Slide : 3- nucleic acids

Dr. Name : Dr. Mamoun Ahram

Sections : 1-2-3

■ Slide □ Sheet

Medical Committee
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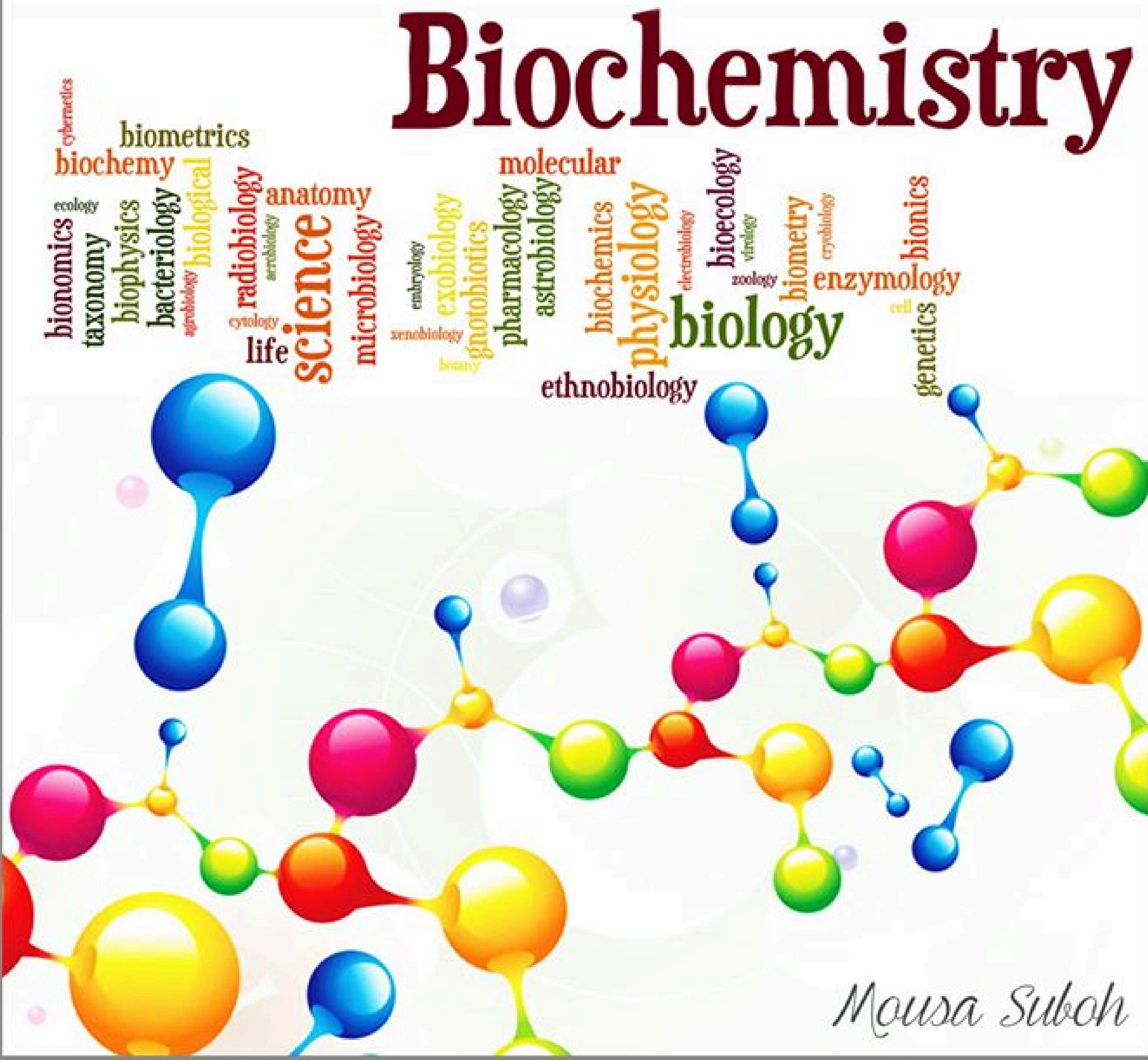


Nucleotides and nucleic acids

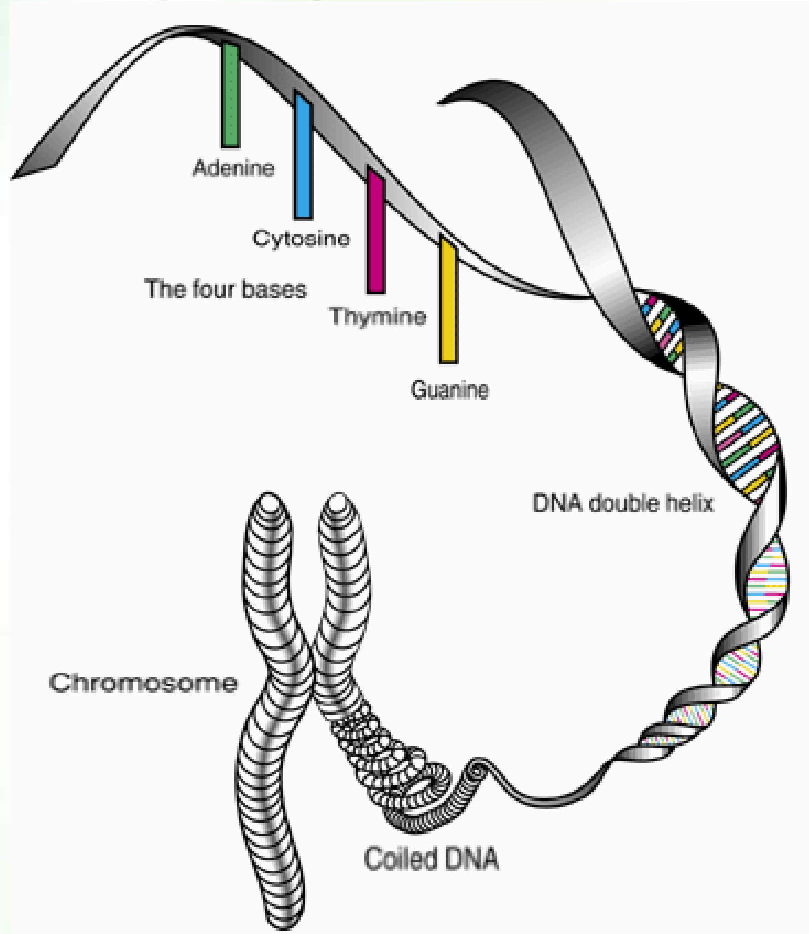
Dr. Mamoun Ahram
Lecture 8
Summer 2013-2014

Resources

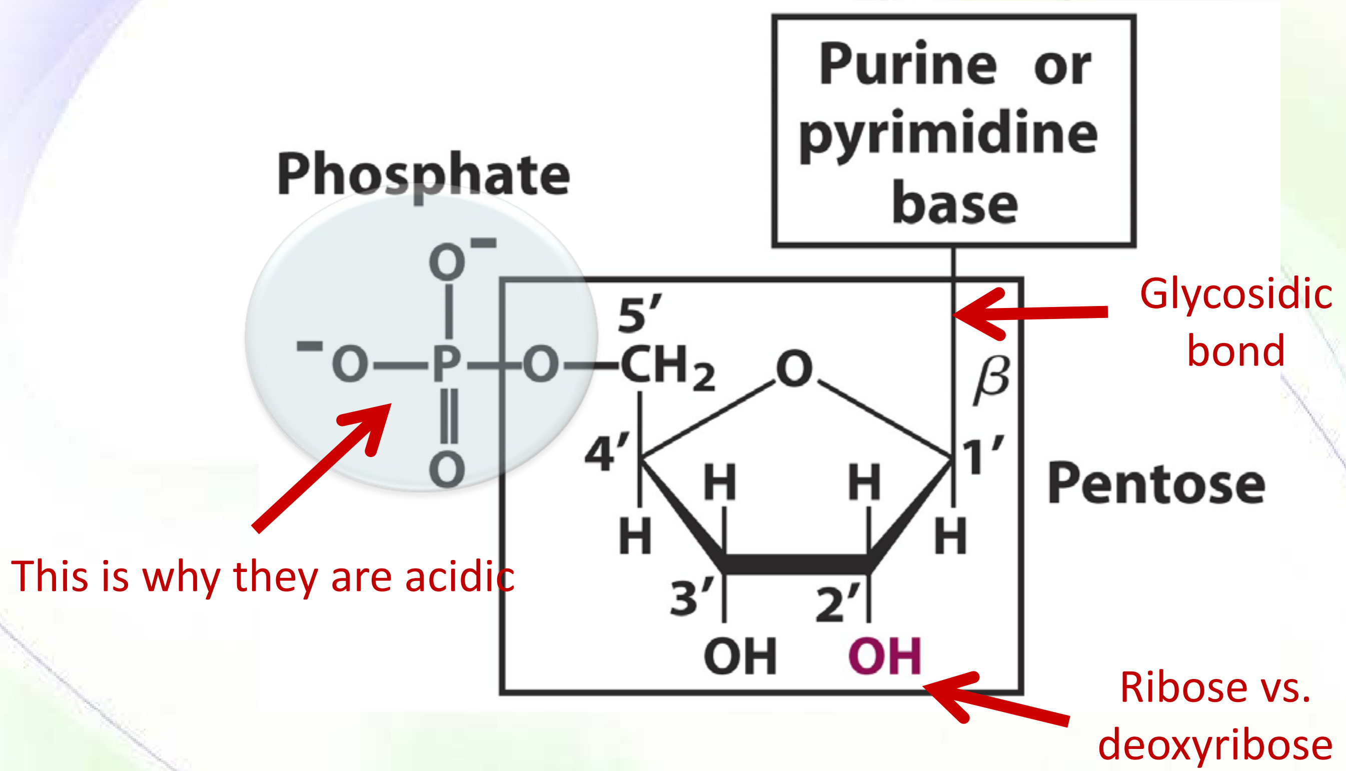
- This lecture
- Campbell and Farrell's Biochemistry, Chapter 9

Nucleic acids

- The primary structure of nucleic acids is the order of bases in the polynucleotide sequence.
- The secondary structure is the three-dimensional conformation of the backbone.
- The tertiary structure is specifically the supercoiling of the molecule.

