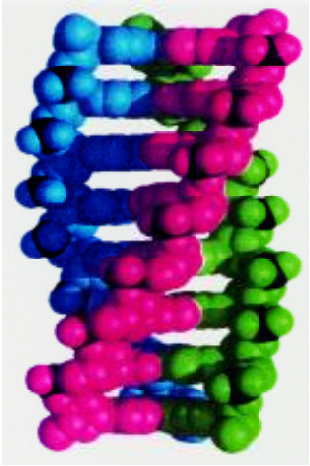

4411-01 BIOCHEMISTRY I Fall 2008

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Core Textbook: Textbook of Biochemistry with Clinical Correlations, Fifth or sixth Edition; Thomas M. Devlin, Editor (Wiley-Liss)

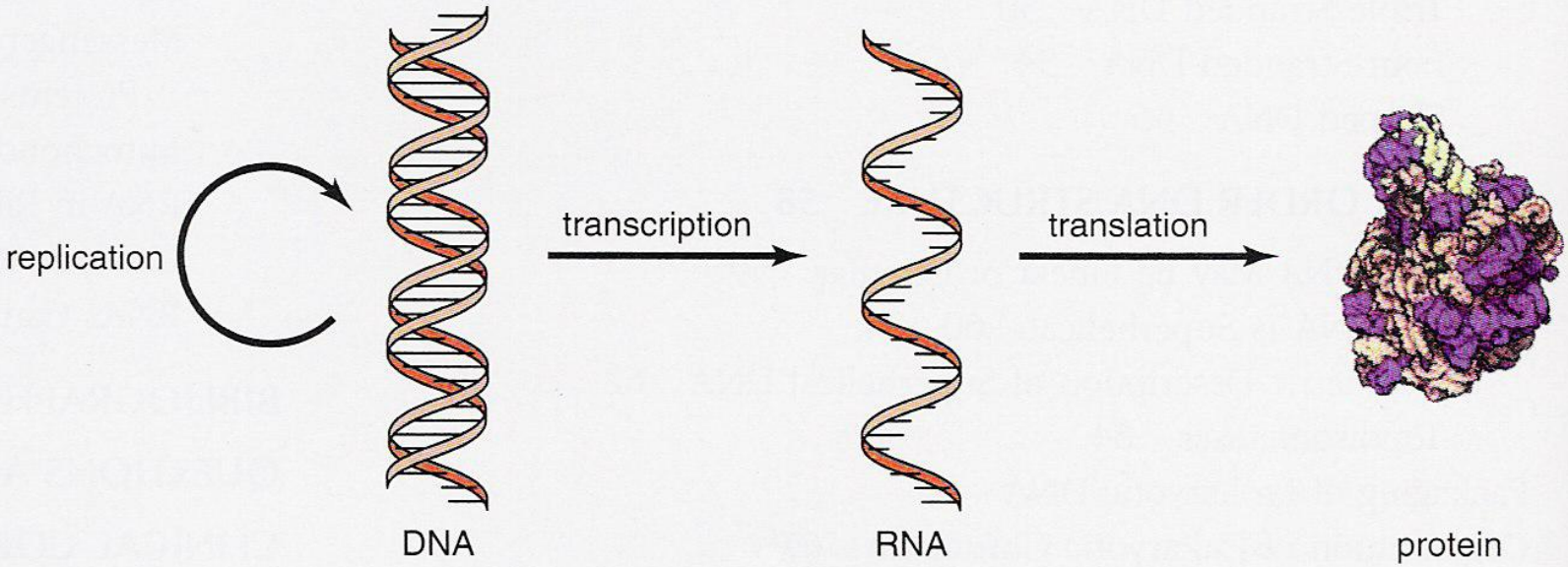


FIGURE 2.1
Central dogma of molecular biology.

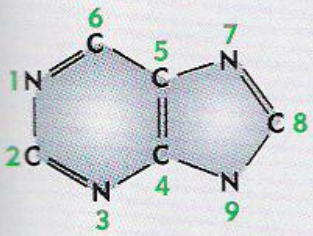
Central Dogma of Molecular Biology holds that DNA stores Information that controls all cellular processes. Information cannot flow directly from DNA to protein, but depends on ribonucleic acid (RNA) to transport the information. Genetic Information is is transmitted from DNA to RNA by transcription. The sequence of RNA is then translated into a protein sequence at the ribosome. Several discoveries have begun to blur the distinct roles of each of these biomolecules; for example, it has been demonstrated that RNA can act as a catalyst in biochemical reactions (ribozymes) and as a template for DNA synthesis (reverse transcription)

The key experiments demonstrating the role of DNA involved pneumococcus, a bacterium that causes a form of pneumonia. When cultured, one strain formed smooth colonies and the other formed rough colonies; these were labeled S- and R-forms, respectively. In addition to their differing appearance, the S-form was virulent while the R-form was nonvirulent. These forms are genetically distinct and cannot interconvert spontaneously. Treatment of R-form bacteria with pure DNA extracted from the S-form resulted in its transformation into the S-form. It was thus demonstrated that DNA was the transforming agent, as well as the material responsible for transmitting genetic information from one generation to the next.

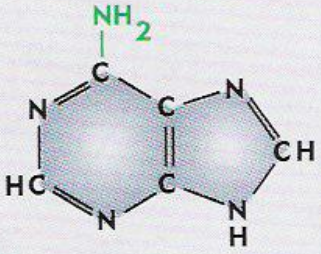
A striking characteristic of DNA is its ability to encode an enormous quantity of biological information. For example, a human cell contains information for synthesis of about 30 000 proteins

Figure 4.4

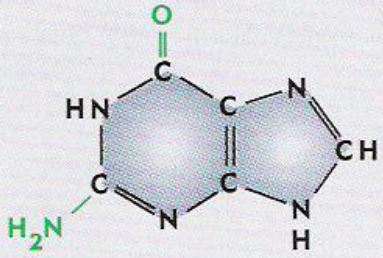
Purines and pyrimidines provide the nitrogenous bases in nucleic acids. The "purine" and "pyrimidine" rings show the general structures of each type of base; the numbers identify the positions on the ring.



Purine

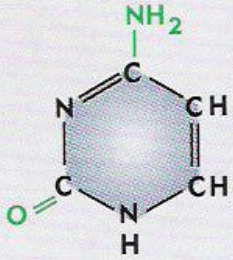


Adenine

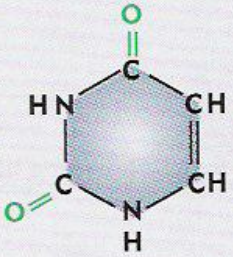


Guanine

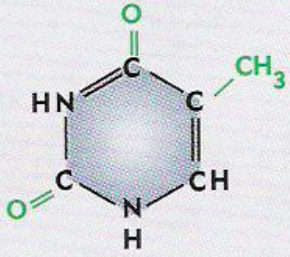
Pyrimidine



Cytosine



Uracil



Thymine

Nucleic acids are linear polymers consisting of repeating nucleotide units. Two classes of major bases found in nucleic acids are purines and pyrimidines. The major purines are adenine and guanine, which are found in both DNA and RNA. There are three major pyrimidine bases: cytosine, uracil, and thymine. Cytosine is present in both DNA and RNA. However, uracil is generally found only in RNA, and thymine is generally found only in DNA.

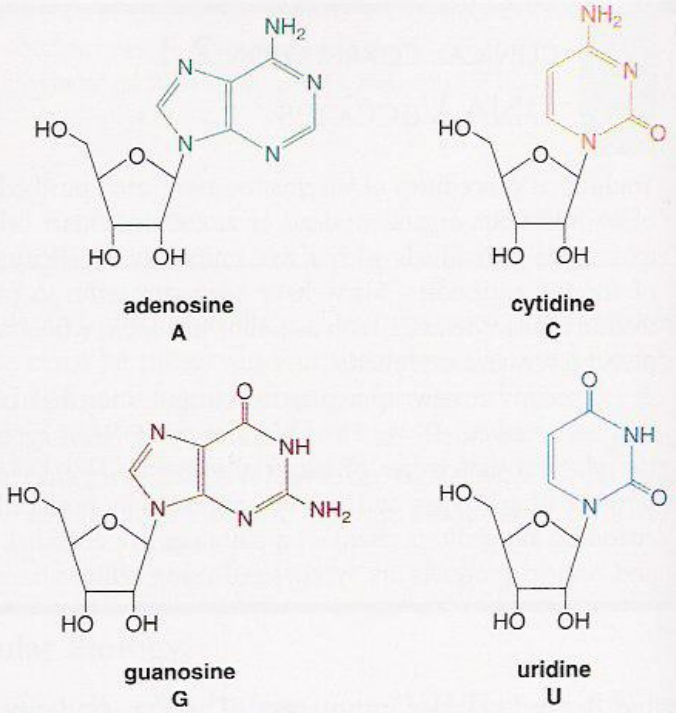


FIGURE 2.3
Structures of ribonucleosides.
Shown are one-letter abbreviations for each compound. These abbreviations are also used for the corresponding bases and nucleotides and, in some instances, for the deoxyribonucleosides.

In nucleic acids, each base is linked to the sugar (through the N1 position in pyrimidines, and N9 position in purines). A base glycosylated with either pentose sugar is referred to as nucleoside. Nucleosides that contain ribose are designated as ribonucleosides, whereas those with deoxyribose are deoxyribonucleosides.

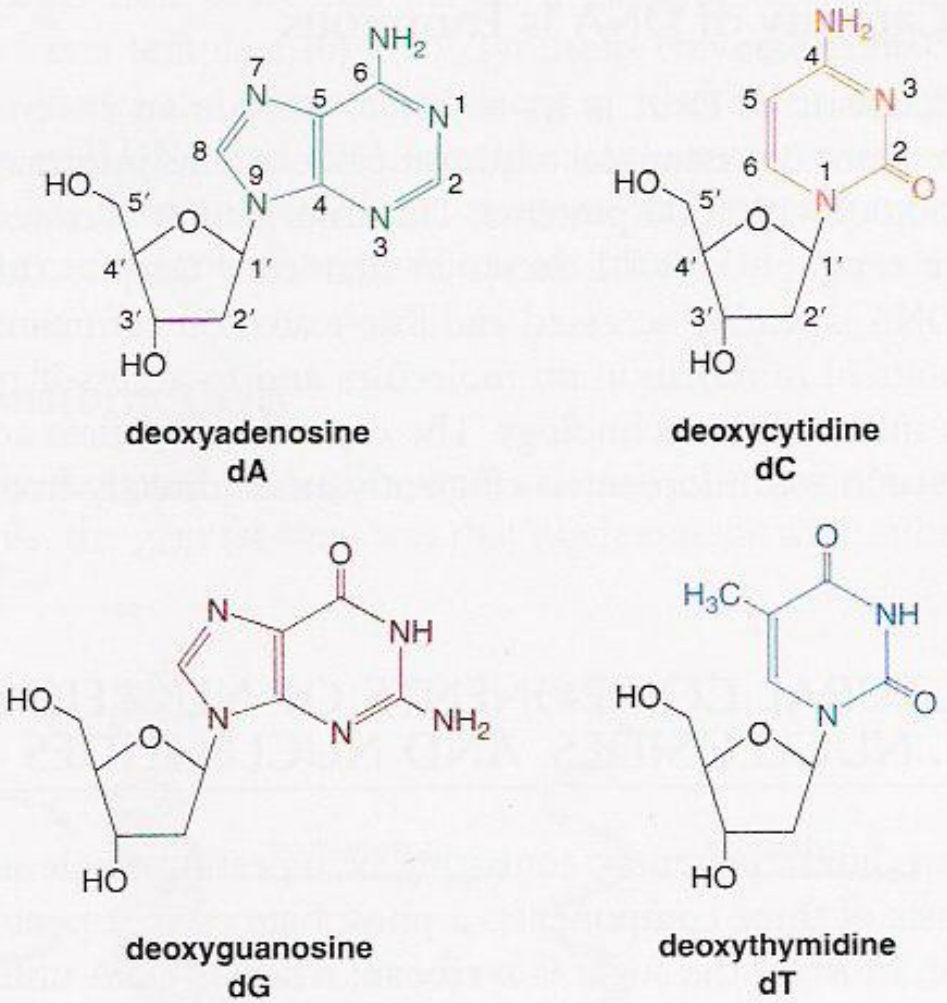
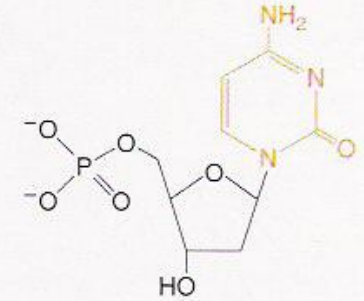


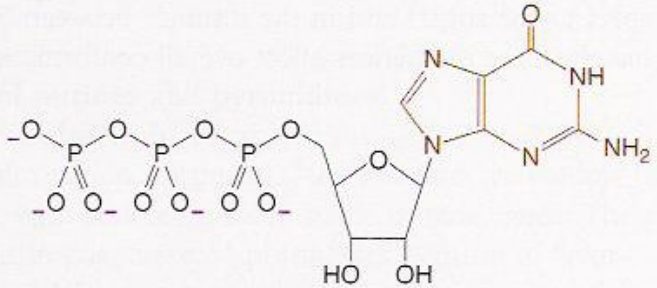
FIGURE 2.4

Structures of deoxyribonucleosides.

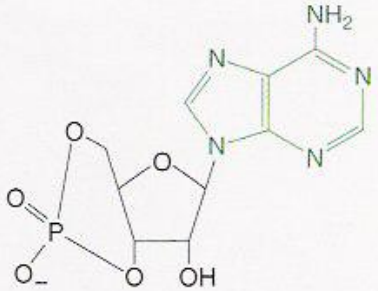
Presence of 2-deoxyribose is abbreviated by "d" preceding the one-letter notation. Note that some sources use thymidine (T) interchangeably with deoxythymidine (dT).



deoxycytidine 5′-monophosphate
dCMP



guanosine 5′-triphosphate
GTP



adenosine 3′,5′-cyclic monophosphate
cAMP

FIGURE 2.5
Structures of some representative nucleotides.

Nucleotides are phosphate esters of nucleosides. Nucleotides often perform as co-factors and ‘second messengers’

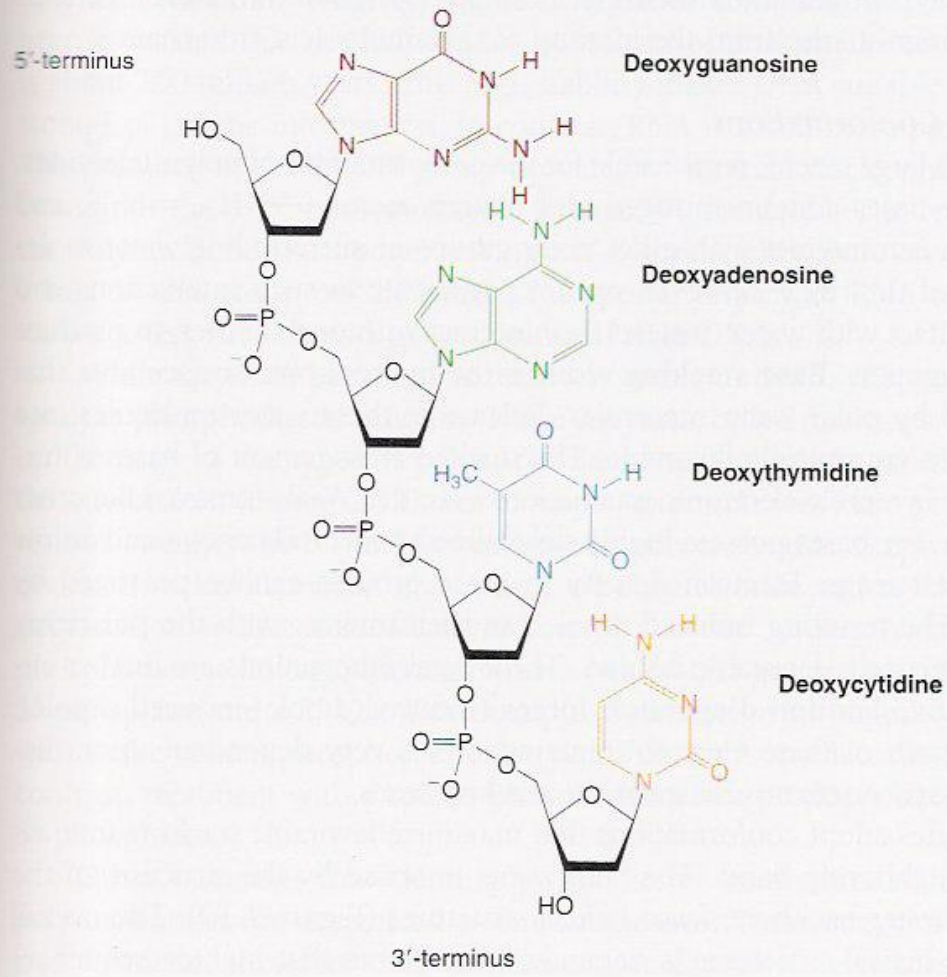


FIGURE 2.8
Structure of a DNA polynucleotide segment.
Shown is a tetranucleotide. Generally, nucleic acids less than 50 nucleotides long are referred to as oligonucleotides. Longer nucleic acids are called polynucleotides.

Nucleic acids are strands of nucleosides linked by phosphodiester bonds. Typically, strands of nucleic acids containing 50 or less nucleotides are called oligonucleotides, whereas those that are longer are called polynucleotides.

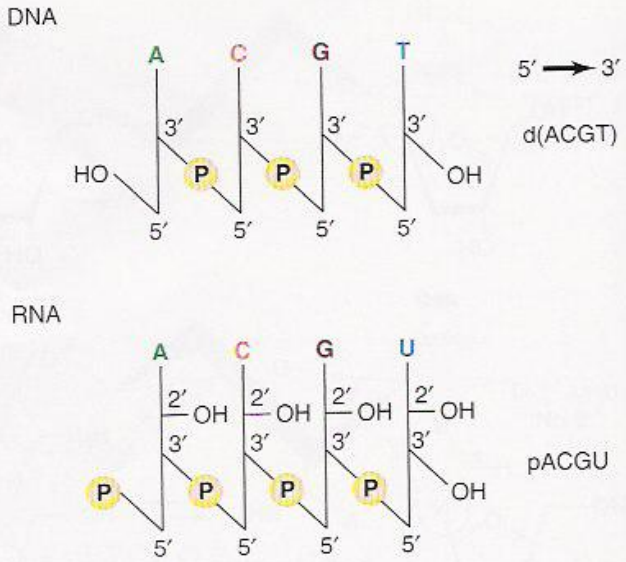


FIGURE 2.9
Shorthand notations for structure of oligonucleotides.
The convention used in writing the structure of an oligo- or polynucleotide is a perpendicular bar representing the sugar moiety, with the 5′-OH position of the sugar located at the bottom of the bar and the 3′-OH (and 2′-OH, if present) at a midway position. Bars joining 3′- and 5′-positions represent the 3′,5′-phosphodiester bond, and the P on the left or right side of the perpendicular bar represents a 5′-phosphate or 3′-phosphate ester, respectively. The base is represented by its initial placed at the top of the bar. An alternative shorthand form is to use the one-letter initials for the bases written in the 5′ → 3′ direction from left to right. Internal phosphodiester groups are assumed, and terminal phosphates are denoted with a "p." Oligonucleotide sequences containing deoxyribose sugars are preceded by a "d."

