Nucleotides and Nucleic Acids

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Outlines

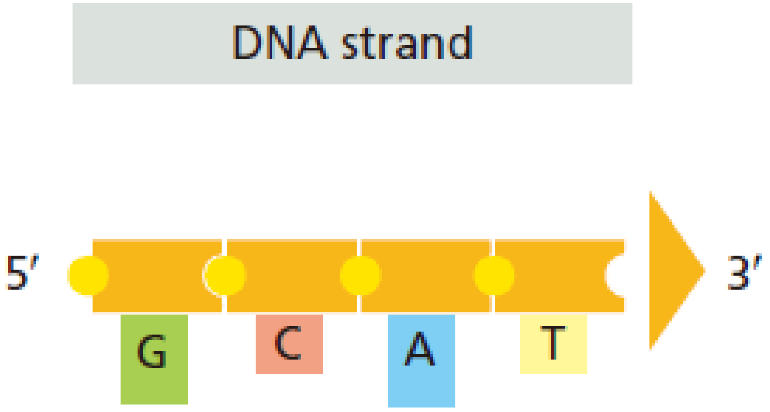
Part I. Some Basics

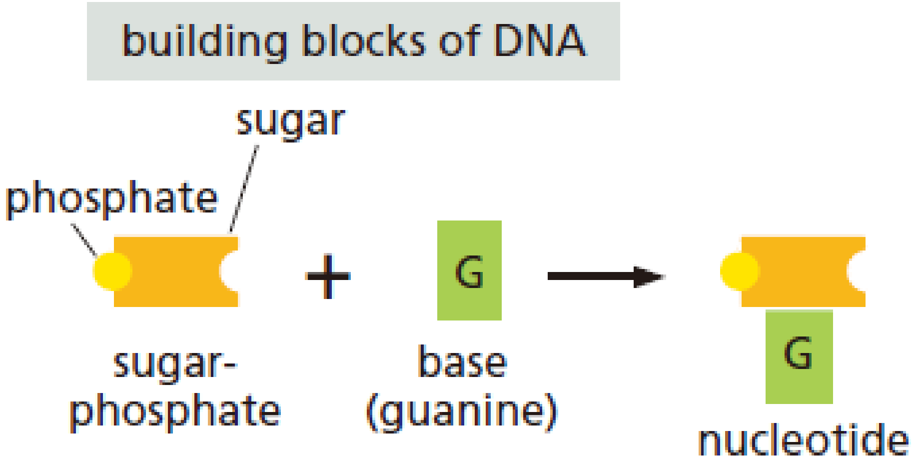
Part II. Nucleic Acid Structures

Part III. Nucleic Acid Chemistry

Part IV. Other Functions of nucleotides

Part I. Some Basics about Nucleic Acids and Nucleotides


DNA strand


building blocks of DNA

Part I

Nucleic Acids

- Deoxyribonucleic acid (DNA)

- Ribonucleic acid (RNA)
  - Ribosomal RNAs (rRNAs)
  - Messenger RNAs (mRNAs)
  - Transfer RNAs (tRNAs)
  - MicroRNAs (miRNA)
  - Non-coding RNAs (ncRNAs)
  - …

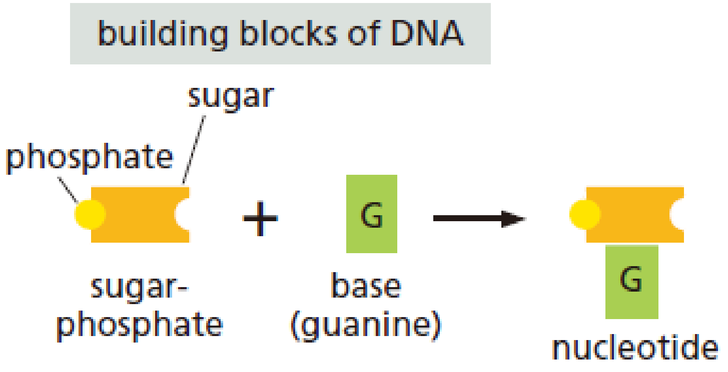
Part I

History of Nucleic Acids

- **Nuclein were discovered by Friedrich Miescher in 1869.**
- **In 1889, Richard Altmann discovered that nuclein have acidic properties and it became called nucleic acid.**
- **In 1938, Astbury and Bell published the first X-ray diffraction pattern of DNA.**
- **In 1953, Watson and Crick determined the structure of DNA.**

Friedrich Miescher
(1844-1895)

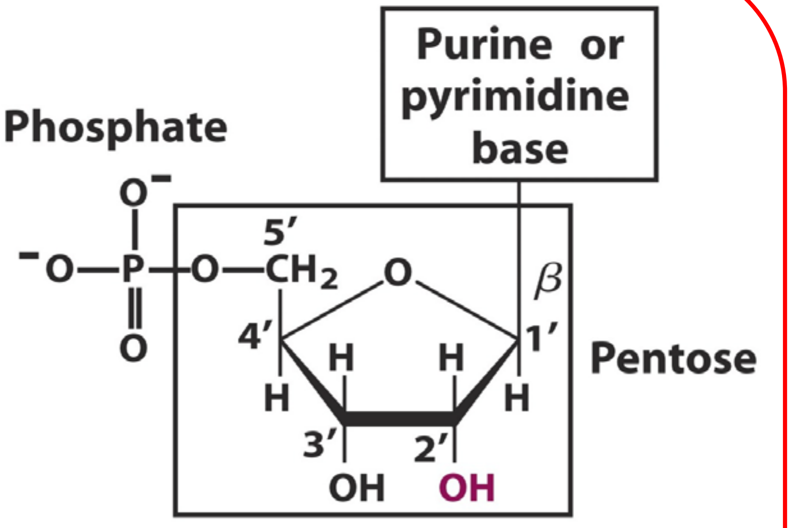
Nucleotides



- Nucleotides are building blocks of nucleic acids
  - Nucleic acids: polynucleotides

- Three characteristic components
  - A nitrogenous base (N-β-glycosyl bond)
  - A pentose
  - A phosphate

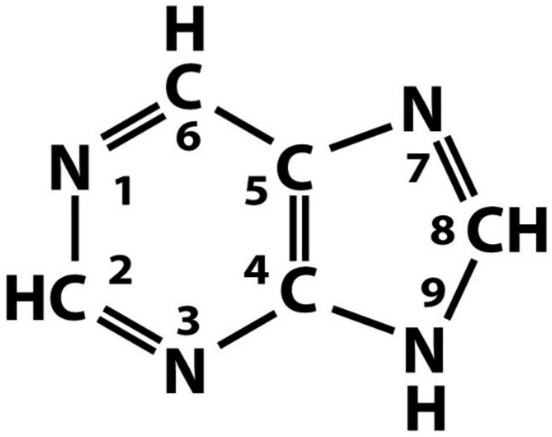
  - Nucleoside: without phosphate



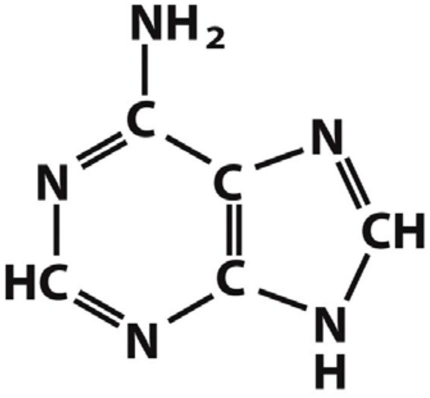
A ribonucleotide

Part I

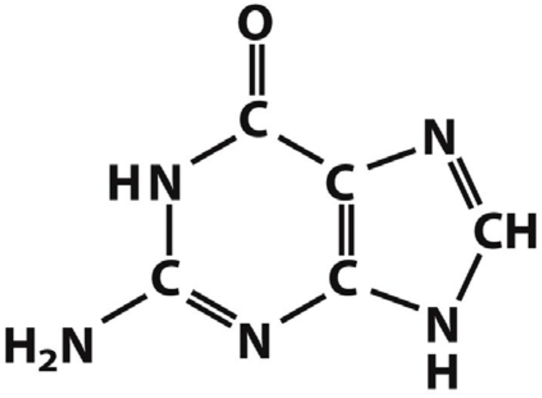
The Heterocyclic Bases



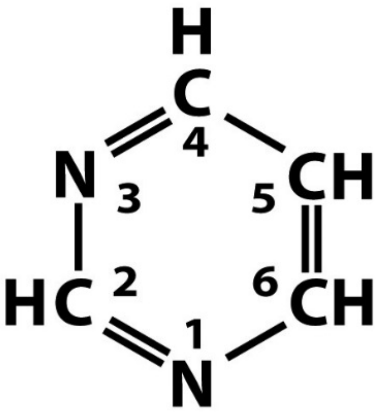
Purine



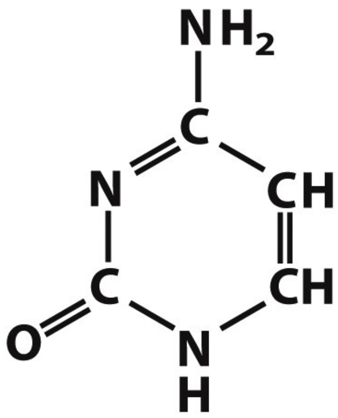
Adenine



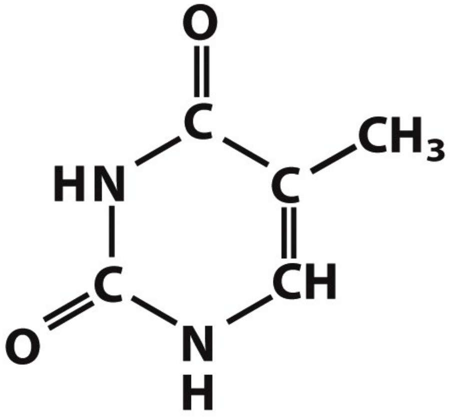
Guanine



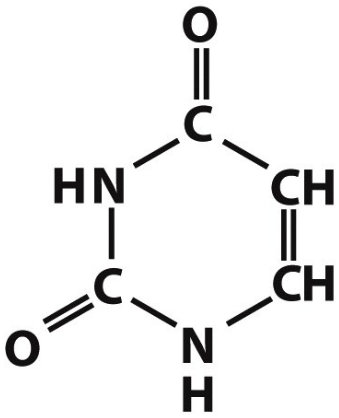
Pyrimidine



Cytosine



Thymine
(DNA)



Uracil
(RNA)

Nomenclature

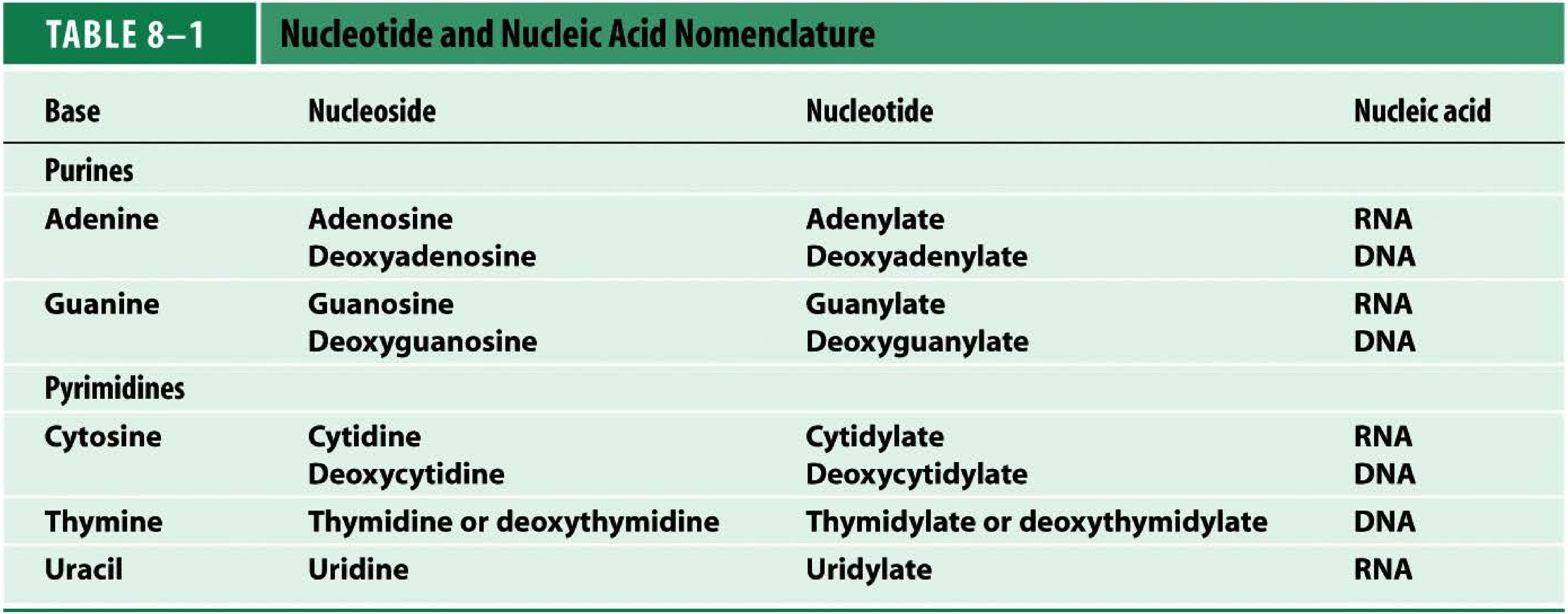


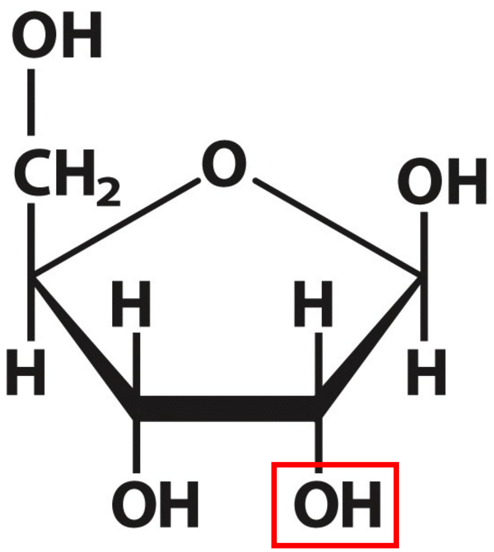
TABLE 8–1 **Nucleotide and Nucleic Acid Nomenclature**

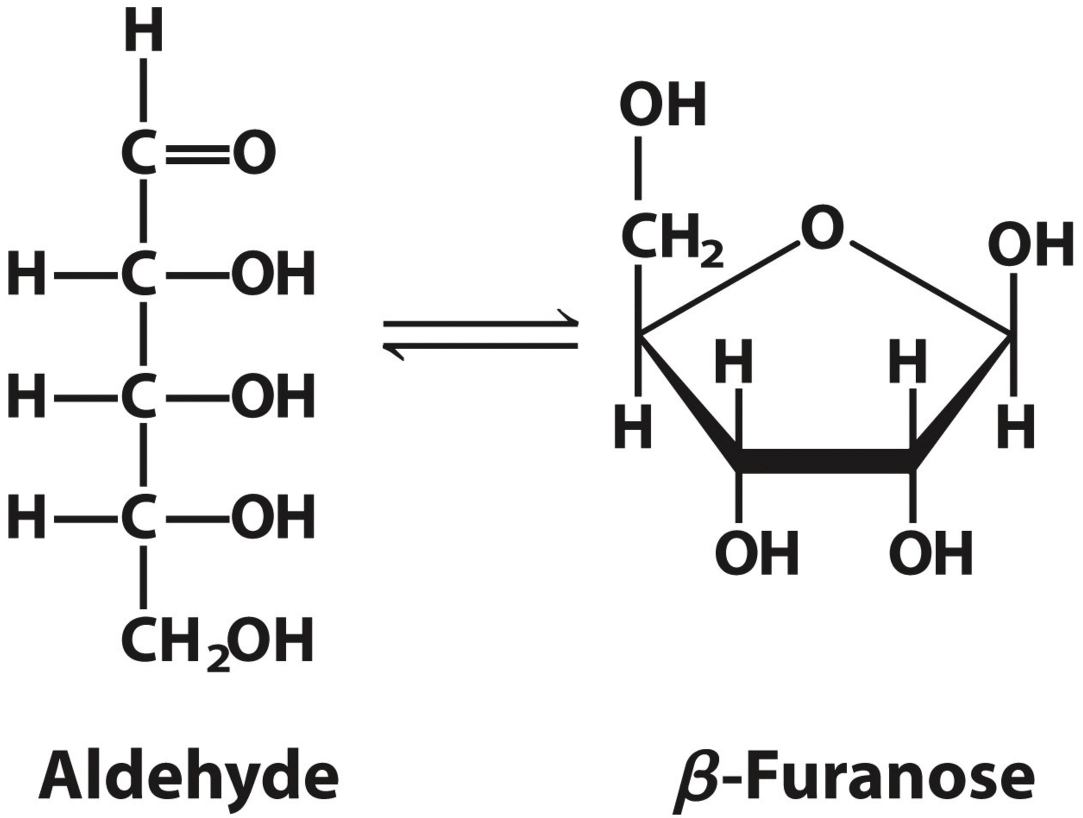
Base	Nucleoside	Nucleotide	Nucleic acid
Purines			
Adenine	Adenosine Deoxyadenosine	Adenylate Deoxyadenylate	RNA DNA
Guanine	Guanosine Deoxyguanosine	Guanylate Deoxyguanylate	RNA DNA
Pyrimidines			
Cytosine	Cytidine Deoxycytidine	Cytidylate Deoxycytidylate	RNA DNA
Thymine	Thymidine or deoxythymidine	Thymidylate or deoxythymidylate	DNA
Uracil	Uridine	Uridylate	RNA

The Pentoses Define the Identity of a Nucleic Acid

DNA: 2’-deoxy-D-ribose

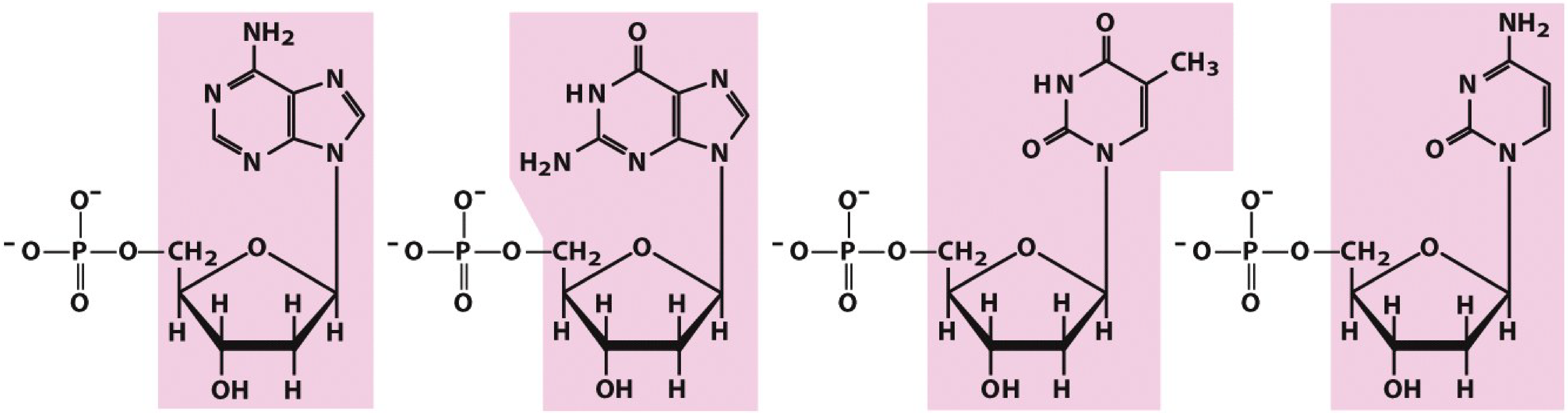
RNA: D-ribose





Conformation of a ribose

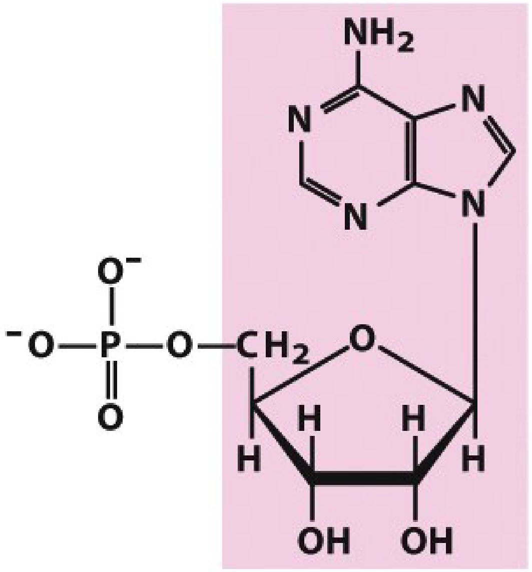
Deoxyribonucleotides Are Structural Units of DNA

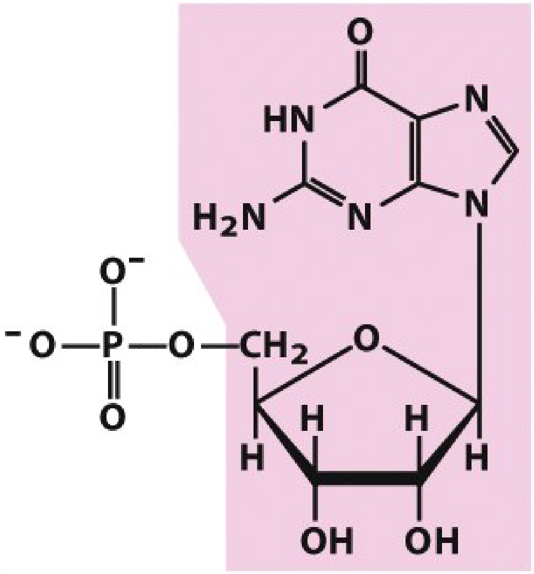


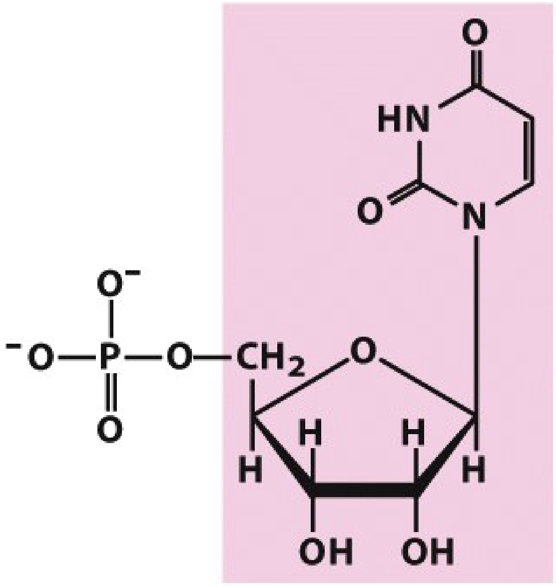
Nucleotide:	Deoxyadenylate (deoxyadenosine 5′-monophosphate)	Deoxyguanylate (deoxyguanosine 5′-monophosphate)	Deoxythymidylate (deoxythymidine 5′-monophosphate)	Deoxycytidylate (deoxycytidine 5′-monophosphate)
Symbols:	A, dA, dAMP	G, dG, dGMP	T, dT, dTMP	C, dC, dCMP
Nucleoside:	Deoxyadenosine	Deoxyguanosine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

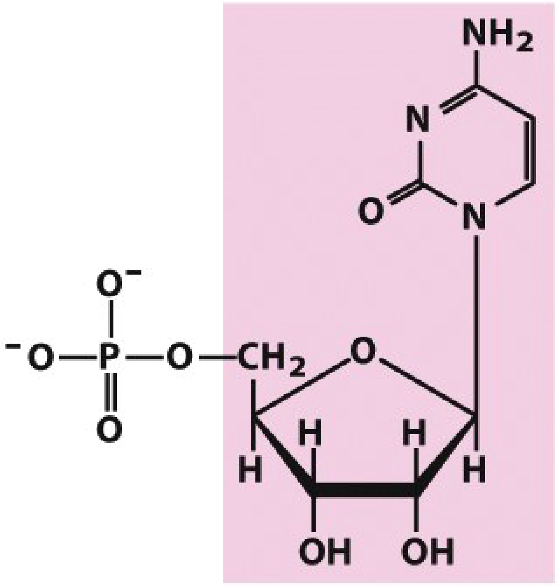
Part I

Ribonucleotides Are Structural Units of RNA







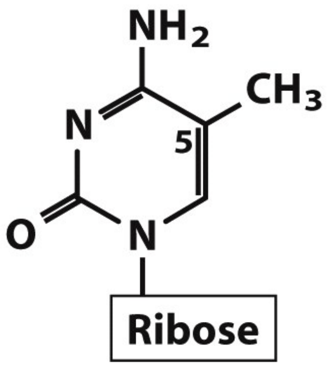


Nucleotide:	Adenylate (adenosine 5′-monophosphate)	Guanylate (guanosine 5′-monophosphate)	Uridylate (uridine 5′-monophosphate)	Cytidylate (cytidine 5′-monophosphate)
Symbols:	A, AMP	G, GMP	U, UMP	C, CMP
Nucleoside:	Adenosine	Guanosine	Uridine	Cytidine

Part I

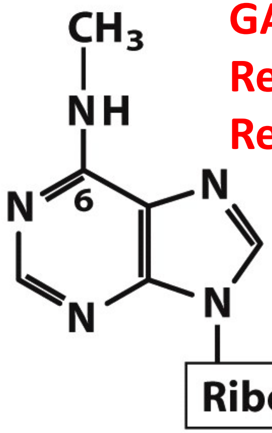
Some Minor Purine and Pyrimidine Bases

CpG Island

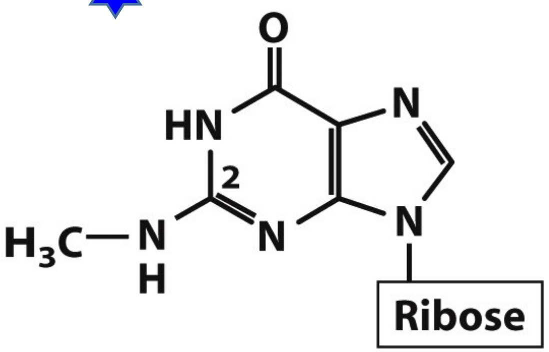


5-Methylcytidine

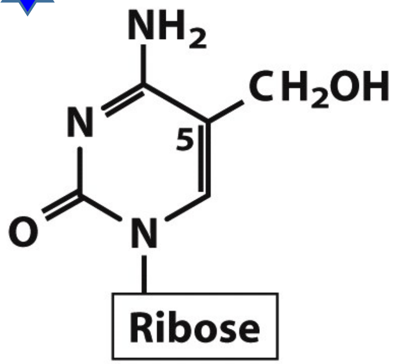
GATC in DNA Replication and Repair



N^6-Methyladenosine

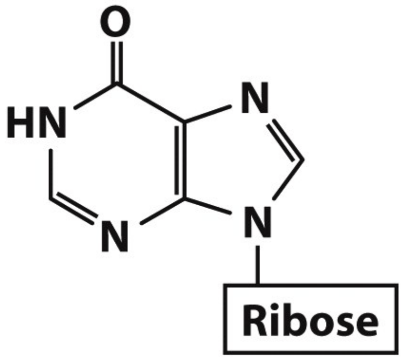


N^2-Methylguanosine

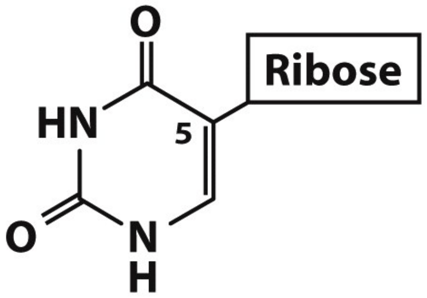


5-Hydroxymethylcytidine

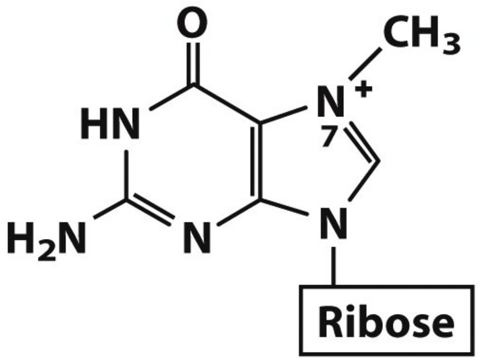
Minor bases of DNA



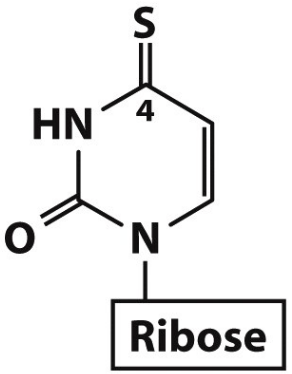
Inosine



Pseudouridine



7-Methylguanosine

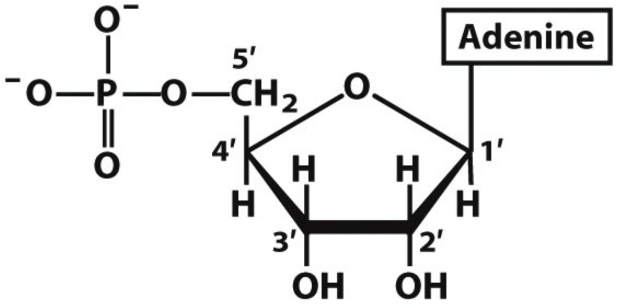


4-Thiouridine

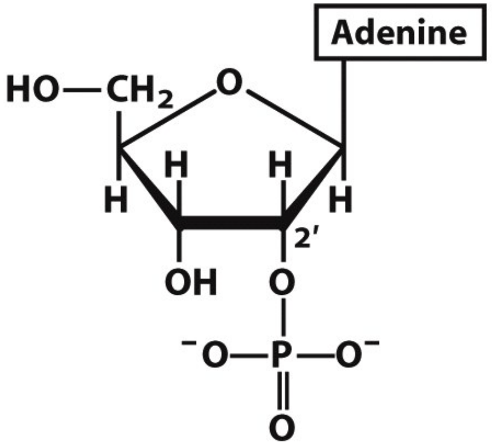
Minor bases of tRNA

Part I

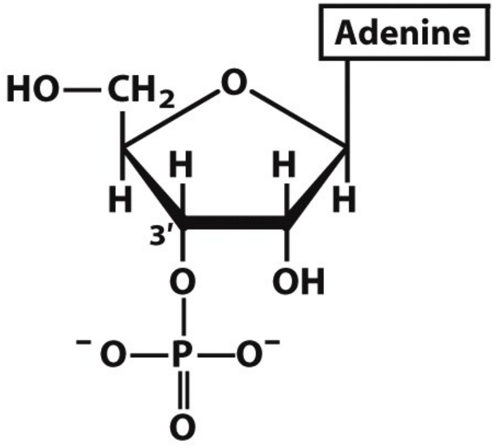
Nucleotides with Phosphate Groups in Positions Other than on the 5’ Carbon



