Section 5.2 DNA Replication

SBI4UP

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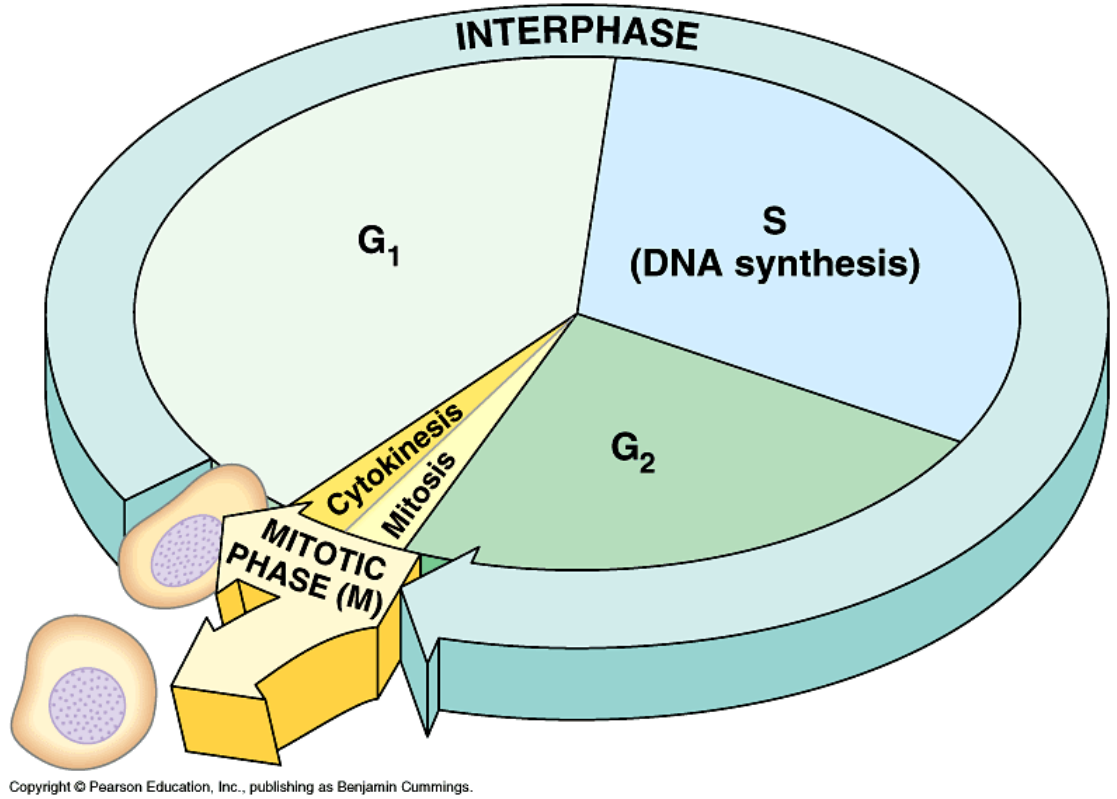
DNA Replication

DNA replication is an essential process in the cell cycle that enables new daughter cells to receive an exact copy of the parental DNA.

DNA replication occurs during the S phase of the cell cycle.



Proposed Models for DNA Replication

There were three models that were proposed by scientists that illustrates DNA replication:

1) Conservative Model

2) Semi-Conservative Model

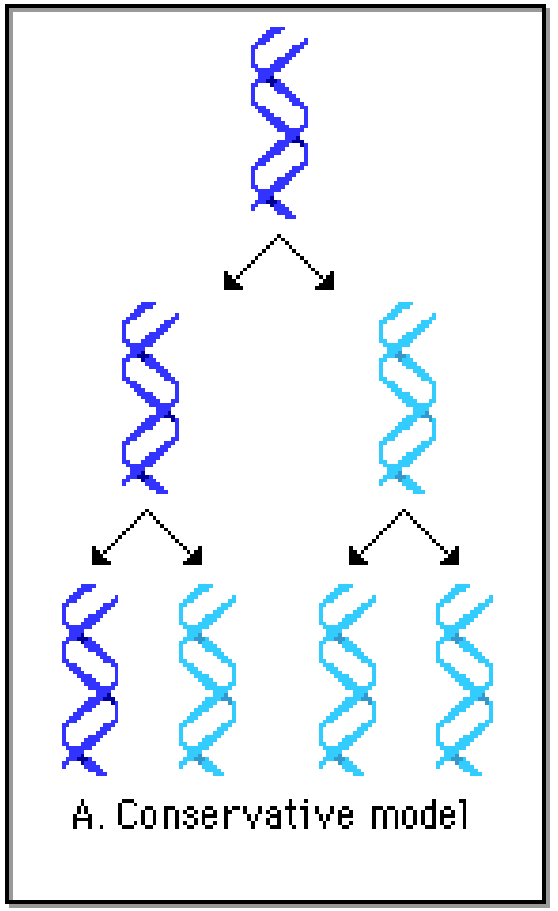
3) Dispersive Model

Each model was researched by scientists to help them determine which was a more accurate representation of DNA replication

1) Conservation Model

Scientists proposed that the parental DNA would make two new DNA strands.

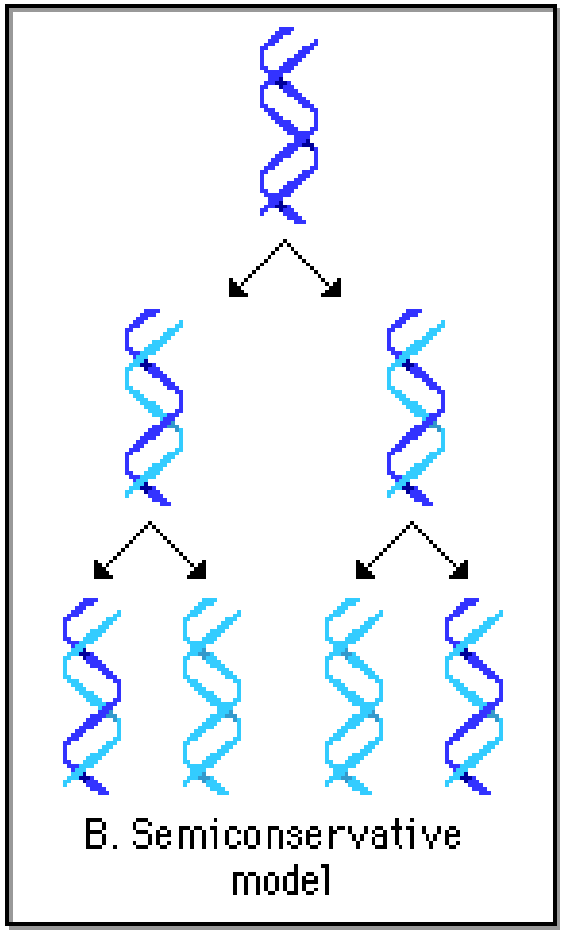
The new daughter strands would join to form one double helix and the original parental DNA would remain as its original double helix.



2) Semi-Conservative Model

Scientists proposed that the two new daughter strands that were replicated would bind to a parental strand and form a double helix.

Thus each DNA molecule of the first replication would contain a parental DNA strand.

