Reaction Kinetics

- Enzyme kinetics: Study of the rates of enzyme-catalyzed reaction
- What are the uses of studying kinetics?

What we will cover:

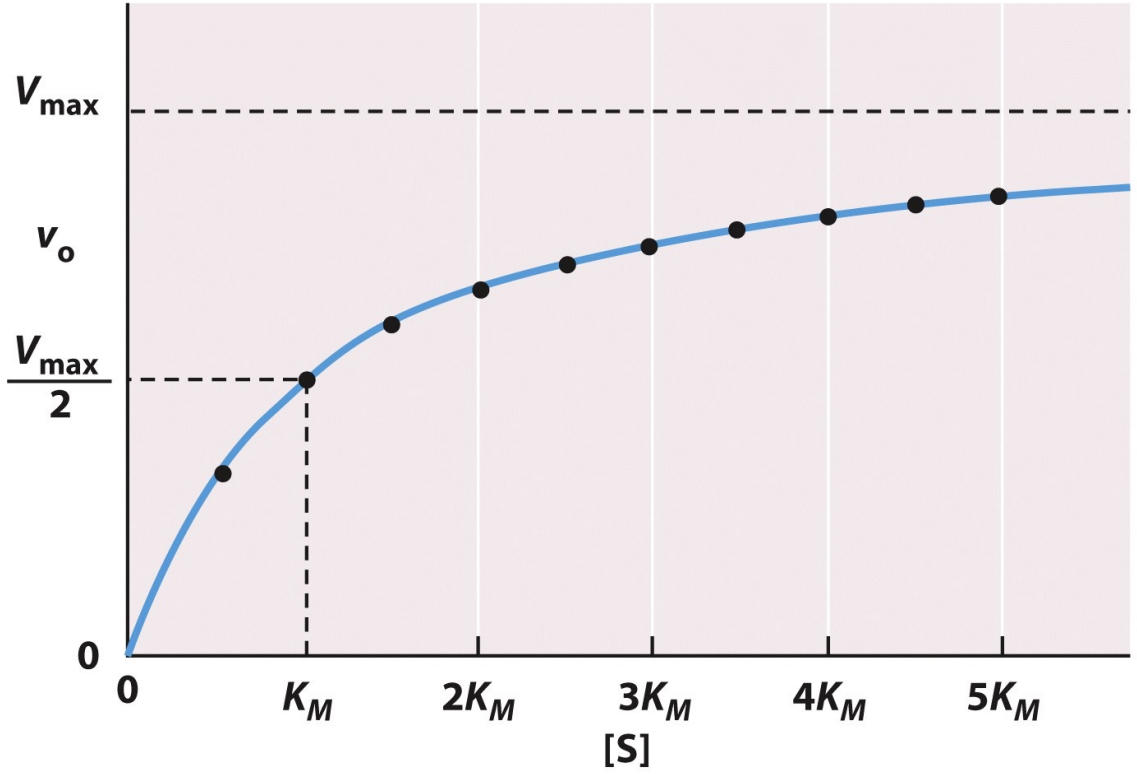
1. Kinetics
2. Inhibition
3. Regulation

Michaelis-Menten Kinetics

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

Three assumptions

1. ES is a necessary intermediate step
2. k_{-2} is negligible due to small [P]
3. Steady state: [ES] is a constant independent on [S] or [P]



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M-M equation

1. Finding K_M

$[E][S]/[ES] = (k_{-1} + k_2)/k_1$

2. Introduce $[E]_T$

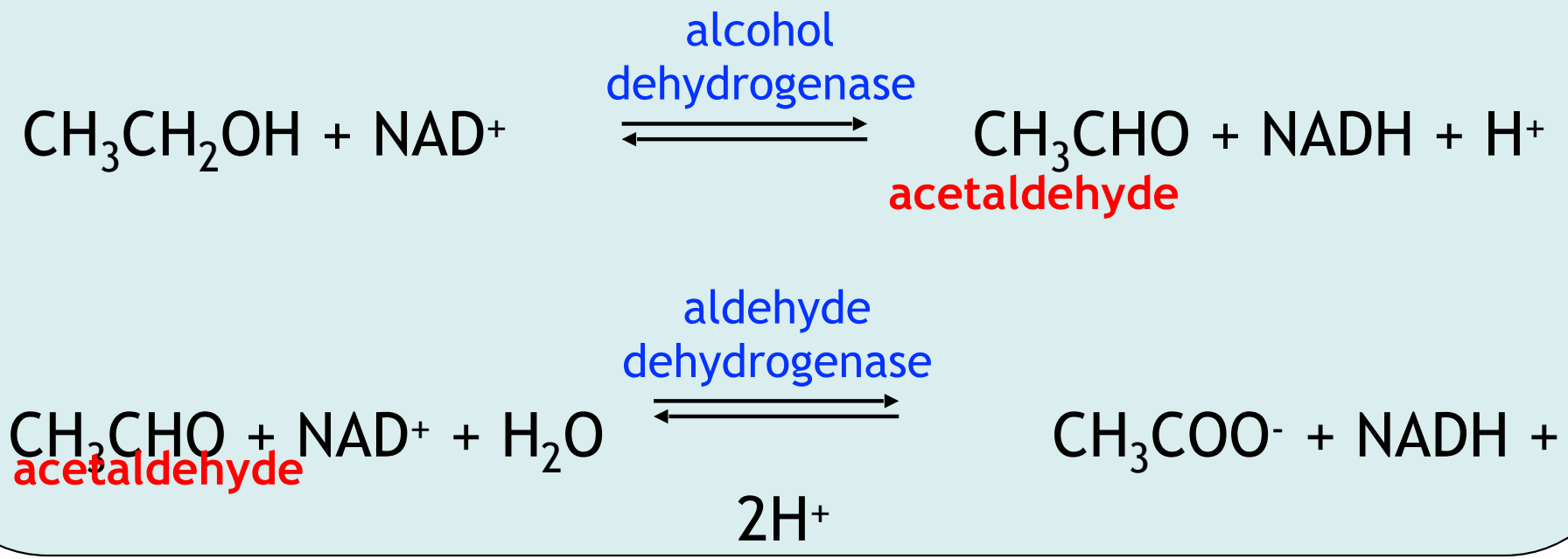
$[E] = [E]_T - [ES]$

3. Introduce $V_0 = k_2[ES]$

4. Find V_{max} when [ES] = $[E]_T$

Meaning of K_M

The physiological consequence of K_M in alcohol sensitivity



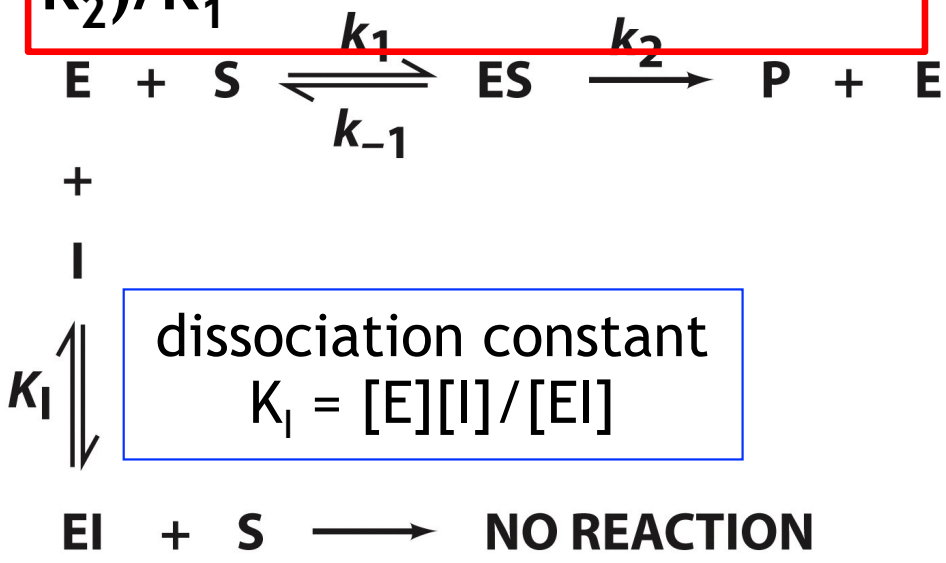
There are two forms of the AD: a low K_M mitochondrial form and a high K_M cytoplasmic form. What happen to the susceptible people?

$$K_M = [E][S]/[ES] = (k_{-1} + k_2)/k_1$$

Competitive Enzyme Inhibition

- Inhibitors mimic the substrate: compete for the same site.
- The inhibition can be overcome by adding more substrate.
- Often act as drugs: e.g. Statins

$K_M = [E][S]/[ES] = (k_{-1} + k_2)/k_1$



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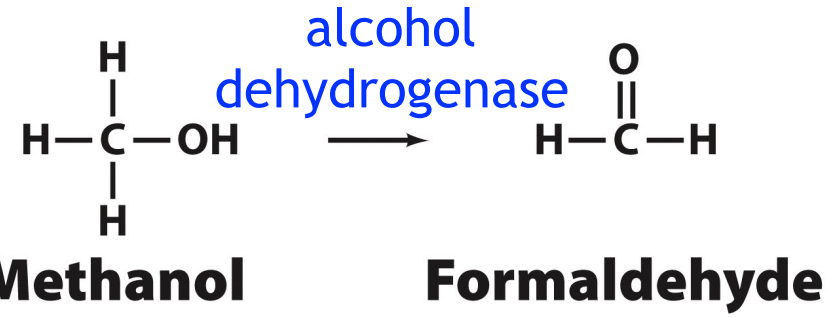
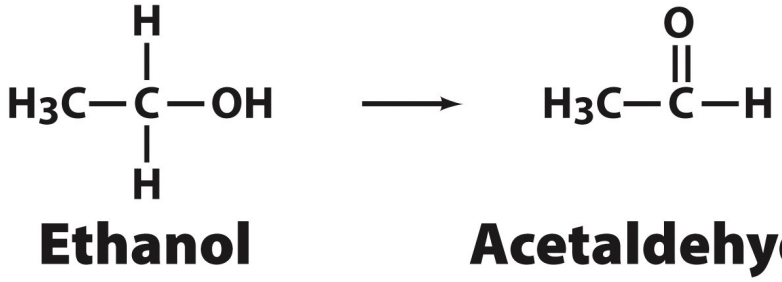
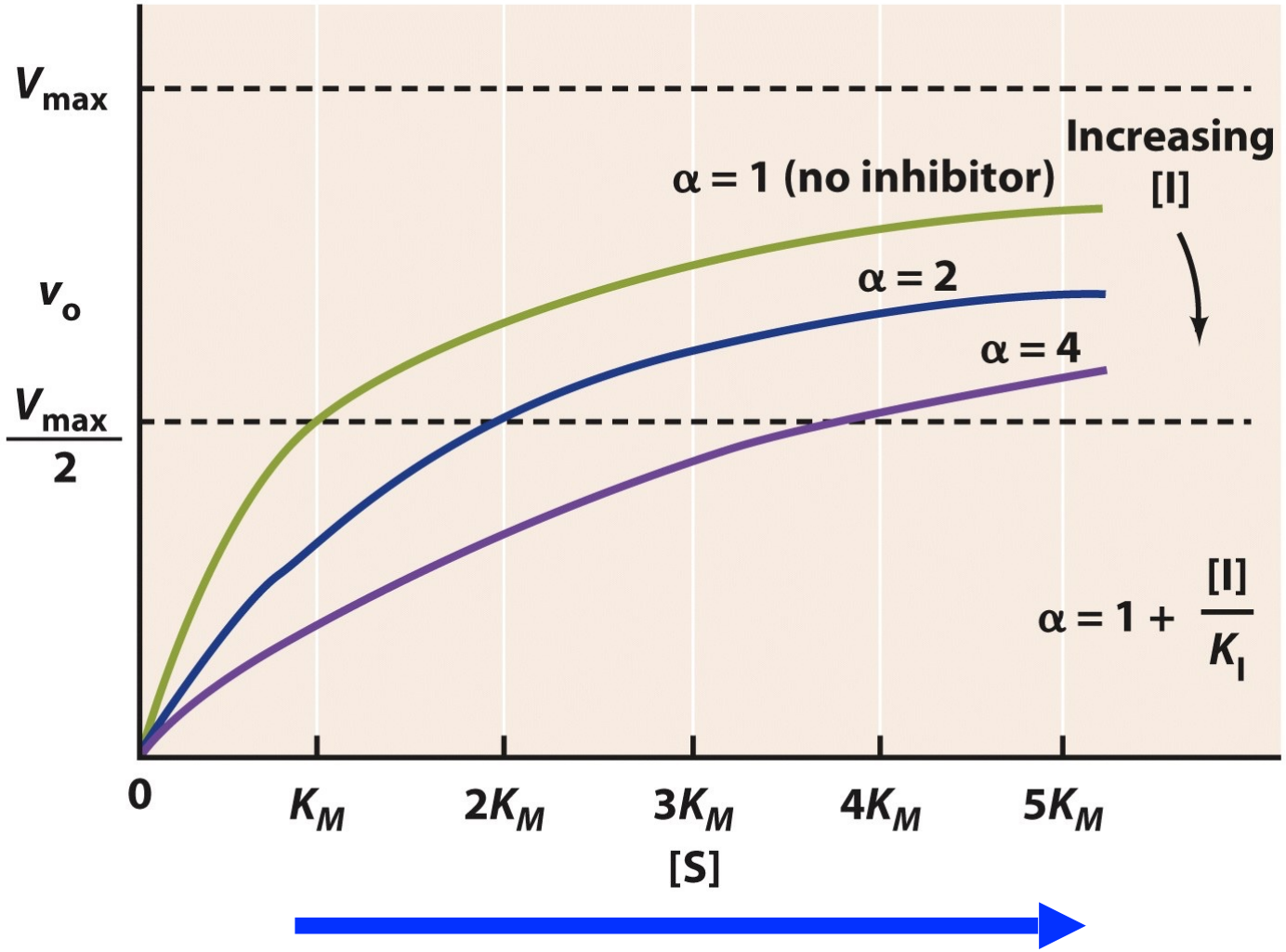
methanol poisoning





Competitive Enzyme Inhibition



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more inhibitors ▸ increases K_M

More substrate is needed to obtain the same reaction rate.