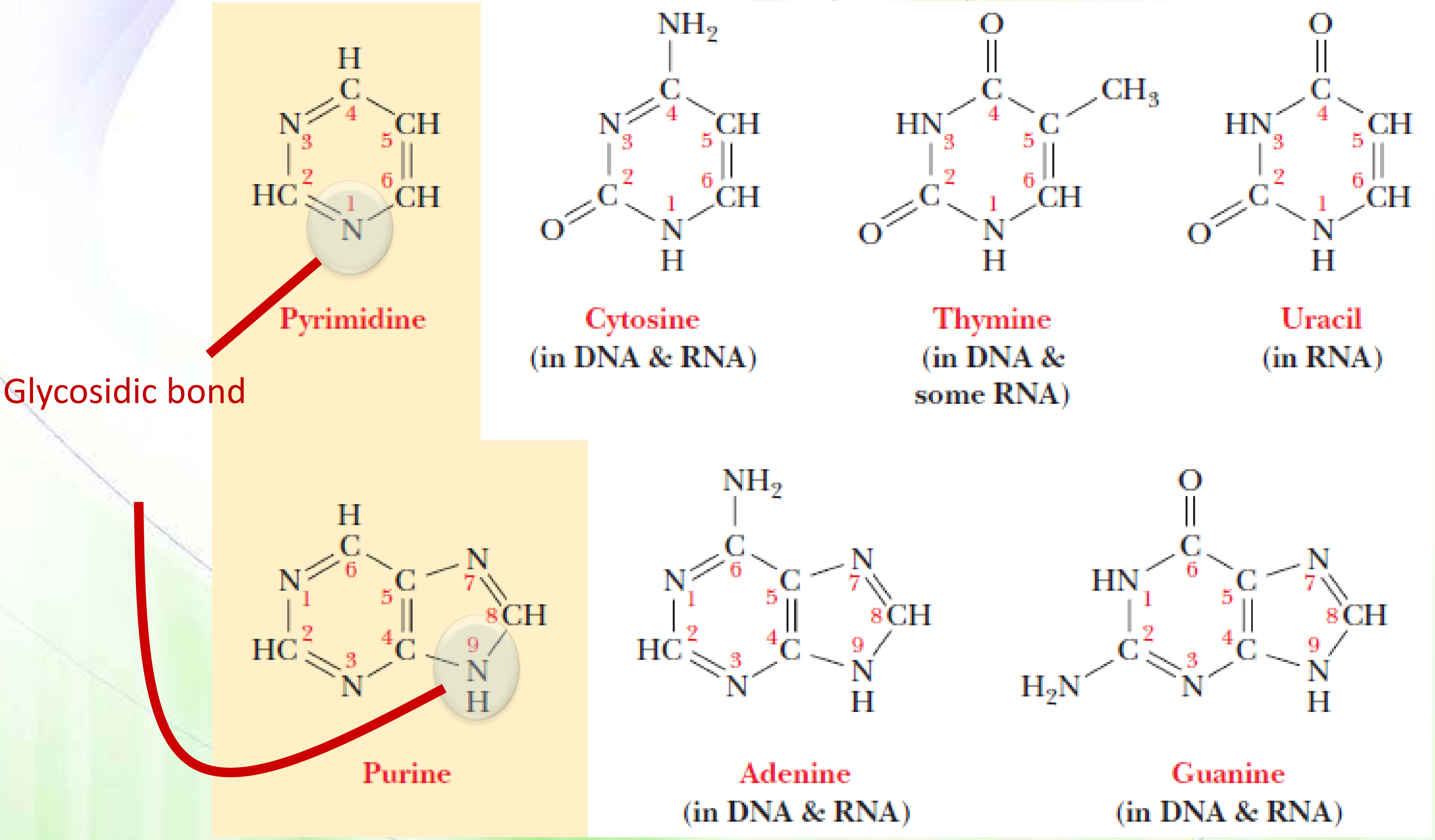


Chemical composition and bonds



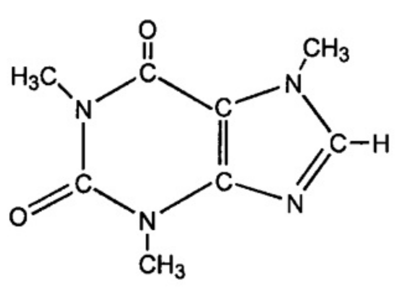
- Positively charged ions (Na+ or Mg2+) and peptides with positively charged side chains can associate with DNA
- Eukaryotic DNA, for example, is complexed with histones, which are positively charged proteins, in the cell nucleus.

Nitrogenous bases

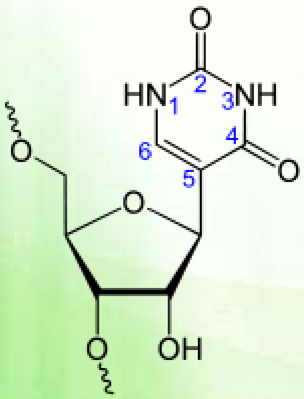


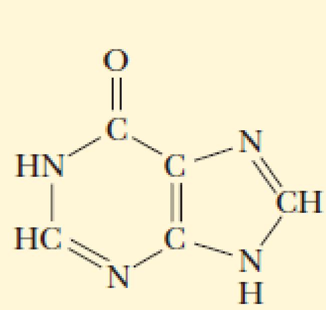
Other nucleotides

- Xanthine, hypoxanthine and uric acid
  - intermediates in purine metabolism
- N6-methyl adenine
- 5-methyl-cytosine (epigenetics)
- Pseudouracil
  - has the ribose attached to C5 instead of N1 of uracil
- 1,3,7-trimethylxanthine (caffeine)

