Compartment	Shape	Dimensions	Type of Nucleic Acid	Length
TMV	filament	0.008 x 0.3 μm	One single-stranded RNA	2 μm = 6.4 kb
Phagefd	filament	0.006 x 0.85 μm	One single-stranded DNA	2 μm = 6.0 kb
Adenovirus	icosahedron	0.07 μm diameter	One double-stranded DNA	11 μm = 35.0 kb
Phage T4	icosahedron	0.065 x 0.10 μm	One double-stranded DNA	55 μm = 170.0 kb
E. coli	cylinder	1.7 x 0.65 μm	One double-stranded DNA	1.3 mm = 4.2×10^3 kb
Mitochondrion (human)	oblate spheroid	3.0 x 0.5 μm	~10 identical double-stranded DNAs	50 μm = 16.0 kb
Nucleus (human)	spheroid	6 μm diameter	46 chromosomes of double-stranded DNA	1.8 m = 6×10^6 kb

FIGURE 9.1 The length of nucleic acid is much greater than the dimensions of the surrounding compartment.

Packing ratio

Length of the DNA divided by the
length of the unit that contains it.

For example, the smallest human chromosome:

mitotic chromosome	=	2 μm
DNA 4,6x 107 bp	=	1,4 cm
packing ratio	=	7000

DNA concentration in different organisms

- Fage T4 = 500 mg/ml
- Bacteria = 10 mg/ml
- Eukaryotic Nuclei = 100 mg/ml

Viral genome is into a preassembled protein shell named capsid

Capsid assembly

- The protein shell can be assembled around the nucleic acid, thereby condensing the DNA or RNA by proteinnucleic acid interactions during the process of assembly.
- The capsid can be constructed from its component(s) in the form of an empty shell, into which the nucleic acid must be inserted, being condensed as it enters.

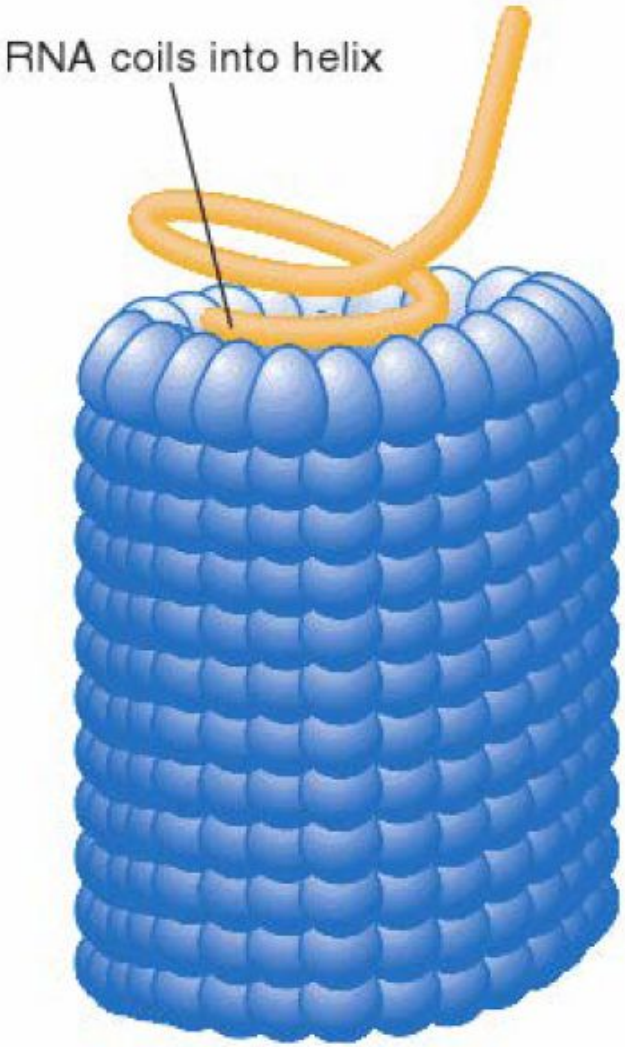
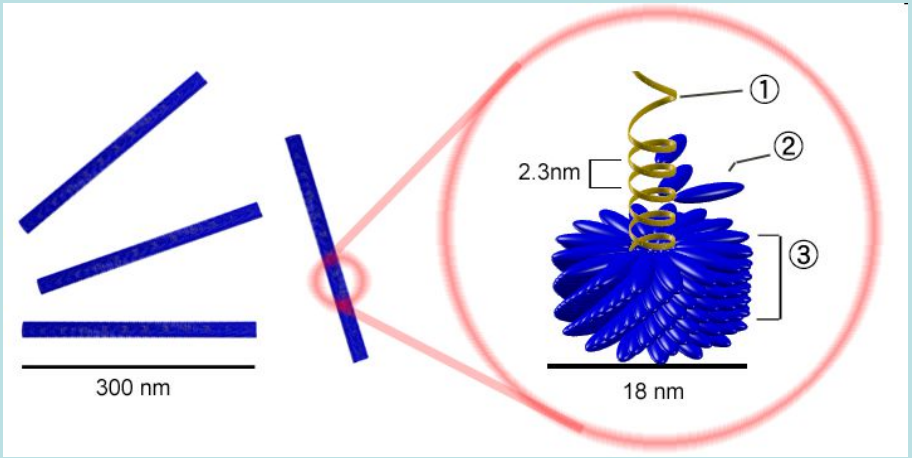


FIGURE 9.2 A helical path for TMV RNA is created by the stacking of protein subunits in the virion (the entire virus particle).



The coat protein self-assembles into the rod like helical structure (16.3 proteins per helix turn) around the RNA which forms a hairpin loop structure

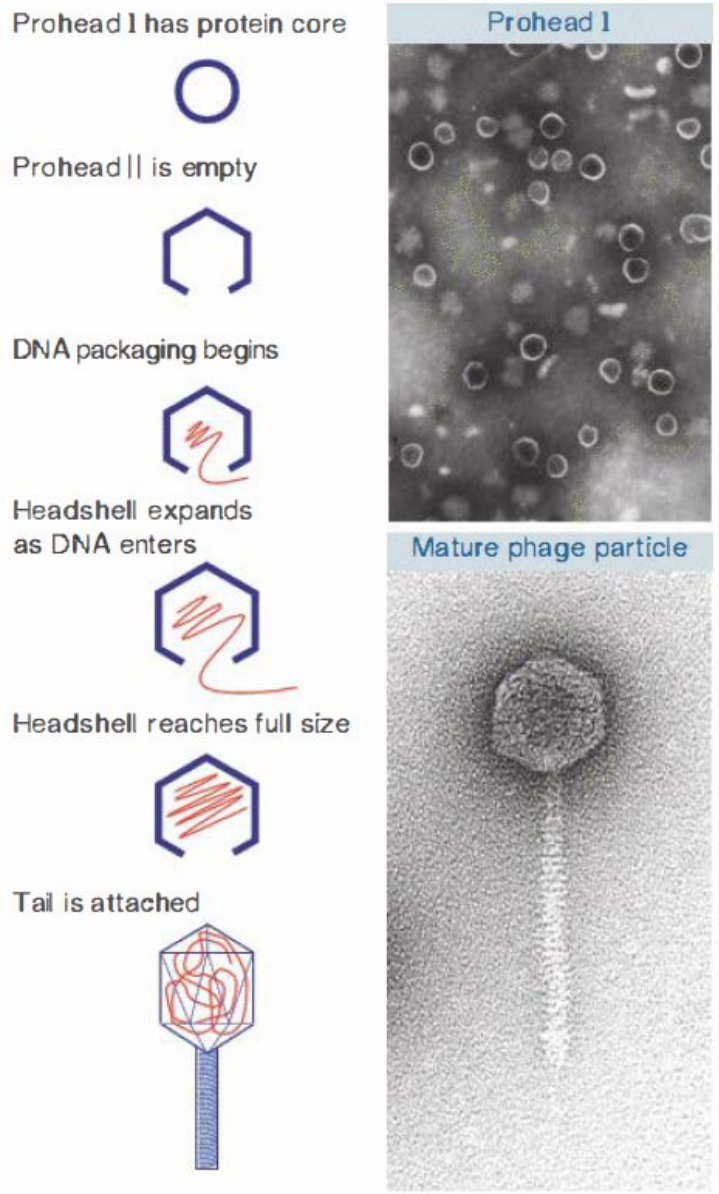
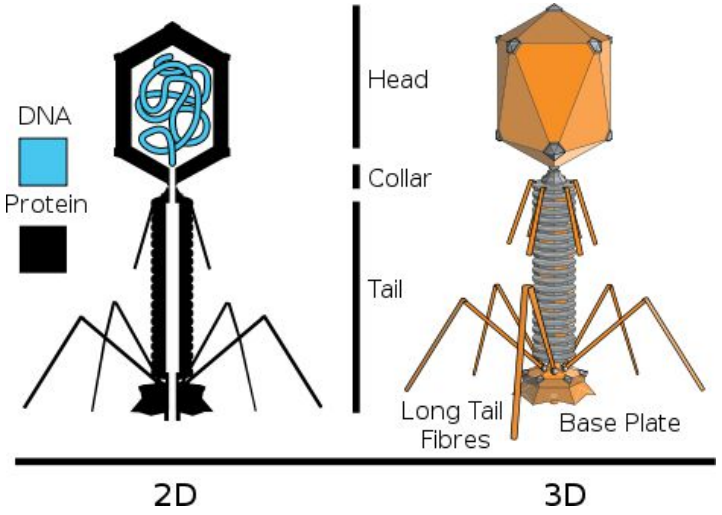
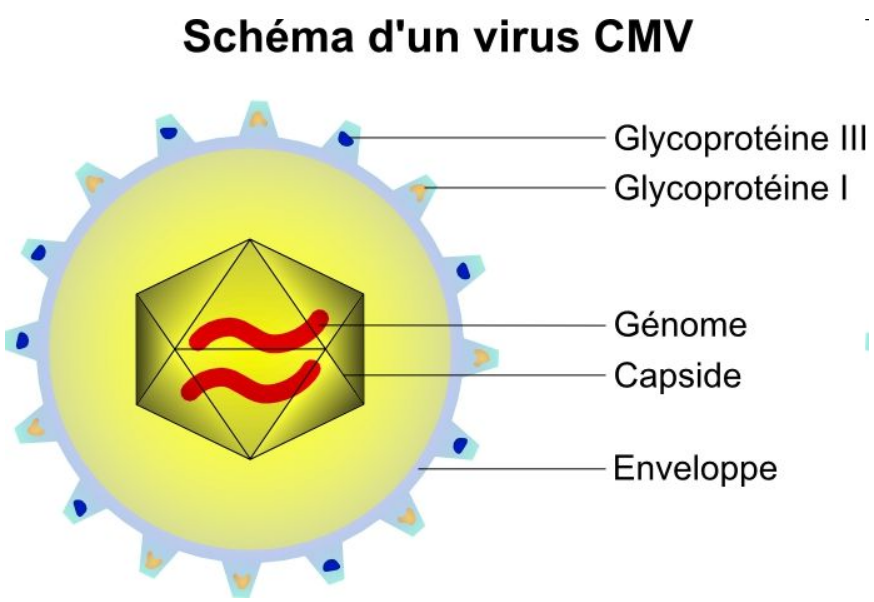


FIGURE 9.3 Maturation of phage lambda passes through several stages. The empty head changes shape and expands when it becomes filled with DNA. The electron micrographs on the right show the particles at the start and the end of the maturation pathway. Top photo reproduced from D. Cue and M. Feiss, *Proc. Natl. Acad. Sci. USA* 90 (1993): 9240–9294. Copyright © 2004 National Academy of Sciences, U.S.A. Bottom photo courtesy of Michael G. Feiss, University of Iowa. Bottom photo courtesy of Robert Duda, University of Pittsburgh.





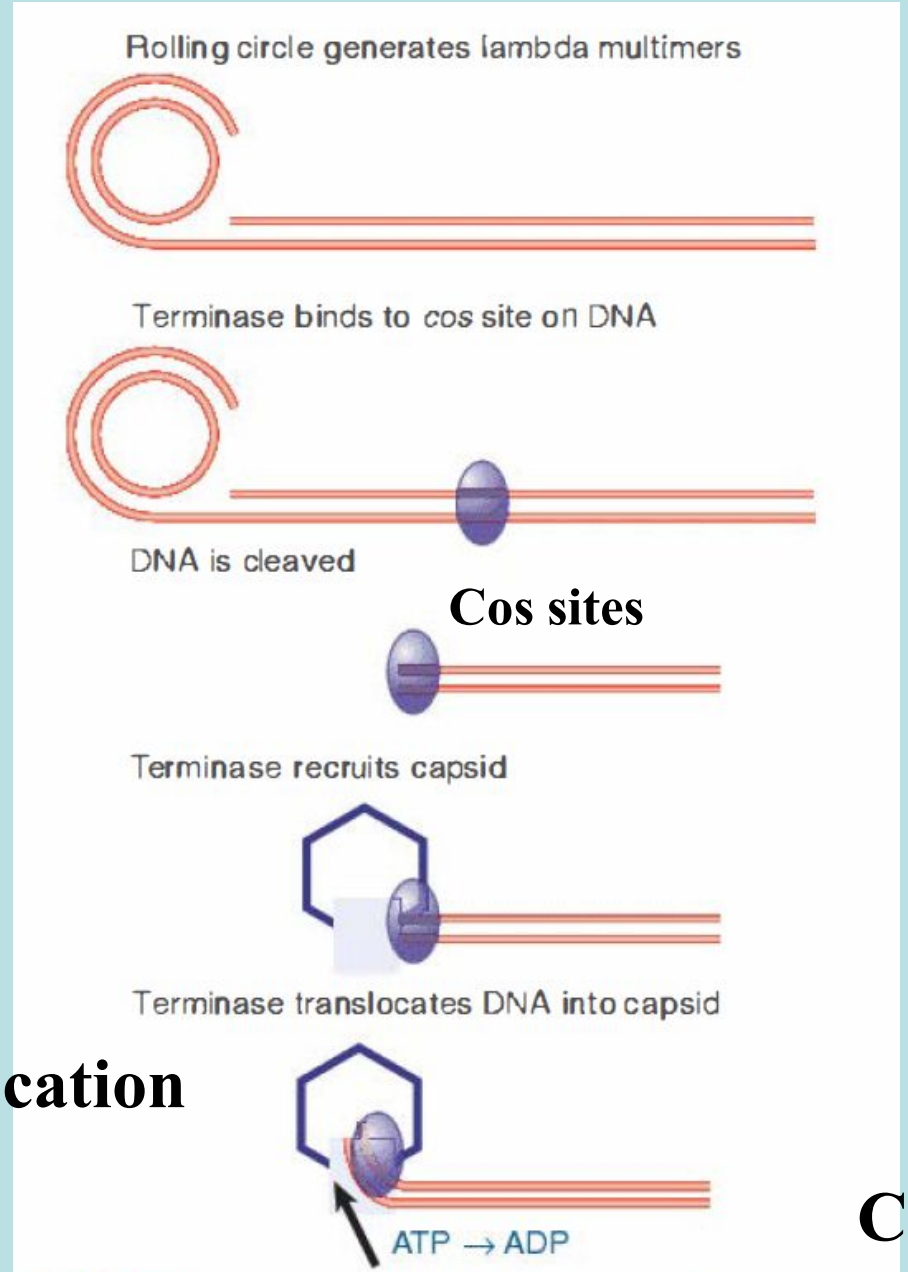
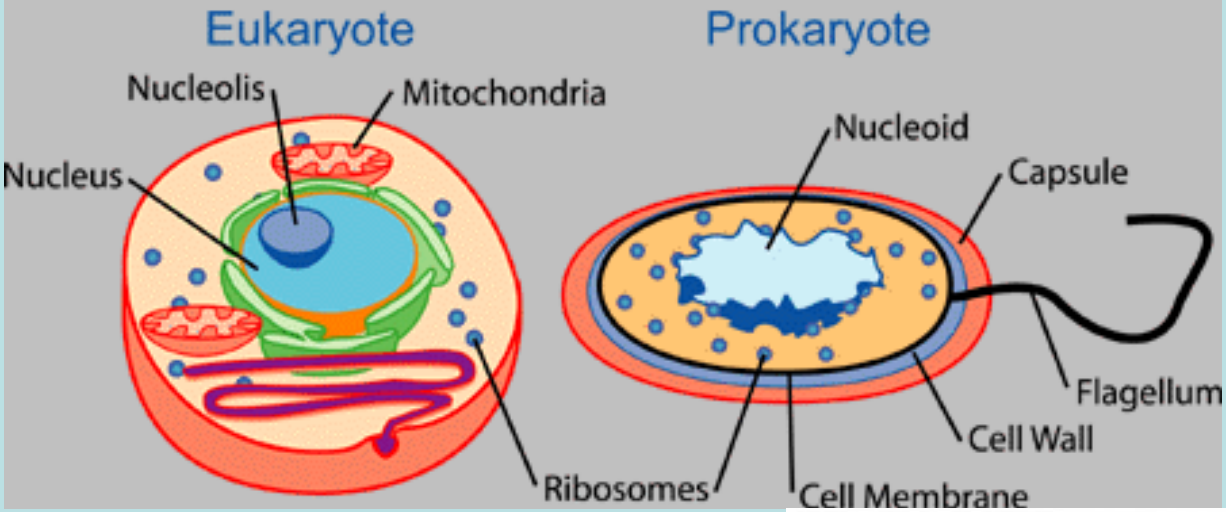


