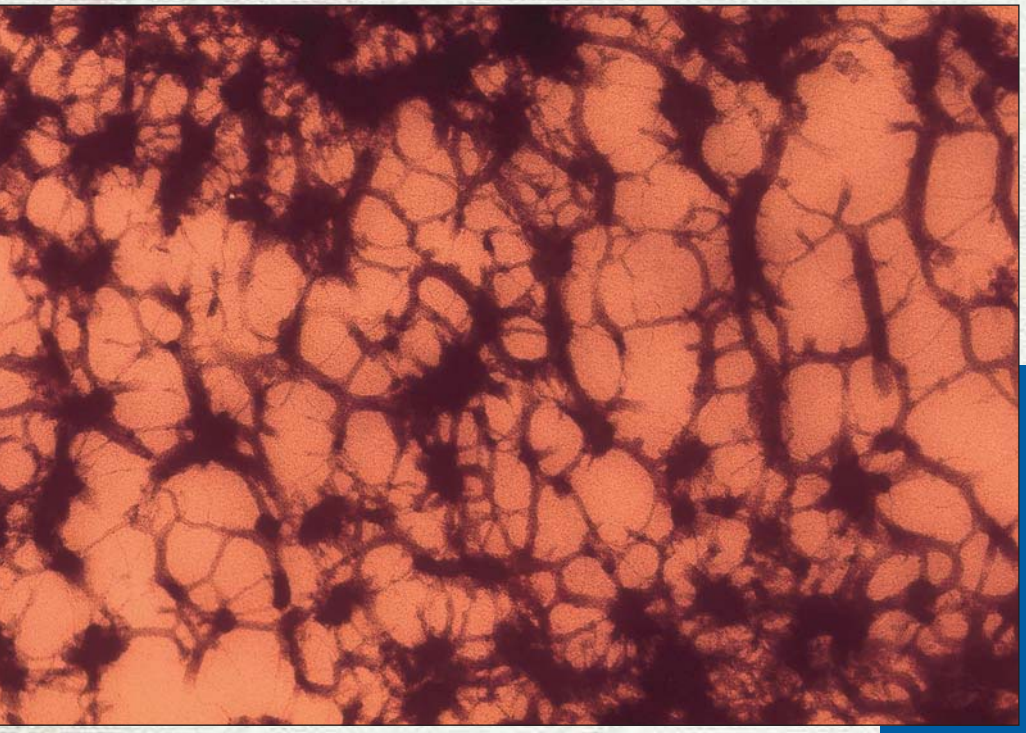
CHAPTER 13

Chromatin Structure and Its Effects on Transcription



Chromatin in developing human spermatid (×300,000).

In our discussion of transcription of eukaryotic genes, we have so far been ignoring an important point: Eukaryotic genes do not exist naturally as naked DNA molecules, or even as DNA molecules bound only to transcription factors. Instead, they are complexed with an equal mass of other proteins to form a substance known as chromatin. As we will see, the chemical nature of chromatin is variable, and these variations play an enormous role in chromatin structure and in the control of gene expression.

13.1 Chromatin Structure

Chromatin is composed of DNA and proteins, mostly basic proteins called **histones** that help chromatin fold so it can pack into the tiny volume of a cell's nucleus. In this section we will examine the structure of histones, and the role they play in folding chromatin. In a later section we will look at the roles histones play in modifying the structure of chromatin and in controlling transcription.

Histones

Most eukaryotic cells contain five different kinds of **histones: H1, H2A, H2B, H3,** and **H4.** These are extremely abundant proteins; the mass of histones in eukaryotic nuclei is equal to the mass of DNA. They are also unusually basic—at least 20% of their amino acids are arginine or lysine—and have a pronounced positive charge at neutral pH. For this reason, they can be extracted from cells with strong acids, such as 1.5 N HCl—conditions that would destroy most proteins. Also because of their basic nature, the histones migrate toward the cathode during nondenaturing electrophoresis, unlike most other proteins, which are acidic and therefore move toward the anode. Most of the histones are also well conserved from one organism to another. The most extreme example of this is histone H4. Cow histone H4 differs from pea H4 in only two amino acids out of a total of 102, and these are conservative changes—one basic amino acid (lysine) substituted for another (arginine), and one hydrophobic amino acid (valine) substituted for another (isoleucine). In other words, in the more than one billion years since the cow and pea lines have diverged from a common ancestor, only two amino acids in histone H4 have changed. Histone H3 is also extremely well conserved; histones H2A and H2B are moderately well conserved; but histone H1 varies considerably among organisms. Table 13.1 lists some of the characteristics of histones.

Low-resolution gel electrophoresis of the histones gives the impression that each histone is a homogeneous species. However, higher resolution separations of the histones have revealed much greater variety. This variety stems from two sources: gene reiteration and posttranslational modification. The histone genes are not single-copy genes like most protein-encoding genes in eukaryotes. Instead, they are repeated many times: 10–20 times in the mouse, and about 100 times in *Drosophila*. Many of these copies are identical, but some are quite different. Histone H1 (the lysine-rich histone) shows the greatest variation, with at least six subspecies in the mouse. One H1 variant is called H1°. Birds, fish, amphibians, and reptiles have another lysine-rich histone that could be an extreme variant of H1, but it is so different from H1 that it is generally called by a distinct name, H5. Histone H4 shows the least variation; only two variant species have ever been reported, and these are rare. It is assumed that the variant species of a given histone all play essentially the same role, but each may influence the properties of chromatin somewhat differently.

The second cause of histone heterogeneity, posttranslational modification, is an exceedingly rich source of variation. The most common histone modification is acetylation, which can occur on N-terminal amino groups and on lysine ε-amino groups. Other modifications include lysine ε-amino methylation and phosphorylation, including serine and threonine O-phosphorylation. These and other histone modifications are summarized in Table 13.2. These modifications are dynamic processes, so modifying groups can be removed as well as added. These histone modifications influence chromatin structure and function, and play important roles in governing gene activity. We will discuss this phenomenon later in this chapter.

Table 13.1 General Properties of the Histones

Histone Type	Histone	Molecular Mass (M_r)
Core histones	H3	15,400
	H4	11,340
	H2A	14,000
	H2B	13,770
Linker histones	H1	21,500
	H1°	~21,500
	H5	21,500

Source: Adapted from Critical Reviews in Biochemistry and Molecular Biology, by Butler, P.J.C., 1983. Taylor & Francis Group. LLC., http://www.taylorandfrancis.com

Table 13.2 Histone Modifications

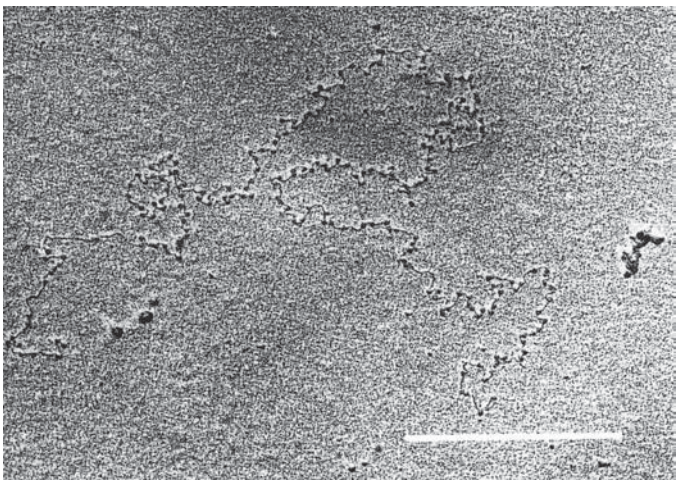
Modification	Amino Acids Modified
Acetylation (ac)	Lysine
Methylation (me)	Lysine (mono-, di-, or tri-me)
Methylation	Arginine (mono- or di-me[symmetric and asymmetric])
Phosphorylation	Serine and threonine
Ubiquitylation	Lysine
Sumoylation	Lysine
ADP ribosylation	Glutamate
Deimination	Arginine → Citrulline
Proline isomerization	Proline (cis → trans)

Nucleosomes

The length-to-width ratio of a typical human chromosome is more than 10 million to one. Such a long, thin molecule would tend to get tangled if it were not folded somehow. Another way of considering the folding problem is that the total length of human DNA, if stretched out, would be about 2 m, and this all has to fit into a nucleus only about 10 μm in diameter. In fact, if you laid all the DNA molecules in your body end to end, they would reach to the sun and back hundreds of times. Obviously, a great deal of DNA folding must occur in your body and in all other living things. We will see that eukaryotic chromatin is indeed folded in several ways. The first order of folding involves structures called **nucleosomes,** which have a core of histones, around which the DNA winds.

Maurice Wilkins showed as early as 1956 that x-ray diffraction patterns of DNA in intact nuclei exhibited sharp bands, indicating a repeating structure larger than the double helix itself. Subsequent x-ray diffraction work by Aaron Klug, Roger Kornberg, Francis Crick, and others showed a strong repeat at intervals of approximately 100 Å. This corresponds to a string of nucleosomes, which are about 110 Å in diameter. Kornberg found in 1974 that he could chemically cross-link histones H3 and H4, or histones H2A and H2B in solution. Moreover, he found that H3 and H4 exist as a tetramer $(H3–H4)_2$ in solution. He also noted that chromatin is composed of roughly equal masses of histones and DNA. In addition, the concentration of histone H1 is about half that of the other histones. This corresponds to one histone octamer (two molecules each of H2A, H2B, H3, and H4) plus one molecule of histone H1 per 200 bp of DNA. Finally, he reconstituted chromatin from H3–H4 tetramers, H2A–H2B oligomers, and DNA and found that this reconstituted chromatin produced the same x-ray diffraction pattern as natural chromatin. Several workers, including Gary Felsenfeld and L.A. Burgoyne, had already shown that chromatin cut with a variety of nucleases yielded DNA fragments about 200 bp long. Based on all these data, Kornberg proposed a repeating structure of chromatin composed of the histone octamer plus one molecule of histone H1 complexed with about 200 bp of DNA.

G.P. Georgiev and coworkers discovered that histone H1 is much easier than the other four histones to remove from chromatin. In 1975, Pierre Chambon and colleagues took advantage of this phenomenon to selectively remove histone H1 from chromatin with trypsin or high salt buffers, and found that this procedure yielded chromatin with a "beads-on-a-string" appearance (Figure 13.1a). They named the beads **nucleosomes.** Figure 13.1b shows some of the nucleosomes that Chambon and coworkers purified from chicken red blood cells, using micrococcal nuclease to cut the DNA string between the beads.



(a)

(b)

Figure 13.1 Early electron micrographs of nucleosomes. **(a)** Nucleosome strings. Chambon and colleagues used trypsin to remove histone H1 from chromatin isolated from chicken red blood cells, revealing a beads-on-a-string structure. The bar represents 500 nm. **(b)** Isolated nucleosomes. Chambon's group used micrococcal nuclease to cut between nucleosomes, then isolated these particles by ultracentrifugation. The arrows point to two representative nucleosomes. The bar represents 250 nm. (*Source:* Oudet P., M. Gross-Bellarard, and P. Chanaban, Electron microscopic and biochemical evidence that chromatin structure is a repeating unit. *Cell* 4 (1975), f. 4b & 5, pp. 286–87. Reprinted by permission of Elsevier Science.)

J.P. Baldwin and colleagues subjected chromatin to neutron-scattering analysis, which is similar to x-ray diffraction, but uses a beam of neutrons instead of x-rays. The pattern of scattering of the neutrons by the sample gives clues to the three-dimensional structure of the molecules in the sample. These investigators found a ring of scattered neutrons corresponding to a repeat distance of about 105 Å, which agreed with the x-ray diffraction analysis. Moreover, the overall pattern suggested that the protein and DNA occupied separate regions within the nucleosomes. Based on these data, Baldwin and coworkers proposed that the core histones (H2A, H2B,

H3, and H4) form a ball, with the DNA wrapped around the outside. Having the DNA on the outside also has the advantage that it minimizes the amount of bending the DNA would have to do. In fact, double-stranded DNA is such a stiff structure that it could not bend tightly enough to fit inside a nucleosome. These workers also placed histone H1 on the outside, in accord with its ease of removal from chromatin. In fact, H1 binds to the linker DNA between nucleosomes, which is why it is called a **linker histone**.

Several research groups have used x-ray crystallography to determine a structure for the histone octamer. According to the work of Evangelos Moudrianakis and his colleagues in 1991, the octamer takes on different shapes when viewed from different directions, but most viewpoints reveal a three-part architecture. This tripartite structure contains a central $(H3–H4)_2$ core attached to two H2A–H2B dimers, as shown in Figure 13.2a and b. The overall structure is shaped roughly like a disc, or hockey puck, that has been worn down to a wedge shape. Notice that this structure is consistent with Kornberg's data on the association between histones in solution and with the fact that the histone octamer dissociates into an $(H3–H4)_2$ tetramer and two H2A–H2B dimers.

Where does the DNA fit in? It was not possible to tell from these data, because the crystals did not include DNA. However, grooves on the surface of the proposed octamer defined a left-handed helical ramp that could provide a path for the DNA (Figure 13.2c). In 1997, Timothy Richmond and colleagues succeeded in crystallizing a nucleosomal core particle that did include DNA. The nucleosome, as originally defined, contained about 200 bp of DNA. This is the length of DNA released by subjecting chromatin to a mild nuclease treatment. However, exhaustive digestion with nuclease gives a **core nucleosome** with 146 bp of DNA and the histone octamer containing all four **core histones** (H2A, H2B, H3, and H4), but no histone H1, which is relatively easily removed because it binds to the

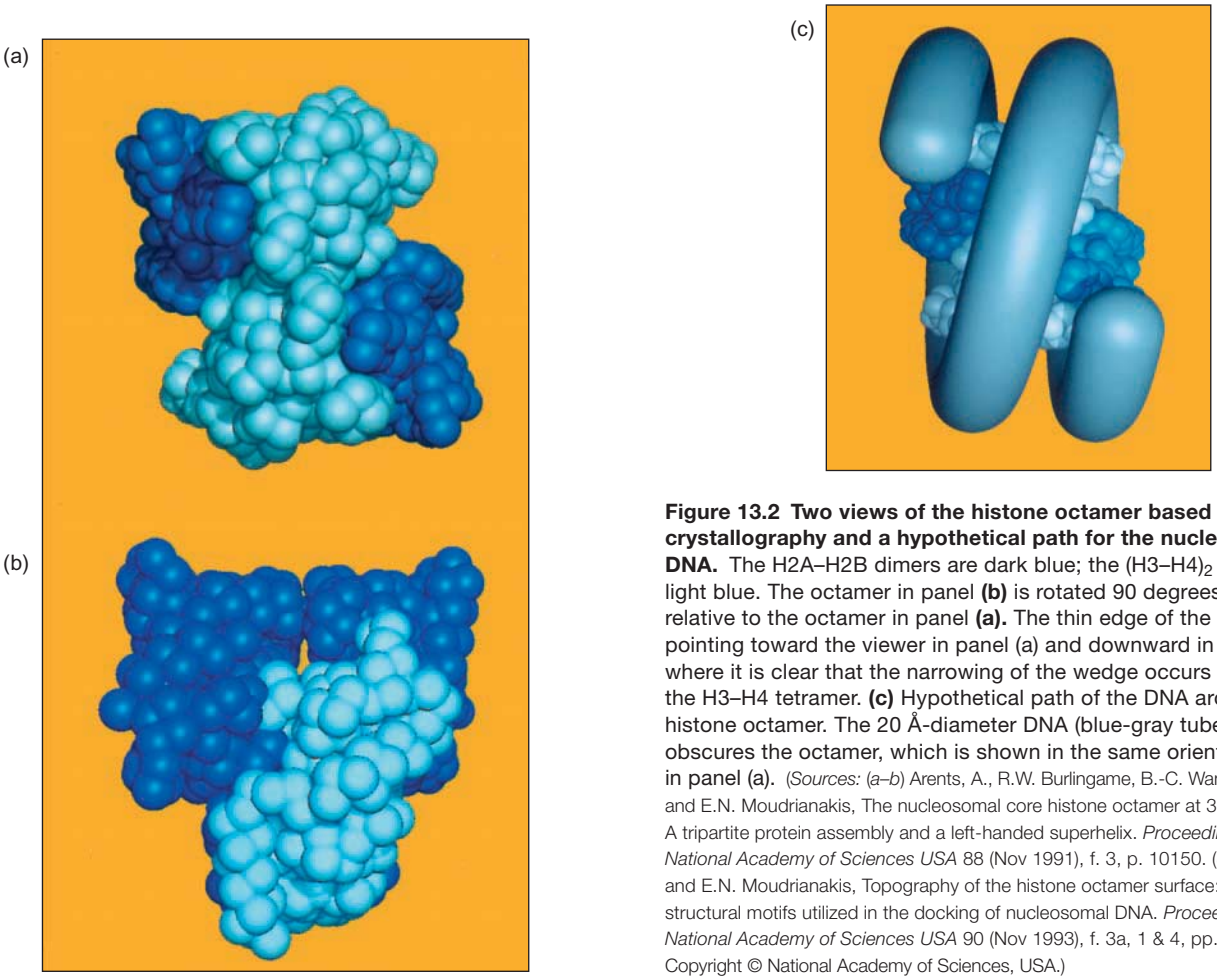
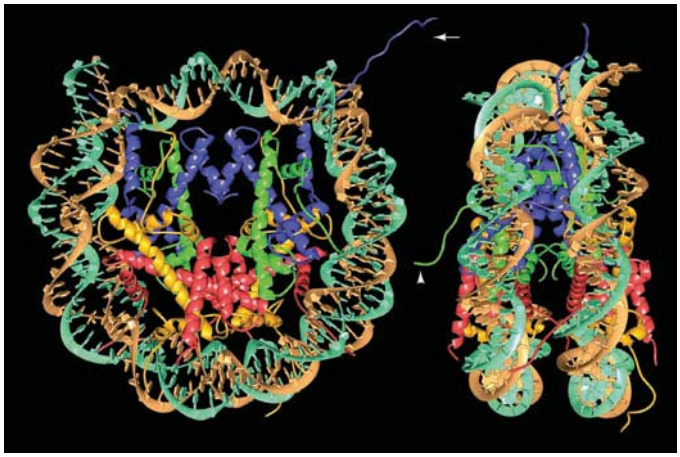
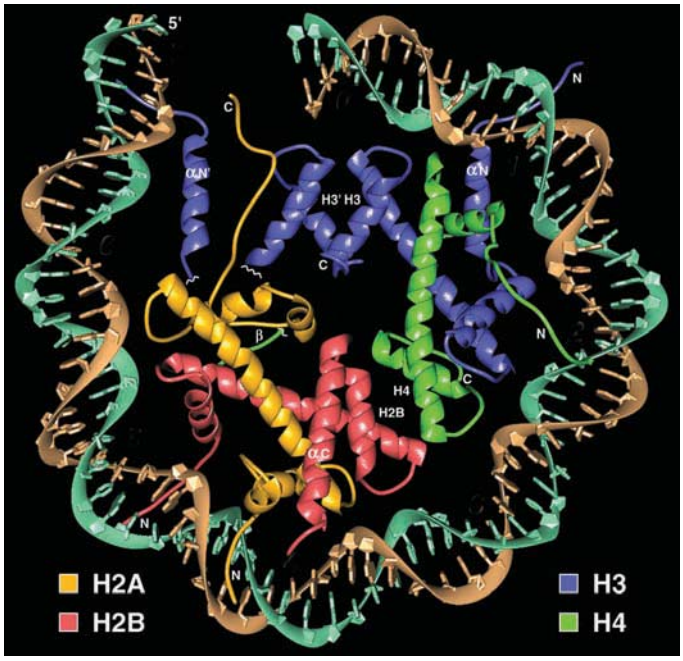


Figure 13.2 Two views of the histone octamer based on x-ray crystallography and a hypothetical path for the nucleosomal DNA. The H2A–H2B dimers are dark blue; the $(H3–H4)_2$ tetramer is light blue. The octamer in panel **(b)** is rotated 90 degrees downward relative to the octamer in panel **(a).** The thin edge of the wedge is pointing toward the viewer in panel (a) and downward in panel (b), where it is clear that the narrowing of the wedge occurs primarily in the H3–H4 tetramer. **(c)** Hypothetical path of the DNA around the histone octamer. The 20 Å-diameter DNA (blue-gray tube) nearly obscures the octamer, which is shown in the same orientation as in panel (a). (*Sources:* (*a–b*) Arents, A., R.W. Burlingame, B.-C. Wang, W.B. Love, and E.N. Moudrianakis, The nucleosomal core histone octamer at 3.1Å resolution: A tripartite protein assembly and a left-handed superhelix. *Proceedings of the National Academy of Sciences USA* 88 (Nov 1991), f. 3, p. 10150. (*c*) Arents, A. and E.N. Moudrianakis, Topography of the histone octamer surface: Repeating structural motifs utilized in the docking of nucleosomal DNA. *Proceedings of the National Academy of Sciences USA* 90 (Nov 1993), f. 3a, 1 & 4, pp. 10490–91.



(a)



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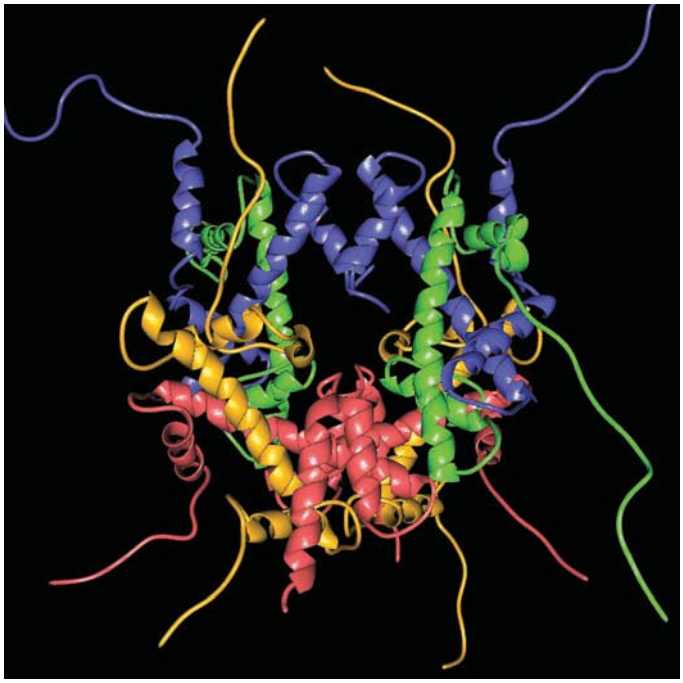


(c)

Figure 13.3 Crystal structure of a nucleosomal core particle. Richmond and colleagues crystallized a core particle composed of a 146-bp DNA and cloned core histones, then determined its crystal structure. **(a)** Two views of the core particle, seen face-on (left) and edge-on (right). The DNA on the outside is rendered in tan and green. The core histones are rendered as follows: H2A, yellow; H2B, red; H3, purple; and H4, green. Note the H3 tail (arrow) extending through a cleft between the minor grooves of the two adjacent turns of the DNA around the core particle. **(b)** Half of the core particle, showing 73 bp of DNA plus at least one molecule each of the core histones. **(c)** Core particle with DNA removed. (*Sources:* (*a–b*) Luger, K., A.W. Mäder, R.K. Richmond, D.F. Sargent, and T.J. Richmond, Crystal structure of the nucleosome core particle at 2.8 Å Resolution. *Nature* 389 (18 Sep 1997) f. 1, p. 252. Copyright © Macmillan Magazines Ltd. (*c*) Rhodes, D., Chromatin structure: The nucleosome core all wrapped up. *Nature* 389 (18 Sep 1997) f. 2, p. 233. Copyright © Macmillan Magazines Ltd.)

linker DNA outside the nucleosome, and this linker DNA is digested by the nuclease.

Figure 13.3 depicts the core nucleosome structure determined by Richmond and colleagues. We can see the DNA winding almost twice around the core histones. We can also see the H3–H4 tetramer near the top and the two H2A–H2B dimers near the bottom. This arrangement is particularly obvious on the right in panel a. The architecture of the histones themselves is interesting. All of the core histones contain the same fundamental **histone fold,** which consists of three α-helices linked by two loops. All of them also contain extended tails that make up about 28% of the mass of the core histones. Because the tails are relatively unstructured, the crystal structure does not include most of their length. The tails are especially evident with the DNA removed in panel c. The tails of H2B and H3 pass out of the core particle through a cleft formed from two adjacent DNA minor grooves (see the long purple tail at the top of the left part of panel a). One of the H4 tails is exposed to the side of the core particle (see the right part of panel a). This tail is rich in basic residues and can interact strongly with an acidic region of an H2A–H2B dimer in an adjacent nucleosome. Such interactions may play a role in nucleosome cross-linking, which we will discuss later in this chapter.

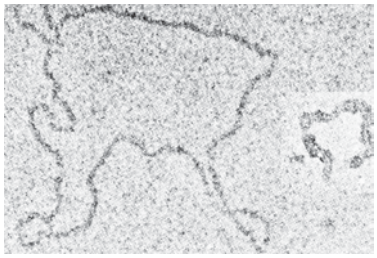


Figure 13.4 Condensation of DNA in nucleosomes. Deproteinized SV40 DNA is shown next to an SV40 minichromosome (inset, right) in electron micrographs enlarged to the same scale. The condensation of DNA afforded by nucleosome formation is apparent. (*Source:* Griffith, J., Chromatin structure: Deduced from a minichromosome. *Science* 187:1202 (28 March 1975). Copyright © AAAS.)

