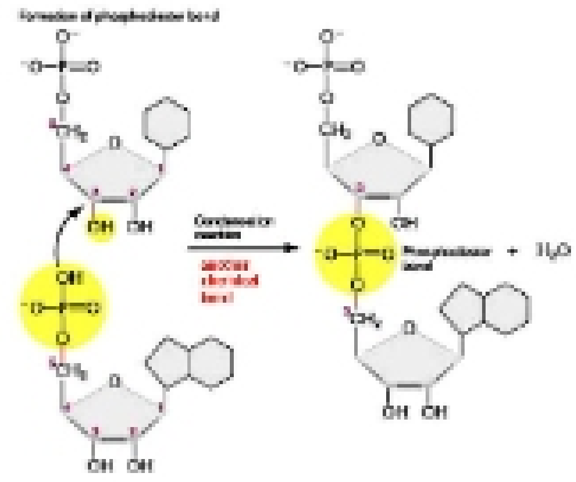
Chapter 12 – DNA Replication & Manipulation (S-Phase of Cell Cycle)

**Happens during Mitosis/Meiosis only; DNA transcription/translation is a different process*

I. **Review of DNA Structure**

2 nucleotides are linked by series of covalent bonds called a **phosphodiester bond** *(C-O-P-O-C)*

A. **The Backbone**
   1. Linear strand of alternating phosphate & sugar groups
   2. Negatively charged
   3. Tail-end phosphate (not connected to a sugar) will be HPO_4 (H attached to top O), instead of PO_4 *(see diagram)*
   4. All sugars in strand are oriented in same direction (5' → 3' or 3' → 5')
   5. Strand has polarity or direction



B. **Base Sequence**
   1. Variable part of DNA – holds info

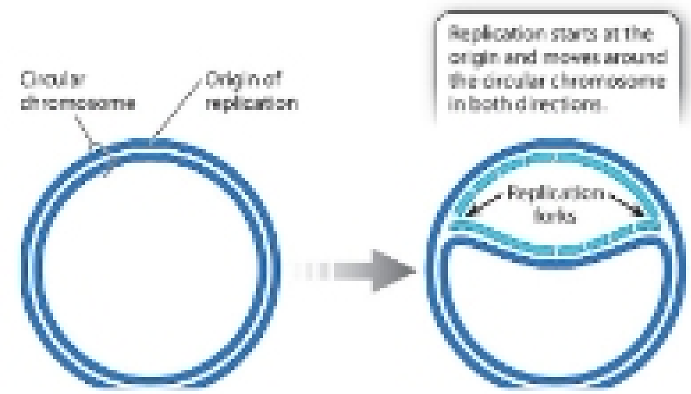
C. **Double-helix**
   1. 2 strands stay together by H-bonds that form between **complimentary bases** (1 purine—1 pyrimidine)
      a. A-T, G-C
         i. This specificity is what keep width of DNA constant
      b. Bases also interact noncovalently with the ones above & below and "stack" (tightly group), which stabilizes helix

