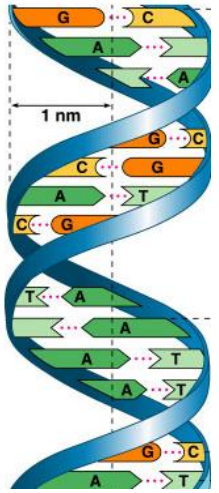
Wednesday, April 9th



DNA
The Genetic Material
Replication

Chapter 16

Modified from Kim Foglia

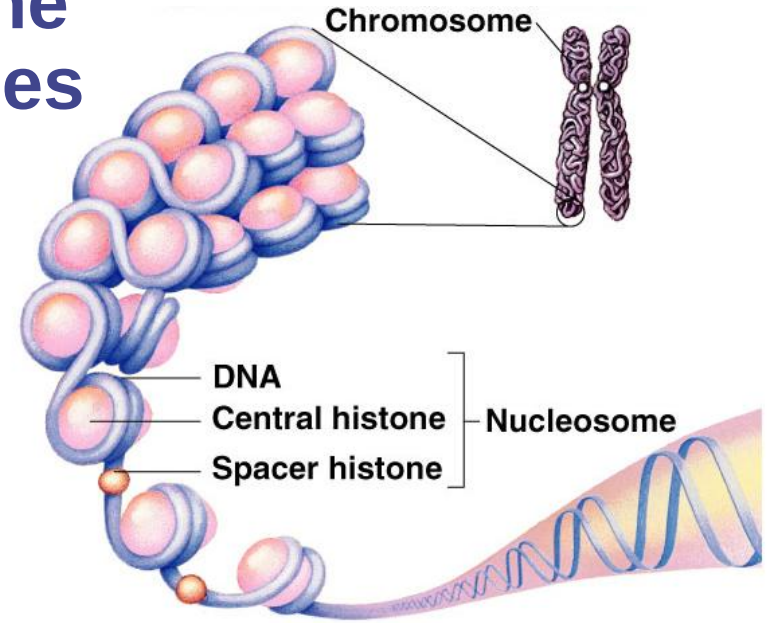
Scientific History

- The march to understanding that DNA is the genetic material
  - T.H. Morgan (1908)
  - Frederick Griffith (1928)
  - Avery, McCarty & MacLeod (1944)
  - Hershey & Chase (1952)
  - Watson & Crick (1953)
  - Meselson & Stahl (1958)

Genes are on chromosomes

1908 | 1933

- **T.H. Morgan**
  - working with *Drosophila* (fruit flies)
  - genes are on chromosomes
  - but is it the **protein** or the **DNA** of the chromosomes that are the genes?
    - through 1940 proteins were thought to be genetic material… Why?



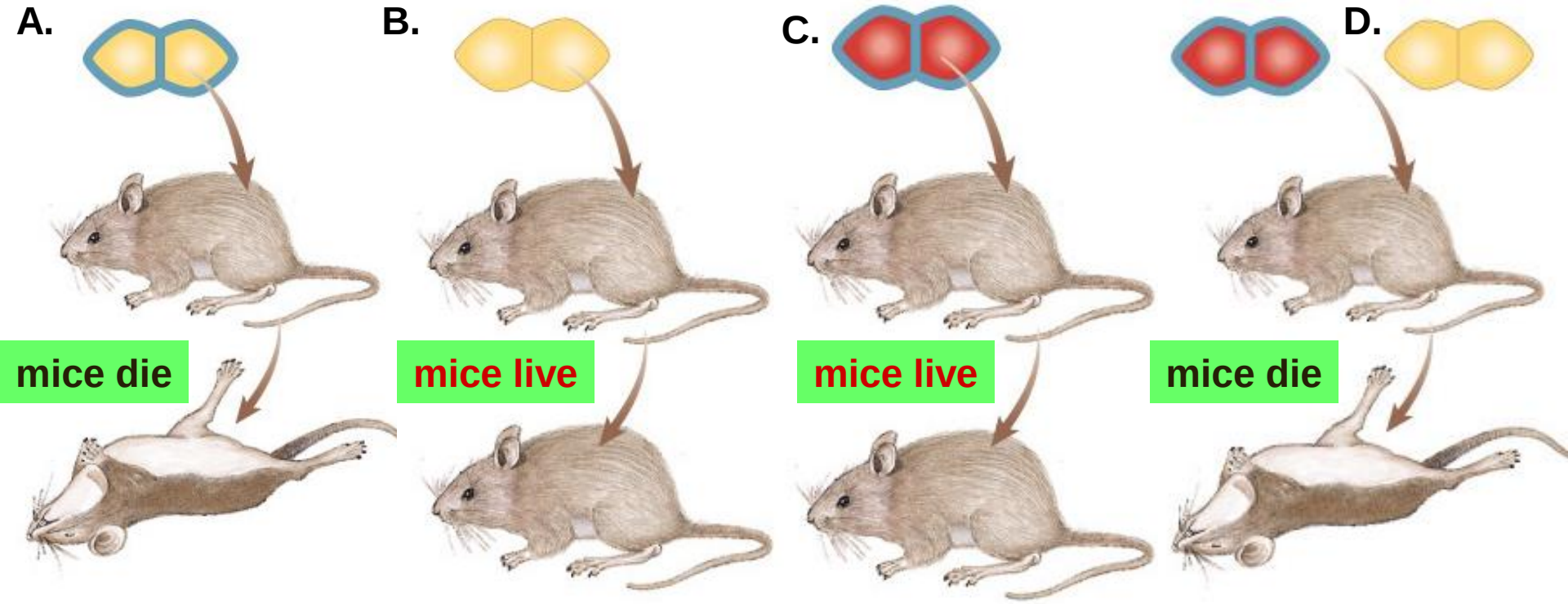
The "Transforming Factor"

1928

- **Frederick Griffith**
  - ***Streptococcus* pneumonia bacteria**
    - **was working to find cure for pneumonia**
  - **harmless live bacteria mixed with heat-killed infectious bacteria causes disease in mice**
  - **substance passed from dead bacteria to live bacteria = "Transforming Factor"**

The "Transforming Factor"

A.	B.	C.	D.
live pathogenic strain of *bacteria*	live non-pathogenic strain of *bacteria*	heat-killed pathogenic *bacteria*	mix heat-killed pathogenic & non-pathogenic *bacteria*
mice die	mice live	mice live	mice die



Transformation?

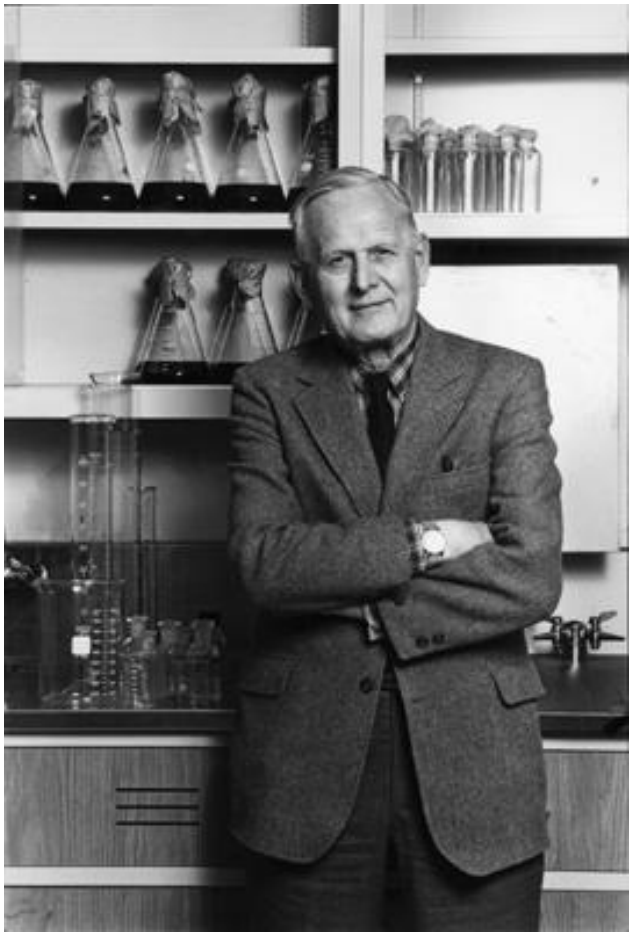
something in heat-killed bacteria could still transmit disease-causing properties

DNA is the "Transforming Factor" 1944

- Avery, McCarty & MacLeod
  - purified both DNA & proteins from *Streptococcus* pneumonia bacteria
    - which will transform non-pathogenic bacteria?
  - injected protein into bacteria
    - no effect
  - injected DNA into bacteria
    - transformed harmless bacteria into virulent bacteria

Avery, McCarty & MacLeod

Oswald Avery



Maclyn McCarty

Colin MacLeod

Hershey and Chase

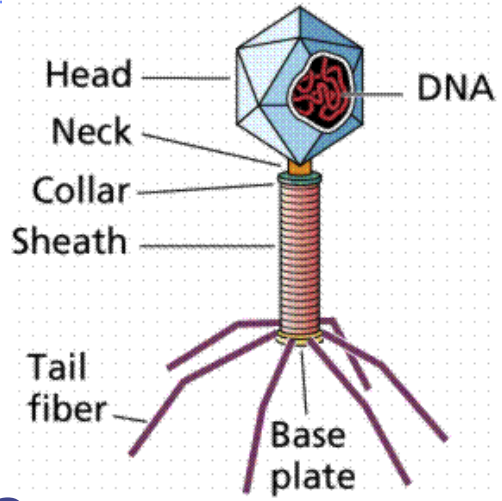
Confirmation of DNA: *animation*

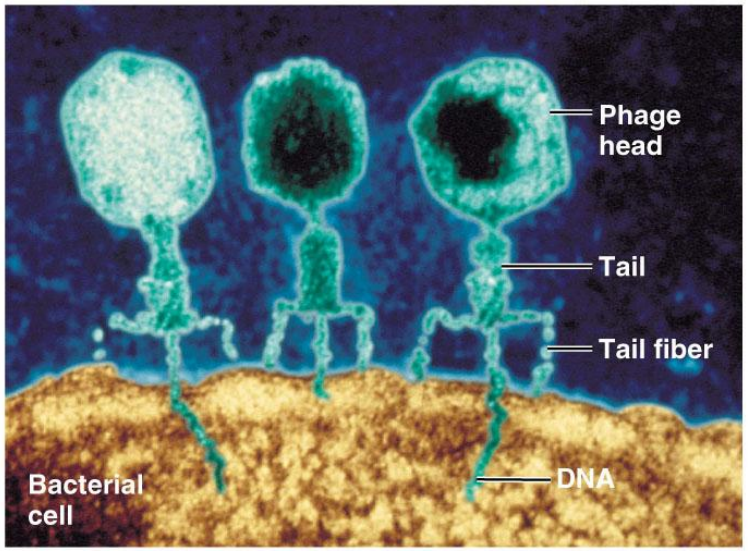
Hershey and Chase experiment

Confirmation of DNA

1952 | 1969

- Hershey & Chase
  - classic "blender" experiment
  - worked with **bacteriophage**
    - viruses that infect bacteria
  - grew phage viruses in 2 media, radioactively labeled with either
    - ^{35}S in their proteins
    - ^{32}P in their DNA
  - infected bacteria with labeled phages





Hershey & Chase

Martha Chase Alfred Hershey

Thursday, April 10th

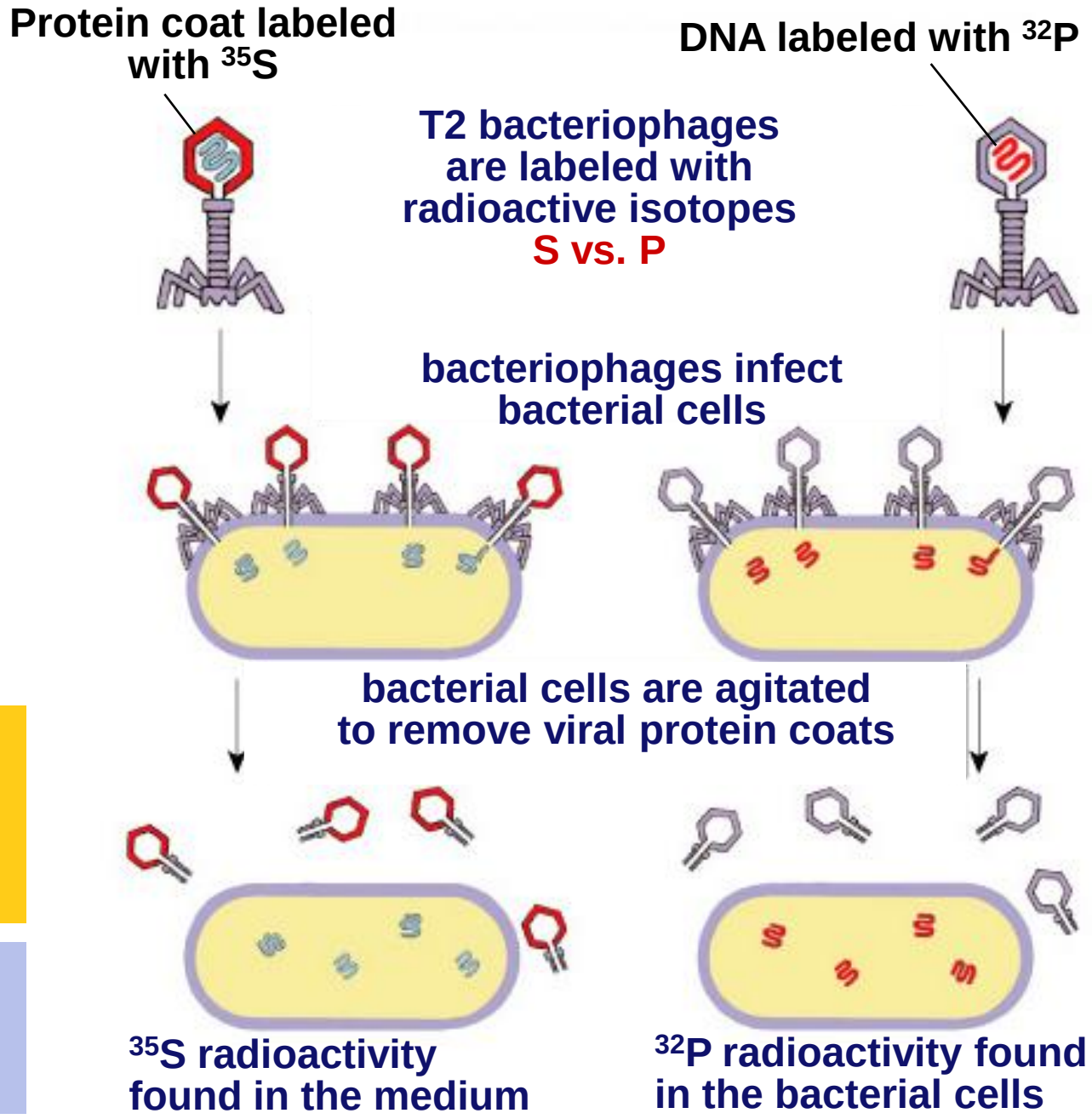
Please explain the experiment of

Frederick Griffith to a peer at your table.

Today I will:

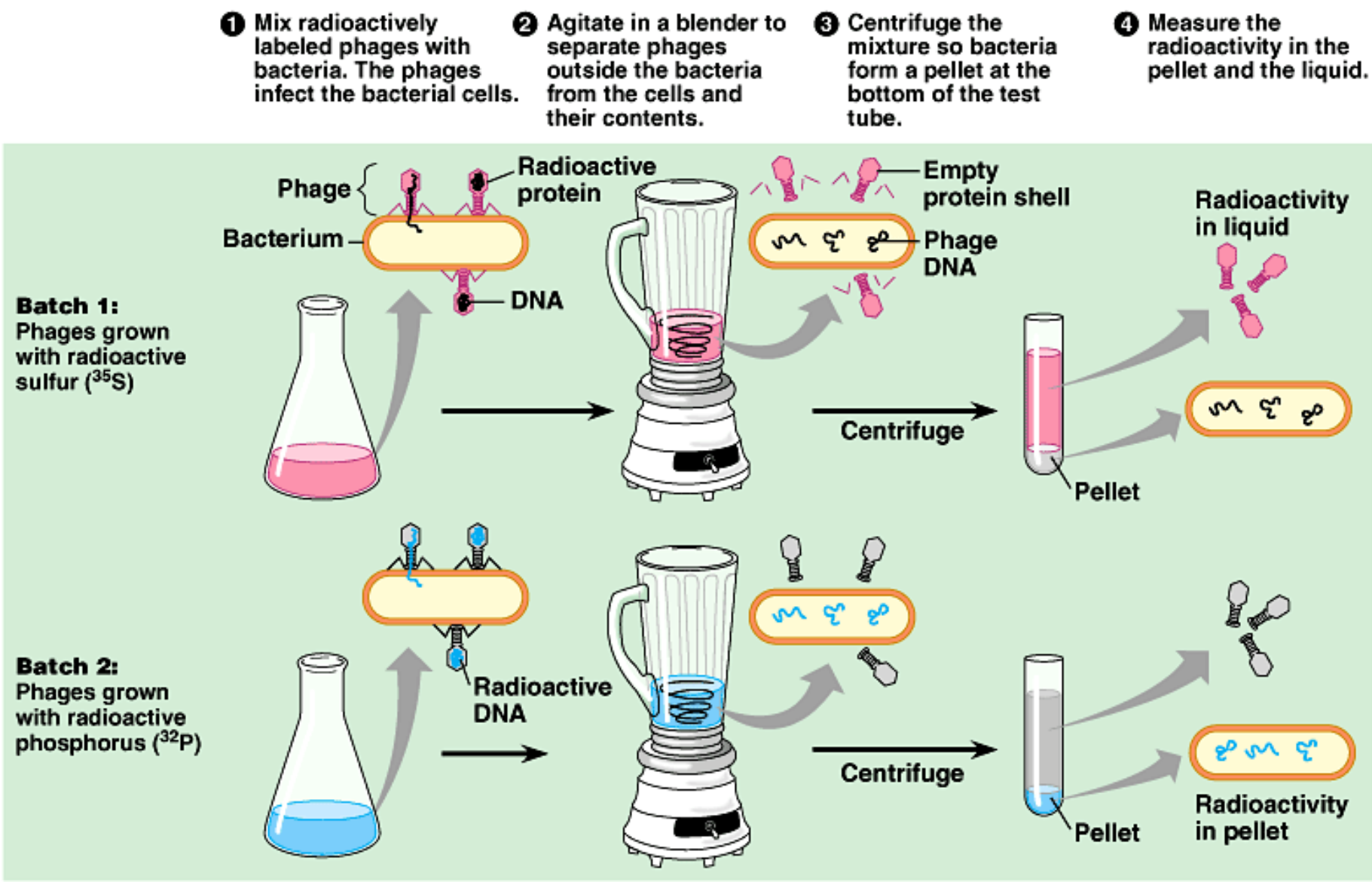
1. **Summarize** the work of Avery, McCarty & MacLeod.
2. **Describe** the Hershey-Chase "blender" experiment.
3. **State** Chargaff's rules and **outline** the structure of a DNA nucleotide.