The phosphodiester links the 5' hydroxyl group of one residue to the 3' hydroxyl group of the next. Linkages between two 5'OH groups or two 3'OHs are are not seen in naturally occurring DNA or RNA. The directionality of the phosphodiester bond means that linear oligo- or polynucleotides have ends that are not structurally equivalent. One end of a polynucleotide must terminate in a 5'OH, the other terminates with 3'OH. These ends are referred to as 5'-terminus and 3'-terminus.

While the DNA backbone is relatively stable to hydrolysis, numerous enzymes catalyze phosphodiester scission. These enzymes, called nucleases, are characterized on the basis of of the types of polynucleotides they hydrolyze and the specific bonds that are broken.

For example, exonucleases cleave the last nucleotide residue at either the 5'- or 3'-terminus. Other nucleases that cleave phosphodiester bonds located in the interior of polynucleotides are designated as endonucleases.

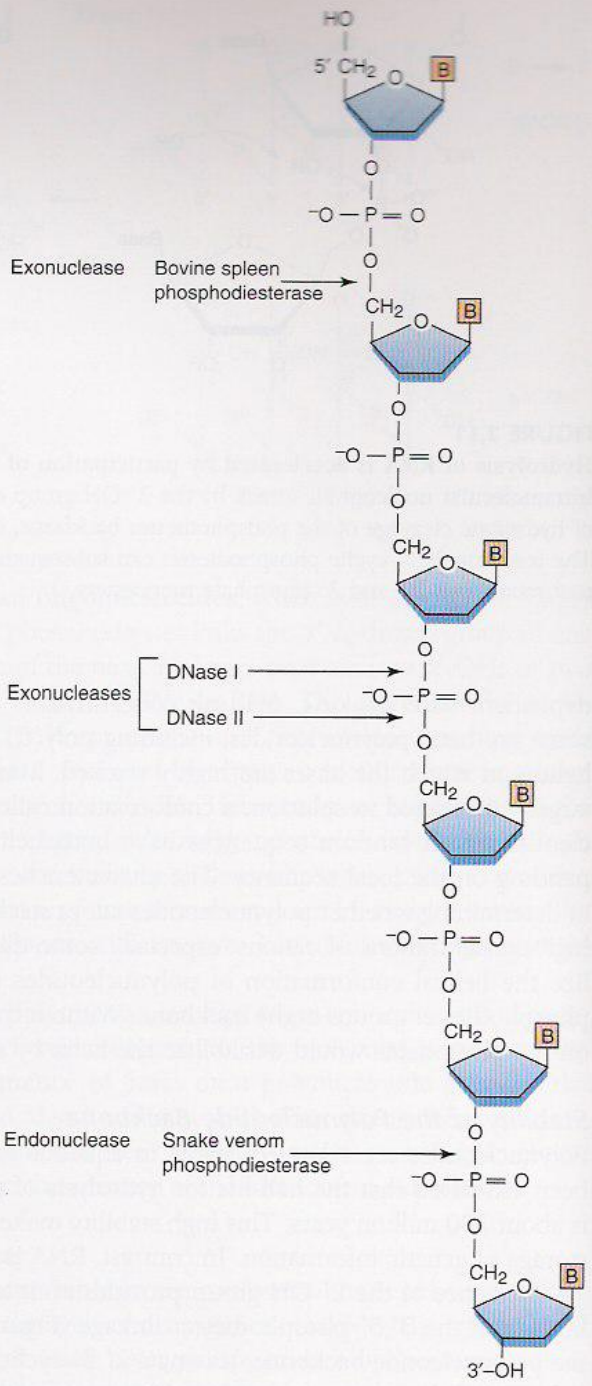
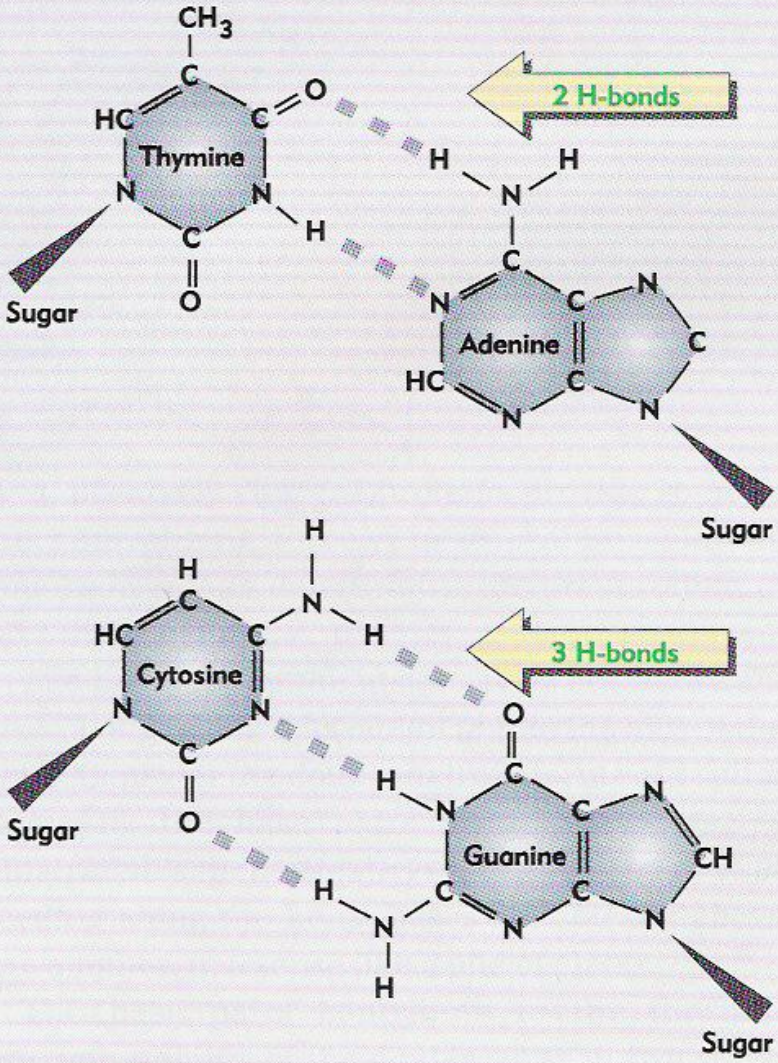


FIGURE 2.12
Specificities of nucleases.
Exonucleases remove nucleotide residues from either end of a polynucleotide, depending on their specificity. Endonucleases hydrolyze interior phosphodiester bonds. Both endo- and exonucleases typically show specificity regarding which oxygen–phosphorus bond is hydrolyzed.

Figure 4.10

Complementary base pairing involves the formation of two hydrogen bonds between A and T, and of three hydrogen bonds between G and C. No other pairs form in DNA.



X-ray diffraction data suggested that DNA contained double-helical structures, and symmetry suggested that the two polynucleotide strands were oriented antiparallel to each other. The structure that Watson and Crick proposed in 1953 for double-helical DNA was quite attractive because of its simplicity and symmetry. The two strands achieve contact through hydrogen bonds, formed at the hydrophilic edges of the bases.

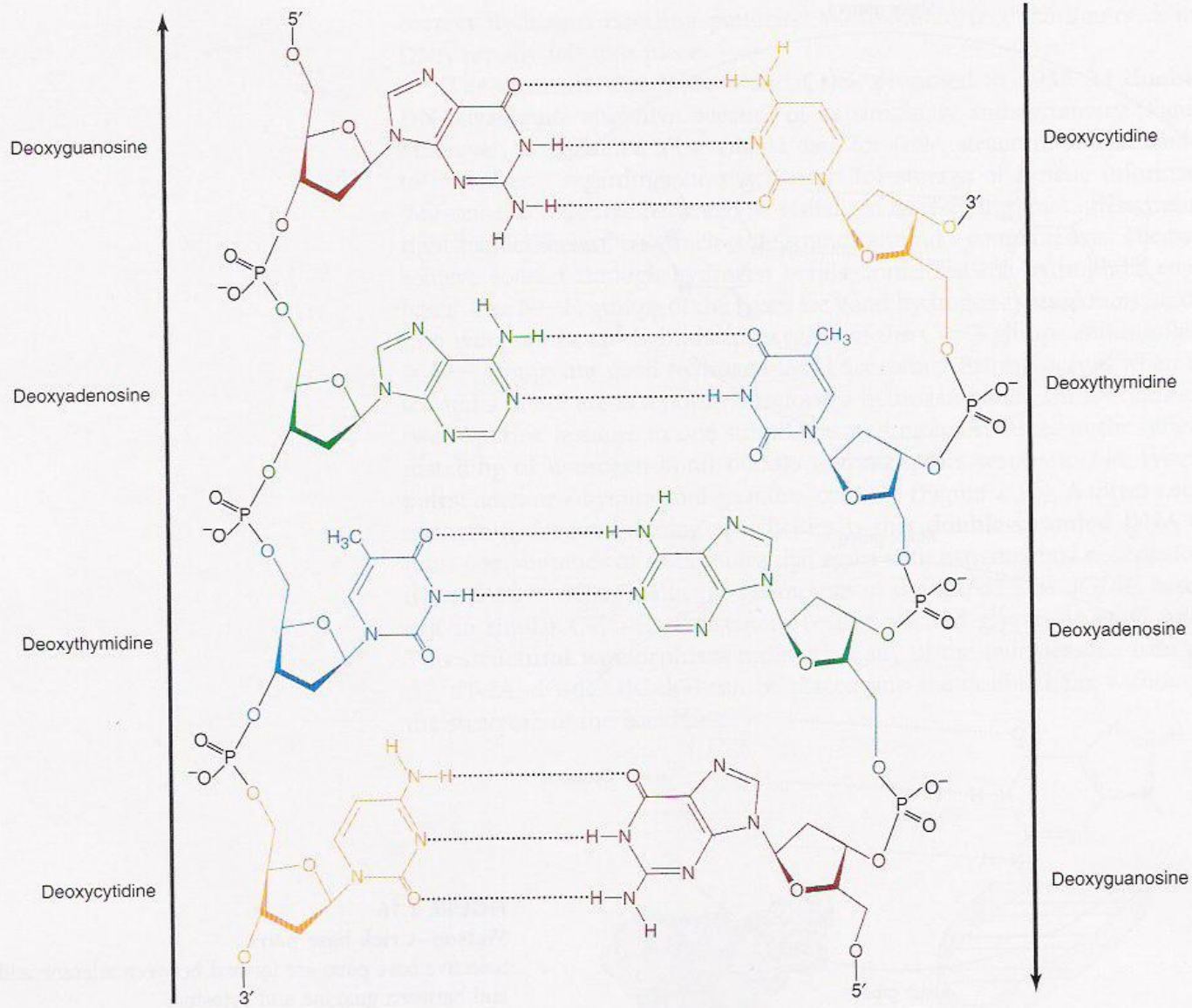


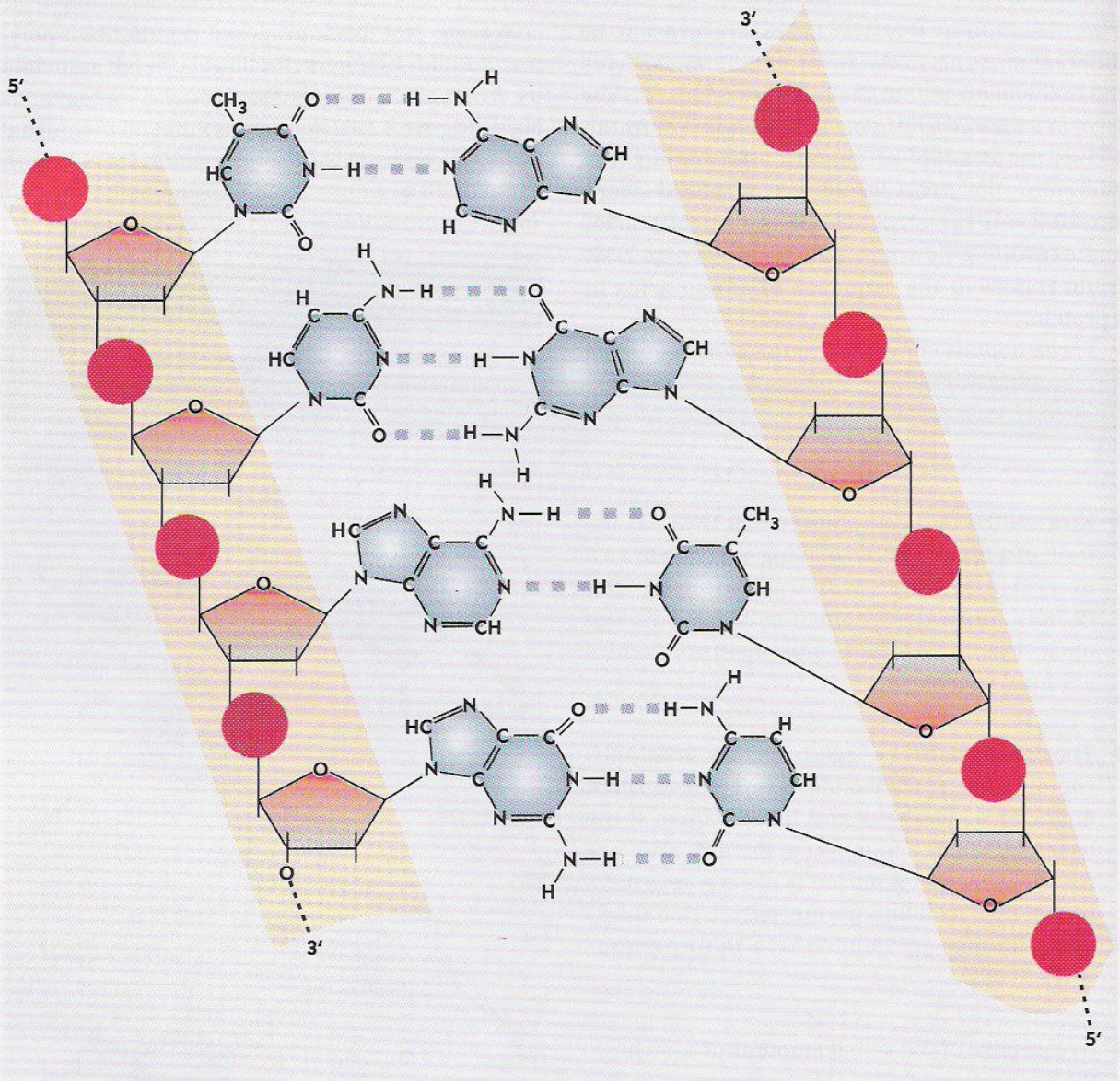
FIGURE 2.17

Formation of hydrogen bonds between complementary bases in double-stranded DNA.

Interaction between polynucleotide strands is highly selective. Complementarity depends not only on the geometric factors that allow the proper fitting between the complementary bases of the two strands, but also on the formation of specific hydrogen bonds between complementary bases. Note the antiparallel orientation of the strands of a double-stranded DNA. Geometry of the helices does not prevent a parallel alignment, but such an arrangement is not found in DNA.

Figure 4.11

The double helix maintains a constant width because purines always face pyrimidines in the complementary A-T and G-C base pairs.



Interwinding of the two antiparallel strands produces a structure that has two distinct helical grooves between the sugar-phosphate backbones. One of the grooves is much wider (major groove) than the other (minor groove); this disparity arises from the geometry of the base pairs.

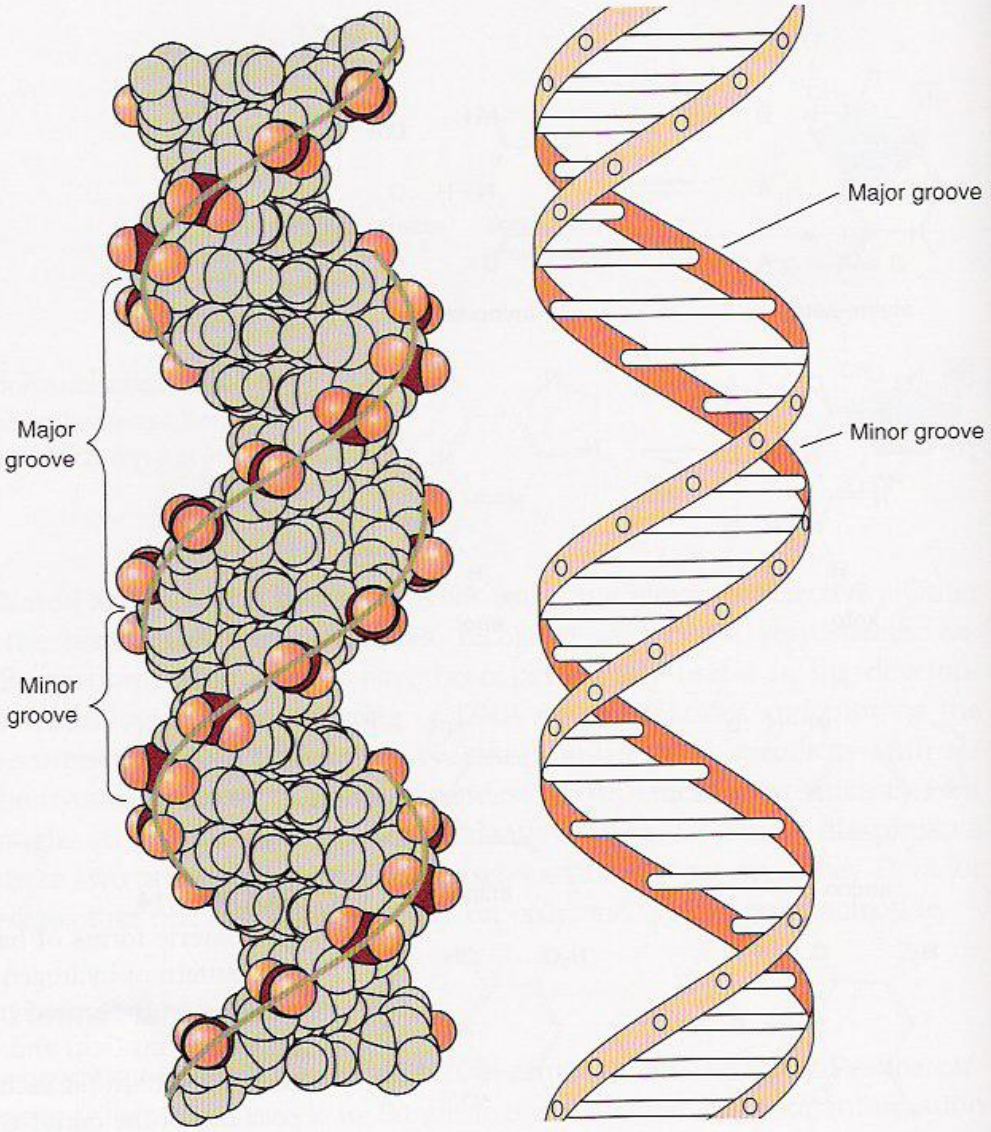


FIGURE 2.15
The Watson–Crick model of DNA.
On the left is a space-filling model of DNA; on the right is an idealized ribbon model. Bases are stacked in the interior of the helix, whereas the hydrophilic sugar–phosphodiester backbone is located on the exterior.
Redrawn from Rich, A. J. Biomol. Struct. Dyn. 1:1, 1983.

