Basic requirements for DNA synthesis

- **Substrates.** The four deoxynucleoside triphosphates (dNTPs)—deoxyadenosine tri-phosphate (dATP), deoxyguanosine triphosphate (dGTP), deoxycytidine triphos-phate (dCTP), and deoxythymidine triphosphate (dTTP)—are needed as substrates for DNA synthesis. Cleavage of the high-energy phosphate bond between the a and (3 phosphates provides the energy for the addition of the nucleotide.
- **Template.** DNA replication cannot occur without a template. A template is required to direct the addition of the appropriate complementary deoxynucleotide to the newly synthesized DNA strand. In semiconservative replication, each strand of parental DNA serves as a template. Then, each template strand and its newly synthesized complementary strand serve as the DNA in daughter cells.
- **Primer.** DNA synthesis cannot start without a primer, which prepares the template strand for the addition of nucleotides. Because new nucleotides are added to the 3' end of a primer that is properly base paired to the template strand of DNA, **new synthesis is said to occur in a 5' to 3' direction.**
- **Enzyme.** The DNA synthesis that occurs during the process of replication is catalyzed by enzymes called **DNA-dependent DNA polymerases.** These enzymes depend on DNA to the extent that they require a DNA template. They are more commonly called DNA polymerases.

THE STRUCTURE OF DNA REVEALS A MECHANISM FOR ITS REPLICATION.

Genetic material must be able to be accurately replicated and passed on from one generation to the next. Although elegant experiments indicated that DNA could carry genetic information from one generation to the next, not until Watson and Crick discovered the structure of DNA was it understood how DNA might be replicated. The **double-helical model** of DNA suggested that the **strands can separate and act as templates for the formation of a new, complementary strand.** However, the structure of DNA did not reveal whether the DNA was replicated conservatively or semiconservatively.

Semiconservative replication was shown in experiments by Meselson and Stahl to be the mechanism by which replication takes place. After replication and cell division, each daughter cell receives one parental DNA strand and one newly synthesized complementary strand for which the parental strand was the template

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Multiple DNA polymerases with multiple enzymatic activities.

	DNA Polymerase		
Feature	I	II	III
5' → 3' exonuclease activity	+	-	-
3' → 5' exonuclease activity	+	+	+
Synthesis rate (nucleotides/minute)	600	30	30,000
Molecules/cell	400	?	10
Replication	+	-	+
Repair	+	+	—

DNA polymerase II (pol II) is a minor DNA polymerase in *E. coli*.

a. Function. Pol II may be involved in some DNA repair processes, but *E coli* mutants lacking this enzyme show no replication or growth deficiencies.

b. Structure. Pol II is a single polypeptide.

c. Other enzymatic activities. Pol II has proofreading (3' to 5' exonuclease) activity but lacks excision-repair (5' to 3' exonuclease) activity.

3. DNA polymerase III (pol III) is the primary DNA polymerase involved in cellular replication.

a. Function. Pol III catalyzes leading and lagging strand synthesis.

b. Structure. Pol III is structurally complex. The **pol III core enzyme,** made of three different polypeptides, was first isolated. Gentle purification schemes and careful reconstitution showed that the active cellular form of pol HI, called **pol III holoenzyme,** is made of 10 subunit polypeptides. The subunits are organized into an asymmetric dimeric structure with two catalytic centers. This is important in understanding how it catalyzes both leading and lagging strand syntheses at the replication fork.

c. Other enzymatic activities. Pol III holoenzyme has proofreading (3' to 5' exonuclease) activity but no excision-repair (5' to 3' exonuclease) activity. The two α subunits both contain the DNA polymerase activity, and the two ε subunits both contain the 3' to 5' exonuclease activity. The two subunits form a dimer that circles around the DNA. This β_2 "DNA clamp" enables the pol III holoenzyme to stay bound to the DNA and facilitates its high rate of replication.

DNA polymerase I (pol I) was the first DNA polymerase discovered.

a. Function. Pol I functions in the **replication of DNA** and in the **repair of damaged DNA**.

b. Structure. Pol I is a single polypeptide.

c. Other enzymatic activities. Pol I has two enzymatic activities—besides DNA polymerase activity—that are important to its cellular function.

(1) Proofreading. Pol I does not typically add a nucleotide to the growing DNA chain that cannot properly base pair with the template strand. If a mismatched nucleotide is added, the enzyme halts polymerization. **A 3'- to 5'-exonuclease** activity removes the mismatched nucleotide, and polymerization resumes. This activity is called proofreading, and it assures the high-level fidelity of replication that is a desirable trait of genetic material.

(2) Excision-repair. **Pol I has a** 5' **to 3' exonuclease activity,** called excision-repair activity, that can hydrolytically remove a segment of DNA from the 5' end of a strand of duplex DNA.

(a) From 1-10 nucleotide segments of DNA can be removed simultaneously.

(b) This activity is essential for the removal of primers in DNA replication.

