DNA Structure: Alternate Forms Of The Double Helix

The Watson–Crick double-helix structure described is called **B-DNA**. B-DNA is the conformation that DNA takes under physiological conditions (in aqueous solutions containing low concentrations of salts). The vast majority of the DNA molecules present in the aqueous protoplasms of living cells exist in the B conformation. However, DNA is not a static, invariant molecule. On the contrary, DNA molecules exhibit considerable conformational flexibility. The structures of DNA molecules change as a function of their environment. The exact conformation of a given DNA molecule or segment of a DNA molecule will depend on the nature of the molecules with which it is interacting. In fact, intracellular B-DNA appears to have an average of 10.4 nucleotide pairs per turn. The helix diameter is 1.9 nm.

In high concentrations of salts or in a partially dehydrated state, DNA exists as **A-DNA**, which is a right-handed helix like B-DNA, but with 11 nucleotide pairs per turn. A-DNA is a shorter, thicker double helix with a diameter of 2.3 nm. DNA molecules almost certainly never exist as A-DNA *in vivo*. However, the A-DNA conformation is important because DNA-RNA heteroduplexes (double helices containing a DNA strand base-paired with a complementary RNA strand) or RNA-RNA duplexes exist in a very similar structure *in vivo*.

Certain DNA sequences have been shown to exist in a **left-handed**, double-helical form called **Z-DNA** (Z for the zigzagged path of the sugar phosphate backbones of the structure). Z-DNA was discovered by X-ray diffraction analysis of crystals formed by DNA oligomers containing alternating G:C and C:G base pairs. Z-DNA occurs in double helices that are G:C-rich and contain alternating purine and pyrimidine residues. In addition to its unique left-handed helical structure, Z-DNA differs from the A and B conformations in having 12 base pairs per turn, a diameter of 1.8 nm, and a single deep groove. The function of Z-DNA in living cells is still not clear.

Chromosome Structure in Eukaryotes

Eukaryotic genomes contain levels of complexity that are not encountered in prokaryotes. Most eukaryotes are diploid, having two complete sets of genes, one from each parent. DNA is packaged into several chromosomes, and each chromosome is present in two (diploids) or more (polyploids) copies. The haploid chromosome complement, or genome, of a human contains about 1000 mm of DNA (or about 2000 mm per diploid cell). Moreover, this meter of DNA is subdivided among 23 chromosomes of variable size and shape, with each chromosome containing 15 to 85 mm of DNA.

Chemical Composition of Eukaryotic Chromosomes

Interphase chromosomes are usually not visible with the light microscope. However, chemical analysis, electron microscopy, and X-ray diffraction studies of isolated chromatin (the complex of the DNA, chromosomal proteins, and other chromosome constituents

isolated from nuclei) provide information about the structure of eukaryotic chromosomes. When chromatin is isolated from interphase nuclei, the individual chromosomes are not recognizable. Instead, one observes an irregular aggregate of nucleoprotein.

Chemical analysis of isolated chromatin shows that it consists primarily of DNA and proteins with lesser amounts of RNA. The proteins are of two major classes:

(1) basic (positively charged at neutral pH) proteins called histones

(2) a heterogeneous, largely acidic (negatively charged at neutral pH) group of proteins collectively referred to as nonhistone chromosomal proteins.

Histones play a major structural role in chromatin.

- They are present in the chromatin of all eukaryotes in amounts equivalent to the amounts of DNA. This relationship suggests that an interaction occurs between histones and DNA that is conserved in eukaryotes.
- The histones of all plants and animals consist of five classes of proteins. These five major histone types, called H1, H2a, H2b, H3, and H4, are present in almost all cell types. A few exceptions exist, most notably some sperm, where the histones are replaced by another class of small basic proteins called protamines.
- The five histone types are present in molar ratios of approximately 1 H1:2 H2a:2 H2b:2 H3:2 H4. Four of the five types of histones are specifically complexed with DNA to produce the basic structural subunits of chromatin, small (approximately 11 nm in diameter by 6.5 nm high) ellipsoidal beads called nucleosomes.
- The histones have been highly conserved during evolution—four of the five types of histone are similar in all eukaryotes.
- The histones are basic because they contain 20 to 30 percent arginine and lysine, two positively charged amino acids. The exposed $-NH^{3+}$ groups of arginine and lysine allow histones to act as polycations.
- The positively charged side groups on histones are important in their interaction with DNA, which is polyanionic because of the negatively charged phosphate groups.
- They are important in chromatin structure (DNA packaging) and are only nonspecifically involved in the regulation of gene expression.

