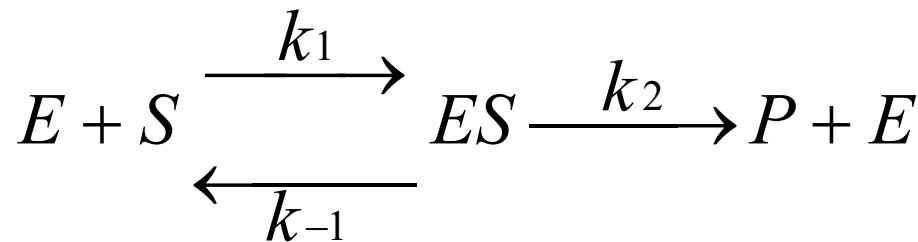


05/02/2024



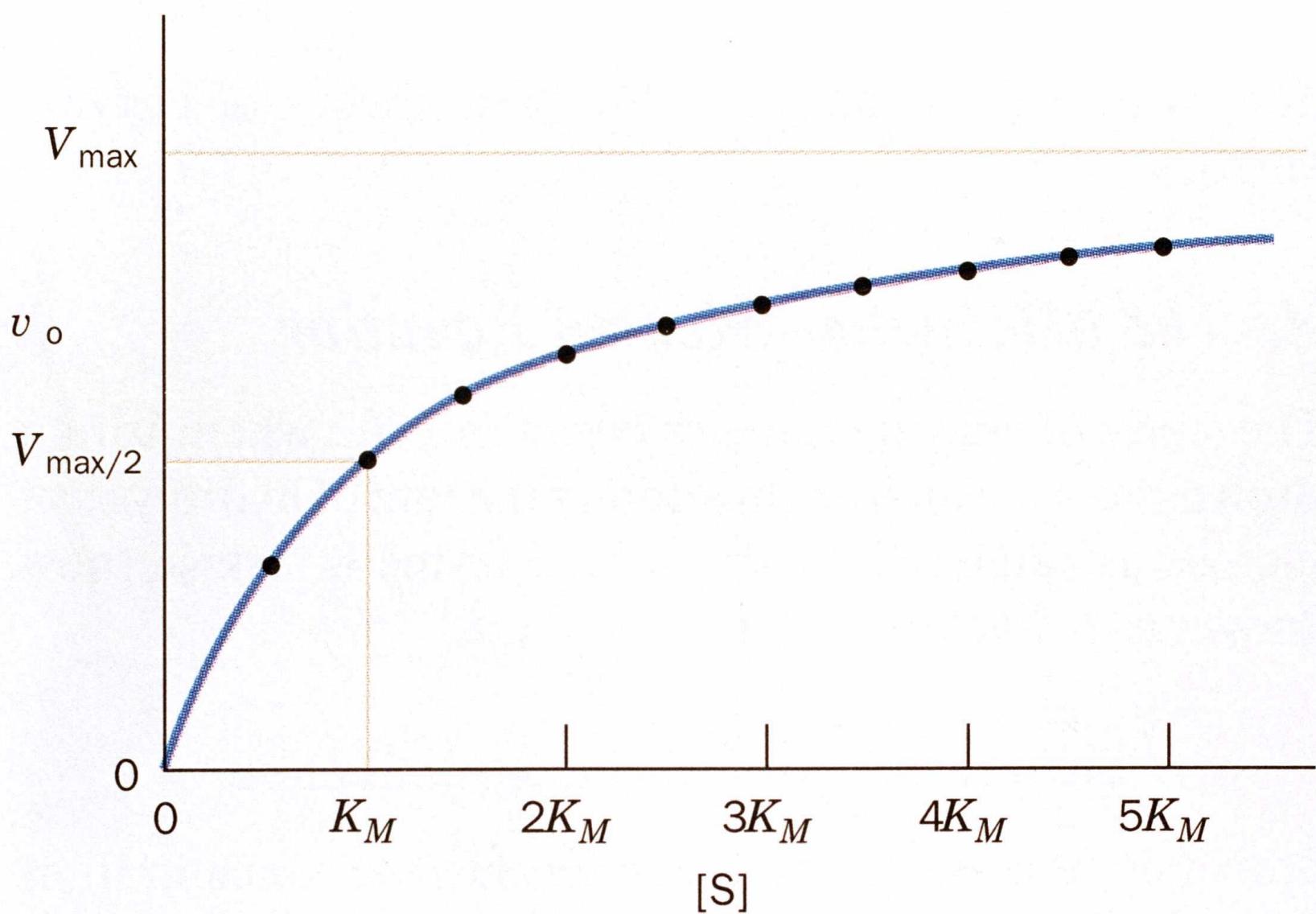
$$v_o = \left(\frac{d[P]}{dt} \right)_{t=0} = k_2 [ES] = \frac{k_2 [E]_T [S]}{K_M + [S]}$$

v_o is the initial velocity when the reaction is just starting out.

And $V_{max} = k_2 [E]_T$ is the maximum velocity

$$v_o = \frac{V_{max} [S]}{K_M + [S]}$$

The Michaelis -
Menten equation



The K_m is the substrate concentration where v_o equals one-half V_{max}

For Michaelis -Menton kinetics $k_2 = k_{cat}$

When $[S] \ll K_M$ very little ES is formed and $[E] = [E]_T$

and $v_o \approx \frac{k_2}{K_M} [E]_T [S] \approx \frac{k_{cat}}{K_M} [E][S]$

K_{cat}/K_M is a measure of catalytic efficiency

K_M is unique for each enzyme-substrate pair

Table 12-1 The Values of K_M , k_{cat} , and k_{cat}/K_M for Some Enzymes and Substrates

Enzyme	Substrate	K_M (M)	k_{cat} (s ⁻¹)	k_{cat}/K_M (M ⁻¹ · s ⁻¹)
Acetylcholinesterase	Acetylcholine	9.5×10^{-5}	1.4×10^4	1.5×10^8
Carbonic anhydrase	CO ₂	1.2×10^{-2}	1.0×10^6	8.3×10^7
	HCO ₃ ⁻	2.6×10^{-2}	4.0×10^5	1.5×10^7
Catalase	H ₂ O ₂	2.5×10^{-2}	1.0×10^7	4.0×10^8
Chymotrypsin	<i>N</i> -Accetylglycine ethyl ester	4.4×10^{-1}	5.1×10^{-2}	1.2×10^{-1}
	<i>N</i> -Acetylvaline ethyl ester	8.8×10^{-2}	1.7×10^{-1}	1.9
	<i>N</i> -Acetyltyrosine ethyl ester	6.6×10^{-4}	1.9×10^2	2.9×10^5
Fumarase	Fumarate	5.0×10^{-6}	8.0×10^2	1.6×10^8
	Malate	2.5×10^{-5}	9.0×10^2	3.6×10^7
Urease	Urea	2.5×10^{-2}	1.0×10^4	4.0×10^5

Table 12-1 Fundamentals of Biochemistry, 2/e

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Enzyme regulation:

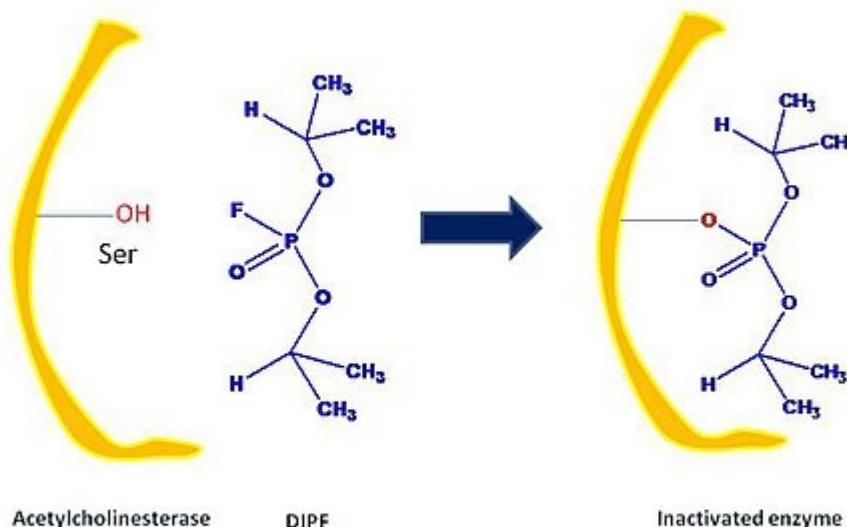
Activity controlled

Continually adjusted

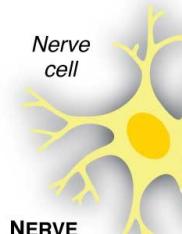
2. Enzyme inhibition

- Substances that reduce an enzyme's activity are known as **inhibitor**.
- Inhibition mechanism:
 - **Irreversible: inactivator**
 - **Reversible:**
 - **Competitive inhibition:** compete directly with the normal substrate for an enzyme's substrate binding site, inhibitor **structurally resembles the substrate.** Product inhibition. Transition state analog may be even better inhibitor.
 - **Uncompetitive inhibition:** the inhibitor binds directly to the enzyme-substrate complex, ES, but not to the free enzyme. Inhibitor need not resemble the substrate.
 - **Mixed inhibition:** inhibitor affects the enzyme in both substrate binding and catalytic activity.

- nerve gases and pesticides, containing organophosphorus, combine with serine residues in the enzyme acetylcholine esterase



How Sarin nerve gas works



SARIN: Man-made nerve gas developed during World War II. Quickly breaks down after release but minuscule amounts can persist in victims' blood for 16-26 days

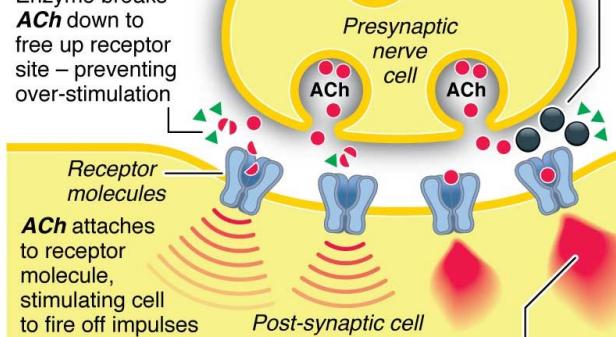
NERVE GAS ACTION

Nervous system relies on transmission of signals through nerve junctions called *synapses*

Electrical impulse

Impulse triggers release of chemical neurotransmitter, **acetylcholine (ACh)**

Enzyme breaks **ACh** down to free up receptor site – preventing over-stimulation



Receptor continually fires off impulses as victim rapidly loses control of vital functions