This and other models of the nucleosome indicate that the DNA winds about 1.65 times around the core, condensing the length of the DNA by a factor of 6 to 7. Jack Griffith also observed this magnitude of condensation in his 1975 study of the SV40 minichromosome. Because SV40 DNA replicates in mammalian nuclei, it is exposed to mammalian histones, and therefore forms typical nucleosomes. Figure 13.4 shows two views of the SV40 DNA. The main panel shows the DNA after all protein has been stripped off. The inset shows the minichromosome with all its protein—at the same scale. The reason the minichromosome looks so much smaller is that the DNA is condensed by winding around the histone cores in the nucleosomes.

SUMMARY Eukaryotic DNA combines with basic protein molecules called histones to form structures known as nucleosomes. These structures contain four pairs of core histones (H2A, H2B, H3, and H4) in a wedge-shaped disc, around which is wrapped a stretch of about 146 bp of DNA. Histone H1 is more easily removed from chromatin than the core histones and is not part of the core nucleosome.

The 30-nm Fiber

After the string of nucleosomes, the next order of chromatin folding produces a fiber about 30 nm in diameter. Until 2005, it had not been possible to crystallize any component of chromatin larger than the nucleosome core, so researchers had to rely on lower-resolution methods such as electron microscopy (EM) to investigate higher-order chromatin structure. Figure 13.5 depicts the results of an EM study that shows how the string of nucleosomes condenses to form the 30-nm fiber at increasing ionic strength. The degree of this condensation is another six- to sevenfold in addition to the approximately six- to sevenfold condensation in the nucleosome itself.

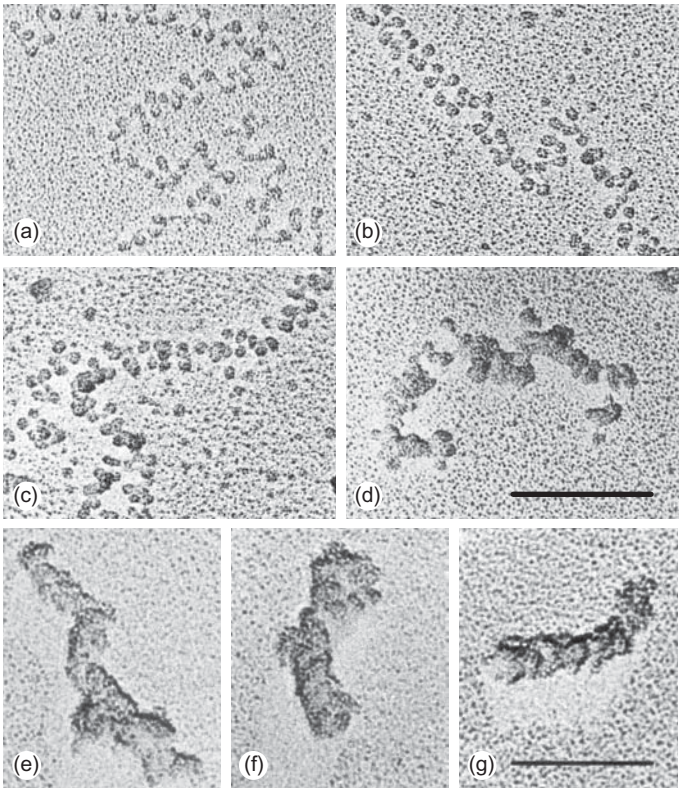


Figure 13.5 Condensation of chromatin on raising the ionic strength. Klug and colleagues subjected rat liver chromatin to buffers of increasing ionic strength, during fixation for electron microscopy. Panels **(a)–(c)** were at low ionic strength, panel **(d)** at moderate ionic strength, and panels **(e)–(g)** at high ionic strength. More specifically, the fixing conditions in each panel were the following, plus 0.2 mM EDTA in each case: (a) 1 mM triethylamine hydrochloride (TEACl); (b and c) 5 mM TEACl; (d) 40 mM NaCl, 5 mM TEACl; (e)–(g) 100 mM NaCl, 5 mM TEACl. The bars represent 100 nm. (*Source:* Thoma, F., T. Koller, and A. Klug, Involvement of histone H1 in the organization of the nucleosome and of the salt-dependent superstructures of chromatin. *Journal of Cell Biology* 83 (1979) f. 4, p. 408. Copyright © Rockefeller University Press.)

What is the structure of the 30-nm fiber? This question has vexed molecular biologists for decades. In 1976, Aaron Klug and his colleagues, on the basis of electron microscopy and small angle x-ray scattering data, proposed a **solenoid** model (Figure 13.6), in which the nucleosomes were arranged in a hollow, compact helix (Greek: *solen* = pipe). But others, not convinced by the data behind the solenoid model, proposed various other schemes: a zigzag ribbon of nucleosomes; a superbead, with relatively disordered nucleosomes; an irregular, open helical arrangement of nucleosomes; and a two-start helix, in which the linker DNA between nucleosomes zigzags back and forth between two helical arrangements of stacked nucleosomes, such that one helix contains the odd-numbered nucleosomes and the other contains the even-numbered ones.

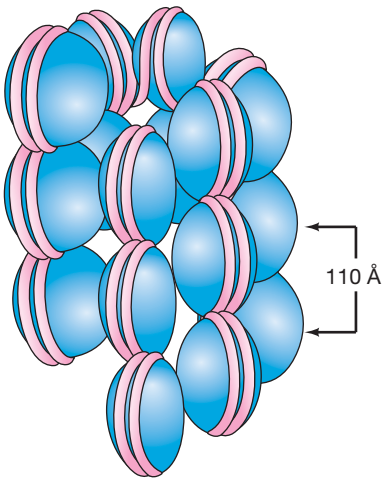


Figure 13.6 The solenoid model of chromatin folding. A string of nucleosomes coils into a hollow tube, or solenoid. Each nucleosome is represented by a blue cylinder with DNA (pink) coiled around it. For simplicity, the solenoid is drawn with six nucleosomes per turn and the nucleosomes parallel to the solenoid axis.
Source: Adapted from Widom, J. and A. Klug. Structure of the 300 Å chromatin filament: X-ray diffraction from oriented samples. Cell 43:210, 1985.

To resolve this long-standing controversy, higher-resolution structural data were needed. Finally, in 2005, Richmond and colleagues achieved a breakthrough by reporting the x-ray crystal structure of a tetranucleosome, or string of four nucleosomes. The resolution of this structure was not very high, only 9 Å, but it was good enough that the high resolution structure of an individual nucleosome could be incorporated. Figure 13.7 illustrates the structure of the tetranucleosome. Panel (a) of this figure starts with a string of nucleosomes, which is constrained only by the number of turns the DNA duplex makes around each nucleosome, and the length of the linker DNA between nucleosomes. One could wind the linker DNA in such a way as to stack the nucleosomes on top of each other. Or one could keep the zigzag arrangement and form two stacks, each containing every-other nucleosome, as shown in panel (b).

In fact, this zigzag arrangement is supported by the crystal structure of the tetranucleosome. This representation of the tetranucleosome structure is complex. The schematic in panel (a) helps interpret it, but it is best viewed in three dimensions. You can do this with a video, using this link:

http://www.nature.com/nature/journal/v436/n7047/suppinfo/nature03686.html

As the video runs, the structure rotates so you can see the connections among all the nucleosomes, which are represented by their DNA only, and appreciate the zigzag nature of the structure.

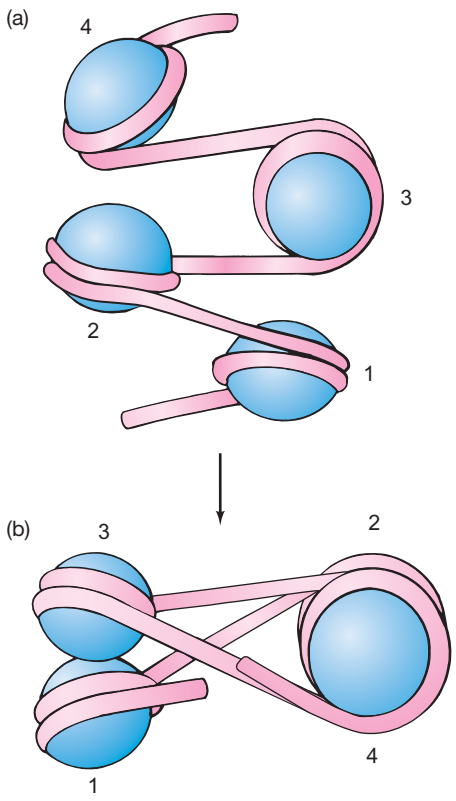


Figure 13.7 Structure of a tetranucleosome. (a) Diagrams of tetranucleosomes in two conformations. **(a)** A hypothetical conformation constrained only by the known degree of winding of DNA around the histone cores. **(b)** The conformation determined by x-ray crystallography. The nucleosomes form two stacks, and the linker DNA zigzags back and forth between nucleosomes in the two stacks. Consequently, consecutive nucleosomes are no longer nearest neighbors. Instead, alternate nucleosomes are nearest neighbors.
(*Source:* Adapted from Woodcock, C.L. *Nature Structural & Molecular Biology* 12, 2005, 1, p. 639.)

The zigzag structure has important implications for the overall structure of chromatin. It is incompatible with most of the previous suggestions, including the solenoid model. But it is consistent with the crossed-linker, two-start helix, in which each of the two stacks of nucleosomes forms a left-handed helix. The exact nature of this double helix of polynucleosomes is not clarified by the tetranucleosome structure, but Richmond and colleagues speculated as follows. First, they built a "direct" model by essentially stacking tetranucleosomes on top of each other. But this led to intolerable steric interference between neighboring tetranucleosomes, so the authors built an "idealized" model by equalizing the angles between each pair of nucleosomes in a stack. This procedure distorted the angles between nucleosomes seen in the tetranucleosome structure, but it avoided steric interference and generated a reasonable model, as illustrated in Figure 13.8. The two helices of polynucleosomes are apparent in this structure, and the zigzags of linker DNA can even be seen between some of the nucleosomes in the two helices.

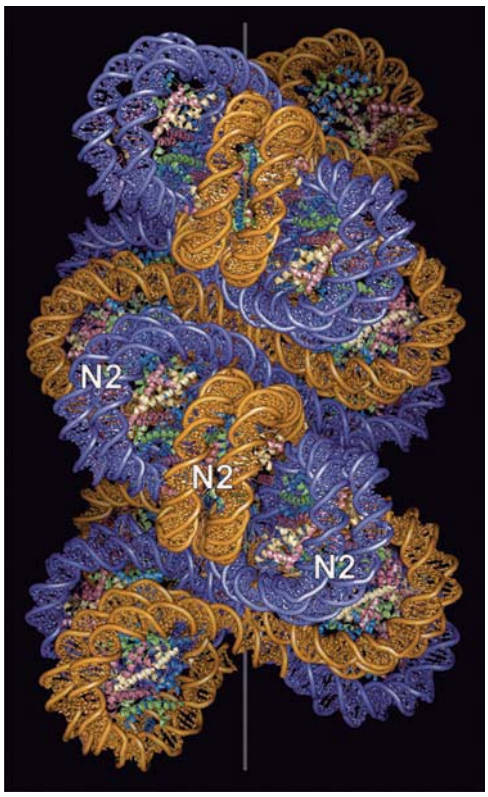


Figure 13.8 A model for the 30-nm fiber. Richmond and colleagues built this "idealized" model based on the tetranucleosome structure. It is arranged so that the dyad axis of each nucleosome (a line through the middle of the nucleosome, between the two coils of DNA) is perpendicular to the axis of the 30-nm fiber (gray vertical line). Also, the angles between any two adjacent nucleosomes are equal. (*Source:* Reprinted by permission from Macmillan Publishers Ltd: *Nature,* 436, 138–141, Thomas Schalch, Sylwia Duda, David F. Sargent and Timothy J. Richmond, "X-ray structure of a tetranucleosome and its implications for the chromatin fibre," fig. 3, p. 140, copyright 2005.)

Two of the models for the 30-nm fiber—the solenoid and the two-start double helix—have considerable experimental support, but which is the right one? In 2009, John van Noort and colleagues presented data that suggested that both models may be right. They proposed that the structure of the 30-nm fiber may depend on the exact nature of the chromatin, and in particular on the **nucleosome repeat length (NRL).** This length of the DNA from the beginning of one nucleosome to the beginning of the next varies between about 165 bp and 212 bp in vivo, but most chromatin has an NRL of about 188 or 196. Chromatin of this type is generally transcriptionally inactive and associated with a linker histone such as H1. A smaller proportion of chromatin has an NRL of 167, tends to be transcriptionally active, and lacks a linker histone. Could it be that one type of chromatin forms one kind of 30-nm fiber, and the other type forms the other?

To answer this question, van Noort and colleagues used a technique called **single-molecule force spectroscopy.** In this method, as applied to chromatin, the experimenter links one end of a 30-nm chromatin fiber to a glass slide, and the other end to a magnetic bead. Then, by applying an attractive magnetic force to the bead, one can stretch the chromatin and note the degree of stretching produced by a given force. One would predict that the simple helical solenoid would be easier to stretch than the two-start double helix.

Indeed, van Noort and colleagues found that chromatin containing 25 nucleosomes with the longer NRL (197 bp) stretches more readily than chromatin containing 25 nucleosomes with the shorter NRL (167 bp). In addition, they found that linker histones did not affect the length or stretchability of the chromatin, but they did stabilize the folding of the chromatin. Thus, it is possible that most of the chromatin in a cell (presumably the inactive fraction) adopts a solenoid shape for its 30-nm fiber, while a minor fraction (at least potentially active) forms a 30-nm fiber according to the two-start double helical model. It is interesting in this regard that Richmond and colleagues, in forming their tetranucleosomes for x-ray crystallography, used an NRL of 167, and found a two-start double helical structure. Such chromatin has also been shown by van Noort and colleagues to conform to the two-start double helical model.

Some have even questioned whether the 30-nm fiber exists in vivo at all. It is well documented in vitro, but has never been visualized in intact nuclei. There are several ways to explain this inability to find the 30-nm fiber in vivo. First, as unlikely as this may seem, it may not exist in vivo. But there are other possibilities: It may exist, but is not seen because higher-order chromatin folding obscures it. Or it may simply be that our tools for visualizing chromatin in intact nuclei are not adequate to detect the 30-nm fiber.

SUMMARY A string of nucleosomes folds into a 30-nm fiber in vitro, and presumably also in vivo. structural studies suggest that the 30-nm chromatin fiber in the nucleus exists in at least two forms: inactive chromatin tends to have a high nucleosome repeat length (about 197 bp) and favors a solenoid folding structure. This kind of chromatin interacts with histone h1, which helps to stabilize its structure. Active chromatin tends to have a low nucleosome repeat length (about 167 bp) and folds according to the two-start double helical model.

Higher-Order Chromatin Folding

The 30-nm fiber probably accounts for most of the chromatin in a typical interphase nucleus, but further

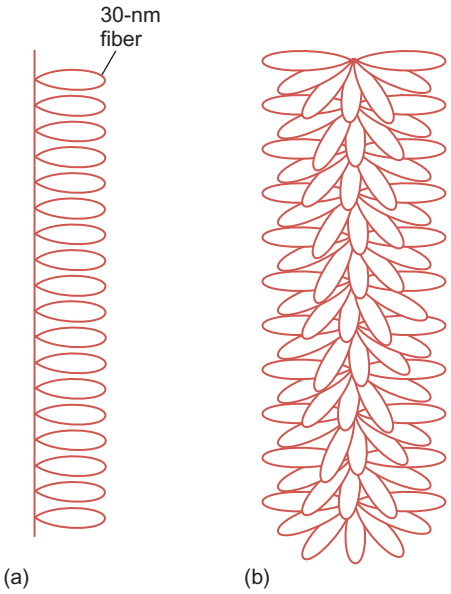


Figure 13.9 Radial loop models of chromatin folding. (a) This is only a partial model, showing some of the loops of chromatin attached to a central scaffold; of course, all the loops are composed of the same continuous 30-nm fiber. **(b)** A more complete model, showing how the loops are arranged in three dimensions around the central scaffold. (*Source:* Adapted from Marsden, M.P.F. and U.K. Laemmli, Metaphase chromosome structure: Evidence of a radial loop model. *Cell* 17:856, 1979.)

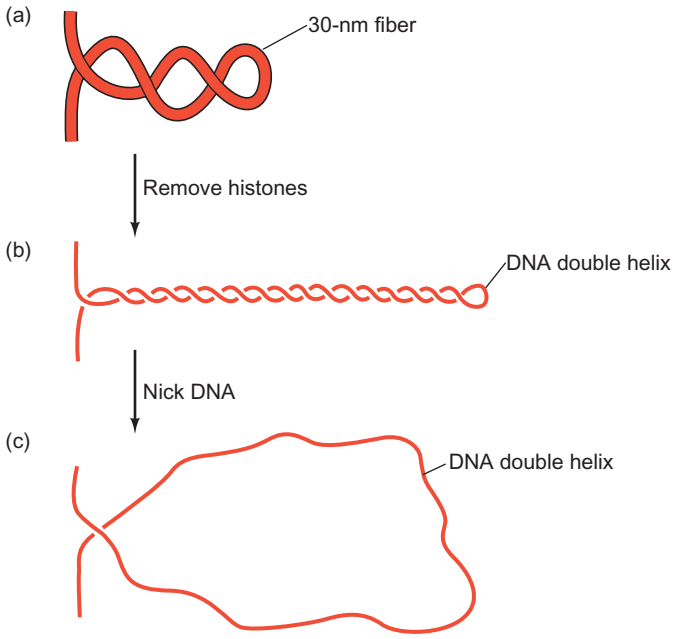


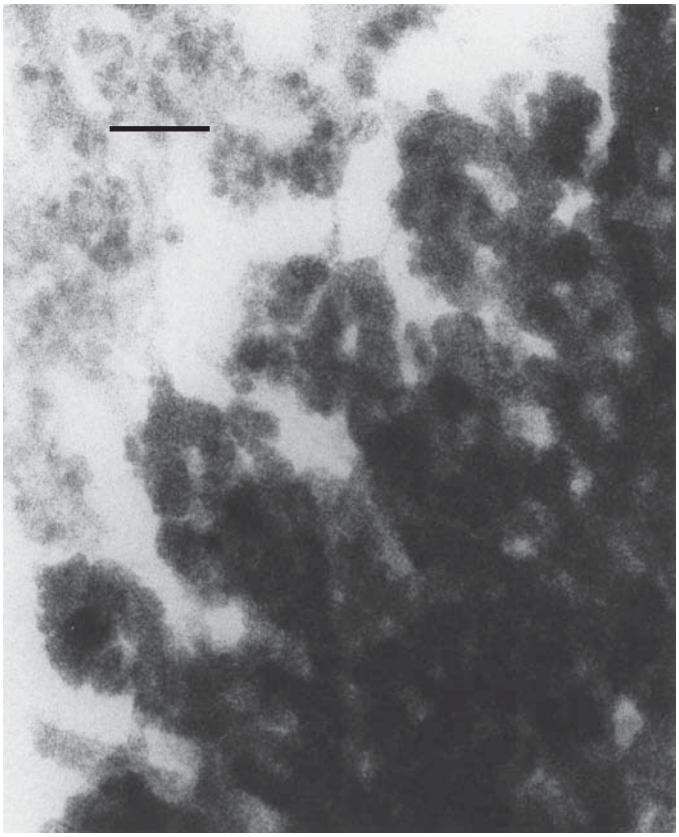
Figure 13.10 Relaxing supercoiling in chromatin loops. (a) A hypothetical chromatin loop composed of the 30-nm fiber, with some superhelical turns. **(b)** The chromatin loop with histones removed. Without histones, the nucleosomes and 30-nm fiber have disappeared, leaving a supercoiled DNA duplex. Note that the helical turns here are superhelices, not ordinary turns in a DNA double helix. **(c)** A relaxed chromatin loop. The DNA has been nicked to relax the superhelix. Now we see a relaxed DNA double helix that forms a loop. With each step from (a) to (c), the apparent length of the loop increases, but these increases are not drawn to scale.

orders of folding are clearly needed, especially in mitotic chromosomes, which have condensed so much that they become visible with a light microscope. The favorite model for the next order of condensation is a series of radial loops, as pictured in Figure 13.9. Cheeptip Benyajati and Abraham Worcel produced the first evidence in support of this model in 1976 when they subjected *Drosophila* chromatin to mild digestion with DNase I, then measured the sedimentation coefficients of the digested chromatin. They found that the coefficients decreased gradually with digestion, then reached a plateau value. Worcel had previously shown that the *E. coli* nucleoid (the DNA-containing complex) exhibited similar behavior, which was caused by the introduction of nicks into more and more superhelical loops of the bacterial DNA. As each loop was nicked once, it relaxed to an open circular form and slightly decreased the sedimentation coefficient of the whole complex. But eukaryotic chromosomes are linear, so how can the DNA in them be supercoiled? If the chromatin fiber is looped as it is in *E. coli* and held fast at the base of each loop, then each loop would be the functional equivalent of a circle and could be supercoiled. Indeed, the winding of DNA in the nucleosomes would provide the strain necessary for supercoiling. Figure 13.10 illustrates this concept and shows how relaxation of a supercoiled loop gives much less compact chromatin in that region, which would reduce the sedimentation coefficient.

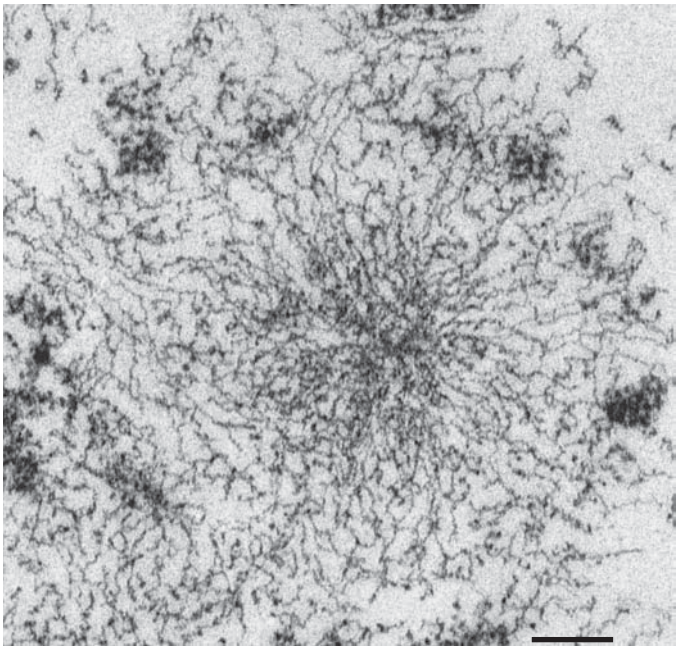
How big are the loops? Worcel calculated that each loop in a *Drosophila* chromosome contains about 85 kb, but other investigators, working with vertebrate species and using a variety of techniques, have made estimates ranging from 35 to 83 kb.

The images of chromosomes in Figure 13.11 also support the loop idea. Figure 13.11a shows the edge of a human metaphase chromosome, with loops clearly visible. Figure 13.11b depicts a cross section of a swollen human chromosome in which the 30-nm fiber is preserved. Radial loops are clearly visible. Figure 13.11c shows part of a deproteinized human chromosome. Loops of DNA are anchored to a central scaffold in the skeleton of the chromosome. All these pictures strongly support the notion of a radially looped fiber in chromosomes.

SUMMARY Sedimentation and EM studies have revealed a radial loop structure in eukaryotic chromosomes. The 30-nm fiber seems to form loops between 35 and 85 kb long, anchored to the central matrix of the chromosome.



(a)



(b)

(c)

Figure 13.11 Three views of loops in human chromosomes. **(a)** Scanning transmission electron micrograph of the edge of a human chromosome isolated with hexylene glycol. Bar represents 100 nm. **(b)** Transmission electron micrograph of cross sections of human chromosomes swollen with EDTA. The chromatin fiber visible here is the 30-nm nucleosome fiber. Bar represents 200 nm. **(c)** Transmission electron micrograph of a deproteinized human chromosome showing DNA loops emanating from a central scaffold. Bar represents 2 μm (2000 nm). (*Sources:* (*a*) Marsden, M.P.F. and U.K. Laemmli, Metaphase chromosome structure: Evidence for a radial loop model. *Cell* 17 (Aug 1979) f. 5, p. 855. Reprinted by permission of Elsevier Science. (*b*) Marsden and Laemmli, *Cell* 17 (Aug 1979) f. 1, p. 851. Reprinted by permission of Elsevier Science. (*c*) Paulson, J.R. and U.K. Laemmli, The structure of histone-depleted metaphase chromosomes. *Cell* 12 (1977) f. 5, p. 823. Reprinted by permission of Elsevier Science.)

13.3 Chromatin Structure and Gene Activity

Enthusiasm for histones as important regulators of gene activity has been inconsistent. When it first became clear that histones could turn off transcription when added to DNA in vitro, molecular biologists got excited. Then, when the role of histones in chromatin structure was elucidated, most investigators tended to focus on this structural role and forget about histones as regulators of genetic activity. Histones were then viewed as mere scaffolding for the DNA. Now we have come full circle and molecular biologists are elucidating the regulatory functions of histones.

The Effects of Histones on Transcription of Class II Genes

In the 1980s, Donald Brown and his colleagues showed that the 5S rRNA genes (class III genes) of *Xenopus laevis* can be selectively repressed in vitro by addition of histone H1, and that this repression increased dramatically as the level of histone H1 reached one molecule per 200 bp of DNA, its natural level in chromatin. In the 1990s, James Kadonaga and his colleagues showed that the same principles concerning the interactions between histones and class III genes also apply to histones and class II genes.