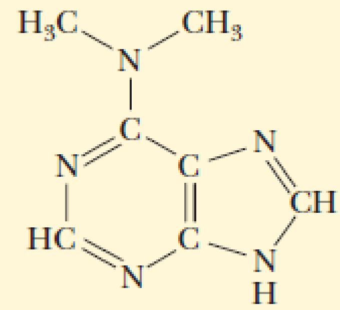


Caffeine

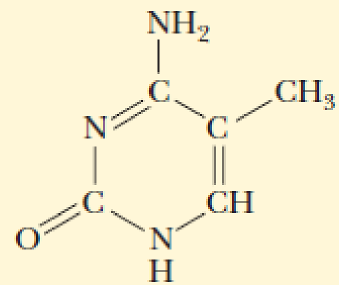




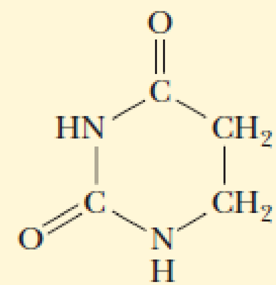
Hypoxanthine



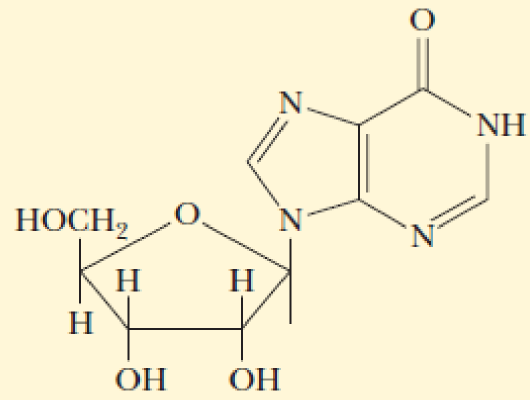
N^6-Dimethyladenine



5-Methylcytosine

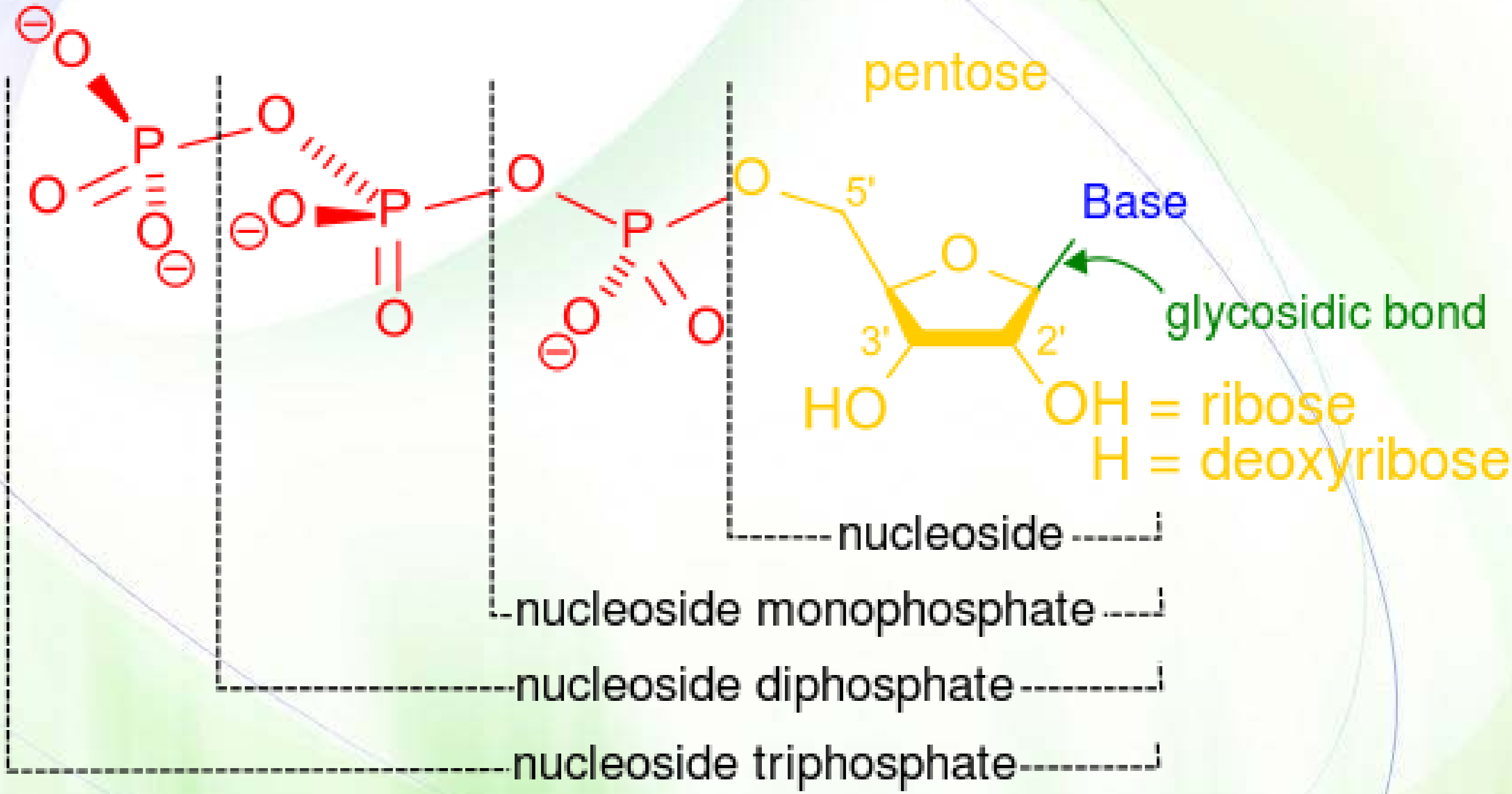


5,6-Dihydrouracil

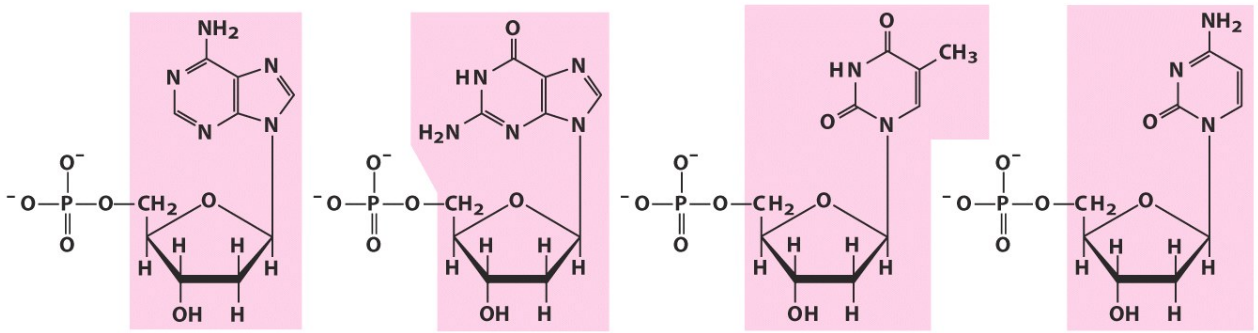


Inosine, an uncommon nucleoside

Nucleotides vs. Nucleosides

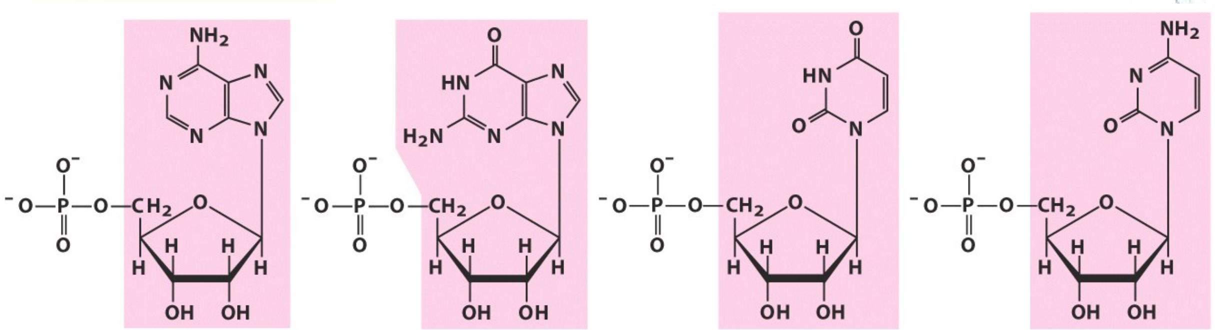


Nucleotides vs. Nucleosides



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

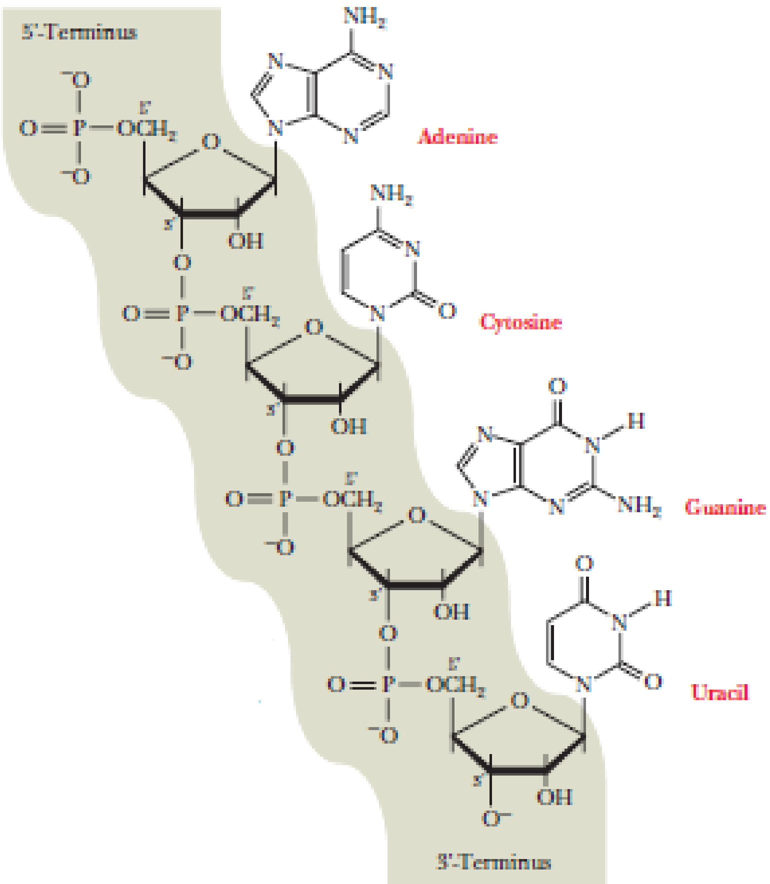
(a) Deoxyribonucleotides

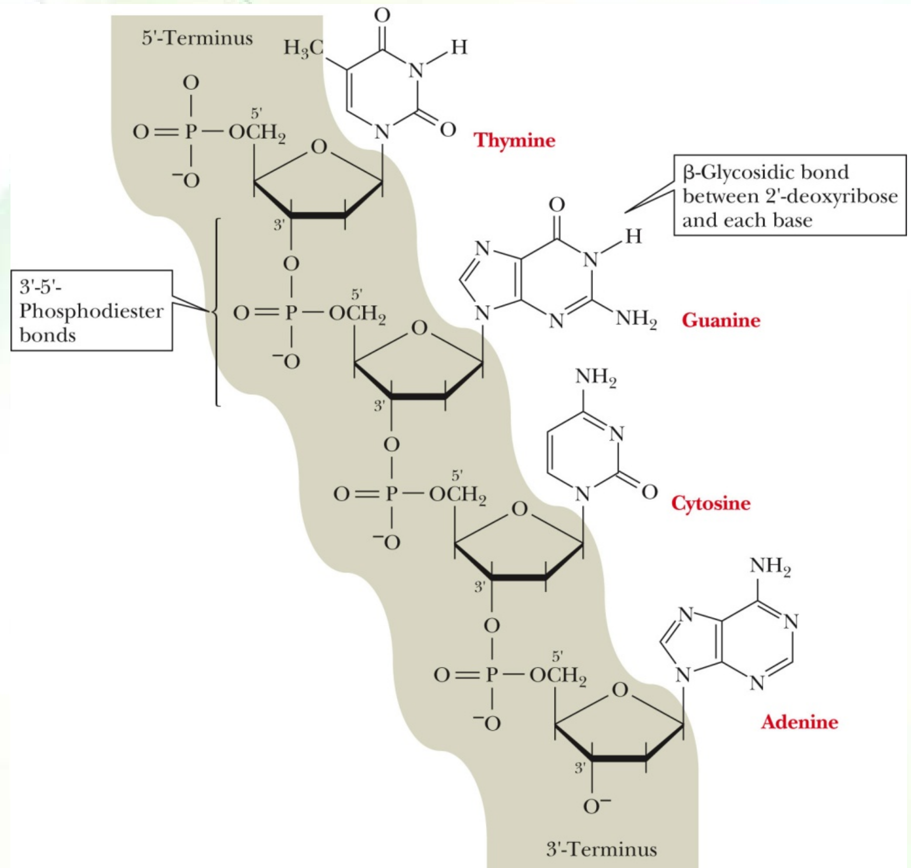


Nucleotide:	Adenylate (adenosine 5′-monophosphate)	Guanylate (guanosine 5′-monophosphate)	Uridylate (uridine 5′-monophosphate)	Cytidylate (cytidine 5′-monophosphate)
Symbols:	A, AMP	G, GMP	U, UMP	C, CMP
Nucleoside:	Adenosine	Guanosine	Uridine	Cytidine

