



Molecular Biology (1)

DNA structure and basic applications

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Second semester, 2018-2019

Resources



- This lecture
- Cooper, pp. 49-52, 118-119, 130

Nucleic acids

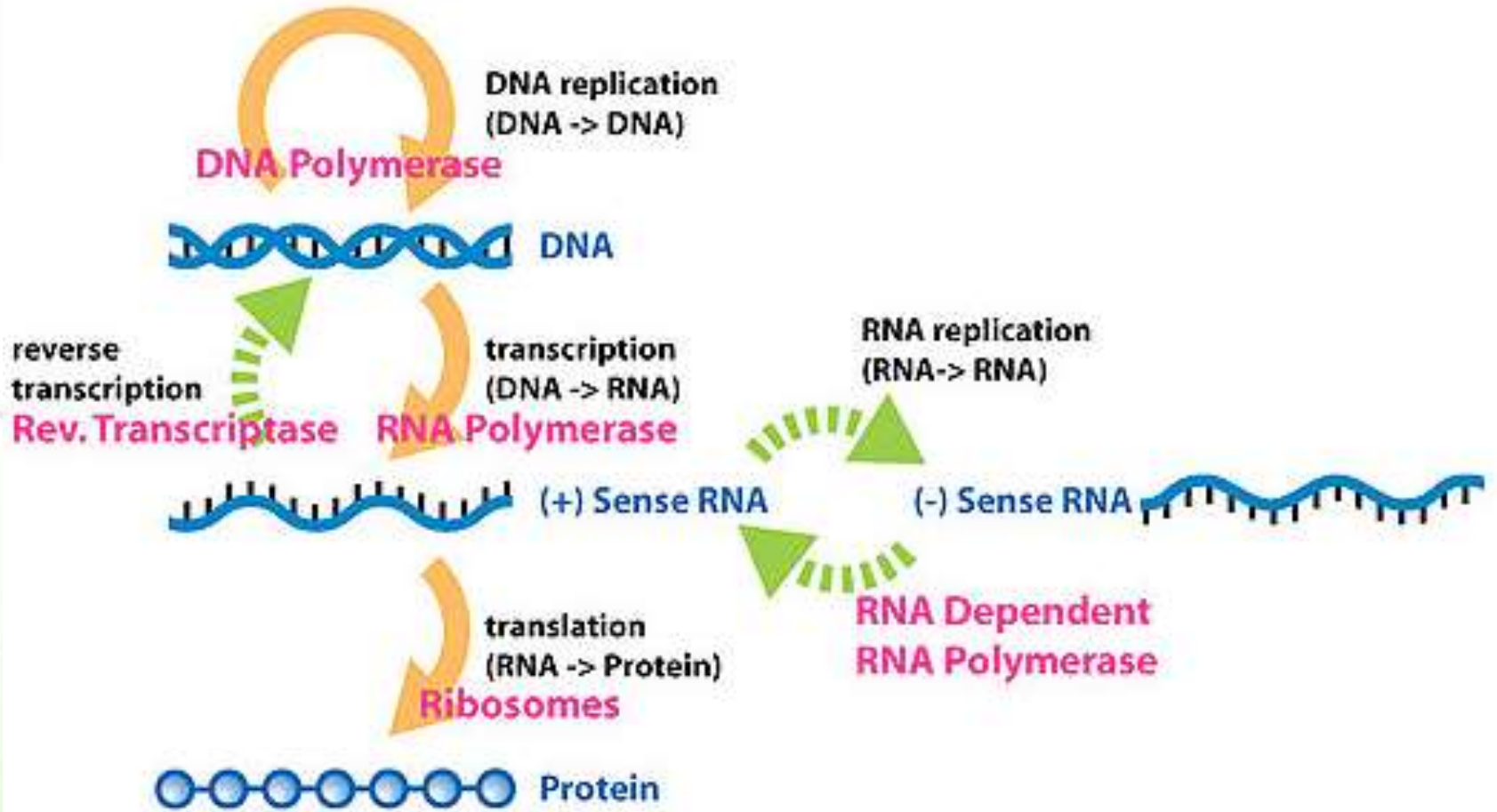


- 2 types:
 - Deoxyribonucleic acid (DNA)
 - Ribonucleic acid (RNA)

What is molecular biology?



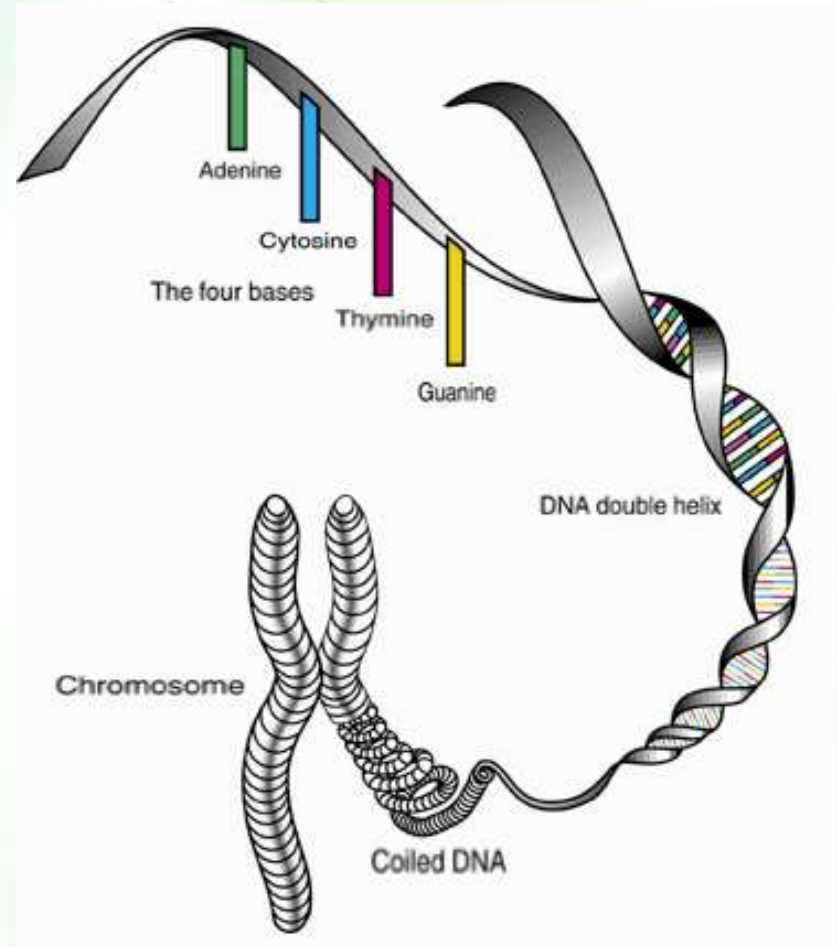
Central dogma of molecular biology



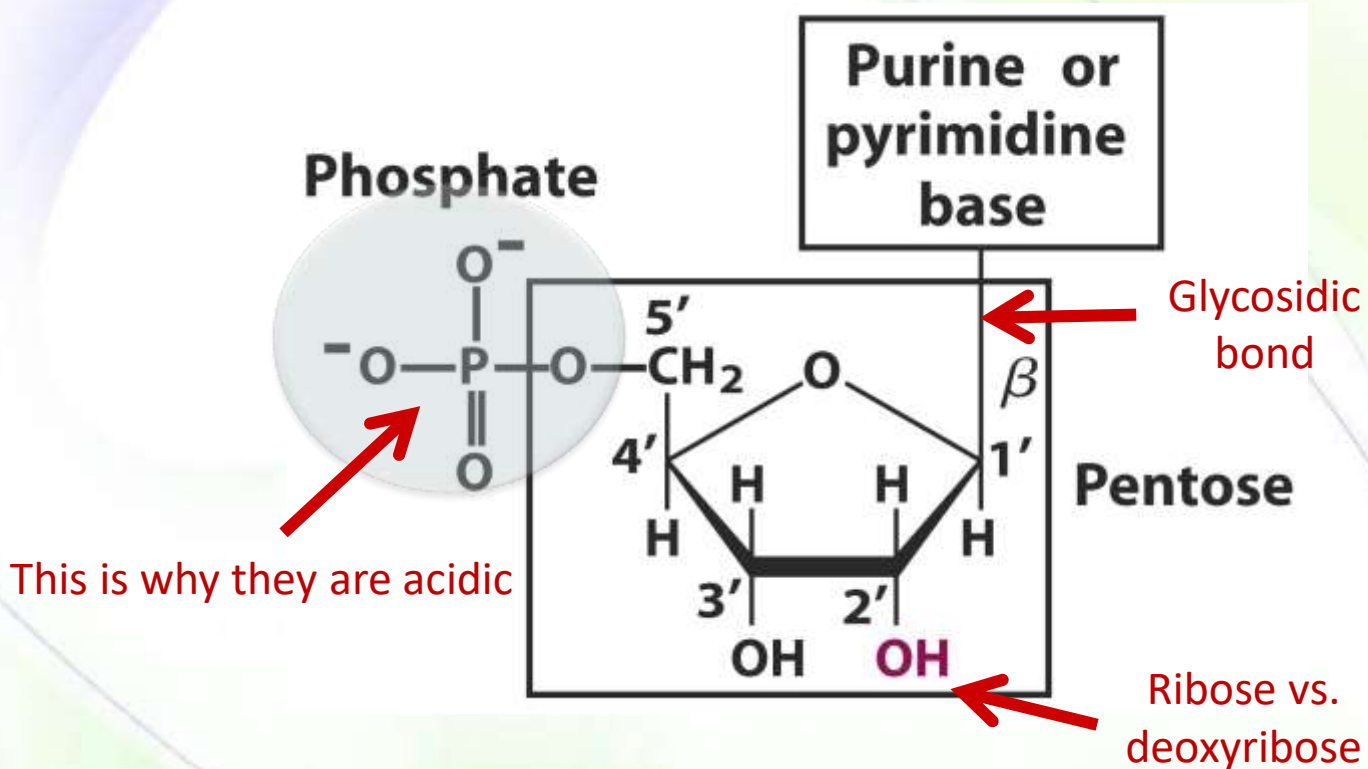
Nucleic acids



- The primary structure of nucleic acids is linear polymers of nucleotides (monomers) bound to each other via phosphodiester bonds.
- DNA is coiled and can be associated with proteins forming chromosomes.

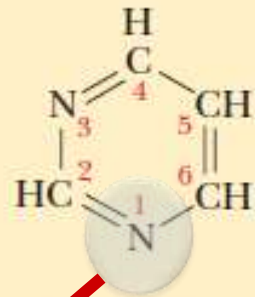


Chemical composition and bonds

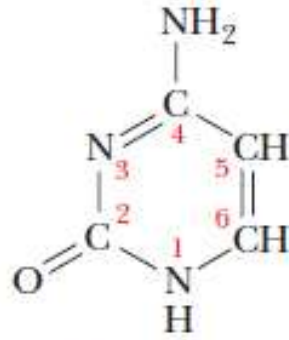


- Positively charged ions (Na⁺ or Mg²⁺) and peptides with positively charged side chains can associate with DNA
- Eukaryotic DNA, for example, is complexed with histones, which are positively charged proteins, in the cell nucleus.

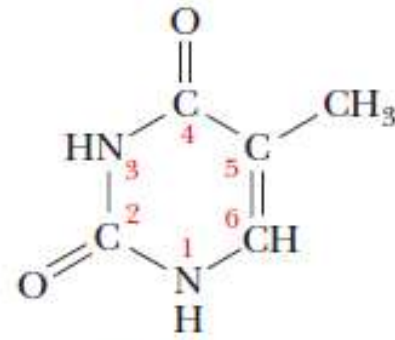
Nitrogenous bases



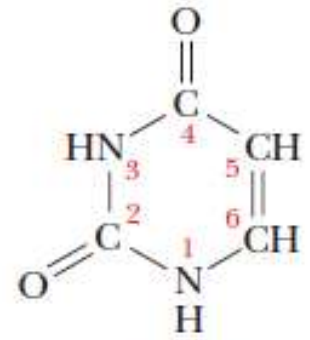
Pyrimidine



Cytosine
(in DNA & RNA)



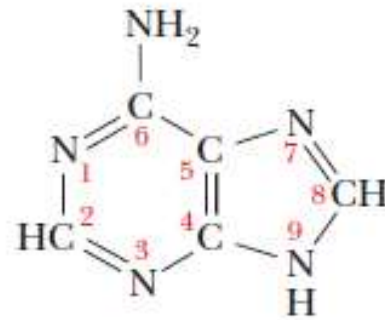
Thymine
(in DNA & some RNA)



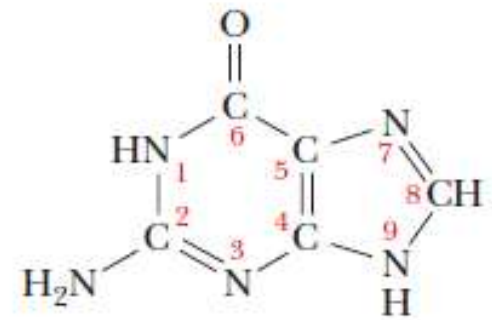
Uracil
(in RNA)



Purine

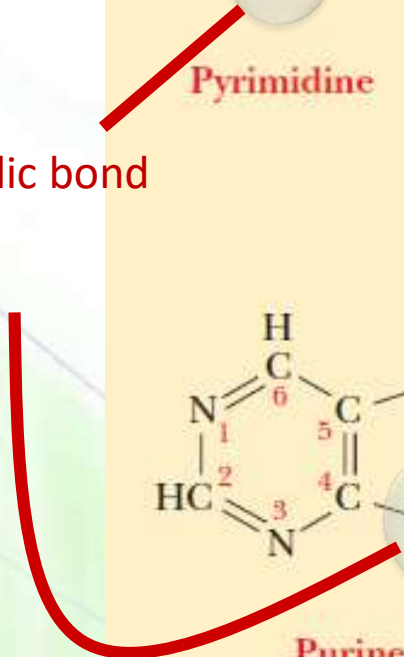


Adenine
(in DNA & RNA)



