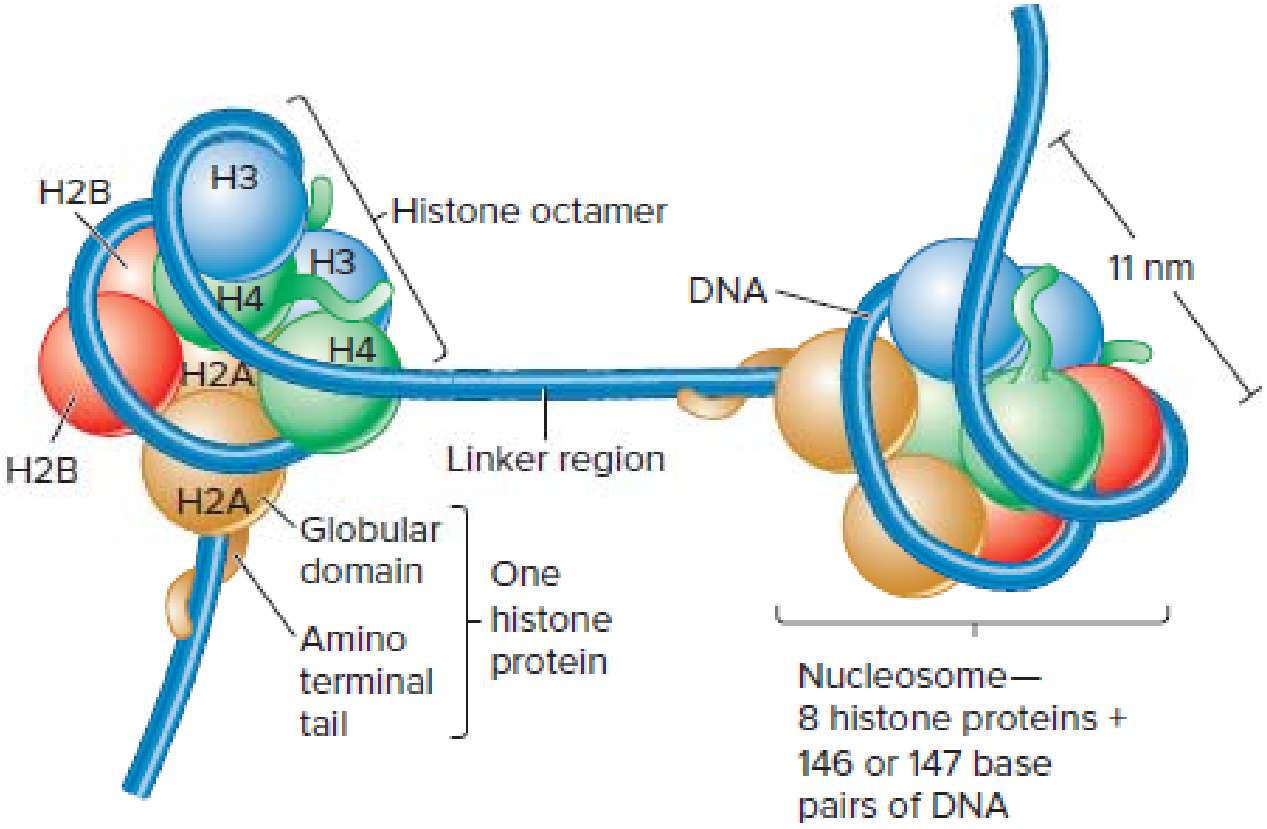

30 nm
(b) 30-nm fiber
Anchoring of the 30-nm fiber to protein filaments to form radial loop domains
300 nm
(c) Radial loop domains
Protein filament
Additional folding in which the radial loops are closely compacted. In a metaphase chromosome, the protein filaments form a scaffold that gives a chromosome its shape.
700 nm
Protein scaffold
1400 nm
(d) Metaphase chromosome
200 nm
180 nm
640 nm

Higher Levels of Chromatin Structure

Nucleosome: A DNA molecule of 2 nm diameter wrapped around nucleosome core particles of 11-nm diameter is the lowest level of chromatin organization.

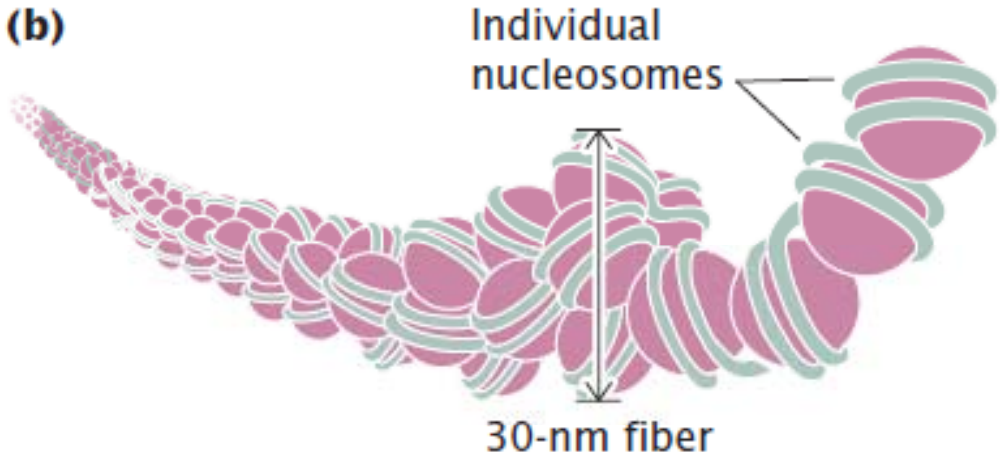
Each nucleosome consists of eight histone proteins around which the DNA wraps 1.65 times.





(a) Nucleosomes showing core histone proteins

30 nm fiber: Nucleosomes fold on themselves to form a dense, tightly packed structure that makes up a fiber with a diameter of about 30 nm.

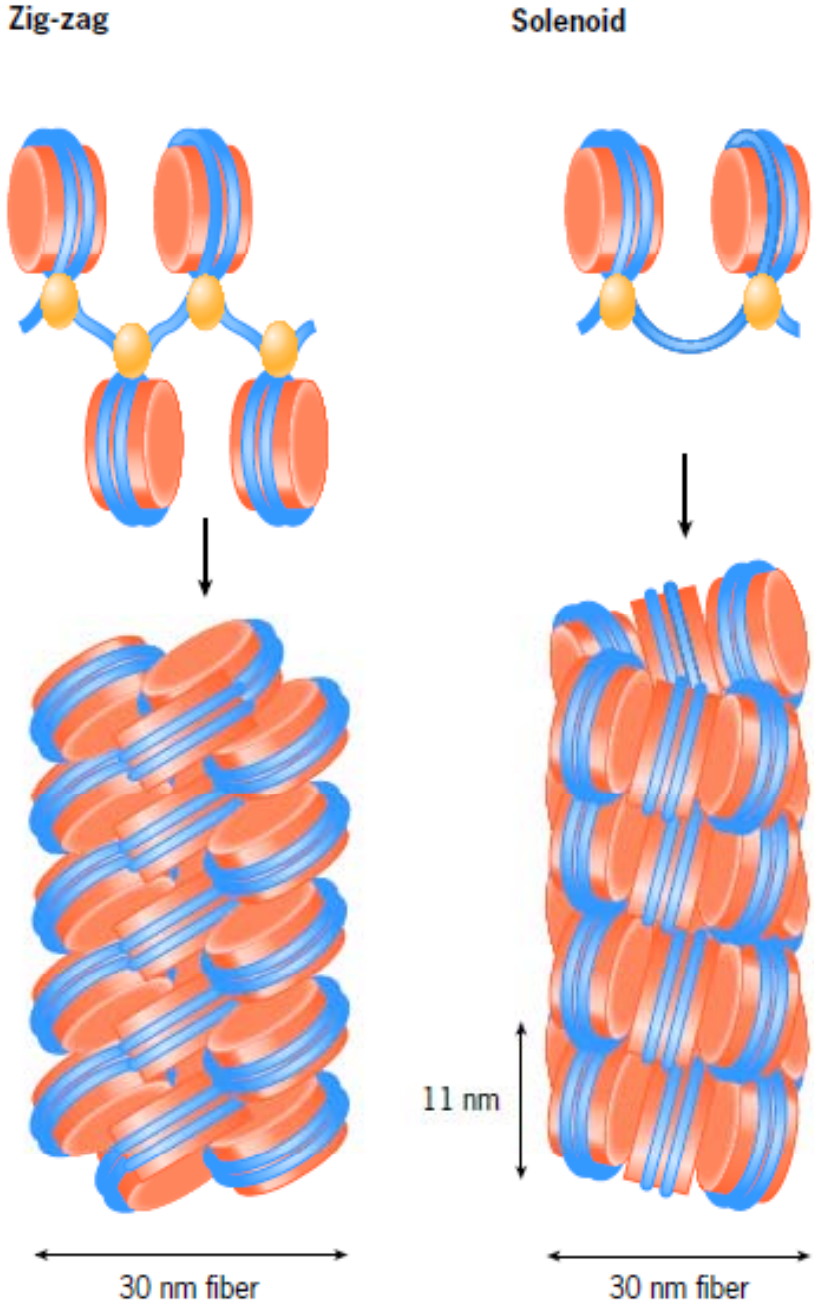


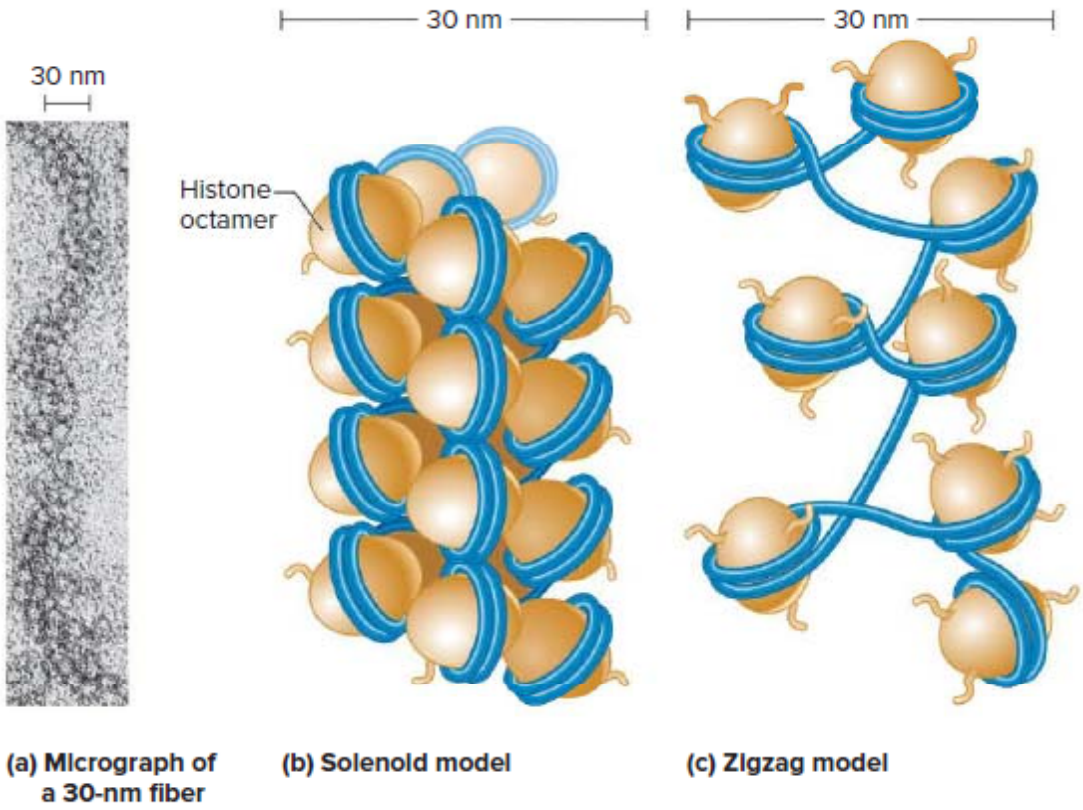
Two different models have been proposed for the 30-nm fiber:

(i) solenoid model, in which a linear array of nucleosomes are coiled

(ii) helix model, in which nucleosomes are arranged in a zigzag ribbon that twists or supercoils.

Recent evidence supports the helix model



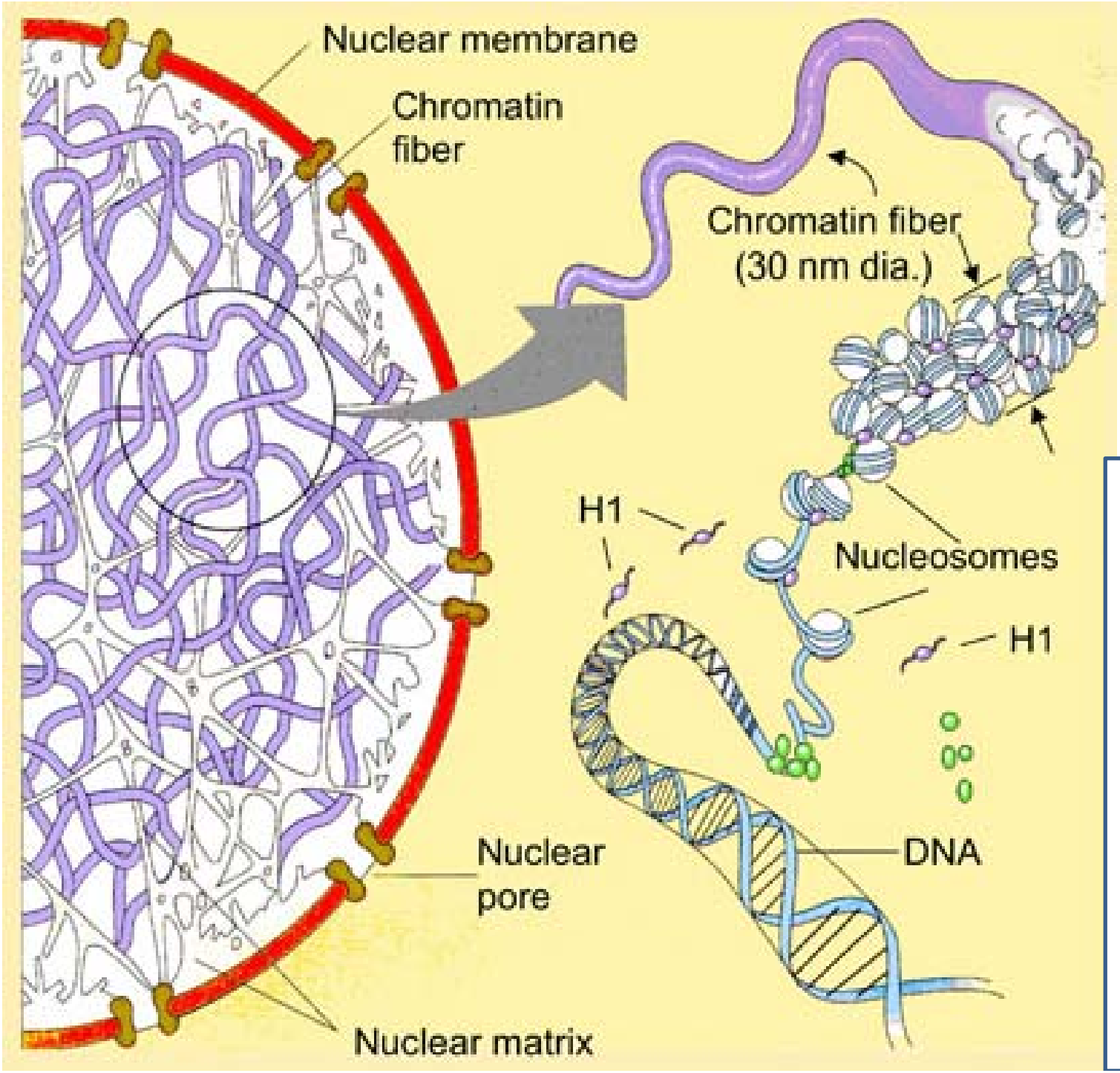


these two processes shorten the DNA nearly 50-fold

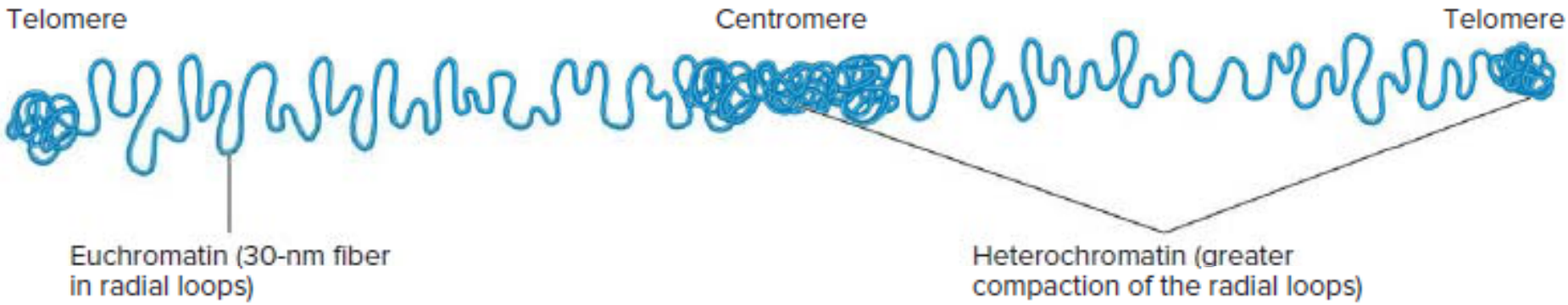
Solenoid model- the nucleosomes are packed in a helical configuration

Zig-zag model- the linker DNA forms a more irregular structure, and less contact occurs between adjacent nucleosomes

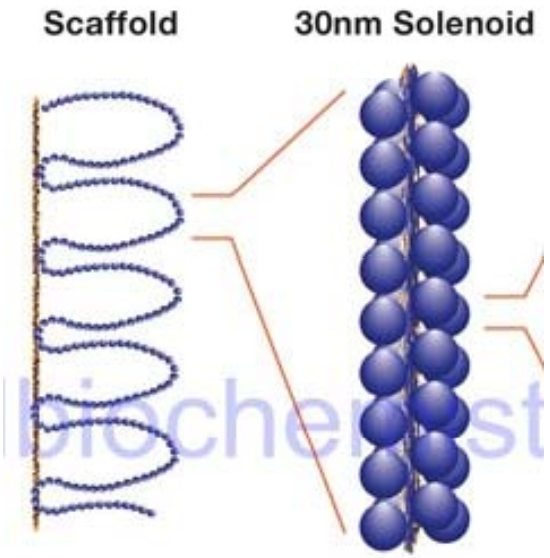
The zigzag model is consistent with more recent data regarding chromatin conformation

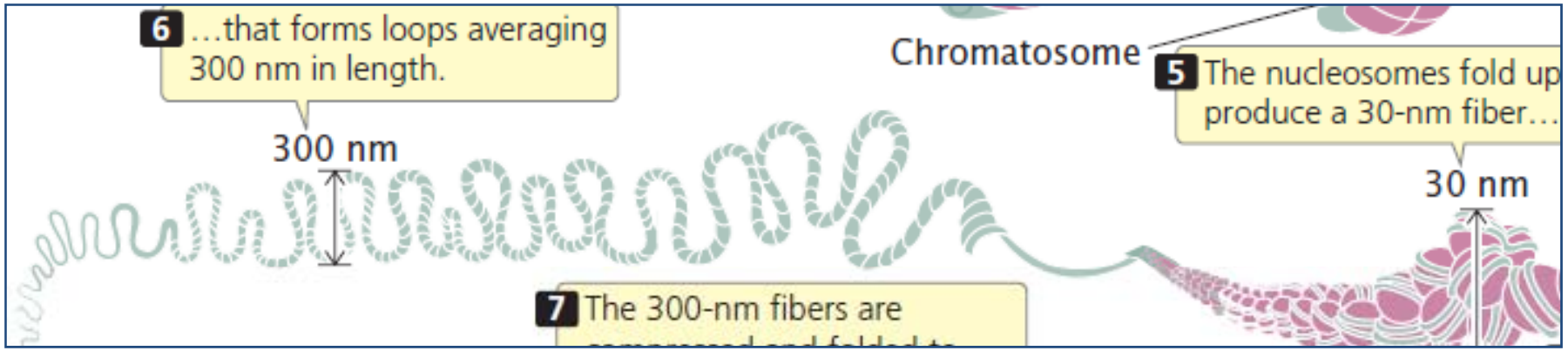


In an interphase stage, the chromatin does exist in the cell in this 30 nm fiber state.

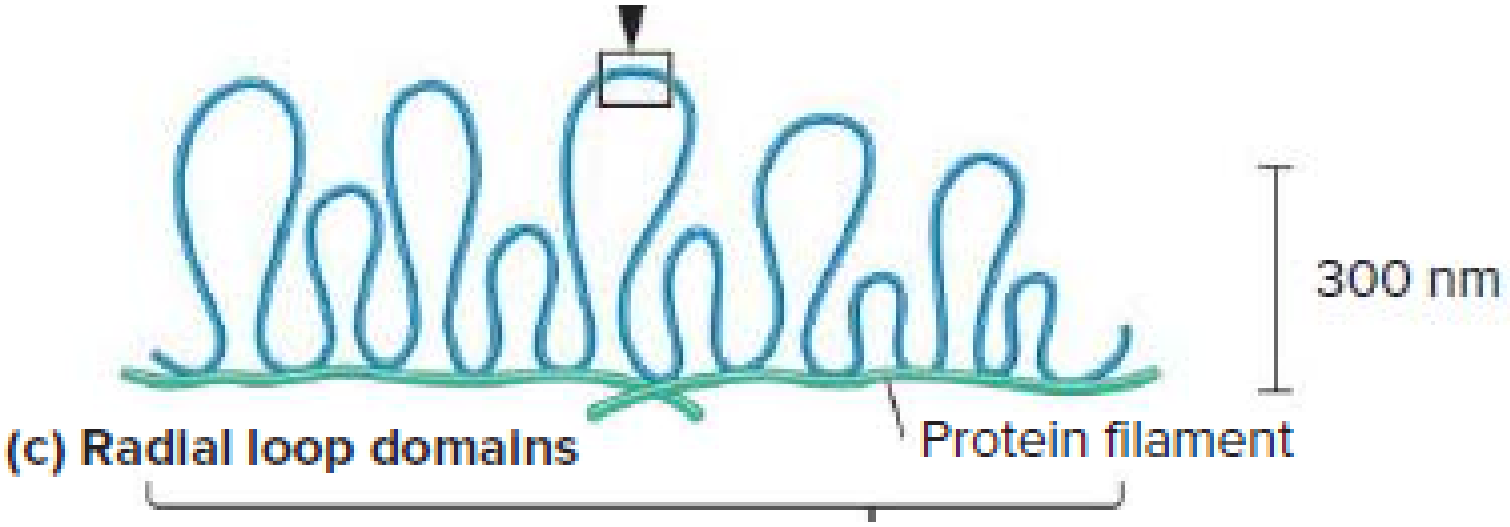

Telomere
Centromere
Telomere
Euchromatin (30-nm fiber in radial loops)
Heterochromatin (greater compaction of the radial loops)

300 nm fiber: The next-higher level of chromatin structure is a series of loops of 30-nm fibers, each anchored at its base by proteins in the nuclear scaffold. Each chromatin loop is about 300 nm in length.

