

What is the molecular basis of inheritance?

- Early 20th century, most scientists assumed proteins.
- 1928—Frederick Griffith discovered that something from heat-killed pathogenic strain of *Streptococcus pneumoniae* could “transform” a nonpathogenic strain to become pathogenic. This pathogenicity was inherited by all subcultures.

EXPERIMENT Bacteria of the “S” (smooth) strain of *Streptococcus pneumoniae* are pathogenic because they have a capsule that protects them from an animal’s immune system. Bacteria of the “R” strain lack a capsule and are nonpathogenic. Frederick Griffith injected mice with the two strains as shown below.

RESULTS Mouse dies, Mouse healthy, Mouse healthy, Mouse healthy, Mouse dies.

CONCLUSION Griffith concluded that the living R bacteria had been transformed into pathogenic S bacteria by an unknown, heritable substance from the dead S cells.

- In 1940s, it was found that the fraction containing DNA extracted from the pathogenic strain was causing the transformation.

What is the molecular basis of inheritance?

- 1952—The Alfred Hershey and Martha Chase experiment

EXPERIMENT In their famous 1952 experiment, Alfred Hershey and Martha Chase used radioactive sulfur and phosphorus to trace the fates of the protein and DNA, respectively, of T2 phages that infected bacterial cells.

- Mixed radioactively labeled phages with bacteria. The phages infected the bacterial cells.
- Agitated in a blender to separate phages outside so that bacteria formed a pellet at the bottom of the test tube.
- Centrifuged the mixture so that bacteria formed a pellet at the bottom of the test tube.
- Measured the radioactivity in the pellet and the liquid.

Batch 1: Phages were grown with radioactive sulfur (³⁵S), which was incorporated into phage protein (pink).

Batch 2: Phages were grown with radioactive phosphorus (³²P), which was incorporated into phage DNA (blue).

RESULTS Phage proteins remained outside the bacterial cells during infection, while phage DNA entered the cells. When cultured, bacterial cells with radioactive phage DNA released new phages with some radioactive phosphorus.

CONCLUSION Hershey and Chase concluded that DNA, not protein, functions as the T2 phage’s genetic material.

What is the molecular basis of inheritance?

- OK, maybe for viruses and bacteria. But what about in “higher” organisms?
- 1947—Erwin Chargaff analyzed the base composition of DNA [%A / %T / %C / %G] from a number of different organisms, both prokaryotes and eukaryotes.
 - Reported that the DNA composition varies among species, but it is very consistent within species.
- 1940s/50s—Others also noted that in dividing eukaryotic cells, the amount of DNA in the cells exactly doubled before division, with exactly half of the amount going to each daughter cell.
- So by 1950, most biologists conceded that DNA is the most likely molecular agent of inheritance. ...
 - ... But how?

Nucleic Acids are polymers of Nucleotide monomers

(a) Nucleotide components

Pyrimidines
 Cytosine (C), Thymine (in DNA) (T), Uracil (in RNA) (U)

Purines
 Adenine (A), Guanine (G)

Deoxyribose (in DNA)
Ribose (in RNA)

(b) Nucleotide
 Nitrogenous base, Phosphate group, Pentose sugar

(c) Polynucleotide

Nitrogen base determines type of nucleotide

- Adenine
- Guanine
- Cytosine
- Thymine
 - DNA only
- Uracil
 - RNA only

Nucleic Acids are polymers of Nucleotide monomers

- Nucleotides: phosphates on 5'-carbon of sugar
- Nucleic Acids: phosphate links 5'-C of sugar to 3'-C of preceding nucleotide sugar
- Nucleic Acid Polymer runs 5' to 3'

5-end

3-end

Nucleic Acids are polymers of Nucleotide monomers

Phosphate group

Nitrogenous base (A, G, C, or U)

Sugar

Nucleotide

Phosphate group

Nitrogenous base (A, G, C, or T)

Thymine (T)

Sugar (deoxyribose)

DNA nucleotide

Polynucleotide

Sugar-phosphate backbone

DNA

Deoxyribose-phosphate backbone

Double-stranded

Bases

Hydrogen bonding occurs between base-pairs

RNA

Ribose-phosphate backbone

Single-stranded

Bases

DNA double strands are anti-parallel (run in opposite directions)