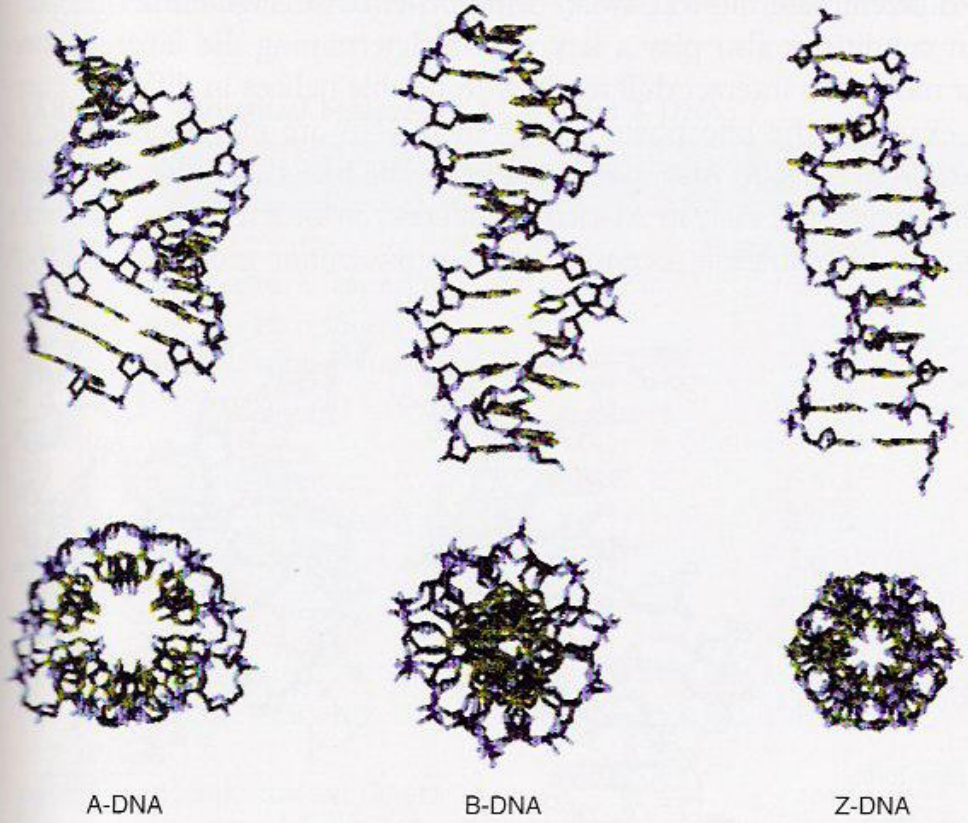


FIGURE 2.23
The varied geometries of double-helical DNA.
Depending on conditions and base sequence, the double helix can acquire various forms of distinct geometries. There are three main families of DNA conformations: A, B, and Z. The right-handed forms, B-DNA and A-DNA, differ in sugar pucker, which leads to differing helical structures. The A-form is underwound compared to the B-form, and the resulting helix is shorter and fatter. The Z-DNA structure is a left-handed helix with a zigzagging backbone. The sugar puckers and glycosidic conformations alternate from one residue to the next, producing a local reversal in the chain direction.

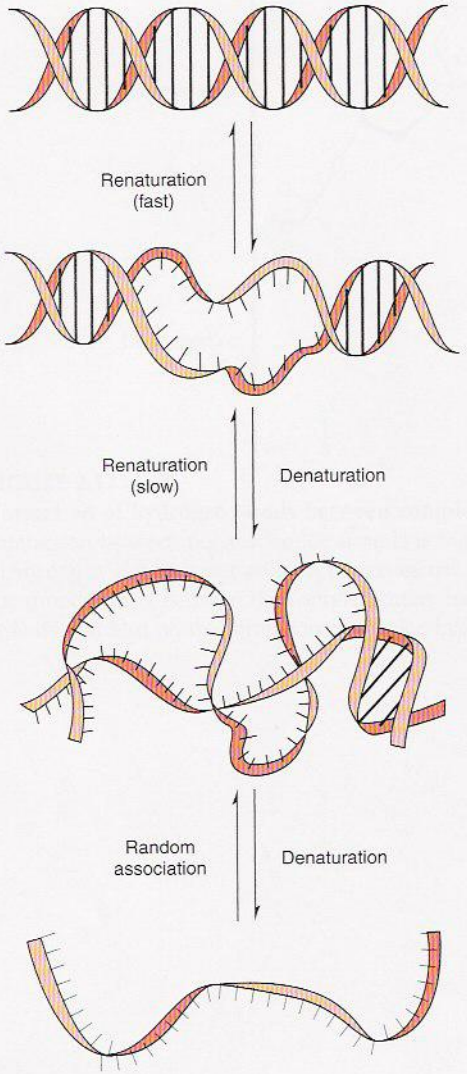


FIGURE 2.19
Denaturation of DNA.
At high temperatures the double-stranded structure of DNA is completely disrupted, with eventual separation of strands and formation of single-stranded open coils. Denaturation also occurs at extreme pH ranges or at extreme ionic strengths.

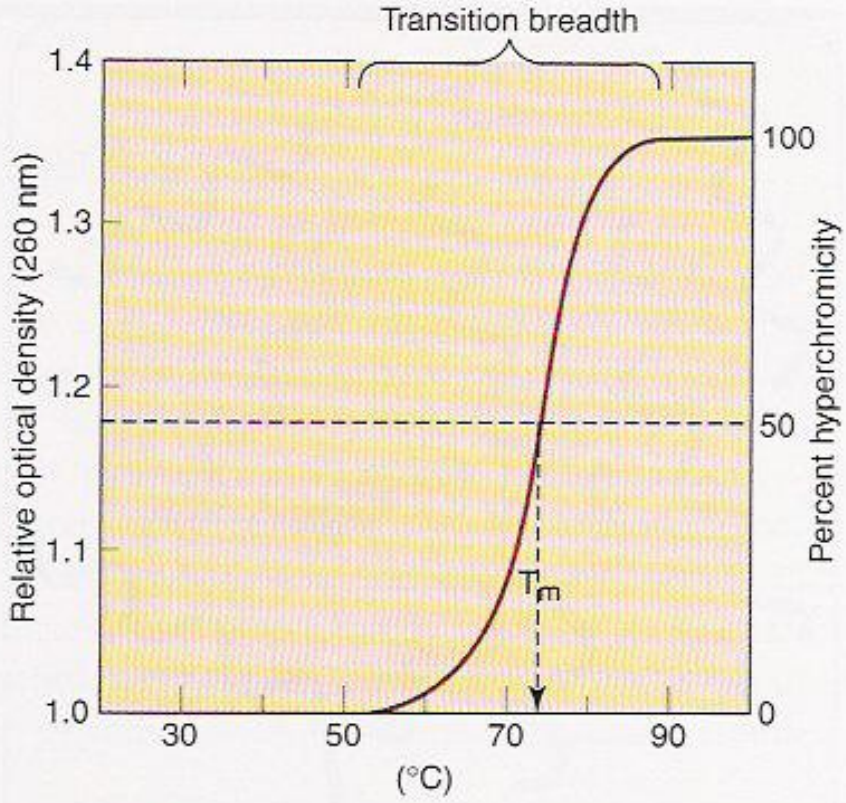


FIGURE 2.20
Temperature–optical density profile for DNA.
When DNA is heated, the absorbance at 260 nm increases with rising temperature. A graph in which absorbance versus temperature is plotted is called a "melting curve." Relative optical density is the ratio of the absorbance at any temperature to that at low temperatures. The temperature at which one-half of the maximum optical density is reached is the midpoint temperature (T_m).

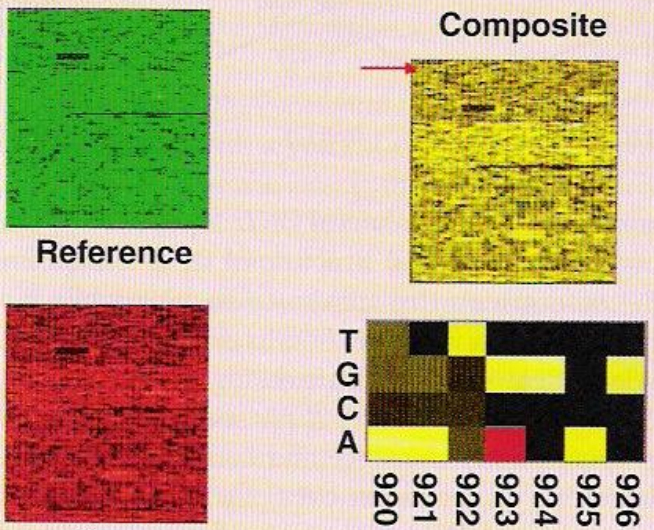
CLINICAL CORRELATION 2.2

Diagnostic Use of DNA Arrays in Medicine and Genetics

With pending completion of the Human Genome Project, a wealth of genetic information is rapidly becoming available. Application of this knowledge to medicine requires development of new techniques to monitor gene expression and rapidly evaluate genes for mutations and other sequence variations. Oligonucleotide arrays have shown great promise for these applications. Such arrays consist of a number of gene-specific oligonucleotide probes immobilized at specific sites on a solid matrix (chip). Arrays can contain thousands of unique probe molecules, each fixed within an "address." Gene chips can then be treated with labeled target nucleic acids (DNA or RNA) derived from cells of an organism. Hybridization of the targets with complementary probe sequences allows for immobilization of the label at specific sites on the chip. In this way, the presence of specific sequences can be determined and the amount of labeled target hybridized to a site can be quantitated, allowing for determination of amount of each target in a sample.

Use of DNA arrays to analyze sequences has led to detection of mutations in human cells. For example, high-density DNA arrays with thousands of oligonucleotide probes have been used to screen for mutations that lead to ataxia telangiectasia, a recessive genetic disease characterized by neurological disorders, recurrent respiratory infections, and dilated blood vessels in the skin and eyes. Similar studies have examined mutations in the hereditary breast and ovarian cancer gene BRCA1 and other genetic markers for disease. In the future, such techniques may be developed into diagnostics for rapid screening of genomic DNA for disease-associated mutations.

Freeman, W. M., Robertson, D. J., and Vrana, K. E. Fundamentals of DNA hybridization arrays for gene expression analysis. *BioTechniques* 29:1042, 2000; and Hacia, J. G., Brody, L. C., Chee, M. S., Fodor, S. P., and Collins, F. S. Detection of heterozygous mutations in *BRCA1* using high density oligonucleotide arrays and two-colour fluorescence analysis. *Nat. Genet.* 14:441, 1996.



High density DNA arrays for mutation analysis. Arrays for the ataxia telangiectasia (A-T) gene are interrogated separately with a reference (unmutated) sample and a test sample. The composite image is made by combination of the images from the two experiments. At sites where the test and reference DNAs have identical sequences, the composite image is yellow (red + green). In this sample, the person has a mutation at position 923, which appears red. This mutation, the replacement of a G with an A, causes premature termination of protein synthesis. Because this sample also has a yellow spot at position 923, the mutation has occurred on only one copy of the gene (e.g., it is heterozygous). In recessive disorders such as A-T, people with heterozygous mutations are carriers, but will not develop the disease themselves.

Noncanonical DNA structures

It is now recognized that DNA is not a straight, monotonous, and uniform structure. Instead, DNA forms unusual structures, such as

-cruciforms

-triple- and, possibly, quadruple-strand complexes

-bends

and also it interacts with miscellaneous DNA-binding proteins. Variations in specific DNA structure or conformation are favored by specific DNA sequence motifs.

-DNA sequences with runs of four to six adenines phased by 10-bp spacers produce bent conformations

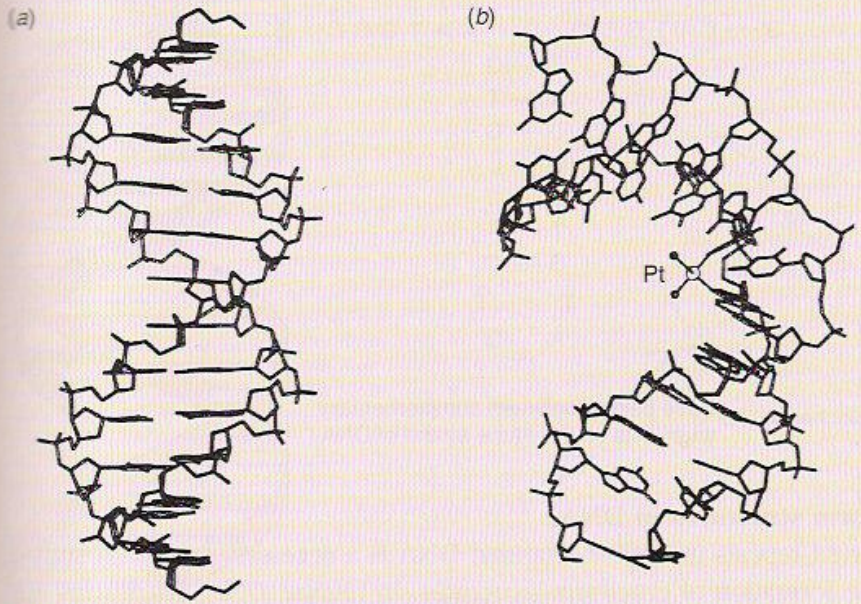
-A number of DNA-binding proteins can bend DNA in a protein-DNA complex

-Inter- and intra-strand cross-linked DNA adducts can also bend DNA (cisplatin)

CLINICAL CORRELATION 2.3

Antitumor Antibiotics that Change the Shape of DNA

The local three-dimensional structure of DNA is important in interactions with proteins involved in repair, transcription, recombination, and chromatin condensation. Recently, it has been proposed that antibiotics can induce formation of DNA structures that can recruit these proteins with cytotoxic results. The best studied example of this phenomenon is the antitumor drug cisplation, a tetracoordinate platinum complex [*cis*-$Pt(NH_2)_2Cl_2$]. Cisplatin is used alone or in combination with other antitumor agents to treat a variety of tumors including testicular, ovarian, bone, and lung cancers. This platinum complex forms inter- and intrastrand cross-links in double-stranded DNA with the latter adduct comprising 90% of DNA lesions. These bonds arise from displacement of chloride ligands on platinum by N-7 atoms of two neighboring guanines. Structural studies on intrastrand cross-linked DNA adduct show that the double helix is strongly bent toward the major groove.



From Zamble, D. B. and Lippard, S. J. In: B. Lippard (Ed.), Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug. *New York: Wiley-VCH 1999, p. 74.*

Bent structures of the DNA–cisplatin adduct are specifically recognized by several DNA-binding proteins that include nucleotide excision repair (NER) proteins and high-mobility-group proteins such as HMG-1. It has been proposed that the cytotoxicity of cisplatin adducts is a complicated process mediated by specific interactions with these proteins. Cellular processes such as transcription and apoptosis (programmed cell death) are affected by formation of cisplatin–DNA adducts. The lesions themselves and the adduct–protein complexes are likely to interfere with transcription. NER proteins are recruited to repair the lesion, but excision repair is prone to introduction of DNA strand breaks. Accumulation of these breaks will ultimately induce apoptosis as the DNA becomes too damaged to function. Similar mechanisms have also been proposed to account for cytotoxicity of other DNA-binding drugs such as ditercalinium. This bifunctional molecule forms noncovalent adducts with DNA that are also highly bent. Cytotoxicity is thought to arise from induction of abortive repair pathways that lead to DNA strand breaks.

Interactions of the cisplatin–DNA adduct with HMG proteins may also contribute to its cytotoxicity. Binding of HMG proteins may incorrectly signal that the damaged region of DNA is transcriptionally active and prevent condensation into folded chromatin structures. These complexes might also perpetuate the lesion by shielding the DNA–cisplatin adduct from repair.

Zamble, D. B. and Lippard, S. J. The response of cellular proteins to cisplatin-damaged DNA. In: B. Lippert (Ed.), *Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug.* New York: Wiley-VCH, 1999, pp. 73–134; and Lambert, B., Segal-Bendirdjian, E., Esnault, C., Le Pecq, J.-B., Roques, B. P., Jones, B., and Yeung, A. T. Recognition by the DNA repair system of DNA structural alterations induced by reversible drug–DNA interactions. *Anti-Cancer Drug Des.* 5:43, 1990.

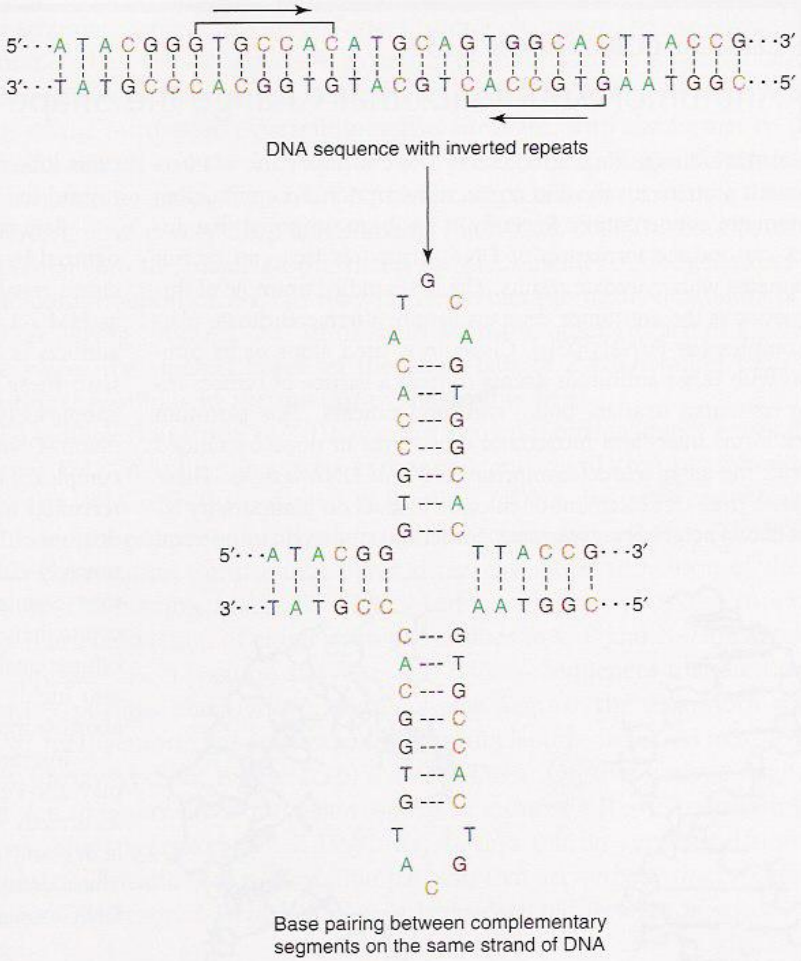
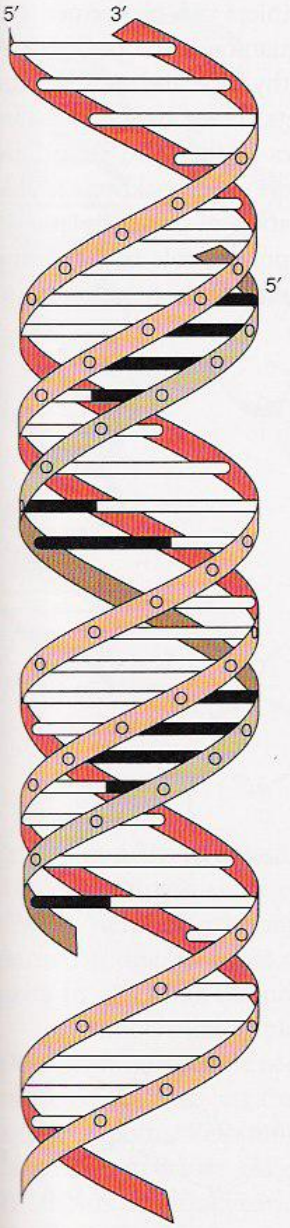
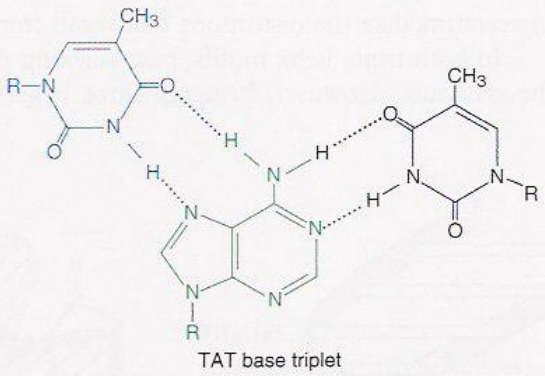


FIGURE 2.28
Formation of cruciform structures in DNA.
The existence of inverted repeats in double-stranded DNA is a necessary but not a sufficient condition for the formation of cruciform structures. In relaxed DNA, cruciforms are not likely to form because the linear DNA accommodates more hydrogen-bonded stacked base pairs than the cruciform structure, making the formation of the latter thermodynamically unfavored. Unwinding is followed by intrastrand hydrogen bond formation between the two symmetrical parts of the repeat to produce the cruciform structure. Formation of cruciform structures is not favored over DNA regions that consist of mirror repeats because such cruciforms would be constructed from parallel rather than antiparallel DNA strands. Instead, certain mirror repeats tend to form triple helices.

