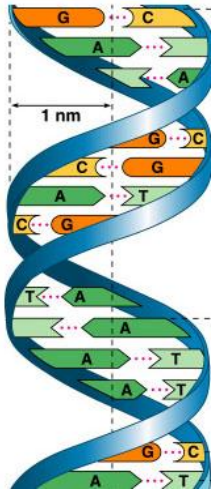


Chapter 10.

The Genetic Material ???

Google™

DNA History

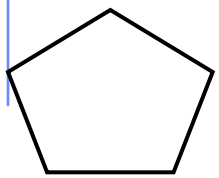
- It took many years and the work of many different scientists to discover that **DNA** is the genetic material
  - Friedrich Meischer (1869)
  - P.A. Levene (1920s)
  - Frederick Griffith (1928)
  - Avery, McCarty & MacLeod (1944)
  - Ervin Chargaff (1947)
  - Hershey & Chase (1952)
  - Rosalind Franklin & Maurice Wilkins (1953)
  - Watson & Crick (1953)

Friedrich Meischer (1869)

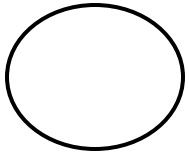
- As he studied **salmon sperm** for clues to understand heredity, he was the first to isolate a material called **nuclein** from the nucleus of the sperm.
- Chemical tests revealed that nuclein was part **protein** and part **DNA**
- Another clue that made him go “Hmmm” was when he later isolated nuclein from the nuclei of white blood cells in **pus**-soaked medical bandages.

P.A. Levene (1920s)

- Discovered that DNA is made from smaller **L**-shaped "building blocks" called **nucleotides** that have **3** chemical parts:

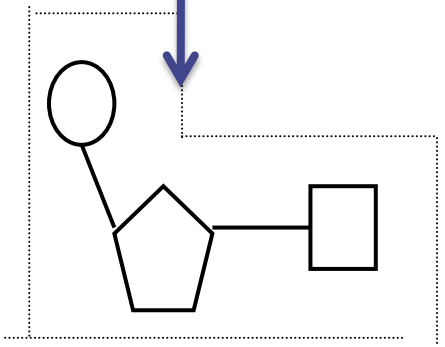


→ 5-C sugar = deoxyribose

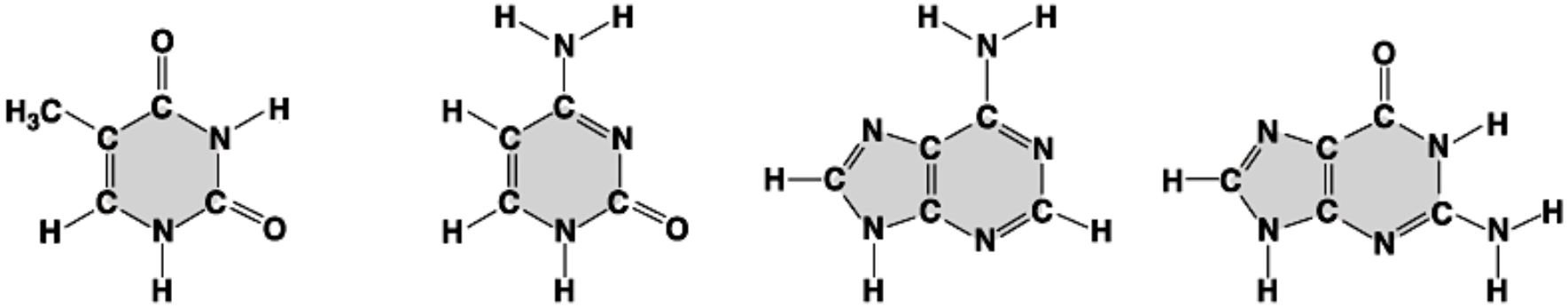


→ phosphate group (PO_4)

→ nitrogen base: 1 of 4



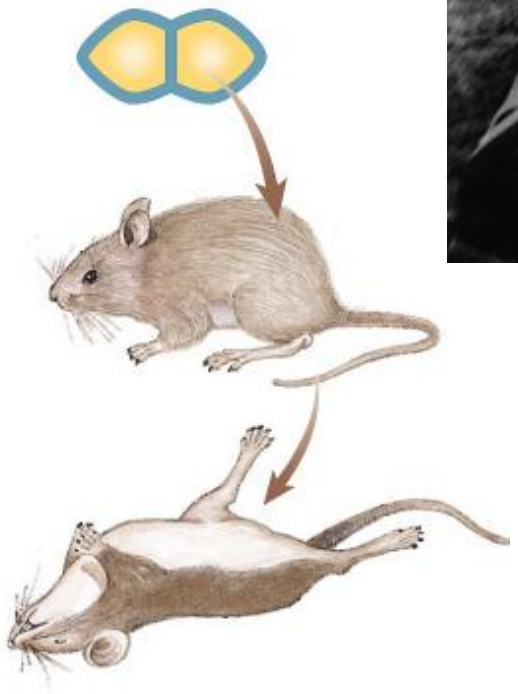
- DNA has four kinds of N-bases, A (Adenine), T (Thymine), C (Cytosine), and G (Guanine)



1928

Fred Griffith (1928)

- While working on a cure for pneumonia with mice, he accidentally discovered the mystery of bacterial transformation



Griffith's 4-part experiments

A. live pathogenic (smooth = S) strain of *bacteria*

mice die

B. live non-pathogenic (rough = R) strain of *bacteria*

mice live

C. heat-killed pathogenic (S) *bacteria*

mice live

D. mix heat-killed pathogenic (S) & living (R) *bacteria*

mice die

Explanation? How could there be living S bacteria??

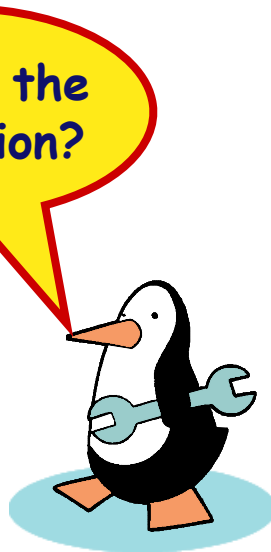
2 Hypotheses:

- 1) **Resurrection**
  - **Somehow the dead S bacteria came back to life ???**
- 2) **Transformation**
  - **Some chemical factor in the dead S bacteria was able to communicate with and transform the non-harmful R bacteria into killer S bacteria**
- Fred Griffith would spend many more years in his lab trying to identify this mysterious "**transforming factor**"

1944

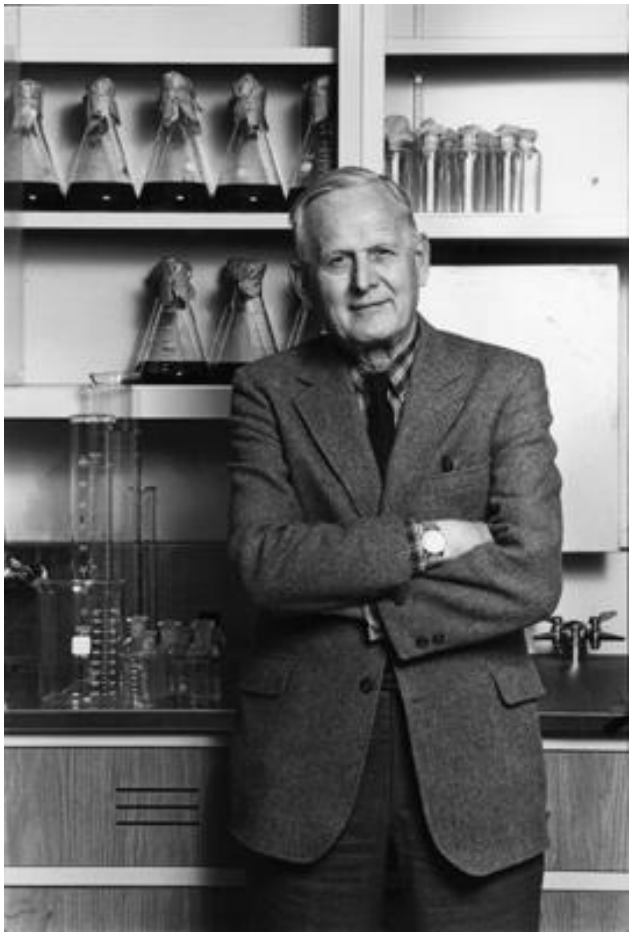
Avery, McCarty & MacLeod (1944)

- 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 (R) bacteria into virulent (S)bacteria
- DNA is the "Transforming Factor"



Avery, McCarty & MacLeod

Oswald Avery

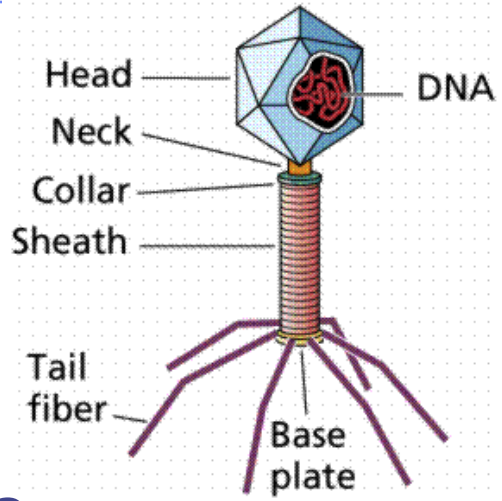


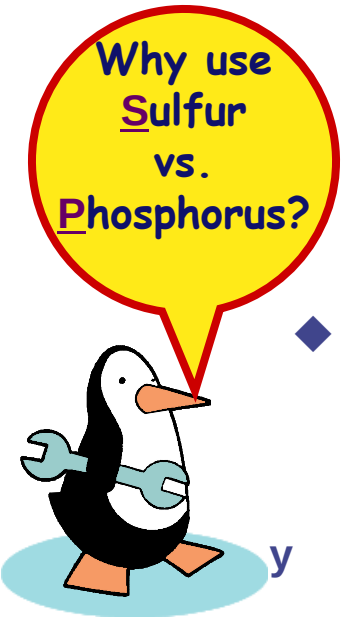
Maclyn McCarty

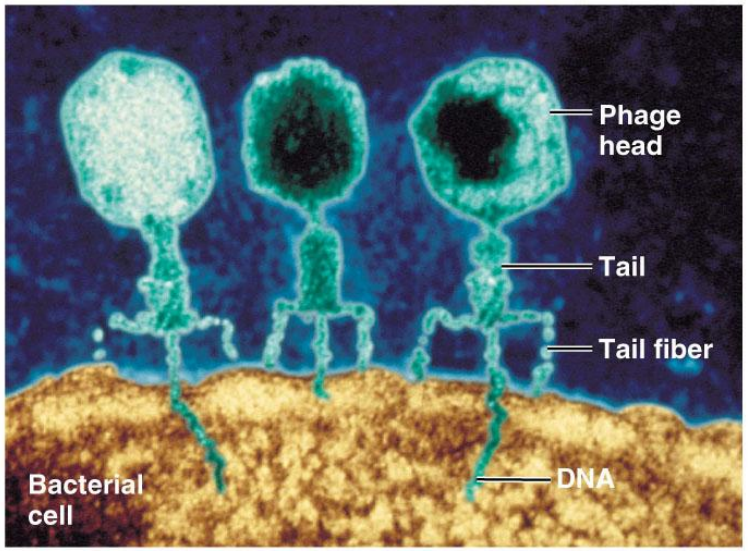
Colin MacLeod

Confirmation of DNA = heredity molecule

- **Hershey & Chase (1952)**
  - 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



