Structure and Function of Biomolecules

BCMB 201

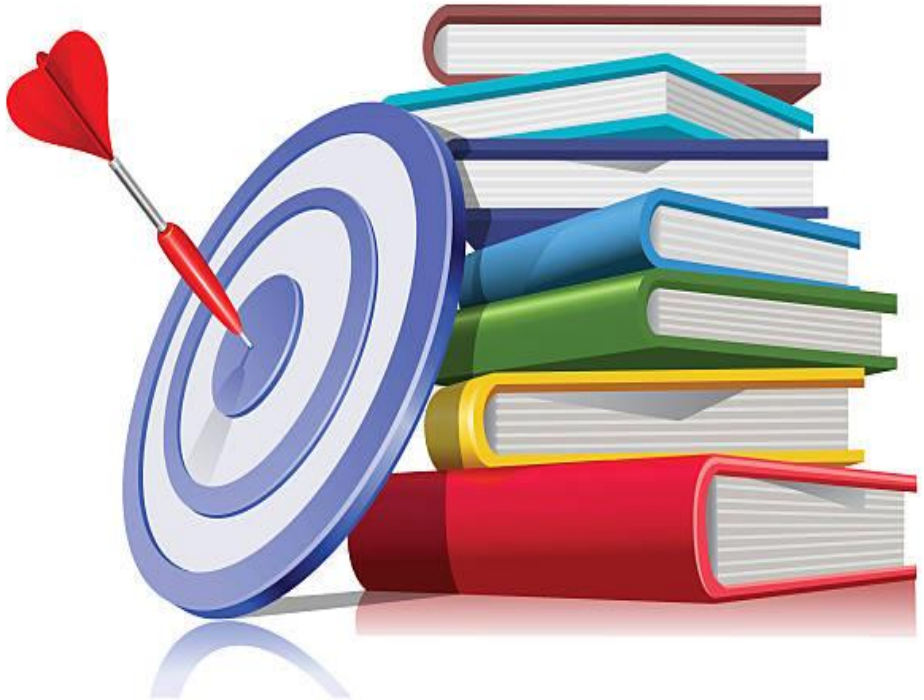
Nucleic Acids

Dr Koby Sarpong

Nucleic Acids

Learning goals:

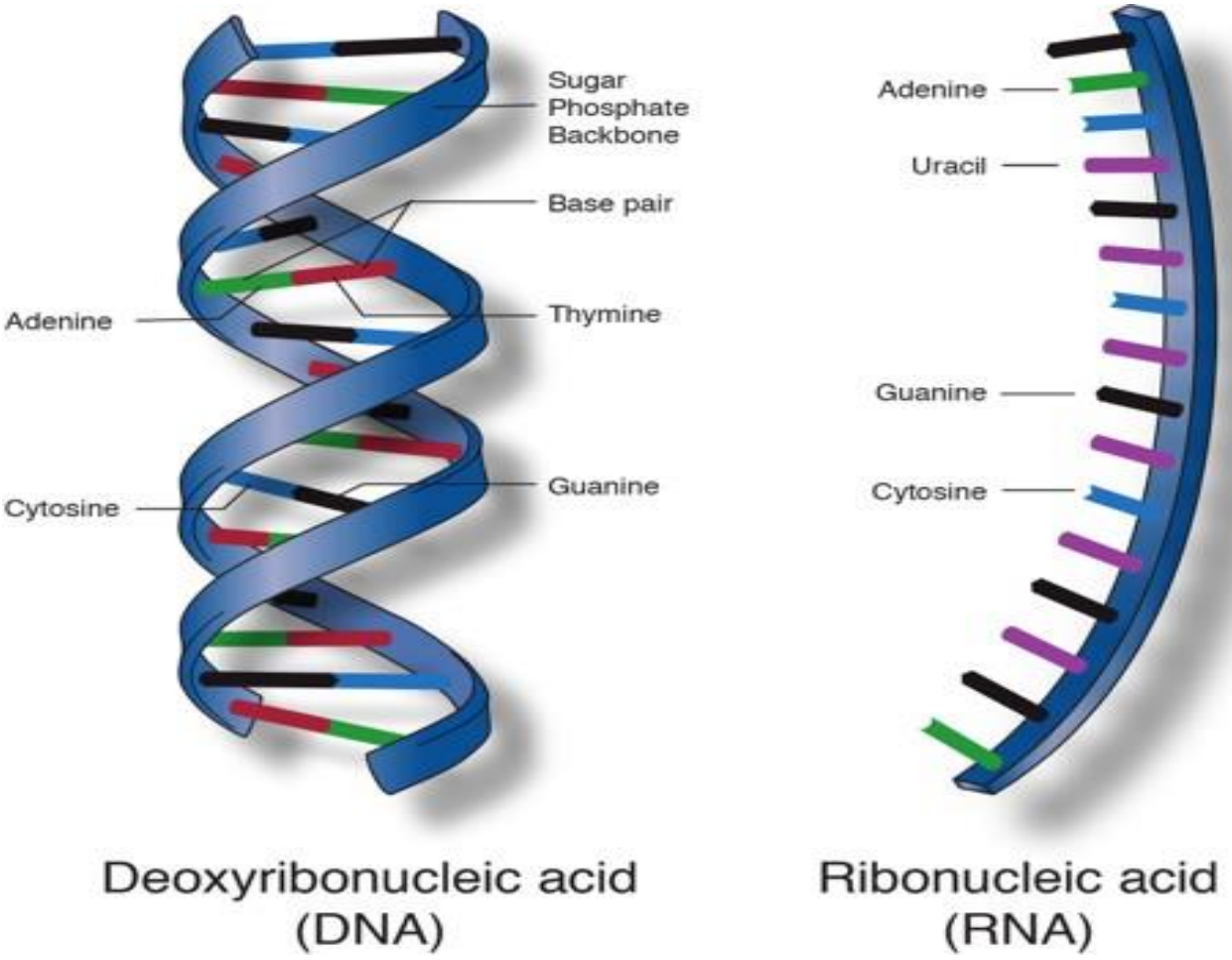
- Biological function of nucleotides and nucleic acids
- Structures of common nucleotides
- Structure of double-stranded DNA
- Structures of ribonucleic acids
- Denaturation and annealing of DNA
- Chemistry of nucleic acids; mutagenesis



What do you know about Nucleic Acids?

HCl
HBr
HI
HNO_3
H_2SO_4
$HClO_4$

WHAT DNA AND RNA LOOK LIKE



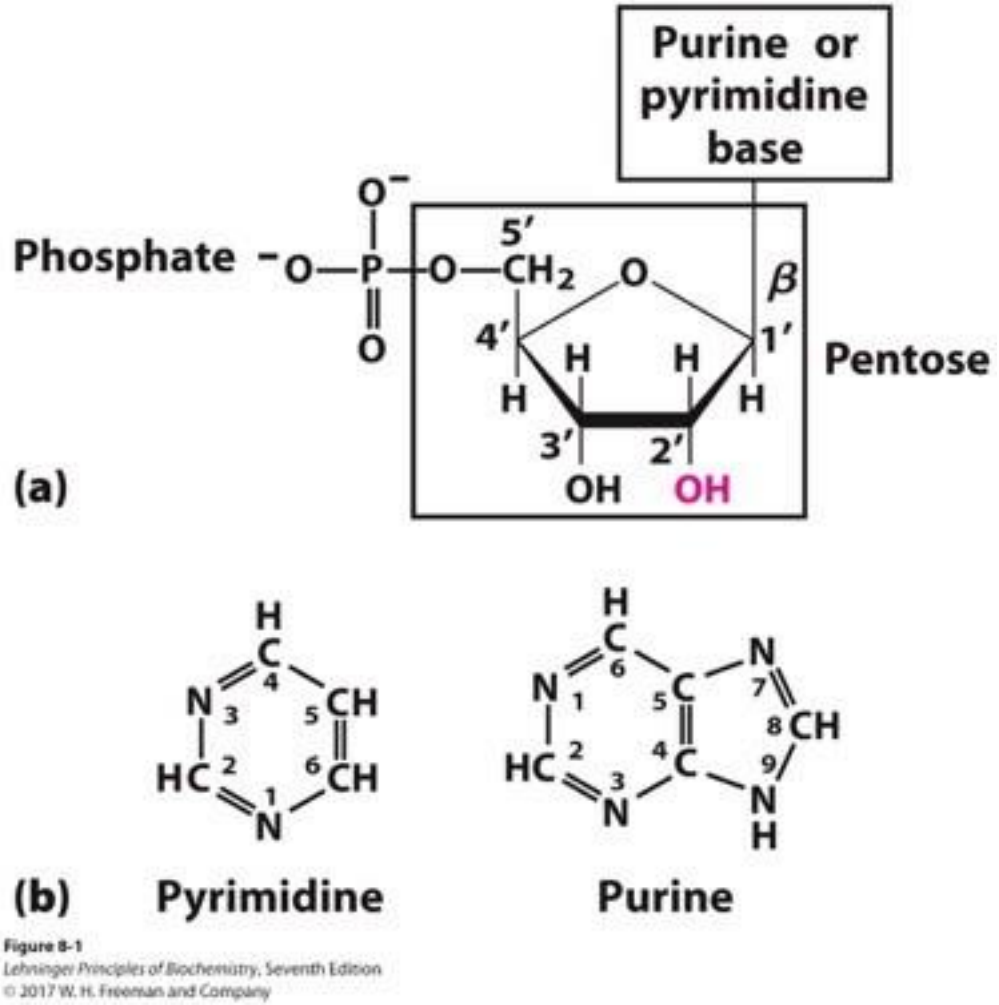
Functions of Nucleotides and Nucleic Acids

- 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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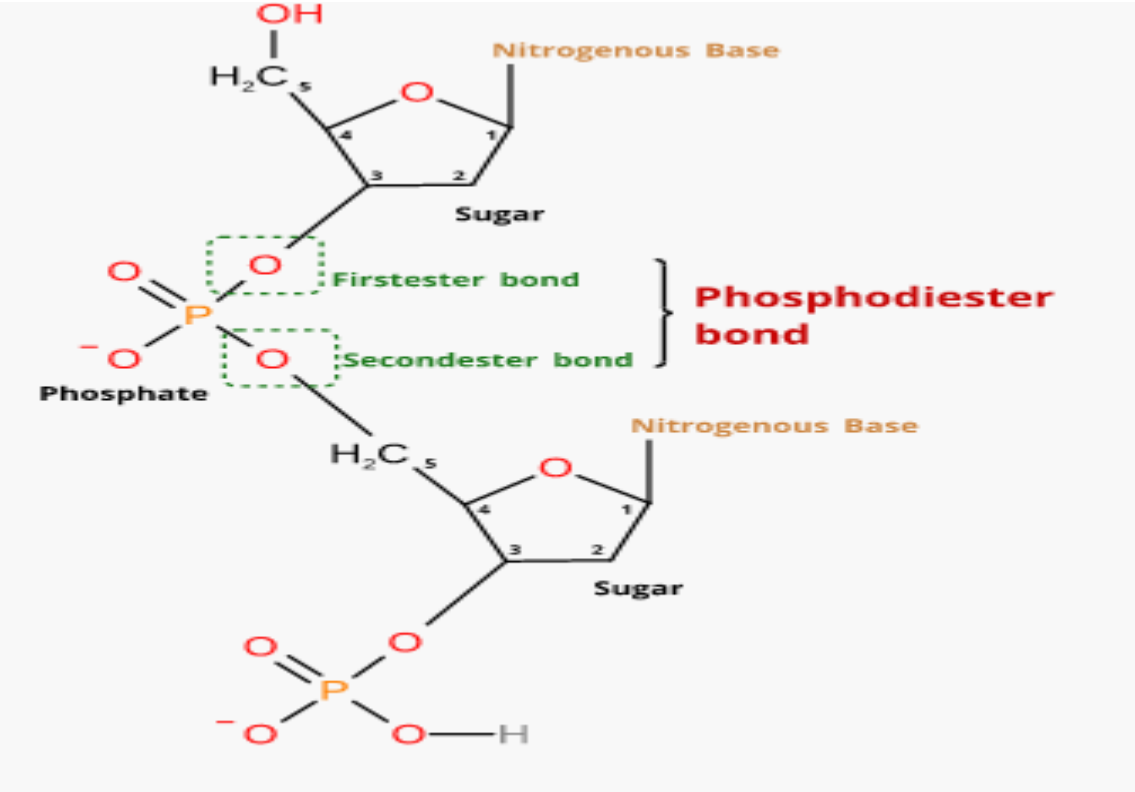
Phosphate Group

- 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

Nucleic Acids

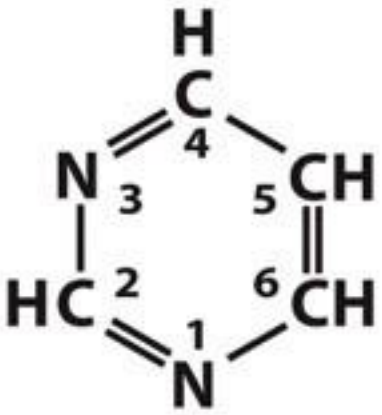
Phosphodiester Linkage



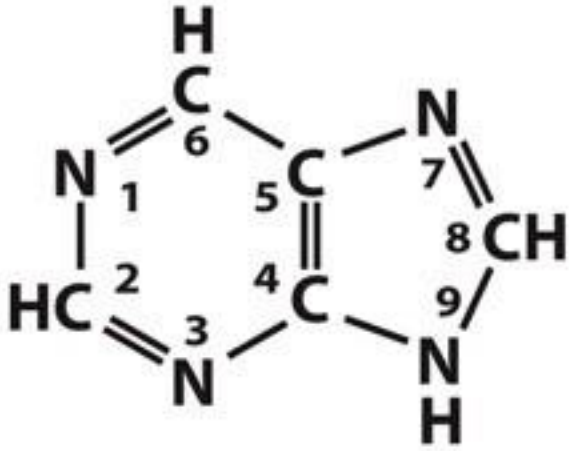
Sugars in DNA and RNA are linked by phosphate through phosphodiester bonds

Nitrogenous Bases

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



Pyrimidine



Purine

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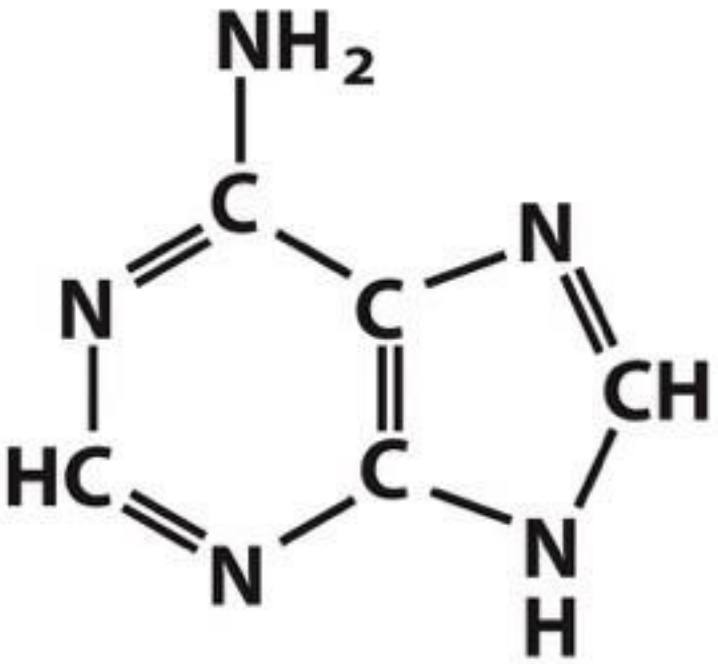
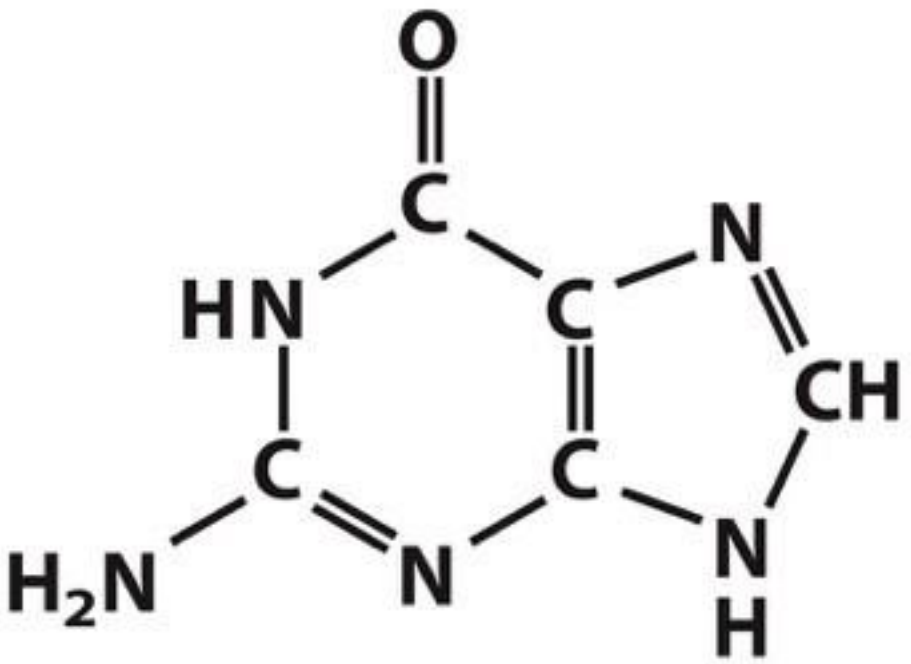
Bases

- 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



Adenine



Guanine

Purines

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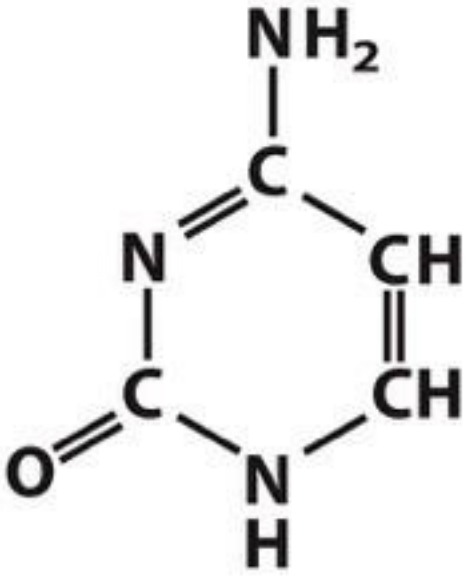
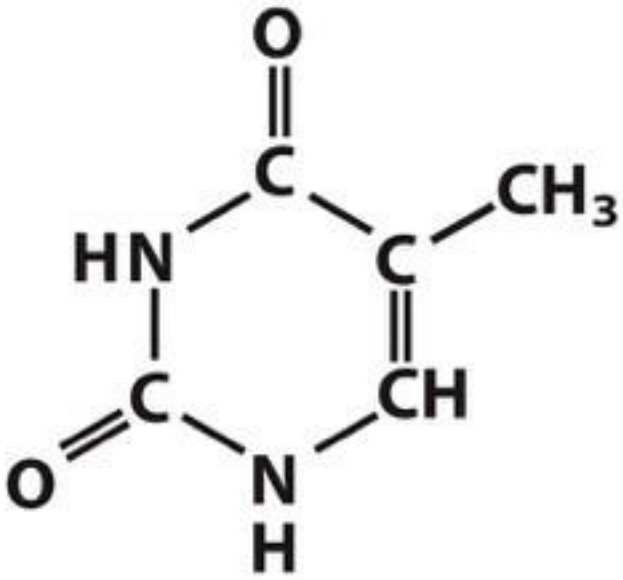
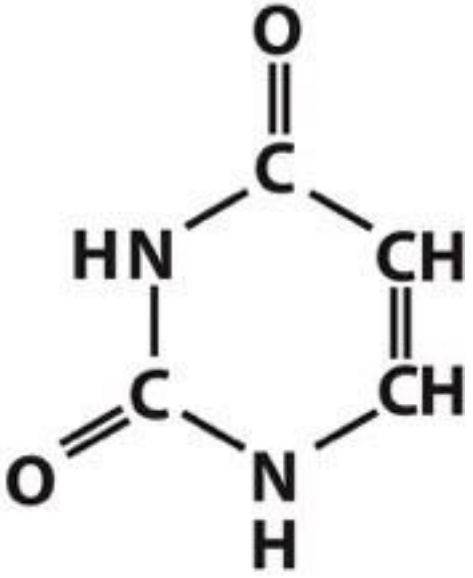


Cytosine



Thymine
(DNA)



Uracil
(RNA)

Pyrimidines

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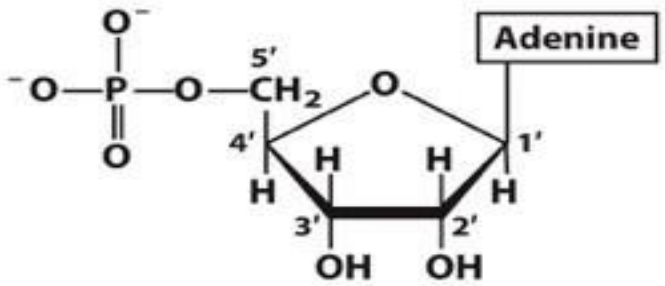
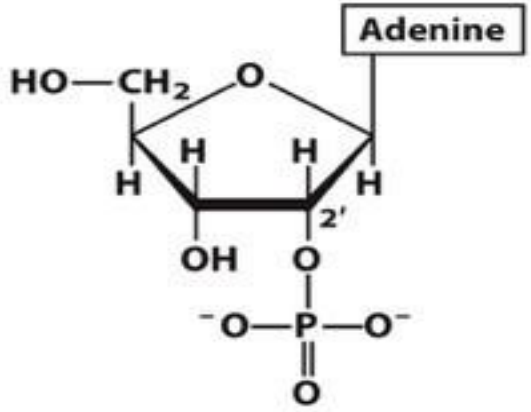
β–*N*-Glycosidic Bond

- 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.
- 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.

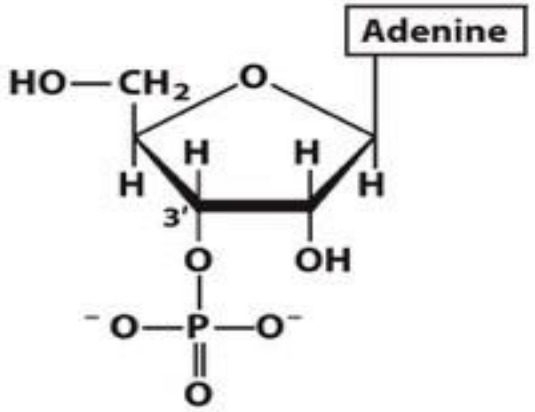
Other Nucleotides: Monophosphate Group in Different Positions



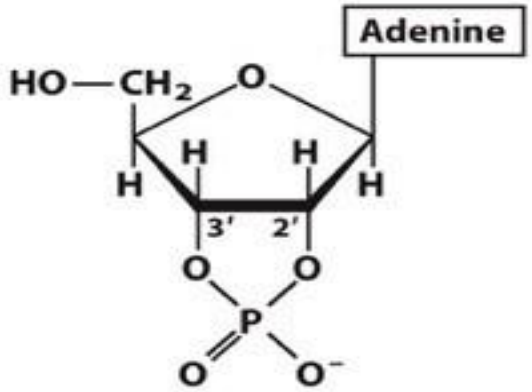
Adenosine 5′-monophosphate



Adenosine 2′-monophosphate



Adenosine 3′-monophosphate



Adenosine 2′,3′-cyclic monophosphate

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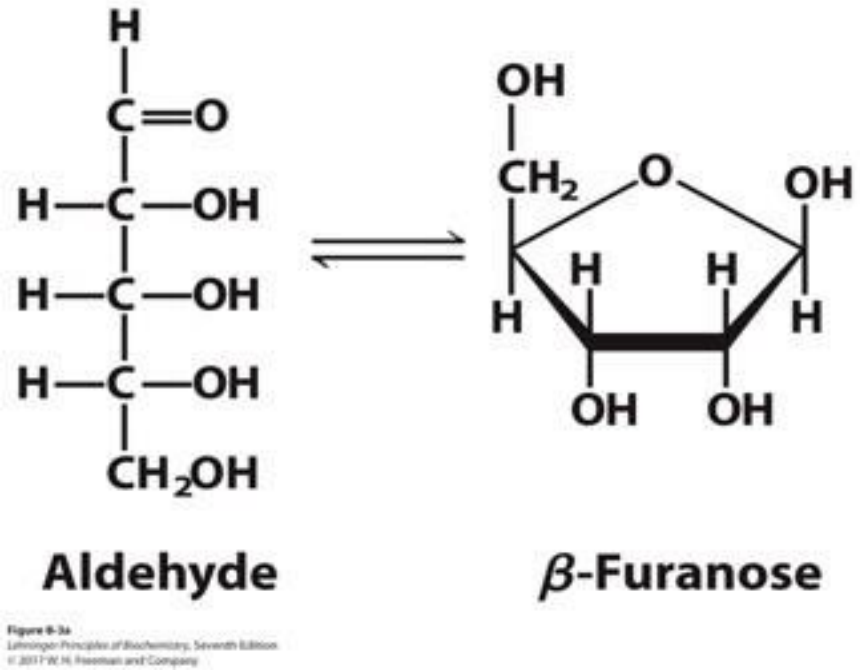
Pentose Forms Differ in Some Nucleic acids and Nucleotides

- β-d-ribofuranose in RNA
- β-2'-deoxy-d-ribofuranose in DNA



- Different puckered conformations of the sugar ring are possible.

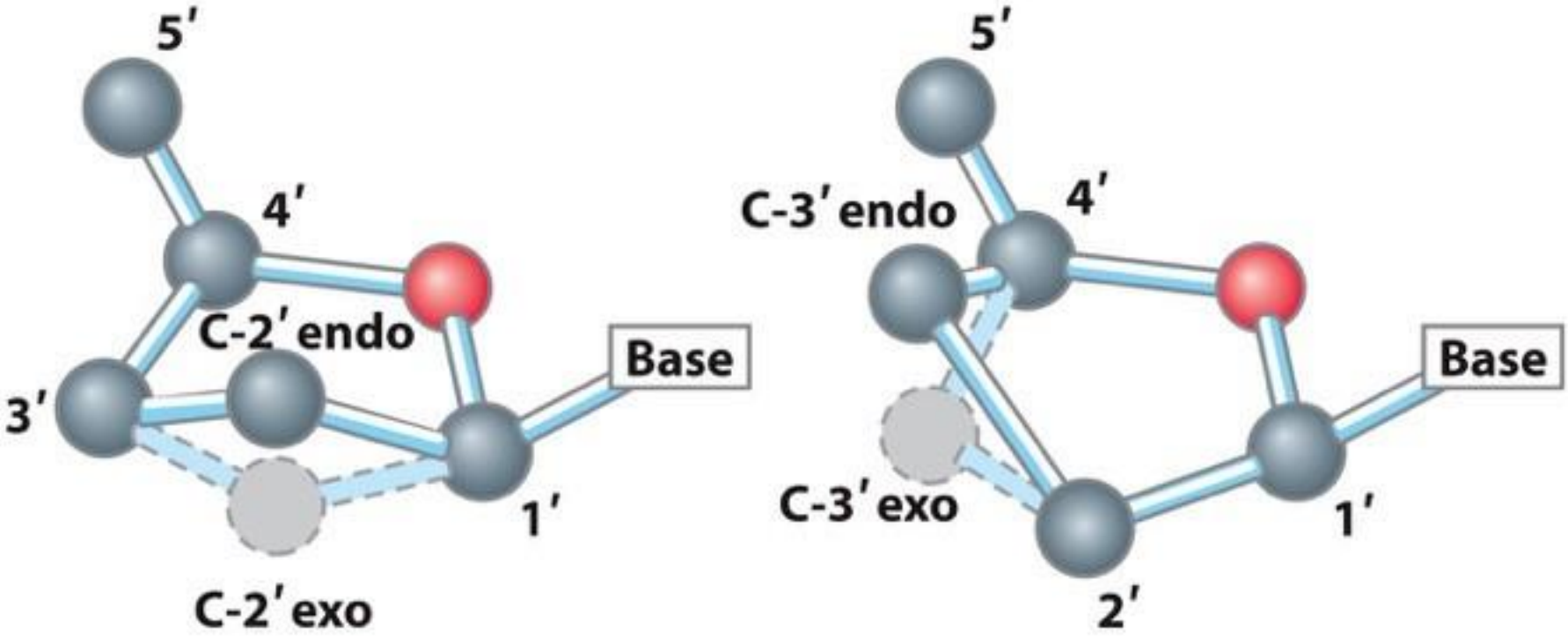


Figure 8-3b
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Nomenclature

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.

Nomenclature: Deoxyribonucleotides

You need to know the structures, names, and symbols (both two-letter (dA) and four-letter (dAMP) codes).

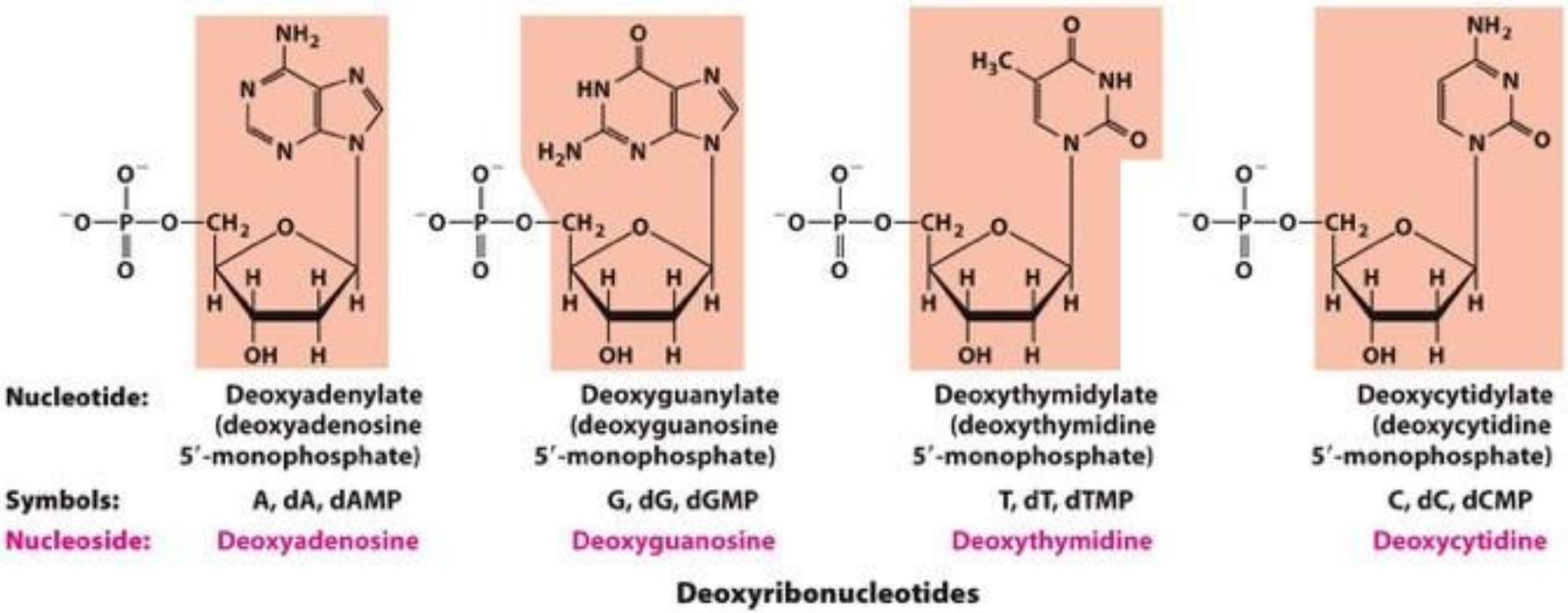

Figure 8-4a
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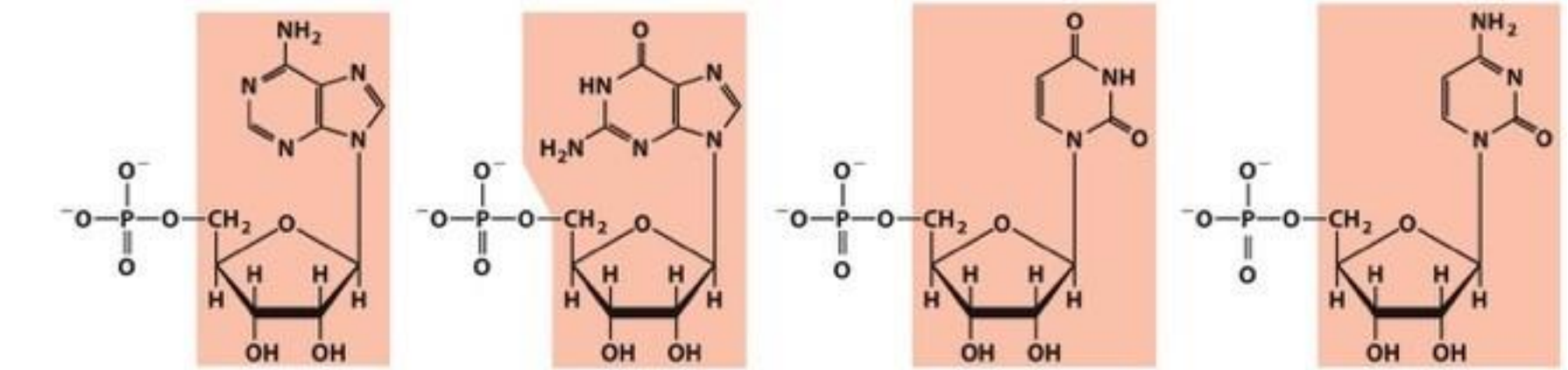
Nomenclature: Ribonucleotides

You need to know structures, names, and symbols (both two-letter (dA) and four-letter (dAMP) codes).



