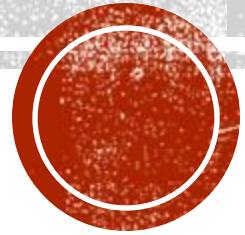
# DNA, RNA AND DNA REPLICATION

Benilda Ramos-Butron



# OBJECTIVES:

- Describe the DNA and RNA molecules
- Recount the history of the elucidation of the structure of the DNA
- Enumerate enzymes and their functions in DNA replication
- Discuss the step-by-step process of DNA replication

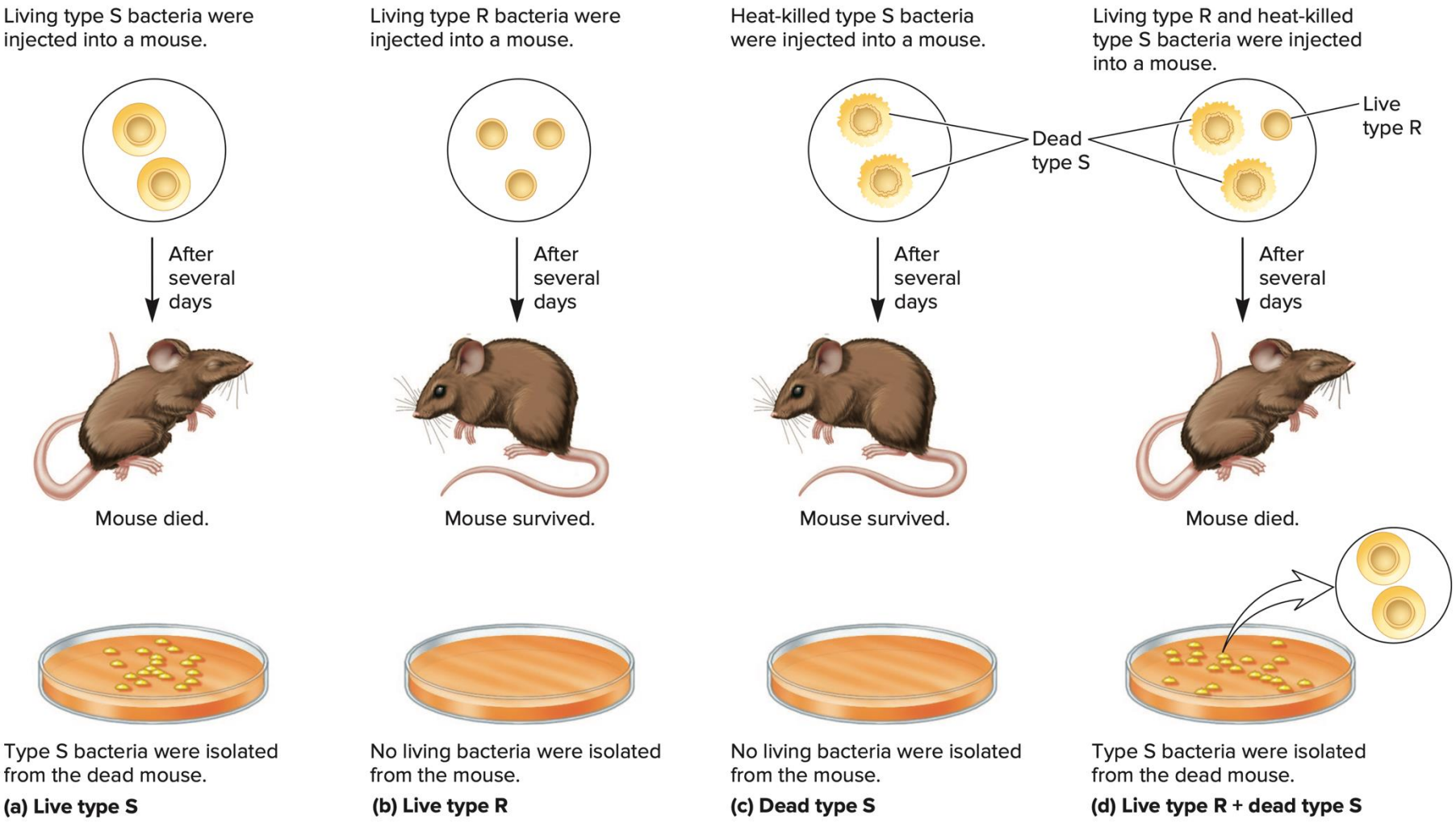
# 4 CRITERIA OF THE GENETIC MATERIAL

1. Information.
2. Transmission.
3. Replication
4. Variation

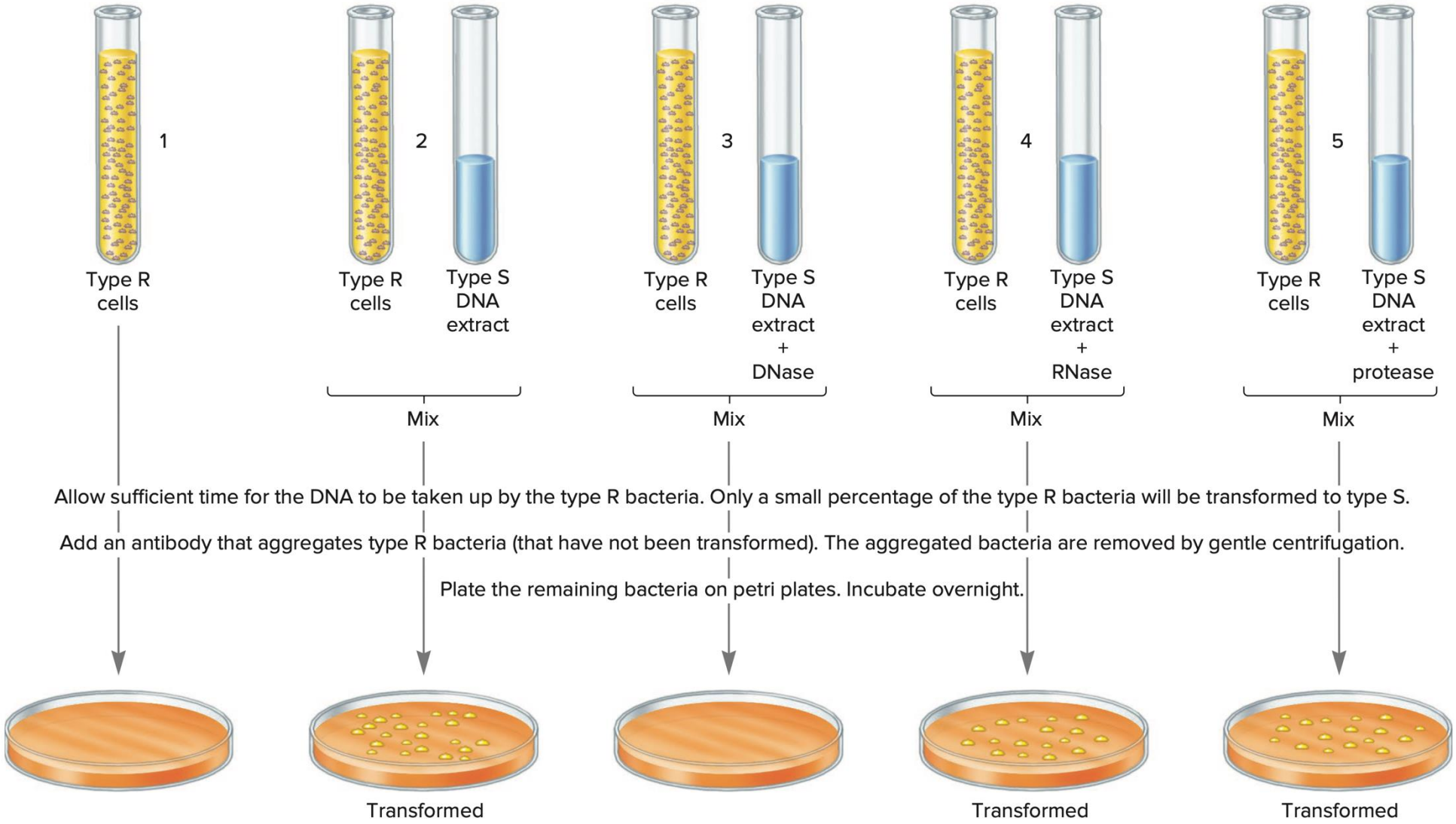
# HISTORY

- **1880**, August Weismann and Carl Nageli floated the idea that a chemical substance within a living cell is responsible for the transmission of traits from parents to offsprings
- **1902-1903**, **Chromosomal Theory of Inheritance**, primarily proposed by Walter Sutton and Theodor Boveri, states that genes are located on chromosomes, and the behavior of chromosomes during meiosis explains Mendel's laws of inheritance

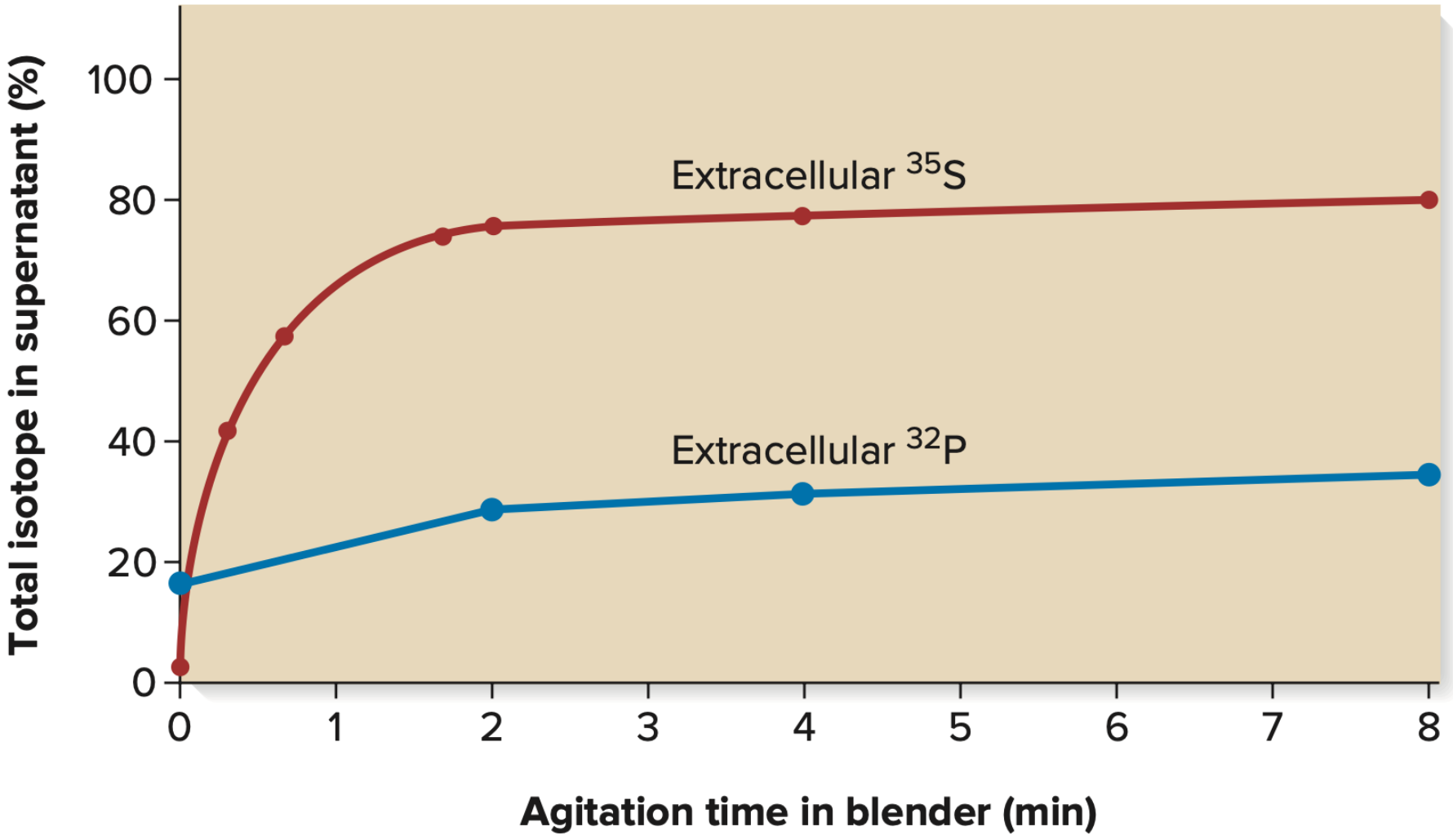
# FREDERICK GRIFFITH EXPERIMENT ON GENETIC TRANSFORMATION IN S. PNEUMONIAE



# AVERY, MACLEOD, AND MCCARTY EXPERIMENT



# ALFRED HERSHEY AND MARTHA CHASE




**80%**
Blending removes 80% of $^{35}S$ from *E. coli* cells.

**35%**
Most of the $^{32}P$ (65%) remains with intact *E. coli* cells.

# OVERVIEW OF THE DNA AND RNA STRUCTURE

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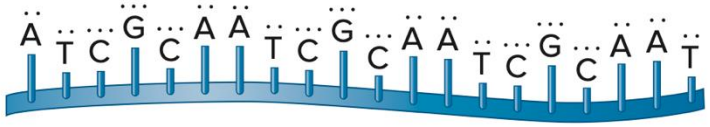
- Nucleic acid – coined by Friedrich Miescher (1869)
- First referred to as "*nuclein*"
- It was determined that they are acidic molecules, which means they release hydrogen ions (H+) in solution and have a net negative charge at neutral pH
- Final term used: **nucleic acid**

# 4 LEVELS OF NUCLEIC ACID COMPLEXITY

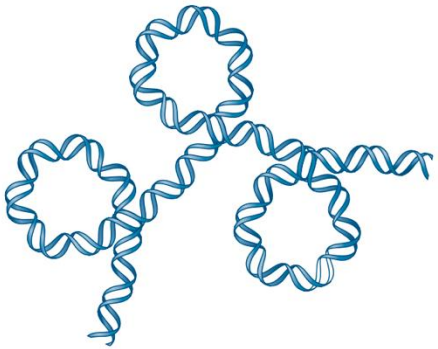
1. **Nucleotides** form the repeating structural unit of nucleic acids.
2. Nucleotides are linked together in a linear manner to form a **strand** of DNA or RNA.
3. Two strands of DNA (or sometimes strands of RNA) interact with each other to form a **double helix**.
4. The three-dimensional structure of DNA results from the folding and bending of the double helix.

Nucleotides



Single strand

Double helix

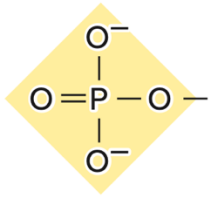


Three-dimensional structure

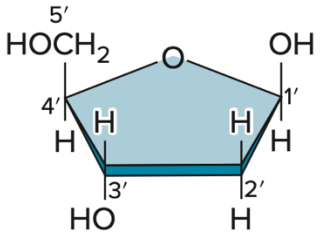
# NUCLEOTIDE STRUCTURE

*What are the components of a nucleotide?*

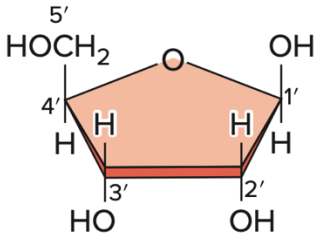
**Phosphate group**



**Sugars**



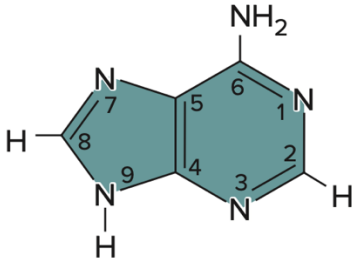
D-Deoxyribose (in DNA)



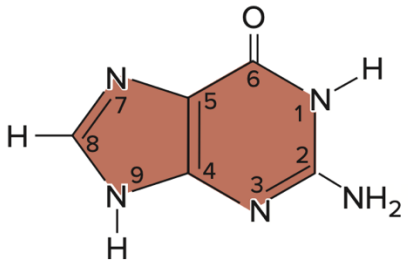
D-Ribose (in RNA)

**Bases**

Purines (double ring)

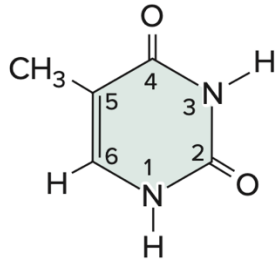


Adenine (A)

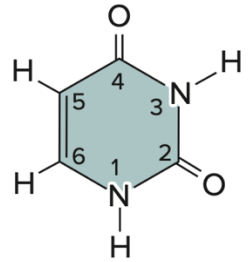


Guanine (G)

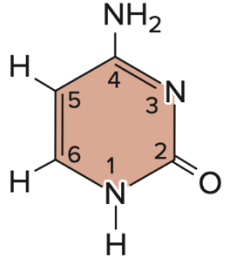
Pyrimidines (single ring)



Thymine (T) (in DNA)

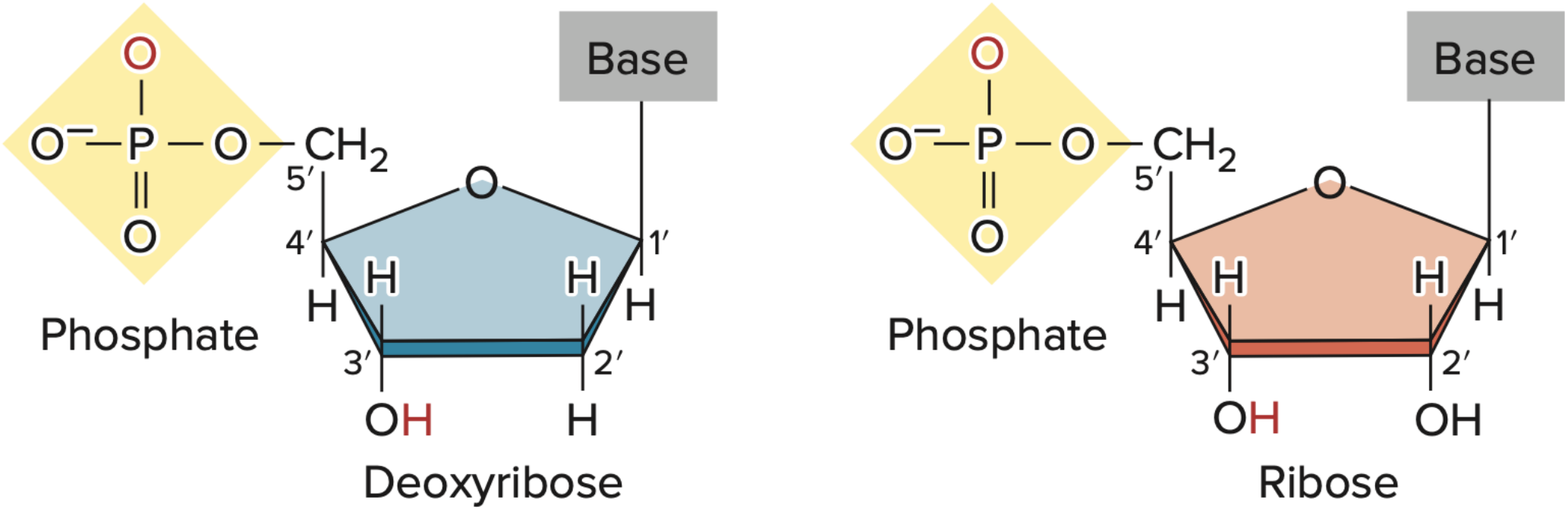


Uracil (U) (in RNA)



