

DNA Replication

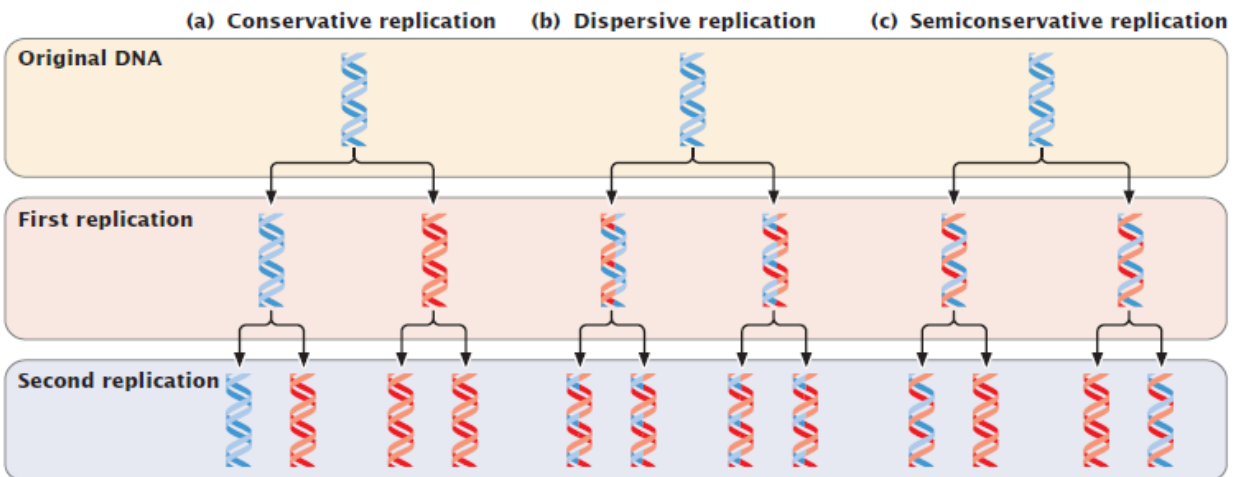
All DNA Replication Takes Place in a Semiconservative Manner

From the three-dimensional structure of DNA proposed by Watson and Crick in 1953, several important genetic implications were immediately apparent. The complementary nature of the two nucleotide strands in a DNA molecule suggested that, during replication, each strand can serve as a template for the synthesis of a new strand. The specificity of base pairing (adenine with thymine; guanine with cytosine) implied that only one sequence of bases can be specified by each template, and so the two DNA molecules built on the pair of templates will be identical with the original. This process is called **semiconservative replication** because each of the original nucleotide strands remains intact (conserved), despite no longer being combined in the same molecule; the original DNA molecule is half (semi) conserved during replication. Initially, three models were proposed for DNA replication.

In **conservative replication** (Figure 12.1a), the entire double-stranded DNA molecule serves as a template for a whole new molecule of DNA, and the original DNA molecule is *fully* conserved during replication.

In **dispersive replication** (Figure 12.1b), both nucleotide strands break down (disperse) into fragments, which serve as templates for the synthesis of new DNA fragments, and then somehow reassemble into two complete DNA molecules. In this model, each resulting DNA molecule is interspersed with fragments of old and new DNA; none of the original molecule is conserved.

Semiconservative replication (Figure 12.1c) is intermediate between these two models; the two nucleotide strands unwind and each serves as a template for a new DNA molecule.



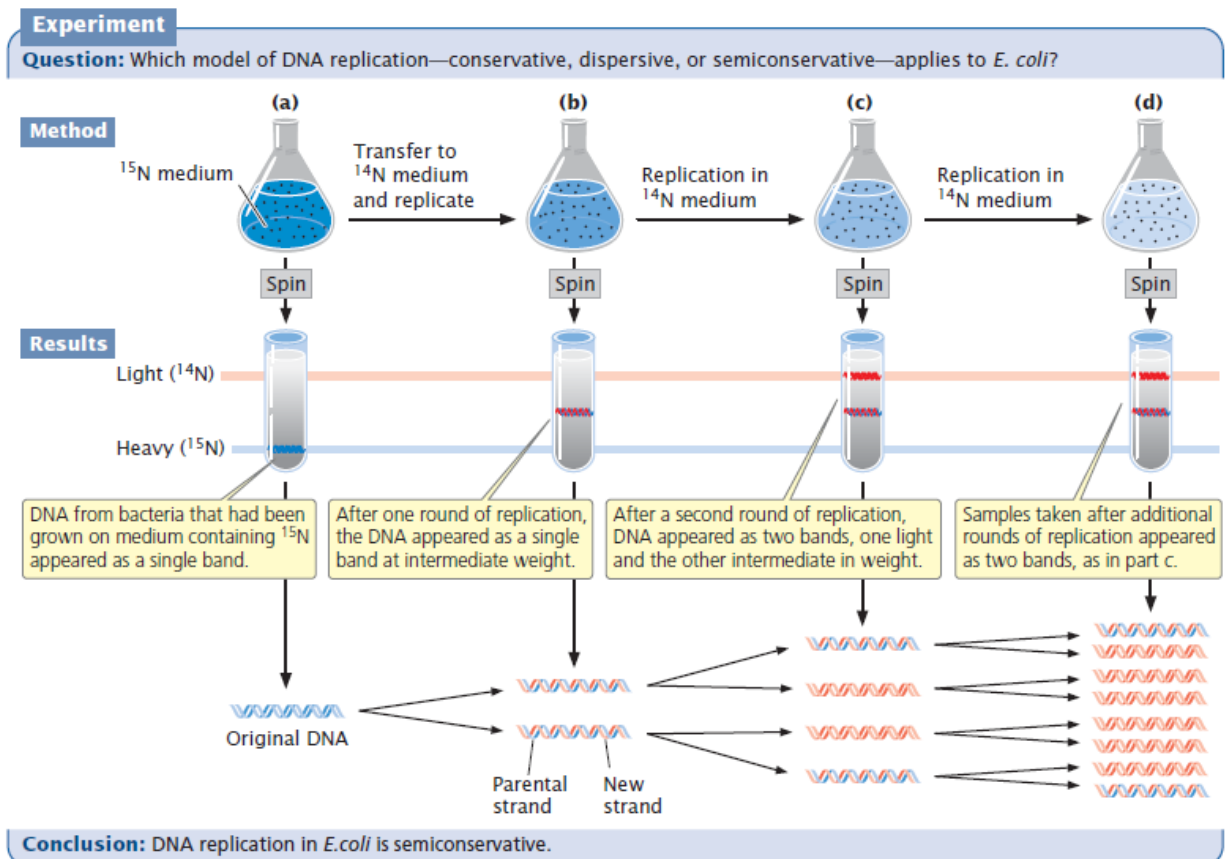
12.1 Three proposed models of replication are conservative replication, dispersive replication, and semiconservative replication.

