

Major Molecular Events of DNA Replication

By: Leslie A. Pray, Ph.D. © 2008 Nature Education

Citation: Pray, L. (2008) Major molecular events of DNA replication. *Nature Education* 1(1):99



Arthur Kornberg compared DNA to a tape recording of instructions that can be copied over and over. How do cells make these near-perfect copies, and does the process ever vary?

Aa Aa Aa

Scientists have devoted decades of effort to understanding how **deoxyribonucleic acid (DNA)** replicates itself. In simple terms, **replication** involves use of an existing strand of DNA as a template for the synthesis of a new, identical strand. American enzymologist and Nobel Prize winner Arthur Kornberg compared this process to a tape recording of instructions for performing a task: "[E]xact copies can be made from it, as from a tape recording, so that this information can be used again and elsewhere in time and space" (Kornberg, 1960).

In reality, the process of replication is far more complex than suggested by Kornberg's analogy. Researchers typically utilize simple bacterial cells in their experiments, but they still do not have all the answers, particularly when it comes to **eukaryotic** replication. Nonetheless, scientists are familiar with the basic steps in the replication process, and they continue to rely on this information as the basis for continued research and experimentation.

The Molecular Machinery of Bacterial DNA Replication

A typical bacterial **cell** has anywhere from about 1 million to 4 million base pairs of DNA, compared to the 3 billion base pairs in the **genome** of the common house mouse (*Mus musculus*). Still, even in **bacteria**, with their smaller genomes, DNA replication involves an incredibly sophisticated, highly coordinated series of molecular events. These events are divided into four major stages: initiation, unwinding, **primer** synthesis, and elongation.

Initiation and Unwinding

During initiation, so-called initiator proteins bind to the **replication origin**, a base-pair sequence of nucleotides known as **oriC**. This binding triggers events that unwind the DNA **double helix** into two single-stranded DNA molecules. Several groups of proteins are involved in this unwinding (Figure 1). For example, the DNA helicases are responsible for breaking the hydrogen bonds that join the **complementary nucleotide** bases to each other; these hydrogen bonds are an essential feature of **James Watson and Francis Crick's three-dimensional DNA model**. Because the newly unwound single strands have a tendency to rejoin, another group of proteins, the single-strand binding proteins, keep the single strands stable until elongation begins. A third family of proteins, the topoisomerases, reduce some of the torsional **strain** caused by the unwinding of the double helix.

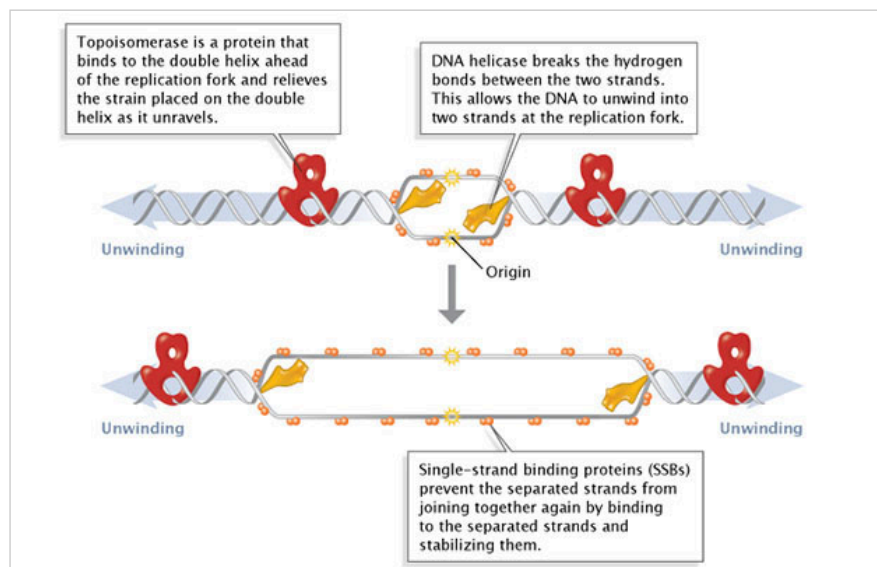


Figure 1: Facilitation of DNA unwinding.

