

DNA Structure & Function (Outline)

1. Historical perspective (DNA as the genetic material):
 - Genetic transformation and DNA
 - DNA is the genetic material in bacterial viruses (phage)
 - The base-pairing rule
 - DNA structure
2. Basis for polarity of SS DNA and anti-parallel complementary strands of DNA
3. DNA replication models
4. Mechanism of DNA replication: steps and molecular machinery
5. Replication and the end of linear chromosomes-
Molecular basis for aging
6. Fidelity of DNA replication

By definition the genetic material of must

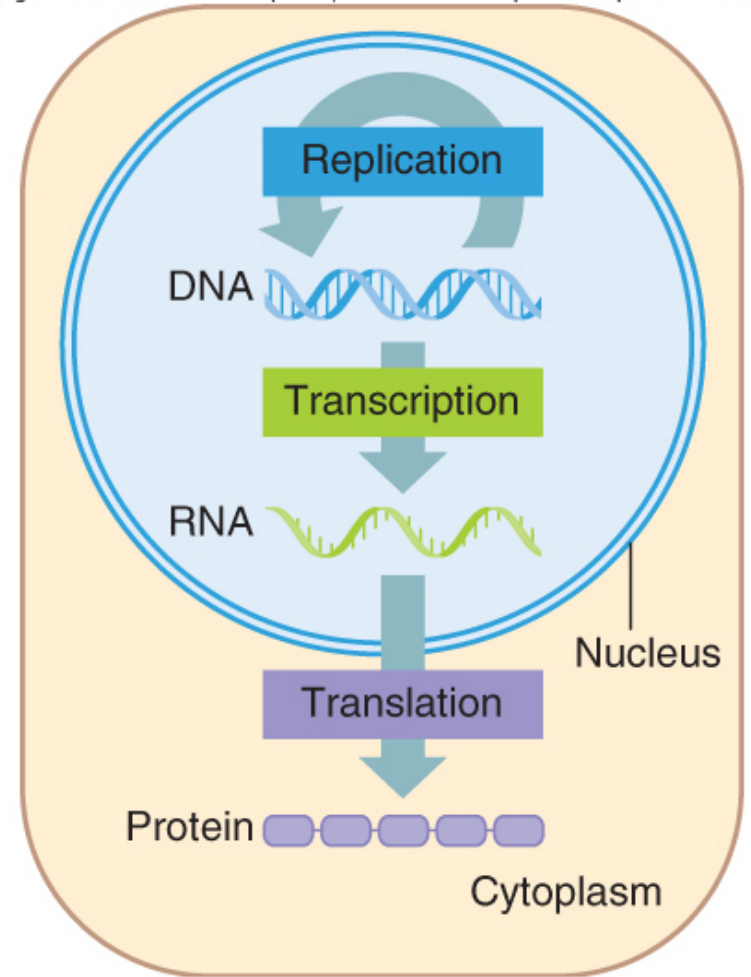
- be replicated

DNA Replication

- direct the cell functions by providing information for production of proteins

*Flow of the genetic information
(Gene Expression)*

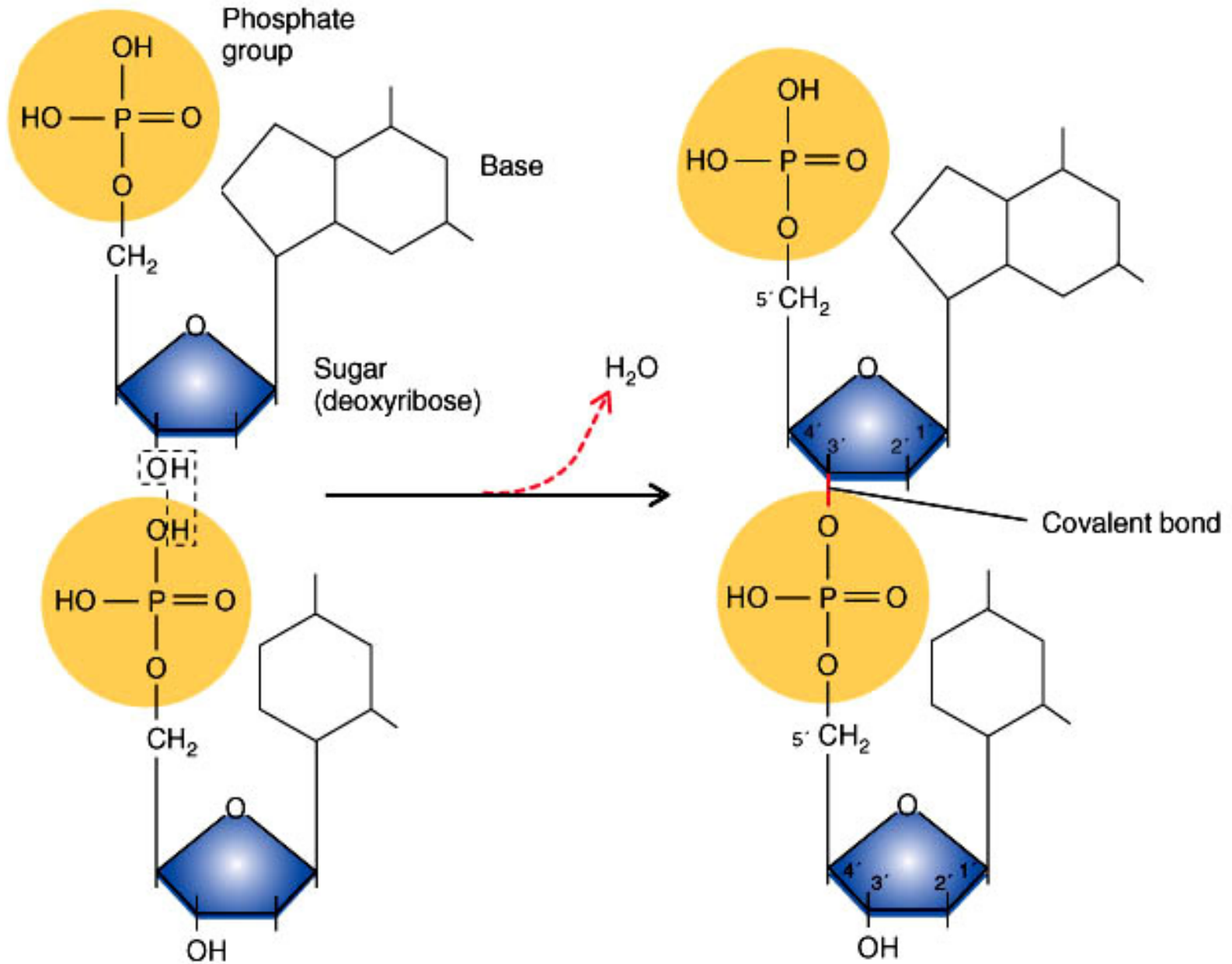
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DNA as the Genetic Material

Time-line

1850's	Mendel
1870-1890	Microscopy: Mitosis and Meiosis
1902	Chromosome basis of inheritance (Thomas H. Morgan)
20 th century	Work with bacteria and viruses

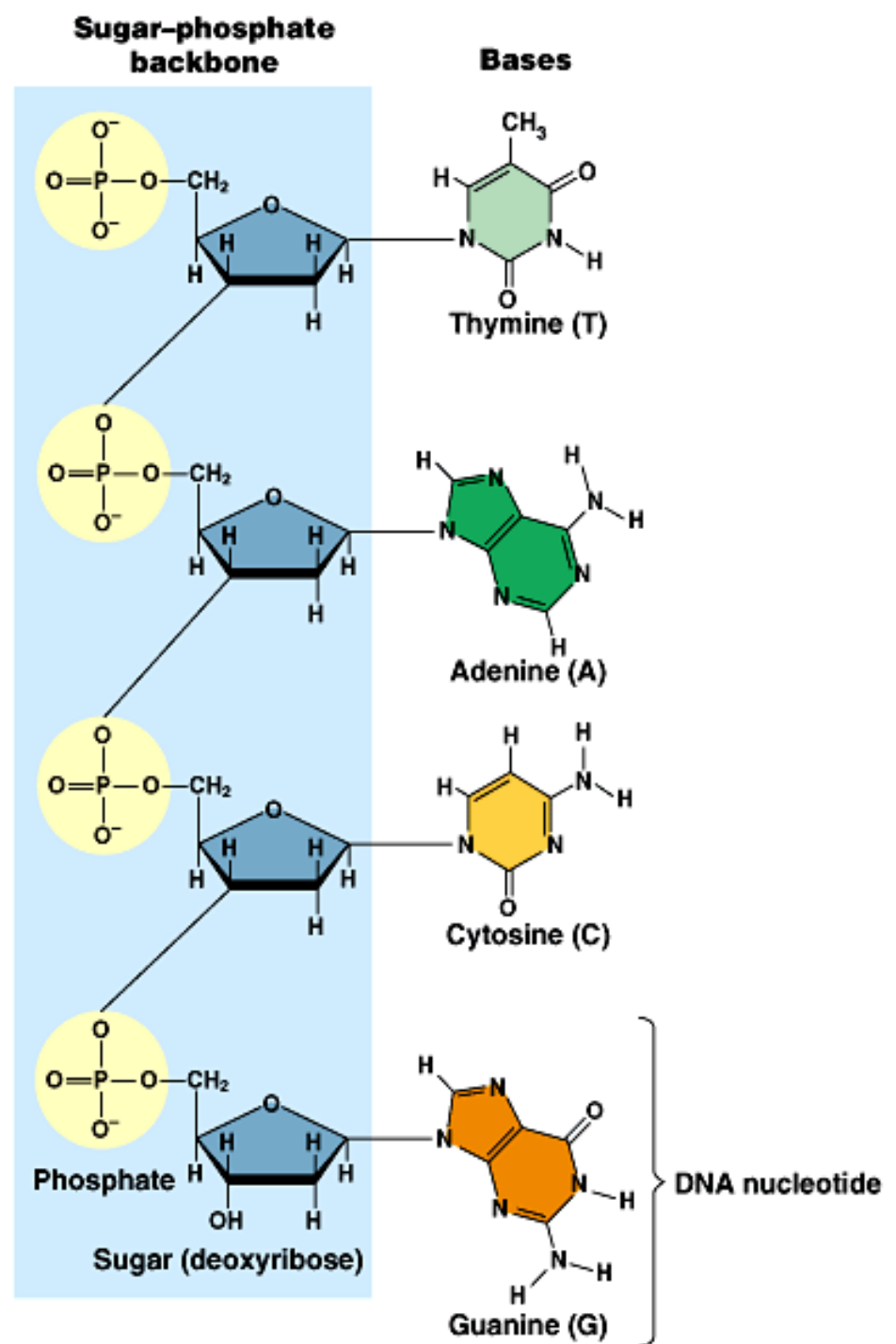


(a)