Core Histones In 1991, Paul Laybourne and Kadonaga performed a detailed study to distinguish between the effects of the core histones and of histone H1 on transcription by RNA polymerase II in vitro. They found that the core histones (H2A, H2B, H3, and H4) formed core nucleosomes with cloned DNA and caused a mild repression (about fourfold) of genetic activity. Transcription factors had no effect on this repression. When they added histone H1, in addition to the core histones, the repression became much more profound: 25- to 100-fold. This repression *could* be blocked by activators. In this respect, these factors resembled the class III factors (presumably TFIIIA, B, and C), which could compete with histone H1 for the control region of the *Xenopus* 5S rRNA gene.

Laybourne and Kadonaga's experimental strategy was to reconstitute chromatin from plasmid DNA containing a well-defined cloned gene, and histones in the presence or absence of activators that were known to affect transcription of the cloned gene in question. They also added topoisomerase I to keep the DNA relaxed. Then they used a primer extension assay to test whether the reconstituted chromatin could be transcribed by a nuclear extract. In the first studies, these workers used only the core histones, not histone H1. They added a mass ratio of histones to DNA of 0.8 to 1.0, which is enough to form an average of one nucleosome per 200 bp of DNA.

Using such reconstituted chromatin that contained the *Drosophila Krüppel* gene, Laybourne and Kadonaga showed that a *Drosophila* nuclear extract could transcribe the *Krüppel* gene (Figure 13.12). However, core histones in quantities that produced nucleosomes at a density of one nucleosome per 200 bp, which is the physiological density, caused partial repression of transcription (down to 25% of the control value; compare lanes 2 and 5). Notice that the transcription start sites as detected by this method are quite heterogeneous in this gene, so we see a cluster of primer extension products.

The authors pointed to two possible explanations for the 75% repression observed with the core histones. First, the nucleosomes could slow the progress of all RNA polymerases by about 75%, but not stop any of them. Second, 75% of the polymerases could be blocked entirely by nucleosomes, but 25% of the promoters might have been left free of nucleosomes and thus could remain available to

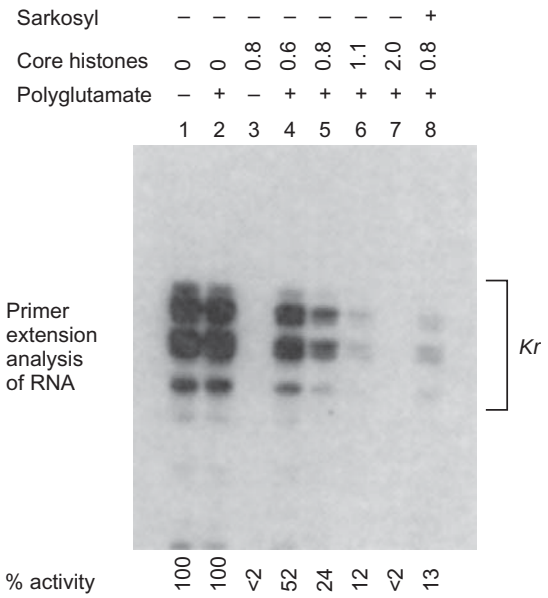


Figure 13.12 In vitro transcription of reconstituted chromatin. Laybourne and Kadonaga reconstituted chromatin with plasmid DNA containing the *Drosophila Krüppel* gene and core histones in varying ratios of protein to DNA, as indicated at top. Then they performed primer extension analysis to measure efficiency of transcription. Diverse signals corresponding to *Krüppel* gene transcription are indicated by the bracket at right. Lane 1, naked DNA; lane 2, naked DNA plus polyglutamate (used as a vehicle to help histones deposit onto DNA); lanes 3–7, chromatin at various core histone–DNA ratios; lane 8, sarkosyl was included to prevent reinitiation, so only one round of transcription occurred. Core histones can apparently inhibit transcription of the *Krüppel* gene in a dose-dependent manner. (*Source:* Laybourn, P.J. and J.T. Kadonaga, Role of nucleosomal cores and histone H1 in regulation of transcription by RNA polymerase II. *Science* 254 (11 Oct 1991) f. 2B, p. 239. Copyright © AAAS.)

RNA polymerase. A control experiment showed that the remaining 25% transcription could be eliminated by cutting the chromatin with a restriction enzyme that cleaves just downstream of the transcription start site. The fact that this site was available indicated that it was nucleosome-free. Thus, hypothesis 2 is the right one.

SUMMARY The core histones (H2A, H2B, H3, and H4) assemble nucleosome cores on naked DNA. Transcription of reconstituted chromatin with an average of one nucleosome core per 200 bp of DNA exhibits about 75% repression relative to naked DNA. The remaining 25% is due to promoter sites not covered by nucleosome cores.

Histone H1 Based on its suspected role as a nucleosome stabilizer, we would expect that histone H1 would add to the inhibition of transcription caused by the core histones in reconstituted chromatin. This is indeed the case, as Laybourne and Kadonaga demonstrated. They reconstituted

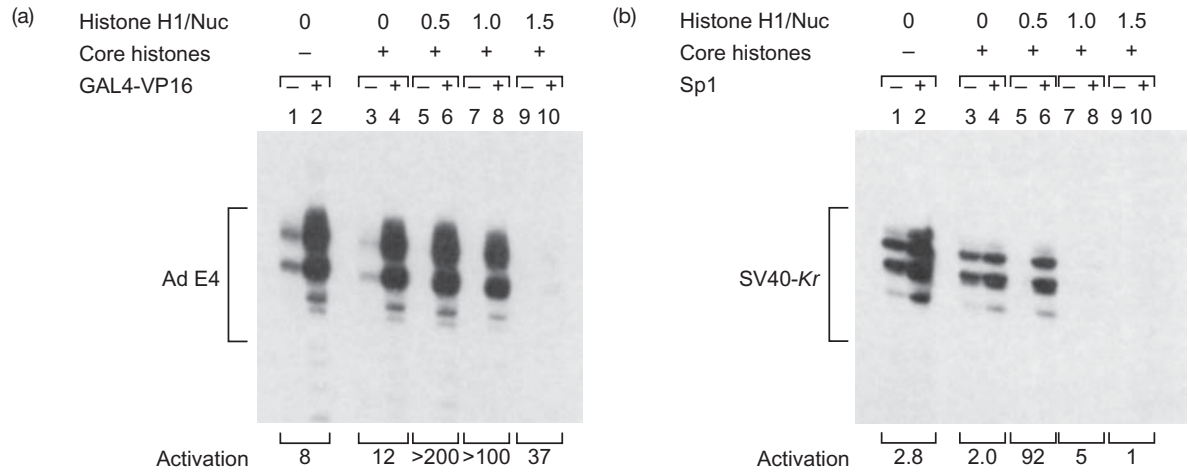


Figure 13.13 Competing effects of histones and activators on transcription. Laybourne and Kadonaga reconstituted chromatin in the presence and absence of core histones and histone H1 as indicated at top. Then they assayed for transcription by primer extension in the presence or absence of an activator as indicated. Apparent degrees of activation by each activator are given below each pair of lanes. The true activation by each activator is seen in lanes 1 and 2 of each panel, where naked DNA was the template. Any higher levels of apparent activation in the other lanes, where chromatin served as the template, were due to antirepression. **(a)** Effect of GAL4-VP16. Chromatin contained the adenovirus E4 promoter with five GAL4-binding sites. The signals corresponding to E4 transcription are indicated by the bracket at left. **(b)** Effect of Sp1. Chromatin contained the *Krüppel* minimal promoter plus the SV40 promoter GC boxes, which are responsive to Sp1. The signals corresponding to *Krüppel* transcription are indicated at left. (*Source:* Laybourn, P.J. and J.T. Kadonaga, Role of nucleosomal cores and histone H1 in regulation of transcription by RNA polymerase II. *Science* 254 (11 Oct 1991) f. 7, p. 243. Copyright © AAAS.)

chromatin with DNA containing two enhancer–promoter constructs: (1) pG_5E4 (five GAL4-binding sites coupled to the adenovirus E4 minimal promoter); and (2) pSV-Kr (six GC boxes from the SV40 early promoter coupled to the *Drosophila Krüppel* minimal promoter). In this experiment, they added not only the core histones, but histone H1 in various quantities, from 0 to 1.5 molecules per core nucleosome. Then they transcribed the reconstituted chromatin in vitro.

The odd lanes in Figure 13.13 show that increasing amounts of histone H1 caused a progressive loss of template activity, until transcription was barely detectable. However, at moderate histone H1 levels (0.5 molecules per core histone), activators could prevent much of the repression. For example, on chromatin reconstituted from the pG_5E4 plasmid, the hybrid activator GAL4-VP16, which interacts with GAL4-binding sites, caused a 200-fold greater template activity. Part of this (eightfold) is due to the stimulatory activity of the activator, observed even on naked DNA. The remaining 25-fold stimulation is apparently due to **antirepression,** the prevention of repression by histones. Similarly, when the reconstituted chromatin contained the pSV-Kr promoter, the activator Sp1, which binds to the GC boxes in the promoter, caused a 92-fold increase in template activity. Because true activation by Sp1 on naked DNA was only 2.8-fold, 33-fold of the 92-fold stimulation was antirepression. The true activation component is what we studied in Chapter 12, in which the experimenters used naked DNAs as the templates in their transcription assays.

These data are consistent with the model in Figure 13.14. Histone H1 can cause repression in the cases studied here by binding to the linker DNA between nucleosomes that happens to contain a transcription start site. Activators, represented by the green oval, can prevent this effect if added at the same time as histone H1. But these factors cannot reverse the effects of preformed nucleosome cores, even without histone H1. In other words, there is a sort of race between these activators and histone H1. If the activators get to the DNA first, they block the repressive action of histone H1. But if histone H1 reaches the DNA first, it stabilizes the nucleosomes and blocks activation. Other activators, represented by the purple oval, when confronted by a nucleosome blocking the promoter, can team up with chromatin-remodeling factors (see later in this chapter) to shoulder nucleosomes aside, at least if the nucleosomes are not stabilized by histone H1.

Kadonaga and colleagues have also studied another protein, called *GAGA factor,* which binds to several GA-rich sequences in the *Krüppel* promoter and to other *Drosophila* promoters. It has no transcription-stimulating activity of its own; in fact it slightly inhibits transcription. But GAGA factor prevents repression by histone H1 when added to DNA before the histone and can therefore cause a significant net increase in transcription rate. Thus, the GAGA factor seems to be a pure antirepressor, unlike the more typical activators we have been studying, which have both antirepression and transcription stimulation activities.

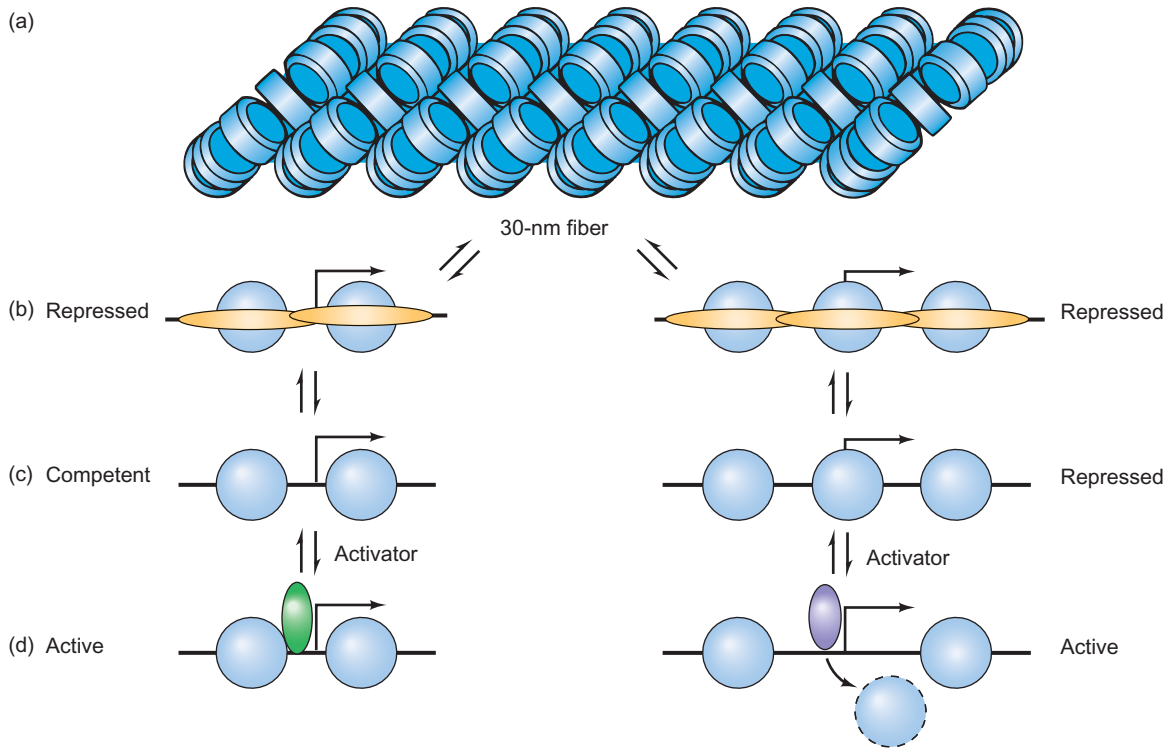


Figure 13.14 A model of transcriptional activation. (a) We start at the top with a 30-nm fiber. **(b)** The 30-nm fiber can open up to give two kinds of repressed chromatin. On the right, a stabilized nucleosome (blue) covers the promoter, keeping it repressed. On the left, no nucleosomes cover the promoter, but histone H1 (yellow) stabilizes nucleosomes flanking the promoter, so the gene is still repressed. **(c)** When we remove histone H1, we can get two chromatin states: On the left the promoter is uncovered, so the gene is competent to be transcribed. On the right, a nucleosome still covers the promoter, so it remains repressed. **(d)** Antirepression. If the gene's control region is not blocked by a nucleosome (left), the activator (green) can bind and, together with other factors, cause transcription initiation. If the gene's control region is blocked by one or more nucleosomes (right), the activator (purple), together with other factors, including chromatin-remodeling factors, can move the nucleosome aside (not necessarily removing it from the DNA, as shown here) and cause transcription to initiate. (*Source:* Adapted from Laybourn, P.J. and J.T. Kadonaga, Role of nucleosomal cores and histone H1 in regulation of transcription by polymerase II. *Science* 254:243, 1991.)

SUMMARY Histone H1 causes a further repression of template activity, in addition to that produced by core nucleosomes. This repression can be counteracted by transcription factors. Some, like Sp1 and GAL4, act as both antirepressors (preventing repression by histones) and as transcription activators. Others, like GAGA factor, are just antirepressors.

Nucleosome Positioning

The model of activation and antirepression in Figure 13.14 asserts that transcription factors can cause antirepression by removing nucleosomes that obscure a promoter or by preventing their binding to the promoter in the first place. Both these scenarios embody the idea of **nucleosome positioning,** in which activators force the nucleosomes to take up positions around, but not within, the promoter.

Nucleosome-Free Zones Several lines of evidence demonstrate nucleosome-free zones in the control regions of active genes. M. Yaniv and colleagues performed a particularly graphic experiment on the control region of SV40 virus DNA. SV40 DNA in an infected mammalian cell exists as a minichromosome, as described earlier in this chapter. Yaniv noticed that some actively transcribed SV40 minichromosomes have a conspicuous nucleosome-free zone late in infection (Figure 13.15). We would expect this nucleosome-free region to include at least one late promoter. In fact, the SV40 early and late promoters lie very close to each other, with the 72-bp repeat enhancer in between. Is this the nucleosome-free zone? The problem with a circular chromosome is that it has no beginning and no end, so we cannot tell what part of the circle we are looking at without a marker of some kind. Yaniv and colleagues used restriction sites as markers. A *Bgl*I restriction site occurs close to one end of the control region, and *Bam*HI and *Eco*RI sites occur on the other side of the circle, as illustrated in Figure 13.16a. Therefore, if the nucleosome-free region includes the control region, *Bgl*I will cut within that zone,

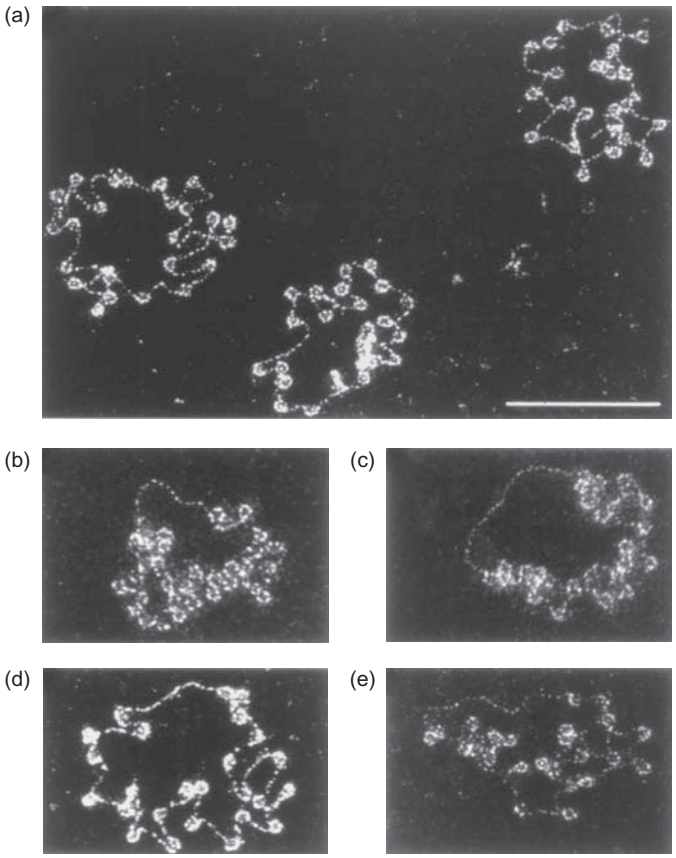


Figure 13.15 Nucleosome-free zones in SV40 minichromosomes. **(a)** Three examples of minichromosomes with no extensive nucleosome-free zones. **(b–e)** Four examples of SV40 minichromosomes with easily detectable nucleosome-free regions. The bar represents 100 nm. (*Source:* Saragosti, S., G. Moyne, and M. Yaniv, Absence of nucleosomes in a fraction of SV40 chromatin between the origin of replication and the region coding for the late leader RNA. *Cell* 20 (May 1980) f. 2, p. 67. Reprinted by permission of Elsevier Science.)

and the other two restriction enzymes will cut at remote sites, as illustrated in Figure 13.16b. Figure 13.17 shows that cutting with *Bam*HI or *Bgl*I produced exactly the expected results. Cutting with *Eco*RI (not shown) also fulfilled the prediction.

We can even tell that *Bgl*I cut asymmetrically within the nucleosome-free region, because it left a long nucleosome-free tail at one end of the linearized minichromosome, but not at the other. This is what we would expect if the nucleosome-free zone corresponds to one of the SV40 promoters, which are asymmetrically arranged relative to the *Bgl*I site. On the other hand, it is not what we would expect if the nucleosome-free zone corresponds to the viral origin of replication, which almost coincides with the *Bgl*I site.

DNase Hypersensitivity Another sign of a nucleosome-free DNA region is hypersensitivity to DNase. Chromatin regions that are actively transcribed are **DNase-sensitive** (≈10-fold more sensitive than bulk chromatin). But the control regions of active genes are **DNase-hypersensitive** (≈100-fold more sensitive than bulk chromatin). For example, the control region of SV40 DNA is DNase-hypersensitive, as we would expect. Yaniv demonstrated this by isolating chromatin from SV40 virus-infected monkey cells, mildly digesting this chromatin with DNase I, then purifying the SV40 DNA, cutting it with *Eco*RI, electrophoresing the fragments, Southern blotting, and probing the blot with radioactive SV40 DNA. Figure 13.16a shows that the *Eco*RI and *Bgl*I sites lie 67% (and 33%) apart on the circle. Therefore, if the nucleosome-free region near the *Bgl*I site is really DNase-hypersensitive, then DNase will cut there and *Eco*RI will cut at its unique site, yielding two fragments containing about 67% and 33% of the total SV40 genome.

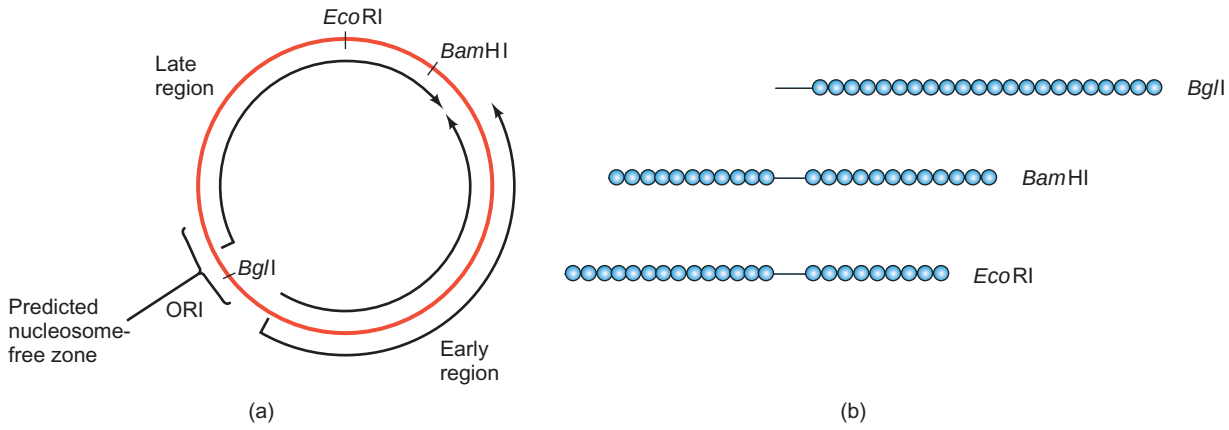


Figure 13.16 Experimental scheme to locate the nucleosome-free zone in the SV40 minichromosome. **(a)** Map of SV40 genome showing the cutting sites for three restriction enzymes *Bgl*I, *Bam*HI, and *Eco*RI. The control region surrounds the origin of replication (ORI), with the late control region on the clockwise side. **(b)** Expected results of cleavage of minichromosome from late infected cells with three restriction enzymes, assuming that the late control region is nucleosome-free. All three enzymes should cut once to linearize the minichromosome. *Bgl*I is predicted to cut near one end of the nucleosome-free zone and should therefore produce a minichromosome with a nucleosome-free zone at one end. *Bam*HI is predicted to cut at a site diametrically opposed to the nucleosome-free zone and should therefore produce a minichromosome with the zone in the middle. In the same way, *Eco*RI should yield a minichromosome with the zone somewhat asymmetrically located.

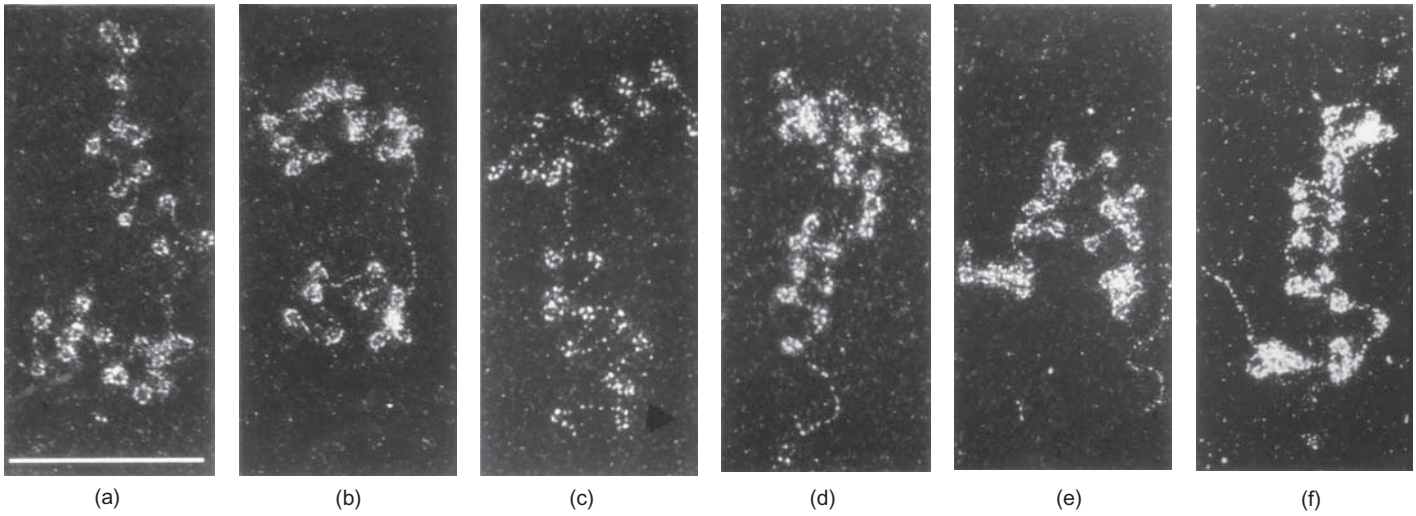


Figure 13.17 Locating the nucleosome-free zone on the SV40 minichromosome. Yaniv and colleagues cut SV40 minichromosomes from late infected cells with either *Bam*HI (panels **a–c**) or *Bgl*I (panels **d–f**). Just as predicted in Figure 13.16, *Bam*HI produced a centrally located nucleosome-free zone, and *Bgl*I yielded a nucleosome-free zone at the end of the minichromosome. The bar represents 100 nm. (*Source:* Saragosti, S., G. Moyne, and M. Yaniv, Absence of nucleosomes in a fraction of SV40 chromatin between the origin of replication and the region coding for the late leader RNA. *Cell* 20 (May 1980) f. 4, p. 69. Reprinted by permission of Elsevier Science.)

