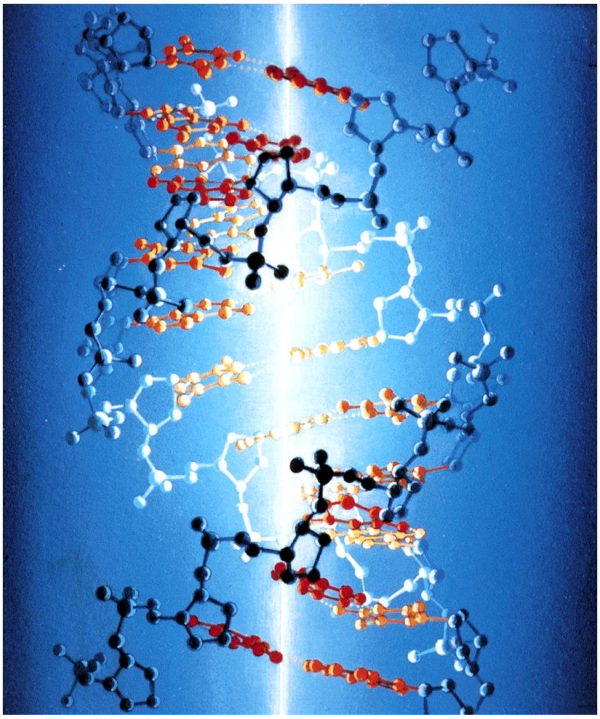
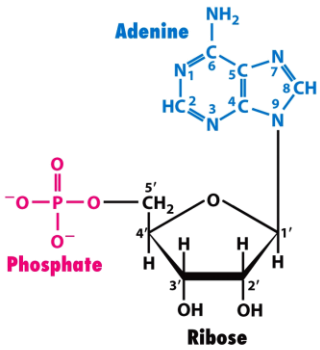


A DNA molecule consists of two strands that wind around a central axis, shown here as a glowing wire. A complete set of genetic instructions contains just four types of monomeric units.

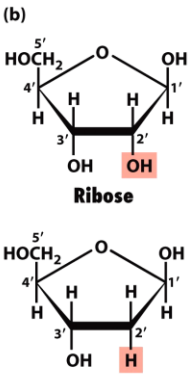
Illustrations, Irving Geis

Five different nucleotides are used to build Nucleic Acids

Two types of chemically similar nucleic acids, DNA and RNA, are *the principal genetic-information-carrying molecules of the cell.* The monomeric building blocks are called nucleotides, all have a common structure: a phosphate group linked by a phosphoester bond to a pentose that in turn is linked to a nitrogen- and carbon-containing ring structure referred as a *base*.

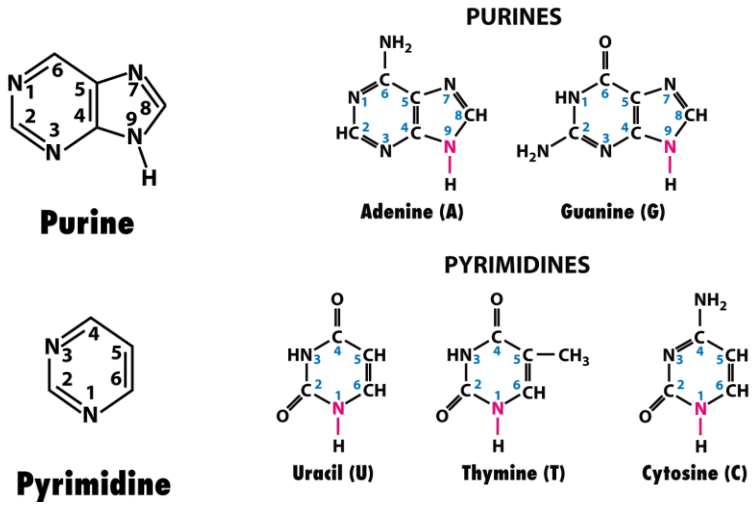


Adenosine 5′-monophosphate (AMP)



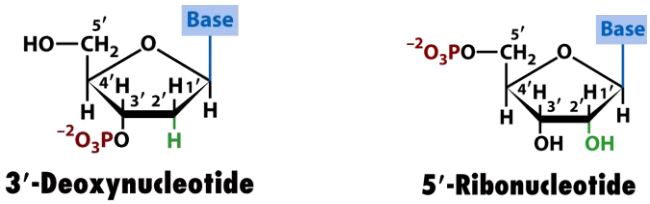
Chemical structures of the principal bases in nucleic acids

In nucleic acids and nucleotides, nitrogen 9 of purines and nitrogen 1 of pyrimidines are bonded to the 1' carbon of ribose or deoxyribose. U is only in RNA, and T is only in DNA. Both RNA and DNA contain A, G, and C.



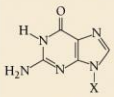
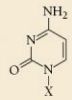
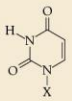
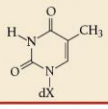
Chemical structures of nucleotides

The purine or pyrimidine base is linked to C1' of the pentose and at least one phosphate is also attached to atom C3' or atom C5' of the pentose. *A nucleoside consists only of a base and a pentose.*



The acidic character of nucleotides is due to the phosphate group, which under normal intracellular conditions releases H^+, leaving the phosphate negatively charged. Most nucleic acids are associated with proteins, which form ionic interactions with the negatively charged phosphates.

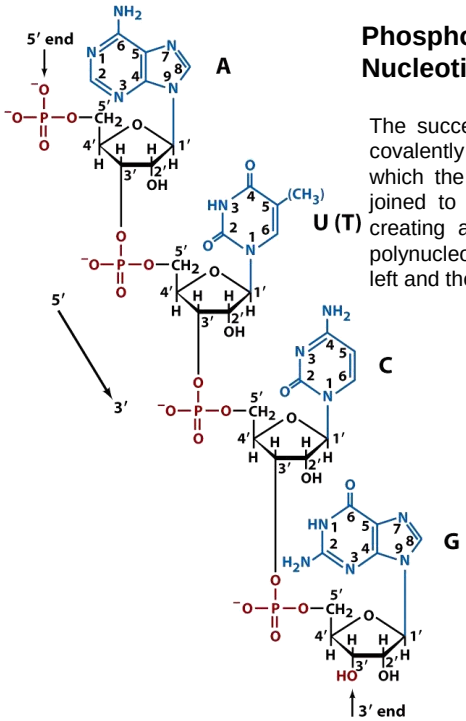
Table 3-1 Names and Abbreviations of Nucleic Acid Bases, Nucleosides, and Nucleotides

Base Formula	Base (X = H)	Nucleoside (X = ribose[a])	Nucleotide[b] (X = ribose phosphate[a])
	Adenine Ade A	Adenosine Ado A	Adenylic acid Adenosine monophosphate AMP
	Guanine Gua G	Guanosine Guo G	Guanylic acid Guanosine monophosphate GMP
	Cytosine Cyt C	Cytidine Cyd C	Cytidylic acid Cytidine monophosphate CMP
	Uracil Ura U	Uridine Urd U	Uridylic acid Uridine monophosphate UMP
	Thymine Thy T	Deoxythymidine dThd dT	Deoxythymidylic acid Deoxythymidine monophosphate dTMP

[a]The presence of a 2′-deoxyribose unit in place of ribose, as occurs in DNA, is implied by the prefixes "deoxy" or "d." For example, the deoxynucleoside of adenine is deoxyadenosine or dA. However, for thymine-containing residues, which rarely occur in RNA, the prefix is redundant and may be dropped. The presence of a ribose unit may be explicitly implied by the prefix "ribo."

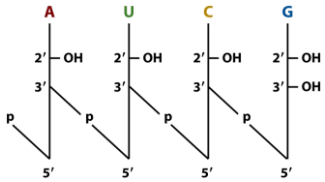
[b]The position of the phosphate group in a nucleotide may be explicitly specified as in, for example, 3′-AMP and 5′-GMP.

