5.2 - DNA Replication

Pages 219-229 (McGraw-Hill Ryerson, 2011)

Subtopics

- Overview
- Three Proposed Models
  - Meselson & Stahl’s Experiment
- The Molecular Events in DNA Replication
  - Initiation, Elongation, Termination
- Correcting Errors during DNA Replication
- Comparing DNA Replication in Eukaryotes and Prokaryotes

https://www.youtube.com/watch?v=C1CRrtkWwu0&ab_channel=Teacher%27sPet

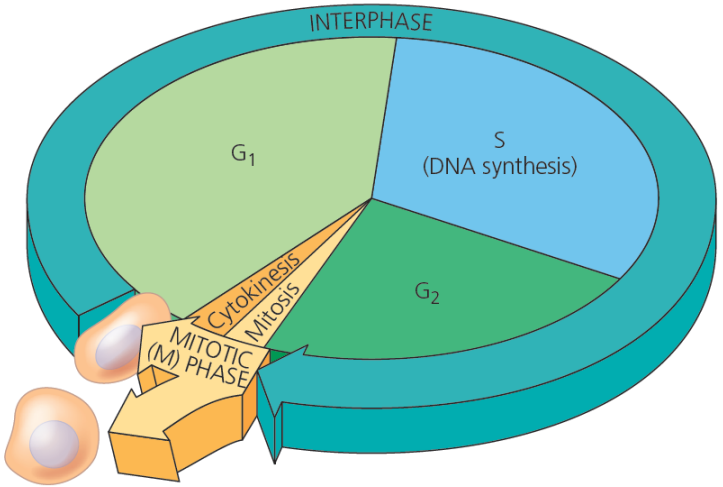
Overview

DNA Replication

→ **The process of producing two identical DNA molecules from an original, parent DNA molecule**

- **Occurs during S phase of Interphase**
- **It is essential for growth and development of an organism**
- **For Watson and Crick, complementary base pairing led**

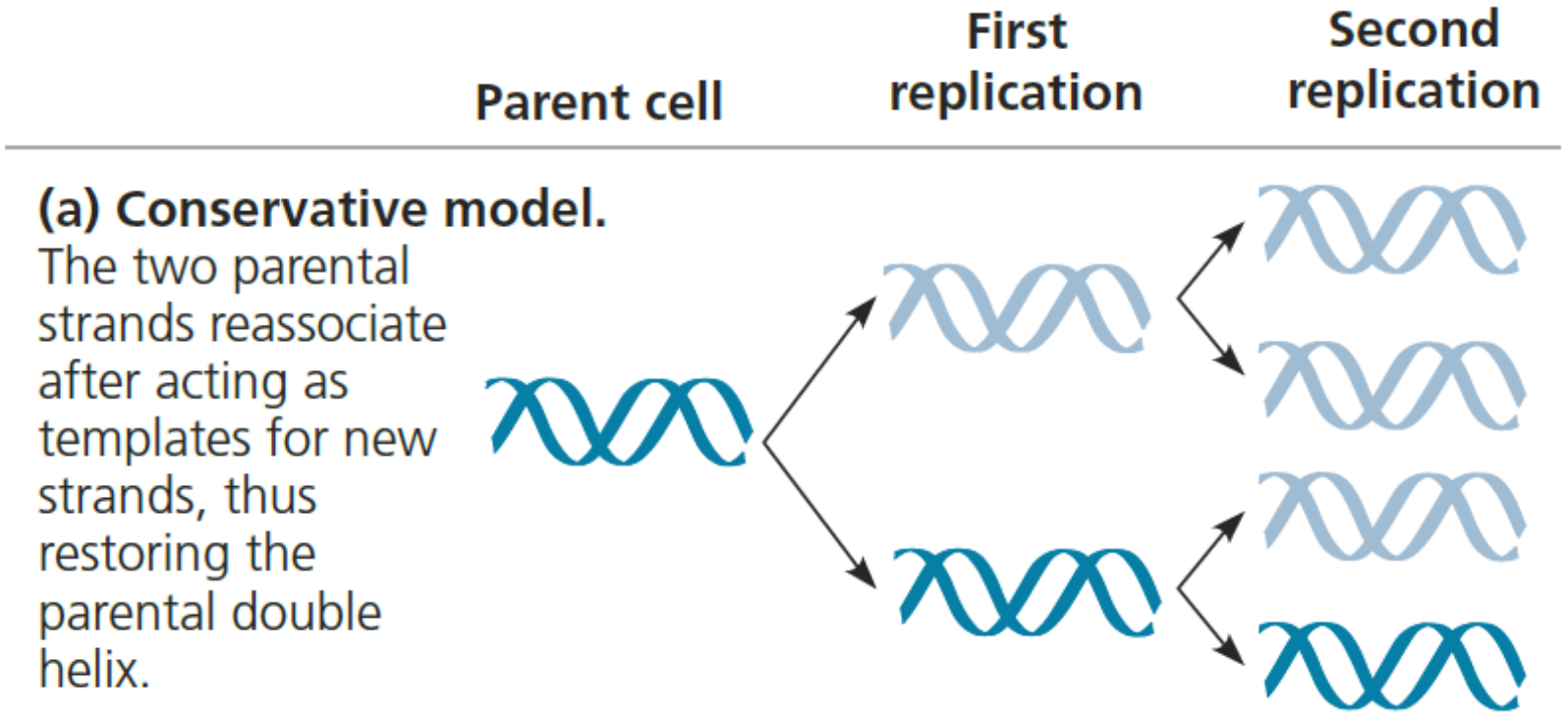
Figure 12.6 The cell cycle. In a dividing cell, the mitotic (M) phase alternates with interphase, a growth period. The first part of interphase (G_1) is followed by the S phase, when the chromosomes duplicate; G_2 is the last part of interphase. In the M phase, mitosis distributes the daughter chromosomes to daughter nuclei, and cytokinesis divides the cytoplasm, producing two daughter cells.