FIGURE 9.4 Terminase protein binds to specific sites on a multimer of virus genomes generated by rolling circle replication. It cuts the DNA and binds to an empty virus capsid, and then uses energy from hydrolysis of ATP to insert the DNA into the capsid.

IHF => Integration Host factor



The nucleoid (meaning *nucleus-like*) is an irregularly-shaped region within the cell of prokaryotes which has nuclear material without a nuclear membrane and where the genetic material is localized.

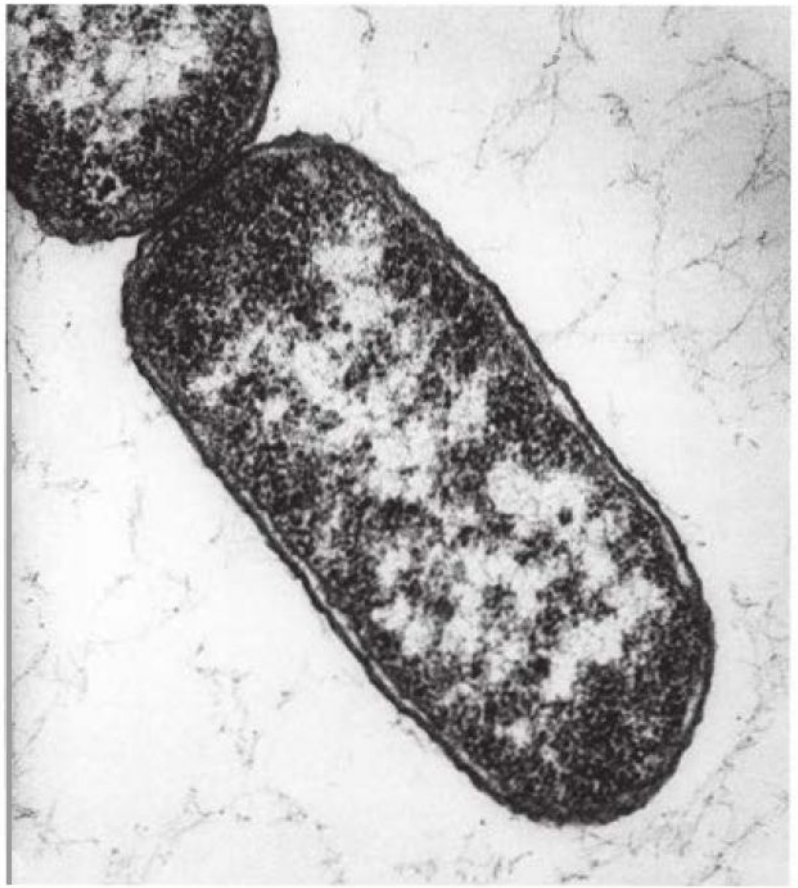


FIGURE 9.5 A thin section shows the bacterial nucleoid as a compact mass in the center of the cell. Photo courtesy of the Molecular and Cell Biology Instructional Laboratory Program, University of California, Berkeley.

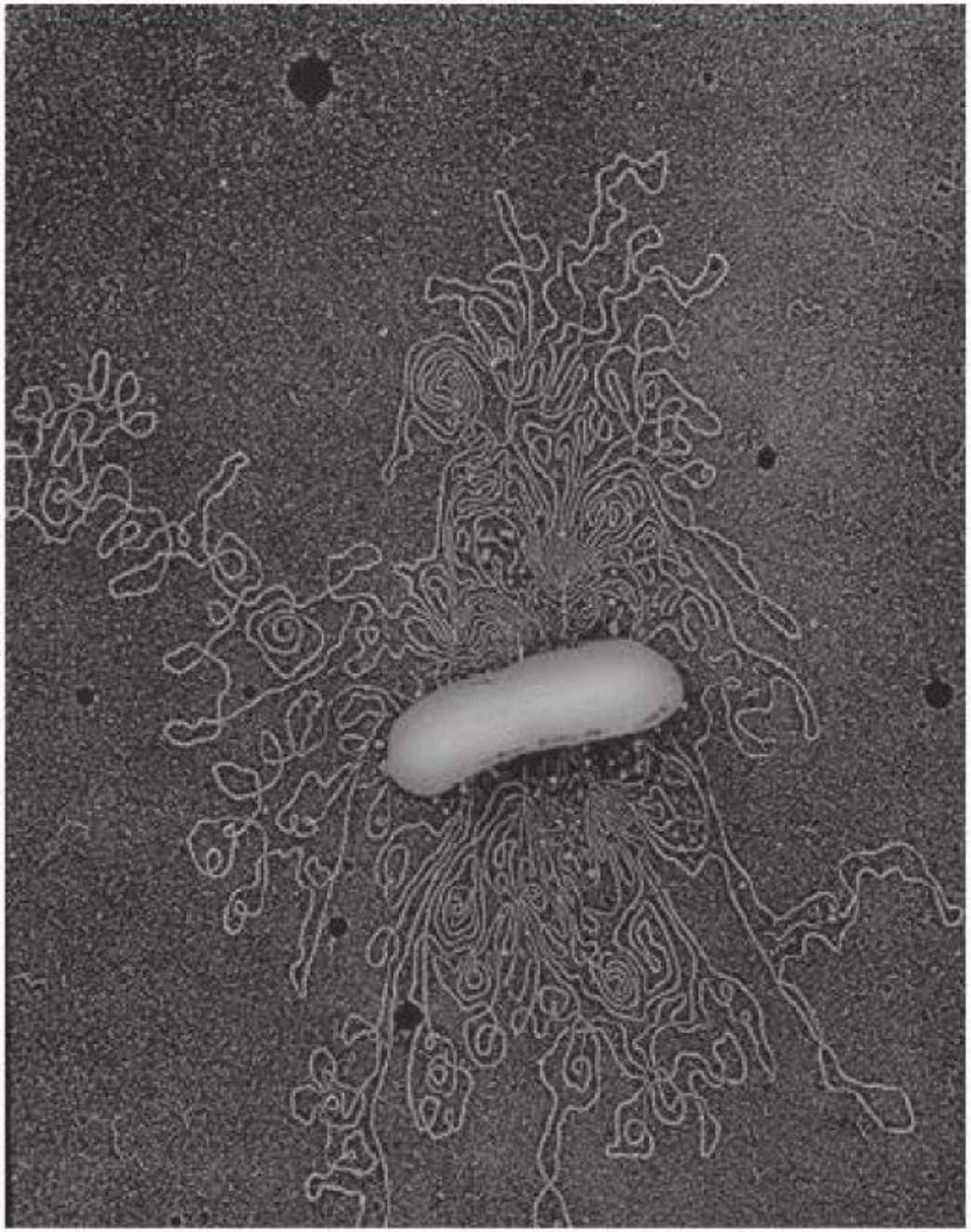


FIGURE 9.6 The nucleoid spills out of a lysed *E. coli* cell in the form of loops of a fiber. © G. Murti/Photo Researchers, Inc.

E.coli has many DNA binding proteins…

Protein	Composition	No./cell	Equivalent in eukaryotes	Locus
HU	α&β subunits, 9000 Da each	40,000 dimers	H2B Histone	*hupA,B*
H	2 identical subunits of 28,000 Da	30,000 dimers	H2A Histone	?
IHF	α subunit = 10,500; β subunit = 9,500	?	?	*himA,D*
H1	subunit of 15,000 Da	10,000 copies	?	?
HLP1	monomer of 17,000 Da	20,000 copies	?	*firA*
P	subunit of 3,000 Da	?	Protamins	?

… and positively charged molecules (poliammines).

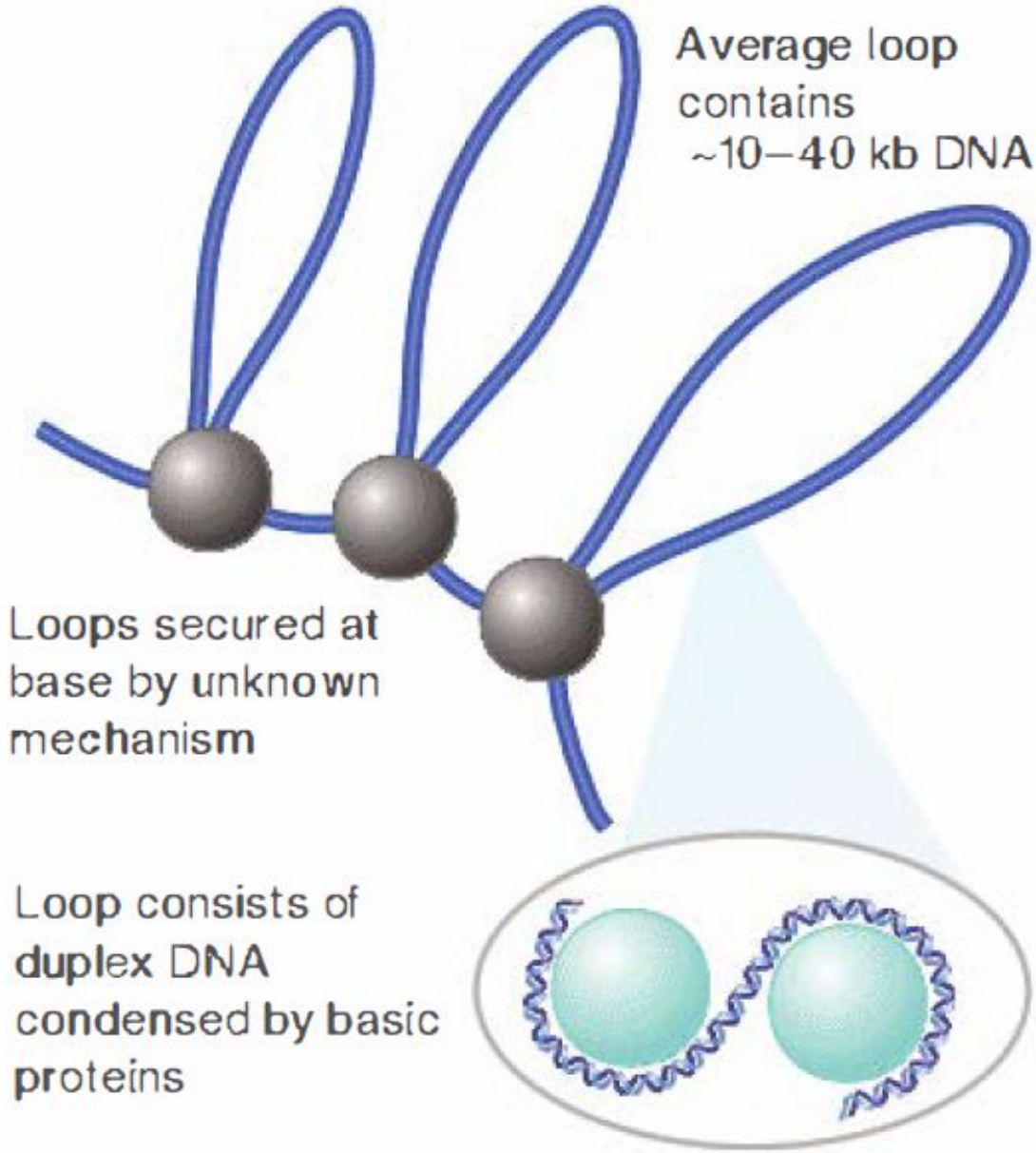


FIGURE 9.7 The bacterial genome consists of a large number of loops of duplex DNA (in the form of a fiber), each of which is secured at the base to form an independent structural domain.

Nuclear DNA organization in eukaryotes


HHMI

Nuclear eukaryotic DNA associates with proteins to form chromatin.

Chromatin is the complex that makes up chromosomes.