Hershey & Chase



Which radioactive marker is found inside the cell?

Which molecule carries viral genetic info?


1 Mix radioactively labeled phages with bacteria. The phages infect the bacterial cells.
2 Agitate in a blender to separate phages outside the bacteria from the cells and their contents.
3 Centrifuge the mixture so bacteria form a pellet at the bottom of the test tube.
4 Measure the radioactivity in the pellet and the liquid.
Batch 1: Phages grown with radioactive sulfur (^{35}S)
Phage
Radioactive protein
Bacterium
DNA
Empty protein shell
Phage DNA
Radioactivity in liquid
Centrifuge
Pellet
Batch 2: Phages grown with radioactive phosphorus (^{32}P)
Radioactive DNA
Centrifuge
Pellet
Radioactivity in pellet

Blender experiment

- Radioactive phage & bacteria in blender
  - ^{35}S phage
    - radioactive proteins stayed in supernatant
    - therefore protein did NOT enter bacteria
  - ^{32}P phage
    - radioactive DNA stayed in pellet
    - therefore DNA did enter bacteria
  - Confirmed DNA is “transforming factor”

1947

Chargaff

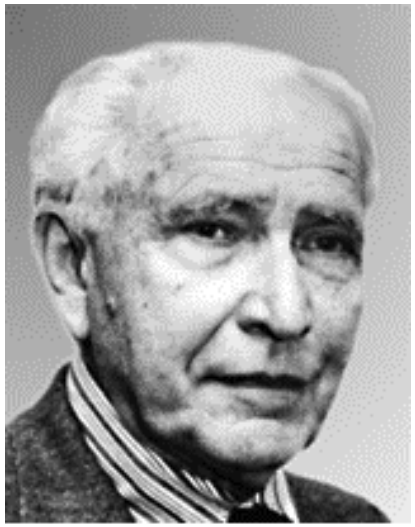
- DNA composition: “Chargaff’s rules”
  - varies from species to species
  - all 4 bases not in equal quantity
  - bases present in characteristic ratio
    - humans:

A = 30.9%

T = 29.4%

G = 19.9%

C = 19.8%

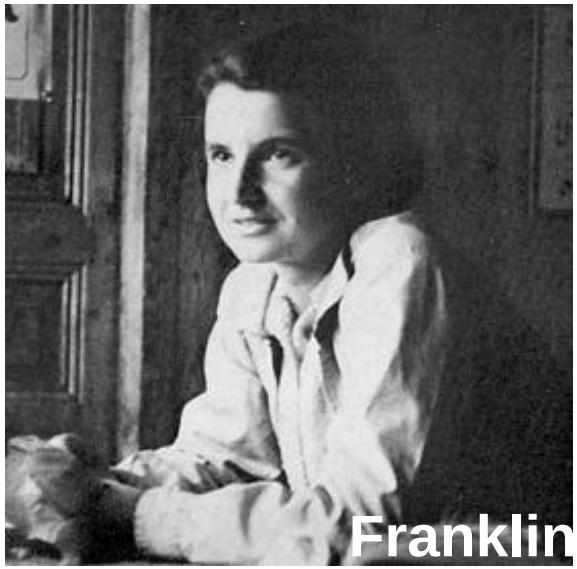


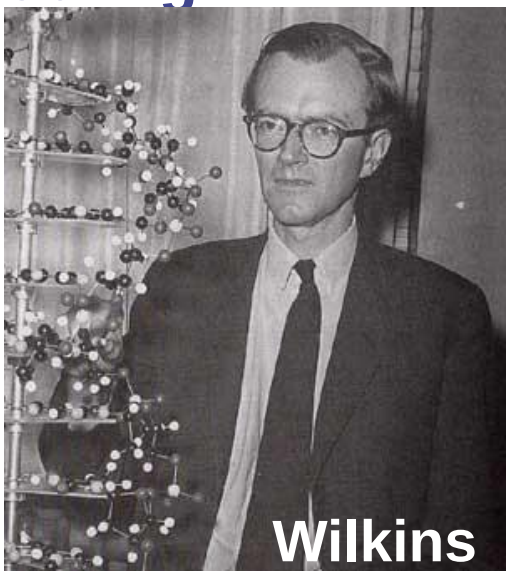
Erwin Chargaff

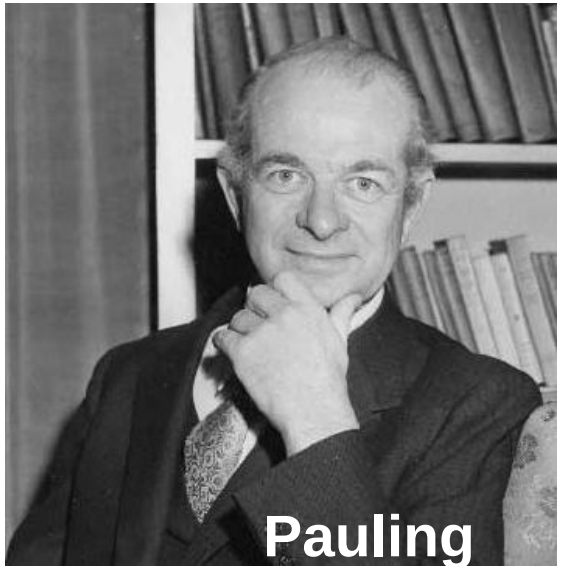
1953 | 1962

Structure of DNA

- **Watson & Crick**
  - **developed double helix model of DNA**
    - **other scientists working on question:**
      - **Rosalind Franklin**
      - **Maurice Wilkins**
      - **Linus Pauling**