Animation: The Cell Cycle

Three Models for DNA Replication

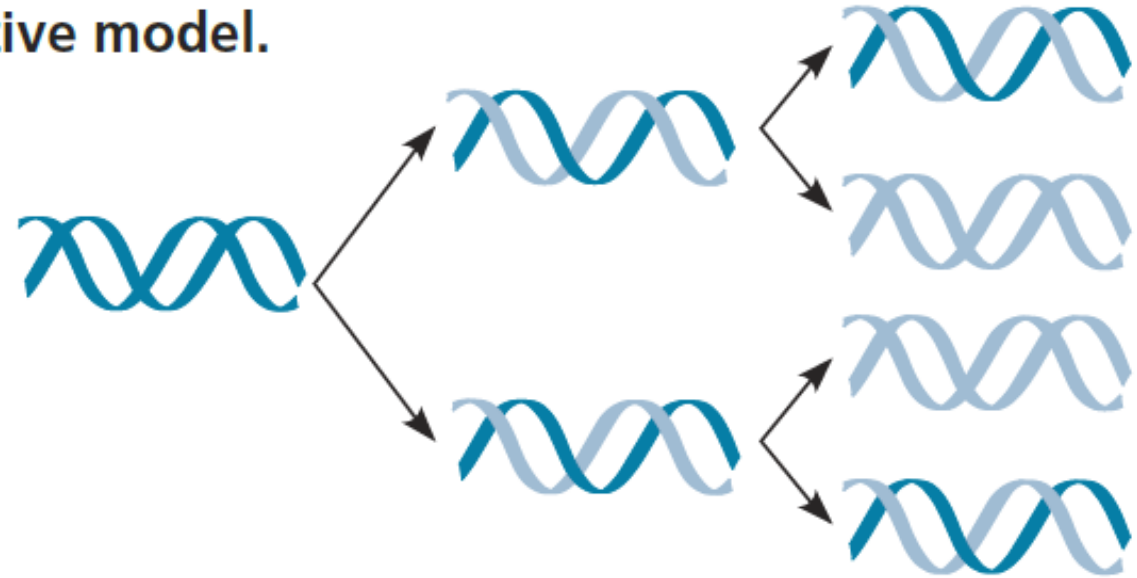
Conservative Model of Replication



Three Models for DNA Replication

Semi-conservative Model of Replication

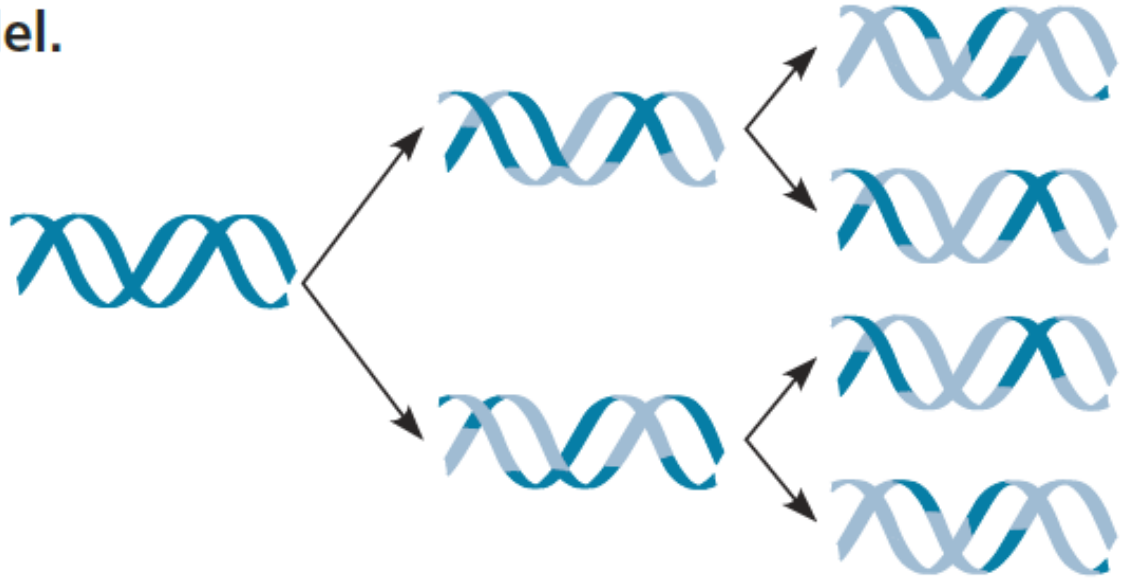
(b) Semiconservative model. The two strands of the parental molecule separate, and each functions as a template for synthesis of a new, complementary strand.



Three Models for DNA Replication

Dispersive Model of Replication

(c) Dispersive model. Each strand of *both* daughter molecules contains a mixture of old and newly synthesized DNA.

