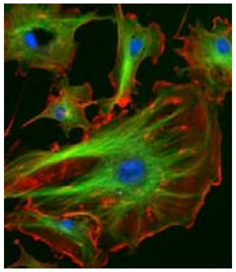
Lecture 4: From Transforming Principle to DNA Structure & Replication



Section A: From Nature to Concepts

Genes & Society
LSM3201 / GEK 1527

The greatest single achievement of nature to date was surely the invention of the molecule DNA.

- Lewis Thomas (Physician, Essayist)

Overview

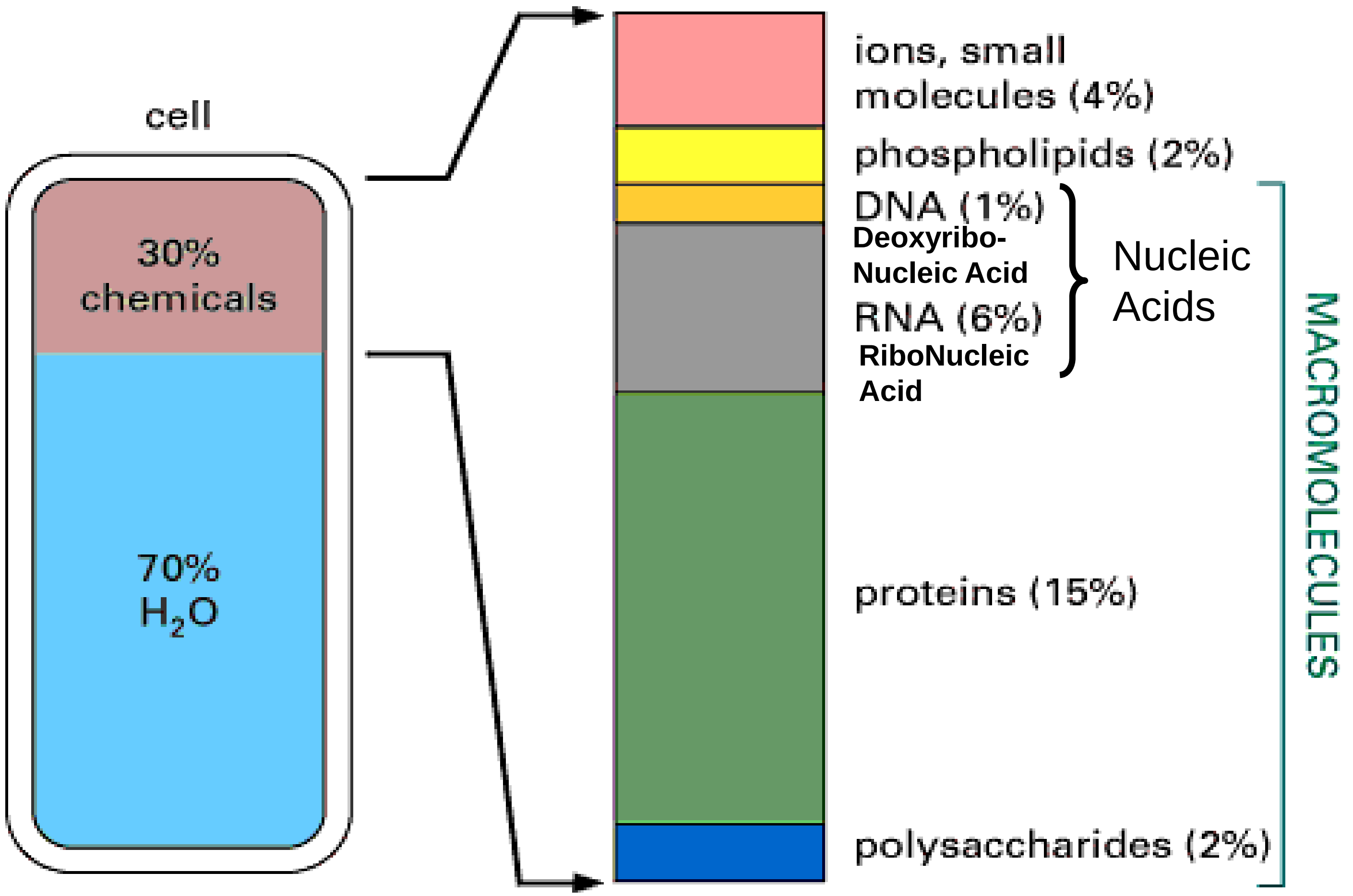
- Experiments that identified DNA (DeoxyriboNucleic Acid) as the genetic material
- Discovery of the DNA structure
- DNA form & properties
- DNA replication

Historical Landmarks in Early DNA Biochemistry

Date	Researcher(s)	Discovery
1868	Friedrich Miescher	Isolated and studied a phosphorus-containing substance from cell nuclei and salmon sperm cells. Miescher called the substance "nuclein" and suggested it was associated with cellular inheritance.
1928	Frederick Griffith	Defined a genetic "transforming principle" in the pneumococcus bacterium.
1929	Phoebus Levine	Used chemical analysis to determine four types of bases (A, T, G, C) as important building blocks of DNA.
1944	Oswald Avery Colin MacLeod Maclyn McCarty	Showed that Griffith's bacterial transforming factor is not protein but DNA. The three published experimental evidence that DNA is a component in chromosomes and the principal agent involved in the transfer of genetic information.
1950	Alfred Hershey Martha Chase	Used viruses in experiments to confirm DNA is the genetic material.
1952	Erwin Chargaff	Studied the composition of DNA from different species and found the ratios of adenine to thymine and of guanine to cytosine to be 1.0.
1952	Maurice Wilkins Rosalind Franklin	Studied X-ray diffraction of DNA crystals and found periodic patterns implying repeating structural units in DNA.
1953	James Watson Francis Crick	Formulated a three-dimensional structure (double helix) for DNA that accounted for X-ray diffraction and A–T and G–C equivalence data.

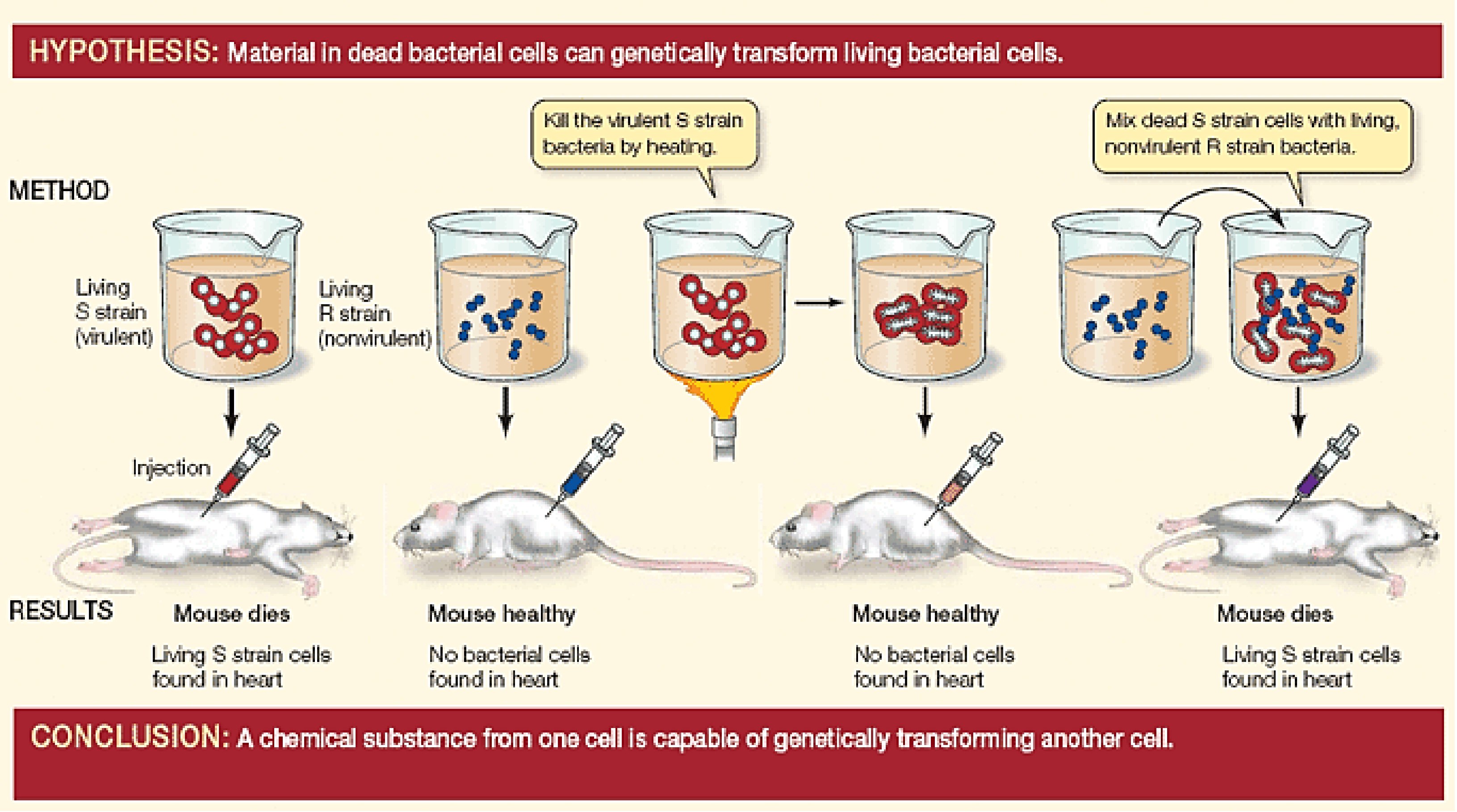
Concepts in Biochemistry 3ed.

How much of genetic materials [nucleic acids i.e. DNA and RNA] are found in a cell?



http://www.nature.com/scitable/topicpage/what-is-a-cell-14023083

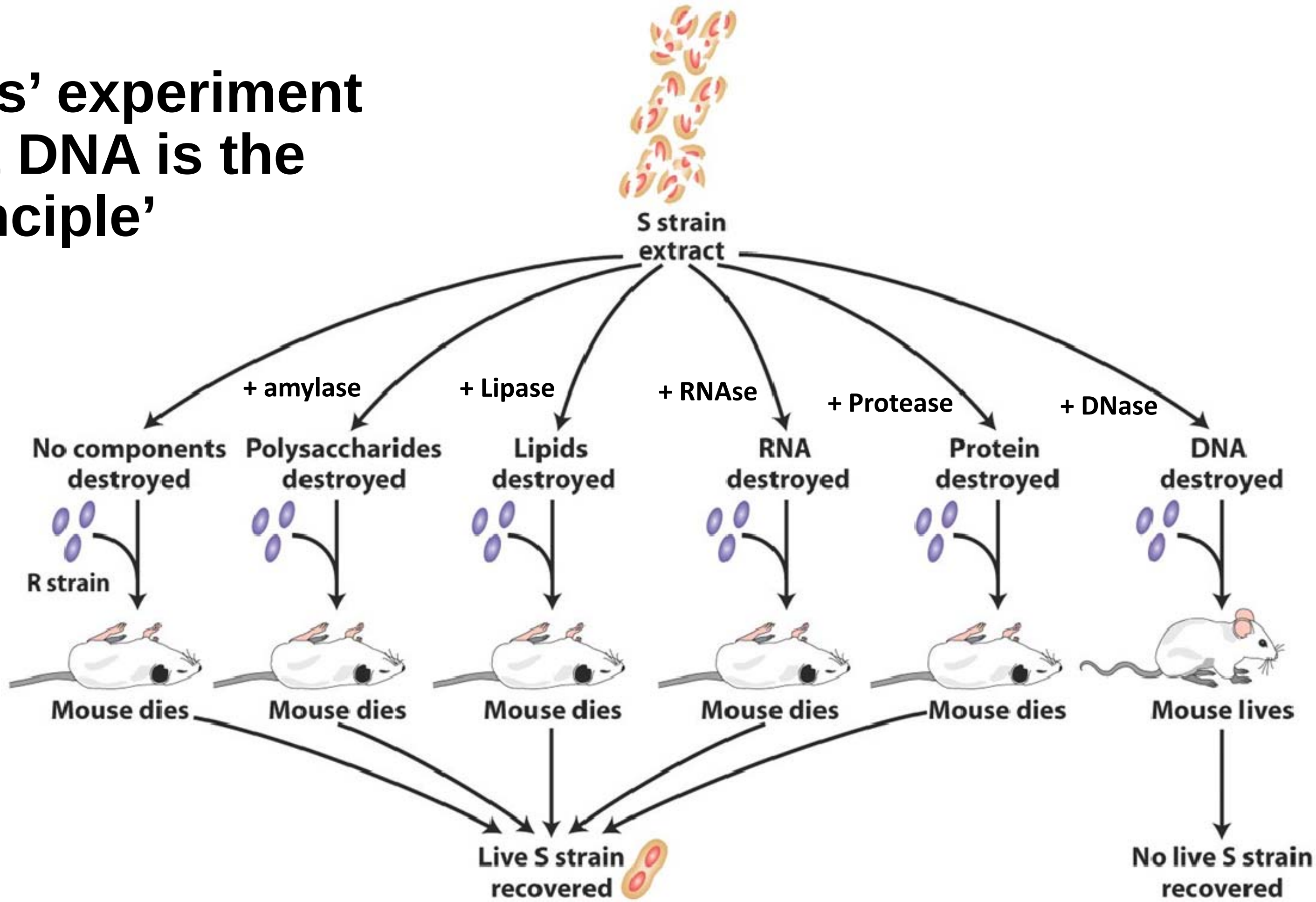
Griffith’s Transformation Experiment (1928)



Heat killed the S cells but did not destroy the factor that causes infection. The factor was transferred from dead S cells to living R cells and transformed living R cells to living S cells. Griffith called this the ‘Transforming Principle’.

https://sites.google.com/site/averymacleodmccarty/griffith-s-experiment

Avery & colleagues' experiment demonstrated that DNA is the 'Transforming Principle' (1944)



However many scientist were still puzzled at how the relatively simple DNA chemical components (compared to more complex proteins) could have produced the complex diversity of life.

http://cpacesclass.blogspot.com/2013/03/advanced-biology-how-do-we-know-that.html

Hershey & Chase experiment (1950)

Is Protein or DNA the Genetic Material?

