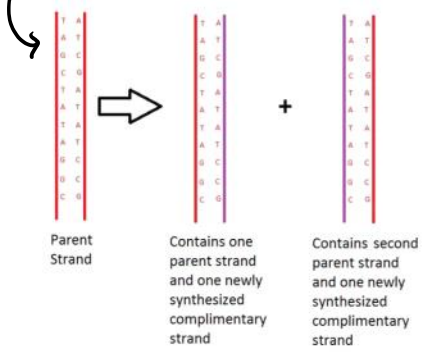
7.1 & 7.2 DNA Replication

Monday, January 7, 2019 12:50 PM

Name:_____ Per:_____ Date:_____

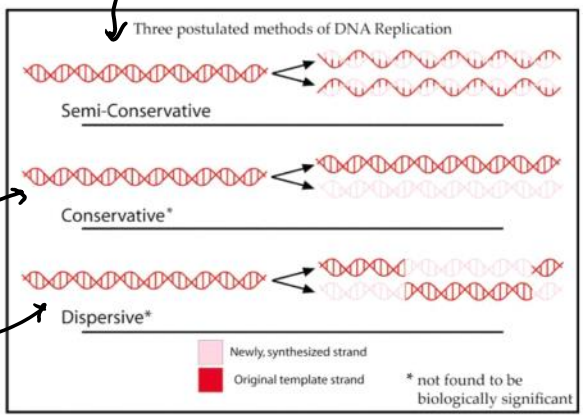
7.1 & 2.7 DNA Replication Notes

Semi-Conservative Replication



What does "semi-conservative" mean?

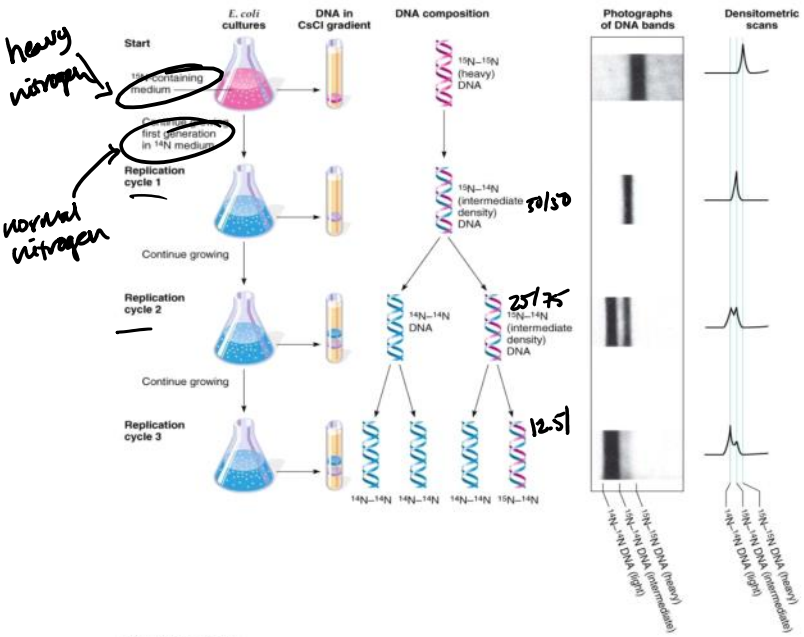
DNA unfolds in the middle and makes a copy of both sides



What are the alternatives to semi-conservative replication?

Conservative: The DNA stays wrapped around histone proteins and it would make a duplicate of itself.

Dispersive:
DNA would shrink itself into 10-20 nucleotide segments, and then the DNA would be copied



How do we know DNA replication is semi-conservative?

Messelson and Stahl Experiment

START: 100% DNA w/ ^{15}N (heavy nitrogen)

CYCLE 1: 50% ^{15}N DNA
50% ^{14}N DNA

CYCLE 2: 25% ^{15}N DNA
75% ^{14}N DNA

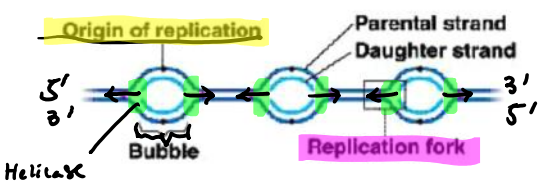
CYCLE 3: 12.5% ^{15}N DNA
rest ^{14}N DNA

