Watson and Crick discovered the double helix by building models to conform to X-ray data

- By the beginnings of the 1950's, the race was on to move from the structure of a single DNA strand to the three-dimensional structure of DNA.
  - Among the scientists working on the problem were Linus Pauling in California, and Maurice Wilkins and Rosalind Franklin in London.

- Maurice Wilkins and Rosalind Franklin used X-ray crystallography to study the structure of DNA.
  - X-rays are diffracted as they passed through aligned fibers of purified DNA.
  - The diffraction pattern can be used to deduce the three-dimensional shape of molecules.
- James Watson learned from their research that DNA was helical in shape and he deduced the width of the helix and the spacing of bases.

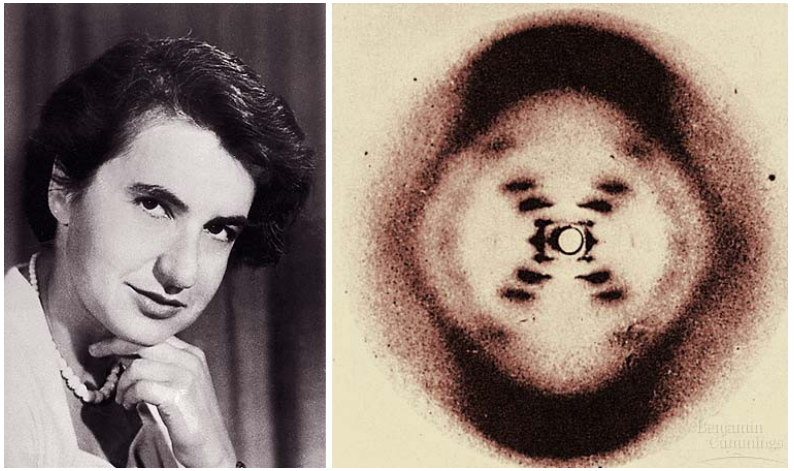
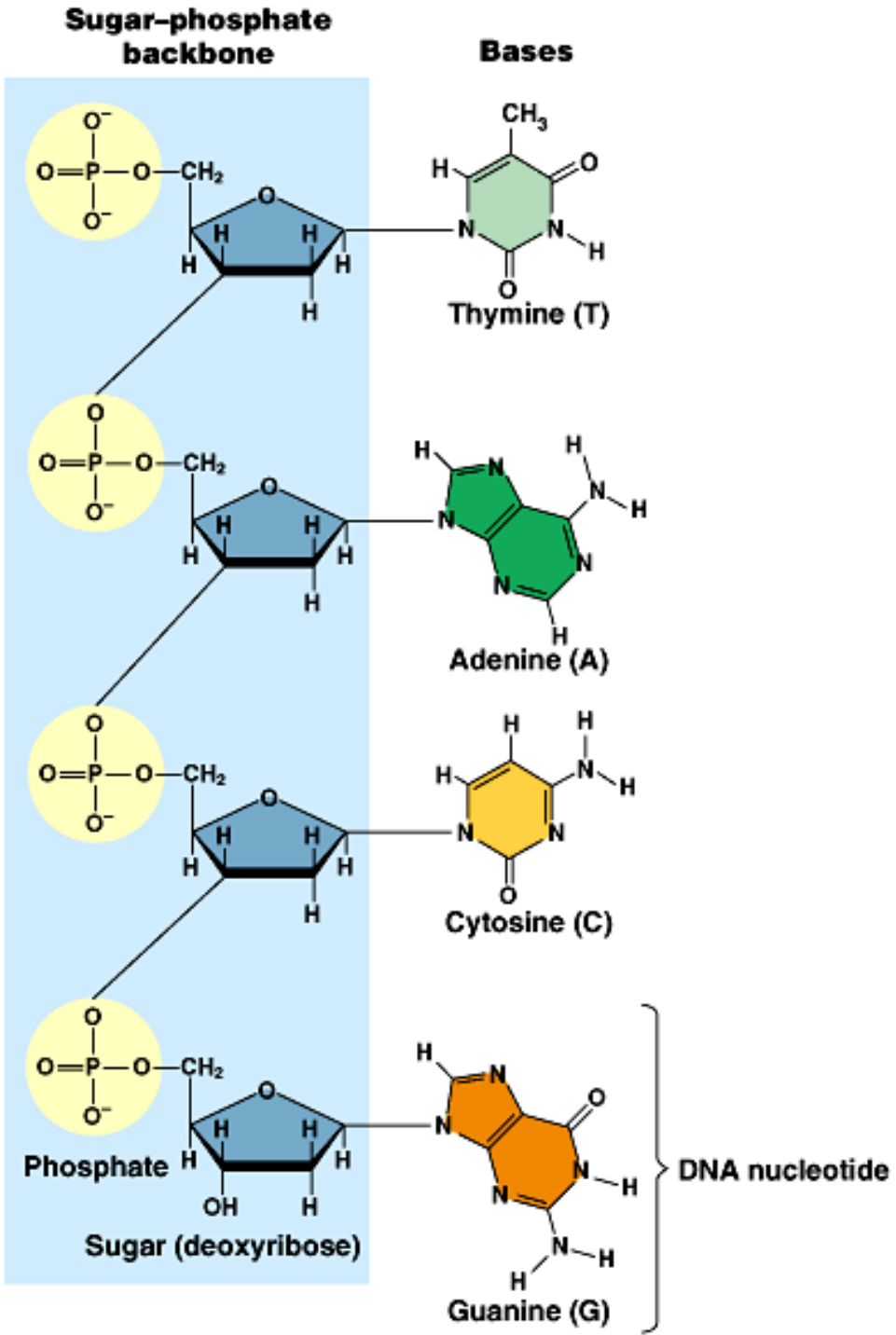
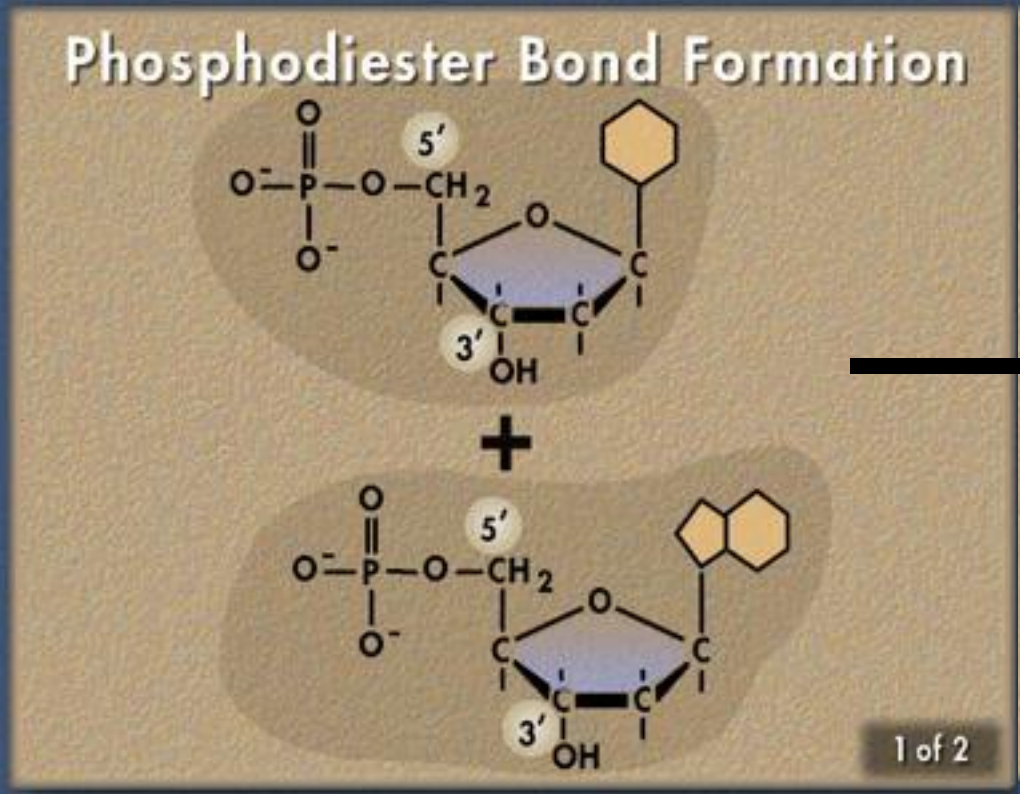


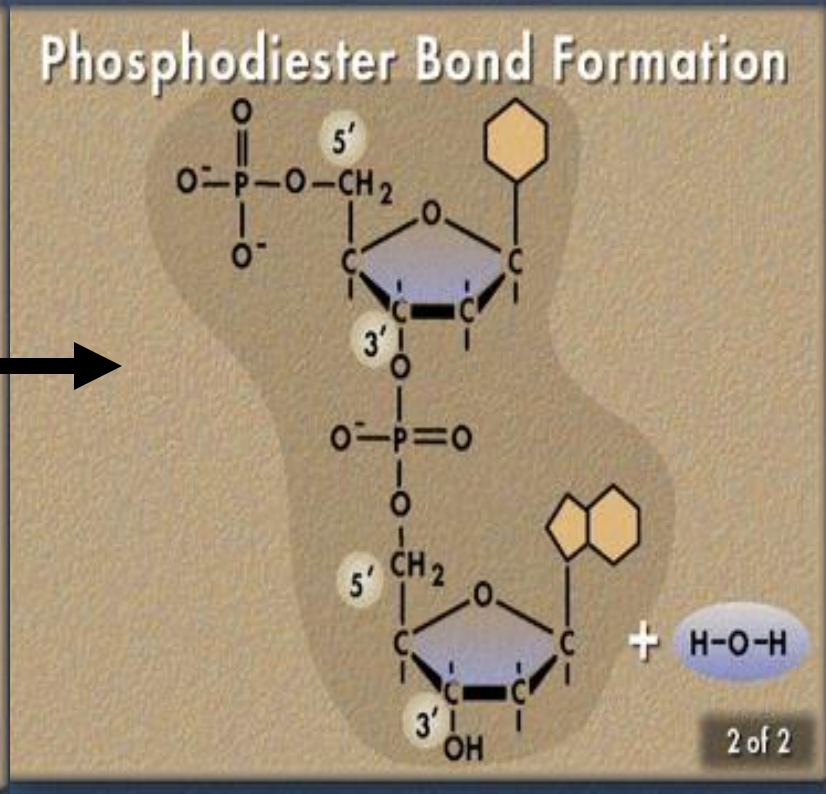
Fig. 16.4

- The phosphate group of one nucleotide is attached to the sugar of the next nucleotide in line.

- The result is a "backbone" of alternating phosphates and sugars, from which the bases project.



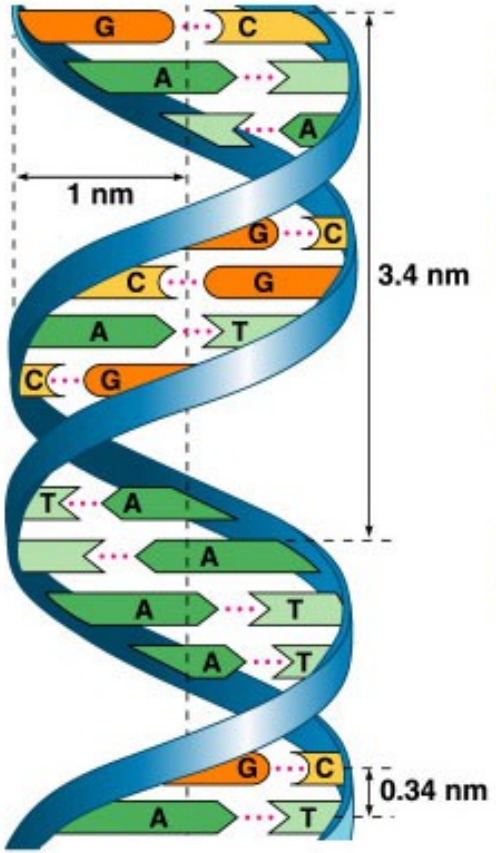




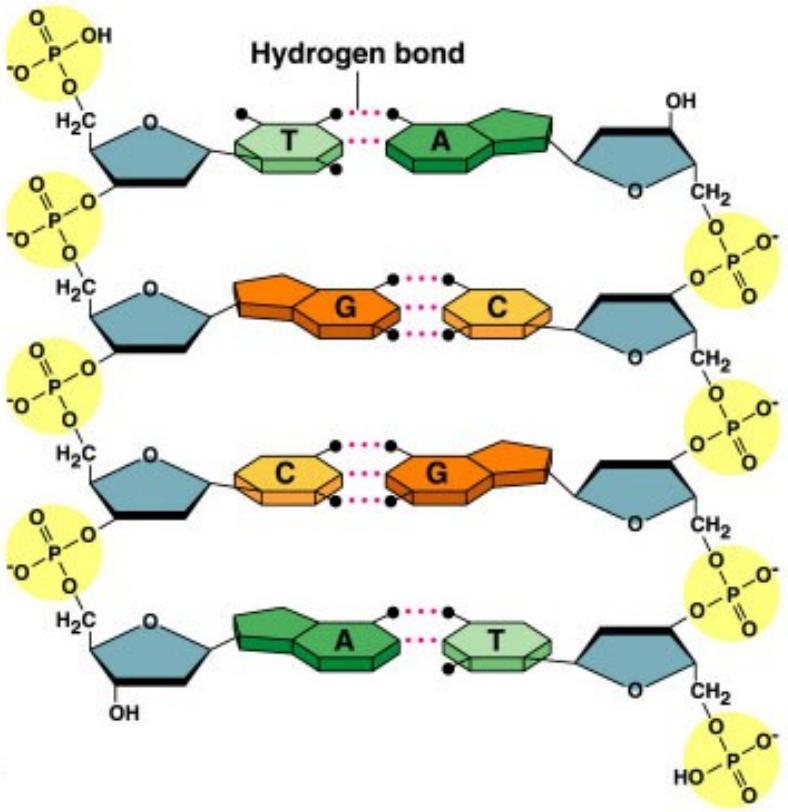
- The phosphodiester bond links the two deoxyribose sugar moleucles together, sandwiching the phosphate group between them.

