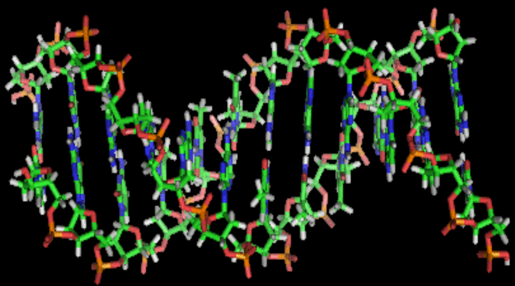

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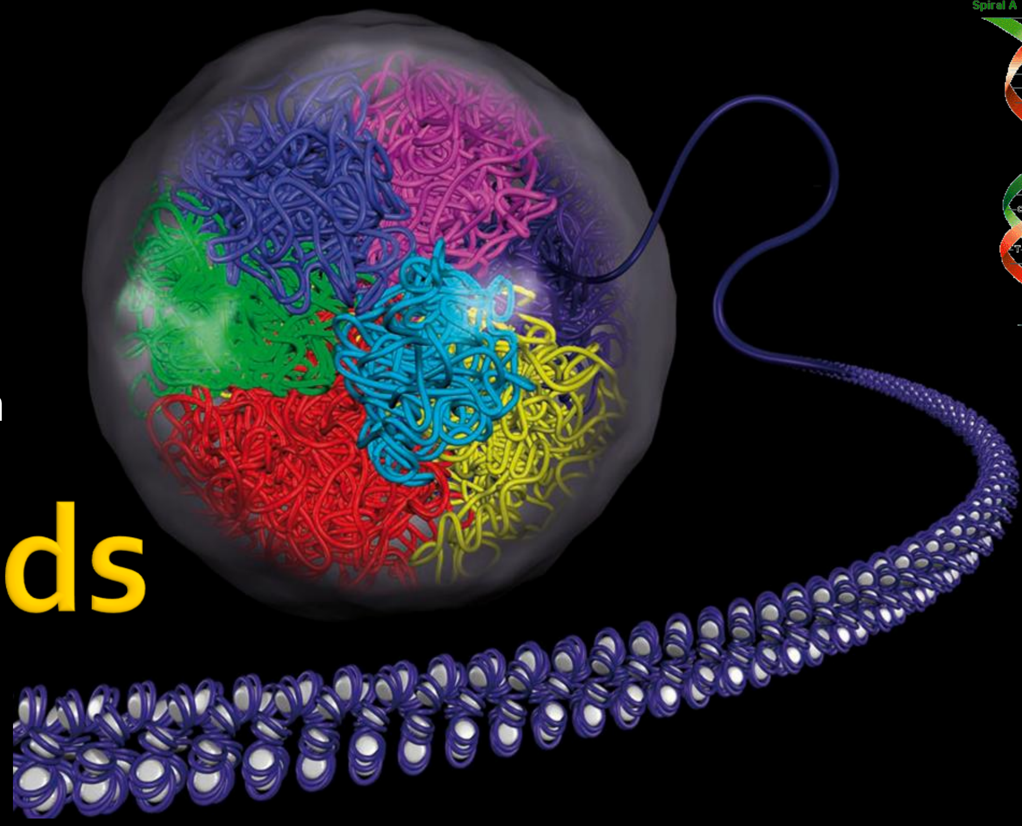

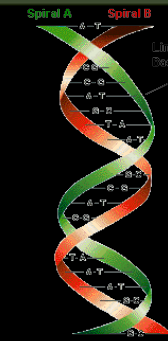



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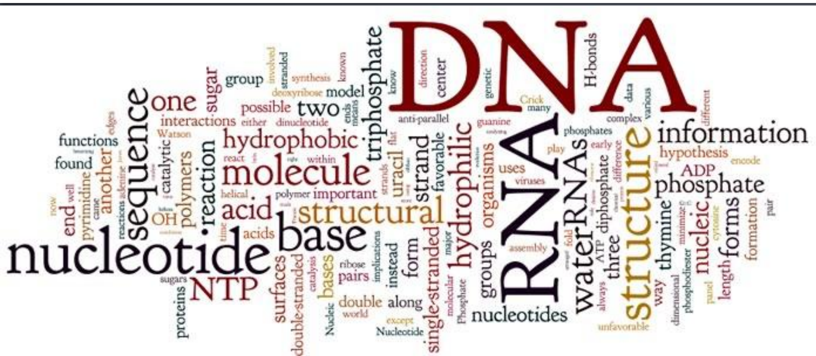
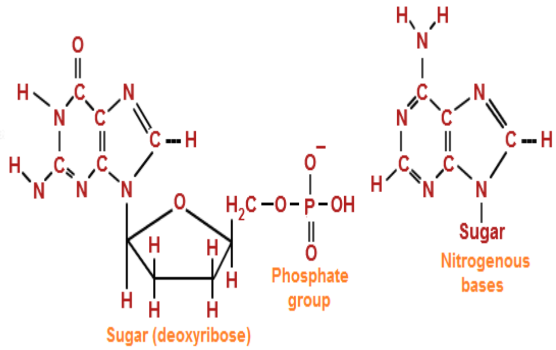
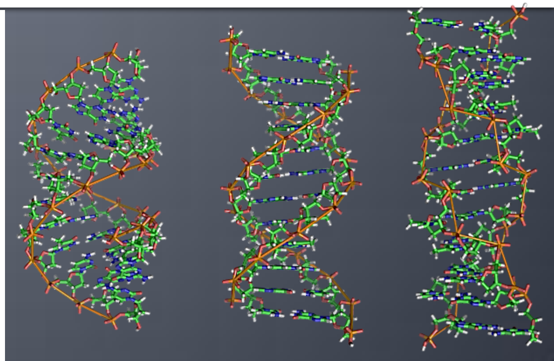
# Nucleic Acids



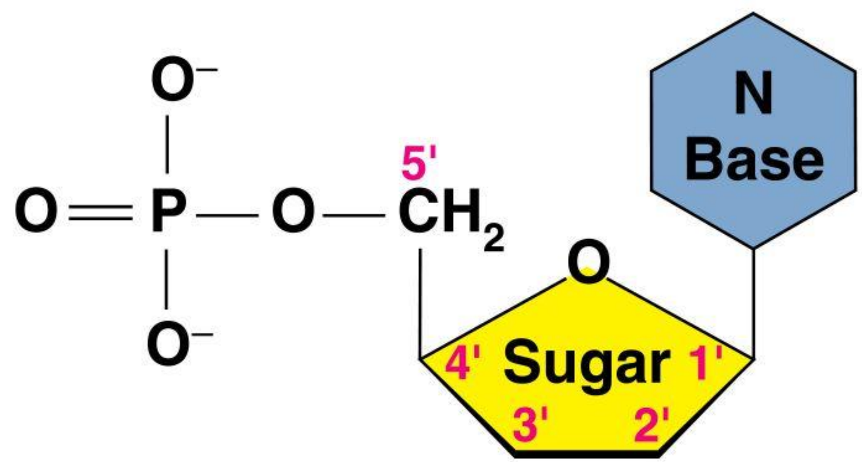



# LECTURE OUTLINE

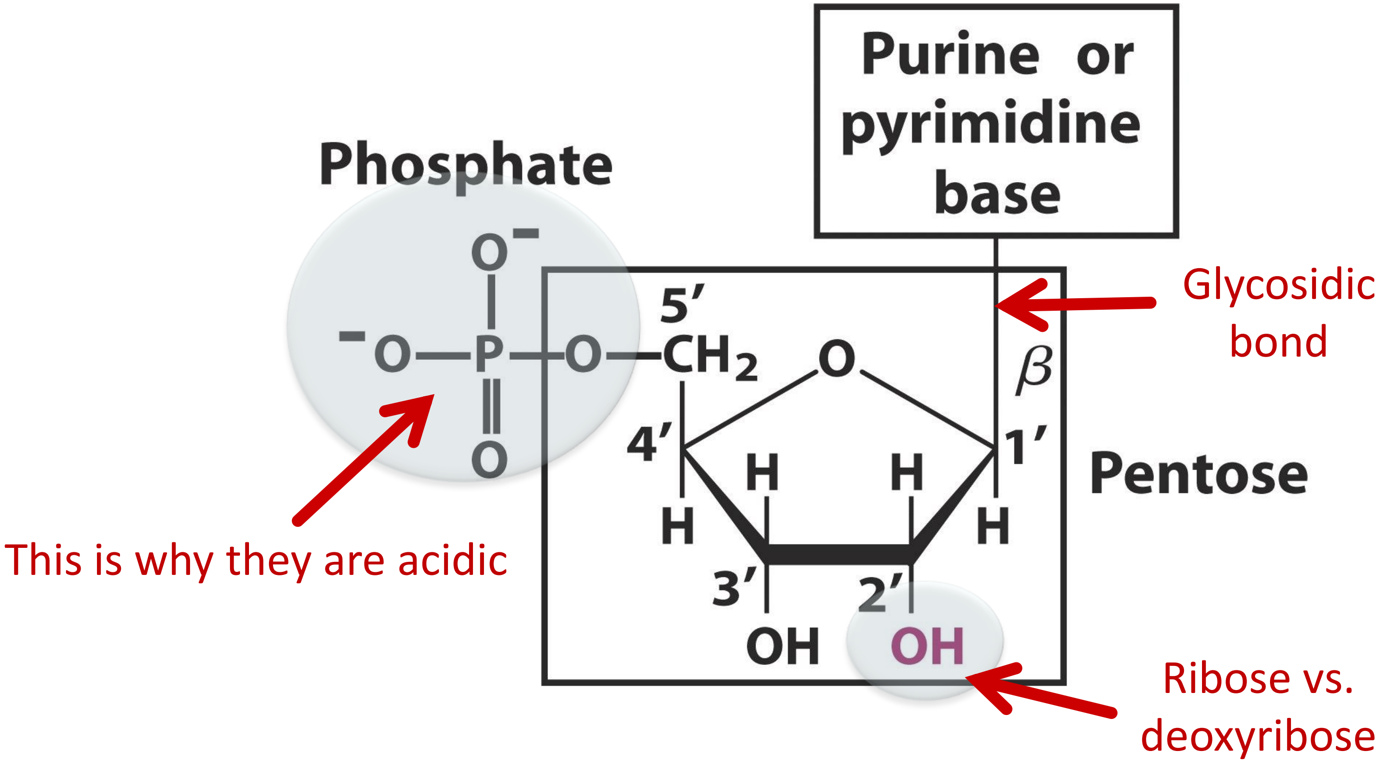
- I. Hierarchical structure of nucleic acids
- II. Structures of nucleotides
  - A. Purines & pyrimidines
  - B. Nucleosides & nucleotides
  - C. Phosphodiester bonds
- III. DNA structure
  - A. The double helix
    - 1. Strand complementarity
    - 2. Major & minor grooves
  - B. Conformational variations
    - 1. A-, B-, and Z-DNA
    - 2. Base stacking & propeller twists
  - C. Supercoiling
    - 1. Prokaryotic supercoiling – topoisomerases & gyrase
    - 2. Eukaryotic supercoiling – chromatin, histones, nucleosomes
  - D. DNA denaturation
- IV. RNA structures & functions
  - A. Sequence dependence on DNA
  - B. Transfer RNA
  - C. Ribosomal RNA
  - D. Messenger RNA
  - E. Small nuclear RNA
  - F. RNA interference

# Nucleic Acids

- Molecules that store information for cellular growth & reproduction
- Biopolymers containing three types of structures in each monomer unit (nucleotides)
  - ✓ A nitrogenous base derived from purine or pyrimidine (nucleobases)
  - ✓ A monosaccharide (pentose), either D-ribose or 2-deoxy-D-ribose
  - ✓ Phosphoric acid
- RNA (Ribonucleic Acid)
  - (throughout the cell)
- DNA (Deoxyribonucleic Acid)
  - (nucleus & mitochondria)