(b) Ribonucleotides

Nucleic acid polymer

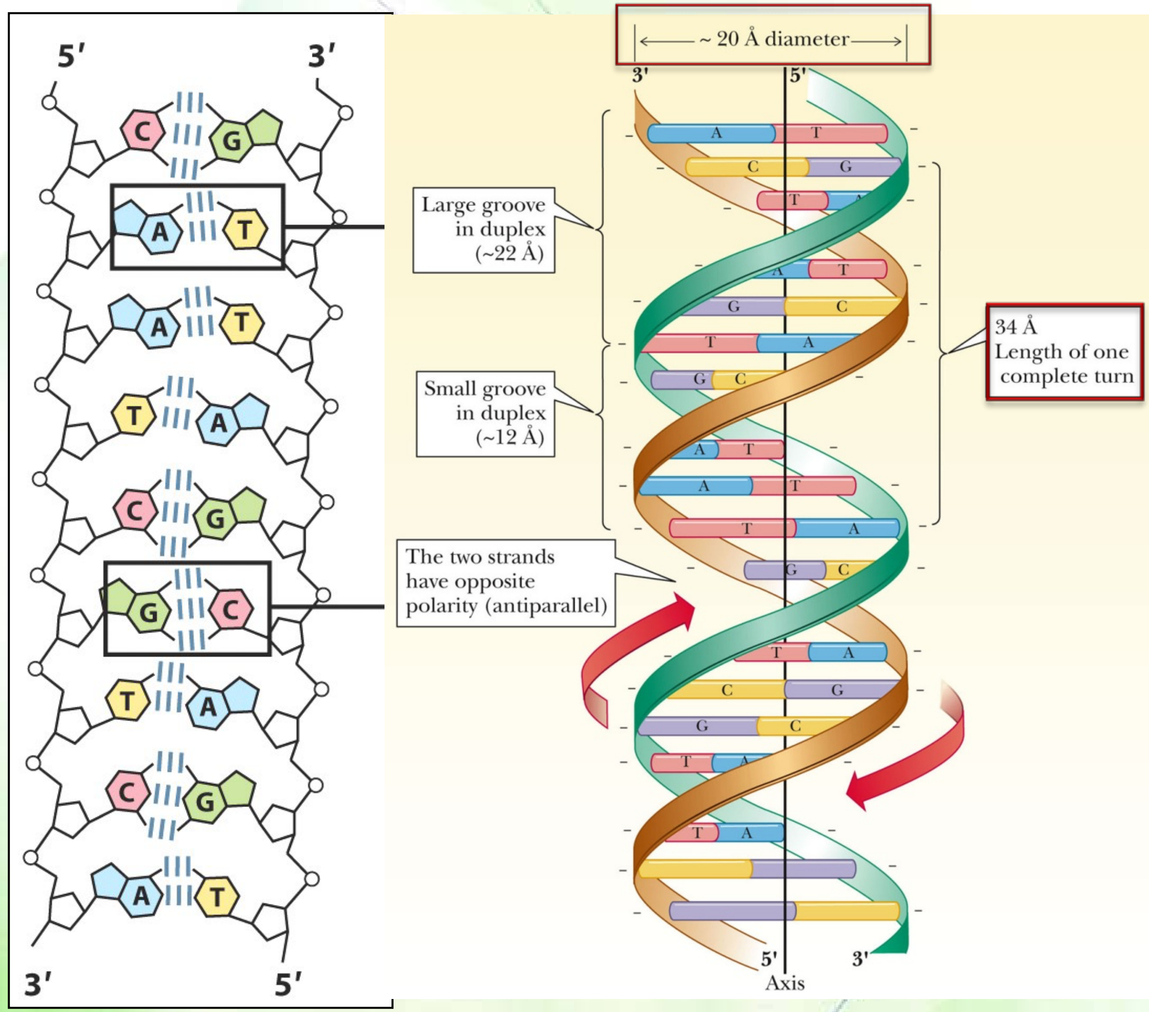


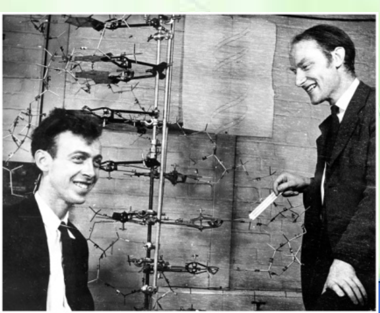


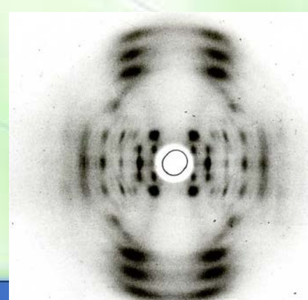
- A letter d can be added to indicate a deoxyribonucleotide residue.
  - for example, dG is substituted for G.
  - The deoxy analogue of a ribooligonucleotide would be d(GACAT).

DNA structure

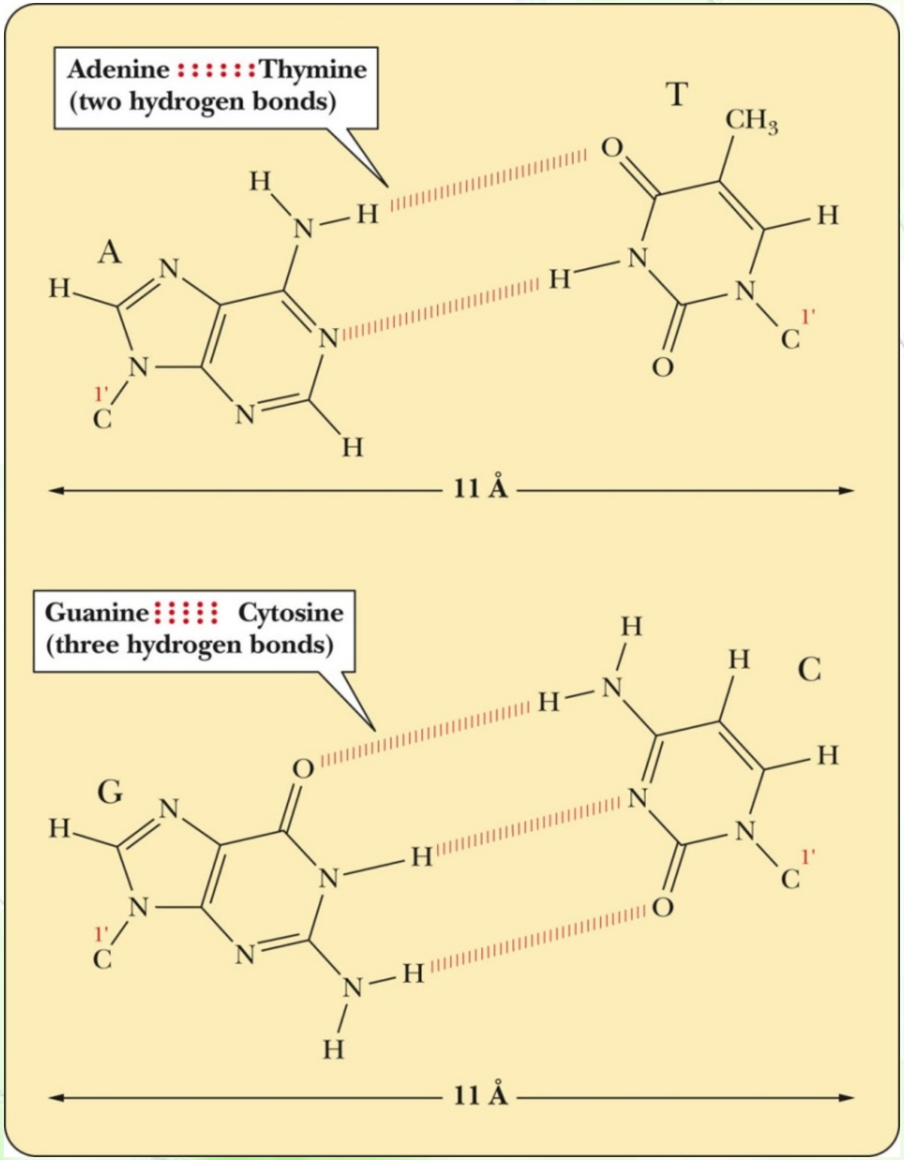
- Specific base-pairing
  - A = T; G = C; Pur = pyr
- Complementary
- A double helix
- Backbone vs. side chains
- Antiparallel
- Stable
- Flexible
- Groovings
- Stability vs. flexibility





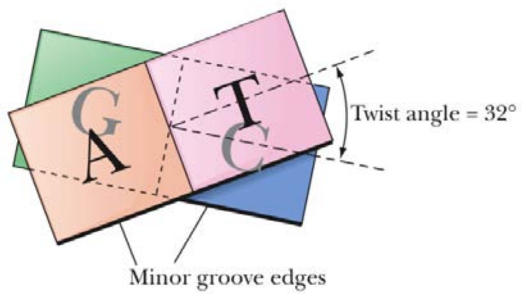


Base pairing

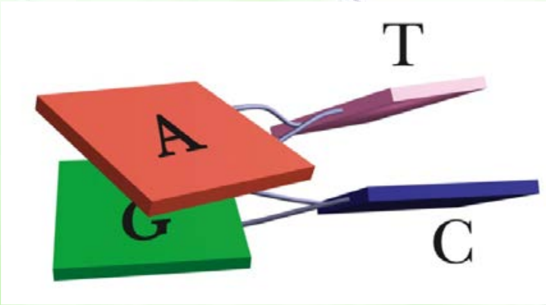


Chemical forces

- Hydrogen bonds
- Ionic interactions (repulsive)
- Both van der Waals and hydrophobic interactions drive the formation of DNA (and RNA) double helices.
  - Base stacking
  - Propeller twist



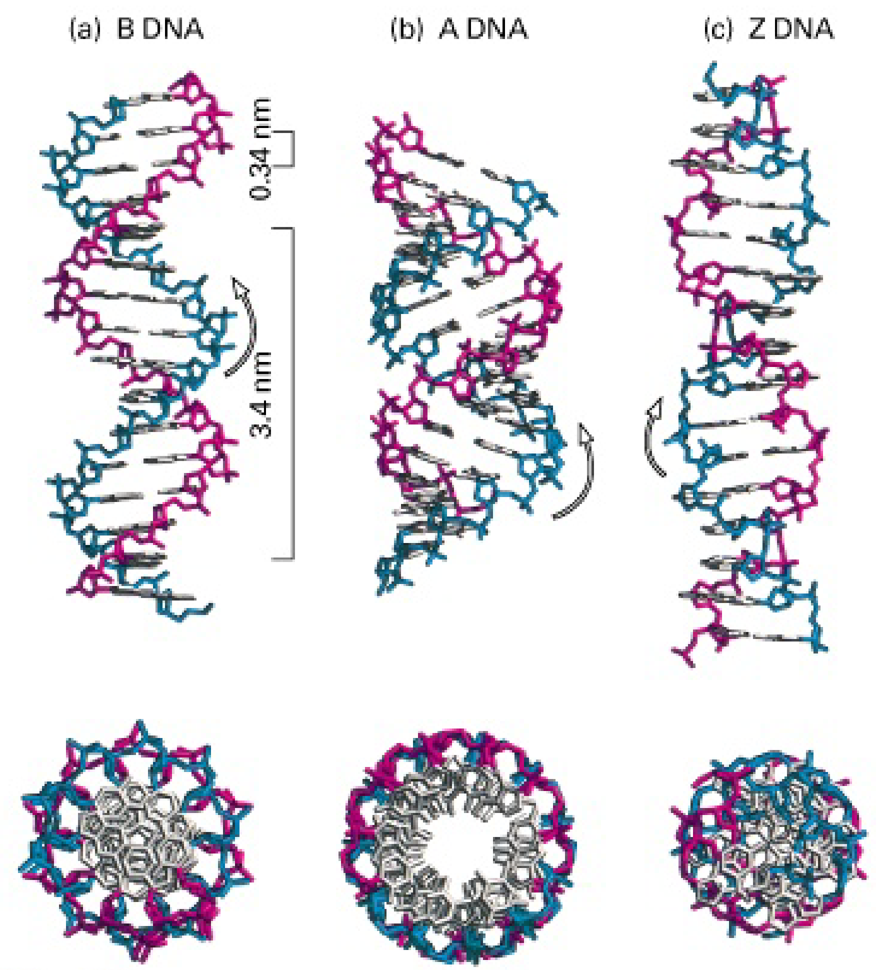
- Maximal base pairing, but
- Not optimal overlap of the bases.
- The edges of the bases that are exposed to the minor groove unfavorably come in contact with water in this form.



- The base-pairing distances are less optimal.
- Base stacking is more optimal.
- Water is eliminated from the minor-groove contacts with the bases.