Table 3-1 Fundamentals of Biochemistry, 2/e



Phosphodiester Bonds Link Successive Nucleotides in Nucleic Acids

The successive nucleotides of both DNA and RNA are covalently linked through phosphate-group "bridges," in which the 5'-phosphate group of one nucleotide unit is joined to the 3'-hydroxyl group of the next nucleotide, creating a **phosphodiester linkage**. By convention, a polynucleotide sequence is written with the 5' end at the left and the 3' end at the right.



The phosphodiester bond links neighboring ribose residues in the 5' ➔3' direction.

The properties of a polymer such as a nucleic acid may be very different from the properties of the individual units, or **monomers,** before polymerization. As the size of the polymer increases from **dimer, trimer, tetramer,** and so on through **oligomer** (Greek: *oligo,* few), physical properties such as charge and solubility may change.

In addition, *a polymer of nonidentical residues as a property that its component monomers lack—namely, it contains information in the form of its sequence of residues*.

Nucleic acid structure

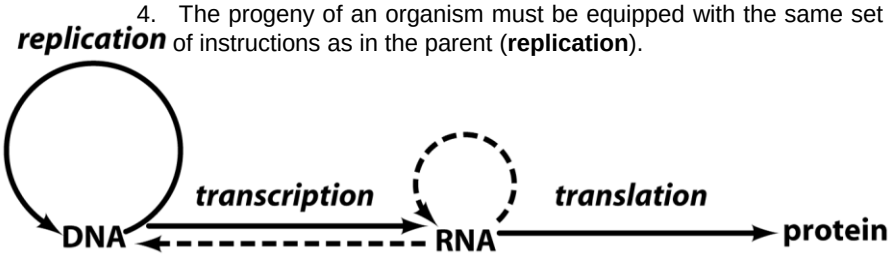
Two kind of nucleic acids, DNA and RNA, store information and make it available to the cell. The structure of these molecules must be consistent with:

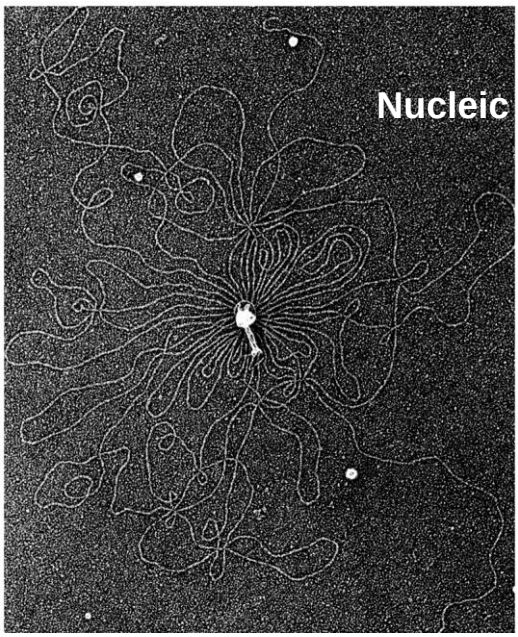
1. Genetic information must be stored in a form that is manageable in size and stable over a long period.

2. Genetic information must be decode in order to be used (**transcription** and **translation**).

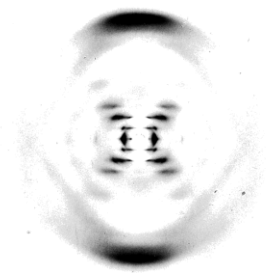
3. Information contained in DNA or RNA must be accessible to proteins and other nucleic acids. These agents must recognize nucleic acids and bind to them in a way that alters their function.

4. The progeny of an organism must be equipped with the same set of instructions as in the parent (**replication**).





Nucleic Acid structure



An X-ray diffraction photograph of a vertically oriented DNA fiber, *taken by Rosalind Franklin.*

From Roth, M.B., and Gall, J.G., *J. Cell Biol*. 105, 1049 (1987)

Chargaff's Rules Describe the Base Composition of DNA

DNA has equal numbers of adenine and thymine residues (A = T) and equal numbers of guanine and cytosine residues (G = C). These relationships, known as **Chargaff's rules.**

DNA's base composition varies widely among different organisms. It ranges from 25 to 75 mol % G + C in different species of bacteria. However, it is more or less constant among related species; for example, in mammals G + C ranges from 39 to 46%.

The significance of Chargaff's rules was not immediately appreciated, but we now know that *the structural basis for the rules derives from DNA's double-stranded nature.*

Tautomeric forms of bases.

The purine and pyrimidine bases of nucleic acids can assume different tautomeric forms (***tautomers*** are easily converted isomers that differ only in hydrogen positions). X-Ray, nuclear magnetic resonance (NMR), and spectroscopic investigations have firmly established that the nucleic acid bases are overwhelmingly in the *keto tautomeric forms*. In 1953, however, this was not generally appreciated!

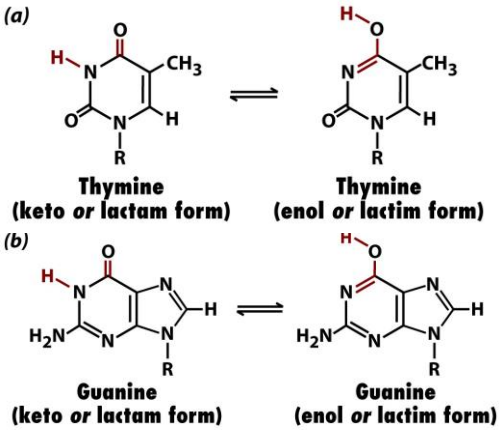


Figure 3-4 Fundamentals of Biochemistry, 2/e
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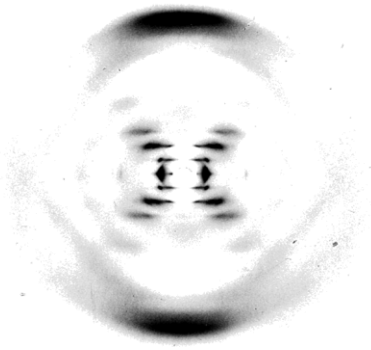
Jerry Donohue

Evidence that DNA is a helical molecule was provided by an X-ray diffraction photograph of a DNA fiber taken by ***Rosalind Franklin***. The appearance of the photograph enabled Crick, an X-ray crystallographer by training, to deduce

(a) that DNA is a helical molecule and

(b) that its planar aromatic bases form a stack that is parallel to the fiber axis.



An X-ray diffraction photograph of a vertically oriented DNA fiber, *taken by Rosalind Franklin.*

The central X-shaped pattern indicates a helix, whereas the heavy black arcs at the top and bottom of the diffraction pattern reveal the spacing of the stacked bases (3.4 Å).

Rosalind Elsie Franklin (1920-1958), química y cristalógrafa inglesa

Although Franklin was aware of Chargaff's rules and Jerry Donohue's work concerning the tautomeric forms of the bases, she did not deduce the existence of base pairs in double-stranded DNA.

In *January 1953, Wilkins showed Franklin's X-ray photograph of B-DNA to Watson, when he visited King's College. Moreover, in February 1953, Max Perutz, Crick's thesis advisor at Cambridge University, showed Watson and Crick his copy of the 1952 Report of the MRC, which summarized the work of all of its principal investigators, including that of Franklin.* ***Within a week*** *(and after 13 months of inactivity on the project),* ***Watson and Crick began building a model of DNA with a backbone structure compatible with Franklin's data****.*

BOX 24-1 **PATHWAYS OF DISCOVERY**

Rosalind Franklin and the Structure of DNA

Rosalind Franklin (1920–1958)

James Watson and Francis Crick were the first to publish an accurate model of the structure of DNA. This seminal discovery was not only based on their own insights but, like all scientific discoveries, built on the work of others. One of the major contributors to this process was Rosalind Franklin, who was probably never fully aware of her role in this discovery and did not share the 1962 Nobel Prize in Physiology or Medicine awarded to Watson, Crick, and Maurice Wilkins.