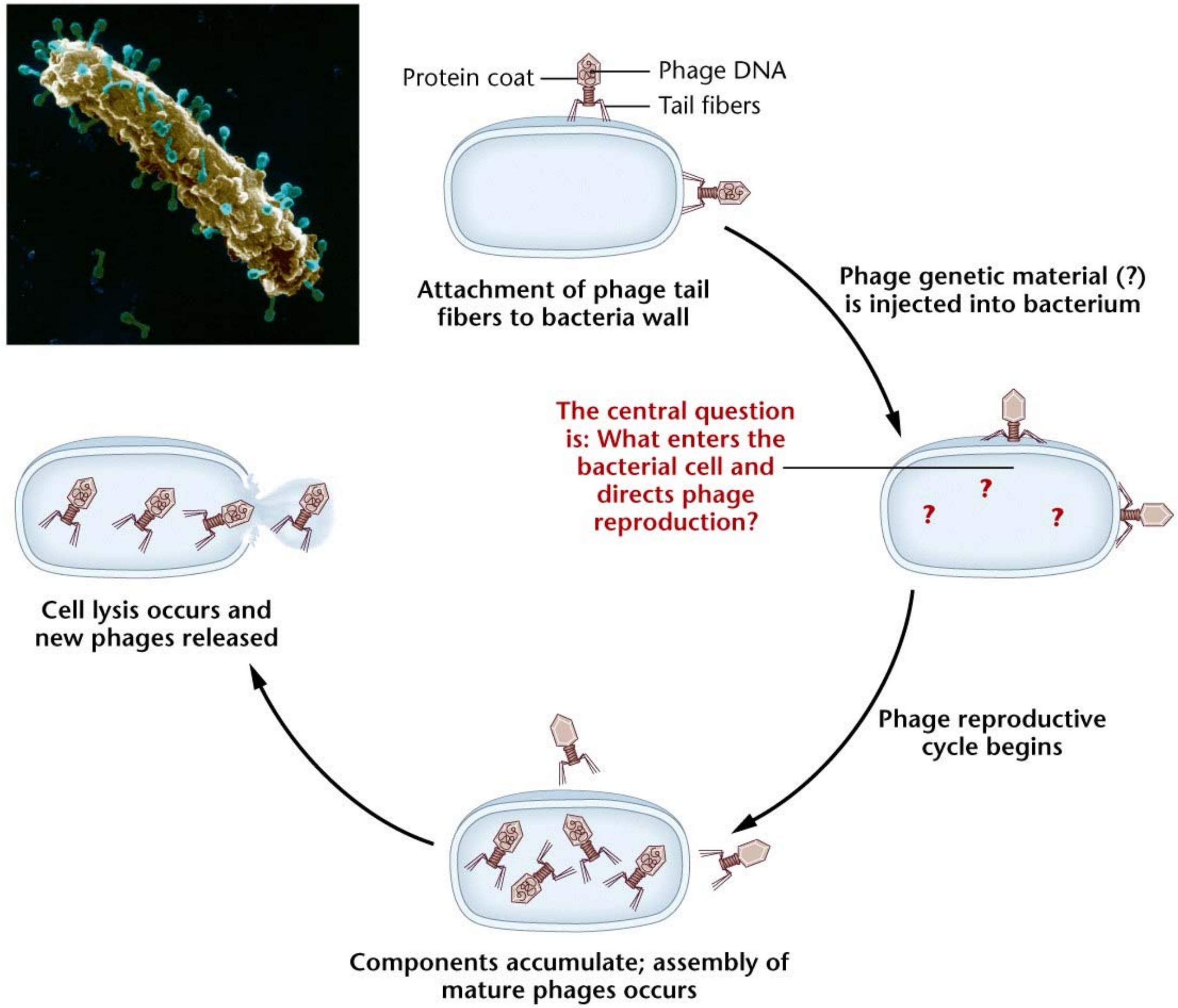
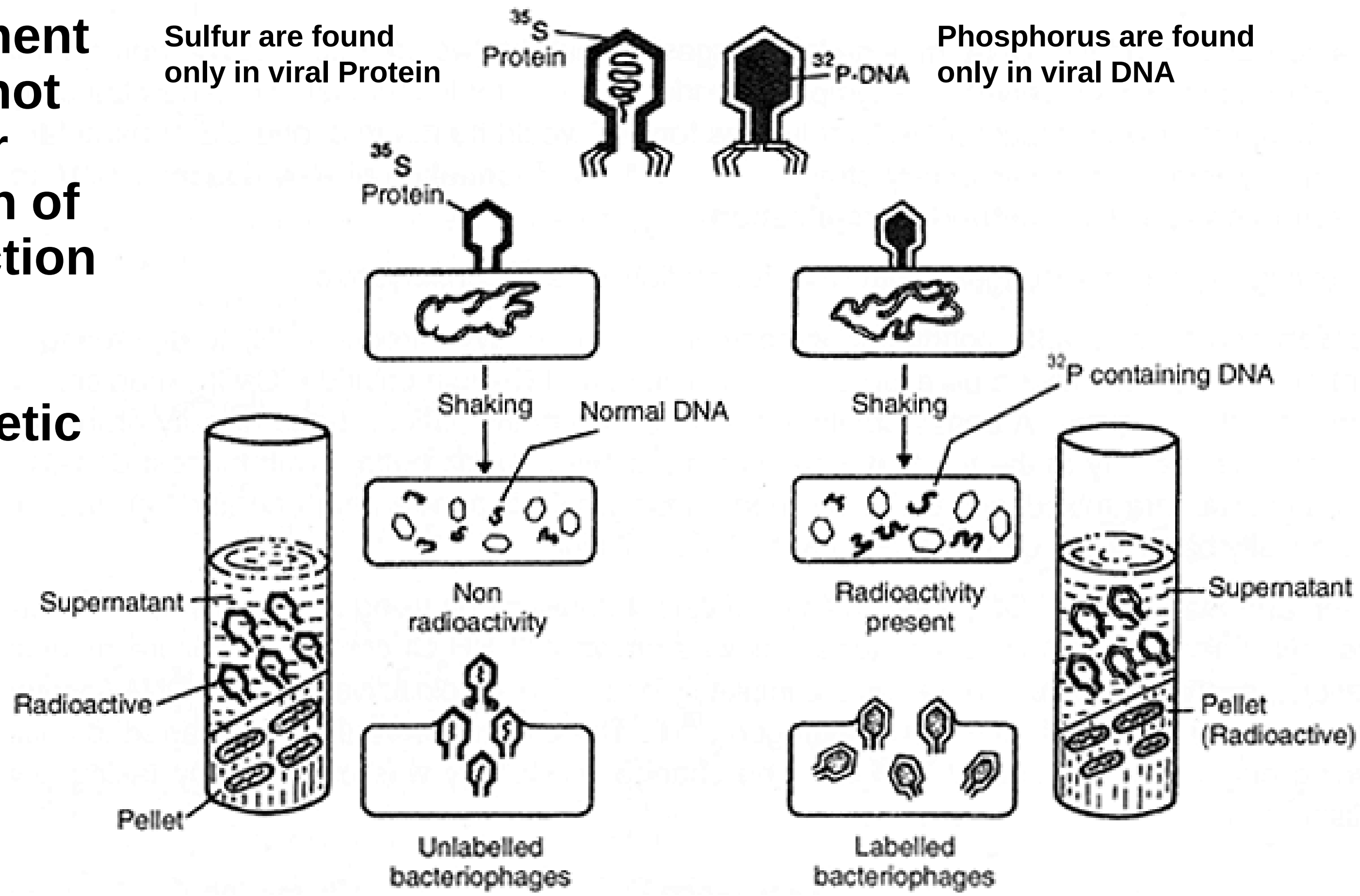


Figure 9-4 Essentials of Genetics, 6/e

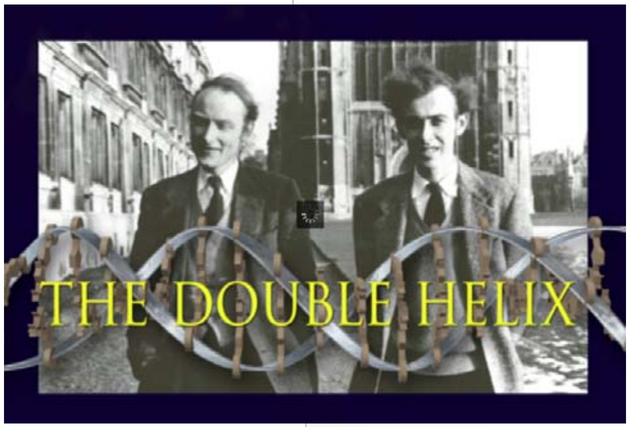
Hershey & Chase experiment demonstrated that DNA, not protein, is responsible for directing the reproduction of phage T2 during the infection of *E. coli*.

Therefore DNA is the genetic material.



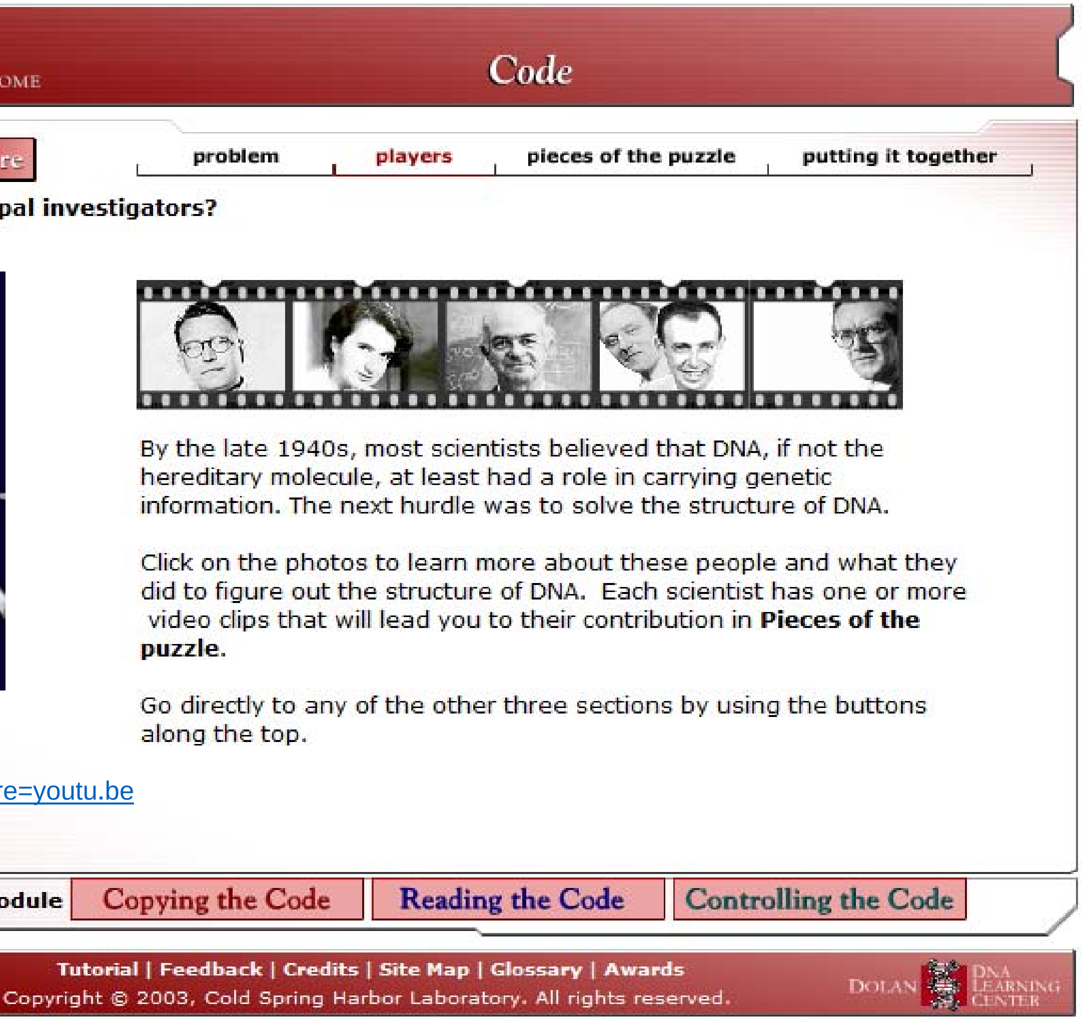
http://www.2classnotes.com/images/12/science/biology/botany/genetic_material/bacteriophages.gif

