David L. Nelson and Michael M. Cox

• LEHNINGER
• PRINCIPLES OF BIOCHEMISTRY
• ***Fifth Edition***

CHAPTER 8
Nucleotides and Nucleic Acids

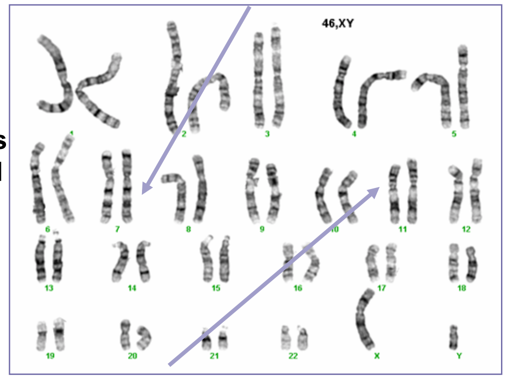
Nucleotides & Nucleic Acids

- Ø Introduction
- Ø General structure of nucleotides
- Ø Minor nucleotides
- Ø Nomenclature of nucleotides
- Ø Nucleotide pairing
- Ø DNA store the genetic information
- Ø Three dimensional structure of DNA
- Ø Unusual structure of DNA
- Ø Complex structure of RNA
- Ø Denaturation of DNA & RNA
- Ø Hybrid of DNA
- Ø Mutation of DNA
- Ø Sequence & Synthesis of DNA
- Ø Functions of nucleotides

Chromosomes

- Chromosomes are made of DNA.
- Each contains genes in a linear order.
- Human body cells contain 46 chromosomes in 23 pairs – one of each pair inherited from each parent
- Chromosome pairs 1 – 22 are called autosomes.
- The 23rd pair are called sex chromosomes: XX is female, XY is male.

Gene for cystic fibrosis (chromosome 7)



Gene for sickle cell disease (chromosome 11)

		Second letter									
		T		C		A		G			
	T	TTT	Phe	TCT	Ser	TAT	Tyr	TGT	Cys	T	
		TTC		TCC		TAC		TGC		C	
		TTA	Leu	TCA		TAA	Stop	TGA	Stop	A	
		TTG		TCG		TAG		TGG	Trp	G	
	C	CTT	Leu	CCT	Pro	CAT	His	CGT	Arg	T	
		CTC		CCC		CAC		CGC		C	
First letter		CTA		CCA		CAA	Gln	CGA		A	Third letter
		CTG		CCG		CAG		CGG		G	
	A	ATT	Ile	ACT	Thr	AAT	Asn	AGT	Ser	T	
		ATC		ACC		AAC		AGC		C	
		ATA		ACA		AAA	Lys	AGA	Arg	A	
		ATG	Met	ACG		AAG		AGG		G	
	G	GTT	Val	GCT	Ala	GAT	Asp	GGT	Gly	T	
		GTC		GCC		GAC		GGC		C	
		GTA		GCA		GAA	Glu	GGA		A	
		GTG		GCG		GAG		GGG		G	

Types of RNA

There are 3 types of RNA, each with a different job

1. **Messenger RNA (mRNA)**
2. **Transfer RNA (tRNA)**
3. **Ribosomal RNA (rRNA)**

Each type of RNA has a different structure that is related to its function.

Why do we need RNA

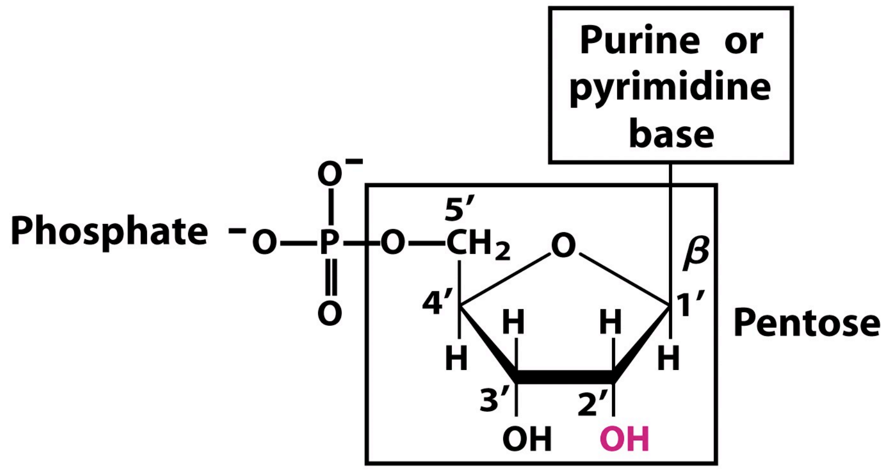
- Our body needs to make proteins in order to carry out cell functions. The instructions on how to make proteins are found in our DNA.
- Enzymes can not read our DNA so it must be converted into RNA which our enzymes can read. This process is called…

Transcription!

Types of RNA

<u>mRNA-</u> single strand that carries messages from the DNA to the cytosol, so that it can be used to make proteins.
<u>tRNA</u>- is a cloverleaf shaped single strand that matches the amino acid to the correct sequence of mRNA
<u>rRNA</u>- is a single strand in globular form, rRNA binds with proteins to make up ribosomes which are then used to make the proteins

Structure of Nucleotides



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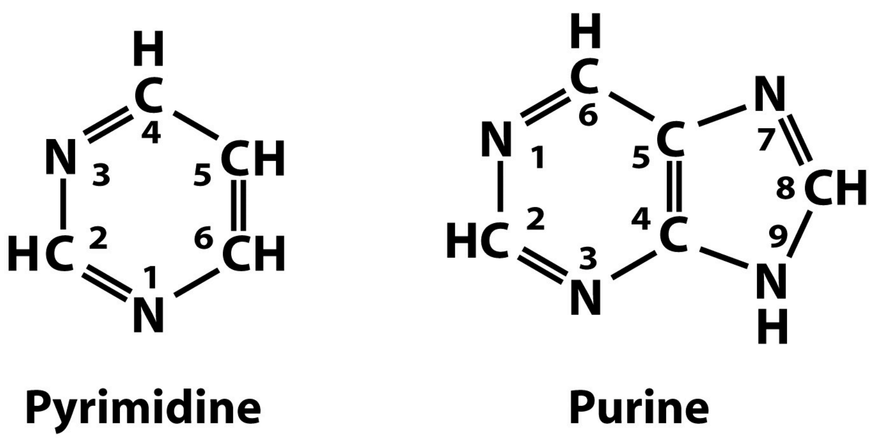

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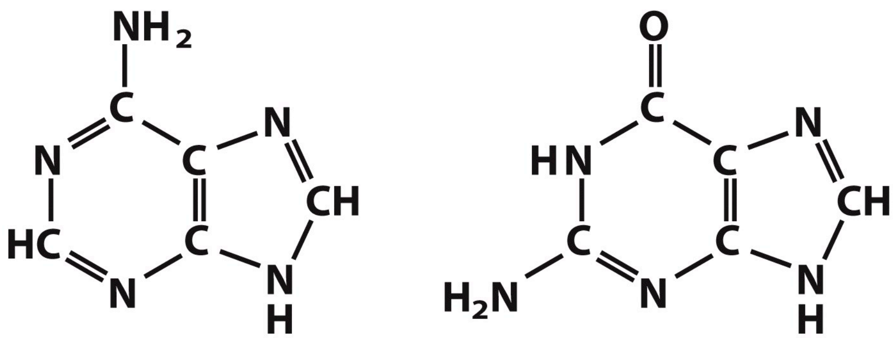

Adenine **Guanine**

Purines

Figure 8-2 part 1
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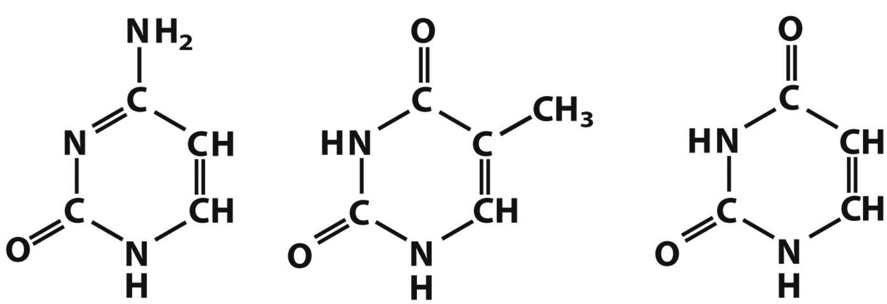

Cytosine **Thymine (DNA)** **Uracil (RNA)**

Pyrimidines

Figure 8-2 part 2
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TABLE 8–1	Nucleotide and Nucleic Acid Nomenclature		
Base	**Nucleoside**	**Nucleotide**	**Nucleic acid**
Purines			
Adenine	Adenosine Deoxyadenosine	Adenylate Deoxyadenylate	RNA DNA
Guanine	Guanosine Deoxyguanosine	Guanylate Deoxyguanylate	RNA DNA
Pyrimidines			
Cytosine	Cytidine Deoxycytidine	Cytidylate Deoxycytidylate	RNA DNA
Thymine	Thymidine or deoxythymidine	Thymidylate or deoxythymidylate	DNA
Uracil	Uridine	Uridylate	RNA

Note: "Nucleoside" and "nucleotide" are generic terms that include both ribo- and deoxyribo- forms. Also, ribonucleosides and ribonucleotides are here designated simply as nucleosides and nucleotides (e.g., riboadenosine as adenosine), and deoxyribonucleosides and deoxyribonucleotides as deoxynucleosides and deoxynucleotides (e.g., deoxyriboadenosine as deoxyadenosine). Both forms of naming are acceptable, but the shortened names are more commonly used. Thymine is an exception; "ribothymidine" is used to describe its unusual occurrence in RNA.

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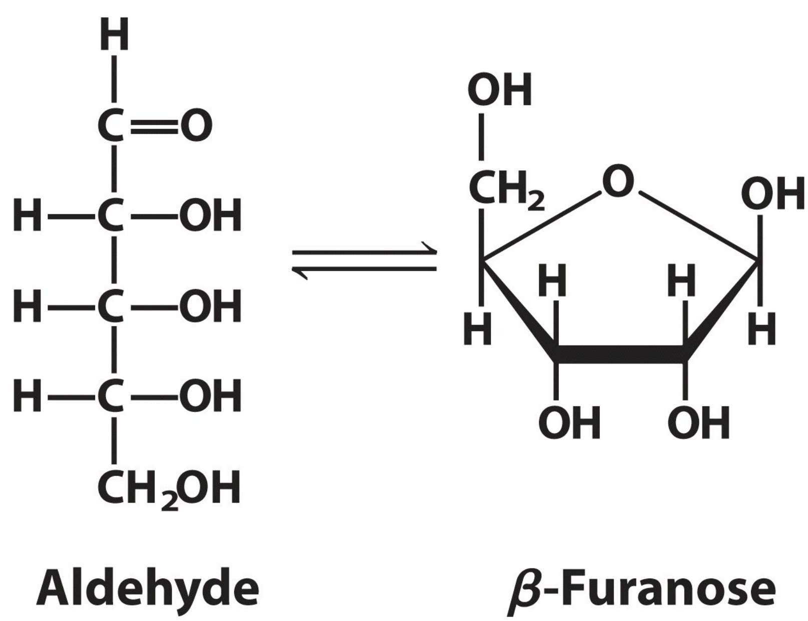