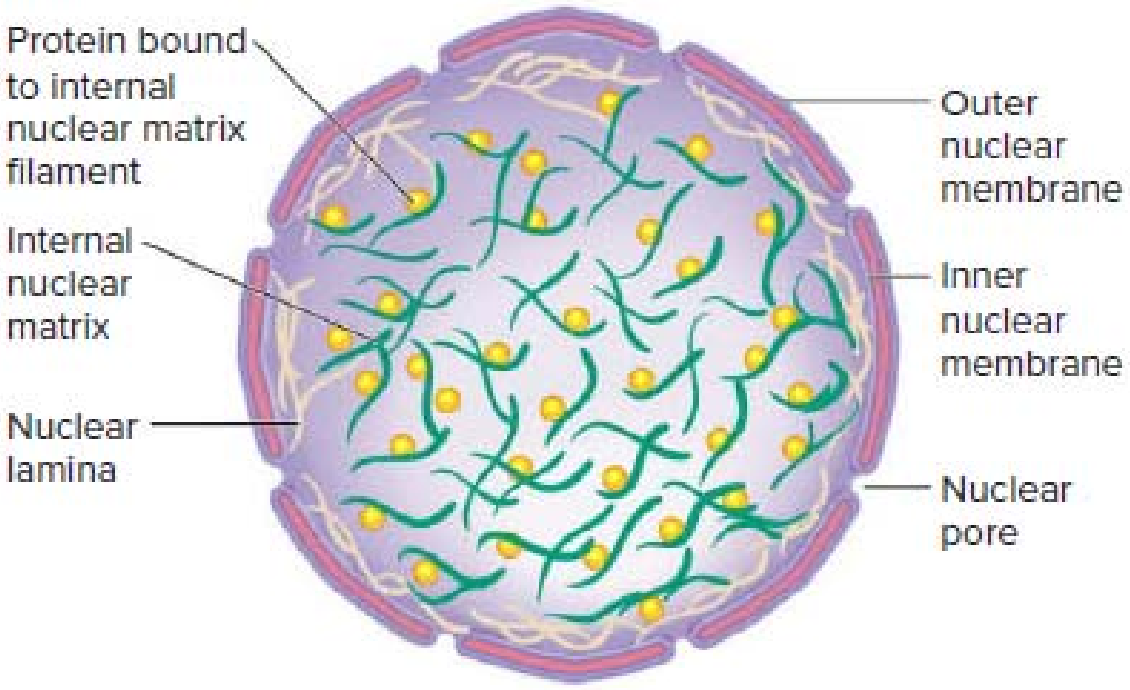




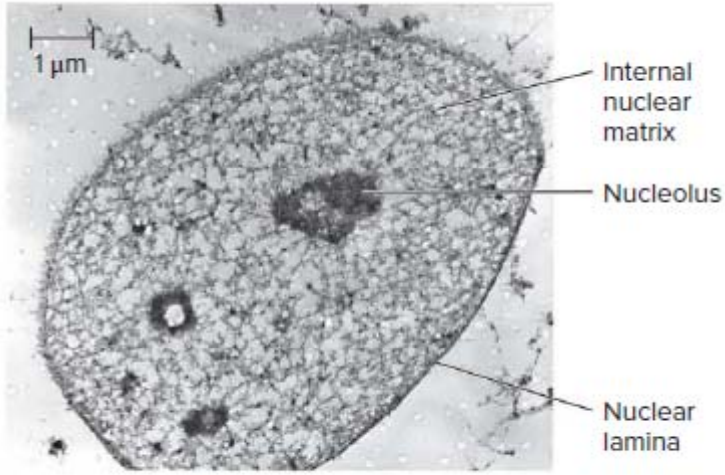
A third level of compaction involves interactions between the 30-nm fibers and a filamentous network of proteins in the nucleus called the nuclear matrix, or nuclear scaffold



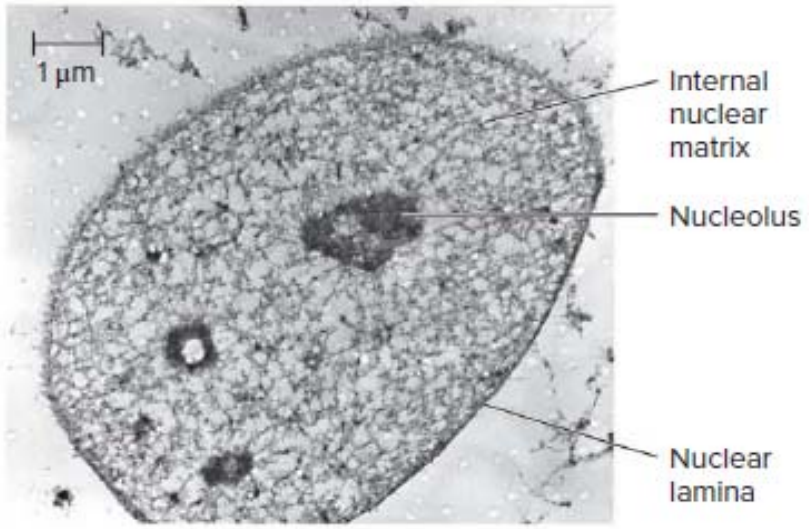
The nuclear matrix consists of two parts: the Nuclear lamina (a collection of filaments that line the inner nuclear membrane; intermediate filament proteins) and Internal nuclear matrix (fine network of irregular protein filaments)



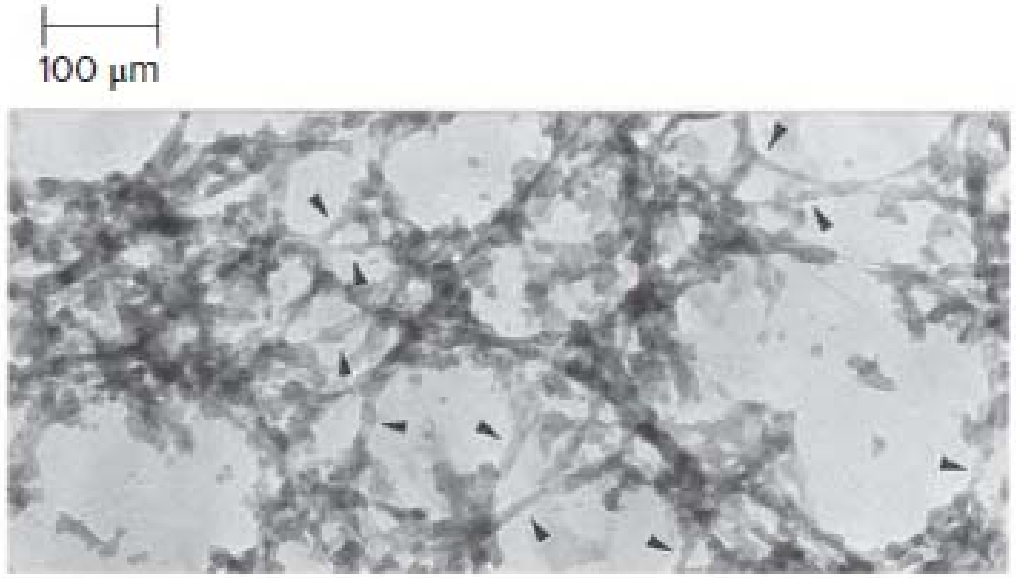
(a) Proteins that form the nuclear matrix



(b) Micrograph of nucleus with chromatin removed



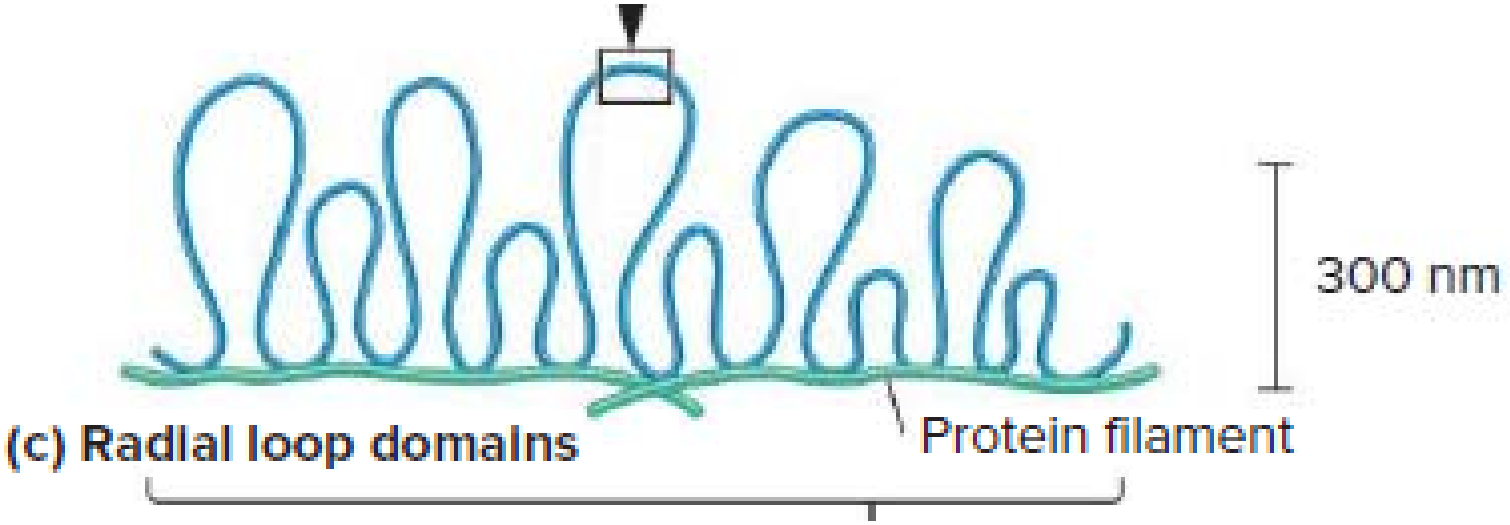
(b) Micrograph of nucleus with chromatin removed



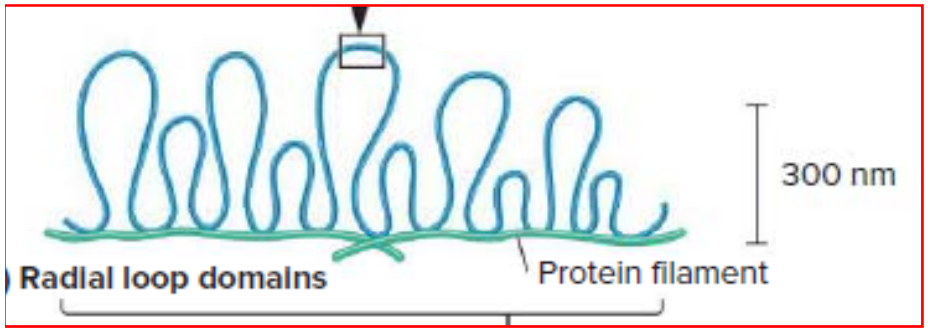
(c) Micrograph showing a close-up of nuclear matrix

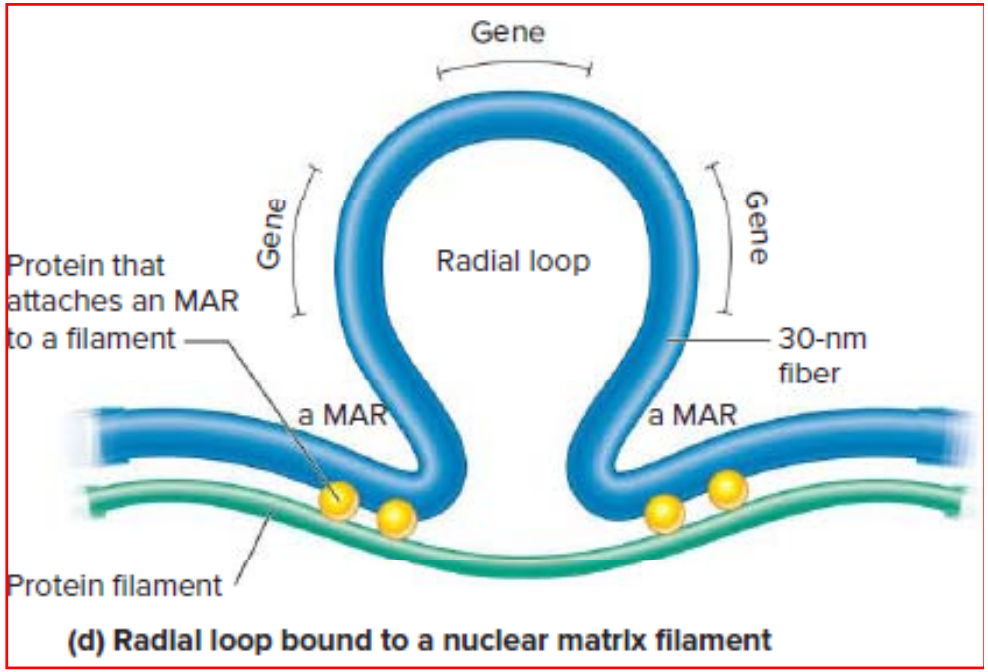
- The proteins of the nuclear matrix are involved in compacting the DNA into radial loop domains

- During interphase, chromatin is organized into loops, often 25,000–200,000 bp in size, which are anchored to the nuclear matrix



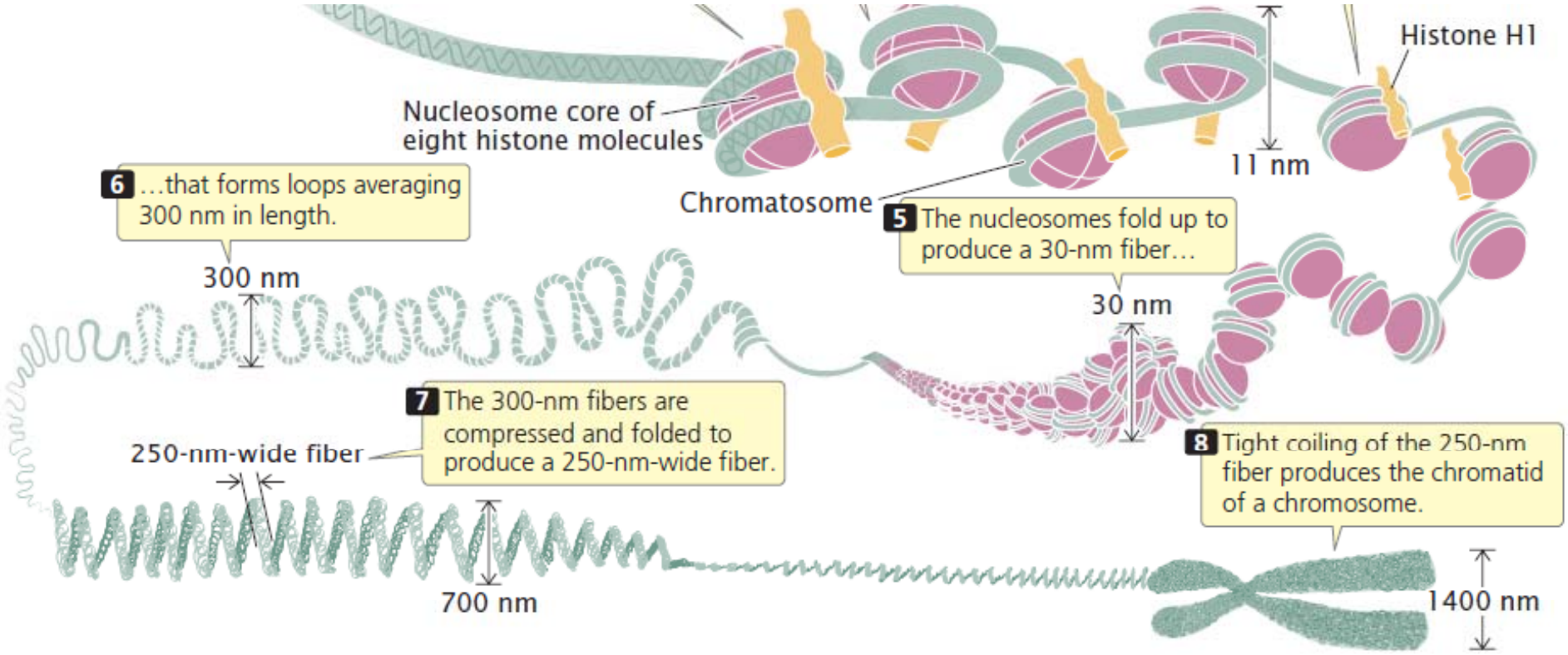
- The chromosomal DNA of eukaryotic species contains sequences called matrix- attachment regions (MARs) or scaffold-attachment regions (SARs), which are interspersed at regular intervals throughout the genome.
- The MARs bind to specific proteins in the nuclear matrix, thus forming chromosomal loops

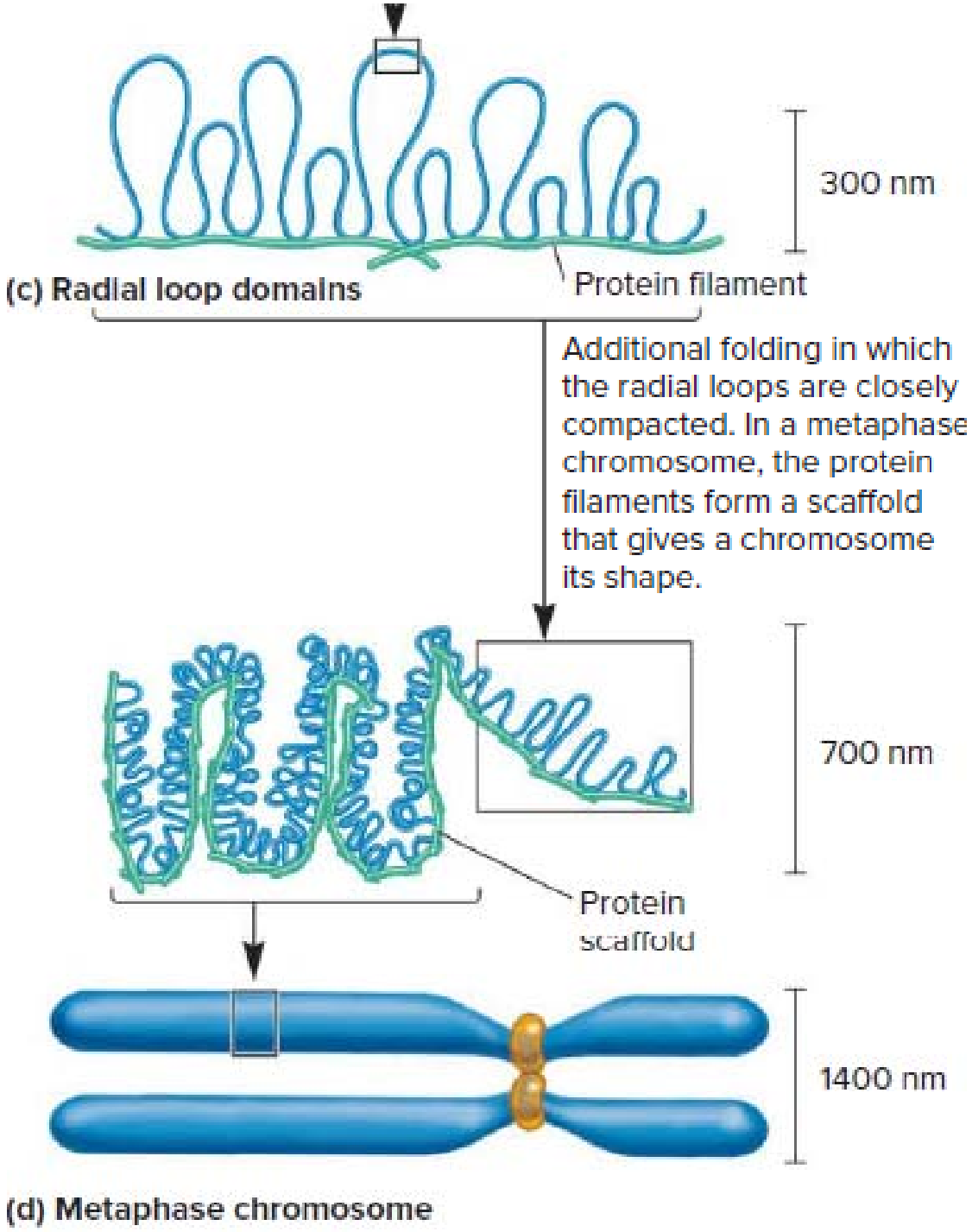




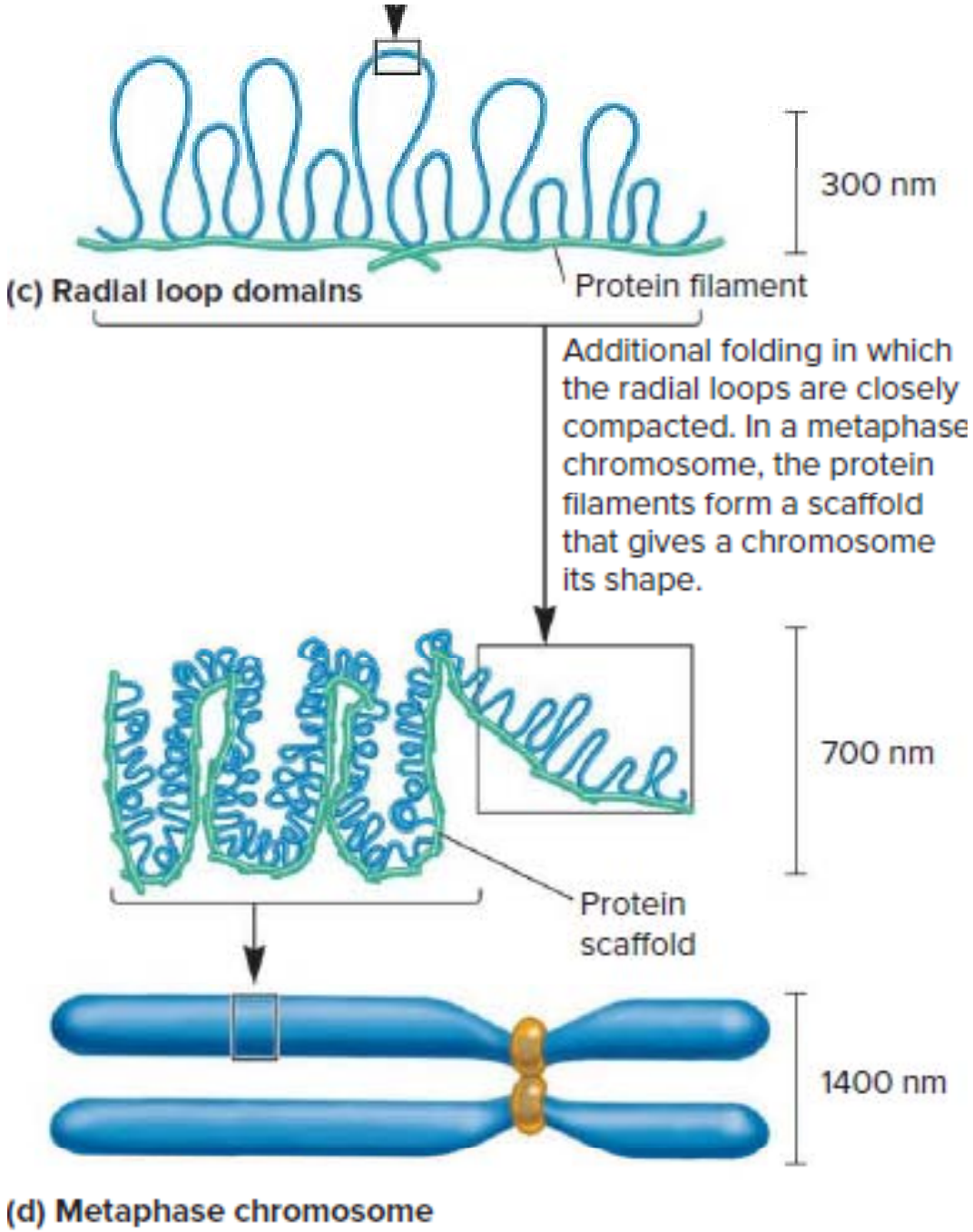
(d) Radial loop bound to a nuclear matrix filament

Chromatid: Tight coiling of the 250 nm fiber produces the 700 nm chromatid of a chromosome.





