Student Guide

Part 2 - Modeling DNA Function: Replication

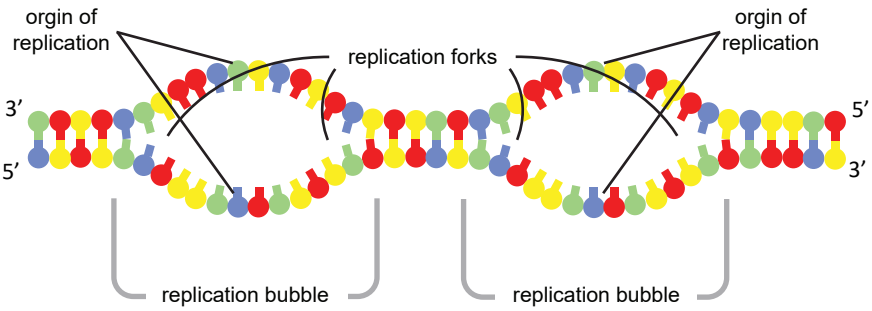
Questions to jumpstart your thinking

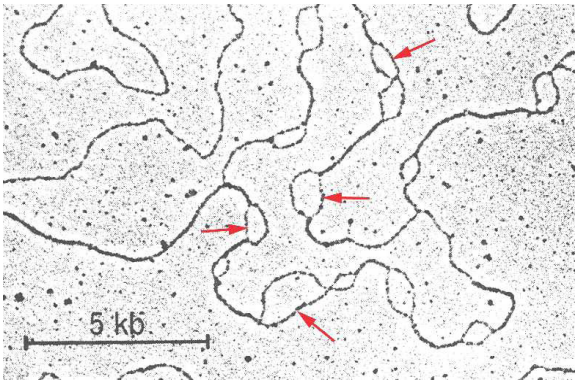
1. Why do cells divide?
2. One of the most common kitchen injuries is cuts from kitchen equipment. When someone sustains such an injury, how do original cells compare to the new cells once the wound has healed?
3. How does your body ensure that new cells for growth and repair contain the same information?
4. How does DNA get into the new cells?

Introduction

The first famous *Nature* paper published by James Watson and Francis Crick in April of 1953, entitled "Molecular Structure of Nucleic Acids," ends with the quote, "It has not escaped our notice that the specific pairing we have postulated immediately suggest a possible copying mechanism for the genetic material." In a second paper published in *Nature* in May of 1953, entitled "Genetical Implications of the Structure of Deoxyribonucleic Acid," Watson and Crick suggest a mechanism for how DNA replicates: "Now our model for deoxyribonucleic acid is, in effect, a pair of templates, each of which is complementary to the other. We imagine that prior to duplication the hydrogen bonds are broken, and the two chains unwind and separate. Each chain then acts as a template for the formation on to itself of a new companion chain, so that eventually we shall have two pairs of chains, where we only had one before. Moreover, the sequence of the pairs of bases will have been duplicated exactly." Since then, many scientists have focused on researching the mechanism of DNA replication. Visit 3dmoleculardesigns.com/Teacher-Resources/Dynamic-DNA-Kit/Watson-and-Crick-Articles.htm to access copies of these papers.

DNA replication begins at specific sites called **origins of replication**. A eukaryotic chromosome may have hundreds or even a few thousand replication origins. Proteins that start DNA replication attach to the DNA and separate the two strands, creating a replication bubble. At each end of the **replication bubble** is a Y-shaped region where the parental strands of DNA are being unwound. This region is referred to as the **replication fork**.





replication bubble

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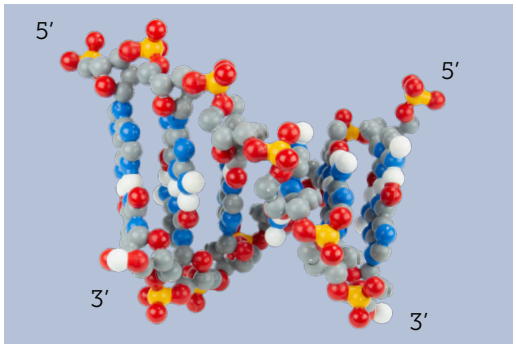
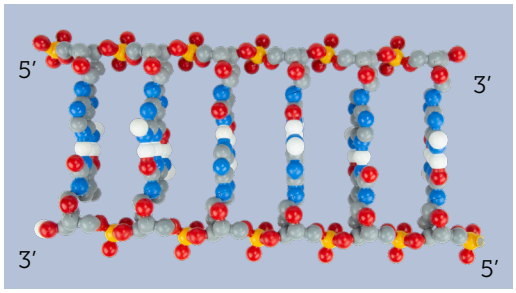
You previously constructed a model of a short sequence of DNA that consists of two anti-parallel strands.

