CHAPTER 6

Controlling Gene Expression

6.2 | Structure and Function of the Cell Nucleus

The contents of the nucleus are enclosed by the *nuclear envelope*.

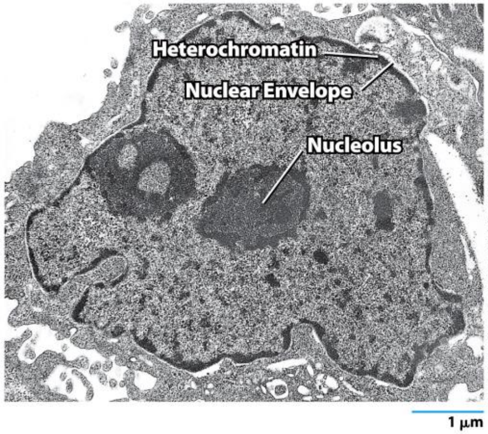
A typical nondividing nucleus includes:

Chromosomes as extended fibers of *chromatin*.

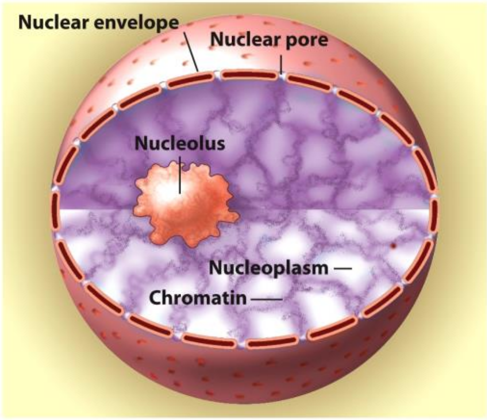
Nucleoli for rRNA synthesis.

Nucleoplasm as the fluid where solutes are dissolved.

The *nuclear matrix*, which is the protein-containing fibrillar network.



The cell nucleus. EM of an interphase HeLa cell nucleus (above) and schematic drawing of major components (below).



6.2 | Structure and Function of the Cell Nucleus

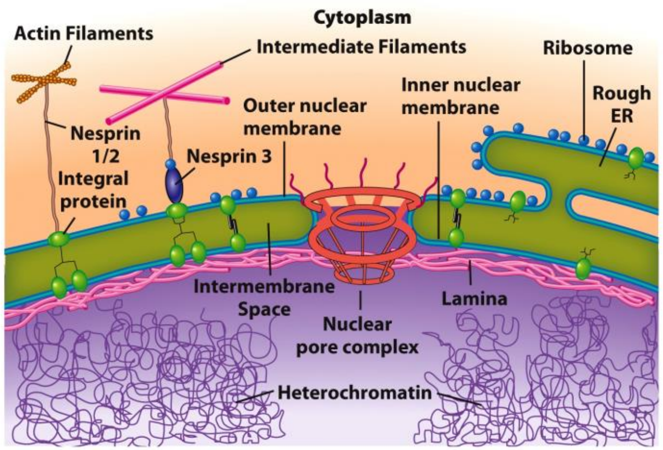
The **nuclear envelope** is a structure that divides the nucleus from its cytoplasm.

It consists of two membranes separated by a nuclear space.

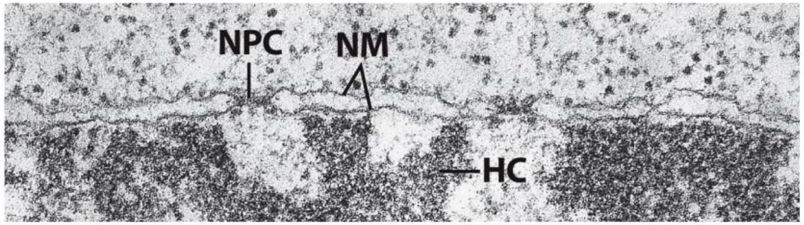
The two membranes are fuses at sites forming a nuclear pore.

The inner surface of the nuclear envelope is lined by the **nuclear lamina**.

Contains around 60 distinct transmembrane proteins.



