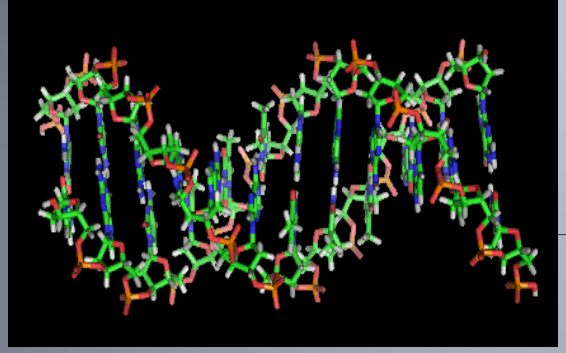

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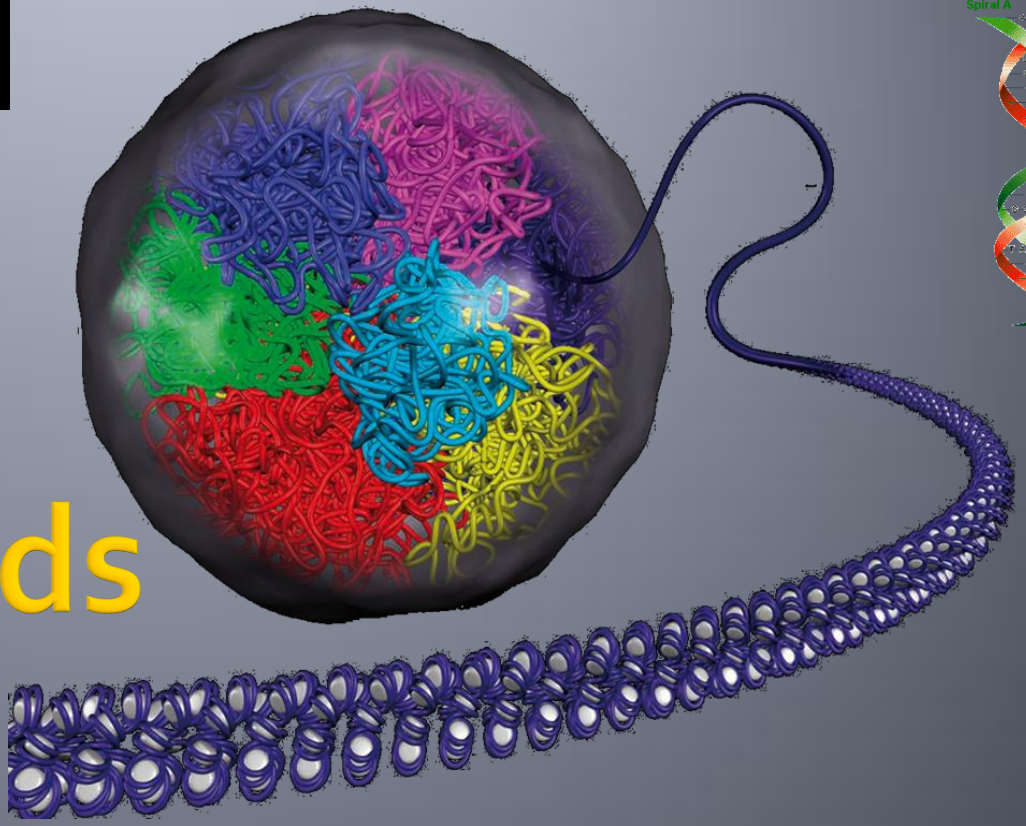
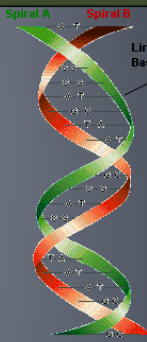
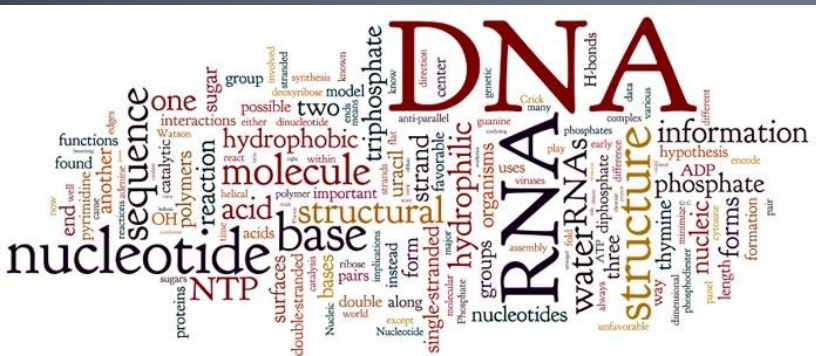
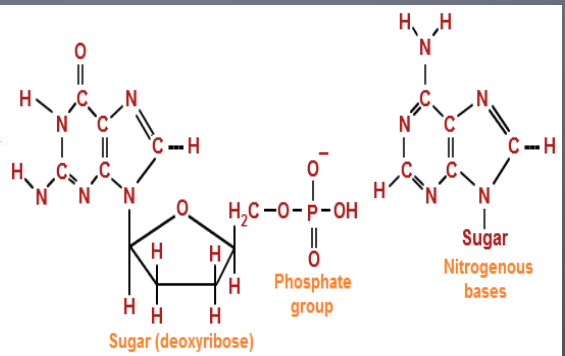
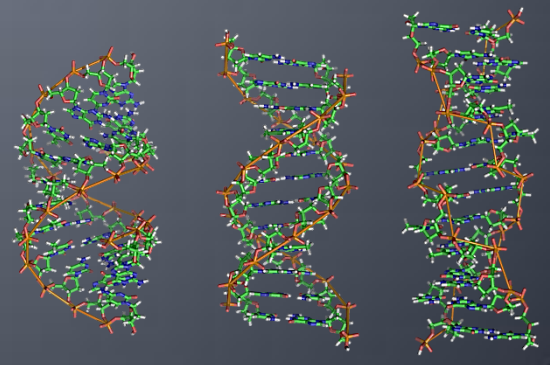
Nucleic Acids









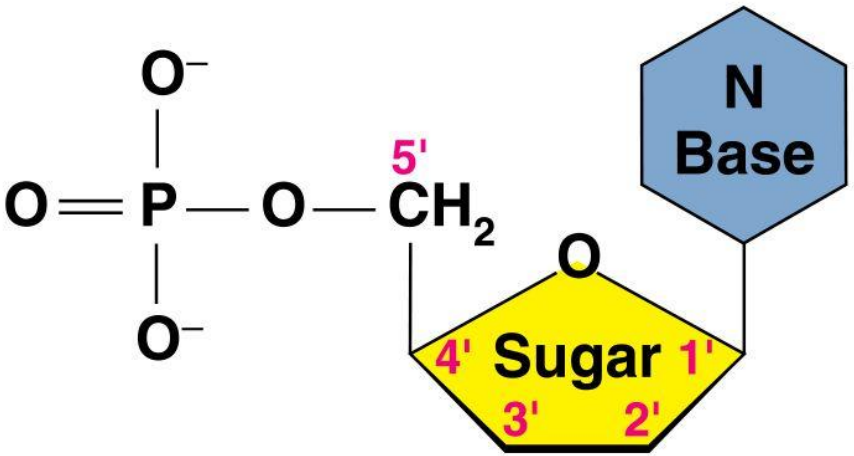


LECTURE OUTLINE

- 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

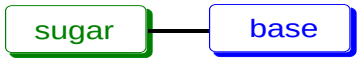
Nucleic Acids

- 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)

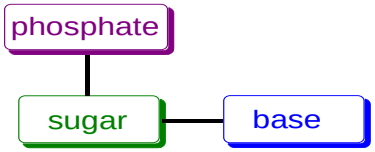


Nucleoside, nucleotides & nucleic acids

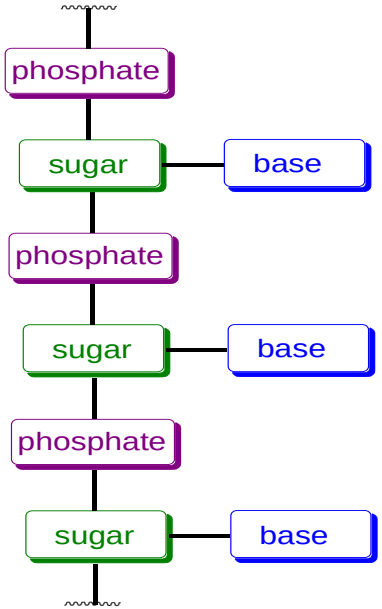
- A nucleoside: N-base linked by a β-glycosidic bond to C1′ of a ribose or deoxyribose
- Nucleosides naming: *-osine* for purines & *–idine* for pyrimidines
- A nucleotide: a nucleoside phosphoric acid esters (C5′ OH of sugar)
- Nucleotides naming: nucleoside followed by *5′-monophosphate (ylate)*