- Watson and his colleague Francis Crick began to work on a model of DNA with two strands, the **double helix**.
- Using molecular models made of wire, they first tried to place the sugar-phosphate chains on the inside.
- However, this did not fit the X-ray measurements and other information on the chemistry of DNA.

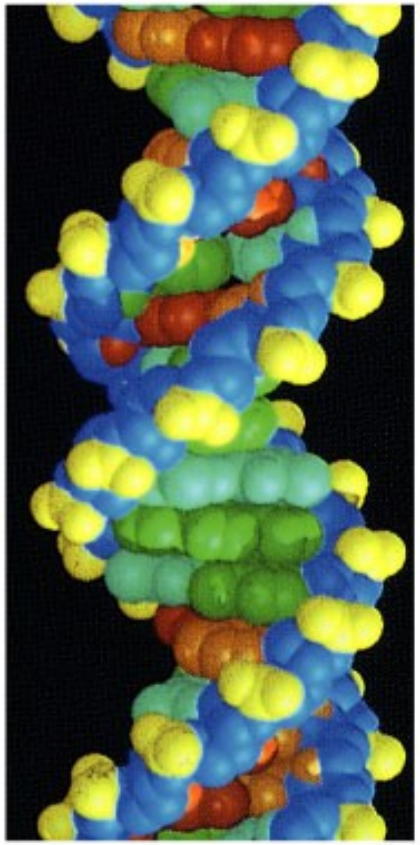
- The key breakthrough came when Watson put the sugar-phosphate chain on the outside and the nitrogen bases on the inside of the double helix.
  - The sugar-phosphate chains of each strand are like the side ropes of a rope ladder.
  - Pairs of nitrogen bases, one from each strand, form rungs.
  - The ladder forms a twist every ten bases.



(a) Key features of DNA structure

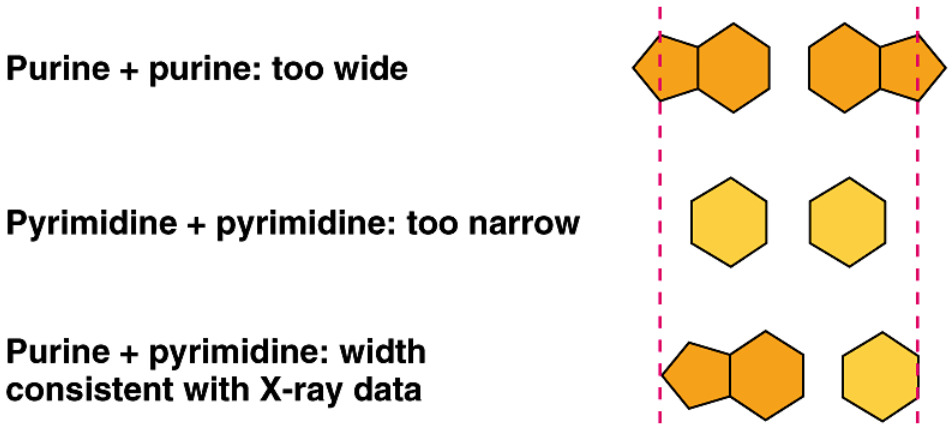


(b) Partial chemical structure



(c) Space-filling model

- The nitrogenous bases are paired in specific combinations: adenine with thymine and guanine with cytosine.
- Pairing "like" nucleotides did not fit the uniform diameter indicated by the X-ray data.
  - A purine-purine pair would be too wide and a pyrimidine-pyrimidine pairing would be too short.
  - Only a pyrimidine-purine pairing would produce the 2-nm diameter indicated by the X-ray data.



- In addition, Watson and Crick determined that chemical side groups off the nitrogen bases would form hydrogen bonds, connecting the two strands.
  - Based on details of their structure, adenine would form two hydrogen bonds only with thymine and guanine would form three hydrogen bonds only with cytosine.
  - This finding explained Chargaff's rules.

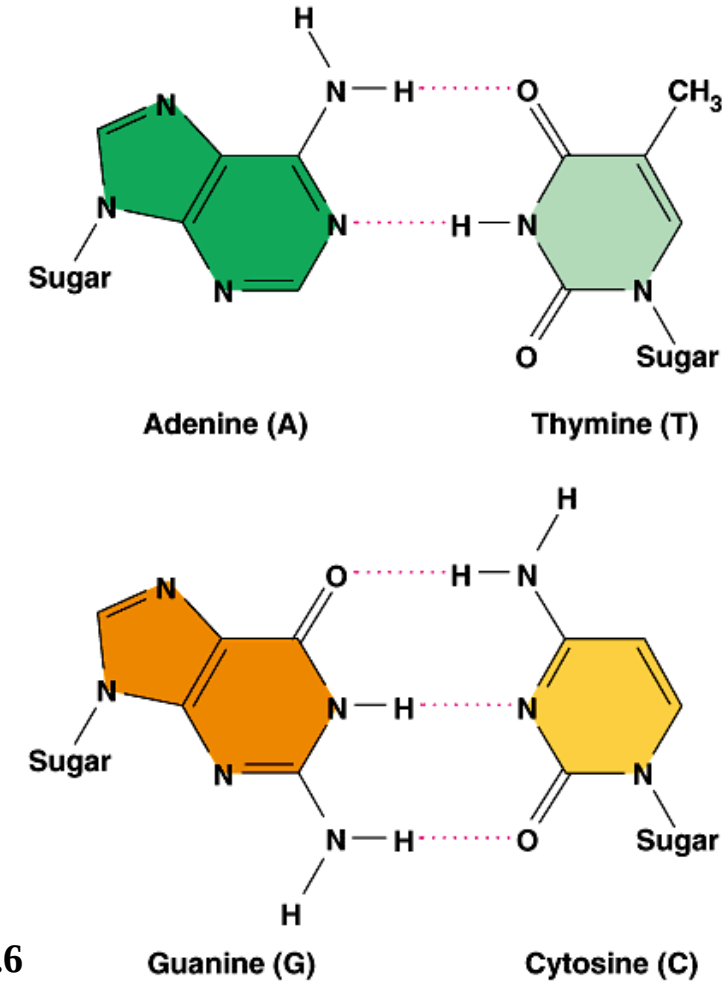


Fig. 16.6

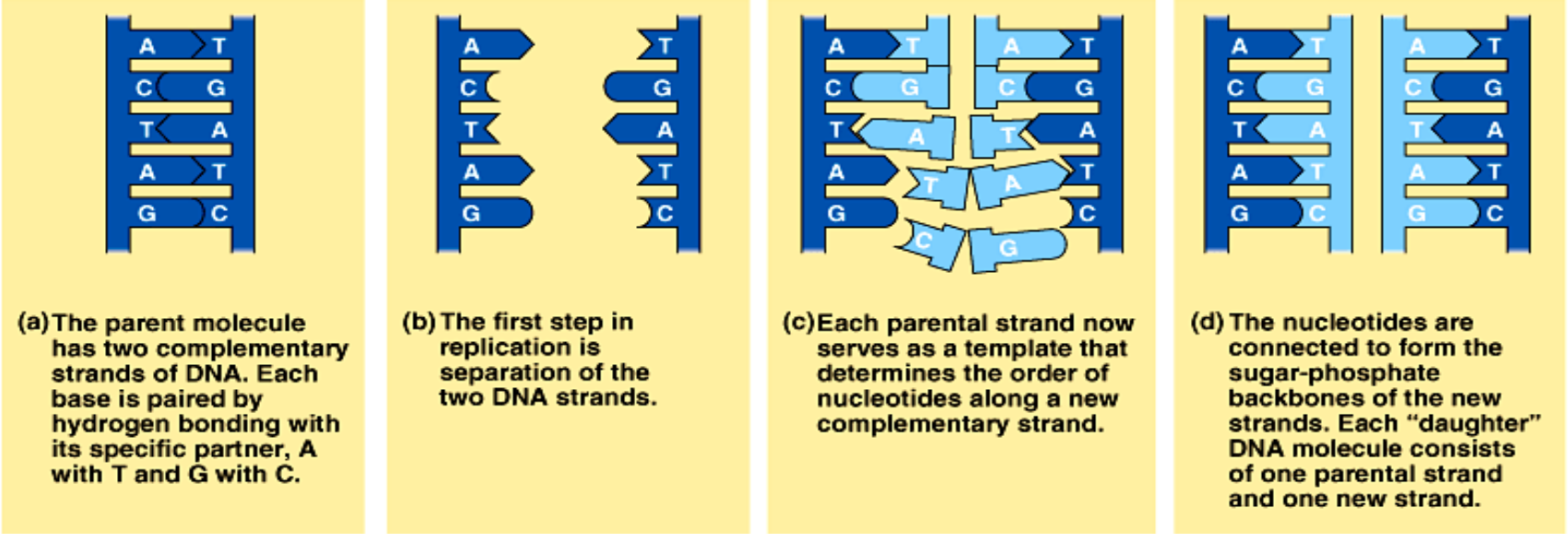
- The base-pairing rules dictate the combinations of nitrogenous bases that form the “rungs” of DNA.
- However, this does not restrict the sequence of nucleotides along *each* DNA strand.
- The linear sequence of the four bases can be varied in countless ways.
- Each gene has a unique order of nitrogen bases.
- In April 1953, Watson and Crick published a succinct, one-page paper in *Nature* reporting their double helix model of DNA.

DNA Replication and Repair

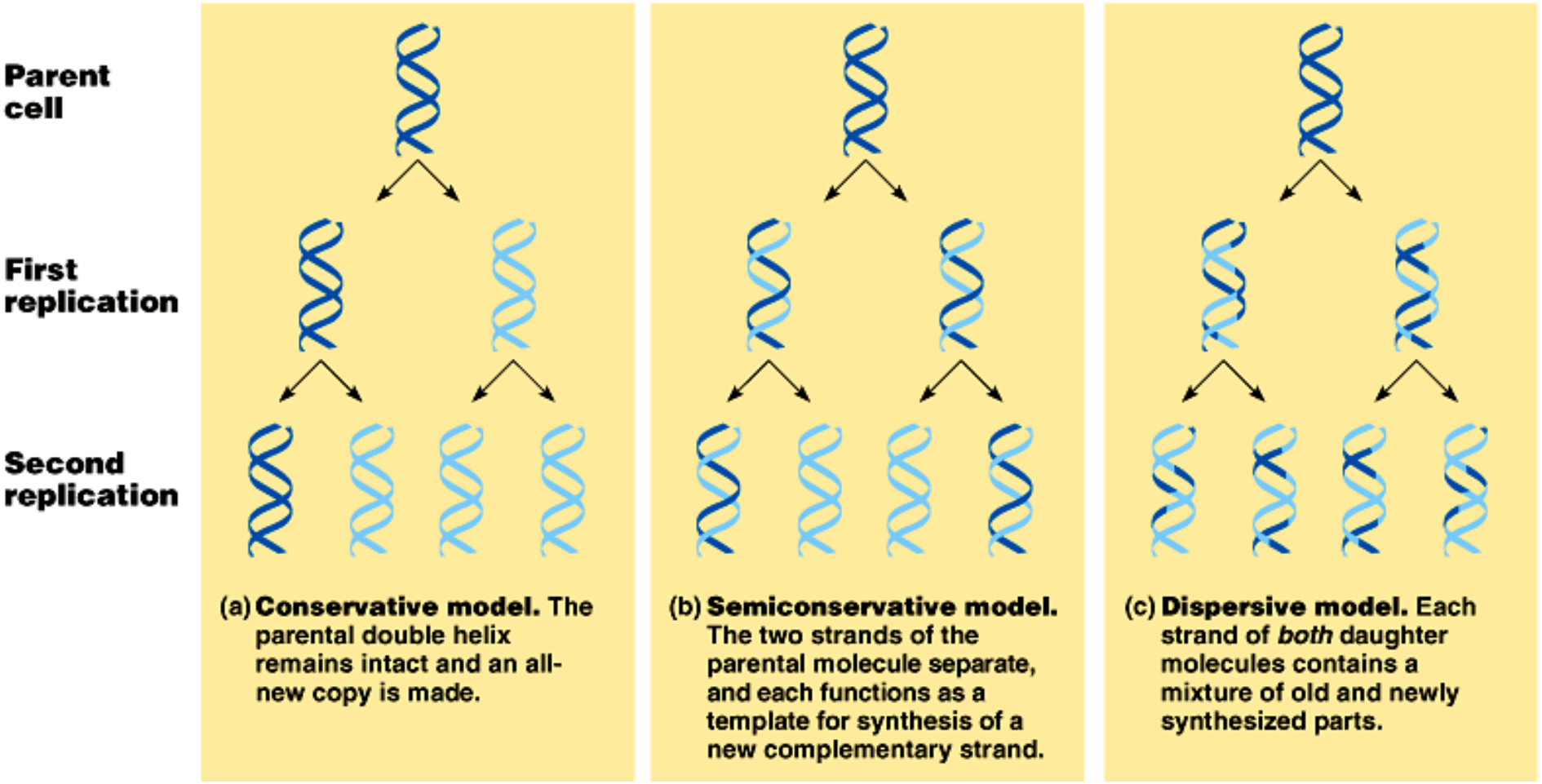
1. Base pairing enables existing DNA strands to serve as templates for new complimentary strands
2. A large team of enzymes and other proteins carries out DNA replication
3. Enzymes proofread DNA during its replication and repair damage to existing DNA
4. The ends of DNA molecules are replicated by a special mechanism

1. During DNA replication, base pairing enables existing DNA strands to serve as templates for new complimentary strands

- In a second paper Watson and Crick published their hypothesis for how DNA replicates.
  - Because each strand is complementary to each other, each can form a template when separated.
  - The order of bases on one strand can be used to add in complementary bases and therefore duplicate the bases exactly.