Cytosine (C)

*Describe the nucleotide.*

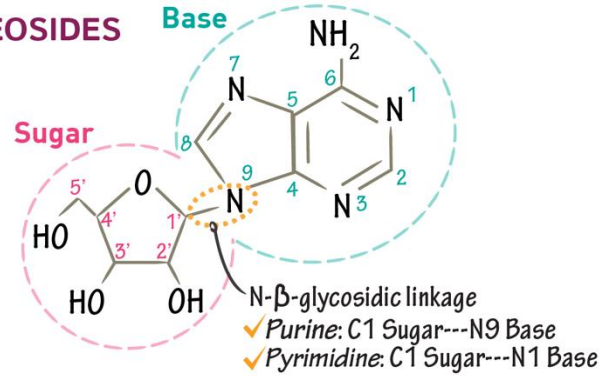



**(a) Repeating unit of deoxyribonucleic acid (DNA)**

**(b) Repeating unit of ribonucleic acid (RNA)**

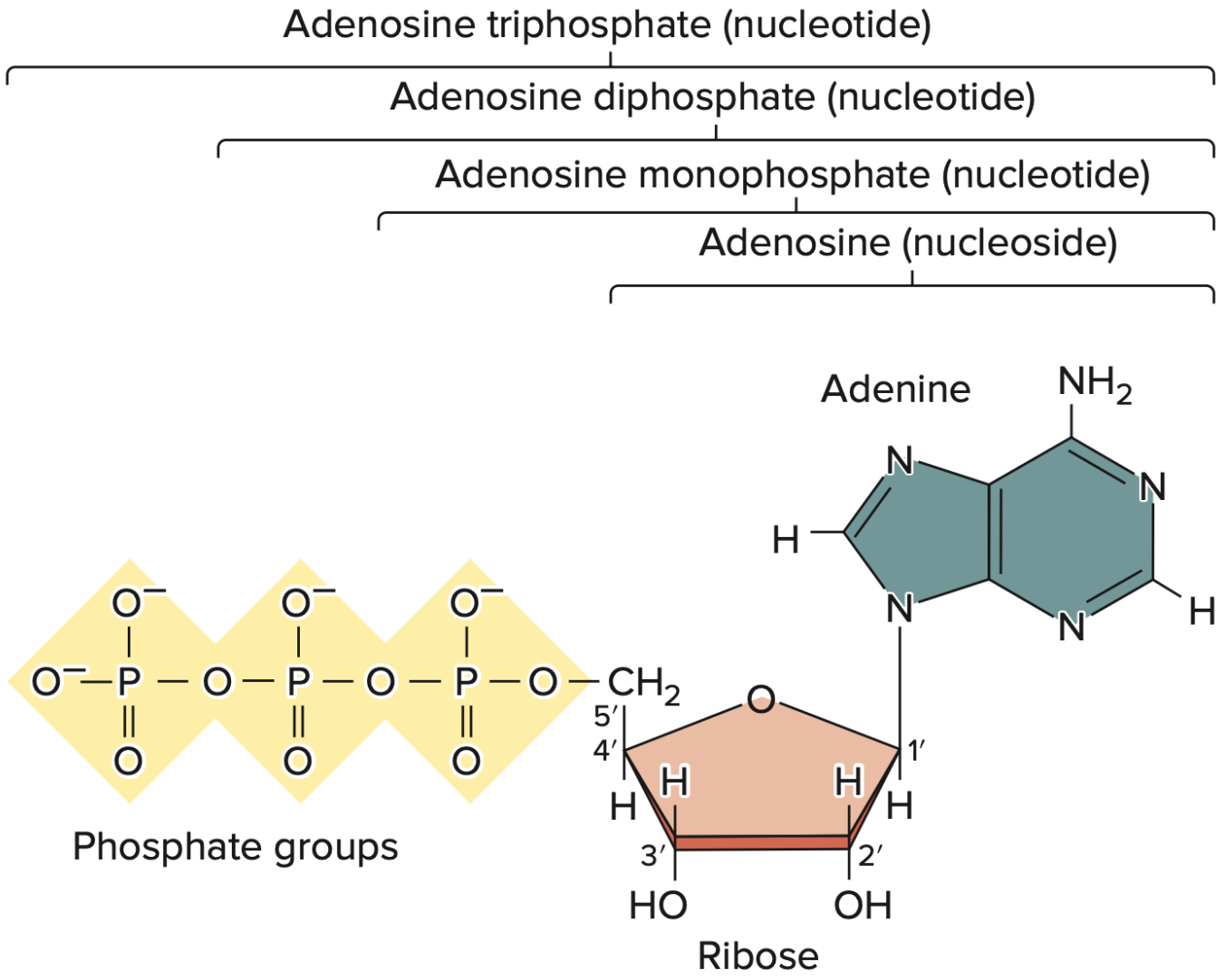
*What if phosphate molecule is removed from the nucleotide?*

## NUCLEOSIDES



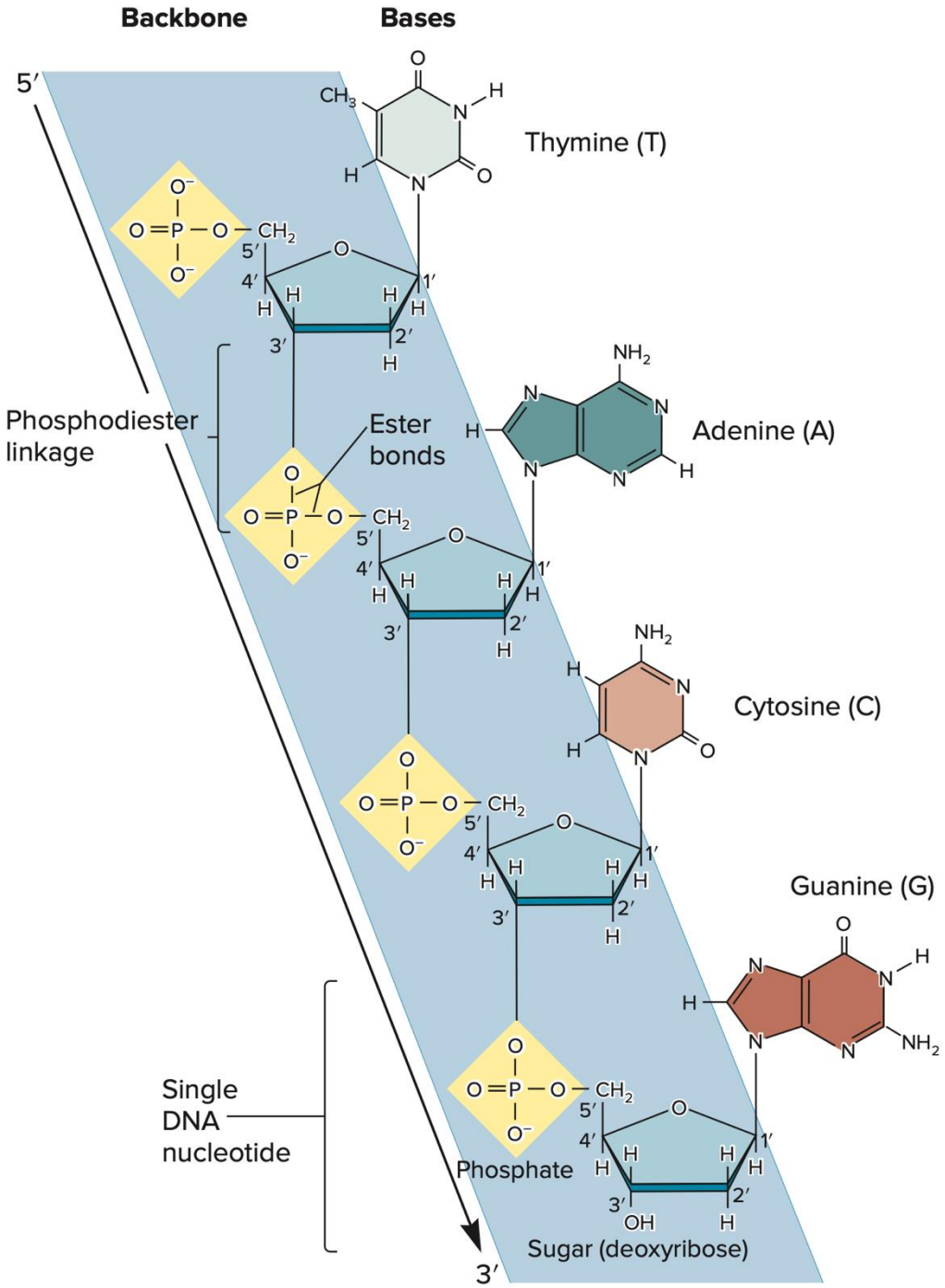

| Base | Ribonucleoside | Deoxyribonucleoside |
|---|---|---|
| Adenine | Adenosine | Deoxyadenosine |
| Cytosine | Cytidine | Deoxycytidine |
| Guanine | Guanosine | Deoxyguanosine |
| Uracil | Uridine | Deoxyuridine |
| Hypoxanthine | Inosine | (does not occur) |
| Thymine | Ribothymidine (rare) | Deoxythymidine<br>Thymidine |

# NUCLEOSIDE TO NUCLEOTIDE



# STRUCTURE OF A DNA STRAND

***Describe the structure of a strand of DNA shown here.***



# STRUCTURAL FEATURES

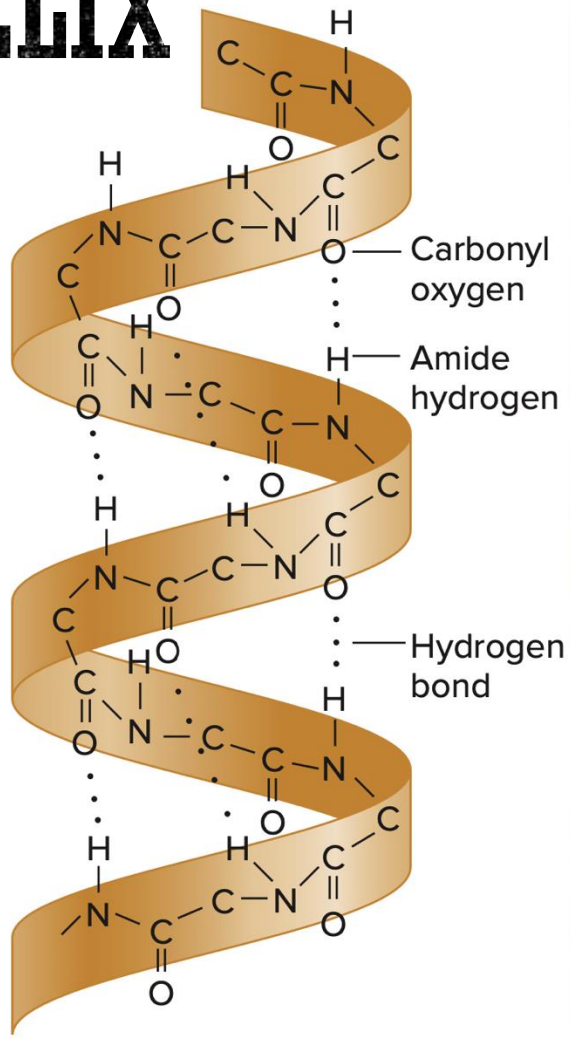
1. A phosphate group connects two sugar molecules via two ester bonds called a **phosphodiester linkage**.
2. The phosphates and sugar molecules form the backbone of the strand. The backbone is negatively charged due to the negative charge on each phosphate.
3. The bases project from the backbone.
4. A phosphodiester linkage involves attachment of a phosphate to the 3′ carbon in one nucleotide and to the 5′ carbon in the next nucleotide.

# DISCOVERY OF THE DOUBLE HELIX

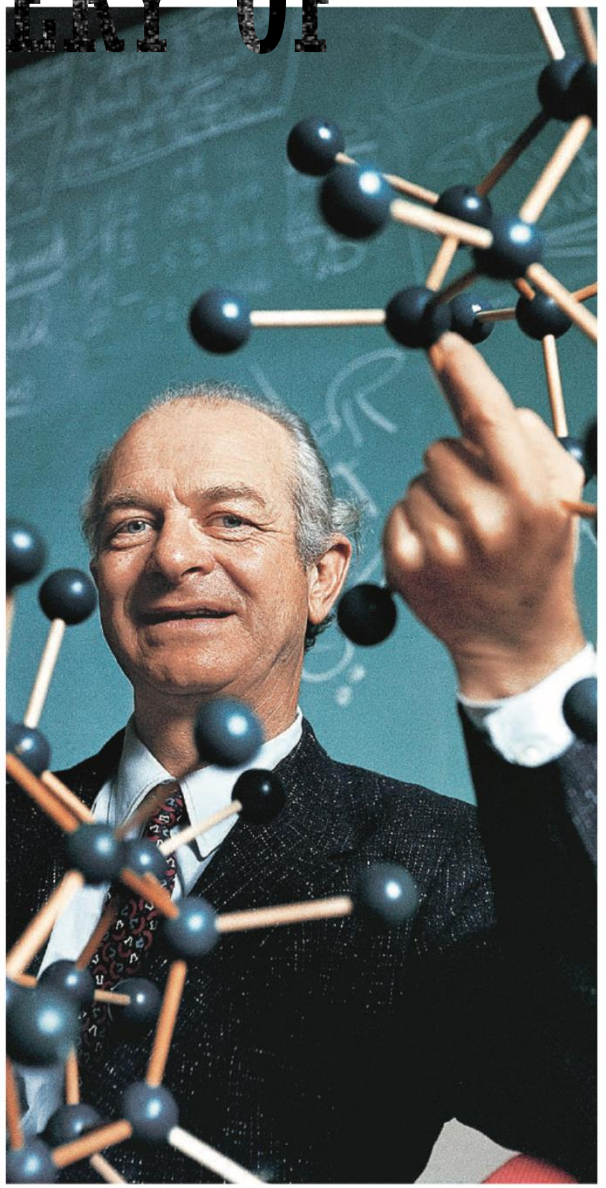
https://www.youtube.com/watch?v=JjO6n85wq8A&t=81s