The apparent $K_M = K_M(1+[I]/K_I)$

Competitive Enzyme Inhibition

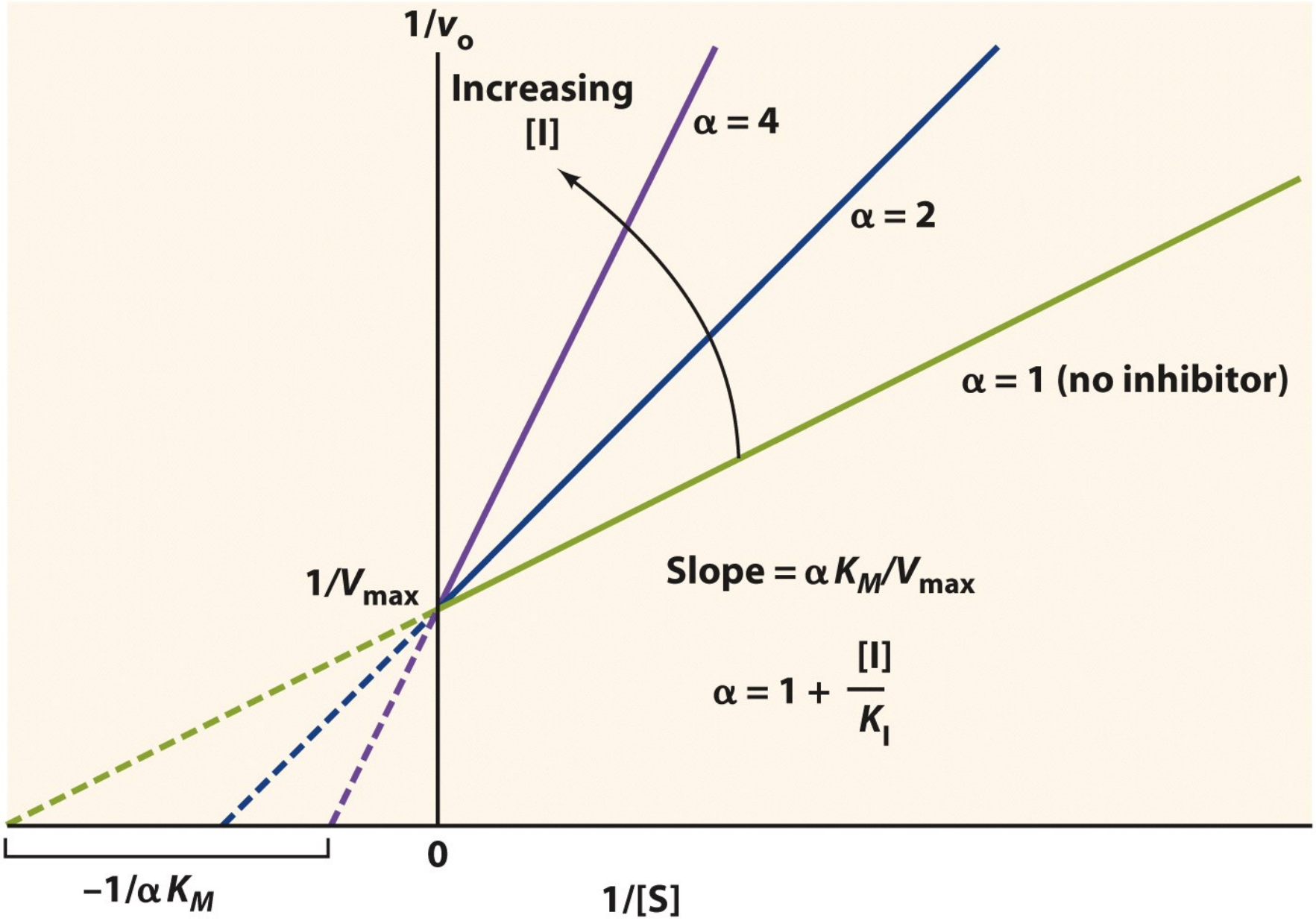

Figure 12-8

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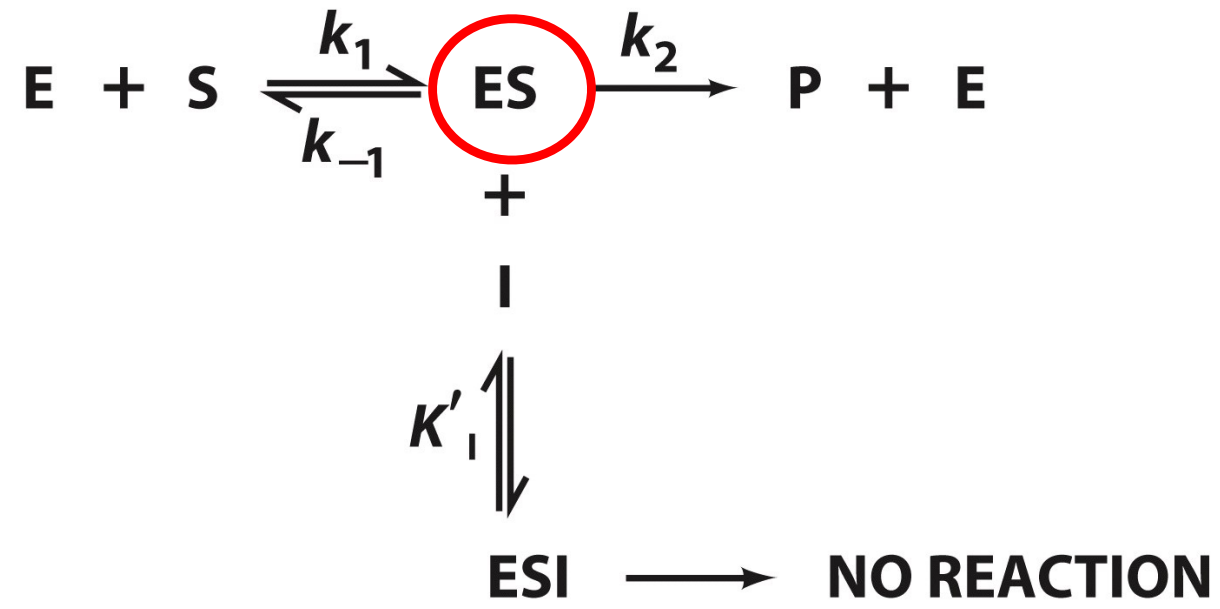
Uncompetitive Enzyme Inhibition



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ESI: Enzyme-Substrate-Inhibitor complex: a certain portion of ESI always exists, thus decreases V_{max} – as if some enzymes are kidnapped in ES form!
What would happen to K_M?

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

$$K_M = (k_{-1} + k_2)/k_1$$

Uncompetitive Enzyme Inhibition

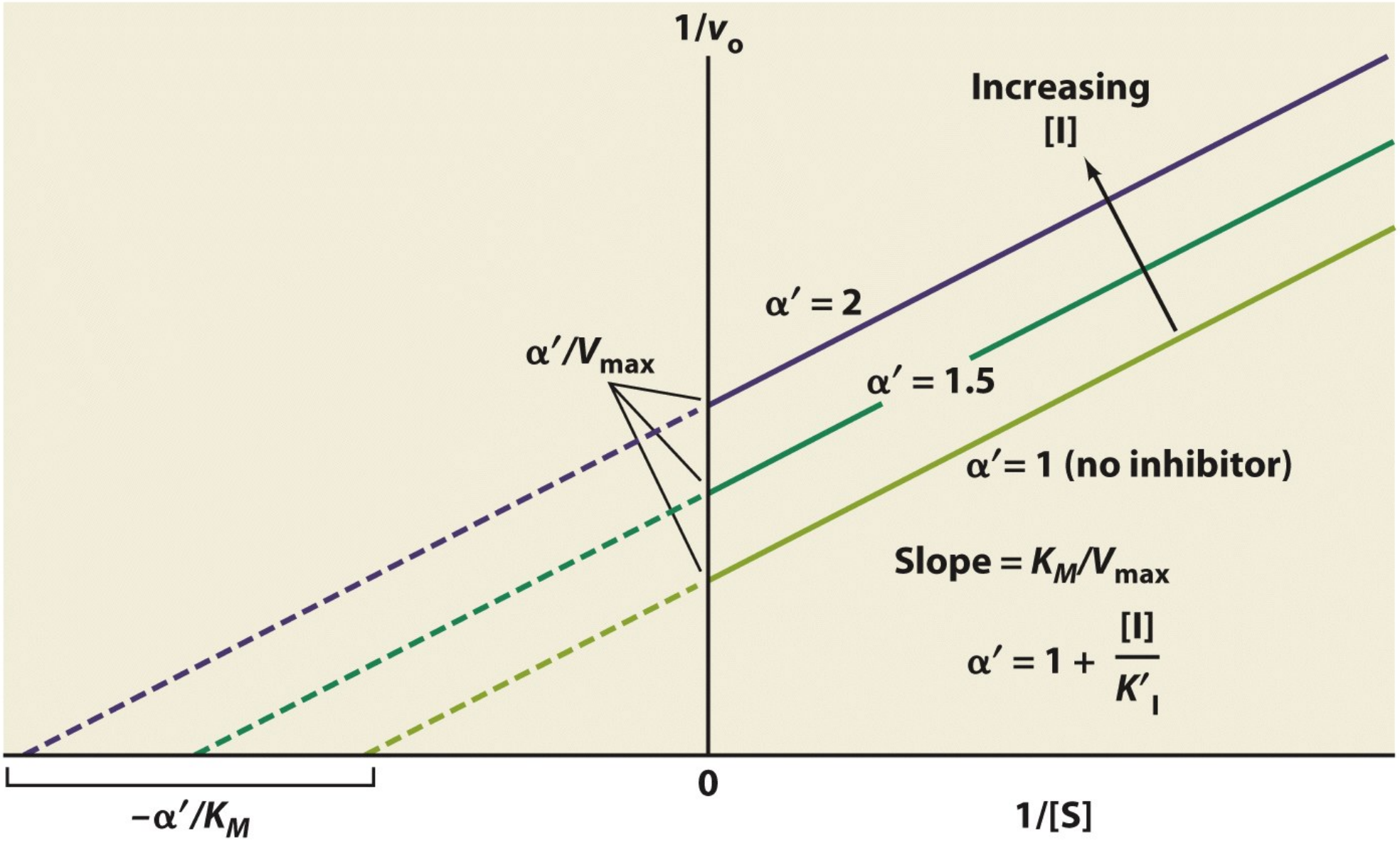

Figure 12-9

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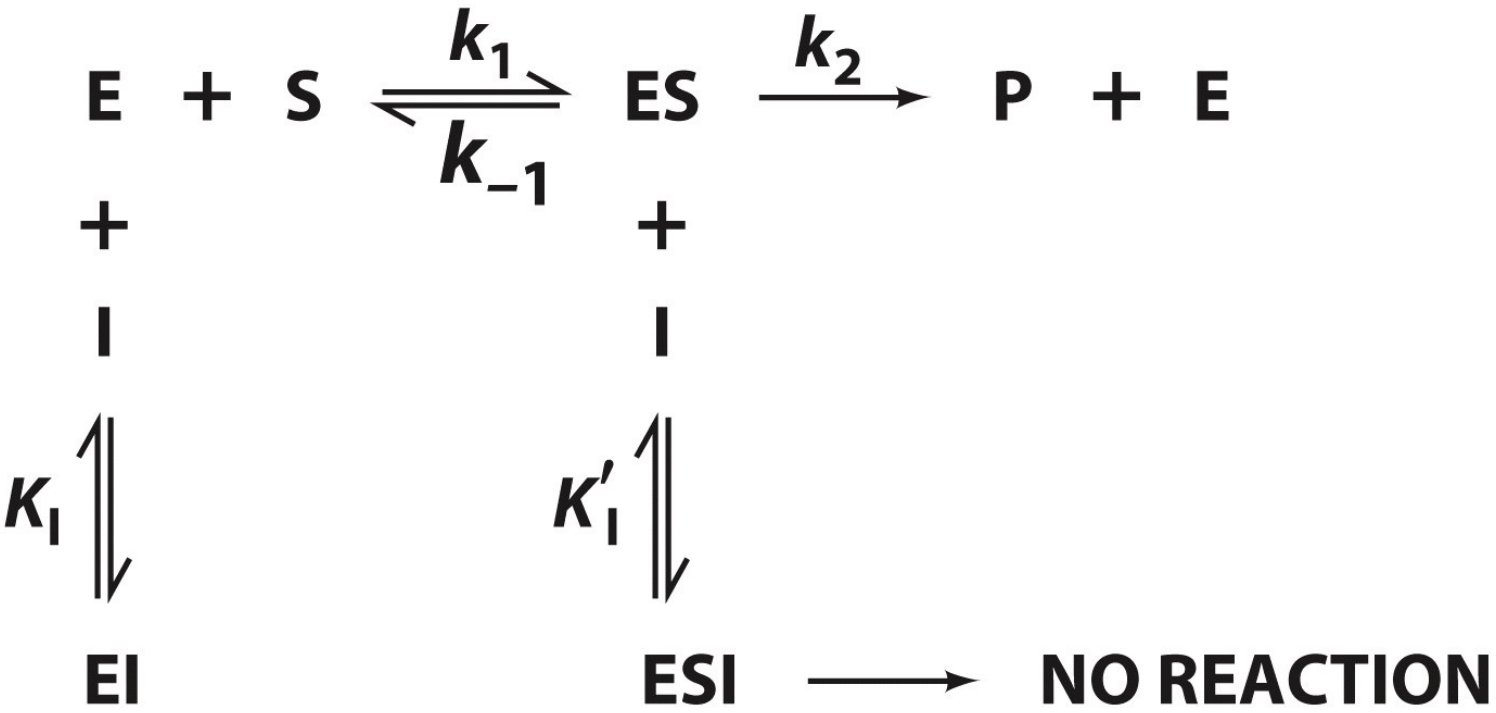
Mixed and Noncompetitive Enzyme Inhibition



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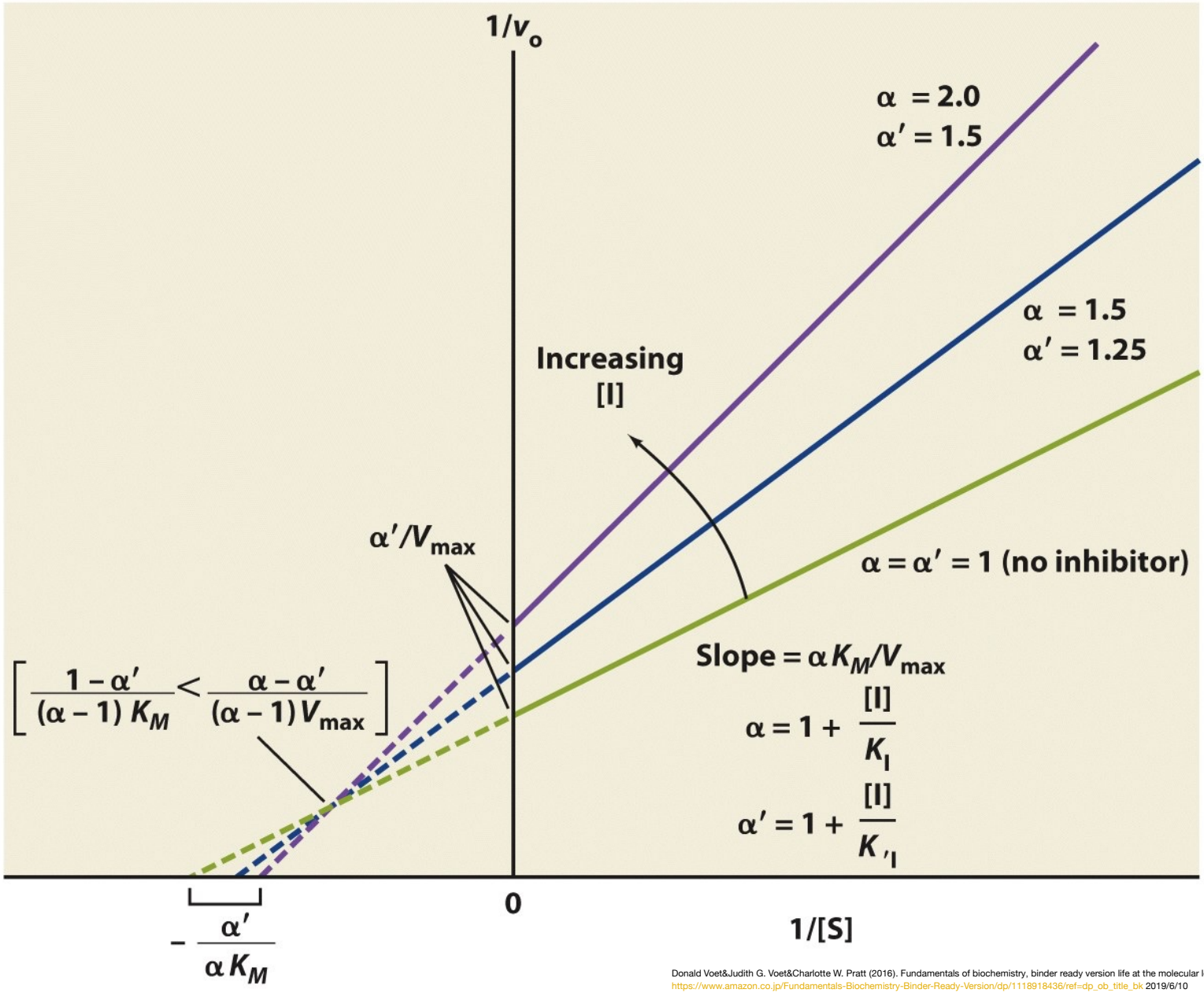
Mixed and Noncompetitive Enzyme Inhibition



