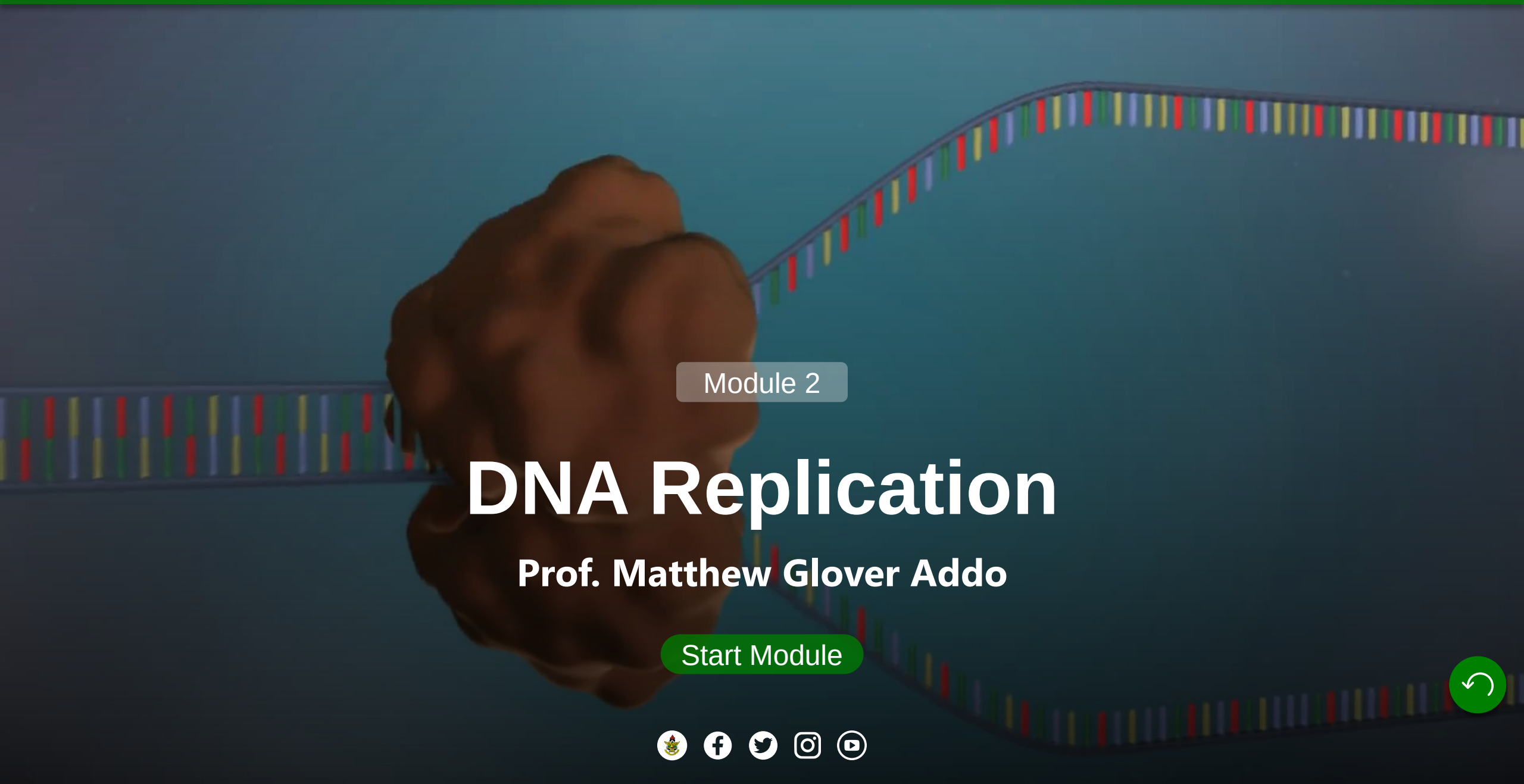
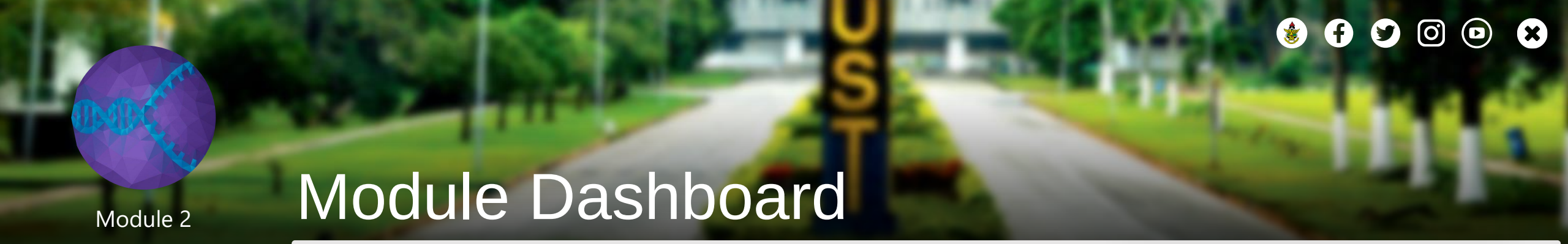


Module 2

DNA Replication

Prof. Matthew Glover Addo

Start Module



Module 2

DNA Replication

Module Dashboard

- Dashboard
- Introduction
- Lessons
- Assignments

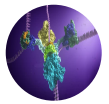
Lessons
3

Assignments
4

Start Learning

Pick a Lesson

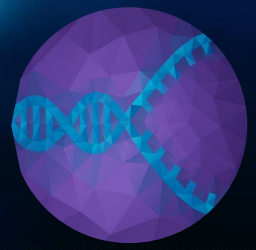
Lesson 1
Method of Replication



Lesson 2
Mechanism of Replication

Lesson 3
Rate of Replication

2



Module 2

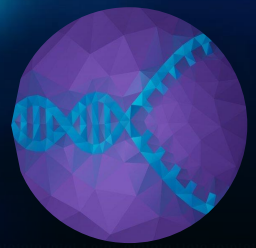
DNA Replication

Module Objectives

1. Consider the modes of replication i.e. dispersive, conservative and semiconservative

2. Consider the experiments of Meselson and Stahl

   Their experiment gave a convincing evidence to support the semiconservative nature of replication



Module 2

DNA Replication

Module Objectives

3 To appreciate how replication is initiated, elongated and terminated

Emphasis will be laid on some of the enzymes involved in these processes

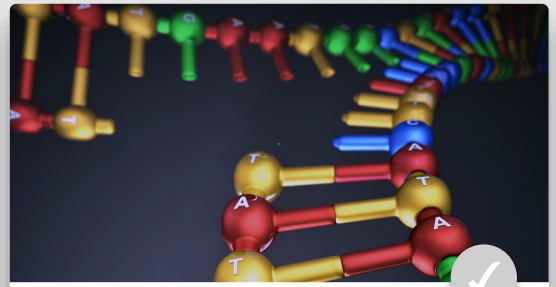
4 DNA repair mechanism

Module 2

DNA Replication

Module Lessons

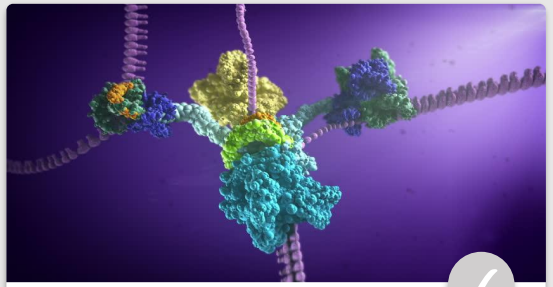
- Dashboard
- Introduction
- Lessons
- Assignments



Lesson 1

Method of Replication

Start ▸



Lesson 2

Mechanism of Replication

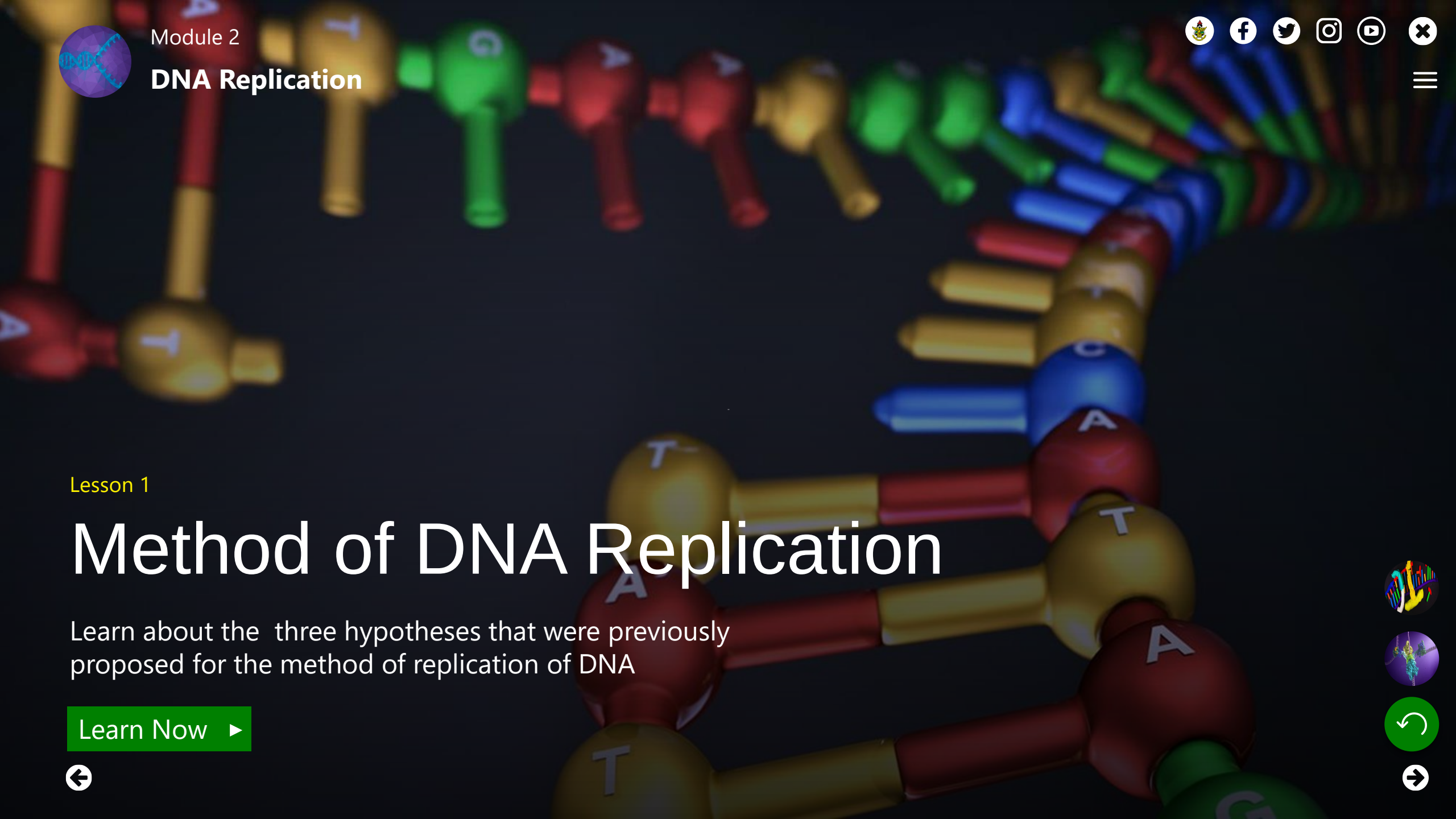
Start ▸

Lesson 3

Rate of Replication

Start ▸

5



Lesson 1

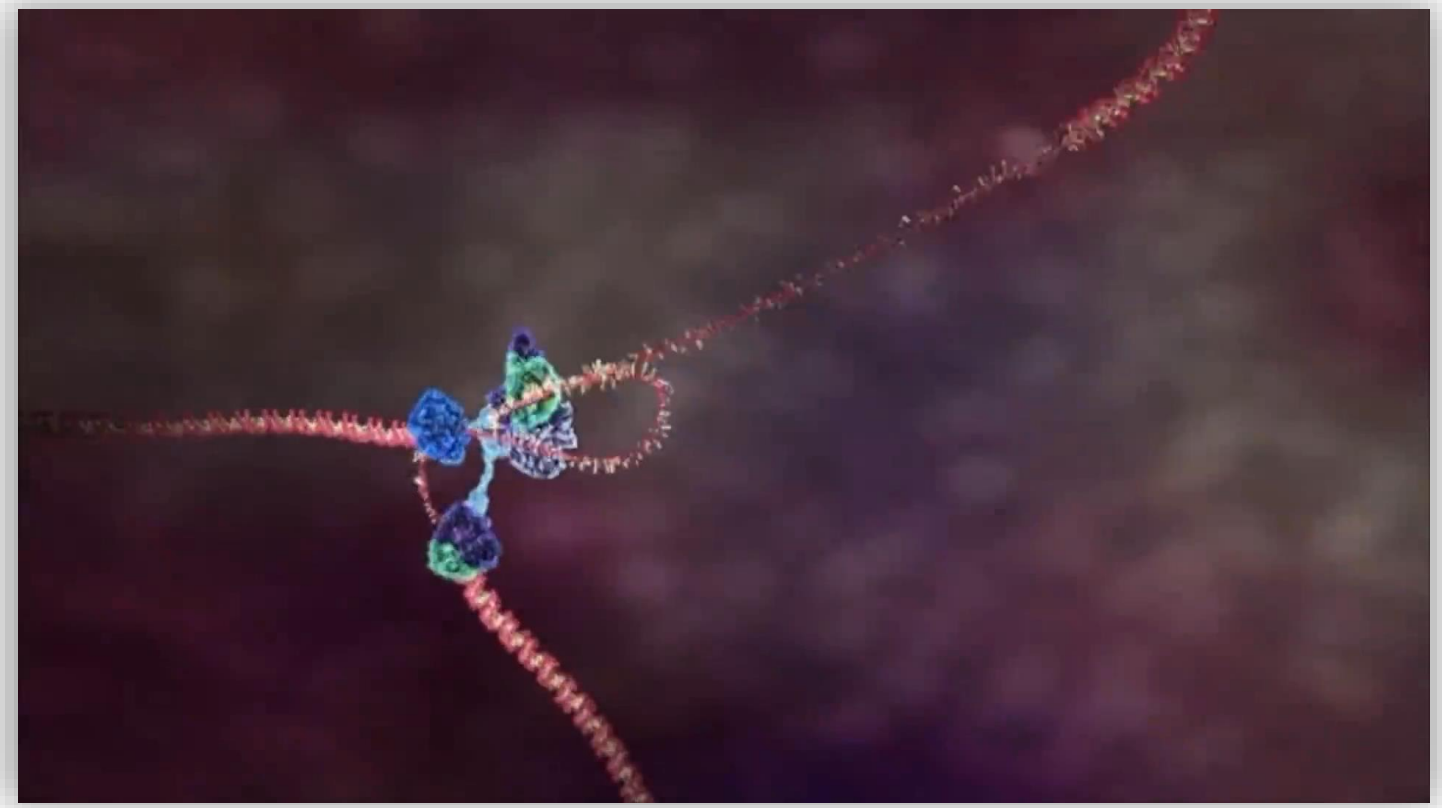
Method of DNA Replication

Learn about the three hypotheses that were previously proposed for the method of replication of DNA

Learn Now ▸

Method of DNA Replication

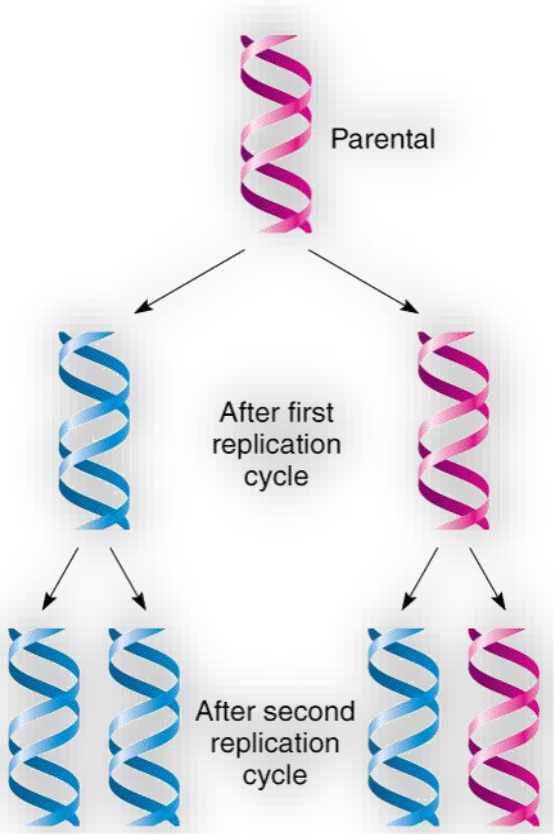
To explain the phenomenon of heredity, biological information must be accurately copied **(replicated)** and transmitted from each cell to all of its progeny



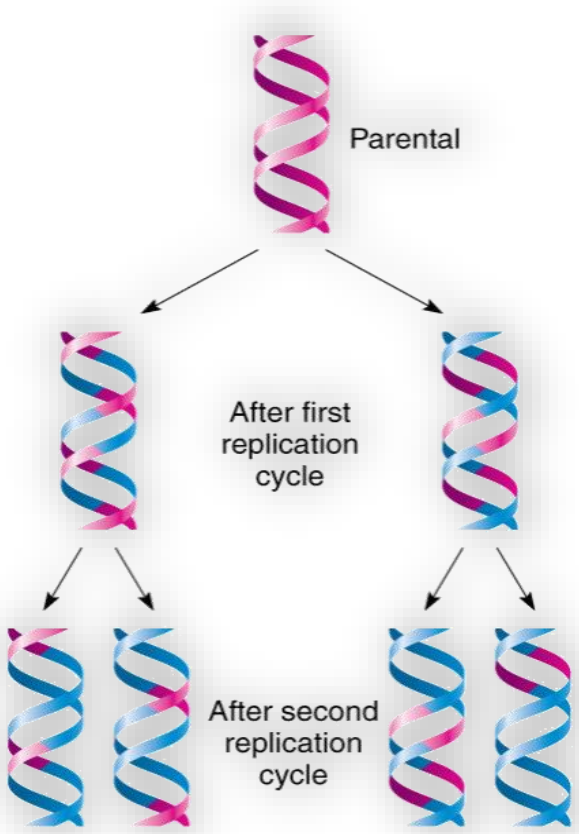
Method of DNA Replication

Proposed Hypotheses

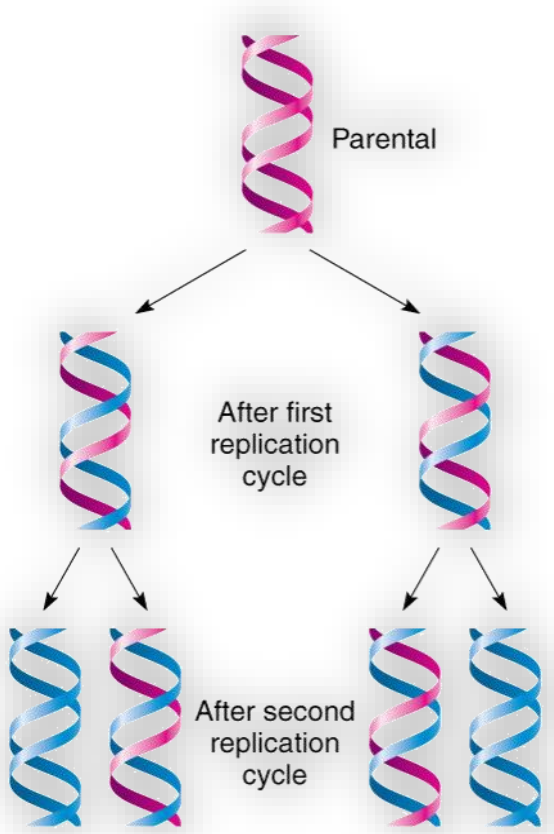
Conservative



Dispersive



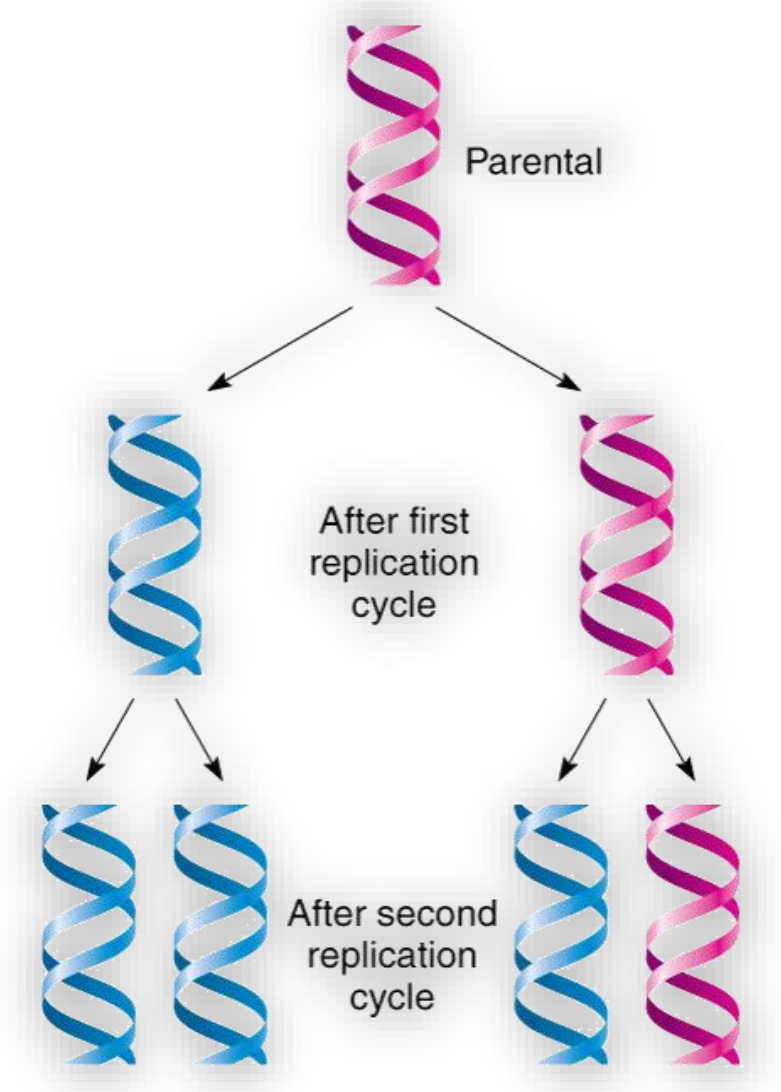
Semiconservative



Method of DNA Replication

Proposed Hypotheses › Conservative

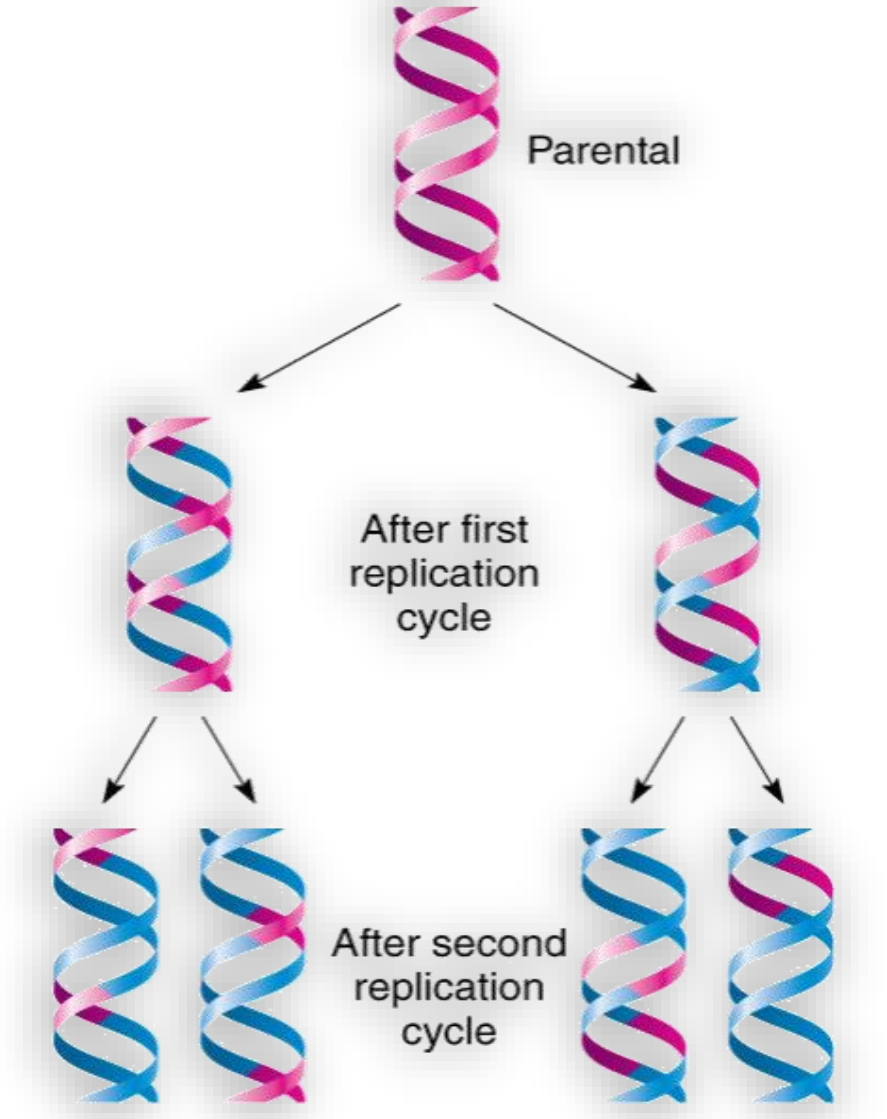
Conservative replication would leave intact the original DNA molecule and generate a completely new molecule



Method of DNA Replication

Proposed Hypotheses › Dispersive

Dispersive replication would produce two DNA molecules with sections of both old and new DNA interspersed along each strand

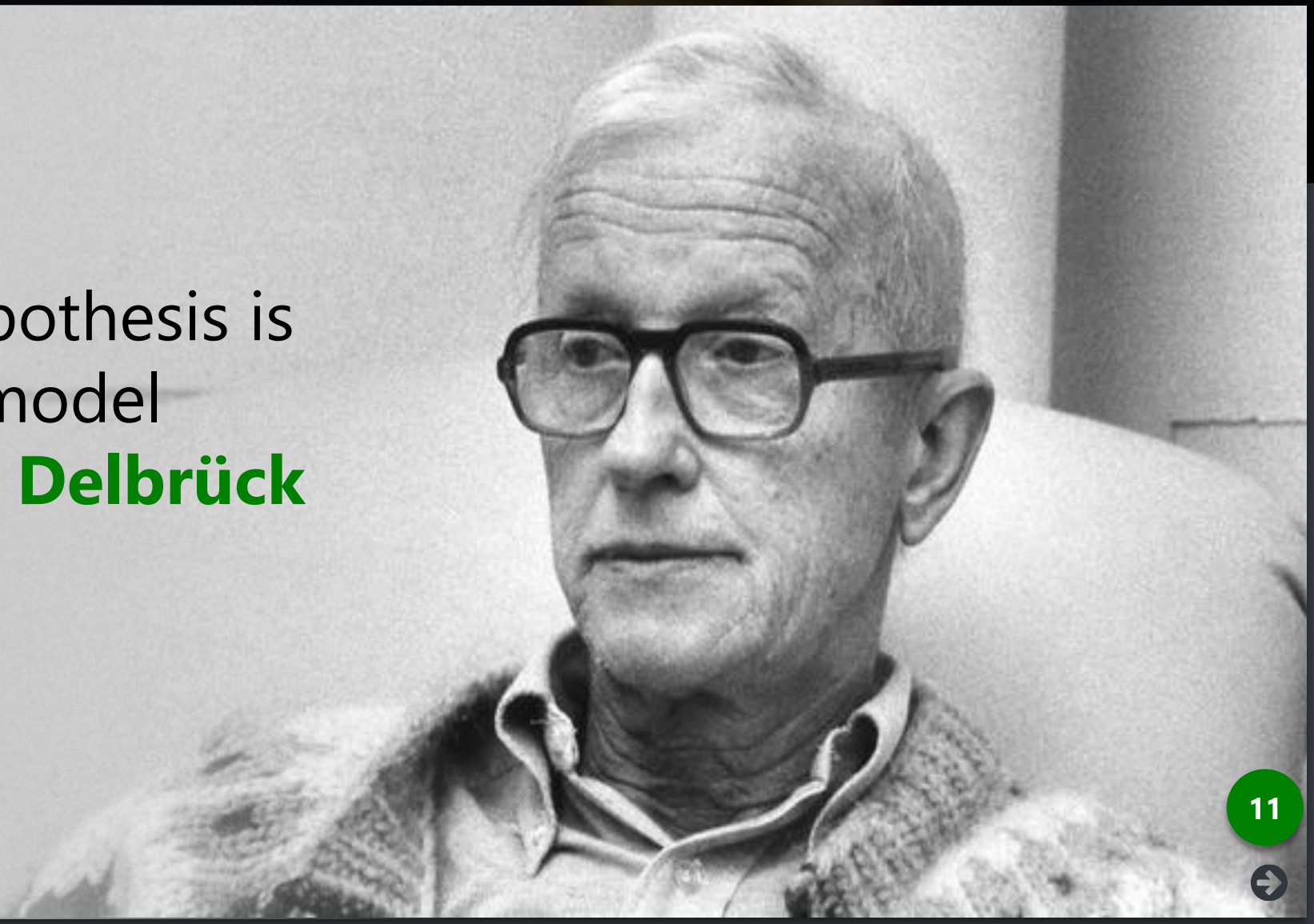


Method of DNA Replication

Proposed Hypotheses › Dispersive

The dispersive hypothesis is exemplified by a model proposed by **Max Delbrück**

Learn More

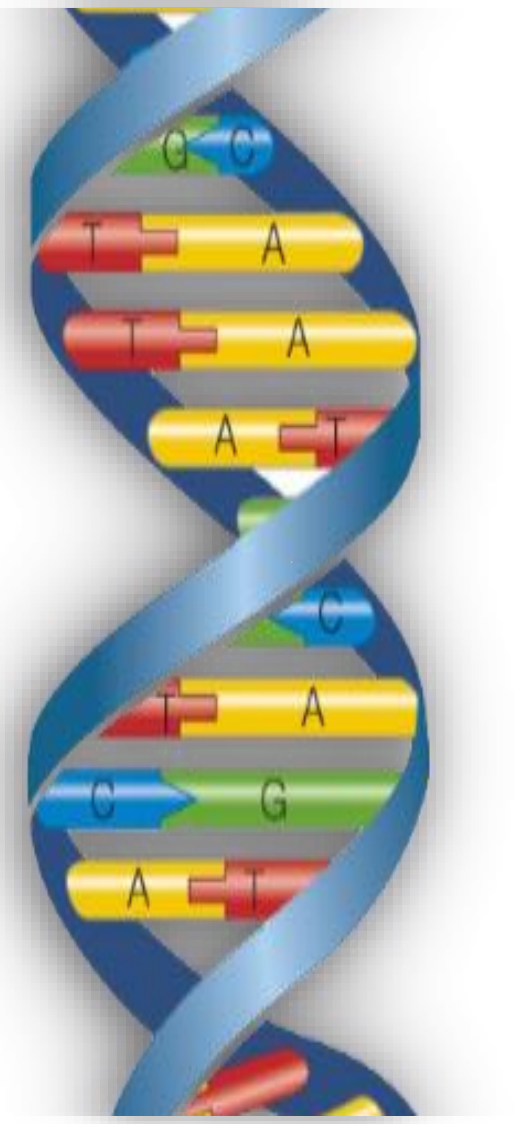


Method of DNA Replication

Proposed Hypotheses › Dispersive

- He attempted to solve the problem of unwinding the two strands of the double helix by a mechanism that breaks the DNA backbone every 10 nucleotides