The most abundant proteins of chromatin are HISTONES

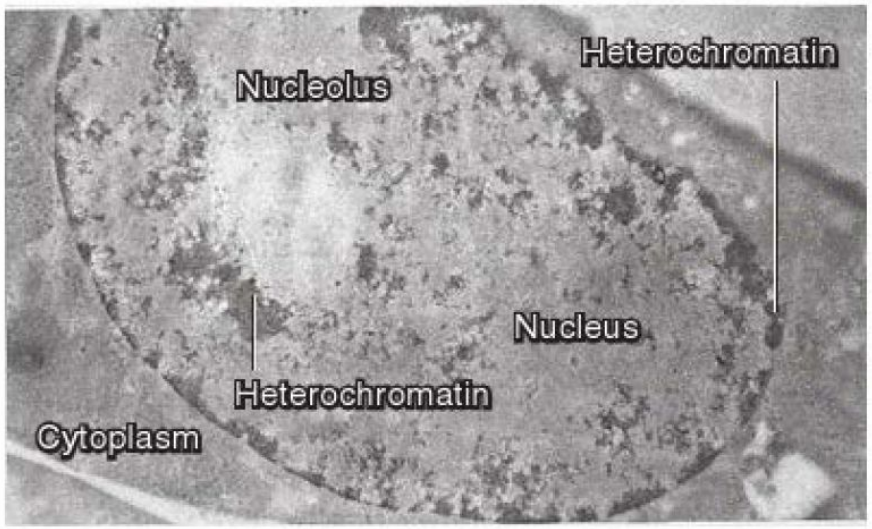


FIGURE 9.12 A thin section through a nucleus stained with Feulgen shows heterochromatin as compact regions clustered near the nucleolus and nuclear membrane. Photo courtesy of Edmund Puvion, Centre National de la Recherche Scientifique.

Euchromatin is a lightly packed form of chromatin (DNA, RNA and protein) that is rich in gene concentration, and is often (but not always) under active transcription.

Heterochromatin is a tightly packed form of DNA, which comes in two varieties: constitutive and facultative.

FIGURE 10.1 Chromatin spilling out of lysed nuclei consists of a compactly organized series of particles. The bar is 100 nm. Reprinted from *Cell*, vol. 4, P. Oudet, M. Gross-Bellard, and P. Chambon, Electron microscopic and biochemical evidence ..., pp. 281–300. Copyright 1975, with permission from Elsevier [http://www.sciencedirect.com/science/journal/00928674]. Photo courtesy of Pierre Chambon, College of France.

Low ionic strength lysis of nuclei

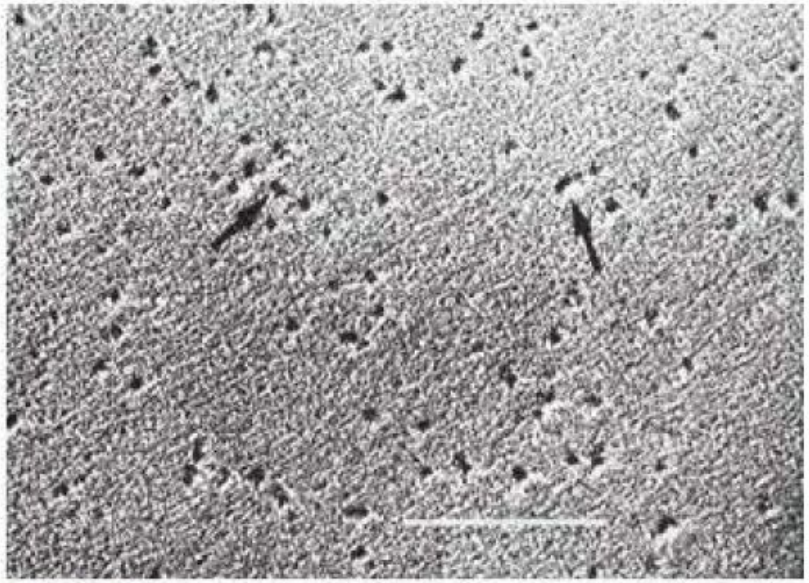


FIGURE 10.2 Individual nucleosomes are released by digestion of chromatin with micrococcal nuclease. The bar is 100 nm. Reprinted from *Cell*, vol. 4, P. Oudet, M. Gross-Bellard, and P. Chambon, Electron microscopic and biochemical evidence ..., pp. 281–300. Copyright 1975, with permission from Elsevier [http://www.sciencedirect.com/science/journal/00928674]. Photo courtesy of Pierre Chambon, College of France.

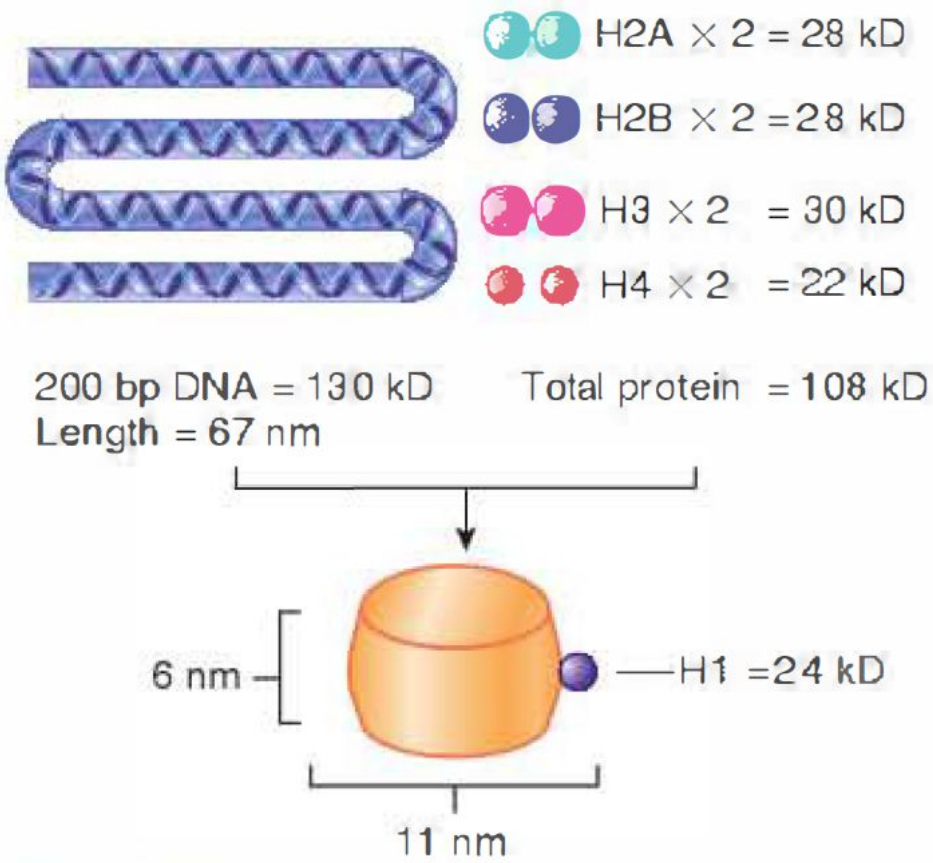


FIGURE 10.6 The nucleosome consists of approximately equal masses of DNA and histones (including H1). The predicted mass of the nucleosome is 262 kD.

Micrococcal nuclease digestion

Histones are very basic proteins

Histones	Basic amino acids		Acidic Amino acids	Basic / acidic ratio	Molecular weight
	Lys	Arg			
H1	29 %	1 %	5 %	5.4	23.000
H2A	11 %	9 %	15 %	1.4	13.960
H2B	16 %	6 %	13 %	1.7	13.774
H3	10 %	13 %	13 %	1.8	15.342
H4	11 %	14 %	10 %	2.5	11.282

data concerning mammalian histones (similar data for other organism)

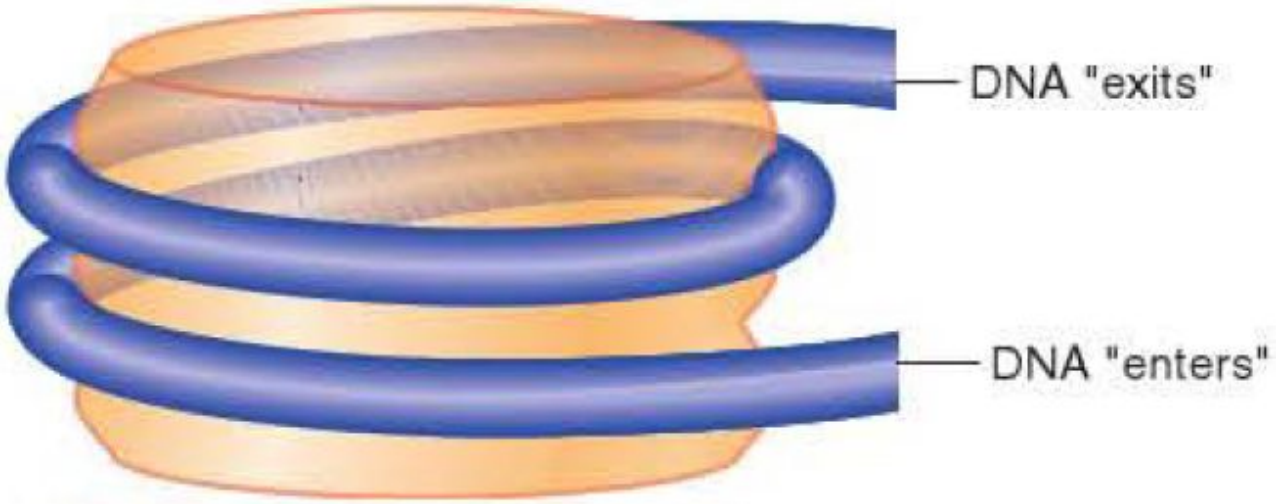


FIGURE 10.7 The nucleosome is a cylinder with DNA organized into ~1⅔ turns around the surface.

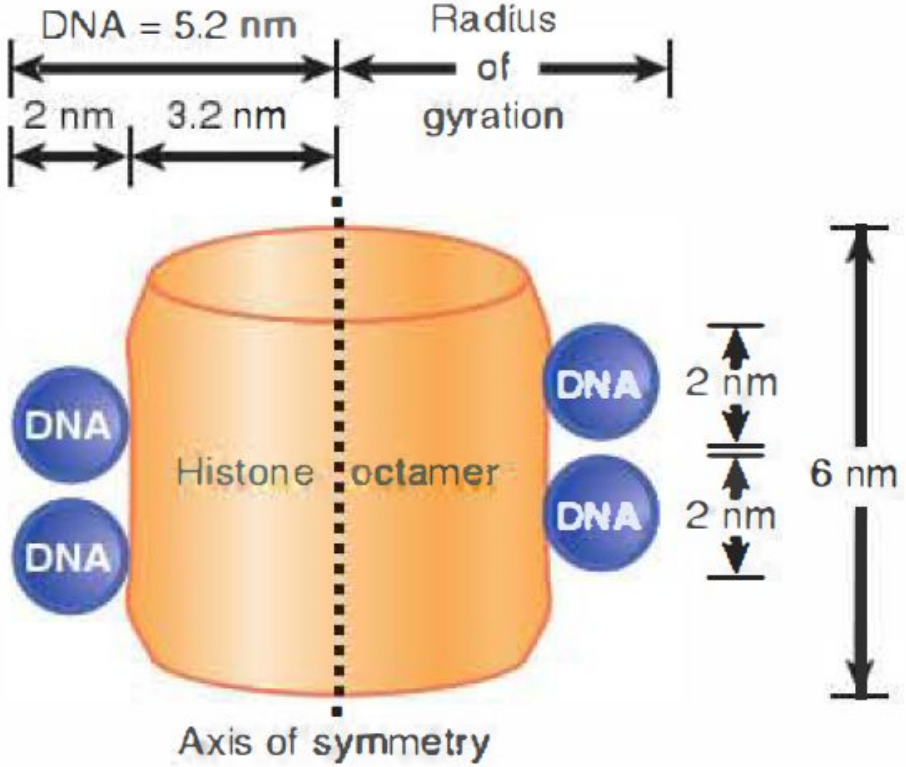


FIGURE 10.8 DNA occupies most of the outer surface of the nucleosome.