In fact, as Figure 13.18 demonstrates, experiments carried out 24 h, 34 h, and 44 h after virus infection all produced a large amount of the 67% product, and lesser amounts of the 33% product and shorter fragments. This suggests that DNase I is really cutting the chromatin in a relatively small region around the *Bgl*I site. Thus, the nucleosome-free region and the DNase-hypersensitive region coincide.

DNase hypersensitivity of the control regions of active genes is a general phenomenon. For example, the 5′-flanking region of the ε-globin gene in red blood cells is DNase-hypersensitive. In fact, the DNase hypersensitivity of the globin genes gives a good indication of the activity of those genes at any given time.

Figure 13.19 illustrates the principle involved in detecting a DNase-hypersensitive gene by Southern blotting. We see at the top of panels a and b the arrangement of nucleosomes on an active and an inactive gene, and the positions of two recognition sites for a restriction endonuclease (RE). If DNase I is used to lightly digest nuclei containing the inactive gene, nothing happens because no DNase-hypersensitive sites are present. On the other hand, if the same thing is done to nuclei containing the active gene, the DNase will attack the hypersensitive site near the promoter. Now the protein is removed from both DNAs, which are then cut with the RE. The restriction fragments are then electrophoresed, Southern blotted, and the blots are probed with a short gene-specific probe (green). DNA from the inactive chromatin will be intact, so the RE will generate a 13-kb fragment that will hybridize to the probe. But DNA from the active chromatin contains a DNase-hypersensitive

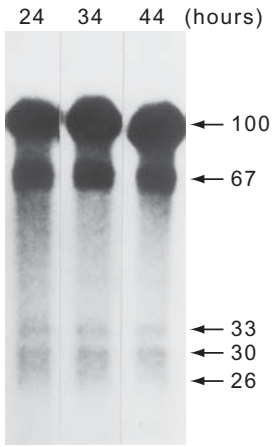


Figure 13.18 Locating the region of DNase hypersensitivity in the SV40 minichromosome. Yaniv and colleagues isolated nuclei from SV40 virus-infected monkey cells at 24, 34, and 44 h after infection and treated them with DNase I. Then they cleaved the treated minichromosomes with *Eco*RI and analyzed the DNA products by electrophoresis, Southern blotting, and probing with radioactive SV40 DNA. Because *Eco*RI cuts 33% of the way clockwise around the circle from the nucleosome-free zone, we would expect to see two fragments, corresponding to 33% and 67% of the whole length of the SV40 genome, assuming that the nucleosome-free zone and the DNase-hypersensitive region coincide. Actually, the 67% fragment is very prevalent, but the 33% fragment is partially degraded into smaller fragments. Thus, the DNase hypersensitive region does correspond to the nucleosome-free zone, which is large enough to produce a range of degradation products. (*Source:* Saragosti, S., G. Moyne, and M. Yaniv, Absence of nucleosomes in a fraction of SV40 chromatin between the origin of replication and the region coding for the late leader RNA. *Cell* 20 (May 1980) f. 7, p. 71. Reprinted by permission of Elsevier Science.)

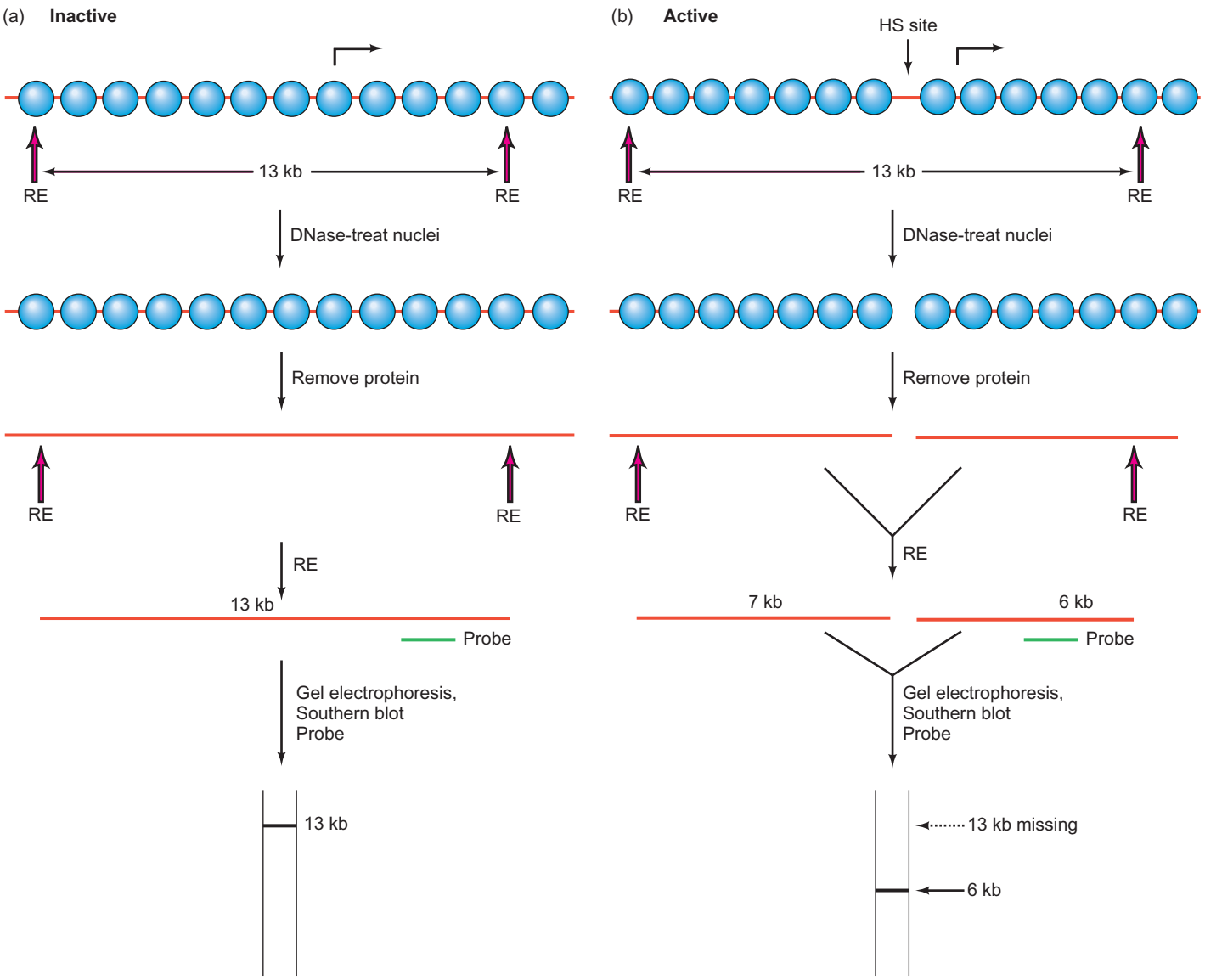


Figure 13.19 Experimental scheme for detecting DNase-hypersensitive regions. (a) Inactive gene, no DNase hypersensitivity. The gene and its control region are complexed with nucleosomes; therefore, no DNA will be degraded when nuclei containing this gene are subjected to mild treatment with DNase I. Next, isolate the DNA from these nuclei, removing all the protein, and digest with a restriction endonuclease (RE). This creates a DNA fragment 13 kb long that spans the gene's control region. Electrophorese the RE digestion products, Southern blot the fragments, and probe the blot with the gene-specific probe (green). This will "light up" the 13-kb fragment. **(b)** Active gene, DNase hypersensitivity. An active gene has one or more nucleosome-free zones that may correspond to a promoter, an enhancer, an insulator, or another control region. Thus, when nuclei containing this active gene are subjected to mild DNase I treatment, that hypersensitive site (HS site) will be digested, as shown. Next, isolate the DNA, remove protein, digest with a restriction endonuclease, electrophorese the fragments, blot, and probe as in panel (a). The 13-kb fragment has disappeared because of its cleavage by DNase, but a new fragment at 6 kb has appeared. The 7-kb fragment will not be detected because it does not hybridize to the probe. This experiment has revealed a DNase-hypersensitive site approximately 6 kb upstream of the downstream RE site. In practice, increasing concentrations of DNase are often used, which would cause a gradual decrease in the intensity of the 13-kb band as the 6-kb band increases in intensity.

site, so two fragments (6 kb and 7 kb) are generated by the combination of DNase I and RE. The 6-kb fragment will be detected by the probe, but the 7-kb fragment will not. And the 13-kb fragment will usually disappear with longer DNase I treatment.

Figure 13.20 shows the results of just such an experiment performed by Frank Grosveld and colleagues in 1987 on the human globin gene cluster, which contains five active globin genes in this order: 5′-ε-^{G}γ-^{A}γ-δ-β-3′. Grosveld and colleagues noted that when the β-globin gene is

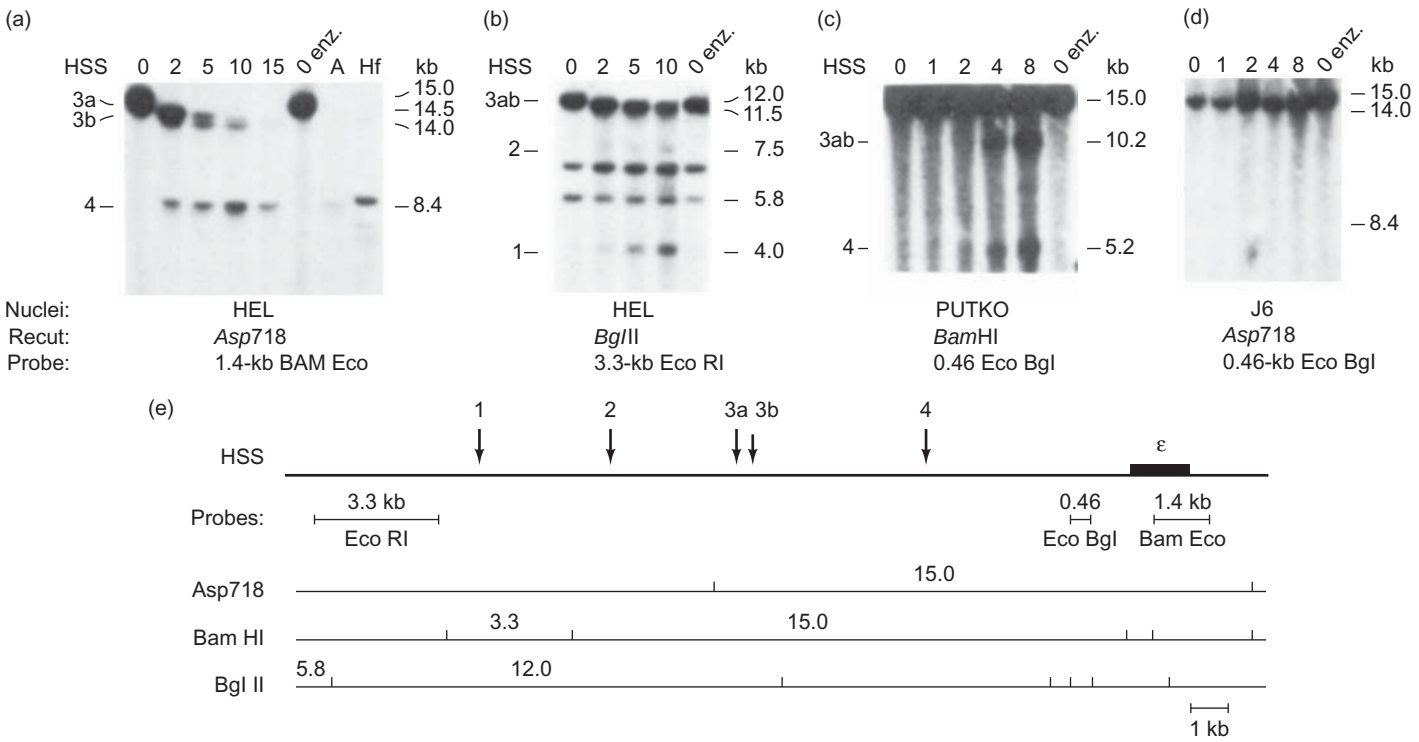


Figure 13.20 Mapping DNase-hypersensitive sites in the 5′-flanking region of the human globin gene. Panels **(a–d)** Grosveld and colleagues treated nuclei from HEL, PUTKO, or J6 cells, as indicated at bottom ("Nuclei:"), with a low concentration of DNase I for the times (in minutes) indicated at top, or with zero enzyme (0 enz.). Then they extracted DNA from the nuclei, deproteinized it with proteinase K, cleaved it with the restriction enzymes indicated at bottom ("Recut:"), electrophoresed the fragments, blotted them, and probed the blots with the probes indicated at bottom ("Probe:"). The fragments corresponding to cleavage at hypersensitive sites (HSS) 1, 2, 3a, 3b, and 4, are indicated at left. The lanes labeled A and Hf in panel (a) contained DNA cut with *Alu*I or *Hin*fI instead of DNase I. **(e)** Map of the 5′-flanking region of the human ε-globin locus, showing the positions of the three probes, and the restriction sites for the three restriction endonucleases used in panels (a–d). (*Source:* Reprinted from *Cell* v. 51, Grosveld et al., p. 976. © 1987, with permission from Elsevier Science.)

transferred by itself to transgenic mice (Chapter 5), it functions at best at only about 10% of its normal level. And when it was inserted into some chromosomal locations it functioned much better than in others. They reasoned that something outside the β-globin gene itself governs efficiency of expression. In fact, several sites contribute to this efficiency, and they are all DNase-hypersensitive.

Five of these sites (1, 2, 3a, 3b, and 4) lie upstream of the ε-globin locus, as shown in Figure 13.20e. Grosveld and colleagues assayed for DNase-hypersensitive sites as described previously in Figure 13.19. The positions of three different probes (Eco RI, Eco Bgl, and Bam Eco) are shown in panel (e). Grosveld and colleagues treated nuclei from two human cell lines that express the β-globin gene—erythroleukemia (HEL) cells, and another human erythroid cell line (PUTKO)—and a cell line that does not express the β-globin gene—human T cells (J6). The "0 enz." lane in each panel shows the results of treatment with no DNase I, and the other numbered lanes show the results of treatment with DNase I for increasing times.

Panel (a) shows the results with HEL cells, the restriction enzyme *Asp*718, and the 1.4-kb Bam Eco probe. DNase I cleavage was readily observed at sites 3a, 3b, and 4. To detect hypersensitive sites farther upstream of the gene, Grosveld and colleagues used the 3.3-kb Eco RI probe, as shown in panel (b). This time, cleavages at sites 1, 2, 3a, and 3b were observed, although cleavage at site 2 was delayed and relatively weak. The 5.8-kb band corresponds to the 5.8-kb fragment that reacts with the probe, as shown in panel (e). The 6.8-kb band came from nonspecific hybridization to an unrelated gene and could be eliminated by hybridization at higher stringency. Panel (c) shows the results with PUTKO cells, the restriction enzyme *Bam*HI and the 0.46 Eco Bgl probe. Cleavage at sites 3a, 3b, and 4 could be observed. Using the same kind of approach, Grosveld and colleagues detected another DNase-hypersensitive site downstream of the β-globin gene.

Finally, Grosveld and colleagues tested for DNase hypersensitivity in J6 T cells, which do not have active globin genes. As panel (d) shows, no DNase hypersensitivity was detected. This result supports the hypothesis that hypersensitivity corresponds to the presence of gene-specific factors that exclude nucleosomes from active genes, but not from inactive genes.

Grosveld and colleagues predicted that these sites corresponded to important gene control regions that are required for optimal expression of transplanted genes. Sure enough, when they transplanted the whole globin gene cluster, including these sites, into transgenic mice, the β-globin gene was expressed just as actively as the resident mouse β-globin gene. And the gene was active no matter where it inserted into the mouse genome. These experiments defined an important control region we now call the globin **locus control region (LCR).**

SUMMARY Active genes tend to have DNase-hypersensitive control regions. At least part of this hypersensitivity is due to the absence of nucleosomes.

Histone Acetylation

Vincent Allfrey discovered in 1964 that histones are found in both acetylated and unacetylated forms. Acetylation occurs on the amino groups on lysine side chains. Allfrey also showed that acetylation of histones correlates with gene activity. That is, unacetylated histones, added to DNA, tend to repress transcription, but acetylated histones are weaker repressors of transcription. These findings implied that enzymes in nuclei acetylate and deacetylate histones and thereby influence gene activity. To investigate this hypothesis, one needs to identify these enzymes, yet they remained elusive for over 30 years, in part because they are present in low quantities in cells.

Finally, in 1996, James Brownell and David Allis succeeded in identifying and purifying a **histone acetyltransferase (HAT),** an enzyme that transfers acetyl groups from a donor (acetyl-CoA) to core histones. These investigators used a creative strategy to isolate the enzyme: They started with *Tetrahymena* (ciliated protozoan) cells because this organism has histones that are heavily acetylated, which suggests that the cells contain relatively high concentrations of HAT. They prepared extracts from macronuclei (the large *Tetrahymena* nuclei that contain the active genes) and subjected them to gel electrophoresis in an SDS gel impregnated with histones. To detect HAT activity, they soaked the gel in a solution of acetyl-CoA with a radioactive label in the acetyl group. If the gel contained a band with HAT activity, the HAT would transfer labeled acetyl groups from acetyl-CoA to the histones. This would create a labeled band of acetylated histones in the gel at the position of the HAT activity. To detect the labeled histones, they washed away the unreacted acetyl-CoA, then subjected the gel to fluorography. Figure 13.21 shows the result: a band of HAT activity corresponding to a protein 55 kD in size. Accordingly, Brownell and Allis named this protein p55.

Allis and colleagues followed this initial identification of the HAT activity with a classic molecular cloning scheme to learn more about p55 and its gene. They began by

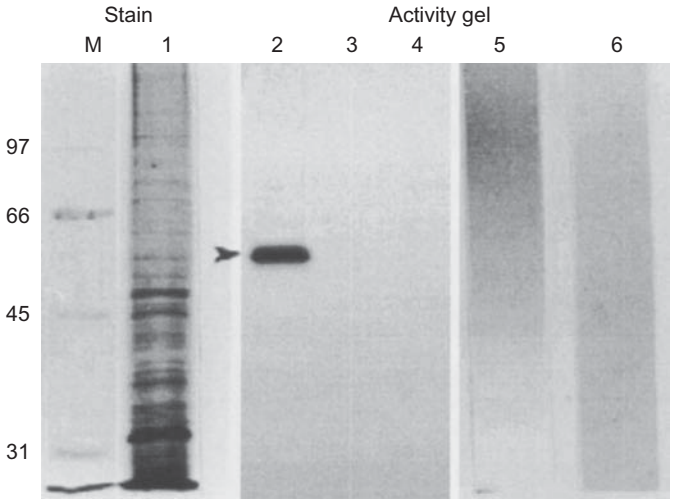


Figure 13.21 Activity gel assay for histone acetyltransferase (HAT) activity. Brownell and Allis electrophoresed a *Tetrahymena* macronuclear extract in an SDS-polyacrylamide gel containing histones (lanes 2–4), bovine serum albumin (BSA, lane 5), or no protein (lanes 1 and 6). After electrophoresis, they either silver-stained the gel to detect protein (lanes M and 1), or treated it with acetyl-CoA labeled in its acetyl group with ^{3}H to detect HAT activity. After washing to remove unreacted acetyl-CoA, they subjected the gel to fluorography to detect ^{3}H-acetyl groups. Lane 2 showed a clear band of ^{3}H-acetylated histones, which indicated the presence of HAT activity. Lanes 3 and 4 failed to show activity because the HAT in the nuclear extracts was inactivated by heating (lane 3) or by treatment with *N*-ethylmaleimide (lane 4) prior to electrophoresis. Lane 5, with BSA instead of histones, also showed no activity, as did lane 6, with no protein substrate. Lane M contained molecular mass marker proteins. (*Source:* Brownell, J.E. and C.D. Allis, An activity gel assay defects a single, catalytically active histone acetyltransferase subunit in *Tetrahymena* macronuclei. *Proceedings of the National Academy of Sciences USA* (July 1995) f. 1, p. 6365. Copyright © National Academy of Sciences, USA.)

purifying the HAT activity further, using standard biochemical techniques. Once they had purified the HAT activity essentially to homogeneity, they isolated enough of it to obtain a partial amino acid sequence. Using this sequence, they designed a set of degenerate oligonucleotides (Chapter 4) that coded for parts of the amino acid sequence and therefore hybridized to the macronuclear genomic DNA (or to cellular RNA). Using these oligonucleotides as primers, and total cellular RNA as template, they performed RT-PCR as explained in Chapter 4, then cloned the PCR products. They obtained the base sequences of some of the cloned PCR products and checked them to verify that the internal parts also coded for known HAT amino acid sequences. None of the PCR clones contained complete cDNAs, so these workers extended them in both the 5′- and 3′-directions, using rapid amplification of cDNA ends (RACE, Chapter 4). Finally, they obtained a cDNA clone that encoded the full 421-amino-acid p55 protein.

The amino acid sequence inferred from the base sequence of the p55 cDNA was very similar to the amino

acid sequence of a yeast protein called Gcn5p. Gcn5p had been identified as a coactivator of acidic transcription activators such as Gcn4p, so the amino acid sequence similarity suggested that both p55 and Gcn5p are HATs that are involved in gene activation. To verify that Gcn5p has HAT activity, Allis and colleagues expressed its gene in *E. coli,* then subjected it and p55 to the SDS-PAGE activity gel assay. Both proteins showed clear HAT activity. Thus, at least one HAT (Gcn5p) has both HAT and transcription coactivator activities. It appears to play a direct role in gene activation by acetylating histones.

It is important to note that p55 and Gcn5p are type A HATs (**HAT A's**) that exist in the nucleus and are apparently involved in gene regulation. They acetylate the lysine-rich N-terminal tails of core histones. Fully acetylated histone H3 has acetyl groups on lysines 9, 14, and 18, and fully acetylated histone H4 has acetyl groups on lysines 5, 8, 12, and 16. Lysines 9 and 14 of histone H3 and Lysines 5, 8, and 16 of histone H4 are acetylated in active chromatin and deacetylated in inactive chromatin. Type B HATs (**HAT B's**) are found in the cytoplasm and acetylate newly synthesized histones H3 and H4 so they can be assembled properly into nucleosomes. The acetyl groups added by HAT B's are later removed in the nucleus by histone deacetylases. All known HAT A's, including p55 and Gcn5p, contain a **bromodomain,** while all known HAT B's lack a bromodomain. Bromodomains allow proteins to bind to acetylated lysines. This is useful to HAT A's, which must recognize partially acetylated histone tails and add acetyl groups to the other lysine residues. But HAT B's have no use for a bromodomain, because they must recognize newly synthesized core histones that are unacetylated.

Since Allis's group's initial discovery of p55, several coactivators besides Gcn5p have been found to have HAT A activity. Among these are CBP/p300 (Chapter 12) and TAF1 (Chapter 11). All three of these coactivators cooperate with activators to enhance transcription. The fact that they have HAT A activity suggests a mechanism for part of this transcription enhancement: By binding near the transcription start site, they could acetylate core histones in the nucleosomes in the neighborhood, neutralizing some of their positive charge and thereby loosening their hold on the DNA (and perhaps on neighboring nucleosomes). This would allow remodeling of the chromatin to make it more accessible to the transcription apparatus, thus stimulating transcription.

It is interesting in this context that TAF1 has a double bromodomain module capable of recognizing two neighboring acetylated lysines, such as we would find on partially acetylated core histones in inactive chromatin. Thus, another role of TAF1 may be to recognize partially acetylated histones in inactive chromatin and to usher its partners, TBP and the other TAFs, into such chromatin to begin the activation process. We will see evidence for this hypothesis later in this chapter.

SUMMARY Histone acetylation occurs in both the cytoplasm and nucleus. Cytoplasmic acetylation is carried out by a HAT B and prepares histones for incorporation into nucleosomes. The acetyl groups are later removed in the nucleus. Nuclear acetylation of core histone N-terminal tails is catalyzed by a HAT A and correlates with transcription activation. A variety of coactivators have HAT A activity, which may allow them to loosen the association of nucleosomes with a gene's control region. Acetylation of core histone tails also attracts bromodomain proteins such as TAF1, which are essential for transcription.