Guanine
(in DNA & RNA)

Glycosidic bond



In prokaryotes and eukaryotes



not viruses

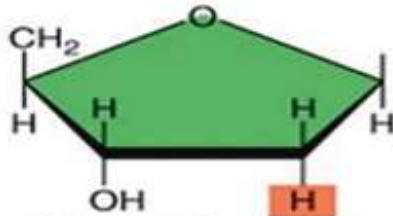
DNA vs. RNA



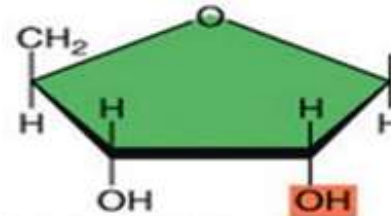
Double-stranded



Generally single-stranded

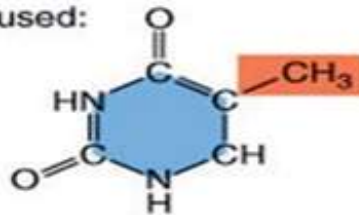


Deoxyribose as the sugar



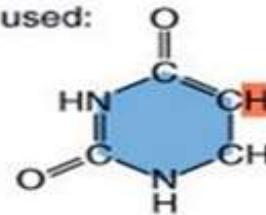
Ribose as the sugar

Bases used:



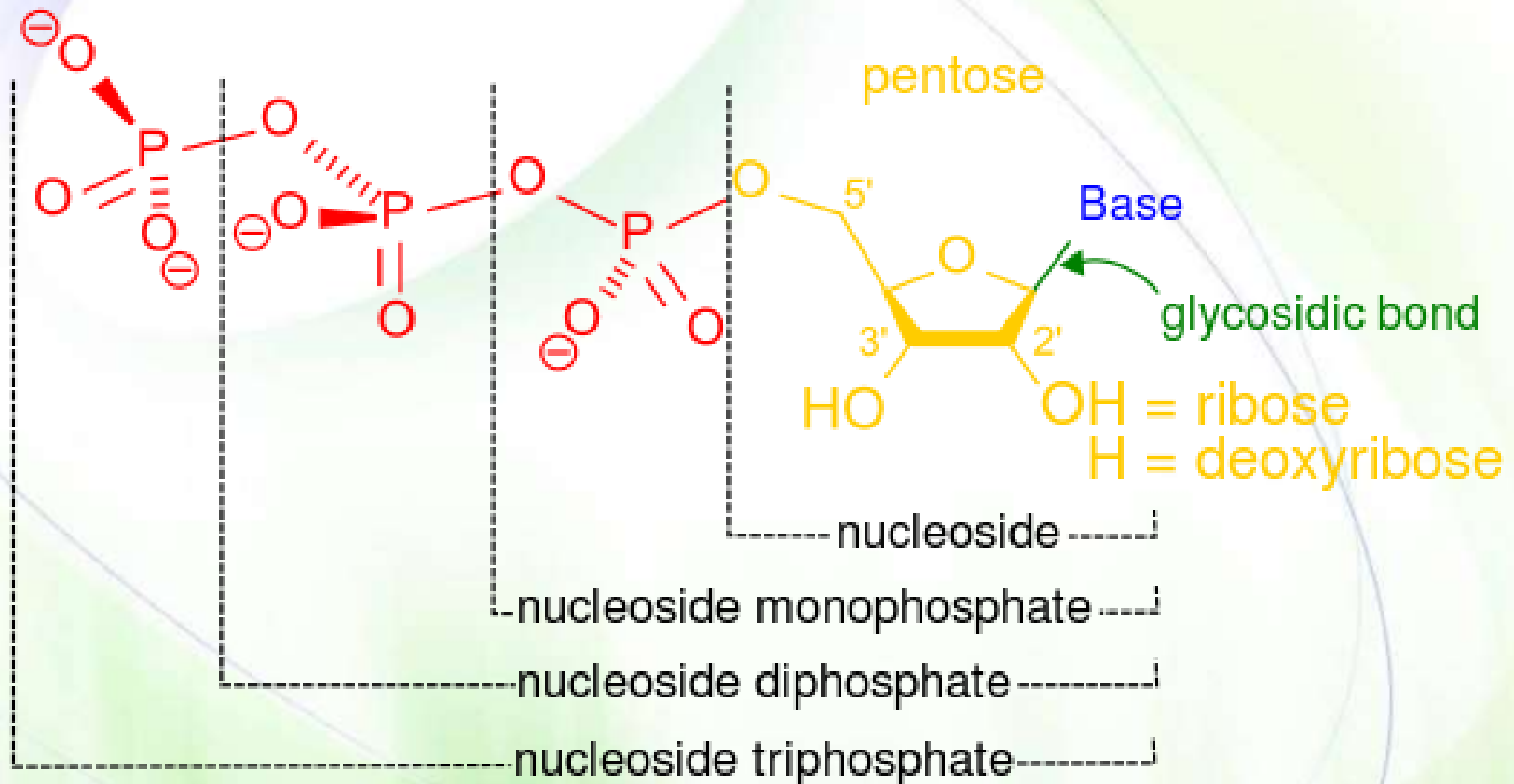
Thymine (T)
Cytosine (C)
Adenine (A)
Guanine (G)

Bases used:

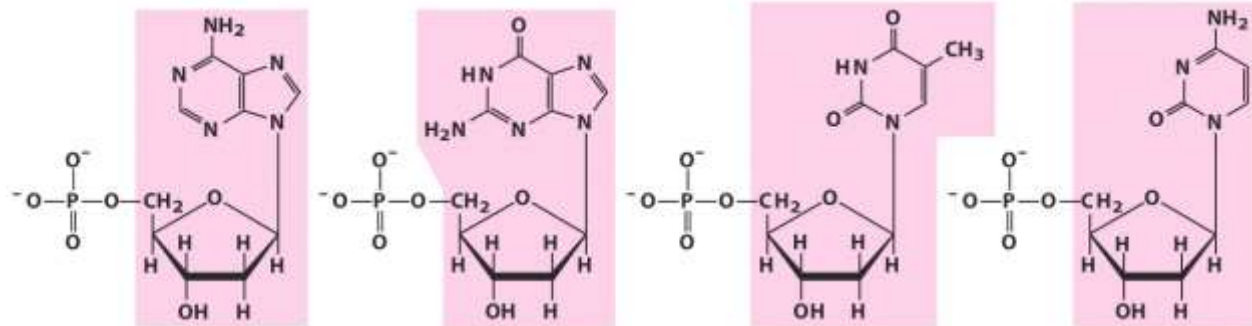


Uracil (U)
Cytosine (C)
Adenine (A)
Guanine (G)

Nucleotides vs. Nucleosides



Nucleotides vs. Nucleosides



Nucleotide: Deoxyadenylate
(deoxyadenosine
5'-monophosphate)

Symbols: A, dA, dAMP

Nucleoside: Deoxyadenosine

Nucleotide: Deoxyguanylate
(deoxyguanosine
5'-monophosphate)

Symbols: G, dG, dGMP

Nucleoside: Deoxyguanosine

Nucleotide: Deoxythymidylate
(deoxythymidine
5'-monophosphate)

Symbols: T, dT, dTMP

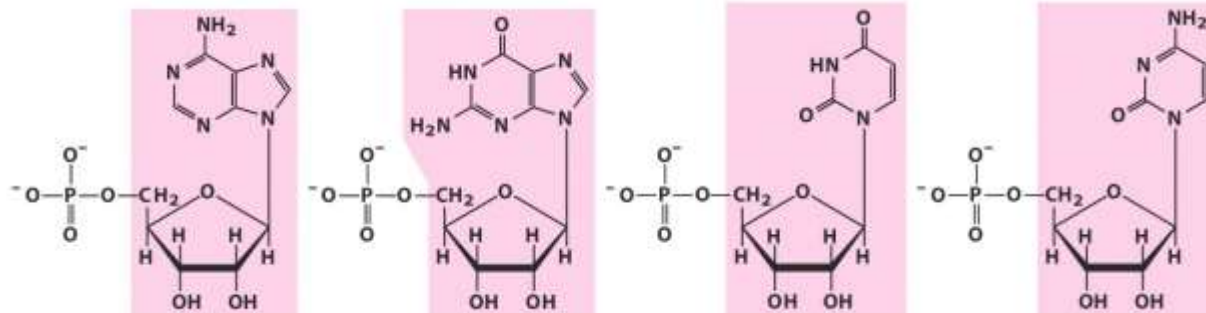
Nucleoside: Deoxythymidine

Nucleotide: Deoxycytidylate
(deoxycytidine
5'-monophosphate)

Symbols: C, dC, dCMP

Nucleoside: Deoxycytidine

(a) Deoxyribonucleotides



Nucleotide: Adenylate (adenosine
5'-monophosphate)

Symbols: A, AMP

Nucleoside: Adenosine

Nucleotide: Guanylate (guanosine
5'-monophosphate)

Symbols: G, GMP

Nucleoside: Guanosine

Nucleotide: Uridylate (uridine
5'-monophosphate)

Symbols: U, UMP

Nucleoside: Uridine

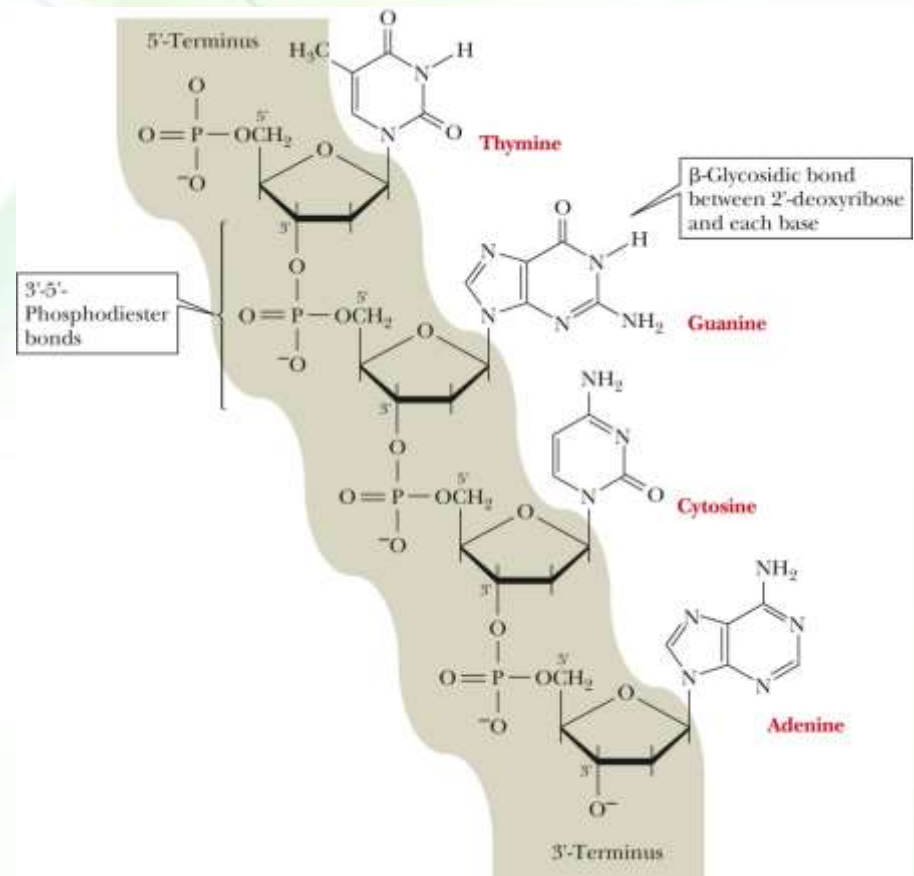
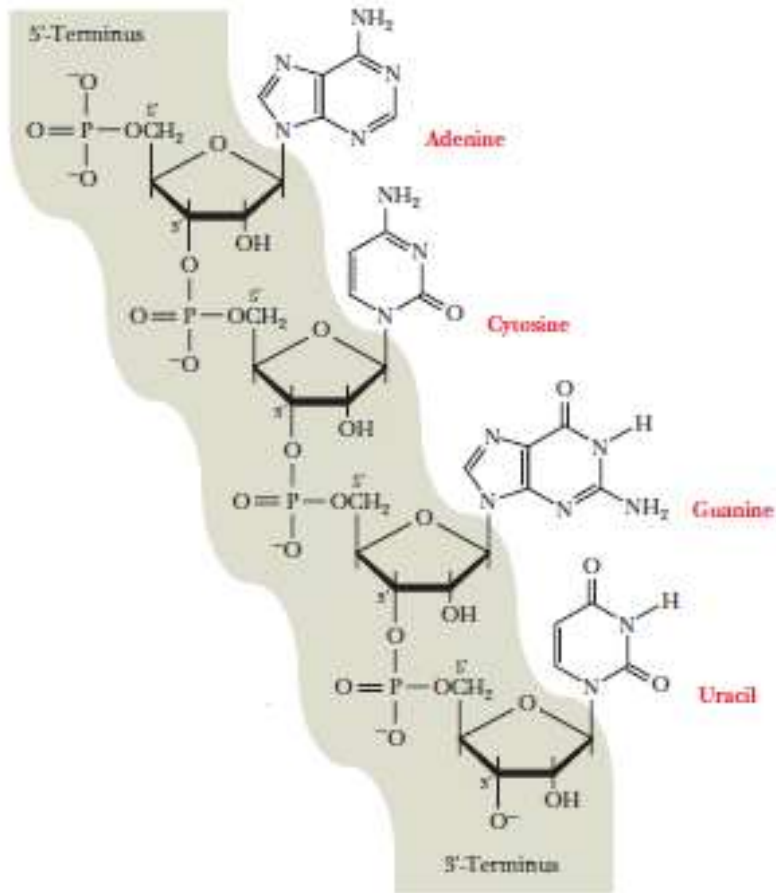
Nucleotide: Cytidylate (cytidine
5'-monophosphate)