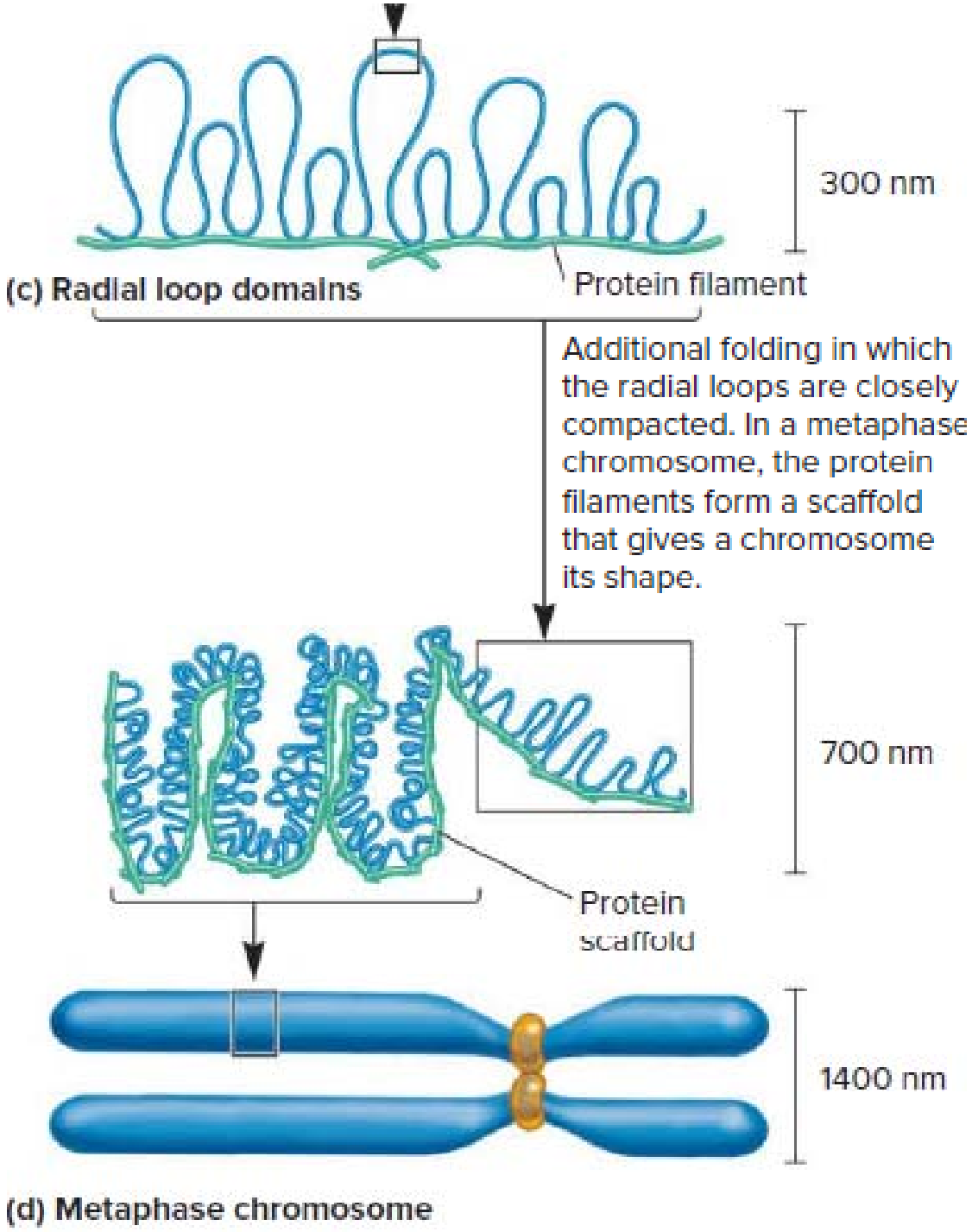
When cells prepare to divide, the protein filaments come closer together and form a more compact scaffold for anchoring the radial loops.



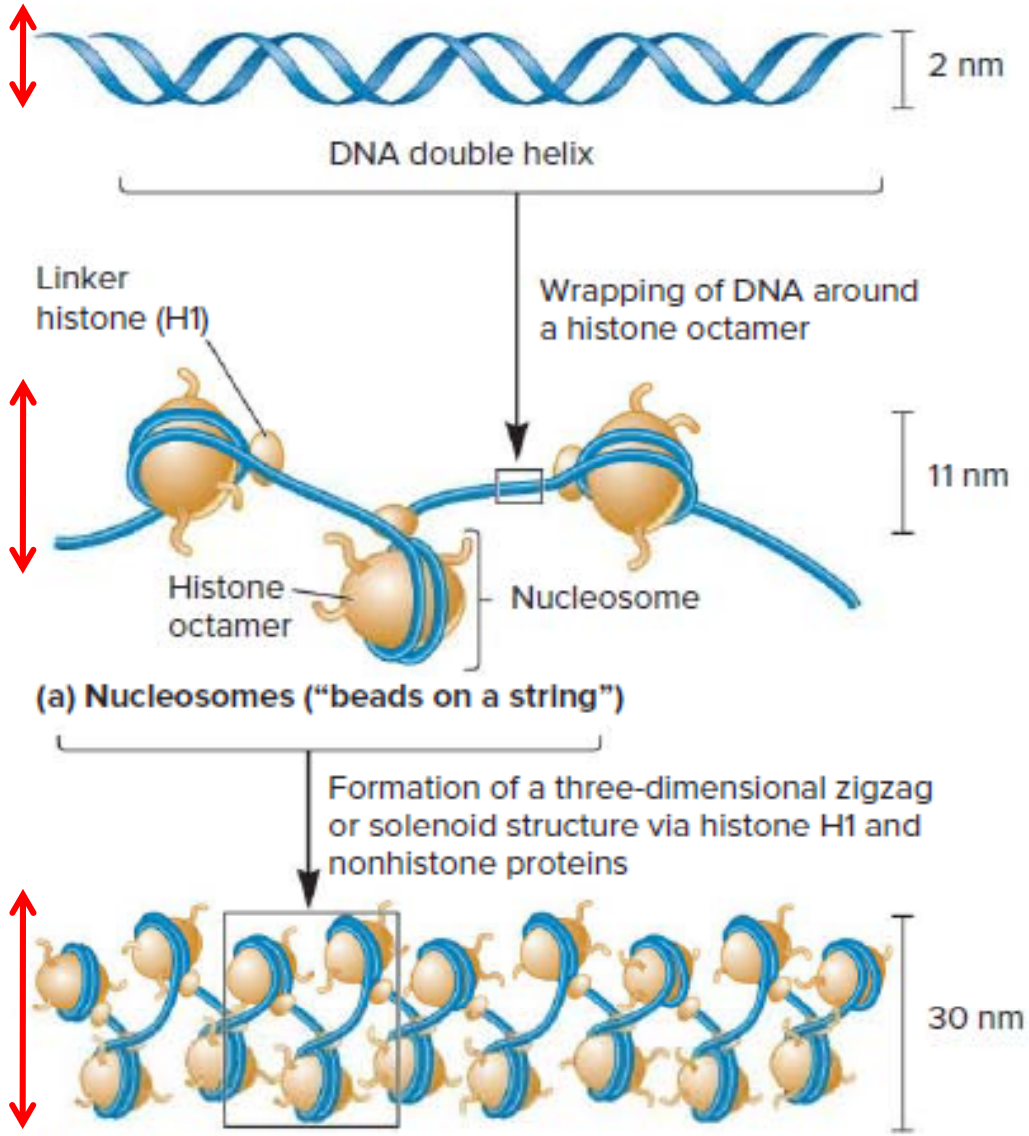
This additional level of compaction greatly shortens the overall length of a chromosome and produces a diameter of approximately 700 nm.

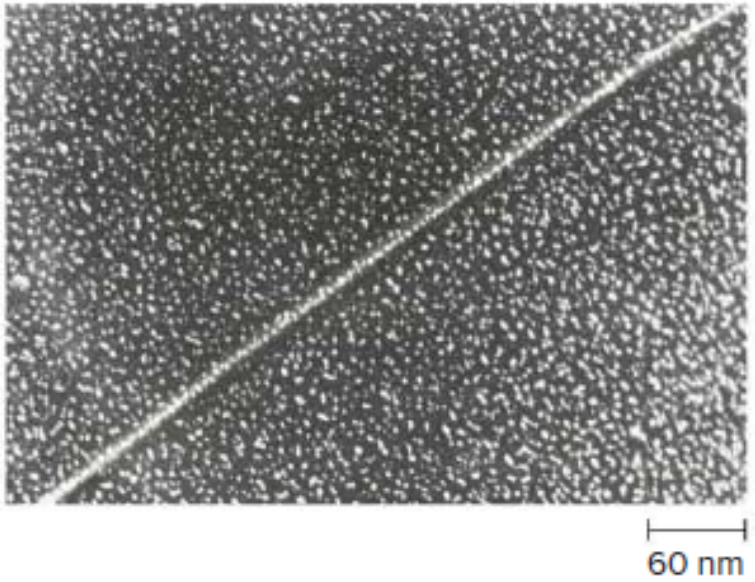
By the end of prophase, sister chromatids are highly compacted.

Two parallel chromatids have a diameter of approximately 1400 nm and a much shorter length than interphase chromosomes.



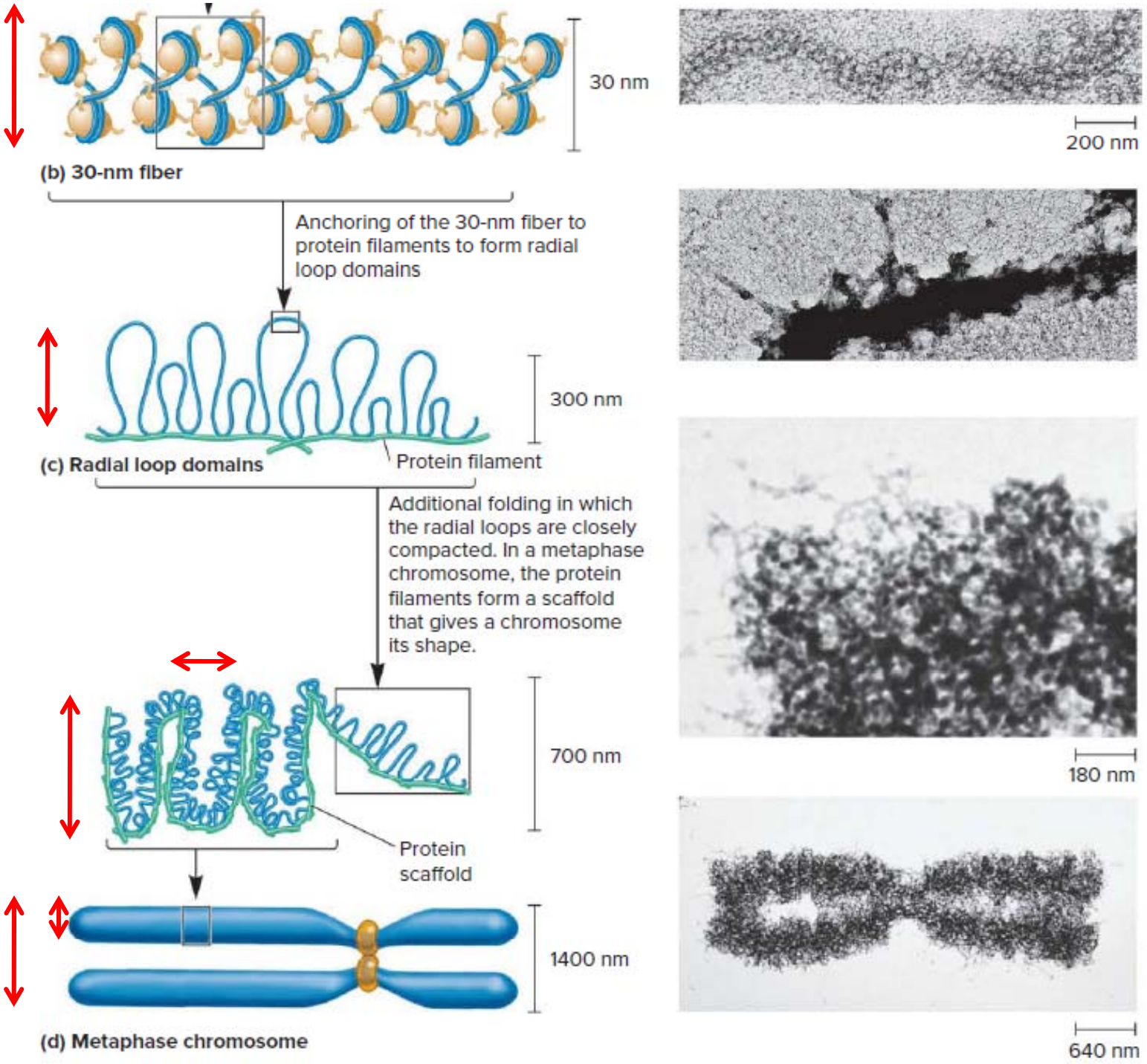
Additional folding in which the radial loops are closely compacted. In a metaphase chromosome, the protein filaments form a scaold that gives a chromosome its shape.

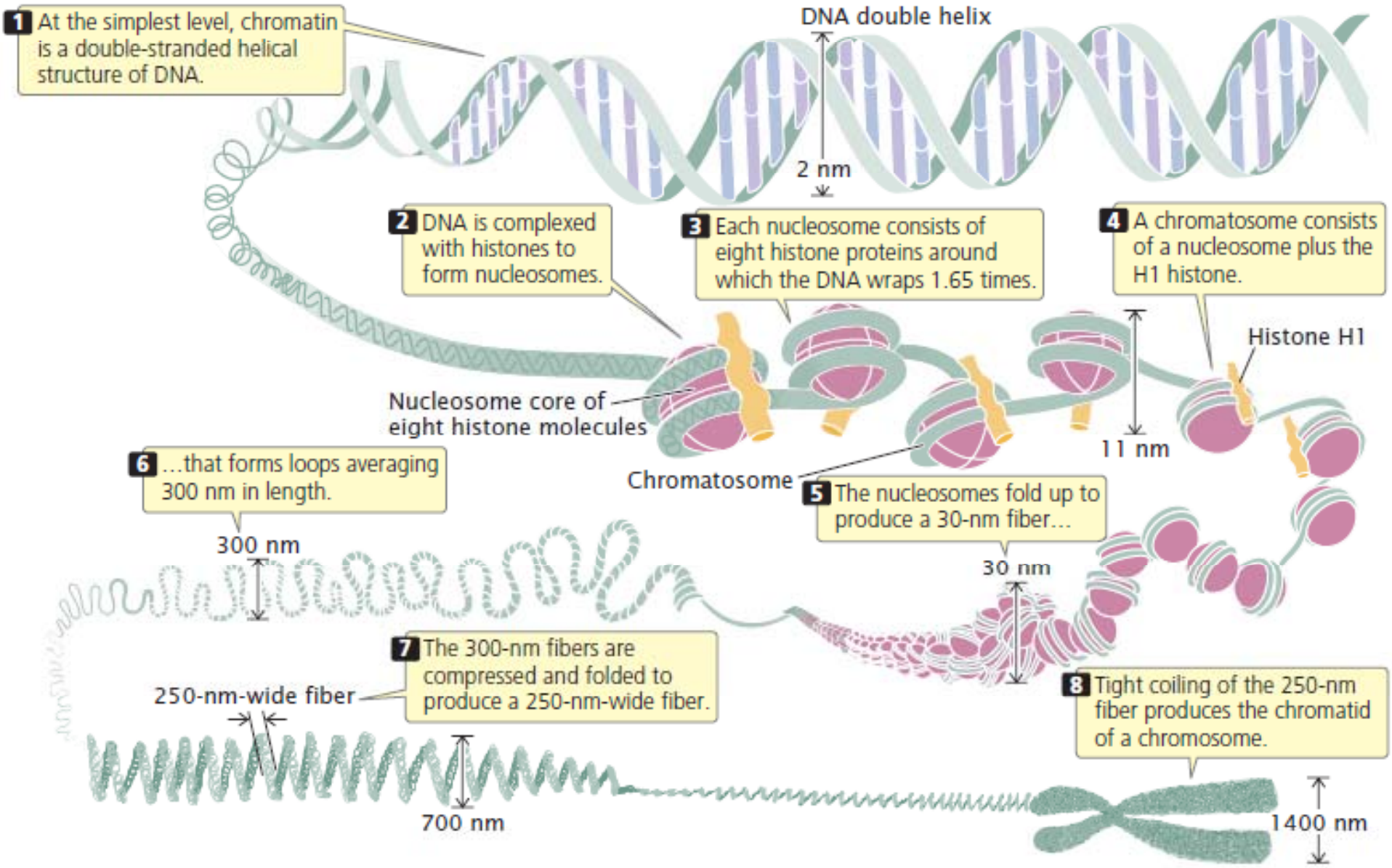

2 nm
DNA double helix
Linker
histone (H1)
Wrapping of DNA around
a histone octamer
11 nm
Histone
octamer
Nucleosome
(a) Nucleosomes ("beads on a string")
Formation of a three-dimensional zigzag
or solenoid structure via histone H1 and
nonhistone proteins
30 nm
(b) 30-nm fiber

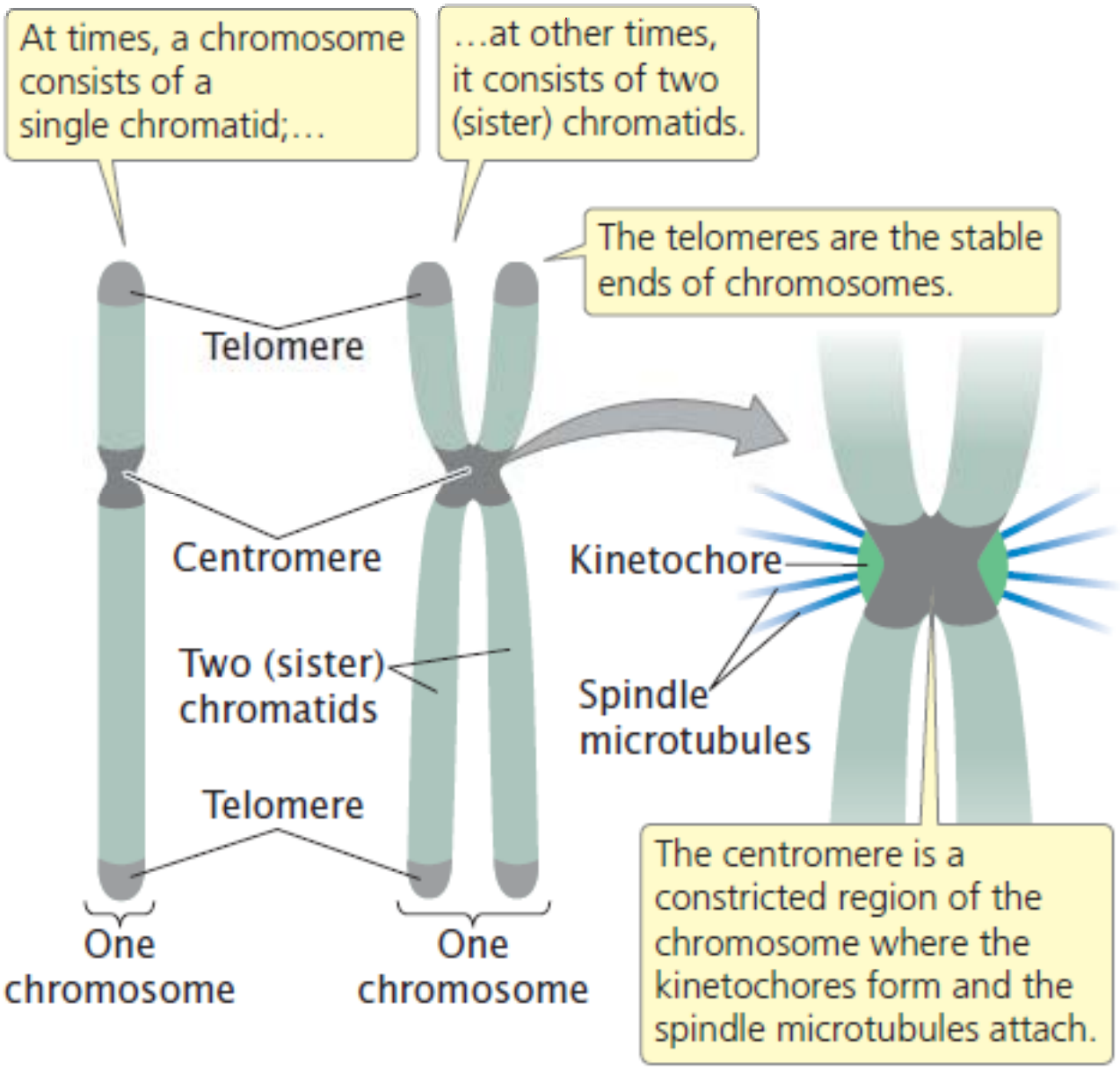

60 nm

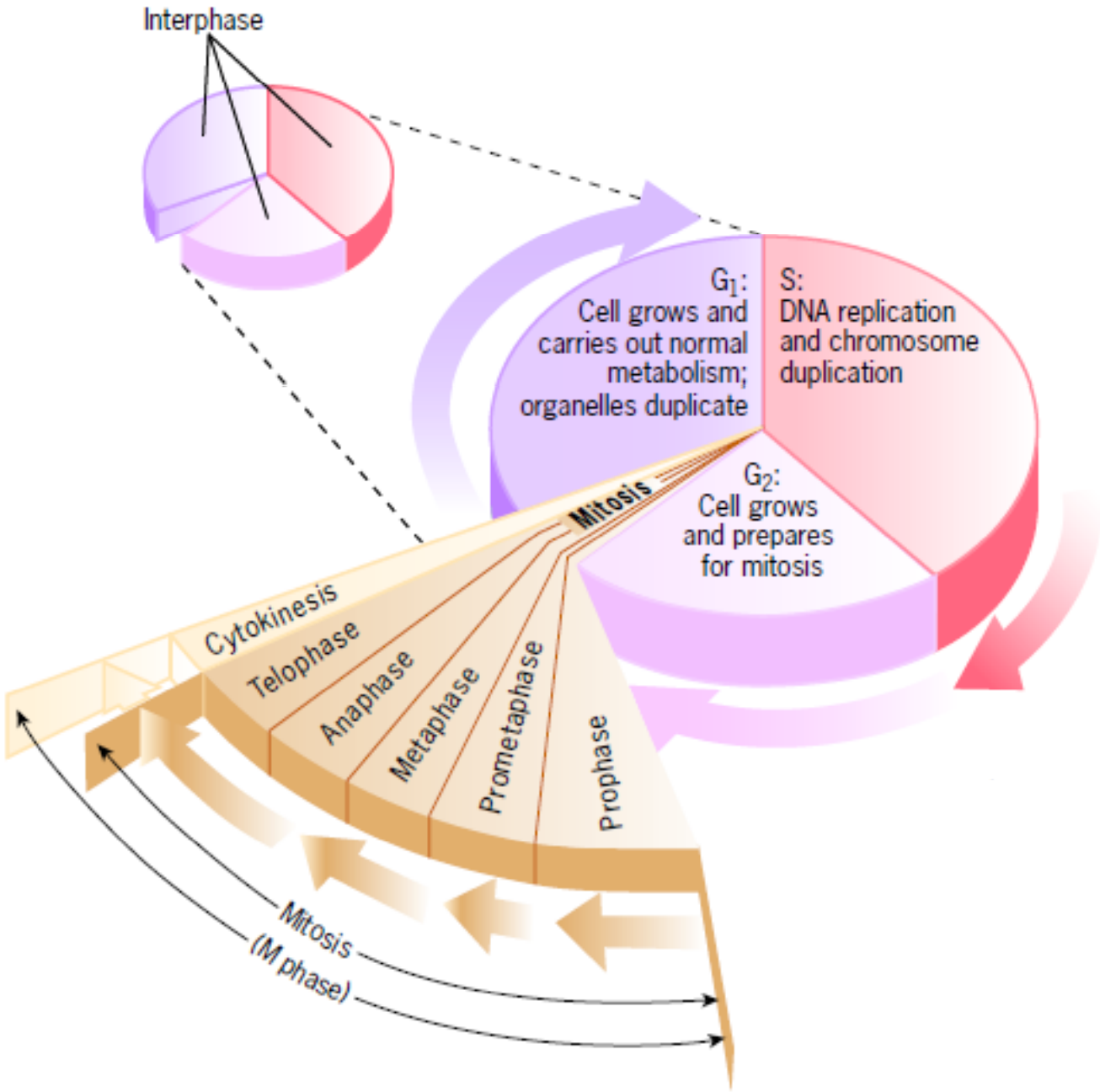
33 nm

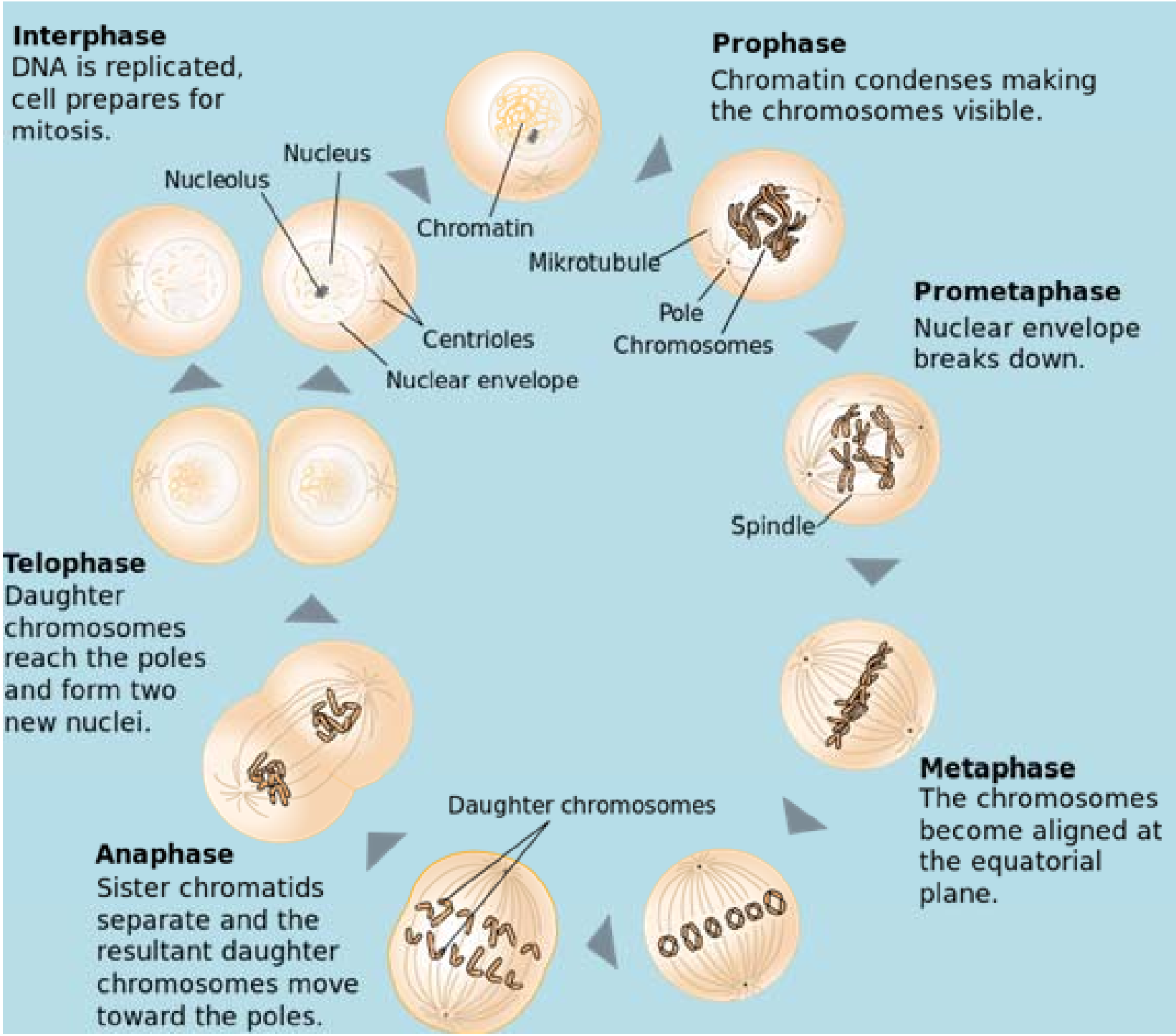
200 nm


30 nm
(b) 30-nm fiber
Anchoring of the 30-nm fiber to protein filaments to form radial loop domains
300 nm
(c) Radial loop domains
Protein filament
Additional folding in which the radial loops are closely compacted. In a metaphase chromosome, the protein filaments form a scaffold that gives a chromosome its shape.
700 nm
Protein scaffold
1400 nm
(d) Metaphase chromosome
200 nm
180 nm
640 nm