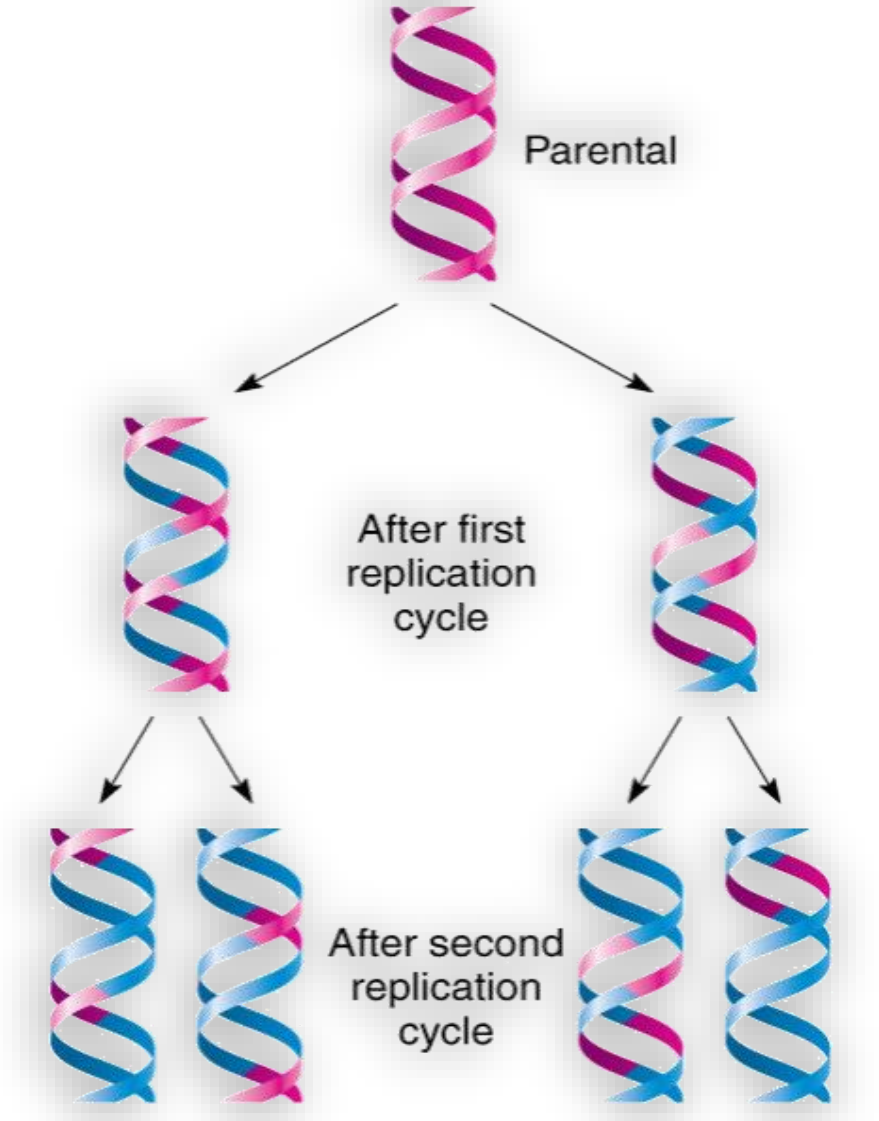
- Untwist the molecule, and attaches the old strand to the newly synthesized one



Method of DNA Replication

Proposed Hypotheses › Dispersive

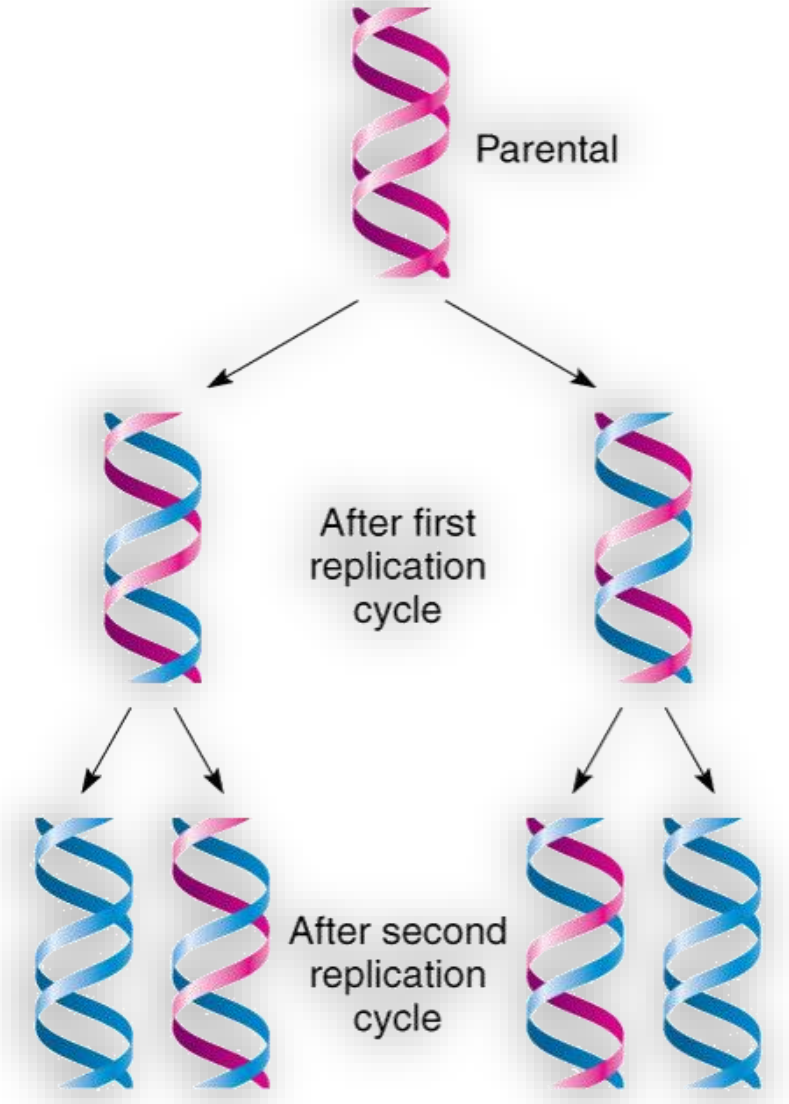
This would synthesize the DNA in short pieces alternating from one strand to the other



Method of DNA Replication

Proposed Hypotheses › Semiconservative

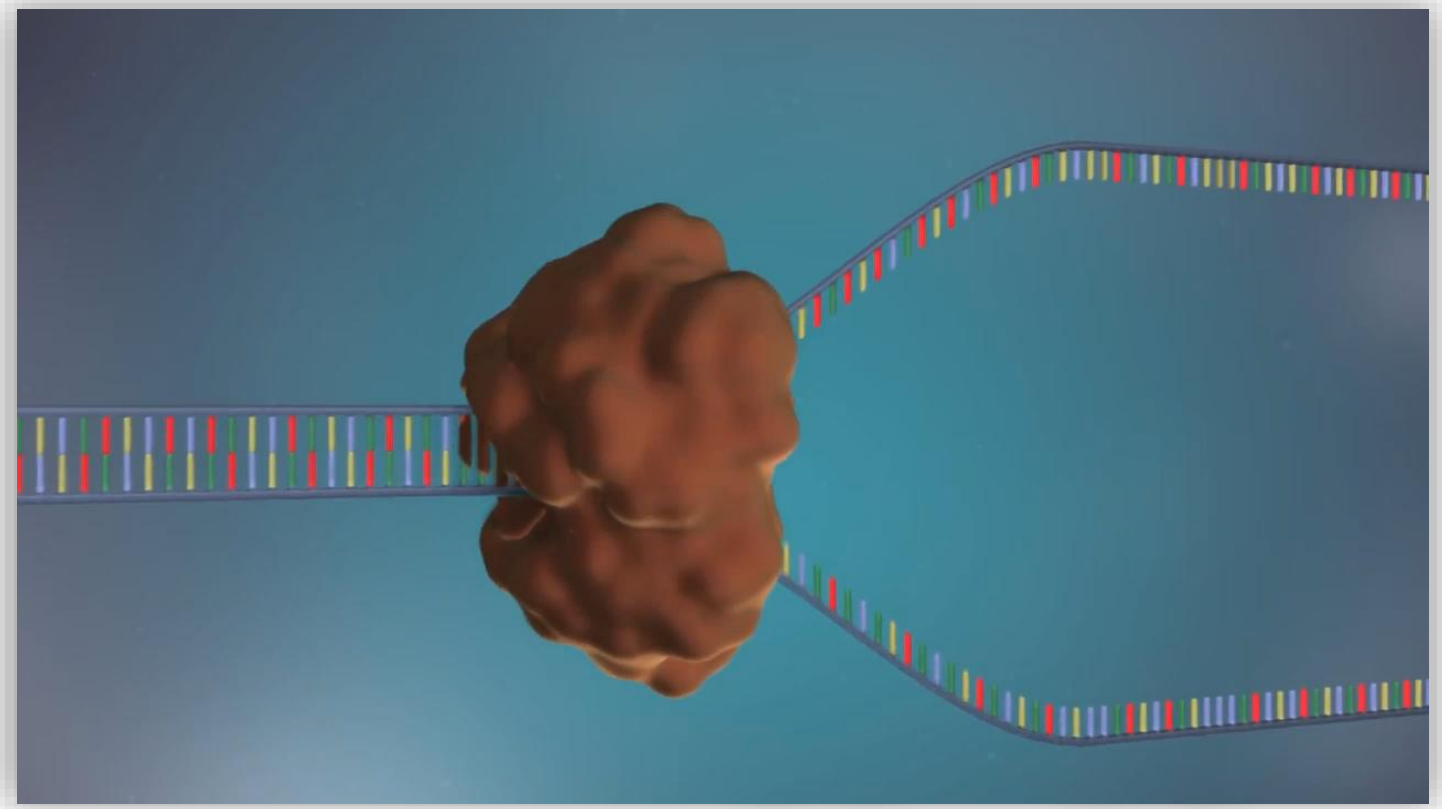
Semiconservative replication would produce molecules with both old and new DNA, but each molecule would be composed of one old strand and one new one



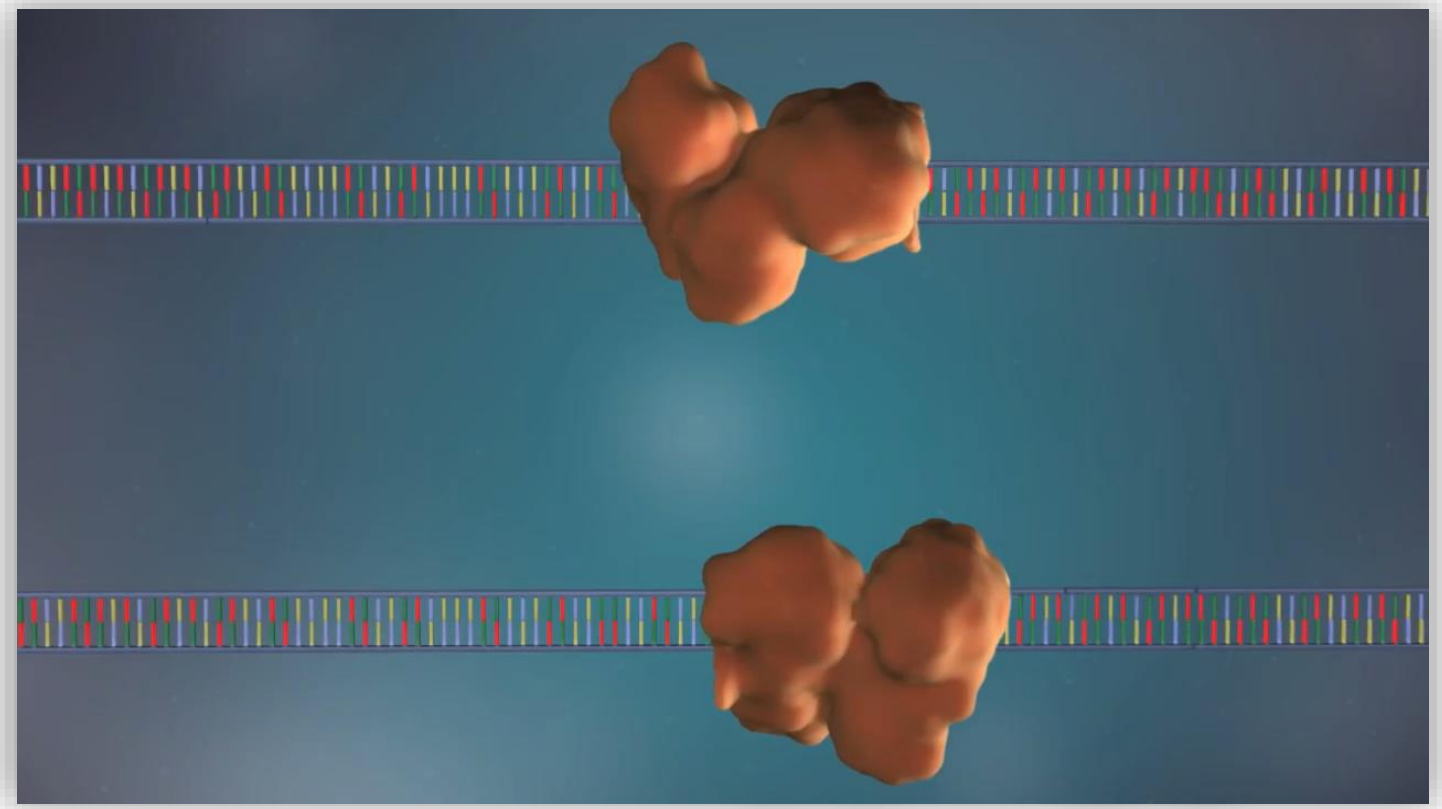
Method of DNA Replication

Proposed Hypotheses › Semiconservative

Each strand acts as a **template** or **guide** for the synthesis of a new DNA molecule by the sequential addition of complementary base pairs



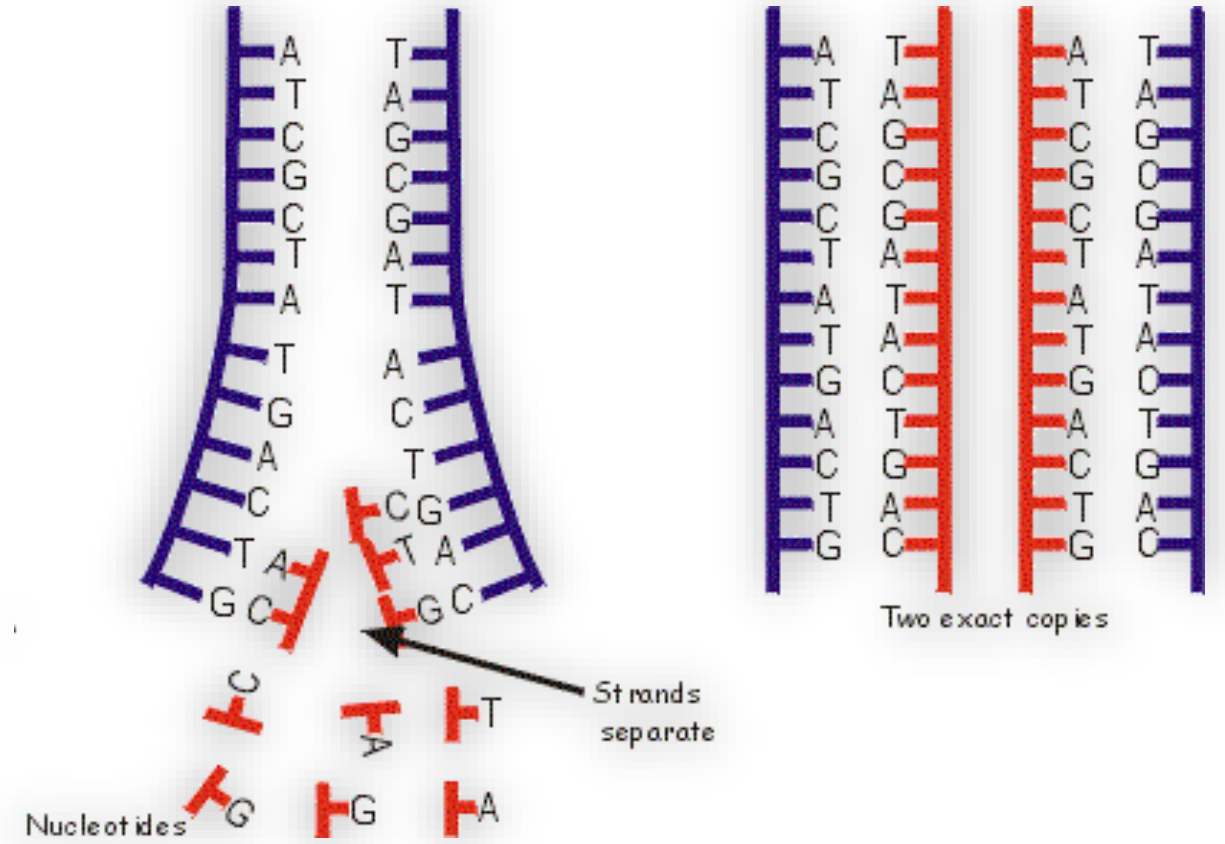
Thereby generating a new DNA strand that is the complementary sequence to the parental DNA



Method of DNA Replication

Proposed Hypotheses › Semiconservative

Each daughter DNA molecule ends up with one of the original strands and one newly synthesized strand

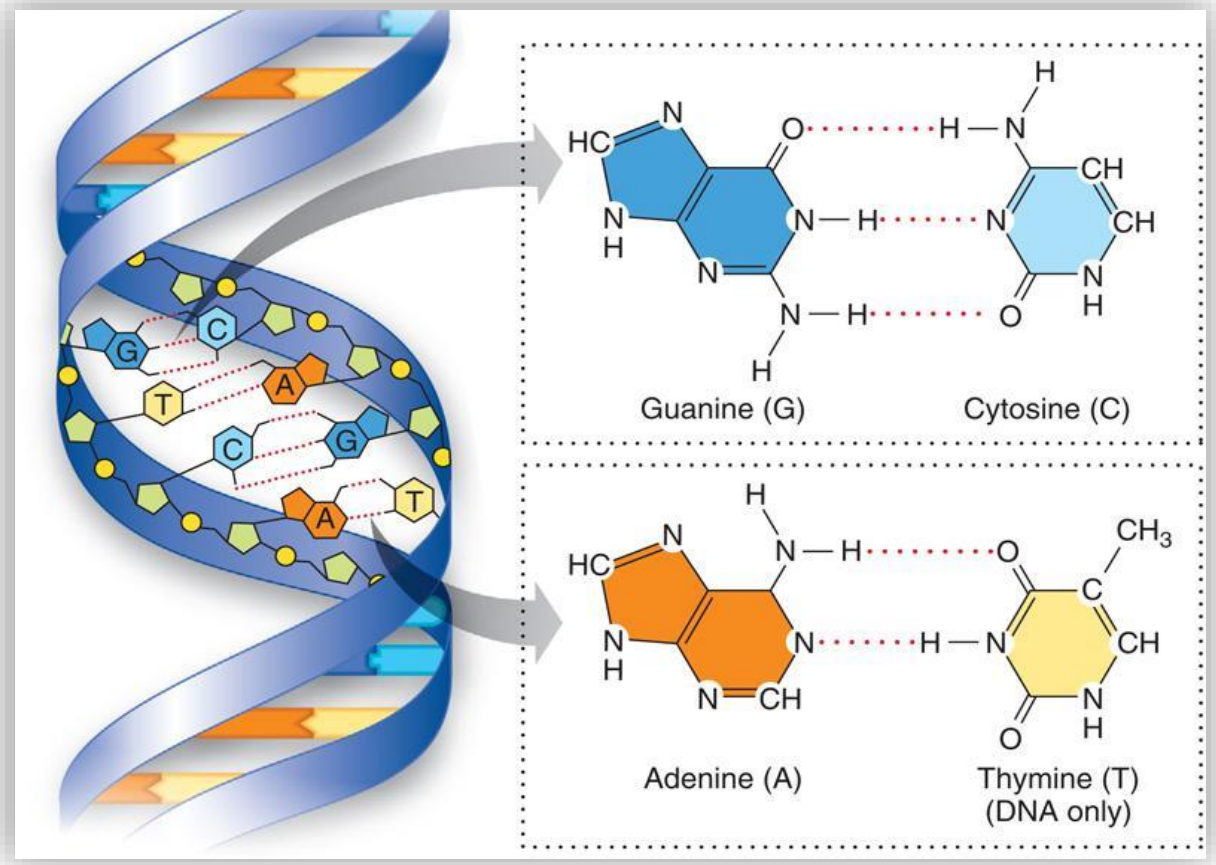


In 1958

Matthew Meselson & Franklin Stahl

worked out a clever procedure to distinguish semi conservative DNA replication from conservative or dispersive replication, using a nonradioactive heavy isotope of nitrogen

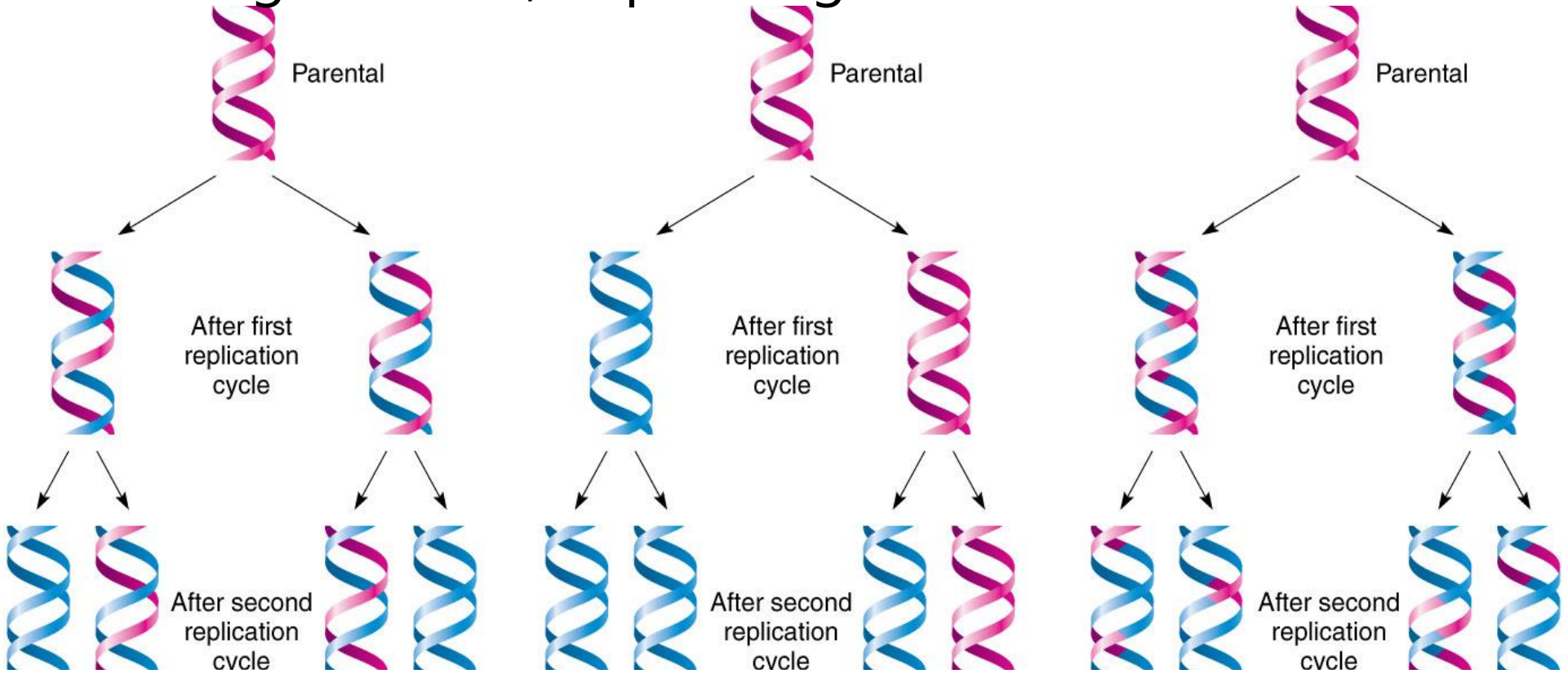
Meselson and Stahl opted for nitrogen because it is an essential chemical component of DNA



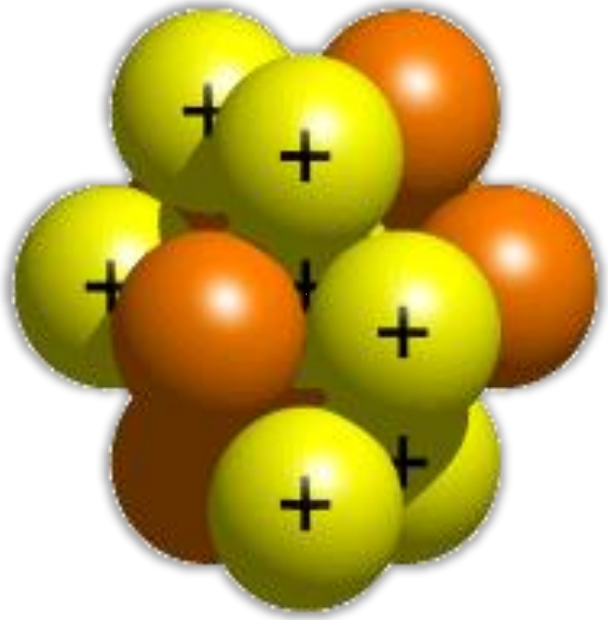
Method of DNA Replication

Proposed Hypotheses › Semiconservative › Experiment

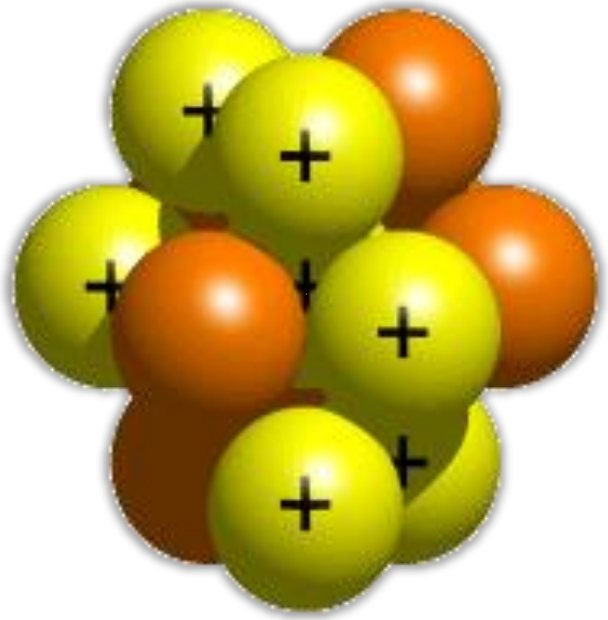
Therefore, every time a cell divides and its DNA replicates, it incorporates new N atoms into the DNA of either one or both of its two daughter cells, depending on which model was correct



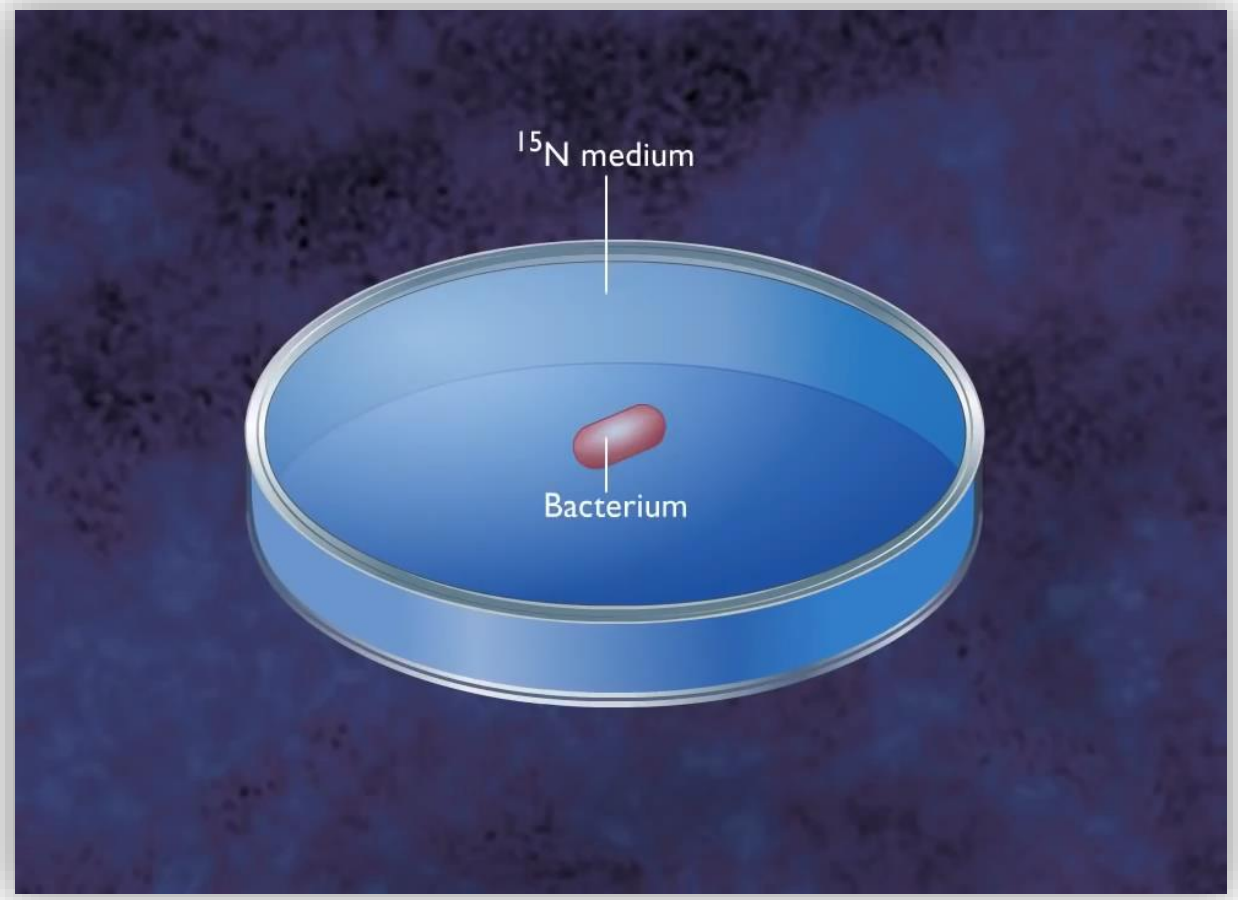
Ordinary nitrogen, the most abundant isotope, has an atomic weight of 14, so it is called ^{14}N