II. **How DNA Replicates**

A. **Semi-Conservative replication**: each new **double** helix has one daughter strand & one parental strand

B. Starts at sites called **"origins"**

C. Bacteria have 1 small, circular chromosome with one origin:

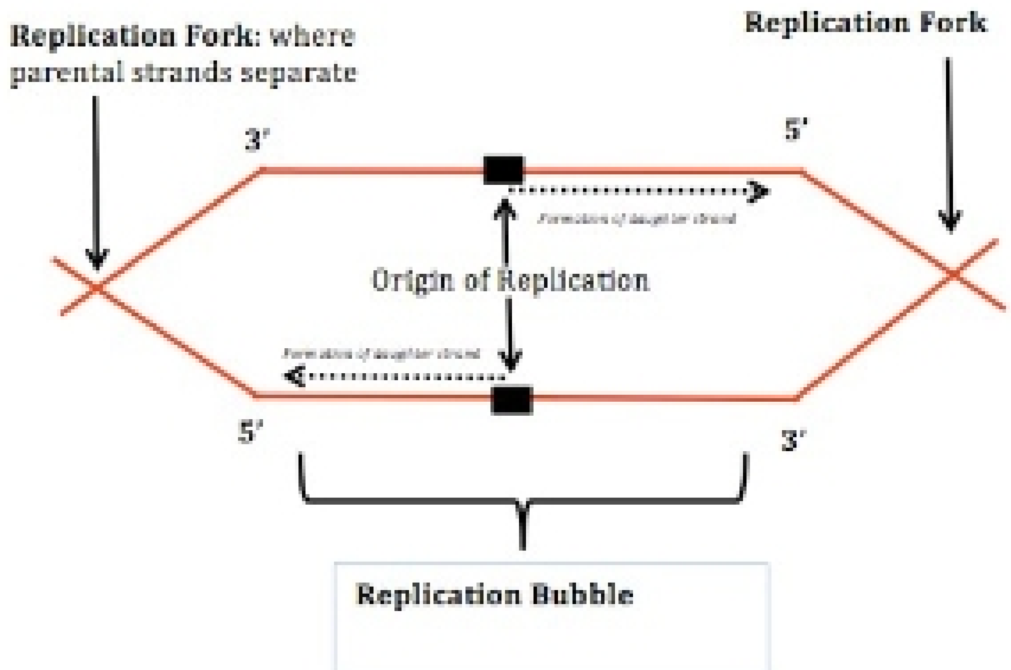


III. **Replication fork Diagram**

A. **Bidirectional replication**
   1. Replication occurs in both directions from the origin

B. Each **new** strand:
   1. Has the opposite orientation from the parent strand
   2. Forms in **its** 5'→3' direction
      a. DNA polymerase can only move from 5'→3' on daughter strand