1 At the simplest level, chromatin is a double-stranded helical structure of DNA.
DNA double helix
2 nm
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.
Histone H1
Nucleosome core of eight histone molecules
11 nm
Chromatosome
5 The nucleosomes fold up to produce a 30-nm fiber...
30 nm
6 ...that forms loops averaging 300 nm in length.
300 nm
7 The 300-nm fibers are compressed and folded to produce a 250-nm-wide fiber.
250-nm-wide fiber
8 Tight coiling of the 250-nm fiber produces the chromatid of a chromosome.
700 nm
1400 nm

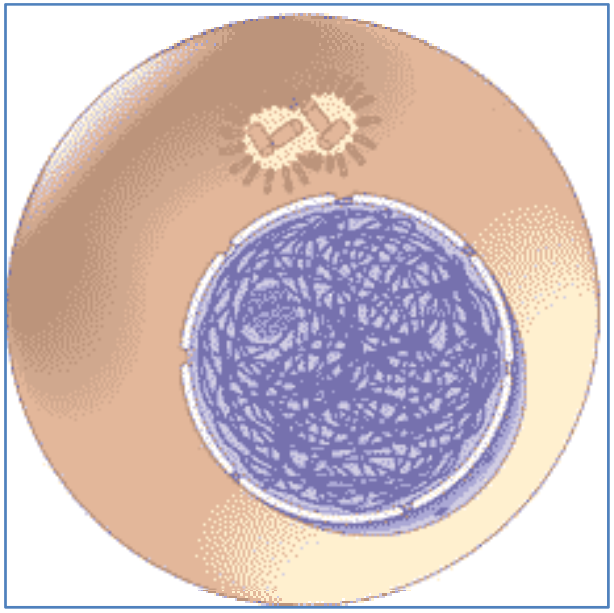

At times, a chromosome consists of a single chromatid;…
…at other times, it consists of two (sister) chromatids.
The telomeres are the stable ends of chromosomes.
Telomere
Centromere
Two (sister) chromatids
Telomere
One chromosome
One chromosome
Kinetochore
Spindle microtubules
The centromere is a constricted region of the chromosome where the kinetochores form and the spindle microtubules attach.

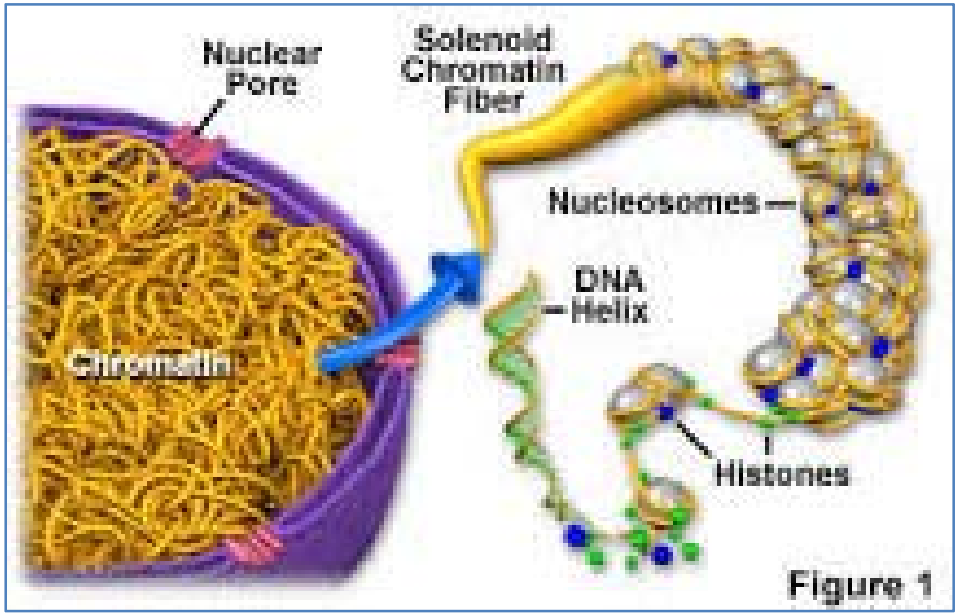

Interphase
G1:
Cell grows and carries out normal metabolism; organelles duplicate
S:
DNA replication and chromosome duplication
G2:
Cell grows and prepares for mitosis
Mitosis
Prophase
Prometaphase
Metaphase
Anaphase
Telophase
Cytokinesis
Mitosis
(M phase)

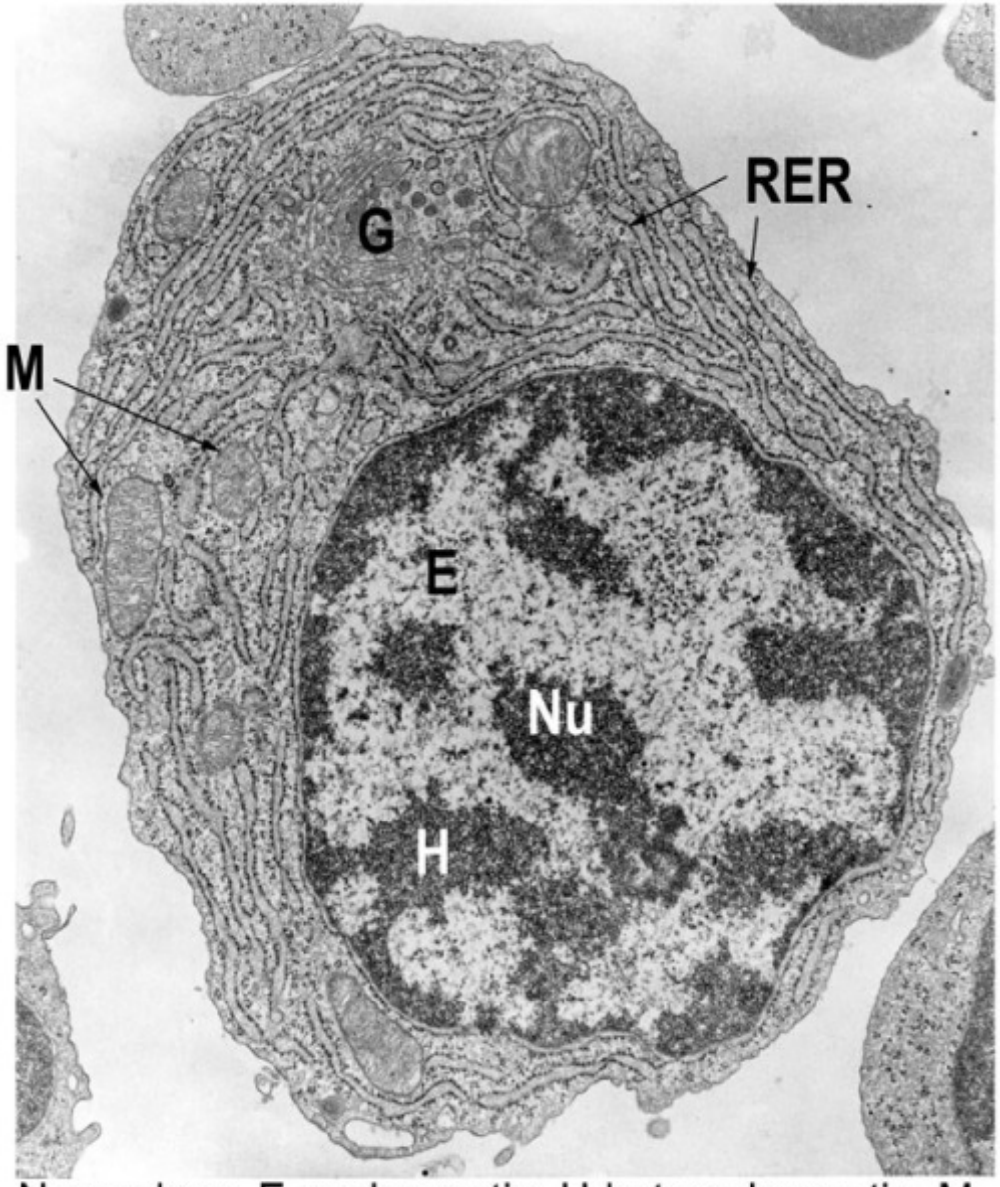

Interphase
DNA is replicated, cell prepares for mitosis.
Nucleus
Nucleolus
Chromatin
Centrioles
Nuclear envelope
Prophase
Chromatin condenses making the chromosomes visible.
Mikrotubule
Pole
Chromosomes
Prometaphase
Nuclear envelope breaks down.
Spindle
Metaphase
The chromosomes become aligned at the equatorial plane.
Daughter chromosomes
Anaphase
Sister chromatids separate and the resultant daughter chromosomes move toward the poles.
Telophase
Daughter chromosomes reach the poles and form two new nuclei.

Heterochromatin and Euchromatin

After mitosis has been completed, most of the chromatin in highly compacted mitotic chromosomes returns to its diffuse interphase condition.

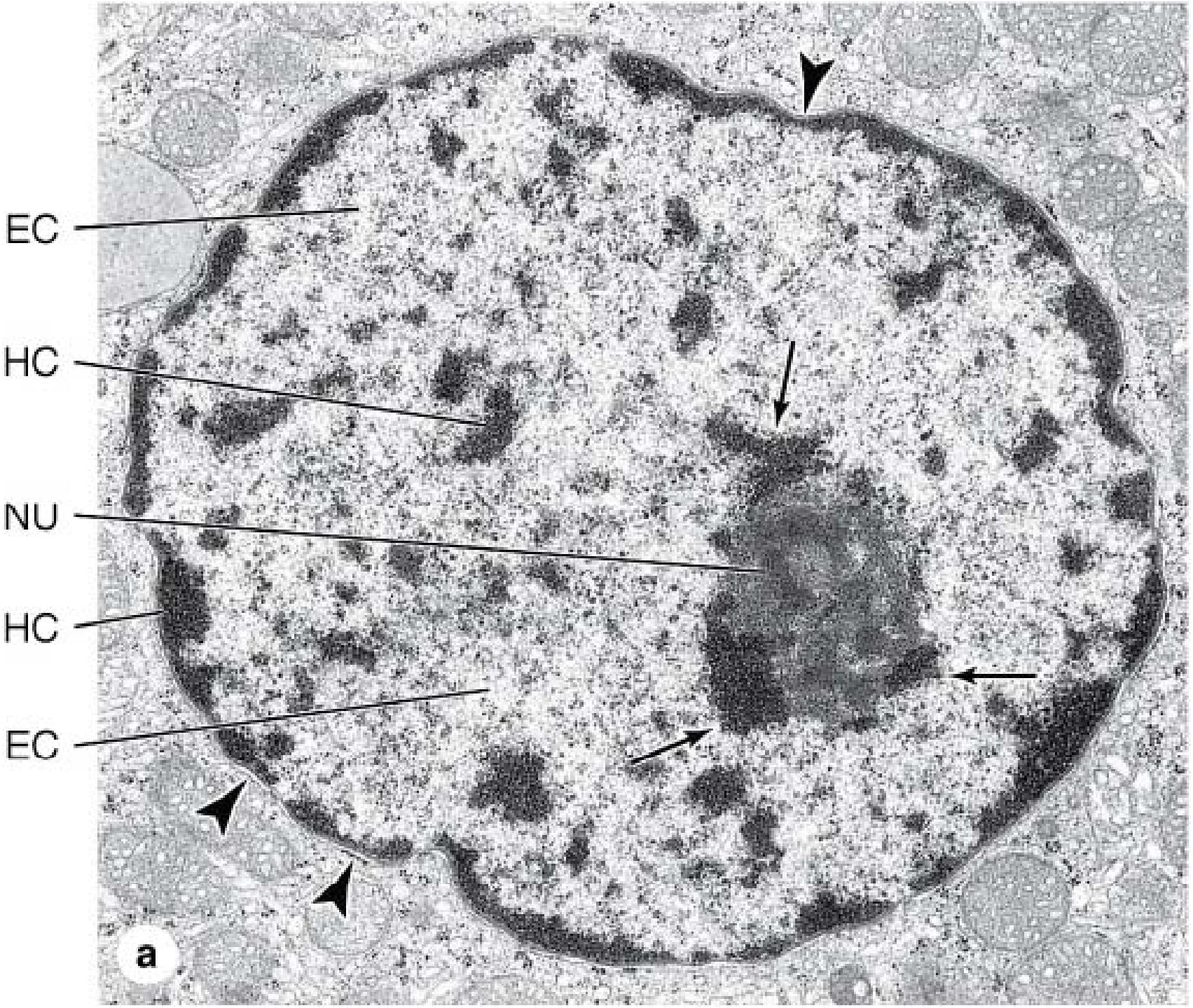


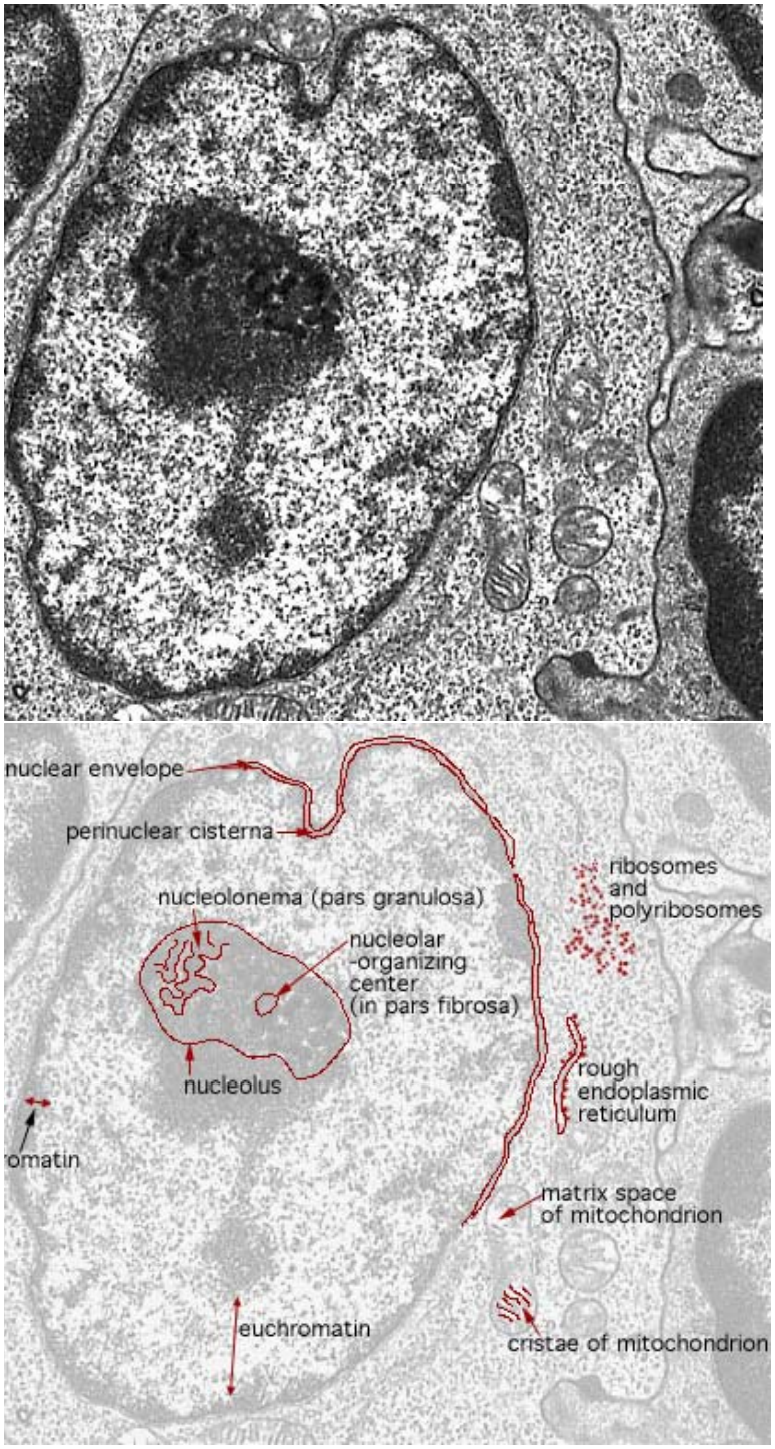


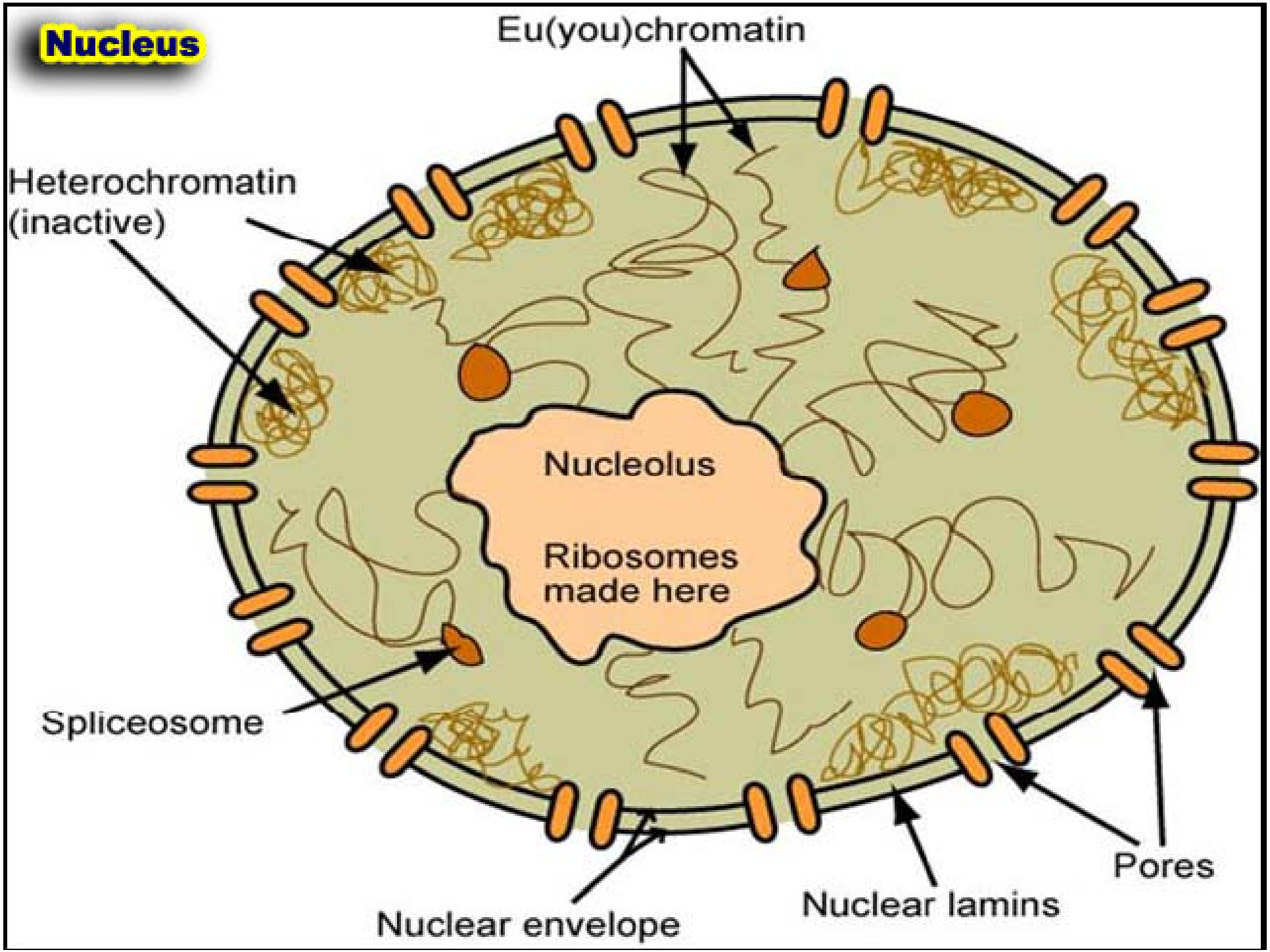


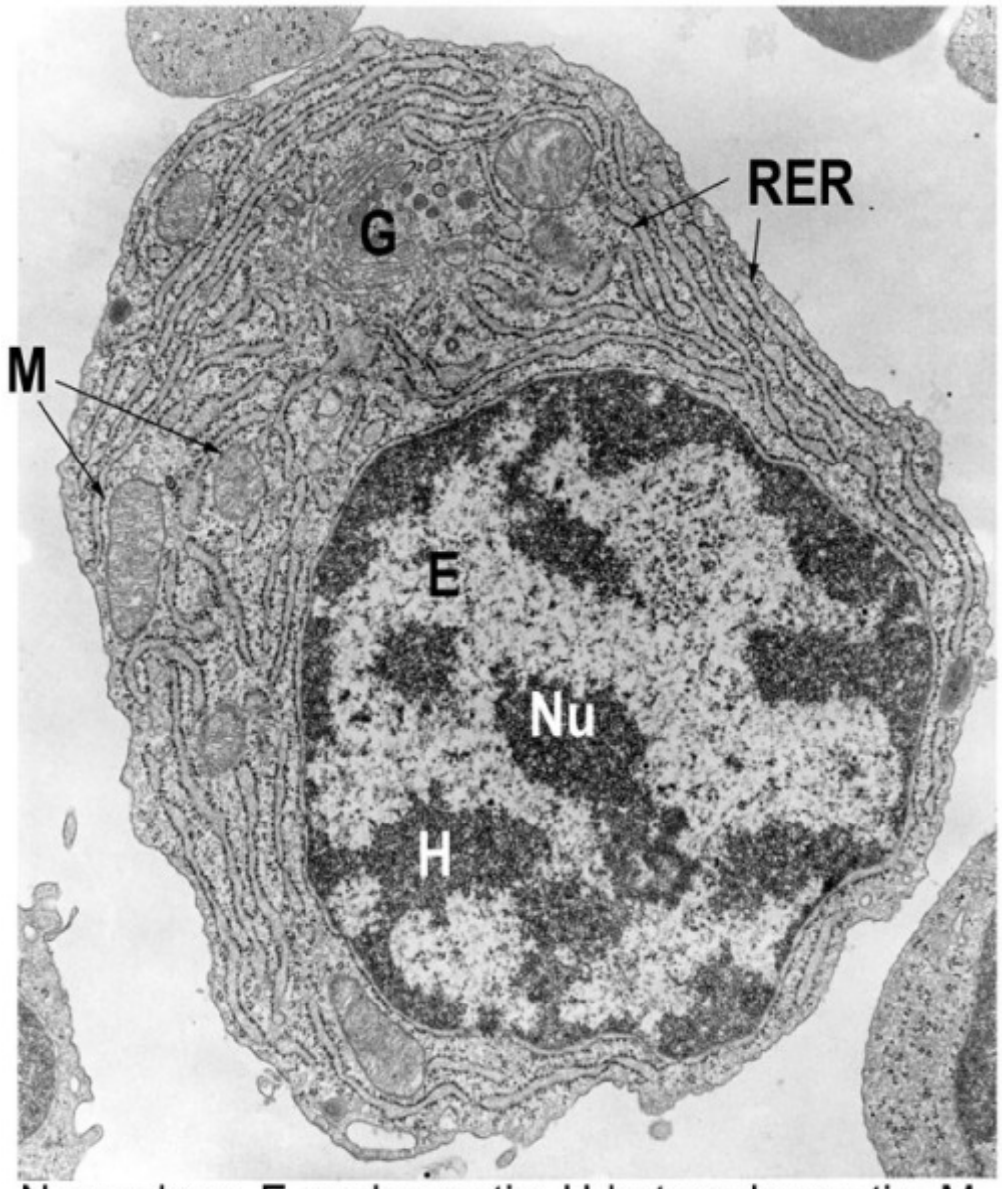
Nu-nucleus, E-euchromatin, H-heterochromatin, M-mitochondria, RER-rough endoplasmic reticulum, G-golgi complex

Approximately 10 percent of the chromatin remains in a condensed and compacted form throughout interphase.