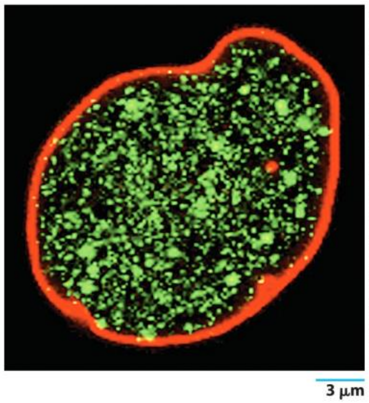
The nuclear envelope. Schematic drawing (top) and EM of the nuclear envelope of an onion root tip cell (bottom)



From Werner W. Franke, et al., J. Cell Biol. 91:47s, Figure 8, 1981. Reproduced with permission of Rockefeller University Press.

0.2 μm

6.2 | Structure and Function of the Cell Nucleus



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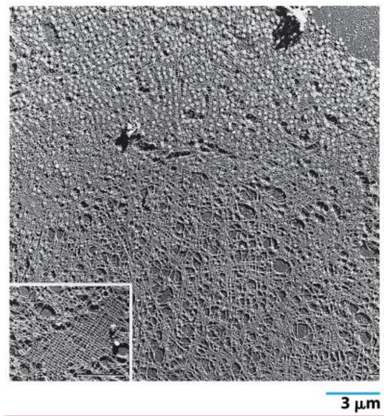
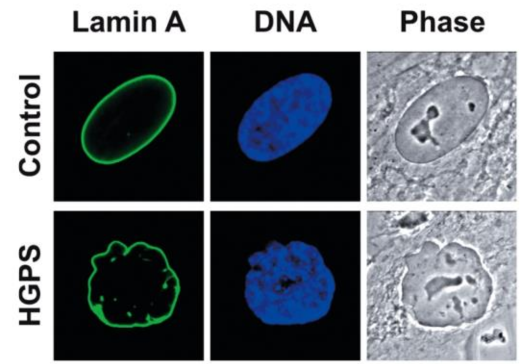
Nucleus stained for nuclear lamina (R) and nuclear matrix (G)



From Ueli Aebi, et al., Nature 323:561, 1986. Reprinted by permission from Macmillan Publishers Ltd.

EM: metal-shadowed nuclear envelope of a *Xenopus* oocyte



From Anna Mattout, et al., courtesy of Yosef Gruenbaum, Curr. Opin. Cell Biol. 18:338, 2006, with permission from Elsevier.

Micrographs of fibroblast nuclei from a patient with HGPS (bottom) or a healthy subject (top).

The nuclear lamina supports the nuclear envelope and is composed of lamins.

Its integrity is regulated by phosphorylation/dephosphorylation of intermediate filaments.

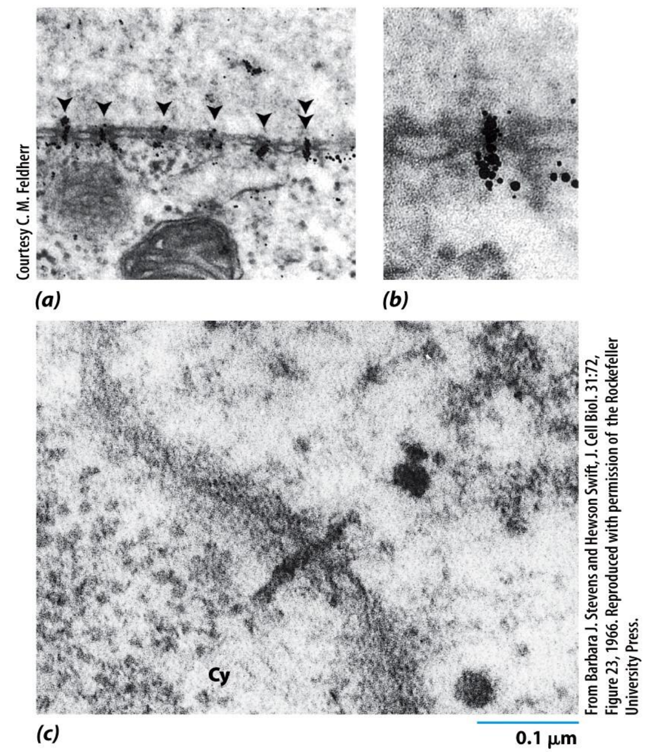
6.2 | Structure and Function of the Cell Nucleus

The Nuclear Pore Complex and Its Role in Nucleocytoplasmic Trafficking

The nuclear envelope is the barrier between the nucleus and cytoplasm, and nuclear pores are the gateways.