Franklin was born in England in an intellectually oriented and well-to-do family. She excelled in mathematics, and although career opportunities for women with her talents were few, she obtained a doctorate in physical chemistry at the University of Cambridge in 1945. From 1947 to 1950 she worked in a French government laboratory, where she became an authority in applying X-ray diffraction techniques to imperfectly crystalline substances such as coal; her numerous publications on the structures of coal and other forms

on the inside. Although Franklin was aware of Chargaff's rules (Section 3-2A) and Jerry Donohue's work concerning the tautomeric forms of the bases (Section 3-2B), she did not deduce the existence of base pairs in double-stranded DNA.

In January 1953, Wilkins showed Franklin's X-ray photograph of B-DNA to Watson, when he visited King's College. Moreover, in February 1953, Max Perutz (Box 7-2), Crick's thesis advisor at Cambridge University, showed Watson and Crick his copy of the 1952 Report of the MRC, which summarized the work of all of its principal investigators, including that of Franklin. Within a week (and after 13 months of inactivity on the project), Watson and Crick began building a model of DNA with a backbone structure compatible with Franklin's data [in earlier modeling attempts, they had placed the bases on the outside of the helix (as did a model published by Linus Pauling; Box 6-1) because they assumed that the bases could transmit genetic information only if they were externally accessible]. On several occasions Crick acknowledged that Franklin's findings were crucial to this enterprise.

Watson and Crick published their model of B-DNA in *Nature* in April 1953. This paper was followed, back-to-back, by papers by

No. 4356 April 25, 1953 NATURE 737

equipment, and to Dr. G. E. R. Deacon and the captain and officers of R.R.S. *Discovery II* for their part in making the observations.

[1] Young, F. B., Gerard, H., and Jevons, W., *Phil. Mag.*, **40**, 149 (1920).

[2] Longuet-Higgins, M. S., *Mon. Not. Roy. Astro. Soc., Geophys. Supp.*, **5**, 285 (1948).

[3] Von Arx, W. S., Woods Hole Papers in Phys. Oceanog. Meteor., **11** (3) (1950).

[4] Ekman, V. W., *Arkiv. Mat. Astron. Fysik.* (*Stockholm*), **2** (11) (1905).

MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

We wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey[1]. They kindly made their manuscript available to us in advance of publication. Their model consists of three intertwined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons: (1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals distances appear to be too small.

Another three-chain structure has also been suggested by Fraser (in the press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it.

This figure is purely diagrammatic. The two ribbons symbolize the two phosphate–sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate diester groups joining β-D-deoxyribofuranose residues with 3′,5′ linkages. The two chains (but not their bases) are related by a dyad perpendicular to the fibre axis. Both chains follow right-handed helices, but owing to the dyad the sequences of the atoms in the two chains run in opposite directions. Each chain loosely resembles Furberg's[2] model No. 1; that is, the bases are on the inside of the helix and the phosphates on the outside. The configuration of the sugar and the atoms near it is close to Furberg's 'standard configuration', the sugar being roughly perpendicular to the attached base. There is a residue on each chain every 3·4 A. in the z-direction. We have assumed an angle of 36° between adjacent residues in the same chain, so that the structure repeats after 10 residues on each chain, that is, after 34 A. The distance of a phosphorus atom from the fibre

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Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.

We are much indebted to Dr. Jerry Donohue for constant advice and criticism, especially on interatomic distances. We have also been stimulated by a knowledge of the general nature of the unpublished experimental results and ideas of Dr. M. H. F. Wilkins, Dr. R. E. Franklin and their co-workers at

740 NATURE April 25, 1953 VOL. 171

We wish to thank Prof. J. T. Randall for encouragement; Profs. E. Chargaff, R. Signer, J. A. V. Butler and Drs. J. D. Watson, J. D. Smith, L. Hamilton, J. C. White and G. R. Wyatt for supplying material without which this work would have been impossible; also Drs. J. D. Watson and Mr. F. H. C. Crick for stimulation, and our colleagues R. E. Franklin, R. G. Gosling, G. L. Brown and W. E. Seeds for discussion. One of us (H. R. W.) wishes to acknowledge the award of a University of Wales Fellowship.

M. H. F. WILKINS
Medical Research Council Biophysics Research Unit,

A. R. STOKES
H. R. WILSON
Wheatstone Physics Laboratory, King's College, London.

April 2.

[1] Astbury, W. T., Symp. Soc. Exp. Biol., 1, Nucleic Acid (Cambridge Univ. Press, 1947).

[2] Riley, D. P., and Oster, G., *Biochim. et Biophys. Acta*, **7**, 526 (1951).

[3] Wilkins, M. H. F., Gosling, R. G., and Seeds, W. E., *Nature*, **167**, 759 (1951).

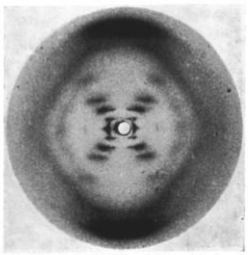
[4] Astbury, W. T., and Bell, F. O., Cold Spring Harb. Symp. Quant. Biol., **6**, 109 (1938).

[5] Cochran, W., Crick, F. H. C., and Vand, V., *Acta Cryst.*, **5**, 581 (1952).

[6] Wilkins, M. H. F., and Randall, J. T., *Biochim. et Biophys. Acta*, **10**, 192 (1953).

Molecular Configuration in Sodium Thymonucleate

SODIUM thymonucleate fibres give two distinct types of X-ray diagram. The first corresponds to a crystalline form, structure *A*, obtained at about 75 per cent relative humidity; a study of this is described in detail elsewhere[1]. At higher humidities a different structure, structure *B*, showing a lower degree of order, appears and persists over a wide



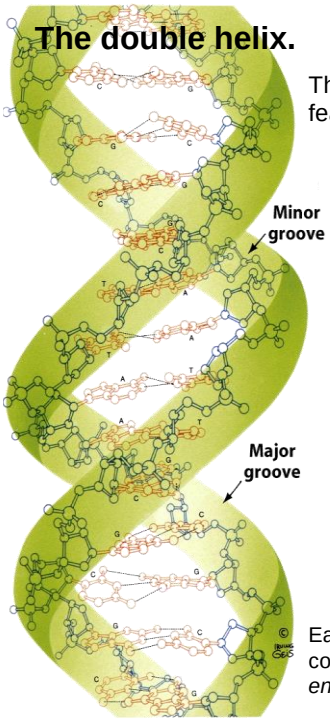
Sodium deoxyribose nucleate from calf thymus. Structure *B*

molecules, each unit being shielded by a sheath of water. Each unit is then free to take up its least-energy configuration independently of its neighbours and, in view of the nature of the long-chain molecules involved, it is highly likely that the general form will be helical[2]. If we adopt the hypothesis of a helical structure, it is immediately possible, from the X-ray diagram of structure *B*, to make certain deductions as to the nature and dimensions of the helix.

The innermost maxima on the first, second, third and fifth layer lines lie approximately on straight lines radiating from the origin. For a smooth single-strand helix the structure factor on the *n*th layer line is given by:

$$F_n = J_n(2\pi rR) \exp i\, n(\psi + \tfrac{1}{2}\pi),$$

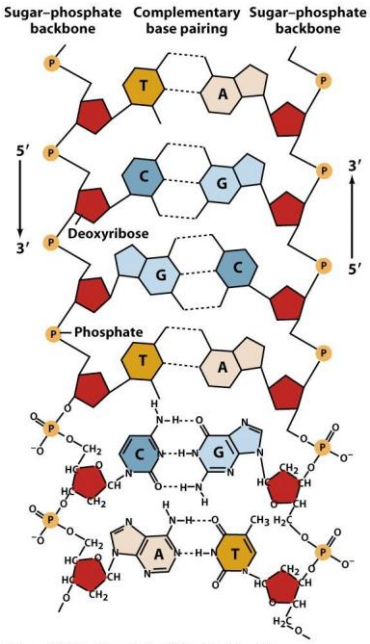
The double helix.



