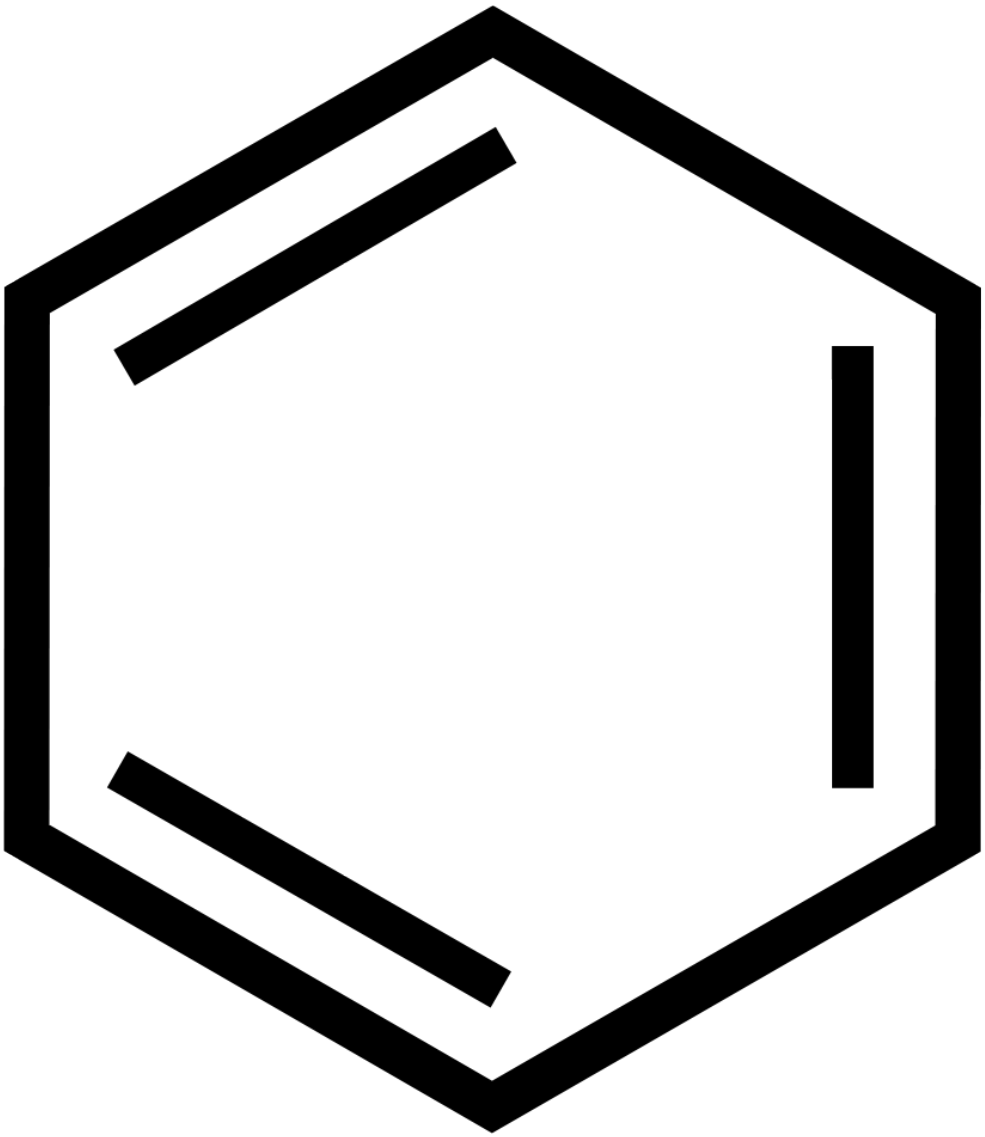

CLUTCH

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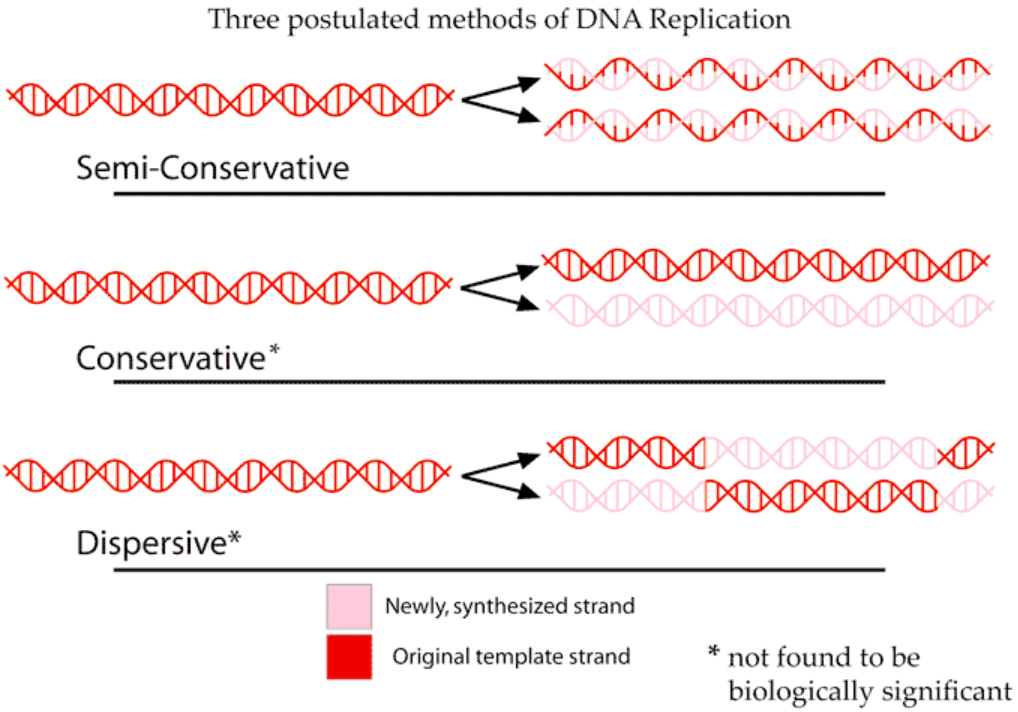
CONCEPT: SEMICONSERVATIVE REPLICATION

- Before replication was understood, there were three _____ of how DNA is replicated
  - □ **Conservative replication** states that after replication, there is one old strand and one new strand
    - The double helix separates, replicates, and the old helix reforms
  - □ **Dispersive replication** states that after replication the strands have some old sections and some new sections
  - □ **Semiconservative replication** states that each strand serves as a template for a new strand
