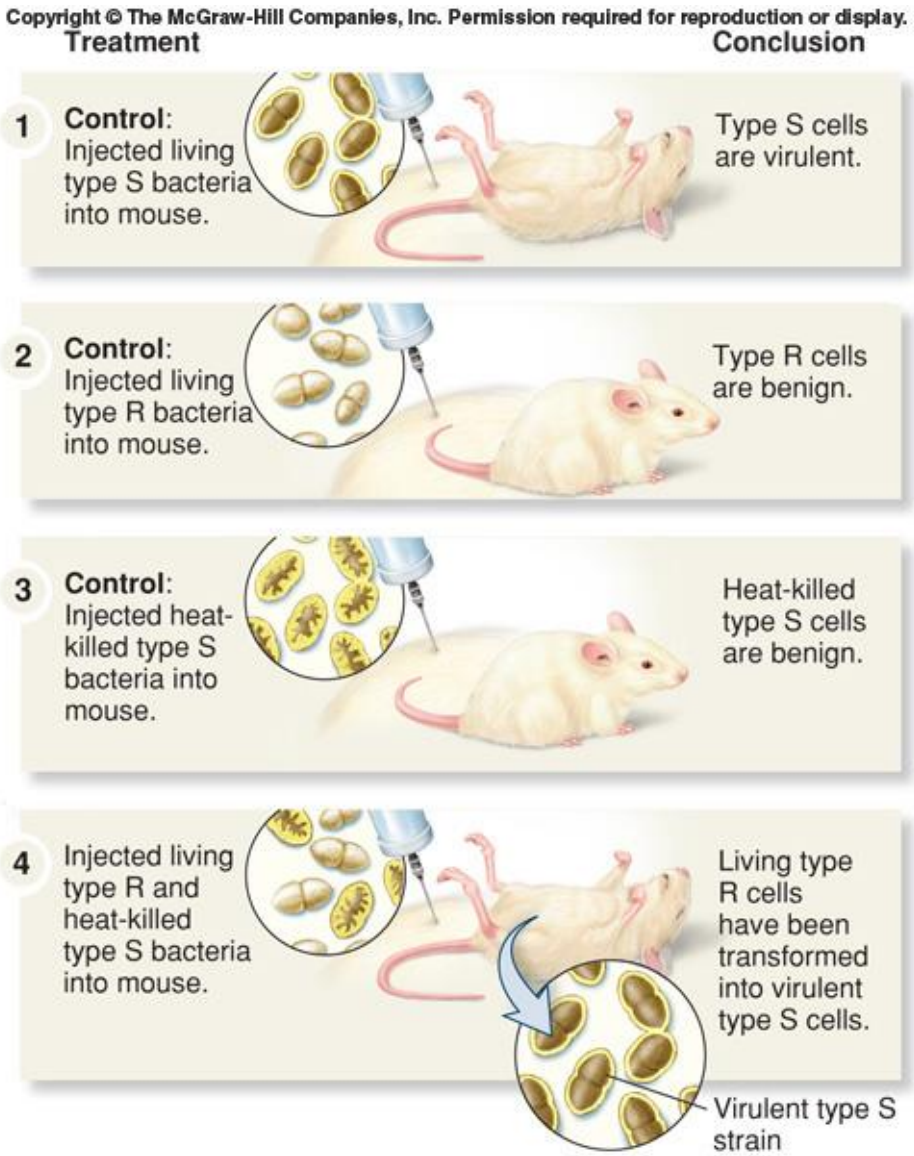
Genetic material must be able to:

- Contain the information necessary to construct an entire organism
- Pass from parent to offspring and from cell to cell during cell division
- Be accurately copied
- Account for the known variation within and between species

Griffith's bacterial transformations

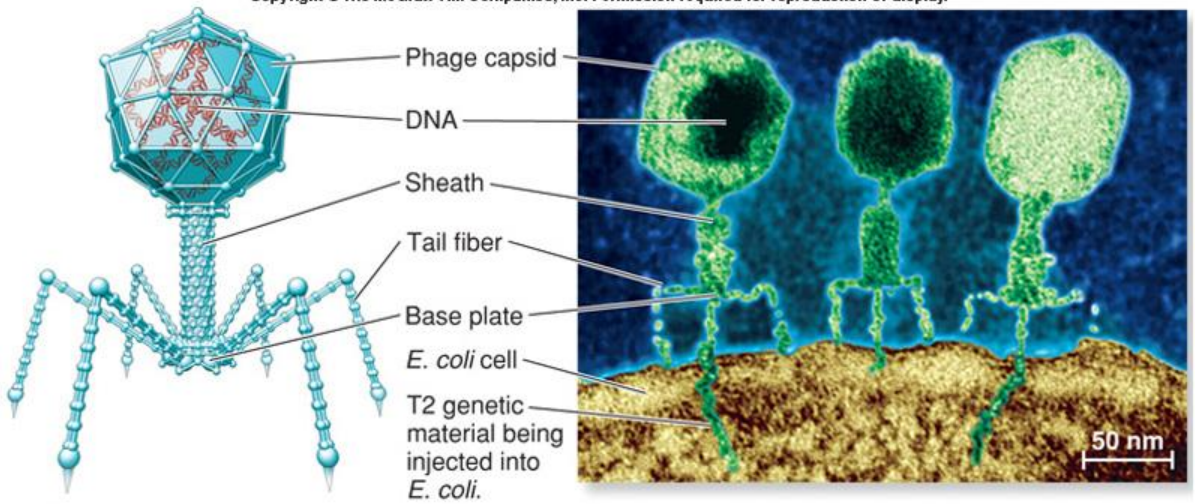
- Late 1920s Frederick Griffith was working with *Streptococcus pneumoniae*
- *S. pneumoniae*
  - Strains that secrete capsules look smooth and can cause fatal infections in mice
  - Strains that do not secrete capsules look rough and infections are not fatal in mice

- Rough strains (R) without capsule are not fatal
  - No living bacteria found in blood
- Smooth strains (S) with capsule are fatal
  - Capsule prevents immune system from killing bacteria
  - Living bacteria found in blood
- If mice are injected with heat-killed type S, they survive
- Mixing live R with heat-killed S kills the mouse
  - Blood contains living S bacteria
  - Transformation



Hershey and Chase

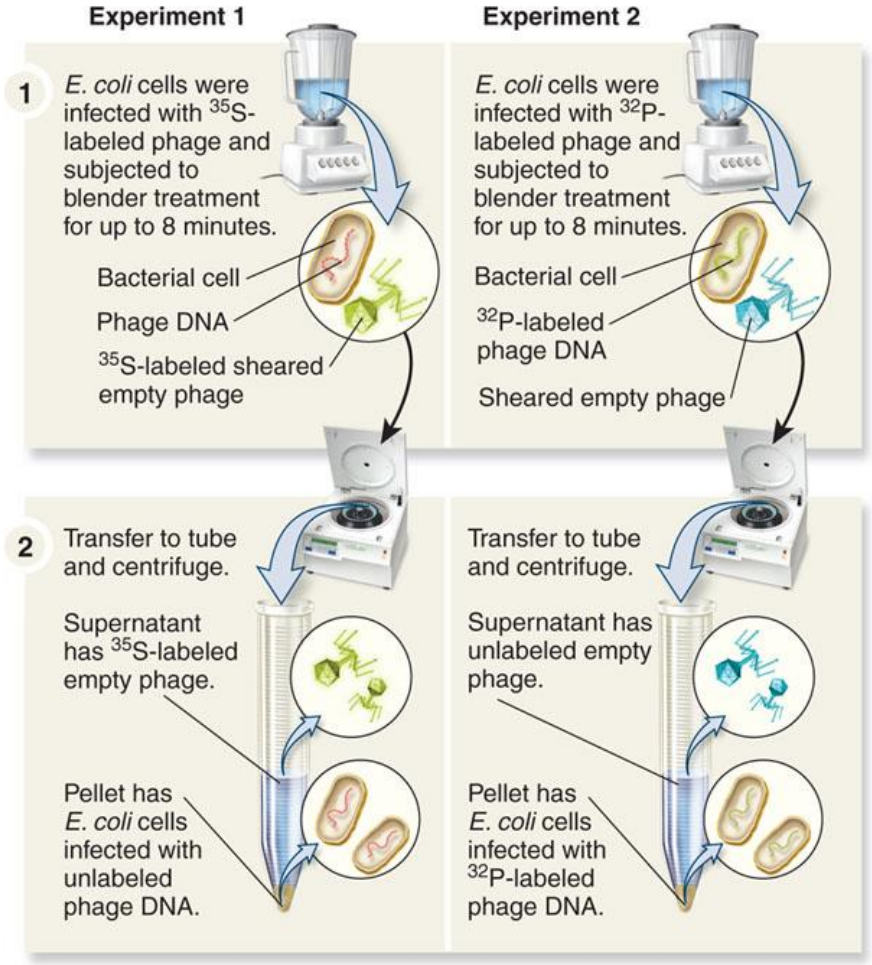
- 1952, studying T2 virus infecting *Escherichia coli*
  - Bacteriophage or phage
- Phage coat made entirely of protein
- DNA found inside capsid

