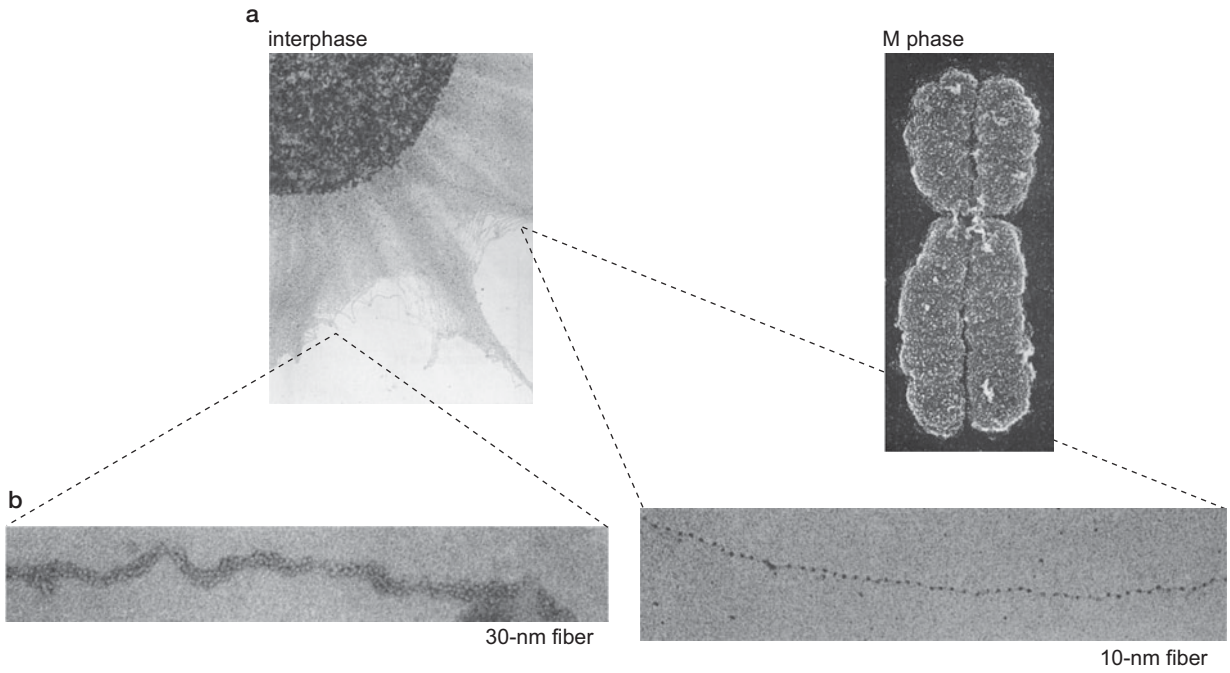


FIGURE 8-17 Forms of chromatin structure seen in the electron microscope. (a) Electron micrographs of interphase and condensed M-phase DNA show the changes in the structure of chromatin. (b) Electron micrographs of different forms of chromatin in interphase cells show the 30-nm and 10-nm chromatin fibers (beads on a string). (a, Reprinted, with permission, from Alberts B. et al. 2002. *Molecular biology of the cell*, 4th ed., Figs. 4-21 and 4-23. Garland Science/Taylor & Francis LLC. © V. Foe.)

how nucleosome-dependent structures are regulated and how they control the accessibility of nuclear DNA.

THE NUCLEOSOME

Nucleosomes Are the Building Blocks of Chromosomes

The majority of the DNA in eukaryotic cells is packaged into nucleosomes. Each nucleosome is composed of a core of eight histone proteins and the DNA wrapped around them. The DNA between each nucleosome (the "string" in the "beads on a string" image in Fig. 8.17b) is called **linker DNA**. By assembling into nucleosomes, the DNA is compacted approximately sixfold. This is far short of the 1000–10,000-fold DNA compaction observed in eukaryotic cells. Nevertheless, this first stage of DNA packaging is essential for all of the remaining levels of DNA compaction.

The DNA most tightly associated with the nucleosome, called the **core DNA**, is wound about 1.65 times around the outside of the histone octamer like thread around a spool (Fig. 8-18). The length of DNA associated with each nucleosome can be determined using nuclease treatment (Box 8-1, Micrococcal Nuclease and the DNA Associated with the Nucleosome). The ~147-bp length of this DNA is an invariant feature of nucleosomes in all eukaryotic cells. In contrast, the length of the linker DNA between nucleosomes is variable. Typically, this distance is 20–60 bp, and each eukaryote has a characteristic average linker DNA length (Table 8-4). The difference

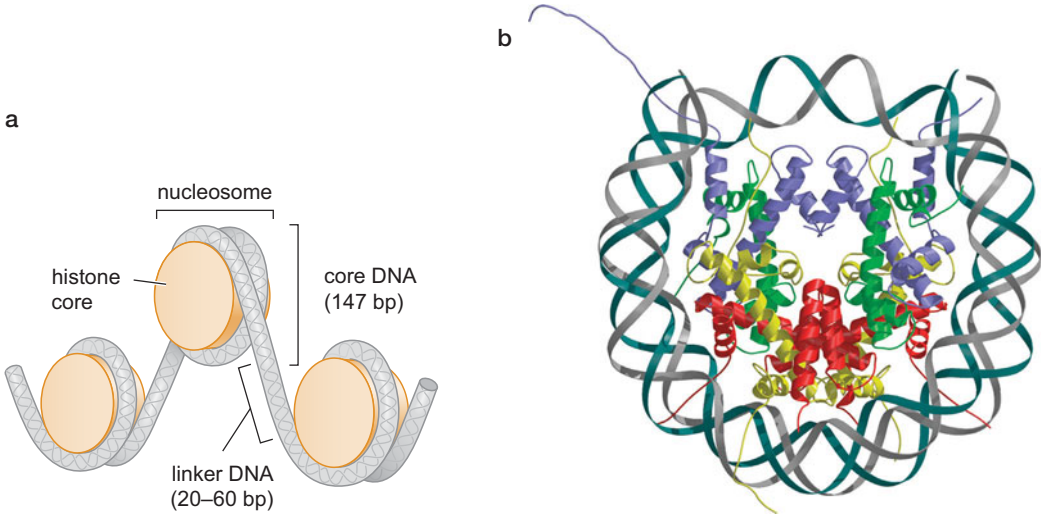


FIGURE 8-18 DNA packaged into nucleosomes. (a) Schematic of the packaging and organization of nucleosomes. (b) Crystal structure of a nucleosome showing DNA wrapped around the histone protein core. (Red) H2A; (yellow) H2B; (purple) H3; (green) H4. Note that the colors of the different histone proteins here and in following structures are the same. (Luger K. et al. 1997. *Nature* **389**: 251–260.) Image prepared with MolScript, BobScript, and Raster3D.

in average linker DNA length is likely to reflect the differences in the larger structures formed by nucleosomal DNA in each organism, rather than differences in the nucleosomes themselves (see the next section on Higher-Order Chromatin Structure).

In any cell, there are stretches of DNA that are not packaged into nucleosomes. Typically, these are regions of DNA engaged in gene expression, replication, or recombination. Although not bound by nucleosomes, these sites are typically associated with nonhistone proteins that are either regulating or participating in these events. We discuss the mechanisms that remove nucleosomes from DNA and maintain such regions of DNA in a nucleosome-free state later and in Chapter 19.

Histones Are Small, Positively Charged Proteins

Histones are by far the most abundant proteins associated with eukaryotic DNA. Eukaryotic cells commonly contain five abundant histones: H1, H2A, H2B, H3, and H4. Histones H2A, H2B, H3, and H4 are the **core histones**, and two copies of each of these histones form the protein core around which nucleosomal DNA is wrapped. Histone H1 is not part of the nucleosome core particle. Instead, it binds to the linker DNA and is referred to as a **linker**

TABLE 8-4 Average Lengths of Linker DNA in Various Organisms

Species	Nucleosome Repeat Length (bp)	Average Linker DNA Length (bp)
Saccharomyces cerevisiae	160–165	13–18
Sea urchin (sperm)	~260	~110
Drosophila melanogaster	~180	~33
Human	185–200	38–53

TABLE 8-5 General Properties of the Histones

Histone Type	Histone	Molecular Weight (M_r)	Lysine and Arginine (%)
Core histones	H2A	14,000	20
	H2B	13,900	22
	H3	15,400	23
	H4	11,400	24
Linker histone	H1	20,800	32

histone. The four core histones are present in equal amounts in the cell. H1 is half as abundant as the other histones, which is consistent with the finding that only one molecule of H1 can associate with a nucleosome.

Consistent with their close association with the negatively charged DNA molecule, the histones have a high content of positively charged amino acids (Table 8-5). At least 20% of the residues in each histone are either lysine or arginine. The core histones are also relatively small proteins ranging in size from 11 to 15 kilodaltons (kDa). Histone H1 is slightly larger at ~21 kDa.

The protein core of the nucleosome is a disc-shaped structure that assembles in an ordered fashion only in the presence of DNA. Without DNA, the core histones form intermediate assemblies in solution. A conserved region found in every core histone, called the **histone-fold domain**, mediates the assembly of these histone-only intermediates (Fig. 8-19). The histone-fold domain is composed of three α-helical regions separated by two short unstructured loops. This domain mediates the formation of head-to-tail heterodimers of specific pairs of histones. H3 and H4 histones first form heterodimers that then come together to form a tetramer with two molecules each of H3 and H4. In contrast, H2A and H2B form heterodimers in solution but not tetramers.

The assembly of a nucleosome involves the ordered association of these building blocks with DNA (Fig. 8-20). First, the H3·H4 tetramer binds to

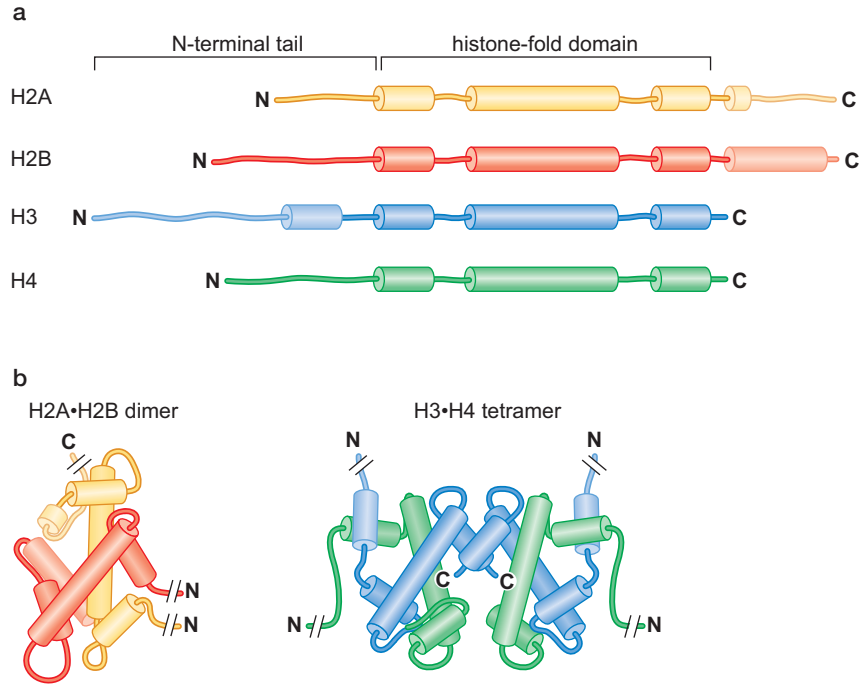


FIGURE 8-19 Core histones share a common structural fold. (a) The four histones are diagrammed as linear molecules. The regions of the histone-fold motif that form α helices are indicated as cylinders. Note that there are adjacent regions of each histone that are structurally distinct including additional α-helical regions. (b) The helical regions of two histones (here H2A and H2B) come together to form a dimer. H3 and H4 also use a similar interaction to form $H3_2{\cdot}H4_2$ tetramers. (Adapted, with permission, from Alberts B. et al. 2002. *Molecular biology of the cell*, 4th ed., p. 209, Fig. 4-26. © Garland Science/ Taylor & Francis LLC.)

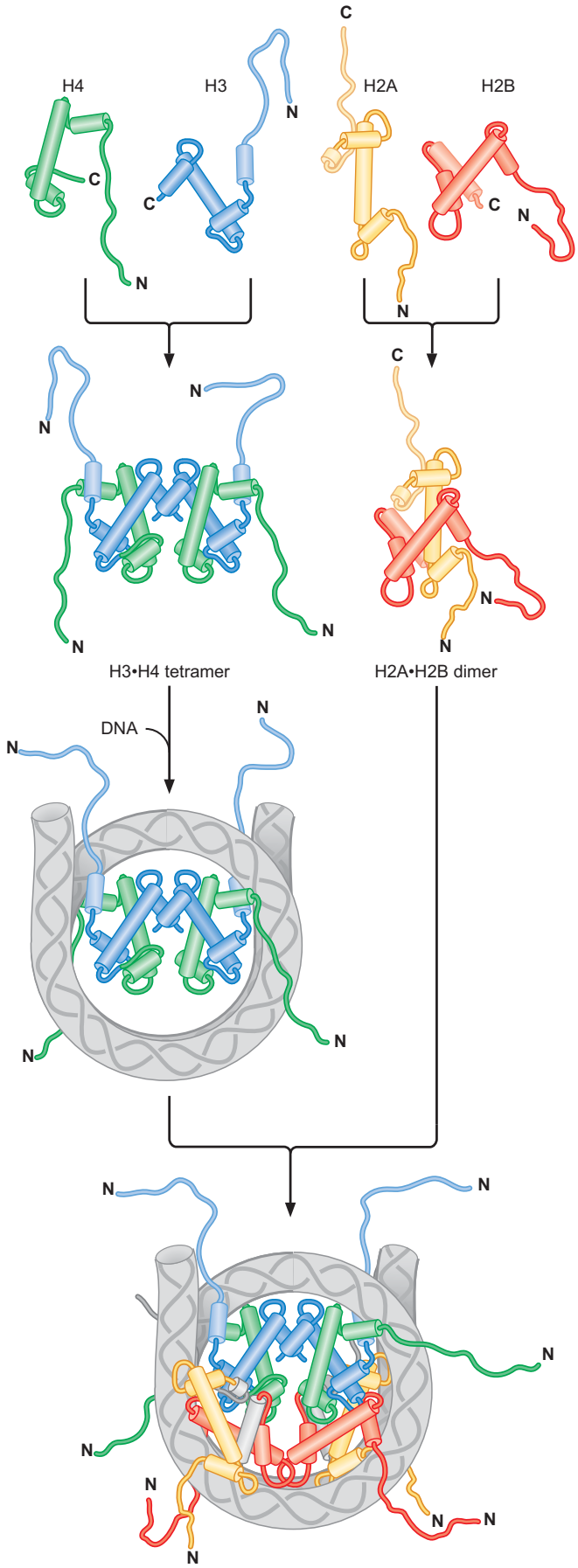


FIGURE 8-20 Assembly of a nucleosome. The assembly of a nucleosome is initiated by the formation of a $H3_2{\cdot}H4_2$ tetramer. The tetramer then binds to double-stranded DNA. The $H3_2{\cdot}H4_2$ tetramer bound to DNA recruits two copies of the H2A·H2B dimer to complete the assembly of the nucleosome. (Adapted, with permission, from Alberts B. et al. 2002. *Molecular biology of the cell*, 4th ed., p. 210, Fig. 4-27. Garland Science/Taylor & Francis LLC, © J. Waterborg.)

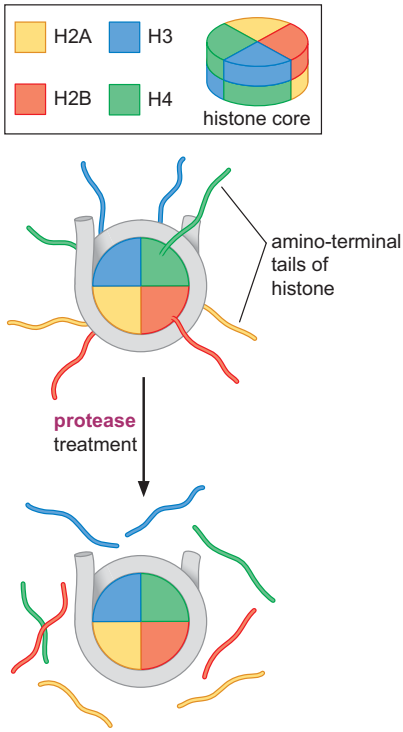


FIGURE 8-21 Amino-terminal tails of the core histones are accessible to proteases. Treatment of nucleosomes with limiting amounts of proteases that cleave after basic amino acids (e.g., trypsin) specifically removes the amino-terminal "tails" leaving the histone core intact.

DNA; then two H2A·H2B dimers join the H3·H4-DNA complex to form the final nucleosome. We discuss how and when this assembly process is accomplished in the cell later in this chapter.

The core histones each have an amino-terminal extension, called a **tail** because it lacks a defined structure and is accessible within the intact nucleosome. This accessibility can be detected by treatment of nucleosomes with the protease trypsin (which specifically cleaves proteins after positively charged amino acids). Treatment of nucleosomes with trypsin rapidly removes the accessible amino-terminal tails of the histones but cannot cleave the tightly packed histone-fold regions (Fig. 8-21). The exposed amino-terminal tails are not required for the association of DNA with the histone octamer, because DNA is still tightly associated with the nucleosome after protease treatment. Instead, the tails are the sites of extensive post-translational modifications that alter the function of individual nucleosomes. These modifications include phosphorylation, acetylation, and methylation on serine, lysine, and arginine residues. We shall return to the role of histone tail modification in nucleosome function later. We now turn to the detailed structure of the nucleosome.

The Atomic Structure of the Nucleosome

The high-resolution three-dimensional (3D) structure of the nucleosome core particle (see Fig. 8-18b) (147 bp of DNA plus an intact histone octamer) has provided many insights into nucleosome function. The high affinity of the nucleosome for DNA, the distortion of the DNA when bound to the nucleosome, and the lack of DNA sequence specificity can each be explained by the nature of the interactions between the histones and the DNA. The structure also sheds light on the function and location of the amino-terminal tails. Finally, the interaction between the DNA and the histone octamer provides insight into the dynamic nature of the nucleosome and the process of nucleosome assembly. We discuss each of these properties of the nucleosome in the following sections.