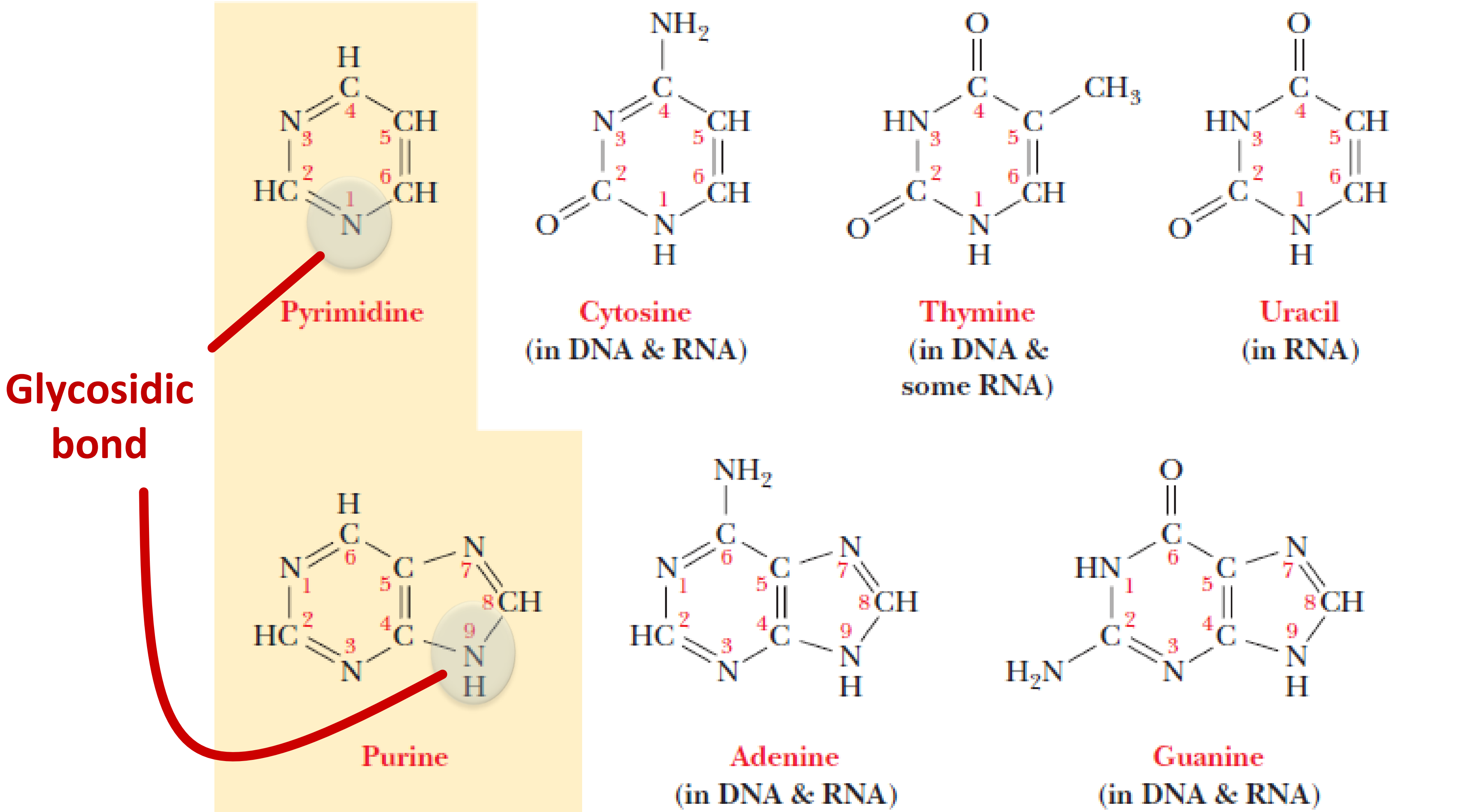


# Chemical composition & bonds

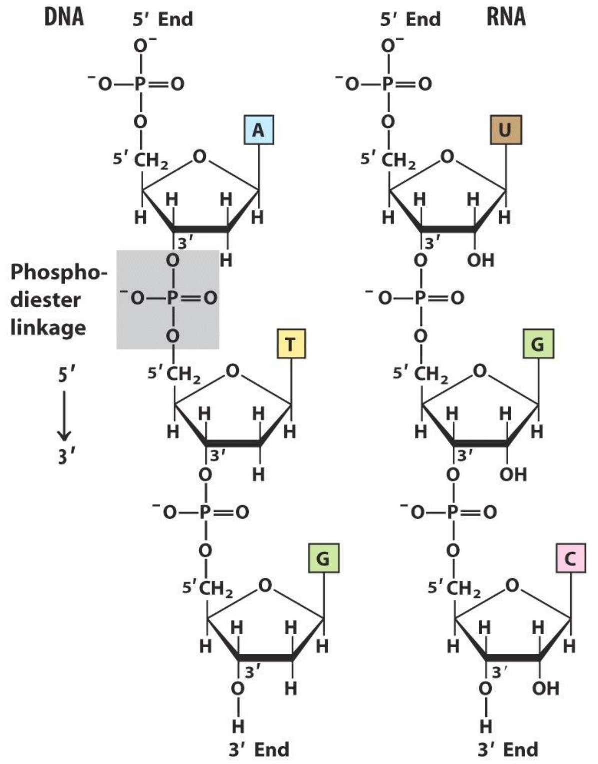



- ❑ Positively charged ions (Na+ or Mg2+) & peptides with positively charged side chains can associate with DNA
- ❑ Eukaryotic DNA, for example, is complexed with histones (positively charged proteins), in the cell nucleus

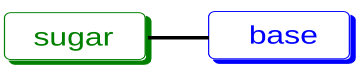
# Nitrogenous bases



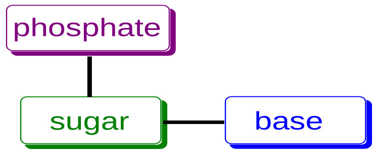
# Nucleoside, nucleotides & nucleic acids



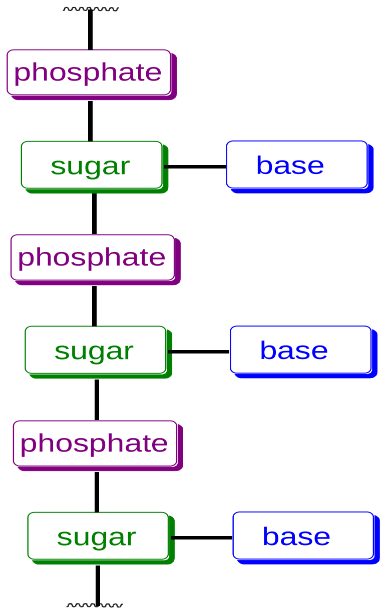

- A nucleoside: N-base linked by a β-glycosidic bond to C1′ of a ribose or deoxyribose
- Nucleosides naming: *-osine* for purines & –*idine* for pyrimidines
- A nucleotide: a nucleoside phosphoric acid esters (C5′ OH of sugar)
- Nucleotides naming: nucleoside followed by *5′-monophosphate (ylate)*



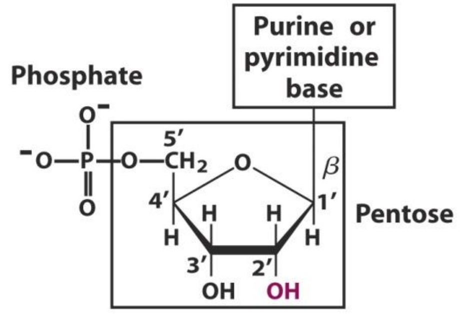

nucleoside




nucleotides




nucleic acids




✓ The chemical linkage between monomer units in nucleic acids is a phosphodiester

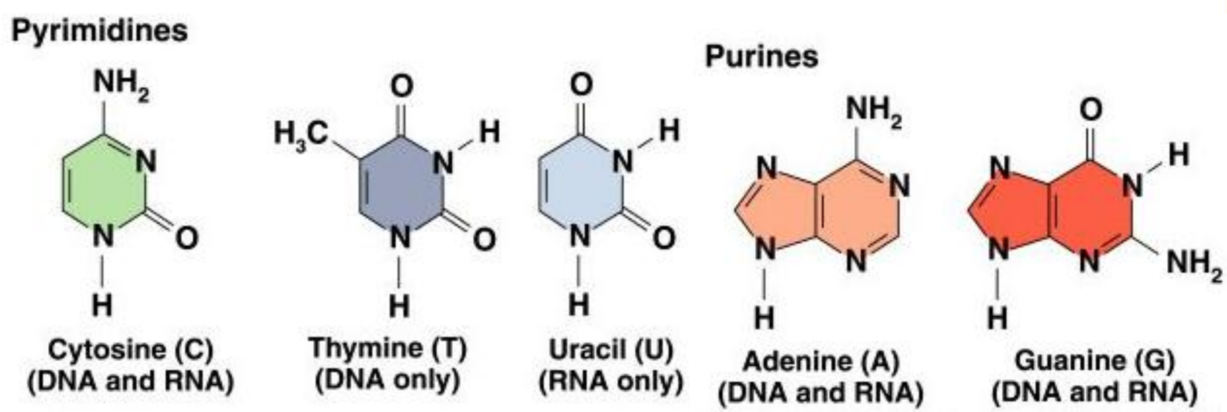
# Nucleotides vs. Nucleosides

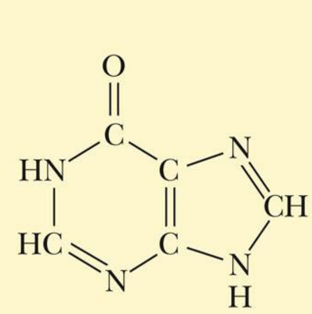
**TABLE 2-2** Terminology of Nucleosides and Nucleotides

| | | Bases | | | |
|---|---|---|---|---|---|
| | | Purines | | Pyrimidines | |
| | | **Adenine** (A) | **Guanine** (G) | **Cytosine** (C) | **Uracil** (U)<br>**Thymine** [T] |
| Nucleosides | in RNA | Adenosine | Guanosine | Cytidine | Uridine |
| | in DNA | Deoxyadenosine | Deoxyguanosine | Deoxycytidine | Deoxythymidine |
| Nucleotides | in RNA | Adenylate | Guanylate | Cytidylate | Uridylate |
| | in DNA | Deoxyadenylate | Deoxyguanylate | Deoxycytidylate | Deoxythymidylate |
| Nucleoside monophosphates | | AMP | GMP | CMP | UMP |
| Nucleoside diphosphates | | ADP | GDP | CDP | UDP |
| Nucleoside triphosphates | | ATP | GTP | CTP | UTP |
| Deoxynucleoside mono-, di-, and triphosphates | | dAMP, etc. | | | |

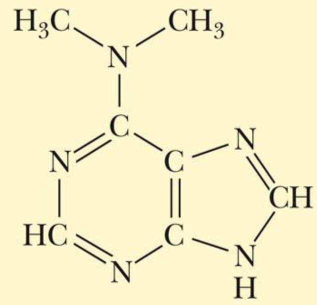
# Nitrogen Bases

- Two general types:
  - ✓ Purines: adenine (A) & guanine (G)
  - ✓ Pyrimidines: cytosine (C), thymine (T) & Uracil (U)
- Less common bases can occur
- Principally but not exclusively, in transfer RNAs

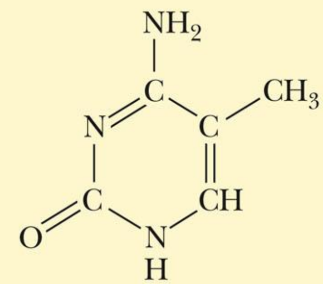


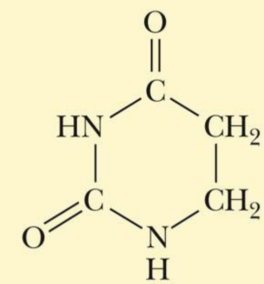
Hypoxanthine



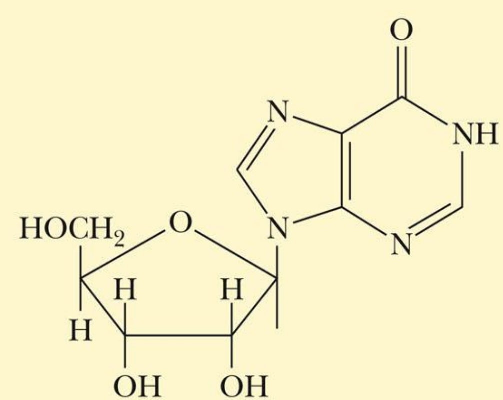
$N^6$-Dimethyladenine



5-Methylcytosine



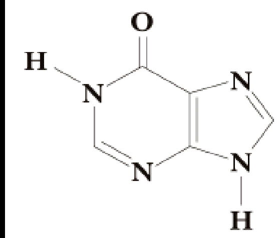
5,6-Dihydrouracil



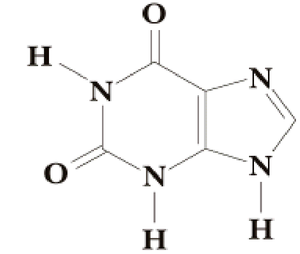
Inosine, an uncommon nucleoside

# Other nucleotides

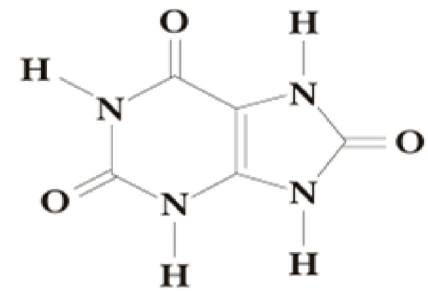
- Xanthine, hypoxanthine & uric acid: intermediates in purine metabolism
- $N_6$-methyl adenine
- 5-methyl-cytosine & $N_4$-methyl cytosine
- Pseudouracil: has the ribose attached to $C_5$ ($N_1$) of uracil (Pseudouridine)
- 1,3,7-trimethylxanthine (caffeine)