# EVENTS LEADING TO THE DISCOVERY OF THE DNA DOUBLE HELIX

1. Model building started by Linus Pauling




(a) An α helix in a protein

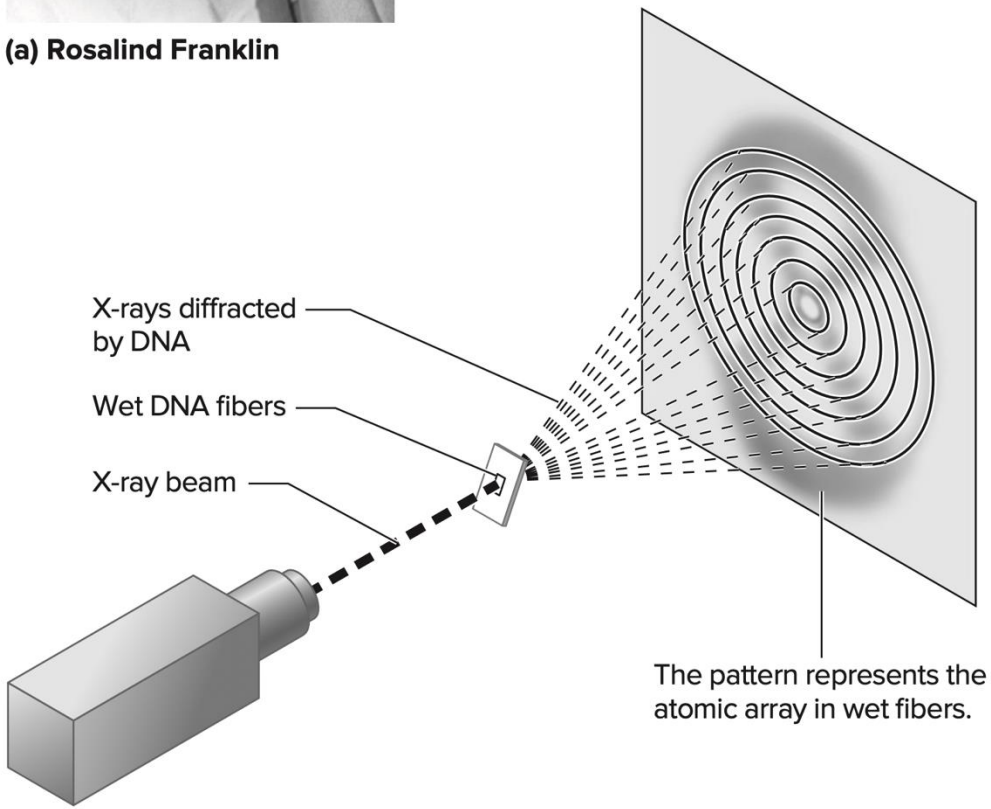


(b) Linus Pauling

# EVENTS LEADING TO THE DISCOVERY OF THE DNA DOUBLE HELIX

(a) Rosalind Franklin

1. Model building started by Linus Pauling
2. Use of X-ray diffraction




(b) X-ray diffraction of wet DNA fibers

***Suggested several features of DNA:***

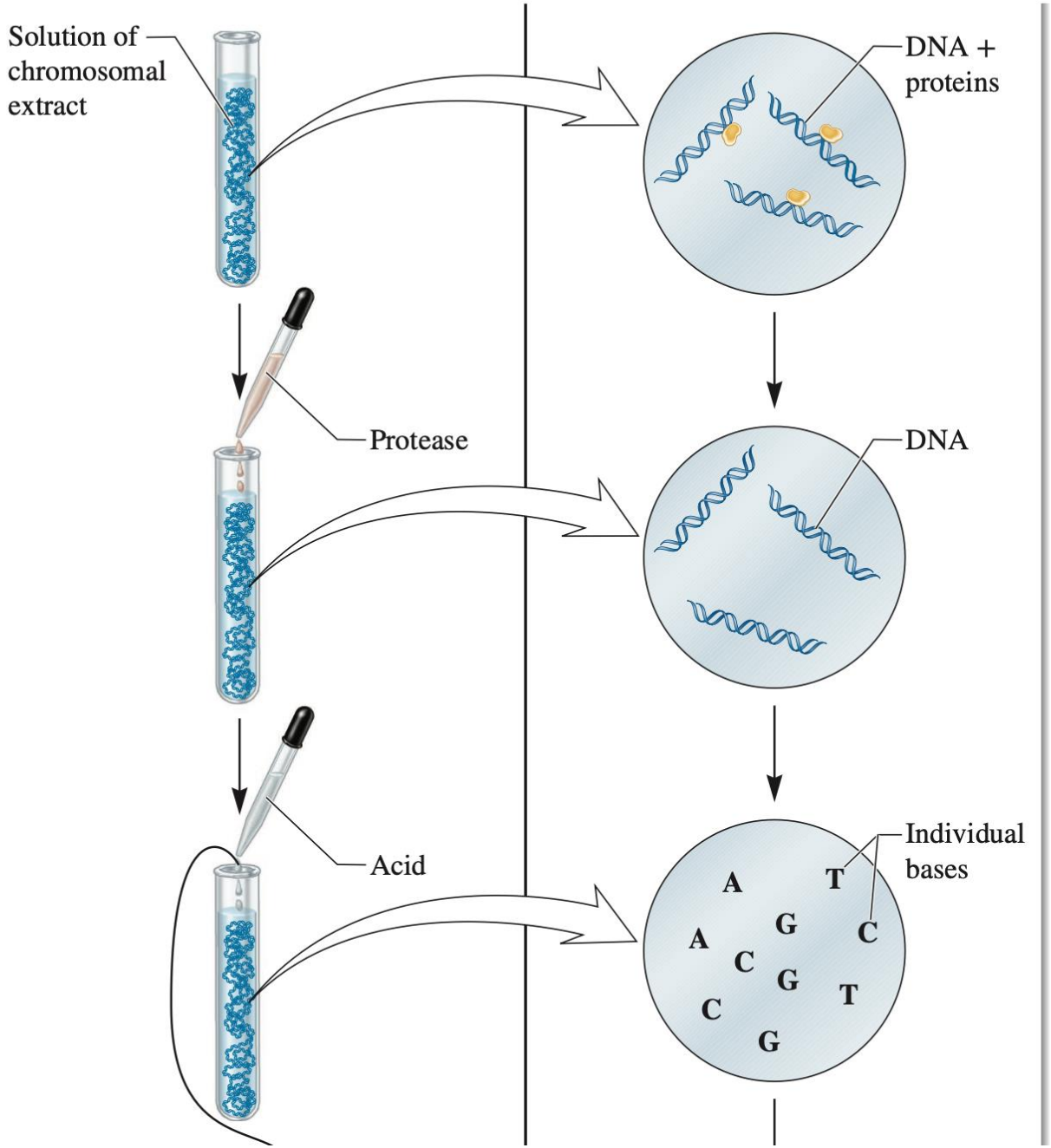
1. *Helical structure*
2. *Diameter is too wide for it to be made only by a single strand helix*
3. *Diffraction pattern indicated that the helix contains about 10 bp per complete turn.*

# ERWIN CHARGAFF (1940-1950)

1. For each type of cell, extract the chromosomal material. This can be done in a variety of ways, including treatment with concentrated salt solution, detergent, or mild alkali. Note: The chromosomes contain both DNA and protein.

2. Remove the protein. This can be done in several ways, including treatment with protease.

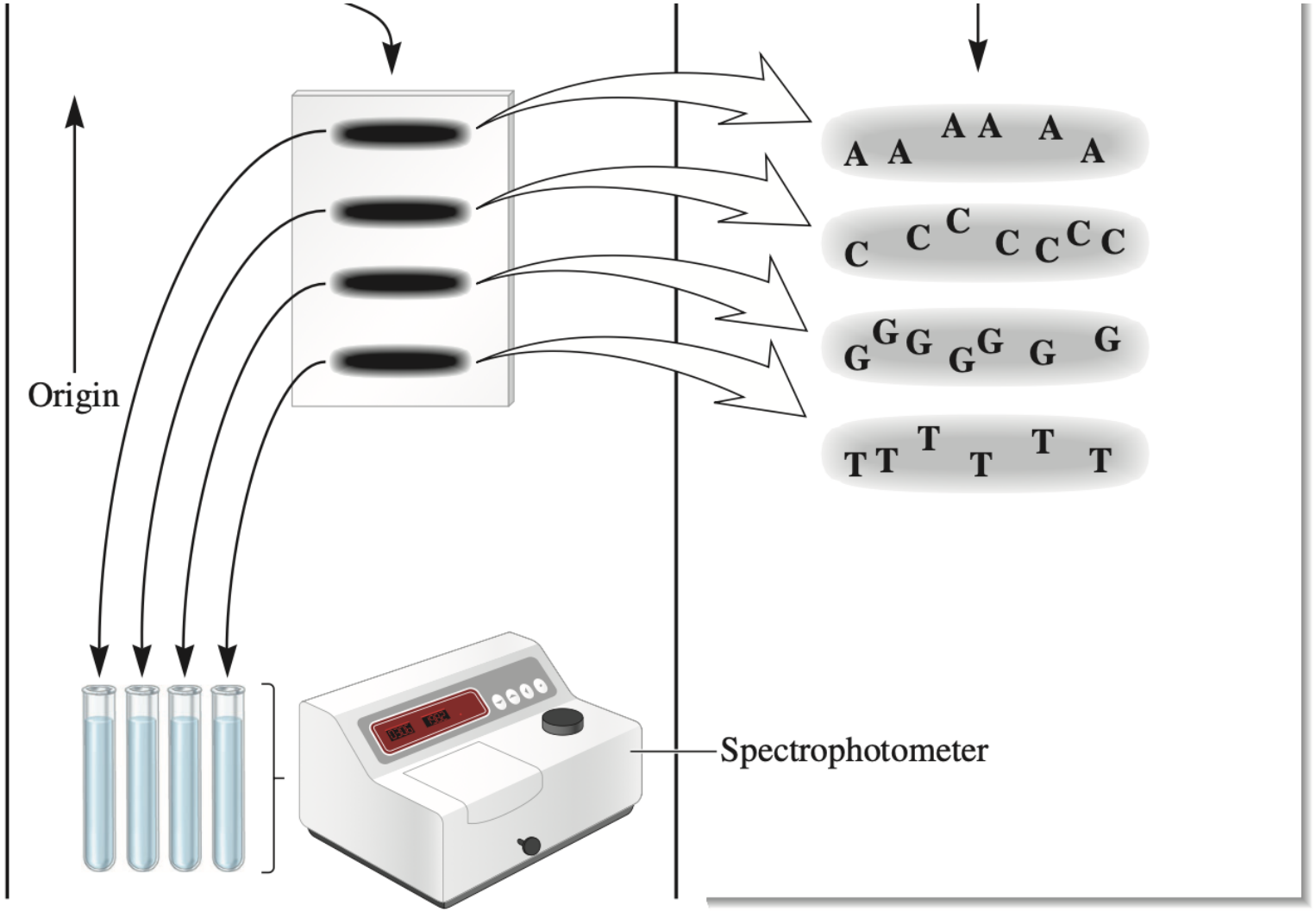
3. Hydrolyze the DNA to release the bases from the DNA strands. A common way to do this is by strong acid treatment.



4. Separate the bases by chromatography. Paper chromatography provides an easy way to separate the four types of bases. (The technique of chromatography is described in Appendix A.)

5. Extract bands from paper into solutions and determine the amounts of each base by spectroscopy. Each base will absorb light at a particular wavelength. By examining the absorption profile of a sample of base, it is then possible to calculate the amount of the base. (Spectroscopy is described in Appendix A.)

6. Compare the base content in the DNA from different organisms.



## THE DATA

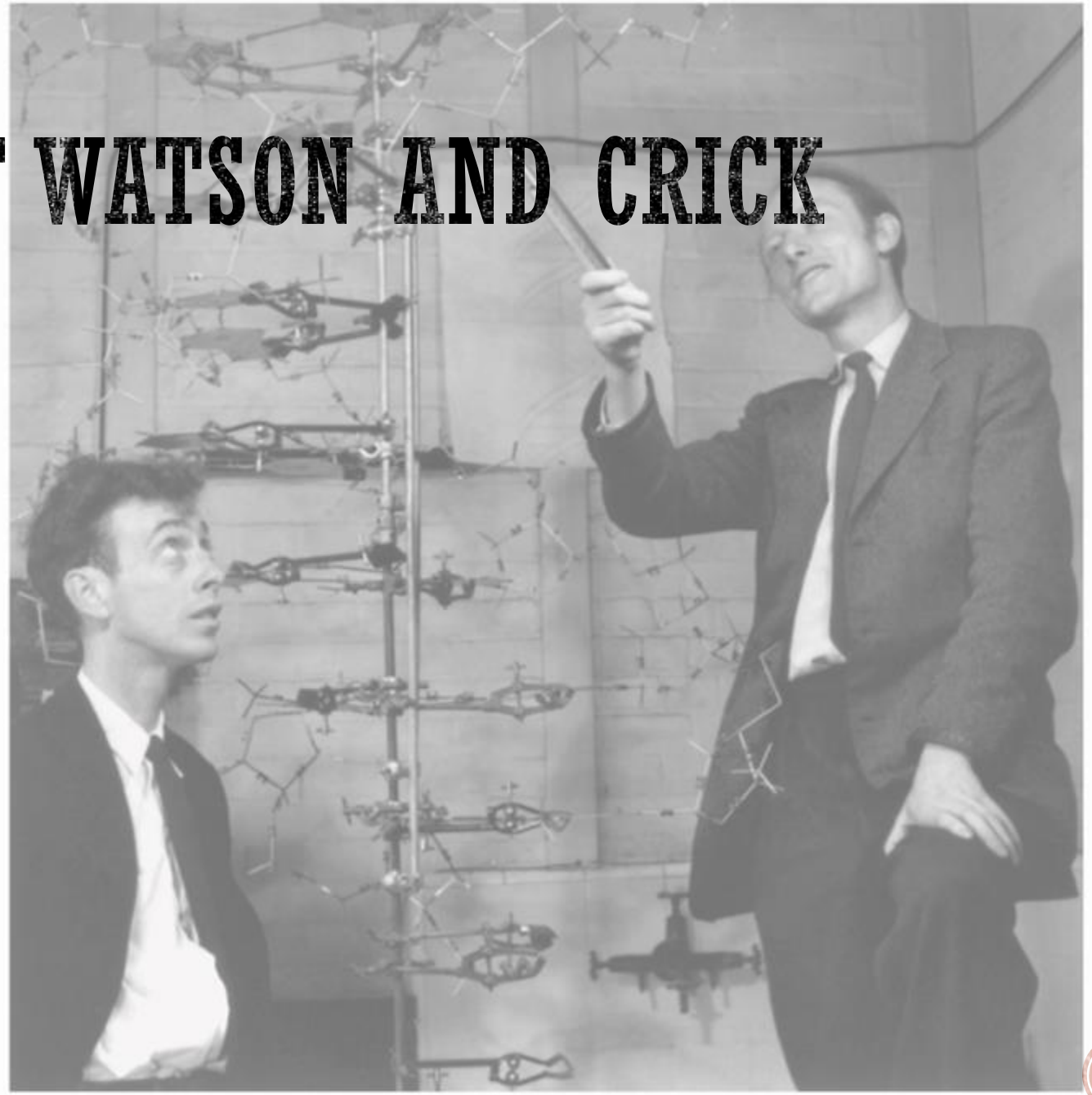
**Base Content in the DNA from a Variety of Organisms***

| | *Percentage of Bases (Based on Molarity)* | | | |
|---|---|---|---|---|
| *Organism* | *Adenine* | *Thymine* | *Guanine* | *Cytosine* |
| *E. coli* | 26.0 | 23.9 | 24.9 | 25.2 |
| *S. pneumoniae* | 29.8 | 31.6 | 20.5 | 18.0 |
| Yeast | 31.7 | 32.6 | 18.3 | 17.4 |
| Turtle red blood cells | 28.7 | 27.9 | 22.0 | 21.3 |
| Salmon sperm | 29.7 | 29.1 | 20.8 | 20.4 |
| Chicken red blood cells | 28.0 | 28.4 | 22.0 | 21.6 |
| Human liver cells | 30.3 | 30.3 | 19.5 | 19.9 |

# WATSON AND CRICK

# ASSUMPTIONS OF WATSON AND CRICK

1. DNA is made up of nucleotides linked together in a linear fashion
2. Chemical linkage between two nucleotides is always the same.



# STRUCTURE OF THE DNA DOUBLE HELIX

# GENERAL STRUCTURAL FEATURES OF A DOUBLE HELIX

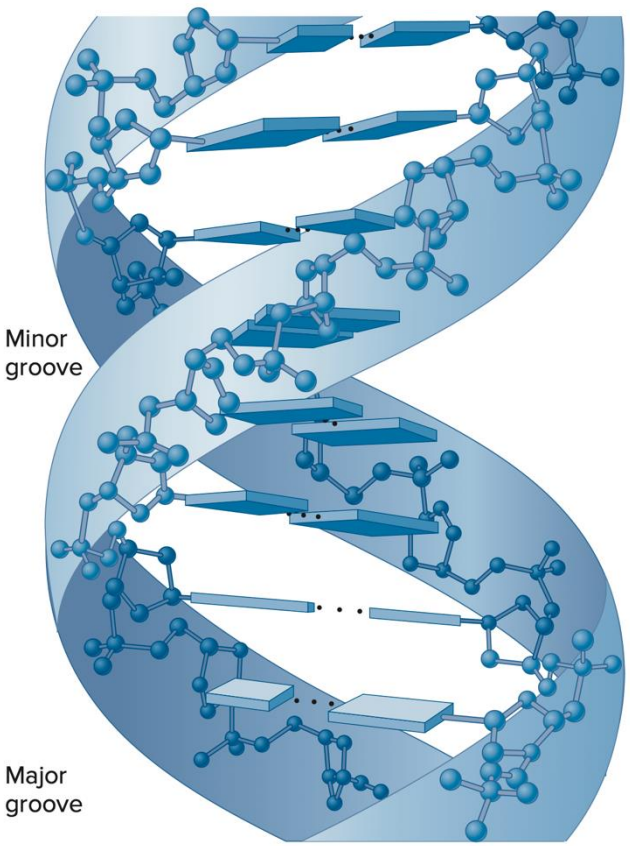
1. In a DNA double helix, two DNA strands are twisted together around a common axis to form a structure that resembles a spiral staircase.
2. The double stranded structure is stabilized by base pairs in opposite strand that are hydrogen bonded to each other. (The linear distance along a strand through such a complete turn is 3.4 nm; each base pair traverse 0.34 nm).
3. AT/GC rule keeps the width of the double helix relatively constant
4. G has 3 hydrogen bonds between C; A has 2 hydrogen bonds between T.