During DNA replication, several proteins facilitate the unwinding of the DNA double helix into two single strands.

Topoisomerases (red) reduce torsional strain caused by the unwinding of the DNA double helix; DNA helicase (yellow) breaks hydrogen bonds between complementary base-pairs; single-strand binding proteins (SSBs) stabilize the separated strands and prevent them from rejoining.

© 2014 **Nature Education** Adapted from Pierce, Benjamin. *Genetics: A Conceptual Approach*, 2nd ed. All rights reserved.



As previously mentioned, the location at which a DNA strand begins to unwind into two separate single strands is known as the **origin of replication**. As shown in Figure 1, when the double helix unwinds, replication proceeds along the two single strands at the same time but in opposite directions (i.e., left to right on one strand, and right to left on the other). This forms two replication forks that move along the DNA, replicating as they go.

Primer Synthesis

Primer synthesis marks the beginning of the actual synthesis of the new DNA **molecule**. Primers are short stretches of nucleotides (about 10 to 12 bases in length) synthesized by an **RNA polymerase enzyme** called **primase**. Primers are required because DNA polymerases, the enzymes responsible for the actual addition of nucleotides to the new DNA strand, can only add deoxyribonucleotides to the 3'-OH group of an existing chain and cannot begin synthesis *de novo*. Primase, on the other hand, can add ribonucleotides *de novo*. Later, after elongation is complete, the primer is removed and replaced with DNA nucleotides.

Elongation

Finally, elongation—the addition of nucleotides to the new DNA strand—begins after the primer has been added. Synthesis of the growing strand involves adding nucleotides, one by one, in the exact order specified by the original (template) strand. Recall that one of the key features of the Watson-Crick DNA model is that **adenine** is always paired with **thymine** and **cytosine** is always paired with **guanine**. So, for example, if the original strand reads A-G-C-T, the new strand will read T-C-G-A.

DNA is always synthesized in the 5'-to-3' direction, meaning that nucleotides are added only to the 3' end of the growing strand. As shown in Figure 2, the 5'-phosphate group of the new nucleotide binds to the 3'-OH group of the last nucleotide of the growing strand. Scientists have yet to identify a polymerase that can add bases to the 5' ends of DNA strands.