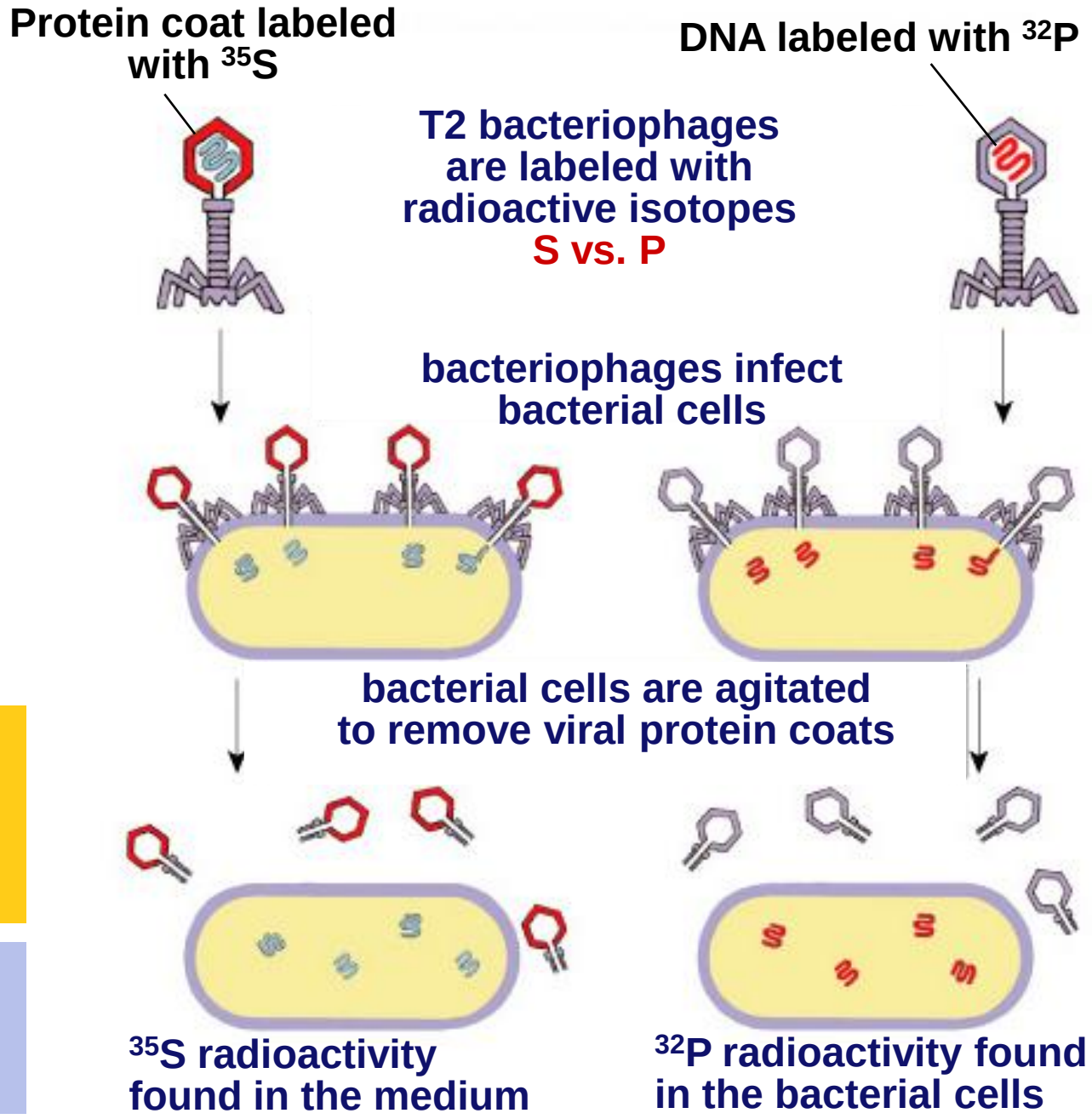




Hershey & Chase

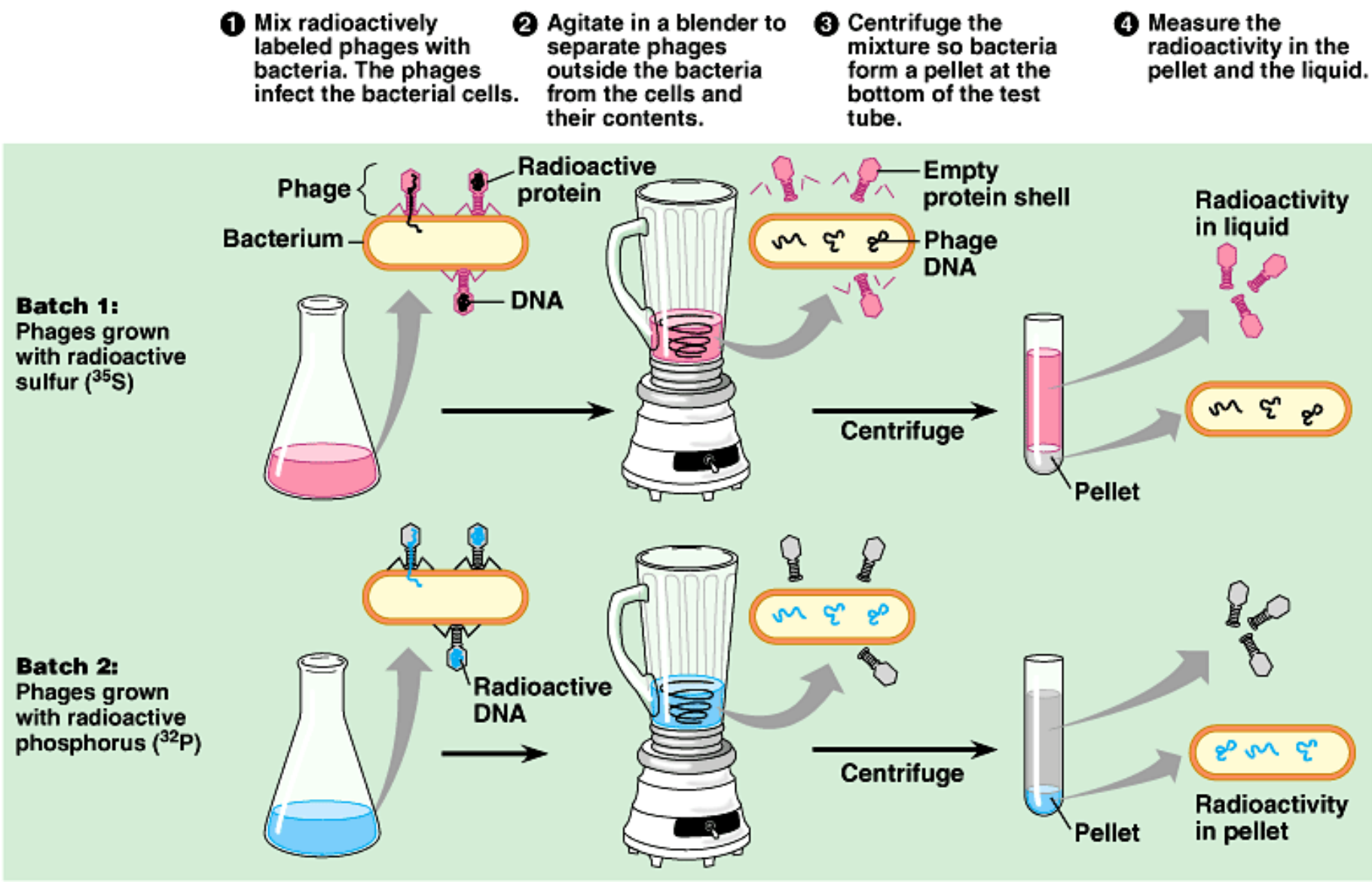
Martha Chase Alfred Hershey

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 conclusions

- **Radioactive phage & bacteria in blender**
  - **^{35}S phage = indicates protein**
    - radioactive proteins stayed in upper liquid
    - therefore protein did NOT enter bacteria
  - **^{32}P phage = indicates DNA**
    - radioactive DNA found in lower pellet
    - therefore DNA did enter bacteria
  - **Confirmed DNA is "transforming factor"**

Chargaff's data:

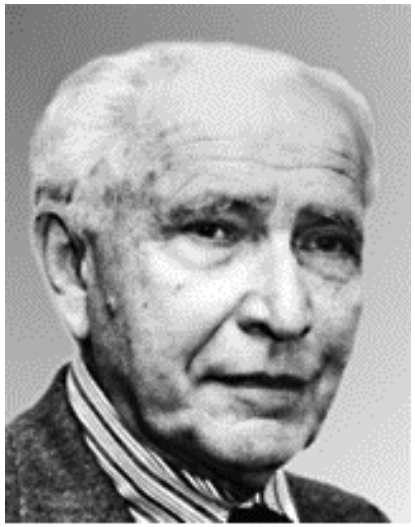
TABLE 11-2 Base Compositions in DNA from Selected Organisms

	Percentage of DNA Bases				Ratios	
Source of DNA	A	T	G	C	A/T	G/C
E. coli	26.1	23.9	24.9	25.1	1.09	0.99
Yeast	31.3	32.9	18.7	17.1	0.95	1.09
Sea urchin sperm	32.5	31.8	17.5	18.2	1.02	0.96
Herring sperm	27.8	27.5	22.2	22.6	1.01	0.98
Human liver	30.3	30.3	19.5	19.9	1.00	0.98
Corn (*zea mays*)	25.6	25.3	24.5	24.6	1.01	1.00

1947

Chargaff (1947)

- **DNA composition: "Chargaff's rules"**
  - varies from species to species
  - all 4 bases not in equal quantity
  - bases present in **characteristic ratio**
    - **humans:**



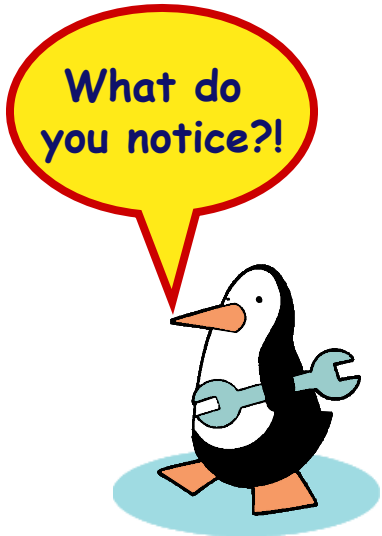
Erwin Chargaff

A = 30.9%

T = 29.4%

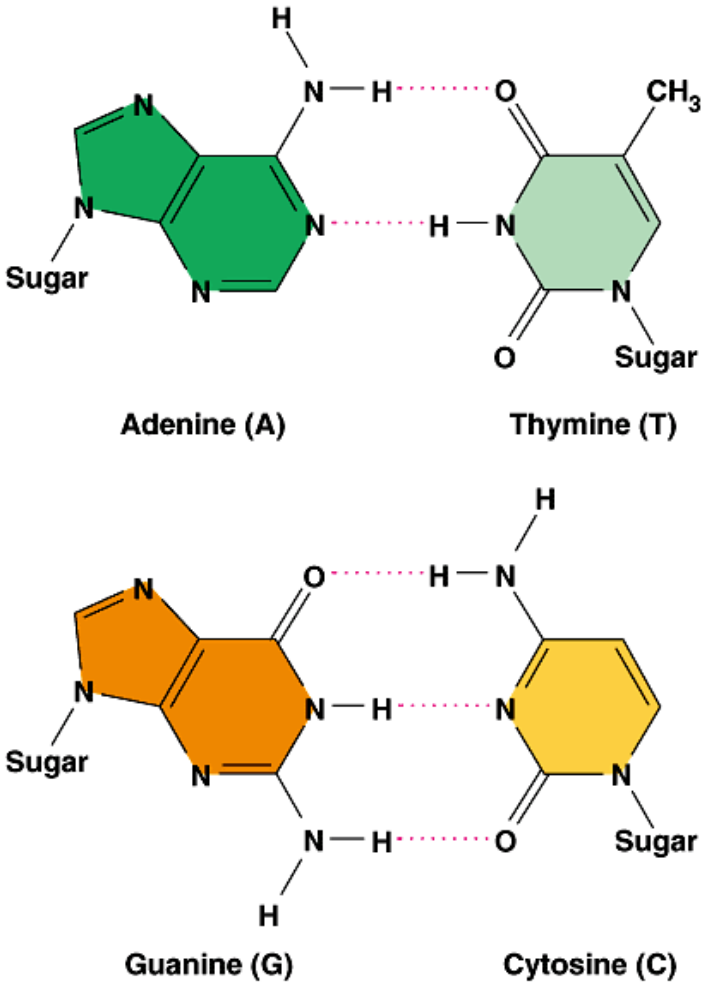
G = 19.9%

C = 19.8%



Chargaff's big clue: Base Pair RATIOS in DNA

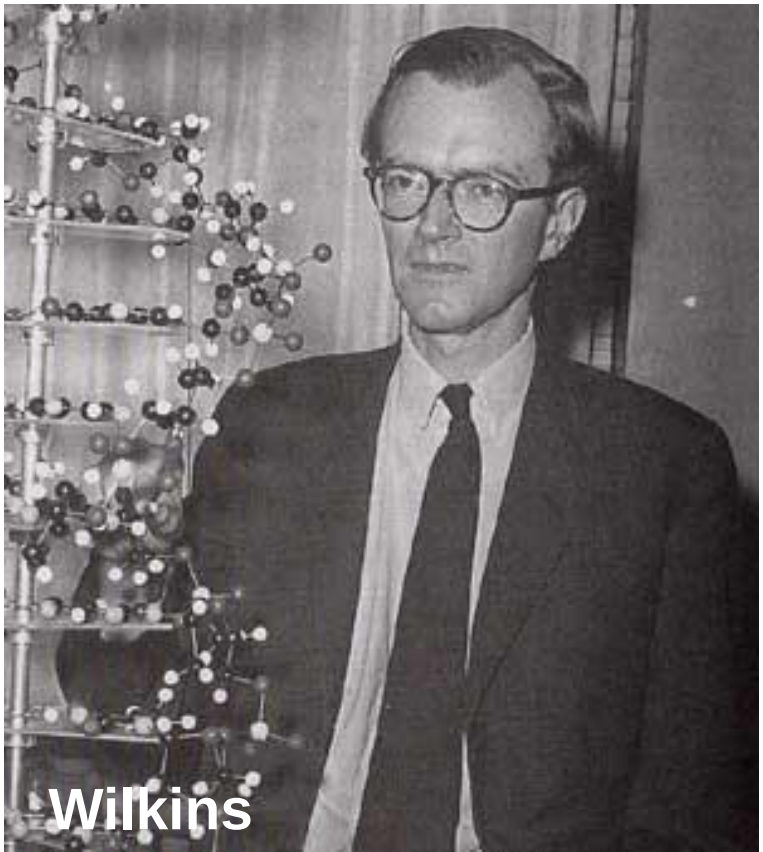
- **Purines**
  - adenine (A)
  - guanine (G)
- **Pyrimidines**
  - thymine (T)
  - cytosine (C)
- **Pairing**
  - A : T
  - C : G



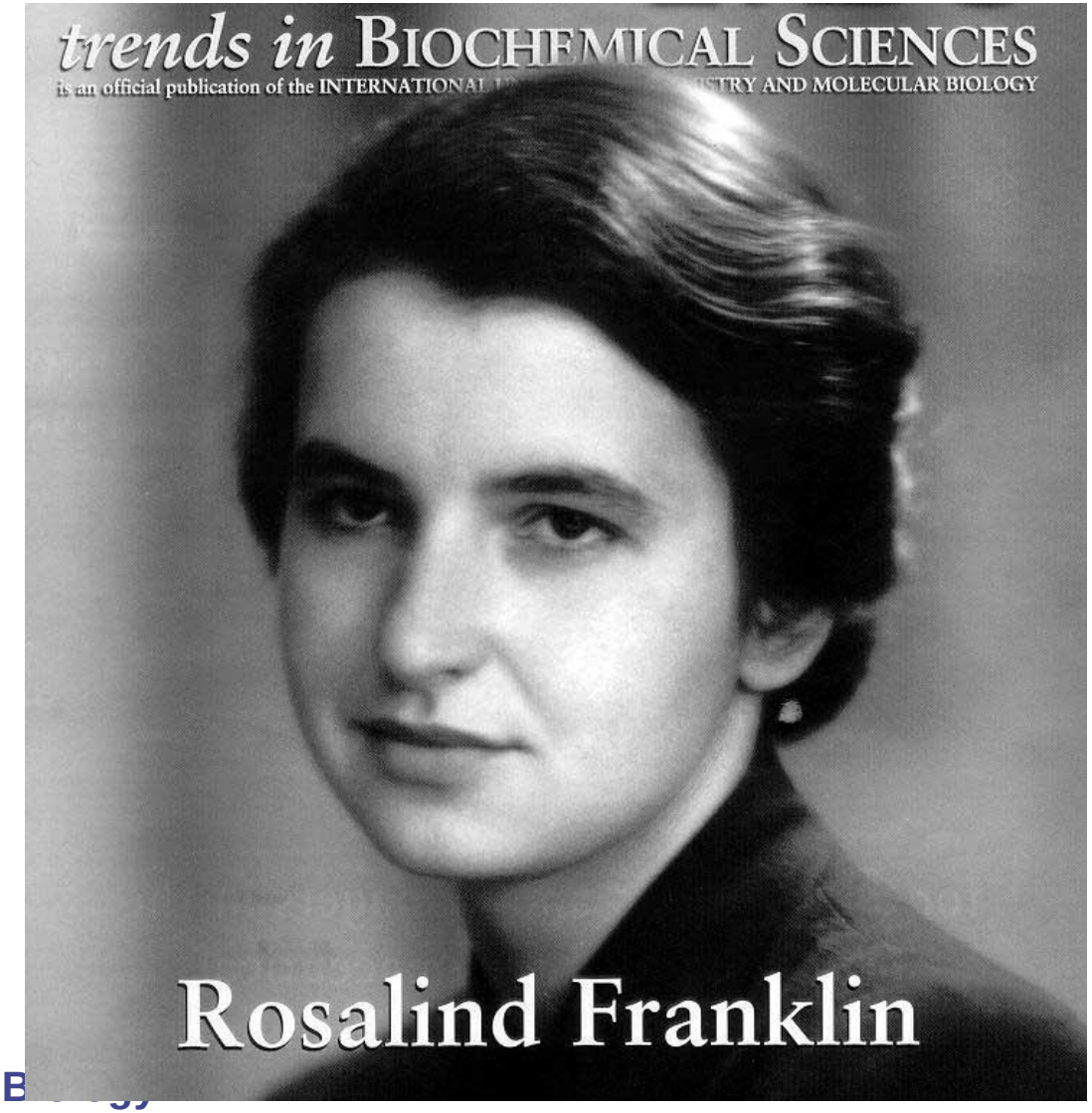
Another clue: Franklin & Wilkins (1953)