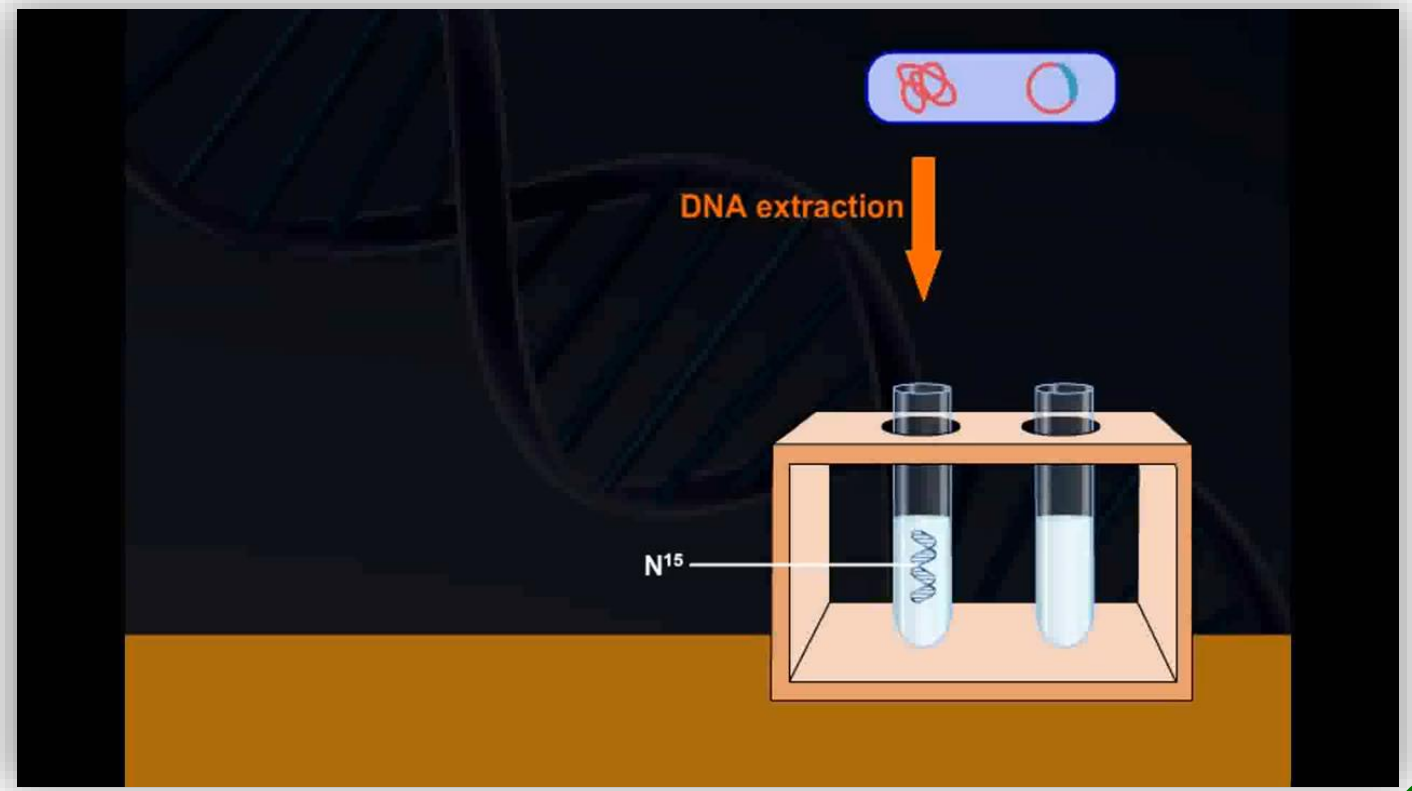
A relatively rare isotope ^{15}N has an atomic weight of 15



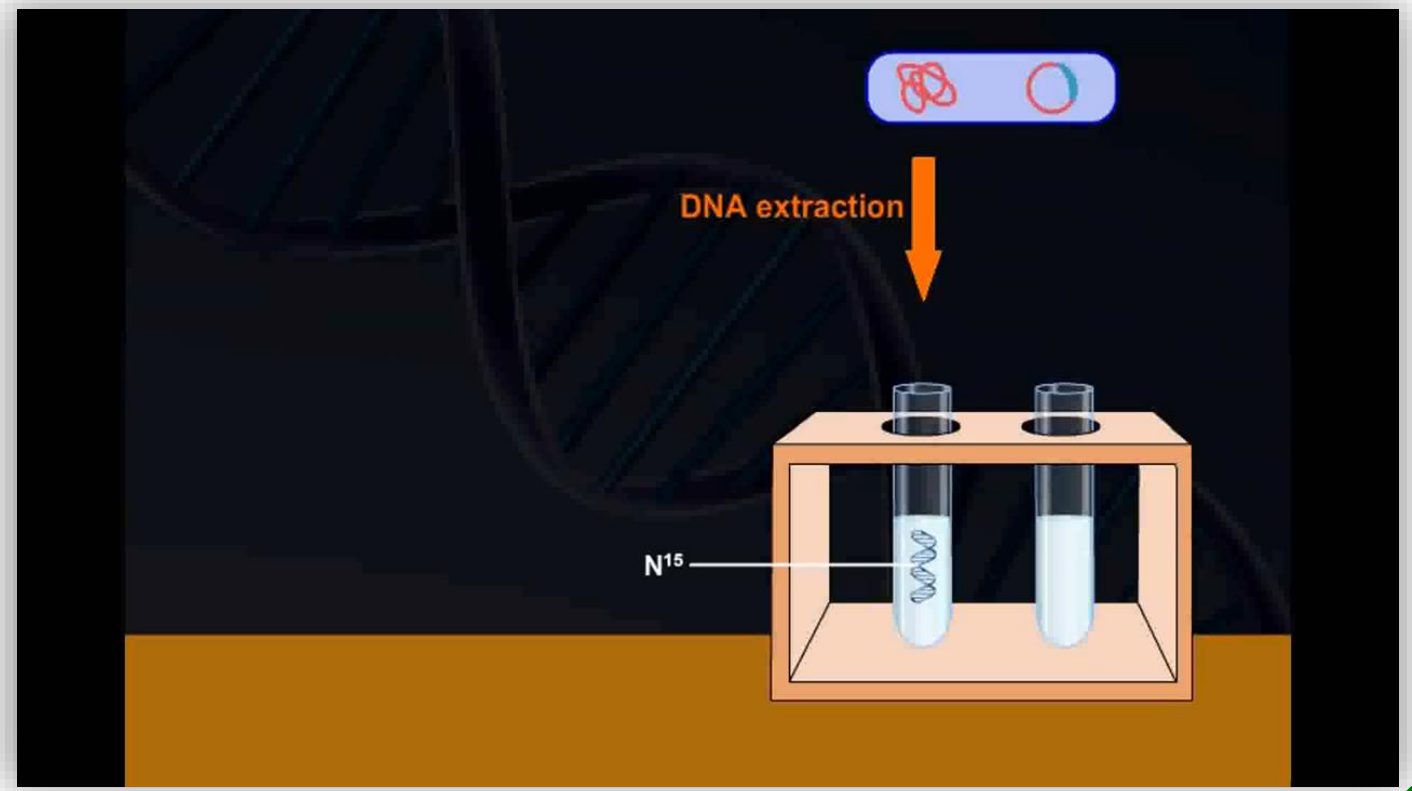
Meselson and Stahl found that if bacteria are grown in a medium enriched in **^{15}N**, they incorporate the heavy isotope into their DNA, which becomes denser than normal



This labeled DNA **clearly** separates from ordinary DNA in gradient of Cesium Chloride (CsCl) spun in an ultracentrifuge



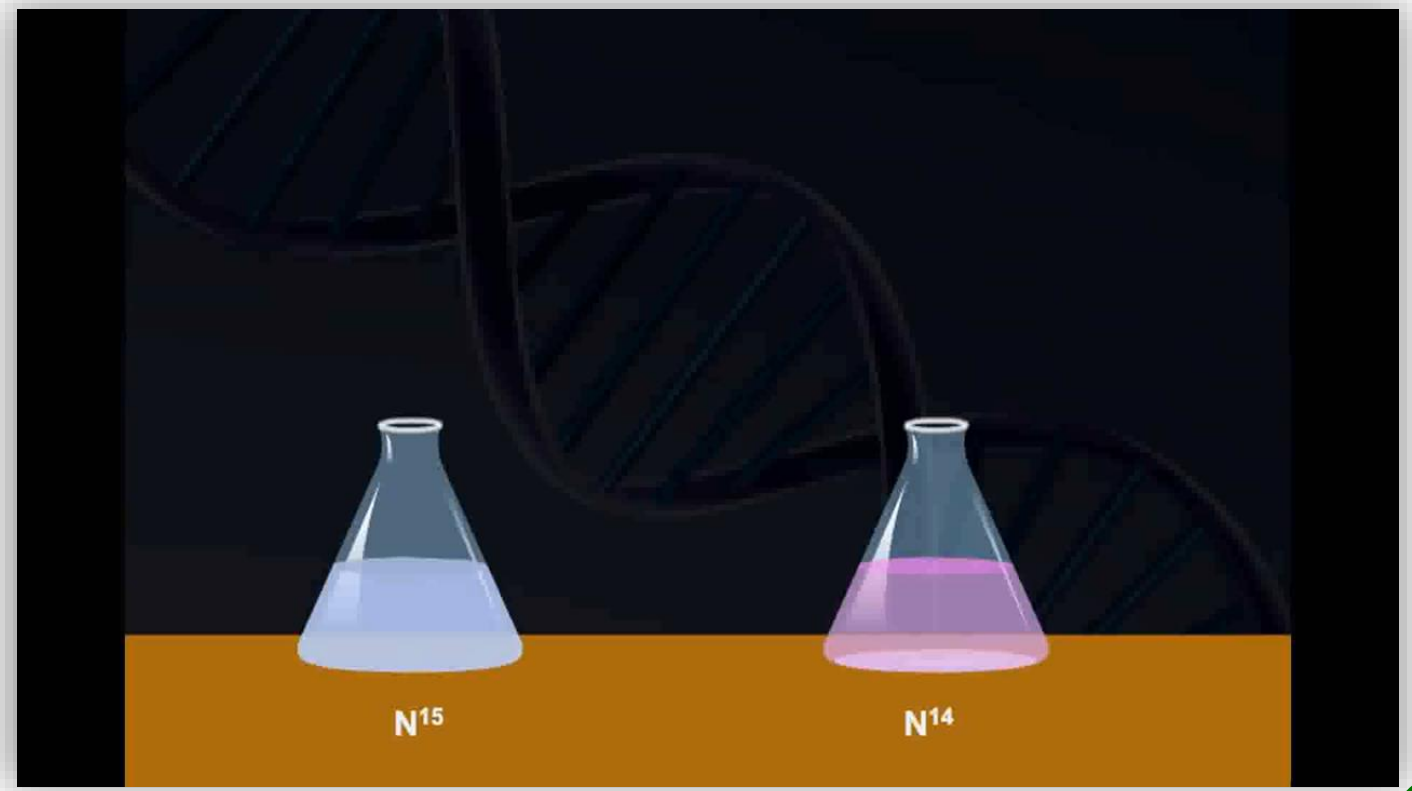
CsCl is used because it is a very dense salt and therefore makes dense enough solution that DNA will float somewhere in the middle rather than sinking to the bottom



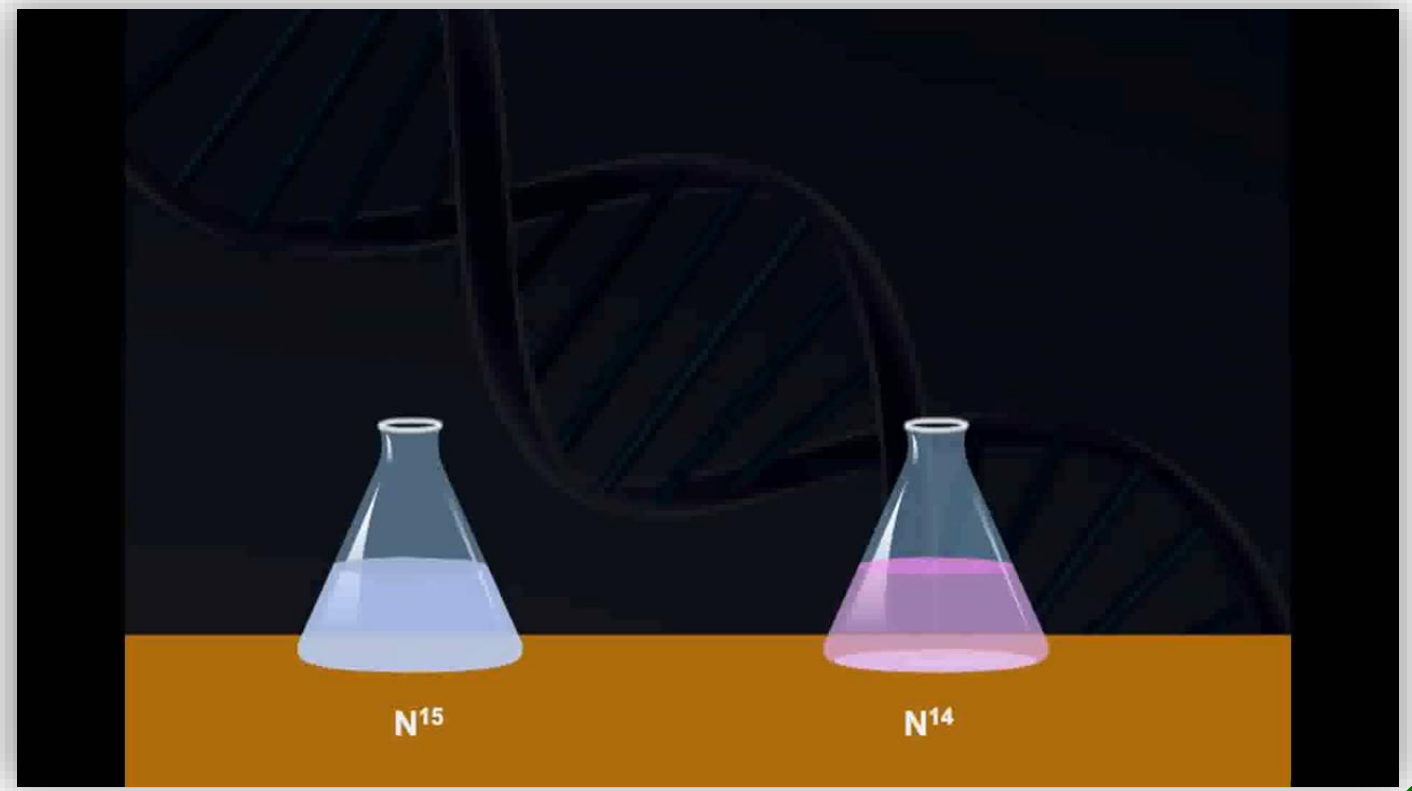
Method of DNA Replication

Proposed Hypotheses › Semiconservative › Experiment

The aim of the experiment was to grow ^{15}N labeled bacteria in ^{14}N-medium and then to look at the density of the DNA products

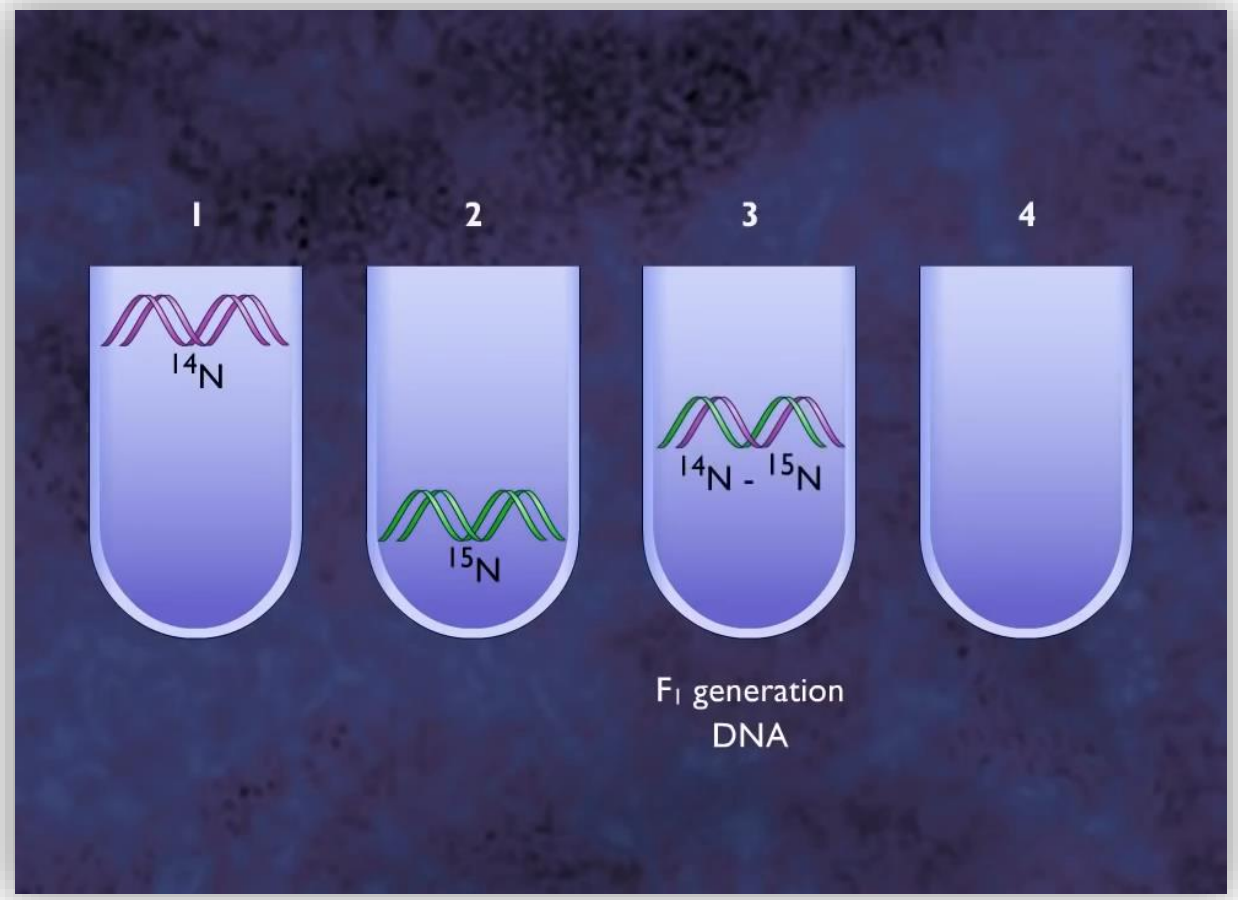


That is, *E. coli* cells with only ^{15}N in their DNA were transferred to a ^{14}N medium and were allowed to divide or replicate



The progress of cell division was monitored by measuring the optical density of the cell suspension

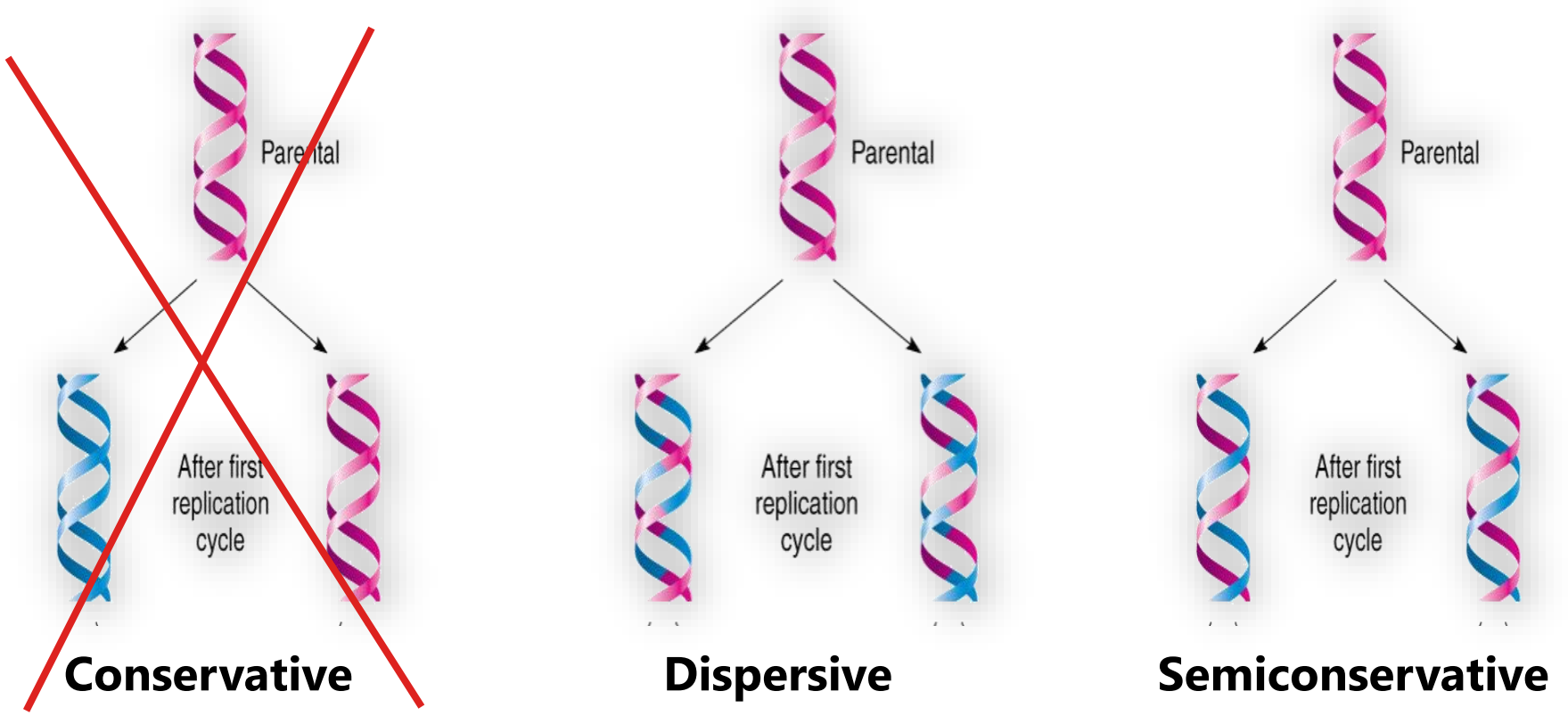
DNA was extracted periodically and was compared to pure ^{14}N DNA and ^{15}N DNA



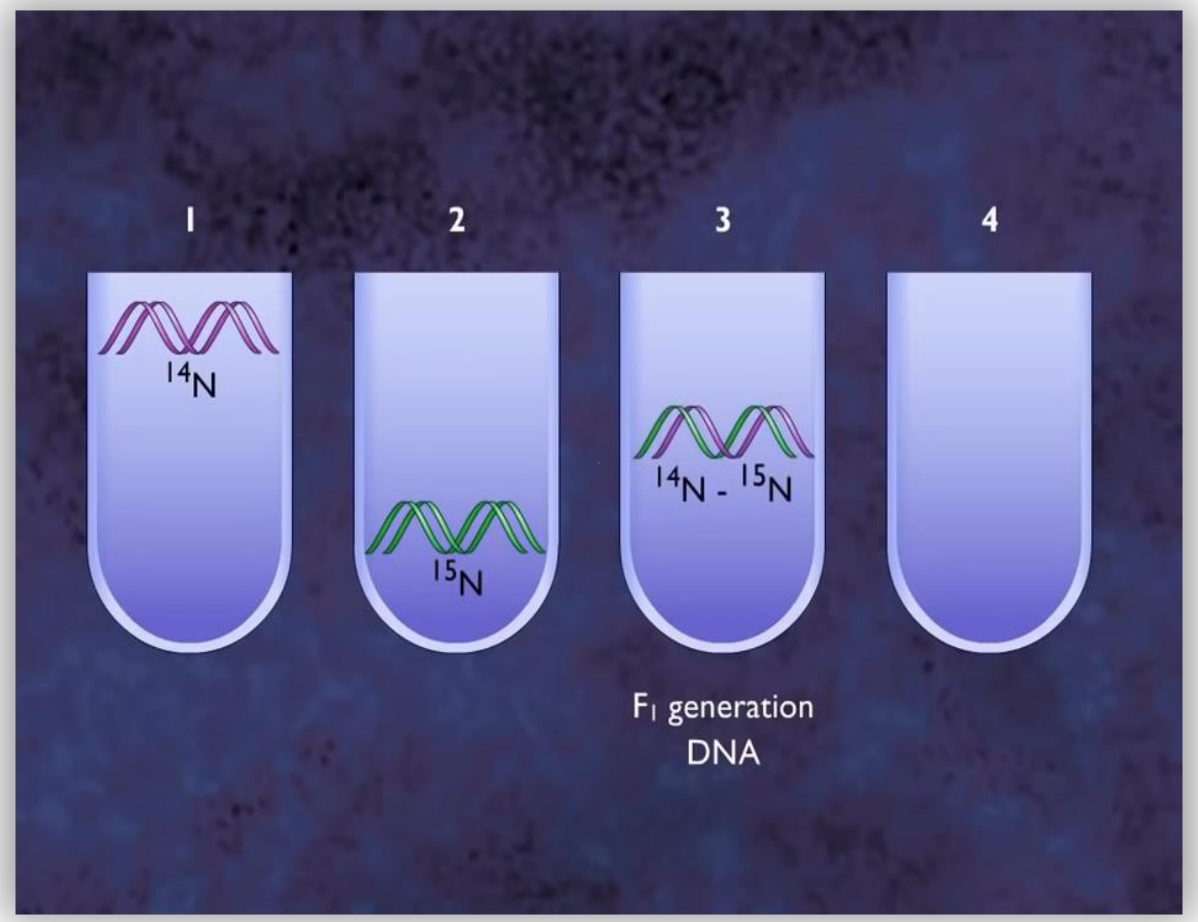
Method of DNA Replication

Proposed Hypotheses › Semiconservative › Results

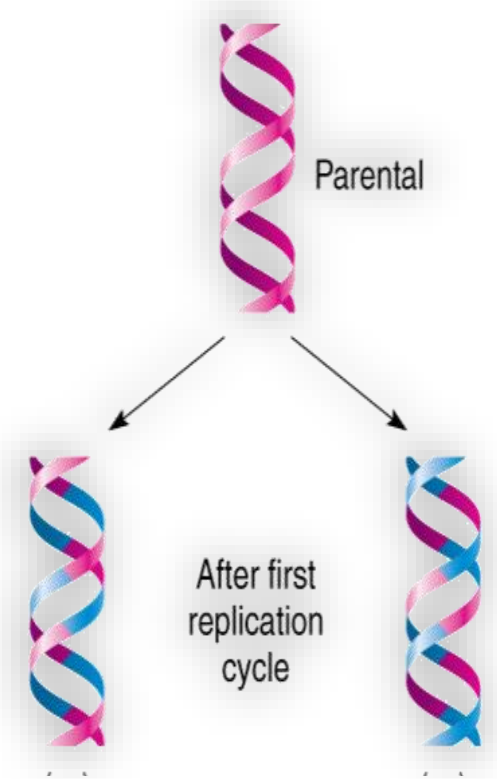
Based on these findings, the scientists were immediately able to **exclude the conservative model of replication as a possibility**



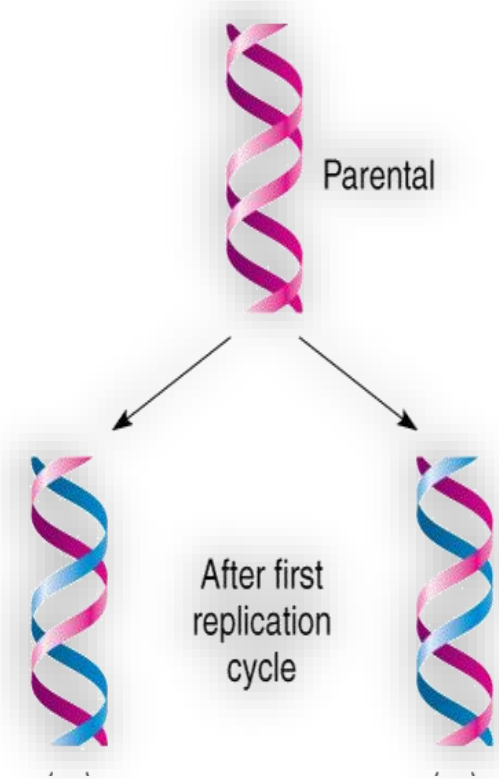
After all, if DNA replicated conservatively, there should have been two distinct bands after a single round of replication



However, this result was consistent with both semiconservative and dispersive replication



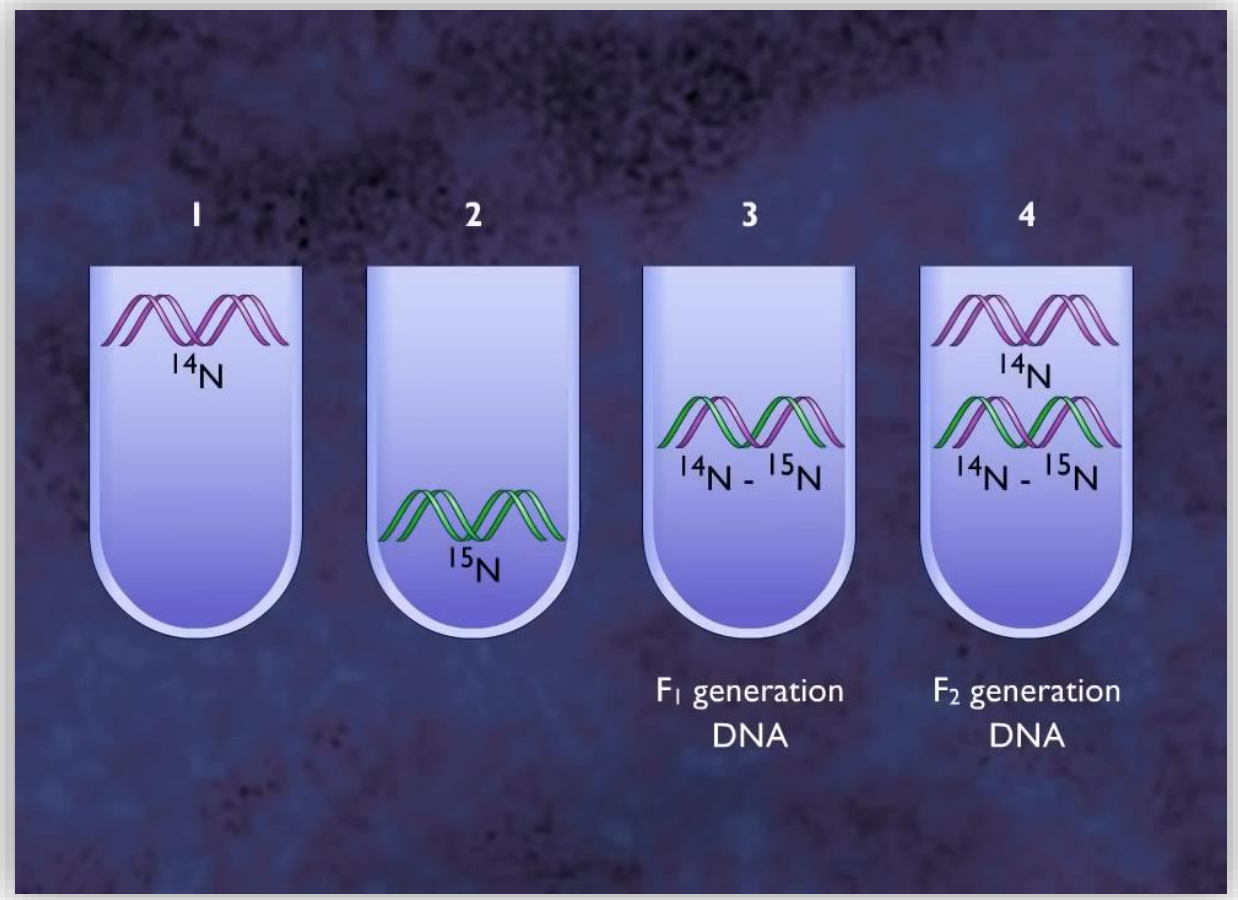
Dispersive



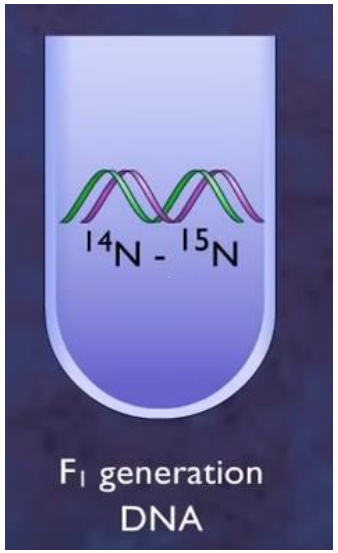
Semiconservative

To differentiate between the two, Meselson and Stahl had to let the cells divide again and then sample the DNA after a second round of replication

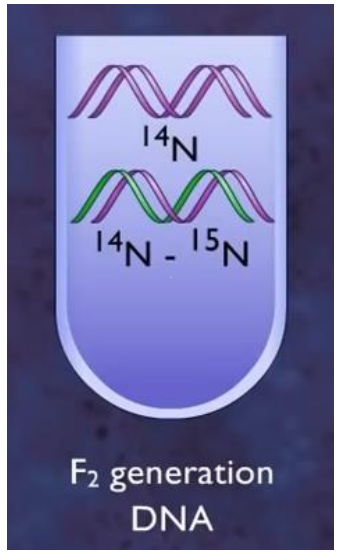
DNA from cells after two replications had been completed was found to consist of equal amounts of DNA with two different densities



Intermediate density of DNA of cells grown for only one division in ^{14}N medium