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Conformation around *N*-Glycosidic Bond

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

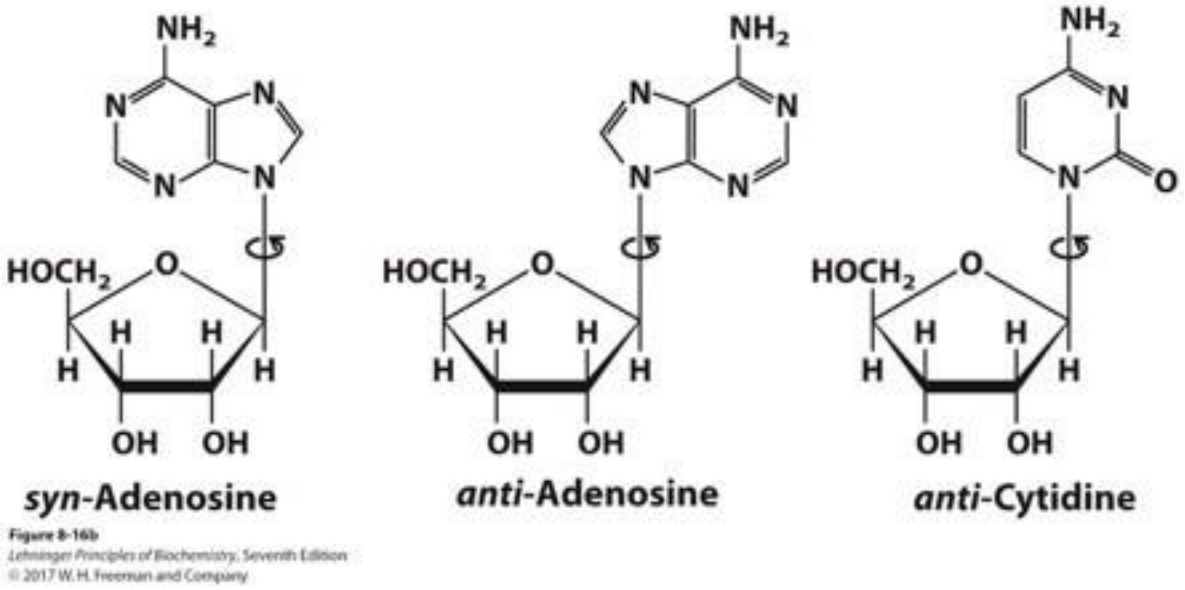

Figure 8-16b
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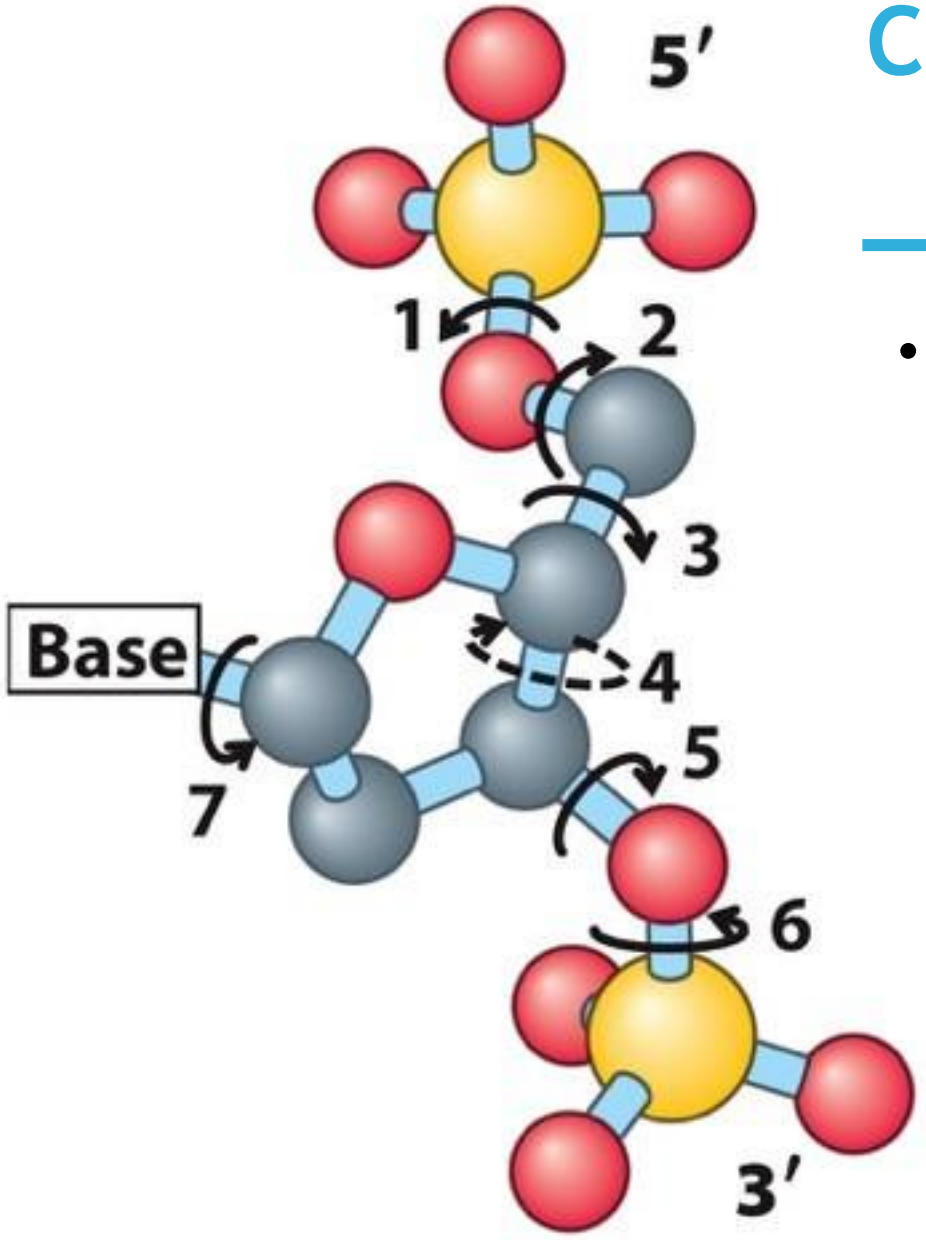
- The sequence of atoms chosen to define this angle is O4'-C1'-N9-C4 for purine, and O4'-C1'-N1-C2 for pyrimidine derivatives.
- Angle near 0° corresponds to *syn* conformation.
- Angle near 180° corresponds to *anti* conformation.
- Anticonformation is found in normal B-DNA.

Conformation Around *N*-Glycosidic Bond



- Ring pucker limits the angle of torsion, *c*, possible for the *N*-glycosidic bond between the nitrogenous base and the pentose.

Figure 8-16a
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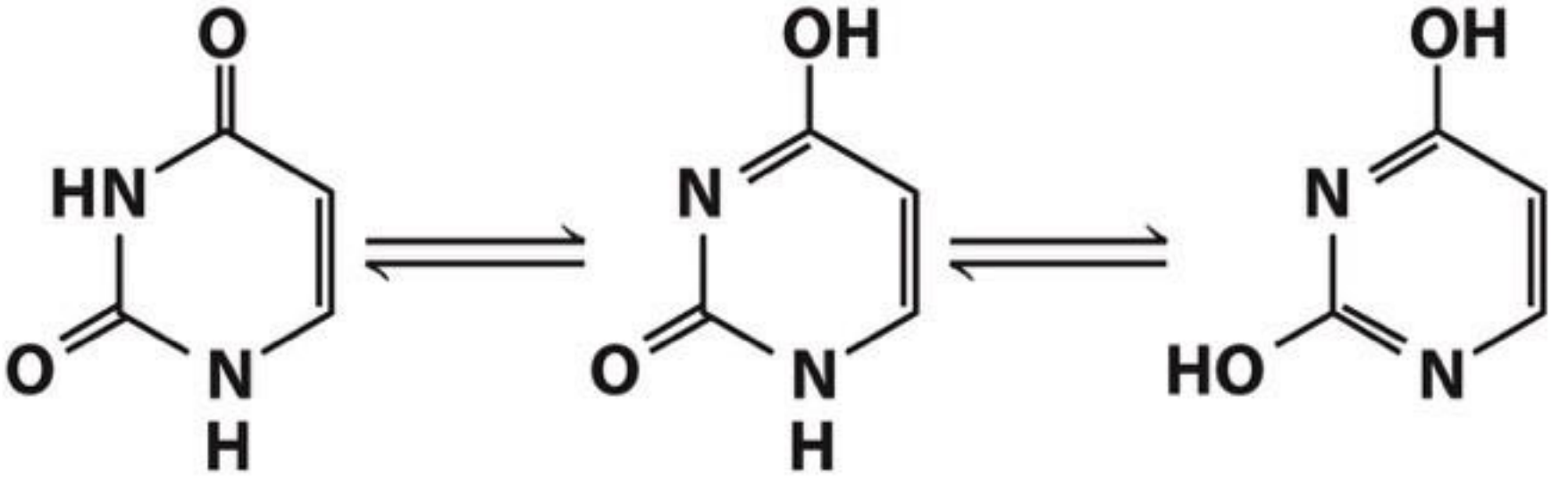
Tautomerism of Nitrogenous Bases

- 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.



Uracil

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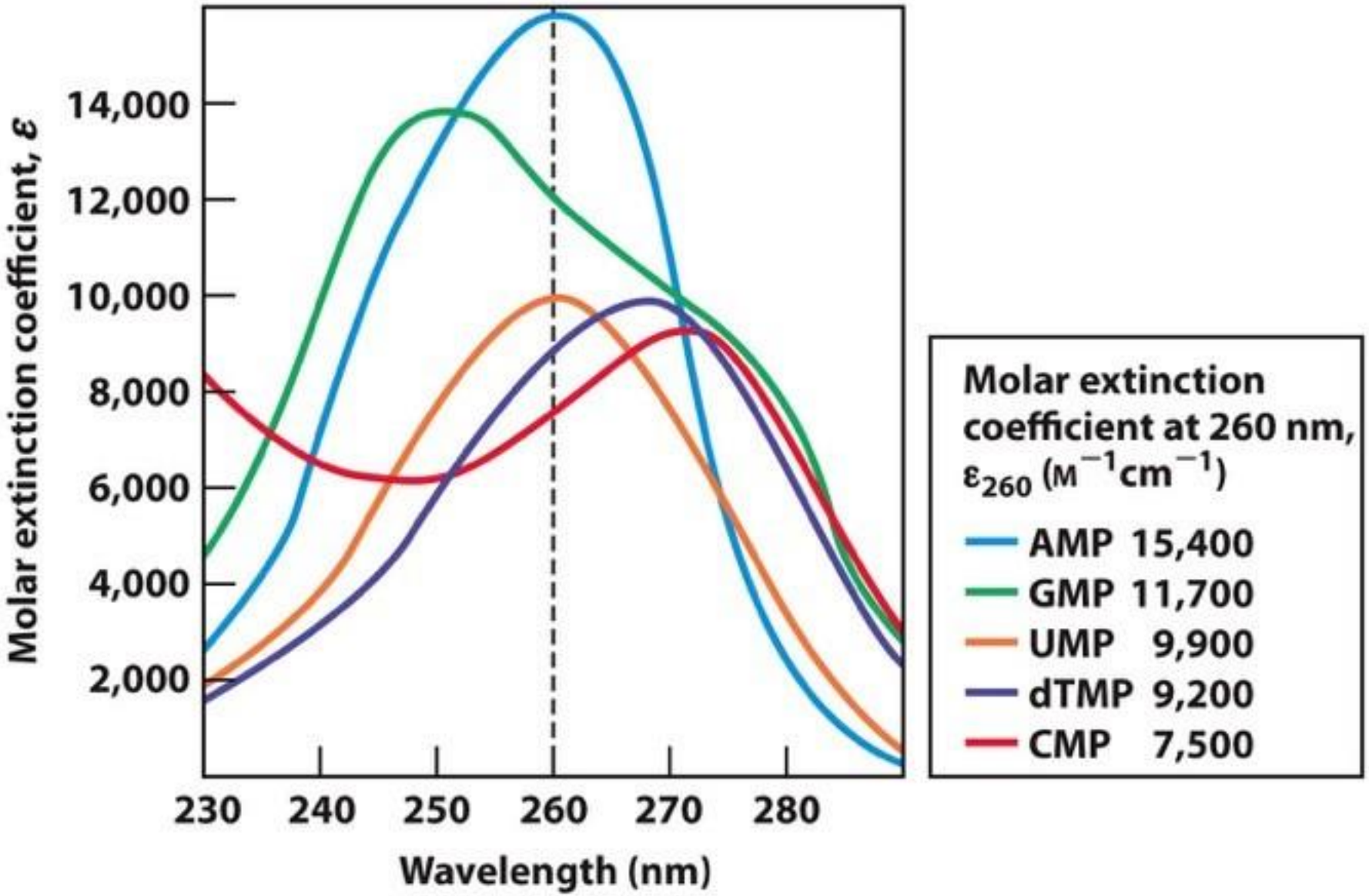
UV Absorption of Nucleobases

- 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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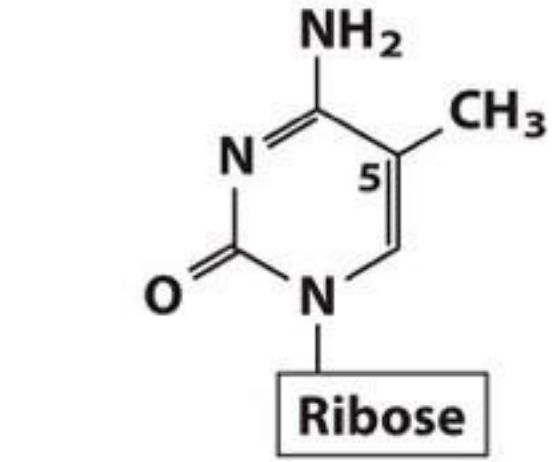
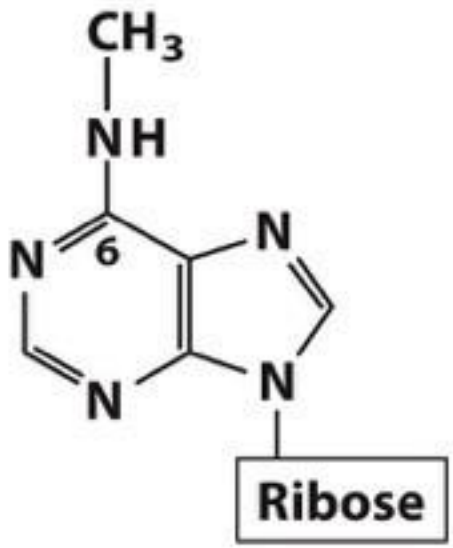
Minor Nucleosides in DNA

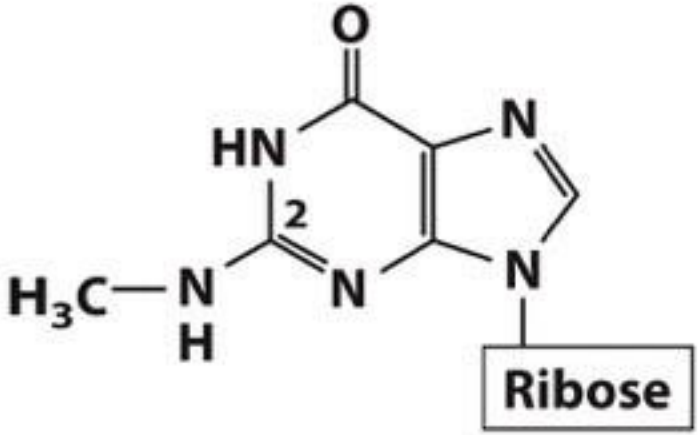
- Modification is done after DNA synthesis.
- 5-Methylcytosine is common in eukaryotes and is also found in bacteria.
- N^6-Methyladenosine is common in bacteria but not found in eukaryotes.
- Epigenetic marker:
- way to mark own DNA so that cells can degrade foreign DNA (prokaryotes)
- way to mark which genes should be active (eukaryotes)
- Could the environment turn genes on and off in an inheritable manner?



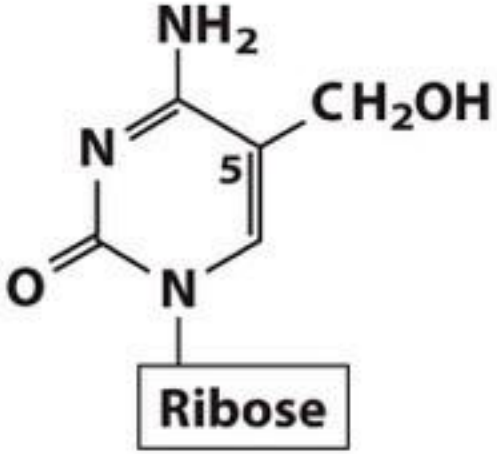
5-Methylcytidine



N^6-Methyladenosine



N^2-Methylguanosine



5-Hydroxymethylcytidine

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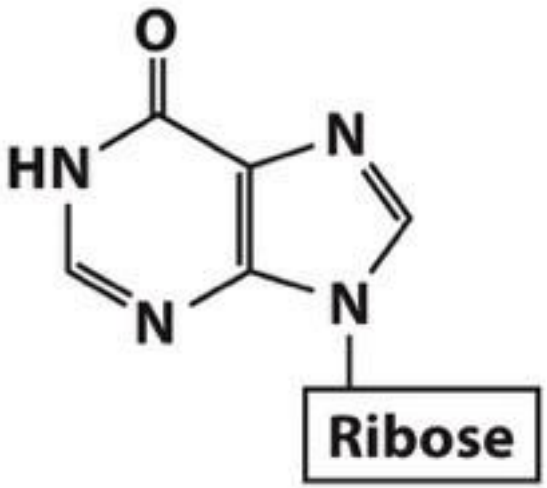
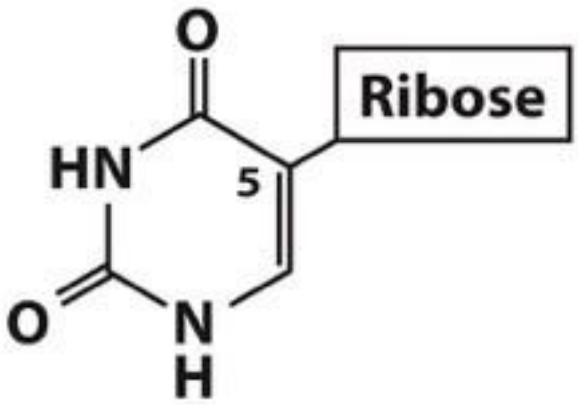
Minor Nucleosides in RNA

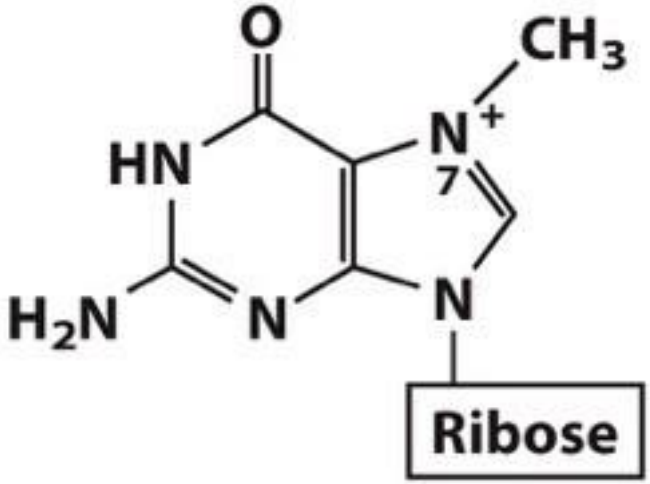
- Inosine is sometimes found in the “wobble position” of the anticodon in tRNA.
  - made by de-aminating adenosine
  - provides richer genetic code
- Pseudouridine (Ψ) is found widely in tRNA and rRNA.
  - more common in eukaryotes but found also in eubacteria
  - made from uridine by enzymatic isomerization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



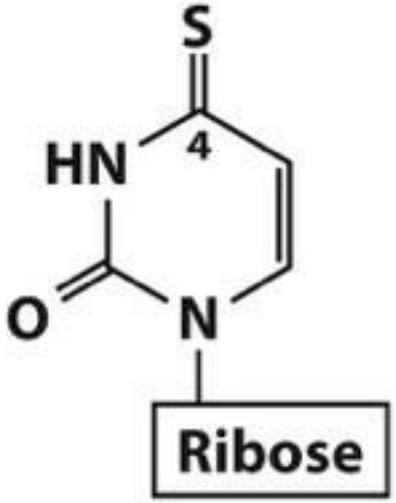
Inosine



Pseudouridine



7-Methylguanosine



4-Thiouridine

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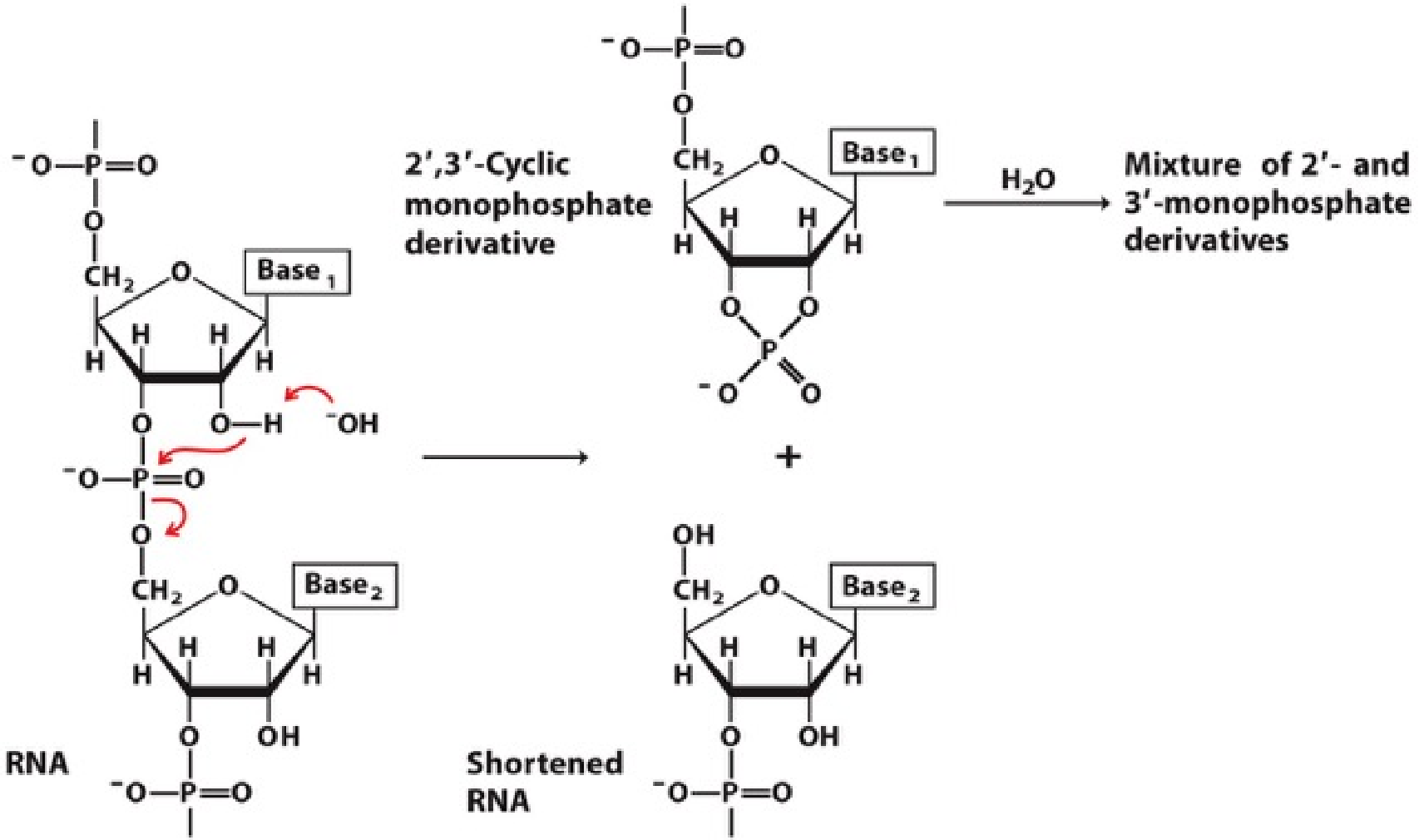
Polynucleotides

- 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'.

Hydrolysis of RNA

- RNA is unstable under alkaline conditions.
- Hydrolysis is also catalyzed by enzymes (RNase).
- RNase enzymes are abundant around us.
  - S-RNase in plants prevents inbreeding.
  - RNase P is a ribozyme (enzyme made of RNA) that processes tRNA precursors.
  - Dicer is an enzyme that cleaves double-stranded RNA into oligonucleotides.
    - protection from viral genomes
    - RNA interference technology

Mechanism of Base-catalyzed RNA Hydrolysis



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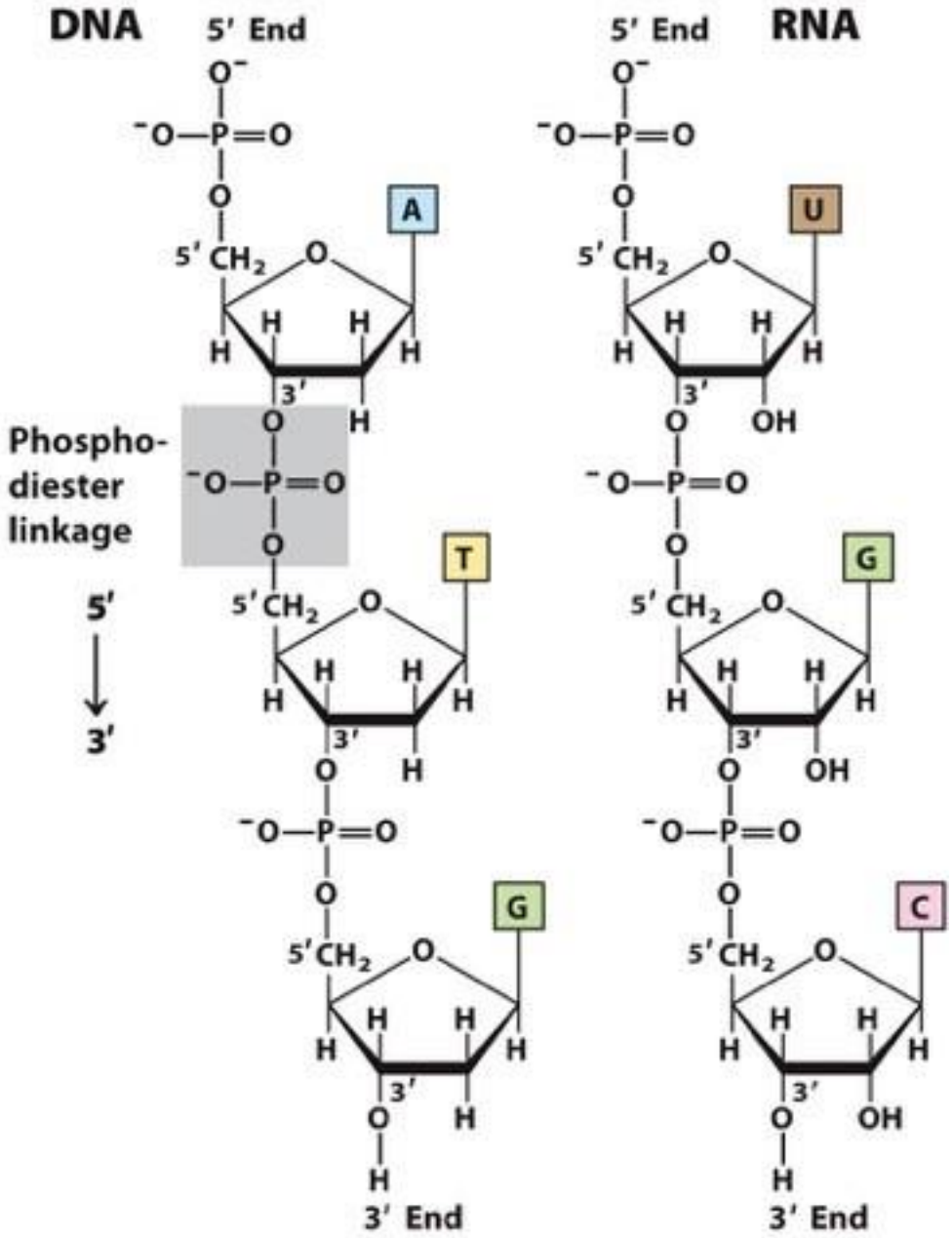

Figure 8-7

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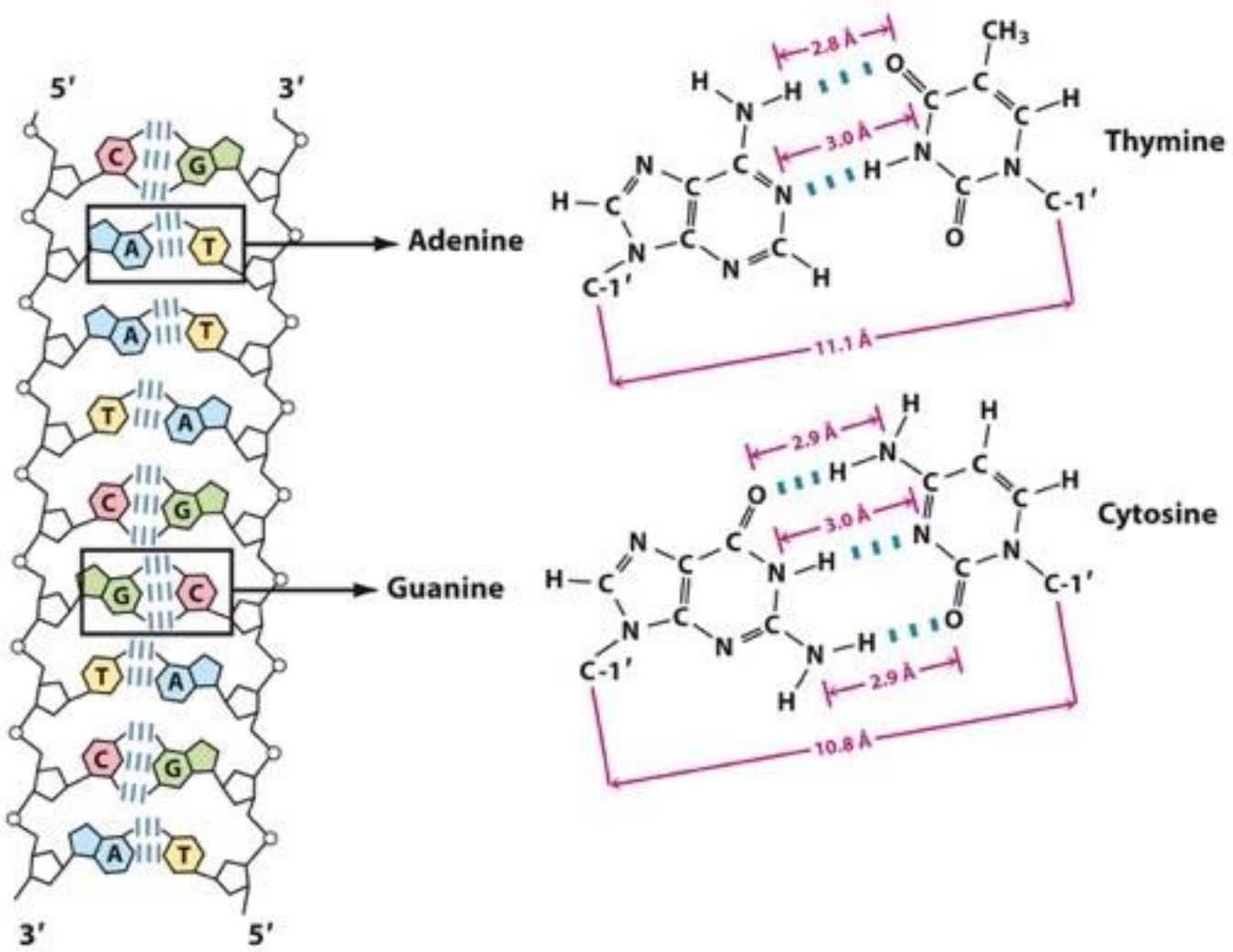
Hydrogen-Bonding Interactions

- 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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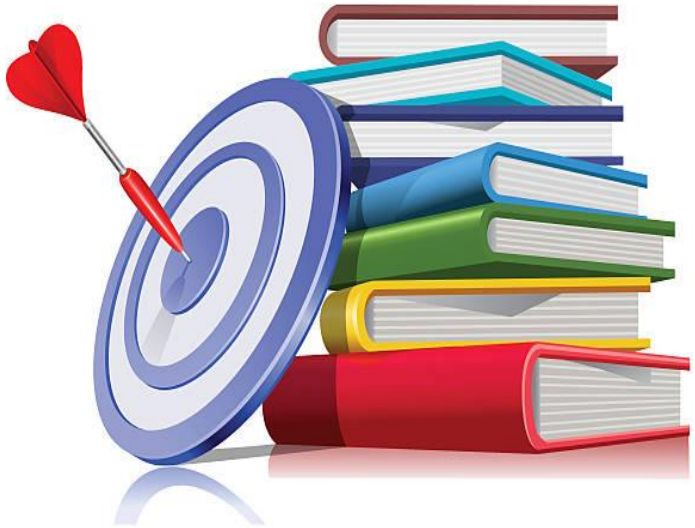
WEEK 1 LECTURE 2

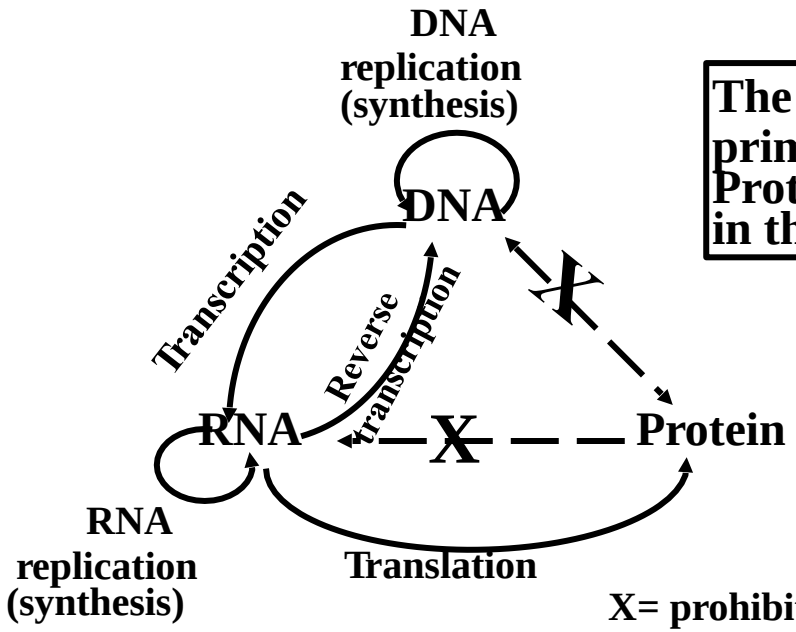
Nucleic Acids

Learning goals:

- Biological function of nucleotides and nucleic acids
- Structures of common nucleotides
- Structure of double-stranded DNA
- Structures of ribonucleic acids
- Denaturation and annealing of DNA
- Chemistry of nucleic acids; mutagenesis



Life's Molecular Trinity



The role of DNA or RNAis primarily to store information. Proteins are the major actors in the biochemistry of the cell.