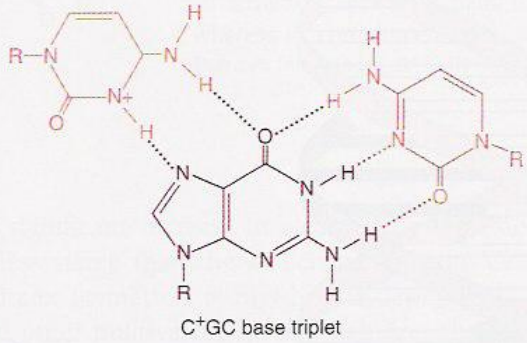
-Inverted repeats are quite widespread within the human genome and often found near putative control regions or at origins of DNA replication

-It has been speculated that inverted repeats may function as molecular switches for replication and transcription





TAT base triplet



C^+GC base triplet

FIGURE 2.29
Hoogsteen triple helix.
Triple helices can be formed by formation of Hoogsteen hydrogen bonds between the homopurine strand of a double helix and a parallel homopyrimidine strand. The resulting isomorphous base triplets, TAT and C^+GC, provide for sequence selective binding.

Work in the 50s and 60s showed that some combination of sequences formed triple-strand complexes. For example, a thymine can selectively form two Hoogsteen H bonds to the adenine of A-T pair. Likewise, a protonated cytosine can form two Hoogsteen H bonds with the guanine of a G-C pair, resulting in a base triplet isomorphous to the T-A-T

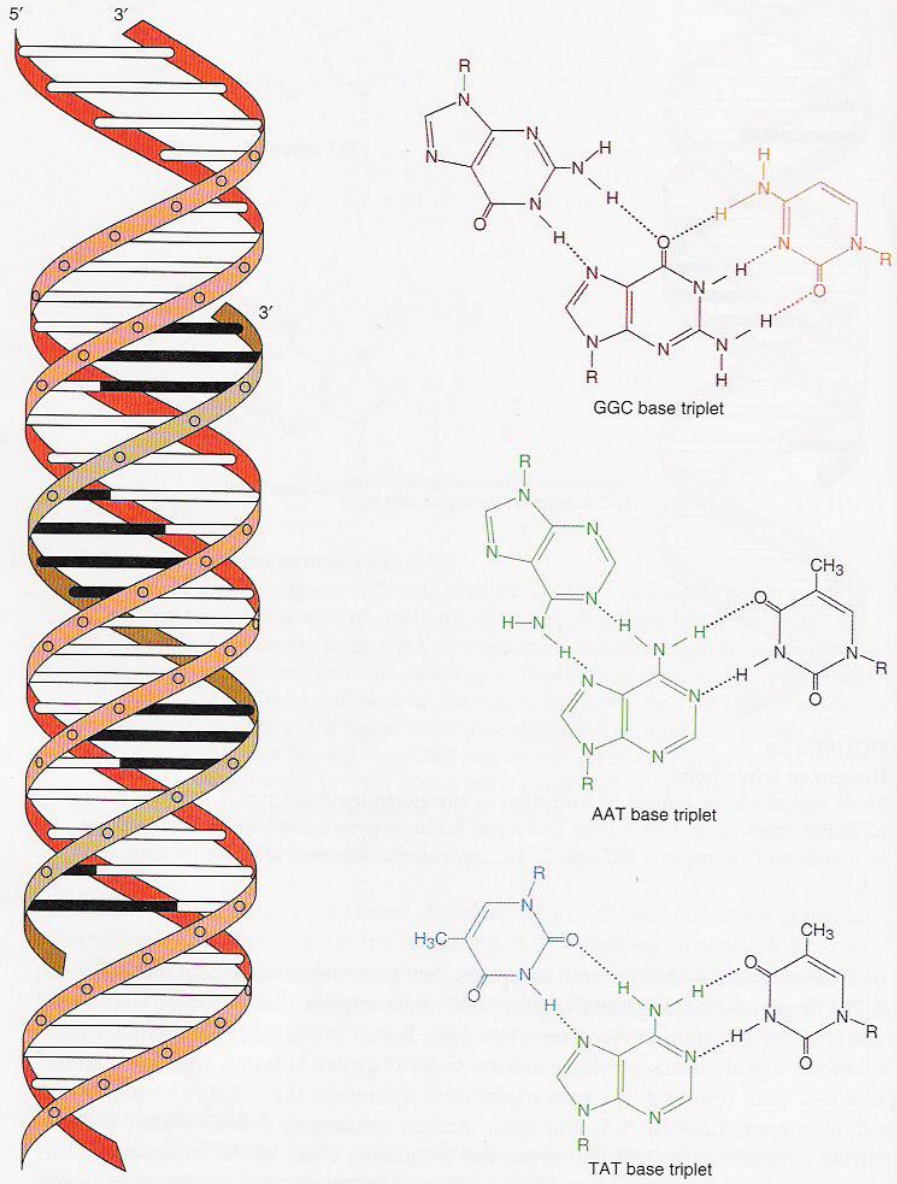


FIGURE 2.30
Reversed Hoogsteen triple helix.
Triple helices can be formed by antiparallel binding of an oligonucleotide to the homopurine strand of a Watson–Crick double helix. Reversed Hoogsteen hydrogen bonding produces three possible triplets: GGC, AAT, and TAT. These triplets are not isomorphous because the sugars (represented by R) are positioned differently with respect to the Watson–Crick base pair.

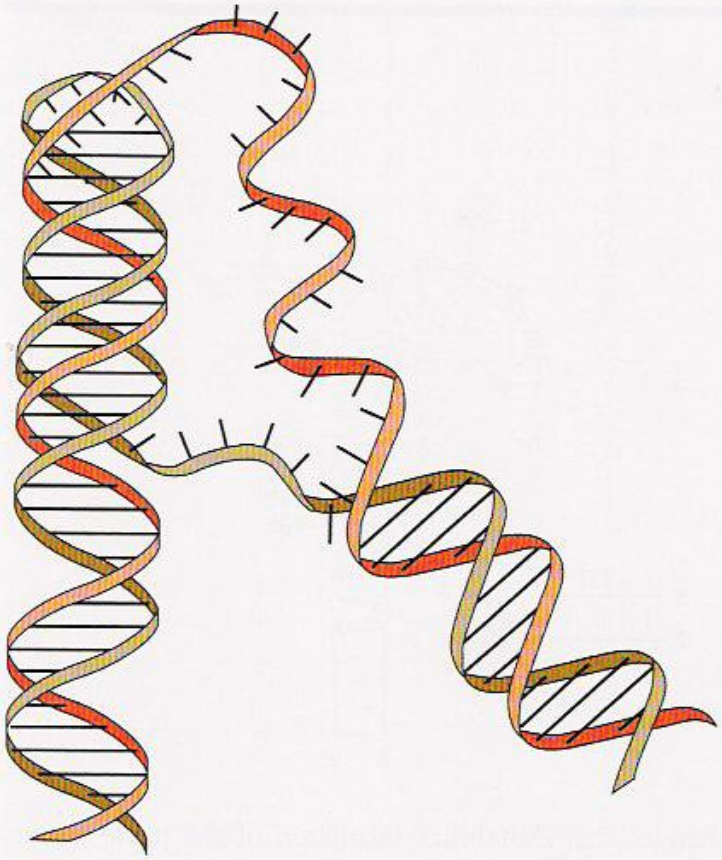


FIGURE 2.31
Intramolecular triple helices—H-DNA.
Polypurine–polypyrimidine regions of DNA with a mirror repeat symmetry can form an intramolecular triple helix in which the third strand lays in the major groove, whereas its complementary strand acquires a single-stranded conformation.
Redrawn from Sinden, R. R. DNA Structure and Function. *New York: Academic Press, 1994.*

-Intramolecular triple-helices can be formed by disruption of double-helical DNA with polypurine sequences in mirror repeats. (A mirror repeat is a region such as AGGGGA that has the same base sequence when read in either direction from a central point). Refolding generates a triple-stranded region and a single-stranded loop in a structure called H-DNA.

-It is believed that triple-strand complexes can be formed during transcription

CLINICAL CORRELATION 2.4

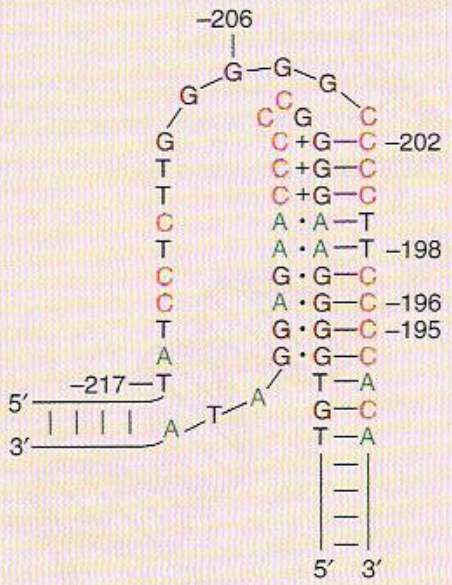
Hereditary Persistence of Fetal Hemoglobin

Hereditary persistence of fetal hemoglobin (HPFH) is a group of conditions in which fetal hemoglobin synthesis is not turned off with development but continues into adulthood. The homozygous form of the disease is extremely uncommon, being characterized by changes in red blood cells similar to those found in heterozygous β-thalassemia. HPFH, in either the homozygous or heterozygous state, is associated with mild clinical or hematologic abnormalities. Mild musculoskeletal pains may occur infrequently, but HPFH patients are generally asymptomatic.

The disease results from failure in control of transcription from human Gγ- and Aγ-globin genes. Affected chromosomes fail to switch from γ- to β-chain synthesis. Expression of these genes appears to be affected substantially by formation of an intramolecular DNA triplex structure located about 200 bp upstream from the initiation site for transcription of genes, specifically between positions −194 and −215.

Hemoglobin genes of patients contain mutations in positions −195, −196, −198, and −202. Mutations at −202 involve changes from C to G and C to T, at −198 from T to C, at −196 from C to T, and at −195 from C to G. These mutations influence the stability of the intramolecular DNA triple helix.

In general, the presence of polypurine–polypyrimidine sequences sufficiently long to form intramolecular triple helices tends to repress transcription, while short polypurine–polypyrimidine segments that are unable to induce triple helix formation have no effect on transcription. In the case of HPFH, a remarkable correspondence is noted between base changes that destabilize formation of the triple helix and presence of the genetic disease.



Ulrich, M. J., Gray, W. J., and Ley, T. J. An intramolecular DNA triplex is disrupted by point mutations associated with hereditary persistence of fetal hemoglobin. *J. Biol. Chem.* 267:18649, 1992; and Bacolla, A., Ulrich, M. J., Larson, J. E., Ley, T. J., and Wells, R. D. An intramolecular triplex in the human gamma-globin 5′-flanking region is altered by point mutations associated with hereditary persistence of fetal hemoglobin. *J. Biol. Chem.* 270:24556, 1995.

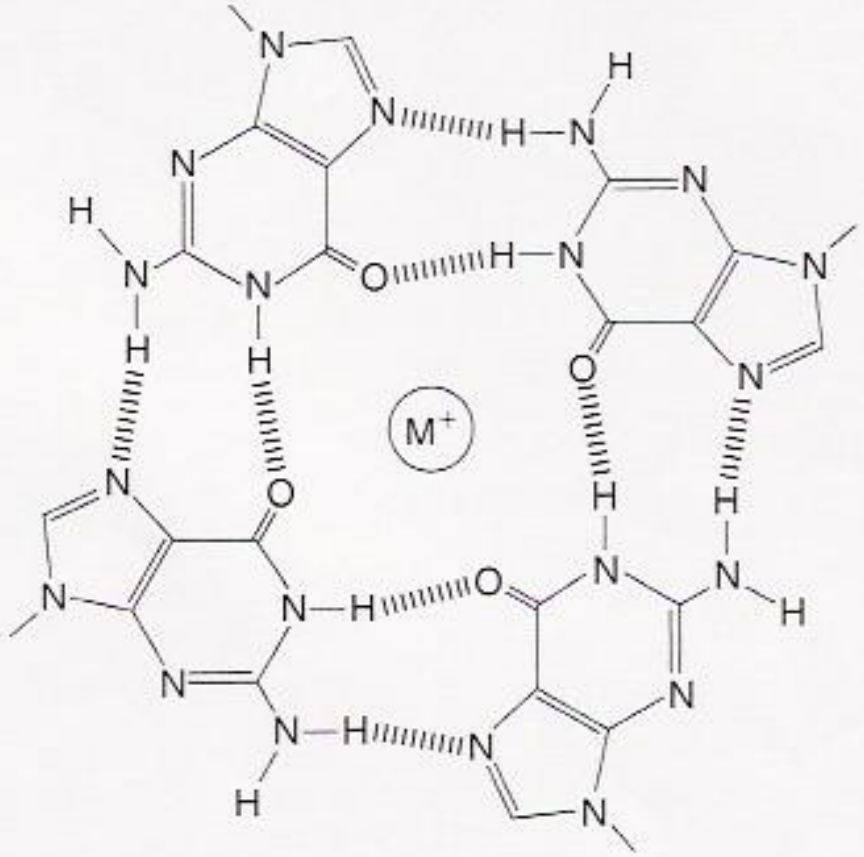
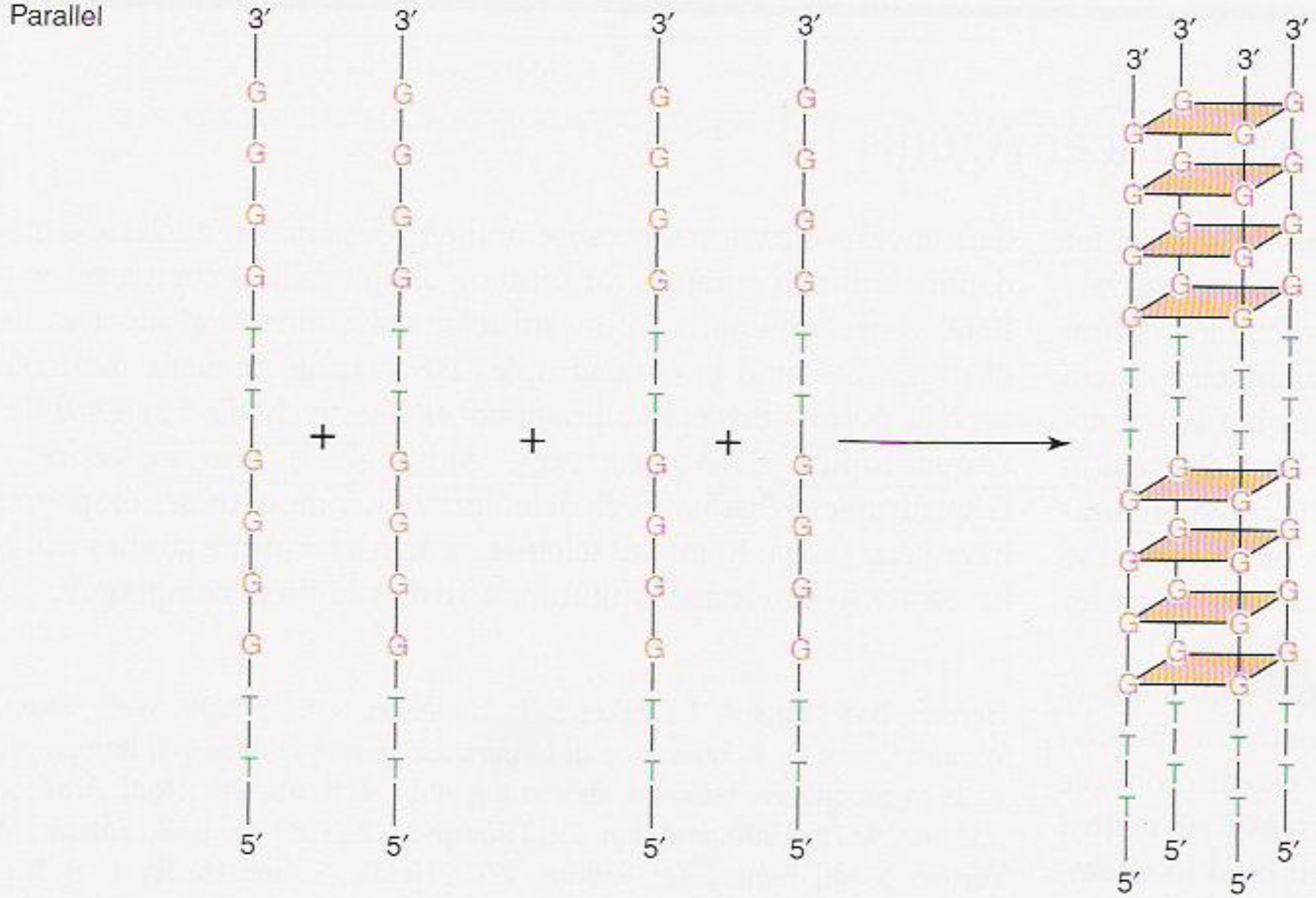


FIGURE 2.32
The structure of a G-quartet.
The four coplanar guanines form a tetrameric structure by formation of Hoogsteen hydrogen bonds. The cavity in the center of the quartet can accommodate a sodium or potassium ion with coordination by the four O-6 oxygens.