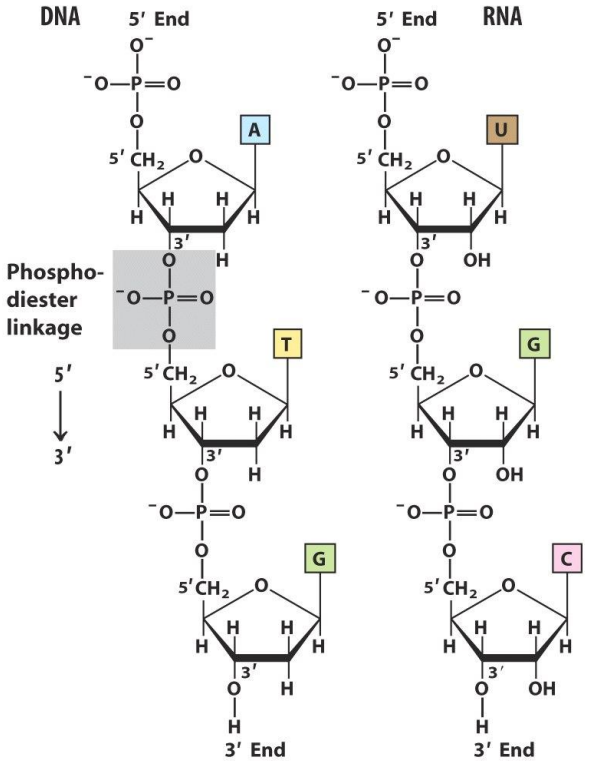
nucleoside

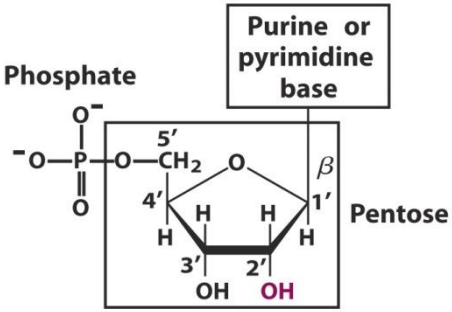


nucleotides



nucleic acids





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

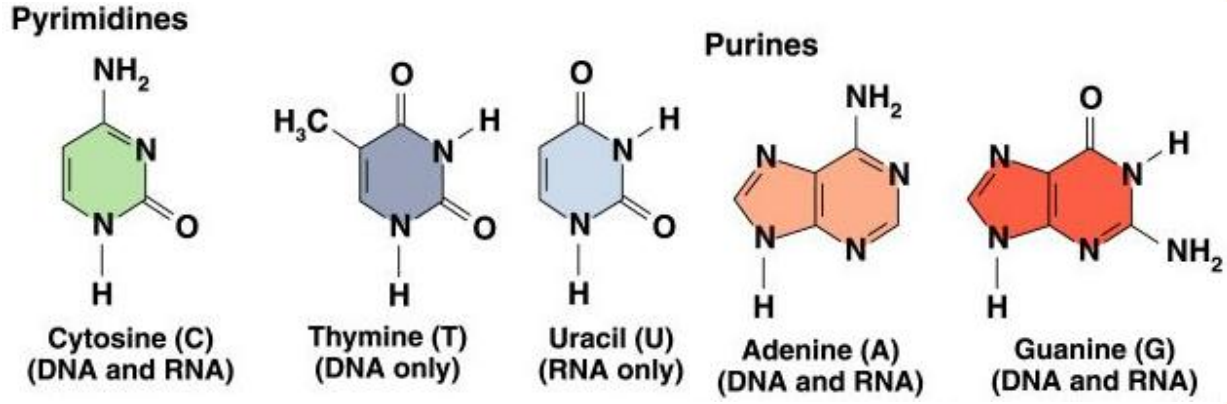
Nucleotides vs. Nucleosides

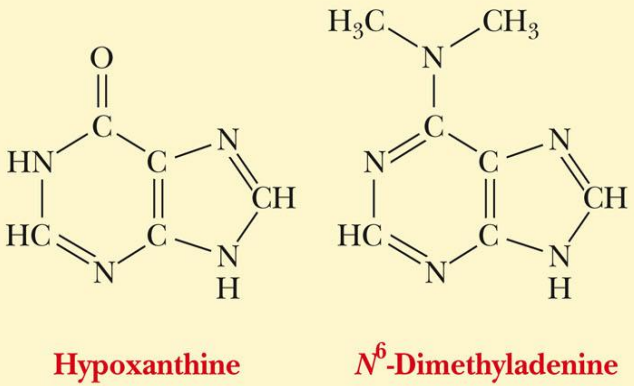
TABLE 2-2 Terminology of Nucleosides and Nucleotides

		Bases			
		Purines		Pyrimidines	
		Adenine (A)	**Guanine (G)**	**Cytosine (C)**	**Uracil (U)** **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.			

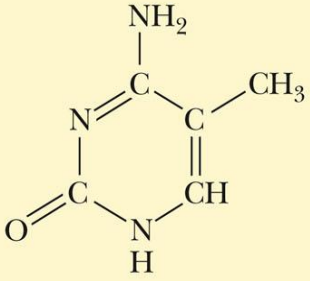
Nitrogen Bases

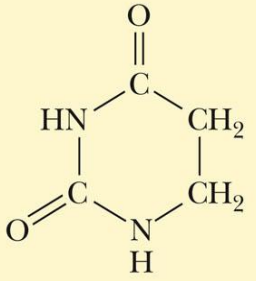
- Two general types:
- ✓ Purines: adenine (A) & guanine (G)
- ✓ Pyrimidines: cytosine (C), thymine (T) & Uracil (U)
- Less common bases can occur
- Principally but not exclusively, in transfer RNAs

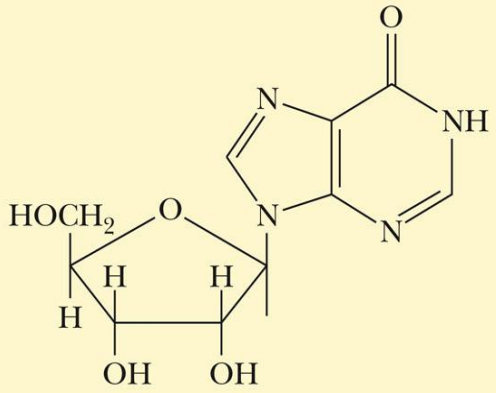



Hypoxanthine

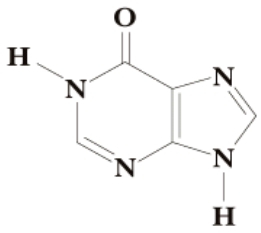
N^6-Dimethyladenine

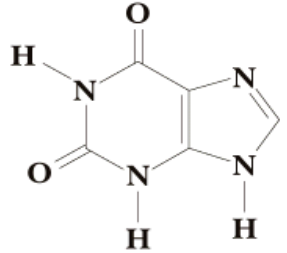

5-Methylcytosine


5,6-Dihydrouracil


Inosine, an uncommon nucleoside

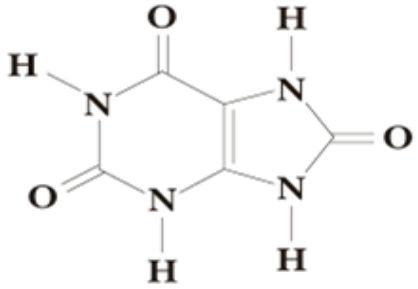
Other nucleotides

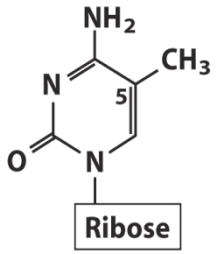

Hypoxanthine

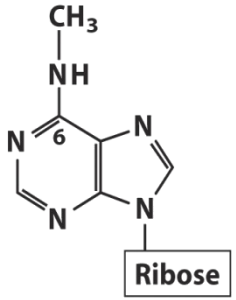

Xanthine

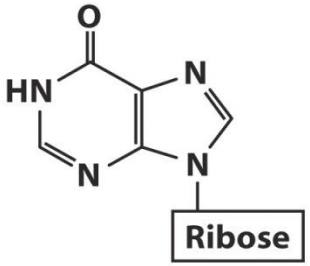
- Xanthine, hypoxanthine & uric acid: intermediates in purine metabolism
- N_6-methyl adenine
- 5-methyl-cytosine & N_4-methyl cytosine

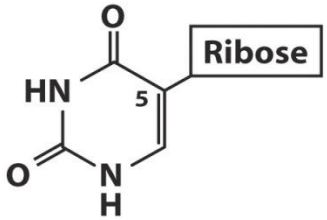
- Pseudouracil: has the ribose attached to C_5 (N_1) of uracil (Pseudouridine)
- 1,3,7-trimethylxanthine (caffeine)

