# Nucleic Acids & Chromosomes

Lecture 17

# Objectives

- Define nucleotide, nitrogenous bases
- Describe structure of a nucleotide and list the types of nitrogen bases
- Compare between DNA & RNA and describe briefly their structure & functions
- List the types of RNA and know function of each type
- Understand Chargaff's rule
- Describe double helix of DNA
- Understand terms minor groove, major groove, B DNA, Z DNA
- Describe the term genome
- Describe briefly the structure and replication of chromosomes
- Define the terms nucleosome, solenoid, centromere, replication bubble, RNA primer

# Nucleic Acids

- Nucleic acids form the structure of genes, which provide the basic blueprint of life
- They are the largest molecules in the body, being polymeric forms. There are two different types of nucleic acids
  - Deoxyribonucleic acid (DNA)
  - Ribonucleic acid (RNA)
- Genes are sequences of DNA that code for proteins or RNA
- All gene action determines the development of the cell and ultimately of the entire organism (if multicellular)

  Genes thus determine the type of organism: species
- Nucleic acids consist of atoms of carbon (C), oxygen (O), hydrogen (H), nitrogen (N) and phosphorus (P)

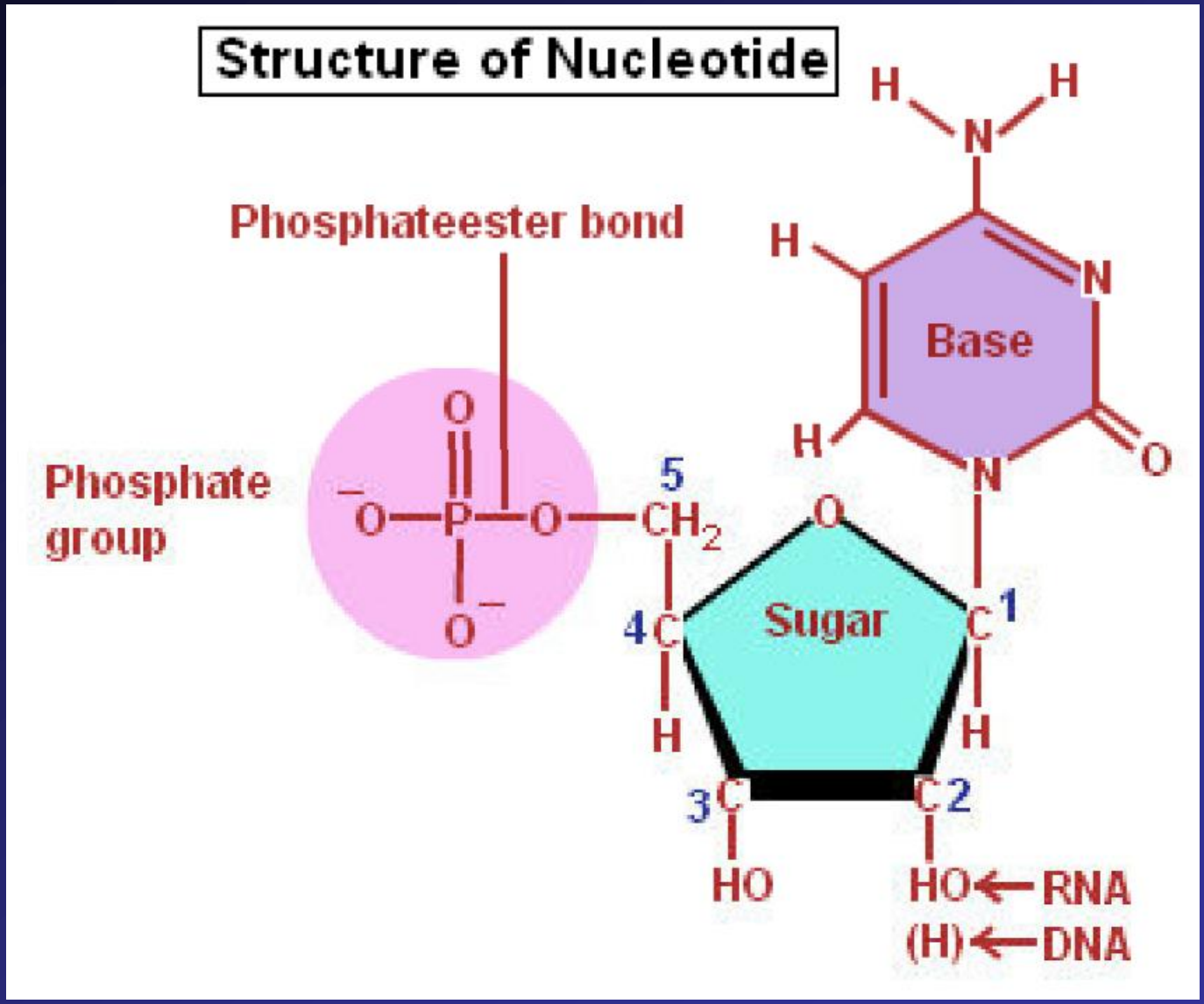
# Nucleotides

- Nucleotides are the monomeric building blocks of nucleic acids: nucleic acids are thus polynucleotides (polymeric nucleotides)
- Each nucleotide has three identifiable moieties (parts)

1. Two types of nitrogen-containing bases
   i. purines: these are 2-aromatic ringed structures
   ii. pyrimidines: these are 1-aromatic ringed structures
2. *keto*pentose sugar
   - 2'-deoxyribose in DNA
   - ribose in RNA
3. phosphate

When DNA and RNA are in solution, the presence of negatively charged phosphate in the DNA and RNA structure makes DNA and RNA very highly negatively charged polymers, and this has tremendous biological significance in their biochemistry

# Nucleotide Structure



# Nitrogenous Base Features

*Within the nucleotide, some detail of the purine and pyrimidine nitrogenous bases*

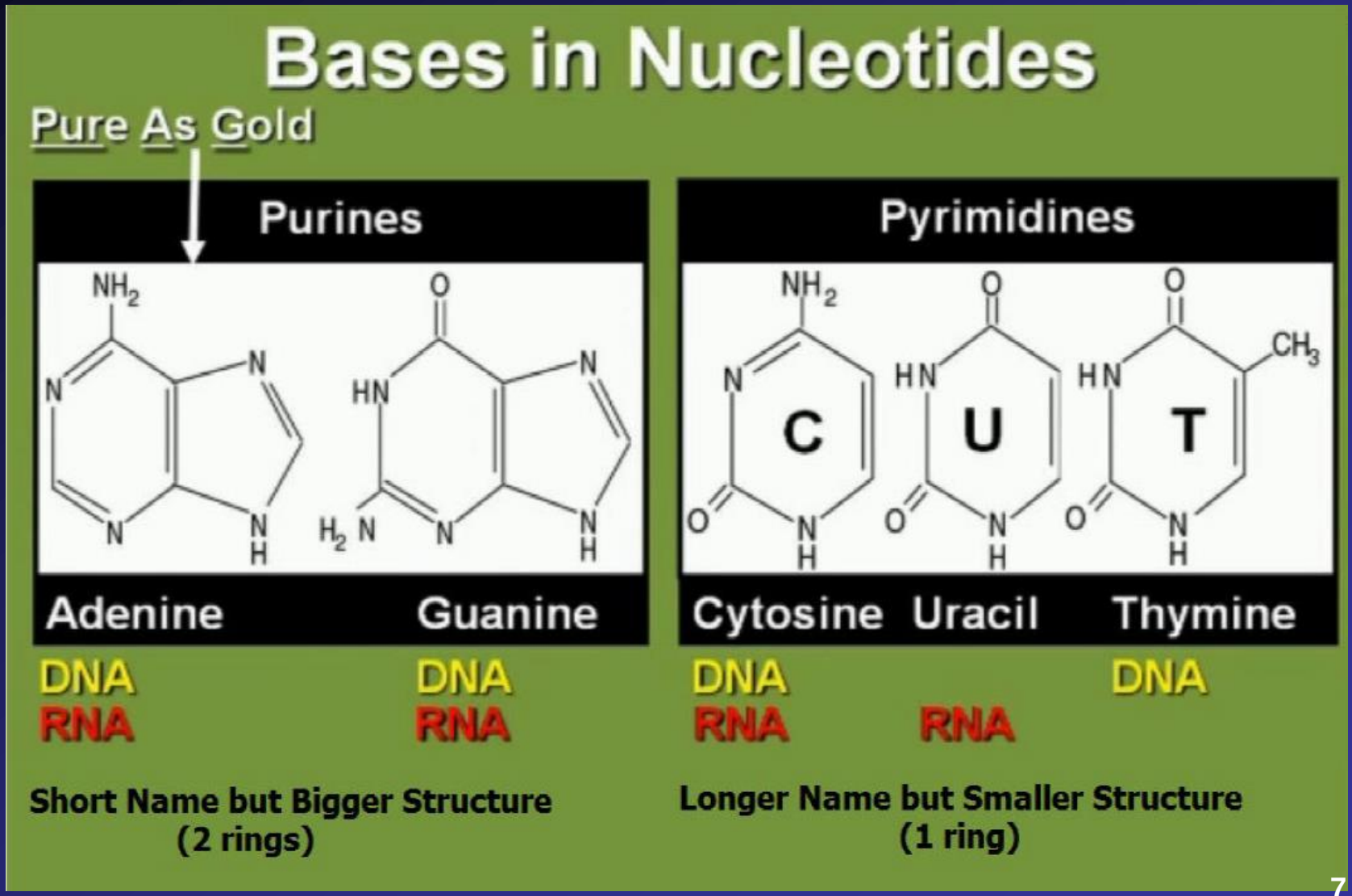
- The 2-ringed purines are in both DNA and RNA
  - adenine (symbolized A)
  - guanine (symbolized G)
- The 1-ringed pyrimidines are
  - cytosine (symbolized C) is common to DNA & RNA
  - thymine (symbolized T) is found only in DNA
  - uracil (symbolized U) is found only in RNA