DNA forms

- **B-DNA**
  - The principal form of DNA.
  - Right-handed
  - Base pairs are perpendicular.
- **A-DNA**
  - 11 base pairs per turn
  - Base pairs lie at an angle
  - Right-handed.
  - Wider than B-DNA
- **Z-DNA**
  - Left-handed
  - Occurs when alternating purine–pyrimidine and sequences with methylated C
  - Narrower than B-DNA



DNA structure

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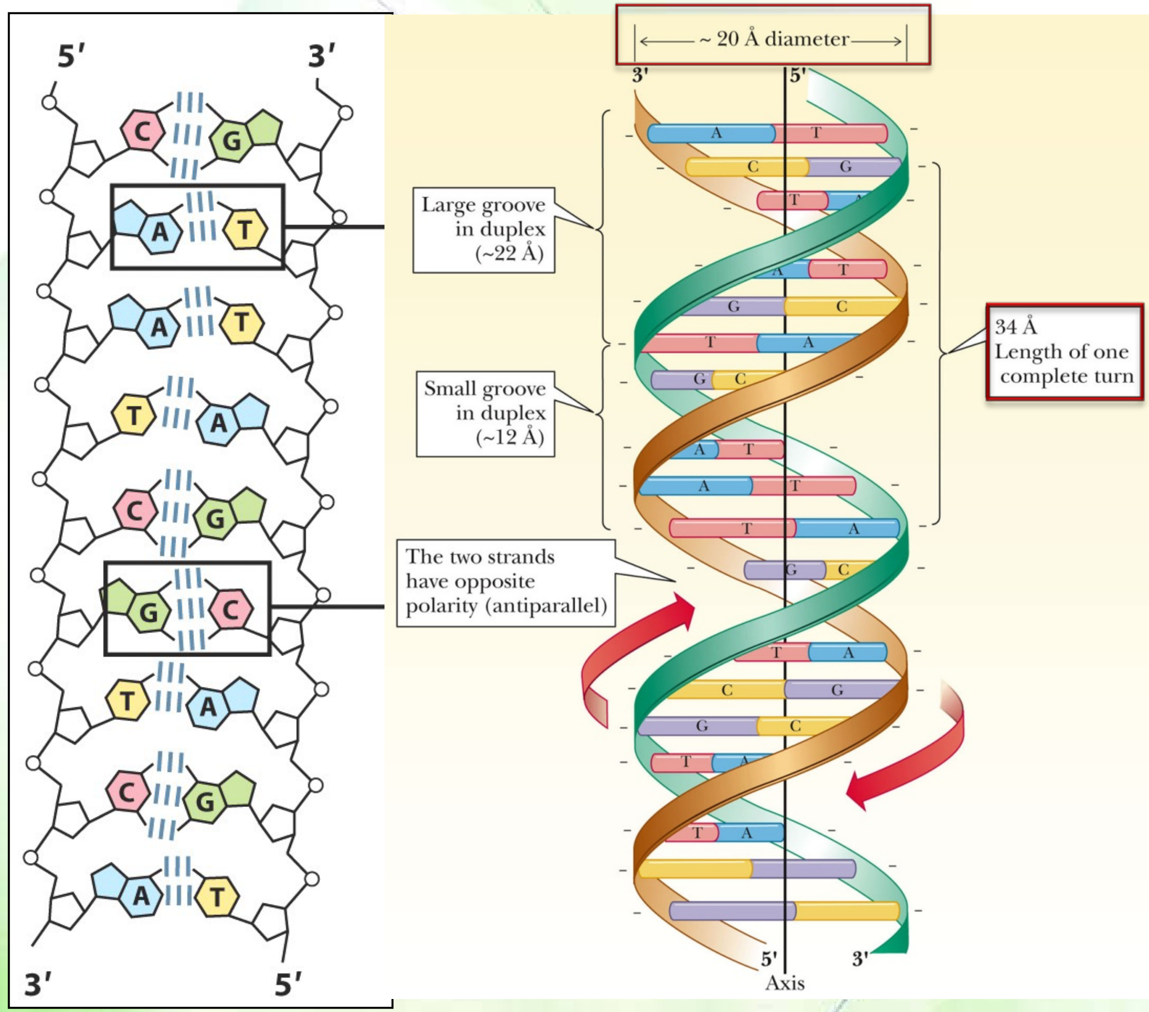
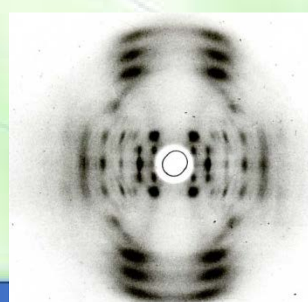
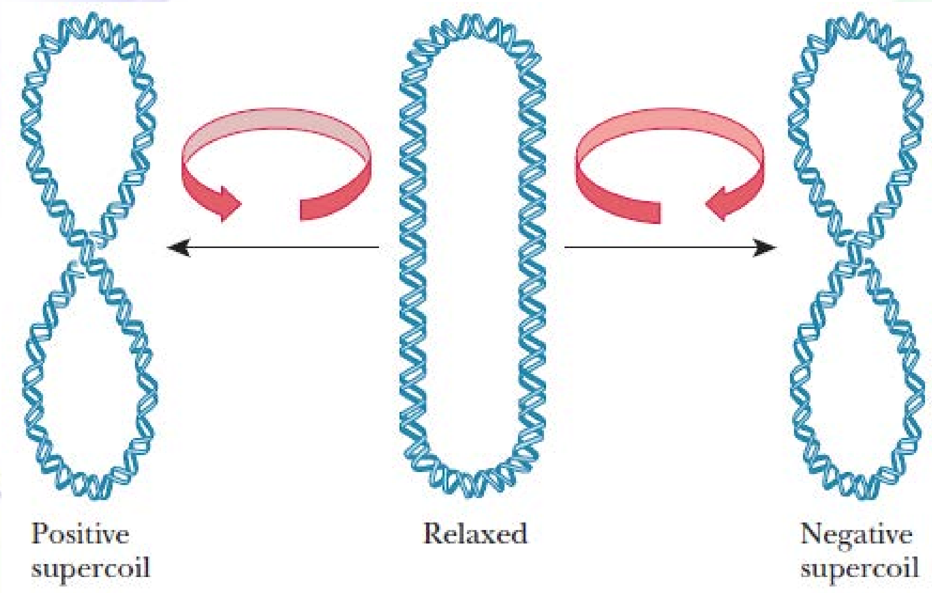


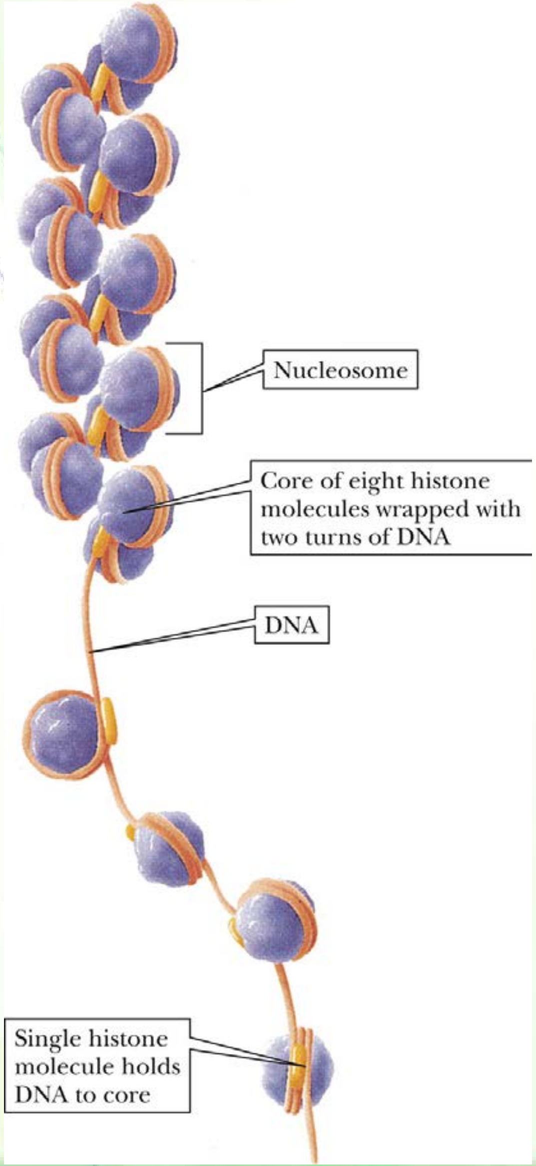


DNA coiling

Prokaryotic DNA



Eukaryotic DNA

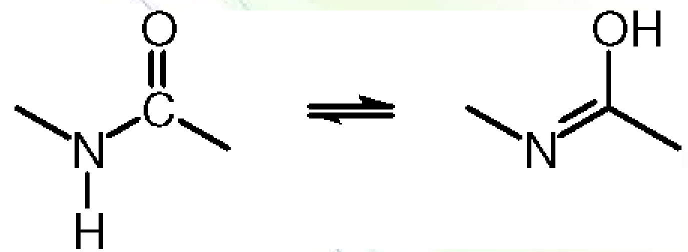


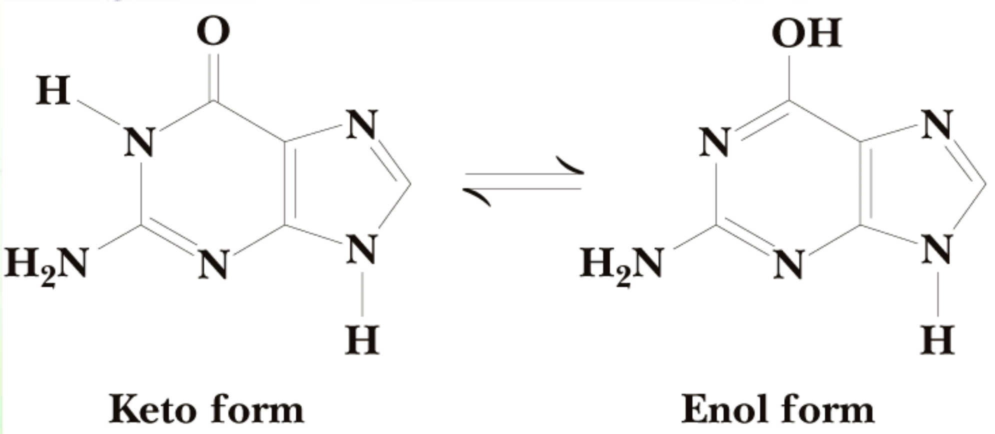
- Chromatin = DNA molecule + proteins
- A nucleosome consists of DNA wrapped around a histone core.
- The protein core is an octamer (two molecules of histones H2A, H2B, H3, and H4).
- Connecting the nucleosome is a *spacer regions that* consists of DNA complexed to H1 histone and nonhistone proteins.
- Positive charges of histones: interaction and charge neutralization