DNA Replication

ENZYMES / FUNCTION

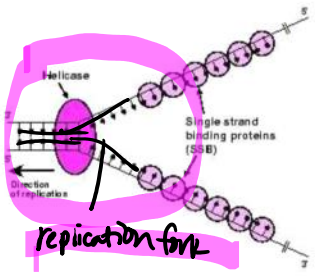
DNA Helicase

1. **Breaks** hydrogen **bonds between bases unzipping the double helix**
   a. Begins to unwind the DNA at the origin of replication (a specific nucleotide sequence)
   b. Helicase enzymes move in both directions from the point of Origin, forming a replication bubble



- helicase

   c. At either end of the replication bubble is a replication fork, a Y-shaped region where the new strands of DNA are elongating

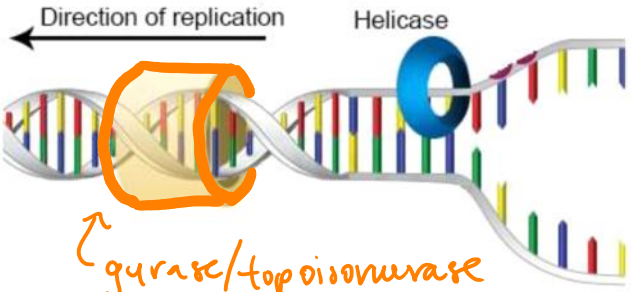


   d. In prokaryotic cells there is one origin

   e. In eukaryotic cells there are 100's to 1000's of origins

Gyrase

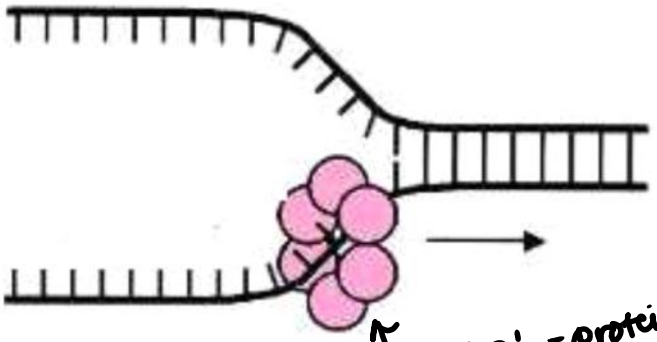
2. **Ahead of the replication fork, unwinds the super coil of DNA. Also known as topoisomerase.**



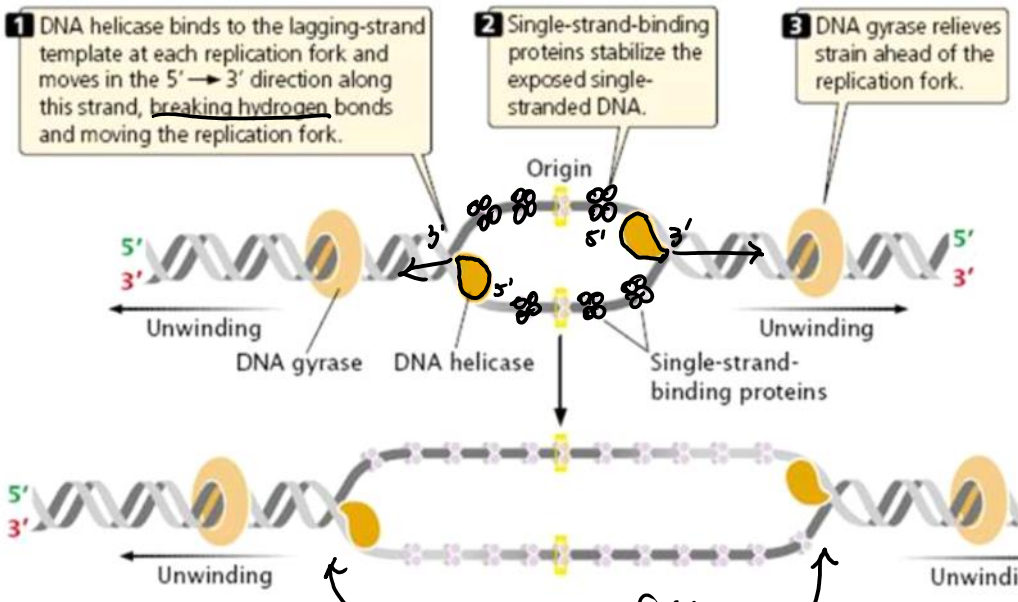
gyrase/topoisomerase
- relieves tension in DNA strand