Symbols: C, CMP

Nucleoside: Cytidine

(b) Ribonucleotides

Nucleic acid polymers

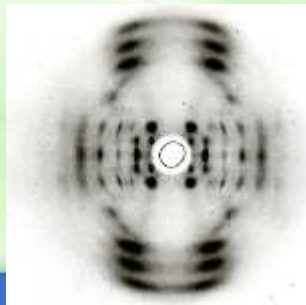
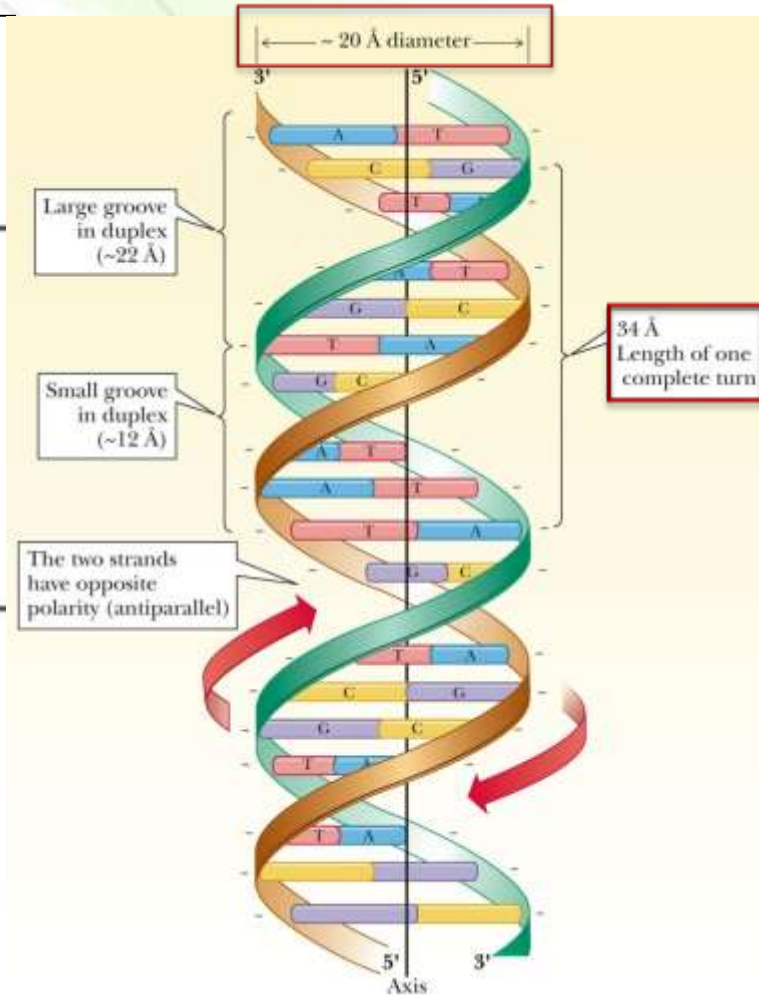
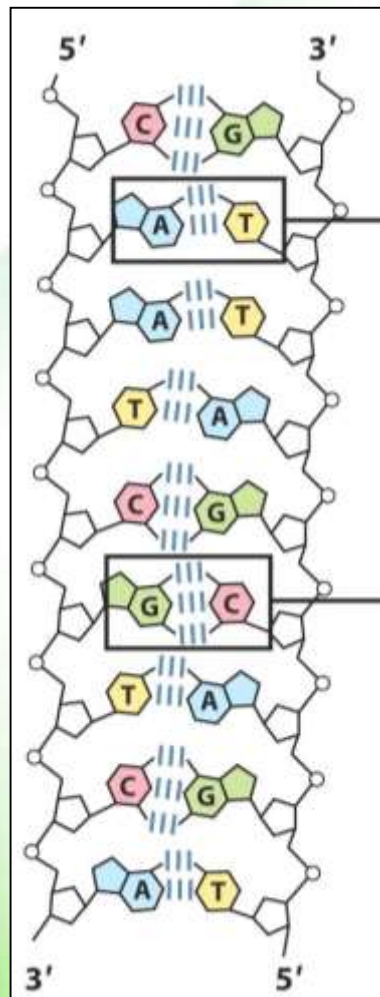


- A letter d can be added to indicate a deoxyribonucleotide residue.
- for example, dG is substituted for G.
- The deoxy analogue of a ribooligonucleotide would be d(GACAT).

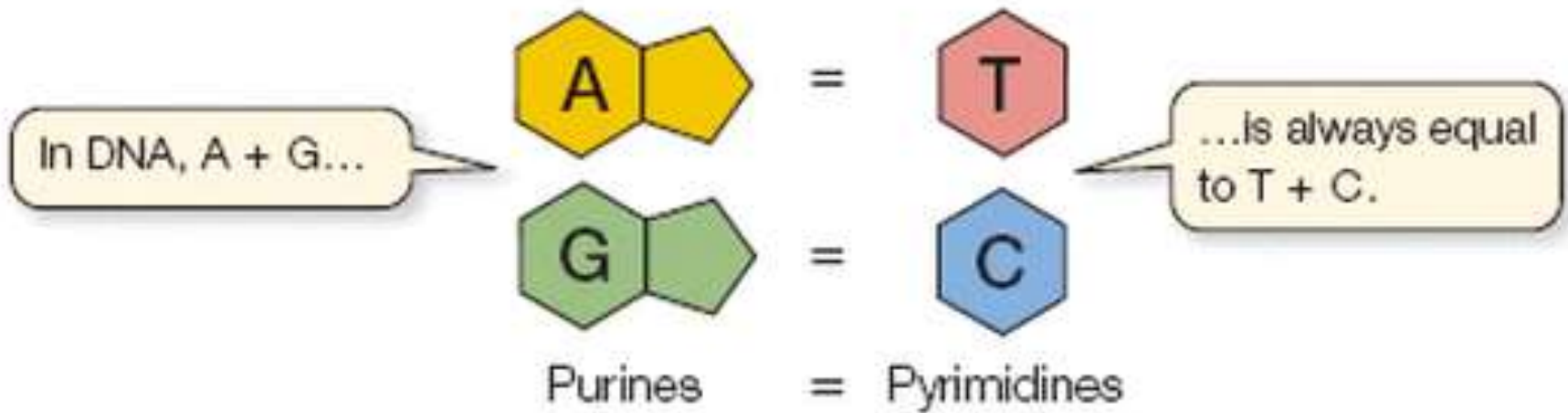
DNA structure



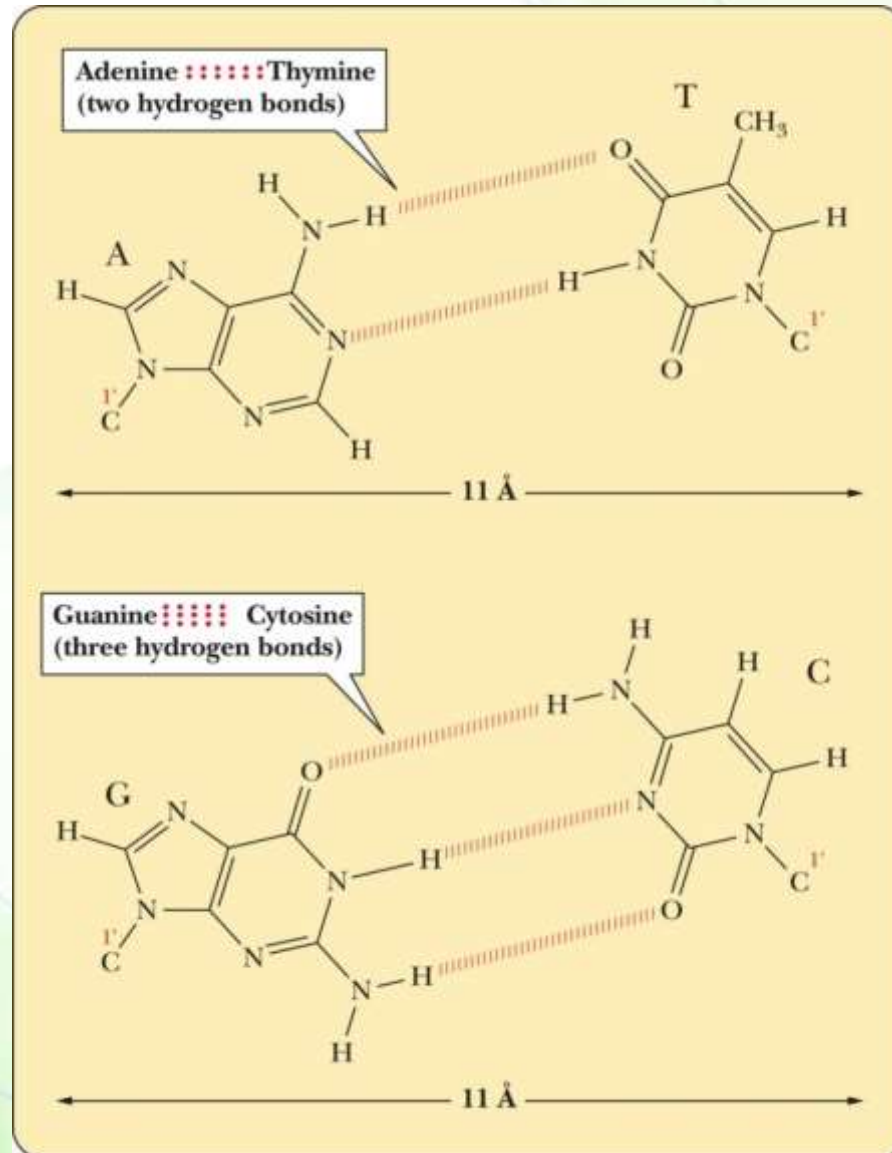
- A double helix
- Specific base-pairing
 - A = T; G = C; Pur = pyr
- Complementary
- Backbone vs. side chains
- Antiparallel
- Stability vs. flexibility
- Groovings



