CONCEPT: DNA REPLICATION

Overview of DNA replication

● DNA replication begins by using one strand as a template (*semiconservative replication*)

□ **Replication origins** are specific DNA sequences where replication begins

- Initiation proteins bind these regions

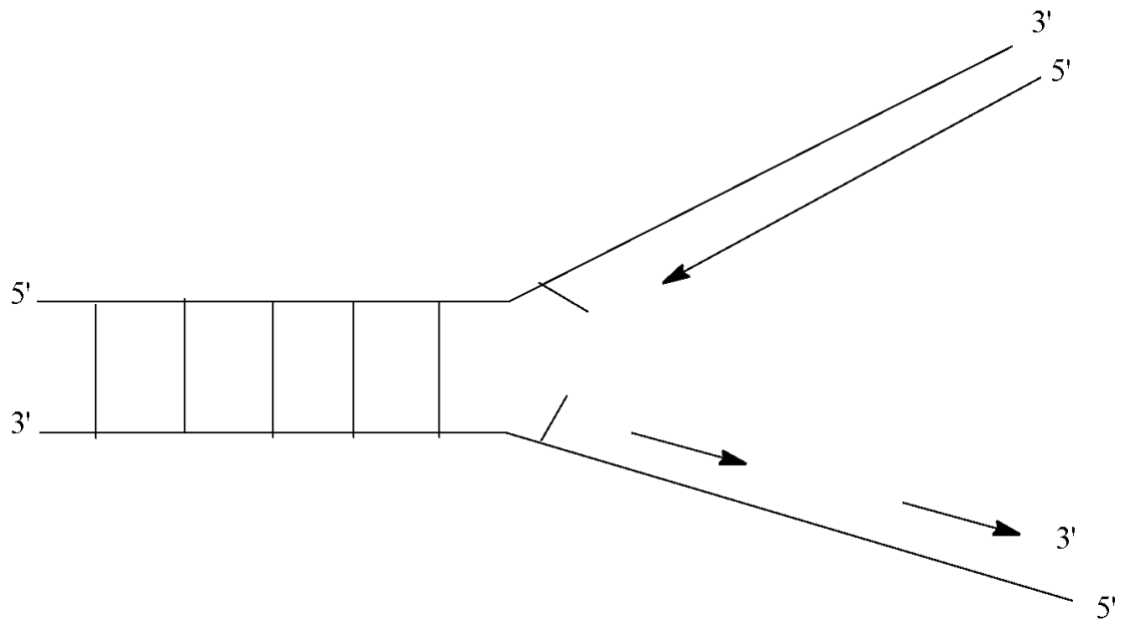
□ Two **replication forks** are formed at each replication origin

- **Bidirectional replication** occurs using each strand is used as a template strand

□ **DNA polymerase** catalyzes the replication of DNA

- Adds nucleotides to the 3' end of a growing DNA strand (replications forms new strand in 5' to 3' direction)