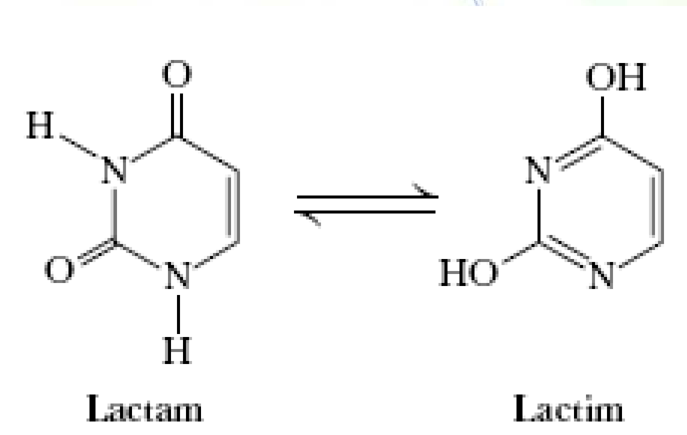
Properties of Pyrimidines & Purines (1)

Keto-enol tautomerism

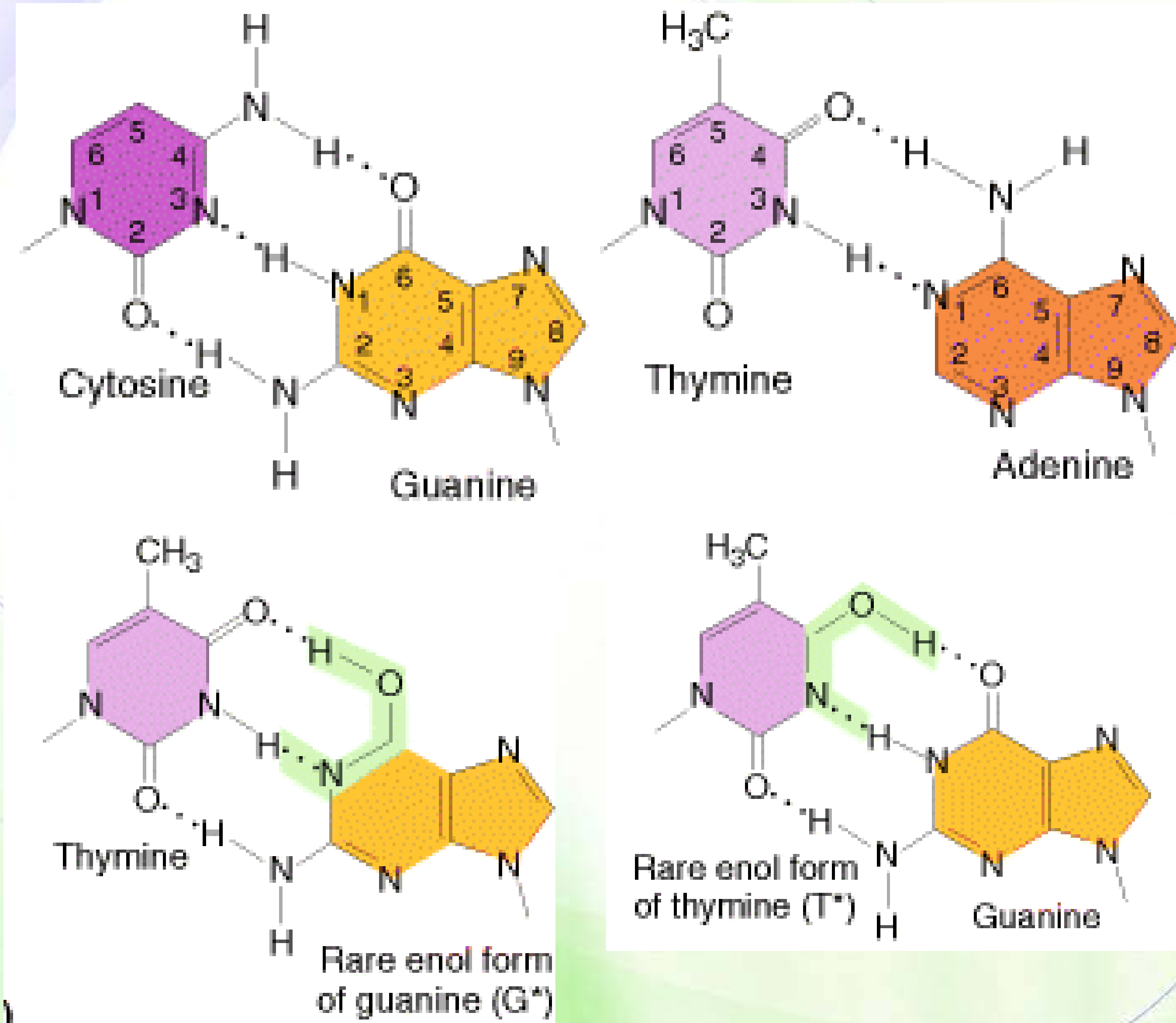
- Tautomers are constitutional isomers of organic compounds that readily interconvert by a chemical reaction.
- The keto tautomer (lactam), whereas the enol form (lactim)
- The lactam form predominates at neutral pH (pKa values for ring nitrogen atoms 1 & 3 in uracil are greater than 8)







Mispairing of tautomers



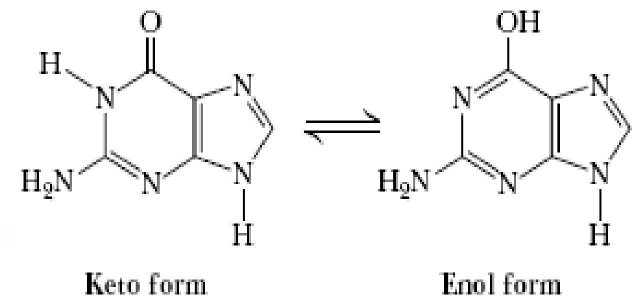
Properties of Pyrimidines & Purines (2)

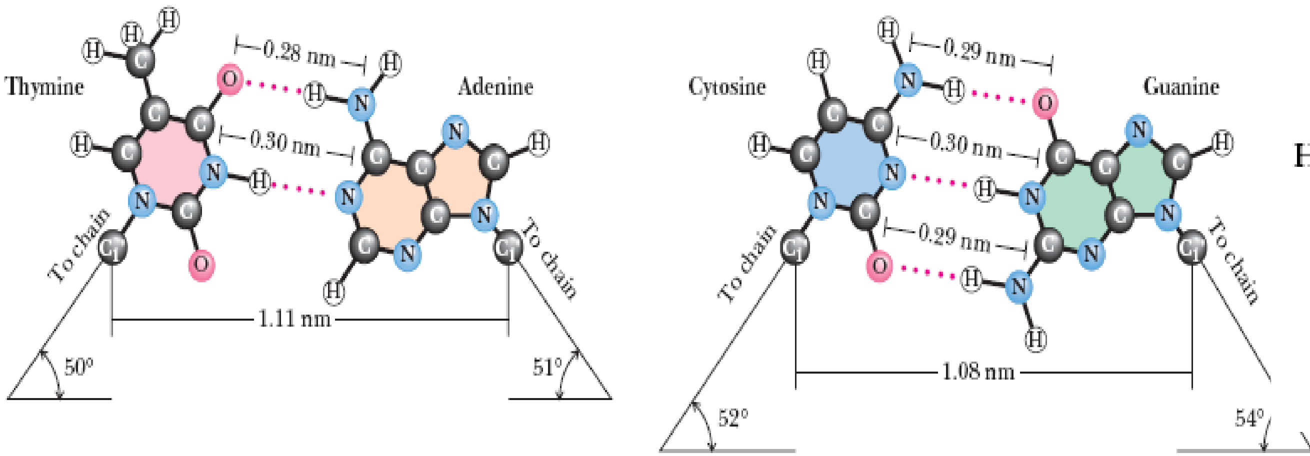
Acid/base dissociations

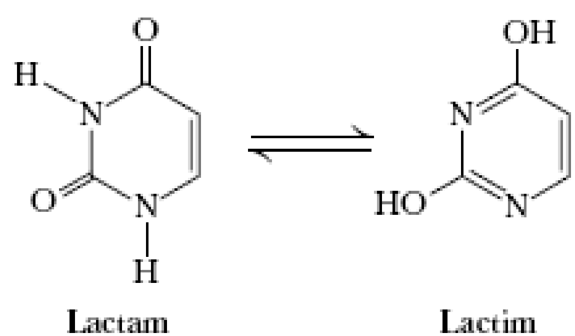
Proton Dissociation Constants (pK_a Values) for Nucleotides

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

- 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

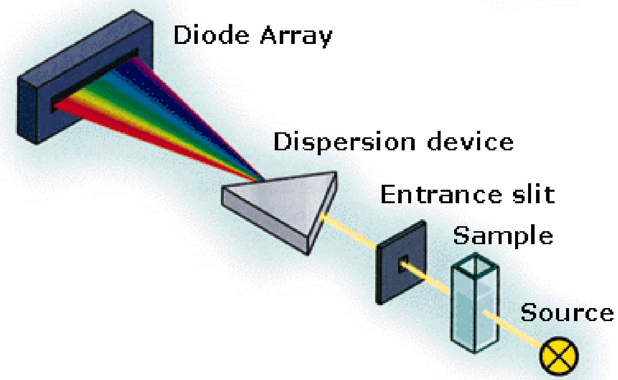






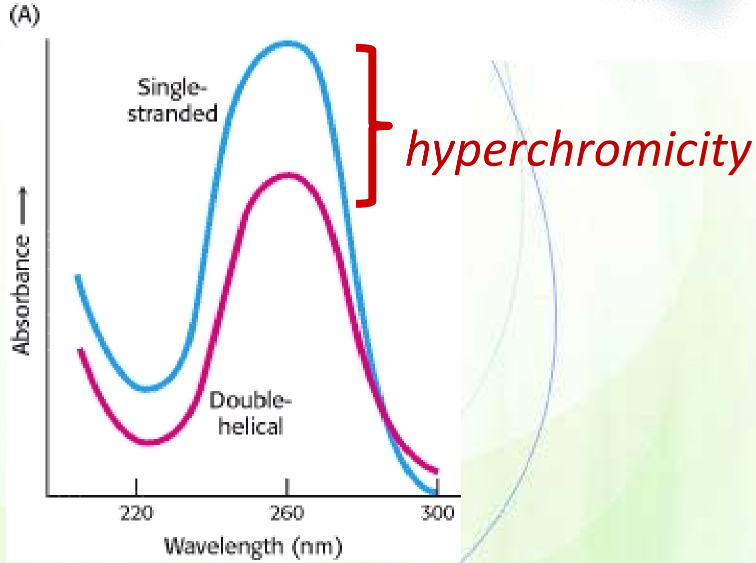
Light absorbance of nucleic acids

- Aromatic pyrimidines and purines can absorb UV light
- The peak absorbance is at 260 nm wavelength