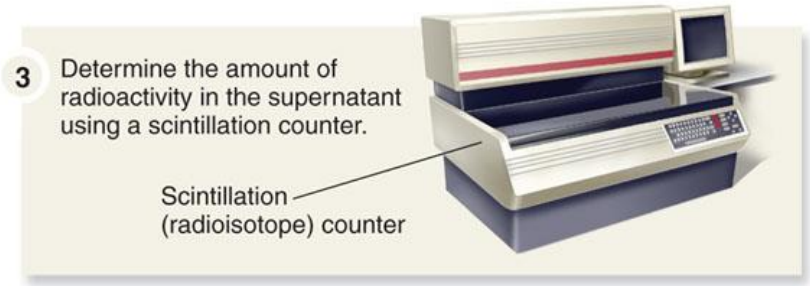


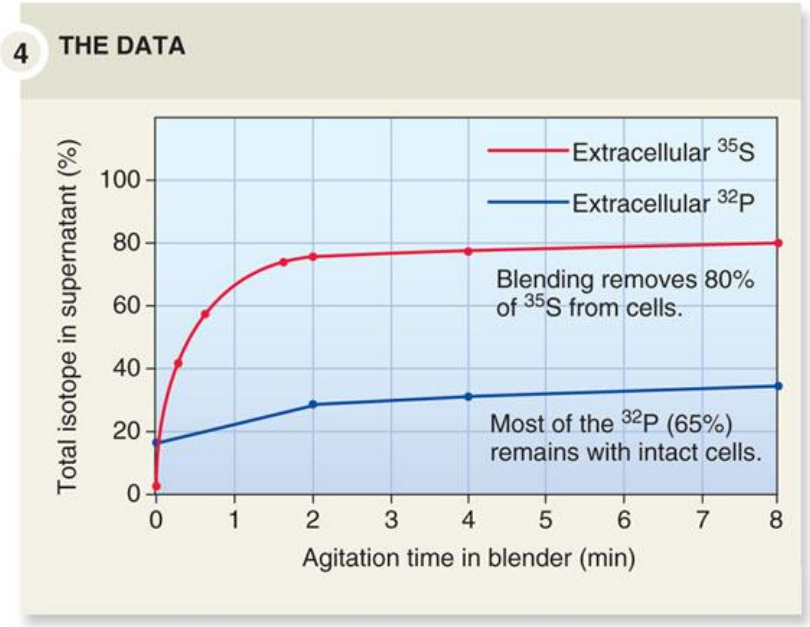
(a) Schematic drawing of T2 bacteriophage

(b) An electron micrograph of T2 bacteriophage infecting *E. coli*

- Shearing force from a blender will separate the phage coat from the bacteria
- ^{35}S will label proteins only
- ^{32}P will label DNA only
- Experiment to find what is injected into bacteria- DNA or protein?
- Results support DNA as the genetic material

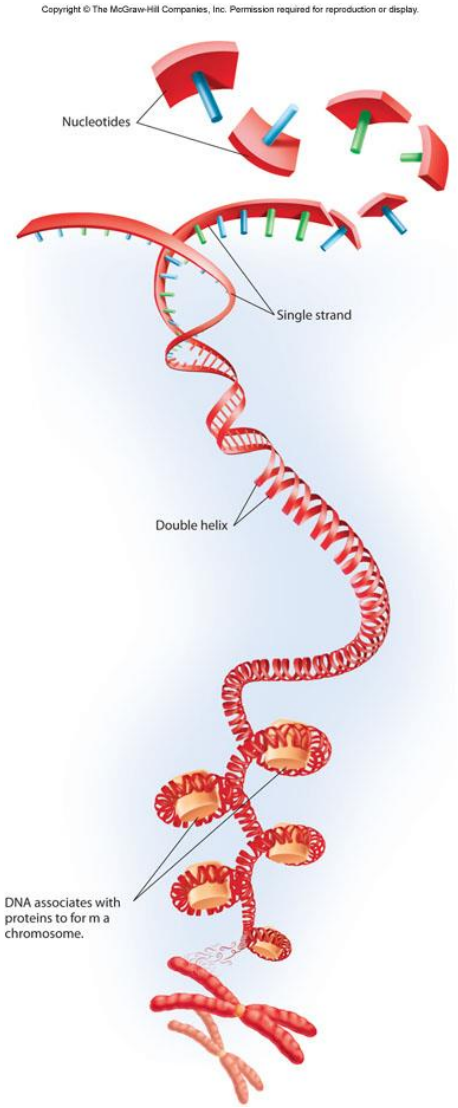






Levels of DNA structure

1. Nucleotides are the building blocks of DNA (and RNA).
2. A strand of DNA (or RNA)
3. Two strands form a double helix.
4. In living cells, DNA is associated with an array of different proteins to form chromosomes.
5. A genome is the complete complement of an organism's genetic material.

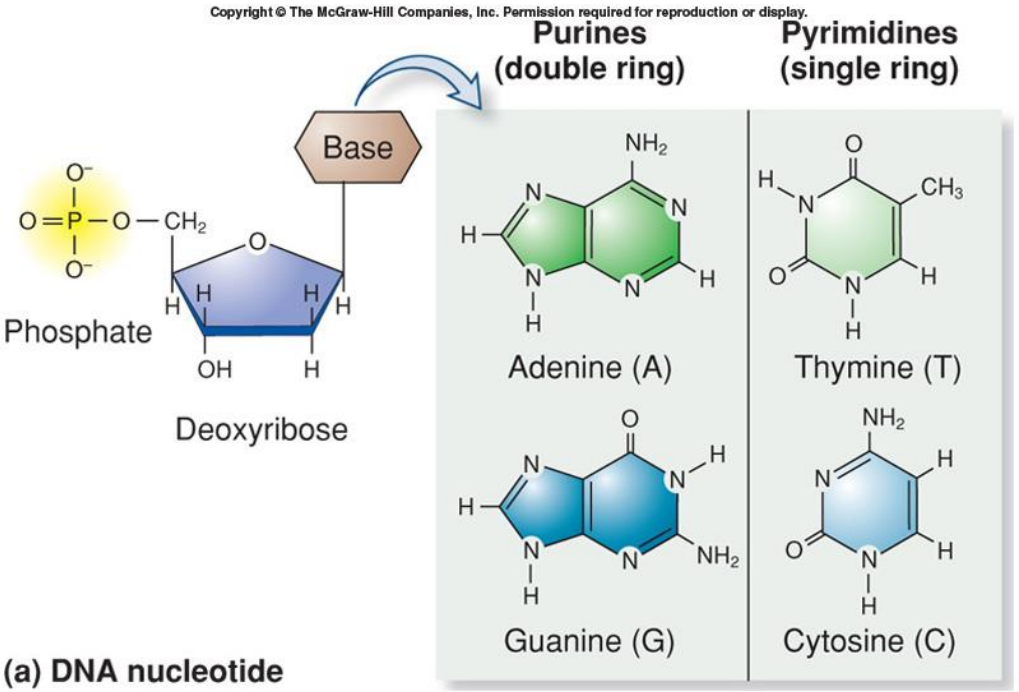


Nucleotides

- 3 components
  - Phosphate group
  - Pentose sugar
  - Nitrogenous base

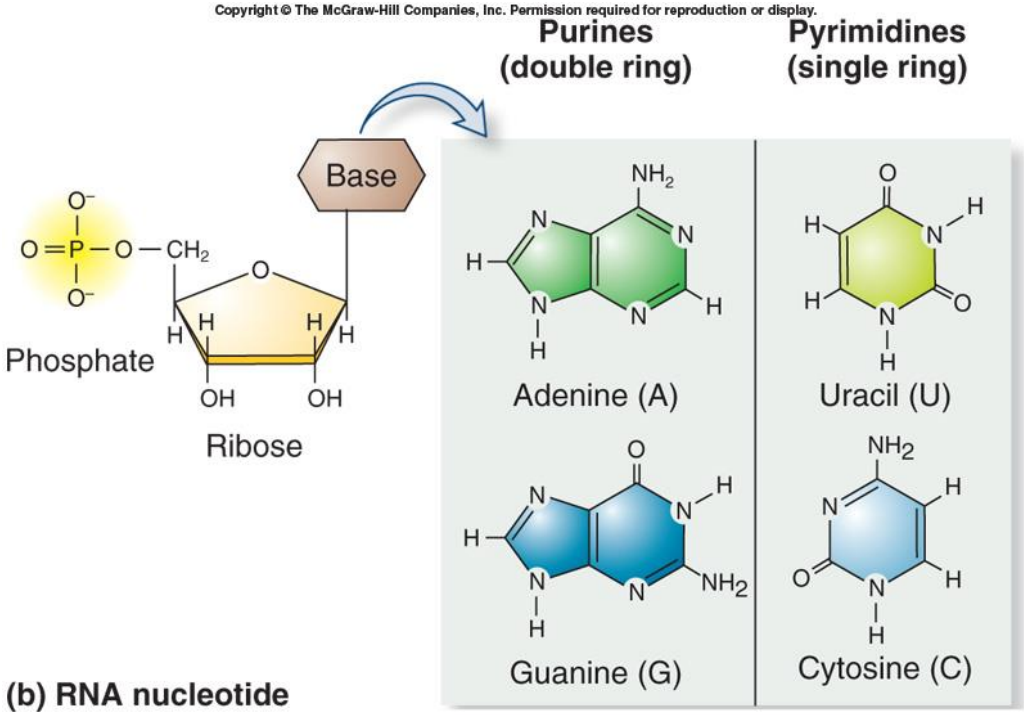
DNA

- 3 components
  - Phosphate group
  - Pentose sugar
    - Deoxyribose
  - Nitrogenous base
    - Purines
      - Adenine (A), guanine (G)
    - Pyrimidines
      - Cytosine (C), thymine (T),