DNA replication and transcription requires large numbers of proteins synthesized in the cytoplasm and transported across the nuclear envelope.

Conversely, mRNAs, tRNAs, and ribosomal subunits are manufactured in the nucleus and transported to the cytoplasm.



Movement of materials through the nuclear pore.

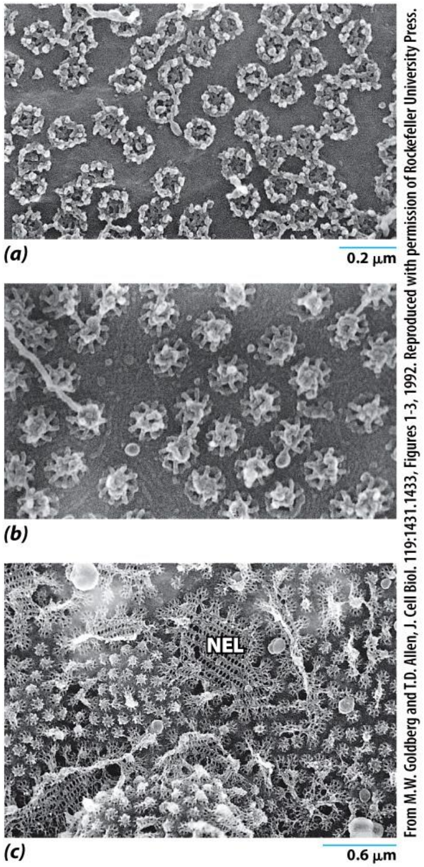
6.2 | Structure and Function of the Cell Nucleus

The Nuclear Pore Complex and Its Role in Nucleocytoplasmic Trafficking

Proteins and RNA are transported in and out of the nucleus by passing single-file through the center of the nuclear pores.

Nuclear pores contain a doughnut-shaped structure called the **nuclear pore complex (NPC).**

The NPC is composed of ~30 proteins called *nucleoporins*.



Scanning electron micrographs of the nuclear pore complex from an amphibian oocyte.

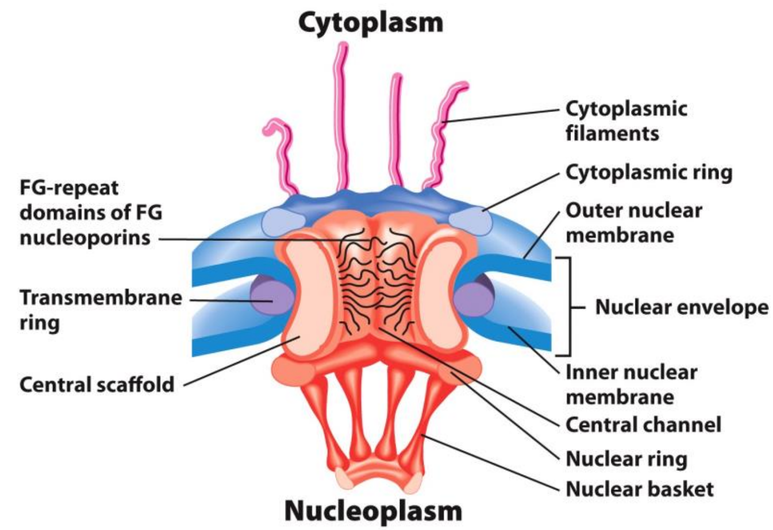
6.2 | Structure and Function of the Cell Nucleus

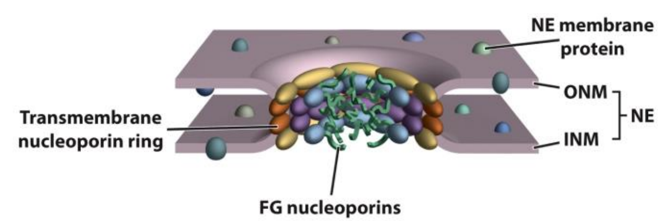
The Nuclear Pore Complex and Its Role in Nucleocytoplasmic Trafficking

The NPC is a huge complex (15-30X mass of a ribosome) that exhibits octagonal symmetry.