SARIN FACTFILE

Appearance: Odourless, tasteless, colourless

Form: Liquid vaporises quickly into gas and spreads

Absorption: Contact with skin, inhalation or ingestion

Effects: Inhalation can cause death within 1-10 minutes of exposure

Principal Ways of Regulating Enzymes

Reversible Inhibitors

Competitive Inhibition

Allosteric Inhibition

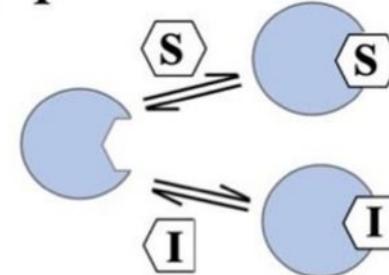


+

I

$$\begin{array}{c} \parallel \\ K_I \end{array}$$

EI



(a) Competitive inhibition

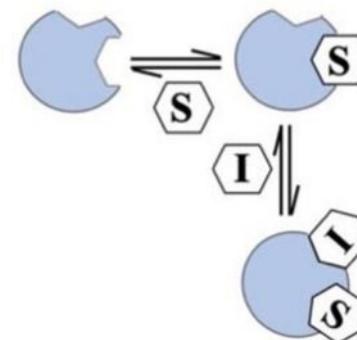


+

I

$$\begin{array}{c} \parallel \\ K_I' \end{array}$$

ESI



(b) Uncompetitive inhibition



+

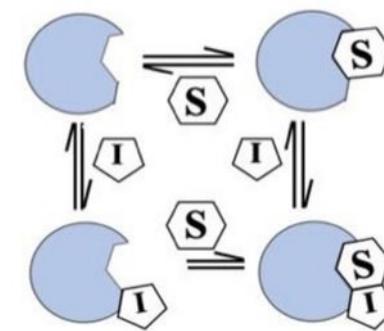
+

I

I

$$\begin{array}{c} \parallel \\ K_I \end{array}$$

$$\begin{array}{c} \parallel \\ K_I' \end{array}$$



(c) Noncompetitive inhibition

Rate of reaction

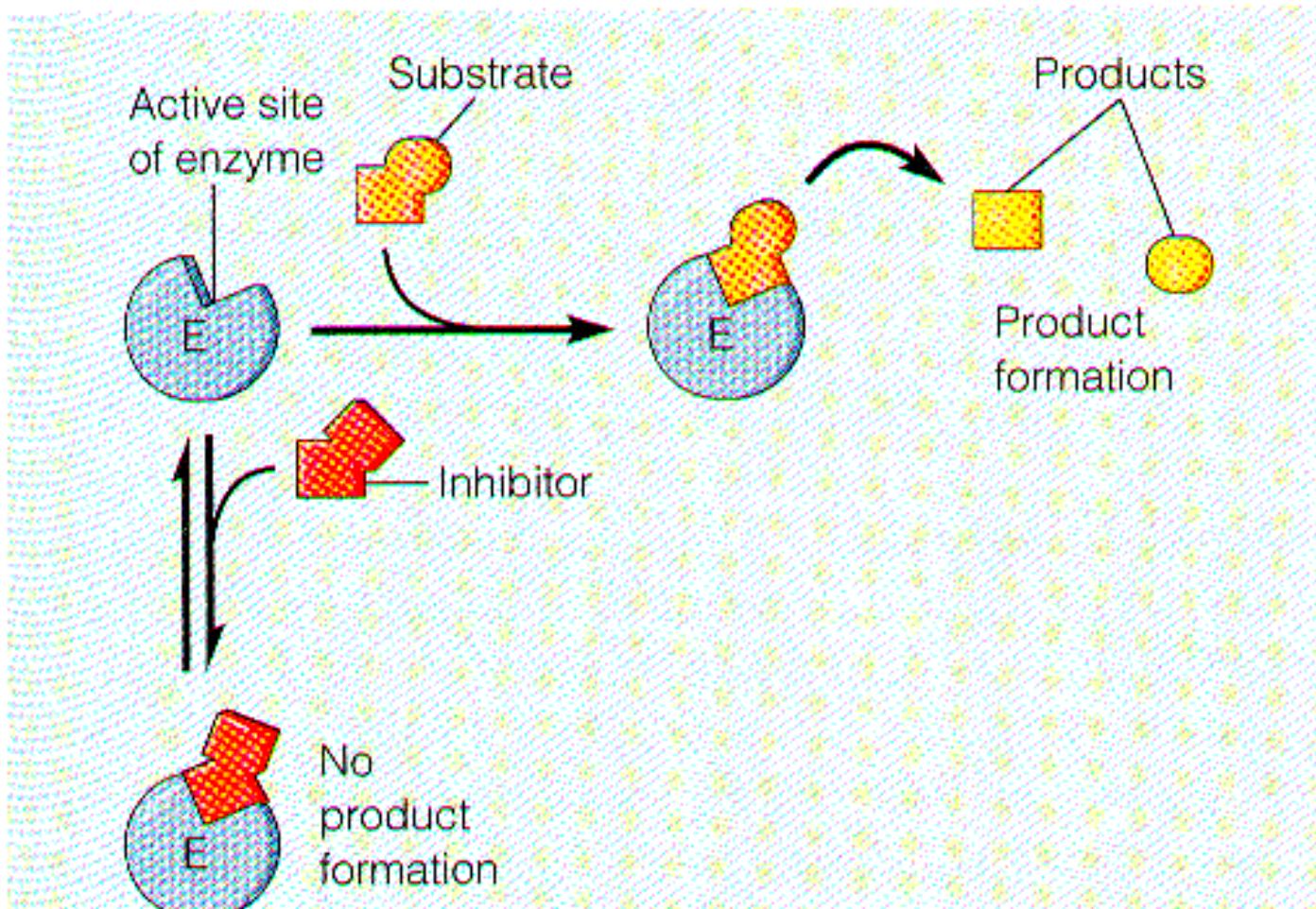
Normal enzyme

Competitive inhibitor

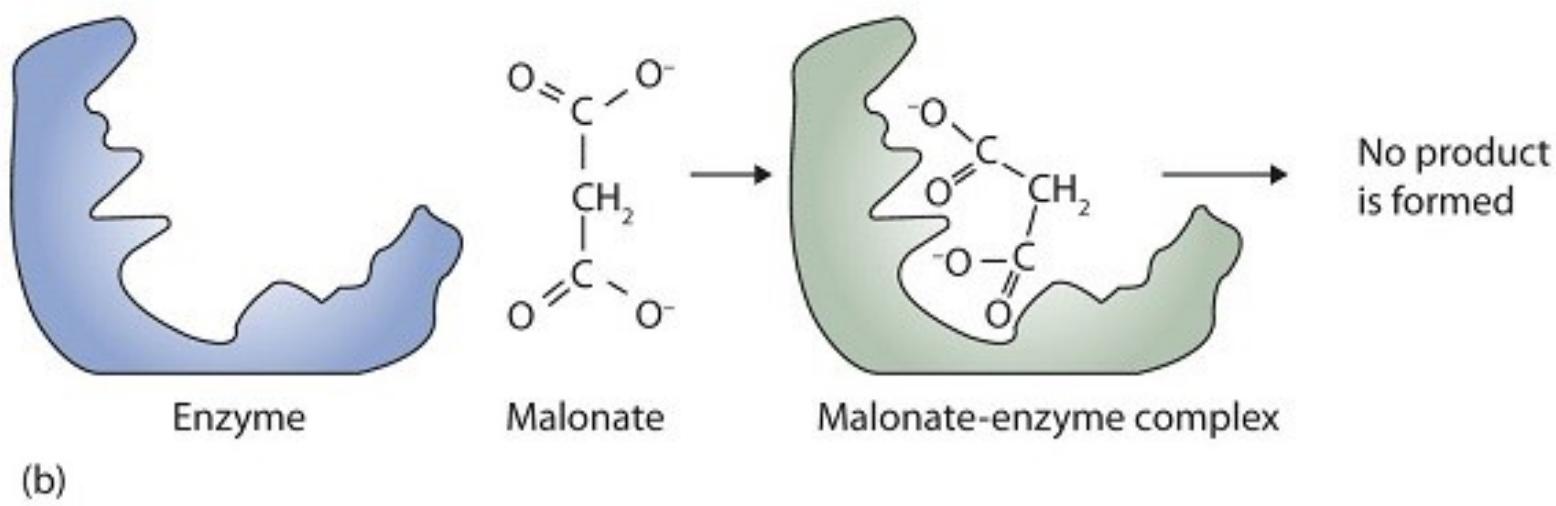
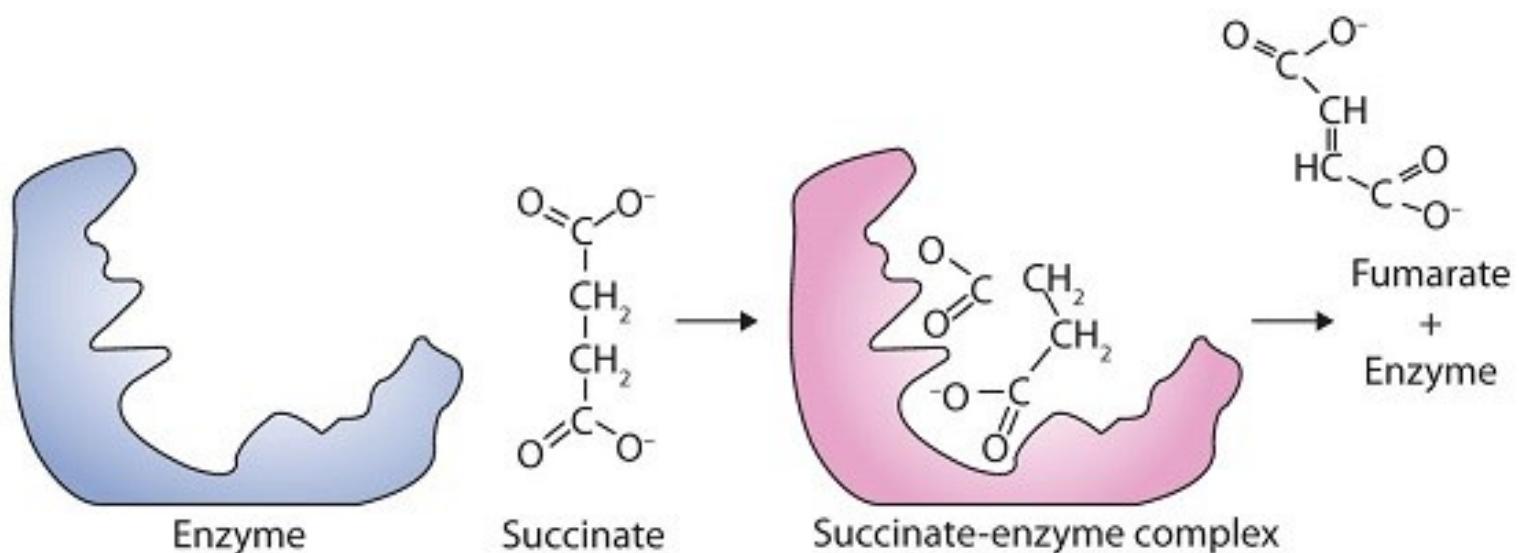
Noncompetitive inhibitor

Substrate concentration

Competitive inhibition



(a) Competitive inhibition. Inhibitor and substrate both bind to the active site of the enzyme. Binding of an inhibitor prevents substrate binding.

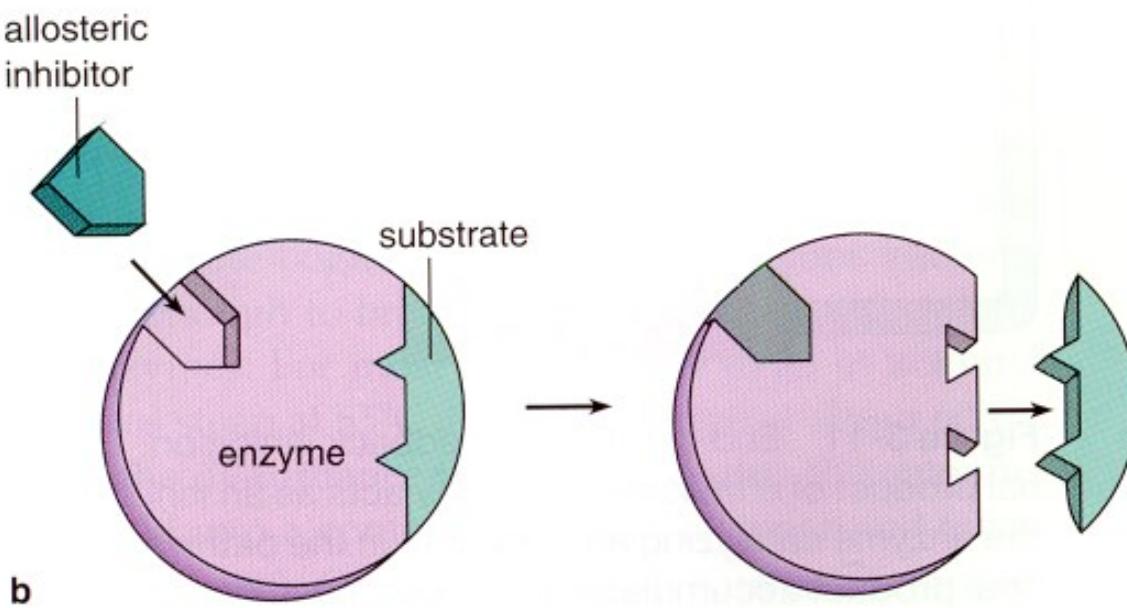


Allosteric Inhibitors

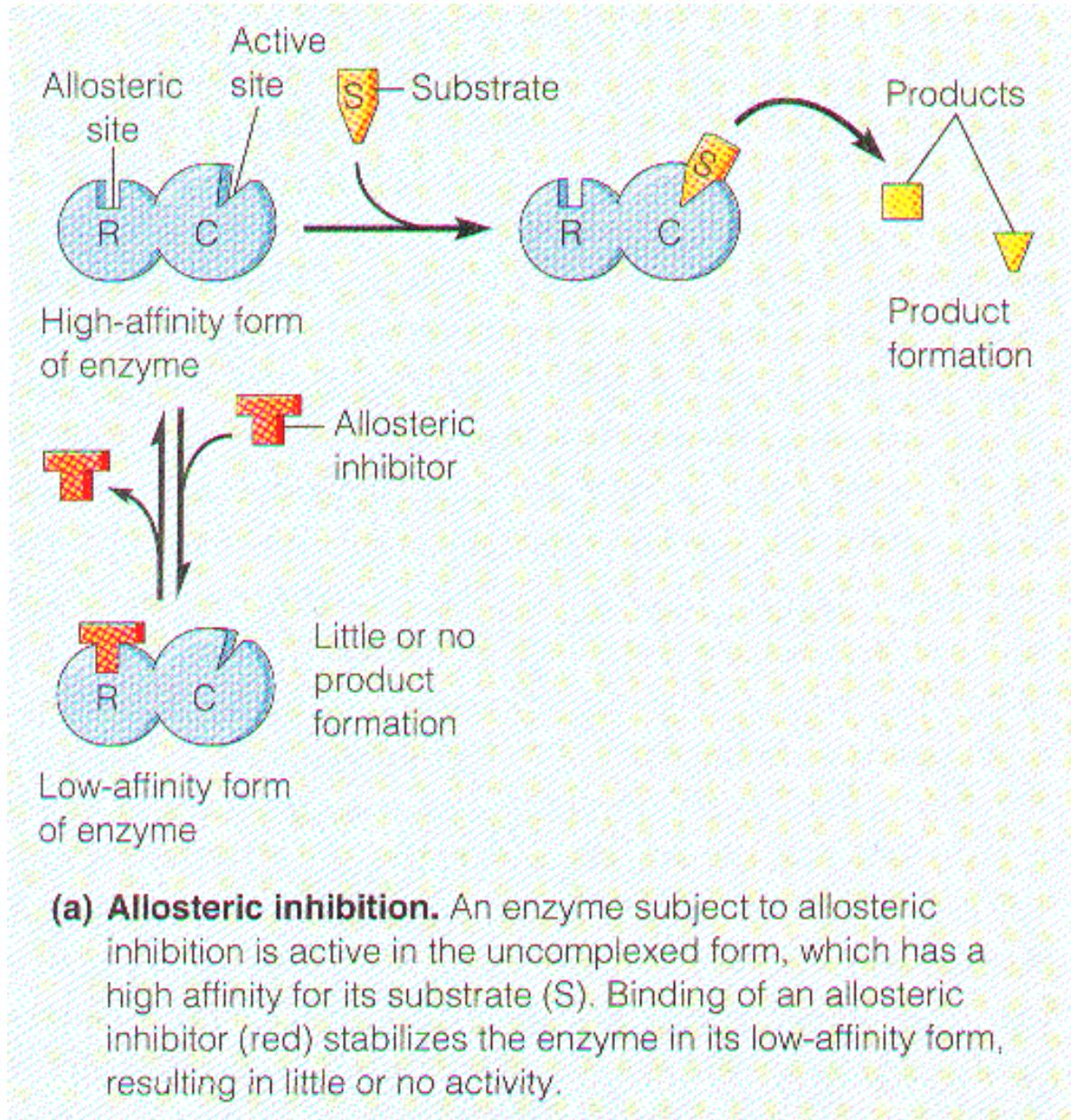
“other” “site”