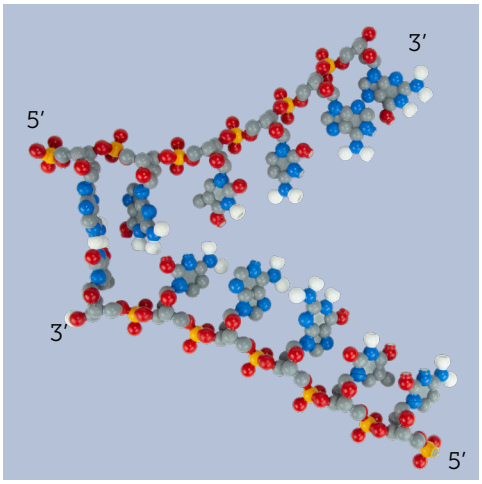


1. To model the replication process with a 12 base pair *Dynamic DNA Kit*©, begin with a DNA sequence using a combination of 6 base pairs to form a double helix. Use 3 A-T base pairs and 3 G-C base pairs in any order to create your sequence. You will use the other 12 nucleotides to form the new DNA strands.



2. You will model DNA replication at one replication fork. Remove the oxygen cap from one 5' phosphate and the hydrogen from the 3' hydroxyl group on one end of your DNA model. This end will be the point of origin for replication in this simulation. Untwist the DNA double helix.

Answer PART 2 questions 1-2 on the student answer sheet.



Separate the two strands leaving the last base pair bound to form a replication fork. In the process of DNA replication, several different proteins participate in the unwinding and separation of the DNA strands. **Helicases** are enzymes that untwist the double helix and separate the parental strands, making them available as template strands for the newly replicated DNA. The untwisting of the double helix causes strain and tighter twisting downstream from the replication fork. **Topoisomerase** is an enzyme that helps to relieve the strain by breaking, swiveling and rejoining the DNA strands. **Single-strand binding proteins** keep the DNA strands from rejoining after they have separated by binding to the unpaired strands during replication.

Answer PART 2 question 3 on the student answer sheet.

FUN FACT

DNA polymerases are enzymes that catalyze the synthesis of DNA by adding nucleotides to the 3′ end of the template strand.* Nucleotides are added at an approximate rate of 50 per second in human cells. The human genome contains 6.4 billion nucleotides (3.2 billion base pairs), which must be copied.

Answer PART 2 question 4-6 on the student answer sheet.

You have already determined that the two ends of a DNA strand are different, giving each strand directionality. These two strands are antiparallel, meaning that the new strands that are generated must also be antiparallel to their template strands. As previously noted, DNA polymerase can only add nucleotides to the 3' end.** The new DNA strands can elongate only in the 5' → 3' direction. This has implications for the process of DNA replication.