    - After replication, each strand has one old strand and one new strand

EXAMPLE:



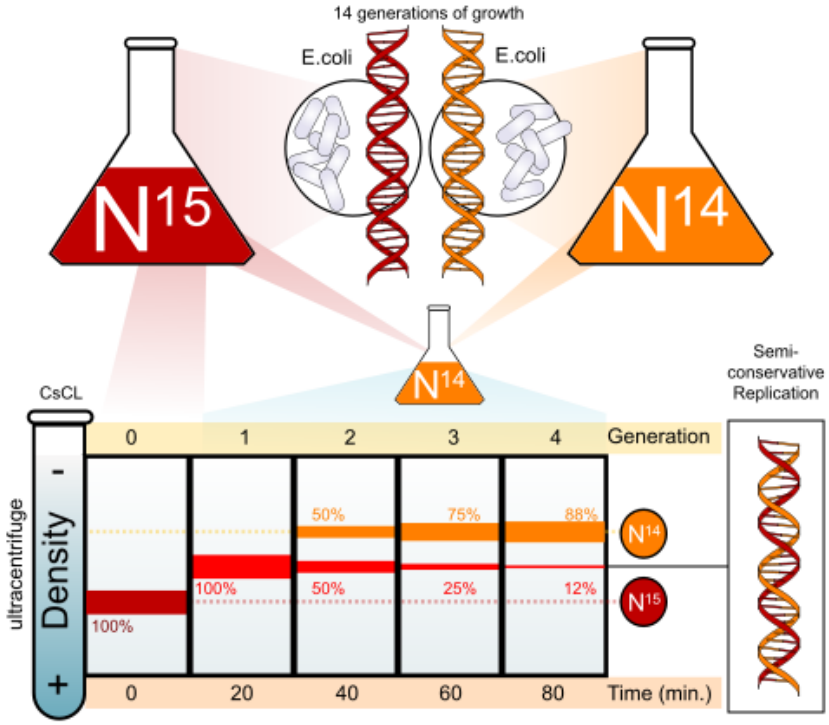
- The **Meselson-Stahl** experiment showed that DNA replicates via _____ replication
  - □ Grew *E.coli* with heavy nitrogen (^{15}N)
    - All Nitrogen incorporated into the DNA was heavy nitrogen
  - □ Moved this *E.coli* into plate with normal nitrogen (^{14}N)
    - New DNA strands will have normal nitrogen and old DNA strand will have heavy nitrogen
  - □ After one round of replication there was only one band of a single, mixed weight
  - □ After two rounds of replication there were two bands: one band of mixed weight and one band of light weight