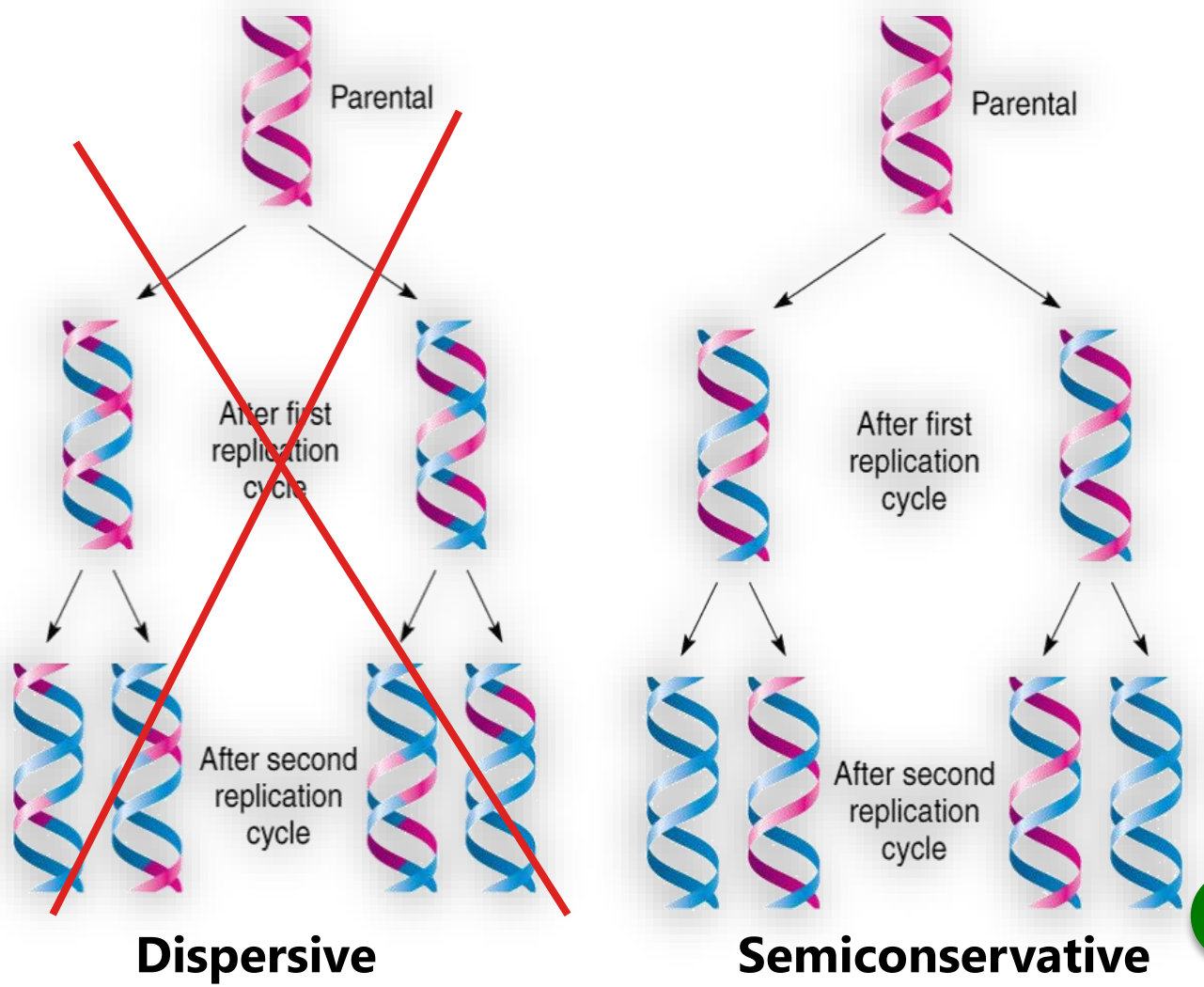


DNA from cells grown exclusively in ^{14}N medium

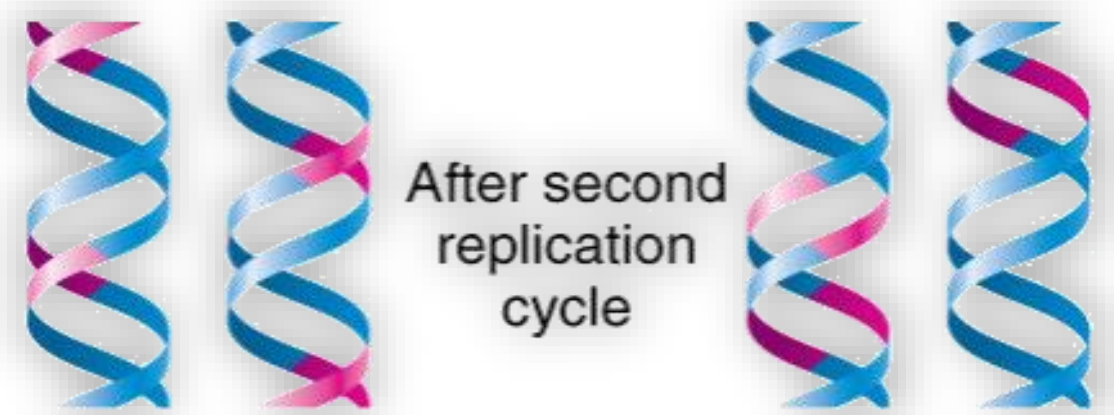


This was inconsistent with dispersive replication

After all, if the dispersive model were the correct model, the scientists would have continued to observe only a single band after every round of replication

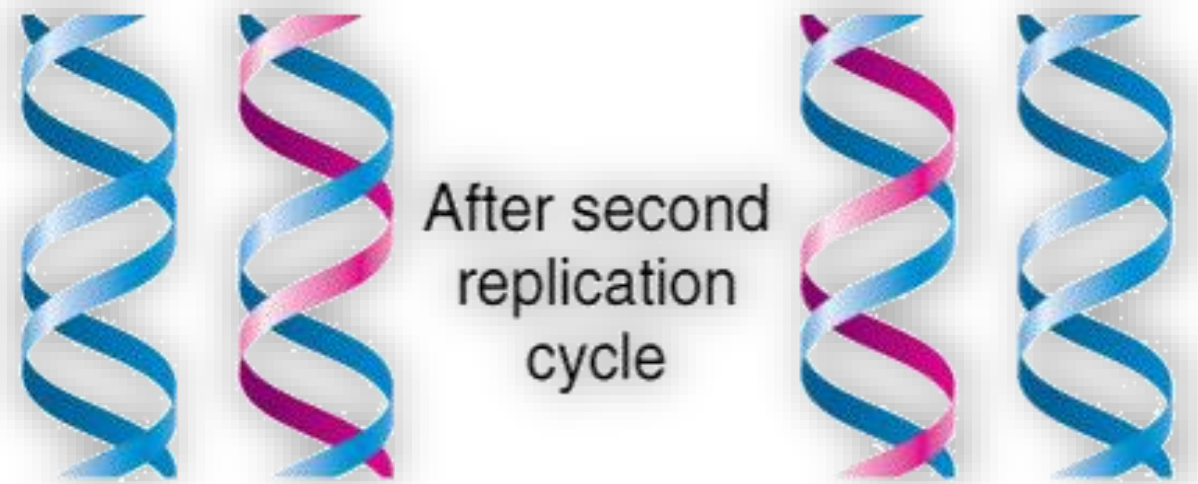


Dispersive replication would have resulted in double-stranded DNA with both strands having mixtures of ^{15}N and ^{14}N DNA, either of which would have appeared as DNA of an intermediate density



Dispersive

Semiconservative replication would result in double-stranded DNA with one strand of ^{15}N DNA, and one of ^{14}N DNA

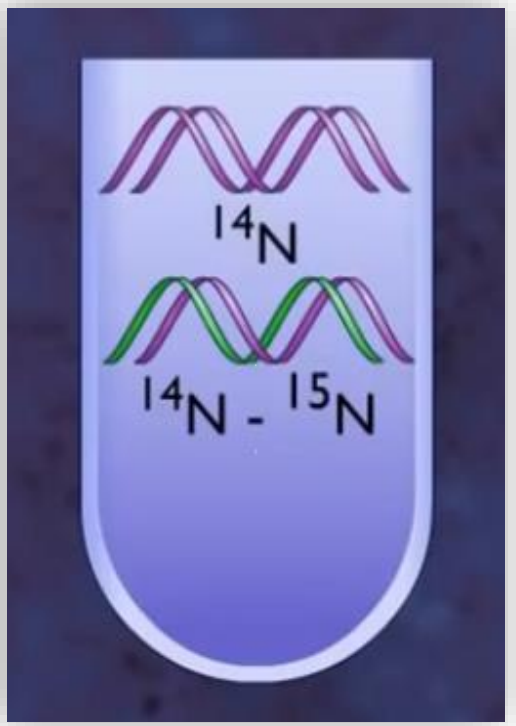


Semiconservative

This will result in equal amounts of DNA with two different densities

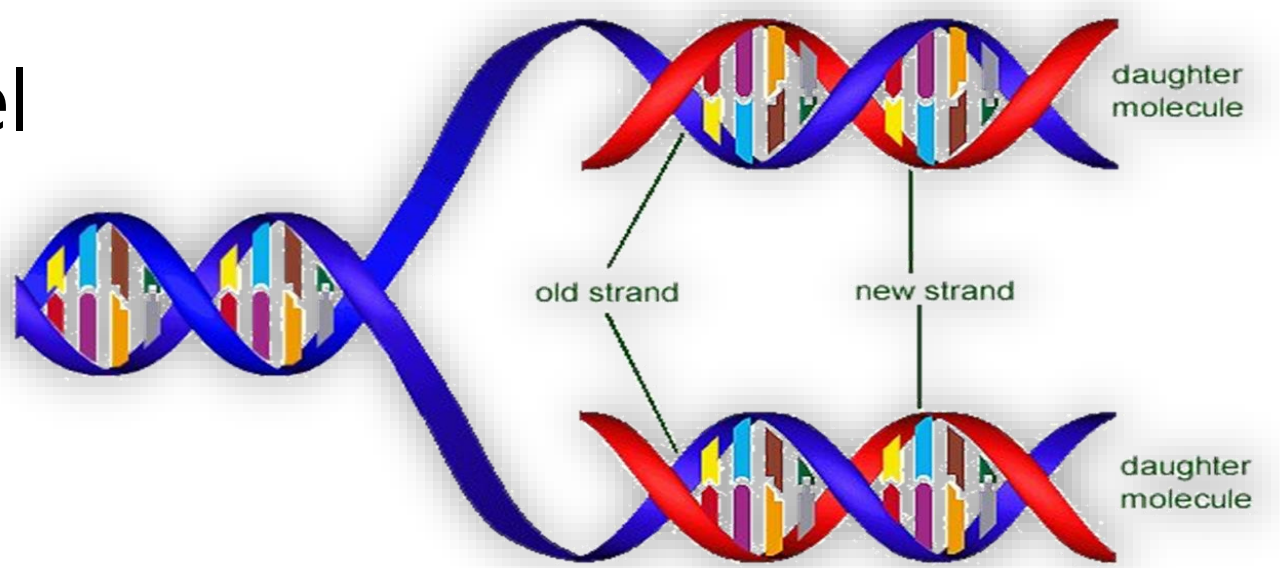
Semiconservative

The scientists continued to observe the same two bands after several subsequent rounds of replication



Semiconservative

These results were consistent with the semiconservative model of replication and the reality that, when DNA replicated, each new double helix was built with one old strand and one new strand

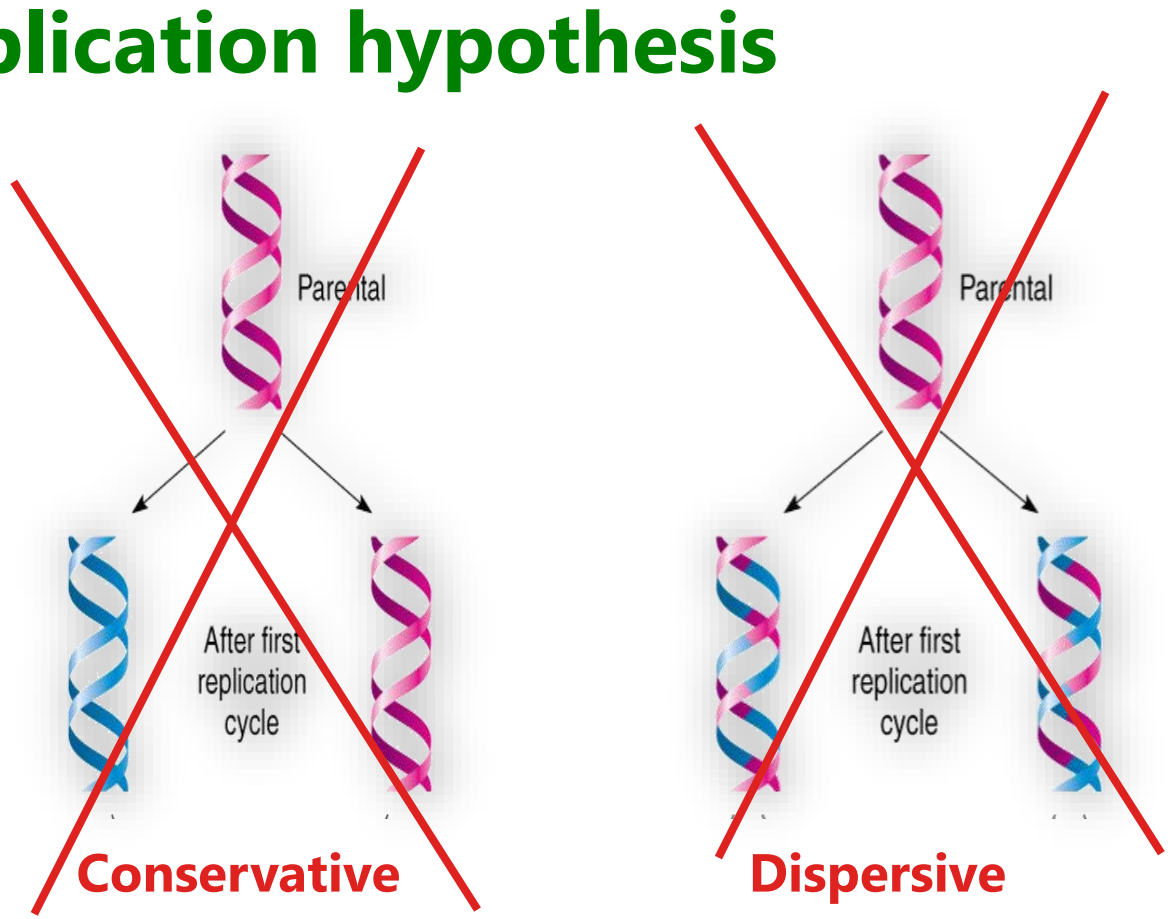


Semiconservative

Method of DNA Replication

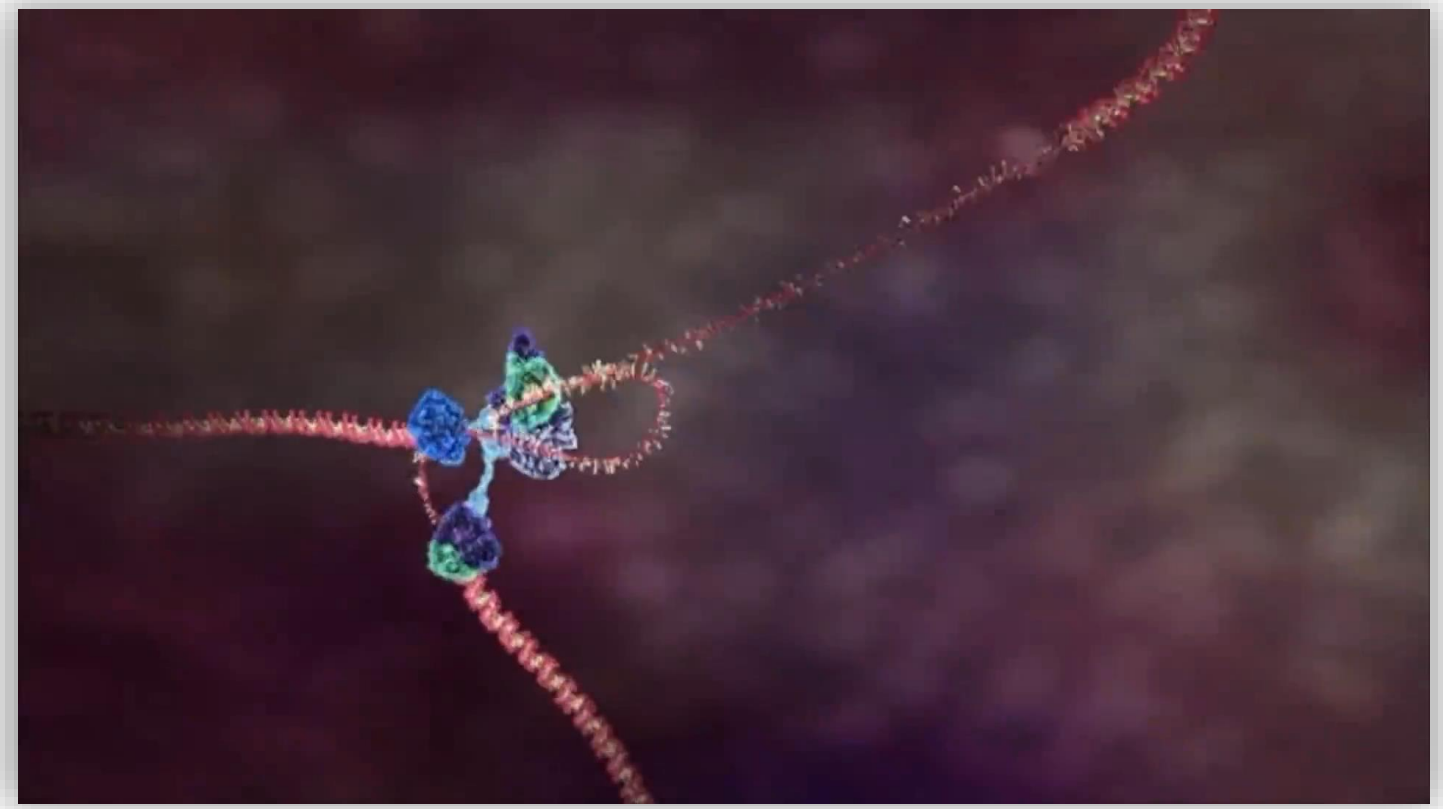
Proposed Hypotheses › Semiconservative › Results

The result was consistent with the semiconservative replication hypothesis



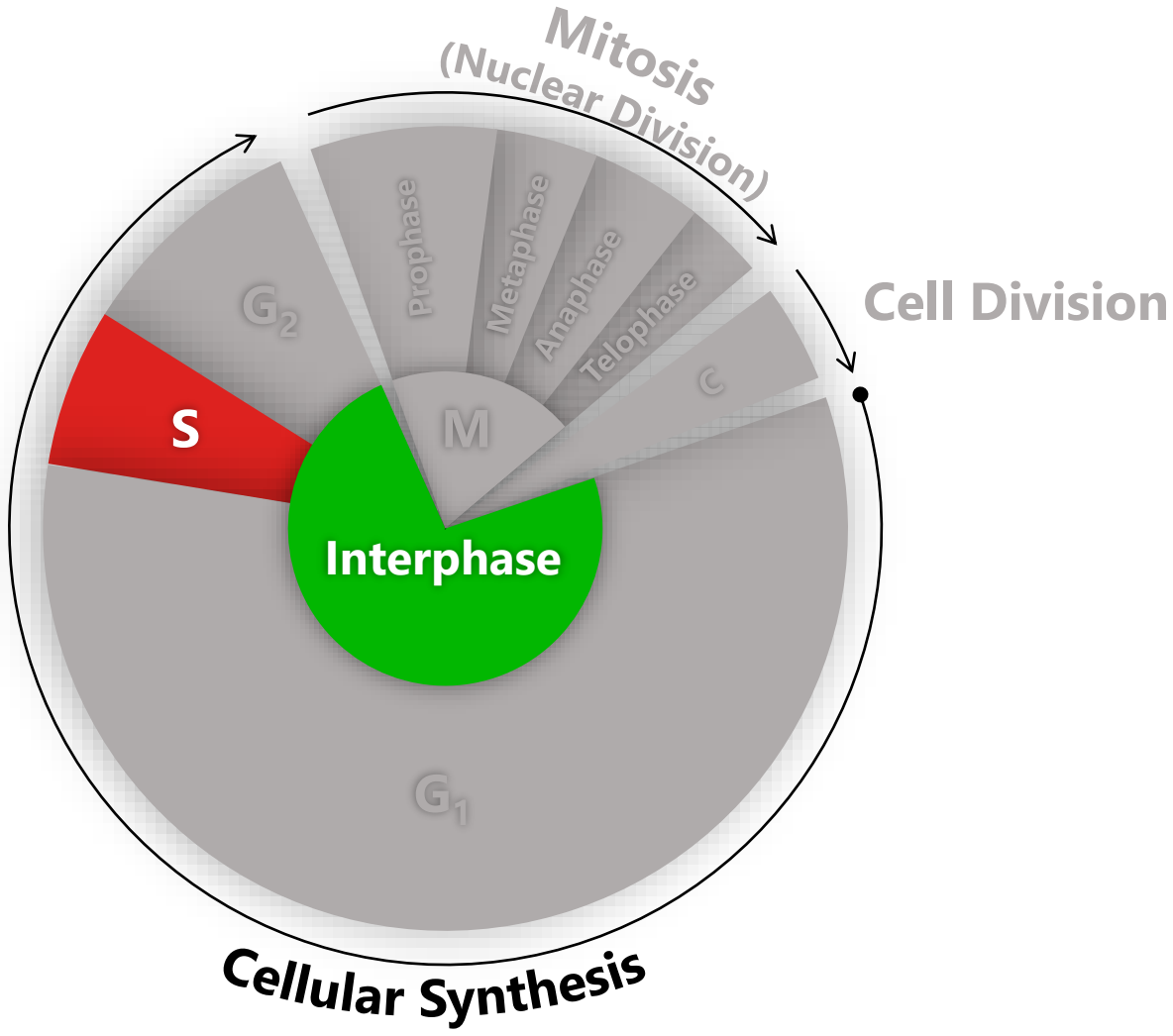
Replication of Eukaryotic Chromosomes

Before a cell can divide, it must duplicate or replicate all of its DNA



Replication of Eukaryotic Chromosomes

In eukaryotes, this occurs during S phase of the cell cycle



Replication of Eukaryotic Chromosomes

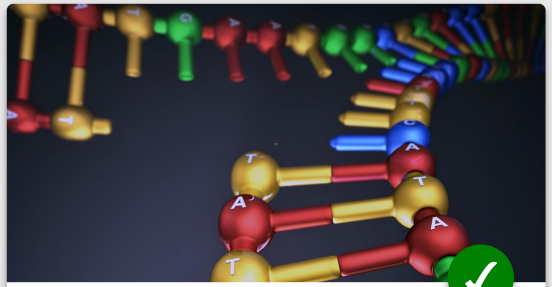
Eukaryotic DNA replication is very slow compared to *E. coli* DNA replication: only about **75 nucleotides/ second**

Module 2

DNA Replication

Module Lessons

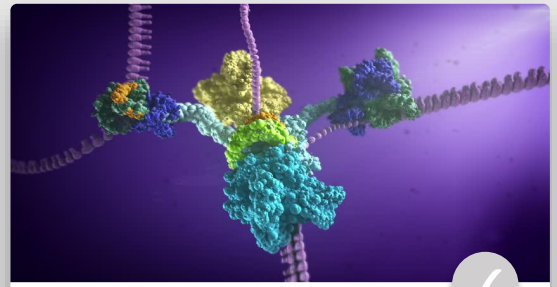
- Dashboard
- Introduction
- Lessons
- Assignments



Lesson 1

Method of Replication

Start ▸



Lesson 2

Mechanism of Replication

Start ▸

Lesson 3

Rate of Replication

Start ▸

46

Lesson 2

Mechanism of DNA Replication

Learn about the mechanism DNA uses to replicate itself to form new DNAs

Learn Now ▶

Mechanism of DNA Replication

Replication is a huge task, whether in bacteria or in eukaryotes and requires many proteins or enzymes to act together

Physical & Biochemical Challenges Cells Overcome

1. **Locating site(s) to begin replication**

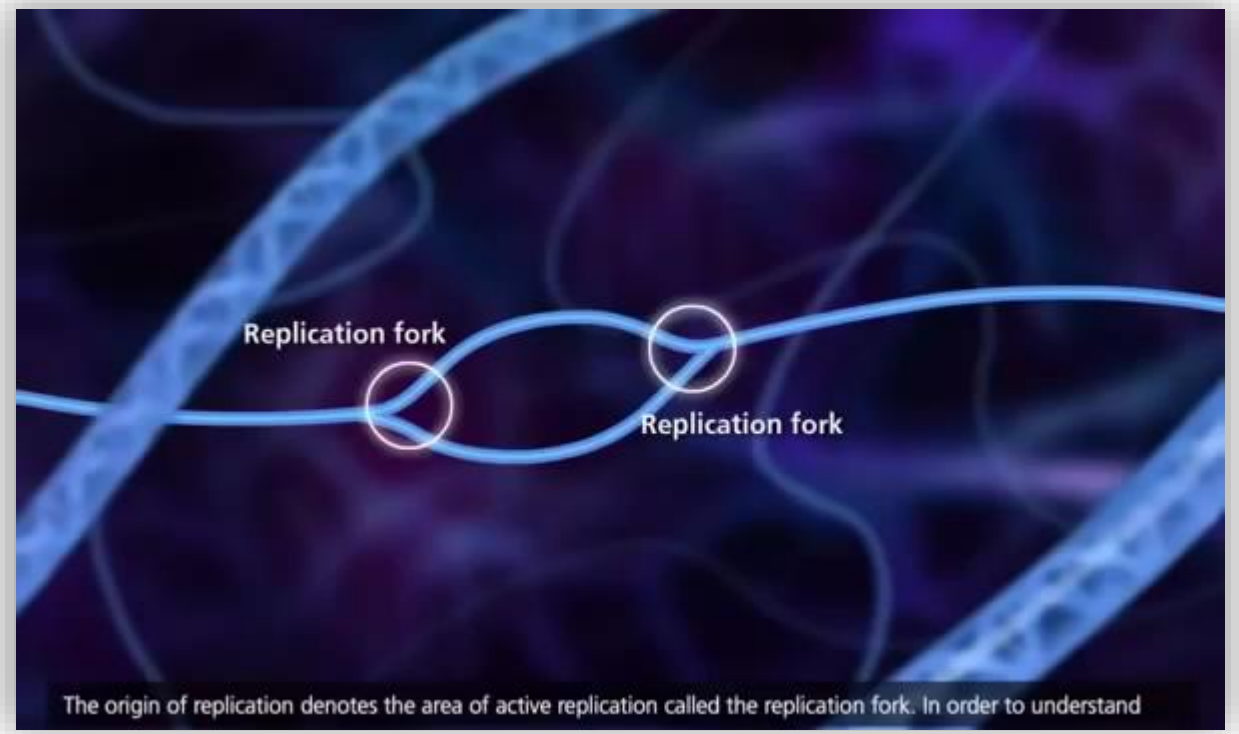
   The proper enzymes must be collected or deposited there

Physical & Biochemical Challenges Cells Overcome

1. Locating site(s) to begin replication

2. **Unwinding the double helix**

   to expose the two strands. This imposes twisting strain on the portions of the helix farther away from the unwinding site

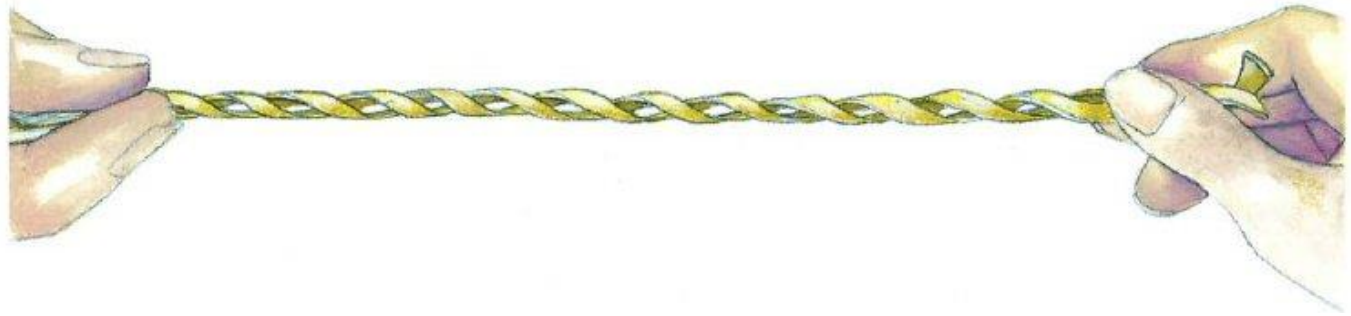


Physical & Biochemical Challenges Cells Overcome

1. Locating site(s) to begin replication

2. **Unwinding the double helix**

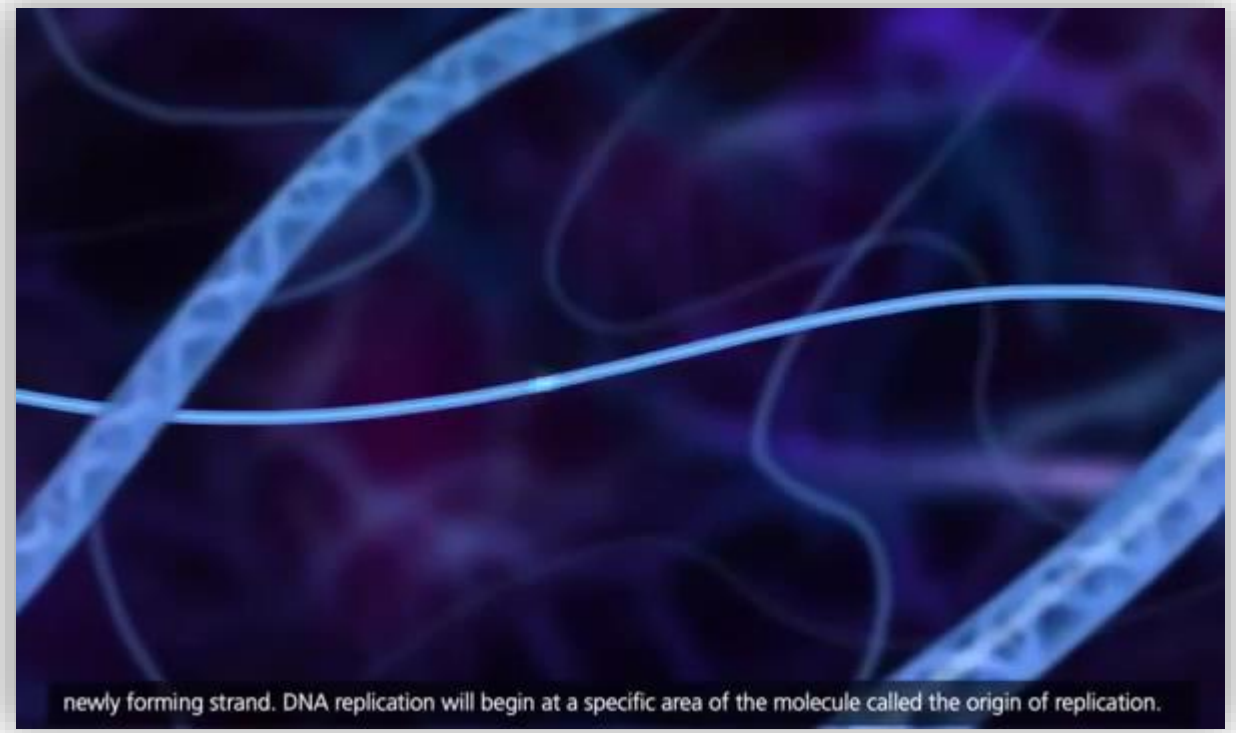
This is much like how untangling a twisted phone cord does, and those forces must be relieved to prevent breakage of the DNA strands