Single Stranded Binding Proteins

3. Hold the DNA strands apart (keeps the separated strands apart and stabilize the unwound DNA).



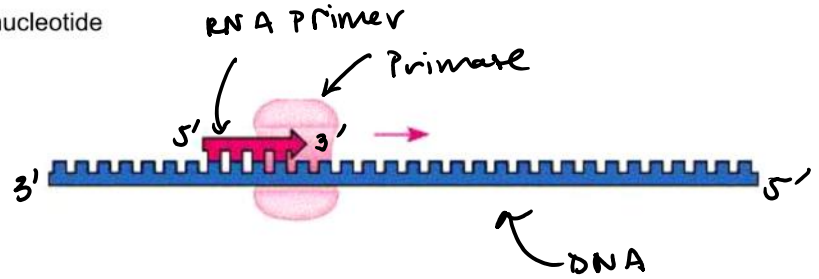
SSB's - proteins that bind to exposed bases to prevent them from annealing (making H bonds)



replication forks move farther from the origin along both sides of the molecule - till replication is finished.

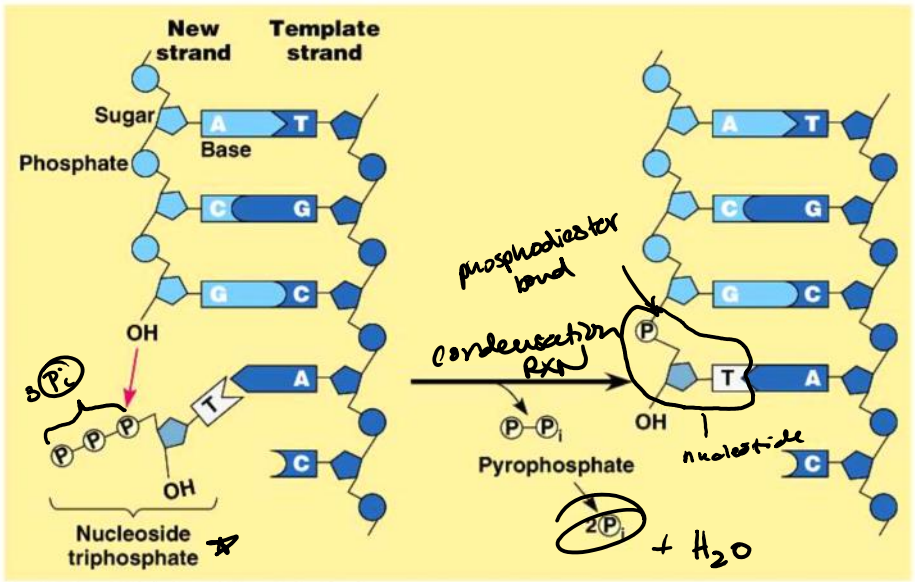
Primase & Primers

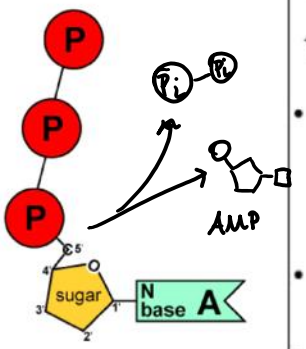
4. The enzyme primase adds primers nucleotides to complementary base sequences. These RNA nucleotides act as a primer for DNA nucleotides. (a place for DNA Polymerase to attatch to)
   a. primers are short segments of RNA, about 10 nucleotides long
   b. Must have a primer because DNA polymerase can only add nucleotides to another nucleotide



DNA Polymerare III

5. Adds DNA nucleoside tri phosphates to the RNA primer sequence in a 5′ → 3′ direction (Reads 3′ → 5′ Builds 5′ → 3′)





Adenine triphosphate (ATP) → Adenine monophosphate (AMP/nucleotide)