- Suggested semiconservative replication

- Conservative replication would have one heavy band, and one light band after one round of replication

- Dispersive replication would have one band of mixed weight after each round of replication

EXAMPLE:



PRACTICE

1. DNA is replicated through which method?

a. Conservative replication
b. Nonconservative replication
c. Dispersive replication
d. Semiconservative replication

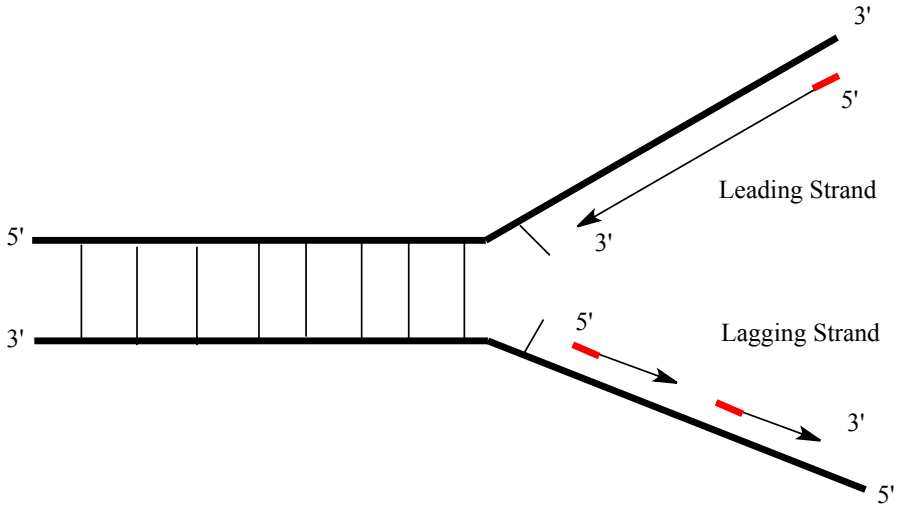
2. Which of the following experiments showed that DNA replicated via semiconservative replication?

a. Hershey/Chase
b. Francis/Crick
c. Meselson-Stahl
d. T.H. Morgan

CONCEPT: DNA REPLICATION

- DNA replication occurs _____ on each strand of DNA
  - □ On the **leading strand** DNA replication proceeds continuously adding nucleotides from 5' to 3'
  - □ On the **lagging strand** DNA replication proceeds continuously adding nucleotides from 5' to 3'
  - □ DNA can only be synthesized from 5' to 3', meaning that the template strand is read from 3' to 5'

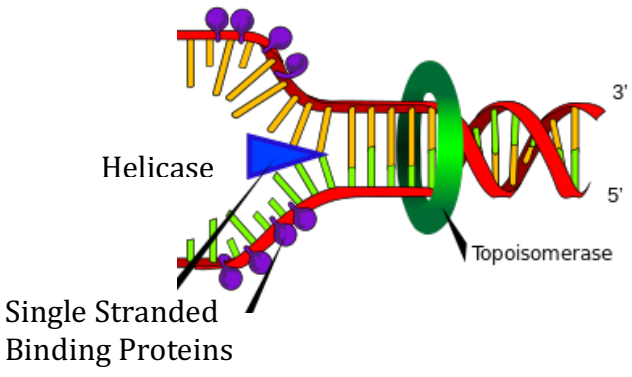
EXAMPLE:



Steps of Replication

- The first step of replication involves _____ the double helix
  - □ **DNA Helicase** attaches to the DNA and unwinds the double helix
    - Breaks hydrogen bonds
  - □ **Single-strand binding proteins** bind to the unwound DNA strands and prevent them from reforming
  - □ **Topoisomerases** relax the supercoiling caused from DNA unwinding

EXAMPLE:



● The second step of replication involves the use of important _____

□ The **Pol III Holoenzyme** consists of **DNA polymerase III** and accessory replication proteins

- **DNA pol III** replicates the DNA

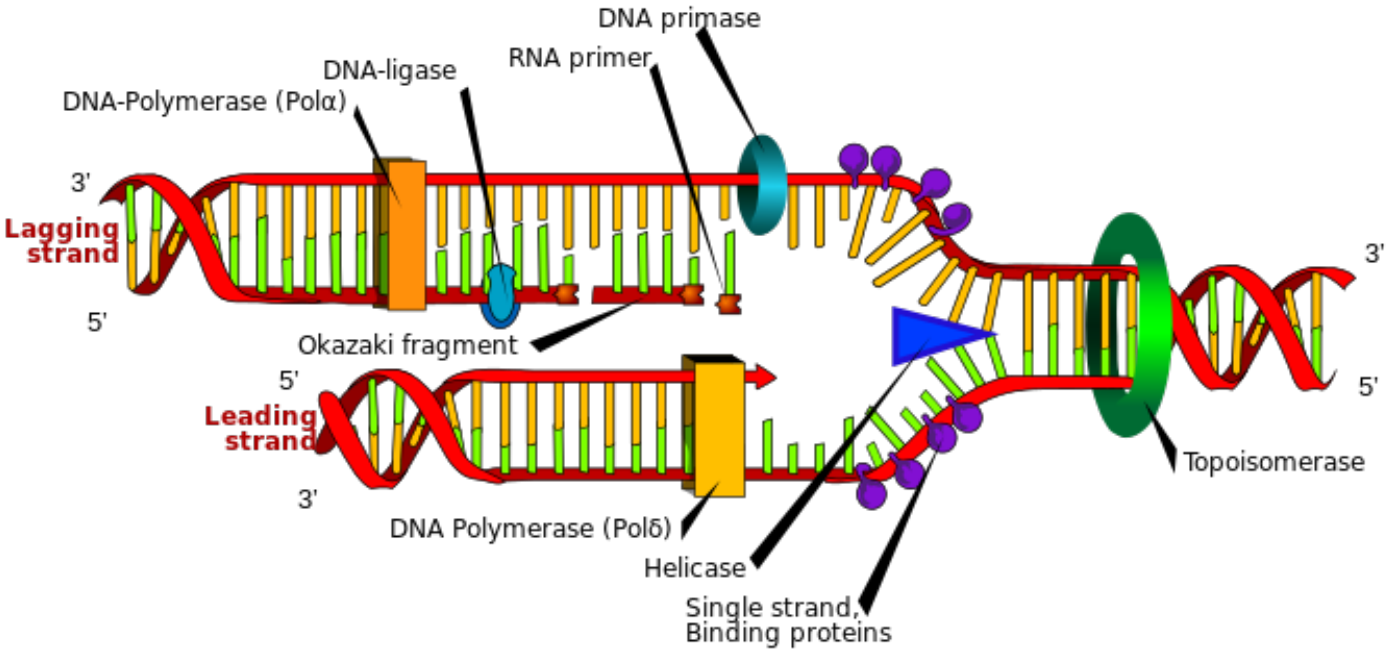
- Without accessory proteins, DNA pol III would only add around 10 nucleotides before falling off the DNA

□ To start replication the **primase (primosome)** enzyme synthesizes RNA primers

- DNA pol III recognizes these primers and starts DNA replication

- **DNA pol I** removes the RNA primers and replaces them with DNA after replication has started