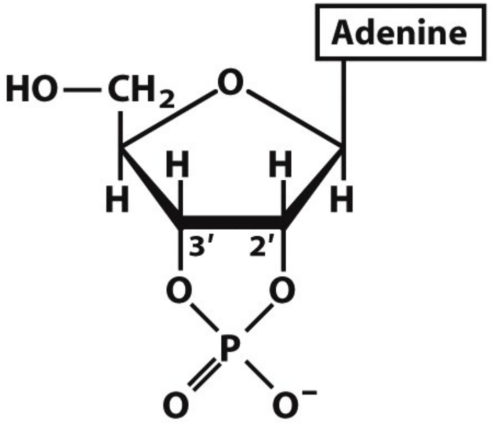
Adenosine 5′-monophosphate



Adenosine 2′-monophosphate



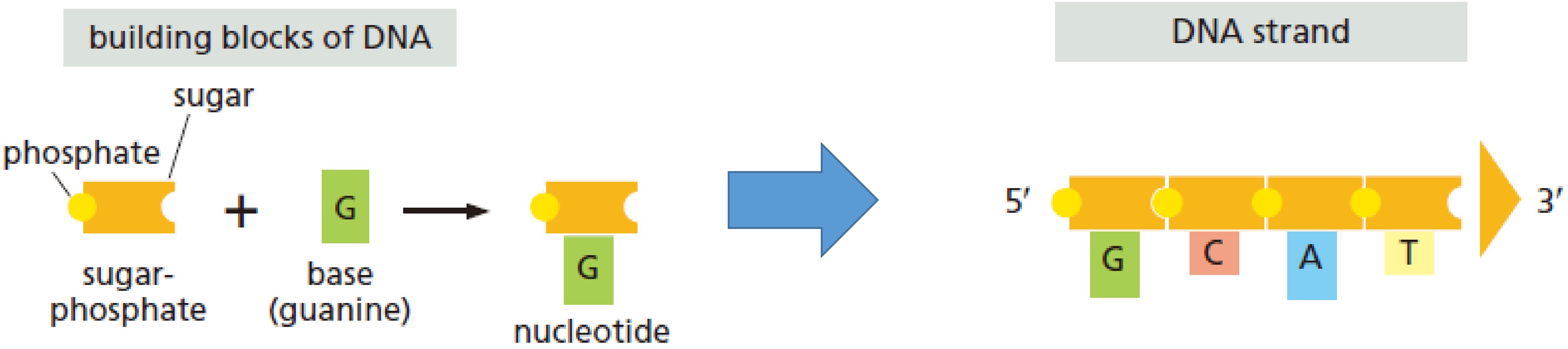
Adenosine 3′-monophosphate



Adenosine 2′,3′-cyclic monophosphate

Part I

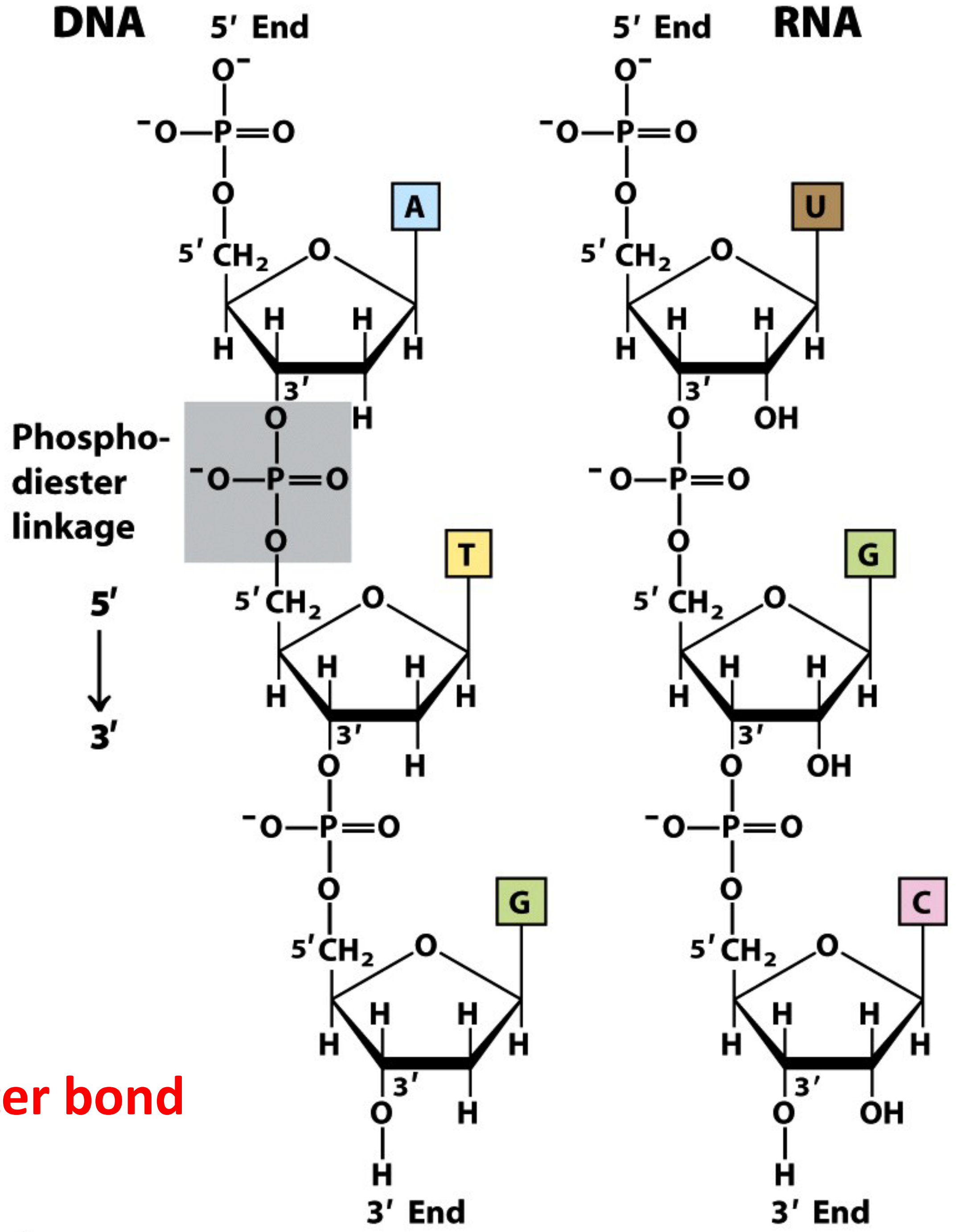
From Nucleotides to Nucleic Acids



Phosphodiester bonds

Part I

Phosphodiester Bonds Link Successive Nucleotides in Nucleic Acids

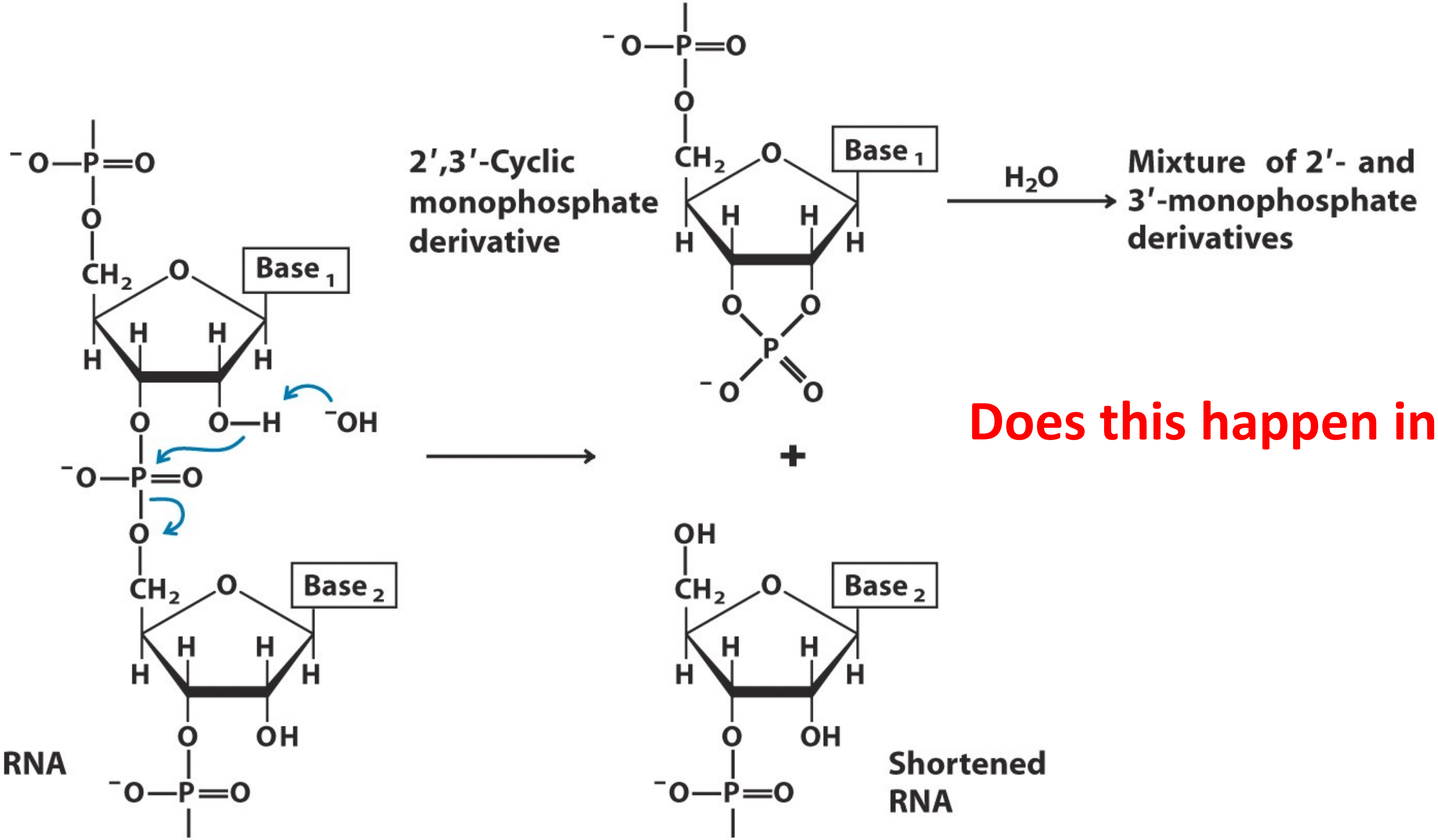


5’ end ⟶ 3’-end

The orientation of a strand,
not the orientation of each phosphodiester bond

Part I

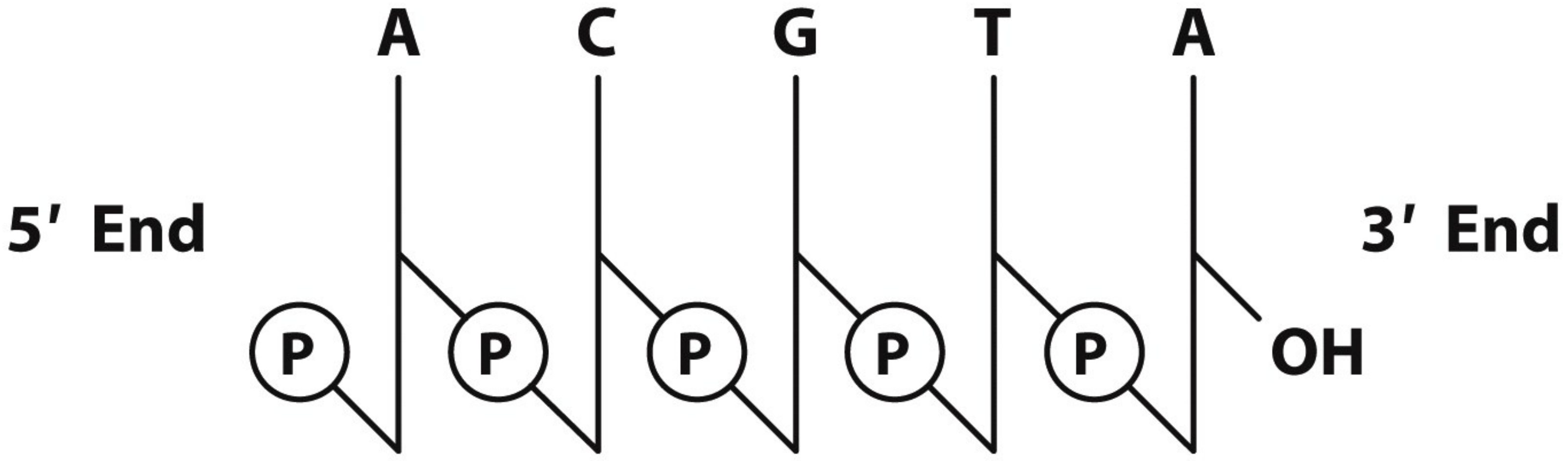
Hydrolysis of the Phosphodiester Bonds in RNA under Alkaline Conditions



Does this happen in DNA?

Part I

Illustration of a Segment of DNA



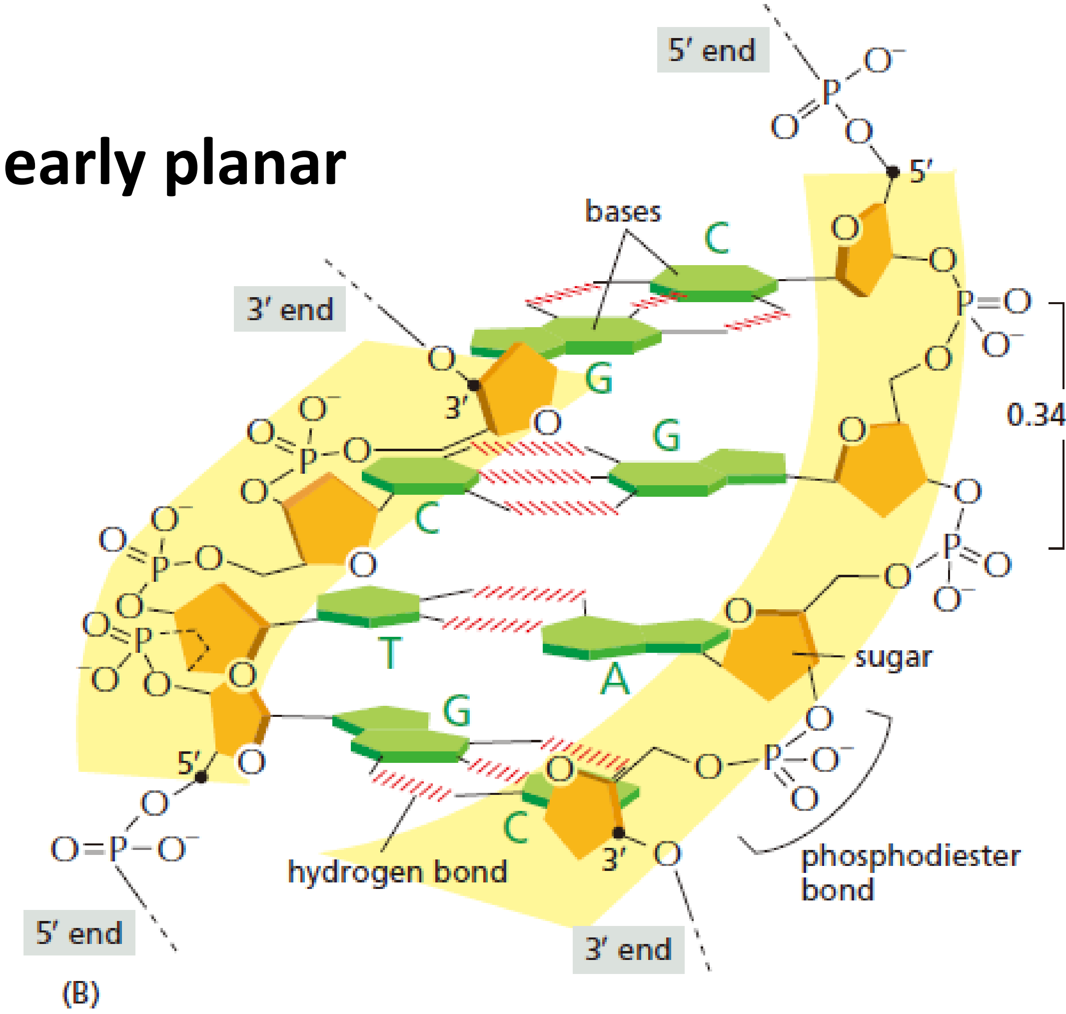
- 5′ - Left to Right - 3′
- pA-C-G-T-A_{OH}
- pApCpGpTpA
- pACGTA

Oligonucleotide: ≤ 50 nucleotides
Polynucleotide: more than 50

Part I

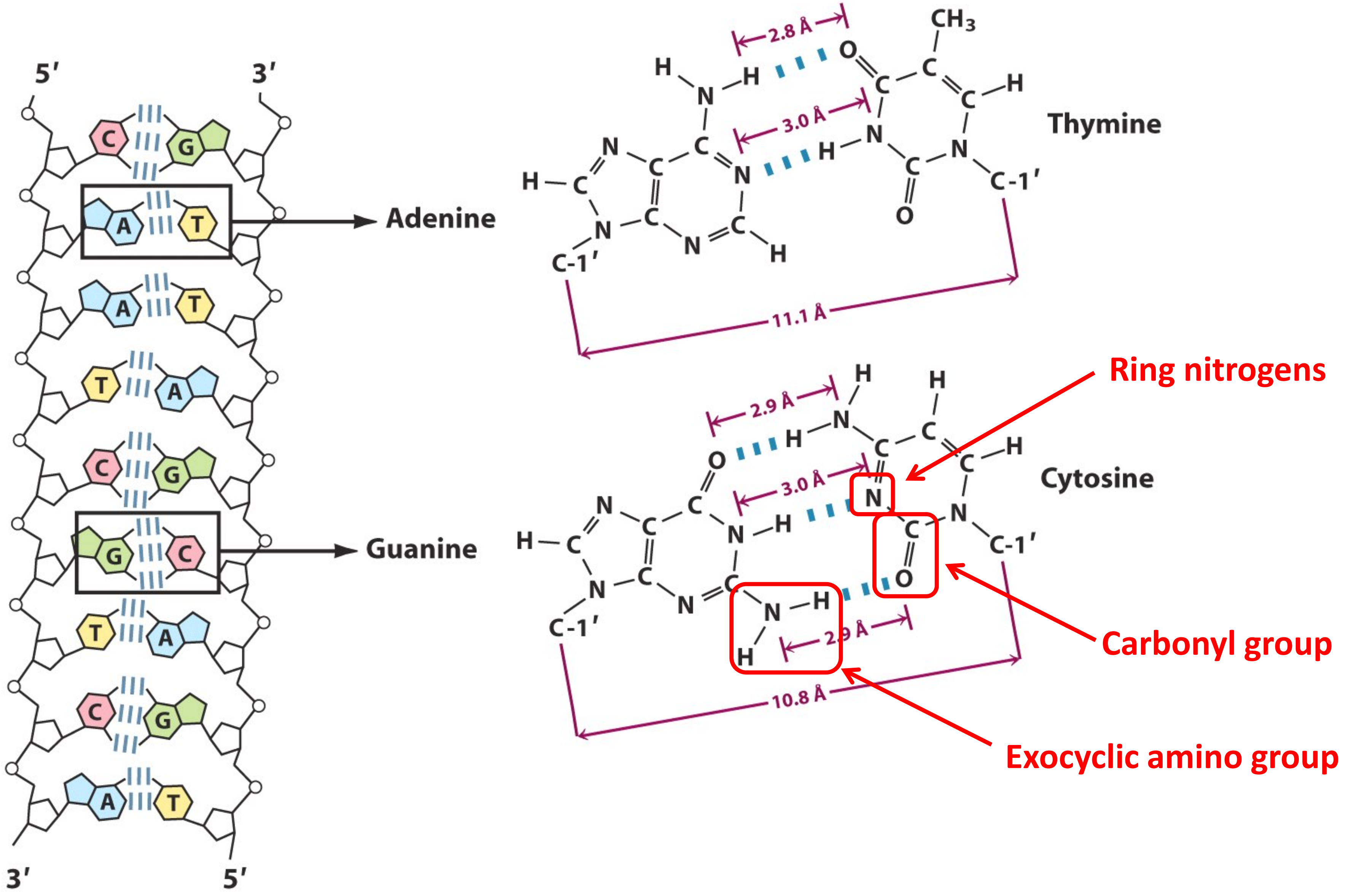
The Properties of Nucleotide Bases

- Purines and pyrimidines in are aromatic
- Pyrimidines are planar, and purines are very nearly planar
- Flat surfaces are hydrophobic
  - Hydrophobic stacking
  - Van de Waals and dipole-dipole interactions
- Hydrogen bonds between two complementary strands



Part I

Hydrogen-Bonding Pattern in the Base Pairs



Ring nitrogens

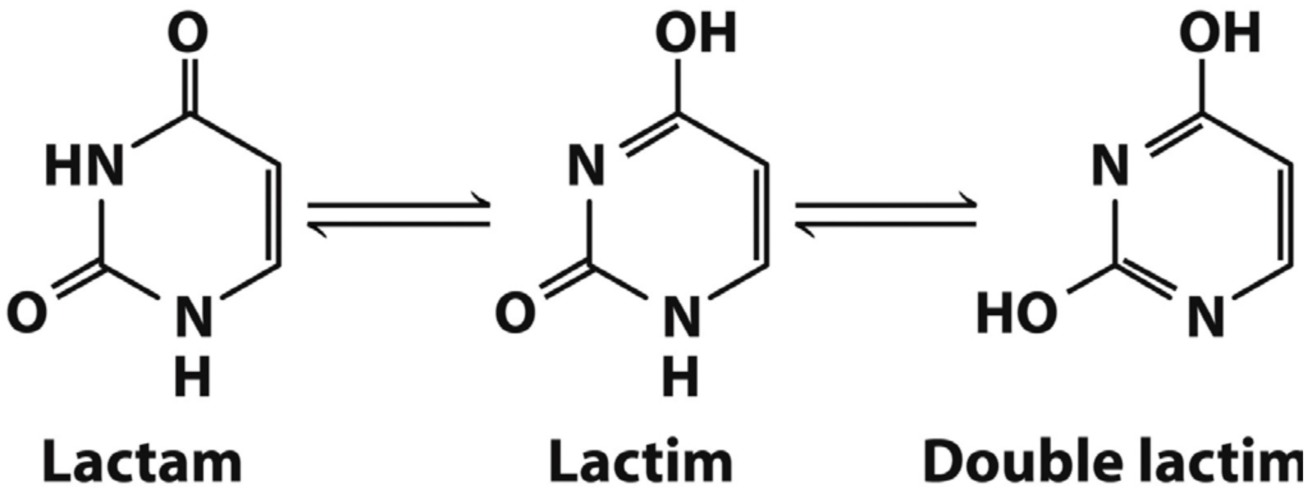
Carbonyl group

Exocyclic amino group

Part I

The Properties of Nucleotide Bases

- Most of the bonds have partial double-bond character
- May exist in two or more tautomeric forms depending on pH



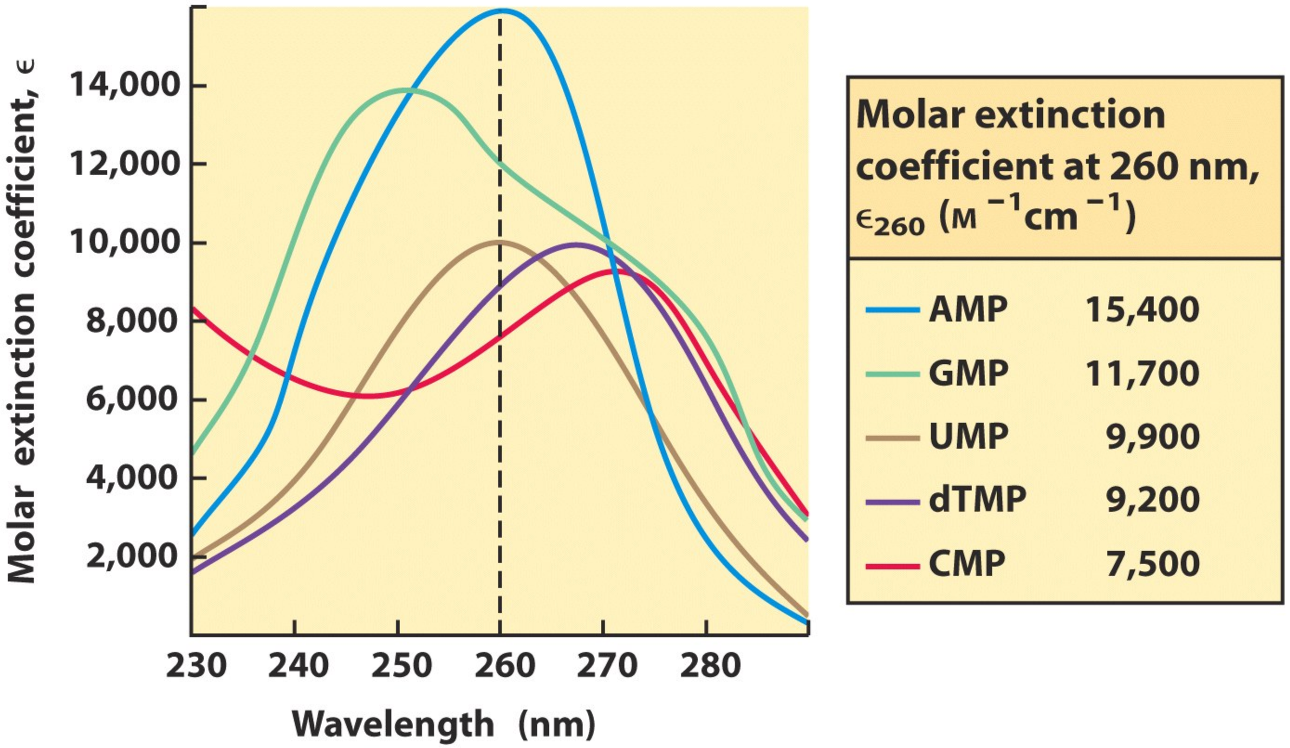
Uracil

pH = 7.0

Part I

The Properties of Nucleotide Bases

- **UV absorbance at 260nm**
- **$\varepsilon_{260} \approx 10{,}000$ (M^{-1} cm^{-1})**
- **The $A_{260} \approx 50$ µg /ml for DS DNA**
- **The $A_{260} \approx 40$ µg /ml for SS DNA or RNA**



Molar extinction coefficient at 260 nm, ϵ_{260} ($M^{-1}cm^{-1}$)	
AMP	15,400
GMP	11,700
UMP	9,900
dTMP	9,200
CMP	7,500

Part I. Some Basics

- What is a nucleotide?
- How are nucleic acids built from nucleotides?
- What is the difference between DNA and RNA?

Part II. Nucleic Acid Structure

Primary, secondary and tertiary

Part II

Important Clues to the Structure of DNA

- **DNA Stores Genetic Information**
- Avery-MacLeod-McCarty experiment（1935-1944）
- Hershey-Chase experiment (1952)

- **Chargaff's Rules**
- %A = %T and %G = %C

- **X-ray diffraction pattern of DNA**

Chargaff's Rules

- The base composition of DNA generally varies from one species to another
- DNAs from different tissues of the same species have the same composition
- DNA composition in a given species does not change with age or environment
- In all cellular DNAs, regardless of species, the number of A equals to that of T (A=T). Similarly, G=C. As a result, the sum of purine residues equals that of the pyrimidine residues, A+G=T+C.

Part II

X-Ray Diffraction Pattern of DNA

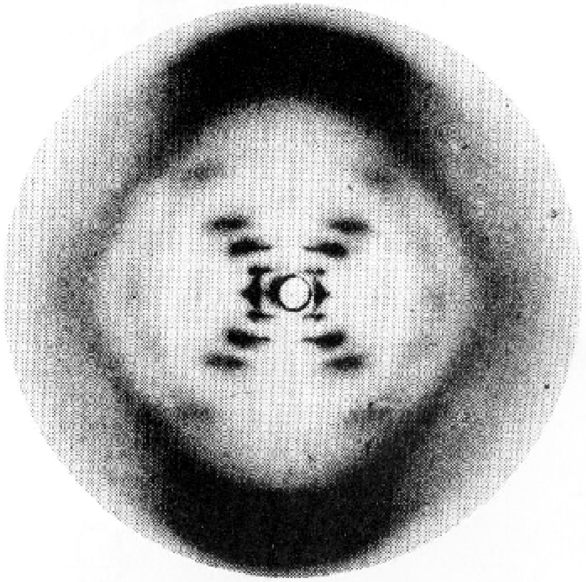


FIGURE **4.9**

Evidence for the structure of DNA. This photograph, taken by Rosalind Franklin, shows the x-ray diffraction pattern produced by wet DNA fibers. It played a key role in the elucidation of DNA structure. The cross pattern indicates a helical structure, and the strong spots at top and bottom correspond to a helical rise of 0.34 nm. The layer line spacing is one-tenth of the distance from the center to either of these spots, showing that there are 10 base pairs per repeat.

Reprinted by permission from R. E. Franklin and R. Gosling, *Nature* (1953) 171:740; © 1953 MacMillan Magazines, Ltd.

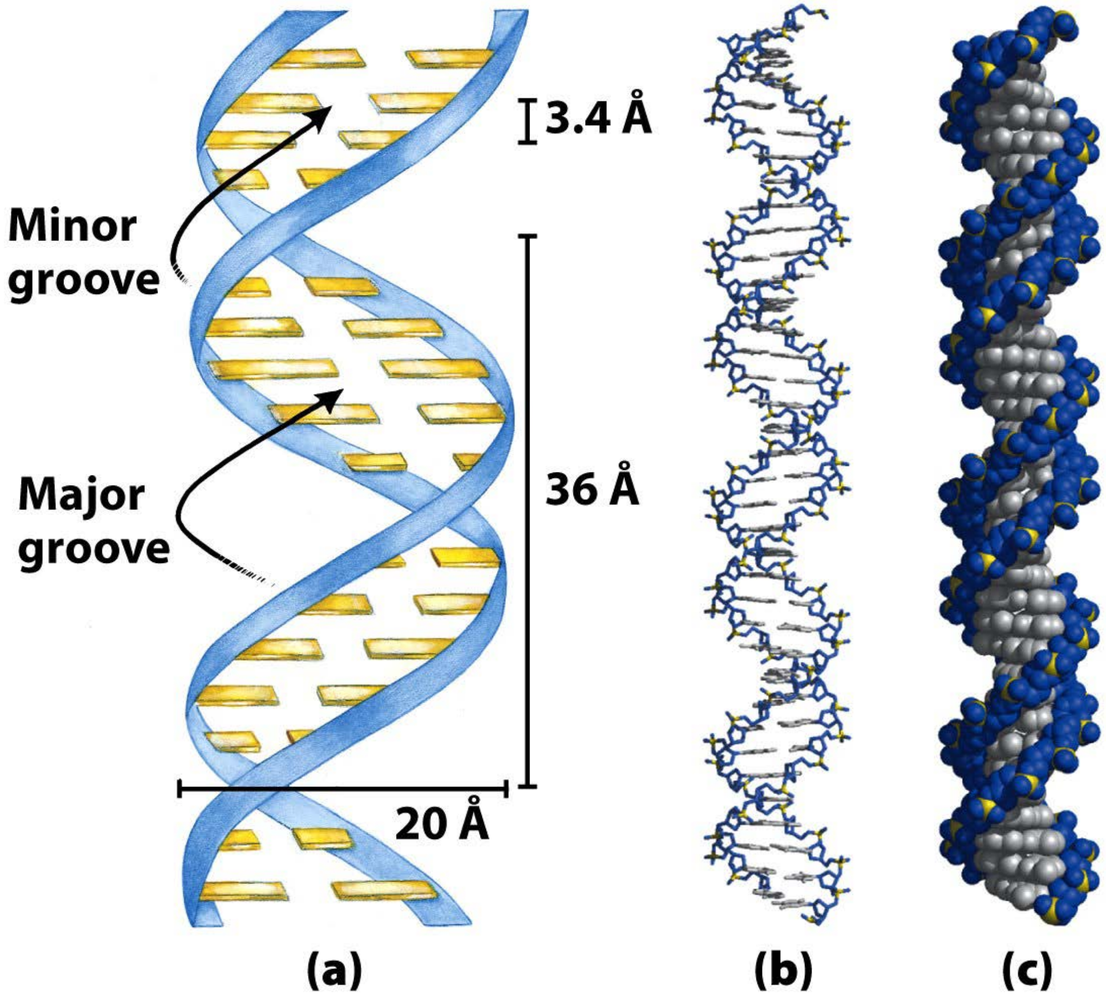
Rosalind Franklin, 1920–1958

Maurice Wilkins, 1916–2004

Helical with two periodicities along the long axis, a primary one of 3.4 Å and a secondary one of 34 Å.