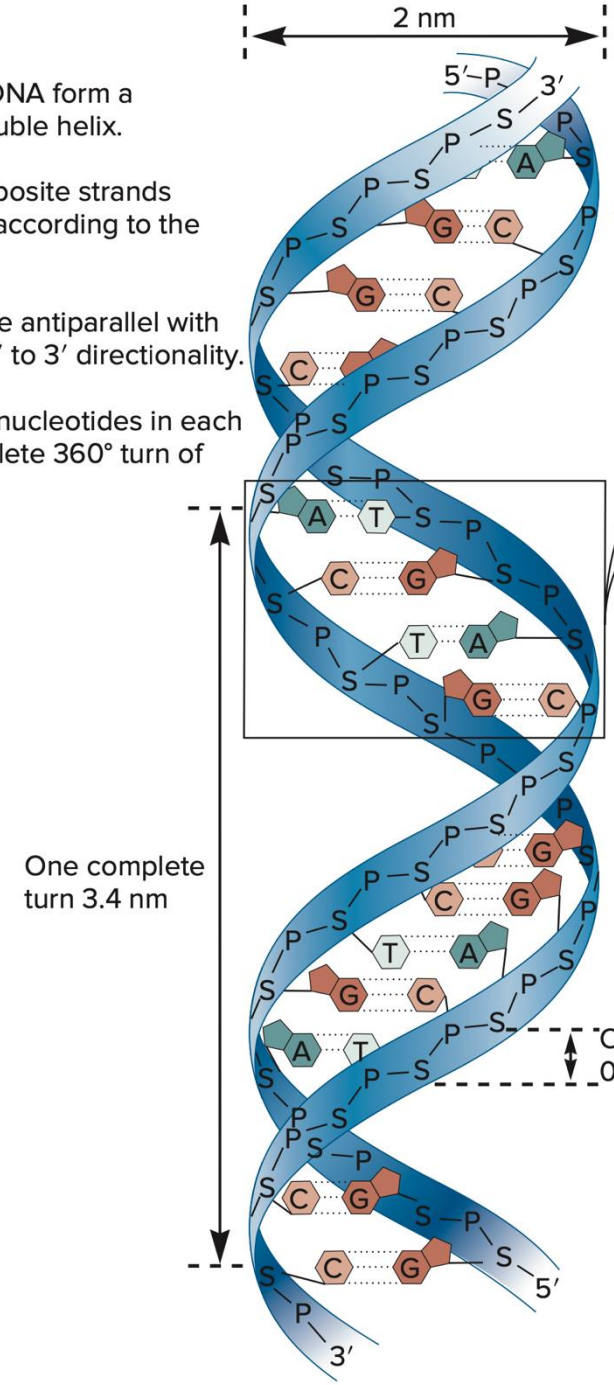
# GENERAL STRUCTURAL FEATURES OF A DOUBLE HELIX

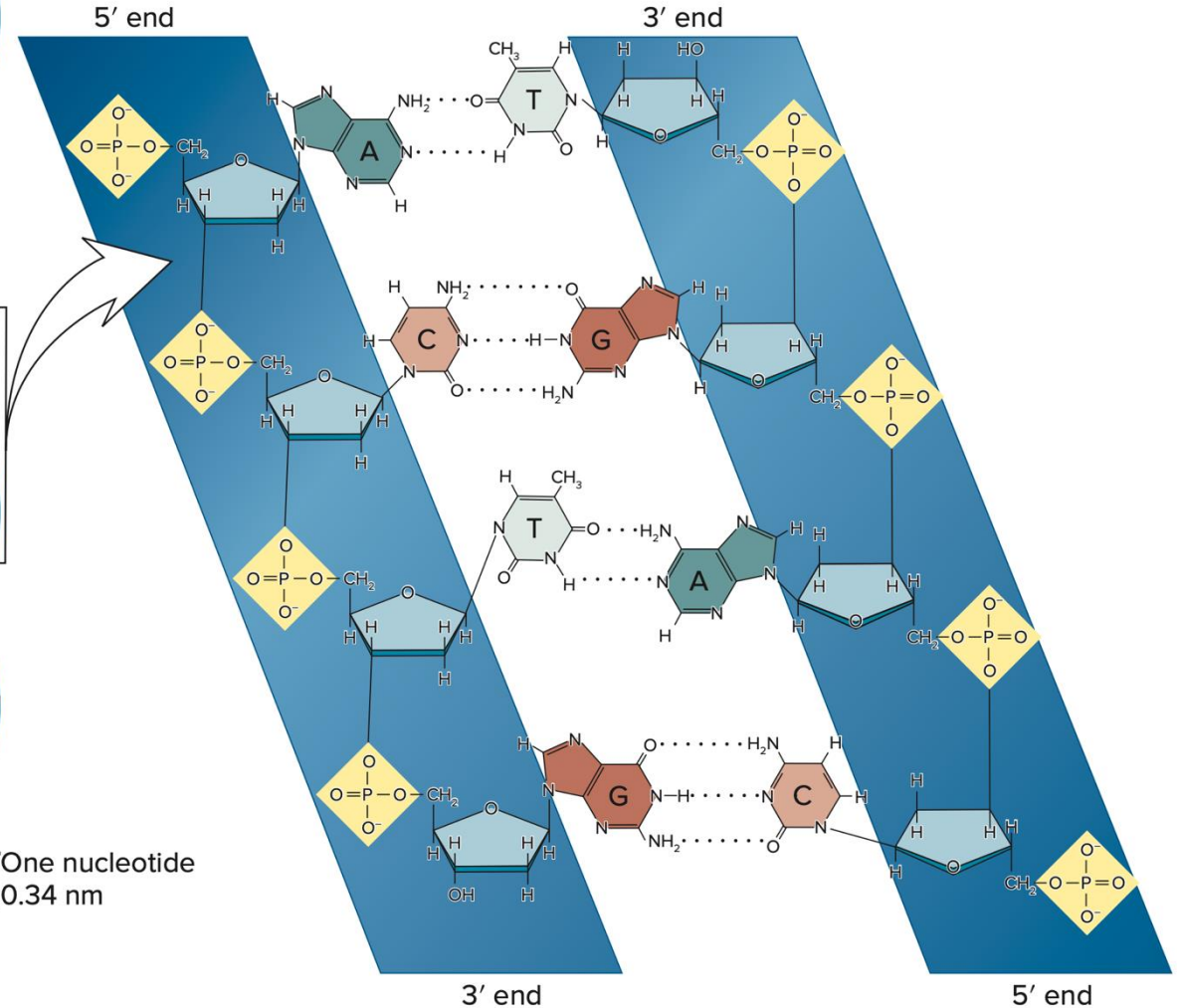
5. AT/GC rule allows the prediction of the sequence in one DNA strand if the sequence in the other strand is known. Called **complementary**.
6. Opposite direction of the two DNA strands is referred to as **antiparallel** arrangement.

**Key Features**

- Two strands of DNA form a right-handed double helix.
- The bases in opposite strands hydrogen bond according to the AT/GC rule.
- The 2 strands are antiparallel with regard to their 5′ to 3′ directionality.
- There are ~10.0 nucleotides in each strand per complete 360° turn of the helix.



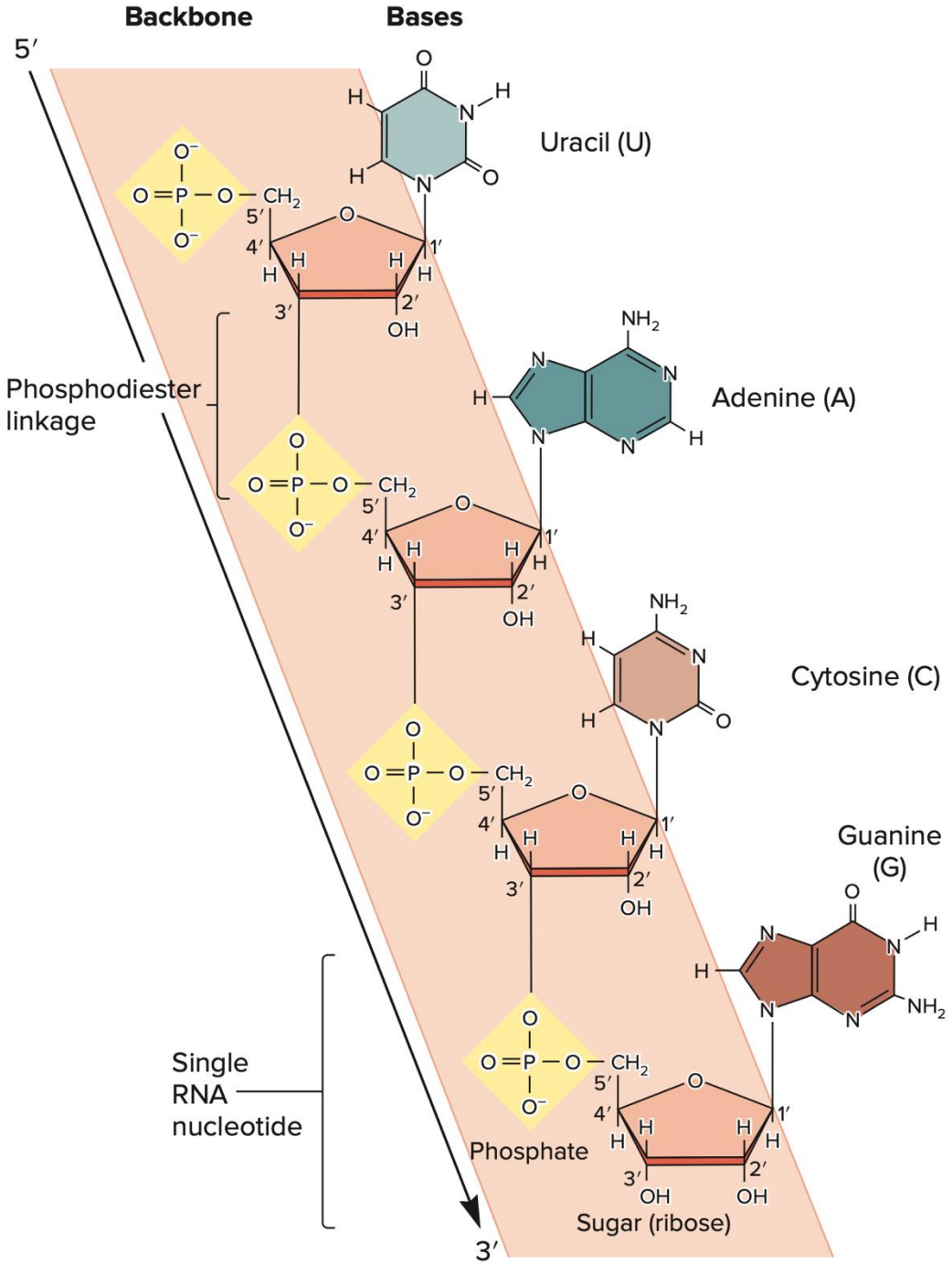
# QUESTION

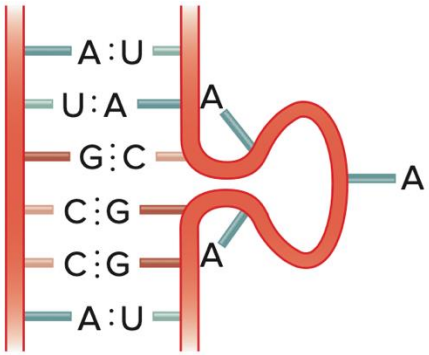
**A double-stranded molecule of B DNA contains 340 nucleotides. How many complete turns occur in this double helix?**

# RNA STRUCTURE

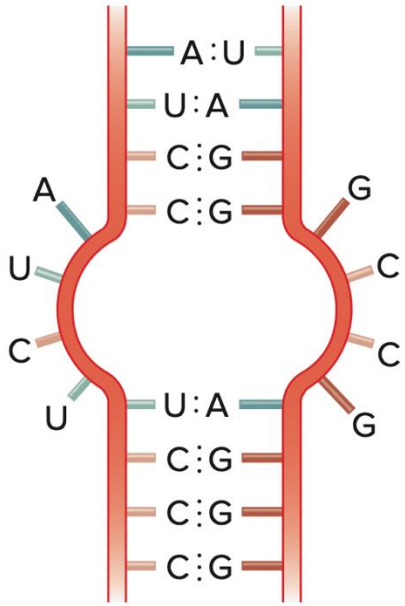
# RNA

- Has shorter strands than DNA
- DNA is used as template for RNA during transcription (only one of the two strands is used)
- Base pairing between A and U and C and G may occur in one RNA molecule or 2 different RNA molecule. This may cause:
  - ✓Formation of double stranded region that is helical
  - ✓Bulge loops, an internal loop, a multibranched junction and a stem-loop (hairpin)