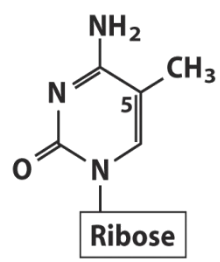
Hypoxanthine



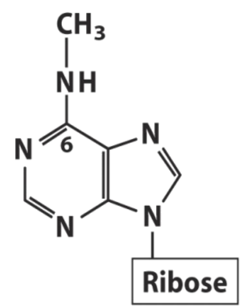
Xanthine



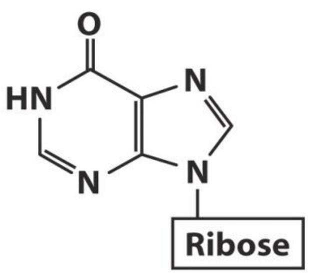
Uric acid



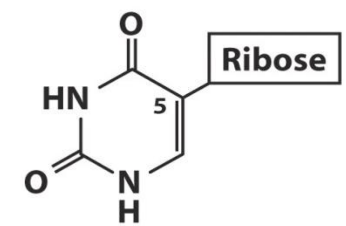
5-Methylcytidine



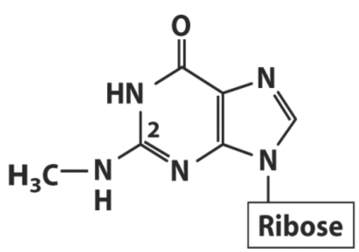
*N*$^6$-Methyladenosine



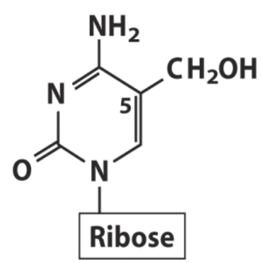
Inosine



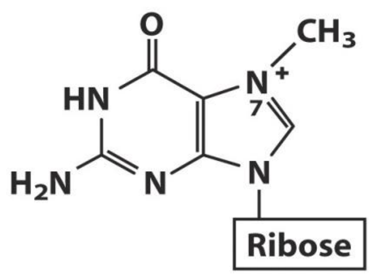
Pseudouridine



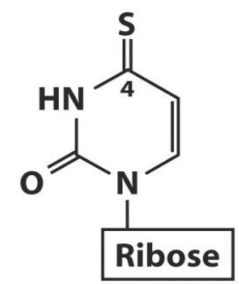
*N*$^2$-Methylguanosine



5-Hydroxymethylcytidine

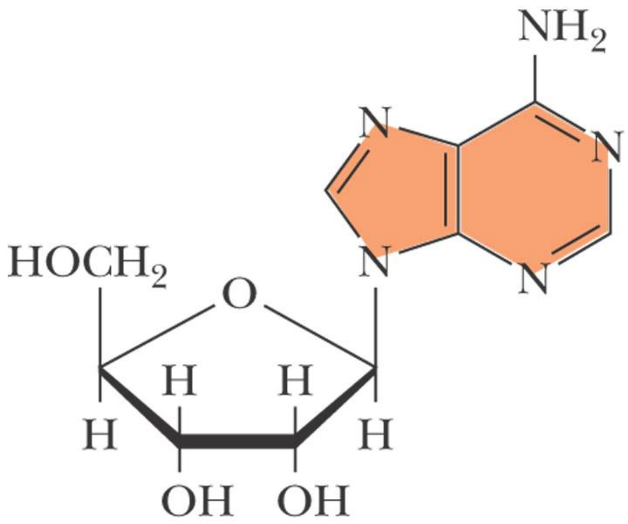


7-Methylguanosine

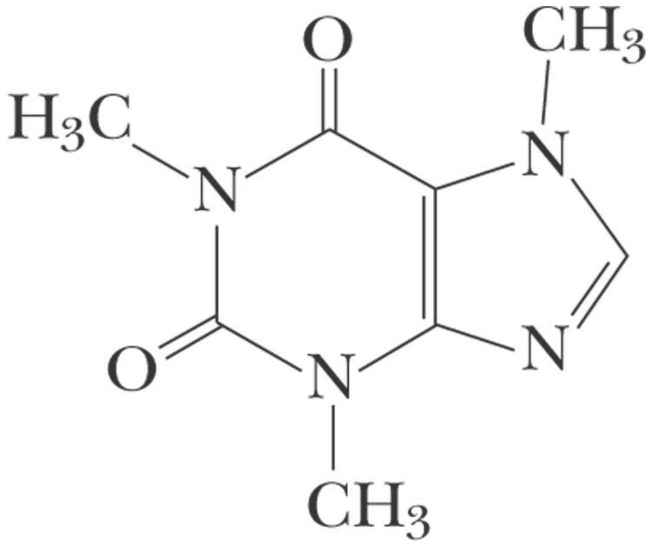


4-Thiouridine

# Adenosine: a nucleoside with physiological activity




**Adenosine**

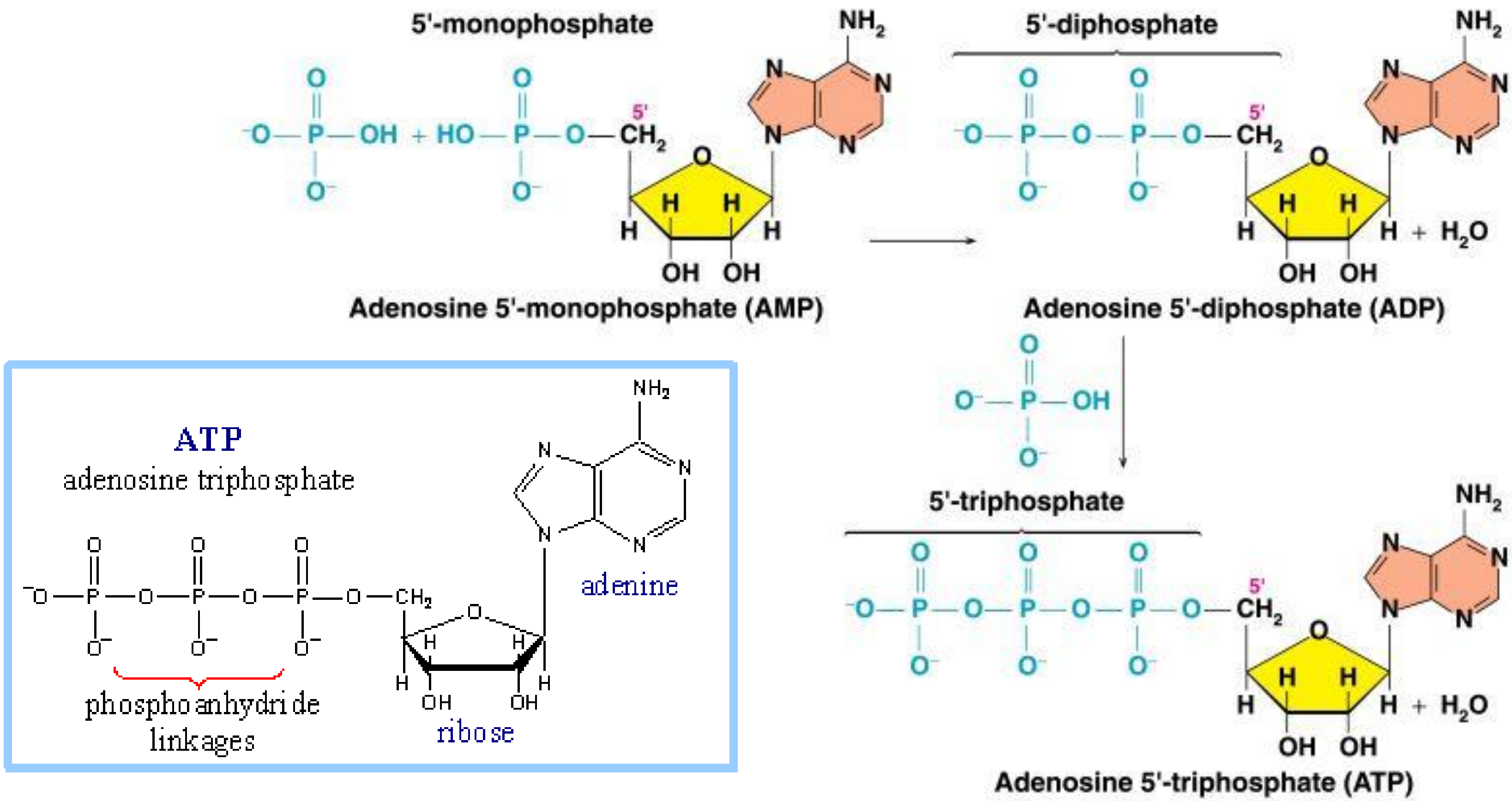



**Caffeine**

- ✓ **High [Ado] promotes sleepiness. Caffeine blocks the interaction of extracellular Ado with its neuronal receptors**

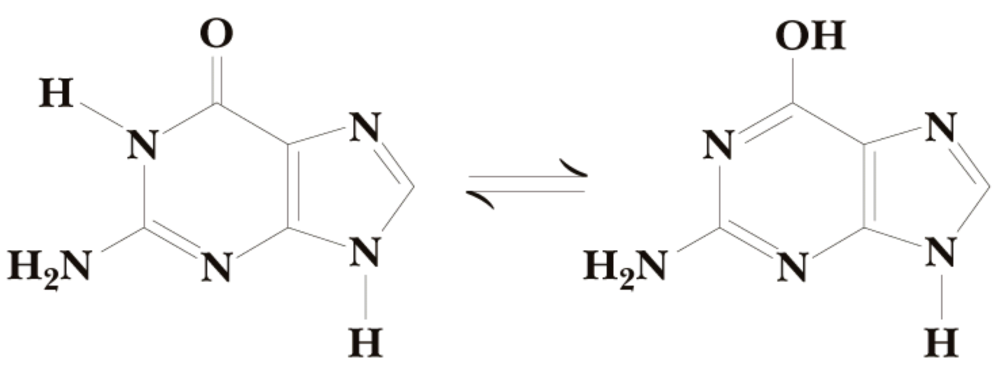
# AMP, ADP & ATP

- Additional phosphate groups can be added to the nucleoside 5'-monophosphates to form diphosphates & triphosphates
- ATP is the major energy source for cellular activity

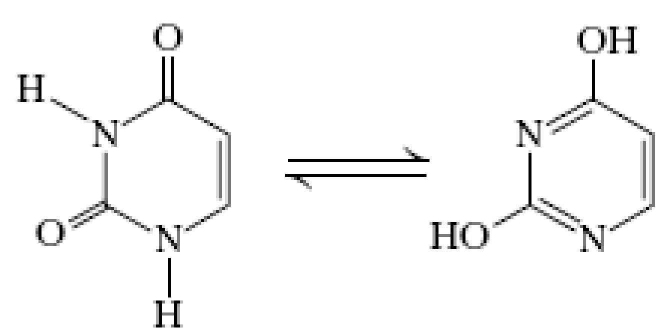


# Properties of Pyrimidines & Purines

- 1. Keto-enol tautomerism:
  - ✓ Tautomers are constitutional isomers of organic compounds that readily interconvert by a chemical reaction
  - ✓ Commonly: migration of a hydrogen atom/proton, accompanied by a switch of a single bond & adjacent double bond
  - ✓ The keto tautomer (lactam), whereas the enol form (lactim)
  - ✓ lactam form vastly predominates at neutral pH (pKa values for ring nitrogen atoms 1 & 3 in uracil are greater than 8)



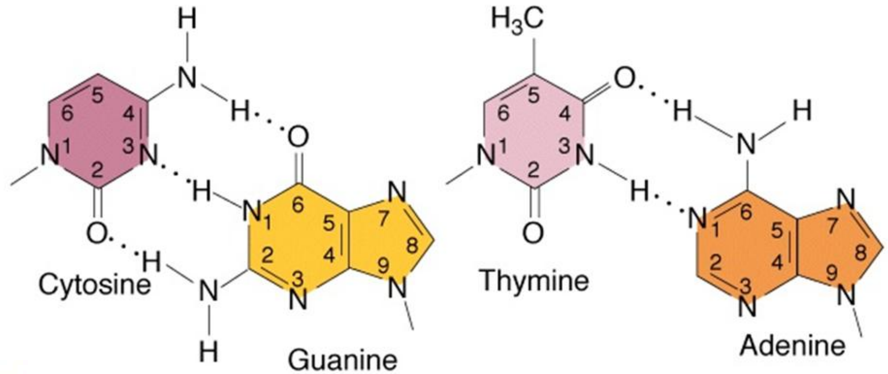
Keto form    Enol form

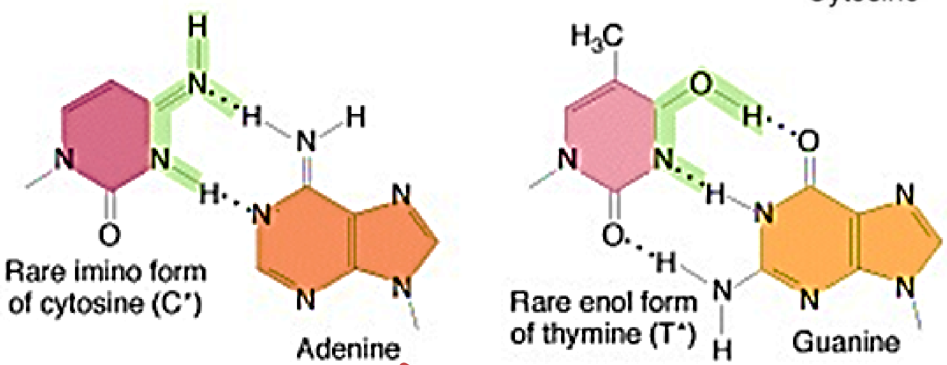


Lactam    Lactim

# Tautomeric shift mutation

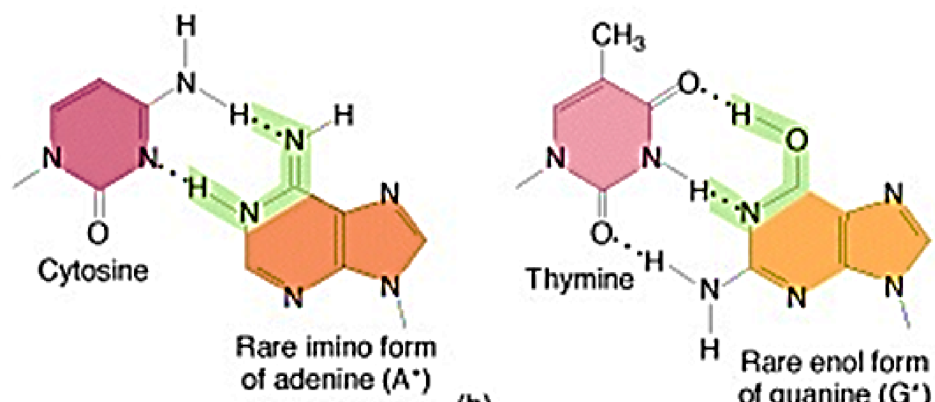
- Normal DNA - most bases are in keto form




(a)

- Rare: enol or imino forms;
  - Tend to cause mispairing



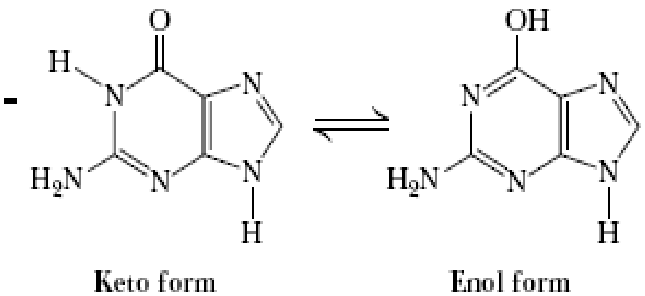

(b)

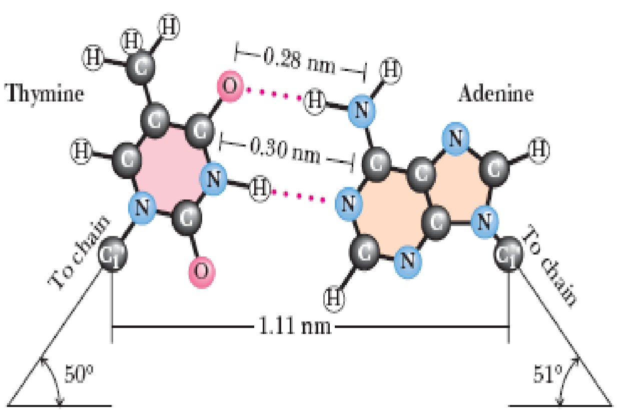
# Properties of Pyrimidines & Purines

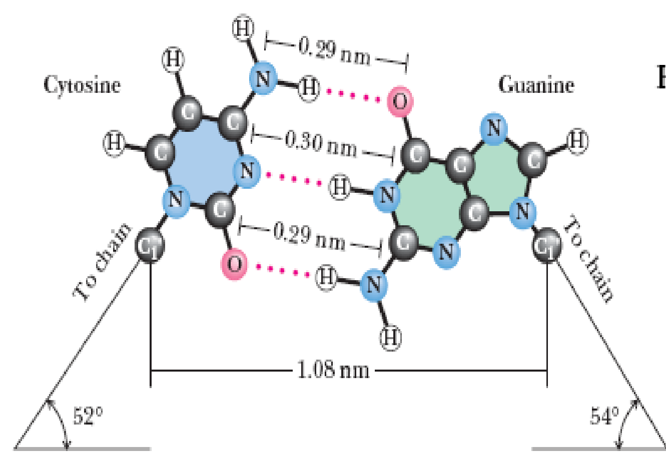
**Proton Dissociation Constants (p$K_a$ Values) for Nucleotides**