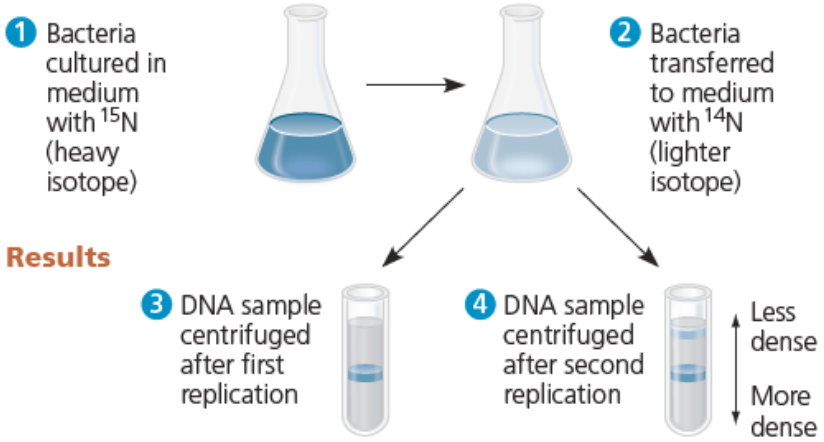


Three Models for DNA Replication

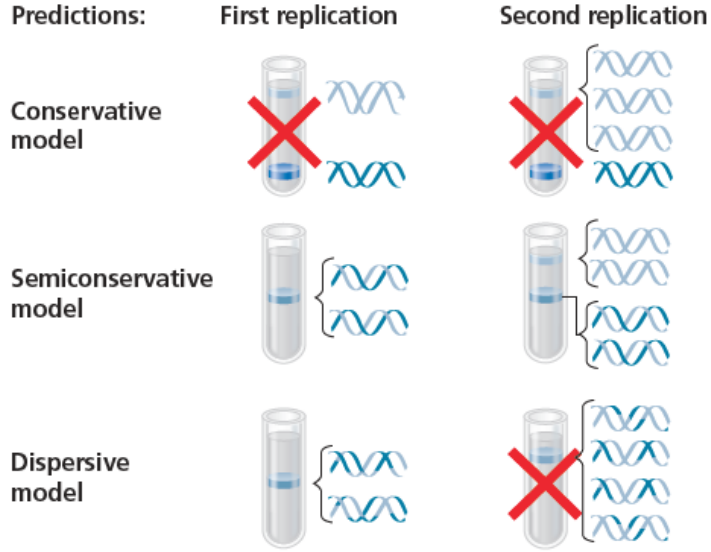
https://www.youtube.com/watch?v=4gdWOWjioBE&ab_channel=YourekaScience

Meselson and Stahl's Experime

Experiment Matthew Meselson and Franklin Stahl cultured *E. coli* for several generations in a medium containing nucleotide precursors labeled with a heavy isotope of nitrogen, ^{15}N. They then transferred the bacteria to a medium with only ^{14}N, a lighter isotope. They took one sample after the first DNA replication and another after the second replication. They extracted DNA from the bacteria in the samples and then centrifuged each DNA sample to separate DNA of different densities.



Conclusion Meselson and Stahl compared their results to those predicted by each of the three models in Figure 16.10, as shown below. The first replication in the ^{14}N medium produced a band of hybrid (^{15}N-^{14}N) DNA. This result eliminated the conservative model. The second replication produced both light and hybrid DNA, a result that refuted the dispersive model and supported the semiconservative model. They therefore concluded that DNA replication is semiconservative.



Data from M. Meselson and F. W. Stahl, The replication of DNA in *Escherichia coli*, *Proceedings of the National Academy of Sciences USA* 44:671–682 (1958).

Molecular Events in DNA Replication

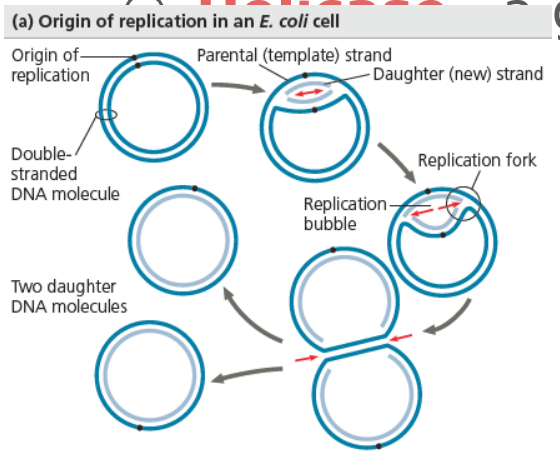
Phases

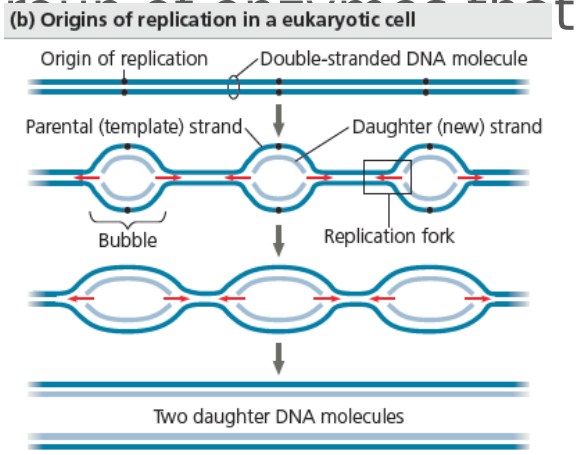
1. Initiation
    - A portion of the DNA double helix is unwound to expose the parent strand for new base pairing
2. Elongation
    - Two new strands of DNA are assembled using the parent strand template
    - The new parent-daughter strands reform into double helices
3. Termination
    - Replication completes, yielding two identical DNA molecules

Molecular Events in DNA Replication

Initiation

- DNA is unwound at the:
  - **Origin of replication** - the DNA sequence where replication begins
- Unwinding is achieved using proteins, specifically:
  - **Helicase** - a group of enzymes that aid in the unwinding of