Distorts the conformation
of the enzyme

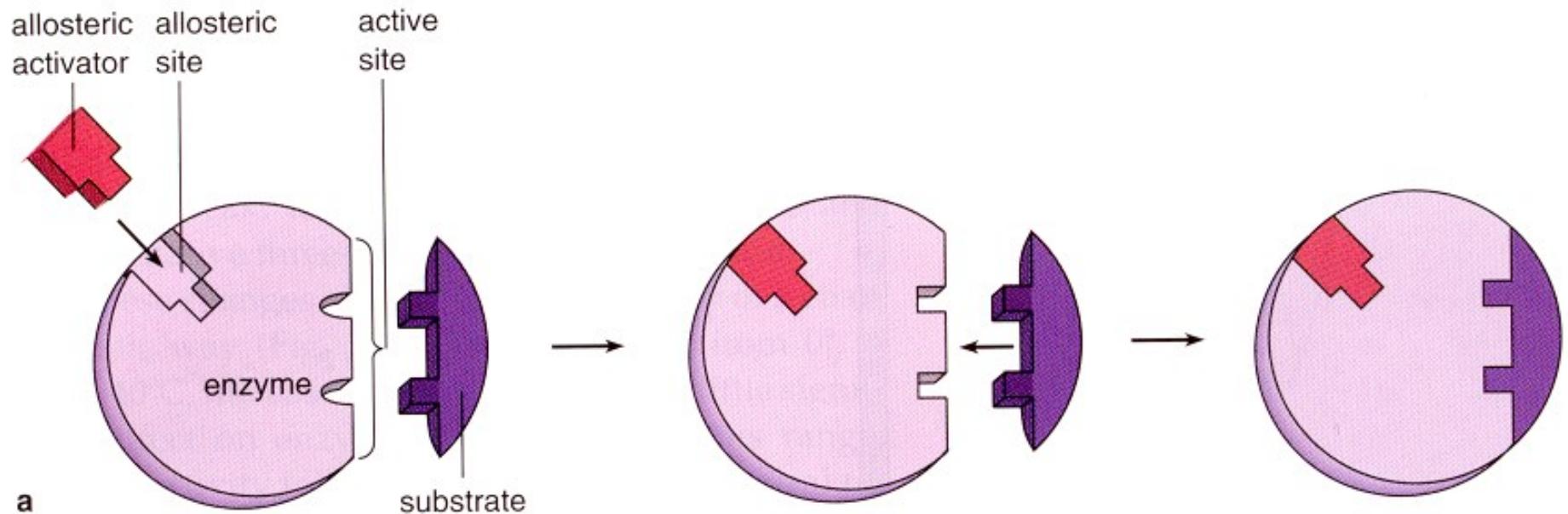
Negative
allosteric
regulator



Allosteric inhibition

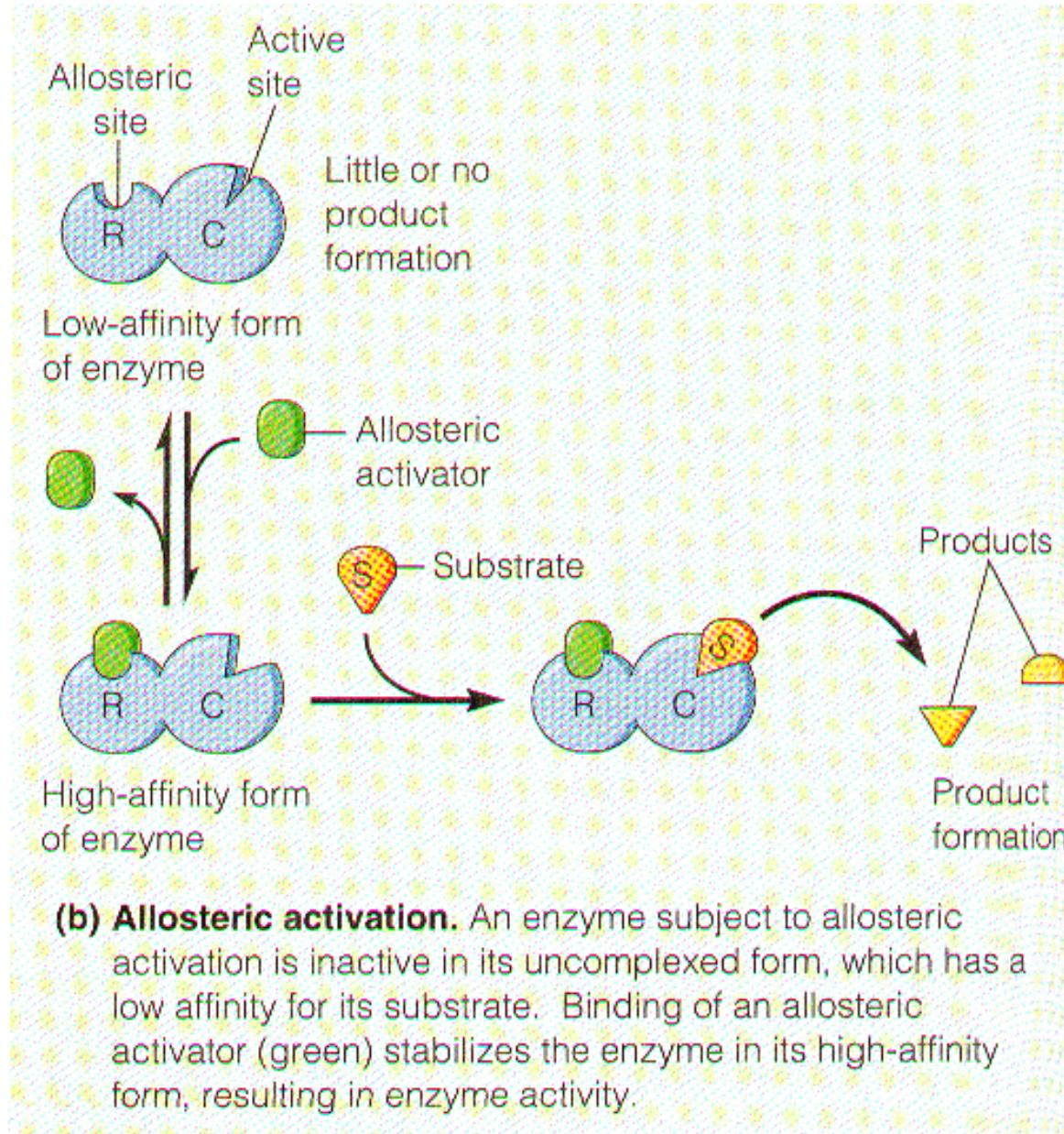


Positive allosteric regulators

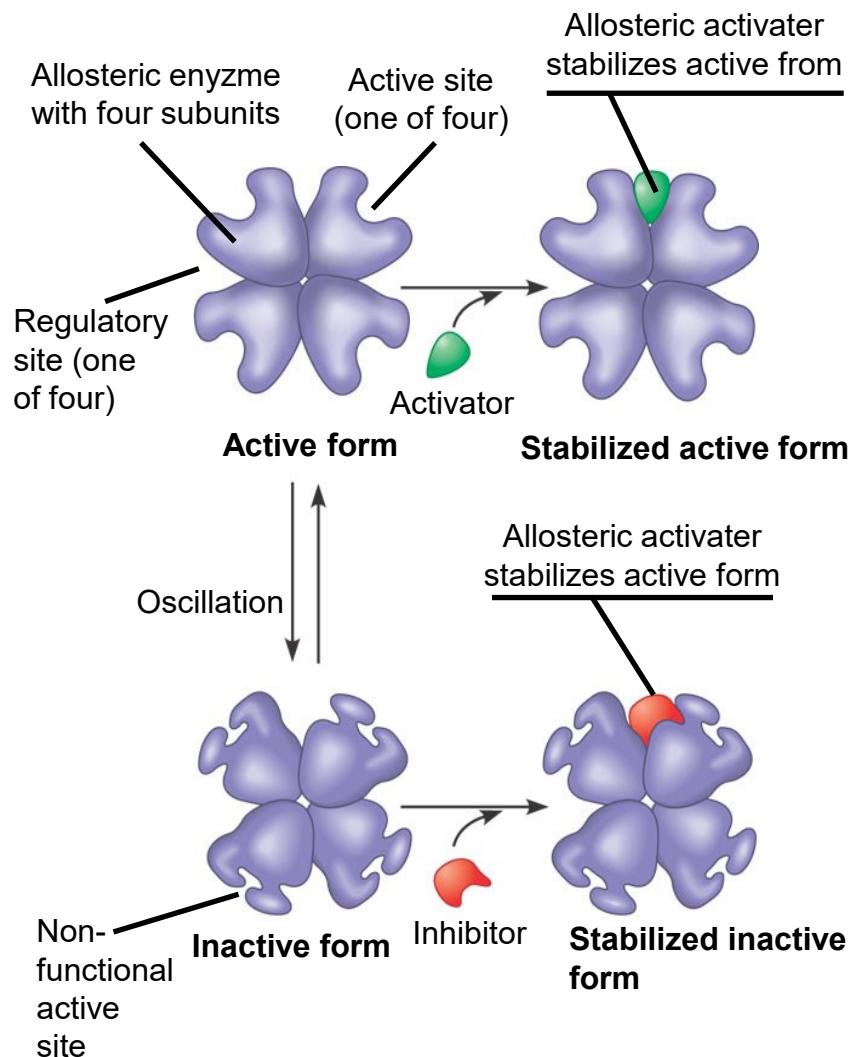


Helps enzyme work **better**
promotes/stabilizes an "active" conformation

Allosteric activation



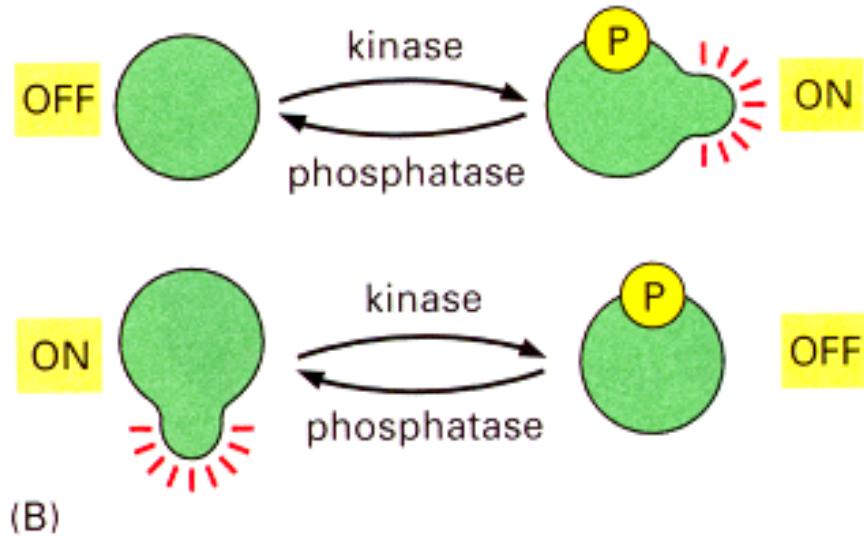
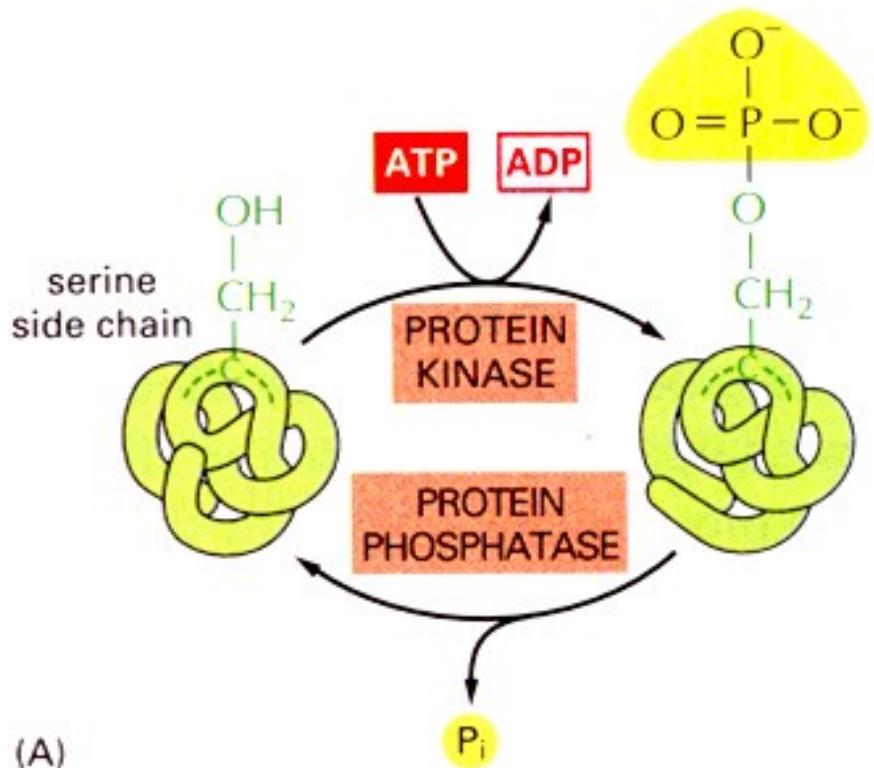
Allosteric regulators change the shape conformation of the enzyme



(a) **Allosteric activators and inhibitors.** In the cell, activators and inhibitors dissociate when at low concentrations. The enzyme can then oscillate again.