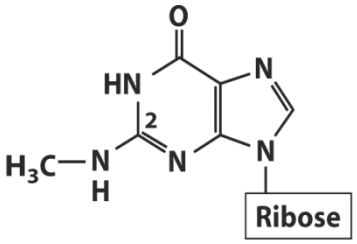

Uric acid

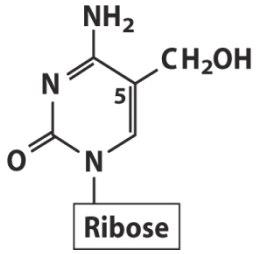

5-Methylcytidine

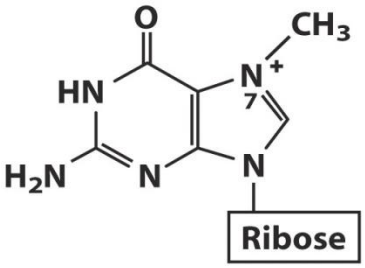

N^6-Methyladenosine

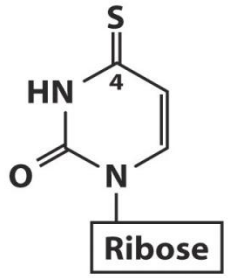

Inosine


Pseudouridine

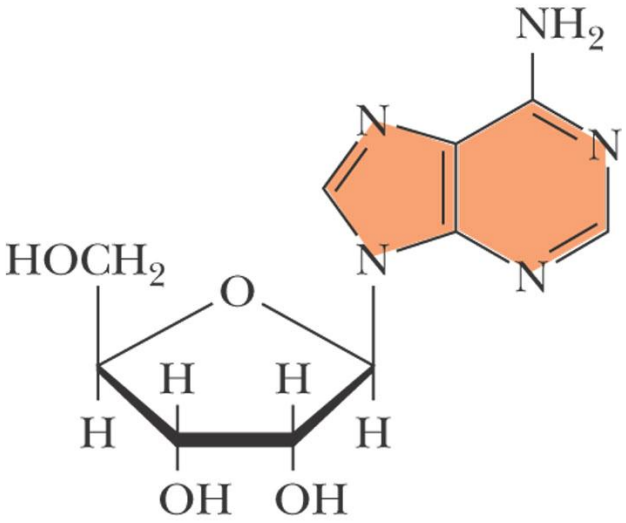

N^2-Methylguanosine


5-Hydroxymethylcytidine

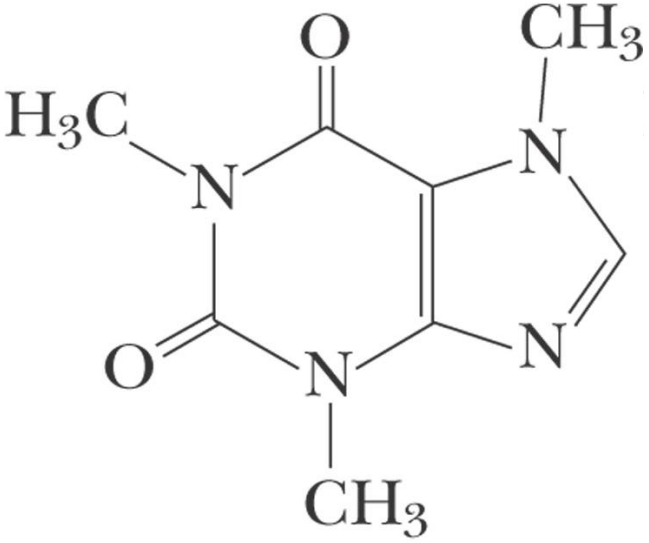

7-Methylguanosine


4-Thiouridine

Adenosine: a nucleoside with physiological activity



Adenosine

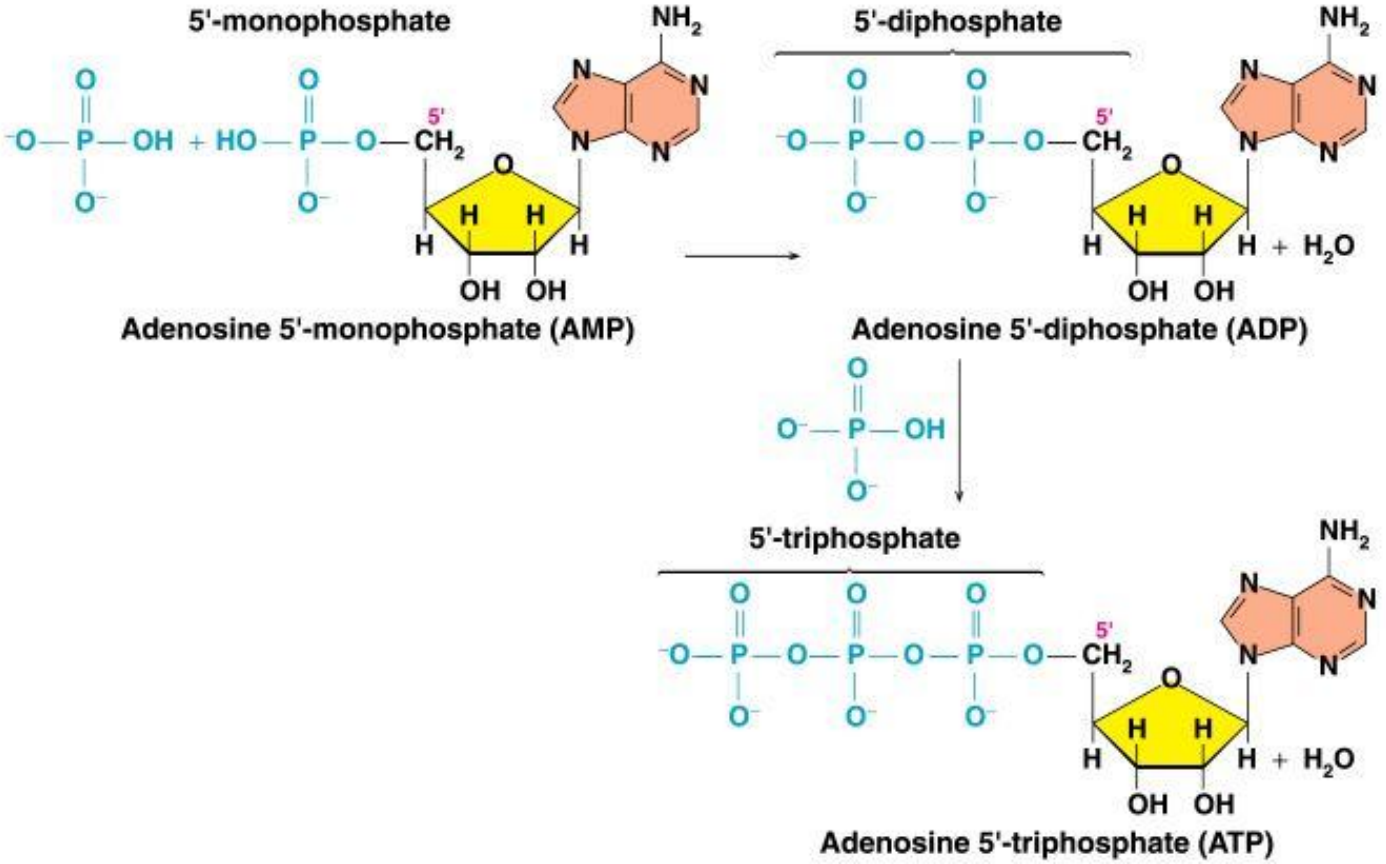


Caffeine

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

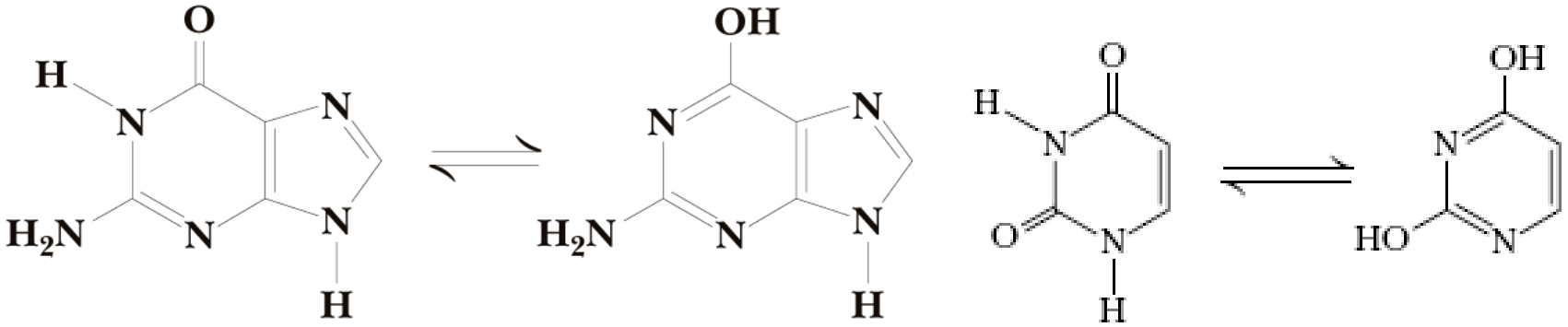
AMP, ADP & ATP

- 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)**

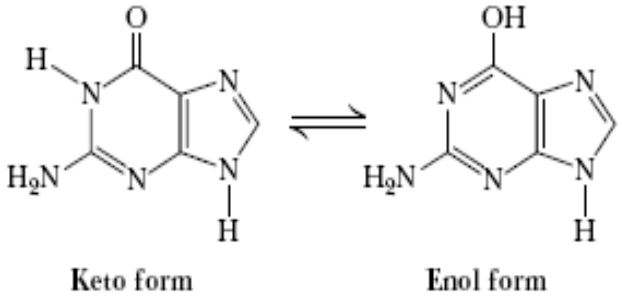


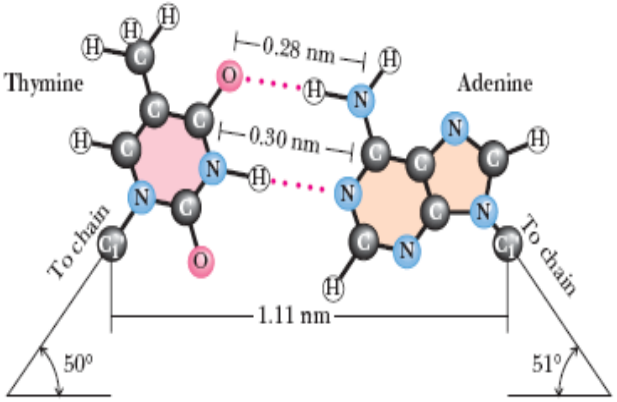
Properties of Pyrimidines & Purines

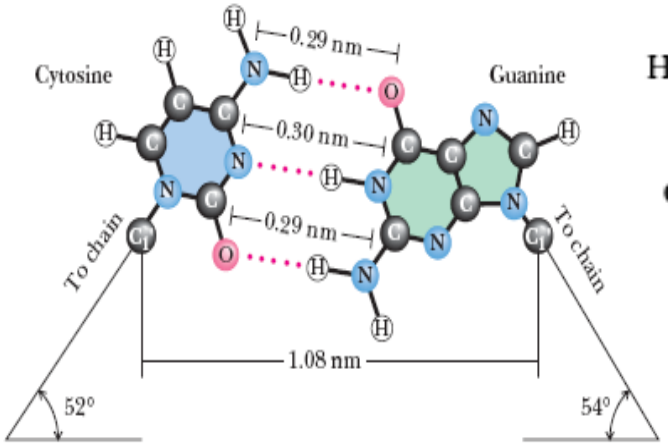
- 2. Acid/base dissociations:
  - ✓ E.g; Uracil, Cytosine, Guanine
- Important in determining if nitrogens are H-bond donors/ acceptors (double helix formation)
- Important functional groups participating in H-bond formation:
  - ✓ Amino groups, Ring Ns, Os

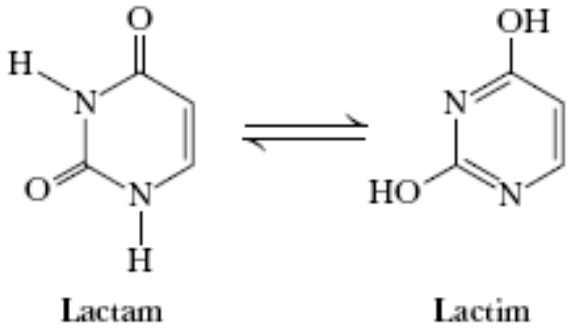
Proton Dissociation Constants (pK_a Values) for Nucleotides

Nucleotide	pK_a Base-N	pK_1 Phosphate	pK_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