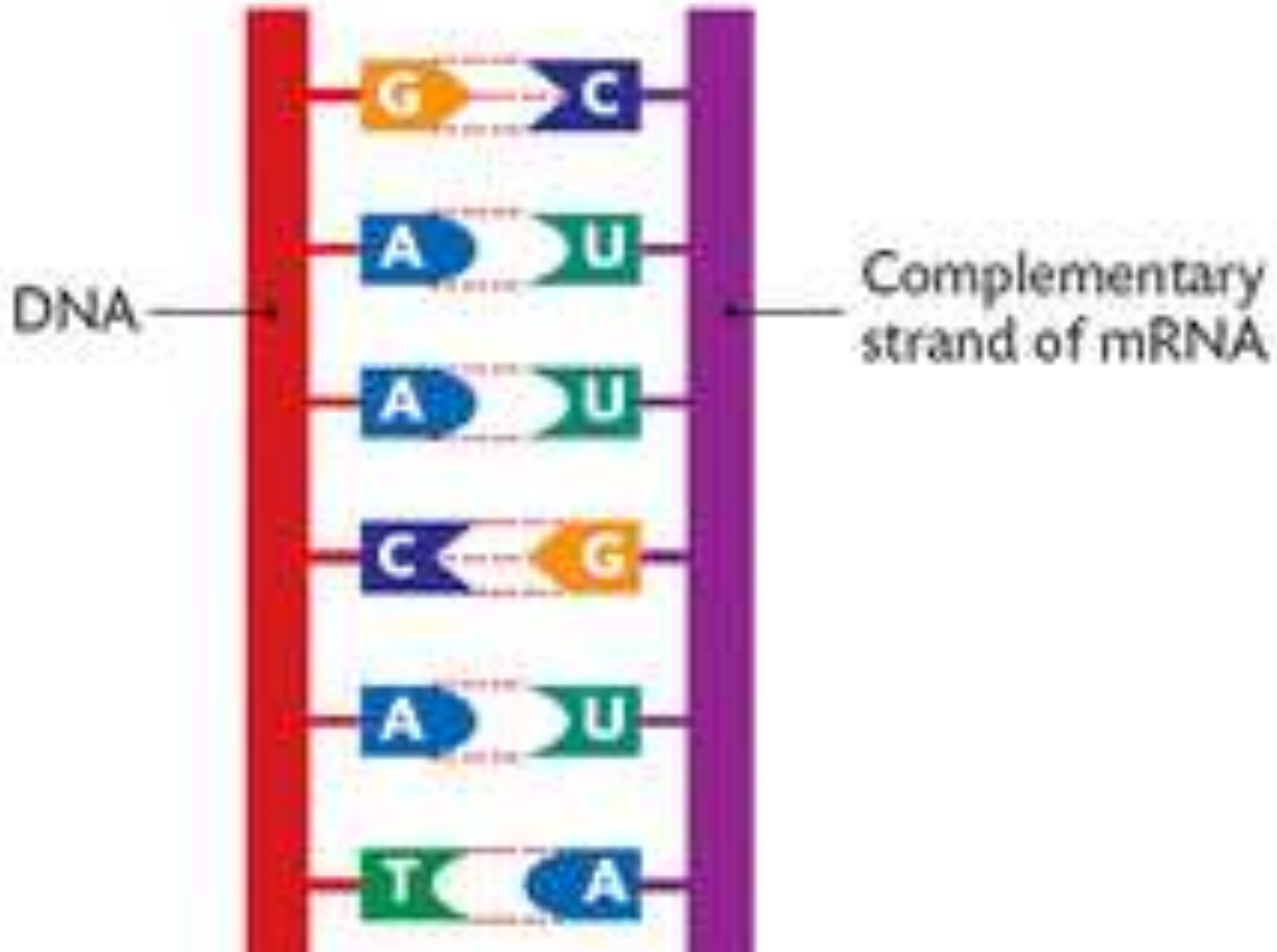
Chargaff's rules



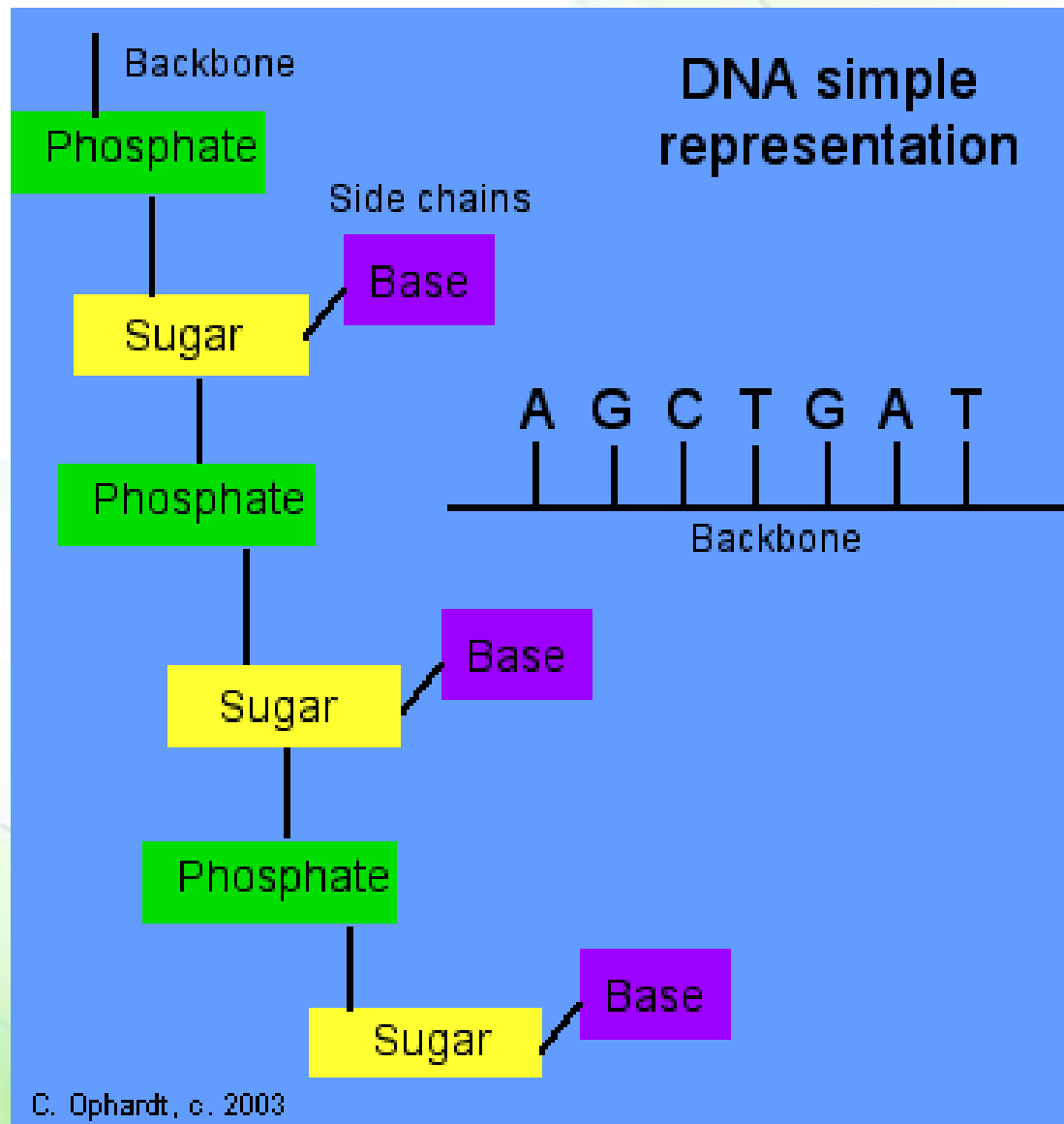
Base pairing



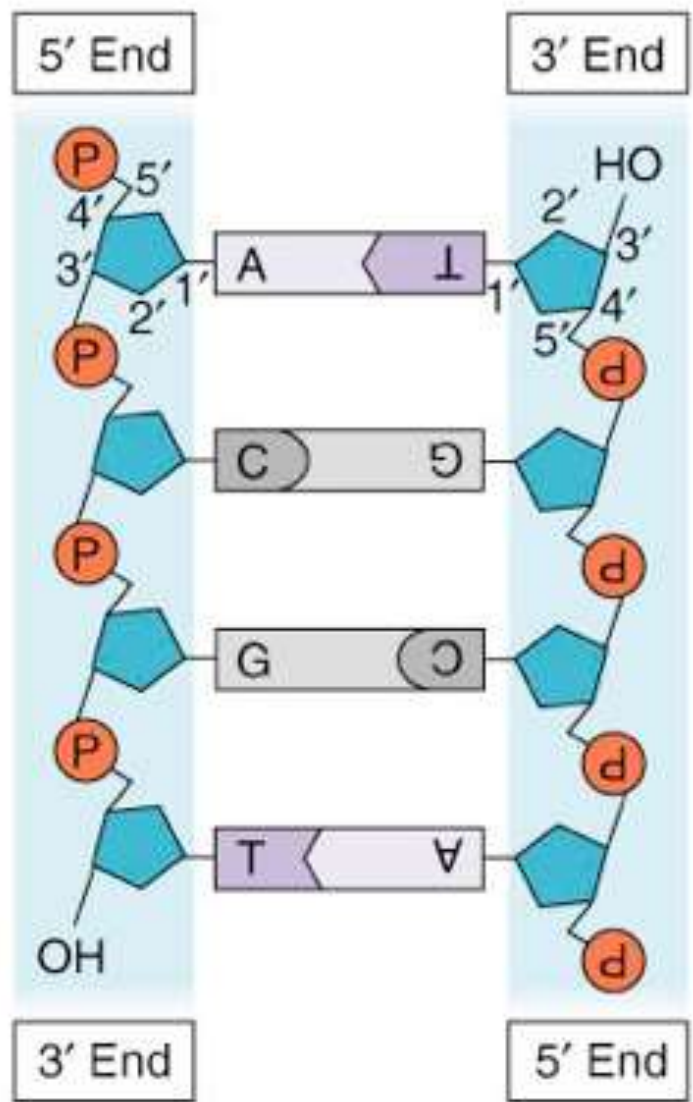
DNA is complementary



Backbone vs. side chains



DNA is anti-parallel



Sequence of nucleic acids

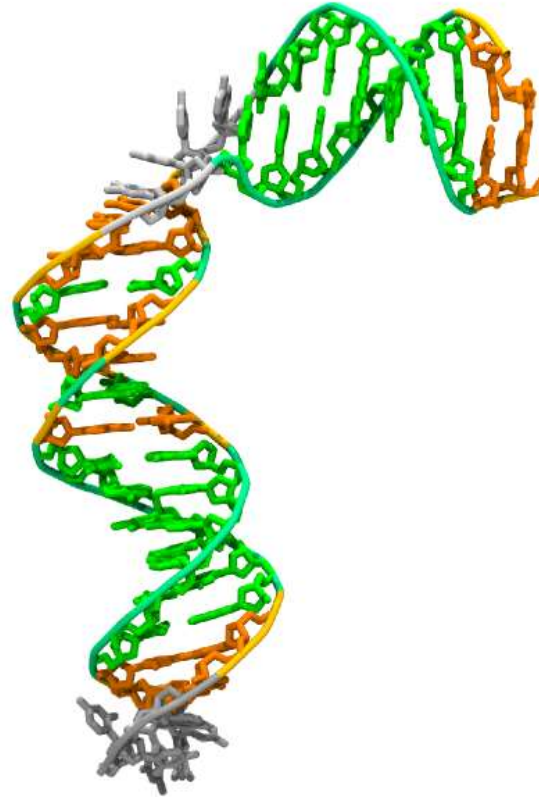
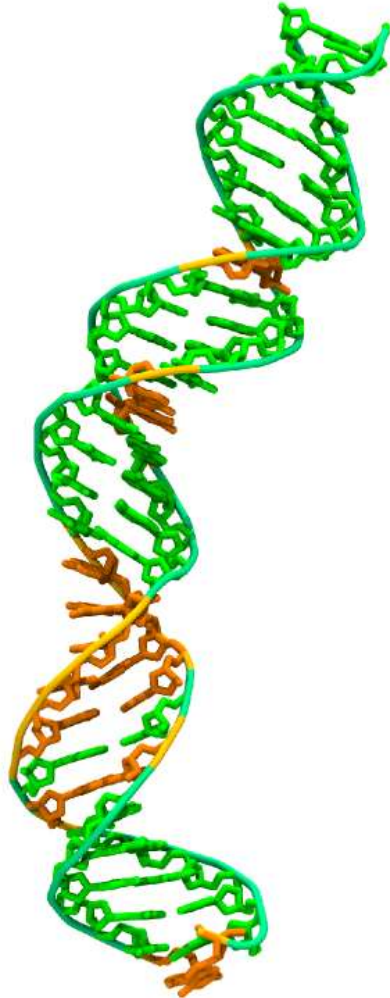
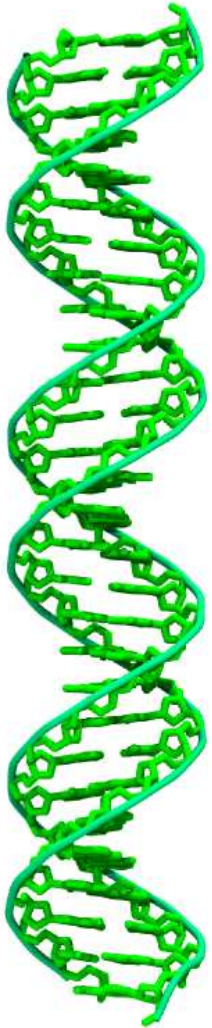


DNA 5' ...A T G G C C T G G A C T T C A... 3'
3' ...T A C C G G A C C T G A A G T... 5'

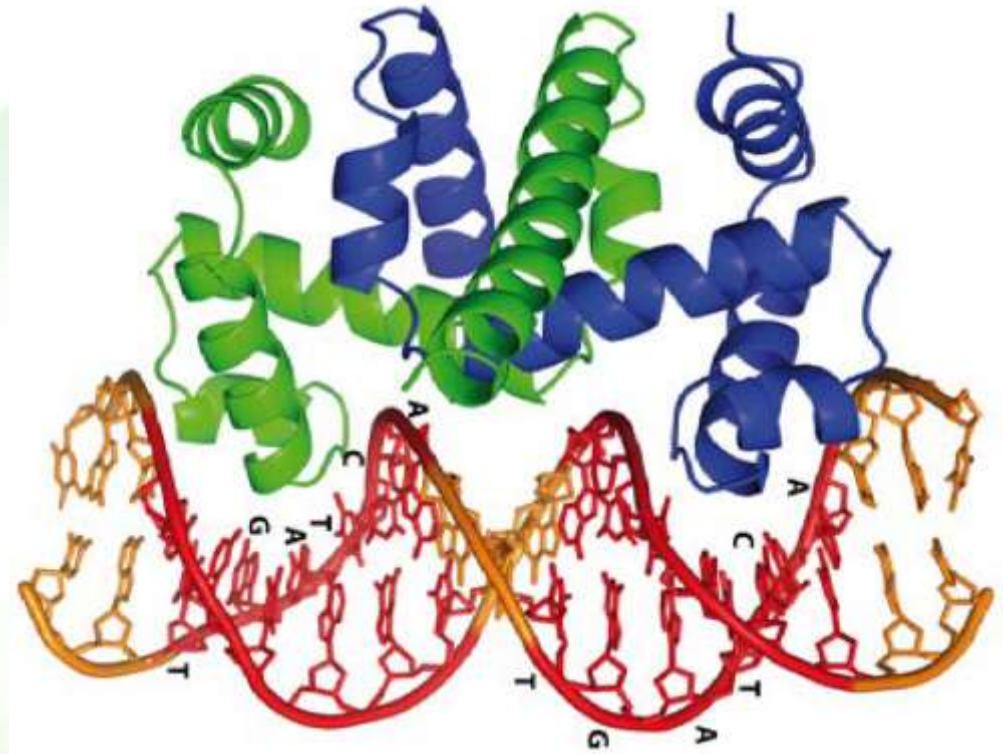
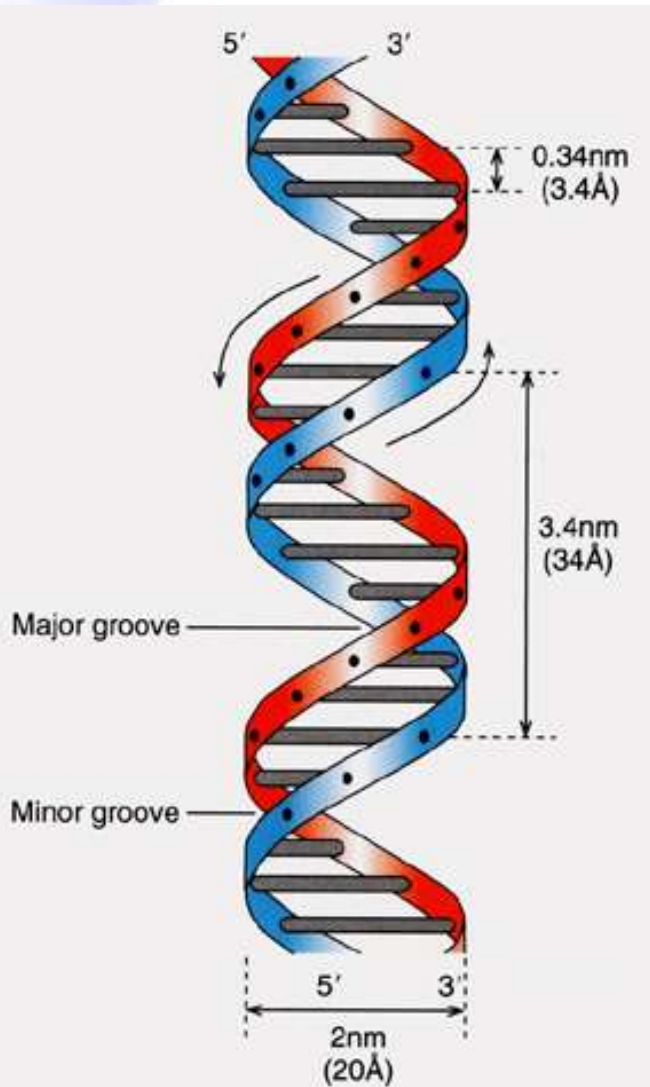
OR A T G G C C T G G A C T T C A.

