Chapter 23 Nucleic Acid Structure

This chapter takes a closer look at the structures of nucleic acids, which were first introduced in Chapter 3. This chapter begins by examining the structure of DNA in some detail, focusing on the different helical conformations and the limited flexibility of DNA that results from restrictions on rotation of various covalent bonds. Next, you are introduced to supercoiling, a structural feature of DNA that has important consequences for the biological activity of DNA. The forces that stabilize double helical structures are considered next. Knowledge of these forces is central to understanding the techniques used to isolate, analyze, and manipulate nucleic acids.

The chapter then turns to the interactions of DNA with proteins, giving as examples the restriction endonucleases and well-characterized prokaryotic and eukaryotic sequence-specific regulators of transcription. These transcription factors include the repressor from bacteriophage 434, the *E. coli trp* repressor, and the *E. coli met* repressor. The eukaryotic transcription factors that are discussed are proteins that contain the zinc finger DNA-binding motif and the leucine zipper dimerization motif (including those containing the helix–loop–helix motif near the DNA-binding region. In all cases, your attention is drawn to the common molecular interactions found in all of these proteins.

Finally, the structure of eukaryotic chromosomes is considered. This section explores how the histone proteins interact with DNA to generate higher-order structures that condense DNA over 50,000-fold. First, the structure of the nucleosome is discussed. This is followed by descriptions of the 300-Å filament of chromatin and the organization of highly condensed metaphase chromosomes.

Essential Concepts

1. DNA and RNA are the two kinds of nucleic acids that store genetic information and make it available to the cell. These molecules must therefore have the following properties:
   (a) Genetic information must be stored in a form that is stable and manageable in size.
   (b) Genetic information must be decoded by transcription and translation, which together convert nucleic acid sequences into protein sequences.
   (c) The information in DNA or RNA must be accessible to proteins and other nucleic acids.
   (d) Replication, in the case of DNA, must be template-driven so that each daughter cell receives the same genetic information.

The DNA Helix

2. DNA occurs in three major structural forms called A-DNA, B-DNA, and Z-DNA. B-DNA is the most common form and has the structural form first described by Watson and Crick.

3. The key structural features of B-DNA include:
   (a) The two antiparallel strands wind in a right-handed manner around a common axis, producing a double helix that is about 20 Å in diameter.

(b) Pairs of nucleotide bases are nearly perpendicular to the axis of the helix. The base pairs are in the interior of the double helix, while the sugar–phosphate backbone is on the outside, thus giving the appearance of a spiral staircase.
(c) The "ideal" B-DNA double helix has ten base pairs (bp) per turn with a pitch of about 34 Å.
(d) The double helix contains a wide and deep major groove and a narrow and deep minor groove.

4. A-DNA is a wider and flatter right-handed helix than B-DNA. The planes of its base pairs are tilted about 20° to the helix axis. Its major groove is deep and narrow, while its minor groove is shallow and wide. A-DNA forms under dehydrating conditions.

5. Z-DNA is a left-handed DNA helix that contains a deep minor groove but no discernible major groove. It forms *in vitro* in nucleic acids containing alternating purines and pyrimidines, but its existence *in vivo* is uncertain.

6. RNA is single-stranded but can form regions of double helix by folding back on itself. 2′-OH groups preclude B-form structures; instead, double helical regions assume conformations resembling A-DNA. DNA–RNA hybrids also show A-form conformations.

7. Segments of B-DNA deviate from the ideal conformation, often in a sequence-dependent manner, which may be important for sequence-specific recognition of DNA by proteins involved in regulating specific gene activity.

8. The conformational flexibility of DNA is limited by steric hindrance at the glycosidic bond between the nitrogenous base and the ribose moiety. Steric hindrance also induces a specific pucker in the ribose and restricts rotation in the bonds of the sugar–phosphate backbone.