DNA Structure

Prior to the 1950s,
DNA is a polymer
of nucleotides
consisting of:

- a nitrogenous base
- a sugar
- a phosphate group



1928 Fredrick Griffith Experiments

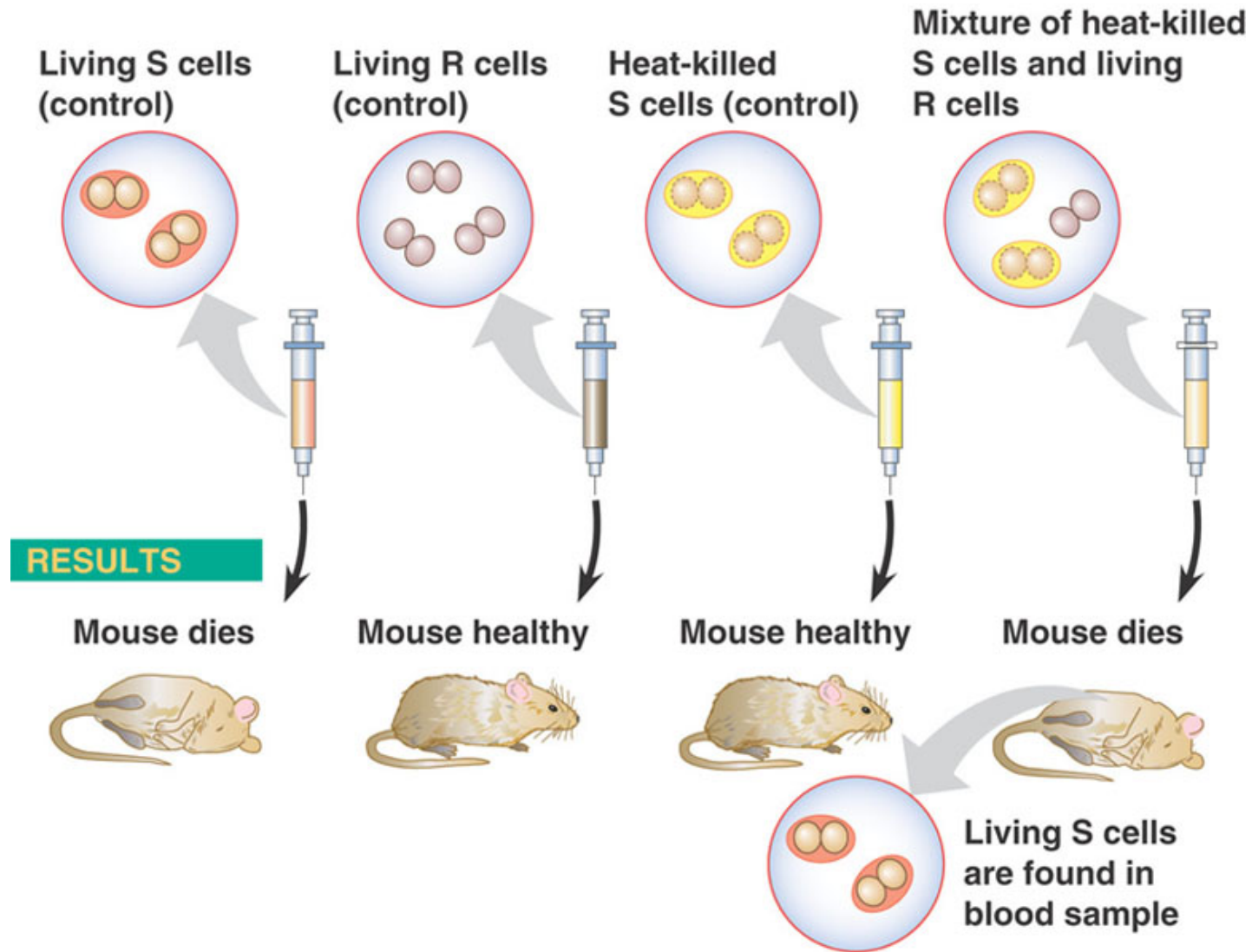
Concept of transformation (using Bacteria that cause pneumonia in mammals)

1944 Avery, McCarty, and MacLeod

The transforming material is DNA
“DNA is the genetic material”

1952 Hershey and Chase

DNA is the genetic material in viruses that infect bacteria



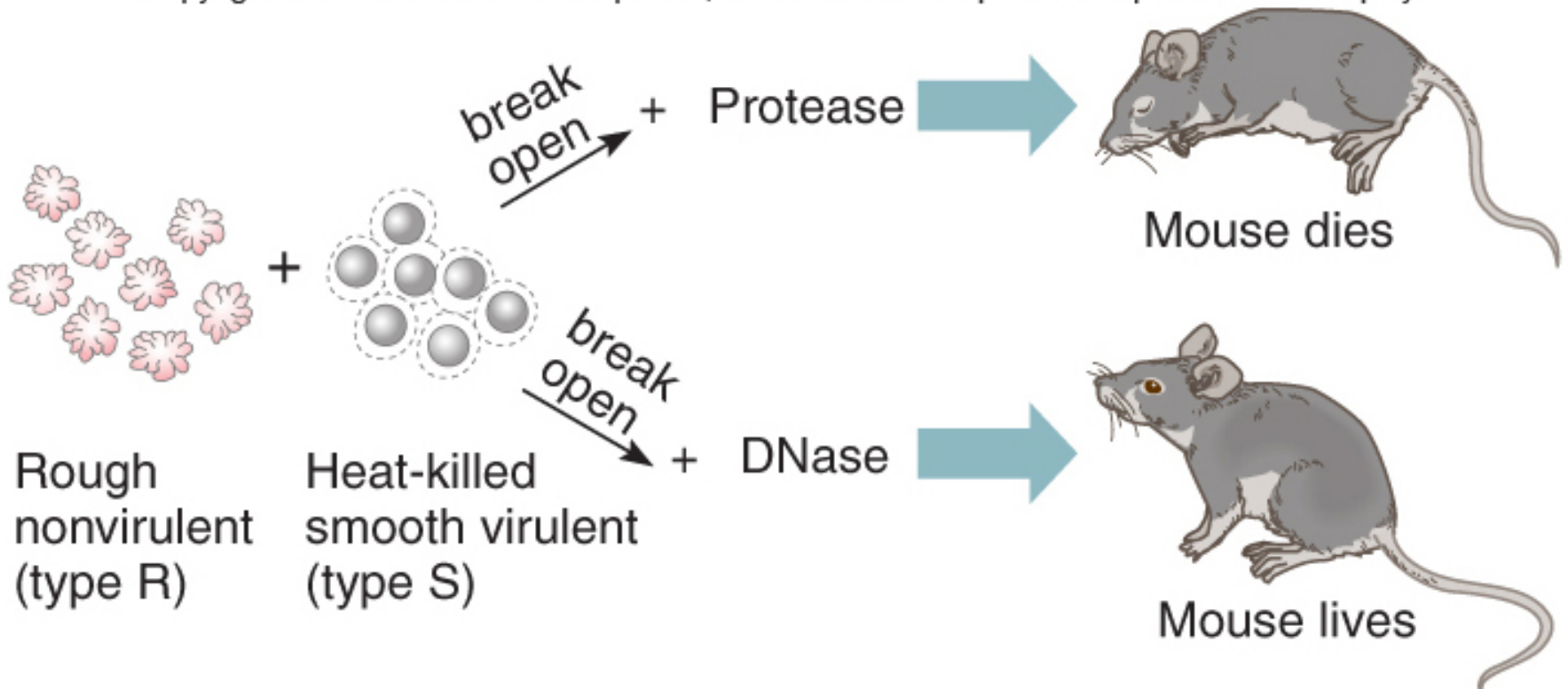
Griffith- Phenomenon of **Transformation**, a change in genotype (*genetic makeup*) by a foreign substance that changes the phenotype (*observed properties*) of the cell