The *Watson- Crick model of DNA* has the following major features:

1. Two polynucleotide chains wind around a common axis to form a ***double helix***.
2. The two strands of DNA are ***antiparallel*** but each forms a right-handed helix.
3. The bases occupy the core of the helix and sugar-phosphate chains run along the periphery. The surface of the double helix contains two grooves of unequal width: the ***major*** and ***minor grooves***.
4. Each base is hydrogen bonded to a base in the opposite strand to form a planar base pair (A-T and C-G). These hydrogen bonding interactions, ***complementary base pairing***, result in the specific association of the two chains of the double helix.

Each DNA strand can act as a template for the synthesis of its complementary strand and hence that *hereditary information is encoded in the sequence of bases on either strand*.



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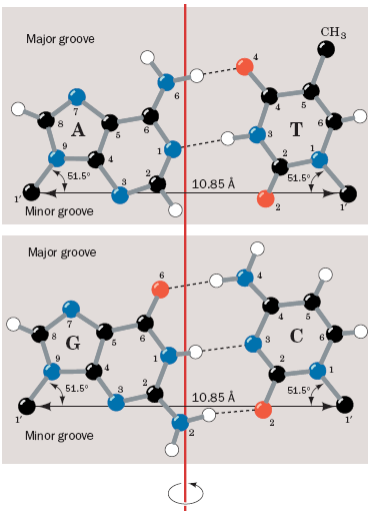
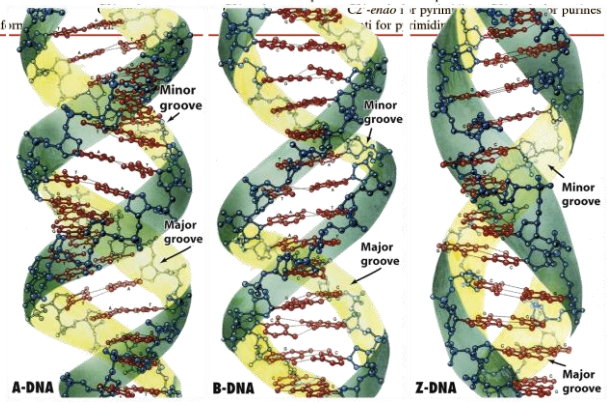


James Dewey Watson (1928-), biólogo estadounidense
Francis Harry Compton Crick, (1916 - 2004), físico, biólogo molecular y neurocientífico británico

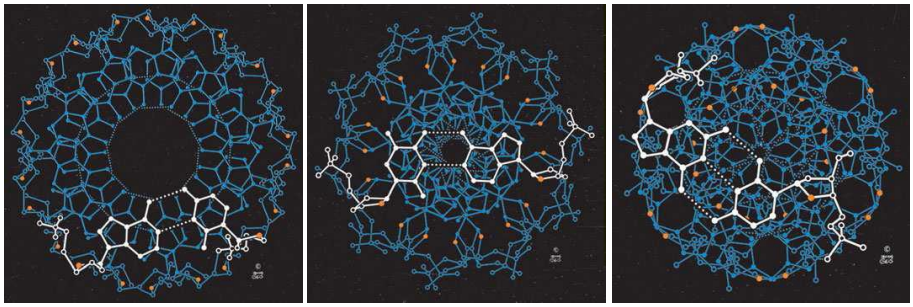
Double-helical DNA can assume several distinct structures depending on the solvent composition and base sequence. The major structural variants of DNA are **A-DNA** and **Z-DNA**.

Key to Structure. **Structural Features of Ideal A-, B-, and Z-DNA**

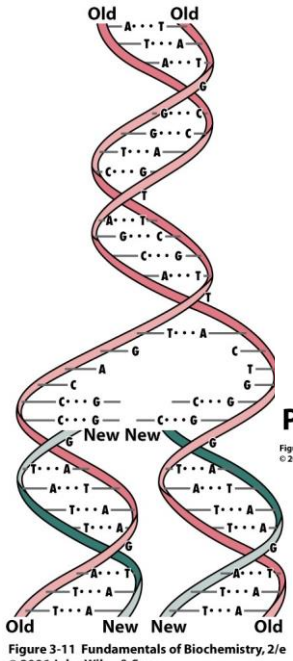
	A	B	Z
Helical sense	Right handed	Right handed	Left handed
Diameter	~26 Å	~20 Å	~18 Å
Base pairs per helical turn	11.6	10	12 (6 dimers)
Helical twist per base pair	31°	36°	60° (per dimer)
Helix pitch (rise per turn)	34 Å	34 Å	44 Å
Helix rise per base pair	2.9 Å	3.4 Å	7.4 Å per dimer
Base tilt normal to the helix axis	20°	6°	7°
Major groove	Narrow and deep	Wide and deep	Flat
Minor groove	Wide and shallow	Narrow and deep	Narrow and deep
Sugar pucker	[illegible]	[illegible]	C2′-*endo* for pyrimidines, C3′-*endo* for purines
Glycosidic bond conformation	[illegible]	[illegible]	*syn* for pyrimidines, *anti* for purines



A-DNA **B-DNA** **Z-DNA**



A-DNA's most striking feature, however, is that the planes of its base pairs are tilted 20 with respect to the helix axis. Since the axis does not pass through its base pairs, A-DNA has a deep major groove and a very shallow minor groove; it can be described as a flat ribbon wound around a 6-Å-diameter cylindrical hole.



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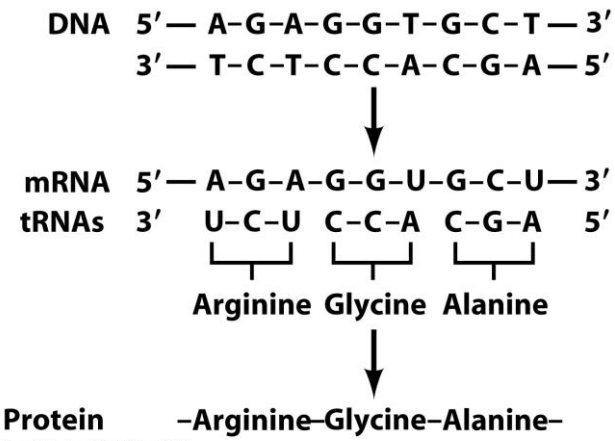

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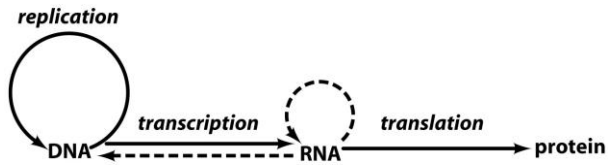

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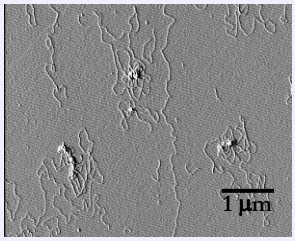
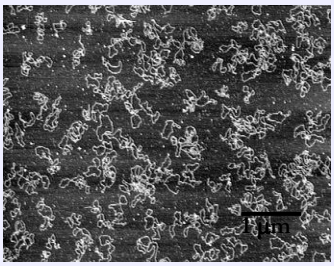
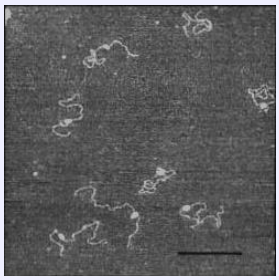
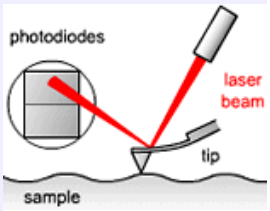
AFM "Seeing is believing"



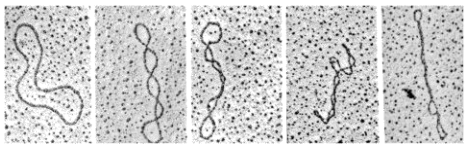


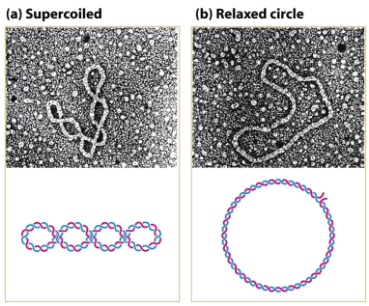




Supercoiled DNA

Circular DNA molecules can be twisted on themselves, forming supercoils. *Naturally occurring DNA is negatively supercoiled*.