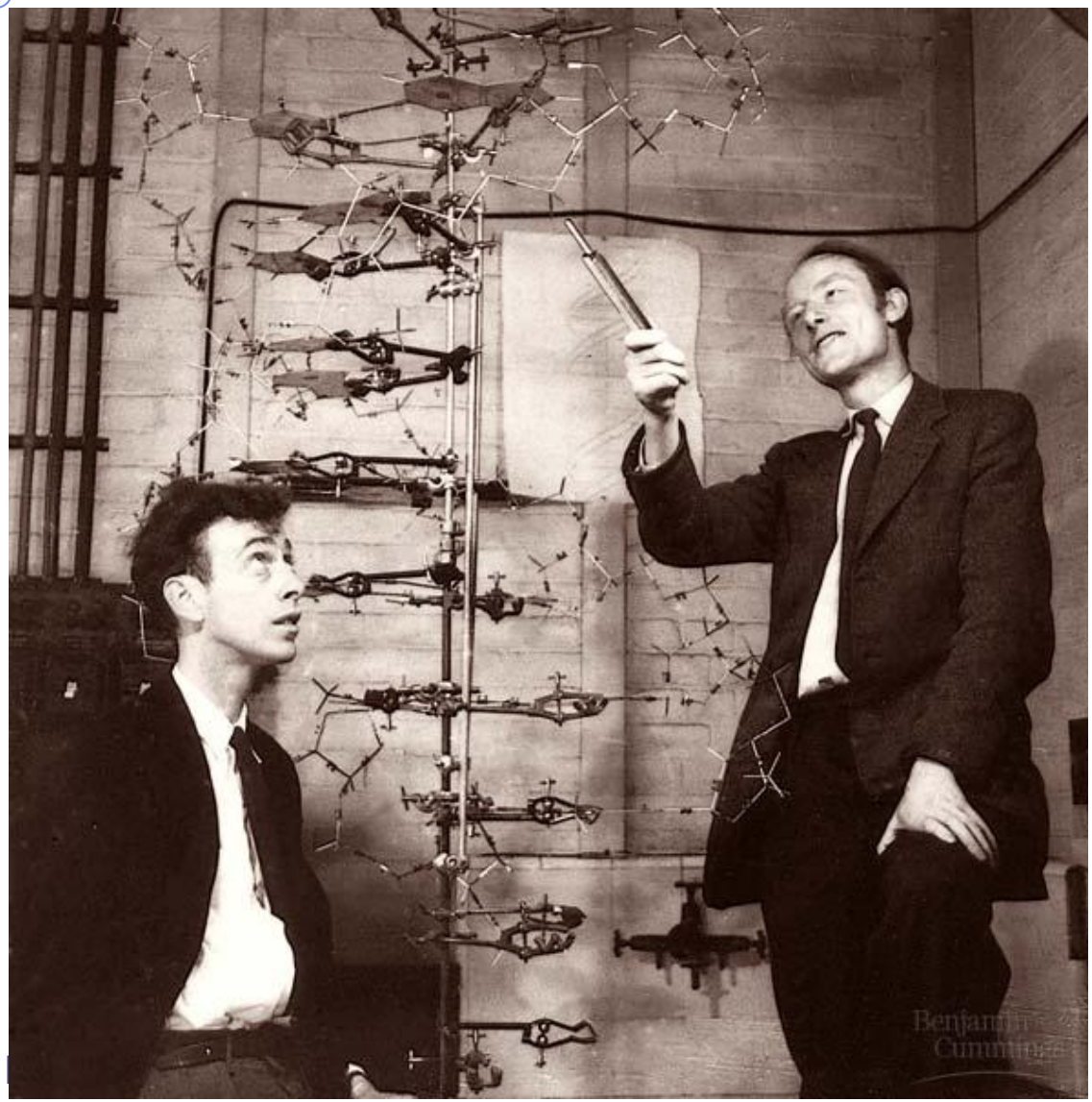

Franklin

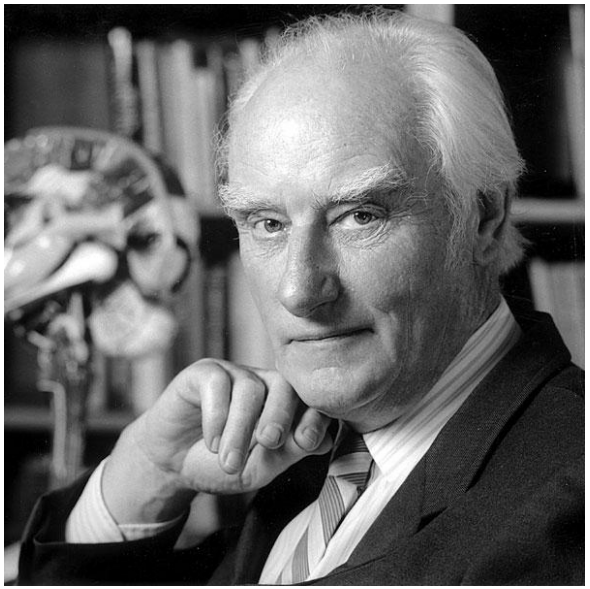

Wilkins


Pauling

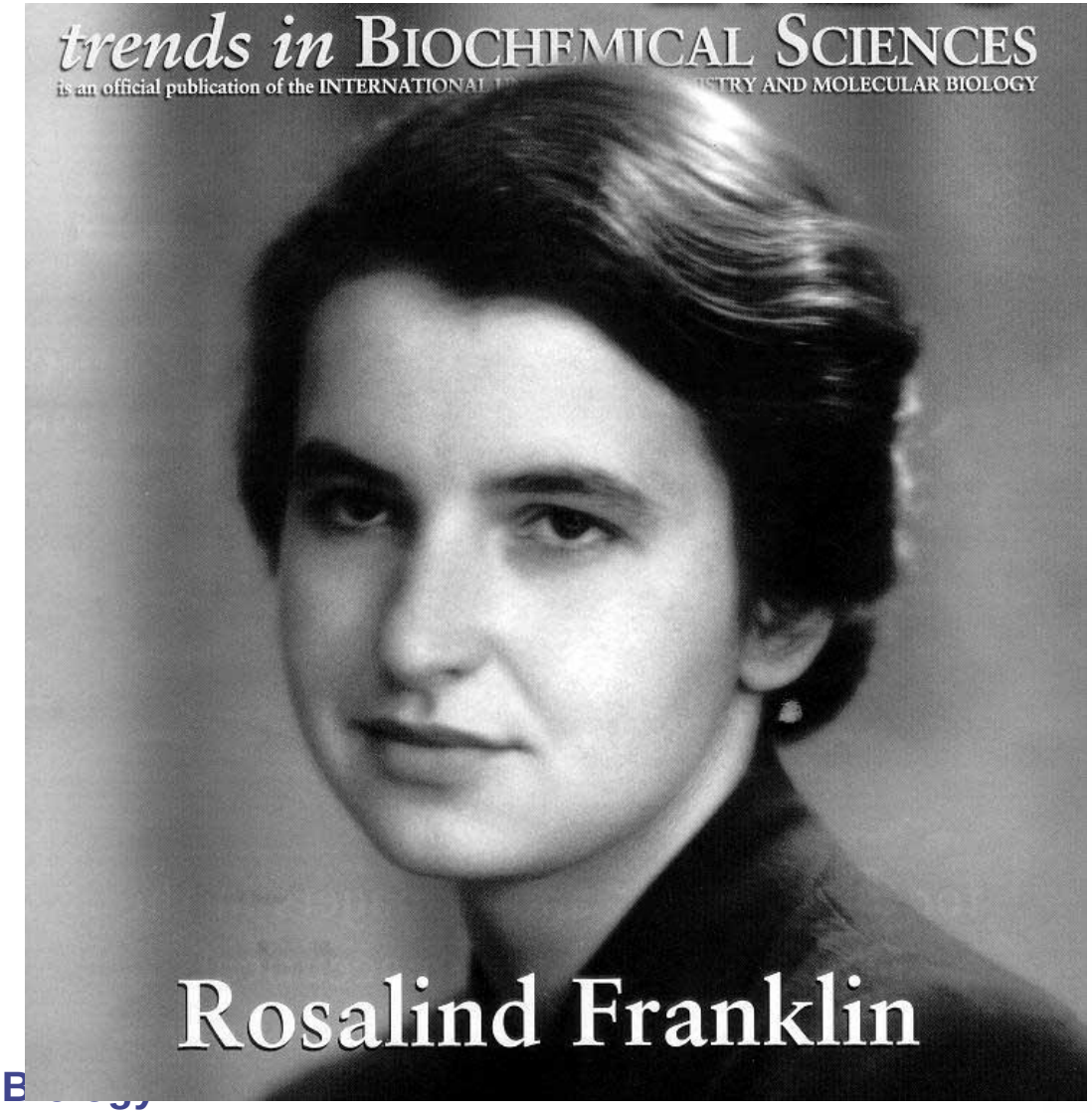
Watson and Crick

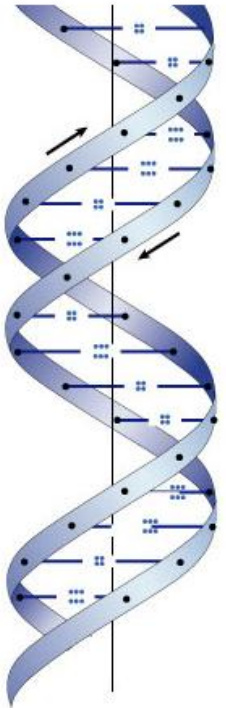
1953 article in Nature

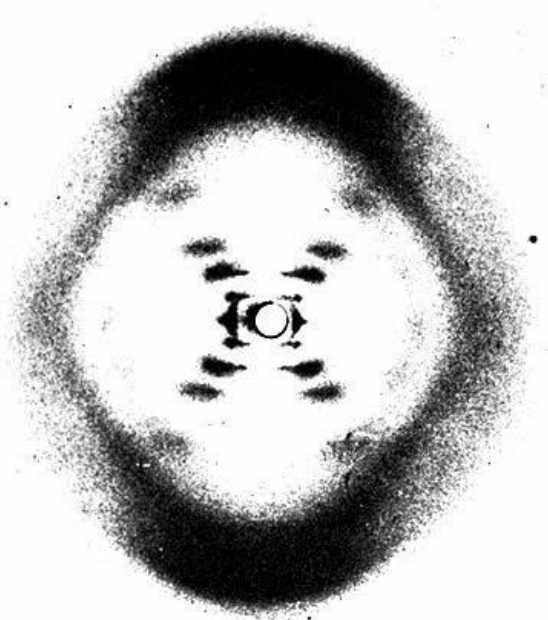




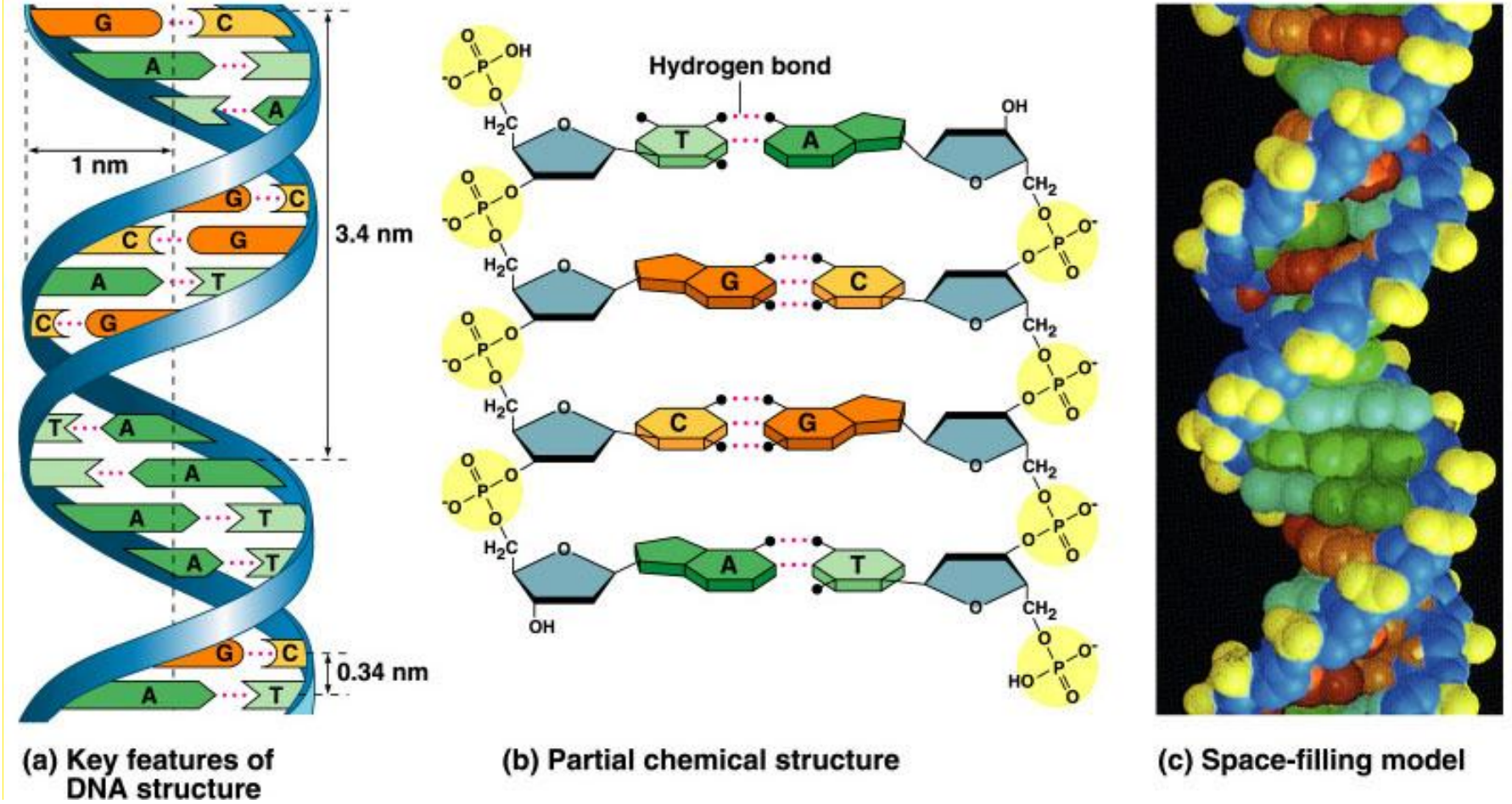
Rosalind Franklin (1920-1958)







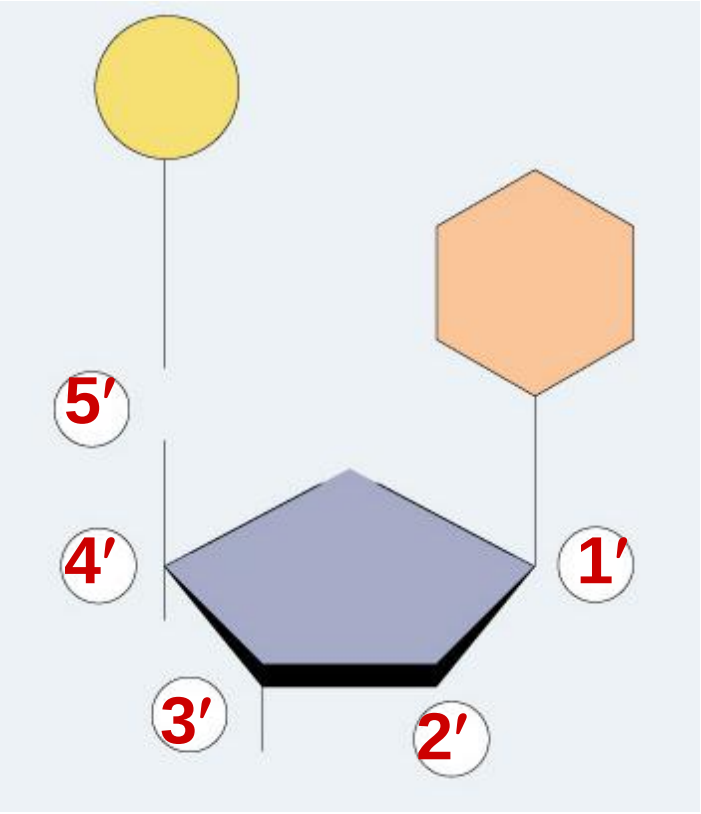
Double helix structure of DNA



the structure of DNA suggested a mechanism for how DNA is copied by the cell

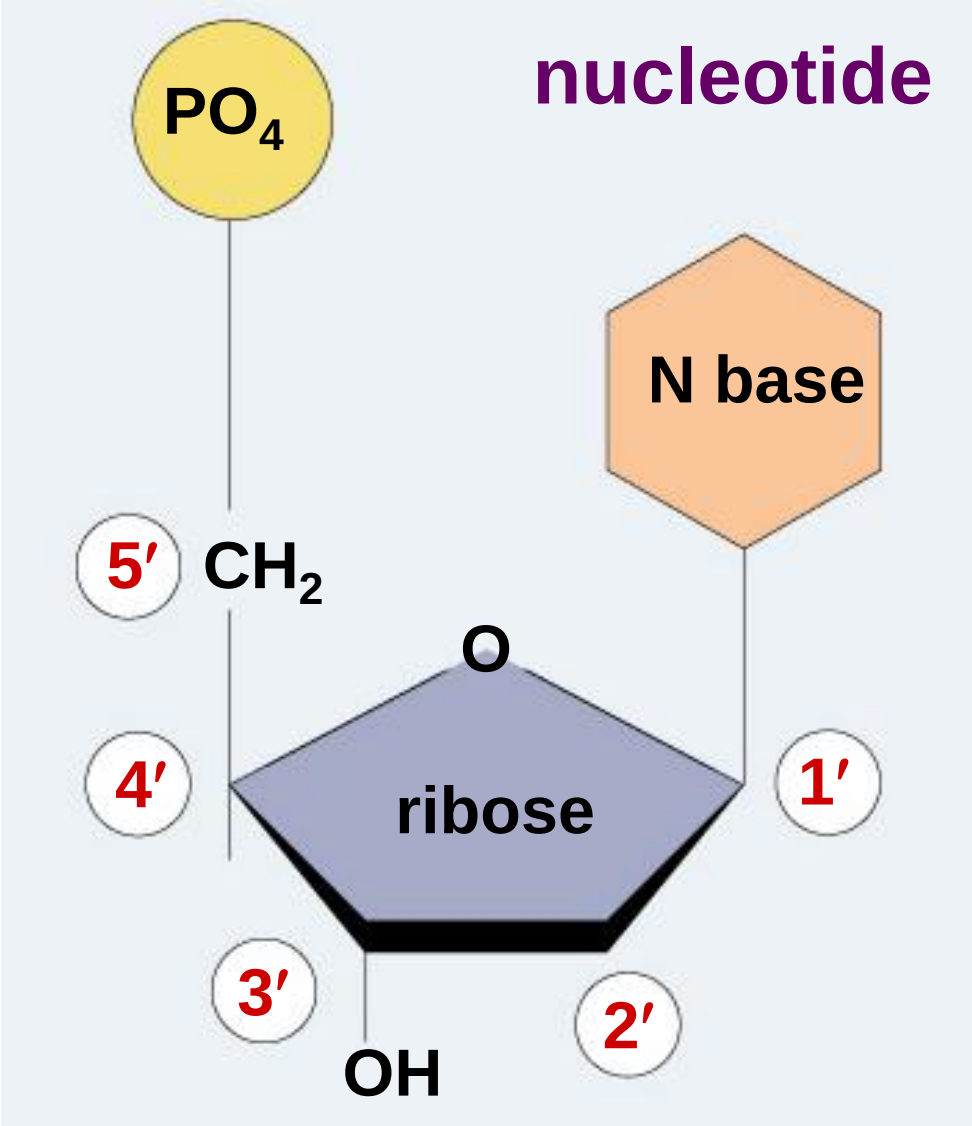
Friday, April 11th

Let's review the NUMBERING of carbons in a **deoxyribose** sugar molecule:



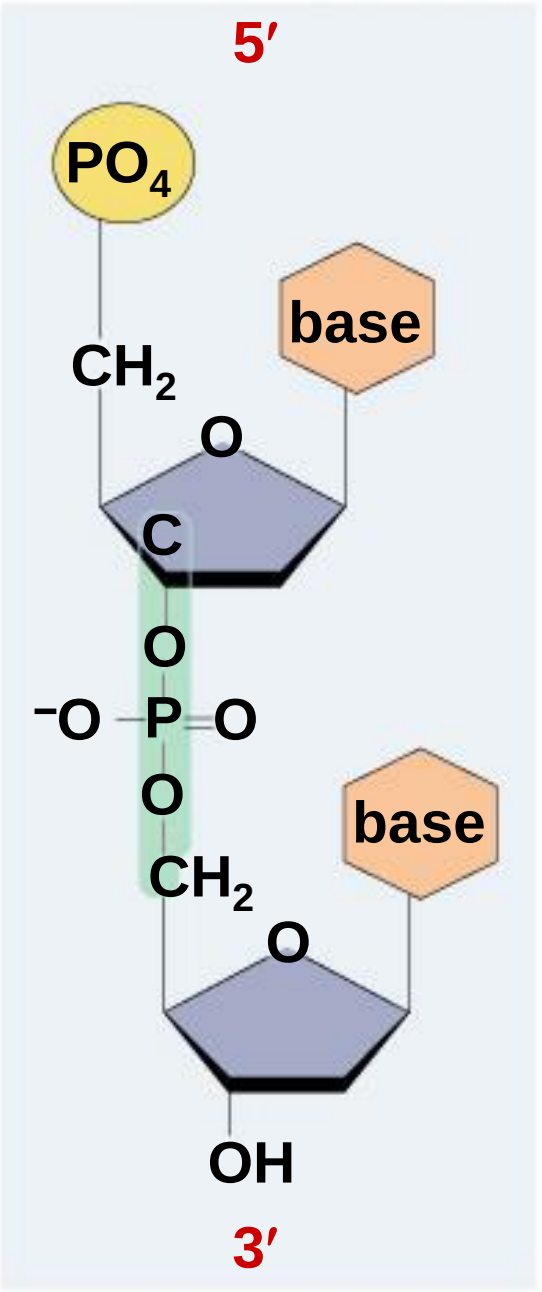
Directionality of DNA

- You need to number the carbons!
  - it matters!



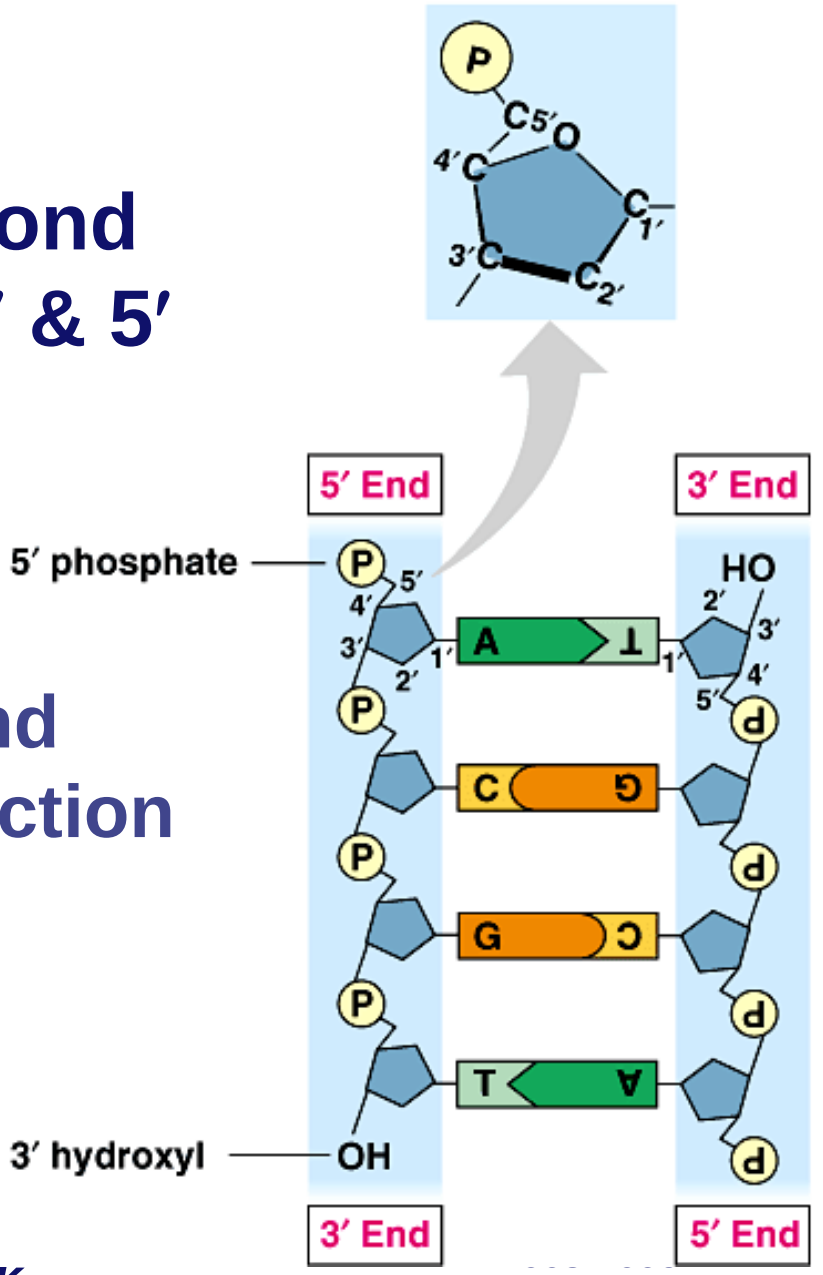
The DNA backbone

- Putting the DNA backbone together
  - refer to the 3′ and 5′ ends of the DNA
    - the last trailing carbon



Anti-parallel strands

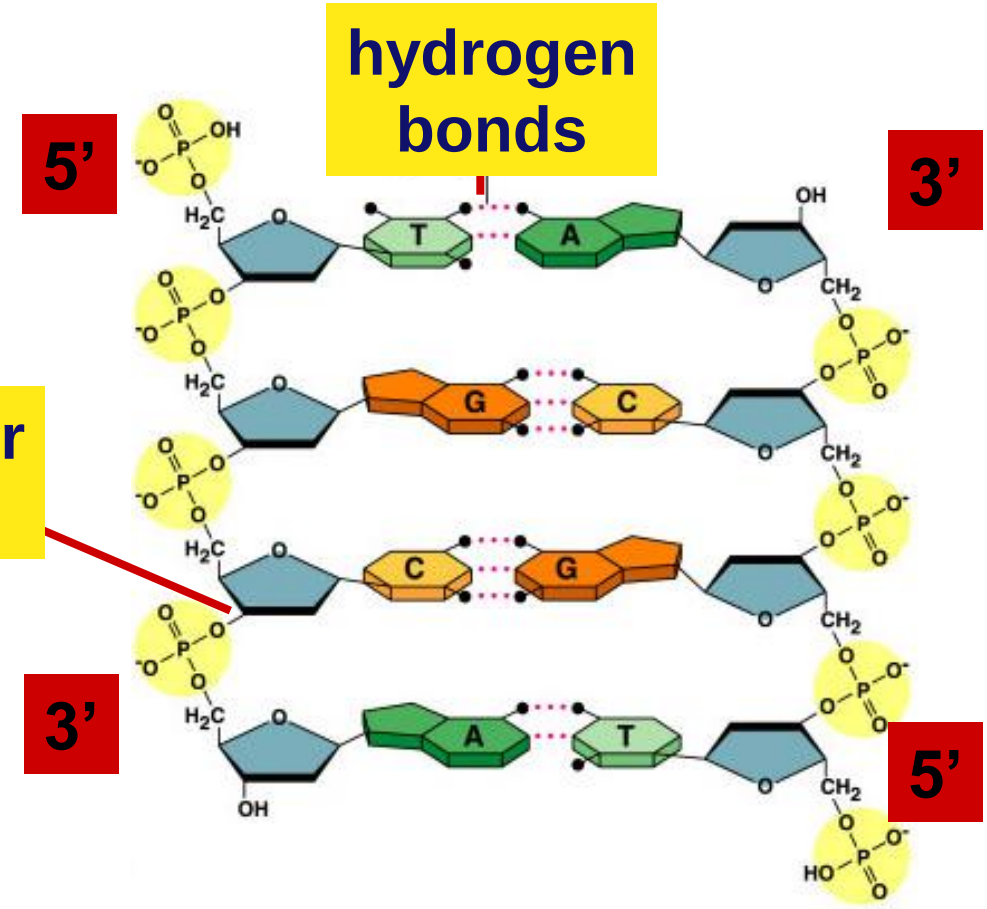
- Phosphate to sugar bond involves carbons in 3′ & 5′ positions
  - DNA molecule has “direction”
  - complementary strand runs in opposite direction