- The absorbance of 260 nm (A260) 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

Reason for ss vs. ds absorbance:
- Stacked bases, vs. unstacked bases

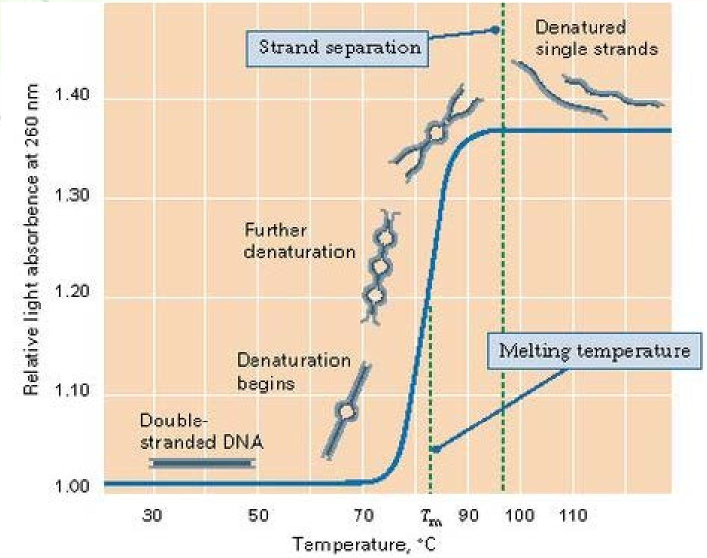


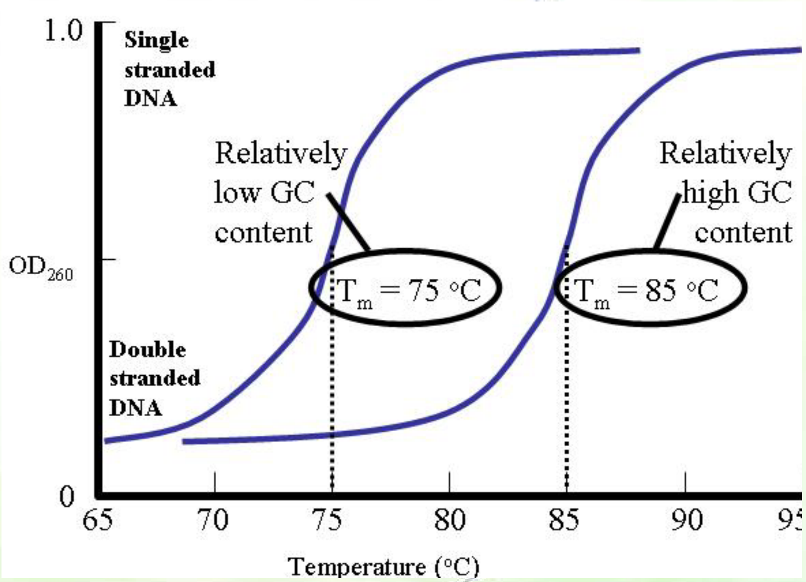
What is the concentration of a double stranded DNA sample diluted at 1:10 and the A260 is 0.1?

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

Observation of denaturation

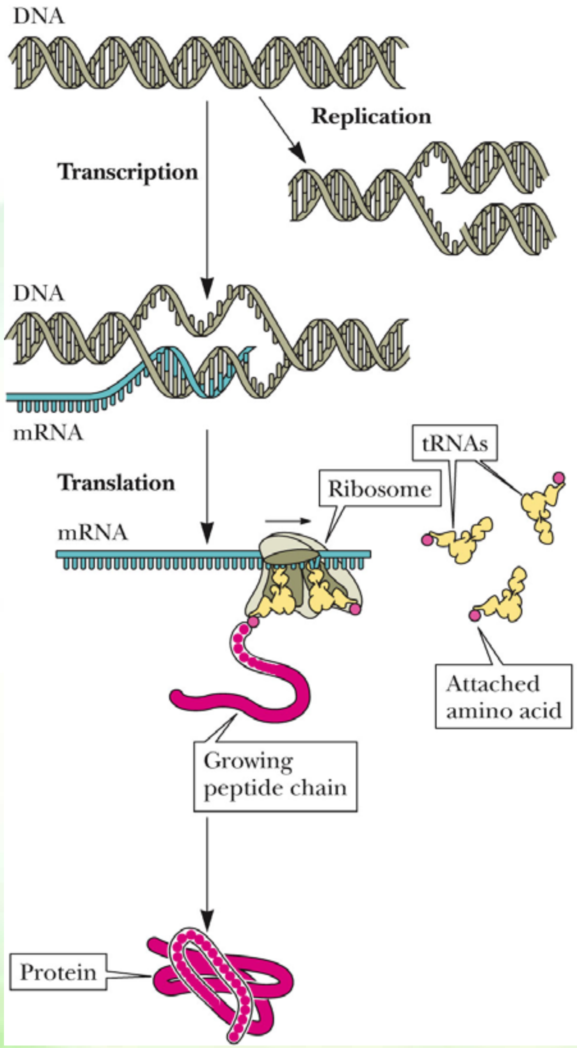
- The transition temperature, or melting temperature (Tm).
- Factors influencing Tm
  - G·C pairs
    - Hydrogen bonds
    - Base stacking
  - pH
  - Salt and ion concentration
  - Destabilizing agents (alkaline solutions, formamide, urea)





Central dogma of biology

- Genetic information must be preserved via DNA replication.
- Information must be translated into action makers (proteins) via transcription and translation.
- RNA Sequence is dictated by DNA sequence.

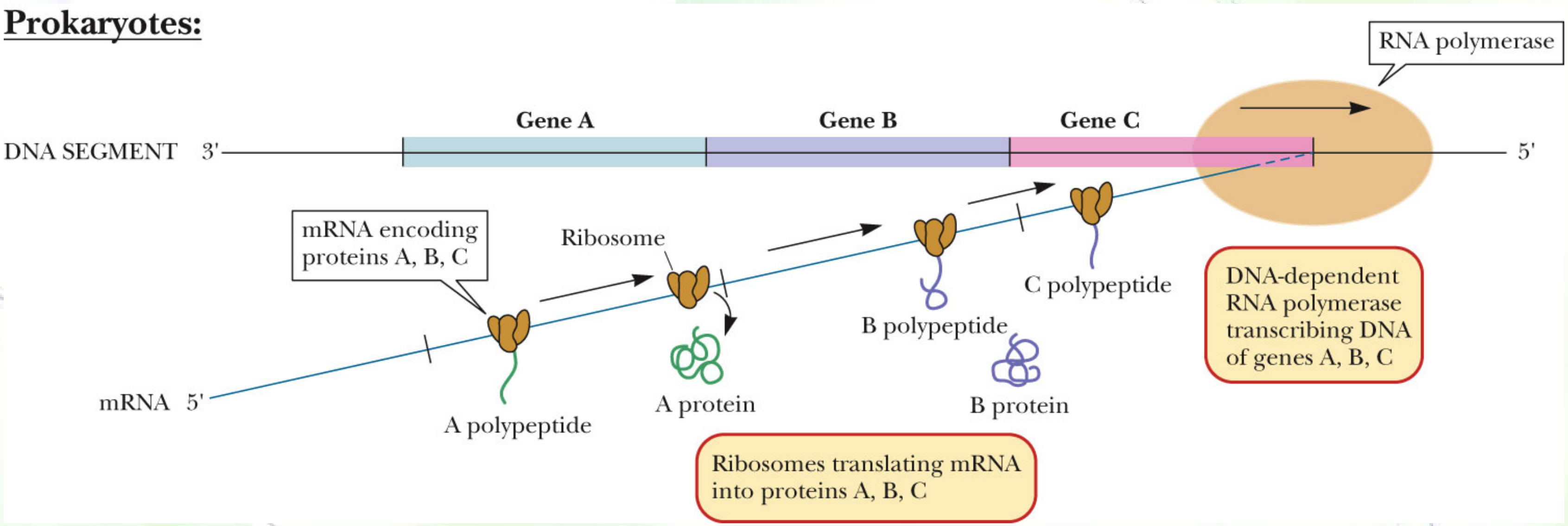


Replication
DNA replication yields two DNA molecules identical to the original one, ensuring transmission of genetic information to daughter cells with exceptional fidelity.

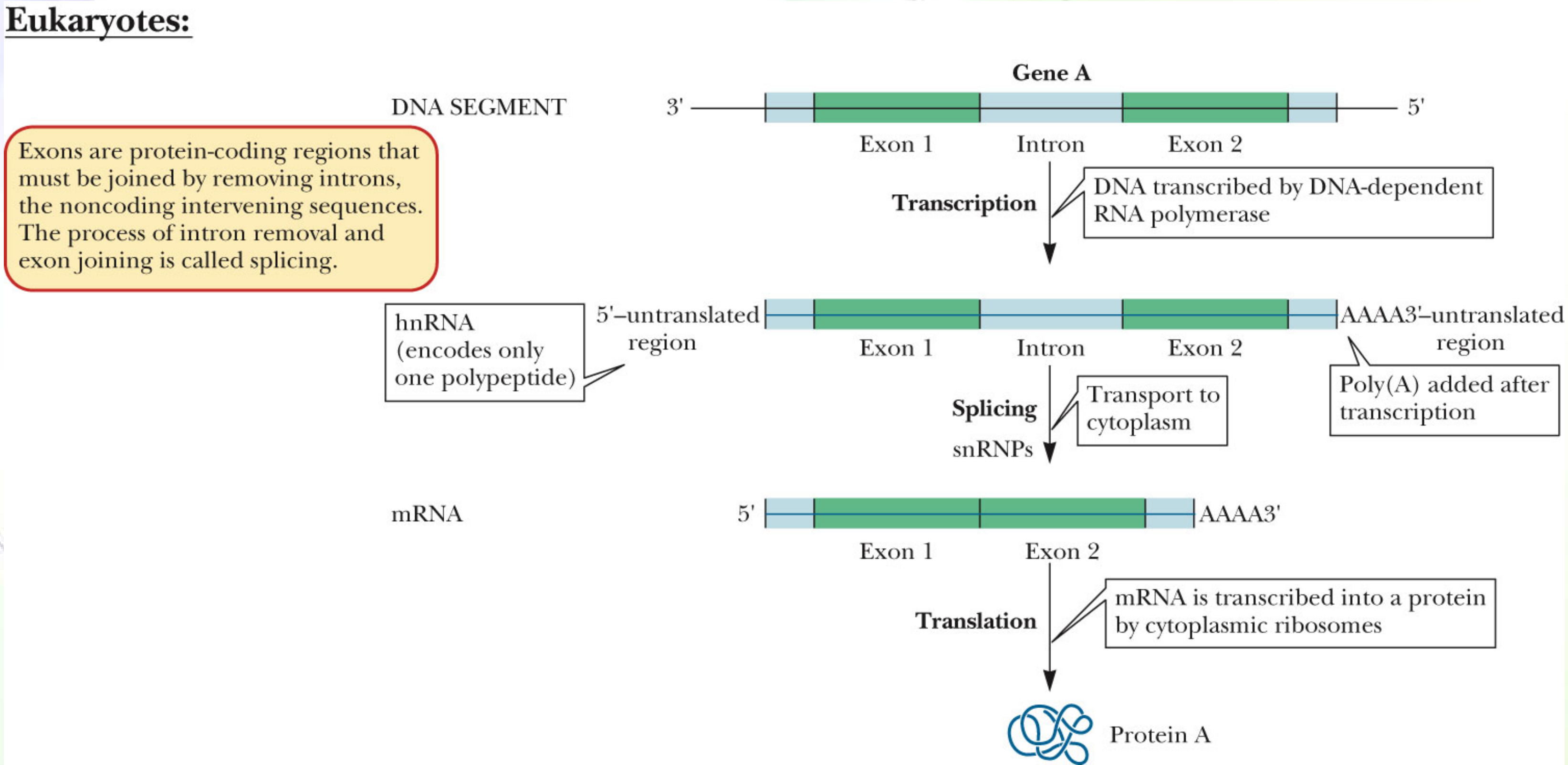
Transcription
The sequence of bases in DNA is recorded as a sequence of complementary bases in a single-stranded mRNA molecule.