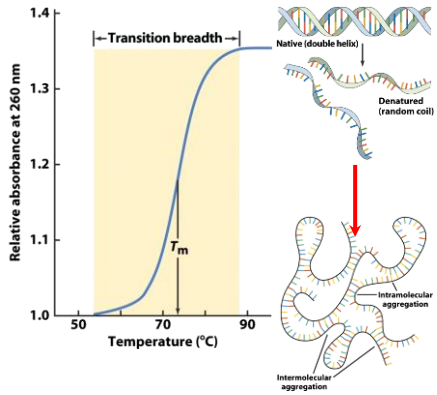




Topoisomerases relax supercoils by cleaving one or both strands of the DNA.

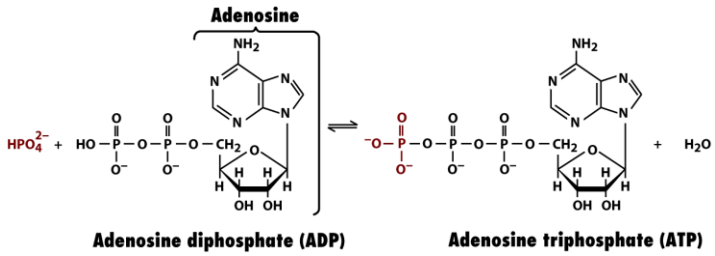
Forces stabilizing DNA

DNA can be denatured by increasing the temperature above their T_m and renatured by lowering the temperature to ~ 25º C.

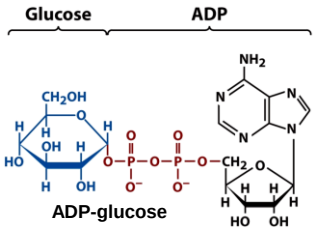


The structures of NA are **stabilized** by Watson-Crick base pairing, by hydrophobic interactions, and by divalent cations.

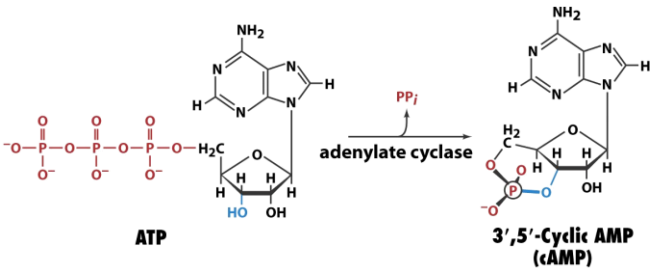
ATP and nucleotide derivatives. Free nucleotides and nucleotide derivatives perform an enormous variety of metabolic functions not related to the management of genetic information. Perhaps the best known nucleotide is **ATP**, ***an energy carrier or energy transfer agent***, which diffuses throughout the cell to provide energy for other cellular work, such as biosynthetic reactions, ion transport, and cell movement.



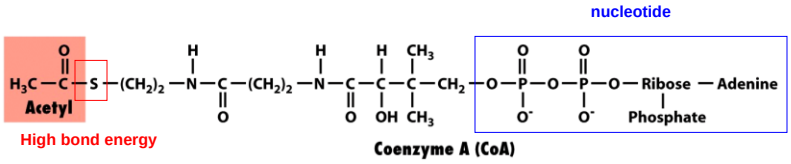
Starch synthesis in plants proceeds by repeated addition of glucose units donated by ADP-glucose. The attached group is linked to the nucleotide through a diphosphate group.



Nucleotides are used as specific signaling molecules in the cell. One nearly universally used *second messenger* is **cyclic AMP** (cAMP) *which regulates the activities of enzymes and nonenzymatic proteins in signaling pathways.*

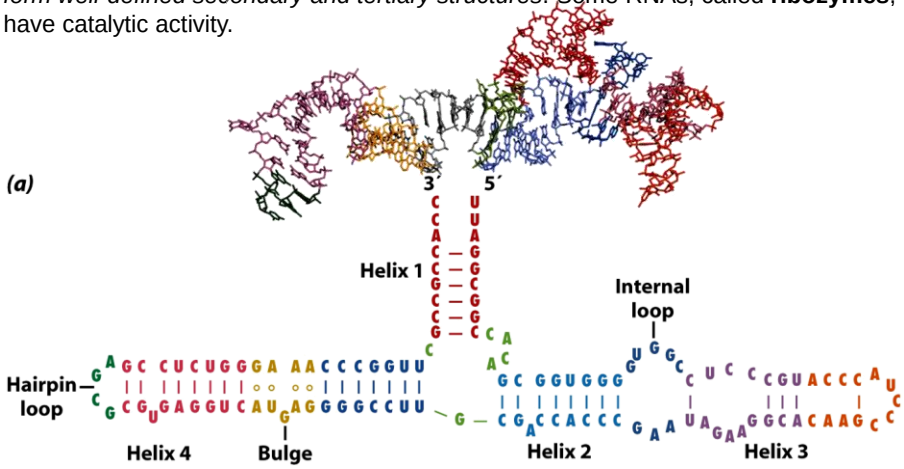


Coenzyme A carries an acetyl group in a readily transferable linkage and in this activated form is known as **acetyl CoA.** Acetyl CoA is an important intermediate in the aerobic oxidation of pyruvate, fatty acids, and many amino acids. It also contributes acetyl groups in many biosynthetic pathways.

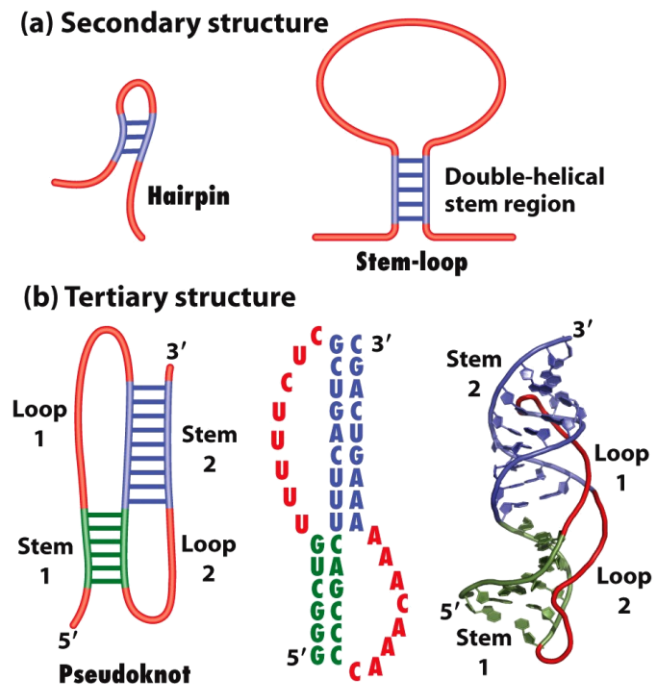


RNA structures

The structure of RNA is stabilized by the same forces that stabilize DNA, and its conformational flexibility is limited by many of the same features that limit DNA conformation. *Cellular RNAs are single-stranded polynucleotides, some of which form well-defined secondary and tertiary structures*. Some RNAs, called **ribozymes**, have catalytic activity.