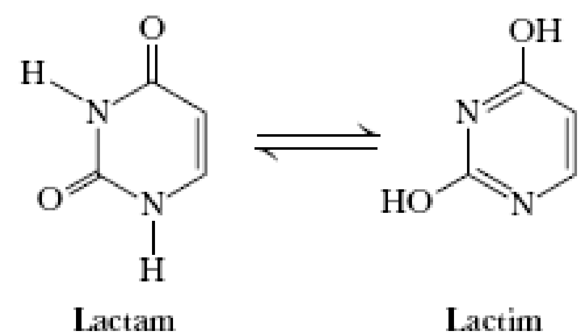
| Nucleotide | p$K_a$ Base-N | p$K_1$ Phosphate | p$K_2$ Phosphate |
|---|---|---|---|
| 5′-AMP | 3.8 (N-1) | 0.9 | 6.1 |
| 5′-GMP | 9.4 (N-1) | 0.7 | 6.1 |
| | 2.4 (N-7) | | |
| 5′-CMP | 4.5 (N-3) | 0.8 | 6.3 |
| 5′-UMP | 9.5 (N-3) | 1.0 | 6.4 |

- 2. Acid/base dissociations:
  - ✓ E.g; Uracil, Cytosine, Guanine
- Important in determining if nitrogens are H-bond donors/ acceptors (double helix formation)
- Important functional groups participating in H-bond formation:
  - ✓ Amino groups, Ring Ns, Os



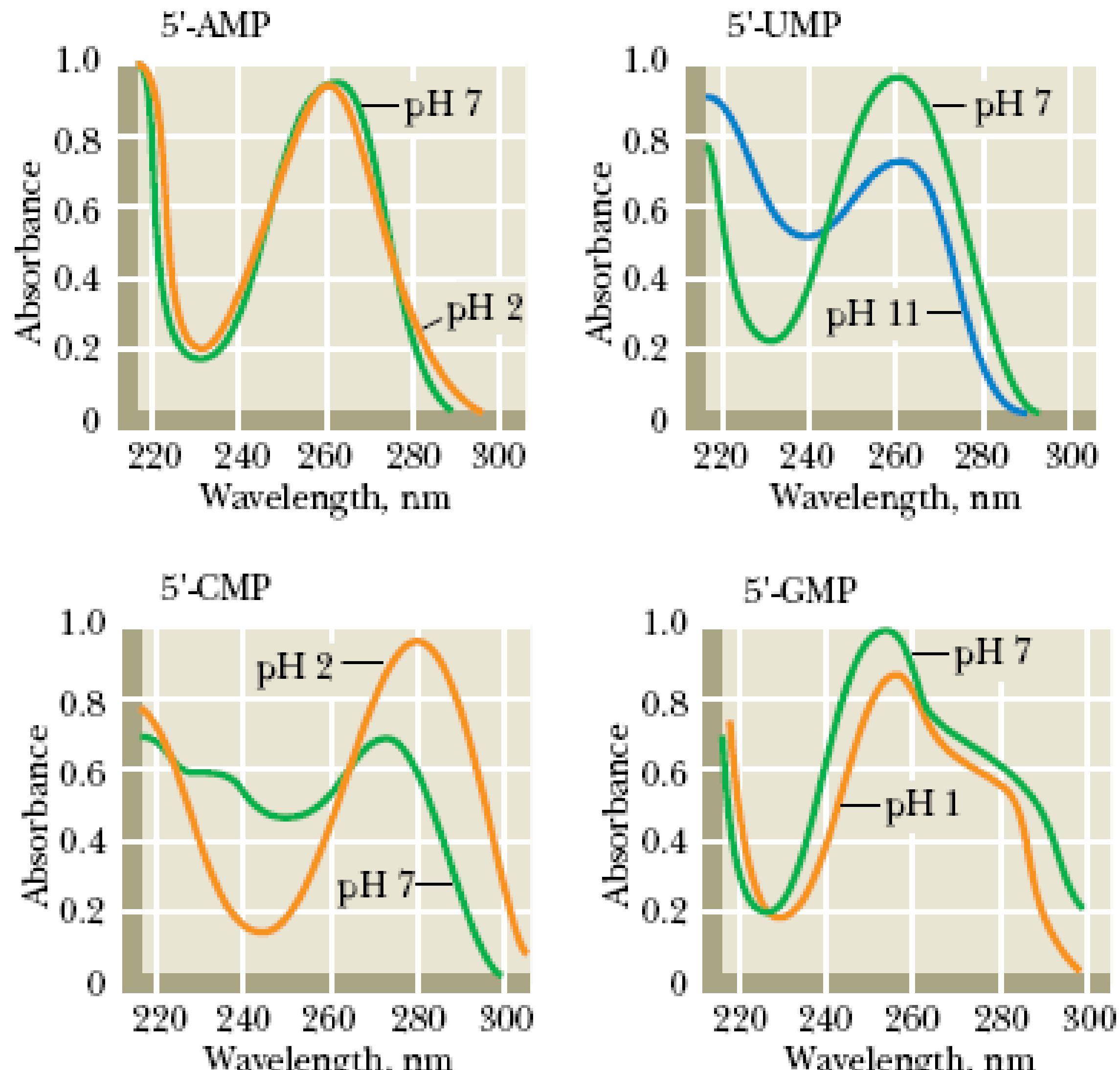



# Properties of Pyrimidines & Purines

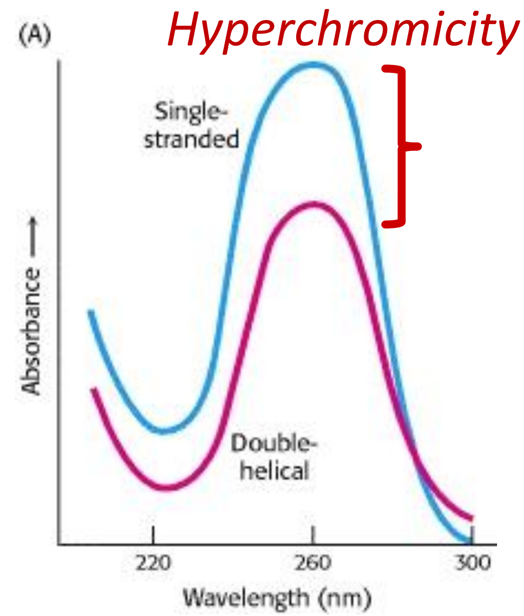
- 3. Strong absorbance of UV light:
  - ✓ A consequence of being aromatic
  - ✓ Particularly useful in quantitative & qualitative analysis of nucleotides & nucleic acids



# Light absorbance of nucleic acids

- **The peak absorbance is at 260 nm wavelength & it is constant**

| $A_{260}$ of 1.0 | dsDNA | ssDNA | ssRNA |
|---|---|---|---|
| | 50 ug/ml | 30 ug/ml | 40 ug/ml |



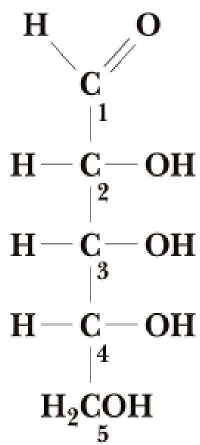

**Stacked bases vs. unstacked bases**

- **What is the concentration of a double stranded DNA sample diluted at 1:10 and the $A_{260}$ is 0.1?**

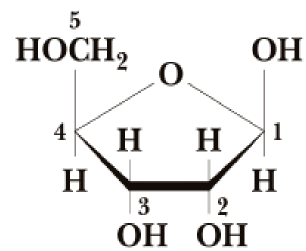
DNA concentration = 0.1 x 10 x 50 µg/ml
= 50 µg /ml

# Pentoses of Nucleotides

- D-ribose (RNA) &
  2-deoxy-D-ribose (DNA)
- Sugars increase solubility (compared to free bases)
- The position of the carbohydrate is followed by a ´ (prime)
- Stereochemistry is β

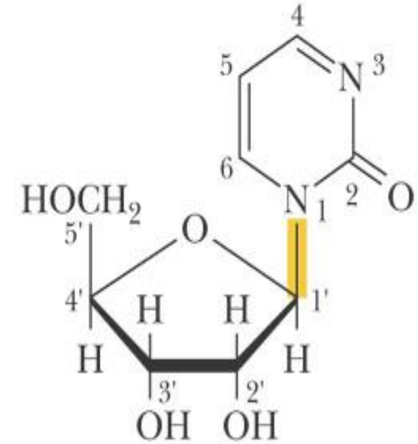


D-Ribose

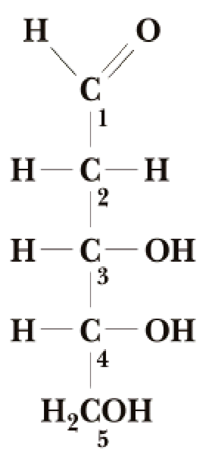


Furanose form of D-Ribose

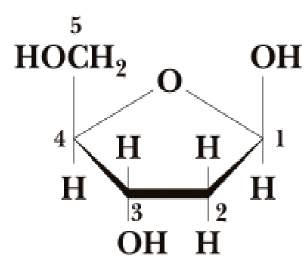
β-D-Ribofuranose



$\beta$-$N_1$-glycosidic bond in pyrimidine ribonucleosides

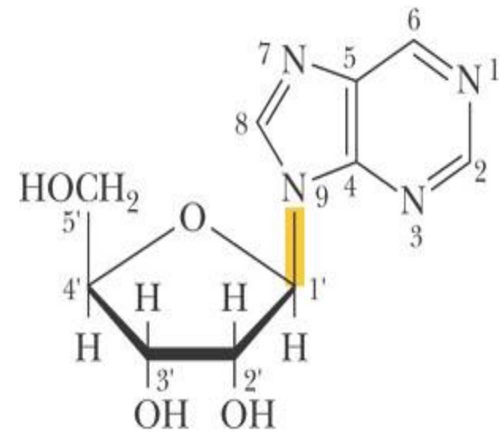


D-2-Deoxyribose



Furanose form of D-2-Deoxyribose

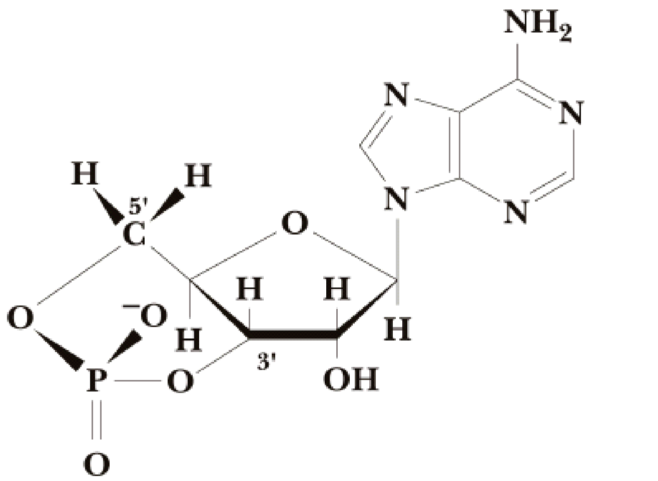
β-D-2-Deoxyribofuranose



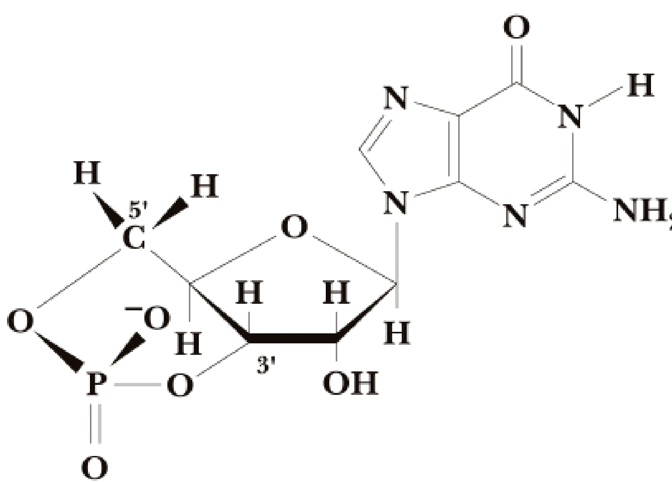
$\beta$-$N_9$-glycosidic bond in purine ribonucleosides

# Functions of Nucleotides

- Nucleoside 5'-triphosphates are <u>carriers of energy</u>
- <u>Cyclic nucleotides are signal molecules & regulators of cellular metabolism & reproduction</u>
- ATP is central to energy metabolism
- GTP drives protein synthesis
- CTP drives lipid synthesis
- UTP drives carbohydrate metabolism