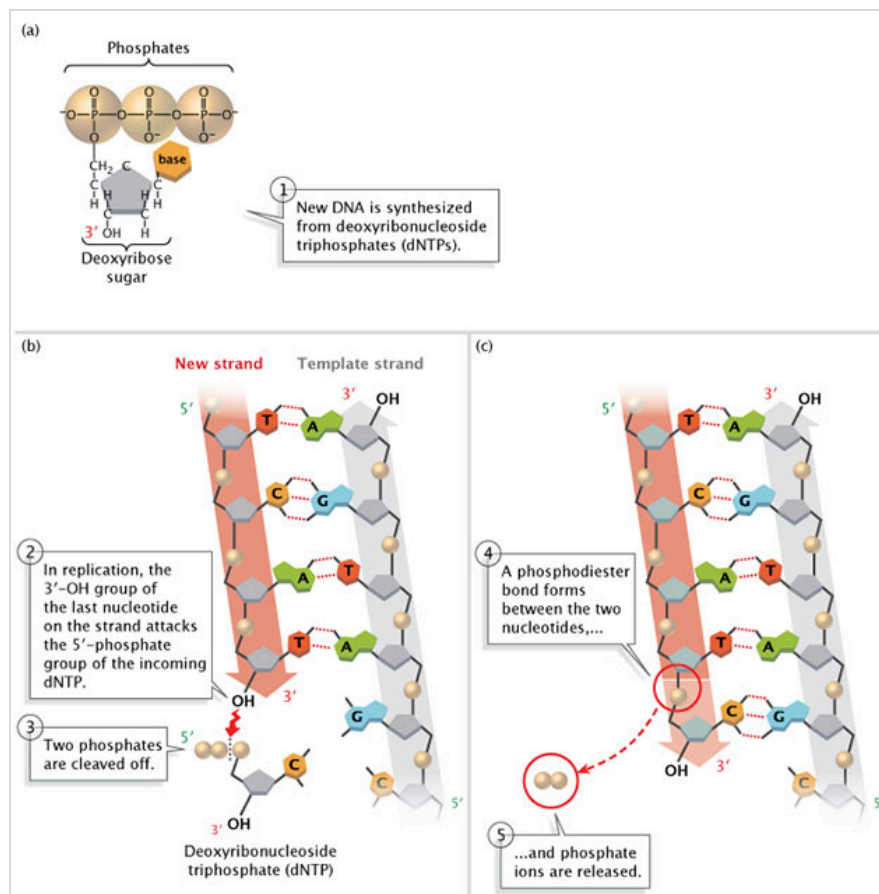


Figure 2: New DNA is synthesized from deoxyribonucleoside triphosphates (dNTPs).

(A) A deoxyribonucleoside triphosphate (dNTP). (B) During DNA replication, the 3'-OH group of the last nucleotide on the new strand attacks the 5'-phosphate group of the incoming dNTP. Two phosphates are cleaved off. (C) A phosphodiester bond forms between the two nucleotides, and phosphate ions are released.

© 2014 **Nature Education** Adapted from Pierce, Benjamin. *Genetics: A Conceptual Approach*, 2nd ed. All rights reserved.



The Discovery of DNA Polymerase

While studying *E. coli* bacteria, enzymologist Arthur Kornberg discovered that DNA polymerases catalyze DNA synthesis. Kornberg's experiment involved mixing all of the basic "ingredients" necessary for *E. coli* DNA synthesis in a test tube, including nucleotides, *E. coli* extract, and **ATP**, and then purifying and testing the enzymes involved. Using this method, Kornberg not only discovered DNA polymerases, but he also performed some of the initial work demonstrating how enzymes add new nucleotides to growing DNA chains (Kornberg, 1959).

Scientists have since identified a total of five different DNA polymerases in *E. coli*, each with a specialized role. For example, **DNA polymerase III** does most of the elongation work, adding nucleotides one by one to the 3' end of the new and growing single strand. Other enzymes, including **DNA polymerase I** and **RNase H**, are responsible for removing the RNA primer after **DNA polymerase III** has begun its work, replacing it with DNA nucleotides (Ogawa & Okazaki, 1984). When these enzymes finish, they leave a nick between the section of DNA that was formerly the primer and the elongated section of DNA. Another enzyme called **DNA ligase** then acts to seal the bond between the two adjacent nucleotides.

DNA Polymerase Only Moves in One Direction

After a primer is synthesized on a strand of DNA and the DNA strands unwind, synthesis and elongation can proceed in only one direction. As previously mentioned, DNA polymerase can only add to the 3' end, so the 5' end of the primer remains unaltered. Consequently, synthesis proceeds immediately only along the so-called **leading strand**. This immediate replication is known as **continuous replication**. The other strand (in the 5' direction from the primer) is called the **lagging strand**, and replication along it is called discontinuous replication. The double helix has to unwind a bit before the synthesis of another primer can be initiated further up on the lagging strand. Synthesis can then occur from the 3' end of that new primer. Next, the double helix unwinds a bit more, and another spurt of replication proceeds. As a result, replication along the lagging strand can only proceed in short, discontinuous spurts (Figure 3).