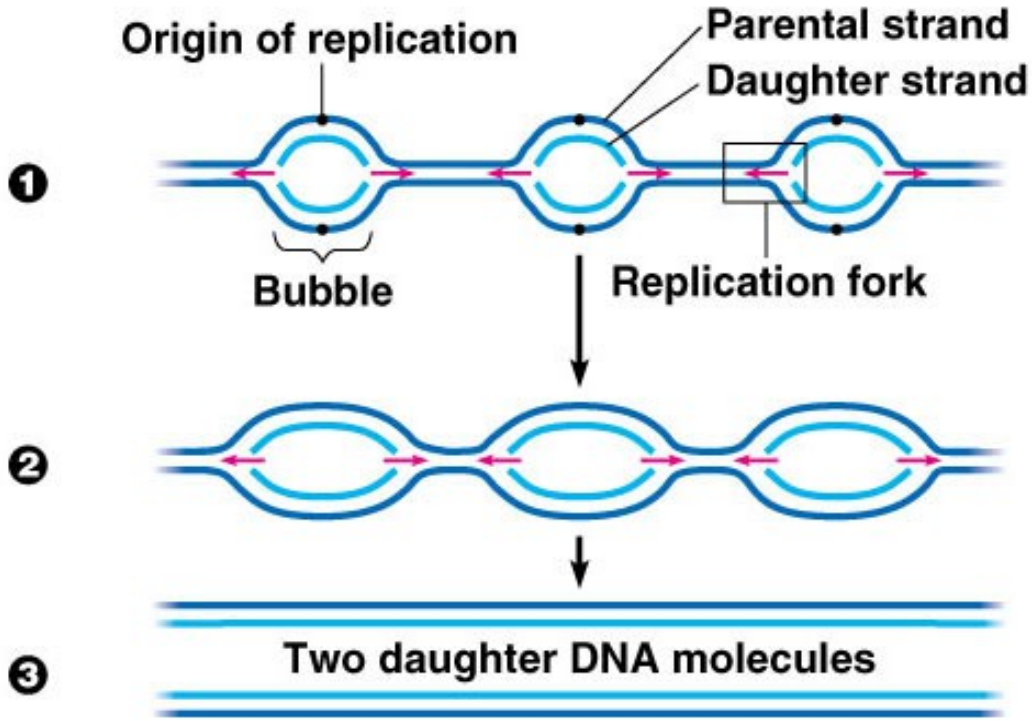
- Other competing models, the conservative model and the dispersive model, were also proposed.

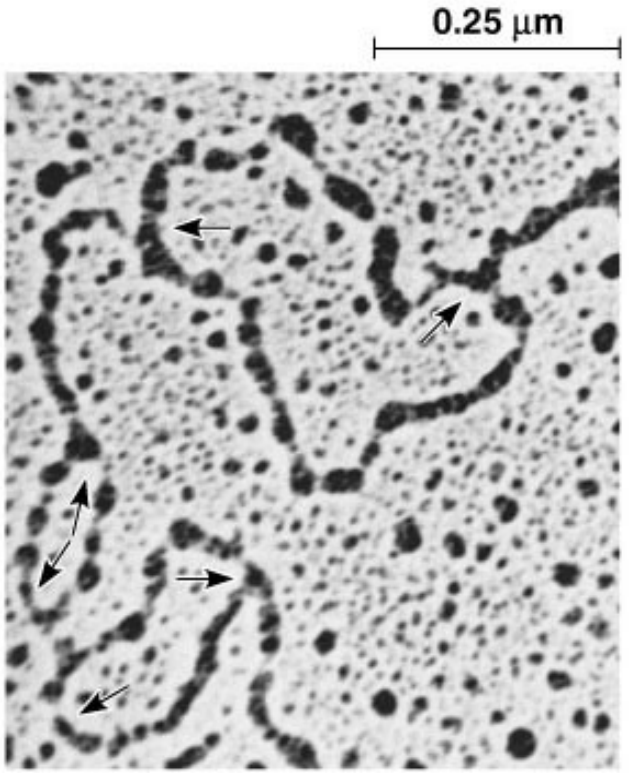


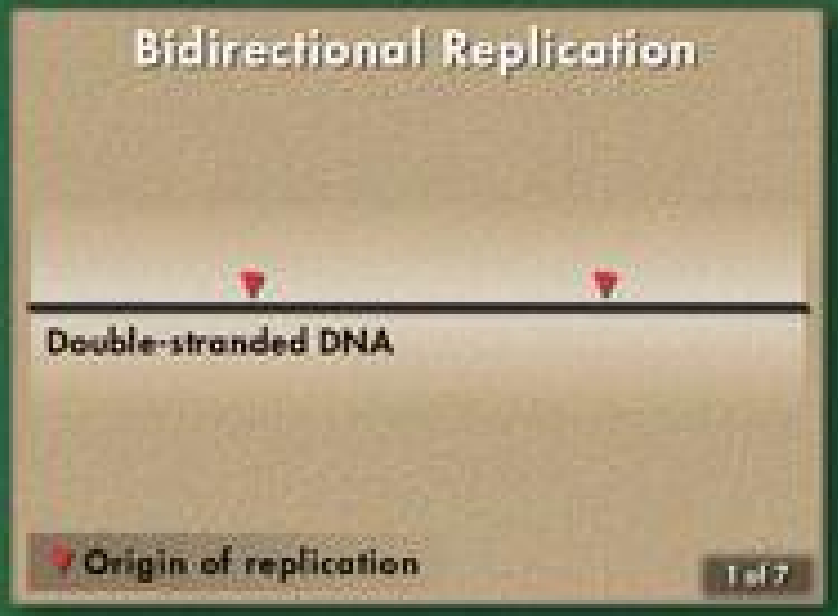
2. A large team of enzymes and other proteins carries out DNA replication

- It takes *E. coli* less than an hour to copy each of the 5 million base pairs in its single chromosome and divide to form two identical daughter cells.
- A human cell can copy its 6 billion base pairs and divide into daughter cells in only a few hours.
- This process is remarkably accurate, with only one error per billion nucleotides.
- More than a dozen enzymes and other proteins participate in DNA replication.

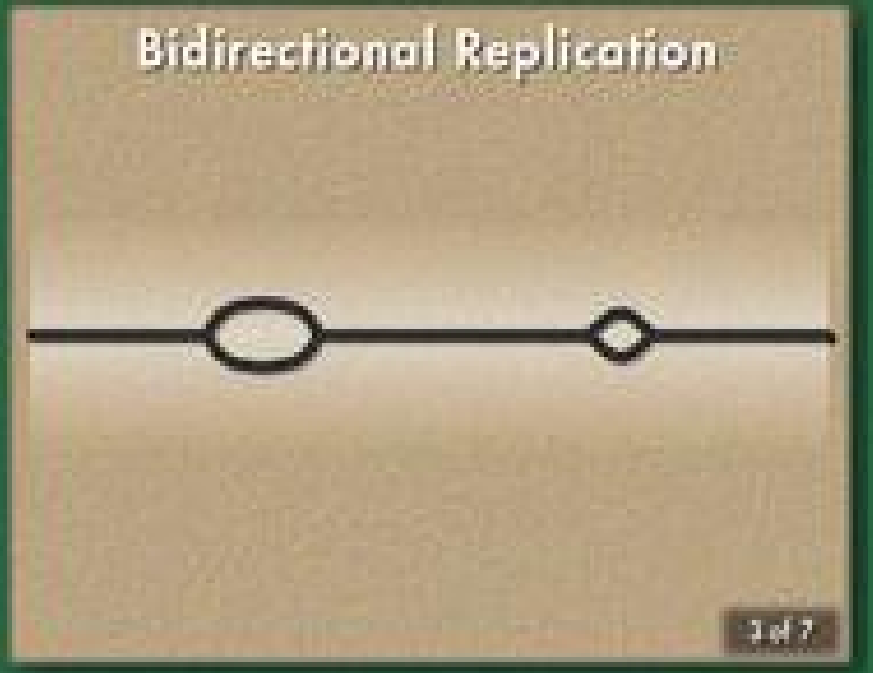
- The replication of a DNA molecule begins at special sites, **origins of replication**.
- In bacteria, this is a single specific sequence of nucleotides that is recognized by the replication enzymes.
  - These enzymes separate the strands, forming a replication "bubble".
  - Proceeds in both directions until the entire molecule is copied.