The NPC is not static, as many of its proteins are replaced over a period of seconds to minutes.

The channel is 20-to 40-nm-wide and contains FG (phenylalanine-glycine) domains that form a hydrophobic sieve to block diffusion of macromolecules greater than 40 KDa.





From Javier Fernandez-Martinez and Michael P. Rout, Curr. Opin. Cell Biol. 21:604, 2009, with permission from Elsevier.

Model of a vertebrate nuclear pore complex (NPC).

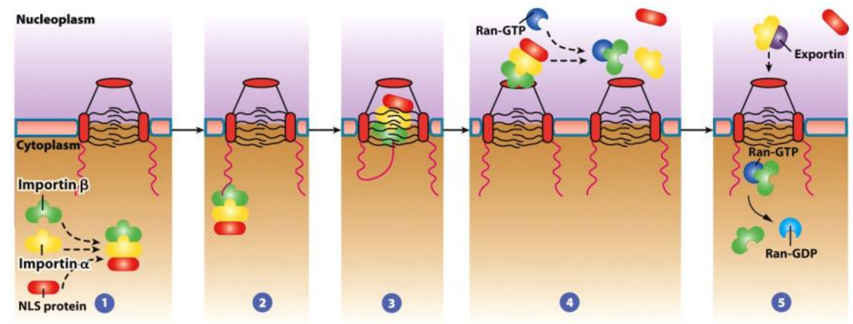
6.2 | Structure and Function of the Cell Nucleus

The Nuclear Pore Complex and Its Role in Nucleocytoplasmic Trafficking

Cytoplasmic proteins are targeted for the nucleus by the **nuclear localization signal** (**NLS**), e.g. P-K-K-K-R-K-V having basic residues.

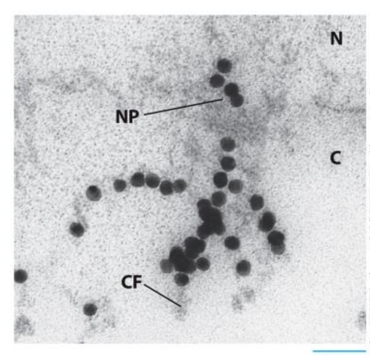
Transport receptors include *importins* to move molecules from the cytoplasm into the nucleus and *exportins* to move molecules in the opposite direction.

RNAs move through NPCs as RNPs and carry *NES* (*nuclear export signals*) to pass.



Based on a model by M. Ohno et al., Cell 92:327, 1998; Cell by Cell Press. Reproduced with permission of Cell Press in the format reuse in a book/textbook via Copyright Clearance Center.

Importing proteins into the nucleus.
Steps in nuclear protein import (top).
Gold particle-nucleoplasmin injection into frog oocytes shows binding to cytoplasmic filaments (bottom).



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6.2 | Structure and Function of the Cell Nucleus

RNA Transport

mRNAs, rRNAs, miRNAs, and tRNAs are synthesized in the nucleus and function in the cytoplasm or are modified in the cytoplasm and return to function in the nucleus.

These RNAs move through the NPC as ribonucleoproteins (RNPs).

Only mature, fully processed mRNAs are capable of nuclear export, as an mRNA with an unspliced intron is retained in the nucleus.

6.3 | Chromosomes and Chromatin

An average human cell contains about 6.4 billion base pairs of DNA divided among 46 chromosomes.

The largest human chromosome would extend 10 cm long.

How is it possible to fit all 46 chromosomes into a nucleus that is only 10 μm in diameter and 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.