Discovery of the DNA Structure

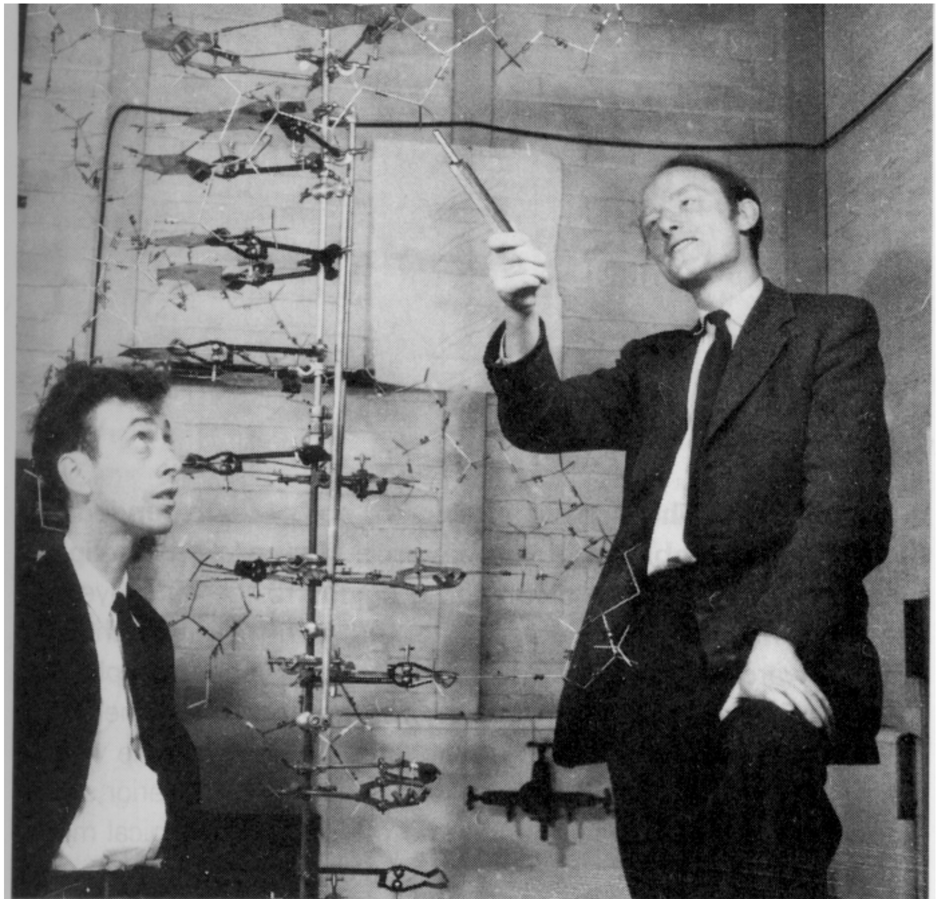


Watch "The Double Helix"

https://www.youtube.com/watch?v=1vm3od_UmFg&feature=youtu.be



DNA Interactive http://www.dnai.org/a/index.html



▲ FIGURE 1-4 James D. Watson *(left)* and Francis H. C. Crick *(right)* with the double-helical model of DNA they constructed in 1952–1953. Their model ultimately proved correct in all its essential aspects. [From J. D. Watson, 1968, *The Double Helix*, Atheneum, Copyright 1968, p. 215; Courtesy of A. C. Barrington Brown.]

1953

Watson & Crick's double helical DNA structure

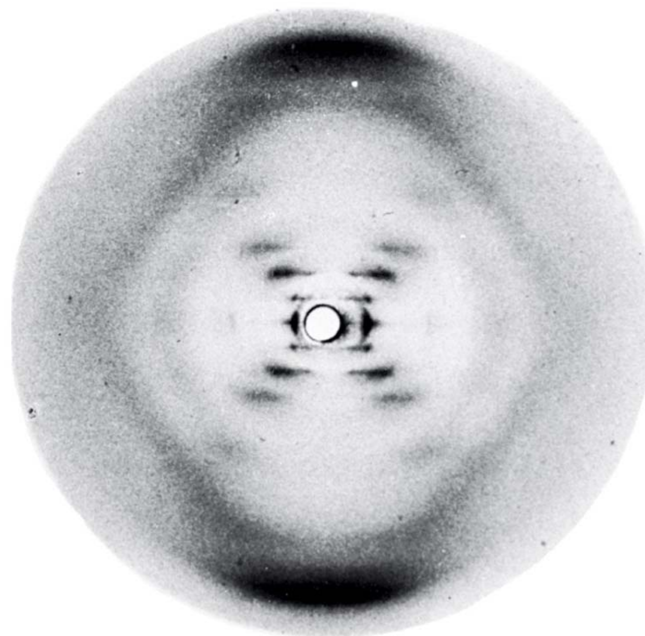
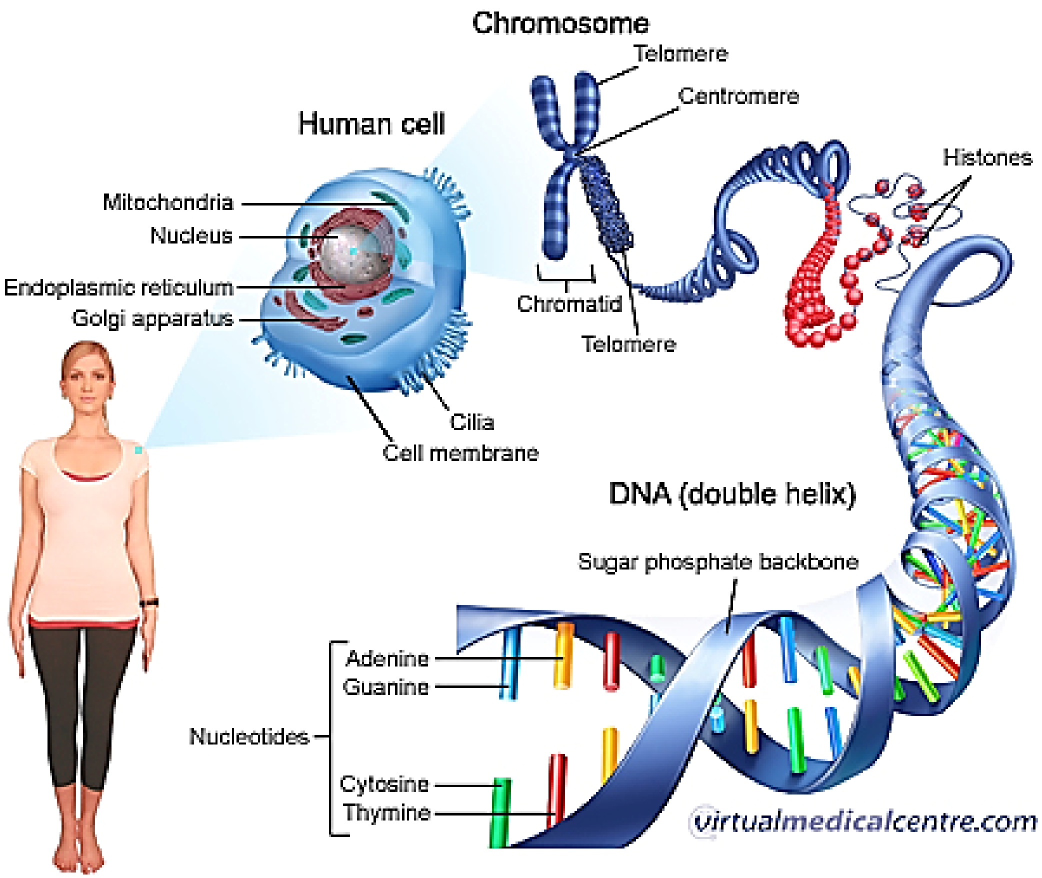
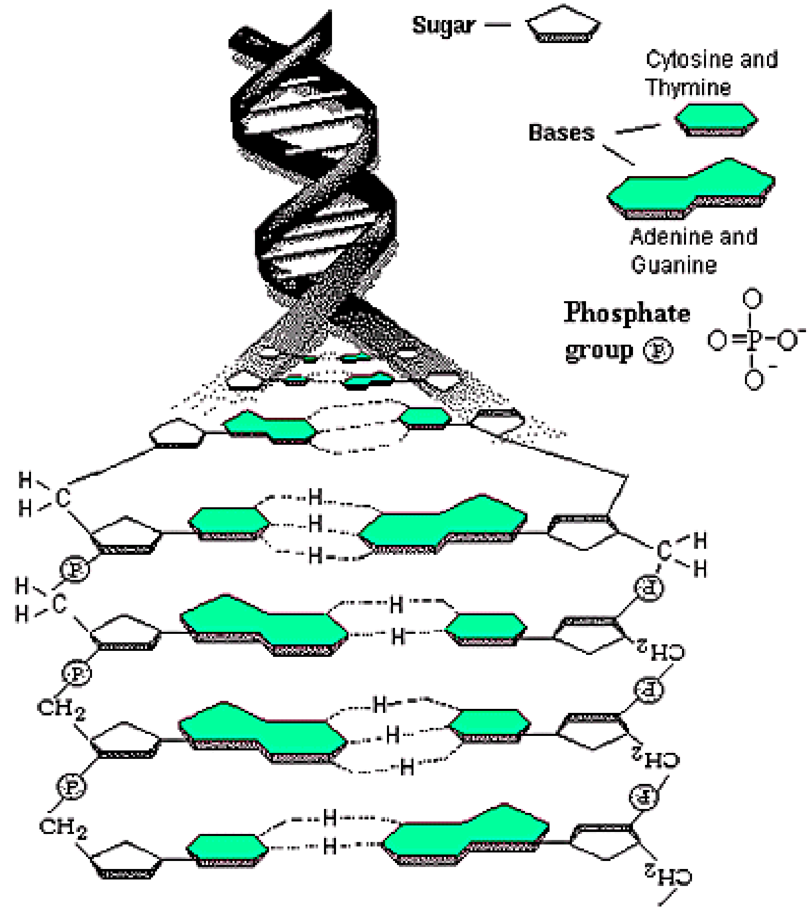


Figure 9-11 Essentials of Genetics, 6/e

Watch "DNA: Secret of Photo 51"
https://www.youtube.com/watch?v=0tmNf6ec2kU

Cell, Nucleus, Chromosome, DNA

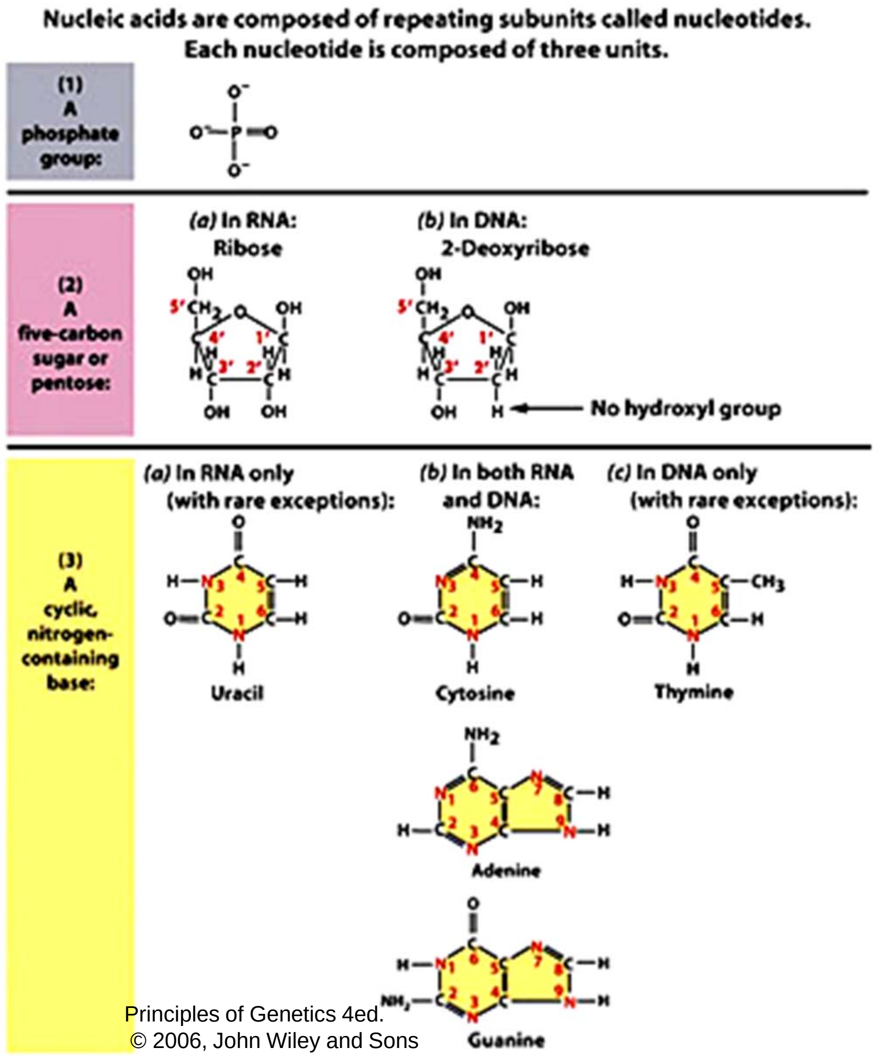


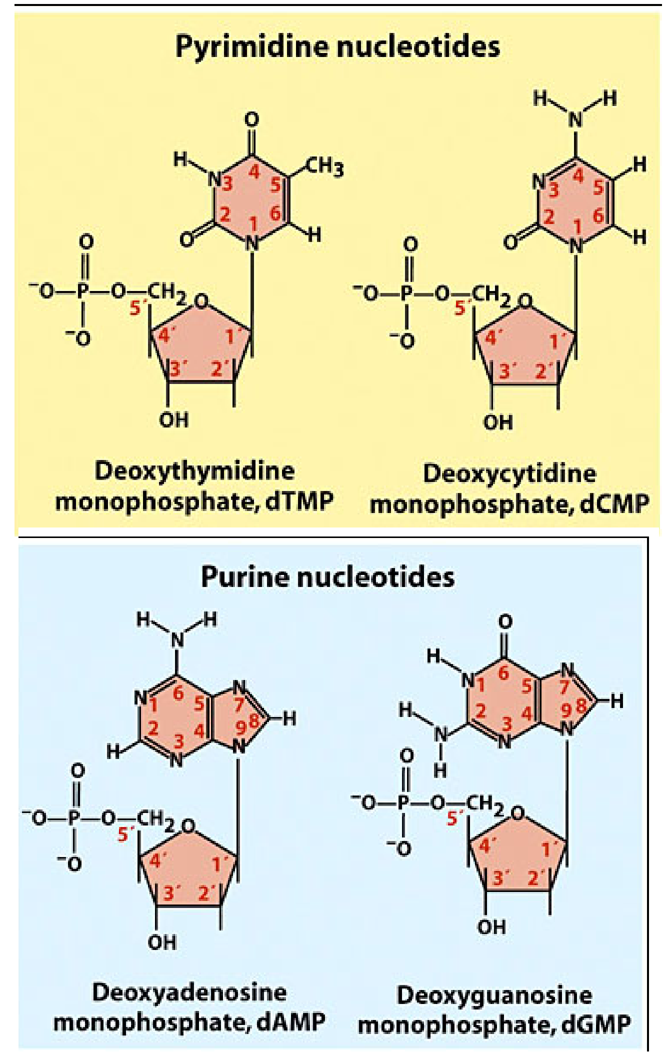


DNA is made up of two strands or chains which are bound to each other. The chains are made up of 4 types of nucleotides