Histones Bind Characteristic Regions of DNA within the Nucleosome

Although not perfectly symmetrical, the nucleosome has an approximate twofold axis of symmetry, called the **dyad axis**. This can be visualized by thinking of the face of the octamer disc as a clock with the midpoint of the 147 bp of DNA located at the 12 o'clock position (Fig. 8-22). This places the ends of the DNA just short of 11 and 1 o'clock. A line drawn from 12 o'clock to 6 o'clock through the middle of the disc defines the dyad axis. Rotation of the nucleosome around this axis by 180° reveals a view of the nucleosome nearly identical to that observed before rotation (see Structural Tutorial 8-1).

The H3·H4 tetramers and H2A·H2B dimers each interact with a particular region of the DNA within the nucleosome (Fig. 8-23). Of the 147 bp of DNA included in the structure, the histone-fold regions of the H3·H4 tetramer interact with the central 60 bp. The amino-terminal region of H3 most proximal to the histone-fold region forms a fourth α helix that interacts with the final 13 bp at each end of the bound DNA (this α helix is distinct from the unstructured H3 amino-terminal tail described above). If we picture the nucleosome with a clock face as described above, the H3·H4 tetramer forms the top half of the histone octamer. Histone H3·H4 tetramers occupy a key position in the nucleosome by binding the middle *and* both ends of the DNA (turquoise DNA in

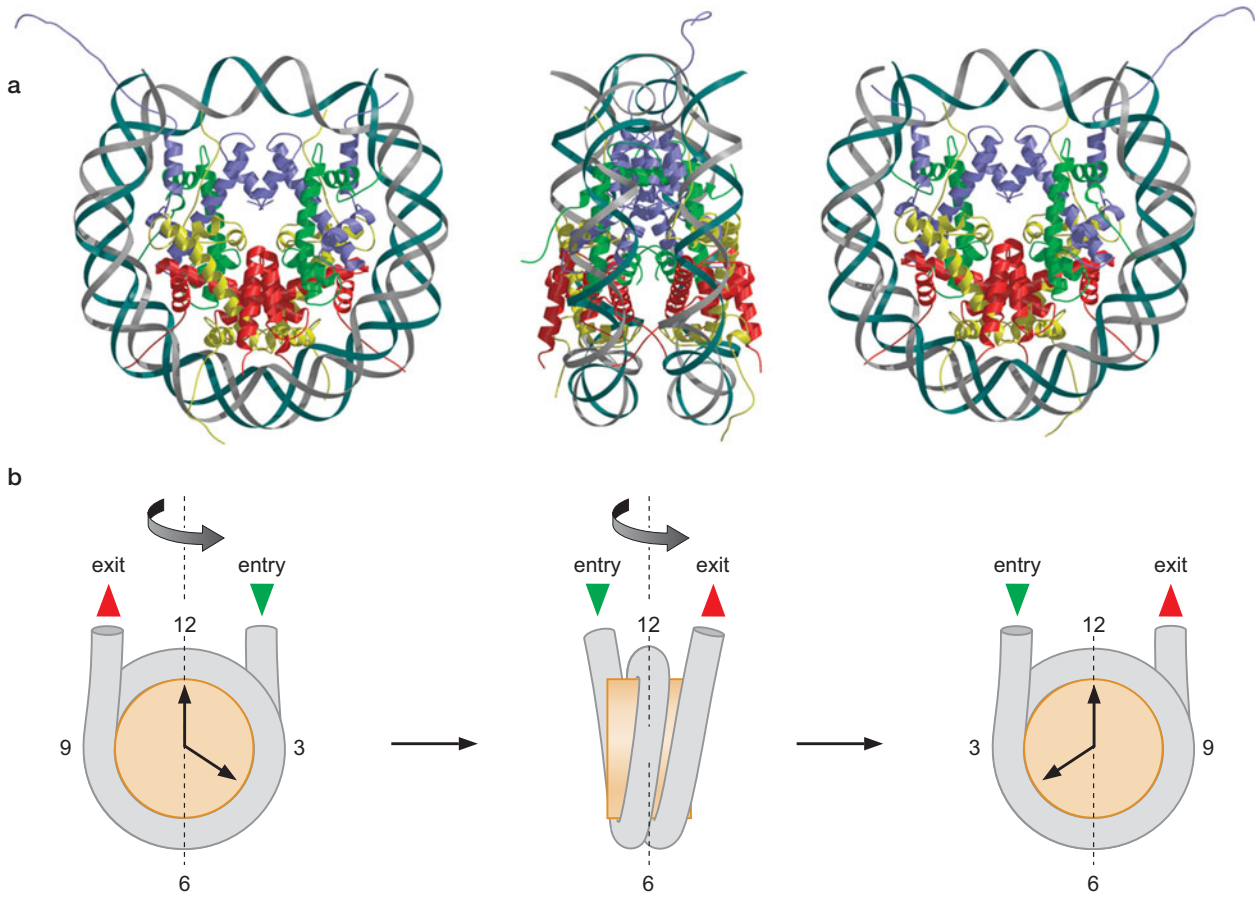


FIGURE 8-22 The nucleosome has an approximate twofold axis of symmetry. (a) Three-dimensional structure. (b) Cartoon illustrating "clock face" analogy to nucleosome. Three views of the nucleosome are shown in each representation. Each view shows a 90° rotation around the axis between 12 and 6 o'clock positions illustrated in the first panel of b. (a, Luger K. et al. 1997. *Nature* **389:** 251–260.) Images prepared with MolScript, BobScript, and Raster3D.

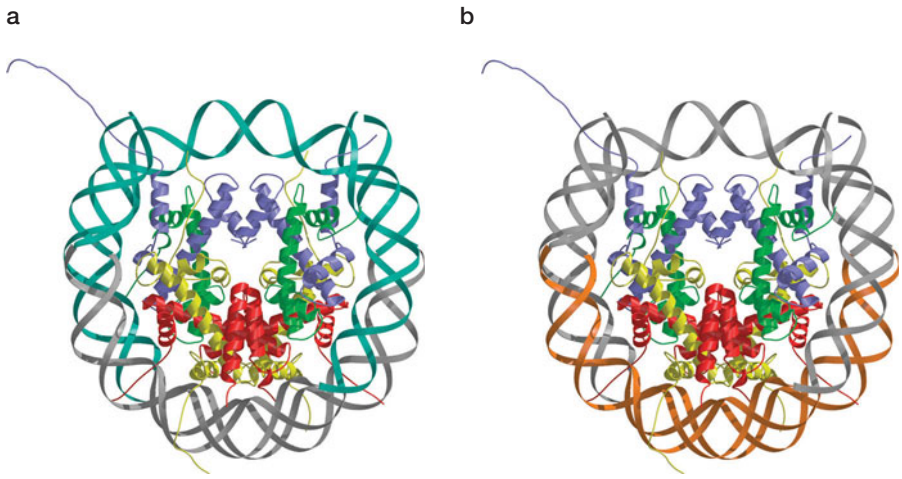


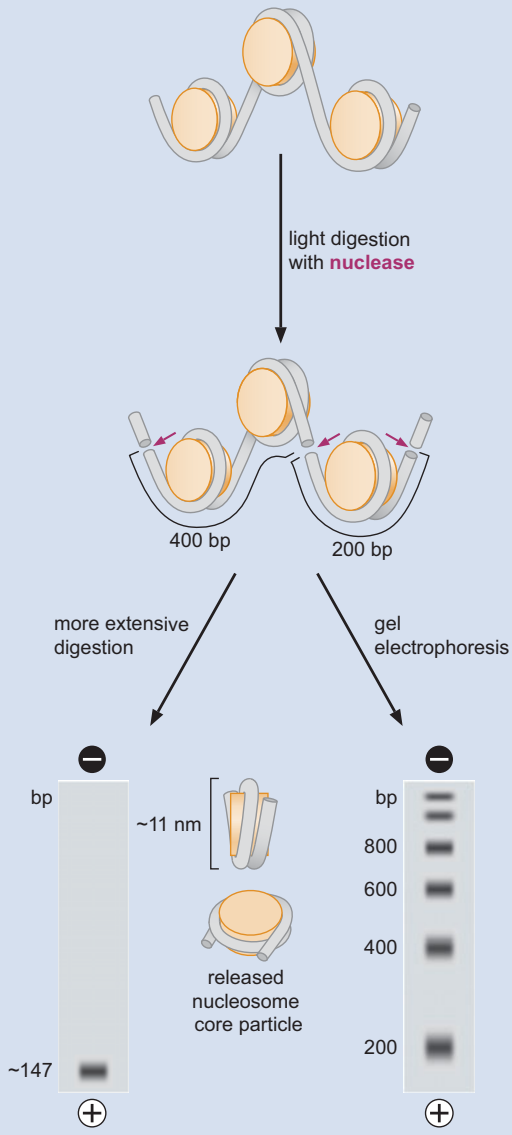
FIGURE 8-23 Interactions of the histones with nucleosomal DNA. (a) H3·H4 binds the middle and the ends of the DNA (turquoise). (b) H2A·H2B binds 30 bp of DNA on one side of the nucleosome (orange). (Luger K. et al. 1997. *Nature* **389:** 251–260.) Images prepared with MolScript, BobScript, and Raster3D.

KEY EXPERIMENTS

BOX 8-1 Micrococcal Nuclease and the DNA Associated with the Nucleosome

Nucleosomes were first purified by treating chromosomes with a sequence-nonspecific nuclease called **micrococcal nuclease** (**MNase**). The ability of this enzyme to cleave DNA is primarily governed by the accessibility of the DNA. Thus, MNase cleaves protein-free DNA sequences rapidly and protein-associated DNA sequences poorly. Limited treatment of chromosomes with this enzyme results in a nuclease-resistant population of DNA molecules that are primarily associated with histones. These DNA molecules are between 160 and 220 bp in length and are associated with two copies each of histones H2A, H2B, H3, and H4. On average, these particles include the DNA tightly associated with the nucleosome as well as one unit of linker DNA. More extensive MNase treatment degrades all of the linker DNA. The remaining minimal nucleosome includes only 147 bp of DNA and is called the **nucleosome core particle**.

The average length of DNA associated with each nucleosome can be measured in a simple experiment (Box 8-1 Fig. 1). Chromatin is treated with the enzyme micrococcal nuclease but this time only gently. This results in single cuts in some but not all linker DNA. After nuclease treatment, the DNA is extracted from all proteins (including the histones) and subjected to gel electrophoresis to separate the DNA by size. Electrophoresis reveals a "ladder" of DNA fragments that are multiples of the average nucleosome-to-nucleosome distance. A ladder of fragments is observed because the MNase-treated chromatin is only partially digested. Thus, sometimes, multiple nucleosomes remain unseparated by digestion, leading to DNA fragments equivalent to all of the DNA bound by these nucleosomes. Further digestion would result in all linker DNA being cleaved and the formation of nucleosome core particles and a single ~147-bp fragment.



BOX 8-1 FIGURE 1 Progressive digestion of nucleosomal DNA with MNase. (Courtesy of R.D. Kornberg.)

Fig. 8-23a). The two H2A·H2B dimers each associate with ~30 bp of DNA on either side of the central 60 bp of DNA bound by H3 and H4. Using the clock analogy again, the DNA associated with H2A·H2B is located from ~5 o'clock to 9 o'clock on either face of the nucleosome disc. Together, the two H2A·H2B dimers form the bottom part of the histone octamer located across the disc from the DNA ends (orange DNA in Fig. 8-23b).

The extensive interactions between the H3·H4 tetramer and the DNA help to explain the ordered assembly of the nucleosome (Fig. 8-24). H3·H4 tetramer association with the middle and ends of the bound DNA would result in the DNA being extensively bent and constrained, making the association of H2A·H2B dimers relatively easy. In contrast, the relatively short length of DNA bound by H2A·H2B dimers is not sufficient to prepare the DNA for H3·H4 tetramer binding.

Many DNA Sequence–Independent Contacts Mediate the Interaction between the Core Histones and DNA

A closer look at the interactions between the histones and the nucleosomal DNA reveals the structural basis for the binding and bending of the DNA within the nucleosome. Fourteen distinct sites of contact are observed, one for each time the minor groove of the DNA faces the histone octamer (Fig. 8-25). The association of DNA with the nucleosome is mediated by a large number (about 40) of hydrogen bonds between the histones and the DNA. The majority of these hydrogen bonds are between the proteins and the oxygen atoms in the phosphodiester backbone near the minor groove of the DNA. Only seven hydrogen bonds are made between the protein side chains and the bases, and all of these are made in the minor groove of the DNA.

The large number of these hydrogen bonds (a typical sequence-specific DNA-binding protein only has about 20 hydrogen bonds with DNA) provides the driving force to bend the DNA. The highly basic nature of the histones further facilitates DNA bending by masking the negative charge of the phosphates that ordinarily resists DNA bending. This is because when DNA is bent, the phosphates on the inside of the bend are brought into unfavorably close proximity. The positively charged nature of the histones also facilitates the close juxtaposition of the two adjacent DNA helices necessary to wrap the DNA more than once around the histone octamer.

The finding that all of the sites of contact between the histones and the DNA involve either the minor groove or the phosphate backbone is consistent with the non-sequence-specific nature of the association of the histone octamer with DNA. Neither the phosphate backbone nor the minor groove is rich in base-specific information. Moreover, of the seven hydrogen bonds formed with the bases in the minor groove, *none* is with parts of the bases that distinguish between G:C and A:T base pairs (see Chapter 4, Fig. 4-10).

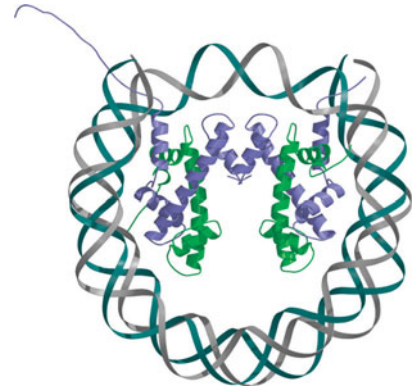


FIGURE 8-24 Nucleosome lacking H2A and H2B. The H2A and H2B histones have been artificially removed from this view of the nucleosome. This structure is likely to resemble the DNA·$H3_2$·$H4_2$ tetramer intermediate in the assembly of a nucleosome (see Fig. 8-20). (Luger K. et al. 1997. *Nature* **389:** 251–260.) Image prepared with MolScript, BobScript, and Raster3D.

The Histone Amino-Terminal Tails Stabilize DNA Wrapping around the Octamer

The structure of the nucleosome also tells us something regarding the histone amino-terminal tails. The four H2B and H3 tails emerge from between

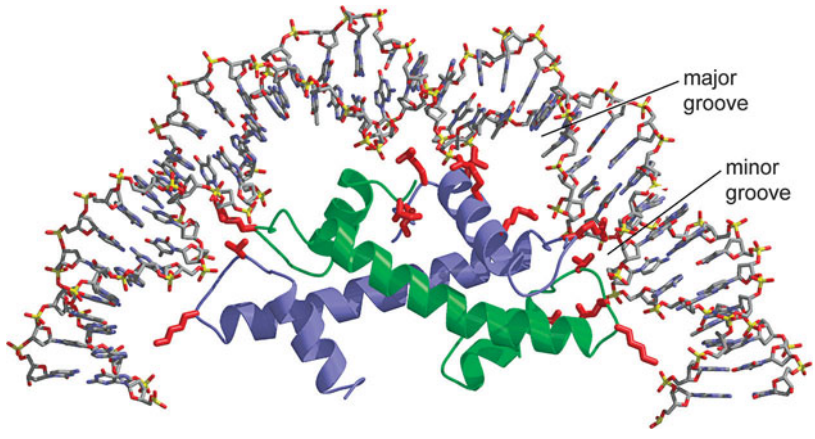


FIGURE 8-25 Sites of contact between the histones and the DNA. For clarity, only the interactions between a single H3·H4 dimer are shown. A subset of the parts of the histones that interact with the DNA is highlighted in red. Note that these regions cluster around the minor groove of the DNA. (Luger K. et al. 1997. *Nature* **389:** 251–260.) Image prepared with MolScript, BobScript, and Raster3D.

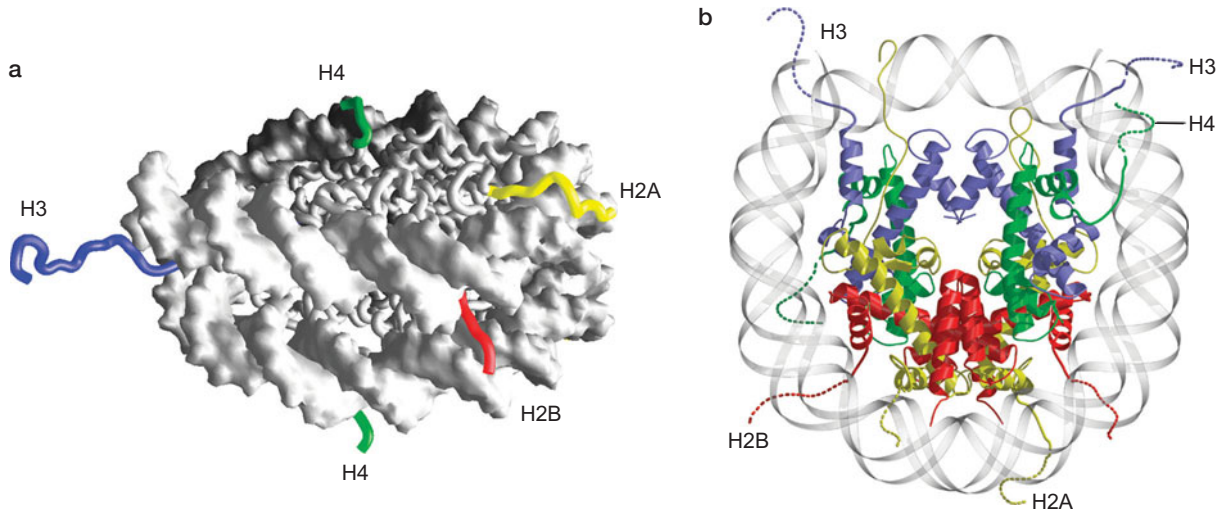


FIGURE 8-26 Histone tails emerge from the core of the nucleosome at specific positions. (a) The side view illustrates that the H3 and H2B tails emerge from between the two DNA helices. In contrast, the H4 and H2A tails emerge either above or below both DNA helices. (Luger K. et al. 1997. *Nature* **389:** 251–260.) Image prepared with GRASP. (b) The position of the tails relative to the entry and exit of the DNA. This view reveals that the histone tails emerge at numerous positions relative to the DNA. (Davey C.A. et al. 2002. *J. Mol. Biol.* **319:** 1097–1113.) Image prepared with MolScript, BobScript, and Raster3D.

the two DNA helices. In each case, their path of exit is formed by two adjacent minor grooves, making a "gap" between the two DNA helices just big enough for a polypeptide chain (Fig. 8-26a). Strikingly, the H2B and H3 tails emerge at approximately equal distances from each other around the octamer disc (at ~1 and 11 o'clock for the H3 tails and 4 and 8 o'clock for H2B). Instead of emerging between the two DNA helices, the H2A and H4 amino-terminal tails emerge from either "above" or "below" both DNA helices (Fig. 8-26a). These tails are also distributed around the face of the nucleosome with the H2A tails emerging at 5 and 7 o'clock and the H4 tails at 3 and 9 o'clock (Fig. 8-26b). By emerging both between and on either side of the DNA helices, the histone tails can be thought of as the grooves of a screw, directing the DNA to wrap around the histone octamer disc in a left-handed manner. As we discussed in Chapter 4, the left-handed nature of the DNA wrapping introduces negative supercoils in the DNA. The parts of the tails most proximal to the histone disc (and therefore not subject to the protease cleavage discussed above) also make some of the many hydrogen bonds between the histones and the DNA as they pass by the DNA.