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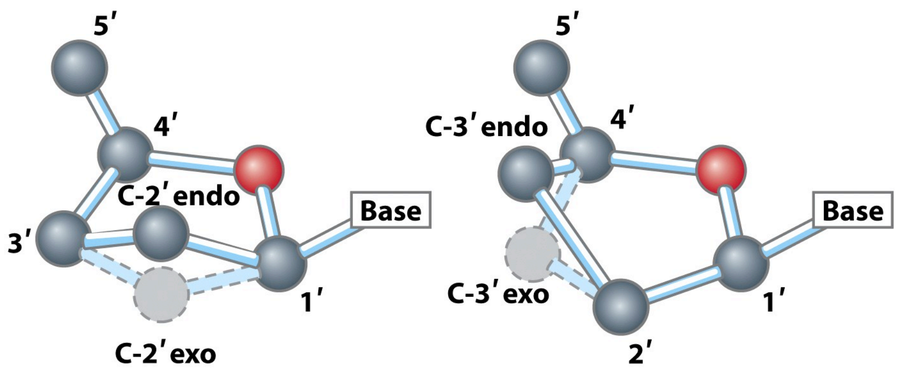

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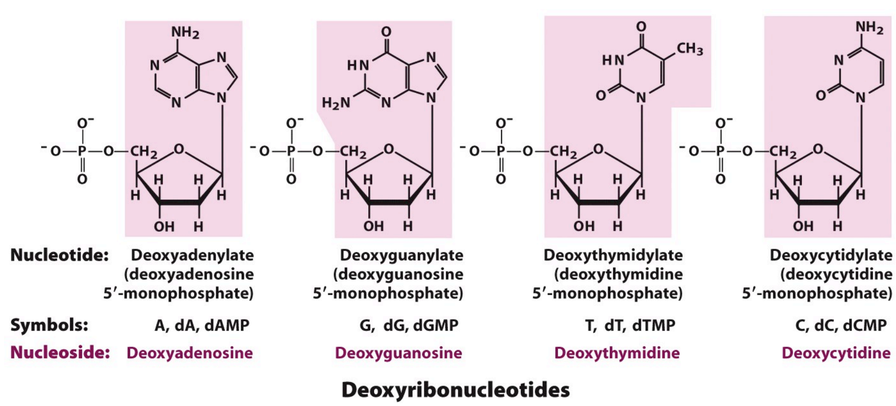

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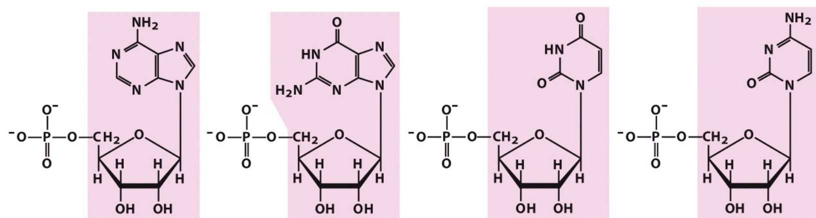

Nucleotide:	Adenylate (adenosine 5′-monophosphate)	Guanylate (guanosine 5′-monophosphate)	Uridylate (uridine 5′-monophosphate)	Cytidylate (cytidine 5′-monophosphate)
Symbols:	A, AMP	G, GMP	U, UMP	C, CMP
Nucleoside:	Adenosine	Guanosine	Uridine	Cytidine

Ribonucleotides

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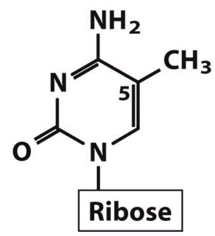
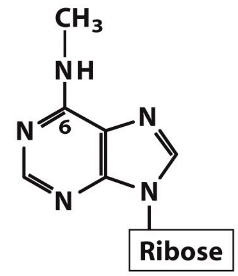
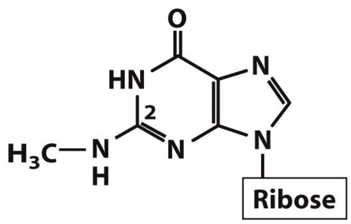
Minor Nucleotides



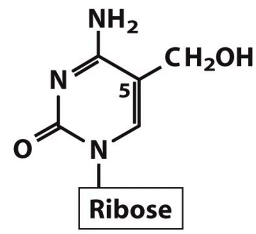
5-Methylcytidine



N^6-Methyladenosine



N^2-Methylguanosine



5-Hydroxymethylcytidine

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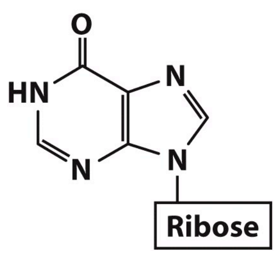
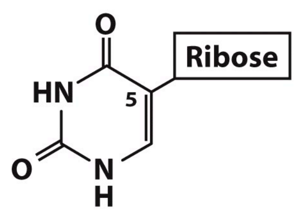
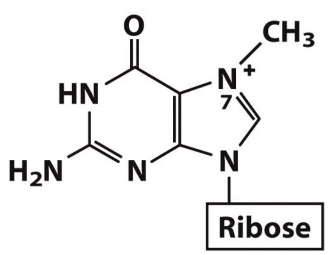


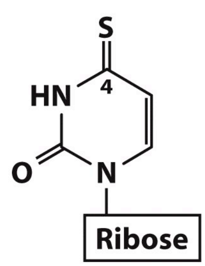
Inosine



Pseudouridine



7-Methylguanosine



4-Thiouridine

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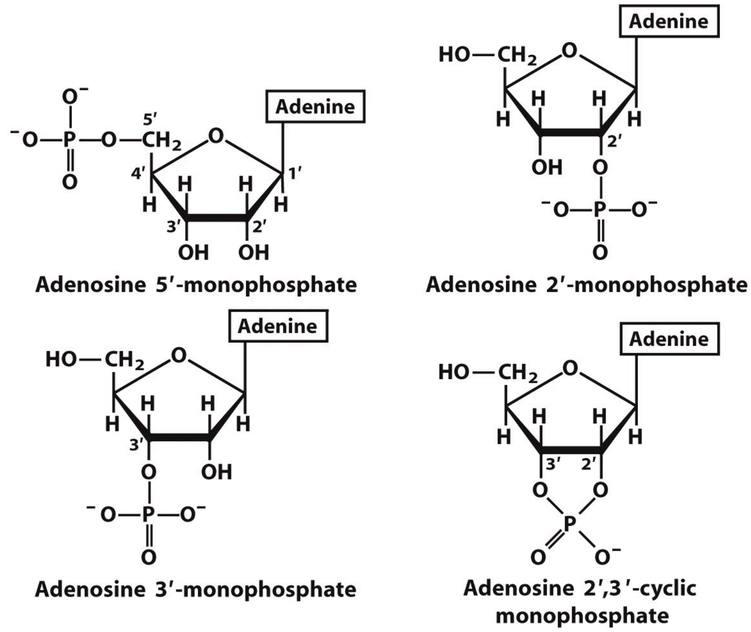

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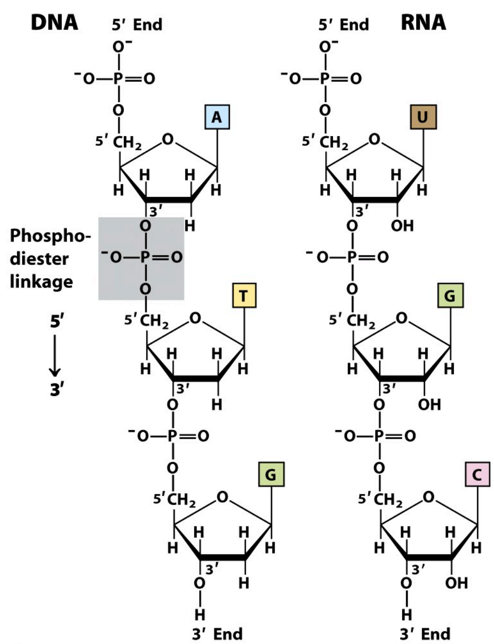
Phosphodiester Bonds Link Successive Nucleotides in Nucleic Acids



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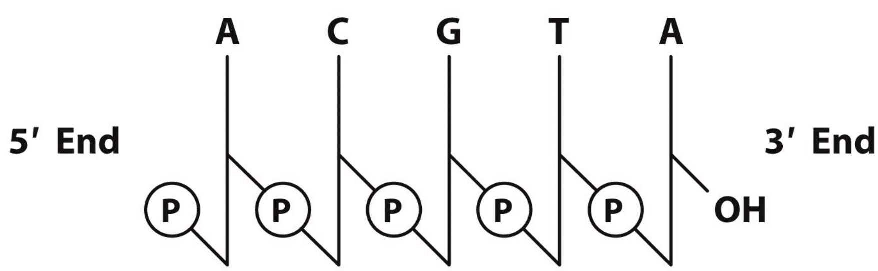

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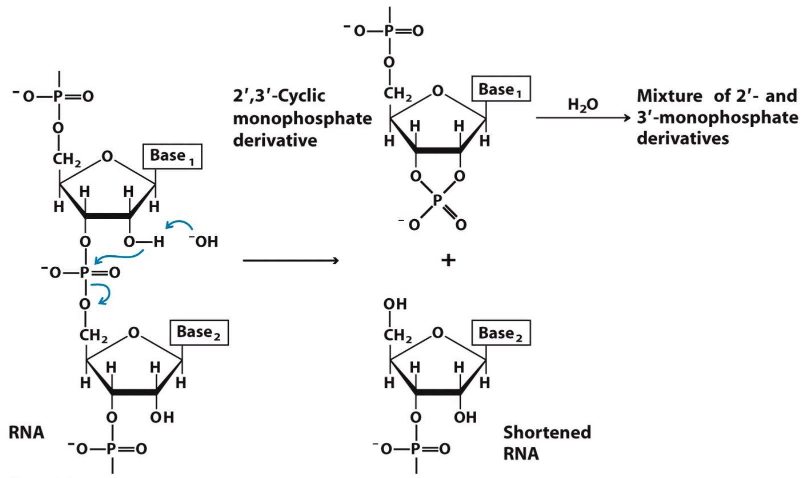
Hydrolysis of RNA under Alkaline Conditions



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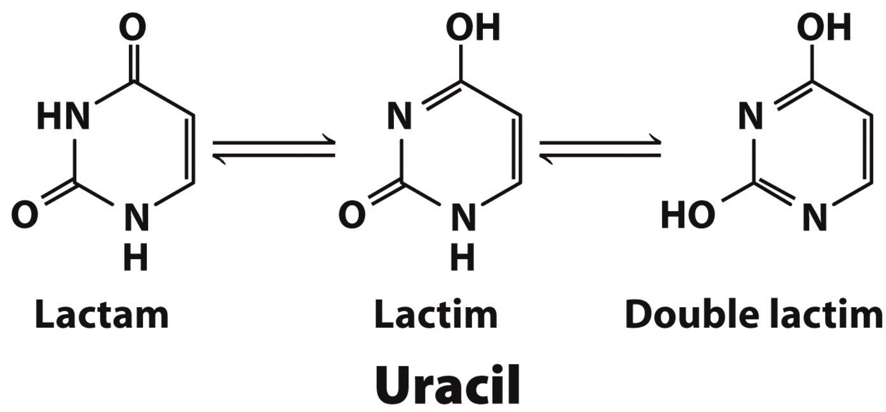
Properties of nucleotide bases affect the structure of nucleic acids