[Check out Learn Genetics>Tour of the Basics>What is DNA?
http://learn.genetics.utah.edu/content/molecules/dna/

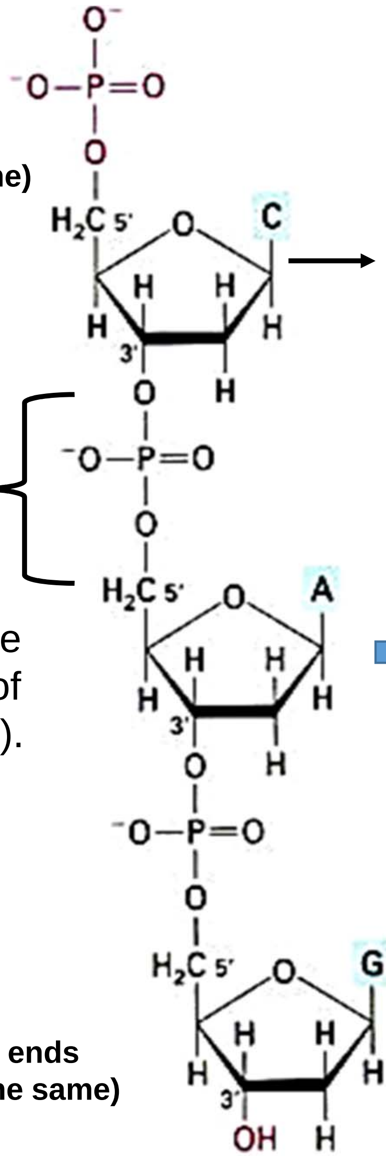
The Components that make Nucleotides





5′ end

(Note the ends
are not the same)

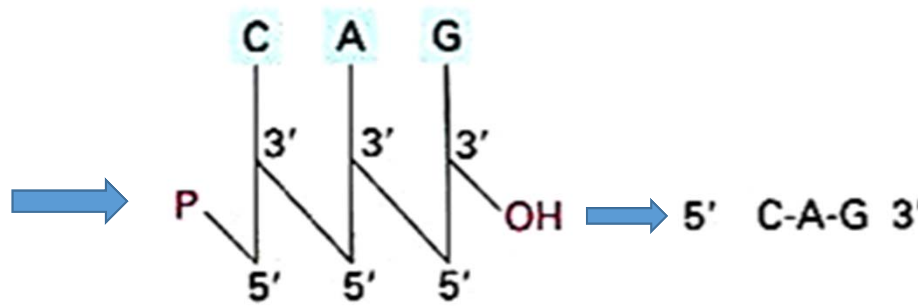


Glycosidic bond (links the base to the sugar)

3’ to 5’ phospho-diester bond

The individual nucleotides are linked through a bond between the sugar of one with the phosphate of the next (phospho-diester linkage).

The ‘backbone’ is made of sugar and phosphate.

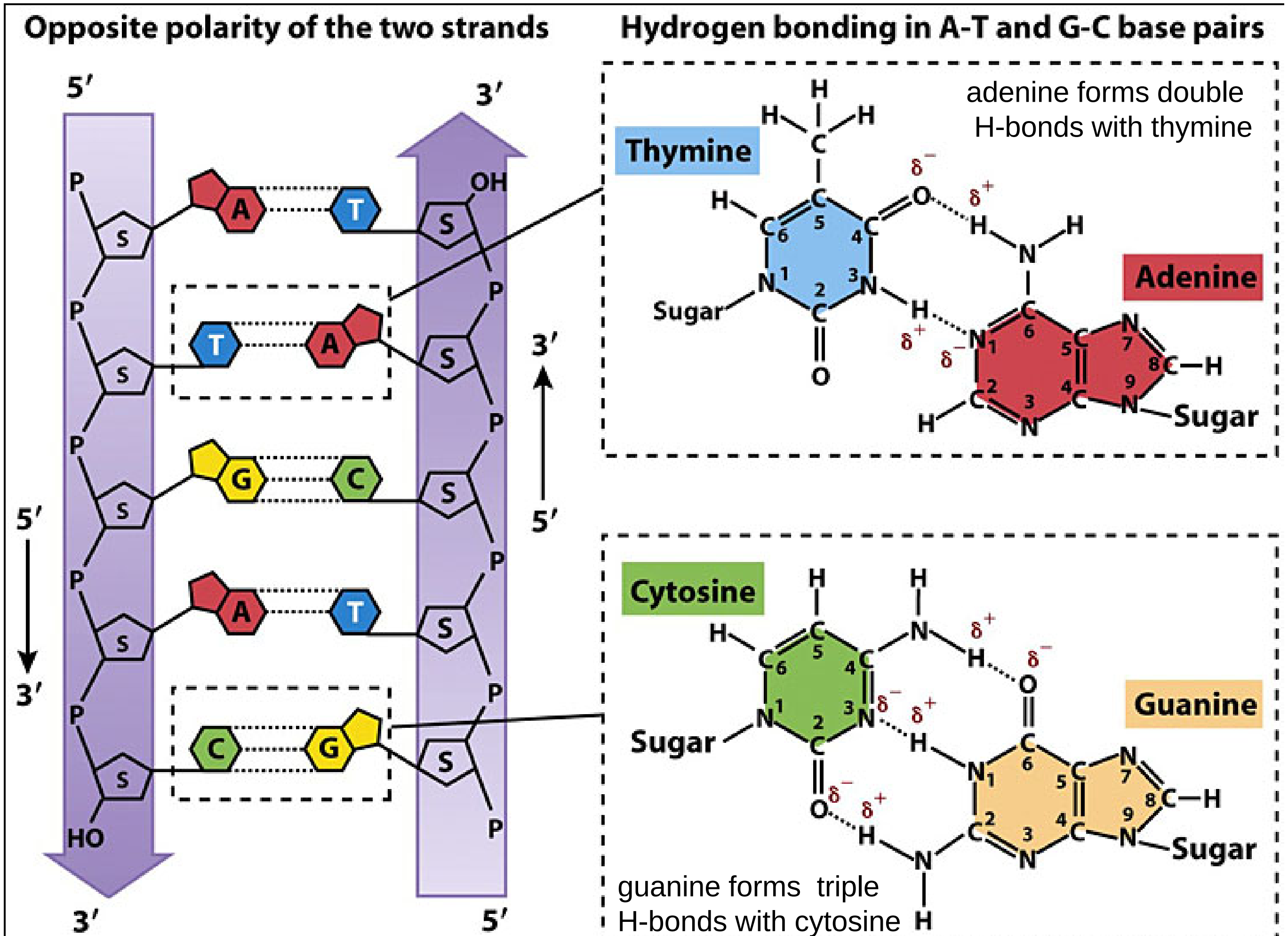


The DNA has a direction.
One end is a 5’ – P (phosphate group)
Another end 5’- OH (hydroxyl group)

(Note the ends
are not the same)

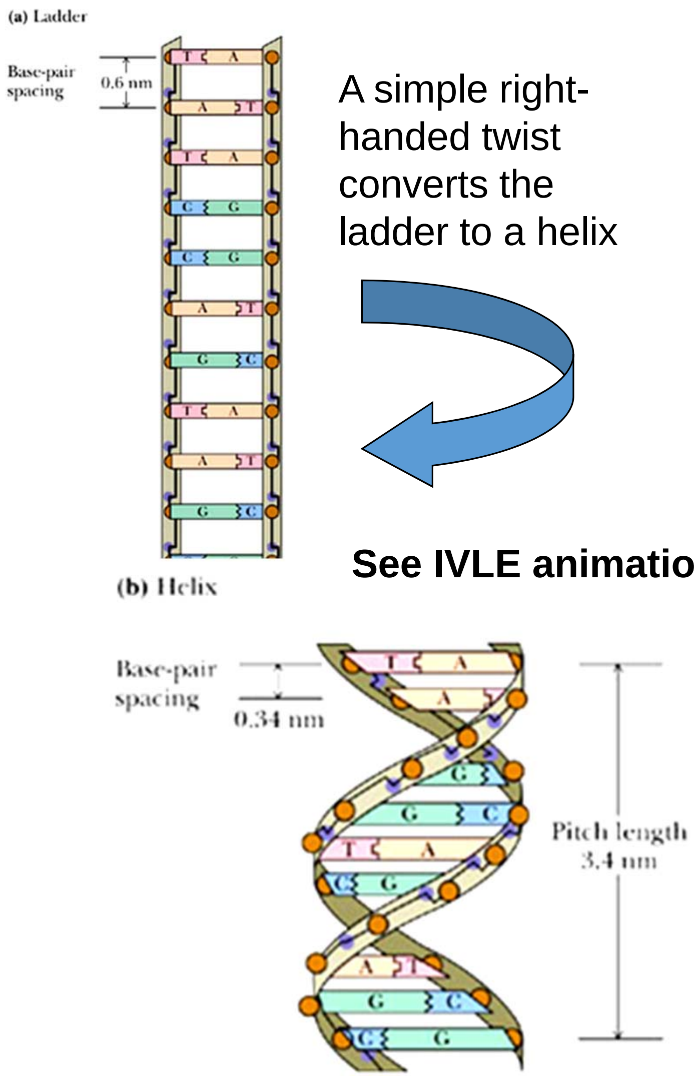
3′ end

DNA: Double Stranded & Anti-parallel



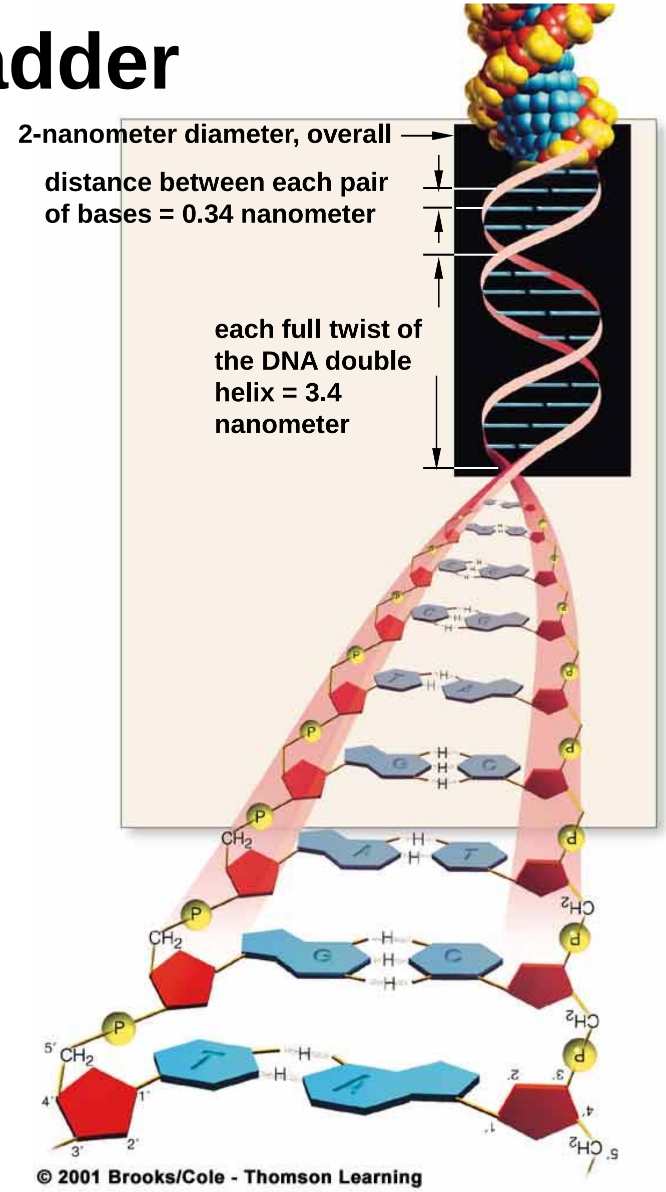
Principles of Genetics 4ed. © 2006, John Wiley and Sons