These three models allow different predictions to be made about the distribution of original DNA and newly synthesized DNA after replication. With conservative replication, after one round of replication, 50% of the molecules would consist entirely of the original DNA and 50% would consist entirely of new DNA. After a second round of replication, 25% of the molecules would consist entirely of the original DNA and 75% would consist entirely of new DNA. With each additional round of replication, the proportion of molecules with new DNA would increase, although the number of molecules with the original DNA would remain constant. Dispersive replication would always produce hybrid molecules, containing some

original and some new DNA, but the proportion of new DNA within the molecules would increase with each replication event. In contrast, with semiconservative replication, one round of replication would produce two hybrid molecules, each consisting of half original DNA and half new DNA. After a second round of replication, half the molecules would be hybrid, and the other half would consist of new DNA only. Additional rounds of replication would produce more and more molecules consisting entirely of new DNA, and a few hybrid molecules would persist.

Meselson and Stahl's Experiment

To determine which of the three models of replication applied to *E. coli* cells, Matthew Meselson and Franklin Stahl needed a way to distinguish old and new DNA. They did so by using two isotopes of nitrogen, ^{14}N (the common form) and ^{15}N (a rare, heavy form). Meselson and Stahl grew a culture of *E. coli* in a medium that contained ^{15}N as the sole nitrogen source; after many generations, all the *E. coli* cells had ^{15}N incorporated into all of the purine and pyrimidine bases of their DNA. Meselson and Stahl took a sample of these bacteria, switched the rest of the bacteria to a medium that contained only ^{14}N , and then took additional samples of bacteria over the next few cellular generations. In each sample, the bacterial DNA that was synthesized before the change in medium contained ^{15}N and was relatively heavy, whereas any DNA synthesized after the switch contained ^{14}N and was relatively light.



12.3 Meselson and Stahl demonstrated that DNA replication is semiconservative.

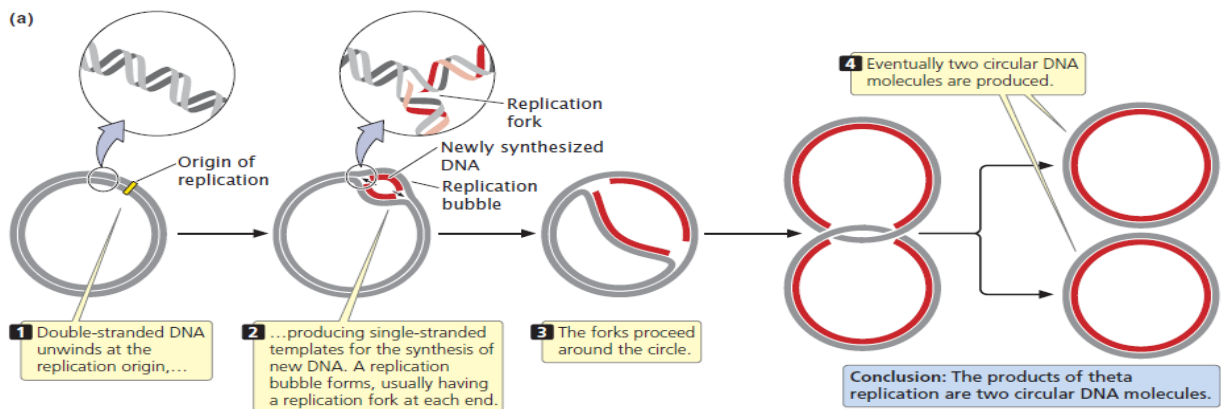
Meselson and Stahl found that DNA from bacteria grown only on medium containing ^{15}N produced a single band at the position expected of DNA containing only ^{15}N (**Figure 12.3a**). DNA from bacteria transferred to the medium with ^{14}N and allowed one round of

replication also produced a single band but at a position intermediate between that expected of DNA containing only ^{15}N and that expected of DNA containing only ^{14}N (**Figure 12.3b**). This result is inconsistent with the conservative replication model, which predicts one heavy band (the original DNA molecules) and one light band (the new DNA molecules). A single band of intermediate density is predicted by both the semiconservative and the dispersive models.

To distinguish between these two models, Meselson and Stahl grew the bacteria in medium containing ^{14}N for a second generation. After a second round of replication in medium with ^{14}N , two bands of equal intensity appeared, one in the intermediate position and the other at the position expected of DNA containing only ^{14}N (**Figure 12.3c**). All samples taken after additional rounds of replication produced the same two bands, and the band representing light DNA became progressively stronger (**Figure 12.3d**). Meselson and Stahl's results were exactly as expected for semiconservative replication and are incompatible with those predicated for both conservative and dispersive replication.