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

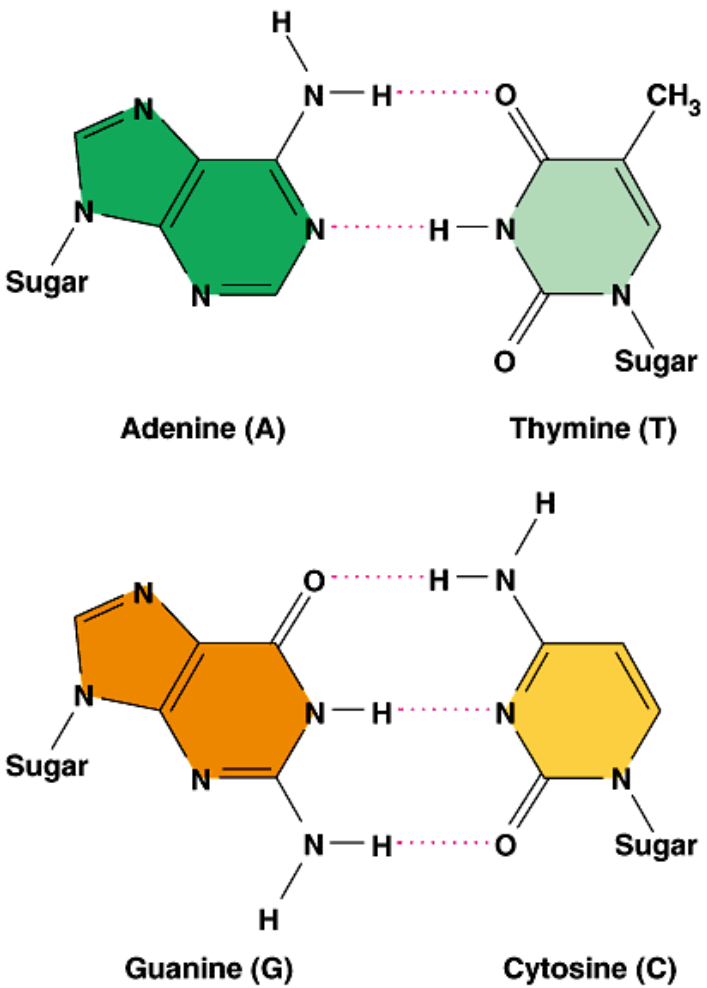
Watson & Crick

Bonding in DNA



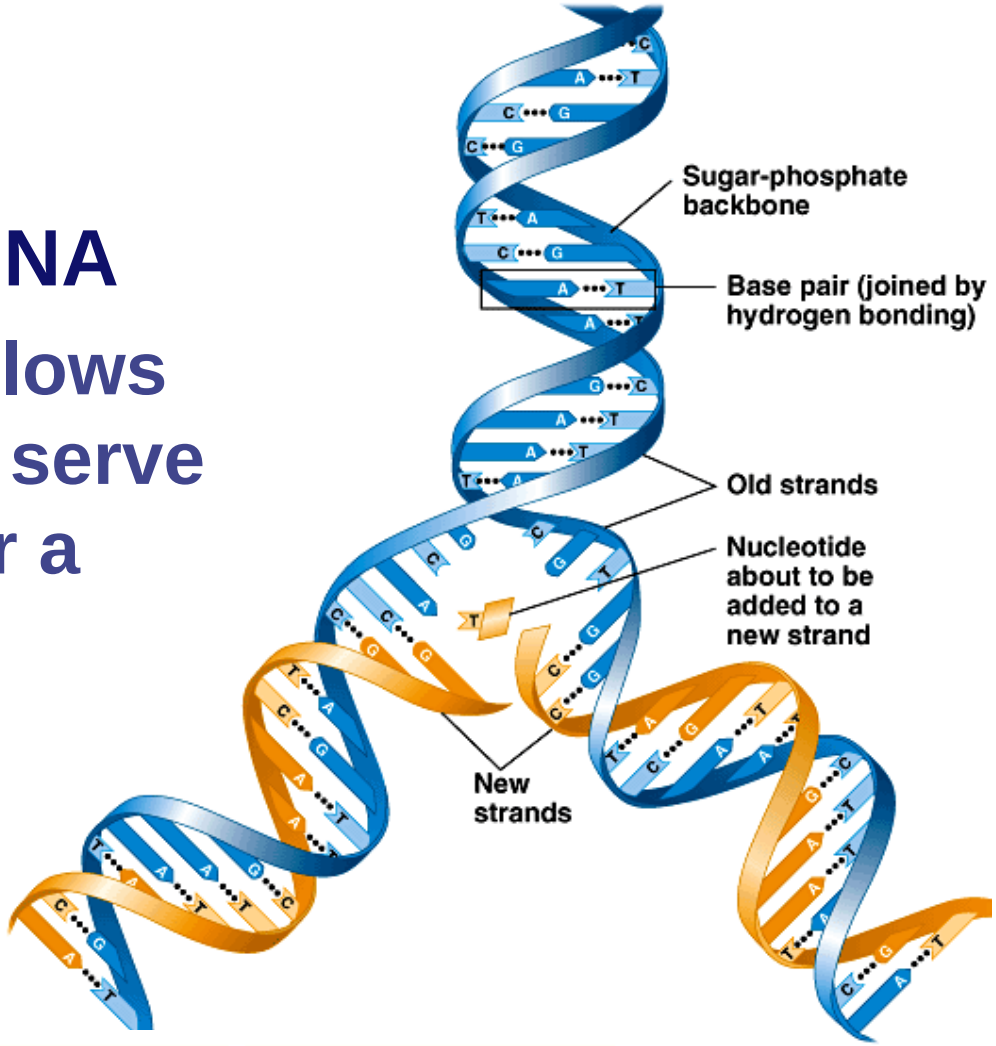
Base pairing in DNA

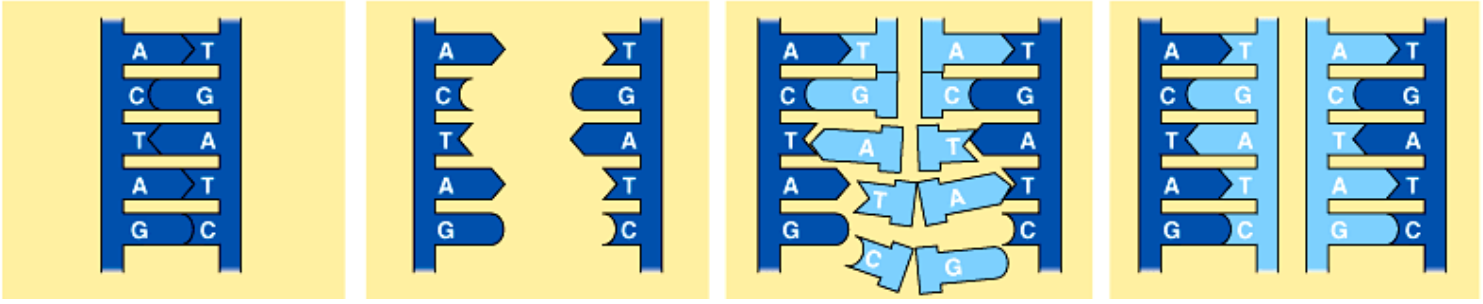
- Purines
  - adenine (A)
  - guanine (G)
- Pyrimidines
  - thymine (T)
  - cytosine (C)
- Pairing
  - A : T
  - C : G



Copying DNA

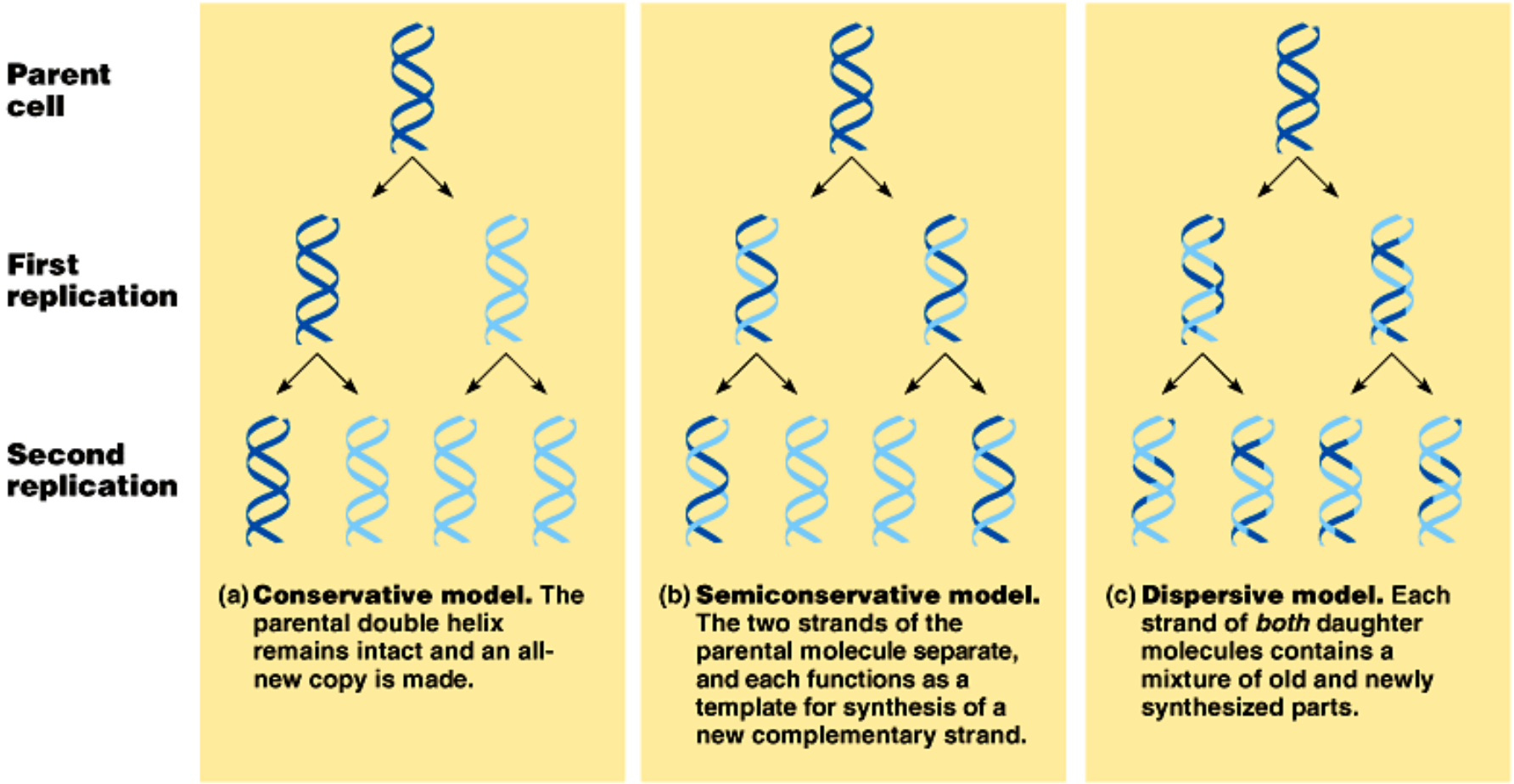
- **Replication of DNA**
  - **base pairing allows each strand to serve as a pattern for a new strand**





Models of DNA Replication

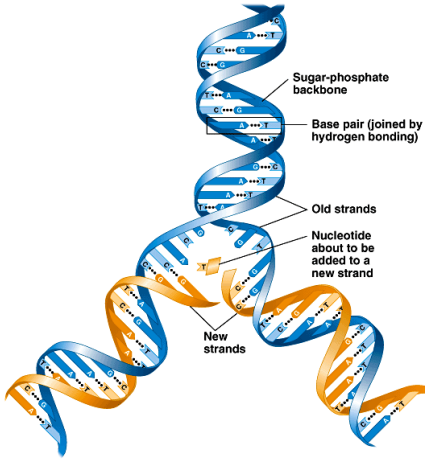
- **Alternative models**
  - **so how is DNA copied?**



Models of DNA Replication

- Meselson and Stahl

Animation: Models of DNA Replication

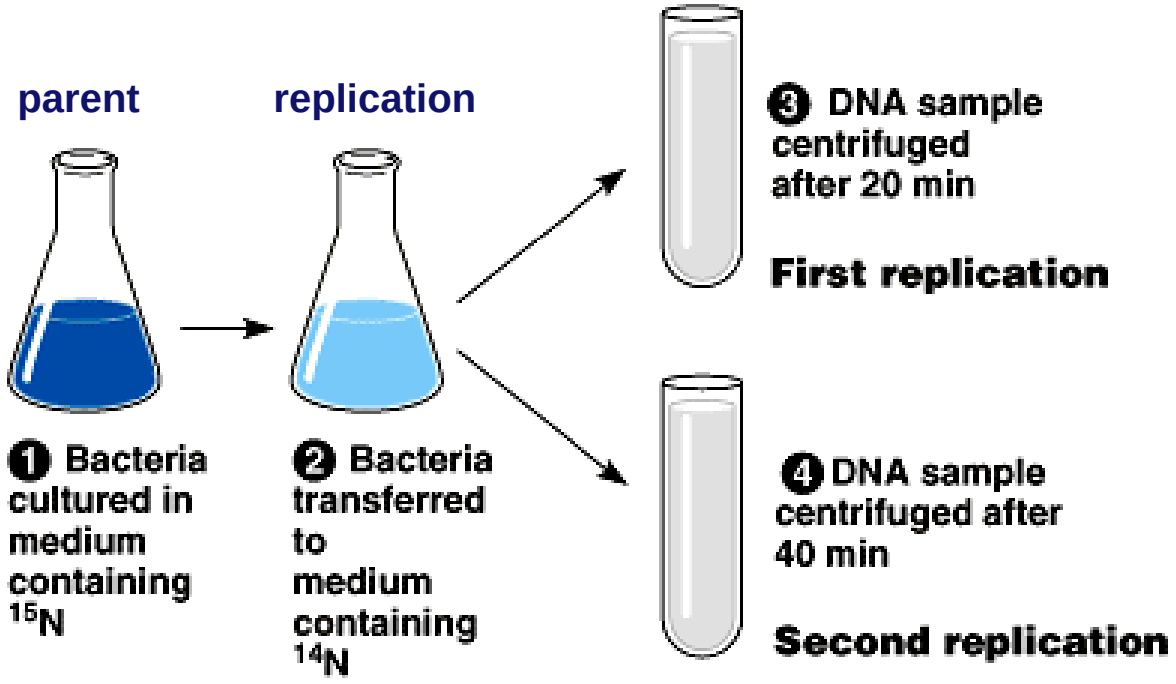


1958

Semi-conservative replication

- Meselson & Stahl
  - label nucleotides of "parent" DNA strands with heavy nitrogen = ^{15}N
  - label new nucleotides with lighter isotope = ^{14}N

"The Most Beautiful Experiment in Biology"