6.3 | Chromosomes and Chromatin

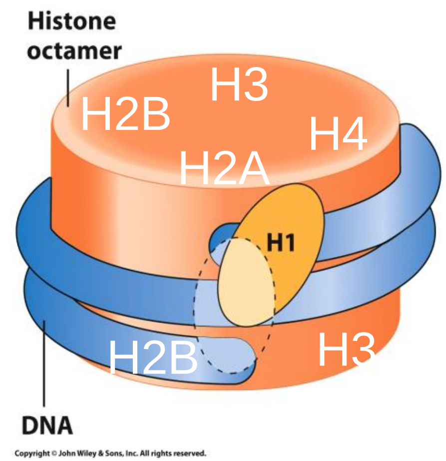
Nucleosomes: The Lowest Level of Chromosome Organization

Each chromosome contains a single, continuous DNA molecule.

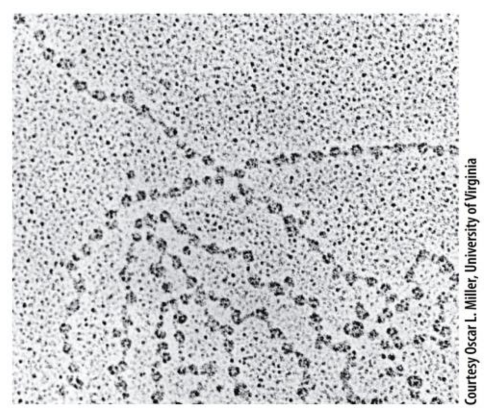
Chromosomes consist of **chromatin** fibers, composed of DNA and associated proteins.

The protein component of chromosomes include **histones**, a group of highly conserved proteins.

Histones have a high content of basic amino acids.



Nucleosomal organization of chromatin: Schematic diagram (top) and EM of *Drosophila* cell nucleus with nucleosomes along DNA strand (bottom)



6.3 | Chromosomes and Chromatin

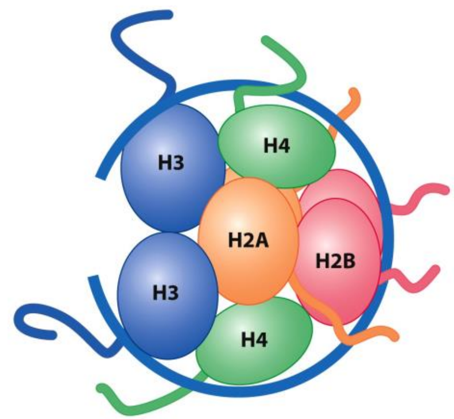
Nucleosomes: The Lowest Level of Chromosome Organization

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

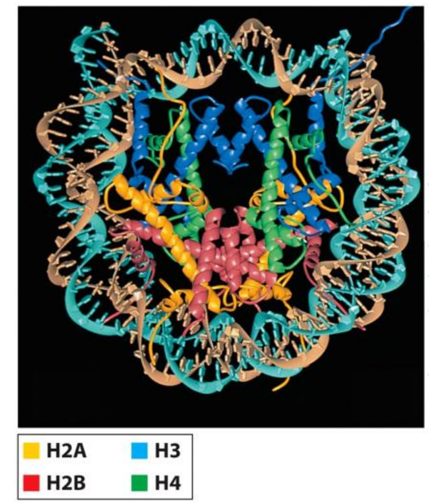
Each nucleosome includes a *core particle* of supercoiled DNA and histone H1 serving as a *linker*.

DNA is wrapped around the core complex.

The histone core complex consists of two molecules each of H2A, H2B, H3, and H4 forming an octamer.

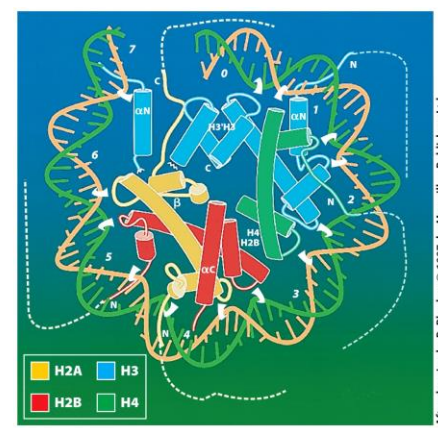


From C. David Allis, et al., Epigenetics, Figure 3.5, p. 30, Copyright 2007. Reprinted with permission from Cold Spring Harbor Laboratory Press.