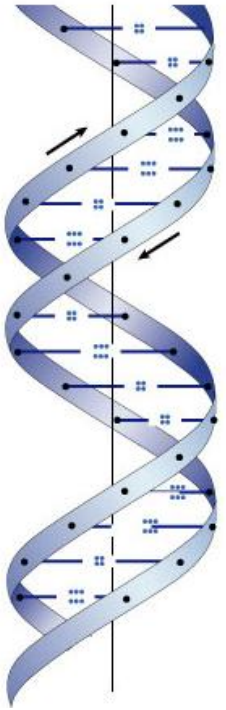
- X-ray pictures of DNA suggest that it consists of 2 chains twisted in a helix

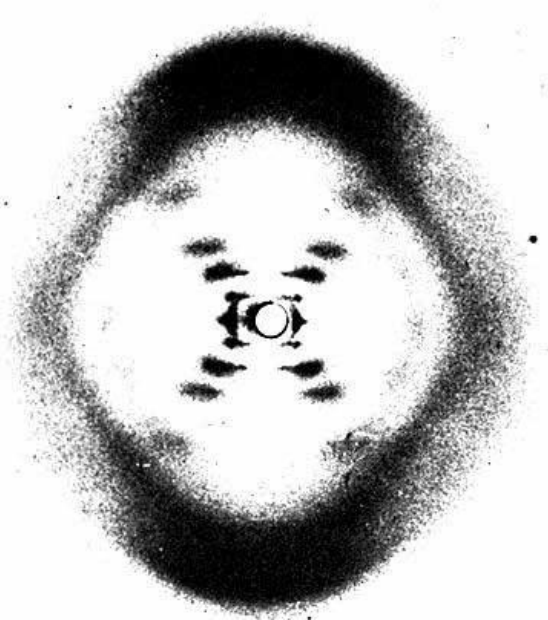

Franklin


Wilkins

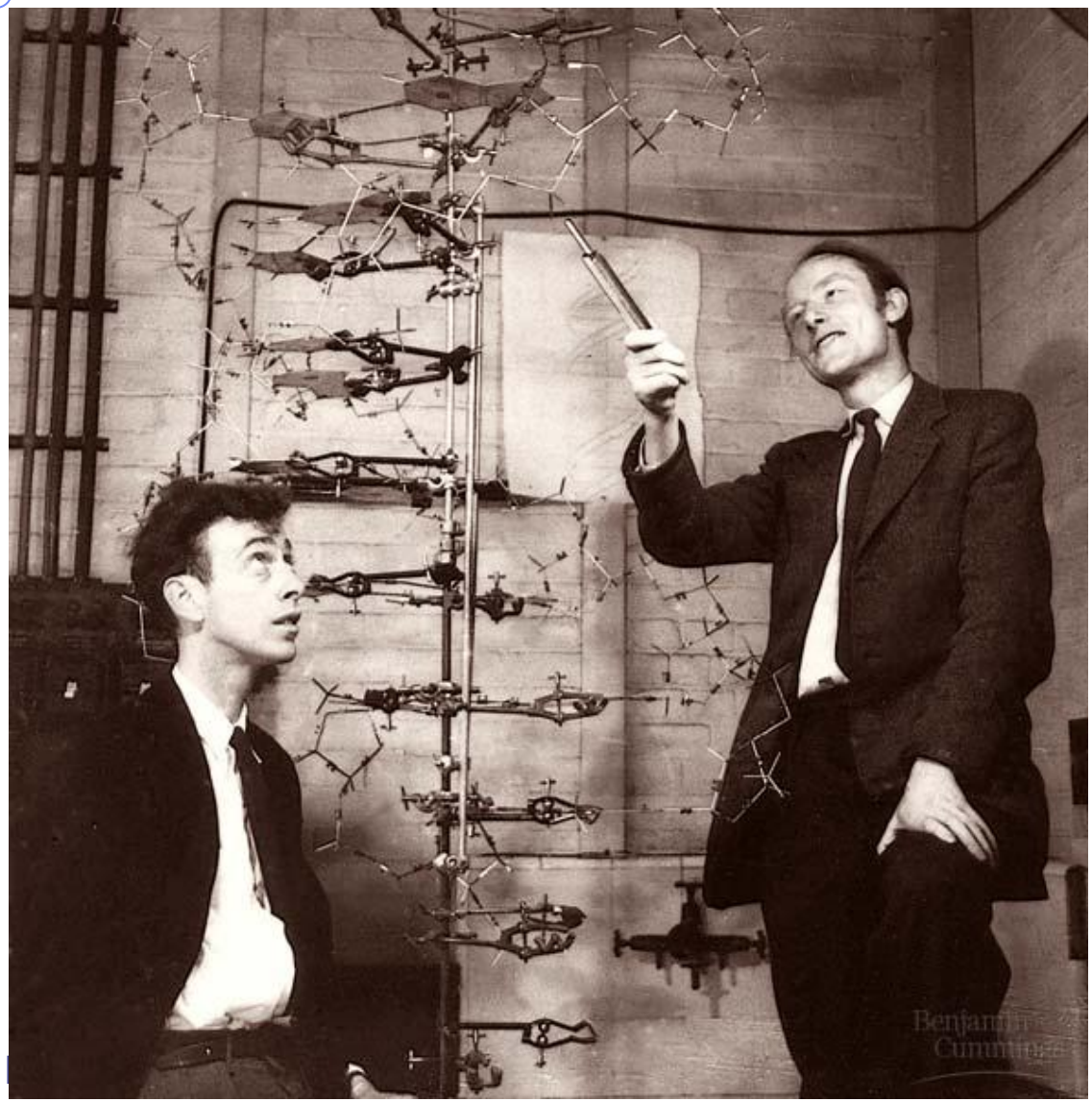
Rosalind Franklin (1920-1958)

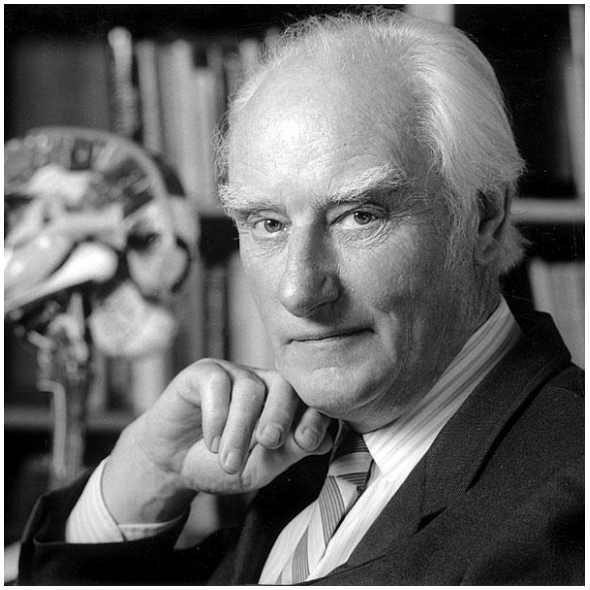






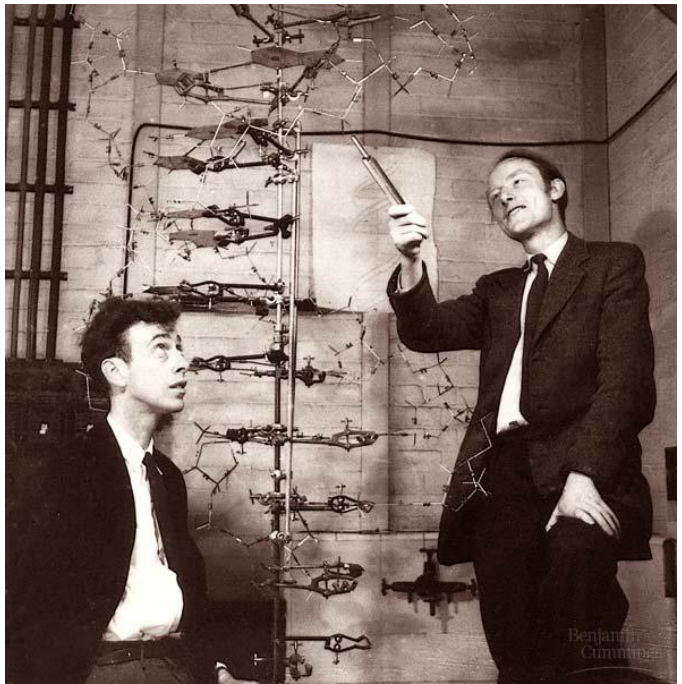
The “Fathers” of DNA Structure

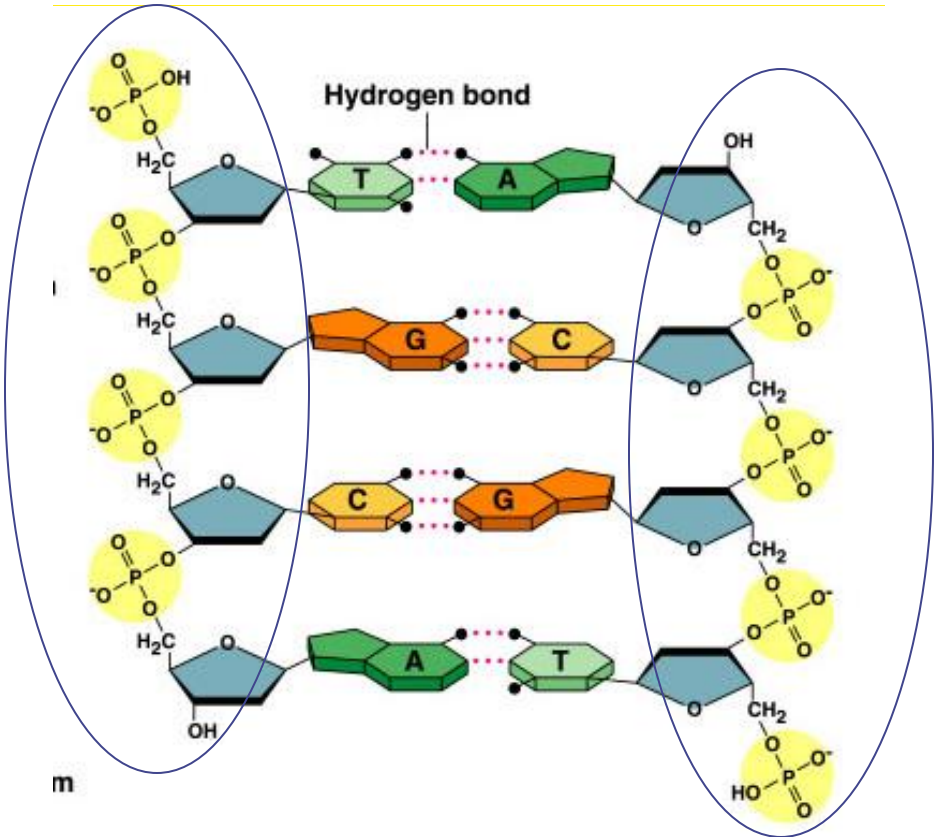




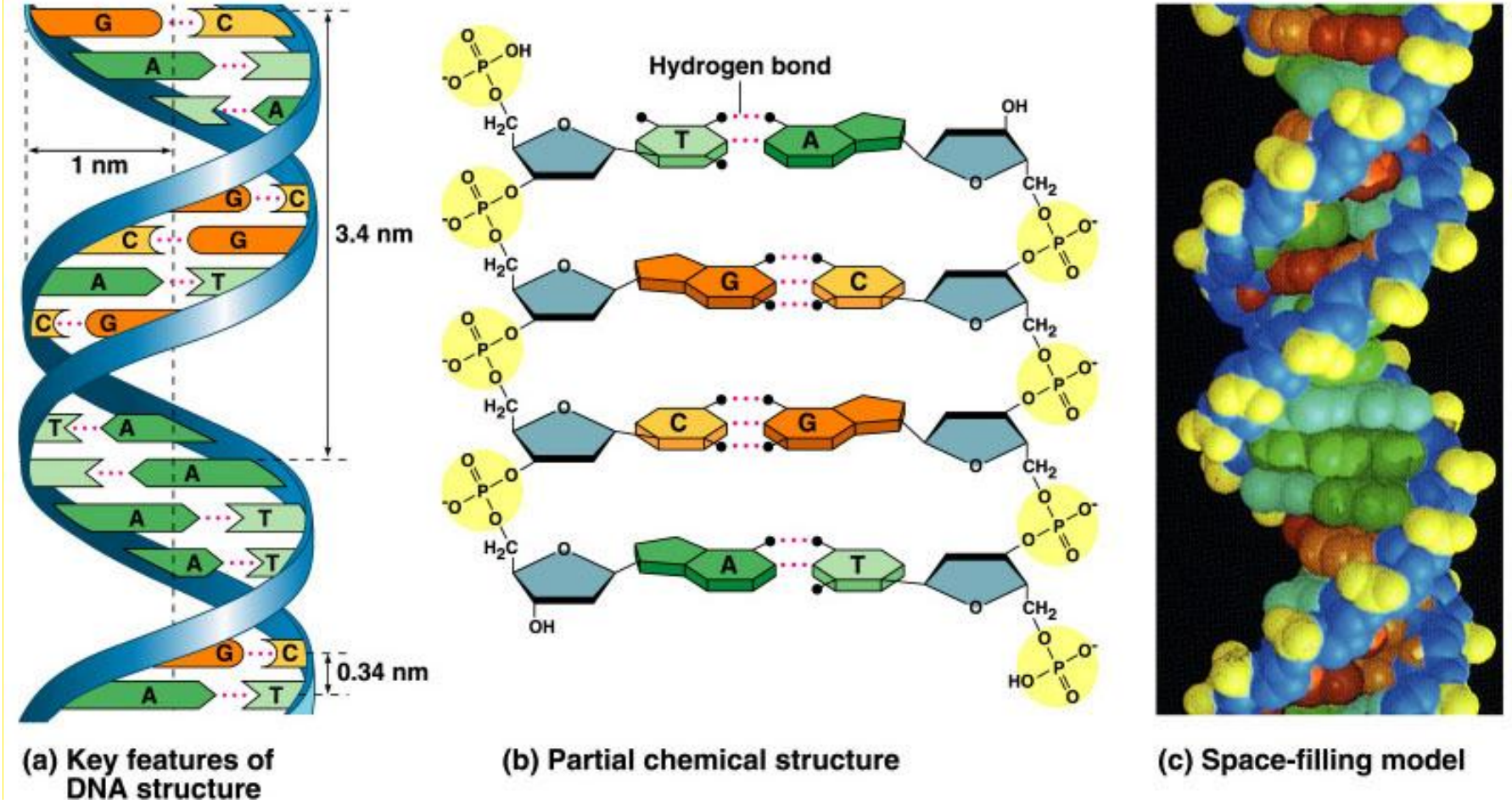
Watson and Crick (1953)