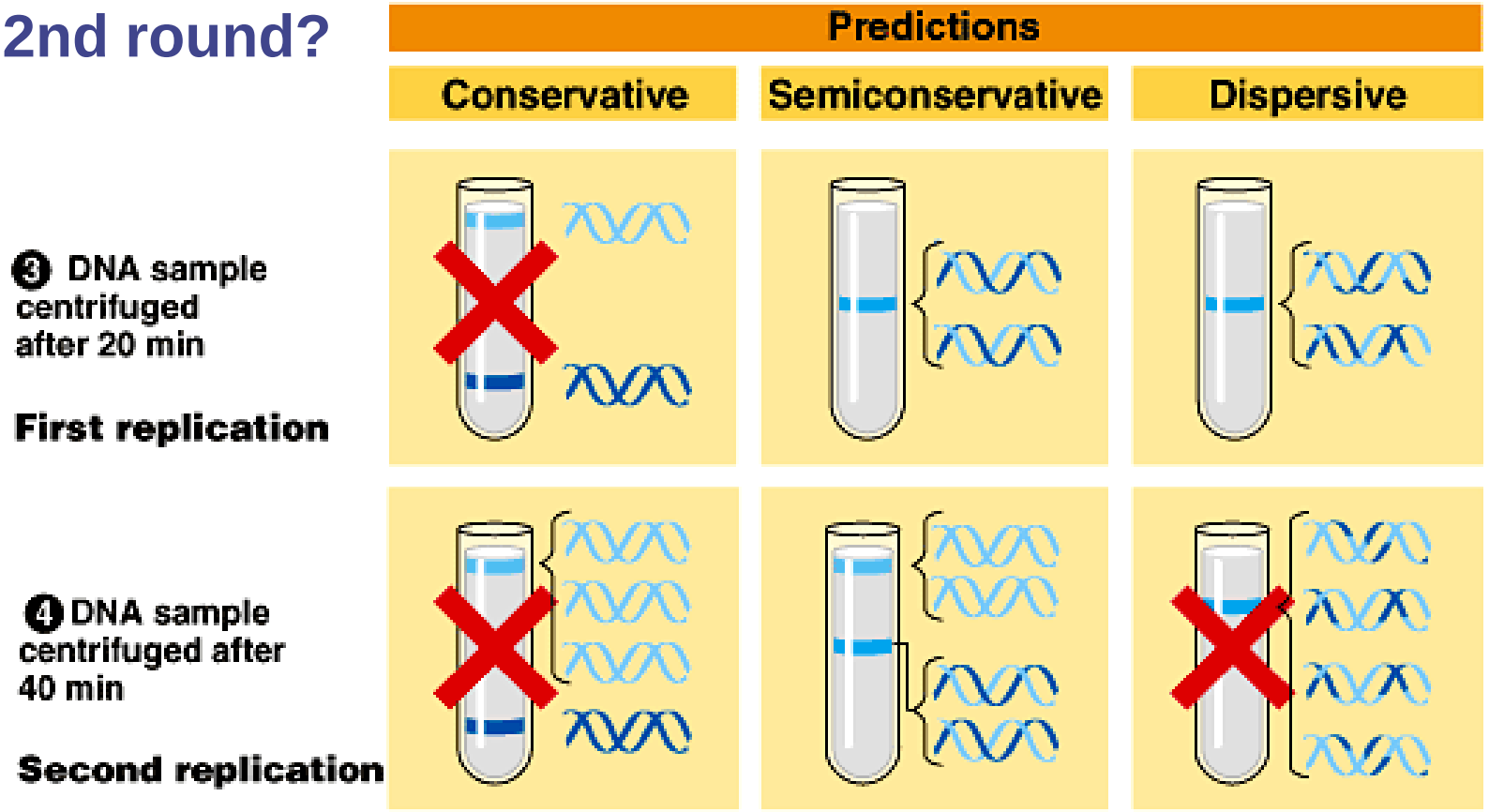


logy

1958

Semi-conservative replication

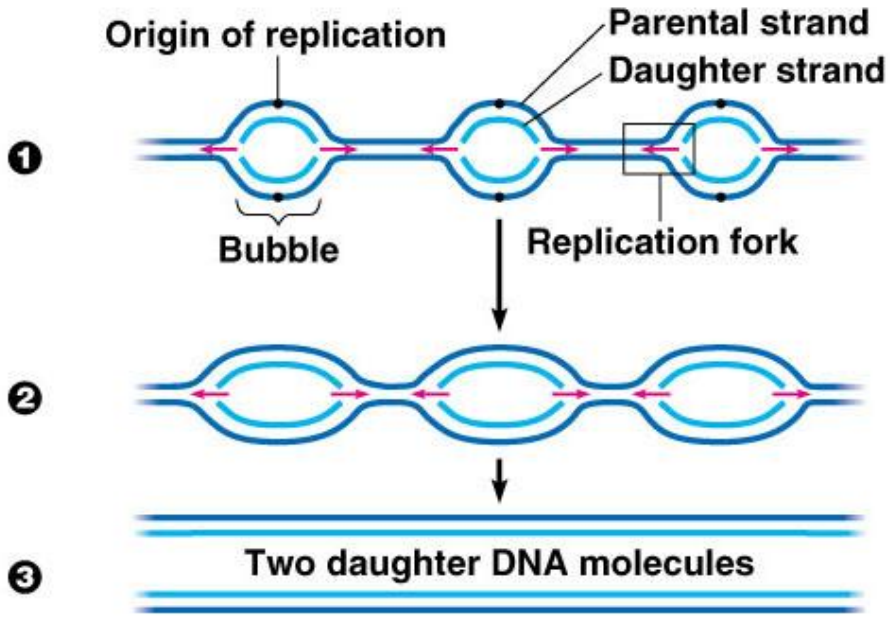
- **Make predictions…**
  - ^{15}N strands replicated in ^{14}N medium
  - 1st round of replication?
  - 2nd round?



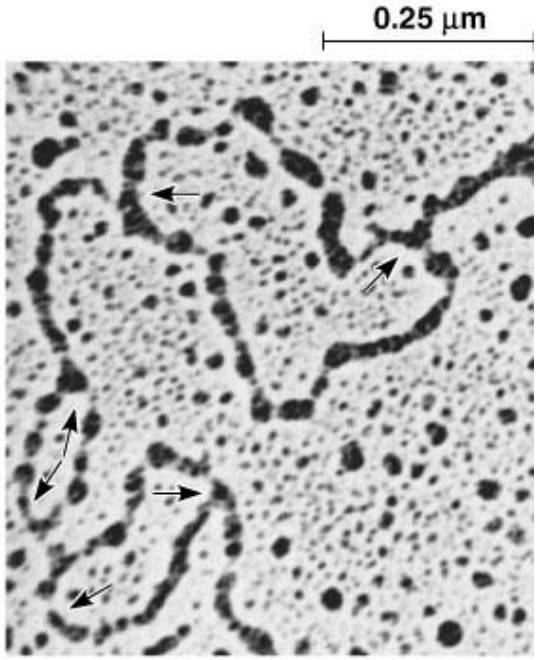
logy

DNA Replication

- Large team of enzymes coordinates replication



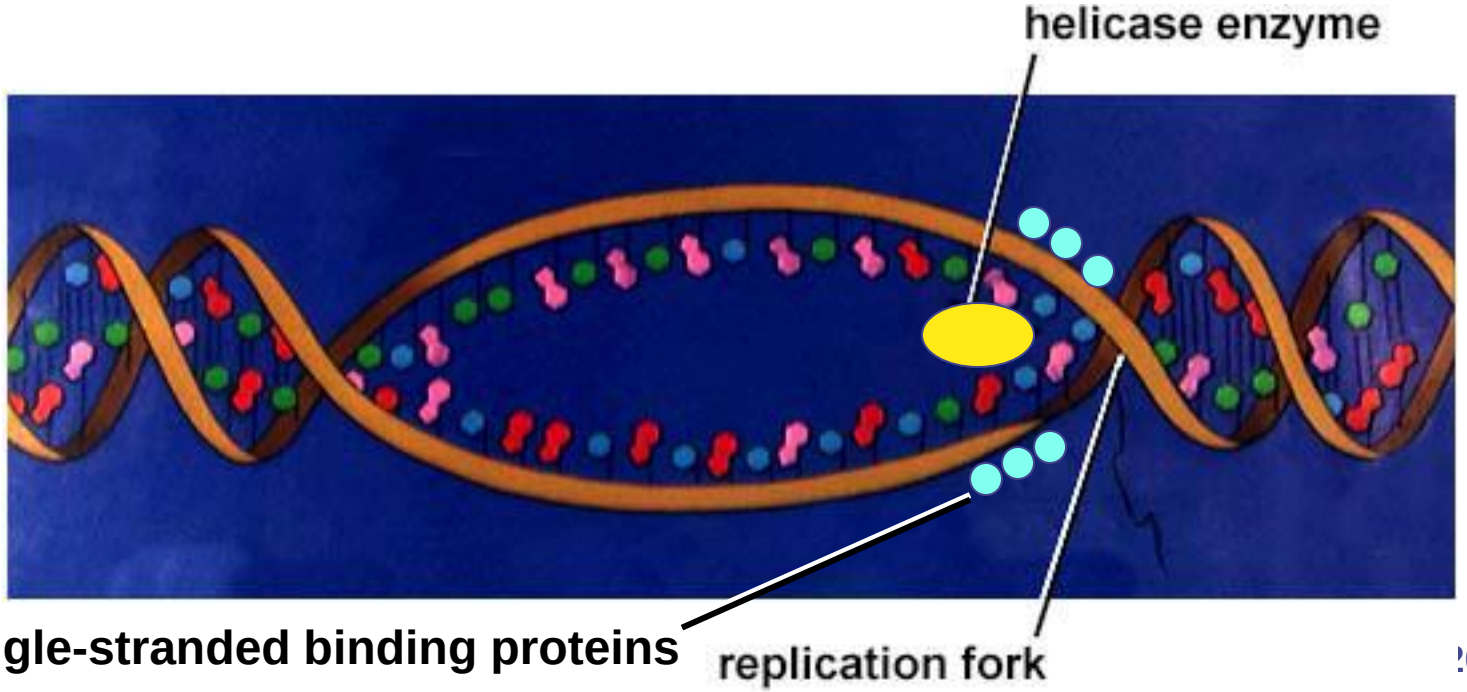
(a) In eukaryotes, DNA replication begins at many sites along the giant DNA molecule of each chromosome.



(b) In this micrograph, three replication bubbles are visible along the DNA of cultured Chinese hamster cells. The arrows indicate the direction of DNA replication at the two ends of each bubble (TEM).

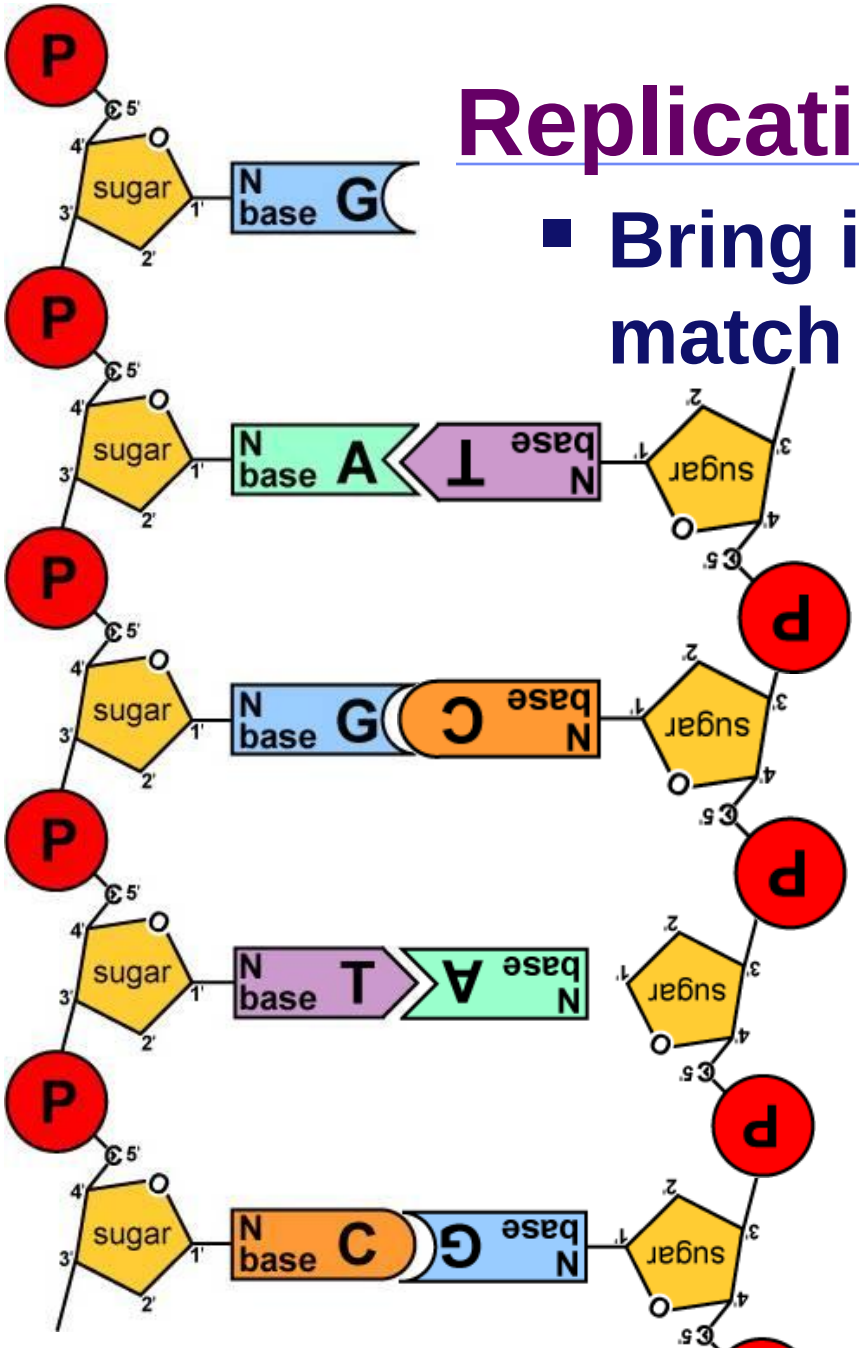
Replication: 1st step

- Unwind DNA
  - helicase enzyme
    - unwinds part of DNA helix
    - stabilized by single-stranded binding proteins



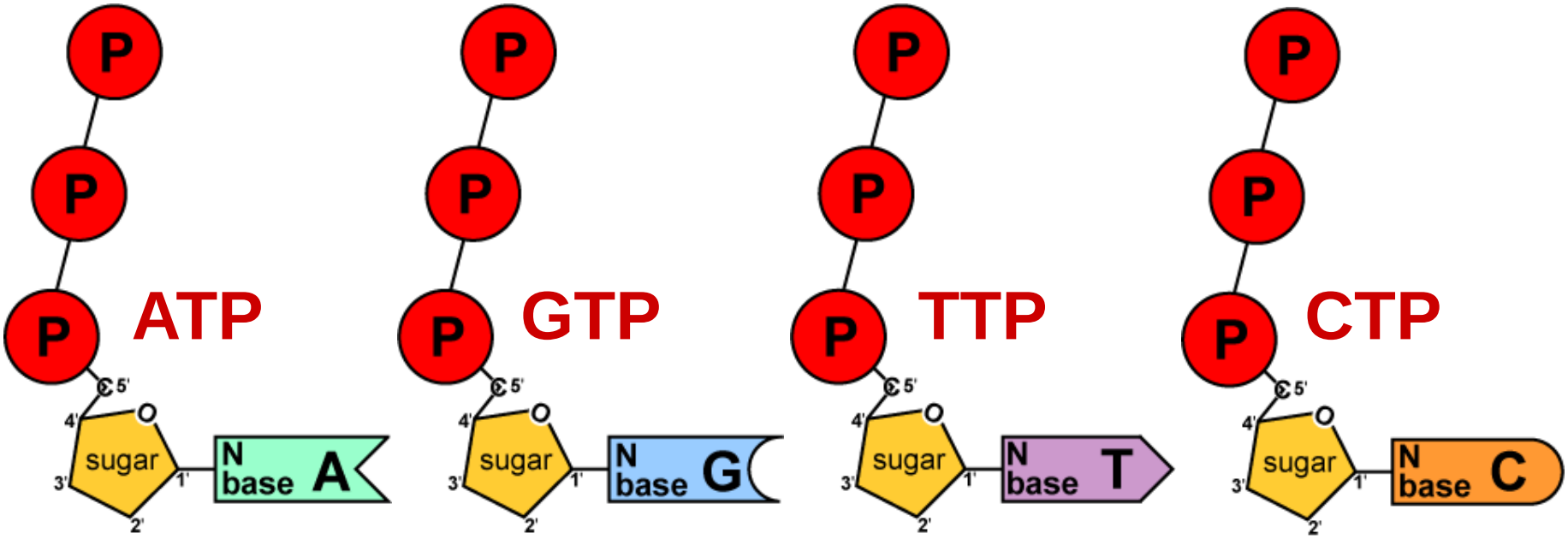
Replication: 2nd step

- Bring in new nucleotides to match up to template strands



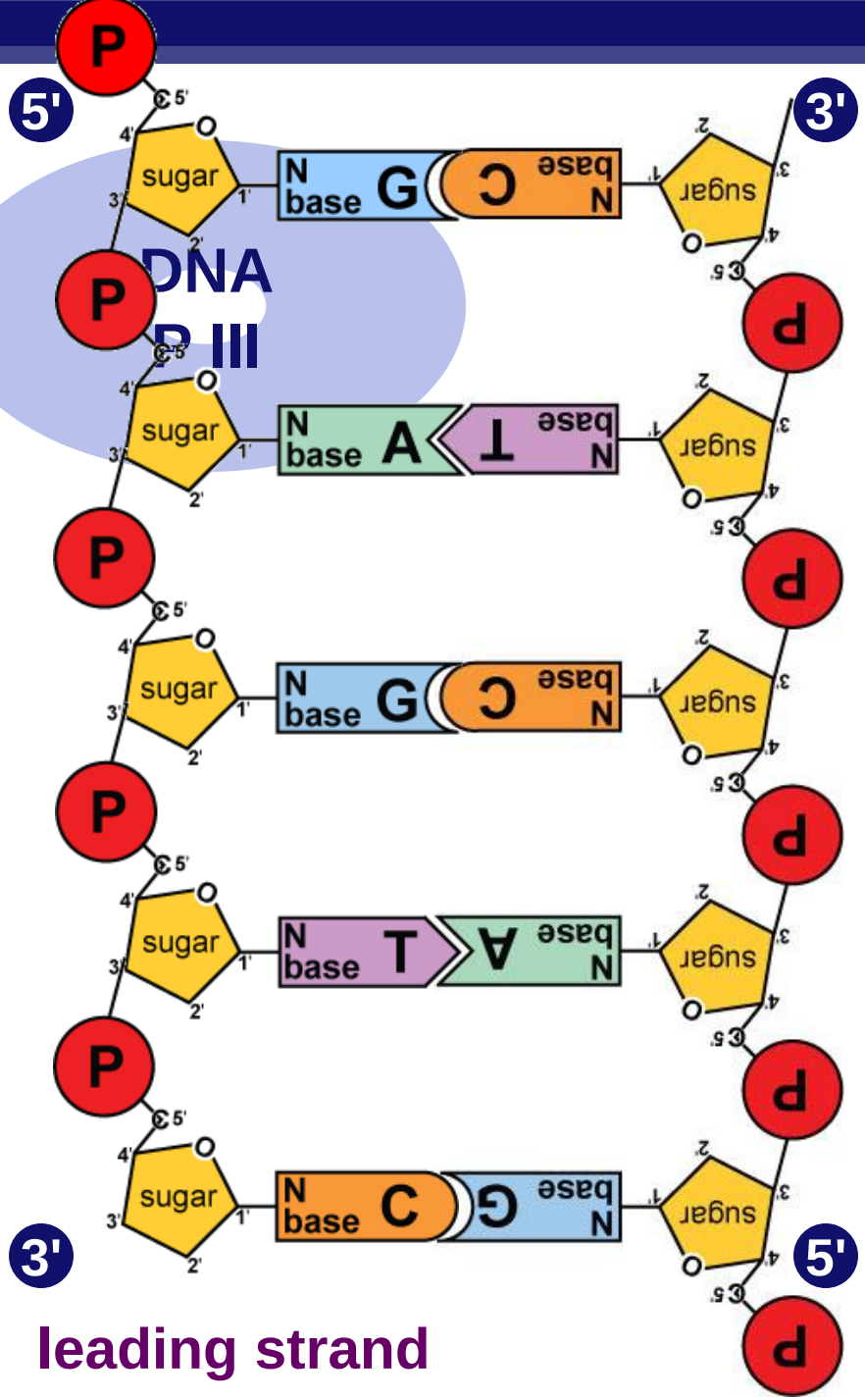
Energy of Replication

- The nucleotides arrive as nucleosides
  - DNA bases with P–P–P
  - DNA bases arrive with their own energy source for bonding
  - bonded by DNA polymerase III