RNA 5' ...A U G G C C U G G A C U U C A... 3'

DNA is flexible, yet stable



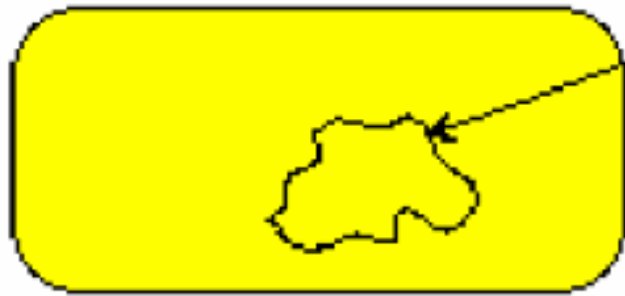
DNA grooves



Prokaryotes versus eukaryotes



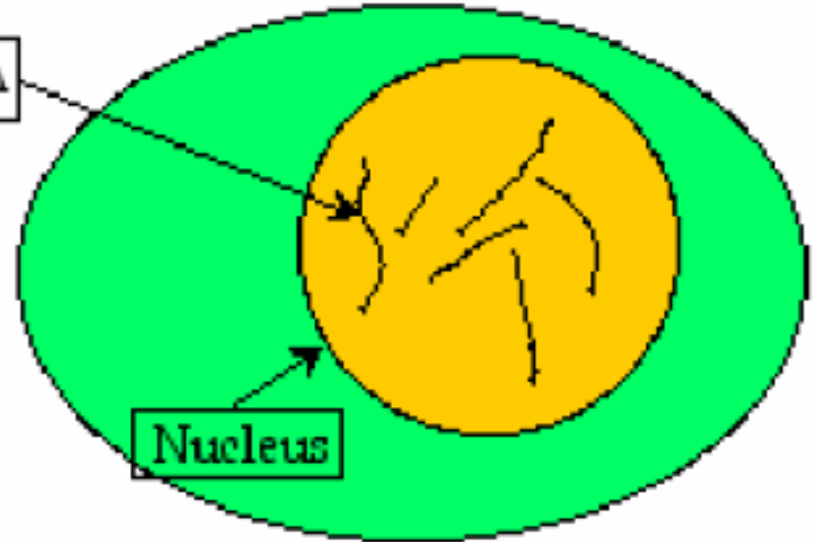
Prokaryote



No nucleus
Single loop of DNA

DNA

Eukaryote

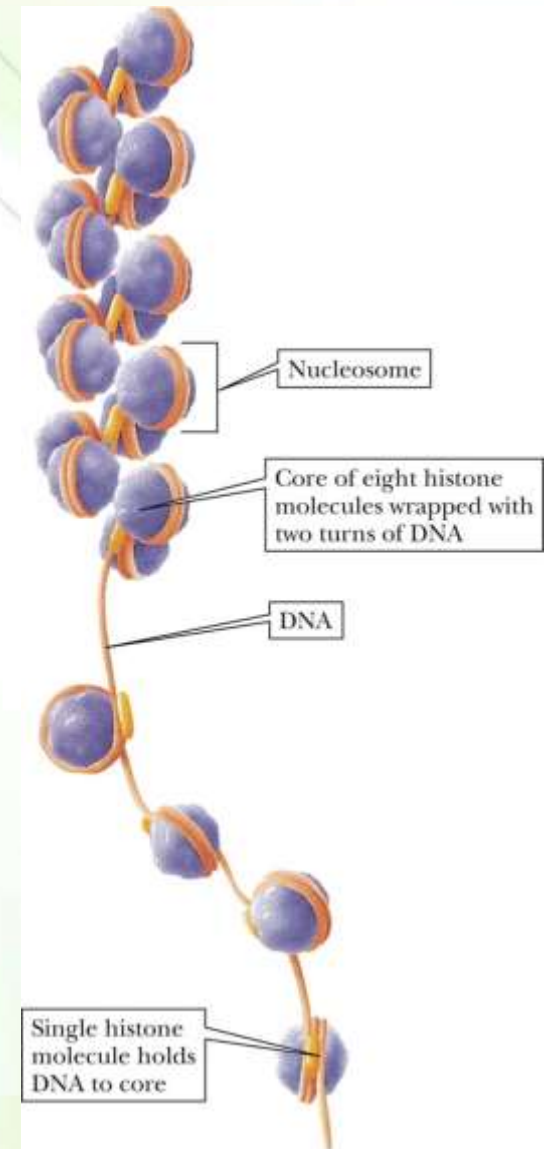


Has a Nucleus with DNA
in non-looped chromosomes

In eukaryotes...

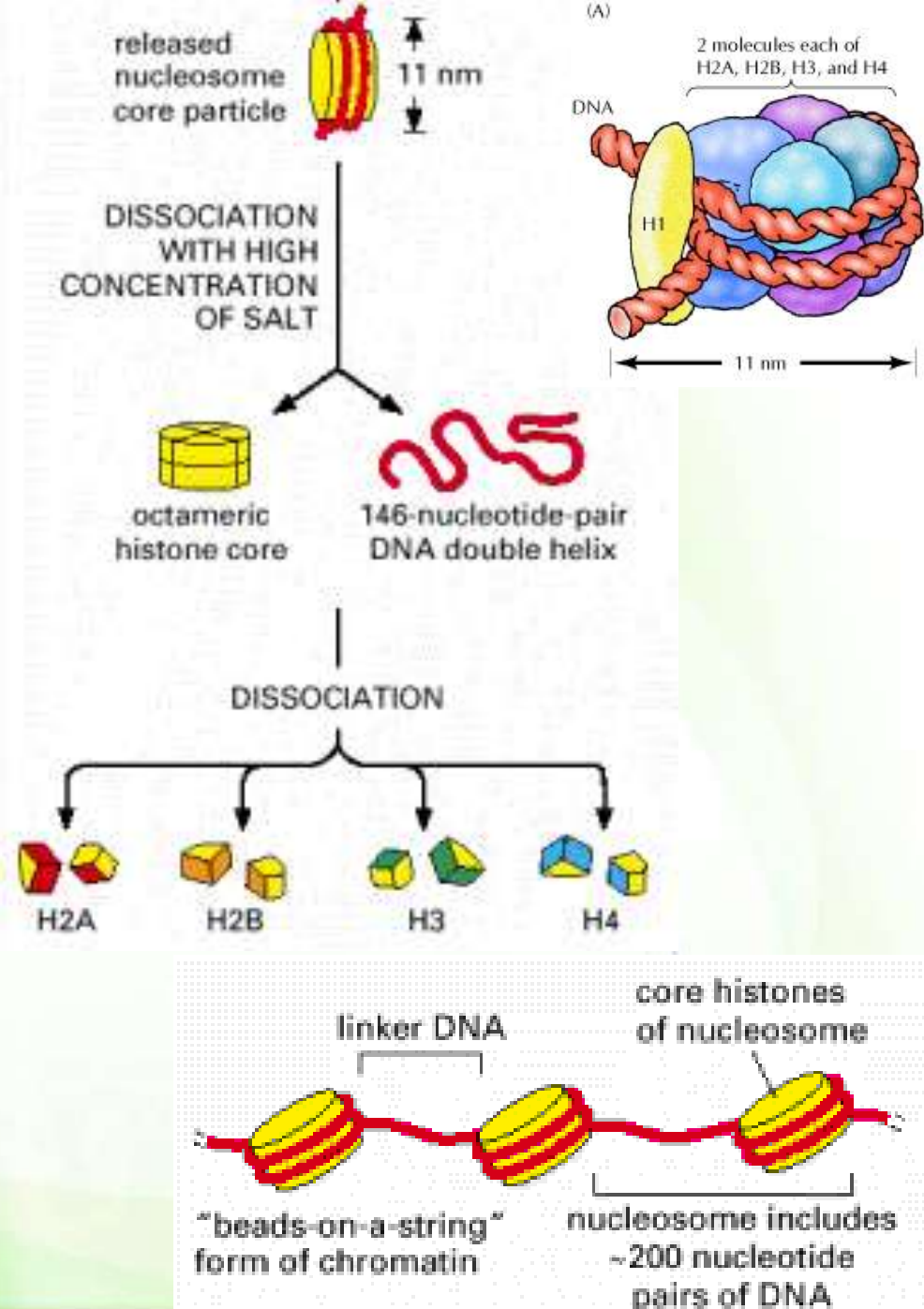


- In eukaryotes, DNA is coiled to package the large DNA.
- Eukaryotic DNA is complexed with a number of proteins, principally histones, which package DNA.
- Chromatin = DNA molecule + proteins.



Nucleosomes

- The histone protein core is an octamer (two molecules of histones H2A, H2B, H3, and H4).
- A linker DNA/spacer region connects the octamer-DNA complexes.
- A **nucleosome** consists of DNA wrapped around a histone core.
- H1 is bound to the the octamer and wrapped DNA (a **chromatosome**).
- Histones are positively charged facilitating DNA interaction and charge neutralization.

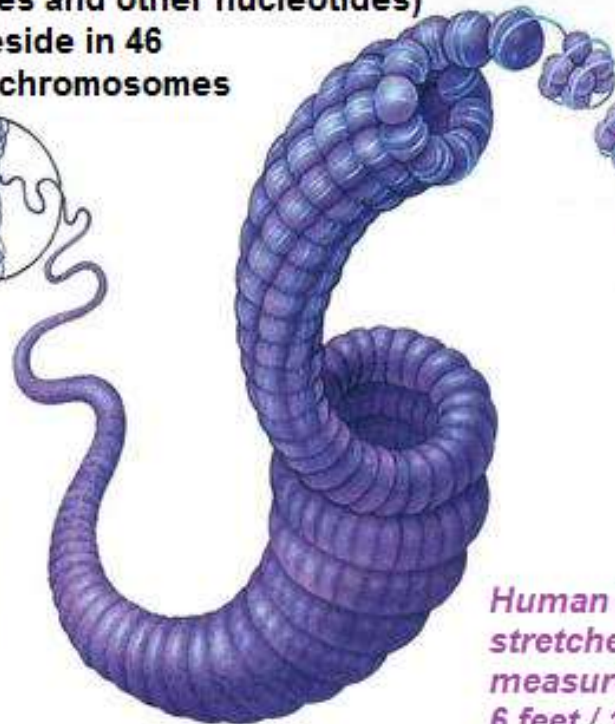


Histone package chromosomes



The Hierarchical Structure of DNA through to the Chromosome

DNA (genes and other nucleotides) reside in 46 chromosomes

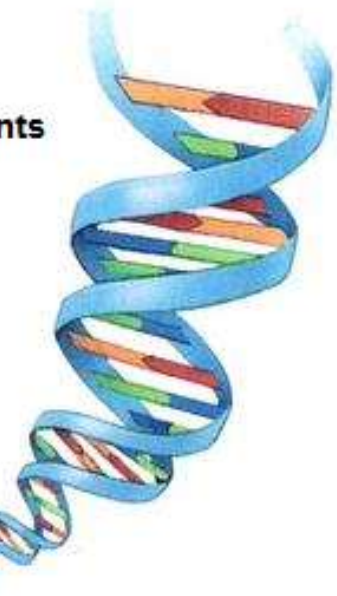


Genes are nucleotides that get expressed in the real world

Nucleotides are multiple segments of DNA base pairs

Human DNA stretched out measures some 6 feet / 1.8 meters

DNA is a combination of 4 possible amino acids, bound in pairs, in a double helix structure

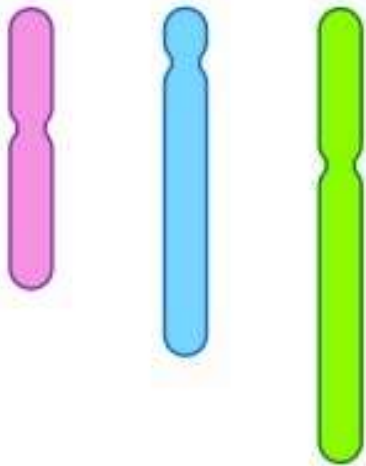


Remember...we are diploid



Haploid (n)