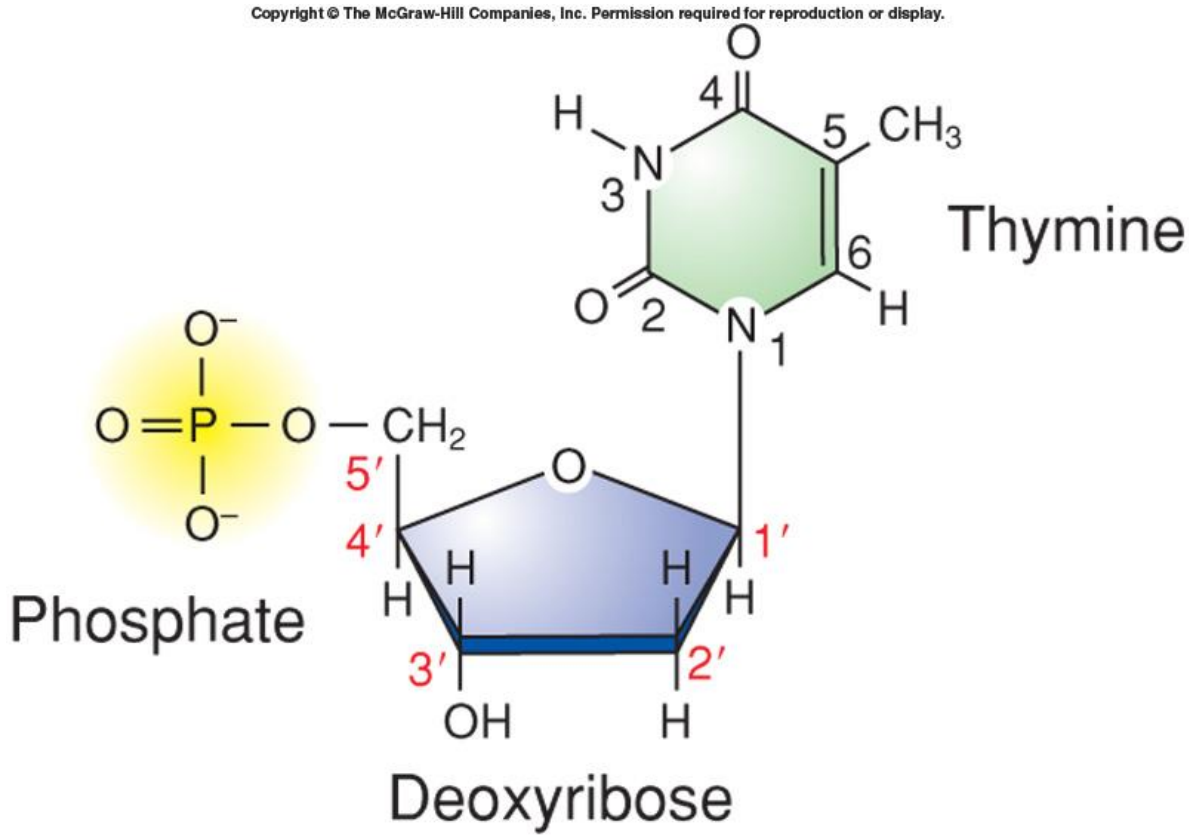


RNA

- 3 components
  - Phosphate group
  - Pentose sugar
    - Ribose
  - Nitrogenous base
    - Purines
      - Adenine (A), guanine (G)
    - Pyrimidines
      - Cytosine (C), uracil (U)

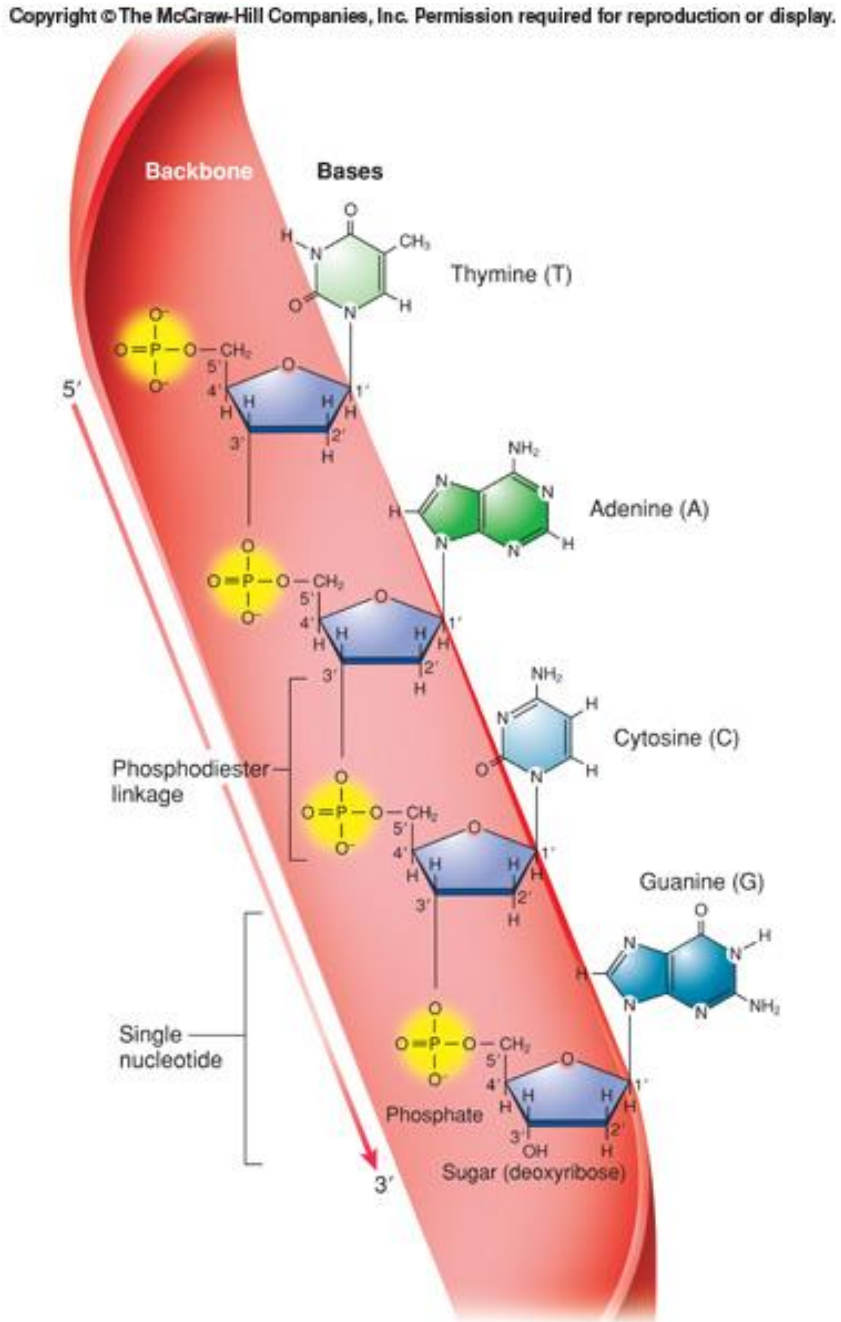


- Conventional numbering system
- Sugar carbons 1’ to 5’
- Base attached to 1’
- Phosphate attached to 5’