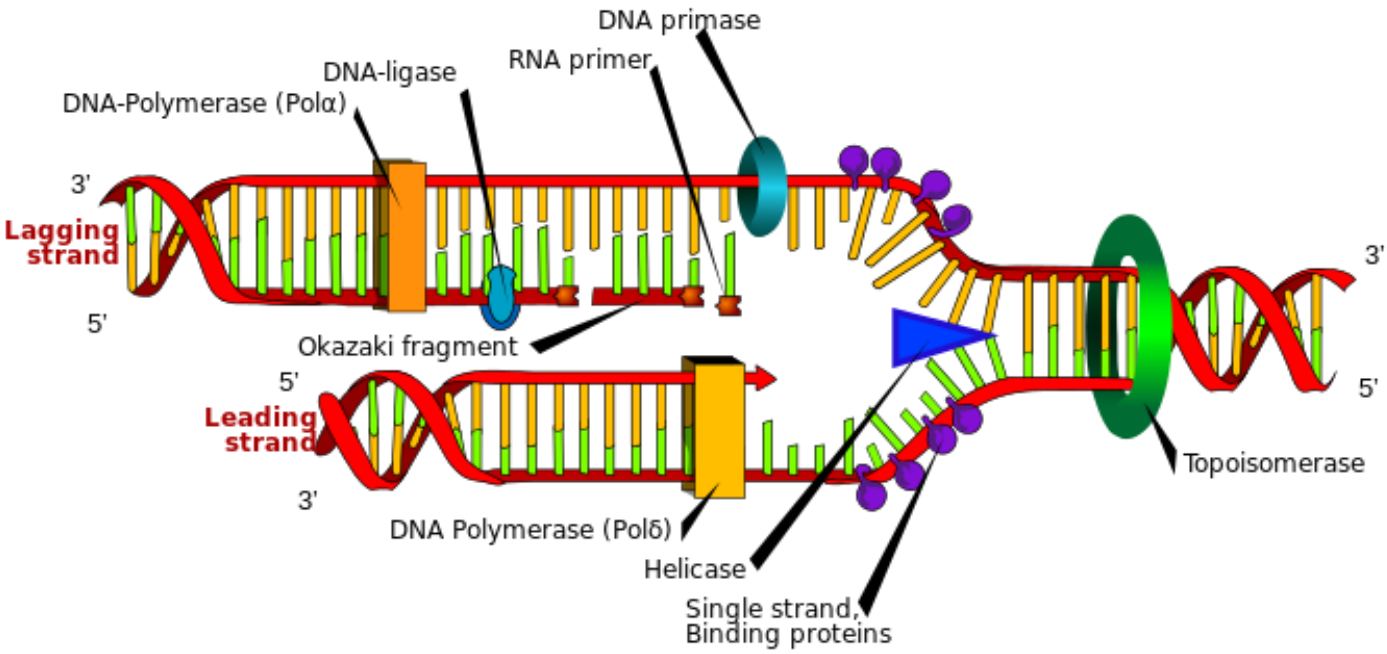
EXAMPLE: Structure of replication



DNA Replication Machinery

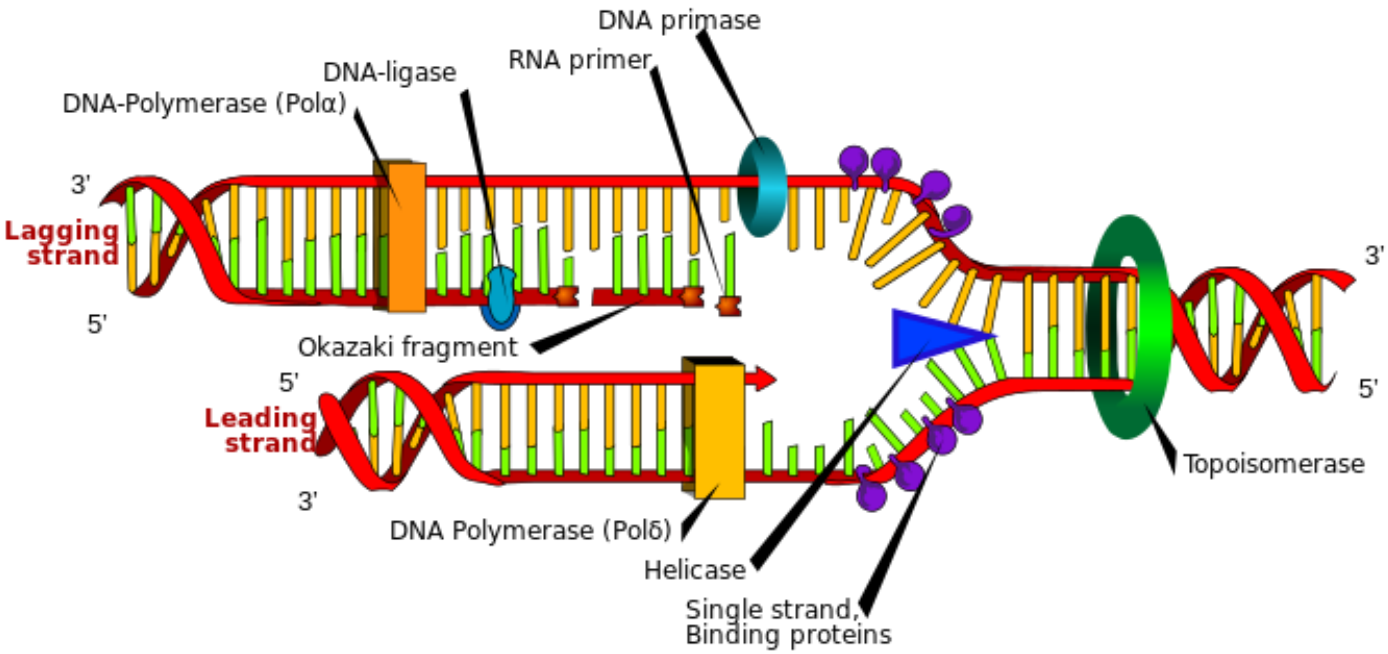
- DNA replication is characterized by bidirectional replication
    - □ Both strands are replicated at the same time
        - DNA Polymerase reads the template DNA strand in a 3’ to 5’ direction and synthesizes the new DNA strand in the 5’ to 3’ direction
        - **Leading strand** is *continuously* synthesized in the 5’ to 3’ direction
        - **Lagging strand** is *discontinuously* synthesized in the 5’ to 3’ direction
        - **Okazaki fragments** are the small fragments of replicated DNA that are bound together to form the lagging strand.
    - □ DNA Replication Machinery:
        - **RNA primer** is composed of around 10 RNA nucleotides and is used to begin DNA replication
        - **Primase** synthesizes the RNA primer utilizing the template DNA strand
        - *Repair polymerase* replaces the RNA with DNA
        - **DNA ligase** joins the Okazaki fragments together