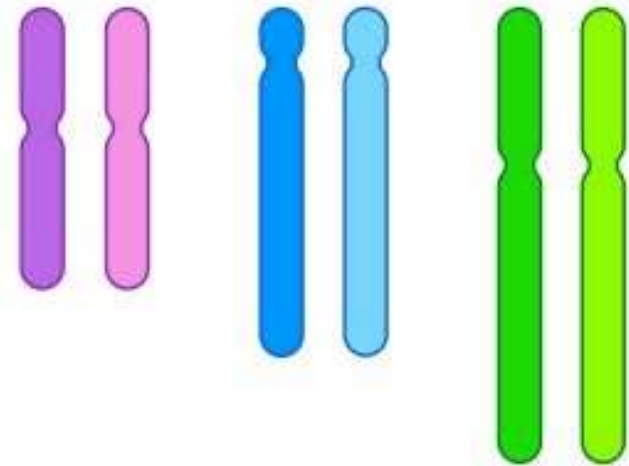
One copy of each chromosome



Three non-homologous chromosomes

Diploid (2n)

Two copies of each chromosome



Three pairs of homologous chromosomes
(of maternal and paternal origin)

Light absorbance of nucleic acids



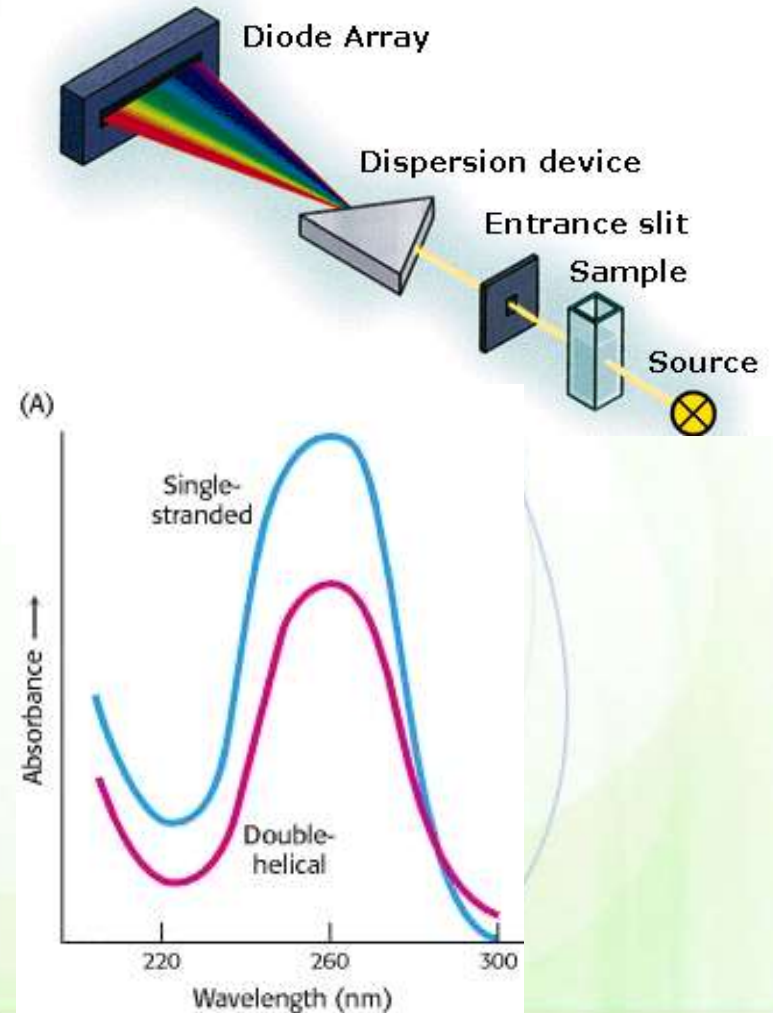
- Aromatic pyrimidines and purines can absorb UV light
- The peak absorbance is at 260 nm wavelength
- The absorbance of nucleic acids at 260 nm (A_{260}) is constant
 - dsDNA: A_{260} of 1.0 = 50 $\mu\text{g/ml}$

Reason for ss vs. ds absorbance:

- Unstacked bases vs. stacked bases

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

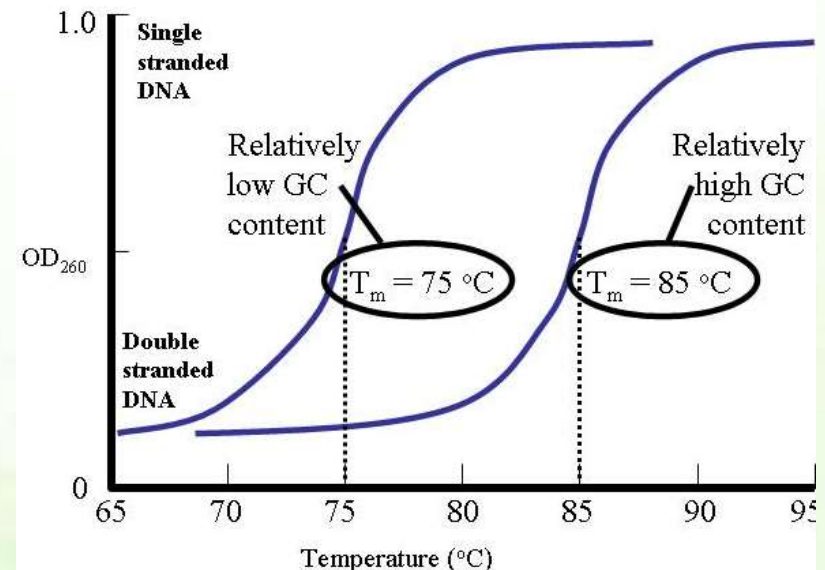
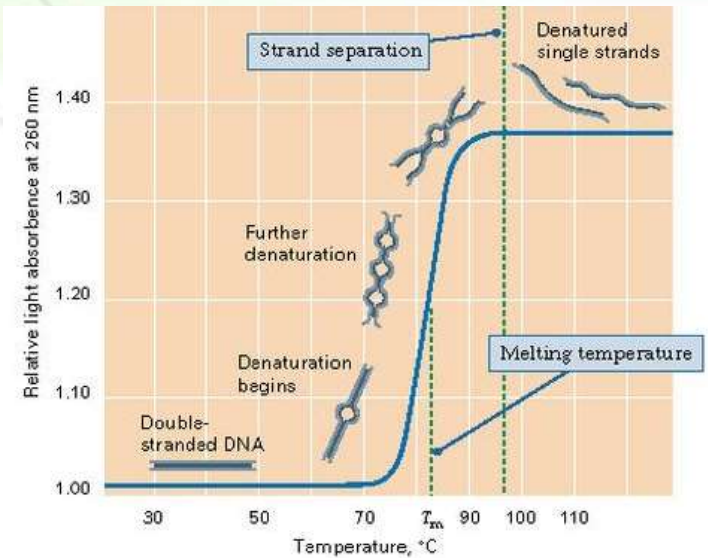
$$\begin{aligned}\text{DNA concentration} &= 0.1 \times 10 \times 50 \mu\text{g/ml} \\ &= 50 \mu\text{g/ml}\end{aligned}$$



Observation of denaturation



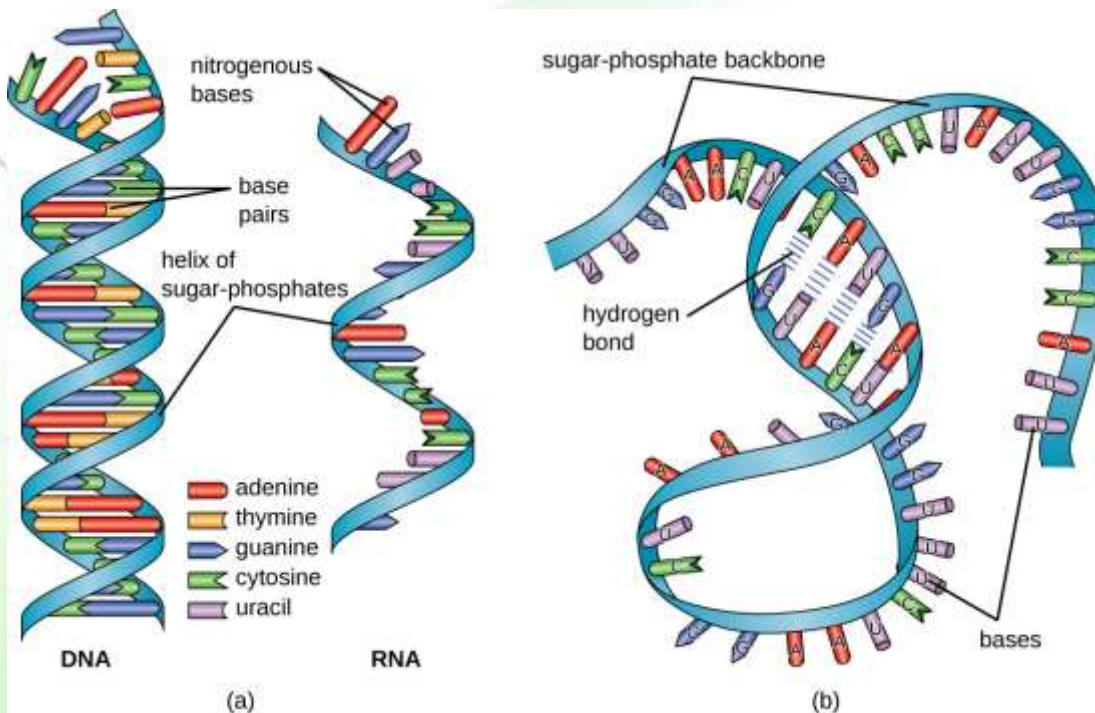
- The transition temperature, or melting temperature (T_m).
- Factors influencing T_m
 - Length
 - G·C pairs
 - Hydrogen bonds
 - Base stacking
 - pH
 - Salts and ions
 - Destabilizing agents (alkaline solutions, formamide, urea)



RNA



- It consists of long, unbranched chains of nucleotides joined by phosphodiester bonds between the 3'-OH of one pentose and the 5'-PO₄⁻ 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 (thymine and cytosine in DNA).
- In general, RNA is single stranded (DNA is double stranded).



RNA does not have a precise structure, but it can fold on itself forming hydrogen bonds within the same molecule.

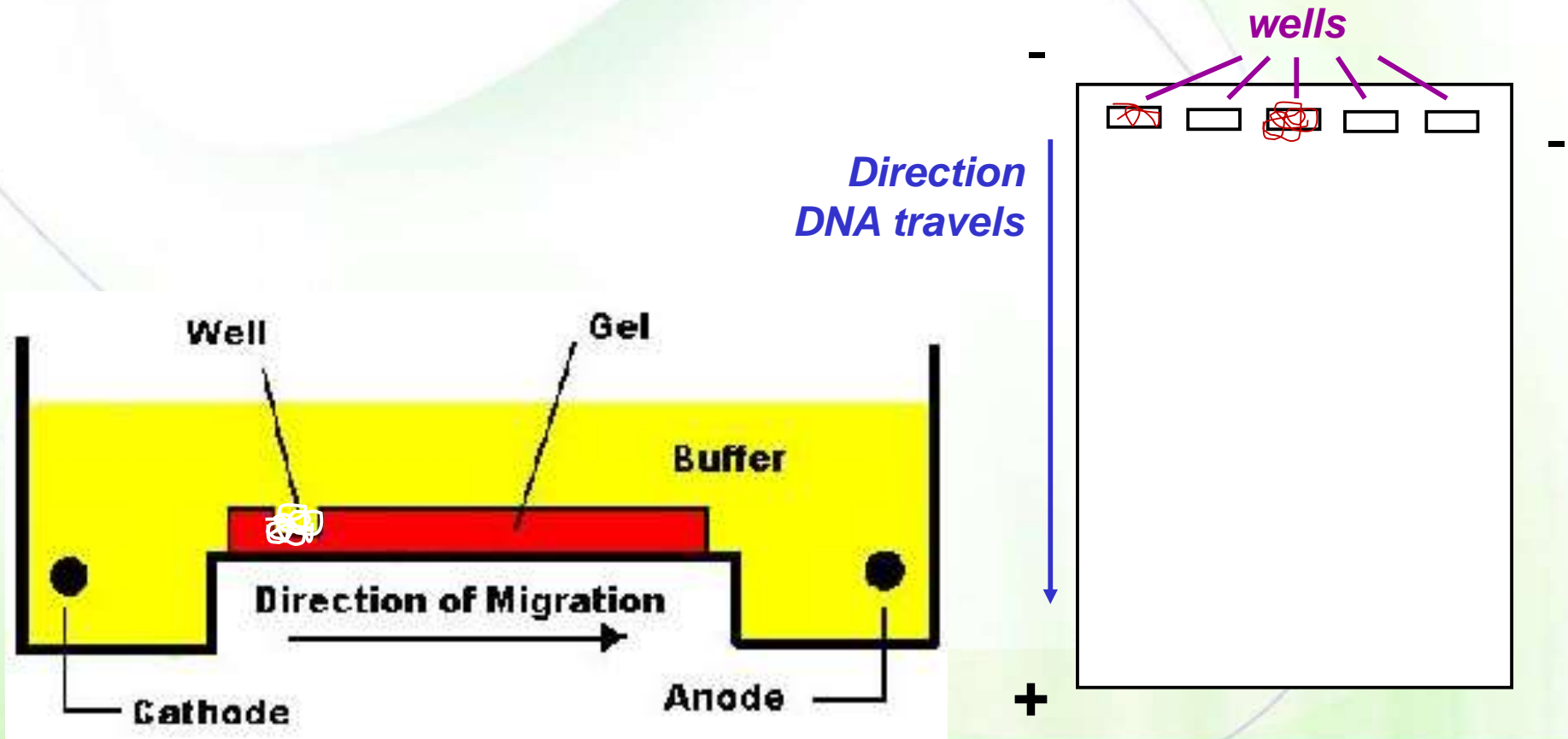
Types of RNA



Non-coding RNA	Length (nt)	Species	Function
Ribosomal RNA (rRNA)	120~4700	All	Translation
Transfer RNA (tRNA)	70~100	All	Translation
Small nuclear RNA (snRNA)	70~350	Eukaryote	Splicing, mRNA processing
Small nucleolar RNA (snoRNA)	70~300	Eukaryote, archaea	RNA modification, rRNA processing
miRNA	21~25	Eukaryote	Translational regulation
siRNA	21~25	Eukaryote	Protection against viral infection
piRNA	24~30	Eukaryote	Genome stabilization
Long ncRNA	several hundreds~ several hundred thousands	Eukaryote	Transcription, splicing, transport regulation

Gel electrophoresis

- The length and purity of DNA molecules can be accurately determined by the gel electrophoresis.