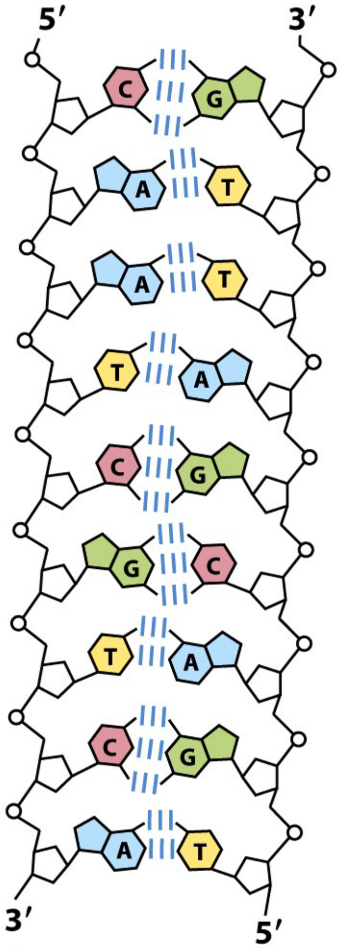
Part II

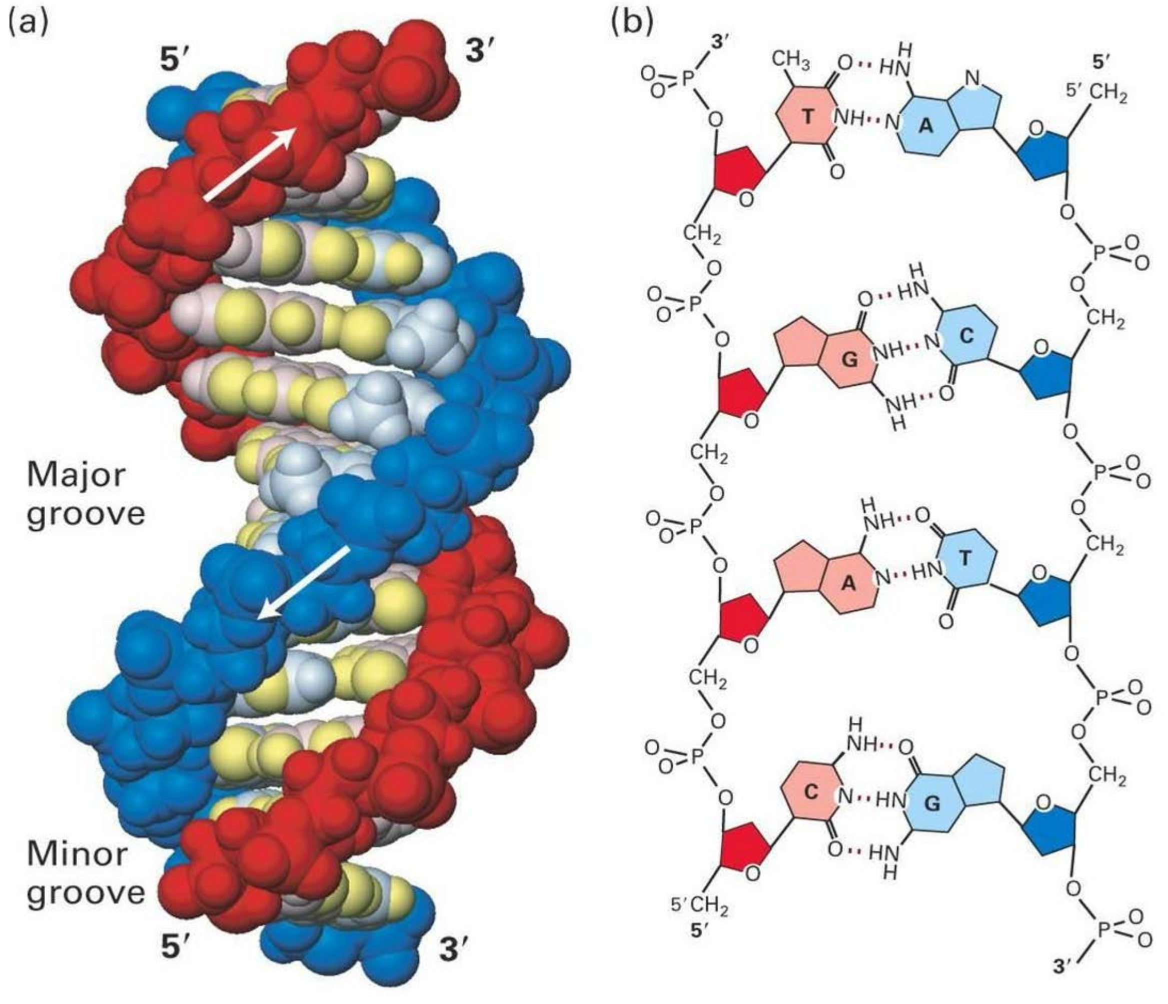
Watson-Crick Model for the Structure of DNA



Part II

Antiparallel and Complementary





Part II

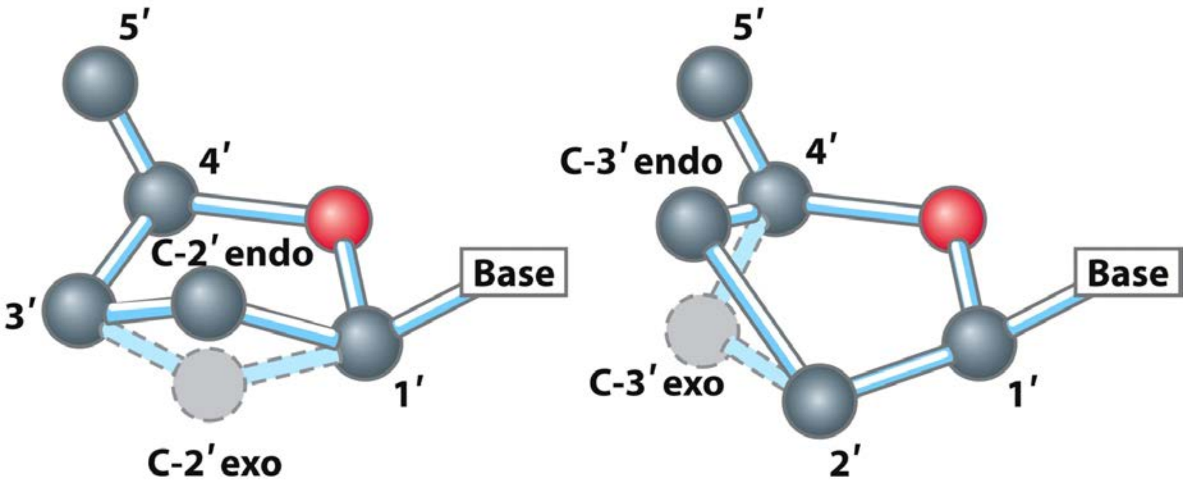
Features of the Watson-Crick Model

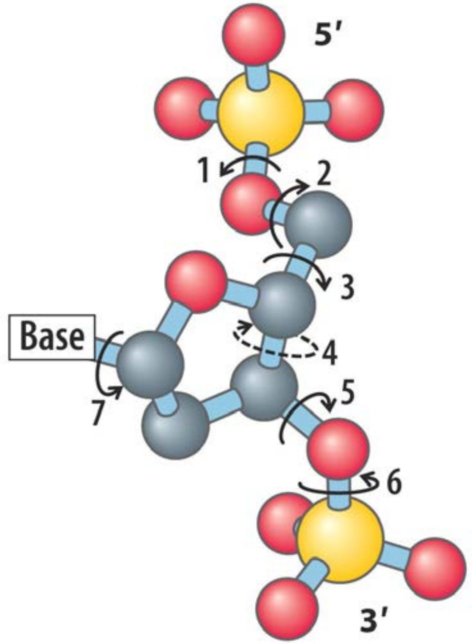
- 3.4 Å per base, 10.5 base pairs per turn, 20 Å in diameter
- Right-handed, with major and minor grooves
- Antiparallel and complementary
- Hydrogen bonding and base stacking
- Provides structure basis for DNA replication (will discuss later)

Part II

DNA Can Occur in Different Three-Dimentional Forms

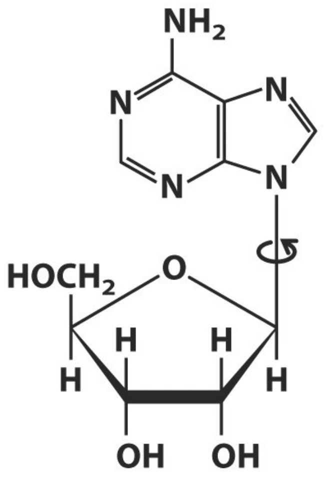
- **Different possible conformations of deoxyribose**



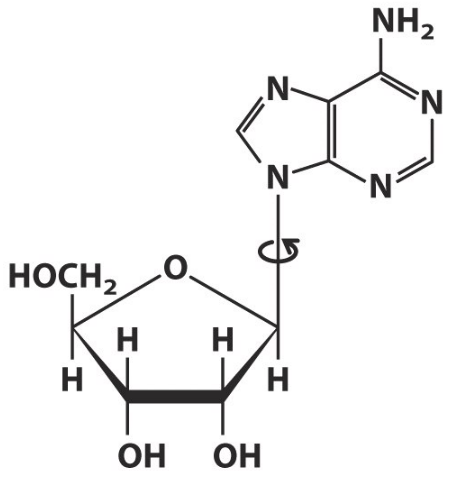


- **Phosphodeoxyribose backbone**

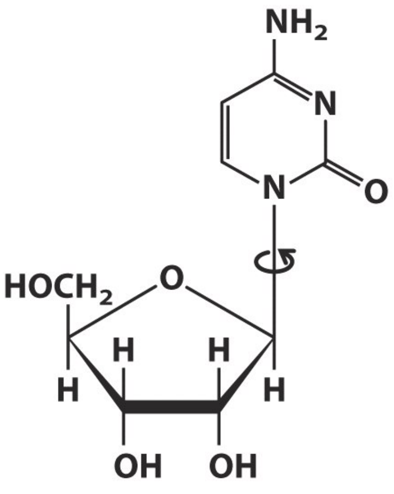
- **C-1’-N-Glycosyl bond**



syn-Adenosine



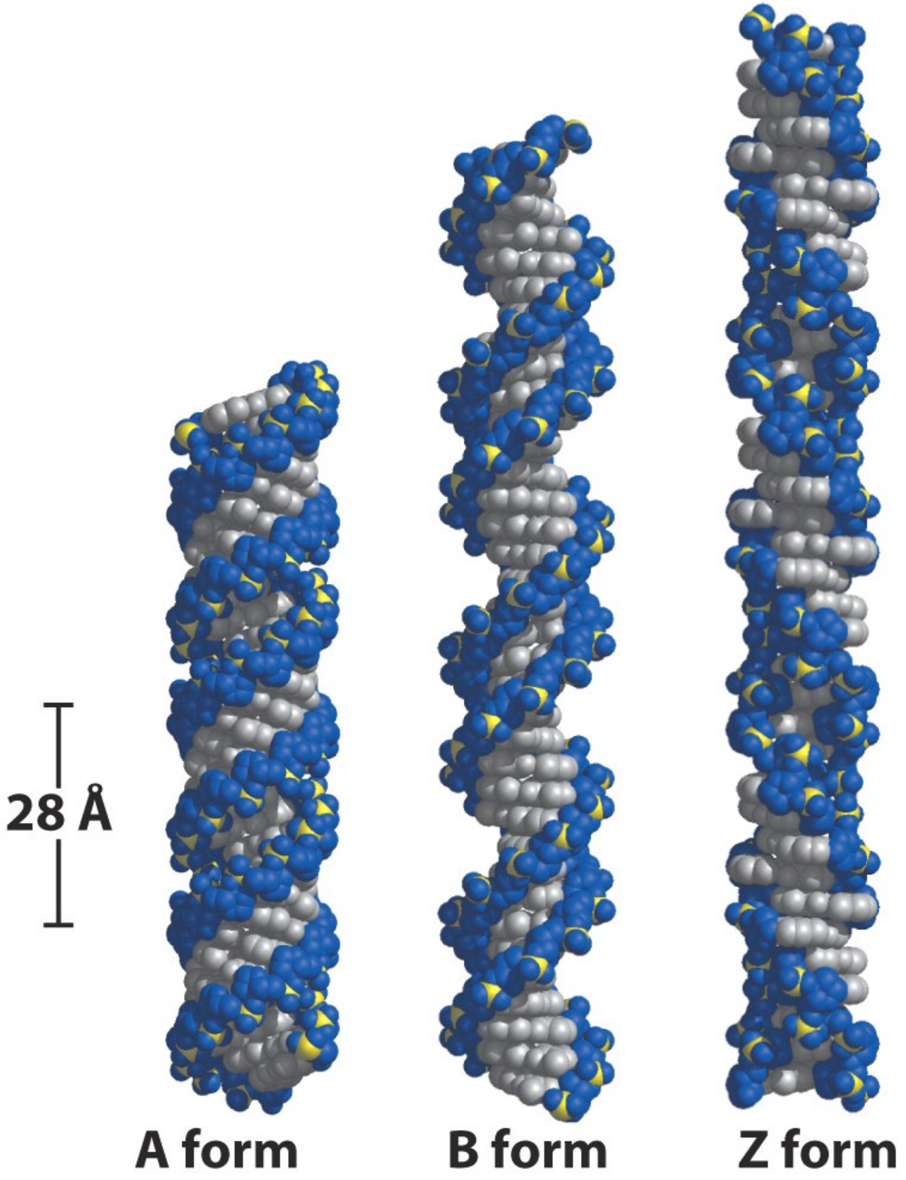
anti-Adenosine



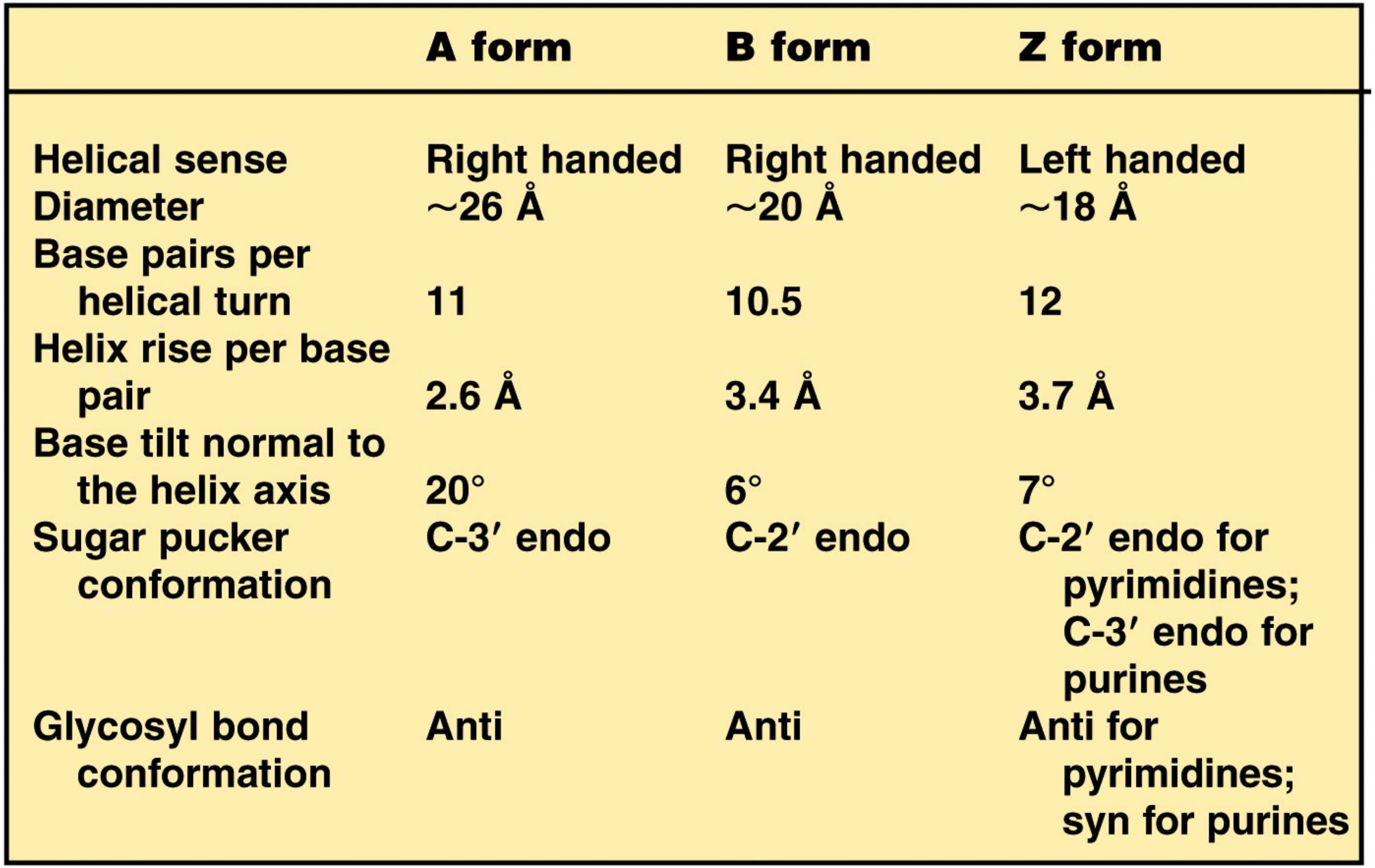
anti-Cytidine

Comparison of A, B, and Z forms of DNA

- A form – devoid of water, favored by RNA
- B form – Standard DNA double helix under physiological conditions
- Z form – laboratory anomaly,
  - Left Handed
  - Requires altered GC
  - High Salt/ Charge neutralization



Comparison of A, B, and Z 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

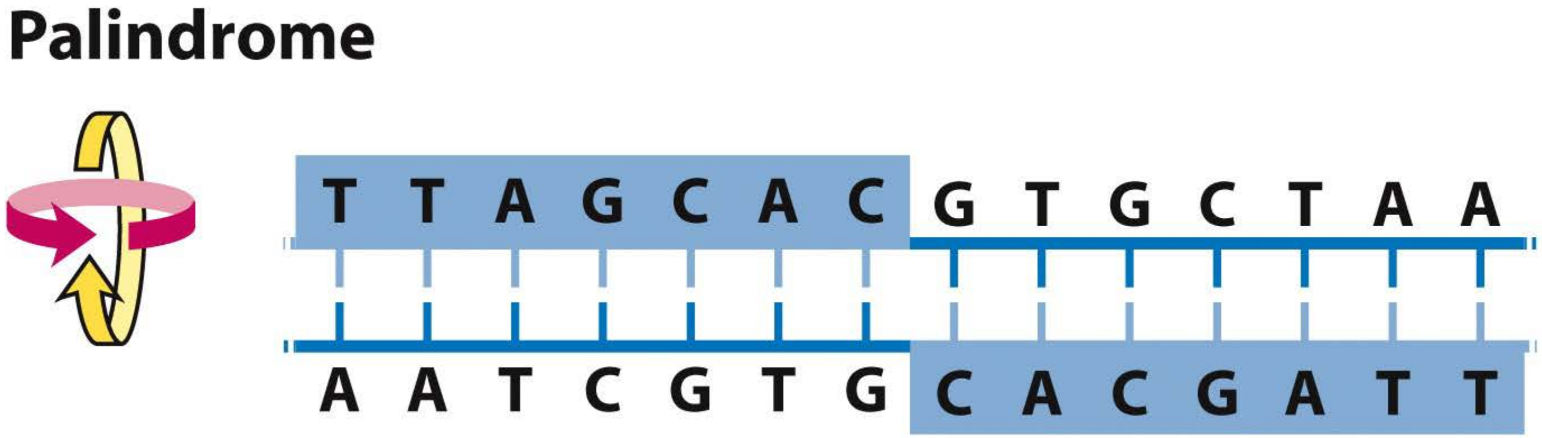
Part II

Unusual DNA Structures

Rotating Tower (Dubai)

Part II

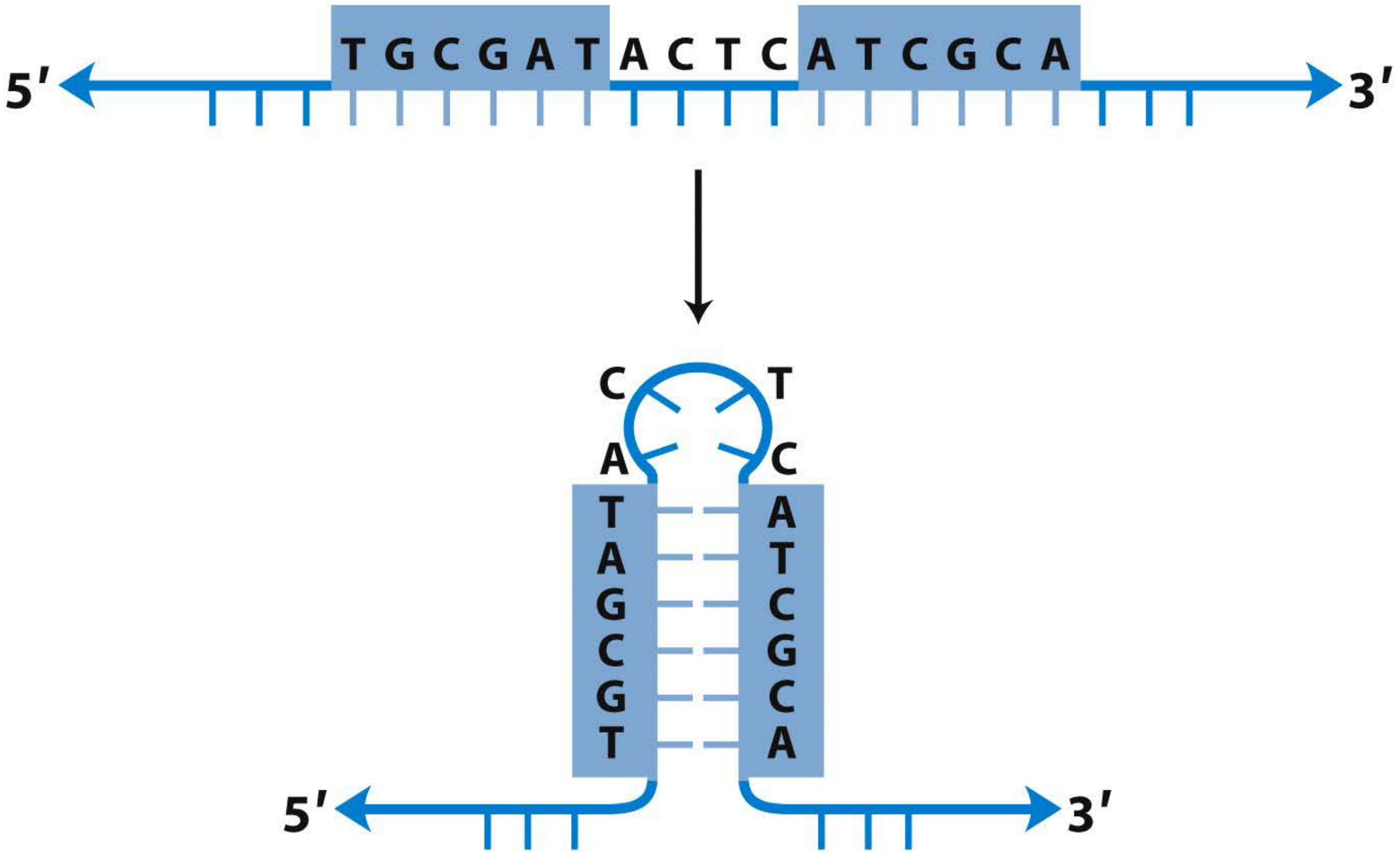
Palindromes



Regions of DNA with inverted repeats.
Self-complementary within each strand,
and able to form hairpin or cruciform.

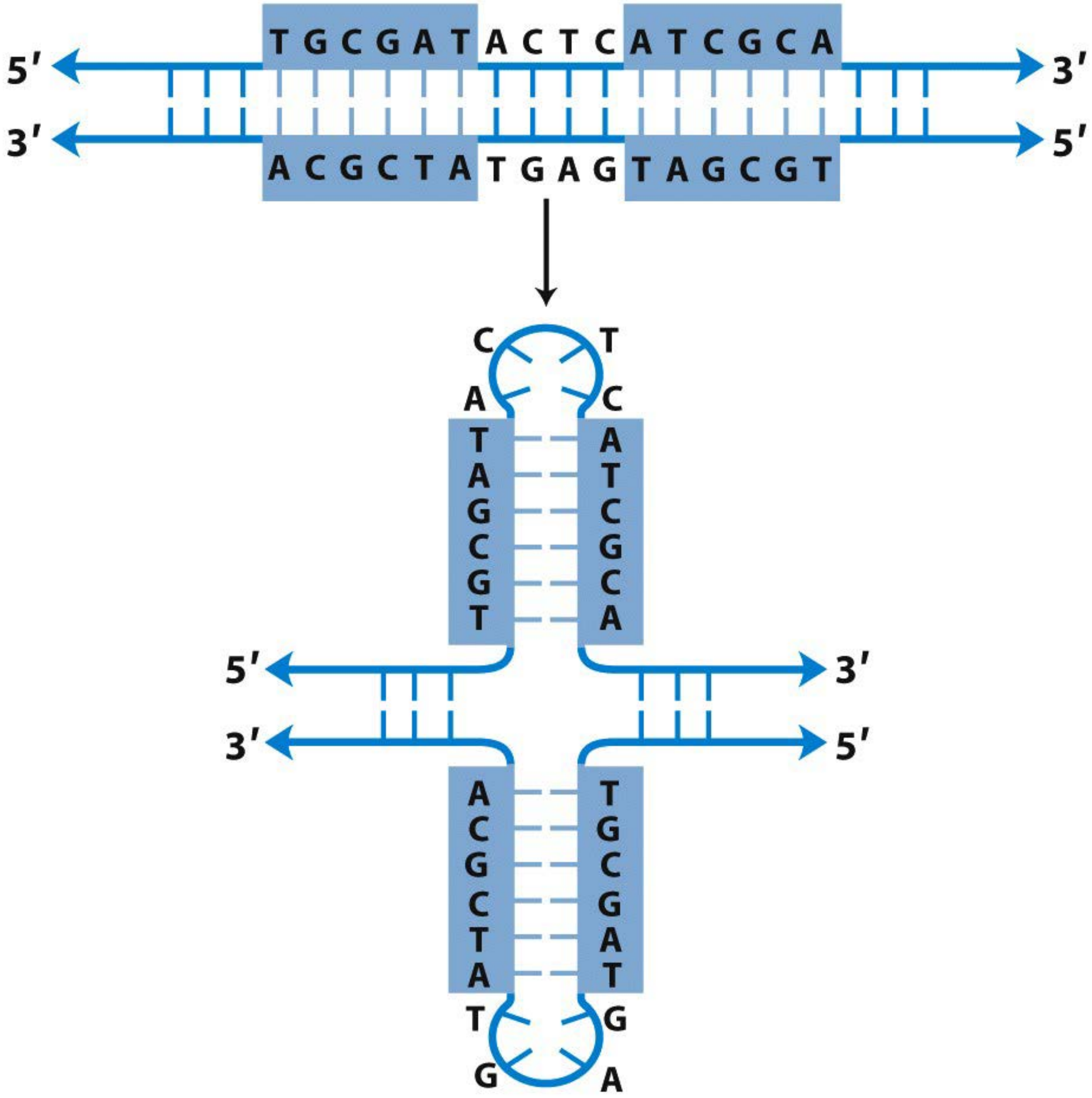
Part II

Hairpin Formation from a Single Strand

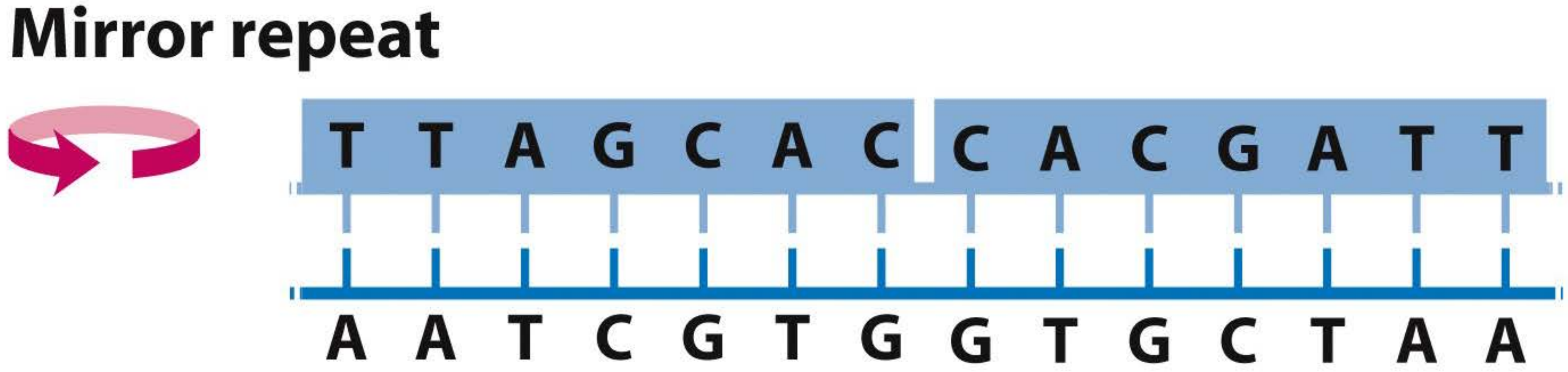


Part II

Cruciform Formation with Both Strands Involved



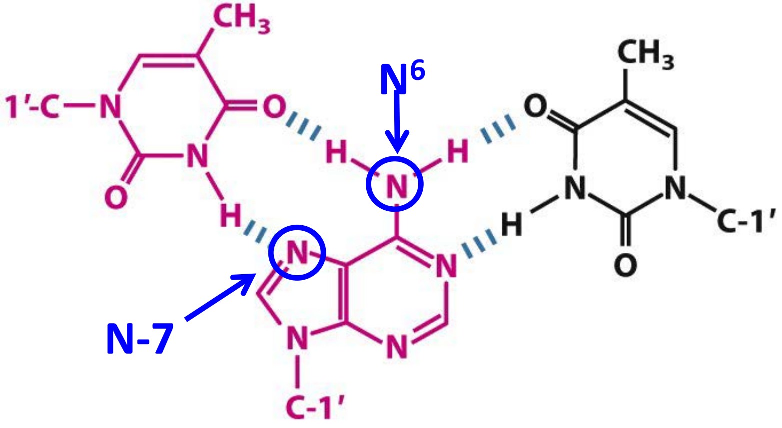
Mirror Repeat



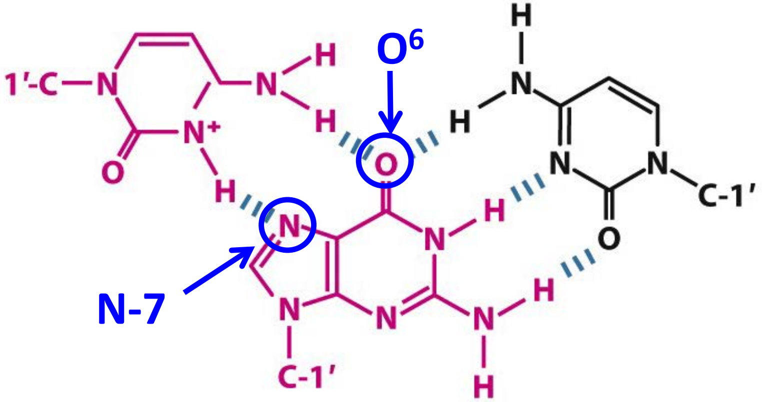
Mirror repeat cannot form hairpin or cruciform structures.