In contrast, the nonhistone protein fraction of chromatin consists of a large number of heterogeneous proteins. Moreover, the composition of the nonhistone chromosomal protein fraction varies widely among different cell types of the same organism. Thus, the nonhistone chromosomal proteins probably do not play central roles in the packaging of DNA into chromosomes. Instead, they are likely candidates for roles in regulating the expression of specific genes or sets of genes.

✓ A typical eukaryotic chromosome contains 1 to 20 cm (10^4 to 2 x 10^5 m) of DNA. During metaphase of meiosis and mitosis, this DNA is packaged in a chromosome with a length of

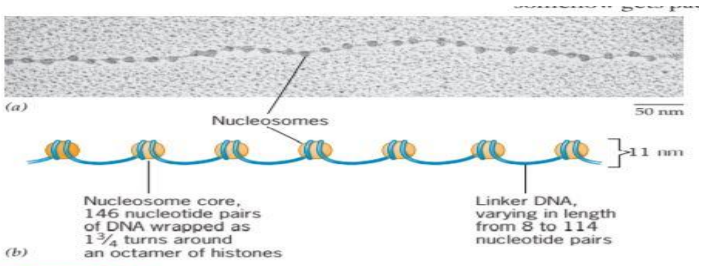
only 1 to 10 m. Each chromosome contains a single, giant molecule of DNA that extends from one end through the centromere all the way to the other end of the chromosome.

Three levels of DNA packaging in eukaryotic chromosomes

The largest chromosome in the human genome contains about 85 mm (85,000 m, or 8.5 x 10^7 nm) of DNA that is believed to exist as one giant molecule. This DNA molecule somehow gets packaged into a metaphase structure that is about 0.5 m in diameter and about 10 m in length—a condensation of almost 10^4 -fold in length from the naked DNA molecule to the metaphase chromosome.

When isolated chromatin from interphase cells is examined by electron microscopy, it is found to consist of a series of ellipsoidal beads (about 11 nm in diameter and 6.5 nm high) joined by thin threads.

Further evidence for a regular, periodic packaging of DNA has come from studies on the digestion of chromatin with various nucleases. Partial digestion of chromatin with these nucleases yielded fragments of DNA in a set of discrete sizes that were integral multiples of the smallest size fragment. Chromatin has a repeating structure, supposedly the bead seen by electron microscopy, within which the DNA is packaged in a nuclease-resistant form. This "bead" or chromatin subunit is called the nucleosome.

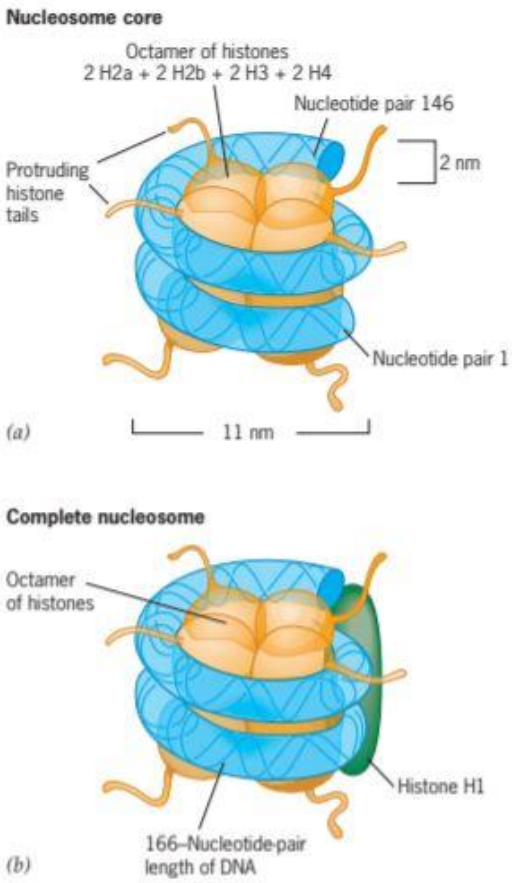


■ **FIGURE 9.17** Electron micrograph (*a*) and low-resolution diagram (*b*) of the beads-on-a-string nucleosome substructure of chromatin isolated from interphase nuclei. *In vivo*, the DNA linkers are probably wound between the nucleosomes forming a condensed 11-nm fiber.