9. The twisting of a double helix that is often observed in covalently closed, circular, double-stranded DNA is called supercoiling or superhelicity. A key topological property of a closed circular double helix is that the number of supercoils cannot be altered without first cutting at least one strand of DNA. In the mathematical relationship

$$L = T + W$$

L is the linking number (the number of times that one strand of the double helix winds around the other), T is the twist (the number of complete revolutions that one strand makes around the axis of the double helix), and W is the writhing number (the number of turns the duplex axis makes around the superhelix axis, a measure of the supercoiling of the circular DNA). When $W < 0$, the DNA is negatively supercoiled; when $W > 0$, the DNA is positively supercoiled. Naturally occurring DNA is negatively supercoiled, which promotes strand separation for processes such as DNA replication and transcription.

10. Topoisomerases alter DNA supercoiling by making transient single-strand breaks (Type I) or double-strand breaks (Type II). Prokaryotic Type I topoisomerases relax negative

supercoils by increasing the linking number in increments of one. Eukaryotic Type I topoisomerases can relax positive supercoils as well. Type II topoisomerases of both prokaryotes and eukaryotes relax negative and positive supercoils in an ATP-dependent manner. Only the prokaryotic version (also called DNA gyrase) can introduce negative supercoils. Negative supercoils in eukaryotes result primarily from packaging DNA into nucleosomes.

Forces Stabilizing Nucleic Acid Structures

11. Like the denaturation of proteins, the denaturation of DNA is cooperative; i.e., the unwinding of one part of DNA destabilizes the remaining double helix. This can be measured by observing the increase in ultraviolet light absorbance in going from double-stranded to single-stranded DNA. The midpoint of this "melting" curve is called the melting temperature, T_m.

12. The T_m of double-stranded DNA depends on the solvent, the kind and concentration of ions in solution, the pH, and the mole fraction of G · C base pairs.

13. While base pairing provides specificity to the structure of DNA, it contributes little to the stability of DNA. Hydrophobic interactions (which tend to cause free base pairs to aggregate) and van der Waals interactions between base pairs (called stacking interactions) contribute the most to the stability of double-stranded nucleic acids. Stacking interactions between G · C base pairs are stronger than those between A · T base pairs. Hence, the greater stability of GC-rich DNA is a reflection not of the greater number of hydrogen bonds but of greater stacking energies.

14. Most RNA is single-stranded and adopts a much wider variety of shapes than does DNA. These varied shapes are due to various sections of RNA forming double-stranded regions via specific base pairing. For example, ribosomal RNA (rRNA) is about 46% double-stranded. The compact shape of transfer RNA (tRNA) is a result of base pairing and extensive stacking interactions.

15. RNA also has catalytic activity. An RNA found in certain plant viruses, called the hammerhead ribozyme, catalyzes the cleavage of a specific RNA during posttranscriptional processing. Synthetic versions of ribozymes can catalyze many other reactions, including phosphoryl group transfer, isomerization at C—C covalent bonds, and hydrolytic reactions.

Fractionation of Nucleic Acids

16. DNA and RNA in cells are invariably associated with proteins, so nucleic acid preparations must be deproteinized as part of their purification. Deproteinization can be accomplished by treating cell lysates with detergents, chaotropic agents, or proteases. The nucleic acid can then be recovered by precipitation with ethanol.

17. Double-stranded DNA can be separated from single-stranded nucleic acid, nucleotides, and other soluble molecules by chromatography using a column of hydroxyapatite, a form of calcium phosphate.

18. Eukaryotic messenger RNA (mRNA) can be separated from total RNA (of which it constitutes no more than 5% by mass), DNA, nucleotides, and other soluble molecules by affinity chromatography. Most eukaryotic mRNAs contain a poly(A) sequence at their 3′ ends, which is not found in rRNA or tRNA. Poly(U) or poly(T) covalently attached to a cellulose or agarose matrix can bind eukaryotic mRNA by complementary base pairing under high salt conditions; the mRNA is subsequently recovered by exposing the matrix to a low salt buffer.

19. Small nucleic acids (< 1000 nucleotides) can be easily resolved by polyacrylamide gel electrophoresis. Larger nucleic acids (up to 100,000 bp) can be separated using less cross-linked agarose gels. For extremely large DNAs (up to 10^7 bp), pulsed-field gel electrophoresis is used. Nucleic acids can be visualized in these gels by adding planar aromatic cations such as ethidium bromide, acridine orange, or proflavin. These dyes are called intercalation agents because they intercalate between the stacked bases, where they exhibit much greater fluorescence than do the free dyes.