Figure 8.20

A frequent regulatory modification Phosphorylation of enzymes



Control of enzyme activity

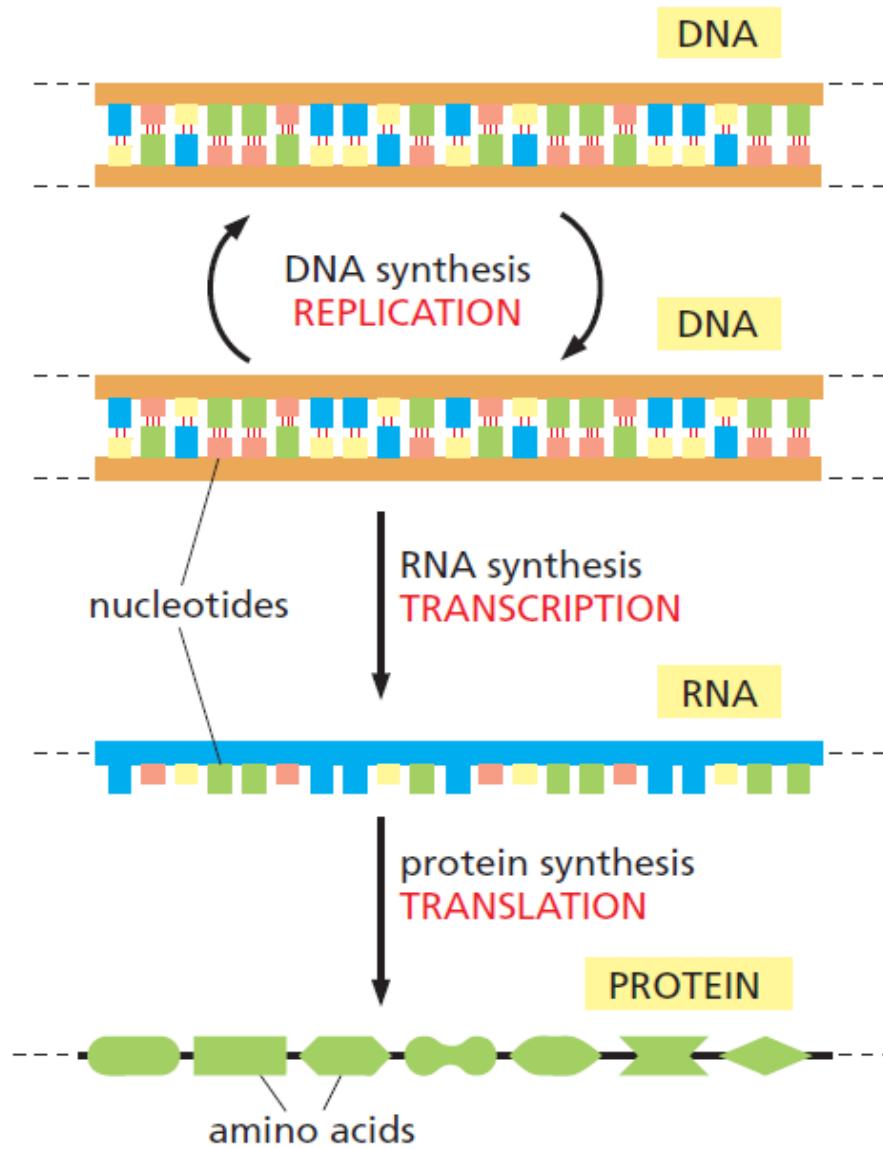
- 1. **control of enzyme availability:** through change of the rate of synthesis and the rate of degradation of the enzyme in the cell.
- 2. **control of enzyme activity:** through structural alteration that influence the enzyme's substrate-binding affinity or turnover number.
 - A. "allosteric regulation". an enzyme's substrate-binding affinity may likewise vary with the binding of small molecules, called **allosteric effectors**.
 - B. covalent modification through phosphorylation or dephosphorylation of specific residues.

Drug design

- Structure-based drug design, also known as rational drug design
- Combinatorial chemistry and high-throughput screening, back to "make many compounds and see what they do" approach.
- Many drugs target to inhibit enzymes or signaling proteins (receptors).

08/02/2024

Central dogma of molecular biology



Nucleotides and Nucleosides

- Nucleotide =
 - nitrogenous base
 - pentose sugar
 - Phosphate
- Nucleoside =
 - nitrogenous base
 - pentose sugar

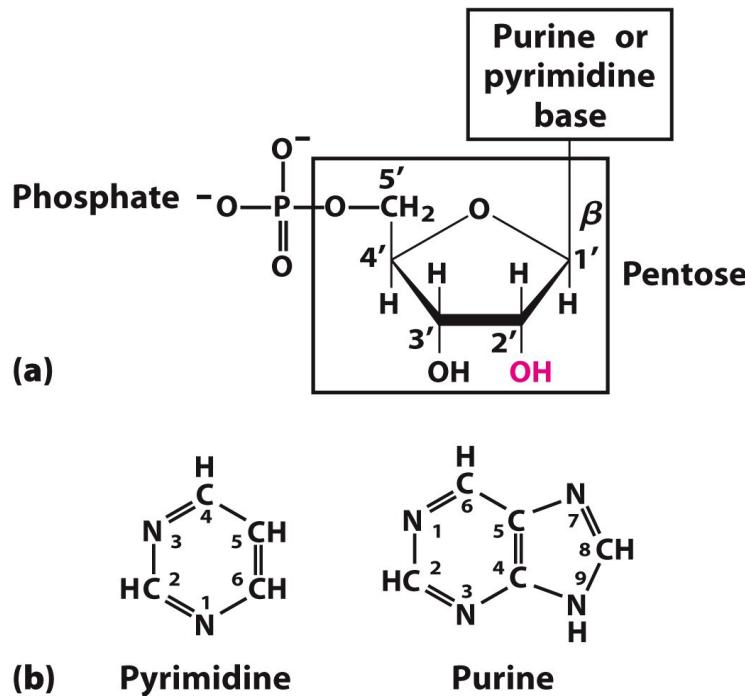


Figure 8-1
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Nucleotides and Nucleosides

- Carbon AND nitrogen atoms on the nitrogenous base are numbered in cyclic format.
- Carbons of the pentose are designated N' to alleviate confusion.

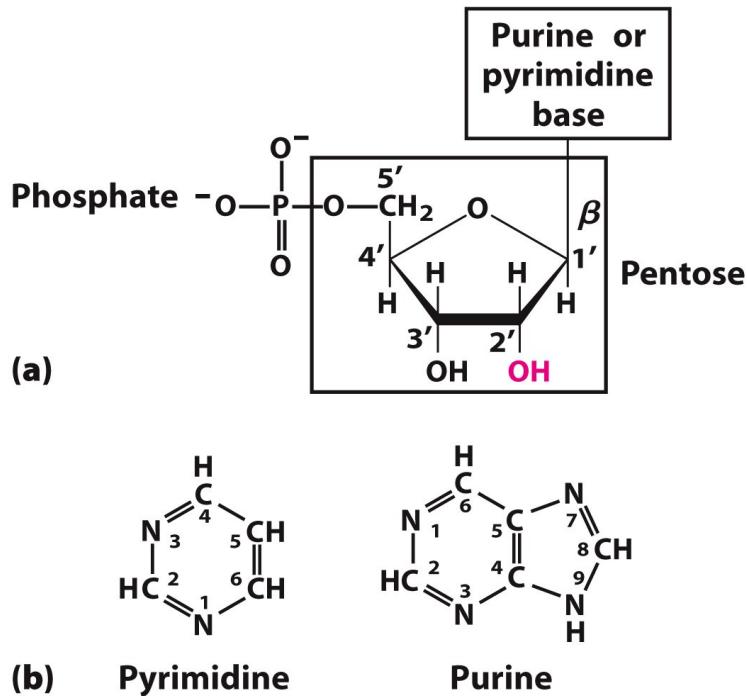
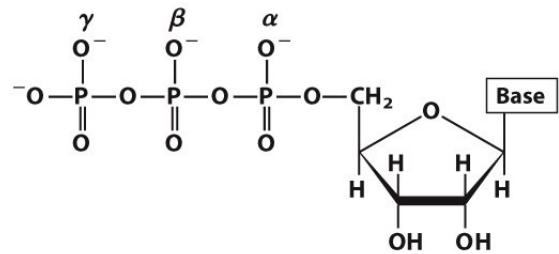


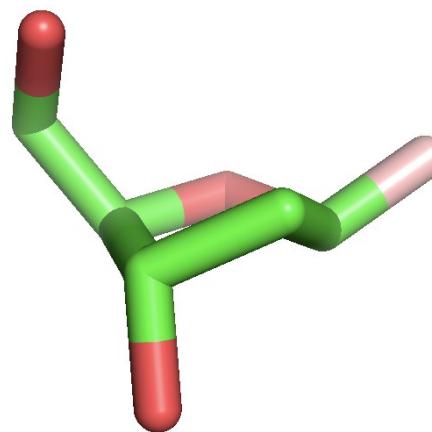
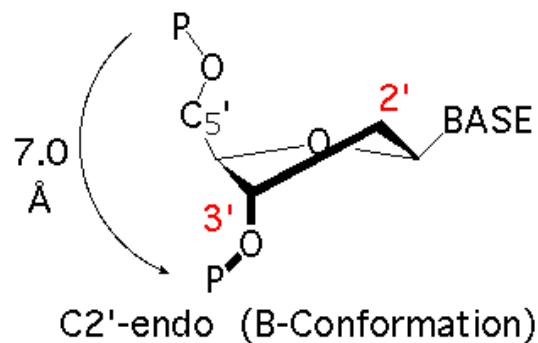
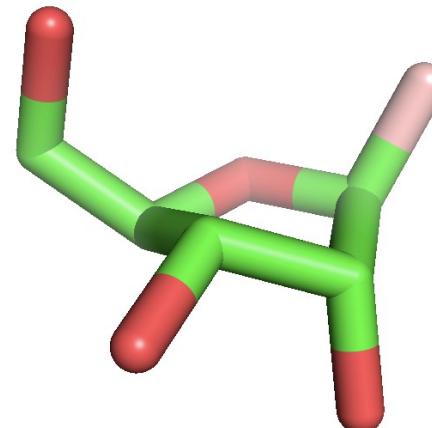
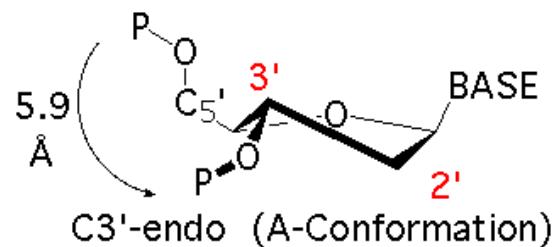
Figure 8-1
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Phosphate Group

- Negatively charged at neutral pH
- Typically attached to 5' position
 - Nucleic acids are built using the 5'-triphosphates version of the nucleotide.
 - ATP, GTP, TTP, CTP
 - Two of the three phosphates used for building nucleic acids form a leaving group, and completed nucleic acids contain one phosphate moiety per nucleotide.
- May be attached to other positions for specialized function

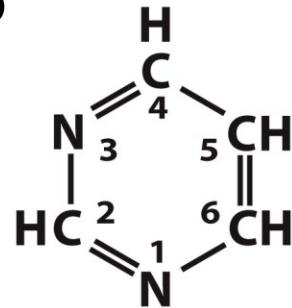


The ribose sugar is “puckered”

