Molecular Genetics

Feb 16, 2001

What is the molecular basis of inheritance?

- 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.

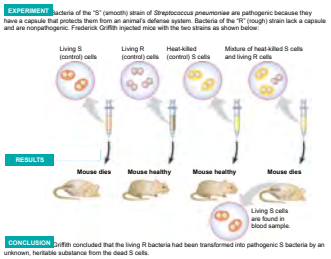
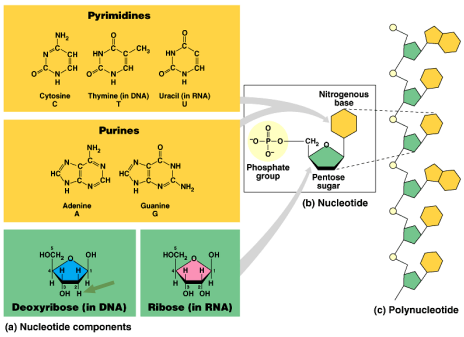


Figure 16.2

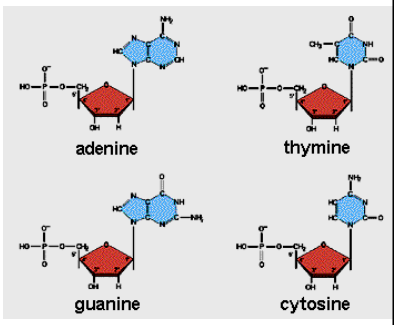
- Early 20th century, most scientists assumed proteins.
- In 1940s, it was found that the fraction containing **DNA** extracted from the pathogenic strain was causing the transformation.

Nucleic Acids are polymers of Nucleotide monomers



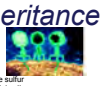
Nitrogen base determines type of nucleotide

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



What is the molecular basis of inheritance?

- 1952—The Alfred Hershey and Martha Chase experiment



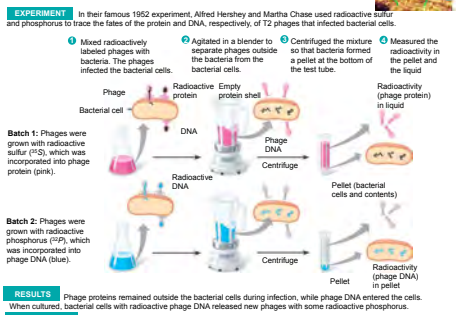


Figure 16.4

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?

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!

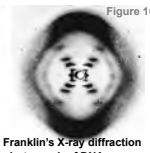
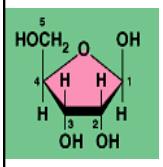


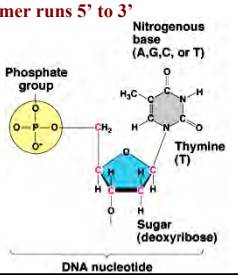
Figure 16.6

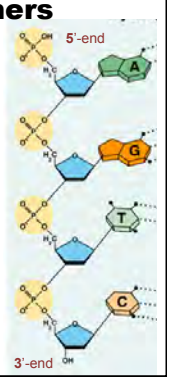
Franklin's X-ray diffraction photograph of DNA

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'**

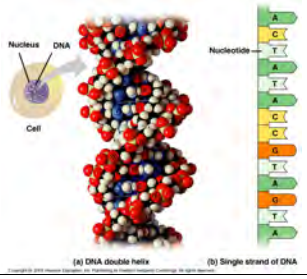


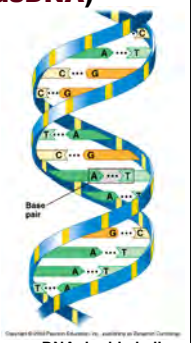




The Double helix: double-stranded Deoxyribo-Nucleic Acid (dsDNA)

- Sequence of bases along the polymer are the "Genetic Information"