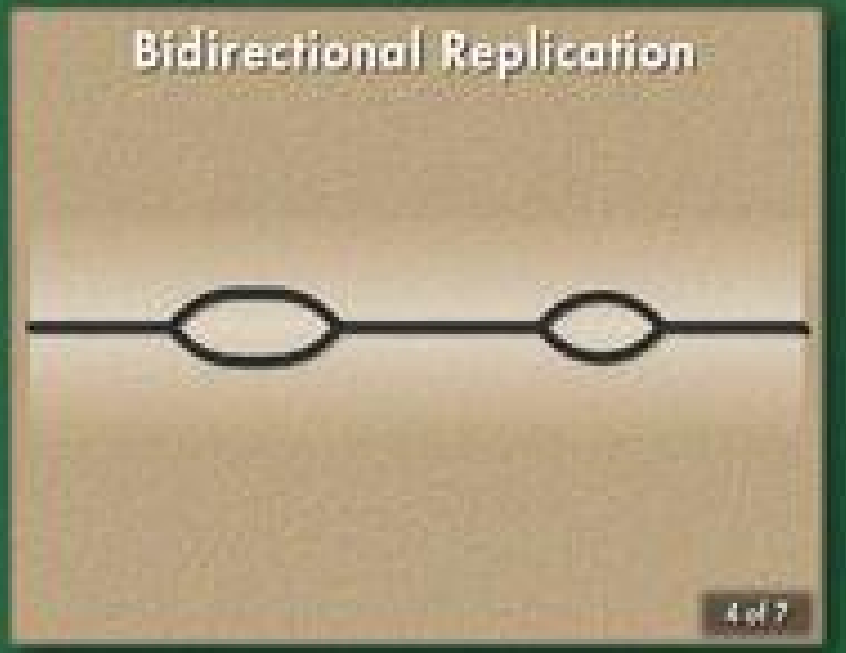


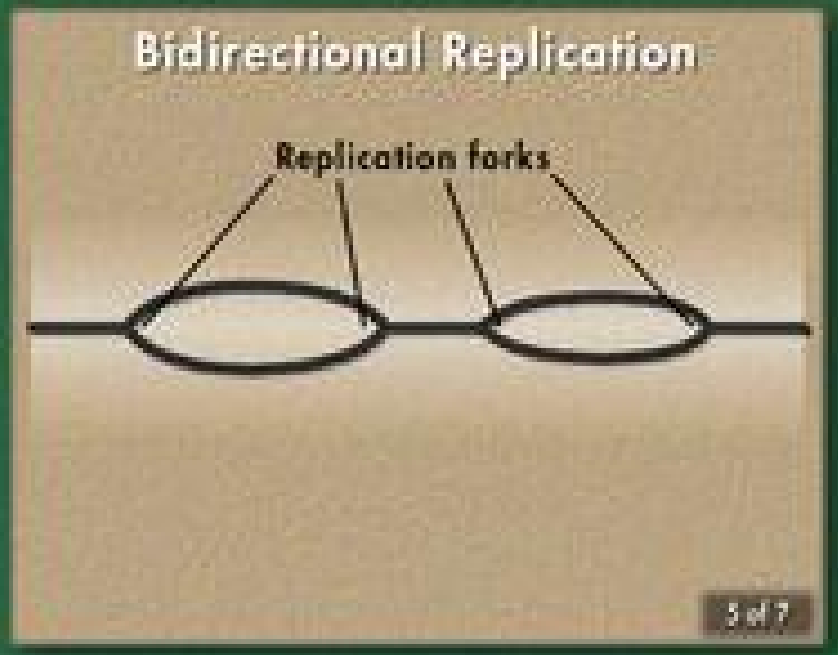


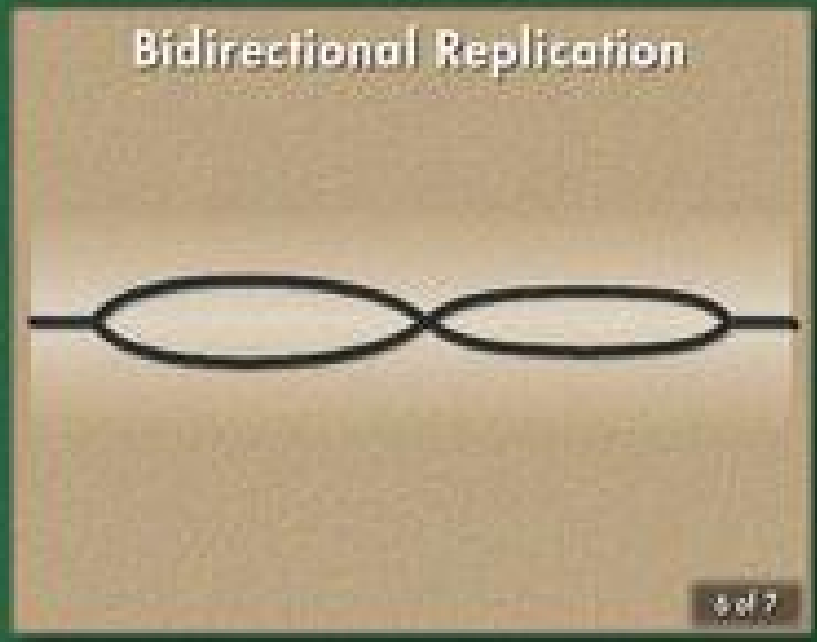

Bidirectional Replication
Double-stranded DNA
Origin of replication
1 of 7

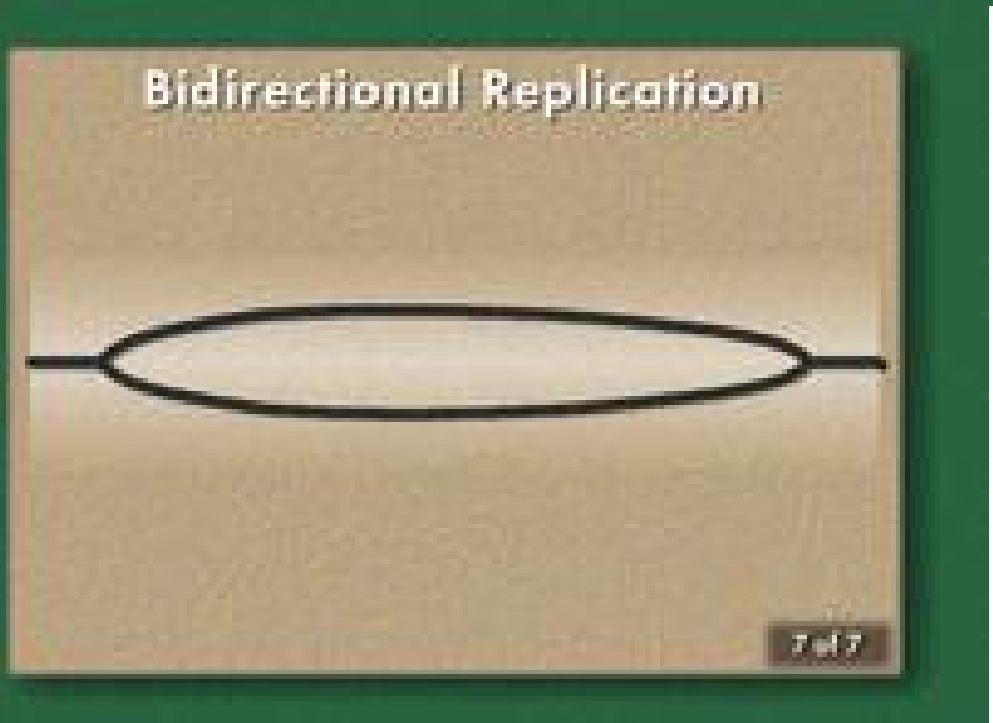

Bidirectional Replication
Origin of replication
2 of 7


Bidirectional Replication
3 of 7


Bidirectional Replication
4 of 7


Bidirectional Replication
Replication forks
5 of 7

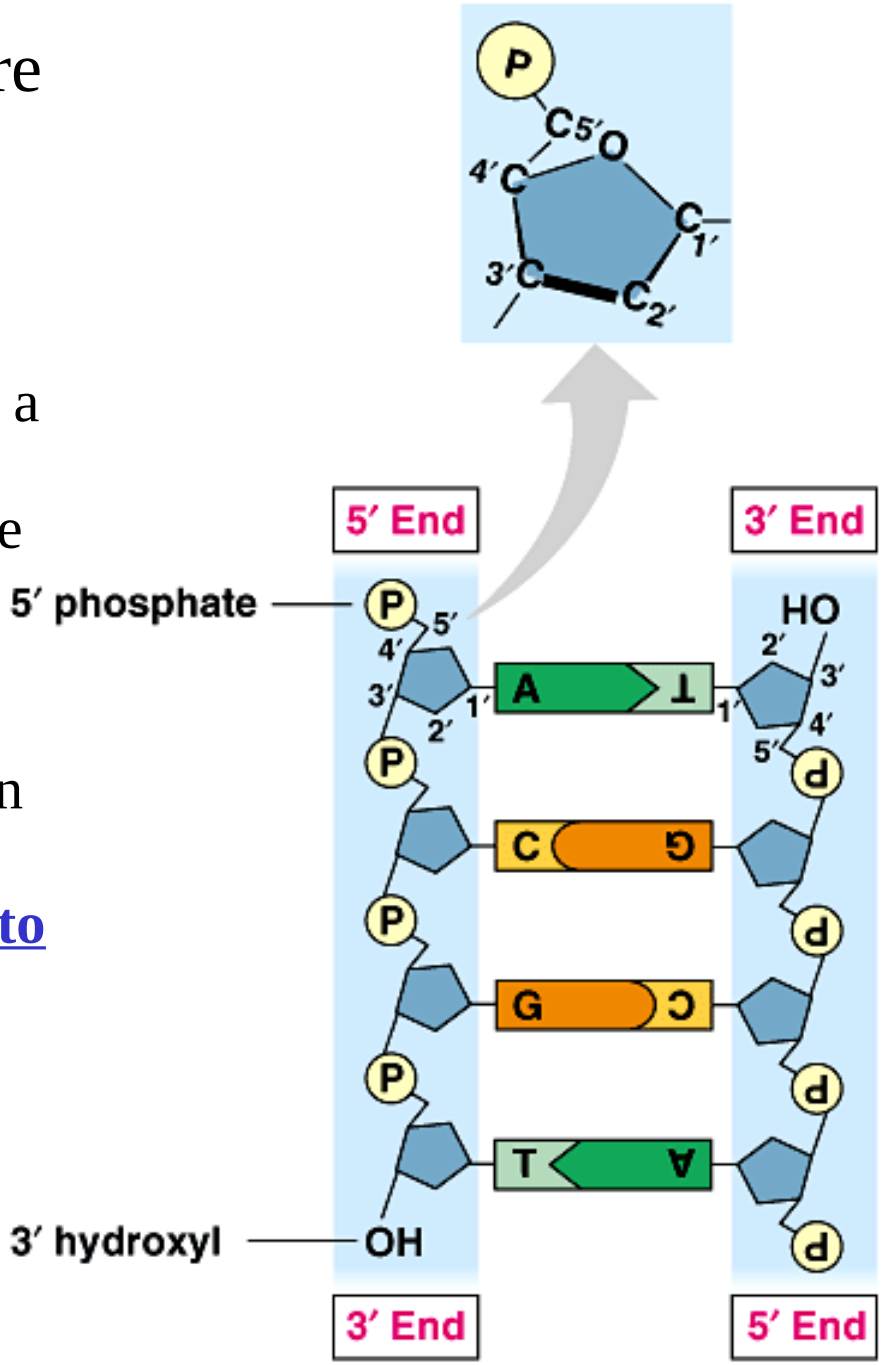

Bidirectional Replication
6 of 7

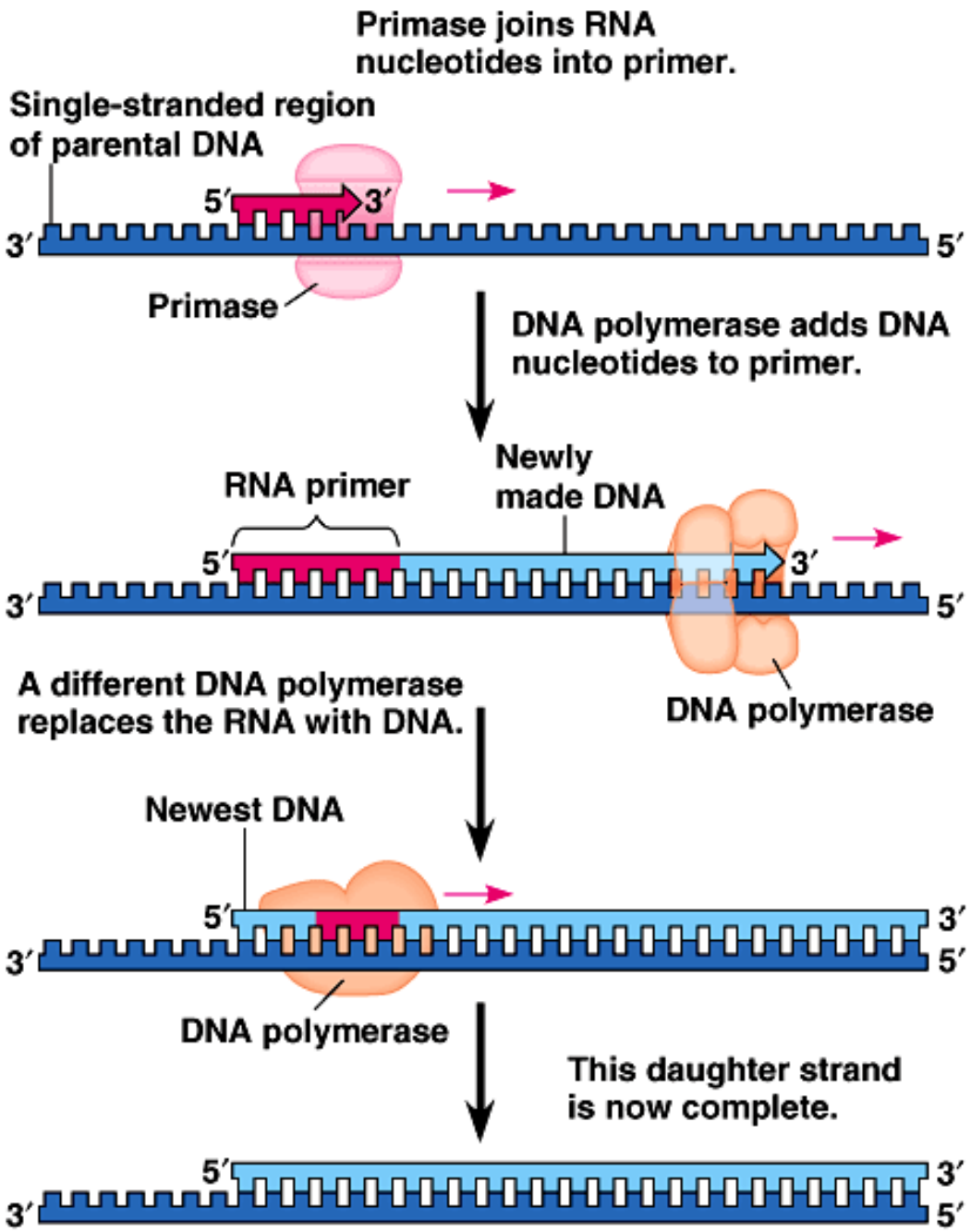

Bidirectional Replication
7 of 7

Parade of proteins/enzymes

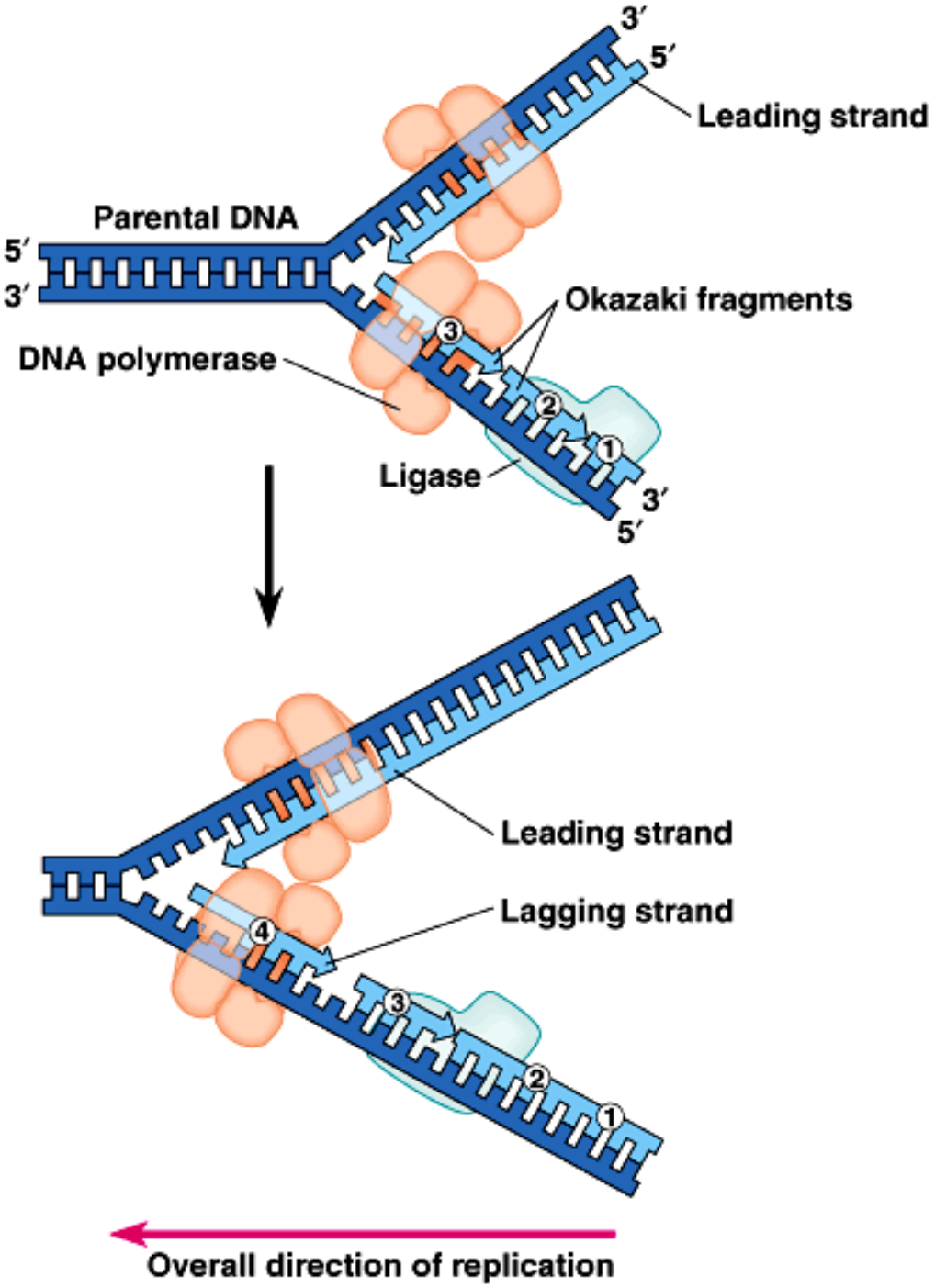
- Origin binding protein—initiates process
- Primase—adds RNA primers
- Helicase—”unzips” the H-bonds
- Single strand binding proteins—keep H-bonds from re-forming; prevents re-zipping of the DNA strand
- DNA polymerase—slots in nucleotides using base-pairing rules; makes a polymer from the nucleotide monomers
- DNA Ligase—ties the Okasaki fragments together
- Proof-reading enzyme—scans for base-pairing “goofs”
- Toposiomerase—keeps the ends of the DNA outside the replication bubble from getting tangled