Origins

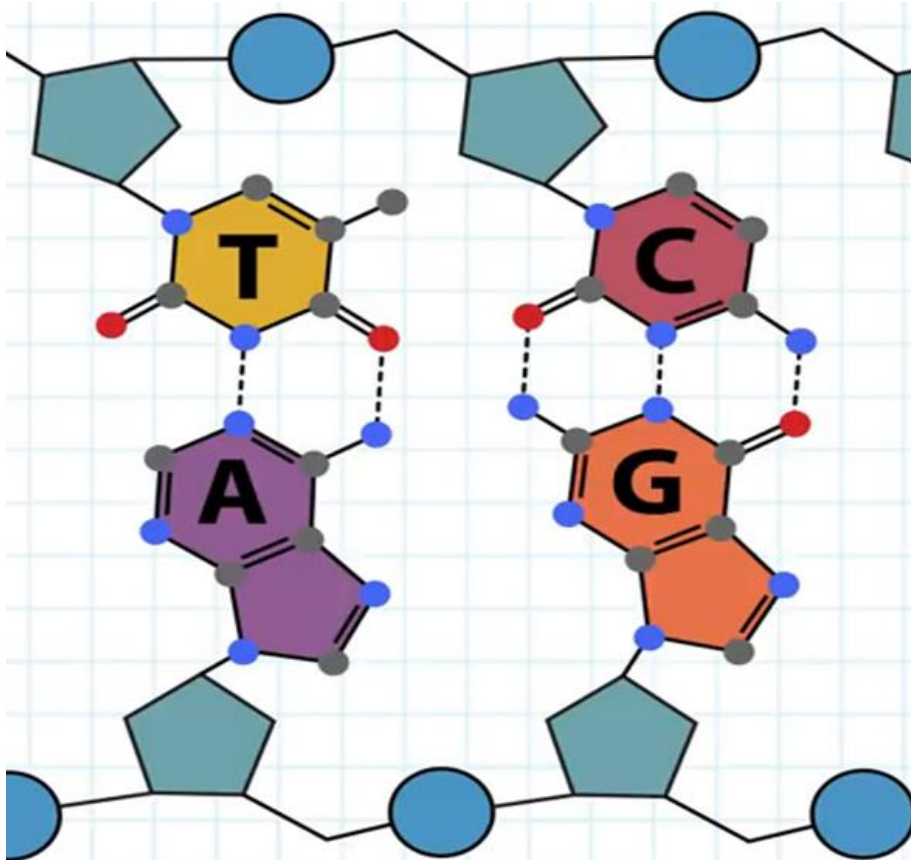
DNA replication begins at specific points known as the **Origins**

These sites are recognized by certain proteins in the cell



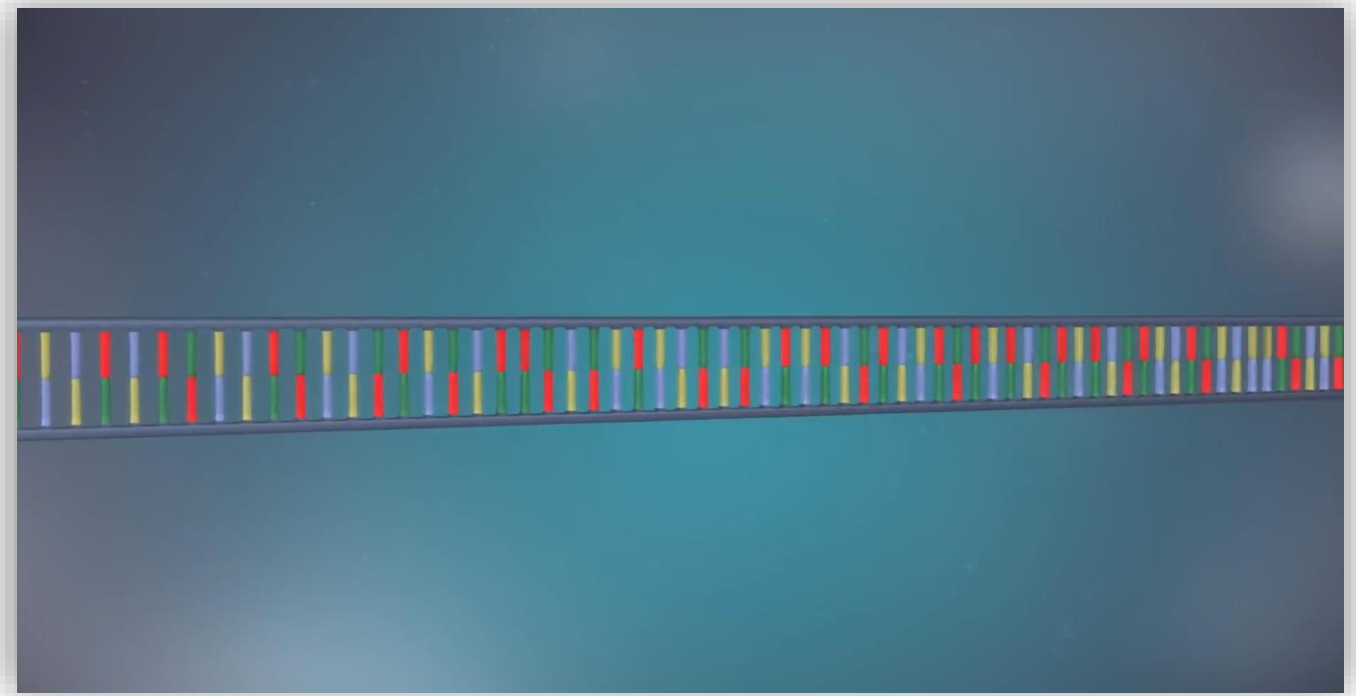
Origins

The Origins are rich in Adenine and Thymine bases, as breaking 2 hydrogen bonds between the Adenine and Thymine is easier than breaking the triple hydrogen bonds between Cytosine and Guanine



Replication Fork

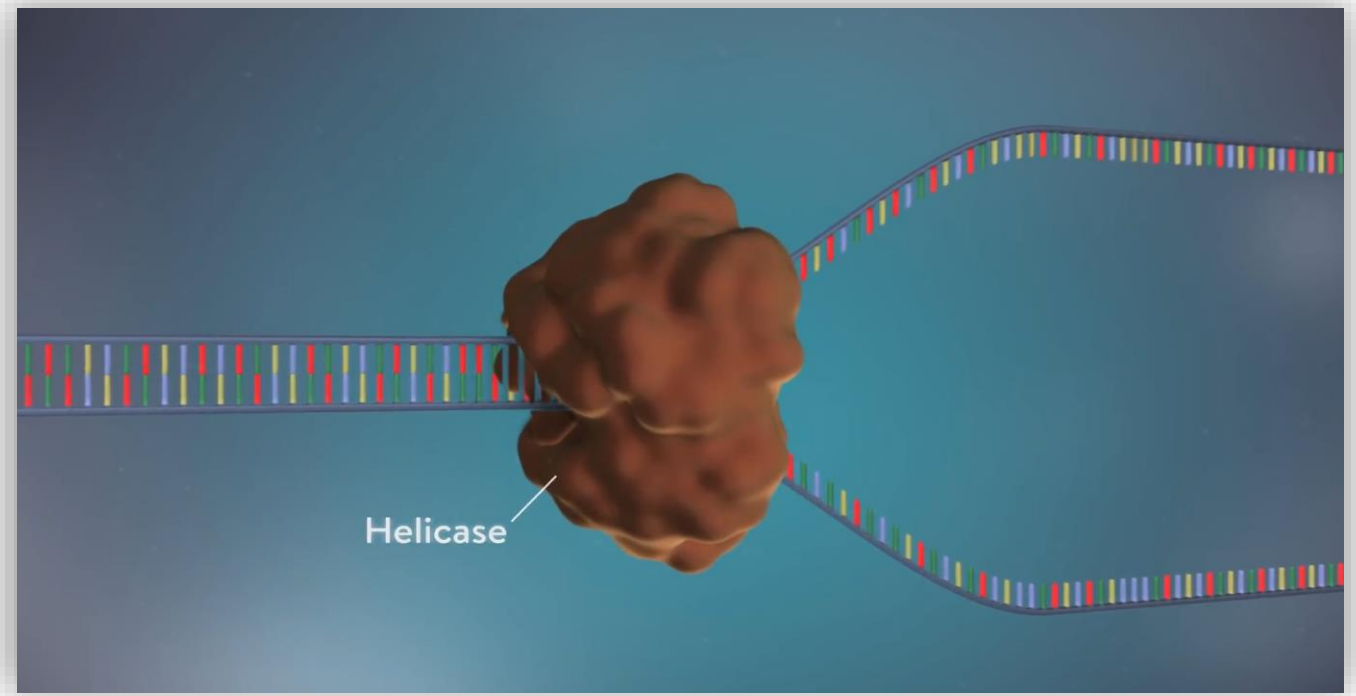
6 proteins arranged in a ring shape known as **Helicase**, unwind the double stranded DNA helix into single strands by breaking the hydrogen bonds between them



Replication Fork

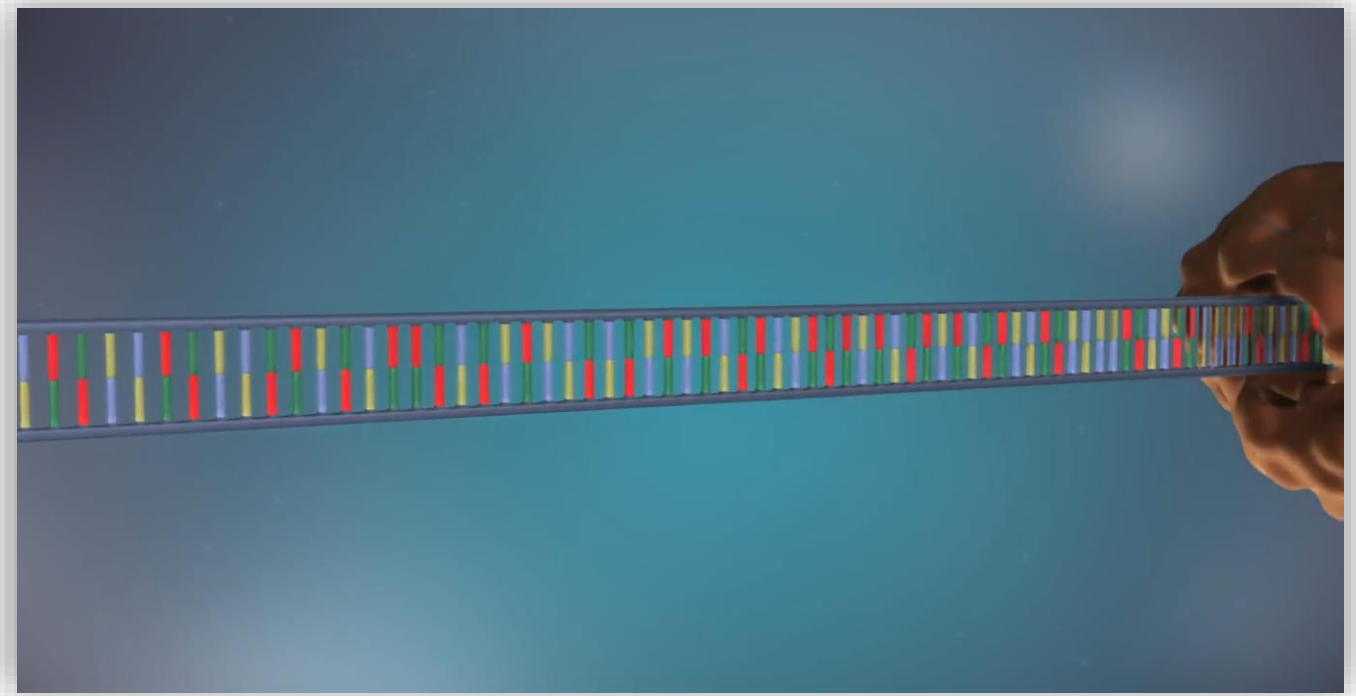
This results in the formation of a **replication fork**

The replication fork is a structure that is formed during the DNA replication process



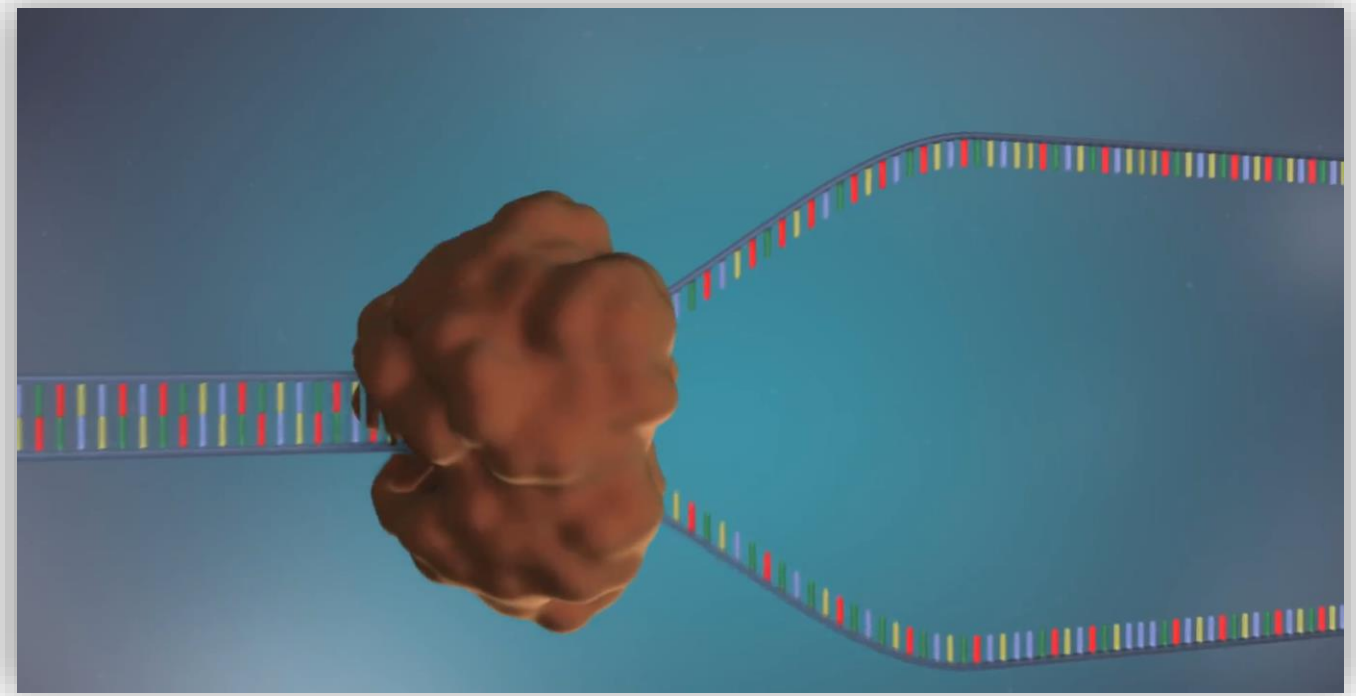
Replication Fork

The fork is made with the action of helicase, that breaks the hydrogen bonds, that hold the two DNA strands together



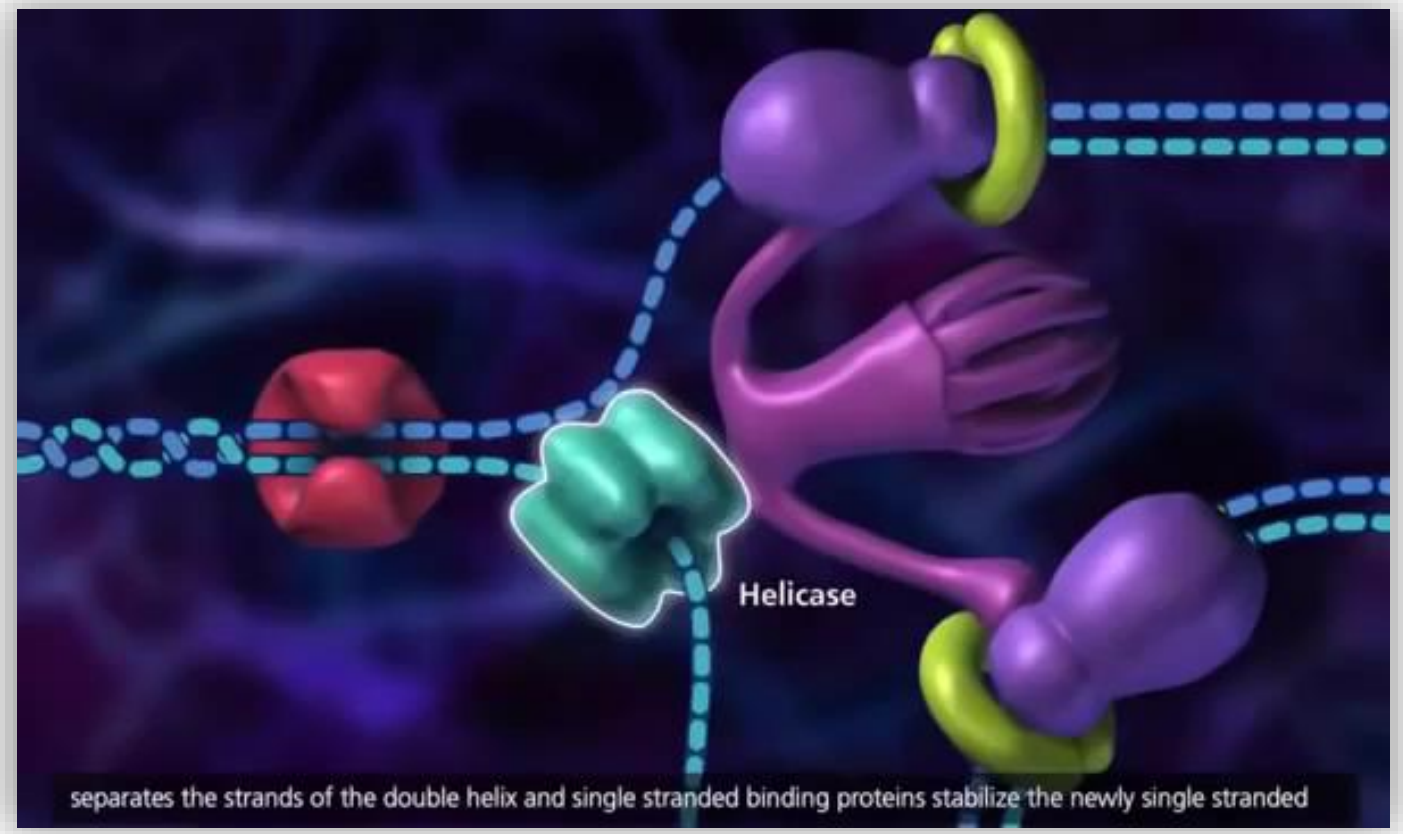
Replication Fork

This results in a structure that has two branching 'prongs' of a single strand DNA each



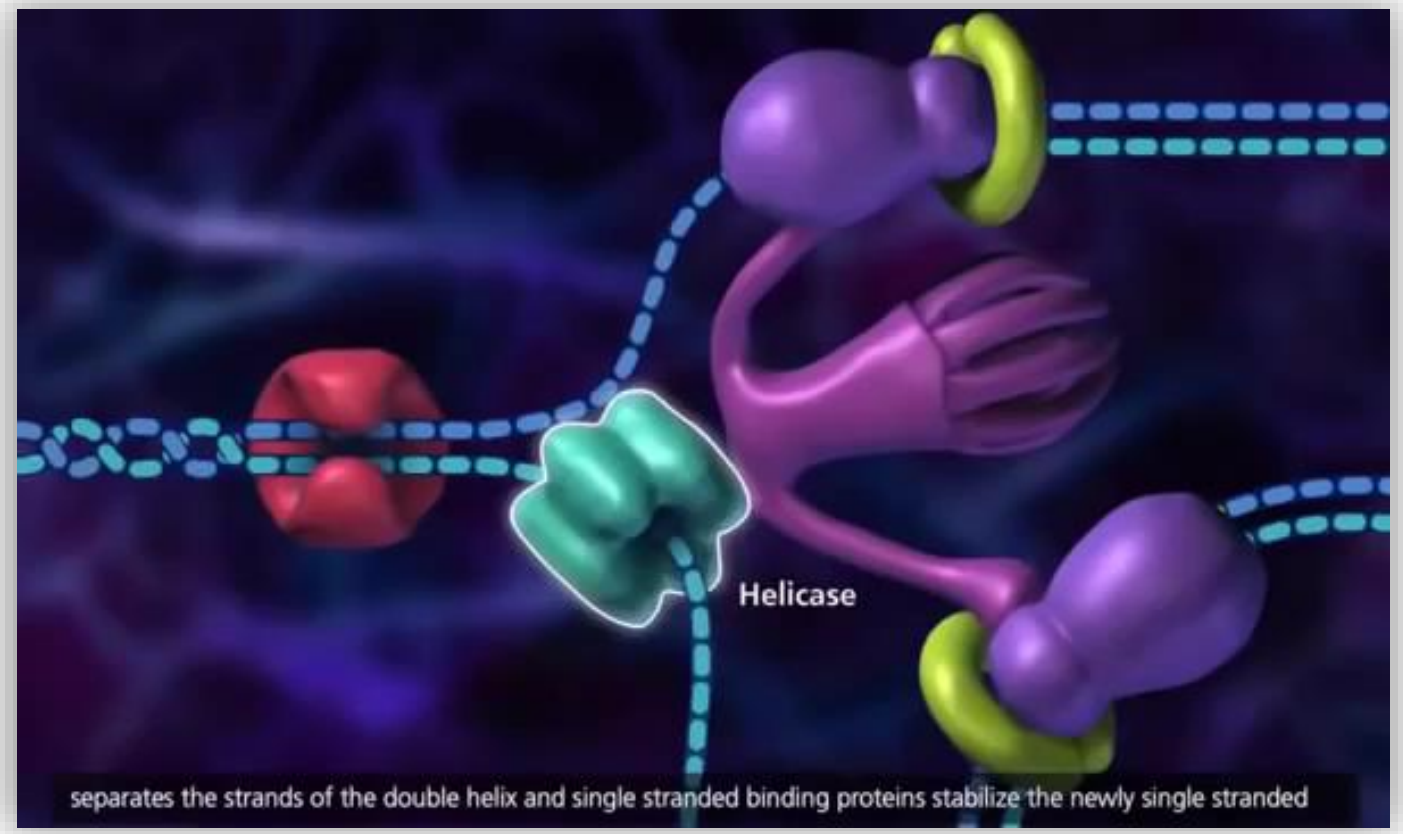
Tetramers

Tetramers, known as the **single stranded binding proteins**, cover the single-stranded DNA



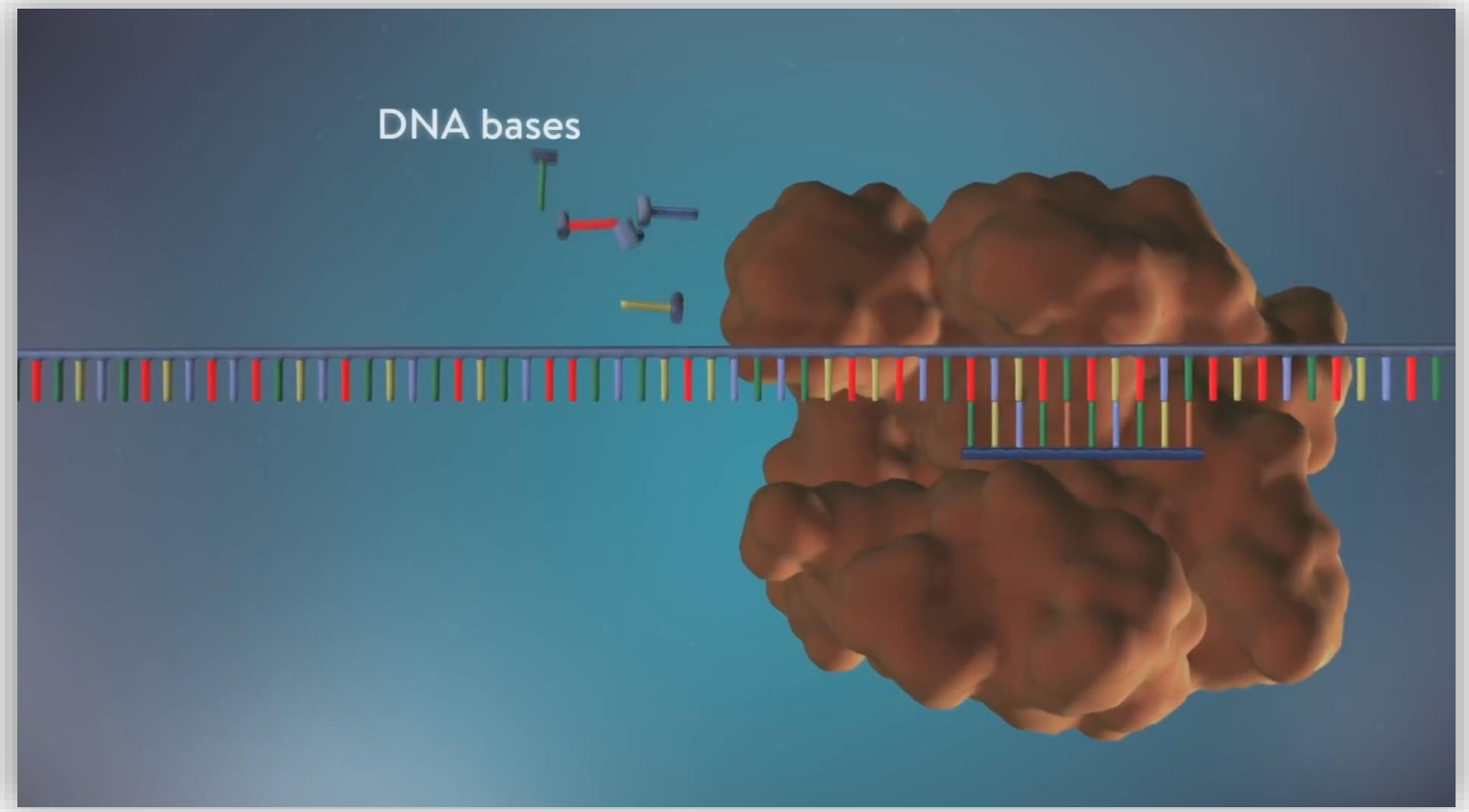
Tetramers

This prevents the DNA strands from re-annealing and forming the double stranded molecule



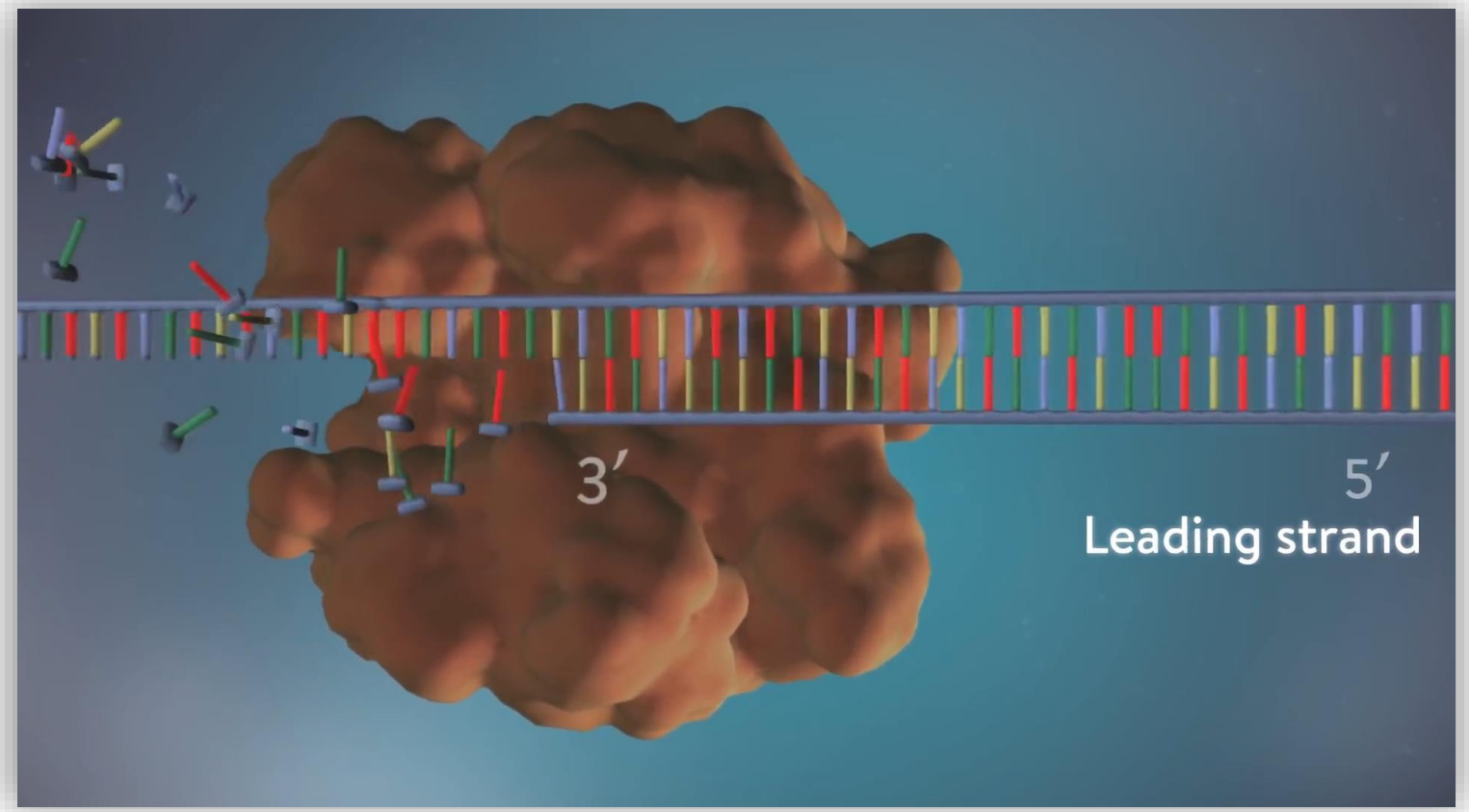
The two single DNA strands

The two single DNA strands act as templates individually, that are used for producing two complementary DNA strands



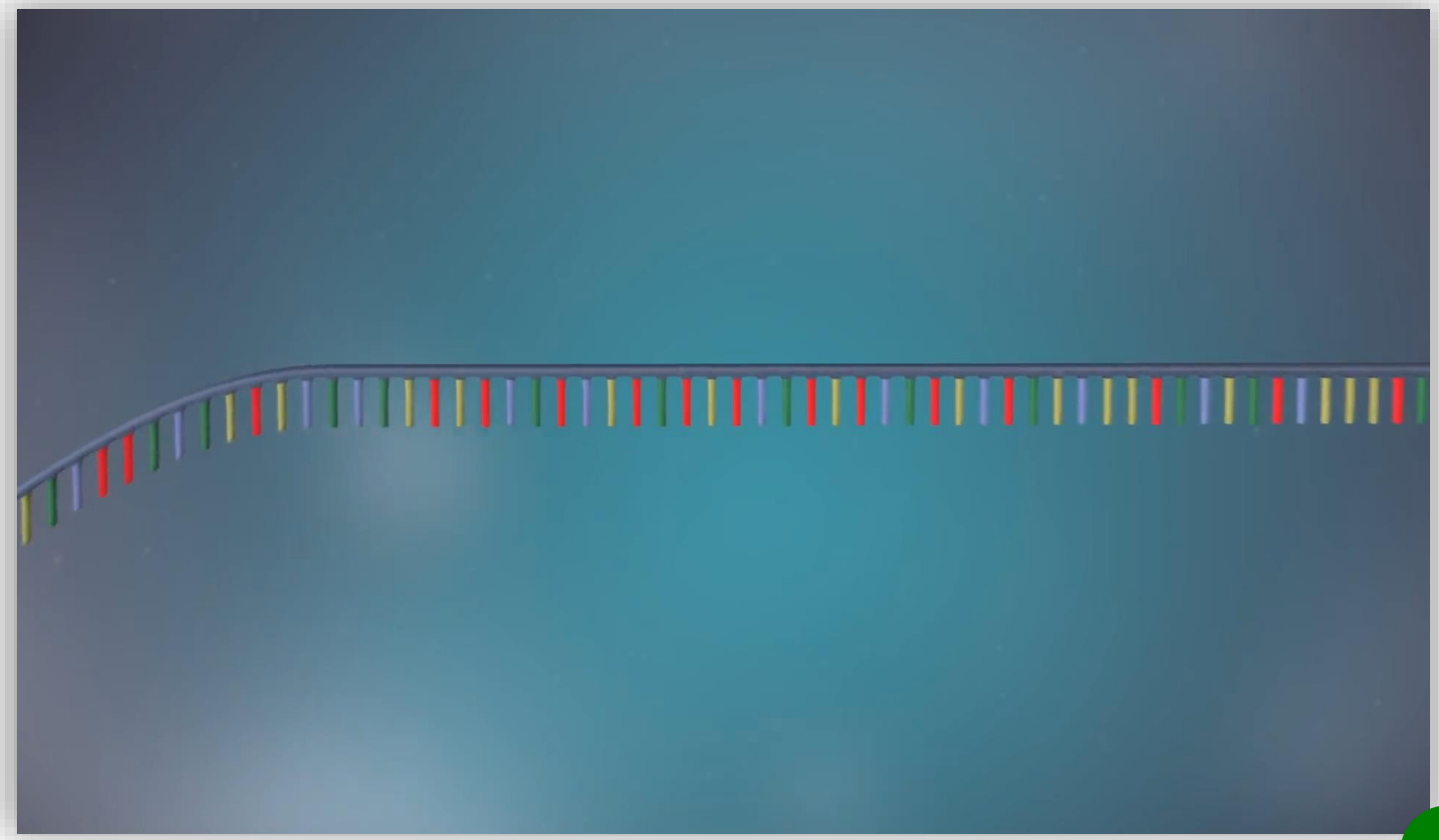
The two single DNA strands

The double helix consists of two anti-parallel DNA strands with complementary 5' to 3' strands