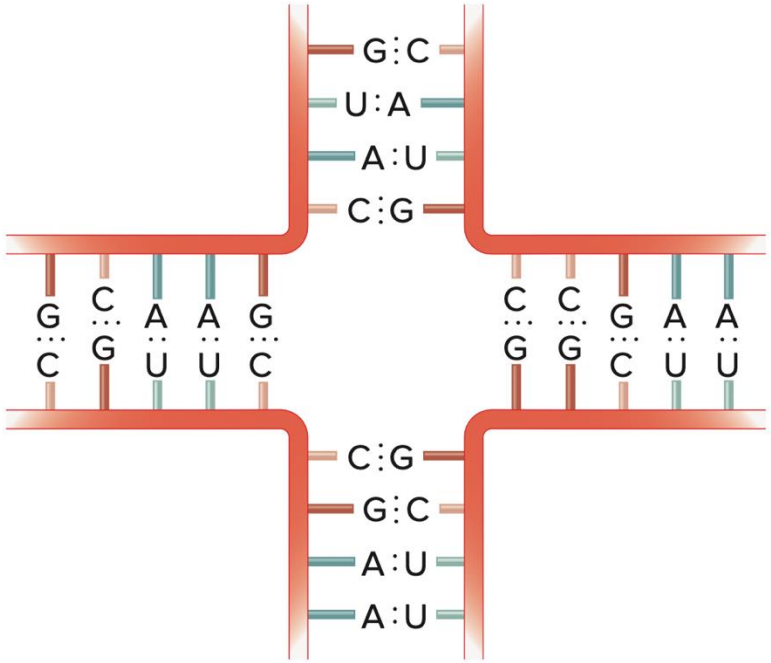




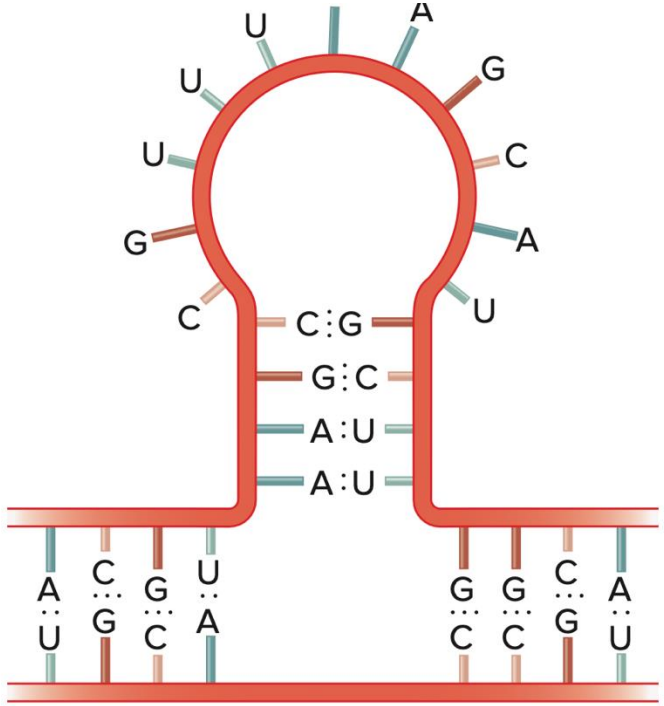

(a) Bulge loop



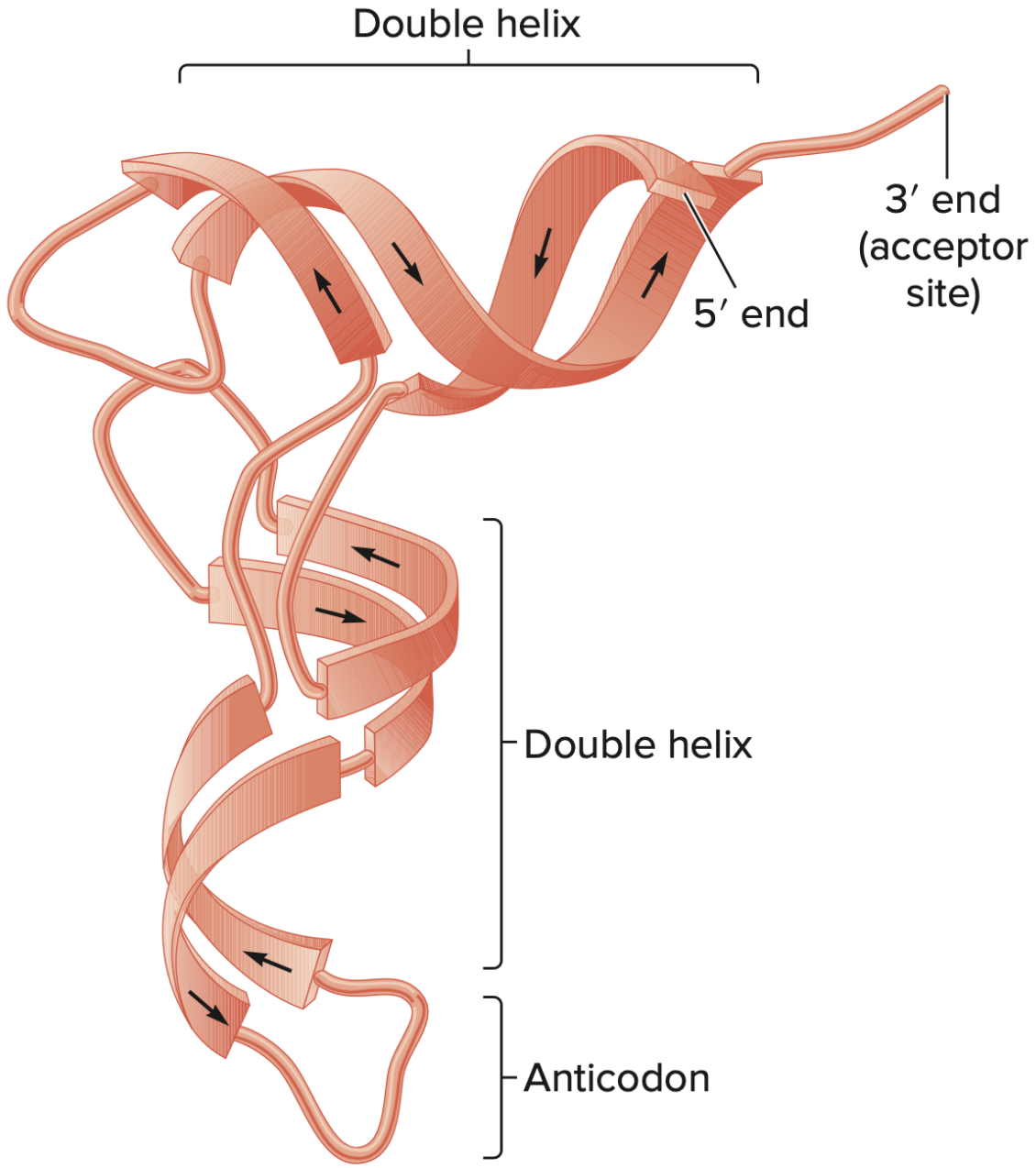

(b) Internal loop




(c) Multibranched junction




(d) Stem-loop




**(a) Ribbon model**

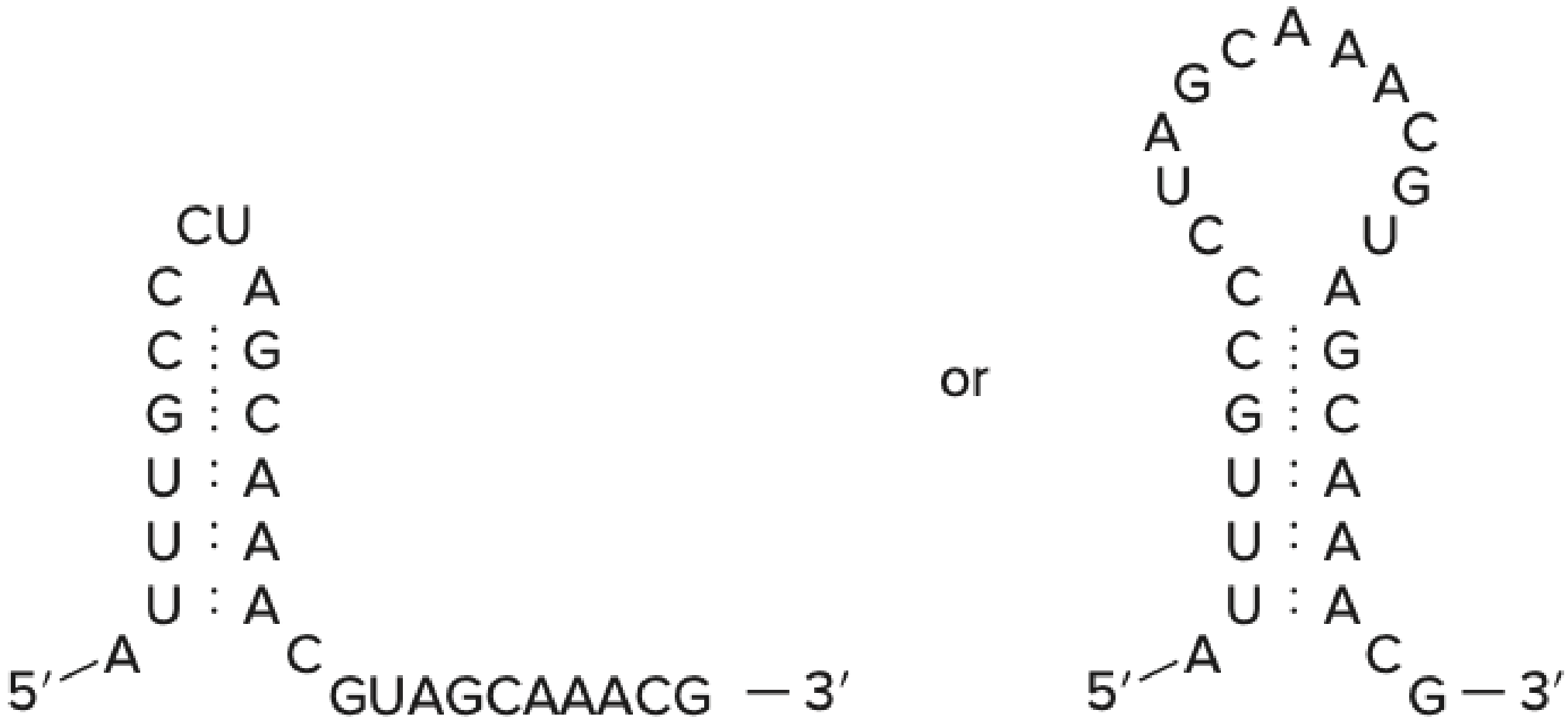
# TASK

A hypothetical base sequence of an RNA molecule is

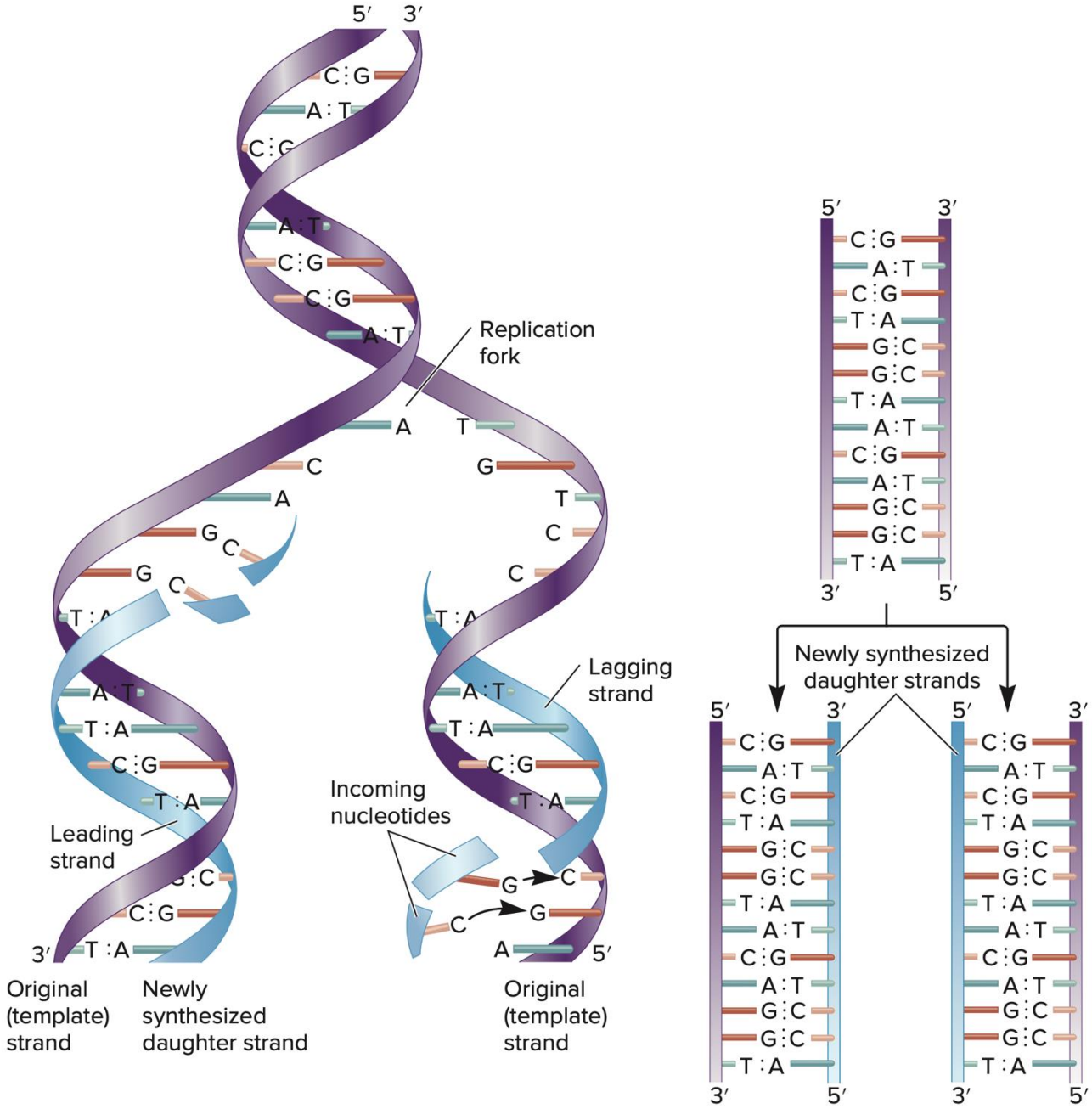
5’-AUUUGCCCUAGCAAACGUAGCAAACG-3’

Using two of the three underlined parts of this sequence, draw two possible stem-loop structures that might form in this RNA.

**ANSWER:**


CU
C A
C G
G C
U A
U A
U A
5′ A
C
GUAGCAAACG — 3′
or
C A A
G A
A C
U G
C U
C A
C G
G C
U A
U A
U A
5′ A
C
G — 3′

# DNA REPLICATION

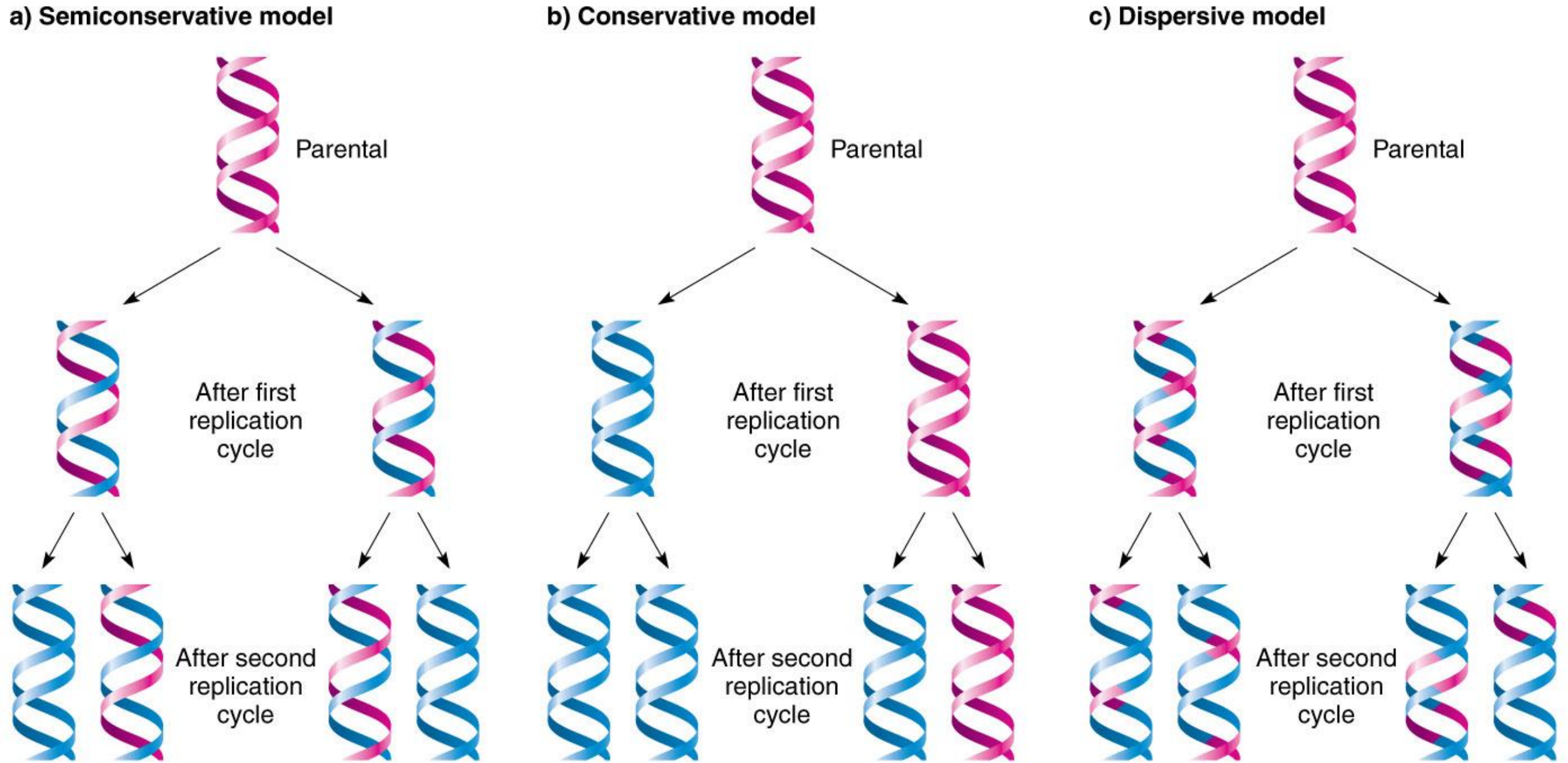



**(a) The mechanism of DNA replication**

**(b) The products of replication**

# 3 MODELS DESCRIBING END RESULTS OF DNA REPLICATION

1. Conservative Model
2. Semiconservative Model
3. Dispersive Model



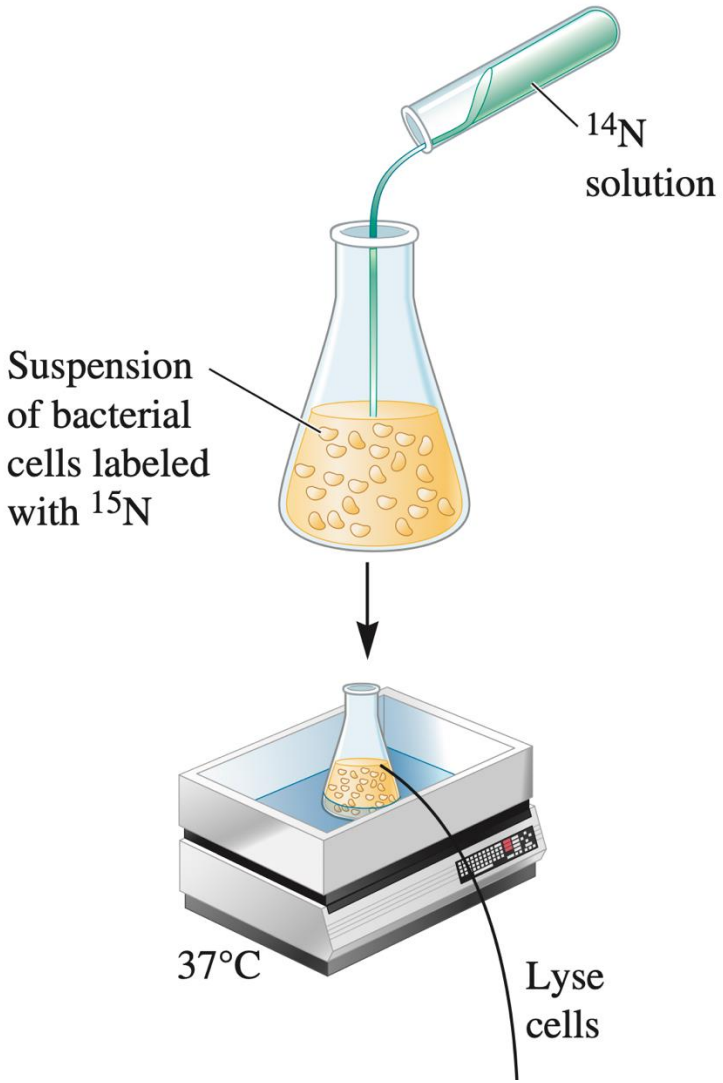
**Starting material:** A strain of *E. coli* that has been grown for many generations in the presence of $^{15}N$. All of the bases in the bacterial DNA are initially labeled with $^{15}N$.

1. Add an excess of $^{14}N$-containing compounds to the growth medium so all of the newly made DNA will contain $^{14}N$.

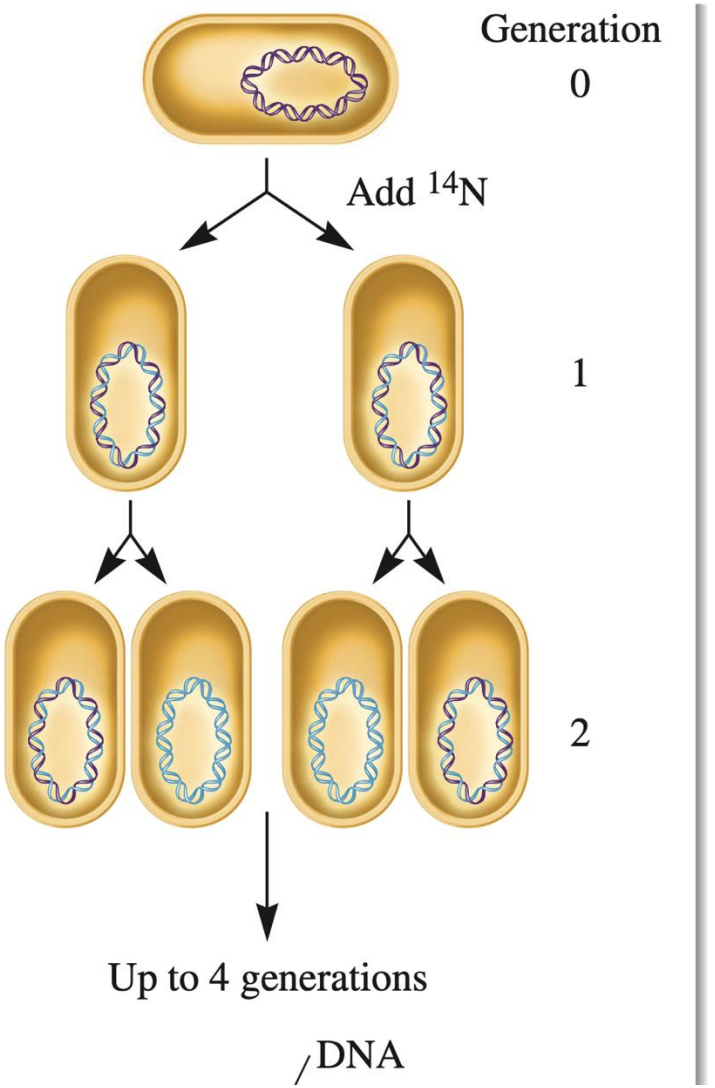
2. Incubate the cells for various lengths of time. Note: The $^{15}N$-labeled DNA is shown in purple, and the $^{14}N$-labeled DNA is shown in blue.

3. Lyse the cells by adding lysozyme and detergent, which disrupt the bacterial cell wall and cell membrane, respectively.