Hydrophobic stacking

Hydrogen bonding



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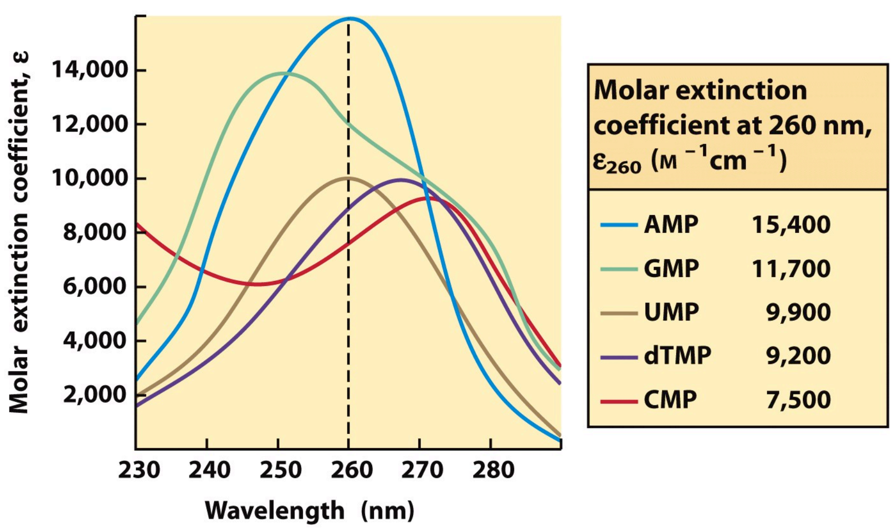

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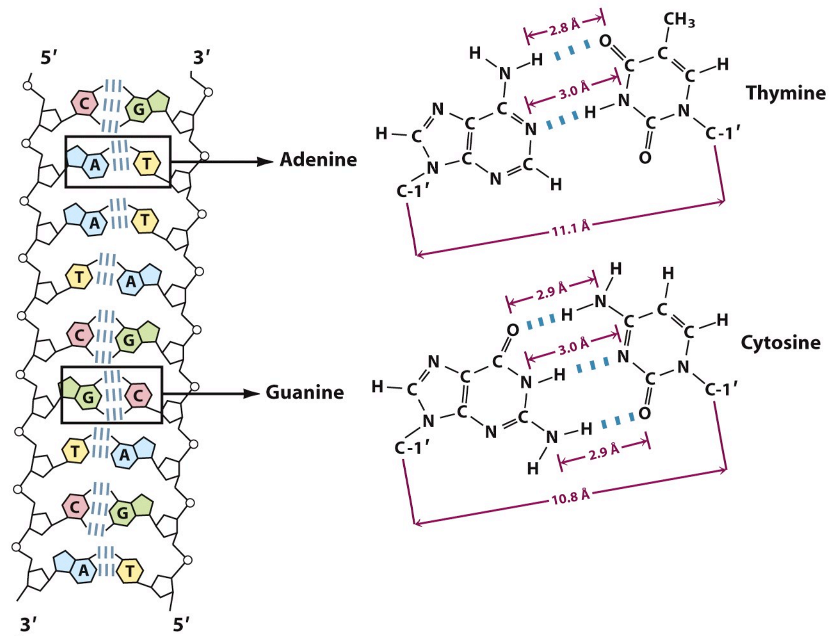

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DNA stores genetic information

1868 Friedrich Miescher isolated nuclein from nuclei of pus cells

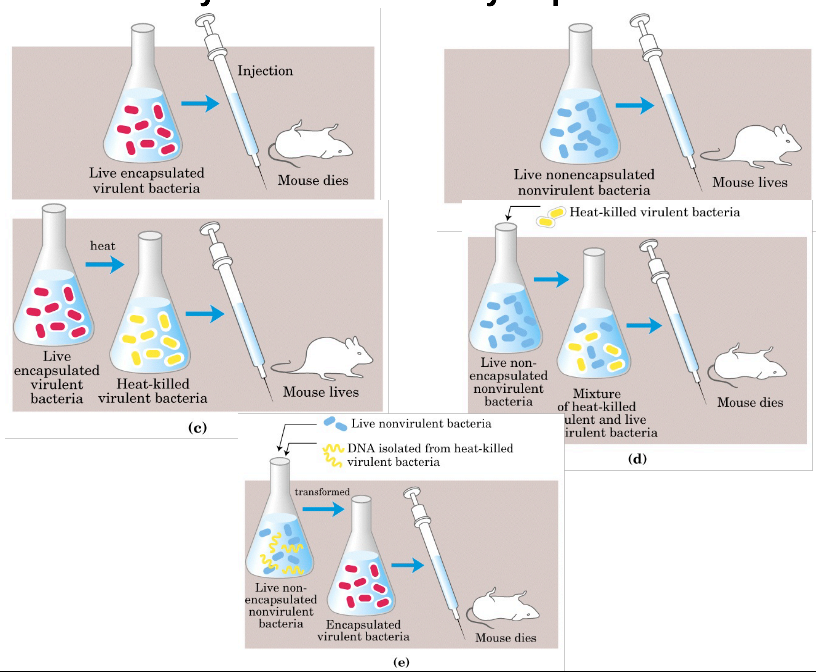
1944 Oswald T. Avery, Colin MacLeod, & Maclyn McCarty

Virulent strain of bacterium *streptococcus pneumoniae*

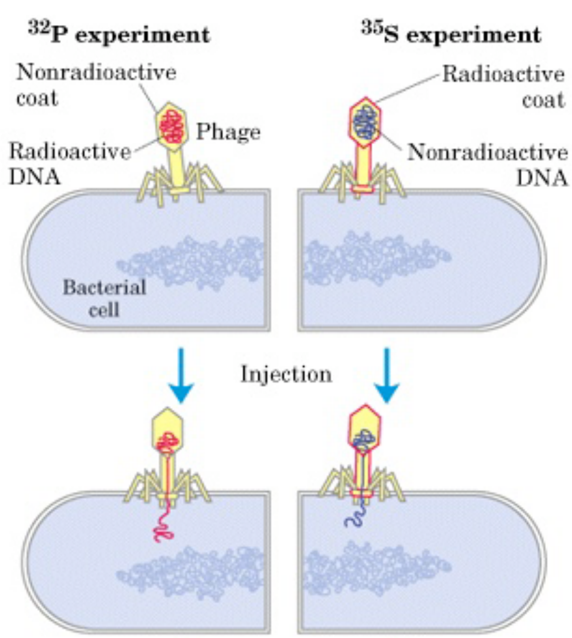
DNA extracted from virulent strain carried inheritable genetic message for virulent

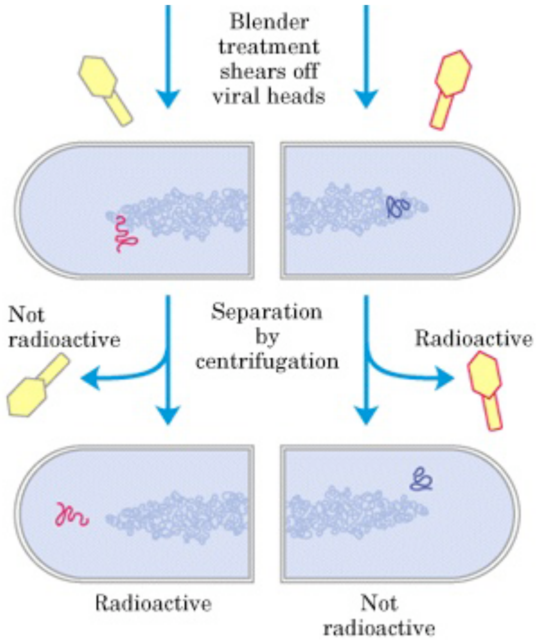
Proteolytic enzyme: active virulent
Deoxyribonucleases: no virulent

Avery-MacLeod-McCarty Experiment



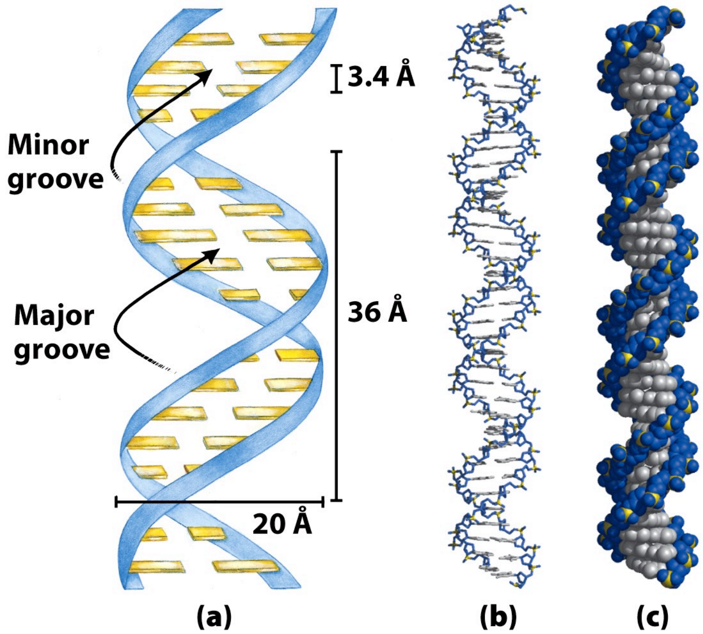
1952 Alfred D. Hershey
Martha Chase
Bacterial virus T2





Chargaff rule:

- The base composition of DNA generally varies from one species to another
- DNA specimens isolated from different tissues of the same species have the same base composition
- The base composition of DNA in a given species does not change with an organism's age, nutritional state, or changing environment
- In all cellular DNAs, regardless of the species, the number of adenosine residues is equal to the number of thymidine residues (that is, A = T), and the number of guanosine residues is equal to the number of cytidine residues (G = C). From these relationships it follows that the sum of the purine residues equals the sum of the pyrimidine residue; that is, A + G = T + C



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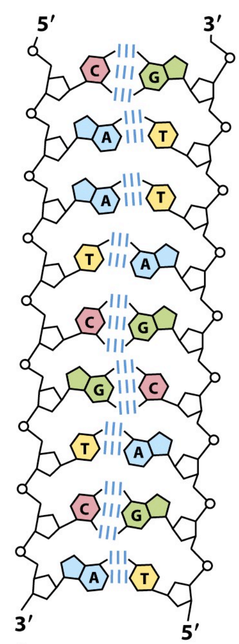
Complementarity of DNA Double Helix



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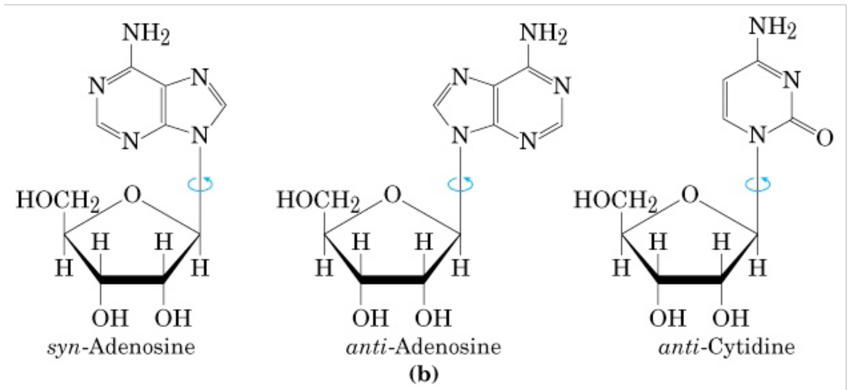
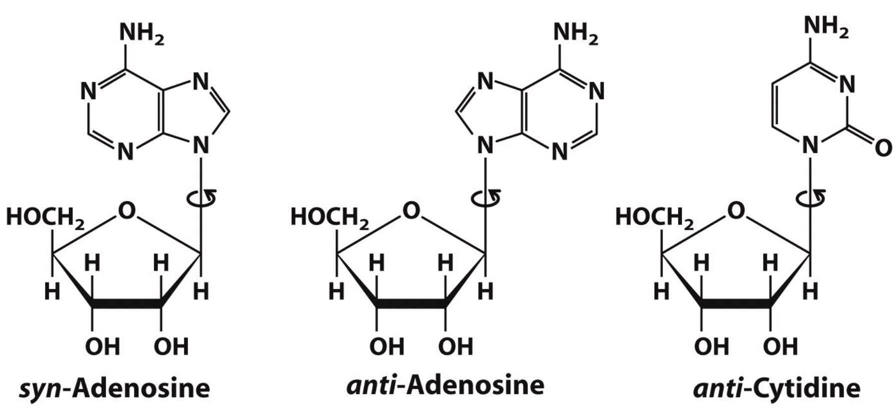
Structural variation in DNA