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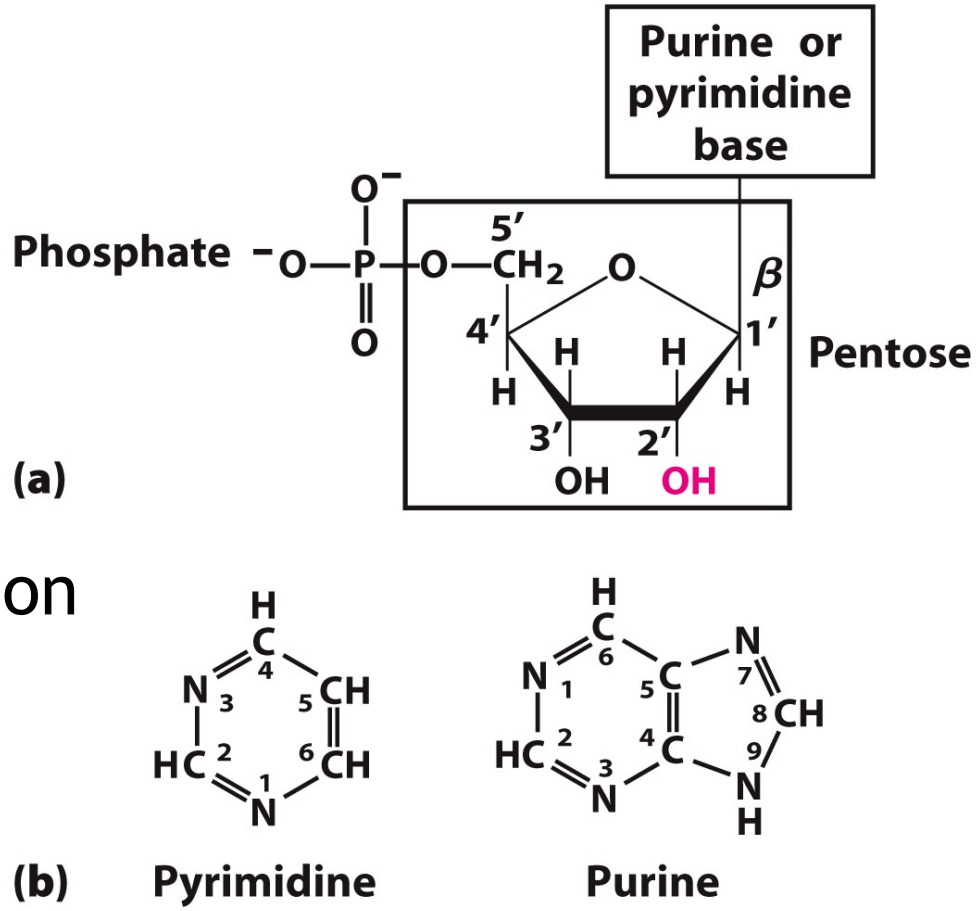
CHAPTER 8
Nucleotides and Nucleic Acids

- Nucleic acids are polymers of nucleotides used for:
  - storage of genetic info (DNA)
  - transmission of genetic info (mRNA)
  - processing of genetic information (ribozymes)
  - protein synthesis (tRNA and rRNA)
- Nucleotides are also used in the monomer form for cellular functions:
  - energy for metabolism (ATP)
  - enzyme cofactors (NAD^+)
  - signal transduction (cAMP)

Nucleotides and Nucleosides

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



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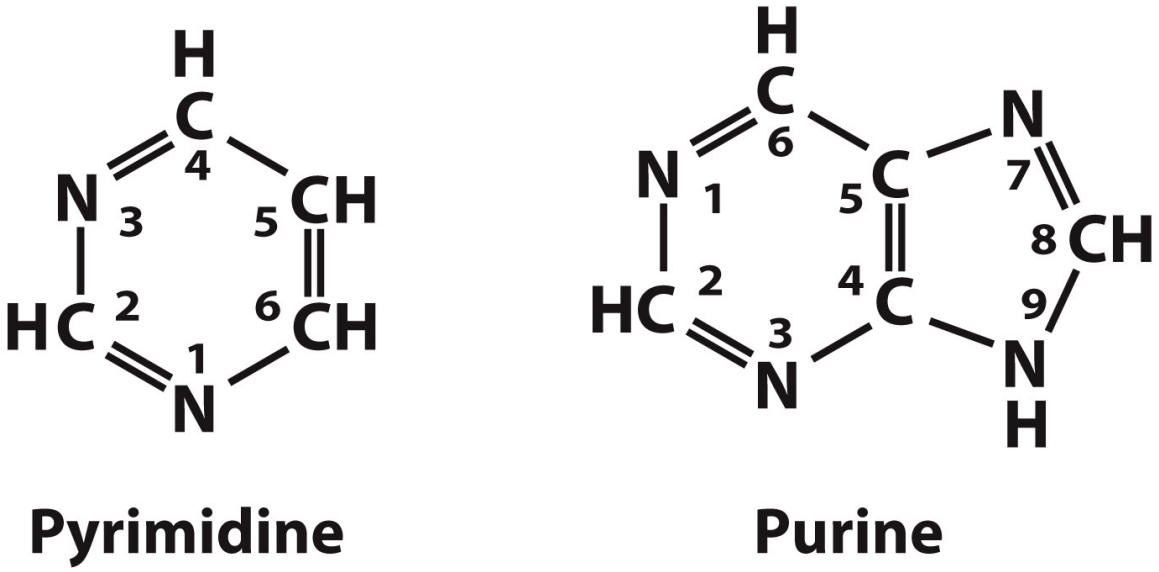
Phosphate Group

- 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

Nitrogenous Bases

- Derivatives of pyrimidine or purine
- Nitrogen-containing heteroaromatic molecules
- Planar or almost planar structures
- Absorb UV light around 250–270 nm



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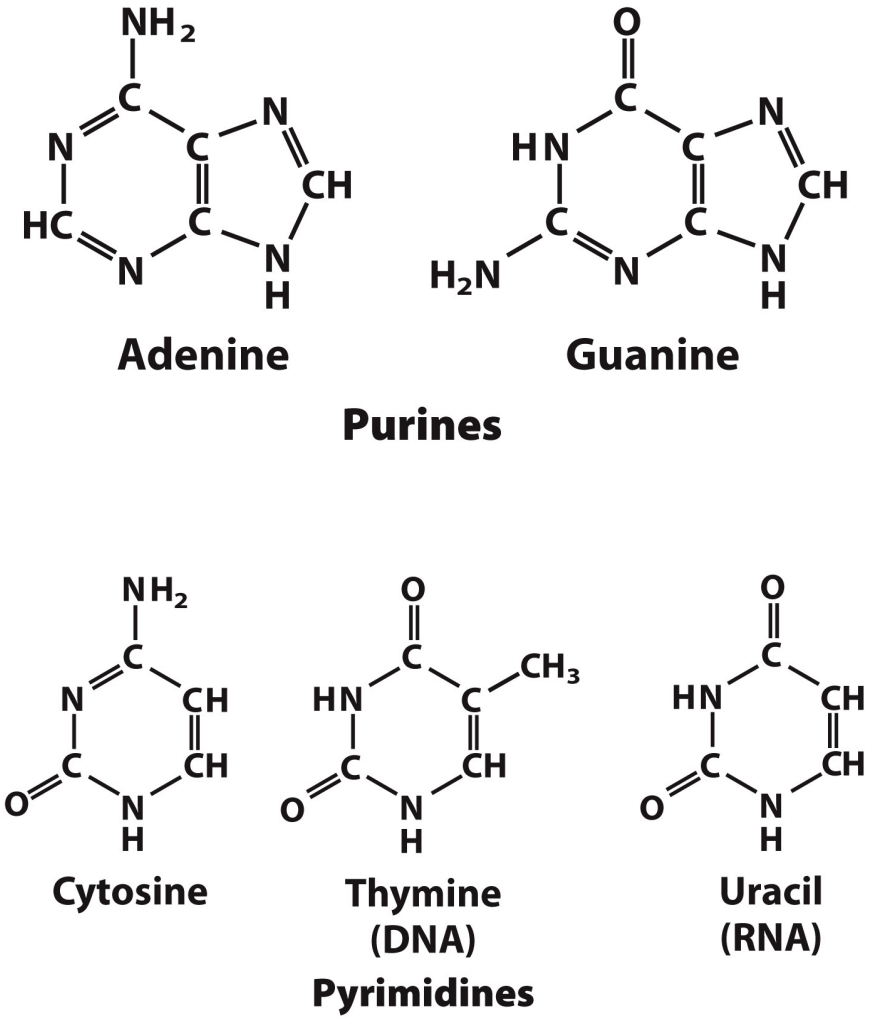
Nitrogenous Bases

- 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



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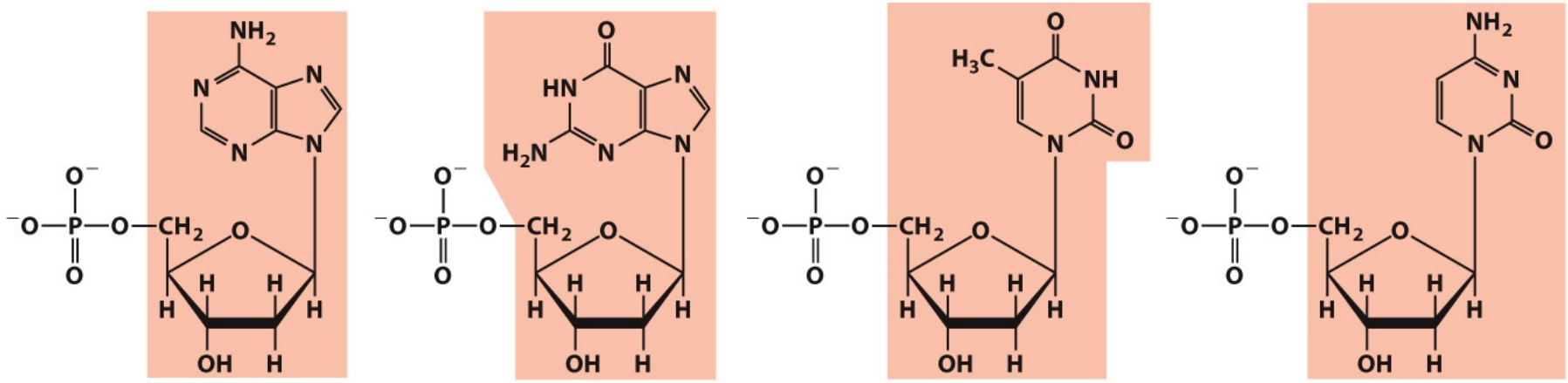
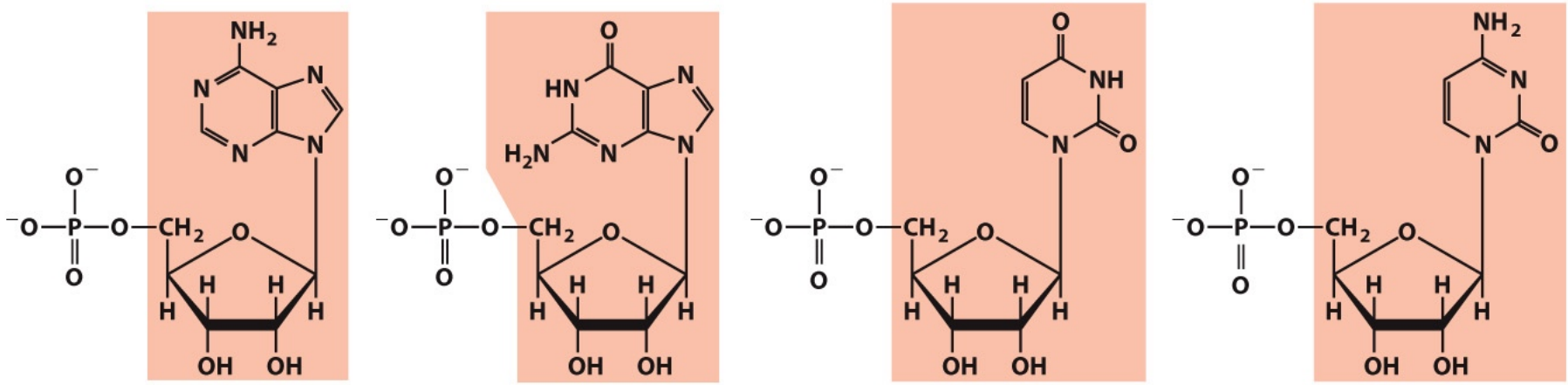
Nomenclature



Nucleotide:	Deoxyadenylate (deoxyadenosine 5′-monophosphate)	Deoxyguanylate (deoxyguanosine 5′-monophosphate)	Deoxythymidylate (deoxythymidine 5′-monophosphate)	Deoxycytidylate (deoxycytidine 5′-monophosphate)
Symbols:	A, dA, dAMP	G, dG, dGMP	T, dT, dTMP	C, dC, dCMP
Nucleoside:	Deoxyadenosine	Deoxyguanosine	Deoxythymidine	Deoxycytidine