C. At each origin, the lagging strand is built in one piece; the lagging strand is built in pieces ***(Fig. 12.5)***



IV. <u>DNA Replication (Fig. 12.5)</u>

A. General

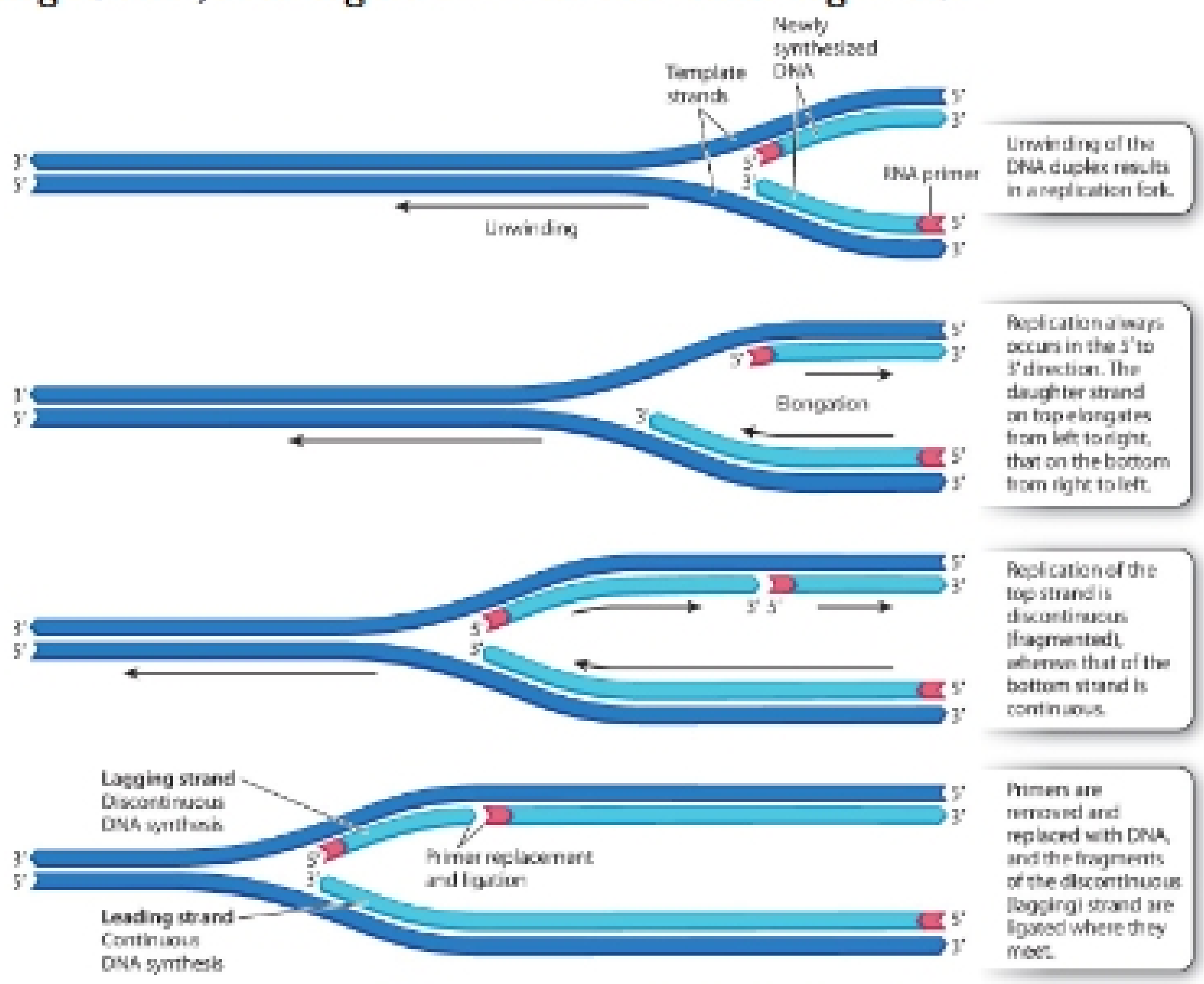
1. Leading Strand: Parent strand that is unzipped in its 3'→5' direction
2. Lagging Strand: Other parent strand that is unzipped in its 5'→3' direction
3. Both daughter strands are synthesized in the 5'→3' direction
   a. For the leading strand, that doesn't cause a problem
   b. For lagging strand, DNA is replicated in fragments
4. Happens during S-phase of cell cycle