Part II

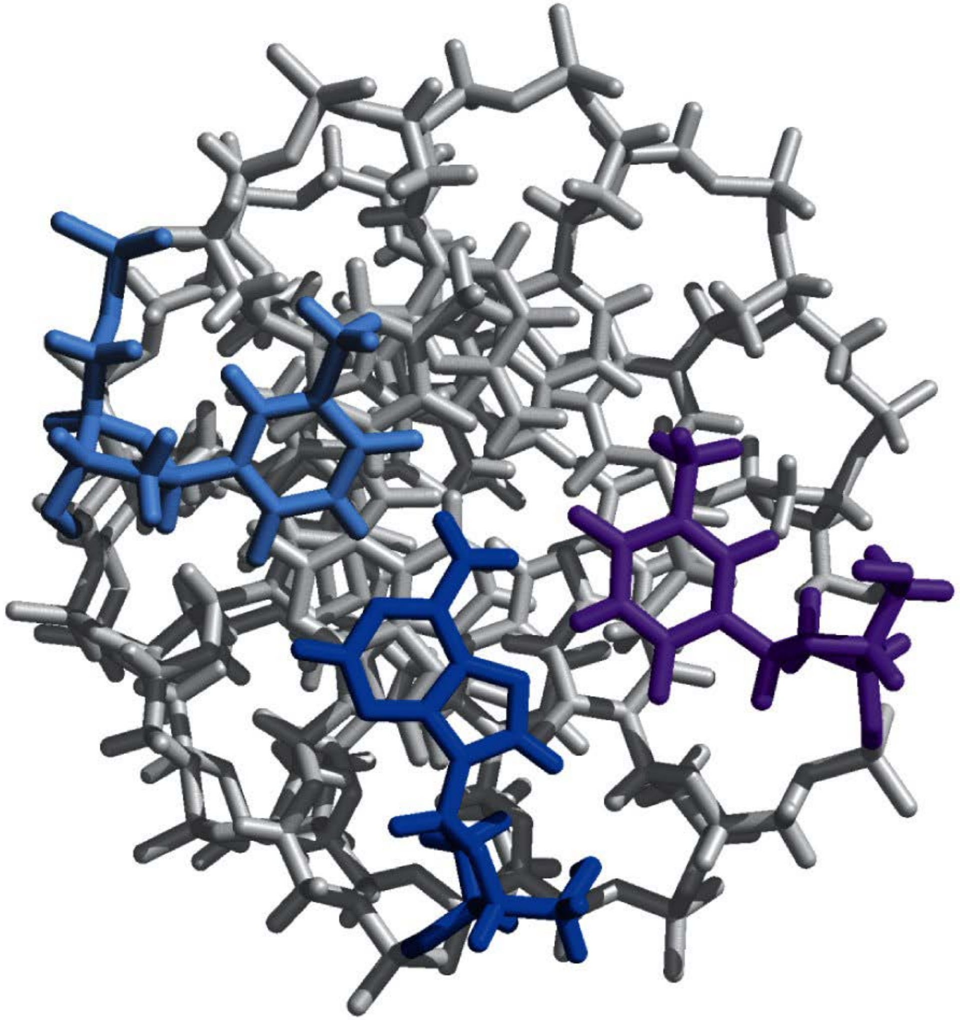
DNA Structures Containing Three Strands



T═A•T



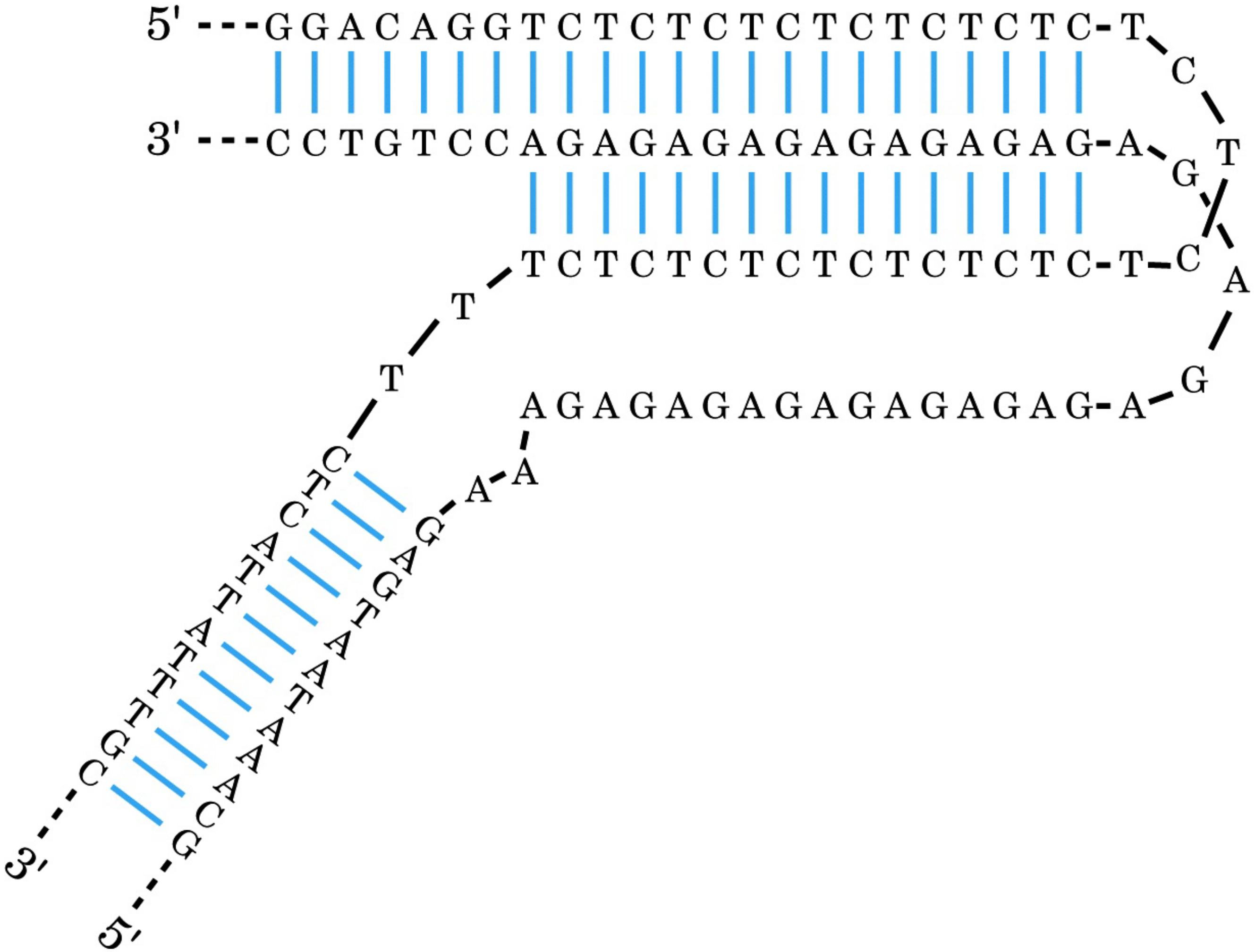
C≡G•C+



Hoogsteen positions
Hoogsteen pairing

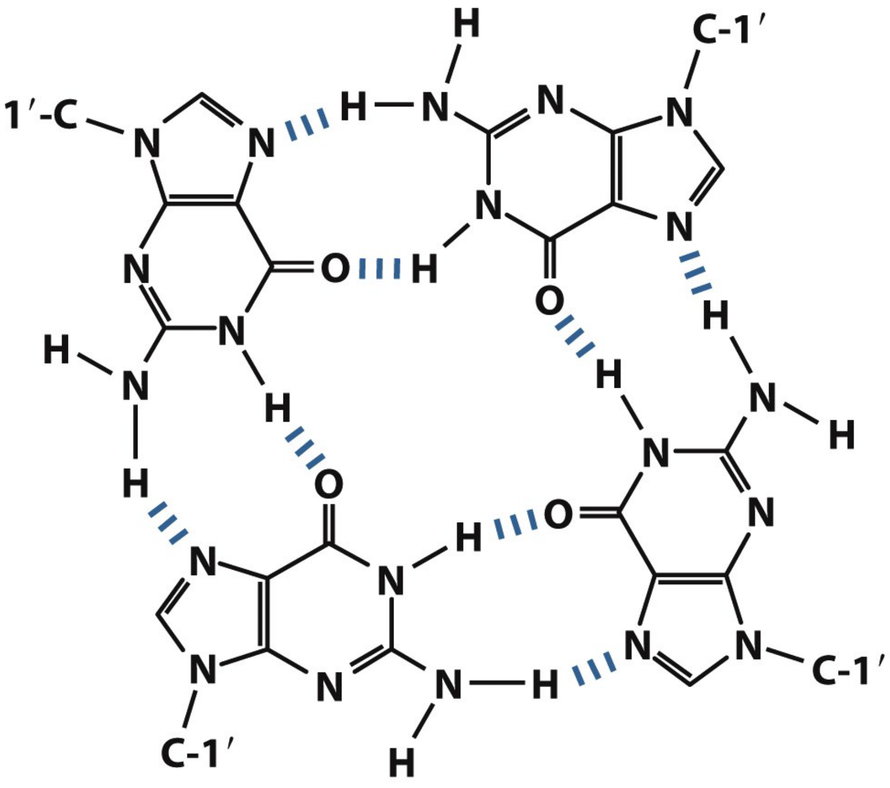
Part II

Triple Helix or H-DNA

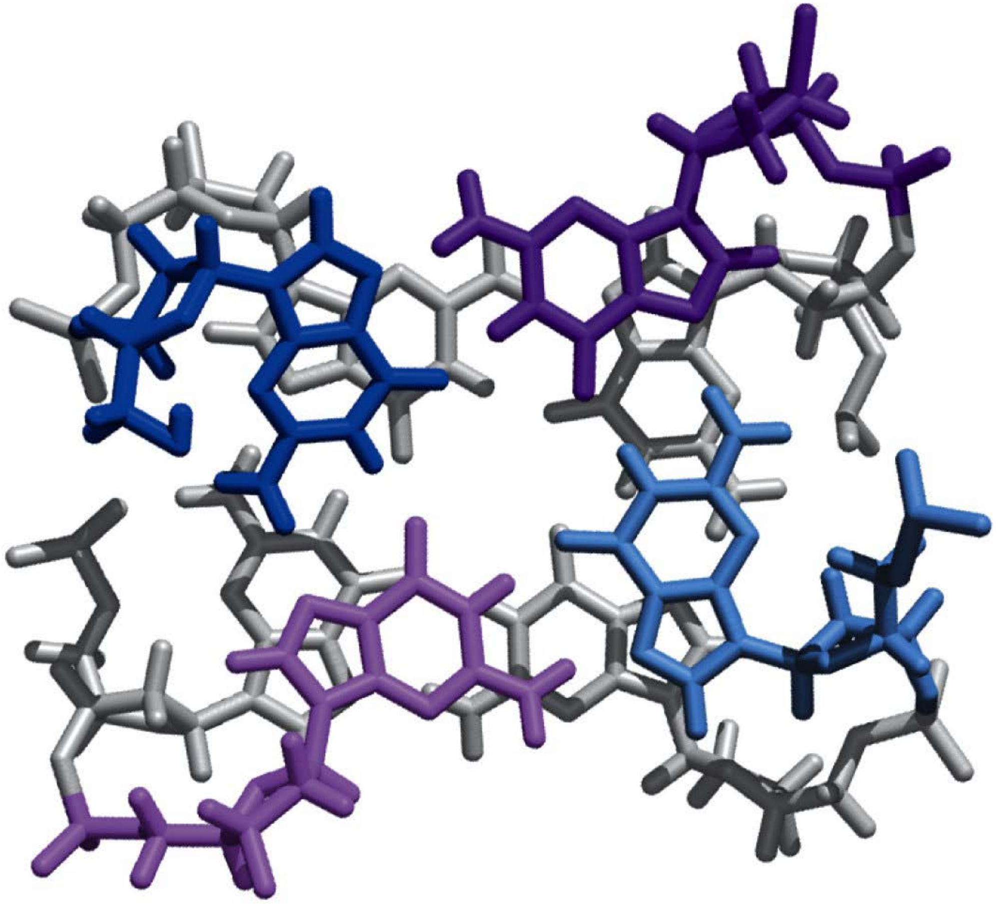


Part II

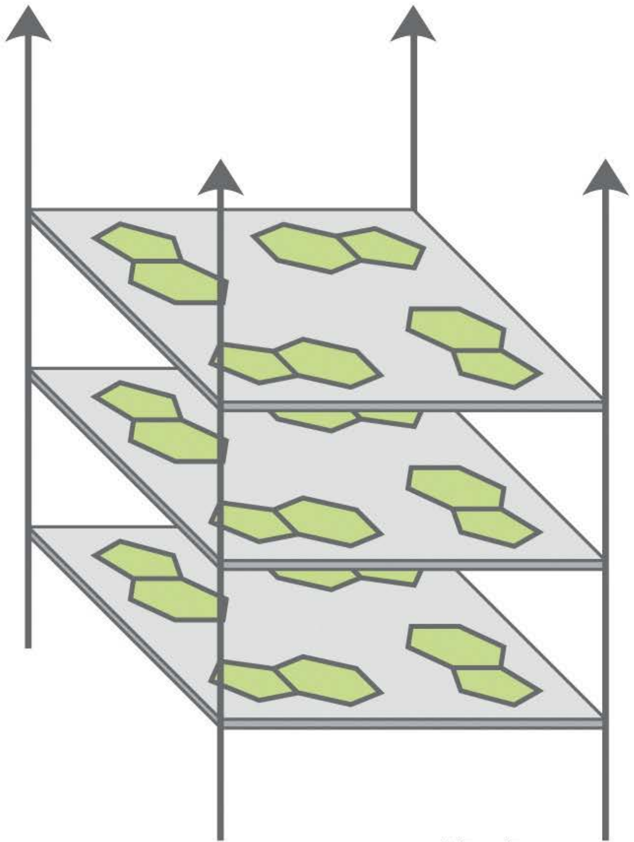
DNA Structures Containing Four Strands



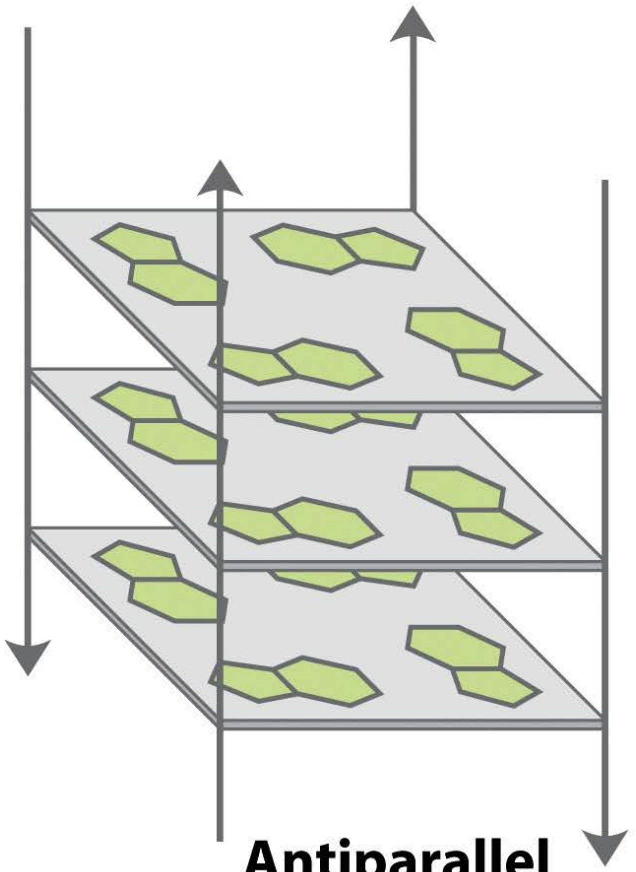
Guanosine tetraplex



Possible Orientations in a G-Tetraplex



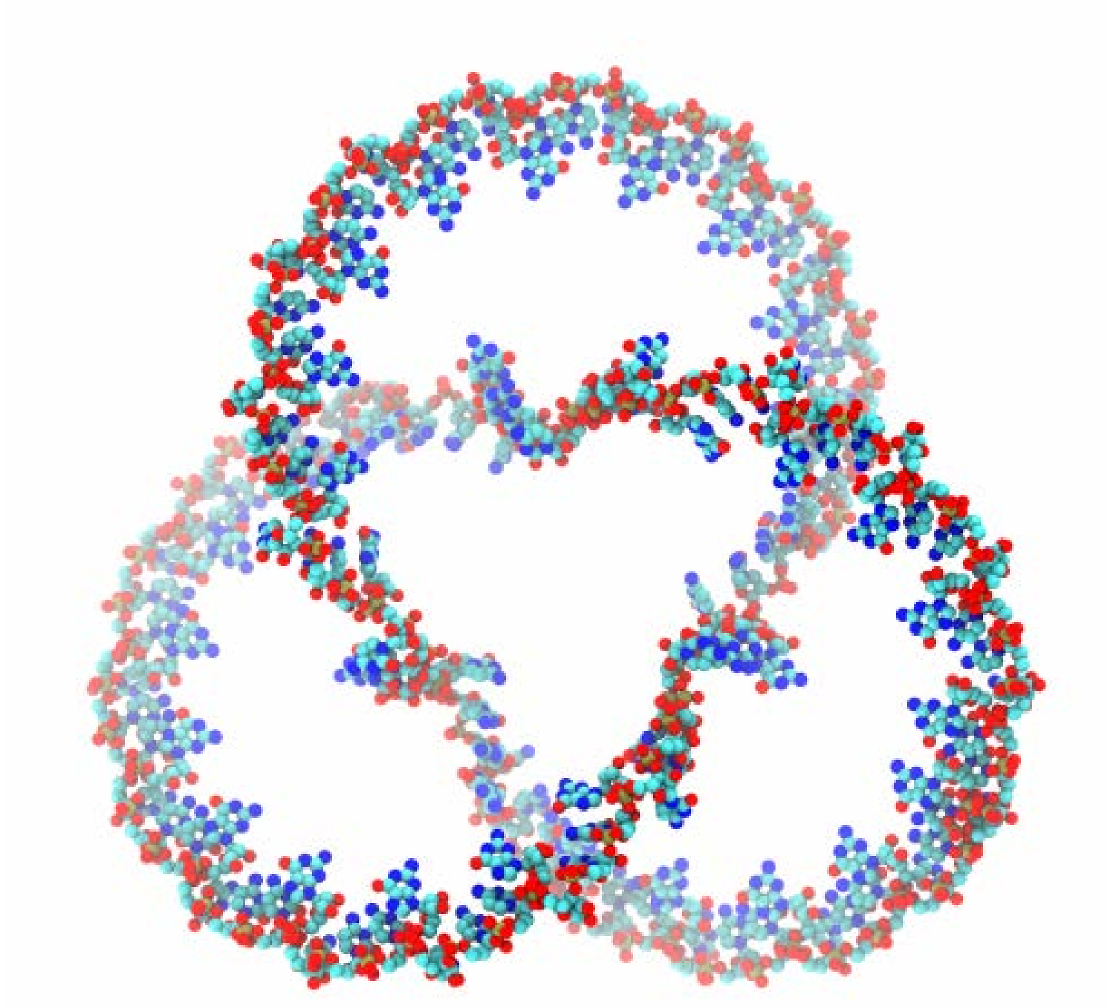
Parallel



Antiparallel

Part II

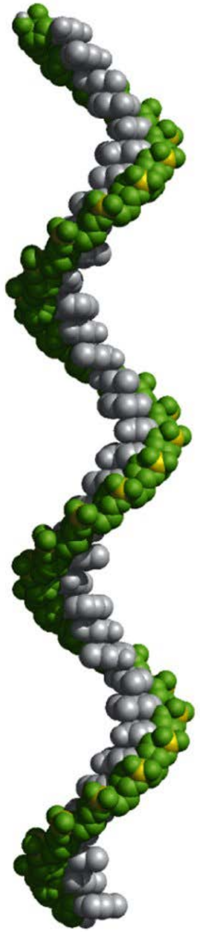
Many RNAs Have More Complex Three-Dimentional Structures

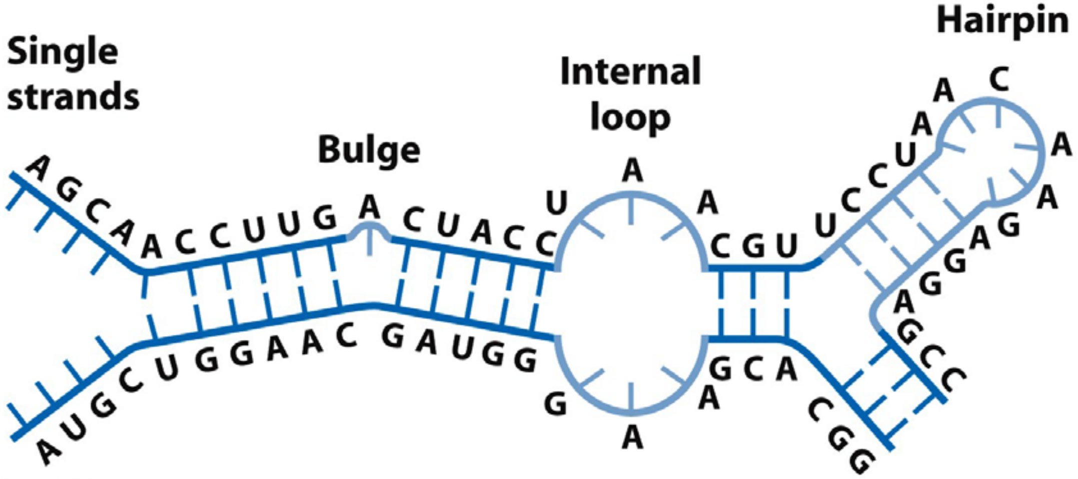


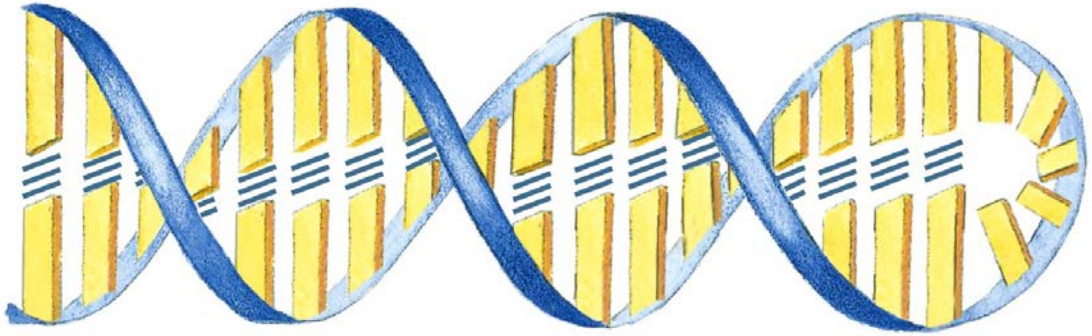
Computer generated RNA structure tied in a trefoil knot.