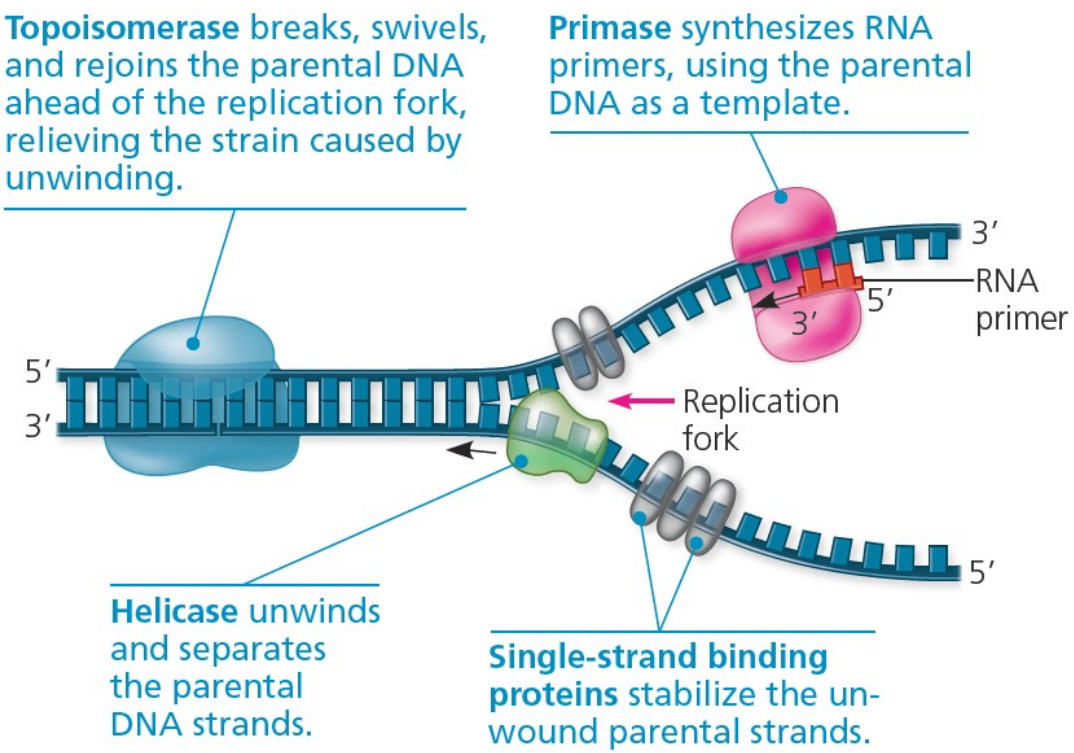


https://www.youtube.com/watch?v=nSH48qHxnSQ&ab_channel=WalterJahn

Initiation

- ***Topoisomerase II*** relieves unwinding strain ahead of the replication fork
- ***Single-strand binding proteins*** hold the unwound DNA apart into separate strands

Figure 16.13 Some of the proteins involved in the initiation of DNA replication. The same proteins function at both replication forks in a replication bubble. For simplicity, only the left-hand fork is shown, and the DNA bases are drawn much larger in relation to the proteins than they are in reality.



Questions 13-18

Learning Check

Learning Check

13. What is the main objective of DNA replication?

14. Why is DNA replication important for cell reproduction?

15. Describe the three proposed models of DNA replication.

16. Why did Meselson and Stahl use two different isotopes of nitrogen in their experiment?

17. What did Meselson and Stahl conclude from their experiment? What results provided convincing evidence for their conclusion?

18. Why is it important for newly replicated daughter strands of DNA to have the same information as the parent strands?

13. The main objective of DNA replication is to produce two identical DNA molecules from a parent DNA molecule.

14. DNA replication occurs during the S-phase of interphase, and prior to cell division. Therefore, DNA replication is important since each new daughter cell must have the same genetic information as the parent cell.

15. • Conservative model: Two new daughter strands form to create a new double helix, and the original DNA strands re-form into the parent molecule.
    • Semi-conservative model: Each new DNA molecule contains one strand of the original DNA and one newly synthesized strand.
    • Dispersive model: Parental DNA is broken into fragments. Therefore, the daughter DNA contains a mix of parental and newly synthesized DNA.

16. Nitrogen is a component of DNA and is incorporated into newly synthesized daughter strands. Having a "light" form (^{14}N) and a "heavy" form (^{15}N) allowed the separation of different DNA strands based on the amount of isotope present in the newly synthesized DNA. DNA with more ^{15}N would be denser than DNA with ^{14}N, and therefore could be separated by centrifugation.

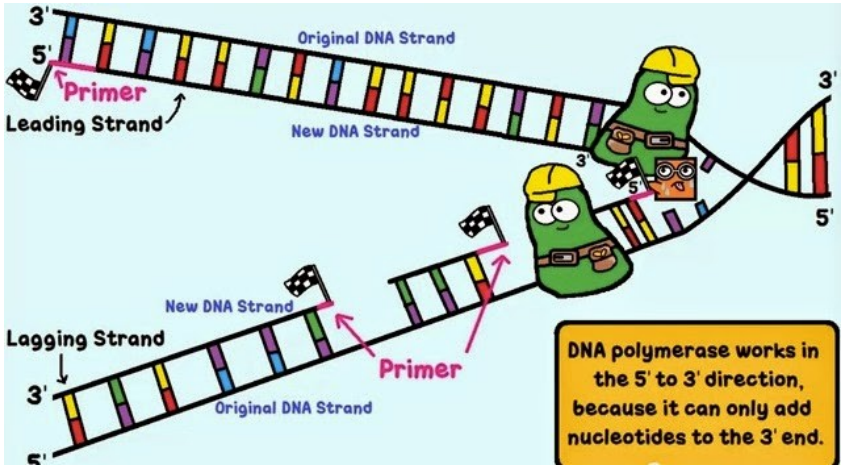
17. Meselson and Stahl concluded that DNA replication is semi-conservative. After one round of replication, DNA appeared as a single band, midway between the expected positions of ^{15}N-labelled DNA and 14N-labelled DNA. After the second round of replication, DNA appeared as two bands, with one band corresponding to 14N-labelled DNA and the other band in the position of hybrid DNA (half ^{14}N and half ^{15}N). In additional rounds of replication, the same two bands were observed, therefore supporting the semi-conservative model.

18. Each new cell that is produced must have an exact copy of parental DNA. The daughter strands of DNA are part of a DNA molecule that will be in the daughter cells. This ensures that newly born cells are similar to parents and maintain their genetic identity.

Molecular Events in DNA Replication

Elongation

- New strands of DNA are synthesized by joining nucleotides; this is achieved by:
  - **DNA polymerase III** - an enzyme that adds nucleotides to the 3’ end of a growing polynucleotide strand
  - **Primers** - these are short strands of RNA that serve as the starting point for polymerization in the 5’ to 3’