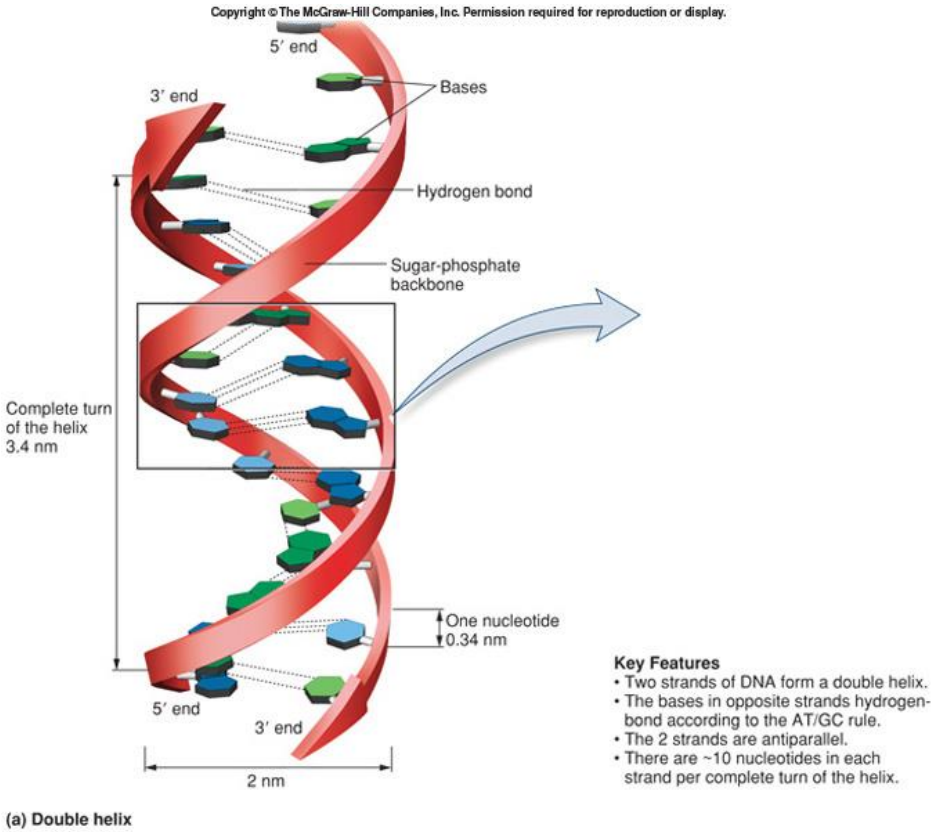


Strands

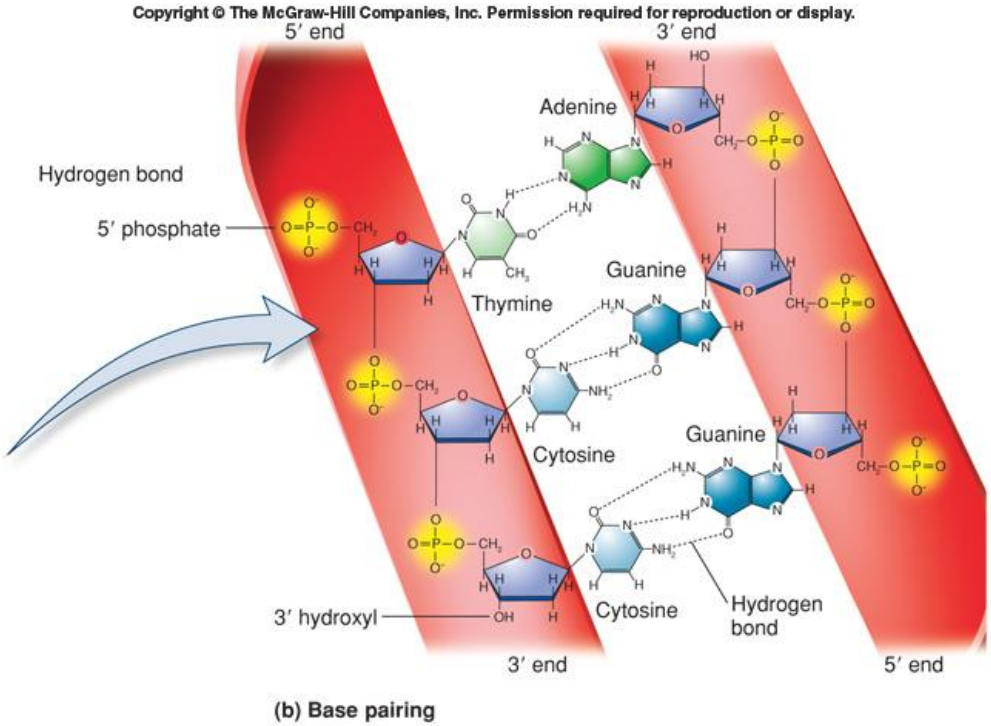
- Nucleotides covalently bonded
- Phosphodiester bond – phosphate group links 2 sugars
- Phosphates and sugars from backbone
- Bases project from backbone
- Directionality- 5’ to 3’
- 5’ – TACG – 3’



- DNA is
  - Double stranded
  - Helical
  - Sugar-phosphate backbone
  - Bases on the inside
  - Stabilized by hydrogen bonding
  - Base pairs with specific pairing



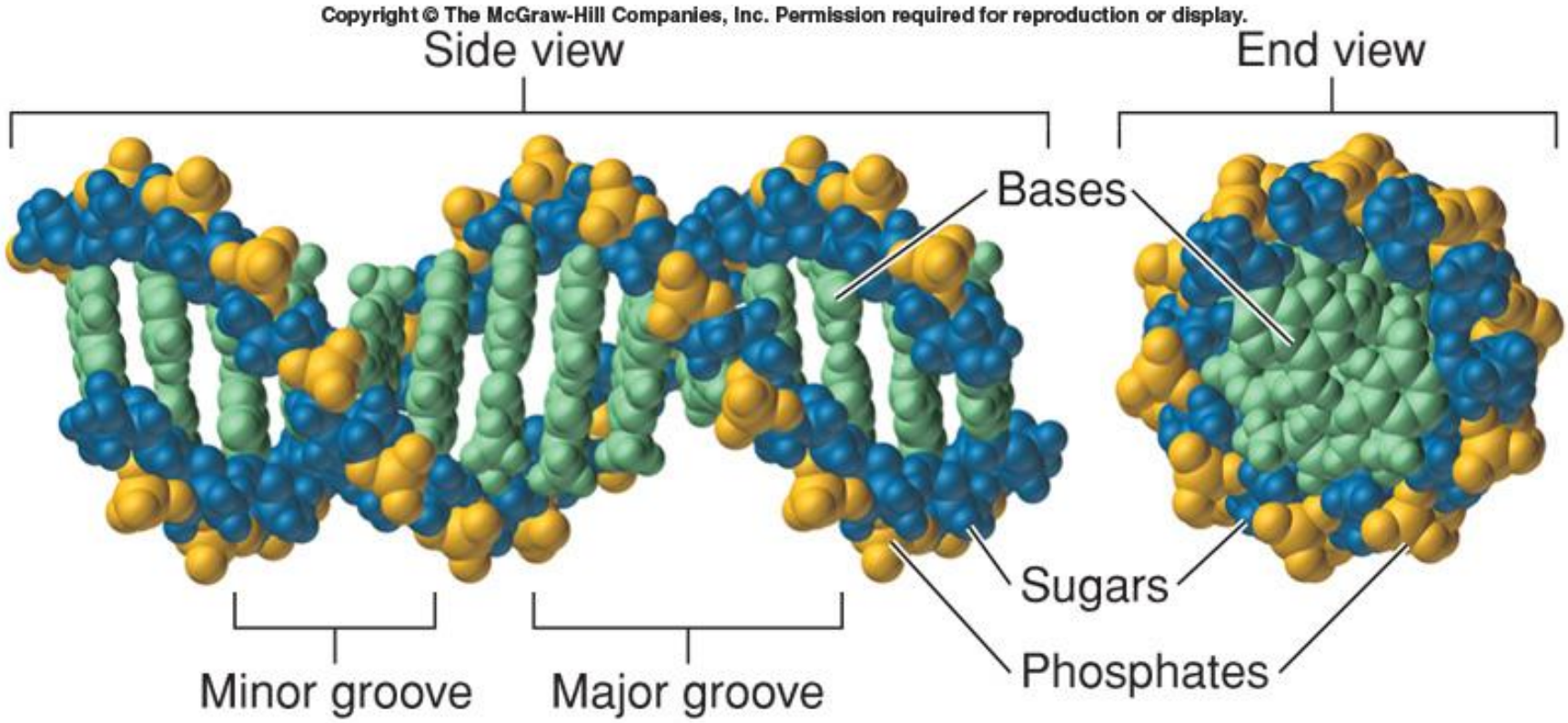
- AT/GC or Chargoff's rule
  - A pairs with T
  - G pairs with C
- Keeps with consistent
- 10 base pairs per turn
- 2 DNA strands are complementary
  - 5' – GCGGATTT – 3'
  - 3' – CGCCTAAA – 5'
- 2 strands are antiparallel
  - One strand 5' to 3'
  - Other stand 3' to 5'



(b) Base pairing

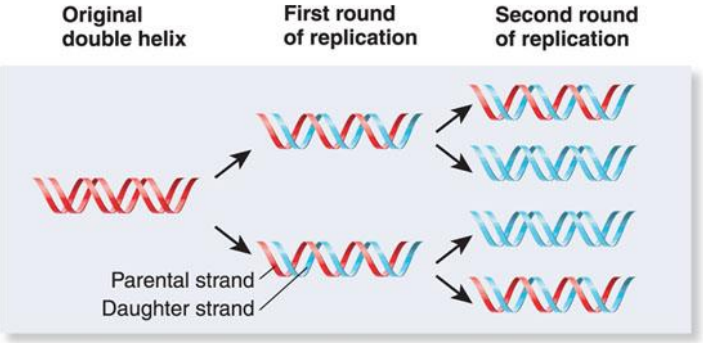
Key Features
- Two strands of DNA form a double helix.
- The bases in opposite strands hydrogen-bond according to the AT/GC rule.
- The 2 strands are antiparallel.
- There are ~10 nucleotides in each strand per complete turn of the helix.

- Space-filling model shows grooves
  - Major groove
    - Where proteins bind
  - Minor groove

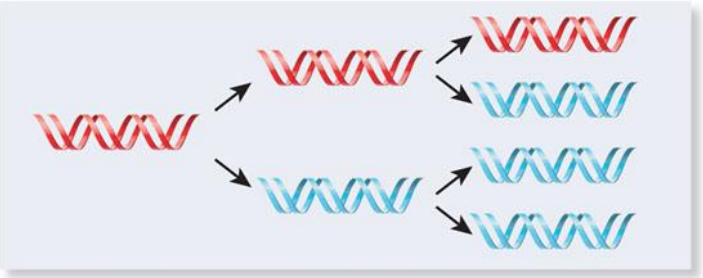


Replication

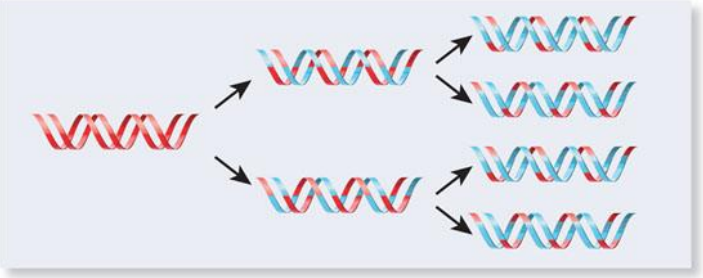
- 3 different models for DNA replication proposed in late 1950s
  - Semiconservative
  - Conservative
  - Dispersive
- Newly made strands are daughter strands
- Original strands are parental strands



(a) Semiconservative mechanism. DNA replication produces DNA molecules with 1 parental strand and 1 newly made strand.