Histone Deacetylation

If core histone acetylation is a transcription-activating event, we would predict that core histone deacetylation would be a repressing event. In accord with this hypothesis, chromatin with underacetylated core histones is less transcriptionally active than average chromatin. Figure 13.22 outlines the apparent mechanism behind this repression: Known transcription repressors, such as nuclear receptors without their ligands, interact with corepressors, which in turn interact with histone deacetylases. These deacetylases then remove acetyl groups from the basic tails of core histones in nearby nucleosomes, tightening the grip of the histones on the DNA, thus stabilizing the nucleosomes and keeping transcription

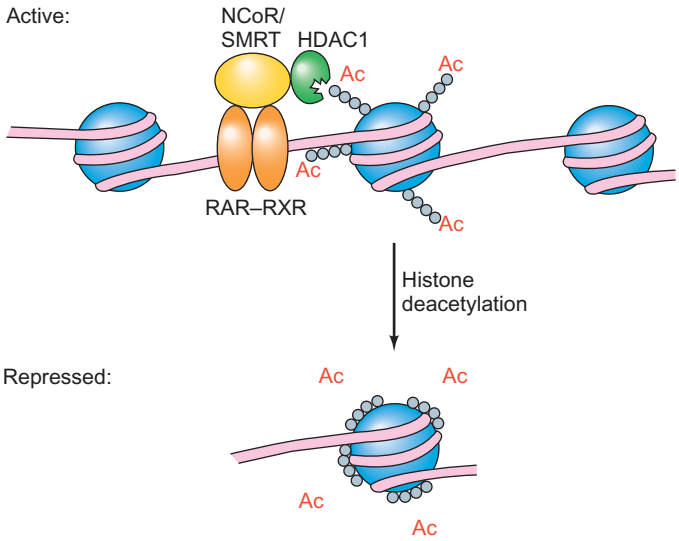


Figure 13.22 Model for participation of histone deacetylase in transcription repression. A heterodimer of retinoic acid receptor (RAR) and retinoic acid receptor X (RXR) binds to an enhancer (top). In the absence of the ligand, retinoic acid, the receptor dimer binds to the corepressor NcoR/SMRT, which binds to the histone deacetylase HDAC1. The deacetylase then removes acetyl groups (red) from the lysine side chains (gray) on core histones of nearby nucleosomes. This deacetylation allows the lysine side chains to associate more closely with DNA (bottom), stabilizing the nucleosomes, and thereby inhibiting transcription.

repressed. This repression can be considered **silencing,** although it is less severe than the silencing seen in heterochromatic regions of chromosomes, such as the ends, or telomeres. Some of the best studied corepressors are **SIN3** (yeast), **SIN3A** and **SIN3B** (mammals), and **NCoR/SMRT** (mammals). NCoR stands for "nuclear receptor corepressor" and SMRT stands for "silencing mediator for retinoid and thyroid hormone receptors." These proteins interact with unliganded retinoic acid receptor (RAR-RXR), a heterodimeric nuclear receptor.

How do we know a physical association exists among transcription factors, corepressors, and histone deacetylases? One way to answer this question has been to add epitope tags to one of the components, then to immunoprecipitate the whole complex with an antibody against the tag. For example, Robert Eisenman and coworkers used epitope tagging to demonstrate a ternary complex among a transcription factor **Mad-Max,** a mammalian Sin3 corepressor (SIN3A), and a histone deacetylase (HDAC2). Max is a transcription factor that can serve as an activator or a repressor, depending on its partner in the heterodimer. If it associates with Myc to form a Myc-Max dimer, it acts as a transcription activator. On the other hand, if it associates with Mad to form a Mad-Max dimer, it acts as a repressor.

Part of the repression caused by Mad-Max comes from histone deacetylation, which suggests some kind of interaction between a histone deacetylase and Mad. By analogy to the RAR-RXR–NCoR/SMRT–HDAC1 interaction illustrated in Figure 13.22, we might expect some corepressor like NCoR/SMRT to mediate this interaction between Mad and a histone deacetylase. To show that this interaction really does occur in vivo, and that it is mediated by a corepressor (SIN3A), Eisenman and coworkers used the following epitope-tagging strategy. They transfected mammalian cells with two plasmids. The first plasmid encoded epitope-tagged histone deacetylase (**HDAC2** tagged with a small peptide called the FLAG epitope [FLAG-HDAC2]). The second plasmid encoded Mad1, or a mutant Mad1 (Mad1Pro) having a proline substitution that blocked both interaction with SIN3A and repression of transcription. Then Eisenman and coworkers prepared extracts from these transfected cells and immunoprecipitated complexes using an anti-FLAG antibody. After electrophoresis, they blotted the proteins and first probed the blots with antibodies against SIN3A, then stripped the blots and probed them with antibodies against Mad1.

Figure 13.23 depicts the results. Lanes 1–3 are negative controls from cells containing a FLAG-encoding plasmid, rather than a FLAG-HDAC2-encoding plasmid. Immunoprecipitation of these lysates with an anti-FLAG antibody should not have precipitated HDAC2 or any proteins associated with it. Accordingly, no SIN3A or Mad1 were found in the blots. Lanes 4–6 contained extracts from cells transfected with a plasmid encoding FLAG-HDAC2, and plasmids encoding: no Mad1 (lane 4); Mad1 (lane 5); and

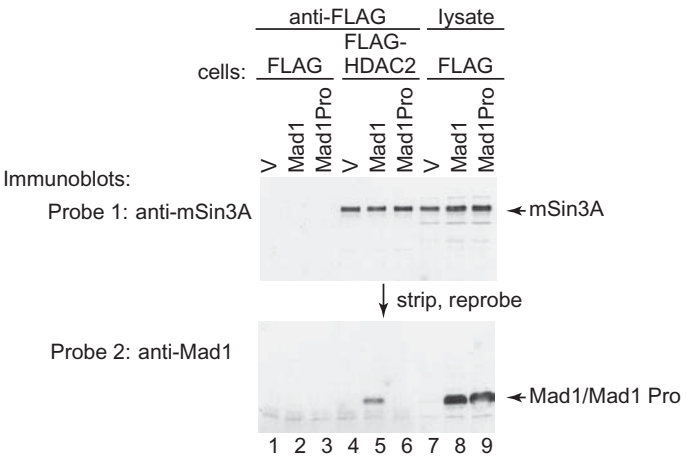


Figure 13.23 Evidence for a ternary complex involving HDAC2, SIN3A, and Mad1. Eisenman and coworkers transfected cells with a plasmid encoding either the FLAG epitope alone, or FLAG–HDAC2, as indicated at the top beside the designation "cells"; and a plasmid encoding either no Mad1 (V), Mad1, or Mad1Pro, also as indicated at top. They immunoprecipitated cell lysates with an anti-FLAG antibody (lanes 1–6, designated "anti-FLAG" at top) or just collected lysates (lanes 7–9, designated "lysate" at top) and electrophoresed the immunoprecipitates or lysates. After electrophoresis, they blotted the proteins to a membrane and probed the immunoblots, first with an anti-SIN3A antibody (top blot). Then, after stripping the first blot, they probed with an anti-Mad1 antibody that reacts with both Mad1 and Mad1Pro (bottom blot). Finally, they detected antibodies bound to proteins on the blot with a secondary antibody conjugated to horseradish peroxidase. They detected the presence of this enzyme with a substrate that becomes chemiluminescent on reaction with peroxidase. The positions of SIN3A and Mad1/Mad1Pro are indicated beside the blots at right. (*Source:* Laherty, C.D., W.-M. Yang, J.-M. Sun, J.R. Davie, E. Seto, and R.N. Eisenman, Histone deacetylases associated with the mSin3 co-repressor mediate Mad transcriptional repression. *Cell* 89 (2 May 1997) f. 3, p. 352. Reprinted by permission of Elsevier Science.)

Mad1Pro (lane 6). All three lanes contained SIN3A, which indicated that this protein coprecipitated with FLAG-HDAC2. However, only lane 5 contained Mad1. It was expected that lane 4 would not contain Mad1 because no Mad1 plasmid was provided. It is significant that lane 6 did not contain Mad1Pro, even though a plasmid encoding this protein was included in the transfection. Because Mad1Pro cannot bind to SIN3A, it would not be expected to coprecipitate with FLAG-HDAC2 unless it interacted directly with HDAC2. The fact that it did not coprecipitate supports the hypothesis that Mad1 must bind to SIN3A, and not to HDAC2. This is another way of saying that the corepressor SIN3A mediates the interaction between the transcription factor Mad1 and the histone deacetylase HDAC2. Lanes 7–9 show the results of simply electrophoresing whole-cell lysates without any immunoprecipitation. The two blots show that these lysates contained plenty of SIN3A and abundant Mad1, if a Mad1-encoding plasmid was given (lane 8), or Mad1Pro, if that was the protein present (lane 9). Thus, the lack of Mad1Pro in lane 6 could

not be explained by the failure of the plasmid encoding Mad1Pro to produce Mad1Pro protein.

We have now seen two examples of proteins that can be either activators or repressors, depending on other molecules bound to them. Some nuclear receptors behave this way depending on whether or not they are bound to their ligands. Max proteins behave this way depending on whether they are bound to Myc or Mad proteins. Figure 13.24 illustrates this phenomenon for a nuclear receptor, **thyroid hormone receptor (TR).** TR forms heterodimers with RXR and binds to the enhancer known as the **thyroid hormone response element (TRE).** In the absence of **thyroid hormone,** it serves as a repressor. Part of this repression is due to its interaction with NCoR, SIN3, and a histone deacetylase known as mRPD3, which deacetylates core histones in neighboring nucleosomes. This deacetylation stabilizes the nucleosomes and therefore represses transcription.

In the presence of thyroid hormone, the TR–RXR dimer serves as an activator. Part of the activation is due to binding to CBP/p300, P/CAF, and TAF1, all three of which are histone acetyltransferases that acetylate histones in neighboring nucleosomes. This acetylation destabilizes the nucleosomes and therefore stimulates transcription. Notice that the significant targets of the histone acetyltransferases and the histone deacetylases are core histones, not histone H1. Thus, the core histones, as well as H1, play important roles in nucleosome stabilization and destabilization.

Acetylation of core histone tails apparently does more than just inhibit binding of these tails to DNA. As we saw earlier in this chapter (see Figure 13.3), Timothy Richmond and colleagues' x-ray crystallography of core nucleosome particles revealed an interaction between histone H4 in one nucleosome core and the histone H2A–H2B dimer in the adjacent nucleosome core in the crystal lattice. In particular, the very basic region of the N-terminal tail of histone H4 (residues 16–25) interacts with an acidic pocket in the H2A–H2B dimer of the adjoining nucleosome. This interaction could help explain the cross-linking of nucleosomes that blocks access to transcription factors and therefore

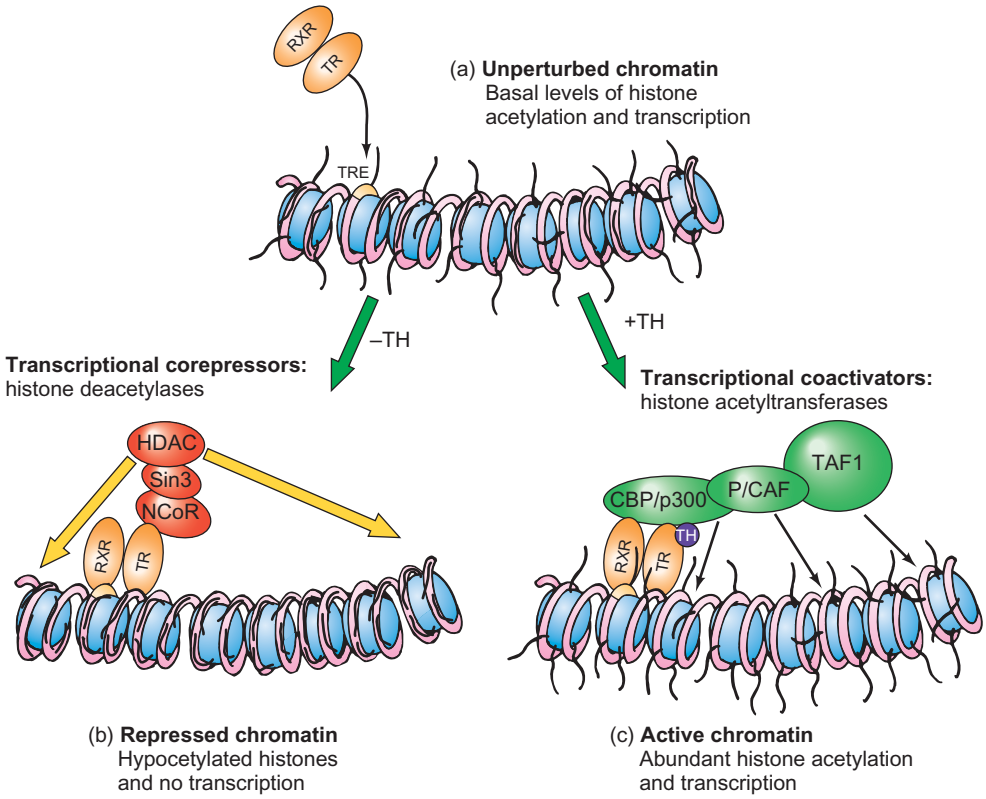


Figure 13.24 A model for activation and repression by the same nuclear receptor. (a) Unperturbed chromatin. No nuclear receptor (TR–RXR dimer) is bound to the thyroid hormone response element (TRE). Core histone tails are moderately acetylated. Transcription occurs at a basal level. **(b)** Repressed chromatin. The nuclear receptor is bound to the TRE in the absence of thyroid hormone (TH). The nuclear receptor interacts with either of the corepressors SIN3 and NCoR, which interact with a histone deacetylase (HDAC). The deacetylase cleaves acetyl groups off of the tails of core histones in surrounding nucleosomes, tightening the binding between histones and DNA, and between histones in neighboring nucleosomes, thereby helping to repress transcription. **(c)** Active chromatin. Thyroid hormone (purple) binds to the TR part of the nuclear receptor dimer, changing its conformation so it binds to one or more of the coactivators CBP/p300, P/CAF, and TAF1. These coactivators are all HAT A's that acetylate the tails of core histones in nearby nucleosomes, loosening the binding between histones and DNA and between histones on neighboring nucleosomes and helping to activate transcription. (*Source:* Adapted from Wolfe, A.P. 1997. Sinful repression. *Nature* 387:16–17.)

represses transcription. This hypothesis would also help explain why acetylating the tails of the core histones has an activating effect: Neutralizing the positive charge of the N-terminal tail of histone H4 by acetylation would help prevent nucleosome cross-linking and therefore help deter repression of transcription.

However, as mentioned in the previous section, simple charge neutralization is only part of the story. Acetylated lysines on core histone tails provide a docking site for bromodomain proteins such as TAF1, which are essential for transcription. In fact, as we will see in the next section, acetylation and other modifications of core histones may constitute a "histone code" that can be interpreted by other proteins that stimulate and repress transcription.

SUMMARY Transcription repressors such as unliganded nuclear receptors and Mad-Max bind to DNA sites and interact with corepressors such as NCoR/SMRT and SIN3, which in turn bind to histone deacetylases such as HDAC1 and 2. This assembly of ternary protein complexes brings the histone deacetylases close to nucleosomes in the neighborhood. The deacetylation of core histones allows the basic tails of the histones to bind strongly to DNA and to histones in neighboring nucleosomes, stabilizing and cross-linking the nucleosomes, and thereby inhibiting transcription. Deacetylation of core histones also removes binding sites for bromodomain proteins that are essential for transcription activation.

Chromatin Remodeling

Histone acetylation is frequently essential for gene derepression but it is not sufficient because it deals only with the tails of the core histones, which lie outside the nucleosome core. Acetylation of these core histone tails can disrupt nucleosome cross-linking, as we will see in the next section, but it leaves the nucleosomes intact. Something else is needed to "remodel" the nucleosome cores to permit access to transcription factors, and this remodeling requires ATP for energy.

Chromatin Remodeling Complexes At least four classes of protein complexes participate in this **chromatin remodeling,** and they are distinguished by their ATPase component, which harnesses the energy of ATP hydrolysis to the task of chromatin remodeling. These are the **SWI/SNF** family (pronounced "switch-sniff"), the **ISWI** ("imitation switch") family, the NuRD family, and the **INO80** family. All four classes of proteins alter the structure of nucleosome cores to make the DNA more accessible, not only to transcription activators, but also to nucleases and other proteins.

SWI/SNF complexes have been isolated from eukaryotic organisms ranging from yeast to human. They were originally identified in yeast, and found to regulate the *HO* endonuclease gene, which was responsible for mating type switching (hence the "SWI" part of the name). They also regulated the *SUC2* gene, which encodes invertase, the enzyme that begins the sucrose fermentation process. Thus, mutants with defects in the genes encoding the subunits of the complex were sucrose non-fermenters (hence the "SNF" part of the name). The SWI/SNF complexes all share an ATPase known as **BRG1** (or Brm in certain organisms). Gerald Crabtree and colleagues used an antibody to BRG1 to immunoprecipitate SWI/SNF complexes from several mammalian species, and found 9–12 **BRG1-associated factors** (**BAFs**) that co-precipitated with BRG1.

There are many similarities between mammalian and yeast BAFs, but some proteins distinct to each. In addition, mammalian BAFs are more diverse than their yeast counterparts. This could reflect the complexity of mammalian development relative to that of yeast, and different mammalian complexes could be devoted to different developmental processes.

One of the BAFs is called BAF 155 or BAF 170, depending on the species. It contains a so-called **SANT domain** ("SANT" is an acronym that refers to four proteins in which the domain is found). This domain has a sequence and three-dimensional structure that resembles that of the DNA-binding domain (DBD) of a transcription factor known as Myb. But some amino acid differences between SANT and the Myb DBD suggest that SANT does not bind DNA. In particular, the putative DNA-binding fold of the domain is lined with acidic residues, rather than basic ones, which is consistent with a role in binding histones, which are basic, and not DNA, which is acidic.

Members of the ISWI class of chromatin remodeling proteins also contain a SANT domain; in fact, they contain two. The first is a canonical SANT domain with a preponderance of acidic residues. The second has a net positive charge at neutral pH and could therefore be involved in DNA binding. This second domain is known as a SANT-like ISWI domain (**SLIDE**) to distinguish it from ordinary SANT domains. Both SANT and SLIDE domains are required for ISWI to bind to nucleosomes, and for its ATPase to be stimulated by nucleosomes. Thus, these domains appear to allow ISWI binding to nucleosomes and to transfer a stimulatory signal to the ATPase domain of ISWI, which then enables chromatin remodeling.

All these families of proteins may yield the nucleosome-free regions around enhancers and promoters that are characteristic of active genes. In fact, we would predict that a nucleosome-free enhancer would be an important early requirement for gene activation. Thus, it is not surprising that SWI/SNF appears to be one of the first co-activators to arrive on the scene when many yeast genes are activated.

SUMMARY Activation of many eukaryotic genes requires chromatin remodeling. Several different protein complexes carry out this remodeling, and all of them have an ATPase that harvests the energy from ATP hydrolysis to use for remodeling. The remodeling complexes are distinguished by their ATPase component, and two of the best-studied complexes are SWI/SNF and ISWI. The SWI/SNF complex in mammals has BRG1 as its ATPase, and 9–12 BRG1-associated factors (BAFs). One of the highly conserved BAFs is called BAF 155 or 170. It has a SANT domain that appears to be responsible for histone binding. This would help SWI/SNF bind to nucleosomes. Members of the ISWI class of remodeling complexes have a SANT domain, and another domain called SLIDE that appears to be involved in DNA binding.

The Mechanism of Chromatin Remodeling It is still not clear exactly what "remodeling" means. Sometimes it involves movement of nucleosomes away from their starting positions, opening up promoters to transcription factors. But remodeling does not necessarily involve simple sliding of nucleosomes. For example, remodeling can occur in chromatin in which nucleosomes are arrayed back-to-back through a promoter, and simply sliding them all in tandem would not open up significant amounts of DNA. Also, as we will see later in this chapter, remodeling sometimes involves a loosening of one or more nucleosomes so they can be moved aside by *other* proteins, such as TFIID. Perhaps the best provisional description of remodeling is that it mobilizes nucleosomes. That is, it allows nucleosomes to move by sliding or by other mechanisms. This movement can be caused by the remodeling complexes themselves, or by other proteins.

Furthermore, the effect of chromatin remodeling is not always activation of transcription; all known remodeling complexes sometimes collaborate in repression. Thus, remodeling of nucleosomes can make it easier to move them away from promoters, activating transcription. But remodeling can also make it easier to move nucleosomes into position to repress transcription. In fact, one of the subunits of the NuRD complex is a histone deacetylase, which can help repress transcription.

Robert Kingston and colleagues examined the nature of chromatin remodeling activity, focusing on the BRG1 subunit of SWI/SNF. They reasoned that one aspect of remodeling is making DNA more accessible, so they studied DNA accessibility as a measure of remodeling activity. They imagined two models for remodeling (Figure 13.25): Model 1 involves the formation of several different conformations of the nucleosomal DNA with respect to the core histones. Model 2 involves the formation of a single remodeled conformation. This would occur if the DNA simply peeled away from the core histones from the point of the DNA's entry to or exit from the nucleosome, as it does in uncatalyzed DNA exposure in mononucleosomes. Model 2 would also apply if the nucleosome simply slid along the DNA, as it does in heated nucleosomes in vitro.

Kingston and colleagues devised several ways to distinguish between the two models, all of which led to the conclusion that model 1 is correct, and remodeled chromatin exists in several different conformations. They started with a model nucleosome, which included a labeled 157-bp

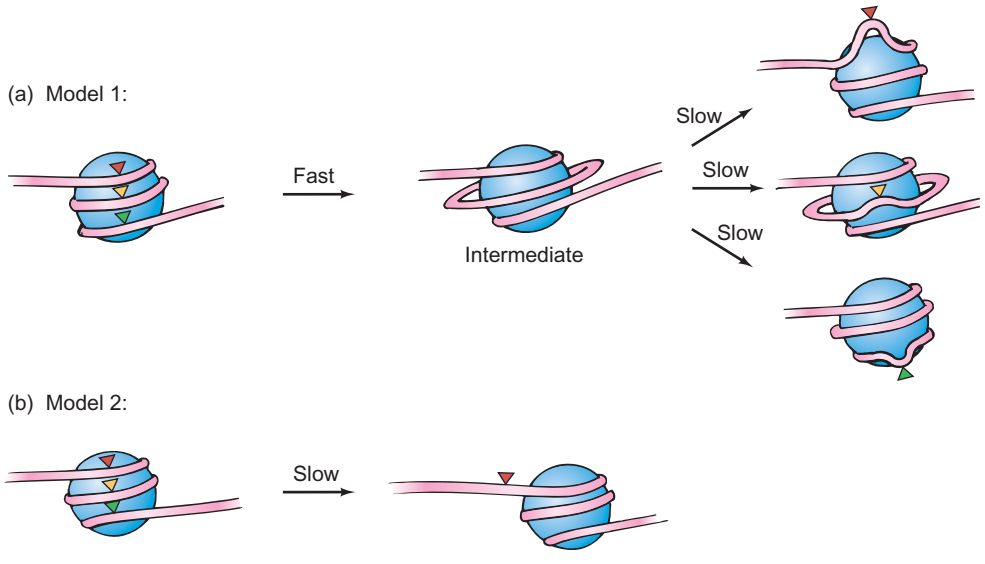


Figure 13.25 Two models for chromatin remodeling by SWI/SNF. **(a)** Model 1. This nucleosome contains three restriction sites, denoted by the colored triangles. In the first (fast) step, the nucleosome may generate an intermediate, which then converts in rate limiting steps to various remodeled conformations. Each of the three conformations illustrated here have opened up one of the restriction sites. **(b)** Model 2. Remodeling yields a single conformation, which, in this case, opens up one of the restriction sites.

DNA fragment that contained cleavage sites for three restriction enzymes, *Pst*I, *Spe*I, and *Xho*I. They reasoned that the two models made different predictions about the rates at which the three restriction sites would become available during remodeling.

Notice that the actual rates of cutting by the restriction enzymes are very fast, so they are not rate limiting. The change in chromatin conformation, which makes the restriction sites accessible, is relatively slow, so that is what limits the rate of cutting. Thus, model 1, in which different conformations are produced, predicts that the rates of cutting by the three enzymes will be different. That is because different conformations will have different accessibilities to the three enzymes, and these different conformations are reached at different rates. Model 2, which produces a single conformation, should yield accessibility to all three enzymes at the same rate, so they should all cut at the same rate.

Thus, Kingston and colleagues added BRG1 and ATP to their labeled model nucleosome and measured the rate of cleavage by each restriction enzyme during remodeling. Figure 13.26 shows that the rates differed by as much as a factor of 9, supporting model 1. Furthermore, the rate of cutting by DNase 1 was 10–20 times faster than the rate of cutting by *Pst*I, which also fits model 1, but not model 2. Finally, Kingston and colleagues repeated their experiments with whole SWI/SNF, instead of just BRG1, and obtained the same results. Thus, model 1 also describes remodeling carried out by intact SWI/SNF, and these experiments make clear that authentic, catalyzed chromatin remodeling is quite different from the simple alterations in chromatin that can occur in the absence of a catalyst.

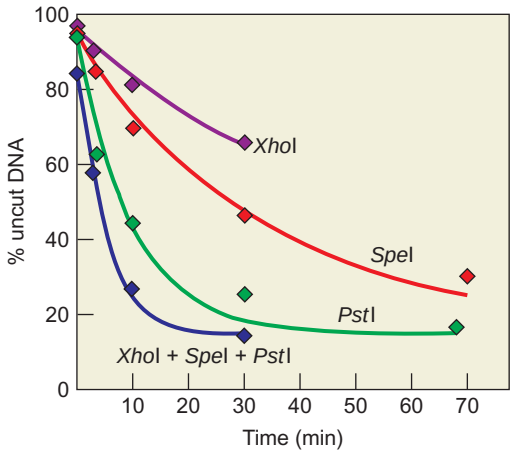


Figure 13.26 Restriction sites are revealed at different rates during BRG1-catalyzed chromatin remodeling. Kingston and colleagues incubated nucleosomes with labeled DNA with BRG1 and ATP for various times up to 70 min and tested the remodeled nucleosomes for susceptibility to cleavage by three restriction enzymes: *Xho*I, *Spe*I, and *Pst*I. They plotted uncut DNA, revealed by electrophoresis of deproteinized DNA, versus time. (*Source:* Adapted from Narlikar G.J. et al., *Molecular Cell* 8, 2001. f. 4A, p. 1224.)