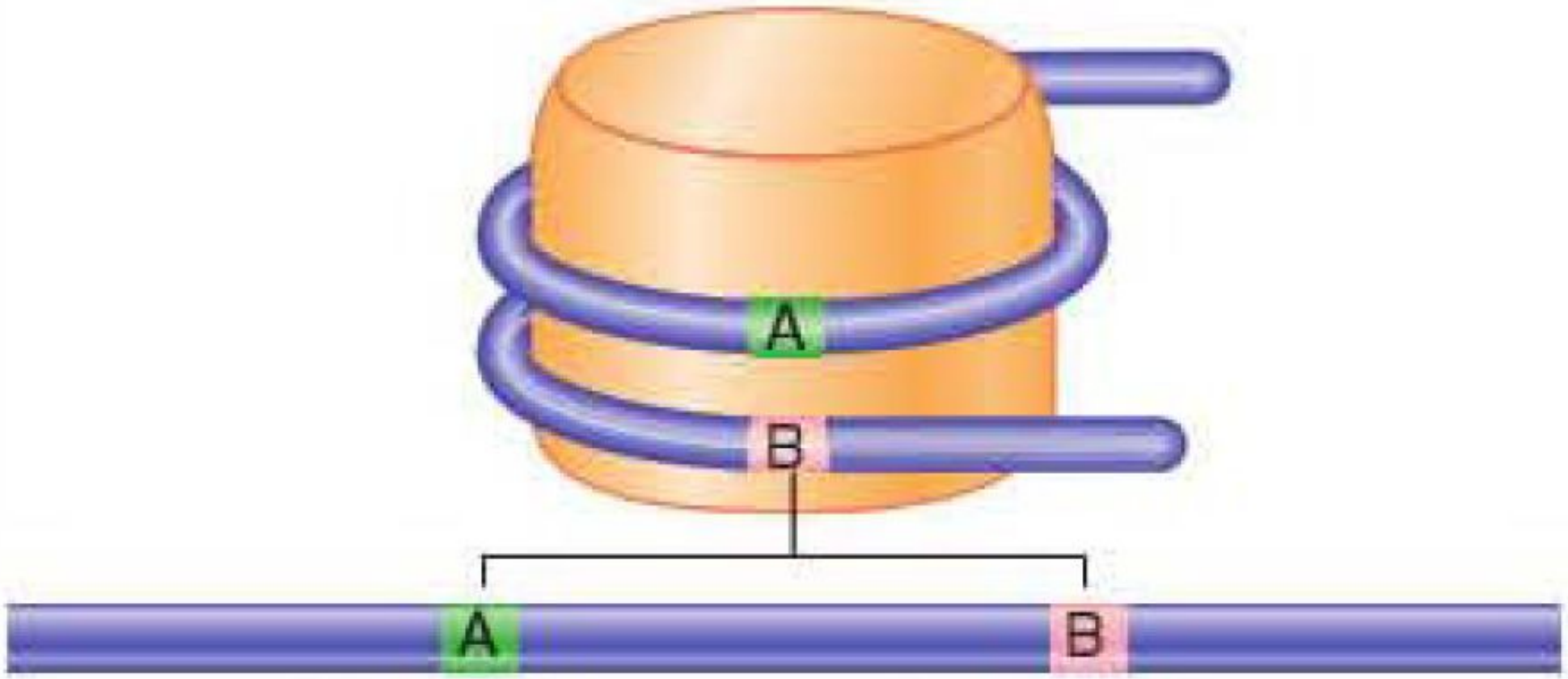
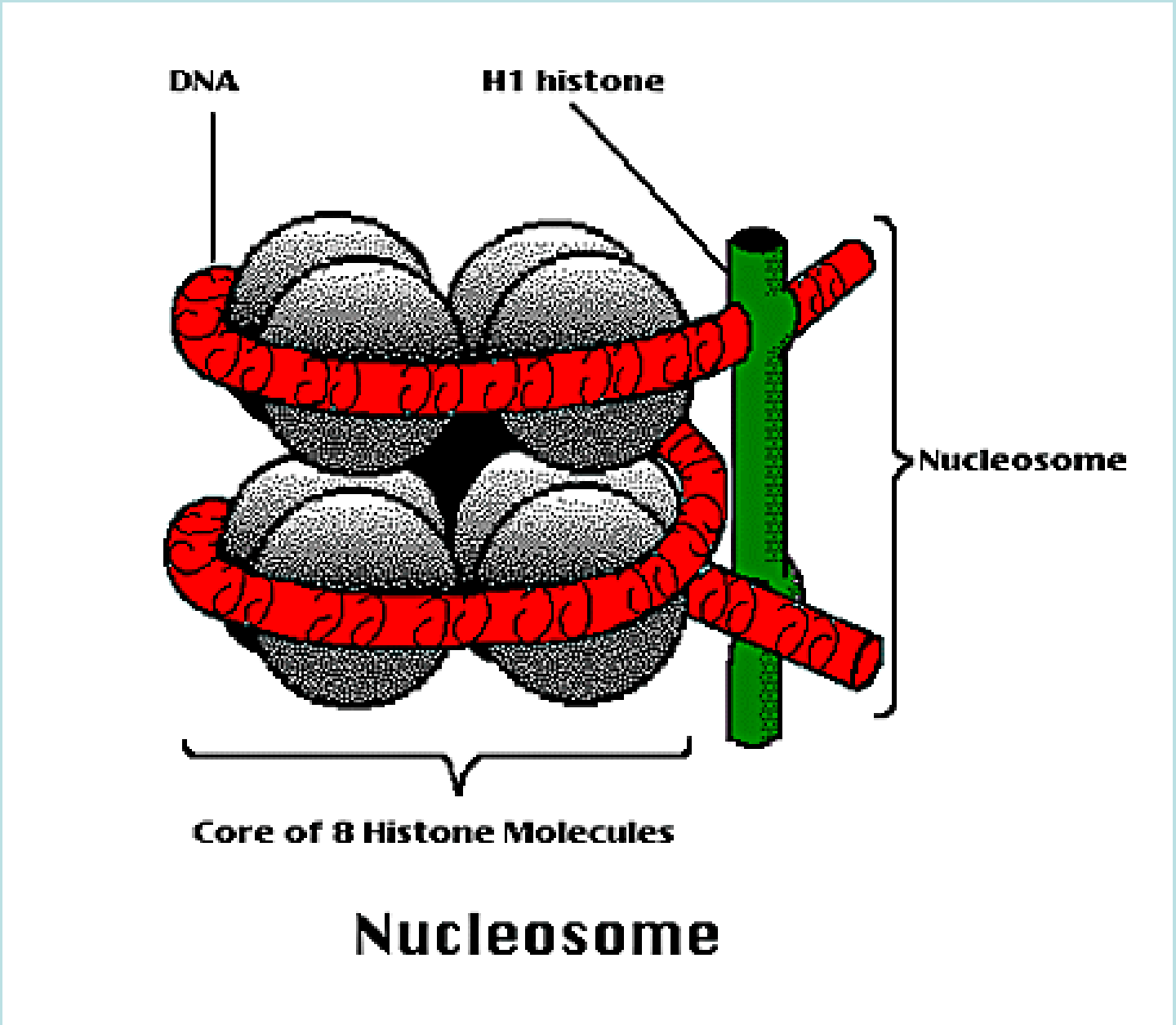


FIGURE 10.9 Sequences on the DNA that lie on different turns around the nucleosome may be close together.



Nucleosome

Nucleases used for chromatin studies

- Micrococcal Nuclease → cuts the double strand
- DNAse I and II → cuts the single strand

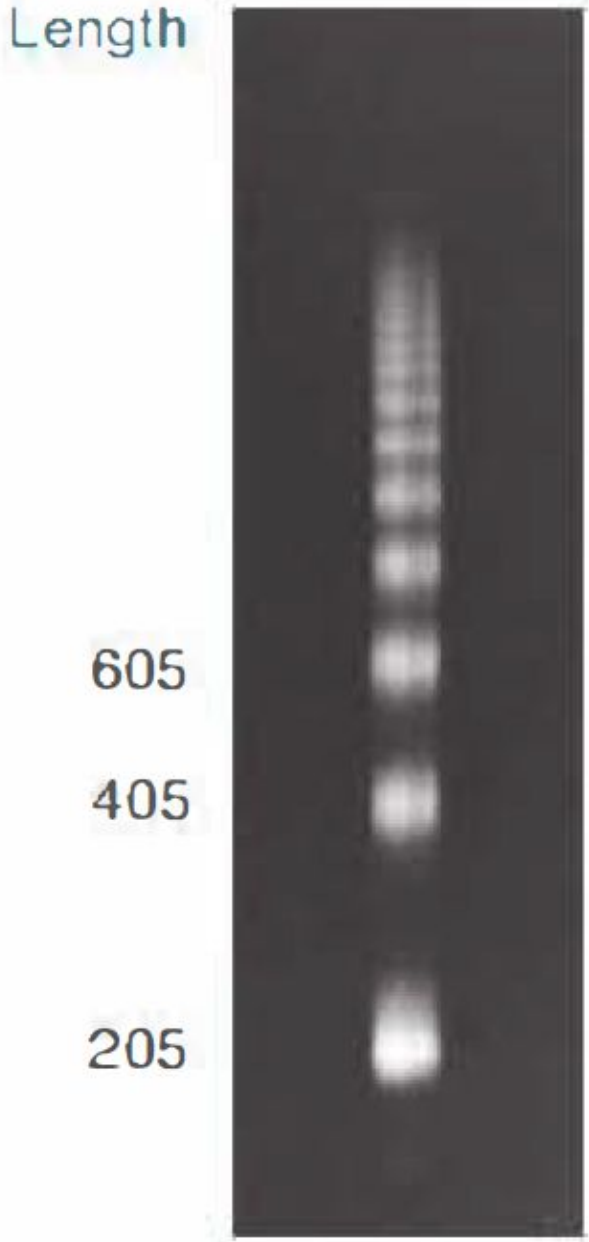


FIGURE 10.3 Micrococcal nuclease digests chromatin in nuclei into a multimeric series of DNA bands that can be separated by gel electrophoresis. Photo courtesy of Markus Noll, Universität Zürich.

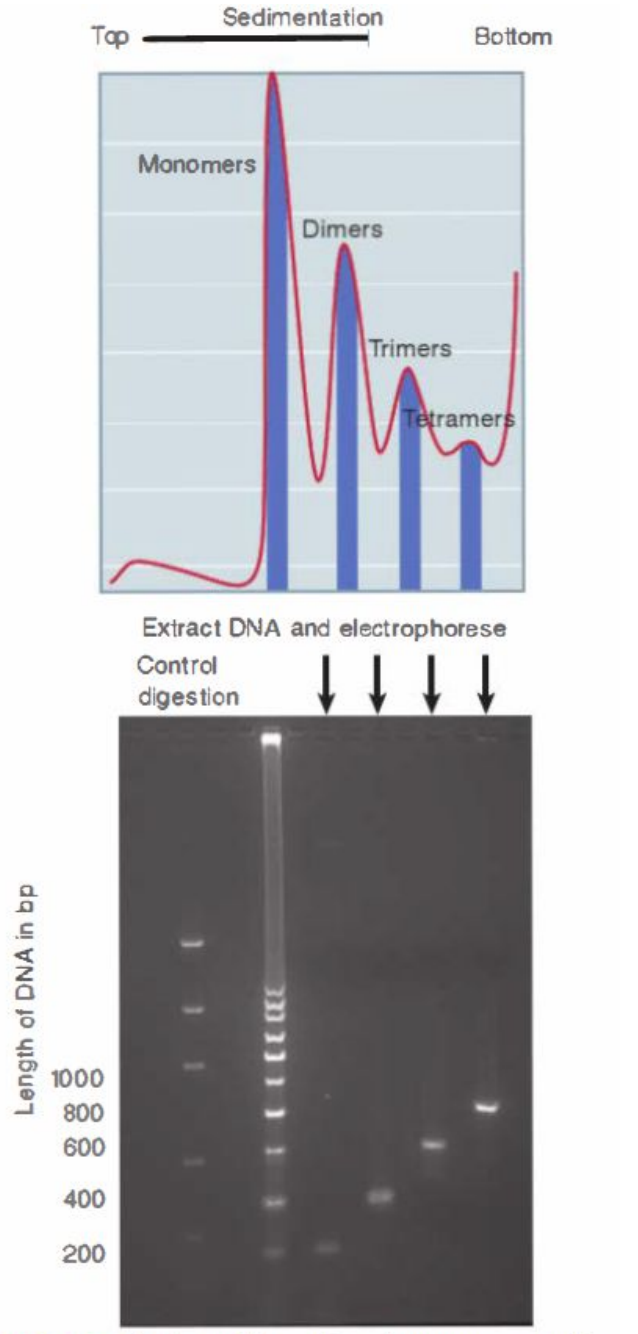


FIGURE 10.4 Each multimer of nucleosomes contains the appropriate number of unit lengths of DNA. In the photo, artificial bands simulate a DNA ladder that would be produced by MNase digestion. The image was constructed using PCR fragments with sizes corresponding to actual band sizes. Photo courtesy of Jan Kieleczawa, Wyzer Biosciences.

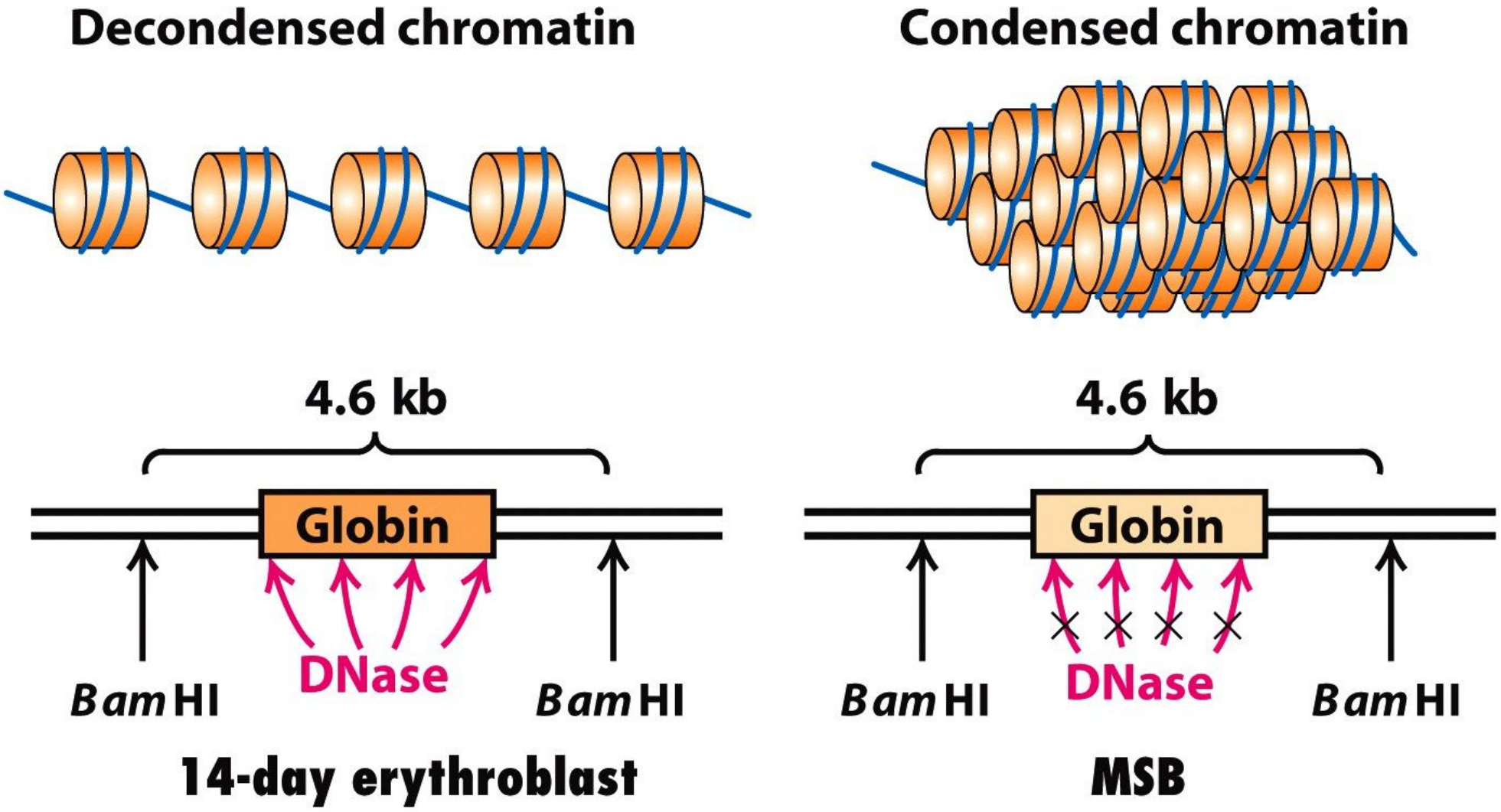


Figure 6-32a
Molecular Cell Biology, Sixth Edition

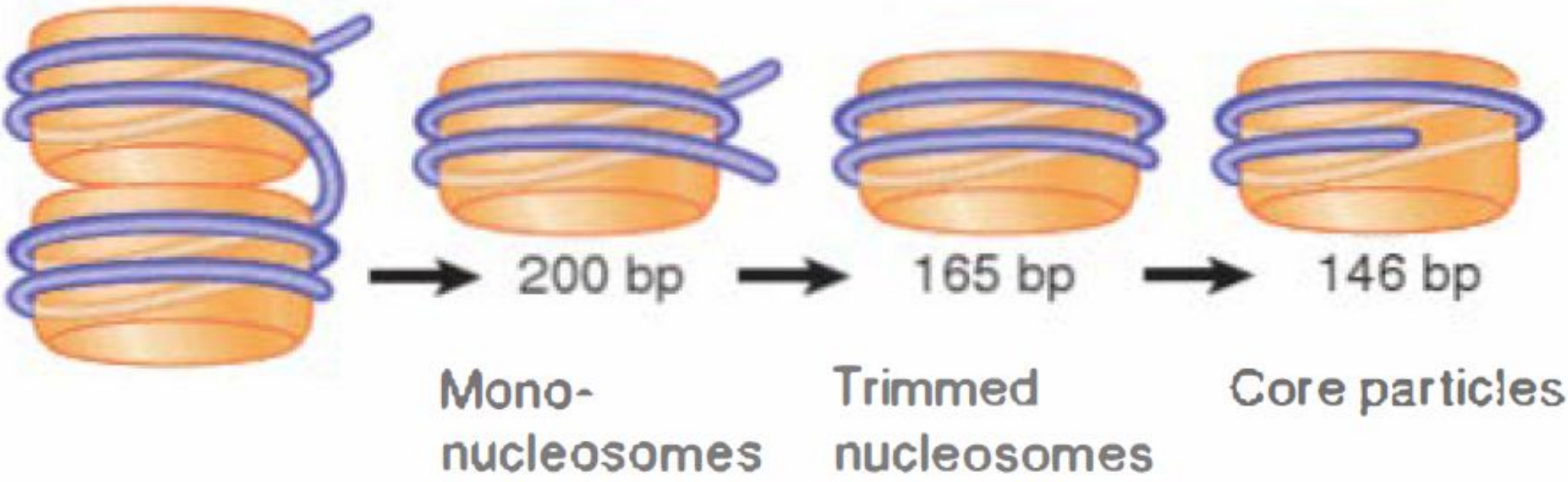


FIGURE 10.5 Micrococcal nuclease initially cleaves between nucleosomes. Mononucleosomes typically have ~200 bp DNA. End-trimming reduces the length of DNA first to ~165 bp, and then generates core particles with 146 bp.