Secondary and tertiary structure of 5S RNA from *Haloarcula marismortui*



Eukaryotic chromosome structure

The DNA of *eukaryotic chromatin* winds around *histone octamers* to *form nucleosome core particles* that further condense in the presence of linker histones. Additional condensation is accomplished by folding chromatin into 30-nm diameter filaments, which are then attached in loops to a fibrous protein scaffold to form a condensed chromosome (metaphase).

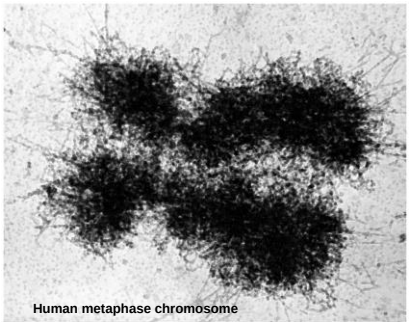
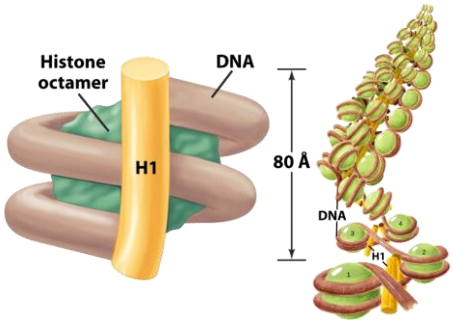
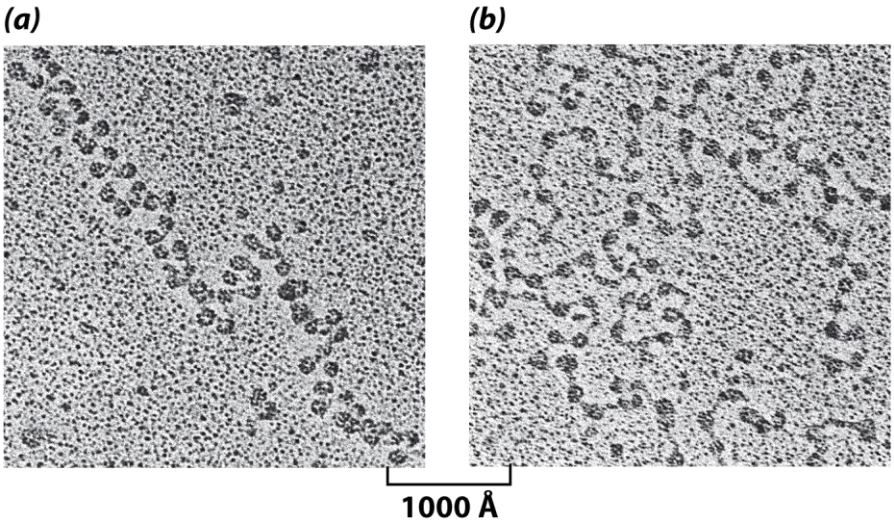

Human metaphase chromosome

Table 23-3 Calf Thymus Histones

Histone	Number of Residues	Mass (kD)	% Arg	% Lys
H1	215	23.0	1	29
H2A	129	14.0	9	11
H2B	125	13.8	6	16
H3	135	15.3	13	10
H4	102	11.3	14	11





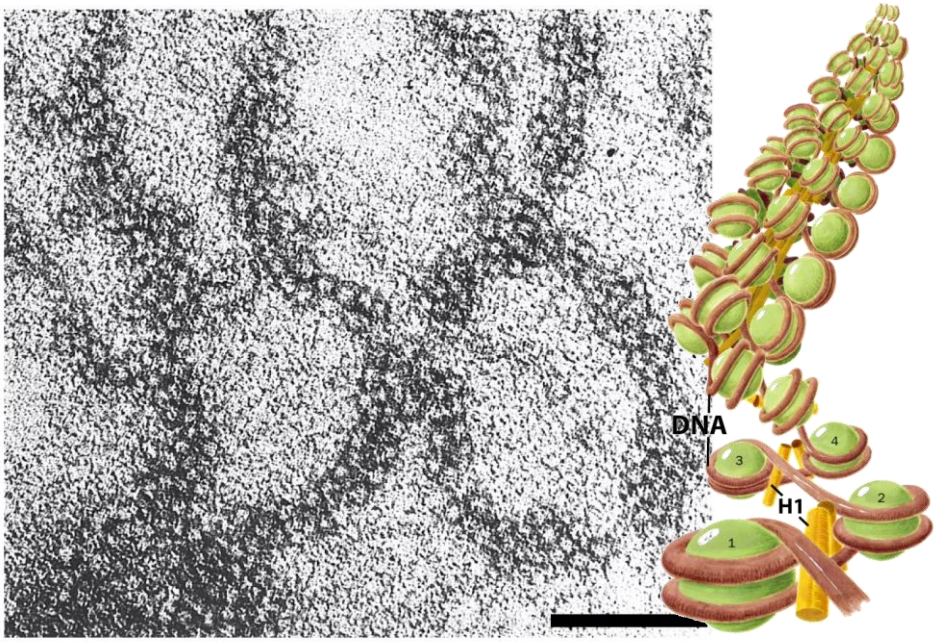


Figure 23-49 Fundamentals of Biochemistry, 2/e

Figure 23-51a Fundamentals of Biochemistry, 2/e

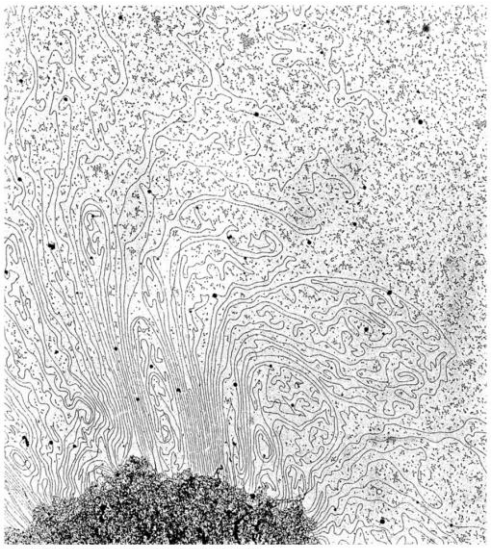


Figure 23-51b Fundamentals of Biochemistry, 2/e

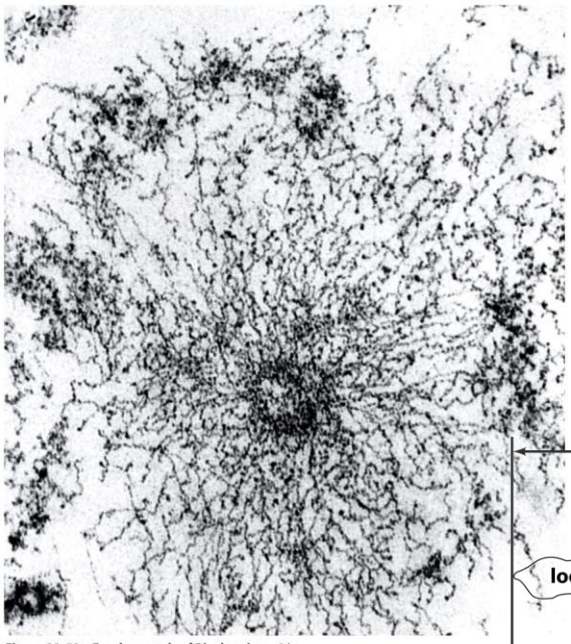
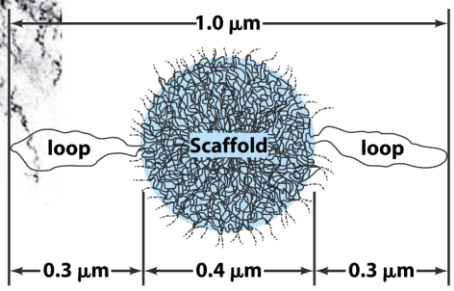


Figure 23-52a Fundamentals of Biochemistry, 2/e



Structure of Nucleic Acids

■ Deoxyribonucleic acid (DNA), the genetic material, carries information to specify the amino acid sequences of
proteins. It is transcribed into several types of ribonucleic acid (RNA), including messenger RNA (mRNA), transfer RNA (tRNA), and ribosomal RNA (rRNA), which function in protein synthesis.