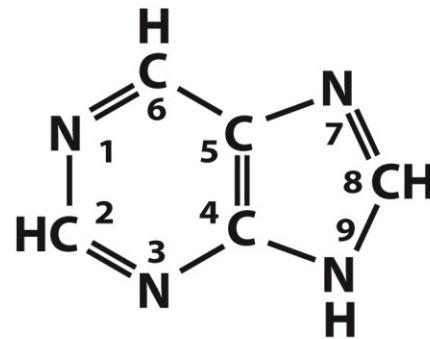


Nitrogenous Bases

- Derivatives of pyrimidine or purine
- Nitrogen-containing heteroaromatic molecules
- Planar or almost planar structures
- Absorbs UV light



Pyrimidine



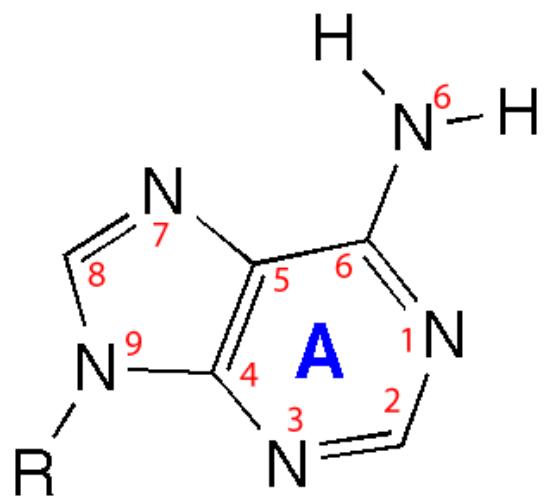
Purine

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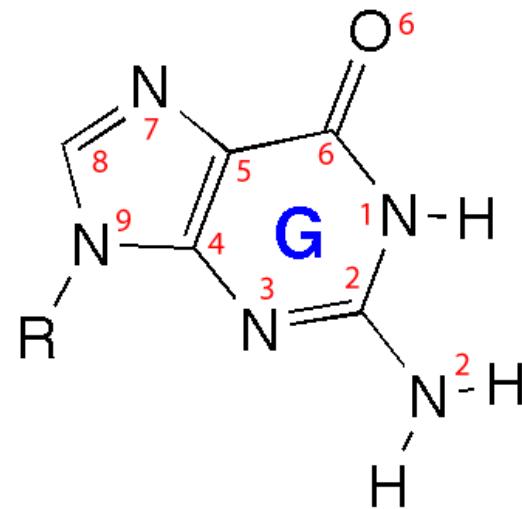
Nucleobases

- Cytosine, adenine, and guanine are found in both DNA and RNA.
- Thymine is found only in DNA.
- Uracil is found only in RNA.
- All are good H-bond donors and acceptors.
- Neutral molecules at pH 7

Purines

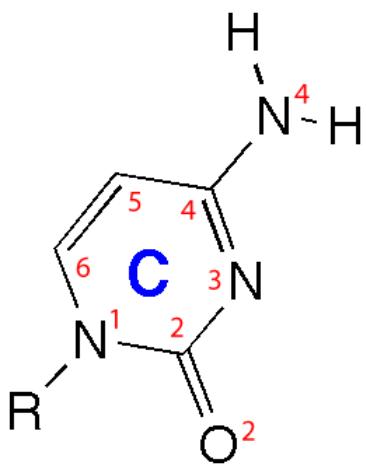


Adenine

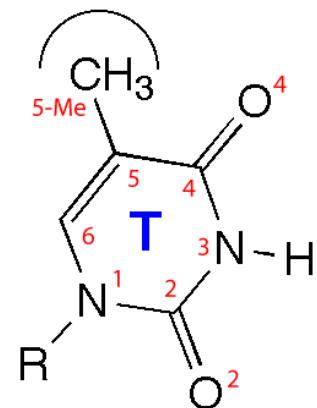


Guanine

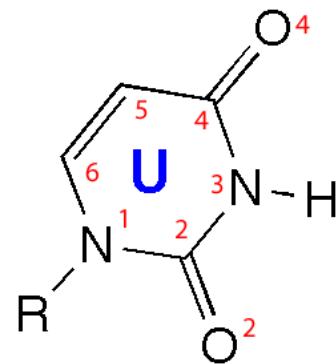
Pyrimidines



Cytosine



Thymine
(DNA)



Uracil
(RNA)

Tautomerism of Nitrogenous Bases

- Prototropic **tautomers** are structural isomers that differ in the location of protons.
- Keto-enol tautomerism is common in ketones.
- **Lactam-lactim** tautomerism occurs in some **heterocycles**.
- Both tautomers exist in solution, but the lactam forms are predominant at neutral pH.

Nomenclature: Deoxyribonucleotides

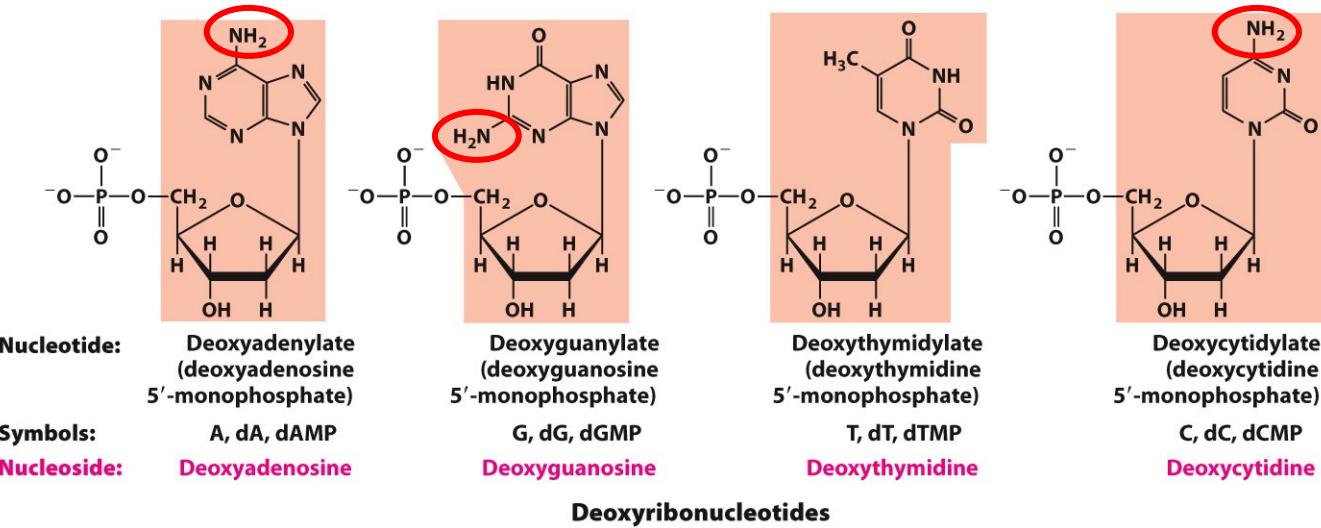


Figure 8-4a

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Nomenclature: Ribonucleotides

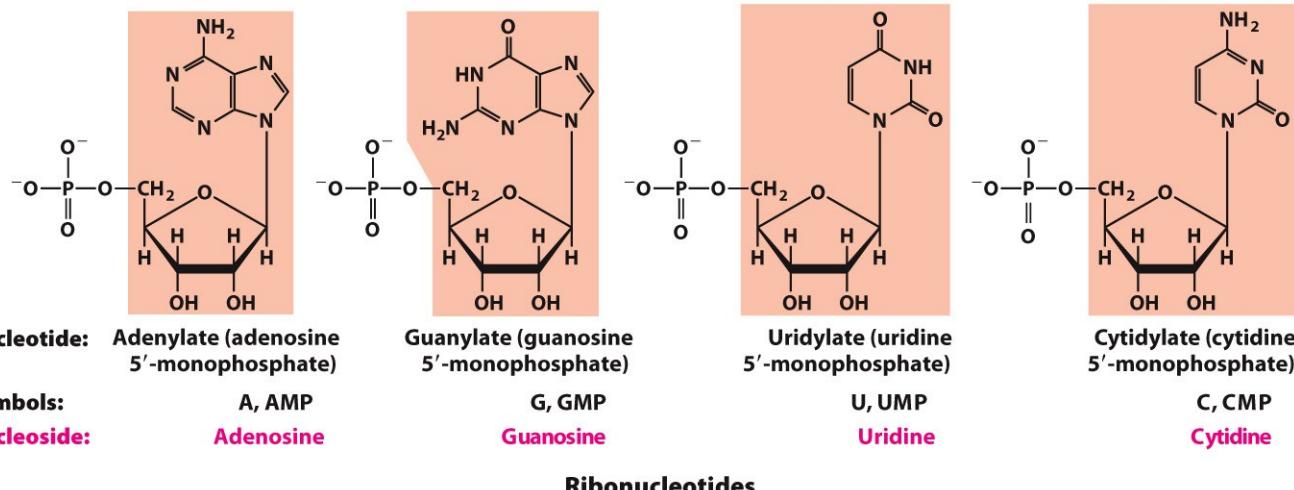


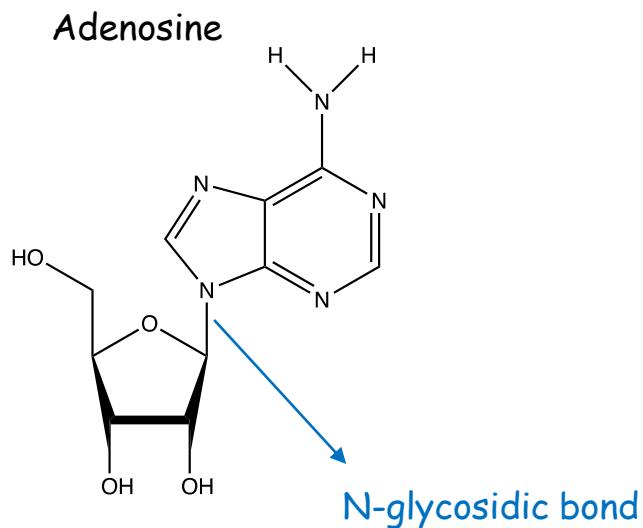
Figure 8-4b

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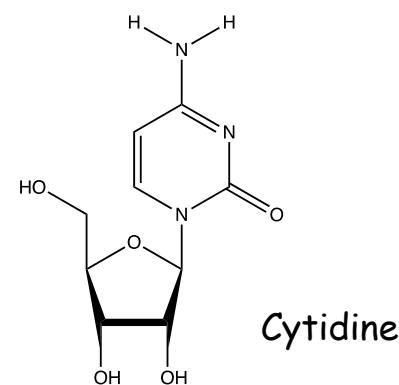
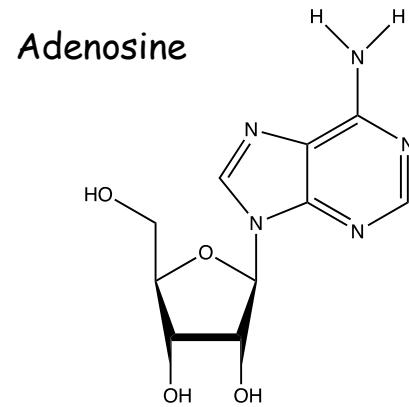
β -N-Glycosidic Bond

- In nucleotides, the pentose ring is attached to the nitrogenous base via a **N-glycosidic bond**.
- The bond is formed to the anomeric carbon of the sugar in β configuration.



β -N-Glycosidic Bond

- The bond is formed:
 - to position N1 in pyrimidines
 - to position N9 in purines
- This bond is quite stable toward hydrolysis, especially in pyrimidines.
- Bond cleavage is catalyzed by acid.



Conformation around N-Glycosidic Bond

- Relatively free rotation can occur around the N-glycosidic bond in free nucleotides.

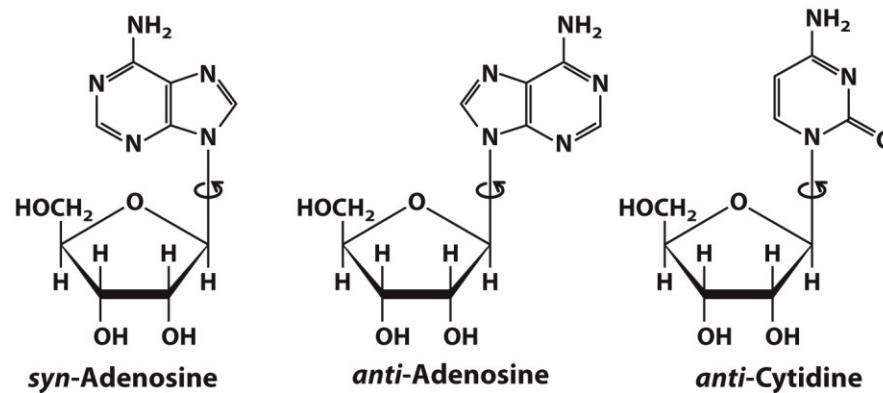


Figure 8-16b
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- The sequence of atoms chosen to define this angle is O4'-C1'-N9-C4 for purine, and O4'-C1'-N1-C2 for pyrimidine derivatives.

Conformation around N-Glycosidic Bond

- Relatively **free rotation** can occur around the N-glycosidic bond in free nucleotides.