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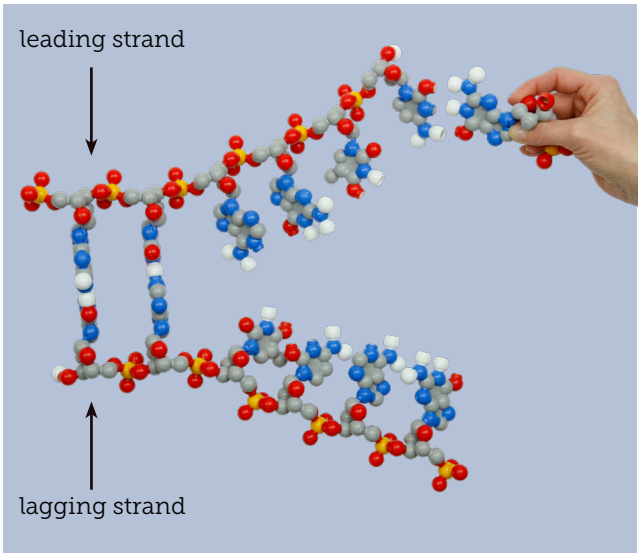
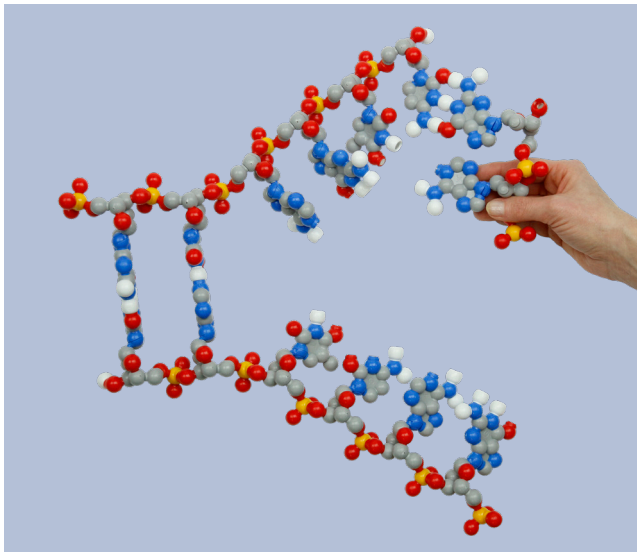
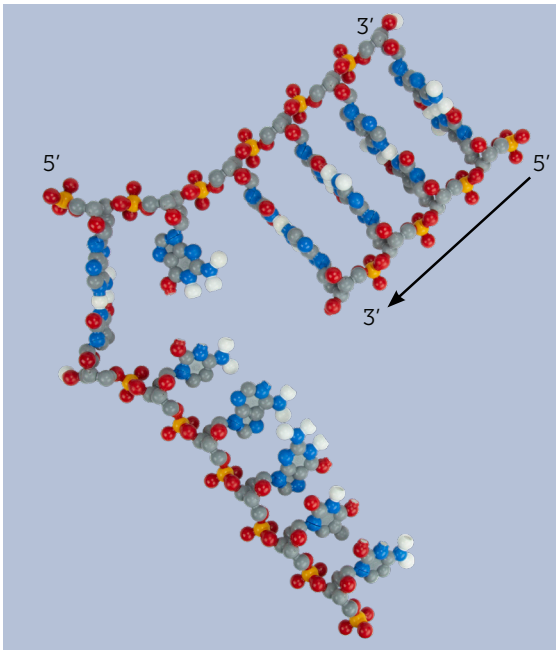
3. Along one template strand, DNA polymerase can synthesize a new strand continuously. Model **continuous replication** by base pairing nucleotides starting at the 3' end of the parental strand. The DNA strand that is synthesized continuously is known as the **leading strand**. See photo to the right.

In order for nucleotides to be added in the mandatory 5' → 3' direction of the other DNA strand, elongation must occur in a series of segments called **Okazaki fragments**. The new strand formed from a series of segments is referred to as the **lagging strand**.

Continuous Replication







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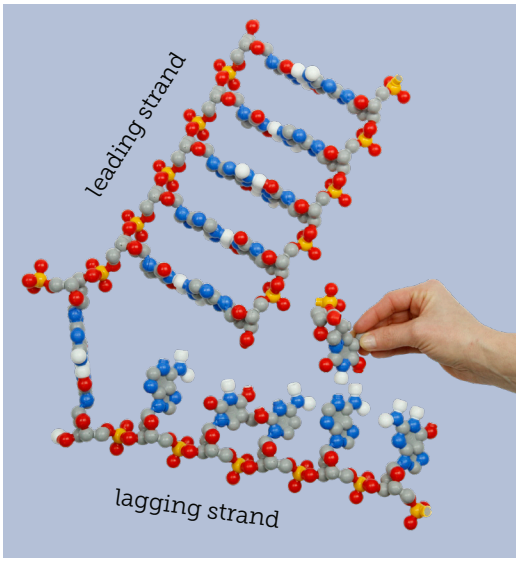
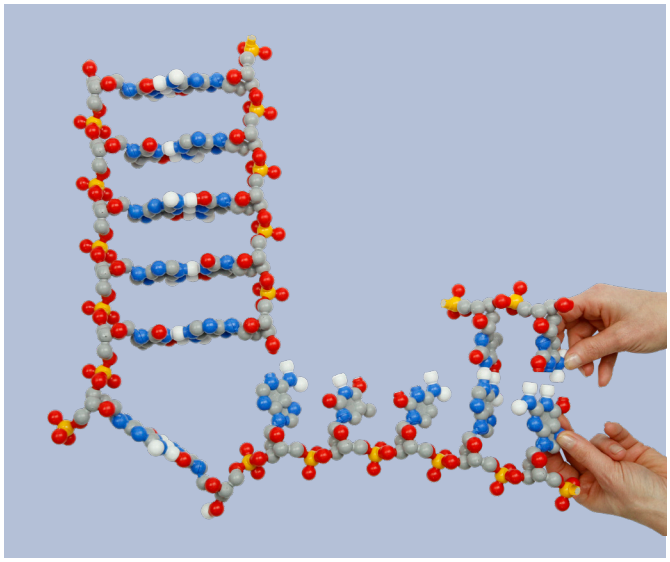
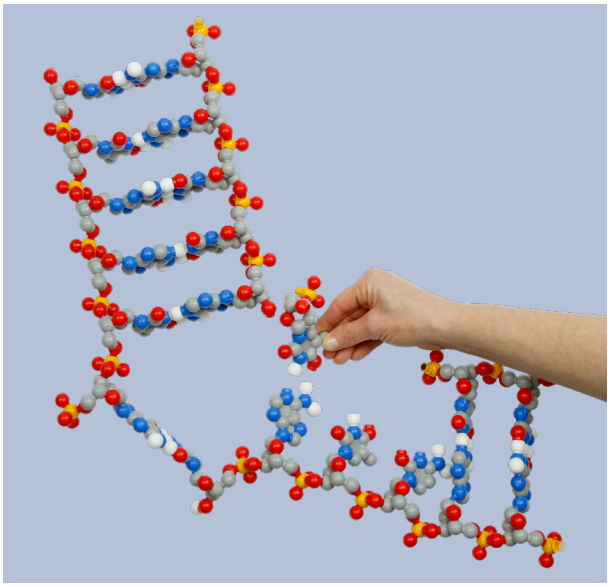
Discontinuous Replication