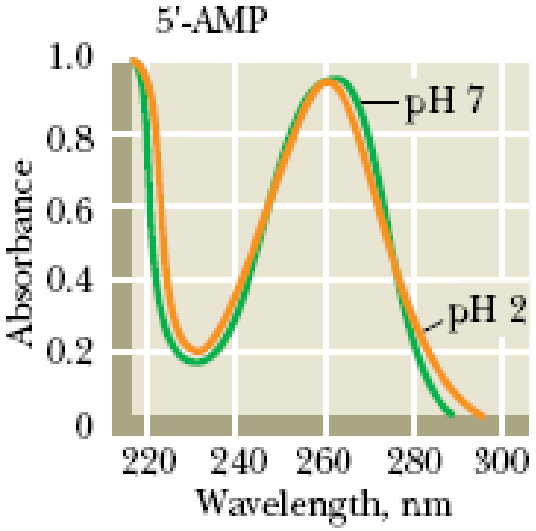


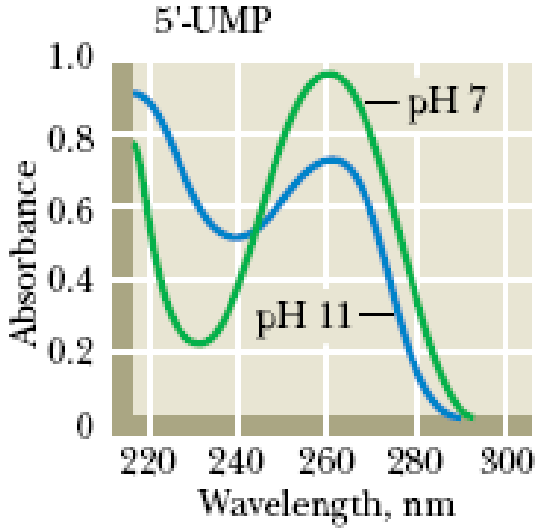


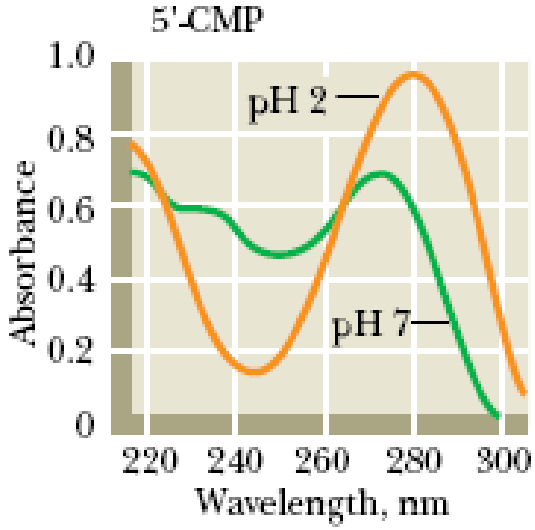


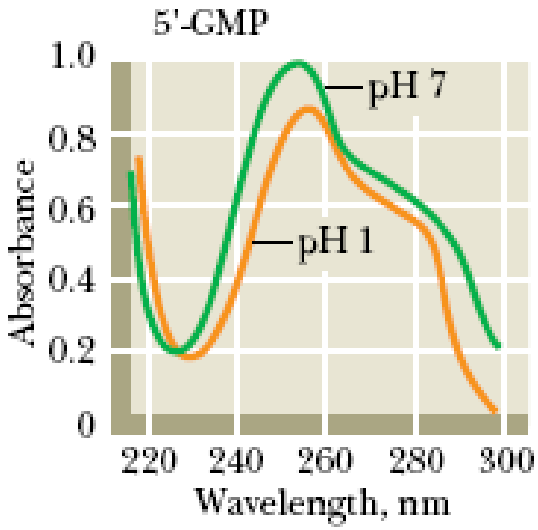
Properties of Pyrimidines & Purines

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



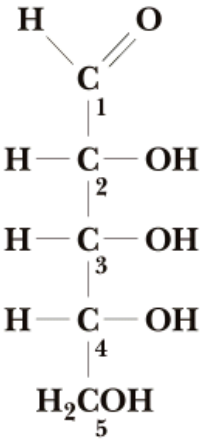




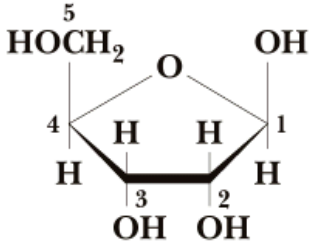


Pentoses of Nucleotides

- D-ribose (in RNA)
- 2-deoxy-D-ribose (in DNA)
- Sugars icreases solubility (compared to free bases)
- The position of the carbohydrate is followed by a′ (prime)
- stereochemistry is β

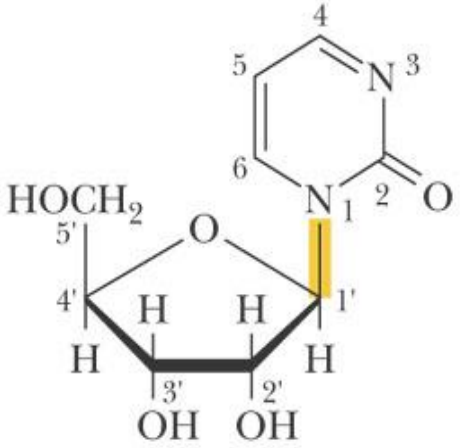


D-Ribose

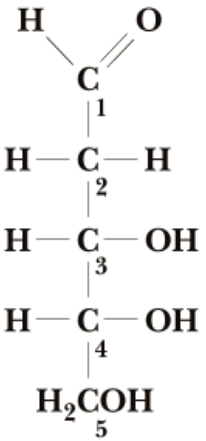


Furanose form of D-Ribose

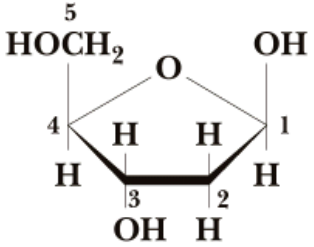
β-D-Ribofuranose



β-N_1-glycosidic bond in pyrimidine ribonucleosides

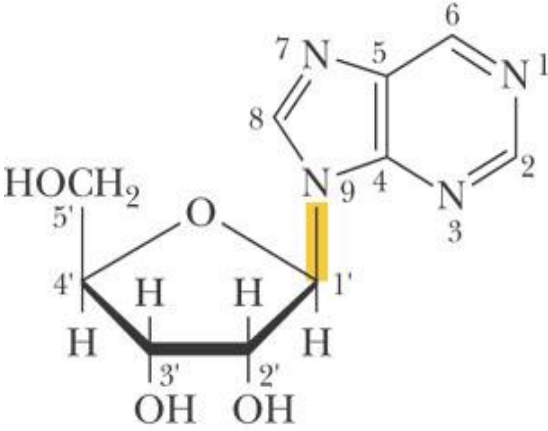


D-2-Deoxyribose



Furanose form of D-2-Deoxyribose

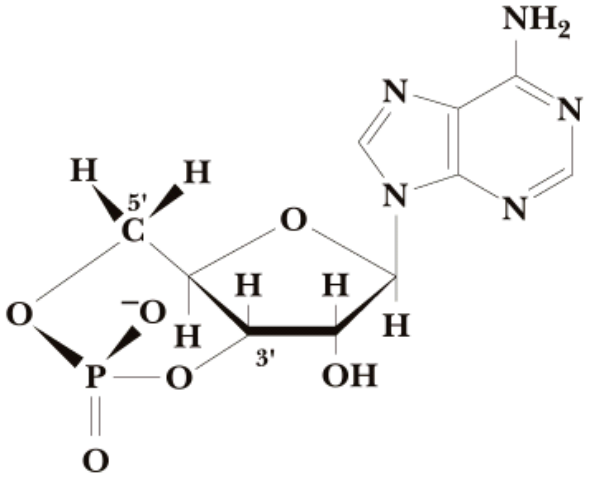
β-D-2-Deoxyribofuranose



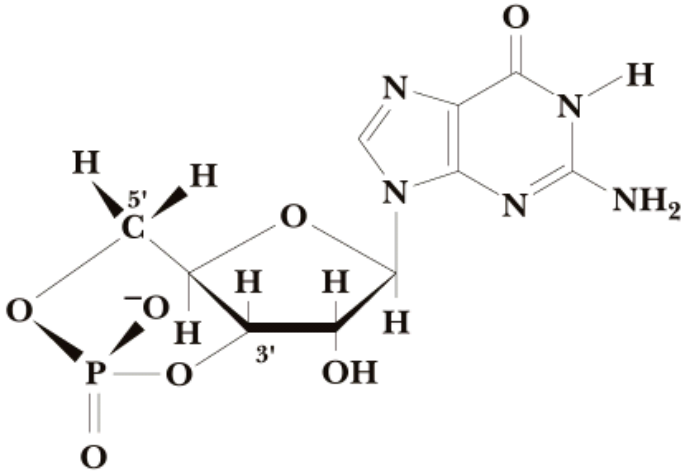
β-N_9-glycosidic bond in purine ribonucleosides