X= prohibited information transfer

The general statement that information is transferred from DNA to RNA to protein is referred to as the *CENTRAL DOGMA OF MOLECULAR BIOLOGY*

Discovery of DNA Structure

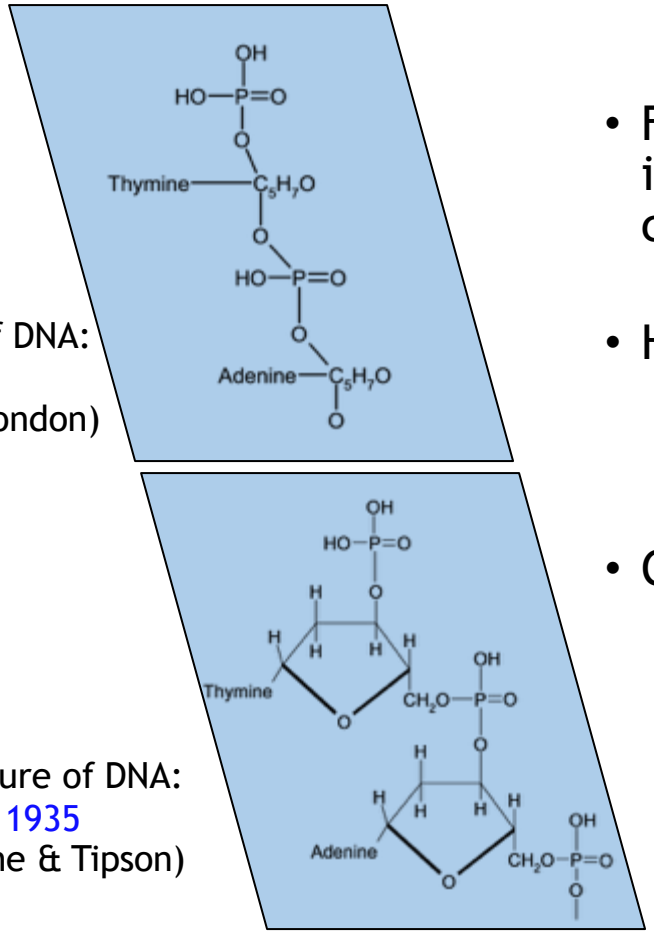
- One of the most important discoveries in biology
- Why is this important?

  "*This structure has novel features which are of considerable biological interest.*"

  —Watson and Crick, *Nature*, 1953

- Good illustration of science in action
  - missteps in the path to a discovery
  - value of knowledge
  - value of collaboration
  - cost of sharing your data too early

Covalent Structure of DNA (1868–1935)



Structure of DNA:
1929
(Levene & London)

Structure of DNA:
1935
(Levene & Tipson)

- Friedrich Miescher isolates “nuclein” from cell nuclei
- Hydrolysis of nuclein
  - phosphate
  - pentose
  - and a nitrogenous base
- Chemical analysis
  - phosphodiester linkages
  - pentose is ribofuranoside

Road to the Double Helix

- Watson and Crick
  - Missing layer means alternating pattern (major and minor groove)
  - Hydrogen bonding: A pairs with T

    G pairs with C

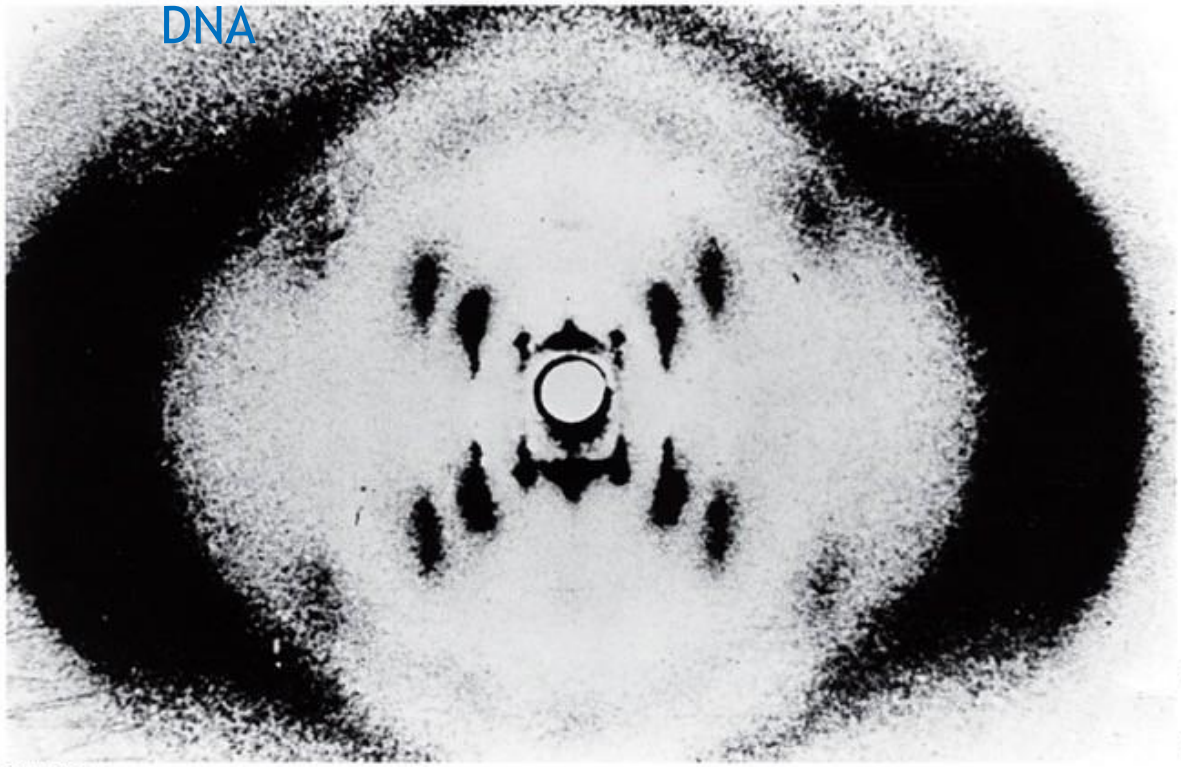
  Double helix fits the data!

- Franklin and Wilkins
  - "Cross" means helix
  - "Diamonds" mean that the phosphate-sugar backbone is outside
  - Calculated helical parameters

Watson, Crick, and Wilkins shared the 1962 Nobel Prize.

Franklin died in 1958.

X-RAY DIFFRACTION PHOTO OF THE DNA



Science Source

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James D. Watson

Francis Crick, 1916–2004

UPI/Bettmann/Corbis

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Science Source

Rosalind Franklin, 1920–1958

UPI/Bettmann/Corbis

Maurice Wilkins, 1916–2004

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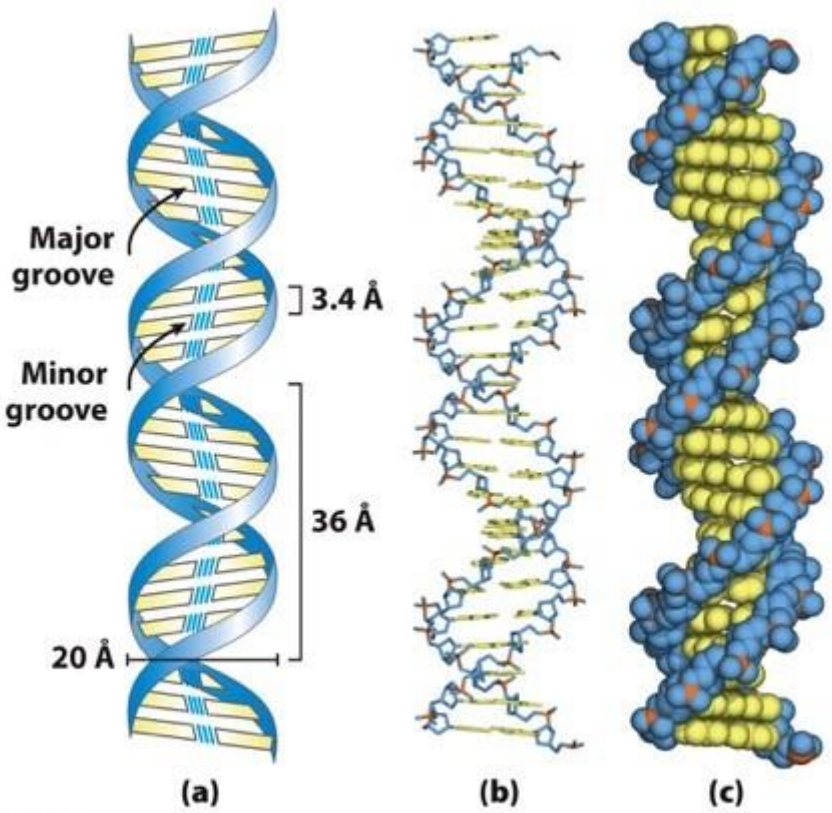
Watson-Crick Model of B-DNA



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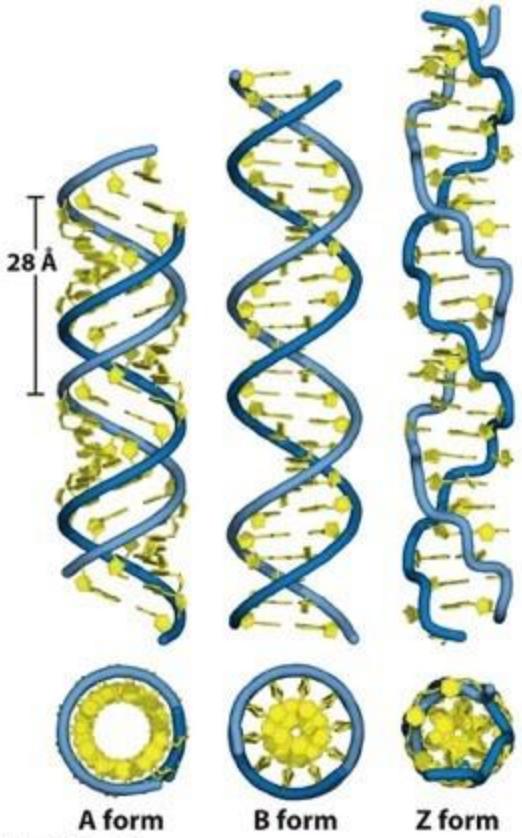
Other Forms of DNA



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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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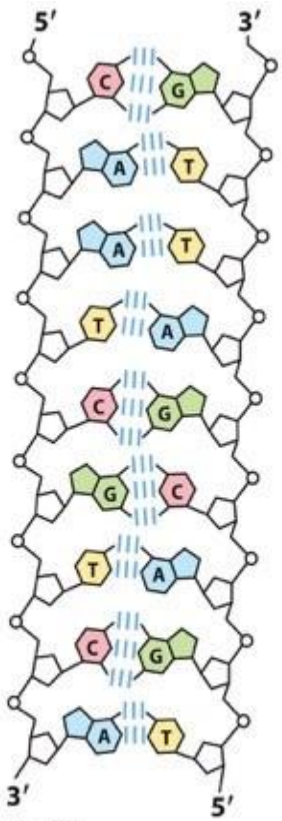
Complementarity of DNA Strands

- 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



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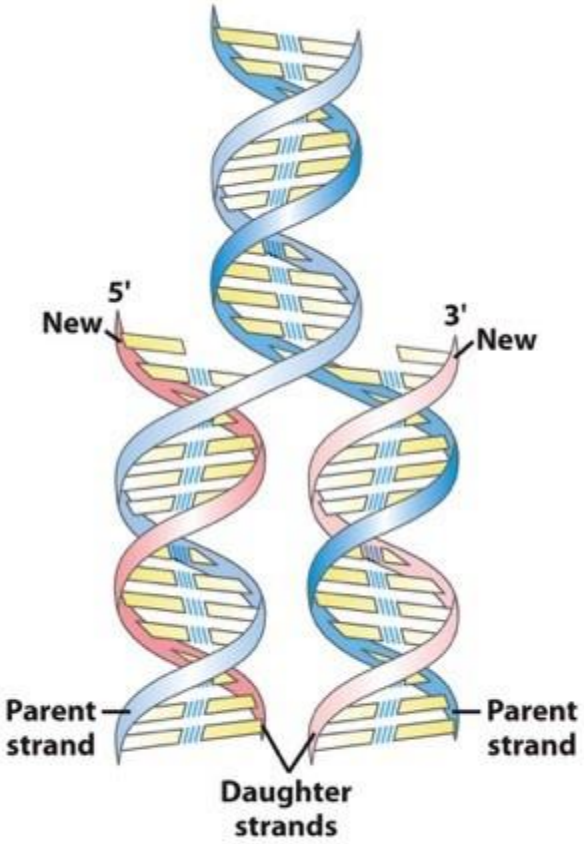
Replication of Genetic Code



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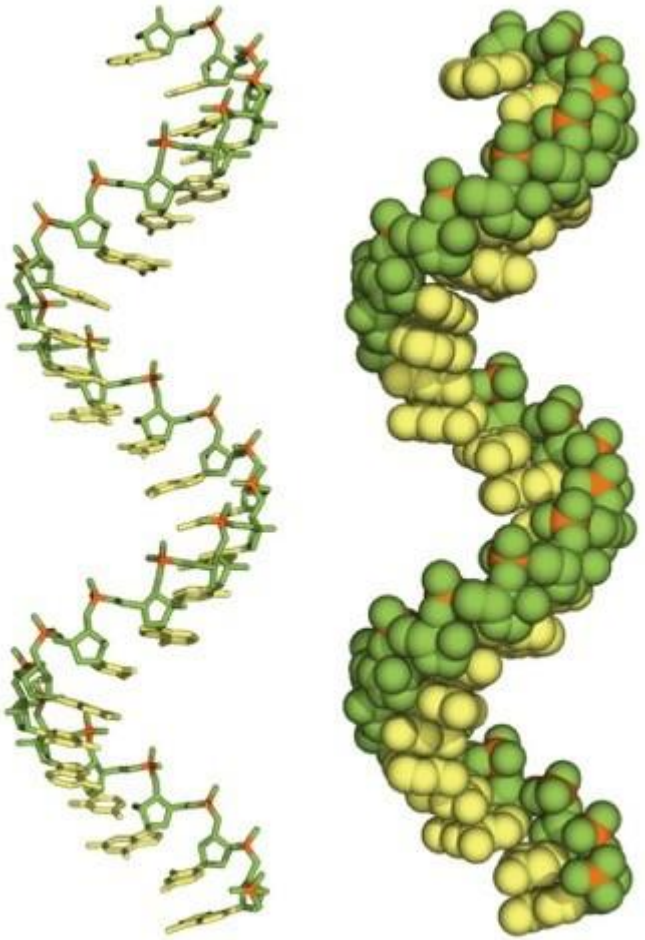
- Strand separation occurs first.
- Each strand serves as a template for the synthesis of a new strand.
- Synthesis is catalyzed by enzymes known as DNA polymerases.
- A newly made DNA molecule has one daughter strand and one parent strand.

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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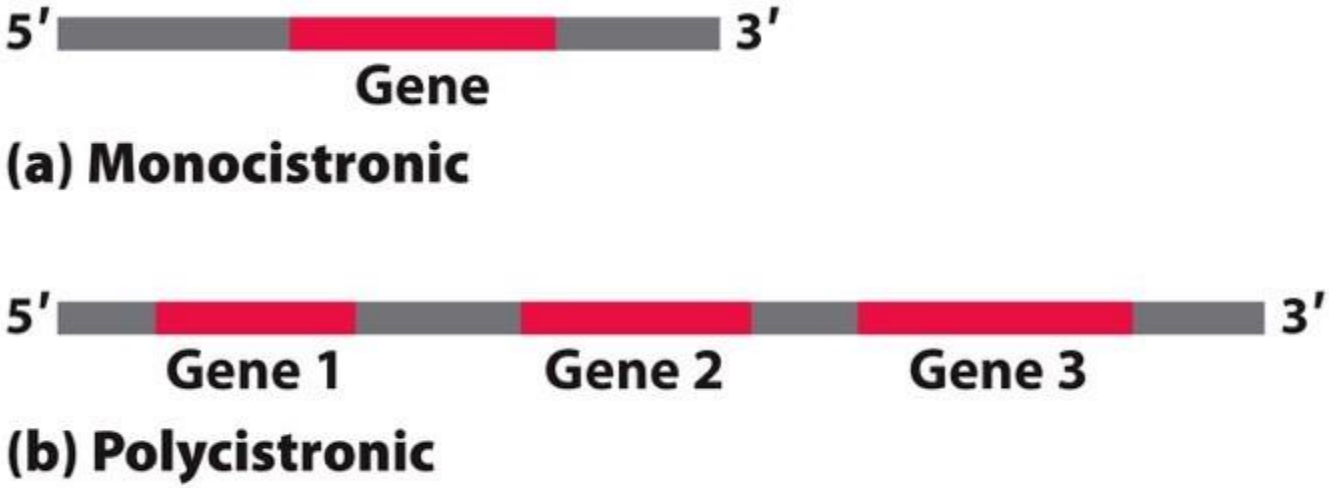

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Palindromic Sequences Can Form Hairpins and Cruciforms

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

Civic

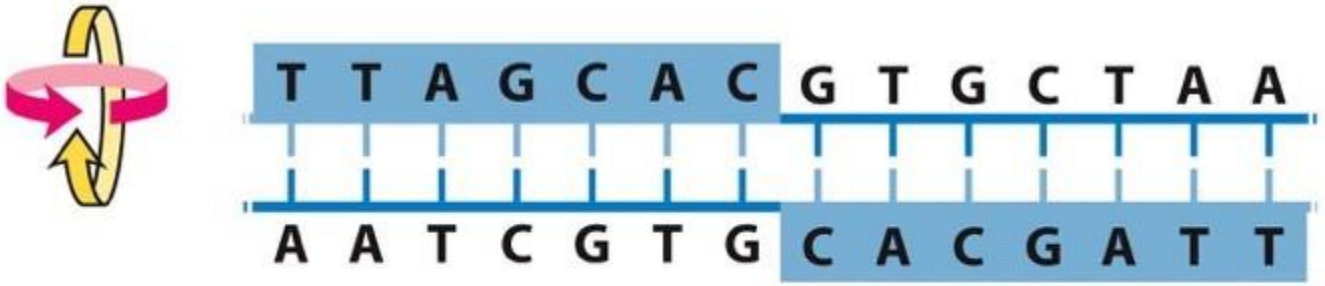
Racecar

Rotator

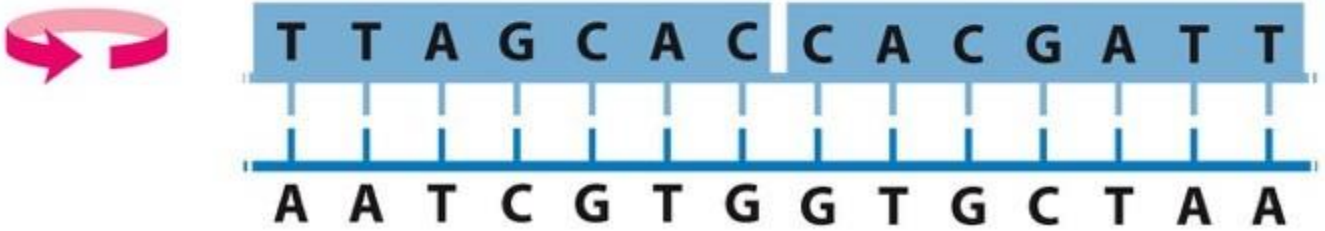
Saippuakuppinippukauppias (Finnish word for "soap cup batch trader")

Νίψον ἀνομήματα, μὴ μόναν ὄψιν (ancient Greek fountain text: "Wash the sin as well as the face")

Palindrome



Mirror repeat



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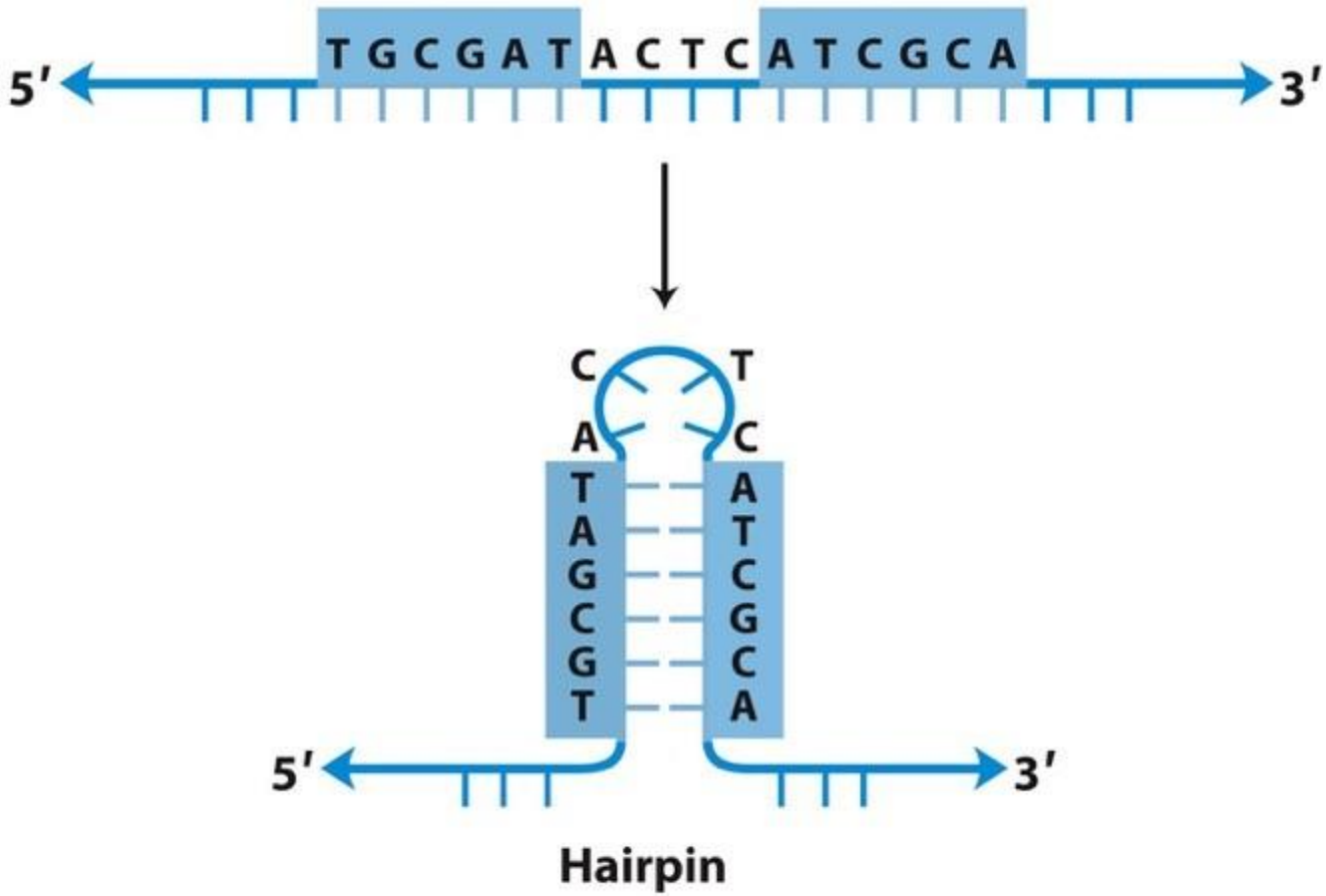

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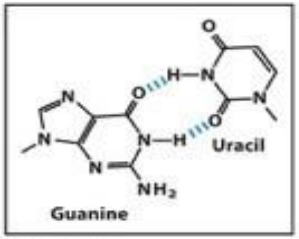
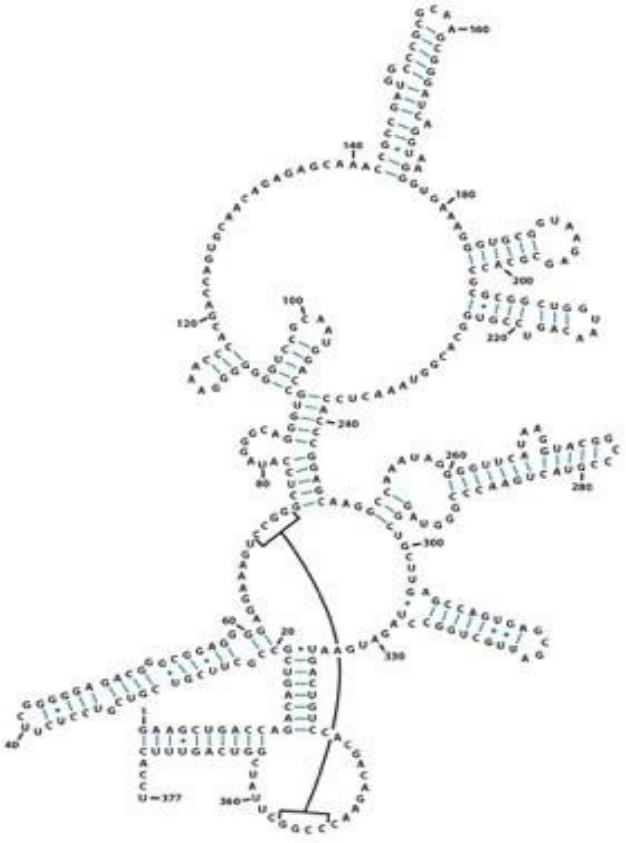
RNA Molecules Have Quite Complex Structures





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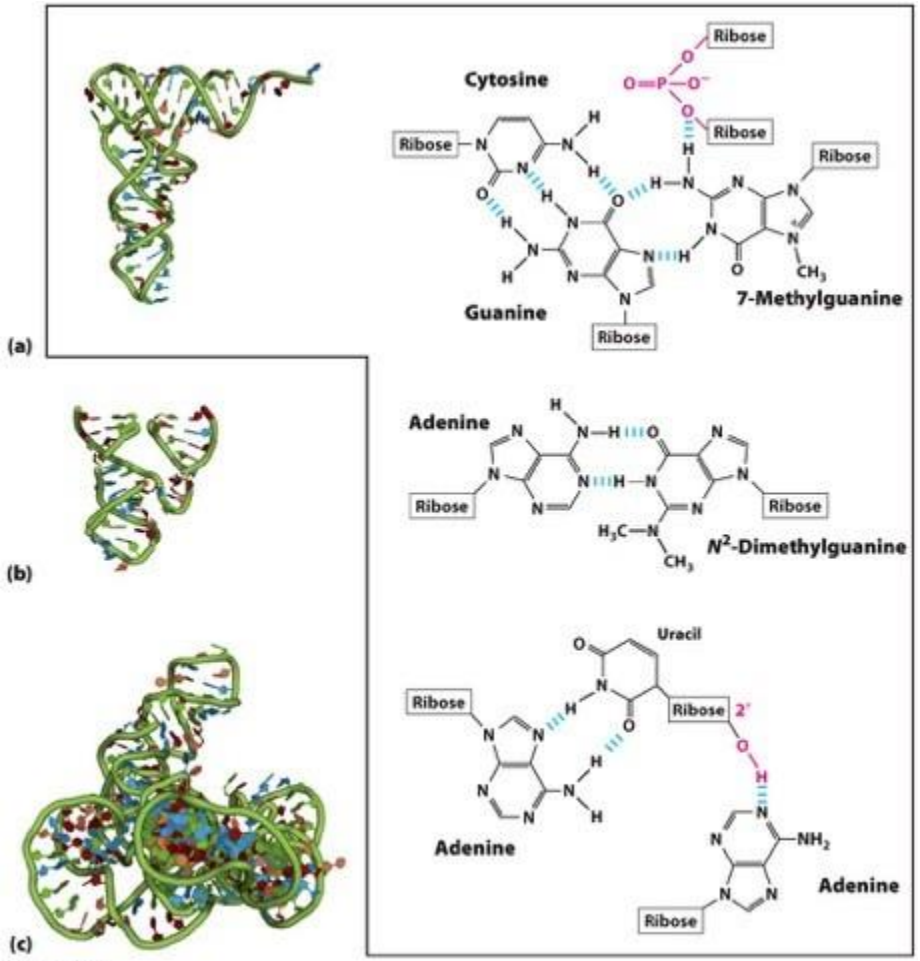
Complex Structures Are Stabilized by Non-Watson-Crick Base-Pair Interactions



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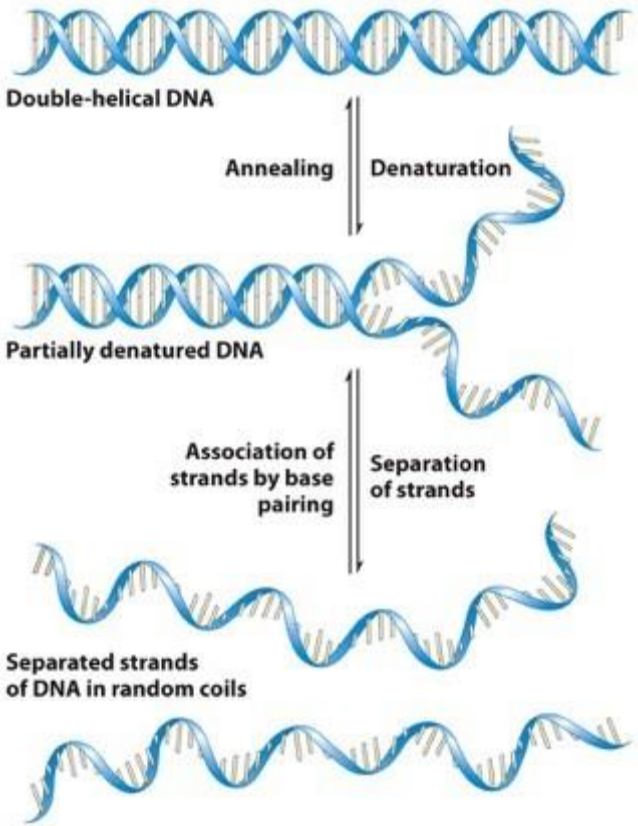
DNA Denaturation

- 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.