Replication

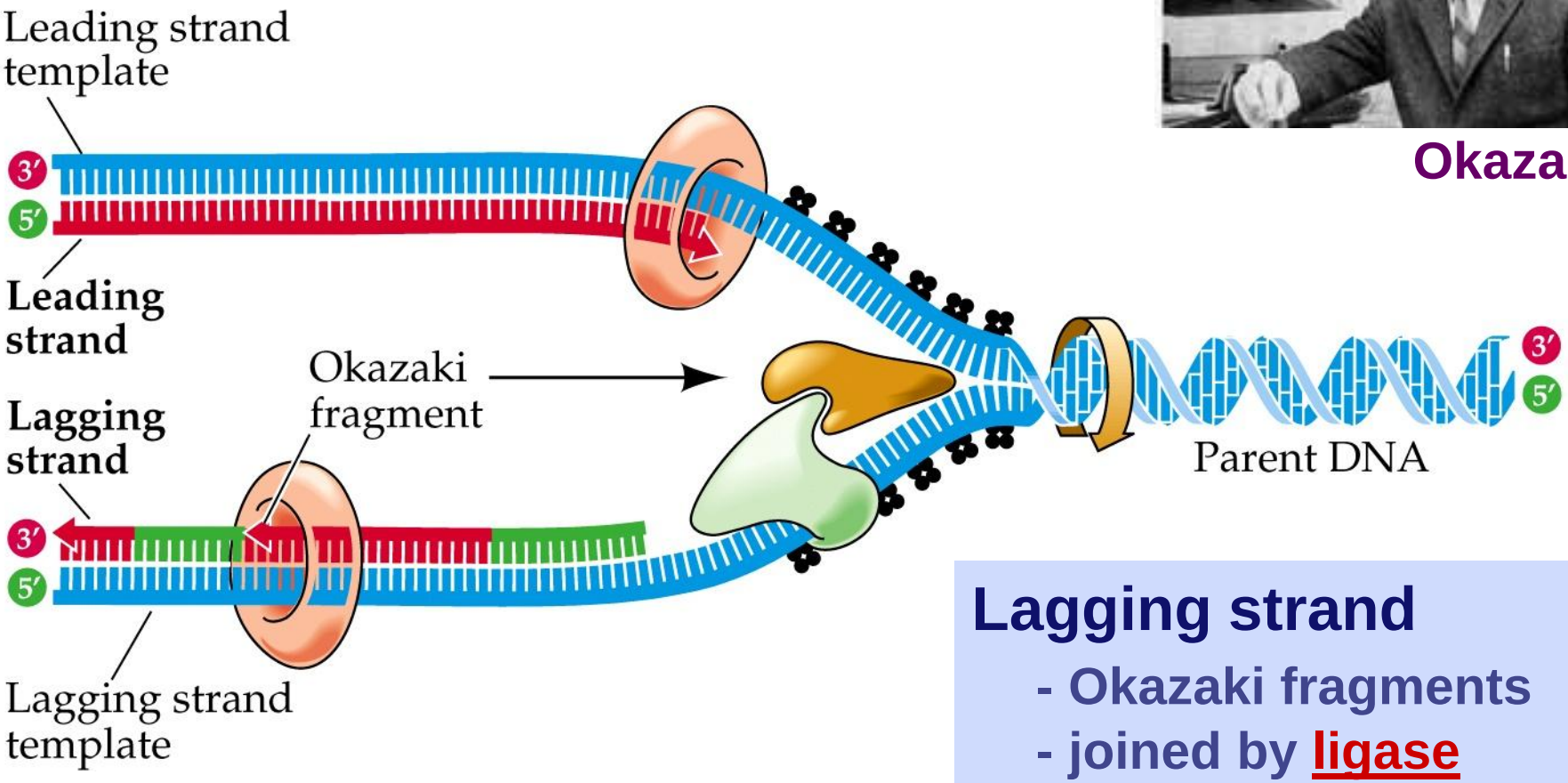
- Adding bases
  - can only add nucleotides to 3′ end of a growing DNA strand
  - strand grow 5'→3’



Leading & Lagging strands

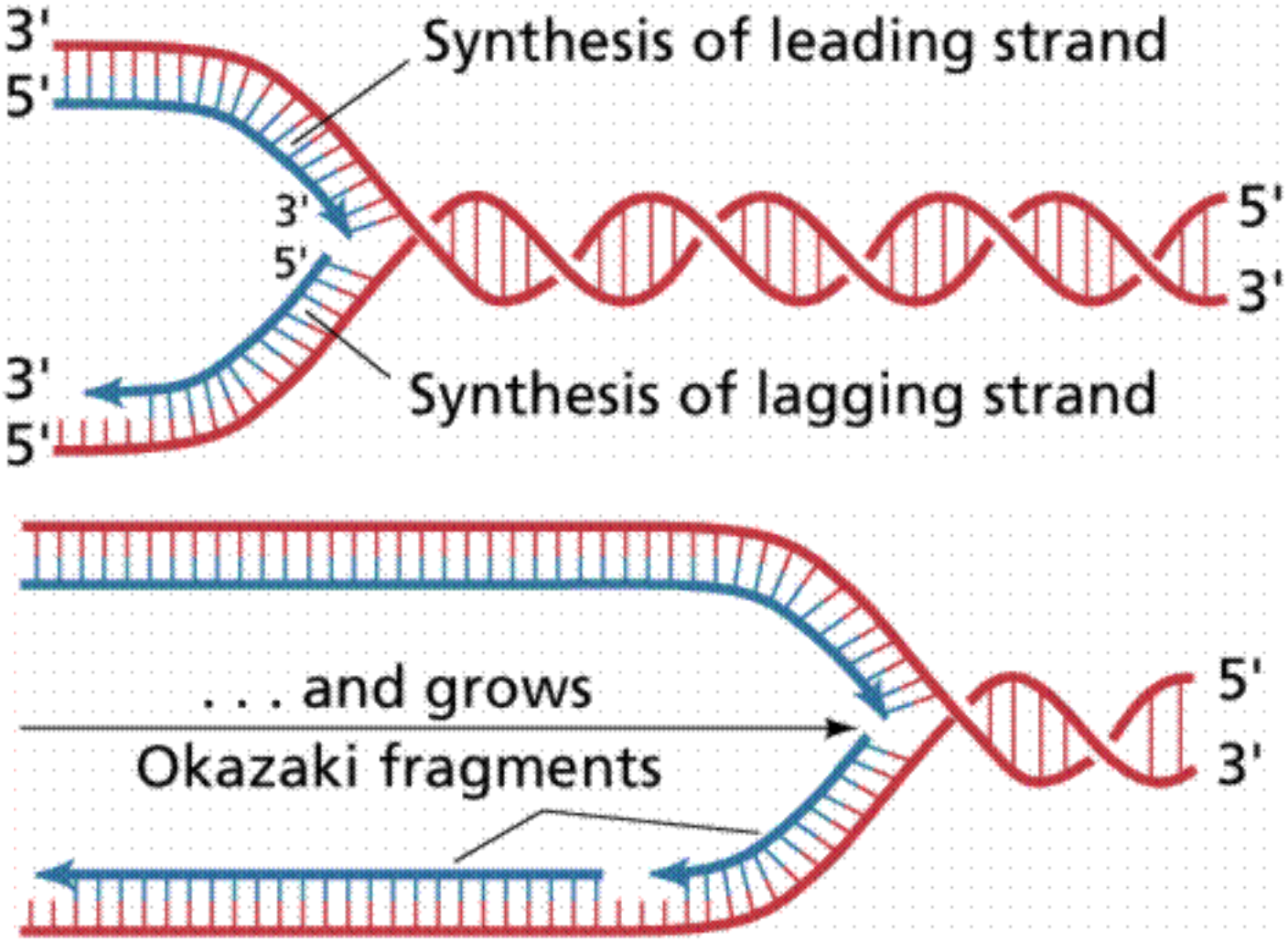
Leading strand
- continuous synthesis

Okazaki



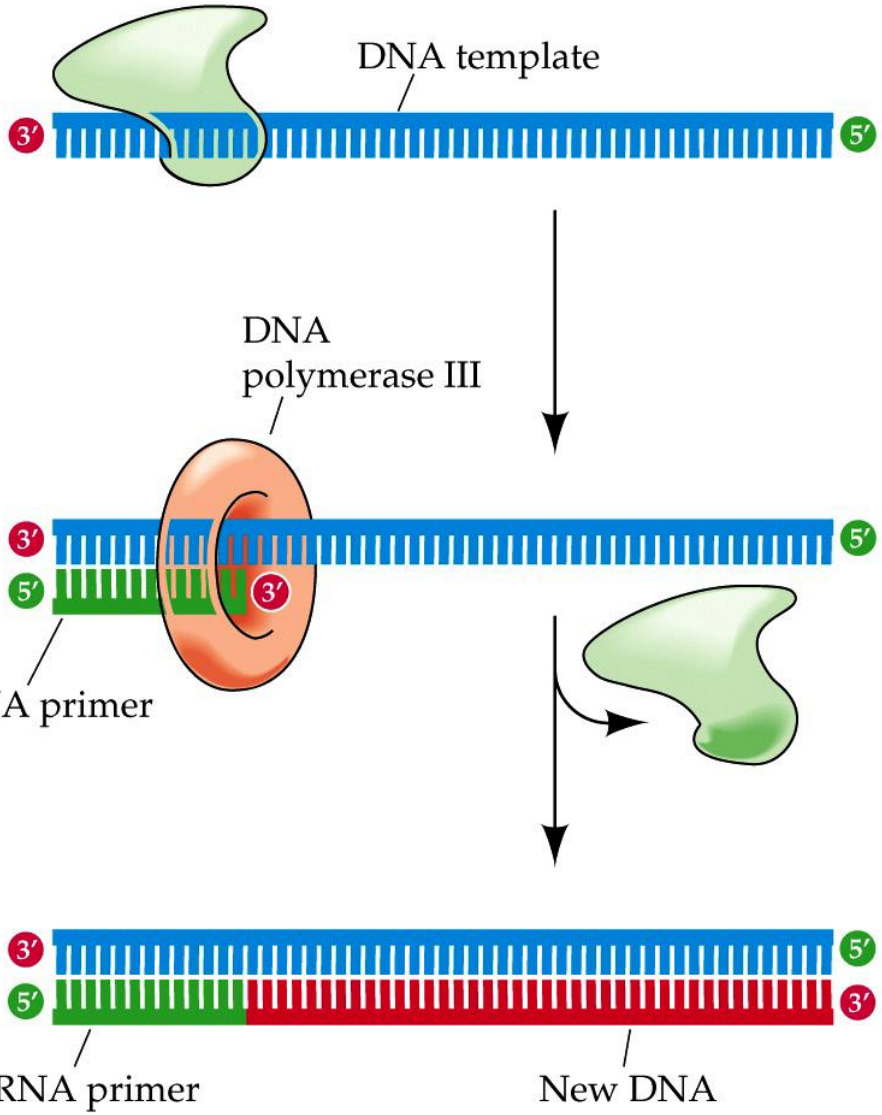
Lagging strand
- Okazaki fragments
- joined by ligase
  - "spot welder" enzyme

Okazaki fragments



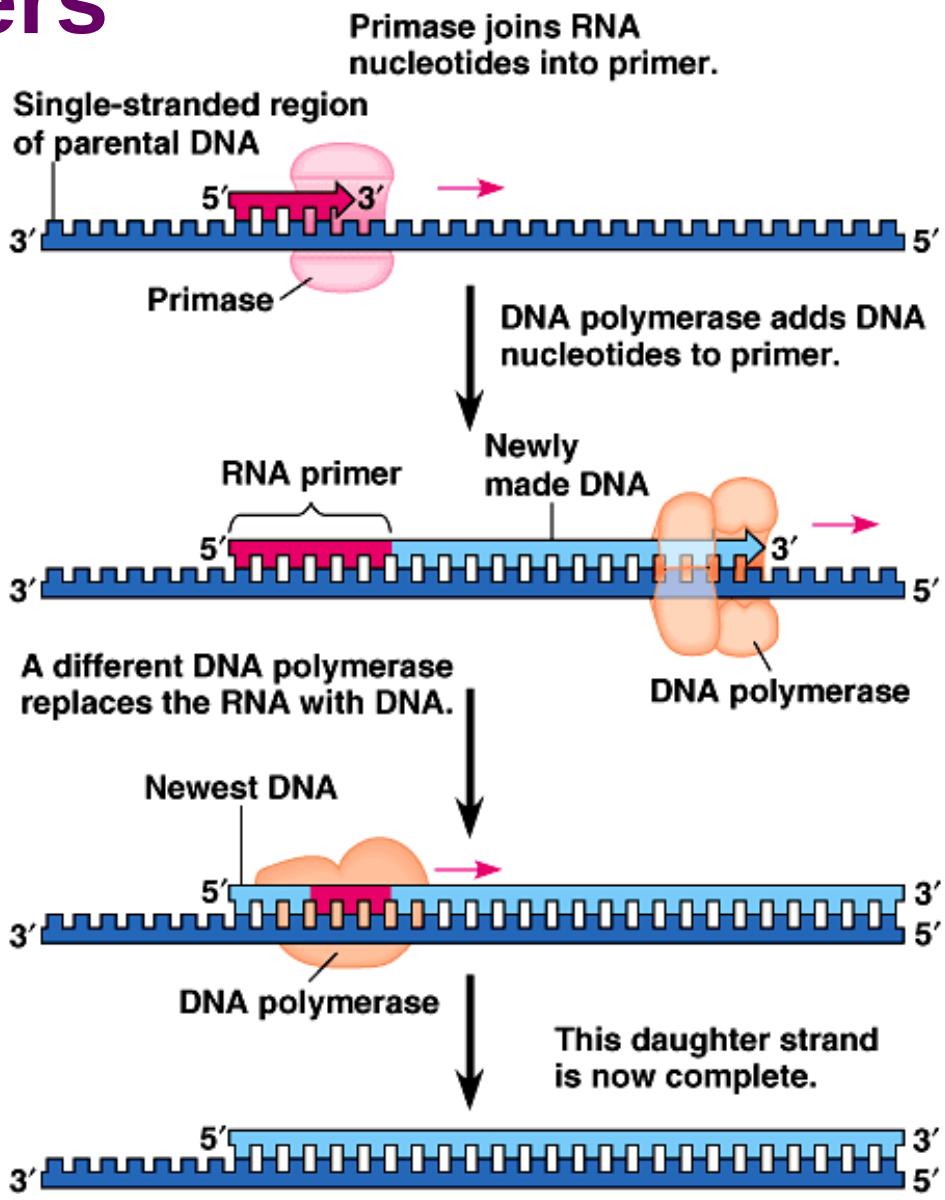
Priming DNA synthesis

- **DNA polymerase III can only extend an existing DNA molecule**
  - **cannot start new one**
    - **cannot place first base**
  - **short RNA primer is built first by primase**
    - **starter sequences**
    - **DNA polymerase III can now add nucleotides to RNA primer**