Hydrogen bond

5-end

3-end

One strand 5' to 3'. The other strand 3' to 5'

DNA is a double helix — two complementary nucleic acid strands

Twist

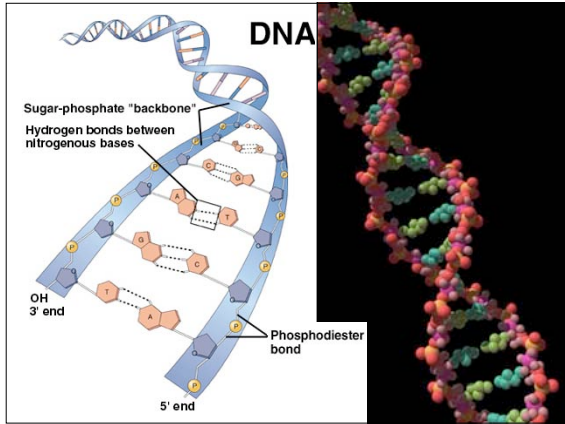
Start the Revolution: Discovering the 3-dimensional structure of DNA

1953 —

- Rosalind Franklin
 - used X-ray crystallography to study the molecular structure of the DNA molecule.
 - concluded that DNA was composed of two anti-parallel sugar-phosphate backbones, with the nitrogenous bases paired in the molecule's interior.
- James Watson and Francis Crick
 - built on Franklin's work to complete the model for the "Double Helix". ...
 - ... and of course got all the credit!

Figure 16.6

Franklin's X-ray diffraction photograph of DNA



Molecular Genetics

- Replication
 - Precisely copying all the genetic information (DNA)
 - S-stage of cell cycle
 - Exact replicas passed to daughter cells
- Gene Expression
 - Using a specific bit of the genetic information
 - Make a “working copy” of the needed bit (gene)
 - Take the working copy to the workshop (ribosome)
 - Use the copied instructions to build a specific protein

The key to molecular genetics: complementary base pairing

G C A C C A A T A

C G T G G T T A T

one base pair

Complementary base pairing in DNA

- C pairs only with G
- A pairs only with T

The key to molecular genetics: complementary base pairing

Adenine (A)

Thymine (T)

Each pair
= 1 purine + 1 pyrimidine

- A=T (2 H-bonds)
- G=C (3 H-bonds)

Guanine (G)

Cytosine (C)

Figure 16.8

DNA Structure

□ the sequence of bases in the two strands are **complementary** to each other (*not* identical).

DNA's complementary base sequence

DNA Replication

Semiconservative

- Each strand serves as a template for a new strand.
- Each “daughter cell” receives one original template strand + one complementary strand.

DNA must be unwound to be read

- Hydrogen bonds “unzip”
- Hydrogen bonds reform between new nucleotides

DNA replication

DNA Replication

Template model for DNA replication

- Replication depends upon base pairing
- Old strands serve as templates determining the sequence of complementary new nucleotides

DNA Replication is Semi-Conservative

Template model for DNA replication

- Replication depends on base pairing
- Old strands serve as templates determining the sequence of complementary new nucleotides
- Both daughter copies have one old and one new strand

Enzymes of Replication

Over a dozen enzymes and other proteins needed for replication

- **DNA Helicase**
 - Unwinds and separates DNA
- **DNA Polymerase**
 - Sequentially adds new nucleotides to 3'-end of growing new DNA strand (Runs 3' to 5' along parental strand.)
- **DNA Ligase**
 - Joins pieces of DNA together

“Replication forks”

