NUCLEOTIDES & NUCLEIC ACIDS

What Constitutes Life?

- Basic requirements for life
  - ability to reproduce
  - ability to acquire specific molecules and use them in controlled chemical rxns
    - need enclosed space (membrane)
    - living entity vs living organism
    - organisms have cells
- Self-replicating RNA molecule was likely 1st living entity
  - RNA = nucleic acid made of nucleotides

Roles of nucleotides

- Building blocks of nucleic acids (RNA, DNA)
  - Analogous to amino acid role in proteins
- Energy currency in cellular metabolism (ATP: adenosine triphosphate)
- Allosteric effectors
- Structural components of many enzyme cofactors (NAD: nicotinamide adenine dinucleotide)

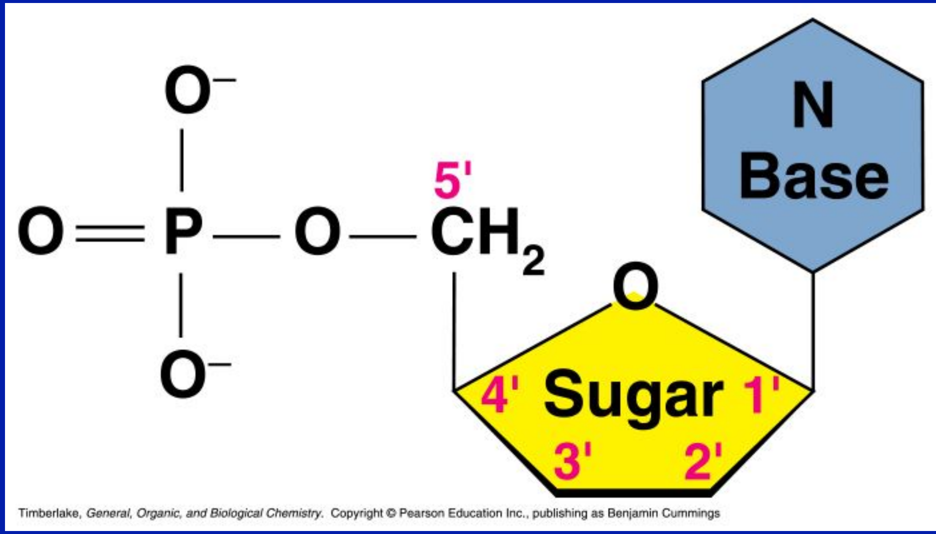
Roles of nucleic acids

- DNA contains genes, the information needed to synthesize functional proteins and RNAs
- DNA contains segments that play a role in regulation of gene expression (promoters)
- Ribosomal RNAs (rRNAs) are components of ribosomes, playing a role in protein synthesis
- Messenger RNAs (mRNAs) carry genetic information from a gene to the ribosome
- Transfer RNAs (tRNAs) translate information in mRNA into an amino acid sequence
- RNAs have other functions, and can in some cases perform catalysis

Nucleic Acids

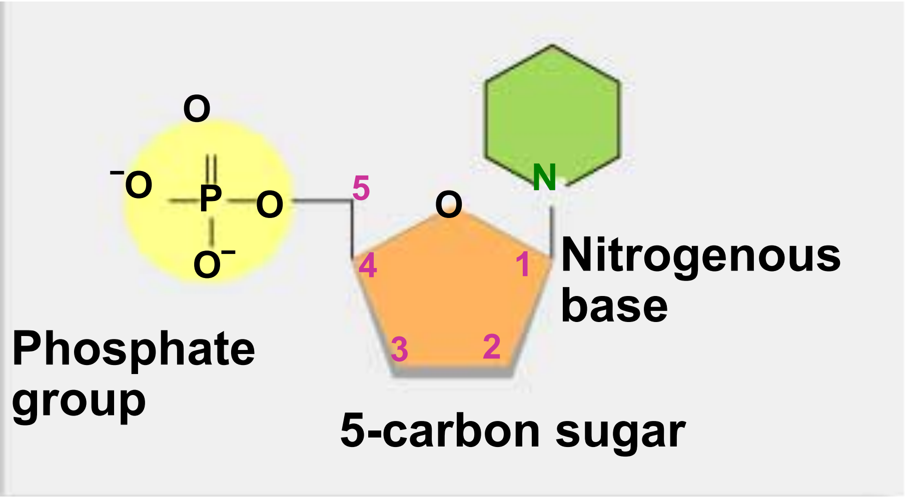
- There are two types of nucleic acids:

  - **deoxyribonucleic acid (DNA)** and **ribonucleic acid (RNA)**
- These are polymers consisting of long chains of monomers called nucleotides
- A **nucleotide** consists of a nitrogenous base, a pentose sugar and a phosphate group:



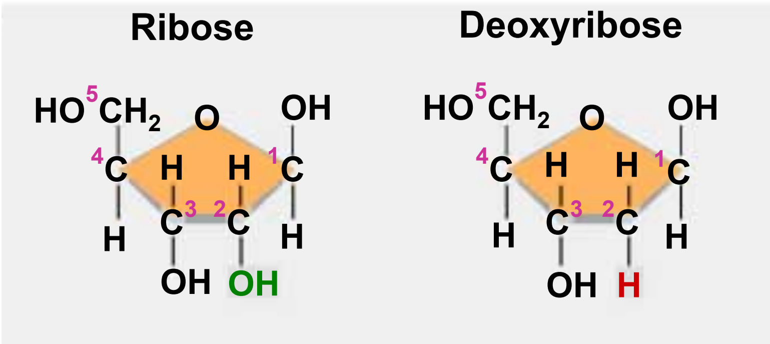
Structure of Nucleotides

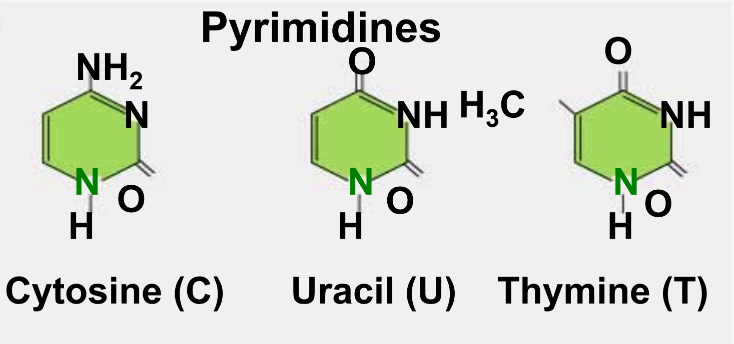
- Nucleotide monomers are building blocks of nucleic acids [deoxyribonucleic acid (DNA) and ribonucleic acid (RNA)]
  - 3 parts of nucleotide
    - phosphate (PO_4^-) and nitrogenous base bound to pentose sugar

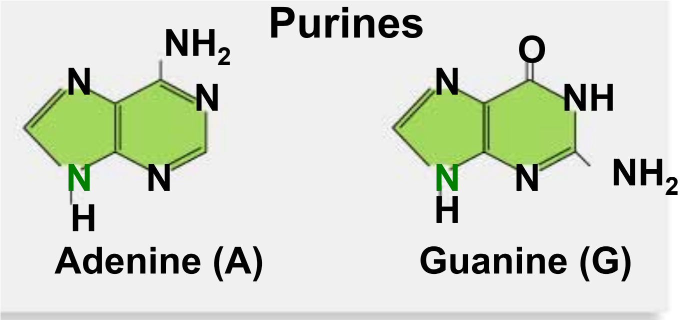


Structure of Nucleotides

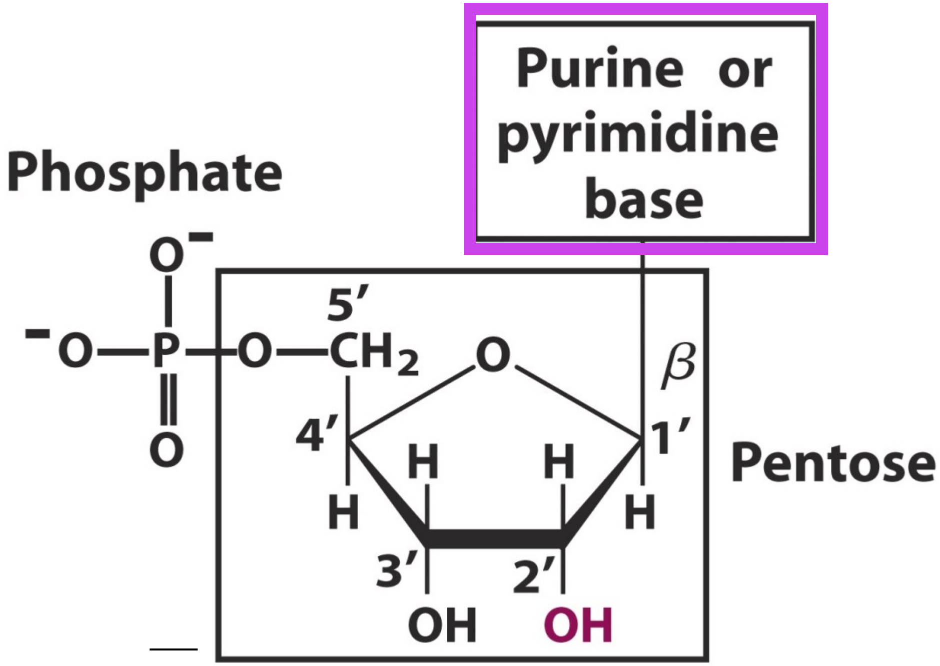
- pentose sugar
  - ribose (RNA) = OH
  - deoxyribose (DNA) = H
  - at C# 2
- nitrogenous bases determine name and identity of nucleotide
  - purines = adenine (A), guanine (G)
    - form from HCN
  - pyrimidines = cytosine (C), thymine (T) (DNA), uracil (U) (RNA)