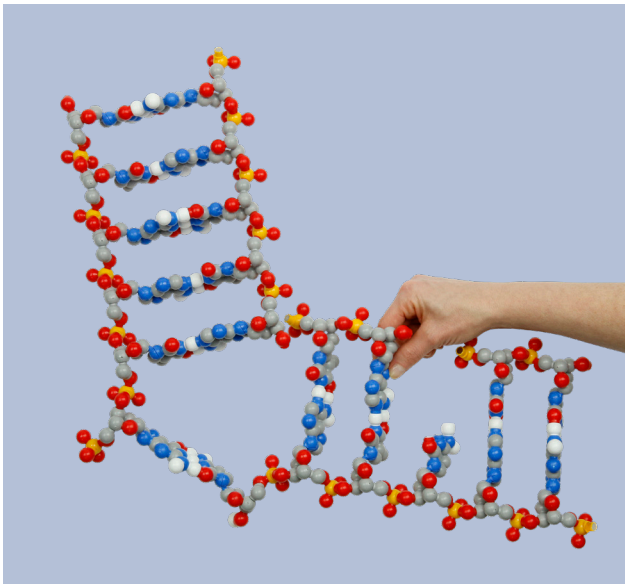
4. You can model **discontinuous replication** by adding the first nucleotide in the new strand to the second to the last position of the 5' end of the parental strand (left image below). Add the second nucleotide in the new strand to the last position of the 5' end of the parental strand (right image below).





5. DNA polymerase will detach and reattach to form the second fragment. You can simulate this by adding a new nucleotide at the base of the fork and then adding subsequent nucleotides until you meet the first fragment.