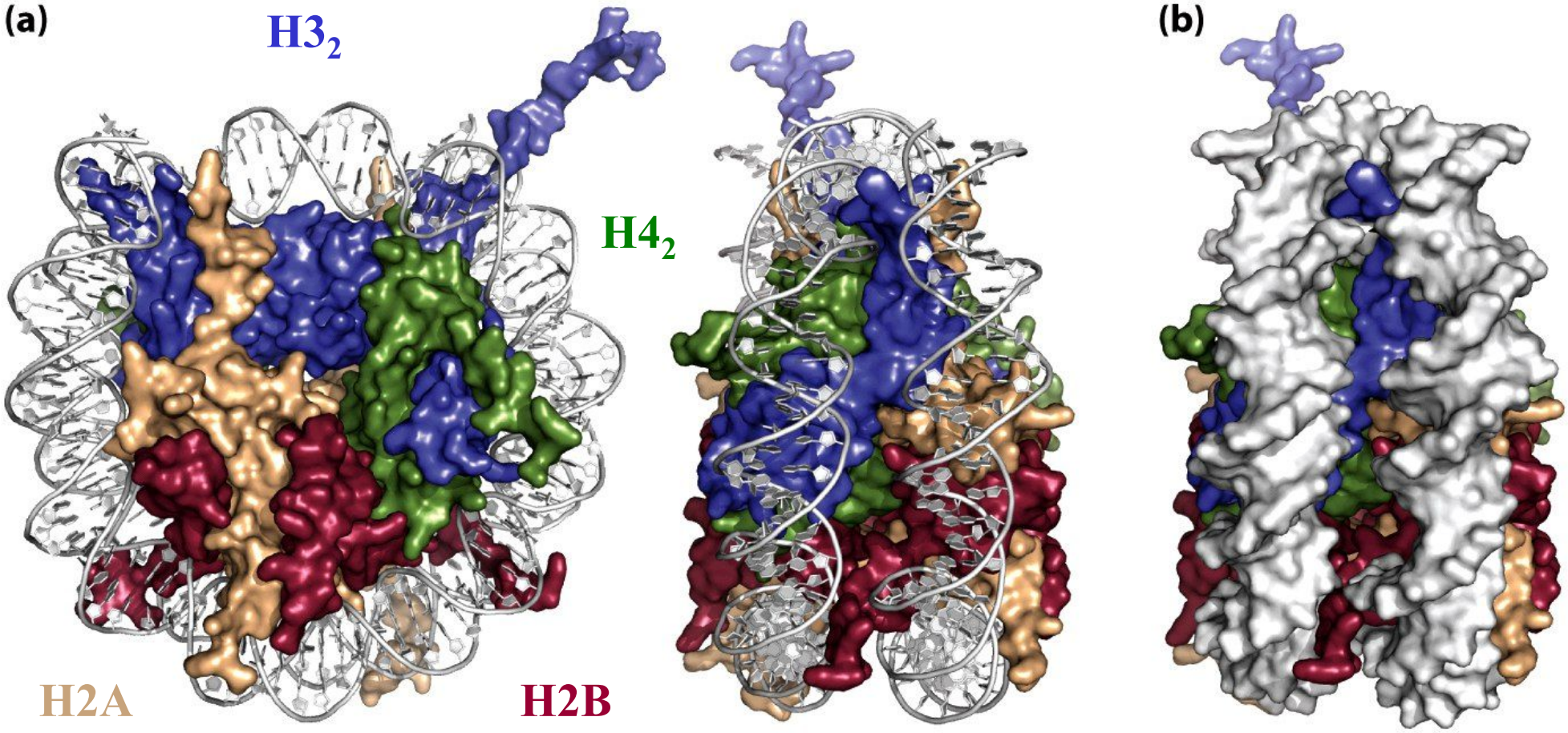


Figure 6-29
Molecular Cell Biology, Sixth Edition

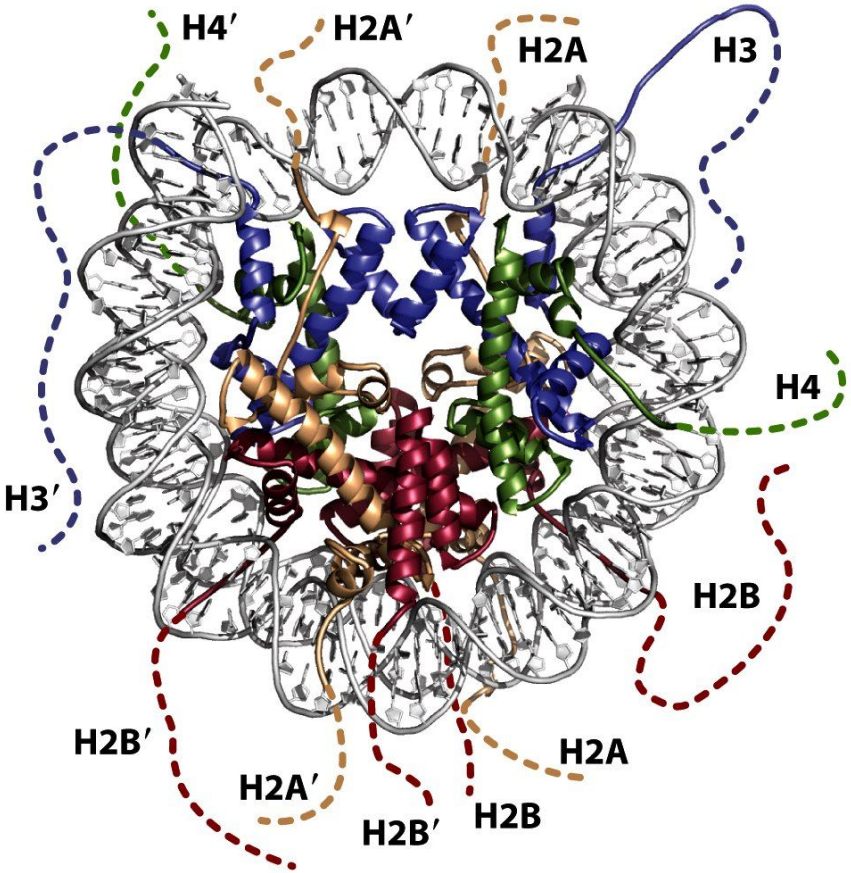


Figure 6-31a
Molecular Cell Biology, Sixth Edition

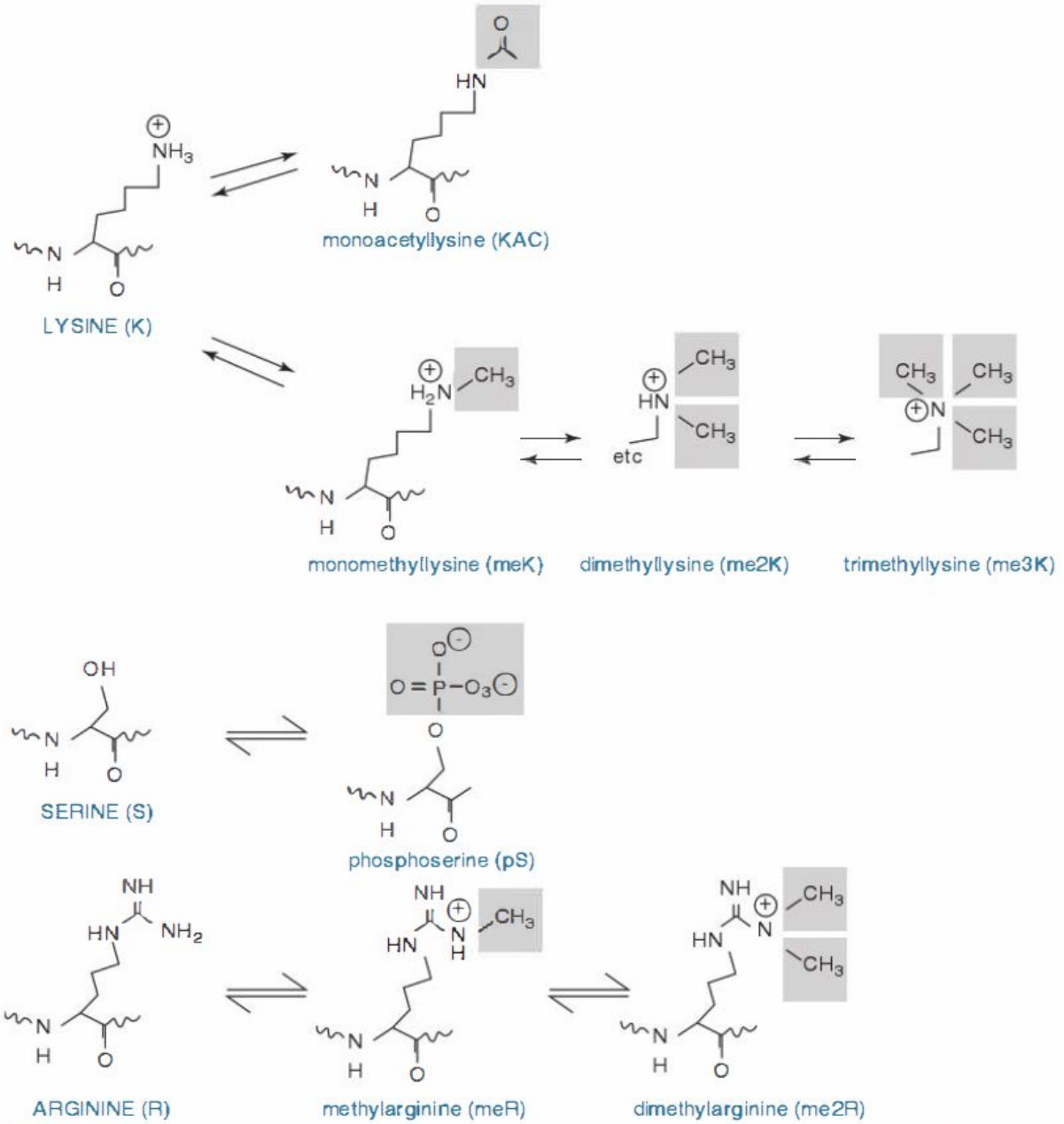


FIGURE 10.17 The positive charge on lysine is neutralized upon acetylation, while methylated lysine and arginine retain their positive charges. Lysine can be mono-, di-, or triacetylated, while arginine can be mono- or diacetylated. Serine or threonine phosphorylation results in a negative charge.

DNA structure changes on the surface of the nucleosome

- If DNA in solution is digested by DNAse I and II it is cutted randomly.
- If DNA is part of nucleosomes it is cutted only in those regions that are accessible with regular gaps. (separated by about 10-11 bp).
- The DNA has more bp/turn than its solution value in the middle, but has fewer bp/turn at the ends.

FIGURE 10.27 Sites for nicking lie at regular intervals along core DNA, as seen in a DNase I digest of nuclei. Photo courtesy of Leonard C. Lutter, Molecular Biology Research Program, Henry Ford Hospital.

Do Nucleosomes Lie at Specific Positions?

- Nucleosomes may form at specific positions as the result of either the local structure of DNA or proteins that interact with specific sequences.
- Nucleosomes are deposited specifically at particular DNA sequences, or are excluded by specific sequences. (INTRINSEC mechanism).
- In other cases the first nucleosome in a region is preferentially assembled at a particular site due to action of other protein(s). (EXTRINSIC mechanism).

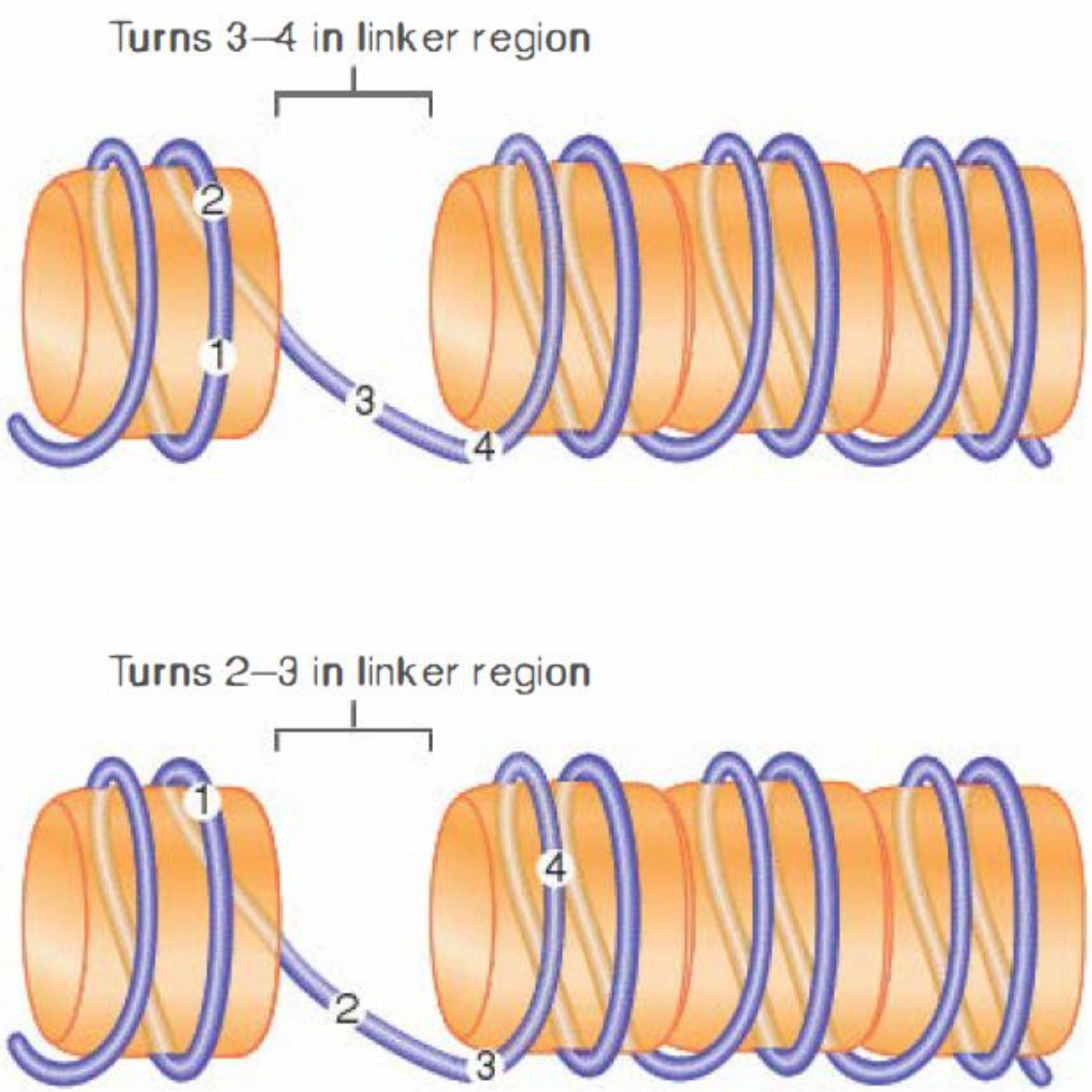


FIGURE 10.41 Translational positioning describes the linear position of DNA relative to the histone octamer. Displacement of the DNA by 10 bp changes the sequences that are in the more exposed linker regions, but does not necessarily alter which face of DNA is protected by the histone surface and which is exposed to the exterior.