DNA : A Twisted Ladder

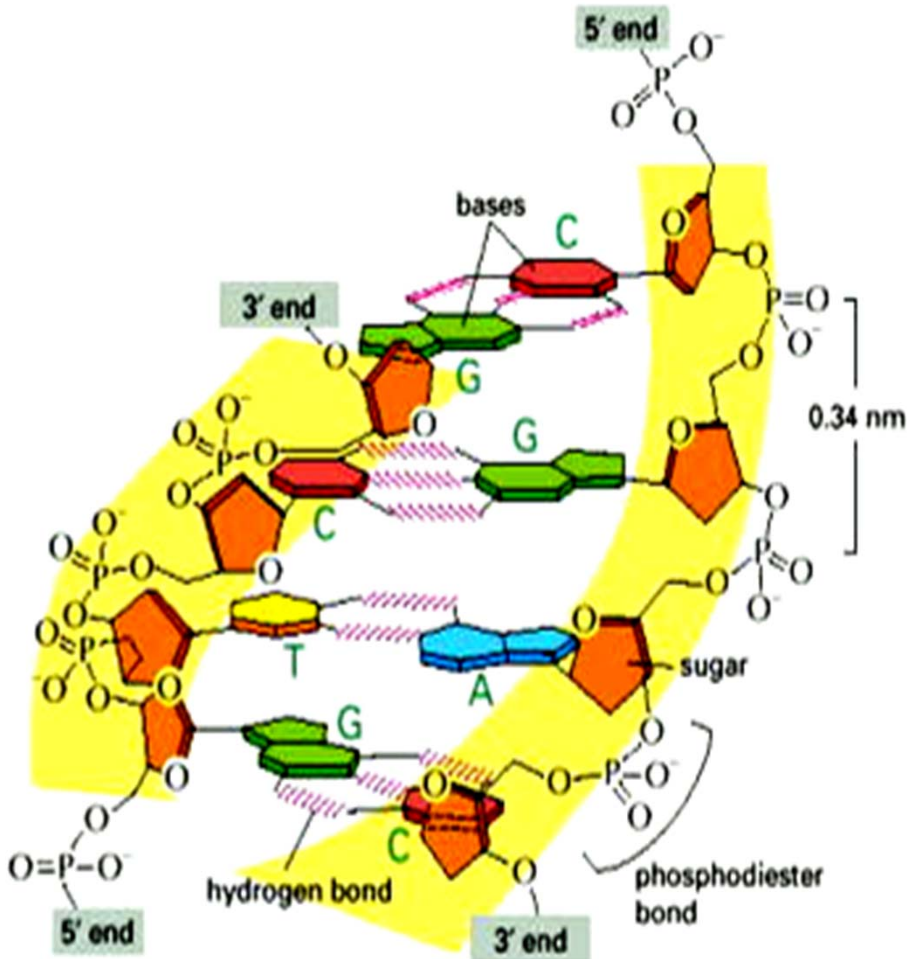


A simple right-handed twist converts the ladder to a helix

See IVLE animation



Forces Stabilizing the DNA Structure



- **Base Pairing**: hydrogen bonds (sensitive to high temperature, pH extremes and strong H-bonding solutes eg. urea and formamide)
- **Base Stacking**: Van Der Waals and hydrophobic interactions (sensitive to temperature and humidity)
- **Ionic Interactions** with cations suppress electrostatic repulsion between negatively charged phosphate groups hence stabilizing it (sensitive to ionic strength of solution e.g. mono- and divalent cations)

DNA has grooves large enough to accommodate parts of a protein. Regulatory proteins (transcription factors) can recognize the pattern of bases and H bonding possibilities in these grooves

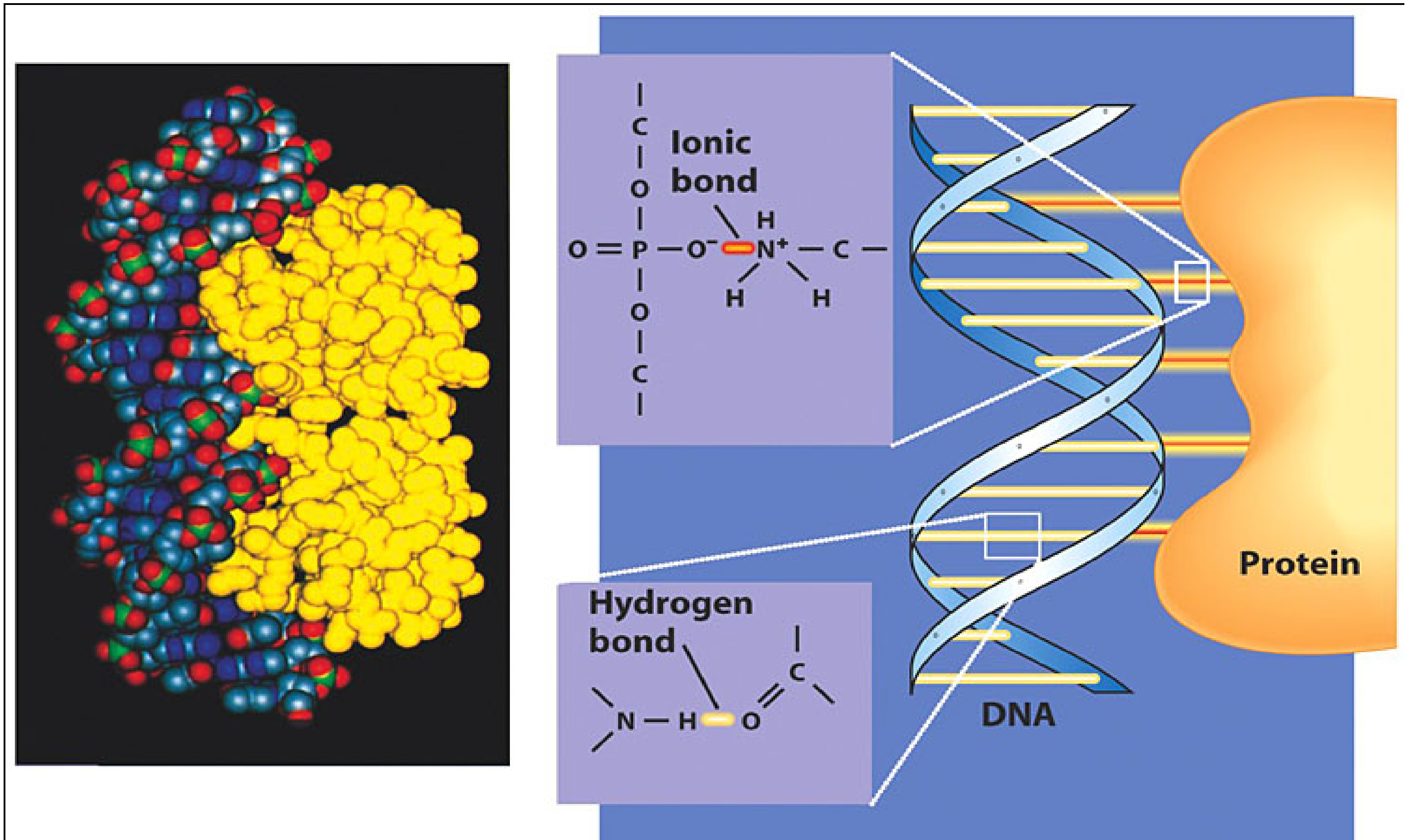
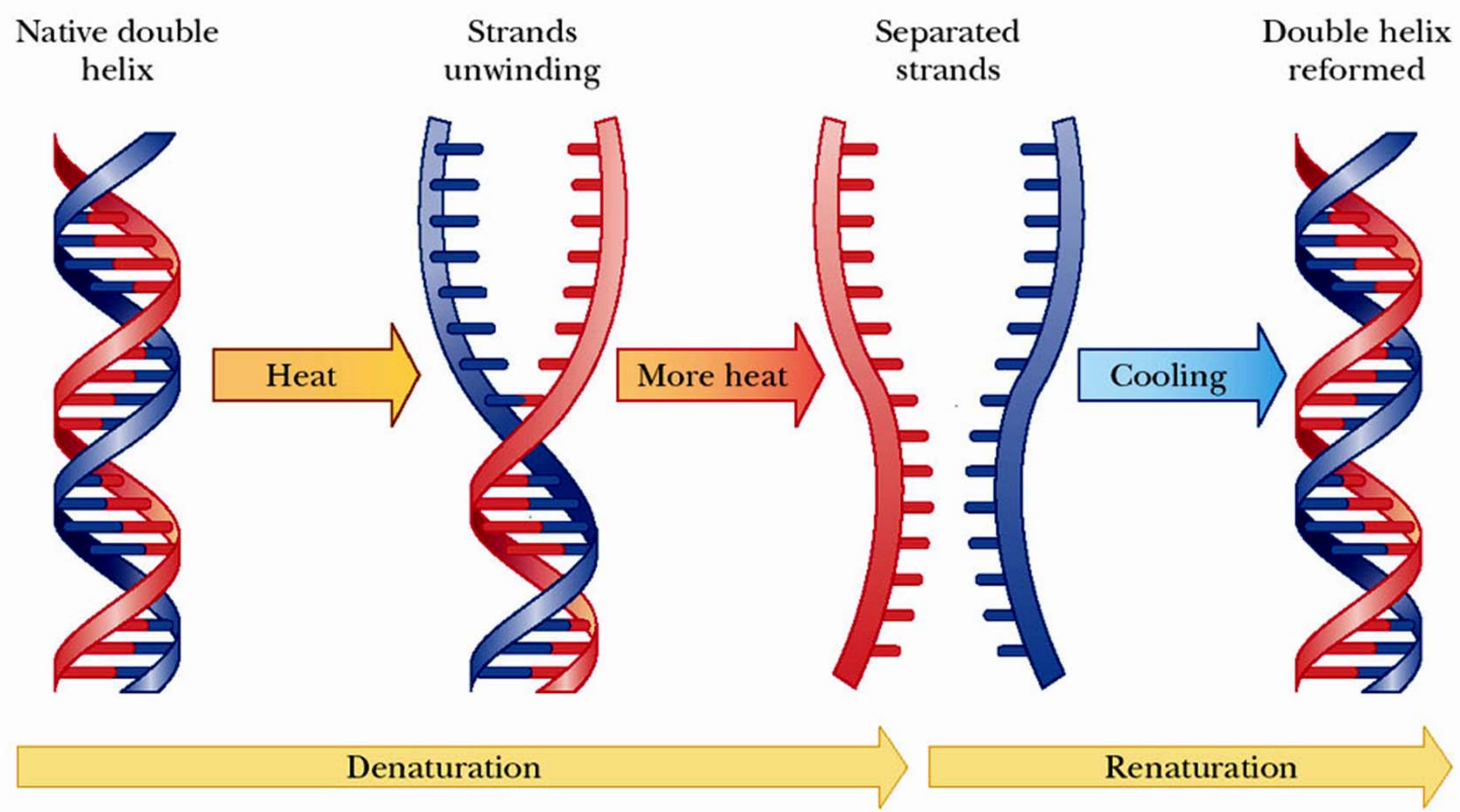


Figure 2-3 Cell and Molecular Biology, 4/e (© 2005 John Wiley & Sons)

Denaturation and Renaturation of DNA



Stability of DNA : Changed Yet Not Destroyed

LARGEST FOSSIL COCKROACH FOUND; SITE PRESERVES INCREDIBLE DETAIL

http://researchnews.osu.edu/archive/bigroach.htm

The Present

The Past

300 million years ago

The Future ?

http://pictxel.com/wall-e-wallpaper/

Climate Change & DNA

E-mail   Share   Comments (0)   Recommend (0)   Print

Ancient DNA Offers Clues About Climate Change

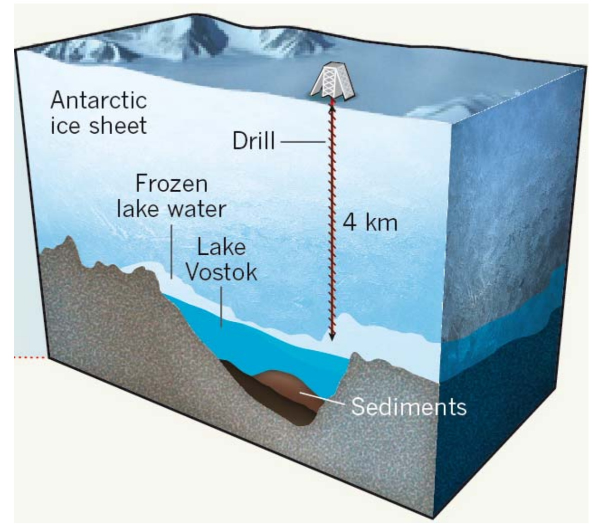
Listen
Talk of the Nation

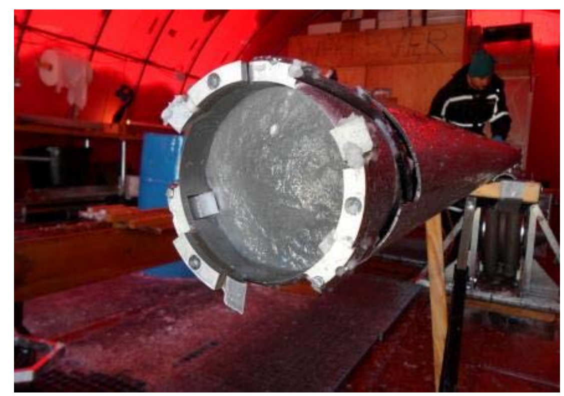
Add to Playlist
Download
Transcript

July 6, 2007   text size A A A

Researchers analyzing ice cores drilled from deep underground have found that Greenland may have once been quite green. The DNA samples included butterflies and spiders, and are thought to be the oldest ever recovered. They indicate that boreal forests once flourished in Greenland.