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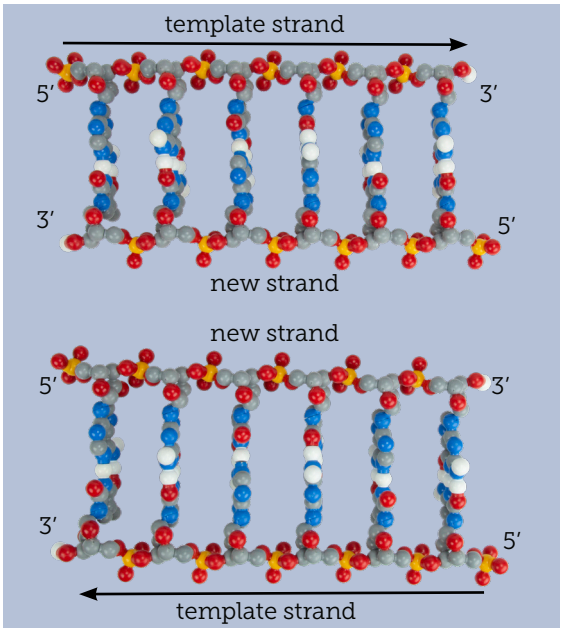
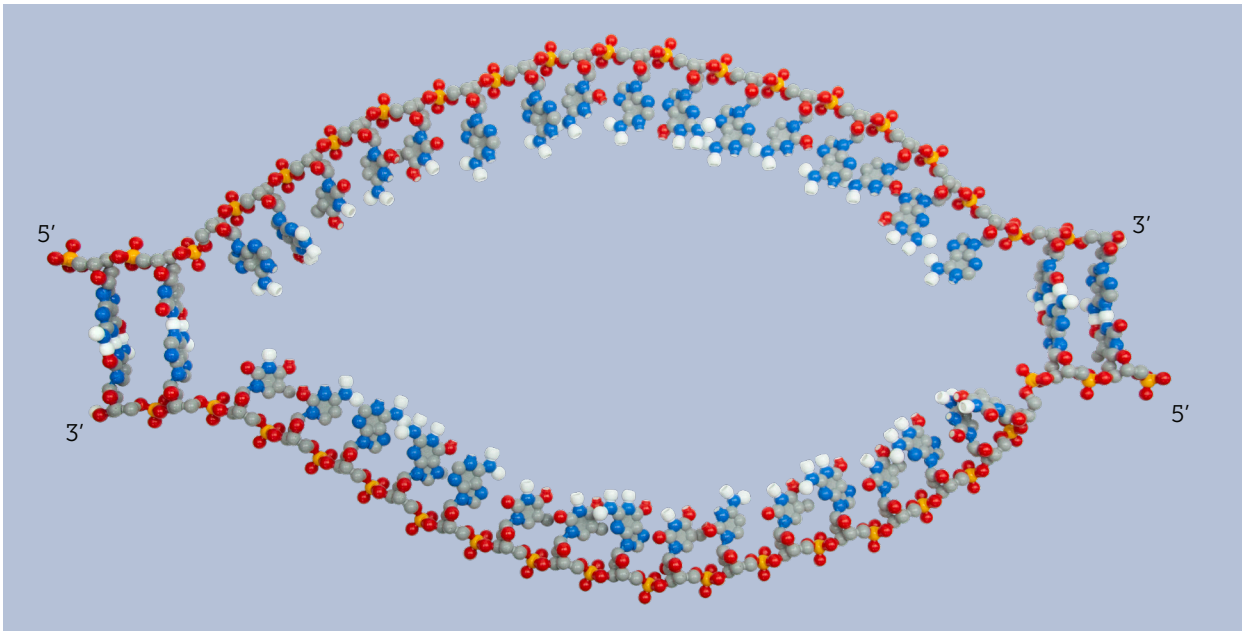
6. Continue adding nucleotides until you have two complete separate DNA molecules. Synthesis of the leading strand and the lagging strand occurs simultaneously. The processes of continuous and discontinuous replication were modeled separately to call your attention to the complications that must be addressed when keeping DNA directionality in mind.

Semiconservatively Replicated DNA



Answer PART 2 questions 7-10 on the student answer sheet.

If 3 or more *Dynamic DNA Kits*© are available, you can create a replication bubble to model DNA replication.