Guanine nucleotides and highly G-rich polynucleotides form novel tetrameric structures called G-quartets


Parallel
3′
G
T
5′
+
+
+

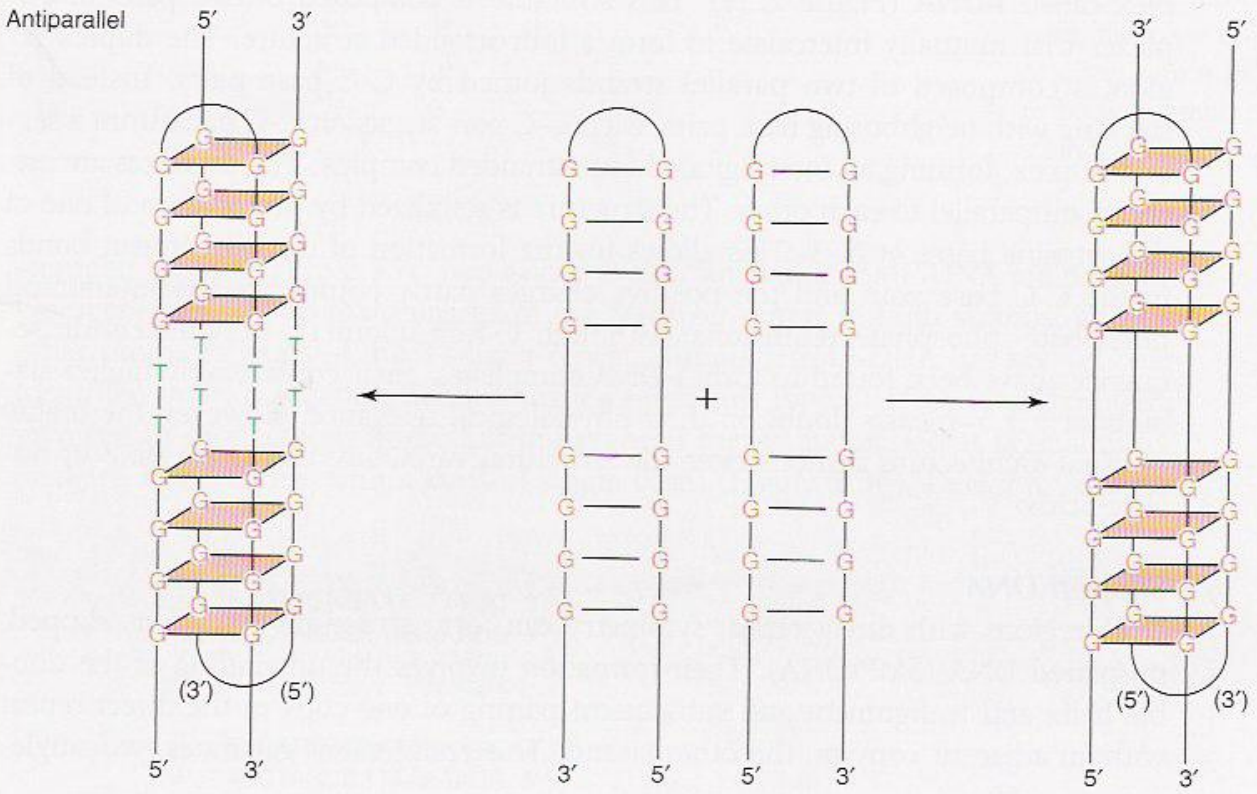
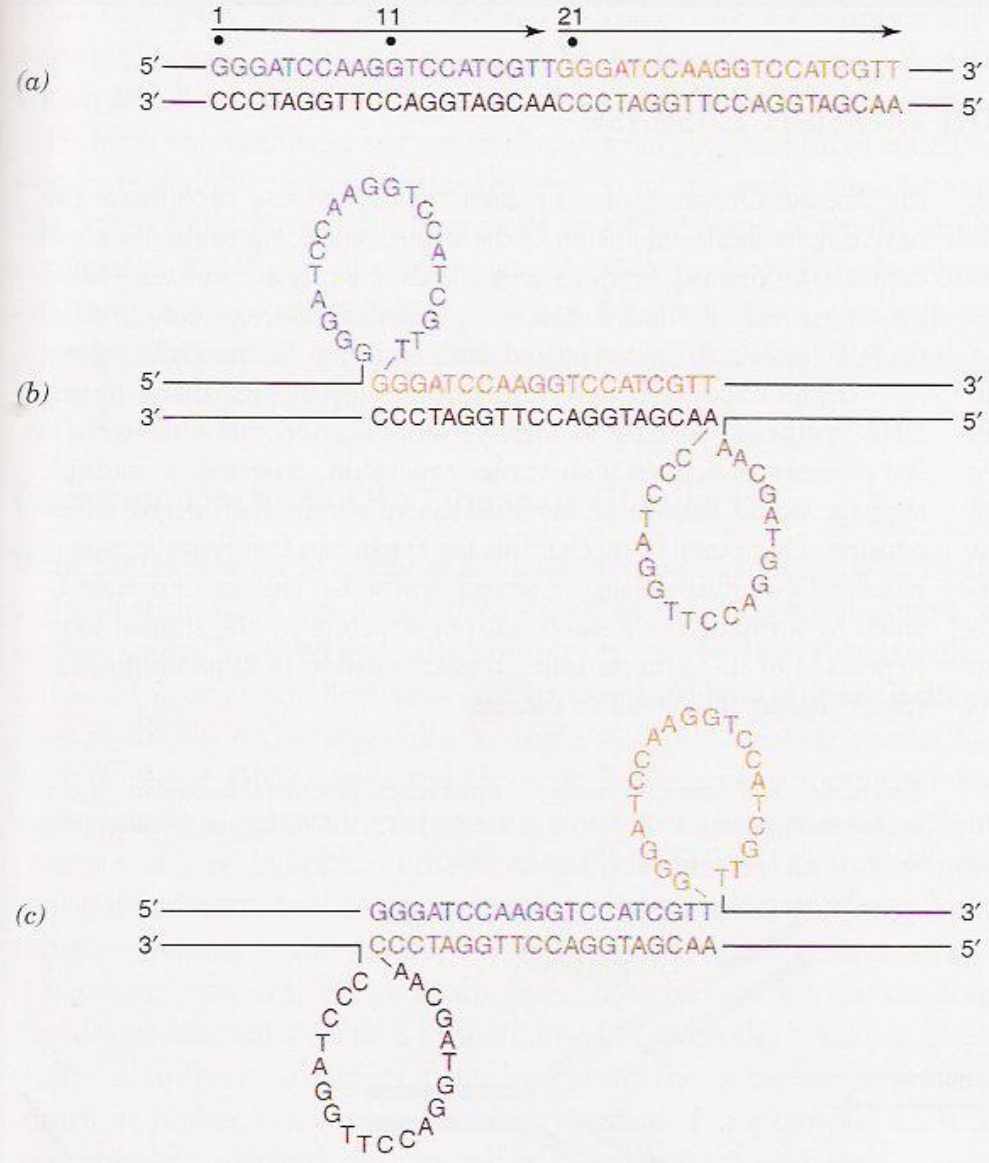


FIGURE 2.33
G-quadruplex DNA.
Four-stranded structures can arise from stacking of G-quartets. Quadruplex structures in which all four strands are parallel can form from four single-stranded G-rich tracts of polynucleotides. Several isomeric quadruplexes can form some strands oriented antiparallel to the others. This arrangement frequently occurs when one polynucleotide provides two or more strands of the quadruplex structure and may be formed by the G-rich sequences of telomeric DNA.
Redrawn from Sinden, R. R. DNA Structure and Function. *New York: Academic Press, 1994.*



-DNA regions with direct repeat symmetry can form structure known as slipped, mispaired DNA (SMP-DNA). Their formation involves the unwinding of a double helix and realignment and subsequent pairing of complimentary regions

-SMP-DNA has not yet been identified in vivo

FIGURE 2.35
Slipped, mispaired DNA.
Presence of two adjacent tandem repeats (*a*) can give rise to one of two isomers of slipped, mispaired DNA. In one of these isomers (*b*) the second copy of the direct repeat in top strand pairs with first copy of repeat on bottom strand. Pairing of first copy of direct repeat in top strand with second copy of direct repeat in bottom strand produces second isomer (*c*). A pair of single-stranded loops is generated in both isomers.

Higher Order DNA Structure

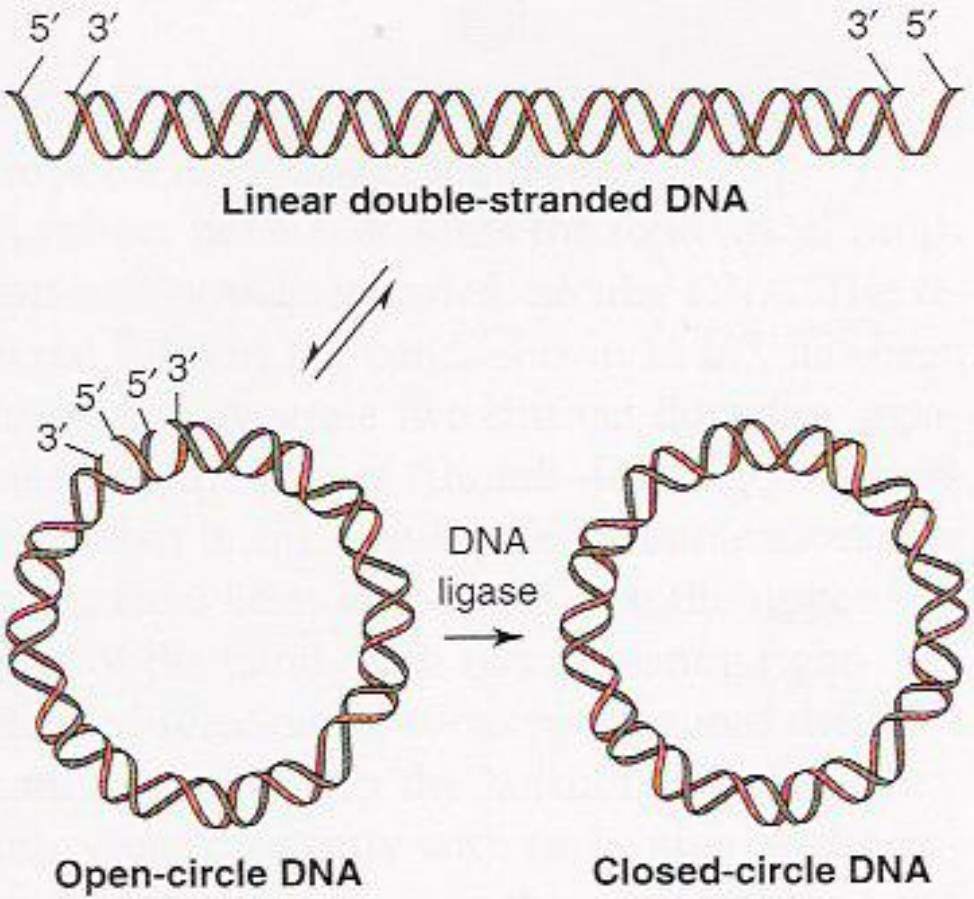


FIGURE 2.37
Circularization of λ DNA.
DNA of bacteriophage λ exists in linear and circular forms, which are interconvertible. Circularization of λ DNA is possible because the 5′-overhangs of the linear form are complementary sequences.

-Genomic DNA may be linear or circular

-Circular DNA results from the formation of phosphodiester bonds between the 3' and 5'-ends of linear polynucleotides, which is carried out by the enzyme called DNA ligase

-Examples of circular DNAs: bacterial, viral genomes, plasmids (small extrachromosomal DNAs in bacteria)

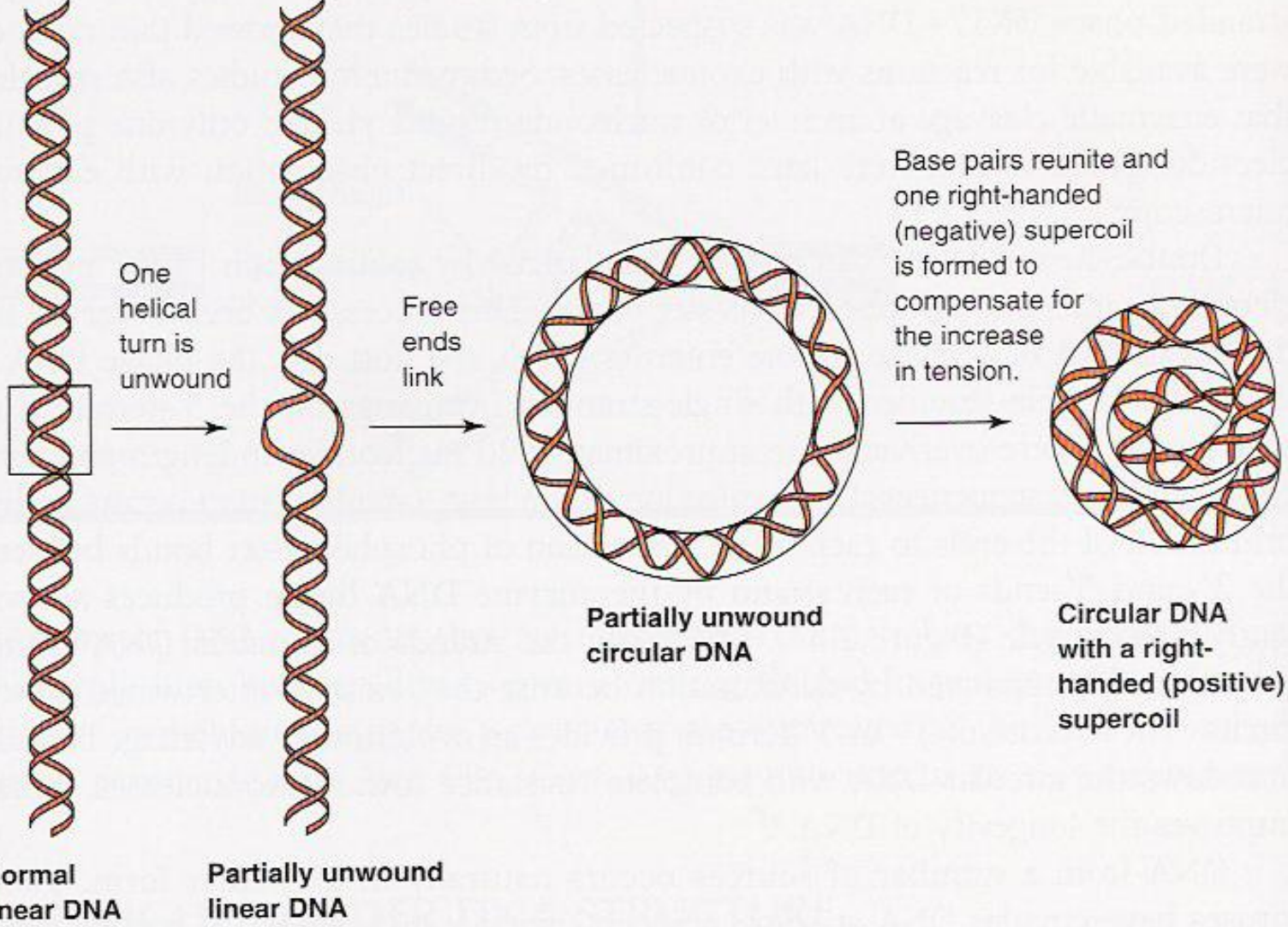


FIGURE 2.38

Negative DNA supercoiling.

Right-handed supercoils (negatively supercoiled DNA) are formed if relaxed DNA is partially unwound. Unwinding may lead to a disruption of hydrogen bonds or alternatively produce negative supercoils. The negative supercoils are formed to compensate for the increase in tension that is generated when disrupted base pairs are reformed.

Redrawn from Darnell, J., Lodish, H., and Baltimore, D. Molecular Cell Biology. *New York: Freeman, 1986.*

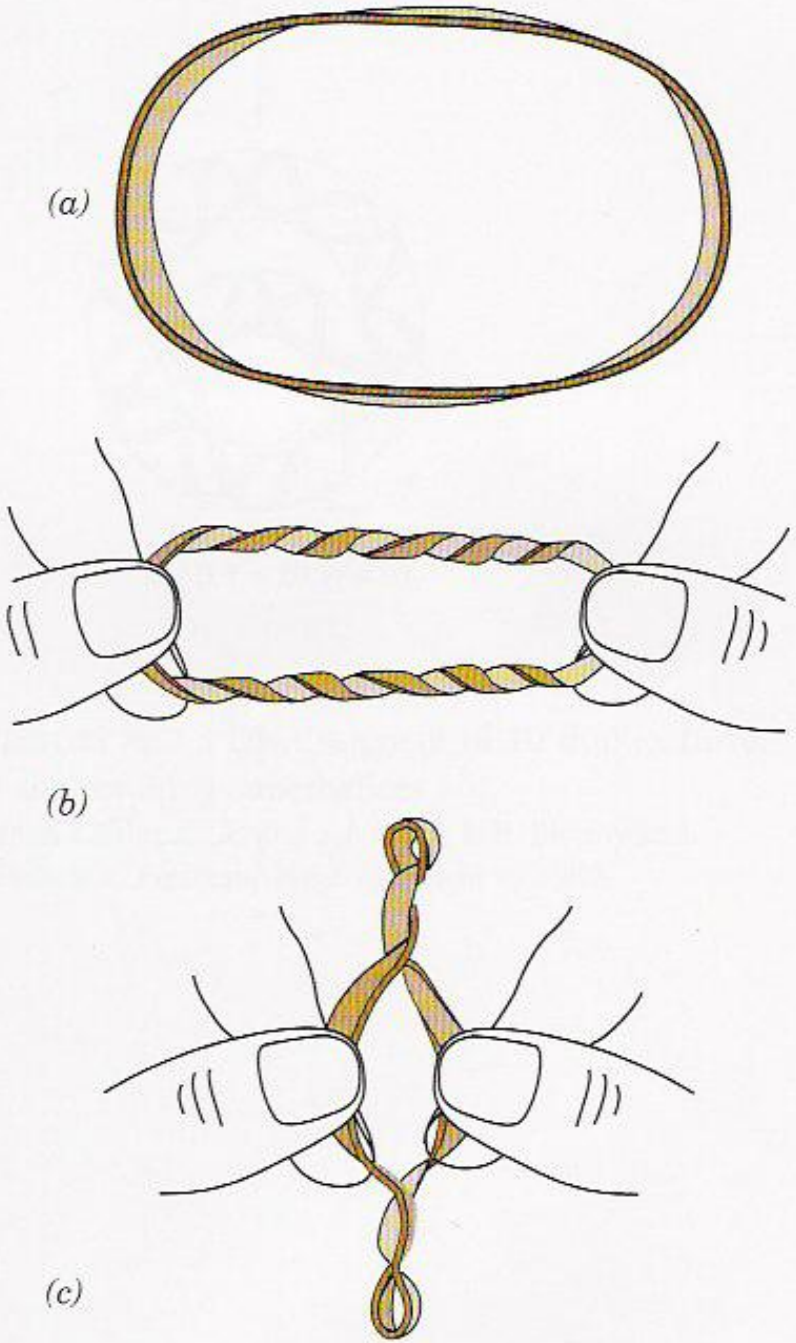


FIGURE 2.40
Superhelical model for DNA.
A rubber band represents the topological properties of double-stranded circular DNA. The relaxed form of the band, shown in (*a*), has been twisted to generate two distinct domains, separated by the pair of "thumb–forefinger anchors," as shown in (*b*). Left-handed (counterclockwise) turns have been introduced into the upper section of the band, with compensating right-handed (clockwise) turns present into the bottom section. When the "anchors" are brought into close proximity with each other as shown in (*c*), the upper section that contained the left-handed turns forms a right-handed superhelix. The bottom section produces a left-handed superhelix. Clearly, superhelicity is not the property of a DNA molecule as a whole but rather a property of specific DNA domains.
Redrawn from Sinden, R. R. and Wells, R. D. Curr. Opin. Biotech. *3:612, 1992.*