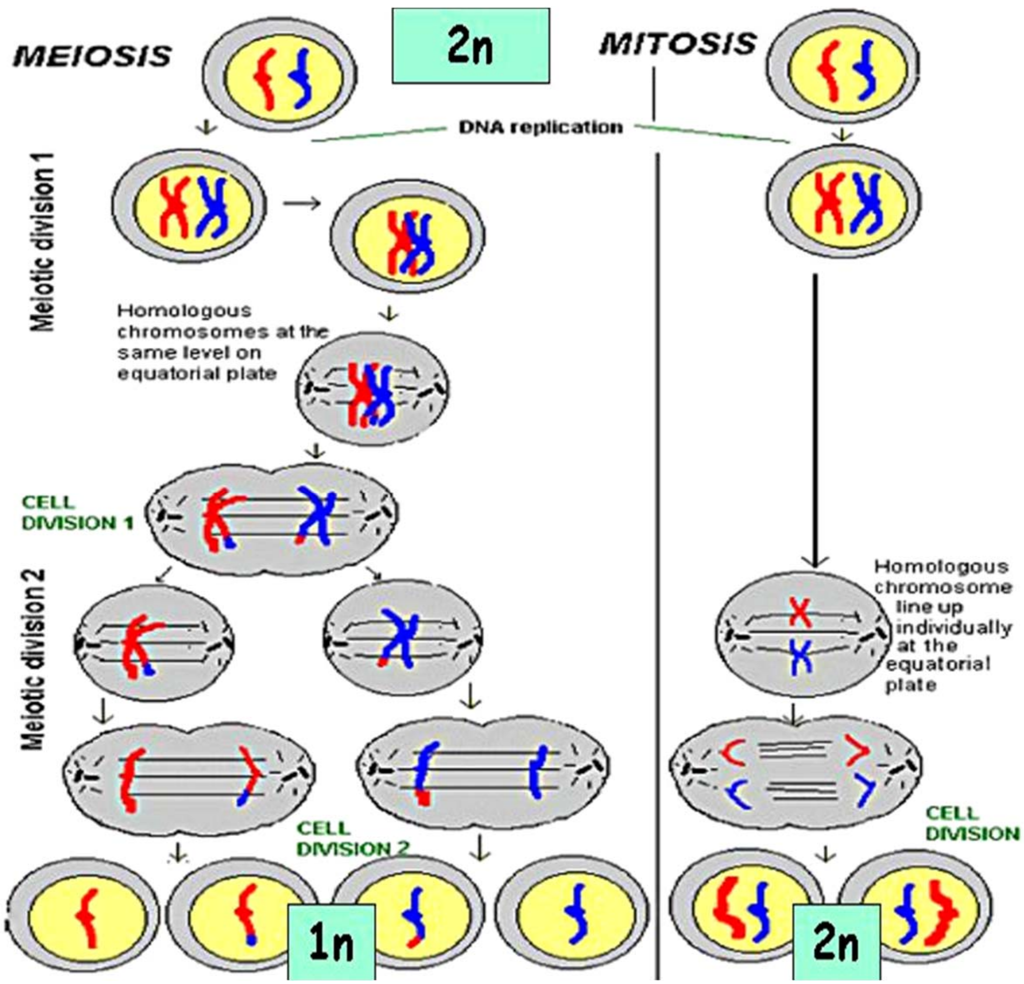
Eske Willerslev, professor, Evolutionary Biology Section Ancient DNA and Evolution Group Director, Center for Ancient Genetics, University of Copenhagen





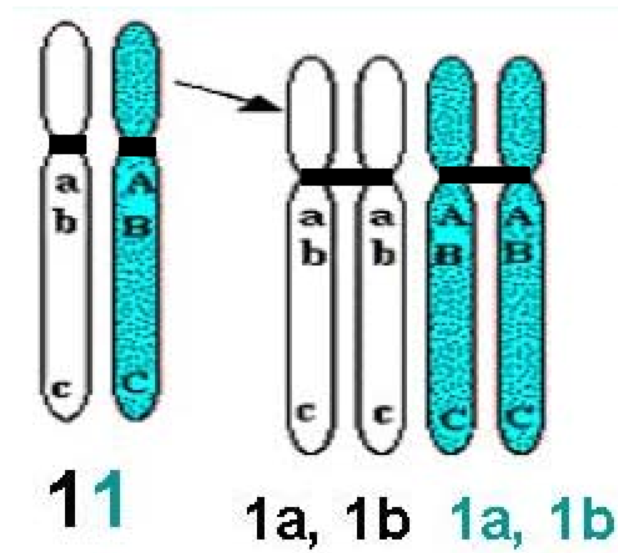
http://sciencenordic.com/dna-drilled-bottom-greenland%E2%80%99s-ice-sheet

You have learned about meiosis and mitosis



DNA are hereditary material involved in the storage and transmission of the genetic information.

Therefore before a cell divides, it has to replicate its DNA in high fidelity so that the genetic information is transmitted to the next generation of cells.

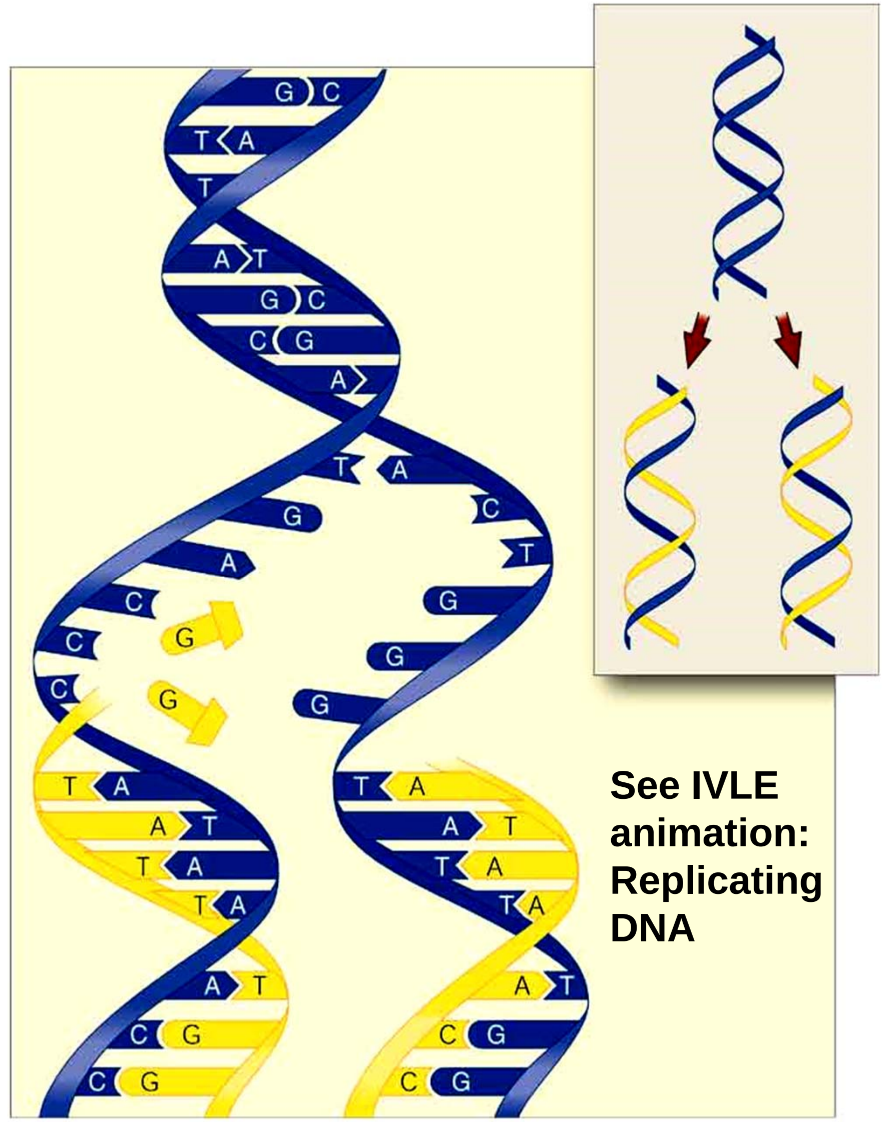


DNA Replication

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

- Concluding remark in the paper by Watson and Crick announcing discovery of the structure of DNA.

Semi-conservative Replication of DNA. Each parental strand acts as template for synthesizing new daughter strand.

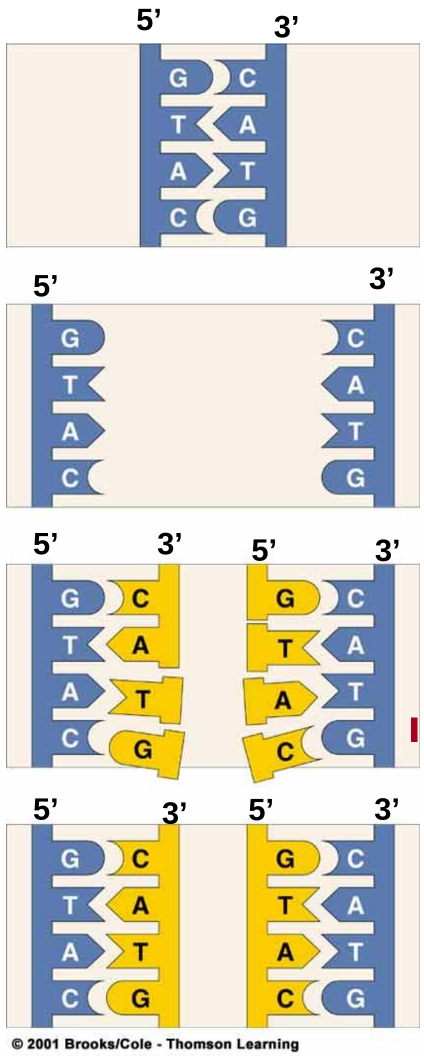


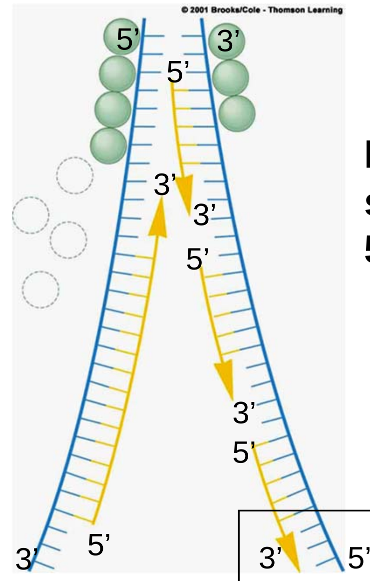
See IVLE animation: Replicating DNA

Semi-conservative Replication of DNA

1) Specific base-pairing between parental template strand and newly forming daughter strand in an antiparallel orientation.

2) DNA is synthesized from 5' to 3' direction

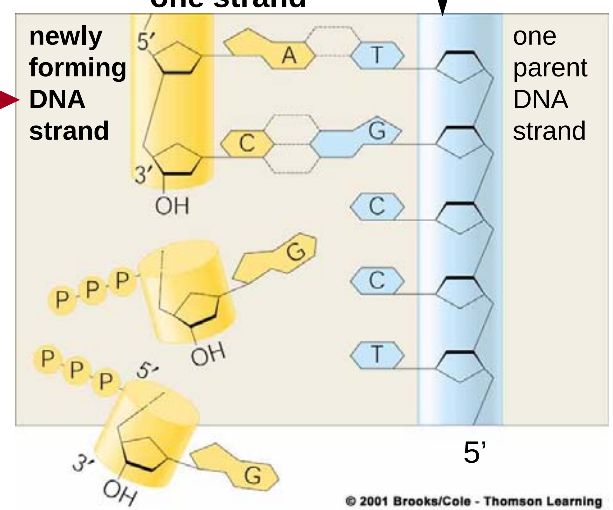




DNA must be synthesized from 5' to 3' direction

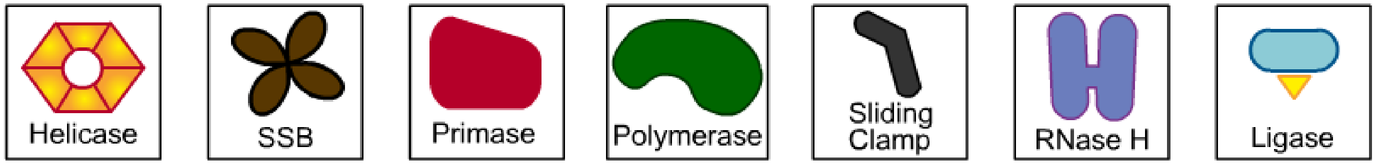
continuous assembly on one strand

discontinuous assembly on other strand



What are needed to replicate DNA in a cell?

http://www.wiley.com/college/pratt/0471393878/student/animations/dna_replication/index.html



1. Proteins involved in DNA replication -:

    (i) **Helicase** unwinds the DNA double helix into two individual strands.

    (ii) **Single-strand binding proteins, or SSBs** coat the single-stranded DNA to prevent the DNA strands from reannealing to form double-stranded DNA.

    (iii) **Primase** is an RNA polymerase that synthesizes the short RNA primers needed to start the DNA strand replication process.

    (iv) **DNA polymerase** is a hand-shaped enzyme that strings nucleotides together to form a DNA strand.

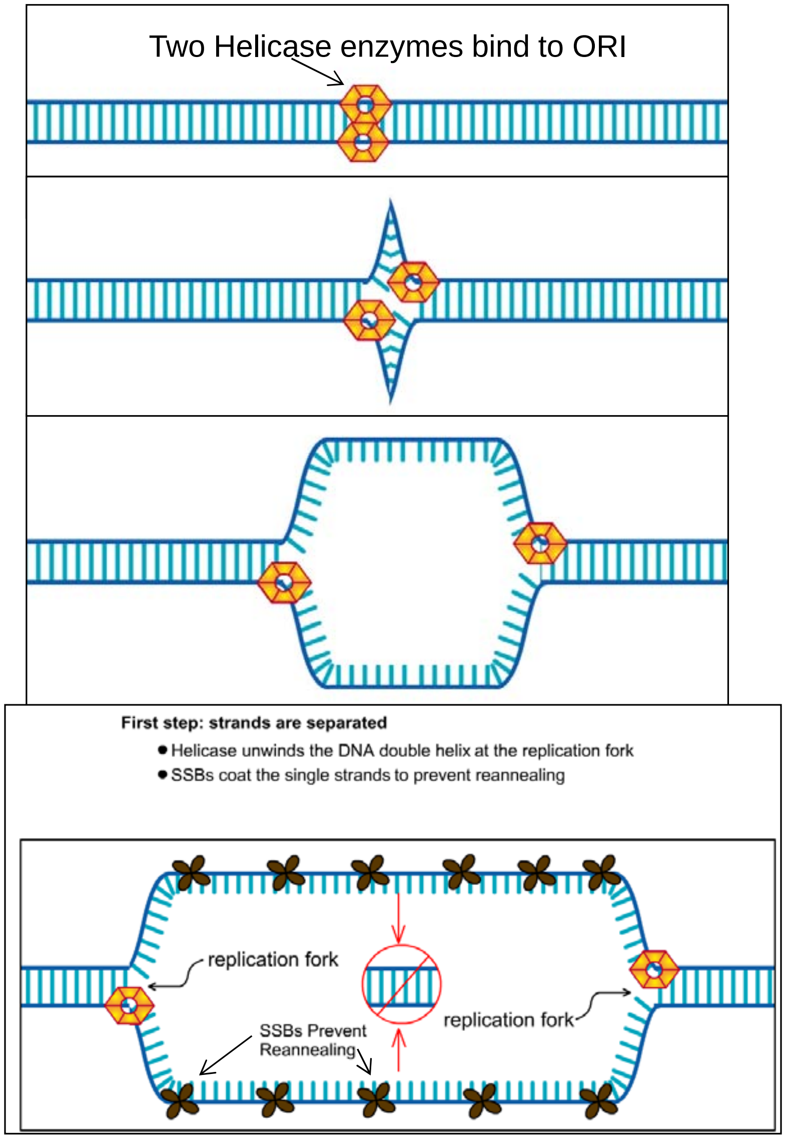
    (v) **Sliding clamp** is an accessory protein that helps hold the DNA polymerase onto the DNA strand during replication.