replication bubble

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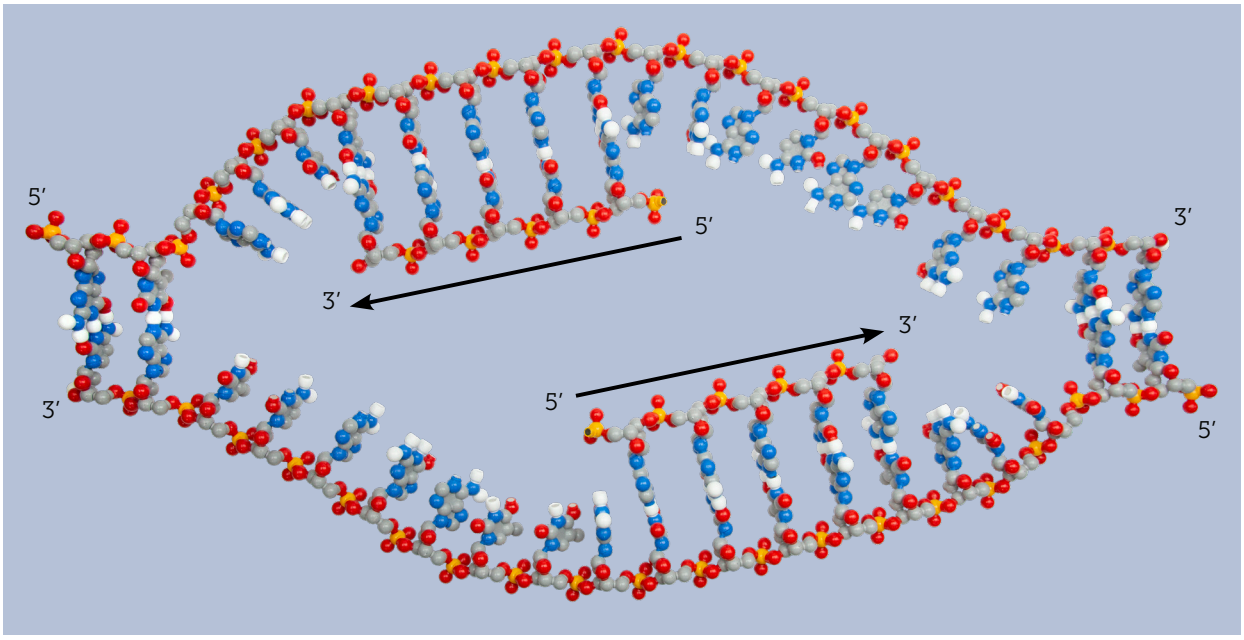
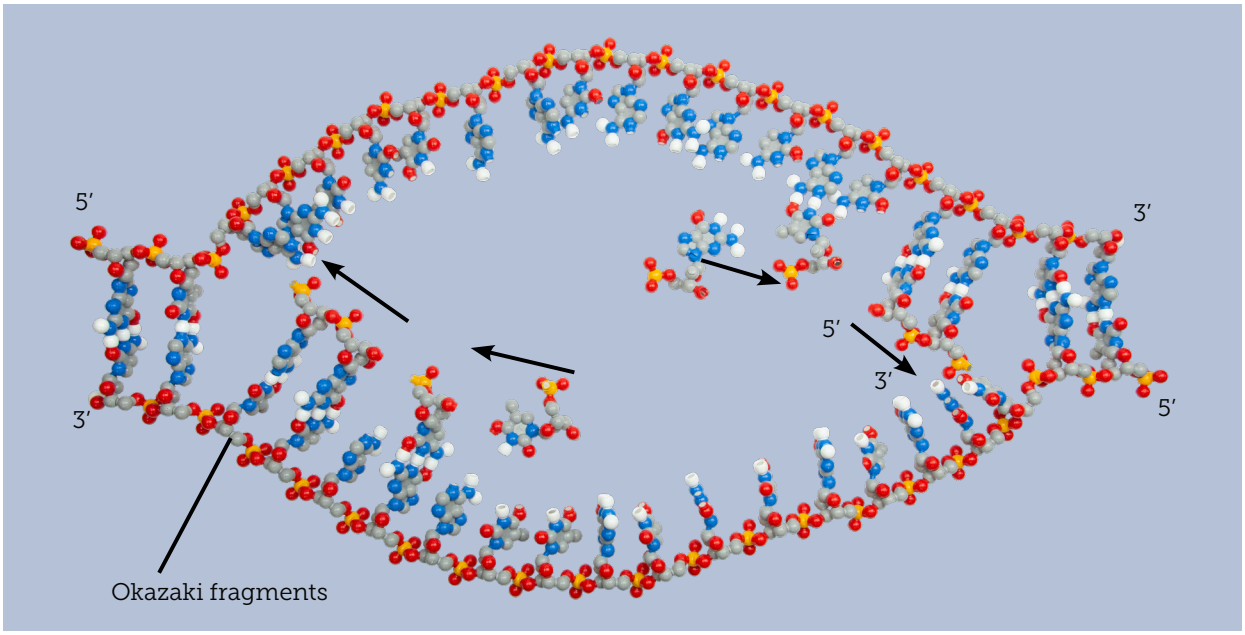