- Win the "race" to build the first accurate model of DNA
- **Double Helix** Model = 2 DNA chains that spiral like a "twisted rope ladder"
- ladder sides formed by alternating 5-C sugars & phosphates = **backbone**
- ladder rungs formed by nitrogen base pairs held together by **hydrogen** bonds
  - A – T (**2** H bonds)
  - C – G (**3** H bonds)





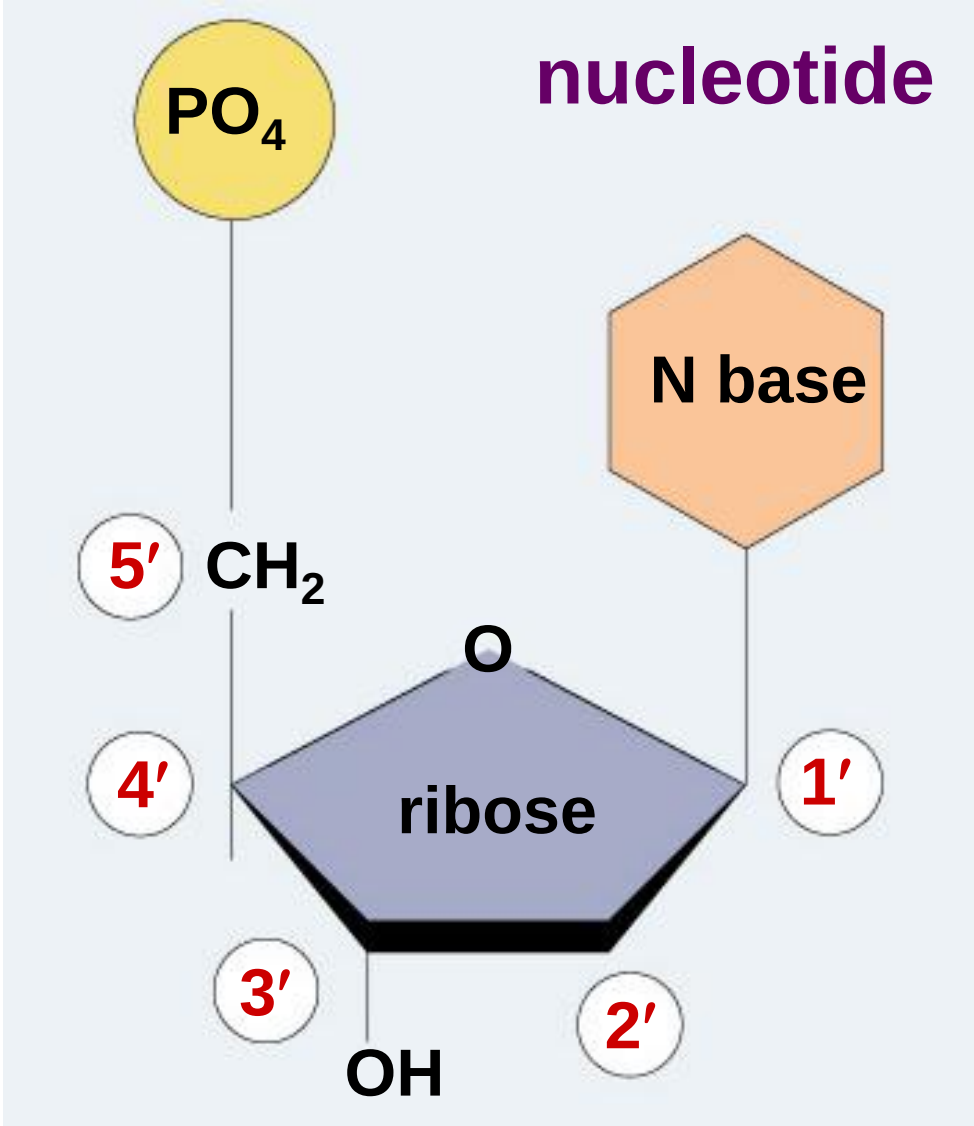
Double helix structure of DNA



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

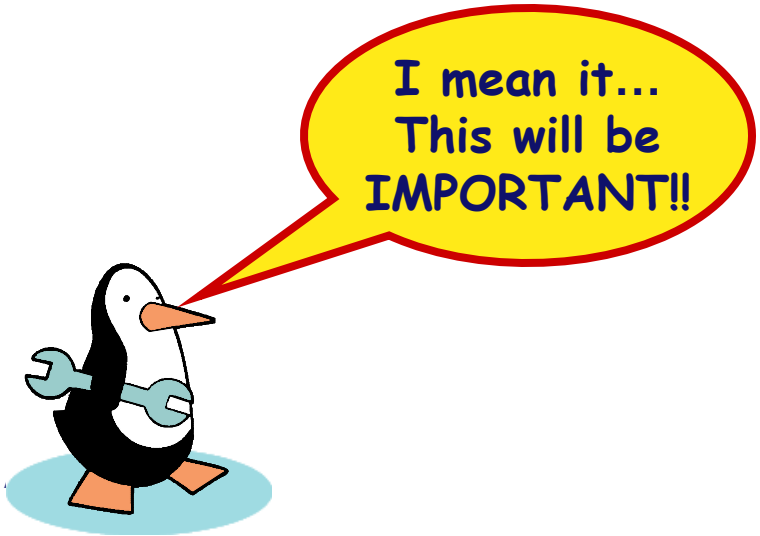
Directionality of DNA

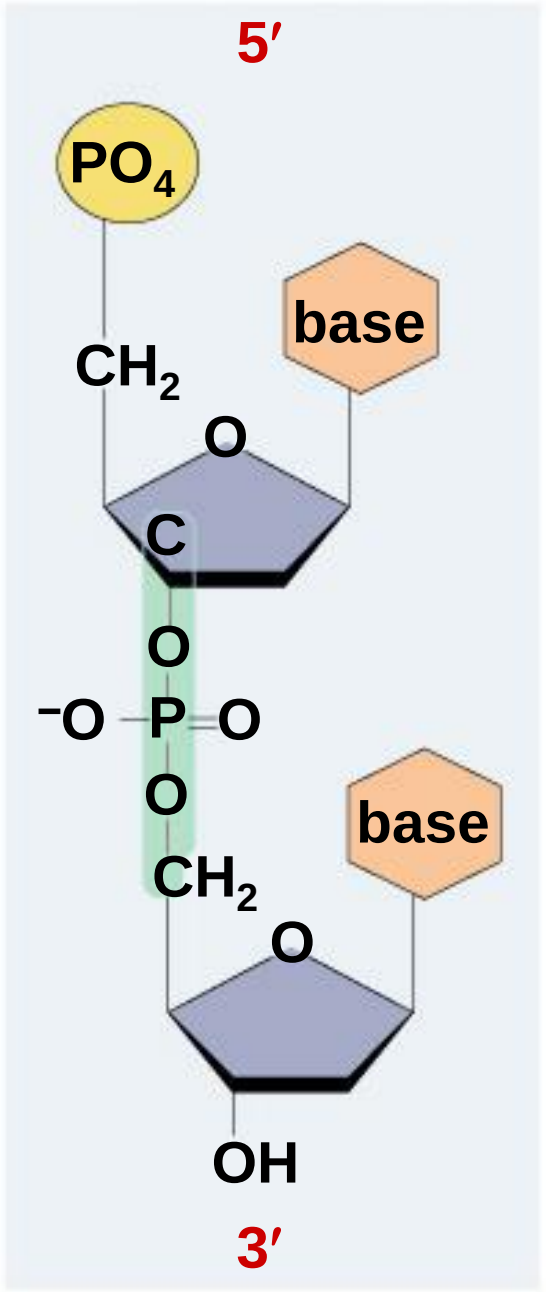
- You need to number the carbons!
  - it matters!



The DNA backbone

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





- **Each strand of the double helix is oriented in the opposite direction =**
  Antiparallel

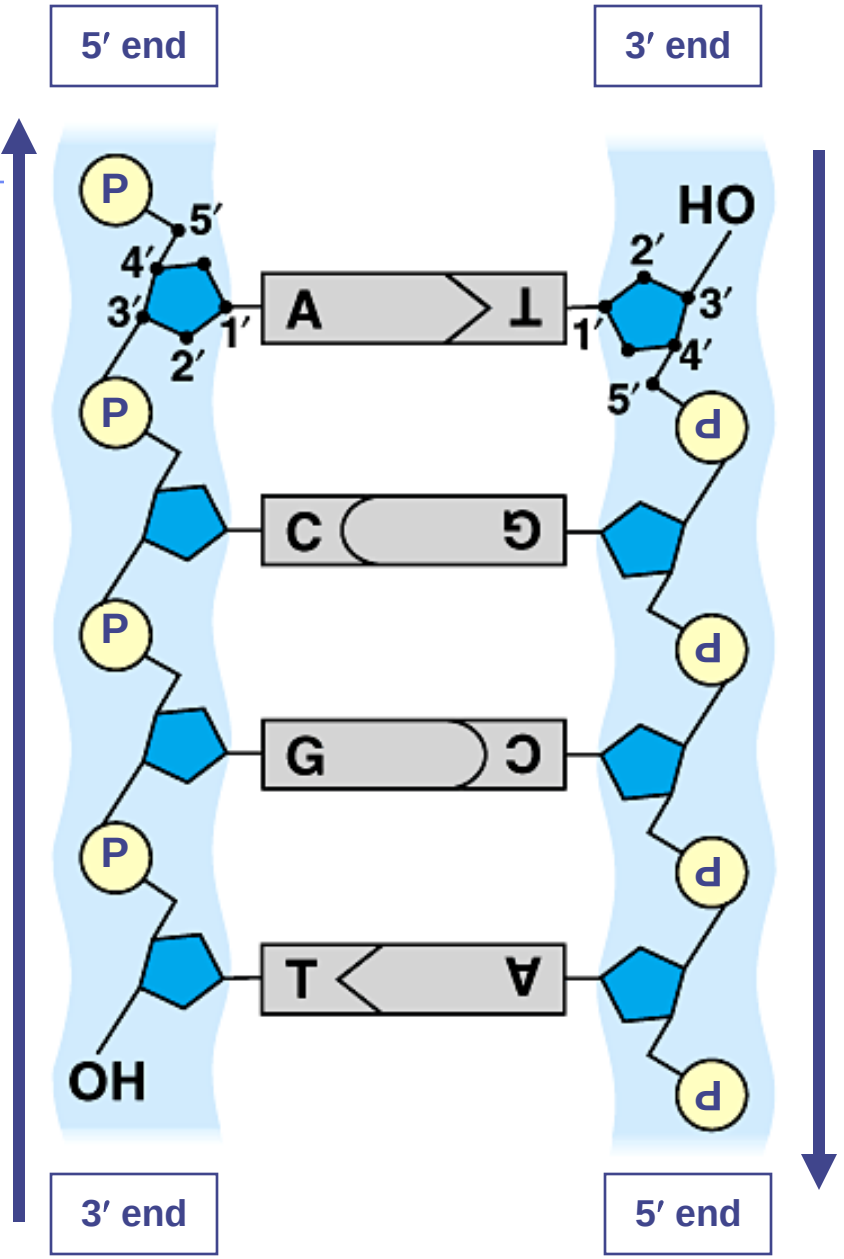
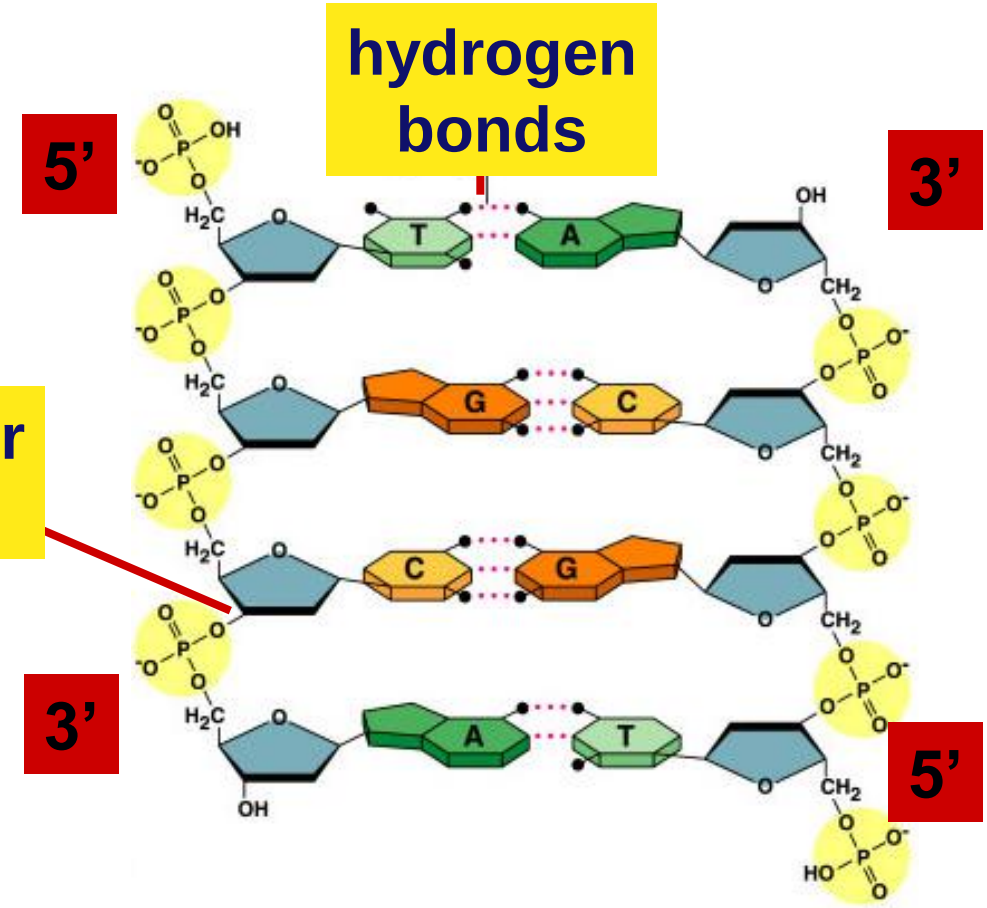


Figure 10.5B

Bonding in DNA



….strong or weak bonds?

How do the bonds fit the mechanism for copying DNA?