Deoxyribonucleotides



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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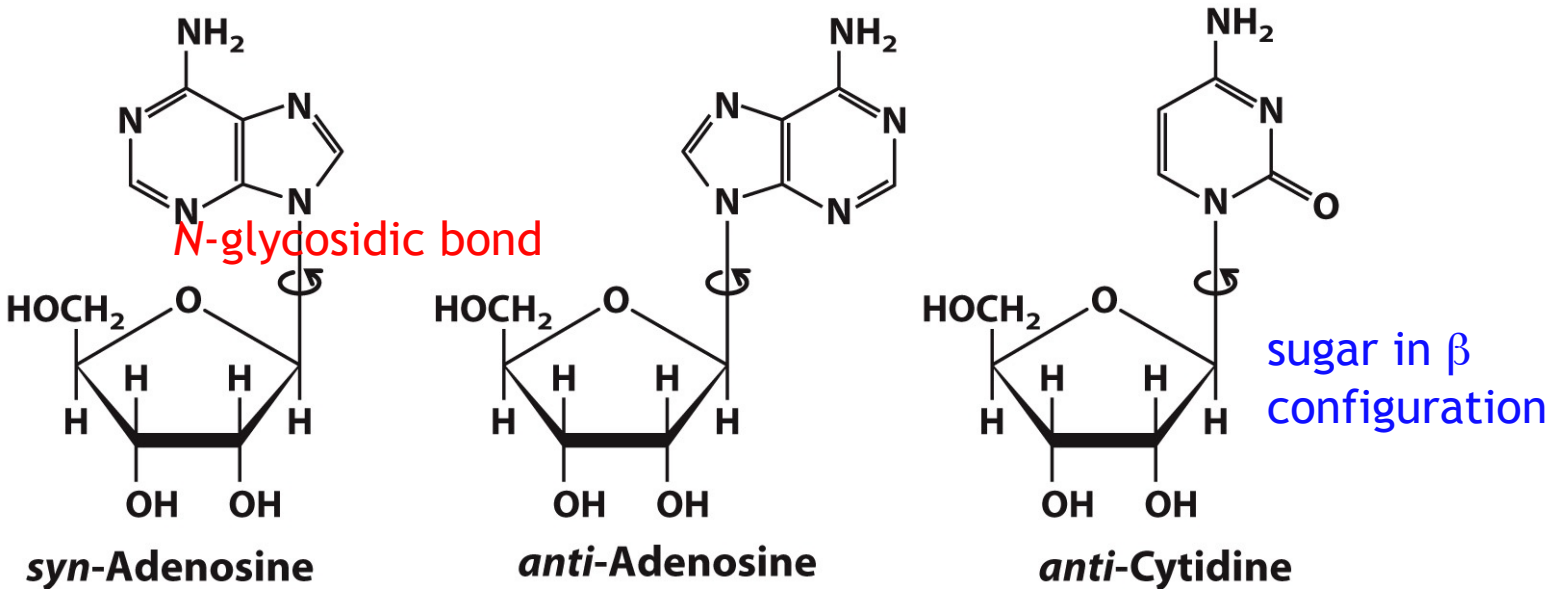
Conformation around *N*-Glycosidic Bond

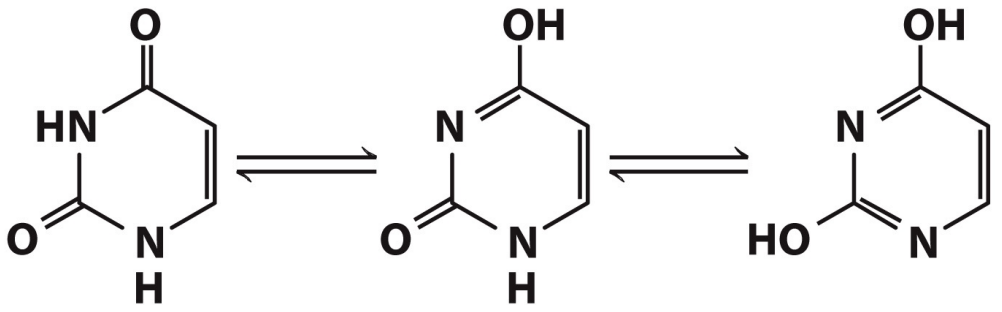
- Relatively free rotation can occur around the *N*-glycosidic bond in free nucleotides.



- Angle near 0° corresponds to *syn* conformation.
- Angle near 180° corresponds to *anti* conformation.
- Anticonformation is found in normal B-DNA.

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.



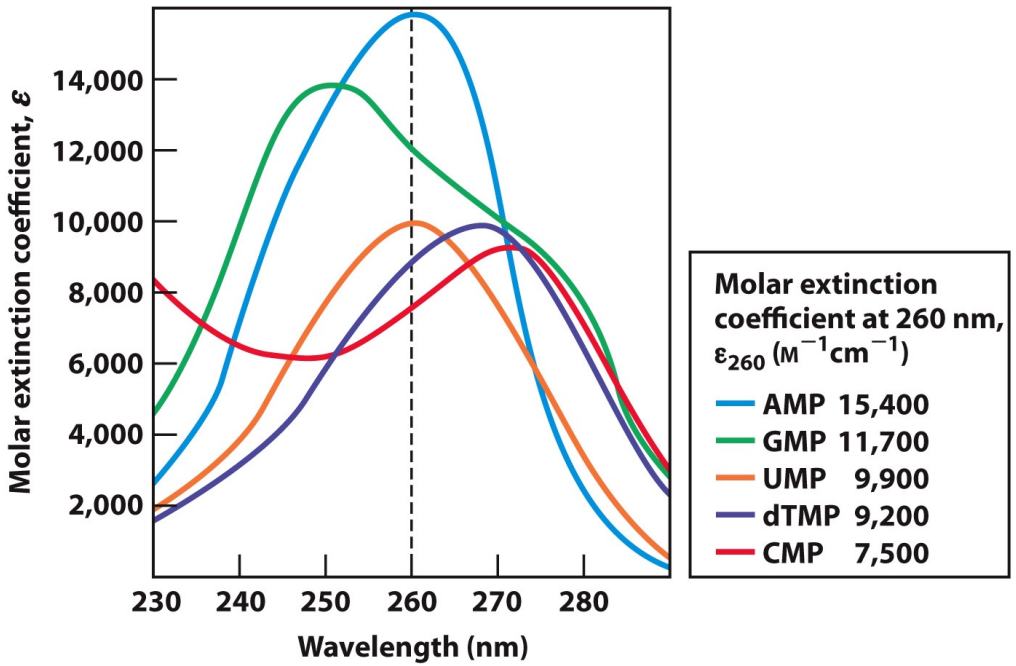
Lactam ⇌ Lactim ⇌ Double lactim

Uracil

Tautomeric forms of uracil. The lactam form predominates at pH 7.0; the other forms become more prominent as pH decreases. The other free pyrimidines and the free purines also have tautomeric forms, but they are more rarely encountered.

UV Absorption of Nucleobases

- Absorption of UV light at 250–270 nm is due to $\pi \rightarrow \pi^*$ electronic transitions.
- Excited states of common nucleobases decay rapidly via radiationless transitions.
  - effective photoprotection of genetic material
  - no fluorescence from nucleic acids



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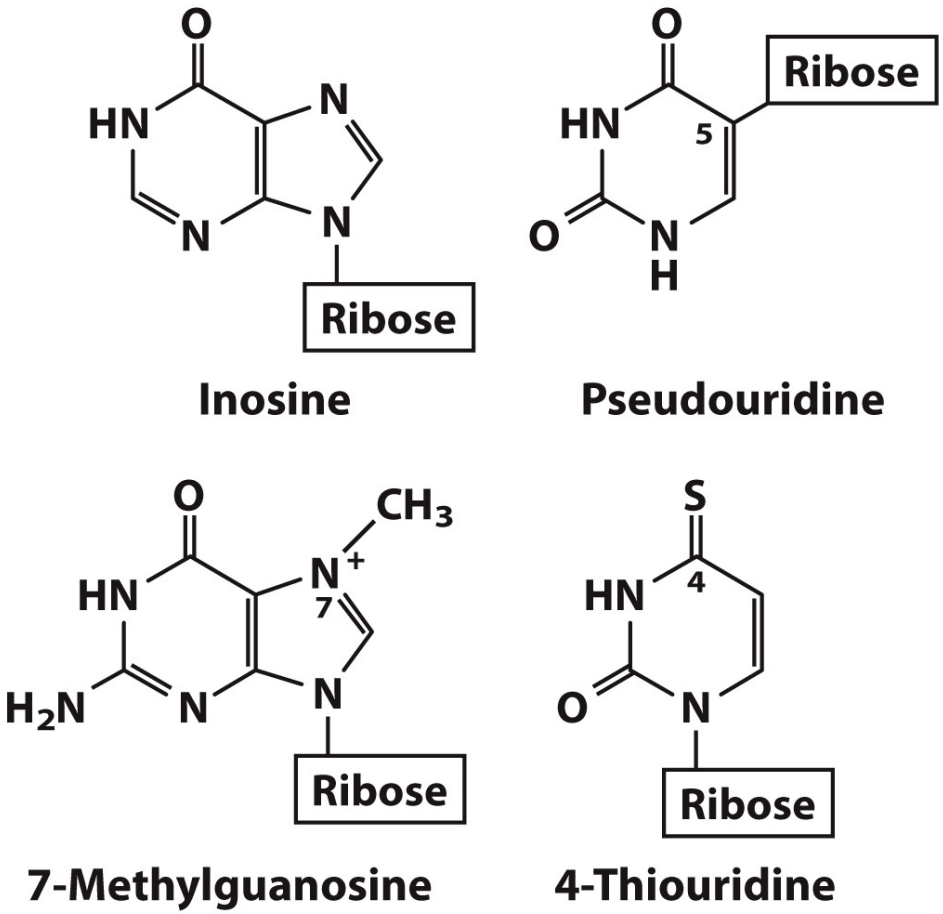
Minor Nucleosides in DNA

- DNA modification
- 5-Methylcytosine: eukaryotes and is also found in bacteria.
- N^6-Methyladenosine: bacteria only
- Epigenetic marker:
  - way to mark own DNA so that cells can degrade foreign DNA (prokaryotes)
  - way to mark which genes should be active (eukaryotes)



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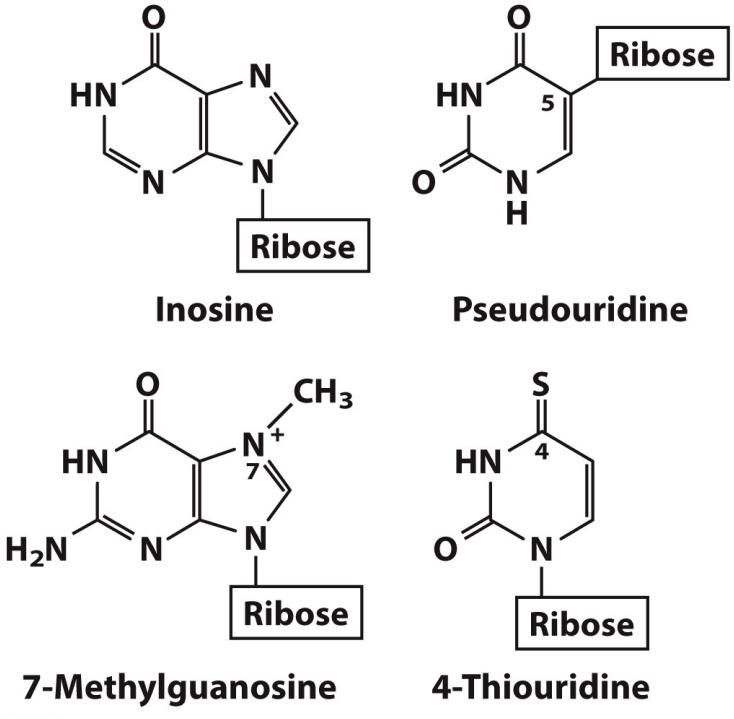
Minor Nucleosides in RNA

- Inosine is sometimes found in the "wobble position" of the anticodon in tRNA.
  - made by de-aminating adenosine
  - provides richer genetic code
- Pseudouridine (Ψ) is found widely in tRNA and rRNA.
  - more common in eukaryotes but found also in eubacteria
  - made from uridine by enzymatic isomerization after RNA synthesis
  - may stabilize the structure of tRNA
  - may help in folding of rRNA



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Polynucleotides

- Covalent bonds are formed via phosphodiester linkages.
  - negatively charged backbone
- DNA backbone is fairly stable.
  - DNA from mammoths?
  - Hydrolysis accelerated by enzymes (DNAse)
- RNA backbone is unstable.
  - In water, RNA lasts for a few years.
  - In cells, mRNA is degraded in a few hours.
- 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’.