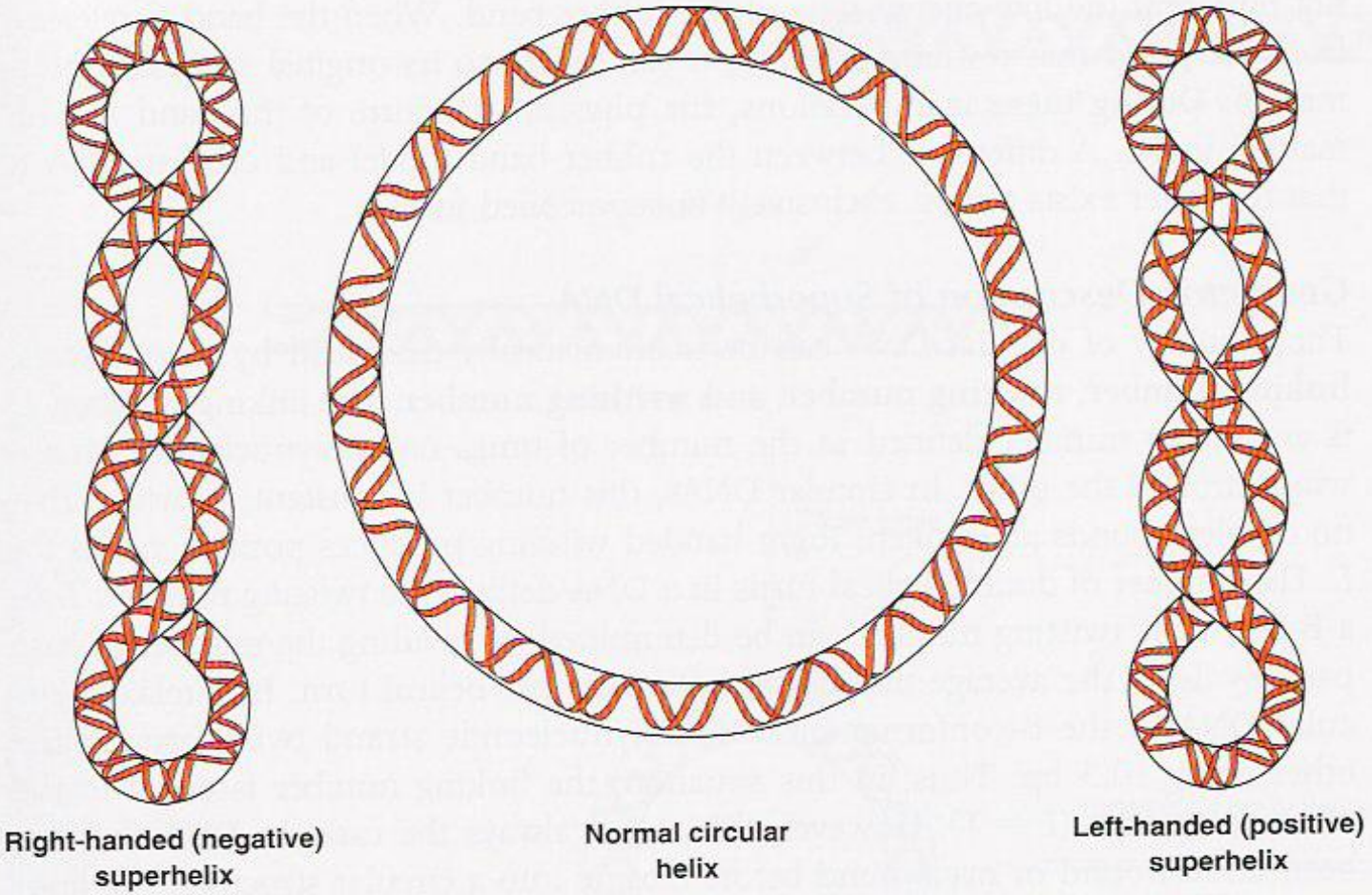


FIGURE 2.39

Relaxed and supercoiled DNA.

Relaxed DNA can be converted to either right- or left-handed superhelical DNA. Right-handed DNA (negatively supercoiled DNA) is the form normally present in cells. Left-handed DNA may also be transiently generated as DNA is subjected to enzymatically catalyzed transformations (replication, recombination, etc.) and it is also present in certain bacterial species. The distinctly different patterns of folding for right- and left-handed DNA superhelices are apparent in this representation.

Redrawn from Dornell, J., Lodish, H., and Baltimore, D. Molecular Cell Biology. *New York: Freeman, 1986.*

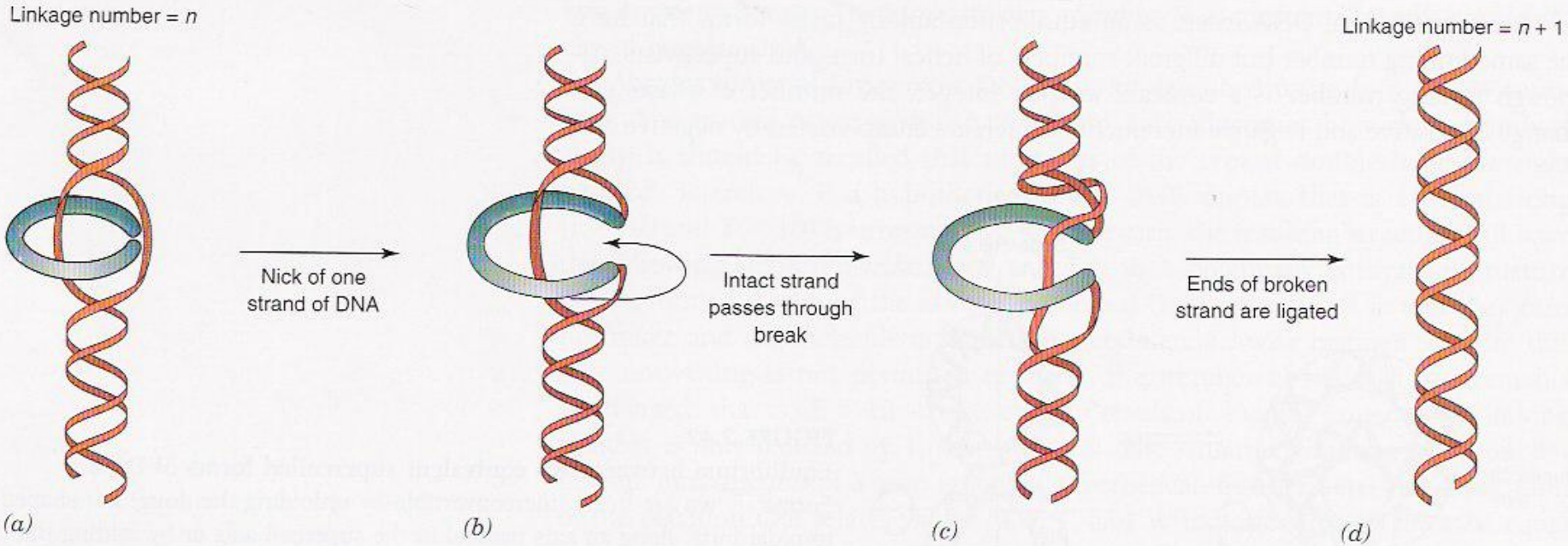


FIGURE 2.43
Mechanism of action of topoisomerases I.
Topoisomerases I relax DNA by (*a*) binding to it and locally separating the complementary strands; then (*b*) nicking one strand and binding to the newly generated termini; and (*c*) passing the intact strand through the gap generated by the nick and closing the gap by restoring the phosphodiester bond. This gives rise to a relaxed structure (*d*).
Redrawn from Dean, F., Kaasnow, M. A., Otter, R., Matzuk, M. M., and Spengler, S. J. Cold Spring Harbor Symp. Quant. Biol. 47:769, 1983.

Enzymes called topoisomerases can regulate the formation of subhelices

Figure 5.9

Supercoiled DNA has a compact twisted structure compared with a non-supercoiled circle or linear molecule. Photograph kindly provided by Svend O. Freytag.

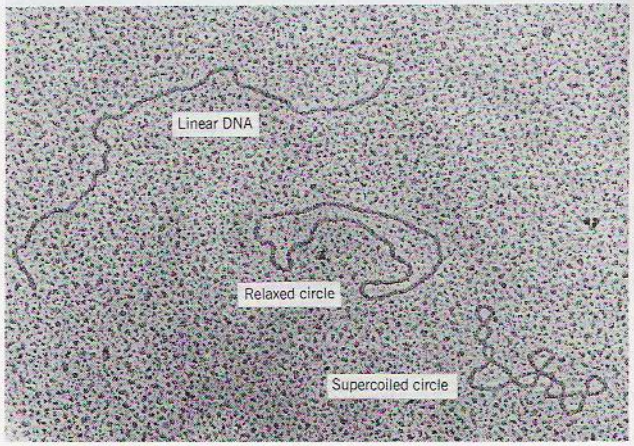
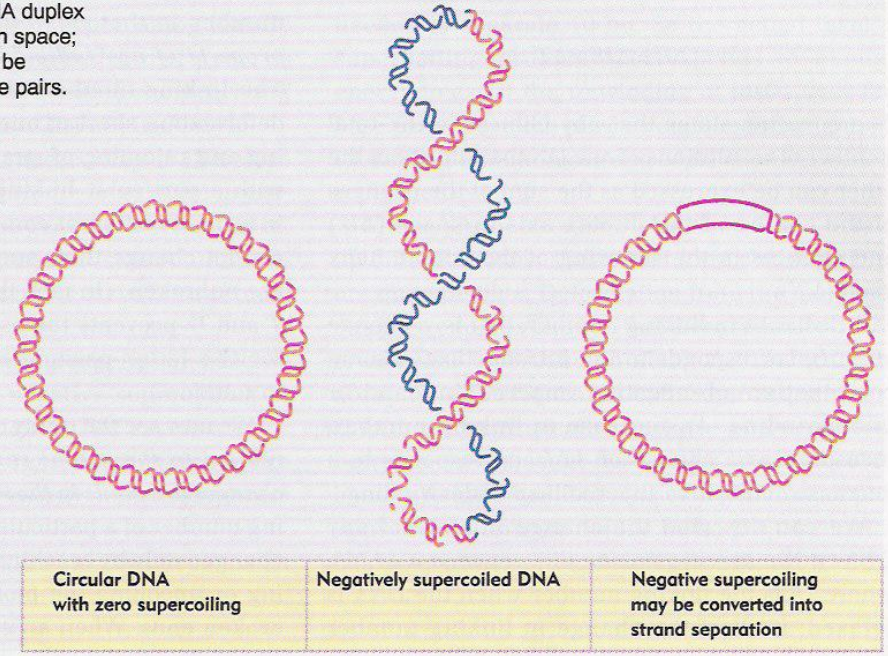


Figure 5.10

Supercoiling causes a DNA duplex to be twisted about itself in space; negative supercoiling can be relieved by disrupting base pairs.



-In mammals, genes for specific proteins and sequences that control gene expression represent only 2-4% of the entire DNA sequence. No specific function has been assigned for the majority of the noncoding DNA ("junk DNA") and it is believed that up to 70-80% of the human DNA is of retroviral origin

-Most eukaryotic genes are interrupted by noncoding intervening nucleotide sequences, called introns. The sequences in the gene that are expressed, either in the final RNA product (mature RNA) or as a protein, are termed exons. The introns are removed during the processing of the RNA transcript, and the remaining exons are ligated together. This tailoring of the original transcript is referred to as splicing.

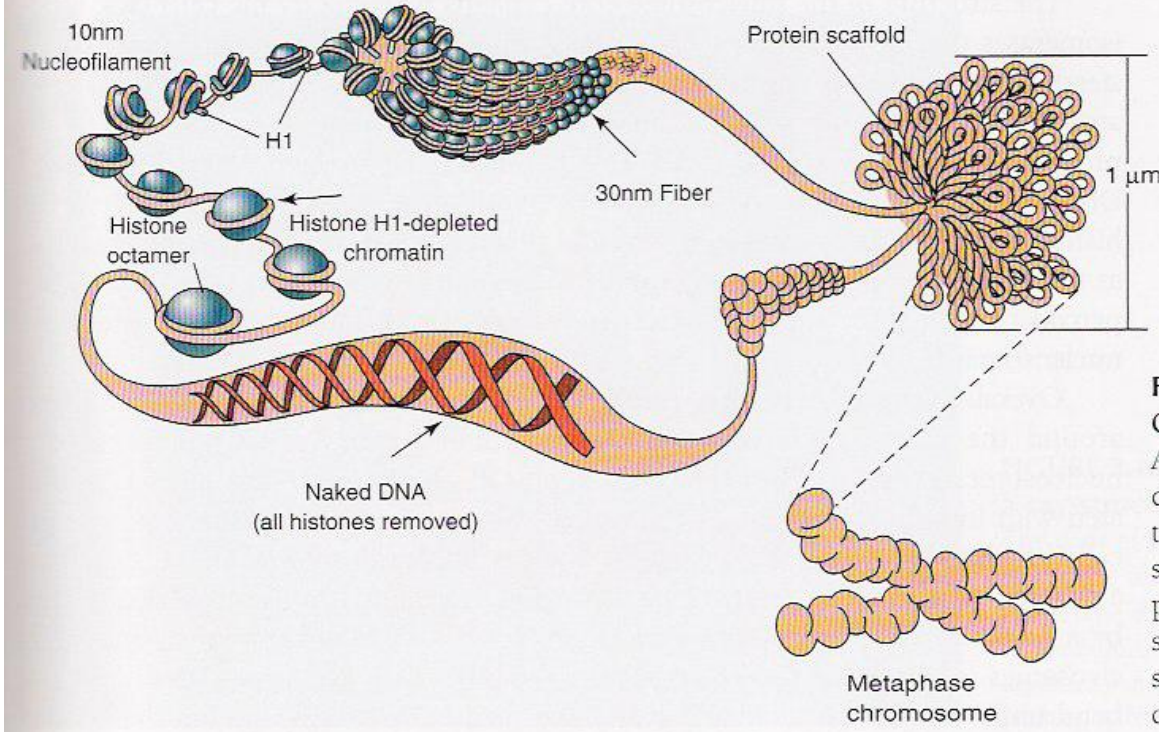


FIGURE 2.46
Organization of DNA into chromosomes.
A speculative drawing showing the stepwise condensation of DNA into chromatin. The DNA initially wraps around the histones of the nucleosome core. Condensation with histone H1 produces the 10-nm nucleofilament, which is subsequently packaged into a twisted, looped structure attached to a protein scaffold within the chromosome.

-DNA in eukaryotic cells is associated with various proteins to form chromatin. Prior to cell division (metaphase) chromatin becomes organized into compact structures called chromosomes

-Early stages of DNA packing lead to formation of 30-nm fibers

-Naked DNA is associated with a class of highly basic proteins called histones

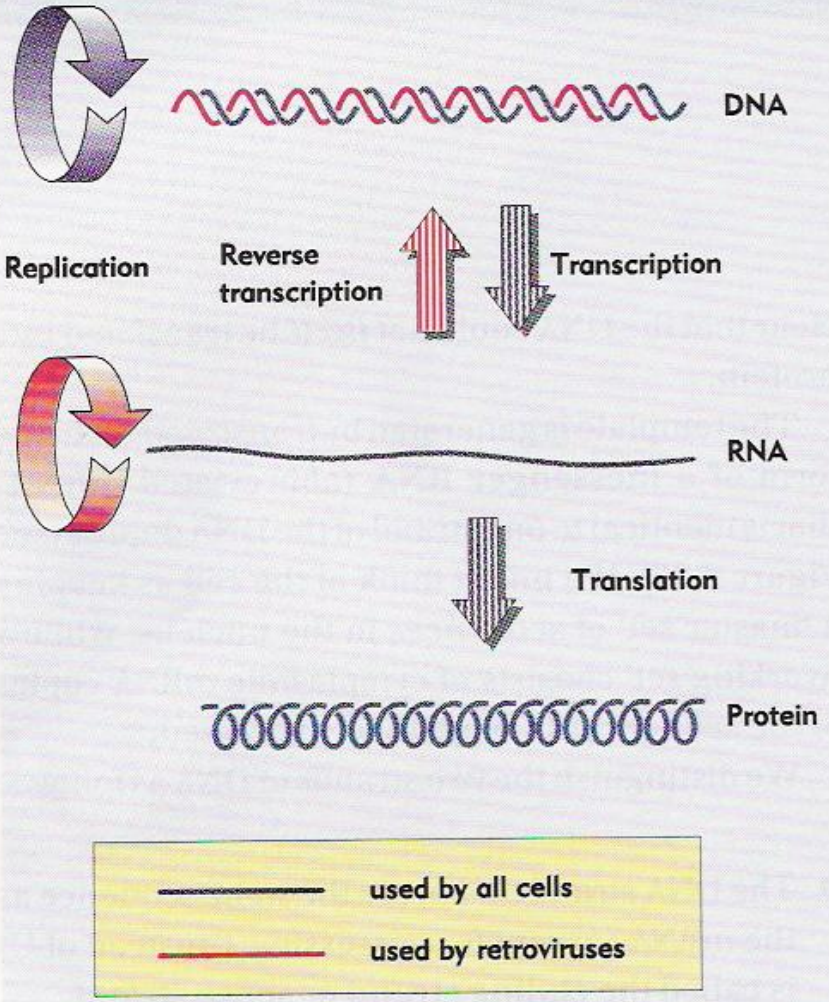
Table 6.2

The amount of nucleic acid in the genome varies over an enormous range.

Genome	Gene Number	Base Pairs
Organisms		
Plants	<50,000?	$<10^{11}$
Mammals	<25,000?	~3×10^8
Worms	~5,000?	~10^8
Fungi	~4,000?	~4×10^7
Bacteria	~2,000?	$<10^7$
Mycoplasma	~750?	$<10^6$
dsDNA Viruses		
Vaccinia	<300	187,000
Papova (SV40)	~6	5,226
Phage T4	~200	165,000
ssDNA Viruses		
Parvovirus	5	5,000
Phage fX174	11	5,387
dsRNA Viruses		
Reovirus	22	23,000
ssRNA Viruses		
Coronavirus	7	20,000
Influenza	12	13,500
TMV	4	6,400
Phage MS2	4	3,569
STNV	1	1,300
Viroids		
PSTV RNA	0	359
Scrapie		
Prion	?	?

Figure 7.1

Overview: the central dogma states that information in nucleic acid can be perpetuated or transferred, but the transfer of information into protein is irreversible.