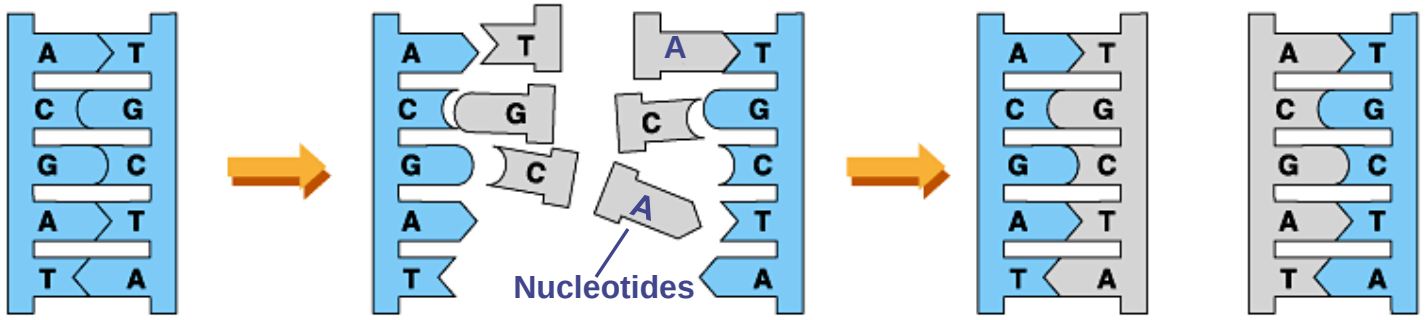
DNA REPLICATION

- **WHAT ? → a process that produces two DNA molecules identical to the original.**
- **WHY? → so when cells divide they can give each daughter cell its own identical copy of the DNA blueprint**
- **WHEN? → right BEFORE cell division as new body cells are made:**
  - **During growth**
  - **Repairing injury**
  - **Replacing old cells that wear out**

    → Producing eggs or sperm (gametes)

DNA REPLICATION: HOW?

1) DNA Helicase enzymes bind to the DNA at different locations and then **unwind** and **unzip** the 2 strands
2) DNA Polymerase enzymes use each old strand as a **template** to assemble the new strand
3) DNA Ligase enzymes **link** together DNA **fragments** until long, continuous strands are formed

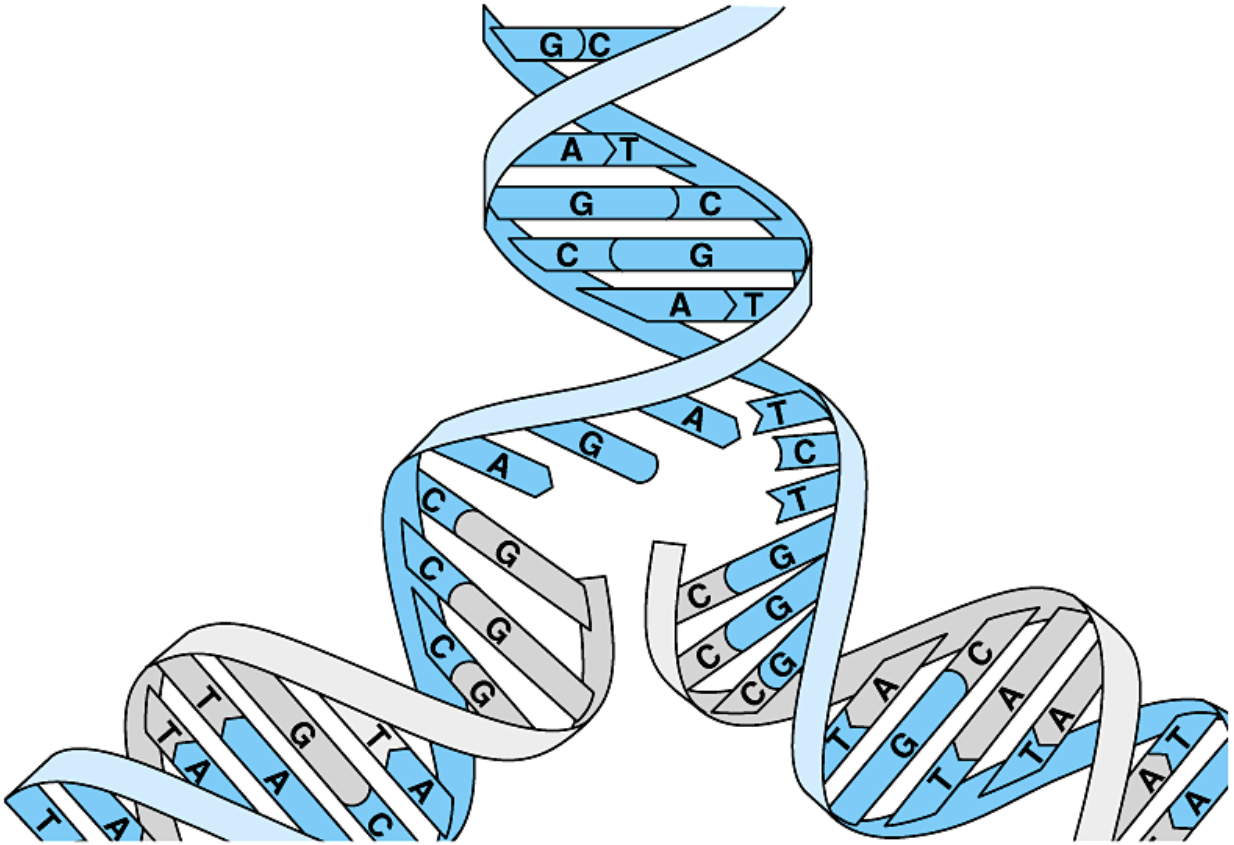


Parental molecule of DNA

Both parental strands serve as templates

Two identical daughter molecules of DNA

Untwisting and replication of DNA

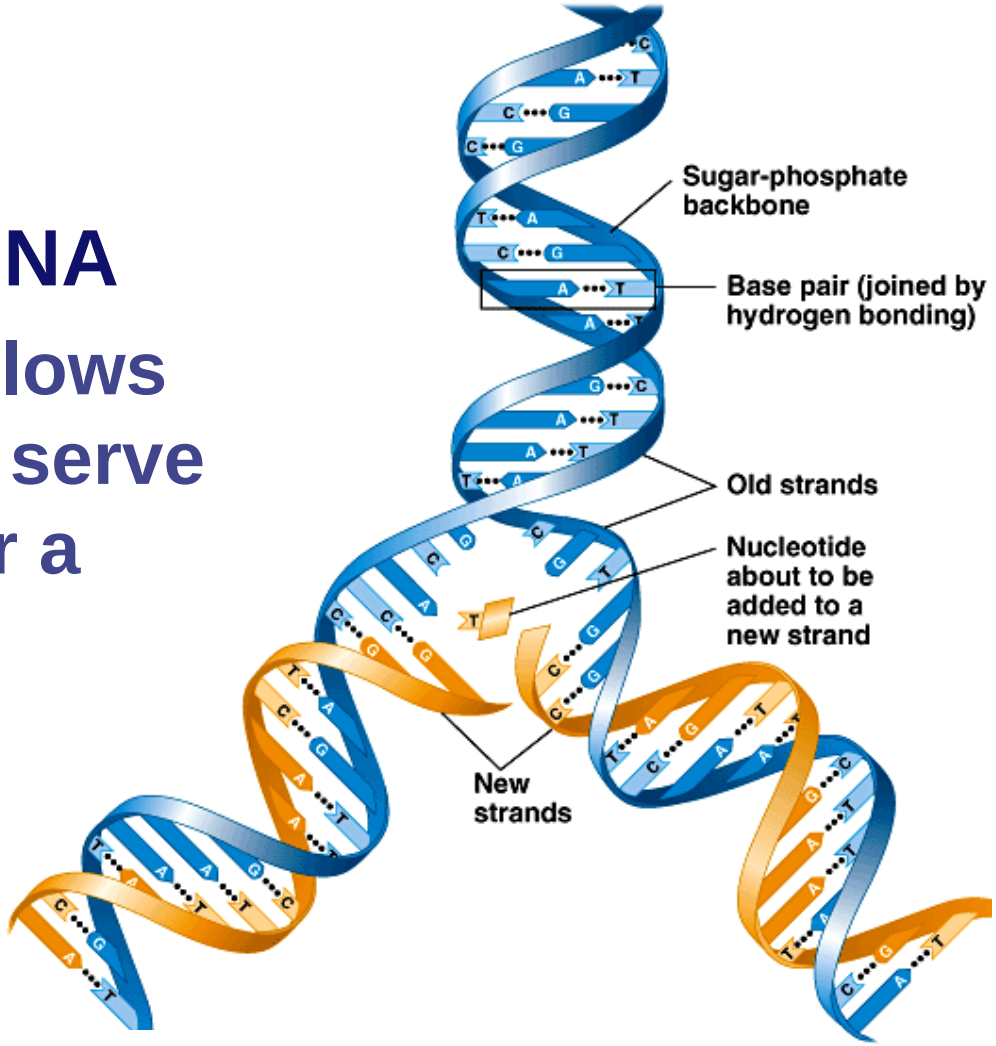


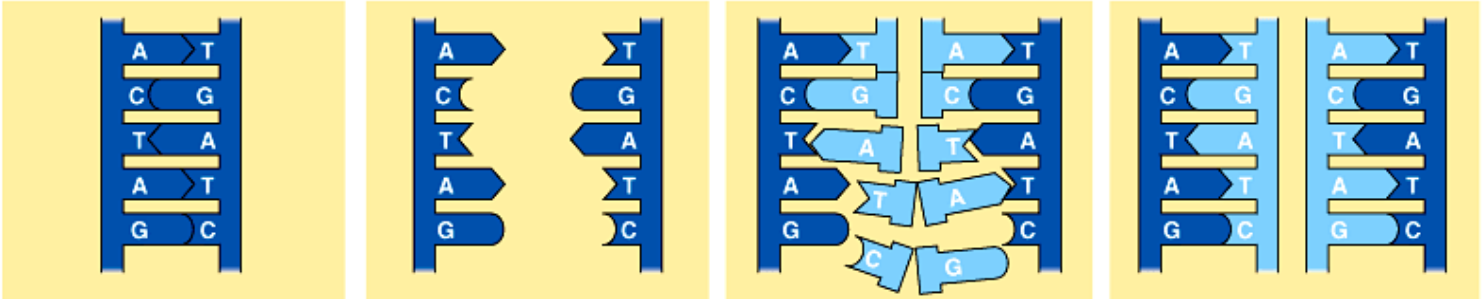
- DNA replication is a semi-conservative process = 1 strand is **old** and 1 strand is **new**

Figure 10.4B

Copying DNA

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





10.5 DNA replication: A closer look

- **DNA replication begins at many different sites, so… the whole molecule can be copied much more quickly**

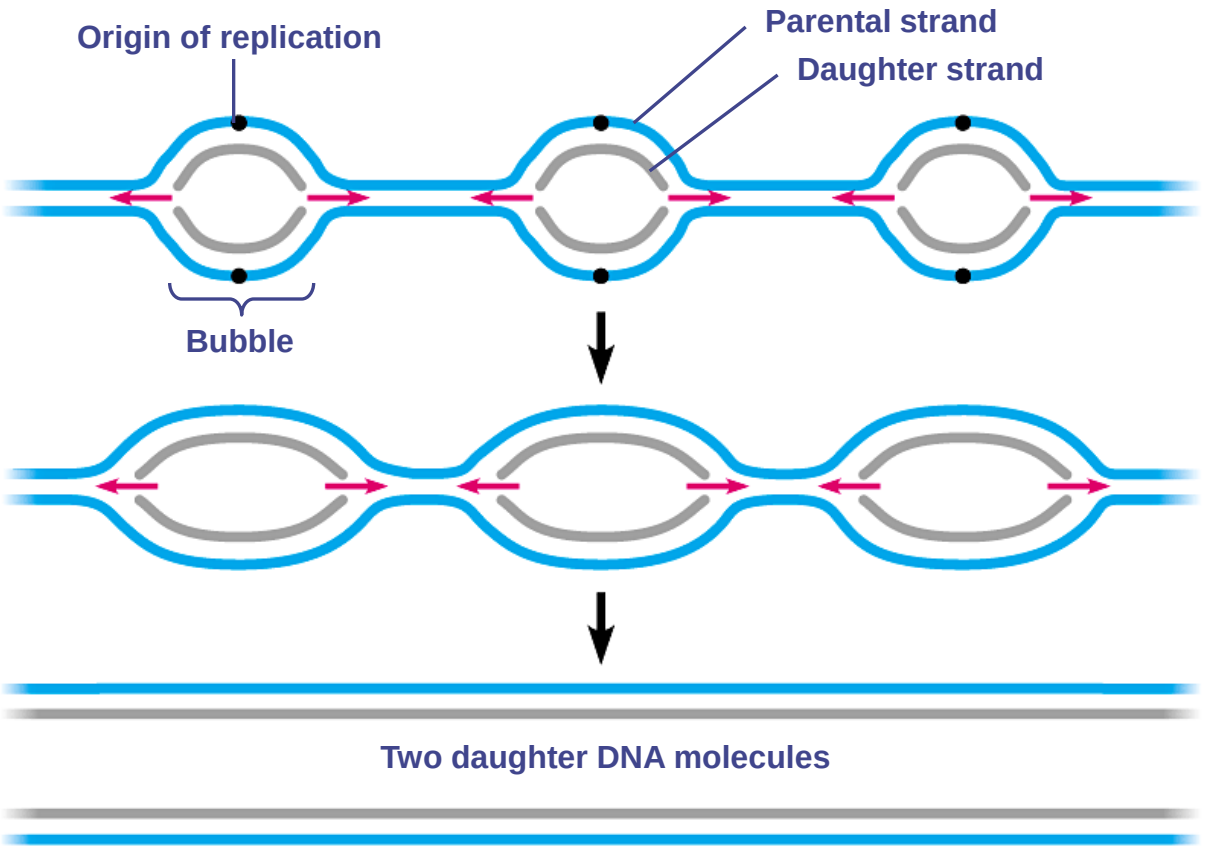


Figure 10.5A

- Because DNA is Antiparallel and Polymerase only builds new DNA in ONE direction (5'→3'), this affects how DNA daughter strands are synthesized:
  - 1 side continuously
  - 1 side in fragments
    - The fragments on one side are linked together by Ligase enzymes.