continuous replication



discontinuous replication

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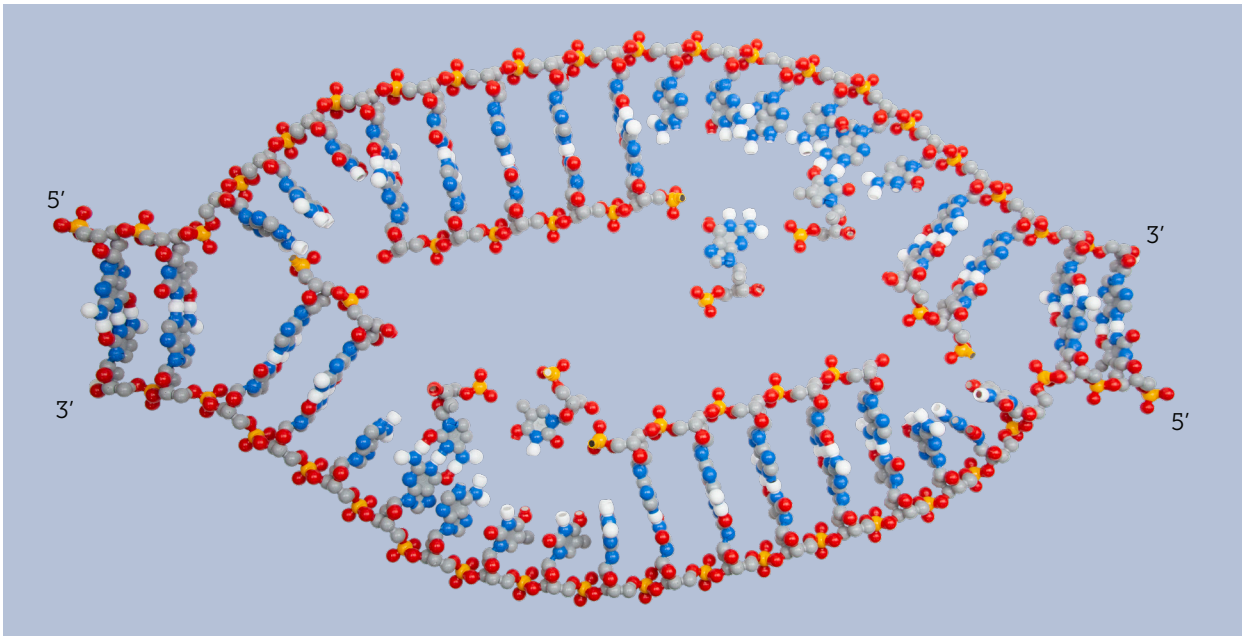
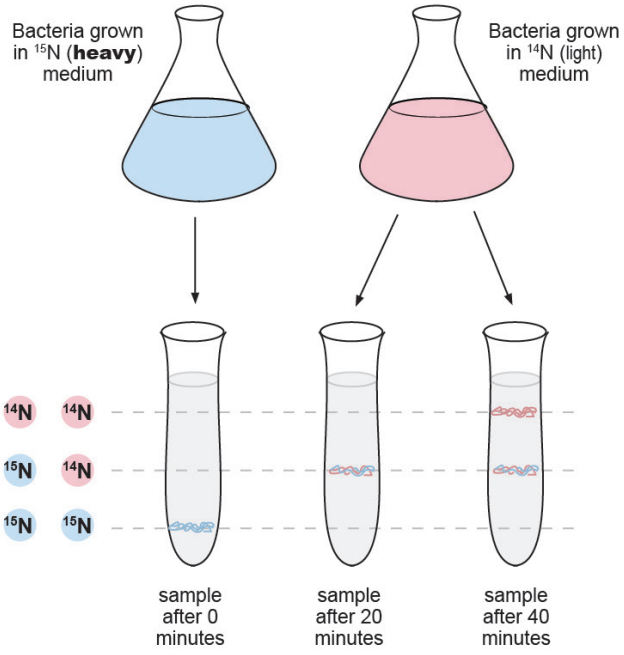


discontinuous and continuous
replication occur simultaneously

Answer PART 2 questions 11-12 on the student answer sheet.

Historically, the mechanism DNA replication was explained by three alternative models: conservative replication, dispersive replication or semiconservative replication. The **conservative model** results in the two parental strands reassociating and restoring the original parental double helix after acting as templates for the new strands. The **dispersive model** results in each strand of both new DNA molecules containing a mixture of old and newly synthesized DNA. The **semiconservative model** results in two new DNA molecules, each with half of the parental DNA and half of newly synthesized DNA. In the late 1950's Matthew Meselson and Franklin Stahl devised an eloquent experiment to determine the mechanism of DNA replication.



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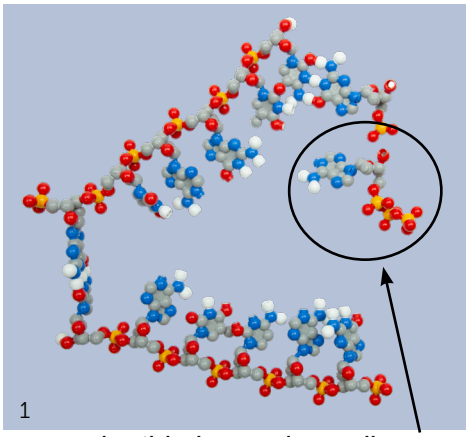
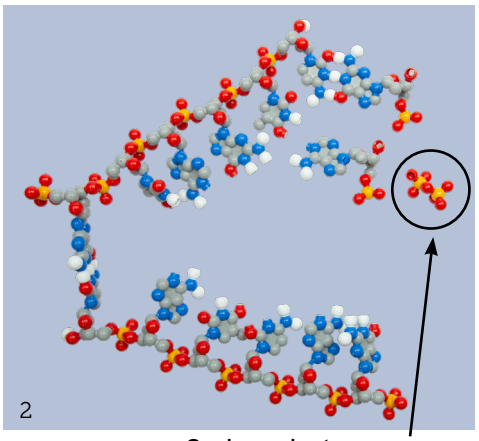
Finer Points