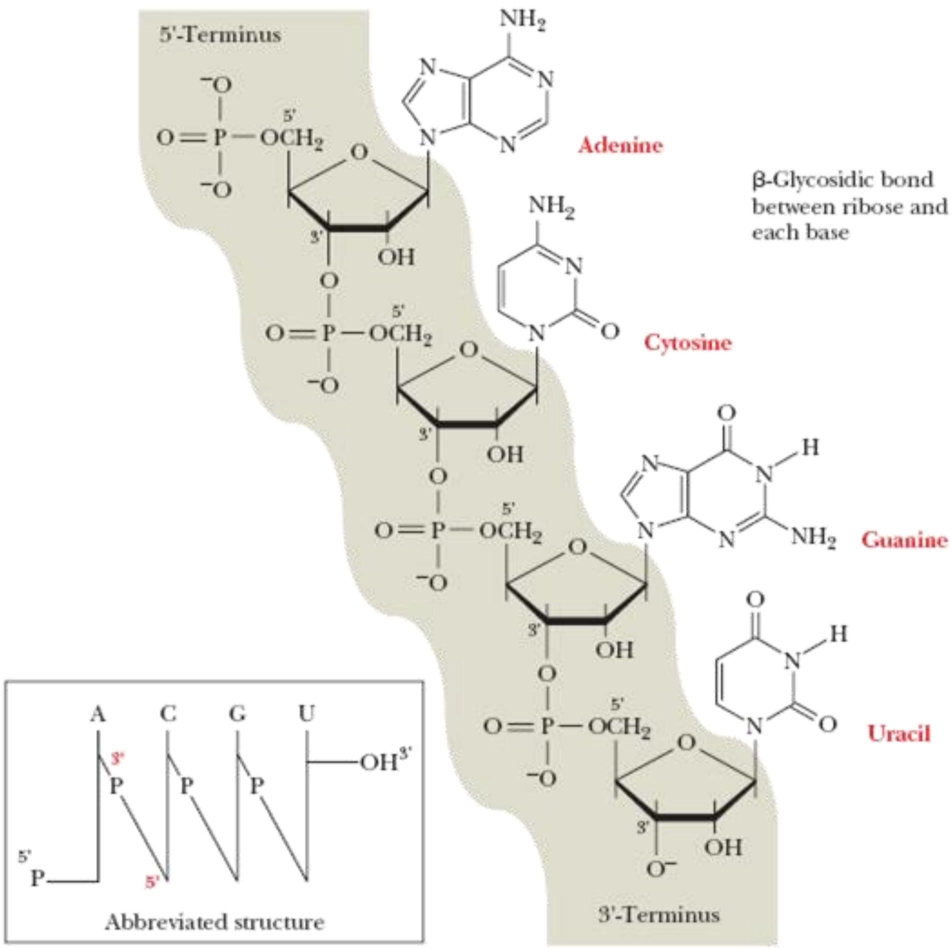
3',5'-Cyclic AMP

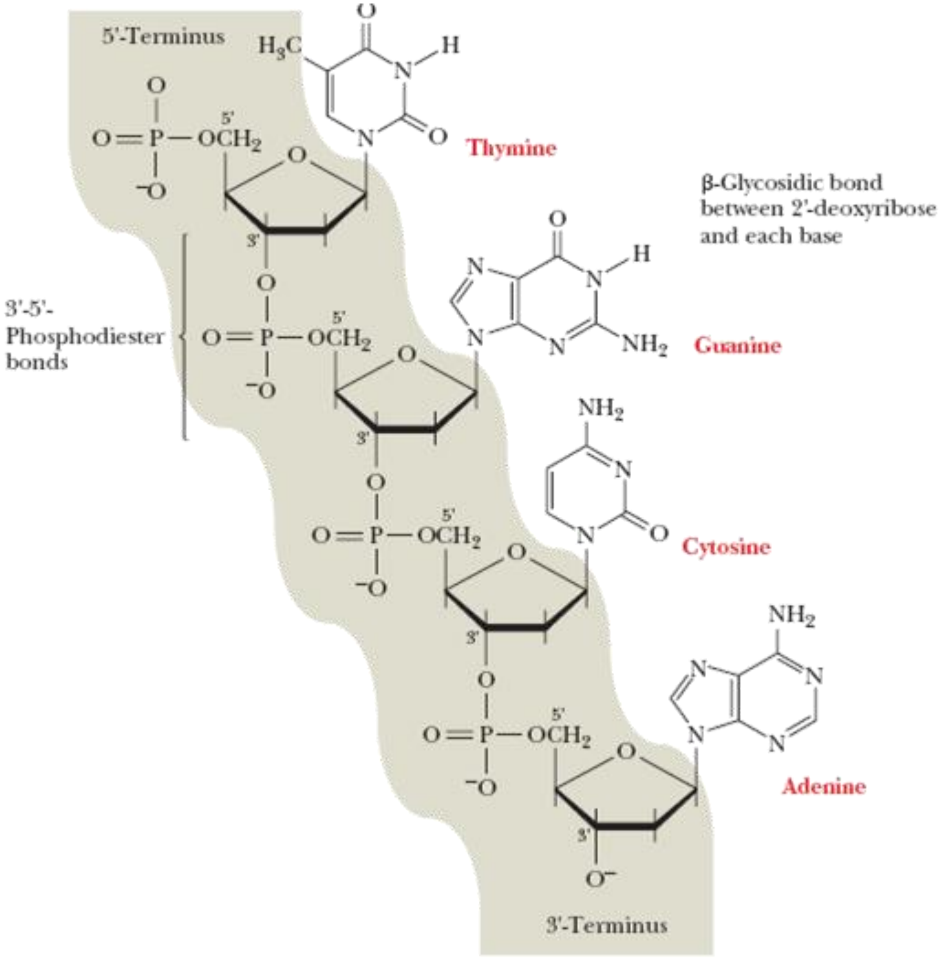


3',5'-Cyclic GMP

# Polymerization

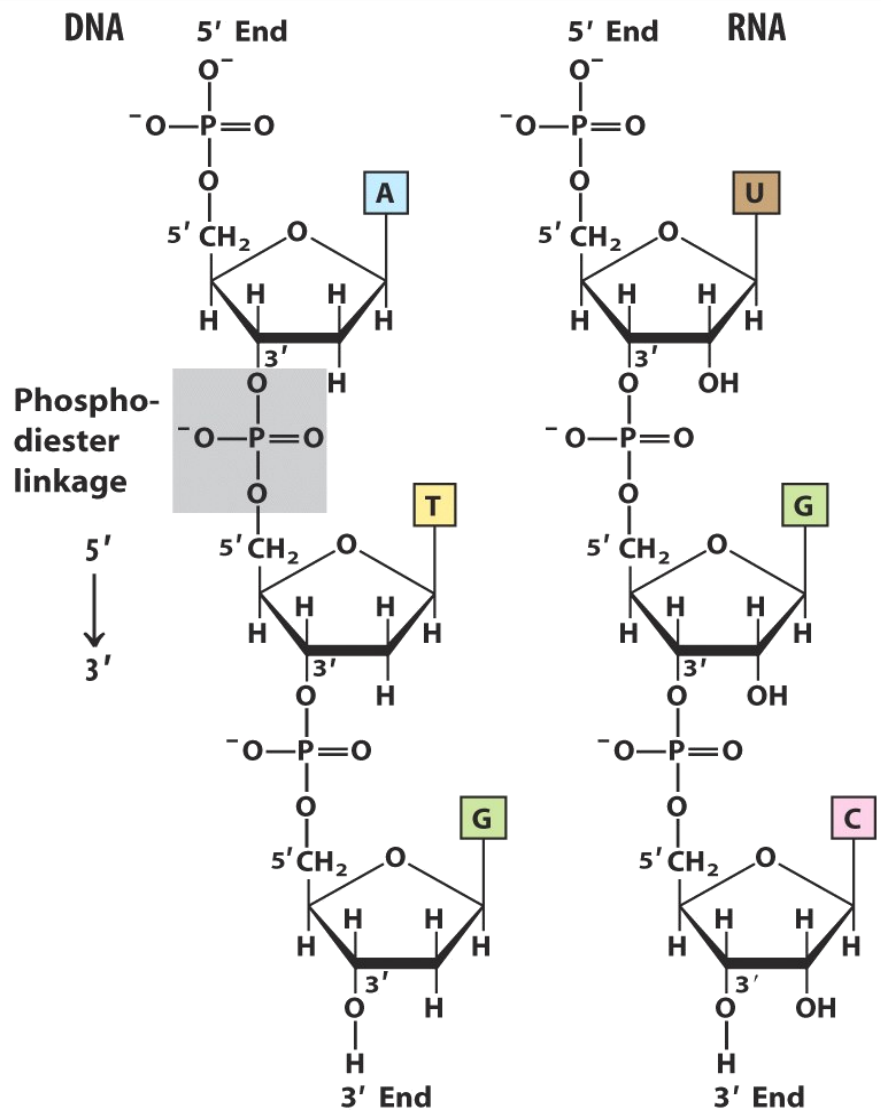
- **Leads to nucleic acids. Linkage is repeated (3′,5′-phosphodiester bond)**



# Phosphodiesters, Oligonucleotides, & Polynucleotides

- Phosphodiester bond: connects the 5′-hydroxyl group of one nucleotide to the 3′-hydroxyl group of the next one
- Formed by Polymerase & Ligase activities
- Phosphate pKa ≈ 0?
- Nucleic acids are negatively charged

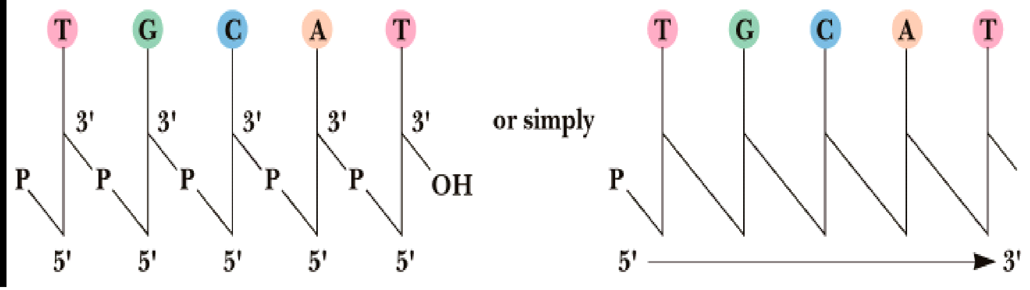


# Classes of Nucleic Acids?

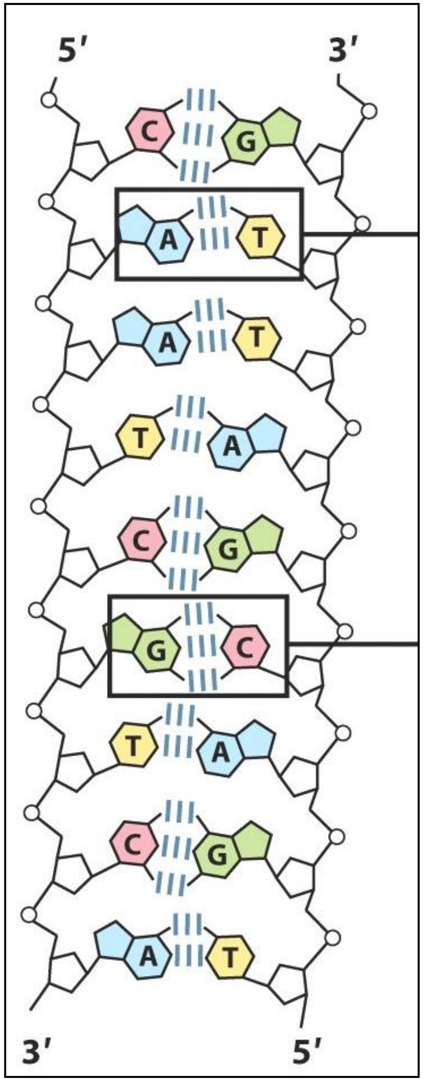
- DNA - one type, one purpose:
  - ✓ A single DNA molecules in virus and bacteria
  - ✓ Eukaryotic cells have many diploid chromosomes mainly in nucleus, but also mitochondria & chloroplasts

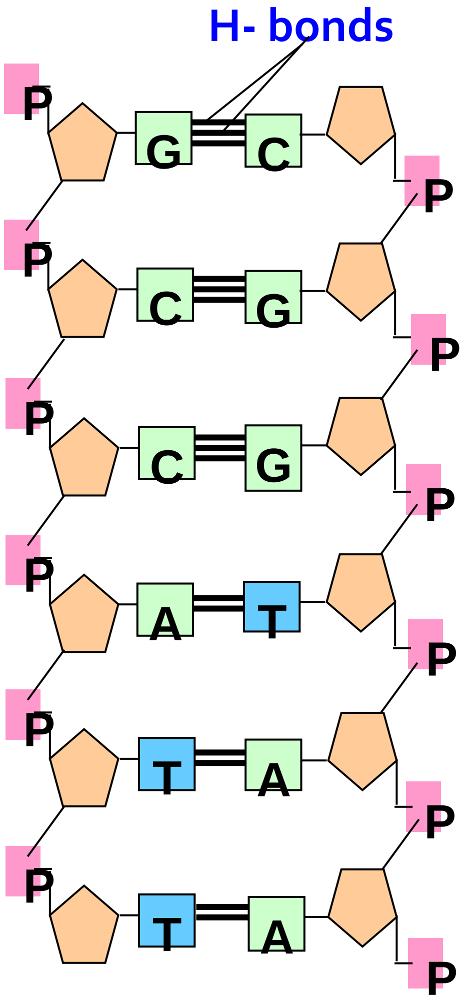
- RNA - 3 (or 4) types, 3 (or 4) purposes
  - ✓ Ribosomal RNA - the basis of structure & function of ribosomes
  - ✓ Messenger RNA - carries the message
  - ✓ Transfer RNA - carries the amino acids
  - ✓ Small nuclear RNA
  - ✓ Small non-coding RNAs