Wrapping of the DNA around the Histone Protein Core Stores Negative Superhelicity

Each nucleosome added to a covalently closed circular template changes the linking number of the associated DNA by approximately −1.2. Because the remainder of the DNA is kept relaxed by topoisomerases, the DNA that is packaged into nucleosomes would become negatively supercoiled if nucleosomes were removed from the DNA. Thus, nucleosomes can be viewed as storing or stabilizing negative superhelicity. Why would the cell want to maintain a stockpile of negative superhelicity? There are many instances when it is useful to drive unwinding of DNA in the cell, including initiation of DNA replication, transcription, and recombination. Importantly,

negatively supercoiled DNA favors DNA unwinding (see Chapter 4, Fig. 4-17). Thus, removal of a nucleosome not only allows increased access to the DNA, but also facilitates DNA unwinding of nearby DNA sequences (Box 8-2, Nucleosomes and Superhelical Density).

If nucleosomes store negative superhelicity in eukaryotic cells, what serves the equivalent function in prokaryotic cells? The answer for many prokaryotic organisms is that the entire genome is maintained in a negatively supercoiled state. This is accomplished by a specialized topoisomerase called **gyrase** that has the ability to introduce negative superhelicity into relaxed DNA by reducing the linking number. For example, in *E. coli* cells, gyrase action results in the genome having an average superhelical density of approximately −0.07. The addition of negative supercoils into otherwise relaxed DNA is an energy-requiring reaction. Consistent with this, gyrase requires ATP to introduce negative supercoils. In the absence of ATP, gyrase can only relax DNA (e.g., reduce the linking number of positively supercoiled DNA).

Not all bacteria need to maintain their DNA in a negatively supercoiled state. Bacteria that prefer to grow at very high temperatures (>80°C) must expend energy to *prevent* their DNA from unwinding due to thermal denaturation. These organisms have a different topoisomerase called **reverse gyrase**. Consistent with its name, reverse gyrase increases the linking number of relaxed DNA in the presence of ATP. By keeping the genome positively supercoiled, reverse gyrase counteracts the effect of thermal denaturation that would ordinarily result in many regions of the genome being unwound.

HIGHER-ORDER CHROMATIN STRUCTURE

Heterochromatin and Euchromatin

From the earliest observations of chromosomes in the light microscope, it was clear that they were not uniform structures. Early studies of chromosomes divided chromosomal regions into two categories: **euchromatin** and **heterochromatin**. Heterochromatin was characterized by dense staining with a variety of dyes and a more condensed appearance, whereas euchromatin had the opposite characteristics, staining poorly with dyes and having a relatively open structure. As our molecular understanding of genes and their expression advanced, it became clear that heterochromatic regions of chromosomes had very limited gene expression. In contrast, euchromatic regions showed higher levels of gene expression, suggesting that these different structures were connected to global levels of gene expression.

Heterochromatic regions show little gene expression, but this does not mean that these regions are not important. As we shall learn when gene expression is discussed, keeping a gene turned off can be just as important as turning a gene on. In addition, heterochromatin is associated with particular chromosomal regions, including the telomere and the centromere, and is important for the function of both of these key chromosomal elements.

Over the years, researchers have gained a more complete molecular understanding of heterochromatin and euchromatin structure. It is clear that DNA in both types of chromatin is packaged into nucleosomes. The difference between heterochromatin structure and euchromatin structure is how the nucleosomes in these different chromosomal regions are (or are not) assembled into larger assemblies. It has become clear that heterochromatic regions are composed of nucleosomal DNA assembled into

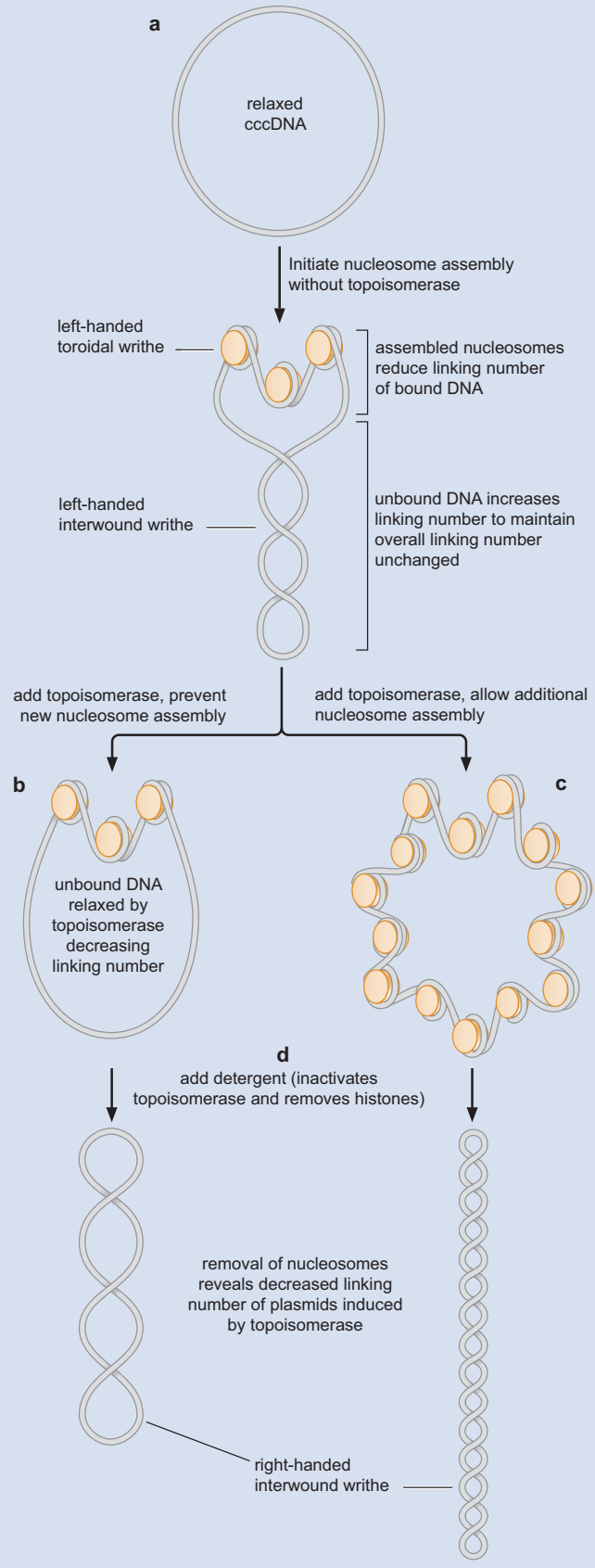
BOX 8-2 Nucleosomes and Superhelical Density

Why do nucleosomes alter the topological state of the DNA they include? As described in Chapter 4, there are two forms of writhe that can contribute to the formation of supercoiled DNA: toroidal and interwound (also referred to as *plectonemic*). The wrapping of DNA around the histone octamer is a form of toroidal writhe. The handedness of the writhe controls whether it introduces positive or negative supercoils (i.e., increases or decreases the linking number of the associated DNA). For toroidal writhe, left-handed wrapping induces negative superhelicity (for interwound writhe, the opposite is true; right-handed pitch is associated with negative superhelicity). Thus, the left-handed toroidal wrapping of DNA around the nucleosome reduces the linking number of the associated DNA. For this reason, nucleosomes preferentially form with DNA that has negative superhelical density. In contrast, assembling nucleosomes on DNA that has positive superhelical density is very difficult.

The assembly of many nucleosomes on covalently closed, circular DNA (cccDNA) requires the presence of a topoisomerase to accommodate changes in the linking number of the DNA bound to histones (see Box 8-2 Fig. 1). Without a topoisomerase present, for every nucleosome formed with the cccDNA, the unbound DNA (not associated with nucleosomes) would have to accommodate an equivalent *increase* in the linking number (remember that the overall linking number of a cccDNA is fixed in the absence of a topoisomerase). Thus, the unbound DNA would accumulate increased linking number and positive superhelical density. The more positively supercoiled the unbound DNA, the more difficult it is for additional nucleosomes to assemble on this DNA.

Addition of a topoisomerase greatly facilitates nucleosome association with cccDNA. When a topoisomerase is present during nucleosome assembly, it cannot act on the DNA bound to the nucleosome. Instead, the topoisomerase relaxes the DNA not included in nucleosomes, reducing the positive superhelical density in these regions by decreasing the linking number. By maintaining the unbound DNA in a relaxed state, topoisomerases facilitate the binding of histones to the DNA and the formation of additional nucleosomes. Importantly, the overall effect on the plasmid is that the linking number is decreased as more nucleosomes are assembled.

The decrease in the linking number caused by topoisomerase during nucleosome assembly can be used as an assay for this



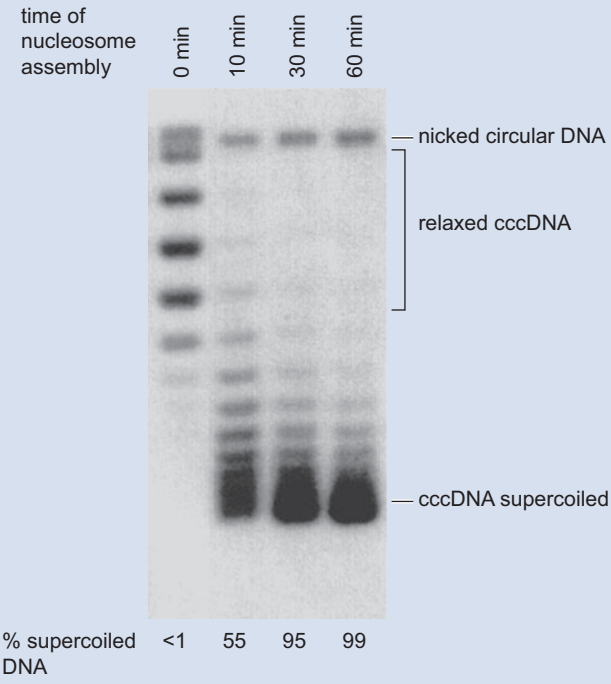
BOX 8-2 FIGURE 1 Topoisomerase is required for nucleosome assembly using covalently closed, circular DNA (cccDNA). (a) Assembly of nucleosomes using cccDNA in the absence of topoisomerase is limited by the accumulation of positive superhelicity in the DNA not associated with nucleosomes. (b) Addition of topoisomerase without additional nucleosome assembly illustrates how topoisomerase reduces the linking number to relax the DNA not incorporated into nucleosomes. (c) Additional nucleosome assembly in the presence of topoisomerase. (d) Simultaneous removal of histones and inactivation of topoisomerase (e.g., by addition of a strong detergent) reveals the reduced linking number associated with nucleosomal DNA.

BOX 8-2 *(Continued)*

event. The assay takes advantage of the ability to distinguish between relaxed and supercoiled cccDNA by gel electrophoresis (see Chapter 4, Fig. 4-27). The first step is to assemble nucleosomes onto a cccDNA in the presence of a topoisomerase. At appropriate times, a strong detergent (e.g., SDS [sodium dodecyl sulfate]) is added to the assembly reaction, rapidly inactivating the topoisomerase and removing histones from the DNA. The resulting DNA is then separated by gel electrophoresis to determine the supercoiled nature of the DNA. Because the detergent inactivates the topoisomerase at the same time as removing the histones from the DNA, the linking number of the DNA assembled into nucleosomes is preserved. On average, the topoisomerase will have decreased the linking number by −1.2 for each nucleosome assembled on the cccDNA. Thus, the more nucleosomes assembled on the cccDNA, the more negatively supercoiled is the cccDNA (Box 8-2 Fig. 1c,d). This can easily be observed by the faster migration of supercoiled DNA during gel electrophoresis (Box 8-2 Fig. 2).

Because nucleosomal DNA wraps around the histone protein 1.65 times, the formation of a single nucleosome using covalently closed, circular plasmid would create a writhe of −1.65 and thus change the linking number by an equivalent amount. As described above, when the change in linking number associated with each nucleosome was measured, the number was lower than this, approximately −1.2 for each nucleosome added. This discrepancy is referred to as the "nucleosome linking number paradox," and the solution to this conundrum was revealed when the high-resolution crystal structure of the nucleosome was solved. Careful analysis of the DNA associated with the histone protein core showed that the number of bases per turn was reduced relative to naked DNA (from 10.5 to 10.2 bp/turn). A reduction in the number of base pairs per turn results in an increase in the linking number for that DNA. Consider the example of a 10,500-bp cccDNA described in Chapter 4. Normal B-form DNA will have 10.5 bp/turn, resulting in a linking number of +1000 for the plasmid (10,500/10.5). In contrast, the same DNA with a pitch of 10.2 bp/turn will have a linking number of approximately +1029 (10,500/10.2). Thus, by decreasing the number of base pairs per turn of the helix, binding to the histone octamer causes a slight increase in the linking number over the length of the nucleosome-bound DNA. This change reduces the change in linking number per nucleosome assembled from −1.65 to −1.2. The difference of approximately +0.4 per nucleosome can be calculated using the difference in the number of base pairs per turn and the length of DNA associated with a nucleosome.

Are these issues relevant to the linear eukaryotic chromosomes? For short linear fragments, superhelicity is not relevant because the ends of the DNA can rotate to accommodate changes in the linking number. But this is not true for the very large linear chromosomes of eukaryotic cells. First, the large size of these chromosomes would not allow rapid enough rotation to dissipate changes in DNA superhelicity easily. More importantly, as we discuss later, the chromosome is not a simple linear strand of DNA. Each chromosomal DNA is folded into a more compact structure composed of large loops that are tethered to a protein structure called the **nuclear scaffold**. These attachments serve to topologically isolate one loop from the next and prevent free rotation of chromosomal DNA.



BOX 8-2 FIGURE 2 Example of a nucleosome assembly assay that measures the associated decrease in linking number. Nucleosome assembly was performed on a relaxed cccDNA in the presence of a topoisomerase. Before the initiation of assembly (0 min) or at various times during the nucleosome assembly reaction, detergent was added and the DNA was separated on a nondenaturing agarose gel and visualized by staining with ethidium bromide. Although an agarose gel does not distinguish between positively and negatively supercoiled cccDNA, the ability of DNA intercalators that increase the linking number to shift the DNA toward the top of the gel (and a more relaxed state) can be used to show that these are negatively supercoiled cccDNAs (not shown). (Reprinted, with permission, from Ito T. et al. 1997. *Cell* **90:** 145–155, Fig. 2c. © Elsevier.)

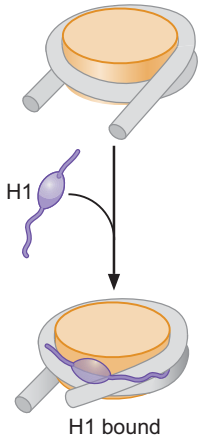


FIGURE 8-27 Histone H1 binds two DNA helices. Upon interacting with a nucleosome, histone H1 binds to the linker DNA at one end of the nucleosome and the central DNA helix of the nucleosome bound DNA (the middle of the 147 bp bound by the core histone octamer).

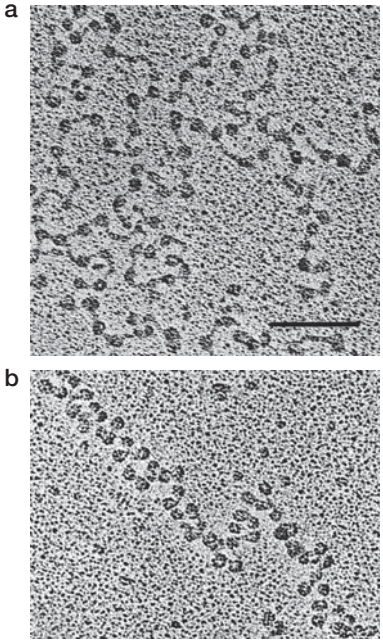


FIGURE 8-28 Addition of H1 leads to more compact nucleosomal DNA. The two images show an electron micrograph of nucleosomal DNA in the absence (a) and presence (b) of histone H1. Note the more compact and defined structure of the DNA in the presence of histone H1. (Reprinted, with permission, from Thoma F. et al. 1979. *J. Cell Biol.* **83:** 403–427, Figs. 4 and 6. © Rockefeller University Press.)

higher-order structures that result in a barrier to gene expression. In contrast, euchromatic nucleosomes are found in much less organized assemblies. In the following sections, we discuss what is known regarding how nucleosomes are assembled into higher-order structures.

Histone H1 Binds to the Linker DNA between Nucleosomes

Once nucleosomes are formed, the next step in the packaging of DNA is the binding of histone H1. Like the core histones, H1 is a small, positively charged protein (see Table 8-5). H1 interacts with the linker DNA between nucleosomes, further tightening the association of the DNA with the nucleosome. This can be detected by the increased protection of nucleosomal DNA from micrococcal nuclease digestion. Thus, beyond the 147 bp protected by the core histones, addition of histone H1 to a nucleosome protects an additional 20 bp of DNA from micrococcal nuclease digestion.

Histone H1 has the unusual property of binding two distinct regions of the DNA duplex. Typically, these two regions are part of a single DNA molecule associated with a nucleosome (Fig. 8-27). The sites of H1 binding are located asymmetrically relative to the nucleosome. One of the two regions bound by H1 is the linker DNA at *one* end of the nucleosome. The second site of DNA binding is in the middle of the associated 147 bp (the only DNA duplex present at the dyad axis). Thus, the additional DNA, protected from the nuclease digestion described above, is restricted to linker DNA on only *one* side of the nucleosome. By bringing these two regions of DNA into close proximity, H1 binding increases the length of the DNA wrapped tightly around the histone octamer.