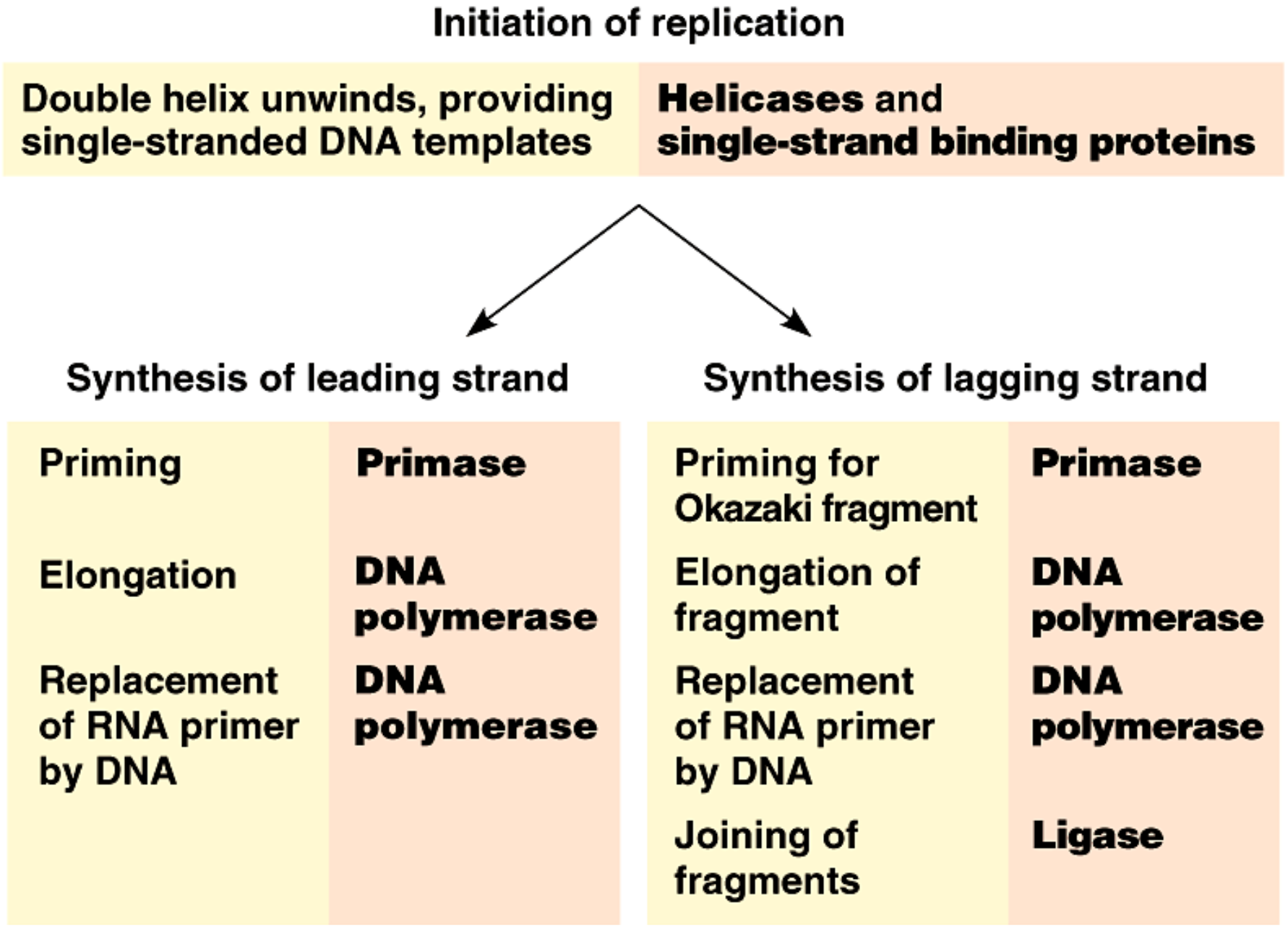
- The strands in the double helix are *antiparallel*.
- The sugar-phosphate backbones run in opposite directions.
  - Each DNA strand has a 3’ end with a free hydroxyl group attached to deoxyribose and a 5’ end with a free phosphate group attached to deoxyribose.
  - The 5’ -> 3’ direction of one strand runs counter to the 3’ -> 5’ direction of the other strand.
  - **Synthesis occurs ONLY in the 5’ to 3’ direction**



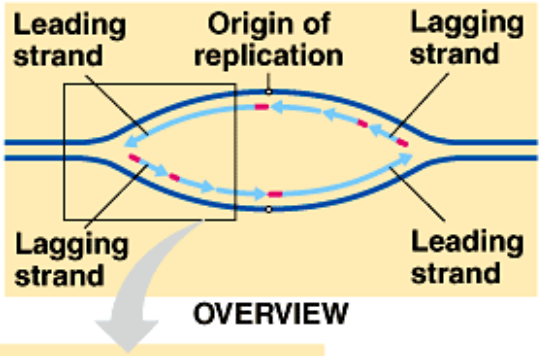


- The other parental strand (5’→3’ into the fork), the **lagging strand**, is copied away from the fork in short segments (Okazaki fragments).
- Okazaki fragments, each about 100-200 nucleotides, are joined by **DNA ligase** to form the sugar-phosphate backbone of a single DNA strand.





- To summarize, at the replication fork, the leading strand is copied continuously into the fork from a single primer.
- The lagging strand is copied away from the fork in short segments, each requiring a new primer.



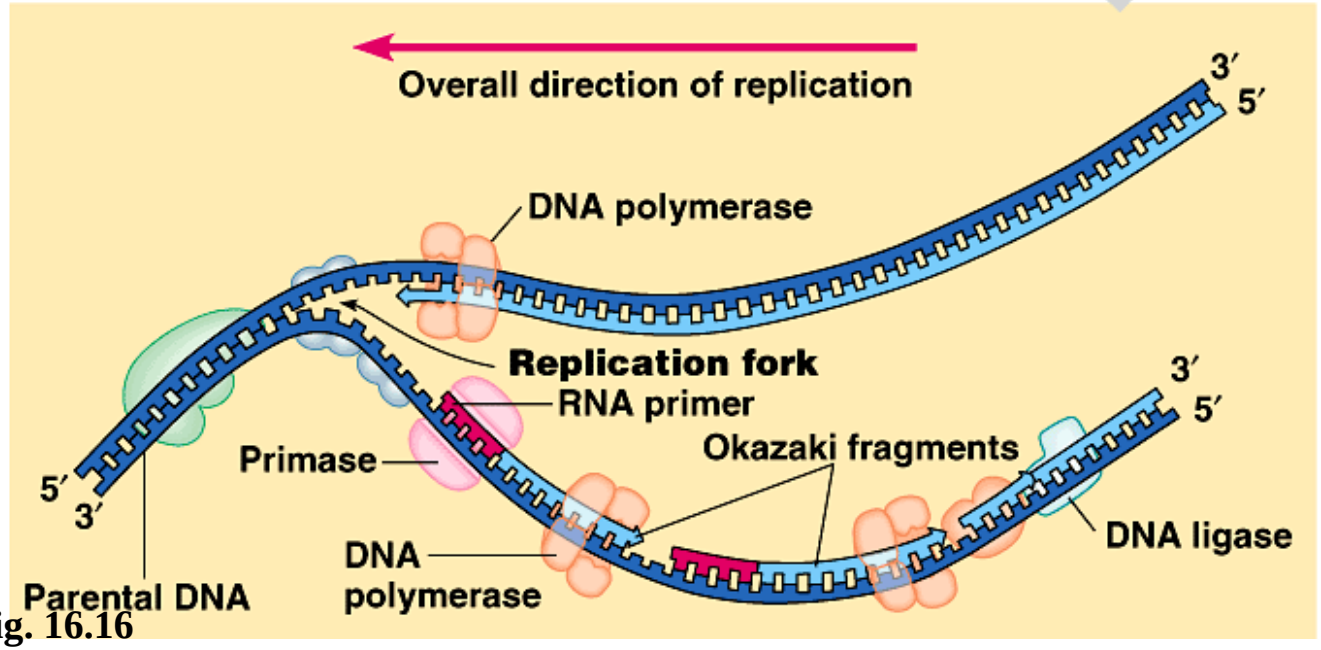
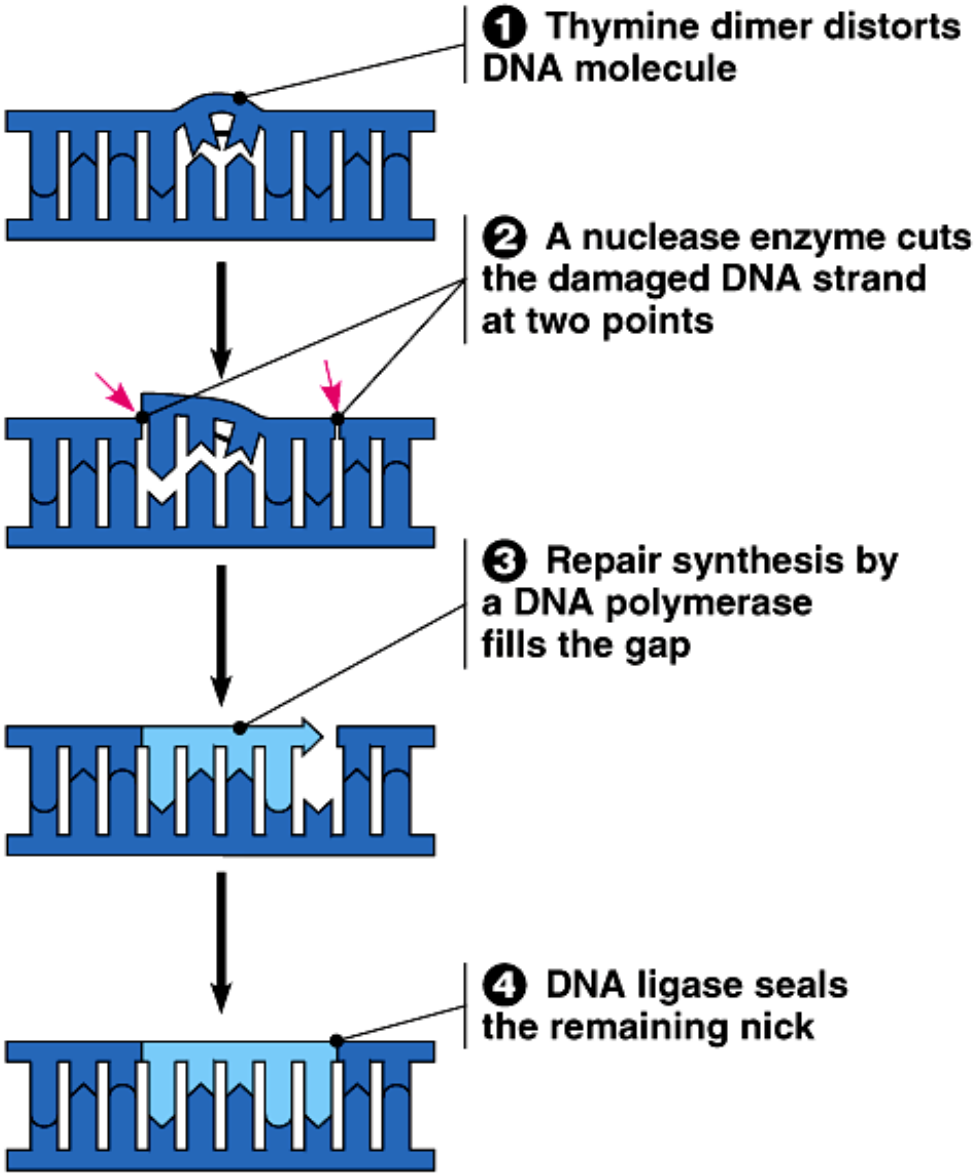


Fig. 16.16

3. Enzymes proofread DNA during its replication and repair damage in existing DNA

- Mistakes during the initial pairing of template nucleotides and complementary nucleotides occurs at a rate of one error per 10,000 base pairs.
- DNA polymerase proofreads each new nucleotide against the template nucleotide as soon as it is added.
- If there is an incorrect pairing, the enzyme removes the wrong nucleotide and then resumes synthesis.
- The final error rate is only one per billion nucleotides.