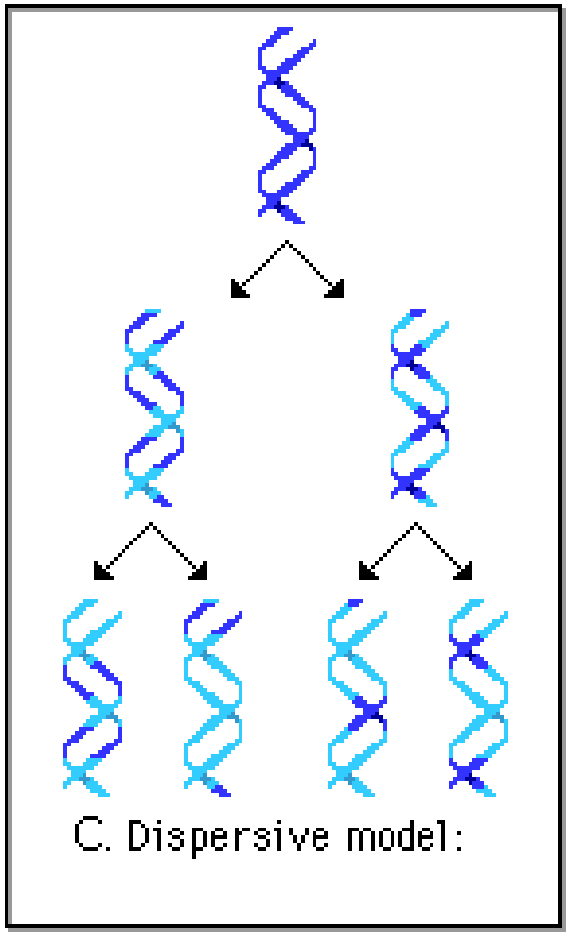


3) Dispersive Model

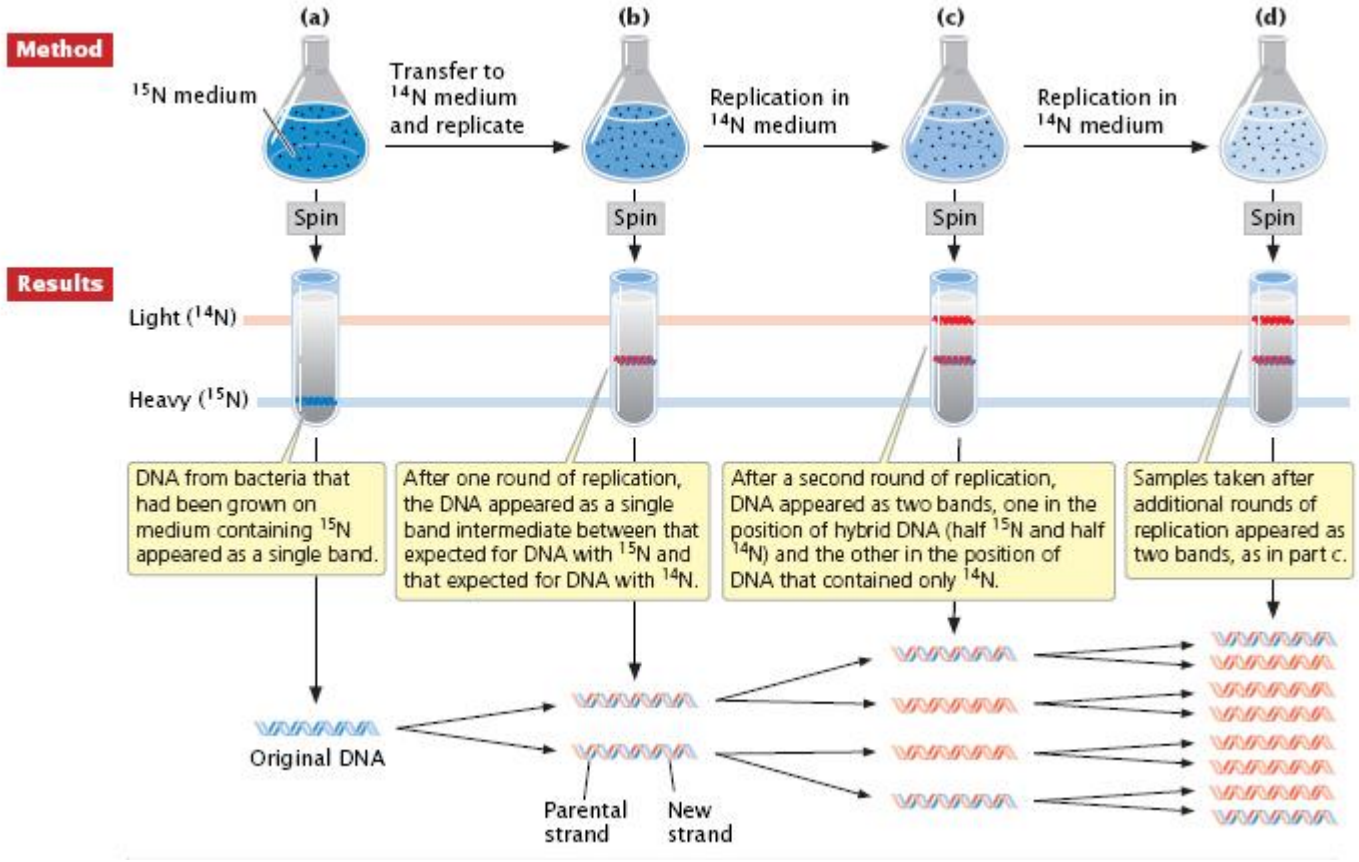
Scientists proposed that during replication the parental DNA strand would be broken into small fragments.

At the end of replication, each small fragment would bind to pieces of the newly copied DNA to form a complete double helix.

Thus, each DNA molecule of the first generation would contain parental fragments and newly synthesized fragments.



Experiment to Determine Replication Model (Meselson & Stahl)

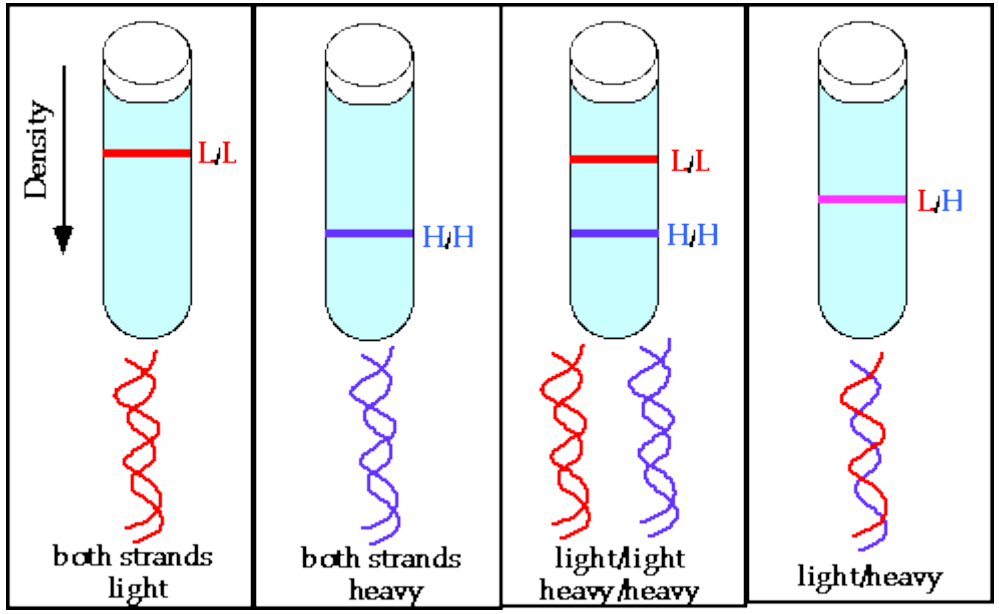


Scientists tested each model by distinguishing the parental strand from the daughter strand. The two strands were tagged with two different isotopes.

N^{14} which is light and N^{15} which is more dense.

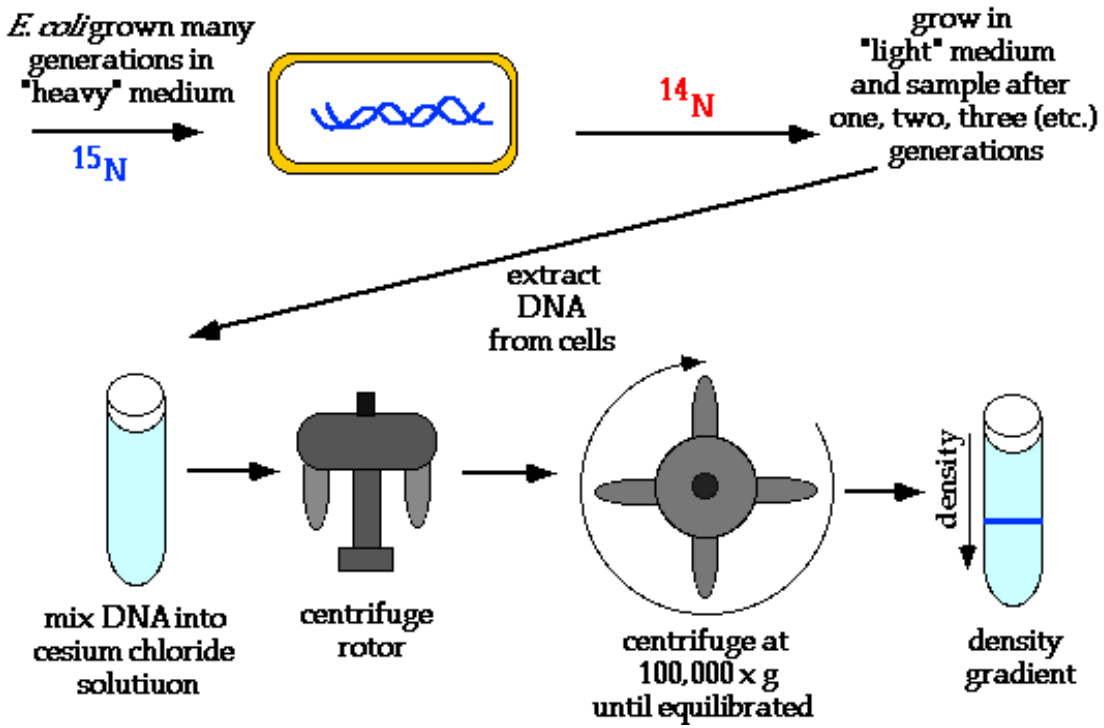
Experiment to Determine Replication Model

Scientists tagged the DNA with both ***nitrogen isotopes*** and separated the content though centrifugation.



Any DNA containing the denser isotope **(N^{15})** would form a band at the ***bottom of the test tube***. The DNA containing the lighter isotope **(N^{14})** would form a band near the ***top of the test tube***.

Experiment to Determine Replication Model

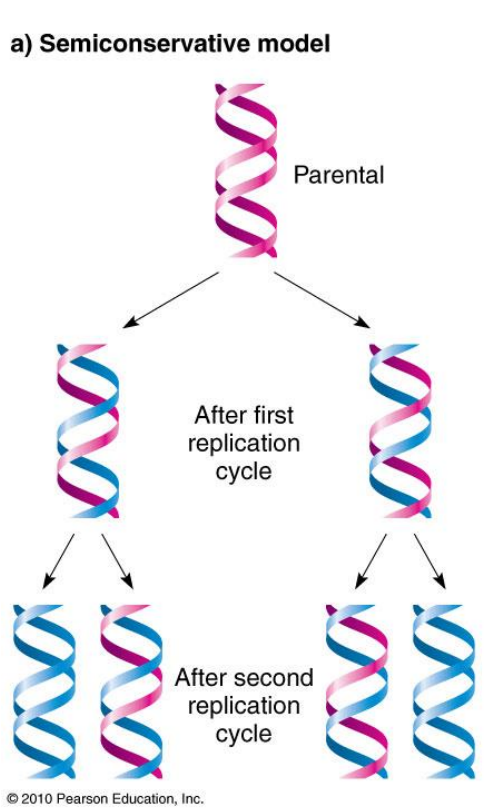


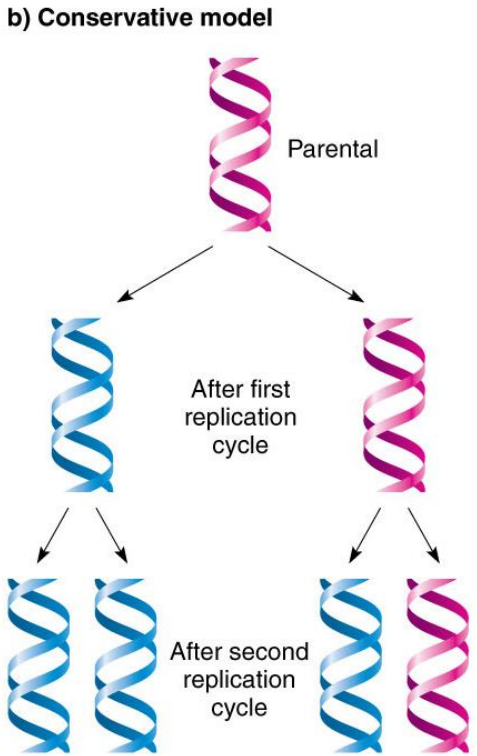
Step 1: E. coli was grown in a medium containing N15 medium.