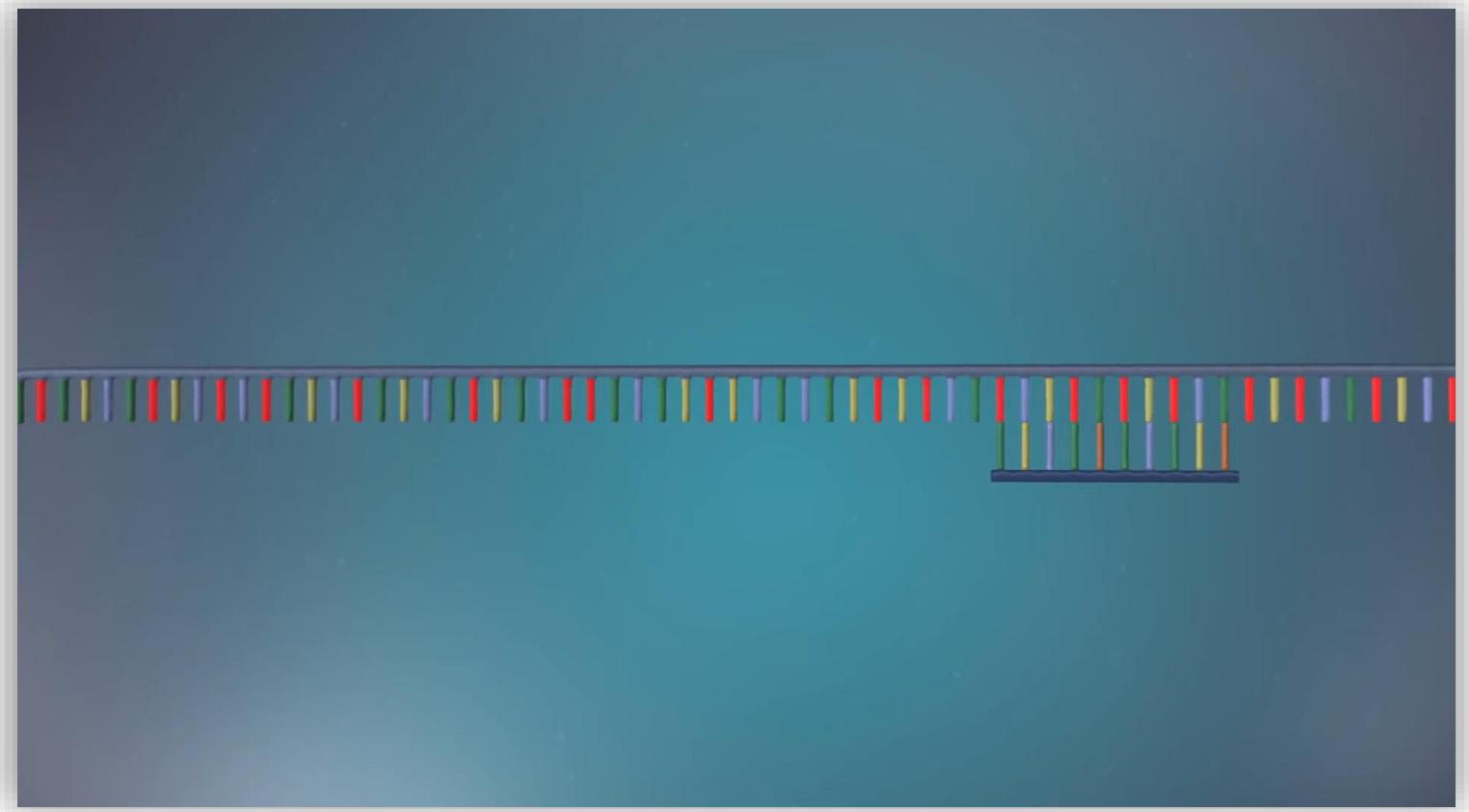
Initiating the Replication Process

An RNA polymerase known as **primase**, synthesizes short **RNA primers** (about 60nt long) that initiate the DNA replication process



Initiating the Replication Process

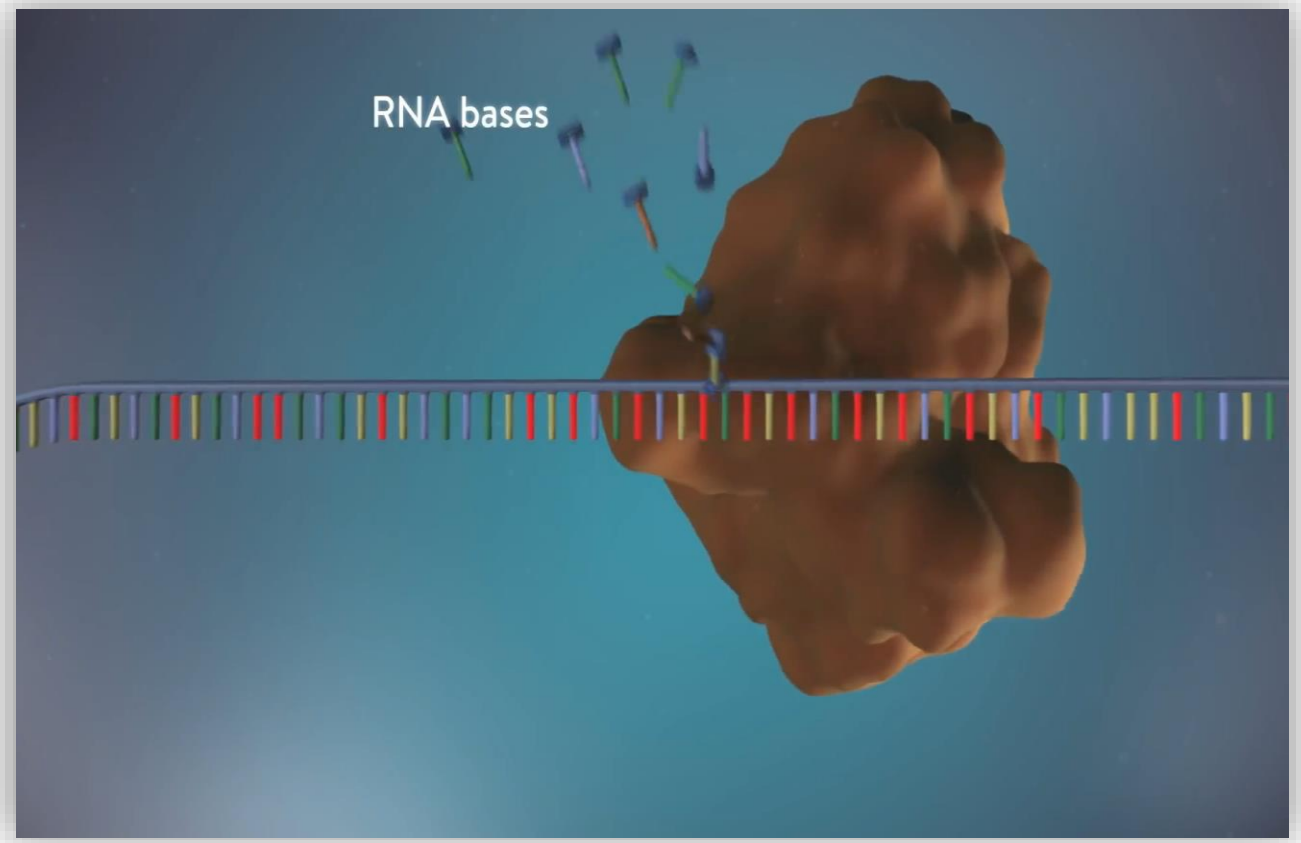
An enzyme called **DNA polymerase** binds to one strand of the DNA and begins moving along it in the 3' to 5' direction



Initiating the Replication Process

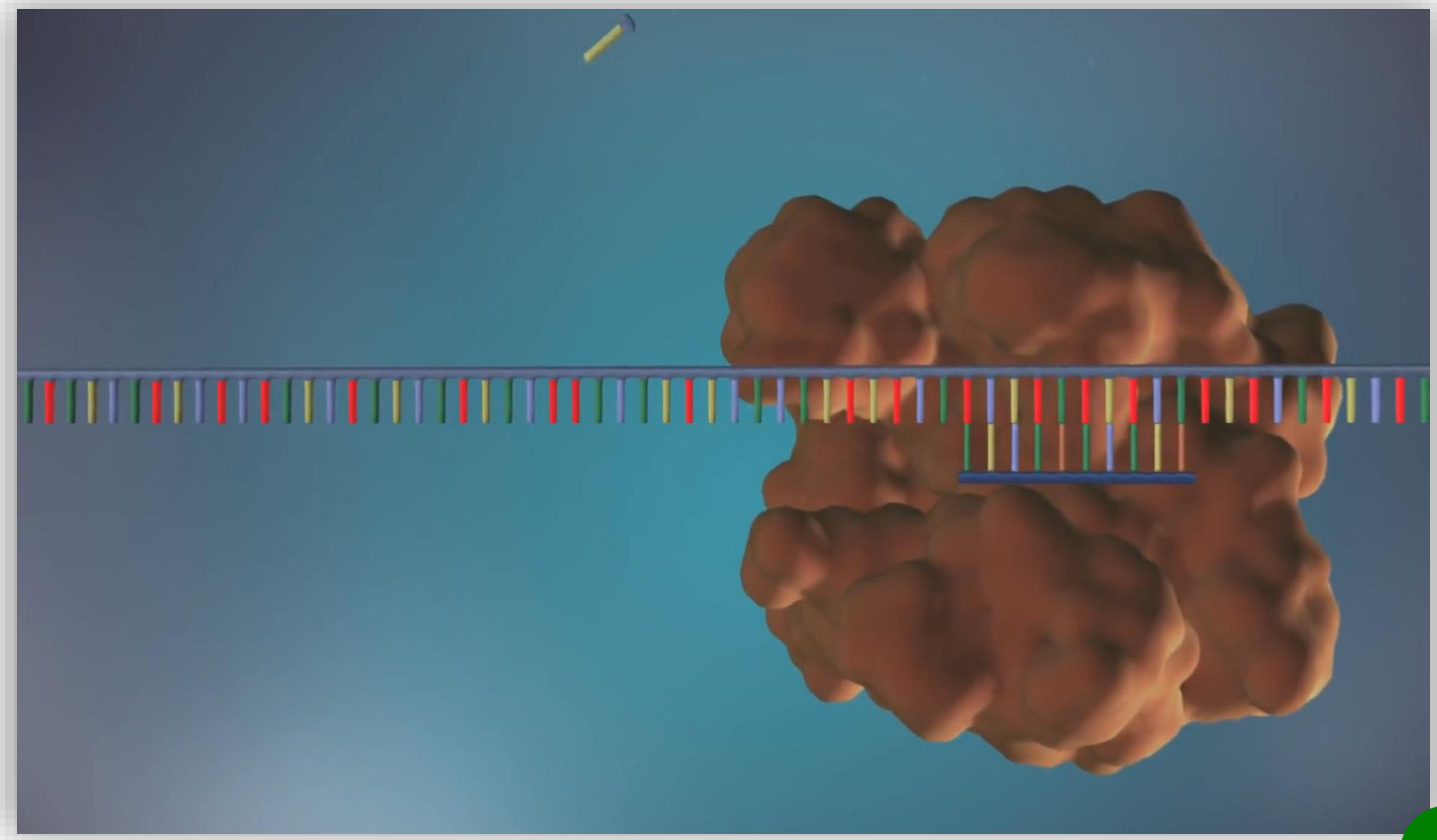
DNA polymerase cannot begin synthesizing the DNA strand initially

It needs a nucleic acid chain in the beginning to begin copying the strand



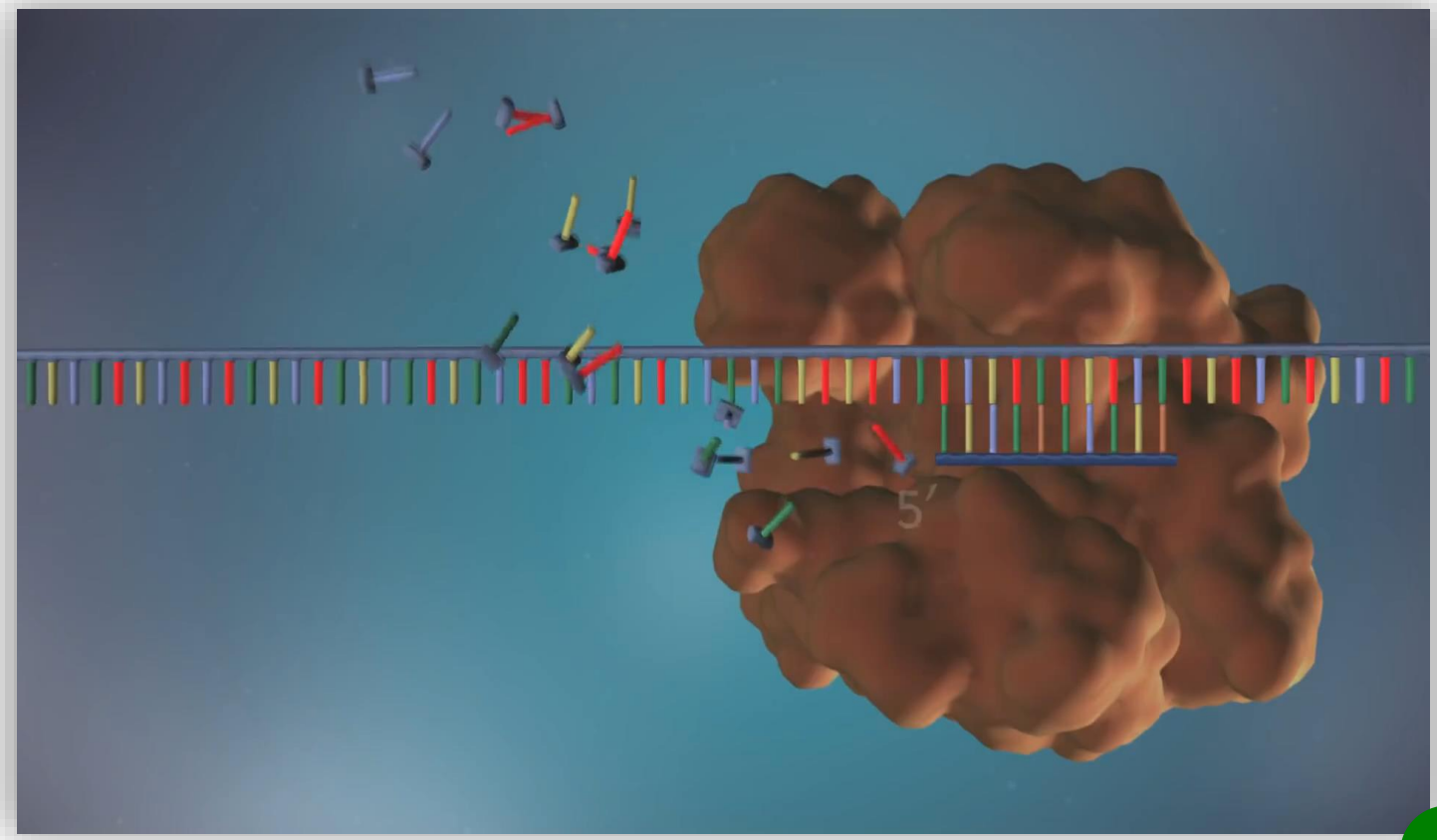
Initiating the Replication Process

The original DNA strand is used as a template to synthesize the DNA strand in the 5' → 3' direction with the help of an extension formed by **RNA primer**



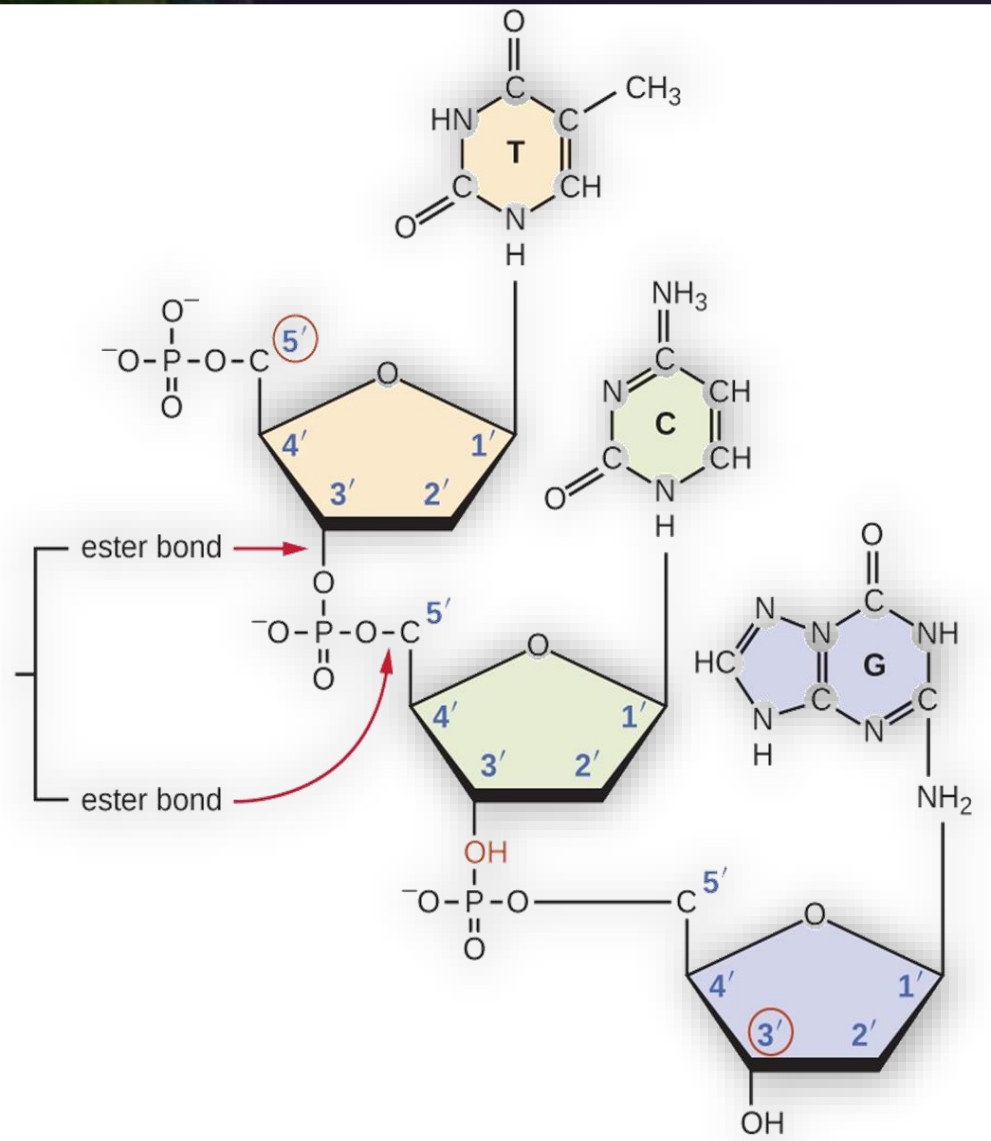
Initiating the Replication Process

DNA polymerase can synthesize the strand in **5' → 3' direction only**



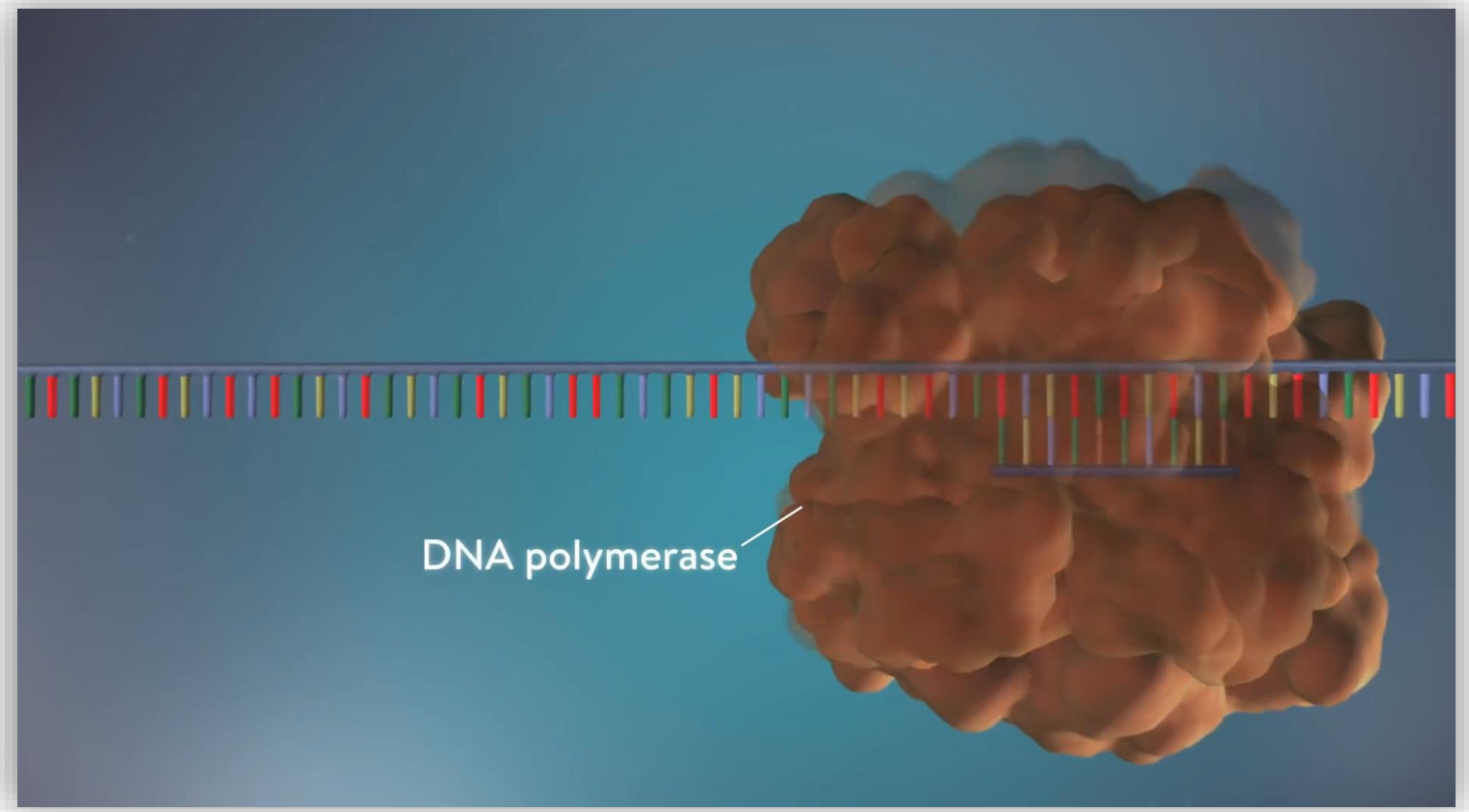
Initiating the Replication Process

It hooks the 5' phosphate group of an incoming nucleotide onto the 3' hydroxyl group at the end of the growing nucleic acid chain



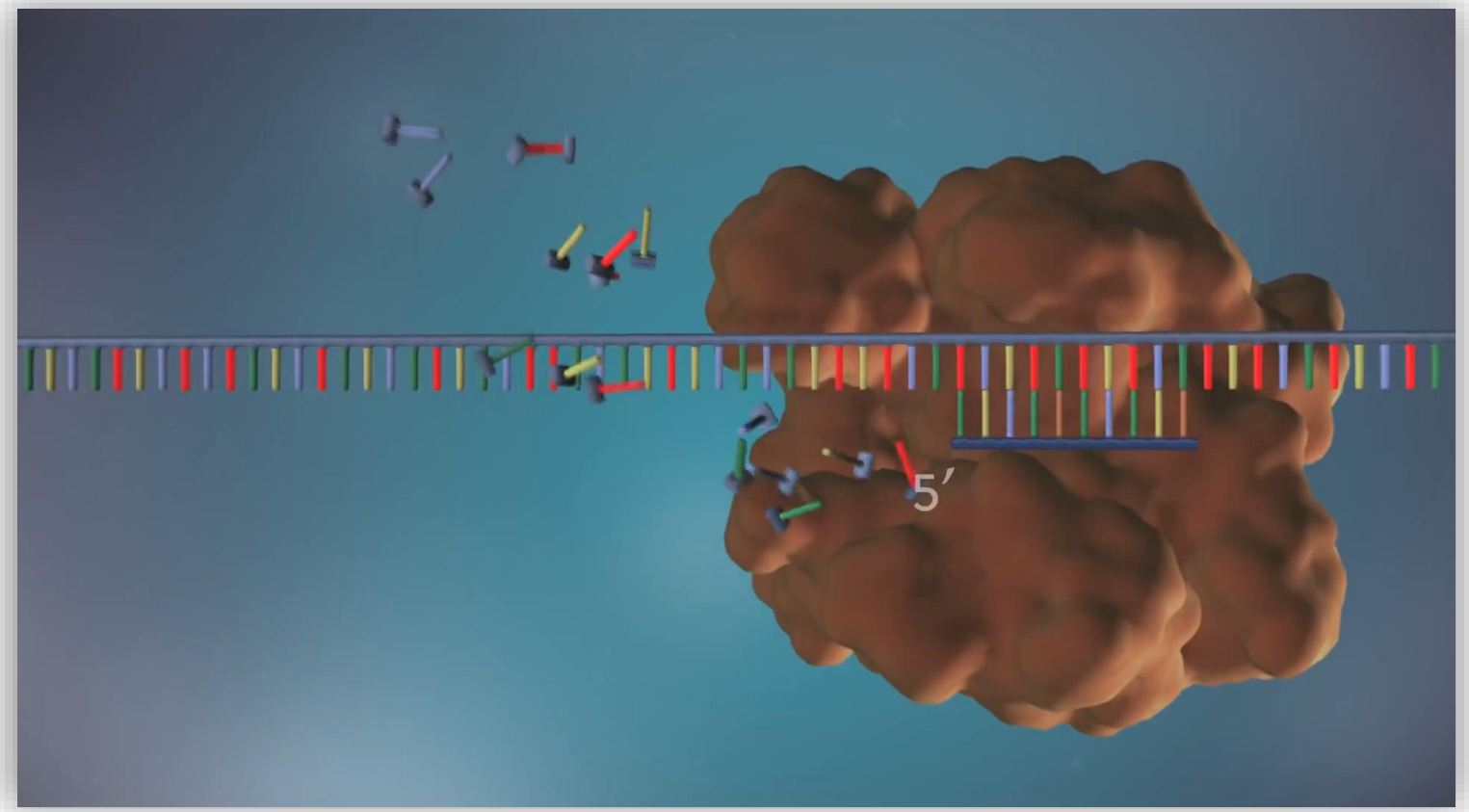
Initiating the Replication Process

In eukaryotes, this molecule is called **DNA polymerase delta (δ)**



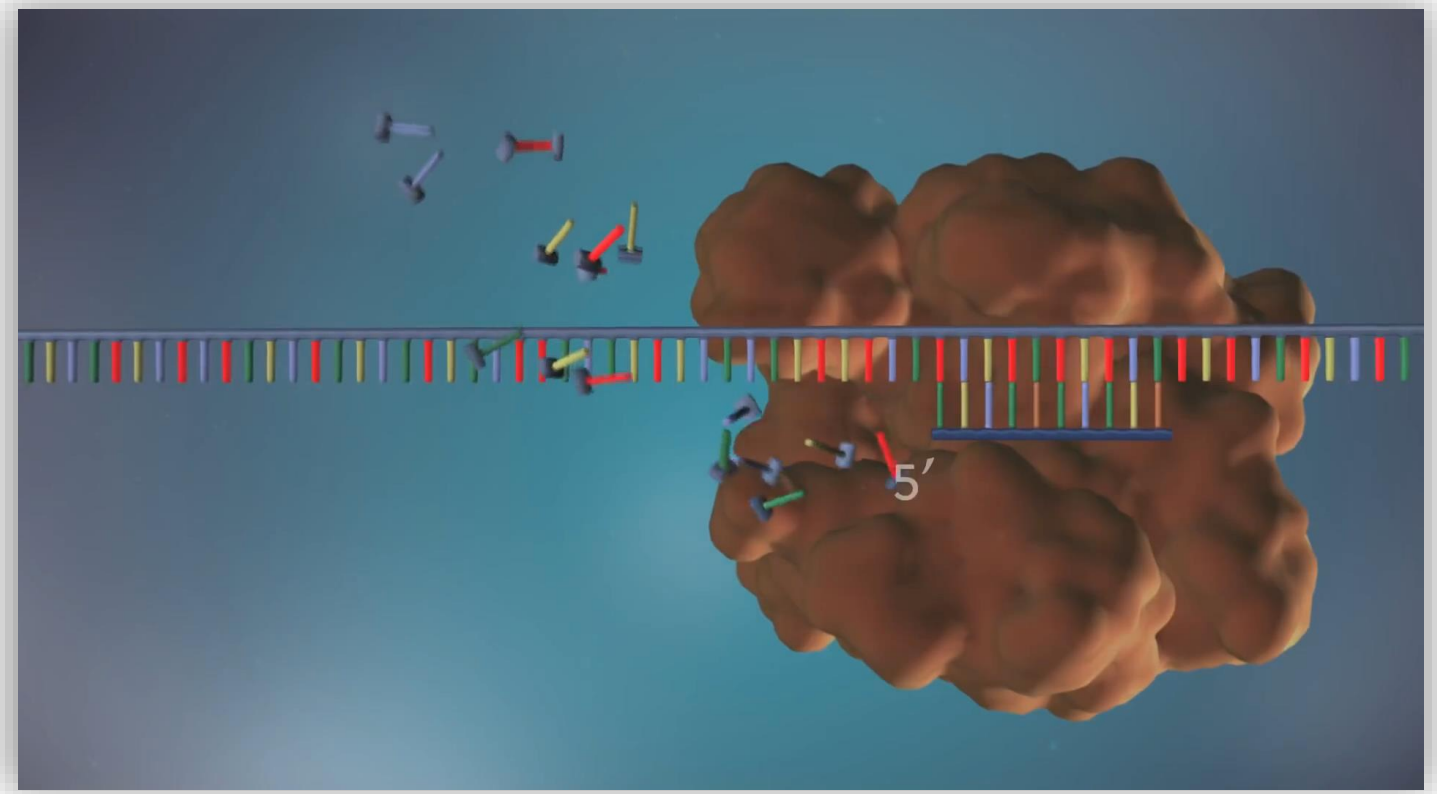
Initiating the Replication Process

The DNA polymerase uses the strand as a template for assembling a **leading strand** of nucleotides and reforming a double helix