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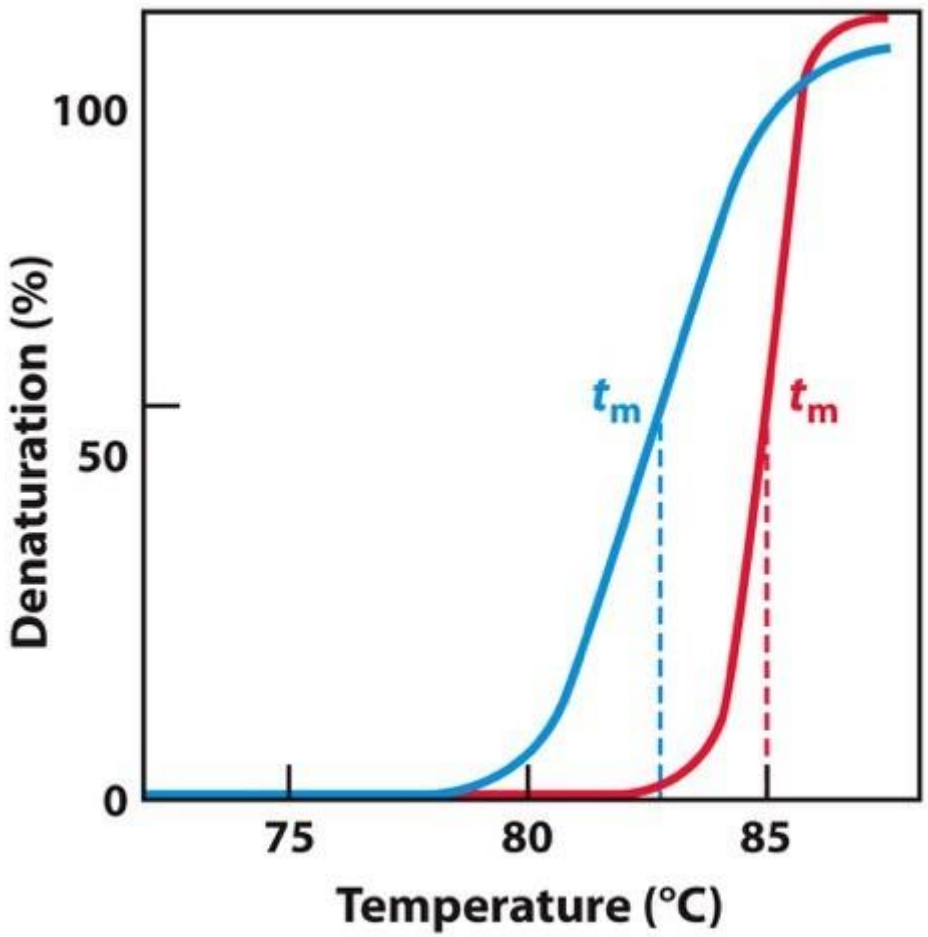
Thermal DNA Denaturation (Melting)

- 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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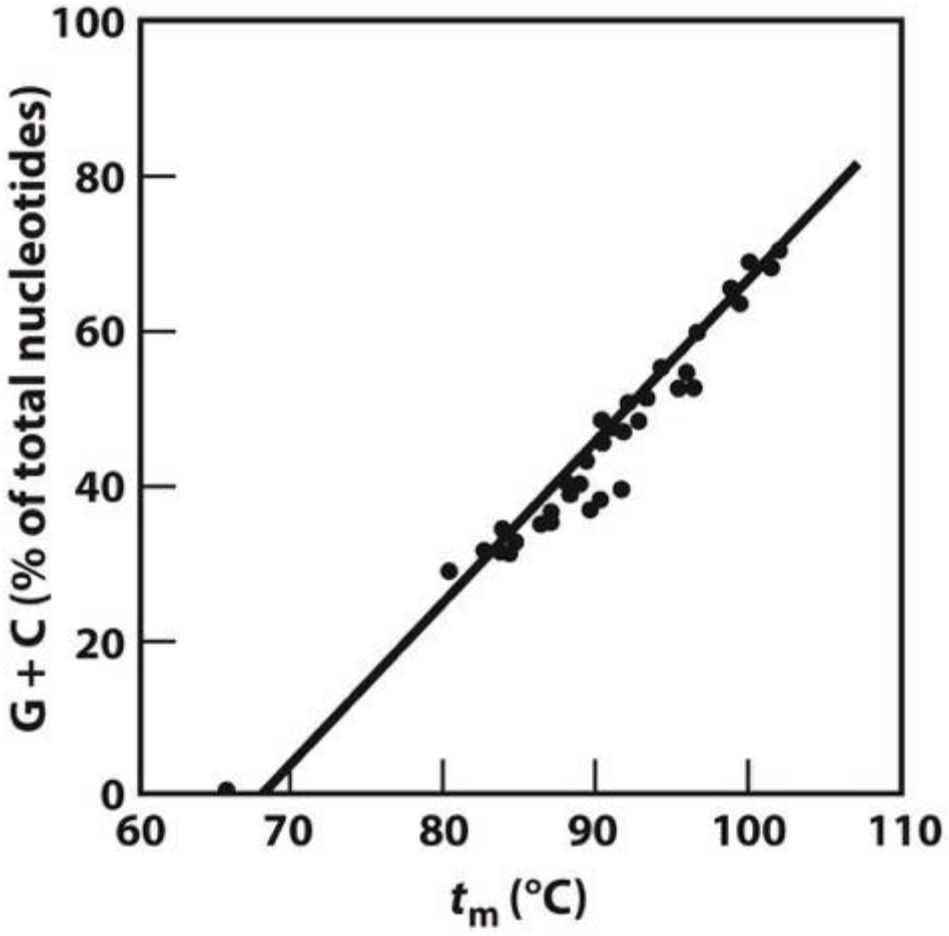
Factors Affecting DNA Denaturation

- 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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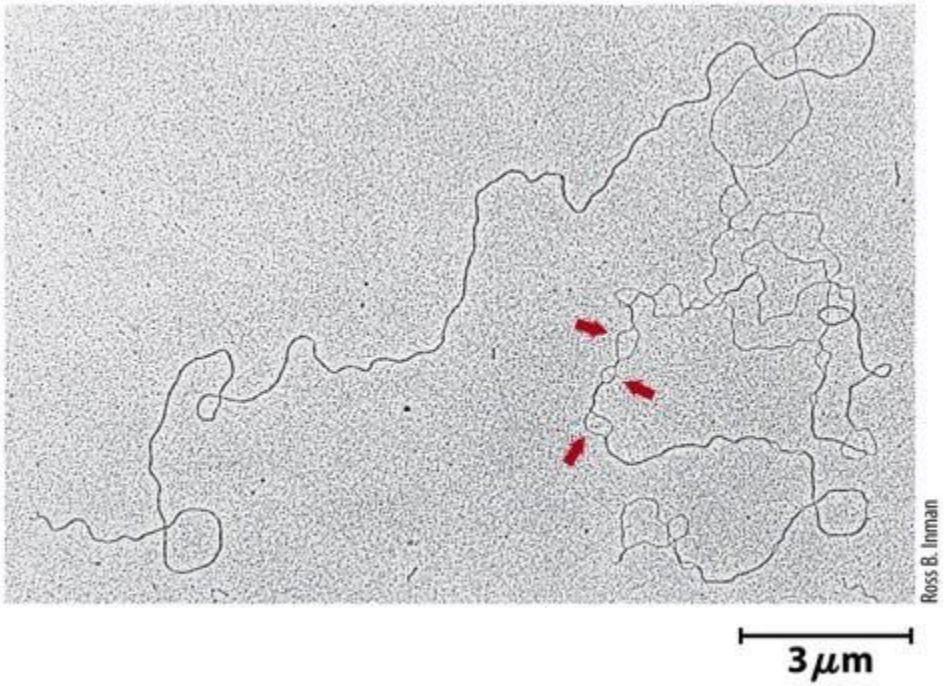
Denaturation of Large DNA Molecules Is Not Uniform

AT-rich regions melt at a lower temperature than GC-rich regions.



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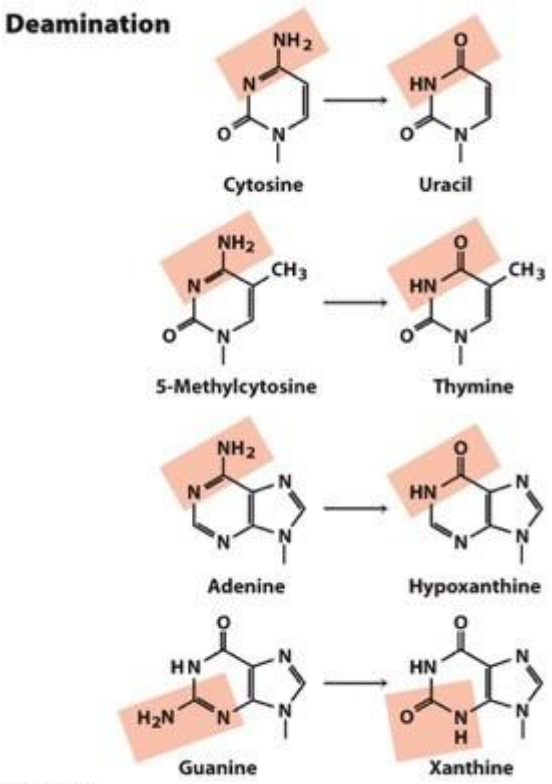
Applications of Near-Complementary Hybrids in DNA and RNA

- Detection of a specific DNA molecule in complex mixture
  - radioactive detection
  - fluorescent DNA chips
- Amplification of specific DNA
  - polymerase chain reaction
  - site-directed mutagenesis
- Evolutionary relationships
- Antisense therapy

Molecular Mechanisms of Spontaneous Mutagenesis: Deamination

- Deamination
  - very slow reactions
  - large number of residues
  - The net effect is significant: 100 C → U events/day in a mammalian cell.



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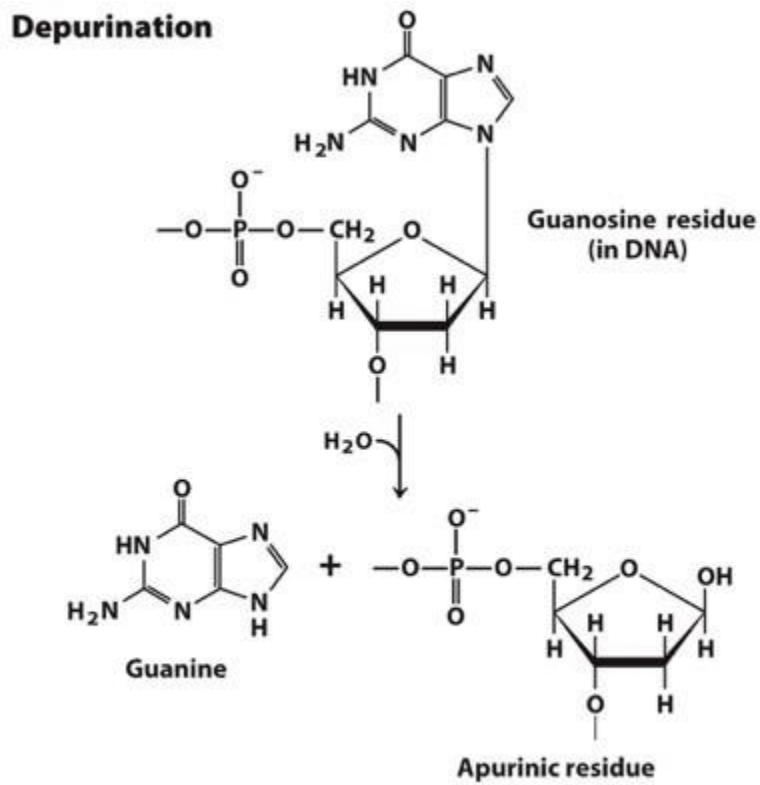
Molecular Mechanisms of Spontaneous Mutagenesis: Depurination

- Depurination
  - *N-glycosidic bond is hydrolyzed*
  - Significant for purines: 10,000 purines lost/day in a mammalian cell



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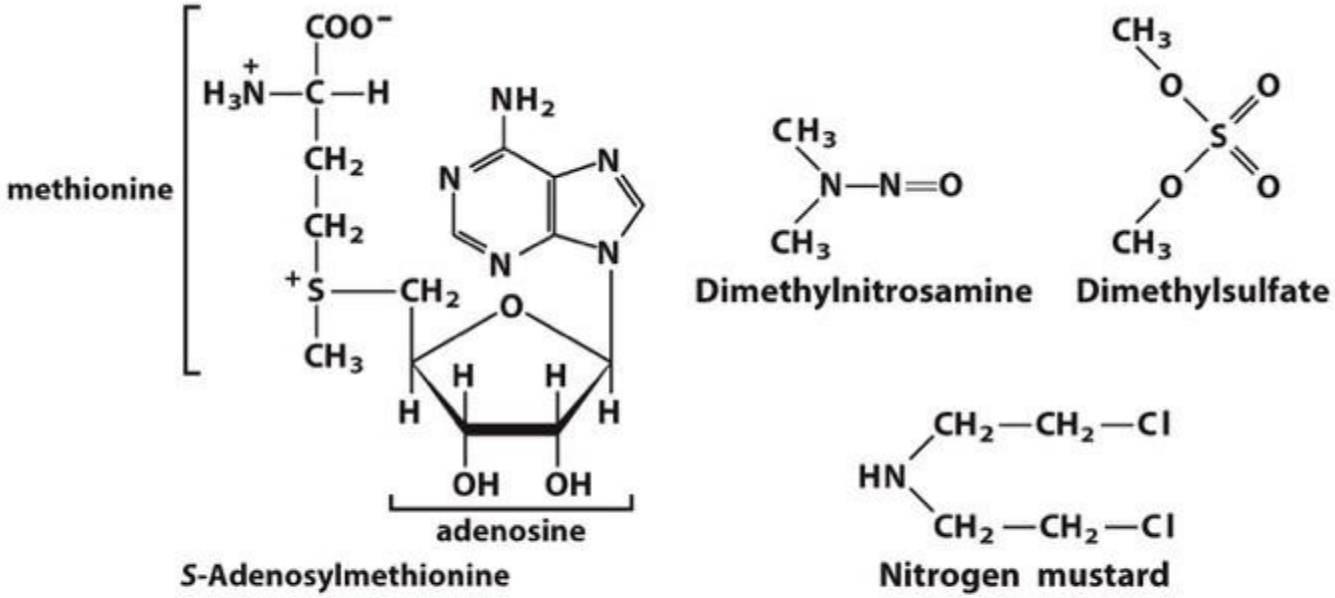
Molecular Mechanisms of Oxidative and Chemical Mutagenesis

- Oxidative damage
- hydroxylation of guanine
- mitochondrial DNA is most susceptible

- Chemical alkylation
- methylation of guanine

- Cells have mechanisms to correct most of these modifications.



Alkylating agents

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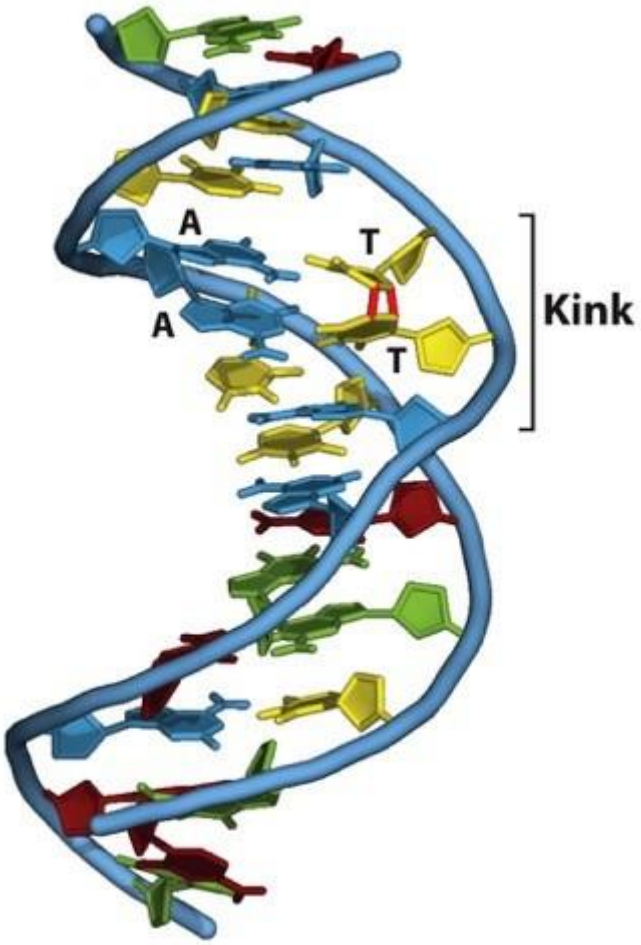
Molecular Mechanisms of Radiation-Induced Mutagenesis

- 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.



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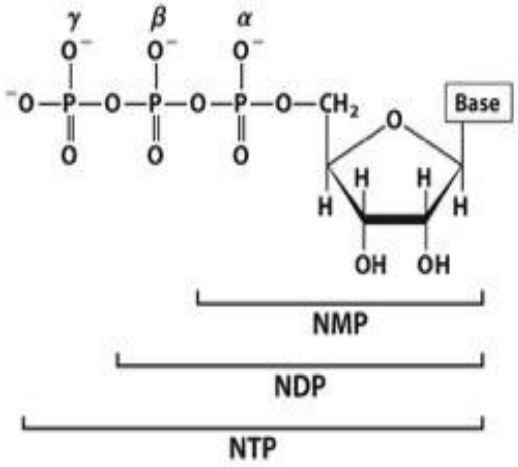
Other Functions of Nucleotides: Energy Source



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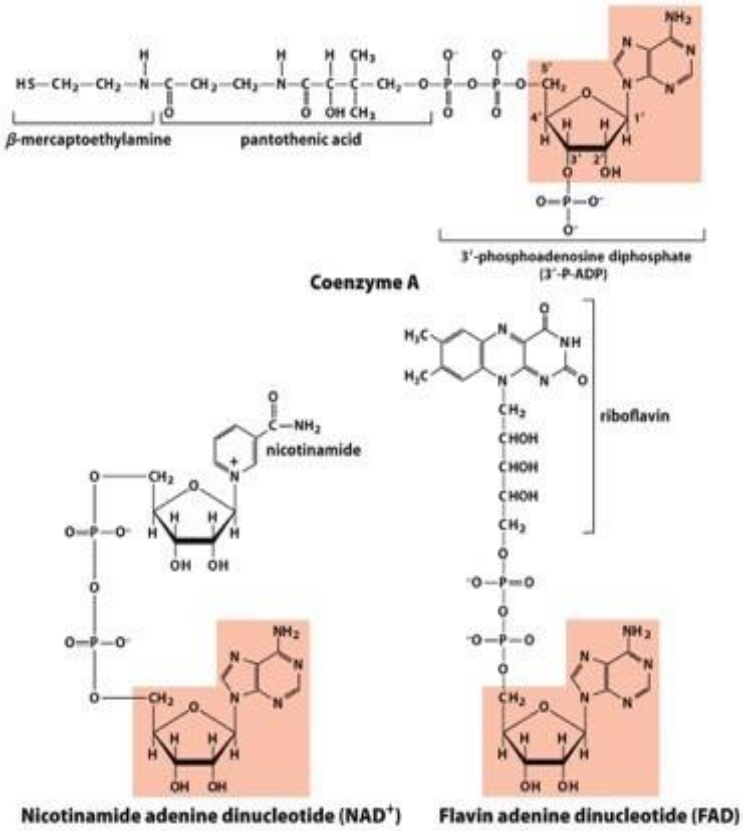
Abbreviations of ribonucleoside 5′-phosphates			
Base	Mono-	Di-	Tri-
Adenine	AMP	ADP	ATP
Guanine	GMP	GDP	GTP
Cytosine	CMP	CDP	CTP
Uracil	UMP	UDP	UTP

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

Other Functions of Nucleotides: Coenzymes



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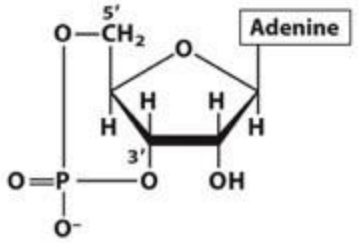
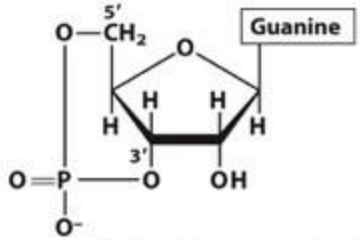
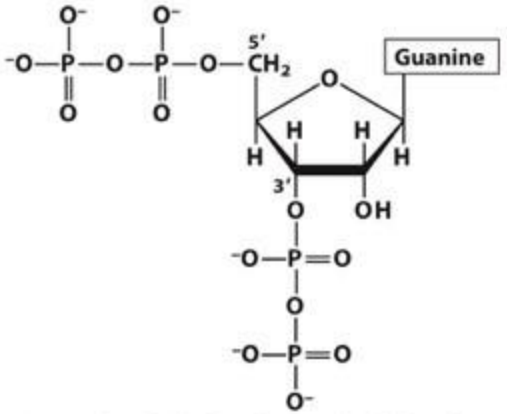
Other Functions of Nucleotides: Regulatory Molecules



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



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



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

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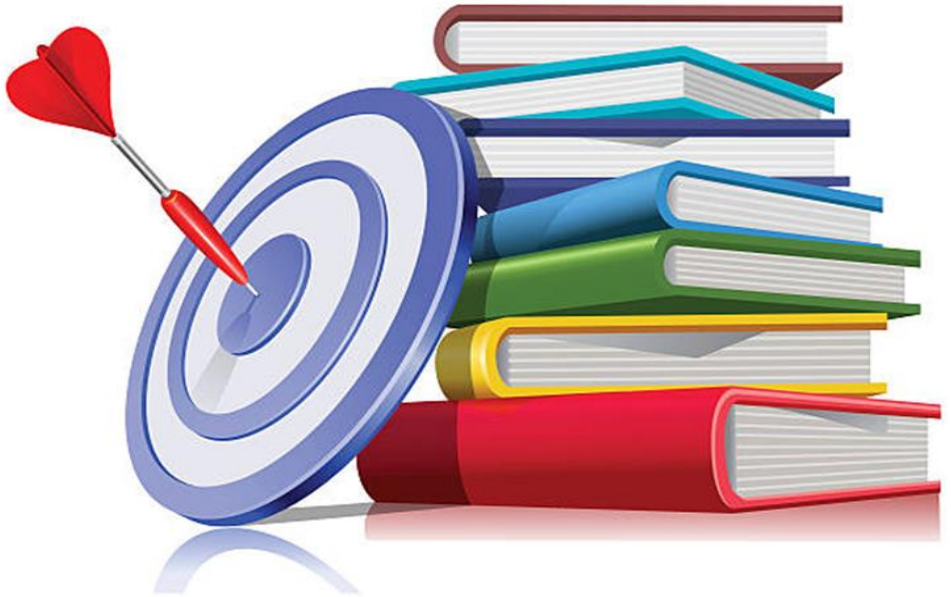
DNA Replication and Repair

Learning goals:

- DNA replication
- DNA repair



What Is DNA Metabolism?

- Although DNA provides stable storage of genetic information, the structure is far from static:
  - A new copy of DNA is synthesized with high fidelity before each cell division.
  - Errors that arise during or after DNA synthesis are constantly checked for, and repairs are made.
  - Segments of DNA are rearranged either within a chromosome or between two DNA molecules (recombination), giving offspring a novel DNA.
- DNA metabolism consists of a set of tightly regulated processes that achieve these tasks.

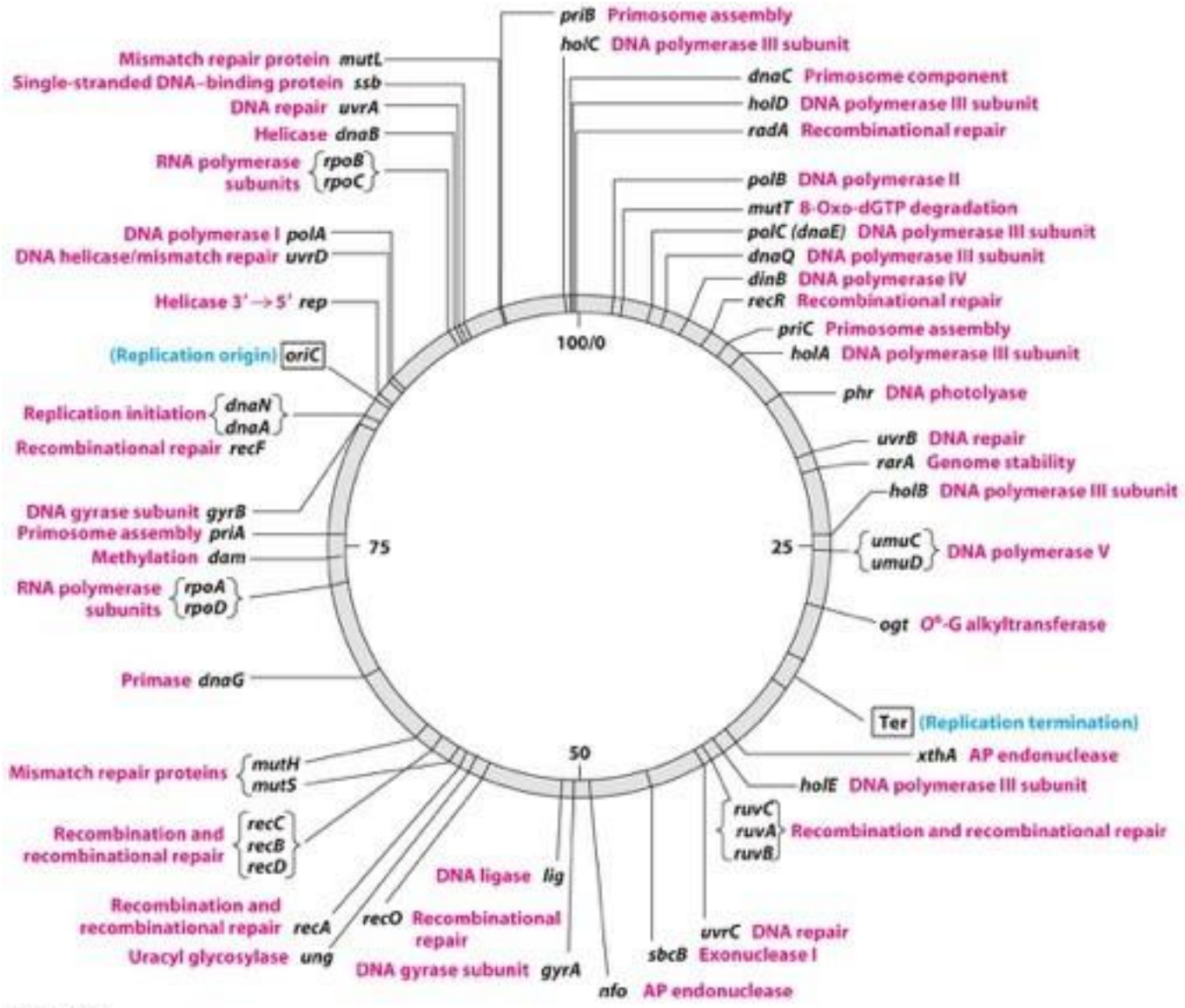
Bacterial Gene Naming

- Three *italicized* lowercase letters
- Example: *uvr*
- Name usually reflects function
  - *uvr* encodes gene for resistance to UV radiation
- Capital letters added to abbreviation reflect order of discovery, not enzymatic order
  - *dnaA*, *dnaB* genes for replication

Bacterial Protein Naming

- Often named after their genes
- Nonitalicized, roman type
- First letter capitalized
- Example: DnaA is the protein encoded by the gene *dnaA*.

Map of the *E. Coli* Chromosome



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Eukaryotic Gene Naming

- Not universal for all eukaryotic organisms
- Can vary with each species
- Name usually reflects function
- In *Saccharomyces cerevisiae*, gene names typically three uppercase italicized letters followed by a number
  - *COX1* – gene that codes for a subunit of cytochrome oxidase

Eukaryotic Protein Naming

- Complex and varies for each species
- May have the same name as the gene but the case of the letters is different
  - In *S. cerevisiae*, the protein has the first letter capitalized followed by two lower case letters, the number, and the letter "p."
  - Example: *RAD51* (gene) – Rad51p (protein)

DNA Replication Properties

- **What is DNA Replication?**

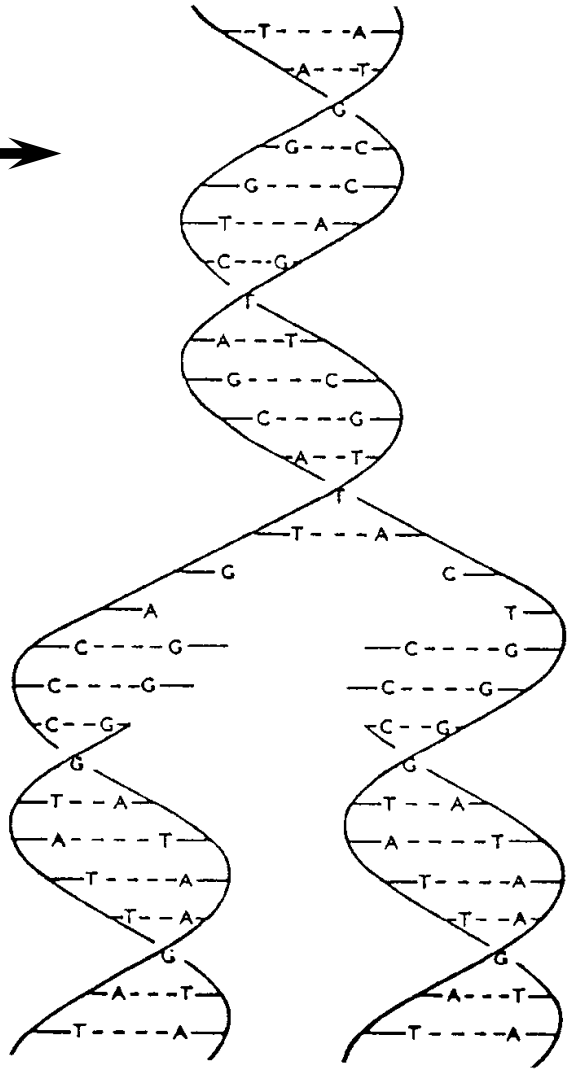
- **Three fundamental rules of replication**
  - Replication is semiconservative
  - Replication begins at an origin and proceeds (usually) bidirectionally
  - Synthesis of new DNA occurs in the 5'→3' direction and is semidiscontinuous

DNA Replication is Semi-conservative

Parental DNA strands

Each of the parental strands serves as a template for a daughter strand

Daughter DNA strands



DNA replication is said to be “semi-conservative” because each strand of the DNA double helix serves as a template for the synthesis of a new complementary DNA strand. Hence, the newly replicated chromosome consists of one old and one new DNA strand

DNA Replication Is Semiconservative

- The Meselson-Stahl experiment (next slide) showed that the nitrogen used for the synthesis of new dsDNA becomes *equally divided between the two daughter genomes*.
- This result suggested a semiconservative replication mechanism.
  - Each new DNA has one old (parent) strand and one new (daughter) strand.

The Meselson-Stahl Experiment

- Proved the hypothesis of semiconservative replication proposed by Watson and Crick
- Cells were grown on a medium containing only ^{15}N isotope (heavy N) until fully labeled.
  - produced **ONE** band when DNA is centrifuged in CsCl
- Cells were then switched to ^{14}N medium and allowed to divide once.
  - ONE band but at a higher position than ^{15}N DNA, but lower than completely ^{14}N DNA (→hybrid DNA)
- Cells were allowed to divide once more.
  - TWO bands, one with all ^{14}N DNA, one hybrid

The Meselson-Stahl Experiment

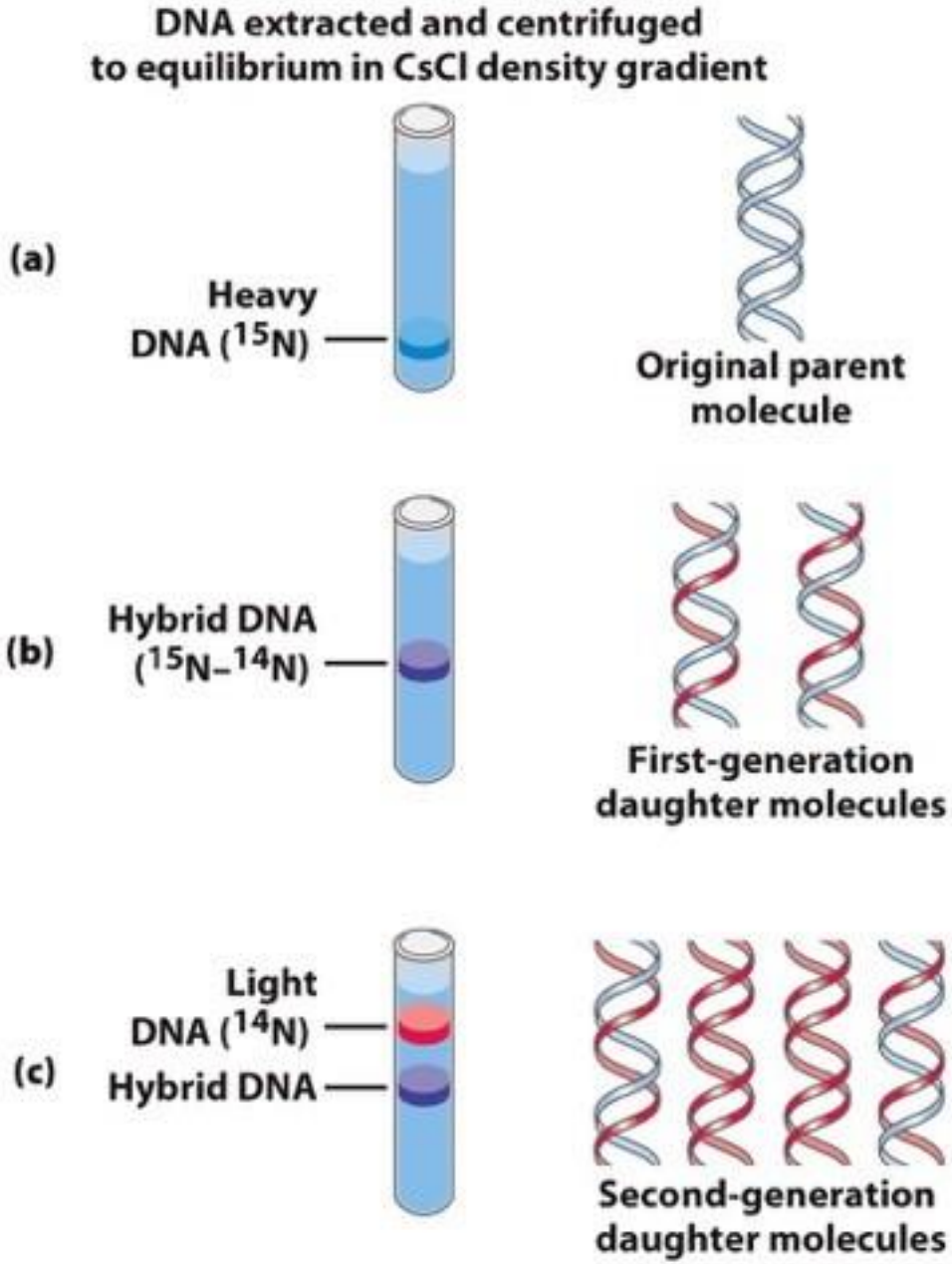


Figure 25-2
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How Did This Experiment Prove Semiconservative Replication?