Part II

Secondary Structures in RNA





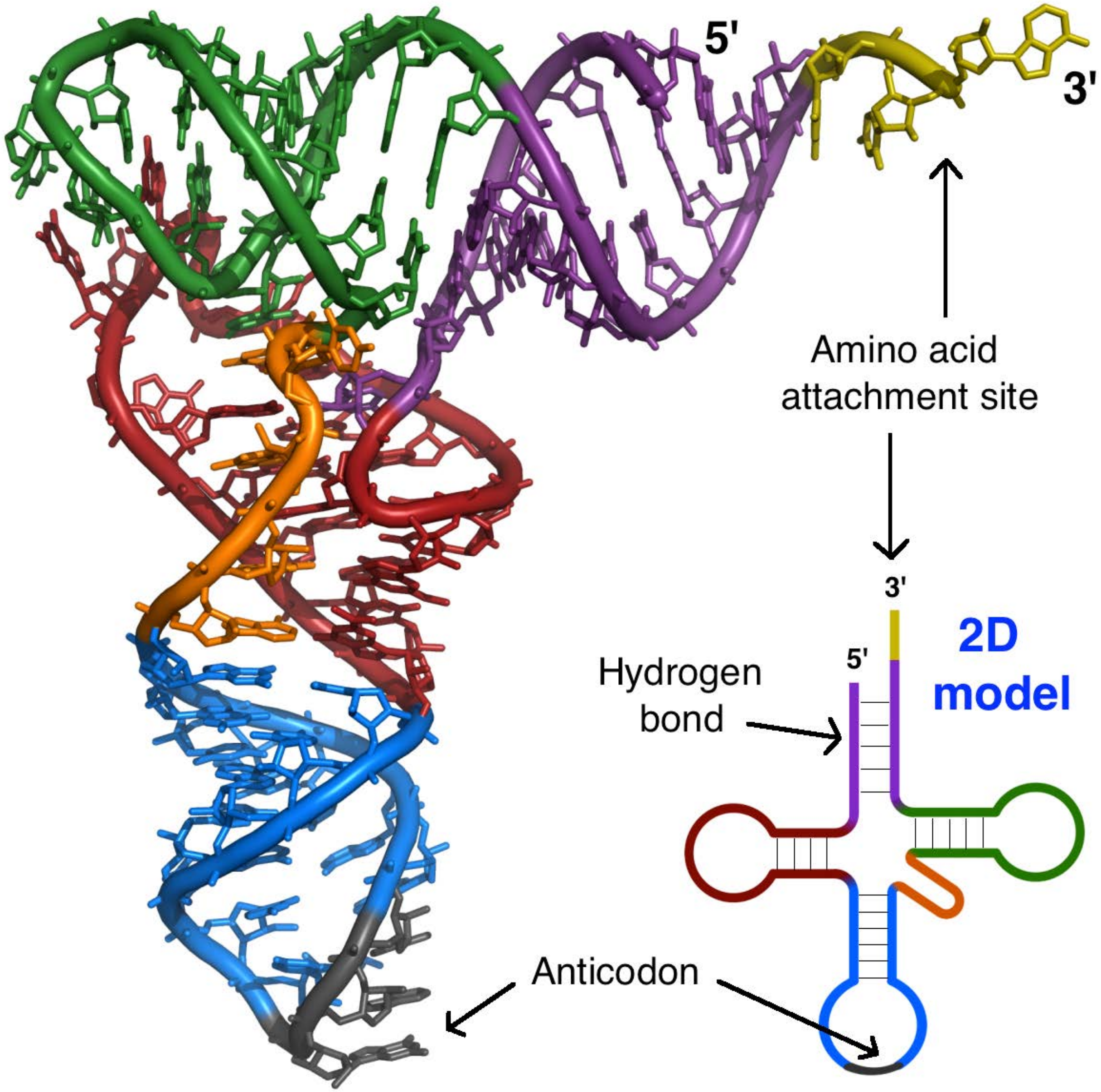


Hairpin double helix

A-form right-handed double helix

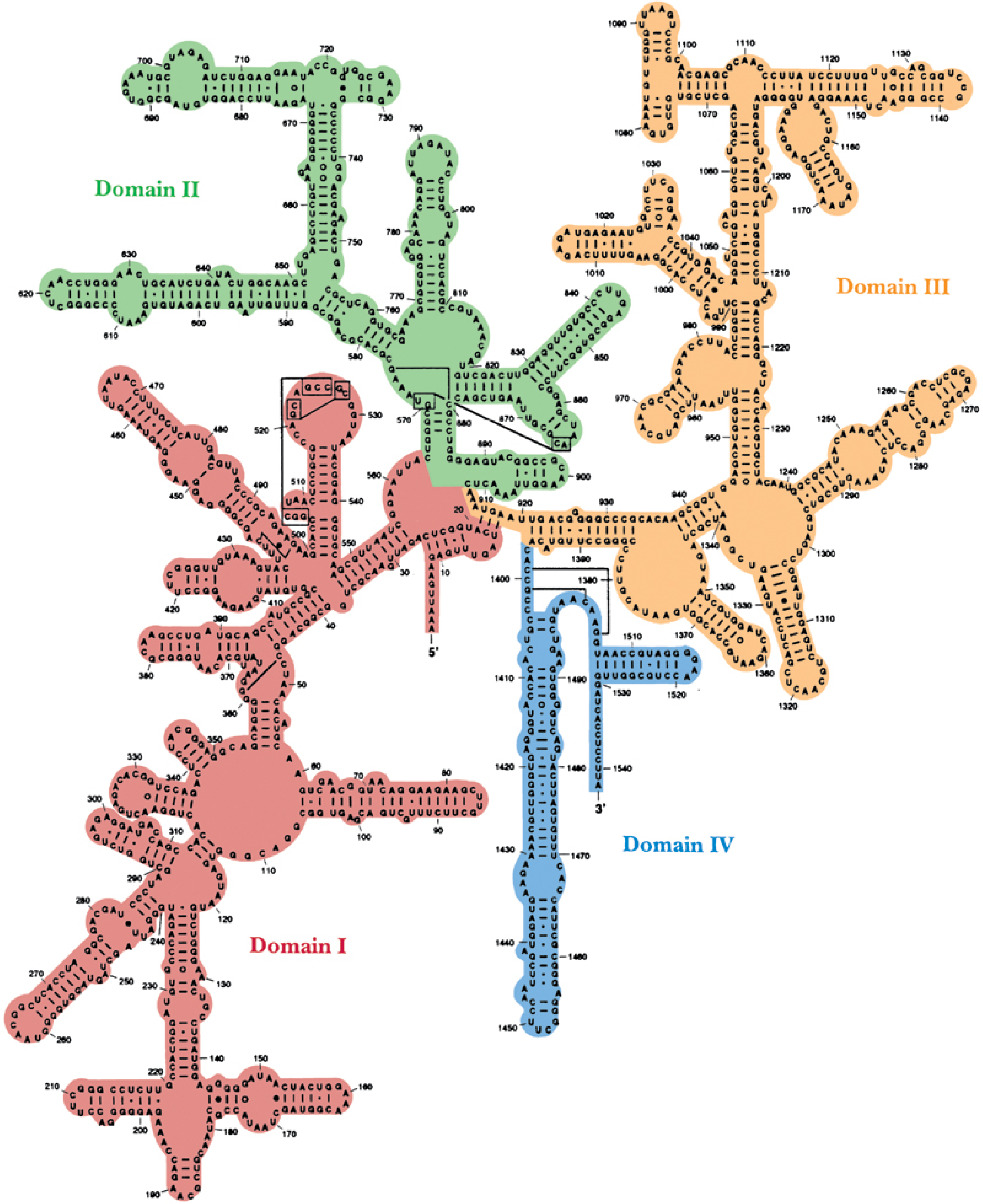
Part II

A Typical tRNA Structure



Part II

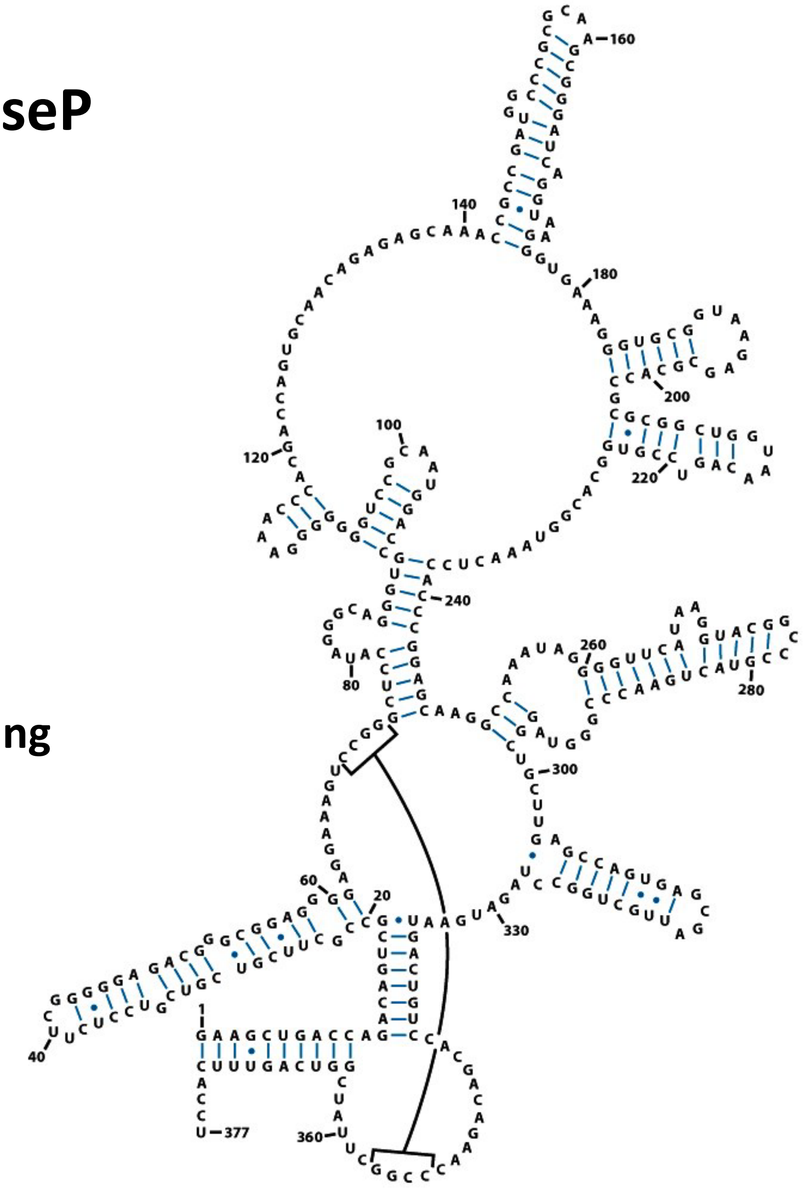
E coli 16S rRNA



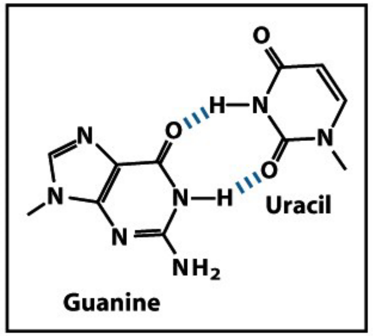
Part II

Base-Paired Helical Structures in an RNA

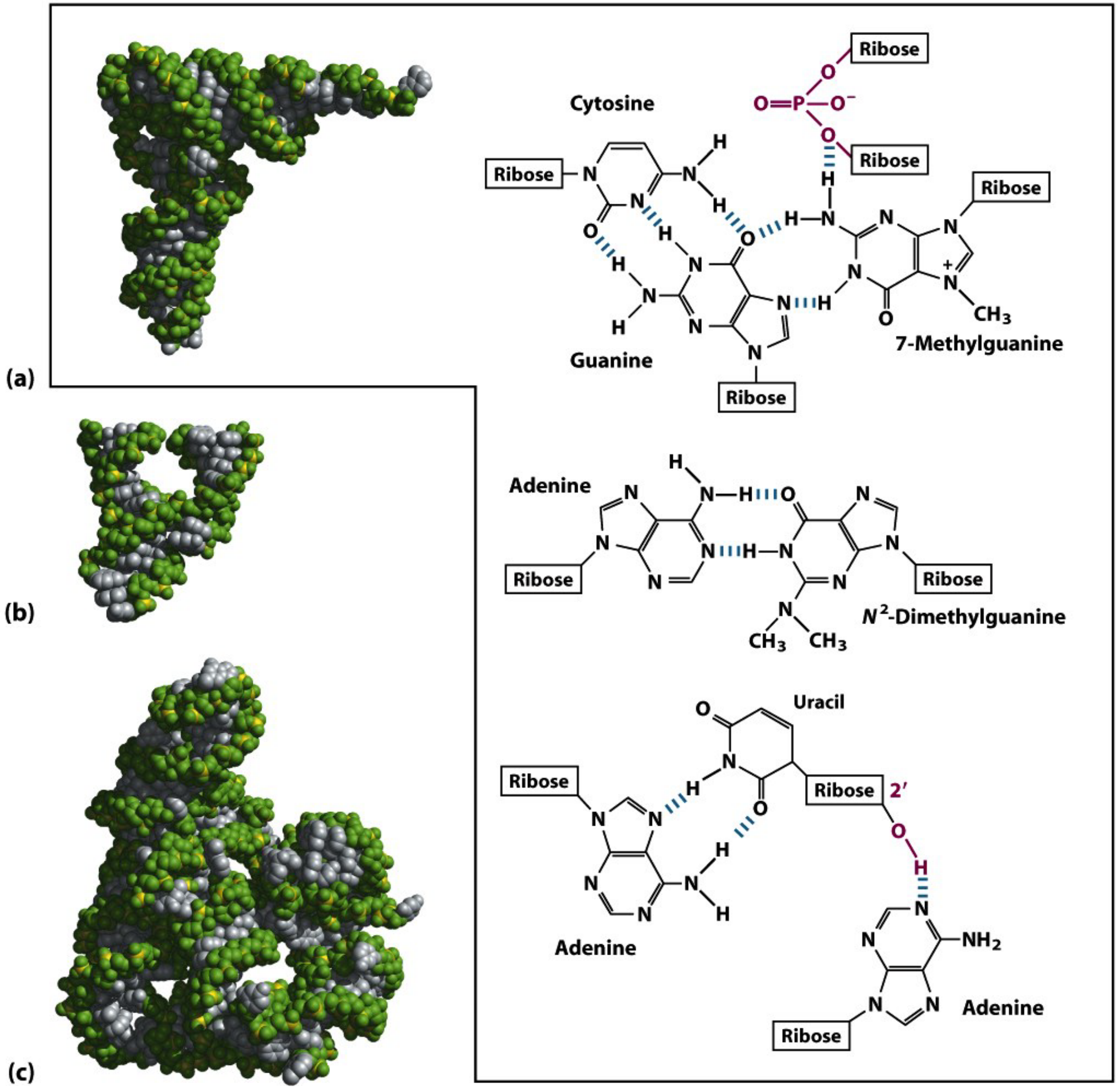
RNA Component of *E. Coli* RNaseP



Non-Waston-Crick Pairing



Three-Dimensional Structure in RNA

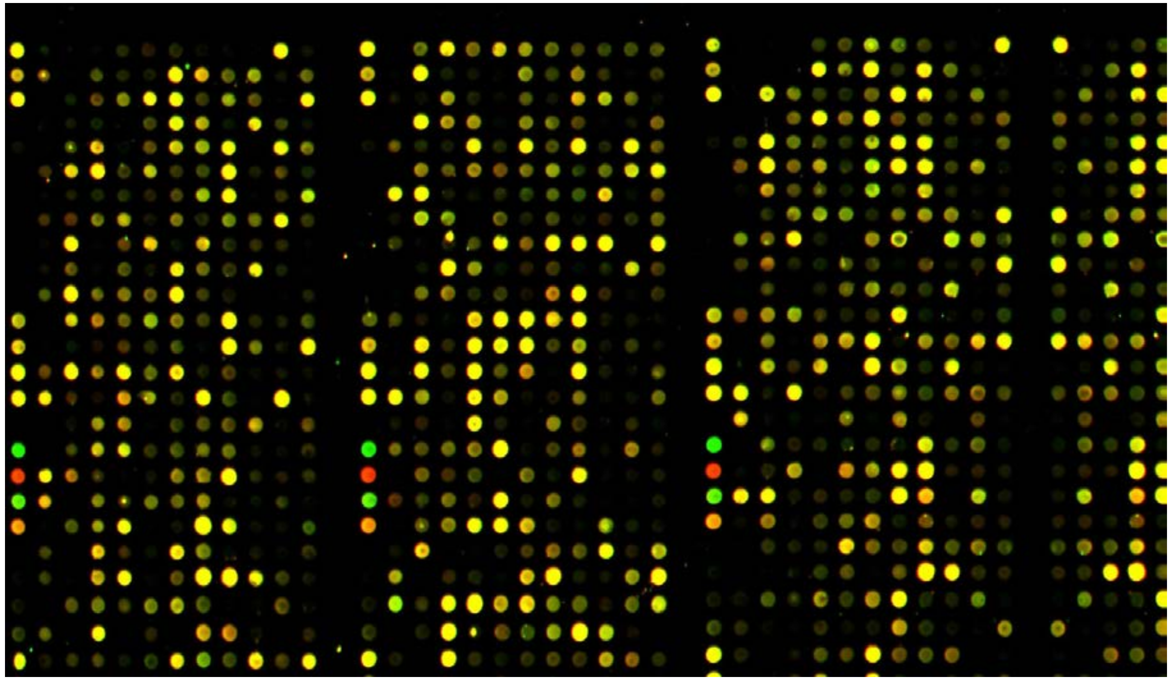


Summary

Part II. Nucleic Acid Structure

- What is the Waston-Crick model?
- What is the difference between B-DNA and A-, or Z-DNA?
- How many unusual DNA structures do you know?
- RNAs can adopt more complex structures.

Part III. Nucleic Acid Chemistry



Microarray

NYC

Part III

Double-Helical DNA and RNA Can Be Denatured

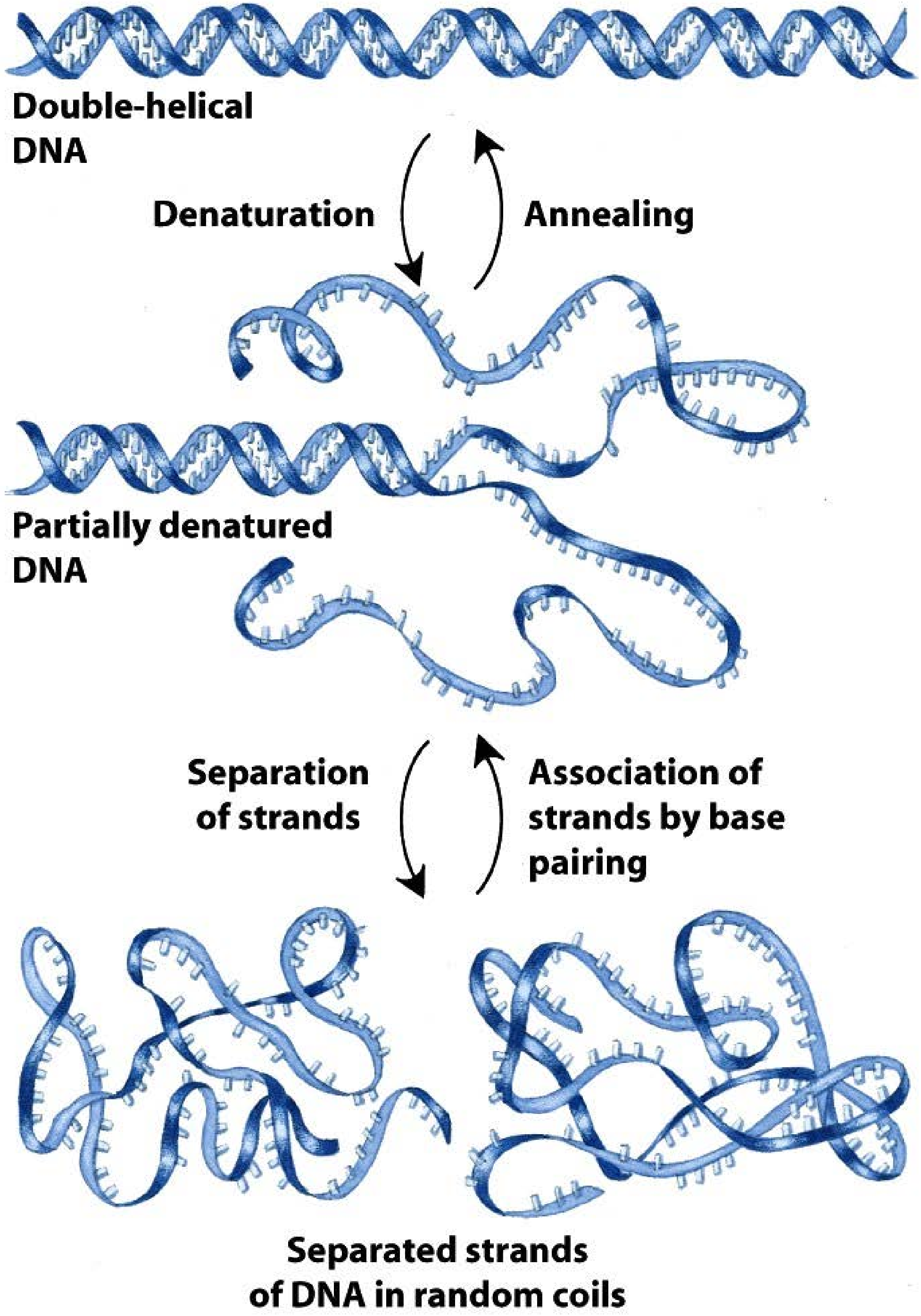
Denaturation

Disruption of the hydrogen bonds and base stacking
Decreased viscosity
Hyperchromic effect

Annealing or Renaturation

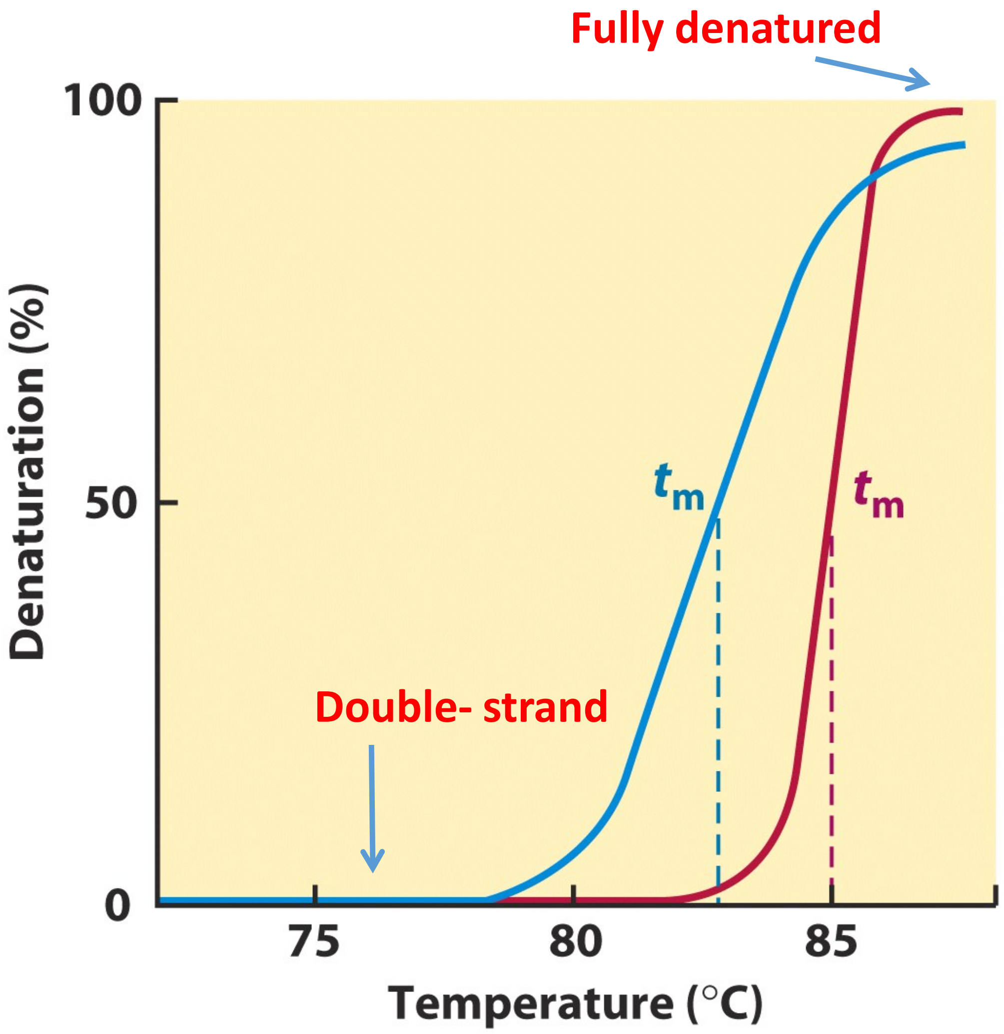
Partially denatured

Fully denatured
Hypochromic effect



Part III

Heat Denaturation of DNA



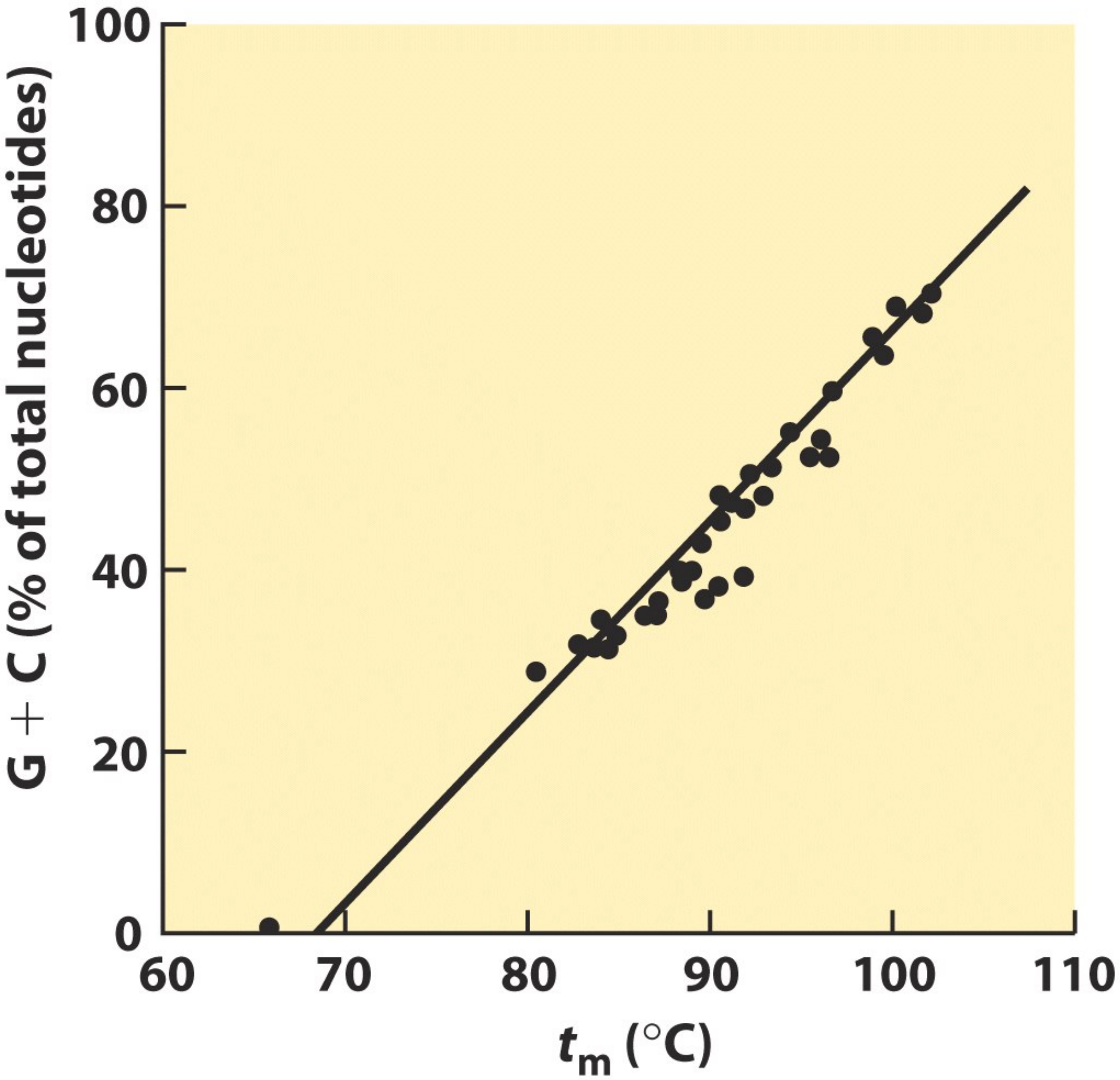
Melting point (t_m)

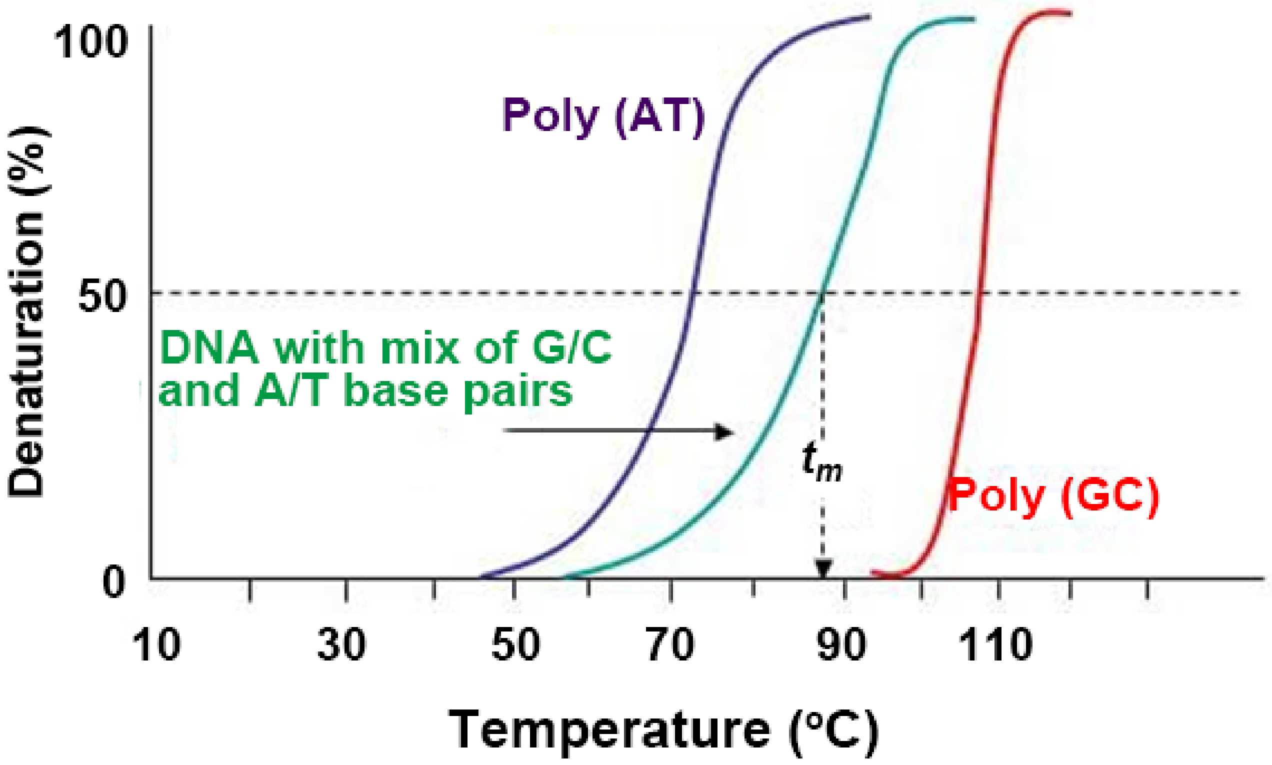
The temperature at the mid-point of the transition

Depends on pH, ionic strength, and size and base composition of the DNA

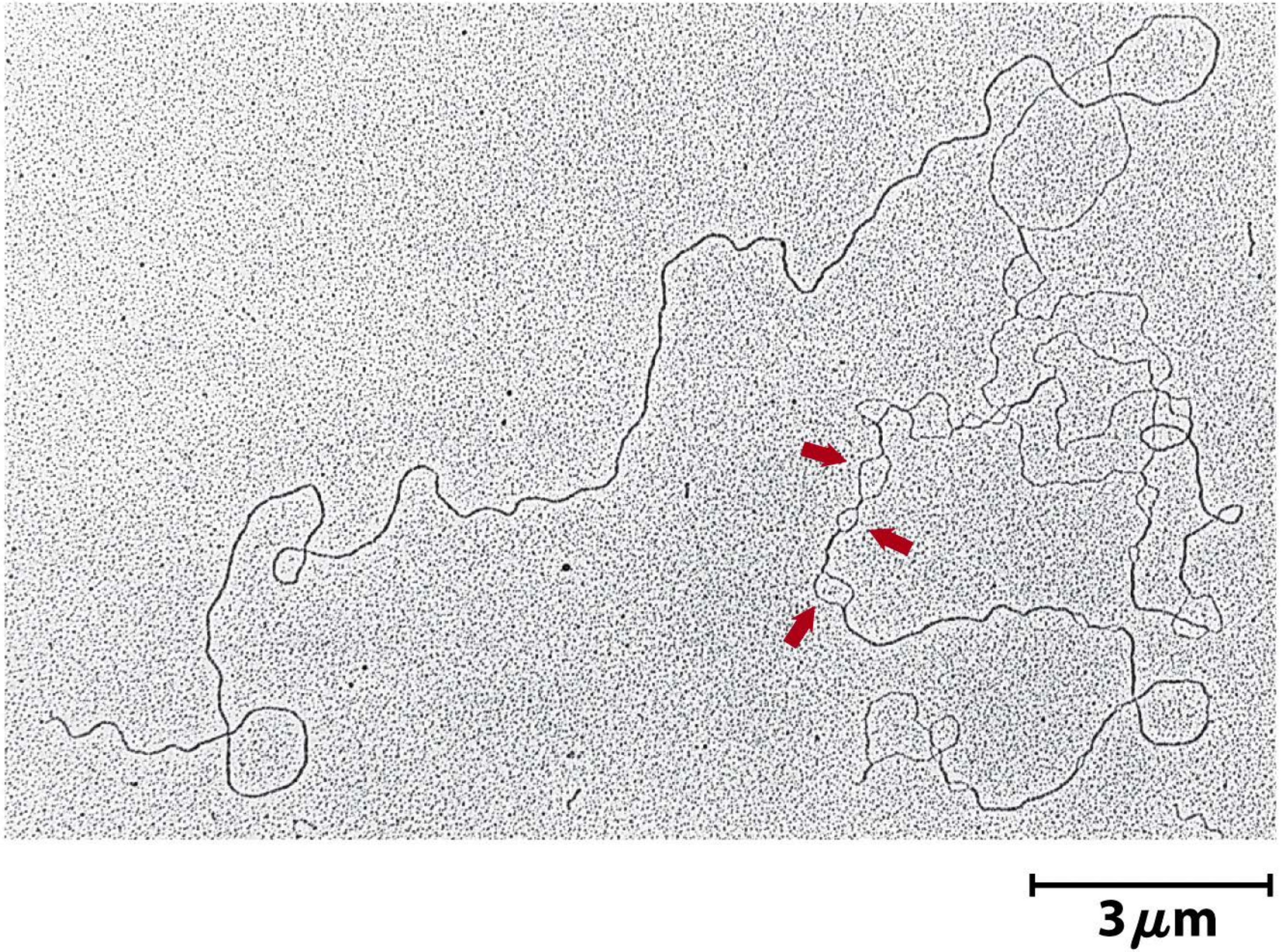
Part III

Relationship between t_m and GC Content of a DNA





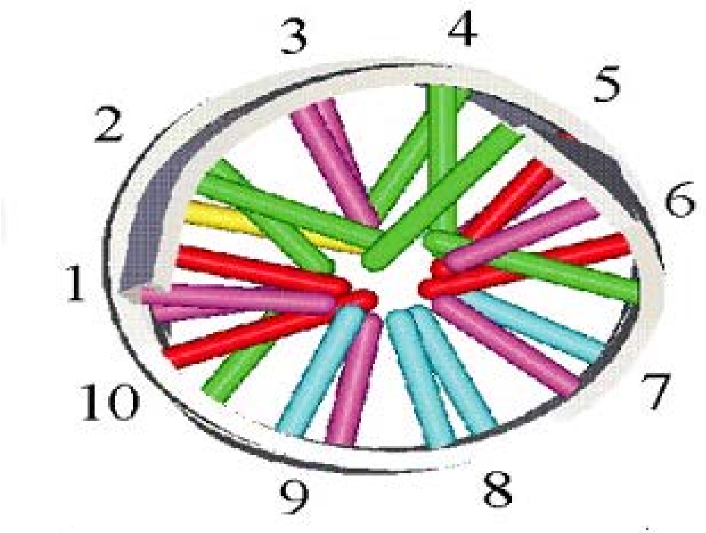
Partially Denatured DNA



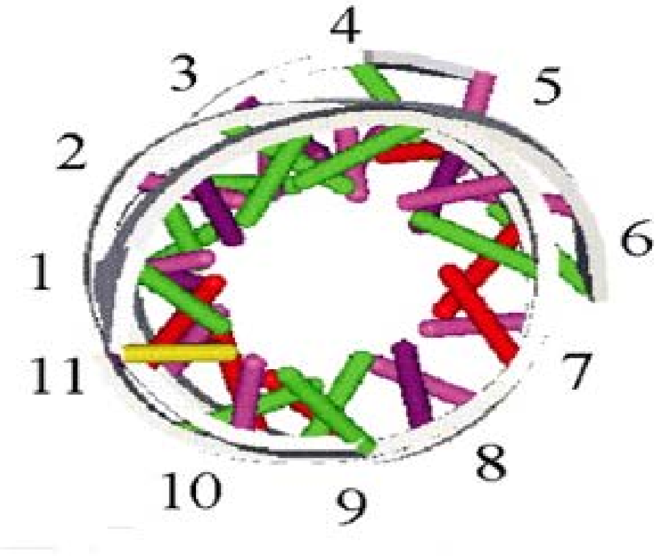
Regions rich in AT denature easily.

Part III

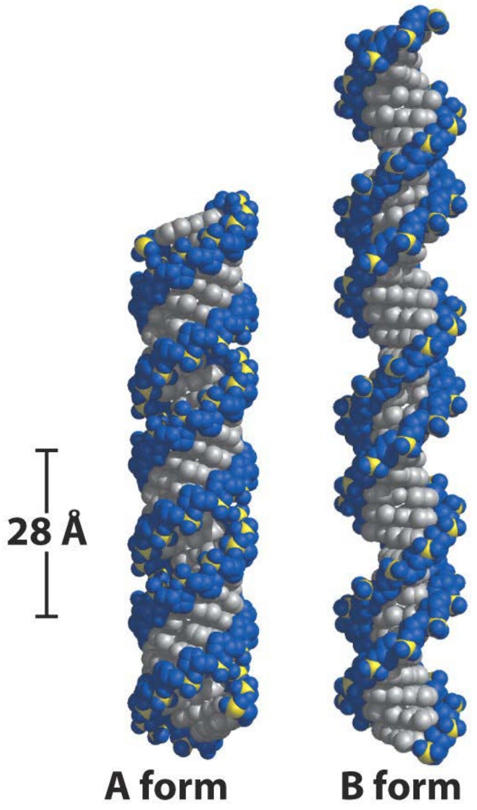
RNA Duplexes Are More Stable than DNA Duplexes



DNA
PDB(1DUF)



RNA
PDB(1QC0)



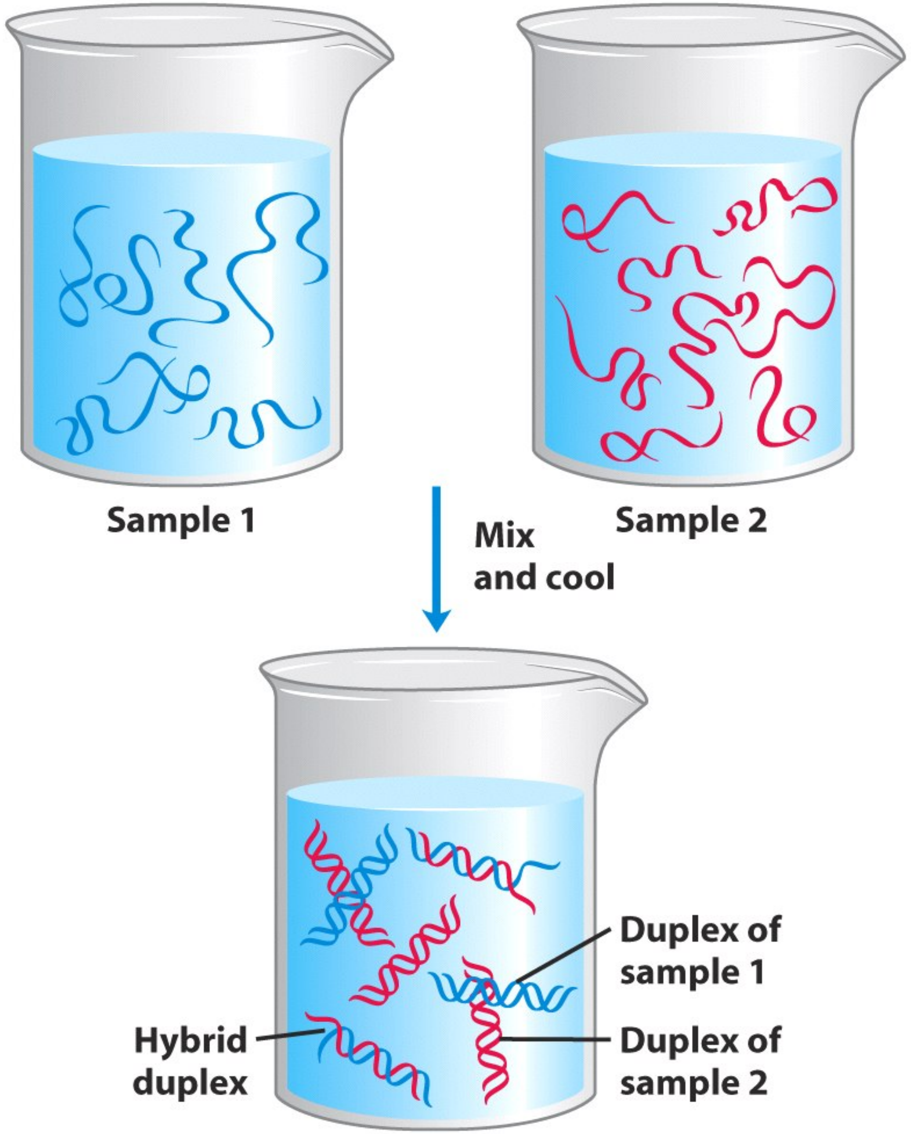
At neutral pH, denaturation of a double-helical RNA requires temperatures 20 °C or more higher than those required for denaturation of a DNA with comparable sequence.