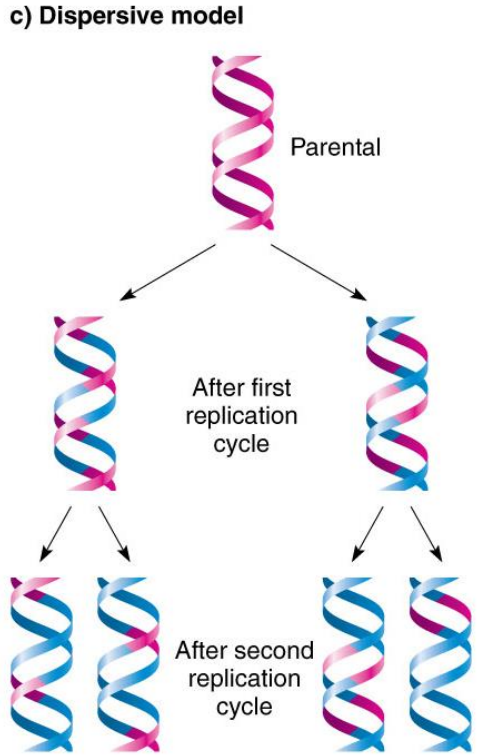
Step 2: The N15 population of E.coli was transferred to a new medium that contained only N14.

Step 3: The content within the test tube were separated by a centrifuge and results were analyzed.

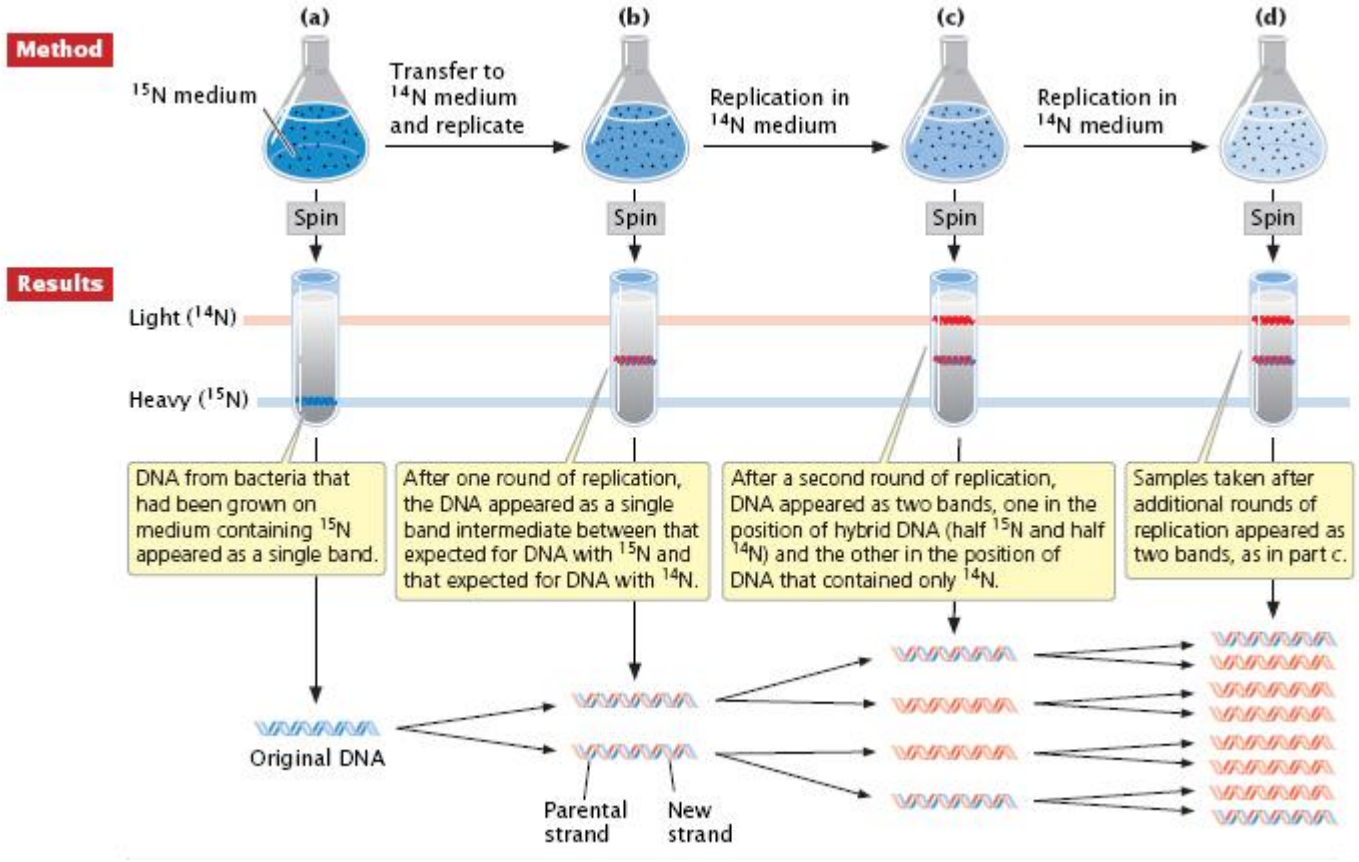
Experiment – Possible Results







Experiment – Interpreting the Results



This experiment proved that the semi-conservative model was the correct representation of DNA replication.

Semi-Conservative Replication

There are 3 main phases involved in replication:

1) Initiation: the double helix is unwound and base pairs are exposed.

2) Elongation: the parental DNA is used as a template and the new strand is copied. The final DNA will have a double helix with one parental and one new strand.

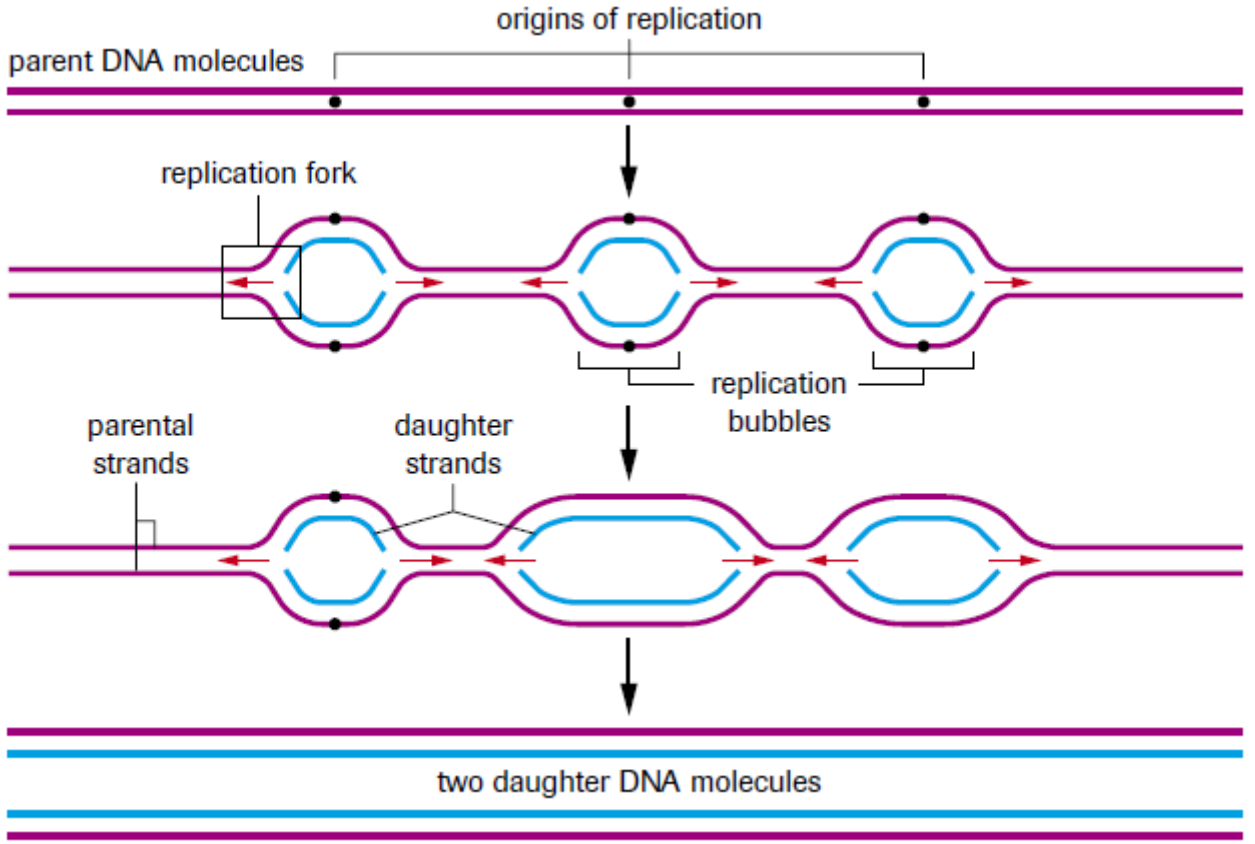
3) Termination: the two new DNA strands are separated.