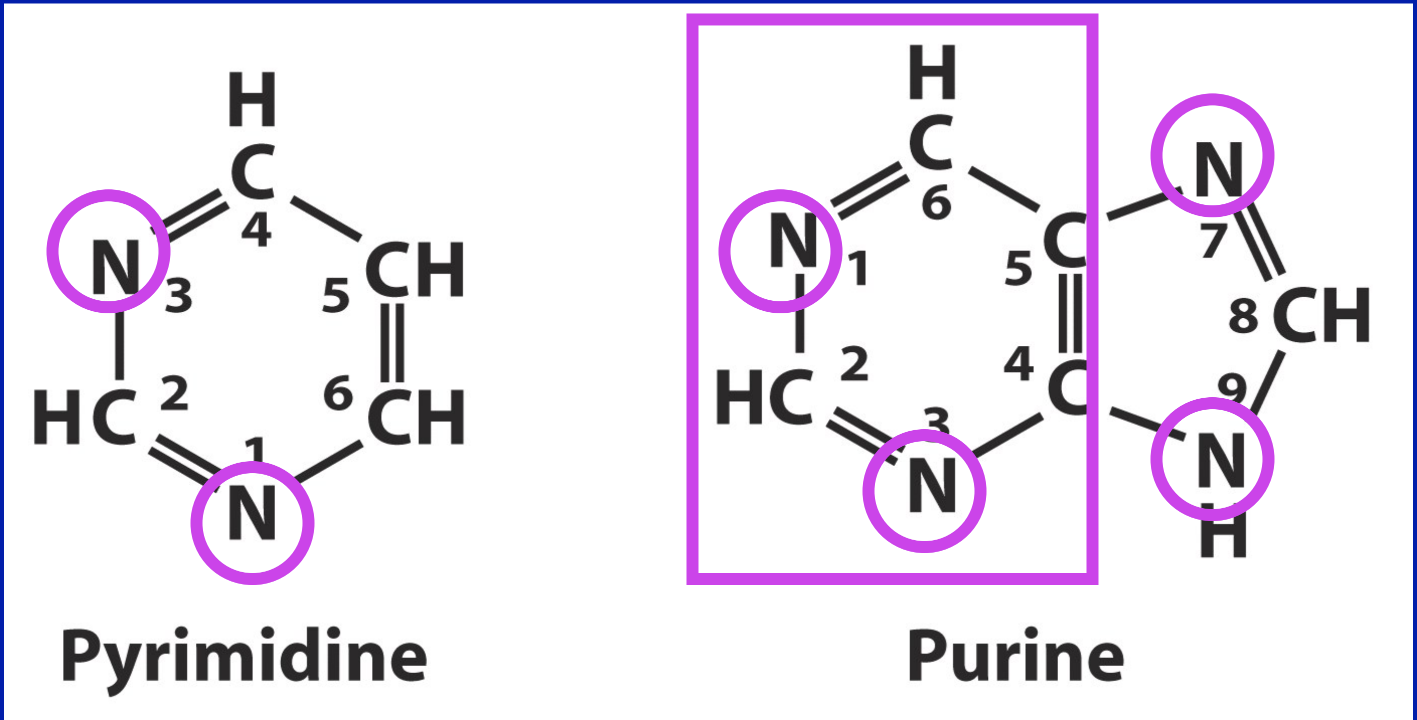


The purine or pyrimidine base



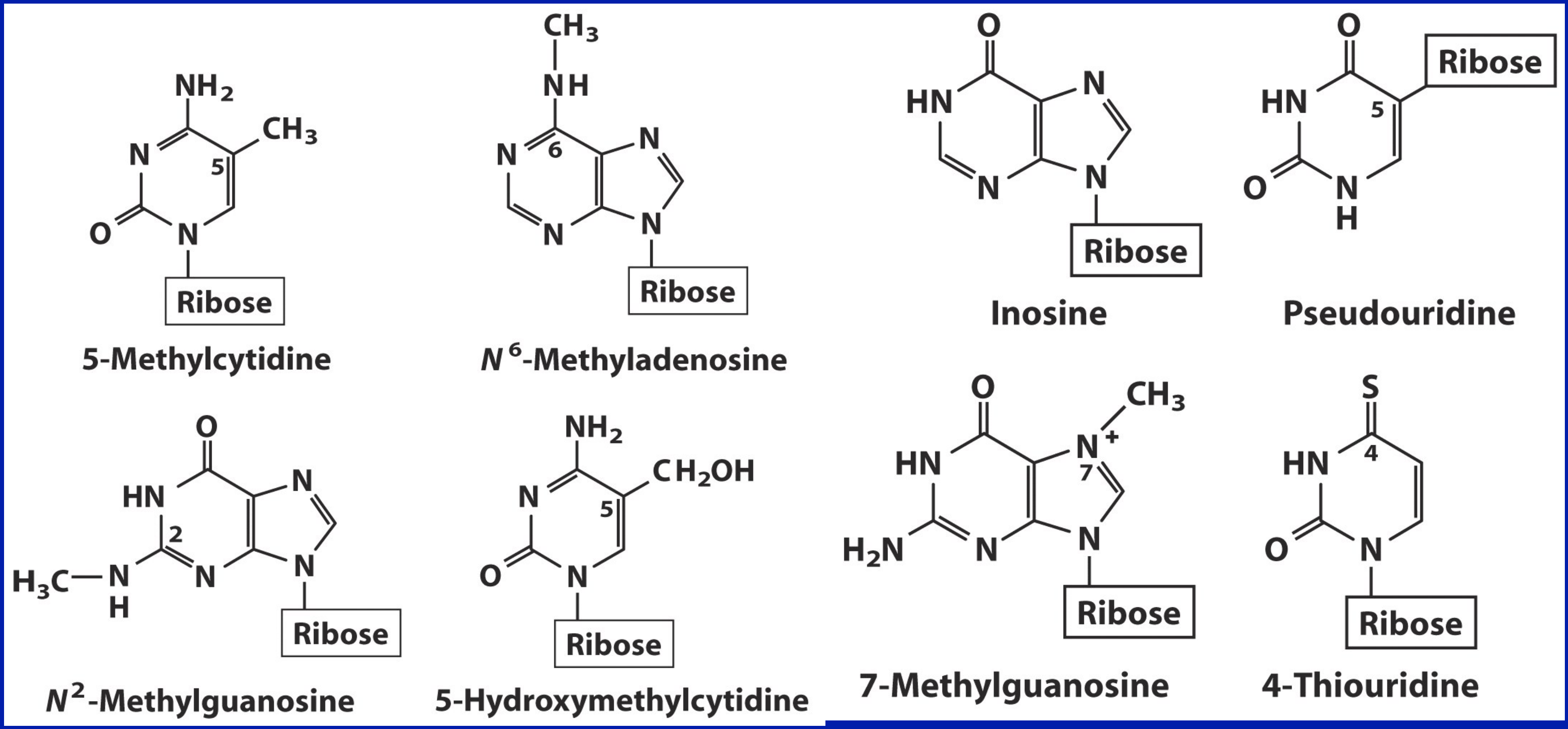
Pyrimidine and purine

Nucleotide bases in nucleic acids are pyrimidines or purines.



Know these!

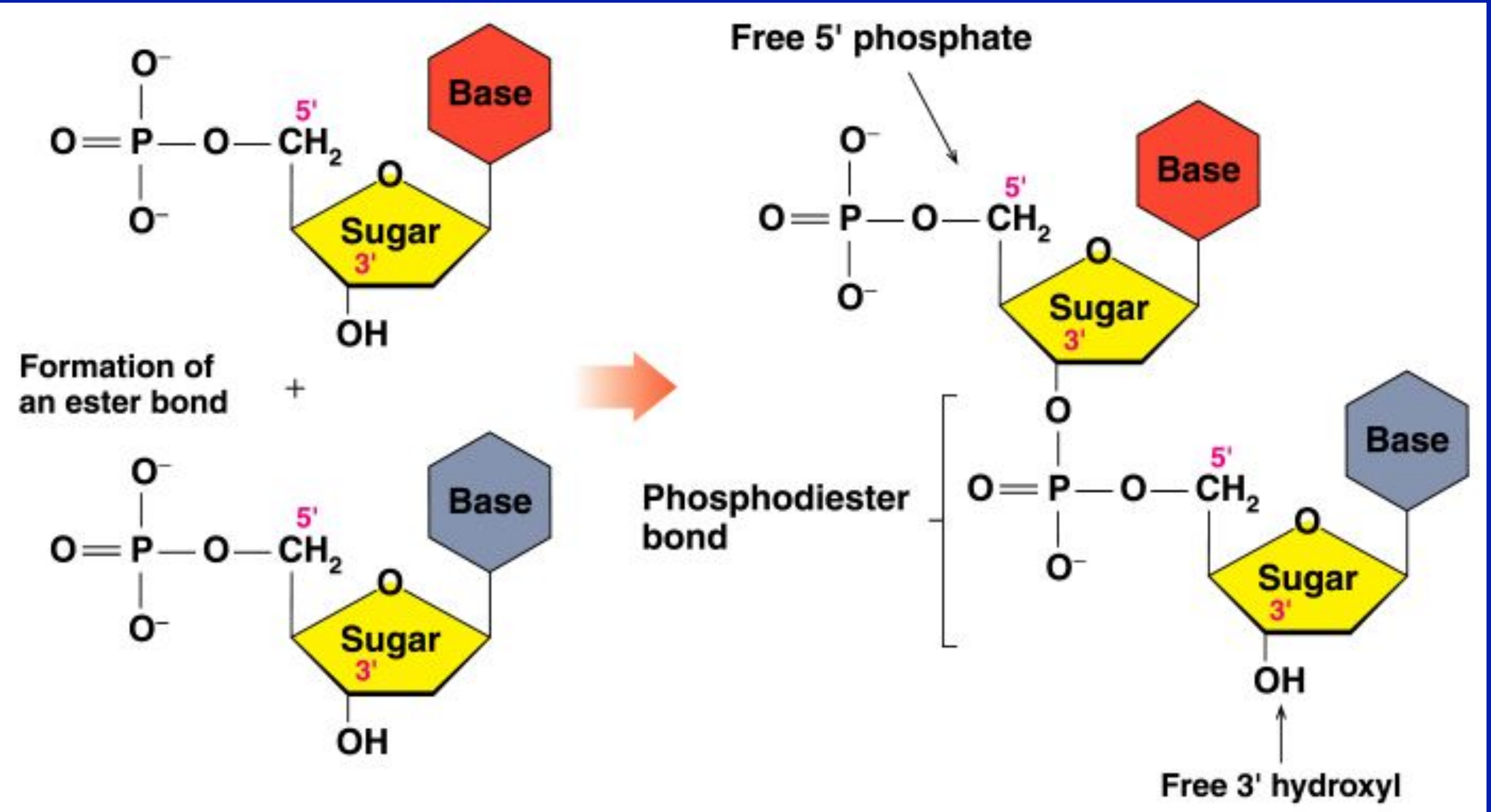
Some minor bases



- 5-Methylcytidine occurs in DNA of animals and higher plants
- N^6-methyladenosine occurs in bacterial DNA

Primary Structure of Nucleic Acids

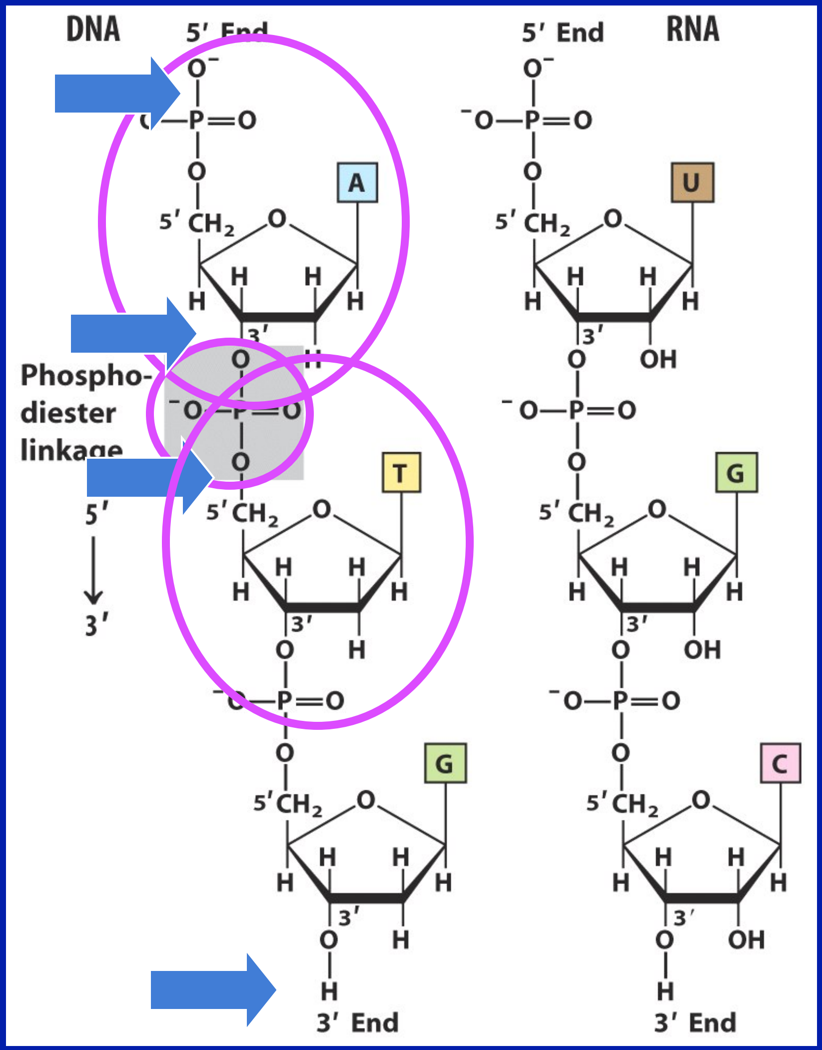
- The **primary structure** of a nucleic acid is the nucleotide sequence
- The nucleotides in nucleic acids are joined by phosphodiester bonds
- The 3'-OH group of the sugar in one nucleotide forms an ester bond to the phosphate group on the 5'-carbon of the sugar of the next nucleotide



Compare polynucleotides and polypeptides

- As in proteins, the sequence of side chains (bases in nucleic acids) plays an important role in function.
- Nucleic acid structure depends on the sequence of bases *and* on the type of ribose sugar (ribose, or 2'-deoxyribose).
- Hydrogen bonding interactions are especially important in nucleic acids.