    When RNA is transcribed (made from template of) DNA, U represents the place of T

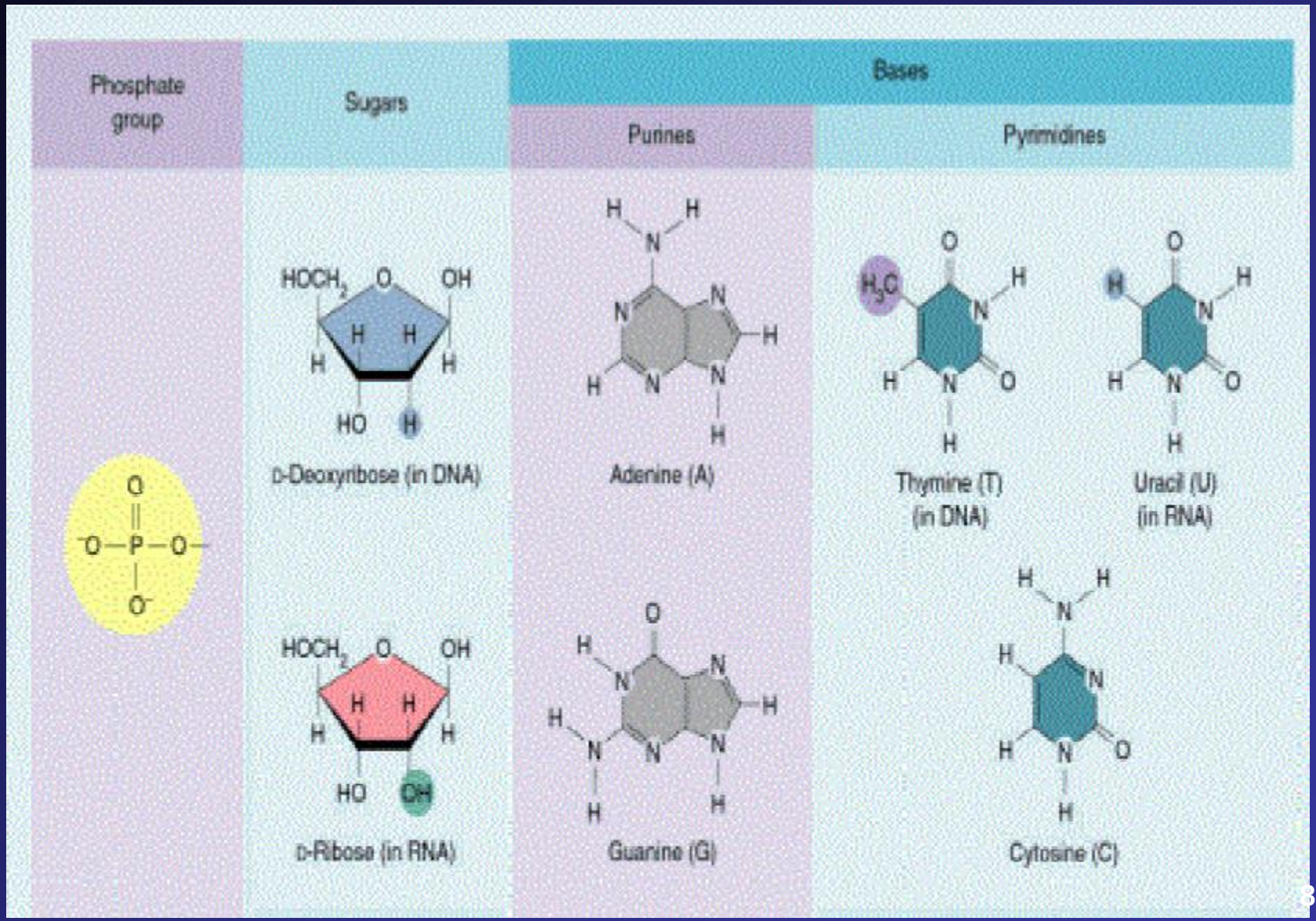
*Mnemonics to assist learning*

purine: *Pure* As Gold, pyrimidine= CUT

# Nitrogenous Bases



# Nucleotide Structure

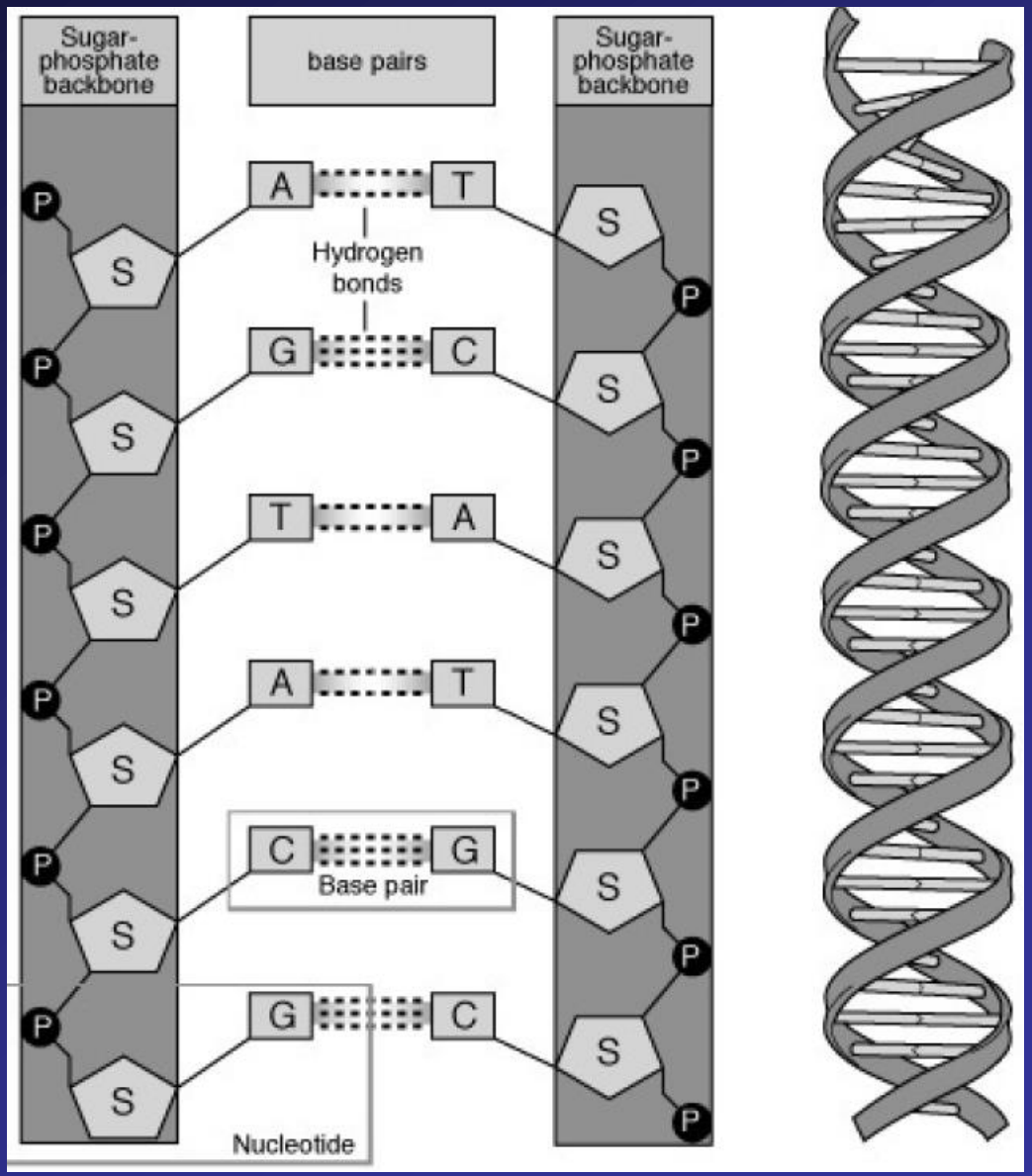


# DNA Structure

- Deoxyribonucleic acid (DNA) is found within nucleus consisting of a spirally winding, double-stranded chain (helix) of polymeric nucleotides (polynucleotides)
- Two polynucleotide chains held together by hydrogen bonds formed between the bases
- A copolymeric chain of deoxyribose sugar & phosphate molecules form "backbone" of the "ladderlike" DNA structure

  The H-bonded pairing of the bases form the "rungs" of the ladder
- The H-bond pairing of bases is very specific
- Purine adenine (A) always pairs with pyrimidine thymine (T), forming TWO hydrogen bonds in the pairing
- Purine guanine (G) always pairs with pyrimidine cytosine (C), forming THREE hydrogen bonds in the pairing

- DNA Structure
- The Ladder & The Double Helix
- Hydrogen bonding
- 2 H-bonds T=A
- 3 H-bonds G≡C



# DNA Function

1. *Machinery to Replicate with Integrity*
   Polymerase enzymes are huge protein complexes that ensure that the polynucleotide sequence of DNA replicates (copies) itself exactly, preparing the complimentary copy strand from a template strand, so all new cells have the information
2. *Codes for all Cell Structure & Function*
   DNA contains the codes producing all proteins and RNA in genes
3. *Regulates Expression of Its Genes*
   DNA also contains codes which are regulatory sequences that direct when genes are expressed, and how much the products of genes are to be expressed

# Chargaff's Rule

- Watson & Crick (1953) use Franklin's x-ray diffraction to deduce the double-stranded helix and propose explanation for how DNA replicates
- Chargaff's rule: Number of purines in DNA is equal to the number of pyrimidines