1) **INITIATION**
2) Elongation
3) Termination

1) Initiation Phase

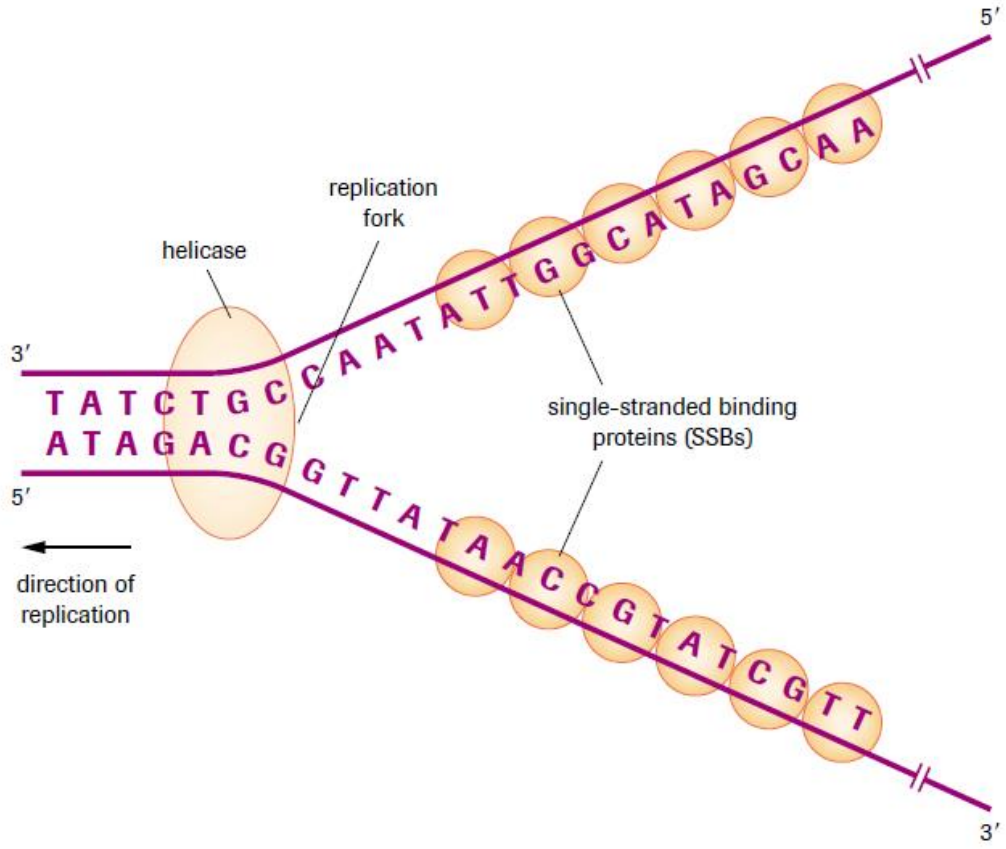
The replication bubbles expand laterally as DNA replication continues on both strands. All of the replication bubbles eventually fuse together.

Replication will begin at the many origin of replications along the DNA strand.



1) **INITIATION**
2) Elongation
3) Termination

1) Initiation Phase



1) Nucleotide sequence indicates the origin of replication.

2) Helicase is an enzyme that recognizes the origin and binds to unwind the DNA.

3) The single-stranded binding proteins (SSB) stabilize the strand that has been unwound.

4) Topoisomerase II (enzyme) will bind at the end of the replication fork to relieve the strain.

1) **INITIATION**
2) Elongation
3) Termination

1) Initiation - Priming for Elongation

DNA polymerase cannot start incorporating nucleotides on its own.

- Needs an existing 3’ end of a nucleic acid.

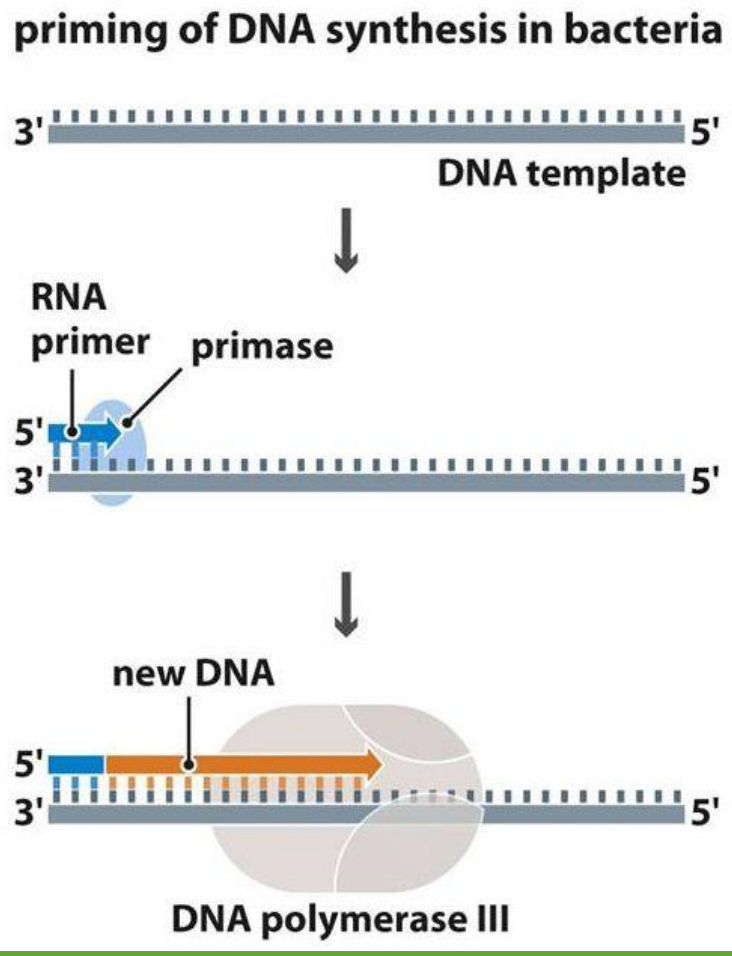
A short segment of RNA (a “primer” – 10 to 60 nucleotides long) provides that 3’ end.

1) **INITIATION**
2) Elongation
3) Termination

1) Initiation - Priming for Elongation

RNA primase *synthesizes the primer and anneals it to the template strand.*

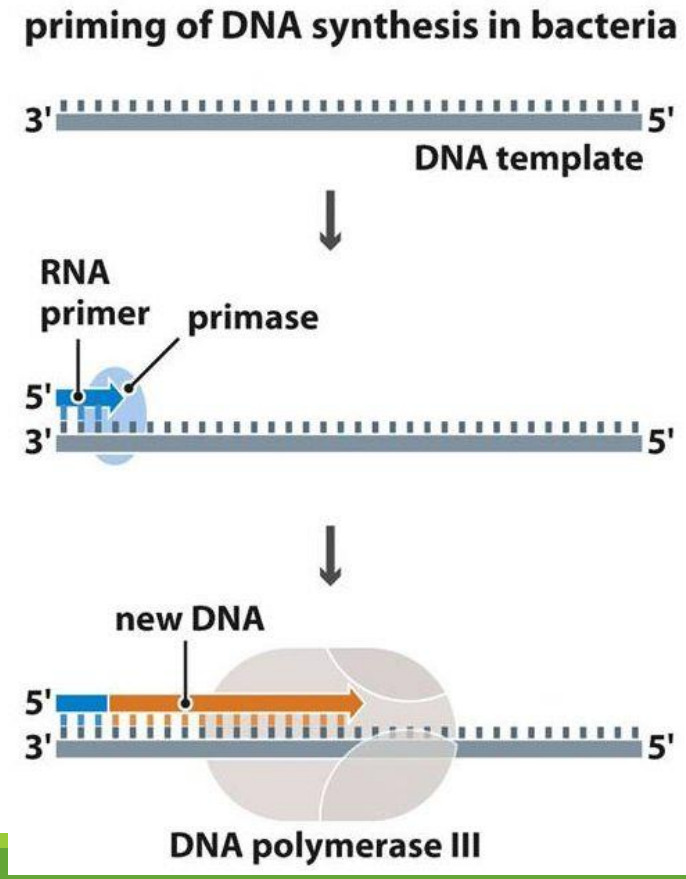
DNA polymerase can then add on DNA nucleotides.



1) Initiation
2) **ELONGATION**
3) Termination

2) Elongation

New DNA strands are synthesized by using the parental strands as a template.



DNA polymerase III (enzyme) *binds to the parental strand and adds matching nucleotides using the parental DNA as a template.*

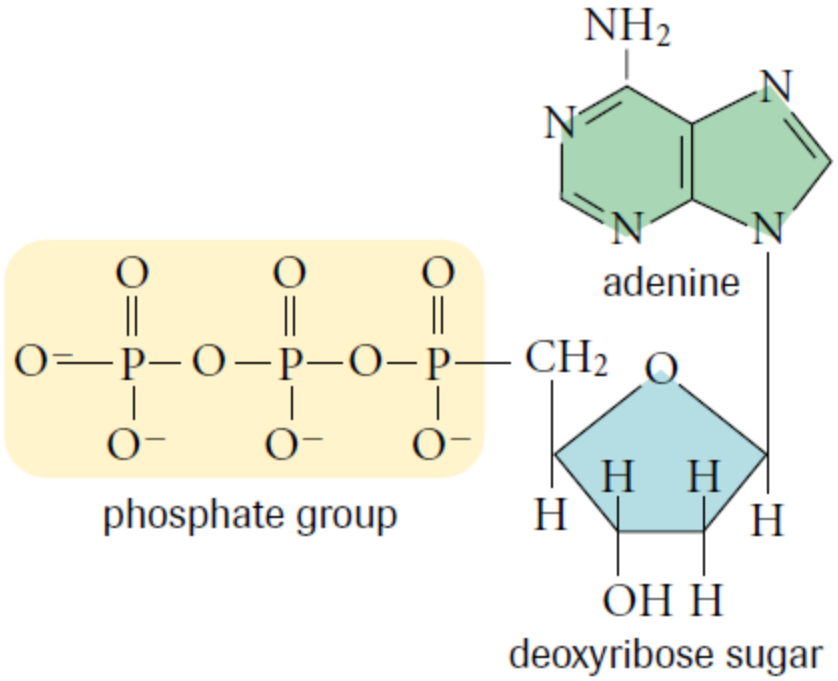
DNA polymerase III will add nucleotides in the 5' to 3' direction (of the new strand) towards the replication fork.