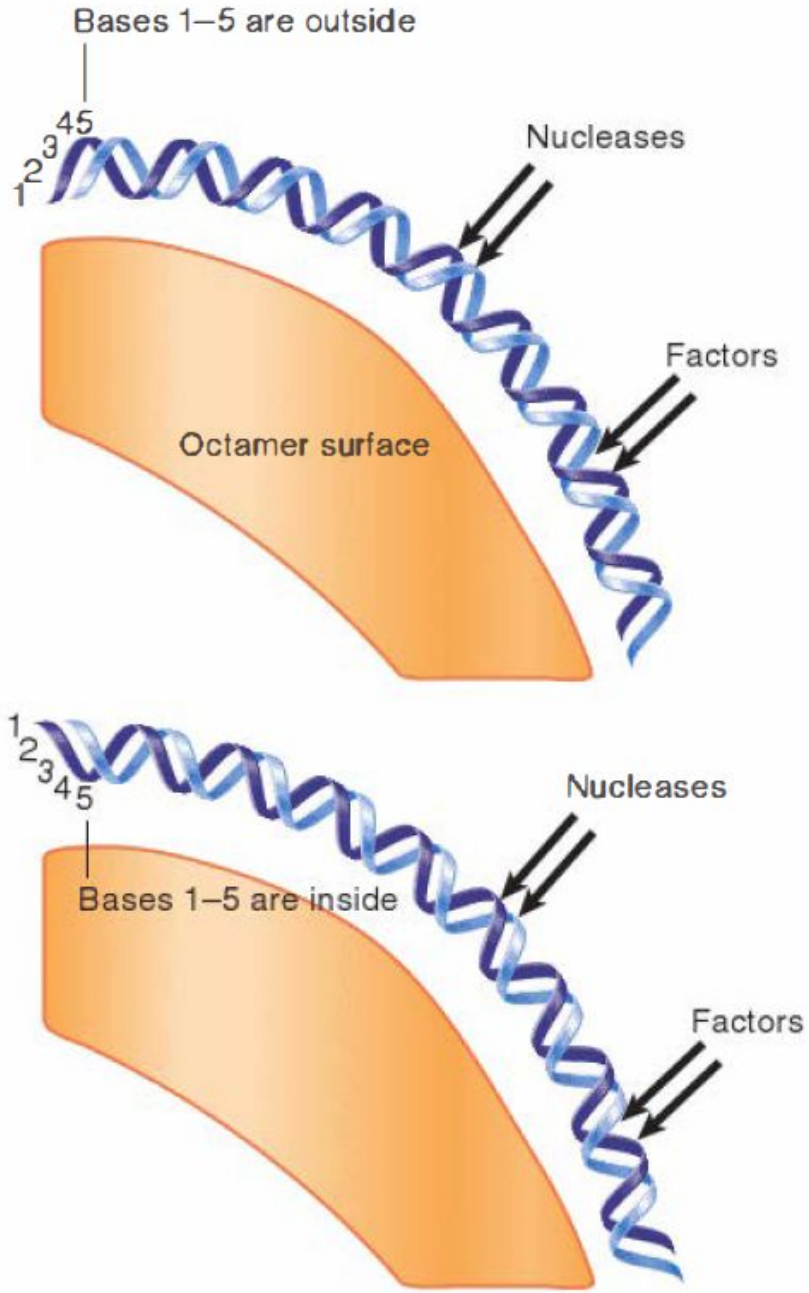
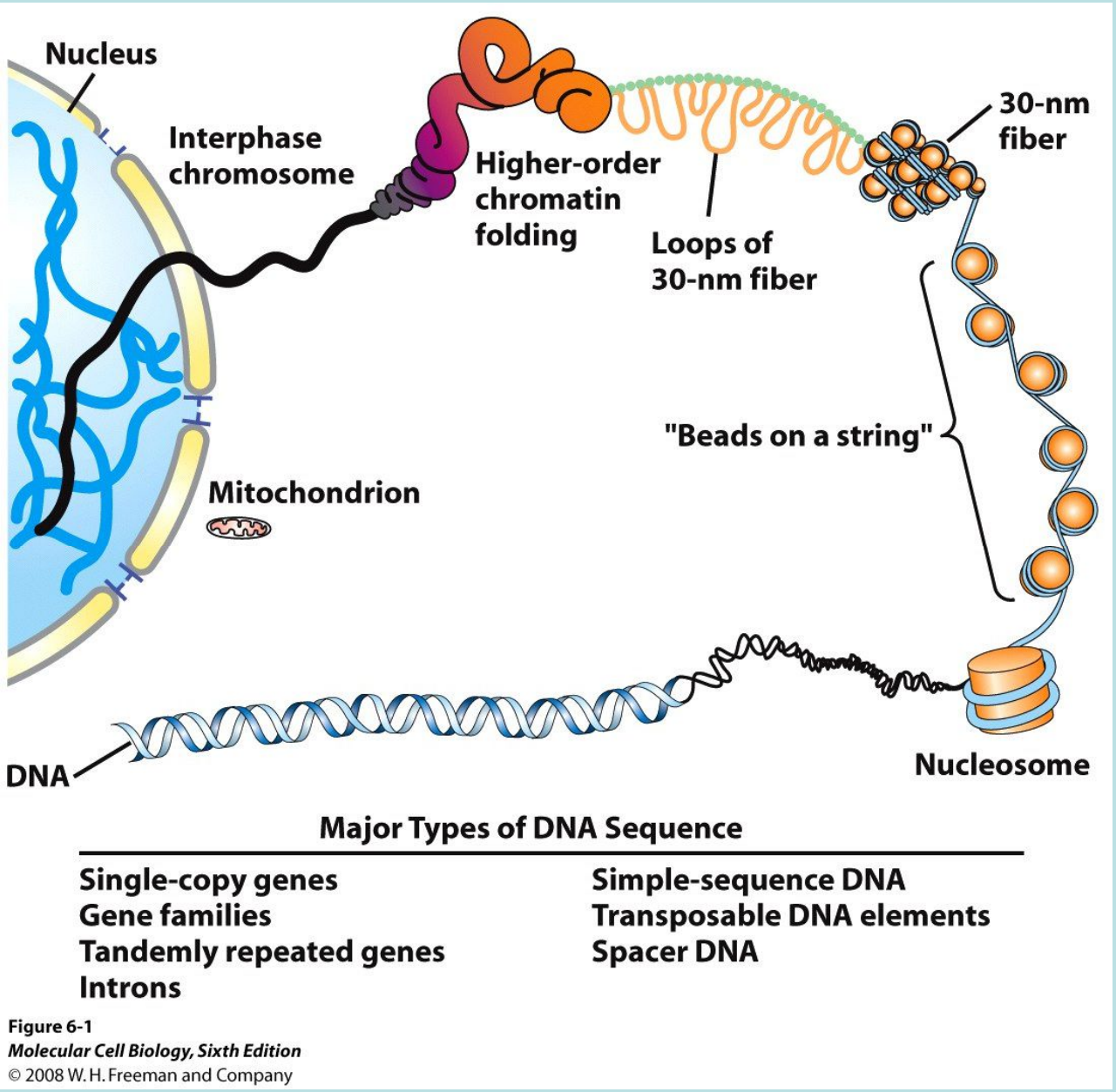


FIGURE 10.42 Rotational positioning describes the exposure of DNA on the surface of the nucleosome. Any movement that differs from the helical repeat (~10.2 bp/turn) displaces DNA with reference to the histone surface. Nucleotides on the inside are more protected against nucleases than nucleotides on the outside.



Major Types of DNA Sequence

Single-copy genes	Simple-sequence DNA
Gene families	Transposable DNA elements
Tandemly repeated genes	Spacer DNA
Introns	

Figure 6-1
Molecular Cell Biology, Sixth Edition

Low ionic strength

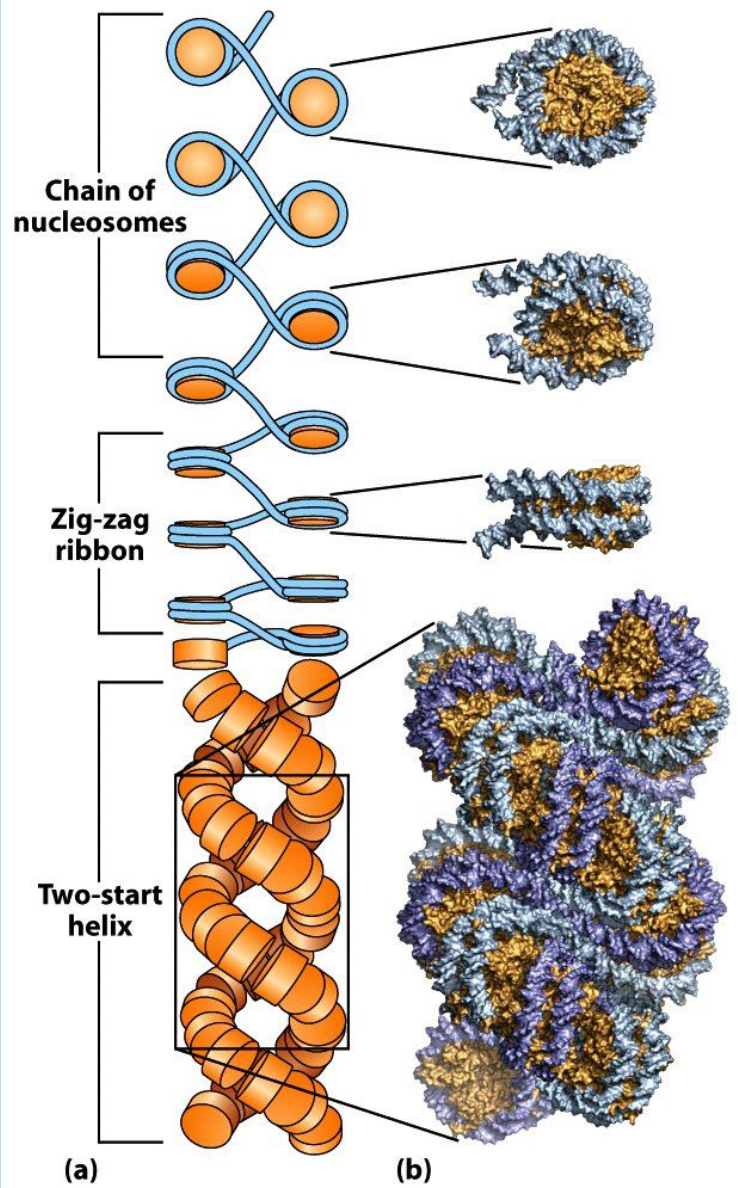
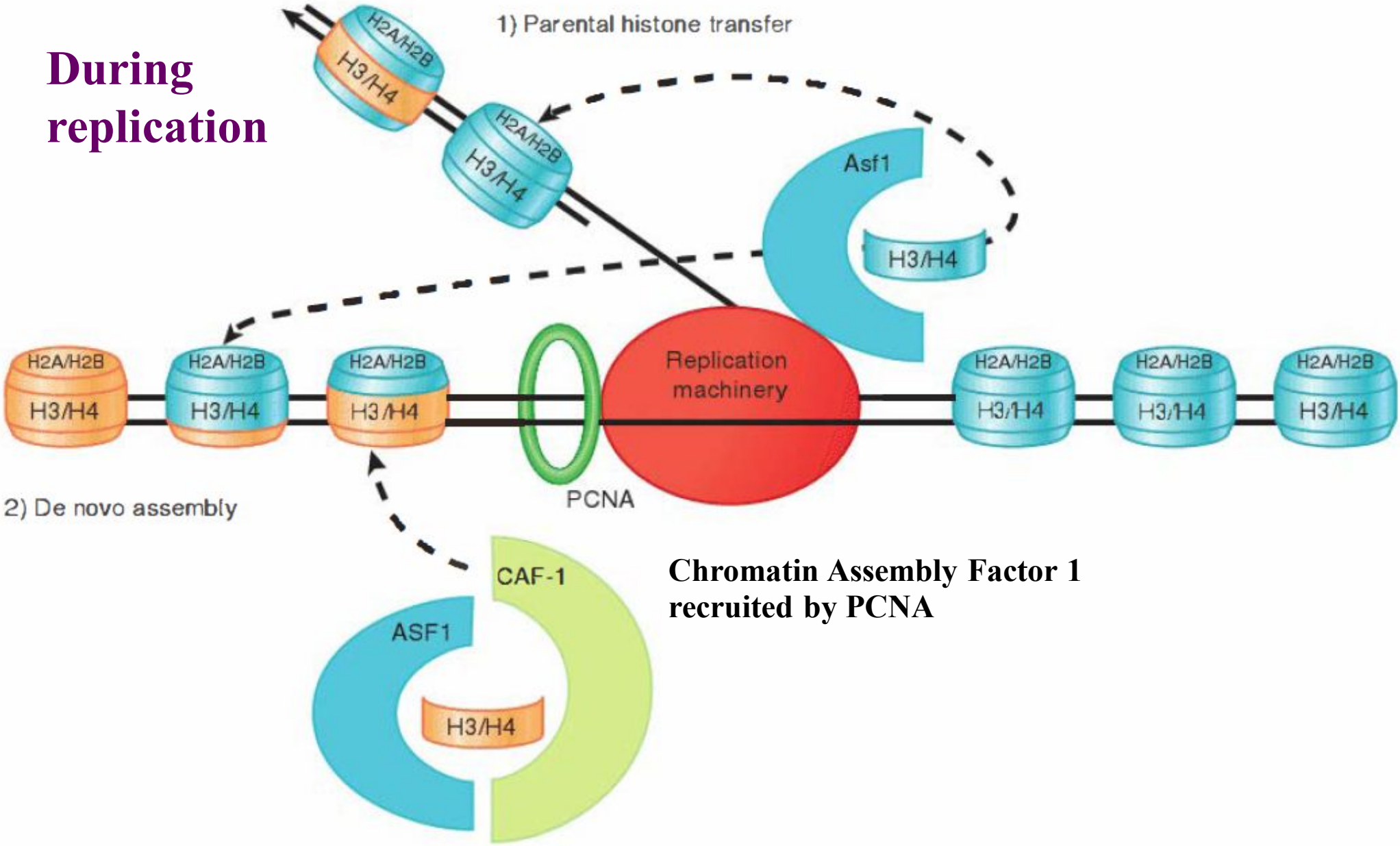


Figure 6-30
Molecular Cell Biology, Sixth Edition

High ionic strength

During replication



Chromatin Assembly Factor 1 recruited by PCNA

FIGURE 10.38 Replication fork passage displaces histone octamers from DNA. They disassemble into H3-H4 tetramers and H2A-H2B dimers. H3-H4 tetramers (blue) are directly transferred behind the replication forks. Newly synthesized histones (orange) are assembled into H3-H4 tetramers and H2A-H2B dimers. The old and new tetramers and dimers are assembled with the aid of histone chaperones into new nucleosomes immediately behind the replication fork. H2A-H2B dimers are omitted from the figure for simplicity; chaperones responsible for dimer assembly have not been identified. Adapted from W. Rocha and A. Verreault, *FEBS Lett.* 582 (2008): 1938–1949.