(c) This activity is essential for the repair of damaged DNA.

(3) Nick-translation. A **nick** is a break in a phosphodiester bond of one strand of DNA in a double helix. A nick leaves a free 3'-hydroxyl and a free 5'-phosphate. Pol I can function at nicks as an exonuclease and a polymerase at the same time. As the 5'-phosphate nucleotide is removed or displaced by pol I, it is replaced with the polymerase activity of pol I. Pol I cannot reseal the nick; therefore, the nick is moved, or **translated,** along the DNA in the direction of synthesis.

Replication forks. After initiation, replication has been observed (using radio-graphic electron microscopic techniques) proceeding away from the origin. In most organisms, replication proceeds **bidirectionally** from the origins as **replication forks**. Replication forks represent unwound parental template DNA strands to which newly synthesized complementary DNA is paired.

Basic molecular events at replication forks. Because the molecular events that occur at replication forks in *E. coli* are well described, there is a good understanding of how new DNA synthesis takes place on both of the parental DNA strands at replication forks.

1. Leading strand synthesis is the continuous synthesis of one of the daughter strands in a 5' to 3' direction. **Pol III catalyzes leading strand synthesis.**

2. Lagging strand synthesis

Okazaki fragments. One of the newly synthesized daughter strands is made **discontinuously.** The resulting short fragments are called Okazaki fragments. These fragments are later joined by DNA ligase to make a continuous piece of DNA. This is called lagging strand synthesis. Discontinuous synthesis of the lagging strand occurs because DNA synthesis always occurs in a 5' to 3' direction**.** **Pol III catalyzes lagging strand synthesis.**

Direction of new synthesis. As the replication fork moves forward, leading strand synthesis follows. A gap forms on the opposite strand because it is in the wrong orientation to direct continuous synthesis of a new strand. After a lag period, the gap that forms is filled in by 5' to 3' synthesis. This means that new DNA synthesis on the lagging strand is actually moving away from the replication fork.

Priming of Okazaki fragment synthesis

(1) Enzyme. An enzyme called **primase** is the catalytic portion of a **primosome** that makes the RNA primer needed to initiate synthesis of Okazaki fragments. It also makes the primer that initiates leading strand synthesis at the origin.

(2) Primers provide a 3'-hydroxyl group that is needed to initiate DNA synthesis. The primers made by primase are small pieces of RNA (4-12 nucleotides) complementary to the template strand.

The role of pol I in replication. On completion of lagging strand synthesis by pol III, **the RNA primer is then removed by pol I and replaced with DNA.** Synthesis of each new Okazaki fragment takes place until it reaches the RNA primer of the preceding Okazaki fragment. This effectively leaves a nick between the newly synthesized Okazaki fragment and the RNA primer. DNA pol I uses its nick-translation properties to hydrolyze the RNA (5' to 3' exonuclease activity) and replace it with DNA.

Joining of Okazaki fragments. After pol I has removed the RNA primer and replaced it with DNA, an enzyme called **DNA ligase** catalyzes the formation of phosphodiester bonds between the adjoining fragments.

Other factors needed for propagation of replication forks

1. **Helicases** are enzymes that catalyze the unwinding of the DNA helix. A helicase derives energy from cleavage of high-energy phosphate bonds of nucleoside tri-phosphates, usually ATP, to unwind the DNA helix. **Helicase activity provides single-strand templates for replication.**
   a. **Proteins with helicase activity.** A number of proteins have been isolated that have helicase activity. Most of these proteins play a role in DNA repair, recombination, or bacteriophage replication.
   b. **The dnaB protein** is the principal helicase of *E. coli* replication and is a component of the primosome.
2. **Gyrase.** Positive supercoils would build up in advance of a moving replication fork without the action of gyrase, which is a topoisomerase.
3. **Single-strand binding protein** (SSBP) is an important component of replication. As its name implies, it binds to single-stranded DNA.
   a. **Function**
      (1) SSBP **enhances the activity of helicase and binds to single-strand template DNA** until it can serve as a template.
      (2) SSBP may serve to protect single-strand DNA from degradation by nucleases, and it may block formation of intrastrand duplexes of hairpins that can slow replication.
   b. **Release.** SSBP is displaced from single-strand DNA when the DNA undergoes replication.

4. Primosome

a. **Definition.** The primosome is a complex of proteins that comprises primase, a hexamer of the helicase dnaB protein, dnaC protein, and several other proteins: n, n', n", and i.

b. **Function.** The primosome complex primes DNA synthesis at the origin. Driven by ATP hydrolysis, the primosome moves with the replication fork, making RNA primers for Okazaki fragment synthesis.

The replisome. It is believed that all the replication enzymes and factors are part of a large macromolecular complex called a replisome. It has been suggested that the replisome may be attached to the membrane and that instead of the replisome moving along the DNA during replication, DNA is passed through the stationary replisome.