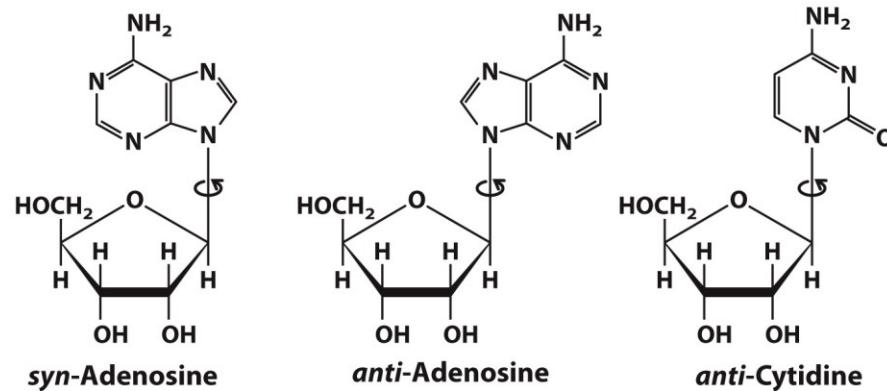


Figure 8-16b
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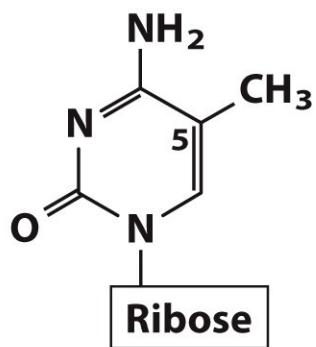
- Angle near 0° corresponds to **syn conformation**.
- Angle near 180° corresponds to **anti conformation**.
- Anti conformation is found in normal B-DNA.

Minor Nucleosides in DNA

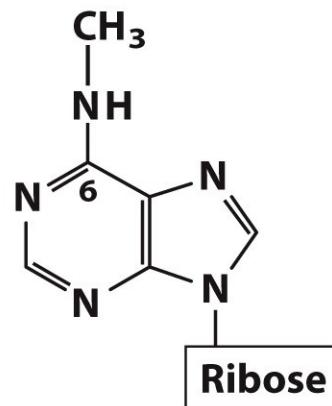
- Modification is done after DNA synthesis.
- **5-Methylcytosine** is common in eukaryotes and is also found in bacteria.
- **N⁶-Methyladenosine** is common in bacteria and eukaryotes

Epigenetic marker:

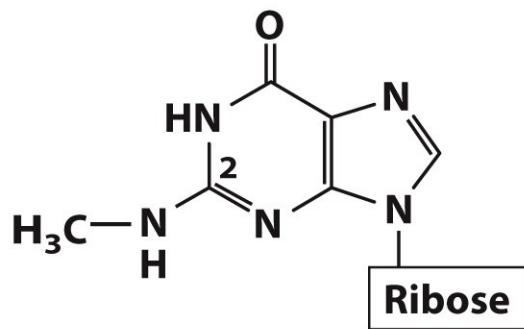
- way to mark own DNA so that cells can degrade foreign DNA (prokaryotes)
- way to mark which genes should be active (eukaryotes)



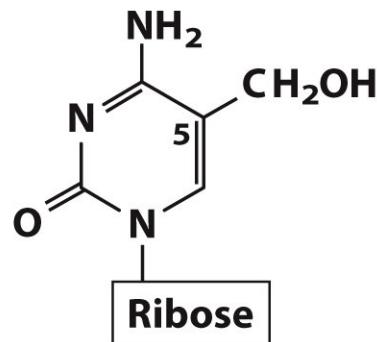
5-Methylcytidine



N^6 -Methyladenosine



N^2 -Methylguanosine



5-Hydroxymethylcytidine

Figure 8-5a

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Polynucleotides

- Covalent bonds are formed via **phosphodiester** linkages.
 - negatively charged backbone

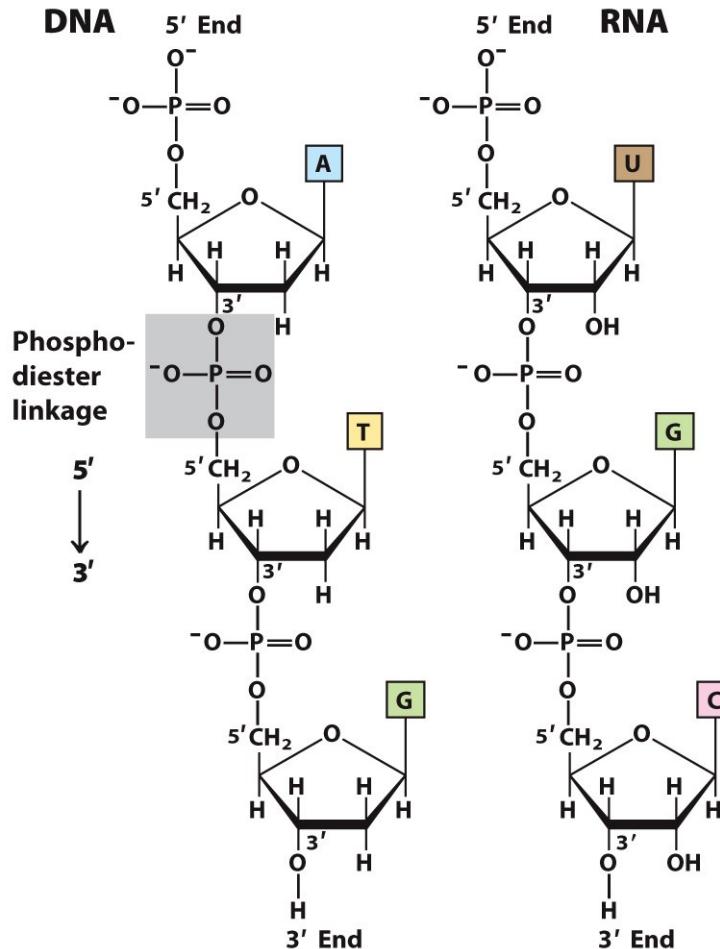


Figure 8-7
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Polynucleotides

- DNA backbone is in a deep kinetic trap.
 - DNA from mammoths?
 - Hydrolysis accelerated by enzymes (DNase)
- RNA backbone is labile
 - In water, RNA lasts for a few years.
 - In cells, mRNA is degraded in a few hours.

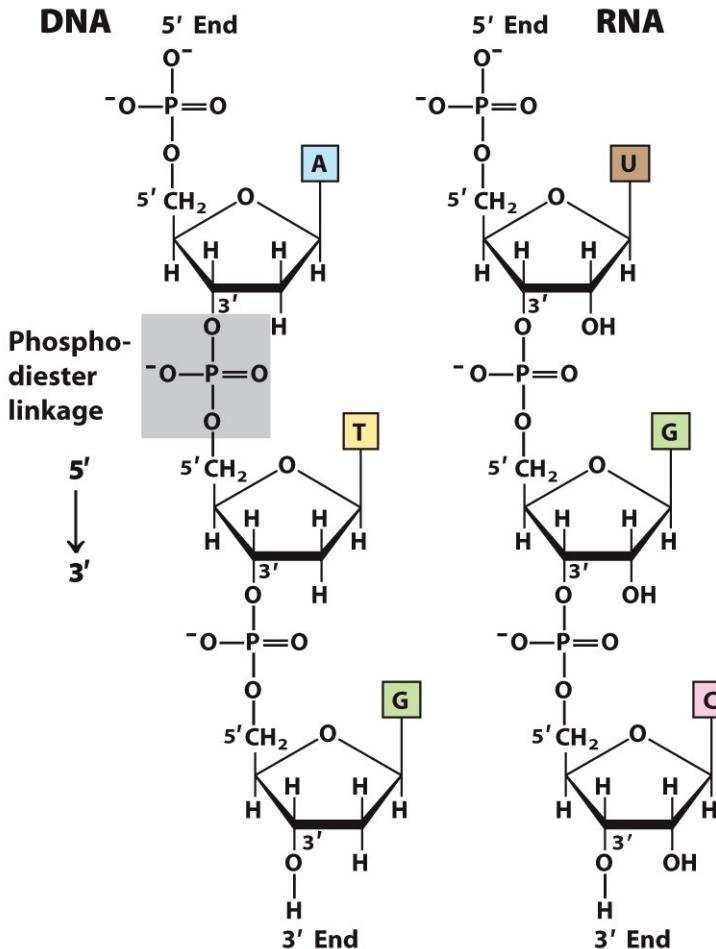


Figure 8-7
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Polynucleotides

- Linear polymers
 - no branching or cross-links
- Directionality
 - The 5' end is different from the 3' end.
 - We read the sequence from 5' to 3'

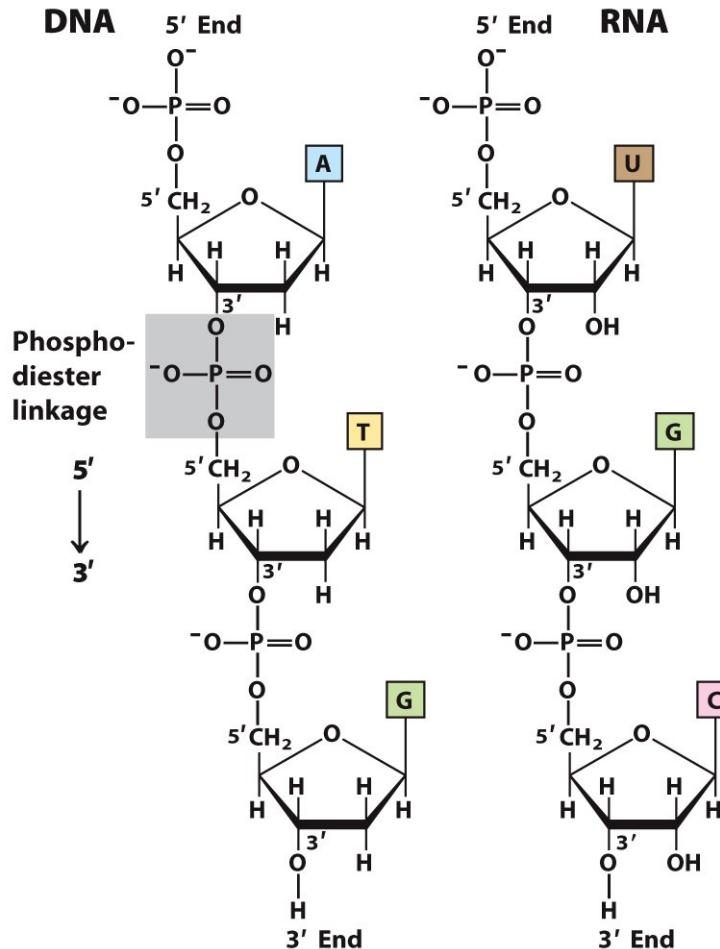


Figure 8-7
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Hydrolysis of RNA

- RNA is unstable under alkaline conditions.
- Hydrolysis is also catalyzed by enzymes (RNase).
- RNase enzymes are abundant around us.