B. Enzymes/Proteins & Functions

1. <u>DNA Polymerase</u> – creates ("elongates") daughter DNA
   a. Also "proofreads" new DNA to make sure there are no mistakes in it
2. <u>DNA Helicase</u> – unzips existing DNA
3. <u>RNA Primase</u> – adds a small RNA sequence to unzipped DNA ("primer")
4. <u>Single-Stranded Binding Proteins</u> – prevent unzipped DNA from coming back together
5. <u>DNA Topoisomerase</u> – relieves stress on DNA during unzipping
6. <u>DNA Ligase</u> – glues together Okazaki fragments on lagging strand

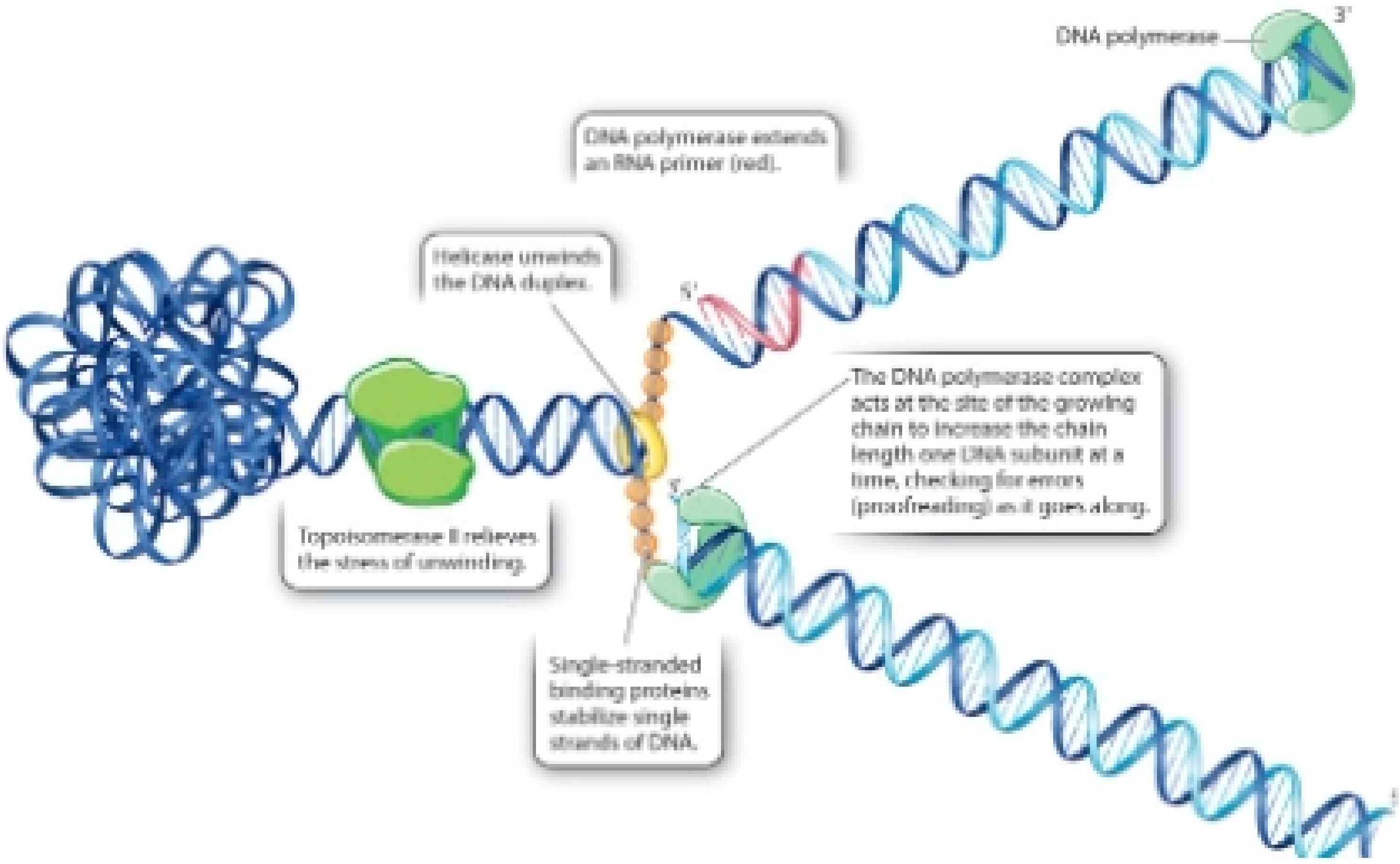
C. Process

1. DNA Helicase hydrolyzes ATP to unwind the existing double helix
   a. As it unwinds, the replication bubble forms
      i. In that bubble, **both** single strands of existing DNA are replicated by DNA polymerase (see diagram)
   b. Single-stranded binding proteins prevent separated parent strands from coming back together
   c. DNA topoisomerase relieves the stress of uncoiling ahead of the replication fork so DNA doesn't break
2. In the bubble, DNA polymerase elongates the existing strands, starting at their 5' ends
3. To start adding nucleotides, DNA polymerase needs a starting sequence
   a. To make DNA polymerase start working, RNA primase makes a short strand of RNA ("primer")
   b. DNA polymerase extends the primer
      i. On the lagging strand, DNA polymerase will run into the primer of the preceding fragment
         - A different form of DNA polymerase replaces these primers with DNA
4. On the lagging strand, DNA ligase anneals Okazaki fragments



V. <u>Lots of Equipment is involved (fig. 12.8) (Process reiterated)</u>

- **A.** Red piece is the starting piece of the light blue strand (starting strand)
  - **1.** You get this from RNA primase
    - **a.** Makes a short strand of RNA called a "primer". DNA polymerase extends the primer (Fig. 12.6)
- **B.** On the lagging strand, DNA polymerase will run into the primer of the preceding fragment (Fig. 12.5.3) (Okazaki fragment)
    - **a.** When that happens, a different DNA polymerase from removes the primer and fills in the DNA (fig. 12.6.3)
- **C.** DNA ligase joins the DNA fragments (Fig. 12.6)



VI. Summary

- **A.** RNA primase makes primers
- **B.** One type of DNA polymerase extends strand
- **C.** MAKE ENZYME/FUNCTION LIST FOR THIS CHAPTER
- **D.** A 23nd type of DNA poly removes any primers & fills in DNA
- **E.** DNA ligase bonds any fragments

VII. Most types of DNA polymerase can "proofread"

- **A.** Checks that the most recently added nucleotide is correct, if not, it will cut it out and add the correct one (fig. 12.7)

Replication Fork: where parental strands separate

Replication Fork