1) Initiation
2) **ELONGATION**
3) Termination

2) Elongation

A ***deoxyribonucleotide triphosphate*** (nitrogenous base with 3 phosphate groups) is added to the newly synthesized strand.

When a new nitrogenous base is added next to it, two phosphate groups are removed and it become a monophosphate.

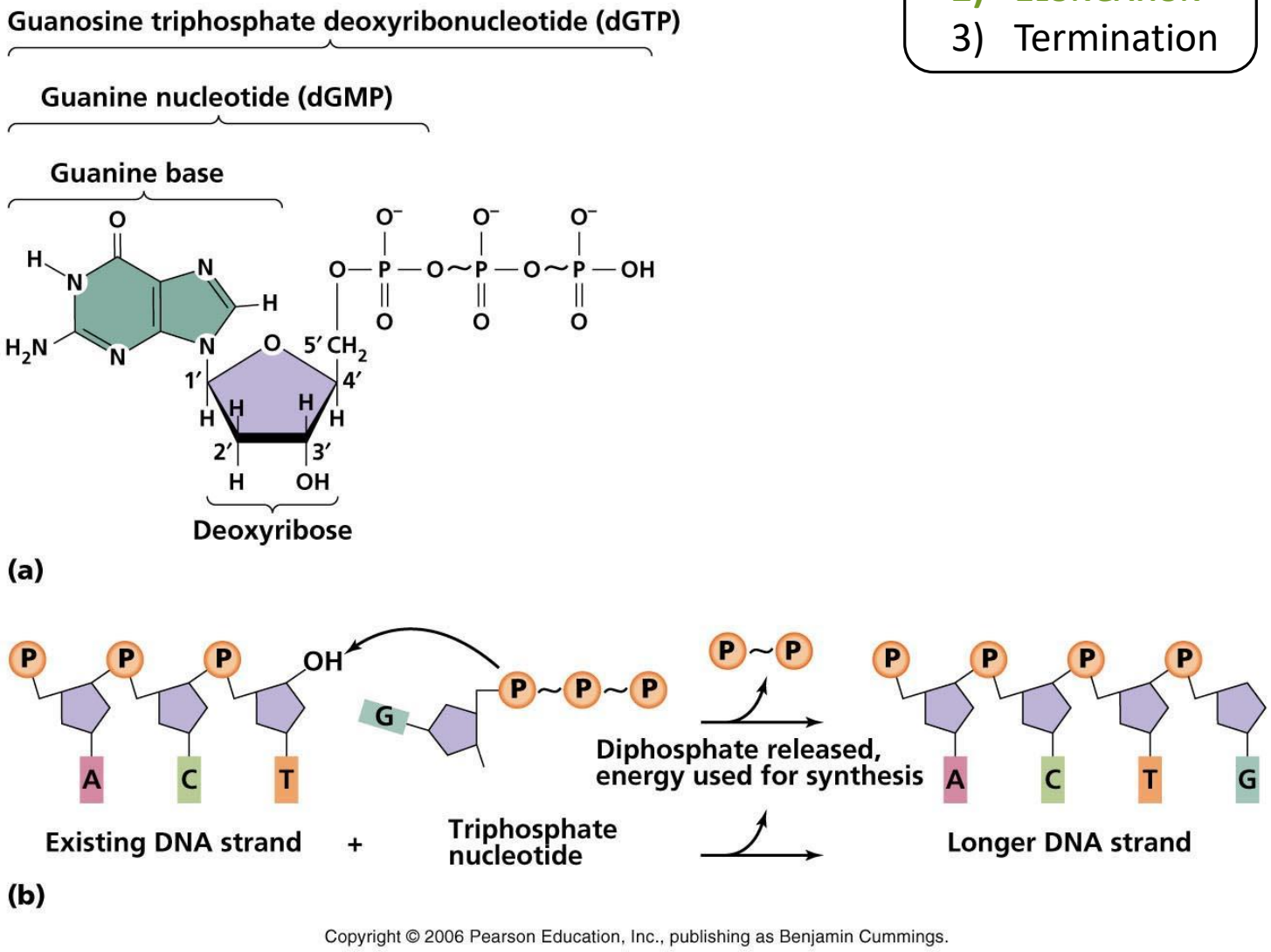


1) Initiation
2) **ELONGATION**
3) Termination

2) Elongation

Free bases are floating in the nucleoplasm as deoxyribonucleoside triphosphates.

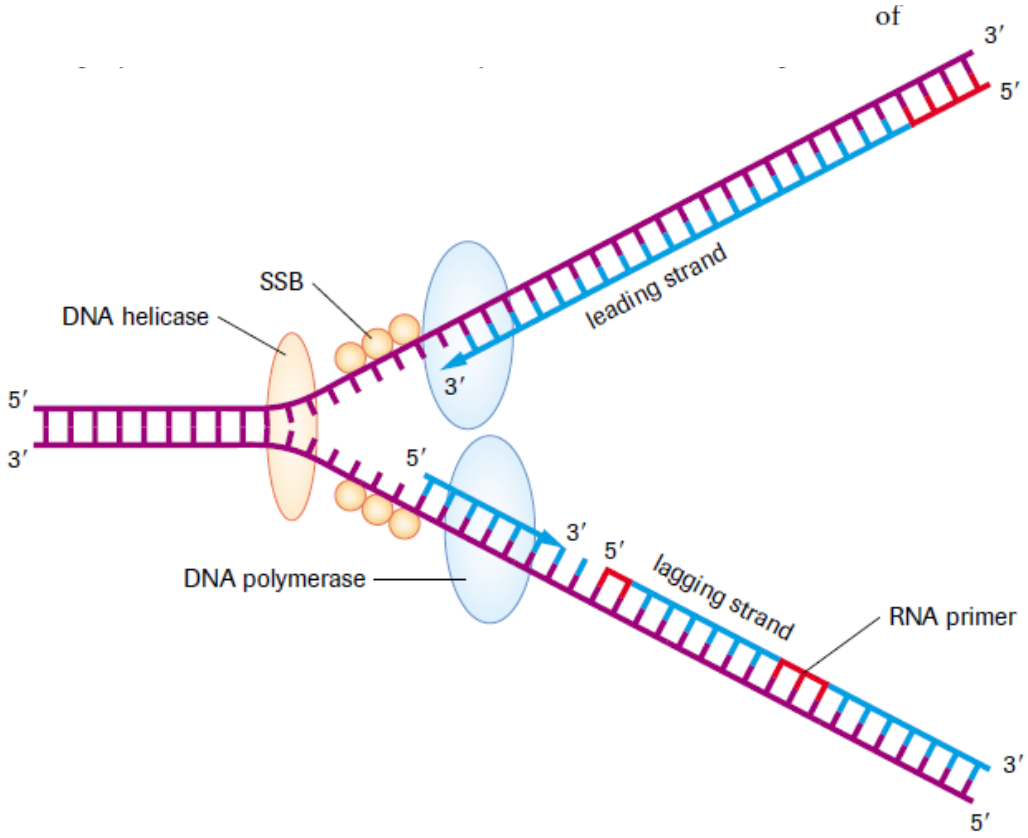
The energy required for DNA synthesis is provided by hydrolyzing the bond between the 1st and 2nd phosphates of the deoxiribonucleoside.



1) Initiation
2) **ELONGATION**
3) Termination

2) Elongation – a bidirectional process

Elongation proceed in two direction, outwards from the origin of replication. DNA polymerase III only replicates in the 5’ to 3’ direction. The two DNA strands are also antiparallel to one another.



*The junction between where the strands are still joined is called the **replication fork.***

1) Initiation
2) **ELONGATION**
3) Termination

2) Elongation – Semi-discontinuous

Leading strand- uses the 3’ to 5’ template strand as its guide.