Nucleic acids



Nucleotide monomers can be linked together via a phosphodiester linkage

formed between the 3' -OH of a nucleotide

and the phosphate of the next nucleotide.

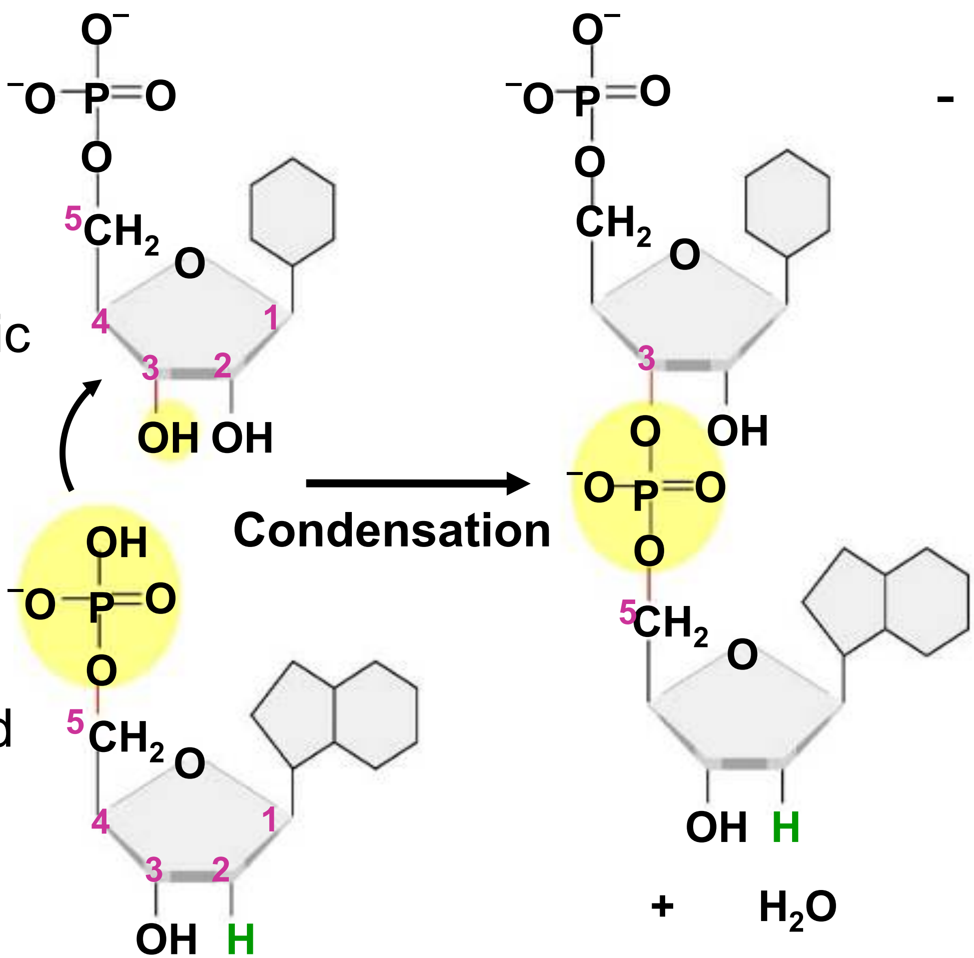
Two ends of the resulting poly- or oligonucleotide are defined:

The 5' end lacks a nucleotide at the 5' position,

and the 3' end lacks a nucleotide at the 3' end position.

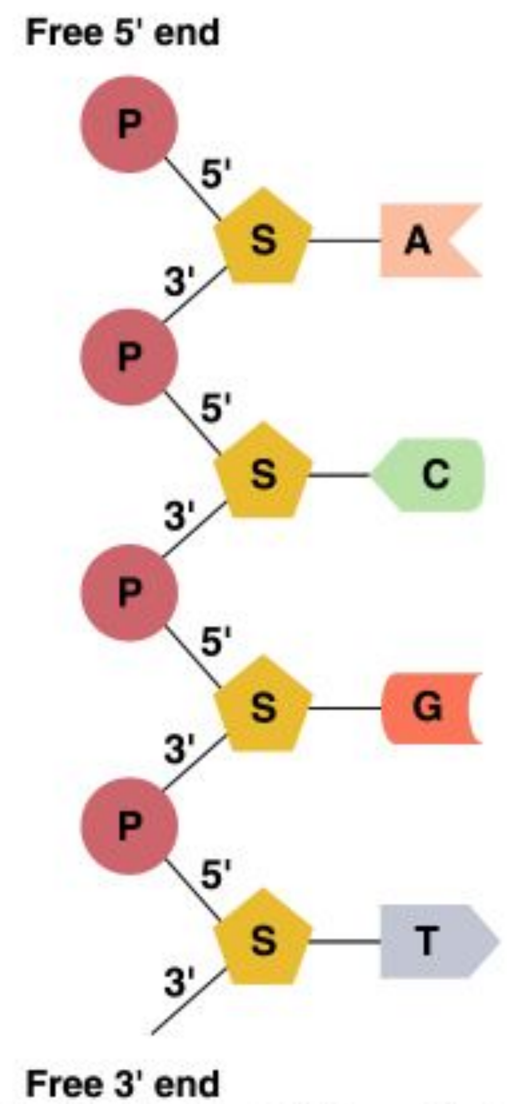
Structure of DNA

- 1° structure = sequence of deoxyribonucleotides (A, T, G, C)
  - condensation rxn btwn PO_4^- group of 5' C to OH group of 3' C via phosphodiester bond
    - polymerization = endergonic
    - presence of clay
  - sugar-phosphate backbone
    - always written 5’ → 3’
    - nucleotides added to 3’ end
  - nitrogenous bases project inward



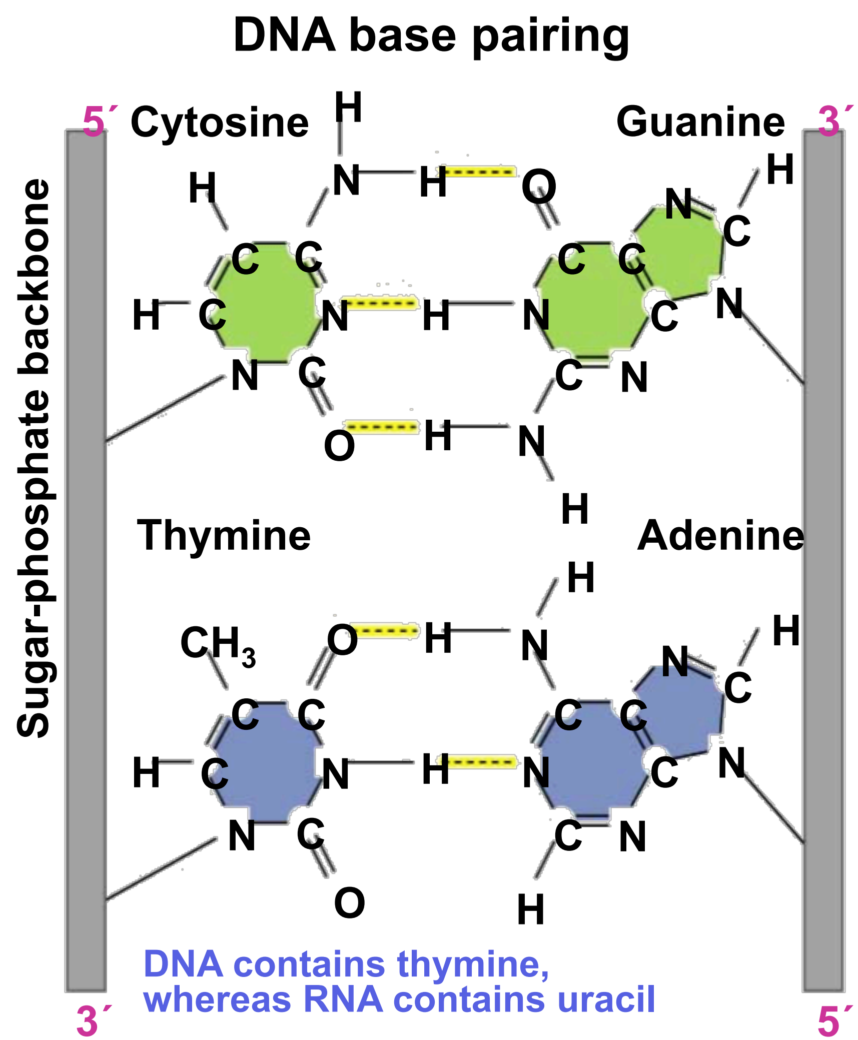
Reading Primary Structure

- A nucleic acid polymer has a free 5’-phosphate group at one end and a free 3’-OH group at the other end
- The sequence is read from the free 5’-end using the letters of the bases
- This example reads 5’—A—C—G—T—3’

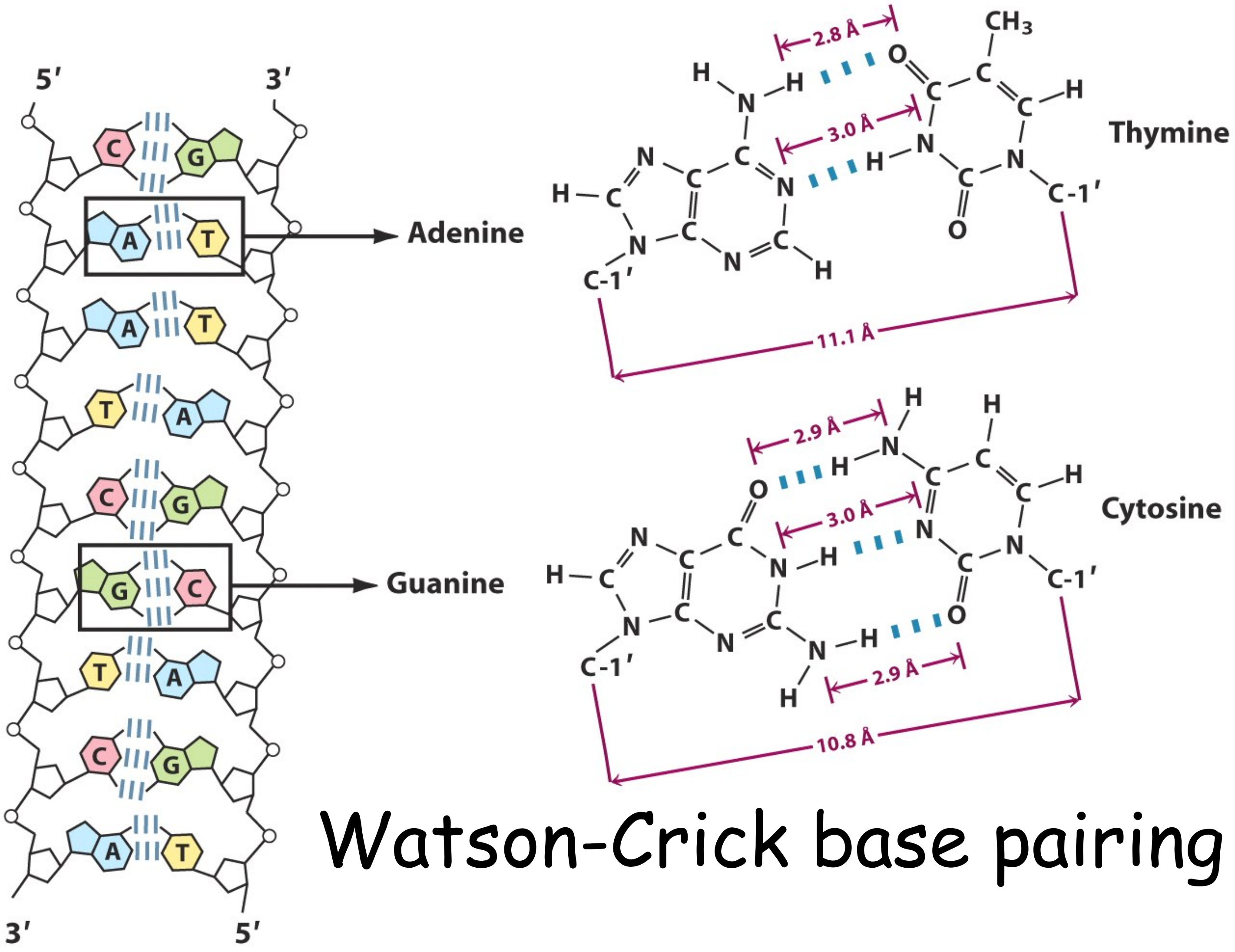


Structure of DNA

- 2° structure = H-bonds btwn nitrogenous bases of 2 DNA strands
  - double helix
  - A bonds to T, C bonds to G
    - complementary base pairs
    - H bonding
    - double bond btwn A and T
    - triple bond btwn G and C