Functions of Nucleotides

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



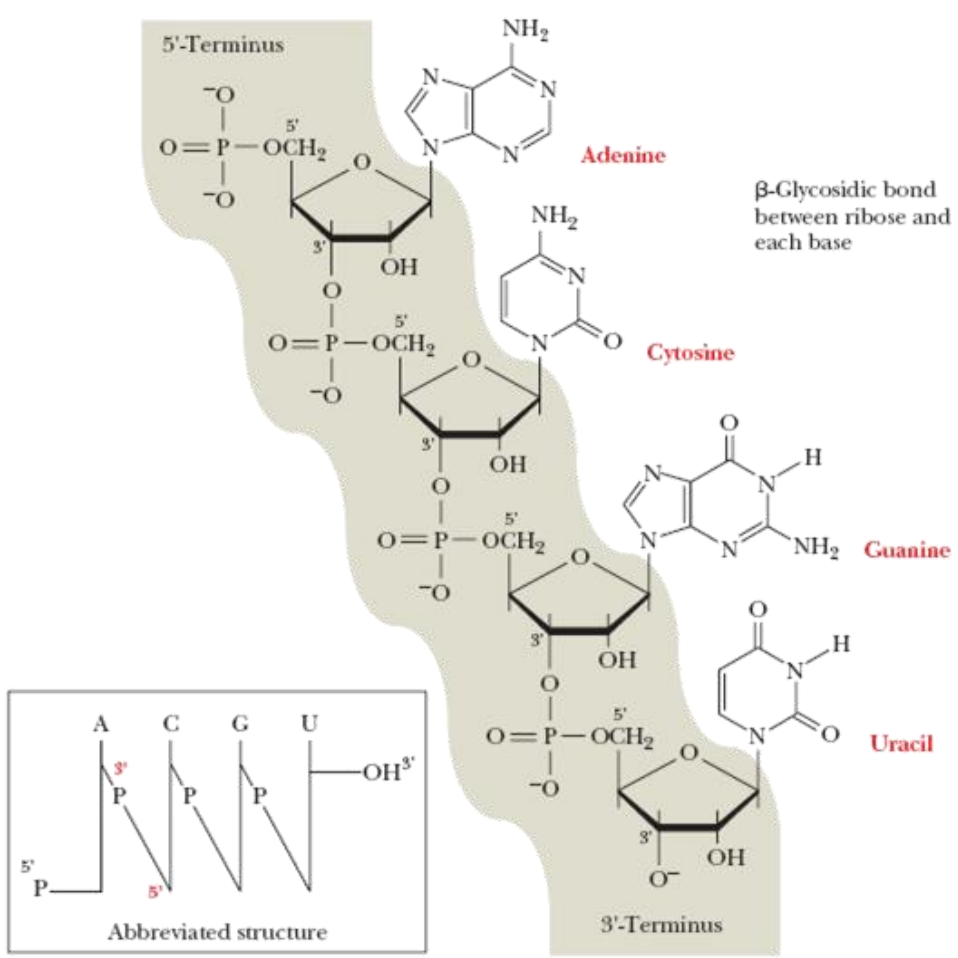
3',5'-Cyclic AMP

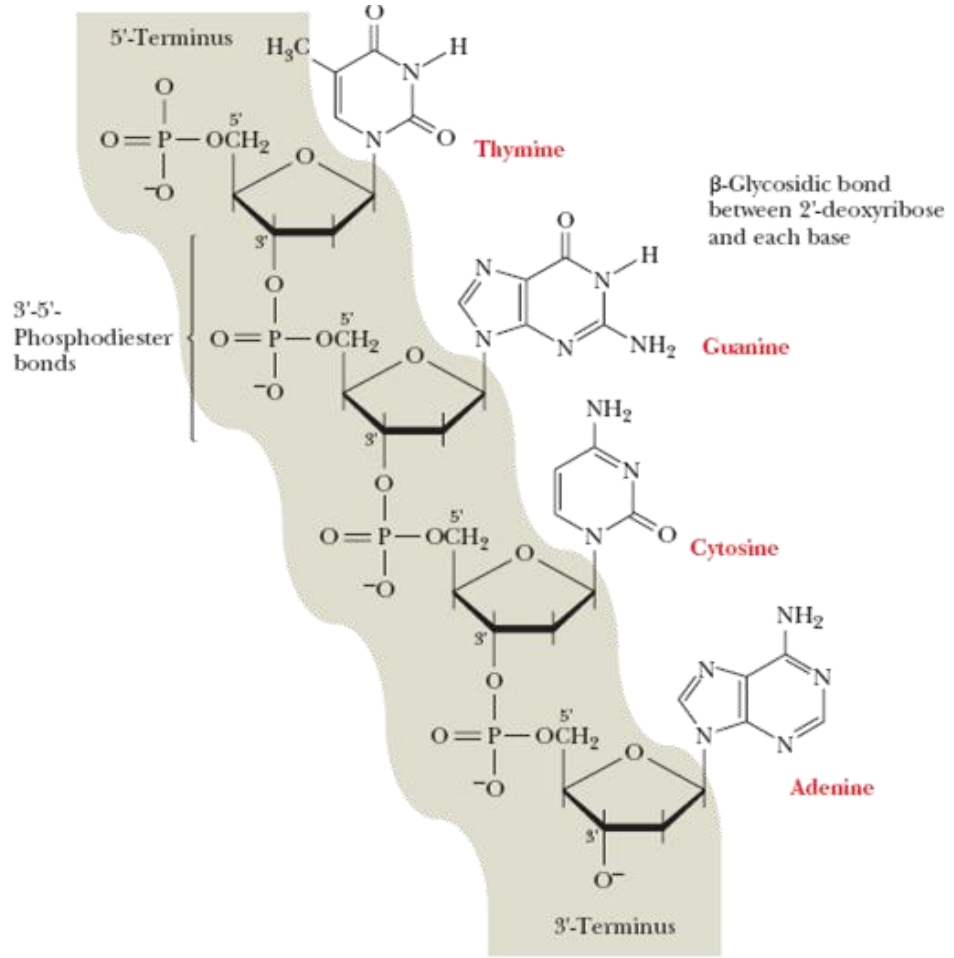


3',5'-Cyclic GMP

Polymerization

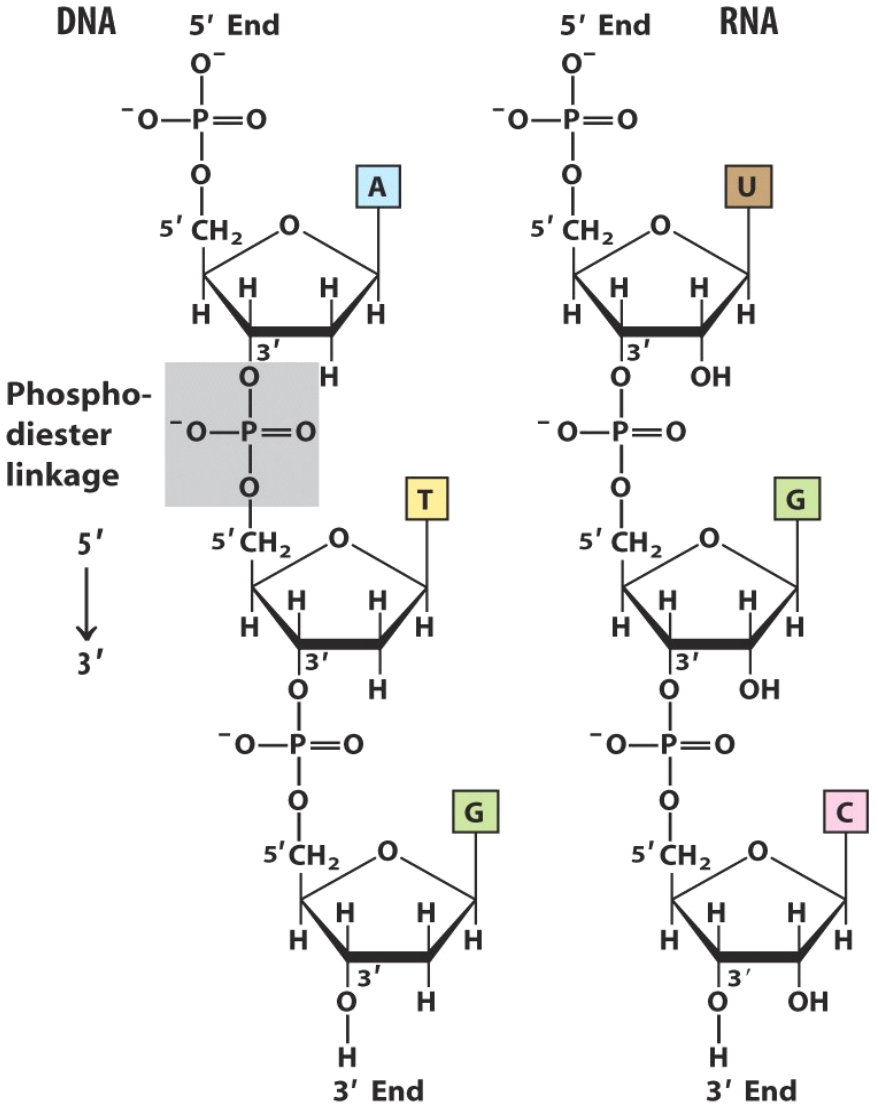
- **Leads to nucleic acids. Linkage is repeated (3′,5′-phosphodiester bond)**





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

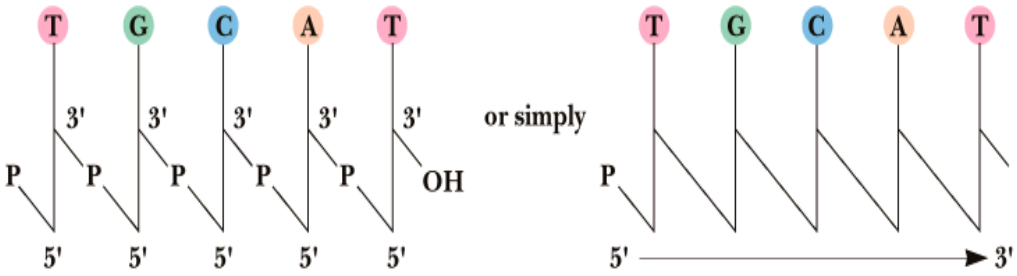


Classes of Nucleic Acids?

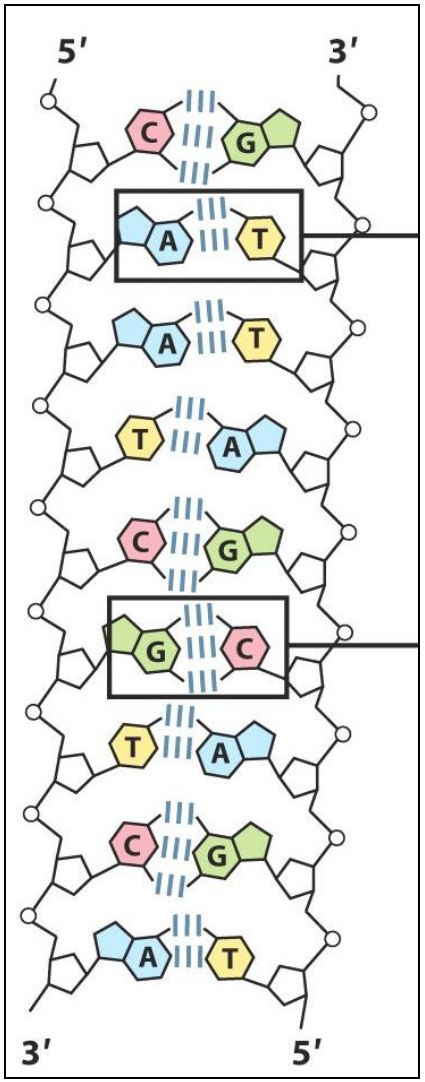
- DNA - one type, one purpose:
  - ✓ A single DNA molecules in virus and bacteria
  - ✓ Eukaryotic cells have many diploid chromosomes mainly in nucleus, but also mitochondria & chloroplasts

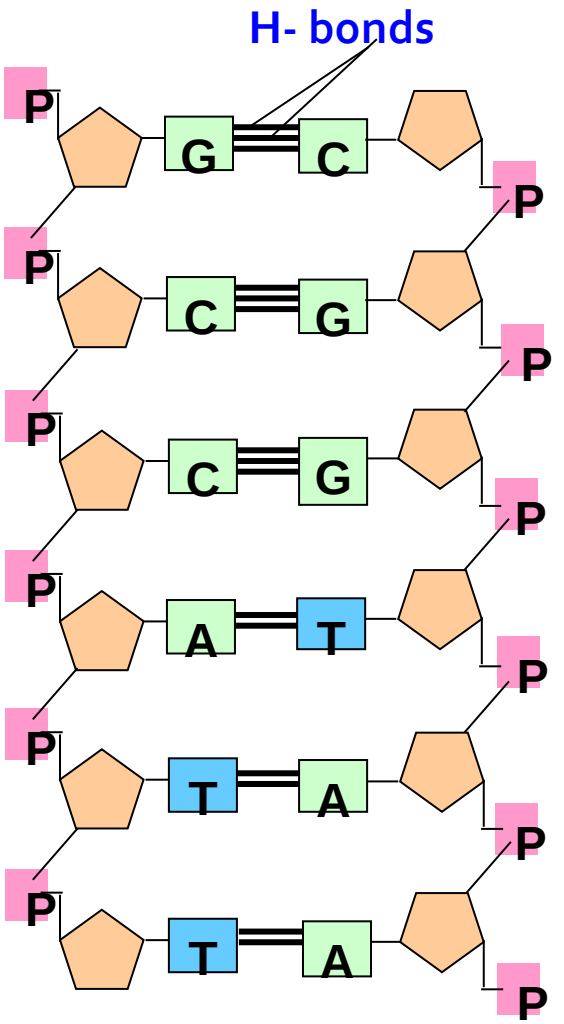
- RNA - 3 (or 4) types, 3 (or 4) purposes
  - ✓ Ribosomal RNA - the basis of structure & function of ribosomes
  - ✓ Messenger RNA - carries the message
  - ✓ Transfer RNA - carries the amino acids
  - ✓ Small nuclear RNA
  - ✓ Small non-coding RNAs