The linkers, or interbead threads of DNA, are susceptible to nuclease attack. After partial digestion of the DNA in chromatin with an endonuclease (an enzyme that cleaves DNA internally), DNA approximately 200 nucleotide pairs in length is associated with each nucleosome (produced by a cleavage in each linker region). After extensive nuclease digestion, a 146-nucleotide-pair-long segment of DNA remains present in each nucleosome. This nuclease-resistant structure is called the nucleosome core. Its structure—essentially invariant in eukaryotes—consists of a 146-nucleotide-pair length of DNA and two molecules each of histones H2a, H2b, H3, and H4. The histones protect the segment of DNA in the nucleosome core from cleavage by endonucleases. Physical studies (X-ray diffraction and similar analyses) of nucleosome-core crystals have shown that the DNA is wound as 1.65 turns of a superhelix around the outside of the histone octamer.

The complete chromatin subunit consists of the nucleosome core, the linker DNA, and the associated nonhistone chromosomal proteins, all stabilized by the binding of one molecule of histone H1 to the outside of the structure. Linkers as short as eight nucleotide pairs and as long as 114 nucleotide pairs have been reported. The complete nucleosome (as opposed to the nucleosome core) contains two full turns of DNA superhelix (a 166-nucleotide-pair length of DNA) on the surface of the histone octamer and the stabilization of this structure by the binding of one molecule of histone H1.

The structure of nucleosomes in transcriptionally active regions of chromatin is known to differ from that of nucleosomes in transcriptionally inactive regions. The tails of some of the histone molecules protrude from the nucleosome and are accessible to enzymes that add and remove chemical groups such as methyl (—CH_3) and acetyl groups. The addition of these groups can change the level of expression of genes packaged in nucleosomes containing the modified histones.



■ **FIGURE 9.18** Diagrams of the gross structure of (*a*) the nucleosome core and (*b*) the complete nucleosome. The nucleosome core contains 146 nucleotide pairs wound as 1.65 turns of negatively supercoiled DNA around an octamer of histones—two molecules each of histones H2a, H2b, H3, and H4. The complete nucleosome contains 166 nucleotide pairs that form almost two superhelical turns of DNA around the histone octamer. One molecule of histone H1 is thought to stabilize the complete nucleosome.

Electron micrographs of isolated metaphase chromosomes show masses of tightly coiled or folded lumpy fibers. These chromatin fibers have an average diameter of 30 nm. The basic structural unit of the metaphase chromosome is the 30-nm chromatin fiber. What is the substructure of the 30-nm fiber seen in chromosomes? The two most popular models are the solenoid model and the zigzag model. *In vivo*, the nucleosomes clearly interact with one another to condense the 11-nm nucleosomes into 30-nm chromatin fibers. Whether these have solenoid structures or zigzag structures, or both, depending on the conditions, is still uncertain. What is certain is that chromatin structure is not static; chromatin can expand and contract in response to chemical modifications of histone H1 and the histone tails that protrude from the nucleosomes.

Metaphase chromosomes are the most condensed of normal eukaryotic chromosomes. Clearly, the role of these highly condensed chromosomes is to organize and package the giant DNA molecules of eukaryotic chromosomes into structures that will facilitate their segregation to daughter nuclei without the DNA molecules of different chromosomes becoming entangled and, as a result, being broken during the anaphase separation of the daughter chromosomes. There is evidence that the gross structure of metaphase chromosomes is not dependent on histones. Electron micrographs of isolated metaphase chromosomes from which the histones have been removed reveal a scaffold, or central core, which is surrounded by a huge pool or halo of DNA. This chromosome scaffold must be composed of nonhistone chromosomal proteins.

In summary, at least three levels of condensation are required to package the 10^3 to 10^5 m of DNA in a eukaryotic chromosome into a metaphase structure a few microns long.

1. The first level of condensation involves packaging DNA as a negative supercoil into nucleosomes, to produce the 11-nm-diameter interphase chromatin fiber. This clearly involves an octamer of histone molecules, two each of histones H2a, H2b, H3, and H4.

2. The second level of condensation involves an additional folding or supercoiling of the 11-nm nucleosome fiber, to produce the 30-nm chromatin fiber. Histone H1 is involved in this supercoiling of the 11-nm nucleosome fiber to produce the 30-nm chromatin fiber.

3. Finally, nonhistone chromosomal proteins form a scaffold that is involved in condensing the 30-nm chromatin fiber into the tightly packed metaphase chromosomes. This third level of condensation appears to involve the separation of segments of the giant DNA molecules present in eukaryotic chromosomes into independently supercoiled domains or loops. The mechanism by which this third level of condensation occurs is not known.