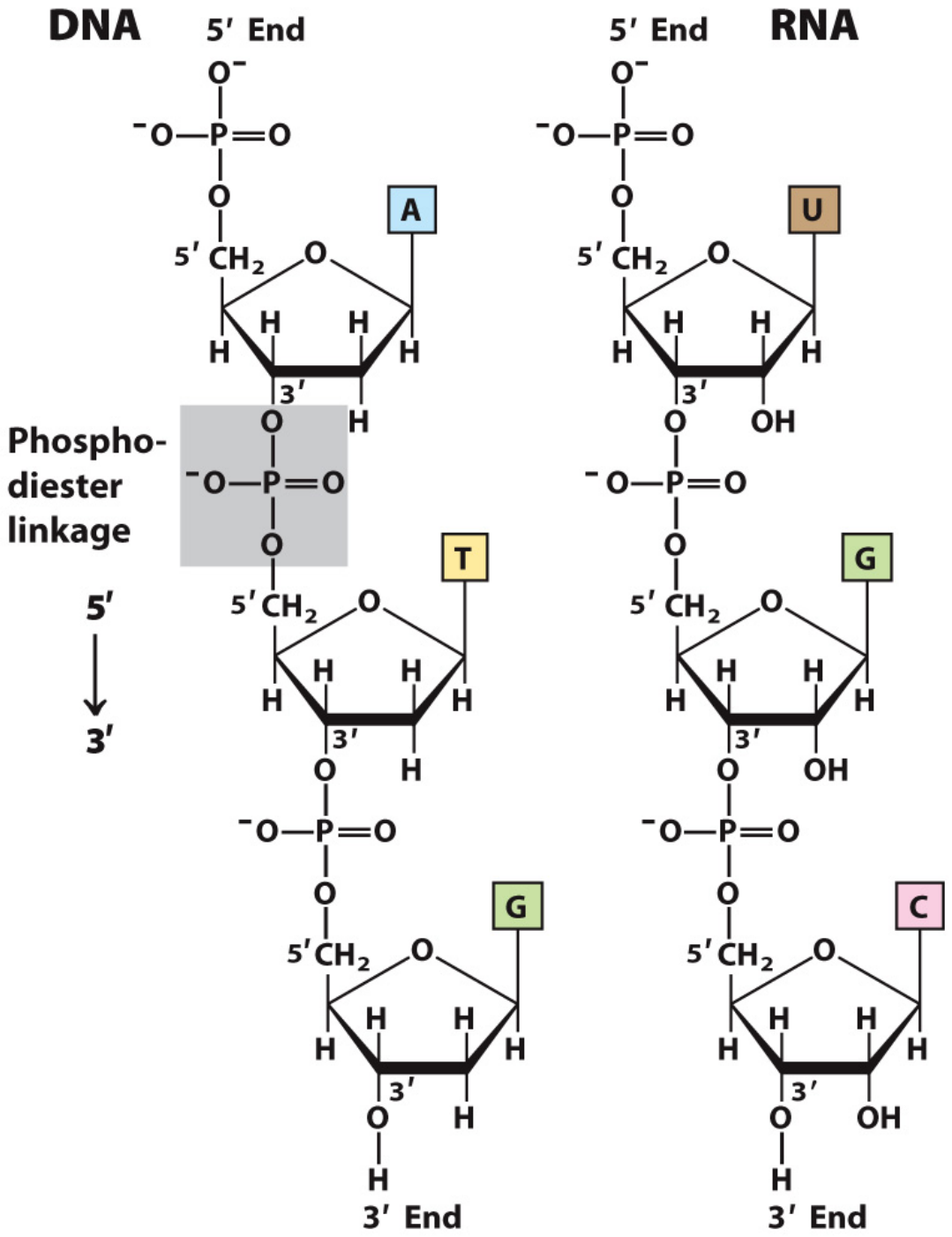

Figure 8-7
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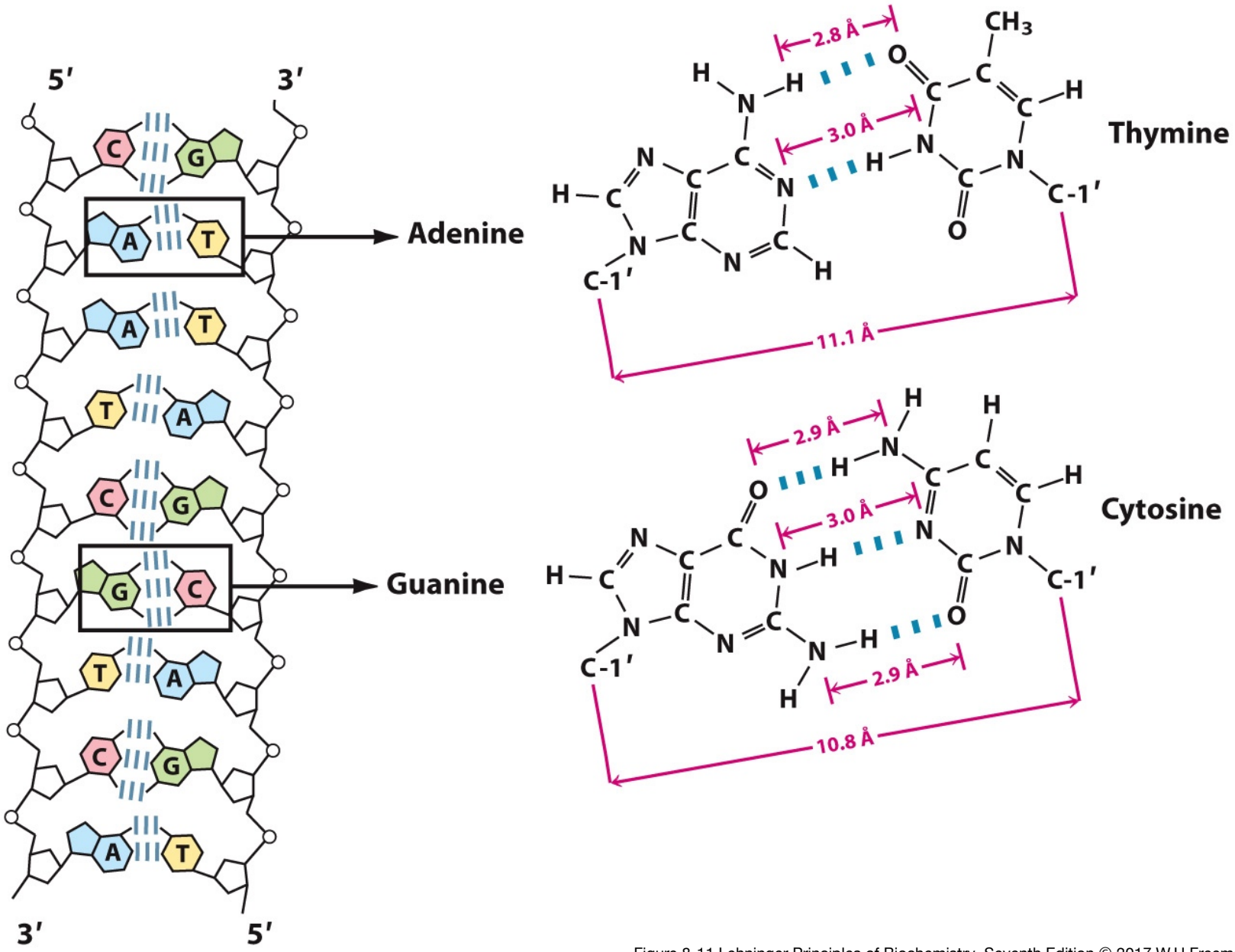
Hydrogen-Bonding Interactions

- Two bases can hydrogen bond to form a base pair.
- For monomers, a large number of base pairs is possible.
- In polynucleotide, 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.

AT and GC Base Pairs



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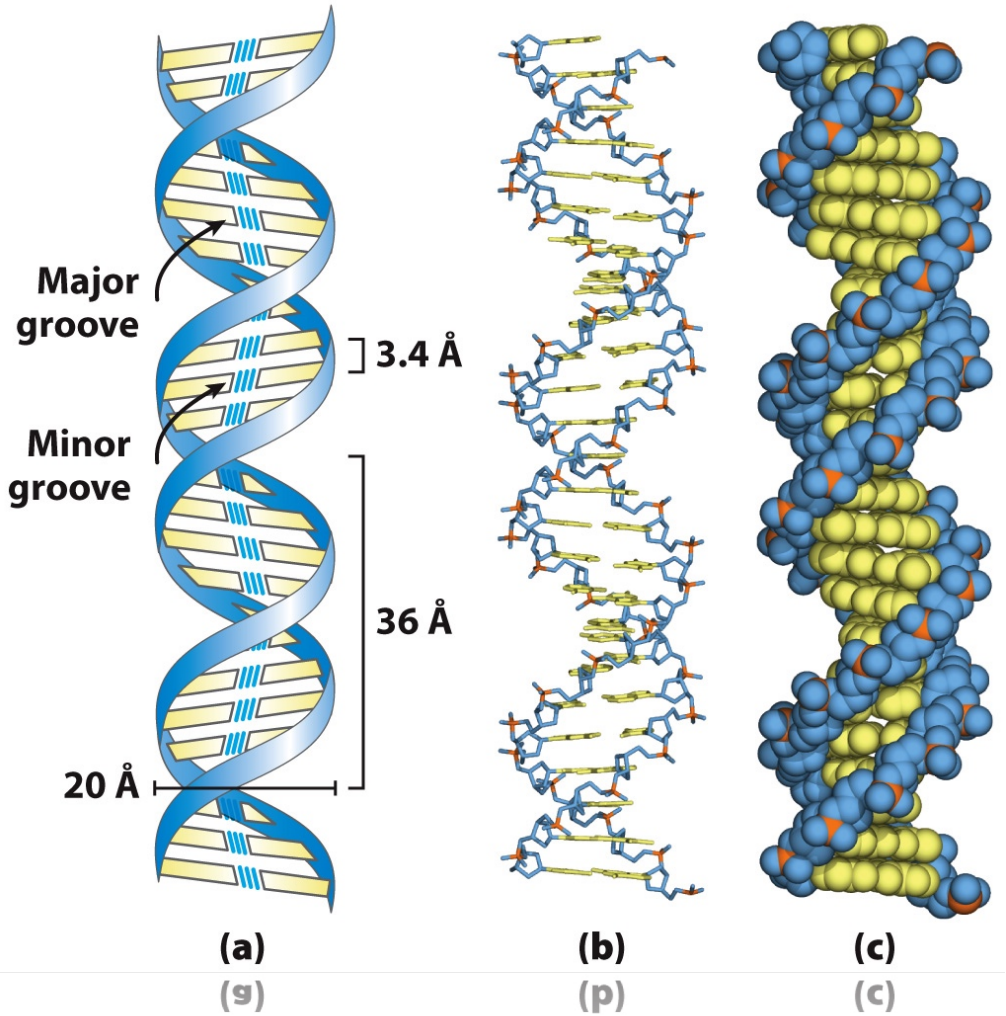
Watson-Crick Model of B-DNA



The original model proposed by Watson and Crick had 10 base pairs, or 34 Å (3.4 nm), per turn of the helix; subsequent measurements revealed 10.5 base pairs, or 36 Å (3.6 nm), per turn.

(a) Schematic representation, showing dimensions of the helix.
(b) Stick representation showing the backbone and stacking of the bases.
(c) Space-filling model.

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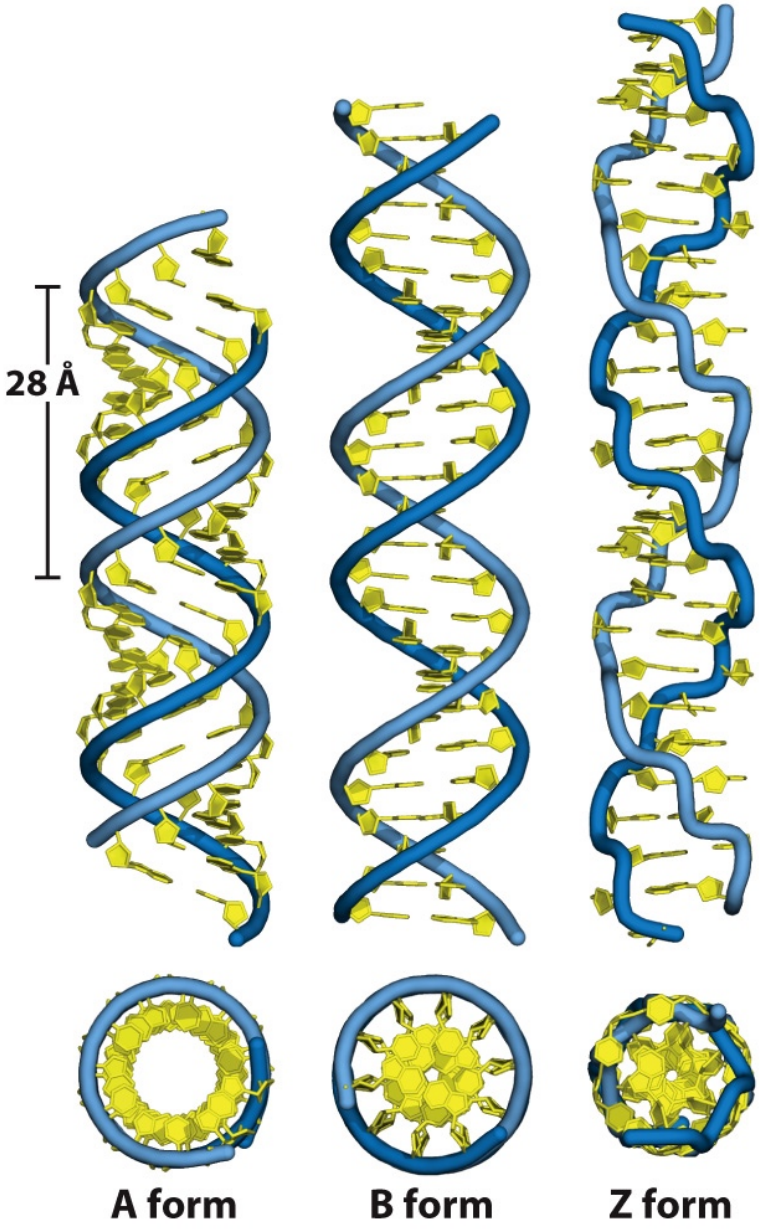
Other Forms of DNA



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Comparison of A, B, and Z forms of DNA.

- Each structure shown here has 36 base pairs.
- The riboses and bases are shown in yellow.
- The phosphodiester backbone is represented as a blue rope.

Other Forms of DNA

	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

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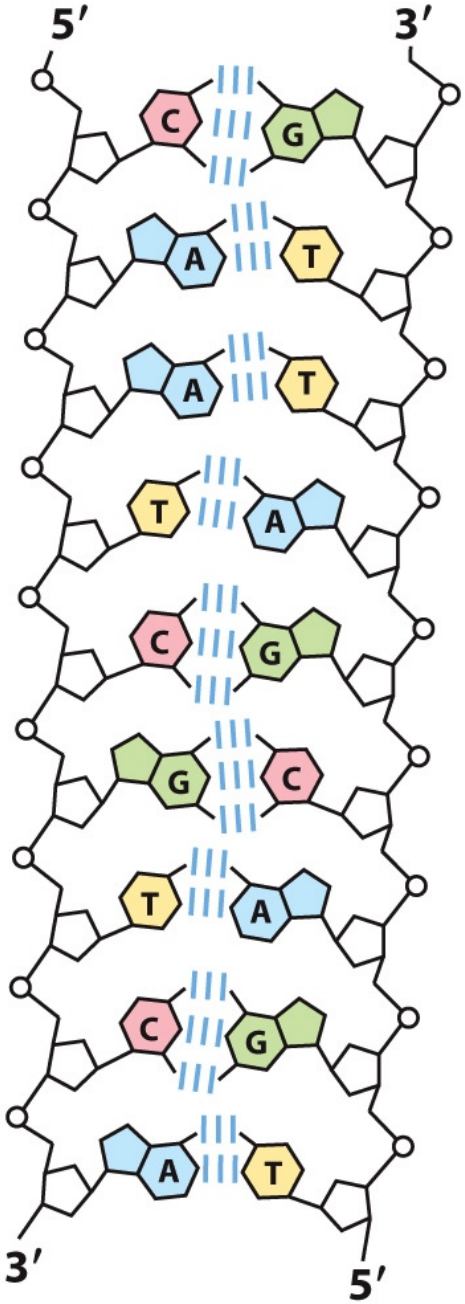
Complementarity of DNA Strands

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



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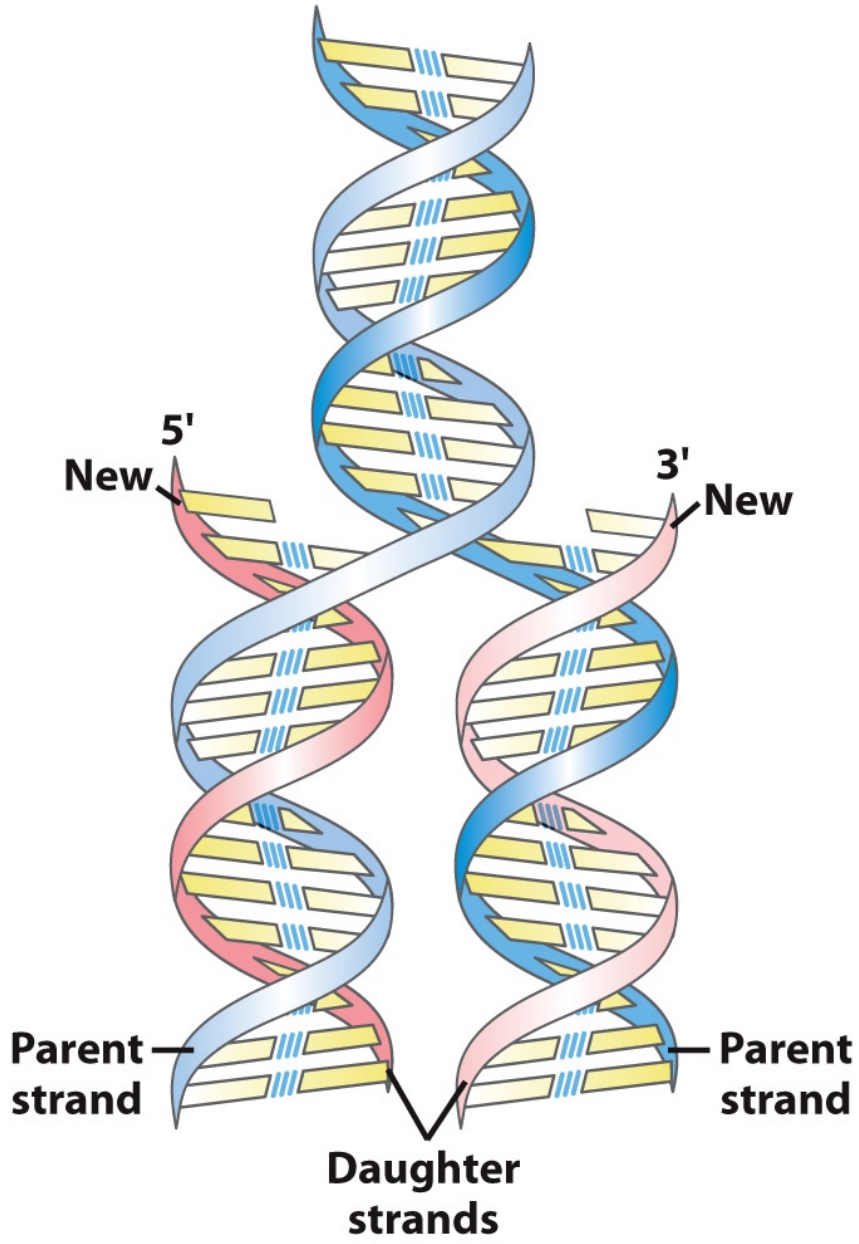
“It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.”