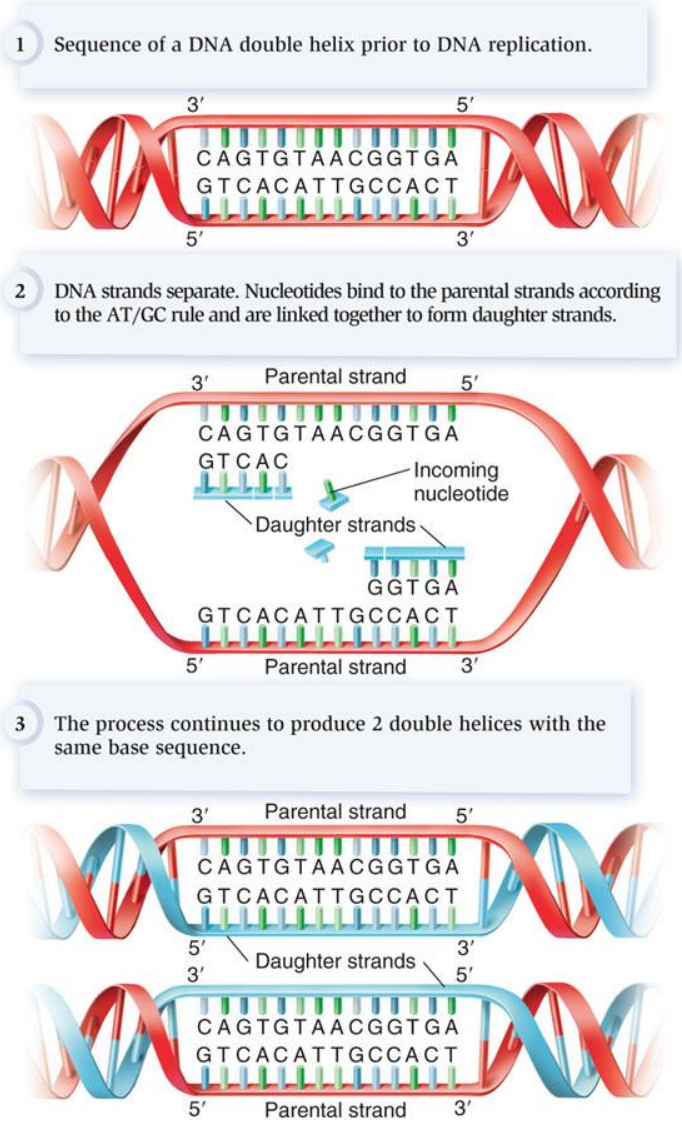
(b) Conservative mechanism. DNA replication produces 1 double helix with both parental strands, and the other with 2 new daughter strands.



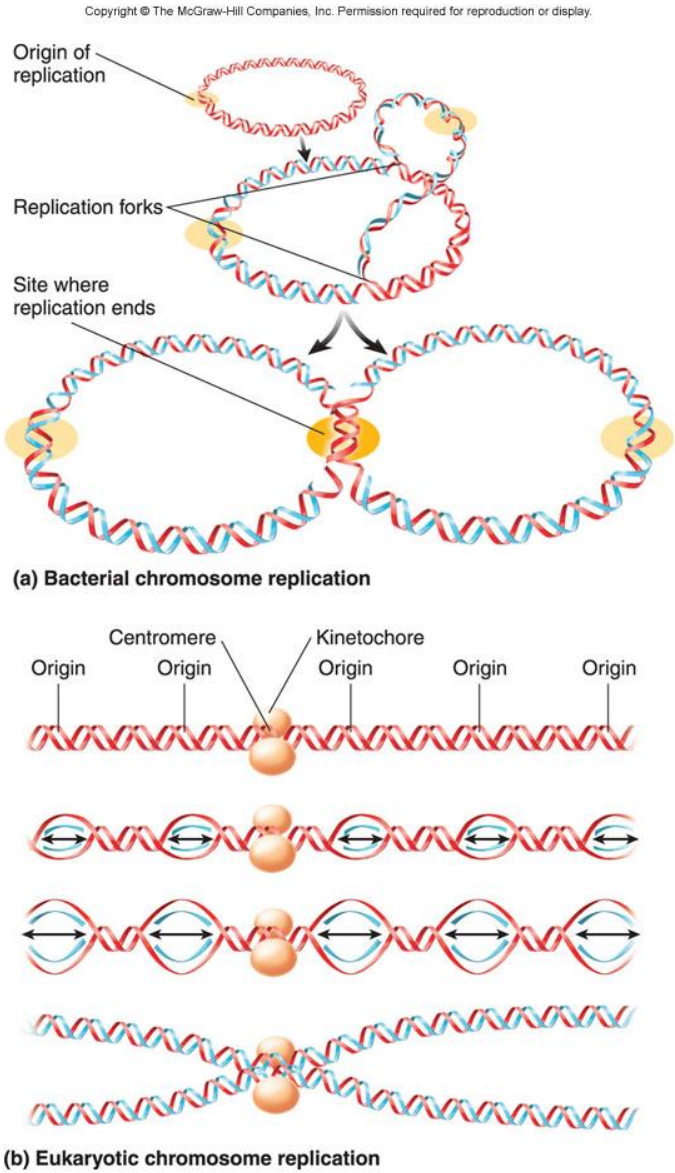
(c) Dispersive mechanism. DNA replication produces DNA strands in which segments of new DNA are interspersed with the parental DNA.

- In 1958, Matthew Meselson and Franklin Stahl devised experiment to differentiate among 3 proposed mechanisms
- Nitrogen comes in a common light form (^{14}N) and a rare heavy form (^{15}N)
- Grew *E.coli* in medium with only ^{15}N
- Then switched to medium with only ^{14}N
- Collected sample after each generation
- Original parental strands would be ^{15}N while newly made strands would be ^{14}N
- Results consistent with semiconservative mechanism

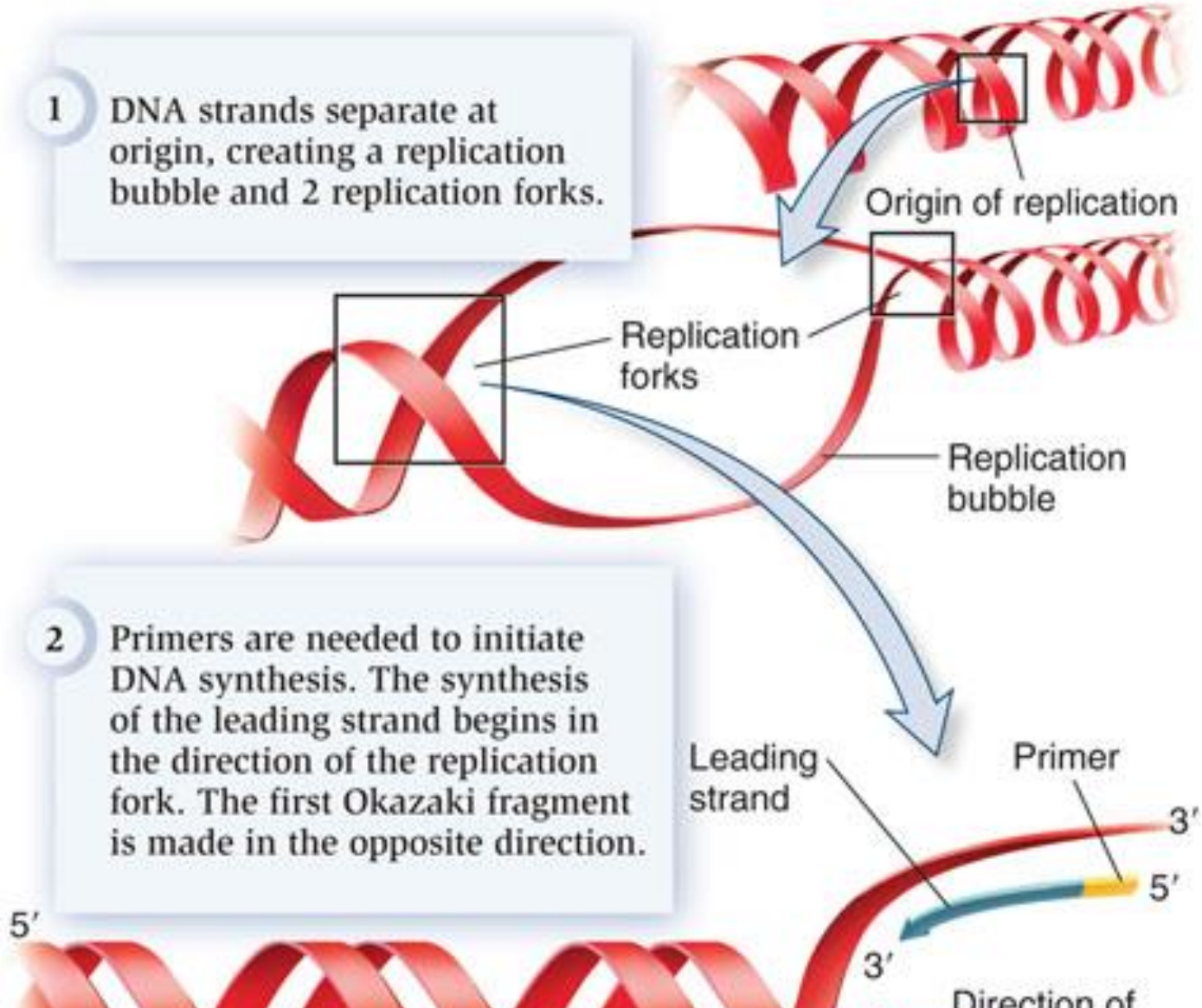
- During replication 2 parental strands separate and serve as template strands
- New nucleotides must obey the AT/GC rule
- End result 2 new double helices with same base sequence as original