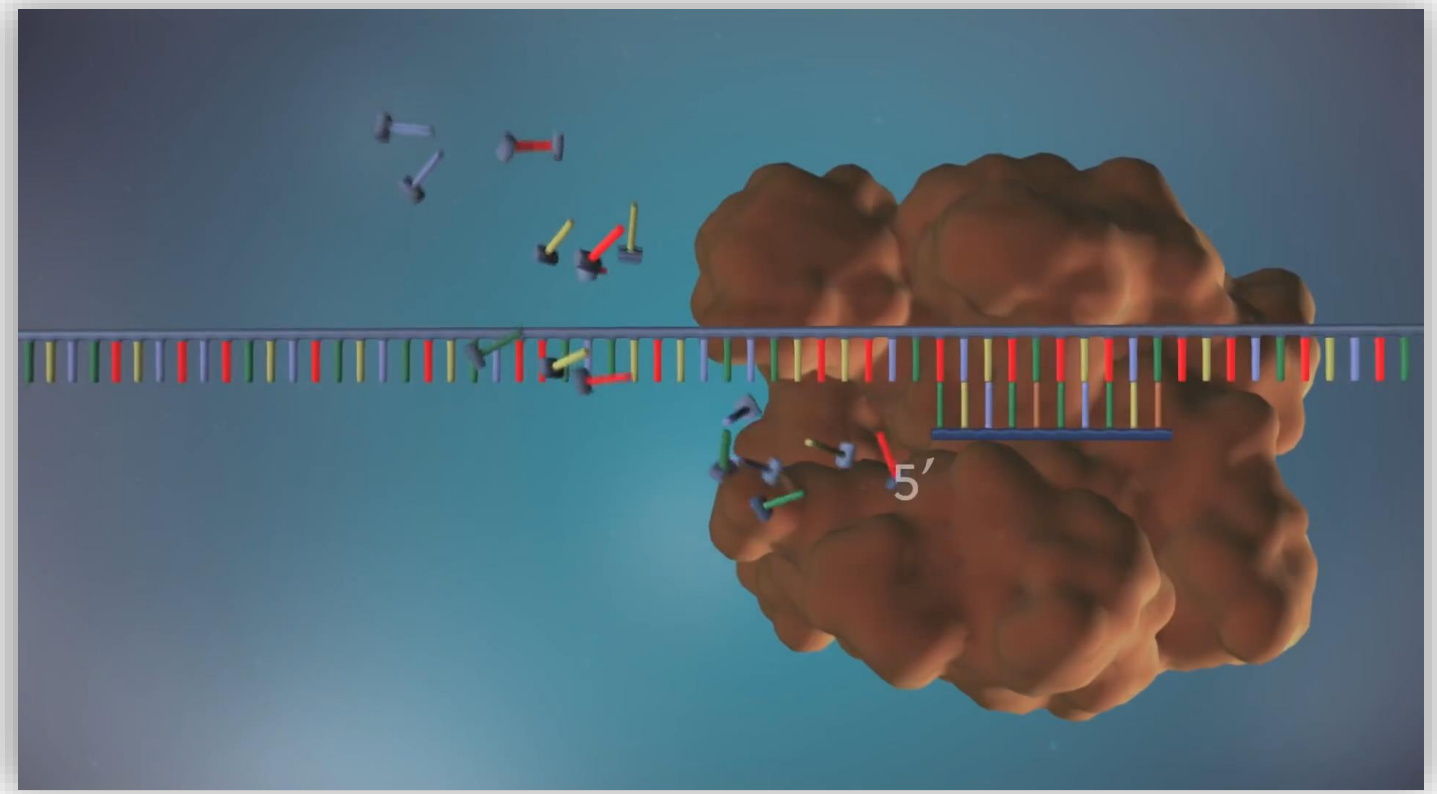
Synthesis of the Leading Strand

The DNA strand that is read in the 3' → 5' and synthesized in the 5' → 3' direction continuously, is known as the **leading strand**



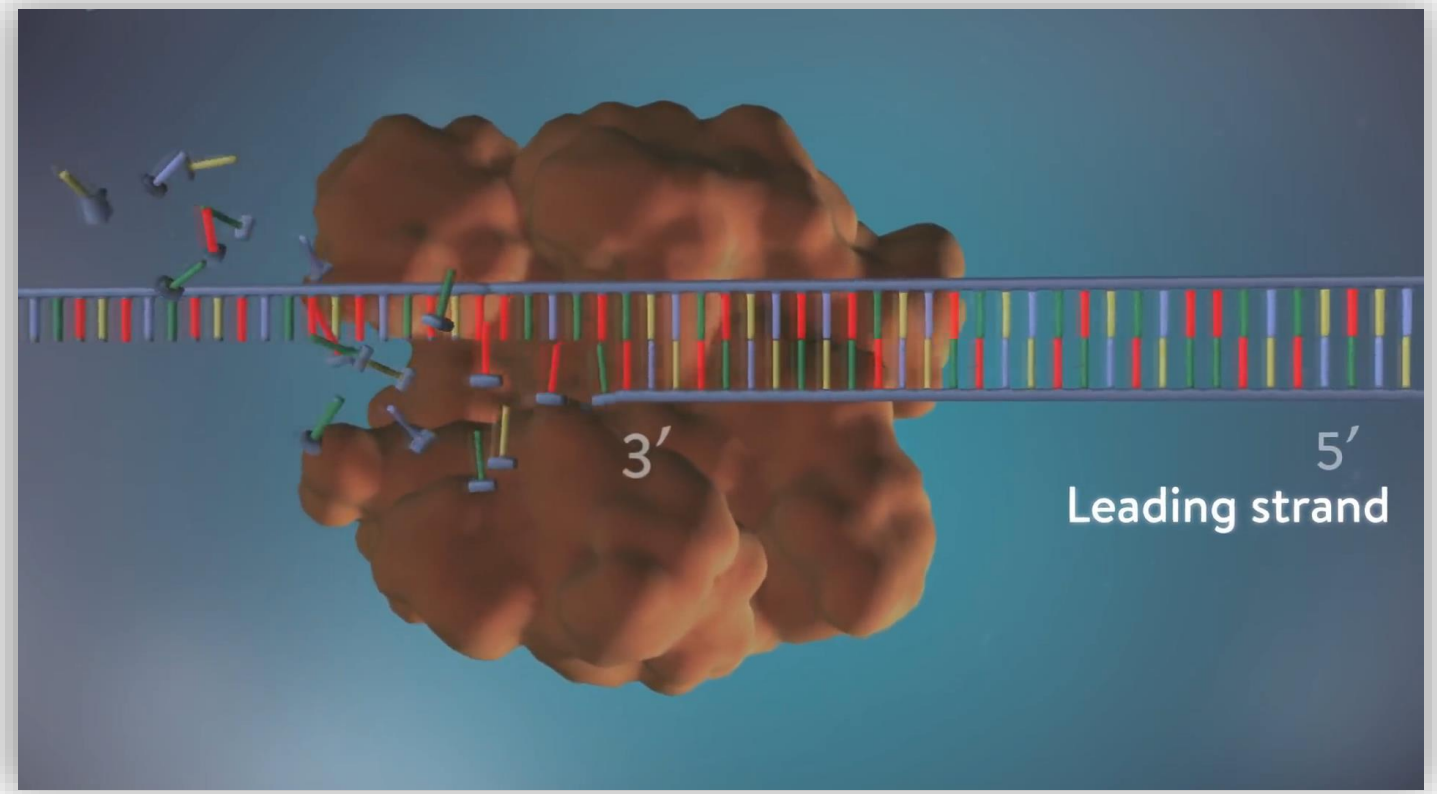
Synthesis of the Leading Strand

DNA polymerase III synthesiSes the DNA using the 3'-OH group, donated by the single RNA primer



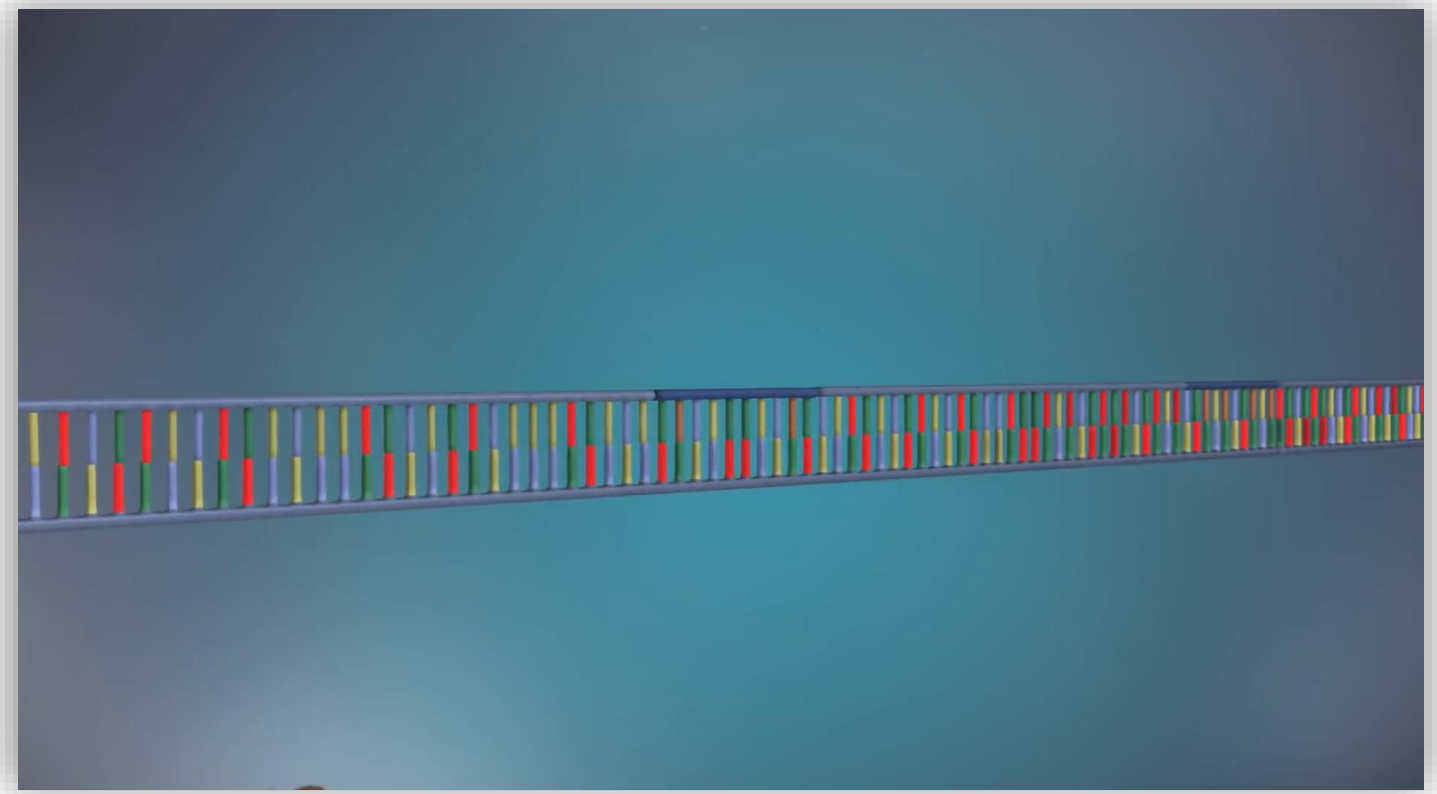
Synthesis of the Leading Strand

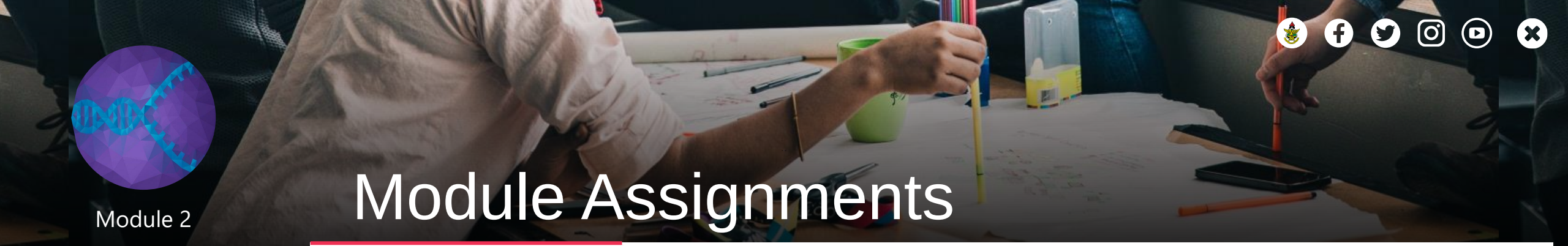
The DNA replication continues in the direction of the replication fork, in a continuous manner



Synthesis of the Leading Strand

RNAse H and DNA polymerase I (exonuclease) recognizes the RNA polymers that are bound to the DNA template and removes the primers by RNA hydrolysis





Module 2

DNA Replication

Module Assignments

Assignment 1

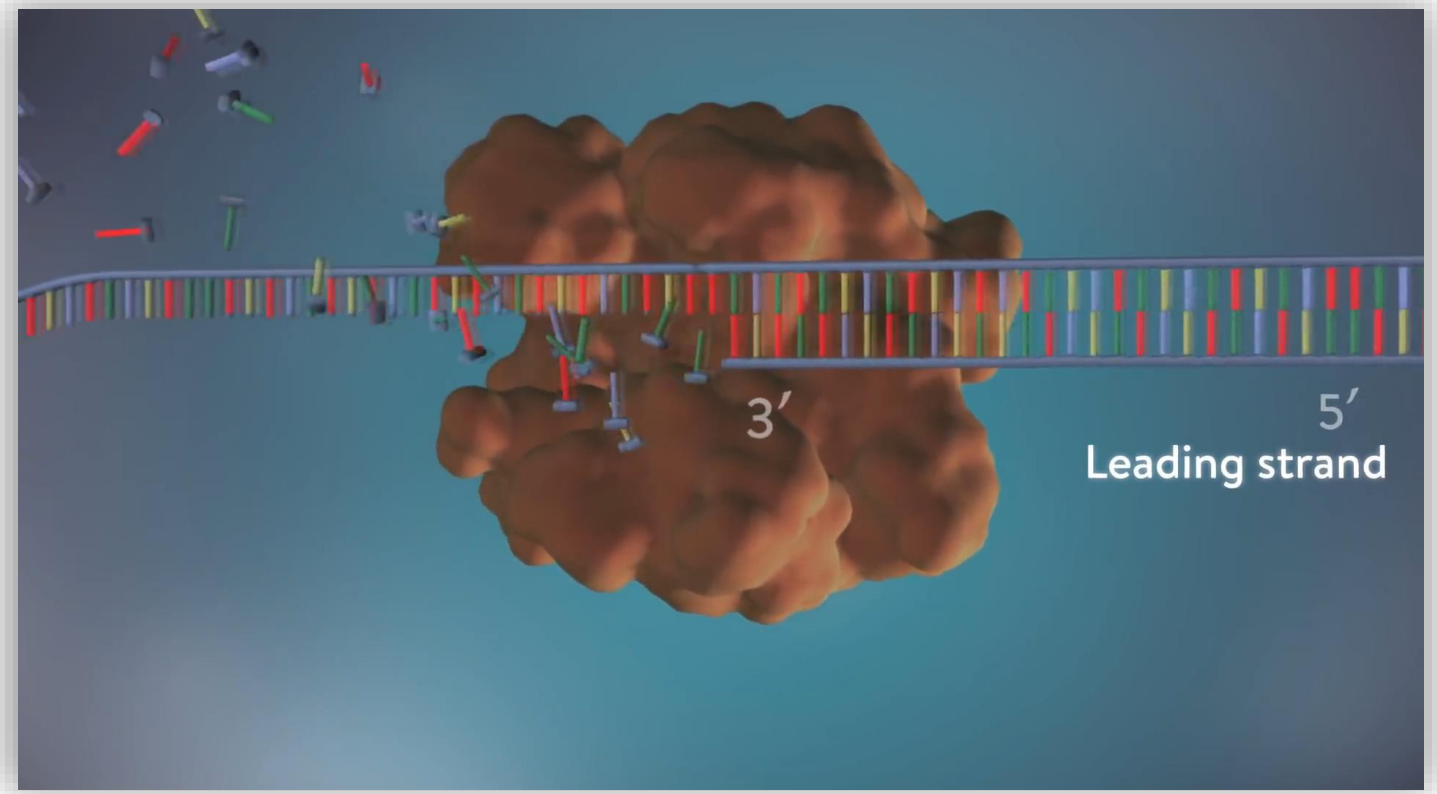
Read on

DNA POL I, POL II and POL III

Dashboard
Introduction
Lessons
Assignments

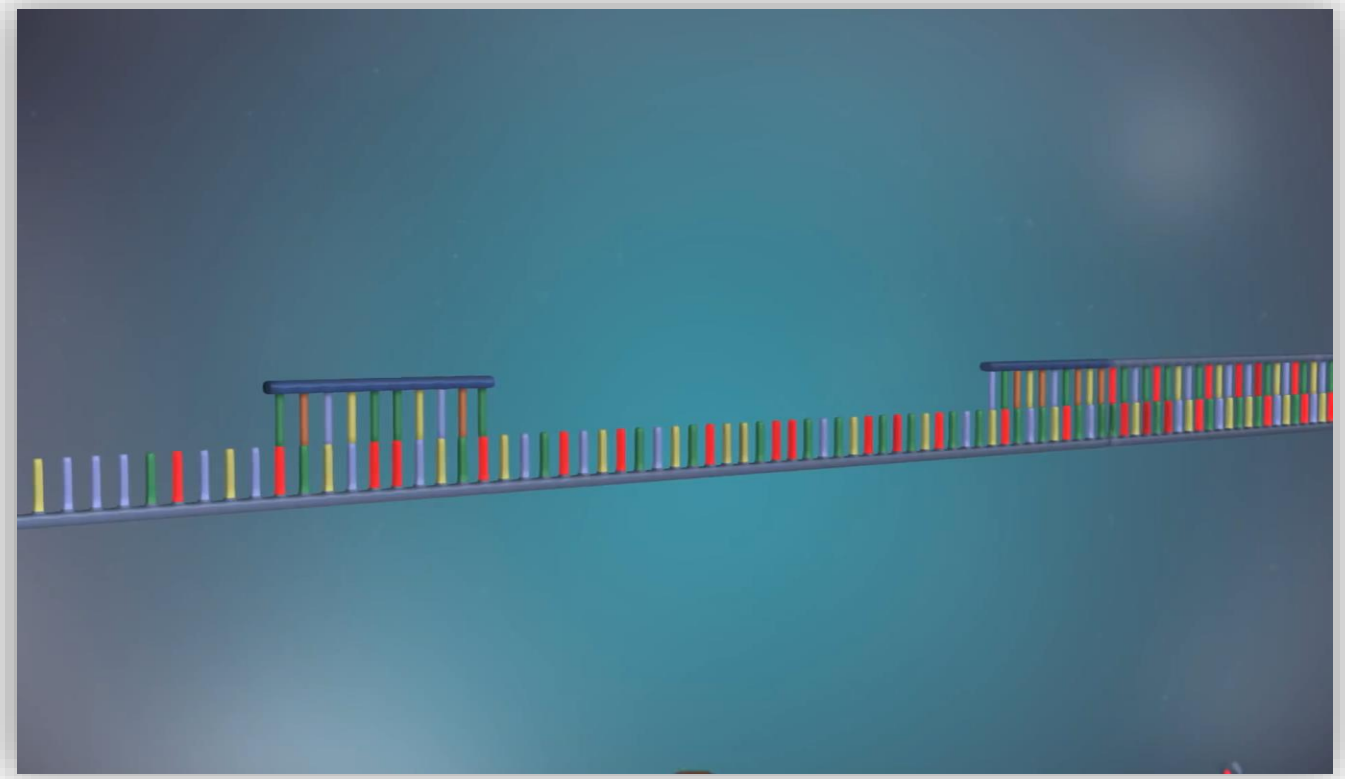
Synthesis of the Lagging Strand

The lagging strand is the DNA strand of the replication fork, that is opposite to the leading strand



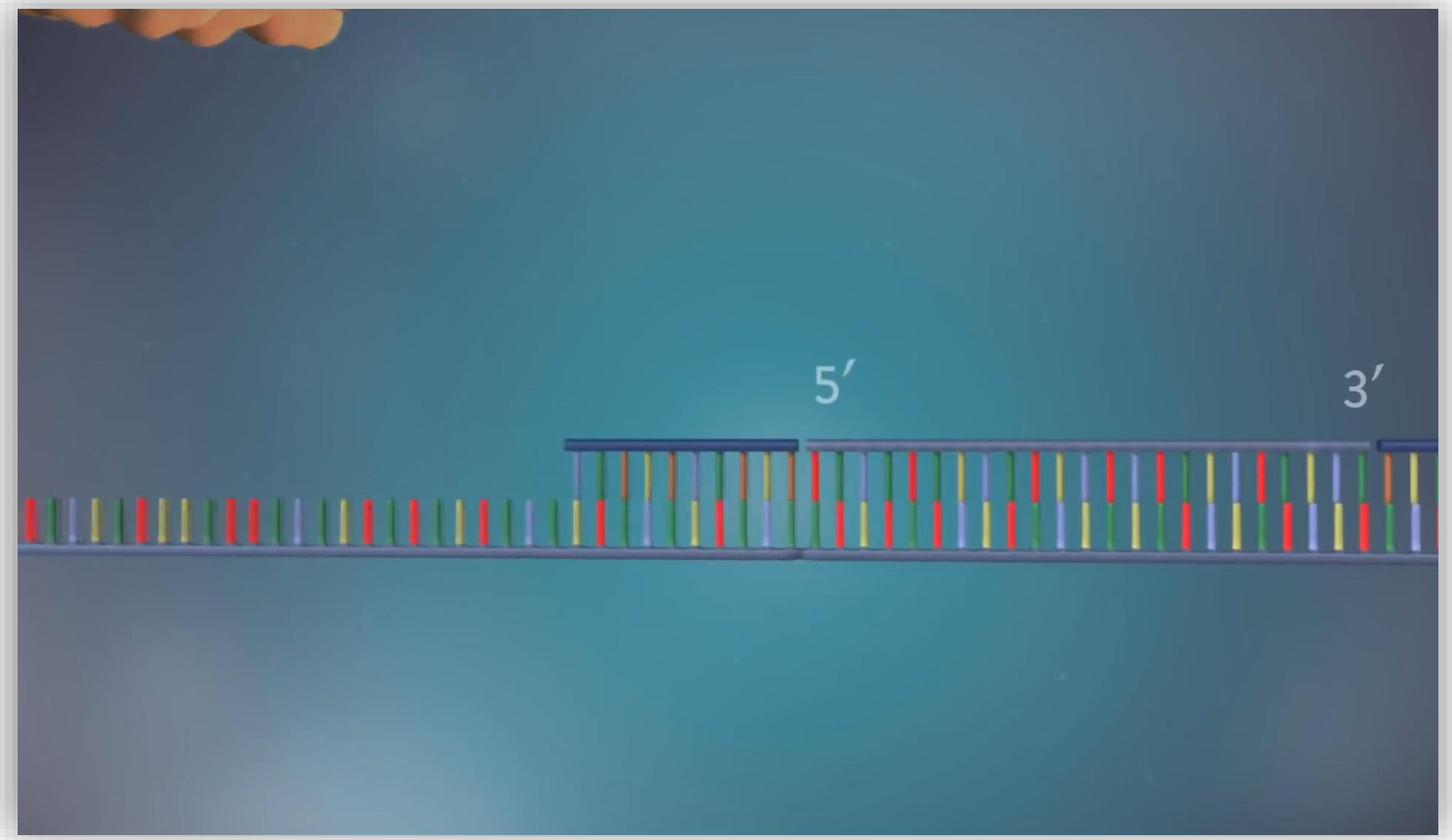
Synthesis of the Lagging Strand

Because DNA synthesis can only occur in 5' to 3' direction, a molecule of a second type of DNA polymerase (**epsilon, ε**, in eukaryotes) binds to the other template strand as the double helix opens



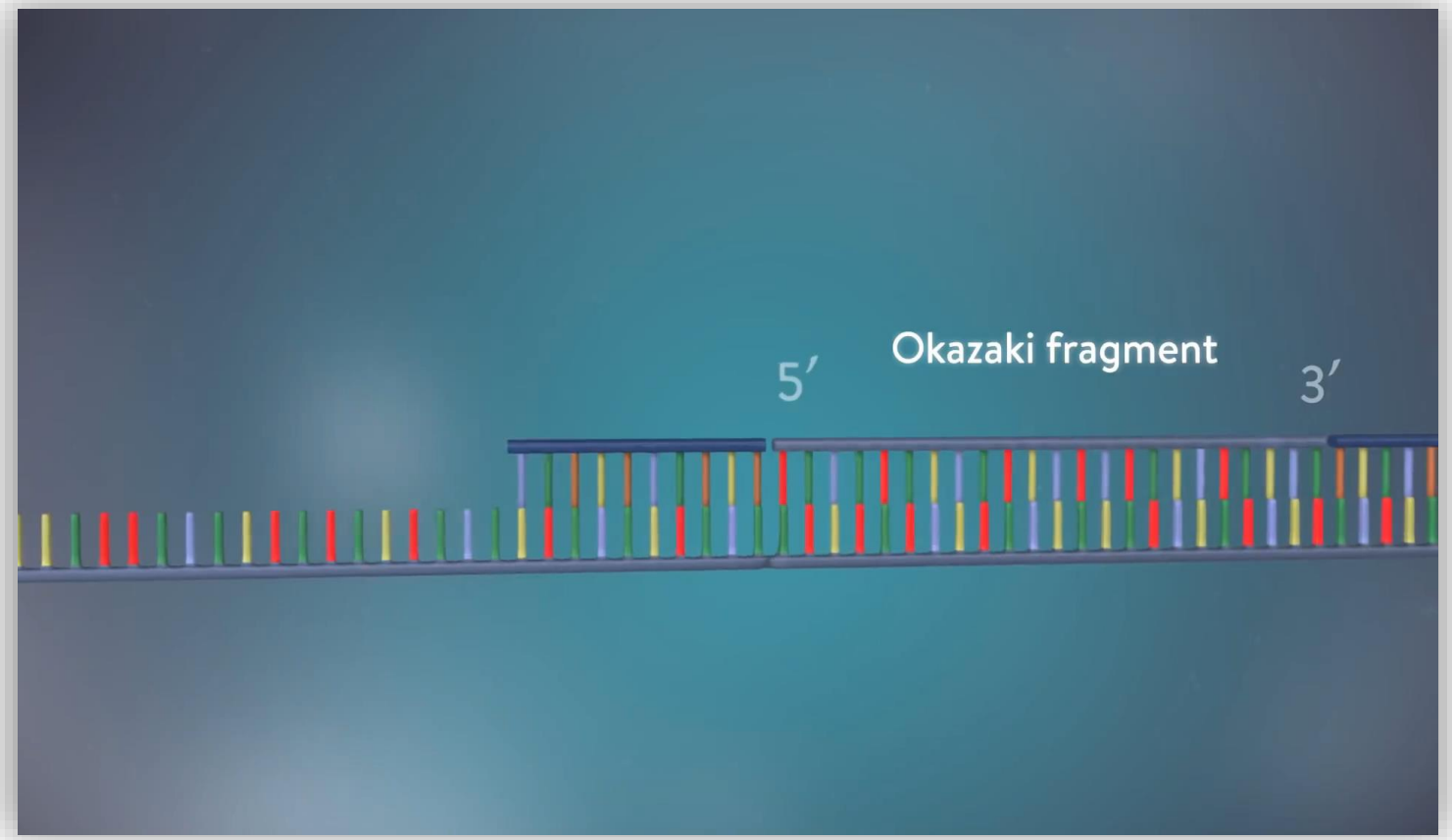
Synthesis of the Lagging Strand

This molecule synthesize discontinuous segments of polynucleotides (called **Okazaki fragments**)



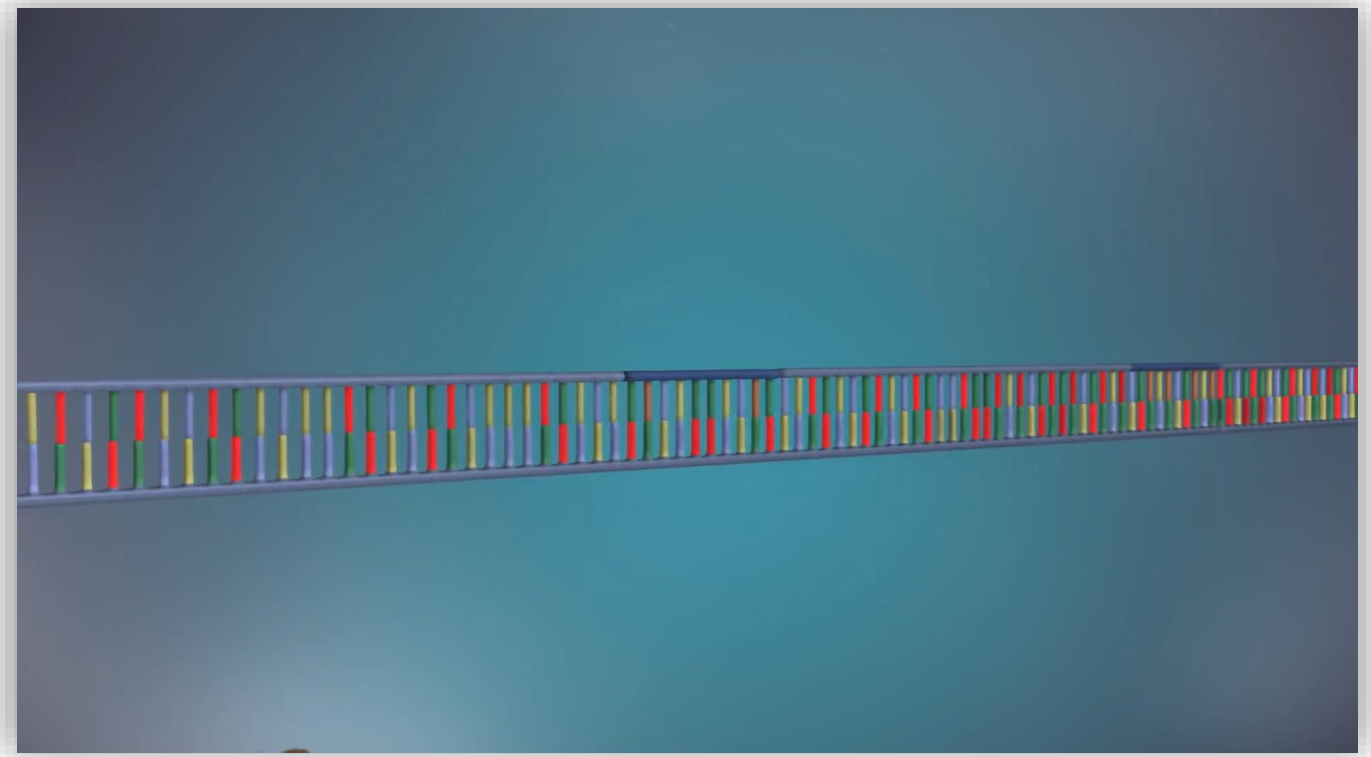
Synthesis of the Lagging Strand

Primase builds RNA primers in short bursts over the lagging strand, which is synthesized in the 5' → 3' by DNA polymerase