DNA structure



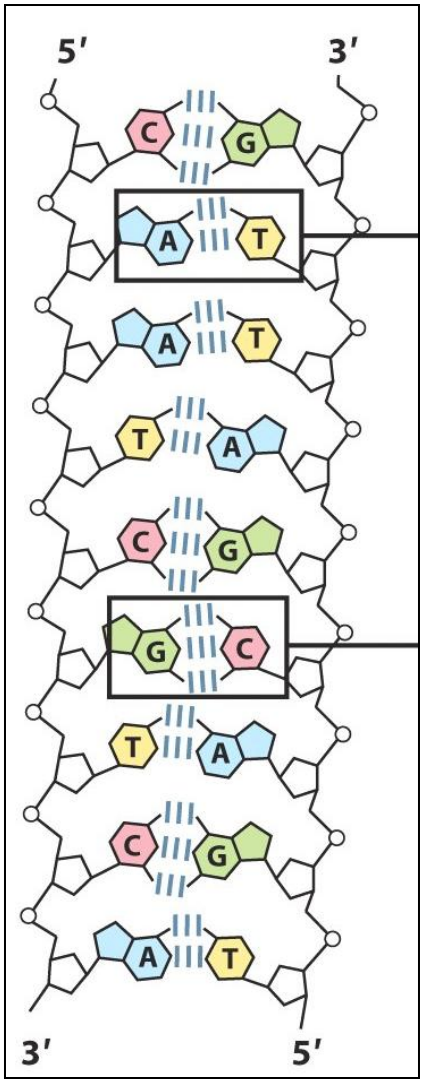
- Diameter: 2 nm
- Length: 1.6 million nm (*E. coli*)
- Compact and folded (*E. coli* cell is only 2000 nm long)
- Antiparallel double helix
- Backbone vs. side chains
- Specific base-pairing
  - ✓ Chargaff's rules (A=T & C=G)
- Strands are joined by the bases (complementary)
- Stable (H-bonds)

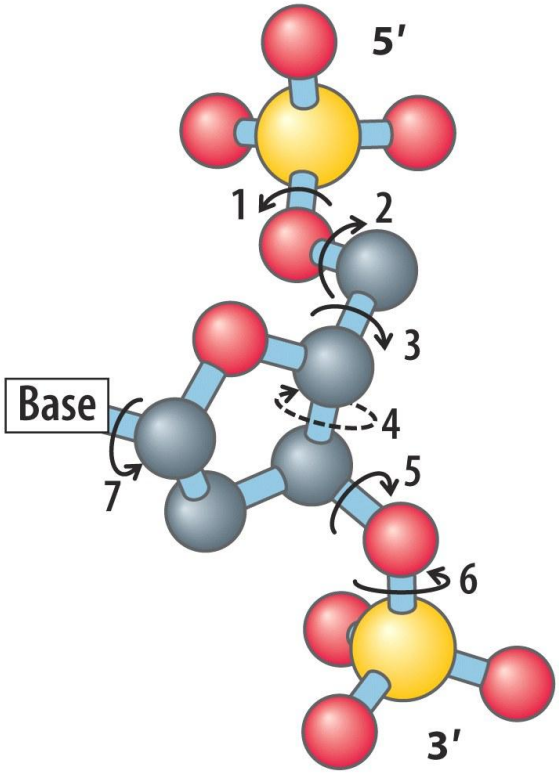




DNA structure – Stability vs. Flexibility

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





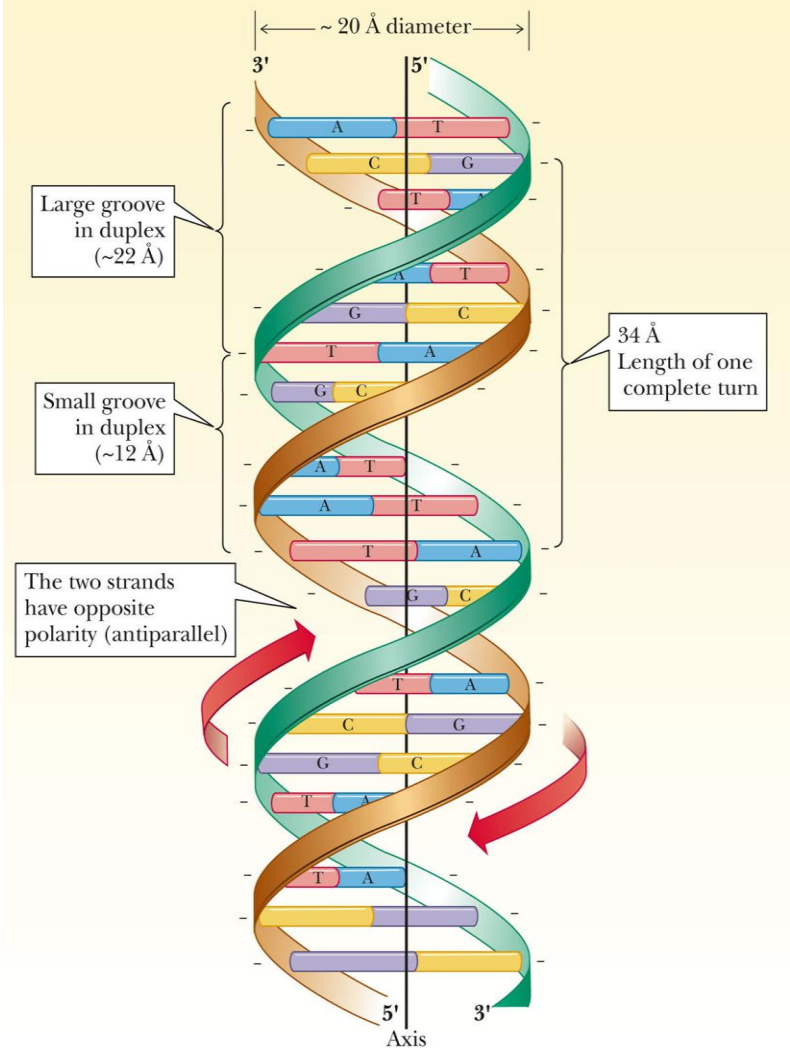
DNA - 1° Structure

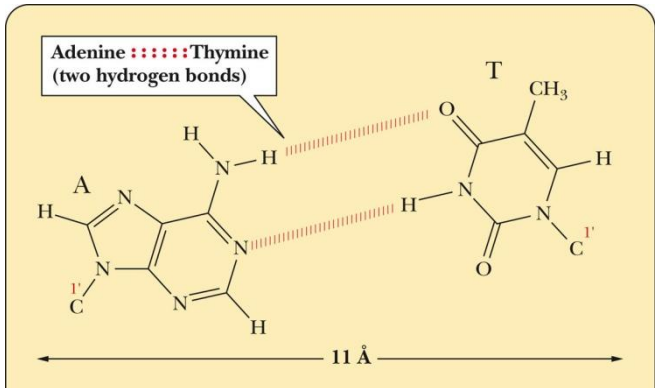
- A biopolymer that consists of a backbone of alternating units of 2-deoxy-D-ribose and phosphate

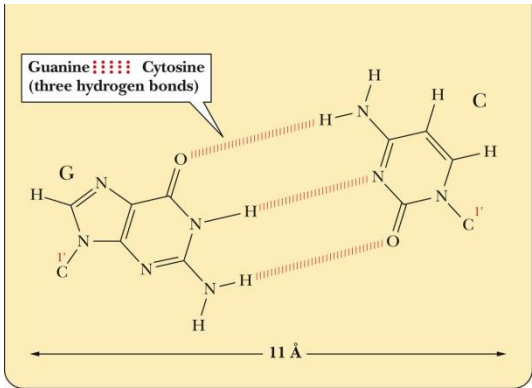
- Primary Structure: the sequence of bases along the pentose-phosphodiester backbone of a DNA molecule
  - ✓ By convention, from left to right, & from the 5′-end to the 3′-end
  - ✓ System of notation single letter (A,G,C,U and T)
  - ✓ More abbreviated notations: d(GACAT); pdApdCpdGpdT pdACGT

DNA - 2° Structure

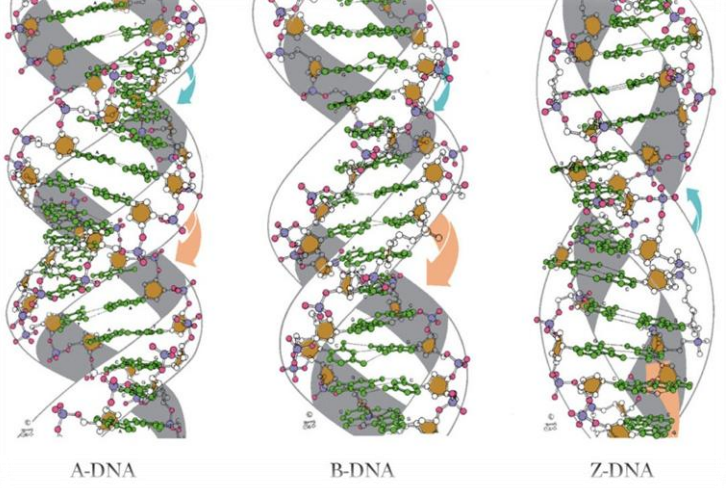
- Secondary structure: the ordered arrangement of nucleic acid strands
- Double helix model (James Watson and Francis Crick): a type of 2° structure of DNA molecules in which two antiparallel polynucleotide strands are coiled in a right-handed manner about the same axis
- Base Pairing: T-A (2 H-bonds) & G-C (3 H-bonds)
- Minor vs. major grooving