- Alternative hypothesis: conservative replication (two daughter strands would be completely new DNA)

→ There would have been no intermediate hybrid band if this hypothesis were true.

Review of Semiconservative Replication

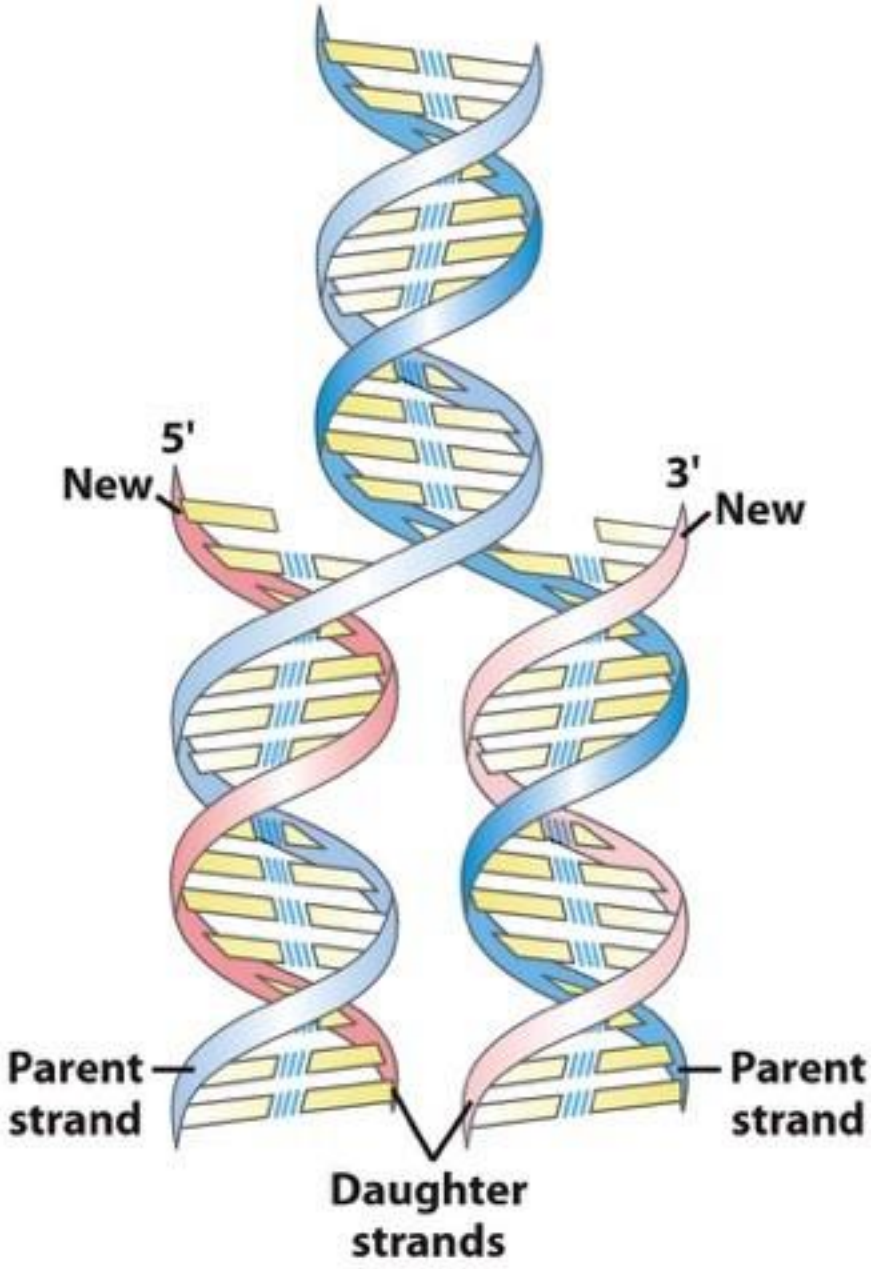

Figure 8-15
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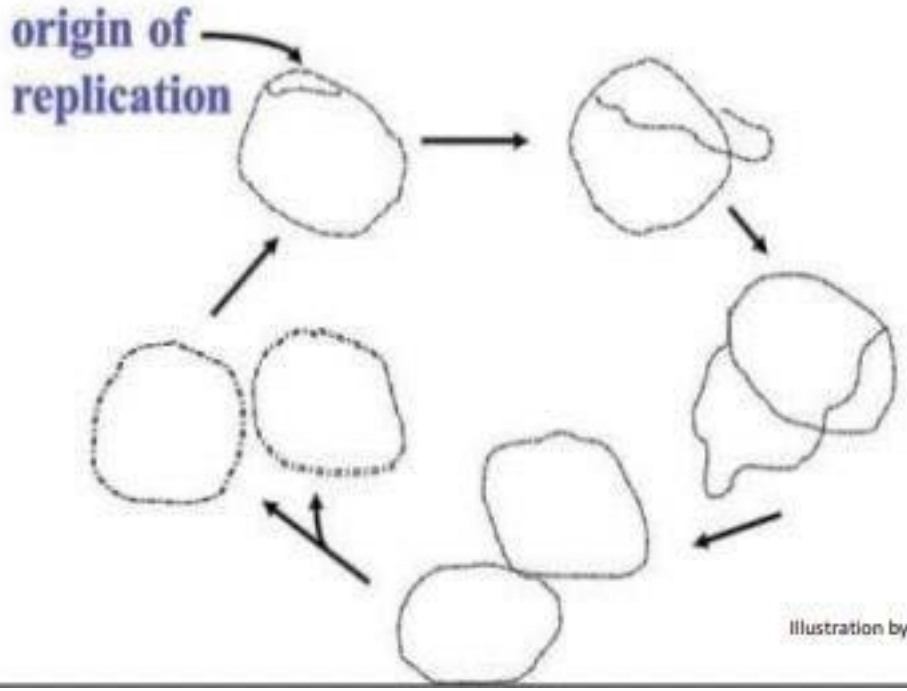
Replication of Circular DNA Is Bidirectional

Once replication begins, does it proceed in the same or opposite directions?

- **Cairns' experiments**—DNA was radiolabeled by growing cells in ^{3}H (tritium); DNA was isolated and spread under a photographic emulsion
  - showed circular DNAs with an extra loop
  - showed that both strands are replicated simultaneously
  - two replication forks, so bidirectional replication

Cairn's interpretation of 'theta' images:

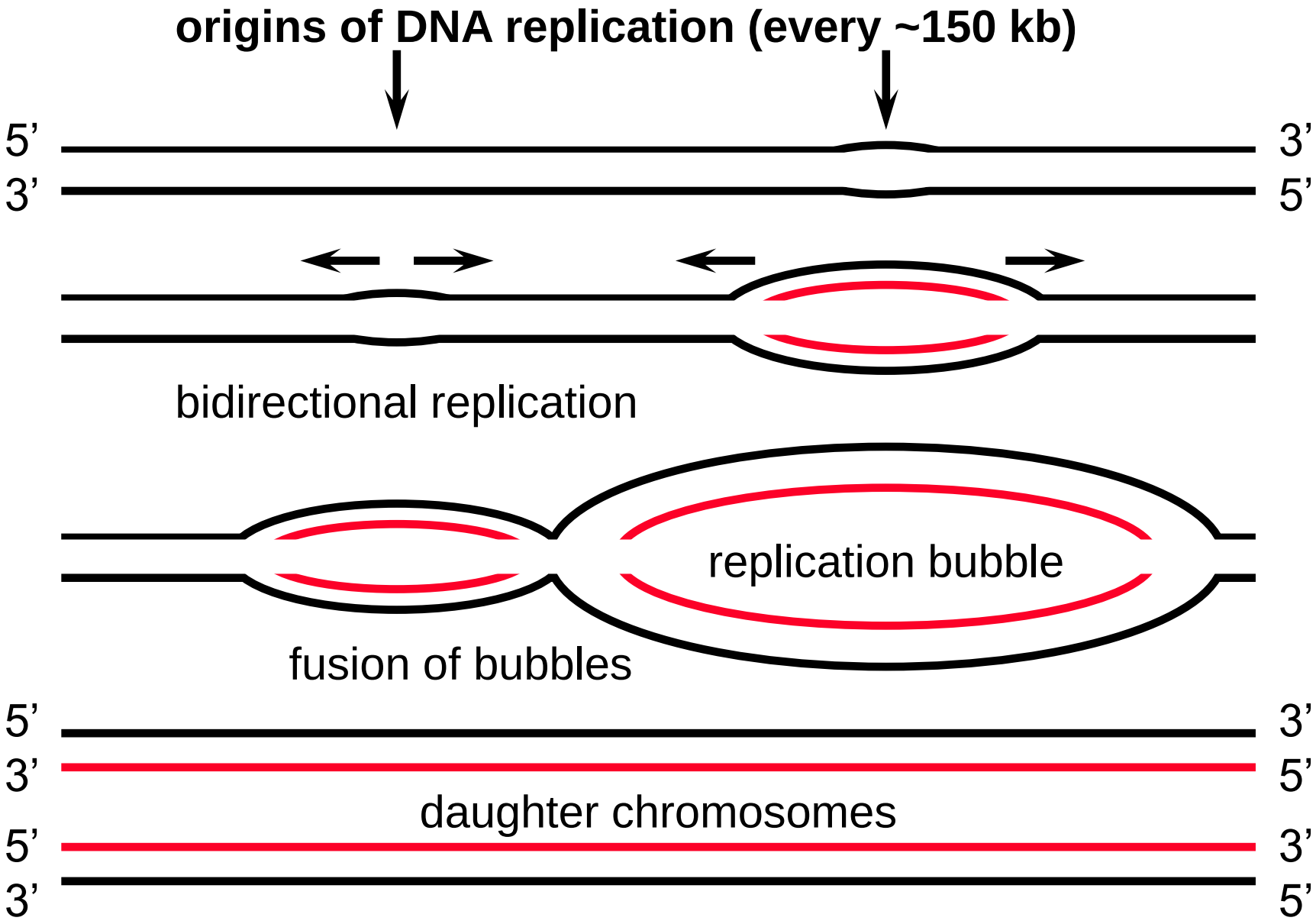


Origin of Replication

Can replication begin anywhere or does it always begin at the same location?

- **Inman's experiments:**
  - denatured $\phi\lambda$ DNA at A = T-rich regions → "bubbles"
  - bubbles were mapped
  - showed that loops always initiate at a unique origin

Origin of Replication

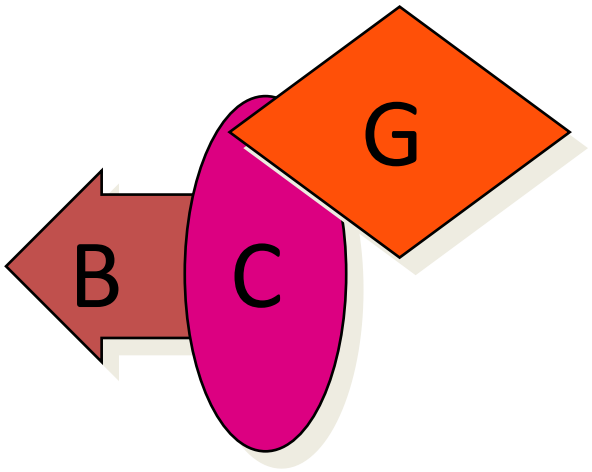


DNA SYNTHESIS IN *E. COLI*

The single, circular *E. coli* chromosome has one origin of replication (ori). Initiation of replication begins with the binding of dnaA proteins to the **ori** sequence.

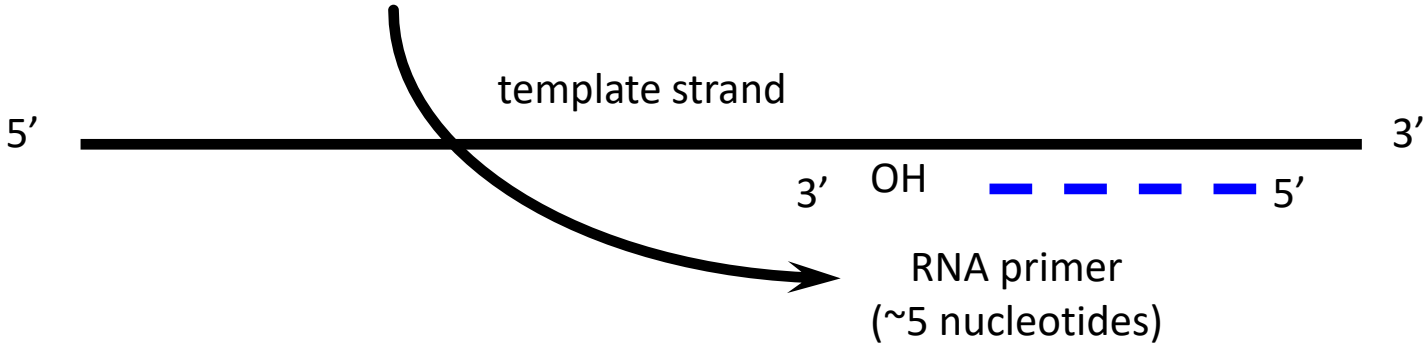
As these proteins coalesce, the adjacent DNA is forced to undergo melting into single strands.

This then allows the dnaB and dnaC proteins to bind the single-stranded DNA and further unwind the double helix, catalyzed by the dnaB protein which is a DNA "helicase" or DNA unwinding enzyme.



Primasome

- dna B (helicase)
- dna C
- dna G (primase)



The “Primasome” consists of the dnaB, dnaC, and dnaG proteins

Leading and Lagging Strand Synthesis

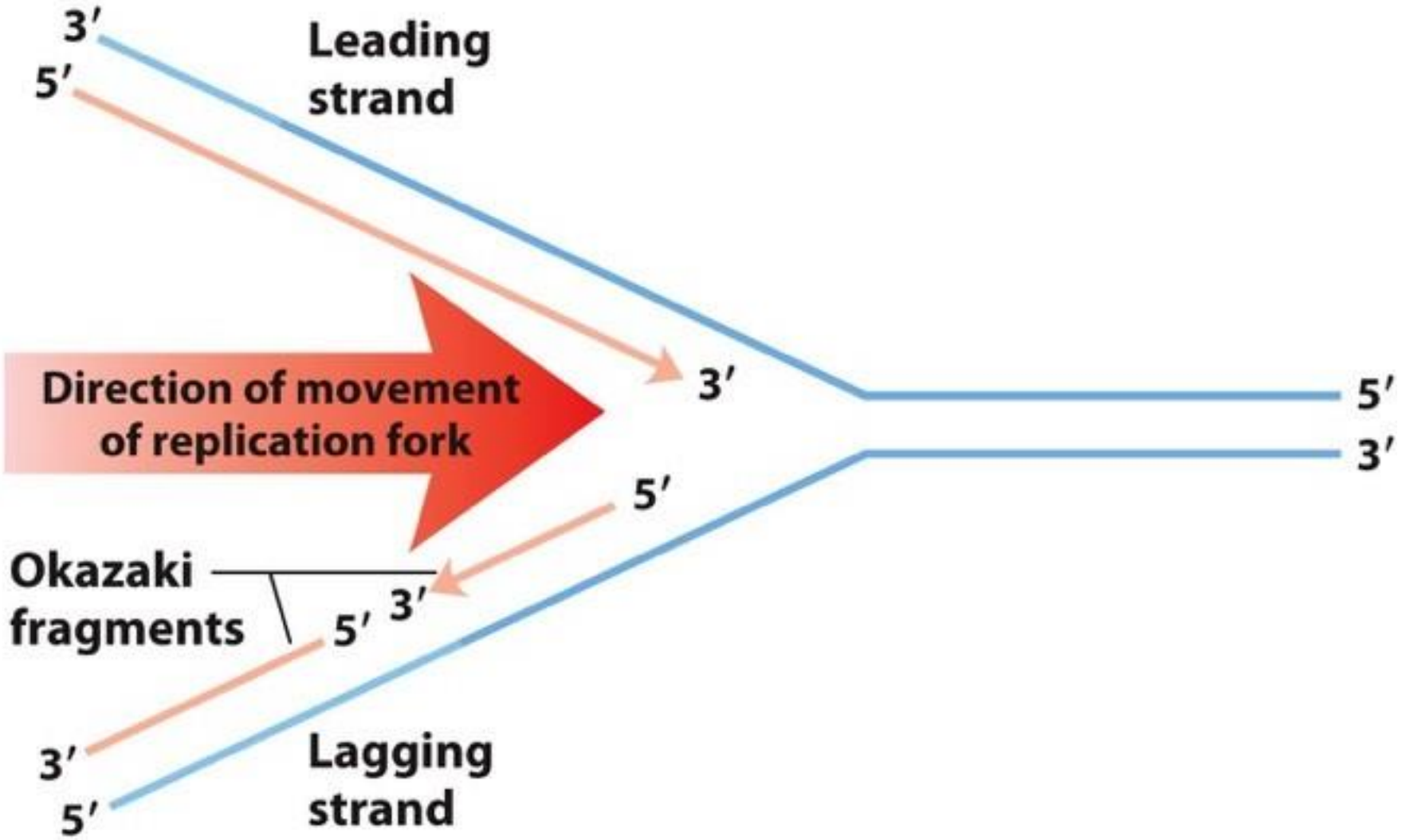


Figure 25-4
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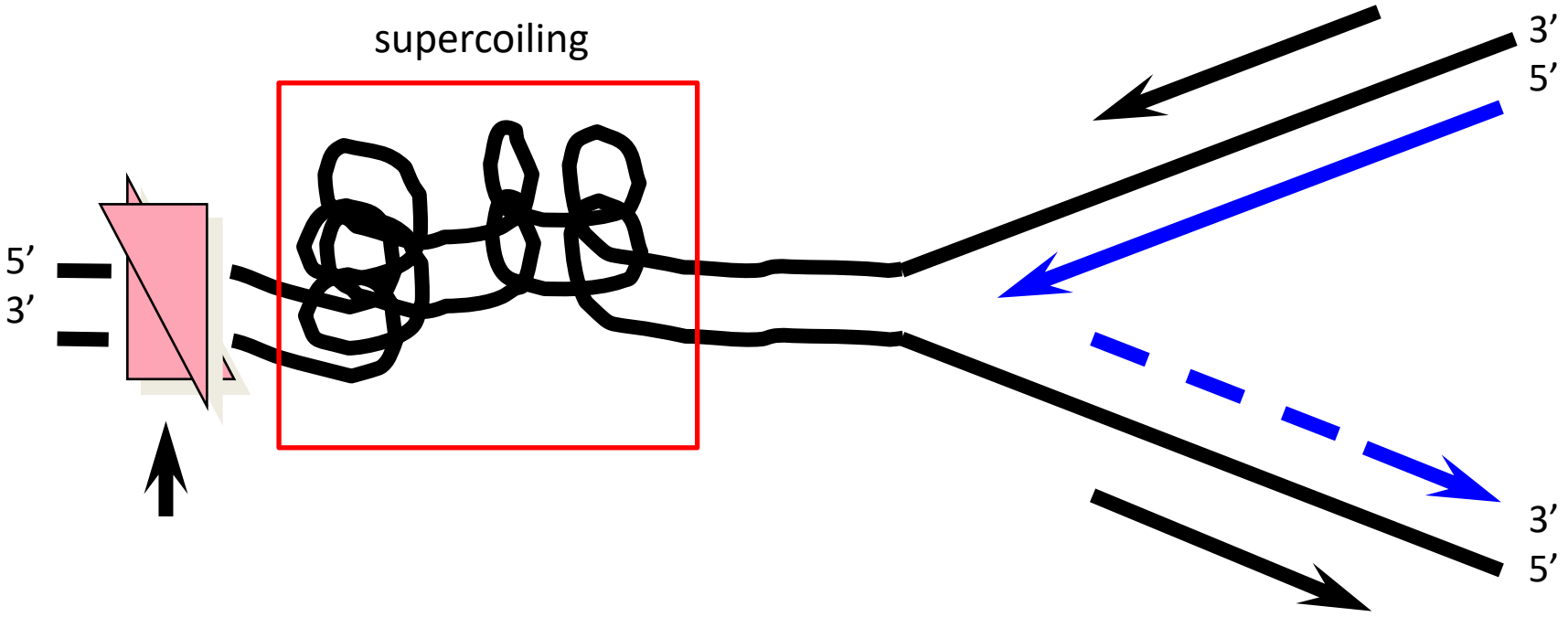
Strand separation at the replication fork causes positive supercoiling of the downstream double helix



• DNA gyrase is a topoisomerase II, which breaks and reseals the DNA to introduce negative supercoils ahead of the fork

UNWINDING THE STRANDS …

- The strand separation process (unwinding the complementary Watson-Crick DNA strands) causes overwinding ahead of the fork. Any DNA that is overwound (or underwound) is said to be “supercoiled.” (*Remember!*)

- Overwound DNA is positively supercoiled.

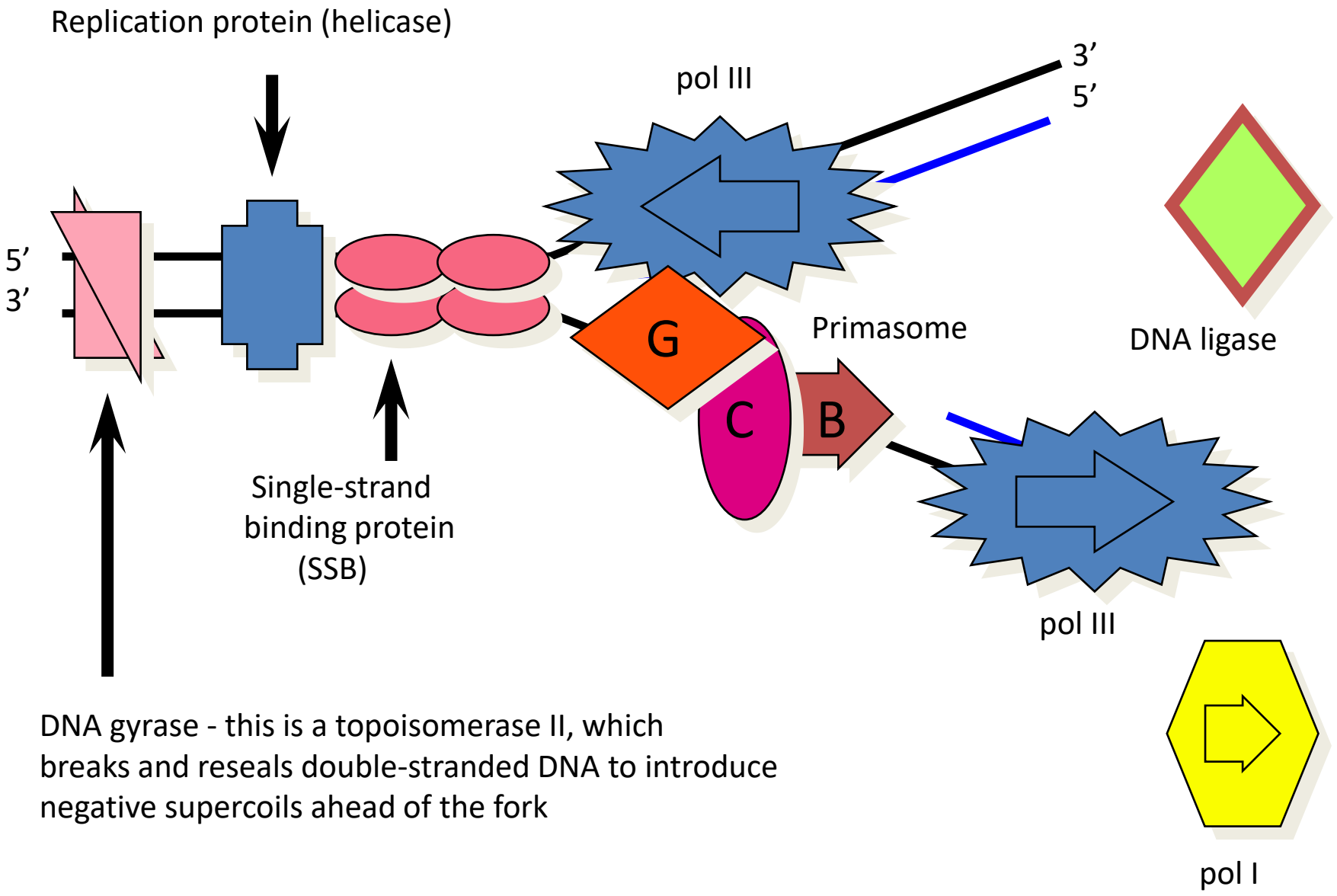
- The increasing torsional stress needs to be dissipated in order for the fork to continue to unwind so that replication can proceed. This is accomplished by DNA topoisomerases, which cut the DNA strands, unwind them and reseal the strands. As they do so they introduce negative supercoiling into the DNA to compensate for the positive supercoiling.

Proteins at the replicative fork

- The act of unwinding the DNA double helix puts torsional stress in the form of positive supercoils in the DNA upstream of the fork.

- To overcome this, DNA gyrase, which is a topoisomerase II, breaks and reseals the DNA in order to introduce negative supercoils in the DNA, thus overcoming the positive supercoils. The unwinding itself is carried out by the Replication protein, which is a helicase. Finally, to keep the unwound strands single-stranded, they bind SSB (single-strand binding protein).

Proteins at the replication fork in *E. coli*



DNA gyrase - this is a topoisomerase II, which breaks and reseals double-stranded DNA to introduce negative supercoils ahead of the fork

Components of the Replication Apparatus

dnaA	binds to origin DNA sequence
Primasome	
dnaB	helicase (unwinds DNA at origin)
dnaC	binds dnaB
dnaG	primase (synthesizes RNA primer)
DNA gyrase	introduces negative supercoils ahead of the replication fork
Rep protein	helicase (unwinds DNA at fork)
SSB	binds to single-stranded DNA (prevents (single strand binding protein) ssDNA from breakage)
DNA pol III	primary replicating polymerase
DNA pol I	removes primer and fills gap
DNA ligase	seals gap by forming 3', 5'-phosphodiester bond

DNA Is Degraded

- Nucleases degrade nucleic acids.
  - Specifically, **DNases** degrade only DNA; **RNases** degrade only RNA.
- **Exo**nucleases cleave bonds that remove nucleotides from the **ends** of DNA.
- **Endo**nucleases cleave bonds **within** a DNA sequence.

5’ CGTAGTGGGCCT 3’

WEEK ....
Lecture 4

DNA Is Synthesized by DNA Polymerases

- First (DNA polymerase I) discovered by Arthur Kornberg in *E. coli*
- *E. coli* contains at least four other DNA polymerases.

DNA Elongation Chemistry

- Parental DNA strand serves as a template.
- Nucleoside triphosphates serve as substrates in strand synthesis.
- The nucleophilic OH group at the 3' end of the growing chain attacks the α-phosphate of the incoming trinucleotide.
  - This 3'-OH is REQUIRED.
  - The 3'-OH is made a more powerful nucleophile by nearby Mg^{2+} ions.
- Pyrophosphate (made of the β and η phosphates) is a good leaving group.

Mechanism of DNA Polymerases

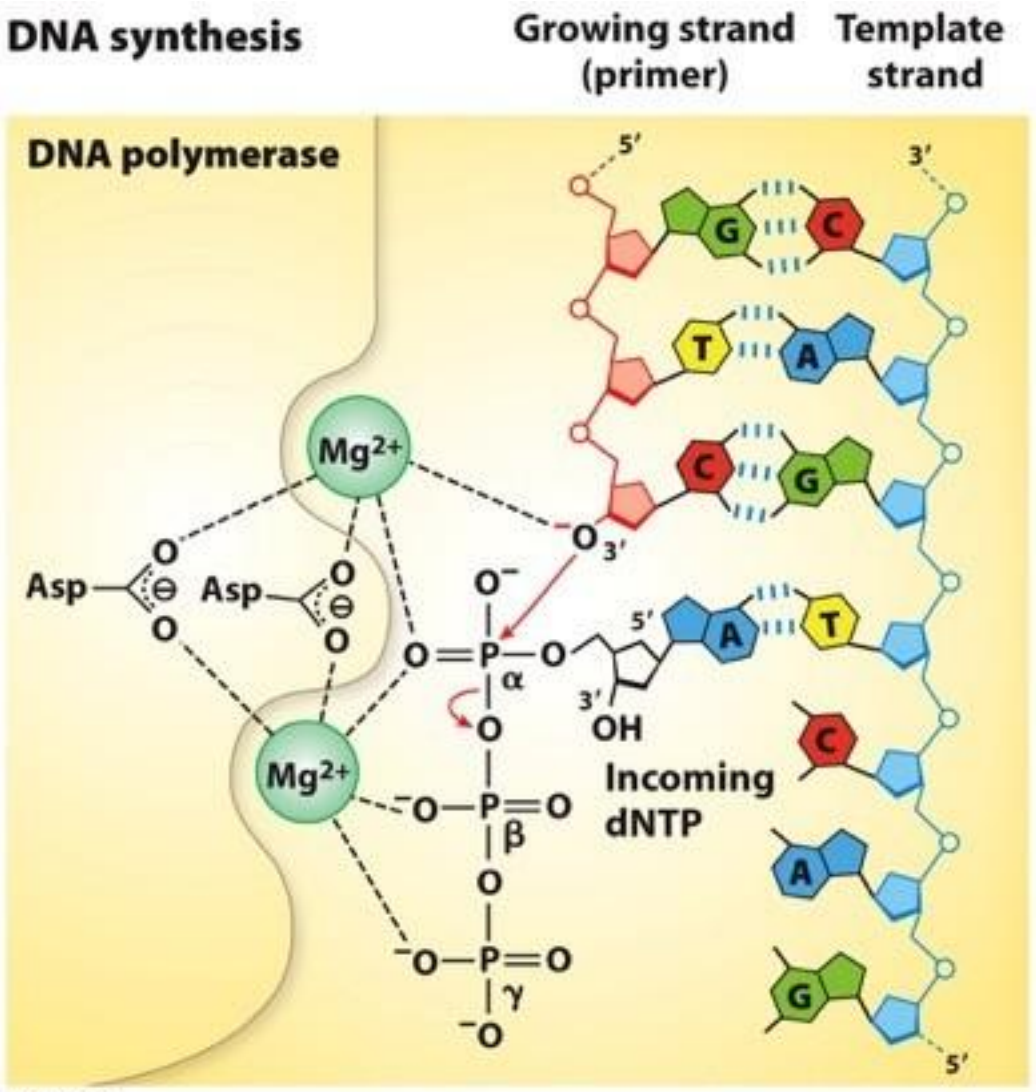


Figure 25-5a
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DNA Polymerase Also Requires a Primer

- Primer = short strand complementary to the template
  - contains a 3’-OH to begin the first DNA polymerase-catalyzed reaction
  - can be made of DNA or RNA (more common)

Importance of a Primer

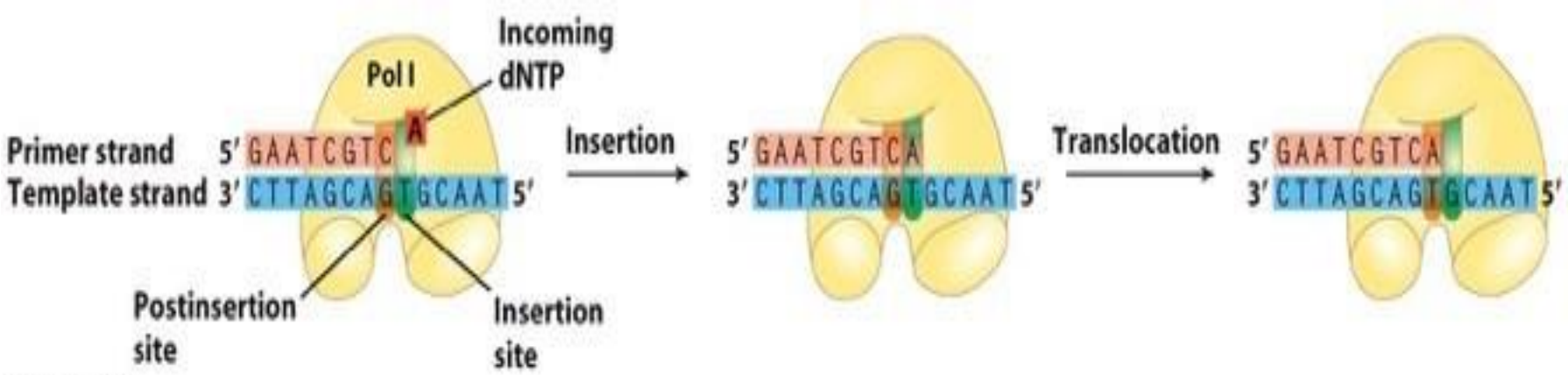

Figure 25-5b

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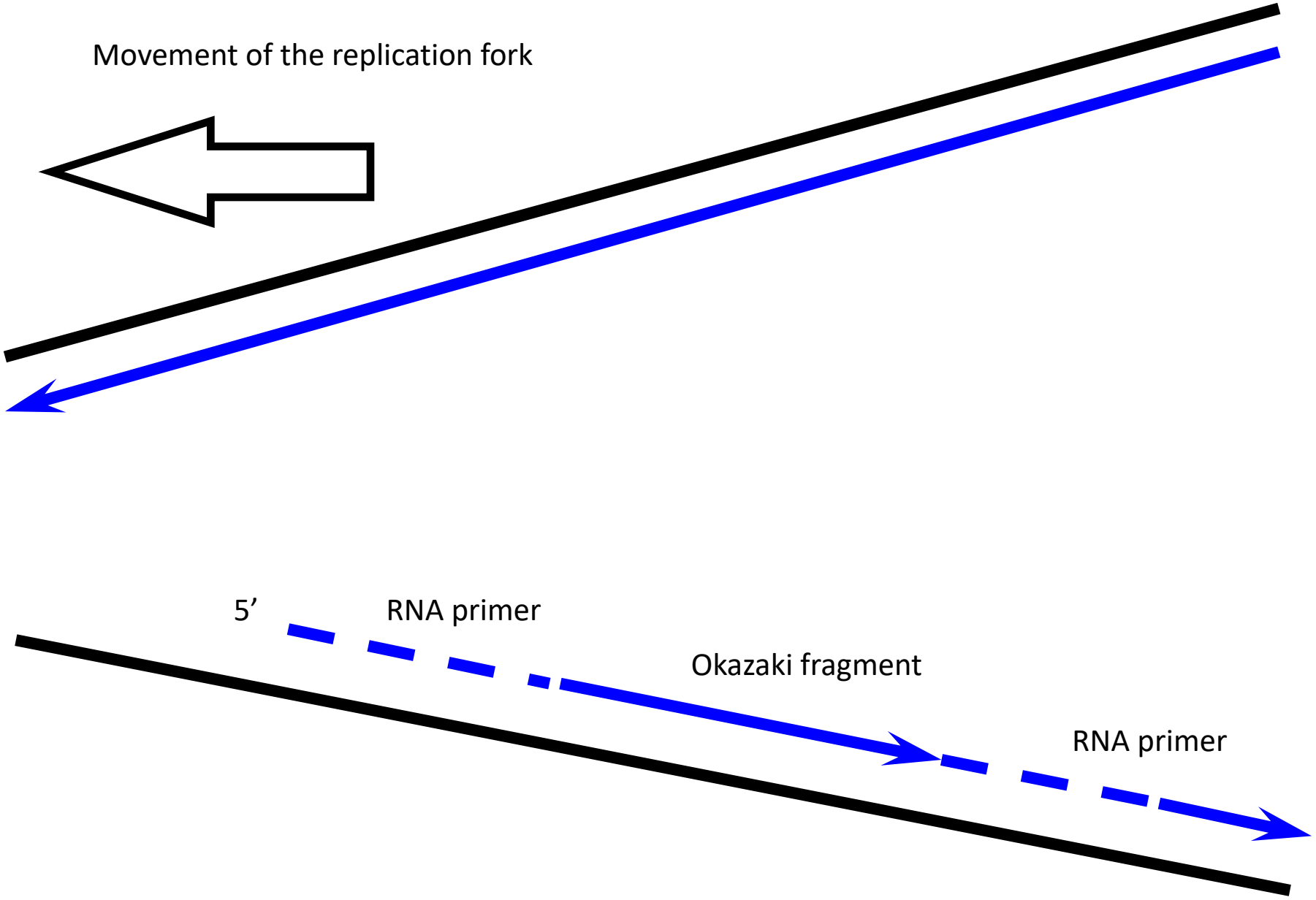

Each RNA primer (dashed red line) on the lagging strand then serves as a starting point for the initiation of DNA synthesis (Okazaki fragment; solid red line).