—Watson and Crick, *Nature*, 1953

Replication of Genetic Code



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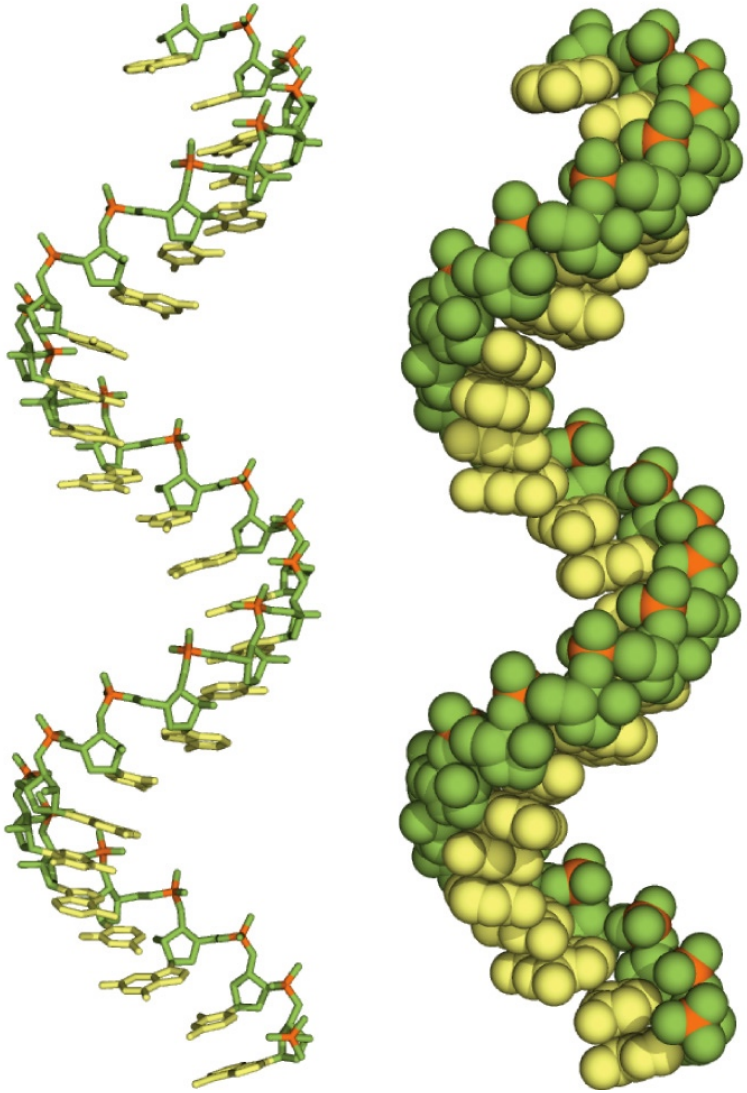
- Strand separation occurs first.
- Each strand serves as a template for the synthesis of a new strand.
- Synthesis is catalyzed by enzymes known as DNA polymerases.
- A newly made DNA molecule has one daughter strand and one parent strand.

Messenger RNA: Code Carrier for the Sequence of Proteins

- Is synthesized using DNA template and generally occurs as a single strand
- Contains ribose instead of deoxyribose
- Contains uracil instead of thymine
- One mRNA may code for more than one protein
- Together with transfer RNA (tRNA), transfers genetic information from DNA to proteins



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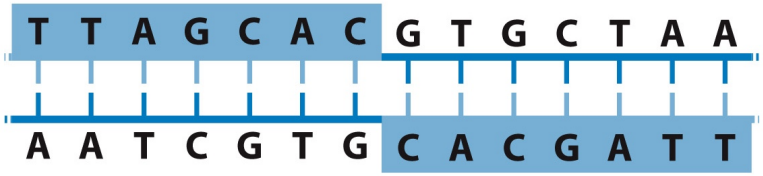
Palindromic Sequences Can Form Hairpins and Cruciforms

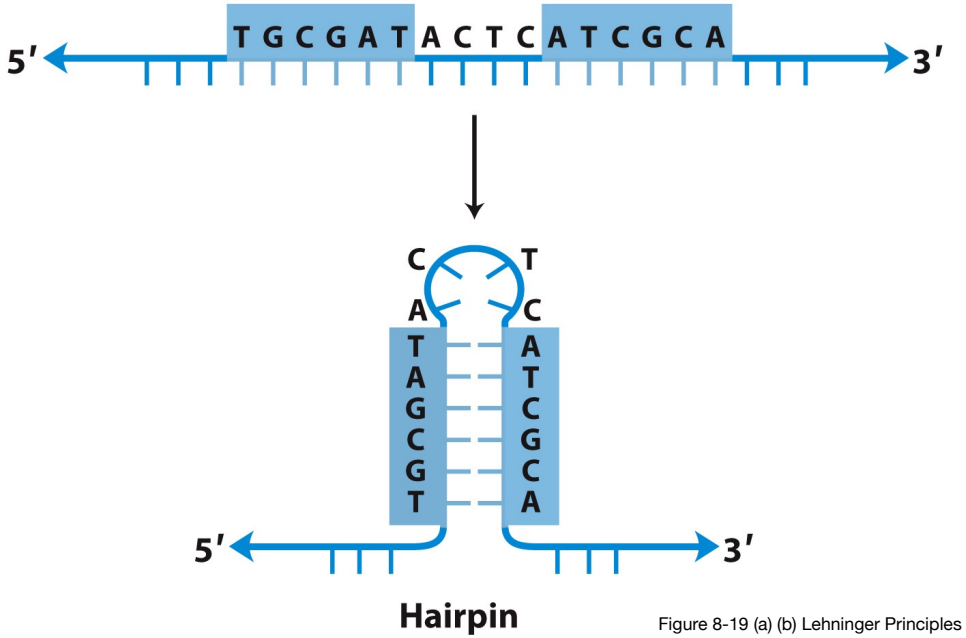
Palindromes: words or phases that are the same when read backward or forward.



Civic, Racecar, Rotator

Saippuakuppinippukauppias (Finnish word for "soap cup batch trader")

Νίψον ἀνομήματα, μὴ μόναν ὄψιν (ancient Greek: "Wash the sin as well as the fa



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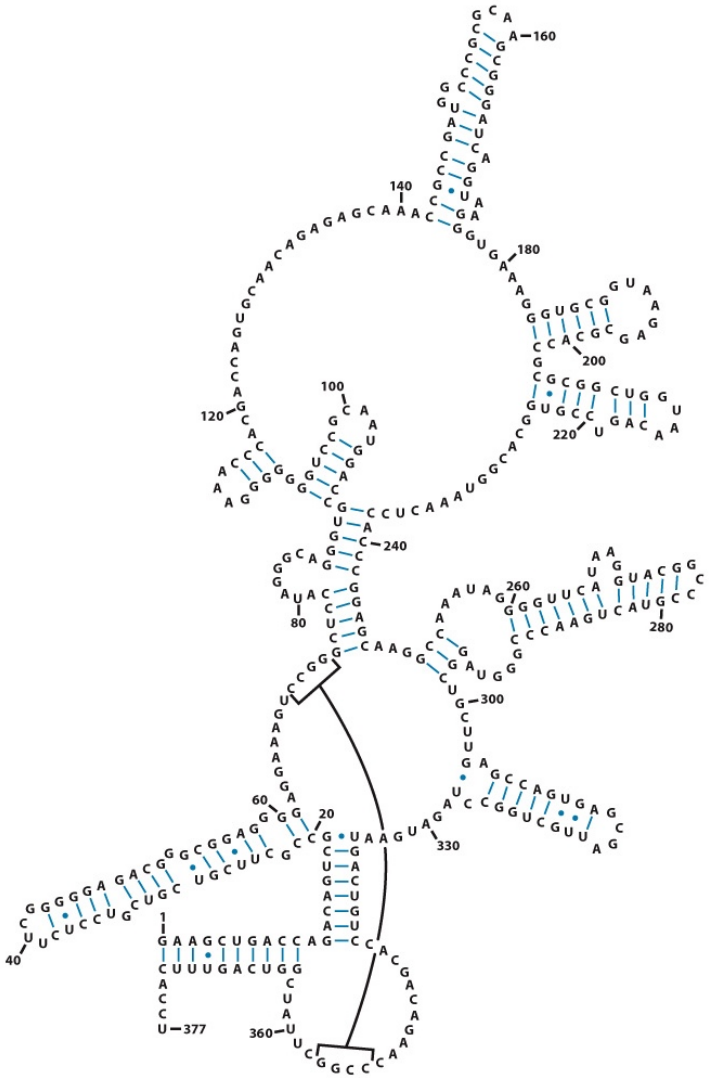
Complex Structures of RNA

M1 RNA component of the enzyme RNase P of *E. coli*



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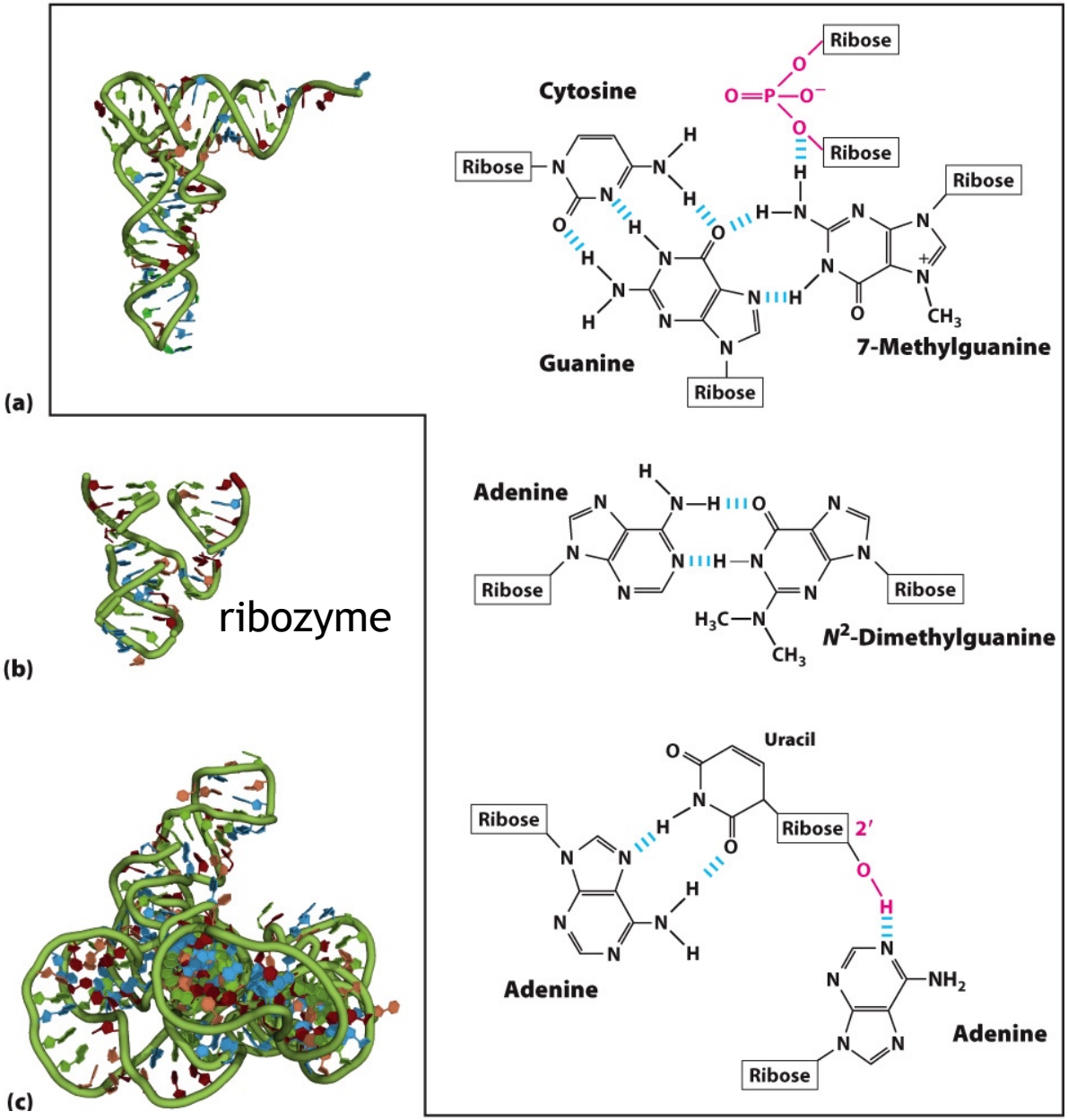
phenylalanine tRNA



ribozyme

intron ribozyme

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DNA Denaturation

- Covalent bonds remain intact.
  - Genetic code remains intact.
- Hydrogen bonds are broken.
  - Two strands separate.
- Base stacking is lost
  - UV absorbance increases.

Denaturation can be induced by high temperature, or change in pH.

Denaturation may be reversible: annealing.