20. Equilibrium density gradient centrifugation in CsCl can be used to separate double-stranded DNA from the denser single-stranded DNA and RNA. Different-sized RNA can be separated by zonal ultracentrifugation through a sucrose gradient.

DNA–Protein Interactions

21. Many proteins bind DNA nonspecifically, primarily through interactions between the protein's functional groups and the sugar–phosphate backbone of DNA. Histones and certain DNA replication proteins are examples of such proteins.

22. Sequence-specific DNA-binding proteins usually interact with base pairs in the major groove of DNA by hydrogen bonding, either directly or indirectly via intervening water molecules. Ionic interactions with the sugar–phosphate backbone also occur, probably to facilitate contact between these proteins and DNA, after which the DNA can be "scanned" for a specific binding site. Dissociation constants for sequence-specific DNA-binding proteins are 10^3 to 10^7 times lower than those for nonspecific DNA-binding proteins.

23. Prokaryotic sequence-specific DNA-binding proteins recognize base pairs directly or through indirect readout, in a manner that depends on the sequence-dependent conformation and/or flexibility of the DNA backbone. Many DNA-binding proteins recognize palindromic (or nearly palindromic) sequences.

24. A large class of eukaryotic sequence-specific DNA-binding proteins are transcription factors. Many of these proteins form dimers and promote transcription at specific sites.

25. Many eukaryotic transcription factors contain structural motifs called zinc fingers. Zinc fingers are compact ~30 residue modules containing one or two Zn^{2+} ions liganded by His and/or Cys residues.

26. Another type of eukaryotic transcription factor contains a structural motif called the leucine zipper. The leucine zipper is a seven-residue pseudorepeating unit $(a\text{-}b\text{-}c\text{-}d\text{-}e\text{-}f\text{-}g)_n$ in which a hydrophobic strip along one side of the α helix formed by these residues promotes dimerization. The DNA-binding domain of such proteins is usually quite separate from the dimerization domain.

Eukaryotic Chromosome Structure

27. Prokaryotic genomes typically consist of a single circular DNA molecule ranging from several hundred thousand bp to several million bp.

28. The 23 chromosomes of the 3-billion-bp human genome have a total extended length of nearly one meter. Individual human chromosomes, with extended lengths between 1.6 and 8.2 cm, are condensed to varying degrees in different cells at different times, down to 1.3 to 10 μm long during mitosis.

29. Eukaryotic DNA is packaged into units called nucleosomes. Each nucleosome contains an octamer of four different basic proteins called histones $[(H2A)_2(H2B)_2(H3)_2(H4)_2]$ and 146 bp of DNA. Approximately 55 bp of DNA (called linker DNA) links two nucleosomes and is associated with a fifth histone, H1.

30. Histones are basic proteins with a large proportion of Arg and Lys residues, which interact with the phosphate oxygens of the sugar–phosphate backbone via salt bridges, hydrogen bonds, and helix dipoles.

31. The winding of DNA in nucleosomes to form the beaded chain seen in electron micrographs reduces its length by sevenfold. Further reduction of length is achieved by coiling the beaded chain into an ~300-Å-diameter filament. Higher-order structures, requiring nonhistone proteins are less well characterized. The most condensed structure is the metaphase chromosome, which has a central scaffold of fibrous protein that anchors loops of DNA and has an overall diameter of ~1.0 μm.

32. Prokaryotic DNA is also packaged into nucleosome-like structures with highly basic proteins that functionally resemble histones.