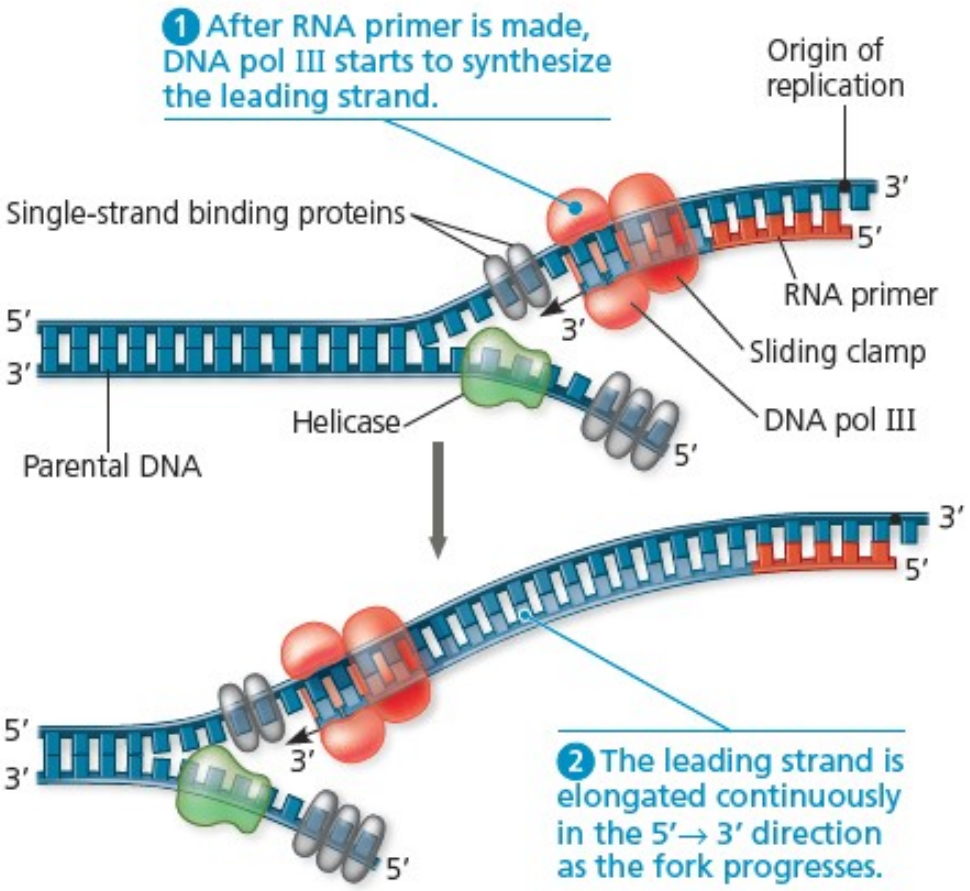
Molecular Events in DNA Replication

Elongation

- *Leading strand* - this is the strand that is polymerized in the 5’ to 3’
  - the template strand is ‘read’ in the 3’-5’ direction - **requires 1 primer** and DNA pol III moves in the same direction as the replication fork

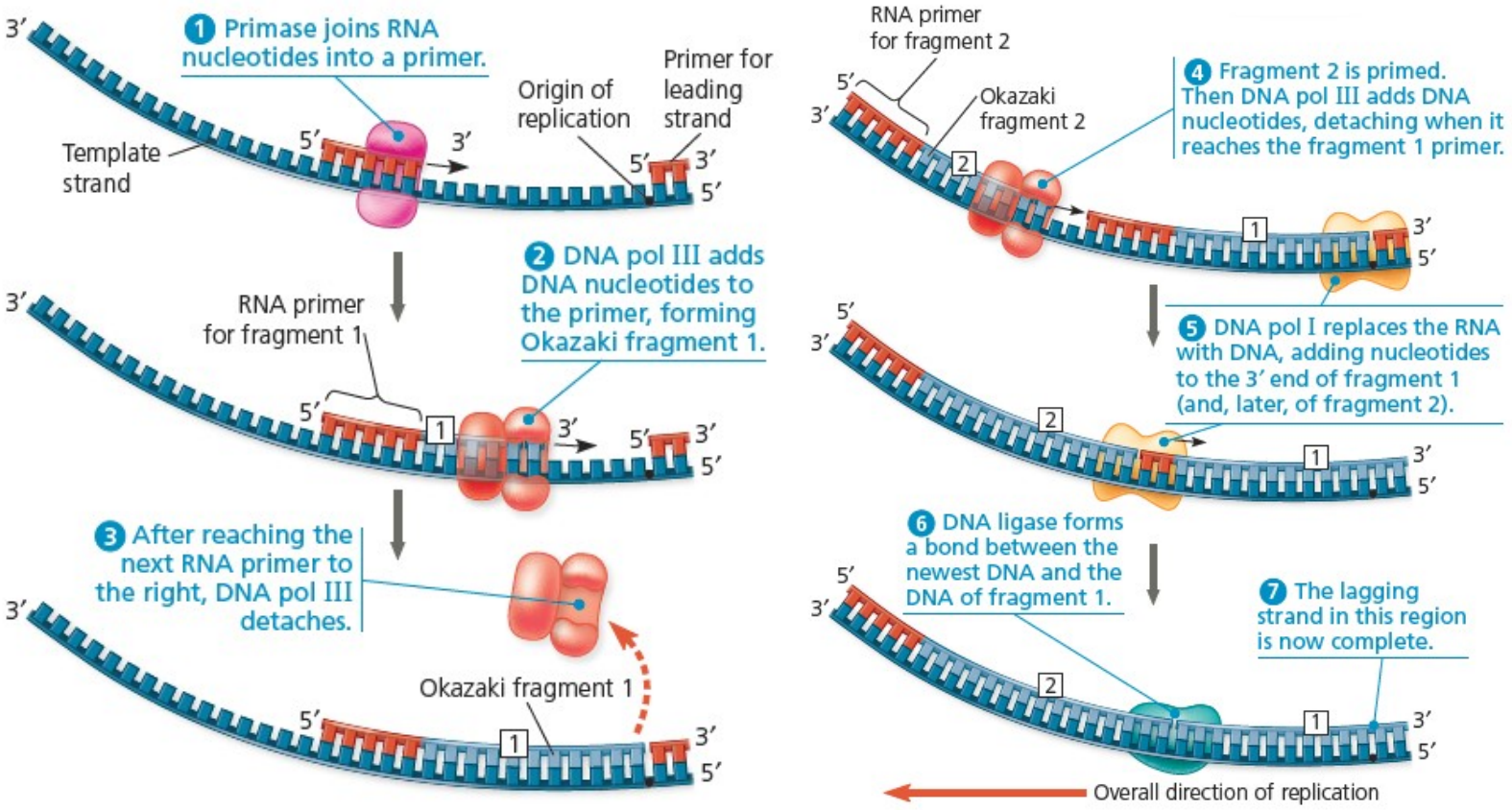
- *Lagging strand* - this strand is also polymerized in the 5’ to 3’ direction
  - the difference here is that the template strand is ‘read’ in the 5’ to 3’ direction so DNA pol III moves in the opposite direction of the replication fork and this **requires many**