**Experimental level**



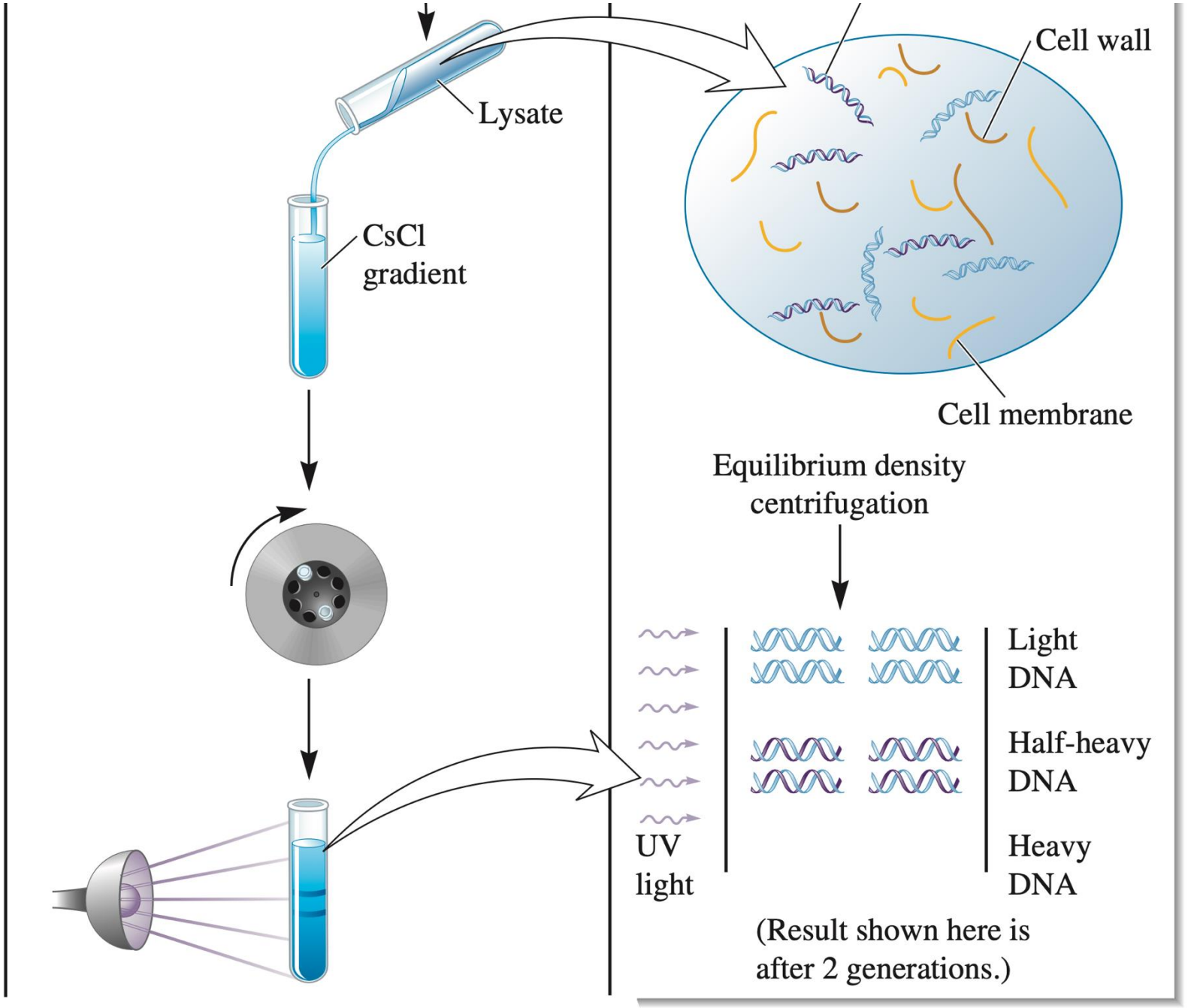

**Conceptual level**



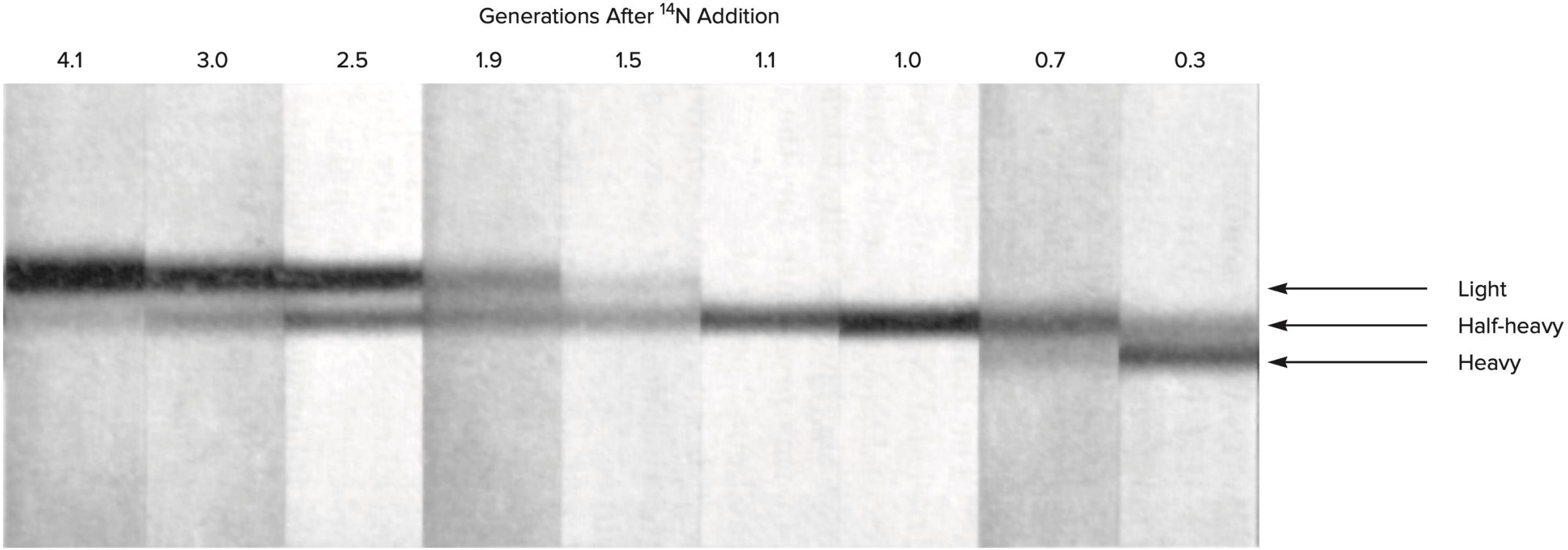
4. Load a sample of the lysate onto a CsCl gradient. Note: The average density of DNA is around 1.7 g/cm$^3$, which is sufficiently different from other cellular macromolecules.

5. Centrifuge the gradients until the DNA molecules reach their equilibrium densities.

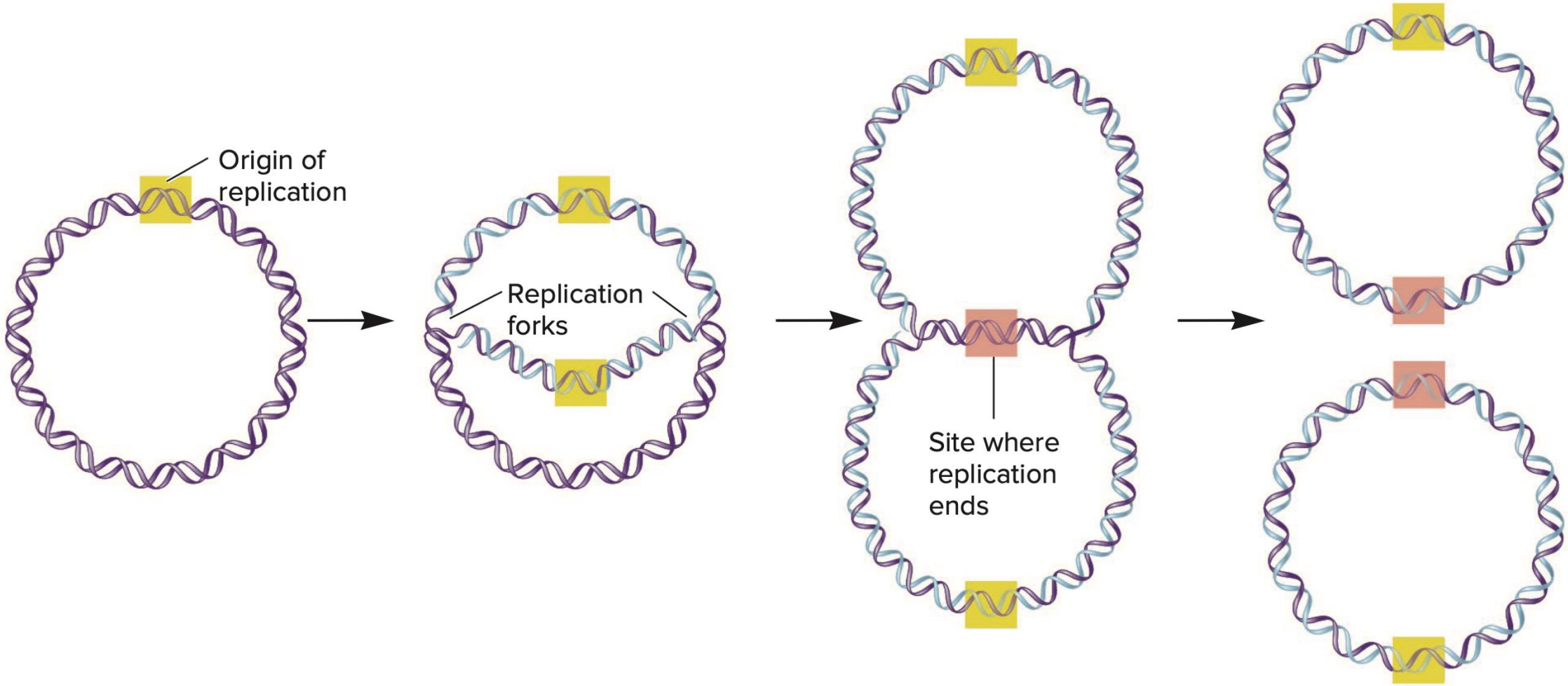
6. DNA within the gradient can be observed under a UV light.



## THE DATA


Generations After $^{14}N$ Addition
4.1
3.0
2.5
1.9
1.5
1.1
1.0
0.7
0.3
Light
Half-heavy
Heavy

# BACTERIAL DNA REPLICATION


Origin of replication
Replication forks
Site where replication ends

# TERMS:

- **Origin of replication** – site on the bacterial chromosome where DNA synthesis begins
- **Bidirectional** – proceeds in both directions
- **Replication fork** – region where the parental DNA strand have separated and new daughter strands are being made

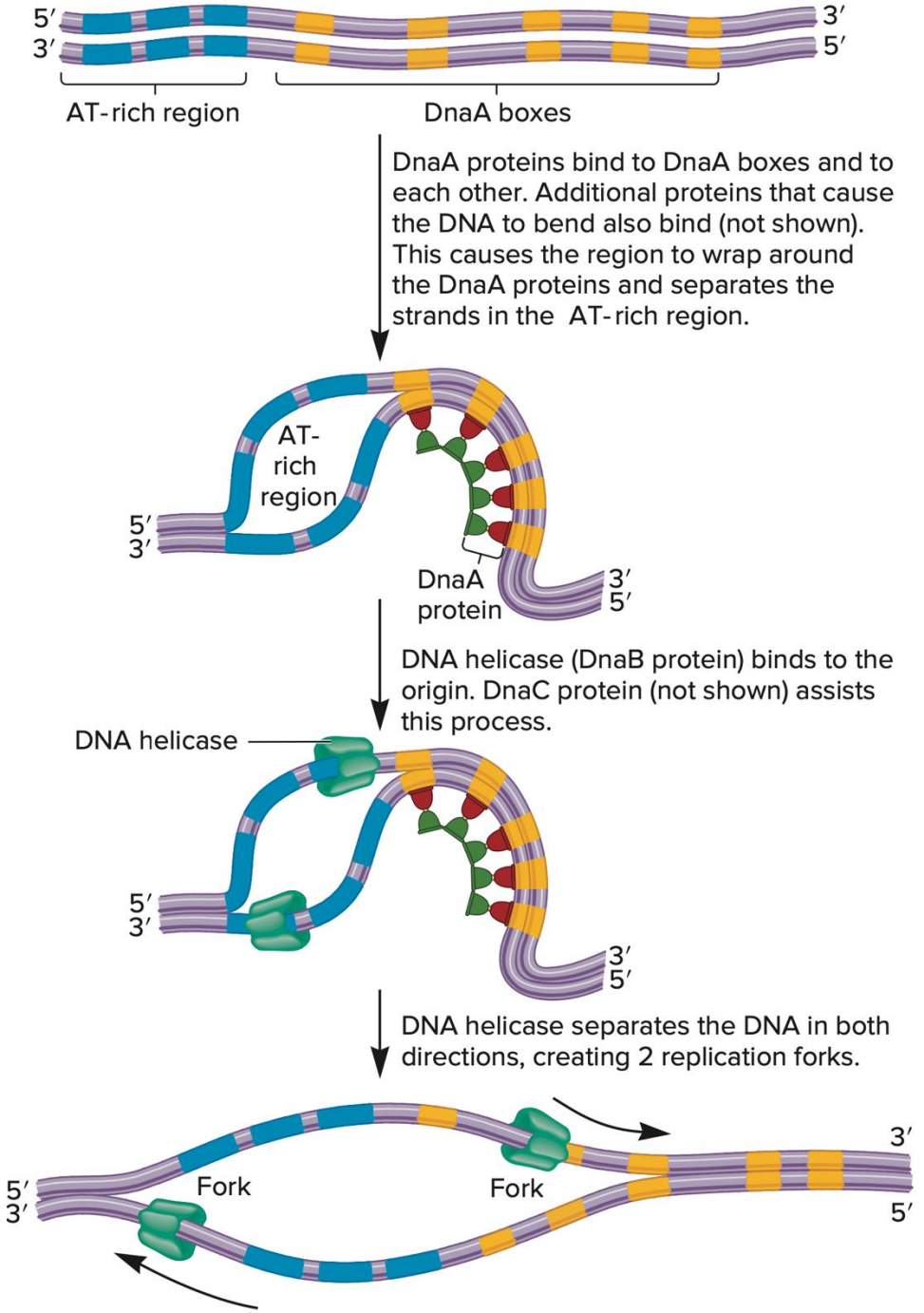
# INITIATION OF REPLICATION

- Termed as the *oriC* (origin of chromosomal replication)
- The 3 types of DNA sequence in oriC
  1. **AT-rich region** – where DNA strands will separate
  2. **DnaA boxes** – provide binding sites for DnaA proteins which initiates the replication fork
  3. **GATC methylation site** – provide mechanism to regulate DNA replication


E. coli
chromosome
oriC
AT-rich region
5′–GGATCCTGGGTATTAAAAAGAAGATCTATTTATTTAGAGATCTGTTCTAT
CCTAGGACCCATAATTTTTCTTCTAGATAAATAAATCTCTAGACAAGATA
1
50
DnaA box
TGTGATCTCTTATTAGGATCGCACTGCCCTGTGGATAACAAGGATCGGCT
ACACTAGAGAATAATCCTAGCGTGACGGGACACCTATTGTTCCTAGCCGA
51
100
DnaA box
TTTAAGATCAACAACCTGGAAAGGATCATTAACTGTGAATGATCGGTGAT
AAATTCTAGTTGTTGGACCTTTCCTAGTAATTGACACTTACTAGCCACTA
101
150
DnaA box
CCTGGACCGTATAAGCTGGGATCAGAATGAGGGTTATACACAGCTCAAAA
GGACCTGGCATATTCGACCCTAGTCTTACTCCCAATATGTGTCGAGTTTT
151
200
DnaA box
ACTGAACAACGGTTGTTCTTTGGATAACTACCGGTTGATCCAAGCTTCCT
TGACTTGTTGCCAACAAGAAACCTATTGATGGCCAACTAGGTTCGAAGGA
201
250
DnaA box
GACAGAGTTATCCACAGTAGATCGC –3′
CTGTCTCAATAGGTGTCATCTAGCG
251
275

**EVENTS**:

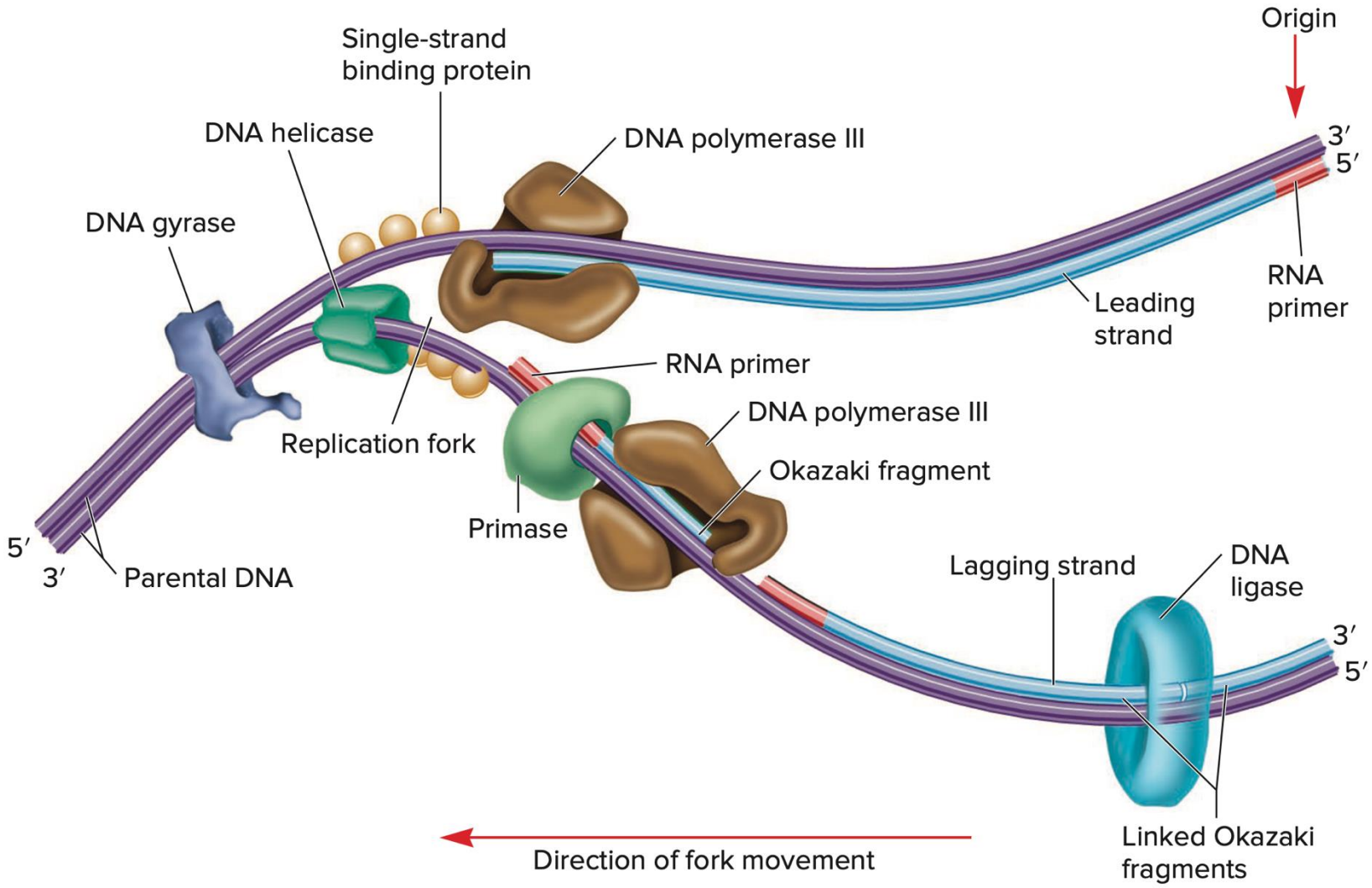
1. DNA replication begins with the binding of DnaA proteins to DnaA boxes within the origin of replication.
2. Other DNA-binding proteins not shown, such as HU and IHF, cause the DNA to bend around the complex of DnaA proteins, which results in the separation of the strands at the AT-rich region.
3. Following separation at the AT-rich region, the DnaA proteins, with the help of DnaC proteins, recruit DNA helicase to this site.
4. Two DNA helicases begin strand separation within the oriC region and continue to separate the DNA strands beyond the origin.