EC
HC
NU
HC
EC
a

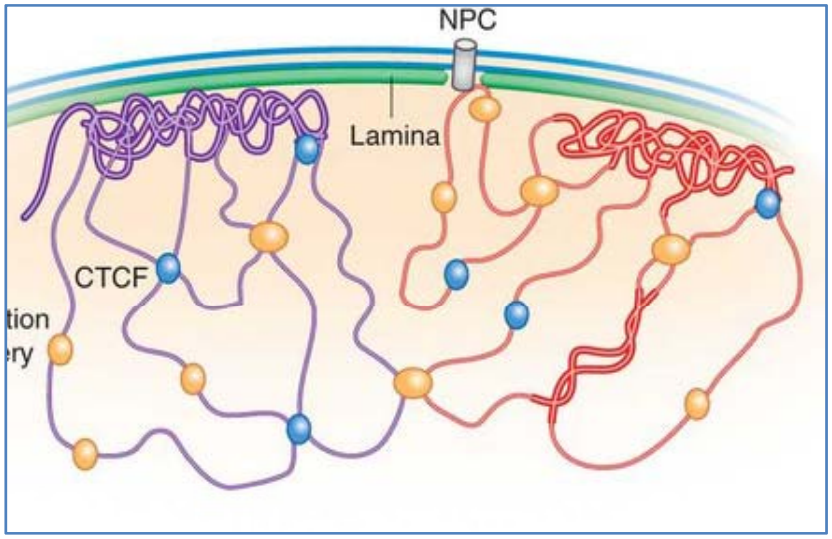

nuclear envelope
perinuclear cisterna
nucleolonema (pars granulosa)
nucleolar
-organizing
center
(in pars fibrosa)
nucleolus
omatin
ribosomes
and
polyribosomes
rough
endoplasmic
reticulum
matrix space
of mitochondrion
euchromatin
cristae of mitochondrion

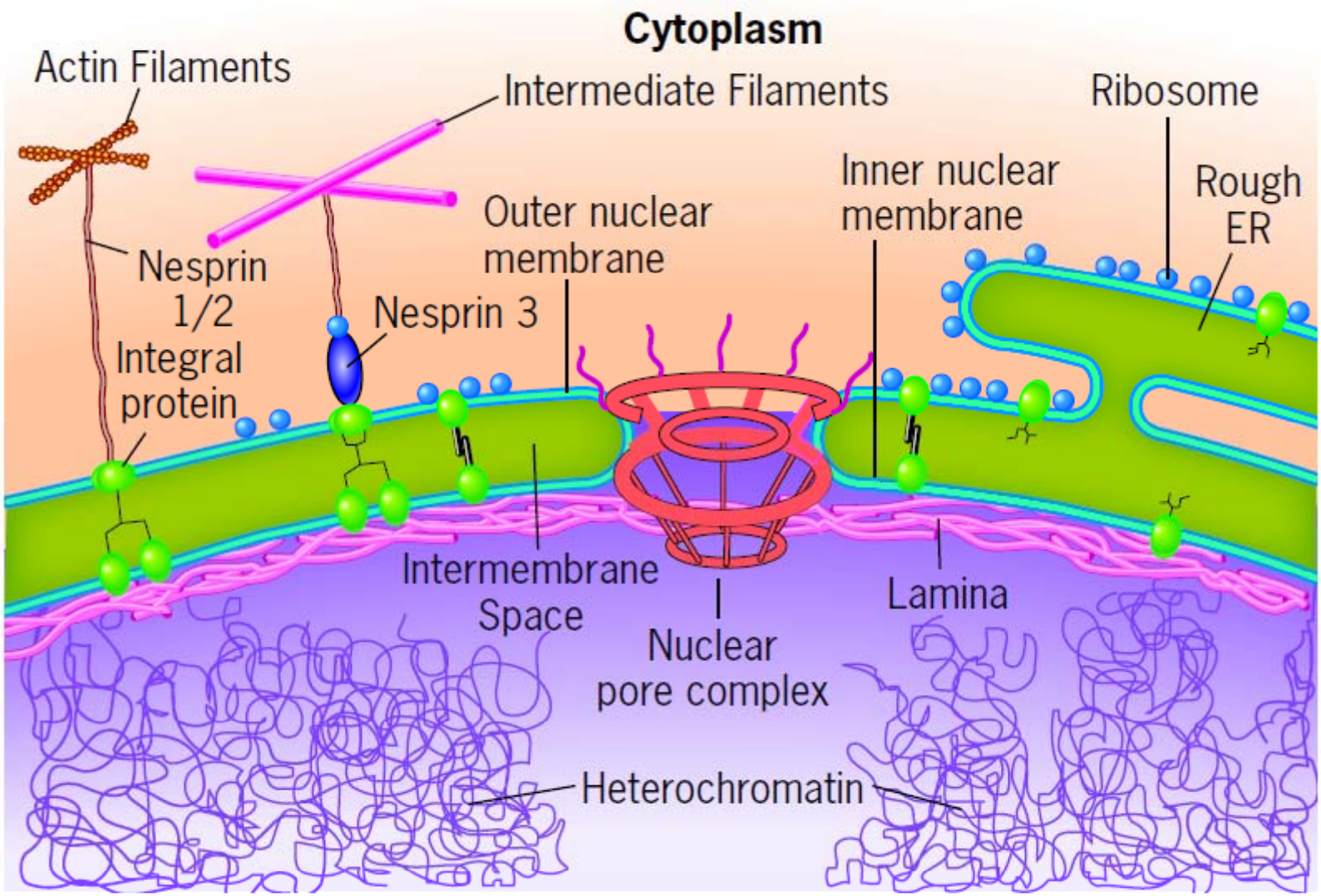

Nucleus
Eu(you)chromatin
Heterochromatin
(inactive)
Nucleolus
Ribosomes
made here
Spliceosome
Nuclear envelope
Nuclear lamins
Pores

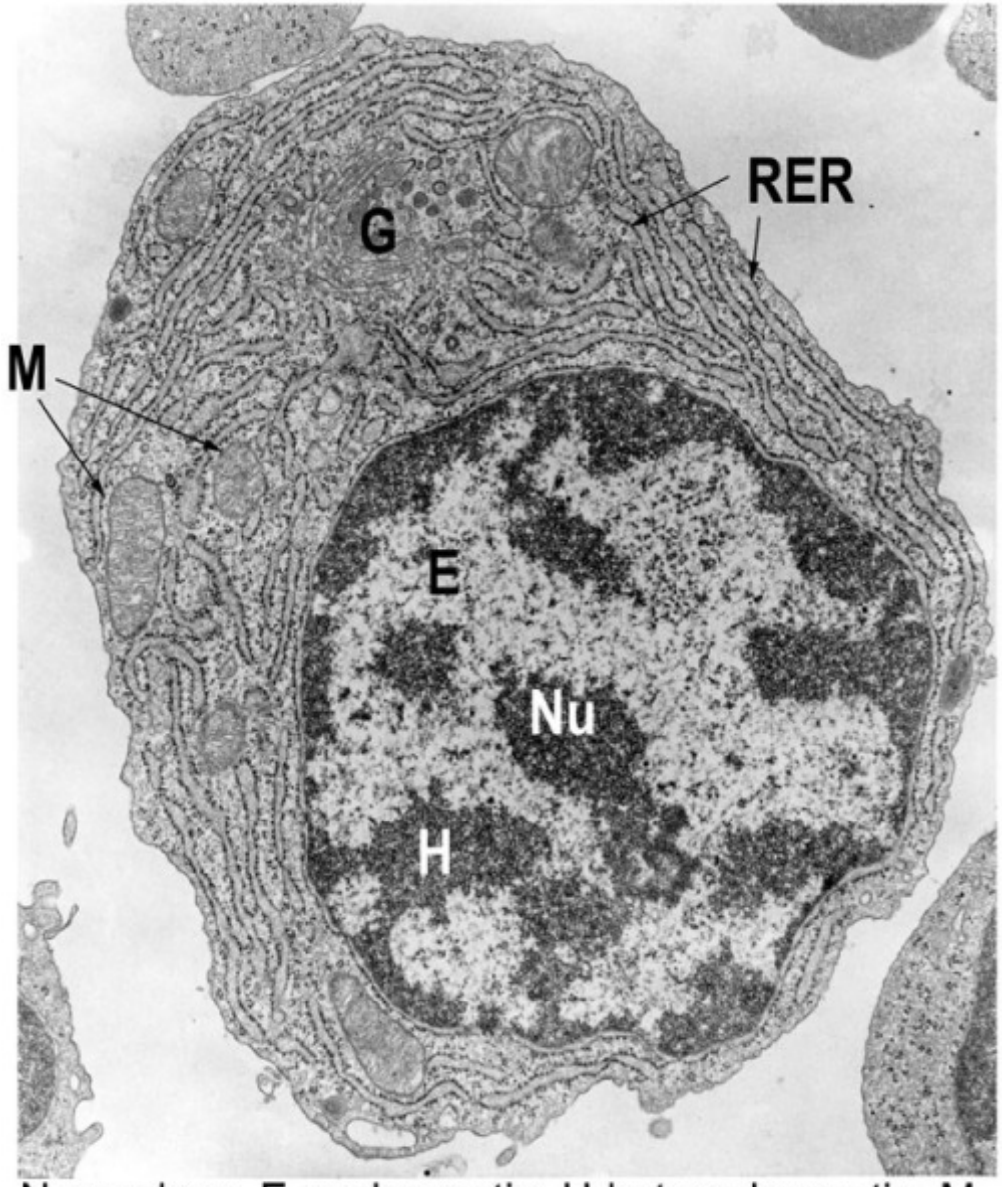


Nu-nucleus, E-euchromatin, H-heterochromatin, M-mitochondria, RER-rough endoplasmic reticulum, G-golgi complex

This compacted, densely stained chromatin is typically concentrated near the periphery of the nucleus, often in proximity with the nuclear lamina.



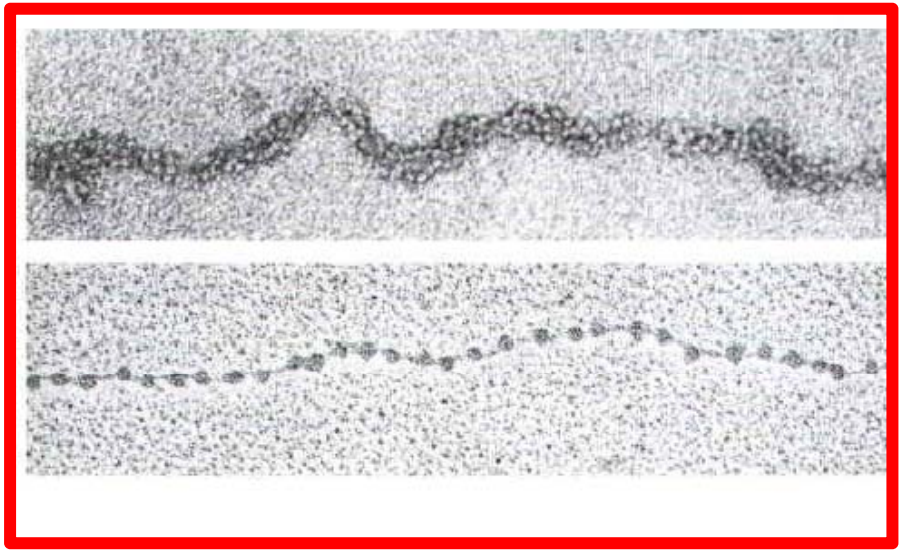

Cytoplasm
Actin Filaments
Intermediate Filaments
Ribosome
Inner nuclear membrane
Rough ER
Outer nuclear membrane
Nesprin 1/2
Nesprin 3
Integral protein
Intermembrane Space
Nuclear pore complex
Lamina
Heterochromatin



Nu-nucleus, E-euchromatin, H-heterochromatin, M-mitochondria, RER-rough endoplasmic reticulum, G-golgi complex

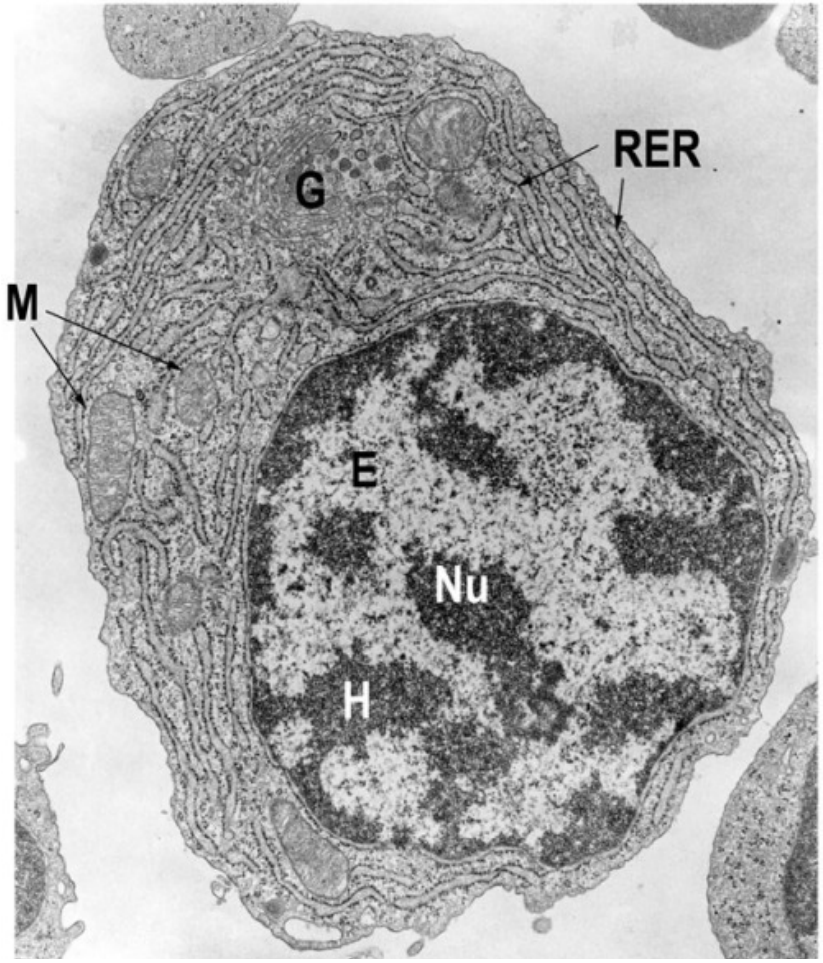
Chromatin that remains compacted during interphase is called heterochromatin;

But euchromatin returns to a dispersed, active state.

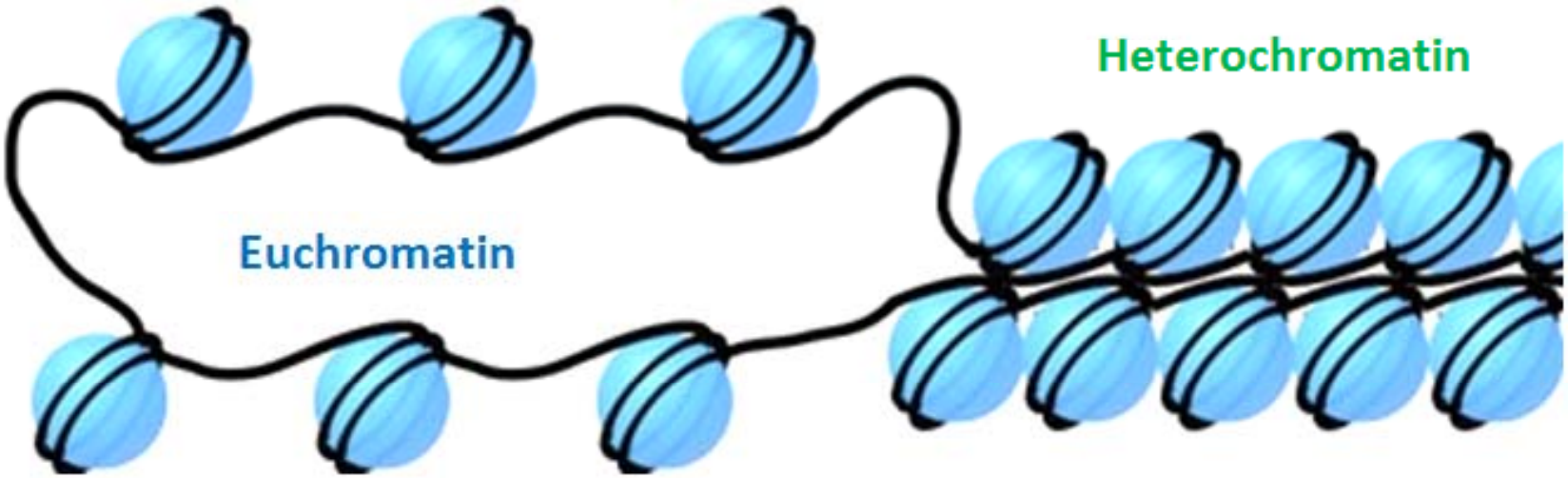


Heterochromatin and Euchromatin

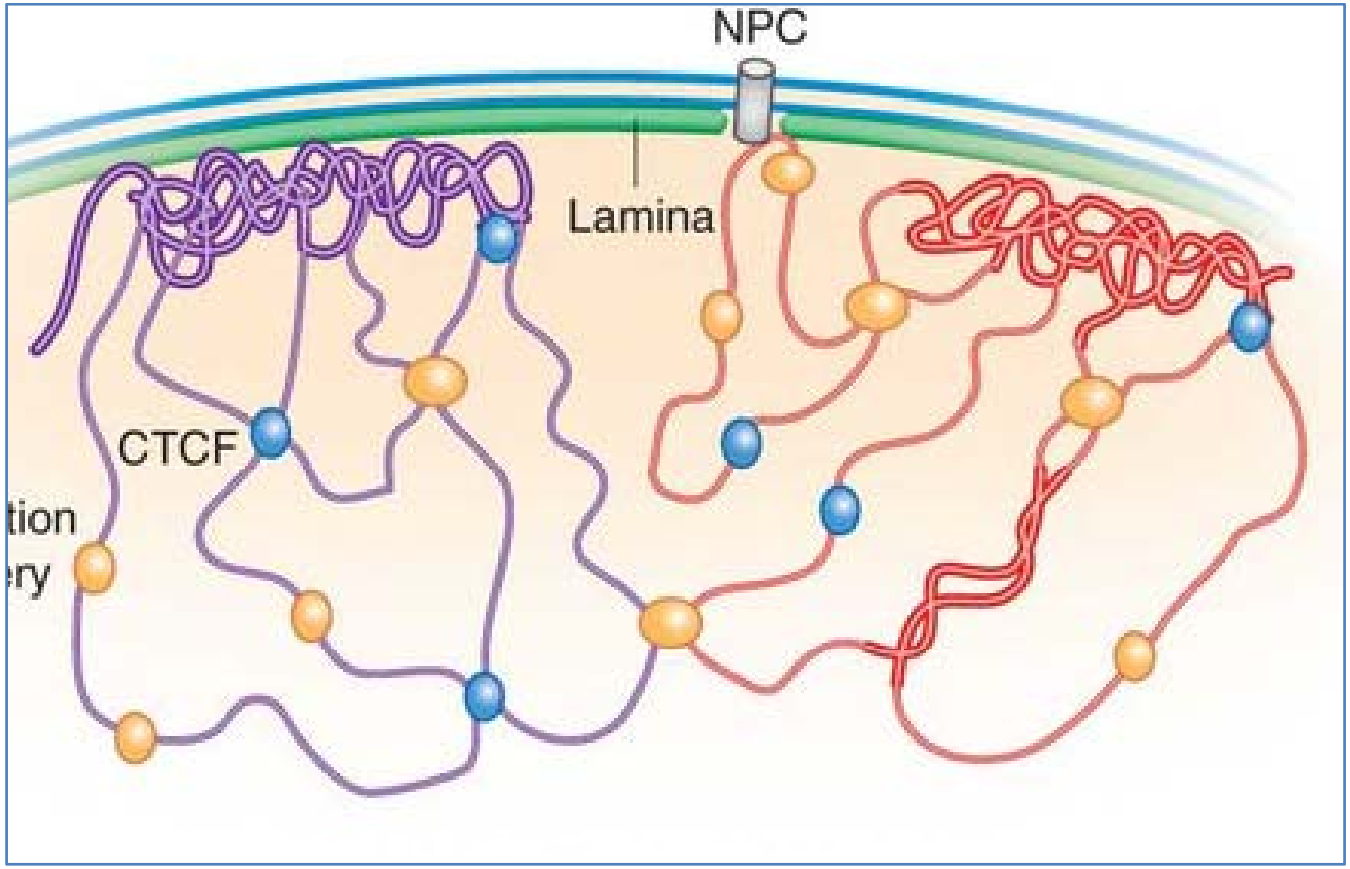
The two basic types of chromatin are euchromatin and heterochromatin.