  %A = %T and %G = %C
- Q: if DNA content has %G=10%, what is %T?
- A: if %G =10%, %G=%C, so %C =10%; thus A+T is 80%, and since A=T so %T=40%
- Watson & Crick structure showed purines & pyrimidines face the center of the helix, forming "steps" in a "spiral staircase," and they were H-bonded to each other

# RNA Structure / Composition

- Ribonucleic acid (RNA) is a single-stranded polynucleotide chain that is transcribed from DNA
- Structure similarity with DNA
  - the nitrogenous bases A, G & C are common to DNA
  - phosphate is part of the backbone
- Structure difference with DNA
  - the nitrogenous base uracil (U) is used in RNA & is the substitute for thymine (T) in DNA
  - the pentose sugar is ribose in RNA and not 2'-deoxyribose in DNA
- while DNA always remain in the nucleus, RNA in all its forms is exported from the nucleus to perform its function

# RNA Main Types & Functions

Three types of RNA, all of which are transcribed from DNA in the nucleus, processed, then exported from nucleus

Messenger RNA (mRNA)

contains the protein-coding type of gene, and is translated to form a polypeptide that is or becomes part of the complete functional protein

Ribosomal RNA (rRNA)

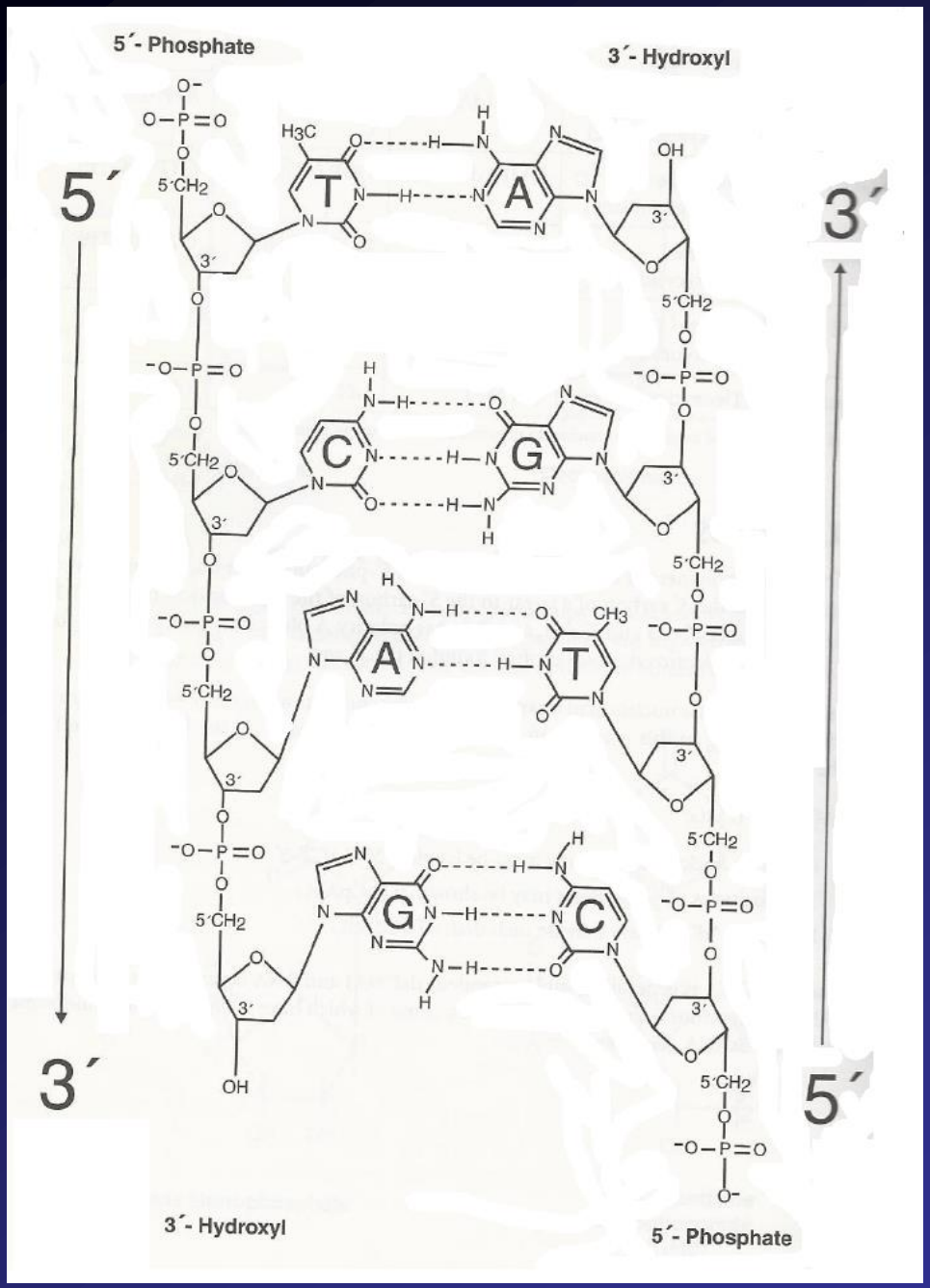
Ribosomes are made of two or three long RNA strands and a couple of dozen proteins: rRNA is the RNA used to make ribosomes, and is heavily involved in protein synthesis

Transfer RNA (tRNA)

A cloverleaf-like short-length RNA that bonds with the amino acids that will become part of polypeptides during protein synthesis

# Comparison of DNA & RNA

| CHARACTERISTIC | DNA | RNA |
|---|---|---|
| Major cellular site | Nucleus | Cytoplasm (cell area outside the nucleus) |
| Major functions | Is the genetic material; directs protein synthesis; replicates itself before cell division | Carries out the genetic instructions for protein synthesis |
| Sugar | Deoxyribose | Ribose |
| Bases | Adenine, guanine, cytosine, thymine | Adenine, guanine, cytosine, uracil |
| Structure | Double strand coiled into a double helix | Single strand, straight or folded |

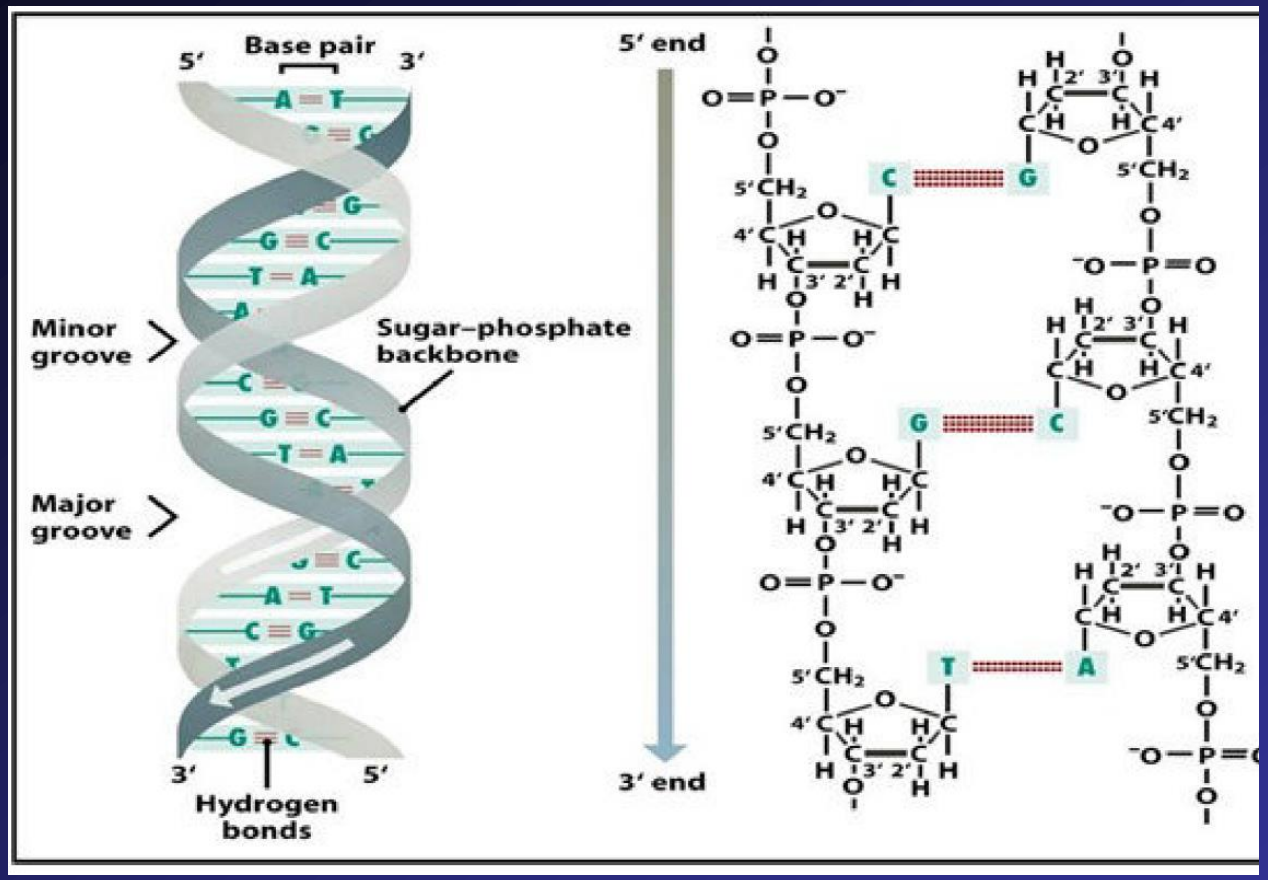



Hydrogen – Bonded Base Pairs in DNA

summary

# Double Helix Features

- The helical formation of the two strands is such that they form a major groove and minor groove
- Proteins having various functions to regulate the reading, synthesis, and repair of DNA depend on differences in this groove formation
- The phosphate bonding of the pentose sugar (deoxyribose) is on hydroxyl functions of the 3' and 5' carbons of the sugar, and DNA shows a polarity in this regard
- The two strands that bond to form the double helix do so in antiparallel fashion: one strand has a directionality of 5'→3', and the H-bonding to the other strand is such that its 5'→3' directionality proceeds in the opposite direction (an antiparallel directionality)
- This antiparallel directionality in the strands has tremendous biological significance in the synthesis and utilization of DNA