Mechanism of Base-catalyzed RNA Hydrolysis

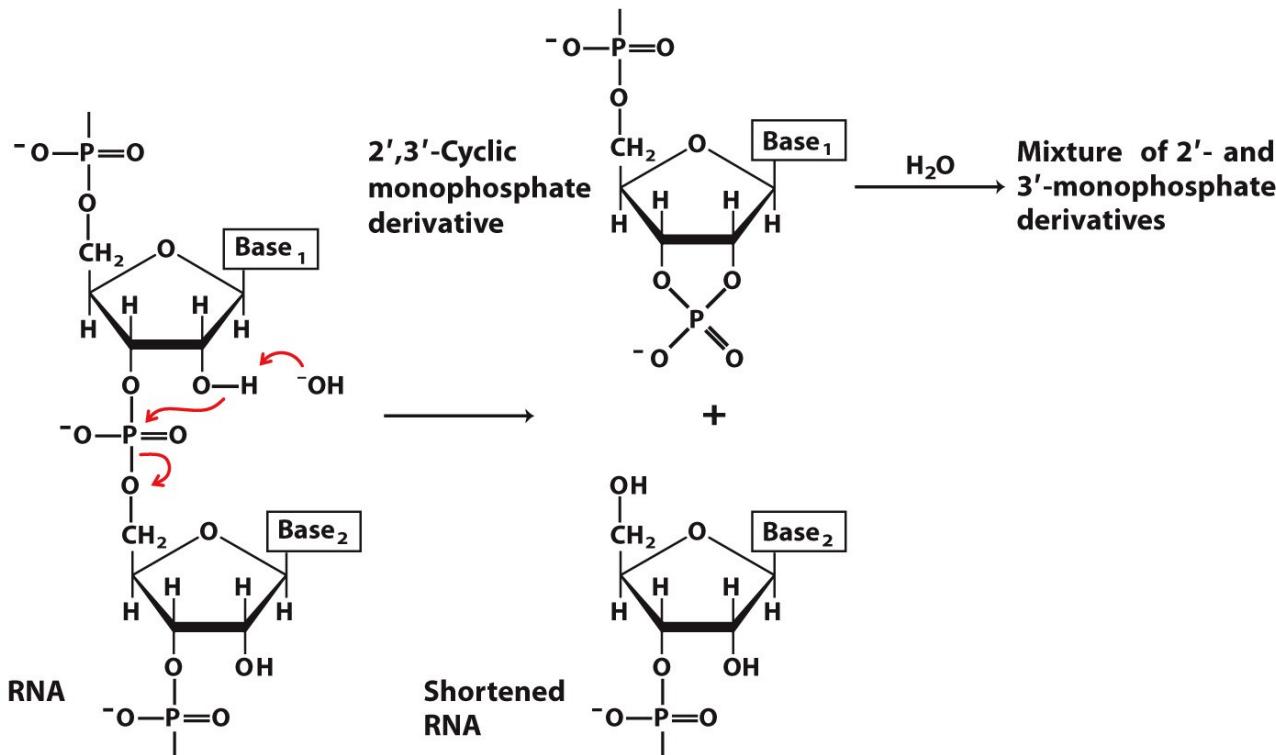
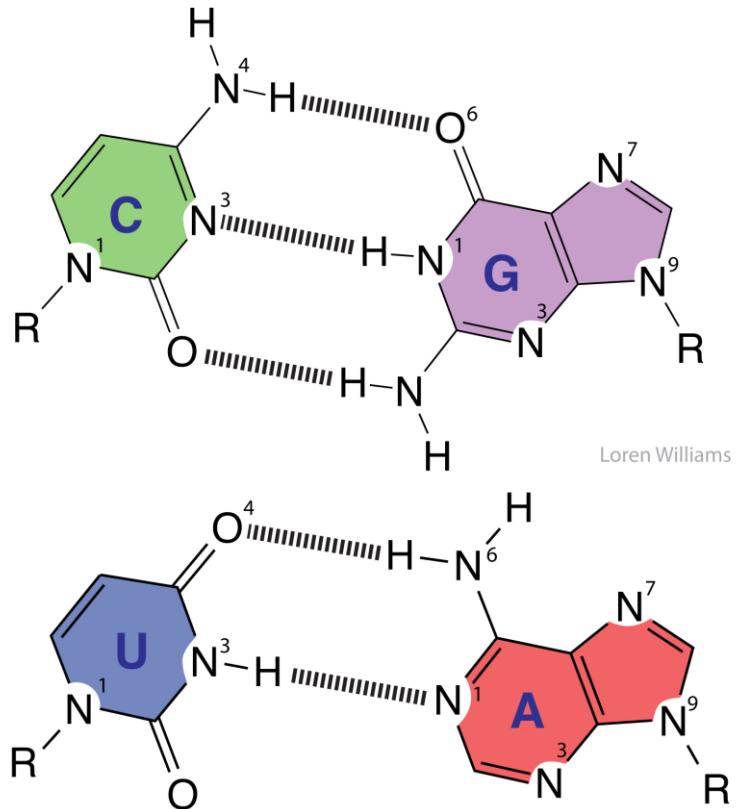


Figure 8-8
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Hydrogen-Bonding Interactions

- Two bases can hydrogen bond to form a base pair.
- For monomers, a large number of base pairs are possible.
- In DNA, only a few possibilities exist.
- Watson-Crick base pairs predominate in double-stranded DNA.
- A pairs with T.
- C pairs with G.
- Purine pairs with pyrimidine.

Base pairing



AT and GC Base Pairs

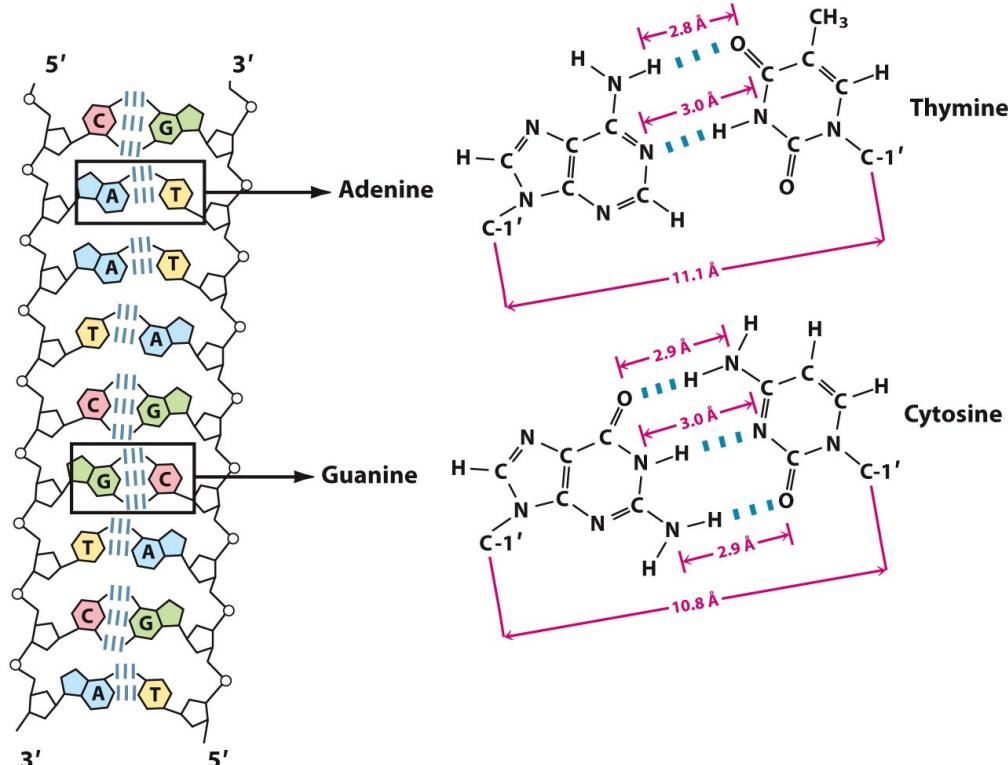


Figure 8-11

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Major and minor groove

major groove

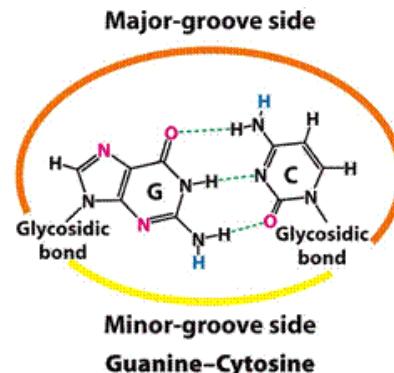
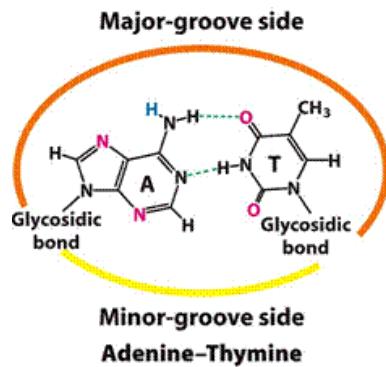
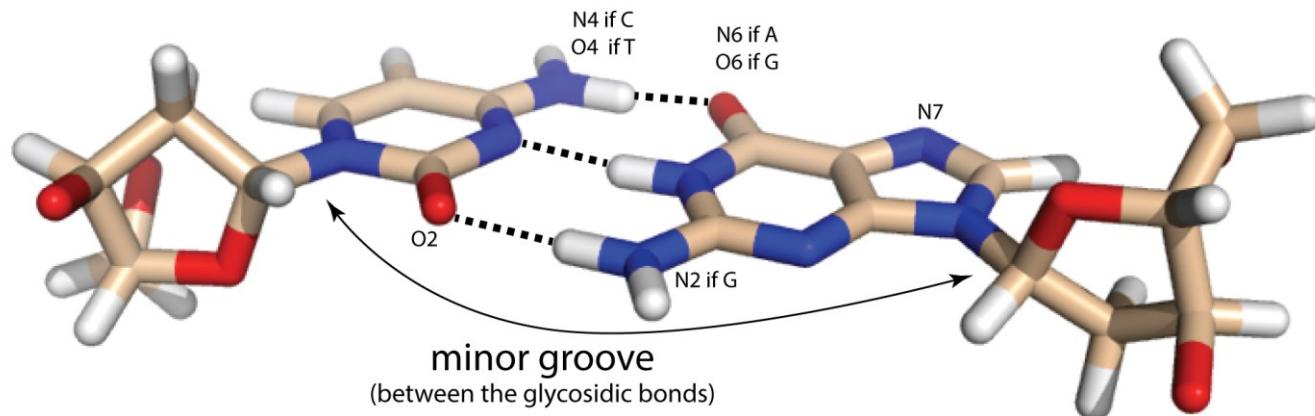
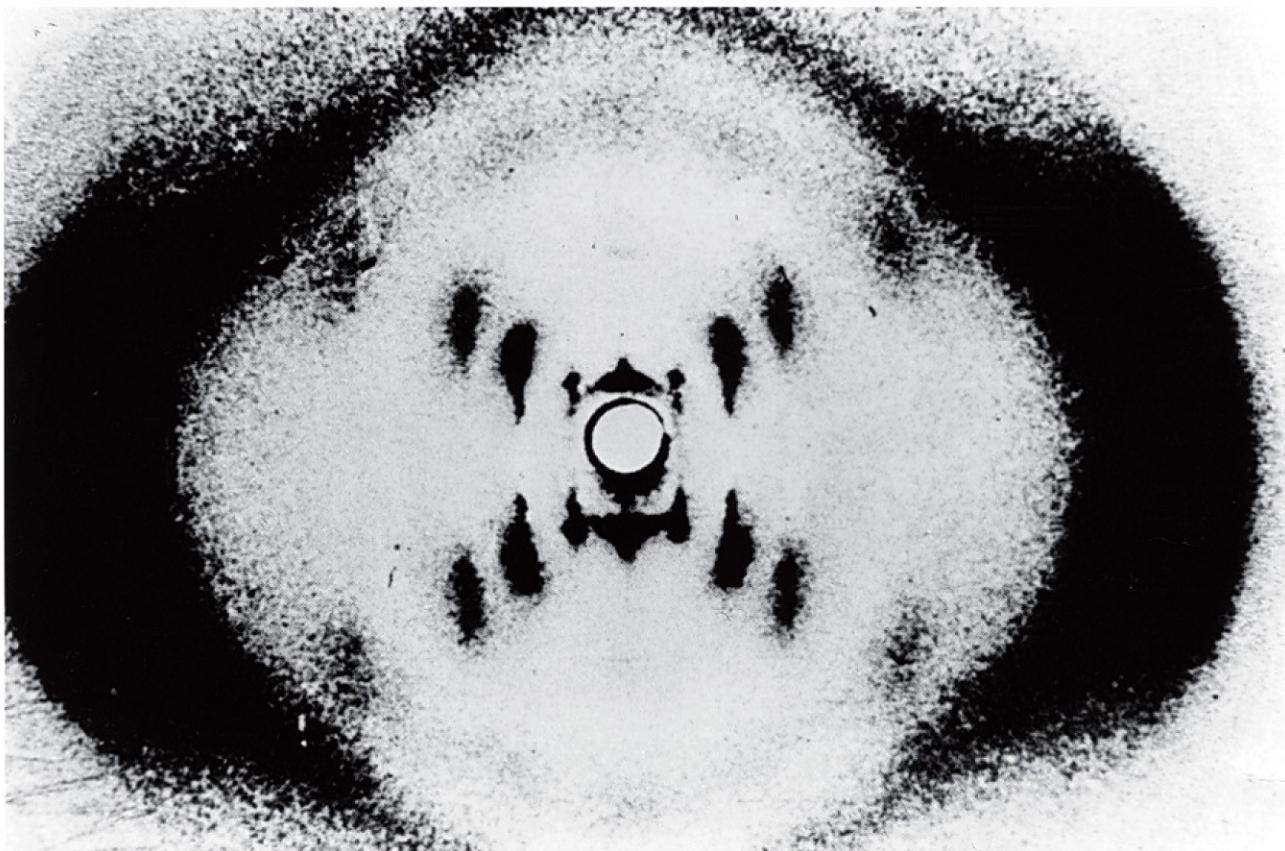


Figure 33.19
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Science Source

Figure 8-12
Lehninger Principles of Biochemistry, Seventh Edition
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James D. Watson

Unnumbered 8 p285

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**Francis Crick,
1916–2004**