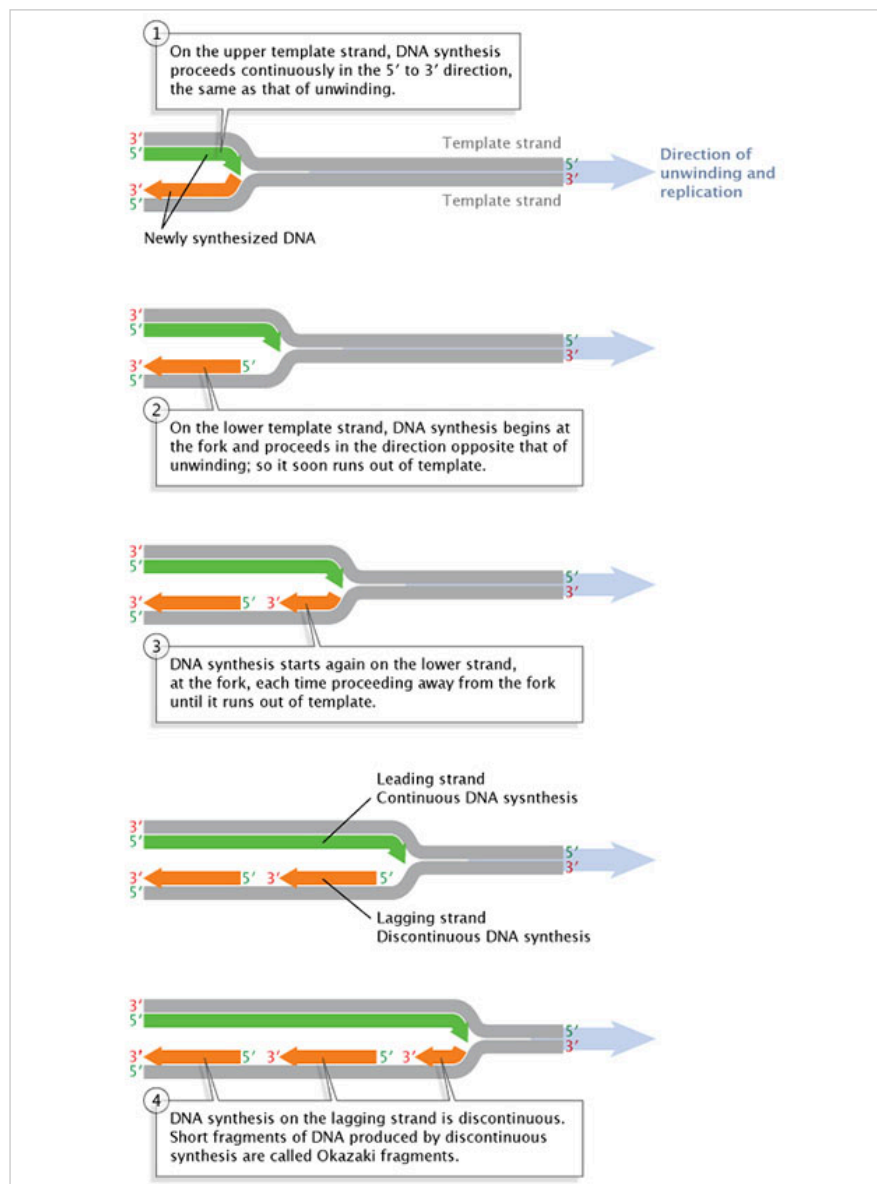


Figure 3: Replication of the leading DNA strand is continuous, while replication along the lagging strand is discontinuous.

After a short length of the DNA has been unwound, synthesis must proceed in the 5' to 3' direction; that is, in the direction opposite that of the unwinding.

© 2014 **Nature Education** Adapted from Pierce, Benjamin. *Genetics: A Conceptual Approach*, 2nd ed. All rights reserved.