The 5-prime (5') and 3-prime (3') ends of DNA refer to the carbons on the deoxyribose sugar on the backbone, where the phosphate group connects in the polynucleotide

This polarity has biological significance in the formation and maintenance of DNA

# Types of DNA

- Watson & Crick originally suggested that all DNA follows a right-handed twisting helix
- 25 years later (1979) scientists discovered DNA a lefthanded twisting DNA occurring in DNA segments (not the whole of the DNA)
- The classical conformation of DNA is called B-DNA, with its major and minor grooves
- The left-handed twist of DNA shows a sugar-phosphate backbone with zig-zag pattern: this is called Z-DNA
- The ability to read (transcribe into RNA) Z-DNA is significantly reduced, so this may be a form of regulating what DNA is readable

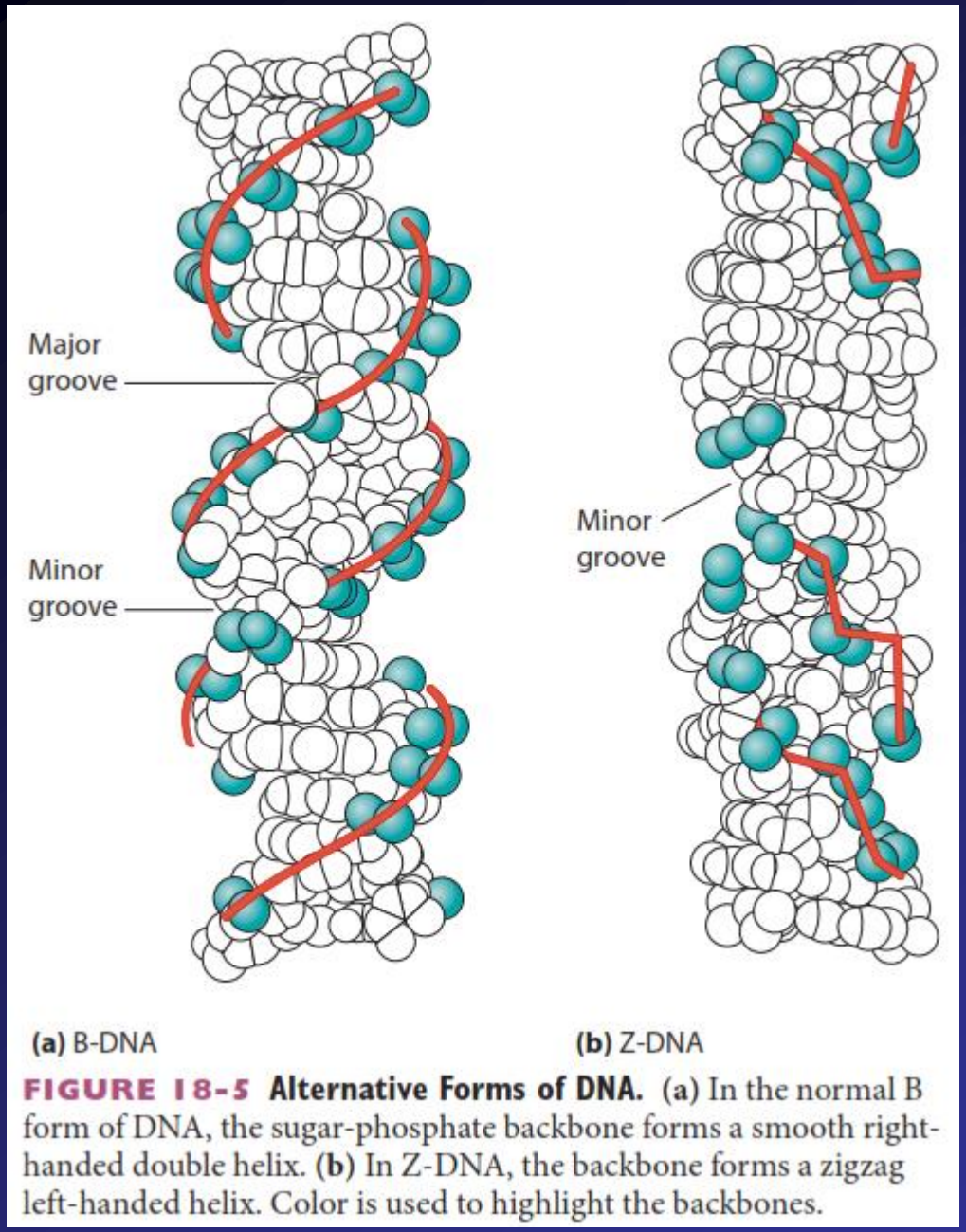
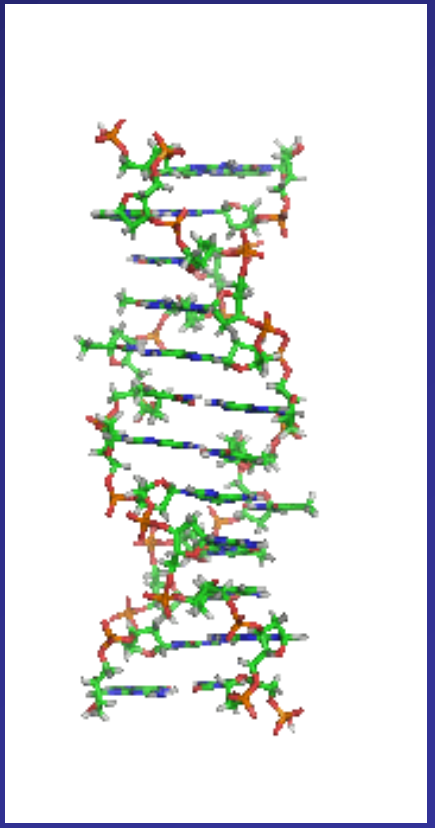



**FIGURE 18-5 Alternative Forms of DNA.** (a) In the normal B form of DNA, the sugar-phosphate backbone forms a smooth right-handed double helix. (b) In Z-DNA, the backbone forms a zigzag left-handed helix. Color is used to highlight the backbones.

Comparing B-DNA (classical right-hand twisting helix) to Z-DNA (zig-zag left-hand twist)

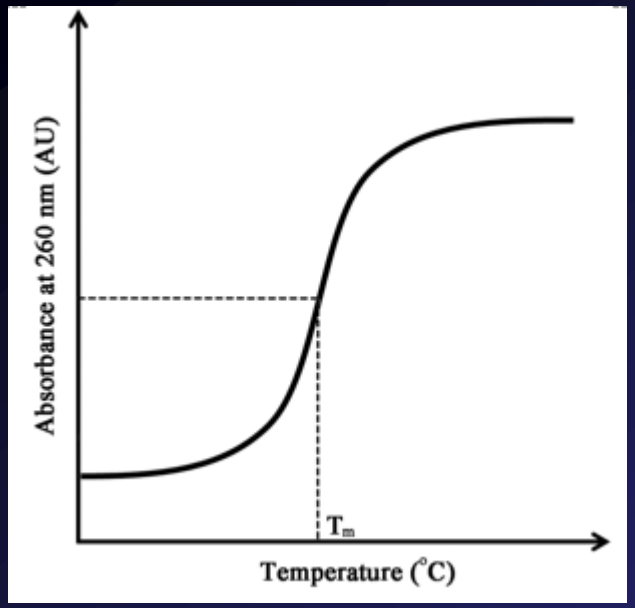


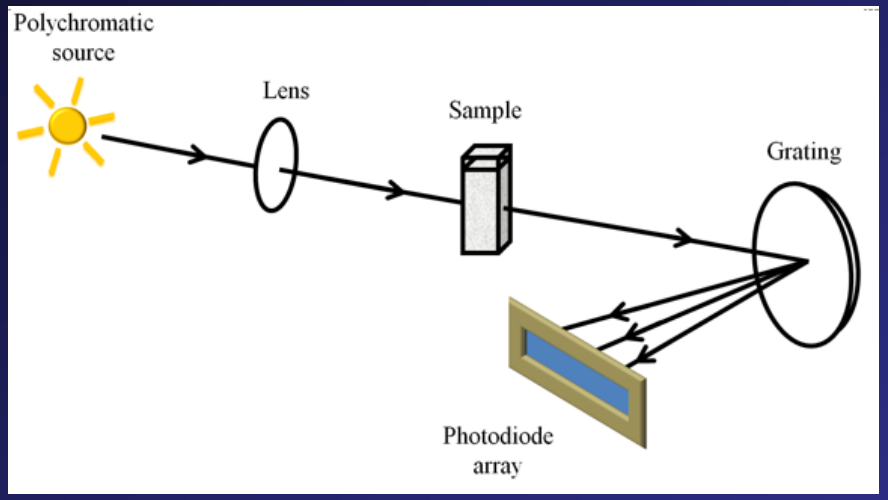
# DNA Denaturation

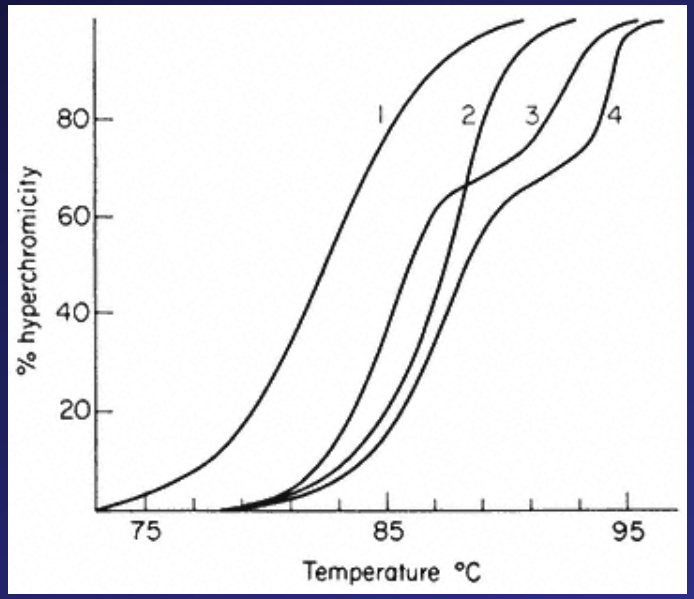
- The hydrogen bonding between bases of the DNA strands holds them in the double helix
- We know that H-bonding is a weaker form of chemical bonding: it holds $H_2O$ molecules together to form liquids (water), but with temperature increases, these bonds can be broken
- Indeed, if DNA is present in near-boiling water, the H-bonds are broken and two separated single strands form with the double helix broken: this is called denaturation