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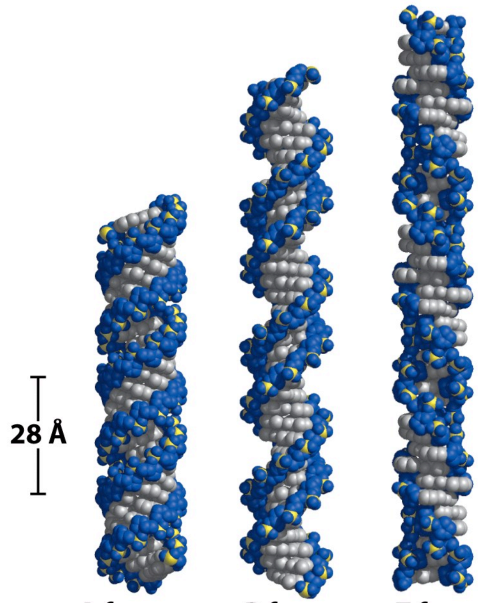

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	A form	B form	Z form
Helical sense	Right handed	Right handed	Left handed
Diameter	~26 Å	~20 Å	~18 Å
Base pairs per helical turn	11	10.5	12
Helix rise per base pair	2.6 Å	3.4 Å	3.7 Å
Base tilt normal to the helix axis	20°	6°	7°
Sugar pucker conformation	C-3′ endo	C-2′ endo	C-2′ endo for pyrimidines; C-3′ endo for purines
Glycosyl bond conformation	Anti	Anti	Anti for pyrimidines; syn for purines

Figure 8-17 part 2
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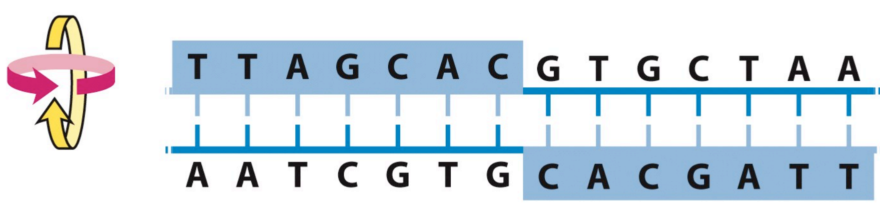
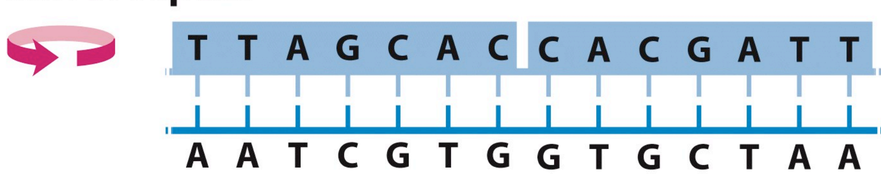
Unusual structures

Palindrome: a word, phrase, or sentence that is spelled identically reading forward or backward.

Palindrome



Mirror repeat



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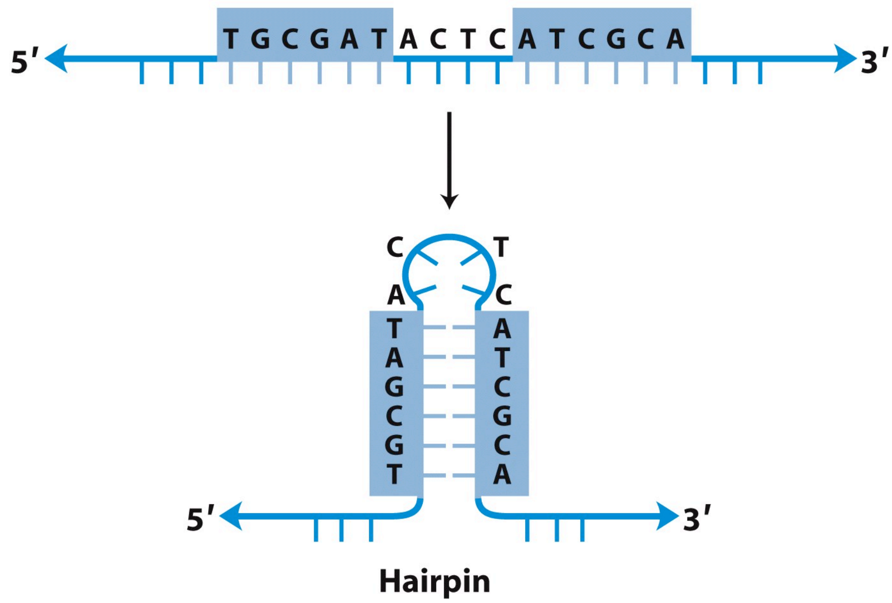

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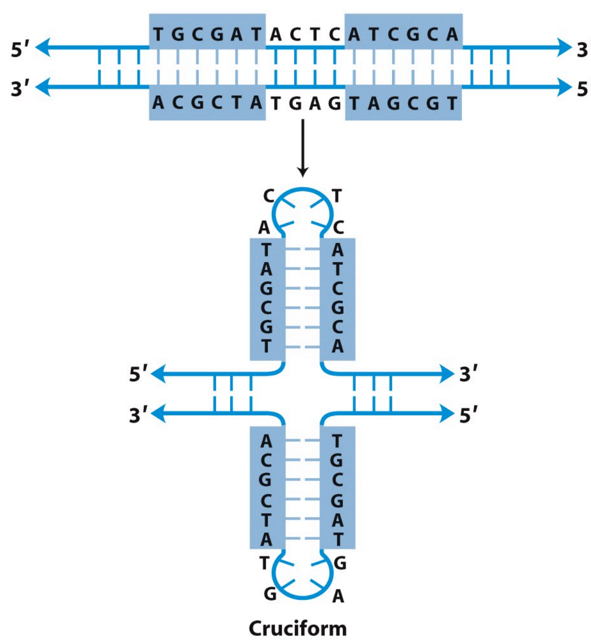

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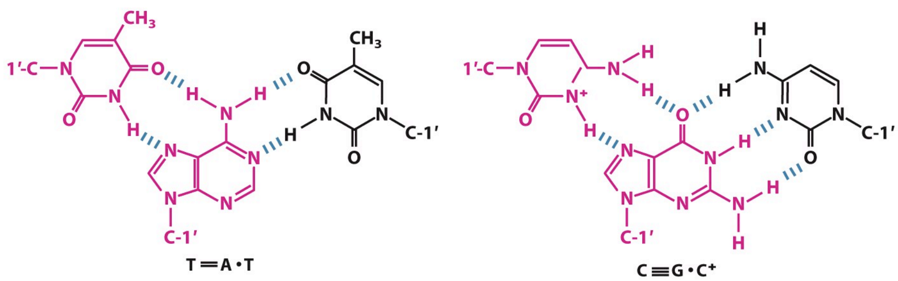

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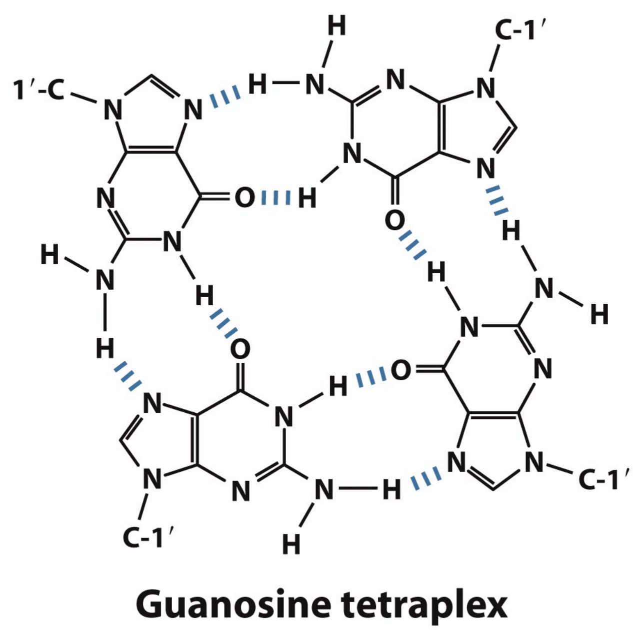

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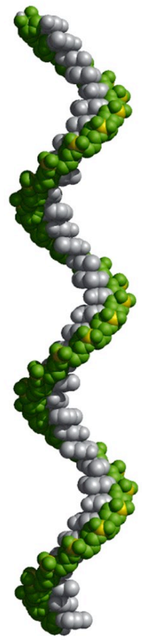

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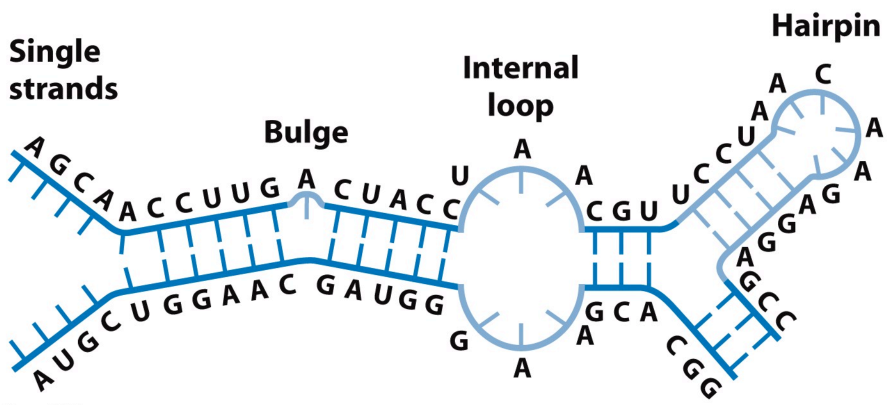

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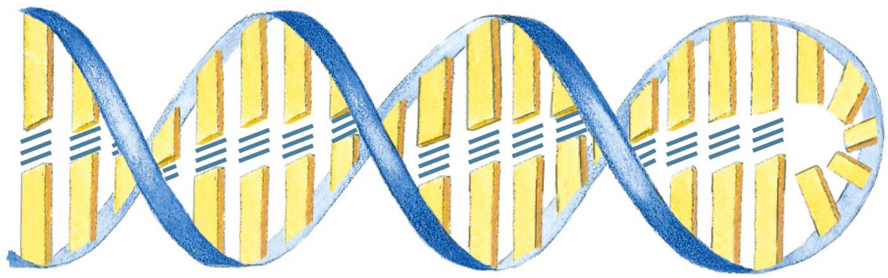