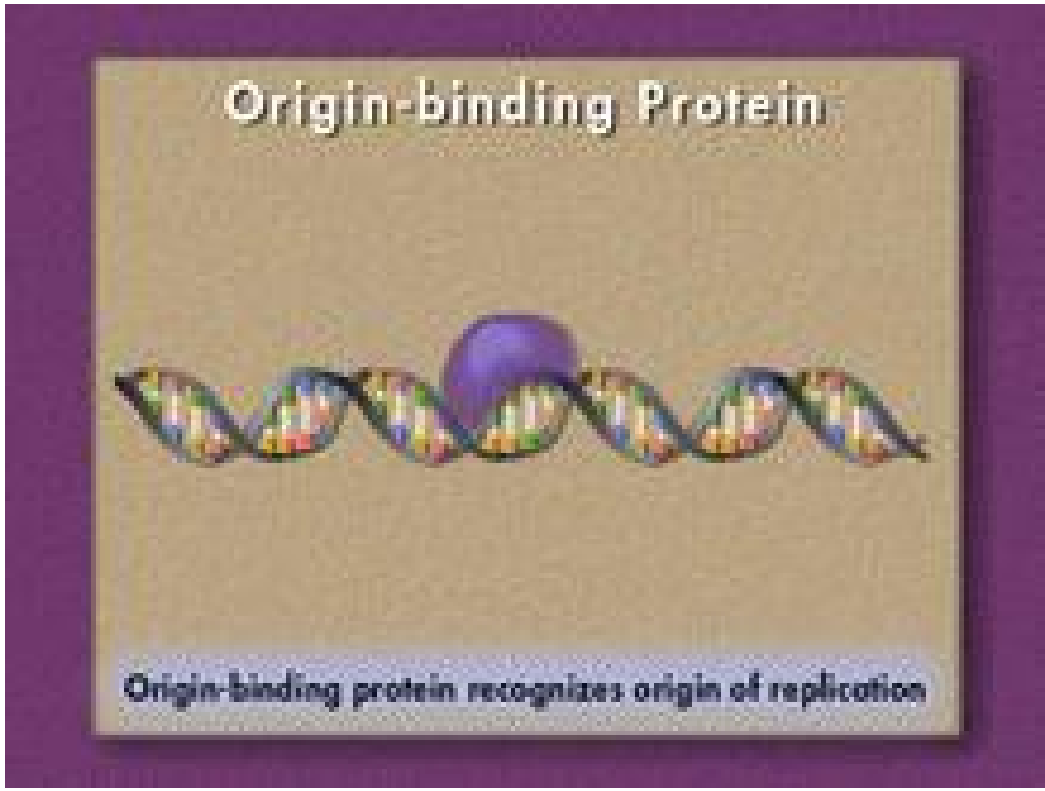
- In **mismatch repair**, special enzymes fix incorrectly paired nucleotides.
  - A hereditary defect in one of these enzymes is associated with a form of colon cancer.
- In **nucleotide excision repair**, a **nuclease** cuts out a segment of a damaged strand.
  - The gap is filled in by DNA polymerase and ligase.



Enzymes and Their Functions in the Replication of DNA

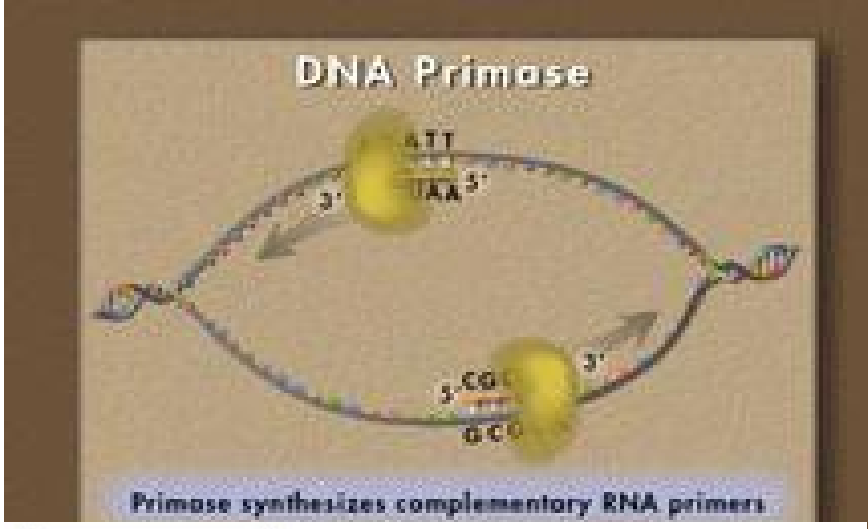
Origin-binding Protein

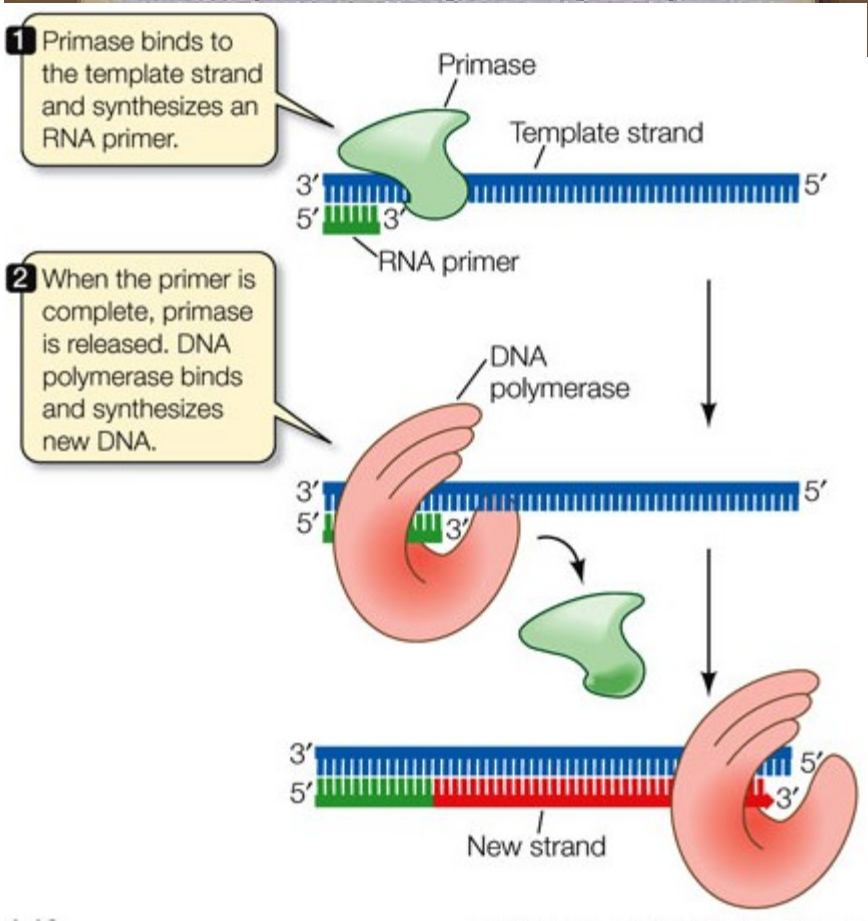
- This protein binds first to begin the replication bubble
- It is chemically attracted to an arrangement of base pairs
- Its function is to break the H-bonds between base pairs so DNA can “unzip”



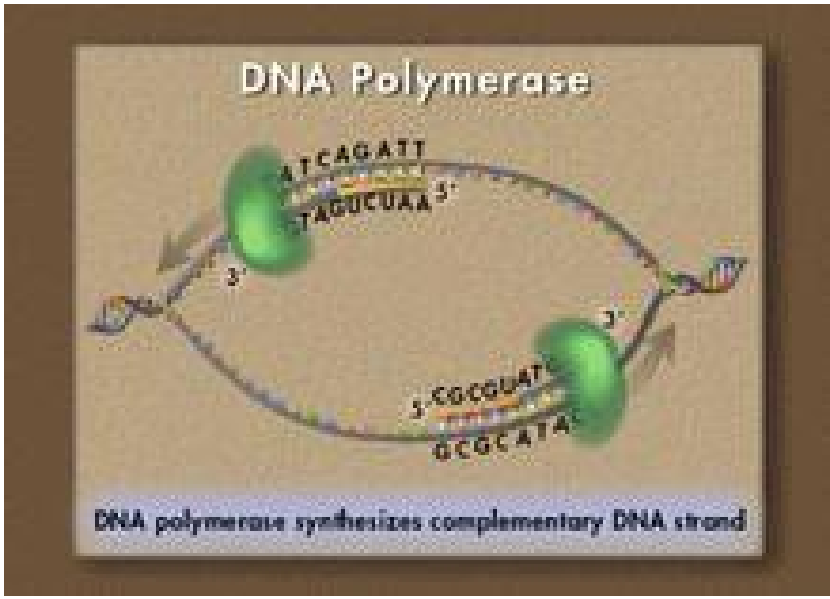
DNA Primase

- This enzyme is the first to act once the replication bubble begins
- It adds about 10 RNA nucleotides to the point of origin in a 5'→3'direction
- Why? DNA polymerase recognizes the primer, NOT the exposed nucleotides

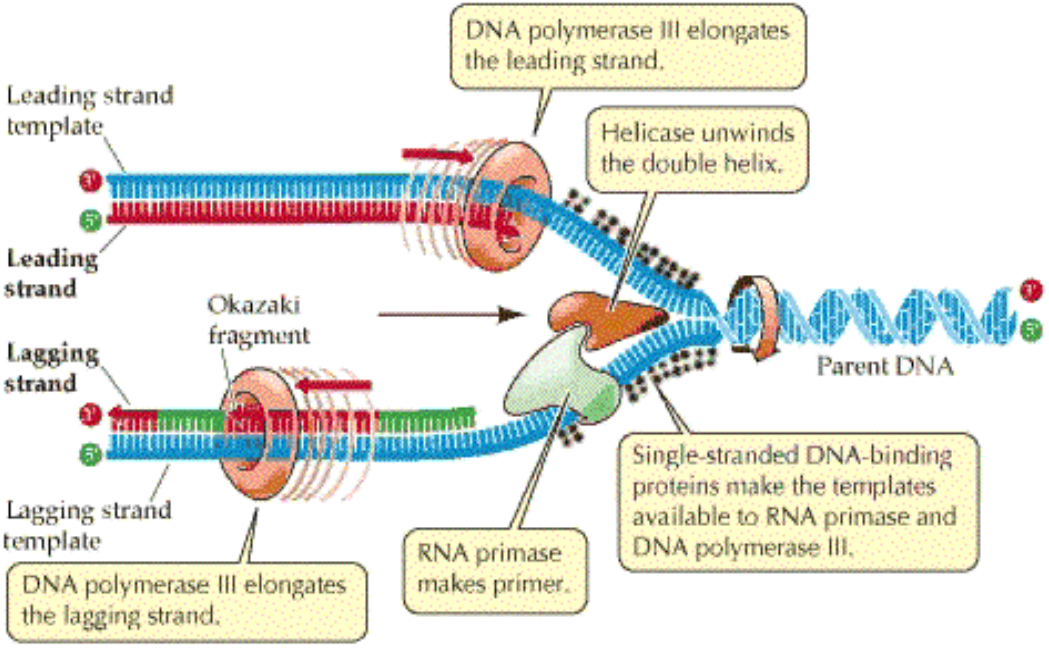




DNA Polymerase

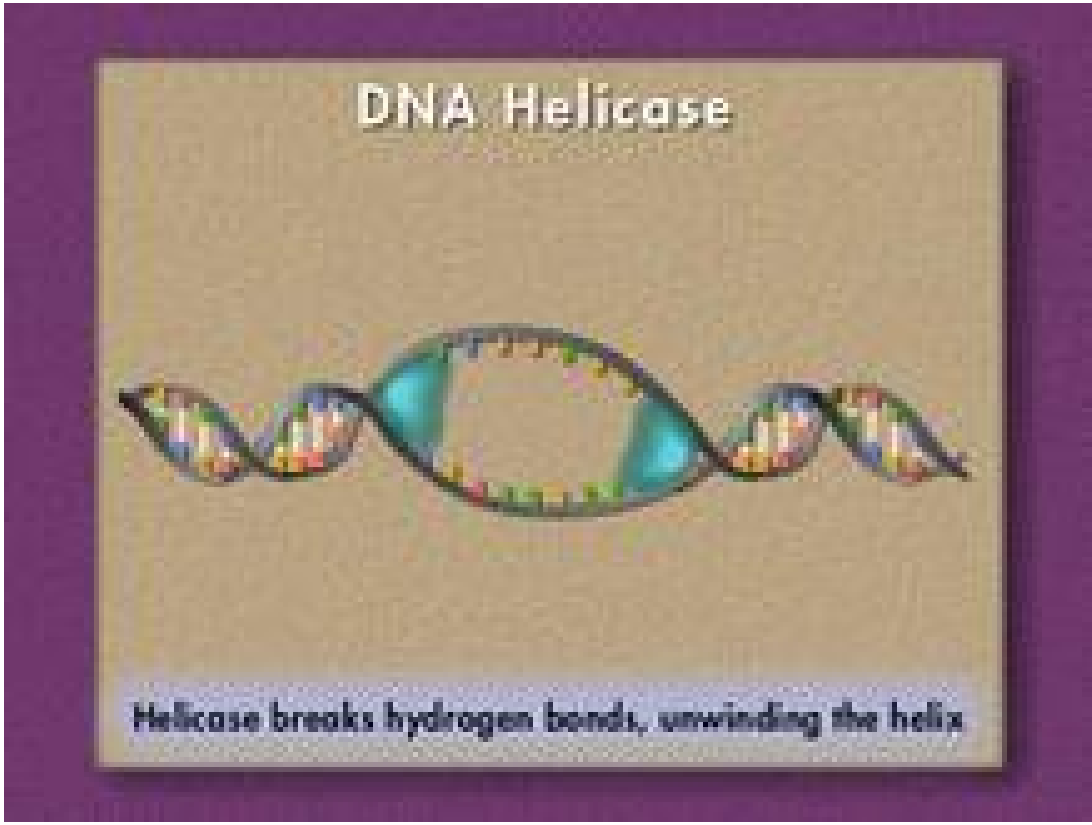


- This enzyme slots the appropriate DNA nucleotides onto the original DNA template synthesizing a new DNA strand.
- Notice, it only synthesizes in a 5'→3' direction
- MANY of these enzymes may be at work within the replication bubble.