Key Equation

$$L = T + W$$

Guide to Study Exercises (text p. 771)

1. See Table 23-1.

2. RNA contains the pentose ribose, whereas DNA has 2′-deoxyribose. Because the 2′-OH group prevents RNA from assuming a B-DNA-like conformation, it adopts a shallower, wider, A-DNA-like helix. Furthermore, because RNA is usually single-stranded, it tends to fold back on itself to satisfy hydrogen-bonding requirements, rather than forming a more linear rodlike molecule typical of double-stranded DNA. Consequently, RNA molecules, which are generally smaller than DNA molecules, exhibit greater conformational variety than DNA and can therefore perform a wider variety of functions than can DNA. (Sections 23-1A and 23-2E)

3. Type I topoisomerases create a single-strand break in DNA through which a DNA strand (or double-stranded DNA) is passed before the nick is sealed. The nicking and closing reactions require no additional source of free energy since the energy of the phosphodiester bond is conserved in the formation of a covalent enzyme–DNA intermediate. Repeated nicking and closing causes supercoiled DNA to fully relax. Prokaryotic Type I topoisomerase can relax only negatively supercoiled DNA; the eukaryotic enzyme can relax both positively and negatively supercoiled DNA.

   Type II topoisomerases cleave both strands of a double-stranded DNA, pass double-stranded DNA through the break, and then reseal the break. These reactions require ATP, whose binding and subsequent hydrolysis provide the free energy that drives enzyme conformational changes required for catalysis. Prokaryotic Type II topoisomerases relax both negative and positive supercoils and can induce negative supercoils into DNA. Eukaryotic Type II topoisomerases can only relax supercoils. (Section 23-1C)

4. During denaturation, the base pairs in nucleic acids separate, yielding single strands that have no fixed conformation. This flexibility results in a decrease in viscosity in the case of a DNA solution. UV absorbance increases by ~40% due to the disruption of electronic interactions among neighboring bases. DNA denaturation typically occurs over a narrow temperature range, because denaturation is a cooperative process in which melting in one part of the molecule destabilizes the rest of the molecule. RNA denaturation is not highly cooperative, because RNA molecules tend to contain many relatively short double-stranded regions that melt independently.

   During renaturation, hydrogen bonds form between complementary regions between strands (in DNA) or between segments of the same strand (in RNA). The temperature must be high enough (only ~25°C below T_m) to provide enough thermal energy to allow short base-paired regions to separate and reanneal until the proper base-pairing interactions are established along the length of the molecule. (Section 23-2A)

5. Nucleic acids are stabilized primarily by Watson–Crick base pairing and van der Waals interactions between stacked bases, particularly G and C bases. Divalent cations, which shield the negative charges of adjacent phosphates, also stabilize nucleic acids. (Section 23-2)

6. Some proteins interact with DNA nonspecifically, that is, through recognition of its overall geometry and sugar–phosphate backbone, without regard to its sequence of bases. Other proteins interact with DNA in a sequence-specific manner, by making contacts with functional groups on the bases or by recognizing subtle base-specific variations in backbone conformation. DNA–protein interactions include hydrogen bonds, often with bridging water molecules, and ionic interactions. Both the protein and the nucleic acid may change conformation on binding. (Section 23-4)

7. A zinc finger unit of a eukaryotic transcription factor binds a short segment of DNA. Typically, multiple zinc fingers are arranged in tandem so that the protein recognizes an extended sequence of bases. The transcription factor often wraps around the DNA, following the surface grooves. Most prokaryotic repressors have a homodimeric structure and thereby recognize DNA sequences that have perfect or nearly perfect palindromic symmetry. Often the repressor interacts with bases in the major grove that are one turn apart. (Sections 23-4B and C)

8. Eukaryotic DNA is dramatically condensed in chromatin, first by being wrapped around a histone octamer to form a nucleosome core particle, which is brought closer to its neighbors through interactions with histone H1. Nucleosomes are then folded in a zigzag fashion to form a 300-Å-diameter solenoid (coil). These filaments are attached as loops to a protein scaffold, producing a metaphase chromosome with a diameter of 1.0 μm. (Section 23-5)

Questions

The DNA Helix

1. What form of DNA might you expect to see in desiccated (but viable!) brine shrimp eggs? Why?

2. Double-stranded DNA is relatively stiff, whereas single-stranded DNA is a flexible coil. What factors influence the structure of single-stranded versus double-stranded DNA?

3. How does the ribose pucker affect DNA structure?

4. A sample of a circular plasmid is digested with Type I topoisomerase and analyzed by agarose gel electrophoresis followed by staining with ethidium bromide. Fifteen bands of DNA are visible. What do these bands represent and how do their linking numbers differ?