Features of DNA Polymerase

- Enzyme has a pocket with two regions:
  - insertion site: where the incoming nucleotide binds
  - postinsertion site: where the newly made base pair resides when the polymerase moves forward

DNA Polymerase

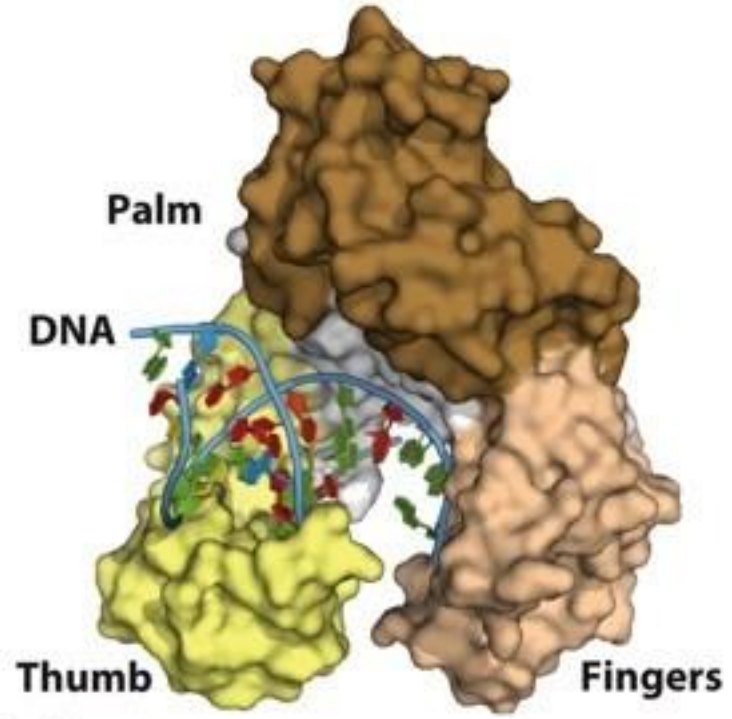

Figure 25-5c
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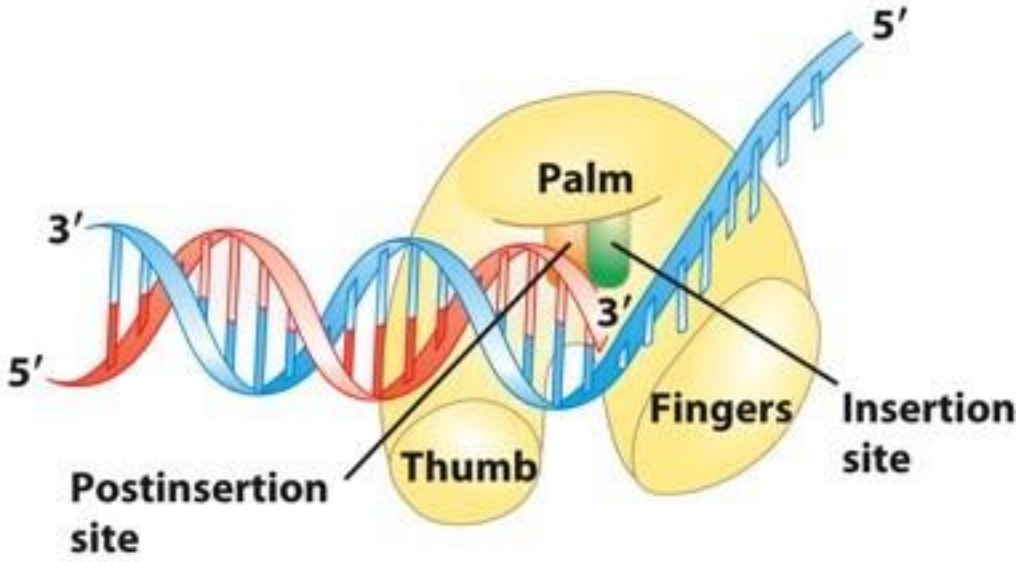

Figure 25-5d
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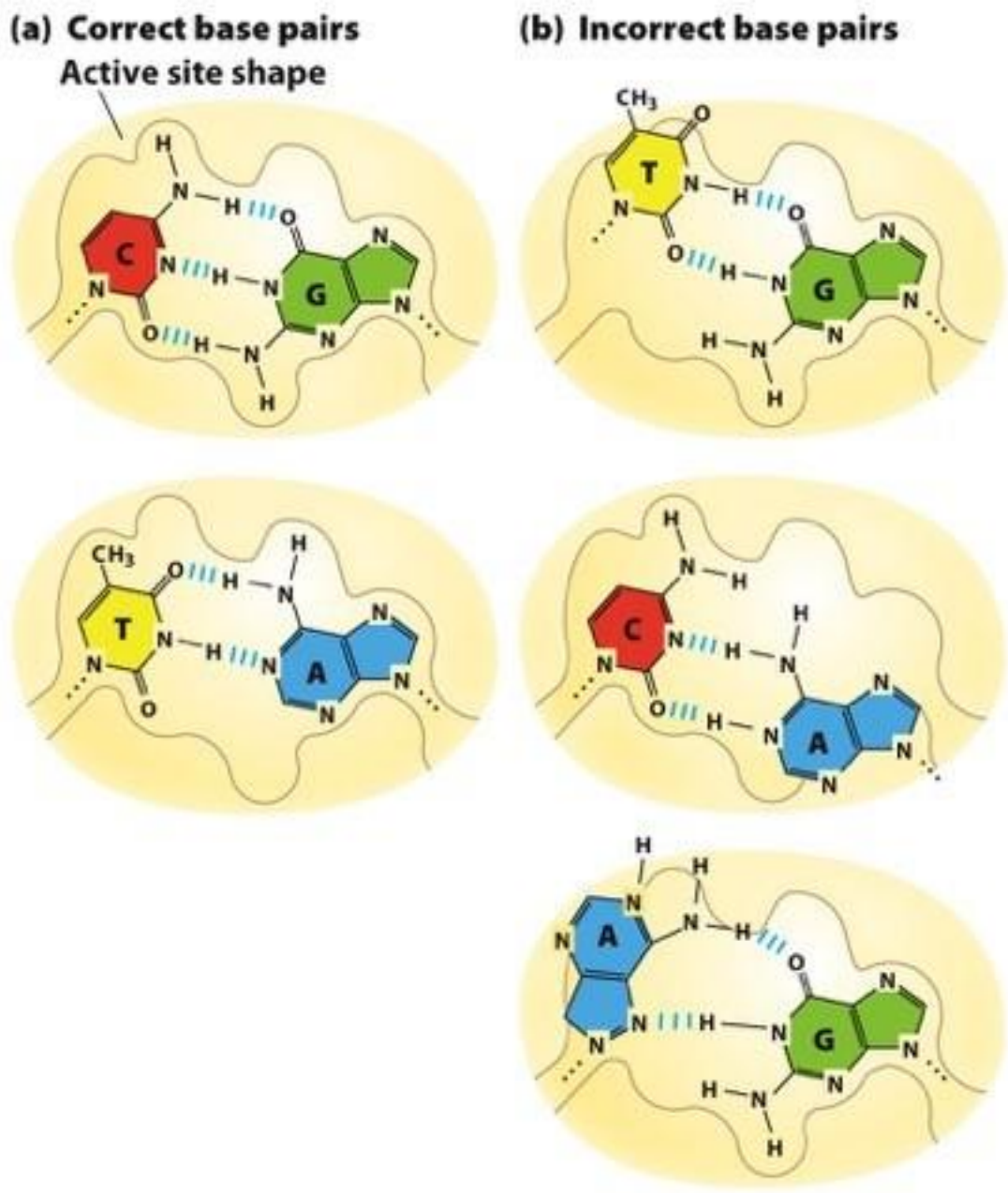
DNA Polymerase Can Add Nucleotides or Dissociate

- The number of nucleotides added before dissociation is called **processivity.**
- The processivity of polymerases, in general, varies widely from a few nucleotides to many thousands.
- Each specific polymerase has its own processivity and polymerization rate.

Geometry of Base Pairing Accounts for High Fidelity

- Errors in *E. coli*: $1/10^9$ – $1/10^{10}$ bp
  - (1 per 1,000–10,000 replications)
- DNA polymerase active site excludes base pairs with incorrect geometry
  - BUT DNA polymerases still insert wrong base $1/10^4$–$1/10^5$ times.
  - Repair mechanisms fix these errors.

Base-Pair Geometry



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Errors During Synthesis Are Corrected by 3'→5' Exonuclease Activity

- ~All DNA polymerases have an additional activity.
- 3'→5'-exonuclease activity "proofreads" synthesis for mismatched base pair
- Translocation of enzyme to next position is inhibited until the enzyme can remove the incorrect nucleotide

Error Correction by 3'→5'-Exonuclease Activity

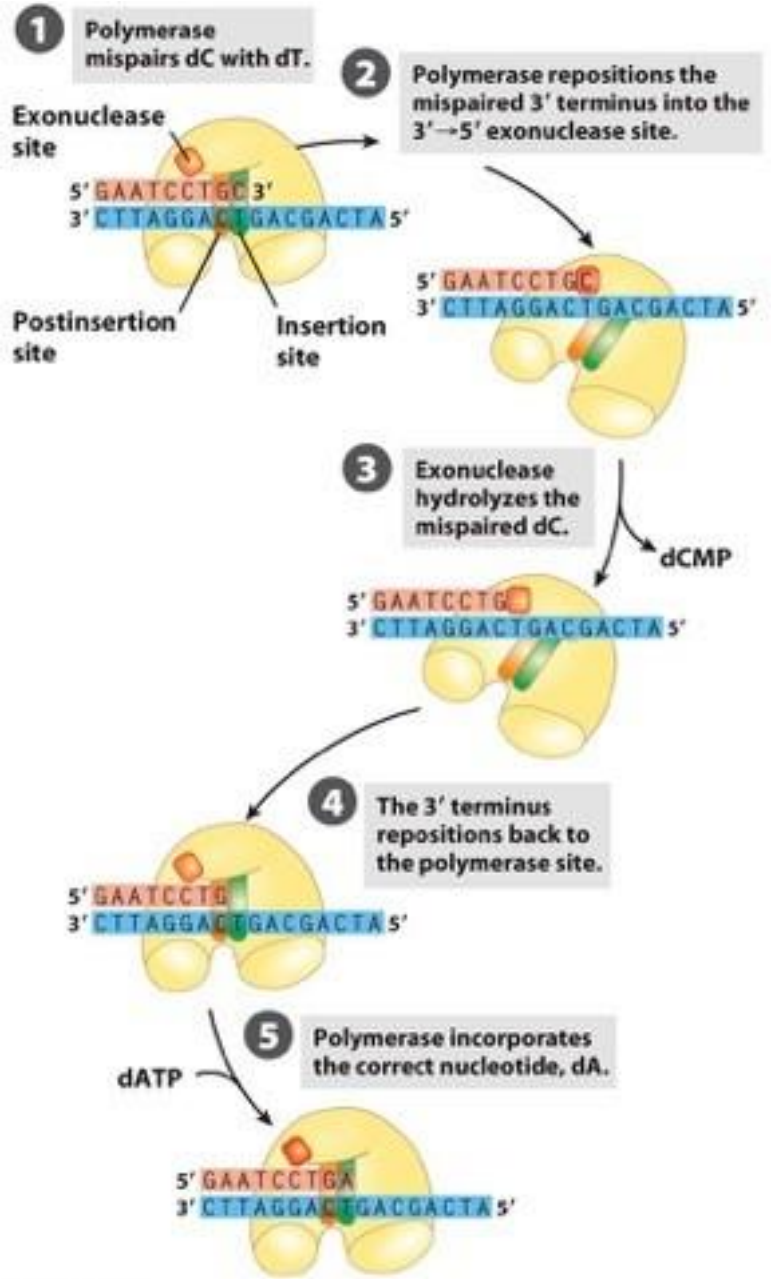

Figure 25-7
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There Are at Least Five DNA Polymerases in *E. Coli*

- DNA polymerase I is abundant but is not ideal for replication.
  - rate (600 nucleotides/min) is slower than observed for replication fork movement
  - has low processivity
  - primary function is in clean-up
- DNA polymerase III is the principal replication polymerase.
- DNA polymerases II, IV, and V are involved in DNA repair.

TABLE 25-1 Comparison of the Five DNA Polymerases of *E. coli*

	DNA polymerase				
	I	**II**[a]	**III**	**IV**[a]	**V**[a]
Structural gene[b]	*polA*	*polB*	*polC* (*dnaE*)	*dinB*	*umuC*
Subunits (number of different types)	1	7	9	1	3
M_r	103,000	88,000[c]	1,065,400	39,100	110,000
3' → 5' exonuclease (proofreading)	Yes	Yes	Yes	No	No
5' → 3' exonuclease	Yes	No	No	No	No
Polymerization rate (nucleotides/s)	10–20	40	250–1,000	2–3	1
Processivity (nucleotides added before polymerase dissociates)	3–200	1,500	≥500,000	1	6–8

[a]Translesion (mutagenic) DNA polymerases. For DNA polymerase IV, processivity is increased substantially by association with a β clamp. These polymerases are slowed when a DNA lesion is present in the DNA template strand.
[b]For enzymes with more than one subunit, the gene listed here encodes the subunit with polymerization activity. Note that dnaE is an earlier designation for the gene now referred to as polC.
[c]Polymerization subunit only. DNA polymerase II shares several subunits with DNA polymerase III, including the β, δ, δ′, χ, and ψ subunits (see Table 25-2).

DNA Polymerase I Also Has 5'→3'-Exonuclease Activity

- In addition to the 3'→5'-exonuclease activity
- Moves ***ahead*** of the enzyme, hydrolyzes things in its path
- Does **nick translation**—a strand break moves along with enzyme
- This activity and the polymerase activity are in the **Klenow fragment**—a distinct domain that can be separated by protease treatment.

Nick Translation

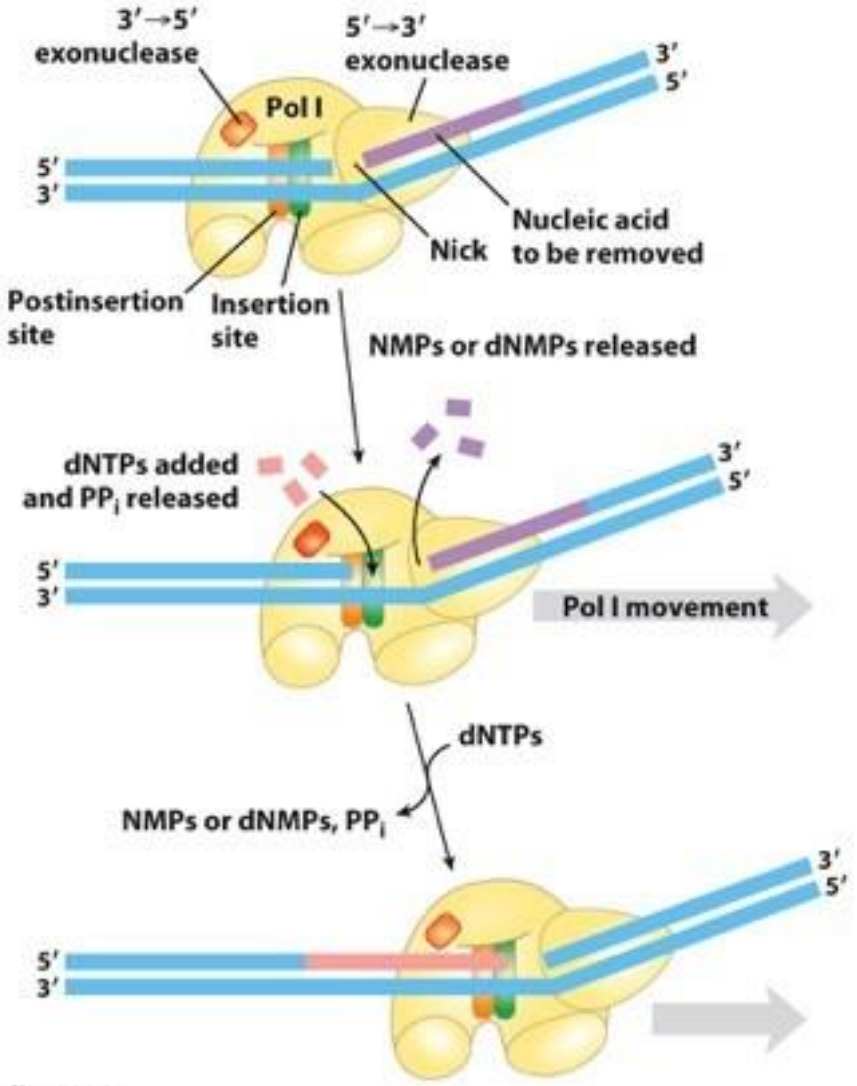

Figure 25-8
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DNA Polymerase III

- Complex structure with 10 types of subunits
- Two core domains of α, ε, and θ subunits
- The core domains are linked by the "clamp-loader" complex $\tau_2\eta\delta\delta'$.
- The core domains each interact with a dimer of β subunits that increase the processivity of the complex.
  - form a sliding clamp the prevents dissociation
  - processivity of DNA Pol III is >500,000 bp because of the β clamps

DNA Polymerase III

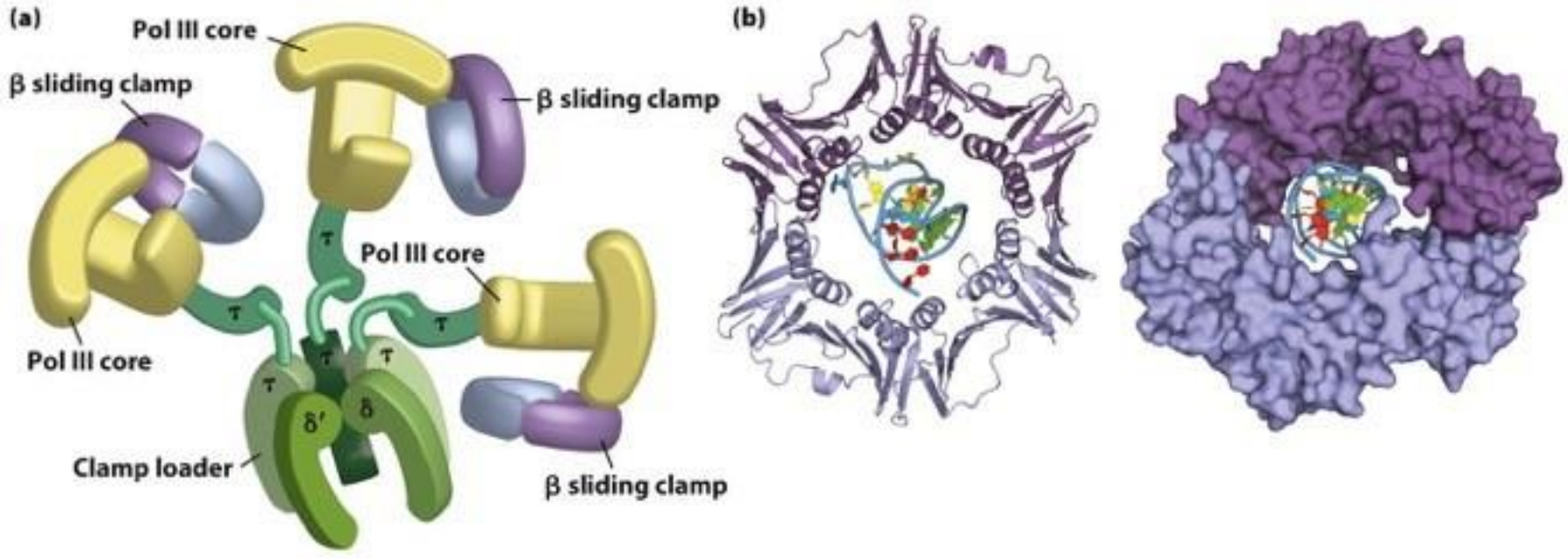

Figure 25-9
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TABLE 25-2 Subunits of DNA Polymerase III of *E. coli*

Subunit	Number of subunits per holoenzyme	M_r of subunit	Gene	Function of subunit	
α	3	129,900	*polC* (*dnaE*)	Polymerization activity	Core polymerase
ε	3	27,500	*dnaQ* (*mutD*)	3' → 5' proofreading exonuclease	
θ	3	8,600	*holE*	Stabilization of ε subunit	
τ	3	71,100	*dnaX*	Stable template binding; core enzyme dimerization	Clamp-loading (η) complex that loads β subunits on lagging strand at each Okazaki fragment[a]
δ	1	38,700	*holA*	Clamp opener	
δ′	1	36,900	*holB*	Clamp loader	
χ	1	16,600	*holC*	Interaction with SSB	
ψ	1	15,200	*holD*	Interaction with τ and χ	
β	6	40,600	*dnaN*	DNA clamp required for optimal processivity	

[a]The clamp-loading complex is also called the η complex, because of the existence of another version of the complex in which three subunits called η replace the τ subunits. The η subunit is encoded by a portion of the gene for the τ subunit (*dnaX*), such that the amino-terminal 66% of the τ subunit has the same amino acid sequence as the η subunit. The η subunit is generated by a translational frameshifting mechanism (p. 1085) that leads to premature translational termination. The η subunit shares the clamp-loading functions of τ but lacks the protein segments that interact with the core polymerase or with DnaB helicase. Clamp-loading complexes incorporating η subunits may operate independently of the DNA polymerase III holoenzyme, promoting the unloading of β clamps discarded on the lagging strand as the replication fork progresses. They may also promote loading of β clamps for some DNA repair processes that require DNA synthesis away from the replication fork.

Requirements for *E. Coli* DNA Replication

- *E. coli* requires over 20 enzymes and proteins.
- **The set is called the replisome.**
- Includes:
  - helicases (use ATP to unwind DNA strands)
  - topoisomerases (relieve the stress caused by unwinding)
  - DNA-binding proteins to stabilize separated strands
  - primases to make RNA primers
  - DNA ligases to seal nicks between successive nucleotides on the same strand (i.e., Okazaki fragments)

Initiation of Replication in *E. Coli*

- Begins at the *oriC* site (245 bp in length)
- Contains highly conserved sequence elements
  - five repeats of a 9-bp sequence (R sites) that form binding site for initiator protein DnaA
  - A = T-rich region (DNA unwinding element (DUE))
  - additional sites include:
    - DnaA (I sites),
    - IHF (integration host factor)
    - FIS (factor for inversion stimulation)

Arrangement of Conserved Sequences of *oriC*

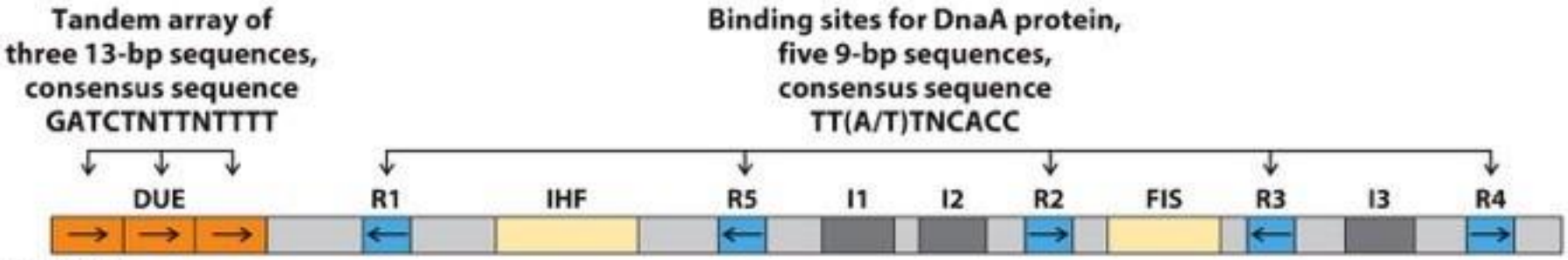

Figure 25-10

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Requirements of Initiation of Replication in *E. Coli*

- Requires at least 10 different proteins
- Goal: open the helix, form prepriming complex

TABLE 25-3 Proteins Required to Initiate Replication at the *E. coli* Origin

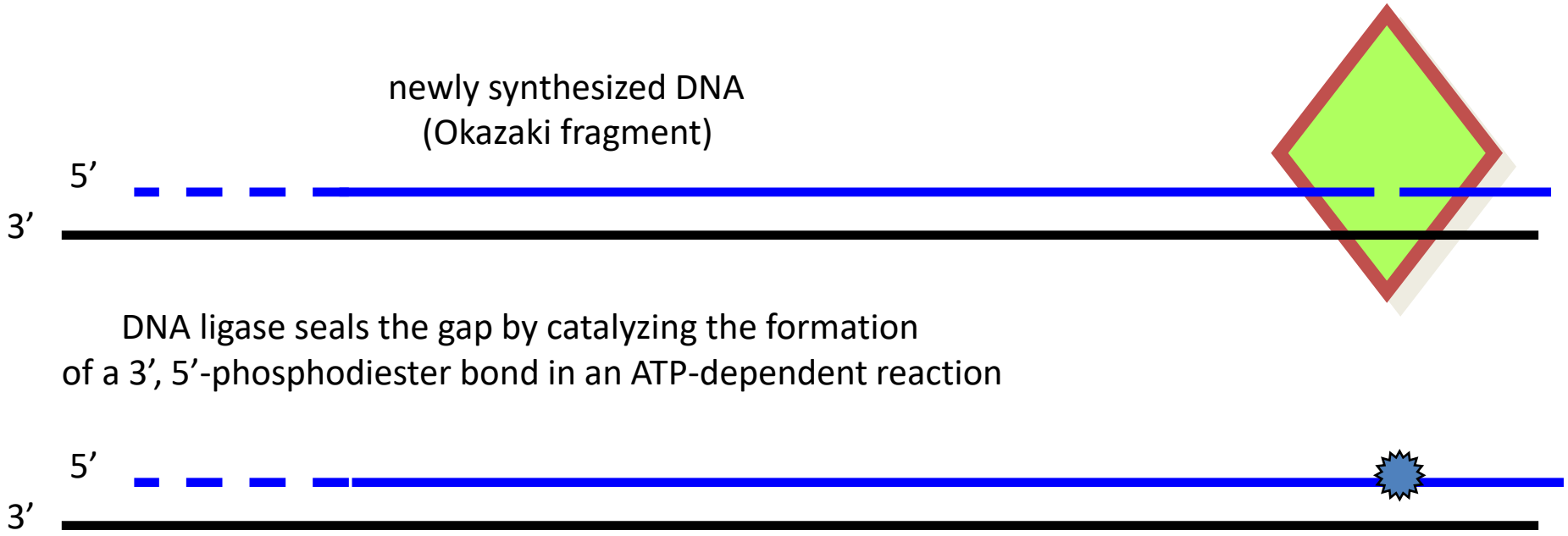
Protein	M_r	Number of subunits	Function
DnaA protein	52,000	1	Recognizes *oriC* sequence; opens duplex at specific sites in origin
DnaB protein (helicase)	300,000	6[a]	Unwinds DNA
DnaC protein	174,000	6[a]	Required for DnaB binding at origin
HU	19,000	2	Histonelike protein; DNA-binding protein; stimulates initiation
FIS	22,500	2[a]	DNA-binding protein; stimulates initiation
IHF	22,000	2	DNA-binding protein; stimulates initiation
Primase (DnaG protein)	60,000	1	Synthesizes RNA primers
Single-stranded DNA-binding protein (SSB)	75,000	4[a]	Binds single-stranded DNA
DNA gyrase (DNA topoisomerase II)	400,000	4	Relieves torsional strain generated by DNA unwinding
Dam methylase	32,000	1	Methylates (5')GATC sequences at *oriC*

[a]Subunits in these cases are identical.

DNA LIGASE

However, DNA polymerase I cannot seal the gap between the two adjacent Okazaki fragments. This job is carried out by DNA ligase, which catalyzes the formation of a 3', 5'-phosphodiester bond in an ATP-dependent reaction.