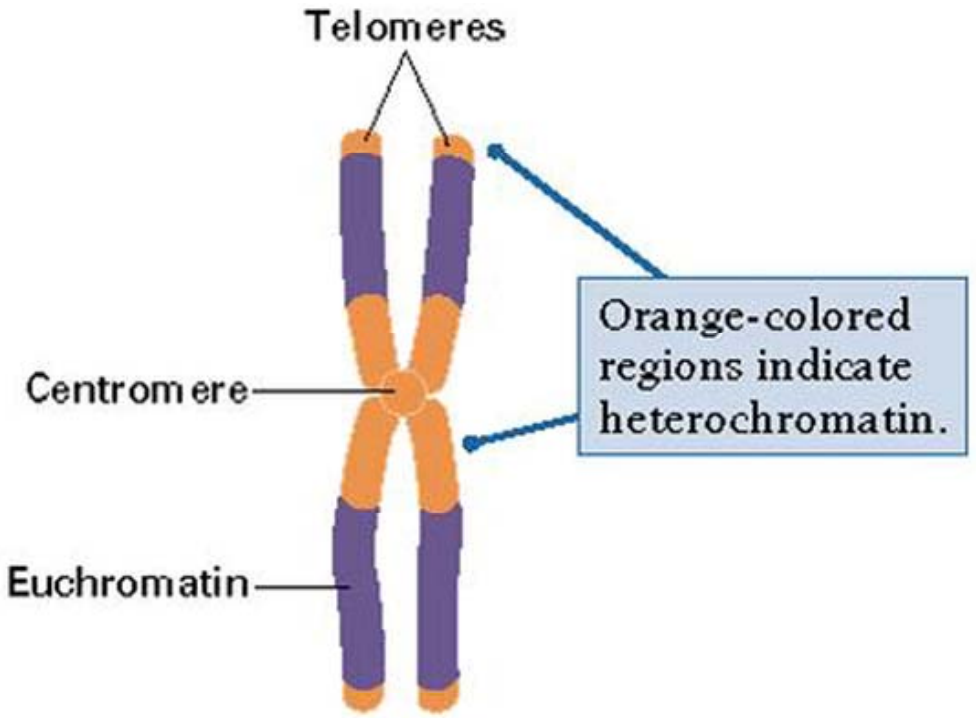
Euchromatin undergoes the normal process of condensation and decondensation in the cell cycle, but heterochromatin remains in a highly condensed state throughout the cell cycle, even during interphase.



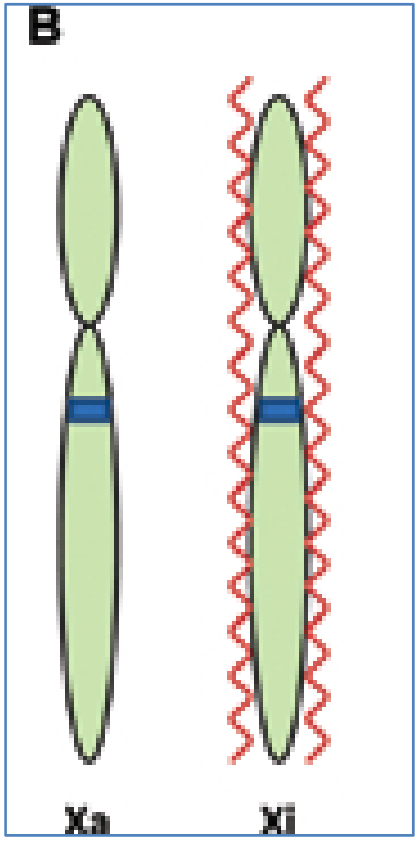
Euchromatin constitutes the majority of the chromosomal material and is where most transcription takes place.

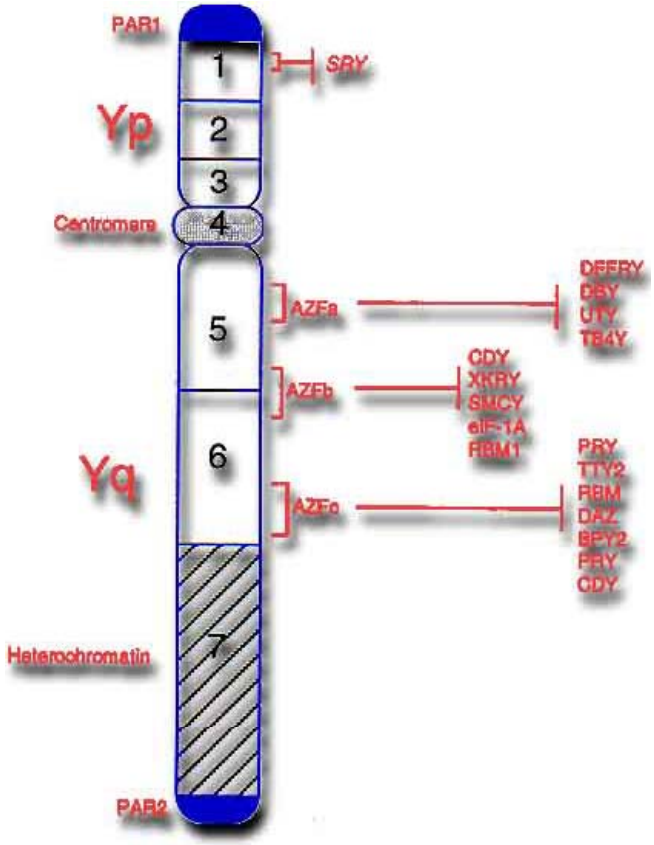


All chromosomes have heterochromatin at the centromeres and telomeres.

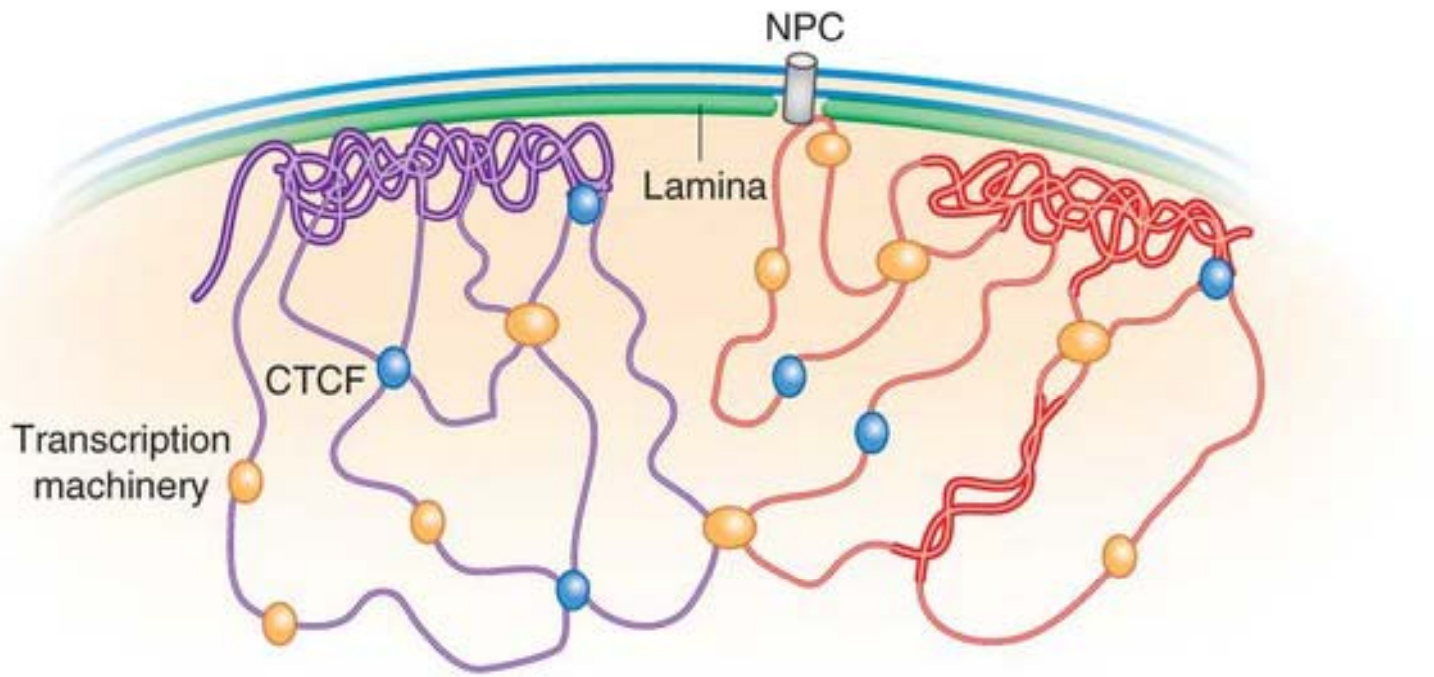


Heterochromatin is also present at the entire inactive X chromosome in female mammals and most of the regions of Y chromosome in males.



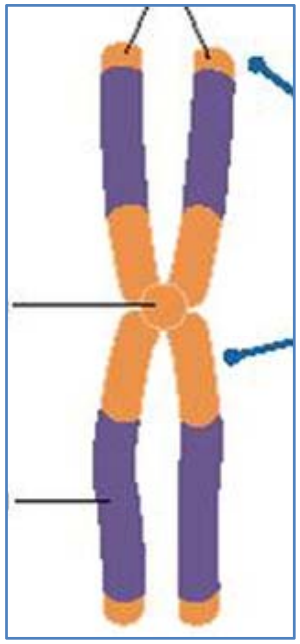


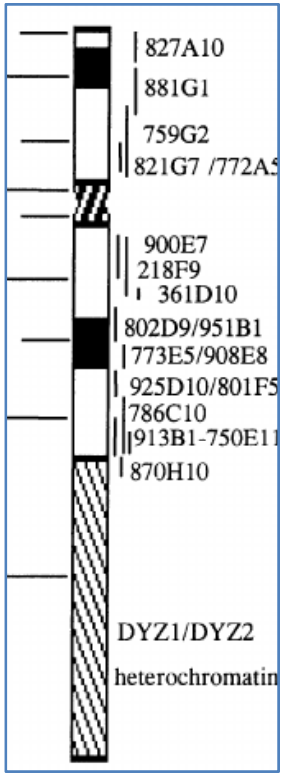
In addition to remaining condensed throughout the cell cycle, heterochromatin is characterized by a general lack of transcription, the absence of crossing over, and replication late in the S stage.



Heterochromatin is divided into two classes.

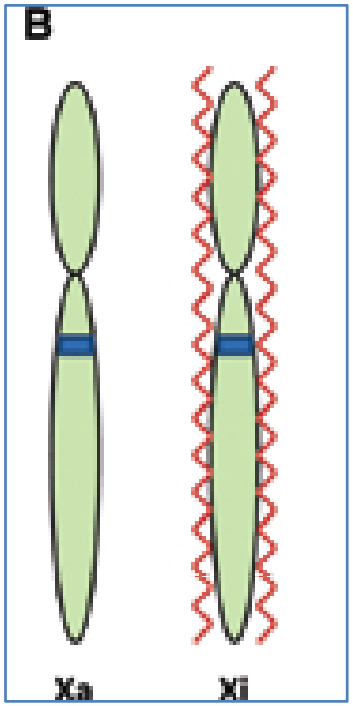
i. **Constitutive heterochromatin:** remains in the compacted state in all cells at all times and, thus, represents DNA that is permanently silenced.
Eg., In mammals constitutive heterochromatin is present at the regions **telomeres** and **centromere** and the distal arm of the Y chromosome.

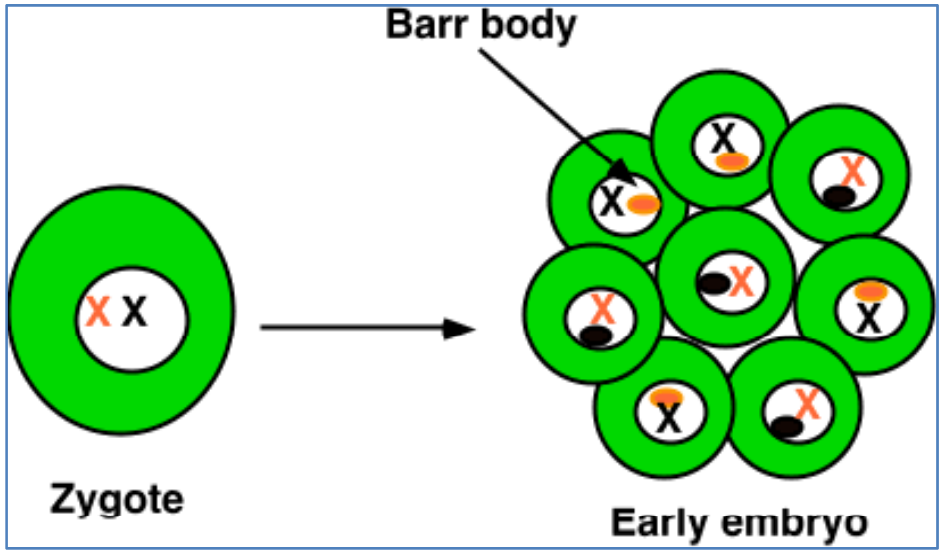




ii. Facultative heterochromatin: It is the chromatin that has been specifically inactivated during certain stages of an organism's life or in certain types of differentiated cells.

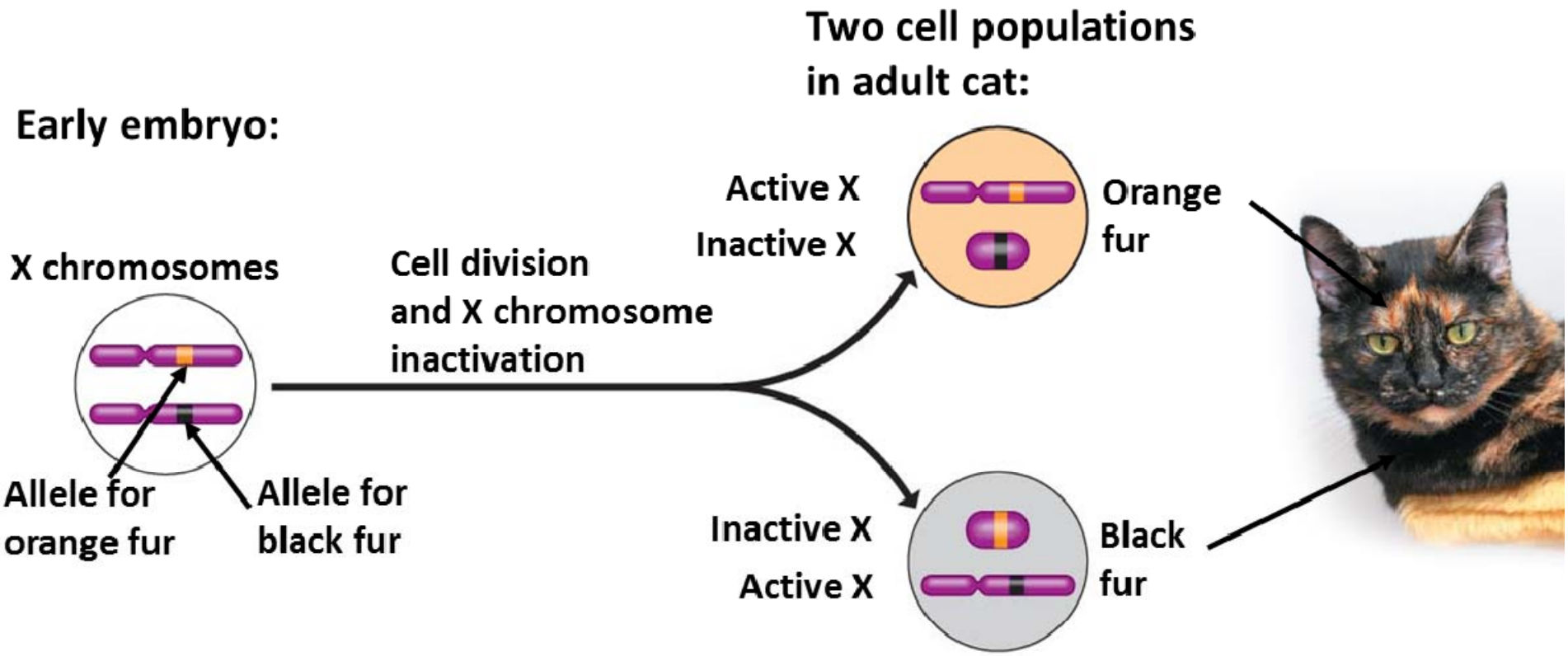
Eg., Cells of females contain two X chromosomes, only one of them is transcriptionally active. The other X chromosome remains condensed as a heterochromatic Clump called as Barr body.





Eg., Random inactivation of either X chromosome in different cells during early embryonic development creates a mosaic of tissue patches and is responsible for the color patterns in calico cats.
Each patch comprises the descendants of one cell that was present in the embryo at the time of inactivation.

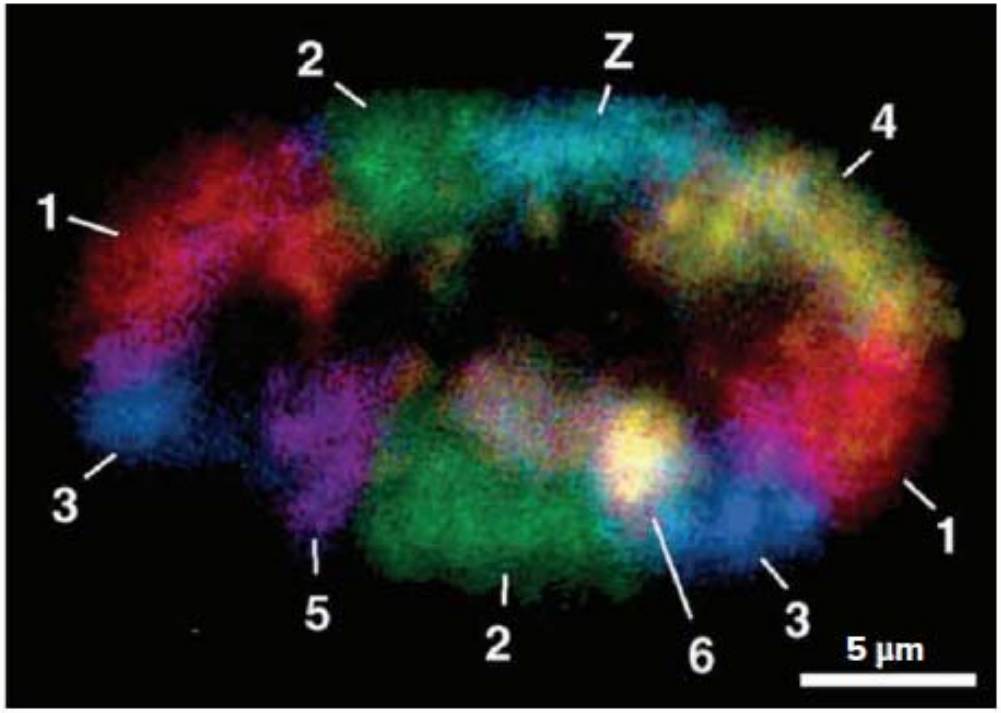
Calico cats are domestic cats with a spotted or particolored coat that is predominantly white, with patches of two other colors.


Two cell populations
in adult cat:
Early embryo:
Active X
Inactive X
Orange
fur
X chromosomes
Cell division
and X chromosome
inactivation
Allele for
orange fur
Allele for
black fur
Inactive X
Active X
Black
fur

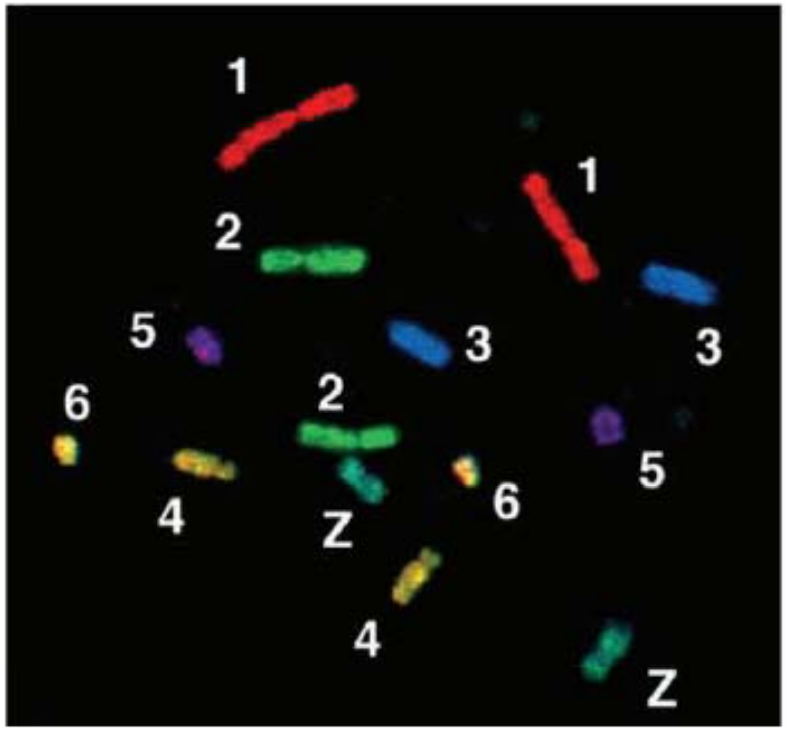
Chromosome territory

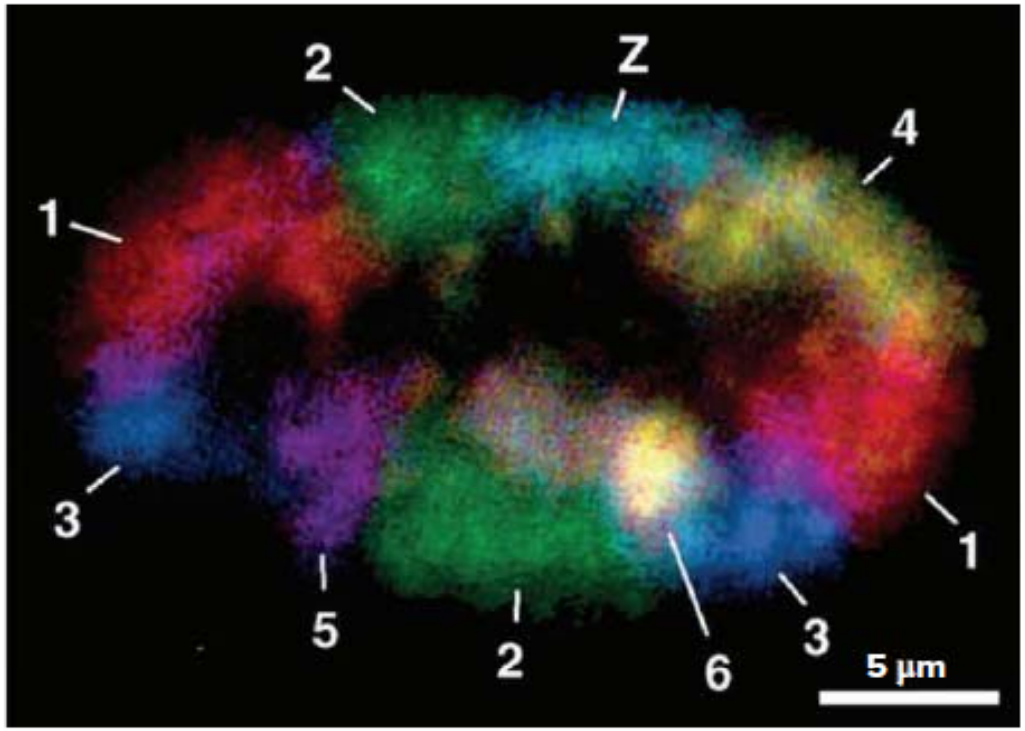
- In addition to being involved in compaction, the nuclear matrix serves to organize the chromosomes within the nucleus.

- Each chromosome in the cell nucleus is located in a discrete chromosome territory.