Termination of replication. Termination sequences (e.g., ter) direct termination of replication. A specific protein—the termination utilization substance (Tus) **protein**—binds to these sequences and prevents the helicase dnaB protein from further unwinding DNA. This facilitates the termination of replication.

EUKARYOTIC REPLICATION. Eukaryotes represent a diversity of organisms that may utilize slightly different mechanisms of replication. However, most of these mechanisms are very similar to those in prokaryotic replication. This section reviews replication only in mammalian cells. As with prokaryotic replication, mammalian replication is semiconservative and proceeds **bidirectionally from many origins.**

Replicons are basic units of replication.

1. Function. A replicon encompasses the entire DNA replicated from the growing replication forks that share a single origin.

2. Size. Replicons may vary in size from 50-120μm. The *E. coll* genome, with its single origin, is essentially one large replicon. There are estimated to be anywhere from 10,000-100,000 replicons per cell in mammals. The large number of replicons is needed to replicate the large mammalian genomes in a reasonable period DNA Replication and Repair of time. It takes approximately 8 hours to replicate the human genome.

3. Replication rate

a. Prokaryotes. An *E. coli* replication fork progresses at approximately 1000 base pairs per second.

b. Eukaryotes. The eukaryotic replication rate is about 10 times slower than the prokaryotic replication rate. Eukaryotic replication forks progress at approximately 100 base pairs per second. Each replicon completes synthesis in approximately 1 hour. Therefore, during the total period of eukaryotic replication, not every replicon is active. The slow rate of eukaryotic replication is likely due to interference of nucleosomes and chromosomal proteins. Heterochromatin is known to replicate slower than euchromatin.

Multiple eukaryotic DNA polymerases. Eukaryotes contain at least four different nuclear DNA polymerases (i.e., α, β, δ, and ε) and one mitochondrial DNA polymerase (γ).

1. **DNA polymerase α is essential for replication.**
    a. DNA polymerase α has three associated subunit polypeptides. Two of these have primase activity, and the function of the third is unknown.
    b. DNA polymerase α is responsible for initiation at origins and of Okazaki fragments. It is therefore required for both leading and lagging strand synthesis.
2. **DNA polymerase δ also is essential for replication.** It is required for both leading and lagging strand synthesis.
    a. Pol δ is associated with another protein called **proliferating cell nuclear antigen** (PCNA). PCNA is analogous to the dimeric β subunits of *E. coli* pol III. It also forms a DNA clamp that enables pol δ to stay bound to DNA and sustain a high rate of replication.
    b. After pol α has initiated replication, a protein called **replication factor C (RF-C)** facilitates the inhibition and replacement of pol a with both PCNA and pol δ in an ATP-dependent manner.
3. **DNA polymerase β** plays **no role in replication and acts only in DNA repair synthesis.**
4. **DNA polymerase ε also is essential for replication,** although its exact role is not clearly defined. It behaves similarly to pol δ and may support some component of lagging strand synthesis.
5. **DNA polymerase γ** resides in and replicates **mitochondrial DNA.**

Telomeres. Unlike prokaryotes, eukaryotic DNA is linear and not circular. Because all DNA synthesis requires a primer, DNA would be lost at the lagging strand ends unless replication of ends proceeds by a different mechanism than all other DNA. Eukaryotic chromosomes have unique sequences at their ends called telomeres. A specialized DNA polymerase called **DNA telomerase** replicates telomeric ends.

- **Structure of telomeres.** Mammalian telomeres are short, tandem repeats of the sequence TTAGGG. The 3' end of these repeats is single stranded.
- **The mechanism of telomere replication** is shown in Figure 8-8. The essence of this mechanism is that the telomerase provides an RNA template complementary to the telomeric repeat, and the free 3' end of the telomere is the primer for new DNA synthesis. After elongation of the telomere by telomerase, normal lagging strand synthesis presumably makes a complementary copy of all but the 3' most terminal sequences.
- **Clinical relevance of telomeres.** The number of telomere repeats varies in the cells of different tissues. This difference in telomere length represents a shortening of telomeres that occurs during the replication in some cells but not in others. This shortening occurs because of the absence of telomerase. There is a correlation in tissue-culture cells of the presence of telomerase and the immortality of the cells. Of potential medical relevance is the fact that cancer cells, which are immortal in culture, have high levels of telomerase.

Properties of Eukaryotic DNA Polymerases

Feature	DNA Polymerase*				
	α	β	δ	γ	ε
Location	Nucleus	Nucleus	Nucleus	Mitochondria	Nucleus
5' → 3' exonuclease activity	-	-	-	-	-
3' → 5' exonuclease activity	-	-	+	+	+
Primase activity	+	-	-	-	-
Replication	+	-	+	+	+
Repair	-	+	-	-	-

The nomenclature of the DNA polymerases is for the mammalian enzymes. Analogous, but differently named, DNA polymerases are present in all eukaryotes.