UPI/Bettmann/Corbis



Science Source

Rosalind Franklin, 1920–1958

Unnumbered 8 p286

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UPI/Bettmann/Corbis

Maurice Wilkins, 1916–2004

Watson-Crick Model of B-DNA

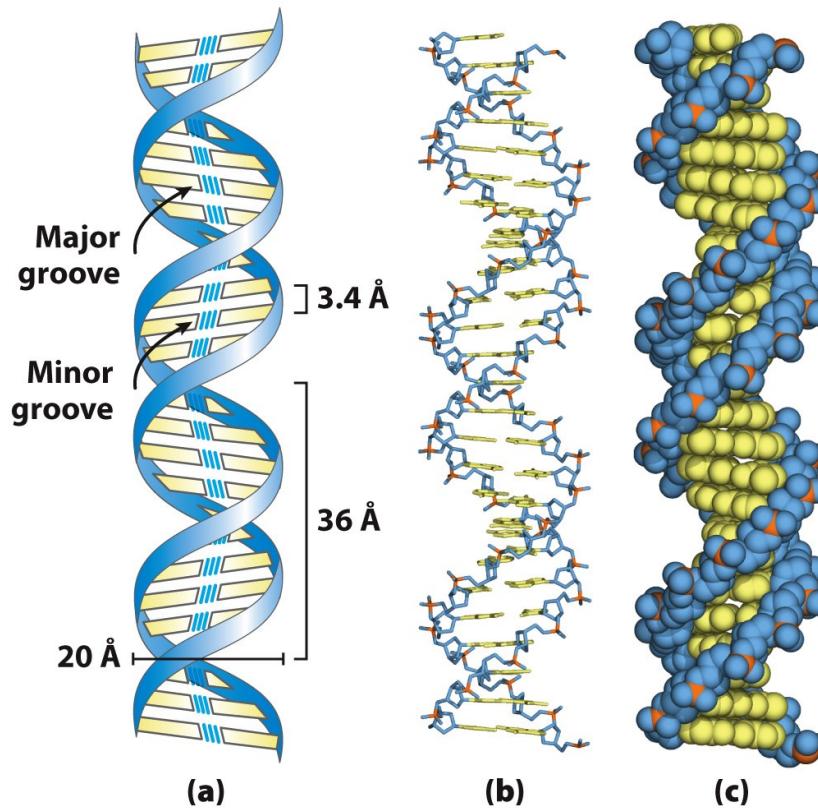


Figure 8-13
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Forms of DNA

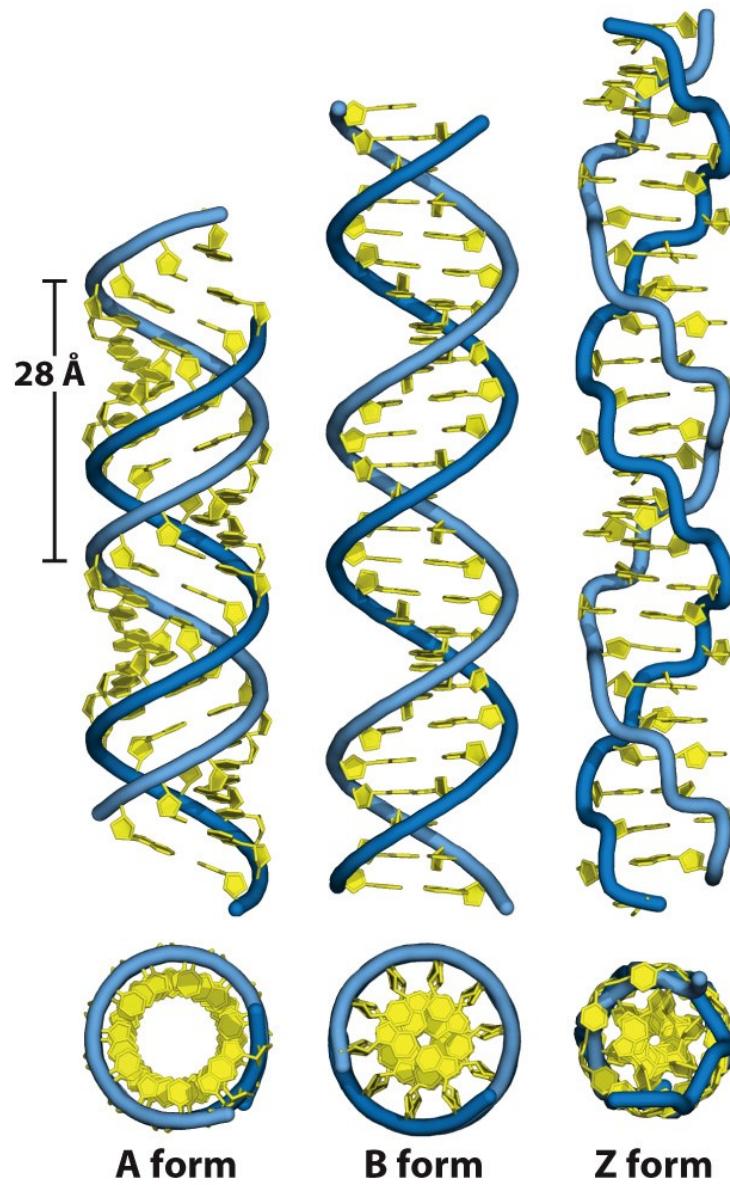
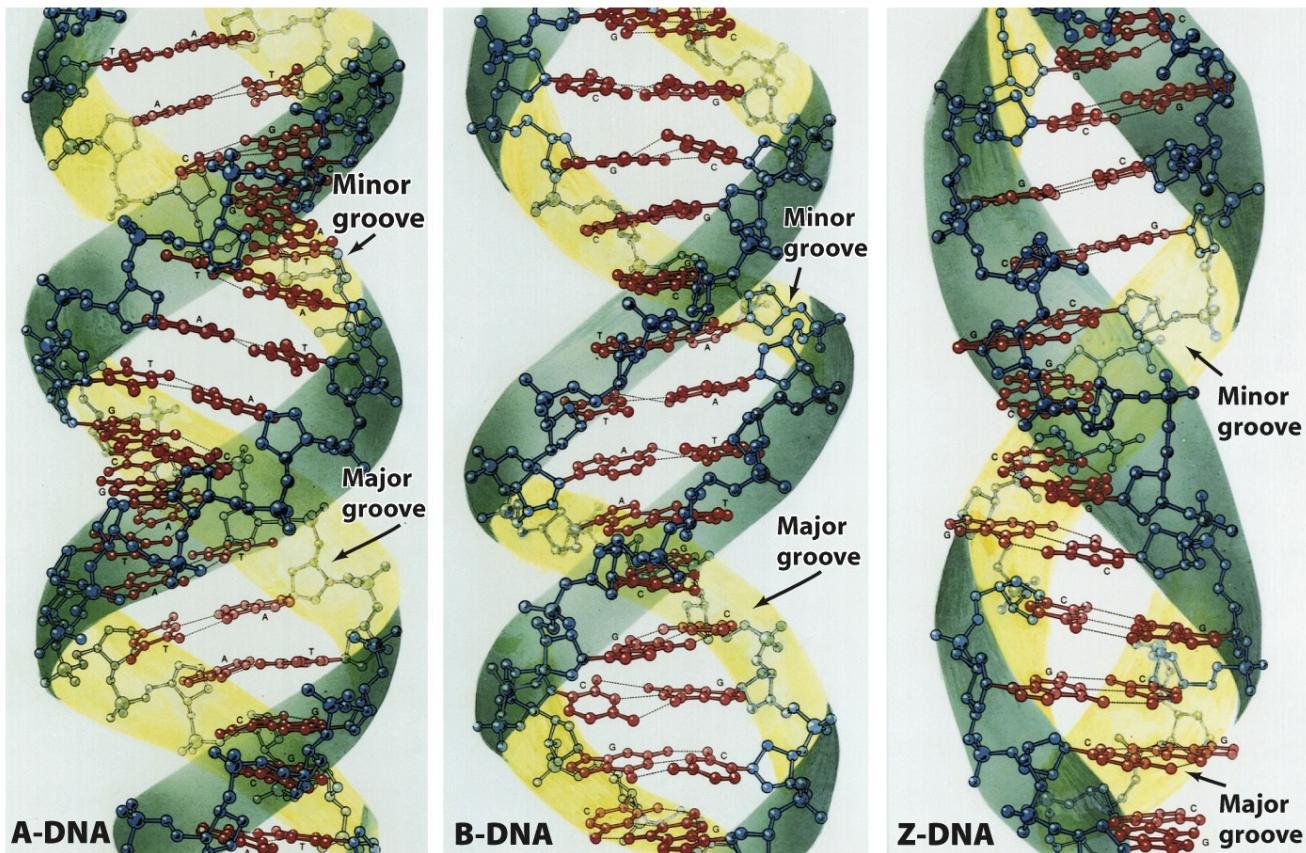


Figure 8-17 part 1
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Which helix is left handed?



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Figure 24-2a

Complementarity of DNA Strands

- Two chains differ in sequence (sequence is read from 5' to 3').
- Two chains are **complementary**.
- Two chains run antiparallel.

“It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.”

—Watson and Crick, *Nature*, 1953

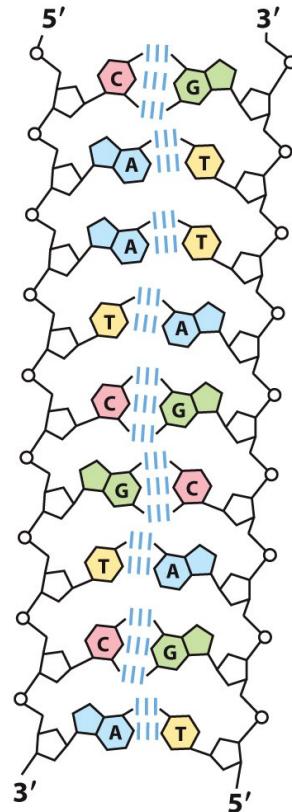


Figure 8-14
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equipment, and to Dr. G. E. R. Deacon and the captain and officers of R.R.S. *Discovery II* for their part in making the observations.

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³ Von Arx, W. S., Woods Hole Papers in Phys. Oceanogr. Meteorol., **13** (1956).

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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 Corey¹. They kindly made their manuscript available to us in advance of publication. Their model consists of two inter-twined 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.

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-deoxyribonose 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 model No. 1; that is, the bases are on the insides of the coils 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 Å. 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 along each chain, that is, after 34 Å. The distance of a phosphorus atom from the fibre axis is 10 Å. As the phosphates are on the outside, cations have easy access to them.

The structure is an open one, and its water content is rather high. At lower water contents we would expect the bases to tilt so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases. The planes of the bases are perpendicular to the fibre axis. They are joined together in pairs, a single base from one chain being hydrogen-bonded to a single base from the other chain, so that the two lie side by side with identical co-ordinates. One of the pair must be a purine and the other a pyrimidine for bonding to occur. The hydrogen bonds are made as follows: purine position 1 to pyrimidine position 1; purine position 6 to pyrimidine position 2.

If it is assumed that the bases only occur in the structure in the most plausible tautomeric forms (that is, with the keto rather than the enol configurations) it is found that only specific pairs of bases can bond together. These pairs are: adenine (purine) with thymine (pyrimidine), and guanine (purine) with cytosine (pyrimidine).

In other words, if an adenine forms one member of a pair, on either chain, then on these assumptions the other member must be thymine; similarly for guanine and cytosine.

It has been found that the amount of guanine to thymine in DNA is approximately 1.0.

It is extremely impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atom would make too close a van der Waals contact.

The previously published X-ray data^{2,3} on deoxyribose nucleic acid are insufficient for a rigorous test of our structure. So far as we can tell, it is roughly compatible with the experimental data, but it must be regarded as unproved until it has been checked against more exact results. Some of these are given in the following communications. We were not aware of the details of the results presented there when we devised our structure, which rests mainly though not entirely on published experimental data and stereochemical arguments.

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material. 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



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

King's College, London. One of us (J. D. W.) has been aided by a fellowship from the National Foundation for Infantile Paralysis.

J. D. WATSON

F. H. C. CRICK

Medical Research Council Unit for the Study of the Molecular Structure of Biological Systems, Cavendish Laboratory, Cambridge. April 2.

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Molecular Structure of Deoxypentose Nucleic Acids

WHILE the biological properties of deoxypentose nucleic acid suggest a molecular structure containing great complexity, X-ray diffraction studies described here (cf. Astbury¹) show the basic molecular configuration has great simplicity. The purpose of this communication is to describe, in a preliminary way, some of the experimental evidence for the polynucleotide chain configuration being helical, and existing in this form when in the natural state. A

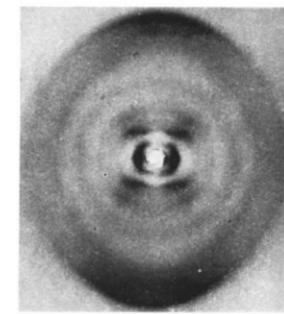


Fig. 1. Fibre diagram of deoxypentose nucleic acid from *E. coli*. Fibre axis vertical.

the innermost maxima of each Bessel function and the origin. The angle this line makes with the equator is roughly equal to the angle between an element of the helix and the helix axis. If a unit repeats n times along the helix there will be a meridional reflexion (J_n) on the nth layer line. The helical configuration

frequency, y distribution, n, on the nth

n terms some

repeating unit

First, if the helical symmetry is, the whole form factor of de consists of a series of points on a radius at right-angles to the helix axis, the phases of radiation scattered by the helices of different diameter passing through each point are the same. Summation of the corresponding Bessel functions gives reinforcement for the inner-

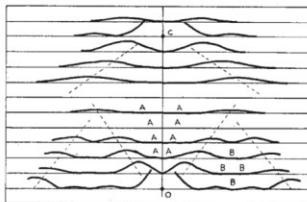


Fig. 2. Diffraction pattern of system of helices corresponding to structure of deoxypentose nucleic acid. The square of Bessel functions are plotted above 0 on the equator, and on the first, second, third and fifth layer lines for half of the nucleotide mass at 20 Å. diameter. The helices are distributed along a radius, the mean of a given radius being proportional to the angle. Above C on the tenth layer line similar functions are plotted for an outer diameter of 12 Å.

Summary

Nucleotides →

- Bases, sugar, phosphate
- Nomenclature

Polynucleotides →

- Phosphodiester bond
- Hydrolysis
- Base-pairing, major and minor groove

Structure →

- Double helix
- A, B and Z forms of DNA