Figure Detail

The fragments of newly synthesized DNA along the lagging strand are called Okazaki fragments, named in honor of their discoverer, Japanese molecular biologist Reiji Okazaki. Okazaki and his colleagues made their discovery by conducting what is known as a pulse-chase experiment, which involved exposing replicating DNA to a short "pulse" of isotope-labeled nucleotides and then varying the length of time that the cells would be exposed to nonlabeled nucleotides. This later period is called the "chase" (Okazaki *et al.*, 1968). The labeled nucleotides were incorporated into growing DNA molecules only during the initial few seconds of the pulse; thereafter, only nonlabeled nucleotides were incorporated during the chase. The scientists then centrifuged the newly synthesized DNA and observed that the shorter chases resulted in most of the radioactivity appearing in "slow" DNA. The sedimentation rate was determined by size: smaller fragments precipitated more slowly than larger fragments because of their lighter weight. As the investigators increased the length of the chases, radioactivity in the "fast" DNA increased with little or no increase of radioactivity in the slow DNA. The researchers correctly interpreted these observations to mean that, with short chases, only very small fragments of DNA were being synthesized along the lagging strand. As the chases increased in length, giving DNA more time to replicate, the lagging strand fragments started integrating into longer, heavier, more rapidly sedimenting DNA.

strands. Today, scientists know that the Okazaki fragments of bacterial DNA are typically between 1,000 and 2,000 nucleotides long, whereas in eukaryotic cells, they are only about 100 to 200 nucleotides long.

The Challenges of Eukaryotic Replication

Bacterial and eukaryotic cells share many of the same basic features of replication; for instance, initiation requires a primer, elongation is always in the 5'-to-3' direction, and replication is always continuous along the leading strand and discontinuous along the lagging strand. But there are also important differences between bacterial and eukaryotic replication, some of which biologists are still actively researching in an effort to better understand the molecular details. One difference is that eukaryotic replication is characterized by many replication origins (often thousands), not just one, and the sequences of the replication origins vary widely among [species](#). On the other hand, while the replication origins for bacteria, *oriC*, vary in length (from about 200 to 1,000 base pairs) and sequence, except among closely related organisms, all bacteria nonetheless have just a single replication origin (Mackiewicz *et al.*, 2004).

Eukaryotic replication also utilizes a different set of DNA polymerase enzymes (e.g., DNA polymerase δ and DNA polymerase ϵ instead of DNA polymerase III). Scientists are still studying the roles of the 13 eukaryotic polymerases discovered to date. In addition, in eukaryotes, [the DNA template is compacted by the way it winds around proteins called histones](#). This DNA-histone complex, called a [nucleosome](#), poses a unique challenge both for the cell and for scientists investigating the molecular details of eukaryotic replication. What happens to nucleosomes during DNA replication? Scientists know from electron micrograph studies that nucleosome reassembly happens very quickly after replication (the reassembled nucleosomes are visible in the electron micrograph images), but they still do not know how this happens (Annunziato, 2005).

Also, whereas bacterial chromosomes are circular, eukaryotic chromosomes are [linear](#). During circular DNA replication, the excised primer is readily replaced by nucleotides, leaving no gap in the newly synthesized DNA. In contrast, in linear DNA replication, there is always a small gap left at the very end of the chromosome because of the lack of a 3'-OH group for replacement nucleotides to bind. (As mentioned, DNA synthesis can proceed only in the 5'-to-3' direction.) If there were no way to fill this gap, the DNA molecule would get shorter and shorter with every generation. However, the ends of linear chromosomes—the [telomeres](#)—have several properties that prevent this.

DNA replication occurs during the [S phase of cell division](#). In *E. coli*, this means that the entire genome is replicated in just 40 minutes, at a pace of approximately 1,000 nucleotides per second. In eukaryotes, the pace is much slower: about 40 nucleotides per second. The coordination of the [protein](#) complexes required for the steps of replication and the speed at which replication must occur in order for cells to divide are impressive, especially considering that enzymes are also [proofreading](#), which leaves very few errors behind.

Summary

The study of DNA replication started almost as soon as the structure of DNA was elucidated, and it continues to this day. Currently, the stages of initiation, unwinding, primer synthesis, and elongation are understood in the most basic sense, but many questions remain unanswered, particularly when it comes to replication of the eukaryotic genome. Scientists have devoted decades to the study of replication, and researchers such as Kornberg and Okazaki have made a number of important breakthroughs. Nonetheless, much remains to be learned about replication, including how errors in this process contribute to human [disease](#).