Cleaning up primers

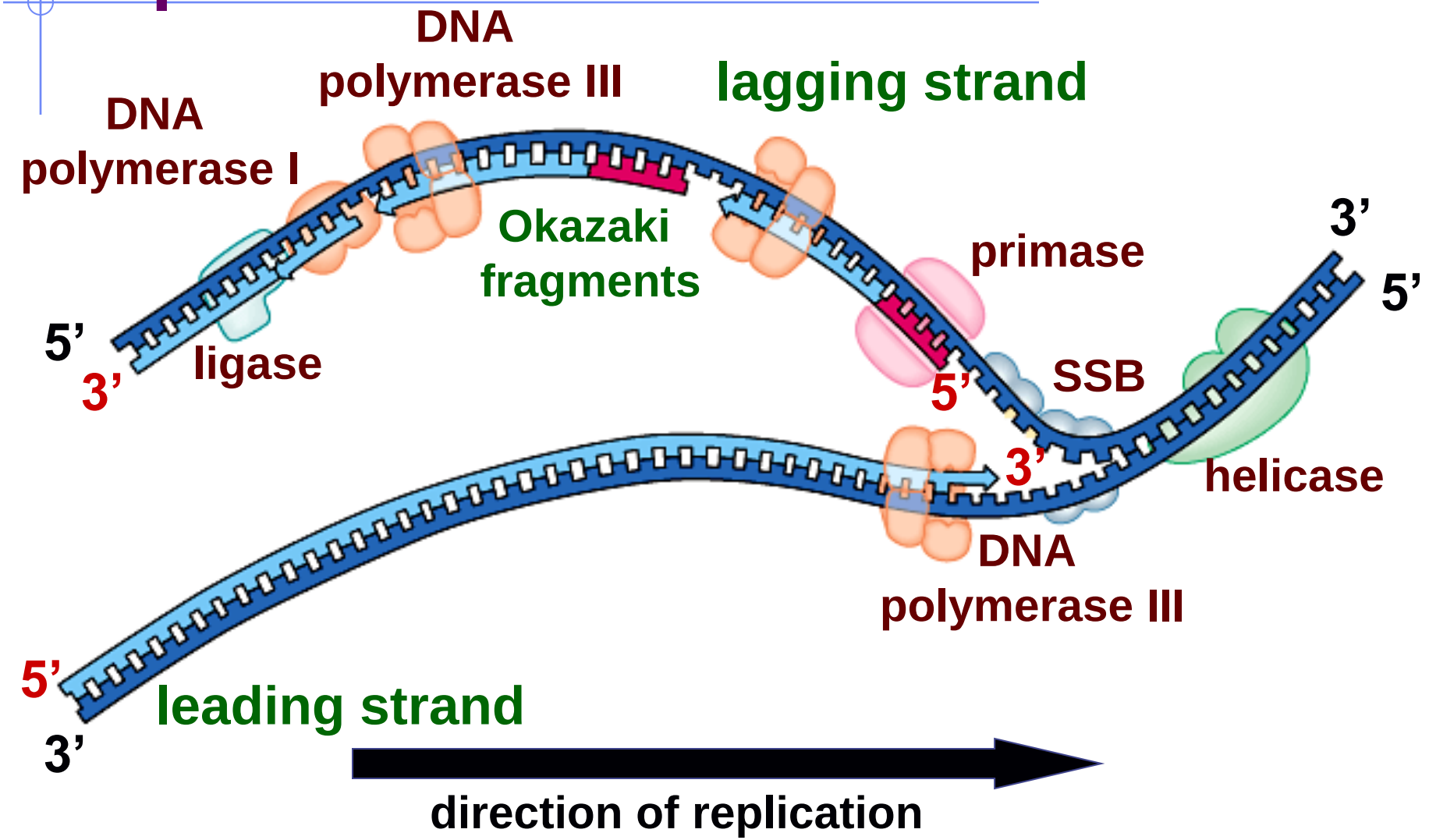
DNA polymerase I removes sections of RNA primer and replaces with DNA nucleotides



McGraw-Hill Replication Fork

Replication Fork animation

Replication fork

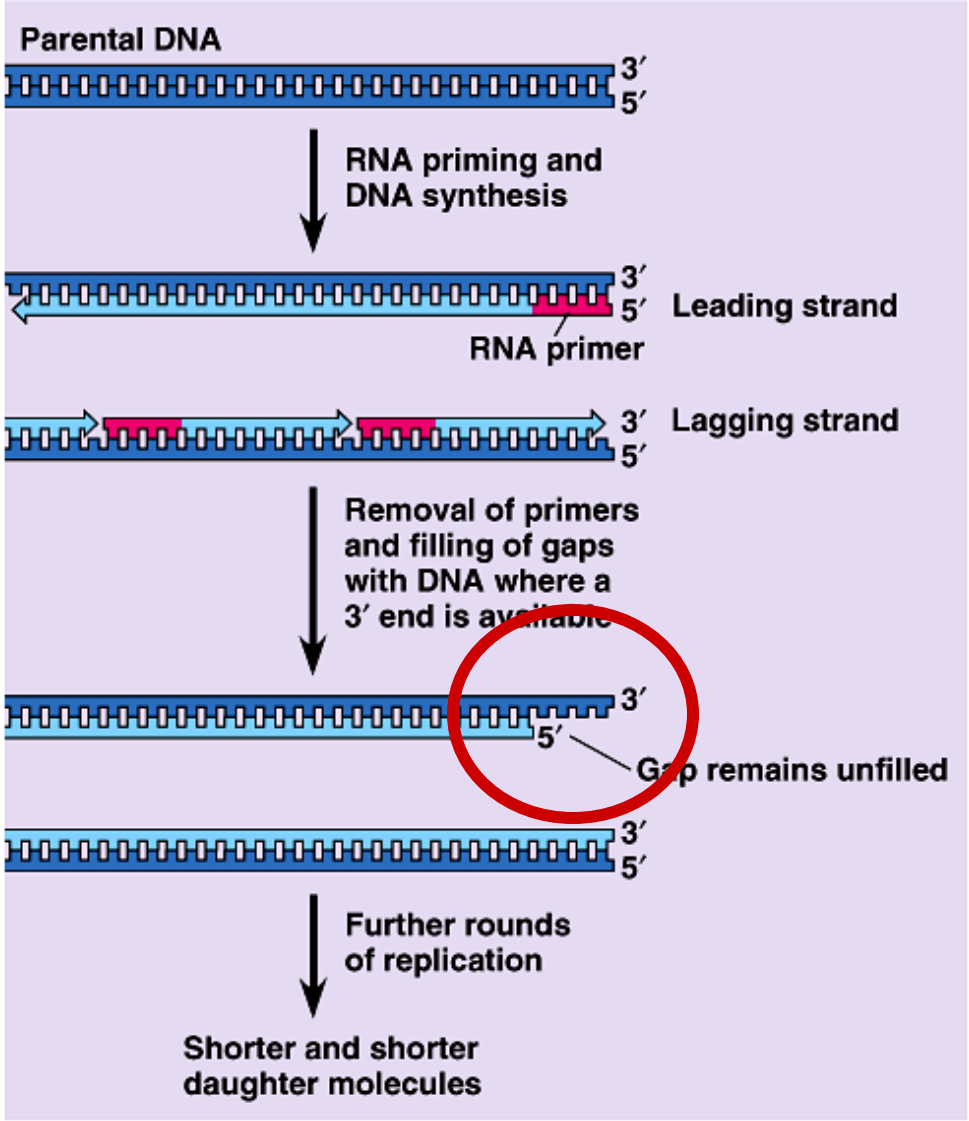


Animation: Replication

- http://www.youtube.com/watch?v=teV62zrm2P0

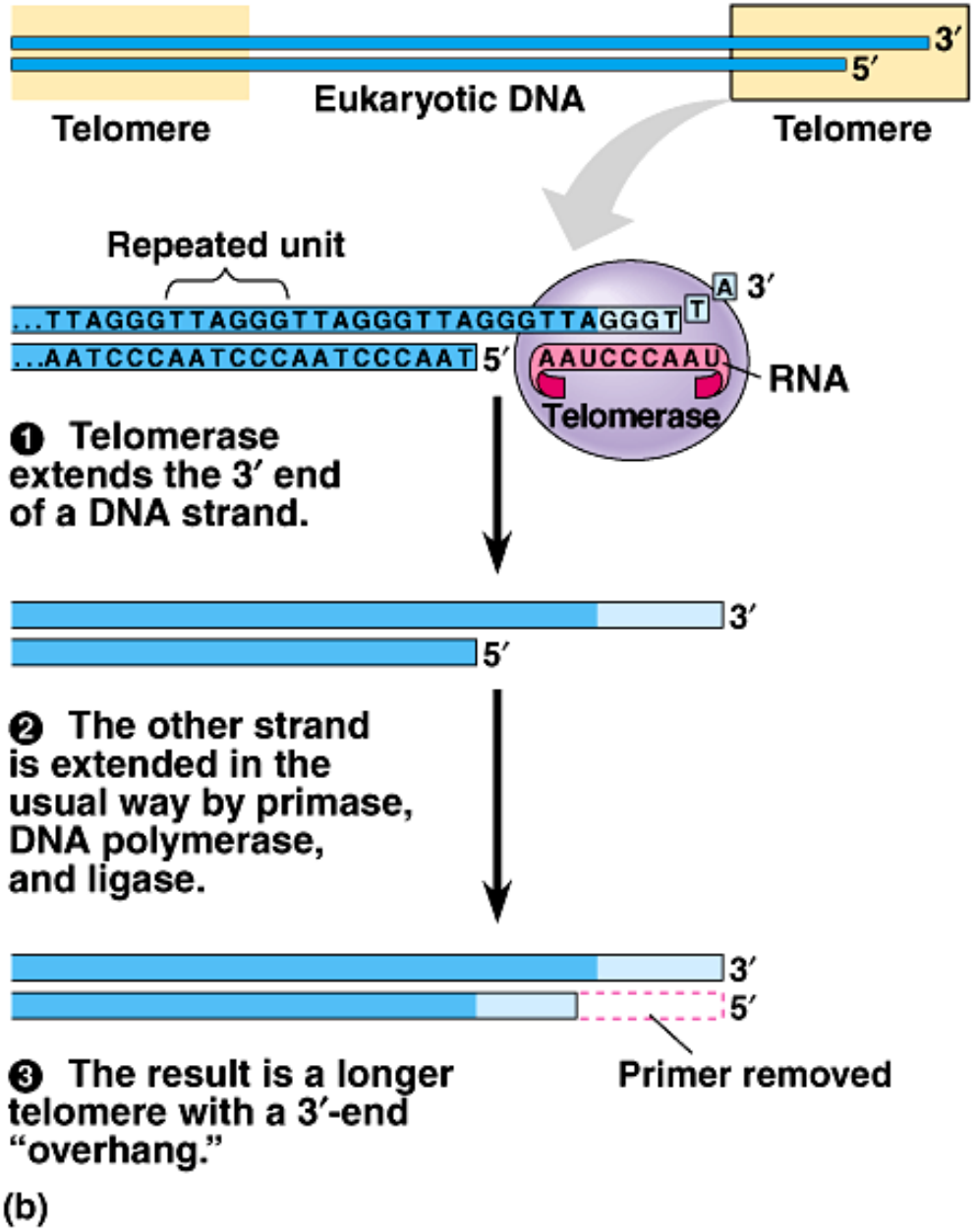
And in the end…

- Ends of chromosomes are eroded with each replication
  - an issue in aging?
  - ends of chromosomes are protected by telomeres



Telomeres

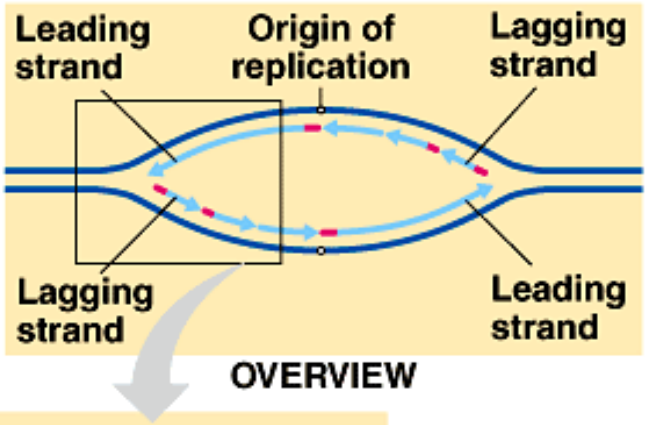
- **Expendable, non-coding sequences at ends of DNA**
  - short sequence of bases repeated 1000s times
  - **TTAGGG** in humans
- **Telomerase enzyme in certain cells**
  - enzyme extends telomeres
  - prevalent in cancers
    - **Why?**

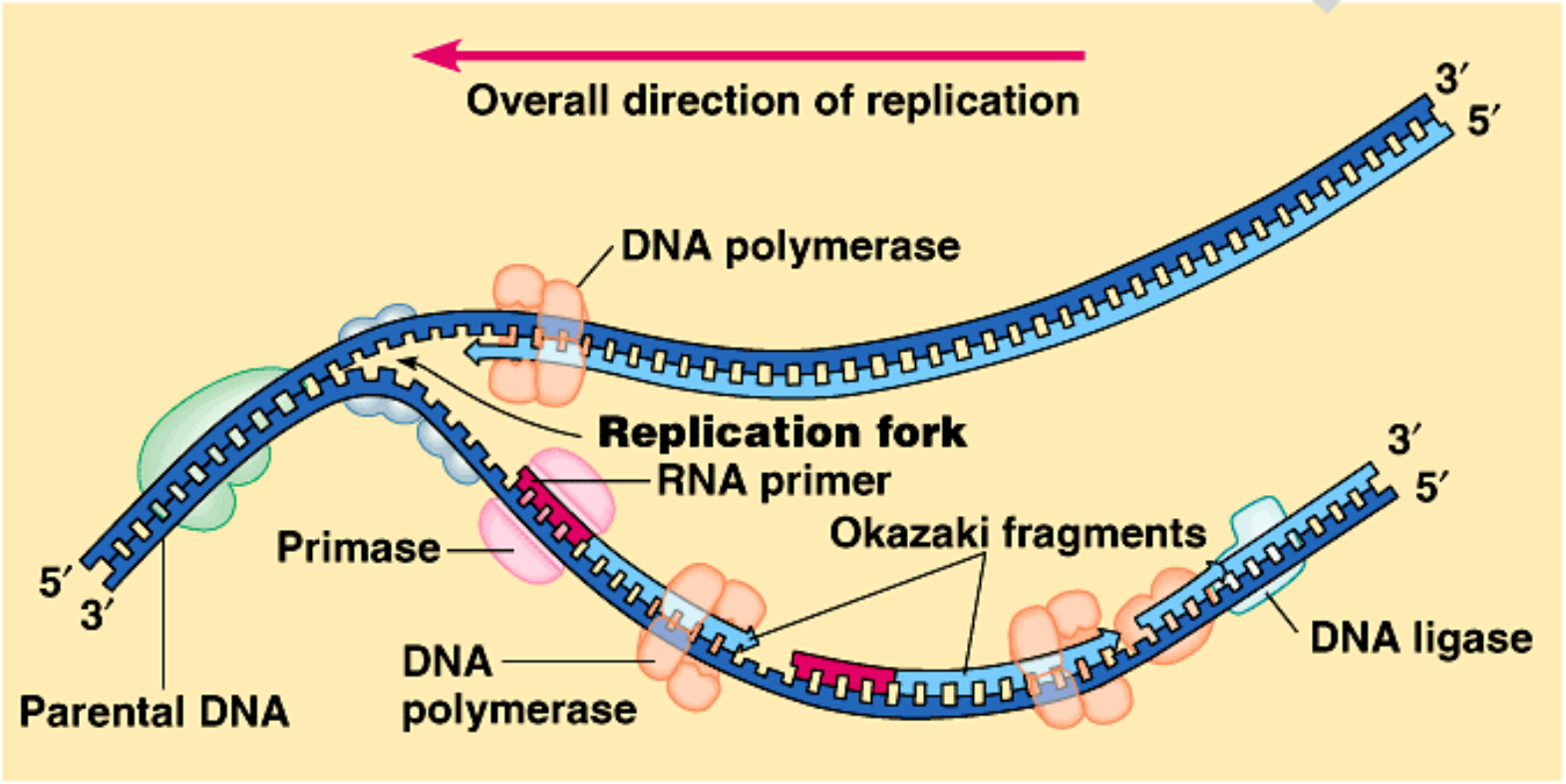


Replication bubble

Adds 1000 bases/second!