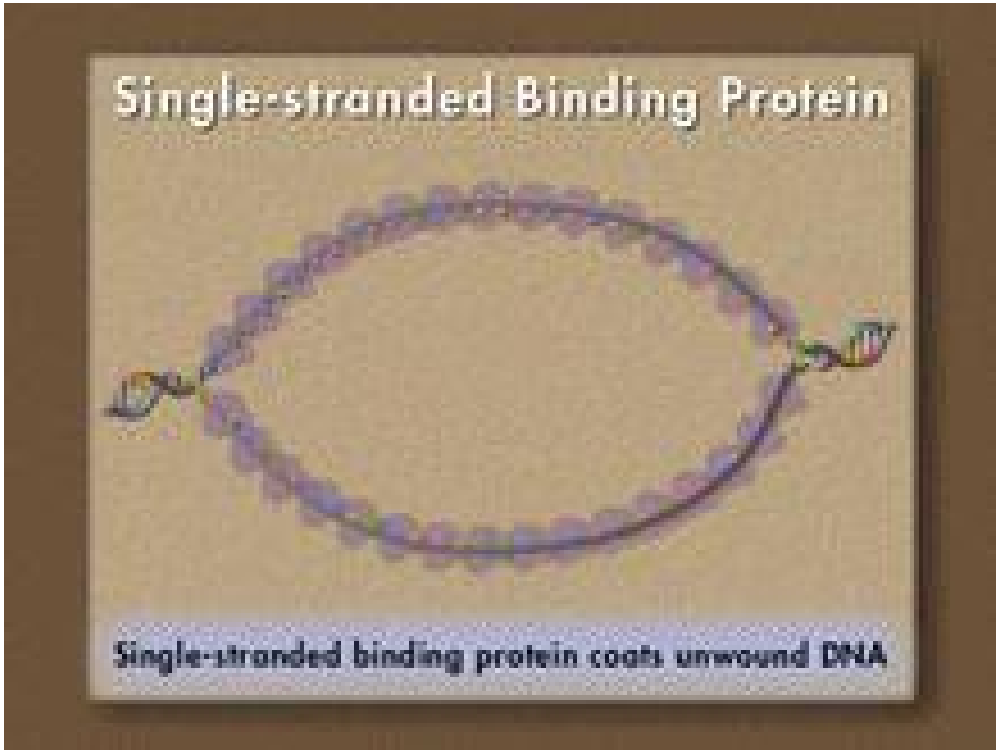
DNA Helicase

- This enzyme unzips the DNA by breaking the H-bonds between the Nitrogen base pairs
- This enlarges the bubble
- Remember, more than one bubble may form at a time and later merge

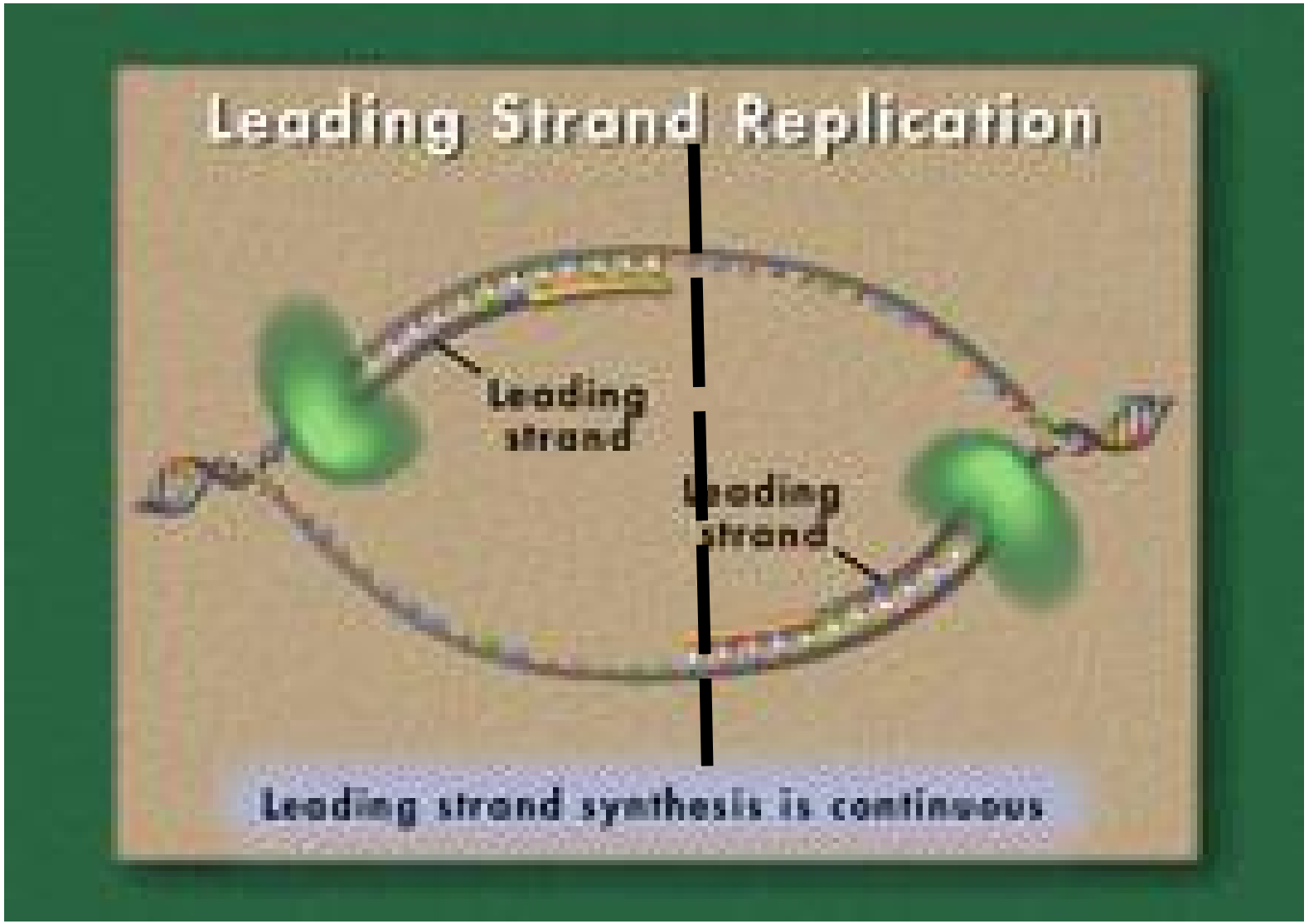


Single-stranded Binding Protein

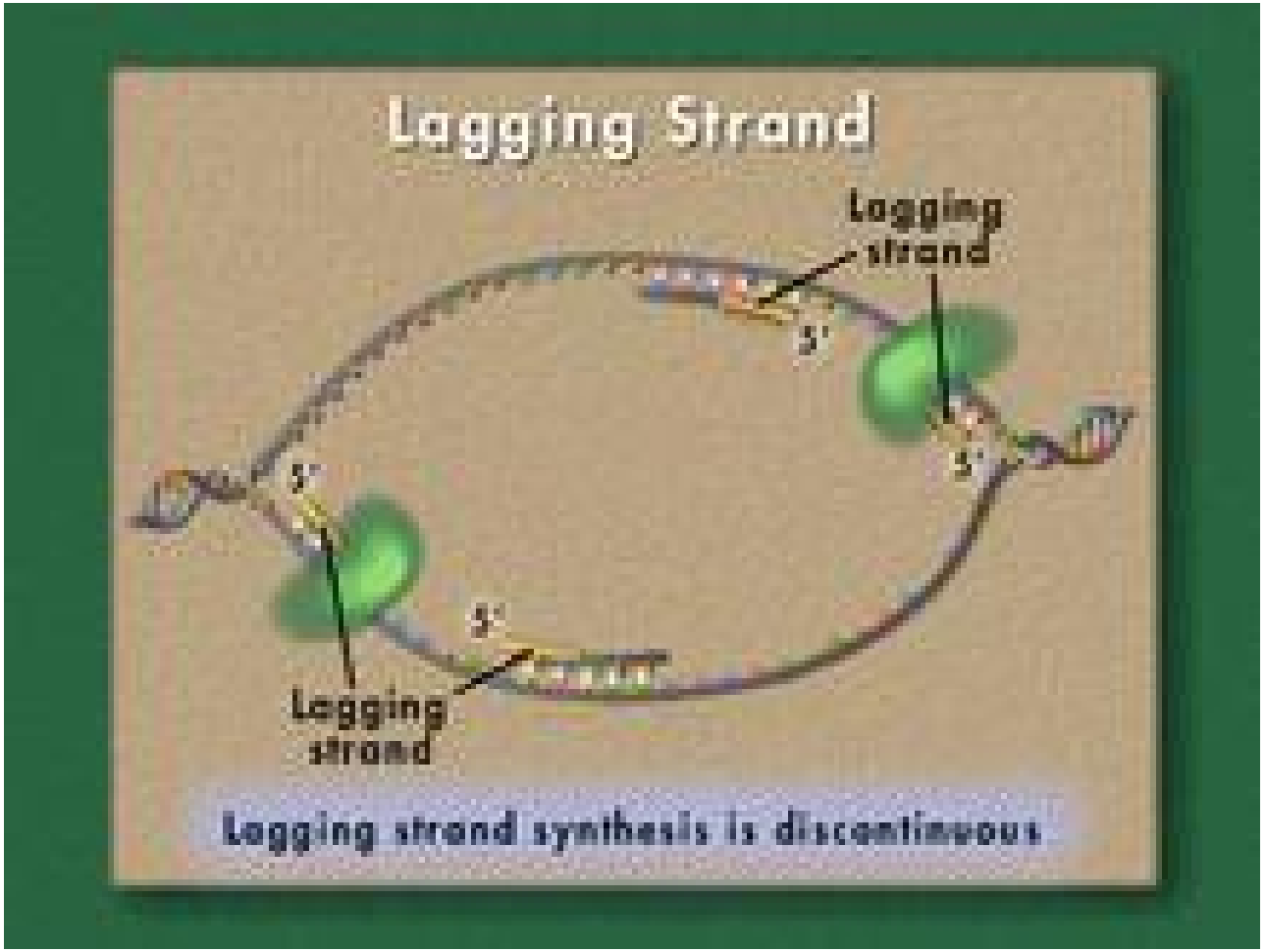
- If this protein did not attach, the H-bonds would re-form, re-zipping the DNA
- Replication could not occur



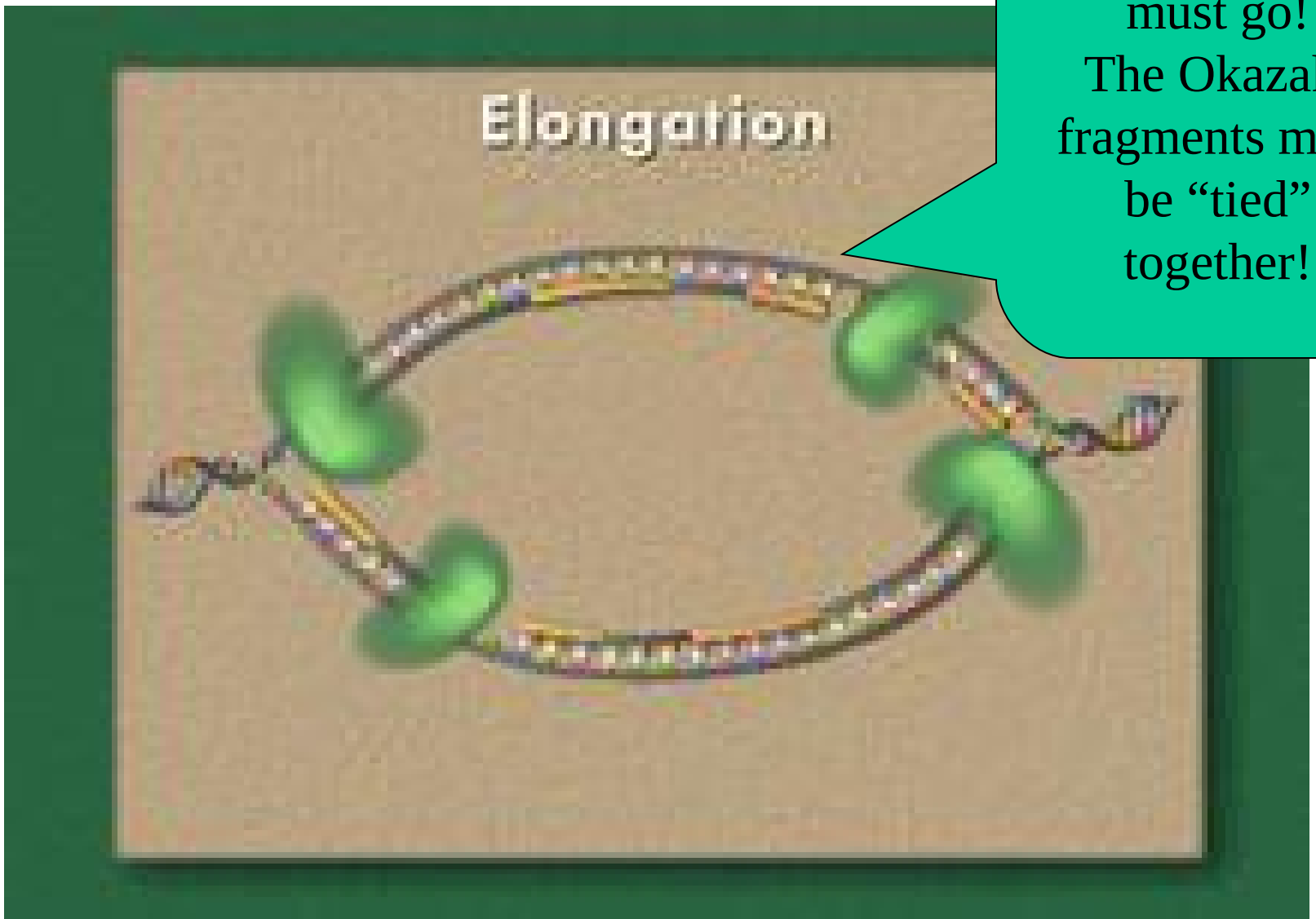
The leading strand forms continuously as the bubble enlarges. Once RNA primase has acted, DNA polymerase "chases the fork" in the 5'→3' direction.