# Synthesis of the New Strand

**Functions of key proteins involved with bacterial DNA replication**

- DNA helicase breaks the hydrogen bonds between the DNA strands.
- DNA gyrase alleviates positive supercoiling.
- Single-strand binding proteins keep the parental strands apart.
- Primase synthesizes an RNA primer.
- DNA polymerase III synthesizes a daughter strand of DNA.
- DNA polymerase I excises the RNA primers and fills in with DNA (not shown).
- DNA ligase covalently links the Okazaki fragments together.



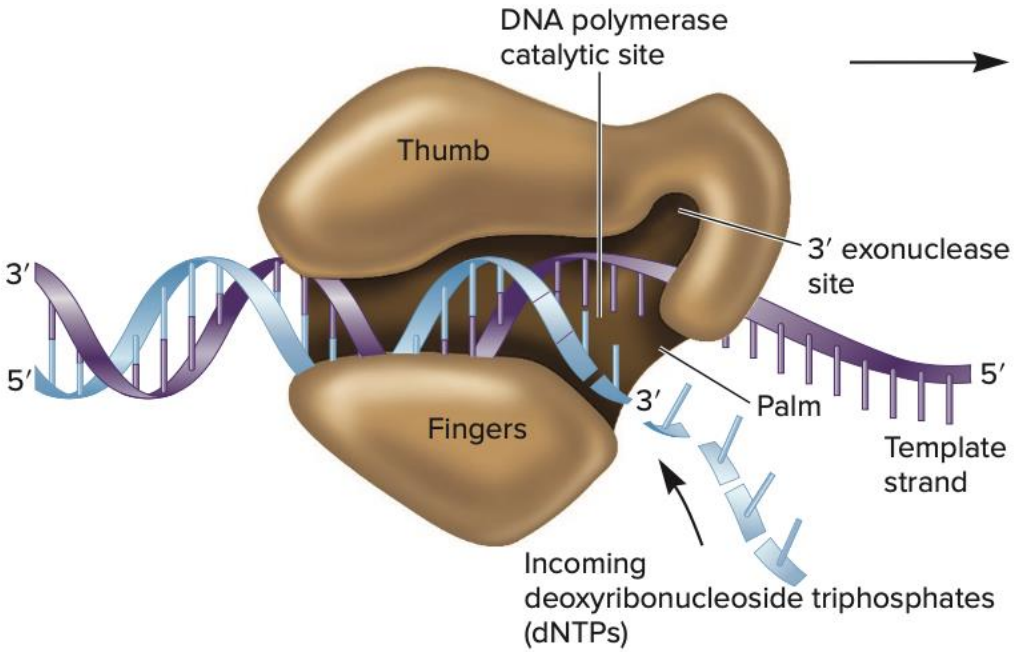
| Proteins Involved in *E. coli* DNA Replication | |
|---|---|
| **Common Name** | **Function** |
| DnaA proteins | Bind to DnaA box sequences within the origin of replication to initiate DNA replication |
| DnaC proteins | Aid DnaA in the recruitment of DNA helicase to the origin |
| DNA helicase (DnaB) | Separates double-stranded DNA |
| DNA gyrase (topoisomerase II) | Removes positive supercoiling ahead of the replication fork |
| Single-strand binding proteins | Bind to single-stranded DNA and prevent it from re-forming a double-stranded structure |
| Primase | Synthesizes short RNA primers |
| DNA polymerase III | Synthesizes DNA in the leading and lagging strands |
| DNA polymerase I | Removes RNA primers, fills in gaps with DNA |
| DNA ligase | Covalently attaches adjacent Okazaki fragments |
| Tus | Binds to ter sequences and prevents the advancement of the replication fork |

# UNWINDING OF THE DNA HELIX AND START OF REPLICATION

- DNA helicase breaks the hydrogen bonds between base pairs and unwinds the strands.
- DNA gyrase or topoisomerase II travels in front of the DNA helicase and relaxes positive supercoiling
- Single-strand binding proteins binds to the strands of parental DNA and prevents them from re-forming a double helix
- RNA primers are synthesized using the enzyme primase which results to 10-12 nucleotides in length
  - **Leading strand** – single primer is made at the origin of replication
  - **Lagging strand** – multiple primers are made

# SYNTHESIS OF DNA

- DNA polymerase catalyzes the formation of covalent bonds between adjacent nucleotides
  - DNA polymerase I and III – involve in DNA replication during cell division
  - DNA polymerase II, IV and V – involve in DNA repair and the replication of damaged DNA
- 2 unusual features of DNA polymerase
  1. Dna polymerase cannot begin DNA synthesis.
  2. DNA polymerase can attach nucleotides only in the 5' to 3' direction, not in the 3' to 5' direction




**(a) Schematic side view of DNA polymerase bound to DNA.**

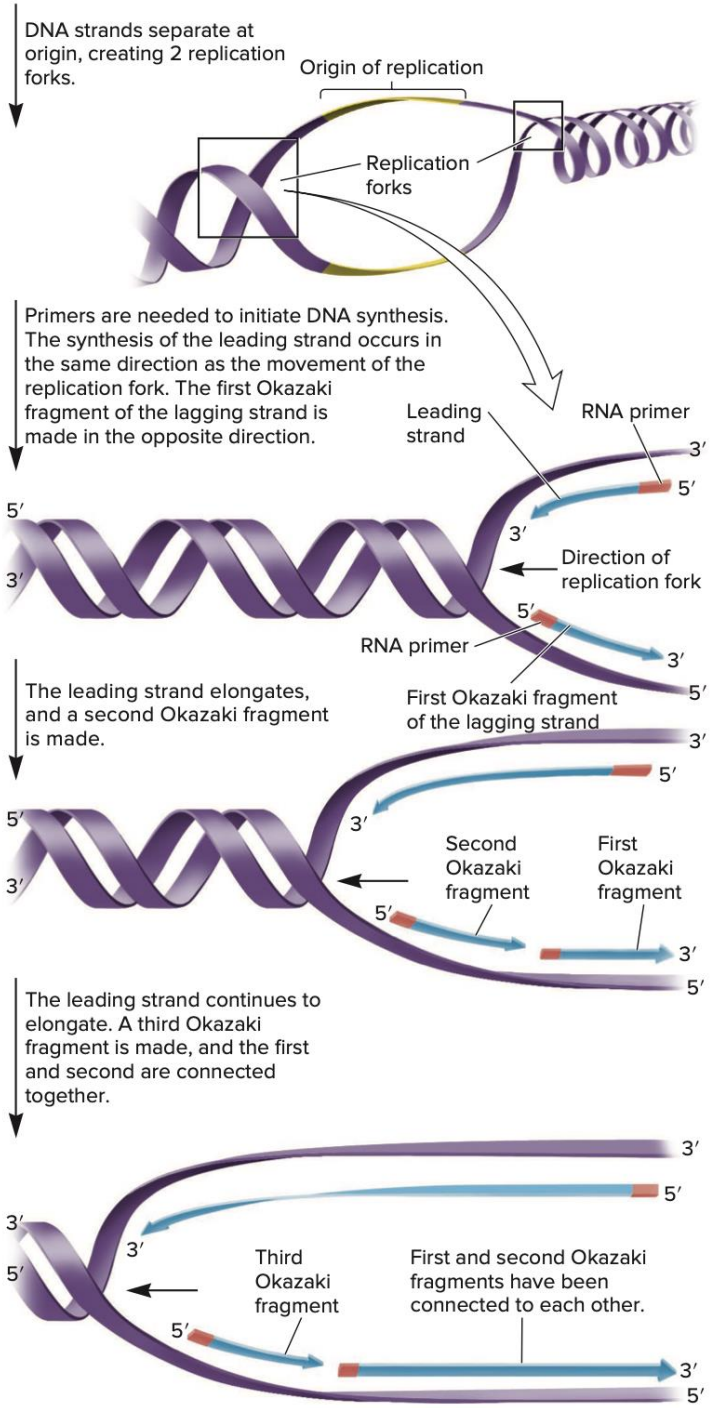
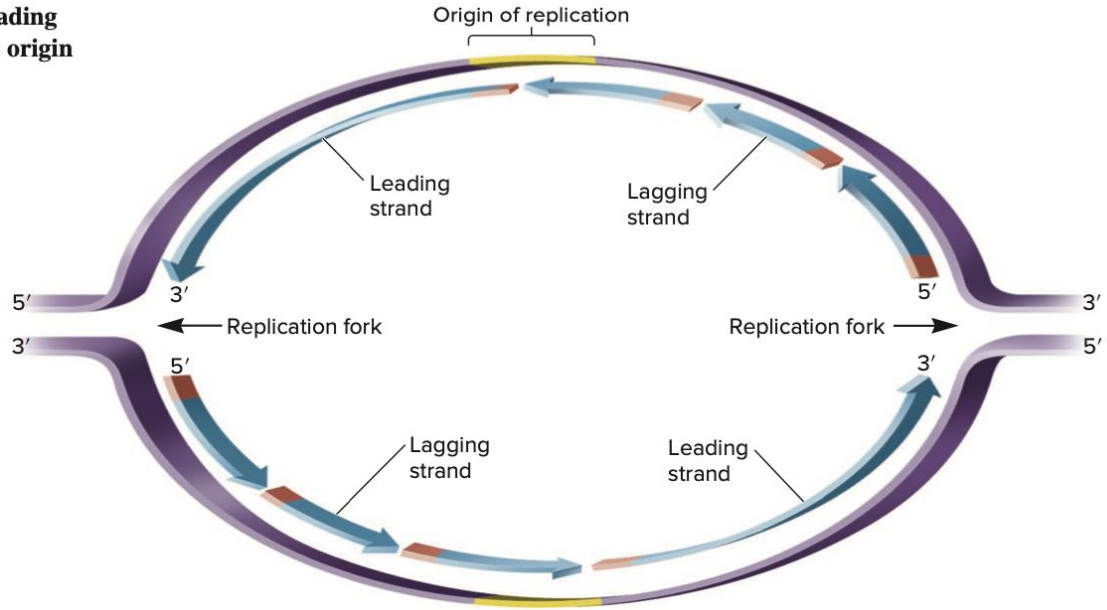


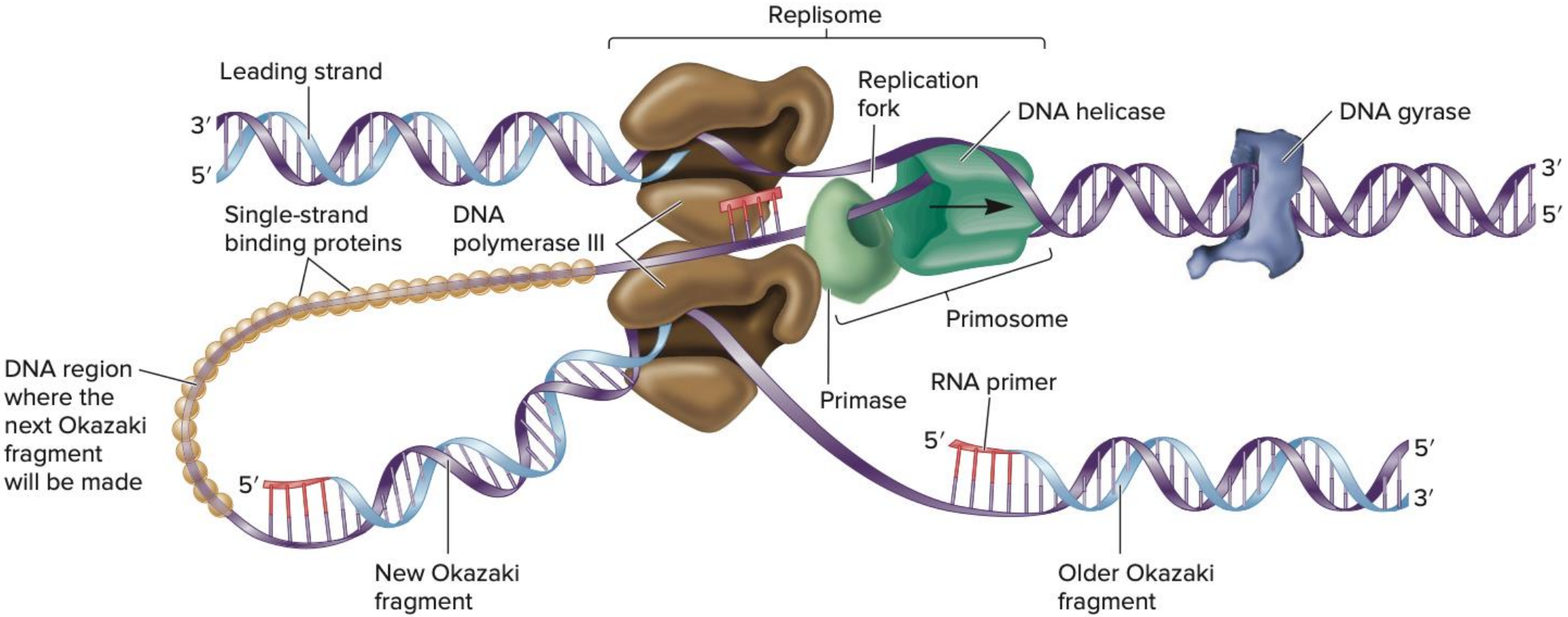

**FIGURE 11.11** **The synthesis of leading and lagging strands outward from a single origin of replication.**



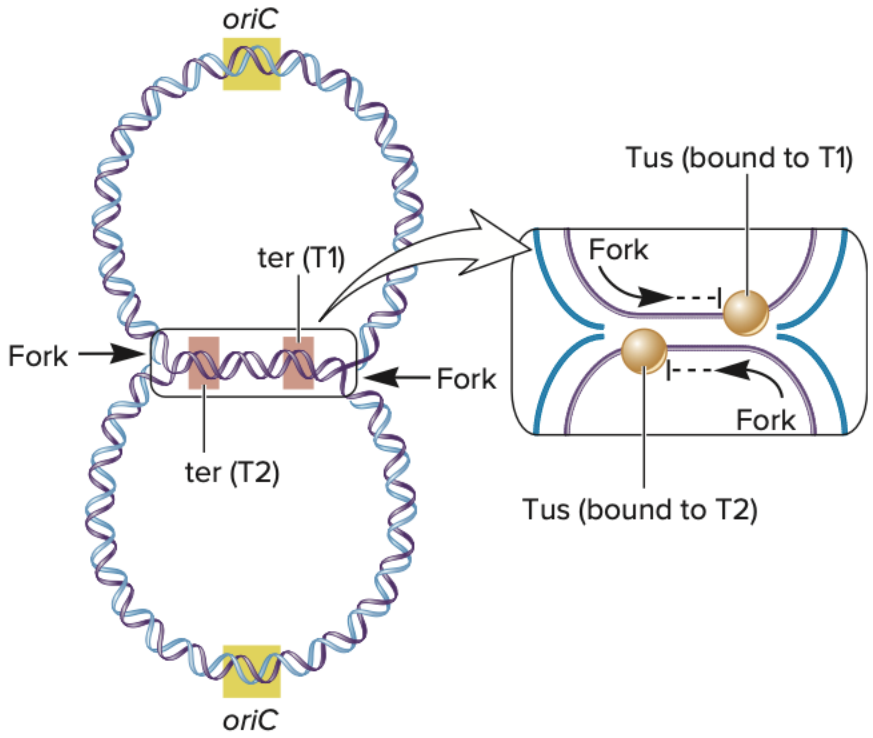

DNA ligase catalyzes the formation of a covalent bond between Adjacent Okazaki fragments to complete the replication process In the lagging strand.