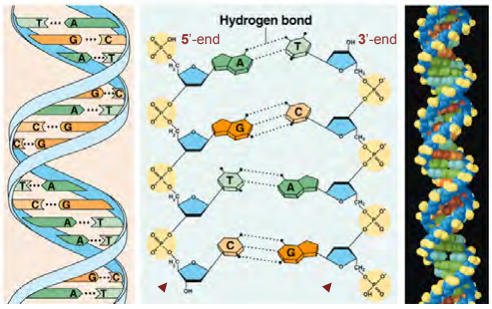




DNA double helix

DNA double strands are anti-parallel

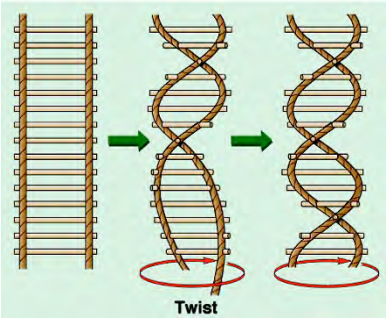
(run in opposite directions)

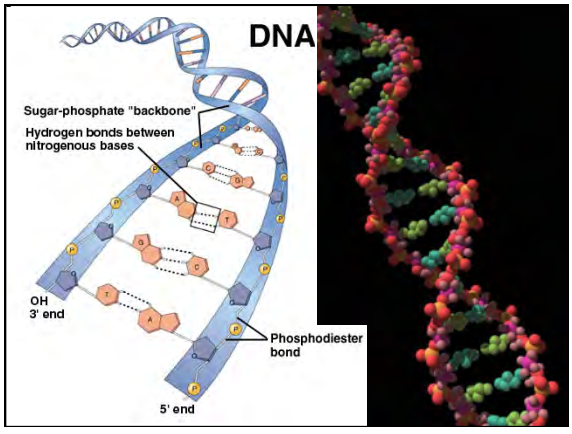


One strand 5' to 3'. The other strand 3' to 5'

DNA is a double helix —

two complementary nucleic acid strands

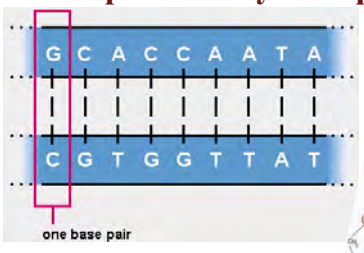


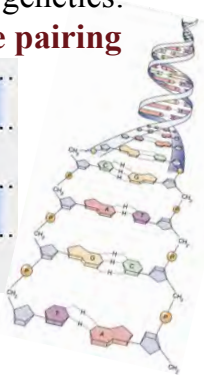


Molecular Genetics

- Replication
  - – **Precisely** copying <u>all</u> the genetic information (DNA)
    - – S-stage of cell cycle
  - – Exact replicas passed to daughter cells
- Gene Expression
  - – Using a <u>specific</u> <u>bit</u> 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





Complementary base pairing in DNA
- **C pairs only with G**
- **A pairs only with T**

The key to molecular genetics: complementary base pairing

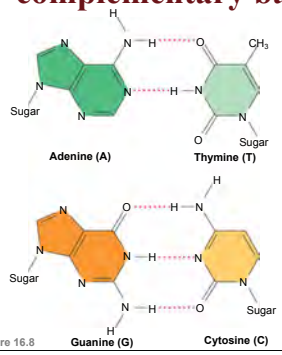
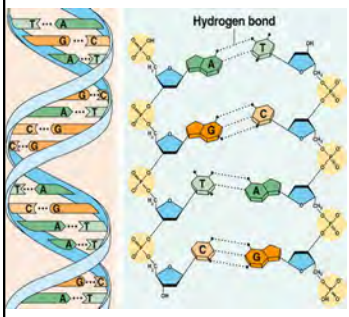


Figure 16.8

Each pair
= 1 purine + 1 pyrimidine

- A=T (2 H-bonds)
- G≡C (3 H-bonds)