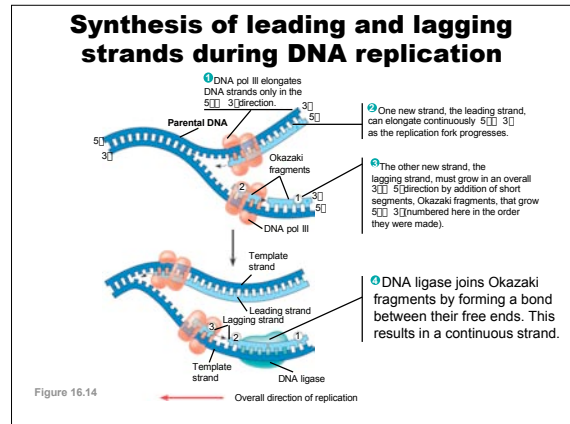
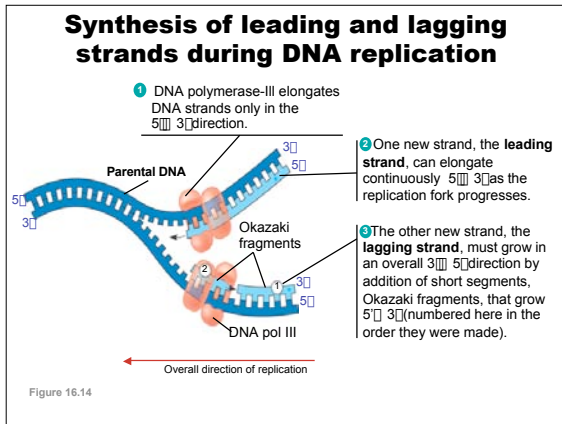
Elongating a New DNA Strand

- Elongation of new DNA at a replication fork is catalyzed by enzymes called DNA polymerases, which add nucleotides to the 3' end of a growing strand.

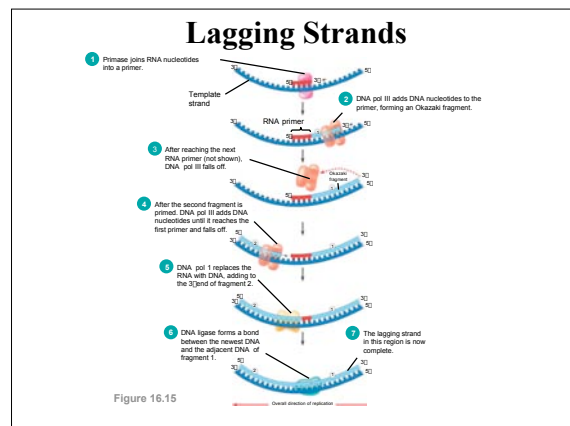
Figure 16.13
Energy for synthesis from hydrolysis of PP_i from nucleotide triphosphate (NTP).

Antiparallel Elongation

- **But** — Remember that polymerase only runs from 3'-to-5' along a parental strand, adding nucleotides to the 3'-end of the elongating strand.
- Elongation of the new **Leading Strand** of DNA along the 3'-to-5' arm of the parental template can proceed continuously 5'-to-3'.
- But elongation of the new **Lagging Strand** of DNA along the antiparallel 5'-to-3' parental template must proceed in 5'-to-3' segments (**Okazaki fragments**), and joined (ligated) later.



- ### Primers & DNA Synthesis
- DNA polymerases cannot initiate the synthesis of a polynucleotide. They can only add more nucleotides to the 3' end of a present oligo- or poly-nucleotide.
 - The initial nucleotide strand to start is called a **primer**.
 - In cells, the primer is a 5–10-nucleotide **RNA-oligomer** synthesized complementary to the parental strand by the enzyme **primase**.
 - In the lab, we can use a synthetic oligonucleotide of either RNA or DNA as a primer to initiate DNA synthesis.
 - For the leading strand, only one primer is needed.
 - For the lagging strand, a new primer is needed for each Okazaki fragment.



Other Proteins That Assist DNA Replication

- Helicase, topoisomerase, single-strand binding protein

Table 16.1 Bacterial DNA replication proteins and their functions	
Protein	Function for Leading and Lagging Strands
Helicase	Unwinds parental double helix at replication forks
Single-strand binding protein	Binds to and stabilizes single-stranded DNA until it can be used as a template
Topoisomerase	Corrects "overwinding" ahead of replication forks by breaking, swiveling, and rejoining DNA strands
Primase	Synthesizes a single RNA primer at the 5' end of the leading strand
DNA pol III	Continuously synthesizes the leading strand, adding on to the primer
DNA pol I	Removes primer from the 5' end of each fragment and replaces it with DNA, adding on to the 3' end of the adjacent fragment
DNA Ligase	Joins the 3' end of the DNA that replaces the primer to the rest of the leading strand

- Most of the various proteins that participate in DNA replication form a single large complex — The DNA replication “machine”
- The DNA replication machine is probably stationary during the replication process