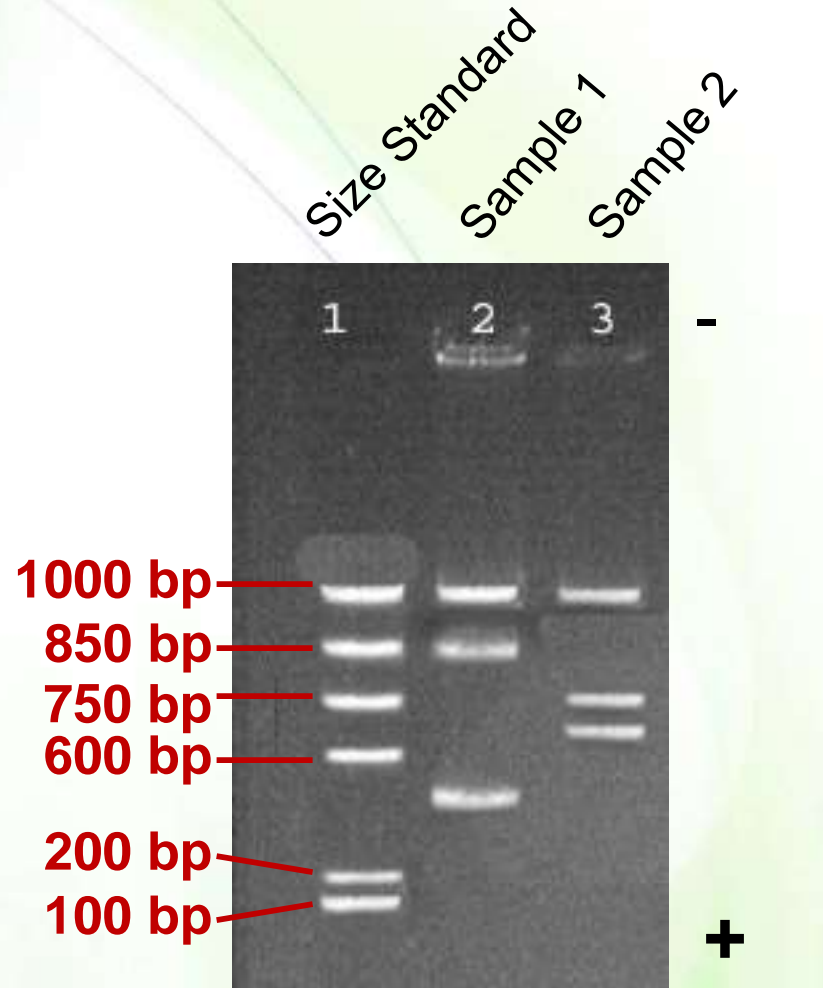
Resources



- <http://www.personal.psu.edu/pzb4/electrophoresis.swf>
- <http://www.sumanasinc.com/webcontent/animations/content/gelectrophoresis.html>
- <http://www.sumanasinc.com/webcontent/animations/content/gelectrophoresis.html>

Detection

- The DNA molecules of different lengths will run as "bands".
- **Each bands contains thousands to millions of copies of DNA fragments of the same length. They can be of same or different type (not one DNA molecule).**
- DNA is **stained** (that is, colored) with a dye (ethidium bromide) or radioactively **labeled** (^{32}P).
- It is common that a DNA standard is used to determine the length of the examined DNA molecule.

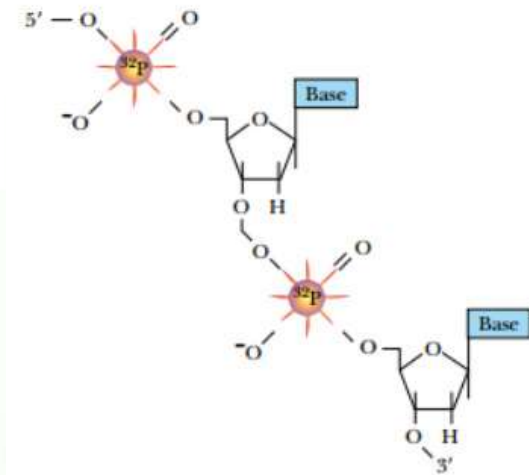
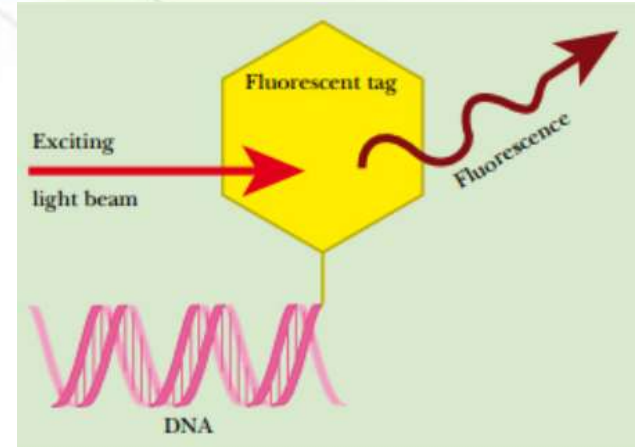
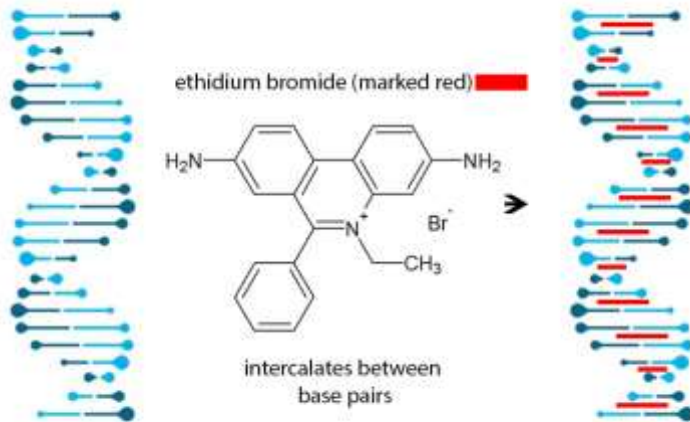


bp: base pair



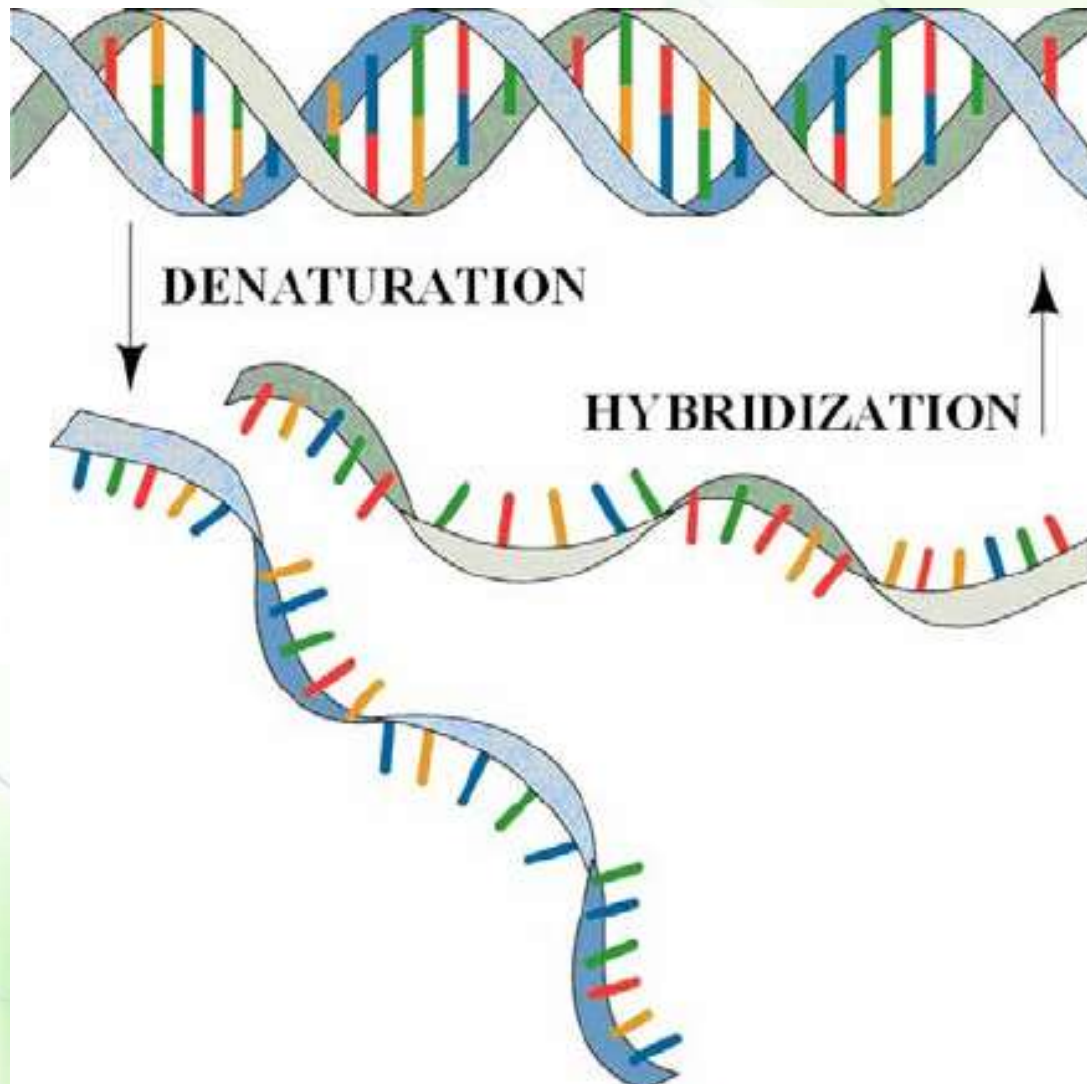
DNA Labeling

DNA staining



³²P-LABELED DNA

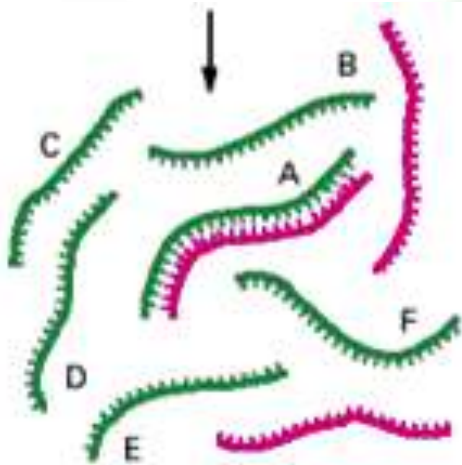
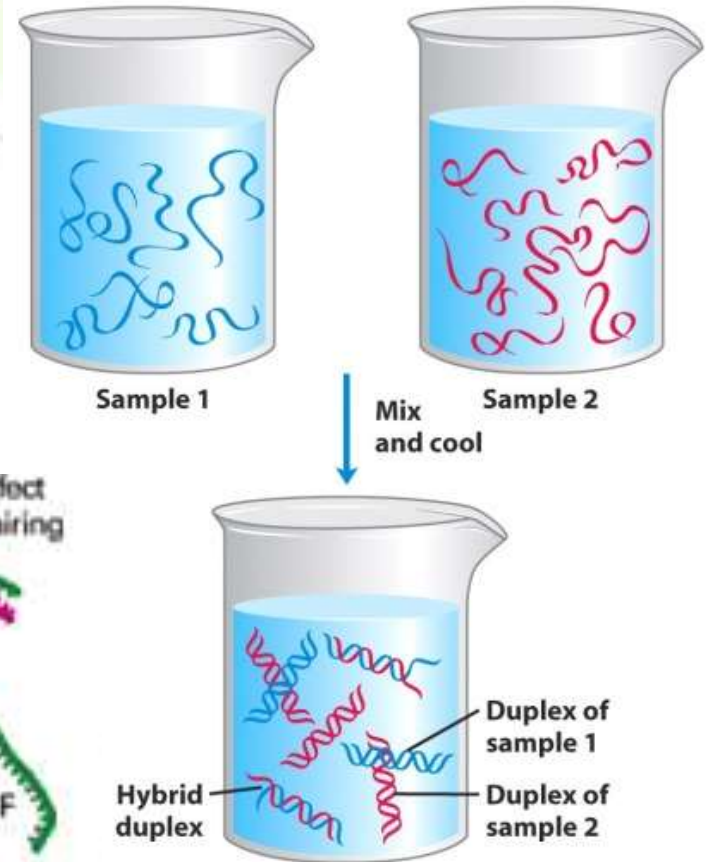
Denaturation versus renaturation (hybridization)