Forces Stabilizing Nucleic Acid Structures

5. Why is DNA not susceptible to hydrolysis by NaOH?

6. High concentrations of denaturing agents such as urea or formamide ($HCONH_2$) tend to favor a rodlike conformation for single-stranded DNA, rather than a flexible coil. What molecular interactions promote this behavior?

7. Explain how the following affect the T_m of double-stranded DNA:
   (a) Increasing the monovalent salt concentration.
   (b) Decreasing the pH.
   (c) Increasing the pH.
   (d) Increasing the concentration of formamide.

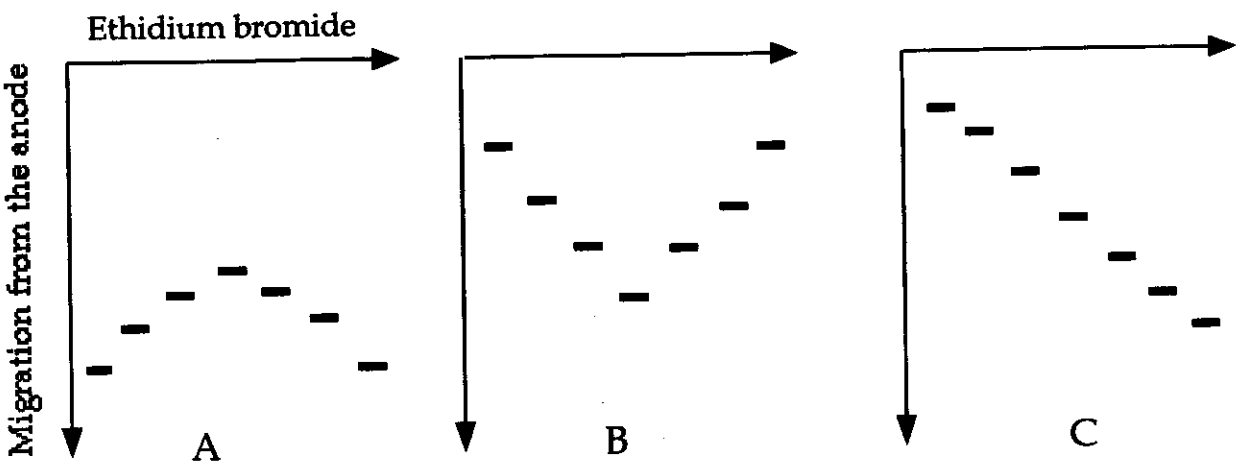
8. Would the UV absorbance of a solution containing partially stacked poly(A) increase or decrease when $[Na^+]$ increases?

9. What forces stabilize the tertiary structure of tRNA?

Fractionation of Nucleic Acids

10. Ice-cold ethanol is used to precipitate DNA. What is the significance of the temperature of the ethanol? Is this more important for shorter or longer DNA strands?

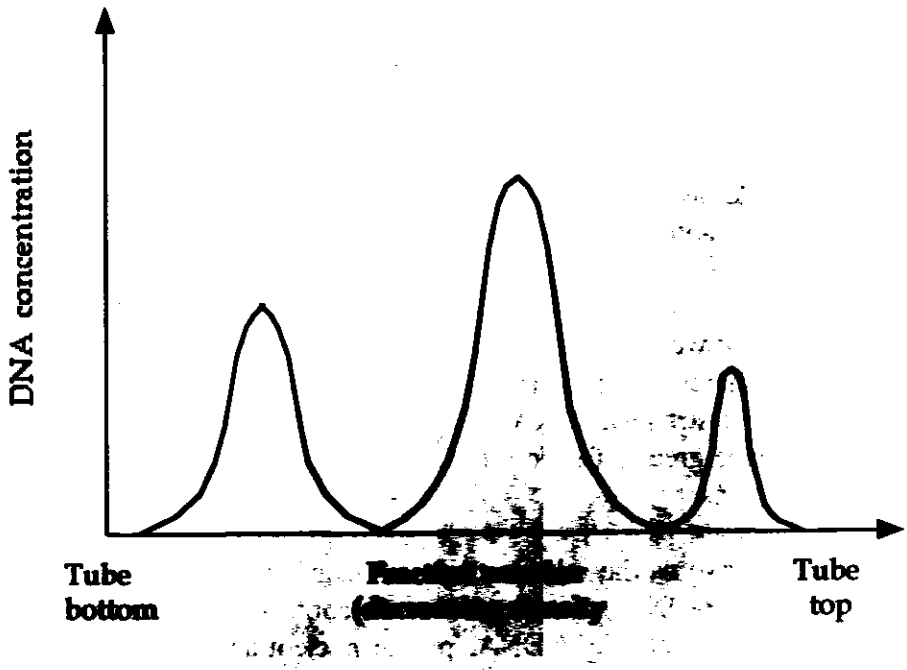
11. Samples of a circular plasmid are incubated with increasing concentrations of ethidium bromide and then analyzed on an agarose gel. Which gel below shows the results of this procedure?