H1 binding produces a more defined angle of DNA entry and exit from the nucleosome. This effect, which can be visualized in the electron microscope (Fig. 8-28), results in the nucleosomal DNA taking on a distinctly zigzag appearance. The angles of entry and exit observed vary substantially depending on conditions (including salt concentration, pH, and the presence of other proteins). If we assume that these angles are $\sim 20^{\circ}$ relative to the dyad axis, this would result in a pattern in which nucleosomes would alternate on either side of a central region of linker DNA bound by histone H1 (Fig. 8-29).

Nucleosome Arrays Can Form More Complex Structures: The 30-nm Fiber

Binding of H1 stabilizes higher-order chromatin structures. In the test tube, as salt concentrations are increased, the addition of histone H1 results in the nucleosomal DNA forming a **30-nm fiber**. This structure, which can also be observed in vivo, represents the next level of DNA compaction. Importantly, the incorporation of DNA into this fiber makes the DNA less accessible to many DNA-dependent enzymes (such as RNA polymerases).

There are two models for the structure of the 30-nm fiber. In the **solenoid model**, the nucleosomal DNA forms a superhelix containing approximately six nucleosomes per turn (Fig. 8-30a). This structure is supported by both electron microscopy and X-ray diffraction studies, which indicate that the 30-nm fiber has a helical pitch of ~11 nm. This distance is also the approximate diameter of the nucleosome disc, suggesting that the 30-nm fiber is composed of nucleosome discs stacked on edge in the form of a helix (see Fig. 8-30a). In this model, the flat surfaces on either face of the histone octamer disc are adjacent to each other, and the DNA surface of the nucleosomes forms the accessible surface of the superhelix. The linker DNA is buried in the center of the superhelix, but it never passes through the axis

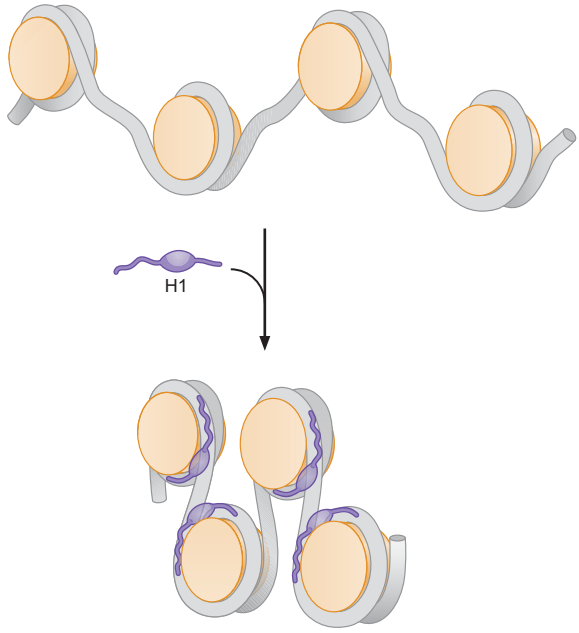


FIGURE 8-29 Histone H1 induces tighter DNA wrapping around the nucleosome. The two illustrations show a comparison of the wrapping of DNA around the nucleosome in the presence and absence of histone H1. One histone H1 can associate with each nucleosome.

of the fiber. Rather, the linker DNA circles around the central axis as the DNA moves from one nucleosome to the next.

An alternative model for the 30-nm fiber is the "zigzag" model (Fig. 8-30b). This model is based on the zigzag pattern of nucleosomes formed upon H1 addition. In this case, the 30-nm fiber is a compacted form of these zigzag nucleosome arrays. A recent X-ray structure of a single DNA molecule participating in four nucleosomes and biophysical studies of the spring-like nature of isolated 30-nm fibers support the zigzag model. Unlike the solenoid model, the zigzag conformation requires the linker DNA to pass through the central axis of the fiber in a relatively straight form (see Fig. 8-30b). Thus, longer linker DNA favors this conformation. Because the average linker DNA varies between different species (see Table 8-4), the form of the 30-nm fiber may not always be the same, and both forms of the 30-nm fiber may be found in cells depending on the local linker DNA length.

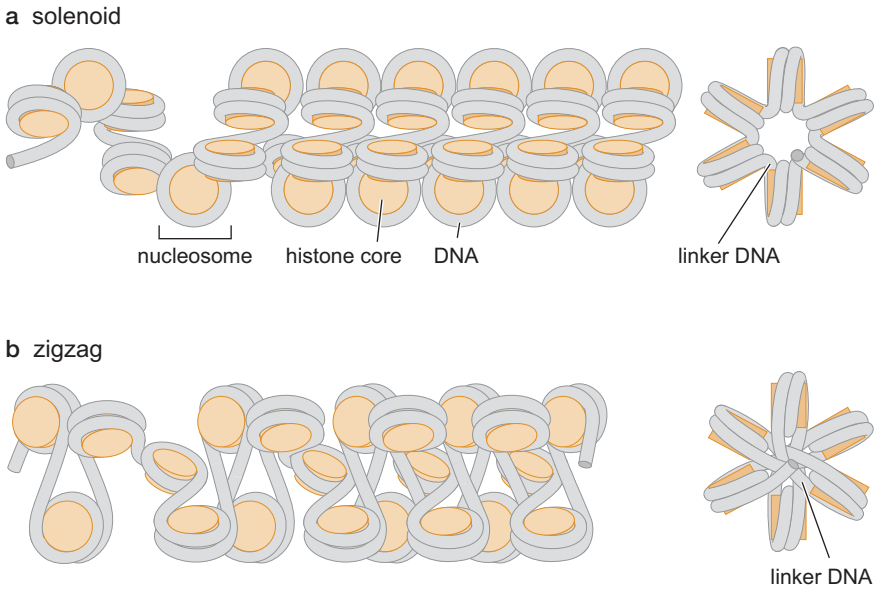


FIGURE 8-30 Two models for the 30-nm chromatin fiber. In each panel, the left-hand view shows the side of the fiber, and the right-hand view shows a view down the central axis of the fiber. (a) The solenoid model. Note that the linker DNA does not pass through the central axis of the superhelix and that the sides and entry and exit points of the nucleosomes are relatively inaccessible. (b) The "zigzag" model. In this model, the linker DNA frequently passes through the central axis of the fiber, and the sides and even the entry and exit points are more accessible. (Reproduced, with permission, from Pollard T. and Earnshaw W. 2002. *Cell biology*, 1st ed., Fig. 13-6. © Elsevier.)

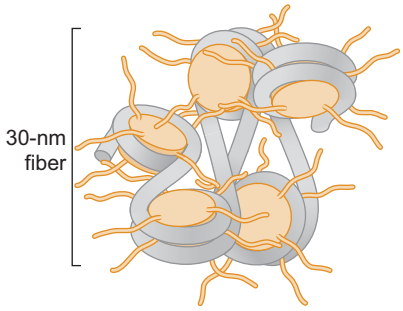


FIGURE 8-31 Speculative model for the stabilization of the 30-nm fiber by histone amino-terminal tails. In this model, the 30-nm fiber is illustrated using the zigzag model. Several different tail–histone core interactions are possible. Here, the interactions are shown as between every alternate histone, but they could also be with adjacent or more distant histones.

The Histone Amino-Terminal Tails Are Required for the Formation of the 30-nm Fiber

Core histones lacking their amino-terminal tails are incapable of forming 30-nm fibers. The most likely role of the tails is to stabilize the 30-nm fiber by interacting with adjacent nucleosomes. This model is supported by the 3D crystal structure of the nucleosome, which shows that each of the amino-terminal tails of H2A, H3, and H4 interacts with adjacent nucleosome cores in the crystal lattice (Fig. 8-31). Recent studies indicate that the interaction between the positively charged amino terminus of histone H4 and a negatively charged region of the histone-fold domain of histone H2A is particularly important for 30-nm fiber formation. Supporting the importance of this interaction, the residues of H2A that interact with the H4 tail are conserved across many eukaryotic organisms but are not involved in DNA binding or formation of the histone octamer. One possibility is that these regions of H2A are conserved to mediate internucleosomal interactions with the H4 tail. As we shall see later, the histone tails are frequent targets for modification in the cell. It is likely that some of these modifications influence the ability to form the 30-nm fiber and other higher-order nucleosome structures.

Further Compaction of DNA Involves Large Loops of Nucleosomal DNA

Together, the packaging of DNA into nucleosomes and the 30-nm fiber results in the compaction of the linear length of DNA by ~40-fold. This is still insufficient to fit 1–2 m of DNA into a nucleus $\sim 10^{-5}$ m across. Additional folding of the 30-nm fiber is required to compact the DNA further. Although the exact nature of this folded structure remains unclear, one popular model proposes that the 30-nm fiber forms loops of 40–90 kb that are held together at their bases by a proteinaceous structure referred to as the **nuclear scaffold** (Fig. 8-32). A variety of methods have been developed to identify proteins that are part of this structure, although the true nature of the nuclear scaffold remains mysterious.

Two classes of proteins that contribute to the nuclear scaffold have been identified. One of these is topoisomerase II (Topo II), which is abundant in both scaffold preparations and purified mitotic chromosomes. Treating cells with drugs that result in DNA breaks at the sites of Topo II DNA binding generates DNA fragments that are ~50 kb in size. This is similar to the size range observed for limited nuclease digestion of chromosomes and suggests that Topo II may be part of the mechanism that holds the DNA at the base of these loops. In addition, the presence of Topo II at the bottom of each loop would ensure that the loops are topologically isolated from one another.

The SMC proteins are also abundant components of the nuclear scaffold. As we discussed above (see above section on Chromosome Duplication and Segregation), these proteins are key components of the machinery that condenses and holds sister chromatids together after chromosome duplication. The associations of these proteins with the nuclear scaffold may serve to enhance their functions by providing an underlying foundation for their interactions with chromosomal DNA.

Histone Variants Alter Nucleosome Function

The core histones are among the most conserved eukaryotic proteins; therefore, the nucleosomes formed by these proteins are very similar in all

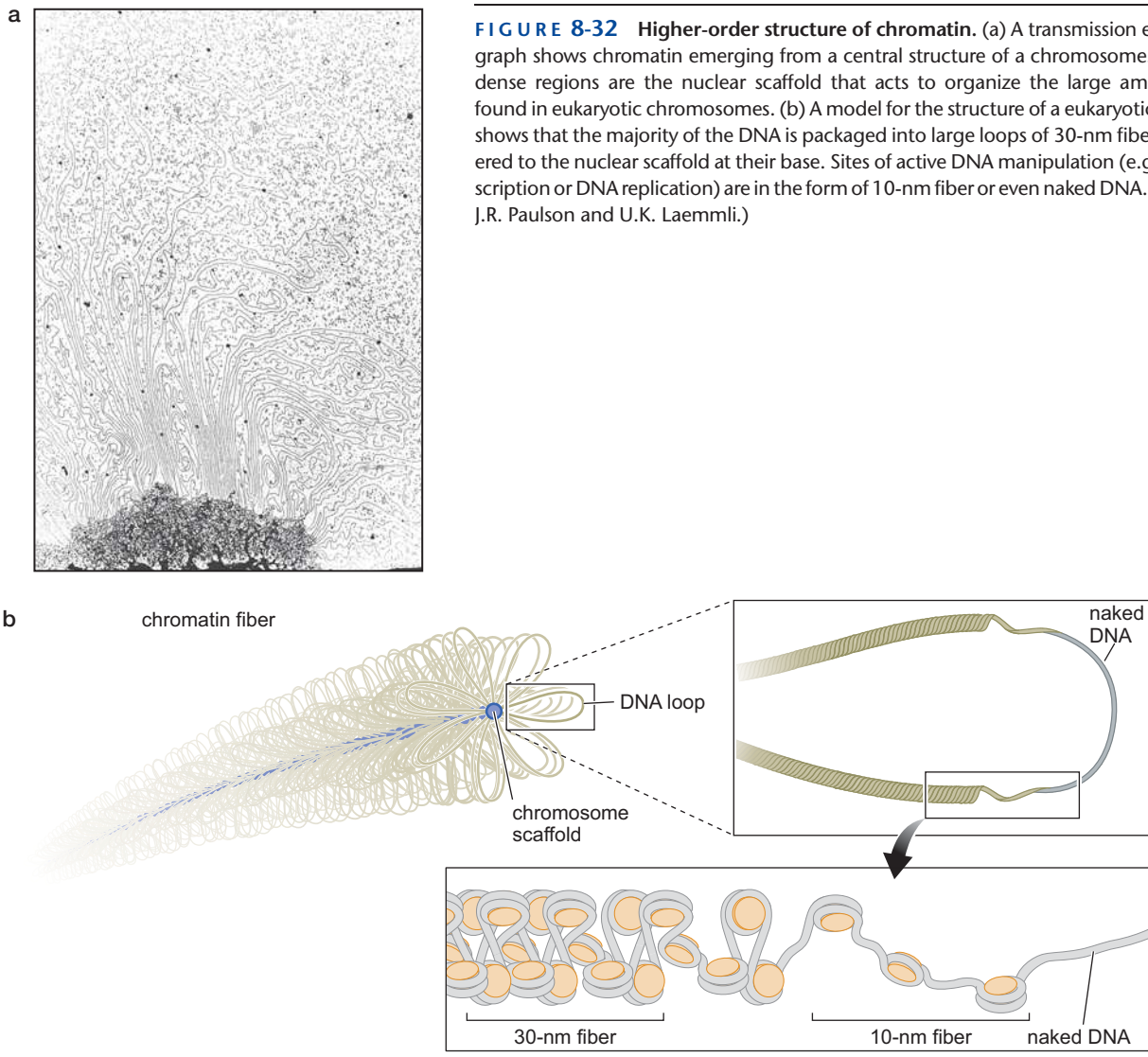


FIGURE 8-32 Higher-order structure of chromatin. (a) A transmission electron micrograph shows chromatin emerging from a central structure of a chromosome. The electron-dense regions are the nuclear scaffold that acts to organize the large amounts of DNA found in eukaryotic chromosomes. (b) A model for the structure of a eukaryotic chromosome shows that the majority of the DNA is packaged into large loops of 30-nm fiber that are tethered to the nuclear scaffold at their base. Sites of active DNA manipulation (e.g., sites of transcription or DNA replication) are in the form of 10-nm fiber or even naked DNA. (a, Courtesy of J.R. Paulson and U.K. Laemmli.)

eukaryotes. But there are numerous histone variants found in eukaryotic cells. Such unorthodox histones can replace one of the four standard histones to form alternate nucleosomes. Such nucleosomes may serve to demarcate particular regions of chromosomes or confer specialized functions to the nucleosomes into which they are incorporated. For example, H2A.X is a variant of H2A that is widely distributed in eukaryotic nucleosomes. When chromosomal DNA is broken (referred to as a double-strand break), H2A.X located adjacent to the break is phosphorylated at a serine residue that is not present in H2A. Phosphorylated H2A.X is specifically recognized by DNA repair enzymes leading to their localization at the site of DNA damage.

A second histone H3 variant, CENP-A, is associated with nucleosomes that include centromeric DNA. In this chromosomal region, CENP-A replaces the histone H3 subunits in nucleosomes. These nucleosomes are incorporated into the kinetochore that mediates attachment of the chromosome to the mitotic spindle (see Fig. 8-12). Compared with H3, CENP-A includes an extended amino-terminal tail region but has an otherwise similar histone-fold region. Thus, it is unlikely that incorporation of CENP-A changes the

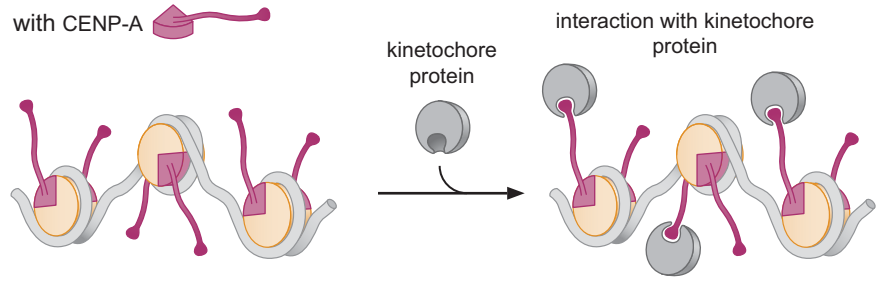


FIGURE 8-33 Alteration of chromatin by incorporation of histone variants. Incorporation of CENP-A in place of histone H3 is proposed to act as a binding site for one or more protein components of the kinetochore.

core structure of the nucleosome. Instead, the extended tail of CENP-A is a binding sites for another protein component of the kinetochore called CENP-C (Fig. 8-33). Consistent with this interaction being critical for kinetochore formation, loss of CENP-A interferes with the association of kinetochore components with centromeric DNA.

REGULATION OF CHROMATIN STRUCTURE

The Interaction of DNA with the Histone Octamer Is Dynamic

As discussed in detail in Chapter 19, the incorporation of DNA into nucleosomes can have a profound impact on the expression of the genome. In many instances, it is critical that nucleosomes can be moved or that their grip on the DNA can be loosened to allow other proteins access to the DNA. Consistent with this requirement, the association of the histone octamer with the DNA is inherently dynamic. In addition, there are factors that act on the nucleosome to increase or decrease the dynamic nature of this association. Together, these properties allow changes in nucleosome position and DNA association in response to the frequently changing needs for DNA accessibility.

Like all interactions mediated by noncovalent bonds, the association of any particular region of DNA with the histone octamer is not permanent: any individual region of the DNA will transiently be released from tight interaction with the octamer now and then. This release is analogous to the occasional opening of the DNA double helix (as we discussed in Chapter 4). The dynamic nature of DNA binding to the histone core structure is important, because many DNA-binding proteins strongly prefer to bind to histone-free DNA. Such proteins can recognize their binding site only when it is released from the histone octamer or is contained in linker or nucleosome-free DNA.