■ Both DNA and RNA are long, unbranched polymers of nucleotides, which consist of a phosphorylated pentose
linked to an organic base, either a purine or pyrimidine.

■ The purines adenine (A) and guanine (G) and the pyrimidine cytosine (C) are present in both DNA and RNA. The pyrimidine thymine (T) present in DNA is replaced by the pyrimidine uracil (U) in RNA.

■ Adjacent nucleotides in a polynucleotide are linked by phosphodiester bonds. The entire strand has a chemical directionality: the 5 end with a free hydroxyl or phosphate group on the 5 carbon of the sugar, and the 3 end with a free hydroxyl group on the 3 carbon of the sugar.

■ Natural DNA (B DNA) contains two complementary antiparallel polynucleotide strands wound together into a regular right-handed double helix with the bases on the inside and the two sugar-phosphate backbones on the outside. Base pairing between the strands and hydrophobic interactions between adjacent bases in the same strand stabilize this native structure.

■ The bases in nucleic acids can interact via hydrogen bonds. The standard Watson-Crick base pairs are G·C, A·T (in DNA), and A·U (in RNA). Base pairing stabilizes the native three-dimensional structures of DNA and RNA.

■ Binding of protein to DNA can deform its helical structure, causing local bending or unwinding of the DNA molecule.

■ Heat causes the DNA strands to separate (denature). The melting temperature Tm of DNA increases with the percentage of G·C base pairs. Under suitable conditions, separated complementary nucleic acid strands will renature.

■ Circular DNA molecules can be twisted on themselves, forming supercoils. Enzymes called topoisomerases can relieve torsional stress and remove supercoils from circular DNA molecules.

■ Cellular RNAs are single-stranded polynucleotides, some of which form well-defined secondary and tertiary structures. Some RNAs, called ribozymes, have catalytic activity.-

METHODS

Fractionation of Nucleic Acids

Nucleic acids in cells are invariably associated with proteins. Once cells have been broken open, their nucleic acids are usually deproteinized. This can be accomplished by shaking the protein–nucleic acid mixture with a phenol solution so that the protein precipitates and can be removed by centrifugation. Alternatively, the protein can be dissociated from the nucleic acids by detergents, guanidinium chloride, or high salt concentration, or it can be enzymatically degraded by proteases.

In all cases, the nucleic acids, ***a mixture of RNA and DNA, can then be isolated by precipitation with ethanol***. The RNA can be recovered from such precipitates by treating them with pancreatic DNase to eliminate the DNA. Conversely, the DNA can be freed of RNA by treatment with RNase.

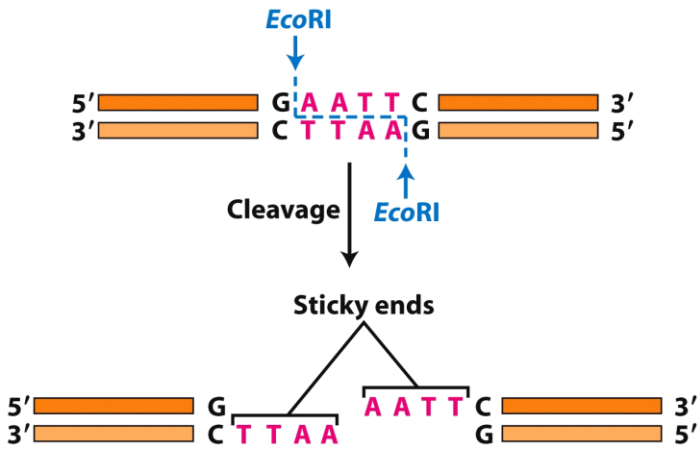
Nucleic Acids Can Be Purified by Chromatography

Many of the chromatographic techniques that are used to separate proteins also apply to nucleic acids. However, **hydroxyapatite,** a form of calcium phosphate [Ca5(PO4)3OH], is particularly useful in the chromatographic purification and fractionation of DNA. Double-stranded DNA binds to hydroxyapatite more tightly than do most other molecules. Consequently, DNA can be rapidly isolated by passing a cell lysate through a hydroxyapatite column, washing the column with a phosphate buffer of concentration low enough to release only the RNA and protein, and then eluting the DNA with a concentrated phosphate solution.

Electrophoresis Separates Nucleic Acids by Size

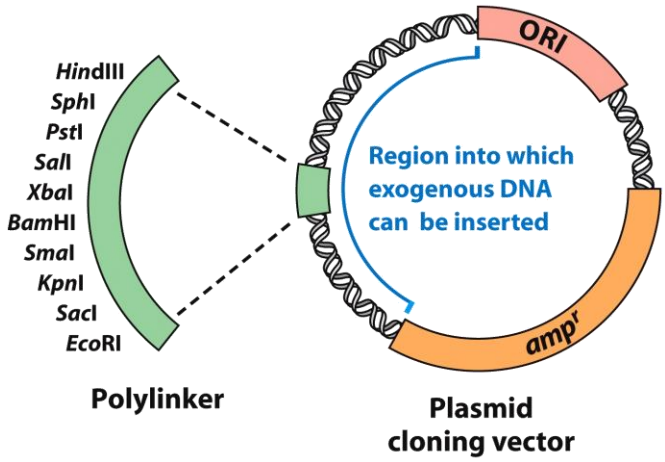
Nucleic acids of a given type can be separated by polyacrylamide gel electrophoresis because their electrophoretic mobilities in such gels vary inversely with their molecular masses. However, DNAs of more than a few thousand base pairs cannot penetrate even a weakly crosslinked polyacrylamide gel and so must be separated in **agarose gels**. Yet conventional gel electrophoresis is limited to DNAs of 100,000 bp, because larger DNA molecules tend to worm their way through the agarose at a rate independent of their size.

Restriction Enzymes and DNA Ligases Allow Insertion of DNA Fragments into Cloning Vectors

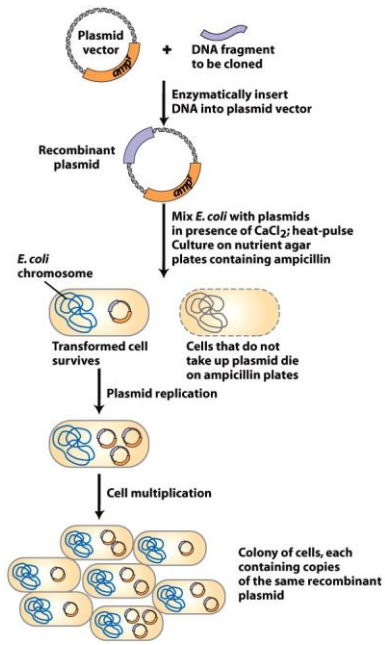


Cleavage of DNA by the restriction enzyme *Eco*RI. This restriction enzyme from *E. coli* makes staggered cuts at the specific 6-bp inverted repeat (palindromic) sequence shown, yielding fragments with single-stranded, complementary "sticky" ends. Many other restriction enzymes also produce fragments with sticky ends.