# $T_m$: Melting Temperature

- Scientists use reversible heat-denaturation of DNA to determine base composition of DNA and for other experiments with DNA
- The temperature at which a segment of DNA "melts" (denatures) is a function of the number of GC and AT pairs and is called the melting temperature (symbolized $T_m$)
- GC pairs are held together by 3 H-bonds, and AT by 2-H-bonds: thus a DNA segment with a higher %GC will be expected to melt at a higher temperature, since it takes more energy to denature it
- The absorption of DNA at 260 nm (UV) light increases when it goes from double-stranded to single-stranded: thus it is possible to follow a 260 nm vs. temperature plot for different DNA segments and see different $T_m$ values for each
- DNA can renature its two strands as the temperature of the solution falls: in many cases, it renatures fully when DNA segments are short, but with long segments, the renaturation is not to original state and can involve mispairing




- The figure above shows the principle of how a UV or visible light spectrophotometer works for looking at the optical properties of molecules, such as detecting the difference between DNA that is denatured or not
- Denatured or single-stranded DNA absorbs at 260 nm more than double stranded DNA, as shown in the plot at top left. The midpoint of the transition or change in absorption of 260 nm from ss DNA to ds DNA is called the "melting temperature" or Tm
- At left, you can see different sequences of DNA with different base compositions (%GC/%AT) with different melting temperatures (%hyperchromicity is just another way of looking at "absorption at 260 nm") Can you tell which have the higher %GC content??

# Breaking the Double Helix

- The H-bonds of the DNA double helix must be broken and the strands separated to perform natural processes such as the synthesis of DNA during its replication or repair, or for the reading of the code by polymerases making RNA during transcription
- The proteins that work on DNA are enzymes, and enzymes allow nature to get around the process of having to raise the temperature of the cell to boiling by lowering the activation energy of a chemical process or reaction
- Thus the proteins do not need to increase temperature to break hydrogen bonds, but rather lower the energy required to do so

# Genomes

- Genome = one complete copy of the genetic information of an organism (and in eukaryotes, of certain organelles)
- The genome of eukaryotic (human) cells is contained within the nucleus of the cell

  Mitochondria also have their own genome, although it only supplies genes for parts of its function (it is thus a limited genome)
- Eukaryotic genomes are organized on chromosomes
- In humans, there are 23 homologous pairs of chromosomes, a set of 23 chromosomes inherited from the male and female of a sexually reproducing species

# Genome Size

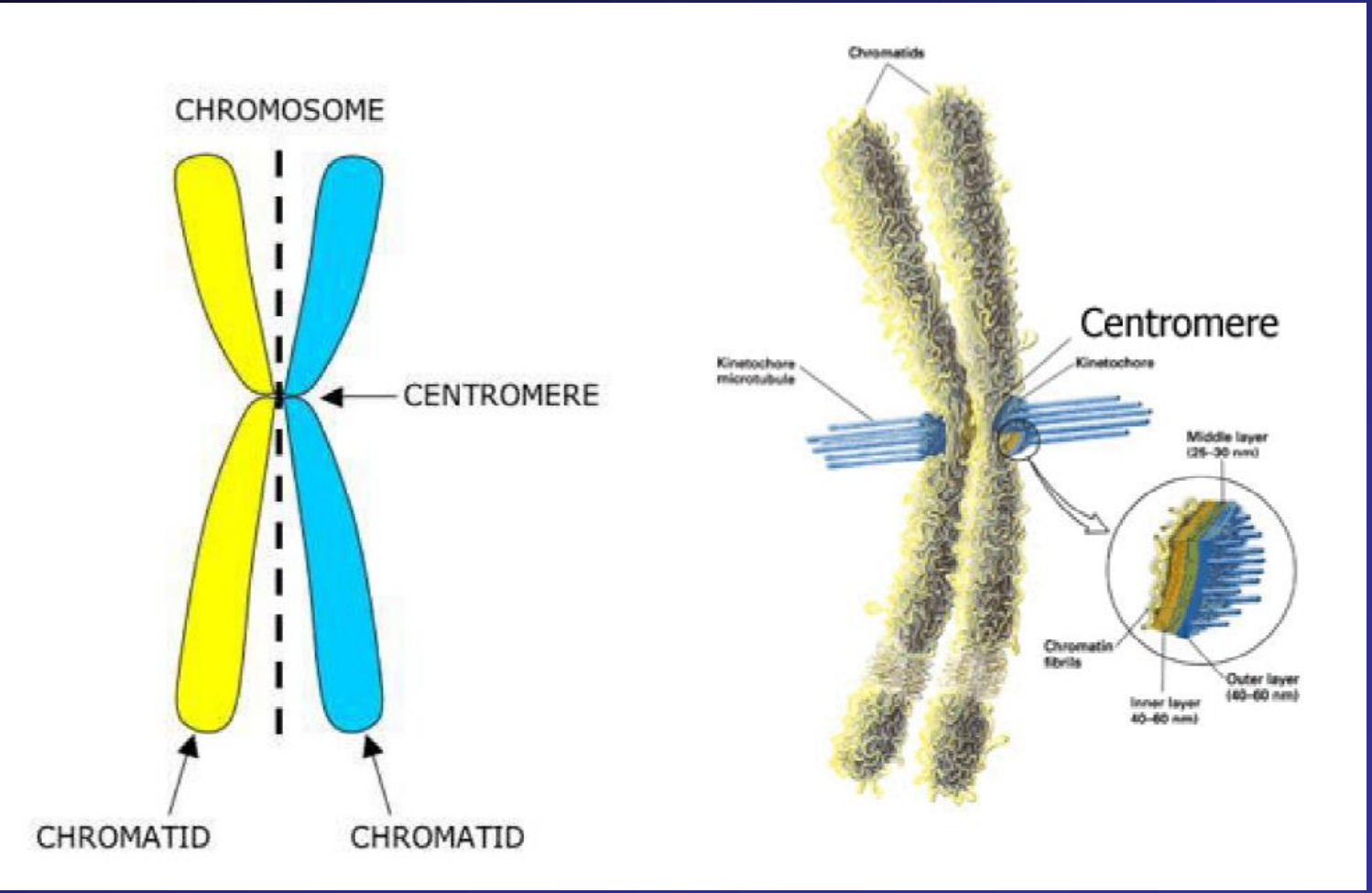
- The size of a genome is given as count of nucleotide pairs per haploid genome
- Genomes for bacteria and some viruses are usually contained in a single circular DNA molecule

  some viruses show linear segments of DNA
- Genome size typically increases with complexity of the organism (with but few exceptions)
- Viruses have enough genomic DNA to code for a few proteins, often those that determine the body of the virus and those that hijack the systems of the cell to copy the virus genome and express its proteins
- Human DNA
  - 3.2 billion ($3.2 \times 10^9$) base pairs (bp, nucleotide pairs)
  - codes for an estimated 21,000 proteins
  - only ~2% of the genome consist of coding sequence (genes)

# Chromosomes, Chromatids

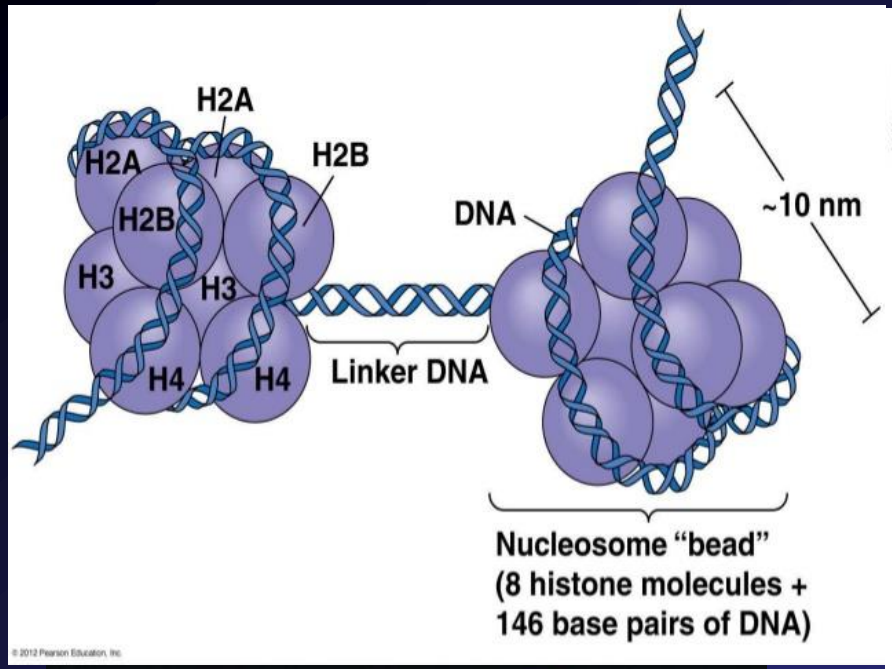
- In eukaryotes, chromosomes are linear segments of DNA and associated proteins that hold part of an organism's genome, and are located in the nucleus
- Chromosomes appear microscopically and typically at metaphase and with staining as compact X-shaped bodies within the nucleus
- The arms along a long axis of the chromosome represent chromatin: single long DNA polynucleotide + RNA + protein
- The long & short arm represent a chromatid
- In most cases, the DNA of the chromosome is duplicated as an exact copy, and the DNA copies are joined at a centromere
- Because they are exact copies, the chromatids joined at the centromere are called sister chromatids
- The creation of an exact copy (a sister chromatid) is important when the cell is to divide into daughter cells