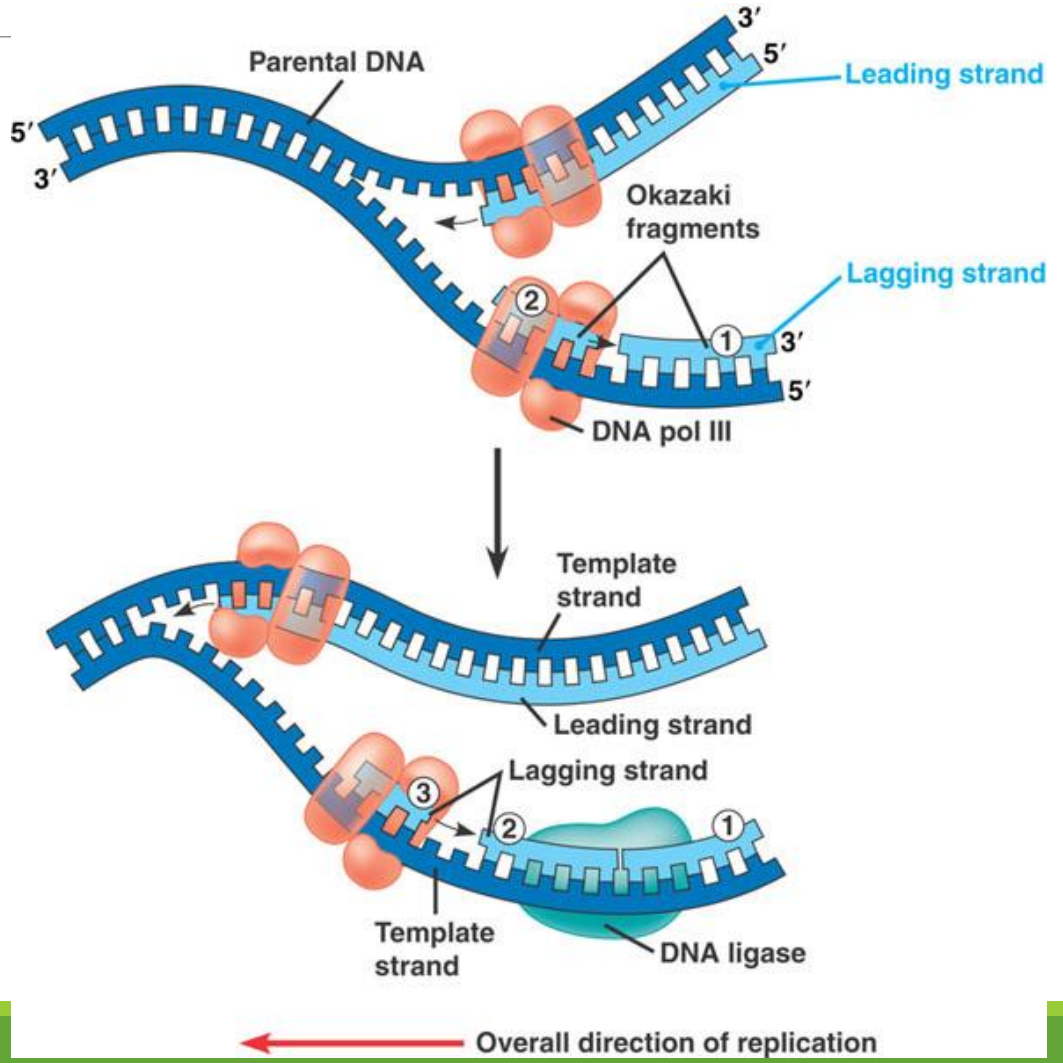
- Is built continuously towards the replication fork.

Lagging strand – uses the 5’ to 3’ template strand as its guide.

- Is built discontinuously in short fragments.

RNA primase constantly adds new RNA primers along the template strand.

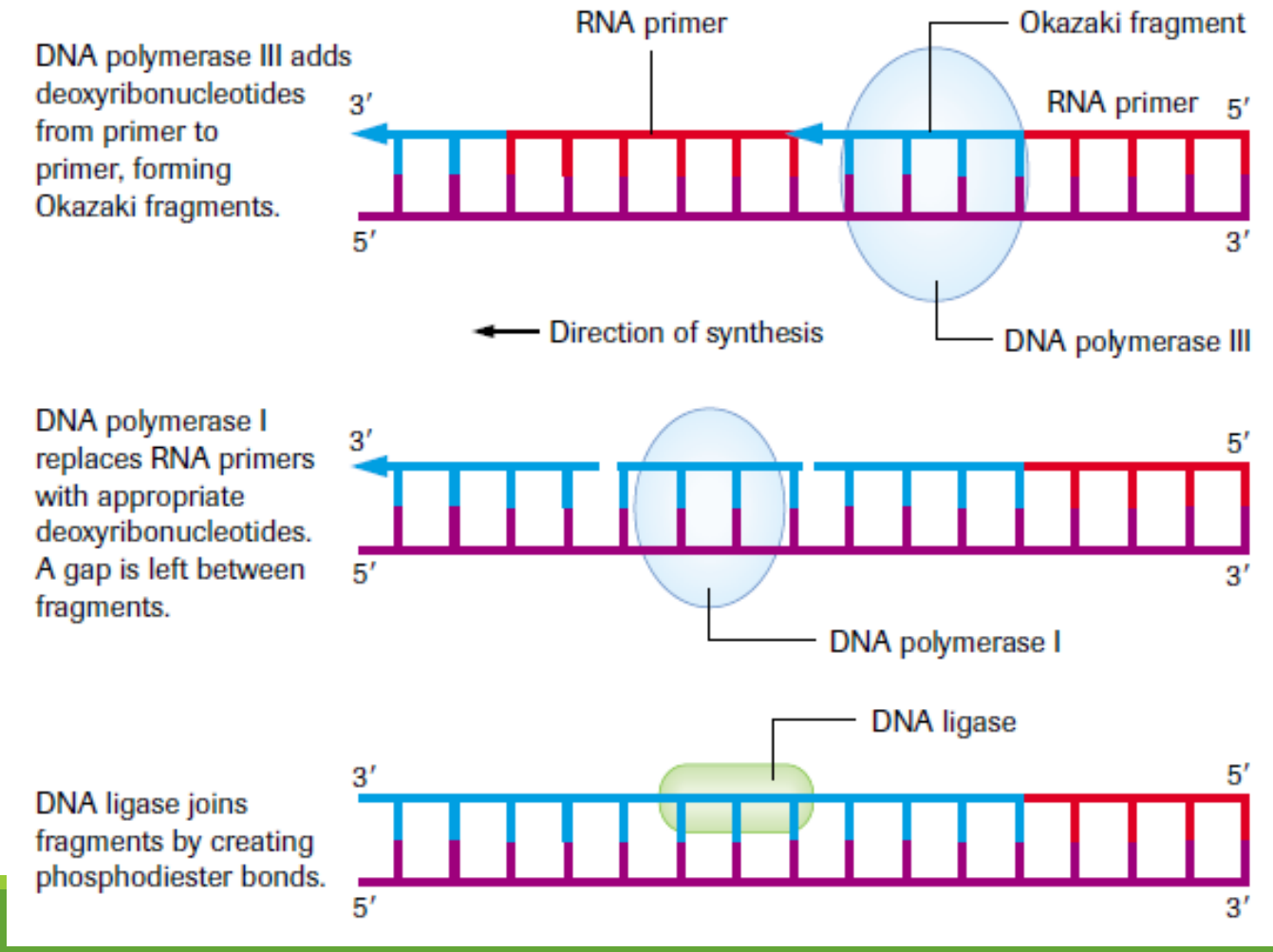
The fragments are known as ***Okazaki fragments.***



1) Initiation
2) **ELONGATION**
3) Termination

2) Elongation

Removal of the RNA primers, and joining of the Okazaki fragments.



1) Initiation
2) **ELONGATION**
3) Termination

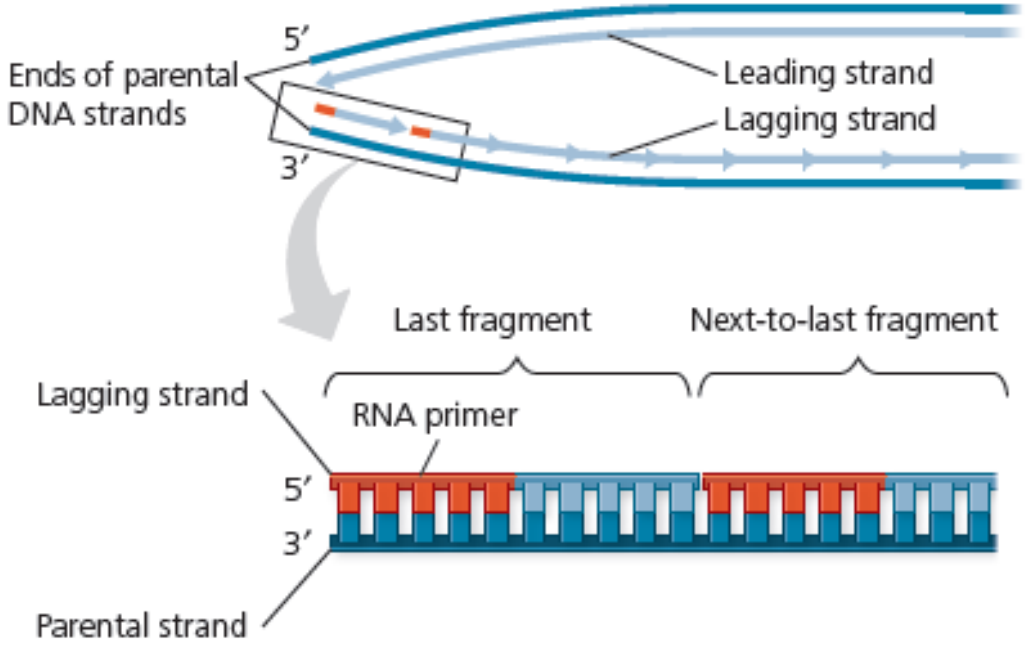
2) Elongation – Okazaki Fragments Summary

Removal of the RNA primers and joining of the Okazaki fragments:

Enzyme	Role
DNA polymerase I	• Removes the RNA primers • Replaces them with the proper deoxyribonucleosides
DNA ligase	• Joins the fragments together (phosphodiester bonds)

Replicating the Ends of DNA

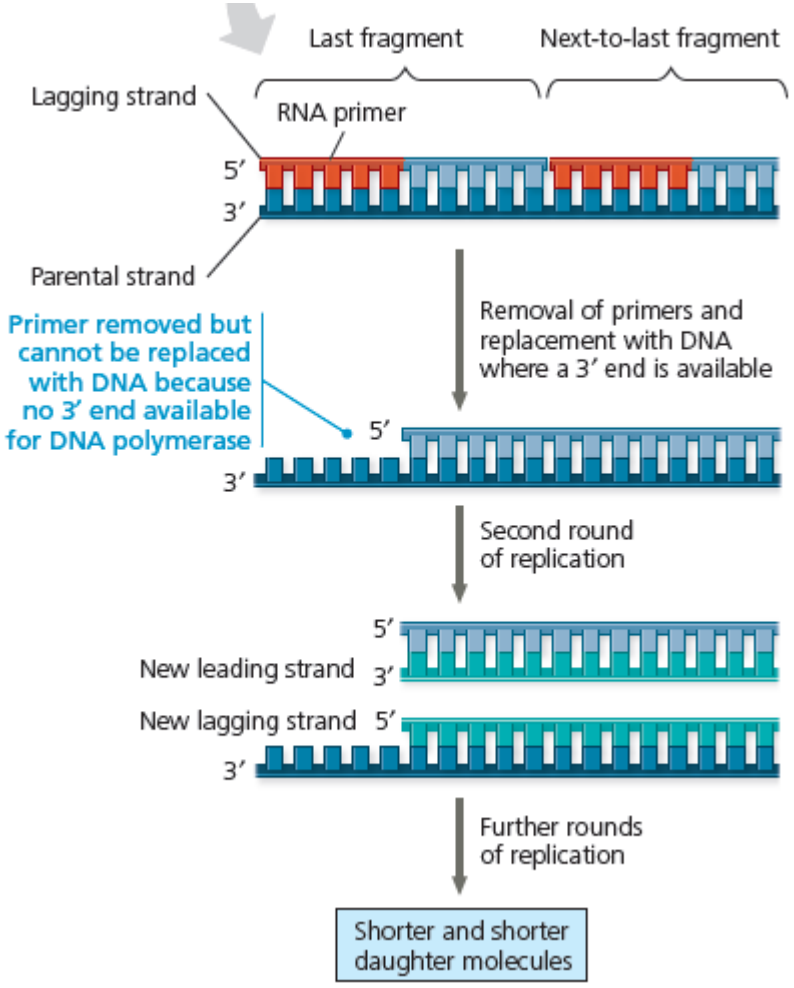
There is a small portion of the DNA strand that DNA polymerase cannot replicate or repair. The end of the ***lagging strand cannot be replicated***.