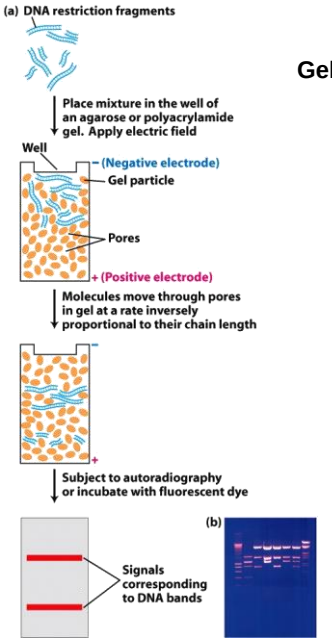
E. coli Plasmid Vectors Are Suitable for Cloning Isolated DNA Fragments



Basic components of a plasmid cloning vector that can replicate within an *E. coli* cell. Plasmid vectors contain a selectable gene such as *amp*r, which encodes the enzyme -lactamase and confers resistance to ampicillin. Exogenous DNA can be inserted into the bracketed region without disturbing the ability of the plasmid to replicate or express the *amp*r gene. Plasmid vectors also contain a replication origin (ORI) sequence where DNA replication is initiated by hostcell enzymes. Inclusion of a synthetic polylinker containing the recognition sequences for several different restriction enzymes increases the versatility of a plasmid vector. The vector is designed so that each site in the polylinker is unique on the plasmid.



Characterizing and Using Cloned DNA Fragments



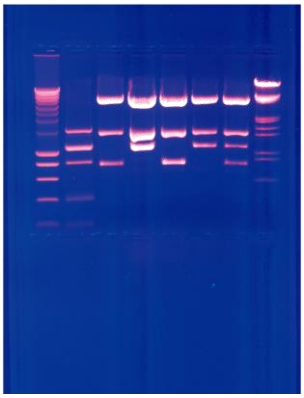
Gel Electrophoresis Allows Separation of Vector DNA from Cloned Fragments

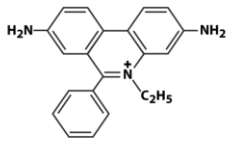
Nucleic acids of a given type can be separated by polyacrylamide gel electrophoresis because their electrophoretic mobilities in such gels vary inversely with their molecular masses. However, DNAs of more than a few thousand base pairs cannot penetrate even a weakly crosslinked polyacrylamide gel and so must be separated in **agarose gels**. Yet conventional gel electrophoresis is limited to DNAs of 100,000 bp, because larger DNA molecules tend to worm their way through the agarose at a rate independent of their size.

Intercalation Agents Stain Duplex DNA.

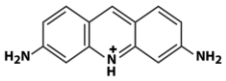
The various DNA bands in a gel can be stained by planar aromatic cations such as **ethidium ion, acridine orange,** or **proflavin.**

The dyes bind to double-stranded DNA by **intercalation** (slipping in between the stacked bases, where they exhibit a fluorescence under UV light that is far more intense than that of the free dye

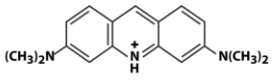




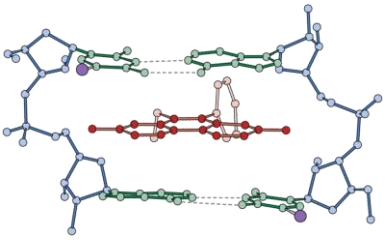
Ethidium



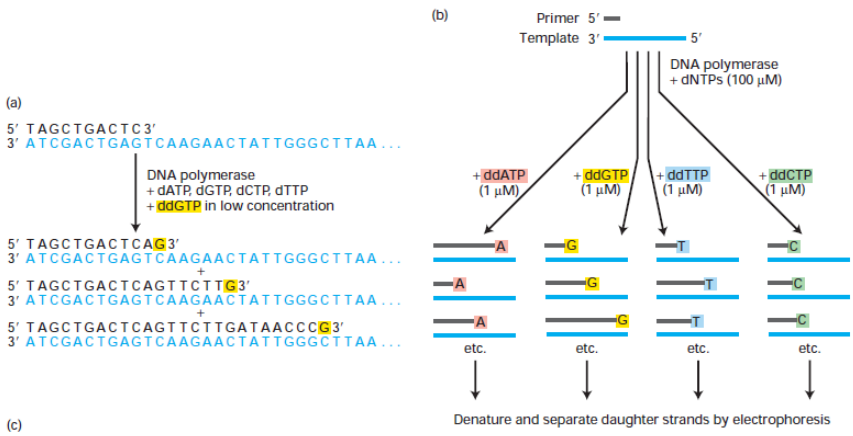
Proflavin



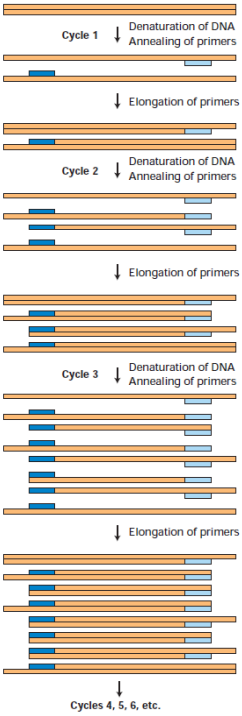
Acridine orange



Cloned DNA Molecules Are Sequenced Rapidly by the Dideoxy Chain-Termination Method



The Polymerase Chain Reaction (PCR) Amplifies a Specific DNA Sequence from a Complex Mixture

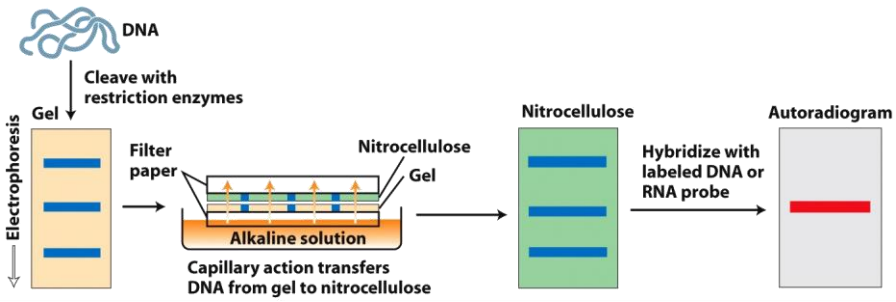


If the nucleotide sequences at the ends of a particular DNA region are known, the intervening fragment can be amplified directly by the **polymerase chain reaction (PCR).**

A typical PCR procedure begins by heat-denaturation of a DNA sample into single strands. Next, two synthetic oligonucleotides complementary to the 3 ends of the target DNA segment of interest are added in great excess to the denatured DNA, and the temperature is lowered to 50–60 C. These specific oligonucleotides, which are at a very high concentration, will hybridize with their complementary sequences in the DNA sample, whereas the long strands of the sample DNA remain apart because of their low concentration. The hybridized oligonucleotides then serve as primers for DNA chain synthesis in the presence of deoxynucleotides (dNTPs) and a temperature-resistant DNA polymerase such as that from *Thermus aquaticus* (a bacterium that lives in hot springs). This enzyme, called *Taq polymerase,* can remain active even after being heated to 95 C and can extend the primers at temperatures up to 72 C. When synthesis is complete, the whole mixture is then heated to 95 C to melt the newly formed DNA duplexes. After the temperature is lowered again, another cycle of synthesis takes place because excess primer is still present. Repeated cycles of melting (heating) and synthesis (cooling) quickly amplify the sequence of interest.

At each cycle, the number of copies of the sequence between the primer sites is doubled; therefore, the **desired sequence increases exponentially—about** a million-fold after 20 cycles—whereas all other sequences in the original DNA sample remain unamplified.

Blotting Techniques Permit Detection of Specific DNA Fragments and mRNAs with DNA Probes



Southern blot technique can detect a specific DNA fragment in a complex mixture of restriction fragments. The diagram depicts three different restriction fragments in the gel, but the procedure can be applied to a mixture of millions of DNA fragments. Only fragments that hybridize to a labeled probe will give a signal on an autoradiogram. A similar technique called *Northern blotting* detects specific mRNAs within a mixture. [See E. M. Southern, 1975, *J. Mol. Biol.* **98**:508.]