# DNA structure



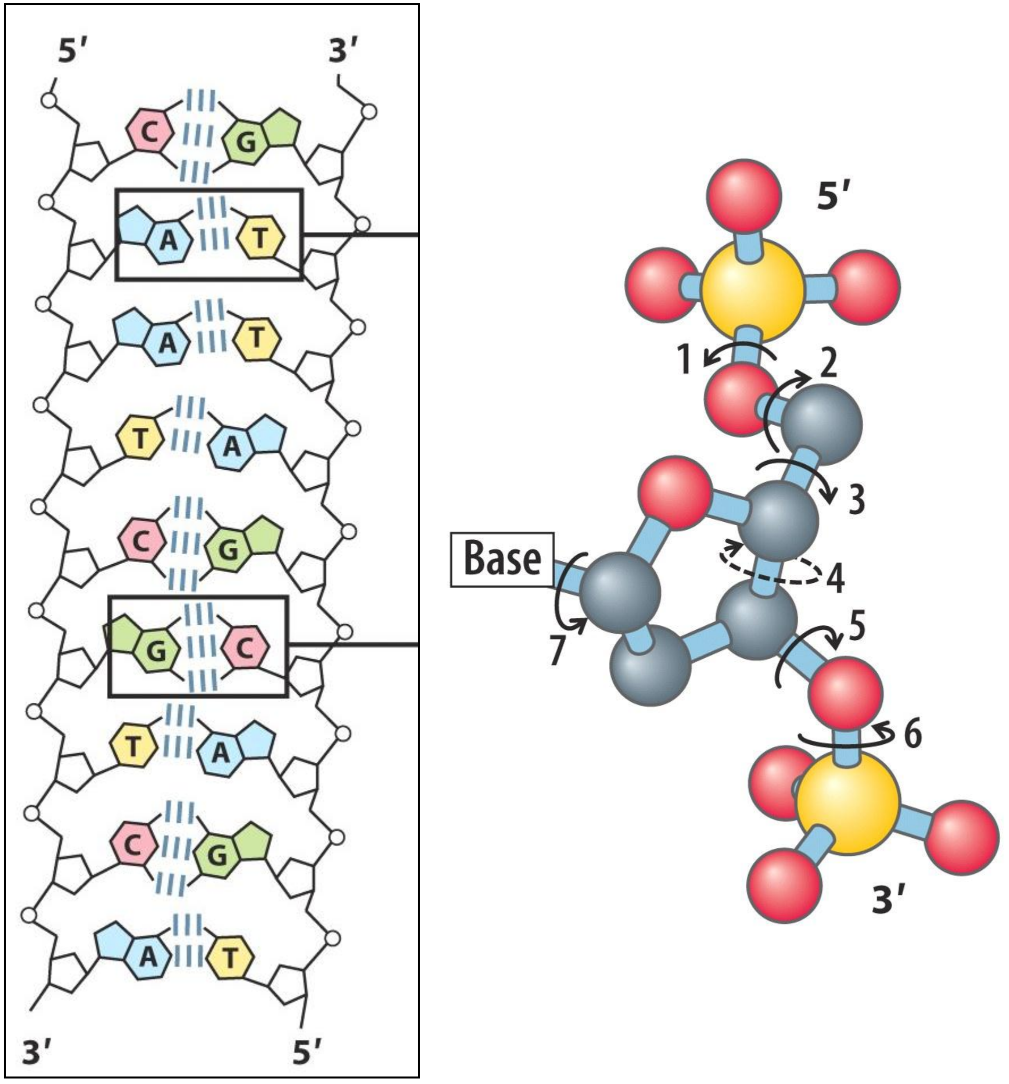

- Diameter: 2 nm
- Length: 1.6 million nm (*E. coli*)
  - Compact and folded (*E. coli* cell is only 2000 nm long)
- Antiparallel double helix
- Backbone vs. side chains
- Specific base-pairing
  - ✓ Chargaff's rules (A=T & C=G)
- Strands are joined by the bases (complementary)
- Stable (H-bonds)



# DNA structure – Stability vs. Flexibility

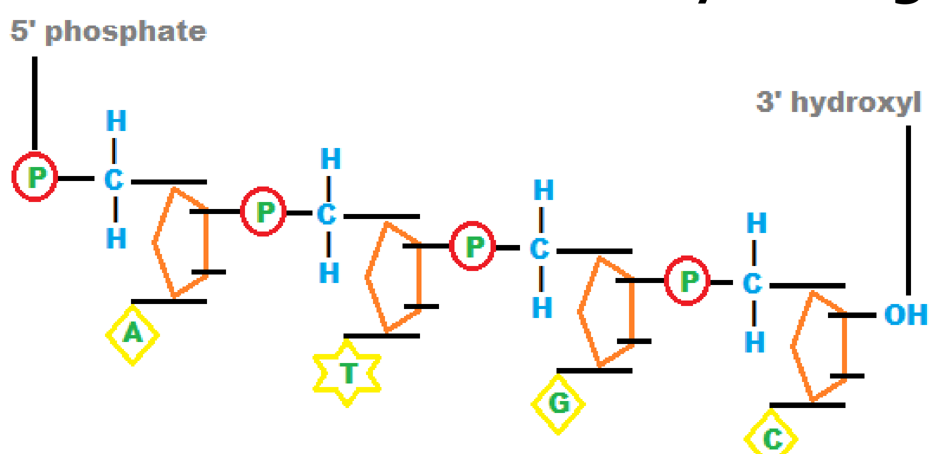
- **DNA Backbone Flexibility:**
  - **Multiple Degrees of Rotational Freedom**



# DNA Structural Levels

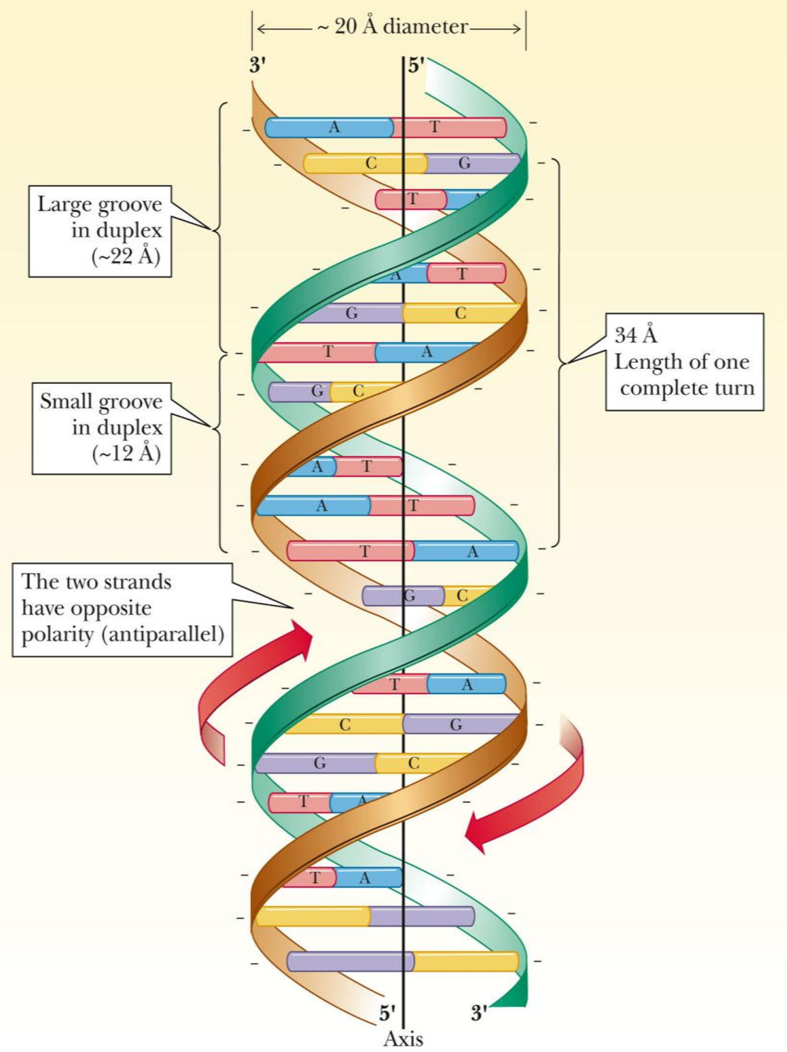
# DNA - 1° Structure

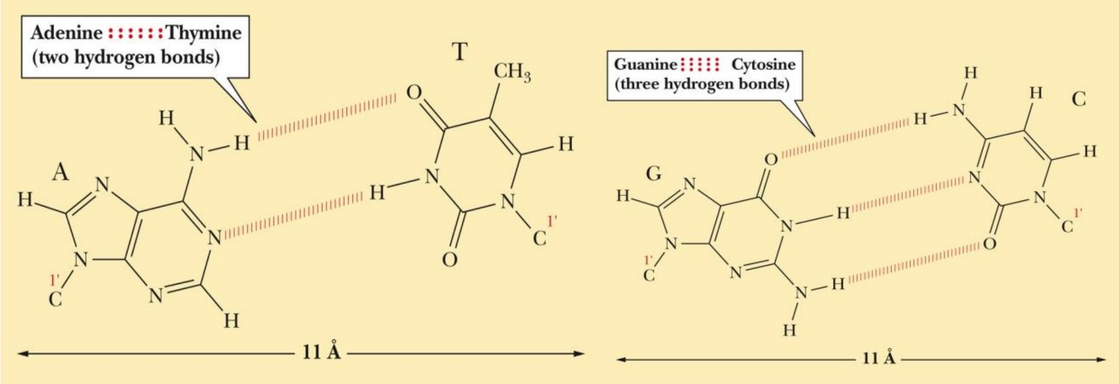
- A biopolymer that consists of a backbone of alternating units of 2-deoxy-D-ribose and phosphate
- Primary Structure: the sequence of bases along the pentose-phosphodiester backbone of a DNA molecule
  - ✓ By convention: left → right, & 5′-end → 3′-end
  - ✓ System of notation single letter (A,G,C, & T)
  - ✓ More abbreviated notations: The deoxy analogue d(GACAT)



# DNA - 2° Structure

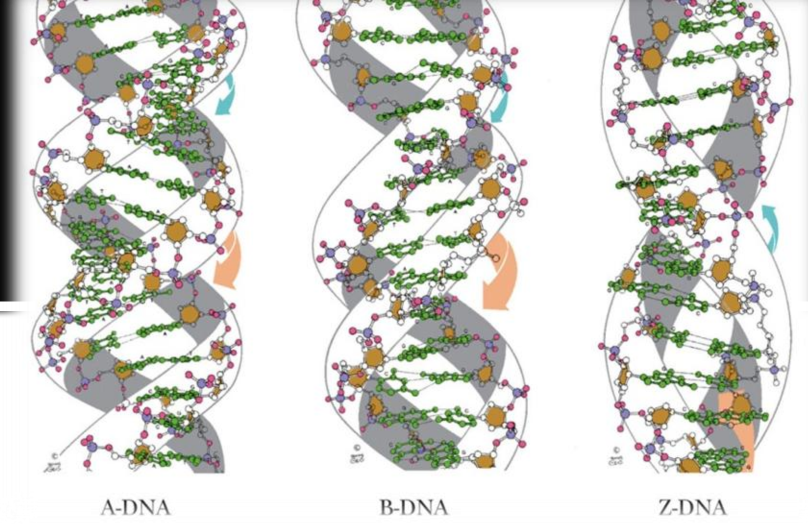
- Secondary structure: the ordered arrangement of nucleic acid strands
- Double helix model (James Watson and Francis Crick):
  - Two antiparallel strands
  - Coiled in a right-handed helix
- Base Pairing:
  - T-A (2 H-bonds)
  - G-C (3 H-bonds)
- Minor vs. major grooving

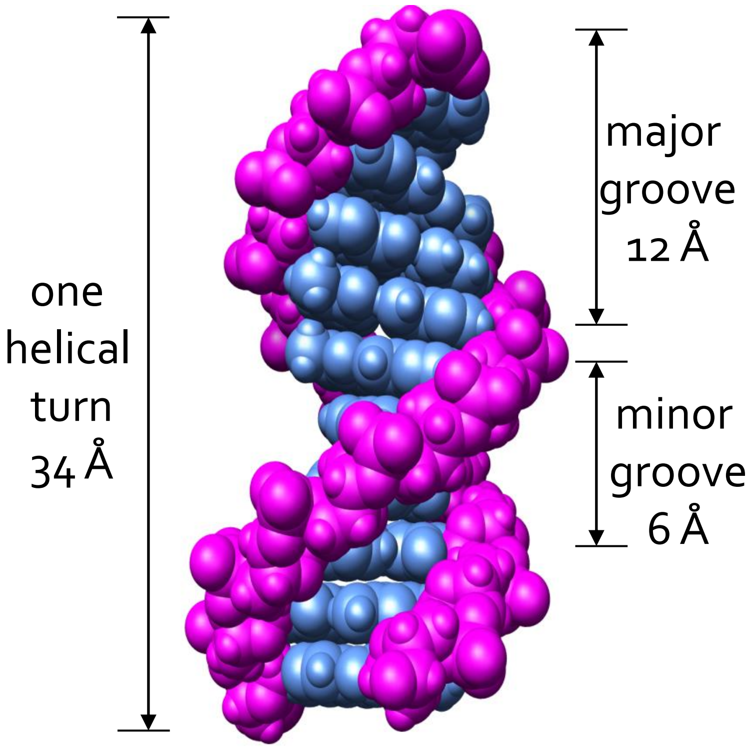


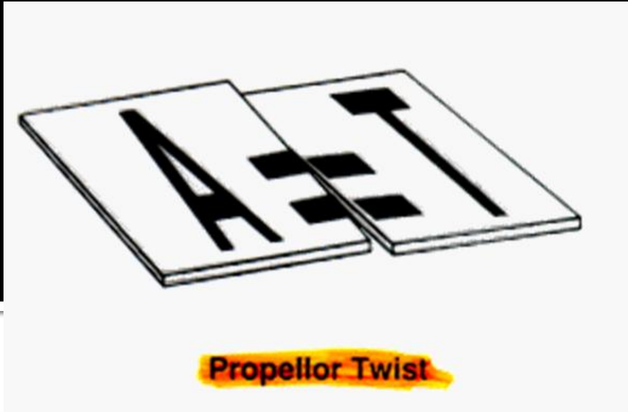
# Forms of DNA

- B-DNA
  - Considered the <u>physiological form</u>
  - <u>Right-handed</u> helix, diameter 11 Å
  - 10 base pairs per turn (34Å)
- A-DNA
  - Right-handed helix
  - Thicker (11 base pairs per turn) & wider
  - Has not been found *in vivo*
- Z-DNA
  - Left-handed helix
  - May play a role in gene expression
  - Narrower than B-DNA



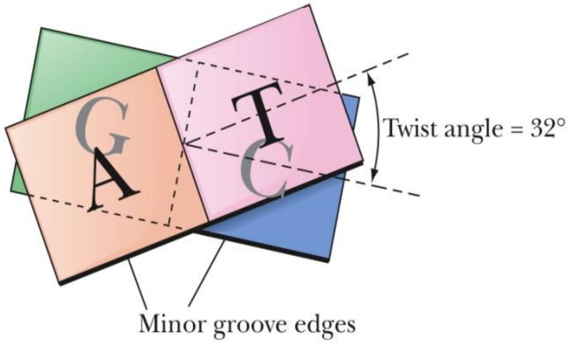



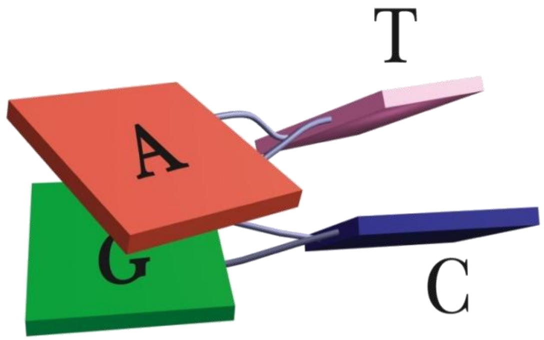
# Features of DNA




- Base stacking
  - Bases are very nearly planar, hydrophobic & interact by hydrophobic interactions
  - In B-DNA, each base rotated by 32° compared to the next (base pairing vs. maximum overlap)
  - Bases exposed to the minor groove come in contact with water
  - Many bases adopt a *propeller-twist* in which base pairing distances are less optimal but base stacking is more optimal & water is eliminated from minor groove contacts