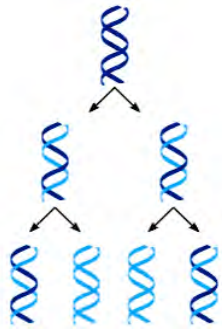
DNA Structure



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

DNA's complementary base sequence

DNA Replication

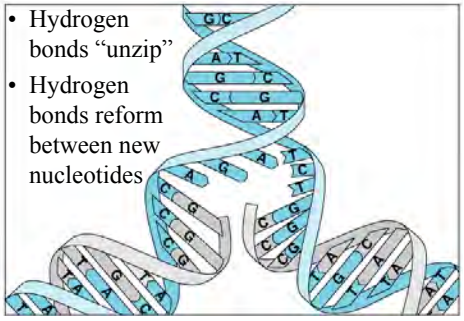


Semiconservative

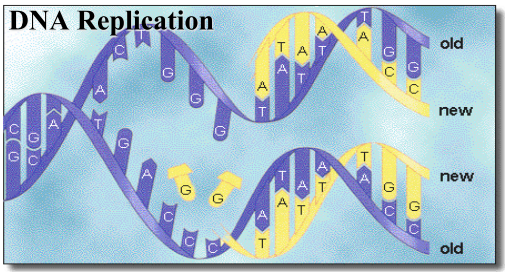
- **Each strand serves as a template for a new strand.**
- **Each "daughter cell" receives one original template strand + one complementary strand.**

DNA must be unwound to be read

- Hydrogen bonds "unzip"
- Hydrogen bonds reform between new nucleotides

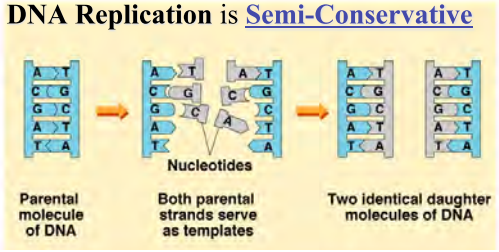


DNA replication



Template model for DNA replication

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



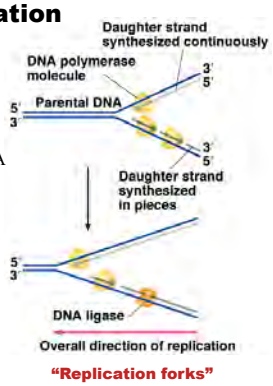
Template model for DNA replication

- Replication depends on base pairing
- Old strands serve as templates determining the sequence of complimentary 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



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.

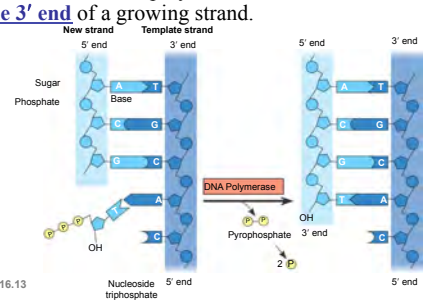
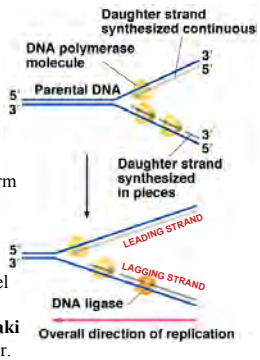


Figure 16.13

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

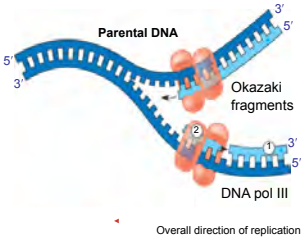
Antiparallel Elongation

- **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.



Synthesis of leading and lagging strands during DNA replication

1 DNA polymerase-III elongates DNA strands only in the 5′→3′ direction.

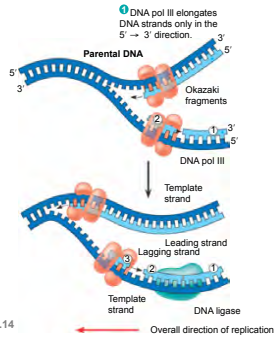


2 One new strand, the **leading strand**, can elongate continuously 5′→3′ as the replication fork progresses.

3 The other new strand, the **lagging strand**, must grow in an overall 3′→5′ direction by addition of short segments, Okazaki fragments, that grow 5′→3′ (numbered here in the order they were made).