EXAMPLE:



- DNA Replication Machinery (continued):
  - □ **DNA helicases** are enzymes that pry the two DNA strand apart (breaks hydrogen bonds between bases)
  - □ **Single-strand DNA binding proteins (SSB)** bind to single stranded DNA to prevent reforming a double helix
  - □ **DNA topoisomerases (DNA gyrase)** help prevent DNA supercoiling during replication
  - □ **Sliding clamp (beta clamp)** keeps DNA polymerase attached while it's replicating DNA
    - **Clamp loader** hydrolyzes ATP to clamp DNA (removed and reattached between Okazaki fragments)

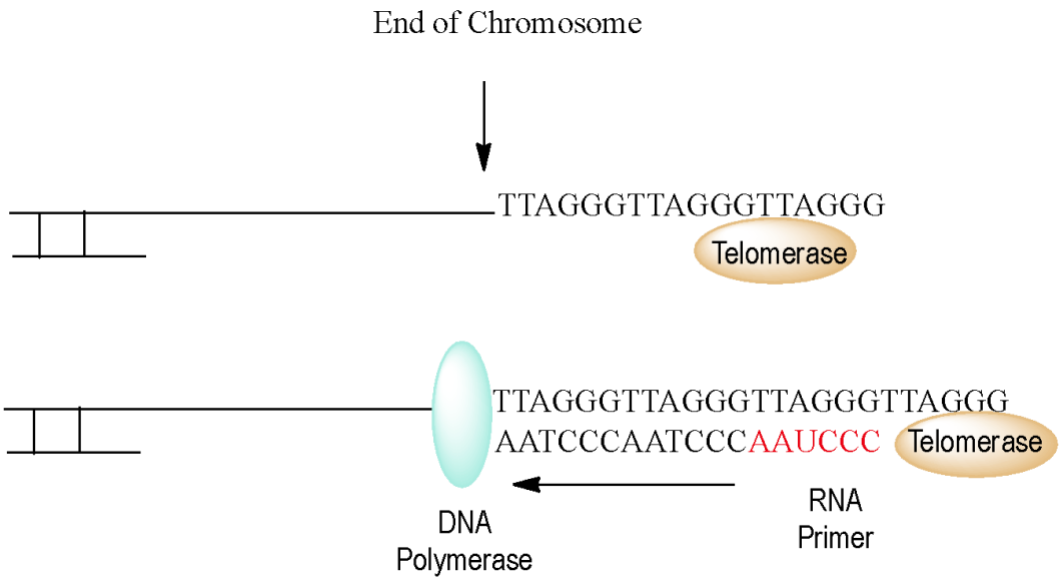
EXAMPLE:



Telomeres

- DNA replication occurs differently at telomeres (ends of the chromosomes)
    - □ Leading strand has no problems replicating the end of the chromosomes
    - □ Lagging strand can't replicate the end of the chromosome because the RNA primer can't bind
    - □ **Telomeres** are long repetitive nucleotide sequences at the end of the chromosome
        - **Telomerase** uses an RNA template (on the enzyme itself) to extend the lagging strand
            - Adds short repetitive DNA sequences to the DNA template – so lagging strand can finish