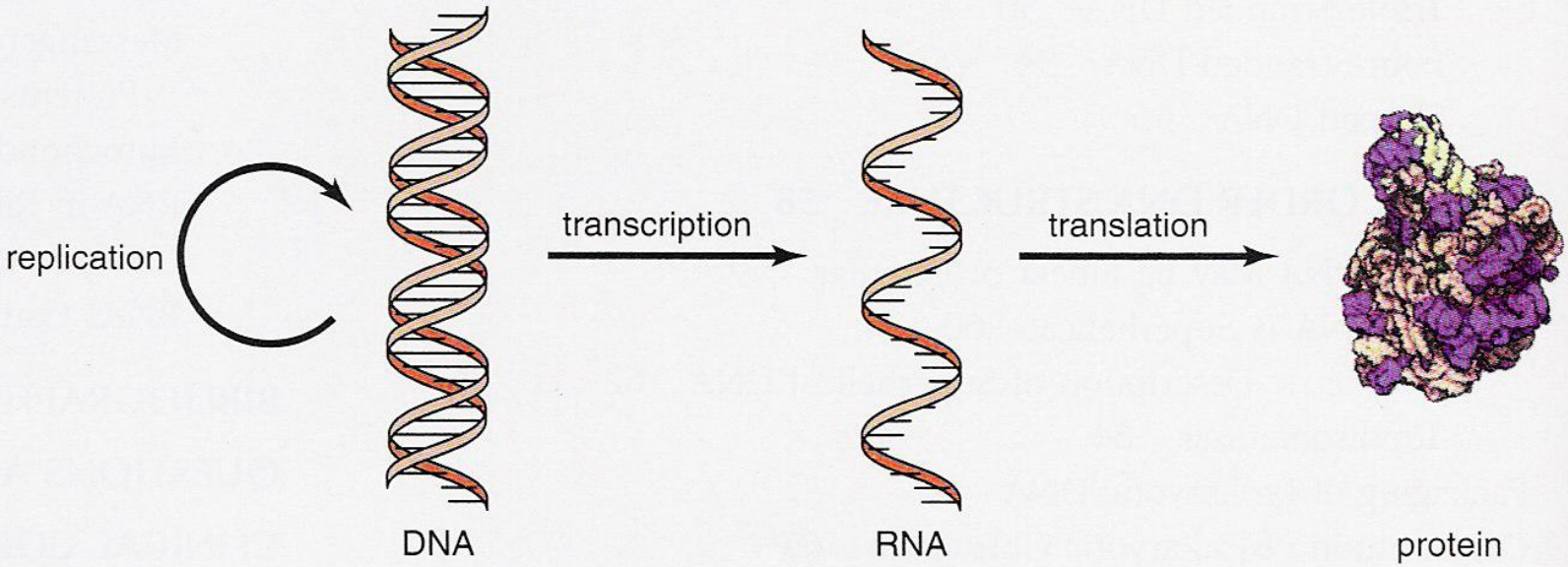


FIGURE 2.1
Central dogma of molecular biology.

Central Dogma of Molecular Biology holds that DNA stores Information that controls all cellular processes. Information cannot flow directly from DNA to protein, but depends on ribonucleic acid (RNA) to transport the information. Genetic Information is is transmitted from DNA to RNA by transcription. The sequence of RNA is then translated into a protein sequence at the ribosome. Several discoveries have begun to blur the distinct roles of each of these biomolecules; for example, it has been demonstrated that RNA can act as a catalyst in biochemical reactions (ribozymes) and as a template for DNA synthesis (reverse transcription)

Primary types of RNA:

-mRNA and its precursors/derivatives (messenger RNA)

-tRNA (transfer RNA)

-rRNA (ribosomal RNA)

-Small stable RNA and catalytic RNA

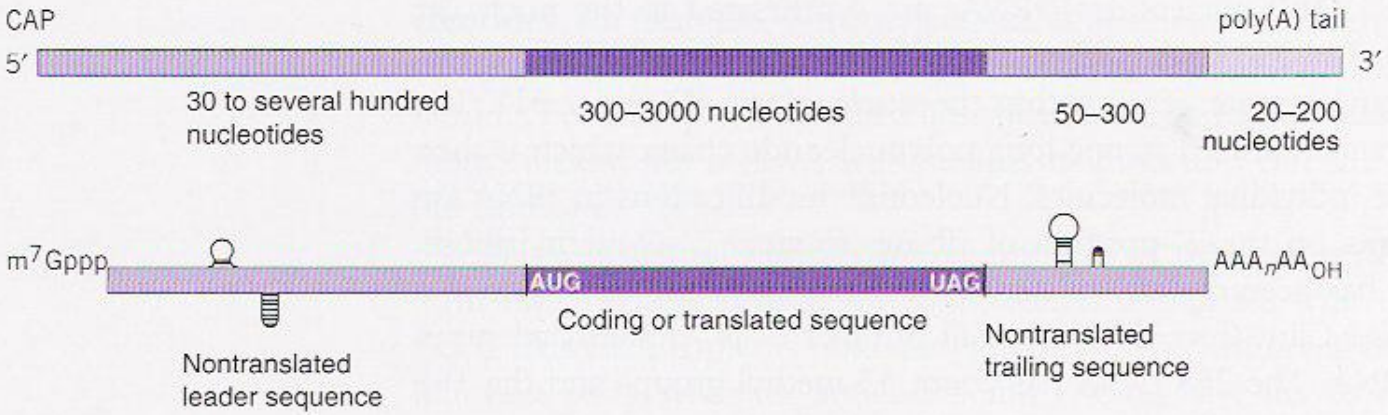


FIGURE 2.62
General structure for a eukaryotic mRNA.
There is a "blocked" 5′-terminus, cap, followed by a nontranslated leader containing a promoter sequence. Coding region usually begins with the initiator codon AUG and continues to the translation termination sequence UAG, UAA, or UGA. This is followed by the nontranslated trailer and a poly(A) tail on the 3′-end.

Messenger RNAs carry the information for the primary structure of proteins

Double-helical stem-loop regions in RNA are often called "hairpins". Each eukaryotic mRNA is monocistronic, that is, contains information for only one polypeptide chain. In eukaryotes, a "cap" structure at the 5'-end of an mRNA specifies where translation should begin.

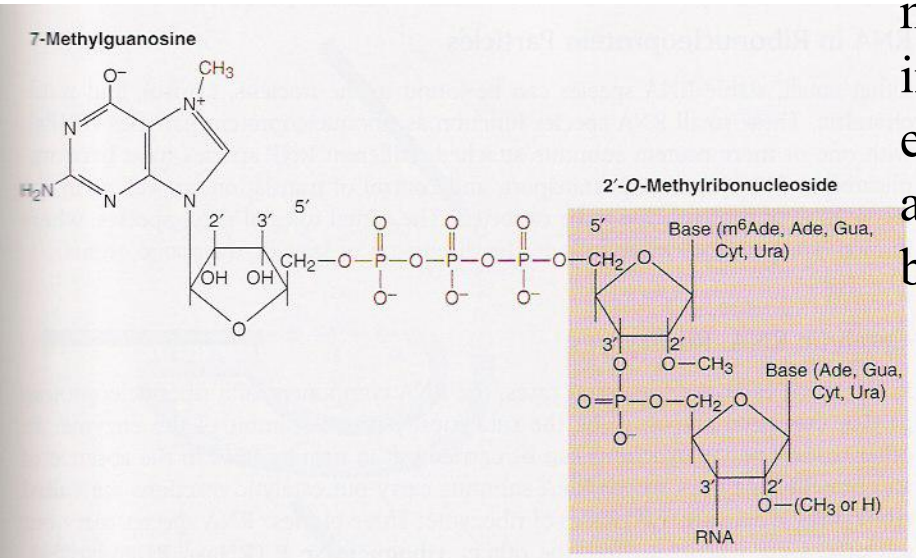


FIGURE 2.63
Diagram of "cap" structure or blocked 5′-terminus in mRNA.
The 7-methylguanosine is inverted to form a 5′-phosphate to 5′-phosphate linkage with the first nucleotide of the mRNA. This nucleotide is often a methylated purine.

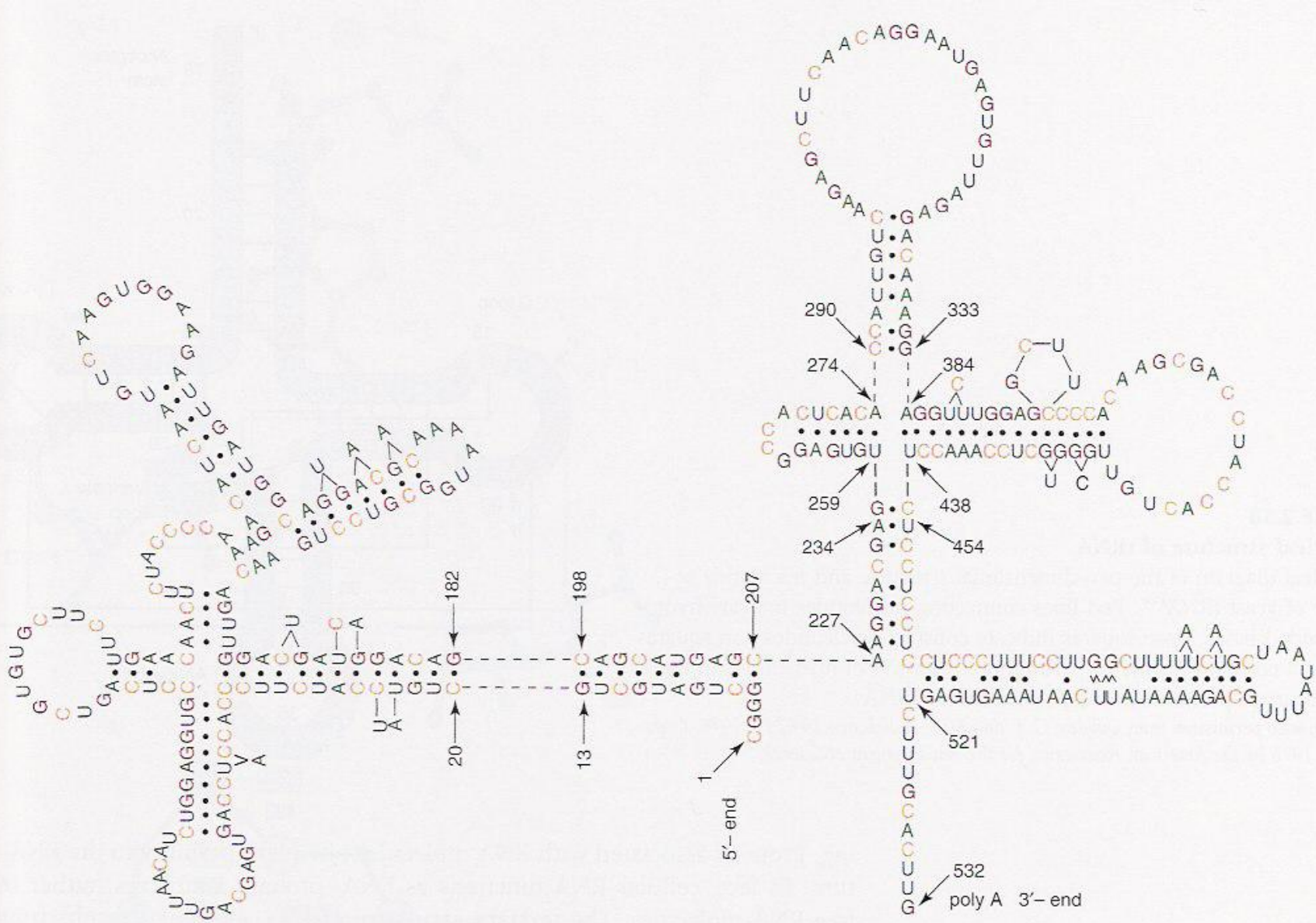


FIGURE 2.57

Proposed base-pairing regions in the mRNA for mouse immunoglobulin light chain. Base-paired structures shown have free energies of at least −5 kcal. Note the variance in loop size and length of paired regions.

Redrawn from Hamlyn, P. H., Browniee, G. G., Cheng, C. C., Gait, M. J., and Milstein, C. Cell 15:1067, 1978.

Transfer RNA has two roles:
-activating amino acids and
-recognizing codons in mRNA

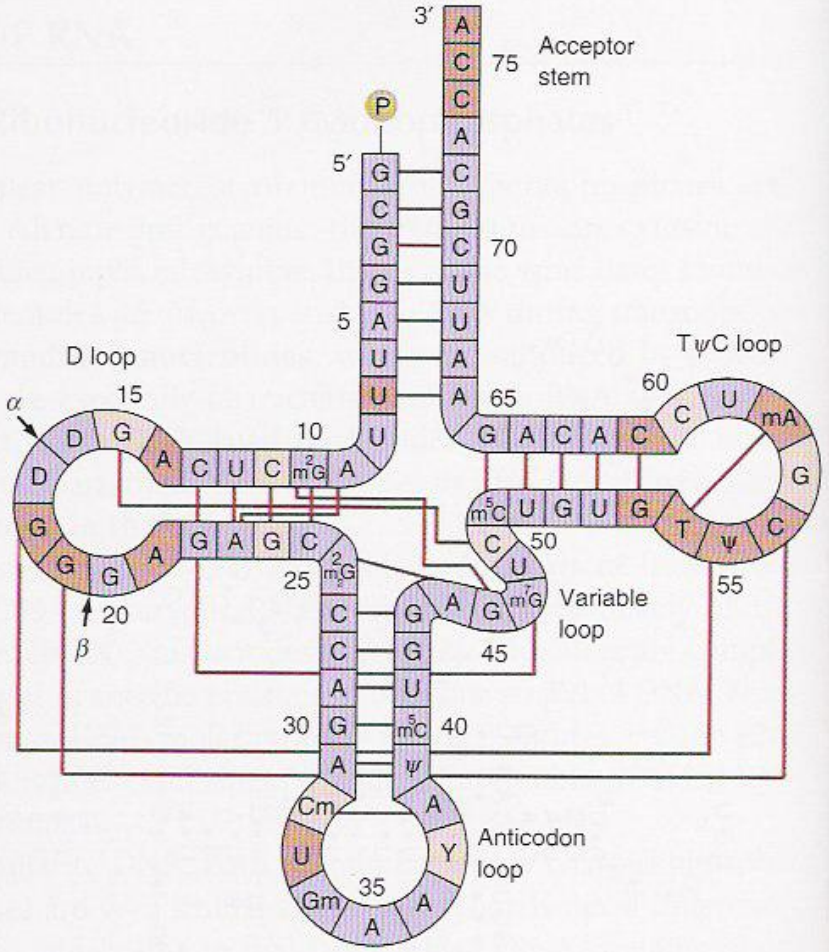
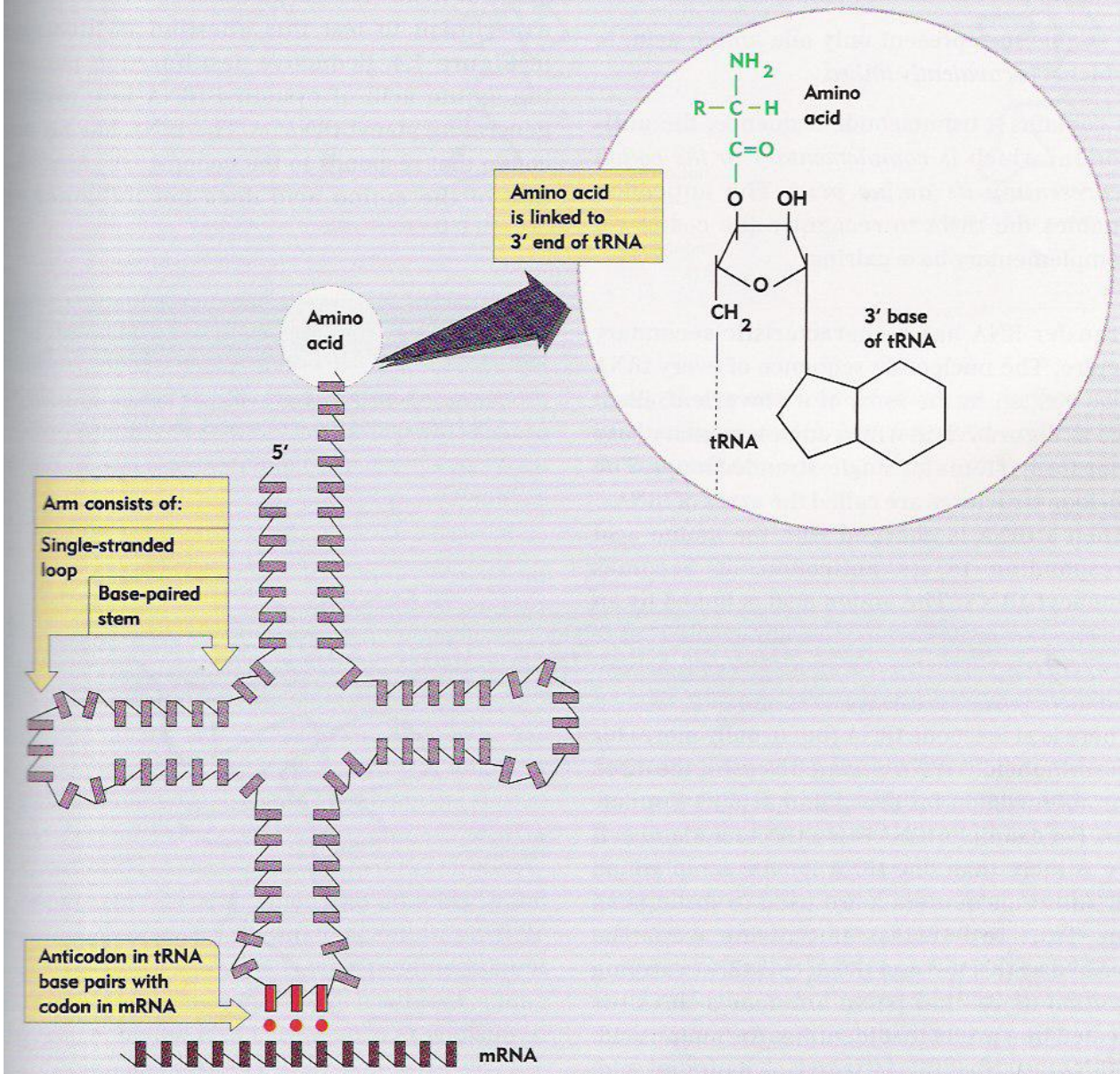


FIGURE 2.58
Cloverleaf structure of tRNA.
Cloverleaf diagram of the two-dimensional structure and nucleotide sequence of yeast $tRNA^{Phe}$. Red lines connecting nucleotides indicate hydrogen-bonded bases. Rose squares indicate constant nucleotides; tan squares indicate a constant purine or pyrimidine. Insertion of nucleotides in the D loop occurs at positions α and β for different tRNAs.
Redrawn with permission from Quigley, G. J. and Rich, A. Science 194:797, 1976. Copyright © 1976 by the American Association for the Advancement of Science.

Figure 7.2

The secondary structure of tRNA has four arms. Each tRNA has the dual properties of an adaptor that recognizes both the amino acid and codon.



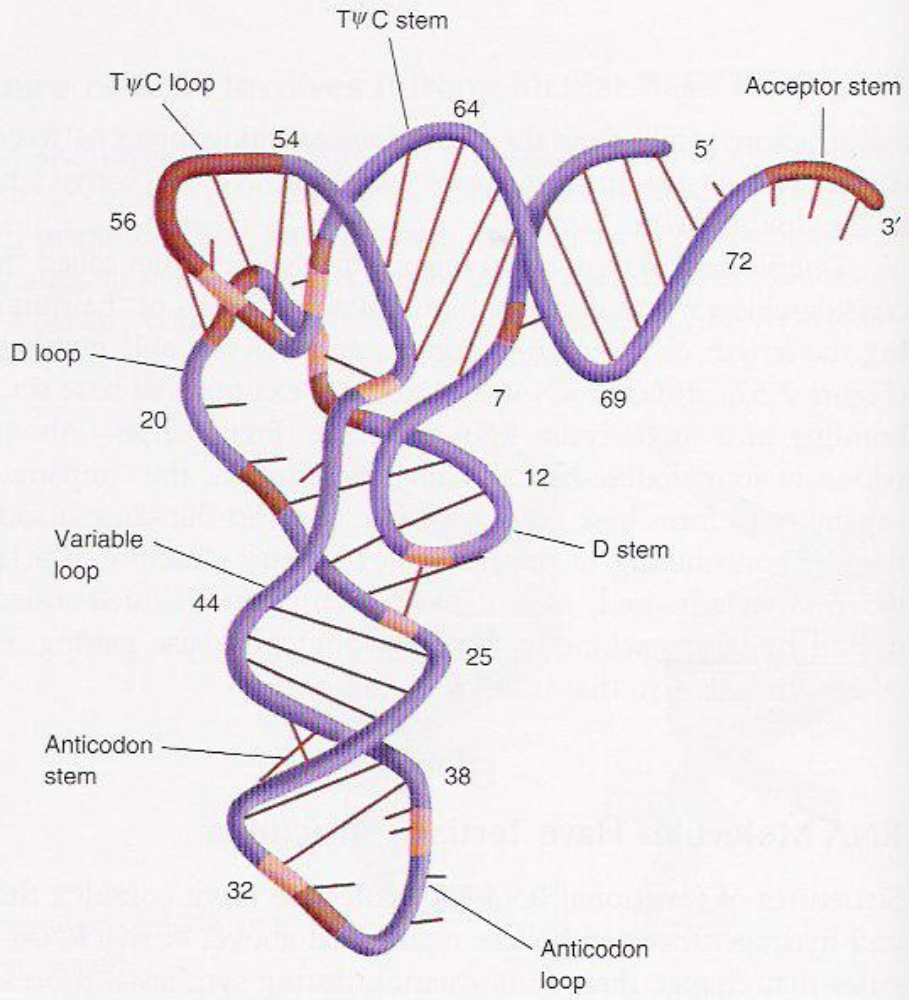


FIGURE 2.59

Tertiary structure on tRNA.