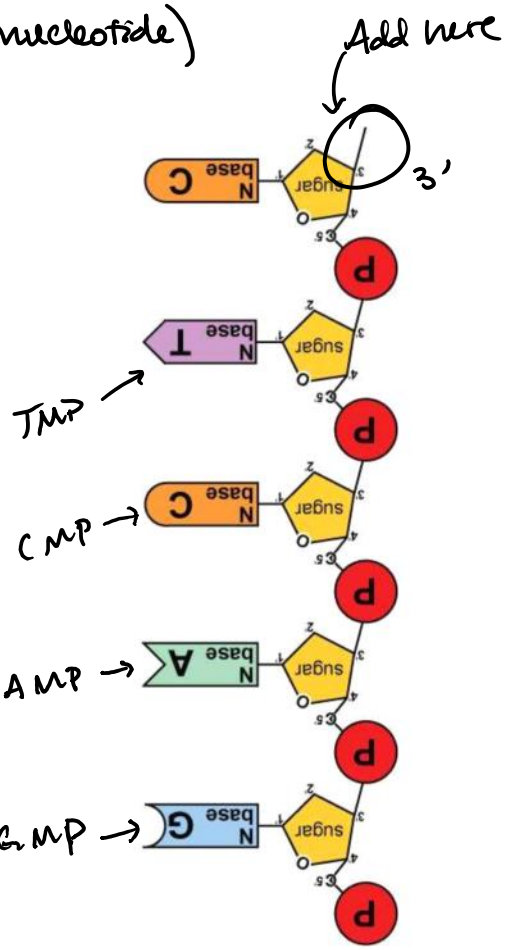
Where does the energy for bonding usually come from?

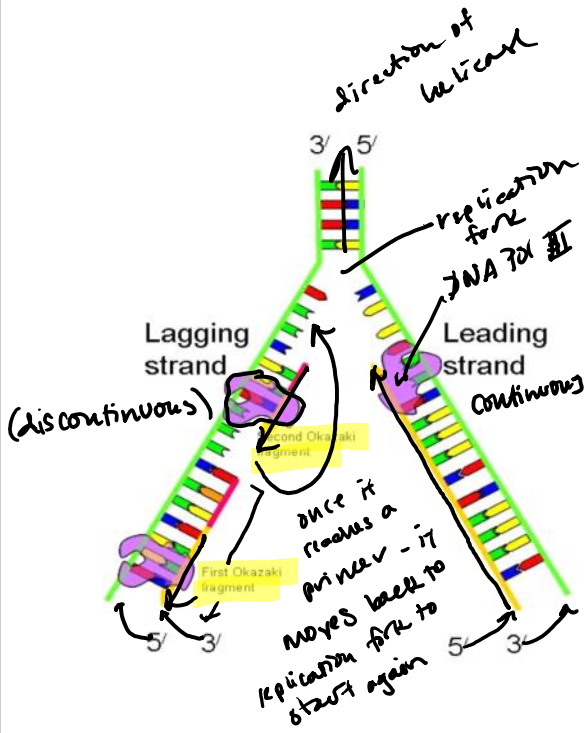
The nucleotides arrive as nucleosides.

- DNA bases with P–P–P (triphosphates)
  - P-P-P = energy for bonding (triphosphates)
- DNA bases arrive with their own stored energy source for bonding
- bonded by enzyme: DNA Polymerase III

ADDING BASES

- DNA polymerasre III can only add nucleotides to 3' of a growing DNA strand
- Need a "starter" nucleotide to bond to .
- Strand only grows 5' → 3'





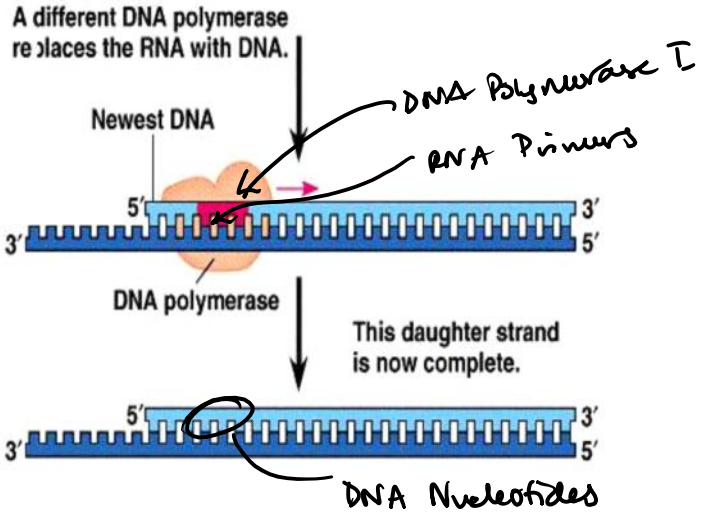
LEADING STRAND: DNA polymerase III can synthesize a complementary strand on one side of the template in the 5' to 3' direction with no problem.