Modes of Replication

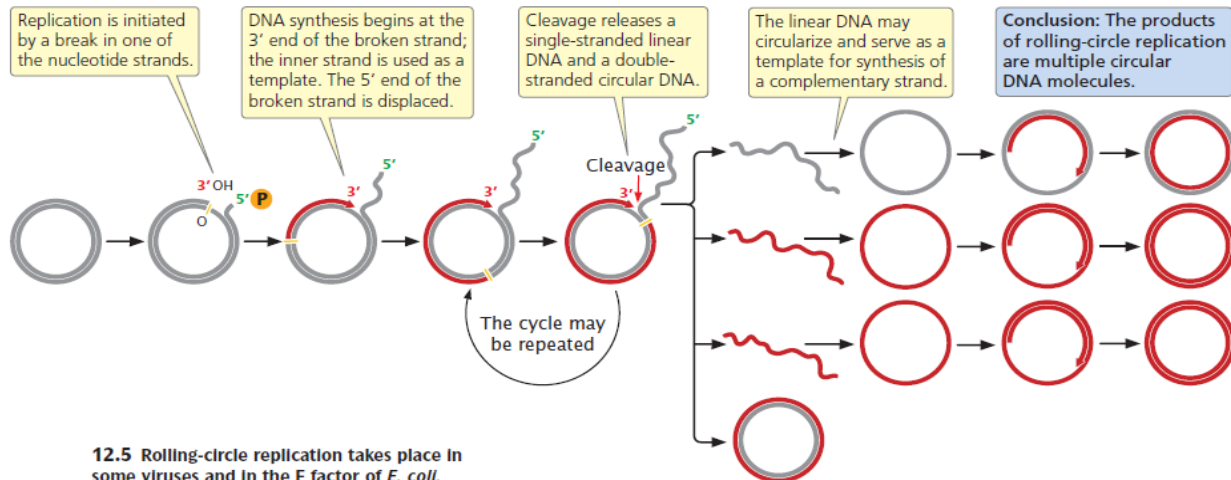
1. Theta replication: A common type of replication that takes place in circular DNA, such as that found in *E. coli* and other bacteria, is called **theta replication** (**Figure 12.4a**) because it generates a structure that resembles the Greek letter theta (θ). In theta replication, double-stranded DNA begins to unwind at the replication origin, producing single stranded nucleotide strands that then serve as templates on which new DNA can be synthesized. The unwinding of the double helix generates a loop, termed a **replication bubble**. Unwinding may be at one or both ends of the bubble, making it progressively larger. DNA replication on both of the template strands is simultaneous with unwinding. The point of unwinding, where the two single nucleotide strands separate from the double-stranded DNA helix, is called a **replication fork**. If there are two replication forks, one at each end of the replication bubble, the forks proceed outward in both directions in a process called **bidirectional replication**, simultaneously unwinding and replicating the DNA until they eventually meet. If a single replication fork is present, it proceeds around the entire circle to produce two complete circular DNA molecules, each consisting of one old and one new nucleotide strand.



12.4. Theta replication is a type of replication common in *E. coli* and other organisms possessing circular DNA.

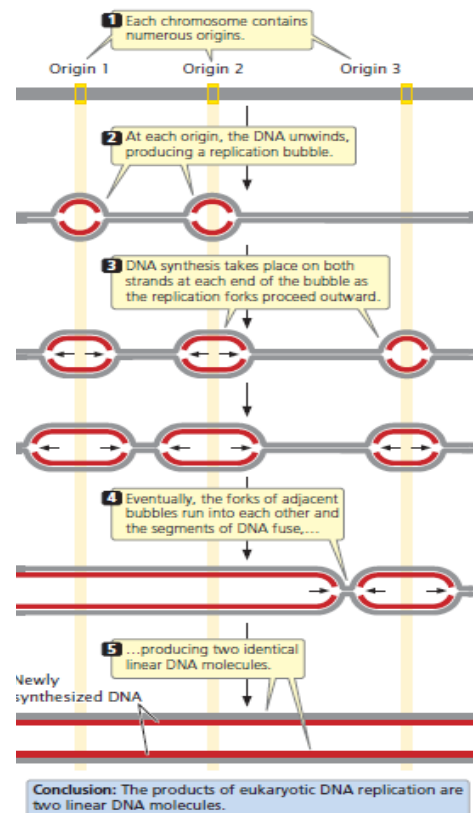
2. Rolling-circle replication: Another form of replication, called **rolling-circle replication** (**Figure 12.5**), takes place in some viruses and in the F factor (a small circle of extra chromosomal DNA that controls mating) of *E. coli*. This form of replication is initiated by a break in one of the nucleotide strands that creates a $3'$ -OH group and a $5'$ -phosphate group. New

nucleotides are added to the 3' end of the broken strand, with the inner (unbroken) strand used as a template. As new nucleotides are added to the 3' end, the 5' end of the broken strand is displaced from the template, rolling out like thread being pulled off a spool. The 3' end grows around the circle, giving rise to the name rolling-circle model. The replication fork may continue around the circle a number of times, producing several linked copies of the same sequence. With each revolution around the circle, the growing 3' end displaces the nucleotide strand synthesized in the preceding revolution. Eventually, the linear DNA molecule is cleaved from the circle, resulting in a double stranded circular DNA molecule and a single-stranded linear DNA molecule. The linear molecule circularizes either before or after serving as a template for the synthesis of a complementary strand.