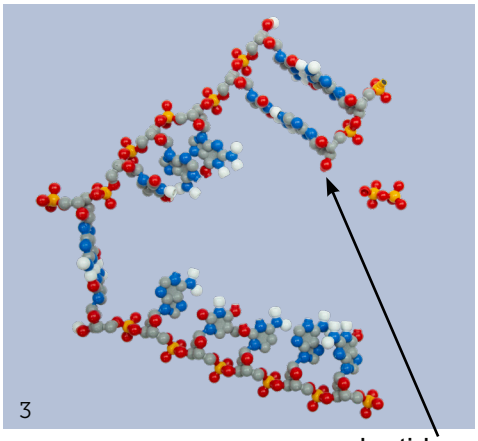
*NOTE: Each nucleotide added to the growing DNA strand consists of a deoxyribose sugar attached to a base and to **three** phosphate groups. The nucleotides used for DNA synthesis are chemically reactive, in part because their triphosphate tails have an unstable cluster of negative charge. In a dehydration synthesis reaction, two phosphate groups are released from the new nucleotide and water is formed when DNA polymerase joins each new nucleotide to the growing DNA strand. It is possible to model this more accurate depiction of the addition of new nucleotides to 6-7 complementary bases without running out of phosphate groups. You will run out of phosphate groups if you try to model the addition of ALL the nucleotides in this manner. However, you will NOT be able to show the formation of water with the kit.



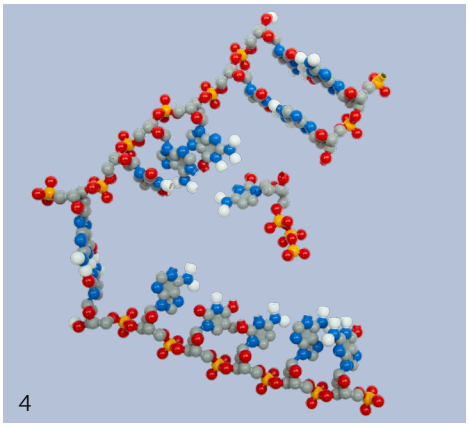
nucleotide base, deoxyribose sugar and 3 phosphate groups



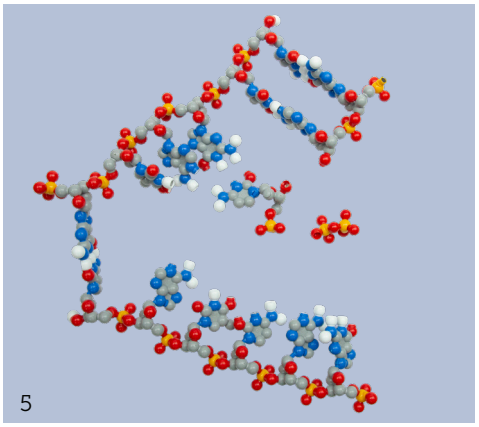
2 phosphate groups are released

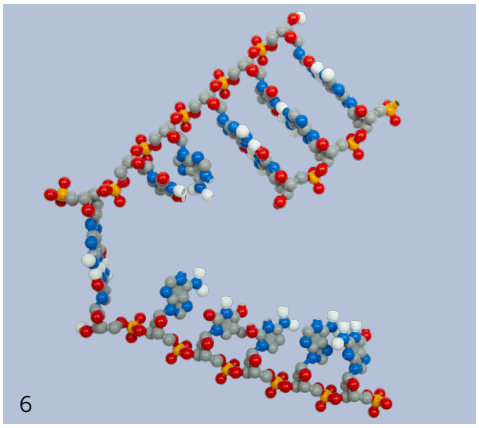


new nucleotide joins DNA strand



repeat





**NOTE: Synthesis of new DNA strands cannot be *initiated* by the enzymes that add DNA nucleotides to the end of an already existing chain that is base-paired with the template strand. The initial nucleotide chain that is produced during DNA synthesis is actually a short segment of RNA, about 5-10 nucleotides in length, by the enzyme primase. The new DNA strand will start from the 3" end of the RNA primer. Later the RNA primer is replaced with DNA nucleotides. DNA ligase joins the DNA segments together.

Answer PART 2 question 13 on the student answer sheet.