Leading strand



Lagging strand

Animation



Molecular Events in DNA Replication

Elongation

Lagging strand synthesis - finishing it off

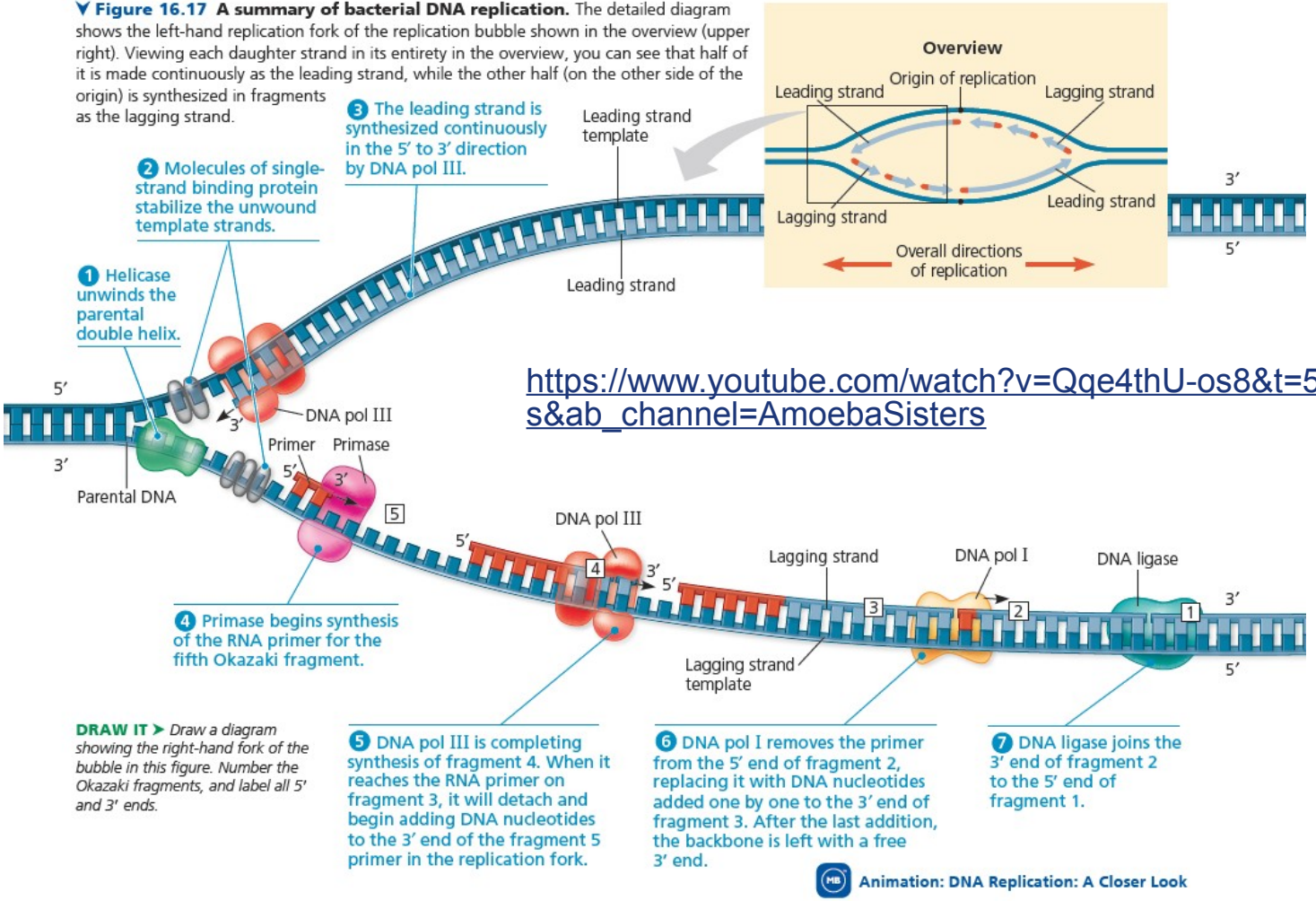
- ***DNA polymerase I*** removes the RNA primers and fills in the gaps by adding the complementary deoxyribonucleotides
- ***DNA ligase*** joins the Okazaki fragments together

Molecular Events in DNA Replication

Termination

- As soon as newly formed strands are created, the DNA automatically rewinds into double helix form
- All the proteins that were involved get disassembled once replication is complete:
  - **Helicase**
  - ***Topoisomerase II***
  - ***Single-strand binding proteins***
  - **DNA polymerase I and III**
  - ***primase***
  - ***DNA ligase***

Figure 16.17 A summary of bacterial DNA replication. The detailed diagram shows the left-hand replication fork of the replication bubble shown in the overview (upper right). Viewing each daughter strand in its entirety in the overview, you can see that half of it is made continuously as the leading strand, while the other half (on the other side of the origin) is synthesized in fragments as the lagging strand.