Tertiary folding of the cloverleaf structure of $tRNA^{Phe}$. Hydrogen bonds are indicated by cross rungs. Compare the presentation with Figure 2.58.

Redrawn with permission from Quigley, G. J. and Rich, A. Science 194:797, 1976.

Ribosomal RNA is part of the protein synthesis apparatus

Ribosomal RNA accounts for most (up to 80%) of cellular RNA and is metabolically stable

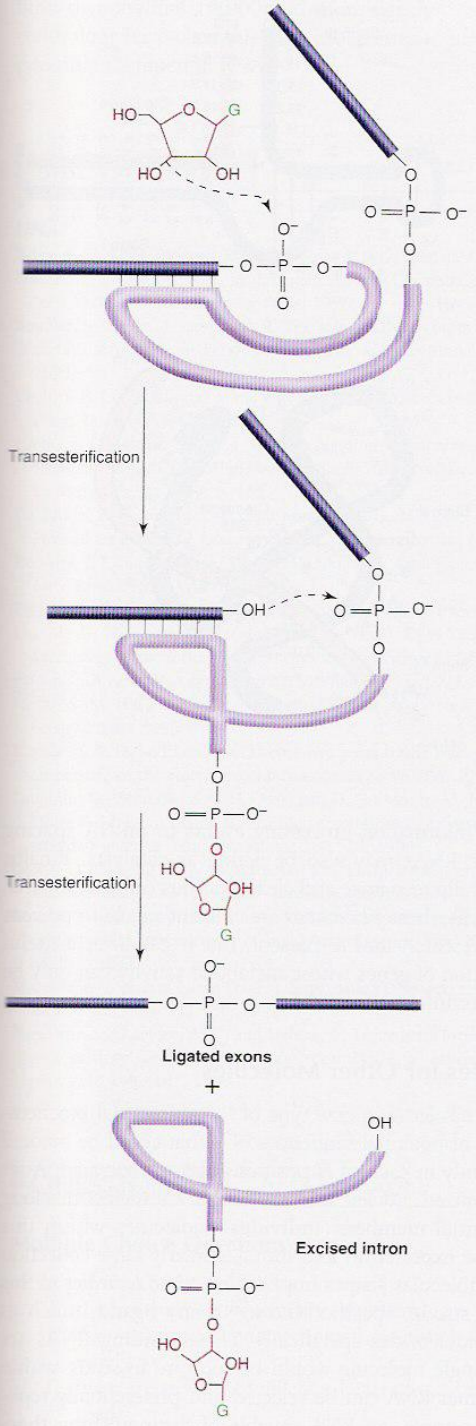


FIGURE 2.64
Mechanism of self-splicing of the rRNA precursor of *Tetrahymena*.
Two exons of rRNA are denoted by dark blue. Catalytic functions reside in the intron, which is purple. This splicing function requires an added guanine nucleoside or nucleotide.
Redrawn from Cech, T. R. JAMA 260:308, 1988.

Enzymes whose RNA subunits carry out catalytic reactions are called ribozymes. Discovery of RNA catalysis has greatly altered our concepts of biochemical evolution and the range of allowable cellular chemistry. First, we now recognize that RNA can serve as both a catalyst and a carrier of genetic information. This has raised the possibility that the earliest living organisms were based entirely on RNA and that DNA and proteins evolved later. This model is sometimes referred to as the "RNA world".

Small stable RNAs have been implicated in:

-RNA processing and transport

-control of translation

-protein recognition

Polymerase Chain Reaction (PCR)

DNA, composed of four different nucleotides is deceptively complex. Complexity is conferred on the DNA molecule by:

-the nonrandom sequence of its bases

-multiple conformations that exist in equilibrium in the biological environment

-specific proteins that recognize and associate with selected regions

Development of DNA sequencing allowed the determination and comparisons of genomes of many diverse life forms

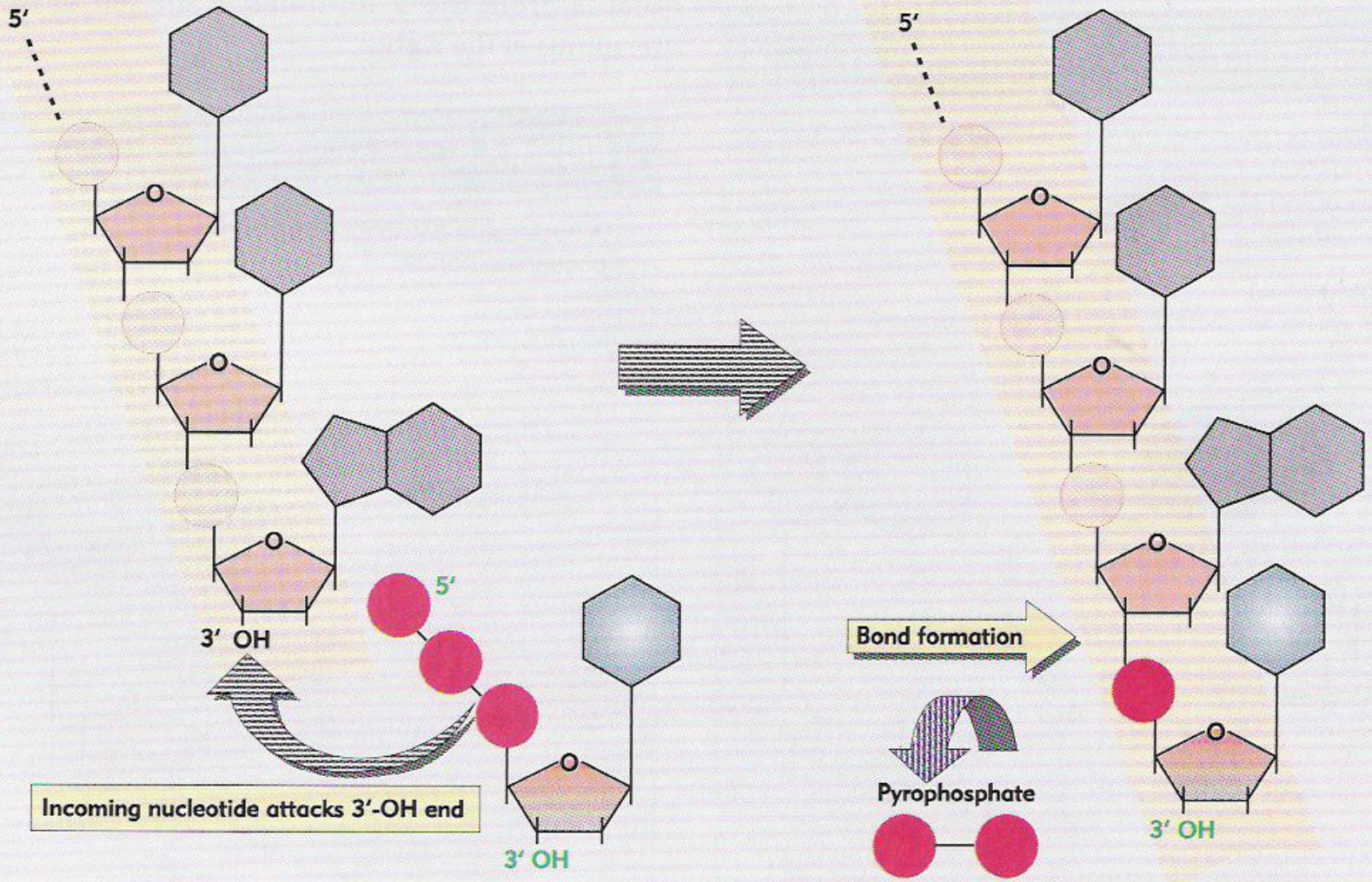
Many methodological approaches in genetic engineering have been greatly simplified by the development of a method that rapidly amplifies selected regions of DNA, the polymerase chain reaction (PCR)

DNA analysis and sequencing requires relatively large amounts of DNA; the rapid production of large quantities of a specific DNA sequence took a leap forward with the development of the polymerase chain reaction (PCR)

-PCR requires two nucleotide oligomers that hybridize to the complimentary DNA strands in a region of interest. These oligomers serve as primers for a DNA polymerase that extends each strand. Repeated cycling of the PCR yields large amounts of each DNA molecule of interest in a matter of hours, as opposed to days and weeks required for cloning techniques

Figure 4.9

Nucleic acid synthesis occurs by adding the nucleoside-5'-monophosphate moiety of a nucleoside triphosphate to the 3'-OH end of the polynucleotide chain.



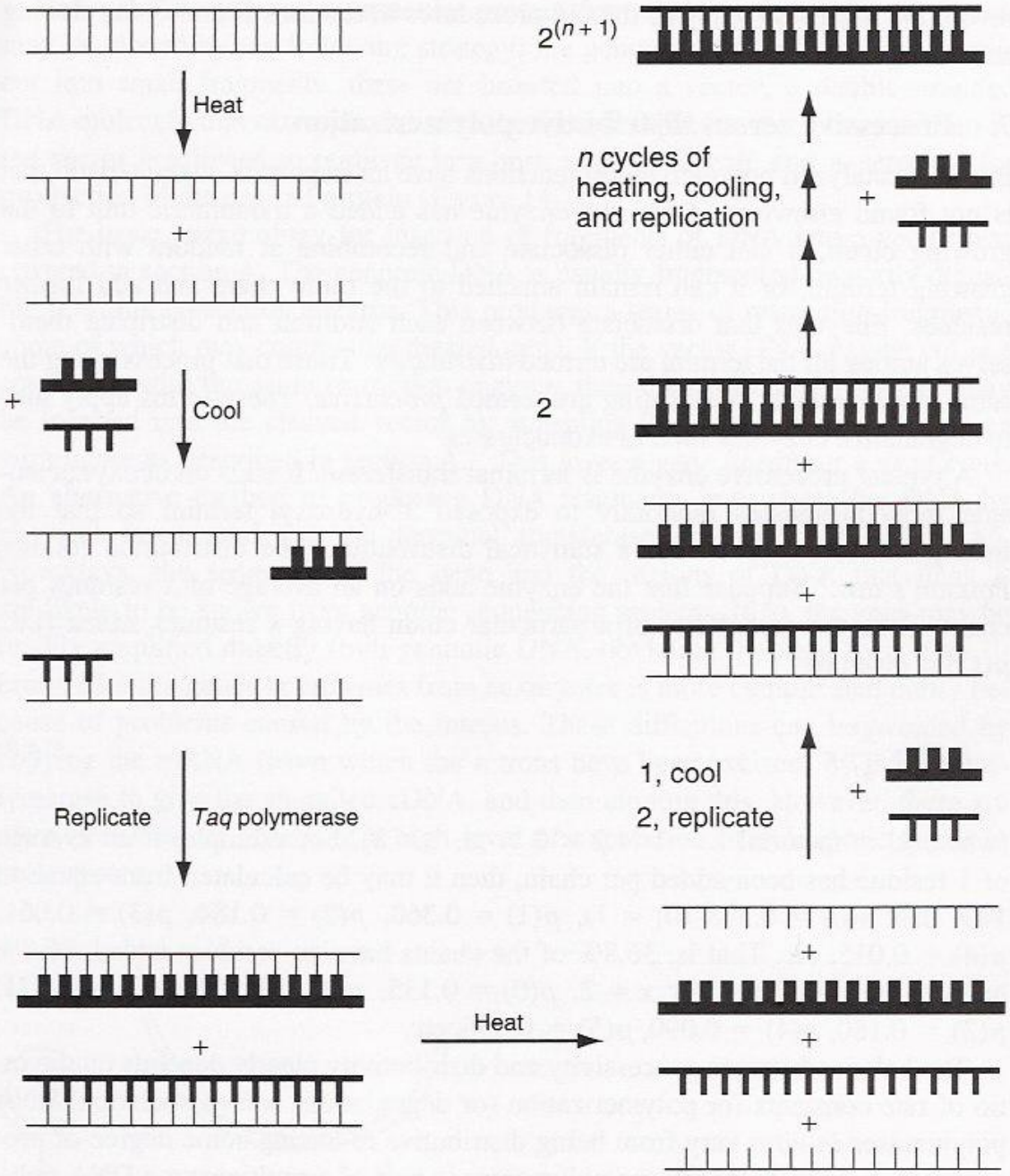
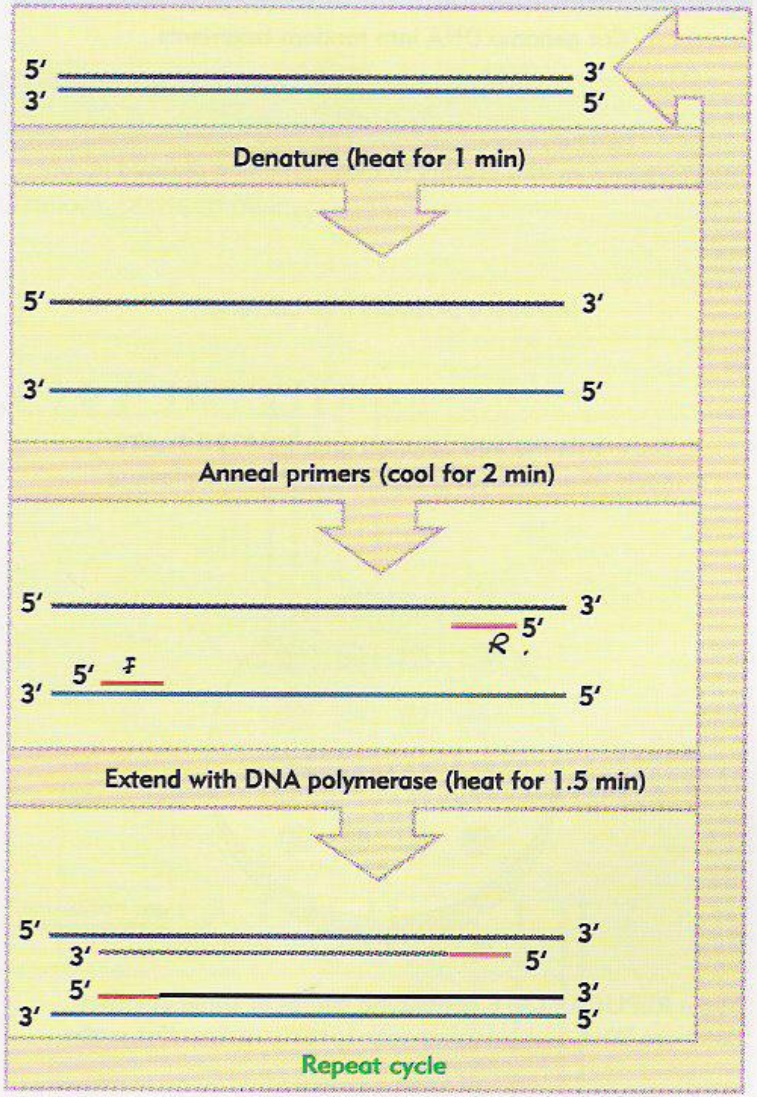
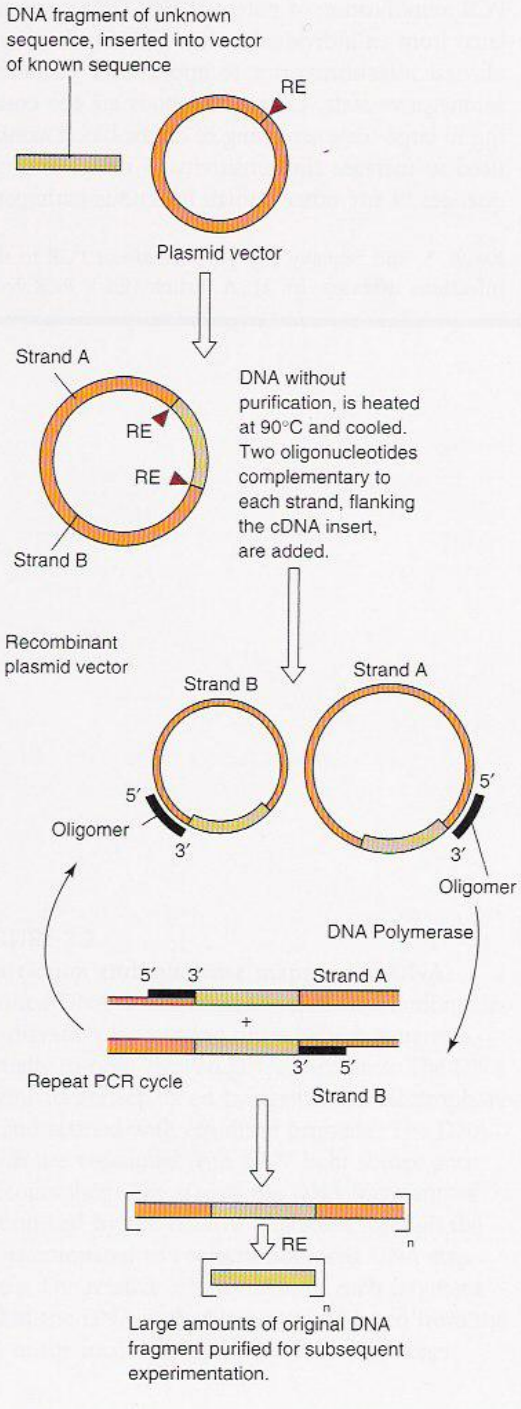


Figure 14.6 The polymerase chain reaction.

Figure 21.10

A single cycle of PCR doubles the number of copies of a target sequence and can be performed in 5 minutes.




DNA fragment of unknown sequence, inserted into vector of known sequence
RE
Plasmid vector
Strand A
RE
RE
Strand B
DNA without purification, is heated at 90°C and cooled. Two oligonucleotides complementary to each strand, flanking the cDNA insert, are added.
Recombinant plasmid vector
Strand B
Strand A
5′
Oligomer
3′
5′
3′
Oligomer
DNA Polymerase
5′ 3′
Strand A
+
3′ 5′
Strand B
Repeat PCR cycle
RE
n
n
Large amounts of original DNA fragment purified for subsequent experimentation.

-Heating to about 90˚C as required for melting DNA inactivates most DNA polymerases, but a heat-stable DNA polymerase (termed *Taq* DNA polymerase) isolated from Thermus aquaticus, obviating the need for fresh polymerase after each PCR cycle. This has permitted the automation of PCR with each DNA molecule capable of being amplified one million-fold.