12.5 Rolling-circle replication takes place in some viruses and in the F factor of *E. coli*.

3. Linear eukaryotic replication: Circular DNA molecules that undergo theta or rolling-circle replication have a single origin of replication. Because of the limited size of these DNA molecules, replication starting from one origin can traverse the entire chromosome in a reasonable amount of time. The large linear chromosomes in eukaryotic cells, however, contain far too much DNA to be replicated speedily from a single origin. Eukaryotic replication proceeds at a rate ranging from 500 to 5000 nucleotides per minute at each replication fork (considerably slower than bacterial replication). Even at 5000 nucleotides per minute at each fork, DNA synthesis starting from a single origin would require 7 days to replicate a typical human chromosome consisting of 100 million base pairs of DNA. The replication of eukaryotic chromosomes actually takes place in a matter of minutes or hours, not days. This rate is possible because replication initiates at thousands of origins. Typical eukaryotic replicons are from 20,000 to 300,000 base pairs in length. At each replication origin, the DNA unwinds and produces a replication bubble. Replication takes place on both strands at each end of the bubble, with the two replication forks spreading outward. Eventually, the replication forks of adjacent replicons run into each other, and the replicons fuse to form long stretches of newly synthesized DNA (**Figure 12.6**).



12.6 Linear DNA replication takes place in eukaryotic chromosomes.

Characteristics of theta, rolling-circle, and linear eukaryotic replication

Replication Model	DNA Template	Breakage of Nucleotide Strand	Number of Replicons	Unidirectional or Bidirectional	Product
Theta	Circular	No	1	Unidirectional or bidirectional	Two circular molecules
Rolling circle	Circular	yes	1	Unidirectional	One circular molecule and one linear molecule that may circularize
Linear eukaryotic	Linear	No	Many	Bidirectional	Two linear molecules

Requirements of Replication:

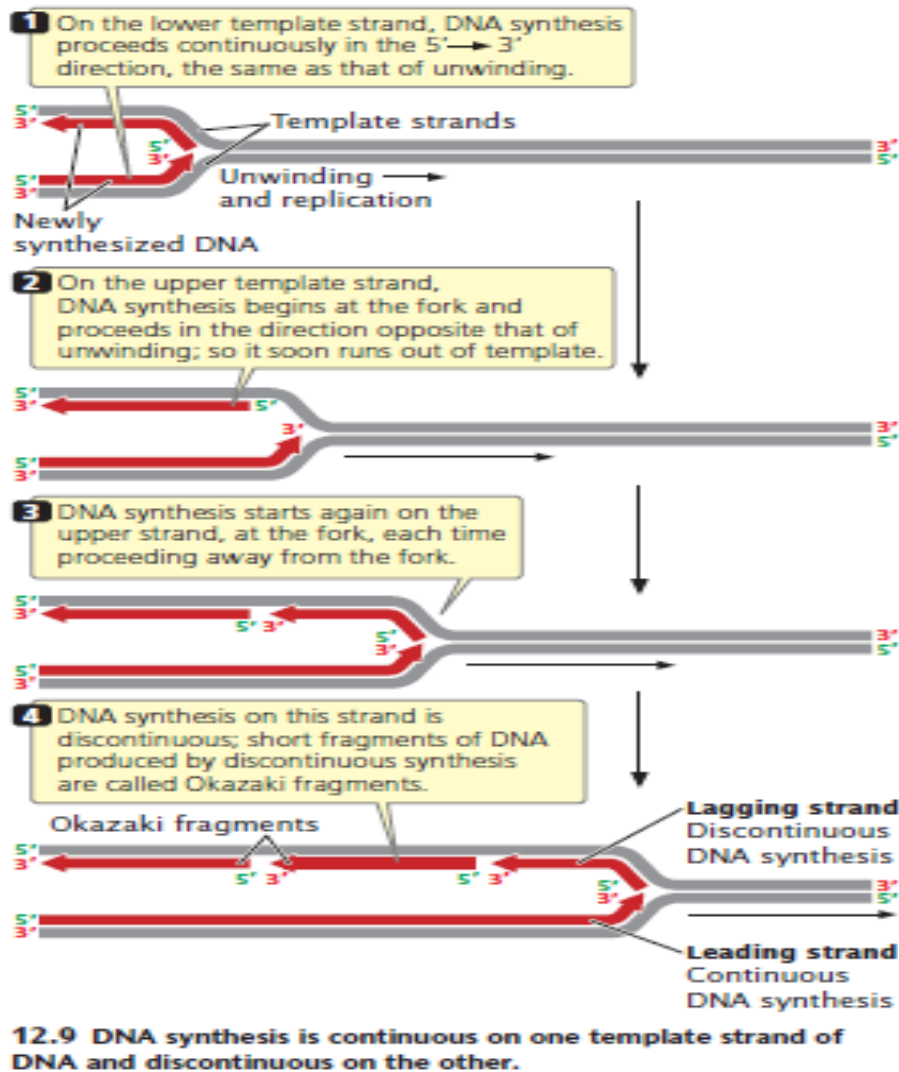
1. A template consisting of single-stranded DNA,
2. Raw materials (substrates) to be assembled into a new nucleotide strand, and
3. Enzymes and other proteins that “read” the template and assemble the substrates into a DNA molecule.

Direction of Replication

In DNA synthesis, new nucleotides are joined one at a time to the 3' end of the newly synthesized strand. **DNA polymerases**, the enzymes that synthesize DNA, can add nucleotides only to the 3' end of the growing strand (not the 5' end), and so new DNA strands always elongate in the same 5'-to-3' direction (5'→3'). Because the two single-stranded DNA templates are antiparallel and strand elongation is always 5'→3', if synthesis on one template proceeds from, say, right to left, then synthesis on the other template must proceed in the opposite direction, from left to right. As DNA unwinds during replication, the antiparallel nature of the two DNA strands means that one template is exposed in the 5'→3' direction and the other template is exposed in the 3'→5' direction; so how can synthesis take place simultaneously on both strands at the fork?