Figure 16.14

Synthesis of leading and lagging strands during DNA replication



2 One new strand, the leading strand, can elongate continuously 5′ → 3′ as the replication fork progresses.

3 The other new strand, the lagging strand, must grow in an overall 3′ → 5′ direction by addition of short segments, Okazaki fragments, that grow 5′ → 3′ (numbered here in the order they were made).

4 DNA ligase joins Okazaki fragments by forming a bond between their free ends. This results in a continuous strand.

Figure 16.14

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.

Lagging Strands

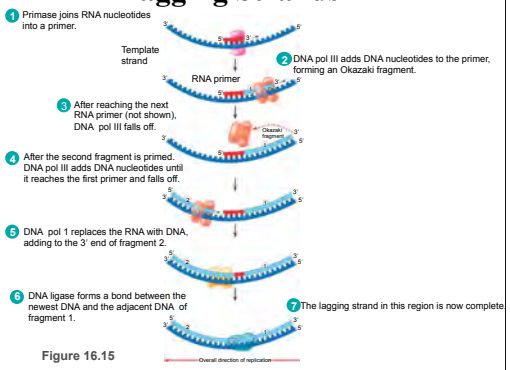


Figure 16.15

Other Proteins That Assist DNA Replication

- Helicase, topoisomerase, single-strand binding protein

Table 16.1 Bacterial DNA replication proteins and their functions

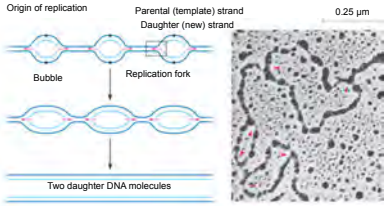
Protein	Function for Leading and Lagging Strands
Helicase	Unwinds parental double helix at replication forks
Single-strand binding protein	Binds to and stabilizes single-stranded DNA until it can be used as a template
Topoisomerase	Corrects "overwinding" ahead of replication forks by breaking, unwinding, and rejoining DNA strands

	Function for Leading Strand	Function for Lagging Strand
Primase	Synthesizes a single RNA primer at the 5′ end of the leading strand	Synthesizes an RNA primer at the 5′ end of each Okazaki fragment
DNA pol III	Continuously synthesizes the leading strand, adding on to the primer	Elongates each Okazaki fragment, adding on to its primer
DNA pol I	Removes primer from the 5′ end of leading strand and replaces it with DNA, adding on to the adjacent 3′ end	Removes the 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	Joins the Okazaki fragments

- 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

Origins of Replication

- The replication of a DNA molecule begins at special sites called origins of replication, where the two strands are separated
- A bacterial chromosome typically has one replication origin
- A eukaryotic chromosome may have hundreds or even thousands of replication origins



1 Replication begins at specific sites where the two parental strands separate and form replication bubbles.

2 The bubbles expand laterally, as DNA replication proceeds in both directions.

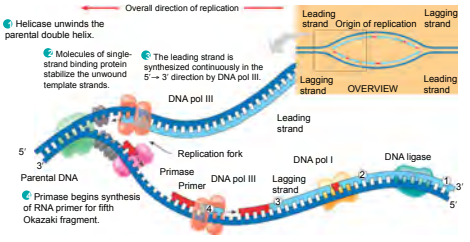
3 Eventually, the replication bubbles fuse, and synthesis of the daughter strands is complete.

(a) In eukaryotes, DNA replication begins at many sites along the giant DNA molecule of each chromosome.

(b) In this micrograph, three replication bubbles are visible along the DNA of a cultured Chinese hamster cell (TEM).

Figure 16.12 a, b

A summary of DNA replication



5 DNA pol III is completing synthesis of the fourth fragment, when it reaches the RNA primer on the third fragment, it will dissociate, move to the replication fork, and add DNA nucleotides to the 3′ end of the fifth fragment primer.