As a result of intermittent, spontaneous unwrapping of DNA from the nucleosome, the DNA-binding site for a given protein will be released from the histone octamer with a probability of 1 in 50 to 1 in 100,000, depending on where the binding site is within the nucleosome. The more central the binding site, the less frequently it is accessible. Thus, a binding site near position 73 of the 147 bp tightly associated with a nucleosome is rarely accessible, whereas binding sites near the ends (position 1 or 147) of the nucleosomal DNA are most frequently accessible. These findings indicate that the mechanism of exposure is due to unwrapping of the DNA from the nucleosome, rather than to the DNA briefly coming off the surface of the histone octamer (Fig. 8-34). It is important to note that these studies were performed on a population of individual nucleosomes in a test tube: the ability of DNA to unwrap from the nucleosome may be different for the large

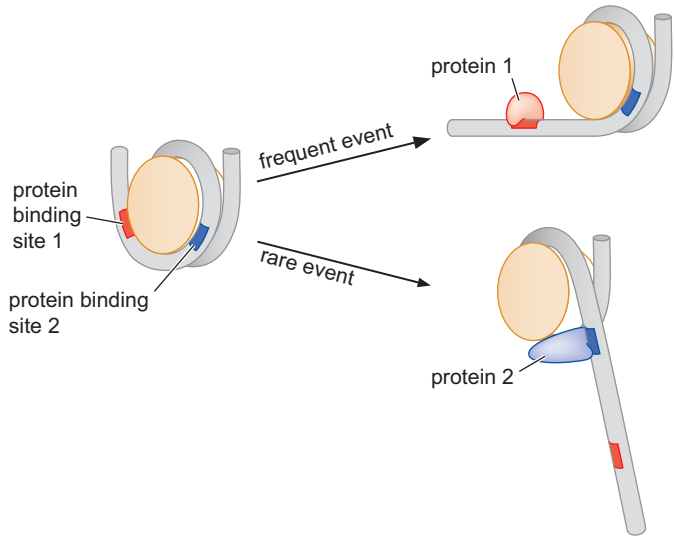


FIGURE 8-34 Model for gaining access to nucleosome-associated DNA. Studies of the ability of sequence-specific DNA-binding proteins to bind nucleosomes suggest that unwrapping of the DNA from the nucleosome is responsible for accessibility of the DNA. DNA sites closest to the entry and exit points are the most accessible, and sites closest to the midpoint of the bound DNA are least accessible.

stretches of DNA participating in many adjacent nucleosomes (called **nucleosome arrays**) present in cells. Association of H1 and incorporation of nucleosomes into the 30-nm fiber will also alter these probabilities. Nevertheless, the dynamic nature of nucleosome structure indicates that nucleosomes only look like the structure revealed in the X-ray crystallography studies for short periods of time and instead spend much of their time in other conformations.

Nucleosome-Remodeling Complexes Facilitate Nucleosome Movement

In addition to the intrinsic dynamics shown by the nucleosome, the stability of the histone octamer–DNA interaction is influenced by large protein complexes called **nucleosome-remodeling complexes**. These multiprotein complexes facilitate changes in nucleosome location or interaction with the DNA using the energy of ATP hydrolysis. There are three basic types of nucleosome changes mediated by these enzymes (Fig. 8-35). All nucleosome-remodeling complexes can catalyze the "**sliding**" of DNA along the surface of the histone octamer. A subset of nucleosome-remodeling complexes can catalyze a second, more extreme change in which a histone octamer is ejected into solution or "**transferred**" from one DNA helix to another. Finally, some of these enzymes can facilitate the exchange of the H2A/H2B dimer within a nucleosome with variants of the dimer (e.g., H2A.X/H2B exchanged for H2A/H2B at double-strand breaks).

Recent studies have begun to reveal how nucleosome-remodeling complexes move DNA on the surface of the histone octamer (Fig. 8-36). Each of these multi-subunit enzymes contains an ATP-hydrolyzing DNA translocase subunit that is capable of moving in a directional manner (also called *translocating*) on double-stranded DNA when separated from the rest of the nucleosome-remodeling complex. Current models suggest that nucleosome-remodeling complexes bind the histone octamer tightly and position the DNA translocase subunit adjacent to the nucleosomal DNA. By holding the translocase in place relative to the histone octamer, the net result of ATP hydrolysis by the nucleosome-remodeling complex is to move the DNA relative to the surface of the histone octamer. DNA translocation generates a loop of DNA that is released from the surface of the nucleosome near the

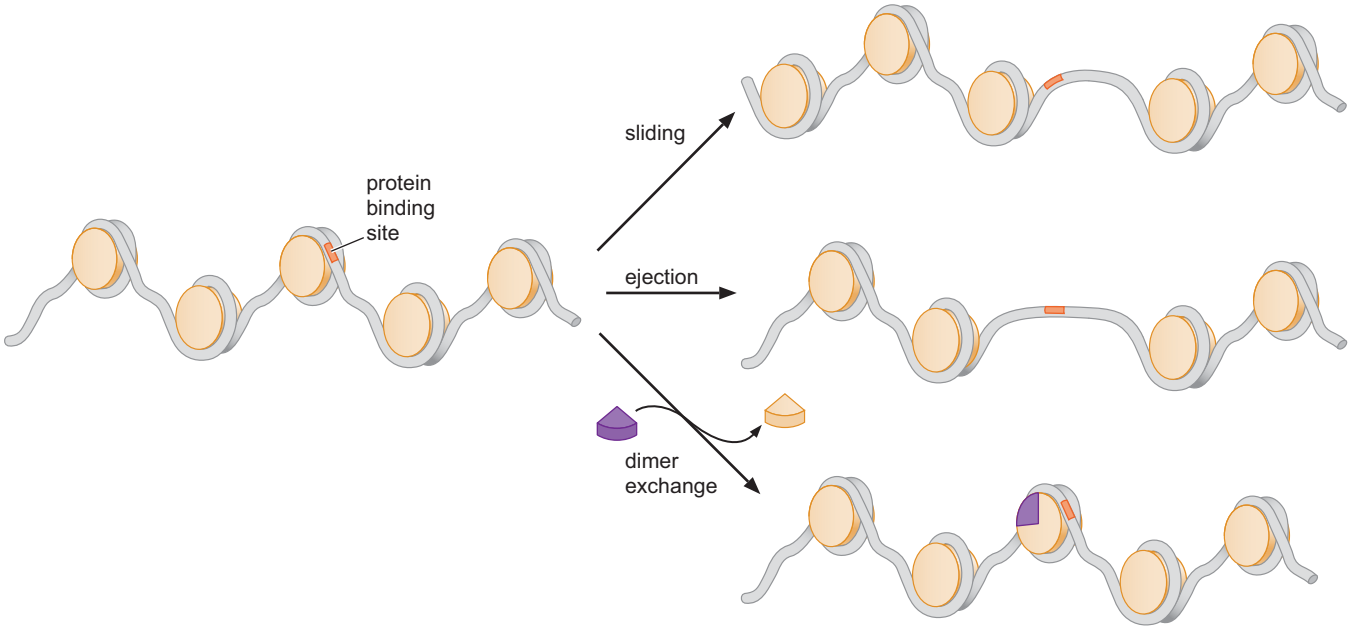


FIGURE 8-35 Nucleosome movement catalyzed by nucleosome-remodeling activities. (Top) Nucleosome movement by sliding along a DNA molecule exposes sites for DNA-binding proteins. (Middle) Nucleosome-remodeling complexes can also eject a nucleosome from the DNA creating larger nucleosome-free regions of DNA. (Bottom) A subset of nucleosome-remodeling complexes catalyzes the exchange of H2A/H2B dimers with either unmodified or variant H2A/H2B dimers (e.g., H2A-X).

site of the translocation. This loop is proposed to propagate on the surface of the histone octamer until it reaches the other end of the nucleosomal DNA. Although this loop movement could potentially proceed in either direction, it is thought that other interactions between the nucleosome-remodeling complex and the nucleosomal DNA prevent propagation toward the proximal DNA linker (which would result in no change in nucleosome positioning). Importantly, this approach does not demand that all interactions between the histone octamer and nucleosomal DNA be broken simultaneously. Instead, the "inchworm-like" movement of the DNA on the surface of the histone octamer allows the majority of the histone DNA interactions to be maintained throughout the remodeling process. It is important to keep in mind that different DNA sequences interact with the histone octamer with roughly equal affinities. Thus, a DNA molecule that is sliding across a histone octamer can be viewed as binding to the octamer in many different energetic equivalent states and the nucleosome-remodeling complex is allowing DNA to access these different states more easily.

There are multiple types of nucleosome-remodeling complexes in any given cell (Table 8-6). They can have as few as two subunits or more than 10 subunits. Each of these complexes contains a related ATP-hydrolyzing subunit that catalyzes the DNA movement described above and in Figure 8-36. Although the ATP-hydrolyzing subunit is similar among the different nucleosome-remodeling complexes, the other subunits associated with each complex modulate their function. For example, these complexes can include subunits that target them to particular chromosomal locations. In some instances, this targeting is mediated by interactions between subunits of the remodeling complex and DNA-bound transcription factors. In other instances, nucleosome-remodeling complexes are localized by subunits that bind to specific histone-tail modifications (via chromodomains or bromodomains, as we discuss later).

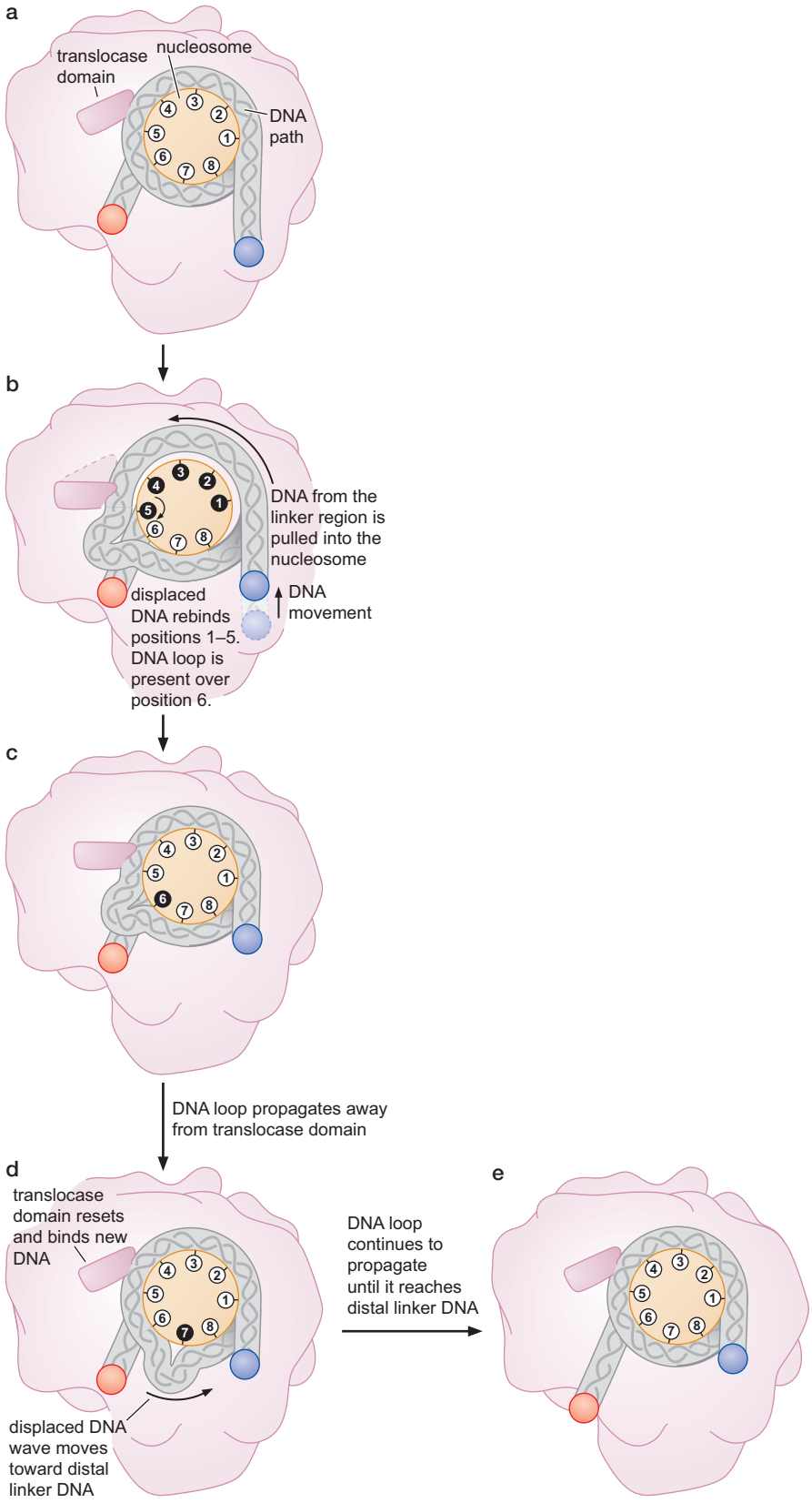


FIGURE 8-36 A model for nucleosomal DNA sliding catalyzed by nucleosome-remodeling complexes. (a) The model proposes that a DNA translocating domain of the ATP-hydrolyzing subunit of the nucleosome-remodeling complex binds the nucleosomal DNA two helical turns from the central dyad (e.g., at position 52 out of the total of 147 bp associated with the nucleosome). Other subunits of the nucleosome-remodeling complex bind tightly to the histones. The illustration shows each of the contacts between the DNA and the histones from the dyad to the closest unbound DNA (one contact per helical turn, seven of the 14 total). (b) Using the ATP-dependent DNA translocating activity, the nucleosome-remodeling complex first pulls the DNA from the nearest linker domain into the nucleosome. This breaks the five histone–DNA contacts between the ATP-hydrolyzing subunit and the linker DNA (broken contacts are shown in black, intact contacts in white) and creates a loop of DNA on the opposite side of the translocase domain. (c) The broken contacts re-form with the translocated DNA (positions 1–5), leaving the loop of DNA next to the ATP-hydrolyzing subunit (disrupting the contacts at position 6). (d) To remove the loop of DNA, the model proposes that the loop moves like a "wave" across the surface of the histones, breaking one or two contacts at a time (first contact 6 and then 7, etc.) until all of the contacts have re-formed with the appropriate amount of DNA between them, at which point the excess DNA is no longer present within the histone-associated DNA and the nucleosome has shifted its position on the DNA. (e) After the loop of DNA has propagated to the distal linker, and the nucleosome has shifted its position on the DNA. (Adapted, with permission, from Saha A. et al. 2006. *Nat. Rev. Mol. Cell Biol.* **7:** 437–447, Fig. 4a. © Macmillan.)

TABLE 8-6 ATP-Dependent Nucleosome-Remodeling Complexes

Type	Number of Subunits	Histone-Binding Domains	Slide	Transfer
SWI/SNF	8–14	Bromodomain	Yes	Yes
ISWI	2–4	Bromodomain, SANT domain, PHD finger	Yes	No
CHD	1–10	Chromodomain, PHD finger, SANT domain	Yes	Yes
INO80	10–16	Bromodomain	Yes	nd

nd, Not determined.

Some Nucleosomes Are Found in Specific Positions: Nucleosome Positioning

Because of their sequence-nonspecific and dynamic interactions with DNA, most nucleosomes are not fixed in their locations. But there are occasions when restricting nucleosome location, or **positioning** nucleosomes as it is called, is beneficial. Typically, positioning a nucleosome allows the DNA-binding site for a regulatory protein to remain in the accessible linker DNA region. In many instances, such nucleosome-free regions are larger to allow the binding sites for multiple regulatory proteins to remain accessible. For example, the regions upstream of active transcription start sites are frequently associated with large nucleosome-free regions.

Nucleosome positioning can be directed by DNA-binding proteins or particular DNA sequences. In the cell, one frequent method involves competition between nucleosomes and DNA-binding proteins. Just as many proteins cannot bind to DNA within a nucleosome, binding of a protein to the DNA can prevent the subsequent association of the core histones with that stretch of DNA. If two such DNA-binding proteins are bound to sites closer than the minimal region of DNA required to assemble a nucleosome (~150 bp), the DNA between the proteins will remain nucleosome-free (Fig. 8-37a). Binding of additional proteins to adjacent DNA can further increase the size of a nucleosome-free region. In addition to this inhibitory mechanism of protein-

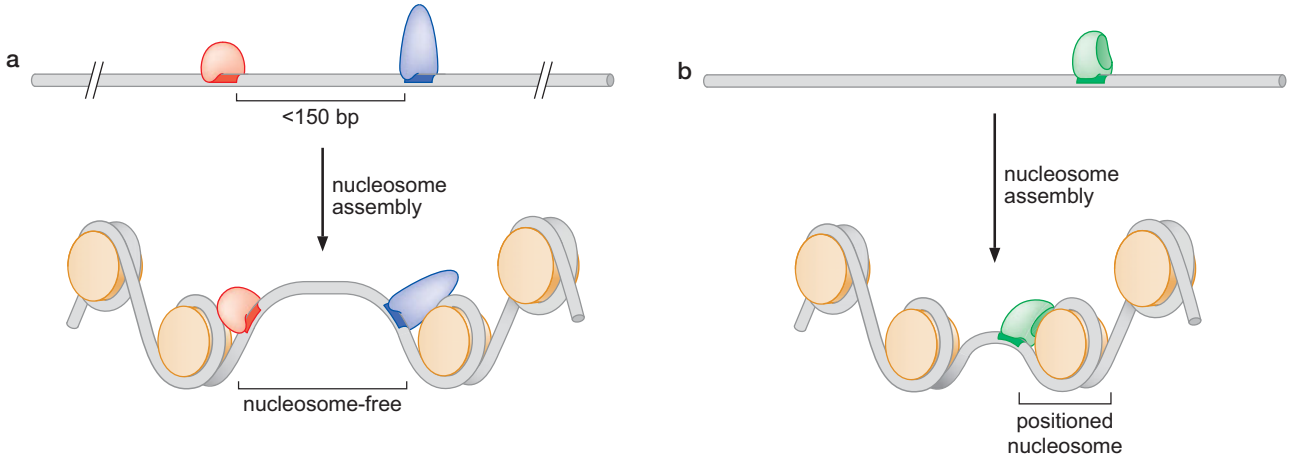


FIGURE 8-37 Two modes of DNA-binding protein-dependent nucleosome positioning. (a) Association of many DNA-binding proteins with DNA is incompatible with the association of the same DNA with the histone octamer. Because a nucleosome requires more than 147 bp of DNA to form, if two such factors bind to the DNA less than this distance apart, the intervening DNA cannot assemble into a nucleosome. (b) A subset of DNA-binding proteins has the ability to bind to nucleosomes. Once bound to DNA, such proteins will facilitate the assembly of nucleosomes immediately adjacent to the protein's DNA-binding site.