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.

Transfer of information in prokaryotes



Transfer of information in eukaryotes



RNA

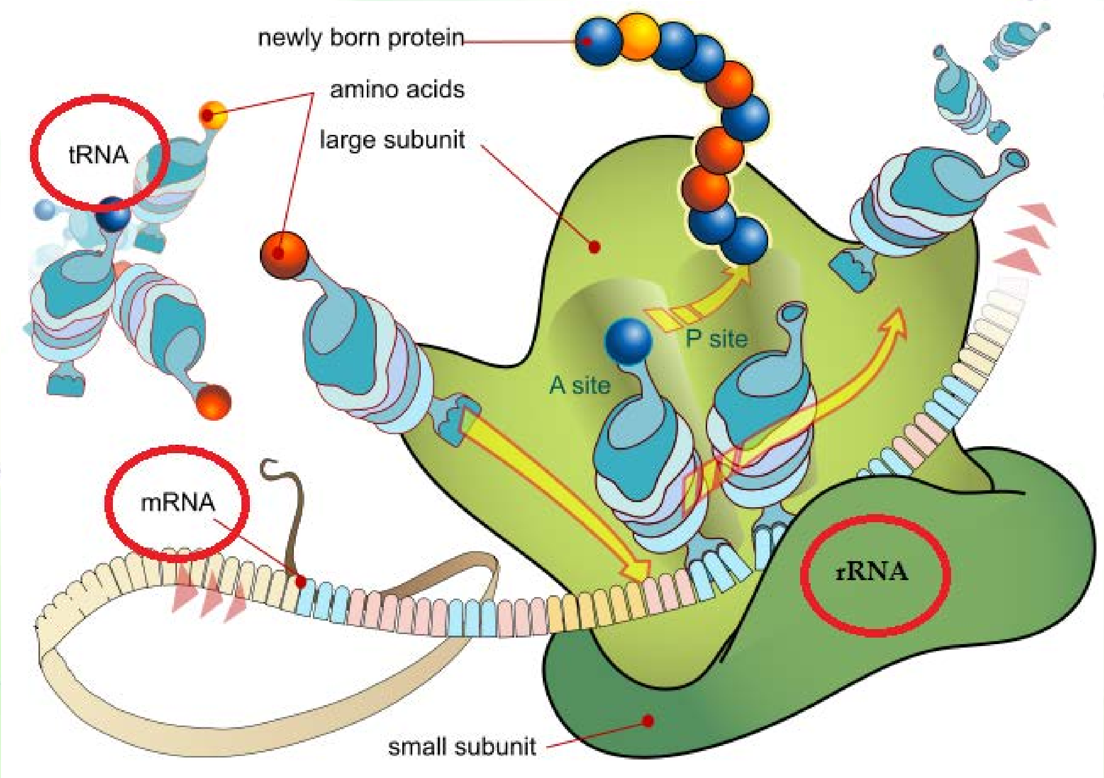
- Consist of long, unbranched chains of nucleotides joined by phosphodiester bonds between the 3’-OH of one pentose and the 5’-OH of the next
- The pentose unit is β-D-ribose (it is 2-deoxy-D-ribose in DNA)
- The pyrimidine bases are uracil and cytosine (they are thymine and cytosine in DNA)
- In general,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

Types of RNA

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

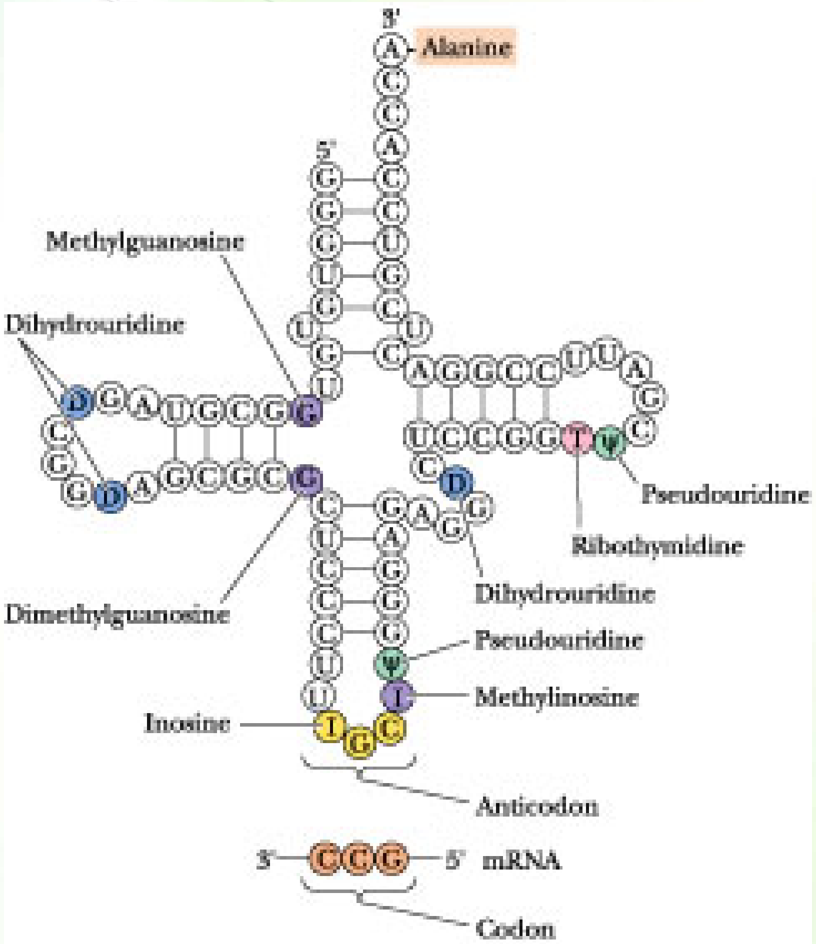
Ribosomal RNA (rRNA)

- Ribosomal RNA molecules comprise 65 to 70% of the mass of the ribosome
- The rRNA maintains the structure of the ribosome and provides sites for the binding of mRNA and protein synthesis



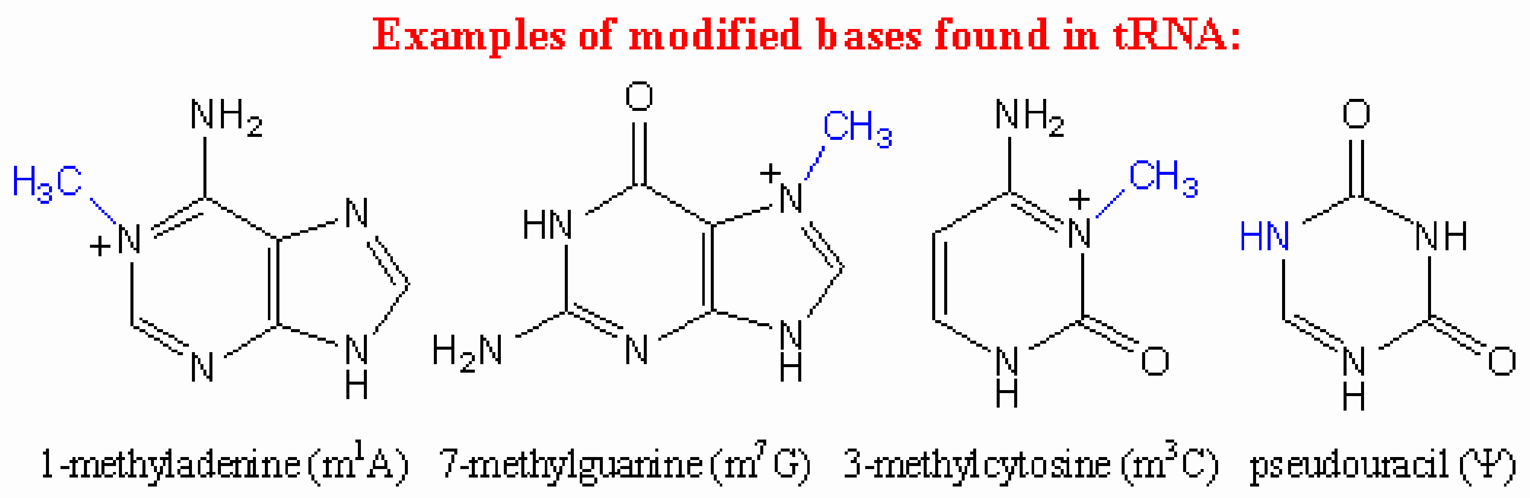
Transfer RNA (tRNA)

- tRNA is a ~75 base molecule that carries the amino acids, and transfers them to the growing protein.
- tRNAs have a common tertiary structure.
- The hydrogen-bonded portions of the molecule are called stems, and the non-hydrogen-bonded portions are loops.
- Some of these loops contain modified bases



Modified nucleotides in tRNA

- tRNAs contain modified bases



Other RNA molecules

- Small nuclear RNA (snRNA)
  - it is complexed with proteins forming small nuclear ribonucleoprotein particles, usually abbreviated snRNPs.
  - RNA processing
- MicroRNA (miRNA)
  - Natural
  - translation regulation
- Small interfering RNA (siRNA)
  - Synthetic
  - Translation regulation