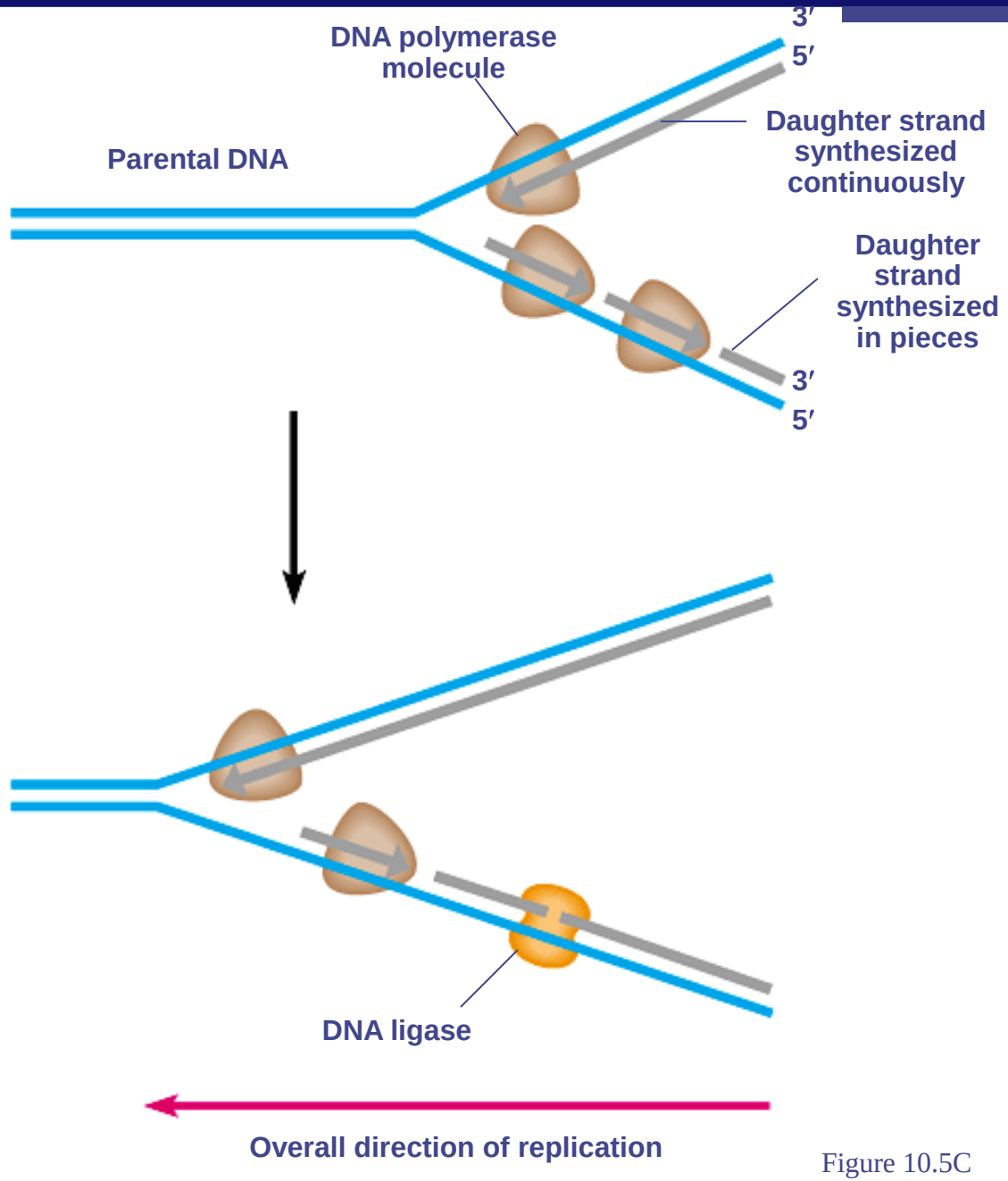
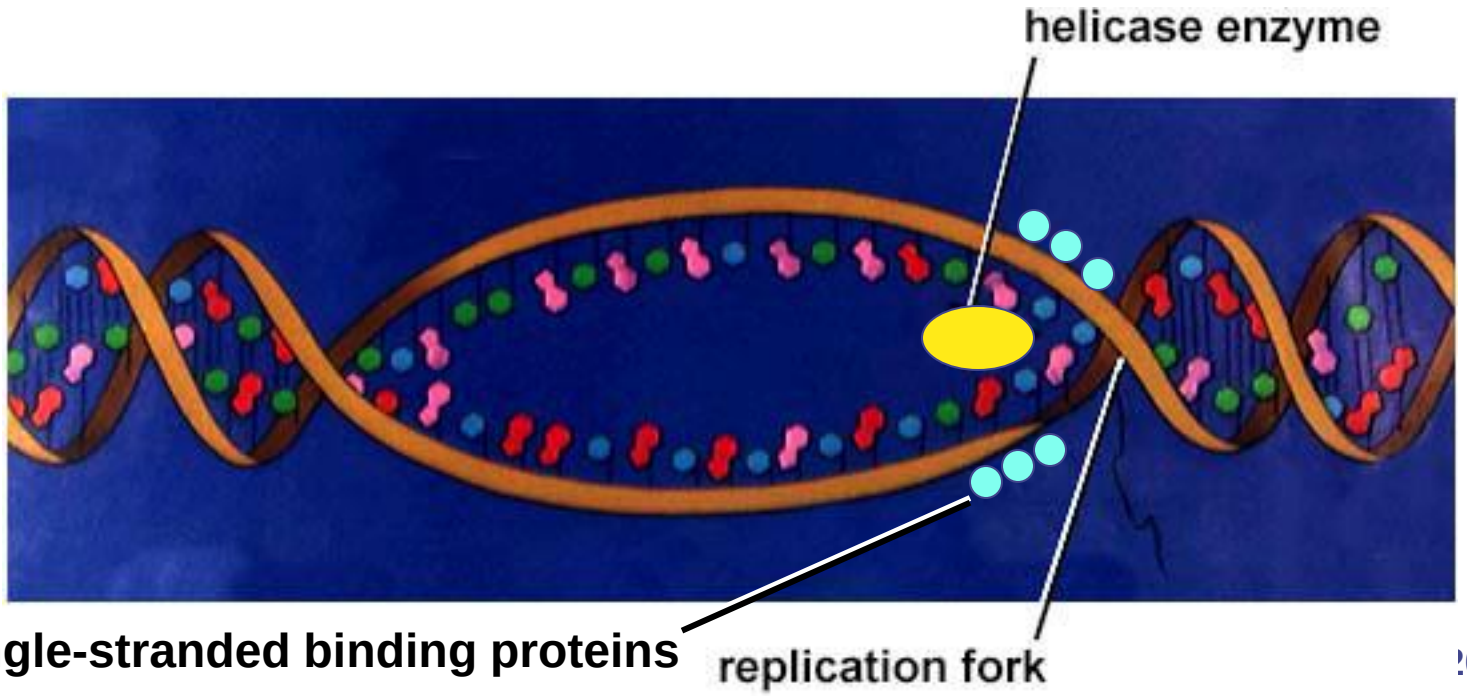


Figure 10.5C

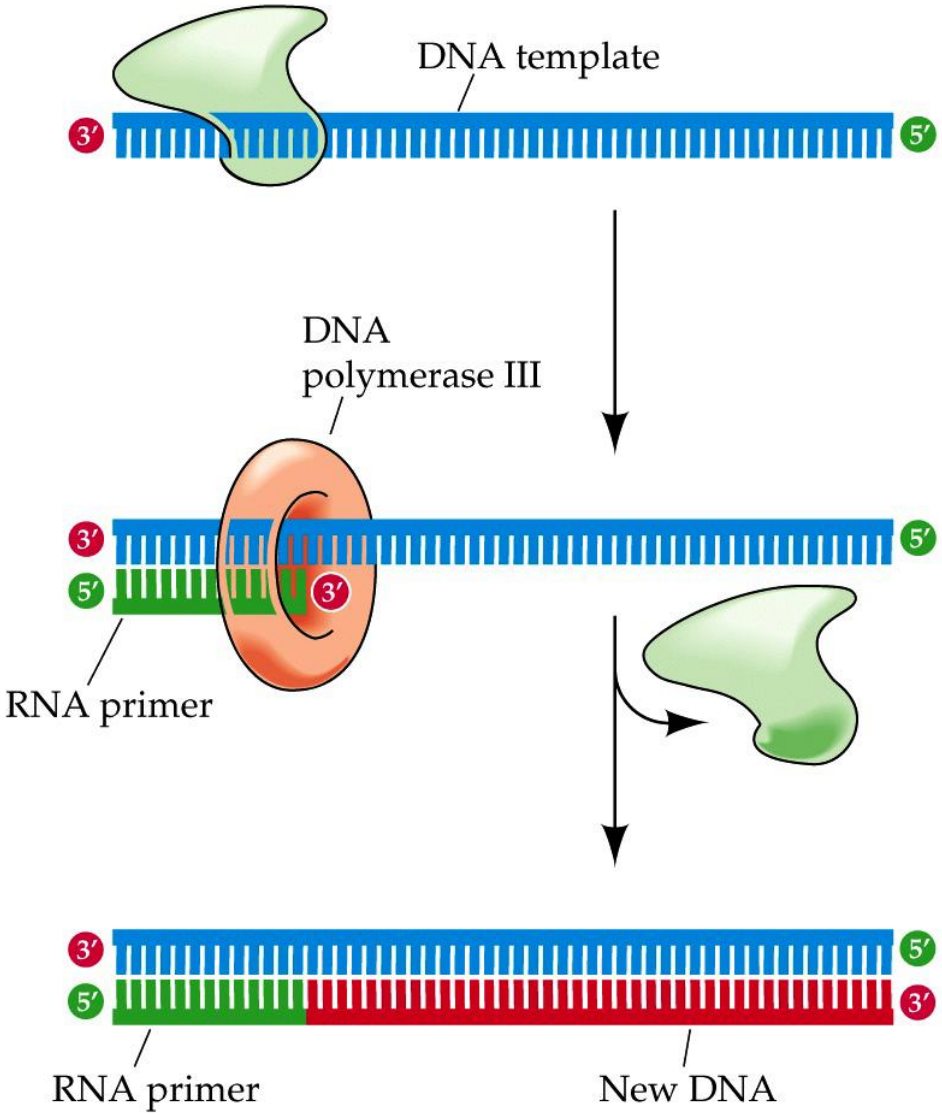
Replication: 1st step

- **Helicase unwinds & unzips the 2 strands**
  - **stabilized by single-stranded binding proteins**



Step 2: Priming DNA synthesis

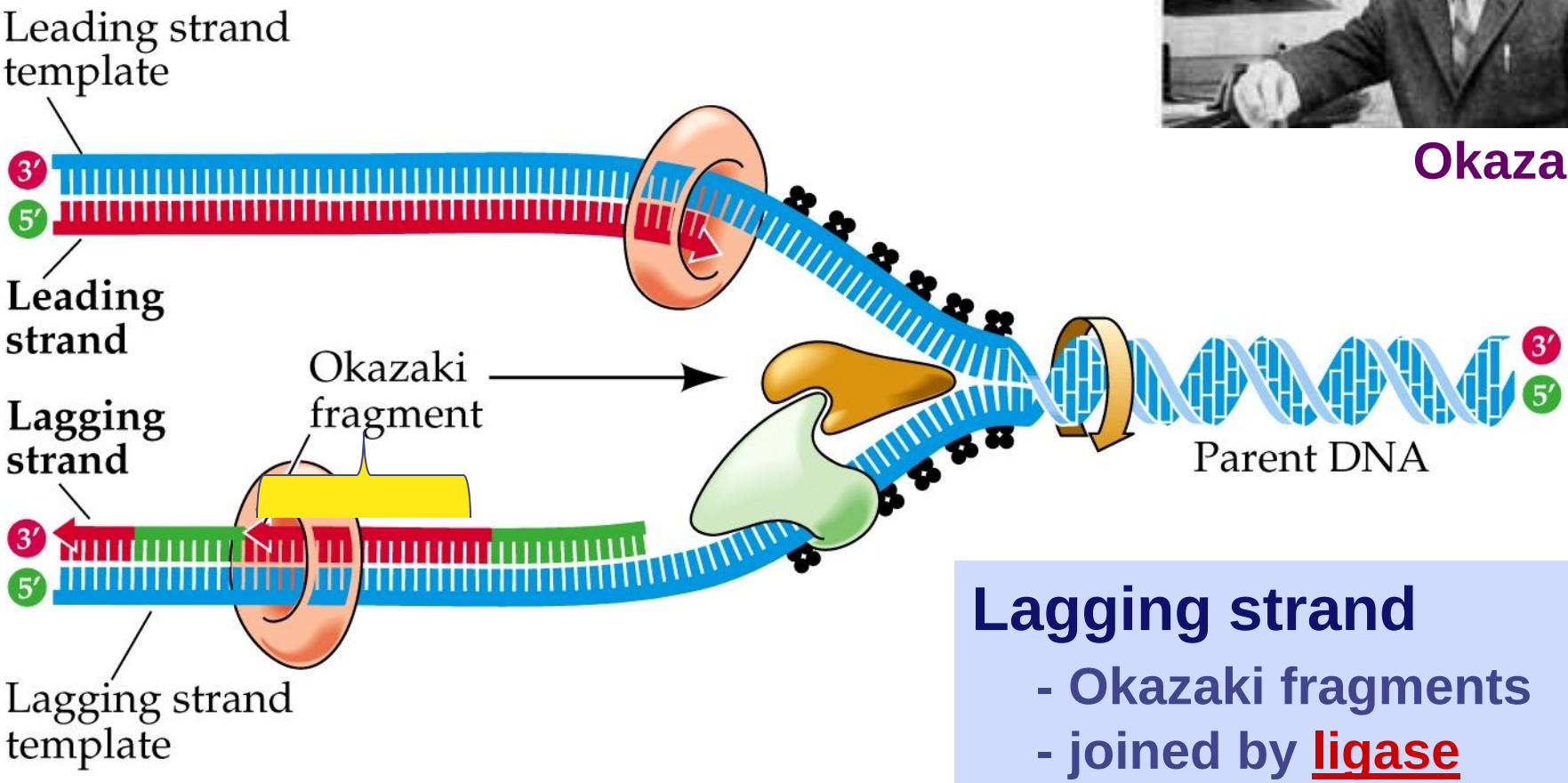
- DNA polymerase I can only attach a new nucleotide on a FREE 3’ end
  - cannot start building a new DNA strand without a free 3’ end
  - short RNA primer with a free 3’ end is built first by primase