When the RNA primer of the lagging strand is removed, there is no 3' OH- group available on the last nucleotide.

As a result, DNA polymerase is not able to add more nucleotides to that last portion of the lagging strand.

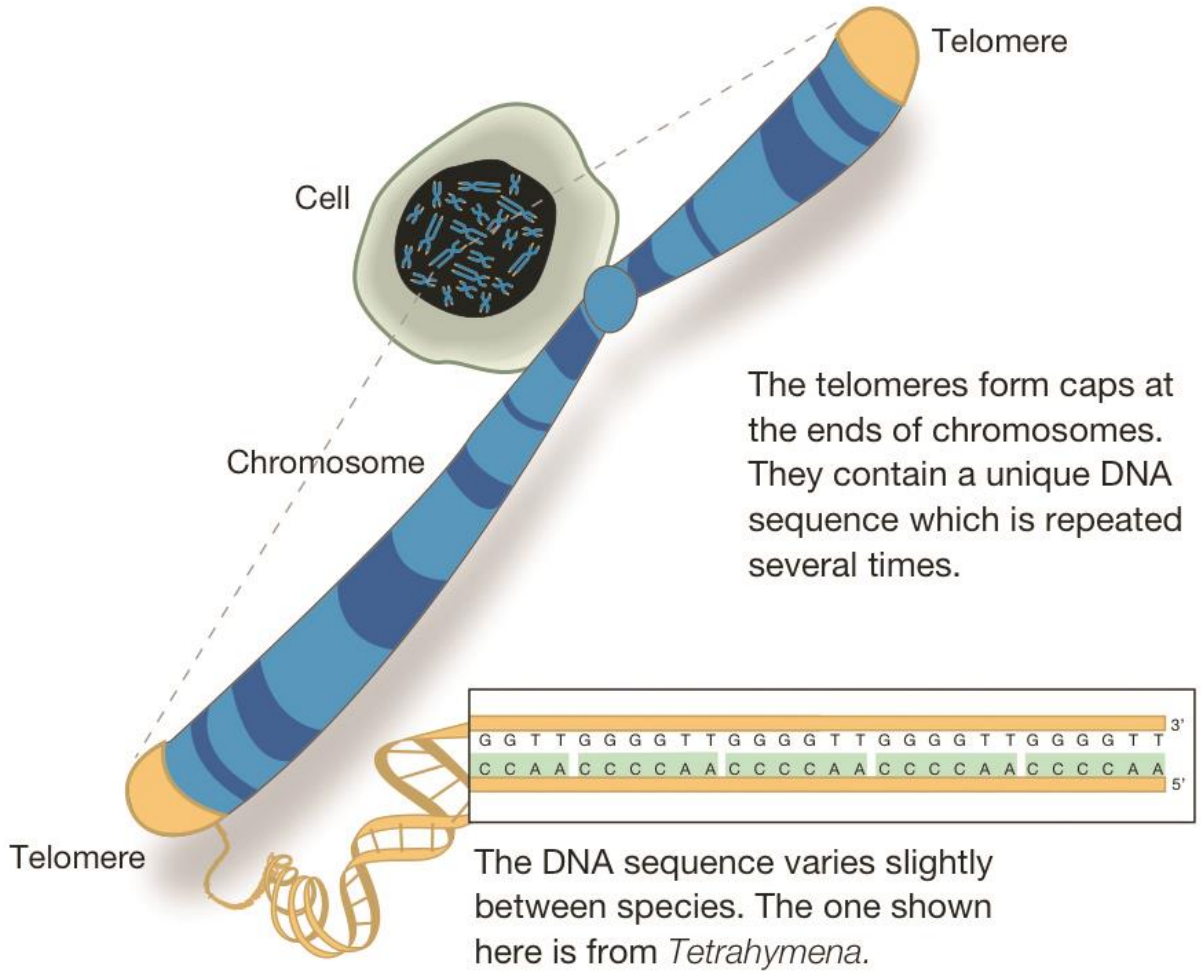
Replicating the Ends of DNA



Considering that DNA polymerase cannot add more nucleotides to the end of the lagging or leading strand, after each round of replication, the new strand of DNA continues to shorten.

This only occurs in Eukaryotic cells because it is linear.

Telomeres



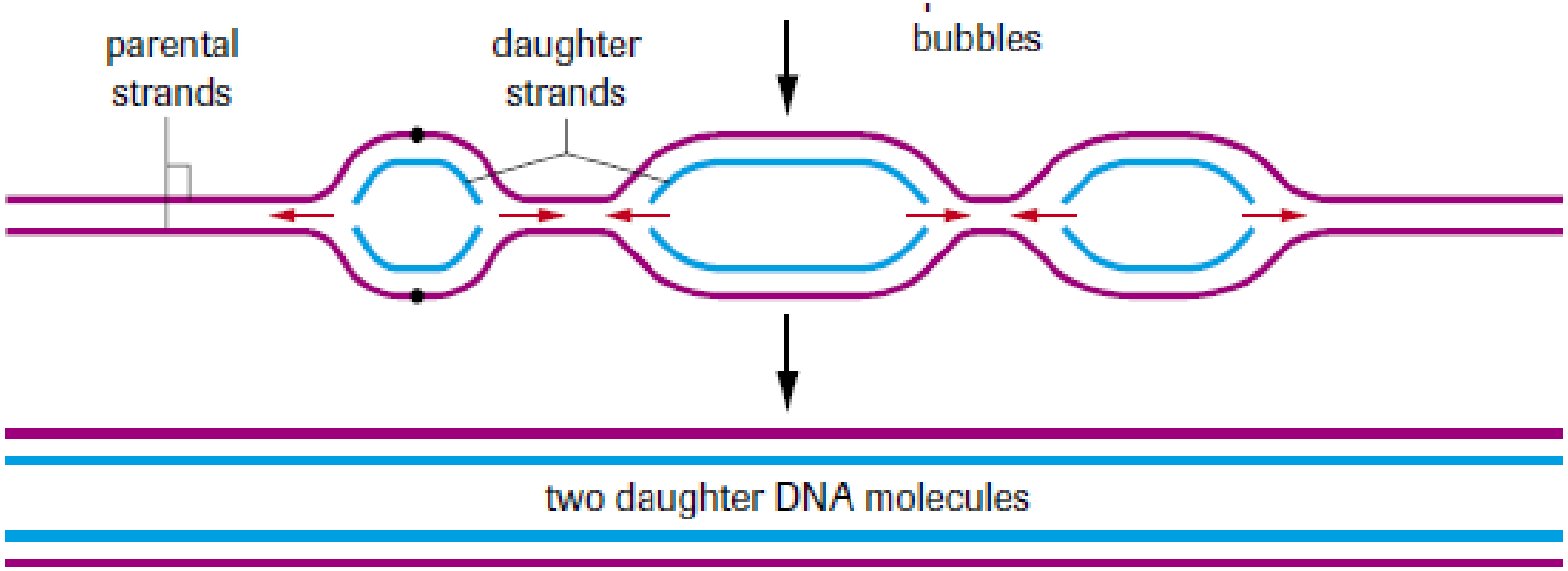
Telomeres are there to protect important genes and prevent them from being degraded during replication. Considering that the ends of the DNA strand shorten with each round of replication, the telomeres will also shorten.

1) Initiation
2) Elongation
3) **TERMINATION**

3) Termination

Eventually two replication forks will fuse together and form a continuous strand of newly synthesized DNA.

The two new daughter DNA molecules will contain a newly synthesized copy of DNA and the parental DNA.



Checking for Understanding

Protein	Functions
Helicase	
Primase	
SSB proteins	
Topoisomerase II	
DNA polymerase I, II and III	
DNA ligase	

Checking for Understanding

In which phase of DNA replication is the replication bubble created?

A) Synthesis

B) Elongation

C) Termination

D) Initiation

E) Mismatch Repair

Checking for Understanding

The leading strand of DNA is synthesized

A) in both 5’ to 3’ and 3’ to 5’ direction

B) Discontinuously in a 5’ to 3’ direction

C) Discontinuously in a 3’ to 5’ direction

D) continuously in a 5’ to 3’ direction

E) Continuously in a 3’ to 5’ direction

Checking for Understanding

How would DNA replication be affected if there were a mutation in the gene that codes for DNA ligase?