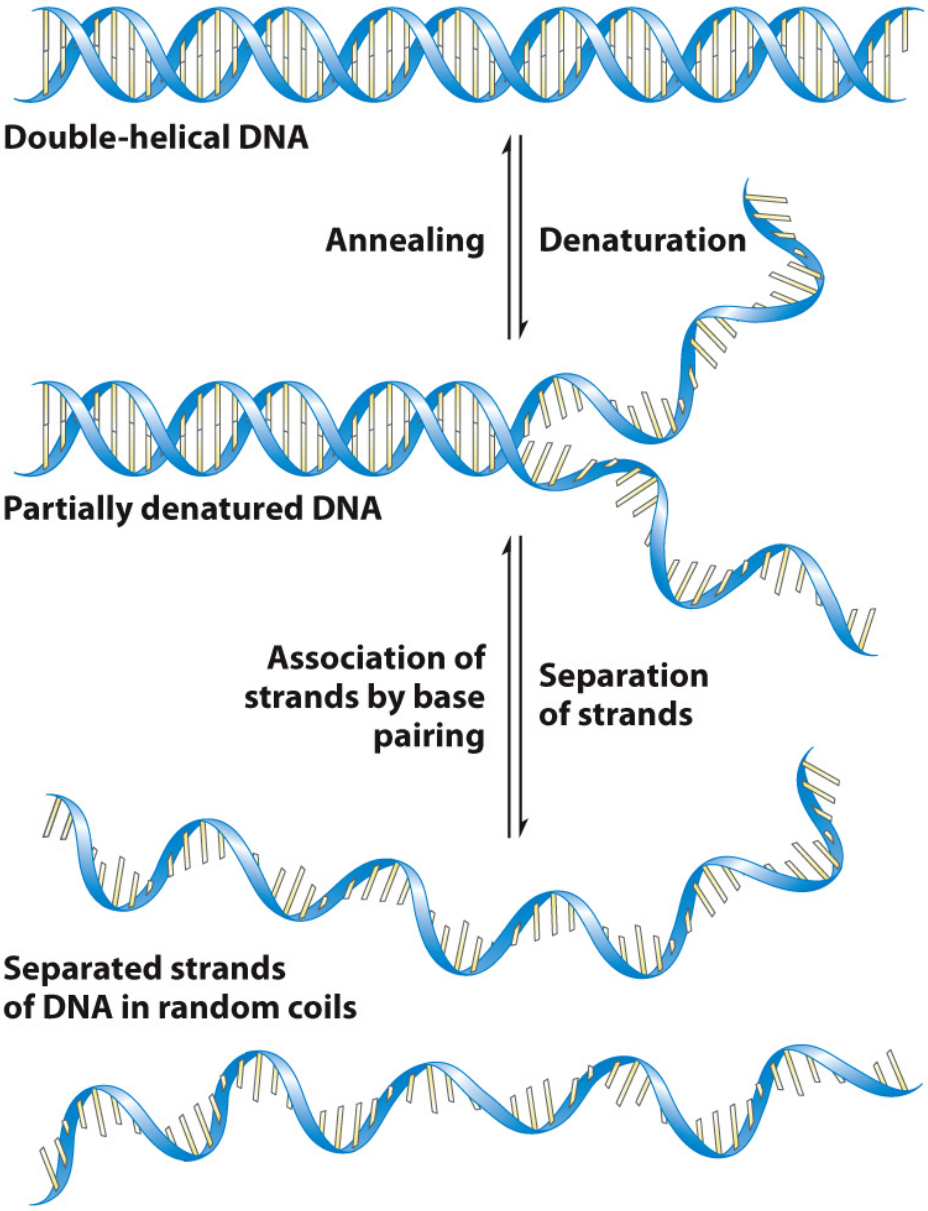

Figure 8-26
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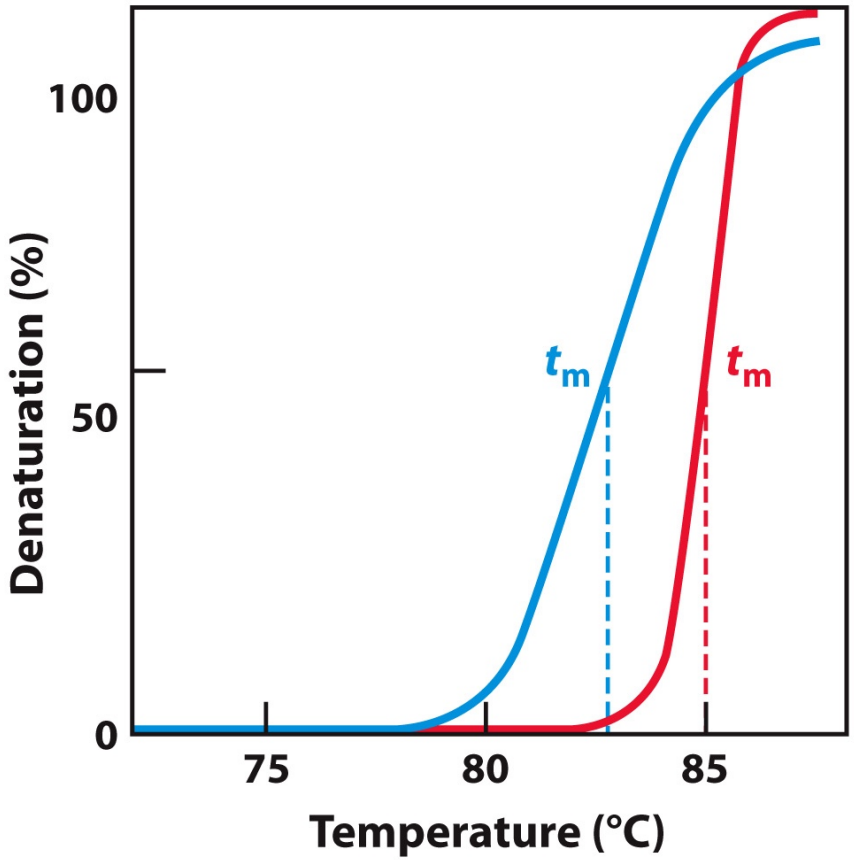
Thermal DNA Denaturation (Melting)

- DNA exists as double helix at normal temperatures.
- Two DNA strands dissociate at elevated temperatures.
- Two strands re-anneal when the temperature is lowered.
- The reversible thermal denaturation and annealing form the basis for the polymerase chain reaction.
- DNA denaturation is commonly monitored by UV spectrophotometry at 260 nm.



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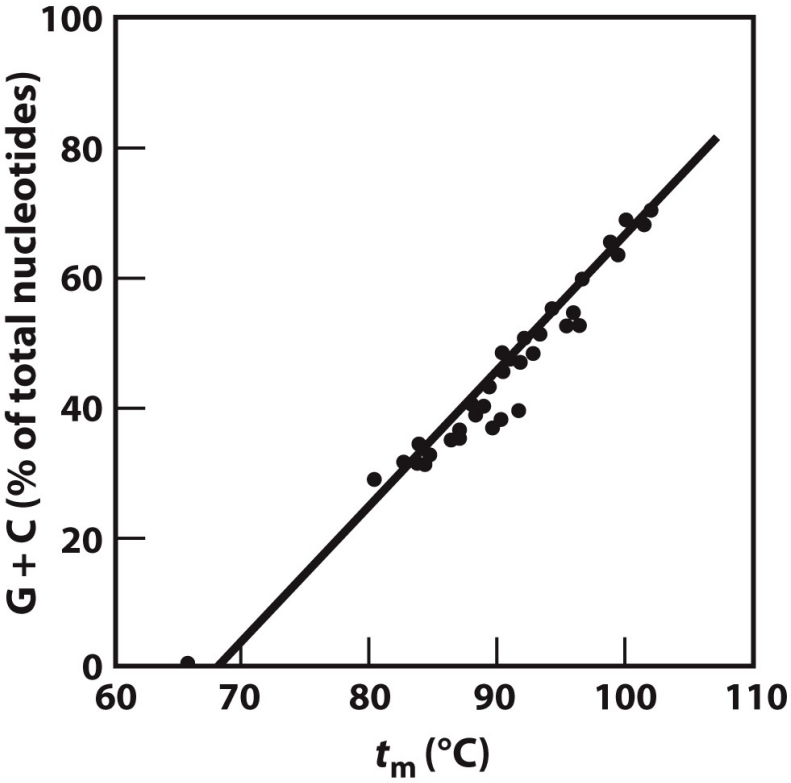
Factors Affecting DNA Denaturation

- The midpoint of melting (T_m) depends on base composition.
  - High CG increases T_m.
- T_m depends on DNA length.
  - Longer DNA has higher T_m.
  - It is important for short DNA.
- T_m depends on pH and ionic strength.
  - High salt increases T_m.



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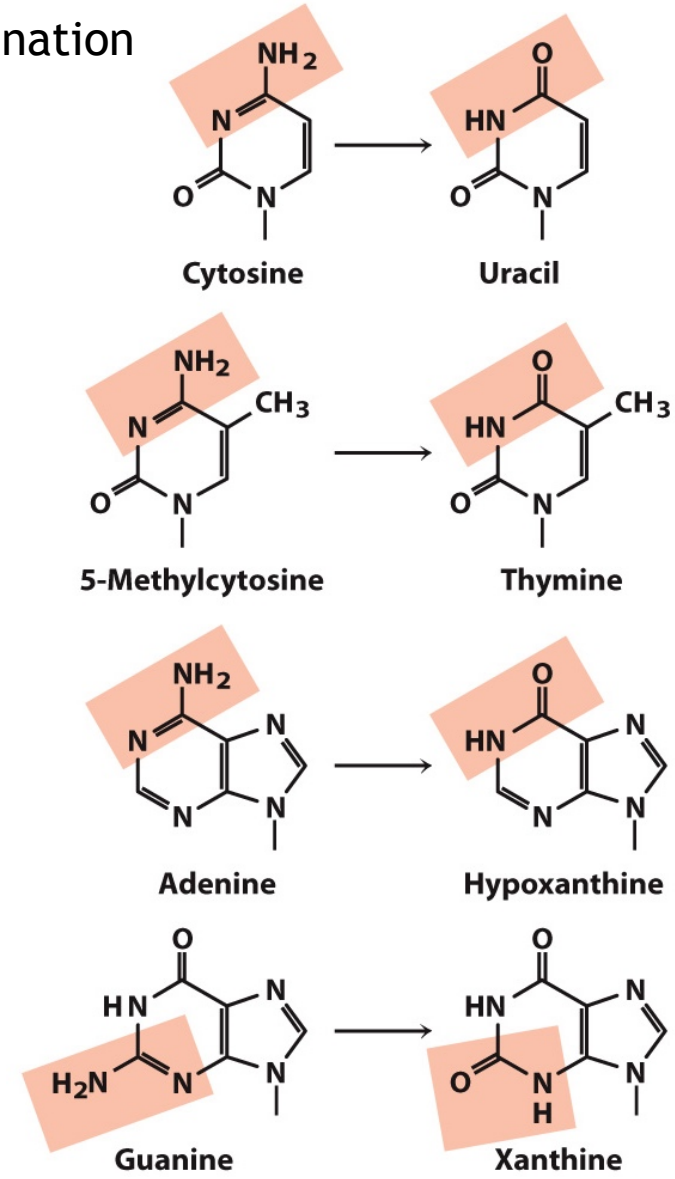
Spontaneous Mutagenesis

100 C → U events/day

Deamination



10,000 purines lost/day

Depurination

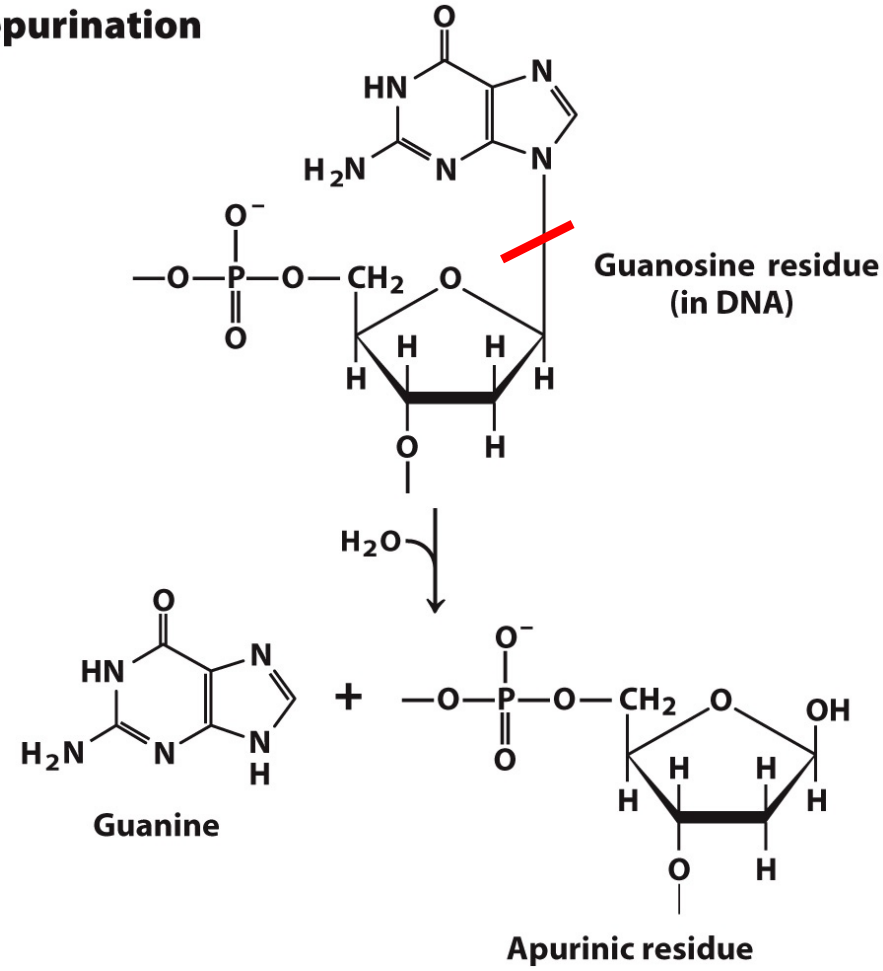


Figure 8-29 (a) (b) Lehninger Principles of Biochemistry, Seventh Edition © 2017 W.H.Freeman and Company

Oxidative & Chemical Mutagenesis

- Oxidative damage: hydroxylation of guanine
  - mitochondrial DNA is most susceptible
- Chemical alkylation: methylation of guanine
- Cells have mechanisms to correct most of these mo[illegible]