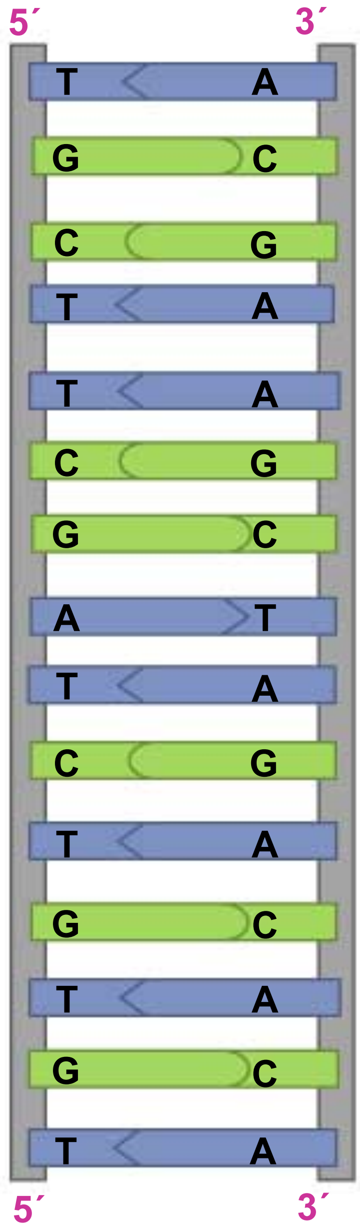


Interstrand H-bonding between DNA bases

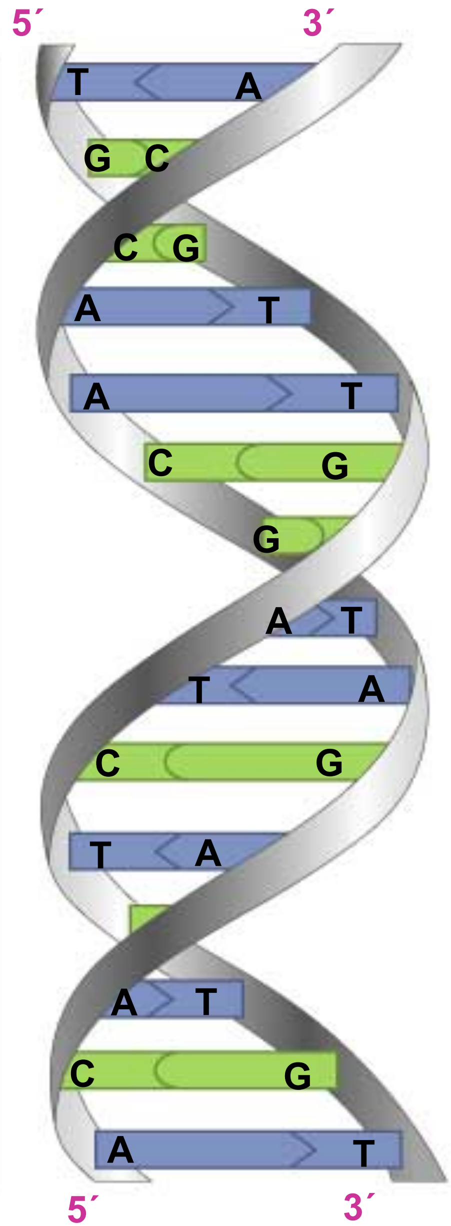


Watson-Crick base pairing

DNA is a double helix



Cartoon of base pairing

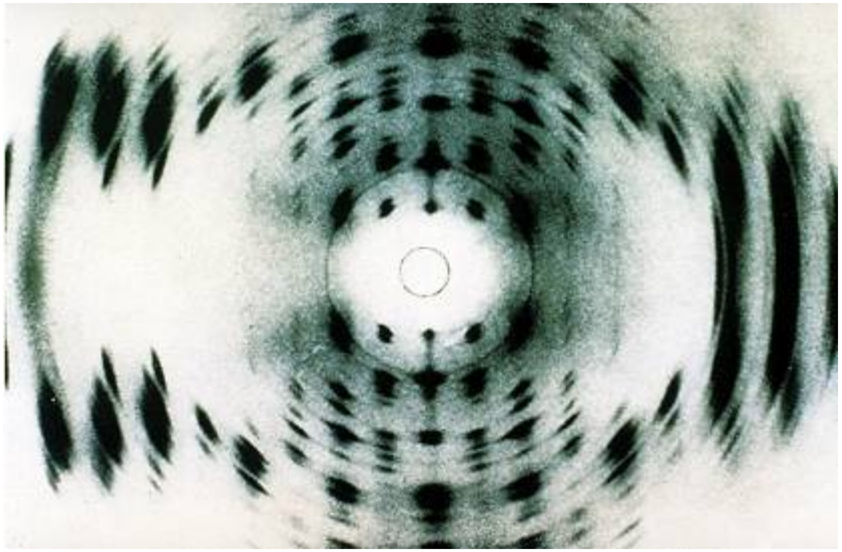


Cartoon of double helix

Space-filling model of double helix

Discovery of DNA Structure

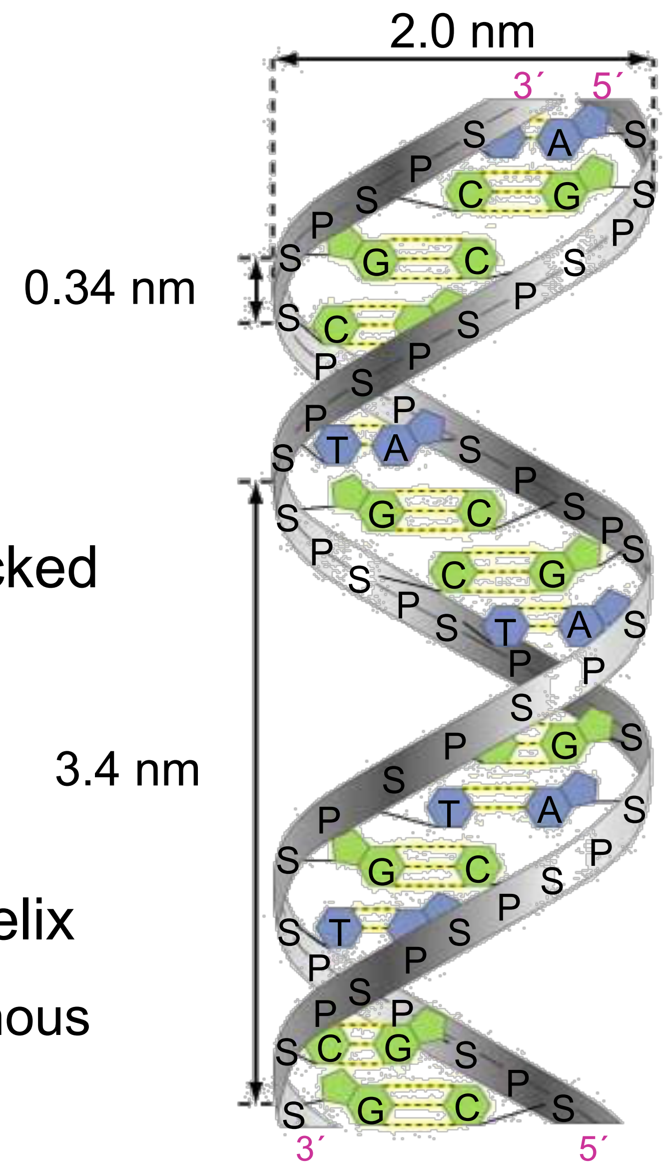
- Chemists determine DNA composed of nucleotides linked by phosphodiesterase bonds
- Chargaff
  - # of purines = # of pyrimidines
  - # T's = # A's, # C's = # G's



- Franklin and Wilkins
  - X-rays of crystalline DNA yield specific/precise pattern, indicating helical shape
  - 3 distances between points in pattern repeated many times (0.34 nm, 2 nm and 3.4 nm)

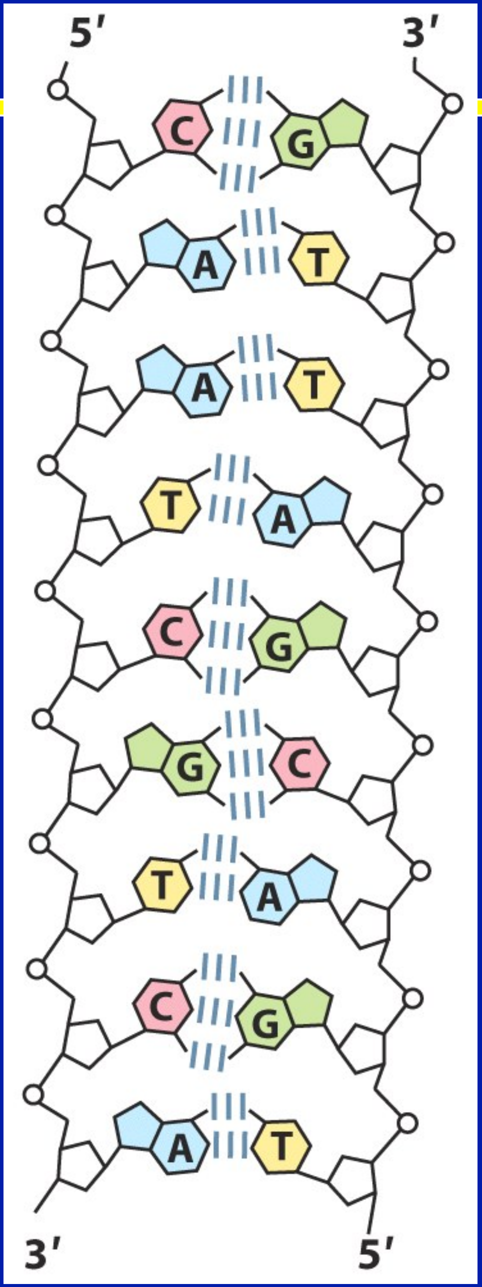
Discovery of DNA

- Watson and Crick
  - built physical models of possible structures
  - 2 nm distance = width of helix
  - 0.34 nm distance = btwn vertically stacked bases
  - 3.4 nm distance = 10x distance btwn bases and length of one turn
  - DNA is double-stranded, anitparallel helix
    - sugar-phosphate backbone w/ nitrogenous bases directed inward