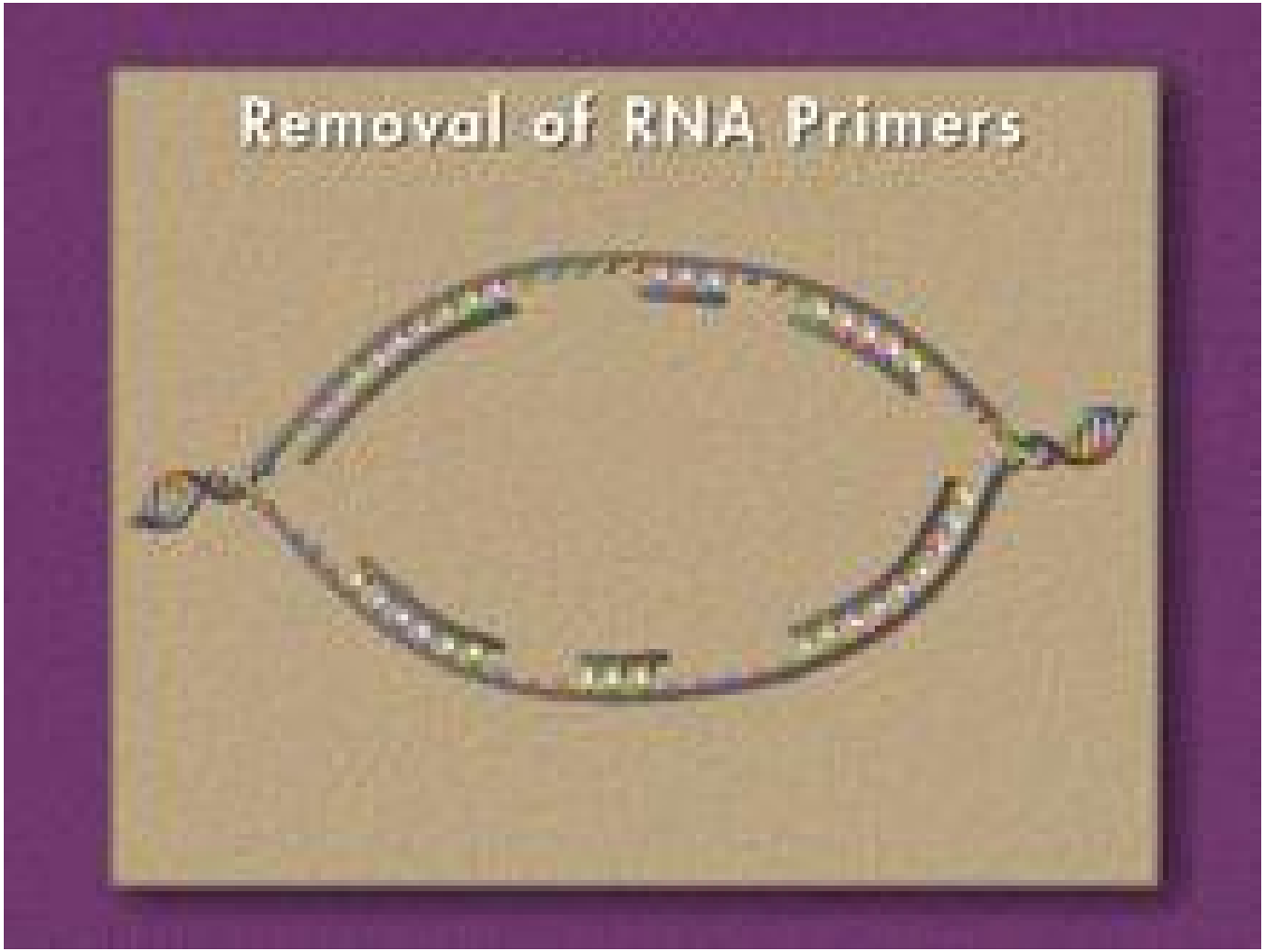
Meanwhile, on the other side of the bubble for each original strand, MANY RNA primases and DNA polymerases are needed to “chase the fork.” This is the discontinuous synthesis that causes it to be referred to as the lagging strand. Okazaki fragments are formed—another enzyme is needed to “tie” them together.



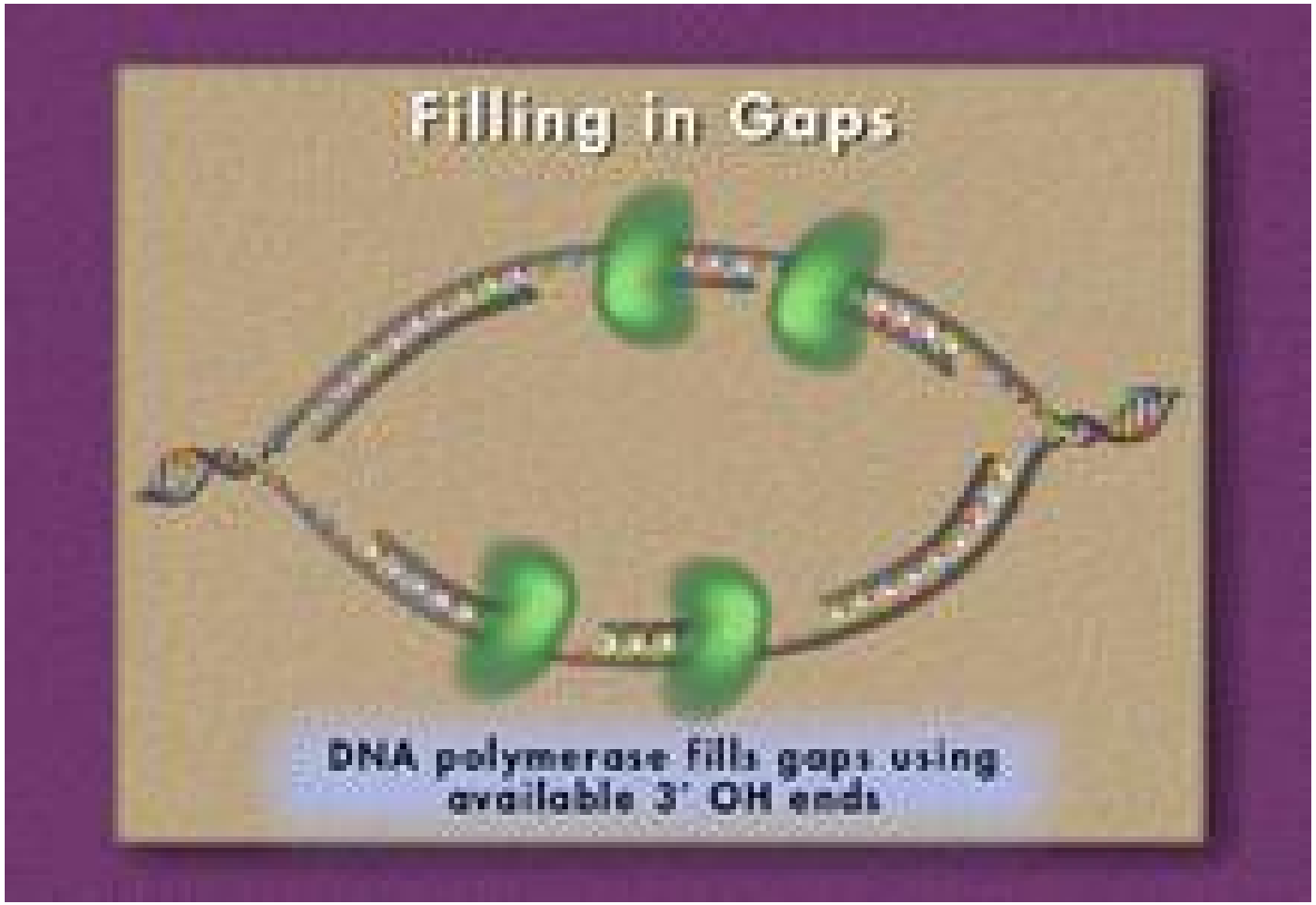
If ALL of the enzymes continue to perform, ELONGATION occurs.



The primers are removed and replaced with DNA nucleotides by DNA polymerase.

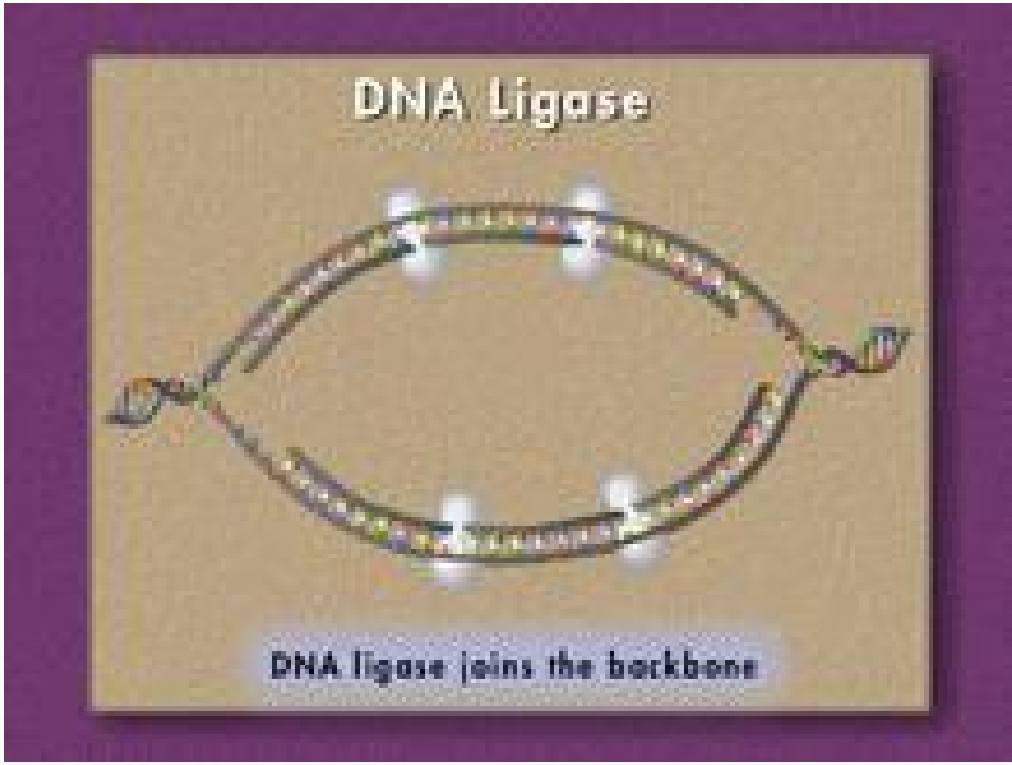


DNA Polymerase fills in the gaps



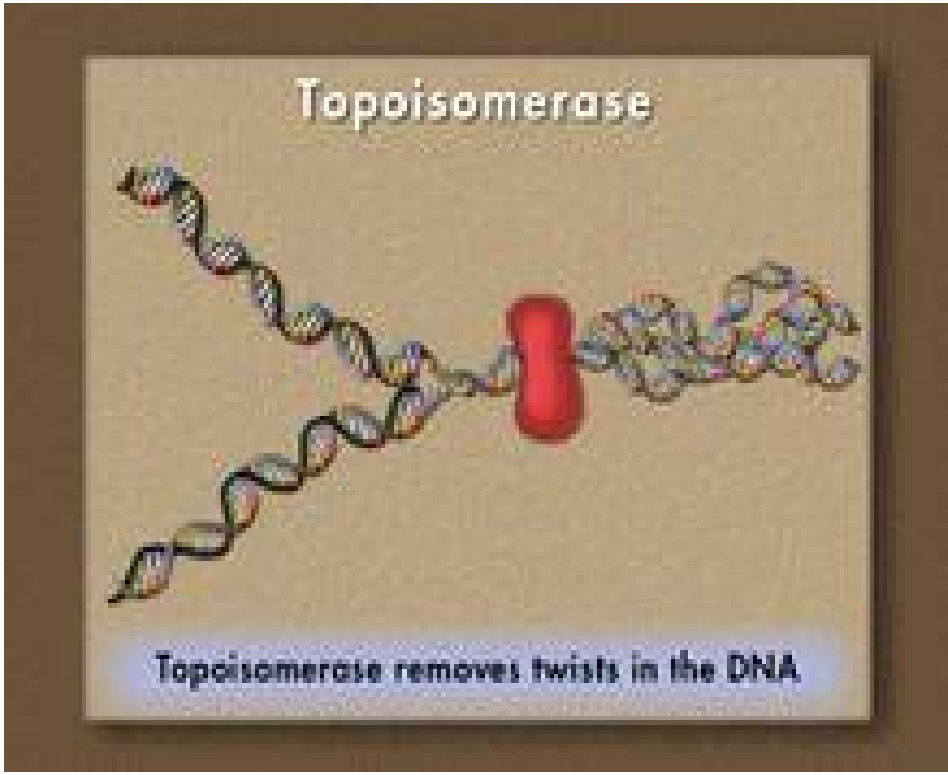
DNA Ligase

- A ligase is an enzyme that ties biochemical units together.
- DNA ligase ties the Okazaki fragments together AND connects the lagging strand to the leading strand.



Topoisomerase

- Have you ever taken a piece of string and unwound it from the center? It tangles on the outside ends.
- Topoisomerase keeps this from happening to the DNA strand during replication. It functions OUTSIDE the replication bubble.



The Proof reading Enzyme

- This is the “Pac Man” enzyme that reads up and down the newly synthesized strand.
- Its function is to locate mismatched base pairs, excise the “new” one [not to mutate the original], and replace it with the correct nitrogen base.