Nucleosomes are present
with the same frequency
into transcribed and
non-transcribed gene sequences

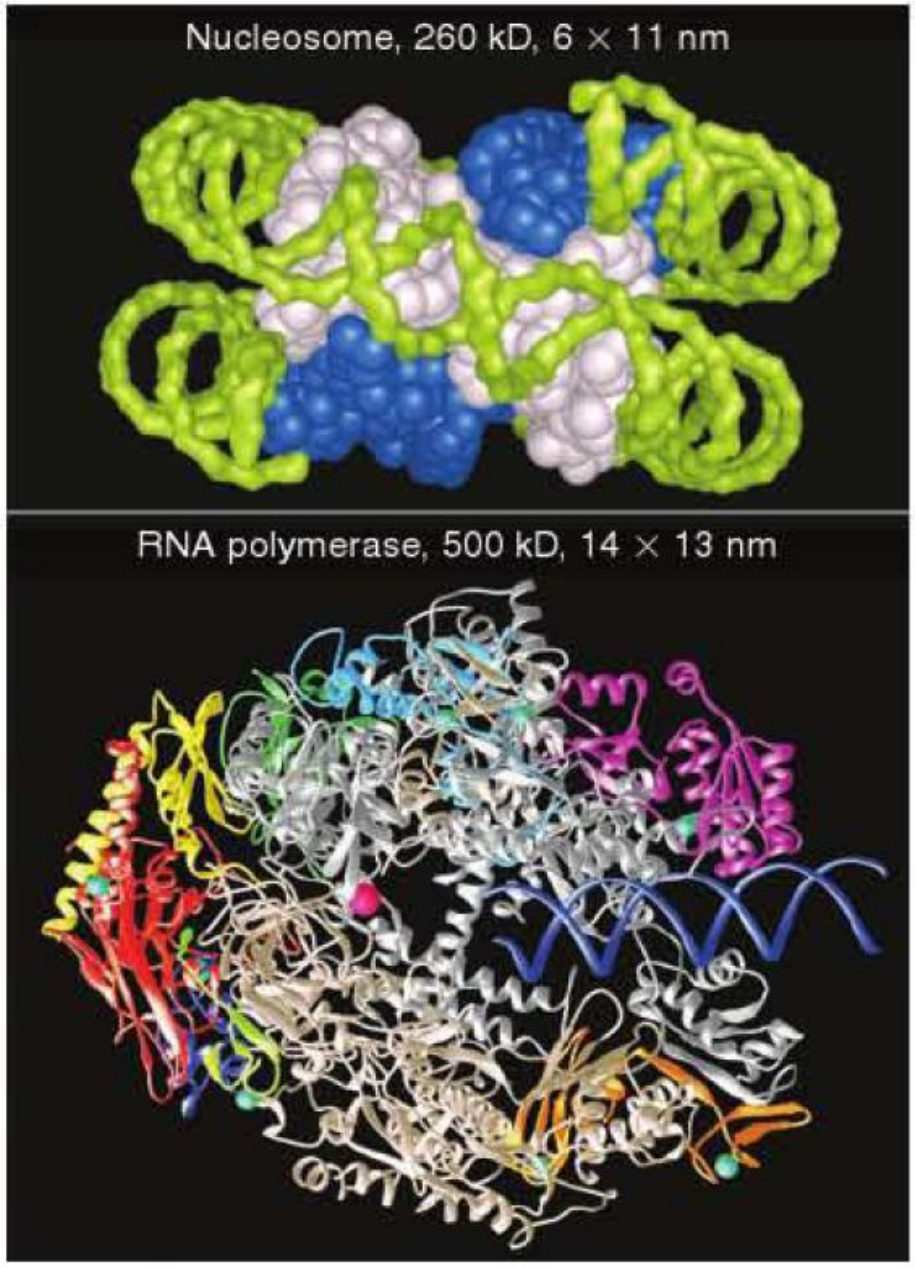


FIGURE 10.45 RNA polymerase is nearly twice the size of the nucleosome and might encounter difficulties in following the DNA around the histone octamer. Top photo courtesy of E. N. Moudrianakis, the Johns Hopkins University. Bottom photo courtesy of Roger Kornberg, Stanford University School of Medicine.

T7 polymerase

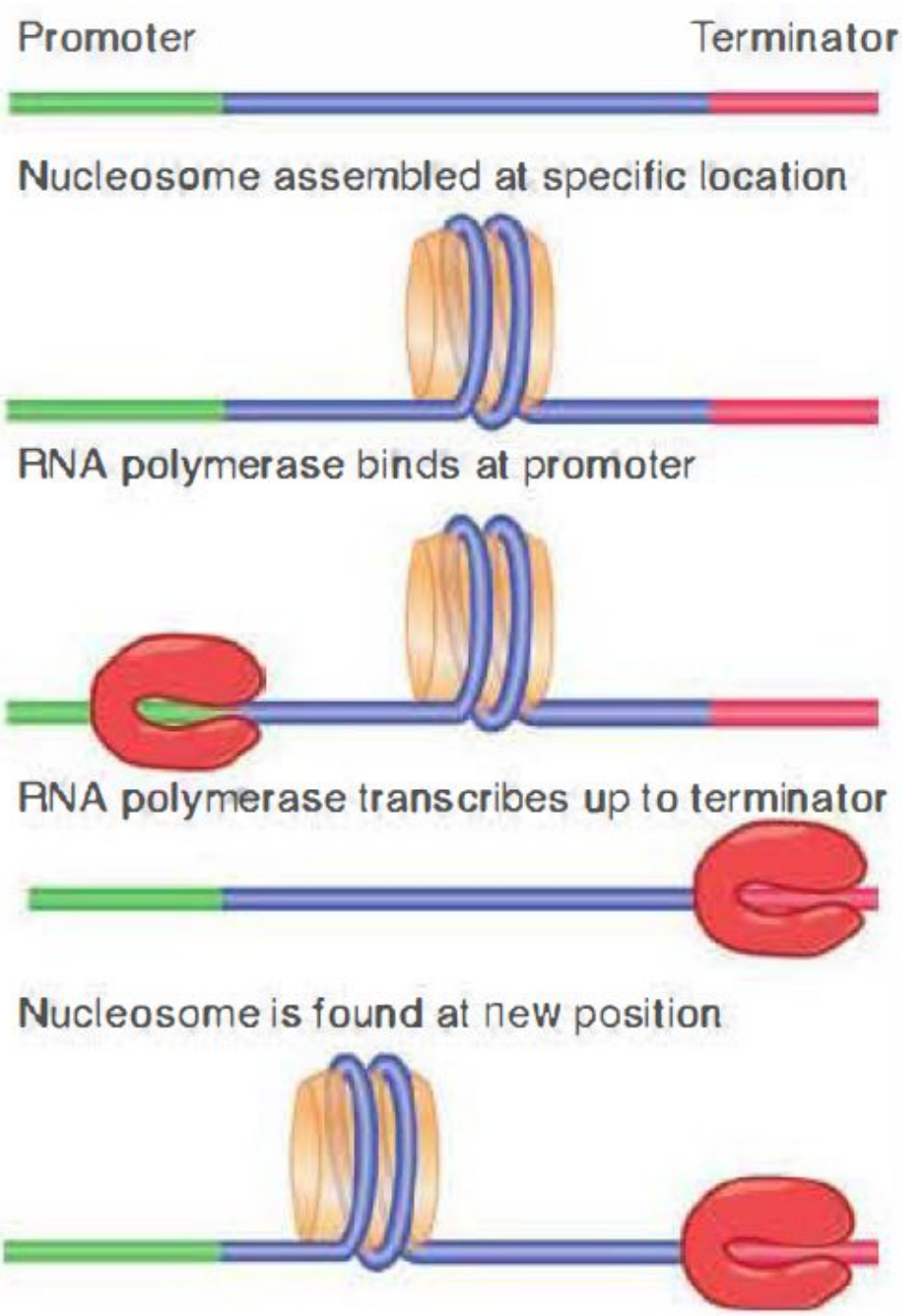
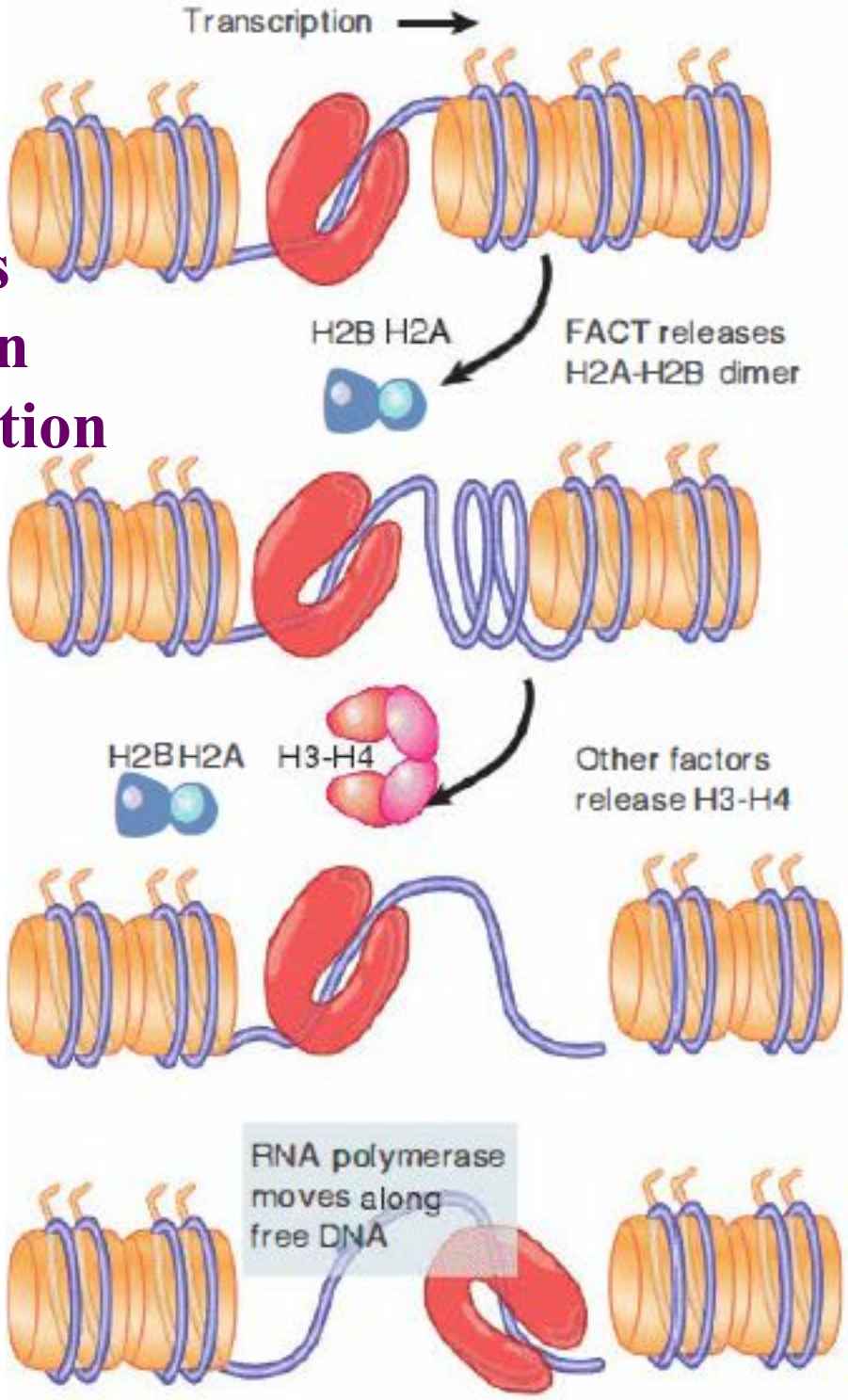


FIGURE 10.46 An experiment to test the effect of transcription on nucleosomes shows that the histone octamer is displaced from DNA and rebinds at a new position.