Part III

DNA Hybridization

- **Nucleic Acids from Different Species Can Form Hybrids**
- The closer the evolutionary relationship between two species, the more extensively their DNAs will hybridize.

- **Basis for many of molecular biology techniques**
- Southern blot
- Northern blot
- Fluorescent In Situ Hybridization (FISH)
- Colony hybridization
- Microarray

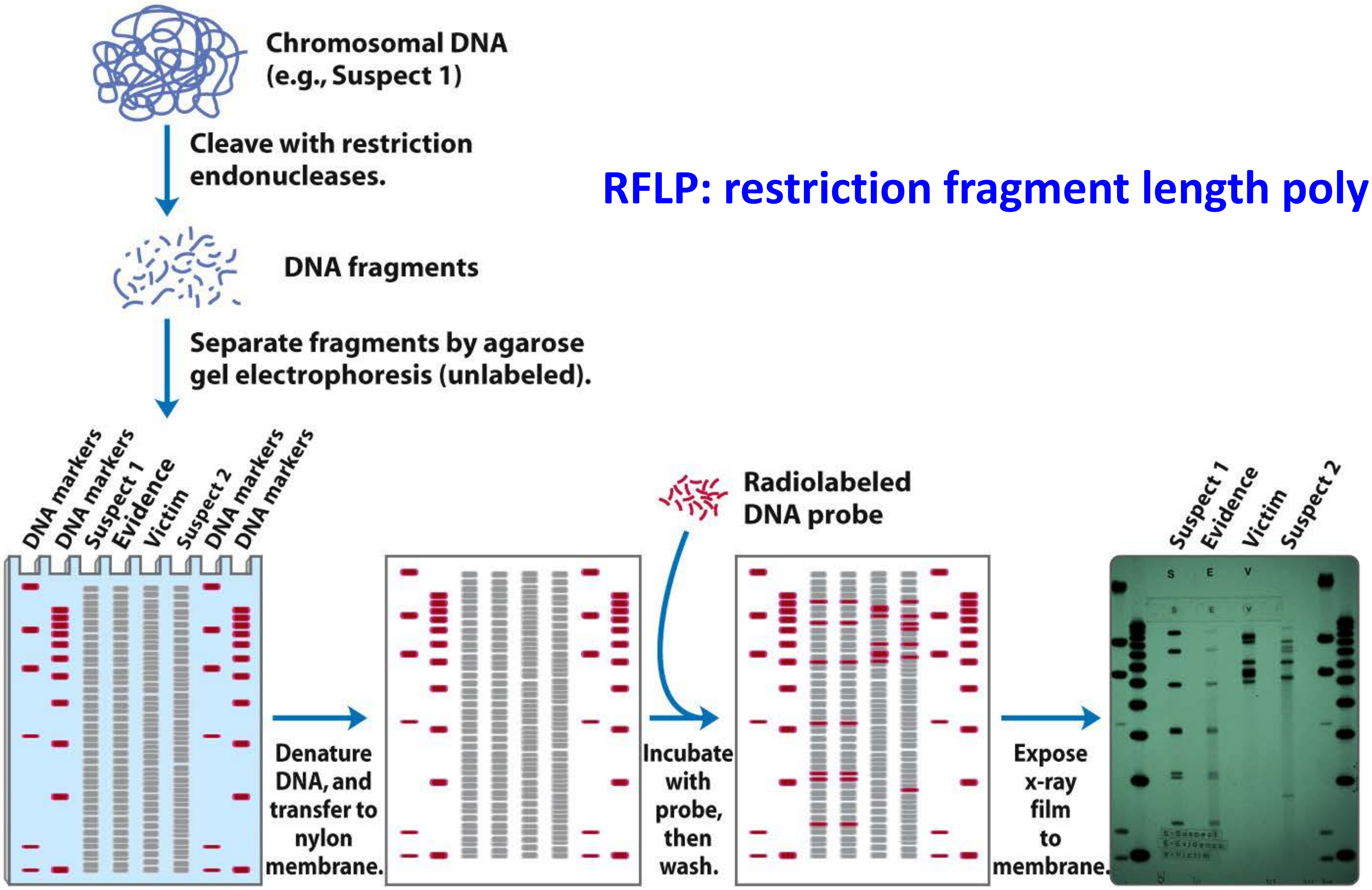


Part III

Southern Blot Procedure

--As Applied to RFLP DNA Fingerprinting

RFLP: restriction fragment length polymorphism


Chromosomal DNA (e.g., Suspect 1)

Cleave with restriction endonucleases.

DNA fragments

Separate fragments by agarose gel electrophoresis (unlabeled).

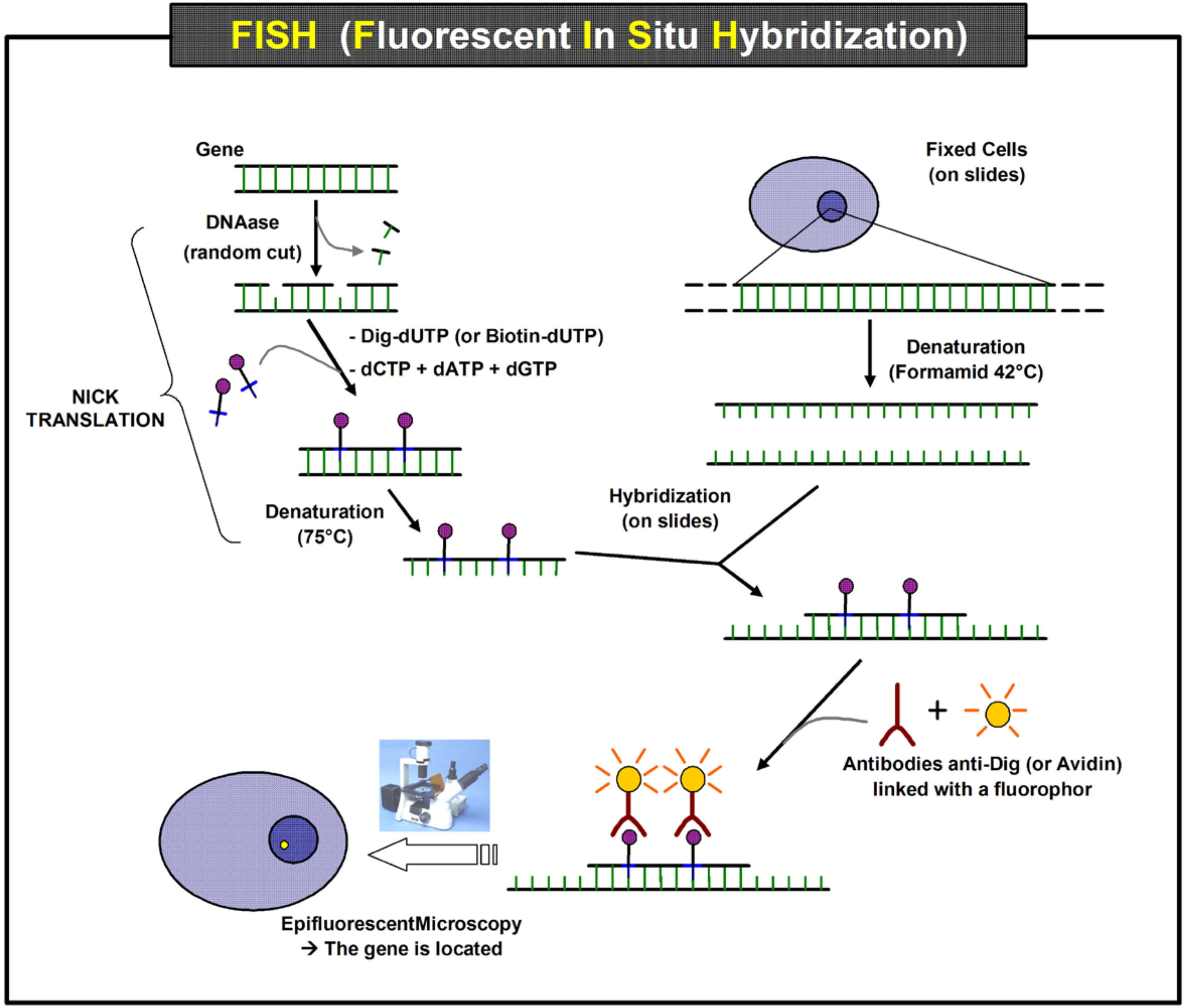
Denature DNA, and transfer to nylon membrane.

Radiolabeled DNA probe

Incubate with probe, then wash.

Expose x-ray film to membrane.

Part III

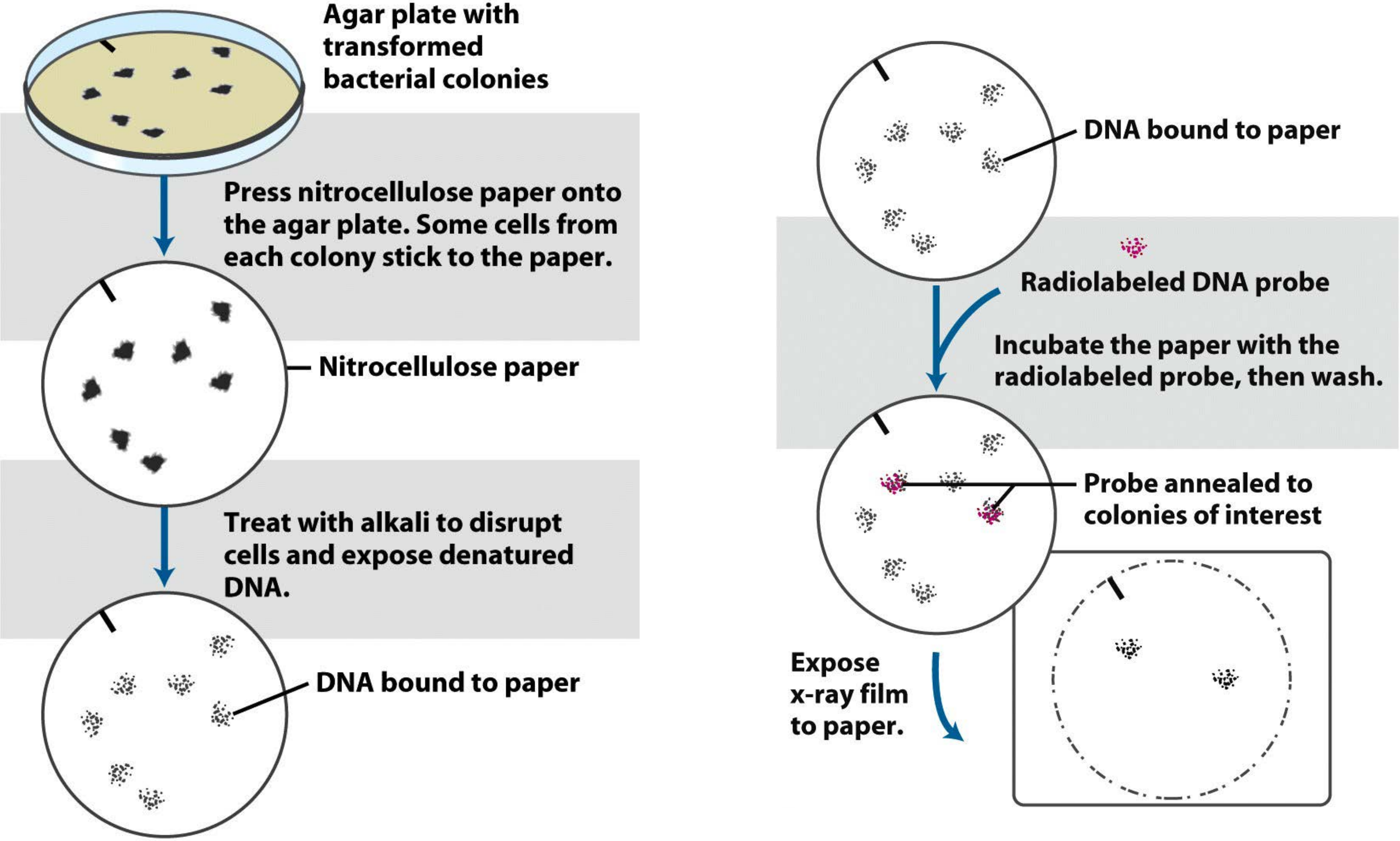


The human **Karyotype**.

Part III

Colony Hybridization

--Use of Hybridization to Identify a Clone with a Particular DNA Segment



Agar plate with transformed bacterial colonies

Press nitrocellulose paper onto the agar plate. Some cells from each colony stick to the paper.

Nitrocellulose paper

Treat with alkali to disrupt cells and expose denatured DNA.

DNA bound to paper

DNA bound to paper

Radiolabeled DNA probe

Incubate the paper with the radiolabeled probe, then wash.

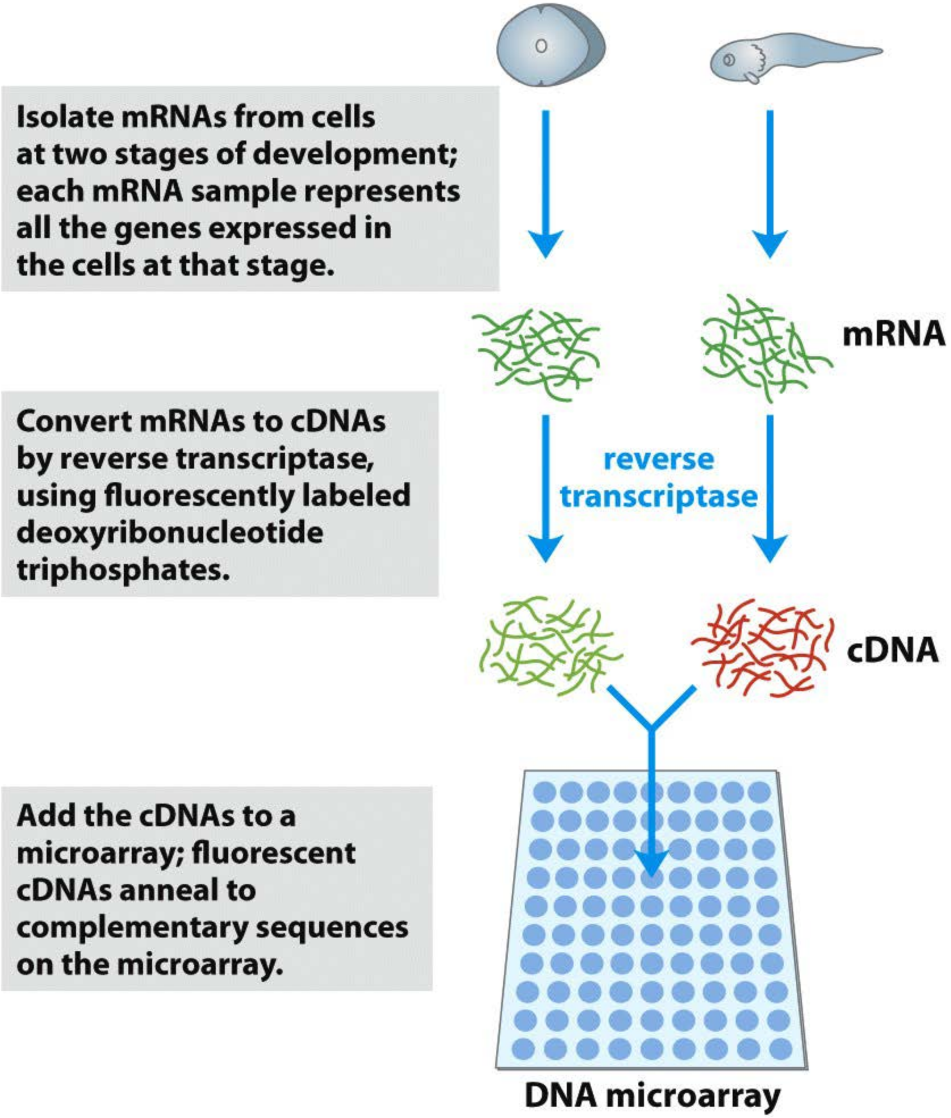
Probe annealed to colonies of interest

Expose x-ray film to paper.

Part III

DNA Microarray

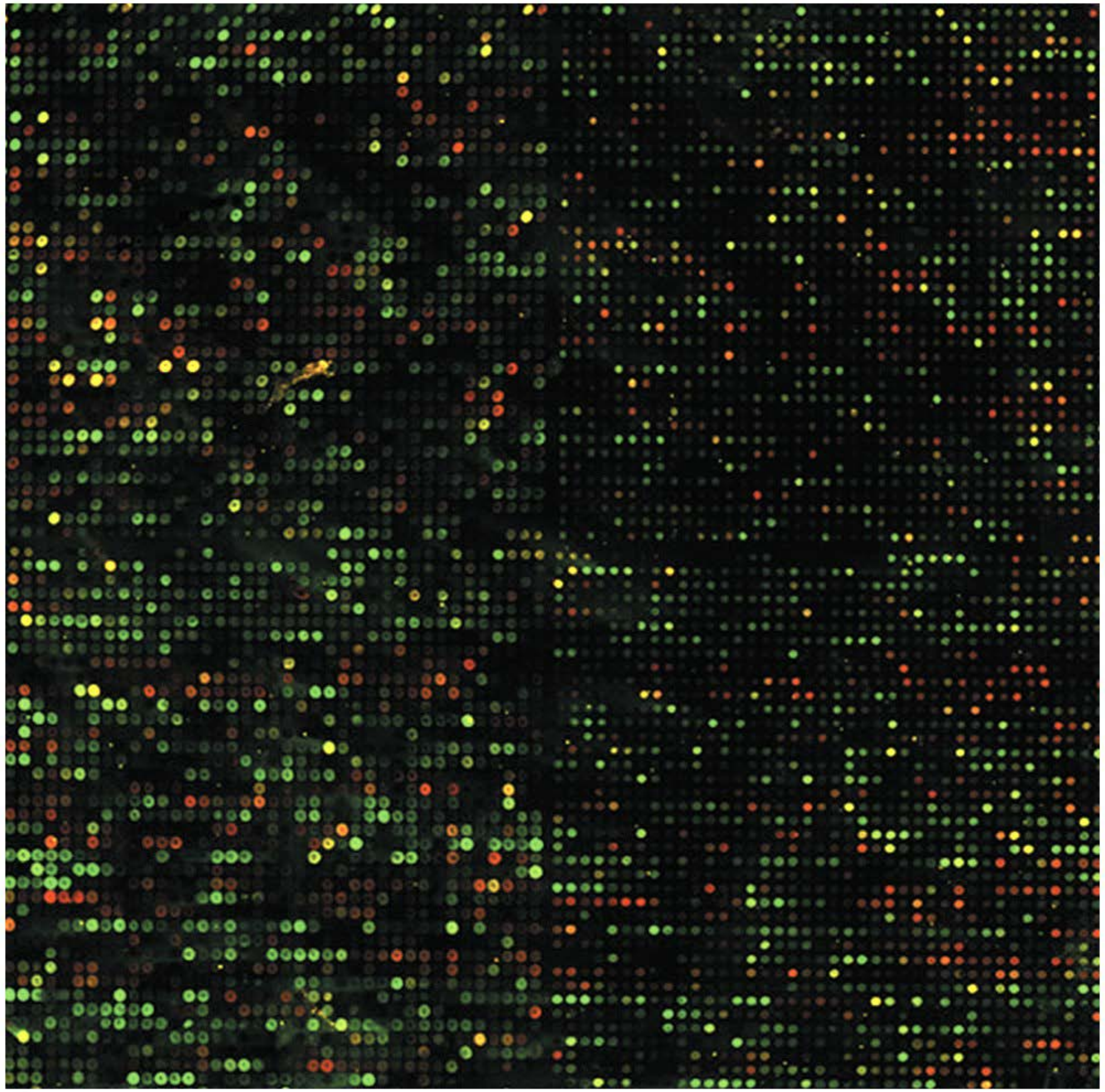
-- A Powerful Tool to Assay Gene Expression Levels of Multiple Genes Simultaneously



Isolate mRNAs from cells at two stages of development; each mRNA sample represents all the genes expressed in the cells at that stage.

Convert mRNAs to cDNAs by reverse transcriptase, using fluorescently labeled deoxyribonucleotide triphosphates.

Add the cDNAs to a microarray; fluorescent cDNAs anneal to complementary sequences on the microarray.



Part III

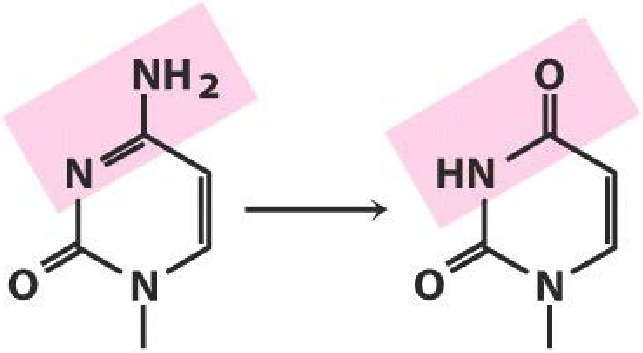
Nucleotides and Nucleic Acids Undergo Nonenzymatic Transformations

- Mutations
  - Alternation in DNA structures that produce permanent changes in the genetics information encoded therein

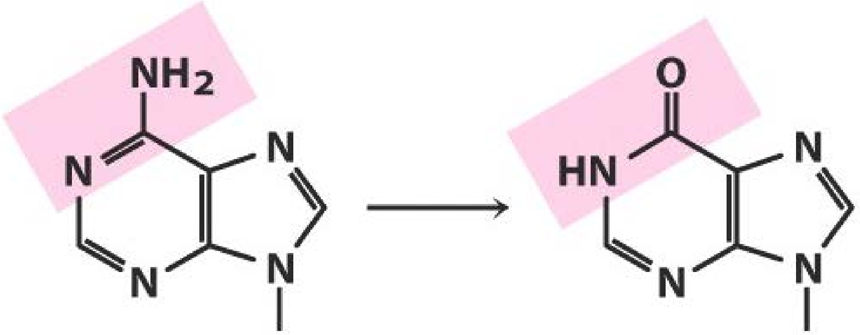
  - Deamination
  - Depurination or depyrimidination
  - Pyrimidine dimers
  - Reactive chemicals, etc.

Part III

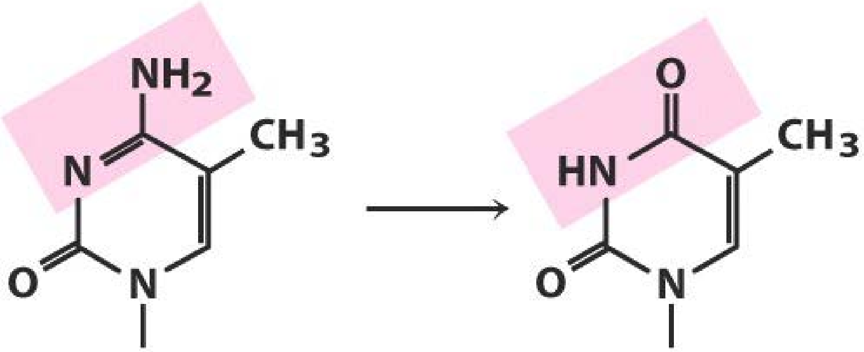
Deamination



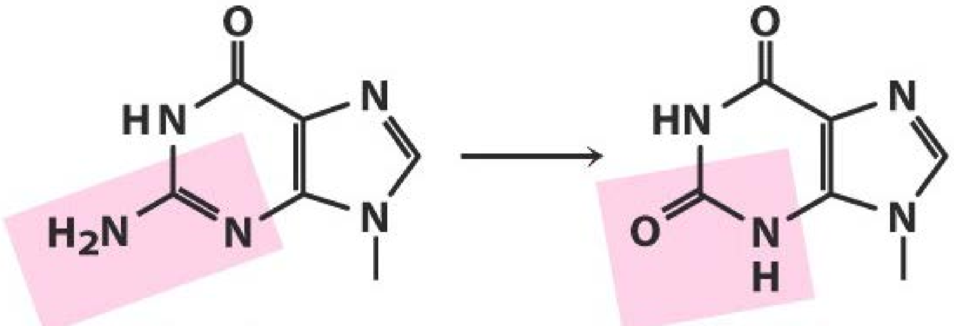
Cytosine Uracil



Adenine Hypoxanthine



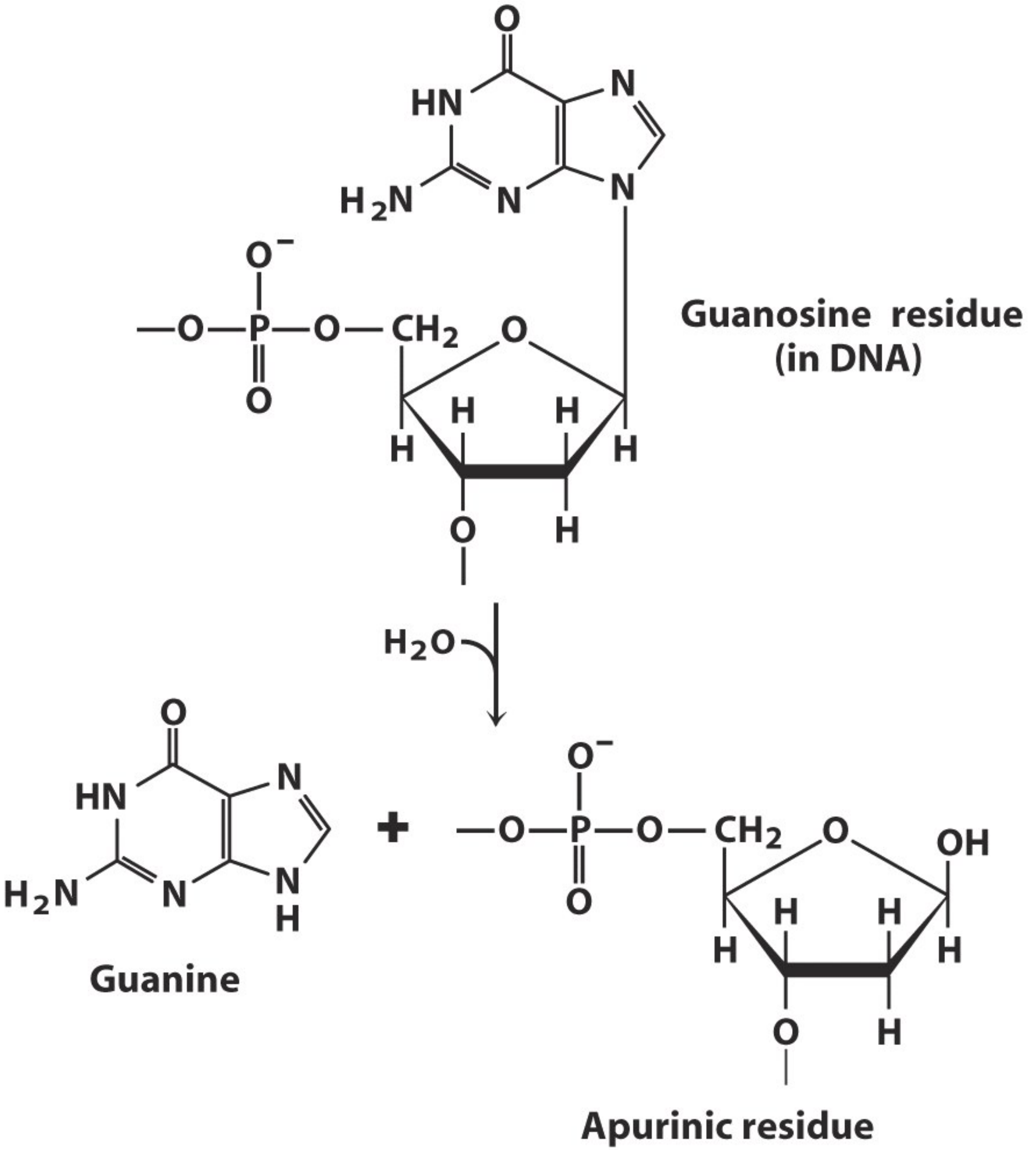
5-Methylcytosine Thymine



Guanine Xanthine

Part III

Depurination



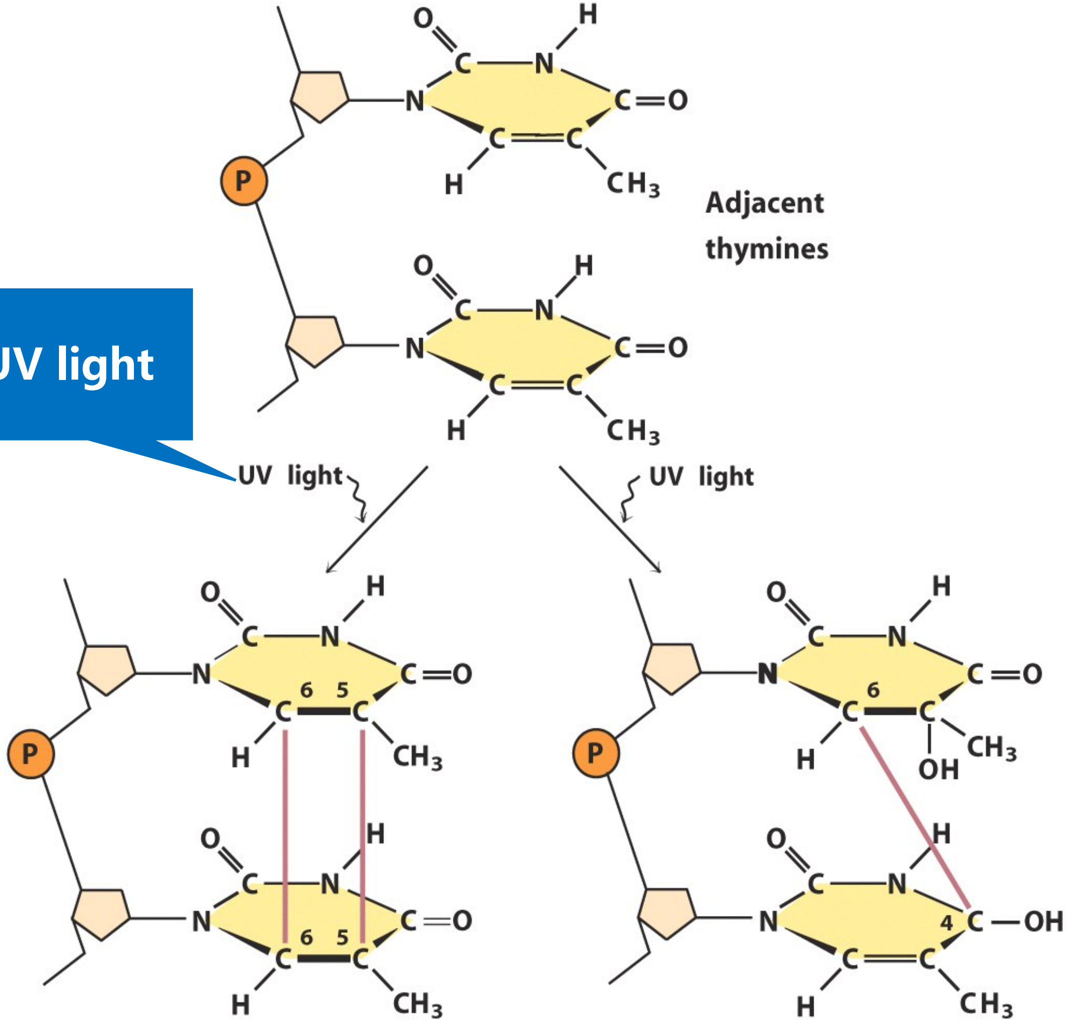
Occurs at higher rate for purines than for pyrimidines.