EXAMPLE:



● The lagging strand is replicated discontinuously, creating many replicated DNA _____

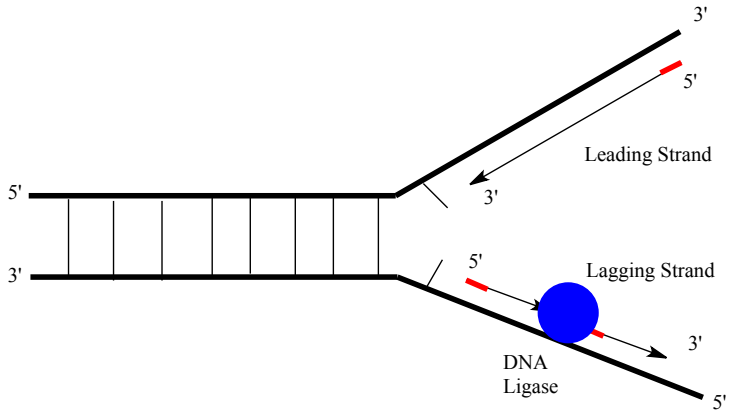
□ As the helix is unwound, primase adds RNA primers onto the 3' end of the template strand

- Replication continuous until it reaches the beginning of the strand, or a previous fragment

- **Okazaki fragments** are created by the discontinuous replication on the lagging strand

□ **DNA ligase** joins all Okazaki fragments together, to create a single new replicated strand of DNA

EXAMPLE:



Proofreading

● DNA replication occurs with extremely high _____

□ There is less than 1 error per every 10^{10} nucleotides replicated

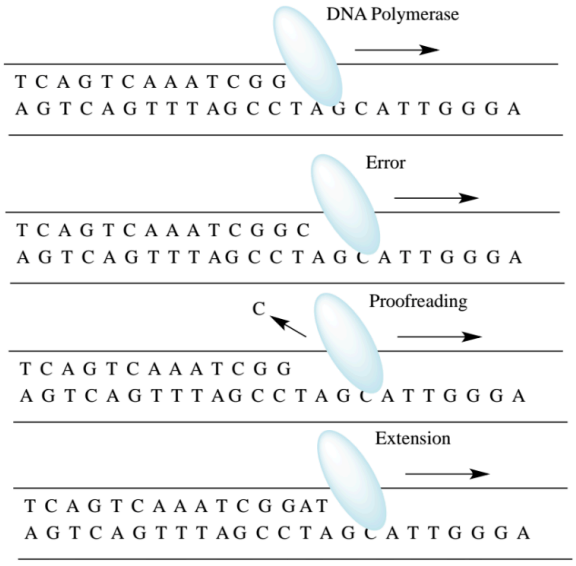
- Nearly 1000 nucleotides are replicated per strand, per second

□ The DNA polymerase has **proofreading** abilities

- If a DNA mismatch is made between two base pairs, it will pause, excise the base, and replace it

- DNA polymerase has 3' to 5' **exonuclease** activity – meaning it can excise a mismatched nucleotide

EXAMPLE:



PRACTICE

1. Which of the following proteins is responsible for unwinding the double stranded DNA?
    a. Topoissomerases
    b. Single-stranded binding proteins
    c. DNA helicase
    d. DNA polymerase III

2. DNA replication synthesizes DNA in which direction?
    a. 5’ to 3’
    b. 3’ to 5’
    c. Leading strand 5’ to 3’, lagging strand 3’ to 5’
    d. Leading strand 3’ to 5’, lagging strand 5’ to 3’

3. What would happen to DNA replication in DNA polymerase lost its 3' to 5' exonuclease activity?
    a. Replication would speed up
    b. Proofreading would stop and replication would stall
    c. Replication would only occur on the leading strand
    d. Replication would only occur on the lagging strand