Continuous and discontinuous replication As the DNA unwinds; the template strand that is exposed in the 3'→5' direction (the lower strand in Figures 12.9) allows the new strand to be synthesized continuously, in the 5'→3' direction. This new strand, which undergoes **continuous replication**, is called the **leading strand**. The other template strand is exposed in the 5'→3' direction (the upper strand in Figures 12.9). After a short length of the DNA has been unwound, synthesis must proceed 5'→3'; that is, in the direction *opposite* that of unwinding (**Figure 12.9**). Because only a short length of DNA needs to be unwound before synthesis on this strand gets started, the replication machinery soon runs out of template. By that time, more DNA has unwound, providing new template at the 5' end of the new strand. DNA synthesis must start anew at the replication fork and proceed in the direction opposite that of the movement of the fork until it runs into the previously replicated segment of DNA. This process is repeated again and again, and so synthesis of this strand is in short, discontinuous bursts. The newly made strand that undergoes **discontinuous replication** is called the **lagging strand**.

Okazaki fragments: The short lengths of DNA produced by discontinuous replication of the lagging strand are called **Okazaki fragments**, after Reiji Okazaki, who discovered them. In bacterial cells, each Okazaki fragment ranges in length from about 1000 to 2000 nucleotides; in eukaryotic cells, they are about 100 to 200 nucleotides long. Okazaki fragments on the lagging strand are linked together to create a continuous new DNA molecule.



Bacterial Replication Requires a Large Number of Enzymes and Proteins

Replication takes place in four stages: initiation, unwinding, elongation, and termination.

Initiation: The circular chromosome of *E. coli* has a single replication origin (*oriC*). The minimal sequence required for *oriC* to function consists of 245 bp that contain several critical sites. An **initiator protein** (known as DnaA in *E. coli*) binds to *oriC* and causes a short section of DNA to unwind. This unwinding allows helicase and other single-strand-binding proteins to attach to the polynucleotide strand (**Figure 12.11**).

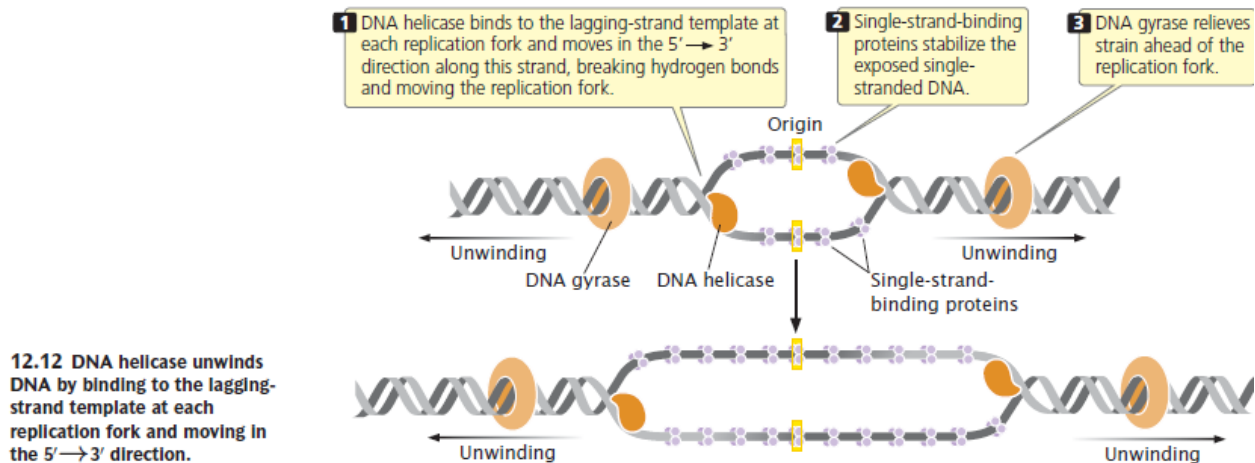
Unwinding: Because DNA synthesis requires a single-stranded template and because double stranded DNA must be unwound before DNA synthesis can take place, the cell relies on several proteins and enzymes to accomplish the unwinding.

DNA helicase: A **DNA helicase** breaks the hydrogen bonds that exist between the bases of the two nucleotide strands of a DNA molecule. Helicase cannot *initiate* the unwinding of double-

stranded DNA; the initiator protein first separates DNA strands at the origin, providing a short template at each replication fork and moves in the 5'→3' direction along this strand, thus also moving the replication fork (**Figure 12.12**).

Single-strand-binding proteins: After DNA has been unwound by helicase, **single-strand-binding proteins** (SSBs) attach tightly to the exposed single-stranded DNA (Figure 12.12). These proteins protect the single-stranded nucleotide chains and prevent the formation of secondary structures such as hairpins that interfere with replication. Unlike many DNA-binding proteins, SSBs are indifferent to base sequence: they will bind to any single-stranded DNA. Single-strand-binding proteins form tetramers (groups of four); each tetramer covers from 35 to 65 nucleotides.

DNA gyrase: Another protein essential for the unwinding process is the enzyme **DNA gyrase**, a topoisomerase (control the supercoiling of DNA). In replication, DNA gyrase reduces the torsional strain (torque) that builds up ahead of the replication fork as a result of unwinding (see Figure 12.12). It reduces torque by making a double-stranded break in one segment of the DNA helix, passing another segment of the helix through the break, and then resealing the broken ends of the DNA. This action removes a twist in the DNA and reduces the supercoiling.