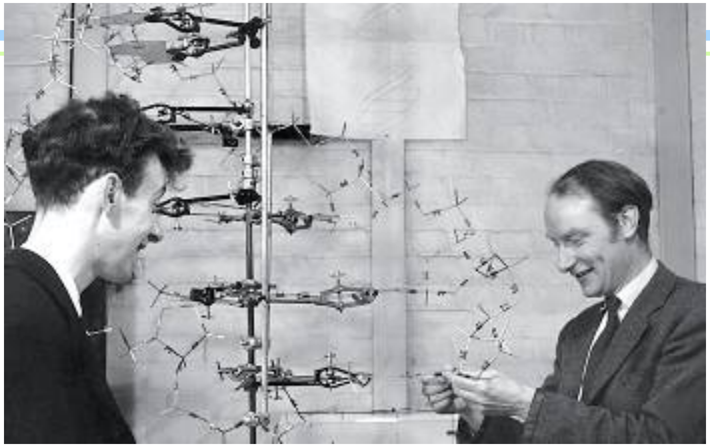


DNA strands

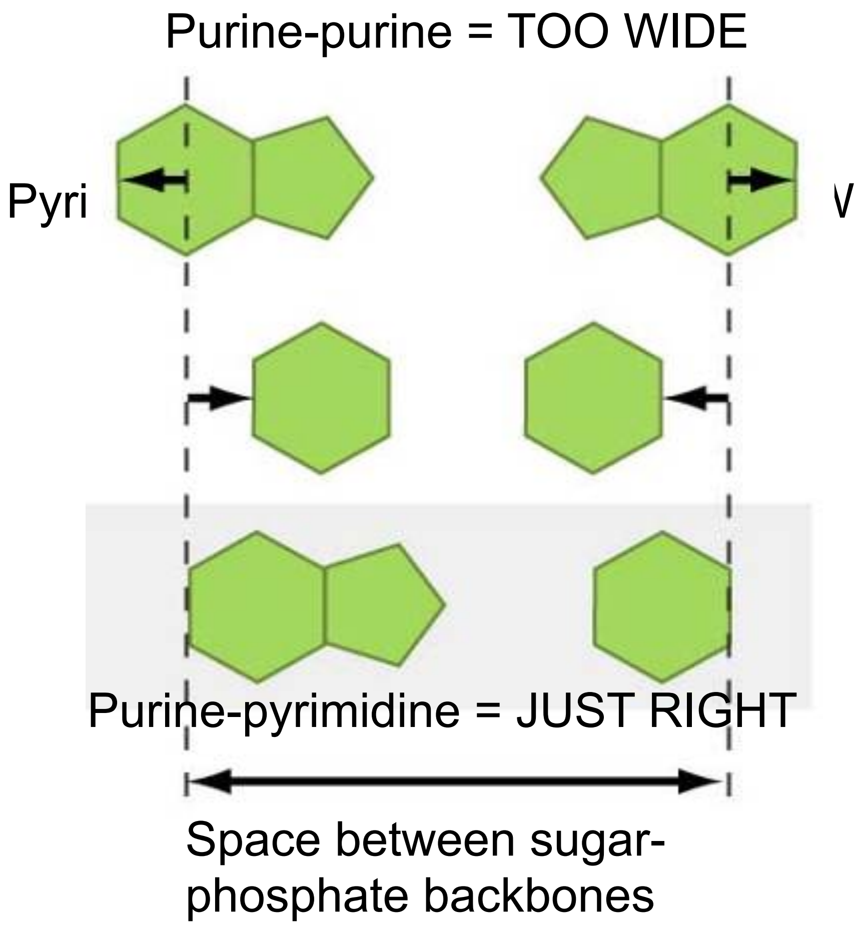
- The antiparallel strands of DNA are not identical, but are complementary.
- This means that they are positioned to align complementary base pairs: C with G, and A with T.
- So you can predict the sequence of one strand given the sequence of its complement.
- Useful for information storage *and* transfer!
- Note sequence conventionally is given from the 5' to 3' end



Discovery of DNA



- purine-pyrimidine pairs (complementary base pairs)
  - space for purines to pair to pyrimidines only
  - H bonds btwn
    - Chargaff's rules suggested A–T and C–G pairings



Only purine-pyrimidine pairs fit inside the double helix.

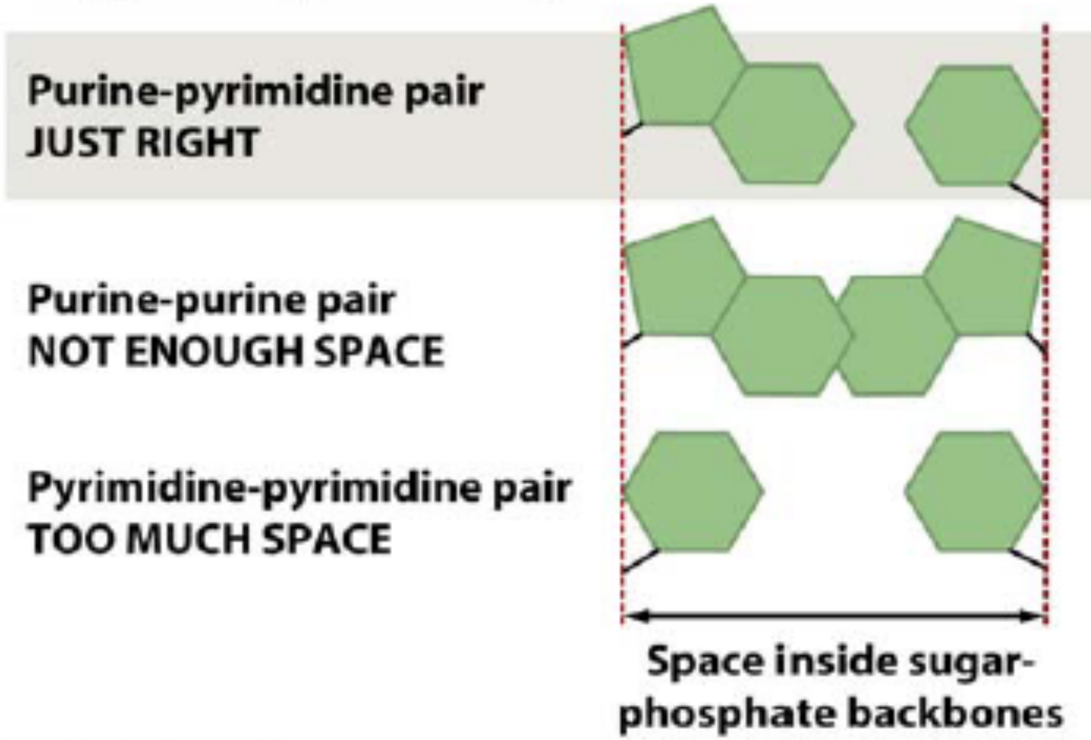
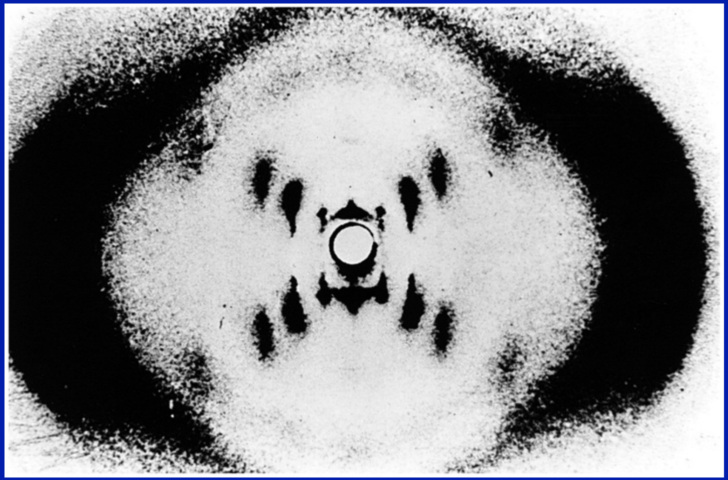
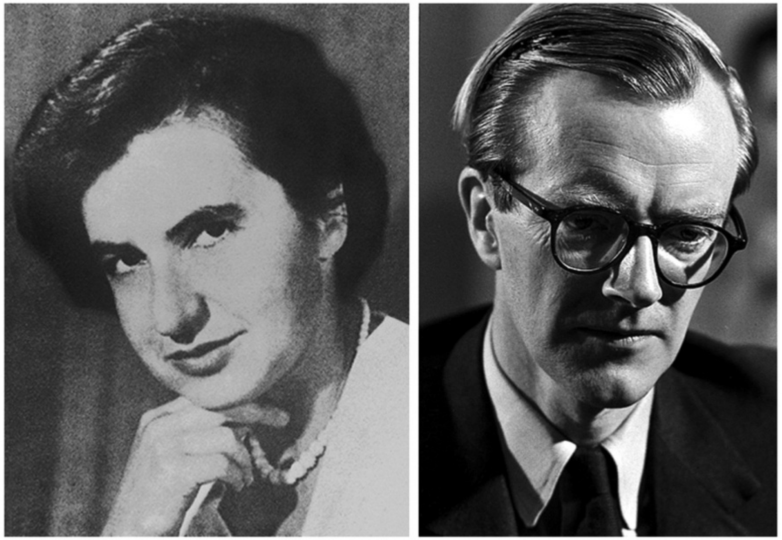


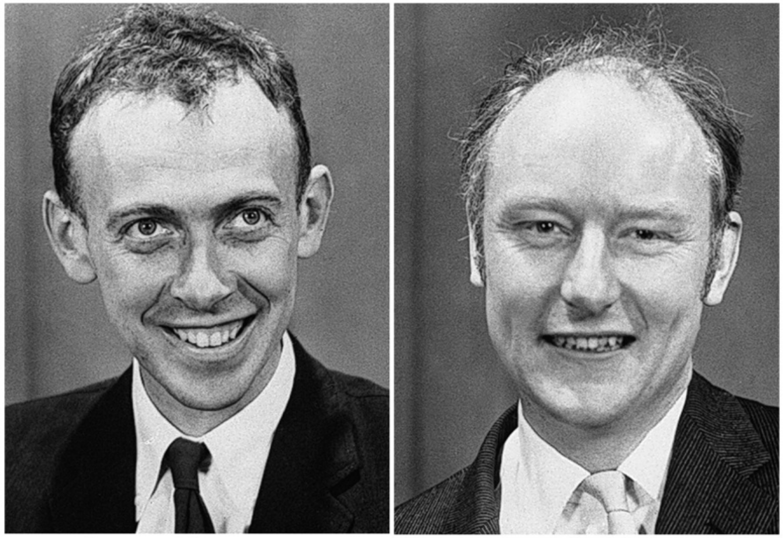
Figure 4-9a Biological Science, 2/e

DNA structure determination





Rosalind Franklin, 1920–1958 **Maurice Wilkins**

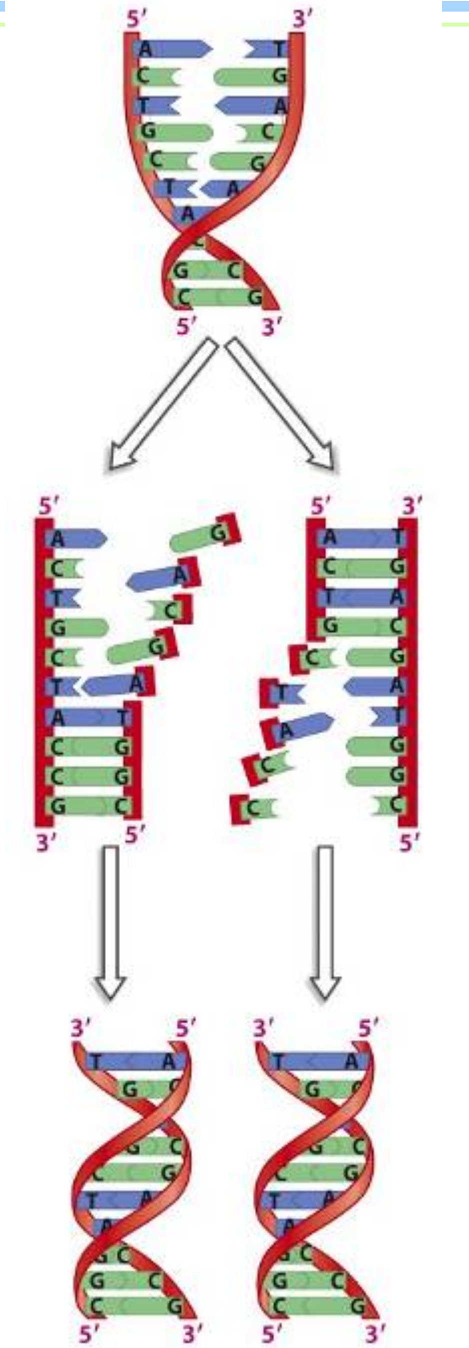


James Watson Francis Crick

- Franklin collected x-ray diffraction data (early 1950s) that indicated 2 periodicities for DNA: 3.4 Å and 34 Å.
- Watson and Crick proposed a 3-D model accounting for the data.