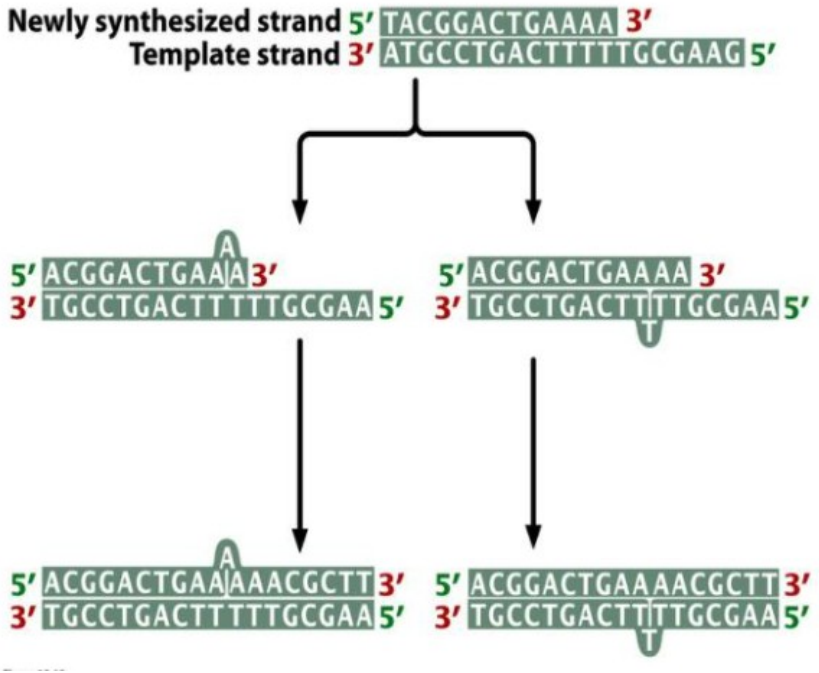
https://www.youtube.com/watch?v=Qqe4thU-os8&t=54s&ab_channel=AmoebaSisters

Proteins and enzymes	Functions
Helicase	Helps to unwind the parent DNA
Primase	Synthesize RNA primer used to generate Okazaki fragments
Single stranded binding protein	Helps to stabilize single-stranded regions of DNA when it unwinds
Topoisomerase II	Helps to relive the strain on the structure of the parent DNA that is generated from the unwinding of the double helix
DNA polymerase I, II and III	A group of enzymes with differing roles that include: addition of nucleotides to the 3’ end of a growing polynucleotide strand, removal of RNA primer and filling gaps between Okazaki fragments, proofreading newly synthesized DNA
DNA ligase	Joins the ends Okazaki fragments in the lagging strand synthesis

Correcting Errors During DNA Replication

- Human Cell Replication Time ~ 1 hour
  - Error rate ~ 1 per 1 billion nucleotide pairs replicated

- Possible Errors
  - **Mispairing** - T might pair with G or C might pair with A
  - **Strand slippage**
    - Template strand slip leads to a missing nucleotide
    - Synthesized strand slip



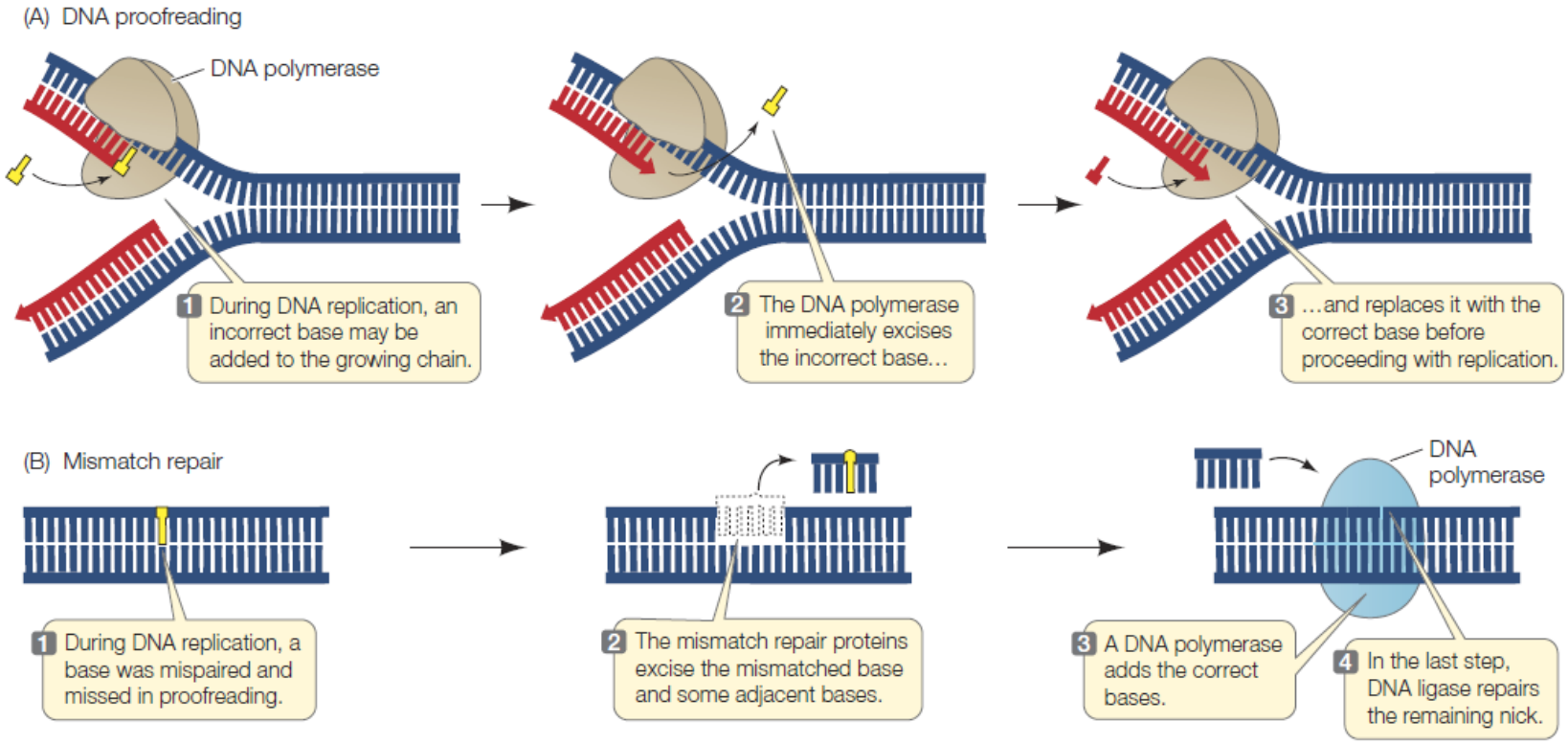


FIGURE 9.14 DNA Repair Mechanisms (A) During replication, the DNA polymerase checks for incorrect bases in the new DNA strand and immediately replaces them with correct ones. This process is called proofreading. (B) After replication, mismatch repair proteins search for incorrect bases that were missed by DNA polymerase and replaces them.