History of DNA

Avery, MacLeod, and McCarty, 1944

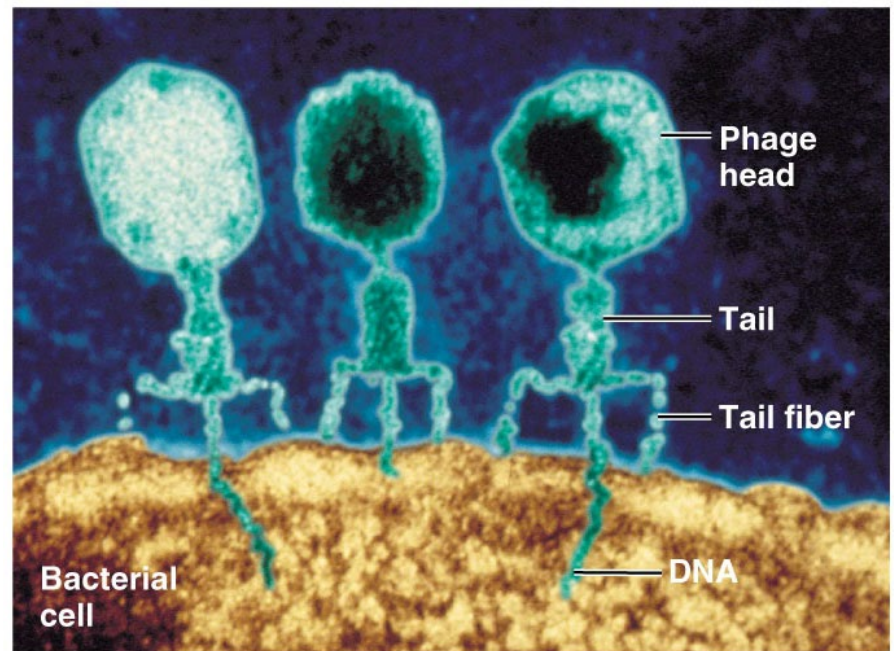
- DNA is the **transforming material**

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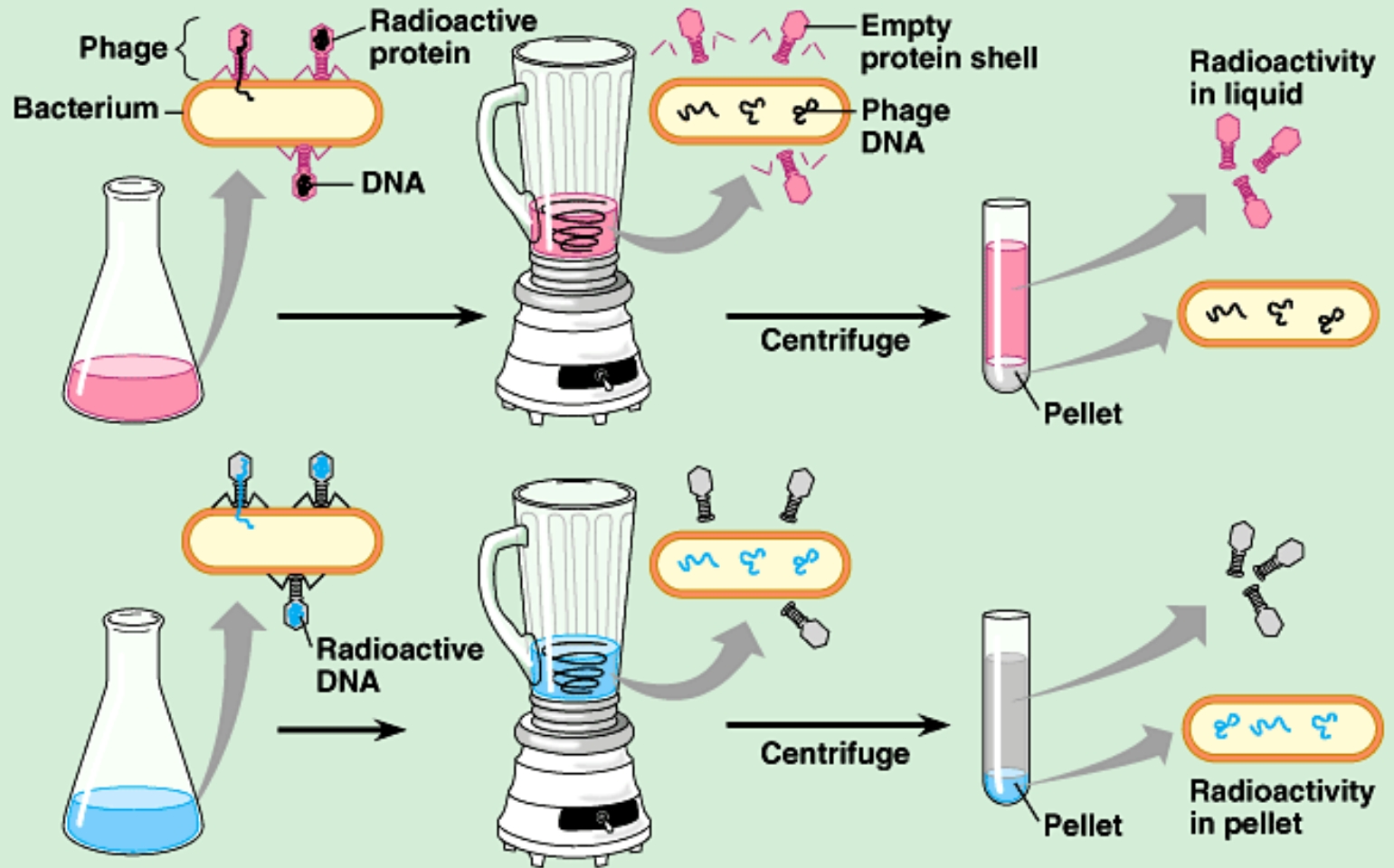


- A phage, is a virus that infects bacteria and is made of DNA and protein.
- Alfred Hershey and Martha Chase- the genetic material of the phage T2 is DNA.

Hershey Chase Experiment Animation



- 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.



Biochemical analysis of DNA: Base-pairing rule

1947 Erwin Chargaff, analysis of DNA from different species %A = %T & %C = %G

Human DNA

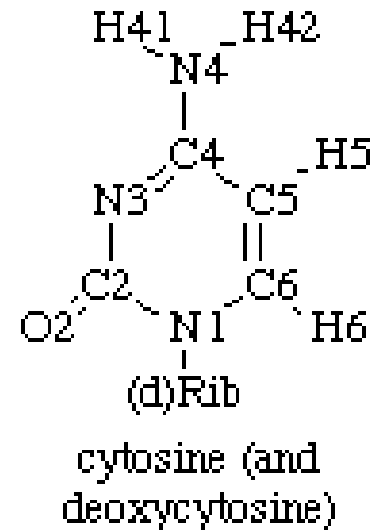
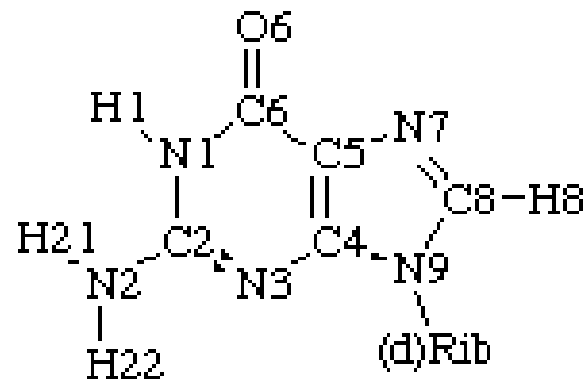
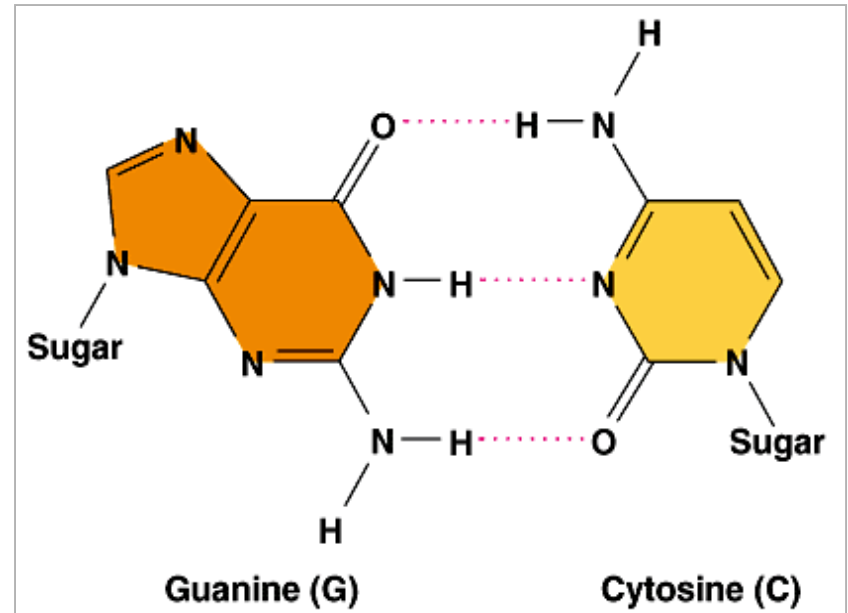
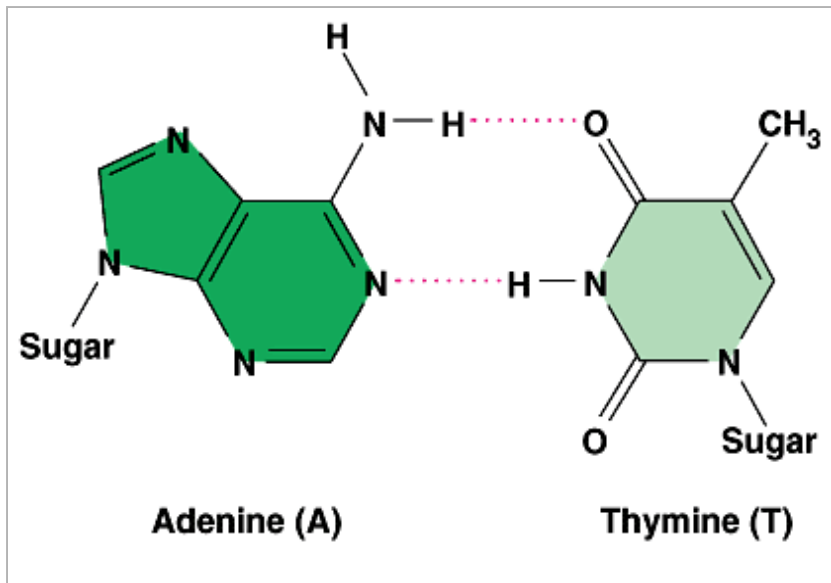
A = 30.9%