Characteristics of DNA

- DNA is excellent template (better than RNA, protein)
  - each strand acts as template for synthesizing opposite strand
    - complementary base-pairing
  - very stable = ↓ chemically reactive, no catalytic capabilities, will not degrade easily
    - lack of –OH group on C#2 = ↓ chemical reactivity
    - double-helix structure = ↑ stability, ↓ chemical reactivity
    - hydrophobic interior difficult to break apart
    - not likely to be 1st living entity



DNA is an INFORMATION carrying molecule

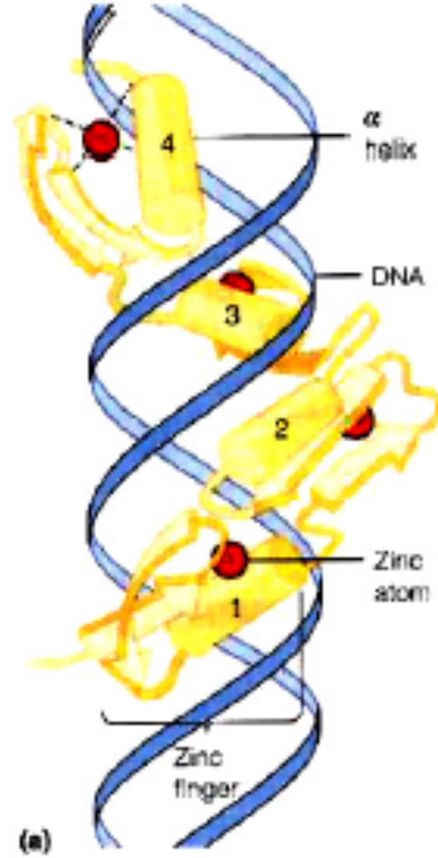
DNA is regular and symmetrical with few chemical groups exposed for further reactions.

Lacks --OH on 2‘C of the sugar: more stable than RNA and highly resistant to degradation

Orderliness and stability ► dependable information carrier (& no evidence of catalytic activity)

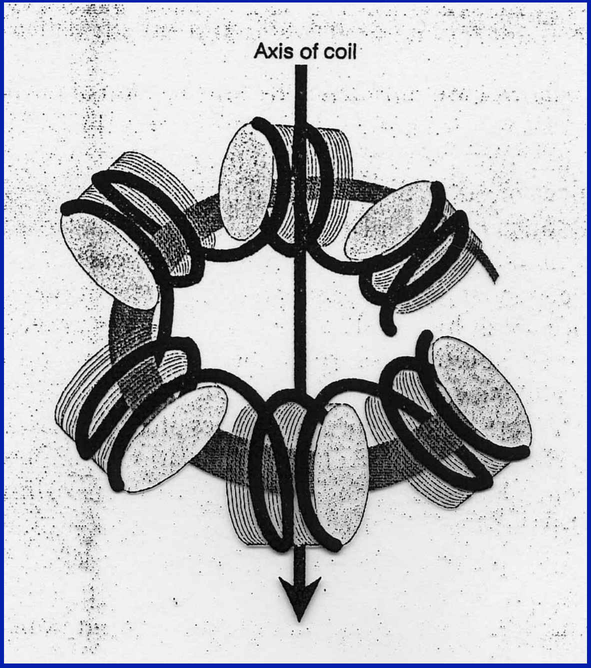
Proteins can bind in grooves

- DNA binding proteins
  - Specific recognition sites
  - major or minor groove

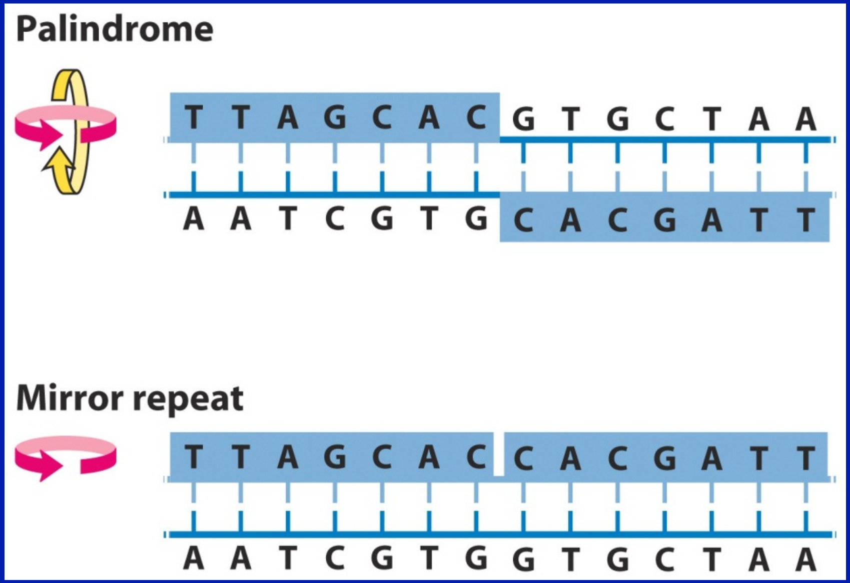


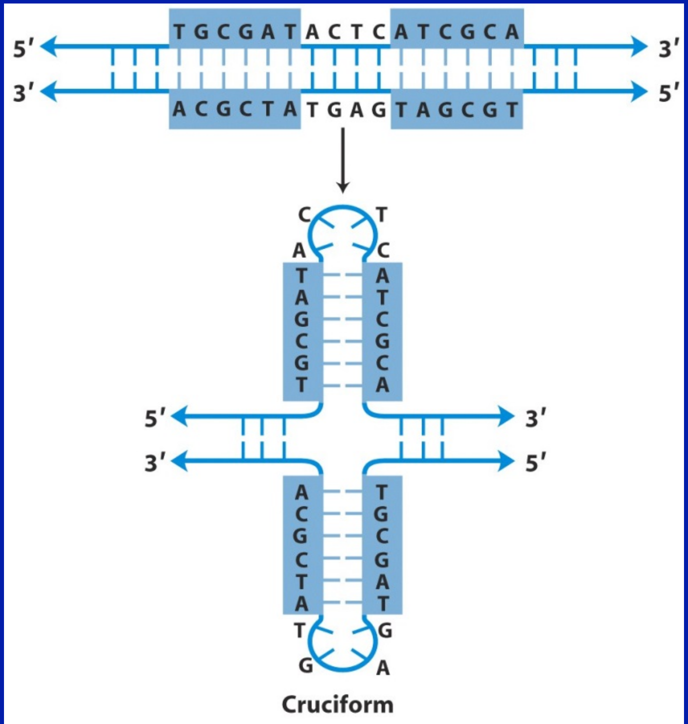
Storage of DNA

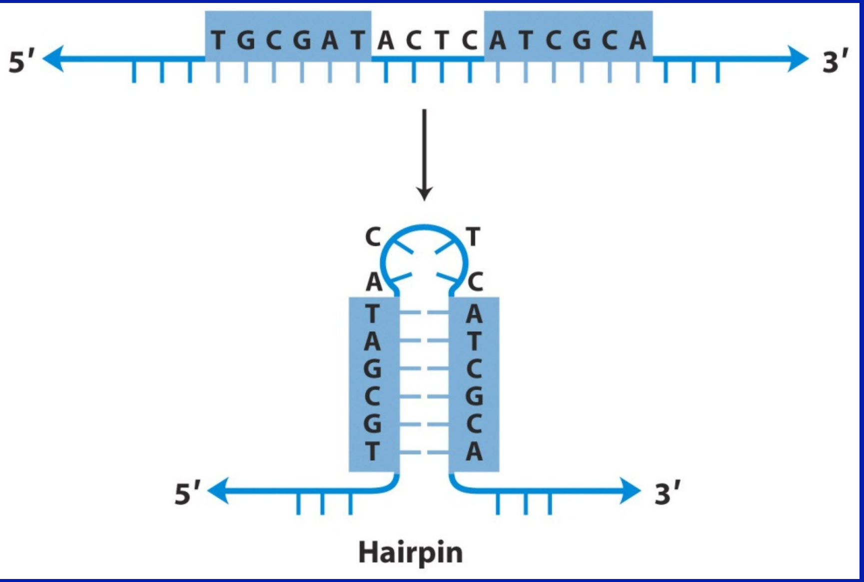
- In **eukaryotic** cells (animals, plants, fungi) DNA is stored in the **nucleus**, which is separated from the rest of the cell by a semipermeable membrane
- The DNA is only organized into **chromosomes** during cell replication
- Between replications, the DNA is stored in a compact ball called **chromatin**, and is wrapped around proteins called **histones** to form **nucleosomes**