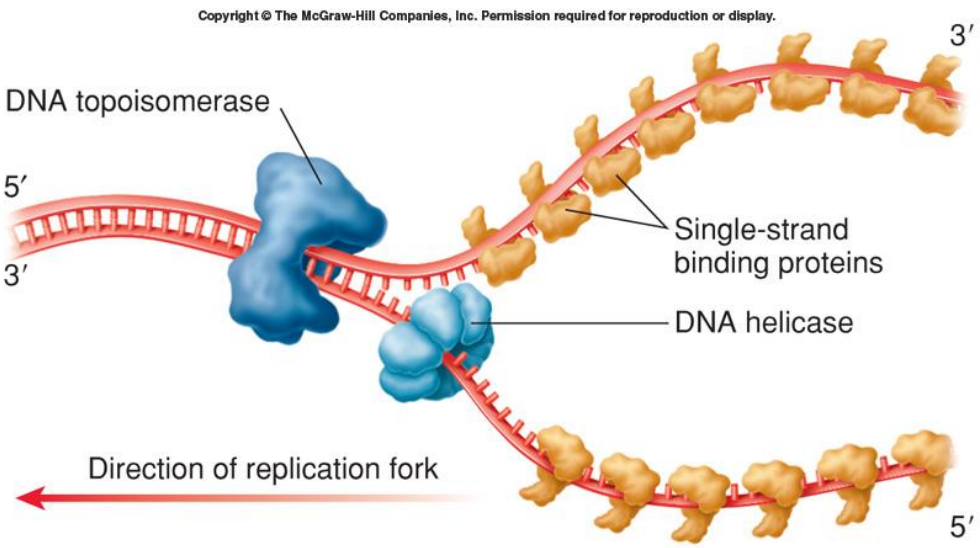
- Origin of replication
  - Site of start point for replication
- Bidirectional replication
  - Replication proceeds outward in opposite directions
- Bacteria have a single origin
- Eukaryotes require multiple origins



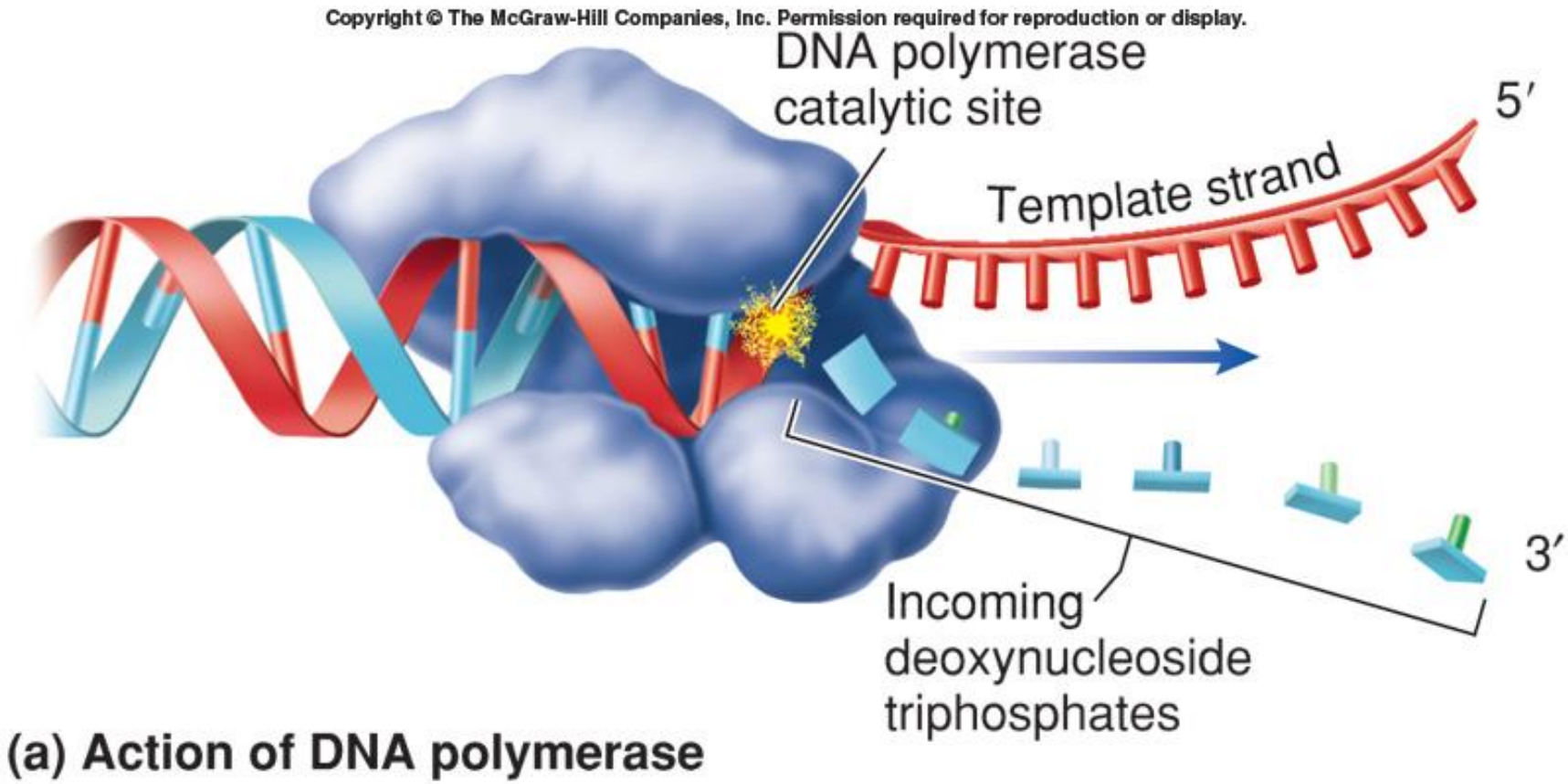
- Origin of replication provides an opening called a replication bubble that forms two replication forks
- DNA replication occurs near the fork
- Synthesis begins with a primer
- Proceeds 5’ to 3’
- Leading strand made in direction fork is moving
  - Synthesized as one long continuous molecule
- Lagging strand made as Okazaki fragments that have to be connected later



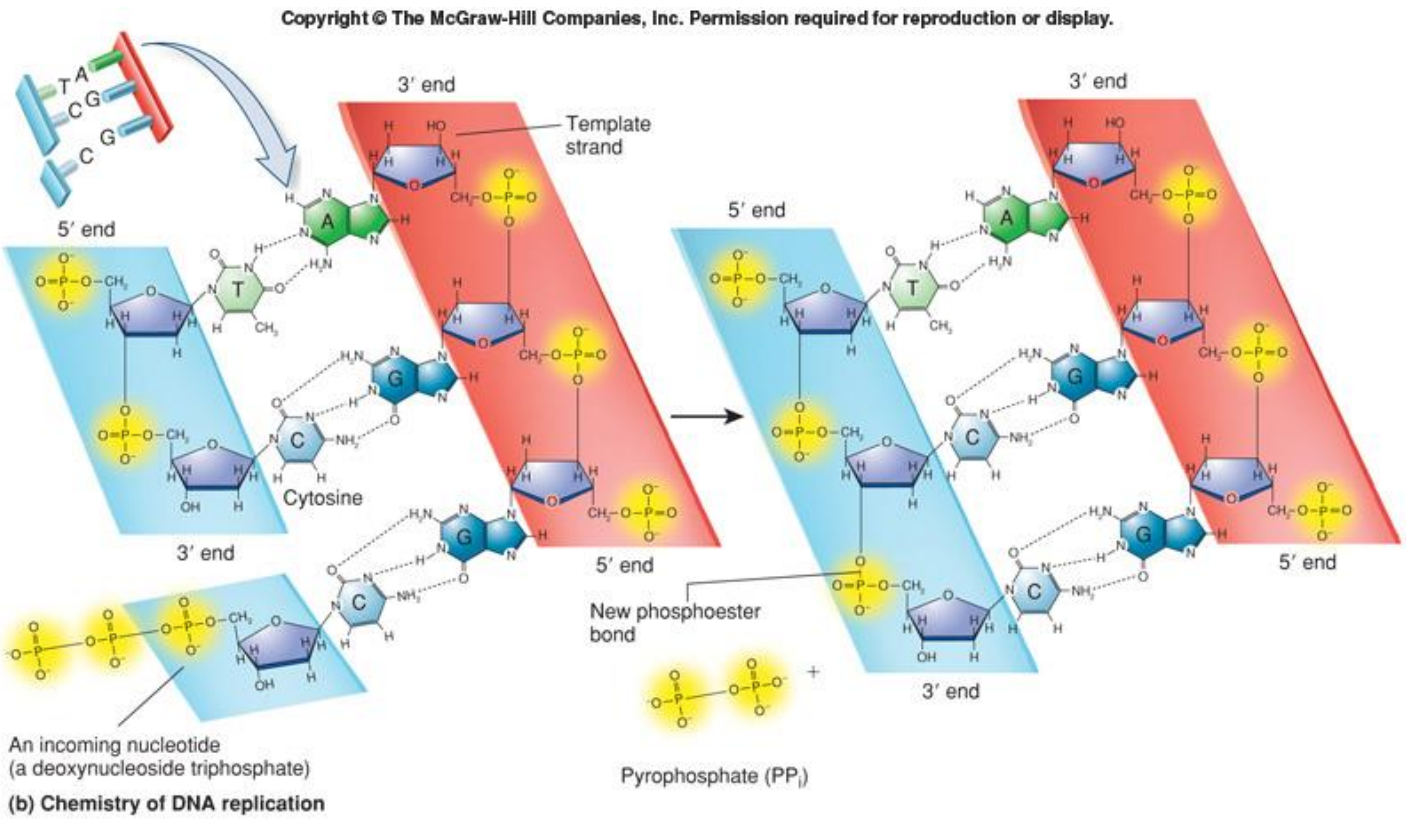
- DNA helicase
  - Binds to DNA and travels 5’ to 3’ using ATP to separate strand and move fork forward
- DNA topoisomerase
  - Relives additional coiling ahead of replication fork
- Single-strand binding proteins
  - Keep parental strands open to act as templates