T = 29.4%

C = 19.9%

G = 19.8%

Base-pairing in DNA

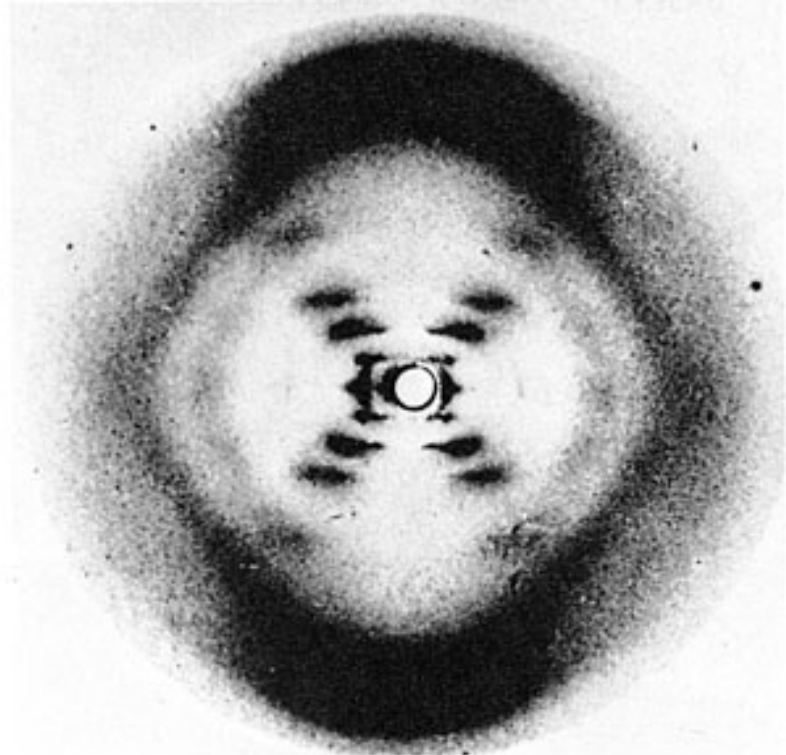


Structural Model of DNA

Maurice Wilkins and Rosalind Franklin- X-ray crystallography: Polynucleotide Helix



(a) Rosalind Franklin

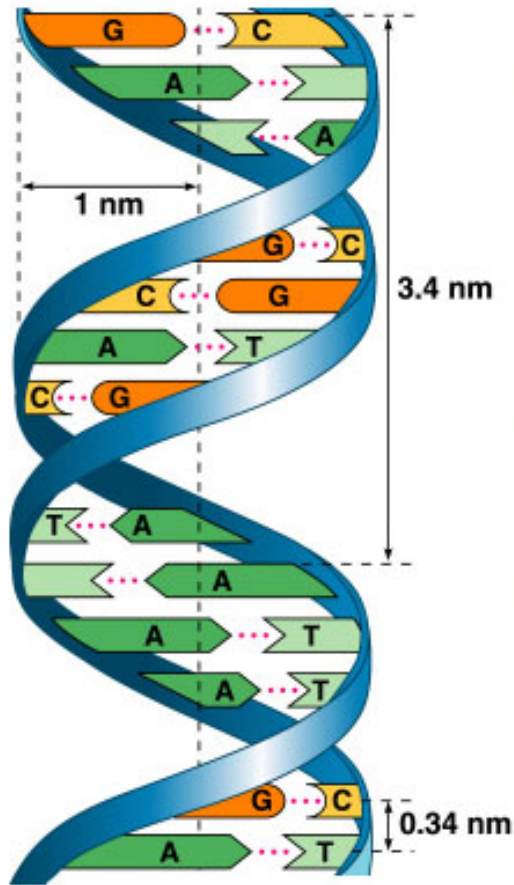


(b)

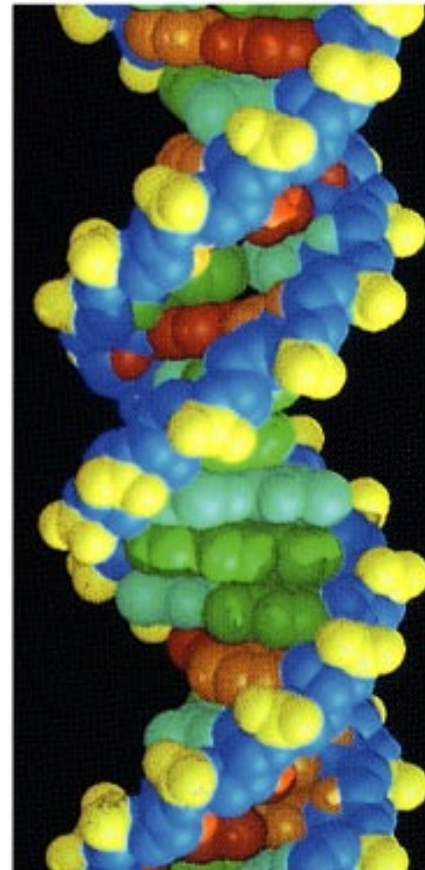
Franklin's X-ray diffraction
Photograph of DNA