Hairpin double helix

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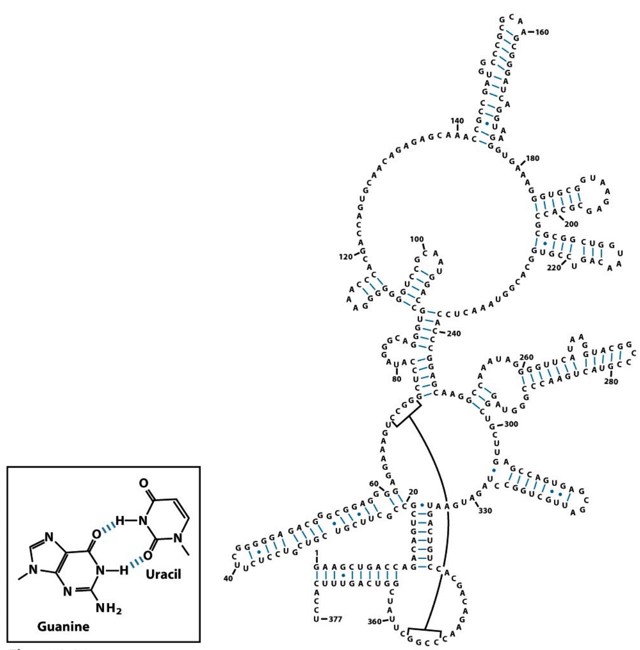

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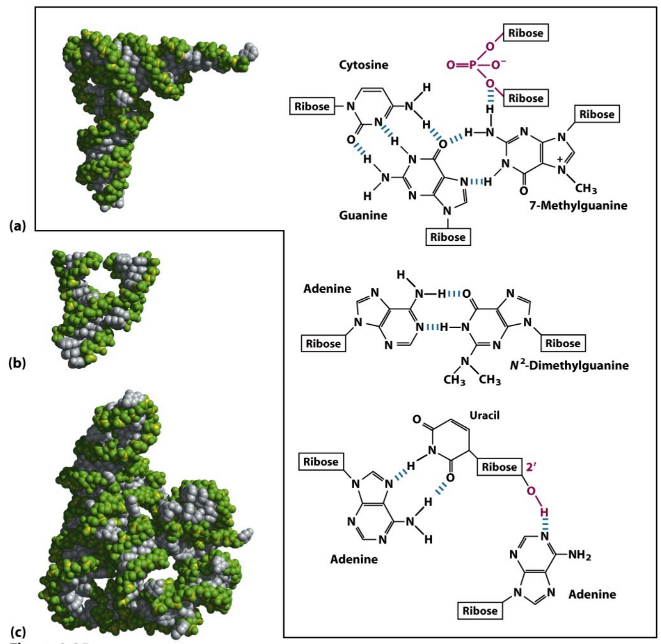

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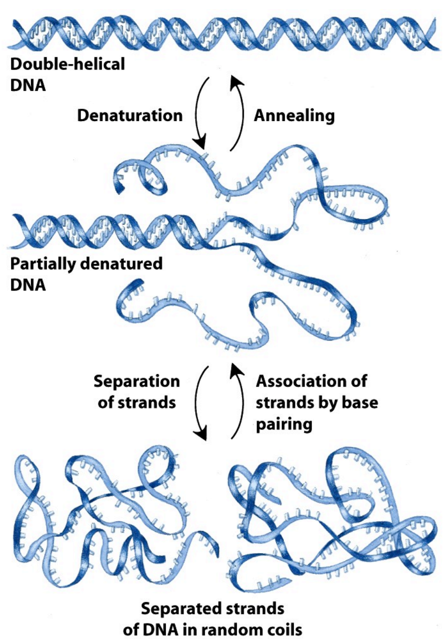

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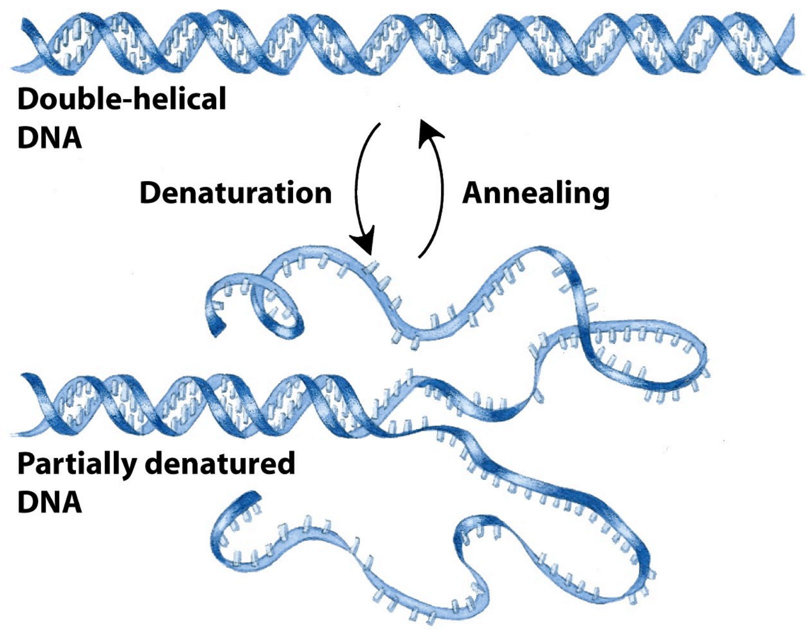

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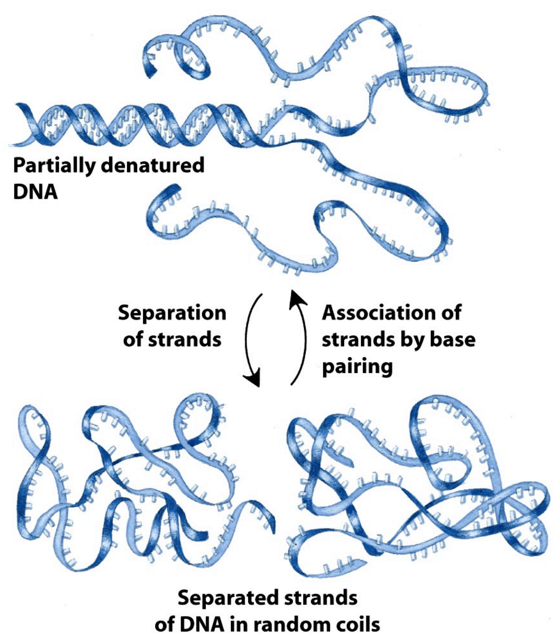

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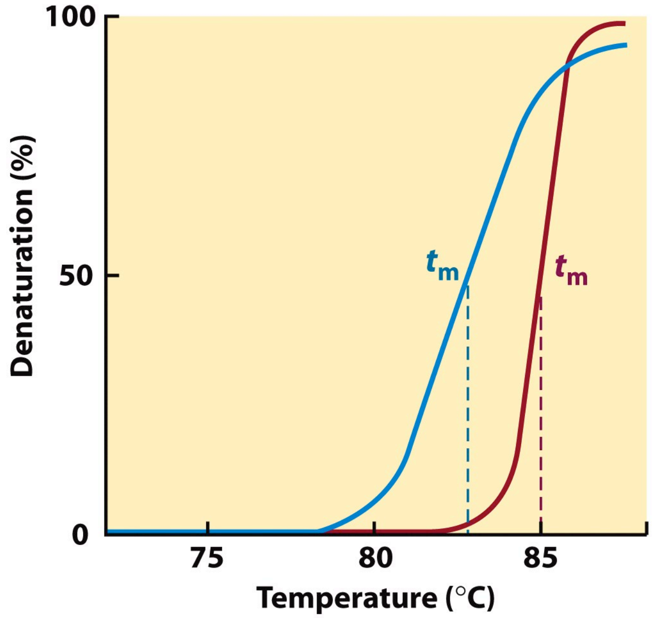

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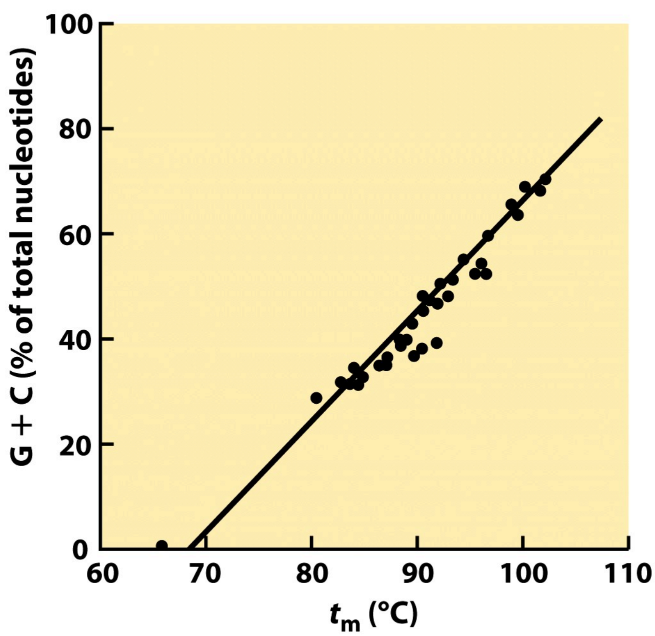

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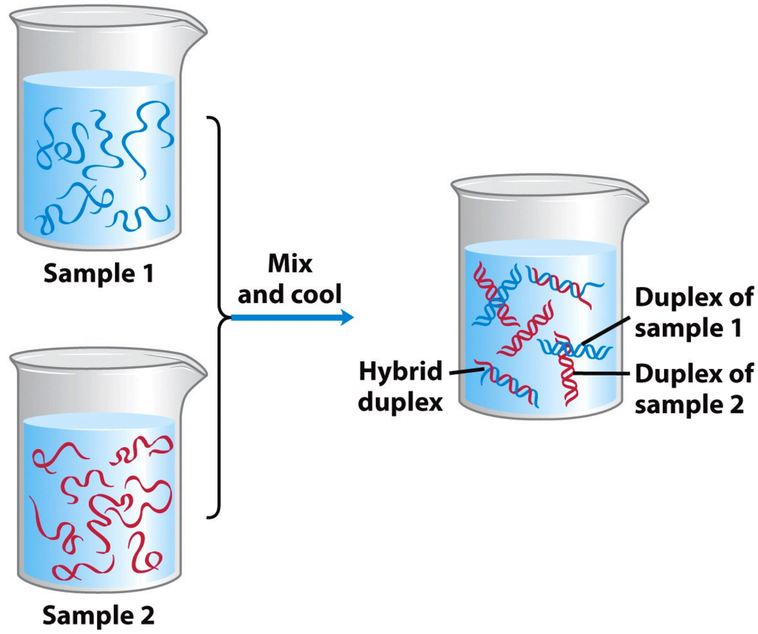

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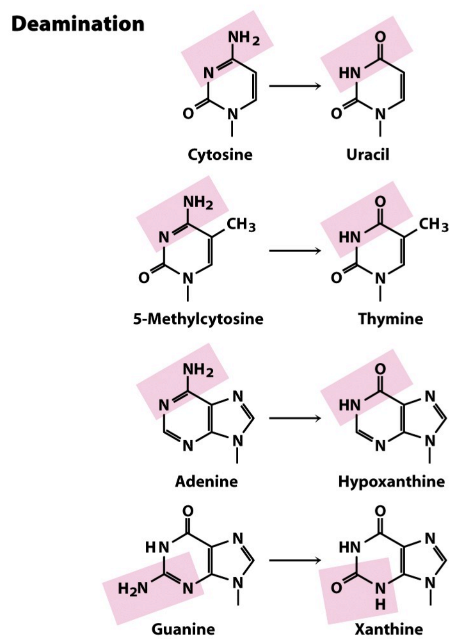