12.12 DNA helicase unwinds DNA by binding to the lagging-strand template at each replication fork and moving in the 5'→3' direction.

Elongation

During the elongation phase of replication, single-stranded DNA is used as a template for the synthesis of DNA. This process requires a series of enzymes.

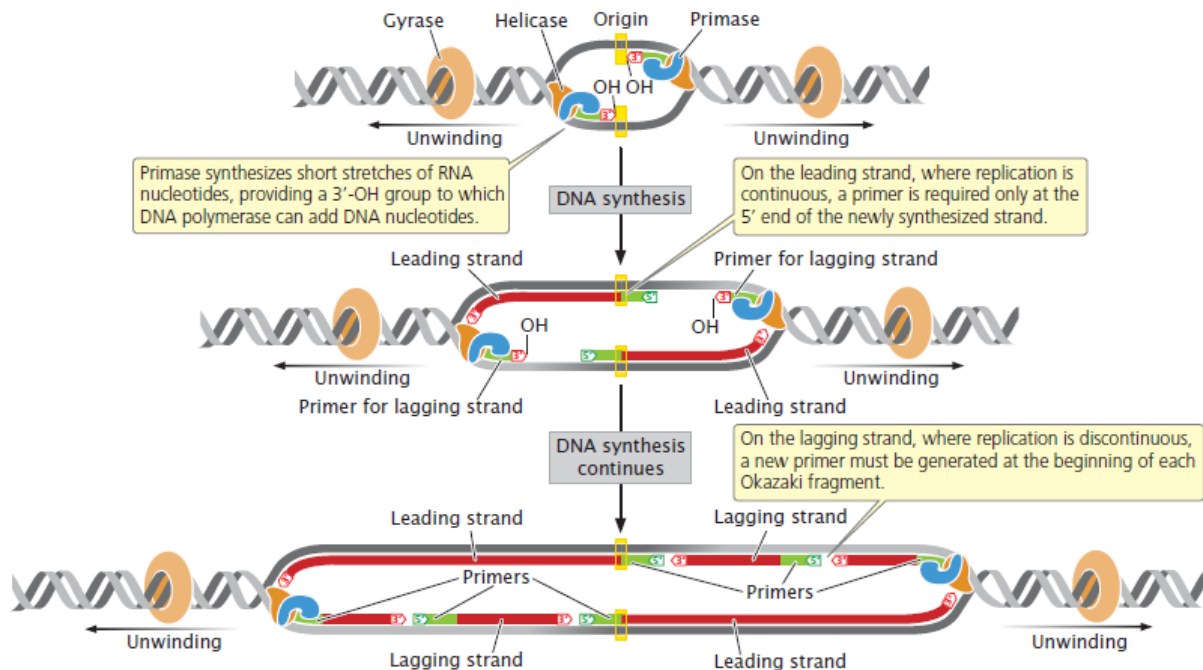
An enzyme called **primase** synthesizes short stretches of nucleotides, or **primers**, to get DNA replication started. Primase synthesizes a short stretch of RNA nucleotides (about 10–12 nucleotides long), which provides a 3'-OH group to which DNA polymerase can attach DNA nucleotides. (Because primase is an RNA polymerase, it does not require a 3'-OH group to which nucleotides can be added). All DNA molecules initially have short RNA primers embedded within them; these primers are later removed and replaced by DNA nucleotides. On the leading strand, where DNA synthesis is continuous, a primer is required only at the 5' end of the newly synthesized strand. On the lagging strand, where replication is discontinuous, a new primer must be generated at the beginning of each Okazaki fragment (**Figure 12.13**). Primase forms a complex with helicase at the replication fork and moves along the template of the lagging strand.

The single primer on the leading strand is probably synthesized by the primase–helicase complex on the template of the lagging strand of the *other* replication fork, at the opposite end of the replication bubble.

DNA polymerases: After DNA is unwound and a primer has been added, DNA polymerases elongate the new polynucleotide strand by catalyzing DNA polymerization. The best-studied polymerases are those of *E. coli*, which has at least five different DNA polymerases. DNA polymerases synthesize DNA in the 5'→3' direction by adding new nucleotides to the 3' end of a growing nucleotide strand. Two of them, DNA polymerase I and DNA polymerase III, carry out DNA synthesis in replication; the other three have specialized functions in DNA repair.

DNA polymerase, characteristics and their function in *E. coli* are presented as follow