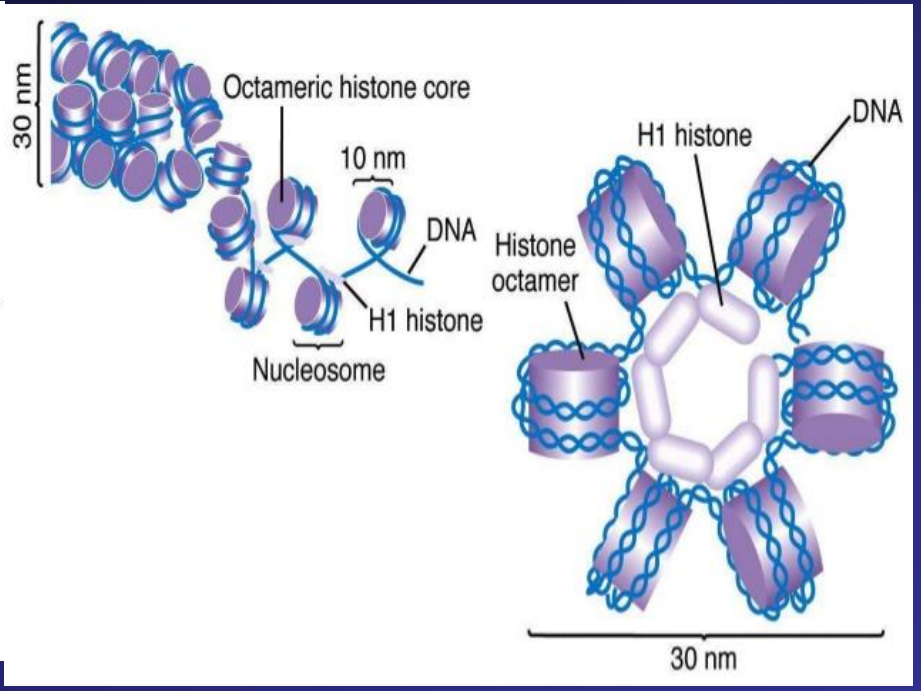
# Chromosome Structure

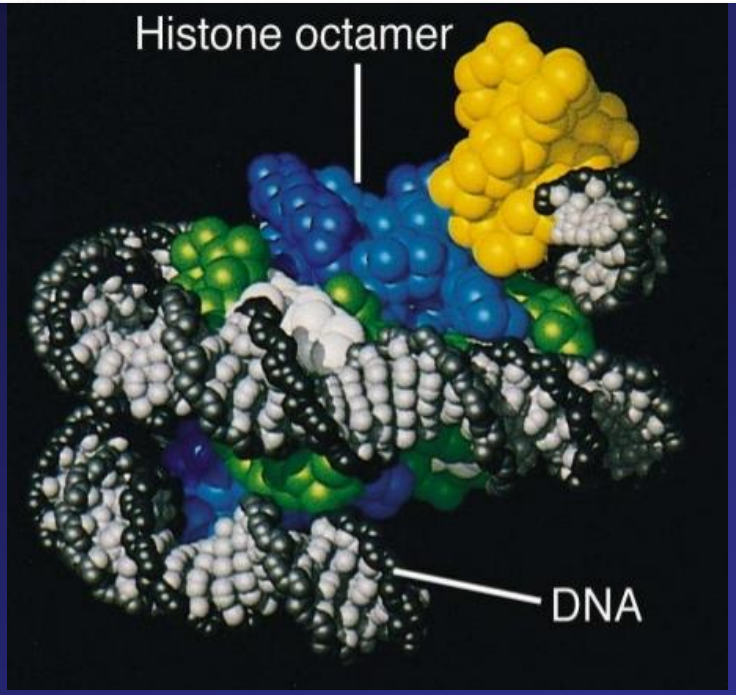


# Nucleosomes

- Chromosome formation starts with a fundamental coiling of DNA called the nucleosome
- Nucleosomes are composed of proteins called histones which form a complex in which the DNA wraps about two times around the complex
- There are five different histone proteins named H1, H2A, H2B, H3 and H4
- An octameric core of histones forms a complex first: this core is two copies each of four histones H2A, H2B, H3, H4
- Histone H1 is not part of the nucleosome core, but acts as a linking spacer between nucleosomes
- The interaction between histones and DNA is all about electric charge: histones have many positively charged amino acids (arginine, lysine side chains) which interact with the negatively charged phosphate groups on DNA




- A look at the histone core schematically and with a space-filling model: note how DNA loops about the core about twice (146 nucleotides)
- Above, the histones are packed into solenoids

# Chromosome Packing Organization

- chromatin fibers: nucleosomes create a long chain called a chromatin fiber about 10 nm thick
- each chromatin fiber forms a helical coil called a solenoid, 30 nm thick; each coil of the solenoid has about 6 nucleosomes per coil
- in turn, a solenoid coils to form a hollow tube about 200-300 nm thick in the interphase chromosome
- with onset of mitosis, further thickening occurs to form 600-700nm thick chromatids
- a chromosome consists of two chromatids attached by a centromere: the two chromatids of a chromosome will separate during mitosis

# Packing Calculations

- The extent of this coiling & compaction of DNA is described quantitatively as the packing ratio

  packing ratio = length of linear DNA molecule divided by length of the coiled structure

- Nucleosome formation makes a 7:1 packing ratio
- Solenoid formation reduces length by 6, so with a solenoid present, packing ratio = 42:1
- Formation of 6 solenoid/turn hollow tubes shortens another 18 times, so packing ratio = 750:1
- When cell division begins in mitosis, the chromosome has a packing ratio = 15,000-20,000:1

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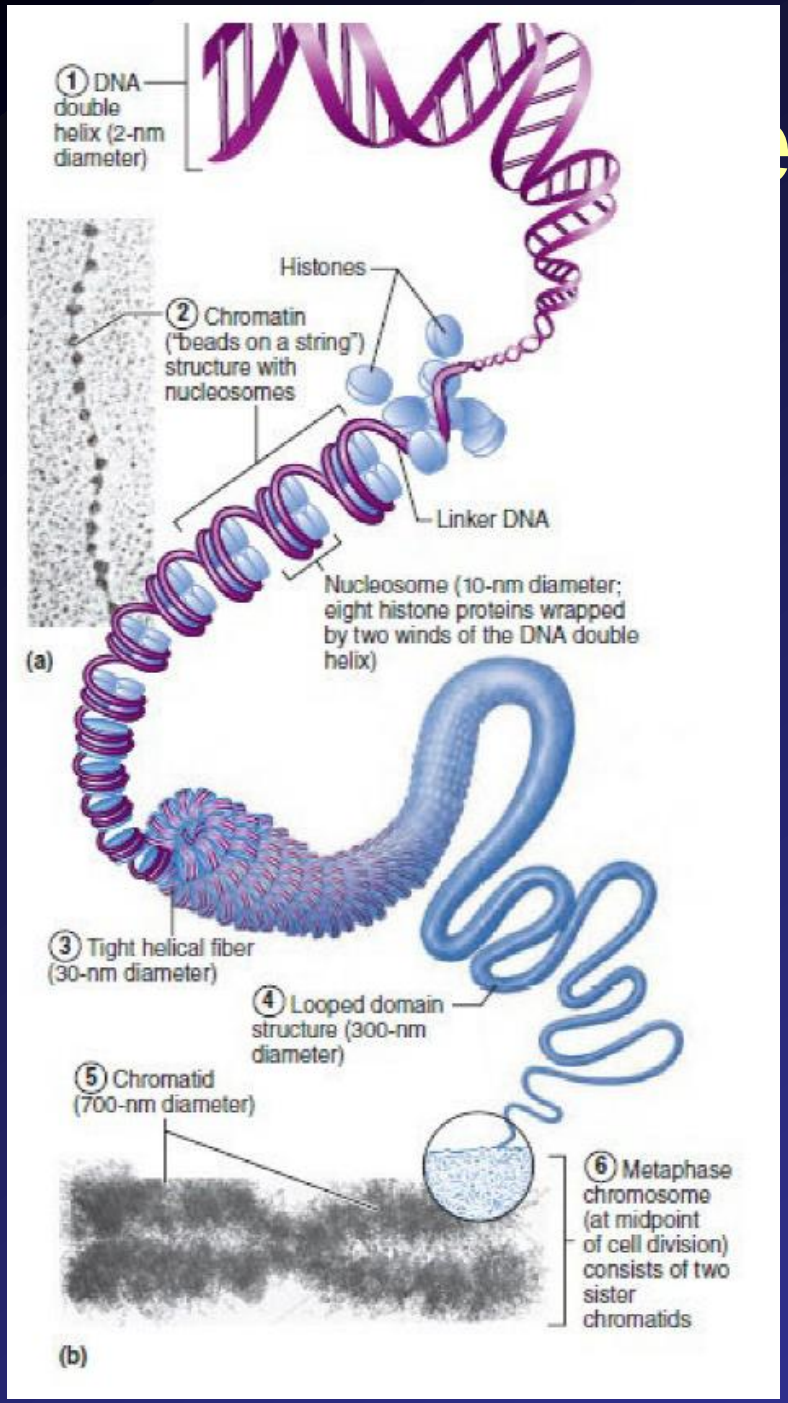