LAGGING STRAND:

- DNA polymerase III must work away from the replication fork.
- Makes a short strand of DNA, called an Okazaki fragment.
- As the bubble widens, it can make another short strand, and so on.

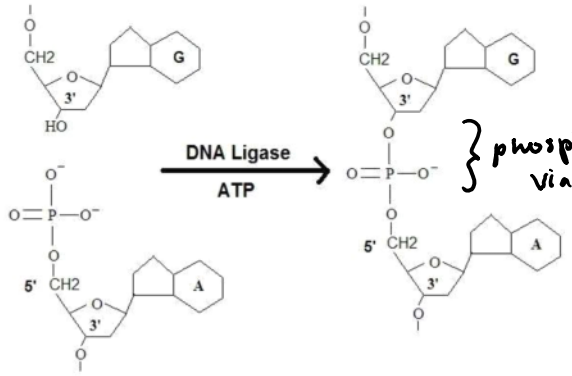
DNA Polymerase I

6. **RNA primers are removed and replaced with** DNA **nucleotides by** DNA Polymerase I.



DNA Ligase

7. **Along the** leading **strand the Okazaki fragments are joined by DNA** ligase **to form a single DNA strand.**



phosphodiester bond via condensation reaction

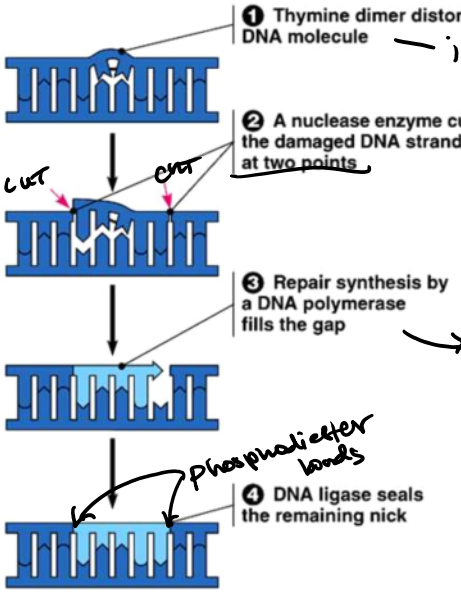
8. **Proofreading of DNA by DNA polymerase I**

a. proofreads & corrects typos

b. repairs unmatched bases

c. removes abnormal bases

d. reduces error rate from 1 in 10,000 to 1 in 100 million bases



irregular hydrogen bond creates a bulge ↳ notifies DNA polymerase I of mistake

DNA polymerase I fixes mistake

phosphodiester bonds

Replication Fork Drawing

Using pencil, you will draw a representation of DNA replication along the leading and lagging strands. Follow the directions below, drawing each element in its proper location along the replicating DNA strand. Once you are sure everything is in the correct place, complete your drawing by adding color to distinguish objects as separate.

- On the diagram below, label the 5' and 3' ends of both parental DNA strands (you can make up which is which)
- Label the replication fork
- Draw and label DNA gyrase
- Draw and label helicase
- Label the overall direction of DNA replication
- Draw and label single stranded binding proteins
- Draw and label the leading strand
- Draw and label a single DNA polymerase III on the leading strand
- Draw and label an RNA primer on the leading strand
- Draw and label a DNA polymerase I on the leading strand
- On the lagging strand, draw and label at least three Okazaki fragments
- On the lagging strand, draw and label at least two DNA polymerase III enzymes
- On the lagging strand, draw and label at least two RNA primers
- On the lagging strand, draw and label at least one primase enzyme
- On the lagging strand, draw and label at least one DNA polymerase I enzyme
- On the lagging strand, draw and label at least one DNA ligase enzyme