    (vi) **RNAse H** removes the RNA primers that previously began the DNA strand synthesis.

    (vii) **DNA ligase** links short stretches of DNA together to create one long continuous DNA strand.

2. Templates (for copying)
3. Nucleotides known as deoxynucleoside triphosphates (dNTPs) as building blocks for the newly synthesized DNA.

Initiation of DNA replication

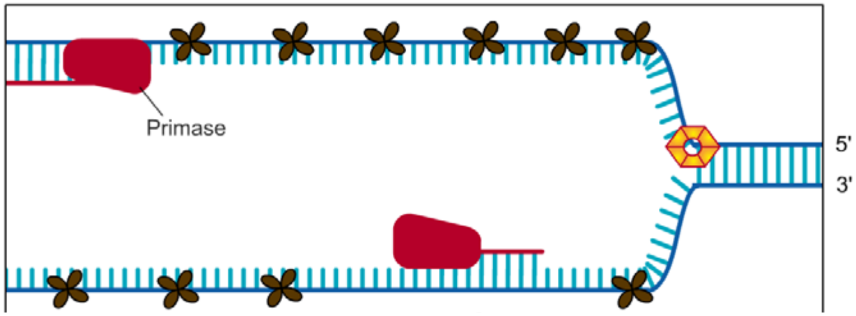


To begin the process of DNA replication, helicase enzymes are loaded onto the Origin Replication Initiation (ORI) site based on specific sequence recognition on the DNA by certain DNA replication initiation proteins (not shown)

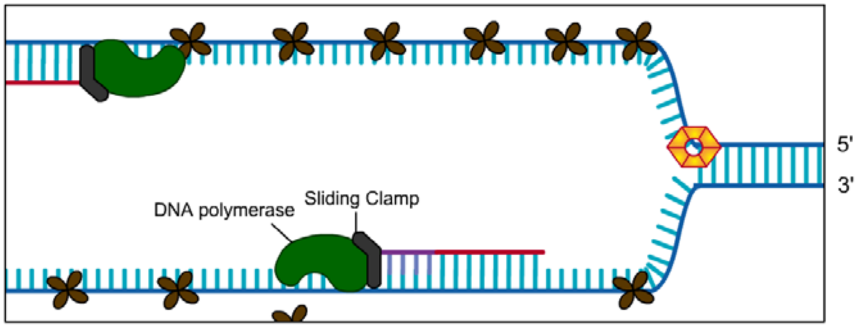
The two double helix strands are unwound and separated from each other by the helicase enzyme.

The point where the DNA is separated into single strands, and where new DNA will be synthesized, is known as the replication fork. Single-strand binding proteins, or SSBs, quickly coat the newly exposed single strands. SSBs maintain the separated strands during DNA replication. Without the SSBs, the complementary DNA strands could easily snap back together.

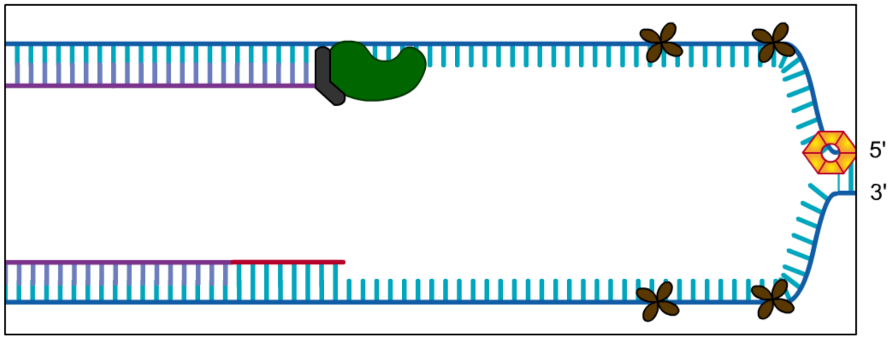
DNA Polymerase needs a Primer to initiate synthesis



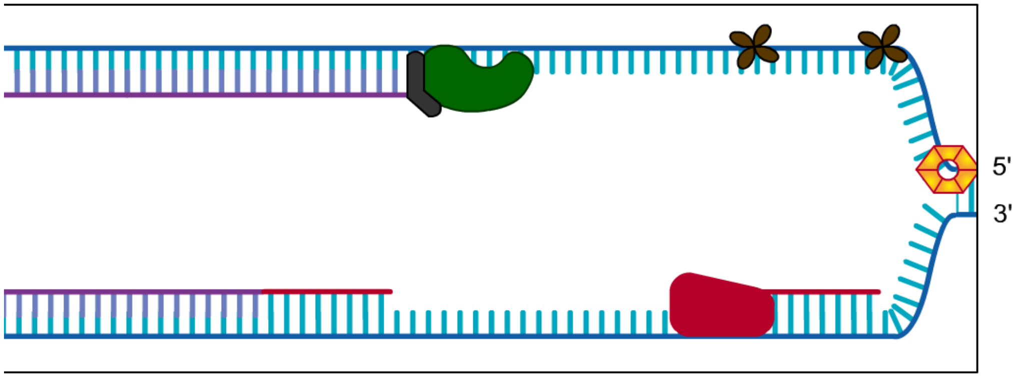
DNA polymerase can only extend a nucleic acid chain but cannot start one from scratch. To give the DNA polymerase a place to start, an RNA polymerase called primase first copies a complementary RNA segment called a primer based on a short stretch of the DNA strand template.

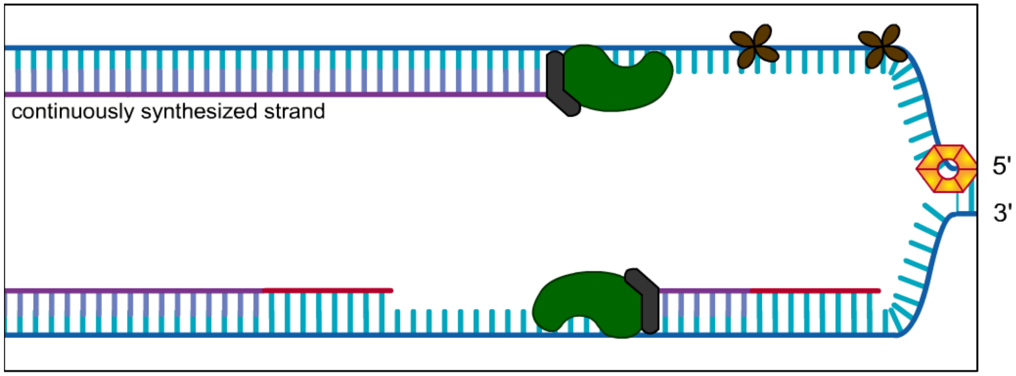


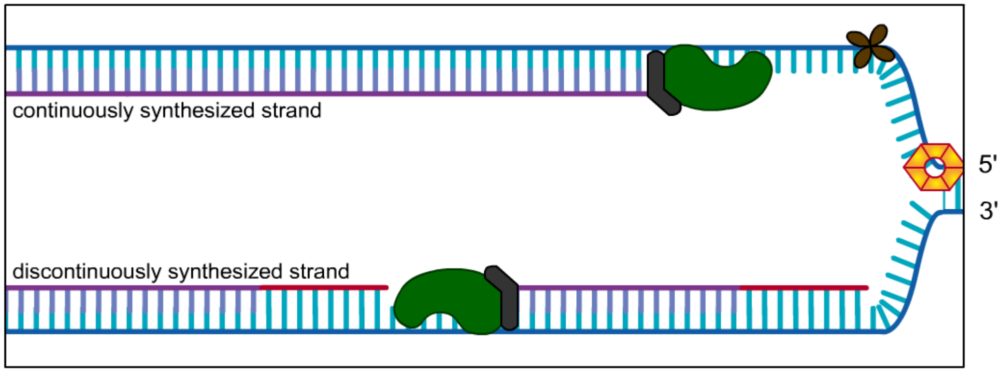
The DNA polymerase starts at the 3’ end of the RNA primer, and, using the original DNA strand as a template, begins to synthesize a new complementary DNA strand.
Two DNA polymerases are required, one for each parental DNA strand. Due to the antiparallel nature of the DNA strands, however, the DNA polymerases on the two strands start to move in opposite directions.



SSBs bind loosely to the DNA, and are displaced when the polymerase enzymes begin synthesizing the new DNA strands.





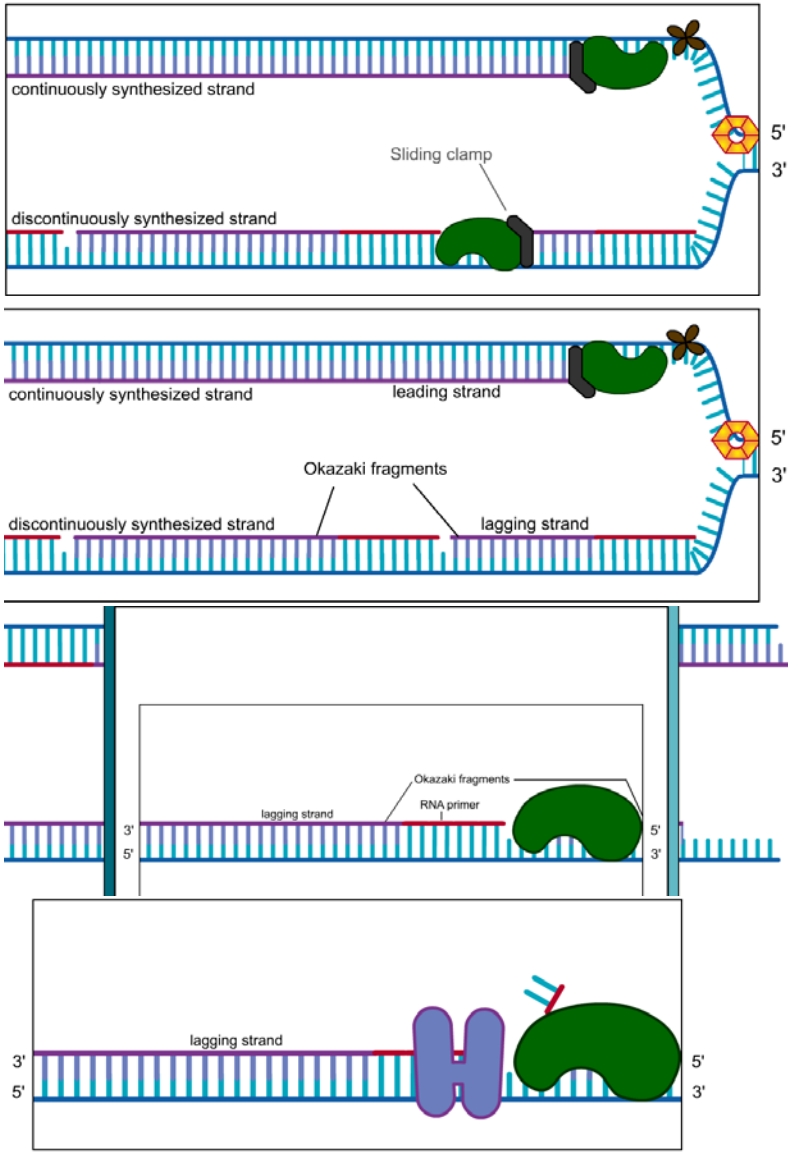


DNA replication is continuous for one strand and discontinuous for another strand.

Both template strands move through the replication factory in the same direction, and DNA polymerase can only synthesize DNA from the 5' end to the 3' end.

Due to these two factors, one polymerase can remain on its DNA template and copy the DNA in one continuous strand while the other complementary DNA strand must be made discontinuously in short pieces which are later joined together.