**Chromatin and Chromosome Structure**

- (a) Electron micrograph of chromatin fiber
  Magnification: 125,000X
- (b) DNA packed in a chromosome. The levels of increasing structural complexity (coiling) from the DNA helix to the metaphase chromosome are indicated in order from the smallest ( "1" DNA double helix) to the largest and most complex ( "6" chromosome)

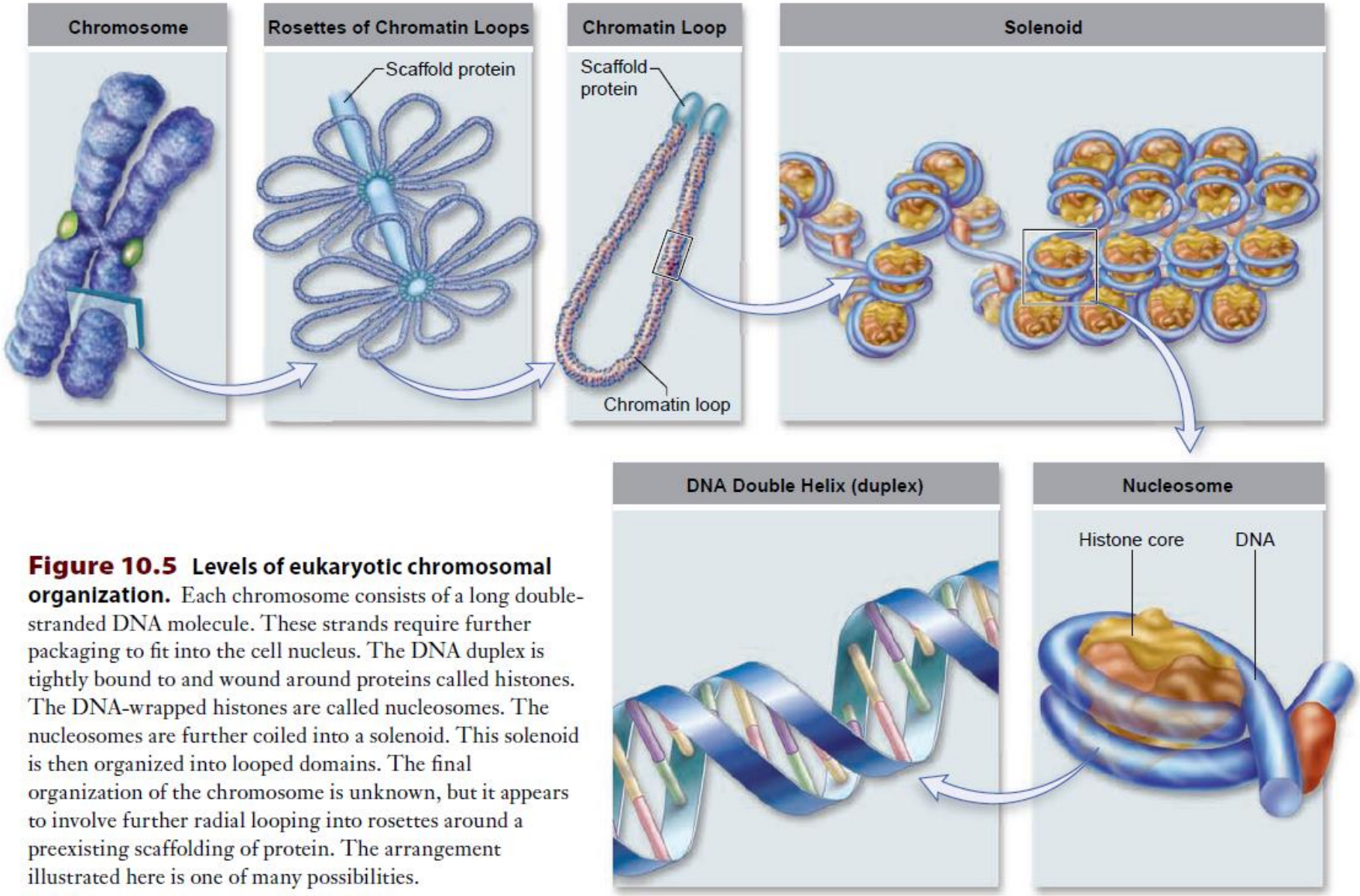



**Figure 10.5 Levels of eukaryotic chromosomal organization.** Each chromosome consists of a long double-stranded DNA molecule. These strands require further packaging to fit into the cell nucleus. The DNA duplex is tightly bound to and wound around proteins called histones. The DNA-wrapped histones are called nucleosomes. The nucleosomes are further coiled into a solenoid. This solenoid is then organized into looped domains. The final organization of the chromosome is unknown, but it appears to involve further radial looping into rosettes around a preexisting scaffolding of protein. The arrangement illustrated here is one of many possibilities.

# Replication of DNA / Chromosome 1 of 3

- S (synthesis) phase: all genomic DNA in all chromosomes has replicated (duplicated)
- DNA replication involves partial denaturation of several sites along chromosome: each start site is called an origin of replication
- DNA polymerase enzymes utilize high energy nucleotides (dATP, dGTP, dCTP, dTTP) and pair them to each of the two existing, partially separated template strands to make a new strand complementary to the template
- DNA polymerases are unable to initiate a new strand however: they must be primed by the presence of a short RNA fragment first synthesized on the open strands; this short RNA fragment synthesis is achieved by enzymes called RNA primases

# Replication of DNA / Chromosome 2 of 3

- DNA polymerases synthesize new DNA in the 5'→3' direction
- This means for one of the open strands, synthesis is continuous and need not be interrupted (leading strands)
- But for the other strand, DNA must be synthesized discontinuously because of the requirement for synthesizing in the 5'→3' direction: thus multiple RNA primers are constructed to enable DNA synthesis for a short stretch (lagging strand)
- Note that bases added to the new strands must pair: where there is a T, an A base is added to the new strand; where there is a G, a C base is added to the new strand

# Replication of DNA / Chromosome 3 of 3

- Two types of DNA polymerase actually do all the DNA synthesis

1. DNA polymerase III works off the RNA primer and synthesizes as much DNA as it can on the continuous (leading) strand;
   on the discontinuous (lagging) strand, it stops at the RNA primer ahead of it (it cannot run through it)
2. DNA polymerase I is about to fill in all the gaps and run right through RNA primers, replacing them with DNA nucleotides; it is thought of as an editing enzyme

3. DNA ligase is a special enzyme that joins any sugar-phosphate bond breaks, which can remain from the synthesis operation