SUMMARY The mechanism of chromatin remodeling is not understood in detail, but it does involve mobilization of nucleosomes, with loosening of the association between DNA and core histones. In contrast to uncatalyzed DNA exposure in nucleosomes, or simple sliding of nucleosomes along a stretch of DNA, catalyzed remodeling of nucleosomes involves the formation of distinct conformations of the nucleosomal DNA with respect to the core histones.

Remodeling in Yeast *HO* Gene Activation Kim Nasmyth and colleagues studied protein association with the *HO* gene of yeast, which plays a key role in switching the mating type. The expression of *HO* depends on a series of protein factors that appear at different phases of the cell cycle. Nasmyth and colleagues used a technique called chromatin immunoprecipitation (ChIP; Chapter 5) as follows: First, they fused DNA fragments encoding short regions (epitopes) of a protein (Myc) to the ends of genes encoding the proteins known to associate with the *HO* gene. This led to the production of fusion proteins with the Myc epitopes at their C-termini. Then they synchronized the yeast cells, so most of them went through the cell cycle together. They obtained cells in various phases of the cell cycle and added formaldehyde to form covalent bonds between DNA and any proteins bound to it. Then they sheared the chromatin by sonication to produce short, double-stranded DNA fragments cross-linked to proteins. Next, they made cell extracts and immunoprecipitated the protein–DNA complexes with antibodies directed against the Myc epitopes. Recall that the Myc epitopes were attached to the proteins known to associate with *HO,* so the immunoprecipitated protein–DNA complexes should contain both these fusion proteins and the *HO* gene. To verify that these complexes contained the *HO* gene, Nasmyth and colleagues performed PCR with *HO*-specific primers. The PCR product should be a band of predictable size if the *HO* gene is really present.

The experimental results showed that a protein known as Swi5 bound first to the control region of *HO*. Next, SWI/SNF bound, followed by the SAGA complex (Chapter 11), which contains the HAT Gcn5p, which then recruited the activator SBF. Other proteins, including general transcription factors and RNA polymerase II bound in turn after SBF. Both SWI/SNF and SAGA are absolutely required for activation of *HO,* and they could act in concert to remodel the chromatin around the *HO* promoter. For example, SWI/SNF could disrupt the core histones around the gene's control region, and SAGA, by acetylating the tails of the core histones, could enhance the disruption and possibly make it permanent. Other work strongly suggests that the factors do not have to act in the order presented here. At other promoters, they can act in many different orders

and can help each other perform their functions. In the next section we will see an example of a gene that recruits a HAT *before* the SWI/SNF complex.

SUMMARY ChIP analysis can reveal the order of binding of factors to a gene during activation. As the yeast *HO* gene is activated, the first factor to bind is Swi5, followed by SWI/SNF and SAGA, which contains the HAT Gcn5p. Next, the general transcription factors and other proteins bind. Thus, chromatin remodeling is among the first steps in activation of this gene, but the order can be different in other genes.

Remodeling in the Human IFN-β Gene: The Histone Code We have seen that the core histone tails are subject to acetylation and deacetylation, which tend to activate, and deactivate transcription, respectively. But histone tails are subject to several other modifications, including methylation, phosphorylation, ubiquitylation, and sumoylation. Each of these modifications affects the transcription levels of nearby genes, which has given rise to the concept of a **histone code.** This concept, elaborated by Thomas Jenuwein and David Allis in 2001, holds that the combination of histone modifications on a given nucleosome near a gene's control region affects the efficiency of transcription of that gene. The histone code is an **epigenetic** code (not affecting the base sequence of DNA itself), which adds to the code written in the base sequence of the gene and its control region. Since 2001, many studies have supported the histone code hypothesis. Let us examine one such study, on the human interferon-β (IFN-β) gene.

Dimitris Thanos and colleagues have investigated a well-studied example of chromatin remodeling that occurs during the activation of the human IFN-β gene. When this gene is activated by viral infection, transcription activators bind to nucleosome-free regions near the promoter, forming an enhanceosome, as we learned in Chapter 12. The activators in the enhanceosome recruit factors that modify and remodel the chromatin around the transcription start site. In particular, one nucleosome is moved out of the way so transcription can initiate.

This process involves the following events: The activators recruit HATs, the SWI/SNF complex, and the general transcription factors. The HATs acetylate core histone tails in the nucleosome, which attracts the CBP–RNA polymerase II holoenzyme via one or more bromodomains in CBP. The SWI/SNF complex in the holoenzyme loosens the association between the nucleosome and the promoter DNA. Then, when TFIID binds to the TATA box and bends it, the remodeled nucleosome slides to a new location 36 bp downstream, allowing transcription initiation to occur.

Thanos and colleagues looked at the ordered acetylation of nucleosome core histones and found that acetylation of lysine 8 of histone H4 causes recruitment of the SWI/SNF complex, and acetylation of lysines 9 and 14 in histone H3 causes recruitment of TFIID.

These investigators began by looking at the time course of histone acetylation after Sendai virus infection of HeLa cells, using ChIP analysis. They immunoprecipitated cross-linked chromatin with antibodies against acetylated and phosphorylated histones H3 and H4. Figure 13.27a shows that chromatin bearing the IFN-β gene could be immunoprecipitated with antibodies against acetylated lysines 8 and 12 on histone H4, and with antibodies against acetylated lysines 9 and 14 and phosphorylated serine 10 on histone H3. But the same chromatin could not be immunoprecipitated with antibodies against acetylated lysines 5 and 16 on histone H4. Thus, the pattern of histone acetylation was not random. In a separate experiment, Thanos and colleagues showed that the antibodies against acetylated lysines 5 and 16 of histone H4 were capable of precipitating chromatin if these lysines really were acetylated.

Furthermore, the timing of histone modification varied from position to position. Thus, lysine 8 of histone H4 was acetylated from 3 to 8 h after virus infection, but lysine 12 of H4 was acetylated only at 6 h. Also, phosphorylation of serine 10 of histone H3 began at about 3 h after infection and peaked strongly at 6 h, whereas acetylation of lysine 14 of H3 began at about 6 h, and acetylation of lysine 9 of H3 began earlier and lasted until at least 19 h.

The timing of serine 10 phosphorylation and lysine 14 acetylation of histone H3 supported an earlier hypothesis that phosphorylation of serine 10 is necessary for lysine 14 acetylation. These results also revealed a perfect correspondence between the timing of acetylation of lysine 14 and the recruitment of TBP to the promoter. (Compare row 9 with row 10, showing immunoprecipitation with an antibody against TBP.) This finding is consistent with the hypothesis that acetylation of lysine 14 of H3 is required to recruit TBP to the promoter.

Thanos and colleagues performed similar experiments in vitro with chromatin reconstituted from histones expressed in bacteria and modified at selected sites in vitro. They found that the sites acetylated in vitro were the same ones acetylated in vivo. Furthermore, they performed the same experiments with extracts missing one or more HATs to see the effects on specific lysine acetylations. Figure 13.27b shows that extracts immunodepleted of the HAT GCN5/PCAF were defective in acetylating lysine 8 of histone H4. On the other hand, extracts immunodepleted of the HAT CBP/p300 or the SWI/SNF component BRG1/BRM could still acetylate lysine 8 of H4. A separate, control experiment demonstrated that depletion of GCN5/PCAF did not cause a depletion of CBP/p300, and vice versa. Thus, it appears that GCN5/PCAF is responsible for acetylating lysine 8 of histone H4, and a separate experiment (not shown) made the same case that this HAT is also responsible for acetylating lysine 14 of

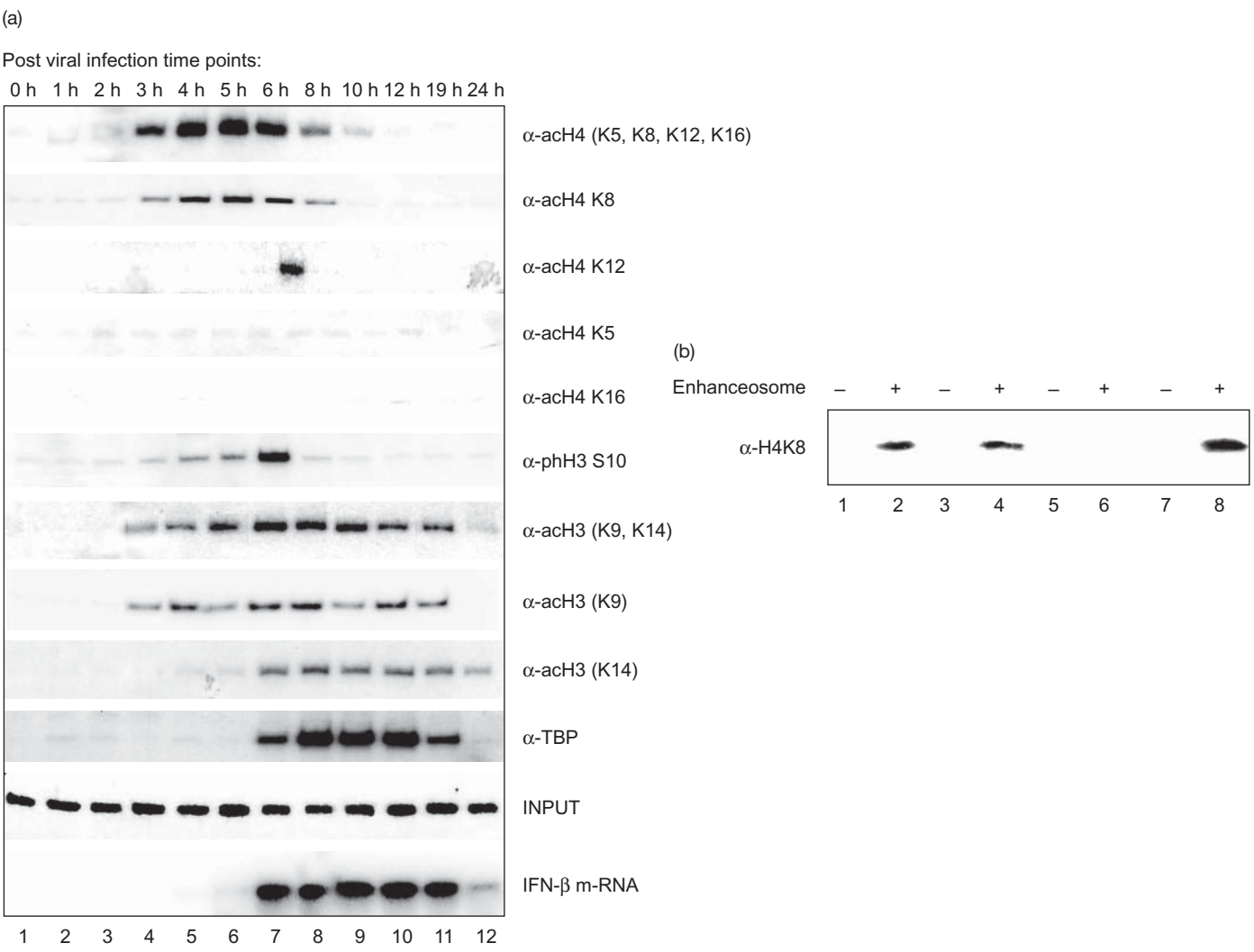


Figure 13.27 Timing of histone acetylation in chromatin at the IFN-β promoter after virus infection. (a) ChIP analysis. Thanos and colleagues performed ChIP with HeLa cell nuclear extracts at various times after infection with Sendai virus, using antibodies (indicated at right) directed against histone H4 acetylated on: lysine 8 (α-acH4 K8), lysine 12 (α-acH4 K12), lysine 5 (α-acH4 K5), or lysine 16 (α-acH4 K16), or all of these antibodies (α-acH4 [K5, K8, K12, K16]); or histone H3 phosphorylated on serine 10 (α-phH3 S10); or histone H3 acetylated on: lysine 9 (α-acH3 K9), or lysine 14 (α-acH3 K14), or both (α-acH3 [K9, K14]). They also performed ChIP with an antibody directed against TBP. Then they performed PCR on all the immunoprecipitated chromatins with primers specific for the IFN-β promoter. These PCR signals are presented, along with an RT-PCR signal that shows the abundance of IFN-β mRNA at the various times. The input lane shows the PCR signal using the input chromatin to show that roughly equal amounts of chromatin were used in each experiment. **(b)** Effects of immunodepletion of HATs on acetylation of lysine 8 of histone H4. Thanos and colleagues assembled the IFN-β enhanceosome on a biotinylated piece of DNA containing the IFN-β promoter and enhancers. Then they incubated the enhanceosome (even lanes) or buffer (odd lanes) with wild-type cell nuclear extracts (lanes 1 and 2), or nuclear extracts depleted of: CBP/p300 (lanes 3 and 4); GCN5/PCAF (lanes 5 and 6); or the SWI/SNF component BRG1/BRM. Then they electrophoresed the proteins, Western blotted the gels, and probed the blots with an antibody directed against histone H4 acetylated on lysine 8. (*Source:* Reprinted from *Cell* v. 111, Agalioti et al., p. 383. © 2002, with permission from Elsevier Science.)

histone H3. (Note that GCN5 is the human homolog of yeast Gcn5p.)

To investigate the effects of core histone tail acetylations on recruitment of SWI/SNF and TFIID, Thanos and colleagues reconstituted chromatin with the IFN-β promoter coupled to resin beads and core histones, then incubated the chromatin with nuclear extracts in the presence or absence of the acetyl donor acetyl-CoA, washed unbound proteins away, then disrupted the chromatin with SDS and subjected the released proteins to Western blotting and probed the blots with antibodies against a SWI/SNF component (BRG1) and a TFIID component (TAF1).

Figure 13.28a, shows that the chromatin bound only small amounts of BRG1 and TAF1 when it was not acetylated (lanes 1 and 2), but larger amounts of both proteins when it was acetylated (lanes 3 and 4). When chromatin was

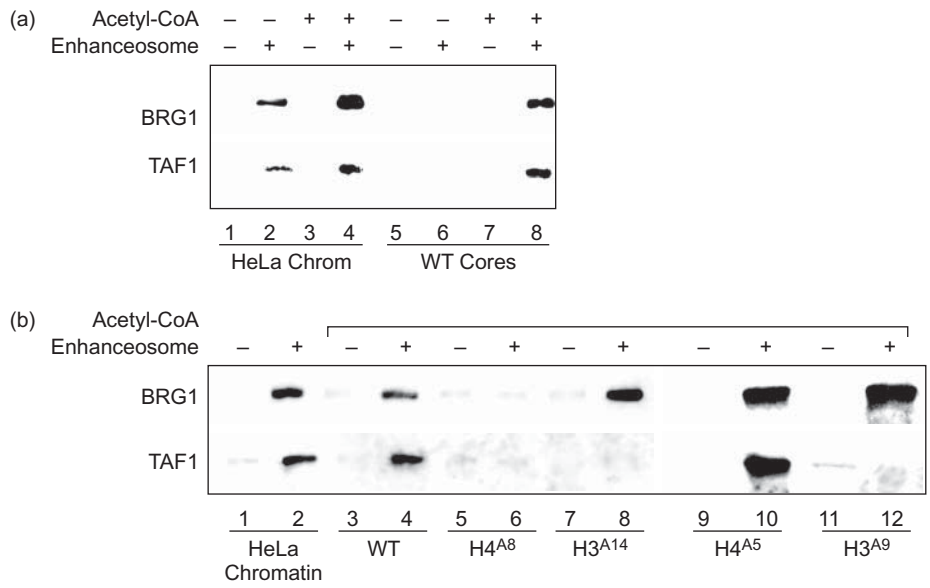


Figure 13.28 Recruitment of SWI/SNF and TFIID to IFN-β promoters: in the presence of wild-type and mutant core histones (a) Thanos and colleagues reconstituted chromatin on an IFN-β promoter attached to Dyna-beads, then incubated it with HeLa cell nuclear extracts, washed away unbound protein, then assayed for bound BRG1 and TAF1 by Western blotting and probing with antibodies against these two proteins. Each lane either contained the enhanceosome, or not, as indicated at top, and acetyl-CoA was included in the nuclear extract incubation to allow acetylation of histones, or not, also as indicated at top. Lanes 1–4 contained chromatin reconstituted from native HeLa cell chromatin. Lanes 5–8 contained chromatin reconstituted from recombinant wild-type core histones expressed in *E. coli* (WT Cores). **(b)** Conditions were as in panel (a) except that mutant core histones were used in some experiments, as indicated below each lane. Again the presence or absence of enhanceosomes was indicated at top, along with presence of acetyl-CoA, indicated by the bracket at top. Examples of mutant nomenclature: $H4^{A8}$ indicates a histone H4 in which lysine 8 has been changed to alanine. (*Source:* Reprinted from *Cell* v. 111, Agalioti et al., p. 386. © 2002, with permission from Elsevier.)

reconstituted with histones produced from cloned genes in *E. coli,* it bound no detectable BRG1 and TAF1 when it was not acetylated (lanes 5 and 6), but abundant quantities of both proteins when it was acetylated (lanes 7 and 8).

To investigate the role of acetylation of specific histone lysines, Thanos and colleagues reconstituted chromatin with mutant histones in which one lysine had been converted to an alanine. Figure 13.28b shows the results. Natural HeLa chromatin bound both BRG1 and TAF1 (lanes 1 and 2), as we have already seen in panel (a). Predictably, chromatin reconstituted with wild-type histones also bound the two proteins (lanes 3 and 4). But chromatin reconstituted with histone H4 lacking lysine 8 (which had been converted to alanine) failed to bind either BRG1 or TAF1 (lanes 5 and 6). This result can be explained by the failure of this mutant chromatin to recruit SWI/SNF (BRG1), which is required to recruit TFIID (TAF1).

When lysine 14 of histone H3 was changed to alanine, the reconstituted chromatin could recruit BRG1, but not TAF1 (lanes 7 and 8). The same behavior was observed when lysine 9 of histone H3 was changed to alanine (lanes 11 and 12). Thus, acetylation of lysines 9 and 14 appear to be required for TFIID recruitment, but not for SWI/SNF recruitment. In a control experiment, lysine 5 of histone H4 was changed to alanine. This lysine is known not to be acetylated on virus infection, so it is not surprising that its mutation had no effect on recruitment of either BRG1 or TAF1 (lanes 9 and 10).

Using the same method, Thanos and colleagues showed that substitution of lysine 12 of histone H3 with alanine did not affect recruitment of either TAF1 or BRG1. This lysine was acetylated in vivo, but only very briefly (Figure 13.27), and this acetylation is apparently not required for recruitment of either TFIID or SWI/SNF. Finally, substitution of serine 10 with alanine blocked recruitment of TAF1, but not BRG1. Thus, loss of serine 10 has the same effect as loss of lysines 9 or 14. The effect of loss of serine 10 is consistent with the hypothesis that phosphorylation of serine 10 is required for acetylation of lysine 14.

All of these results can be summarized by a model like the one in Figure 13.29. The core idea of the model is that the enhancer has all the genetic information needed to assemble the enhanceosome, and the enhanceosome can then recruit the appropriate factors to remove the nucleosome blocking initiation of transcription. Thus, information flows from the enhancer to the nucleosome, and not in the reverse direction.

In particular, the model calls for the following sequence of events: On virus infection, activators appear and assemble the enhanceosome on the enhancer. The enhanceosome then recruits the HAT GCN5, which acetylates lysine 8 of histone H4 and lysine 9 of histone H3. The enhanceosome also recruits an unknown protein kinase that

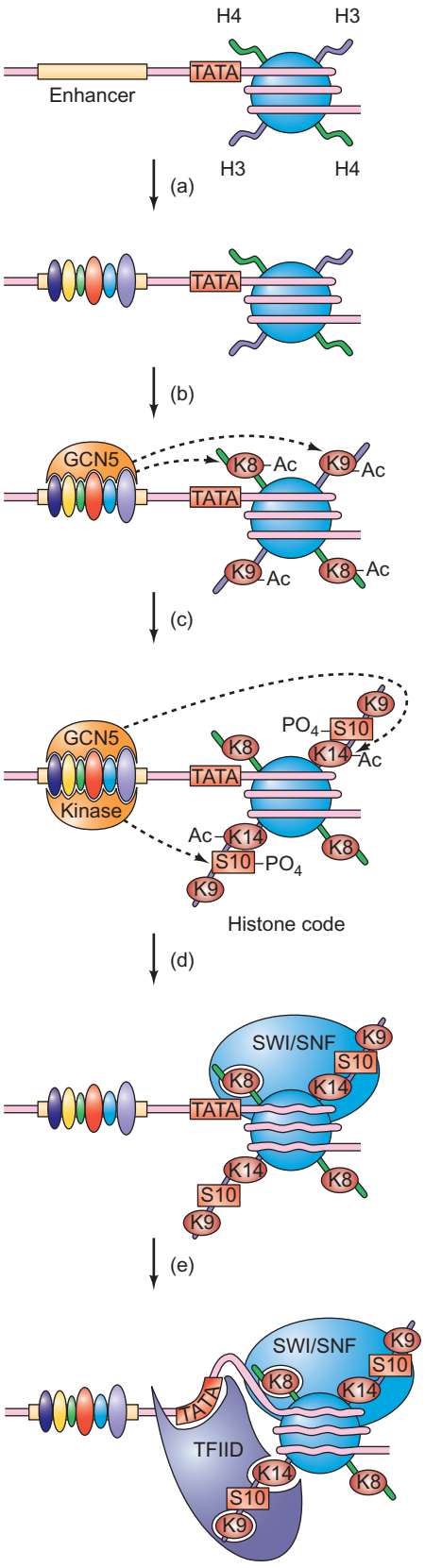


Figure 13.29 Model for the histone code at the human IFN-β promoter. **(a)** The enhanceosome assembles at the promoter according to the DNA code (the collection of enhancer elements). **(b)** The activators in the enhanceosome recruit GCN5, and this HAT acetylates lysine 8 (K8) on the tail of histone H4 and lysine 9 (K9) on the tail of histone H3. Arrows indicate acetylation only on the upper histone tails, but acetylation occurs on all four tails. **(c)** The enhanceosome also recruits a protein kinase that phosphorylates serine 10 (S10) of histone H3. Again, phosphorylation occurs on both H3 tails. This phosphorylation allows GCN5 to acetylate lysine 14 (K14) of histone H3. This completes the histone code, which is interpreted in the last two steps of the model. **(d)** Acetylated lysine 8 of histone H4 attracts the SWI/SNF complex, which remodels the nucleosome. This remodeling is represented by the wavy DNA lines in the nucleosome. **(e)** The remodeled nucleosome can now permit the binding of TFIID, which is attracted not only by the TATA box, but by the acetylated lysines 9 and 14 on the tail of histone H3. TFIID bends the DNA and moves the remodeled nucleosome 36 bp downstream. Now transcription can begin. (*Source:* Adapted from Agalioti, T., G. Chen, and D. Thanos, Deciphering the transcriptional histone acetylation code for a human gene. *Cell* 111 [2002] p. 389, f. 5.)

phosphorylates serine 10 of histone H3. Once that serine is phosphorylated, lysine 14 of histone H3 can be acetylated by GCN5. At this point, the histone code is complete.

Next, bromodomain-containing proteins interpret the histone code as follows: The single-bromodomain protein BRG1 binds to the acetylated lysine 8 of histone H4, bringing the whole SWI/SNF complex along with it. The rest of the polymerase II holoenzyme is presumably also recruited at this time but, for simplicity's sake, it is not shown. SWI/SNF then remodels the nucleosome in such a way that the double-bromodomain protein TAF1 can bind to histone H3, with its two acetylated lysines (9 and 14), and TAF1 brings the whole TFIID along with it. The binding of TFIID bends the DNA and causes the remodeled nucleosome to move out of the way downstream. The complex can now associate with the coactivator CBP, and transcription can begin.

In this context, it is worth mentioning another activity of TAF1 that has the potential to activate transcription, though it probably does not do so at the IFN-β promoter. That is, TAF1 has ubiquitin-conjugating activity, and one of its targets appears to be histone H1. Thus, when TAF1 is recruited to a promoter, possibly by binding to acetylated core histone tails, it can ubiquitylate a neighboring histone H1, targeting it for degradation by the 26S proteasome (Chapter 12). Because histone H1 helps repress transcription by cross-linking nucleosomes, the destruction of histone H1 would tend to activate neighboring genes.

SUMMARY The activators in the IFN-β enhanceosome can recruit a HAT (GCN5), which acetylates some of the lysines on histones H3 and H4 in a nucleosome at the promoter. A protein kinase also phosphorylates one of the serines on histone H3 of the same nucleosome, and this permits acetylation of

one more lysine on histone H3, completing the histone code. One of the acetylated lysines then recruits the SWI/SNF complex, which remodels the nucleosome. This remodeling allows TFIID to bind to two acetylated lysines in the nucleosome through the dual bromodomain in TAF1. TFIID binding bends the DNA and moves the remodeled nucleosome aside, paving the way for transcription to begin.