Step 3: Polymerase I builds Leading & Lagging strands

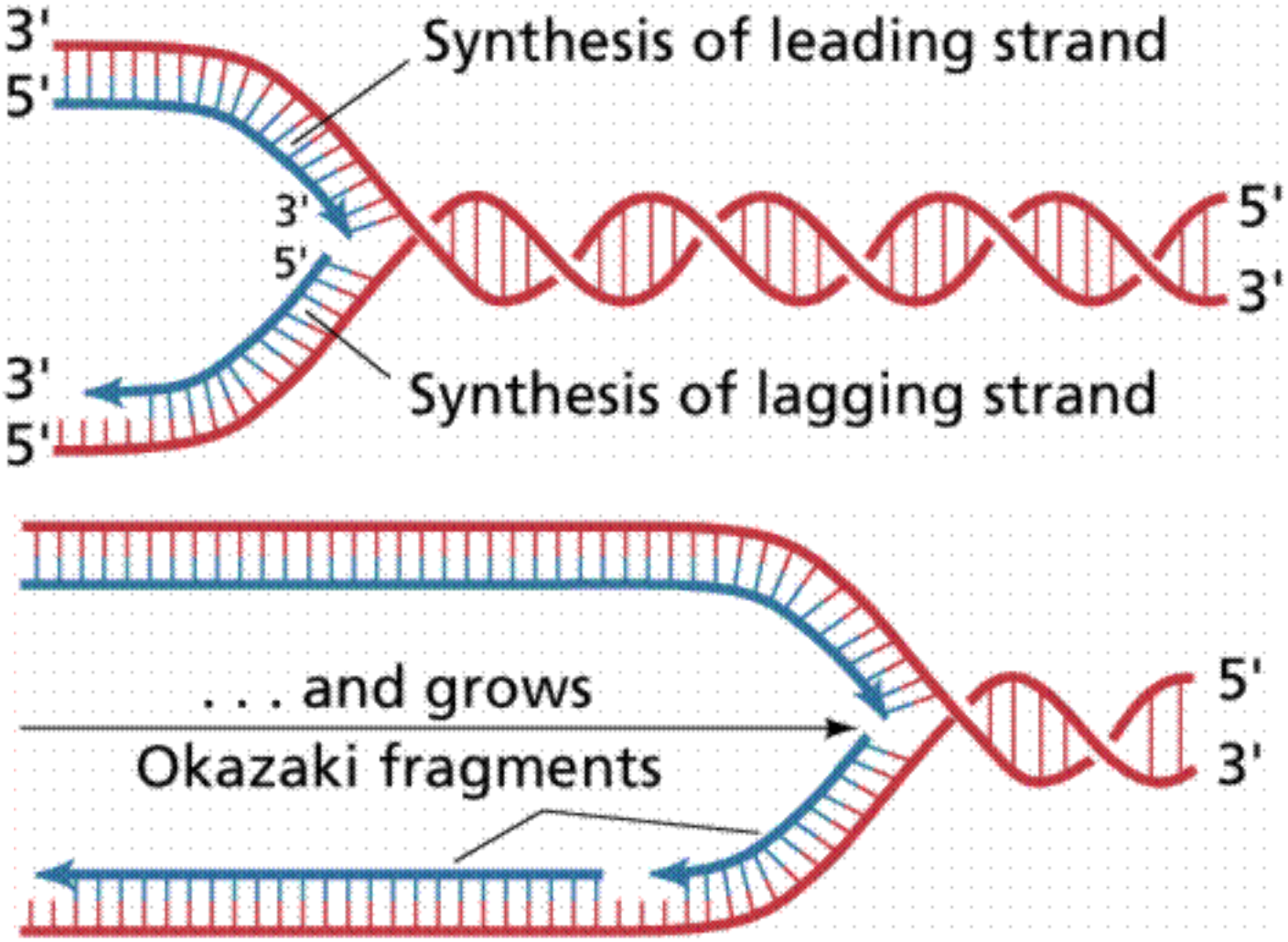
Leading strand
- continuous synthesis

Okazaki



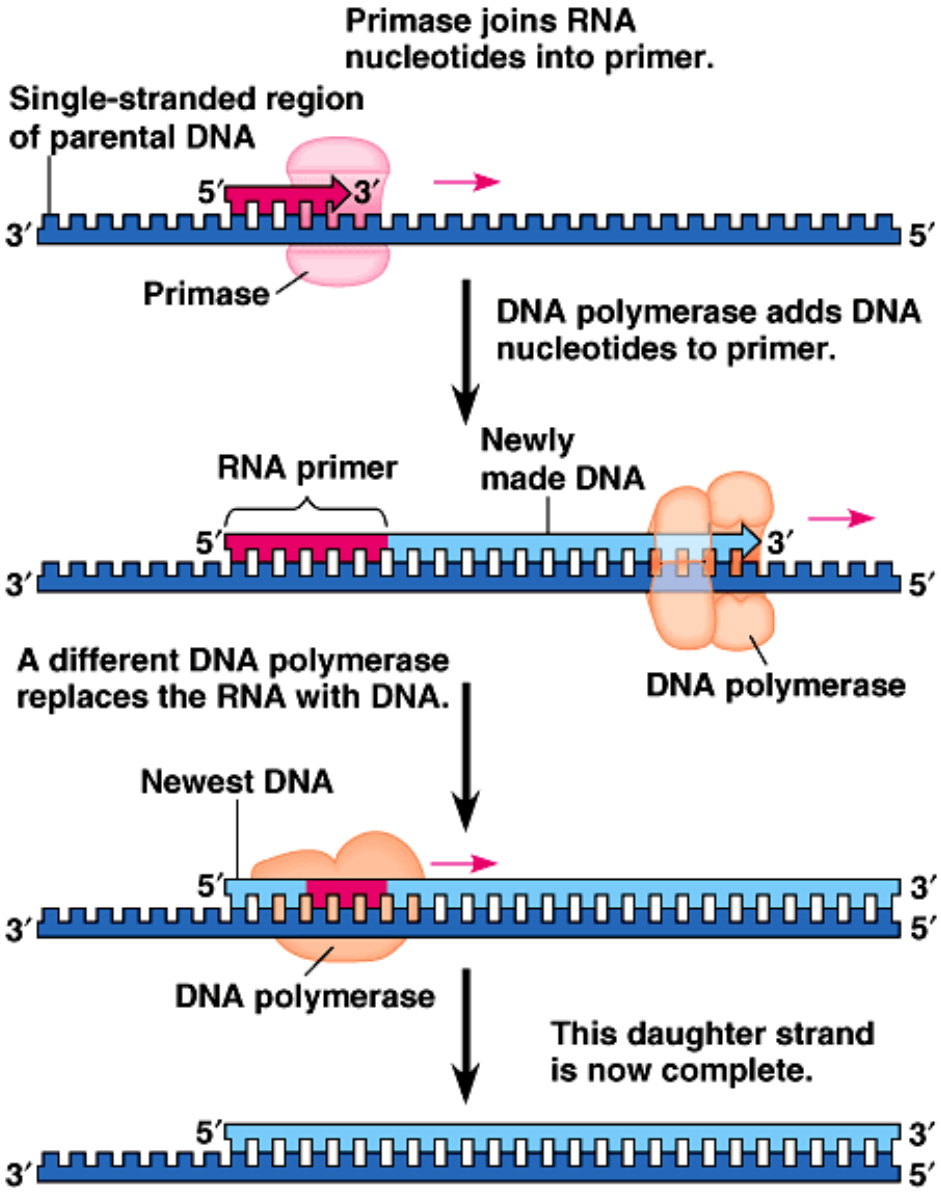
Lagging strand
- Okazaki fragments
- joined by ligase
  - "spot welder" enzyme

Okazaki fragments

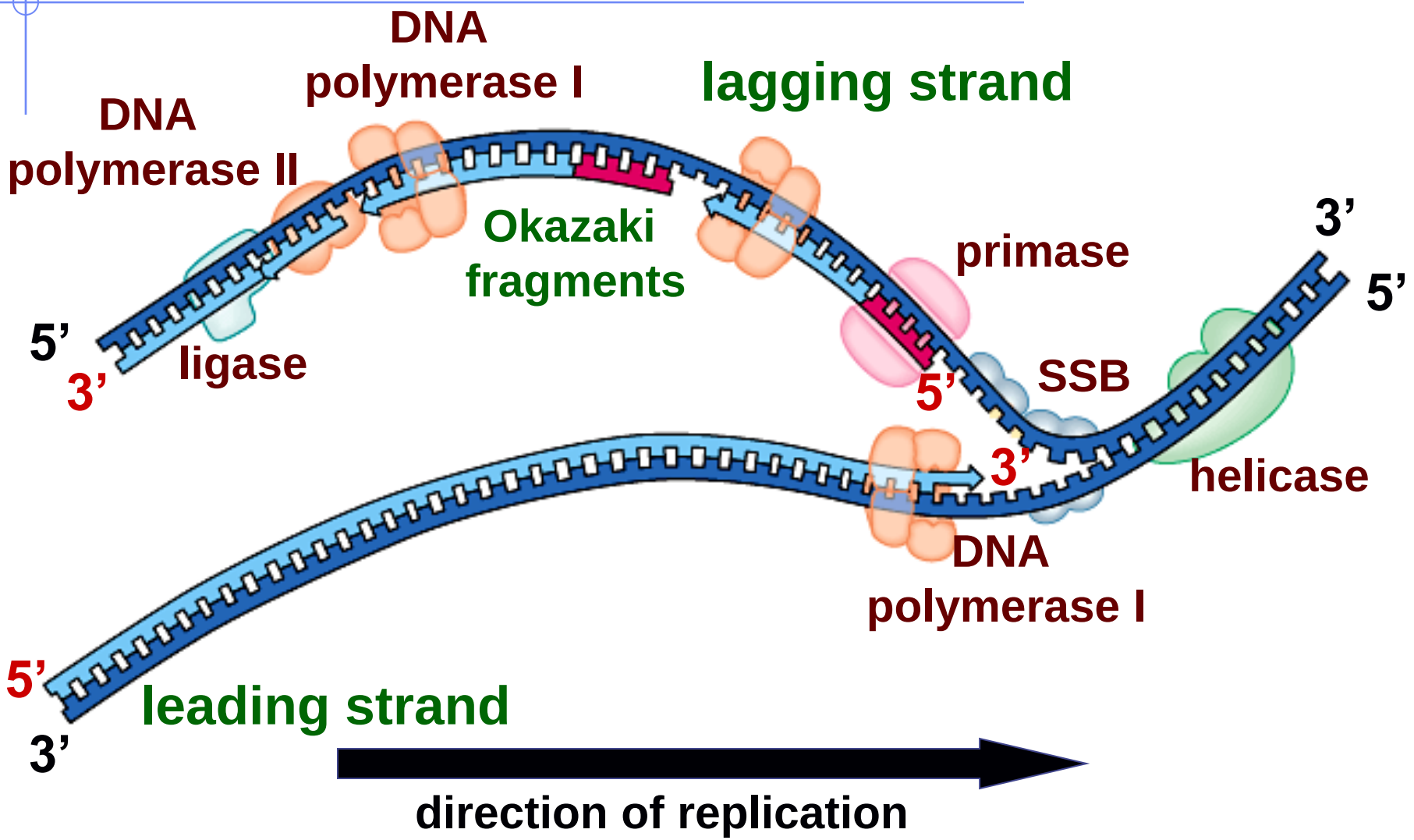


Step 4: Polymerase II cleans up primers

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



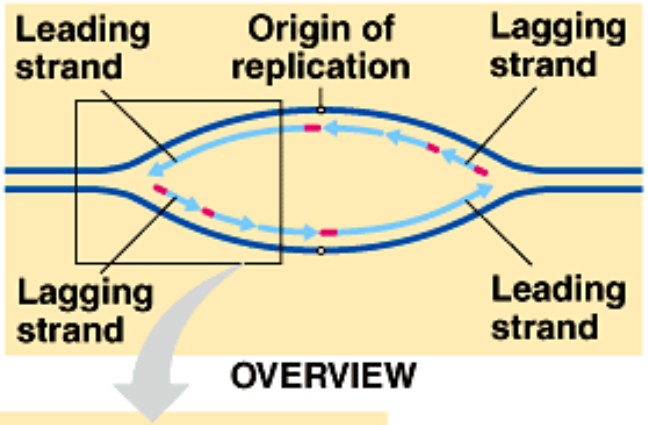
Step 5: Ligase links all DNA fragments

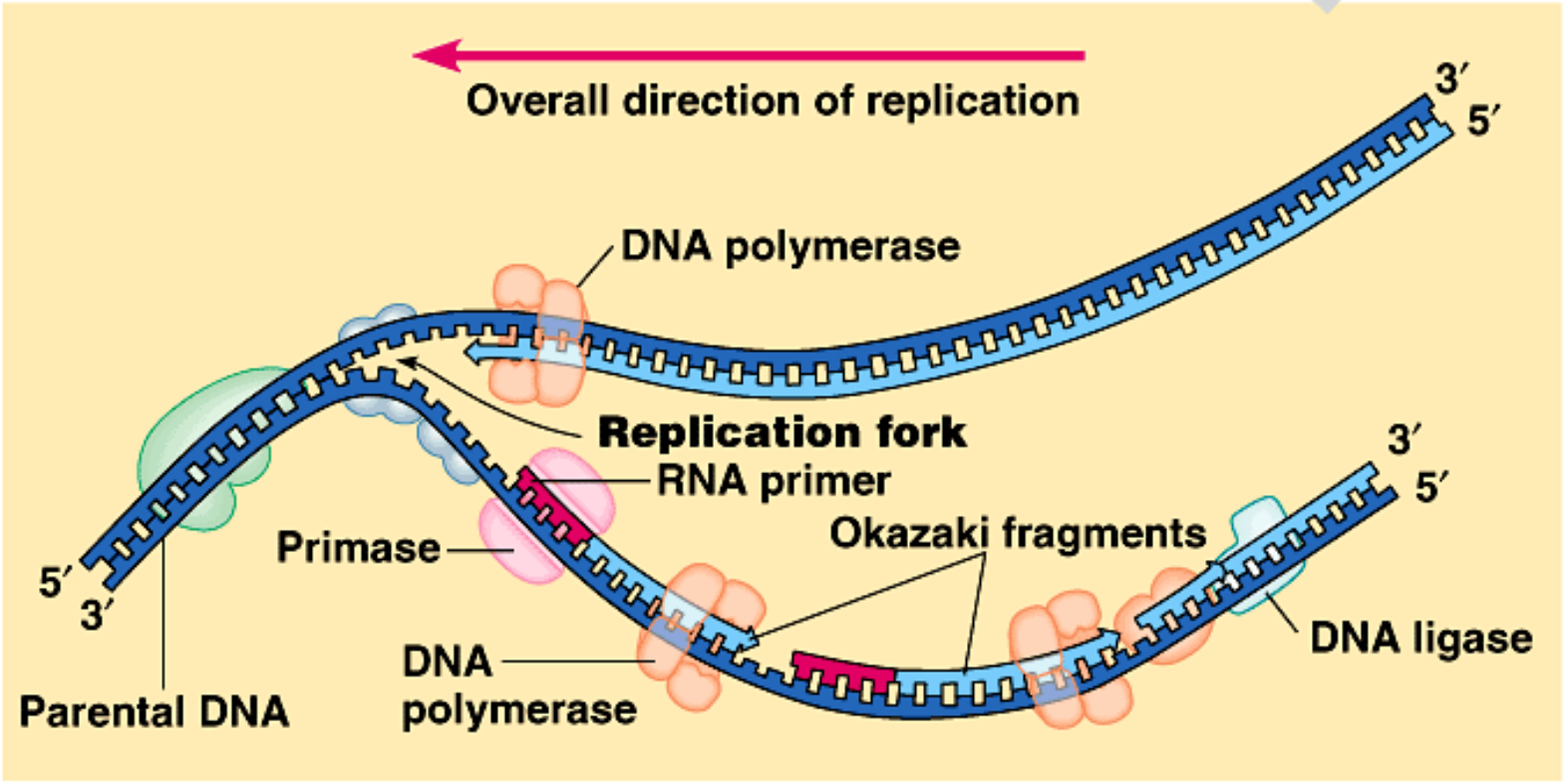


Replication bubble

Adds 1000 bases/second!