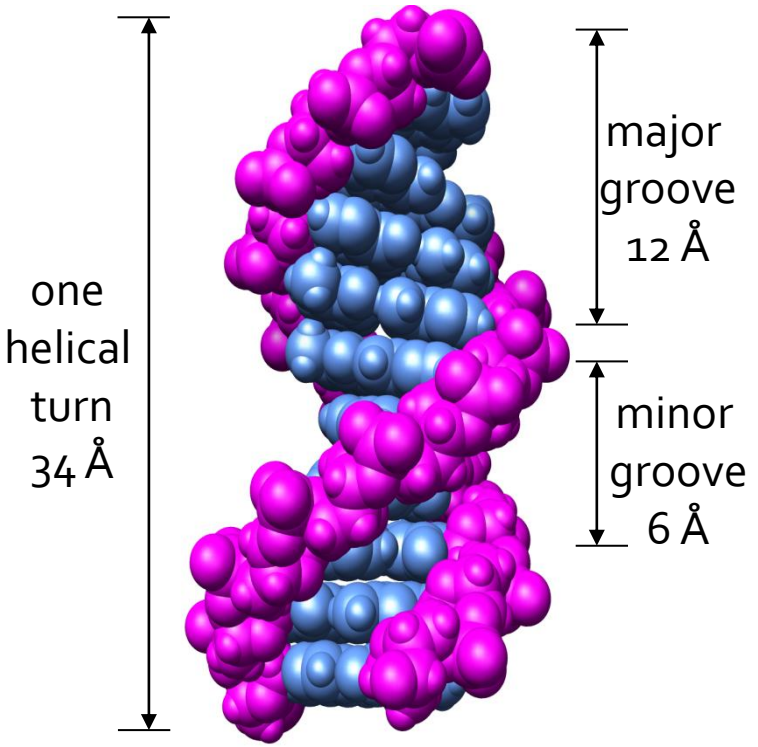




Forms of DNA

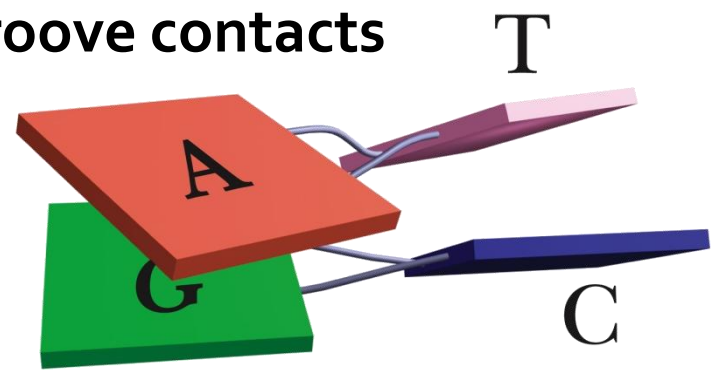


- B-DNA
  - Considered the physiological form
  - A right-handed helix, diameter 11 Å
  - 10 base pairs per turn (34Å)
- A-DNA
  - A right-handed helix, but thicker than B-DNA
  - 11 base pairs per turn of the helix
  - Has not been found *in vivo*
- Z-DNA
  - A left-handed double helix
  - May play a role in gene expression



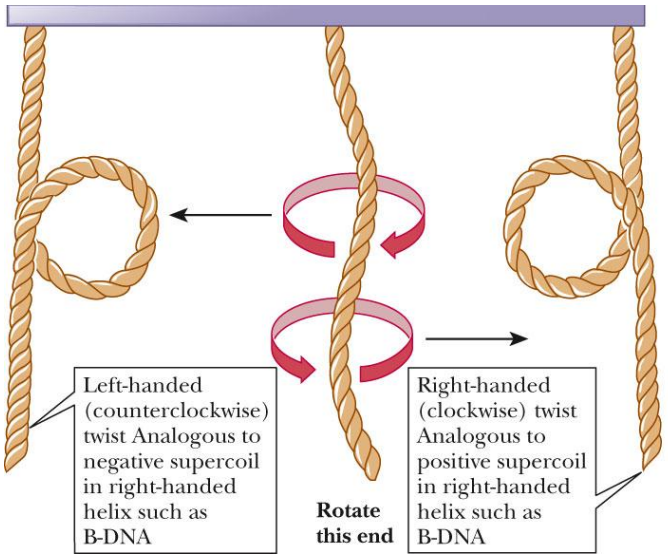
Features of DNA

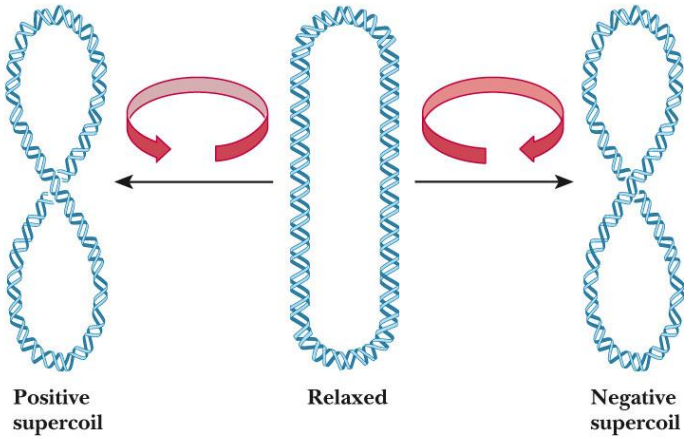
- Base stacking
  - Bases are very nearly planar, hydrophobic & interact by hydrophobic interactions
  - In B-DNA, each base rotated by 32° compared to the next and (base pairing vs. maximum overlap)
  - Bases exposed to the minor groove come in contact with water
  - Many bases adopt a ***propeller-twist***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



DNA - 3° Structure

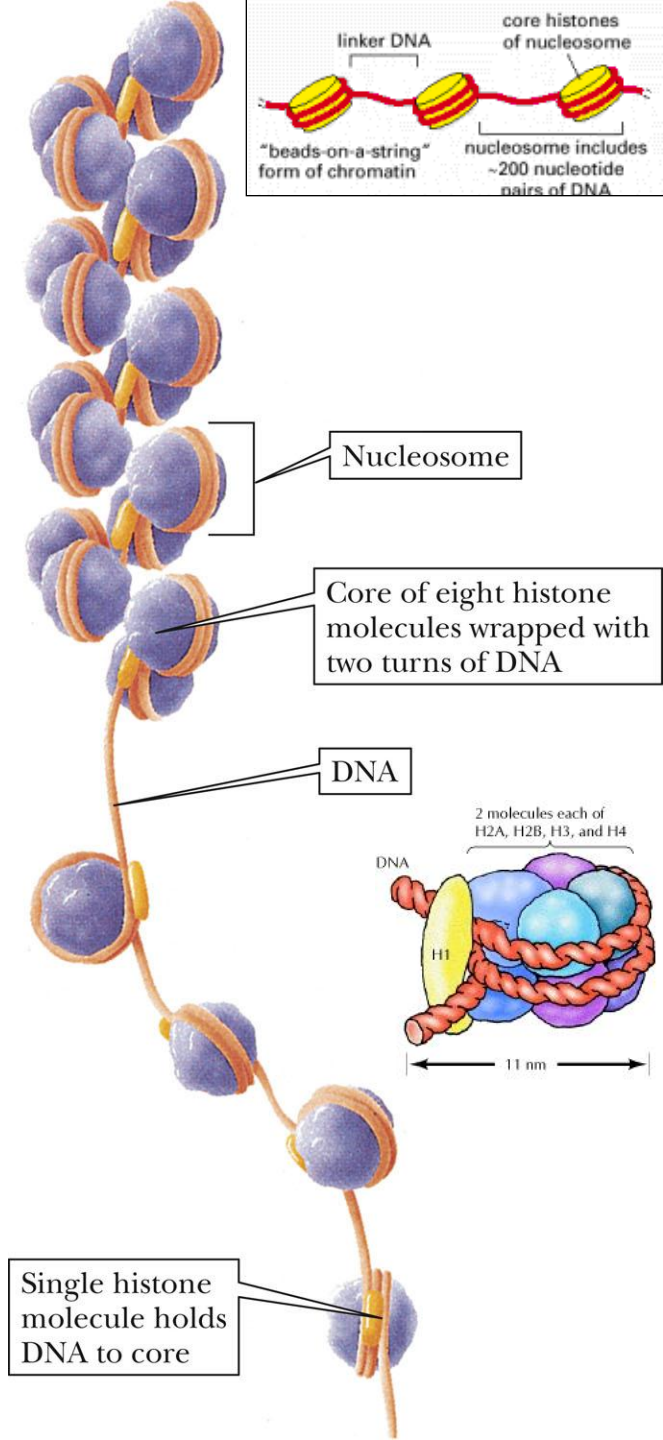
- The three-dimensional arrangement of all atoms of a nucleic acid; commonly referred to as supercoiling
- Circular DNA: a type of double-stranded DNA in which the 5′ and 3′ ends of each stand are joined by phosphodiester bonds
- Supercoiling: further coiling and twisting of DNA helix
- Topoisomerases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





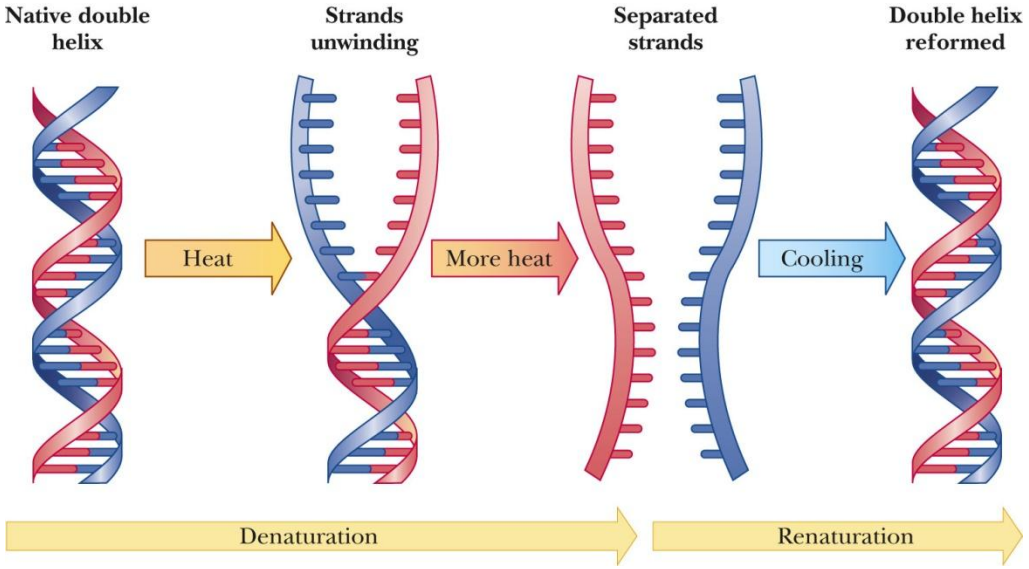
Supercoiling in Eukaryotic DNA

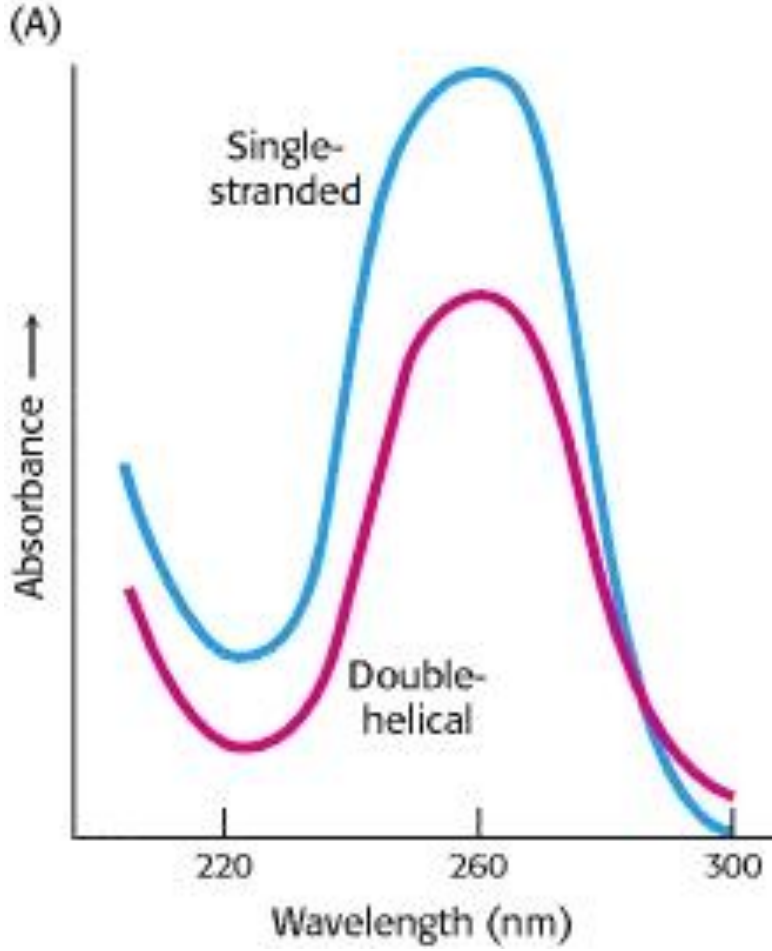
- Histone: a protein, particularly rich in the basic amino acids Lys and Arg; found associated with eukaryotic DNA
  - ✓ Five main types: H1, H2A, H2B, H3, H4
- Chromatin: DNA molecules wound around particles of histones in a beadlike structure
- Each "Bead" is a nucleosome: DNA wrapped around histone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