Thus, it takes one RNA polymerase (primase), two DNA polymerases (III and I), and DNA ligase to synthesize the lagging strand. Once initiation occurs on the RNA primer, the leading strand only requires DNA polymerase III for its synthesis.


newly synthesized DNA
(Okazaki fragment)
5’
3’
DNA ligase seals the gap by catalyzing the formation
of a 3’, 5’-phosphodiester bond in an ATP-dependent reaction
5’
3’

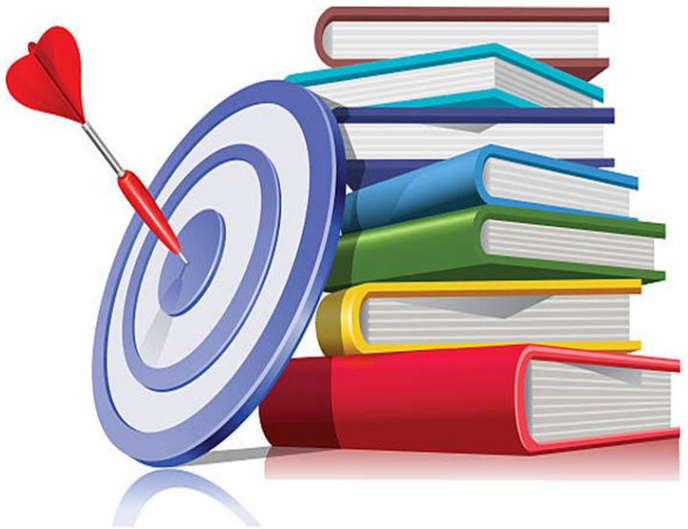
TRANSCRIPTION

LECTURE 4

TRANSCRIPTION

Learning goals:

- Know what transcription is
- Know what reverse transcription is
- Know what the genetic code is
- Know how to read the genetic code



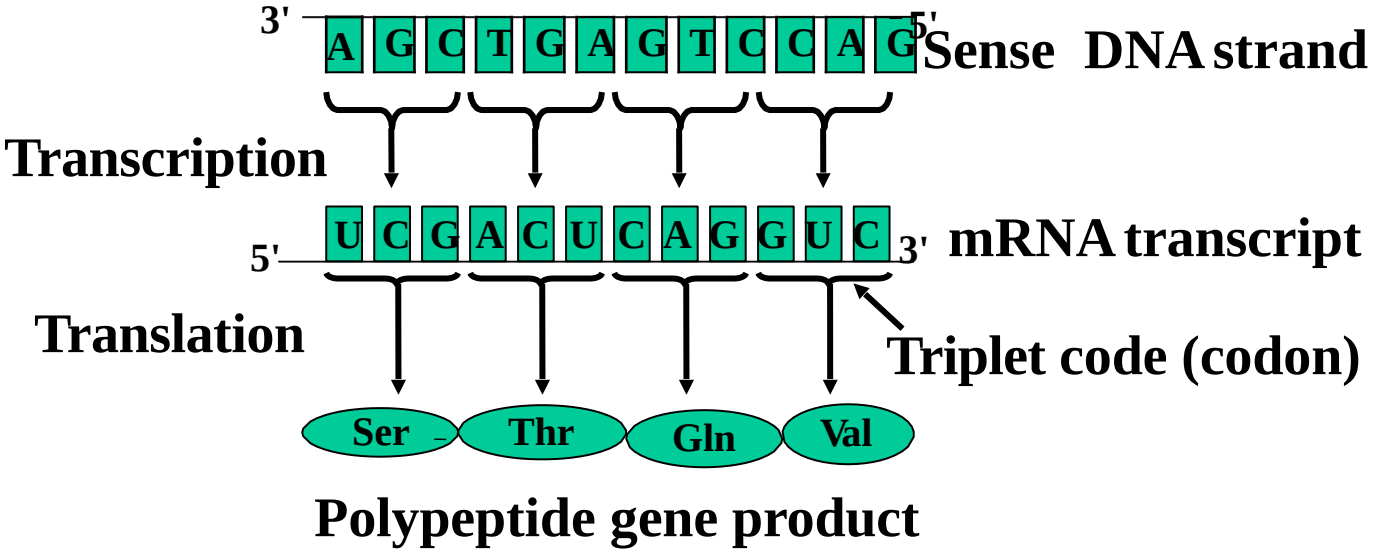
Transcription (RNA Synthesis)

- In eukaryotes, genes remain in the nucleus whilst protein synthesis takes place in the cytosol.
- The DNA cannot therefore serve directly as a template for protein synthesis.
- Information stored in the nucleotide-pair sequence in DNA is transferred by transcription to a nucleotide sequence in mRNA.
- mRNA, therefore, carries information from genes in the chromosomes to ribosomes in cytosol for protein synthesis.

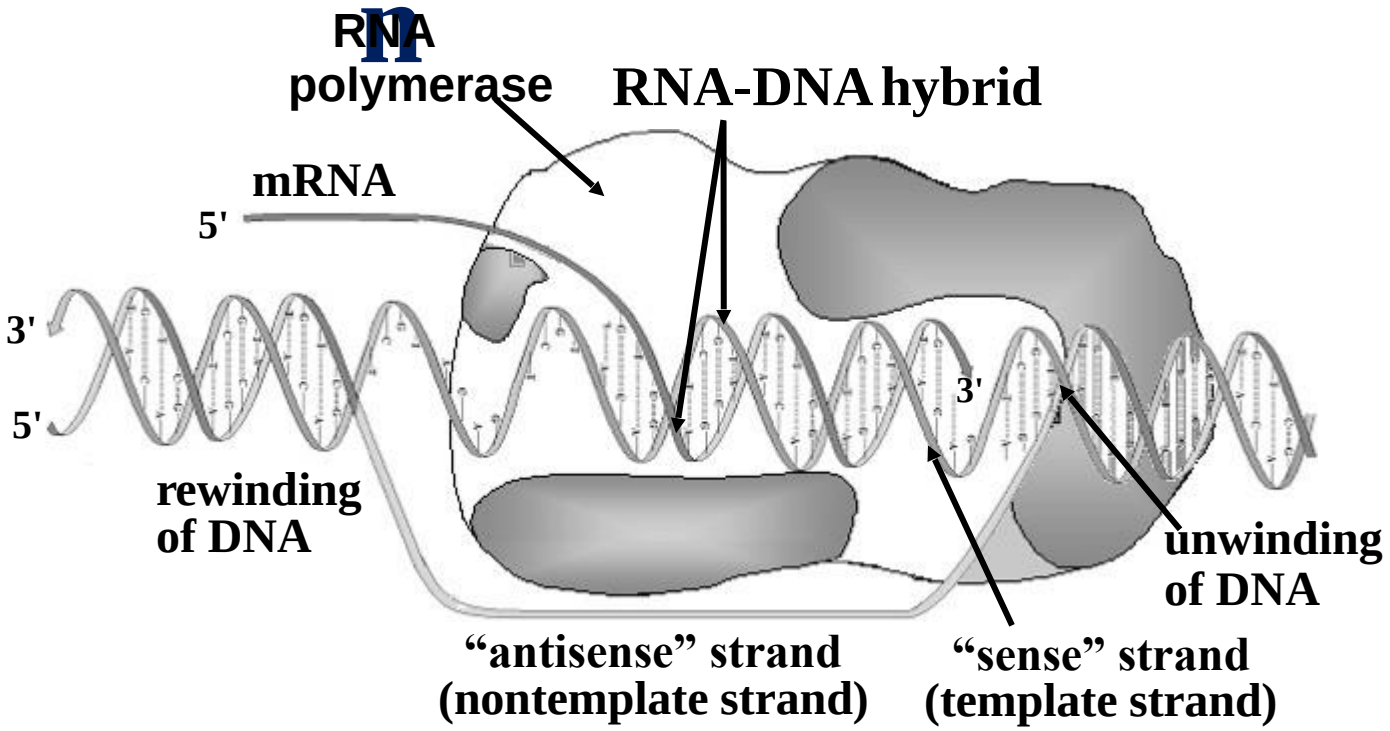
Transcription

- DNA codes for several types of RNA
- **Sense strand**: the strand of DNA that contains the code that is transcribed into mRNA
- **Ribosomal RNA** (rRNA):RNA in the structure of the ribosome (site of protein synthesis)
- **Messenger RNA** (mRNA): carries instruction for protein synthesis in codes called **codons (**3-nucleotide sequences in mRNA that code for one amino acid)
- **Transfer RNA** (tRNA): binds amino acid and carries (transfers) it to site of protein synthesis.

Transcription and Translation



Transcriptio n



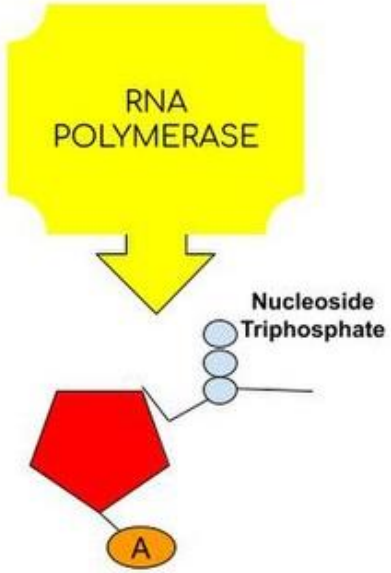
Double strands of DNA unwind and only one strand the "sense" strand is transcribed at a given time into RNA.

Direction of transcription

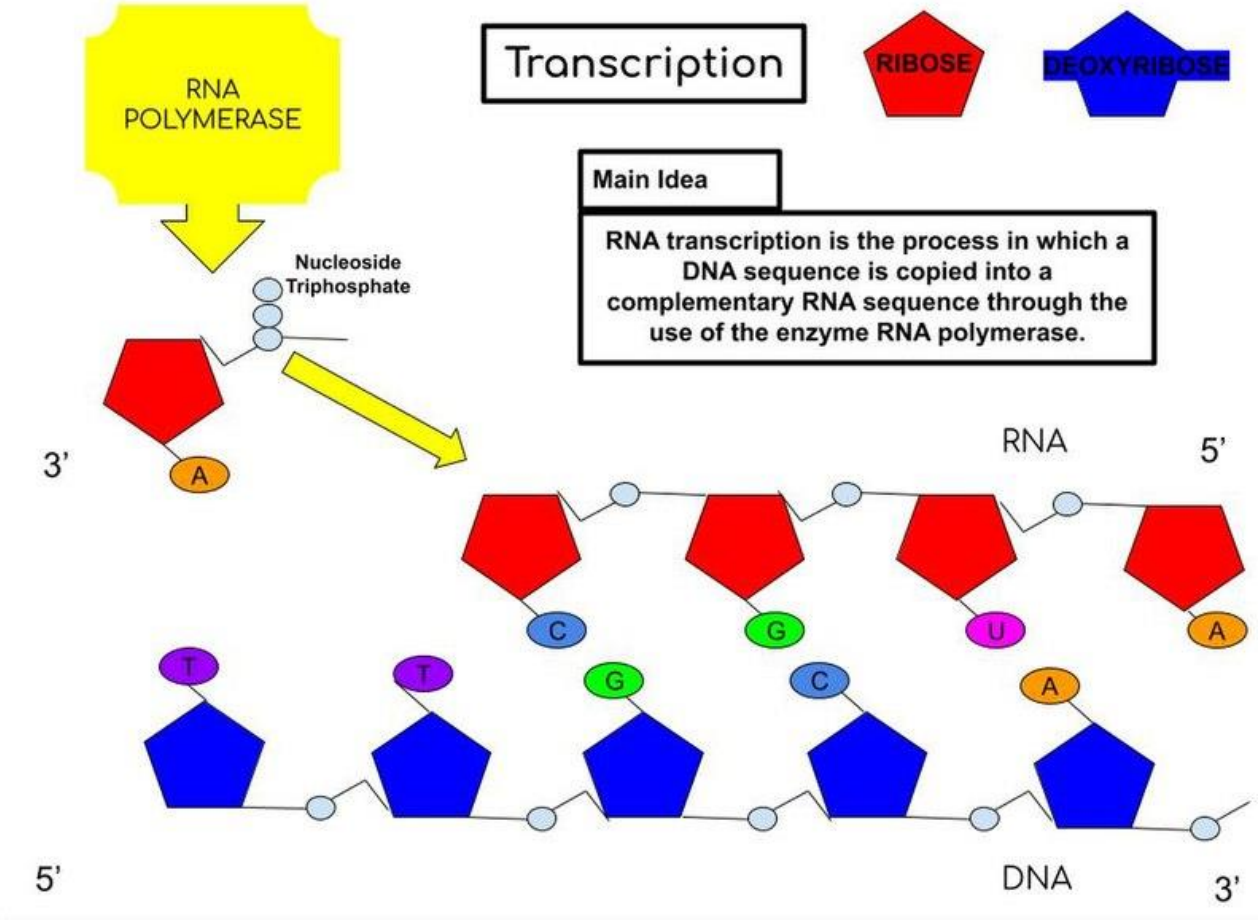
Types of RNA in *E.coli*

Type	Molecular Shape	Function	# of different kinds	# of nucleotides	% of total RNA in cell	Stability ($T_{1/2}$)
mRNA	extended	messenger	thousands	500-6000	3	1 to 3 min
rRNA	extended to compact	structure and function of ribosomes	23S 16S 5S	2800 1540 120	90	stable
tRNA leaf	clover	Adaptor	50-60	75-90	7	stable
RNA primers		DNA replication	?	< 50	< 1	
Ribozymes		?	1 or 2	250-350	< 1	?

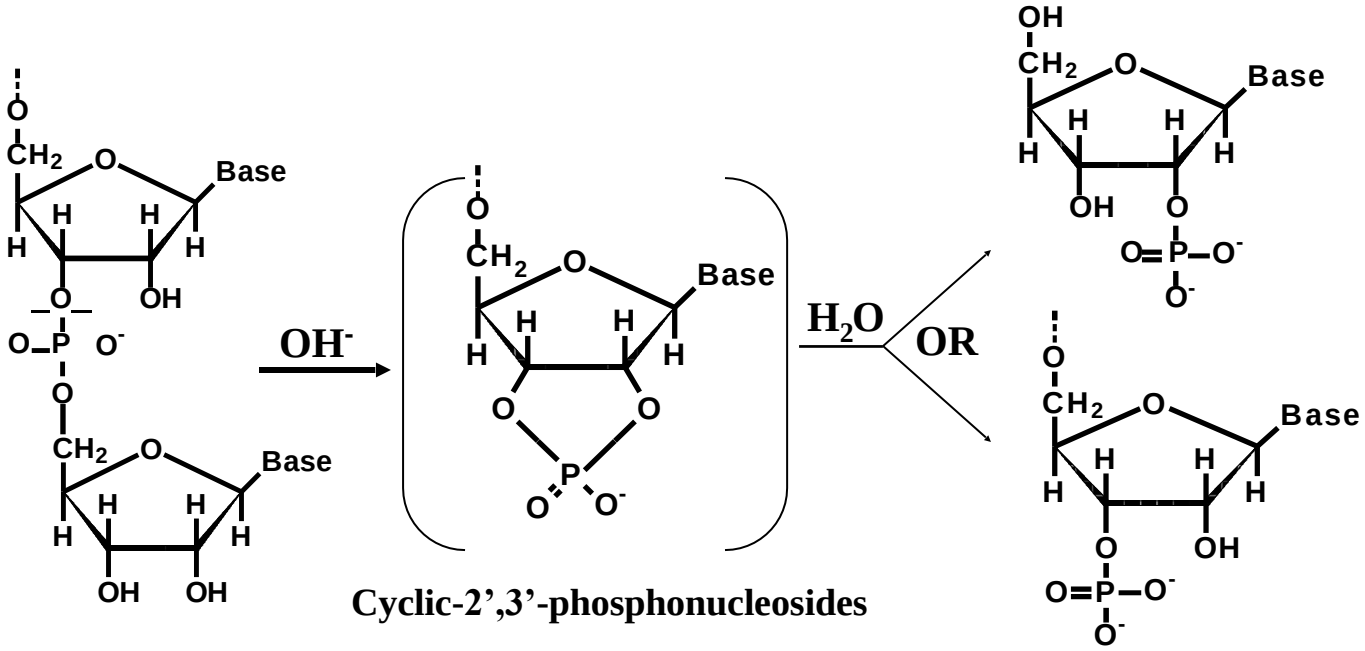
Source:Adapted from Zubay, G. (1983).Biochemistry



Nucleoside Triphosphates are free nucleotides are present in the cell. The RNA Polymerase enzyme covalently bonds the Nucleoside triphosphates together.


Transcription
RIBOSE
DEOXYRIBOSE
RNA POLYMERASE
Main Idea
RNA transcription is the process in which a DNA sequence is copied into a complementary RNA sequence through the use of the enzyme RNA polymerase.
Nucleoside Triphosphate
3'
A
RNA
5'
C
G
U
A
T
T
G
C
A
5'
DNA
3'

Alkaline Hydrolysis of RNA



Unlike DNA, RNA can be hydrolyzed with alkali. This is possible because of the existence of the extra OH group at position 2 of ribose. A cyclic 2',3'-phosphate is an obligatory intermediate for base hydrolysis, and only RNA is capable of forming such a structure. The 2',3'-phosphate can be hydrolyzed to produce either the 2' or 3'monophosphate.

STEPS

Transcription has three main steps: Initiation, Elongation and Transcription.

Initiation	RNA polymerase binds to the promoter. The unwinding of DNA follows.
Elongation	RNA polymerase synthesises RNA in the 5' to 3' direction.
Termination	If RNA polymerase reaches the terminator, the enzyme and the RNA strand detach causing the DNA to go back to its original state.

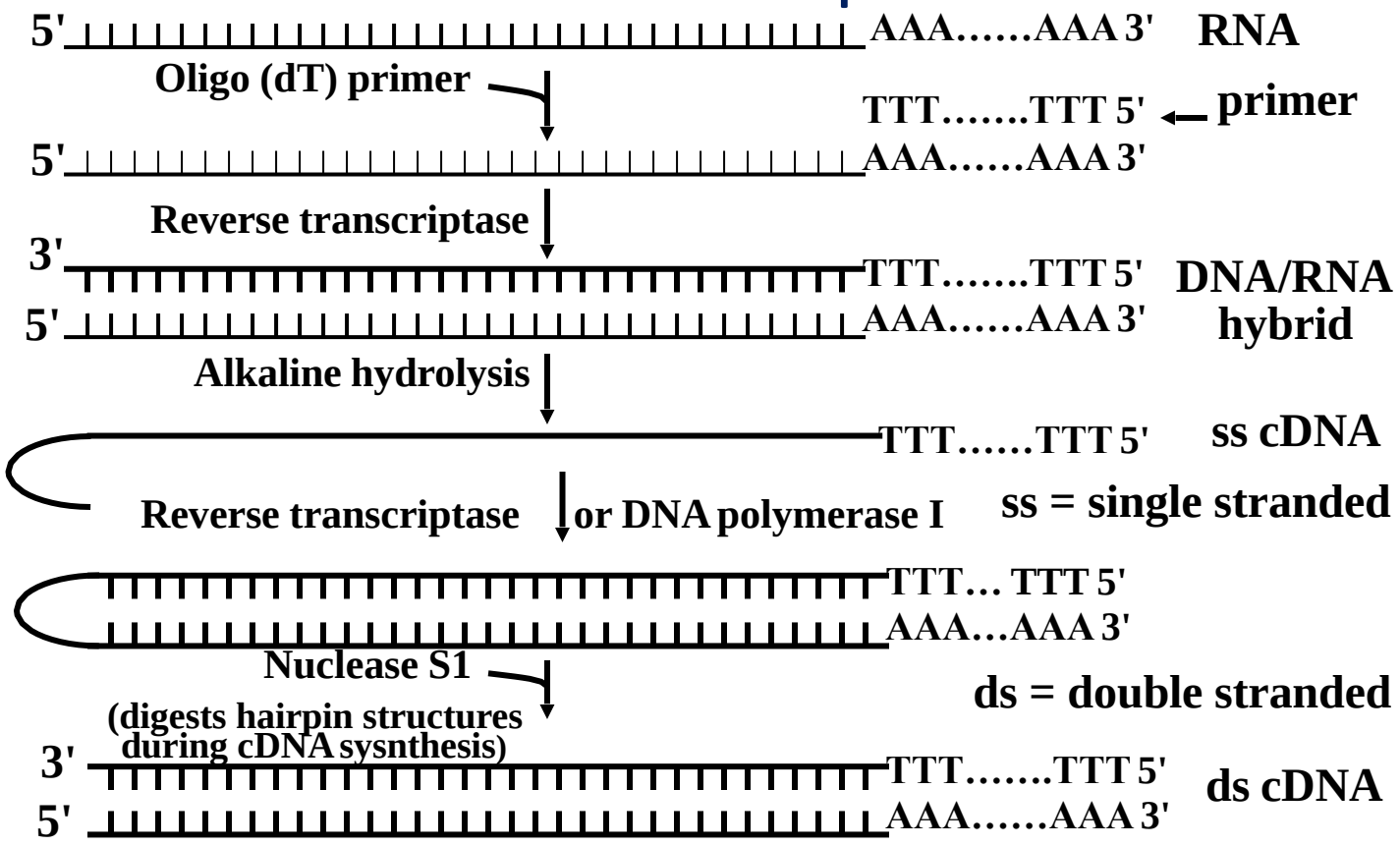
Reverse Transcription

- Some RNA viruses (**retroviruses**) contain an unusual DNA polymerase (**Reverse Transcriptase or** RNA-dependent DNA-polymerase).
- Reverse transcriptase also contains a DNA-dependent DNA polymerase activity which is responsible for second-strand formation in cDNA (complementary DNA) synthesis.
- The virus is therefore able to transcribe its RNA into cDNA which can be integrated into the host chromosome.

Reverse Transcription

- Synthesis of cDNA is an important tool for the isolation of functional DNA sequences.
- The cloning of cDNA copies of the genomes of RNA viruses has been used to study the genomic structure of the viruses.
- cDNA transcripts are used for analysis of eukaryotic gene structure, organization, and expression.
- Comparison of cDNA and genomic DNA is used in the study of intervening sequences and gene splicing events.

Reverse Transcription



Source:Adapted from Boehringer Mannhein GmbH (1985) Biochemicals for Molecular Biology

Inhibitors of Transcription

Actinomycin D- an antibiotic that binds DNA and blocks template function

α-amanitin – a synthetic antibiotic that inhibits bacterial polymerases

Cordycepin – a toxin in poisonous mushroom (Amanita phalloides). It Inhibits eukaryotic RNA polymerase but not bacterial enzyme.

Rifampicin- a 3'-deoxy substrate analog. Causes chain termination

Translation (Protein Synthesis)

- Genetic information stored in sequence of nucleotides in mRNA is translated following the dictates of the **Genetic Code** into a sequence of amino acids in a polypeptide gene product.

- Translation involves 3 RNAs (tRNA, rRNA, mRNA), all transcribed from DNA.

- Other macromolecules involved in the process include:
  - At least 20 aminoacyl-tRNA synthetases (amino acid activating enzymes).
  - At least 9 soluble protein factors involved in chain **initiation**, **elongation** and **termination.**

- Proteins are synthesized in the cytosol on the ribosomes.

The Genetic Code

- Is a catalogue of base sequences in the mRNA that specify amino acids in proteins.
- Code is a **triplet codon**
  - a sequence of 3 nucleotides specify one amino acid.
- Code is **non-overlapping** and **comma free**
  - triplets follow in immediate sequence, no intervening sequences.
- Code is **almost universal**
  - same or nearly so in all organisms
    - Termination codon “UGA” directs tryptophan incorporation in yeast and human mitochondrion. In some protozoa, UAA and UAG are not stop signals.

The Genetic Code

- Code is **degenerate**
  - more than one triplet code for the same amino acid.
- The genetic code has 64 codons. 61 of which specify amino acids. The other 3 specify stop codons.

- The 1st and 2nd nucleotides are often sufficient to specify an amino acid, the 3rd nucleotide is redundant.

- This is designed to minimize effects of mutations.
- The flexibility in base pairing at the 3rd nucleotide position is responsible for the degeneracy of the Genetic Code.

The Genetic Code

	U	C	A	G	
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Try	Cys	C
	Leu	Ser	STOP	STOP	A
	Leu	Ser	STOP	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

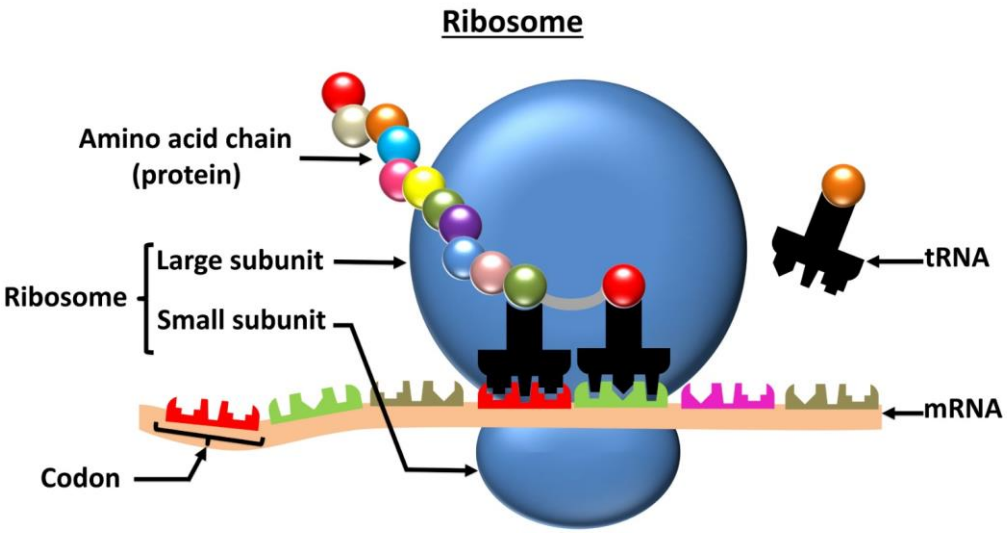
The Genetic Code

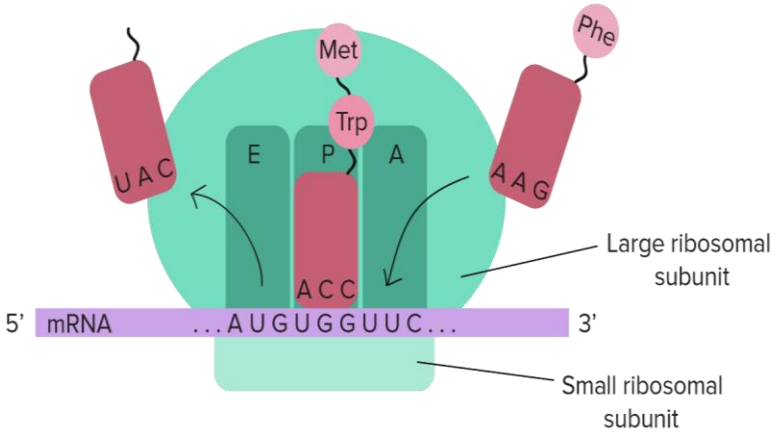
- There are now 22 known amino acids that are found in proteins.

- Two additional amino acids, selenocysteine and pyrrolysine, have been discovered in the past 40 years.

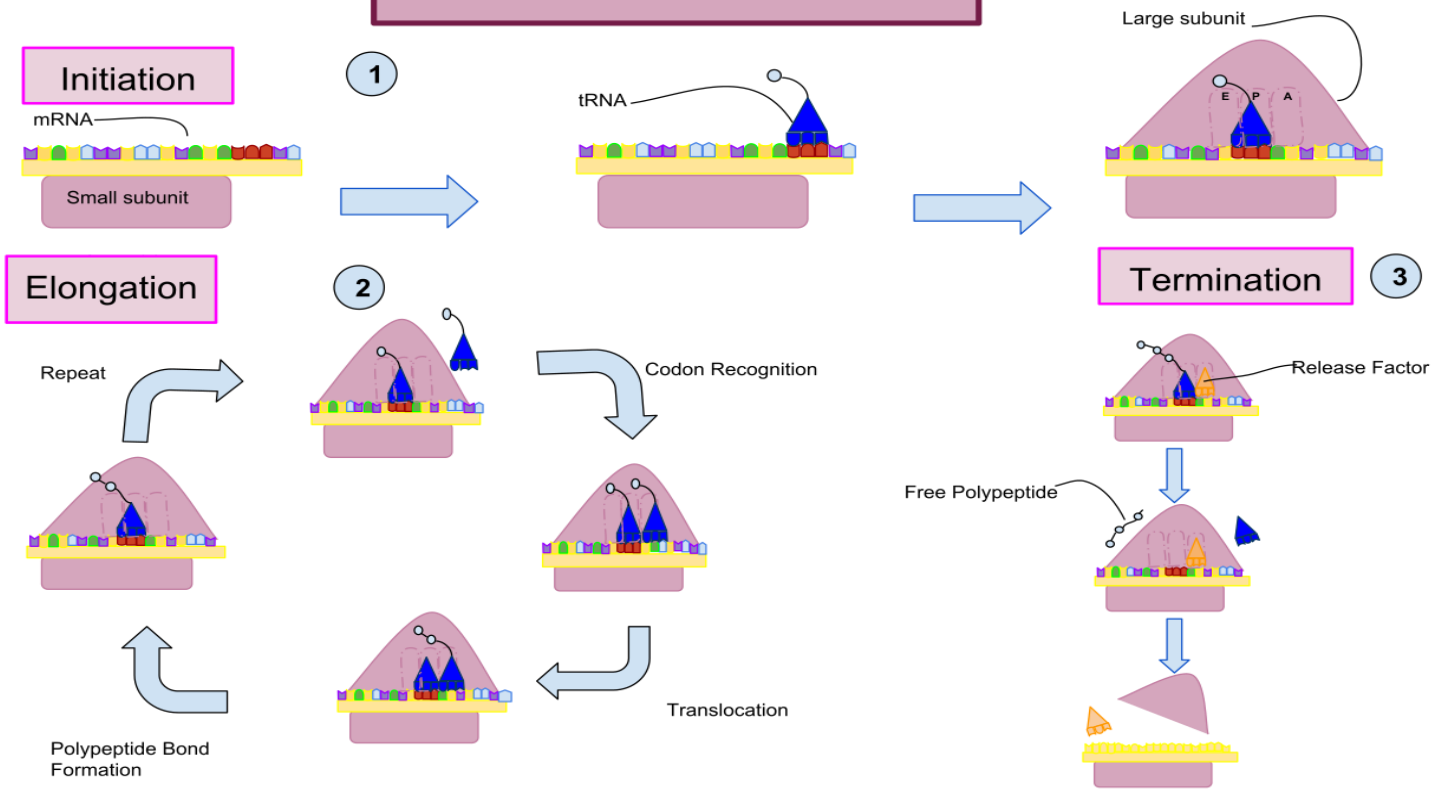
Translation

- All stages require translation factors in addition to ribosomes, mRNA, and aa-tRNAs





Phases of Translation

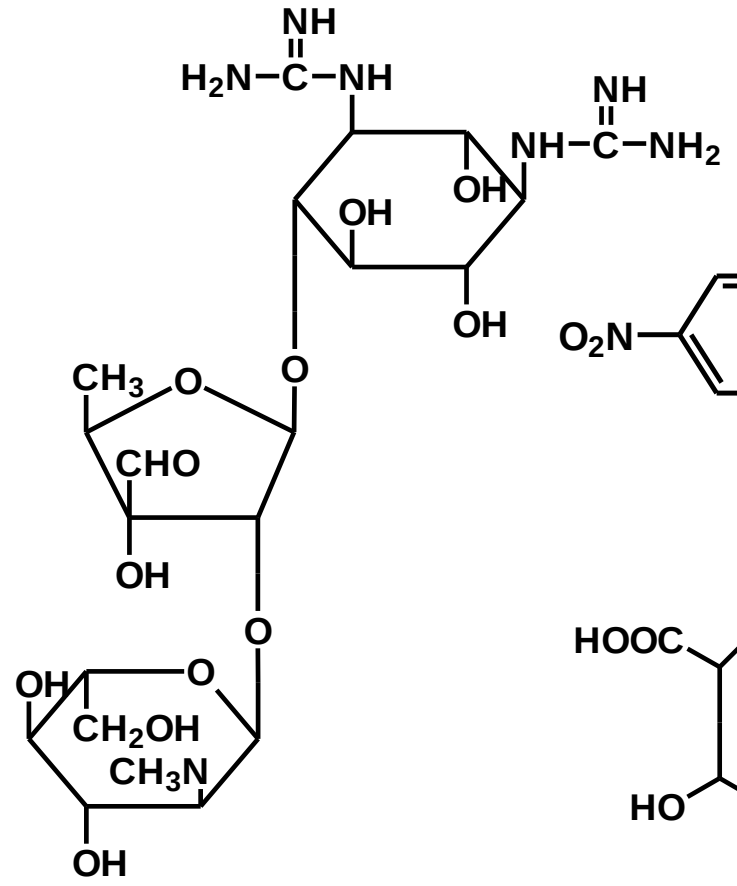


Antibiotics

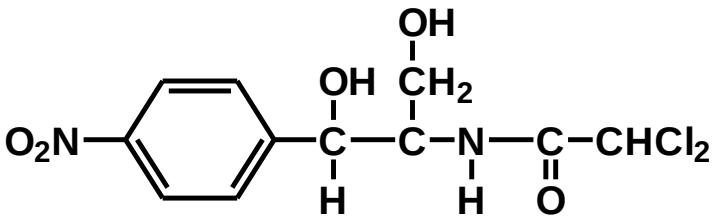
Structures of Antibiotics

- Many antibiotics act by interfering with bacterial protein synthesis.
- They inhibit either transcription or translation.
- Puromycin for example inhibits translation because it structurally resembles a tRNA-amino acid complex.
- During translation, puromycin binds to mRNA and thus halts protein synthesis.