- DNA polymerase
  - Covalently links nucleotides
- Deoxynuceloside triphosphates



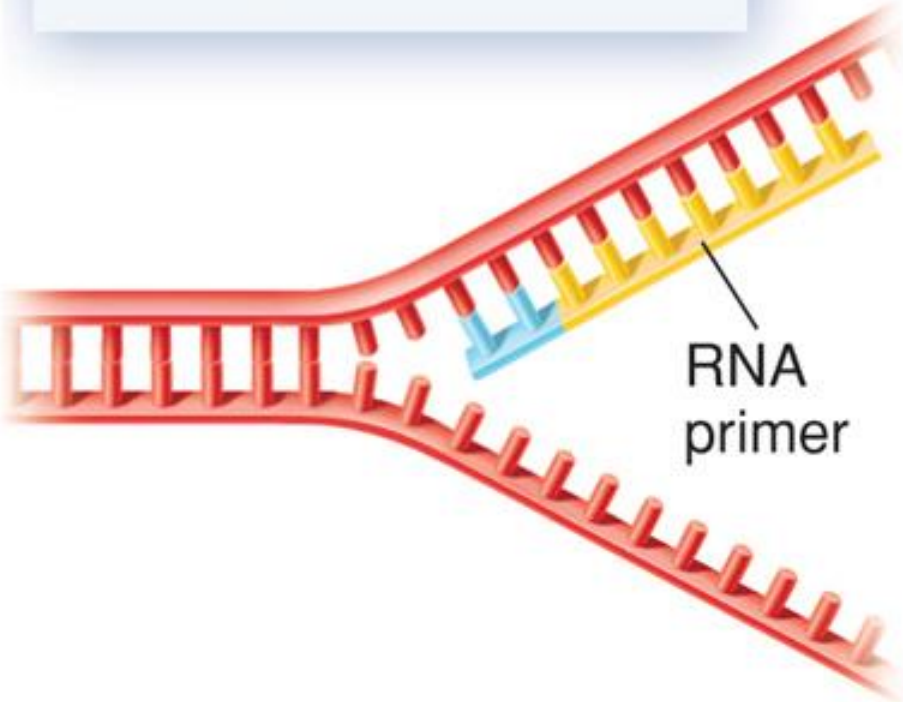
- Deoxynuceloside triphosphates
  - Free nucleotides with 3 phosphate groups
  - Breaking covalent bond to release pyrophosphate (2 phosphate groups) provides energy to connect adjacent nucleotides



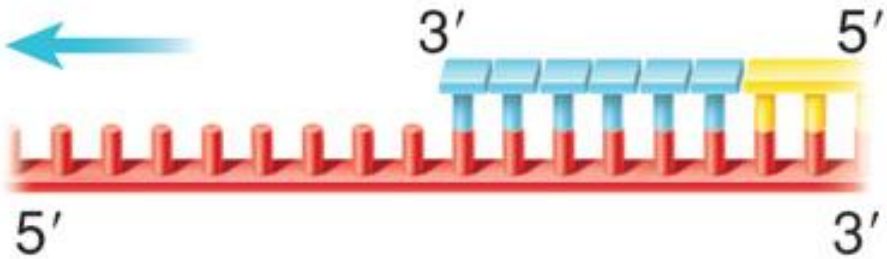
- DNA polymerase has 2 enzymatic features to explain leading and lagging strands
  1. DNA polymerase unable to begin DNA synthesis on a bare template strand
     - DNA primase must make a short RNA primer
       - RNA primer will be removed and replaced with DNA later
  2. DNA polymerase can only work 5’ to 3’

DNA polymerase is able to covalently link nucleotides together from a primer.

DNA polymerase can only link nucleotides in the 5′ to 3′ direction.

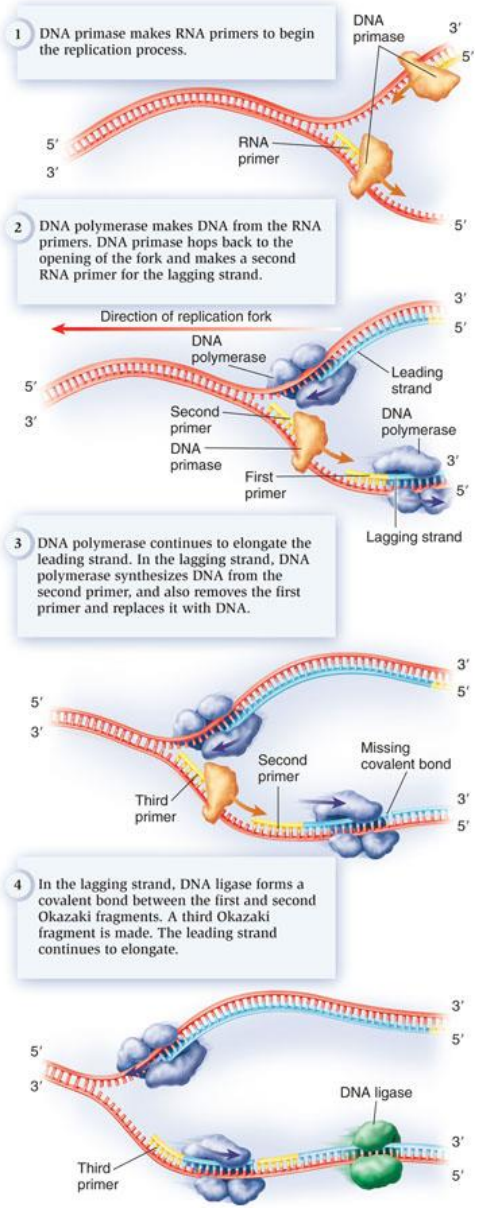


(a) Need for a primer



(b) Directional synthesis

- In the leading strand
  - DNA primase makes one RNA primer
  - DNA polymerase attaches nucleotides in a 5’ to 3’ direction as it slides forward
- In the lagging strand
  - DNA synthesized 5’ to 3’ but in a direction away from the fork
  - Okazaki fragments made as a short RNA primer made by DNA primase at the 5’ end and then DNA laid down by DNA polymerase
  - RNA primers will be removed by DNA polymerase and filled in with DNA
  - DNA ligase will join adjacent DNA fragments



DNA replication is very accurate

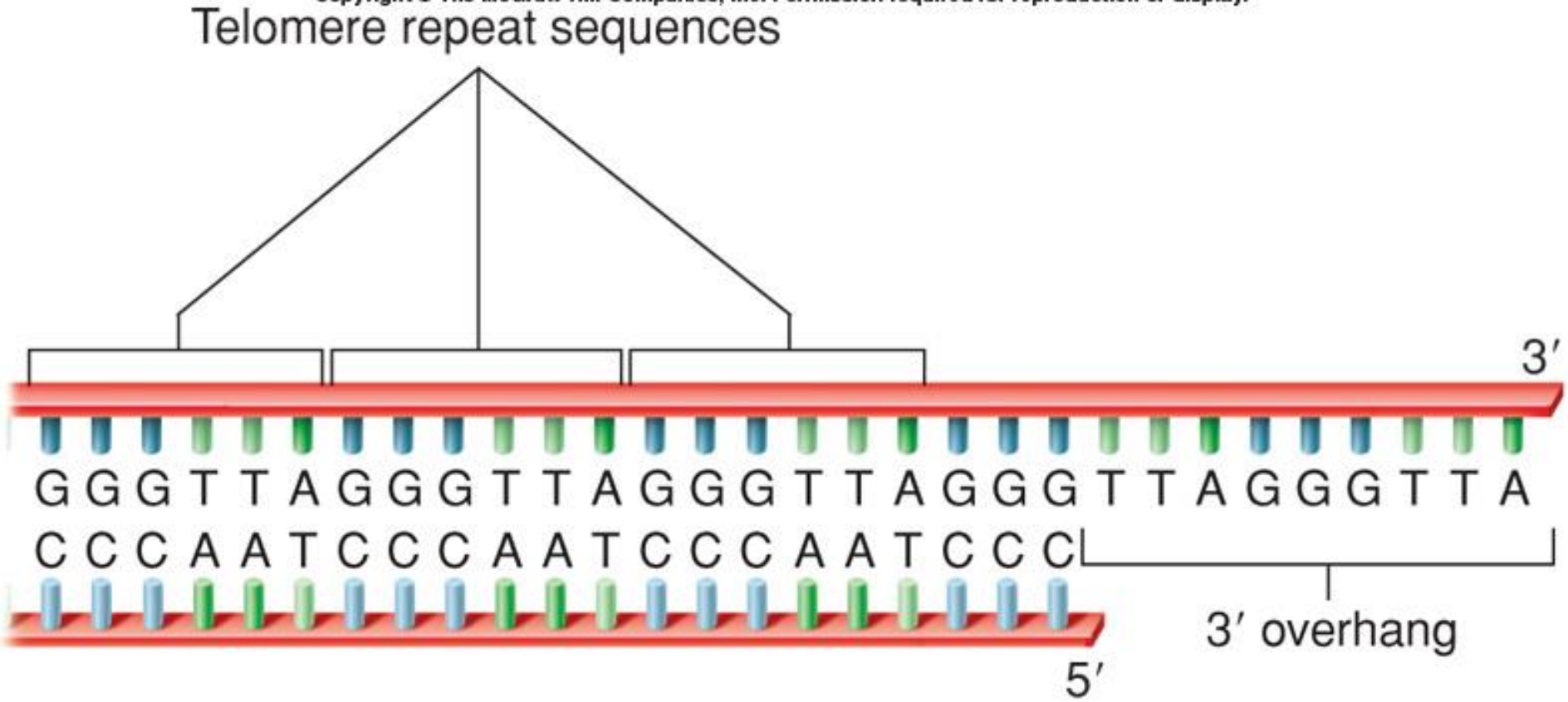
- 3 reasons
  1. Hydrogen bonding between A and T or G and C more stable than mismatches
  2. Active site of DNA polymerase unlikely to form bonds if pairs mismatched
  3. DNA polymerase removes mismatched pairs
     - Proofreading results in DNA polymerase backing up and digesting linkages
     - Other DNA repair enzymes