From Karolin Luger and Timothy Richmond, et al., Nature 389:252, 1997, Fig. 1. Reprinted by permission from Macmillan Publishers Ltd.

3D structure of a nucleosome from X-ray crystallography. Core particle at two views (top) and schematic of half of a core particle (side)



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6.3 | Chromosomes and Chromatin

Nucleosomes: The Lowest Level of Chromosome Organization

Histone modification is one mechanism to alter the character of nucleosomes.

DNA and histones are held together by noncovalent bonds.

Ionic bonds between negatively charged phosphates of the DNA backbone and positively charged residues of the histones.

Histones, regulatory proteins, and enzymes dynamically mediate DNA transcription, replication, recombination, and repair.

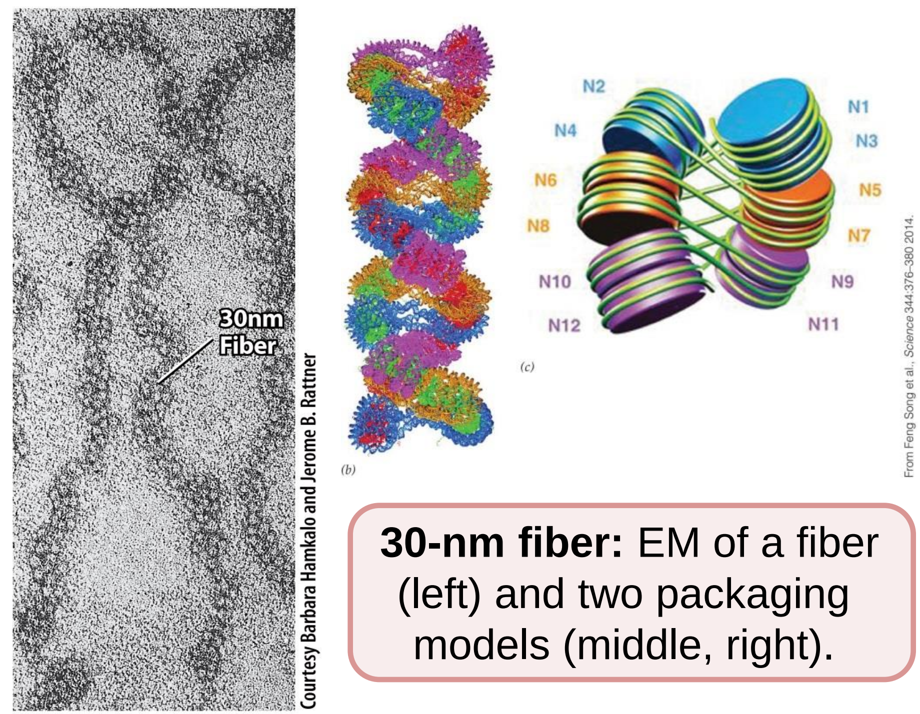
6.3 | Chromosomes and Chromatin

Higher Levels of Chromatin Structure

Nucleosomes line up end-to-end into two stacks of nucleosomes that form a double helical structure.

Assembly of the 30-nm fiber increases the DNA- packing ratio an additional 6-fold, or about 40-fold altogether.

Maintenance of the 30-nm fiber depends on interactions between histone molecules of neighboring nucleosomes.