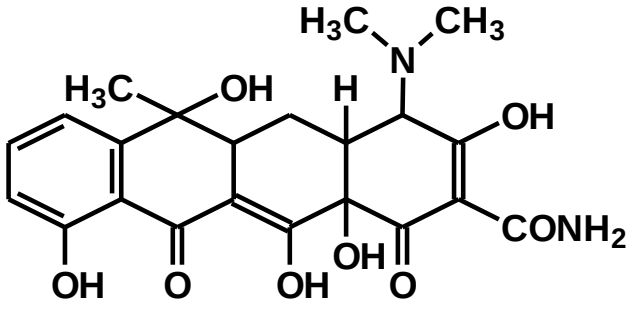
Structures of Antibiotics



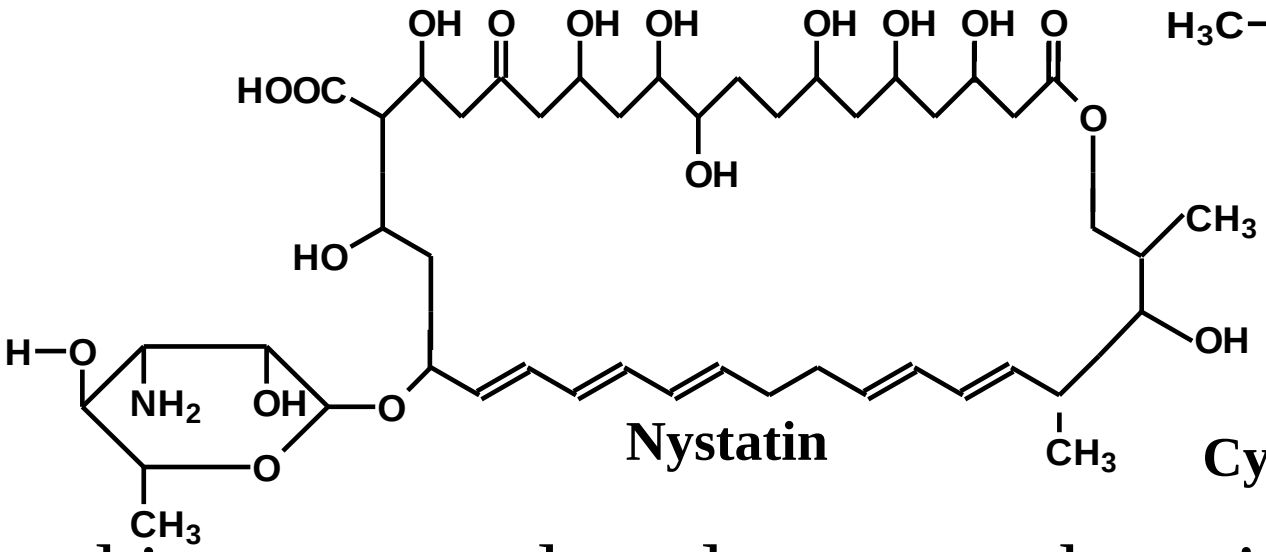
Streptomycin



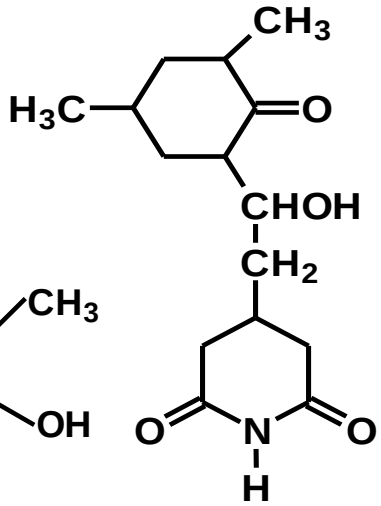
Chloramphenicol



Tetracycline



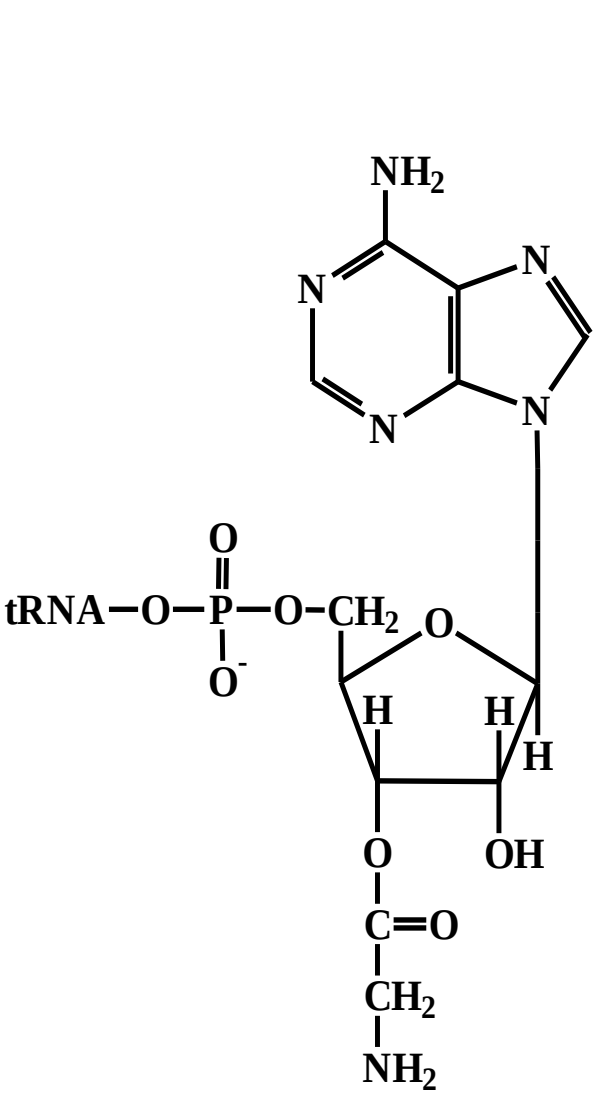
Nystatin



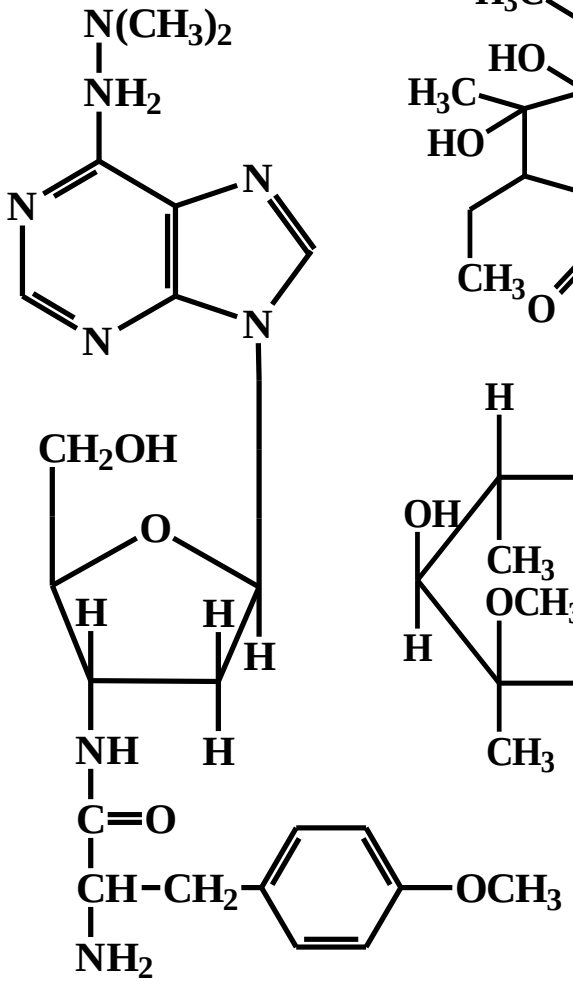
Cycloheximide

Nystatin and its structural analogue amphotericin B are antifungal agents

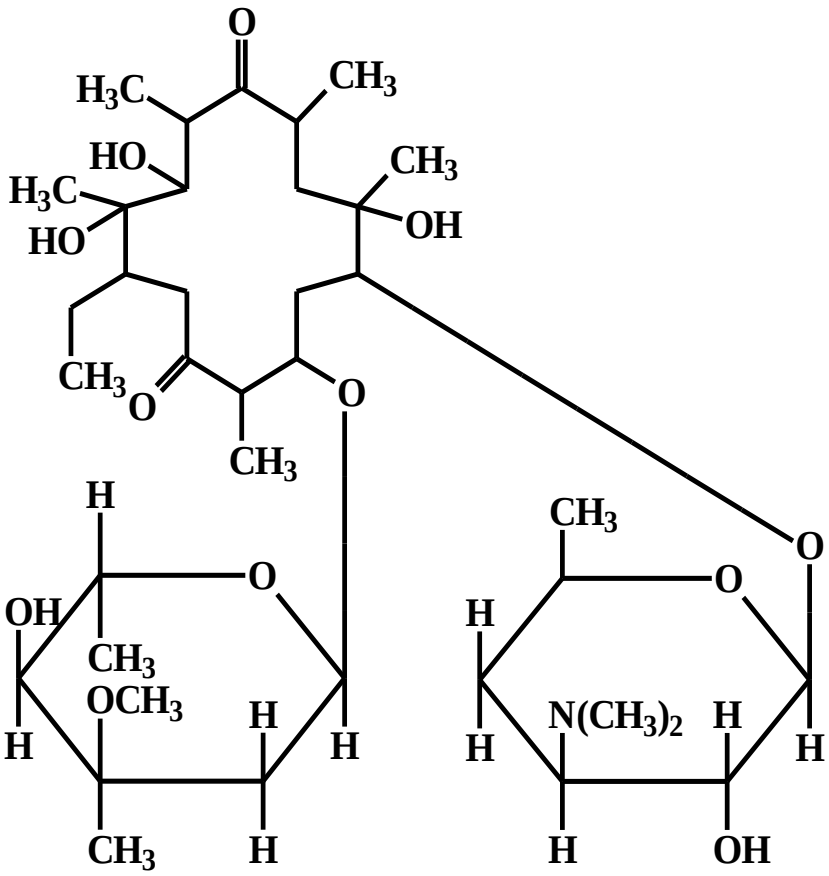
Structures of Antibiotics



Aminoacyl-tRNA



Puromycin



Erythromycin

Mode of Action of Antibiotics

Antibiotic	Prokaryote	Eukaryote		Mode of Action
		Cyto	Mito	
Chloramphenicol	+	-	+	Binds to 50S;blocks chain elongation
Cyclohexamide	-	+	-	Binds 60S; blocks chain elongation
Puromycin	+	+	+	Binds to A site of larger subunit, causes premature chain termination
Streptomycin	+	+	+	Binds to smaller ribosomal subunit, blocks chain initiation and elongation.
Erythromycin	+	-	+	Binds 50S, prevents translocation
Tetracycline	+	-	+	Binds to 30S ribosomal subunit, blocks A site
Sparsomycin	+	+	+	Blocks peptidyl transferase

Source: Adapted from Wood, W. B. et al (1974)Biochemistry, A Problems Approach.

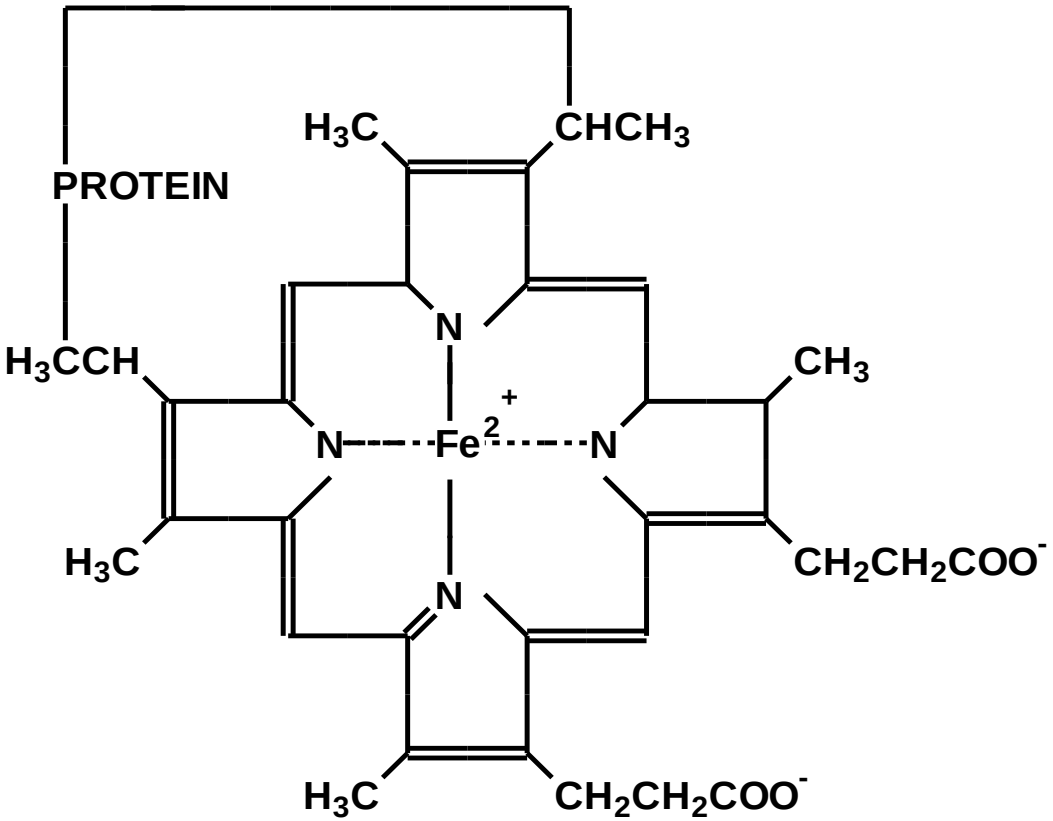
Porphyrin Compounds

Porphyrins

- Consist of four pyrrole rings linked through methene groups (-CH_2-).
- The porphyrin ring system is present in:
  - Heme proteins (e.g. hemoglobin, myoglobin, cytochromes, peroxidase, catalase)
  - Chlorophyll of green plants
- Porphyrins are classified according to the side chain attached to individual pyrrole rings
- The conjugated double bonds in the pyrrole ring system impart certain characteristics to porphyrin- containing compounds e.g.
  - Absorption of light in the visible range
  - Fluorescence
- The porphyrin molecule is very heat stable.

Porphyrin Compounds

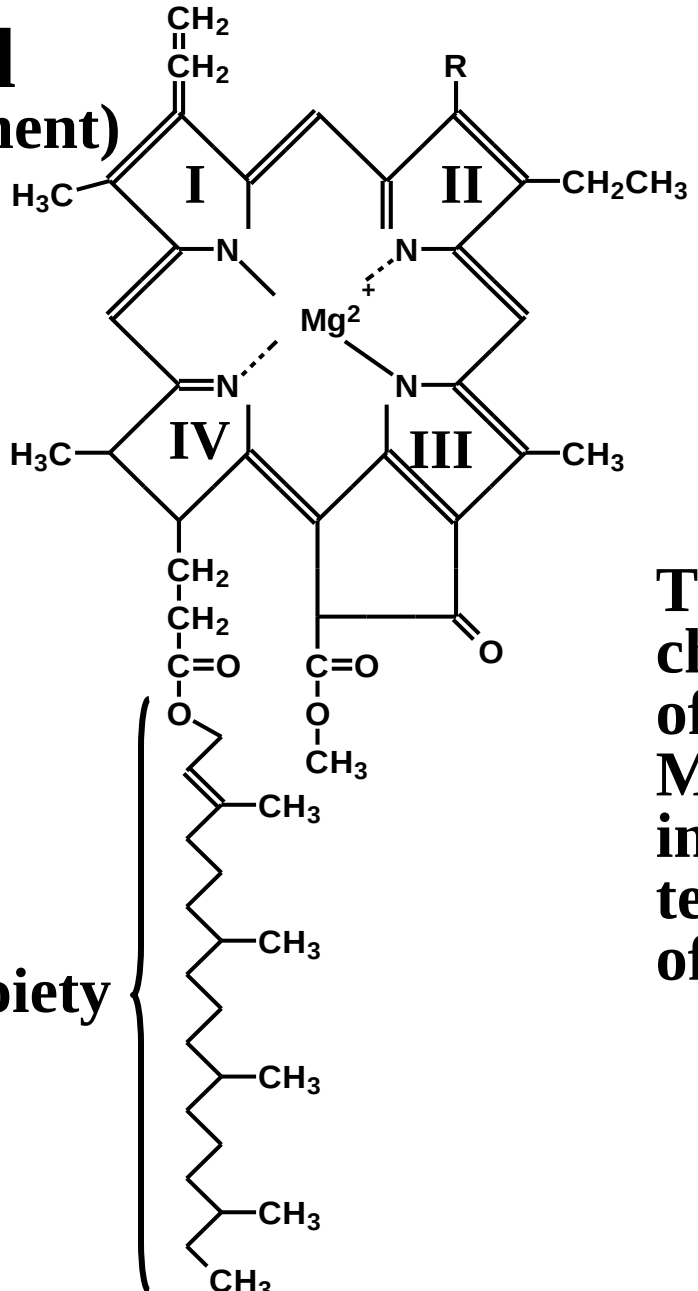
Heme containing compounds



Heme pyrrole ring system is found in heme proteins such as cytochrome C, hemoglobin and myoglobin.

Porphyrin Compounds

Chlorophyll
(green plant pigment)



Phytol moiety

The structure of chlorophylls *a* and *b*.
in *a*, R = CH_3;
in *b*, R = -CHO

The pyrrole system of chlorophylls resembles that of the heme except that, Mg^{2+} is coordinately bound in the centre of the tetrapyrrole ring instead of Fe^{2+}

Secondary Plant Metabolites

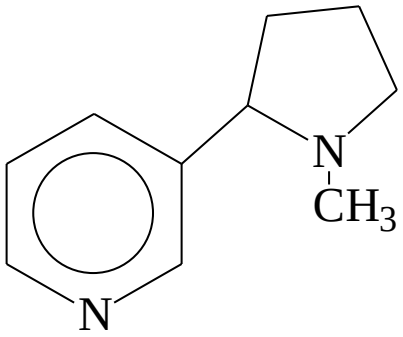
Alkaloids

- The name alkaloids means **“like an alkali”**.
- They are heterocyclic nitrogen compounds found in plants.
- Are basic and can be extracted by dilute acid and regenerated by subsequent treatment with aqueous base.
- They have profound physiological activity.
- They are used as:
  - anesthetics
  - anti-depressants
  - stimulants
- They are habit forming (addictive)

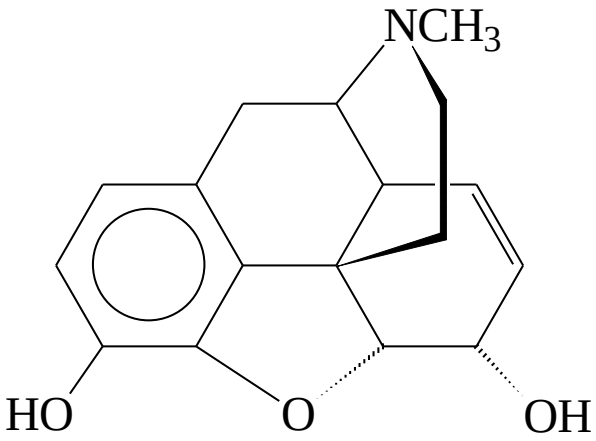
Alkaloids

- **Nicotine**
  - Is toxic. Nicotine sulfate is used as an insecticide.
  - Acts by stimulating nervous system in small doses. Continuous doses can depress the nervous system. It is responsible for the addiction of smoking.
- **Morphine**
  - Obtained from the gum and seeds of opium poppy.
  - **Codeine** (an analgesic), is a naturally occurring methoxy derivative of morphine.
  - **Heroin** (an analgesic), is a synthetic diacetyl derivative of morphine. A much more addictive form of morphine.

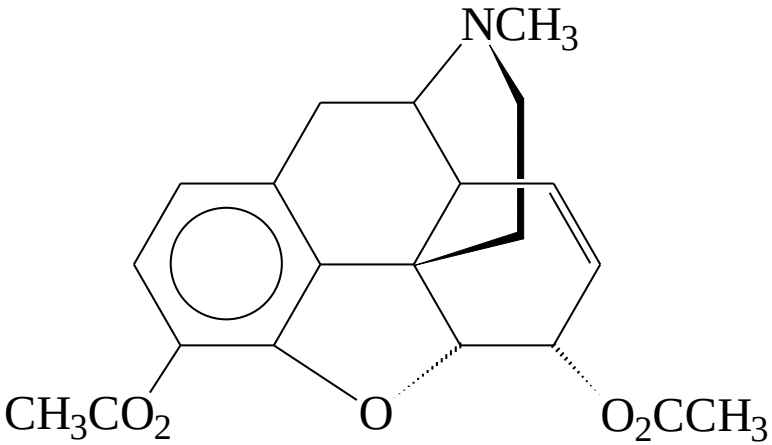
Alkaloids



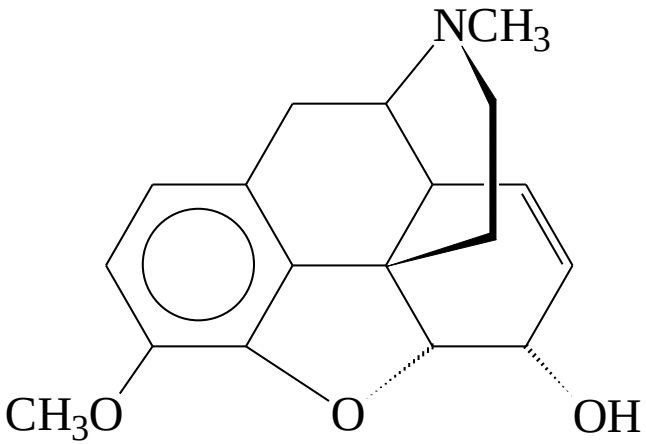
nicotine



morphine



heroin



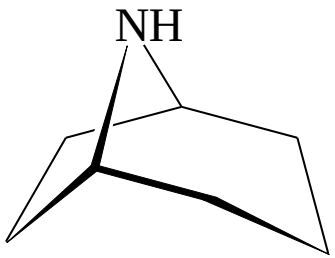
codeine

Alkaloids

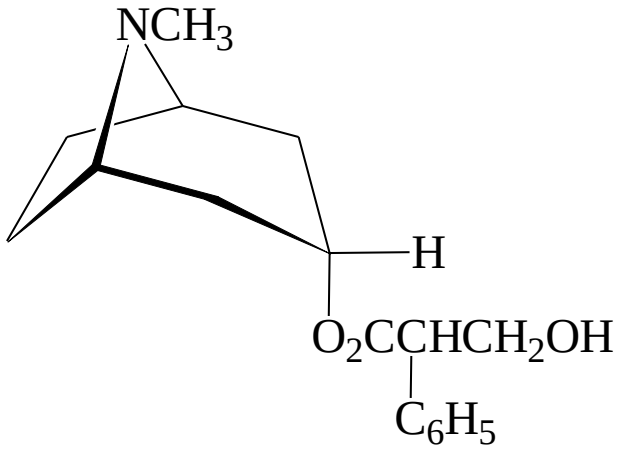
Tropane alkaloids

- They contain the tropane ring system
  - **Atropine**
    - used in eye drops to dilate pupils
    - used as anesthetic for eye examination.
    - used to accelerate slow heart rates.
  - **Cocaine**
    - a habituating stimulant and pain reliever.

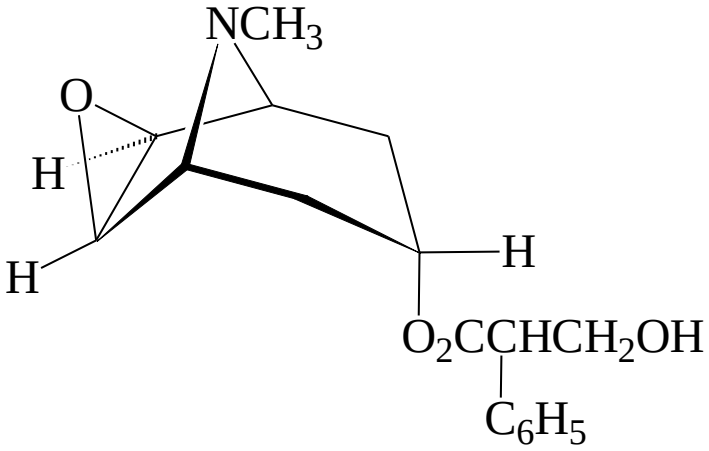
Alkaloids



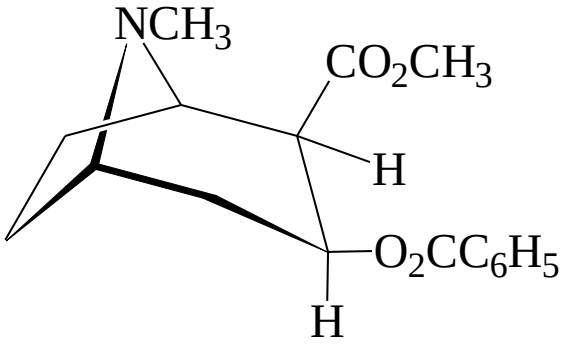
tropane



atropine



scopolamine



cocaine

Saponins

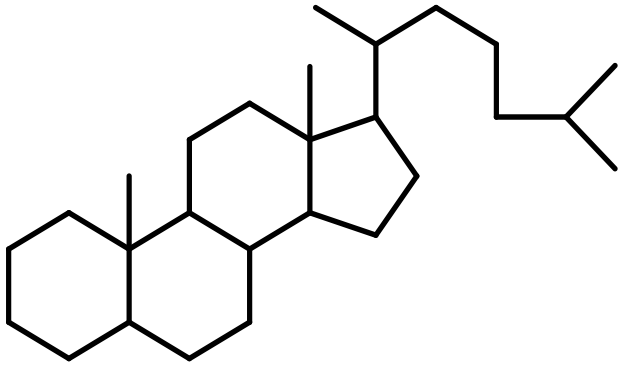
- Plants contain a high percent of glycosides called **saponins** (Latin *sapo*, soap)
- Have detergent properties and produce frothing in aqueous solution.
- Are toxic and have hemolytic properties.
- Differ in the nature of the sugar(s) present or the aglycone (sapogenin) structure. The sugar moieties are attached at carbon-3
- Two types of saponins are known depending on the nature of the aglycone
  - tetracyclic triterpenoids (steroidal saponins)
  - pentacyclic triterpenoids

Saponins

- Steroidal saponins are pharmaceutically important because of their relationship to other steroidal compounds e.g. sex hormones.
- Saponins in powder form have been used as foaming and emulsifying agents in the manufacture of toothpastes, foam fire extinguishers, foam in beverages, shampoo, liquid soaps and cosmetics.

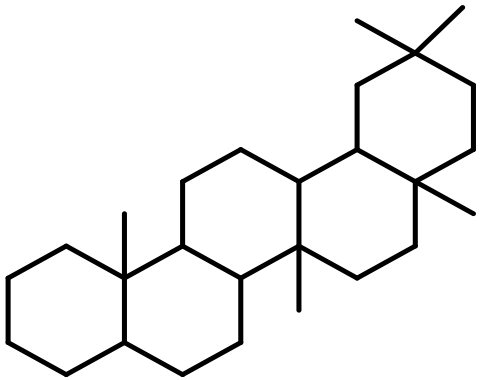
Saponins

General Structure



Skeleton of tetracyclic triterpenoid

(steroidal saponins)



Skeleton of pentacyclic triterpenoid

Saponins

Steroidal Saponin	Constituent Sugars
Gitonin	1 glucose, 2 galactose, 1 xylose
Digitonin	2 glucose, 2 galactose, 1 xylose
Dioscin	1 glucose, 2 rhamnose

Flavonoids

- Flavonoids (from Latin *flavus* meaning yellow)
- They are plant metabolites with various functions.
- Constitute the largest group of naturally occurring phenolic compounds (more than 2000 known).
- Occur in the free state and as glycosides
- Function as medicinal agents and are common constituents of herbal remedies. They are known for:
  - anti-inflammatory, antithrombitic, vasoprotective, anti-allergic properties.
- They are believed to inhibit cell proliferation.
- Activity thought to be due to effects of flavonoids on arachidonic acid metabolism.

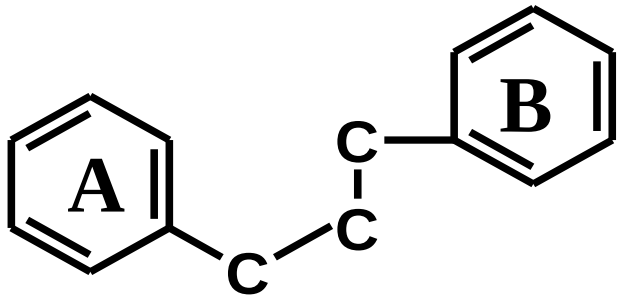
Flavonoids

- Some flavonoids are also known for antifungal, antibacterial and antitumour properties.
- Intensity of yellow color increases with pH and the number of hydroxyl groups.

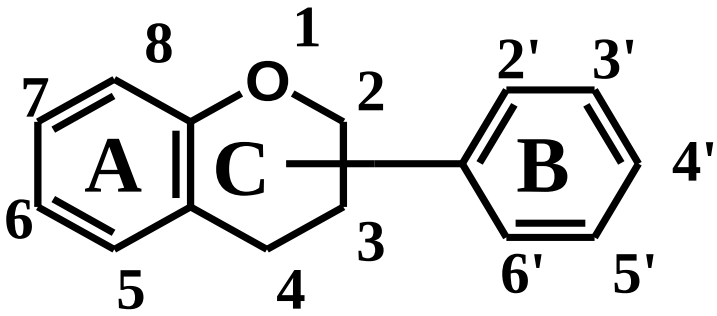
Flavonoids

Flavonoid Skeletons

Rare



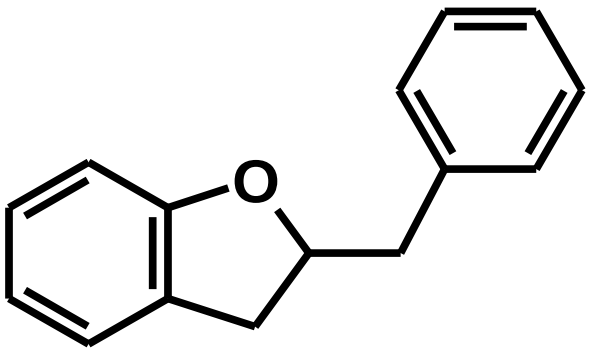
Commonest forms



Chromane ring

B ring can be in positions 2 or 3

Rare

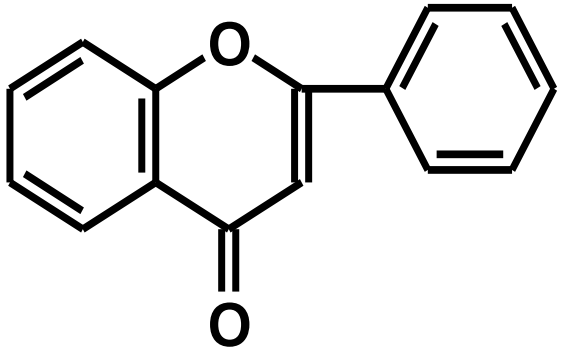


Flavanones, flavones, flavonols and anthocyanins have a B ring in position 2. In isoflavonoids the B ring is in position 3. Neoflavonoids have the B ring in position 4. Isoflavonoids and neoflavonoids are said to be abnormal flavonoids.

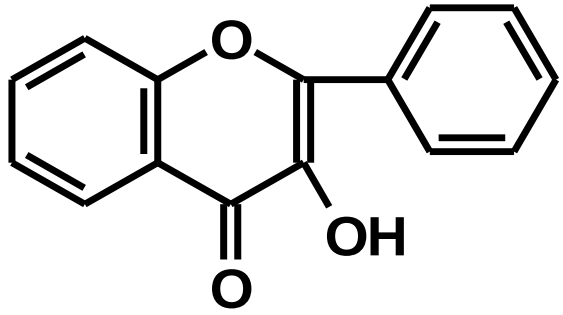
Flavonoids

- **Subgroups of flavonoids**
  - **Anthocyanins**
  - Chalcones
  - Flavone
  - Flavonol
  - Flavanone
  - Isoflavanoids (e.g. rotenone is an insecticide fish poison and mitochondrial electron transport inhibitor)
  - Neoflavonoids

Flavonoids



Flavone



Flavonol

Anthocyanins

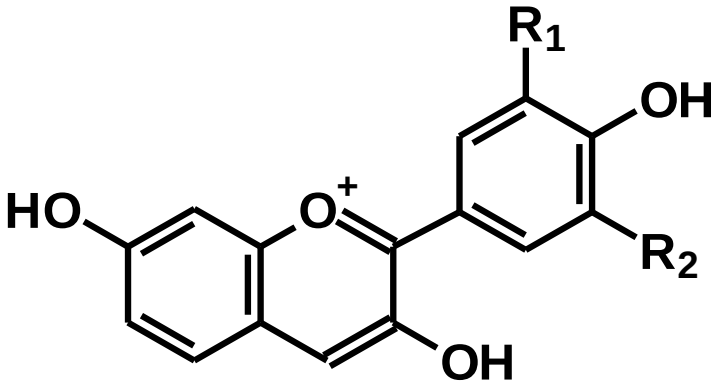
- From the Greek *antho*-, flower, and *kyanos*, blue
- They are flavonoid glycosides of great economic importance
- Occur in practically all parts of most higher plants.
- Most red and blue flowers owe their colors to anthocyanins.
- They serve as,
  - attractants in pollination.
  - natural antioxidants, anti-inflammatory agents
  - promoters of healthy vision, skin and brain function.
  - preventers of premature aging
  - colorants for fruit juices, wine and some beverages.

Anthocyanins

- Cocoa and chocolate products have a high flavonol content, which makes them good at protecting the body against cardiovascular diseases.
- Anthocyanins vary in different types of fruit.
- Differences in R_1, and R_2 substituents on the B ring distinguishes anthocyanins into three main families of compounds, namely; the **dephinins**, the **cyanins** and the **pelargonins**.
- R_1 and R_2 can be one of the following H, OH, or OCH_3
- Anthocyanins usually have the sugar moiety attached in a glycosidic linkage to positions 3,5 or 7.
- **Anthocyanidins** are anthocyanins without sugar attachments and are referred to as **delphinidin**, **cyanidin**, and **perlargonidin**.

Anthocyanins

General Structure of Anthocyanidin



the aglycone core of anthocyanins

Anthocyanins

- The number and types of sugars attached to the aglycone core affect the polarity and electrochemical properties of anthocyanins.
- The modified properties cause the varieties of reds and blues in flowers and fruits.
- The actual colour created by an anthocyanin depends on pH (red in acid, violet in neutral and blue in a basic environment).
- Anthocyanins with sugars attached to position 7 are not common.
- Those with a sugar attached only to position 3 are called monoglycosides, whereas those with sugar moieties attached at both positions 3 and 5 are called diglycosides.

Anthocyanins

- Sugar(s) can be removed chemically by acid hydrolysis to give the agylcone, anthocyanidin.
- It has been postulated that anthocyanins when injested by the body are converted into anthocyanidins at the site of use.
- When many anthocyanidins are linked, the polymer is called a proanthocyanidin or more technically, oligomeric proanthocyanidins.