Heterochromatin and Silencing

Most of the chromatin we have discussed in this chapter is in a class known as **euchromatin.** This chromatin is relatively extended and open and at least potentially active. By contrast, **heterochromatin** is very condensed and its DNA is inaccessible. In higher eukaryotes it even appears as clumps when viewed microscopically (Figure 13.30). In the yeast *Saccharomyces cerevisiae*, the chromosomes are too small to produce such clumps, but heterochromatin still exists, and it has the same repressive character as in higher eukaryotes. In fact, it can silence gene activity up to 3 kb away. Yeast heterochromatin is found at the telomeres, or tips of the chromosomes, and in the permanently repressed mating loci mentioned at the end of Chapter 10. Generally speaking, heterochromatin is found at the telomeres and the centromeres of chromosomes.

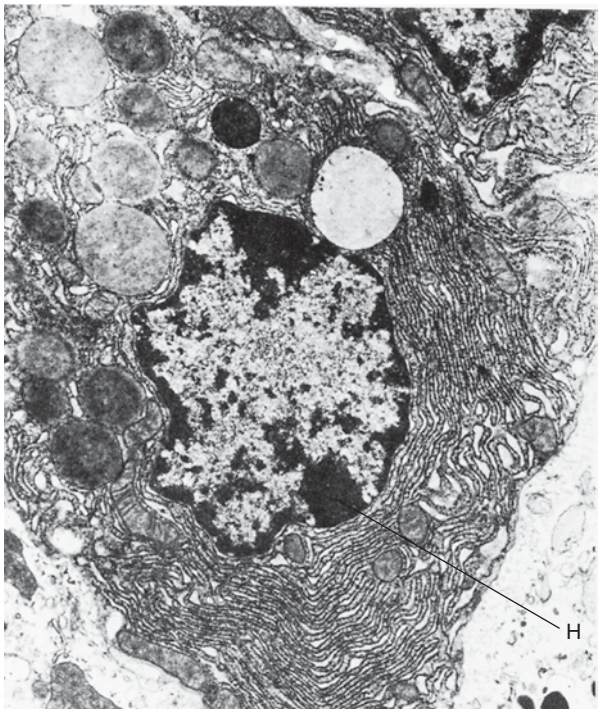


Figure 13.30 Interphase nucleus showing heterochromatin. Bat stomach lining cell with nucleus at center. Dark areas around periphery of nucleus are heterochromatin (H). (*Source:* Courtesy Dr. Keith Porter.)

It is particularly convenient to do genetic and biochemical experiments in yeast, so molecular biologists have exploited this organism to learn about the structure of heterochromatin and the way in which it silences genes, not only within the heterochromatin, but in neighboring regions of the chromosome. The silencing of genes near the telomere is called the **telomere position effect** (**TPE**) because the silencing of a gene is dependent on its position in the chromosome: If it is within about 3 kb of the telomere, it is silenced; if it is farther away, it is not.

Studies on yeast telomeric heterochromatin have shown that several proteins bind to the telomeres and are presumably involved in forming heterochromatin. These are **RAP1, SIR2, SIR3, SIR4,** and histones H3 and H4. (SIR stands for silencing information regulator.) Yeast telomeres consist of many repeats of this sequence: $C_{2–3}A(CA)_{1–5}$. (Of course, the opposite strand of the telomere has the complementary sequence.) This sequence, commonly called $C_{1–3}A$, is the binding site for the RAP1 protein, the only telomeric protein that binds to a specific site in DNA. RAP1 then recruits the SIR proteins to the telomere in this order: SIR3-SIR4-SIR2. As we have already seen, histones H3 and H4 are core histones of the nucleosome. Both SIR3 and SIR4 bind directly to the N-terminal tails of these two histones at residues 4–20 of histone H3 and residues 16–29 of histone H4.

Because RAP1 binds only to telomeric DNA, we might expect to find it associated only with the telomere, but we find it in the "subtelomeric" region adjacent to the telomere, along with the SIR proteins. To explain this finding, Michael Grunstein and his colleagues have proposed a model similar to the one in Figure 13.31: RAP1 binds to

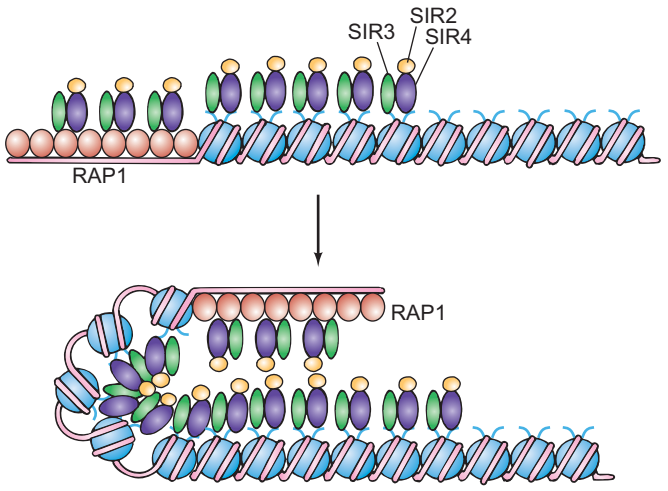


Figure 13.31 Model of telomere structure. RAP1 (red) binds to the telomere, and recruits SIR3 (green) and SIR4 (purple), which in turn attract SIR2 (yellow). SIR3 and SIR4 also bind to the N-terminal tails of histones H3 and H4 (thin blue lines). Interaction among the SIR proteins then causes the end of the chromosome to fold back on itself, so RAP1 is associated with the subtelomeric part of the chromosome. (*Source:* Adapted from Grunstein, M. 1998. Yeast heterochromatin: Regulation of its assembly and inheritance by histones. *Cell* 93: 325–28. Cell Press, Cambridge, MA.)

the telomeric DNA, the SIR proteins bind to RAP1 and to histones in the nucleosomes of the subtelomeric region. Then, protein–protein interactions cause the telomere to fold back on the subtelomeric region.

Earlier in this chapter, we learned that removing acetyl groups from core histones has a repressive effect on gene activity. Thus, we would predict that core histones in silenced chromatin would be poor in acetyl groups, or hypoacetylated. Indeed, whereas histone H4 in euchromatin is acetylated on lysines 5, 8, 12, and 16, histone H4 in yeast heterochromatin is acetylated only on lysine 12. What role might this hypoacetylation play in silencing? We know that lysine 16 of histone H4 is part of the domain (residues 16–29) that interacts with the SIR proteins (SIR3 in particular). Thus, acetylation of lysine 16 of histone H4 may block its interaction with SIR3, averting the formation of heterochromatin, and therefore preventing silencing.

Genetic experiments in yeast provide support for this hypothesis. Changing lysine 16 of histone H4 to a glutamine mimics the acetylation of this residue by removing its positive charge. This mutation also mimics acetylation in blocking the silencing of genes placed close to yeast telomeres and mating loci. On the other hand, changing lysine 16 to an arginine preserves the positive charge of the amino acid and thus mimics to some extent the deacetylated form of lysine. As expected, this mutation has less of an effect on silencing.

Because deacetylation of lysine 16 of histone H4 appears to attract the silencing complex, it is interesting that the SIR2 component of the yeast silencing complex has histone deacetylase activity (an NAD-dependent HDAC called N-HDAC). Thus, SIR2 is a good candidate for the enzyme that deacetylates lysine 16 of histone H4. If this hypothesis is valid, then SIR2 attracted to a nucleosome with a deacetylated lysine 16 of histone H4 could then deacetylate lysine 16 of histone H4 on a neighboring nucleosome and so propagate the silencing process.

SUMMARY Euchromatin is relatively extended and potentially active, whereas heterochromatin is condensed and genetically inactive. Heterochromatin can also silence genes as much as 3 kb away. Formation of heterochromatin at the tips of yeast chromosomes (telomeres) depends on binding of the protein RAP1 to telomeric DNA, followed by recruitment of the proteins SIR3, SIR4, and SIR2, in that order. Heterochromatin at other locations in the chromosome also depends on the SIR proteins. SIR3 and SIR4 also interact directly with histones H3 and H4 in nucleosomes. Acetylation of lysine 16 of histone H4 in nucleosomes prevents its interaction with SIR3 and therefore blocks heterochromatin formation. This is another way in which histone acetylation promotes gene activity.

Histone Methylation In addition to the other modifications we have seen, core histone tails are also subject to methylation, and methylation can have either an activating or a repressing effect. As we have seen, certain proteins, such as HATs, interact with specific acetylated lysines in core histone tails through acetyl-lysine-binding domains known as bromodomains. Thomas Jenuwein and colleagues noted that certain proteins involved in forming heterochromatin have conserved regions called **chromodomains.** One such protein is a **histone methyltransferase** (**HMTase**) whose human form is known as SUV39H HMTase. Another is a histone methyltransferase-associated protein called **HP1.**

Jenuwein and colleagues, and another group led by Tony Kouzarides, tested these and other proteins for binding to methylated and unmethylated peptides that included lysine 9 of histone H3, which is a target for methylation. Both groups found that HP1 binds to these peptides, but only if lysine 9 was methylated. This finding suggested a mechanism for spreading of methylated, and therefore repressive, chromatin: When lysine 9 of one histone H3 is methylated, it attracts HP1 through the latter's chromodomain. HP1 could then recruit SUV39H HMTase, which could methylate another nearby histone H3 on its lysine 9. In this way, the process could continue until many nucleosomes had become methylated. This methylation could lead to spreading of the heterochromatin state, as illustrated in Figure 13.32.

Lysine 9 of histone H3 is by no means the only histone target for methylation. All the core histones can be methylated on lysines and arginines, and the amino groups of lysines can accept up to three methyl groups each. Another favorite methylation site on histone H3 is lysine 4, and methylation of this site generally has an activating effect on transcription, owing to at least two mechanisms. First, it inhibits binding of the NuRD chromatin-remodeling and histone deacetylase complex to the histone H3 tail. This interferes with histone deacetylation, which would have a repressive effect. Second, methylation of lysine 4 of histone H3 blocks methylation of the nearby lysine 9, which would also be repressive. By inhibiting both of these repressive events, methylation of H3 lysine 4 has a net activating effect. Just as histone acetylation can be reversed by deacetylases, methylation of histone lysines and arginines can be reversed by demethylases, which reverse whatever repressive or stimulatory effect the methylation had.

Methylation of lysine 4 of histone H3 is generally trimethylation (designated H3K4Me3), and is usually associated with the 5′-end of an active gene. Thus, this modification appears to be a sign of transcription initiation. By contrast, trimethylation of lysine 36 of histone H3 (H3K36Me3) is usually associated with the 3'-end of an active gene, and therefore is taken as a marker for transcription elongation.

In a 2007 genome-wide ChIP-chip assay (Chapter 24) of these, as well as other markers, in human stem cell chromatin, Richard Young and colleagues made the following interesting discovery: Many protein-encoding genes are

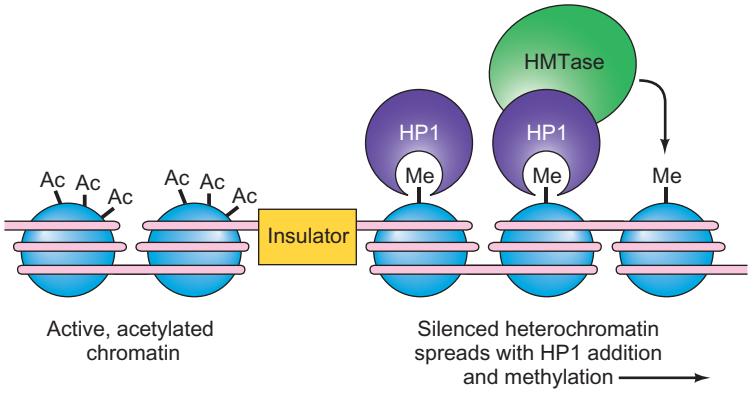


Figure 13.32 Model for involvement of histone methylation in chromatin repression. Nucleosomes to the right of the insulator have become methylated on lysine 9 of the histone H3 tails. This recruits HP1 (purple), which binds to a methylated lysine 9 on one nucleosome and recruits a histone methyltransferase (HMTase, green), to methylate lysine 9 on a neighboring nucleosome. Thus, the methylated, repressive state is propagated from one nucleosome to the next. (*Source:* Adapted from Bannister, S.D., P. Zegerman, J.F. Partridge, E.A. Miska, J.O. Thomas, R.C. Allshire, and T. Kouzarides, Selected recognation of methylated lysine 9 on histone H3 by the HP1 chromodomain. *Nature* 410 [2001] p. 123, f. 5.)

associated with nucleosomes having H3K4Me3, and therefore have presumably experienced transcription initiation, but they are not associated with nucleosomes having H3K36Me3, and therefore have probably not experienced transcription elongation. The simplest way to reconcile these two findings is to propose that many human genes contain RNA polymerase paused a short distance downstream of their promoters. This condition would open up a new potential means of controlling gene expression by controlling the restarting of paused RNA polymerase.

So far, we have dealt with individual methylations in isolation, but they do not really occur that way. Instead, many histone residues in a given nucleosome can be modified in various ways. Some will be acetylated, others will be methylated, others will be phosphorylated, and still others will be ubiquitylated. Figure 13.33 summarizes the modifications that can happen to the core histones.

As we have already seen, there is evidence for a histone code in which histone acetylation and phosphorylation can participate in a cascade of events leading to gene activation. Some investigators have wondered whether this histone code idea can be generalized to all histone modifications. A cell could read the different combinations of histone modifications in a given nucleosome as a combinatorial code that tells how much to express or silence genes in the neighborhood.

To address this question in the context of histone methylation, Frank Sauer and colleagues investigated the combined effects of methylations on three lysines in two histones: lysines 4 and 9 of histone H3 and lysine 20 of histone H4. They found that this combination of methylated lysines, created by a single HMTase called Ash1, had two effects in *Drosophila*, both of them positive. First, these methylations stimulated the binding of an activator called Brahma. Second, they inhibited the binding of the repressors HP1 and polycomb. Thus, the normal repressive effect of methylated histone H3 lysine 9 is masked in the context of the other two histone methylations. The cell must be able to read the whole combination of histone modifications, not just one.

Histone modifications not only mark chromatin for either activation or repression, they also affect other histone modifications. For example, methylation of histone H3 lysine 9 can be inhibited by several modifications on the same histone tail, including acetylation of lysine 9 (and perhaps lysine 14), methylation of lysine 4, and phosphorylation of serine 10.

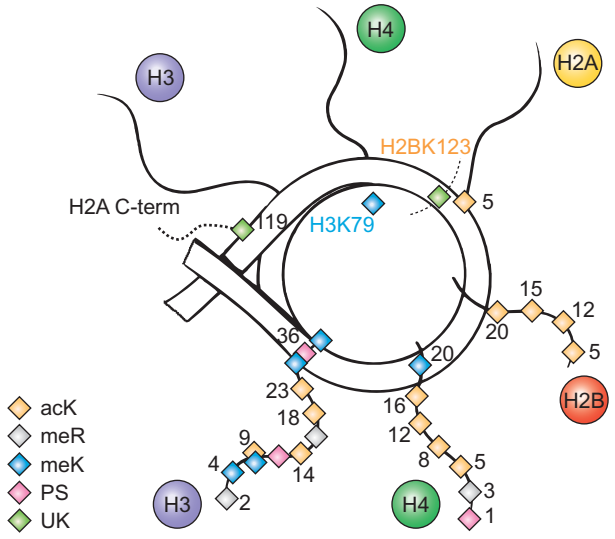


Figure 13.33 Summary of core histone modifications. Modifications are coded as shown at lower left: yellow, acetylated lysine (acK); gray, methylated arginine (meR); blue, methylated lysine (meK); pink, phosphorylated serine (PS); green, ubiquitylated lysine (UK). Modifications are shown on only one of the two histone H3 and H4 tails. Only one tail each is shown for histones H2A and H2B. The C-terminal tails of H2A and H2B are illustrated by dotted lines. The position of histone H3 lysine 79 (H3K79) is shown, though it is not on a histone tail. (*Source:* Adapted from Turner, B.M., Cellular memory and the histone code. *Cell* 111 [2002] p. 286, f. 1.)

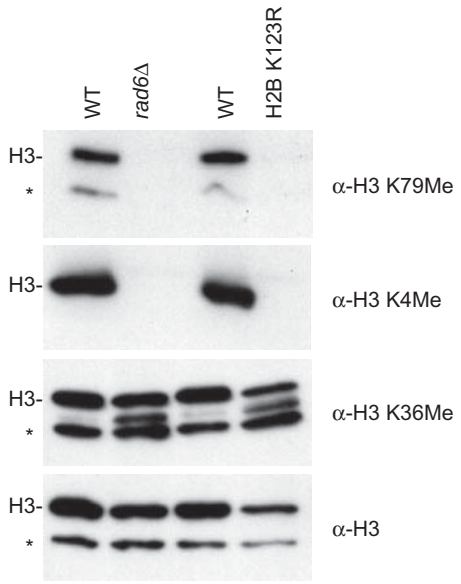


Figure 13.34 Effect of ubiquitylation of histone H2B on methylation of histone H3. Strahl and colleagues tested wild-type and mutant strains of yeast for the ability to methylate lysine 79 of histone H3. One mutant (*rad6*Δ) had the *rad6* gene deleted, so it could not ubiquitylate lysine 123 of histone H2B. In the other mutant (H2B K123R), lysine 123 of histone H2B was changed to arginine, so it could not be ubiquitylated, even with Rad6 functioning. Nuclear extracts from wild-type (lanes 1 and 3), and *rad6*Δ (lane 2) and H2B K123R (lane 4) were subjected to Western blotting by electrophoresis, followed by blotting and probing with antibodies against: methylated lysine 79 in histone H3 (top row); methylated lysine 4 in histone H3 (second row); lysine 36 in histone H3 (third row); and histone H3 (bottom row). The last row, with anti-H3 antibody, served as a positive control to make sure all lanes contained histone H3. The mutants did not support methylation of either lysine 4 or 79, but they did support methylation of lysine 36 of histone H3. The asterisk denotes a proteolytic product of H3 that removes the lysine 4 methylation site. (*Source:* Reprinted with permission from *Nature* 418: from Briggs et al., fig. 1, p. 498. © 2001 Macmillan Magazines Limited.)

Modifications in one histone can also affect modifications in another histone in the same nucleosome. For example, Brian Strahl and coworkers tested the effects of deleting the yeast gene *rad6*, which encodes the ubiquitin ligase **Rad6.** This enzyme is required for ubiquitylation of lysine 123 of histone H2B. This mutation blocked methylation of lysines 4 and 79 but had no effect on methylation of lysine 36 of histone H3 (Figure 13.34). Changing lysine 123 of histone H2B to arginine prevented ubiquitylation in cells with wild-type *rad6* and had the same negative effect on methylation of lysines 4 and 79 in histone H3. Thus, ubiquitylation of a lysine on one histone (H2B) can profoundly affect methylation of at least two sites on another (H3). By the way, lysine 79 is not on a histone tail. But it is on the surface of the nucleosome, as illustrated in Figure 13.33, and is accessible to the methylation machinery.

Finally, let us consider a regulatory interaction among modifications of three amino acids in the tail of histone H3: lysine 9, serine 10, and lysine 14. As we have seen, acetylation of lysine 14 is required for activation of some genes, including the human IFN-β gene. But, as we have also seen, this acetylation depends on phosphorylation of serine 10. Furthermore, phosphorylation of serine 10 is inhibited by methylation of lysine 9. Thus, methylation of lysine 9 can repress transcription by blocking phosphorylation of serine 10, thus blocking the needed acetylation of lysine 14. But the other side of the coin is that phosphorylation of serine 10, and probably acetylation of lysine 14, block methylation of lysine 9. Thus, once serine 10 and lysine 14 are appropriately modified, they tend to perpetuate the active state by preventing the repressive methylation of lysine 9. Moreover, acetylation of lysine 9 prevents methylation of the same residue, so that acetylation also works against repression. Figure 13.35 illustrates these interactions, interactions

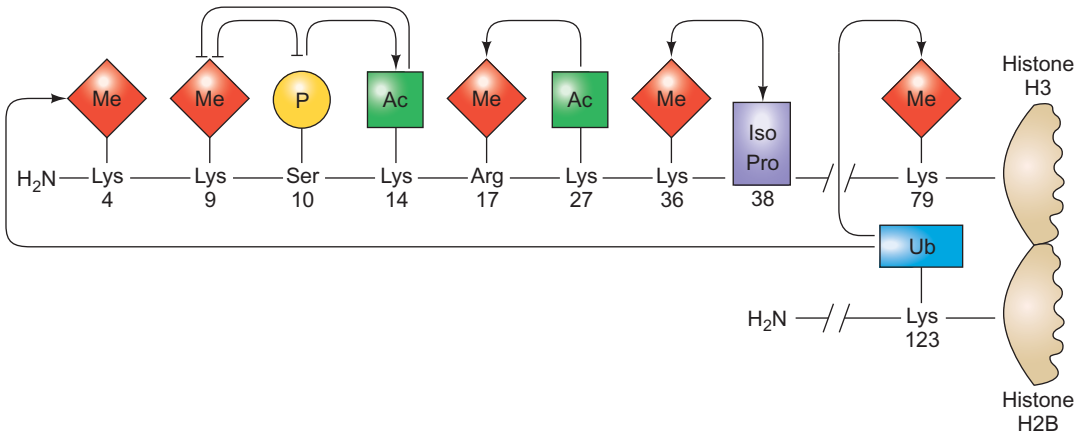


Figure 13.35 A model for the crosstalk among modifications on histone tails. The known interactions among modified residues on histones H3 and H2B are shown, but some crosstalk with at least histone H2A is also known. Activating interactions are shown with arrows, and inhibiting interactions are shown with a blocking symbol. For example, phosphorylation on serine 10 activates acetylation of lysine 14 and inhibits methylation of lysine 9. Me, methylation; Ac, acetylation; P, phosphorylation; Iso, proline isomeration; Ub, ubiquitylation.

with other histone H3 modifications, and crosstalk among modifications on histones H3 and H2A.

So far in this section we have learned that histone modifications can affect gene activity by two mechanisms: First, by altering the way histone tails interact with DNA and with histone tails in neighboring nucleosomes, and thereby altering nucleosome cross-linking. Second, by attracting proteins that can affect chromatin structure and activity. For example, acetylated lysines attract bromodomain proteins; methylated lysines attract proteins with chromodomains and chromo-like domains such as tudor and MBT, or other domains such as PHD fingers; and phosphorylated serines attract so-called **14-3-3 proteins** (this uninformative name derives from the electrophoretic mobilities of these proteins). These proteins frequently have catalytic activities of their own and can further modify histones or remodel chromatin. They can also recruit other proteins with their own activities.

For example, two of the subunits of the Rpd3C(S) histone deacetylase complex are the chromodomain protein Eaf3 and the PHD finger protein Rco1. Together, these proteins recognize histone H3 molecules methylated on lysine 36 downstream of promoters, and assure association of the Rpd3C(S) deacetylase with this downstream chromatin. The resulting histone deacetylation slows transcription elongation, which can be counteracted by one or more positive elongation factors. This deacetylation also prevents transcription initiation at any cryptic class II promoters that happen to lie within the body of the gene.

SUMMARY Methylation of lysine 9 in the N-terminal tail of histone H3 attracts the protein HP1, which in turn recruits a histone methyltransferase, which presumably methylates lysine 9 on a neighboring nucleosome, propagating the repressed, heterochromatic state. Methylation of other lysine and arginine side chains in the core histones can have either repressive or activating effects. These effects are achieved by proteins that recognize and bind to nucleosomes with specific patterns of histone methylation, and further modify the chromatin or directly affect transcription. Methylations occur in a given nucleosome in combination with other histone modifications, including acetylations, phosphorylations, and ubiquitylations. In principle, each particular combination can send a different message to the cell about activation or repression of transcription. A given histone modification can also influence other, nearby modifications.

Nucleosomes and Transcription Elongation

We have seen that nucleosomes must be absent from a gene's control region, or at least nudged aside as activators and general transcription factors bind to their respective DNA sites. But how does RNA polymerase deal with the nucleosomes that lie within the transcribed region of a gene?

The Role of FACT One important factor is a protein called FACT (facilitates chromatin transcription), which expedites elongation through nucleosomes by RNA polymerase II in vitro. Human FACT is composed of two polypeptides: the human homolog of the yeast Spt16 protein, and SSRP1, which is an HMG-1-like protein. FACT has been shown to interact strongly with histones H2A and H2B, which leads to the hypothesis that it can remove these two histones from nucleosomes, at least temporarily, and thereby destabilize the nucleosomes so RNA polymerase can transcribe through.

Several early lines of evidence supported this hypothesis. First, cross-linking the histones so none can be removed from the nucleosome blocks the action of FACT. Second, mutations in the yeast gene encoding histone H4 that alter histone–histone interactions have the same phenotype as mutations in the gene for the Spt16 subunit of FACT. Finally, actively transcribed chromatin is poor in histones H2A and H2B.

In 2003, Danny Reinberg and colleagues provided direct evidence that FACT facilitates chromatin transcription by RNA polymerase II by removing at least a histone H2A–H2B dimer from nucleosomes. They also showed that these proteins have a **histone chaperone** activity that can deposit histones back onto chromatin, reconstituting nucleosomes after the transcription machinery has passed through.

First, these workers used co-immunoprecipitation experiments to show that the Spt16 subunit of FACT binds to histone H2A–H2B dimers, and that the SSRP1 subunit binds to H3–H4 tetramers. The Spt16 subunit has a very acidic C-terminus, and Reinberg and colleagues demonstrated that recombinant FACT with an Spt16 subunit lacking this C-terminus (FACTΔC) can neither interact with histones in nucleosomes, nor facilitate transcription through chromatin.