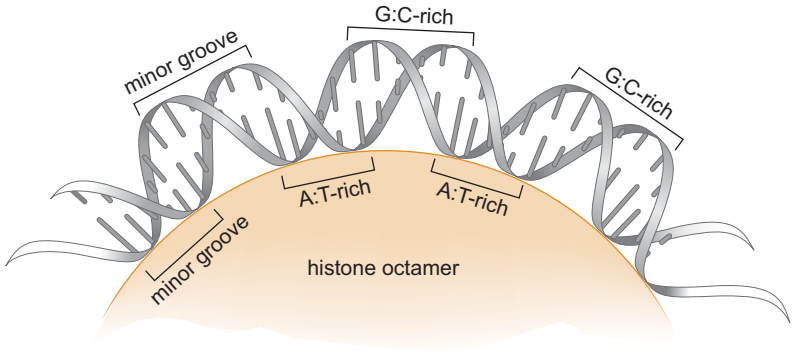


FIGURE 8-38 Nucleosomes prefer to bind bent DNA. Specific DNA sequences can position nucleosomes. Because the DNA is bent severely during association with the nucleosome, DNA sequences that position nucleosomes are intrinsically bent. A:T base pairs have an intrinsic tendency to bend toward the minor groove and G:C base pairs have the opposite tendency. Sequences that alternate between A:T- and G:C-rich sequences with a periodicity of ~5 bp will act as preferred nucleosome-binding sites. (Adapted, with permission, from Alberts B. et al. 2002. *Molecular biology of the cell,* 4th ed., Fig. 4-28. © Garland Science/Taylor & Francis LLC.)

dependent nucleosome positioning, some DNA-binding proteins interact tightly with adjacent nucleosomes, leading to nucleosomes *preferentially* assembling immediately adjacent to these proteins (Fig. 8-37b).

A second method of nucleosome positioning involves particular DNA sequences that have a high affinity for the nucleosome. Because DNA bound in a nucleosome is bent, nucleosomes preferentially form on DNA that bends easily. A:T-rich DNA has an intrinsic tendency to bend toward the minor groove. Thus, A:T-rich DNA is favored in positions in which the minor groove faces the histone octamer. G:C-rich DNA has the opposite tendency and is therefore favored when the minor groove is facing away from the histone octamer (Fig. 8-38). Each nucleosome will try to maximize this arrangement of A:T-rich and G:C-rich sequences. Recent studies of nucleosome positioning in the yeast *S. cerevisiae* suggest that as many as 50% of tightly positioned nucleosomes can be attributed to preferential binding of the histone core to the sequences they include. It is important to note that, despite being favored, such sequences are not required for nucleosome assembly, and the action of other proteins including chromatin-remodeling and transcription factors can move nucleosomes from such preferred positions.

These mechanisms of nucleosome positioning influence the organization of nucleosomes in the genome. Despite this, many nucleosomes are not tightly positioned. As discussed in the chapters on eukaryotic transcription (Chapters 13 and 19), tightly positioned nucleosomes are most often found at sites directing the initiation of transcription. Although we have discussed positioning primarily as a method to ensure that a regulatory DNA sequence is accessible, a positioned nucleosome can just as easily prevent access to specific DNA sites by being positioned in a manner that overlaps the same sequence. Thus, positioned nucleosomes can have either a positive or negative effect on the accessibility of nearby DNA sequences. An approach to mapping nucleosome locations is described in Box 8-3, Determining Nucleosome Position in the Cell.

The Amino-Terminal Tails of the Histones Are Frequently Modified

When histones are isolated from cells, a subset of their amino-terminal tails is typically modified with a variety of small molecules (Fig. 8-39). Lysines in

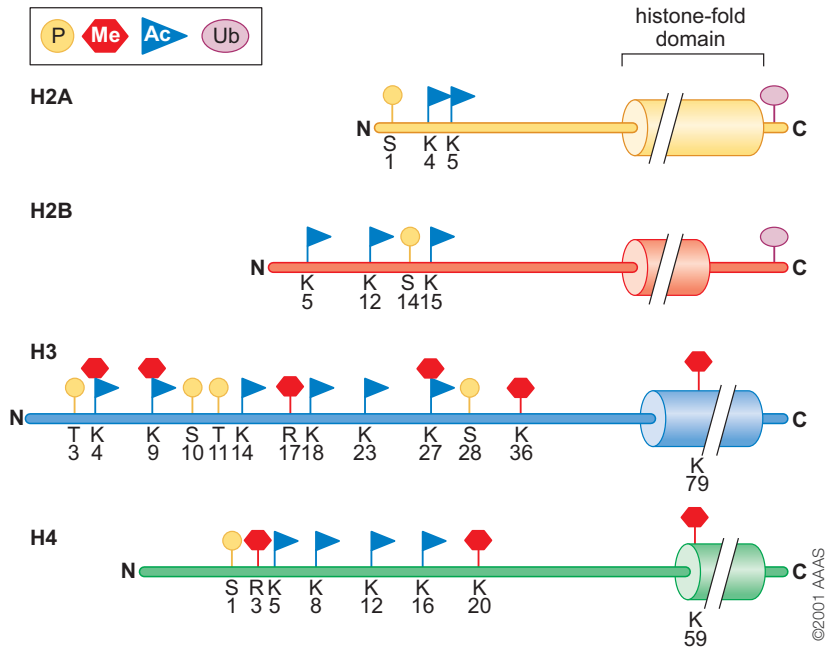


FIGURE 8-39 Modifications of the histone amino-terminal tails alters the function of chromatin. The sites of known histone modifications are illustrated on each histone. Although the types of histone modifications continue to grow, for simplicity, only sites of acetylation, methylation, phosphorylation, and ubiquitinylation are shown. The majority of these modifications occur on the tail regions, but there are occasional modifications within the histone fold (e.g., methylation of lysine 79 of histone H3). (Adapted, with permission, from Alberts B. et al. 2002. *Molecular biology of the cell*, 4th ed., Fig. 4-35. © Garland Science/Taylor & Francis LLC; and, with permission, from Jenuwein T. and Allis C.D. 2001. *Science* **293:** 1074–1080, Figs. 2 and 3. © AAAS.)

the tails are frequently modified with a single acetyl or methyl group, and arginines are found to be modified with one, two, or three methyl groups (Fig. 8-40). Similarly, serines and threonines (and one tyrosine) are subject to modification with phosphate. Although less common, other modifications with larger moieties including ADP-ribose and the small proteins ubiquitin and sumo are also found attached to histones.

Importantly, specific modifications are associated with histones involved in different cellular events. For example, acetylation of lysines at positions 8 and 16 of the histone H4 amino-terminal tail is associated with the start sites of expressed genes, but acetylation at lysines 5 and 12 is not. Instead, acetylation of these other lysines (5 and 12) marks newly synthesized H4 molecules that are ready to be deposited onto DNA as part of a new nucleosome. Similarly, methylation of lysines 4, 36, or 79 of histone H3 typically is associated with expressed genes, whereas methylation of lysines 9 or 27 of the same histone frequently is associated with transcriptional repression. The observation that particular histone modifications have a high probability of occurring at specific functional regions of chromatin (e.g., transcription start sites) has led to the hypothesis that histone tail modifications constitute a biological code that can be written, read, and erased by specific proteins in the cell. For a full discussion of this hypothesis, see Box 19-5.

How does histone modification alter nucleosome function? One obvious change is that acetylation and phosphorylation each acts to reduce the overall positive charge of the histone tails; acetylation of lysine neutralizes its positive charge (Fig. 8-41). This loss of positive charge reduces the affinity of the tails for the negatively charged backbone of the DNA. More importantly, modification of the histone tails affects the ability of nucleosome arrays to form more repressive higher-order chromatin structure. As we described above, histone amino-terminal tails are required to form the 30-nm fiber, and modification of the tails modulates this function. For example, consistent with the association of some types of acetylated histones with expressed regions of the genome, acetylation of the H4 amino-terminal tail interferes with the ability of nucleosomes to be incorporated into the

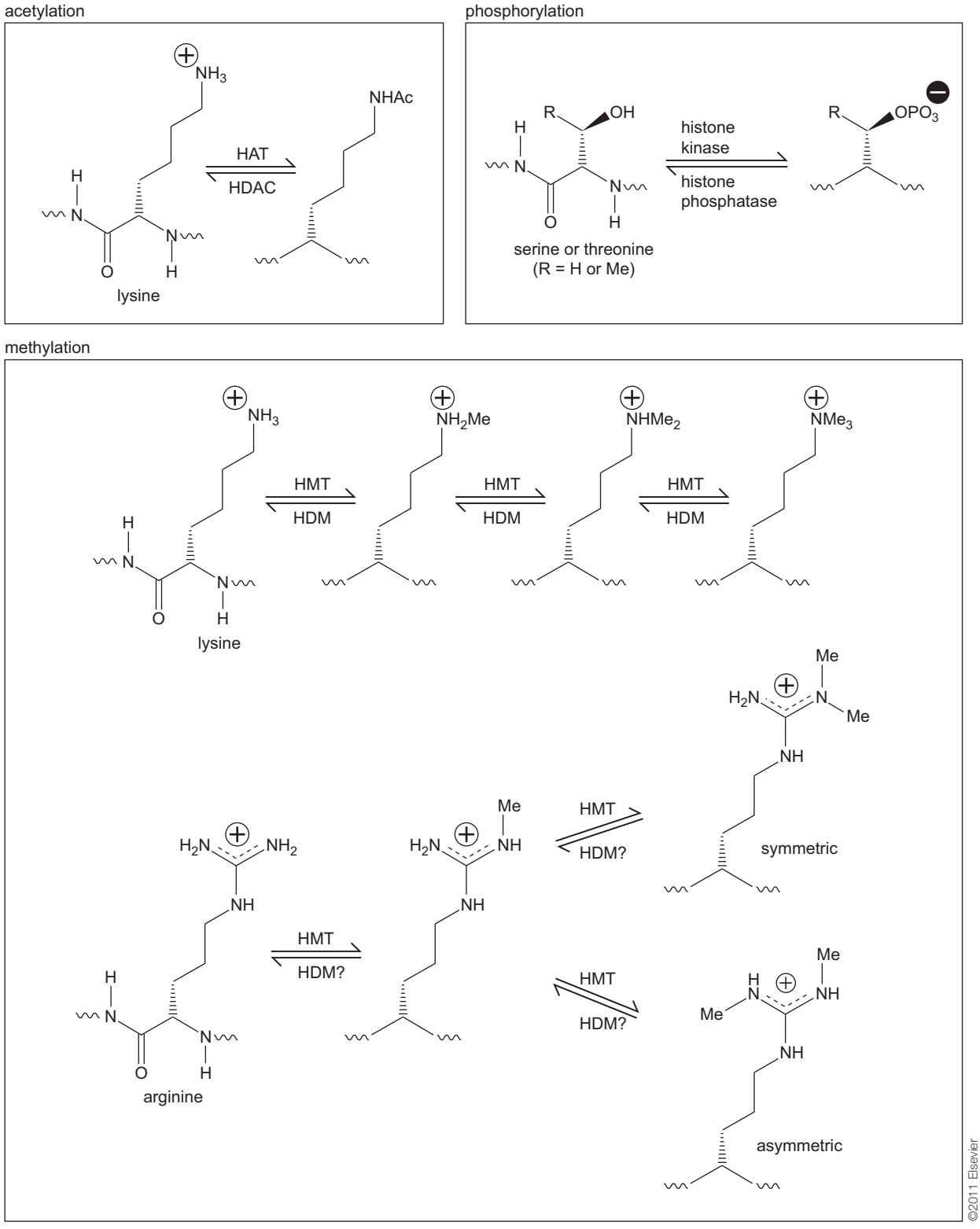


FIGURE 8-40 Structure of histone tail modifications. The molecular structure of the small molecule histone modifications and the class of enzyme responsible are illustrated (histone acetyl transferase [HAT]; histone deacetylase [HDAc]; histone methyltransferase [HMT]; histone demethylase [HDM]). Only the affected amino acid is shown. Currently there is no known histone demethylase for arginine methylation, suggesting that these marks are only lost when the histone is removed from the DNA. (Adapted, with permission, from Lohse B. et al. 2011. *Bioorg. Med. Chem.* **19:** 3625–3636, Fig. 1. © Elsevier.)

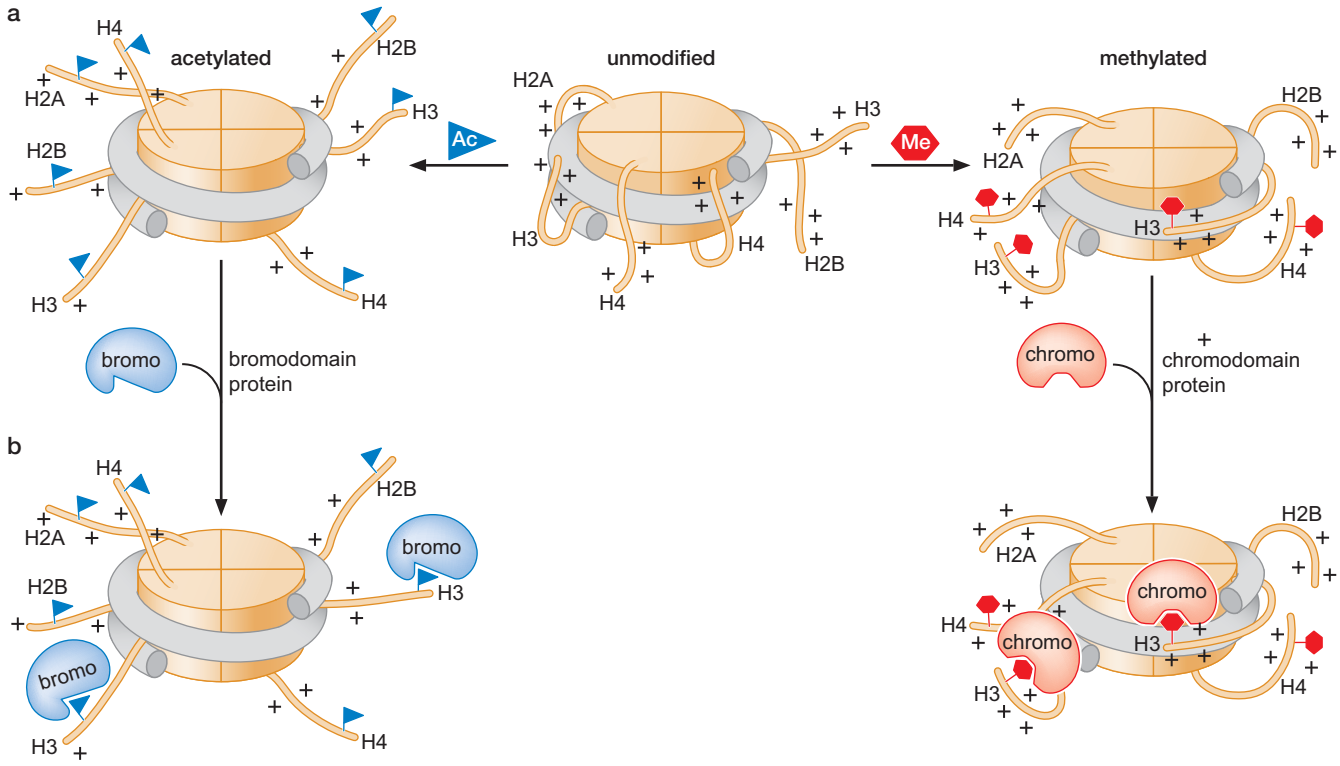


FIGURE 8-41 Effects of histone tail modifications. (a) The effect on the association with nucleosome-bound DNA. Unmodified and methylated histone tails are thought to associate more tightly with nucleosomal DNA than acetylated histone tails. (b) Modification of histone tails creates binding sites for chromatin-modifying enzymes.

repressive 30-nm fiber. As we described above, formation of the 30-nm fiber is facilitated by an interaction between the positively charged H4 amino-terminal tail and the negatively charged surface of the H2A histone-fold domain. Acetylation interferes with this association by altering the charge of the H4 tail.

Protein Domains in Nucleosome-Remodeling and -Modifying Complexes Recognize Modified Histones

Modified histone tails can also act to recruit specific proteins to the chromatin (Fig. 8-41b). Protein domains called **bromodomains**, **chromodomains**, **TUDOR domains**, and **PHD** (for plant homeodomain) **fingers** specifically recognize modified forms of histone tails. Bromodomain-containing proteins interact with acetylated histone tails, and chromodomain-TUDOR domains and PHD-finger-containing proteins interact with methylated histone tails. Yet another protein domain, called a **SANT domain**, has the opposite property. SANT-domain-containing proteins interact preferentially with unmodified histone tails. Consistent with these protein domains being important for interpreting histone modifications, in many instances proteins containing these domains specifically recognize the modified form of only one of the many possible sites of histone modification. For example, the protein HP1 contains a chromodomain that will bind to methylated lysine 9 of histone H3 but not to any other site of histone methylation. Intriguingly, there are proteins that include more than one of these domains,

suggesting that they are specialized for recognizing histone tails that are multiply modified. For example, there are proteins that contain a PHD finger specific for methylated lysine 4 of histone H3 immediately next to a bromodomain capable of recognizing an acetylated lysine.

How do the domains that recognize modified histones alter the function of the associated nucleosomes? One important way is that modified histones recruit enzymes that will further modify adjacent nucleosomes. For example, many of the enzymes that acetylate histone tails (called histone acetyltransferases or HATs) include bromodomains that recognize the same histone modifications that they create (Table 8-7). In this case, the bromodomain facilitates the maintenance and propagation of acetylated histones by modifying nucleosomes that are adjacent to the already acetylated histones (as we shall discuss later).

Modified histones can also recruit other proteins that act on chromatin. Many nucleosome-remodeling complexes include one or more subunits with domains that recognize modified histones (see Table 8-6) allowing modified histones to recruit these enzymes. Several proteins involved in

KEY EXPERIMENTS

Box 8-3 Determining Nucleosome Position in the Cell

The significance of the location of nucleosomes adjacent to important regulatory sequences has led to the development of methods to monitor the location of nucleosomes in cells. Many of these methods exploit the ability of nucleosomes to protect DNA from digestion by micrococcal nuclease. As described in Box 8-1, micrococcal nuclease has a strong preference to cleave DNA between nucleosomes, rather than DNA tightly associated with nucleosomes. This property can be used to map nucleosomes that are associated with the same position throughout a cell population (Box 8-3 Fig. 1).