The family of DNA polymerases

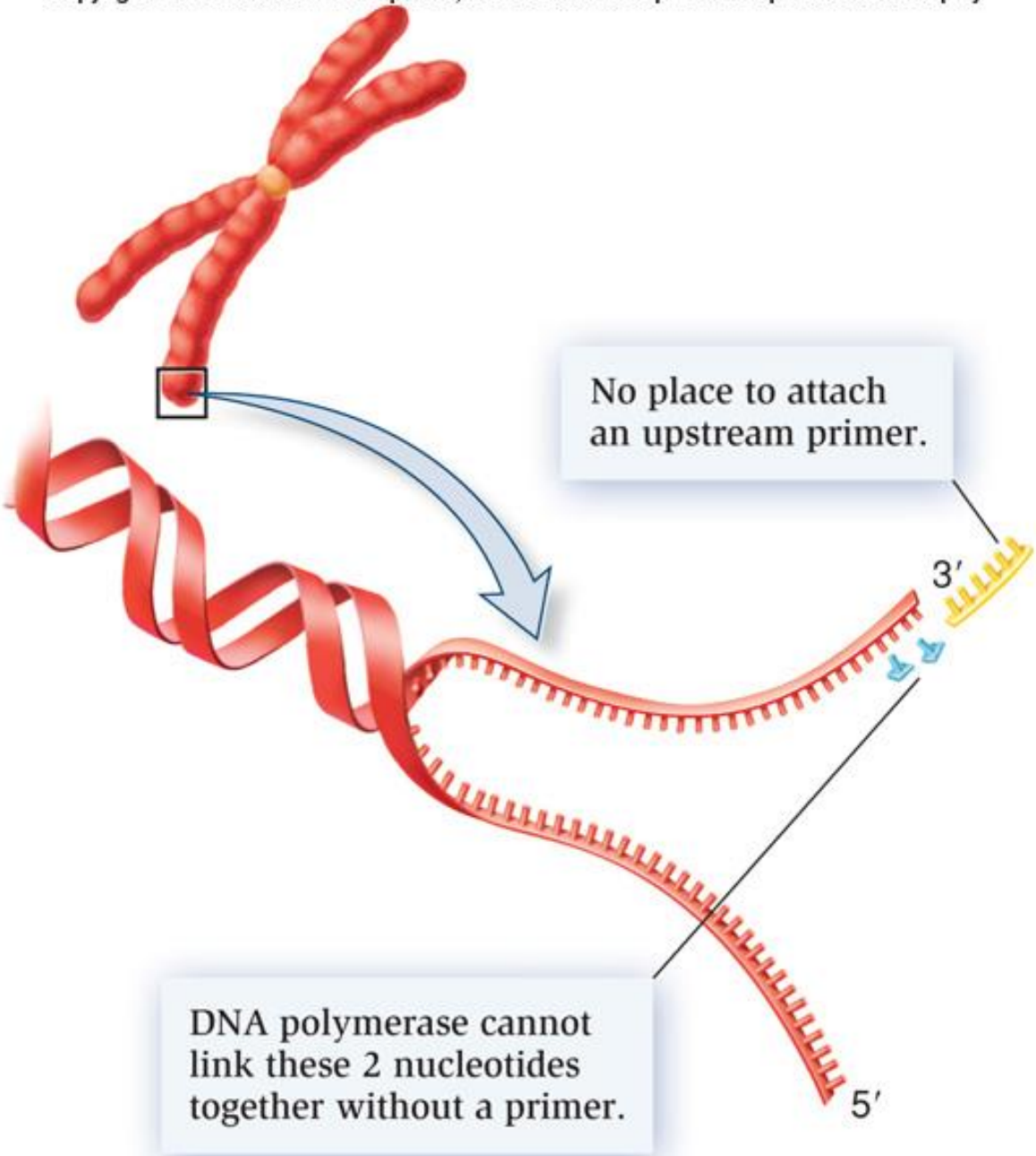
- 3 important issues for DNA polymerase are speed, fidelity, and completeness
- Nearly all living species have more than 1 type of DNA polymerase
- Genomes of most species have several DNA polymerase genes due to gene duplication
- Independent genetic changes produce enzymes with specialized functions suited to the organism

- *E. coli* has 5 DNA polymerases
  - DNA polymerase III with multiple subunits responsible for majority of replication
  - DNA polymerase I has a single subunit whose job is to rapidly remove RNA primers and fill in DNA
  - DNA polymerases II, IV and V are involved in DNA repair and replicating damaged DNA
    - DNA polymerases I and III stall at DNA damage
    - DNA polymerases II, IV and V don’t stall but go slower and make sure replication is complete

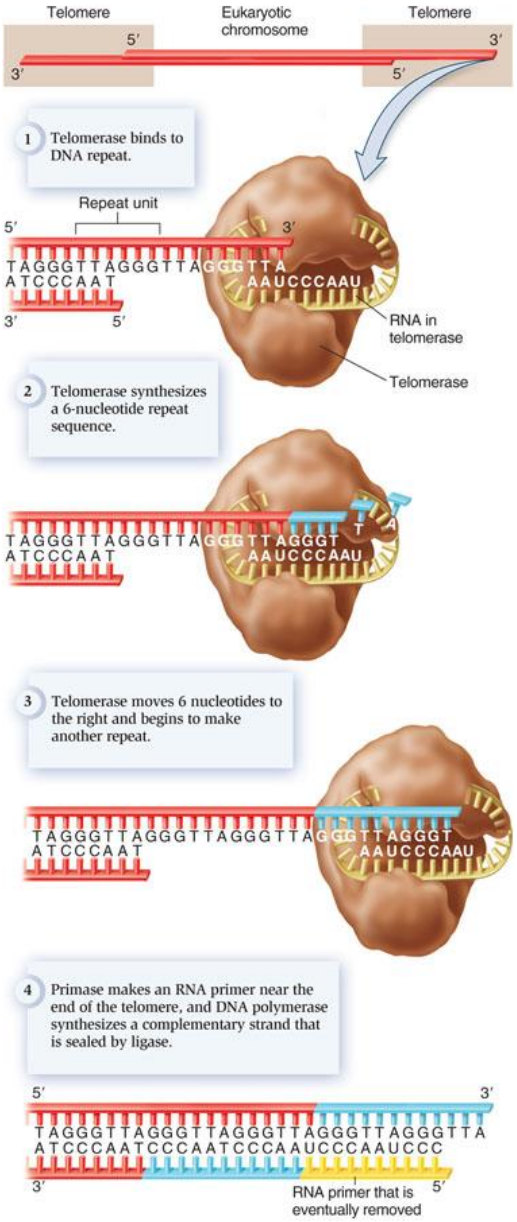
- Specialized form of DNA replication only in eukaryotes in the telomeres
- Telomeres are a series of repeat sequences within DNA and special proteins
- Telomere at 3’ does not have a complementary strand and is called a 3’ overhang



- DNA polymerase cannot copy the tip of the DNA strand with a 3’ end
  - No place for upstream primer to be made
- If this replication problem were not solved, linear chromosomes would become progressively shorter



- Telomerase prevents chromosome shortening
- Attaches many copies of repeated DNA sequences to the ends of the chromosomes
- Provides upstream site for RNA primer



Telomeres and aging

- Body cells have a predetermined life span
- Skin sample grown in a dish will double a finite number of times
  - Infants, about 80 times
  - Older person, 10 to 20 times
- Senescent cells have lost the capacity to divide

- Progressive shortening of telomeres correlated with cellular senescence
- Telomerase present in germ-line cells and in rapidly dividing somatic cells
- Telomerase function reduces with age
- Inserting a highly active telomerase gene into cells in the lab causes them to continue to divide

Telomeres and cancer

- When cells become cancerous they divide uncontrollably
- In 90% of all types of human cancers, telomerase is found at high levels
- Prevents telomere shortening and may play a role in continued growth of cancer cells
- Mechanism unknown