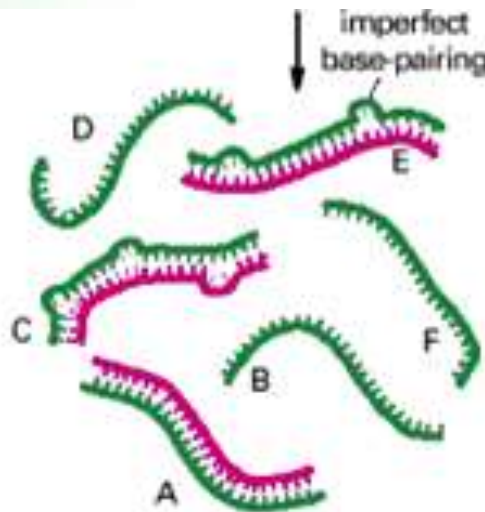
Hybridization



- DNA from different sources can form double helix as long as their sequences are compatible (hybrid DNA).
- Hybridization can be imperfect.



only A forms stable double helix



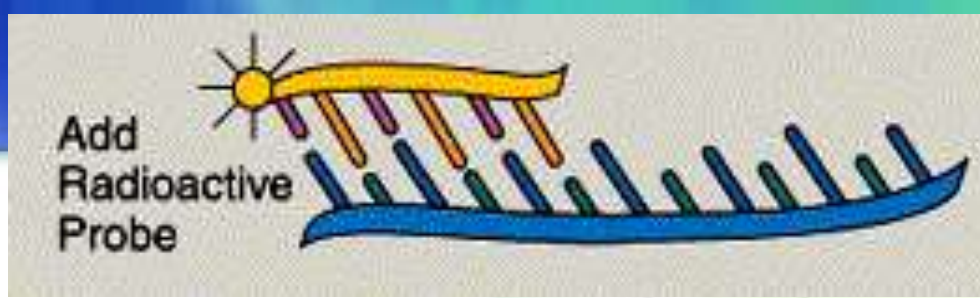
A, C, and E all form stable double helices

Hybridization techniques



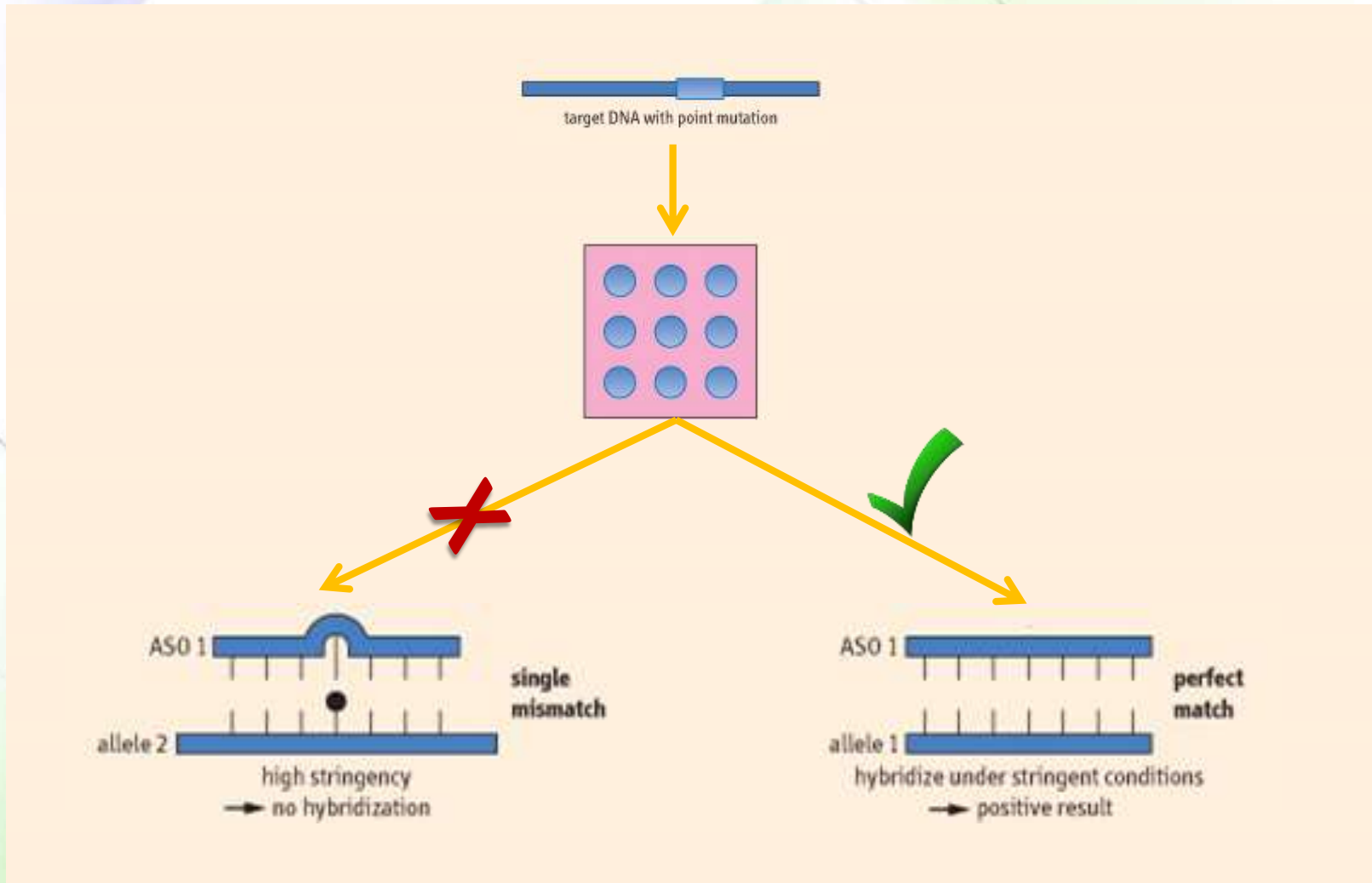
- Hybridization reactions can occur between any two single-stranded nucleic acid chains provided that they have complementary nucleotide sequences
- Hybridization reactions are used to detect and characterize specific nucleotide sequences

Probes



- A probe is a short sequence of single stranded DNA (an oligonucleotide) that is complementary to a small part of a larger DNA sequence.
- Hybridization reactions use labeled DNA probes to detect larger DNA fragments.

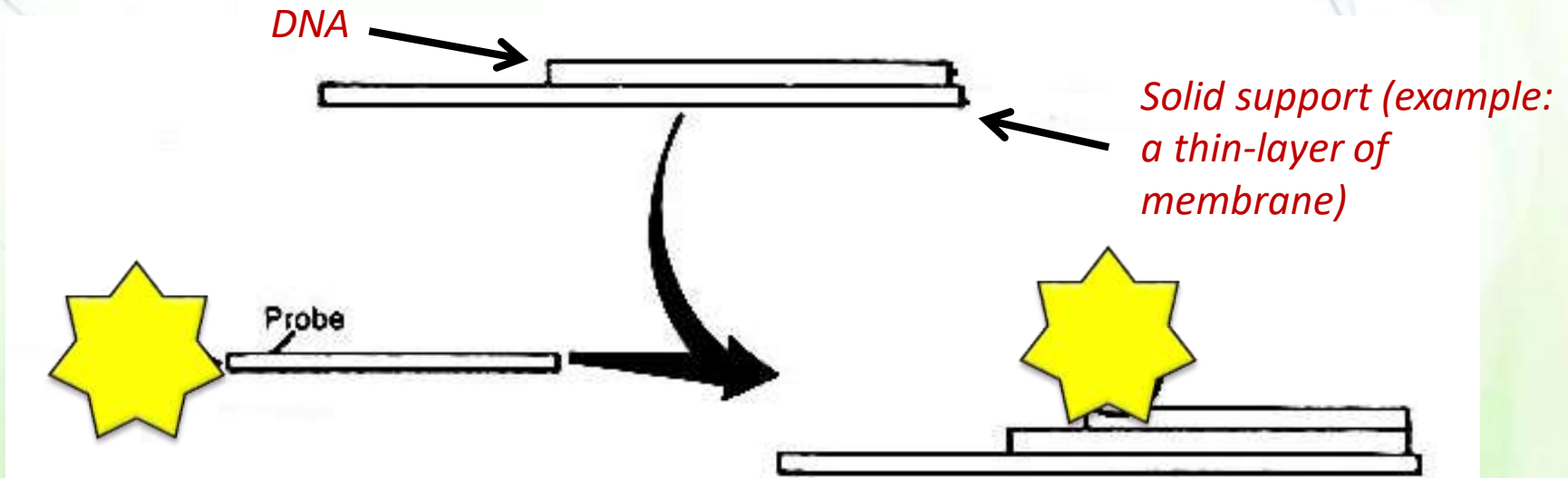




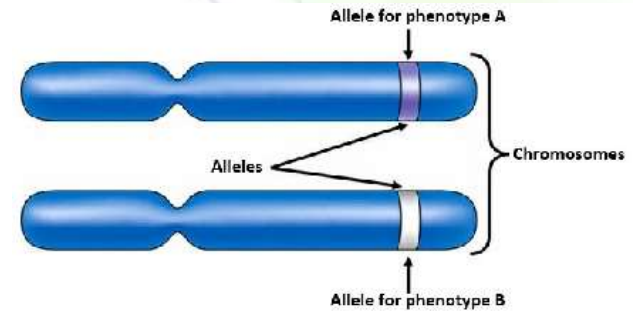
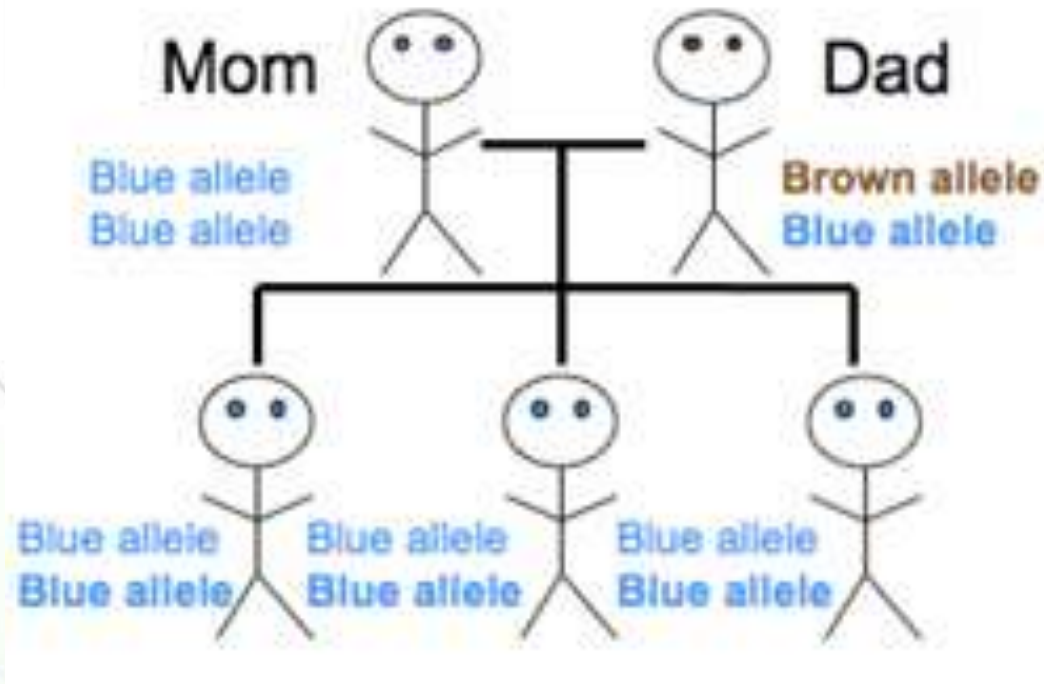
Dot blot



- This is a technique that informs us if a specific sequence that is complementary to a probe of a known sequence exists in a larger DNA.
- DNA is bound to a solid support and a labeled probe is added. If binding occurs, the sequence exists.



Concepts to know...



Pedigree

Allele

Dominant vs. recessive

Oligonucleotide

Disease detection by ASO (Cystic fibrosis)



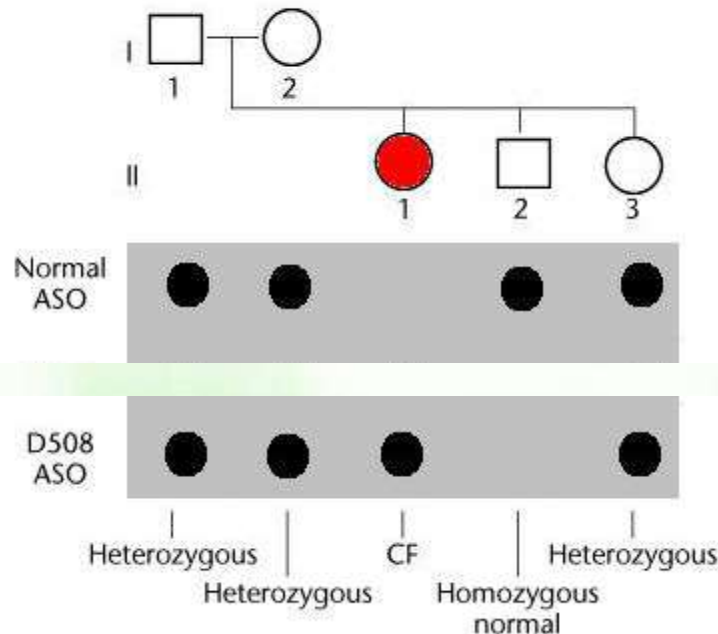
ASO: Allele-specific oligonucleotide

The whole genomic DNA is spotted on a solid support (like a nylon membrane) and hybridized with two ASO's, one at a time.

Cystic Fibrosis allele $\Delta 508$ has 3bp deletion [AGA]

ASO for normal DNA 5' CACCAA[AGA]GATATTTTC-3'

ASO for DNA sequence of $\Delta 508$ mutation 5' CACCAATGATATTTTC-3'



Southern blotting



- This technique is a combination of DNA gel electrophoresis and hybridization
- Used to detect:
 - the presence of a DNA segment complementary to the probe
 - the size of the DNA fragment