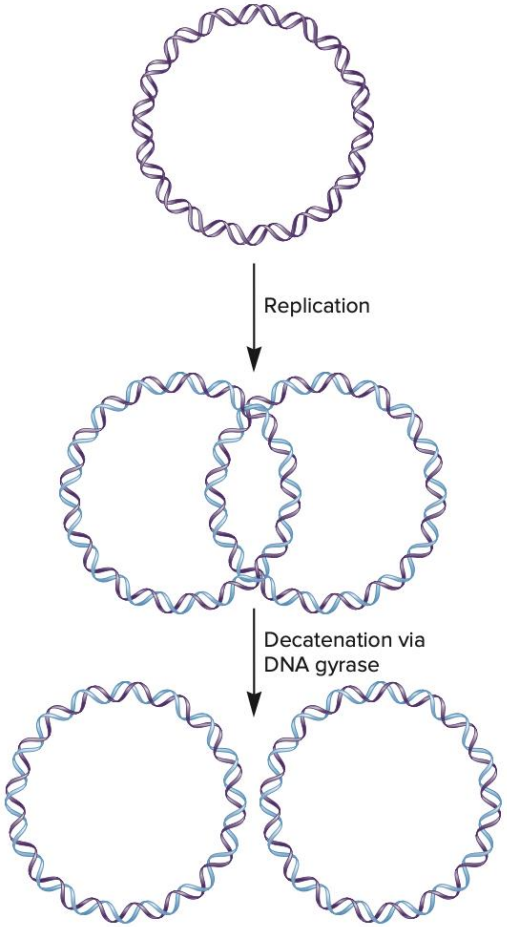
*DNA ligase in bacteria requires NAD+ to carry out reaction

*DNA ligase in archae and eukaryotes require ATP


Replisome
Leading strand
3′
5′
Replication fork
DNA helicase
DNA gyrase
3′
5′
Single-strand binding proteins
DNA polymerase III
Primosome
DNA region where the next Okazaki fragment will be made
Primase
RNA primer
5′
5′
3′
5′
New Okazaki fragment
Older Okazaki fragment

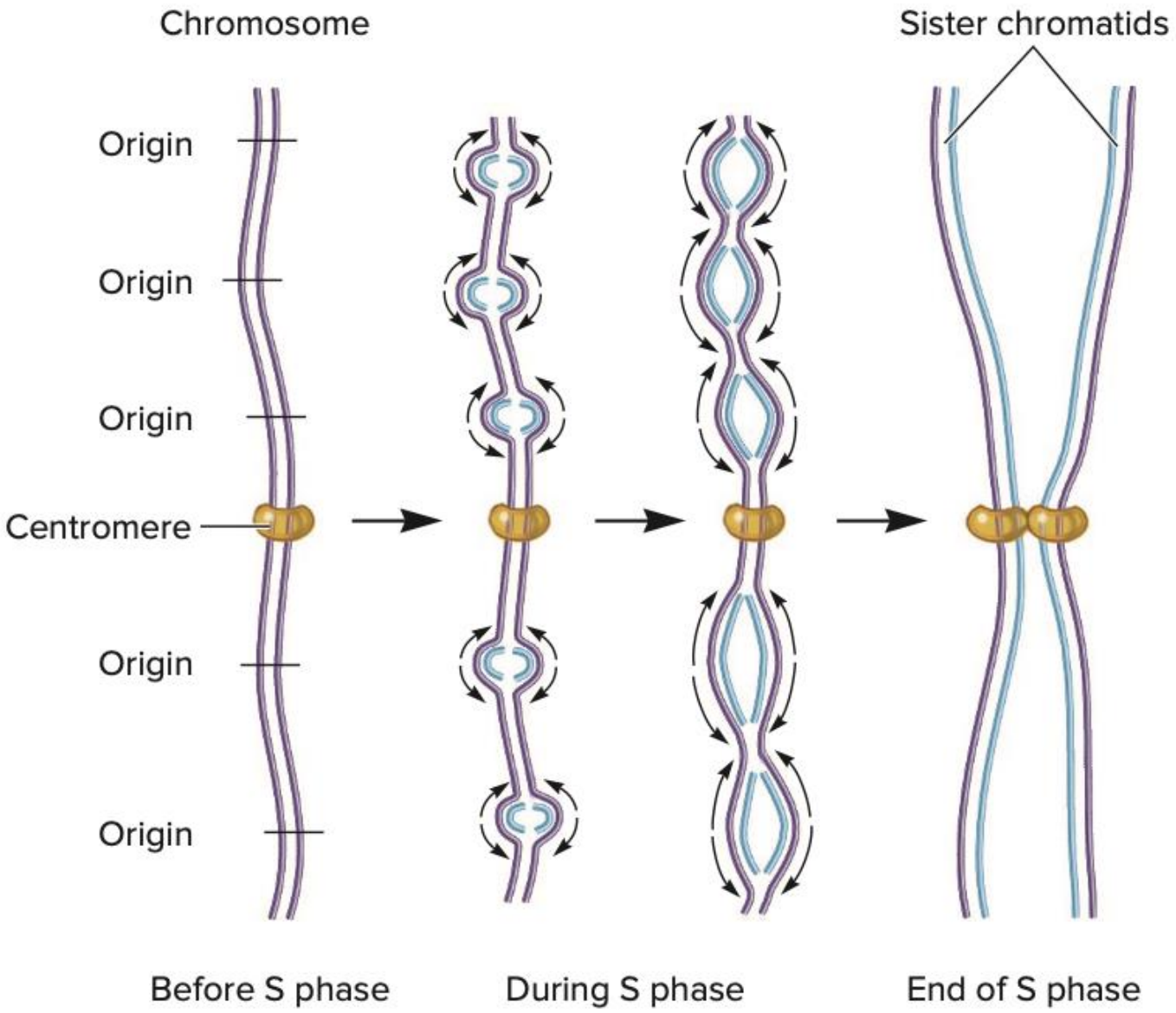
# TERMINATION OF DNA REPLICATION



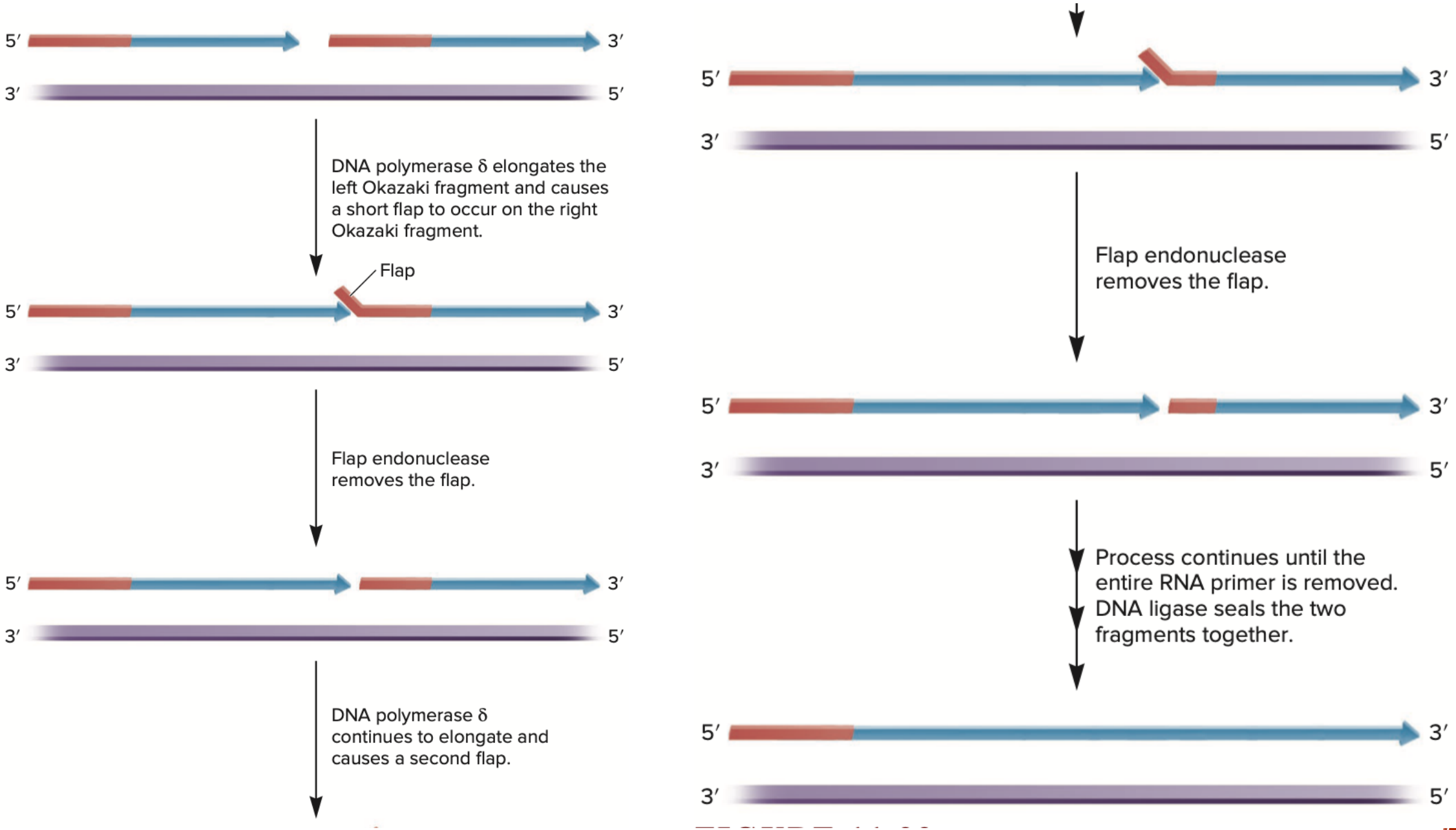
# EUKARYOTIC DNA REPLICATION

# OVERVIEW


Chromosome
Sister chromatids
Origin
Origin
Origin
Centromere
Origin
Origin
Before S phase
During S phase
End of S phase

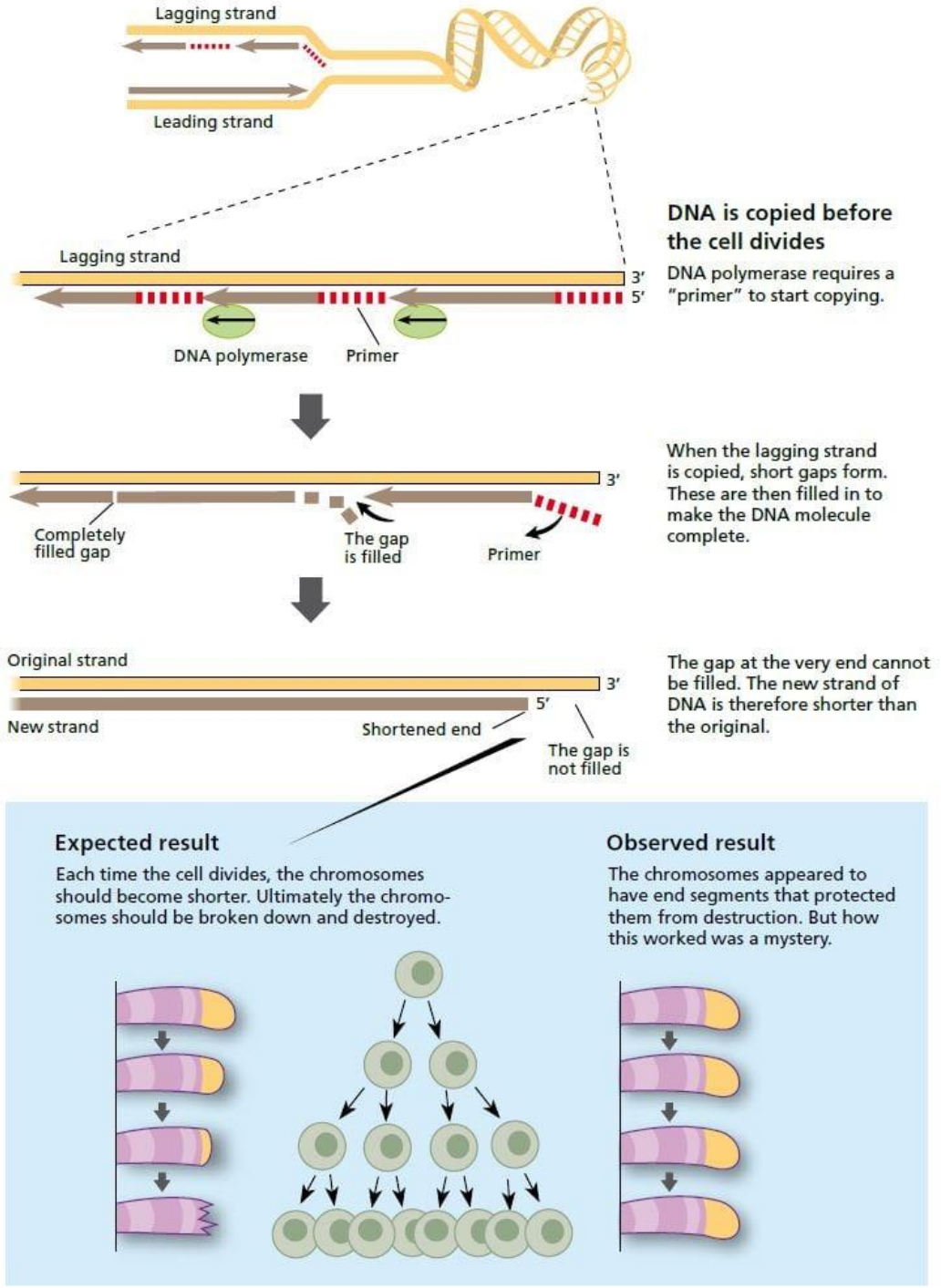
# DNA POLYMERASES IN EUKARYOTES

| Eukaryotic DNA Polymerases | |
|---|---|
| **Polymerase Type(s)*** | **Function** |
| α | Initiates DNA replication in conjunction with primase |
| ε | Replication of the leading strand |
| δ | Replication of the lagging strand |
| γ | Replication of mitochondrial DNA |
| η, κ, ι, ξ (translesion-replicating polymerases) | Replication of damaged DNA |
| α, β, δ, ε, σ, λ, μ, φ, θ, η | DNA repair or other functions† |


5′
3′
3′
5′
DNA polymerase δ elongates the left Okazaki fragment and causes a short flap to occur on the right Okazaki fragment.
Flap
Flap endonuclease removes the flap.
DNA polymerase δ continues to elongate and causes a second flap.
Flap endonuclease removes the flap.
Process continues until the entire RNA primer is removed. DNA ligase seals the two fragments together.

# TELOMERES OF EUKARYOTIC CHROMOSOMES

- Telomere - refers to the telomeric repeat sequences within the DNA and the specific protein that are bound to those sequences. It consist of tandemly repeated sequence and a 3' overhang region that is 12-16 nucleotides in length
- Why are telomeric repeat sequences needed? *Because DNA polymerase is unable to replicate the 3' ends of DNA strands.*
- What can result from this? *The chromosome would become progressively shorter with each round of DNA replication.*
- What is the solution for this? *Additional DNA sequences are attached to the ends of telomeres.*



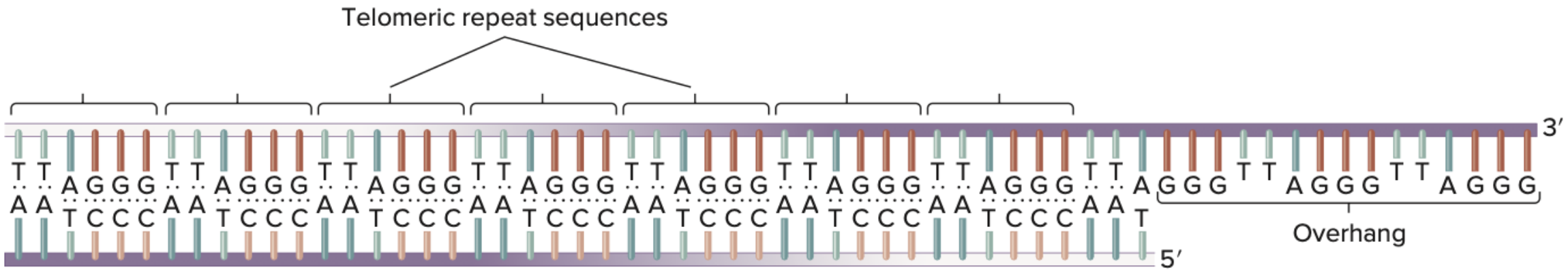
# CAROL GREIDER AND ELIZABETH BLACKBURN AND JACK W. SZOSTAK

Elizabeth H. Blackburn

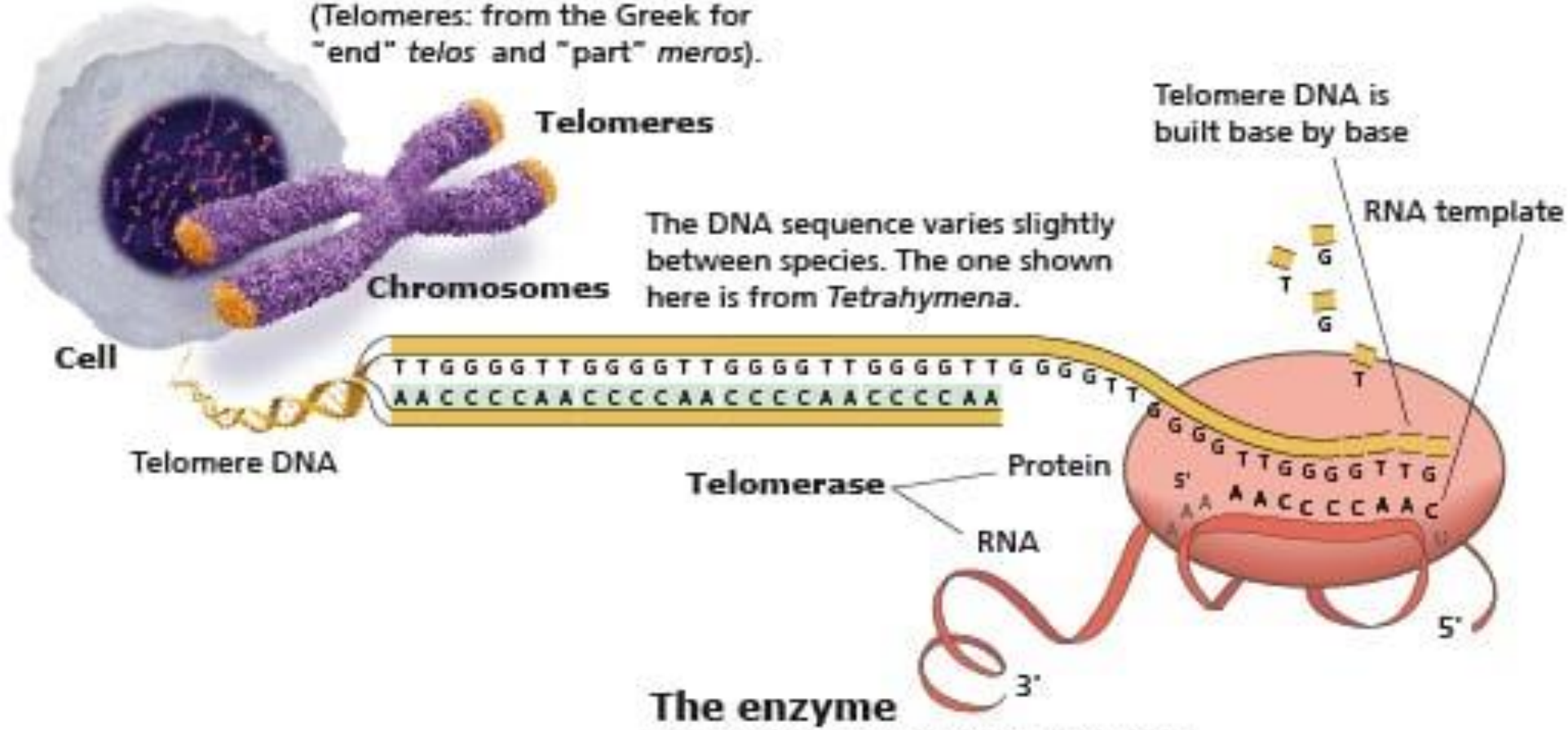
Carol W. Greider

Jack W. Szostak

# GENERAL STRUCTURE OF TELOMERIC SEQUENCES



The telomeres form caps at the ends of chromosomes. They contain a unique DNA sequence which is repeated several times. (Telomeres: from the Greek for "end" *telos* and "part" *meros*).




## The enzyme

Telomerase operates at the end of the chromosome. It is an enzyme consisting of a protein and an RNA component. The RNA serves as a template for synthesizing telomere DNA.