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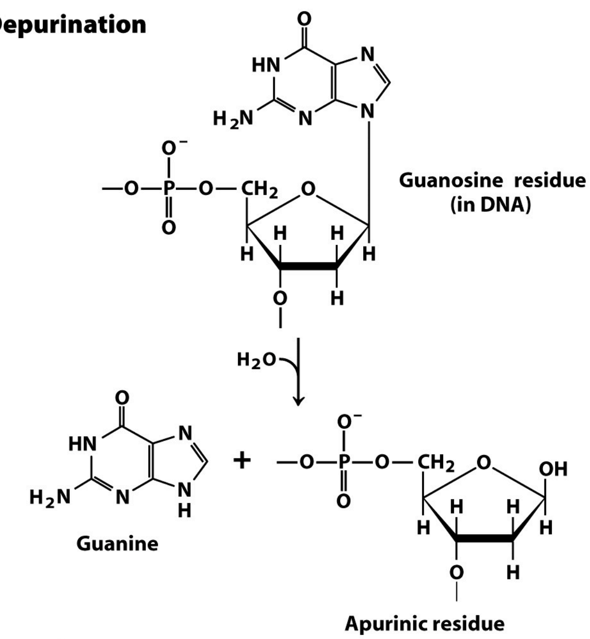
Depurination



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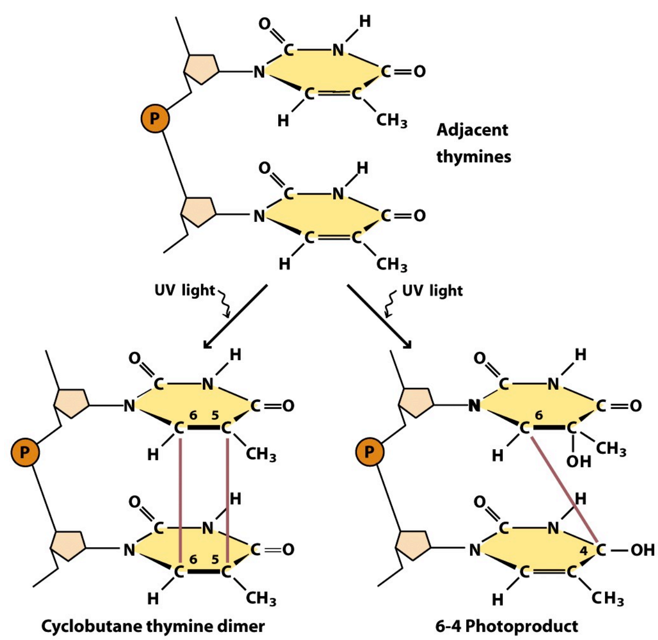

Figure 8-31a
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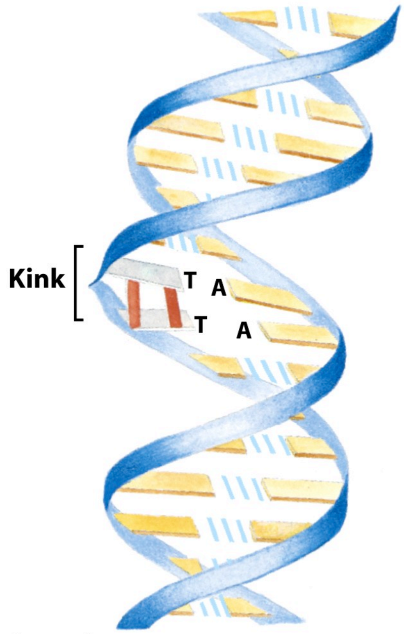

Figure 8-31b
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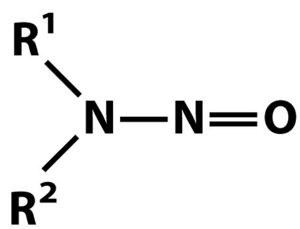
$NaNO_2$ **$NaNO_3$**

Sodium nitrite **Sodium nitrate**



Nitrosamine

Nitrous acid precursors

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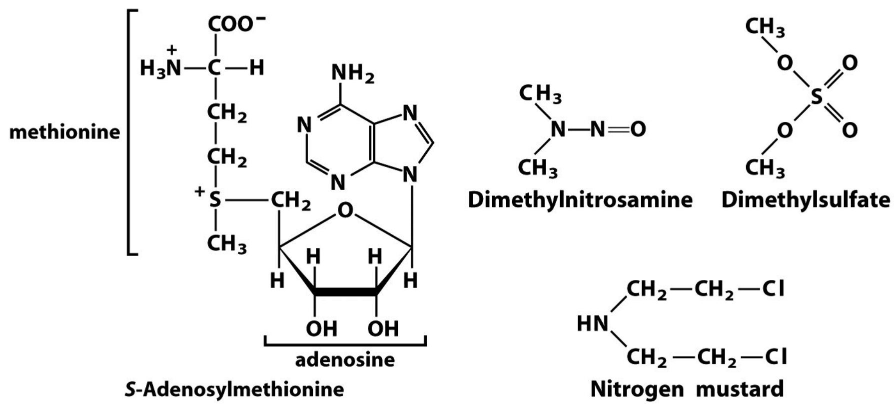

Alkylating agents

Figure 8-32b
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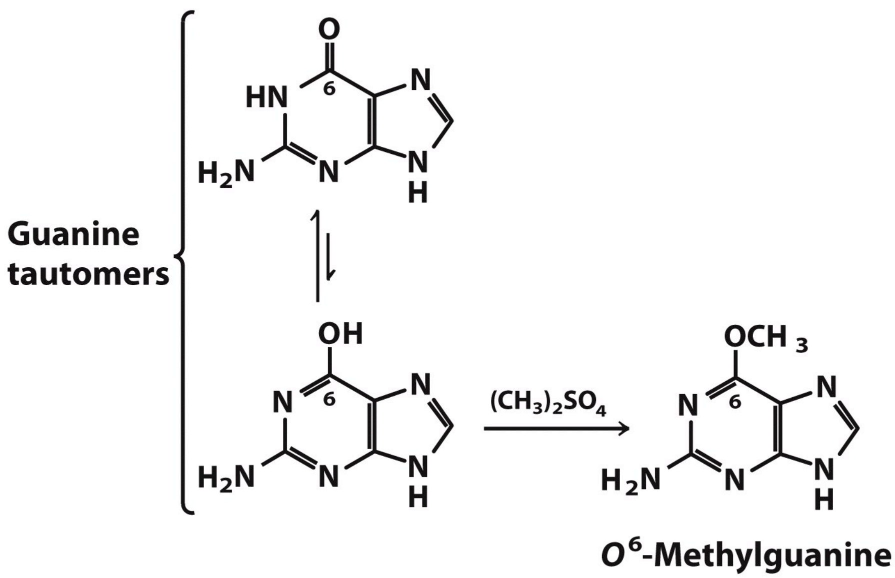

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DNA sequencing

Sanger method

- DNA sequencing
  - Based on chain termination method
  - Yields information about
    - Structure of gene
    - Probable amino acid sequences of its encoded proteins

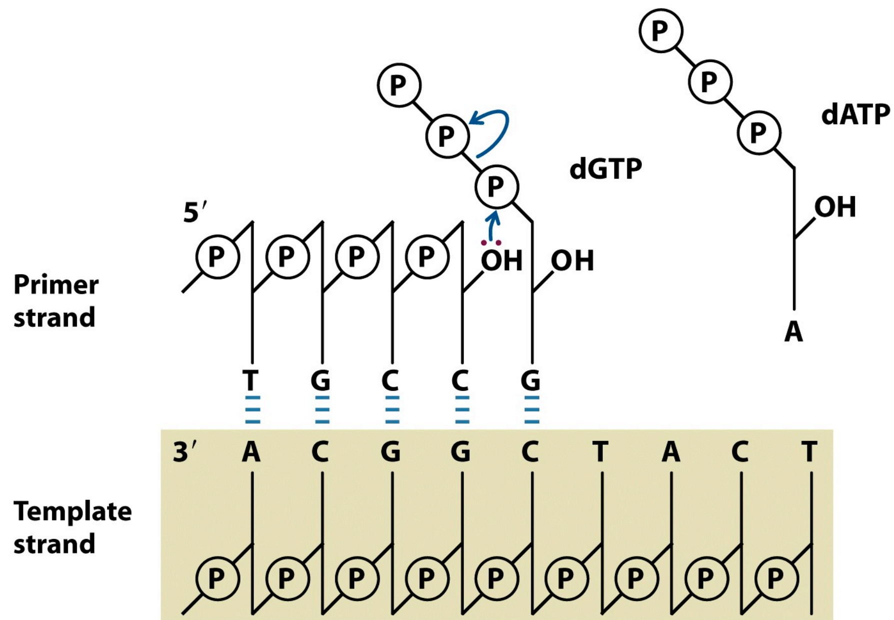

Figure 8-33a
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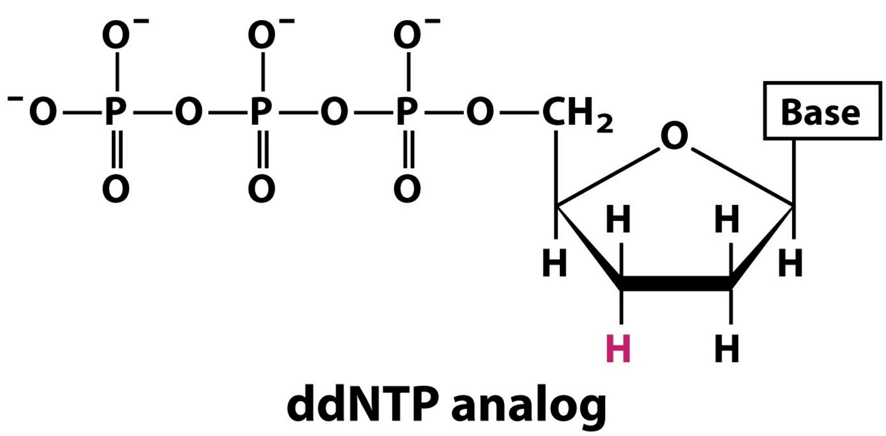