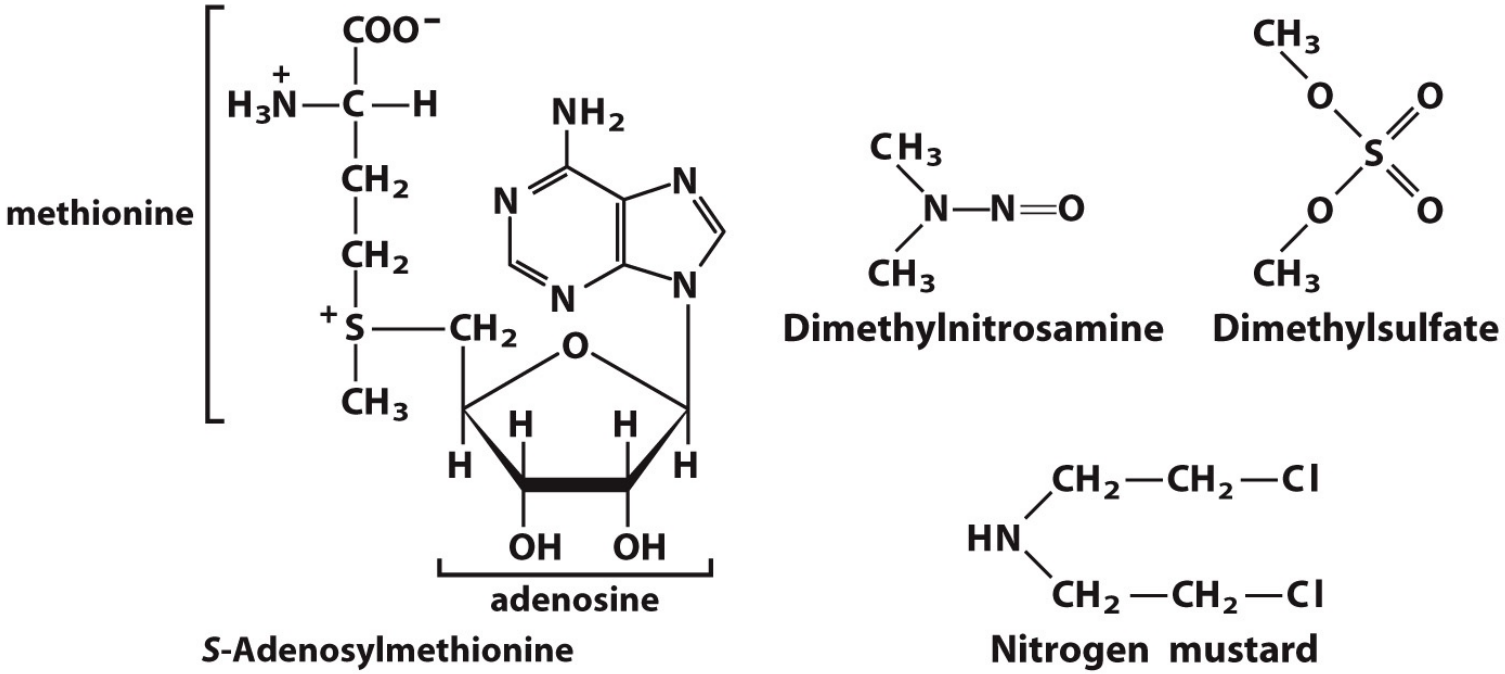


Figure 8-31(b) Lehninger Principles of Biochemistry, Seventh Edition © 2017 W.H.Freeman and Company

Radiation-Induced Mutagenesis

- UV light induces dimerization of pyrimidines; this may be the main mechanism for skin cancers.
- Ionizing radiation (x rays and γ rays) causes ring opening and strand breaking. These are difficult to fix.
- Cells can repair some of these modifications, but others cause mutations. Accumulation of mutations is linked to aging and carcinogenesis.

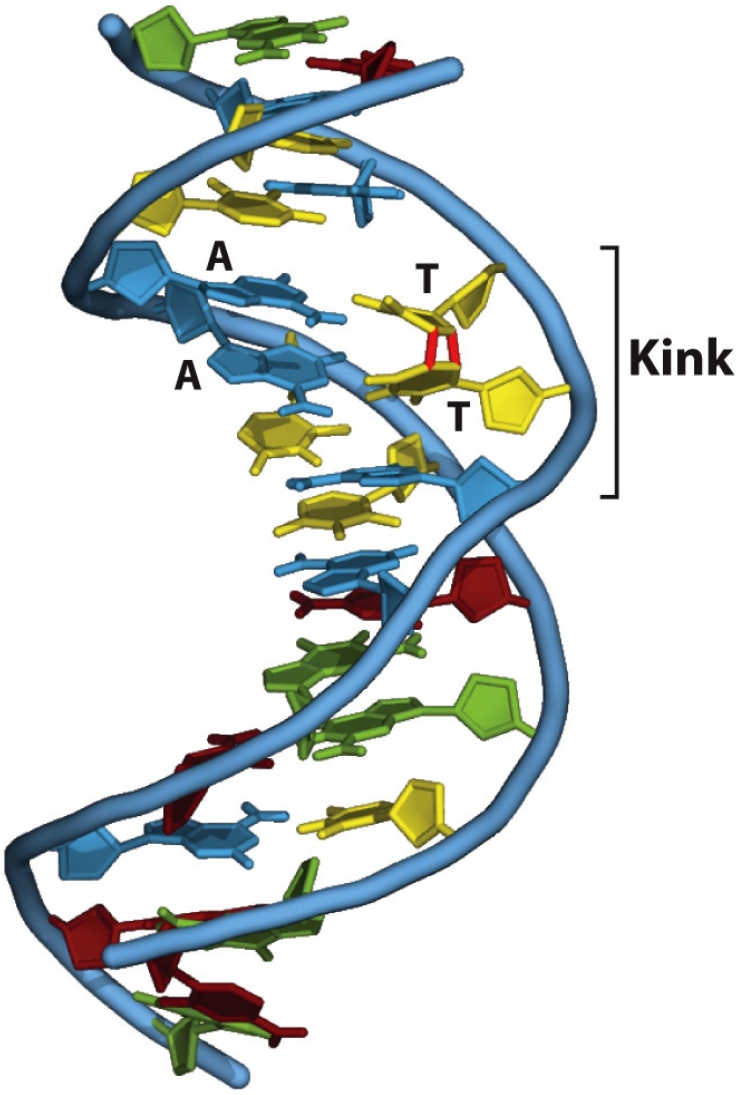
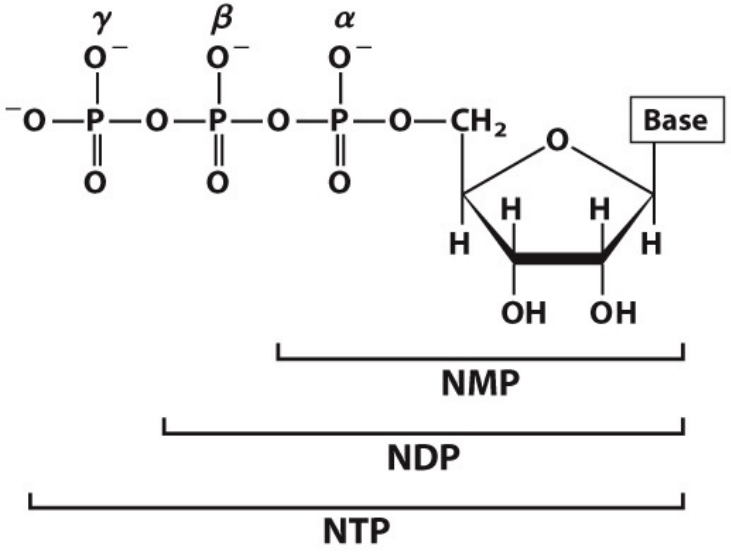


Figure 8-30(b) Lehninger Principles of Biochemistry, Seventh Edition © 2017 W.H.Freeman and Company

Formation of pyrimidine dimers by UV light

Other Functions of Nucleotides:

Energy Source



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

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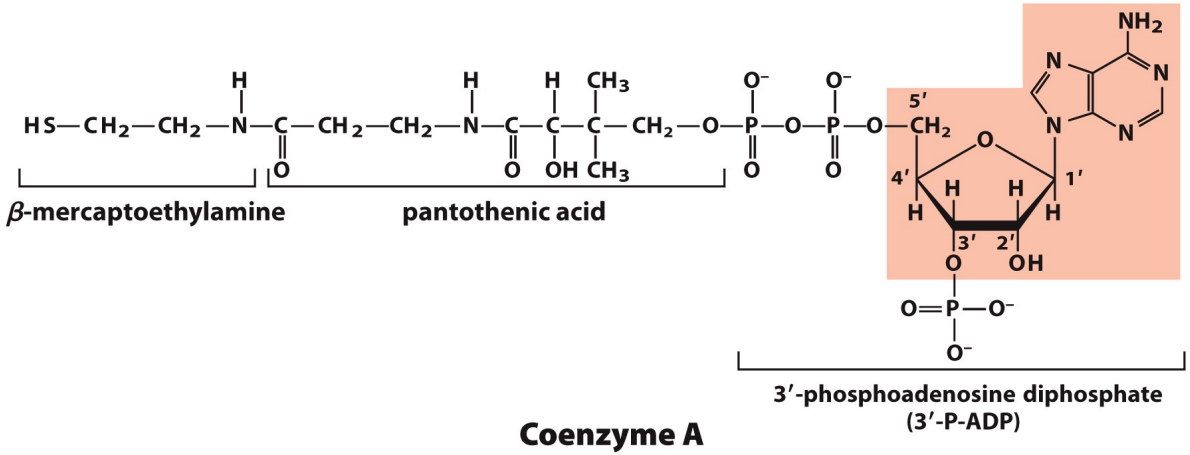
Nucleoside phosphates

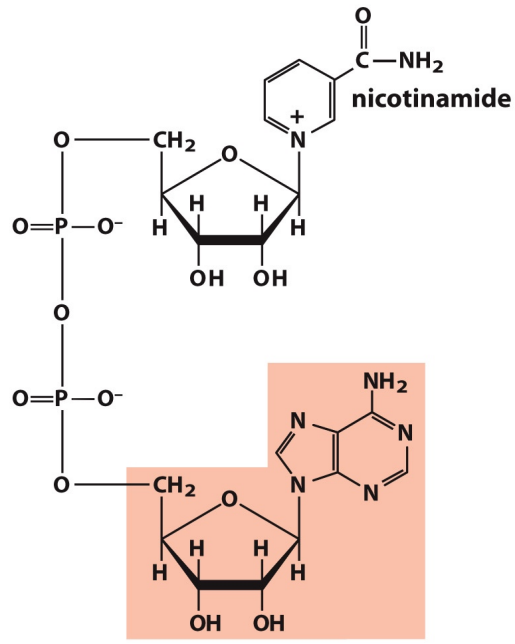
- General structure of the nucleoside 5’-mono-, di-, and triphosphates (NMPs, NDPs, and NTPs) and their standard abbreviations.
- In the deoxyribonucleoside phosphates (dNMPs, dNDPs, and dNTPs), the pentose is 2’-deoxy-D-ribose.

Other Functions of Nucleotides:

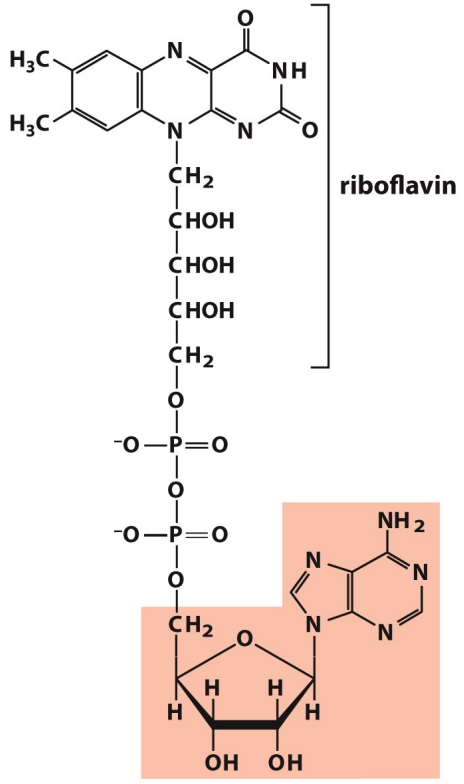
Coenzymes



Coenzyme A



Nicotinamide adenine dinucleotide (NAD^+)



Flavin adenine dinucleotide (FAD)

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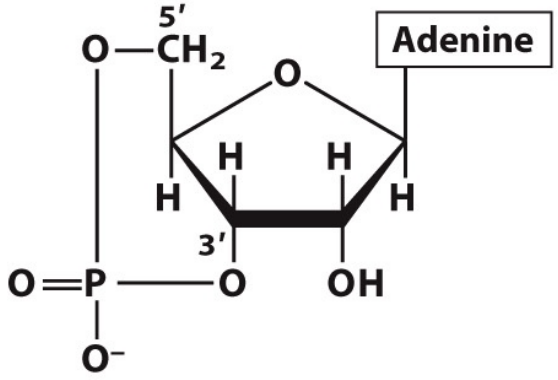
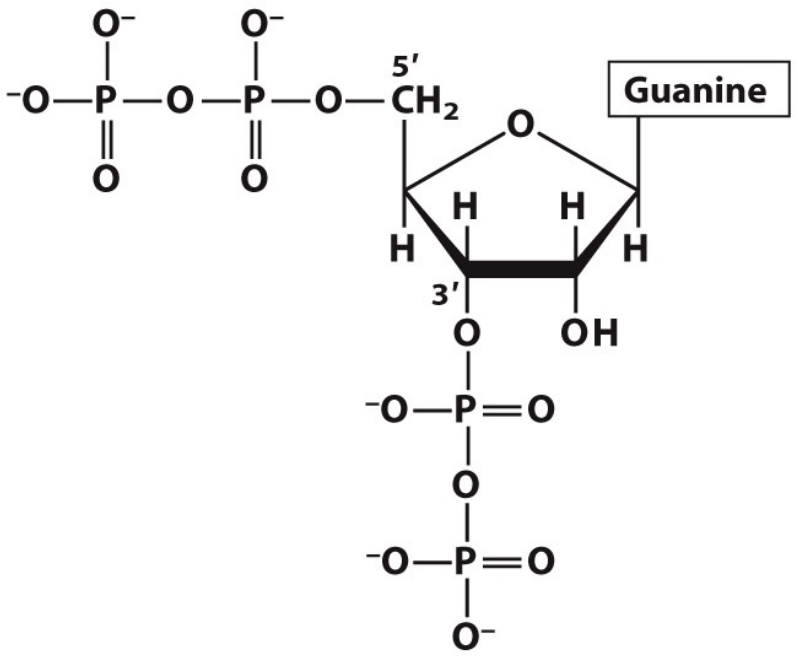
Other Functions of Nucleotides:

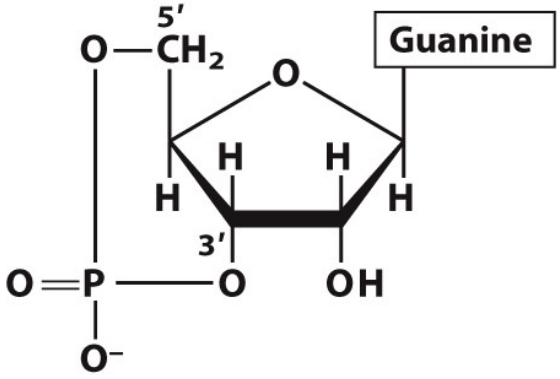
Regulatory Molecules



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



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



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

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Tools to Study Genes

Topics	Team 1	Team 2
gene editing	Cindy & Olivia	Catherine & Nadia
Recombinant DNA	Moeri & Buddhini	Yu Min & Yam
RNA interference	Ronny & Anh	Yui & Kazuma
PCR	Viet & Ryuki	Steve & Chee Ming
DNA sequencing	Bach & Hayato	Maydelene & Poh

- Recombinant DNA & Cloning
- Gene editing (e.g. CRISPR)
- RNA interference
- DNA Sequencing
- PCR