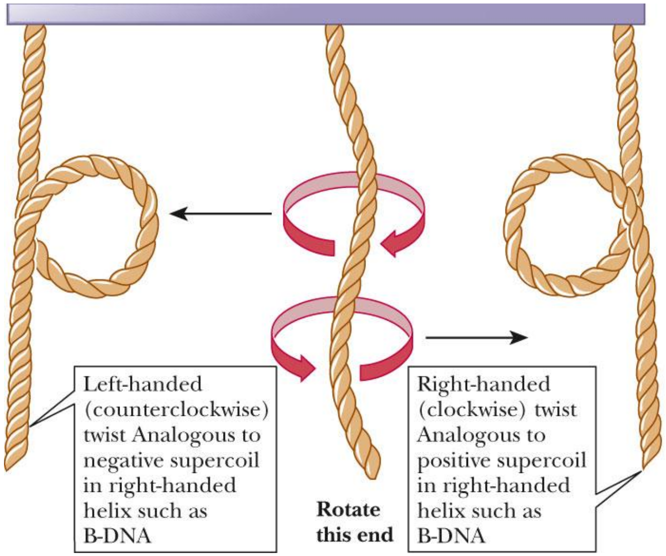
| Dinucleotide pair (5′–3′)–(3′–5′) | Stacking energy per stacked pair / kJ $mol^{-1}$ |
|---|---|
| (GC)–(GC) | −61.0 |
| (AC)–(GT) | −44.0 |
| (TC)–(GA) | −41.0 |
| (CG)–(CG) | −41.3 |
| (GG)–(CC) | −34.6 |
| (AT)–(AT) | −27.5 |
| (TG)–(CA) | −27.5 |
| (AG)–(CT) | −27.1 |
| (AA)–(TT) | −22.5 |
| (TA)–(TA) | −16.0 |

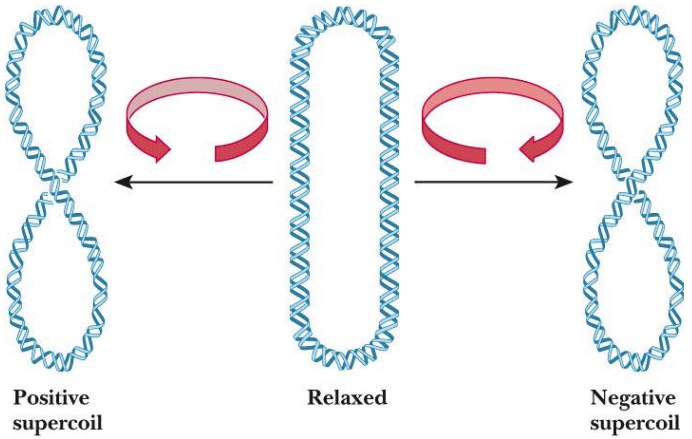


# DNA - 3° Structure

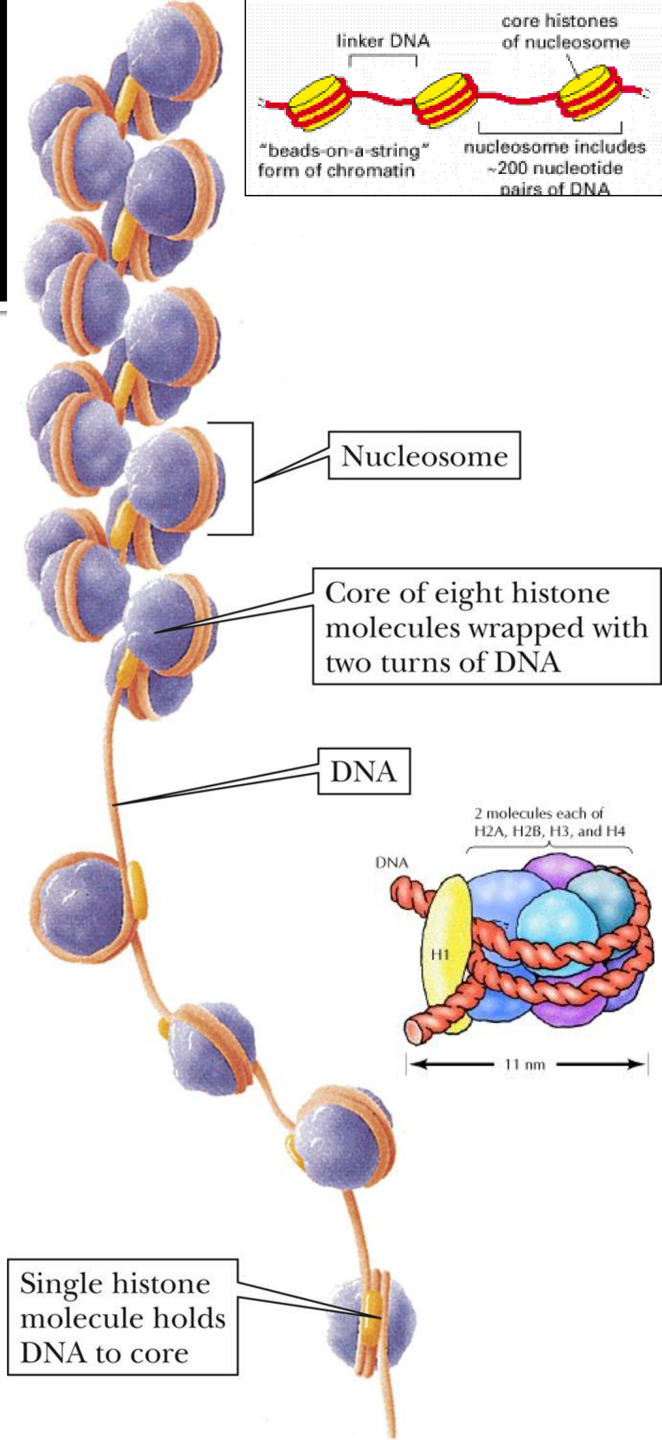
- The three-dimensional arrangement of all atoms of a nucleic acid; commonly referred to as supercoiling
- Circular DNA: a type of double-stranded DNA in which the 5′ & 3′ ends of each stand are joined by phosphodiester bonds
- Supercoiling: further coiling and twisting of DNA helix



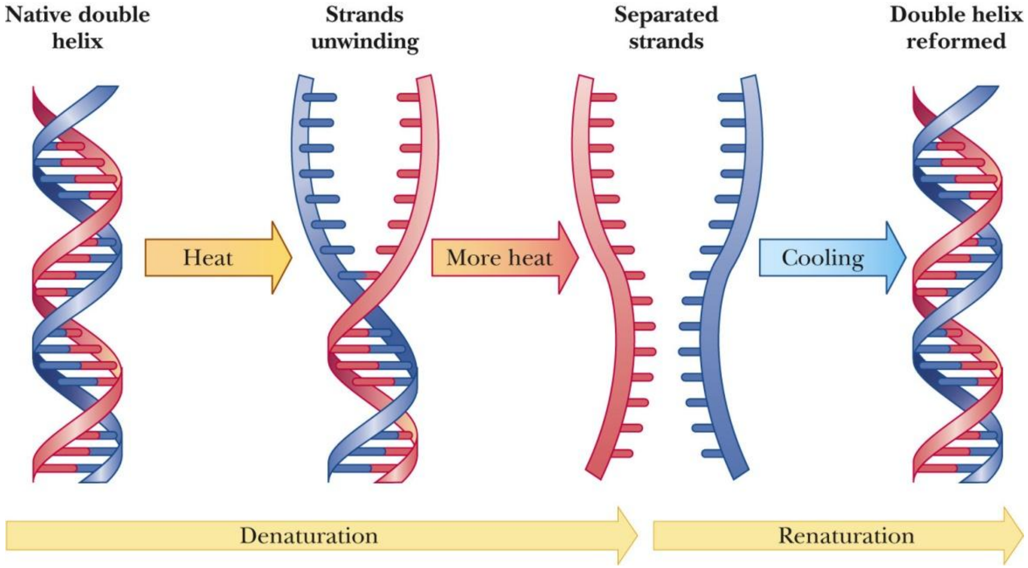
# Supercoiling in Eukaryotic DNA

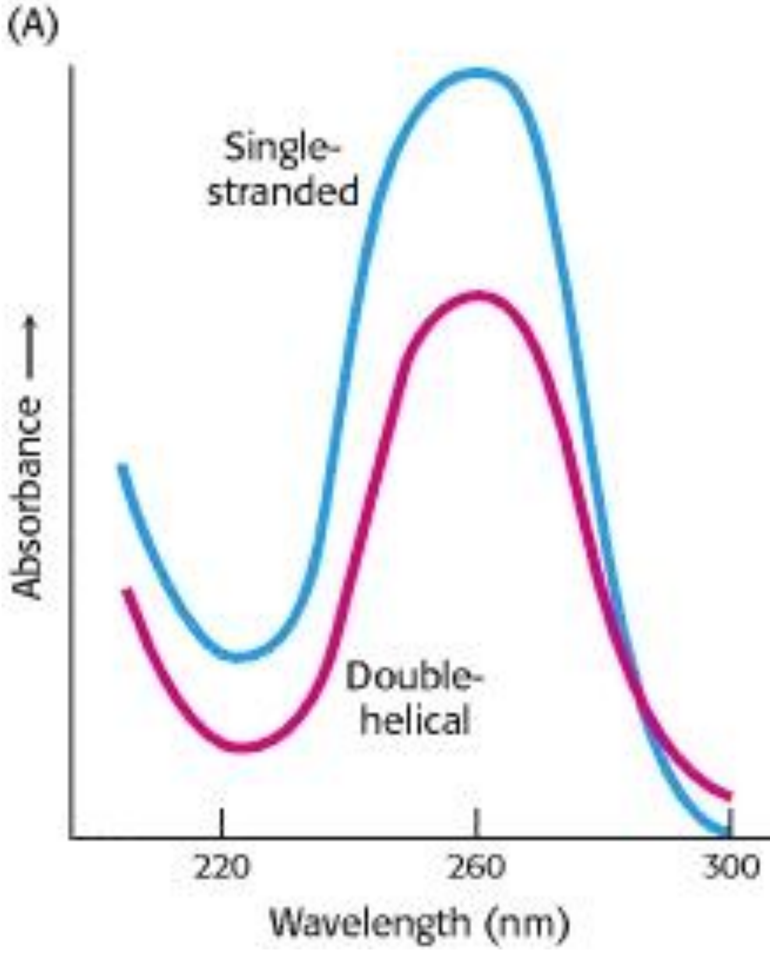
- Histone: a protein, particularly rich in the basic amino acids Lys and Arg; found associated with eukaryotic DNA
  - ✓ Five main types: H1, H2A, H2B, H3, H4
- Chromatin: DNA molecules wound around particles of histones in a beadlike structure
- Each "Bead" is a nucleosome: DNA wrapped around histone core
- Histones are positively charged:
  - ✓ Interaction
  - ✓ Charge neutralization



# Denaturation of DNA

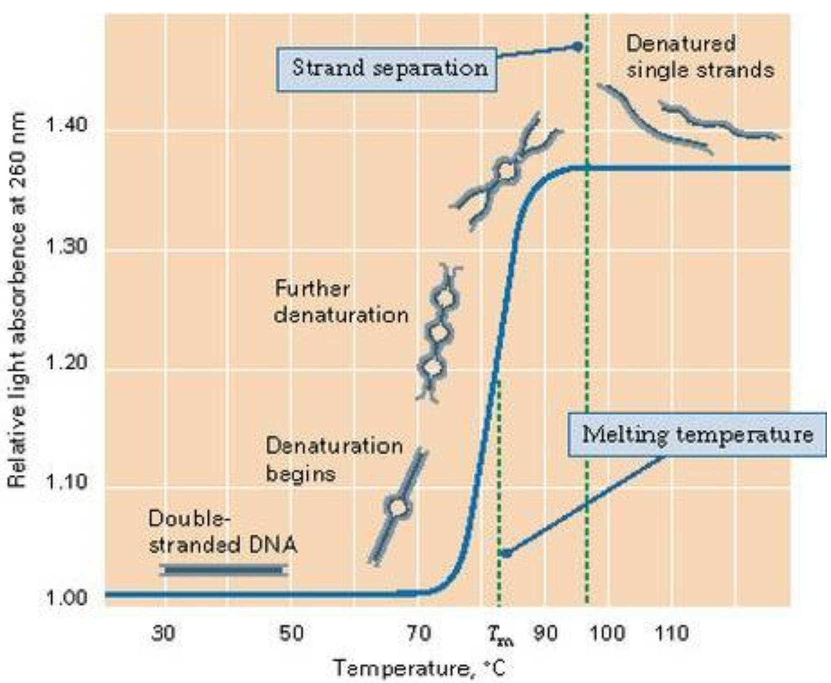
- Disruption of 2° structure
  - ✓ Most commonly by heat
  - ✓ Absorbance at 260 nm increases (hyperchromicity)
  - ✓ Renaturation (annealing) is possible on slow cooling