Figure 8-33b
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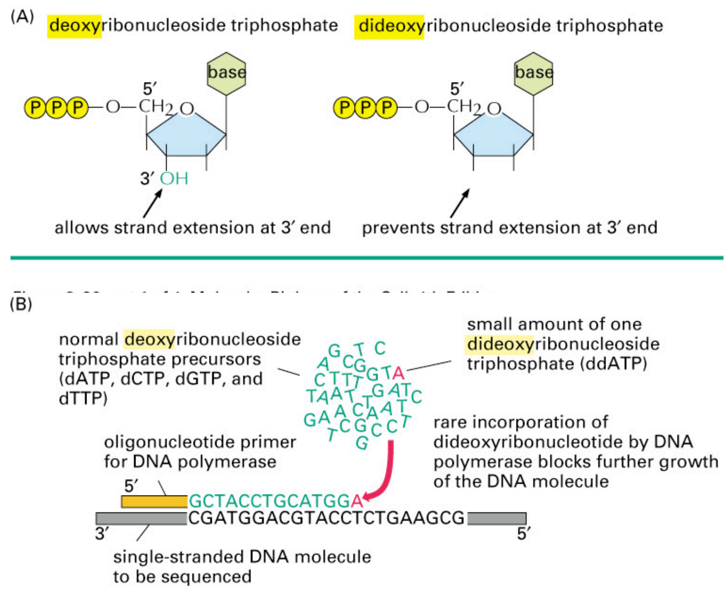
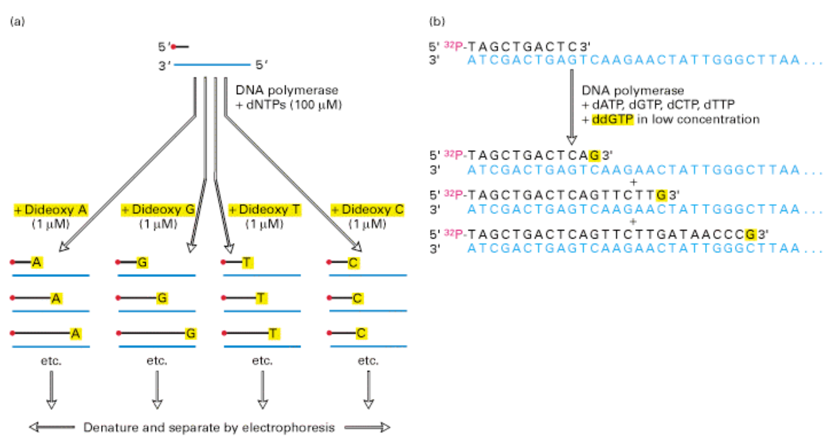
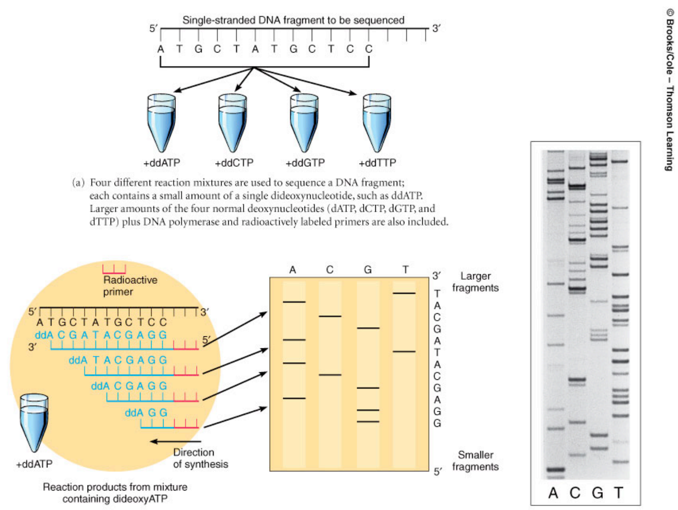


Figure 8–36 part 2 of 4. Molecular Biology of the Cell, 4th Edition.



Chain Termination Method

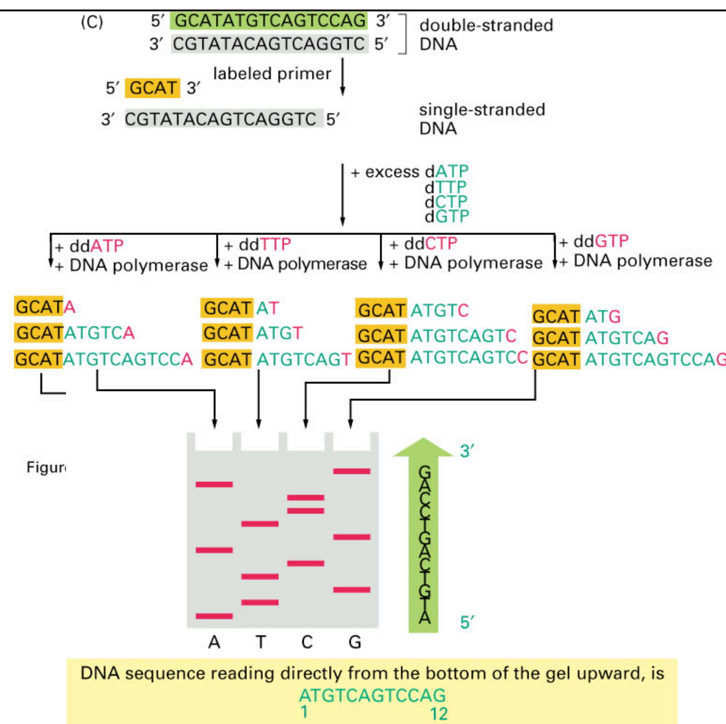


(a) Four different reaction mixtures are used to sequence a DNA fragment; each contains a small amount of a single dideoxynucleotide, such as ddATP. Larger amounts of the four normal deoxynucleotides (dATP, dCTP, dGTP, and dTTP) plus DNA polymerase and radioactively labeled primers are also included.

(b) The random incorporation of dideoxyATP (ddATP) into the growing chain generates a series of smaller DNA fragments ending at all the possible positions where adenine is found in the newly synthesized fragments. These correspond to positions where thymine occurs in the original template strand.

(c) The radioactive products of each reaction mixture are separated by gel electrophoresis and located by exposing the gel to x-ray film. The nucleotide sequence of the newly synthesized DNA is read directly from the film (5′ → 3′). The sequence in the original template strand is its complement (3′ → 5′).

(d) An exposed x-ray film of a DNA sequencing gel. The four lanes represent A, C, G, and T dideoxy reaction mixtures, respectively.



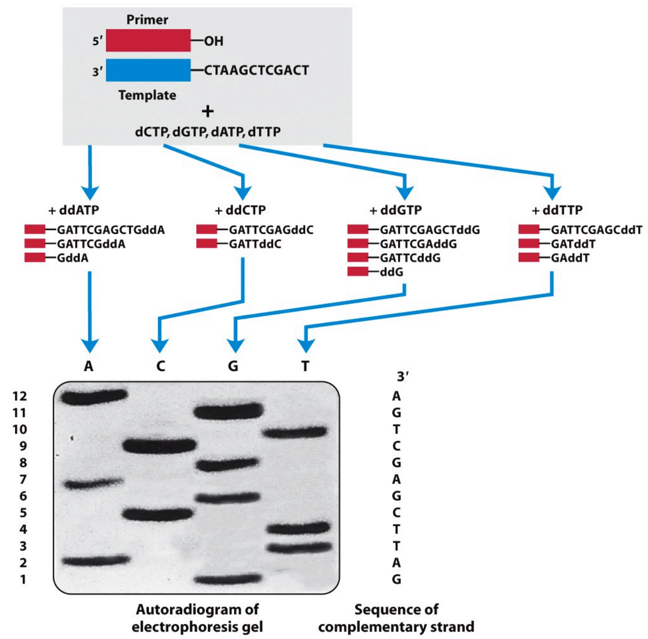


Figure 8-33c
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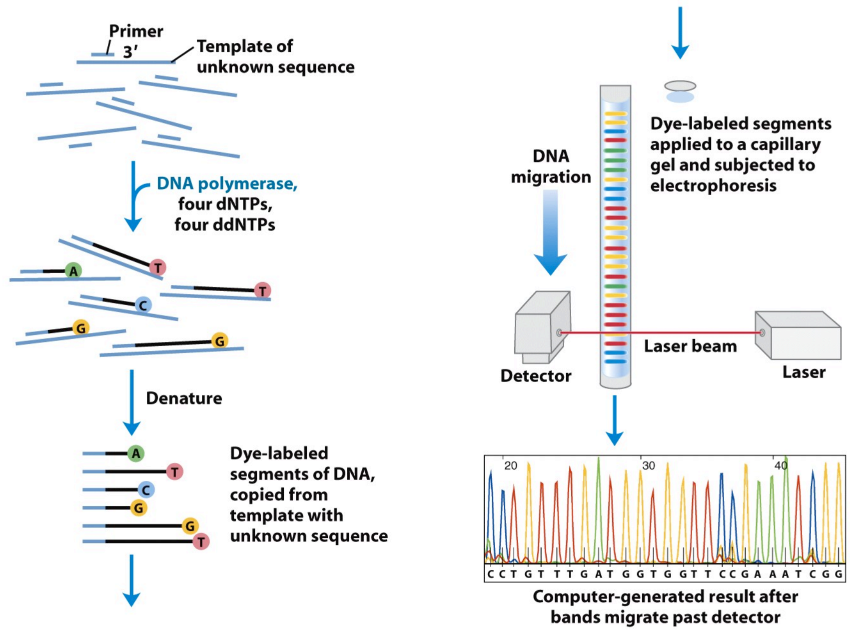

Figure 8-34
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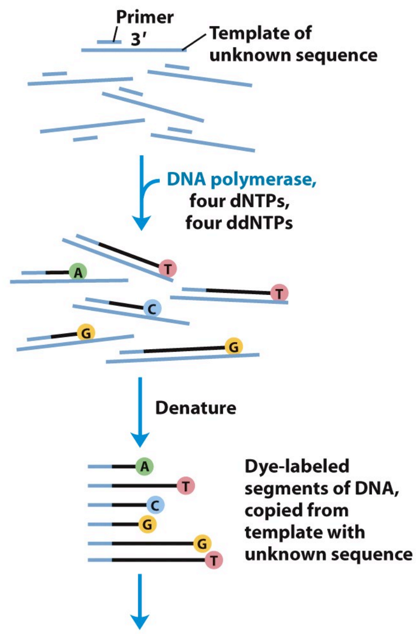

Figure 8-34 part 1
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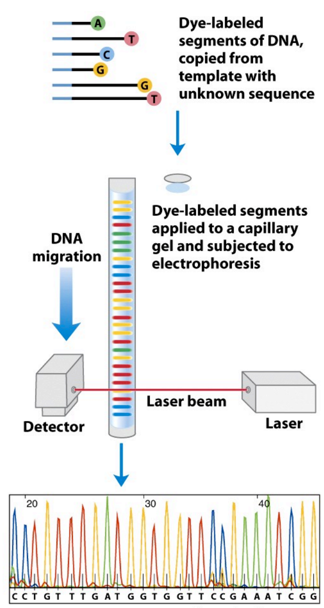
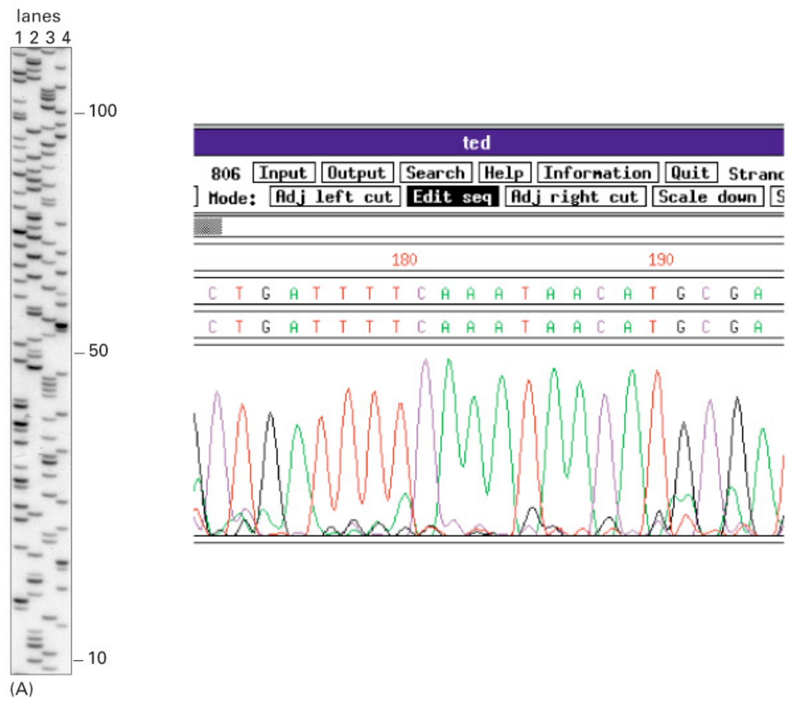


Figure 8-34 part 2
Lehninger Principles of Biochemistry, Fifth Edition



DNA sequencing: The following DNA fragment was sequenced by the Sanger method. The asterisk indicates a fluorescent label. (12 points)

5’—3’-OH

3’—ATTACGCAAGGACATTAGAC---5’

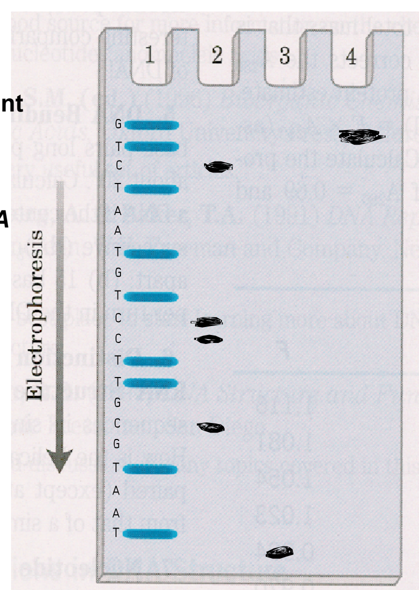
A sample of the DNA was reacted with DNA polymerase and each of the nucleotide mixtures (in an appropriate buffer) listed below. Dideoxynucleotides (ddNTPs) were added in relatively small amounts.