Next, they labeled H2A–H2B dimers and H3–H4 tetramers with two different fluorescent tags. Then, after treatment with FACT or FACTΔC, they washed with buffer containing 350 mM KCl and detected the loss of dimers from nucleosomes by measuring the dimer/tetramer ratio by SDS-PAGE, followed by fluorimaging. (A fluorimager quantitatively measures the fluorescence of bands in a gel.) Figure 13.36 shows that FACT caused up to a 50% loss of H2A–H2B dimers from treated nucleosomes, but FACTΔC caused no more loss than washing with buffer alone (about 20%). Thus, FACT appears to weaken the association between H2A–H2B dimers and H3–H4 tetramers, and this effect depends on the C-terminus of the Spt16 subunit.

Reinberg and colleagues also demonstrated that FACT stimulated transcription through nucleosomes, and that the transcribed templates contained so-called hexasomes, which are nucleosomes lacking one H2A–H2B dimer.

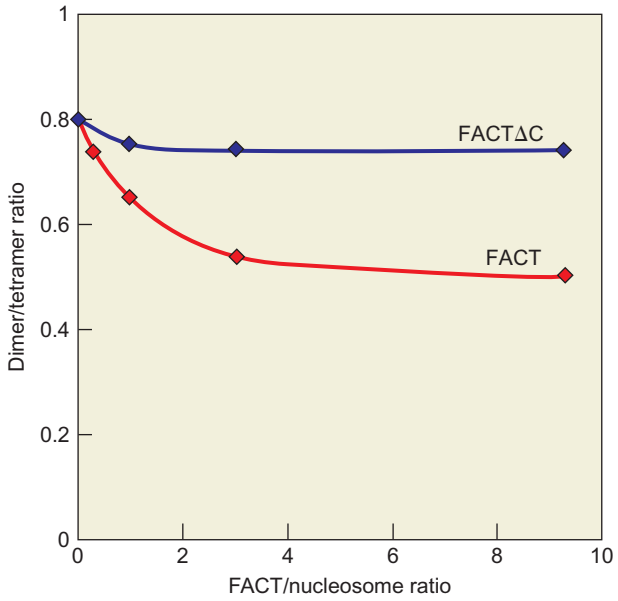


Figure 13.36 FACT stimulates loss of histone H2A–H2B dimers from nucleosomes. Reinberg and colleagues labeled H2A–H2B dimers and H3–H4 tetramers in nucleosomes with two different fluorescent tags, added FACT or FACTΔC for a one-hour incubation, then washed the nucleosomes to remove any loosely bound histones. Then they followed the loss of H2A–H2B dimers by measuring the ratio of dimers to tetramers using SDS-PAGE. The fluorescent tags were detected quantitatively in the SDS-PAGE gel with a fluorimager. (*Source:* Adapted from Belotserkovskaya, et al., *Science* 301, 2003, f. 3, p. 1092.)

To do this experiment, the investigators used a template with a single nucleosome positioned downstream of the transcription start site. They assembled transcription complexes on this template, and tethered the complexes to beads through a tag on RNA polymerase II. Then they carried out transcription with labeled nucleotides, in the presence of FACT or FACTΔC. When they electrophoresed the transcripts, they found that FACT, but not FACTΔC, stimulated transcription through the nucleosomes to form full-length run-off transcripts. That is, transcription with no FACT, or with FACTΔC, yielded a number of transcripts that stalled in the region of DNA involved in the nucleosome, but that FACT reduced such stalling, and yielded a higher percentage of full-length transcripts.

This experiment also allowed Reinberg and colleagues to examine the templates released, along with full-length run-off transcripts, from RNA polymerase. They labeled the DNA prior to transcription, and then electrophoresed the released templates, which had presumably been fully transcribed. These templates contained hexasomes if transcription was done in the presence of FACT, but not in the presence of FACTΔC. Furthermore, adding H2A and H2B back to the hexasomes converted them to full-size nucleosomes, indicating that the hexasomes really are nucleosomes lacking an H2A–H2B dimer. Thus, FACT appears to facilitate transcription through nucleosomes, at least in part, by loosening nucleosome structure enough to allow loss of at least one H2A–H2B dimer.

But, as we have mentioned, FACT is more than a nucleosome-disrupter. It can also deposit histones on DNA to reconstitute nucleosomes. Reinberg and colleagues demonstrated this histone chaperone effect of FACT with two experiments. First, they mixed core histones with labeled DNA with no FACT, FACT, or FACTΔC, and then electrophoresed the products. Without FACT, an aggregate formed that would not enter the electrophoretic gel. But with FACT, a well-behaved DNA-histone complex formed. Predictably, this complex did not form with FACTΔC. In the second experiment, Reinberg and colleagues labeled H2A–H2B dimers and H3–H4 tetramers with two different fluorescent tags, and then visualized the histone-DNA complexes on the electrophoretic gel with a fluorimager to see whether they contained the fluorescent tags associated with both sets of histones. Indeed they did, showing that FACT, but not FACTΔC, has histone chaperone activity. They also showed that neither of the FACT subunits alone has this activity.

If FACT really does play the role of a chromatin remodeler during transcription elongation, it should be found on chromatin along with RNA polymerase. Reinberg and John Lis and their colleagues demonstrated this behavior using the *Drosophila* heat shock gene *hsp70* as their experimental system. In the salivary gland cells of fruit fly larvae, the chromosomes replicate repeatedly without cell division, giving rise to large **polytene chromosomes,** with many sister chromatids packed side by side. These polytene chromosomes are visible with the aid of a light microscope, and active transcription sites are visible as swollen sites, or **chromosome puffs.** In particular, raising the temperature creates puffs at heat shock loci, such as *Hsp70*.

First, Reinberg and Lis isolated *Drosophila* polytene chromosomes before and after a 20-min heat shock and stained them with fluorescently labeled antibodies directed against RNA polymerase II and Spt16. After heat shock, the two antibodies co-localized over two chromosome puffs containing *hsp70* loci.

If FACT really does accompany RNA polymerase II, remodeling chromatin as transcription progesses, then FACT should be recruited to the heat shock gene as rapidly as polymerase II is, and it should be found downstream of promoter-associated transcription factors soon after transcription begins. To test this hypothesis, Reinberg, Lis, and colleagues examined chromatin stained with antibodies against the two subunits of FACT and against HSF, an activator that binds to the control region upstream of the *hsp70* gene. They looked before, and at 2.5 and 10 min after heat shock.

Figure 13.37 shows the results. Even at 2.5 min after heat shock, the two subunits of FACT are associated with the *hsp70* gene, just as HSF is. However, the FACT subunits

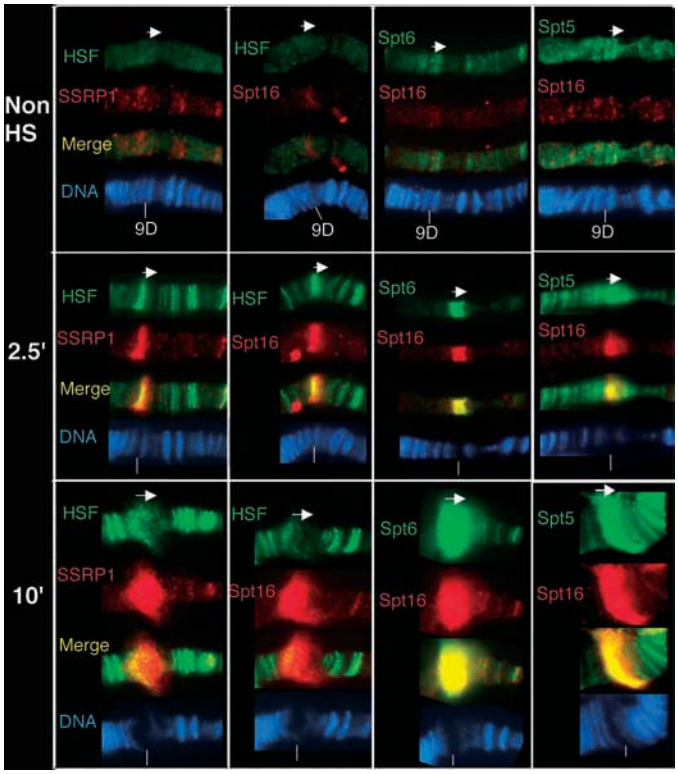


Figure 13.37 FACT is recruited rapidly to a transcribed gene and localizes downstream of an activator bound to the promoter. Reinberg, Lis, and colleagues stained *Drosophila* chromosomes with fluorescent antibodies in nonstimulated cells, and in cells 2.5 and 10 min after heat shock, as indicated at left. The antibodies used are indicated beside each stained chromosome, in the same color as the fluorescent antibody. Thus, the antibodies specific for HSF and Spt6 fluoresce green, and the antibody for SSRP1 and Spt16 fluoresce red. They also merged the two fluorescence images to check for overlap. Wherever the red and green fluorescence overlapped, it appeared yellow. Wherever there was not perfect overlap, some red fluorescence appeared to the right (downstream) of the yellow. This was especially evident in the merger of HSF and SSRP1 fluorescence at 10 min after heat shock (lower left panel). The chromosomes were also stained with Hoechst dye, which stains DNA violet (bottom of each panel.) (*Source:* Reprinted with permission from *Science,* Vol. 301, Abbie Saunders, Janis Werner, Erik D. Andrulis, Takahiro Nakayama, Susumu Hirose, Danny Reinberg, and John T. Lis, "Tracking FACT and the RNA Polymerase II Elongation Complex Through Chromatin in Vivo," Fig. 2, p. 1095. Copyright 2003, AAAS.)

are both located significantly further downstream than HSF. We can see this separation by comparing the red staining due to either SSRP1 or Spt16 and the green staining due to HSF. Separately, they are hard to distinguish, but when the two images are merged, we can see a leading edge of red (FACT fluorescence) downstream of the yellow, which corresponds to overlapping red (FACT) and green (HSF) fluorescence. This effect is also apparent 10 min after heat shock, especially with SSRP1.

By contrast, when another putative chromatin remodeler, Spt6, is stained with a green fluorescent tag, it colocalizes perfectly with FACT (see the yellow bands in the third panel either 2.5 or 10 min after heat shock). This behavior suggests that Spt6 and FACT both travel along with RNA polymerase II so they are in a position to help remodel chromatin to facilitate transcription.

SUMMARY FACT is a transcription elongation facilitator composed of two subunits, Spt16 and SSRP1. Spt16 binds to histone H2A–H2B dimers, and SSRP1 binds to H3–H4 tetramers. FACT can facilitate transcription through a nucleosome by promoting the loss of at least one H2A–H2B dimer from the nucleosome. It can also act as a histone chaperone by promoting the re-addition of an H2A–H2B dimer to a nucleosome that has lost such a dimer. The Spt16 subunit of FACT has an acid-rich C-terminus that is essential for both of these nucleosome remodeling activities.

The Role of PARP-1 The heat shock genes of *Drosophila* provide another example of removing nucleosomes to allow transcription. In 2008, Stephen Petesch and John Lis presented data elucidating the loss of nucleosomes from the *Hsp70* locus in *Drosophila* polytene chromosomes. They found that nucleosomes begin to disappear across the *Hsp70* locus only 30 s after heat shock, and this disappearance intensifies within two minutes. Thirty seconds is too short a time to allow for transcription of the whole locus, suggesting that loss of nucleosomes is not dependent on transcription. This hypothesis is supported by the finding that nucleosomes are lost even when transcription elongation is blocked by drugs. But nucleosome loss does require three proteins: heat shock factor (HSF), GAGA factor (discussed earlier in this chapter), and a **poly(ADP-ribose) polymerase (PARP)** known as **PARP1.**

PARP extracts ADP-ribose units from the substrate nicotinamide adenine dinucleotide (NAD) and links them together in a polymer [**poly(ADP-ribose), (PAR)**] attached through a glutamate carboxyl group to a protein, usually PARP itself (Figure 13.38). The polymer typically branches (by links between the ribose parts of the ADP-ribose units) every 40 to 50 units. The formation of PAR can be reversed by the enzyme **poly(ADP-ribose) glycohydrolase, (PARG)**, which breaks the bonds between ADP-ribose units.

How does PARP1 participate in nucleosome removal? First of all, PARP1 is able to bind to core nucleosomes much as histone H1 does, and that has a repressive effect. Activation of PARP1 causes it to poly(ADP-ribosyl)ate itself, which causes it to dissociate from nucleosomes, which should have an activating effect. Second, the PAR produced by PARP1 resembles a polynucleotide, particularly in its acidic nature. Thus, PAR can presumably compete with

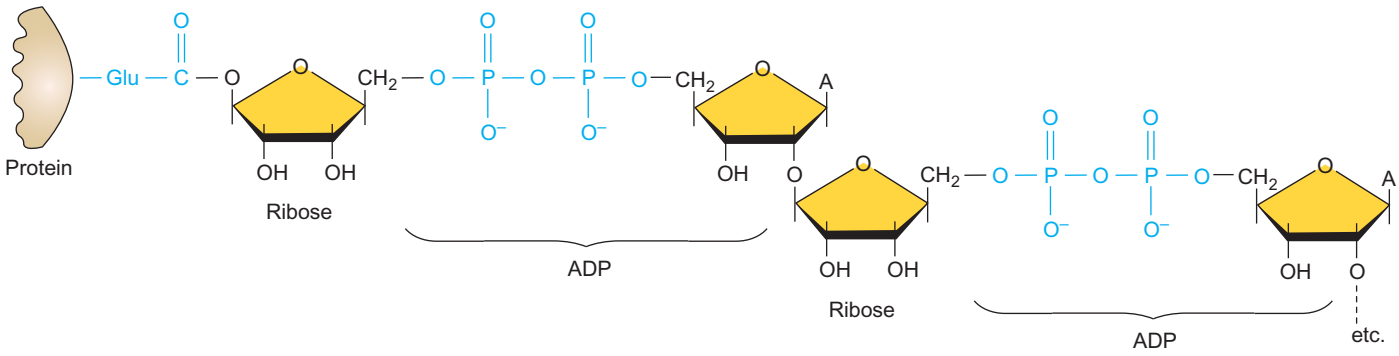


Figure 13.38 Poly(ADP-ribose). The first ADP-ribose unit is linked to a protein glutamate via an ester bond. The remaining ADP-ribose units are linked together via glycosidic bonds between the 2′-carbon of an ADP on one unit and the 1-carbon of the ribose on the next unit. The enzyme PARP forms these glycosidic linkages, and PARG breaks them.

DNA for association with the basic histones, thereby loosening the binding between histones and DNA and facilitating the breakup of nucleosomes.

SUMMARY Heat shock causes rapid loss of nucleosomes from chromatin in *Drosophila* polytene chromosome puffs. One of the agents required for this nucleosome loss is a poly(ADP-ribose) polymerase (PARP1). In response to heat shock, this enzyme poly(ADP-ribosyl)ates itself, removing it from its histone H1-like binding to core nucleosomes, thereby helping to destabilize the nucleosomes. Also, the poly(ADP-ribose), which is a polyanion, could bind directly to histones, further destabilizing the nucleosomes.

SUMMARY

Eukaryotic DNA combines with basic protein molecules called histones to form structures known as nucleosomes. These structures contain four pairs of histones (H2A, H2B, H3, and H4) in a wedge-shaped disc, around which is wrapped a stretch of 146 bp of DNA. Histone H1 is more easily removed from chromatin than the core histones and is not part of the core nucleosome.

In the second order of chromatin folding in vitro, and presumably also in vivo, a string of nucleosomes folds into a 30-nm fiber. Structural studies suggest that the 30-nm chromatin fiber in the nucleus exists in at least two forms: Inactive chromatin tends to have a high nucleosome repeat length (about 197 bp) and favors a solenoid folding structure. This kind of chromatin interacts with histone H1, which helps to stabilize its structure. Active chromatin tends to have a low nucleosome repeat length (about 167 bp) and folds according to the two-start double helical model.

The third order of chromatin condensation appears to involve formation of a radial loop structure in eukaryotic chromosomes. The 30-nm fiber seems to form loops between 35 and 85 kb long, anchored to the central matrix of the chromosome.

The core histones (H2A, H2B, H3, and H4) assemble nucleosome cores on naked DNA. Transcription of a class II gene in reconstituted chromatin with an average of one nucleosome core per 200 bp of DNA exhibits about 75% repression relative to naked DNA. The remaining 25% is due to promoter sites not covered by nucleosome cores. Histone H1 causes a further repression of template activity, in addition to that produced by core nucleosomes. This repression can be counteracted by transcription factors. Some, like Sp1 and GAL4, act as both antirepressors (preventing repression by histone H1) and as transcription activators. Others, like GAGA factor, are just antirepressors. The antirepressors presumably compete with histone H1 for binding sites on the DNA template.

Active genes tend to have DNase-hypersensitive control regions. At least part of this hypersensitivity is due to the absence of nucleosomes.

Histone acetylation occurs in both the cytoplasm and nucleus. Cytoplasmic acetylation is carried out by a HAT B and prepares histones for incorporation into nucleosomes. The acetyl groups are later removed in the nucleus. Nuclear acetylation is catalyzed by a HAT A and correlates with transcription activation. A variety of coactivators have HAT A activity, which may allow them to loosen the association of nucleosomes with each other and with a gene's control region. Acetylation of core histone tails also attracts bromodomain proteins such as TAF1, which are essential for transcription.

Transcription repressors such as unliganded nuclear receptors and Mad-Max bind to DNA sites and interact with corepressors such as NCoR/SMRT and SIN3, which in turn bind to histone deacetylases such as HDAC1 and 2. This assembly of ternary protein complexes brings the histone deacetylases close to nucleosomes in the

neighborhood. The deacetylation of core histones allows the basic tails of the histones to bind strongly to DNA and to histones in neighboring nucleosomes, stabilizing the nucleosomes and inhibiting transcription.

Activation of many eukaryotic genes requires chromatin remodeling. Several different protein complexes carry out this remodeling, and all of them have an ATPase that harvests the energy from ATP hydrolysis to use for remodeling. The remodeling complexes are distinguished by their ATPase component, and two of the best-studied complexes are SWI/SNF and ISWI. The SWI/SNF complex in mammals has BRG1 as its ATPase, and 9-12 BRG1-associated factors (BAFs). One of the highly conserved BAFs is called BAF 155 or 170. It has a SANT domain that appears to be responsible for histone binding. This would help SWI/SNF bind to nucleosomes. Members of the ISWI class of remodeling complexes have a SANT domain, and another domain called SLIDE that appears to be involved in DNA binding.

The mechanism of chromatin remodeling is not understood in detail, but it does involve mobilization of nucleosomes, with loosening of the association between DNA and core histones. In contrast to uncatalyzed DNA exposure in nucleosomes, or simple sliding of nucleosomes along a stretch of DNA, catalyzed remodeling of nucleosomes involves the formation of distinct conformations of the nucleosomal DNA with respect to the core histones.

ChIP analysis can reveal the order of binding of factors to a gene during activation. As the yeast *HO* gene is activated, the first factor to bind is Swi5, followed by SWI/SNF and SAGA, which contains the HAT Gcn5p. Next, the general transcription factors and other proteins bind. Thus, chromatin remodeling is among the first steps in activation of this gene, but the order can be different in other genes.

The pattern of core histone modifications in a given nucleosome appears to constitute a histone code that can determine what happens to the nucleosome. For example, the activators in the IFN-β enhanceosome can recruit a histone acetyltransferase, which acetylates some of the lysines on the tails of histones H3 and H4 at the promoter. One of the serines on histone H3 also becomes phosphorylated, which allows acetylation of another lysine on histone H3, completing the histone code. One of the acetylated lysines on histone H4 then recruits the SWI/SNF complex, which remodels the nucleosome. Then TFIID can bind to two acetylated lysines on histone H3. TFIID binding bends the DNA and moves the remodeled nucleosome aside, paving the way for transcription to begin.

Euchromatin is relatively extended and potentially active, whereas heterochromatin is condensed and genetically inactive. Heterochromatin can also silence genes as much as 3 kb away. Formation of heterochromatin at the tips of yeast chromosomes (telomeres) depends on binding of the protein RAP1 to telomeric DNA, followed by recruitment of the proteins SIR3, SIR4, and SIR2, in that order. Heterochromatin at other locations in the chromosome also depends on the SIR proteins. SIR3 and SIR4 also interact directly with histones H3 and H4 in nucleosomes. Acetylation of lysine 16 of histone H4 in nucleosomes prevents its interaction with SIR3 and therefore prevents heterochromatin formation. This is another way in which histone acetylation promotes gene activity.

Methylation of lysine 9 in the N-terminal tail of histone H3 attracts the protein HP1, which in turn recruits a histone methyltransferase, which presumably methylates lysine 9 on a neighboring nucleosome, propagating the repressed, heterochromatic state. Methylation of other lysine and arginine side chains in the core histones can have either repressive or activating effects, and these methylations occur in a given nucleosome in combination with other histone modifications, including acetylations, phosphorylations, and ubiquitylations. In principle, each particular combination can send a different message to the cell about activation or repression of transcription. A given histone modification can also influence other, nearby modifications.

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Heat shock causes rapid loss of nucleosomes from chromatin in *Drosophila* polytene chromosome puffs. One of the agents required for this nucleosome loss is a poly(ADP-ribose) polymerase (PARP1). In response to heat shock, this enzyme poly(ADP-ribosyl)ates itself, removing it from its histone H1-like binding to core nucleosomes, thereby helping to destabilize the nucleosomes. Also, the poly(ADP-ribose), which is a polyanion, could bind directly to histones, further destabilizing the nucleosomes.

REVIEW QUESTIONS

1. Diagram a nucleosome as follows: (a) On a drawing of the histones without the DNA, show the rough positions of all the histones. (b) On a separate drawing, show the path of DNA around the histones.
2. Cite electron microscopic evidence for a six- to sevenfold condensation of DNA in nucleosomes.

3. Cite electron microscopic evidence for formation of a condensed fiber (30-nm fiber) at high ionic strength.
4. Diagram the solenoid model of the 30-nm chromatin fiber.
5. Diagram the structure of a tetranucleosome revealed by x-ray crystallography. What structure for the 30-nm fiber does this tetranucleosome structure suggest?
6. How can single-molecule force spectroscopy shed light on the structure of the 30-nm chromatin fiber? What conclusions does it suggest?
7. Draw a model to explain the next order of chromatin folding after the 30-nm fiber. Cite biochemical and microscopic evidence to support the model.
8. Describe and give the results of an experiment that shows the competing effects of histone H1 and the activator GAL4-VP16 on transcription of the adenovirus E4 gene in reconstituted chromatin.
9. Present two models for antirepression by transcription activators, one in which the gene's control region is not blocked by a nucleosome, the other in which it is.
10. Describe and give the results of an experiment that shows that the nucleosome-free zone in active SV40 chromatin lies at the viral late gene control region.
11. Describe and give the results of an experiment that shows that the zone of DNase hypersensitivity in SV40 chromatin lies at the viral late gene control region.
12. Diagram and describe a general technique for detecting a DNase-hypersensitive DNA region.
13. Describe and give the results of an activity gel assay that shows the existence of a histone acetyltransferase (HAT) activity.
14. Present a model for the involvement of a corepressor and histone deacetylase in transcription repression.
15. Describe and give the results of an epitope-tagging experiment that shows interaction among the following three proteins: the repressor Mad1, the corepressor SIN3A, and the histone deacetylase HDAC2.
16. Present a model for activation and repression by the same protein, depending on the presence or absence of that protein's ligand.
17. Present models for uncatalyzed nucleosomal DNA exposure and for catalyzed nucleosome remodeling. Present evidence for the catalyzed model.
18. Describe how you could use a chromatin immunoprecipitation procedure to detect the proteins associated with a particular gene at various points in the cell cycle.
19. Describe and give the results of an experiment using chromatin immunoprecipitation to discover the timing of acetylation and phosphorylation of particular sites on core histones in a nucleosome at the IFN-β promoter.
20. Describe and give the results of an experiment to measure recruitment of SWI/SNF and TFIID to the IFN-β promoter with wild-type and mutant histones.
21. Present a model depicting the establishment and decoding of a histone code at the IFN-β promoter.
22. Present a model to explain why lysine 16 in histone H4 is thought to be critical for silencing. What evidence supports this hypothesis?
23. Present a model depicting the spread of chromatin repression via histone methylation.
24. Present a model of the interactions among the modifications of lysines 9 and 14, and serine 10 in the N-terminal tail of histone H3. Show both positive and negative interactions.
25. Present evidence that FACT causes a loss of histone H2A–H2B dimers from nucleosomes, and that this activity depends on the C-terminus of the Spt16 subunit of FACT.

ANALYTICAL QUESTIONS

1. If the globin locus did have the same DNase-hypersensitive sites in J6 cells as in HEL cells, approximately what size fragments would have been detected in Figure 13.20d? Which hypersensitive sites would not be detected?
2. Explain why brief digestion of eukaryotic chromatin with micrococcal nuclease gives DNA fragments about 200 bp long, but longer digestion yields 146-bp fragments.
3. The amino acid sequences of the core histones are highly conserved between plants and animals. Present a hypothesis to explain this finding.
4. Type A histone acetyltransferases (HAT A's) contain a bromodomain and HAT B's do not. What do you predict would occur if HAT A's were missing this bromodomain? What if HAT B's possessed this bromodomain? If the bromodomains were reversed so that all HAT B's gained bromodomains and all HAT A's lost them, would HAT A's take over the role of HAT B's and vice versa? Why or why not? How would you answer this question experimentally?

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