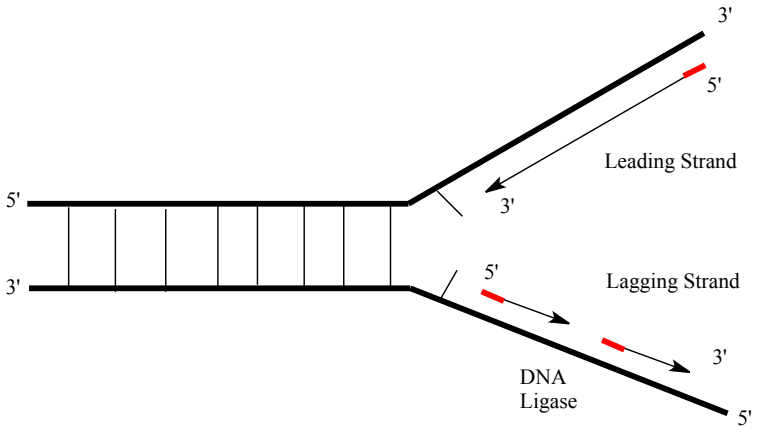
4. Which of the following proteins is responsible for synthesizing RNA primers?
    a. Topoisomerases
    b. Single-stranded binding proteins
    c. Primase
    d. DNA polymerase III

5. The short DNA fragments created during lagging strand replication are called what?
    a. Primers
    b. Okazaki Fragments
    c. Replicates
    d. Exonucleases

CONCEPT: TELOMERES AND TELOMERASE

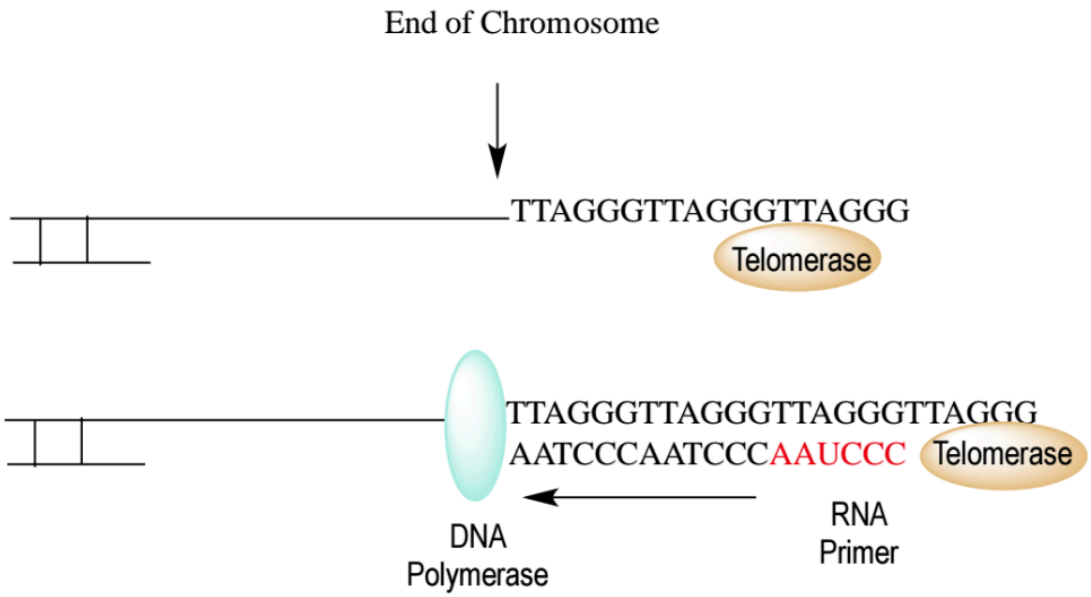
- The ends of chromosomes (**telomeres**) are replicated _____
  - □ In the leading strand, replication continuous all the way to the end
  - □ In the lagging strand, primers are required to create Okazaki fragments – but what about after the last primer?
    - If the end was replicated normally the chromosome would shorten after each replication

EXAMPLE:



- **Telomerase** is the enzyme responsible for replicating the telomeres on the lagging strand
  - □ Telomerase _____ onto the 3' end of DNA molecules
    - Telomerase contains a short RNA molecule that reads 3' AAUCCC 5' – this is a template for replication
      - Called **reverse transcription** when RNA is used as a template for DNA
  - □ Telomerase then repeatedly adds 5' TTAGGG 3' onto the end of the telomere
    - This prevents the telomere ends from shortening
  - □ Telomeres contain lots of TTAGGG repeats