Some common local DNA motifs

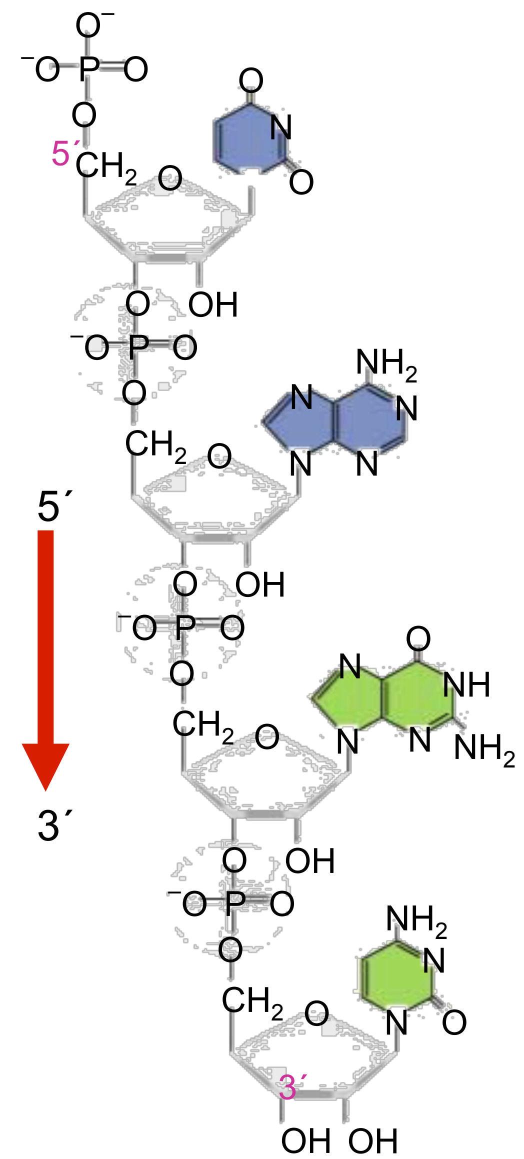






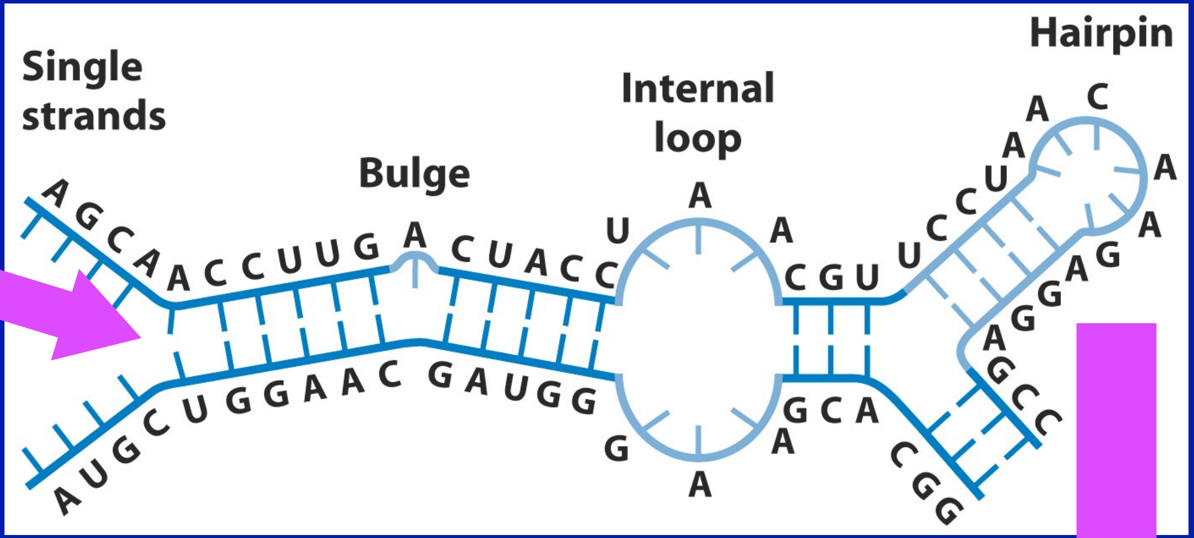
Structure of RNA

- 1° structure = sequence of ribonucleotides (A, U, G, C)
  - condensation rxn, phosphodiester bond
  - sugar-phosphate backbone w/ nitrogenous bases projecting off
  - written 5’ to 3’

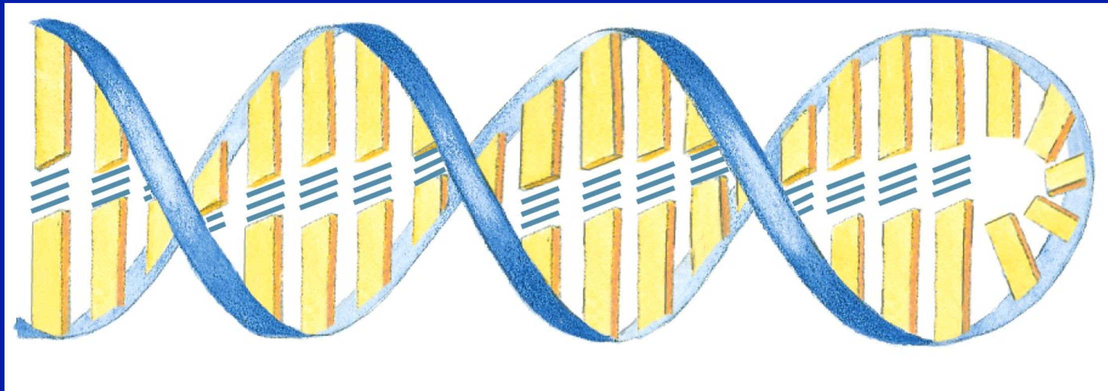


RNA has a rich and varied structure

Watson-
Crick base pairs
(helical segments;
Usually A-form).
Helix is secondary
structure.
Note A-U pairs in
RNA.



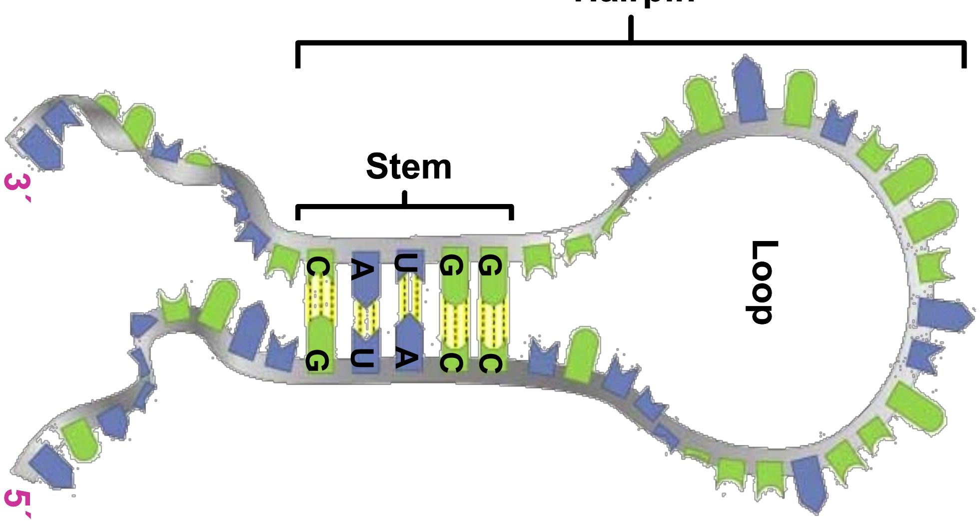
DNA can
form
structures
like this as
well.

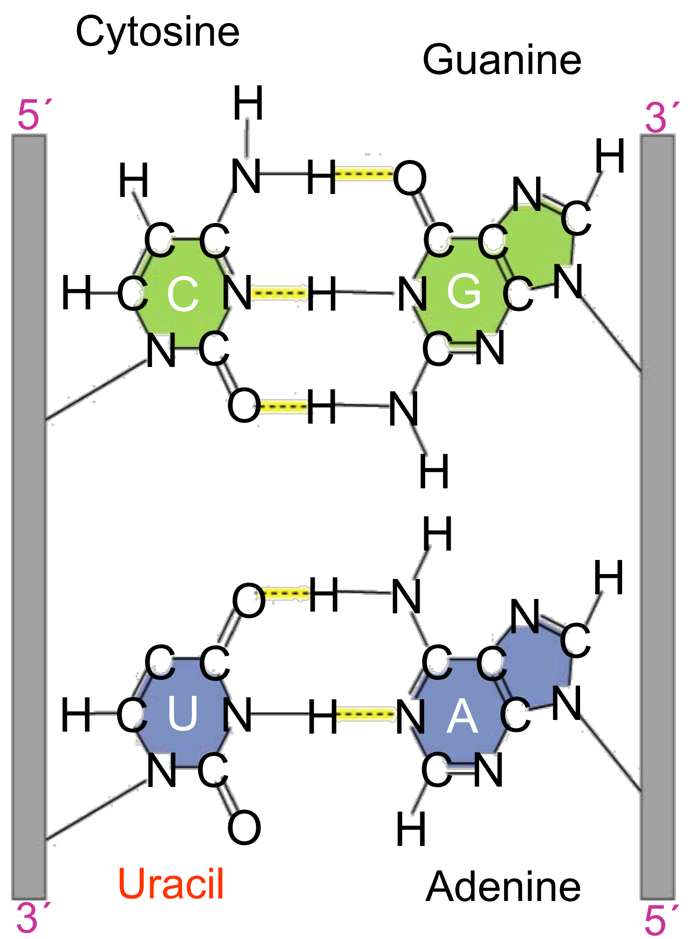


Hairpin double helix

Structure of RNA

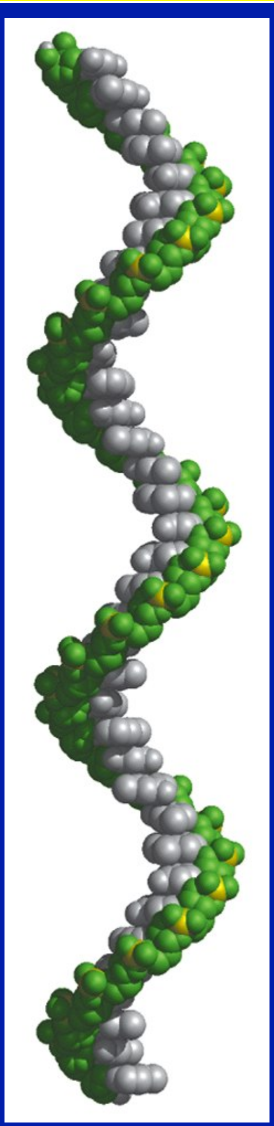
- 2° structure = stable stem-loop hairpins
  - H bonding btwn bases on ***same*** strand
    - ↑ stability
  - double bond btwn A and U
  - triple bond btwn G and C

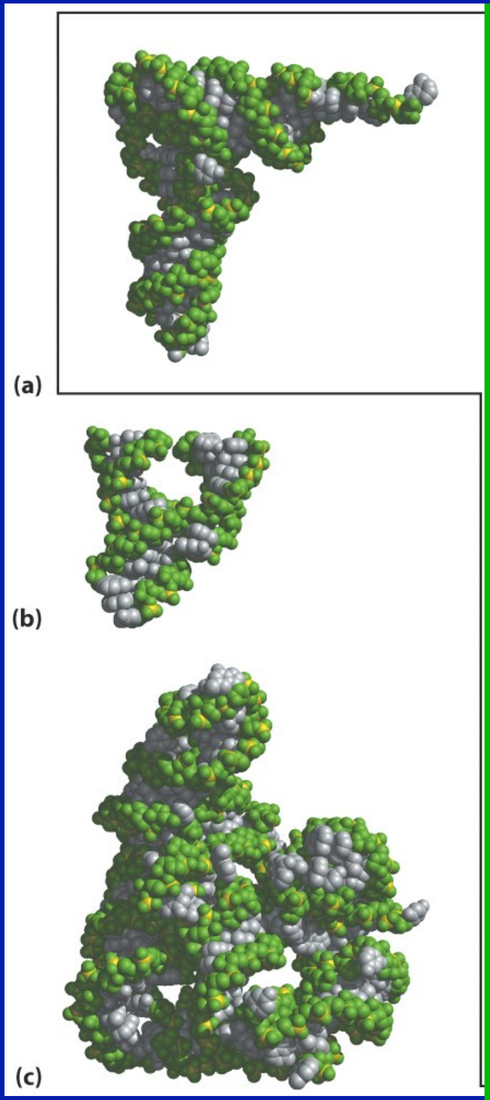




RNA displays interesting tertiary structure

Single-stranded RNA right-handed helix





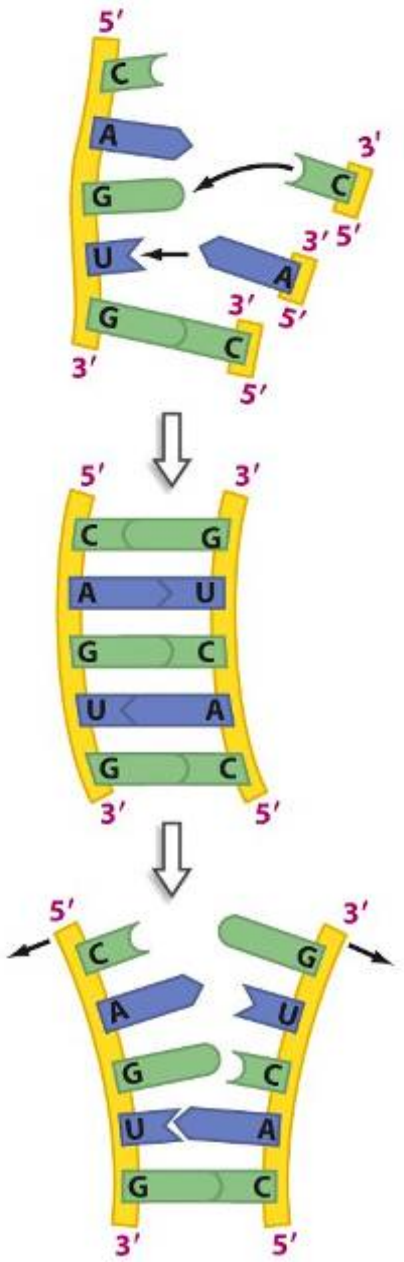
Yeast $tRNA^{Phe}$ (1TRA)