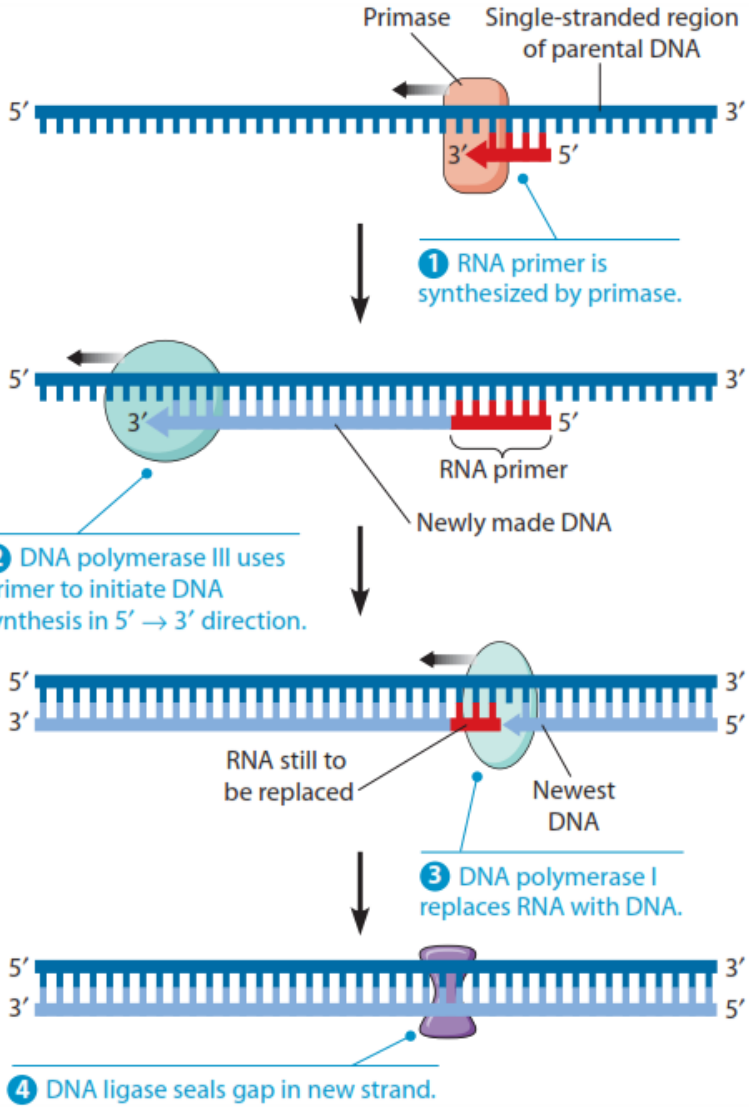
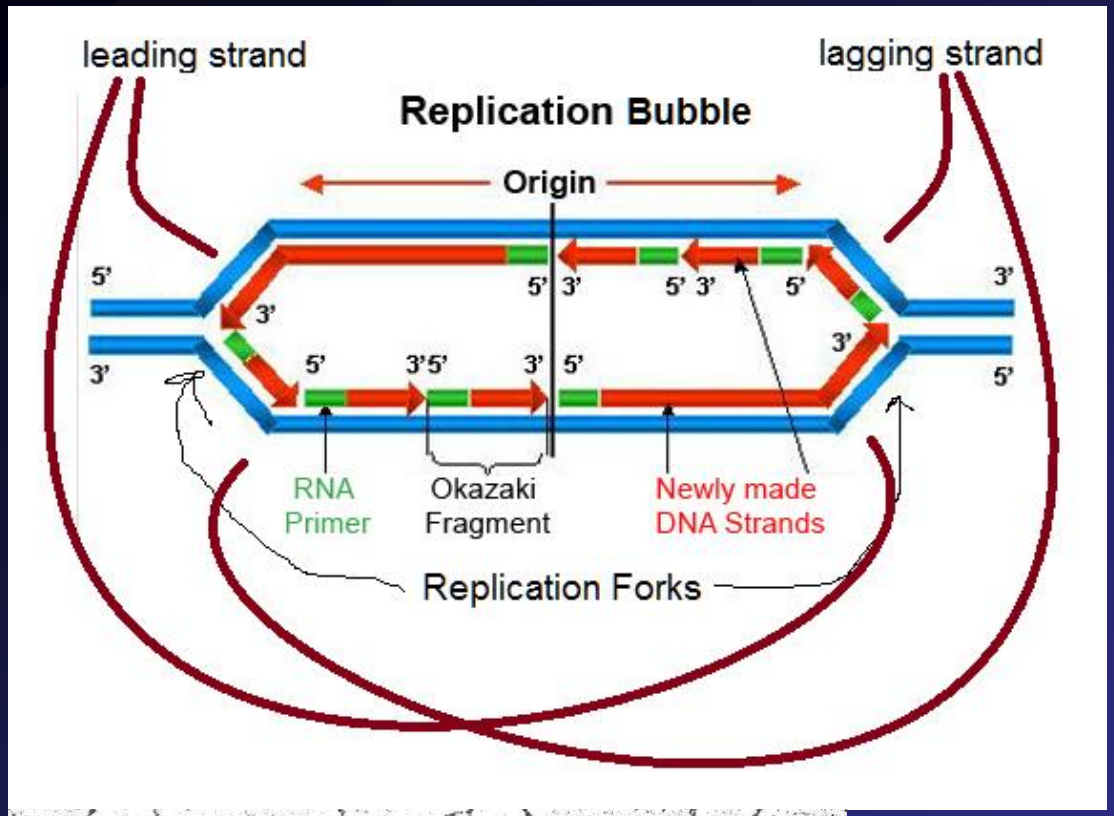


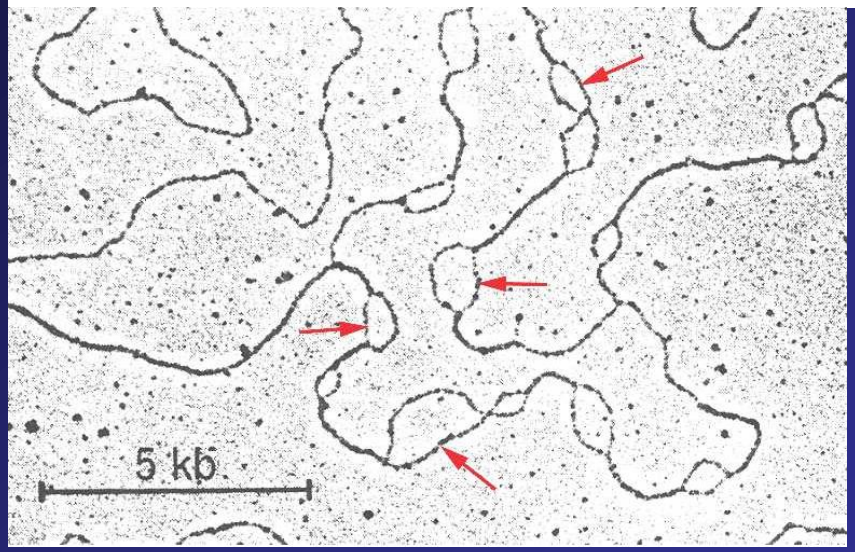

**FIGURE 19-11 The Role of RNA Primers in DNA Replication.** DNA synthesis is initiated with a short RNA primer in both bacteria and eukaryotes. This figure shows the process as it occurs for the lagging strand in *E. coli*.

1. Form the RNA primer with the RNA primase enzyme
2. Extend with making DNA using the DNA polymerase III enzyme
3. DNA polymerase I fills in gaps and runs ("eats") through the RNA primers
4. DNA ligase fills in sugar-phosphate bond break gaps

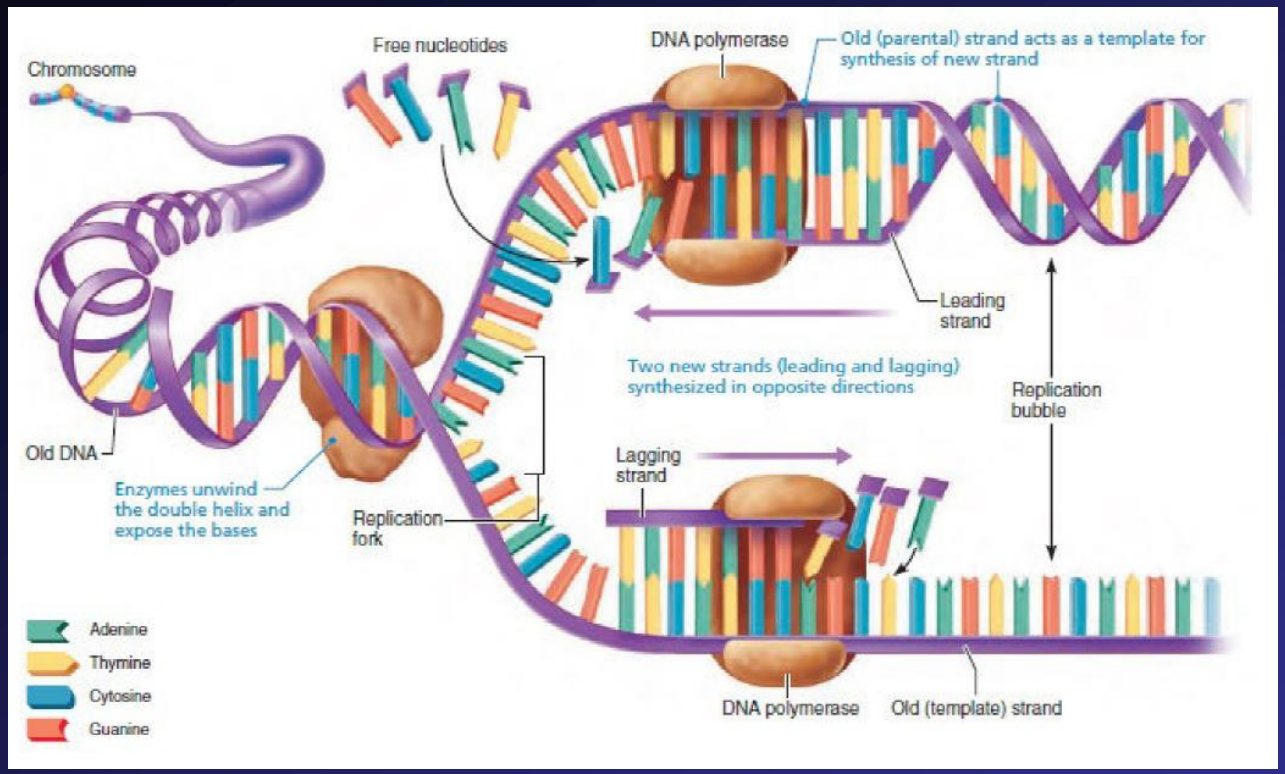
# Replication Forks, Bubbles, etc

- Replication Bubble: The opening of double-stranded DNA to two single strands has the appearance of a "bubble" under electron microscopes. Each bubble has two Y-shaped replication forks which are the points where the double strand helix are separated to achieve semiconservative synthesis of two new strands on templates
- Each eukaryotic chromosome has hundreds of initiation points (origins of replication), forming bubbles. Adjacent bubbles fuse to generate larger bubbles: the two new double strands being separated
- Semiconservative replication refers to the fact that the two new "daughter" strands are the other strand of the parent strand




- The terms used to refer to the features of DNA replication
- The "leading strand" refers to continuous DNA synthesis in the 5'→3' direction, while the "lagging strand" refers to the need for interrupted or discontinuous synthesis to complete its strand
- Replication bubbles as seen with EM at lower left




- **Replication of DNA Summary:** Once the DNA helix is uncoiled, and the hydrogen bonds between its base pairs are broken, each nucleotide strand of the DNA acts as a template for constructing a complementary strand, as illustrated on the right-hand side of the diagram. (The step in which RNA primers are formed to start the process at replication bubbles is not shown.)
- DNA polymerases work in one direction only, so the two new strands (leading and lagging) are synthesized in opposite directions. (The DNA ligase enzymes that join the DNA fragments on the lagging strand are not illustrated.) Each DNA molecule formed consists of one old (template) strand and one newly assembled strand and constitutes a chromatid of a chromosome.

# Reading (Sources)

- Becker's WotC:  pp 510-514, 515-518, 530-533, 553-559, 561-563
- Raven:  Chap 14.3, 14.5, 15.1, 15.2, 16.5