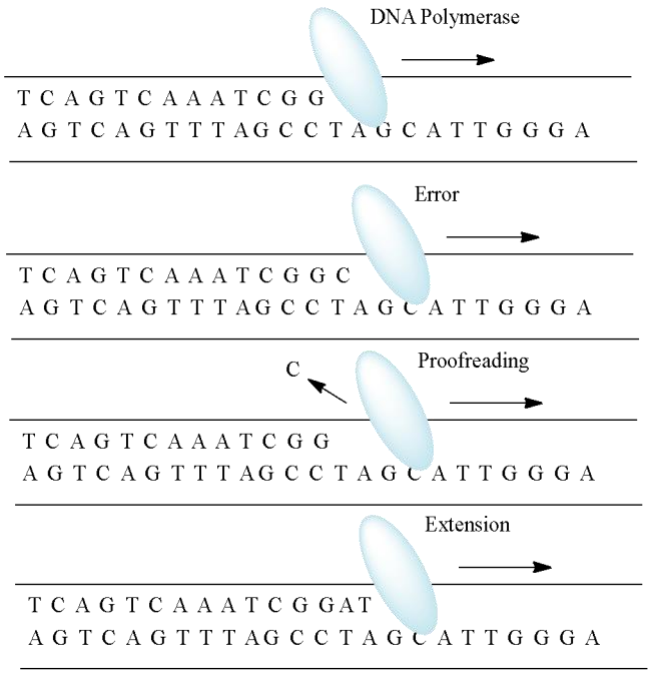
EXAMPLE: Telomerase allowing for replication of end of chromosome

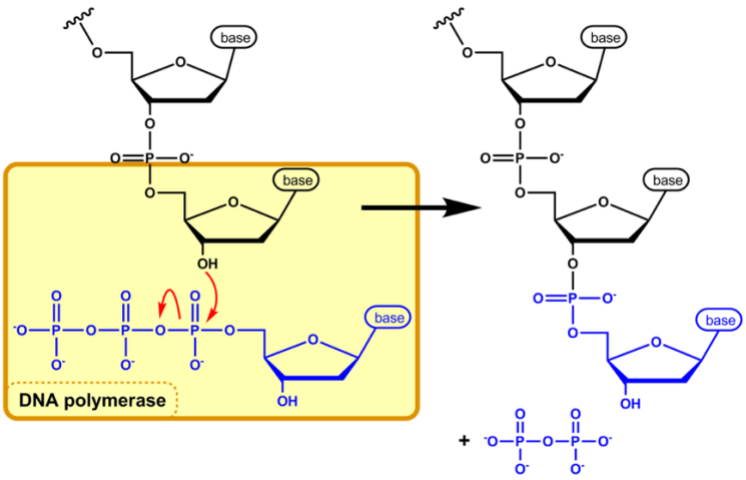


Replication Fidelity and Proofreading

- DNA replication is highly accurate
  - □ There is one error per every 10^7 (ten million) replicated nucleotide bases (human genome ~ 3 billion base pairs)
    - Very rarely, DNA Polymerase will match base pair incorrectly
    - **Proofreading** is the ability of the polymerase to double check and correct mismatched bases
  - □ Proofreading occurs before the next nucleotide is added
    - If the previous match was incorrect then it removes it and replaces it with the correct one
      - removal of mismatched bases is done by the DNA Polymerase's *3' to 5' exonuclease activity*
  - □ DNA can only be synthesized in the 5' to 3' direction:
    - **dNTPs** (deoxyribose nucleoside triphosphates) have 3 phosphate groups attached to a DNA nucleoside
      - 2 of the phosphate groups are removed releasing energy to power DNA synthesis
      - the 3' end of the growing DNA strand helps remove the 2 phosphates of the dNTPs
    - The 5' end wont undergo this reaction so the phosphate remains on the nucleoside instead of hydrolyzing

EXAMPLE: DNA polymerase proofreading & the addition of new dNTPs





PRACTICE

1. DNA replication occurs differently at telomeres.
    a. True
    b. False

2. DNA is replicated in which of the following directions?
    a. 5’ to 3’
    b. 3’ to 5’
    c. 5’ to 5’
    d. 3’ to 3’

3. Which of the following proteins are responsible for unwinding the DNA double helix for replication?
    a. DNA helicases
    b. Single stranded binding proteins
    c. DNA Topoisomerases
    d. Sliding Clamp

4. Only the lagging strand uses telomerase to replicate the ends of the telomeres.
    a. True
    b. False