Denaturation of DNA

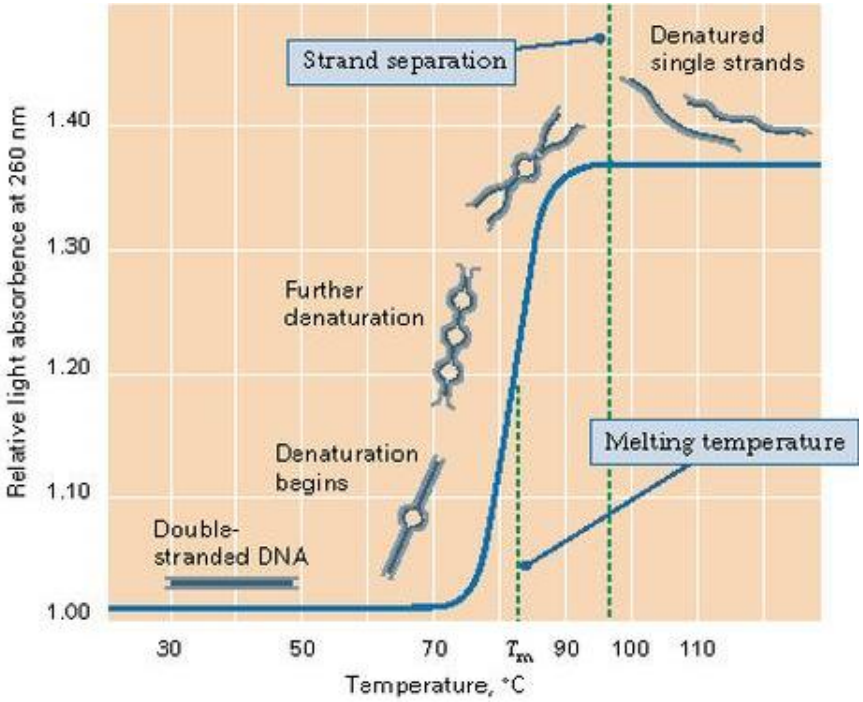
- Disruption of 2° structure
  - ✓ Most commonly by heat
  - ✓ Absorbance at 260 nm increases (hyperchromicity)
  - ✓ Renaturation (annealing) is possible on slow cooling

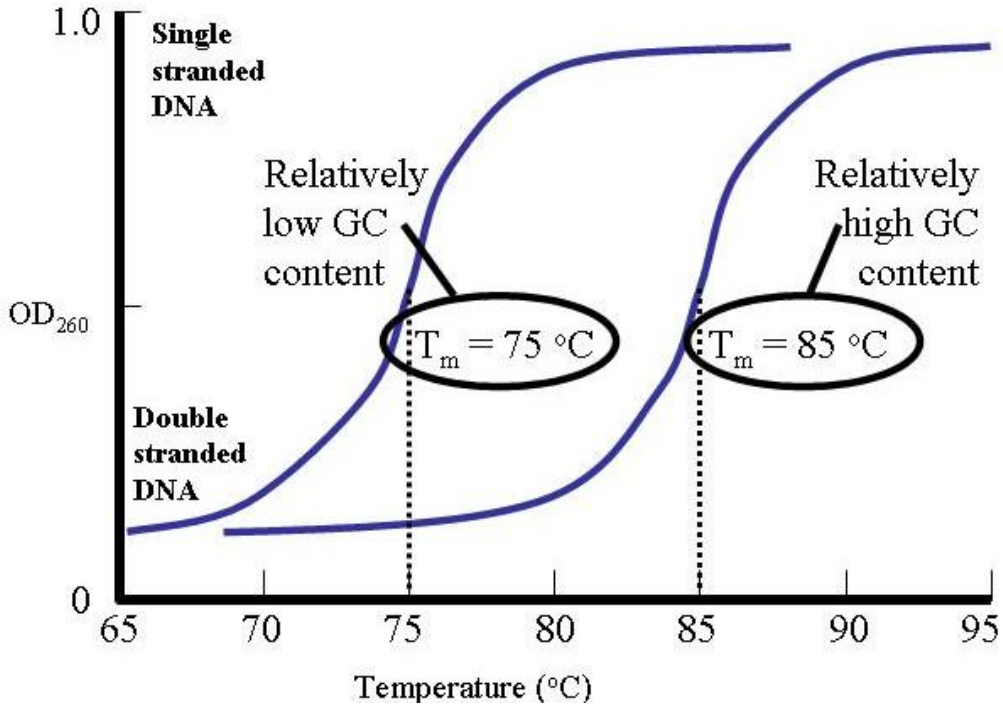




Denaturation of DNA

- ✓ **Midpoint of transition (melting) curve = T_m**
- ✓ **The higher the % G-C, the higher the T_m**





RNA

- Consist of long, unbranched chains of nucleotides joined by phosphodiester bonds between the 3′-OH of one pentose and the 5′-OH of the next
- The pentose unit is β-D-ribose (it is 2-deoxy-D-ribose in DNA)
- The pyrimidine bases are uracil and cytosine (they are thymine and cytosine in DNA)
- In general, RNA is single stranded (DNA is double stranded)

RNA - Classification

- **According to their structure and 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, rRNA & mRNA

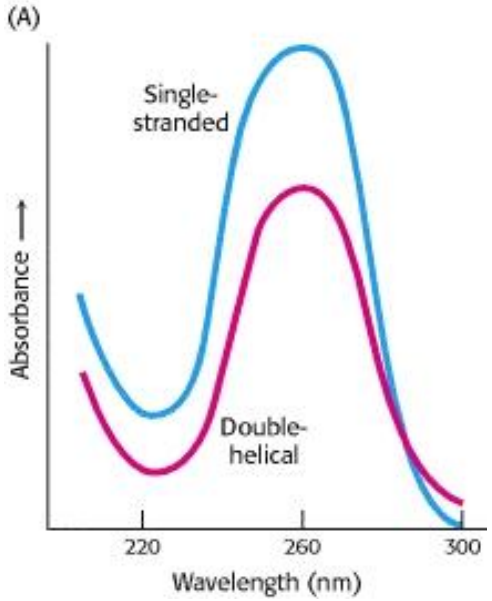
- Transfer RNA, tRNA:
  - ✓ The smallest of the 3
  - ✓ Single-stranded
  - ✓ Carries an amino acid at its 3′ end
  - ✓ Intramolecular hydrogen bonding occurs in tRNA
- Ribosomal RNA, rRNA:
  - ✓ Found in ribosomes (protein synthesis)
  - ✓ Of few types
  - ✓ Constitute ≈ 60% of the ribosomes
- Messenger RNA, mRNA:
  - ✓ Carries coded genetic information
  - ✓ Relatively small amounts & very short-lived
  - ✓ Single stranded

snRNA & others

- Small nuclear RNA (snRNA):
  - ✓ Found in nucleus of eukaryotes
  - ✓ Small (100-200 nucleotides long)
  - ✓ Forms complexes with protein - small nuclear ribonucleoprotein particles (snRNPs)
    - ○ snRNPs help with processing of initial mRNA transcribed from DNA
- MicroRNA (miRNA)
  - ✓ Natural
  - ✓ Translation regulation
- Small interfering RNA (siRNA)
  - ✓ Synthetic
  - ✓ Translation regulation

Light absorbance of nucleic acids

- The peak absorbance is at 260 nm wavelength & it is constant
  - ✓ dsDNA: A260 of 1.0 = 50 ug/ml
  - ✓ ssDNA: A260 of 1.0 = 30 ug/ml
  - ✓ ssRNA: A260 of 1.0 =40 ug/ml



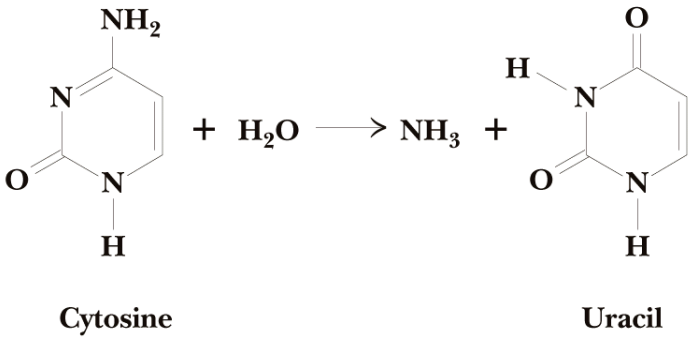
- What is the concentration of a double stranded DNA sample diluted at 1:10 and the A260 is 0.1?

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

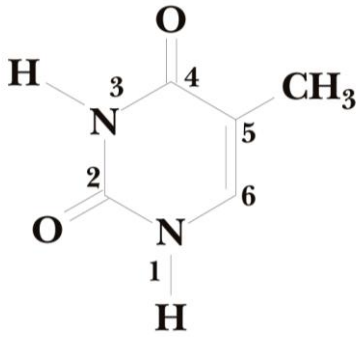
DNA & RNA Differences

1. Thymine vs. Uracil?

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

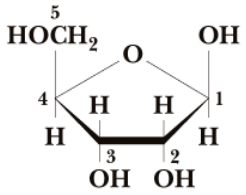


Cytosine Uracil



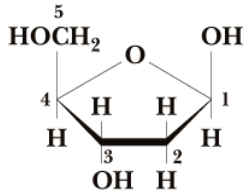
2. The 2'-deoxy sugar?

- Stability
  - ✓ -OH groups (2' and 3') in RNA make it 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