A) Okazaki fragment would not be joined

B) Error in DNA replication would not be corrected

C) Unwinding of the DNA would be stalled

D) Elongation of the leading strand would not occur

E) RNA primers would not be synthesized

Homework

Textbook: pg. 222 # 15, 16 & 17

Pg. 229 # 2, 3, 5 & 7

Errors in DNA Replication

Types of Errors:

A) Mispairing of nucleotides:

- During replication, nucleotides may be paired with a non-base pair nucleotide (i.e A-C, T-G)
- Mispairing causes the DNA to change its shape and become more unstable. This halts the replication process.
- DNA polymerase recognizes the mismatched pairs and repairs them.

Errors in DNA Replication

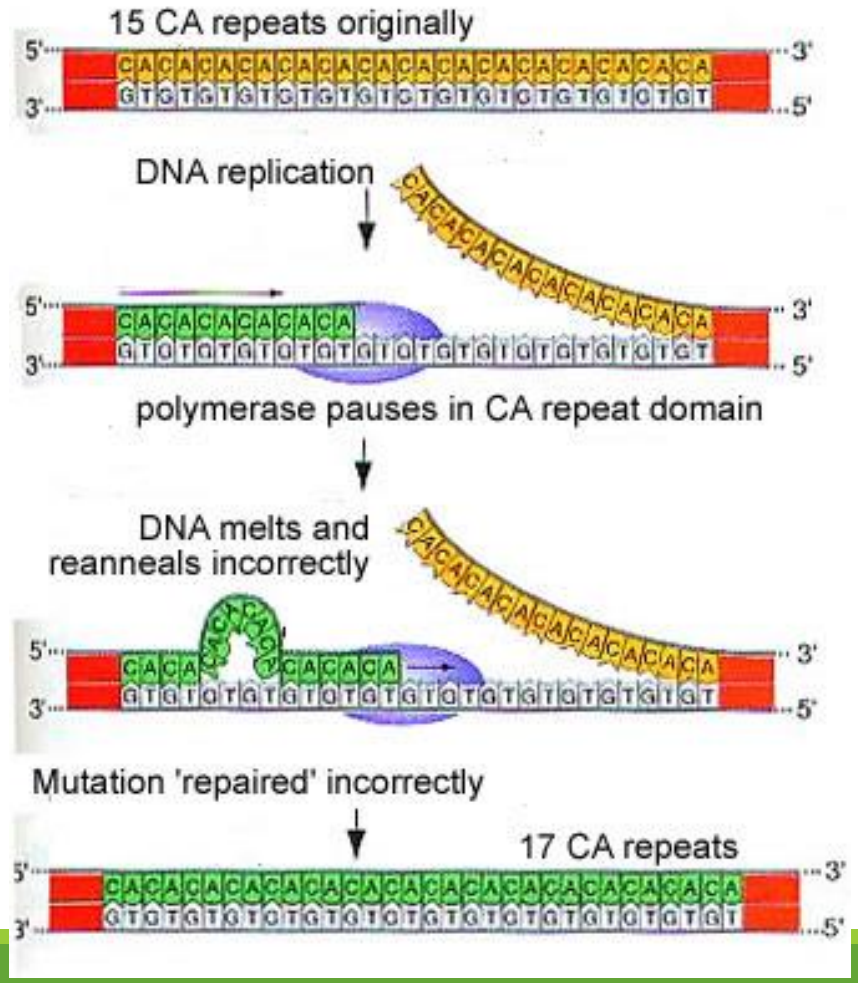
Types of Errors:

B) STAND SLIPPAGE:

- At time either the newly synthesized strand or the template strand may loop out during the replication process. This may cause additional nucleotides to be added or deleted.

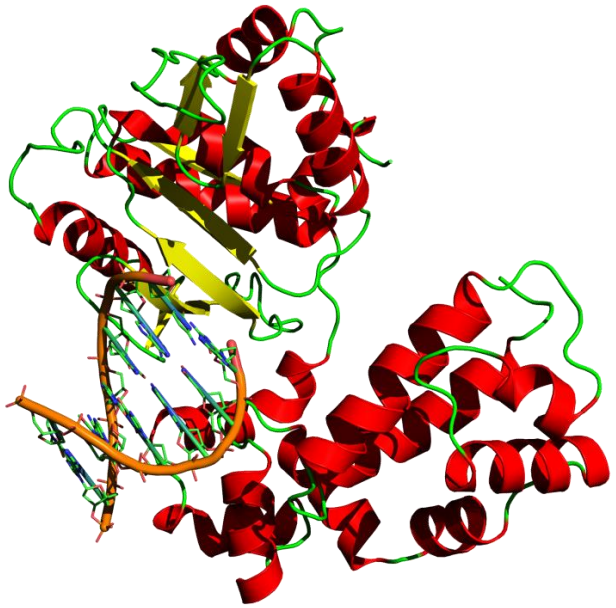
i) Template strand loops: the newly synthesized strand will be missing certain nucleotides

ii) New DNA strand loops: additional nucleotides are added as a result.



Errors in DNA Replication

When the DNA is being replicated there are many proteins involved in the process. Due to the large number of proteins there is a chance that a mistake can occur.



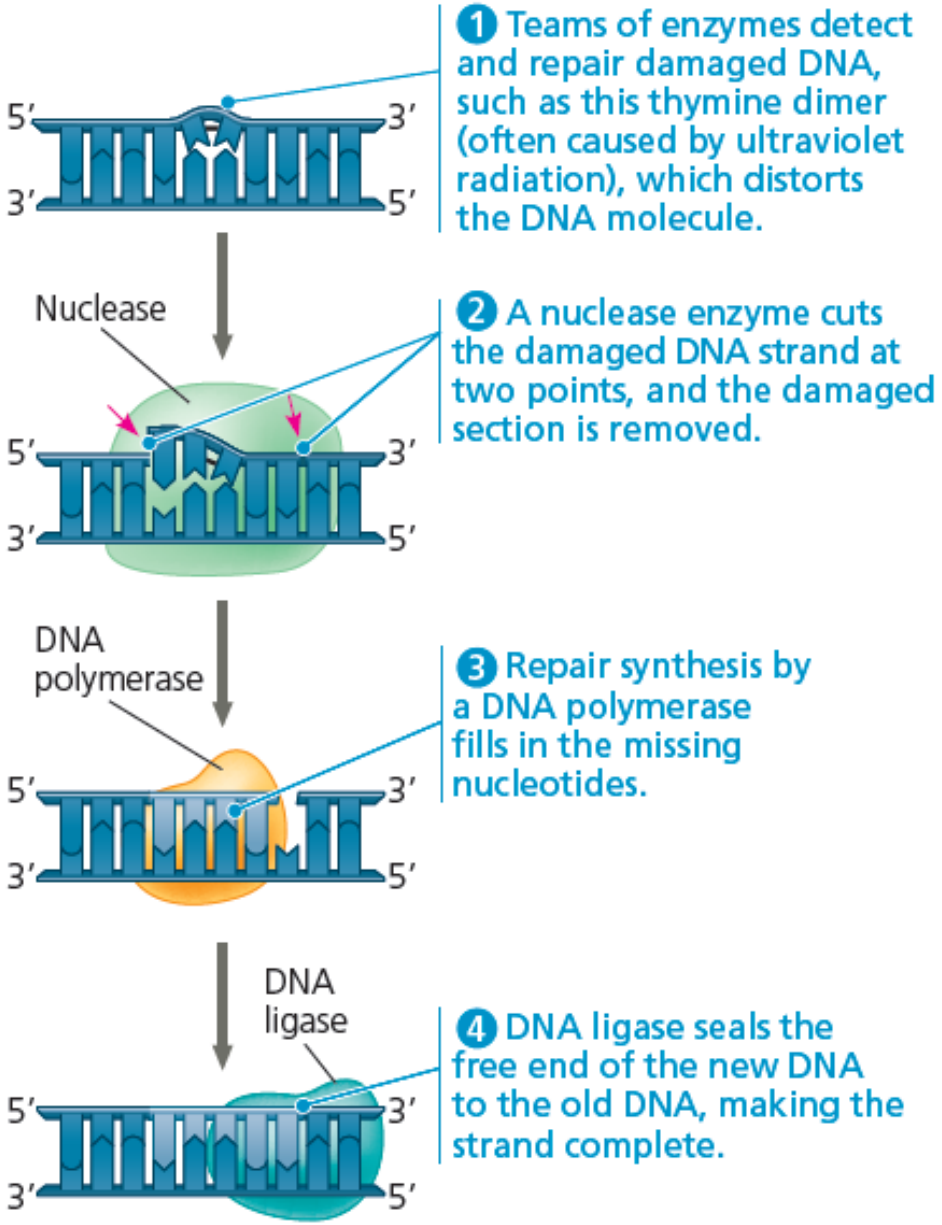
DNA polymerase II is an enzyme that proofreads the new DNA strands for any nucleotide error.

It replaces the mismatched nucleotide with the correct base pair.

Other proteins must be present to check over the replicated DNA and repair any mistake in the newly synthesized DNA strand.

Repairing Errors in DNA

DNA is misshapen when the incorrect nucleotide is paired with the template strand. This can be recognized by the DNA polymerase II.



Checking for Understanding

In which phase of DNA replication is the replication bubble created?

A) Synthesis

B) Elongation

C) Termination

D) Initiation

E) Mismatch Repair

Checking for Understanding

The leading strand of DNA is synthesized

A) in both 5’ to 3’ and 3’ to 5’ directions

B) Discontinuously in a 5’ to 3’ direction

C) Discontinuously in a 3’ to 5’ direction

D) Continuously in a 5’ to 3’ direction

E) Continuously in a 3’ to 5’ direction

Checking for Understanding

How would DNA replication be affected if there was a mutation in the gene that codes for DNA ligase?

A) Okazaki fragments would not be joined

B) Error in DNA replication would not be corrected

C) Unwinding of the DNA would be stalled

D) Elongation of the leading strand would not occur

E) RNA primers would not be synthesized

Homework

Textbook: pg. 222 # 15, 16, 17 & pg. 229 # 2,3,5 & 7