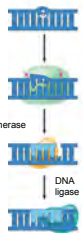
6 DNA pol I removes the primer from the 5′ end of the second fragment, replacing it with DNA nucleotides that it adds one by one to the 3′ end of the third fragment. The replacement of the last RNA nucleotide with DNA leaves the sugar-phosphate backbone with a free 3′ end.

7 DNA ligase bonds the 3′ end of the second fragment to the 5′ end of the first fragment.

Figure 16.16

Proofreading and Repairing DNA

- DNA polymerases proofread newly made DNA, replacing any incorrect nucleotides.
- In mismatch repair of DNA, repair enzymes correct errors in base pairing.
- In nucleotide excision repair, nucleases cut out and replace damaged stretches of DNA.



1 A thymine dimer distorts the DNA molecule.
2 A nuclease enzyme cuts the damaged DNA strand at two points and the damaged section is removed. Nuclease
3 Repair synthesis by a DNA polymerase fills in the missing nucleotides.
4 DNA ligase seals the Free end of the new DNA To the old DNA, making the strand complete.

Figure 16.17

Replicating the Ends of DNA Molecules

- The ends of eukaryotic chromosomal DNA get shorter with each round of replication

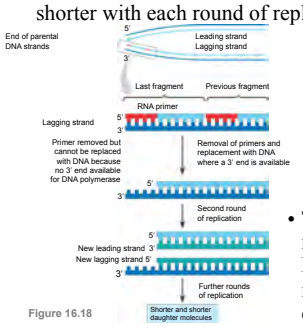


Figure 16.18

- **This could limit the number of times DNA could be replicated and cells could divide.**

Replicating the Ends of DNA Molecules

- Eukaryotic chromosomal DNA molecules have at their ends nucleotide sequences, called telomeres, that postpone the erosion of genes near the ends of DNA molecules.
- Telomere-binding-proteins "tie off" the ends to protect them from unraveling or degrading.

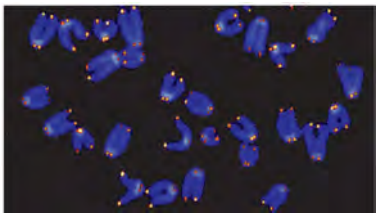
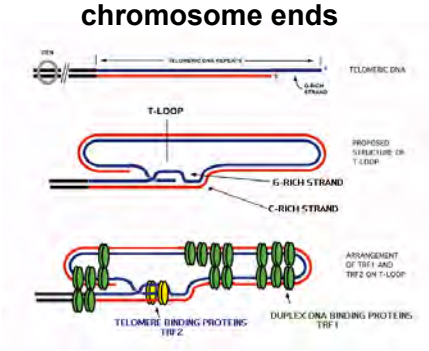


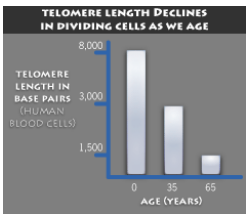
Figure 16.19

1 μm

Telomeres form loops on chromosome ends

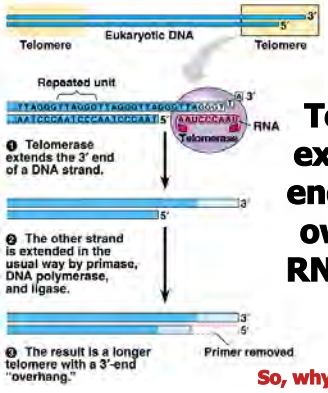


Telomeres shorten with age



- If the chromosomes of germ cells became shorter in every cell cycle essential genes would eventually be missing from the gametes they produce.
- In germ cells, an enzyme called **telomerase** catalyzes the lengthening of telomeres so no genes are lost in gametes.

- **Factor in aging and maximum life span?**
- **Long-term chronic stress increases rate of telomere shortening!**



Telomerase extends DNA ends using its own built-in RNA template

So, why only in germ cells?