EXAMPLE:



□ Different cell types contain different _____ of telomerase

- *Germ cells* have a lot of telomerase

- *Somatic cells* have little to no telomerase

□ In somatic cells, the telomeres continue to get shorter, until the cell dies

PRACTICE

1. True or False: Lagging strand telomeres are replicated in the same way as the rest of the chromosome
    a. True
    b. False

2. Which cell type contains the most telomerase
    a. Kidney cells
    b. Brain cells
    c. Egg cells
    d. Skin cells

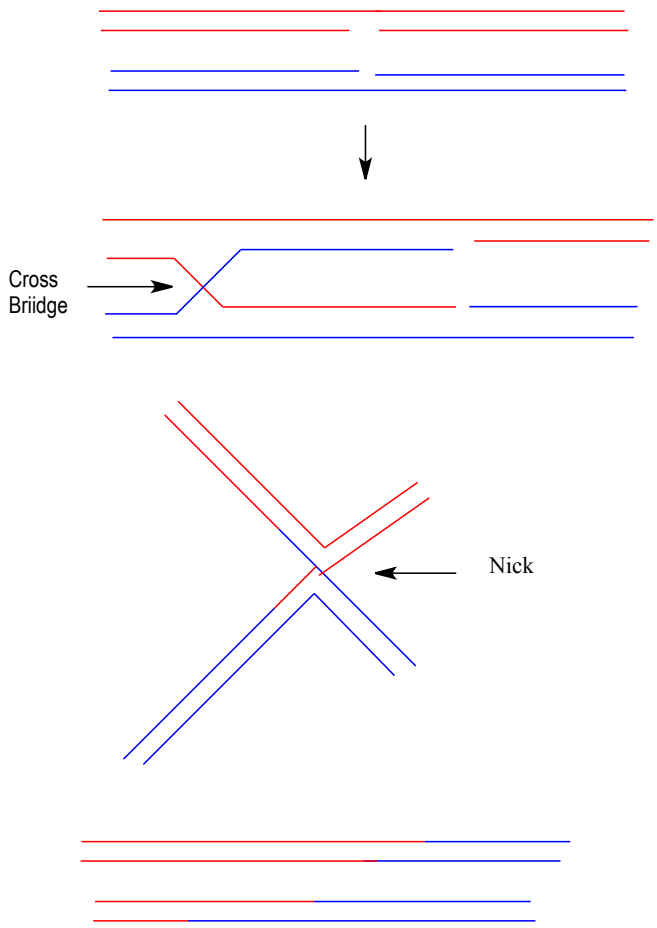
3. Telomeres are composed of what type of DNA molecule?
    a. Single A repeats
    b. Repeats of a 5’ TTAGGG 3’ sequence
    c. Repeats of a 3’ AAUCCC 5’ sequence
    d. Single T repeats

CONCEPT: RECOMBINATION

Recombination

- **Homologous recombination** is an exchange that occurs at equivalent positions along 2 homologous chromosomes
  - □ It can be _____ by single strand breaks or double strand breaks
  - □ Single strand breaks follow certain steps
    - DNA duplex pairs together and there is a nick on a single strand of both homologous chromosomes
    - The nicked strands are displaced, and paired with the other nicked strands. Complex is sealed by a *ligase*
    - **Branch migration** occurs when the paired cross-bridge structures moves down the chromosome
      - Hydrogen bonds are broke and reformed
    - A nick occurs in the other, non-paired, strands
    - A recombinant duplex is formed

EXAMPLE:



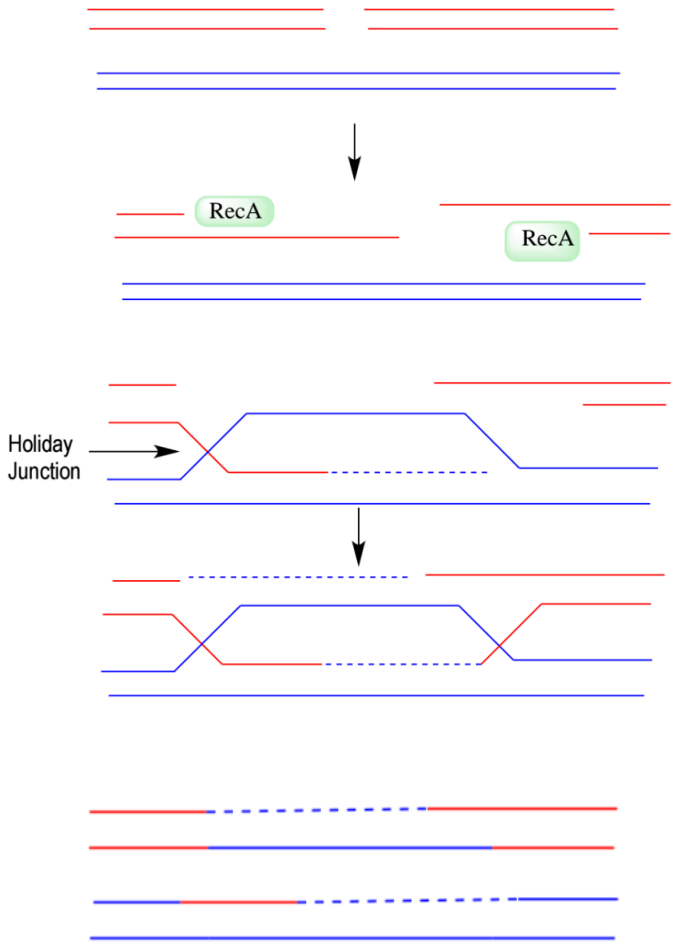
Recombinant Duplexes

Double Strand Breaks

□ Double strand breaks follow certain _____

- *Endonucleases* remove nucleotides at the place where the DNA break has occurred
    - Creates a 3' overhang, which is protected by **RecA**
- Broken strands invade the other double helix on the homolog
- DNA repair fills the gaps
    - Forms **holiday junctions**, which are structures made from four DNA strands
- End result: recombination between two strands

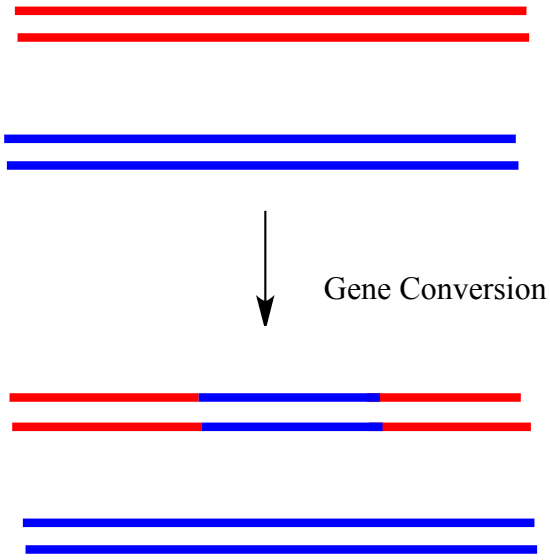
EXAMPLE:



Gene Conversion

- **Gene Conversion** is the nonreciprocal genetic exchange between two closely linked genes
  - □ Caused due to a _____ of base pairs during the duplex formation
    - Has the ability to convert one allele into another allele
  - □ Normal allocation of Aa genotype into gametes: ½ get A and ½ get a
    - Gene conversion will cause ¾ to get A and ¼ to get a (or vise versa)

EXAMPLE:



PRACTICE

1. Homologous recombination is defined as what?
    a. Exchange of alleles from two different genes
    b. Exchange of regulatory regions from two different genes
    c. Exchange that occurs at equivalent positions in two non-homologous chromosomes
    d. Exchange that occurs at equivalent positions in two homologous chromosomes

2. True or False: Homologous recombination repairs double-strand breaks only
    a. True
    b. False

3. Gene conversion can cause a Aa genotype to be sorted into gametes in which way?
    a. All gametes get A allele
    b. All gametes get a allele
    c. ½ gametes get A and ½ gametes get a
    d. ¾ gametes get a and ¼ gametes get A