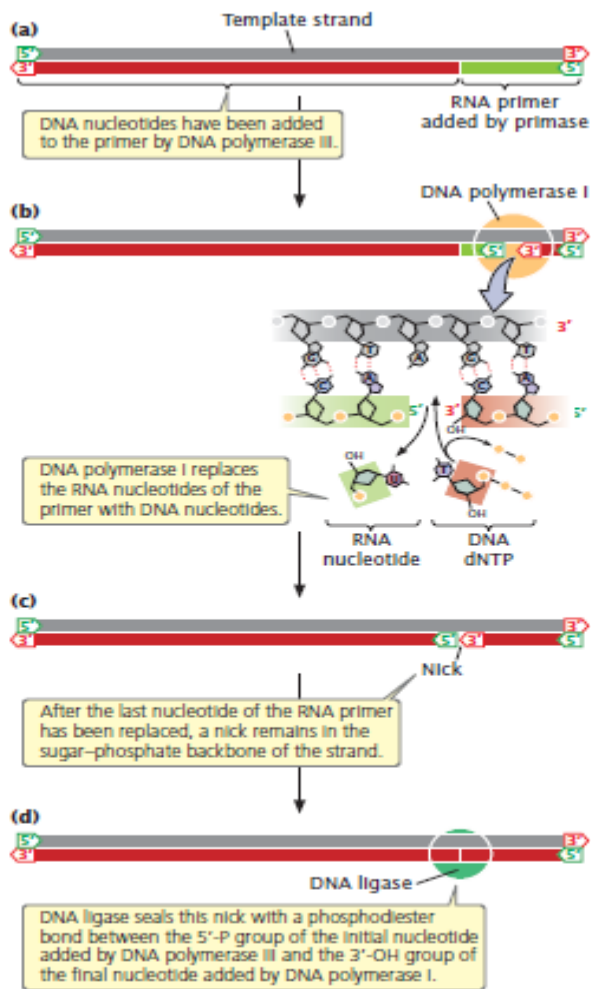
DNA polymerase	5'→3' Polymerization	3'→5' Exonuclease	5'→3' Exonuclease	Function
I	Yes	Yes	Yes	Removes and replace primers
II	Yes	Yes	No	DNA repair, restarts replication after damaged DNA halts synthesis
III	Yes	Yes	No	Elongates DNA
IV	Yes	No	No	DNA repair
V	Yes	No	No	DNA repair, translation, DNA synthesis



12.13 Primase synthesizes short stretches of RNA nucleotides, providing a 3'-OH group to which DNA polymerase can add DNA nucleotides.

DNA ligase: After DNA polymerase III attaches a DNA nucleotide to the 3'-OH group on the last nucleotide of the RNA primer, each new DNA nucleotide then provides the 3'-OH group needed for the next DNA nucleotide to be added. This process continues as long as template is

available (**Figure 12.14a**). DNA polymerase I follows DNA polymerase III and, using its 5'→3' exonuclease activity, removes the RNA primer. It then uses its 5'→3' polymerase activity to replace the RNA nucleotides with DNA nucleotides. DNA polymerase I attaches the first nucleotide to the OH group at the 3' end of the preceding Okazaki fragment and then continues, in the 5'→3' direction along the nucleotide strand, removing and replacing, one at a time, the RNA nucleotides of the primer (**Figure 12.14b**). After polymerase I has replaced the last nucleotide of the RNA primer with a DNA nucleotide, a nick remains in the sugar–phosphate backbone of the new DNA strand. The 3'-OH group of the last nucleotide to have been added by DNA polymerase I is not attached to the 5'-phosphate group of the first nucleotide added by DNA polymerase III (**Figure 12.14c**). This nick is sealed by the enzyme **DNA ligase**, which catalyzes the formation of a phosphodiester bond without adding another nucleotide to the strand (**Figure 12.14d**).



12.14 DNA ligase seals the nick left by DNA polymerase I in the sugar–phosphate backbone.

Some of the major enzymes and proteins required for prokaryotic

Component	Function
Initiator protein	Binds to origin and separates strands of DNA to initiate replication
DNA helicase	Unwinds DNA at replication fork
Single-strand-binding proteins	Attach to single-stranded DNA and prevent secondary structures from forming
DNA gyrase	Moves ahead of the replication fork, making and resealing breaks in the double-helical DNA to release the torque that builds up as a result of unwinding at the replication fork
DNA primase	Synthesizes a short RNA primer to provide a 3'-OH group for the attachment of DNA nucleotides
DNA polymerase III	Elongates a new nucleotide strand from the 3'-OH group provided by the primer
DNA polymerase I	Removes RNA primers and replaces them with DNA
DNA ligase	Joins Okazaki fragments by sealing nicks in the sugar–phosphate backbone of newly synthesized DNA

Elongation at the replication fork: Now that the major enzymatic components of elongation — DNA polymerases, helicase, primase, and ligase—have been introduced, let's consider how these components interact at the replication fork. Because the synthesis of both strands takes place simultaneously, two units of DNA polymerase III must be present at the replication fork, one for each strand. In one model of the replication process, the two units of DNA polymerase

III are connected (**Figure 12.15**); the lagging-strand template loops around so that it is in position for 5'→3' replication. In this way, the DNA polymerase III complex is able to carry out 5'→3' replication simultaneously on both templates, even though they run in opposite directions. After about 1000 bp of new DNA has been synthesized, DNA polymerase III releases the lagging-strand template, and a new loop forms. Primase synthesizes a new primer on the lagging strand and DNA polymerase III then synthesizes a new Okazaki fragment. In summary, each active replication fork requires five basic components

1. helicase to unwind the DNA,
2. single-strand-binding proteins to protect the single nucleotide strands and prevent secondary structures,
3. the topoisomerase gyrase to remove strain ahead of the replication fork,
4. primase to synthesize primers with a 3'-OH group at the beginning of each DNA fragment, and
5. DNA polymerase to synthesize the leading and lagging nucleotide strands.

Termination

In some DNA molecules, replication is terminated whenever two replication forks meet. In others, specific termination sequences block further replication. A termination protein, called **Tus** in *E. coli*, binds to these sequences. Tus blocks the movement of helicase, thus stalling the replication fork and preventing further DNA replication.