Hammerhead ribozyme (1MME)

T. thermophila intron, A ribozyme (RNA enzyme) (1GRZ)

Characteristics of RNA

- RNA can be both catalyst and template
  - catalytic RNAs (ribozymes) in some sp.
  - -OH more reactive, less stable
  - template for new strand by base-pairing rules
    - free ribonucleotides pair with complementary bases on existing template RNA
    - phosphodiester bonds form btwn newly added ribonucleotides
      - nucleotides added to 3’ end
    - H-bonds joining complementary strand to template are broken by heating/catalysis, releasing new RNA strand
- Self-replicating RNA likely 1st living entity



RNA function(s)

Information containing molecule (but less stable than DNA)

Information <u>carrying</u> molecule (RNA ▶ protein)

Catalytic molecule (ribozyme = “RNA enzyme”)

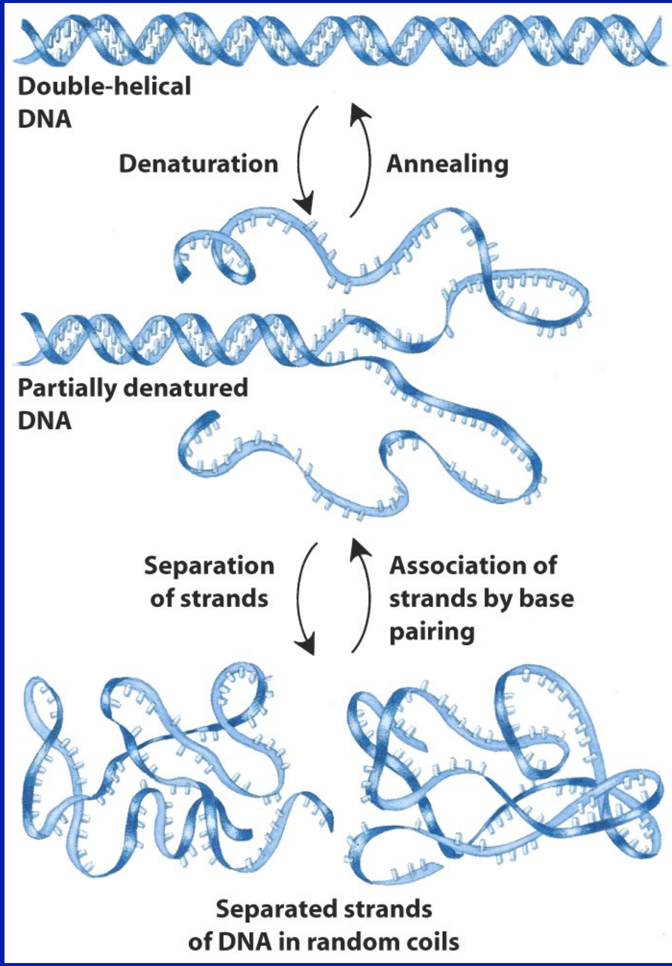
Previously thought that proteins were the only molecule capable of catalyzing reactions

Differences between DNA and RNA

- DNA is double-stranded vs. RNA is typically single-stranded
- DNA has pyrimidine thymine vs. RNA has pyrimidine uracil
- Sugar in DNA is deoxyribose (-H at C# 2) vs. sugar in RNA is ribose (-OH at C# 2)
- DNA forms complementary base pairs btwn 2 DNA molecules making double helix vs. RNA forms complementary base pairs btwn different parts of same molecule resulting in stem-loop structures

Physical and Chemical Properties of Nucleic Acids

- Heat denatures (melts) double-stranded nucleic acids
- Structure is maintained NOT by hydrogen bonds but by the hydrophobic effect, although details are poorly understood: Base stacking



Heat (and changes in pH/ionic strength) disrupts hydrogen bonds and van der Waals and stacking interactions, causing denaturation. (Covalent bonds remain intact.)
If complete separation does not occur, the duplex can re-anneal (spontaneously) under proper conditions.
If strands completely separate, the process is slower and step-wise.

Hyperchromicity: Free nucleotides have a higher A_{260} than an identical concentration assembled into a single-stranded polynucleotide. Double stranded nucleic acids have a lower A_{260} than single stranded polynucleotides - used to monitor assembley or denaturation of nucleic acids *in vitro*.

	λmax	**Em x 10^{-3} @ λmax**
Adenine	**260.5**	**E = 13.4**
Guanine	**275**	**E = 8.1**
Cytosine	**267**	**E = 6.1**
Thymine	**264.5**	**E = 7.9**
Uracil	**259.5**	**E = 8.2**
NADH	**340**	**E = 6.23**
NAD	**260**	**E = 18**

Purine & pyrimidine bases bases in nucleic acids:

the hyperchromic effect

The absorption of nucleic acids arises from n to π^* & π^* to π^* transitions. The spectra of purine & pyrimidine bases occur between 200 and 300nm, are sensitive to pH & contain contributions from several electronic transitions. The absorption spectra of the bases when they are in polymers is greatly influenced by electronic interactions between bases

↑heat : ↑UV absorbance of DNA

The heat is used to denature the DNA and the nucleotides are then more exposed to theUV light.

More U.V. light is absorbed when DNA is in the denatured state

The sequence of DNA affects the hyperchromic effect and transitional temperature (T_m)

All organisms have a characteristic T_m (also called midpoint temperature, transitional temp. or melting temp.)

↑ C≡G content : ↑ T_m

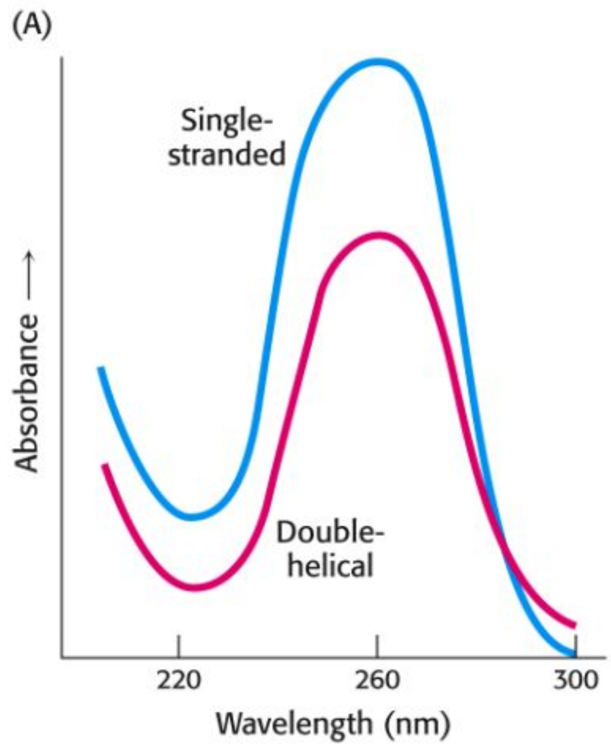
There is a direct correlation between T_m and G≡C percent (%) in DNA,

Tm is important for knowing that we can separate DNA and renature it.

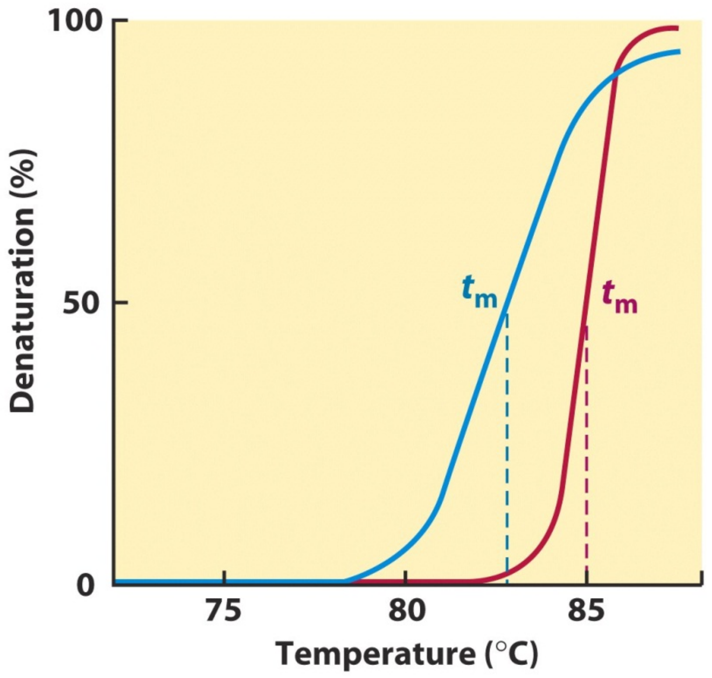
Different sequences in DNA will renature faster.

The Hypo(er)chromic Effect

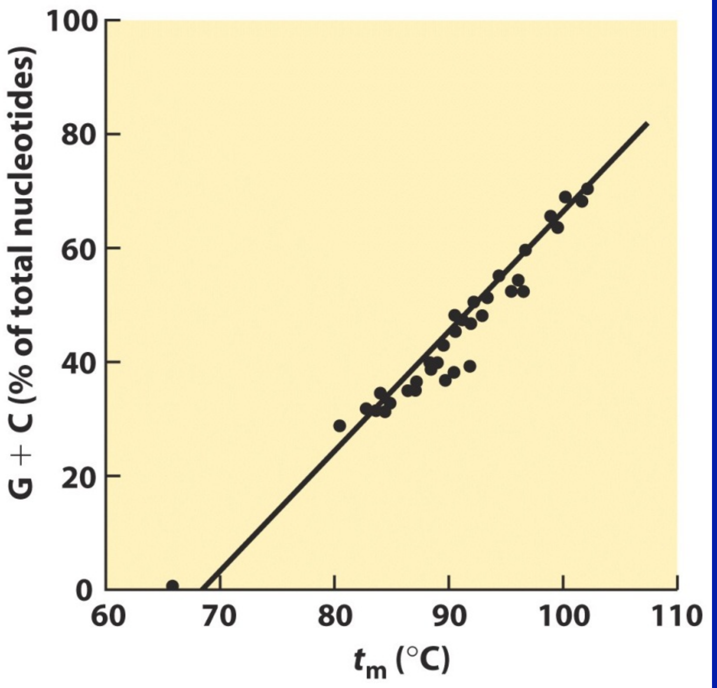
- Because of high degree of conjugation, pi electrons cause high molar extinction coefficients
- Polynucleotides absorb LESS than monomeric nucleotides, but at the same wavelength (~ 260 nm) (***<u>Hypo</u>***chromic)
- Also, single stranded DNA absorbs MORE than double stranded
- This can be used to monitor the kinetics of DNA melting



(A)
Single-
stranded
Double-
helical
Absorbance ⟶
220
260
300
Wavelength (nm)



100
50
0
Denaturation (%)
t_m
t_m
75
80
85
Temperature (°C)



100
80
60
40
20
0
G + C (% of total nucleotides)
60
70
80
90
100
110
t_m (°C)