12. What method of nucleic acid fractionation, other than gel electrophoresis, might be useful in separating a native supercoiled plasmid from genomic bacterial DNA or relaxed circular plasmid?

13. In one procedure for isolating RNA, the contents of a cell are homogenized (forcefully mixed) with detergents and chaotropic agents and then subjected to ultracentrifugation in a CsCl equilibrium density gradient. What is the purpose of the detergents and chaotropic agents? Where in the CsCl gradient do you expect to find the RNA and DNA (the protein forms a band at the top of the gradient)?

14. You have obtained 100–1000 bp DNA fragments from a dinosaur bone and have purified them by CsCl equilibrium density ultracentrifugation. Shown below is the banding pattern of DNA. Which band is the richest in G + C? Which band most likely represents mitochondrial DNA?



DNA–Protein Interactions

15. Specific DNA-binding proteins mainly contact DNA through hydrogen bonds in the major groove of B- DNA.
    (a) Why might sequence-specific binding be more common in the major groove than in the minor groove?
    (b) What hydrogen-bond contacts can proteins make with the bases in the major groove? Are these different from those in the minor groove?

16. In what ways do HTH proteins and the *met* repressor represent two general modes of DNA–protein interaction?

Eukaryotic Chromosome Structure

17. What insight into the structure of the eukaryotic chromosome was obtained from nuclease digestion studies?

18. Is histone H1 present in the electron micrograph shown in Figure 23-43? What is the relationship of H1 to the nucleosome structure?

19. Estimate the packing ratio of the DNA in human metaphase chromosomes, which have a total length of 200 μm and contain 6×10^9 bp.

20. Limited digestion of chromatin with a bacterial nuclease yields a 166-bp DNA fragment, whereas limited digestion with pancreatic DNase I yields 10-bp fragments. Explain the difference in these results.

21. Why is it important for the nucleic acid in certain RNA viruses to form a double-stranded A-type helix?

Answers to Questions

1. The A form of DNA, favored under dehydrating conditions, is the most likely form to occur in desiccated cysts or spores or in such things as brine shrimp eggs, which survive for years in a desiccated form.

2. Hydrogen bonding between base pairs and the limited rotation of the bases with respect to the sugar residues impose limitations on the rotation of the bonds in the ribose–phosphate backbone of double-stranded DNA. However, in single-stranded DNA, the ribose–phsophate bonds have greater freedom to rotate, allowing the polymer to take up a greater range of conformations.

3. The ribose pucker governs the relative orientations of the phosphate groups to each sugar residue. Residues in B-DNA have the C2′-*endo* conformation; in A-DNA, they have the C3′-*endo* conformation; and in Z-DNA, purine nucleotides are C3′-*endo* and pyrimidine nucleotides are C2′-*endo*.

4. The fifteen bands represent the native, negatively supercoiled plasmid (migrating the fastest because it is the most compact) and progressively less supercoiled DNA molecules (with the most relaxed DNA migrating the slowest because it is the least compact). Because Type I topoisomerase relaxes DNA by making single-strand cuts, the linking numbers of the 15 DNA bands change by increments of one from bottom to top.

5. DNA does not contain a 2′-OH group that can be deprotonated and then serve as a nucleophile to attack the phosphate group at the 3′ position.