Watson and Crick deduced that DNA was a double-stranded helix

Through observations of the X-ray crystallographic images of DNA



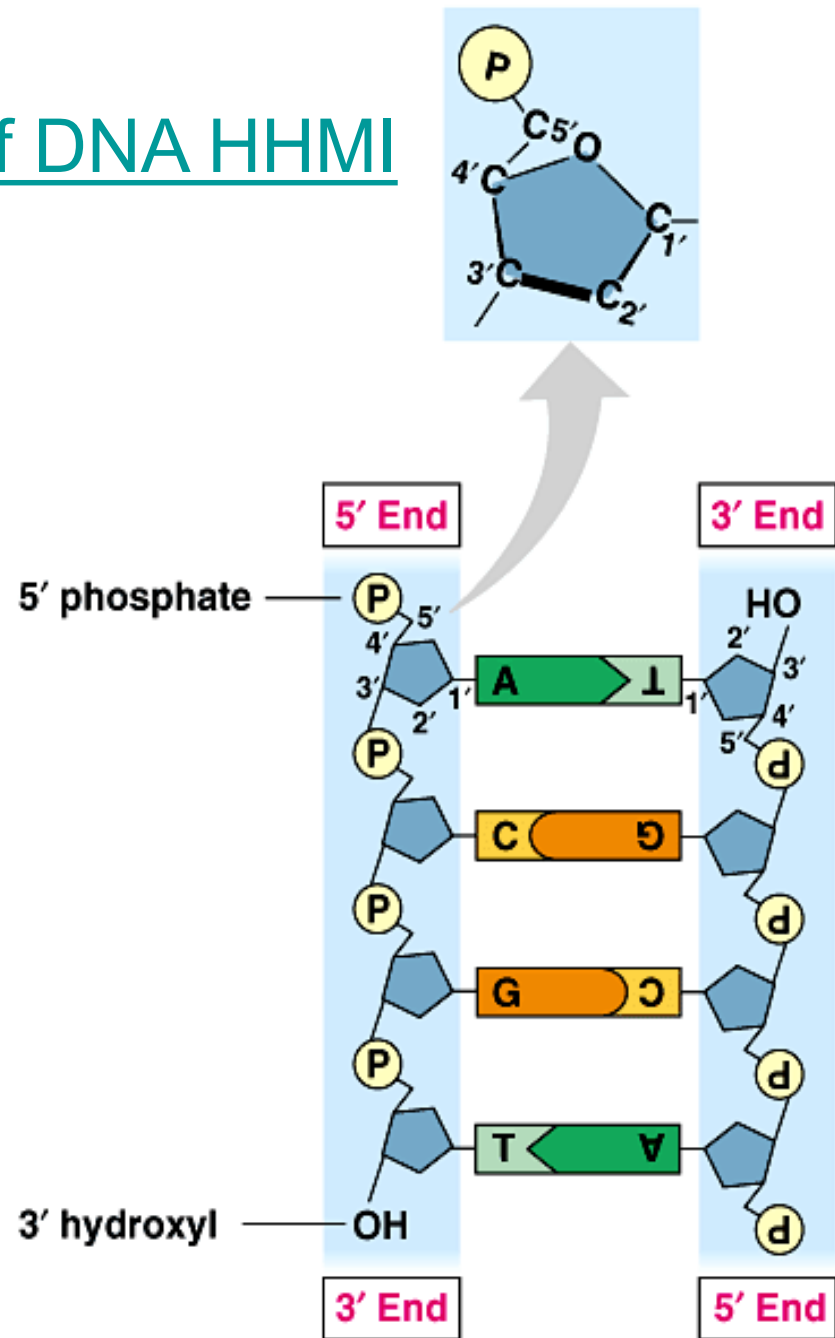
(a) Key features of DNA structure



(c) Space-filling model

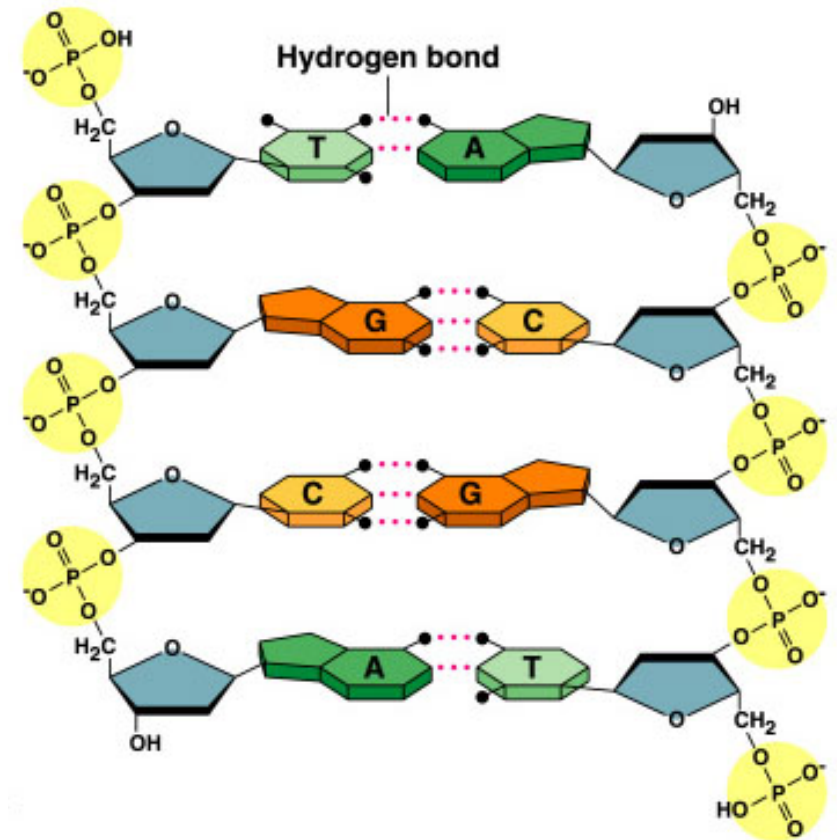
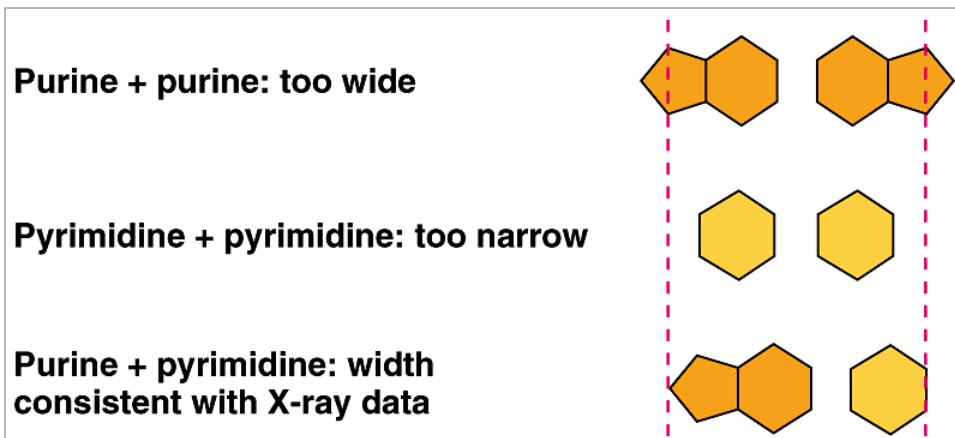
Chemical Structure of DNA HHMI

Polarity and anti-parallel nature of the two DNA strands (5' and 3' ends)



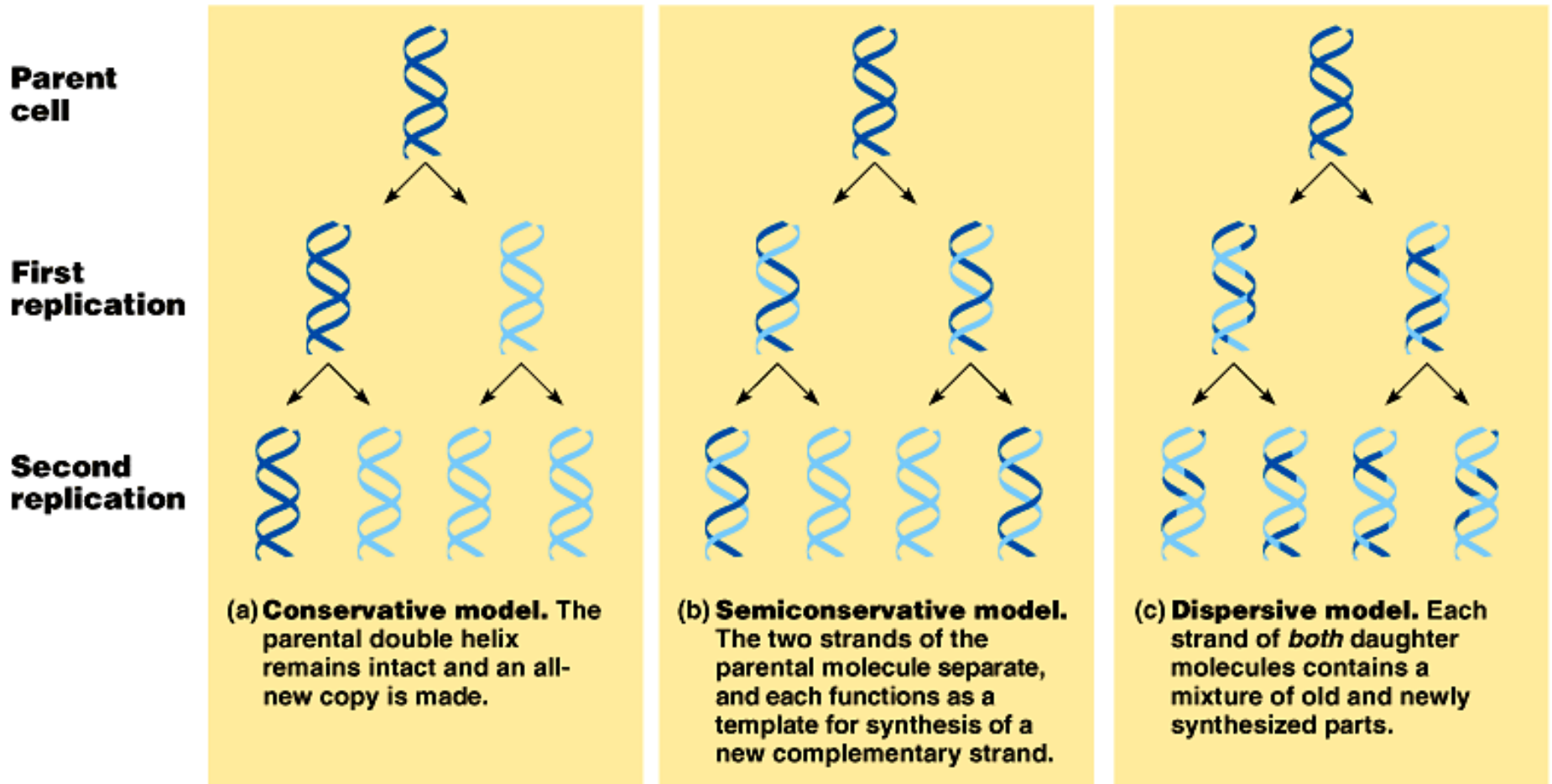
Watson and Crick

- Specificity of pairing is dictated by the structure of the bases



Example

(b) Partial chemical structure



Three models for DNA replication:
 Conservative model
 Semi-conservative model
 Dispersive model

Meselson-Stahl Experiment

1 Bacteria cultured in medium containing ^{15}N



2 Bacteria transferred to medium containing ^{14}N



3 DNA sample centrifuged after 20 min (after first replication)



4 DNA sample centrifuged after 40 min (after second replication)

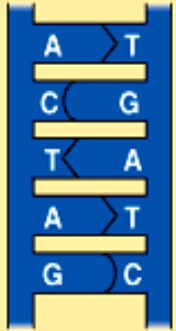


Less dense
More dense

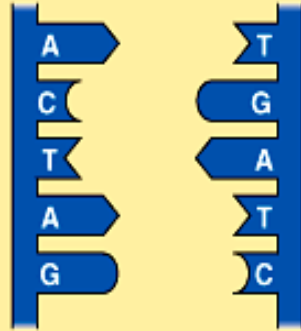
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[Meselson-Stahl Experiment](#) animation

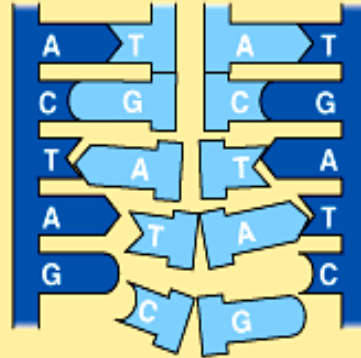
The Basic concept of DNA replication



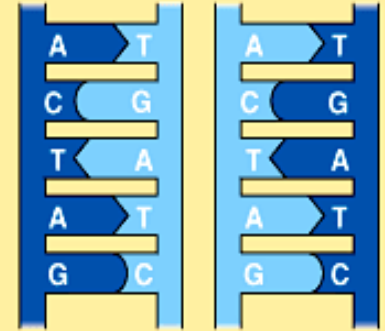
(a) The parent molecule has two complementary strands of DNA. Each base is paired by hydrogen bonding with its specific partner, A with T and G with C.



(b) The first step in replication is separation of the two DNA strands.



(c) Each parental strand now serves as a template that determines the order of nucleotides along a new complementary strand.