1/10^5 purines (1/10^4 in mammalian cells) are lost every 24 h.

Part III

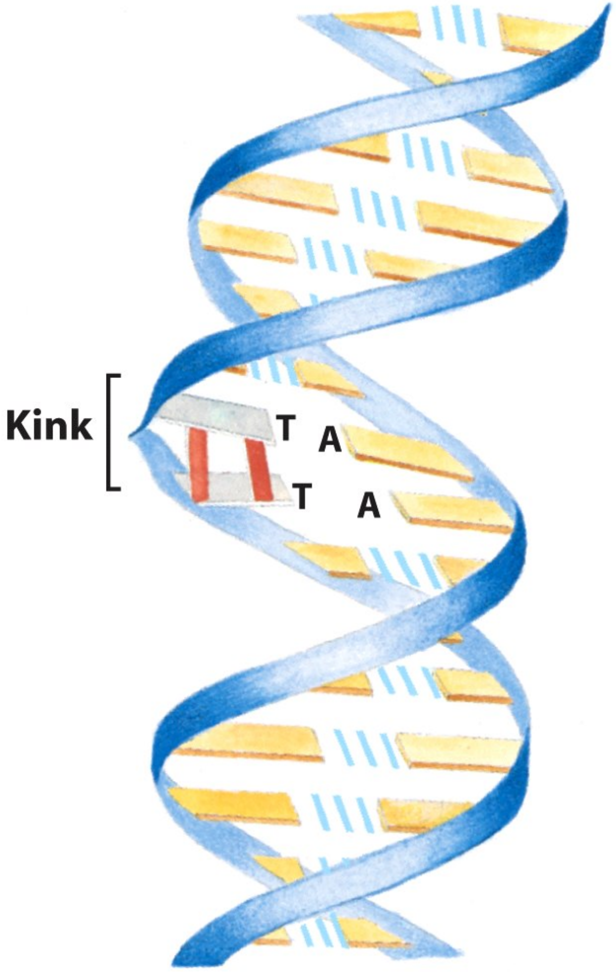
Formation of Pyrimidine Dimers

UV light



Cyclobutane thymine dimer

6-4 Photoproduct

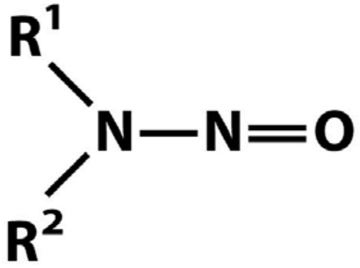


UV and ionizing radiations (cosmic rays):
10% DNA damage caused by environmental agents

Reactive Chemicals—Deaminating Agents

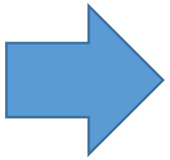
$NaNO_2$
Sodium nitrite

$NaNO_3$
Sodium nitrate



Nitrosamine

Nitrous acid precursors



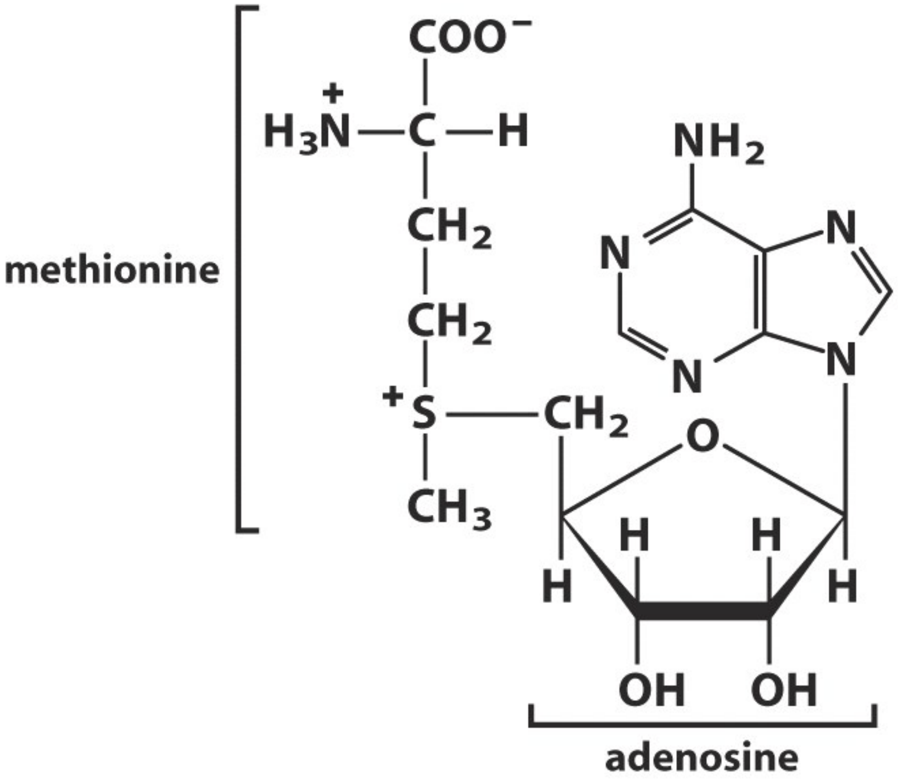
HNO_2

Food Preservatives.

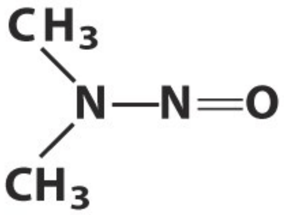
Bisulfite has similar effects.

Bisulfite sequencing is used to determine DNA methylation pattern.

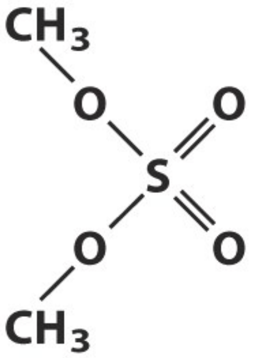
Reactive Chemicals—Alkylating Agents



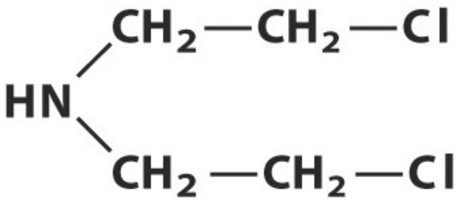
S-Adenosylmethionine



Dimethylnitrosamine

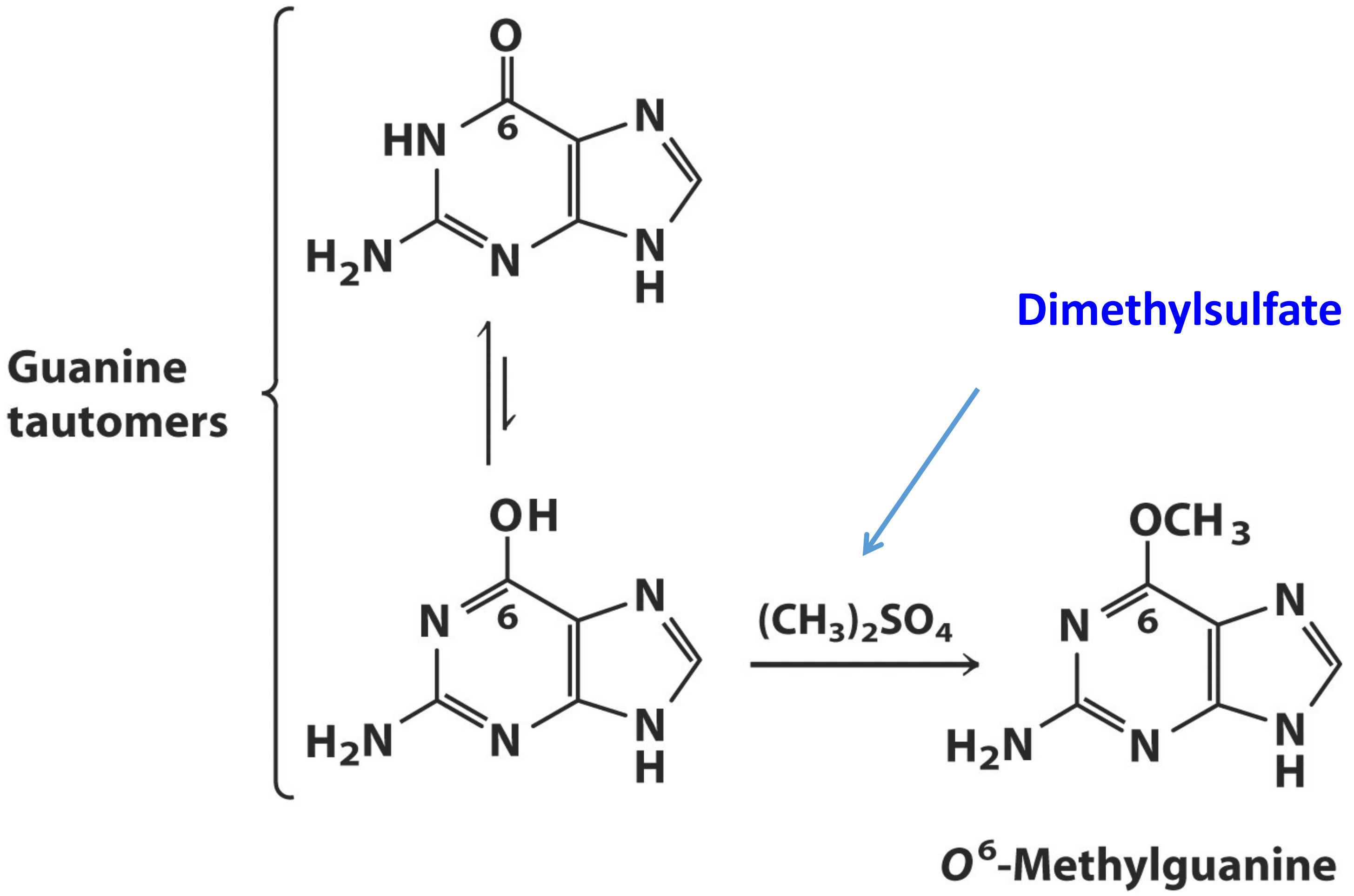


Dimethylsulfate



Nitrogen mustard

Reactive Chemicals—Alkylating Agents

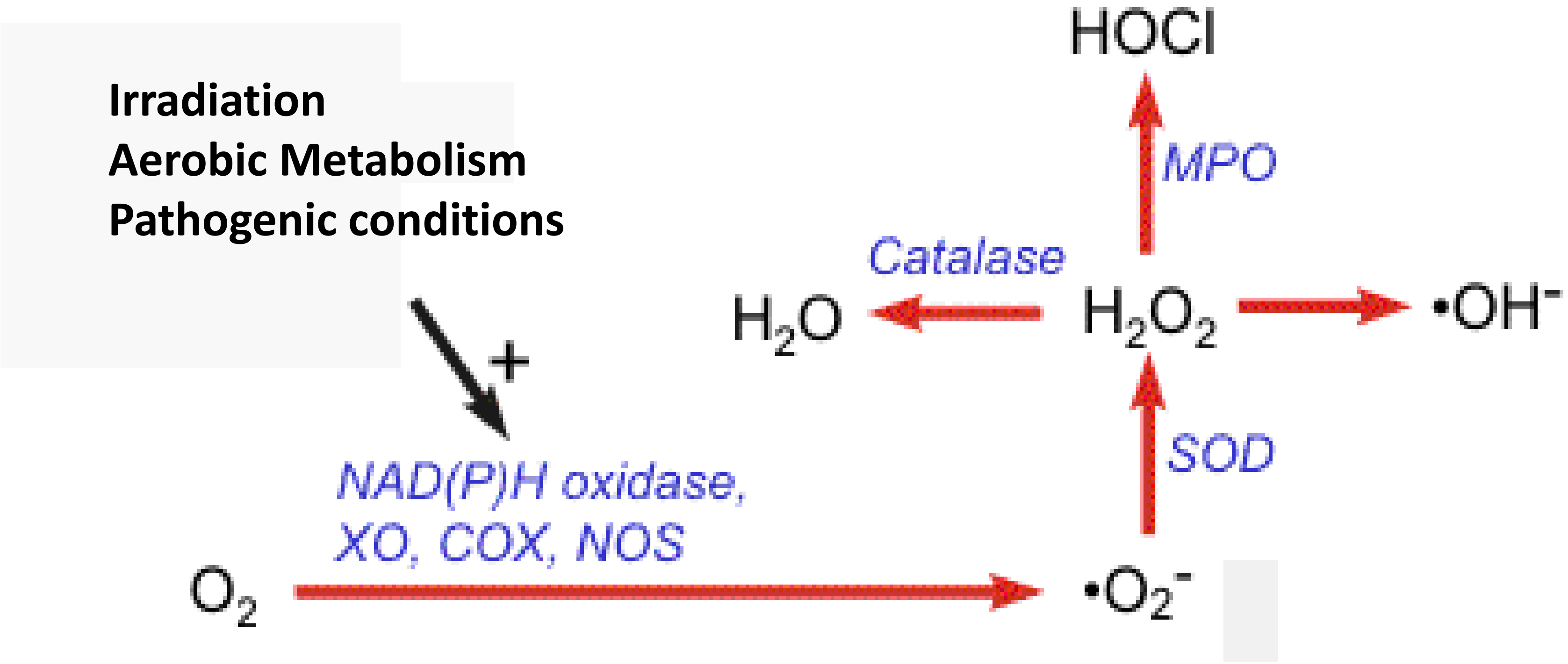


Dimethylsulfate

O^6-Methylguanine

Part III

Oxidative Damage Is the Most Important Source of Mutagenic Alternations in DNA

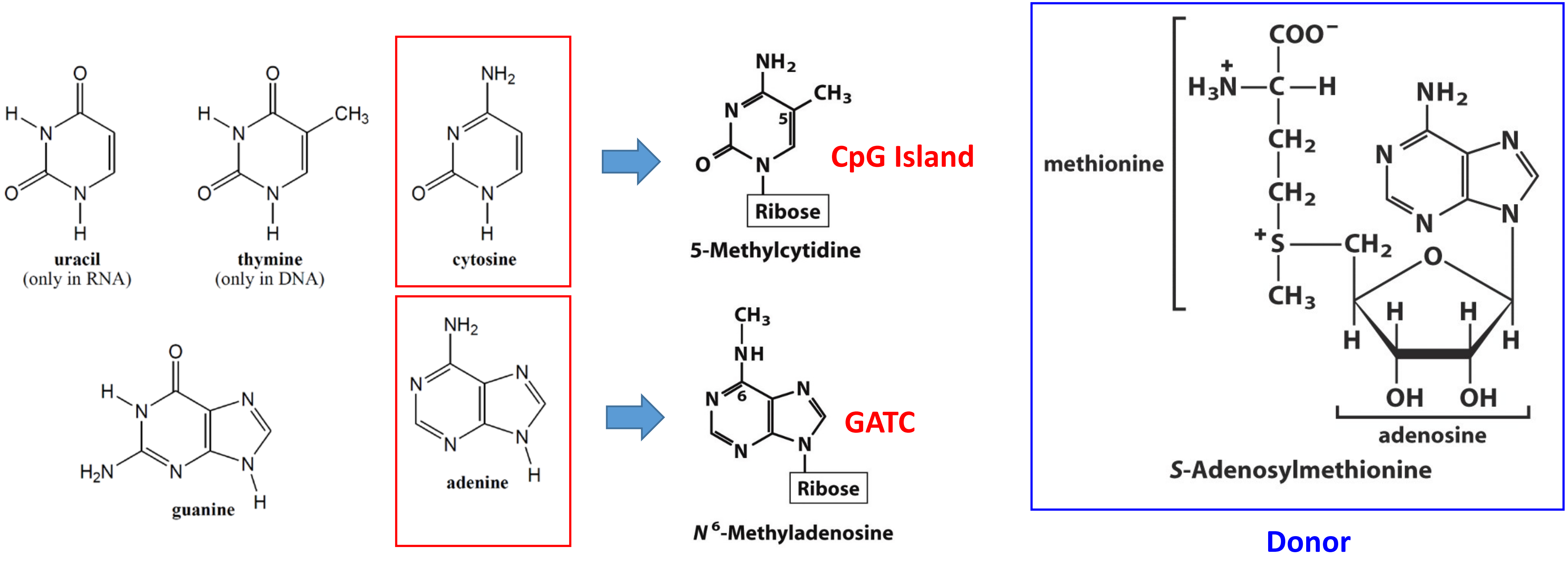

Irradiation
Aerobic Metabolism
Pathogenic conditions

Excited-Oxygen Species (Reactive Oxygen Species, ROS)

Hydrogen Peroxide, Hydroxyl Radicals, and Superoxide radicals

Part III

Some Bases of DNA Are Enzymatically Methylated



Adenine and **cytosine** are methylated more often than guanine and thymine.

All known DNA methylases use **S-adenosylmethionine** as a methyl group donor.

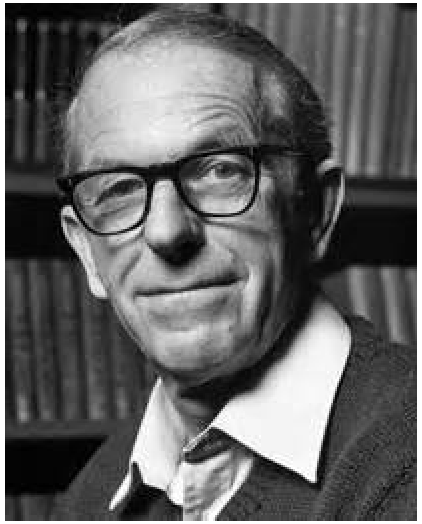
Part III

The Sequence of Long DNA Strands Can Be Determined

Development of two techniques in 1977

Alan Maxam and Walter Gilbert

Frederick Sanger



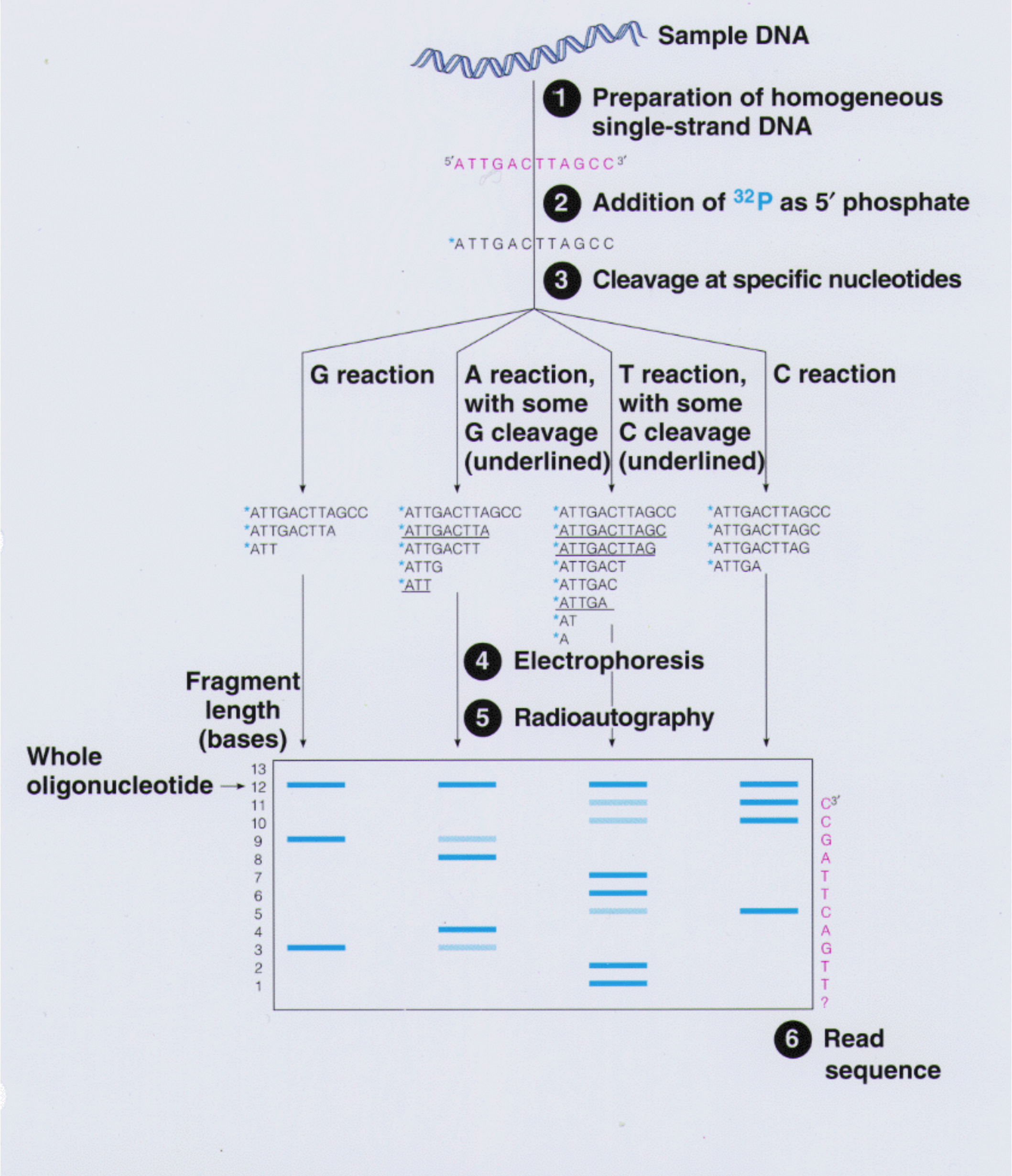
Frederick Sanger

Walter Gilbert

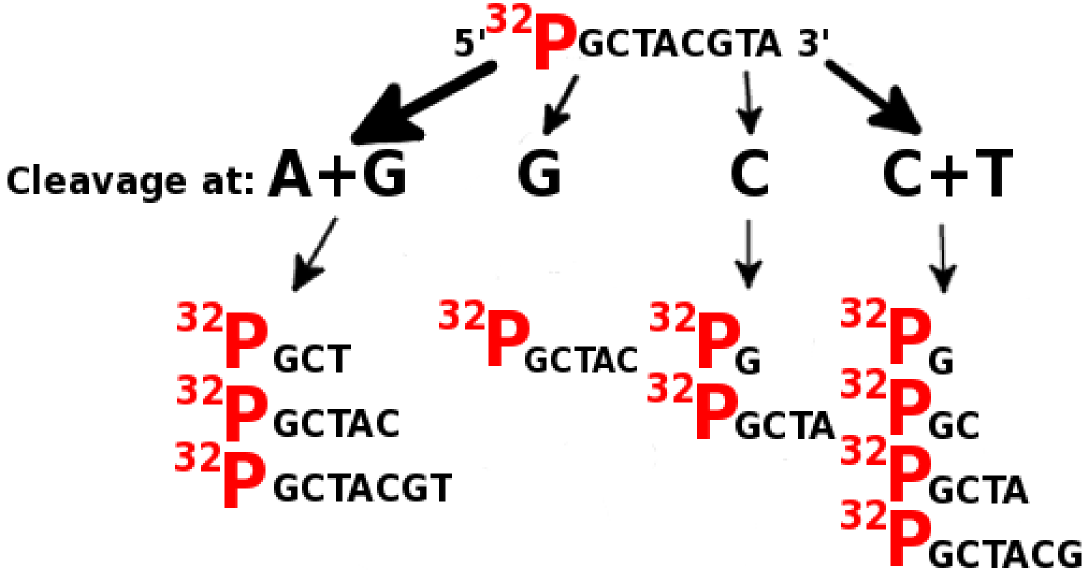
Nobel Prize in Chemistry in 1980

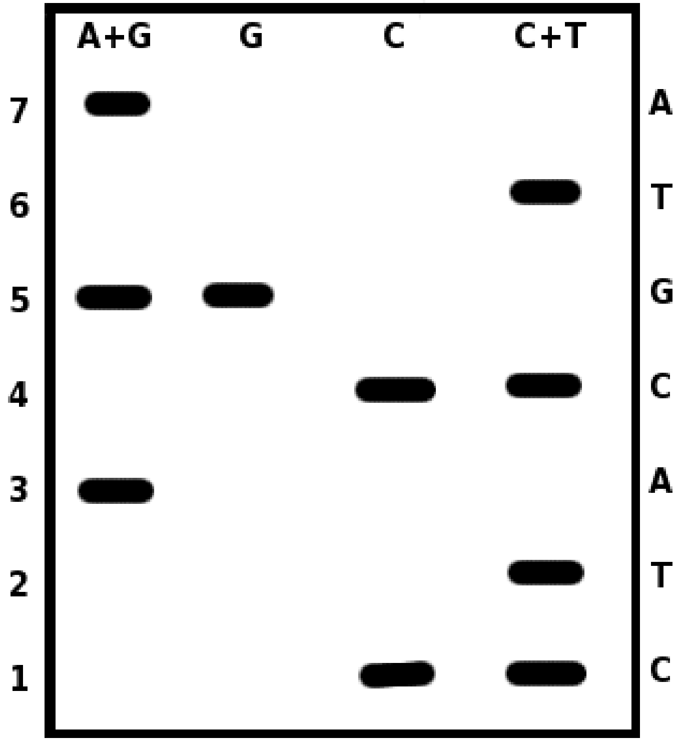
Part III

Maxam-Gilbert Sequencing



From Mathews and van Holde: *Biochemistry 2/e.* © The Benjamin/Cummings Publishing Co., Inc.