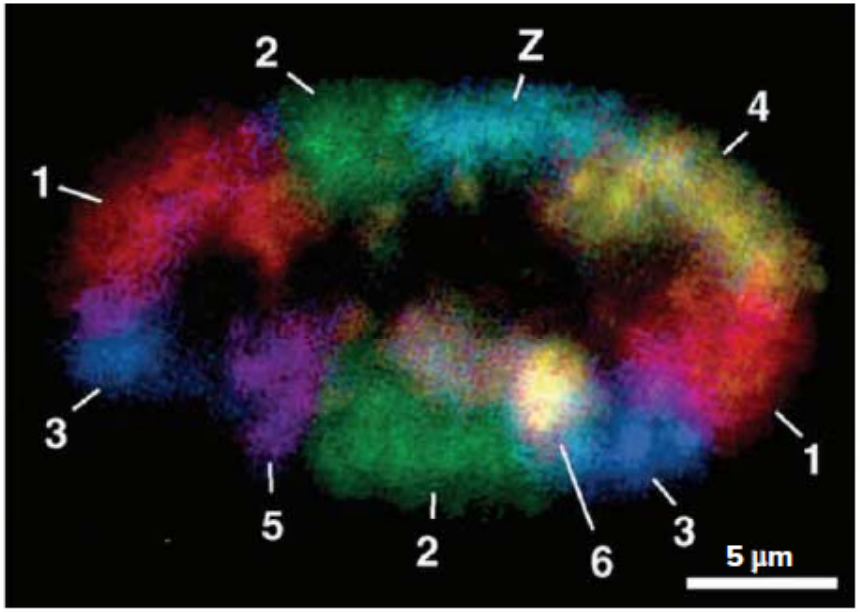


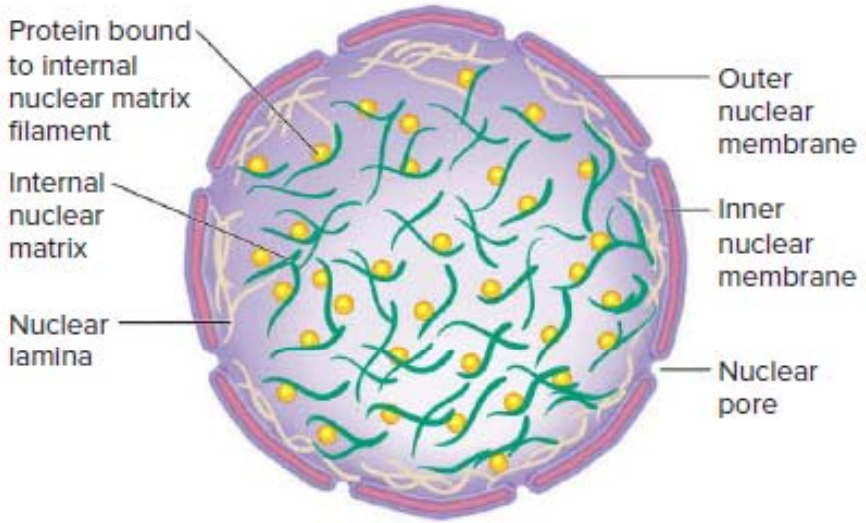
- the territories can be viewed when interphase cells are exposed to multiple fluorescent molecules that recognize specific sequences on particular chromosomes.



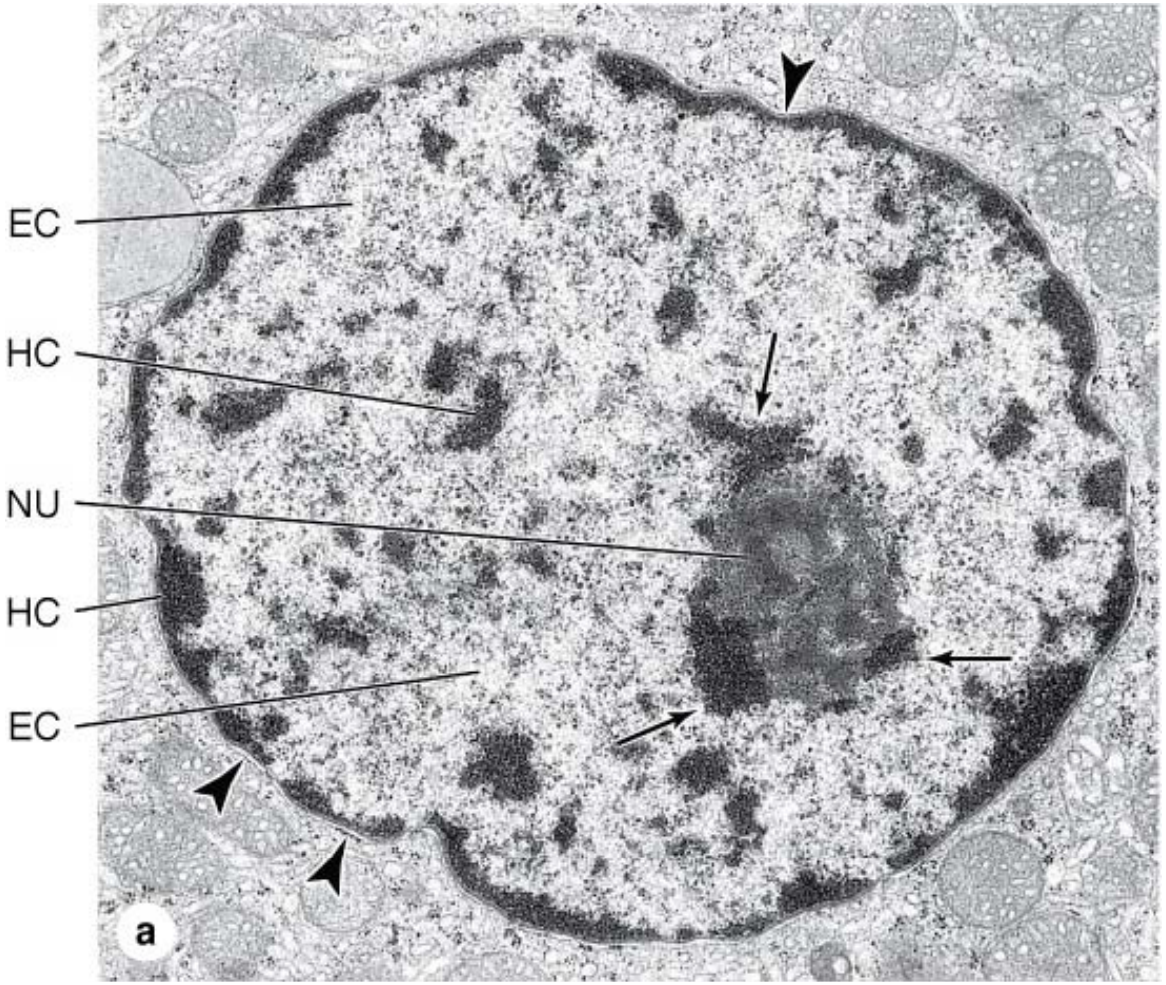


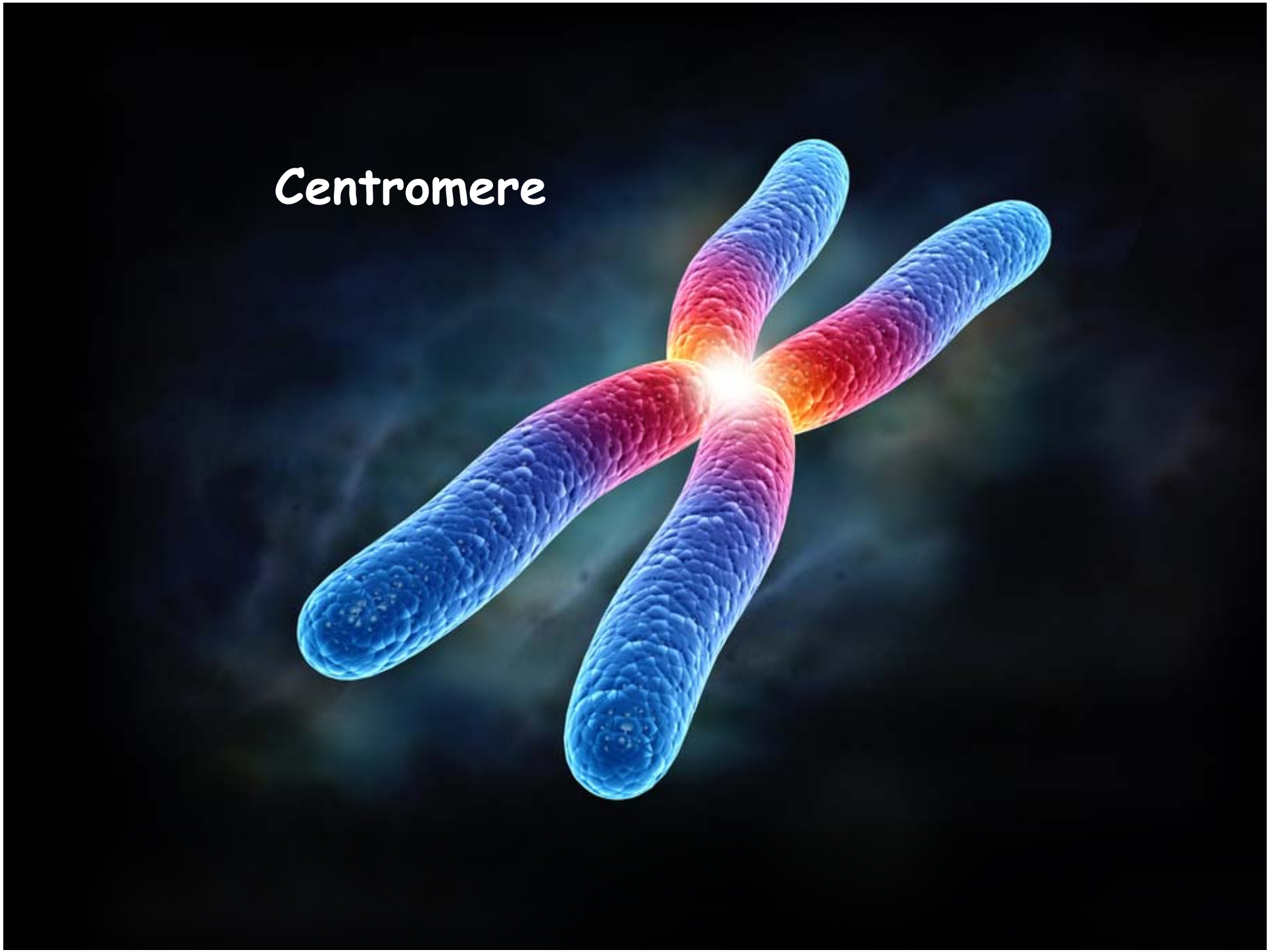
The binding of each chromosome to the nuclear matrix is thought to play a key role in forming these chromosome territories





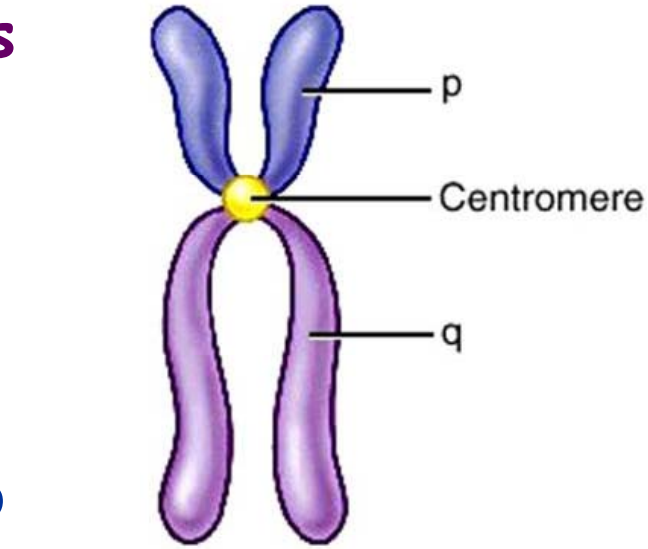
(a) Proteins that form the nuclear matrix


EC
HC
NU
HC
EC
a


Centromere

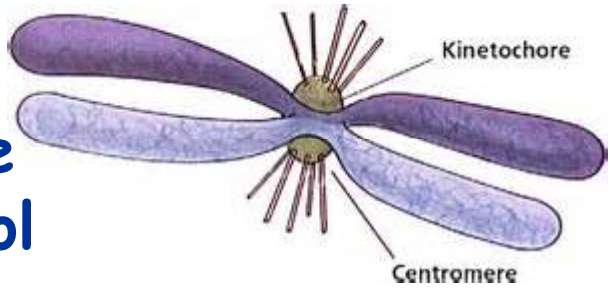
Centromere

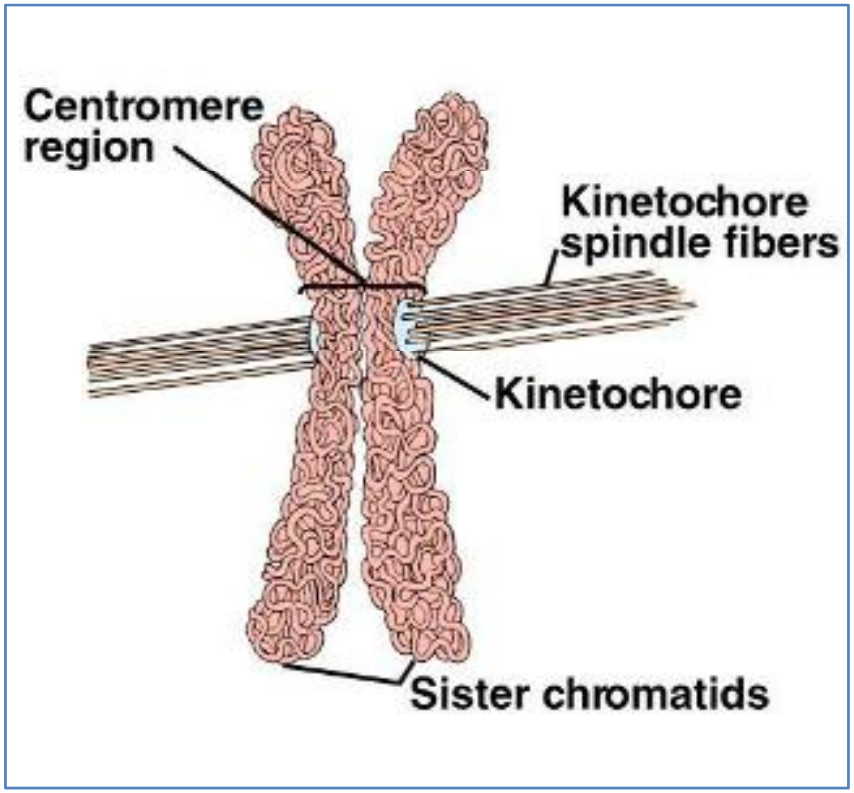
The centromere is a constricted region of the chromosome to which spindle fibers attached.

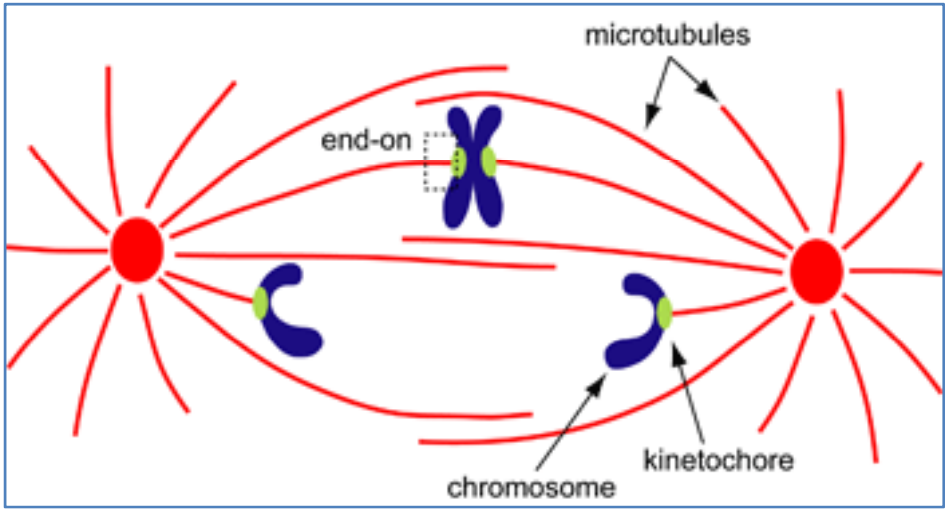


It is essential for:

(i) proper movement of chromosome in mitosis and meiosis.
(ii) binding sites for the kinetochore to which spindle fibers attach
(iii) Correct orientation of each chromosome on the metaphase plate
(iv) Centromere is involved in the control the cell cycle



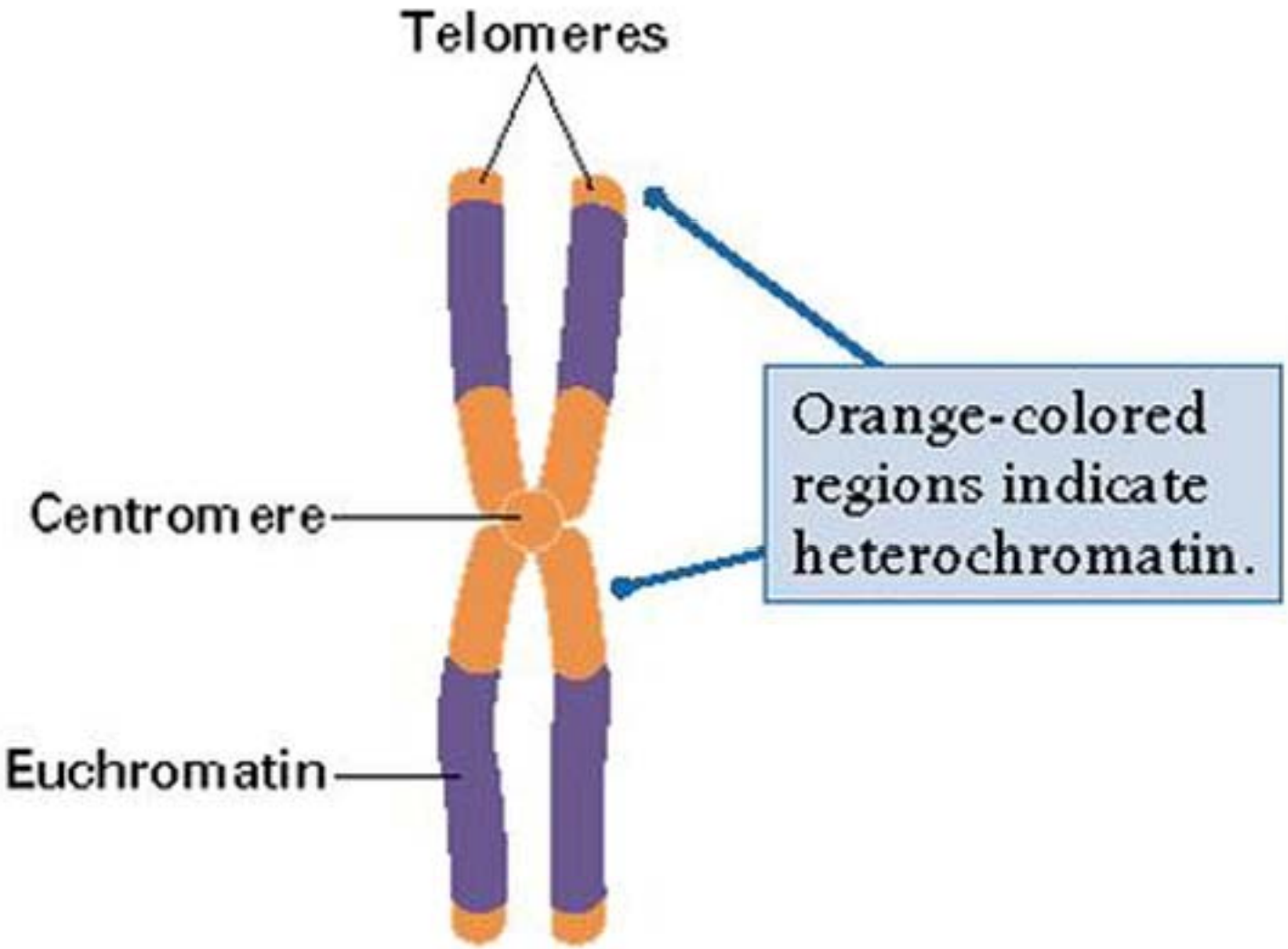




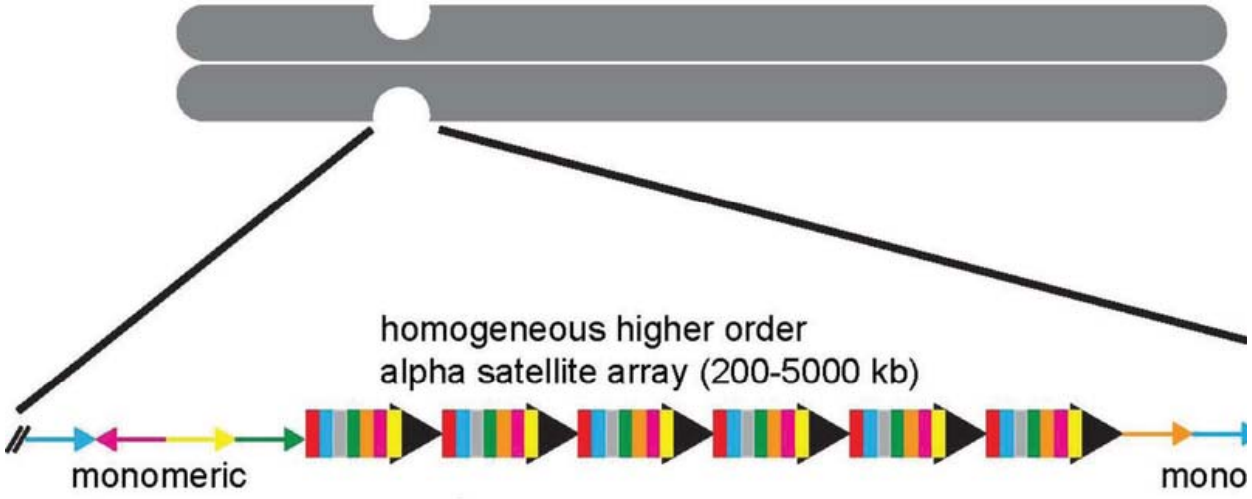
Kinetochore is the protein complex assembled at each centromere and serves as the attachment site for spindle microtubules

The centromeric sequences are the binding sites for the kinetochore proteins, to which spindle fibers attach.

Most of the centromere is made up of heterochromatin



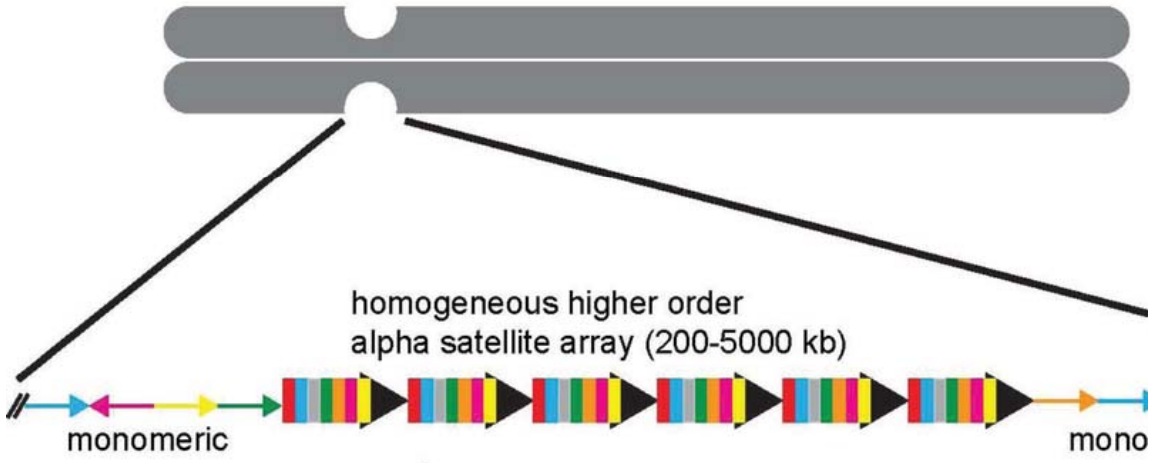
The centromeres of different organisms exhibit considerable variation in centromeric sequences.



The centromeric sequnce consisting of short sequences of DNA that are repeated thousands of times in tandem.

In humans the centromere contains a tandemly repeated, 171-base-pair DNA sequence that extends for at least 500 kilobases (about 3000 times repeated)

This stretch of DNA associates with specific proteins that distinguish it from other parts of the chromosome



- There are no specilic sequences that are found in all centrcmeres.
- Which raises the question of what exactly determines where the centromere is.
- Research suggests that most centromeres are not defined by DNA sequence but rather by epigenetic changes in chromatin structure.