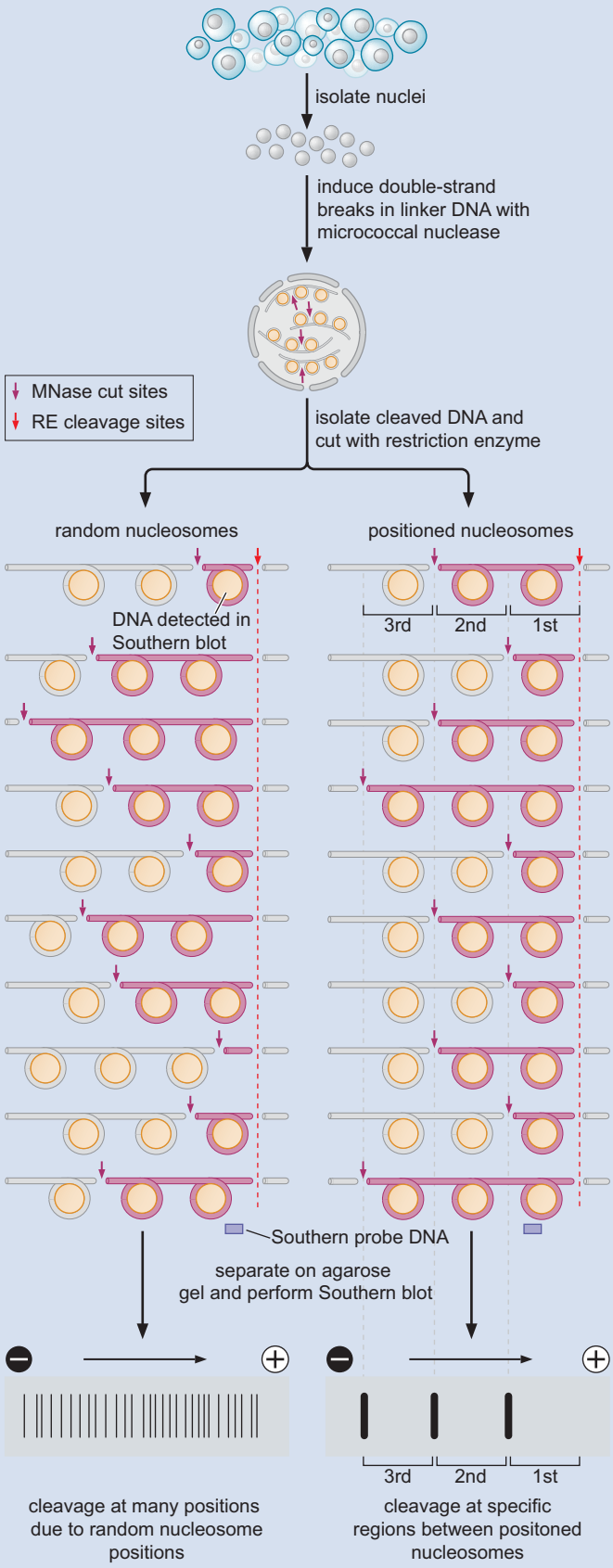
To map nucleosome location accurately, it is important to isolate the cellular chromatin and treat it with the appropriate amount of micrococcal nuclease with minimal disruption of the overall chromatin structure. This is typically achieved by gently lysing cells while leaving the nuclei intact. The nuclei are then briefly treated (typically for 1 min) with several different concentrations of micrococcal nuclease, a protein small enough to diffuse rapidly into the nucleus. The goal of the titration is for micrococcal nuclease to cleave the region of interest only once in each cell. Once the DNA has been digested, the nuclei can be lysed, and all of the protein can be removed from the DNA. The sites of cleavage (and, more importantly, the sites not cleaved) leave a record of the protein bound to DNA.

To identify the sites of cleavage in a particular region, it is necessary to create a defined end point for all of the cleaved fragments and exploit the specificity of DNA hybridization. To create a defined end point, the purified DNA from each sample is cut with a restriction enzyme known to cleave adjacent to the site of interest. After separation by size using agarose gel electrophoresis, the DNA is denatured and transferred to a nitrocellulose membrane such that its position in the gel is retained. A labeled DNA probe of specific sequence is then hybridized to the nitrocellulose-bound DNA (this is called a Southern blot and is described in more detail in Chapter 7). In this case, the DNA probe is chosen to hybridize immediately adjacent to the restriction enzyme cleavage site at the site of interest. After hybridization and washing, the DNA probe will show the size of the fragments generated by micrococcal nuclease in the region of interest.

How do the fragment sizes reveal the location of positioned nucleosomes? DNA associated with positioned nucleosomes will be resistant to micrococcal nuclease digestion, leaving an ~160–200-bp region of DNA that is not cleaved. This will appear as a gap in the ladder of DNA bands detected on the Southern blot. The location of these gaps reveals the position of the nucleosomes adjacent to the restriction site/labeled DNA probe.

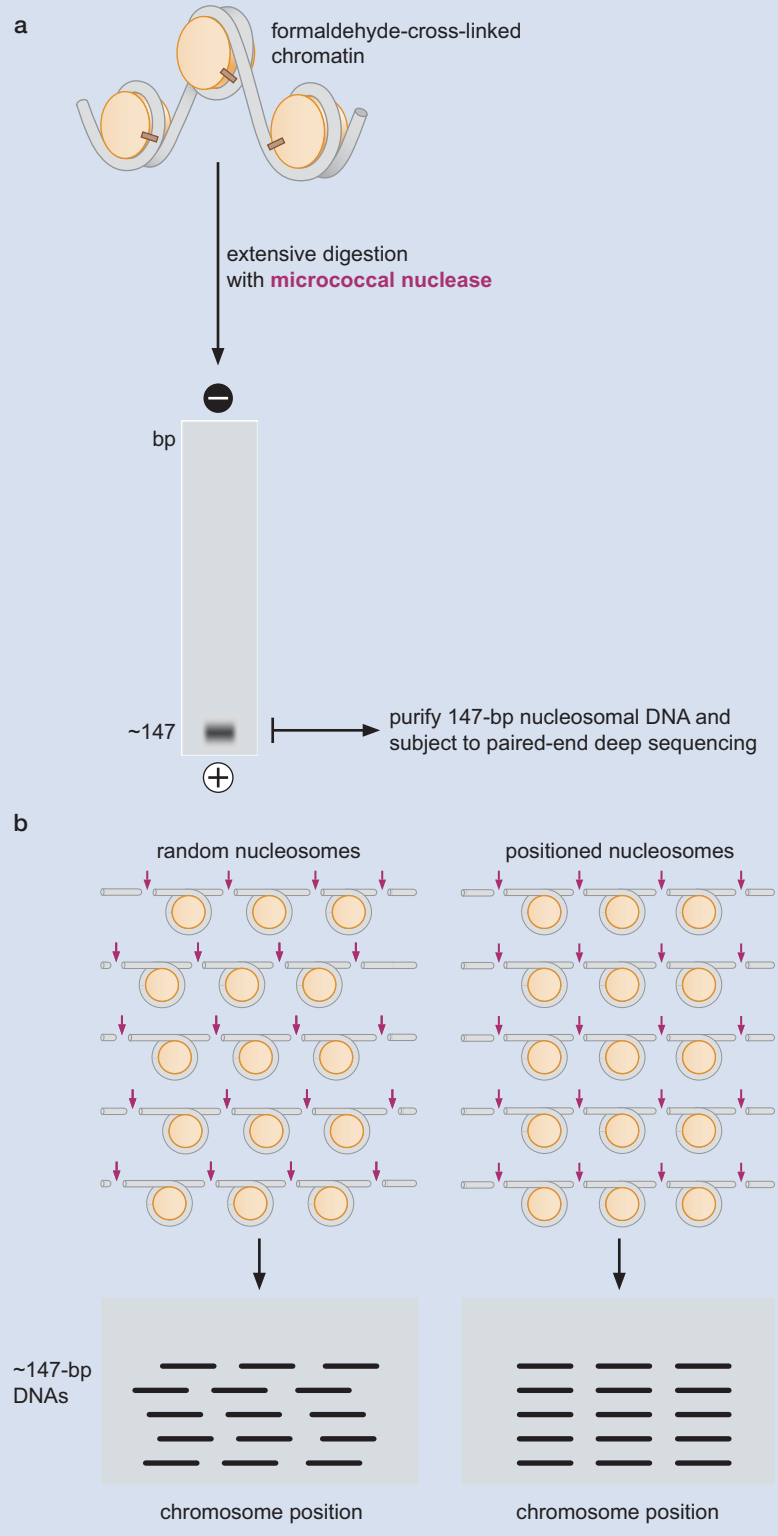
More recently, a related approach has been developed to identify positioned nucleosomes across entire genomes. This method starts by cross-linking histones to the DNA by treating the cells of interest with formaldehyde (Box 8-3 Fig. 2a). Next, the cells are lysed, and chromatin is isolated and treated with micrococcal nuclease until the majority of the DNA is the size of a mononucleosome (~147 bp). After reversing the cross-linking, the DNA is separated using gel electrophoresis, and the resulting 147-bp DNA fragments are purified and subjected to paired-end deep sequencing. This method of deep sequencing not only sequences both ends of each DNA fragment but also keeps track of which ends are from the same DNA fragment. Thus, paired-end sequencing reveals both the genomic location and the length of the sequenced DNA fragment. The nucleosome-sized DNA fragments sequenced reveal the location of a nucleosome. These locations can then be plotted along the length of each chromosome. The location of positioned nucleosomes is revealed by sites with many DNA fragments that are derived from the same 147-bp region (Box 8-3 Fig. 2b). Using this approach, all of the positioned nucleosomes across an entire genome can be mapped.

BOX 8-3 *(Continued)*



BOX 8-3 FIGURE 1 Analysis of nucleosome positioning at a defined chromosomal position. The experimental steps in determining nucleosome positioning in the cell are illustrated. See box text for details.

BOX 8-3 *(Continued)*



BOX 8-3 FIGURE 2 Genome-wide analysis of nucleosome positioning. (a) After cells are cross-linked with formaldehyde and chromatin is isolated, extensive treatment of the cross-linked chromatin with micrococcal nuclease results in the generation of predominantly nucleosome-core particles. Following the reversal of the cross-links, the predominant band of 147-bp DNA is isolated using gel electrophoresis and subjected to paired-end deep sequencing. (b) Illustration of the chromosomal mapping of nucleosome-associated DNAs at a site with random and positioned nucleosomes.

TABLE 8-7 Histone-Modifying Enzymes

Histone Acetyltransferase Complexes				
Type	**Number of Subunits**	**Catalytic Subunit**	**Histone-Binding Domains**	**Target Histones**
SAGA	15	Gcn5	Bromodomain, chromodomain	H3 and H2B
PCAF	11	PCAF	Bromodomain	H3 and H4
NuA3	5	Sas3	PHD finger	H3
NuA4	6	Esa1	Chromodomain, SANT domain, PHD finger	H4 and H2A
P300/CBP	1	P300/CBP	Bromodomain, PHD finger	H2A, H2B, H3, and H4

Histone Deacetylase Complexes			
Type	**Number of Subunits**	**Catalytic Subunit(s)**	**Histone-Binding Domains**
NuRD	9	HDAC1/HDAC2	Chromodomain, PHD finger
SIR2 complex	3	Sir2	Neither
Rpd3 large	12	Rpd3	PHD finger
Rpd3 small	5	Rpd3	Chromodomain, PHD finger

Histone Methyltransferases		
Name	**Histone-Binding Domains**	**Target Histone**
SET1	None	H3 (lysine 4)
SUV39/CLR4	Chromodomain	H3 (lysine 9)
SET2	None	H3 (lysine 36)
DOT1	None	H3 (lysine 79)
PRMT	None	H3 (arginine 3)
SET9/SUV4-20	None	H4 (lysine 20)

Histone Demethylases		
Name	**Histone-Binding Domains**	**Target Methylated Histone**
LSD1	PHD finger, SANT domain	H3 (lysine 4)
JHDM1	PHD finger	H3 (lysine 36)
JHDM3	PHD finger, TUDOR domain	H3 (lysines 9 and 36)

regulating transcription also include these domains. For example, a key component of the eukaryotic transcription machinery called TFIID includes a bromodomain. This domain directs the transcription machinery to sites of histone acetylation, which is an additional way that histone acetylation contributes to the increased transcriptional activity of the associated DNA. Chromodomains that recognize sites of histone methylation associated with transcriptionally repressed genes are found in several proteins that are important for the establishment of heterochromatin, including the HP1 protein and Polycomb proteins (see Chapters 19 and 21, respectively).

Specific Enzymes Are Responsible for Histone Modification

The histone modifications we have just described are dynamic and are catalyzed by specific enzymes (Fig. 8-40). Histone acetyltransferases (HATs) catalyze the addition of acetyl groups to histones, whereas histone deacetylases (HDACs) remove these modifications. Similarly, histone methyltransferases (HMTs) add methyl groups to histones, and histone demethylases (HDMs) remove these modifications. A number of different histone acetyltransferases and deacetylases have been identified and are distinguished by their abilities

to target a different subset of histones or in some cases specific lysines in one histone. Histone methyltransferases and demethylases appear to be much more specific, always targeting only one of the many lysines or arginines on a specific histone (Table 8-7). Because these different modifications have different effects on nucleosome function, the modification of a nucleosome with different histone acetyltransferases or methyltransferases (or the removal of modifications by histone deacetylases or demethylases) can modulate chromatin structure and influence a wide array of DNA transactions.

Like their nucleosome-remodeling complex counterparts, these modifying enzymes are part of large multiprotein complexes. Additional subunits play important roles in recruiting these enzymes to specific regions of the DNA. Similar to the nucleosome-remodeling complexes, these interactions can be with transcription factors bound to DNA or directly with specifically modified nucleosomes. The recruitment of these enzymes to particular DNA regions is responsible for the distinct patterns of histone modification observed along the chromatin and is a major mechanism for modulating the levels of gene expression along the eukaryotic chromosome (see Chapter 19).

Nucleosome Modification and Remodeling Work Together to Increase DNA Accessibility

The combination of amino-terminal tail modifications and nucleosome remodeling can dramatically change the accessibility of the DNA. As discussed in Chapters 13 and 19, the protein complexes involved in these modifications are frequently recruited to sites of active transcription. Although the order of their function is not always the same, the combined action can result in a profound, but localized, changes in DNA accessibility. Modification of amino-terminal tails can reduce the ability of nucleosome arrays to form repressive structures. This change creates sites that can recruit other proteins, including nucleosome remodelers. Remodeling of the nucleosomes can then further increase the accessibility of the nucleosomal DNA to allow DNA-binding proteins access to their binding sites. In combination with the appropriate DNA-binding proteins or DNA sequences, these changes can result in the positioning or release of nucleosomes at specific sites on the DNA (Fig. 8-42).

NUCLEOSOME ASSEMBLY

Nucleosomes Are Assembled Immediately after DNA Replication

The duplication of a chromosome requires replication of the DNA *and* the reassembly of the associated proteins on each daughter DNA molecule. The latter process is tightly linked to DNA replication to ensure that the newly replicated DNA is rapidly packaged into nucleosomes. In Chapter 9, we discuss the mechanisms of DNA replication in detail. Here, we discuss the mechanisms that direct the assembly of nucleosomes after the DNA is replicated (see Interactive Animation 8-2).

Although the replication of DNA requires the nucleosome disassembly, the DNA is rapidly repackaged into nucleosomes in an ordered series of events. As discussed above, the first step in the assembly of a nucleosome is the binding of an H3·H4 tetramer to the DNA. Once the tetramer is bound, two H2A·H2B dimers associate to form the final nucleosome. H1 joins this

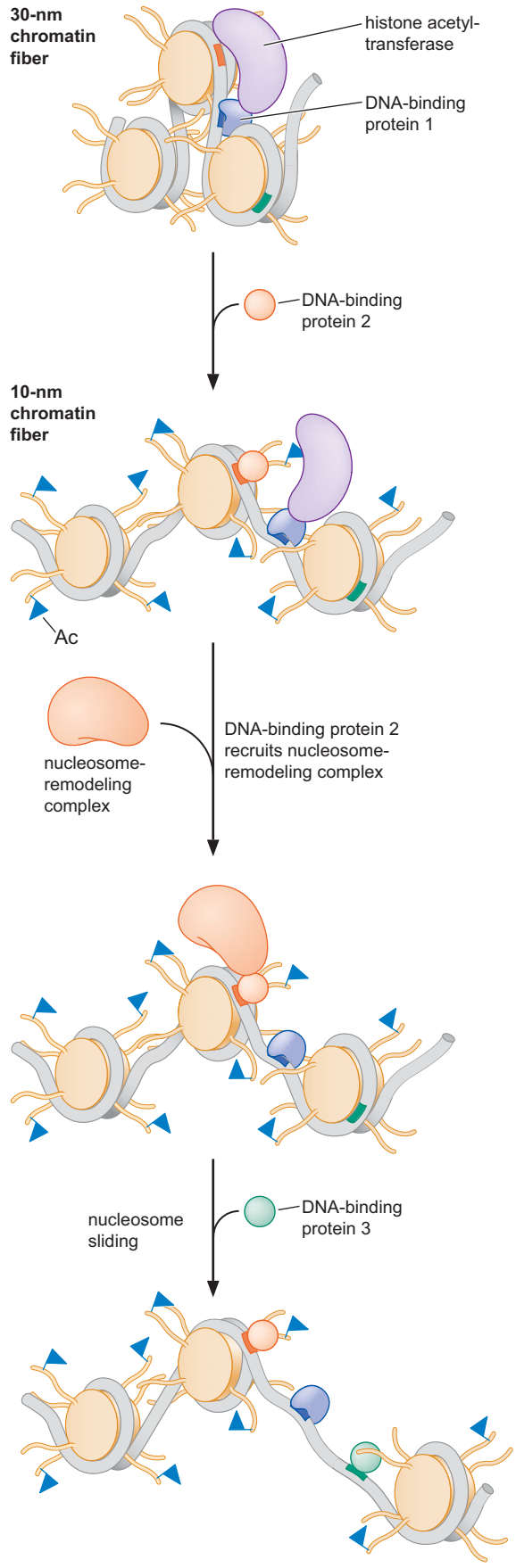


FIGURE 8-42 Chromatin-remodeling and histone-modifying complexes work together to alter chromatin structure. Sequence-specific DNA-binding proteins typically recruit these enzymes to specific regions of a chromosome. In the illustration, the blue DNA-binding protein first recruits a histone acetyltransferase that modifies the adjacent nucleosomes, increasing the accessibility of the associated DNA by locally converting the chromatin fiber from the 30-nm fiber to the more accessible 10-nm form. This increased accessibility allows the binding of a second DNA-binding protein (orange) that recruits a nucleosome-remodeling complex. Localization of the nucleosome-remodeling complex facilitates the sliding of the adjacent nucleosomes, which allows the binding site for a third DNA-binding protein (green) to be exposed. For example, this could be the binding site for the TATA-binding protein at a start site of transcription. Although we show the order of association as histone acetylation complex and then nucleosome-remodeling complex, both orders are observed and can be equally effective. It is also true that recruitment of a different histone-modifying complex could result in the formation of more compact and inaccessible chromatin.

complex last, presumably during the formation of higher-order chromatin assemblies.