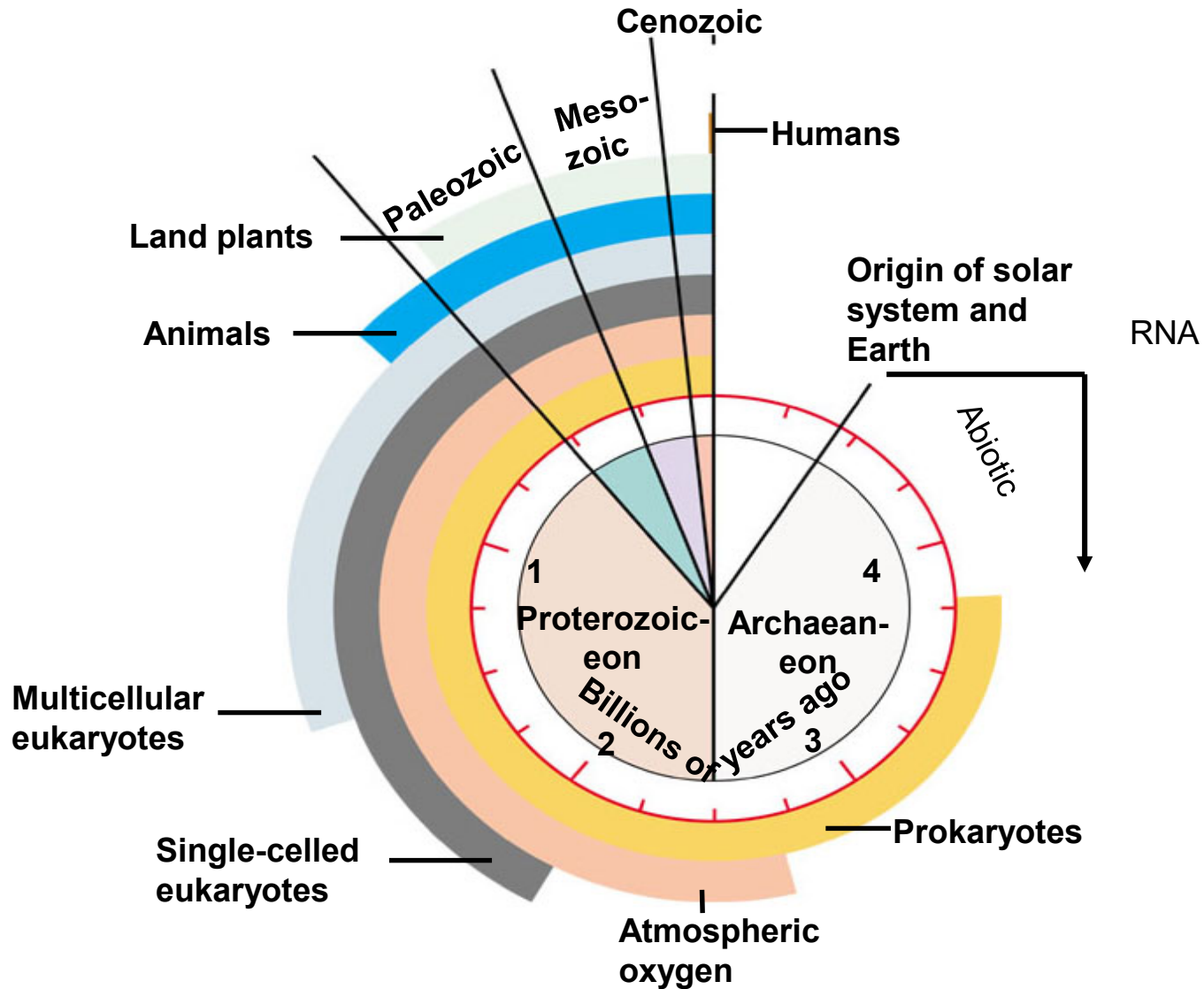


(d) The nucleotides are connected to form the sugar-phosphate backbones of the new strands. Each "daughter" DNA molecule consists of one parental strand and one new strand.

Each strand of DNA act as a template for synthesis of new complementary strands

Major Events in the History of Earth

Role of RNA in DNA Replication



Molecular Mechanism of DNA Replication

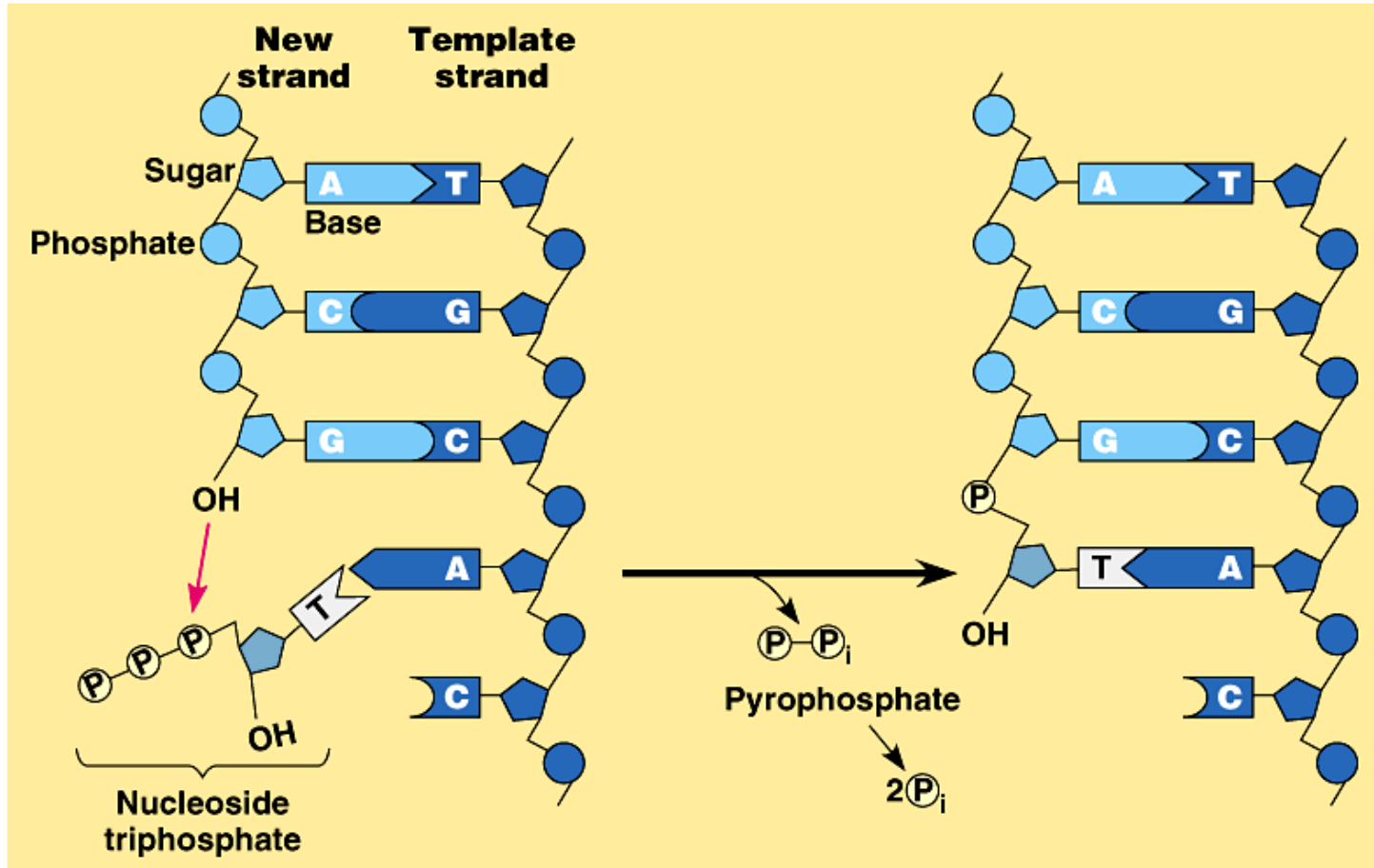
Collective action of several macro-molecules:

- DNA
- Proteins (enzymes & others)
- RNA
- Ribo-protein (for linear chromosomes)

Direction of replication of new strands; 5'-----3'

[How nucleotides are added in DNA replication](#) (Active link)

DNA polymerase adds deoxyribonucleotides in a 5' to 3' direction, it adds nucleotides to the 3' end of a growing strand.



The new strand always starts with the 5' end, the template starts with the 3' end.

Primase, an RNA polymerase, uses the DNA template strand to polymerize a short complementary RNA chain (RNA primer)

Two different **DNA polymerases** both

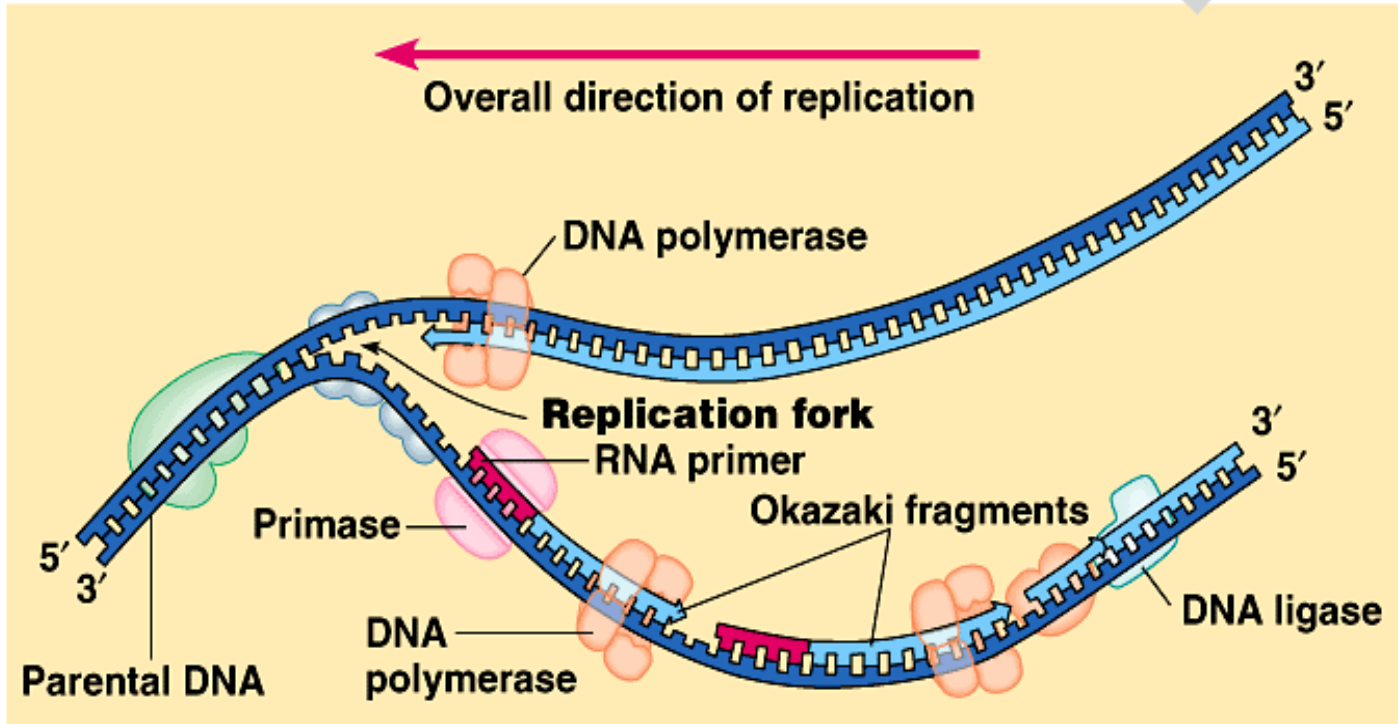
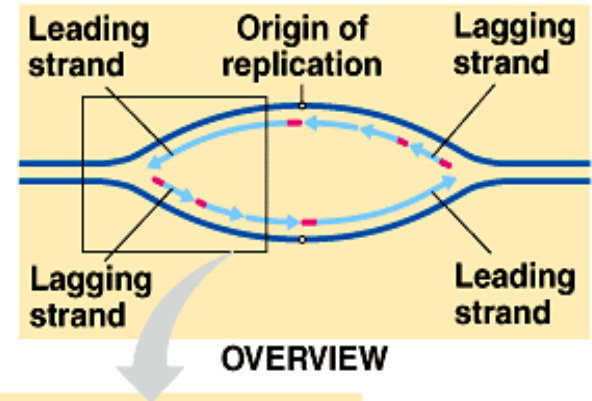
- cannot initiate the synthesis of a polynucleotide
- can only add nucleotides to an existing 3' end

Summary of DNA Replication

- Semi-conservative
- Initiation: Origin of replication
- Primase and RNA primer
- Template strand vs. new strand
- 5' to 3' direction
- DNA polymerase (III and I)
- Base-pairing rules
- dNTPs: deoxy-ATP, deoxy-GTP, deoxy-CTP, deoxy-TTP
- Leading and lagging strands
- Okazaki fragments
- DNA ligase
- Bidirectional
- Fidelity of DNA replication is maintained by activity of DNA polymerase and other proof-reading systems.