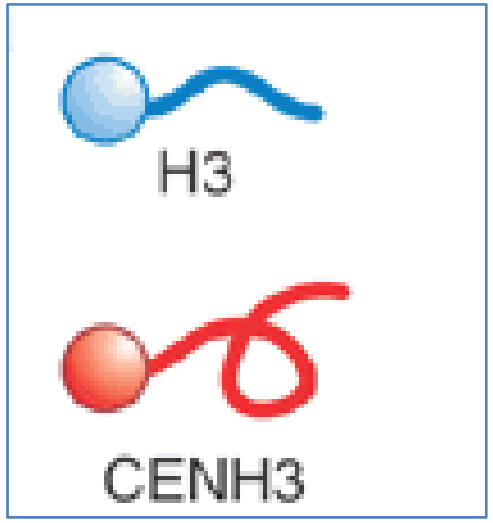
Epigenetics is the study of heritable phenotype changes that do not involve alterations in the DNA sequence.

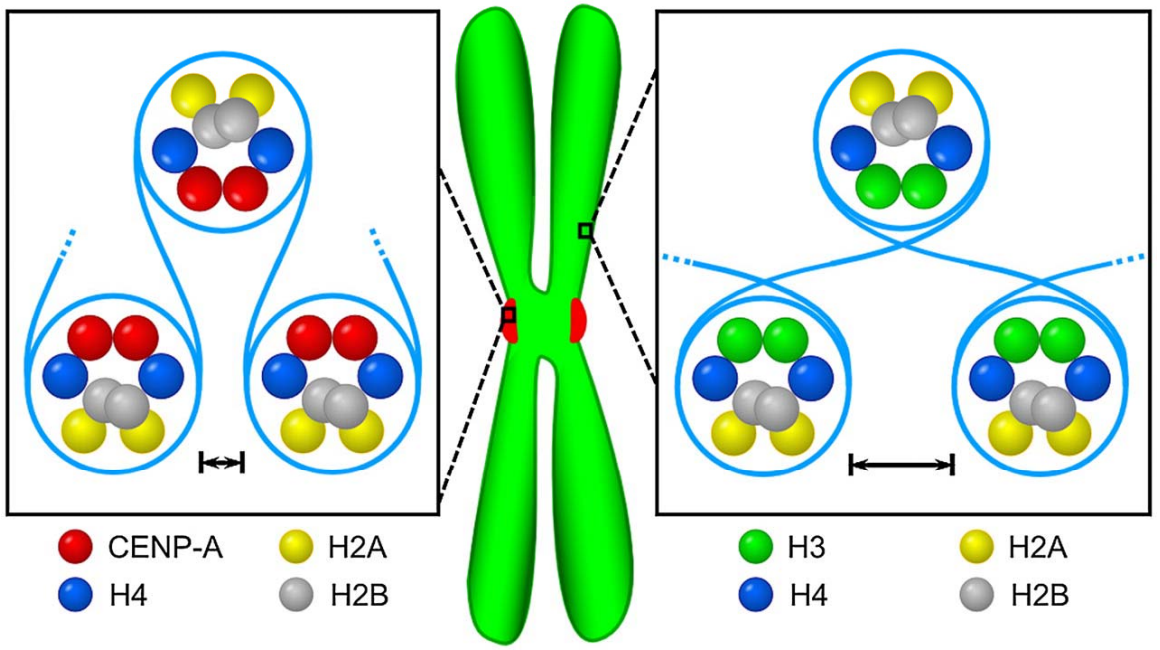
Epigenetic modifications to histone proteins such as methylation / demethylation and acetylation / deacetylation can alter the structure of chromatin resulting in transcriptional activation or repression

Nucleosomes in the centromeres of most eukaryotes have a variant histone protein called CenH3, which takes the place of the usual H3 histone.

CenH3 histones promote the binding of kinetochore proteins

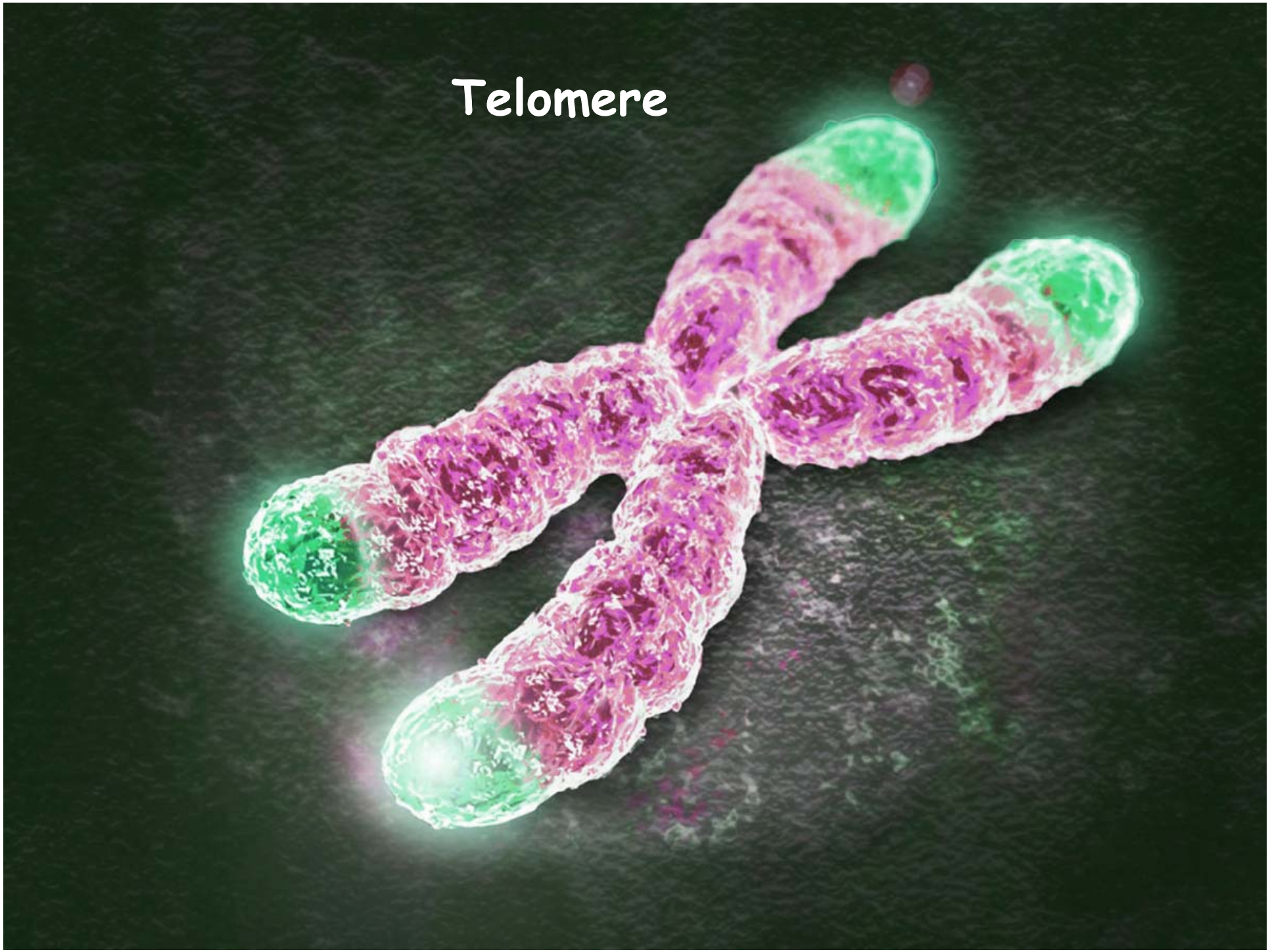
CenH3 in mammals is known as CENP-A





Some organisms have chromosomes with diffuse centromeres, and spindle fibers attach along the entire length of each chromosome (polycentric chromosome).

Most have chromosomes with localized centromeres; in these organisms, spindle fibers attach at a specific place on the chromosome

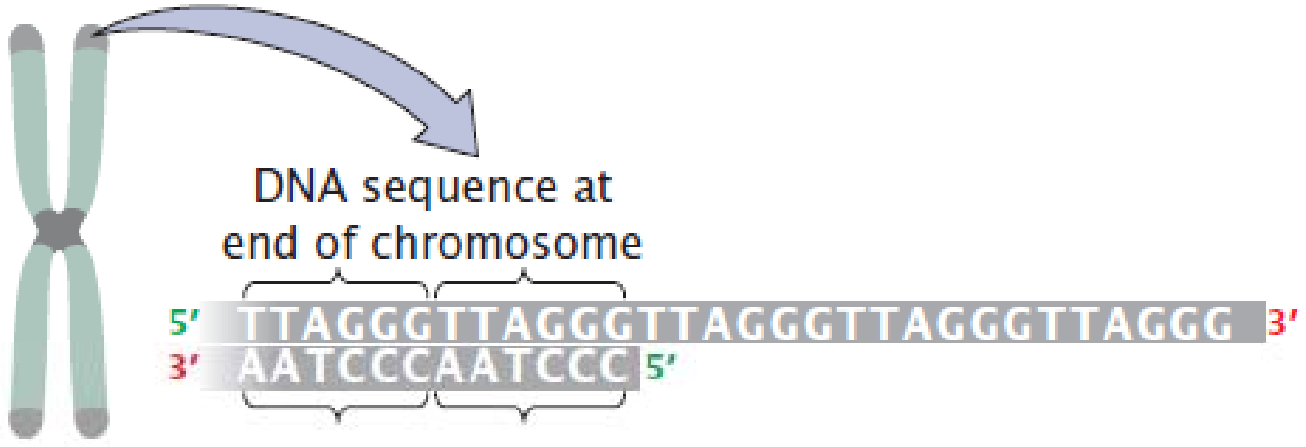

Telomere

Telomere

Telomeres are the natural ends of a chromosome.

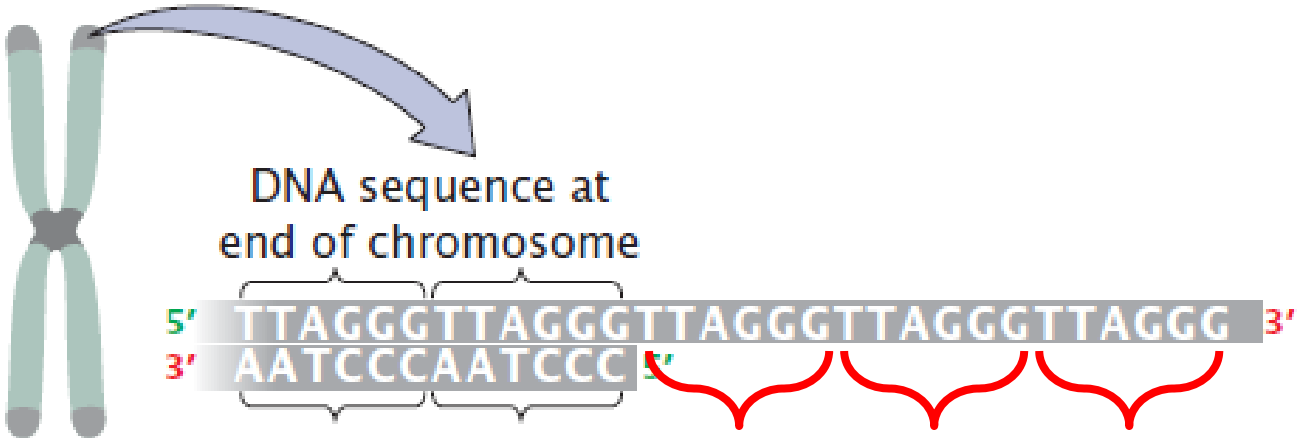
Telomeres in almost all eukaryotic organisms have similar structure.

The telomeric DNA is heterochromatic



The sequences confined to the telomeric region is known as telomeric sequences.

Usually contains direct tandem repeats of a series of adenine or thymine nucleotides followed by several guanine nucleotides.

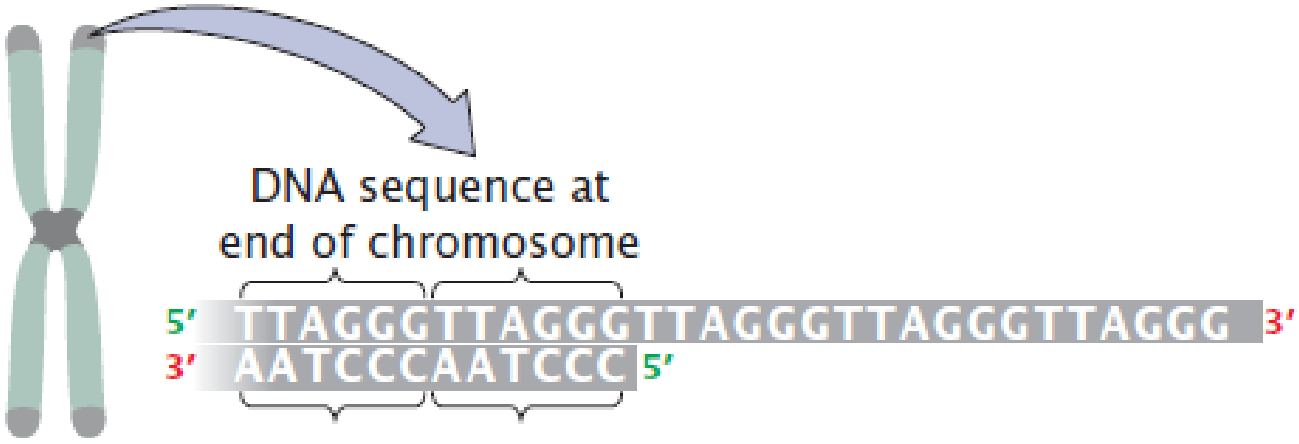


Species	Repeat Sequence
Arabidopsis	TTTAGGG
Human	TTAGGG
Oxytricha	TTTTGGGG
Slime Mold	TAGGG
Tetrahymena	TTGGGG
Trypanosome	TAGGG
Yeast	$(TG)_{1-3}TG_{2-3}$

In human chromosomes, the telomere is about 10-15 kb in length, composed of the tandem repeat: TTAGGG

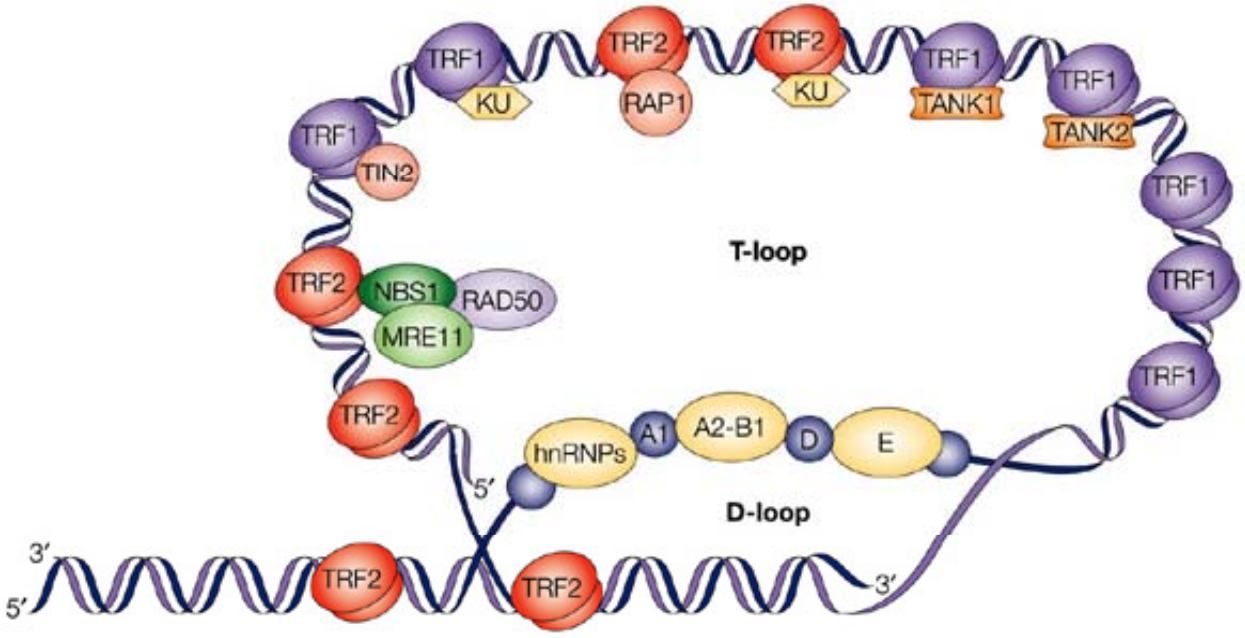
The G-rich strand protrudes beyond the complementary strand at the end of the chromosome and is called the **3' overhang**.

The 3' overhang in the telomeres of mammals is from 50 to 500 nucleotides long.



Special proteins bind to the G-rich single-stranded sequence, protecting the telomere from degradation and preventing the ends of chromosomes from sticking together.

A multiprotein complex called **shelterin** binds to mammalian telomeres and protects the ends of the DNA



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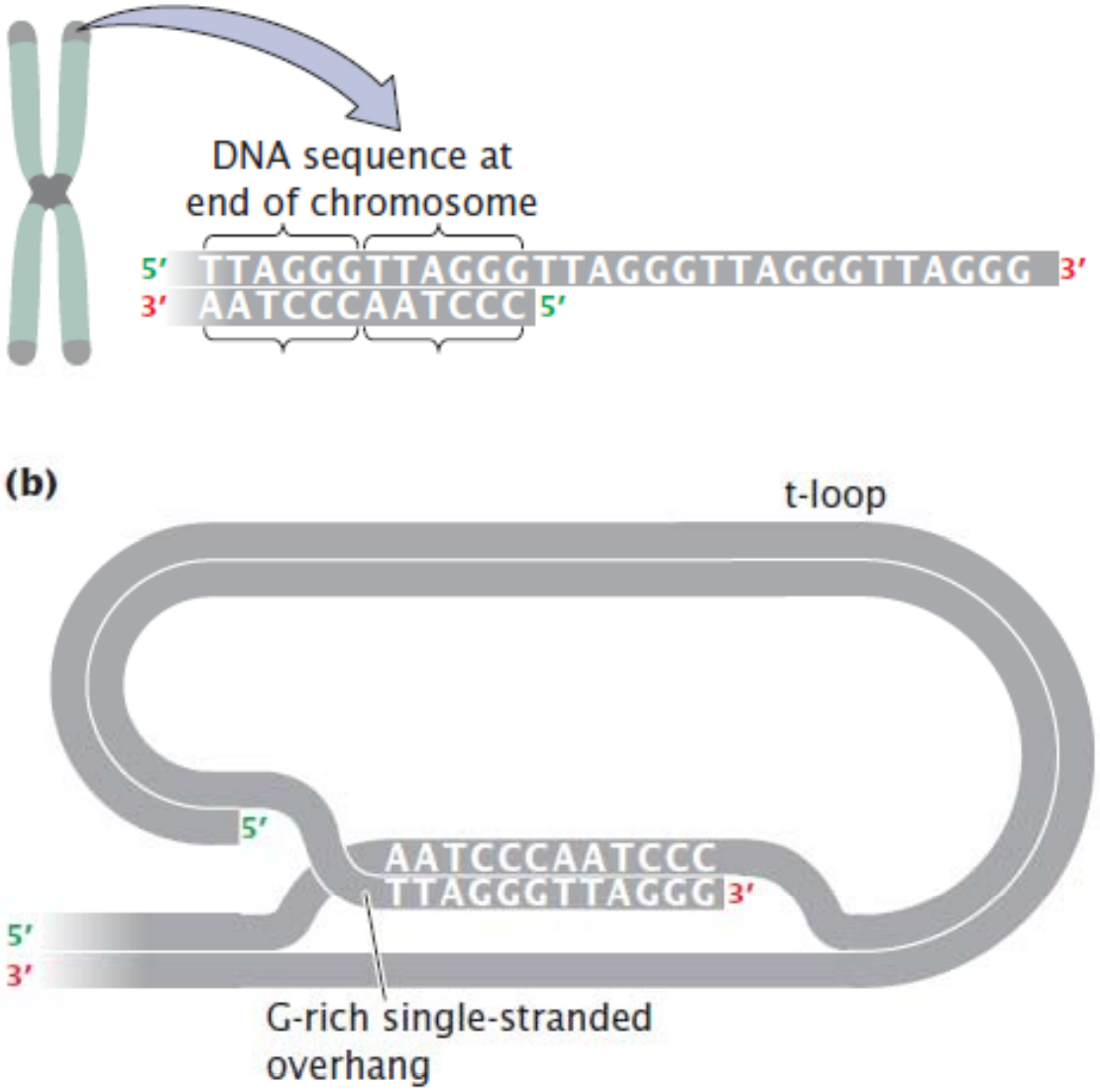
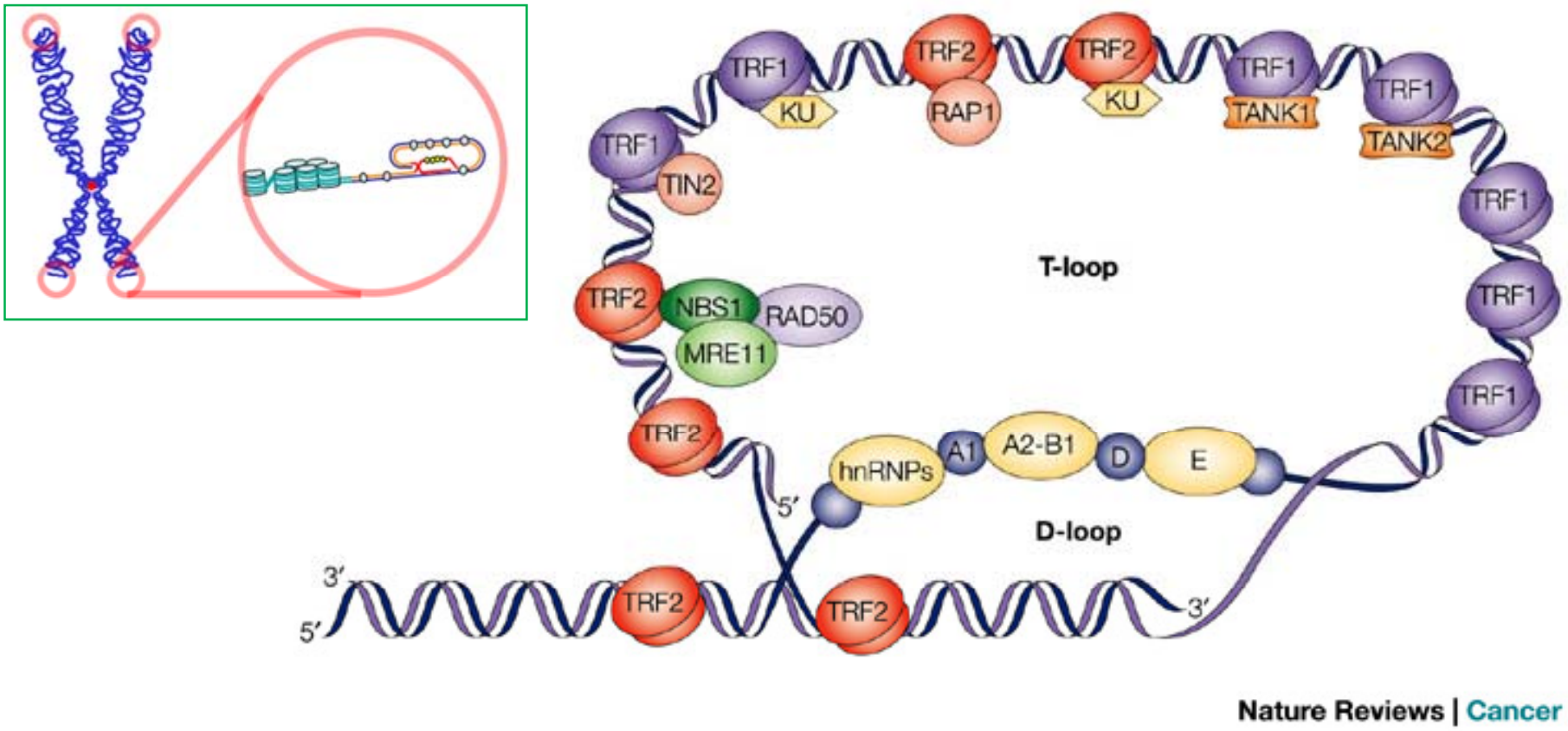
In mammalian cells, the single-stranded 3′ overhang may fold over and anneal with part of the duplex DNA forms a structure called t-loop, which also functions in protecting the end of the telomere from degradation.





The single-stranded DNA at the end invade and anneal with part of the duplex DNA forms a t-loop.

Several proteins (shelterin) bind specifically to telomeric DNA

Telomeres have several functions:

- protect chromosomes from fusing with each other
- protect from harm to the DNA within
- solve the end-replication problem