The Fidelity of DNA Replication: Overall, the error rate in replication is less than one mistake per billion nucleotides. How is this incredible accuracy achieved? DNA polymerases are very particular in pairing nucleotides with their complements on the template strand. Errors in nucleotide selection by DNA polymerase arise only about once per 100,000 nucleotides. Most of the errors that do arise in nucleotide selection are corrected in a second process called **proofreading**. When a DNA polymerase inserts an incorrect nucleotide into the growing strand, the 3'-OH group of the mispaired nucleotide is not correctly positioned in the active site of the DNA polymerase for accepting the next nucleotide. The incorrect positioning stalls the polymerization reaction, and the 3'→5' exonuclease activity of DNA polymerase removes the incorrectly paired nucleotide. DNA polymerase then inserts the correct nucleotide. Together, proofreading and nucleotide selection result in an error rate of only one in 10 million nucleotides. A third process, called **mismatch repair** corrects errors after replication is complete. Any incorrectly paired nucleotides remaining after replication produce a deformity in the secondary structure of the DNA; the deformity is recognized by enzymes that excise an incorrectly paired nucleotide and use the original nucleotide strand as a template to replace the incorrect nucleotide. Mismatch repair requires the ability to distinguish between the old and the new strands of DNA, because the enzymes need some way of determining which of the two incorrectly paired bases to remove. In *E. coli*, methyl groups (-CH₃) are added to particular nucleotide sequences, but only *after* replication. Thus, immediately after DNA synthesis, only the old DNA strand is methylated. It can therefore be distinguished from the newly synthesized strand, and mismatch repair takes place preferentially on the unmethylated nucleotide strand. No single process could produce this level of accuracy; a series of processes are required, each process catching errors missed by the preceding ones.

Eukaryotic DNA Replication Is Similar to Bacterial Replication but Differs in Several Aspects

Although eukaryotic replication resembles bacterial replication in many respects, replication in eukaryotic cells presents several additional challenges. First, the much greater size of eukaryotic genomes requires that replication be initiated at multiple origins. Second, eukaryotic chromosomes are linear, whereas prokaryotic chromosomes are circular. Third, the DNA template is associated with histone proteins in the form of nucleosomes, and nucleosome assembly must immediately follow DNA replication.

Eukaryotic Origins: Eukaryotic DNA contains many origins of replication. At each origin, a multiprotein origin-recognition complex binds to initiate the unwinding of the DNA. The **autonomously replicating sequences** (ARSs) enabled any DNA to which they were attached to replicate. The origins of replication of different organisms vary greatly in sequence, although they usually contain numerous A–T base pairs. In yeast, origins consist of 100 to 120 bp of DNA. A multiprotein complex, the origin-recognition complex (ORC), binds to origins and unwinds the DNA in this region. Interestingly, ORCs also function in regulating transcription.

The Licensing of DNA Replication: Eukaryotic cells utilize thousands of origins, and so the entire genome can be replicated in a timely manner. The use of multiple origins, however, creates a special problem in the timing of replication: the entire genome must be precisely replicated once and only once in each cell cycle so that no genes are left unreplicated and no genes are replicated more than once. How does a cell ensure that replication is initiated at thousands of origins only once per cell cycle? The precise replication of DNA is accomplished by the separation of the initiation of replication into two distinct steps. In the first step, the origins are licensed, meaning that they are approved for replication. This step is early in the cell cycle when a **replication licensing factor** attaches to an origin. In the second step, the replication machinery initiates replication at each *licensed* origin. The key is that the replication machinery functions only at licensed origins. As the replication forks move away from the origin, the licensing factor is removed, leaving the origin in an unlicensed state, where replication cannot be initiated again until the license is renewed. To ensure that replication takes place only once per cell cycle, the licensing factor is active only after the cell has completed mitosis and before the replication is initiated. The eukaryotic licensing factor is a complex called MCM (for minichromosome maintenance), which contains a DNA helicase that unwinds a short stretch of DNA in the initiation of replication. MCM must bind to the DNA for replication to initiate at an origin. After replication has begun at an origin, a protein called Geminin prevents MCM from binding to DNA and reinitiating replication at that origin. At the end of mitosis, Geminin is degraded, allowing MCM to bind once again to DNA and relicense the origin.

Unwinding: Several different helicases that separate double-stranded DNA have been isolated from eukaryotic cells, as have single-strand-binding proteins and topoisomerases (which have a function equivalent to the DNA gyrase in bacterial cells). These enzymes and proteins are assumed to function in unwinding eukaryotic DNA in much the same way as their bacterial counterparts do.

Eukaryotic DNA Polymerases: Some significant differences in the processes of bacterial and eukaryotic replication are in the number and functions of DNA polymerases. Eukaryotic cells contain a number of different DNA polymerases that function in replication, recombination, and DNA repair given in below table. Three DNA polymerases carry out most of nuclear DNA synthesis during replication: DNA polymerase α , DNA polymerase δ , and DNA polymerase ϵ .

DNA polymerases in Eukaryotic cell

DNA Polymerase	5'→3' Polymerase Activity	3'→5' Exonuclease Activity	Cellular Function
α (alpha)	Yes	No	Initiation of nuclear DNA synthesis and DNA repair; has primase activity
β (beta)	Yes	No	DNA repair and recombination of nuclear DNA
γ (gamma)	Yes	Yes	Replication and repair of mitochondrial DNA
δ (delta)	Yes	Yes	Lagging-strand synthesis of nuclear DNA, DNA repair, and translesion DNA synthesis
ϵ (epsilon)	Yes	Yes	Leading-strand synthesis
ξ (zeta)	Yes	No	Translesion DNA synthesis
η (eta)	Yes	No	Translesion DNA synthesis
θ (theta)	Yes	No	DNA repair
ι (iota)	Yes	No	Translesion DNA synthesis
κ (kappa)	Yes	No	Translesion DNA synthesis
λ (lambda)	Yes	No	DNA repair
μ (mu)	Yes	No	DNA repair
σ (sigma)	Yes	No	Nuclear DNA replication (possibly), DNA repair, and sister-chromatid cohesion
ϕ (phi)	Yes	No	Translesion DNA synthesis
Rev1	Yes	No	DNA repair