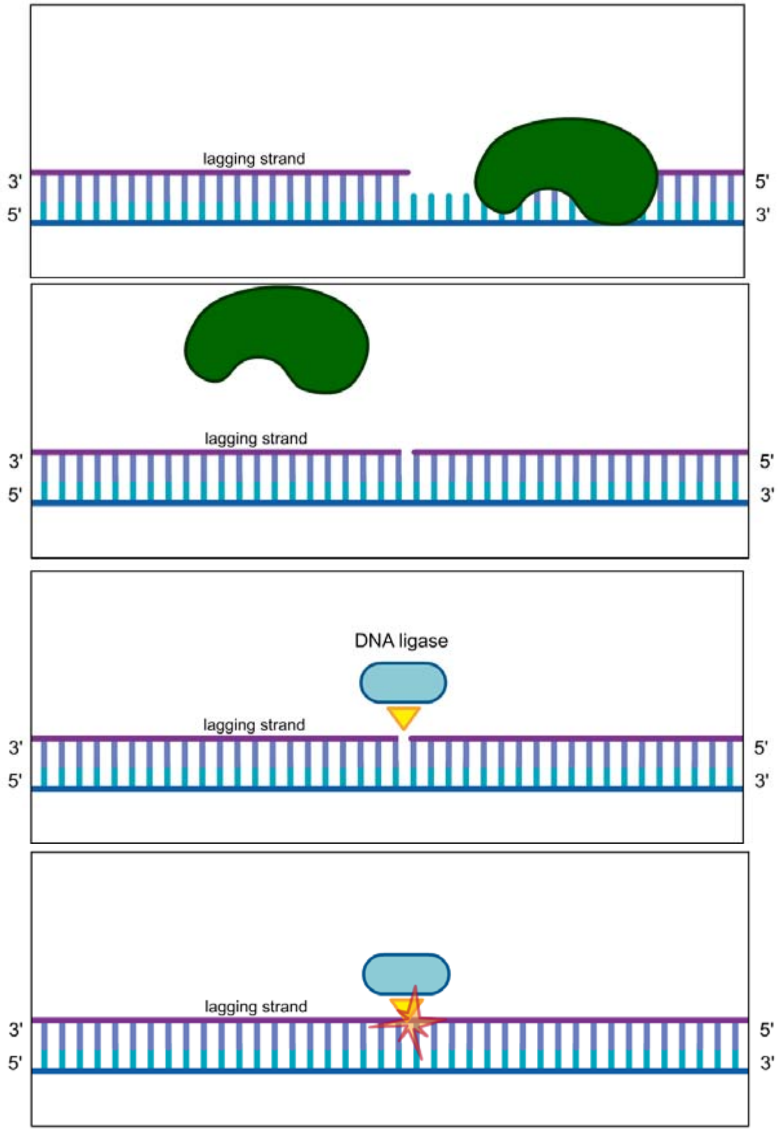
Leading strand, Lagging strand, Okazaki fragments & removal of primers



The continuously synthesized strand is known as the leading strand, while the strand that is synthesized in short pieces is known as the lagging strand. The short stretches of DNA that make up the lagging strand are known as Okazaki fragments.

Before the lagging-strand DNA exits the replication factory, its RNA primers must be removed and the Okazaki fragments must be joined together to create a continuous DNA strand.

The first step is the removal of the RNA primer. RNAse H, which recognizes RNA-DNA hybrid helices, degrades the RNA by hydrolyzing its phosphodiester bonds.

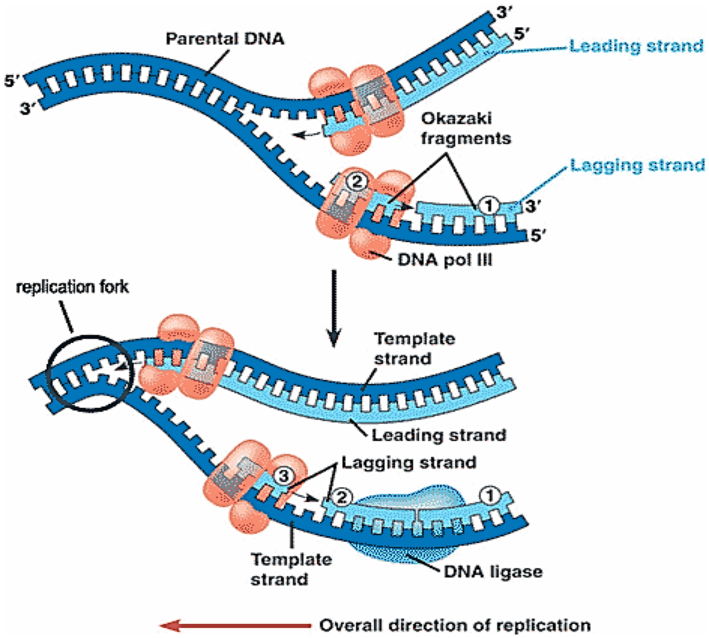


The replacement of RNA primer with DNA by DNA polymerase and joining of Okazaki fragments by ligase transform the fragmented lagging strand to a continuous strand.

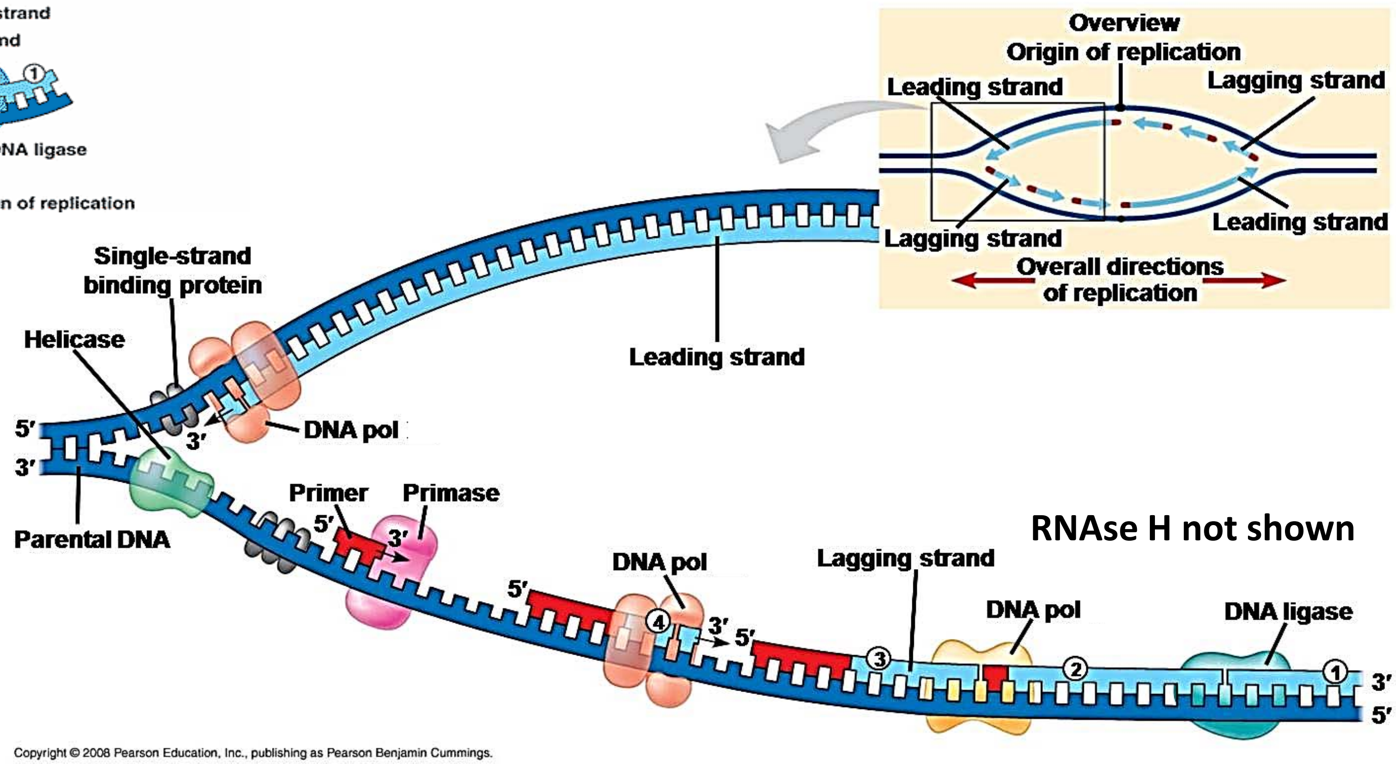
Next, the sequence gap created by RNAse H is then filled in by DNA polymerase which extends the 3' end of the neighboring Okazaki fragment.

Finally, the Okazaki fragments are joined together by DNA ligase that hooks together the 3' end of one fragment to the 5' phosphate group of the neighboring fragment in an ATP- dependent reaction.

DNA Replication Overview: Putting All Together

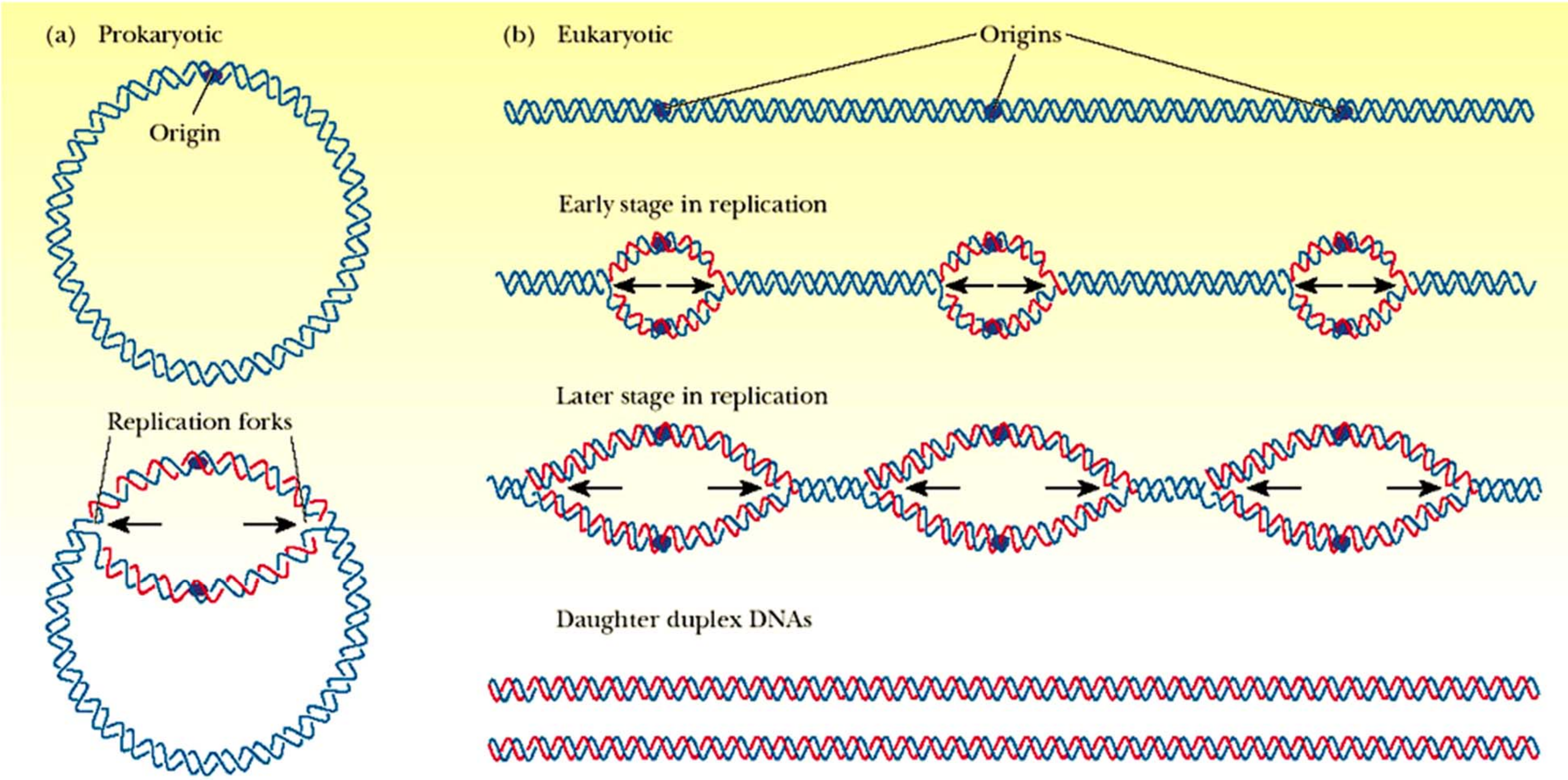


http://www.quia.com/jg/1266941list.html



RNAse H not shown

At the origin of replication site (multiple origins for eukaryotes), bidirectional replication involves two replication forks, which move in opposite directions.



Summary of DNA Structure & Properties

• Double-stranded helix. Anti-parallel (opposite directionality 5’ to 3 between the two strands).

• The Nucleotide constituents: sugar, phosphate, “bases” (nucleotides).

• Phosphodiester bond between adjacent nucleotides (form the sugar-phosphate backbone).

• Glycosidic bond between sugar and base.

• Hydrogen bonds between “bases” following complementary base pairing between A&T and C&G.

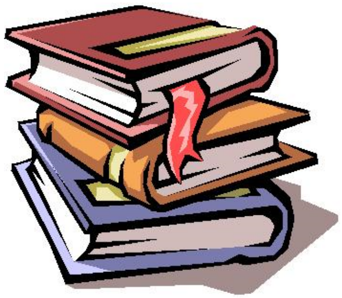
• DNA replication is semiconservative. Each parental strand is used as a template to produce a daughter strand.

• DNA synthesis require a DNA template, primers, free nucleotides, and appropriate proteins (e.g. helicase, SSBs, primase, Sliding clamp, DNA polymerase, RNAse H, ligase)

• The direction of DNA synthesis is from 5’ to 3’ of the newly forming strand.

• One strand is formed continuously (leading strand) while the other strand is formed discontinuously (lagging strand). On the lagging strand, small DNA fragments (Okazaki fragments) are subsequently linked by ligase.

Additional Enrichment Materials



- **DNA Art Forms (In additional reading material workbin on IVLE)**
- **IVLE Animations: DNA Close-up; DNA structure; Replicating DNA; Replication Video, DNA polymerase**
- **Useful Weblinks:**
- **Short film on "The Double Helix":**
- https://www.youtube.com/watch?v=1vm3od_UmFg&feature=youtu.be
- **Short film on "DNA: Secret of Photo 51":**
- **https://www.youtube.com/watch?v=0tmNf6ec2kU**
- **DNA interactive for Discovery of DNA structure:**
- **http://www.dnai.org/a/index.html**
- **DNA Learn Genetics: Tour of the basic on DNA and other related basic concepts:**
- **http://learn.genetics.utah.edu/content/basics/**
- **http://learn.genetics.utah.edu/content/molecules/dna/**
- **DNA replication:**

http://www.wiley.com/college/pratt/0471393878/student/animations/dna_replication/index.html