Origin of Replication

[DNA Replication Tutorial \(active link\)](#)

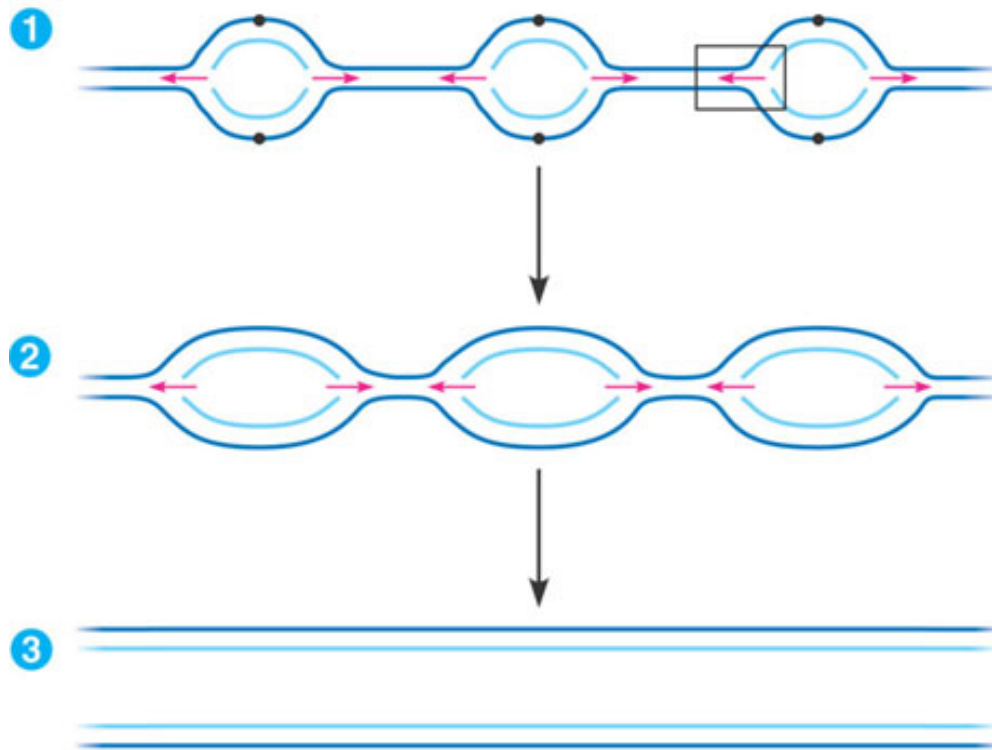


Other proteins participate in DNA replication including: Helicase, topoisomerase, single-strand binding protein

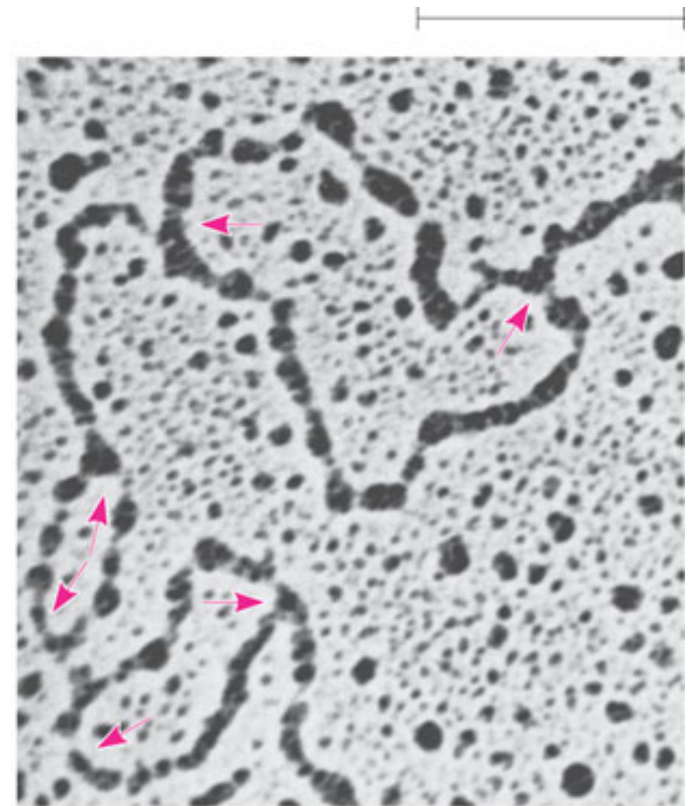
Table 16.1 Bacterial DNA replication proteins and their functions

Protein	Function for Leading and Lagging Strands	
Helicase	Unwinds parental double helix at replication forks	
Single-strand binding protein	Binds to and stabilizes single-stranded DNA until it can be used as a template	
Topoisomerase	Corrects “overwinding” ahead of replication forks by breaking, swiveling, and rejoining DNA strands	
	Function for Leading Strand	Function for Lagging Strand
Primase	Synthesizes a single RNA primer at the 5′ end of the leading strand	Synthesizes an RNA primer at the 5′ end of each Okazaki fragment
DNA pol III	Continuously synthesizes the leading strand, adding on to the primer	Elongates each Okazaki fragment, adding on to its primer
DNA pol I	Removes primer from the 5′ end of leading strand and replaces it with DNA, adding on to the adjacent 3′ end	Removes the primer from the 5′ end of each fragment and replaces it with DNA, adding on to the 3′ end of the adjacent fragment
DNA Ligase	Joins the 3′ end of the DNA that replaces the primer to the rest of the leading strand	Joins the Okazaki fragments

Replication of long DNA molecules begins at multiple origins of replication simultaneously and is bidirectional



(a)

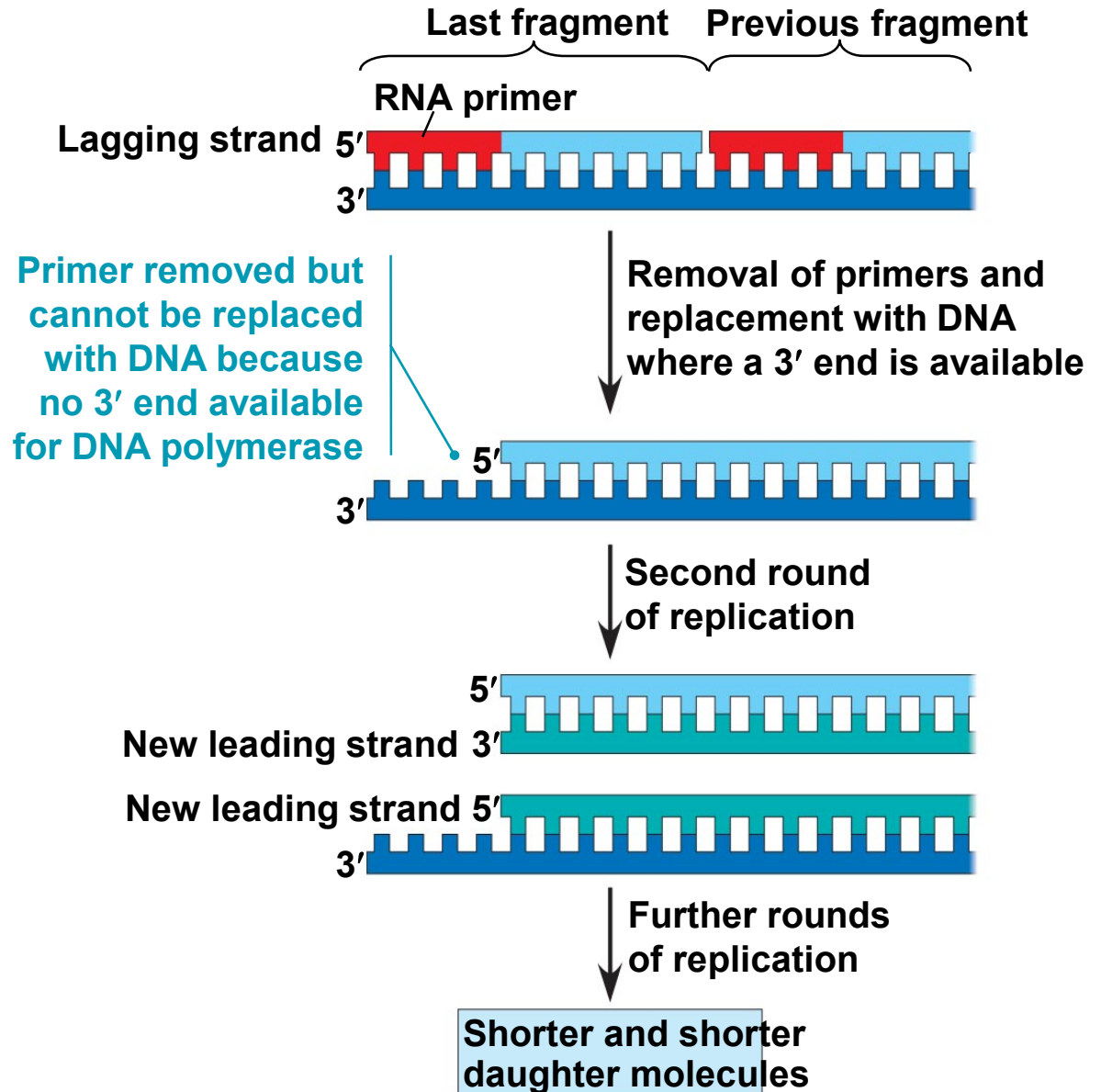


(b)