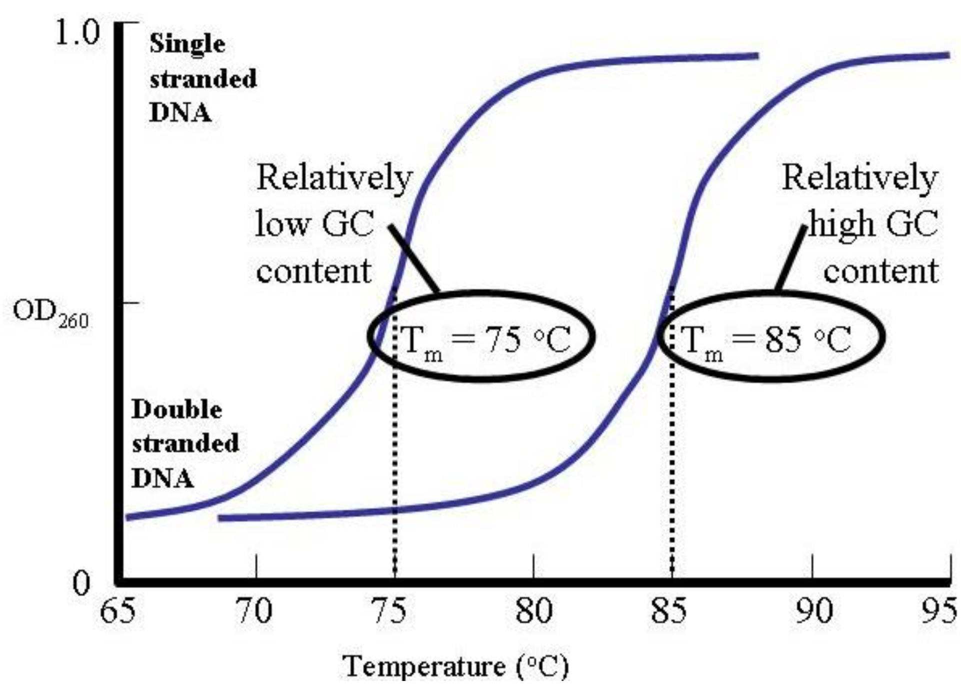


# Denaturation of DNA

- ✓ Midpoint of transition (melting) curve = $T_m$
- ✓ The higher the % G-C, the higher the $T_m$
- ✓ pH
- ✓ Salt & ion concentration
- ✓ Destabilizing agents (alkaline solutions, formamide, urea)



# RNA

- Long, unbranched chains of nucleotides
- Phosphodiester bonds: 3′-OH → 5′-OH of the next pentose
- The pentose: β-D-ribose
- Bases: uracil and cytosine
- generally, it is single stranded

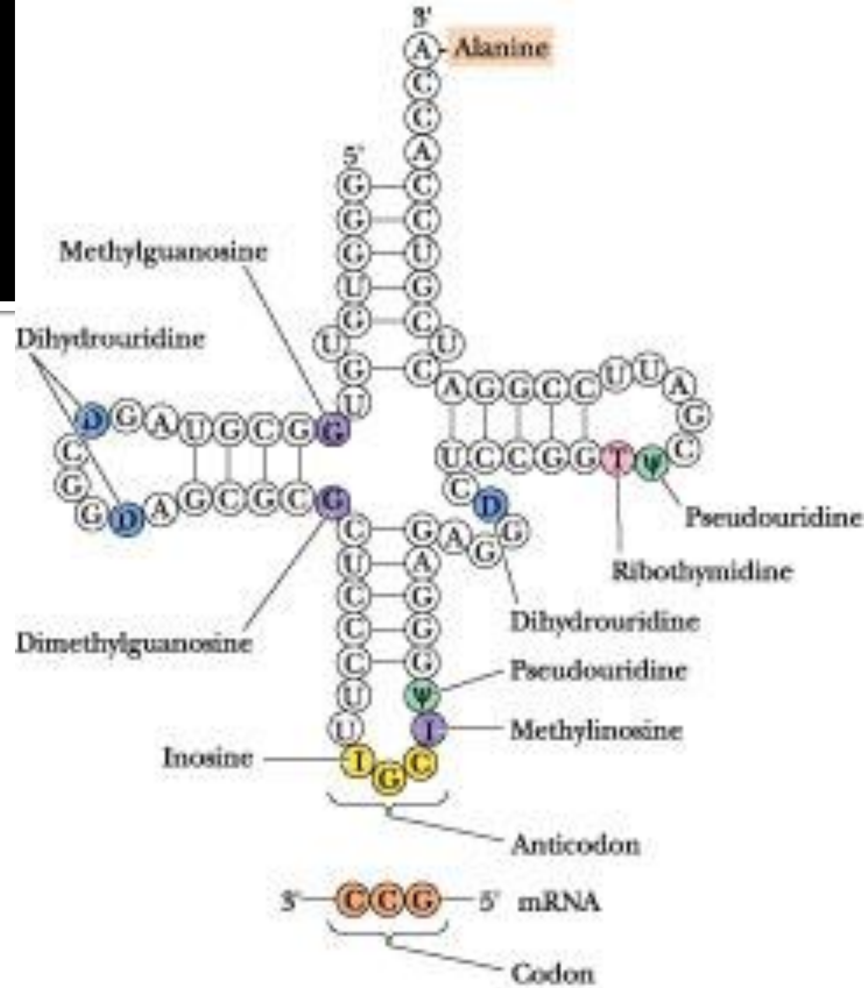
**According to their structure & function**

**The Roles of Different Kinds of RNA**

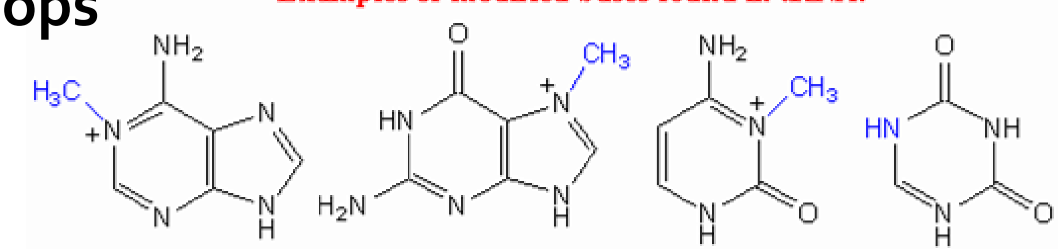
| RNA Type | Size | Function |
|---|---|---|
| Transfer RNA | Small | Transports amino acids to site of protein synthesis |
| Ribosomal RNA | Several kinds—variable in size | Combines with proteins to form ribosomes, the site of protein synthesis |
| Messenger RNA | Variable | Directs amino acid sequence of proteins |
| Small nuclear RNA | Small | Processes initial mRNA to its mature form in eukaryotes |
| Small interfering RNA | Small | Affects gene expression; used by scientists to knock out a gene being studied |
| Micro RNA | Small | Affects gene expression; important in growth and development |

# tRNA

- Transfer RNA, tRNA:
  - ✓ The smallest of the 3
  - ✓ ~75 base molecule
  - ✓ <u>Single-stranded</u>
  - ✓ Carries an amino acid at its 3′ end
  - ✓ <u>Intramolecular hydrogen bonding</u>
  - ✓ has a common tertiary structure
  - ✓ Stems (H-bonded) vs. Loops (non-H bonded)
  - ✓ Modified bases occurs in loops




Examples of modified bases found in tRNA:

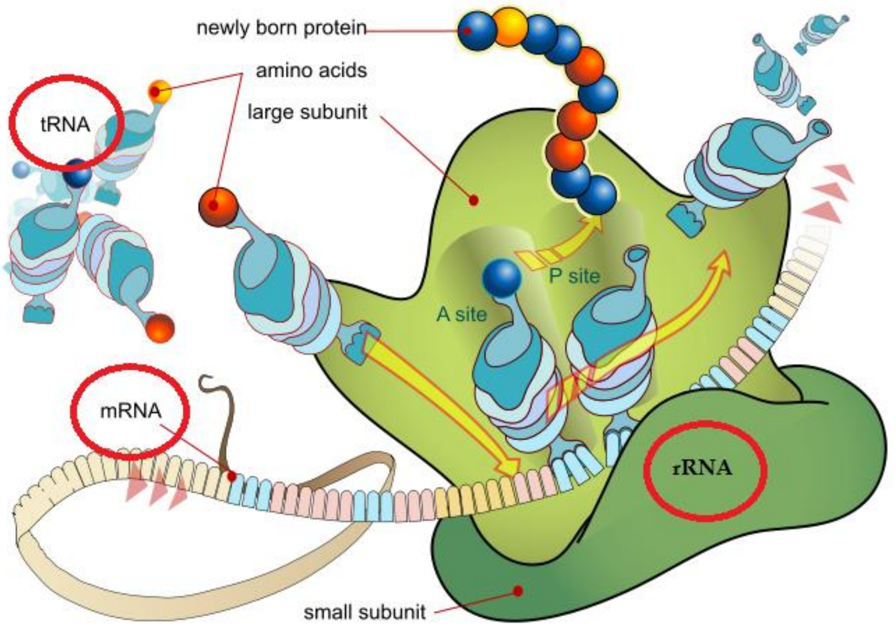


1-methyladenine ($m^1A$) 7-methylguanine ($m^7G$) 3-methylcytosine ($m^3C$) pseudouracil ($\Psi$)

# rRNA & mRNA

- **Ribosomal RNA, rRNA:**
  - ✓ Found in ribosomes
  - ✓ Constitute ≈ 60% of the ribosomes
  - ✓ Maintains ribosomes structure & provides sites for mRNA binding & protein synthesis

- **Messenger RNA, mRNA:**
  - ✓ Carries coded genetic information
  - ✓ Relatively small amounts & very short-lived



# Non-Coding RNAs: snRNA, miRNA, & siRNA

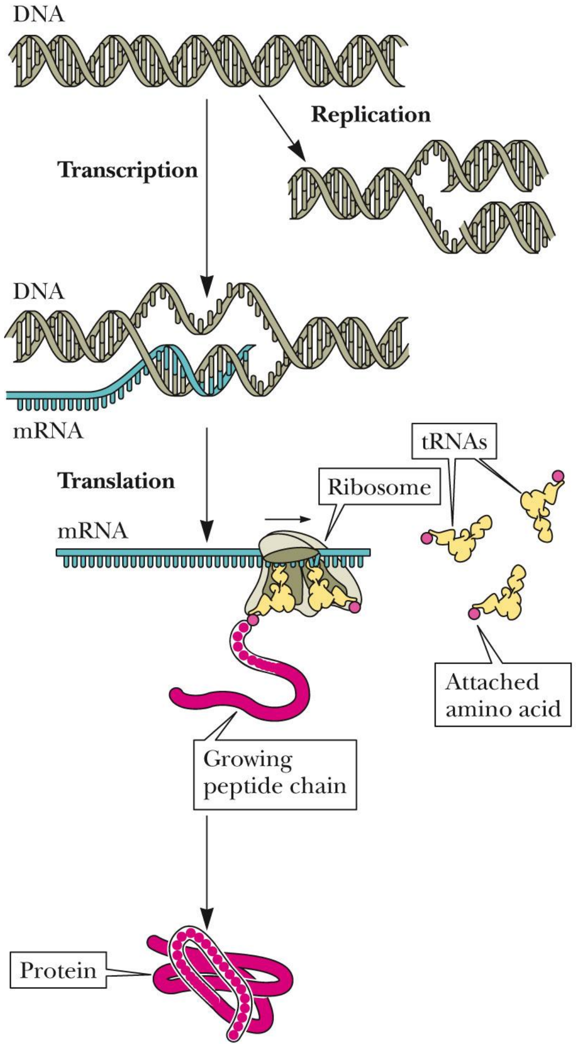
- Small nuclear RNA (snRNA):
  - ✓ Found in nucleus of eukaryotes
  - ✓ Small (100-200 nucleotides long)
  - ✓ Forms complexes with protein - small nuclear ribonucleoprotein particles (snRNPs)
    - snRNPs: processing of initial mRNA transcribed from DNA

- MicroRNA (miRNA)
  - ✓ Natural
  - ✓ Translation regulation

- Small interfering RNA (siRNA)
  - ✓ Synthetic
  - ✓ Translation regulation

# Information Transfer in Cells

- Information encoded in nucleotide sequence of DNA is transcribed through mRNA synthesis
- Protein sequence then dictated by DNA sequence
- Known as:
  - Central dogma of biology




**Replication**
DNA replication yields two DNA molecules identical to the original one, ensuring transmission of genetic information to daughter cells with exceptional fidelity.

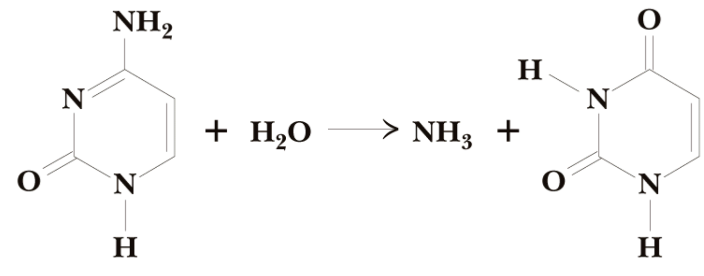
**Transcription**
The sequence of bases in DNA is recorded as a sequence of complementary bases in a single-stranded mRNA molecule.

**Translation**
Three-base codons on the mRNA corresponding to specific amino acids direct the sequence of building a protein. These codons are recognized by tRNAs (transfer RNAs) carrying the appropriate amino acids. Ribosomes are the “machinery” for protein synthesis.

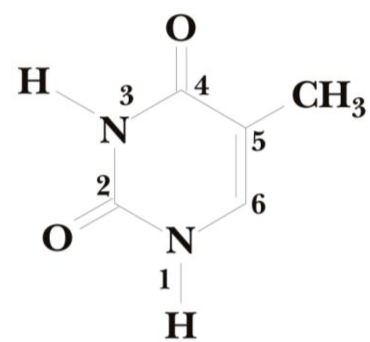
# DNA & RNA Differences

*1. Thymine vs. Uracil?*

- To distinguish natural U from mutant U
  - ✓ Cytosine undergoes spontaneous deamination (uracil)
  - ✓ Recognized by repair enzymes (mutations)

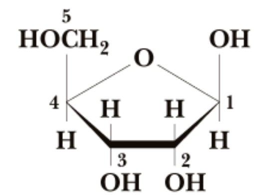



Cytosine Uracil



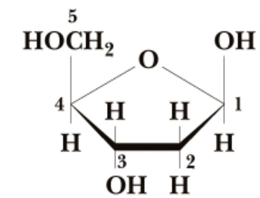

*2. The 2'-deoxy sugar?*

- Stability
  - ✓ -OH groups (2' & 3') in RNA: more susceptible to hydrolysis
  - ✓ DNA, lacks 2'-OH (stability)
  - ✓ Does it make sense?




Furanose form of
D-Ribose

β-D-Ribofuranose




Furanose form of
D-2-Deoxyribose

β-D-2-Deoxyribofuranose