1. dATP, dTTP, dCTP, dGTP, ddTTP

2. dATP, dTTP, dCTP, dGTP, ddCTP

3. dATP, dCTP, dGTP, ddTTP

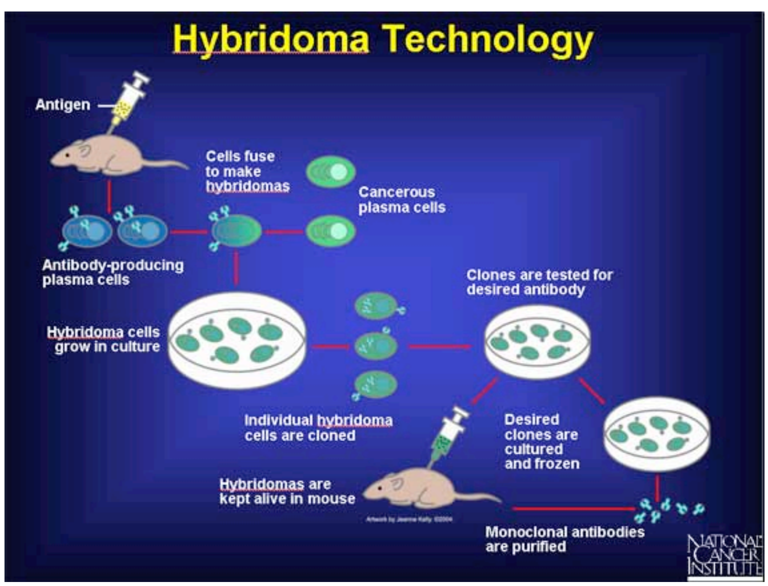
4. dATP, dTTP, dCTP, dGTP

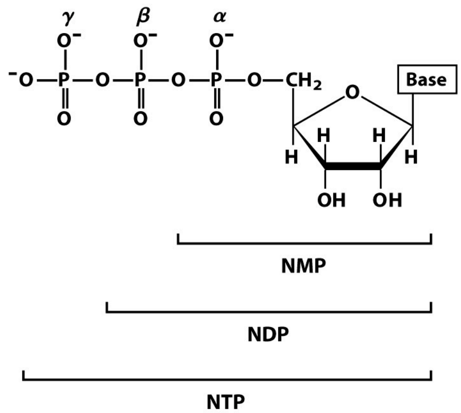


Hybridoma Technology

Hybridoma: A cell hybrid resulting from the fusion of a cancer cell and a normal lymphocyte (a type of white blood cell). The hybridoma is immortal in the laboratory and makes the same products as its parent cells forever

A hybridoma is a hybrid cell produced by injecting a specific antigen into a mouse, collecting an antibody-producing cell from the mouse's spleen, and fusing it with a long-lived cancerous immune cell called a myeloma cell. Individual hybridoma cells are cloned and tested to find those that produce the desired antibody. Their many identical daughter clones will secrete, over a long period of time, millions of identical copies of made-to-order "monoclonal" antibodies.Thanks to hybridoma technology, scientists are now able to make large quantities of specific antibodies.





Abbreviations of ribonucleoside 5′-phosphates			
Base	**Mono-**	**Di-**	**Tri-**
Adenine	AMP	ADP	ATP
Guanine	GMP	GDP	GTP
Cytosine	CMP	CDP	CTP
Uracil	UMP	UDP	UTP

Abbreviations of deoxyribonucleoside 5′-phosphates			
Base	**Mono-**	**Di-**	**Tri-**
Adenine	dAMP	dADP	dATP
Guanine	dGMP	dGDP	dGTP
Cytosine	dCMP	dCDP	dCTP
Thymine	dTMP	dTDP	dTTP

Figure 8-36
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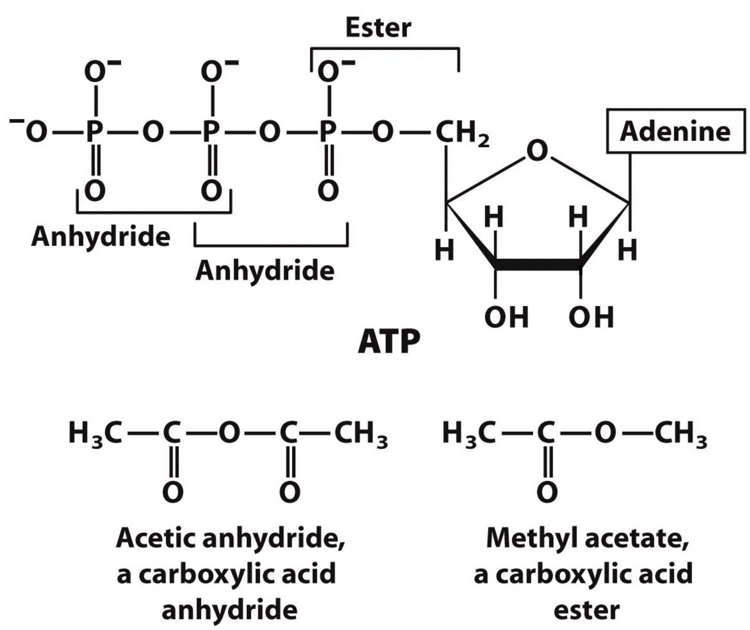

Figure 8-37
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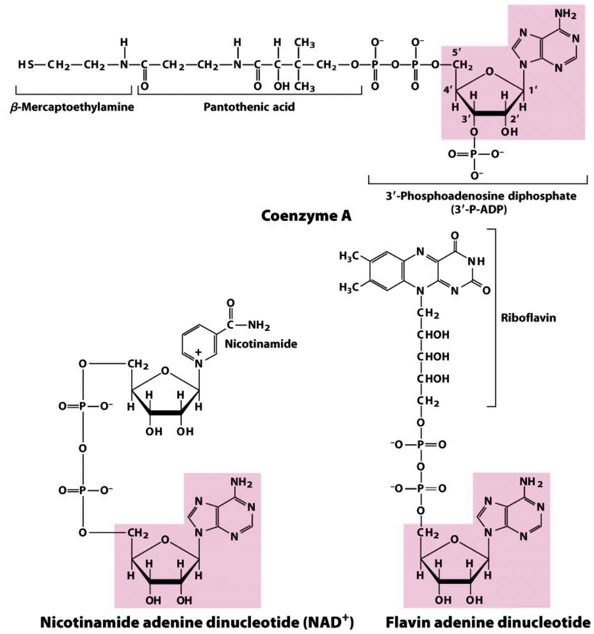

Figure 8-38
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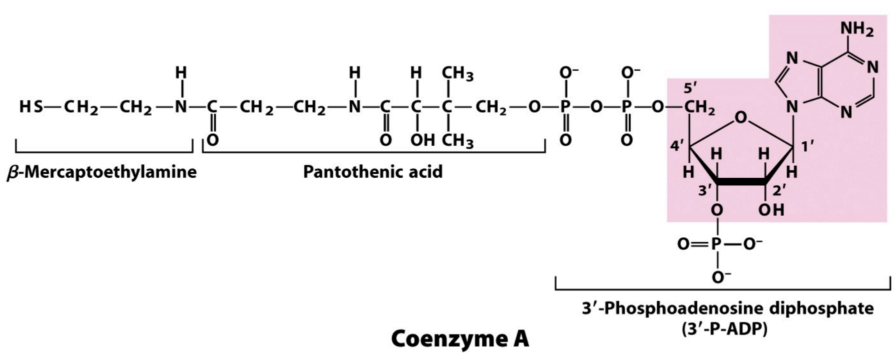

Figure 8-38 part 1
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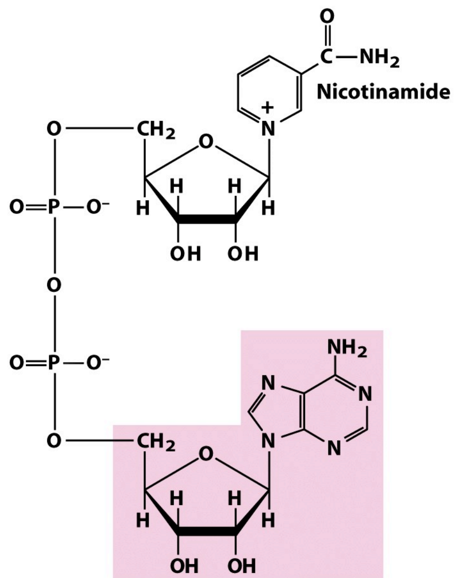

Nicotinamide adenine dinucleotide (NAD^+)

Figure 8-38 part 2
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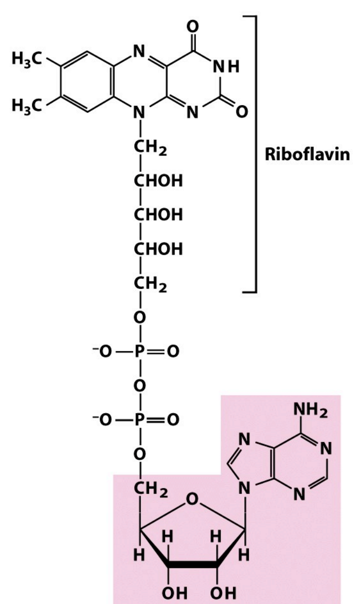

Flavin adenine dinucleotide (FAD)

Figure 8-38 part 3
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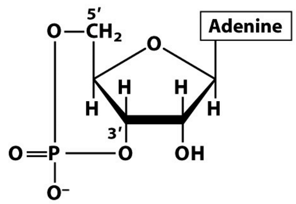
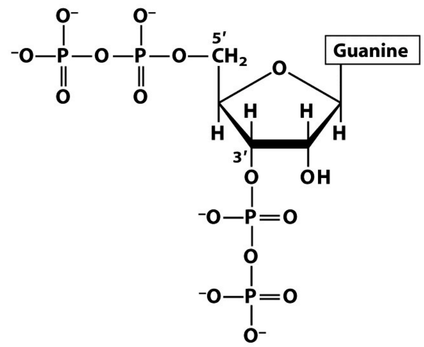
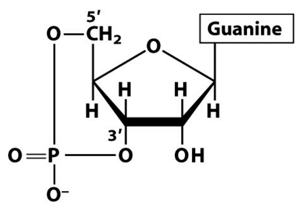


Adenosine 3′,5′-cyclic monophosphate (cyclic AMP; cAMP)



Guanosine 5′-diphosphate, 3′-diphosphate (guanosine tetraphosphate) (ppGpp)



Guanosine 3′,5′-cyclic monophosphate (cyclic GMP; cGMP)

Figure 8-39
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