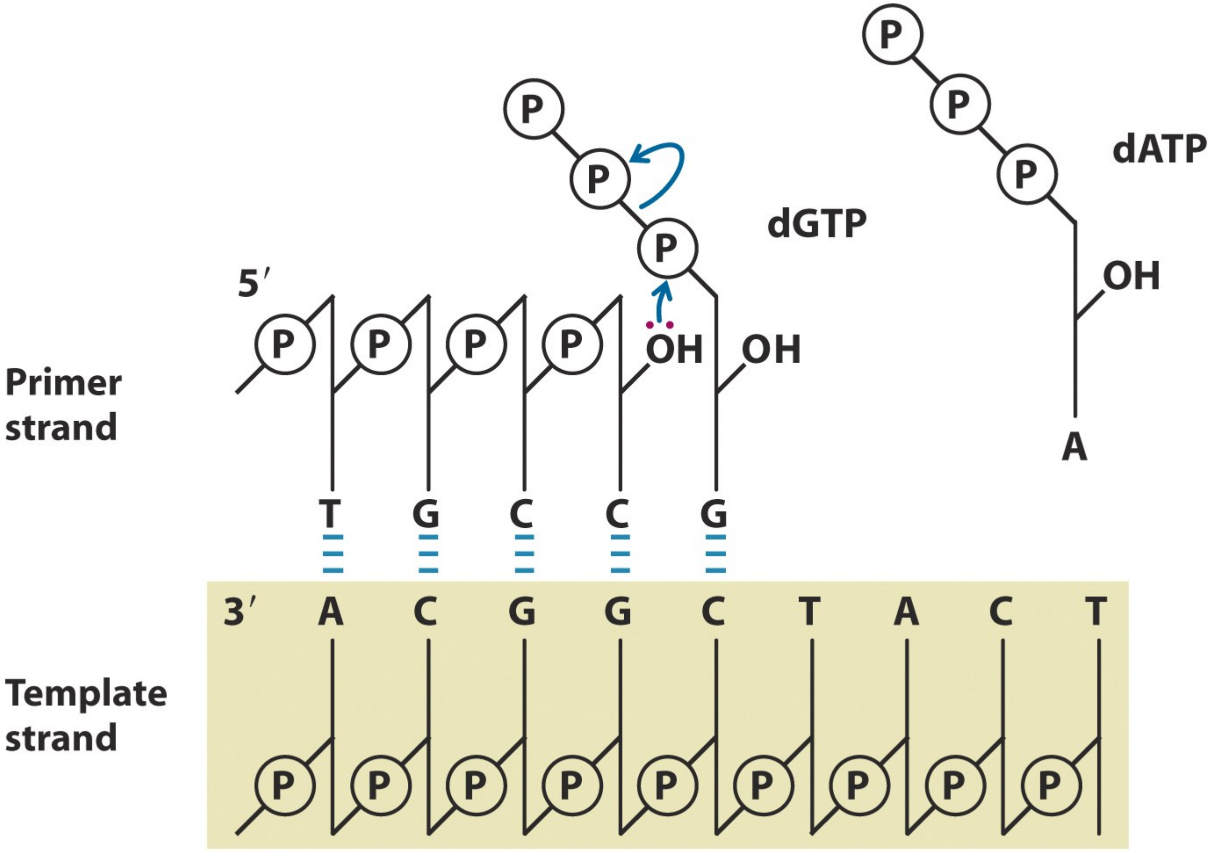


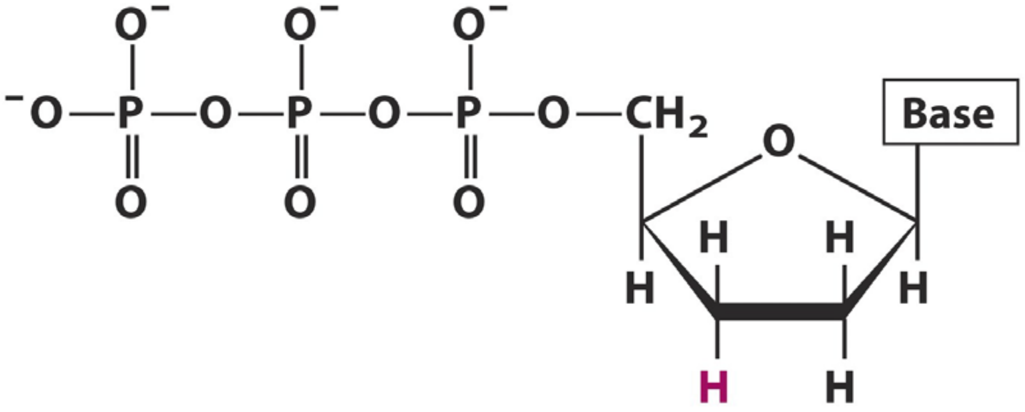


Sequencing Gel

Part III

Sanger Sequencing

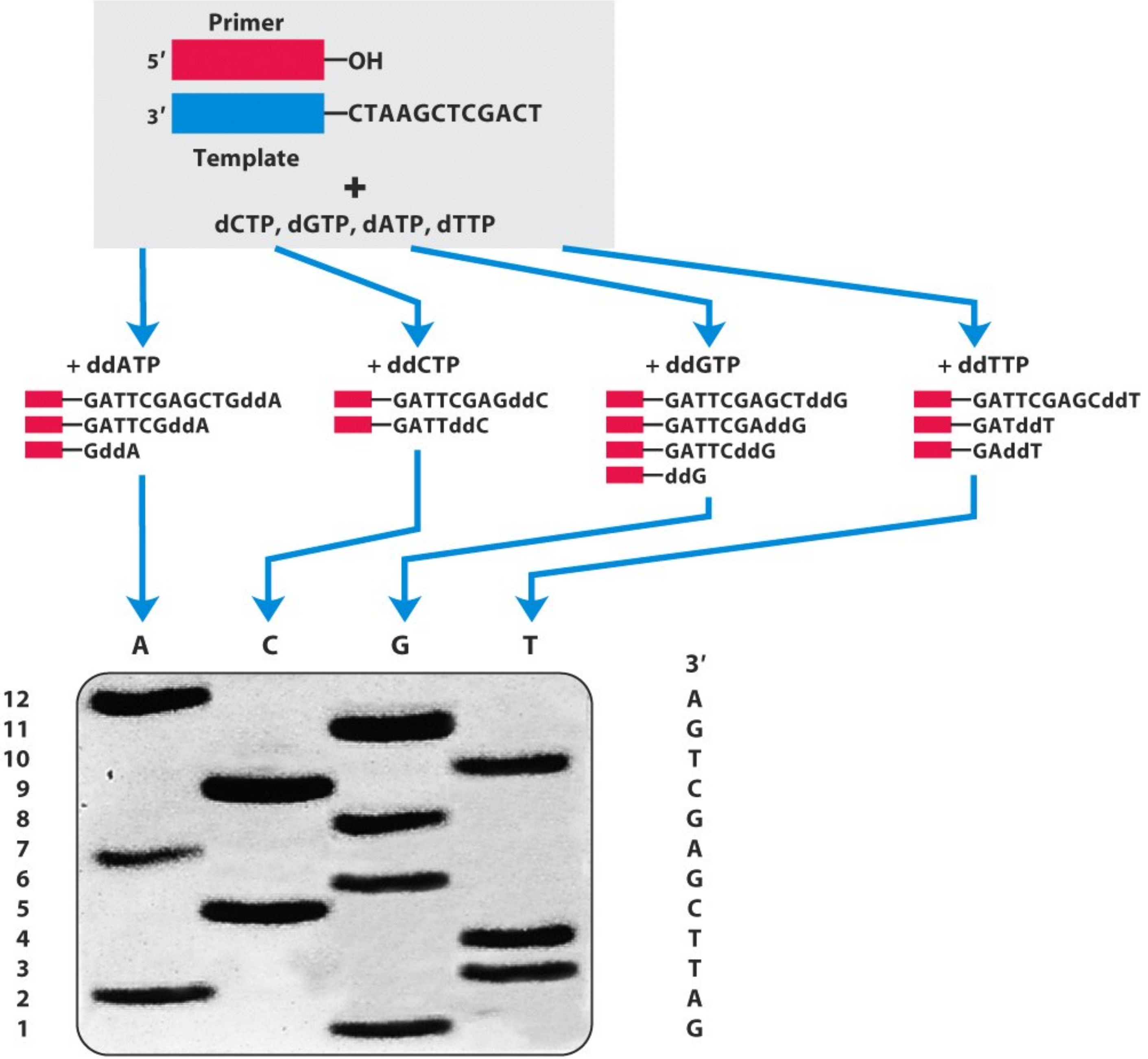




ddNTP analog

Part III

Sanger Sequencing



Primer

5′ —OH

3′ —CTAAGCTCGACT

Template

+

dCTP, dGTP, dATP, dTTP

+ ddATP

—GATTCGAGCTGddA
—GATTCGddA
—GddA

+ ddCTP

—GATTCGAGddC
—GATTddC

+ ddGTP

—GATTCGAGCTddG
—GATTCGAddG
—GATTCddG
—ddG

+ ddTTP

—GATTCGAGCddT
—GATddT
—GAddT

A C G T

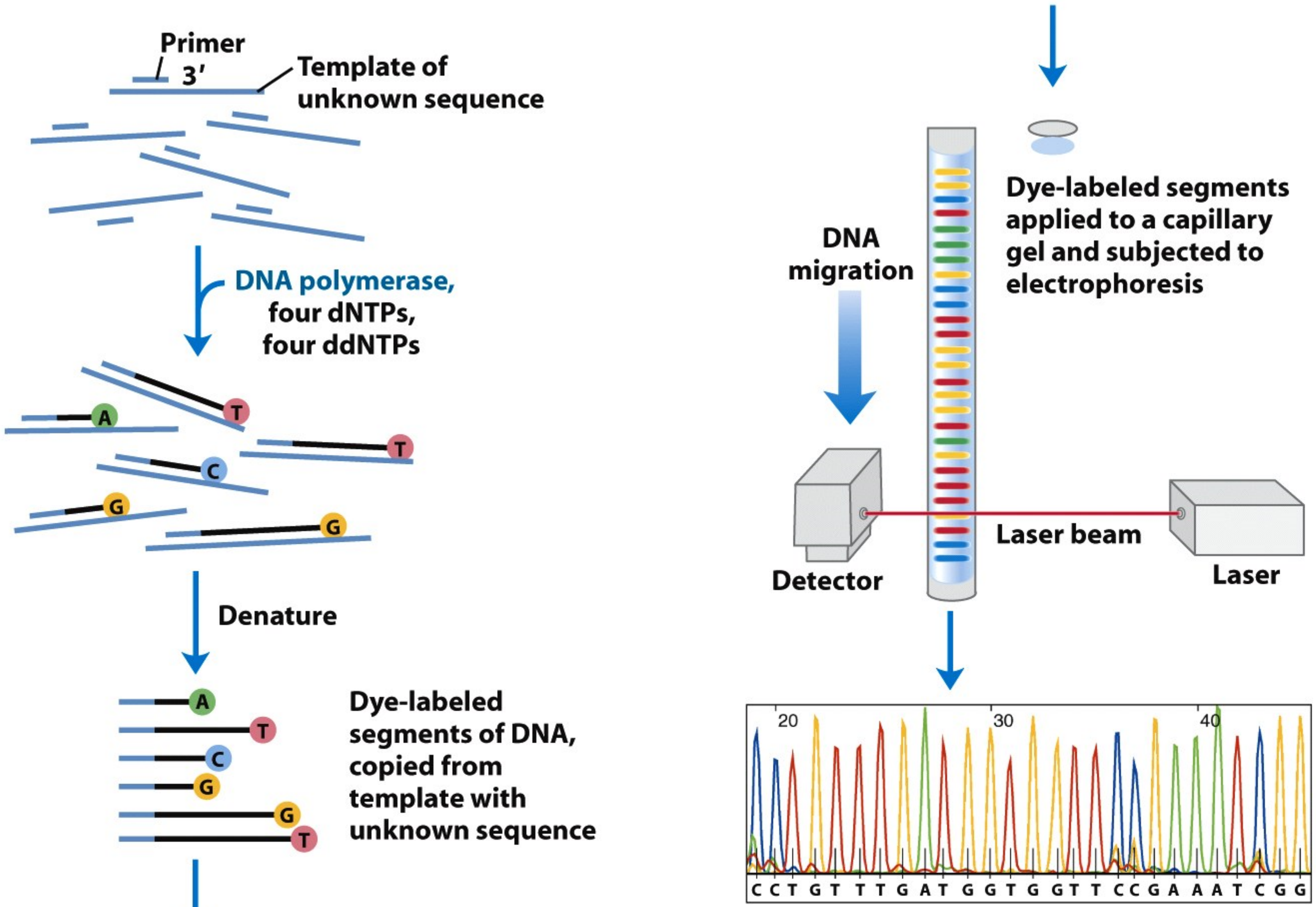
	Sequence (3′)
12	A
11	G
10	T
9	C
8	G
7	A
6	G
5	C
4	T
3	T
2	A
1	G

Autoradiogram of electrophoresis gel

Sequence of complementary strand

Part III

Strategy for Automating DNA Sequencing



DNA polymerase, four dNTPs, four ddNTPs

Denature

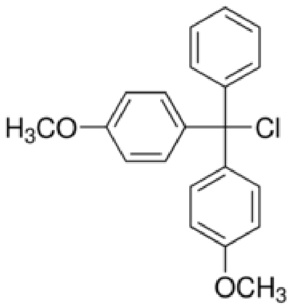
Dye-labeled segments of DNA, copied from template with unknown sequence

Dye-labeled segments applied to a capillary gel and subjected to electrophoresis

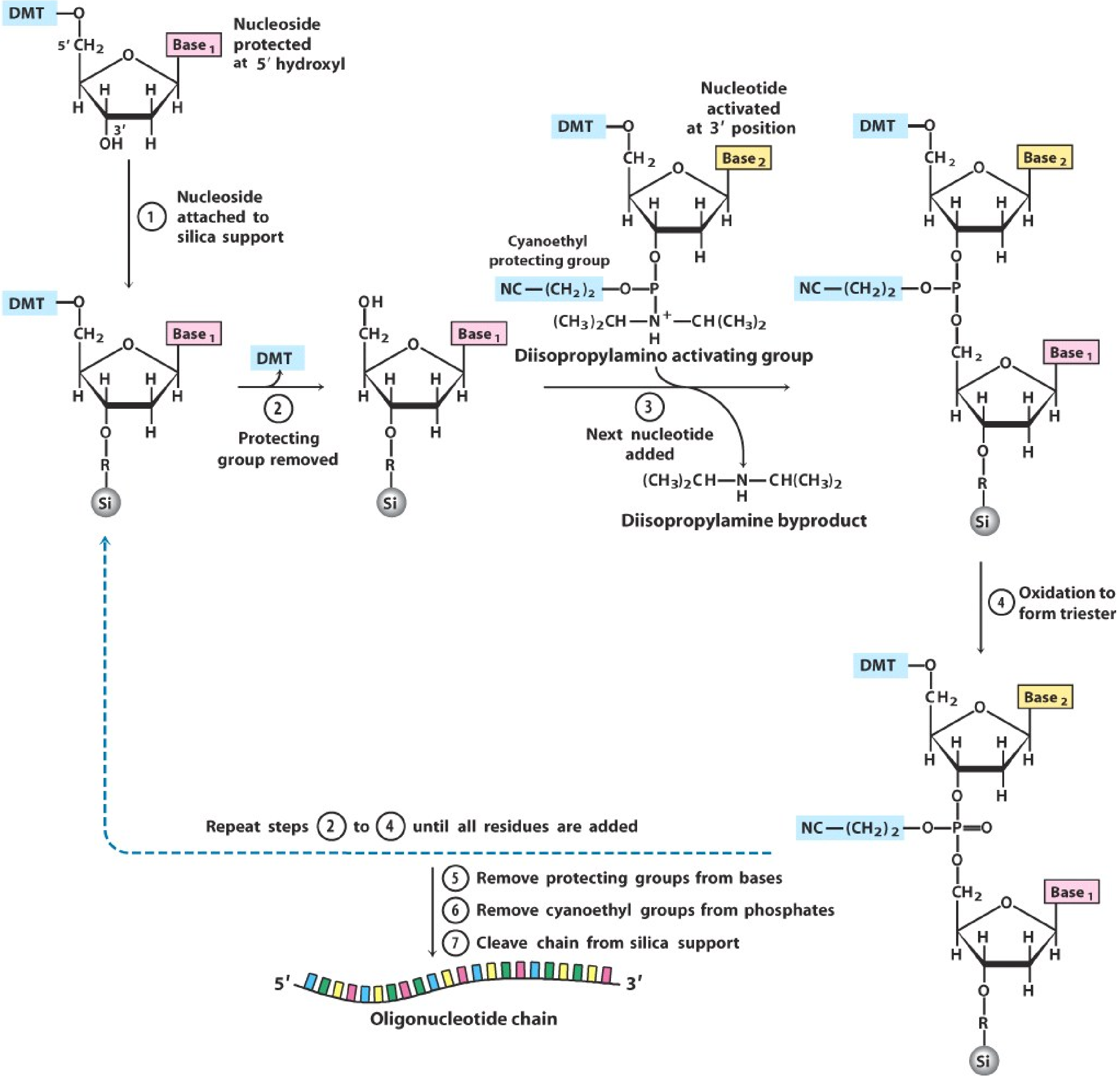
Computer-generated result after bands migrate past detector

Part III

Automated DNA Synthesis



DMT



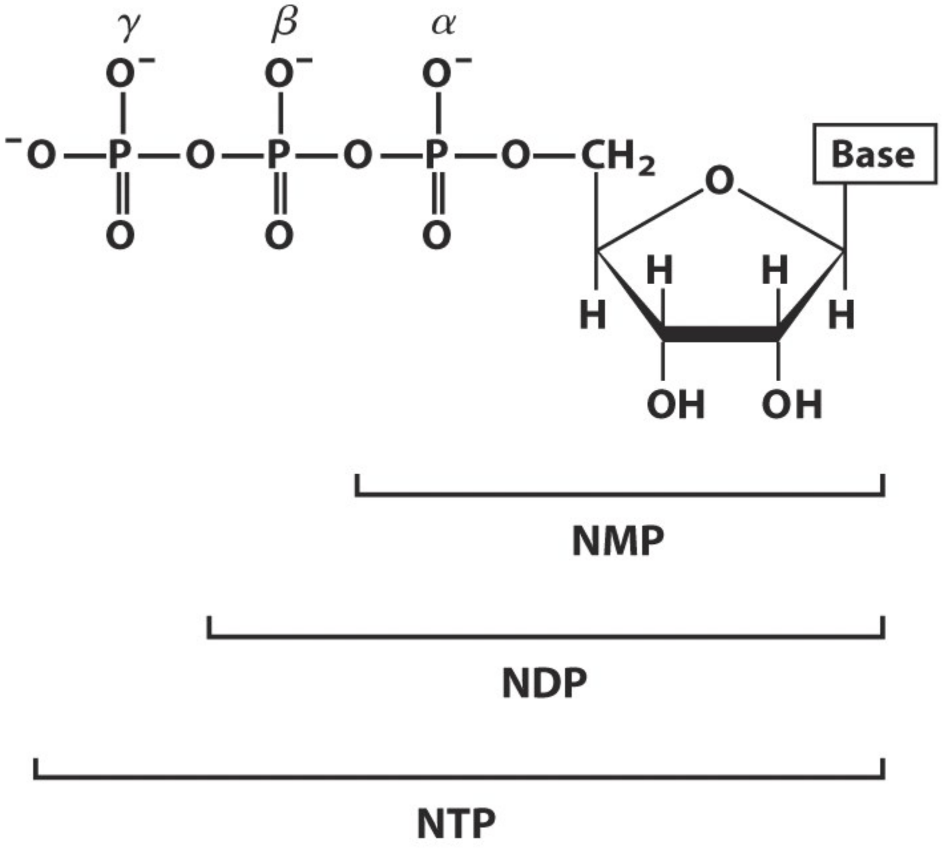
Summary

Part III. Nucleic Acid Chemistry

- What is the denaturation and annealing?
- What techniques rely on DNA hybridization?
- Spontaneous reaction happens.
- What is Sanger sequencing?

Part IV. Other Functions of Nucleotides

Nucleotides Carry Chemical Energy in Cells

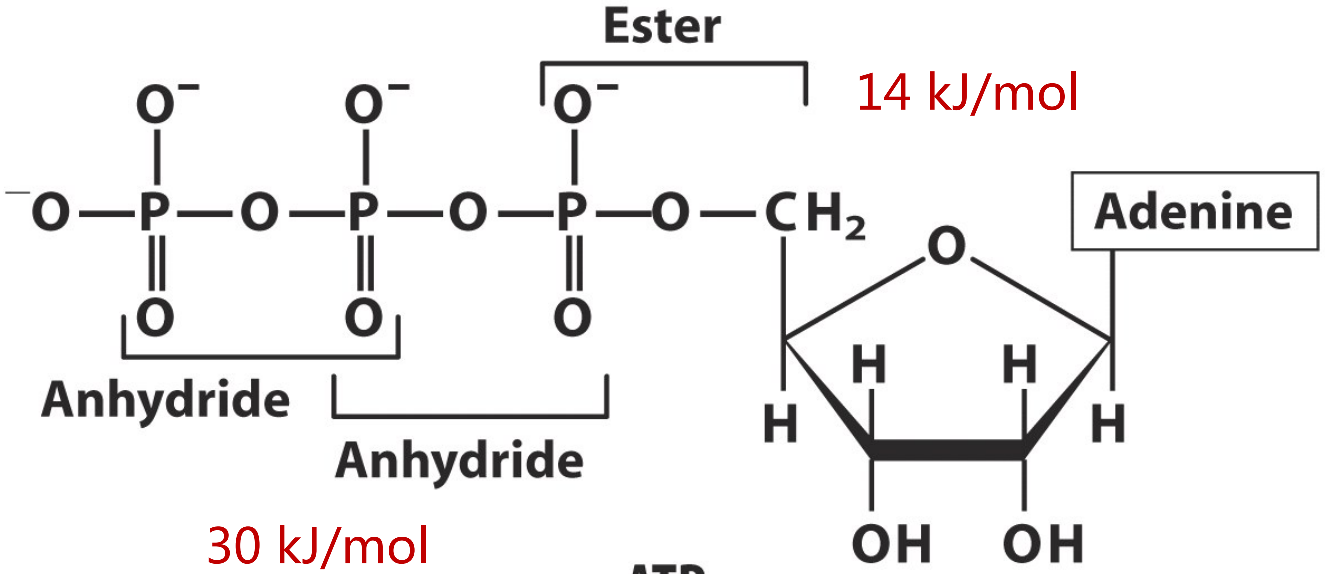


Abbreviations of ribonucleoside 5′-phosphates			
Base	**Mono-**	**Di-**	**Tri-**
Adenine	AMP	ADP	ATP
Guanine	GMP	GDP	GTP
Cytosine	CMP	CDP	CTP
Uracil	UMP	UDP	UTP

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

Part IV

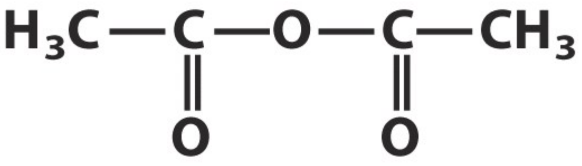
Phospho-Anhydrides and Phosphate Esters – High Energy Bonds



14 kJ/mol

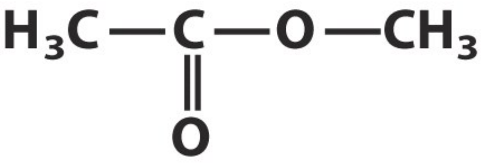
30 kJ/mol

ATP



$$H_3C-\underset{\underset{O}{\|}}{C}-O-\underset{\underset{O}{\|}}{C}-CH_3$$

Acetic anhydride,
a carboxylic acid
anhydride

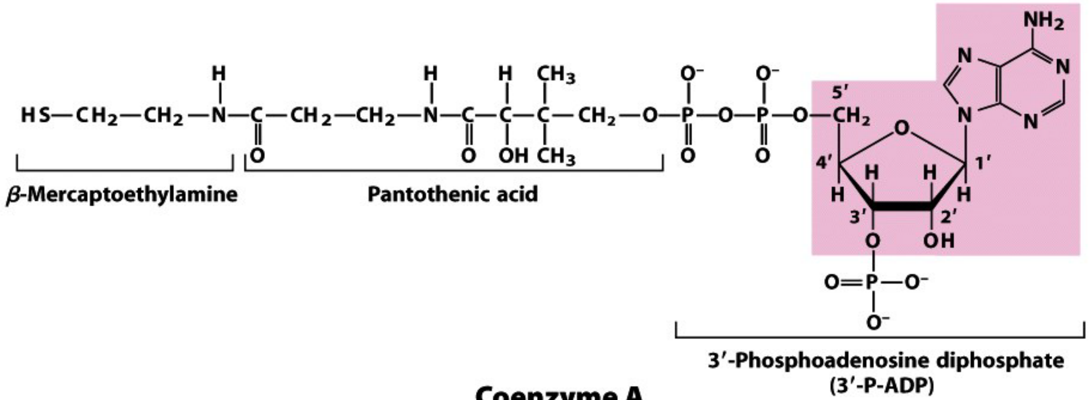


$$H_3C-\underset{\underset{O}{\|}}{C}-O-CH_3$$

Methyl acetate,
a carboxylic acid
ester

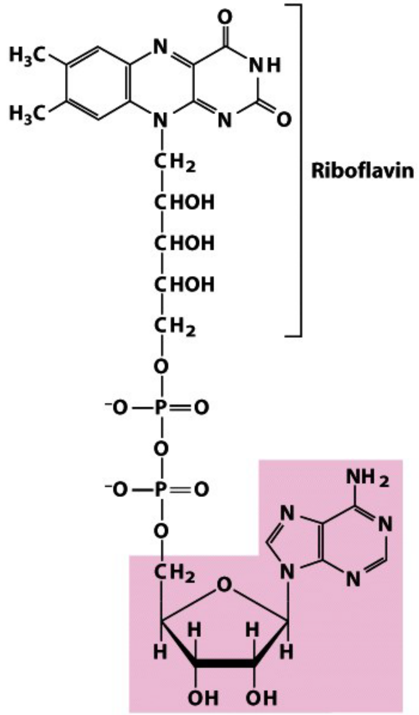
Part IV

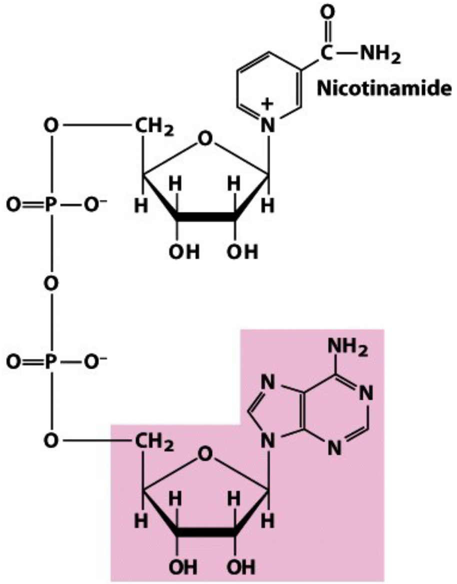
Adenine Nucleotides Are Components of Many Enzyme Cofactors



Coenzyme A

Acyl group transfer reactions;
Thioester bonds.





NAD⁺/NADH
Hydride transfers

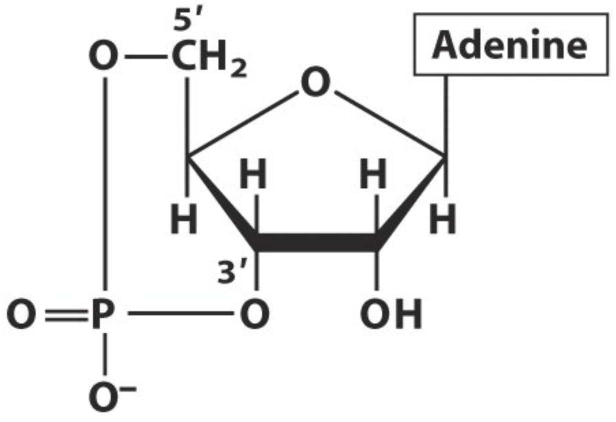
Nicotinamide adenine dinucleotide (NAD^+)

Flavin adenine dinucleotide

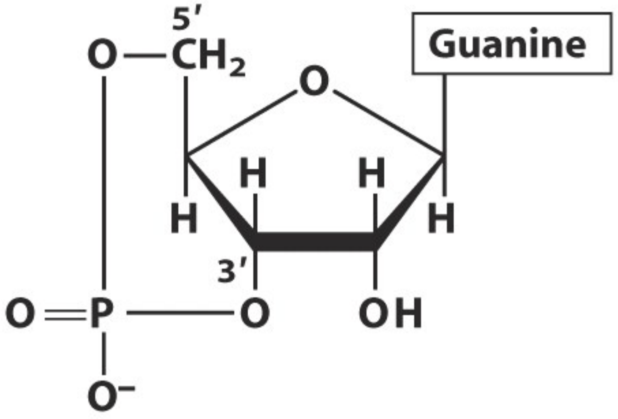
Electron transfers

Part IV

Some Nucleotides Are Regulatory Molecules



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

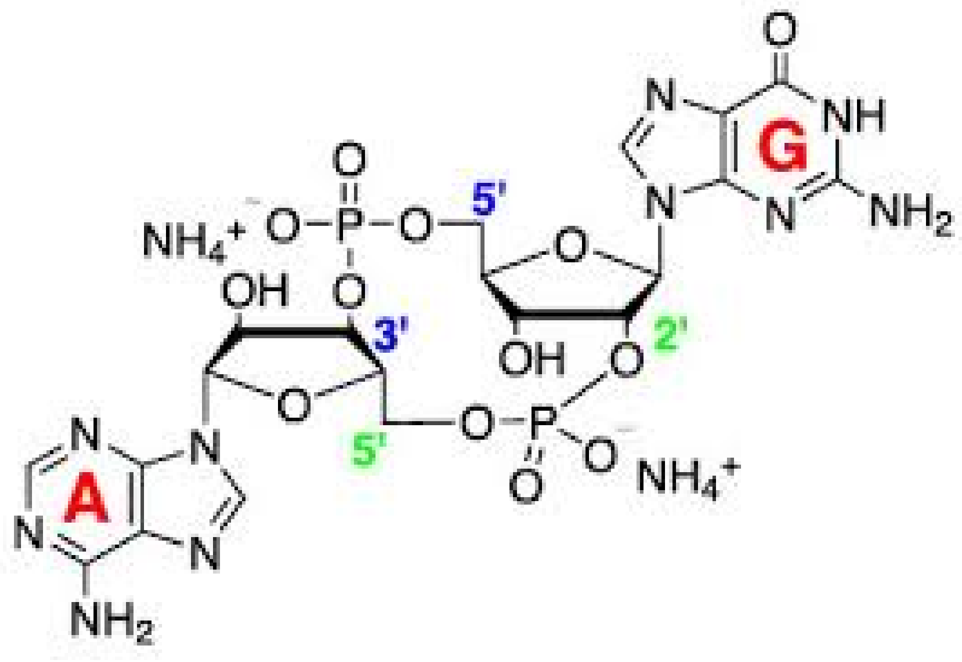


Guanosine 3′,5′-cyclic monophosphate
(cyclic GMP; cGMP)

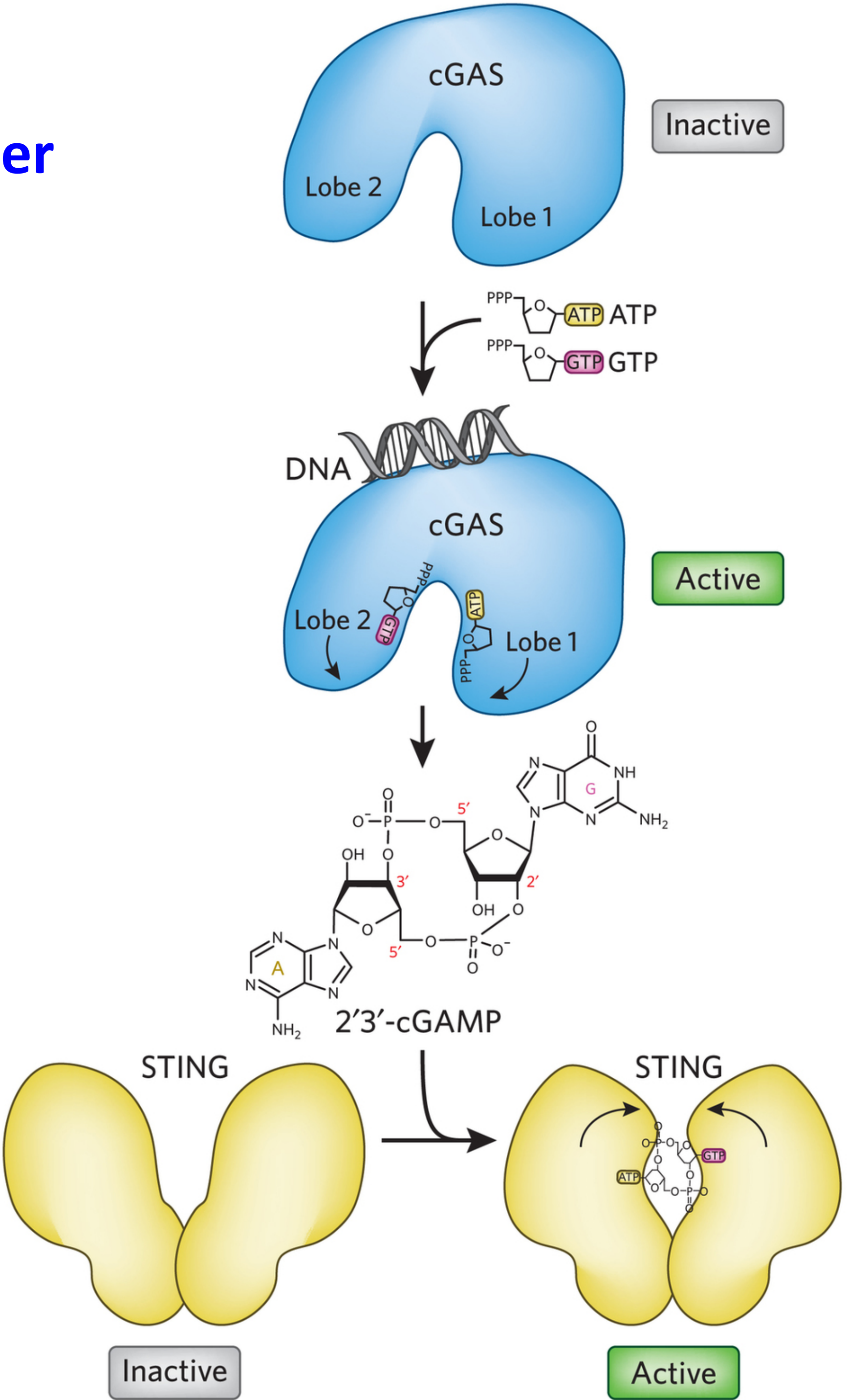
second messengers

Part IV

cGAMP as a Newly Identified Sencond Messenger



cGAMP

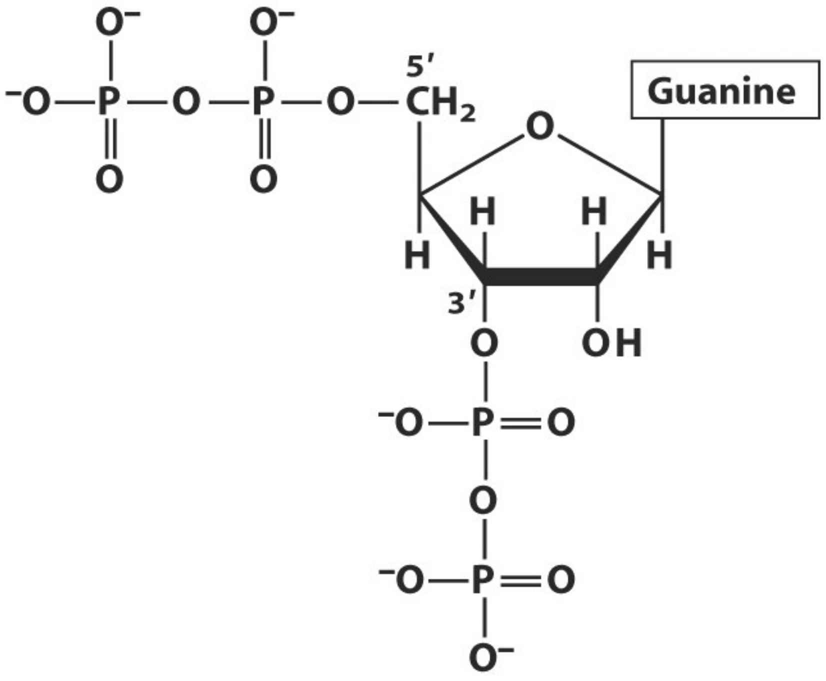


Science **2013**, *339*, 786–791.

Nat. Chem. Biol. **2013**, 533-534.

Part IV

ppGpp Is Produced in Bacteria in Response to a Slowdown in Protein Synthesis During Amino Acid Starvation



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

Inhibits rRNA and tRNA snthesis

Question Time

Come back for Genes and Chromosomes.

References:

Lehninger Principle of Biochemistry (fifth edition)
Molecular Biology of the Cell (sixth edition)

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