References and Recommended Reading

Annunziato, A. T. Split decision: What happens to nucleosomes during DNA replication? *Journal of Biological Chemistry* **280**, 12065–12068 (2005)

Bessman, M. J., *et al.* Enzymatic synthesis of deoxyribonucleic acid. II. General properties of the reaction. *Journal of Biological Chemistry* **233**, 171–177 (1958)

Kornberg, A. The biological synthesis of deoxyribonucleic acid. Nobel Lecture, December 11, 1959. ([link to transcript](#))

———. Biological synthesis of deoxyribonucleic acid. *Science* **131**, 1503–1508 (1960)

Lehman, I. R., *et al.* Enzymatic synthesis of deoxyribonucleic acid. I. Preparation of substrates and partial purification of an enzyme from *Escherichia coli*. *Journal of Biological Chemistry* **233**, 163–170 (1958)

Losick, R., & Shapiro, L. DNA replication: Bringing the mountain to Mohammed. *Science* **282**, 1430–1431 (1998)

Mackiewicz, P., *et al.* Where does bacterial replication start? Rules for predicting the *oriC* region. *Nucleic Acids Research* **32**, 3781–3791 (2004)

Okawa, T., & Okazaki, T. Function of RNase H in DNA replication revealed by RNase H defective mutants of *Escherichia coli*. *Molecular and General Genetics* **193**, 231–237 (1984)

Okazaki, R., *et al.* Mechanism of DNA chain growth. I. Possible discontinuity and unusual secondary structure of newly synthesized chains. *Proceedings of the National Academy of Sciences* **59**, 598–605 (1968)

[Outline](#) | [Keywords](#) | [Add Content to Group](#) [FEEDBACK](#)



Explore This Subject

APPLICATIONS IN BIOTECHNOLOGY

Genetically Modified Organisms (GMOs):
Transgenic Crops and Recombinant DNA
Technology
Recombinant DNA Technology and
Transgenic Animals
Restriction Enzymes
The Biotechnology Revolution: PCR and
the Use of Reverse Transcriptase to
Clone Expressed Genes

DNA REPLICATION

DNA Damage & Repair: Mechanisms for
Maintaining DNA Integrity
DNA Replication and Causes of Mutation

DISCOVERY OF GENETIC MATERIAL

Barbara McClintock and the Discovery of
Jumping Genes (Transposons)
Discovery of DNA as the Hereditary
Material using *Streptococcus pneumoniae*
Discovery of DNA Structure and Function:
Watson and Crick
Isolating Hereditary Material: Frederick
Griffith, Oswald Avery, Alfred Hershey,
and Martha Chase

GENE COPIES

Copy Number Variation
Copy Number Variation and Genetic
Disease

Genetic Mutation
Genetic Mutation
Major Molecular Events of DNA Replication
Semi-Conservative DNA Replication: Meselson and Stahl

Copy Number Variation and Human Disease
DNA Deletion and Duplication and the Associated Genetic Disorders
Tandem Repeats and Morphological Variation

JUMPING GENES

Barbara McClintock and the Discovery of Jumping Genes (Transposons)
Functions and Utility of *Alu* Jumping Genes
Transposons, or Jumping Genes: Not Junk DNA?
Transposons: The Jumping Genes

TRANSCRIPTION & TRANSLATION

DNA Transcription
RNA Transcription by RNA Polymerase: Prokaryotes vs Eukaryotes
Translation: DNA to mRNA to Protein
What is a Gene? Colinearity and Transcription Units

RNA

Chemical Structure of RNA
Eukaryotic Genome Complexity
Genome Packaging in Prokaryotes: the Circular Chromosome of *E. coli*
RNA Functions
RNA Splicing: Introns, Exons and Spliceosome
RNA Transcription by RNA Polymerase: Prokaryotes vs Eukaryotes
What is a Gene? Colinearity and Transcription Units