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





Replication Speed & Accuracy

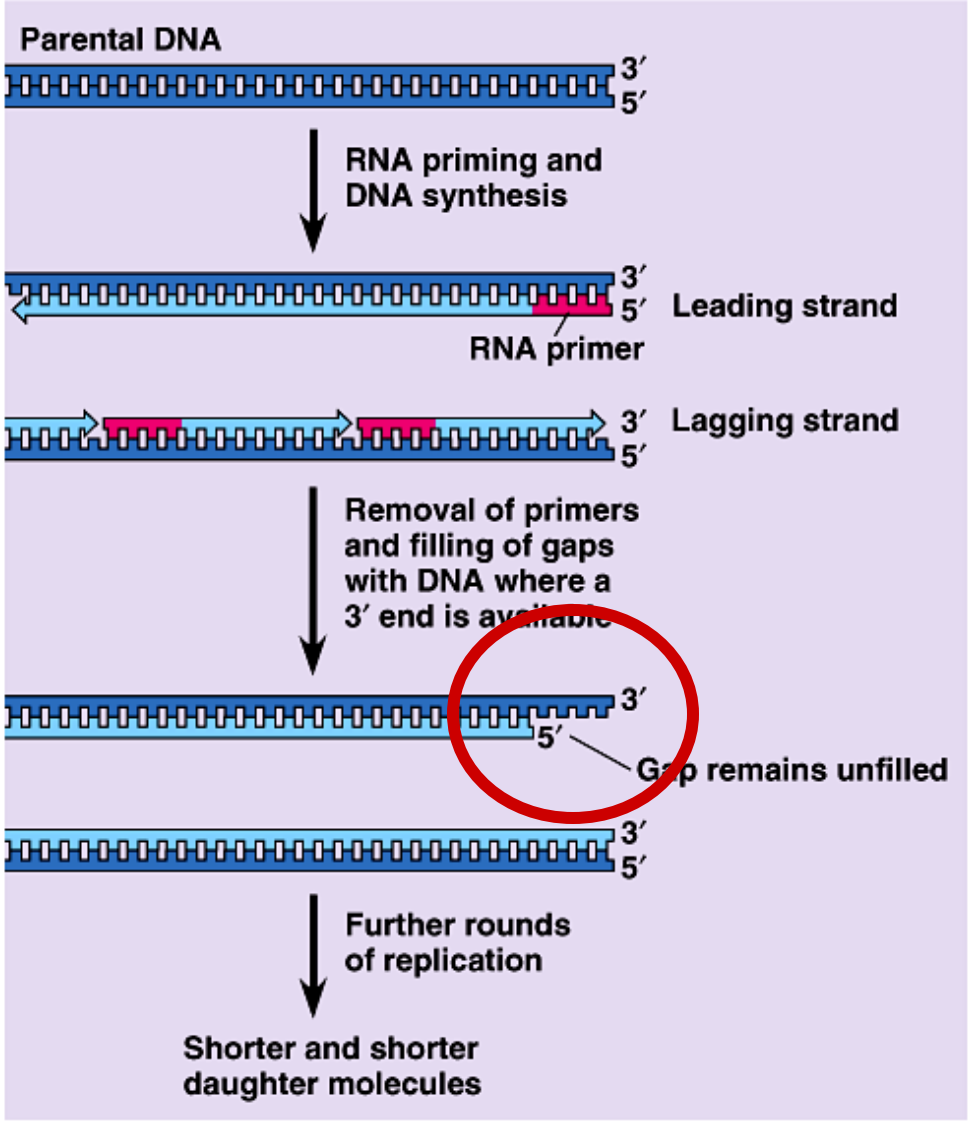
- Because the DNA molecule is copied so quickly (~50 new letters per second in mammals), occasionally the wrong letters are paired together
  - Most of these mismatch errors are fixed by the Polymerase enzyme as it backs up and inserts the correct matching base.
  - However, 1 mistake or mismatch every 1 billion letters does not get fixed and remains to be copied
- An army of repair enzymes constantly proofread the new DNA strands and fix most (99.9%) mistakes.
- Any unfixed DNA mistake = mutation

Replication enzymes

- ***helicase***
- ***DNA polymerase I***
- ***primase***
- ***DNA polymerase II***
- ***ligase***
- ***Repair Enzymes***

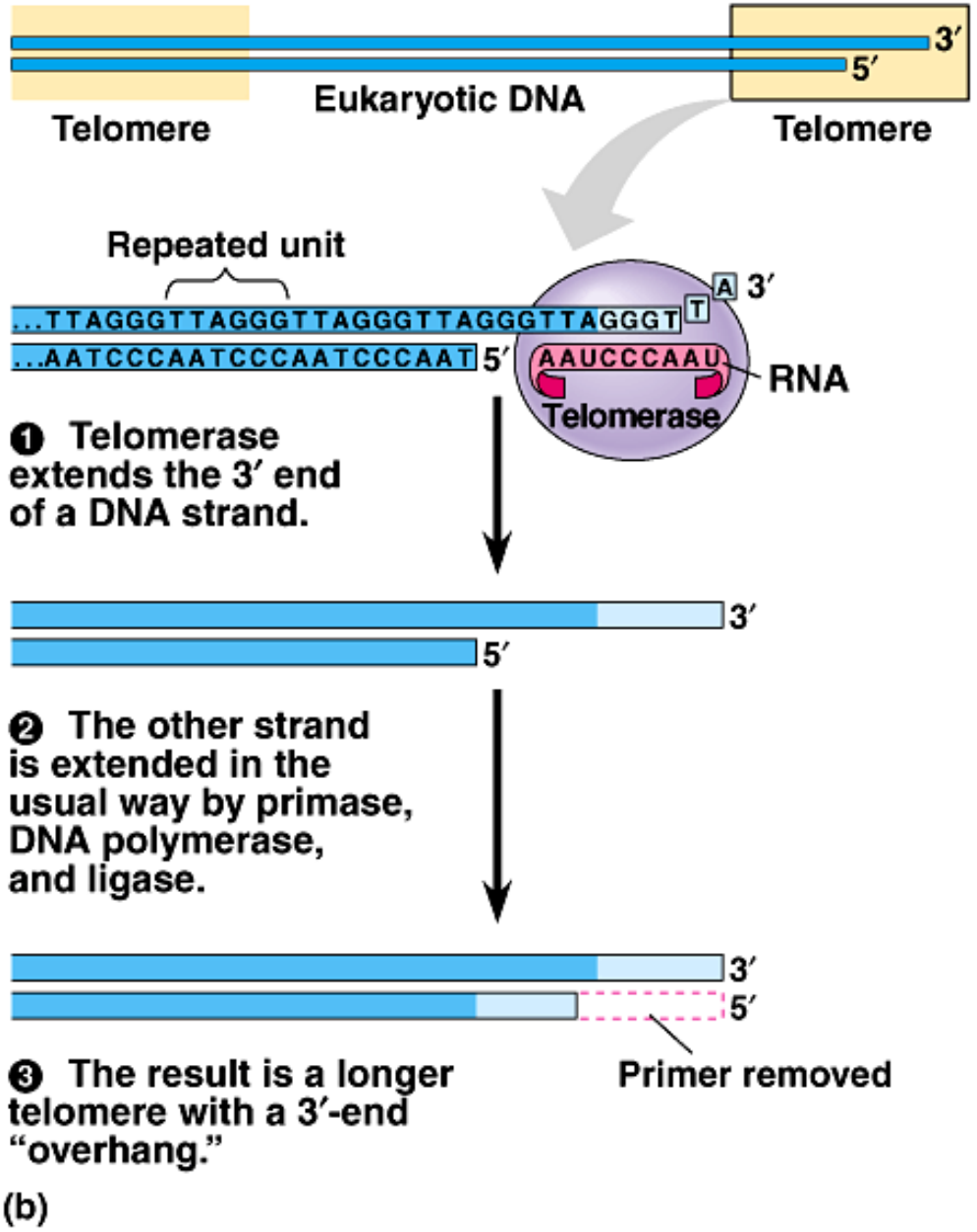
And in the end…

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



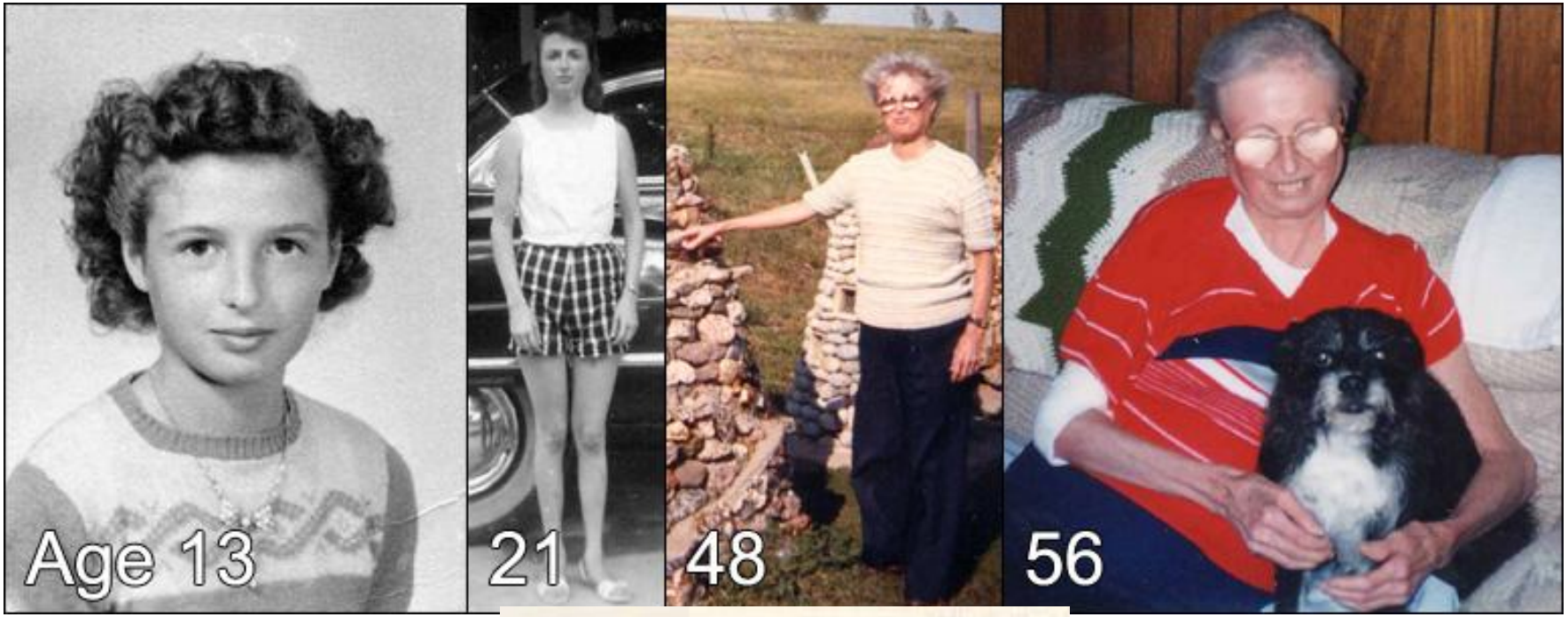
Telomeres

- **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?**



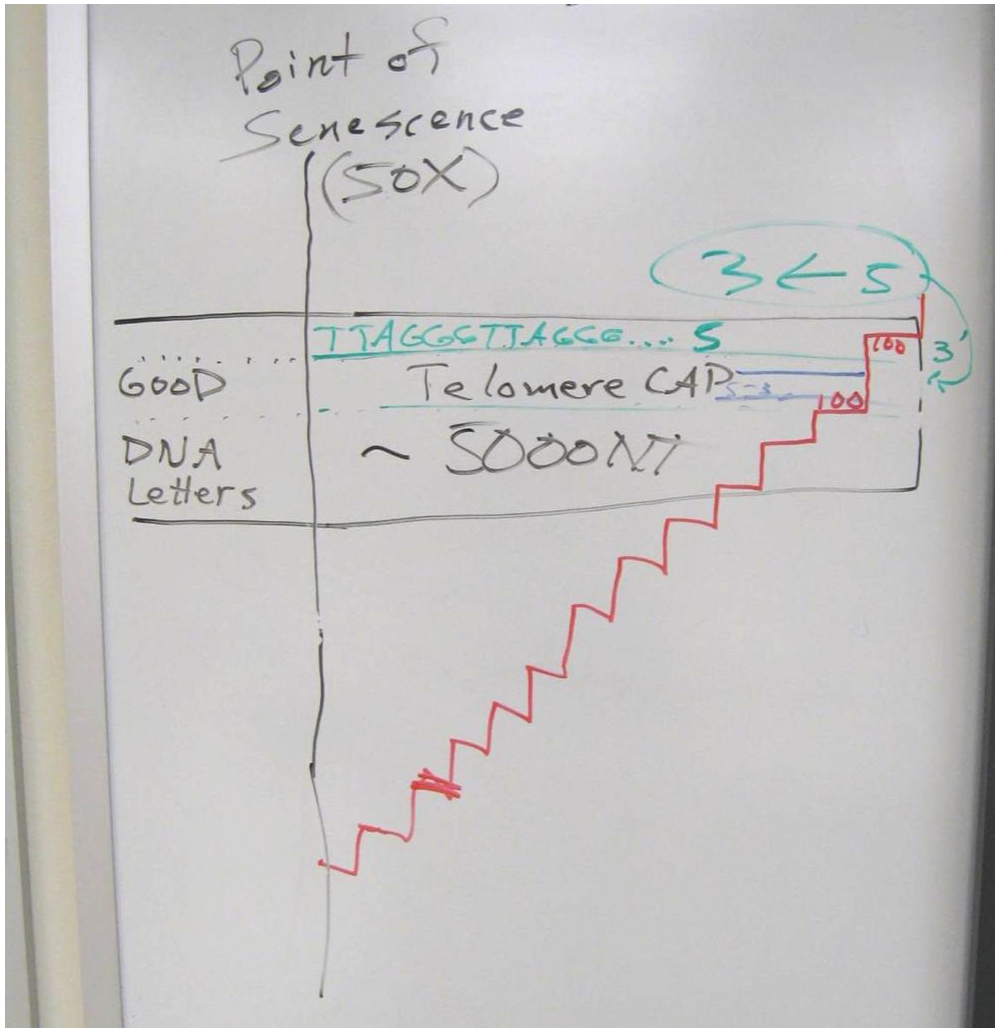
Progeria: One type of premature aging

Werner Syndrome: another type of premature again



Taking its toll. As a teenager (left) this Japanese American looked normal, but by age 48, the effects of Werner's syndrome were readily apparent. [Image credit: William and Wilkens Publishing Inc.]

Measuring Antioxidant levels in the skin


Point of
Senescence
(50X)
3←5
TTAGGGTTAGGG... 5
GOOD
Telomere CAP
DNA
Letters
~ 5000 NT
100
100
3'