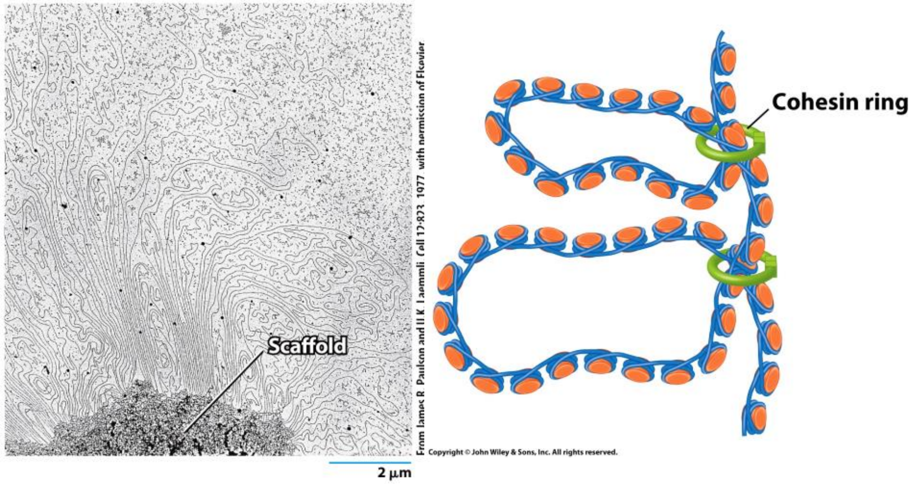
30-nm fiber: EM of a fiber (left) and two packaging models (middle, right).

6.3 | Chromosomes and Chromatin

Higher Levels of Chromatin Structure

The 30-nm chromatin fiber is gathered into a series of large, supercoiled loops, or domains, compacted into thicker (80–100 nm) fibers.

Among the proteins that may maintain these DNA loops is **cohesin**, best known for holding replicated DNA molecules together during mitosis.



Chromatin loops: a higher level of chromatin structure. EM: of a mitotic chromosome (left) and model for cohesin in maintaining loops (right)

6.3 | Chromosomes and Chromatin

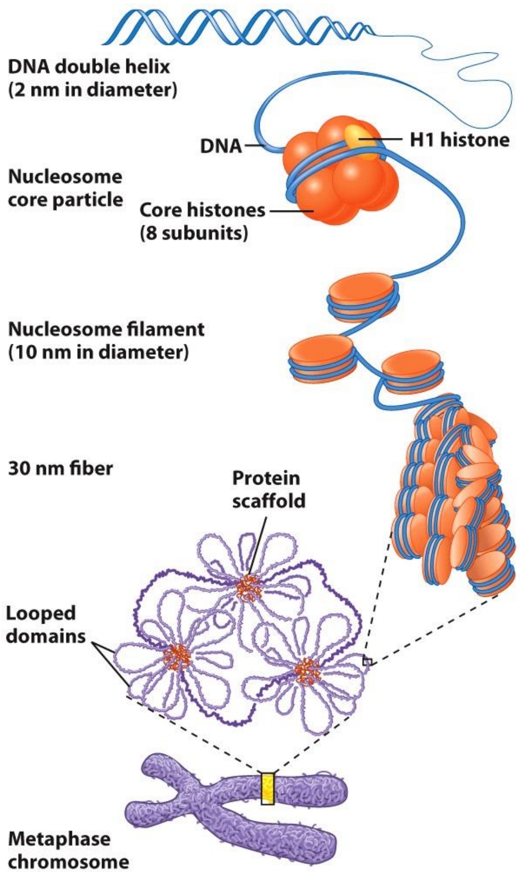
Higher Levels of Chromatin Structure

A nucleus 10 mm in diameter can pack 200,000 times this length of DNA within its boundaries.

Packing ratio of the DNA in nucleosomes is approximately 7:1.

Assembly of the 30-nm fiber increases the DNA-packing ratio to 40:1.

Mitotic chromosomes represent the ultimate in chromatin compactness with a ratio of 10,000:1.



Levels of organization of chromatin.

6.4 | Heterochromatin and Euchromatin

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

Euchromatin returns to a dispersed state after mitosis, **heterochromatin** is condensed during interphase.

Constitutive heterochromatin remains condensed all the time. It is found mostly around centromeres and telomeres and consists of highly repeated sequences and few genes.

Facultative heterochromatin is inactivated during certain phases of the organism's life. It is found in one of the X chromosomes as a Barr body through X inactivation, a random process that makes adult females genetic mosaics.