Replicating the Ends of linear DNA Molecules

Mechanism of DNA replication causes telomeres to get shorter with each round of replication

<http://www.learner.org/courses/biology/units/cancer/images.html>



Current Connections *to* *DNA structure and replication*

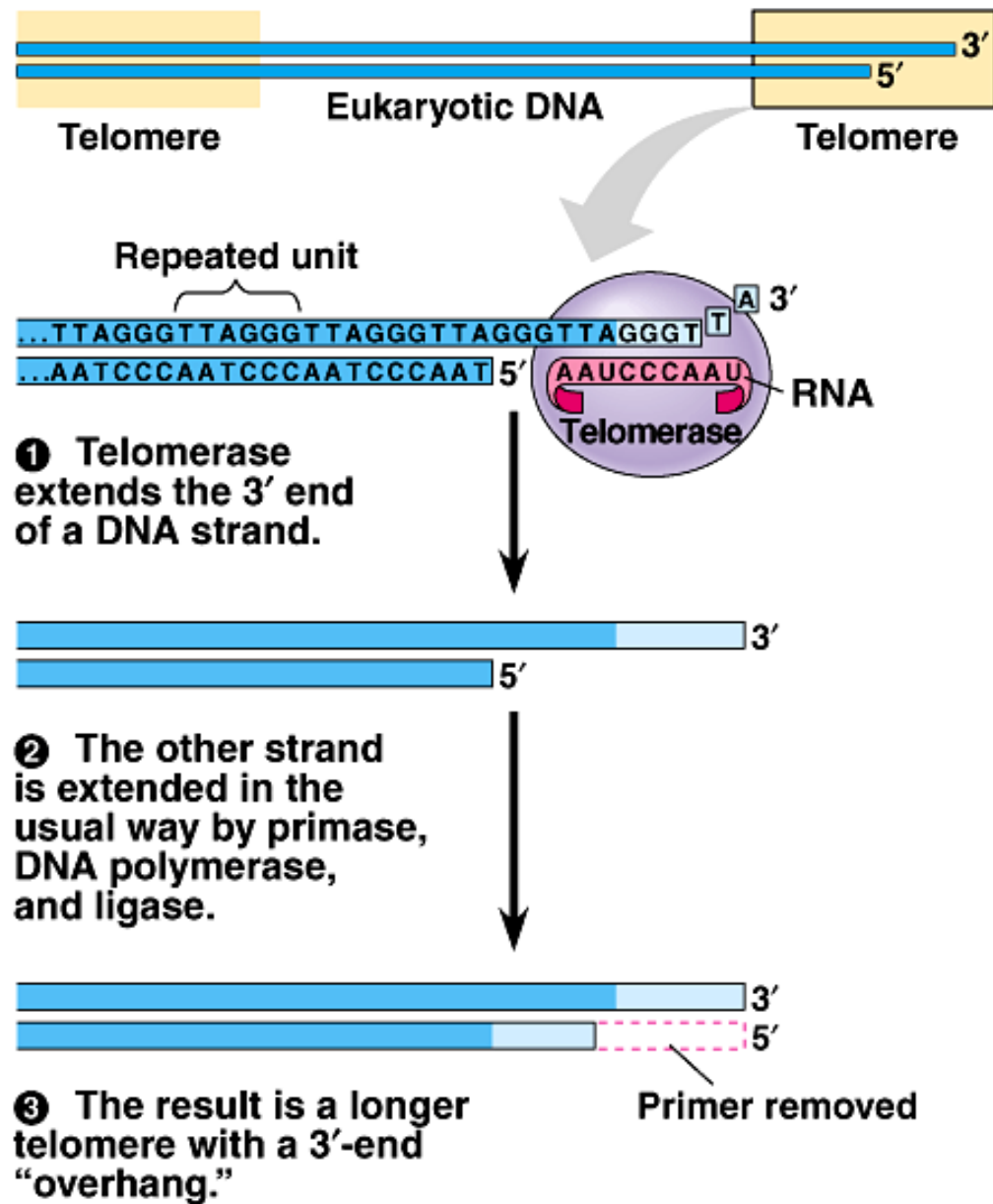
Q: Why are we mortal with a limited life span?

A: Our cells have a limited life span (# of cell divisions)

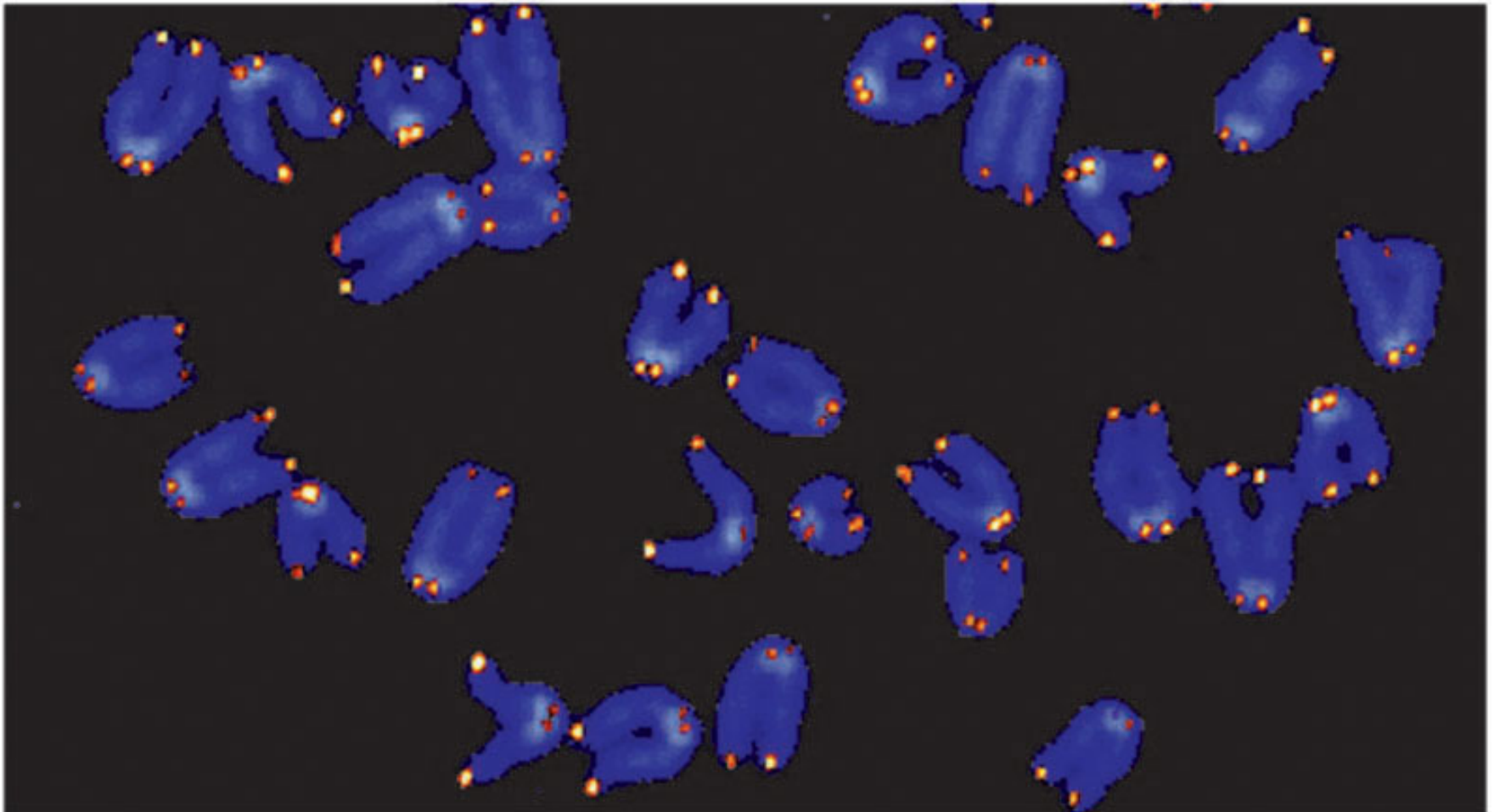
Telomerase- an enzyme (riboprotein) that extends the 3' end of the DNA strand by adding a repeated sequence of 6-nucleotides typically TTAGGG (100-1000 times)

<https://www.youtube.com/watch?v=AJNoTmWsE0s>

<https://www.youtube.com/watch?v=vtXrehpCPEE>



Ends of linear chromosomes have special DNA sequences and are known as **telomeres** added by an enzyme known as **telomerase** after DNA replication is completed



Life span of dividing cells

- Telomerase is active in sperm, eggs, stem cells (bone marrow), and cancer cells but not in somatic tissues
- Most cells lose 50-200 endmost bases after each cell division
- After about 50 divisions, shortened telomeres signal the cell to stop dividing

Fidelity of DNA replication & maintaining DNA integrity

Maintained by:

1. Proof-reading function of DNA polymerase
2. DNA repair systems

http://www.hhmi.org/biointeractive/media/mismatch_repair-lg.mov

DNA damage and repair in general

<http://www.youtube.com/watch?v=y16w-CGAa0Y&feature=related>

<http://www.youtube.com/watch?v=nPS2jBq1k48>

Genetic Integrity and Diversity

- Need for maintaining genetic integrity is balanced by having enough genetic variability for natural selection to act on
- Few errors of DNA replication are not corrected!