6. Chaotropic agents such as urea and formamide tend to disrupt the structure of water. Hence, in their presence, water's ability to solvate DNA's anionic phosphate groups is reduced. Consequently, the phosphate groups repel one another mores strongly than they do in the absence of the chaotropic agents, which induces the DNA to take up an extended rodlike conformation.

7. (a) Monovalent salts increase the T_m because they attenuate the repulsions between the negatively charged phosphate groups.
   (b) A large decrease in pH disrupts hydrogen bonding between base pairs by protonating some of the bases and therefore decreases T_m.

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(c) A large increase in pH disrupts hydrogen bonding between base pairs by deprotonating some of the base pairs and therefore decreases T_m.
(d) Formamide disrupts water structure and thereby promotes denaturation, leading to a decrease in T_m.

8. An increase in ionic strength reduces the repulsions between phosphate groups and hence promotes base stacking. UV absorbance, which increases when the bases melt apart, would therefore decrease.

9. The tertiary structure of tRNA is stabilized by non-Watson–Crick hydrogen bonding and by stacking interactions.

10. Ethanol decreases the T_m of the DNA as well as decreasing its solubility, so it is important to keep the solution cold to keep the duplex intact. Shorter DNA molecules are more vulnerable to melting and hence strand separation, since T_m depends in part on the length of the DNA.

11. Gel A best represents the expected banding pattern. With increasing ethidium bromide, the plasmid unwinds, becoming progressively less supercoiled until it is a relaxed open circle. The relaxed open circle is less compact than the native plasmid; therefore, it migrates more slowly during electrophoresis. Further increases in ethidium bromide induce positive supercoils, so the plasmid again becomes more compact and migrates faster.

12. CsCl equilibrium density centrifugation would also be useful, since the native supercoiled plasmid is likely to be denser than the other two species of DNA.

13. The detergent and chaotropic agents denature proteins and destroy nucleic acid–protein interactions. The denatured protein collects at the top of the gradient, the DNA forms a band in the gradient, and the RNA, which is too dense to form a band in the CsCl, collects as a pellet at the bottom of the tube.

14. The band nearest the bottom of the tube contains the DNA with the highest G + C content. Because the DNA fragments are so small, it is not possible to tell whether the DNA is nuclear or mitochondrial. Mitochondrial DNA would band separately from nuclear DNA if it were intact, but not when it is mixed with nuclear DNA in small pieces whose differences in base composition are nearly indistinguishable.

15. (a) The bases are more exposed in the major groove of B-DNA and are therefore more accessible to binding proteins. In addition, more base-specific hydrogen bonding donors and acceptors are exposed in the major groove.
(b) In the major groove, the groups available for hydrogen bonding are N7 and N6 of adenine, N7 and O6 of guanine, N4 of cytosine, and O4 of thymine. In the minor groove, the groups available for hydrogen bonding are N3 of adenine, N3 and N2 of guanine, O2 of cytosine, and O2 of thymine.

16. Both kinds of protein exhibit a two-fold symmetry that is reflected in the palindromic DNA sequence at their binding sites. The α helices of HTH proteins contact the major groove of DNA directly, or indirectly via H_2O bridges. In the *met* repressor-like proteins, β strands contact the major groove of DNA.

17. Studies using micrococcal nuclease digestion indicated that the nucleosome contains ~200 bp of DNA. Further digestion trims this DNA to ~146 bp, leaving the nucleosome core particle.

18. Histone H1 is probably absent from the preparation shown in Figure 23-43; compare with Figure 23-46*a*. H1 appears to compact the DNA by binding to the ends of the DNA entering and leaving the nucleosome core.

19. The uncondensed DNA would have a length of $(6 \times 10^9$ bp)(0.34 nm/bp) = 2.04 m. The packing ratio is therefore 2.04 m/200 μm = ~10,000

20. The bacterial nuclease does not cleave the DNA of the nucleosome; however, DNase I appears to be able to cleave nucleosome-bound DNA, cutting it once per helical turn.

21. The RNA must be packaged efficiently within the viral protein capsid. The formation of a double-stranded A helix compacts the RNA in a regular fashion so that it can fit inside the regular shape of the capsid.