To duplicate a chromosome, at least half of the nucleosomes on the daughter chromosomes must be newly synthesized. Are all of the old histones lost and only new histones assembled into nucleosomes? If not, how are the old histones distributed between the two daughter chromosomes? The fate of the old histones is a particularly important issue given the effects that histone modification can have on the accessibility of the resulting chromatin. If the old histones were lost completely, then chromosome duplication would erase any "memory" of the previously modified nucleosomes. In contrast, if the old histones were retained on a single chromosome, that chromosome would have a distinct set of modifications relative to the other copy of the chromosome.

In experiments that differentially labeled old and new histones, it was found that the old histones are present on both of the daughter chromosomes (Fig. 8-43). Mixing is not entirely random, however. H3·H4 tetramers and H2A·H2B dimers are composed of either all new or all old histones. Thus, as the replication fork passes, nucleosomes are broken down into their component subassemblies. H3·H4 tetramers appear to remain bound to one of the two daughter duplexes at random and are never released from DNA into the free pool of histones. In contrast, the H2A·H2B dimers are released and enter the local pool, available for new nucleosome assembly.

The distributive inheritance of old histones during chromosome duplication provides a mechanism for the propagation of the parental pattern of histone modification. By this mechanism, old modified histones will tend to rebind one of the daughter chromosomes at a position near their previous position on the parental chromosome (Fig. 8-44). The old histones have an equal probability of binding either daughter chromosome. This localized inheritance of modified histones ensures that a subset of the modified histones is located in similar positions on each daughter chromosome.

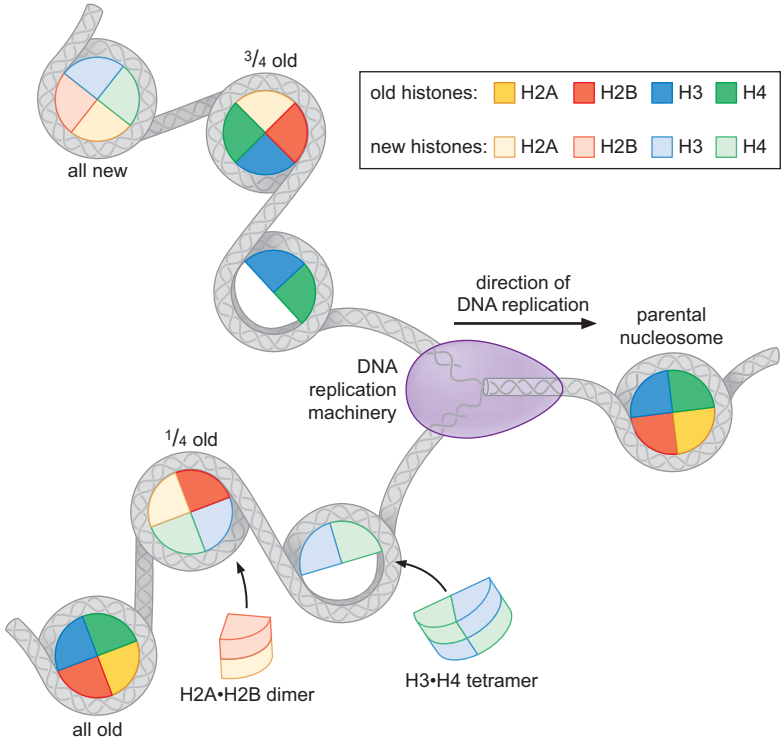


FIGURE 8-43 Inheritance of histones after DNA replication. As the chromosome is replicated, histones that were associated with the parental chromosome are differently distributed. The histone H3·H4 tetramers are randomly transferred to one of the two daughter strands but do not enter into the soluble pool of H3·H4 tetramers. Newly synthesized H3·H4 tetramers form the basis of the nucleosomes on the strand that does not inherit the parental tetramer. In contrast, H2A and H2B dimers are released into the soluble pool and compete for H3·H4 association with newly synthesized H2A and H2B. As a consequence of this type of distribution, on average, every second H3·H4 tetramer on newly synthesized DNA will be derived from the parental chromosome. These tetramers will include all of the modifications added to the parental nucleosomes. The H2A·H2B dimers are more likely to be derived from newly synthesized protein.

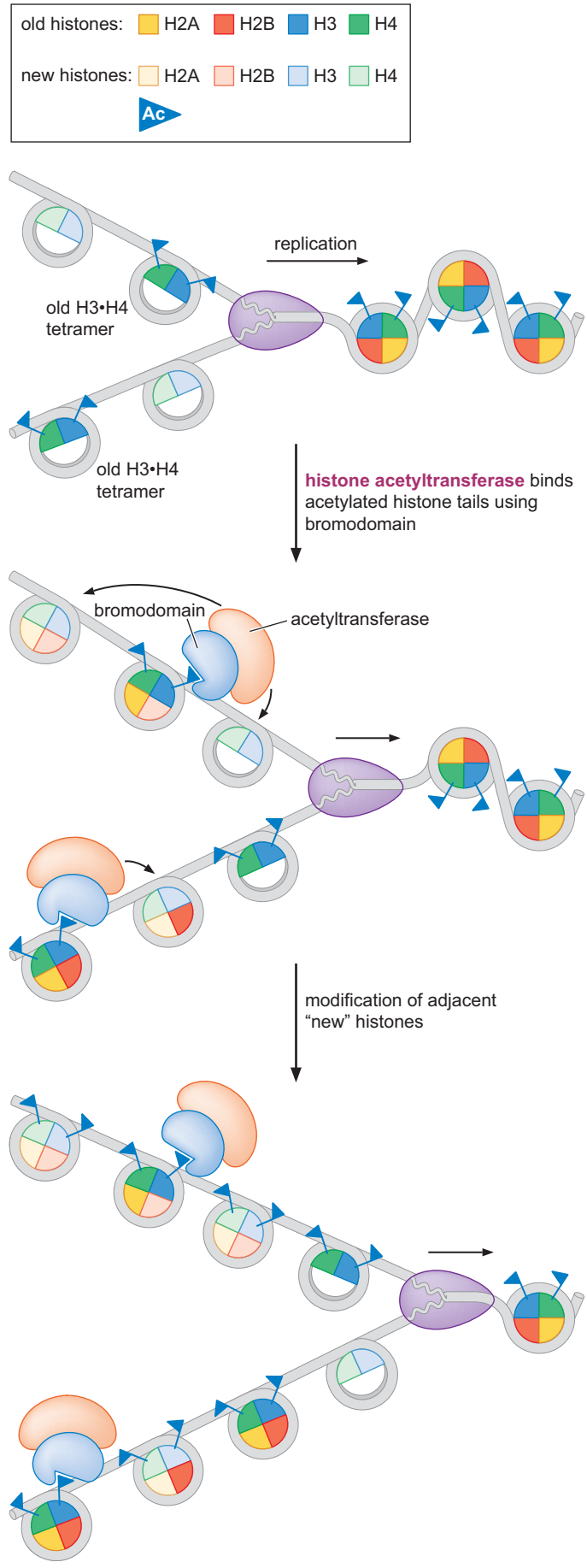


FIGURE 8-44 Inheritance of parental H3·H4 tetramers facilitates the inheritance of chromatin states. As a chromosome is replicated, the distribution of the parental H3·H4 tetramers results in the daughter chromosomes receiving the same modifications as the parent. The ability of these modifications to recruit enzymes that perform the same modifications facilitates the propagation of the modification to the two daughter chromosomes. For simplicity, acetylation is shown on the core regions of the histones. In reality, this modification is generally on the amino-terminal tails.

The ability of these modified histones to recruit enzymes that add similar modifications to adjacent nucleosomes (see the discussion of modified histone-binding domains above) provides a simple mechanism to maintain states of modification after DNA replication has occurred (Fig. 8-44). Such mechanisms are likely to play a critical role in the inheritance of chromatin states from one generation to another. Given the importance of histone modification in controlling gene expression (see Chapter 19) as well as other DNA transactions, the maintenance of such modification states is critical to maintaining cell identity as cells replicate their DNA and divide.

Assembly of Nucleosomes Requires Histone "Chaperones"

The assembly of nucleosomes is not a spontaneous process. Early studies found that the simple addition of purified histones to DNA resulted in little or no nucleosome formation. Instead, the majority of the histones aggregate in a nonproductive form. For correct nucleosome assembly, it was necessary to raise salt concentrations to very high levels (>1 M NaCl) and then slowly reduce the concentration over many hours. Although useful for assembling nucleosomes for in vitro studies (such as for the structural studies of the nucleosome described above), elevated salt concentrations are not involved in nucleosome assembly in vivo.

Studies of nucleosome assembly under physiological salt concentrations identified factors required to direct the assembly of histones onto the DNA. These factors are negatively charged proteins that form complexes with either H3·H4 or H2A·H2B dimers (see Table 8-8) and escort them to sites of nucleosome assembly. Because they act to keep histones from interacting with the DNA nonproductively, these factors have been referred to as **histone chaperones** (see Fig. 8-45).

How do the histone chaperones direct nucleosome assembly to sites of new DNA synthesis? Studies of the histone H3·H4 chaperone CAF-I reveal a likely answer. Nucleosome assembly directed by CAF-I requires that the target DNA be replicating. Thus, replicating DNA is marked in some way for nucleosome assembly. Interestingly, this mark is gradually lost after replication is completed. Studies of CAF-I-dependent assembly have determined that the mark is a ring-shaped sliding clamp protein called PCNA. As we discuss in detail in Chapter 9, this factor forms a ring around the DNA duplex and is responsible for holding DNA polymerase on the DNA during DNA synthesis. After the polymerase is finished, PCNA is released from the DNA polymerase but still encircles the DNA. In this condition, PCNA is available to interact with other proteins. CAF-I associates with the released PCNA and assembles H3·H4 preferentially on the PCNA-bound DNA. Thus, by associating with a component of the DNA replication machinery, CAF-I is directed to assemble nucleosomes at sites of recent DNA replication.

TABLE 8-8 Properties of Histone Chaperones

Name	Number of Subunits	Histones Bound	Interaction with Sliding Clamp
CAF-1	4	H3·H4	Yes
HIRA	4	H3·H4	No
RCAF	1	H3·H4	No
NAP-1	1	H2A·H2B	No

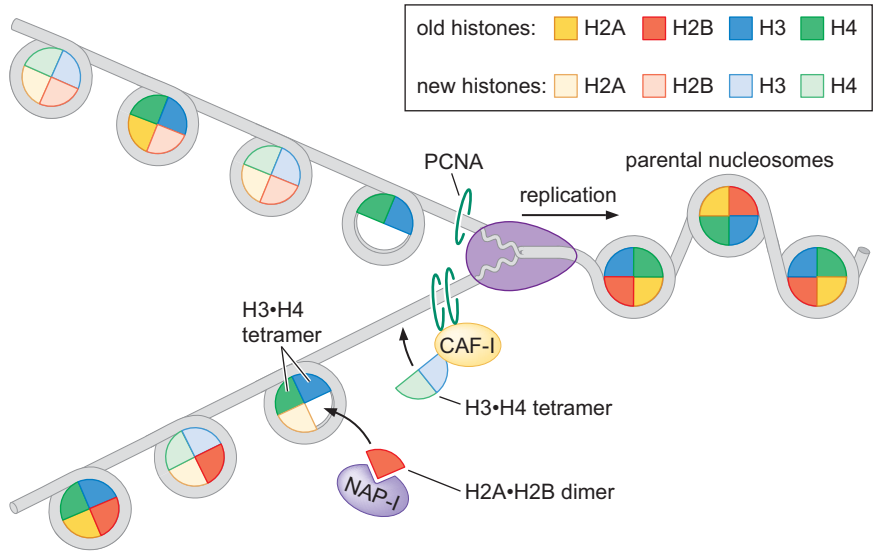


FIGURE 8-45 Chromatin assembly factors facilitate the assembly of nucleosomes. After the replication fork has passed, chromatin assembly factors chaperone free H3·H4 tetramers (e.g., CAF-I) and H2A·H2B dimers (NAP-I) to the site of newly replicated DNA. Once at the newly replicated DNA, these factors transfer their associated histone to the DNA. CAF-I is recruited to the newly replicated DNA by interactions with DNA sliding clamps. These ring-shaped, auxiliary replication factors encircle the DNA and are released from the replication machinery as the replication fork moves. For a more detailed description of DNA sliding clamps and their function in DNA replication, see Chapter 9.

SUMMARY

Within the cell, DNA is organized into large structures called chromosomes. Although the DNA forms the foundations for each chromosome, approximately half of each chromosome is composed of protein. Chromosomes can be either circular or linear; however, each cell has a characteristic number and composition of chromosomes. We now know the sequence of the entire genome of thousands of organisms. These sequences have revealed that the underlying DNA of each organism's chromosomes is used more or less efficiently to encode proteins. Simple organisms tend to use the majority of DNA to encode protein; however, more complex organisms use only a small portion of their DNA to encode proteins. The increased complexity of regulatory sequences, the appearance of introns, and the presence of additional regulatory RNAs (e.g., miRNAs) all contribute to the expansion of the noncoding regions of the genomes of more complex organisms.

Cells must carefully maintain their complement of chromosomes as they divide. Each chromosome must have DNA elements that direct chromosome maintenance during cell division. All chromosomes must have one or more origins of replication. In eukaryotic cells, centromeres play a critical role in the segregation of chromosomes, and telomeres help to protect and replicate the ends of linear chromosomes. Eukaryotic cells carefully separate the events that duplicate and segregate chromosomes as cell division proceeds. Chromosome segregation can occur in one of two ways. During mitosis, a highly specialized apparatus ensures that one copy of each duplicated chromosome is delivered to each daughter cell. During meiosis, an additional round of chromosome segregation (without DNA replication) reduces the number of chromosomes in the resulting daughter cells by half to generate haploid gametes.

The combination of eukaryotic DNA and its associated proteins is referred to as chromatin. The fundamental unit of chromatin is the nucleosome, which is made up of two copies each of the core histones (H2A, H2B, H3, and H4) and ~147 bp of DNA. This protein–DNA complex serves two important functions in the cell: it compacts the DNA to allow it to fit into the nucleus, and it restricts the accessibility of the DNA. This latter function is extensively exploited by cells to regulate many different DNA transactions including gene expression.

The atomic structure of the nucleosome shows that the DNA is wrapped about 1.7 times around the outside of a disc-shaped, histone protein core. The interactions between the DNA and the histones are extensive but uniformly base-nonspecific. The nature of these interactions explains both the bending of the DNA around the histone octamer and the ability of virtually all DNA sequences to be incorporated into a nucleosome. This structure also reveals the location of the amino-terminal tails of the histones and their role in directing the path of the DNA around the histones.

Once DNA is packaged into nucleosomes, it has the ability to form more complex structures that further compact the DNA. This process is facilitated by a fifth histone called H1. By binding the DNA both within and adjacent to the nucleosome, H1 causes the DNA to wrap more tightly around the octamer. A more compact form of chromatin, the 30-nm fiber, is readily formed by arrays of H1-bound nucleosomes. This structure is more repressive than DNA packaged into nucleosomes alone. The incorporation of DNA into this structure results in a dramatic reduction in its accessibility to the enzymes and proteins involved in transcription of the DNA.

The interaction of the DNA with the histones in the nucleosome is dynamic, allowing DNA-binding proteins intermittent access to the DNA. Nucleosome-remodeling complexes increase the accessibility of DNA incorporated into nucleosomes by increasing the mobility of nucleosomes. Two forms of mobility can be observed: sliding of the histone octamer along the DNA or complete release of the histone octamer from the DNA. In addition, these complexes facilitate the

exchange of H2A/H2B dimers. Nucleosome-remodeling complexes are recruited to particular regions of the genome to facilitate alterations in chromatin accessibility. A subset of nucleosomes is restricted to fixed sites in the genome and is said to be "positioned." Nucleosome positioning can be directed by DNA-binding proteins or particular DNA sequences.

Modification of the histone amino-terminal tails also alters the accessibility of chromatin. The types of modifications include acetylation and methylation of lysines, methylation of arginines, and phosphorylation of serines, threonines, and tyrosines. Acetylation of amino-terminal tails is frequently associated with regions of active gene expression and inhibits formation of the 30-nm fiber. Histone modifications alter the properties of the nucleosome itself, as well as acting as binding sites for proteins that influence the accessibility of the chromatin. In addition, these modifications recruit enzymes that perform the same modification, leading to similar modification of adjacent nucleosomes and facilitating the stable propagation of regions of modified nucleosomes/chromatin as the chromosomes are duplicated.

Nucleosomes are assembled immediately after the DNA is replicated, leaving little time during which the DNA is unpackaged. Assembly involves the function of specialized histone chaperones that escort the H3·H4 tetramers and H2A·H2B dimers to the replication fork. During the replication of the DNA, nucleosomes are transiently disassembled. Histone H3·H4 tetramers and H2A·H2B dimers are randomly distributed to one or the other daughter molecule. On average, each new DNA molecule receives half old and half new histones. Thus, both chromosomes inherit modified histones that can then act as "seeds" for the similar modification of adjacent histones.

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QUESTIONS

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For answers to even-numbered questions, see Appendix 2: Answers.

Question 1. List at least three properties that differ between the chromosome makeup in *E. coli* compared to human cells.

Question 2. Explain where the chromosomal DNA is located in prokaryotic versus eukaryotic cells.

Question 3. Does genome size correlate directly with organism complexity? Explain your reasoning.

Question 4. Intergenic sequences make up >60% of the human genome. Where do these intergenic sequences come from and what are some of their functions?

Question 5. Explain why each chromosome in a eukaryotic cell contains multiple origins of replication but includes one and only one centromere.

Question 6. How does the sister chromatid cohesion ensure that each daughter cell receives one copy of each chromosome?