FACT - Facilitates Chromatin Transcription



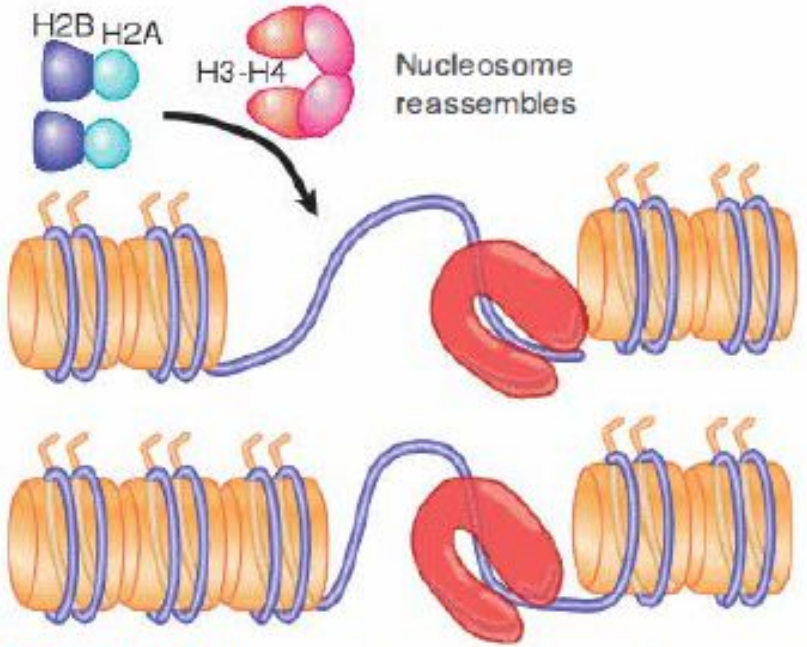


FIGURE 10.47 Histone octamers are disassembled ahead of transcription to remove nucleosomes. They reform following transcription. Release of H2A-H28 dimers probably initiates the disassembly process.

Hypersensitive sites

DNA sequences without nucleosome structures hypersensitive to digestion with nucleases and chemical agents.

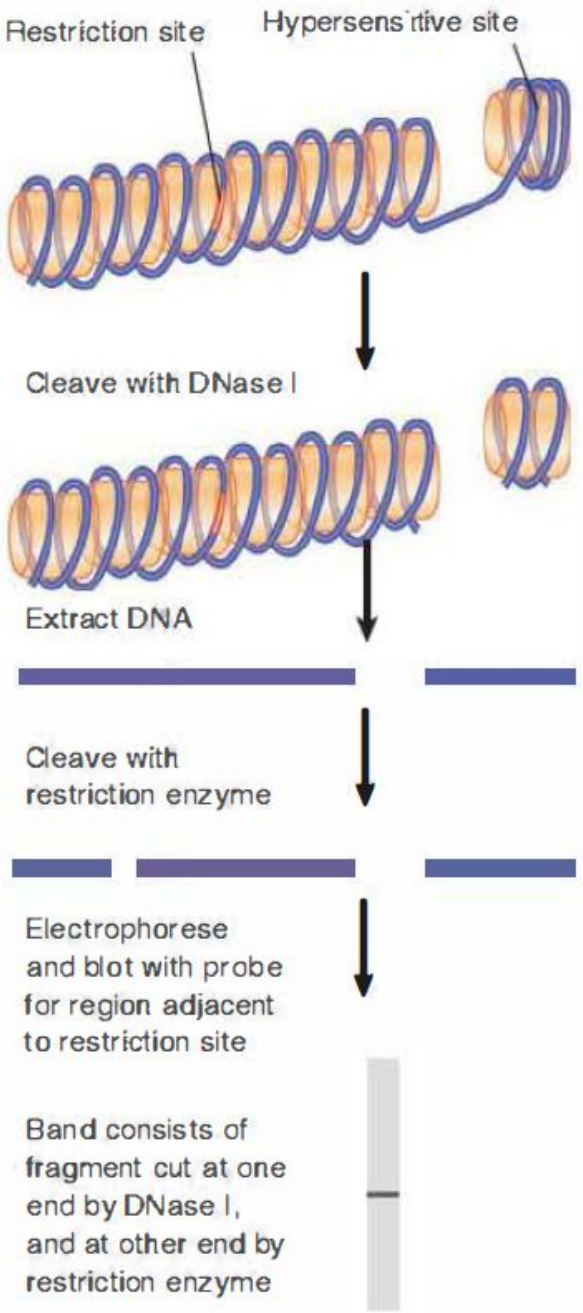


FIGURE 10.48 Indirect end labeling identifies the distance of a DNase hypersensitive site from a restriction cleavage site. The existence of a particular cutting site for DNase I generates a discrete fragment, whose size indicates the distance of the DNase I hypersensitive site from the restriction site.

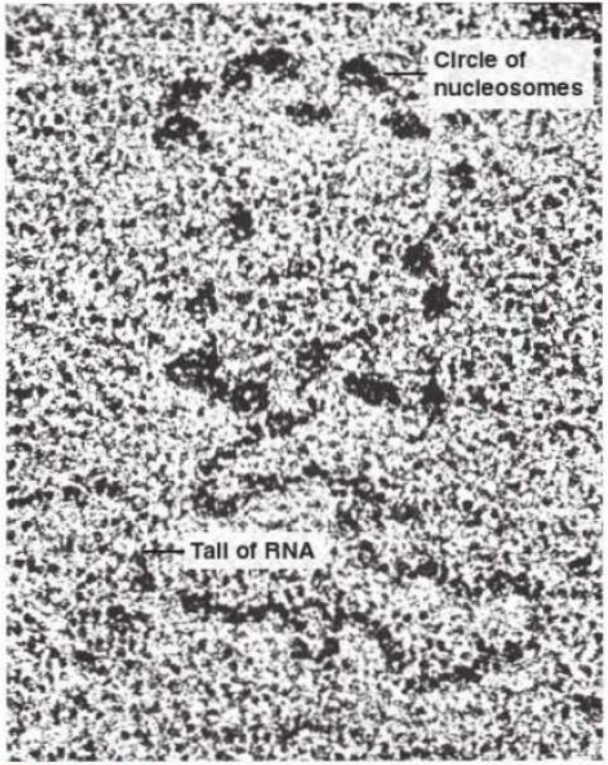
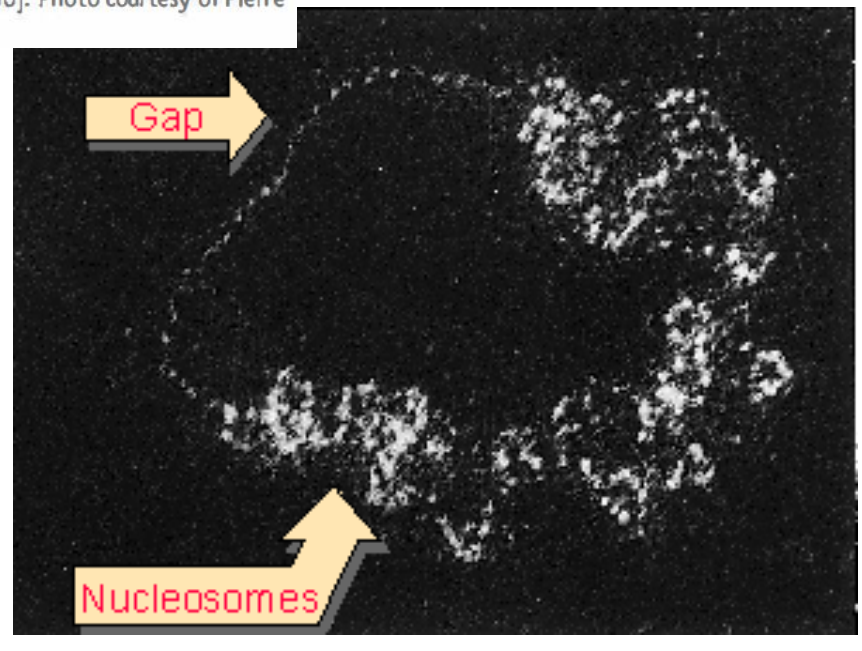


FIGURE 10.44 An SV40 minichromosome is transcribed while maintaining a nucleosomal structure. Reprinted from *J. Mol. Bio.*, vol. 131, P. Gariglio, et al., The template of the isolated native ..., pp. 75–105. Copyright 1979, with permission from Elsevier [http://www.sciencedirect.com/science/journal/00222836]. Photo courtesy of Pierre Chambon, College of France.



LCR-Locus Control Region

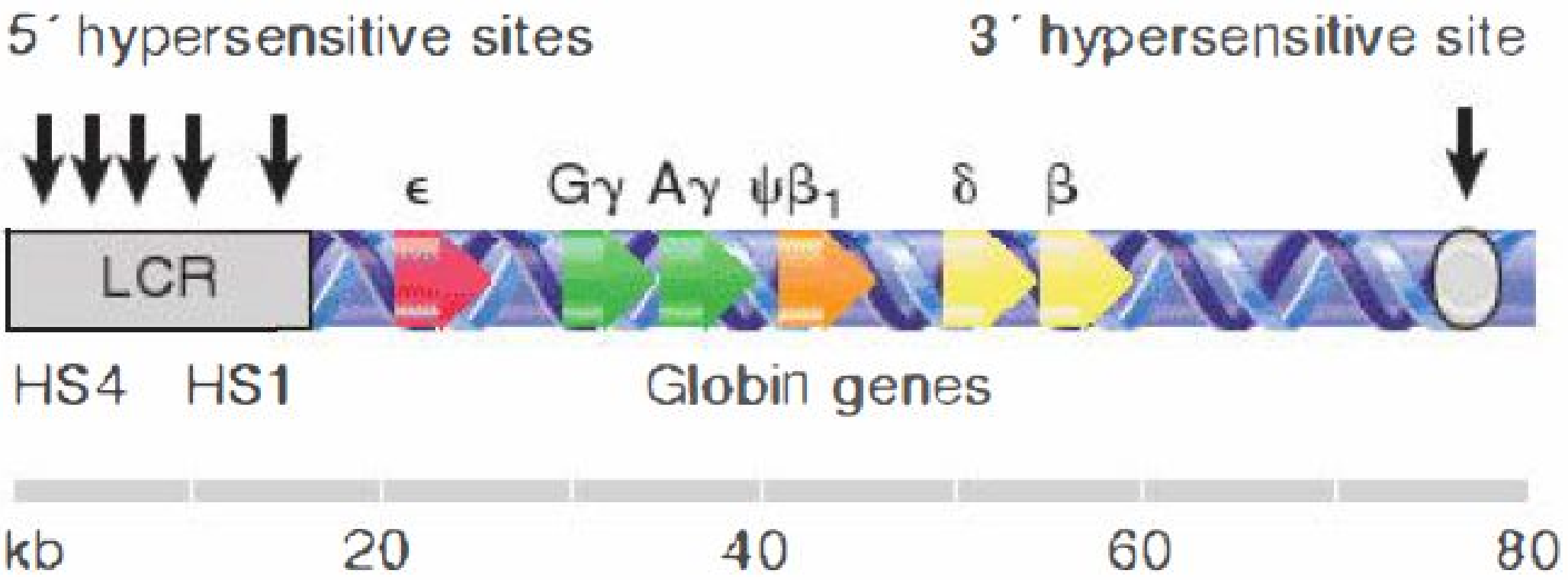


FIGURE 10.57 The β-globin locus is marked by hypersensitive sites at either end. The group of sites at the 5′ side constitutes the LCR and is essential for the function of all genes in the cluster.

Hypersensitive sites are generated by the binding of factors (regulatory proteins) that exclude histone octamers.

Chromosomal domain

- Independent structural entity, where the supercoiling in each domain is not affected by events in the other domains

- A region of altered structure including at least one active transcription unit, and sometimes extending farther with enhanced sensibility to DNAse I digestion. (Note that use of the term domain does not imply any necessary connection with the structural domains identified by the loops of chromatin or chromosomes.)

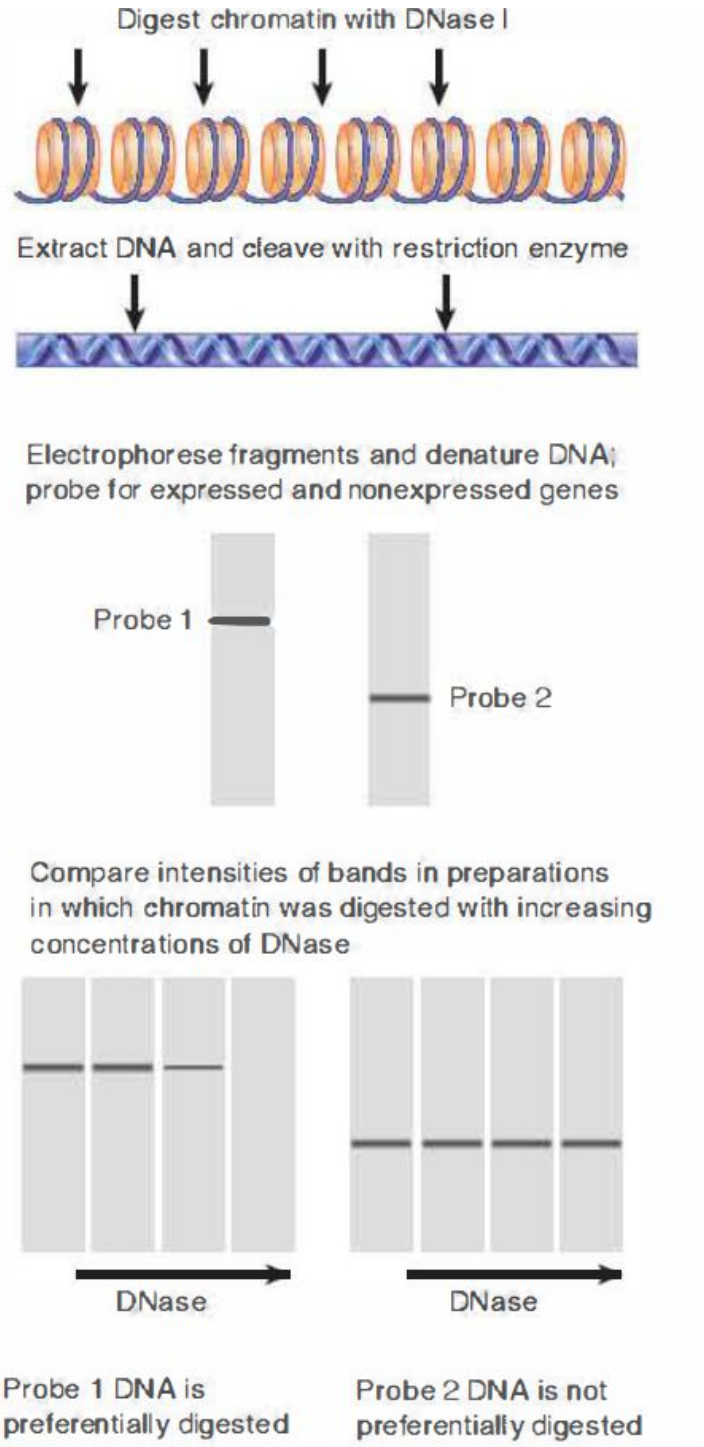


FIGURE 10.49 Sensitivity to DNase I can be measured by determining the rate of disappearance of the material hybridizing with a particular probe.

Chromatin capable of being transcribed has a generally increased sensitivity to DNase I, reflecting a change in structure over an extensive region that can be defined as a domain containing active or potentially active genes.

Hypersensitive sites in DNA occur at discrete locations, and are identified by greatly increased sensitivity to DNase I.

Nucleosome positioning may be important in controlling access of regulatory proteins to DNA.