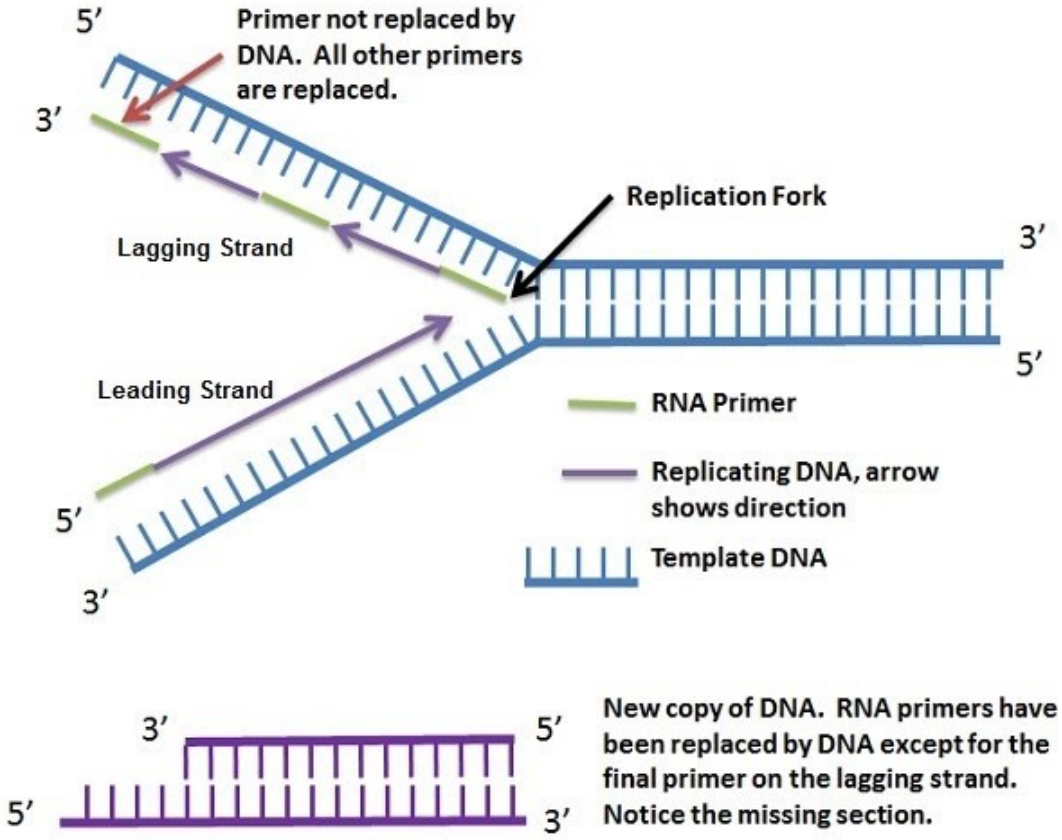
Correcting Errors During DNA Replication

- Methods for Fixing Errors
  - **Proofreading**
    - Mismatches can be fixed by DNA Polymerase I & II “proofreading”
    - 99% of these errors are fixed in this way
  - **Mismatch repair**
    - Incorrect complementary base pairing leads to structural deformities - caused by unexpected hydrogen bonding alignment
    - A group of proteins will recognize mismatches and will remove the incorrect base

Comparing DNA Replication in Eukaryotes and Prokaryotes

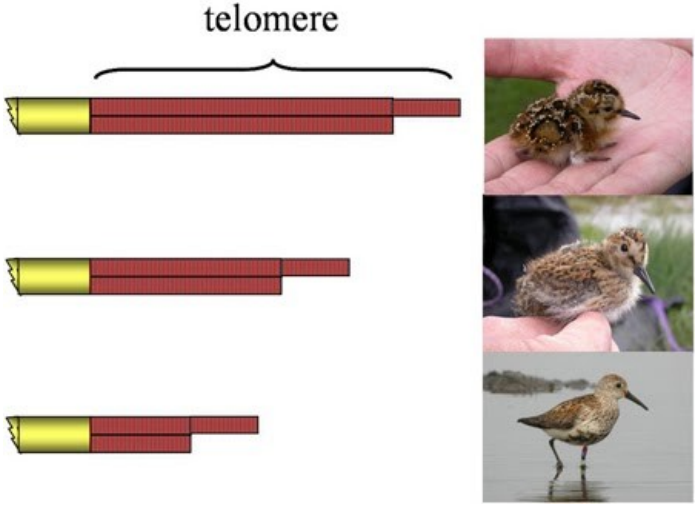
Eukaryotes	Both	Prokaryotes
- Relatively slower (40 nucleotides per second) - Approximately 13 different kinds of DNA polymerase enzymes - Multiple origins of replication - At the end, when the final primer is removed by DNA polymerase 1, the DNA gets shortened	- Require origins of replication - Elongation from 5’ to 3’ - Continuous leading strand and discontinuous lagging strand - Use primers for the synthesis of Okazaki fragments - Use DNA polymerase enzymes	- Relatively faster (1000 nucleotides per second) - 5 different kinds of DNA polymerase enzymes - Single origin of replication

Problem with DNA replication in Eukaryotes



Comparing DNA Replication in Eukaryotes and Prokaryotes

Telomere - a repetitive section of DNA, near each end of a chromosome; the presence of this sequence helps to protect from the loss of important genetic information during replication of the linear DNA in eukaryotic cells



- *Telomerase* is responsible for creating this region of DNA redundancy. It creates a region of expendable DNA that doesn't lead to coding sequence loss from DNA shortening as

https://www.youtube.com/watch?v=uqaR1q2trG8&ab_channel=Dr.EricBergDC

References

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