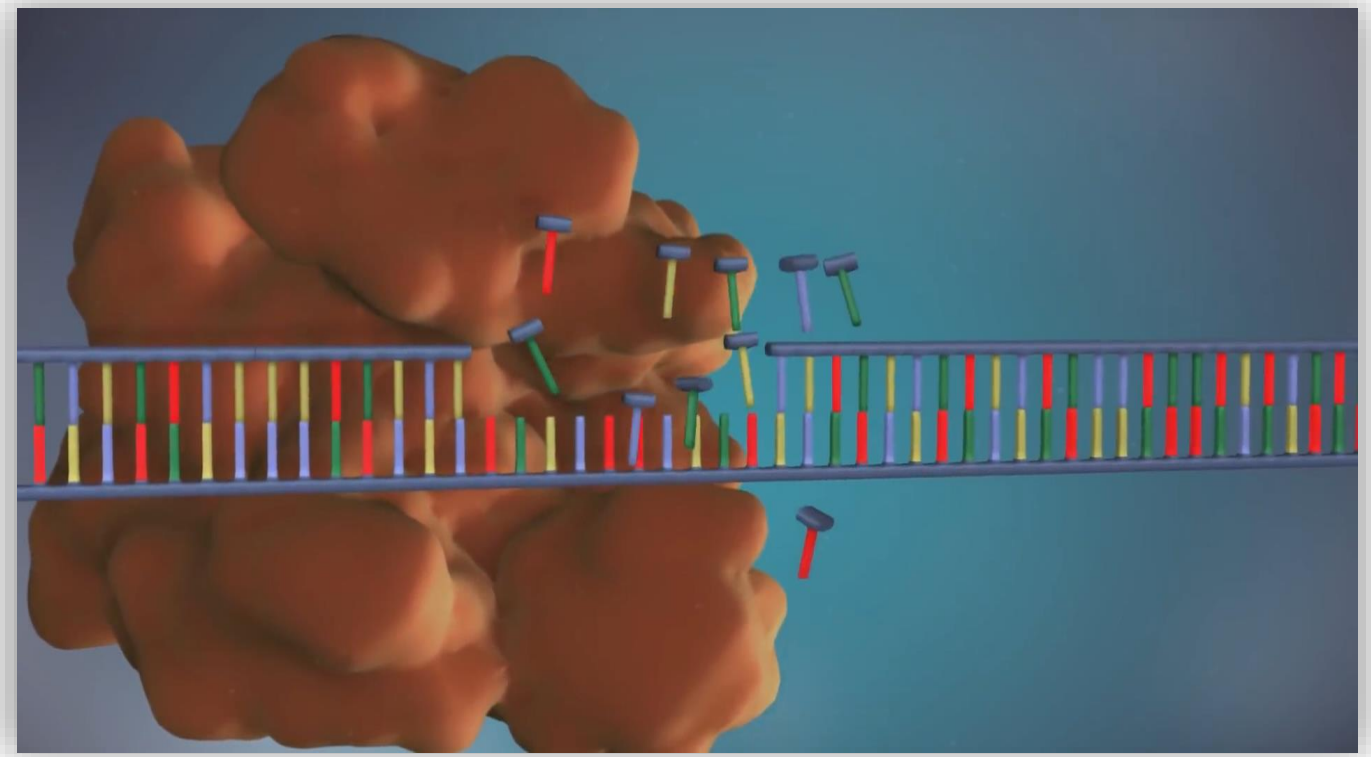
Synthesis of the Lagging Strand

The RNA primers are then removed and new deoxyribonucleotides are added to the gaps, where the RNA was present



Synthesis of the Lagging Strand

DNA Polymerase continues with the synthesis of the new DNA strand

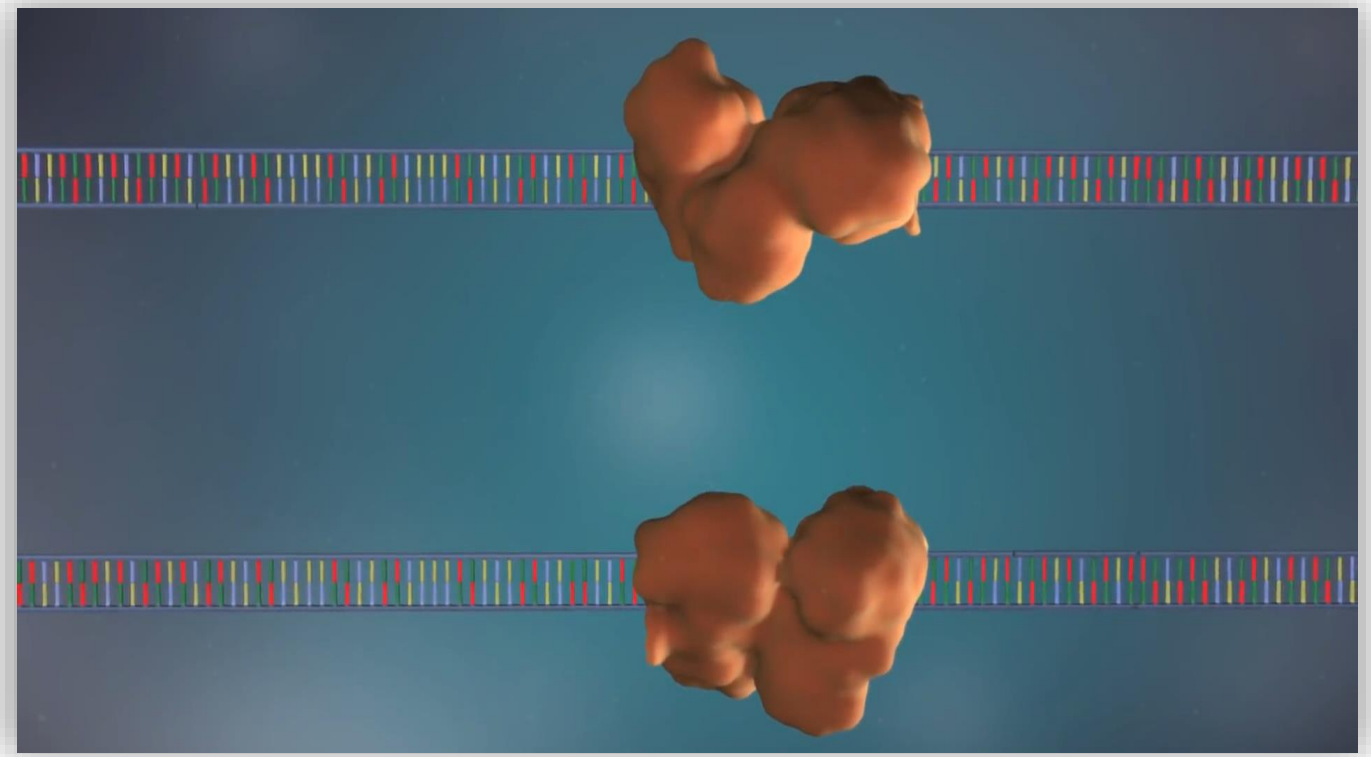


Synthesis of the Lagging Strand

Finally, DNA ligase (an enzyme) joins the deoxyribonucleotides together, thus completing the lagging strand

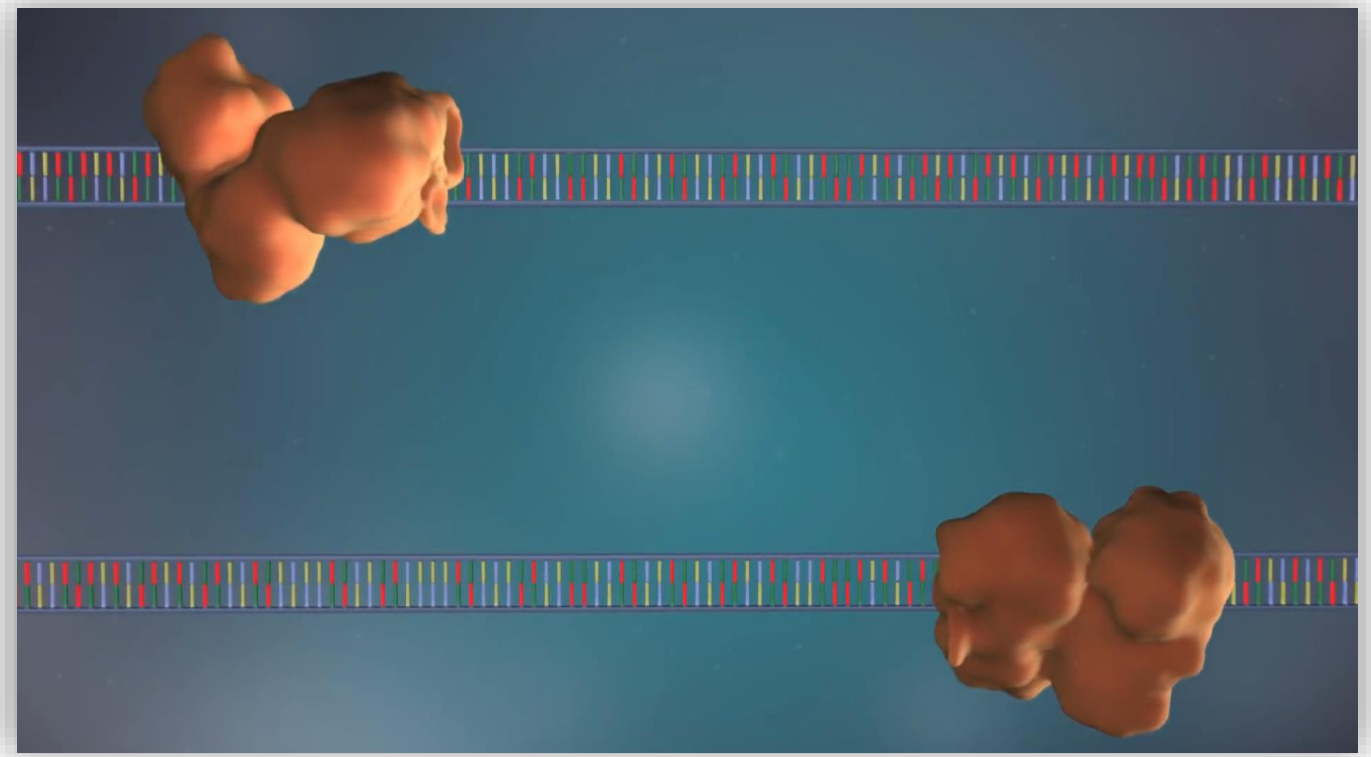
Termination of Replication

In eukaryotes, termination of replication is poorly understood



Termination of Replication

Eukaryotes have linear DNA, and therefore use telomeres, which are GT rich repeating units that 'protect' the end of the DNA

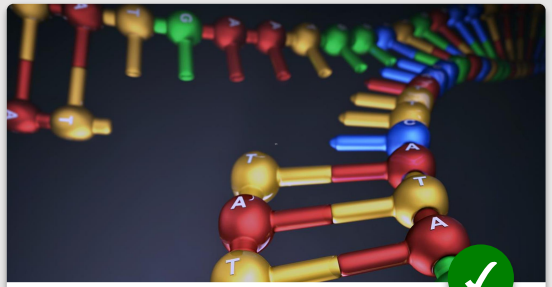


Module 2

Module Lessons

DNA Replication

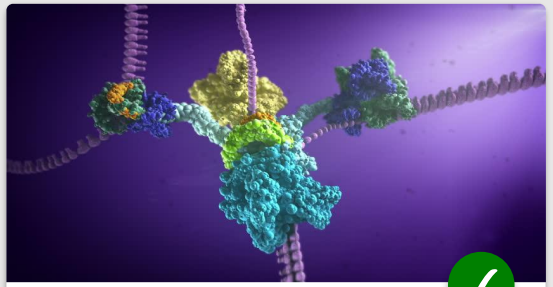
- Dashboard
- Introduction
- Lessons
- Assignments



Lesson 1

Method of Replication

Start ▸



Lesson 2

Mechanism of Replication

Start ▸

Lesson 3

Rate of Replication

Start ▸

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Lesson 3

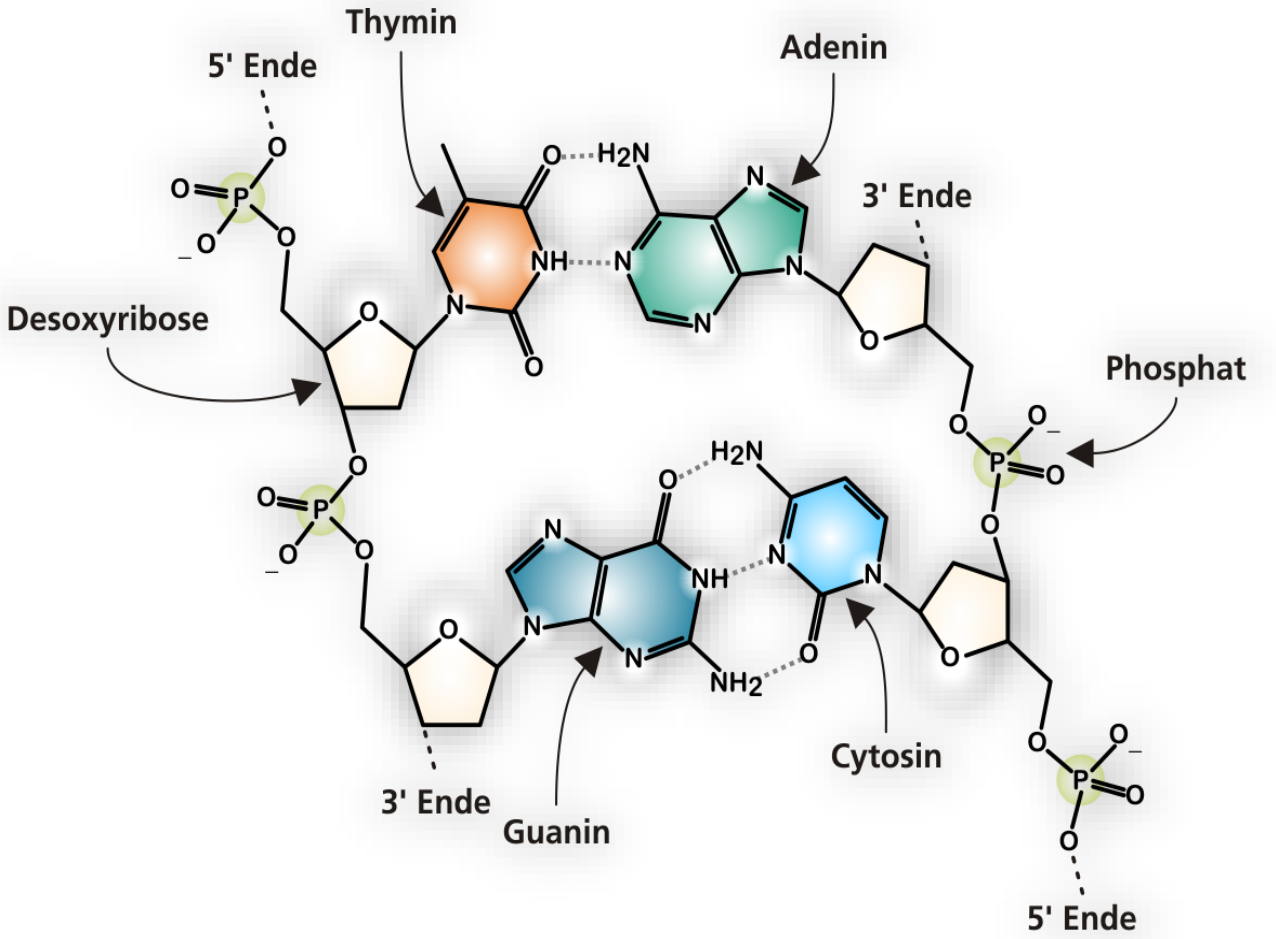
Rate of Replication

Find out how long it takes for DNA to replicate

Learn Now ▶

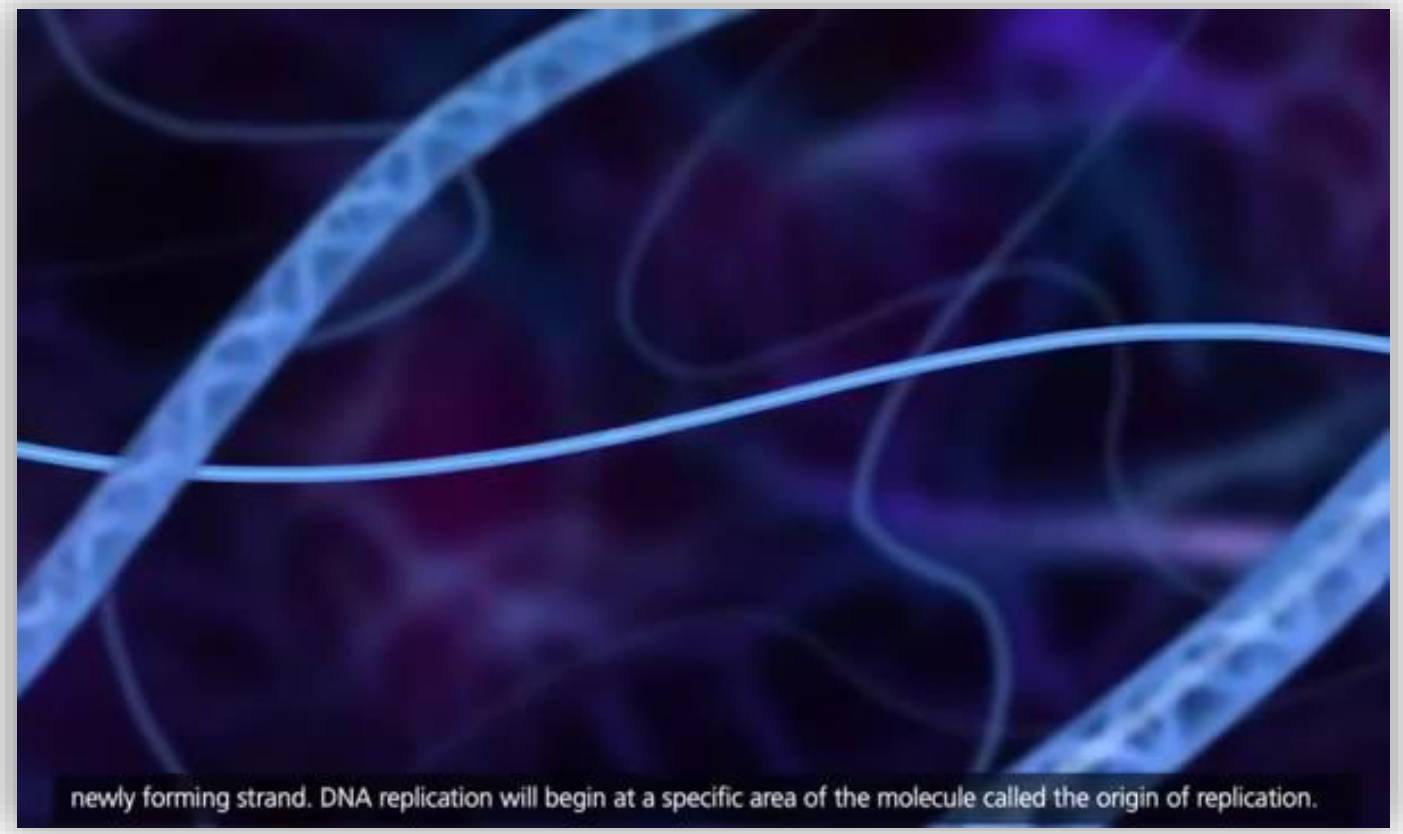
Rate of Replication

The single molecule of DNA, that is the *E. coli* genome contains 4.7 x 10^6 nucleotide pairs



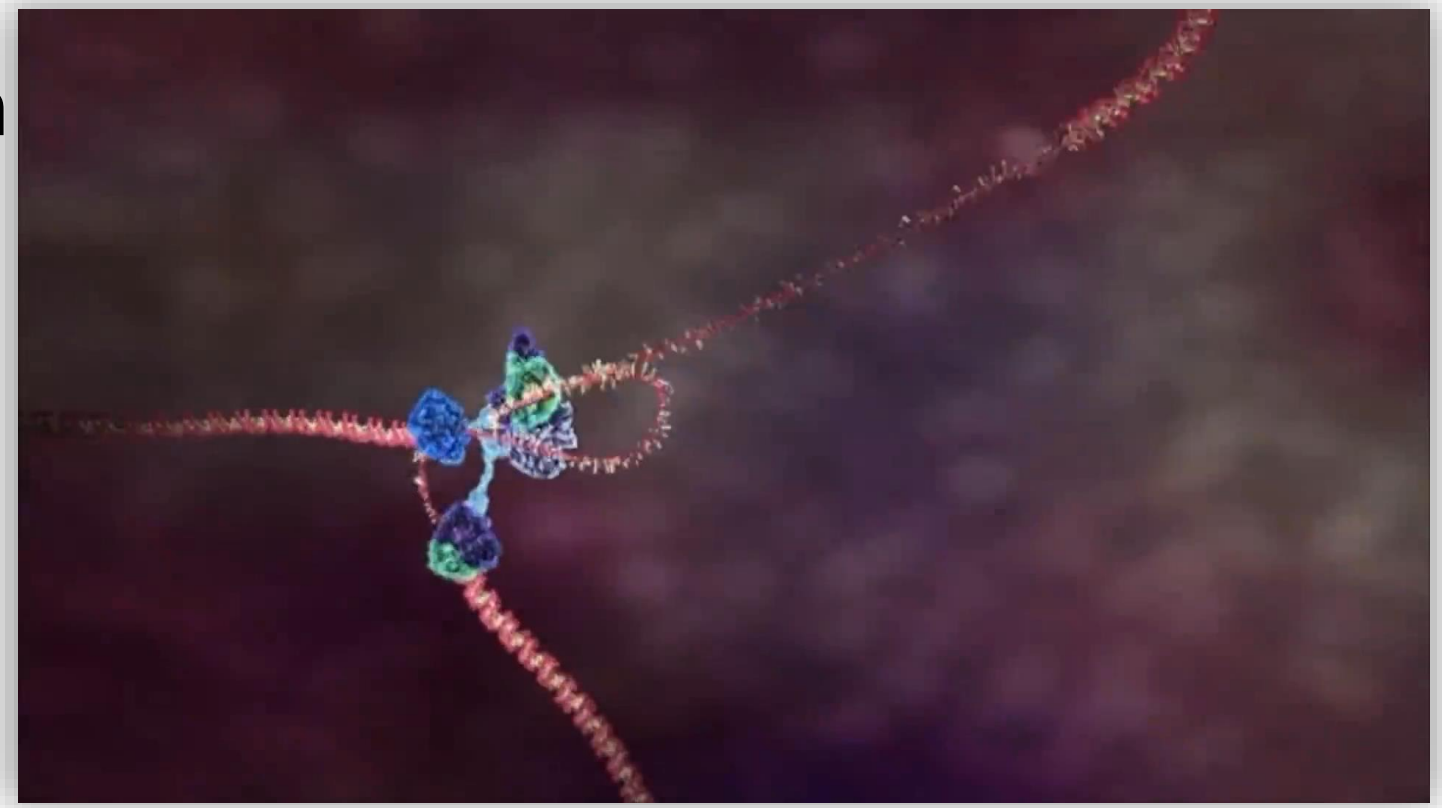
Rate of Replication

DNA replication begins at a single, fixed location in this molecule, called the **replication origin**, proceeds at about 1000 nucleotides per second, and thus is done in no more than 40 minutes



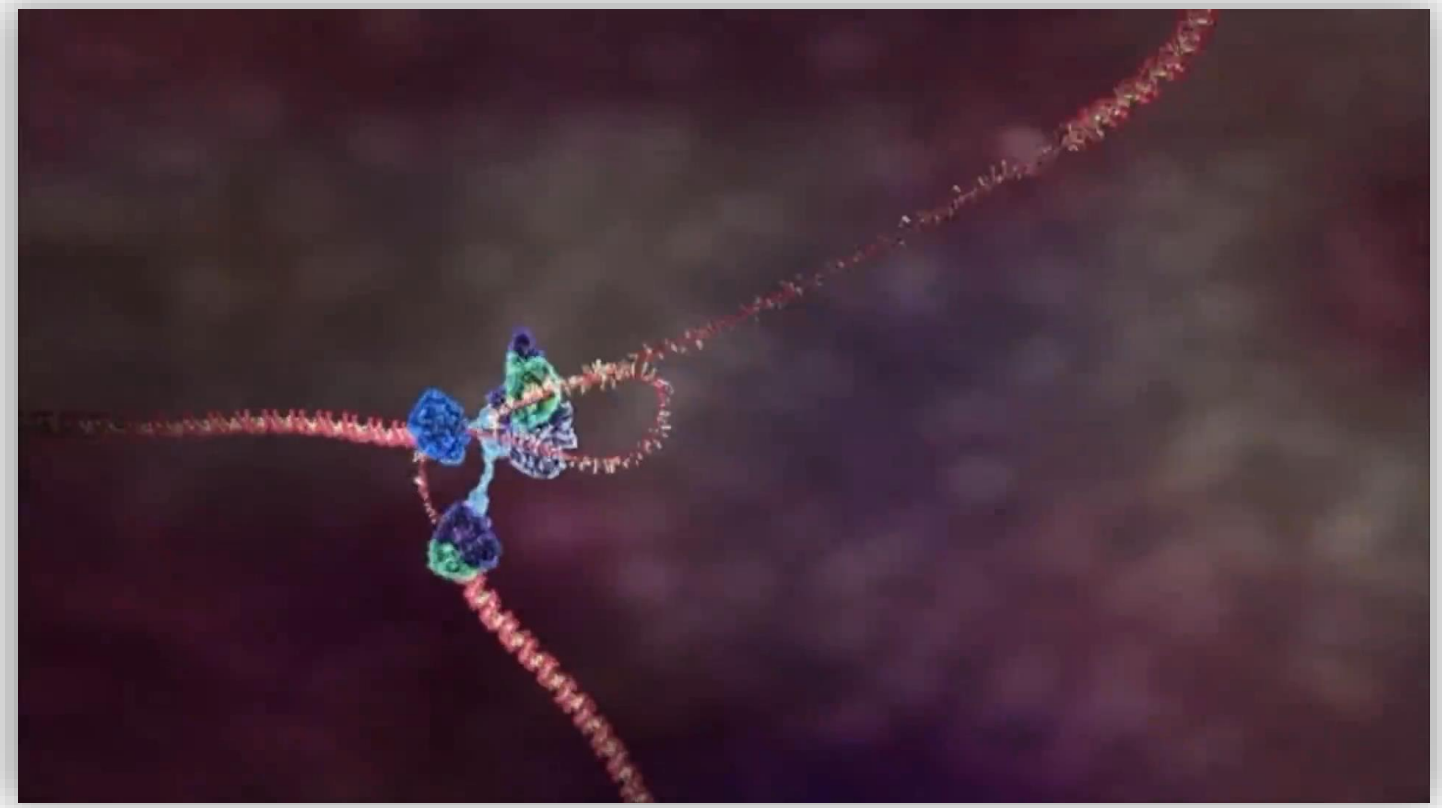
Rate of Replication

Due to the precision of the process (which includes a "proof-reading" function), the job is done with only about one incorrect nucleotide for every 10^9 nucleotides inserted



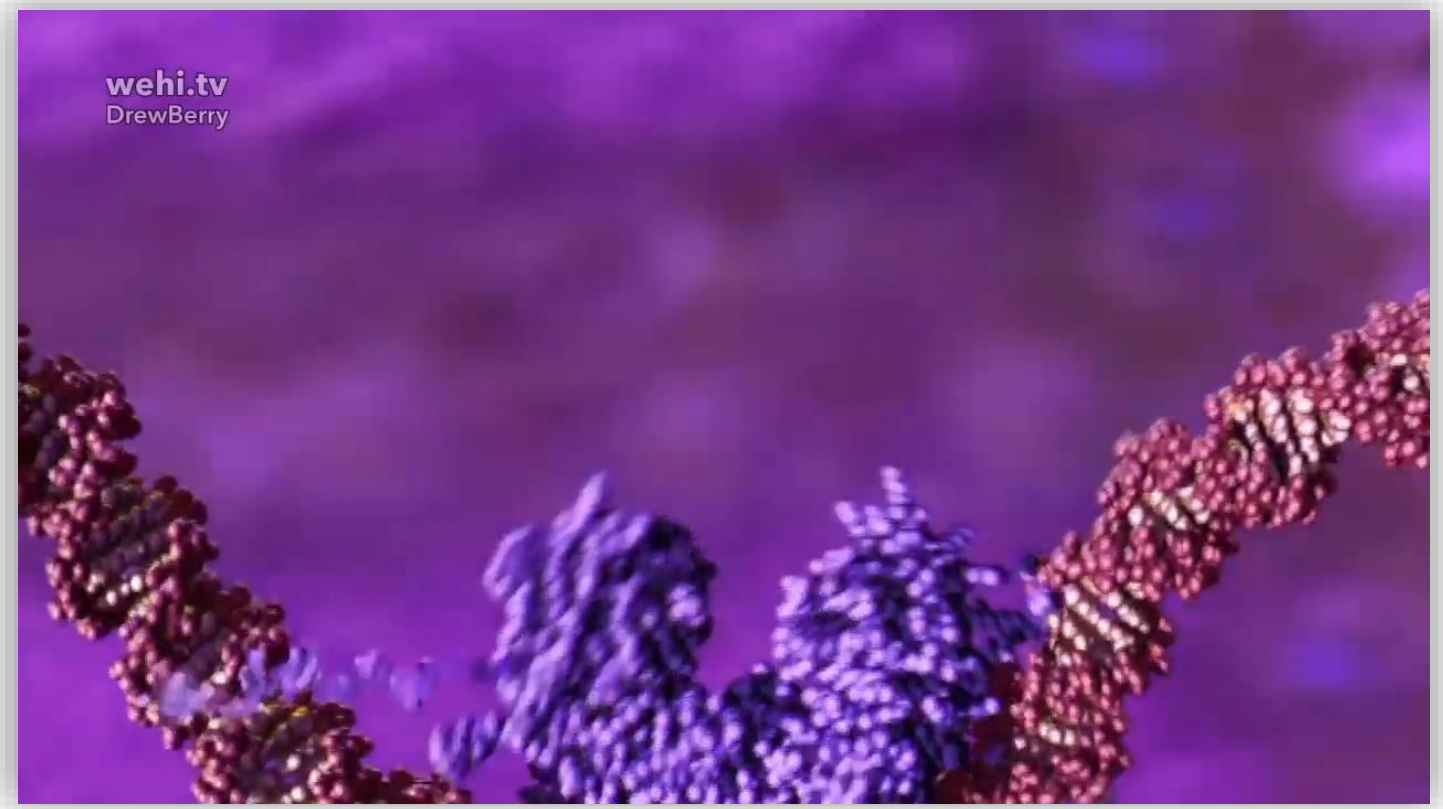
Rate of Replication

In other words, more often than not, the E. coli genome (4.7×10^6) is copied without error!



Rate of Replication

The Genome of complex eukaryotes is huge as compared to procaryotes



Rate of Replication

The speed of DNA replication for human is about 50-75 nucleotides per second per replication fork

Rate of Replication

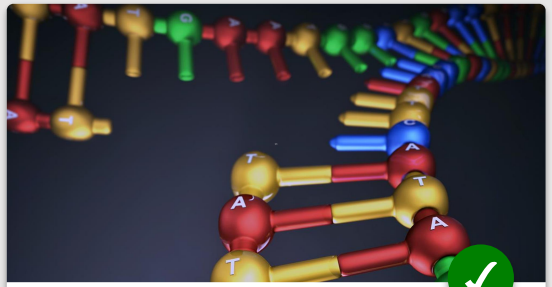
However, the genome can be copied only in a few hours because many replication forks take place at the same time (multiple initiation sites)

Module 2

DNA Replication

Module Lessons

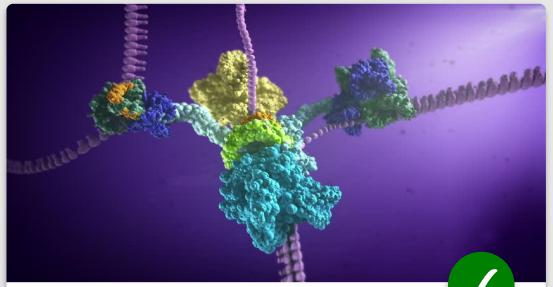
- Dashboard
- Introduction
- Lessons
- Assignments



Lesson 1

Method of Replication

Start ▸



Lesson 2

Mechanism of Replication

Start ▸

Lesson 3

Rate of Replication

Start ▸

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End of Module 2

Previous Module

Next Module