→ Which direction does DNA build?
→ List the enzymes & their role

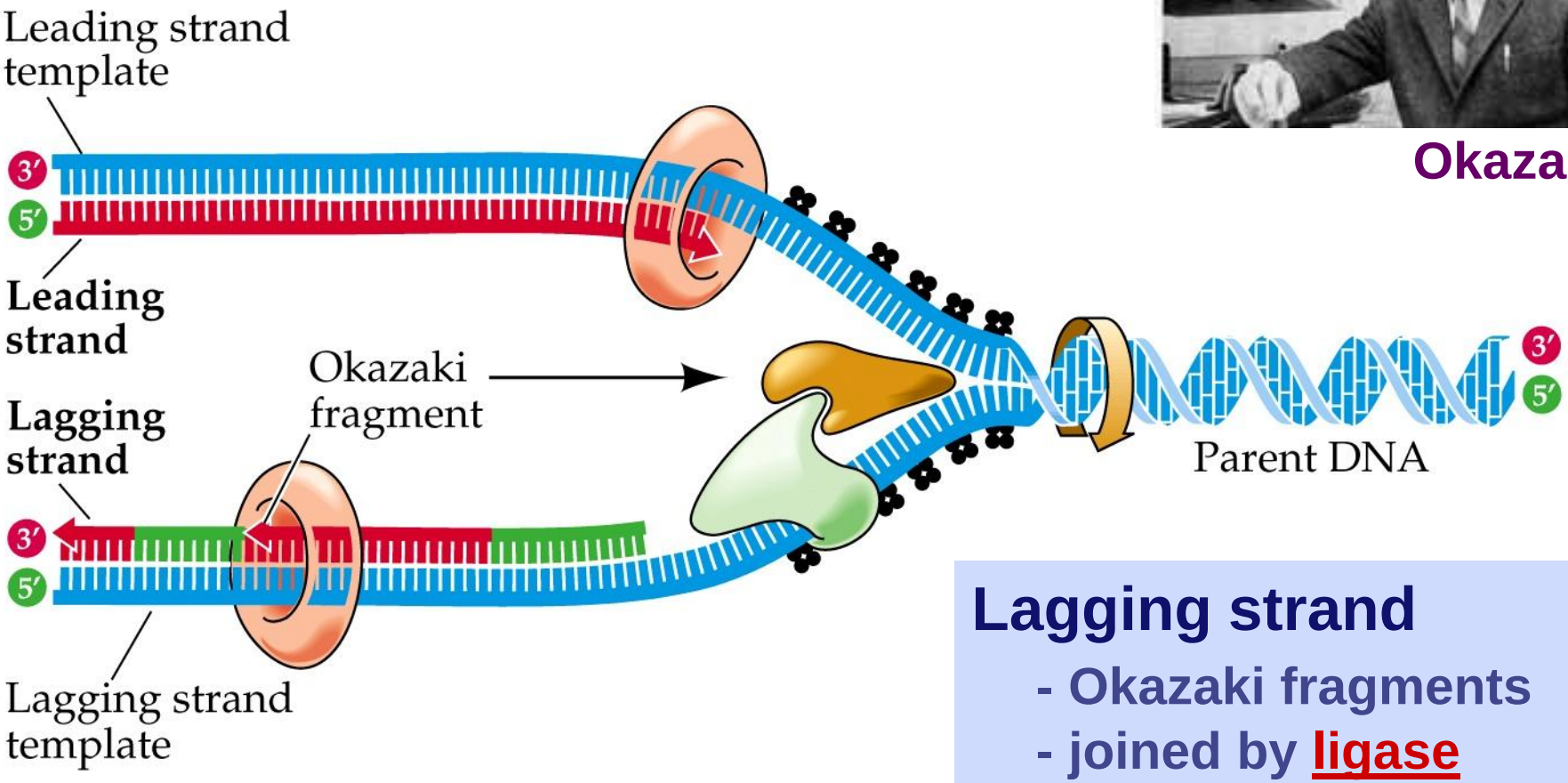




Replication: A Review

Leading strand
- continuous synthesis

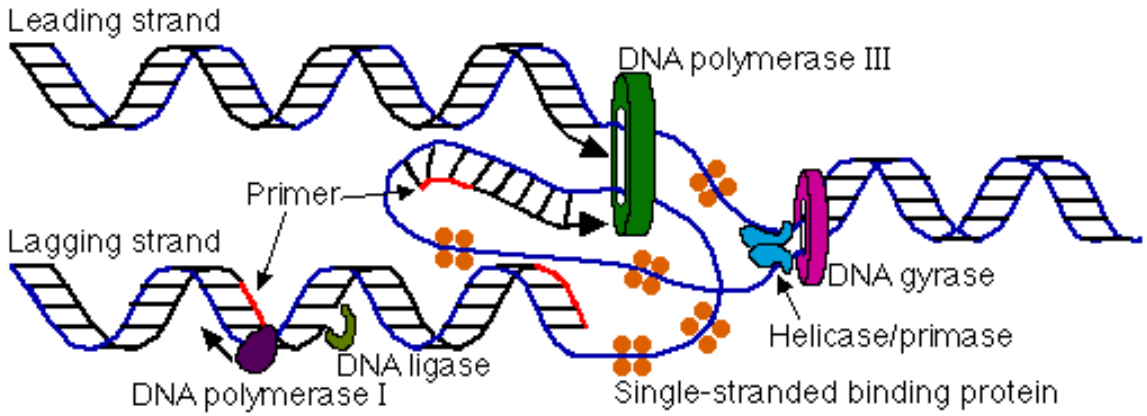
Okazaki

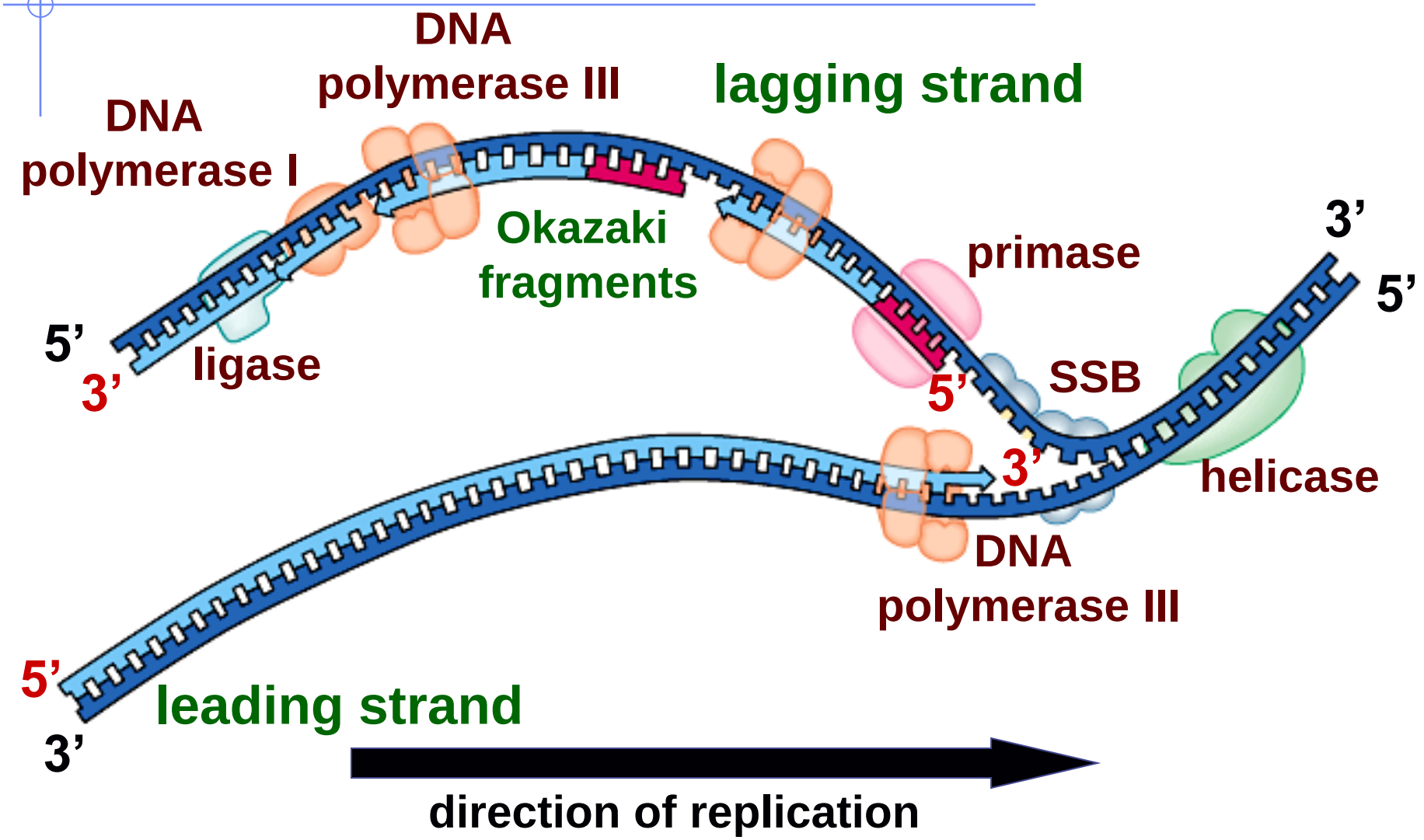


Lagging strand
- Okazaki fragments
- joined by ligase
  - "spot welder" enzyme

Replication enzymes

- helicase
- DNA polymerase III
- primase
- DNA polymerase I
- ligase
- single-stranded binding proteins

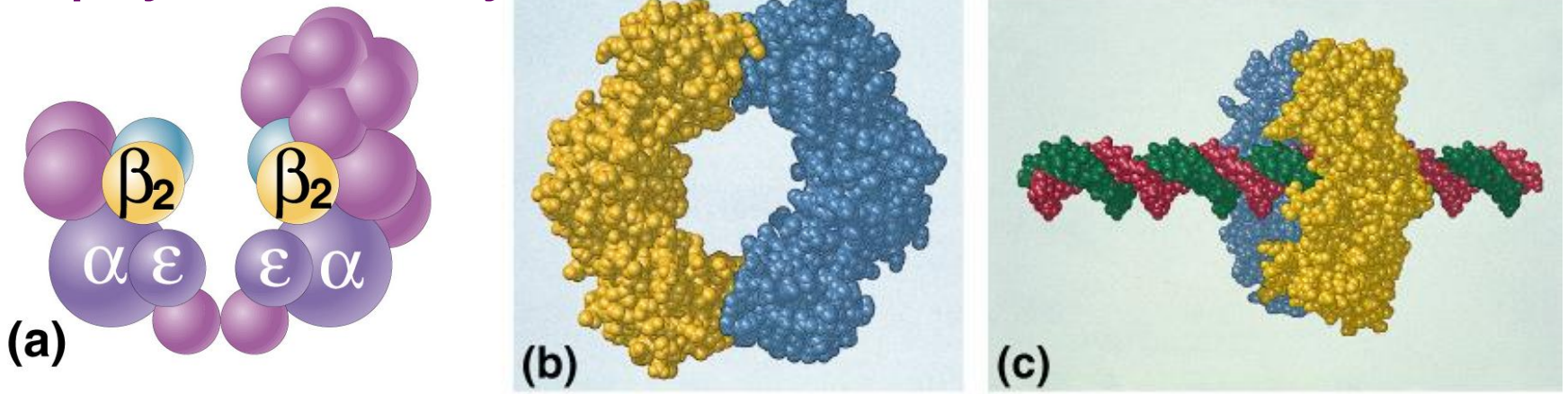



DNA
polymerase III
lagging strand
DNA
polymerase I
Okazaki
fragments
primase
3’
5’
5’
3’
ligase
5’
SSB
3’
helicase
DNA
polymerase III
5’
3’
leading strand
direction of replication

DNA polymerases

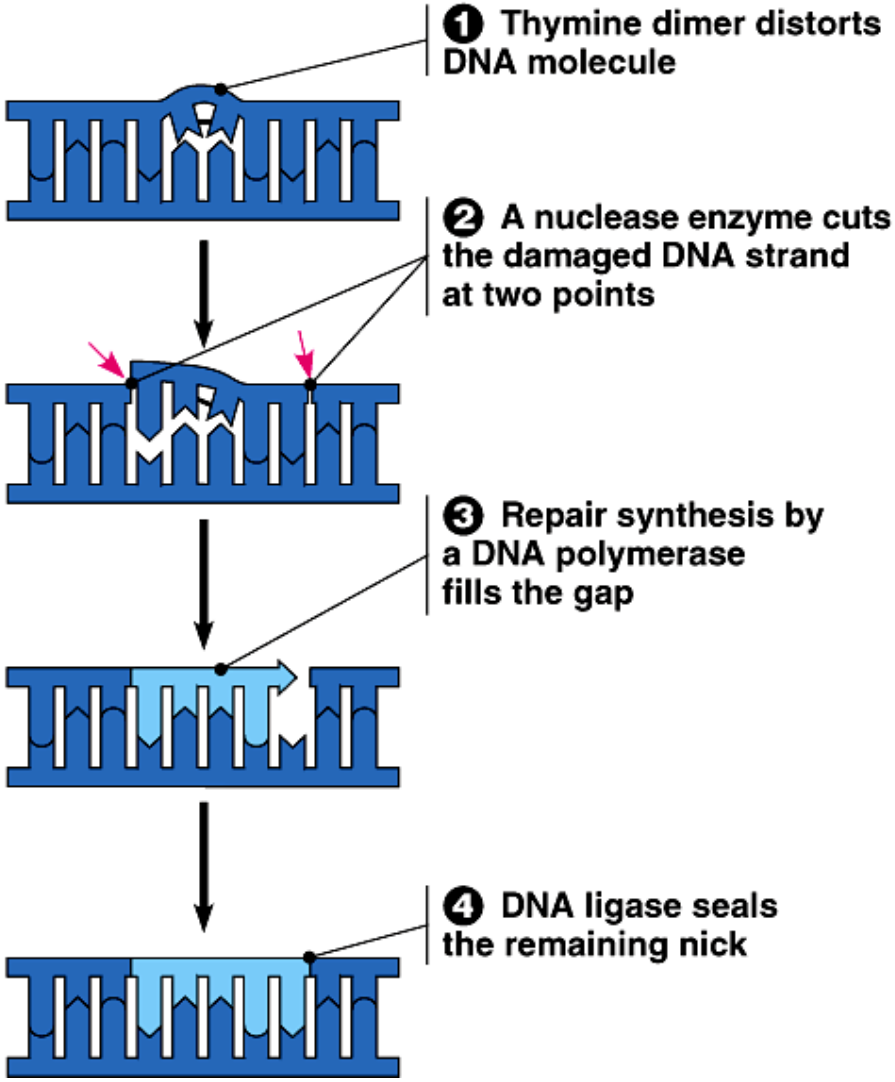
- DNA polymerase III
  - 1000 bases/second
  - main DNA building enzyme
- DNA polymerase I
  - 20 bases/second
  - editing, repair & primer removal

DNA polymerase III enzyme



Editing & proofreading DNA

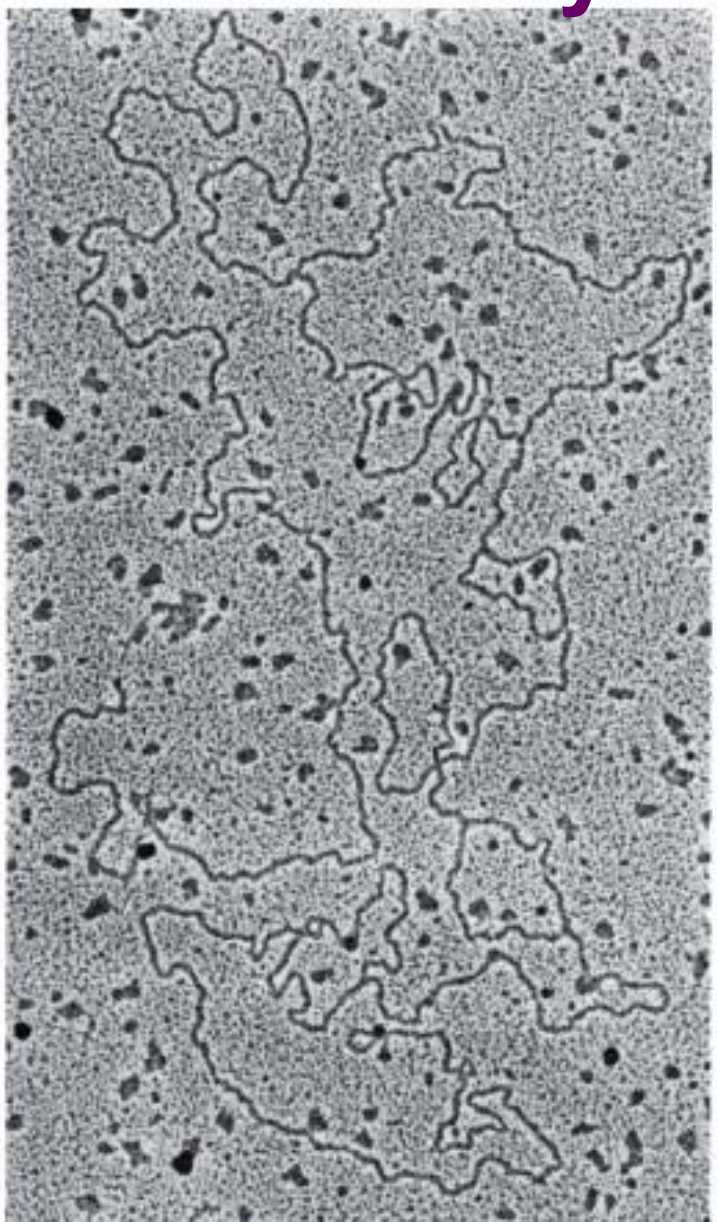
- 1000 bases/second = lots of typos!
- DNA polymerase I
  - proofreads & corrects typos
  - repairs mismatched bases
  - excises abnormal bases
    - repairs damage throughout life
  - reduces error rate from 1 in 10,000 to 1 in 100 million bases



Fast & accurate!

- It takes *E. coli* <1 hour to copy 5 million base pairs in its single chromosome
  - divide to form 2 identical daughter cells
- Human cell copies its 6 billion bases & divide into daughter cells in only few hours
  - remarkably accurate
  - only ~1 error per 100 million bases
  - ~30 errors per cell cycle

What's it really look like?



The “Central Dogma”

- flow of genetic information within a cell